Chemical proteomics strategies for elucidation of cellular steroid hormone targets

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102 5,7-Dimethyl-2,3-dihydro-1H-1,4-diazepin-1-ium perchlorate

103 {12-[[[8,9-Dihydro-1-[2-[2-[2-[[3-(*tert*.-butoxycarbonyl)amino-2-[(9-fluorenyl)methoxycarbonyl]aminopropanamido]ethoxy]ethoxy]ethoxy]ethyl]-1H-dibenzo[3,4:7,8]cycloocta[1,2-d]triazol-9-yl]oxy]carbonyl]amino}-3,6,10-trioxadodecanoic acid amide; {12-[[[8,9-Dihydro-3-[2-[2-[2-[[3-(*tert*.-butoxycarbonyl)amino-2-[(9-fluorenyl)methoxycarbonyl]aminopropanamido]ethoxy]ethoxy]ethoxy]ethyl]-1H-dibenzo[3,4:7,8]cycloocta[1,2-d]triazol-9-yl]oxy]carbonyl]amino}-3,6,10-trioxadodecanoic acid amide

2 Abbreviations

9-BBN	9-borabicyclo[3.3.1]nonane
7TM	seven-transmembrane
aa	α-amino acid
AcOH	acetic acid
ACTH	adrenocorticotropic hormone
Akt	protein kinase B
Alloc	Allyloxycarbonyl
AP	acetophenone
Ar	aryl
AR	androgen receptor
aq.	aqueous
Boc	*tert.*-butoxycarbonyl
BP	benzophenone
Bu	butyl
calcd	calculated
CMA	*O*-(carboxymethyl)oxyamine
CMO	*O*-(carboxymethyl)oxime
CYP	cytochrome P450
Cys	cysteine
d	days
d	doublet (NMR)
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCC	dicyclohexylcarbodiimide
DIC	diisopropylcarbodiimide
DCM	dichloromethane
DC-urea	N,N`-dicyclohexylurea
dd	duplet from duplet (NMR)
Dex	dexamethasone
DHT	5α-dihydrotestosterone
DIPEA	N,N-diisopropylethylamine
DMAP	4-N,N-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide

DMP	Dess-Martin periodinane
DMSO	dimethylsulfoxide
DTT	dithiothreitol
E_2	estradiol
EA	ethyl acetate
EDC	*N*-(3-dimethylaminopropyl)-*N`*-ethylcarbodiimide
EL	electrophoresis
EG	ethylene glycol
ER	estrogen receptor
ERK	extracellular-signal regulated kinase
Et	ethyl
eq.	equivalents
FAB	fast atom bombardment
FKBP	FK-506 binding protein
Fmoc	(9-fluorenyl)methoxycarbonyl
FT-ICR	Fourier-transform ion cyclotron resonance
GR	glucocorticoid receptor
GnRH	gonadotropin releasing hormone
HBTU	*O*-(benzotriazol-1-yl)-*N*,*N*,*N'*,*N'*,-tetramethyluronium hexafluorophosphate
HC	hydrocortisone
HOBt	1-hydroxybenzotriazole
Hsp	heat shock protein
Im	imidazole
LBD	ligand binding domain
LC	liquid chromatography
LH	luteinizing hormone
Lys	lysine
MALDI	matrix-assisted laser-desorption/ionization
MAPK	mitogen-activated protein kinase
Me	methyl
min	minutes
MR	mineralocorticoid receptor
MS	mass-spectrometry
NLS	nuclear localization signal

NMR	nuclear magnetic resonance
NR	nuclear receptor
P_4	progesterone
PAL	photoaffinity labeling
PAT	palmitoyl acyl-transferase
PBS	phosphate buffered saline
PE	light petroleum ether
PFP	pentafluorophenol
Ph	phenyl
*p*NP	*para*-nitrophenyl
PR	progesterone receptor
Py	pyridine
PyBop	(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
q	quartet (NMR)
quant.	quantitative
R_t	retention time
R_f	retardation factor
RT	room temperature
SAR	structure activity relationship
sat.	saturated
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SH	sex-hormone
soln.	solution
T	testosterone
t	triplet (NMR)
TBAF	tetrabutylammonium fluoride
TBDMS	*tert*-butyldimethylsilyl
TFA	trifluoroacetic acid
TFAc	trifluoroacetyl
THF	tetrahydrofurane
TLC	thin layer chromatography
Vim	vimentin

3 Introduction

In recent years, it has become more and more realized that for successful drug development the accurate knowledge of the structures interacting with the drug candidate in the cellular environment is of crucial importance. Assembling the "interactome" of a drug candidate does not only facilitate the prediction of side effects caused by specific interaction with so called "off-targets", but also enables the researcher to explore new pathways of reactivity. Hereby, new druggable targets and leads for drug optimization or multi-target drug approaches can be revealed.[1-3]

Likewise, information about the physiological targets of small molecules is of special interest in chemical biology. Chemical biologists assume that for every structure in living cells there exists an affector molecule e.g. a synthetic small molecule, a complex synthetic construct or a natural product, promoting or inhibiting its function.[4] Hereby, these molecular affectors are used as tools for directed manipulation of cellular functions. The ultimate goal is to gain a deeper understanding of the molecular mechanisms of action on a cellular level. Prominent examples for small molecular tools frequently used in biochemical investigations are the inhibitors of transcription actinomycin D and cycloheximide, semi-synthetic nuclear receptor modulators like mifepristone (RU486) or methyltrienolone (R1881) and the carcinogenic phorbol esters.

Both, medicinal chemistry and chemical biology, heavily rely on large libraries of synthetic molecules and natural products to obtain active compounds. Accordingly, both disciplines suffer from the same "target-ID" problem, namely discovering the suitable affector for the target structure examined or *vice versa*.[4] This search for the "needle in a haystack" necessitates the access to appropriate methods for target deconvolution in a high throughput manner.

Indeed, drug discovery underwent several quantum leaps in the recent past owing to the development of high throughput screening technologies like chemical micro-arrays[4] and chemical genetics methods like the yeast- and mammalian-three-hybrid assay[3-6] and the phage-display assay[3, 4, 7]. Likewise, the rapid evolution of mass spectrometry (MS)-based bio-analytical methods in combination with affinity purification techniques crucially contributed to the developments in the field.[8, 9] In this regard, affinity purification resembles the most simple and widespread approach for the identification of cellular target structures.

Developed in the late 1960s, affinity purification based techniques are enjoying sustained popularity since these methods are unbiased of assumed drug targets and produce large

amounts of data about possible targets. However, confirmation of the direct drug-target interaction and its physiological relevance are very time-consuming and thus considered to be the bottleneck for a high throughput workflow.[10]

Vast compound libraries for affector screenings are typically derived from diversity-oriented solid phase organic synthesis or natural sources. Both of them allow access to an immense number of small molecules. Mutant synthesis of novel natural products in genetically altered microorganisms further augments the resource of potential bioactive compounds.[11] Nonetheless, for many endogenous small molecules and their metabolites showing hormonal or second messenger activity some of the actual cellular target structures they affect remain unknown. In particular this holds true for the endogenous steroid hormones. They unfold their physiological effects either in a classical genomic manner through corresponding nuclear receptors or via activation of membrane-bound nuclear receptors, non-classical receptors and other cellular target structures.[12, 13]

The endogenous steroid hormones play important roles in the regulation of physiological functions in mammals. Such functions comprise among others the development and maintenance of male and female phenotype (sex-hormones), the electrolyte and blood pressure homeostasis (mineralocorticoids) and the metabolism of carbohydrates, fatty acids and amino acids (glucocorticoids). Moreover, the steroid hormones or their synthetic analogs are important agents in clinical therapy.

The receptor-mediated molecular actions of the male sex hormone testosterone, the female sex hormones estradiol and progesterone are linked to major pathological conditions like prostate- and breast- cancer, respectively. Sex hormones also deploy anti-inflammatory and immunosuppressive properties in a gender dependent manner.[14] Likewise, the vital glucocorticoids exhibit anti-inflammatory and immunosuppressive properties. In fact, the family of glucocorticoids covers some of the most potent agents with anti-inflammatory activity currently available and thus is indispensable to the treatment of inflammation-associated diseases like rheumatism or asthma.[15] When administered as drugs, all steroid hormones cause systemic side effects by interfering with the physiological hormone balance. The severity of side effects varies with the type of steroid hormone applied, the dosage and formulation of the agent and not least with the physiological status of the patient.

Steroid hormone actions mediated by classical nuclear receptors are comparatively well characterized.[12, 15] On the contrary, effects mediated by non-genomic reaction pathways are poorly understood, mostly due to the lack of knowledge about the molecular targets responsible for this type of steroid hormone action. In their 2010 position paper Wendler et al.

[16] indicated the need for an increased research effort on identification and validation of the physiological mediators of steroid hormone action. Of special interest ought to be the elucidation of underlying reaction mechanisms with emphasis on clinical relevance of the cellular effects.

Building on the experience of some of our colleagues with the pharmacology and biochemistry of steroid hormones, an interdisciplinary research unit was launched consisting of pharmacists, biochemists and synthetic chemists. The objective of this research unit is the development of new methologies for target identification and the examination of the non-genomic mechanisms of steroid hormone action.

In this regard, the aim of the given work was to develop strategies for the synthesis of biological active derivatives of the steroid hormones progesterone, testosterone, hydrocortisone and dexamethasone suitable for affinity purification of steroid-binding proteins. Furthermore, "catch-and-release"-strategies employing photoreactive steroidal probes should be developed in order to increase the efficiency of target identification and gain additional information about the target structures. In combination with modern MS-based bio-analytics these affinity-based approaches ought to yield the framework of the cellular "interactome" of the mentioned steroid hormones. The insights gained into protein-steroid interaction were not only intended to offer new impetus for pharmacological research on hormonally active agents, but were also meant to deliver a broader understanding of the complexity of steroid hormone regulation in a cellular context.

4 General considerations

4.1 "Target-fishing"

The development of affinity purification-based methods for isolation and characterization of cellular target structures (in laboratory parlance "target-fishing") with affinity matrices bearing bioactive small molecules, proteins or oligonucleotides was initiated in the late 1960s.[17] Since then, these methods have constantly been refined and applied successfully for the target identification of numerous important bioactive compounds.[3, 4, 10]

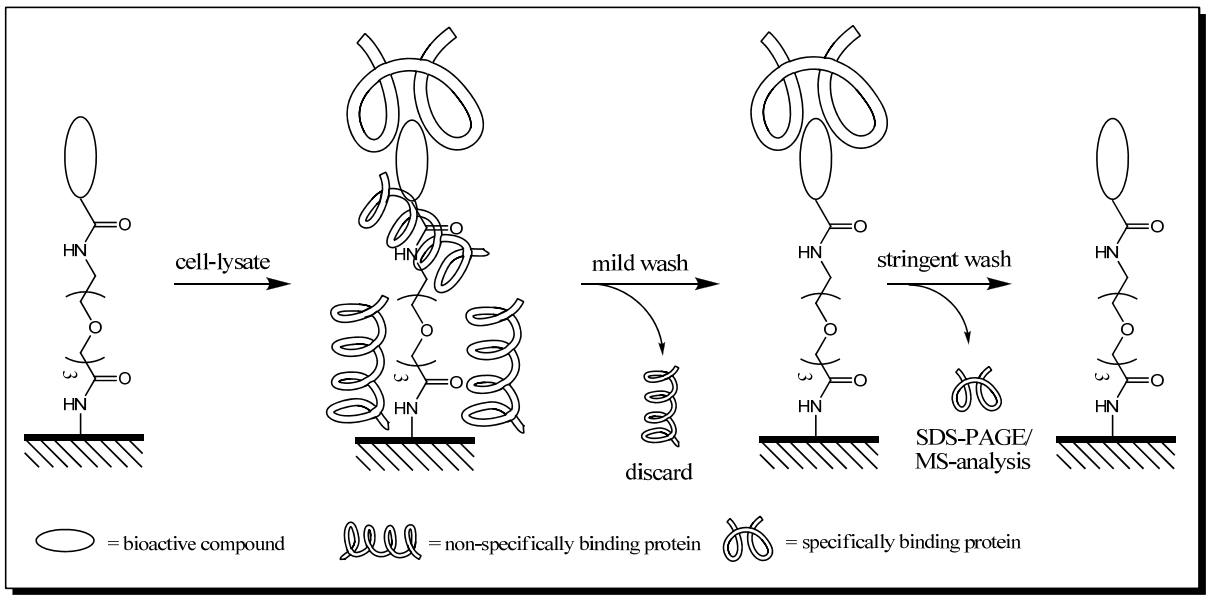
This rather simple approach is based on chemical modification and subsequent covalent attachment of the bioactive molecule to a solid support (immobilization) e.g. sepharose or suitable synthetic polymers (see section **4.1.1**). The obtained affinity matrices are then exposed to a protein source like a fractionated or whole cell lysate. Proteins with a certain affinity to the immobilized bioactive compound are thought to be adsorbed more tightly than unspecifically bound proteins. The specifically (strongly) bound proteins can thus be isolated by first eluting the non-specifically (weakly) bound proteins by mild washing of the solid support and afterward eluting under more abrasive conditions (Scheme 1). The protein samples obtained from the affinity purification step are then analyzed by 1D- or 2D-gel electrophoresis and mass spectrometry (MS) to yield target candidates.

Since proteins do not exclusively adsorb to the functionalized beads due to specific interaction by means of the "key-lock" principle the ideal case is oftentimes not attained in the laboratory. Proteins are also strongly adsorbed due to physical affects like increased lipophilicity of the matrices or charged groups leading to precipitation.[18, 19] Accordingly, often large numbers of proteins are obtained, especially of the highly abundant ones, complicating the analysis of 1D- and 2D-gels obtained from cell lysates. Another major drawback of the method is the necessity to chemically modify the applied ligand. Ligand derivatization involves the proper study of its structure-activity relationship (SAR) and regularly considerable synthetic effort to modify an appropriate functional group on the ligand. Since in some cases neither SAR-data nor appropriate functional groups are available, a "target-fishing"-project can turn out to be a challenging task. Nonetheless, this approach led to the identification of the physiological targets of a series of important bioactive small molecules like the natural products FK-506 (FK506 binding protein, FKBP12),[20] Trapoxin (histone-deacetylase, HDAC) [21] and the boswelic acids (Cathepsin G),[22] among others.

One of the pre-eminent advantages of affinity purification-based methods are their impartiality for certain target structures that are known to be related to the bioactive

compounds action. Thus, completely new and unexpected target structures can be identified, leading to the discovery of new druggable targets and bioactive compounds.

In order to overcome the disadvantages, various approaches have been developed over the last decades including more sophisticated solid supports, linker systems and synthetic methods for ligand derivatization. Development and application of cleavable linkers, reactive affinity- or activity-based probes and especially the avidin-biotin system for tagging, identification, purification and isolation of target proteins further broadened the scope of affinity purification-based methods (see below).



Scheme 1: Schematic course of a "target-fishing"-experiment. The bioactive compound is immobilized on a solid support and incubated with a protein source. Non-specifically bound proteins are washed off in a mild washing step. Specifically bound proteins are eluted by a more stringent washing step and analyzed by gel-electrophoresis/MS-analysis.

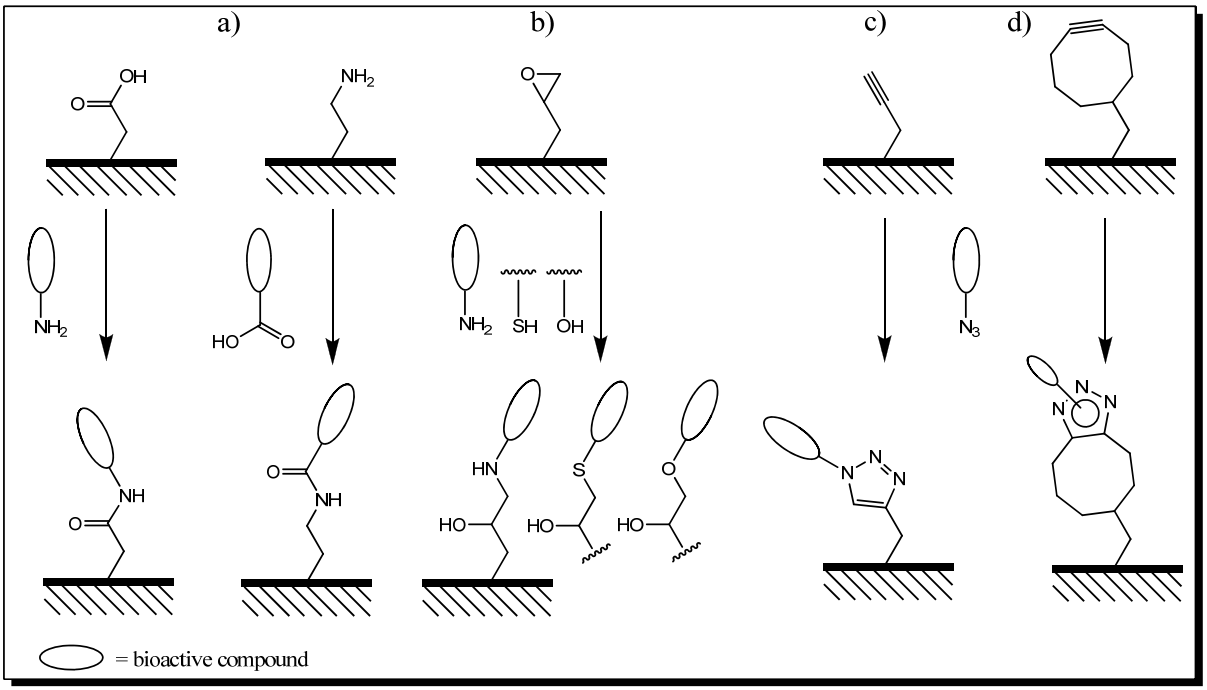
4.1.1 Matrices for affinity purification

In recent years various natural as well as synthetic polymers were proposed as suitable solid supports for affinity purification and some of them are now commercially available. Among the most prominent are cross-linked agarose (Affi-Gel®, Sepharose®), ethylene glycol conjugated poly styrene (Tentagel®)[23] and poly(methacrylate)-based polymers (e.g. TOYOPEARL®). Most recently polymer coated, paramagnetic nanoparticles were introduced as suitable solid supports for "target-fishing".[4, 24, 25] The hydrophilic agarose resins perform very well in affinity purification, especially regarding the reduction of non-specific binding.[18, 19] However, their applicability is restricted to water or aqueous organics as solvents, precluding the use of protocols regularly applied in solid phase organic chemistry.

Other attractive options are ethylene glycol-conjugated poly styrene beads like Tentagel® and the poly(methacrylate)-based TOYOPEARL®. Since these types of polymers were originally developed for solid phase organic synthesis, they exhibit good resilience for a wide scope of synthetic reagents and conditions. On the other hand, due to its relative hydrophobic nature, enhanced unspecific binding of abundant proteins e.g. actin and tubulin was observed for TOYOPEARL®.[18] The hydrophobicity of poly(methacrylate)-beads can be partially countered by use of hydrophilic spacer groups conjugated to the bioactive compound (see below). The highly hydrophilic Tentagel® does not show the tendency for enhanced unspecific protein binding. Therefore it should be an ideal solid support for affinity purification.

4.1.2 Immobilization techniques

The classical methods to immobilize bioactive compounds on solid supports like the above mentioned Affi-Gel®, Sepharose® and TOYOPEARL®, are based on peptide coupling chemistry or epoxide opening with an appropriate nucleophile (Scheme 2). Most of these resins are commercially available as their amino-, carboxy- and epoxy functionalized variants.



Scheme 2. Selection of coupling reactions suitable for immobilization of macromolecules and small molecules. a) and b) Amide coupling techniques. c) Epoxide ring opening with appropriate nucleophiles. c) Copper-catalyzed Huisgen-cycloaddition between an azide and a terminal alkyne (archetypical "click-reaction"). d) Strain promoted Huisgen-cycloaddition reaction with a cyclooctyne derivative.

The epoxy-resins can be coupled to ligands bearing either a hydroxyl function under basic conditions (pH ~13) or at milder pH-values with thioles and amines (pH ~10).

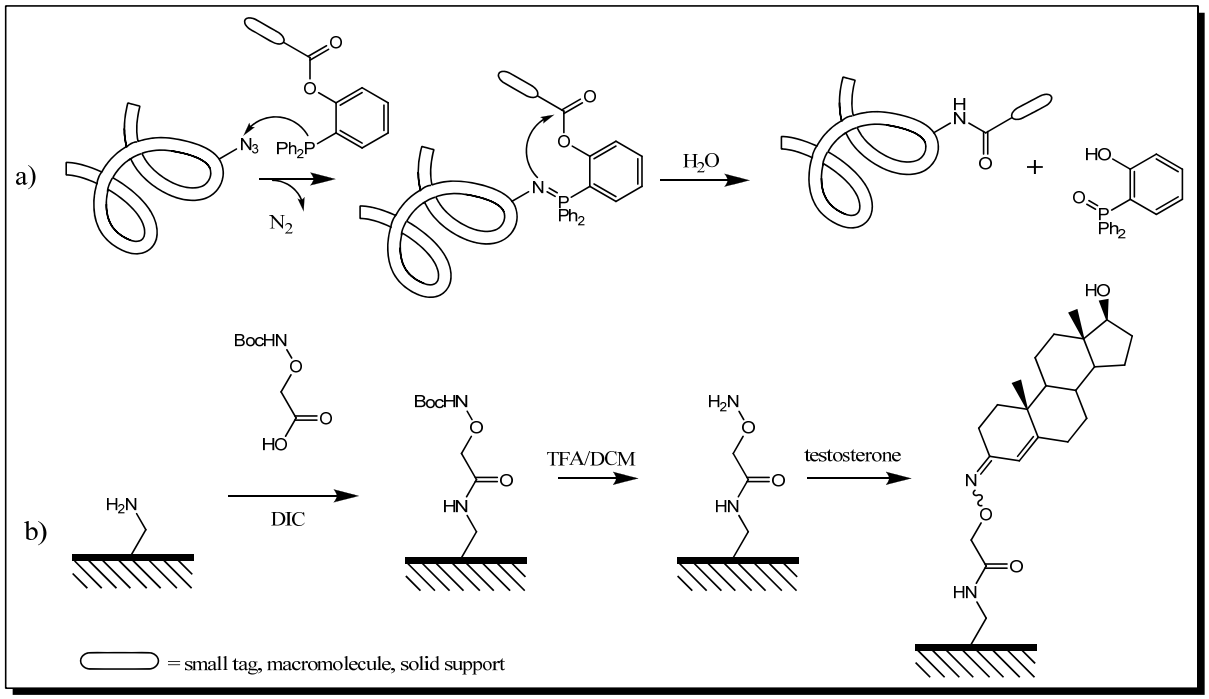
In recent years a series of more sophisticated coupling methods, e.g. the copper catalyzed Huisgen-cycloaddition reaction (archetypical "click-reaction"),[26, 27] the Staudinger-ligation reaction[28-30] and the oxime-ligation reaction[31] (Scheme 3) have successfully been applied for immobilization of macromolecules as well as small bioactive compounds.

The copper catalyzed Huisgen-cycloaddition reaction between an azide moiety and a terminal alkyne offers the advantage of mild reaction conditions and leads usually to high yields (>90%). Additionally, since azides and terminal alkynes are not present in natural products or endogenous molecules, the reaction can be considered highly chemoselective and was hence termed "bioorthogonal". The same applies to the Staudinger-ligation reaction between an azide and a suitable phosphine derivative. Staudinger-ligation offers the additional advantage of working without any additive. Due to the assumed bioorthogonality, the Staudinger-ligation reaction was applied in the chemo- and regioselective derivatization of biomolecules containing unnatural azide-functionalized glycosides and amino acids in live cells.[29, 30, 32, 33] However, since it is known for a long time that phosphine reagents rapidly cleave disulfides, especially in aqueous media, some doubts remain about the true bioorthogonality of Staudinger-ligation reaction.[34]

Application of the copper catalyzed Huisgen-reaction in live systems is limited due to the cytotoxicity of copper(I). Quite recently a copper free strain-promoted version of the Huisgen-cycloaddition between azides and suitable cyclooctyne-derivatives has been developed to overcome this disadvantage. The strain promoted "click-reaction" was successfully applied for real time fluorescence imaging of live cells containing azide-functionalized glycans[35, 36] with fluorescent dyes bearing a strained alkyne residue. The convenience of the coupling protocols and the high yields obtained (usually >90 % in homogenous phase) led to adaption of the strain promoted "click-reaction" into oligonucleotide synthesis,[37, 38] immobilization techniques for fluorescent probes[39] and not least the catch-and-release approach presented in this work (see section **4.1.6**).

Though not truly bioorthogonal, the chemoselective oxime-ligation between an aldehyde or a ketone and a suitable aminooxy ether derivative has been used for biomolecule modification. Especially the anilinium ion catalyzed variant was exploited to modify proteins,[31, 40, 41] glycans[42] and glycolipids.[43] Oxime ligation has also been applied previously for the immobilization of aldehyde/ketone-bearing macromolecules on aminooxy-functionalized surfaces.[44] The latter principle was also tested for immobilization steroidal ketones on

(carboxymethyl)oxyamine-(CMA-) functionalized TOYOPEARL® to form affinity matrices suitable for affinity purification (Scheme 3).[46]



Scheme 3. Selection of coupling reactions suitable for immobilization of macromolecules and small molecules. **a)** Schematic course of the Staudinger-ligation reaction with nucleophilic attack of the phosphine on the azide moiety, loss of nitrogen, formation of the phosphazene intermediate and intramolecular capture of the acyl group forming a stable amide. Shown is the attachment of a variable tag to an azide functionalized macromolecule using an *O*-acyl-*o*-hydroxyphenylphosphine-derivative.[45] **b)** Preparation of an aminooxy-functionalized solid support by coupling of *N*-Boc-*O*-(carboxymethyl)oxyamine to an amino-functionalized matrix and subsequent Boc-deprotection. Ketones or aldehydes (e.g. testosterone) can be coupled directly to the solid support via oxime-ligation reaction.

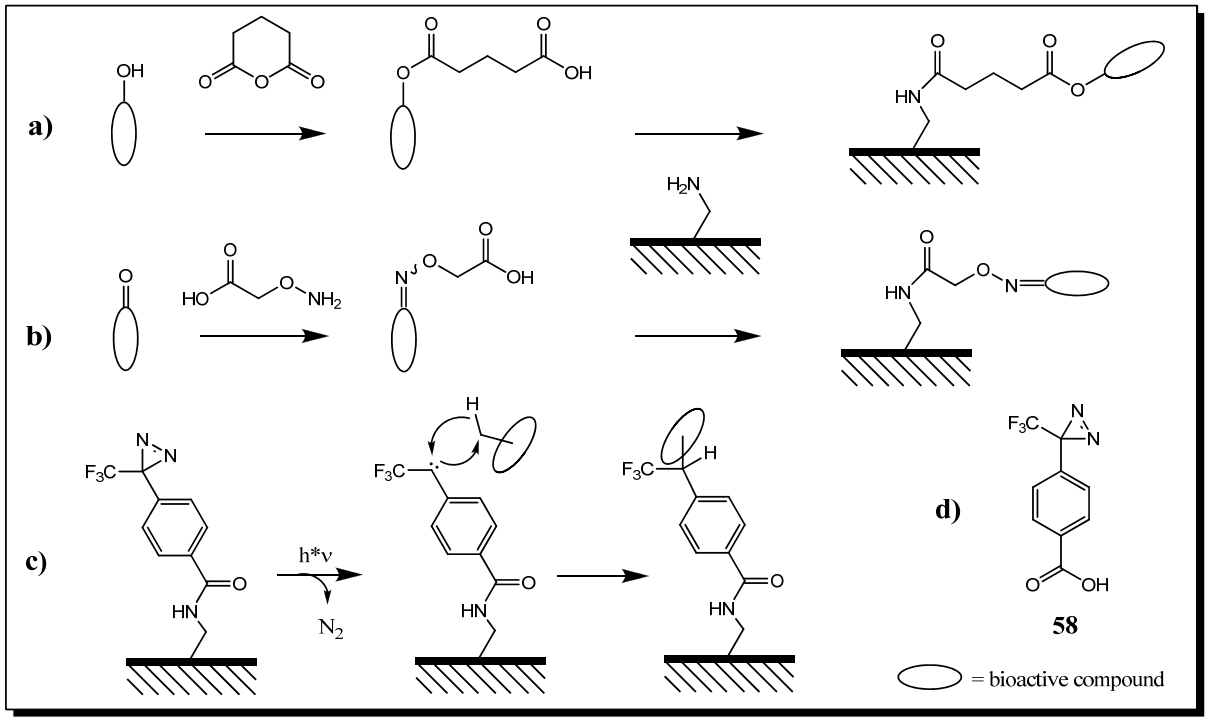
4.1.3 Ligand derivatization

Regarding the derivatization of bioactive compounds, one has to keep in mind that usually the chemical modification diminishes bioactivity to a certain extent. In order to minimize this effect the site of derivatization should be chosen distinctly from the essential pharmacophore of the molecule.[10, 47] Furthermore, the chemical nature of the functional group introduced at the site of derivatization should, if possible, resemble the polarity and hydrogen donor-acceptor properties of the parent compound e.g. alkyl groups on oxygen and nitrogen should not be replaced with acyl groups and so on.

In case sufficient SAR-data for the bioactive molecule is available and a suitable functional group is present on the ligand, its derivatization resembles a minor challenge to the synthetic

organic chemist. If available, amine or carboxyl moieties can be coupled to a linker or directly to the solid support via standard peptide coupling procedures. Suitable alcohol moieties can be esterified with succinic or glutaric anhydride to obtain the corresponding ester acids for peptide coupling[22] or can be reacted with an appropriate epoxide (Scheme 4; see also section **4.1.2**).

As outlined above, compounds bearing sufficiently reactive ketone or aldehyde groups can be derivatized by oxime ligation with e.g. with CMA. The obtained carboxylic acids can be further conjugated by peptide coupling. CMA performs exceptionally well in oxime ligation reactions. Thus, even ketones of low electrophilicity like the dienone moiety found in the dexamethasone A-ring or α-ketols react smoothly with CMA to yield the corresponding oximes. Most likely the neighboring carboxylic acid function facilitates loss of water from the primary adduct of CMA and the ketone in the rate-determining step of the condensation reaction.[48] The same reaction with an aminooxy ether lacking the carboxylic function results in incomplete conversion and complex product mixtures (see section **5.1.6**).



Scheme 4. Selection of strategies for ligand derivatization and immobilization. **a)** Esterification of a ligand bearing a hydroxyl function with glutaric anhydride for subsequent immobilization via amide coupling. **b)** Oxime ligation with CMA for subsequent immobilization via amide coupling. **c)** Photochemically induced carbene formation and random insertion for direct immobilization of small organic compounds and macromolecules. **d)** Diazirine **58** used for preparation of photoreactive solid supports.

Since for many natural products and synthetic compounds no SAR-data is available, it is often unavoidable to synthesize arrays of different derivatives and test them on their biological activity. The situation worsens if there is no suitable functional group for derivatization present on the molecule, making it necessary to newly build up the compound and introduce an appropriate functionality in course of the synthesis. Both of these factors, the lack of SAR data and of adequate functional groups, can raise the efforts of a target identification project significantly.

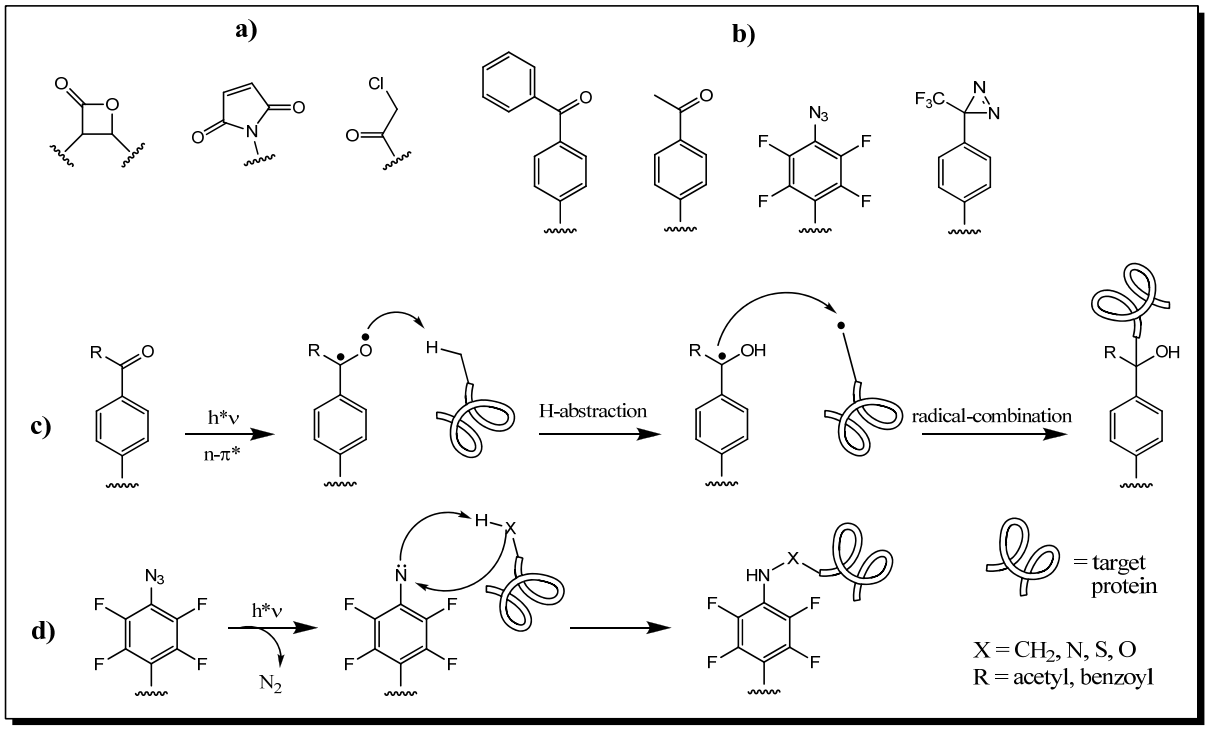
An intriguing solution to this problem is the random derivatization of bioactive compounds via a photoimmobilization reaction using an appropriate photophore.[49, 50] Osada et al. proposed the aromatic diazirine **58** (Scheme 4) as suitable photoreactive carbene precursor for random derivatization of arbitrary organic compounds. Thereby, the random orientation of the bioactive compound on the solid support is supposed to ensure a certain fraction of ligands to retain their biological activity. It has recently been shown that FK-506 adsorbed on a solid support functionalized with the diazirine **58** yielded upon irradiation affinity matrices capable of specifically binding the endogenous ligand of FK-506, namely FKBP12.[51] The preference for carbene insertion into heteroatom-hydrogen bonds observed in solution reactions is circumvented due to fixation by adsorption on the solid support.[52] A drawback of this random derivatization approach is that only a hardly assessable small fraction of cross-linked compound retains biological activity. The biological inactive immobilized molecules account to enhancement of unspecific protein binding. This is of special relevance if the bioactive compound binds rather weakly (K_D ~nM-μM range) to its physiological target/s. To increase the loading of the solid support with active compound as a countermeasure could further increase non-specific binding, thus complicating the analysis of the obtained protein samples. Extensive non-specific binding would have the highest impact on very sensitive analytical methods e.g. techniques exclusively based MS/LC-MS-analysis. In this regard, one approach to uncover the specifically bound proteins among the vast number of non-specifically bound ones could be a soluble competition assay in stable isotope labeled cell lysates (see section **4.2**).

4.1.4 Protein targeting with chemically reactive probes

Both, target identification as well as proteome mapping approaches exploit techniques to specifically place a tag on the proteins examined, facilitating their quantification and isolation/identification. For this purpose some very sophisticated methods for targeting and covalent crosslinking have been developed in recent years. The most popular tags introduced

into target proteins are thereby terminal alkynes and azides for subsequent modification via "click"-chemistry or biotin. The exceptionally strong non-covalent interaction of biotin and avidin led to the development of biotin as the affinity tag of choice for a plethora of biochemical and analytical applications.[53, 54] Thus, biotinylated proteins can be selectively captured and purified using avidin-functionalized solid supports or further examined by tagging with avidin fusion constructs. Immobilized proteins can be liberated by eluting with a biotin solution or under denaturing conditions.

For covalent crosslinking of proteins with small molecular probes generally two different types of reactive moieties are used. These are electrophilic groups e.g. β-lactones,[55] α-halo-carbonyls, maleimides[56] and epoxides, among others. These electrophiles are usually employed to target nucleophilic moieties present in an enzyme´s active site.



Scheme 5. Reactive groups for covalent crosslinking of targeted proteins and mechanism of photo-activation of arylketones and arylazides. **a)** Electrophilic residues suitable for capturing nucleophilic residues present on the target protein, here (from left to right) a β-lactone-, maleimide- and α-chloroacetyl residue. **b)** Benzophenone-, acetophenone-, tetrafluoroarylazide- and diazirine-based photophores widely used for photoaffinity labeling (PAL). **c)** Mechanism of photo-activation, H-abstraction and radical-combination in protein-probe crosslinking with arylketones. **d)** Nitrene formation from the arylazide precursor by irradiation and insertion reaction into a CH- or hetero atom-H bond on the target protein.

Photoreactive moieties like aryl-azides, diazirines and arylketones (Scheme 5)[57-60] are employed when the enzyme does not exhibit a nucleophile for irreversible capture of an electrophilic probe.

Reactive probes are exploited in various approaches to target proteins. Activity-based protein profiling (ABPP) utilizes target directed, activity-based probes, consisting of a known enzyme-substrate of an enzyme or enzyme family and a reactive residue for capturing appropriate functional groups present in the catalytic center of the target.[61] With this so-called "warhead" a tag like an alkyne, azide or biotin is attached to the enzyme in order to facilitate subsequent examination of the target. Certain classes of proteins with enzymatic activity towards the probe are hereby distinguished by their activity.

A compound-centered chemical proteomics approach (CCCP)[1] distinguishes targets by their affinity to the probe. Covalent crosslinking of the protein-probe complex can be achieved with a target identification probe (TIP) consisting of the affinity bait and a suitable photophore.[60] Exceptions are irreversible inhibitors which are covalently attached to the target upon binding as e.g. has been found for ligands possessing a Michael-acceptor moiety. A clear distinction between ABPP and CCCP cannot easily be made since the techniques applied in both strategies widely coincide. Quantitation of proteome populations by these methods can be achieved via stable isotope labeling with various methods (see section **4.2**).

As mentioned above, among the photophores proposed as practical for photoaffinity labeling (PAL) are benzophenone and acetophenone derivatives.[62] Benzophenone (BP) absorbs UV-light in the range of 330-360 nm, acetophenone (AP) at slightly shorter wavelengths of 310-320 nm leading to n-π*-transition and formation of an exited triplet-state. n-π*-Triplets behave like alkoxy radicals, capable of quickly abstracting hydrogen from a proximal hydrocarbon. Radical combination then leads to C-C bond formation allowing covalent attachment to a biomolecule (Scheme 5). BP photophores are known for their high cross coupling yields (up to 70 %[62]) but sometimes lead to poor results due to the steric bulkiness of the group. In these cases the smaller AP moiety was successfully applied as a substitute.[63] Tetrafluoroarylazides (Scheme 5) were proposed as adequate nitrene precursors for PAL.[64] Contrary to their non-fluorinated analogs they lack the tendency of rearranging to the corresponding cyclic ketene imines,[65] which are still good electrophiles, but react considerably slower with targeted biomolecules as compared to singlet nitrenes (CH-insertion). Aryl(trifluoromethyl)diazirines were already discussed in section **4.1.3**. They are widely used for PAL experiments due to their high reactivity and ease of handling. Nonetheless, both the nitrene- and carbene-based photophores are inferior to BP photophores

regarding the cross-coupling yields because the corresponding reactive intermediates are subject of many side reactions or are rapidly quenched by water in a physiological environment. This can result in cross-coupling yields significantly lower than 30 %.[60]

4.1.5 Cleavable linker systems

When the target proteins of an affinity purification experiment are covalently cross-linked to the solid support via an immobilized reactive probe, application of a cleavable linker is crucial in order to recover the protein samples for subsequent analysis. The same applies if the protein was tagged with e.g. an azide or alkyne, which is captured on the solid support via "click"-chemistry and can hence only be recovered by breaking a covalent bond (see also section **4.1.6**).

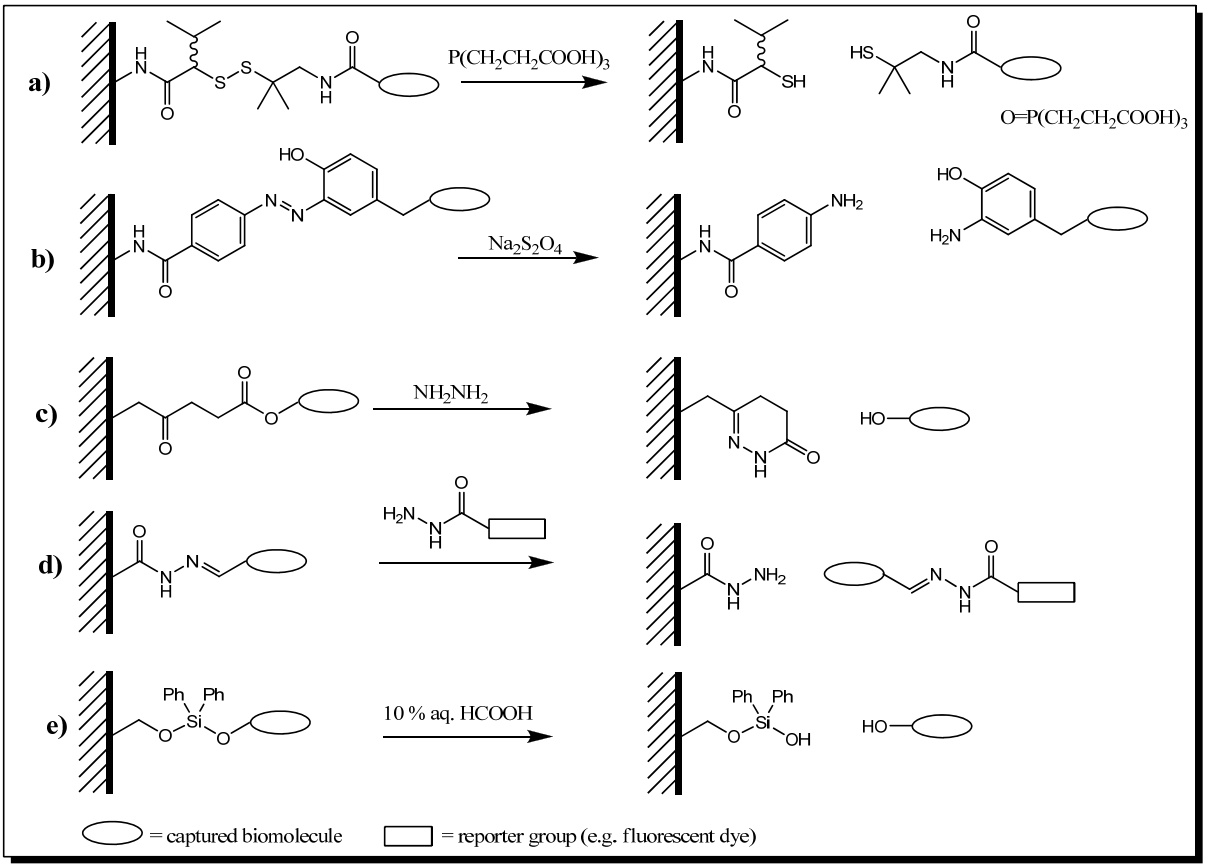
Likewise, liberation of biotinylated proteins from avidin matrices necessitates abrasive elution conditions (e.g. boiling in SDS-buffer) due to the strong biotin-avidin interaction. Consequently, loss and damage of captured proteins or contamination of the protein samples with avidin is an often-encountered problem when using avidin-biotin techniques.

In order to facilitate elution of covalently cross-linked or biotin tagged protein samples from the corresponding matrices under mild conditions, various chemically or photochemically cleavable linker molecules have been developed and found widespread application in chemical proteomics protocols.[61, 66]

Chemically cleavable linkers developed for the purpose of sample recovery are based for instance on disulfide linkages,[67, 68] peptide sequences specifically targeted by proteases e.g. trypsin,[69] azo aromatics,[70-72] γ-keto esters,[73, 74] acylhydrazones[75] and acid labile linkages e.g. based on the dialkoxydiphenylsilyl (DADPS)-moiety[66], *p*-alkoxybenzyl derivatives[76] or cobalt-alkyne complexes.[77] Photochemically cleavable linkers like *o*-nitrobenzyl linkers[66, 78, 79] and phenacyl linkers[80] adapted from solid phase organic synthesis and protective group chemistry[81] have also been suggested for chemical proteomics applications (Scheme 7). Cleavable linkers based on the disulfide moiety have been used in proteomic approaches since over a decade[67] and are easily cleaved by treatment with an excess of a thiol-reagent like mercaptoethanol or Cleland's-reagent (dithiothreitol, DTT). Unfortunately, simple disulfides were found to be prone to sulfide exchange in physiological environment and liable in reducing buffer media leading to premature cleavage of the linkers. For this reason, Gygi et al.[65] introduced a sterically hindered disulfide linker, which is more stable under physiological conditions and withstands thiol-reagents like DTT. It can be selectively cleaved

under mild reaction conditions with the water soluble phosphine reagent tris-(2-carboxyethyl)phosphine (TCEP; Scheme 6).

Aromatic azo compounds have also been used as cleavable linkers for chemical proteomic applications. They are rapidly reduced by treatment with sodium dithionite in aqueous media (<30 min) and exhibit no obvious drawback.[70-72]

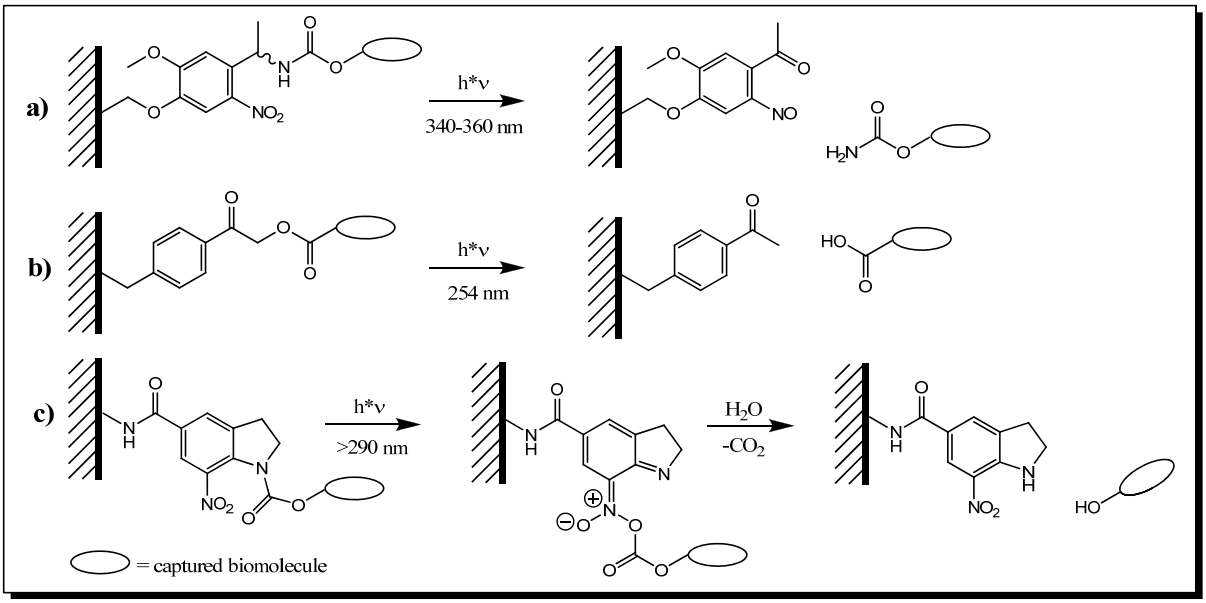


Scheme 6. Chemically cleavable linkers used for liberation of captured biomolecules from the solid support. **a)** Phosphine-cleavable disulfide linker. **b)** Sodium dithionite cleavable azo linker. **c)** Hydrazine-cleavable γ-ketoester linker. **d)** Acylhydrazide based linker cleavable by hydrazone exchange with acylhydrazides. Here a reporter group can be attached to the biomolecule when liberated from the solid support. **e)** Acid cleavable dialkoxydiphenylsilyl (DADPS)-based linker.

4-Oxopimelic acid-derived γ-keto esters found application as hydrazine-cleavable linkers in solid phase organic synthesis[73] and chemical proteomics[74] and exhibit good stability in physiological media. They can be cleaved by treatment with aqueous hydrazine solution. Liberation of biological samples conjugated to an acylhydrazone-linker from a solid support is established by treatment with excess acylhydrazide at nearly neutral pH. Cleavage rates are convincing with 85 % protein recovery after one hour treatment with an acylhydrazide at 50°C.[75] This approach additionally facilitates conjugation of the biomolecule with a suitable reporter group, e.g. a fluorescent dye, in the cleavage step.

Among the acid labile linkers mentioned above, DADPS-derivatives (Scheme 6) seem to be the most practical option, being chemoselectively cleaved in <30 min by treatment with 10 % aqueous formic acid.[66] In contrast, *p*-alkoxybenzyl-derivatives[76] and cobalt-alkyne complexes[77] necessitate aqueous TFA for liberation of immobilized biomolecules.

An obvious advantage of photocleavable linkers is that no chemical reagent has to be added, rendering the cleavage procedures exceptionally mild and convenient. Photocleavable linkers of the *o*-nitrobenzyl type are frequently used for solid phase organic synthesis[78], and in some instances for cleavable biotin probes.[63, 79] They absorb UV-light of 340-360 nm wavelength, leading to oxygen transfer from the nitro group to the proximal benzylic CH-group and thus to liberation of the residue attached to the benzylic amino- or oxy-moiety (Scheme 7). The veratryl-based *o*-nitrobenzyl linker introduced by Holmes [78] is rapidly cleaved in homogenous organics-buffer solution (<10 min), but it was found that cleavage rates on solid support significantly extend due to light scattering and shadowing on the beads (see also section **5.2**). Linkers based on the phenacyl ester moiety exhibit faster cleavage rates (<5 min), but adsorb at wavelengths critical for biological samples (~250-260 nm).[80] Holmes et al.[82, 83] and others[84] previously elucidated that proteins and oligonucleotides are relatively stable to irradiation with light of >350 nm wavelength, but can take severe damage by photons of higher energy. Hence, for irradiation of the photolabile samples discussed in this work a <350 nm cut-off filter was applied (see section **5.2**).



Scheme 7. Photochemically cleavable linkers suitable for solid phase organic synthesis and/or chemical proteomics applications. **a)** *o*-Nitrobenzyl-based linker. **b)** phenacyl-based linker. **c)** 7-Nitroindoline-based linker.

To conclude, although obviously an ideal choice for chemical proteomics applications, it would be worthwhile to develop photocleavable linkers with faster cleavage rates from solid supports absorbing above 350 nm wavelength. A suitable functionality to be tested for matching these criteria could be the 7-nitroindoline-moiety previously used in photolabile amide protecting groups[85] and linkers for solid phase organic synthesis[86] (Scheme 7).

4.1.6 A biotin-free catch-and-release approach

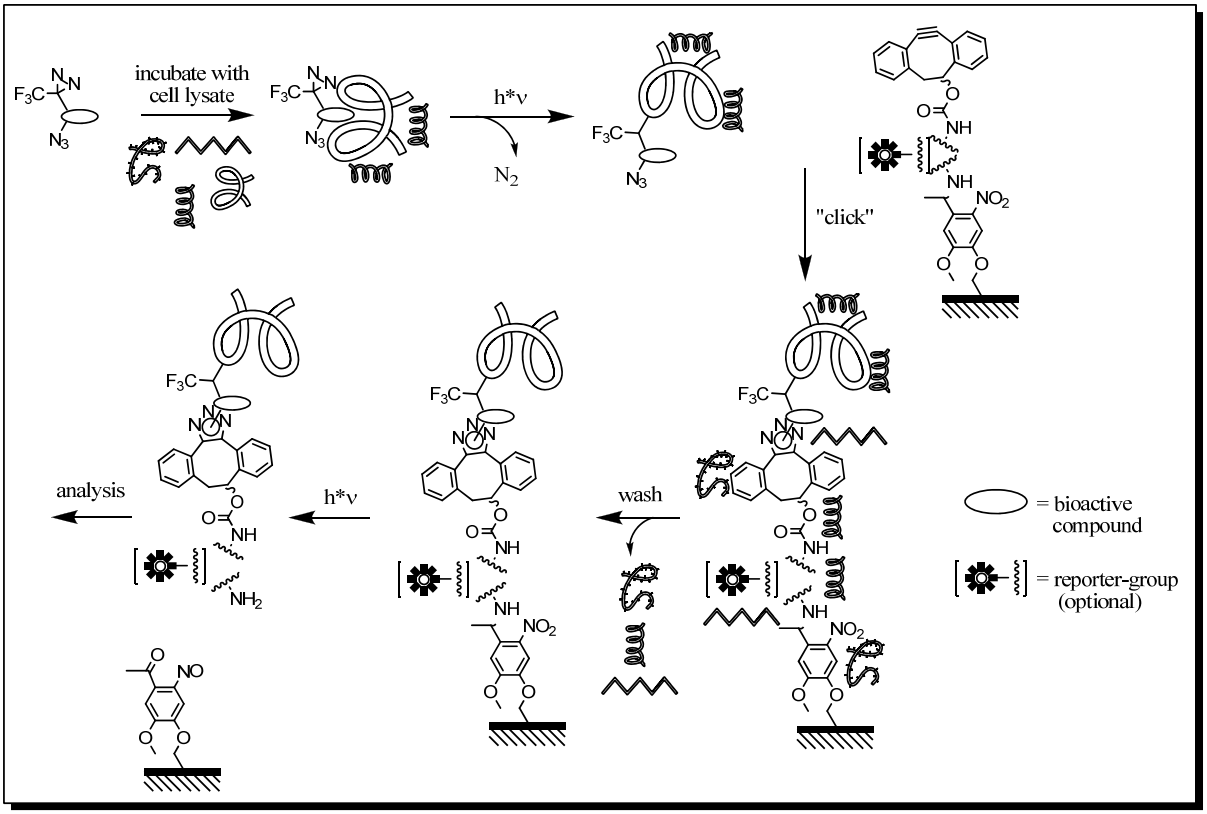
The azide moiety resembles a bioorthogonal, small functional group that can be chemoselectively coupled with an appropriate terminal alkyne ("click"-chemistry) or phosphine reagent (Staudinger-ligation) to yield stable triazoles and amides, respectively. Further, the development of strain-promoted "click"-chemistry involving a cyclooctyne derivative and an azide obviates the use of any additional chemical reagent (see section **4.1.2**). Accordingly, the biotin tag widely used in chemical proteomics approaches might be replaced by the smaller azide tag. Cleavable linkers utilized for liberation of biotin-tagged proteins from avidin-beads can also be applied to cleave the biomolecules captured by strain-promoted "click"-reaction from the corresponding solid support.

A striking advantage of this approach is that the cleavable moiety can be moved from the photoaffinity probe to the solid support. Hence, the probe`s molecular weight can be significantly reduced by consisting solely of the bait, the photophore and the small azide tag. This could lead to less unspecific binding as compared to the very large cleavable biotin constructs.[66] Furthermore, since the cleavable moiety is placed on the solid support, it should be possible to place a reporter group, e.g. a fluorescent dye, between the predetermined breaking point and the strained alkyne which is then liberated with the biomolecule in the "release"-step. Since the biomolecules are attached covalently to the solid support via a highly stable triazole moiety, the beads can be washed abrasively in order to remove all non-specifically binding proteins and protein complexes. The higher purity should contribute to simplify the analysis of the protein samples obtained. If a photocleavable linker, e.g. Holmes`s linker[78] is employed, a sequence of irradiation in the first part of the "catch"-step, a strain-promoted "click" in the second part of the "catch"-step and again irradiation in the "release"-step can be realized (Scheme 8). No chemical reagent has to be added in course of the procedure.

An additional advantage of the given approach is, as with other reactive affinity probes, that interaction of the ligands with the target structures occurs in homogenous phase, avoiding non-specific protein binding on the phase boundary to the solid support (precipitation).

Subsequent sequence analysis of peptide fragments by ESI-MS/MS can reveal the location of the binding site of the probe on the biomolecule by the specific mass-tag attached to the biomolecule. Particularly, in case a stable isotope-label was introduced in course of the PAL experiments (light and heavy) the binding site can be pin-pointed conveniently since labeled peptides appear as doublets in the MS-spectrum (see section 5).

Finally, size reduction of the photoaffinity constructs could allow the probes to penetrate through physiological membranes by diffusion, thus allowing PAL in live cells and a binding event under exact physiological conditions. The retained intracellular localization of target structures and intactness of cell organelles should further contribute to simplify the analysis of the obtained protein samples.



Scheme 8. Catch-and-release approach developed in this work, utilizing photoaffinity labeling of a target protein, capture onto solid support by strain-promoted "click"-reaction and release of the captured proteins by cleavage of a photolabile linker. The use of a reporter group released with the biomolecule upon cleavage of the photo-labile linker is optional.

The whole sequence for PAL and pull-down resembles a two-step photochemical process, devoid of the necessity to add any chemical reagent making it very economical and suitable for automation. Photoaffinitylabeling with diazirines and strain promoted "click"-reaction are both rather fast processes (≤ 30 min). However, in order to increase the time economy of

photolabile linker cleavage, development of a linker with faster cleavage rates or more suitable solid supports for photochemistry would have top priority.

4.2 Mass-spectrometry in chemical proteomics

Mass spectrometry (MS)-based methods became the by far most important analytical tools in proteomic research. In the last two decades MS-techniques underwent several quantum-leaps now providing devices to examine intact protein complexes in the gas phase by natural MS,[87] the expression pattern and quantity of proteins in different cells and different states of the cell cycle,[8] quantitative measurements of the phosphorylation state of the proteome for phospho-proteomics[88] and the high throughput identification of target proteins in drug research.[89]

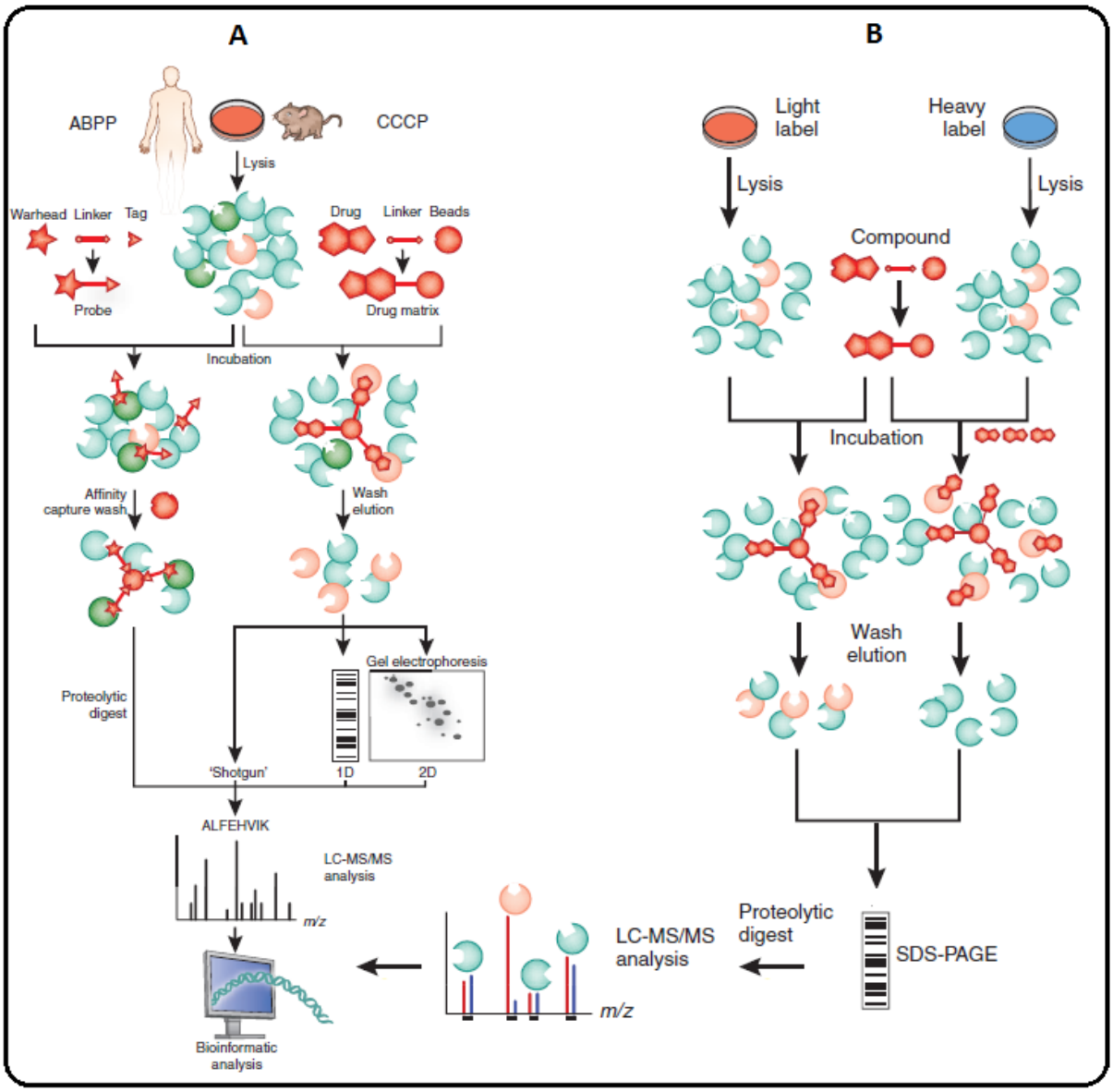
For MS-analysis of large biomolecules, the ionization techniques of choice are either MALDI or ESI. MALDI allows detection of intact proteins from simple protein mixtures. ESI coupled with liquid-chromatography (LC) is used to detect proteins in complex protein mixtures in a high throughput manner. In the latter case, identification of target proteins is achieved by ESI-MS/MS methods, which allow sequential analyses of peptide fragments. The original protein can be traced by the alignment of the peptide fragments detected with protein databases.

For drug target identification, protein samples are usually obtained by affinity purification of cell lysates. Prior to MS-analysis the protein samples are fractionated by gel electrophoresis. Then, either single protein bands or broader strips of the gel are cut out, extracted and subjected to tryptic digestion. The peptides obtained are analyzed by LC-MS/MS and the original proteins are traced with the identified peptides (see above).

In case that an affinity probe was covalently attached to the identified protein e.g. in course of a PAL experiment, the binding site of the bioactive compound on the protein can be revealed by the specific mass tag introduced.[56, 90] Particularly convenient is the use of stable isotope labeled probes (biochemically identical but different in mass) facilitating detection of the labeled peptides as doublets in the MS-spectrum. Sequential analysis then yields the amino acids the reactive probe was attached to.[91-93]

Likewise, quantification of proteins isolated by different bioactive compounds in “fishing”-experiments can be achieved by stable isotope labeling of the protein samples. For instance, if the affinity of two agents for a protein has to be compared, two fishing experiments can be performed. One of the experiments is performed in a stable isotope labeled proteome (heavy), the other in a non-labeled proteome (light). The protein samples obtained from both experiments are then combined and subjected to the standard procedure of electrophoresis, digestion and LC-MS/MS analysis. Proteins from the two different proteomes can then be

distinguished by the characteristic mass-shift introduced by the stable isotope label (see above).



Scheme 9: MS-based chemical proteomics workflow (Scheme adapted from[1]). **A**: Comparison of ABPP and a compound centered approach (see section 2.1.4) with covalent tagging of target proteins vs. regular affinity purification. The recovered proteins can be separated by gel-electrophoresis, digested with trypsin and subjected to MS-analysis. Comparison with protein databases reveals the identity of the "fished" proteins. Alternatively the protein samples can be analyzed via "Shotgun"-MS. **B**: SILAC-based soluble competition assay. Two protein pools, one of them stable isotope labeled (light and heavy), are subjected to affinity purification with and without a soluble competitor. Specific binding proteins are displaced from the affinity matrix and can be quantified via MS-Analysis by differences in abundance compared to labeled/non-labeled analogs.

Integration of a peptide`s doublet MS-signal yields a quantitative value for the amount of peptide (protein) isolated with the bioactive compound from one of the proteomes. In case the

affinity of the reference compound to the target is known, this value correlates to the affinity of the ligand to the target structure (Scheme 9).[94] Similarly, the experiment can be performed in a competitive manner. Here one protein sample is obtained by a standard "fishing" experiment and the other sample from an experiment where binding of the immobilized bioactive molecule to target proteins is in competition with of a soluble analog of the bioactive compound. Proteins that fail to bind to the solid support are likely to interact specifically with the soluble competitor. These specific binding proteins can easily be distinguished from unspecific binders by identification and quantification via MS-analysis. Since the early 1990s a variety of methods have been developed to introduce stable isotope labels into proteins.[94] These are, among others (see PAL-techniques above), metabolic labeling with heavy salts and amino acids (^{15}N),[95] enzymatic labeling with $H_2{}^{18}O$,[96] chemical modification based on reductive amination with formaldehyde[97] and the isobaric tag for relative and absolute quantitation (iTRAQ)[98].

Mann, Ong and others introduced "stable isotope labeling with amino acids in cell culture" (SILAC), allowing nearly homogenous labeling of the entire cellular proteome (up to 95 %) by raising cell cultures with heavy amino acids (D^3-Leu, $^{13}C_6$-lysin and $^{13}C_6$-arginine, $^{15}N_4$-Arg).[99, 100] Only very recently this technique has been adapted to target identification of small, pharmacologically relevant molecules. In a case study published by Ong et al.[9] a target screening of kinase inhibitors yielded accurate assignment and quantitation of known kinase inhibitor targets and several unknown targets. Since then several studies have been published relying on SILAC for target identification and quantitation of biomolecules and small bioactive compounds.[101, 102]

4.3 Biochemistry of steroid hormones

4.3.1 Physiological and Pharmacological considerations

Steroid hormones play important roles in the regulation of physiological functions in humans. The sex-hormones (SHs) testosterone (T), progesterone (P_4) and estradiol (E_2) are essential for development and maintenance of the male and female phenotype, respectively. Disordered sex hormone balance associates with reproductive behavior, fertility, immune response and SH-dependent cancers e.g. breast and prostate cancer.[103] The gender dependent anti-inflammatory and immunosuppressive properties have already been mentioned above.[14] Sex-steroid hormones and their synthetic analogs are important agents in clinical therapy with applications like hormone replacement therapy, contraception, treatment of hormone-dependent cancers and not least in competitive sports as anabolic agents.[103]

In Contrast to sex-hormones, the corticosteroids are vital for maintenance of bodily functions. Mineralocorticoids, with the most important representative aldosterone, regulate electrolyte balance and are therefore responsible for e.g. blood pressure homeostasis.[104]
Glucocorticoids, like the endogenous hydrocortisone (HC), regulate the metabolism of carbohydrates, fatty acids and amino acids and deploy strong immunosuppressive and anti-inflammatory properties[104, 105] as well and are thus currently indispensable as agents in clinical therapy.[15, 106, 107] High doses of potent glucocorticoids are administered in life-threatening conditions like acute spinal trauma and after organ transplantation.[103]
Hormonally active steroidal and non-steroidal agents cause a plethora of adverse effects related to the function of their endogenous analogs.[15, 103, 108] Due to this fact, the research on hormonally active agents with a higher specificity for their cellular targets is still a dynamic field of research. In particular, the development of agents that specifically direct their cognate nuclear receptors transcriptional activity towards transcription of beneficial gene products (specific nuclear receptor modulators) has made considerable progress.[16, 109]

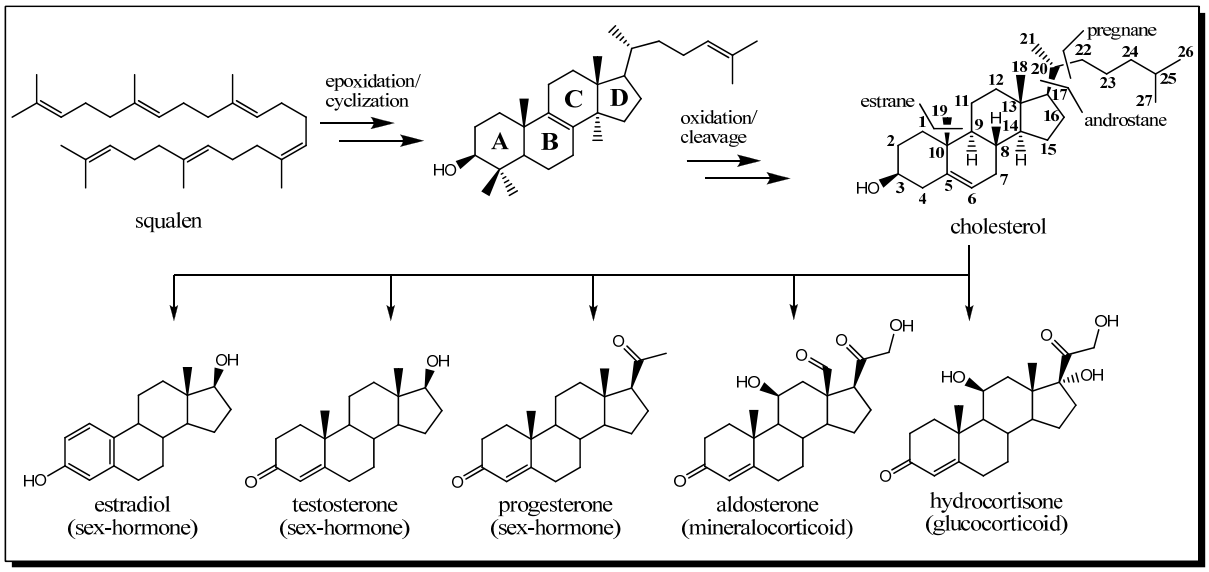
4.3.2 Steroid biosynthesis

Endogenous steroid hormones, with exception of the cardiac glycosides[110] and the bile acids, share the common structural features of three six membered and one five membered all-*trans* fused carbocycles (A, B, C and D-ring, respectively; Scheme 10). All steroid hormones have the common precursor cholesterol, which is processed in the body by cytochrome P450 (CYP) enzymes and other oxidoreductases in a sequence of oxidation and cleavage steps to steroids possessing the pregnane, androstane and estrane scaffold (C21-, C19-, C18-body, respectively).
Cholesterol itself is synthesized from squalen, a triterpene, by squalen mono oxigenase mediated 2,3-epoxidation and subsequent cationic domino cyclization accomplished by squalen cyclase. The primary product of cyclization is lanosterol (C30-body), which is processed in several steps to the C27-body cholesterol. Breakdown of the cholesterol side chain is achieved by CYP11A via dihydroxylation at position 20/22 and bond rupture between C20/22 yielding pregnenolone. Pregnenolone is converted to progesterone by 3β hydroxysteroid dehydrogenase (3β-HSD). Both of these steroids are the common precursors for the sex-hormones, glucocorticoids and mineralocorticoids.[111, 112] Hydroxylation at position 21, 18, 17 and 11 by CYP-enzymes, mainly expressed in the adrenal cortex, leads to formation of the glucocorticoids and mineralocorticoids. Further breakdown of the C17 side chain by CYP17 17,20 lyase yields the androstane scaffold for biosynthesis of the androgens

and the estrogens. For formation of the estrogens the C19-methyl group is removed and the A-ring is aromatized by CYP19A1 (aromatase).

As outlined above, most of the synthetic steps in course of the steroid biosynthesis are catalyzed by members of the cytochrome P450-family. Many of the cytochrome-enzymes involved in steroid biosynthesis and their further processing can catalyze a variety of reactions on a broad spectrum of substrates.[112] Thus, although the mechanisms of steroid biosynthesis are relatively well understood, elucidation of the exact mechanisms of oxygenation- and cleavage-reactions is still subject of current investigations.[113, 114]

Production of the hormonally active steroids is a tightly regulated process and, as implied above, takes place in highly specialized tissues. Since in contrast to peptide hormones, steroid hormone release occurs by diffusion, physiological hormone levels are regulated generally by affecting expression of the proteins responsible for their biosynthesis. Responsible for regulation of steroid biosynthesis are hereby the pituitary peptide hormones luteinizing hormone (LH) and adrenocorticotropic hormone (ACTH) which are in turn regulated by the hypothalamic gonadotropin releasing hormone (GnRH).



Scheme 10: Cholesterol biosynthesis and further processing to the endogenous steroid hormones. Shown are representatives of the glucocorticoids (hydrocortisone), mineralocorticoids (aldosterone) and the sex-hormones (estradiol, testosterone, progesterone).

Testosterone biosynthesis, occurring mainly in the gonadal Leydig-cells, is regulated by LH. Likewise, LH regulates production of the female sex-hormones mainly proceeding in the ovaries. The corticosteroids and to a lesser extend estradiol and the androgens DHEA and androstenedione are synthesized in the adrenal cortex under control of ACTH. The regulation

cycle is closed by a negative feedback loop via steroid hormone induced inhibition of pituitary peptide hormone biosynthesis.[104]

4.3.3 Genomic mechanism of steroid hormone action

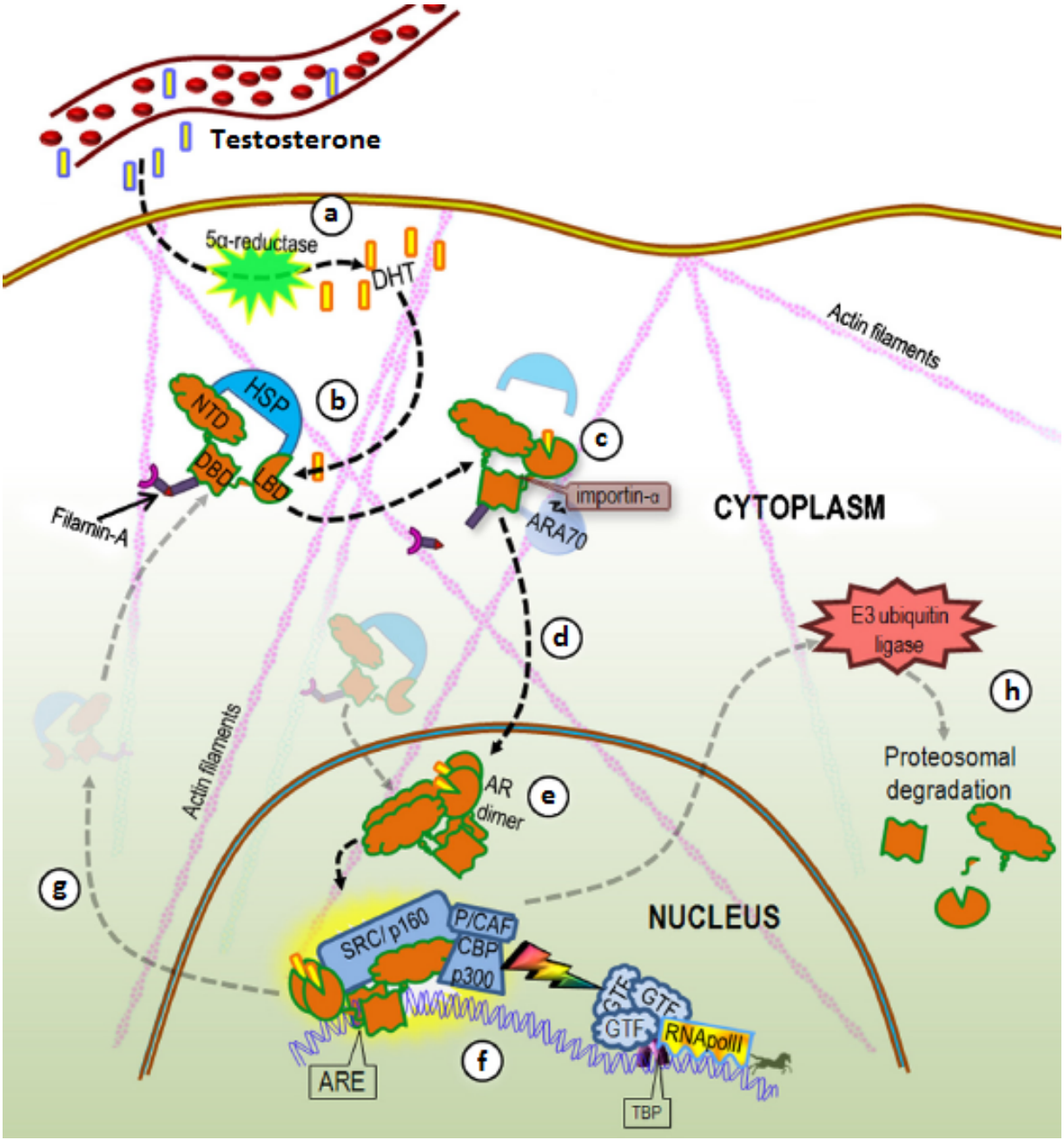
Two distinct reaction patterns can be discerned for steroid hormone action. These are the rapidly occurring non-genomic actions of steroid hormones, discussed in the next section, and the genomic effects, discussed in this section.

Steroid hormones serve as mediators of gene transcription by interacting with their specific nuclear receptors (NRs).[115] This mode of steroid hormone action is termed "genomic" or "classical". The influence of NRs on transcription is known for a long time and extensive knowledge about their target genes exists.

NRs are ligand mediated transcription factors and the nuclear receptor superfamily includes the intracellular receptors for sex-hormones (SHs), glucocorticoids (GCs), mineralocorticoids (MCs), thyroxine (T_4), retinoic acid, vitamin D and other small lipophilic hormones.[109, 116] The androgen receptor (AR), progesterone receptor (PR), glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR) are closely related to each other. The estrogen receptor (ER) shows less homology to the named NRs.

All NRs posses a typical assembly of domains, namely a C-terminal ligand binding domain (LBD), exhibiting a moderate degree of homology throughout the receptor family, and a hinge region next to the LBD bearing the activation domain, a sequence for Hsp-binding and the nuclear localization signal (NLS). In the central domain of the polypeptide a highly conserved approx. 70 amino acid-(aa-) long sequence containing two zinc-finger motives for DNA-binding can be found. The N-terminal domain is highly variable in sequence and length containing activation functions (AF) responsible for binding of various co-activators.[116-118] Variations in the N-terminal domain lead to the occurrence of various NR-subtypes. Thereby, each of these NR-subtypes has access to different pools of co-activator and co-repressor proteins resulting in the plethora of transcriptional functions displayed by the NRs.

A characteristic of genomic steroid hormone action is that the effect (protein biosynthesis) becomes noticeable after several hours following treatment with a steroid agent. Genomic actions are thus considered to operate slowly displaying long term effects.[12]



Scheme 11: Transcription regulation by activated nuclear hormone receptors exemplified by activation and action of the AR (adapted from[121]). Basically the glucocorticoid and progesterone receptor follow the same mechanism of action. **a**) Testosterone is transported from the Leydig-cells to target tissues, diffuses freely through the cell membrane and is converted to the more active androgen dihydrotestosterone (DHT). **b**) Ligand binding to the AR causes complex dissociation and exposure of the NLS. **c**) The NLS is recognized by importin-α. **d**) The activated AR is translocated into the nucleus. **e**) Homo-dimer formation enables the interaction with the corresponding androgen response elements (AREs) on the DNA. **f**) Various co-activators facilitate interaction with the transcription machinery. **g**) Dissociation of DHT from the AR causes exposure of the nuclear export signal (NES) leading to export of the AR. The AR is either recycled to bind new hormone ligands (**g**) or ubiquitinylated and delivered to the proteasome (**f**).

The binding event with the steroid hormone leads to translocation of the corresponding receptors from the cytosol into the nucleus. There the NRs can bind to specific (negative and positive) hormone receptor response elements on the DNA and promote or inhibit expression of the respective genes.

Glucocorticoids (GCs) unfold their effects by binding to the GR which resides dormant in the cytosol as a complex with various heat shock proteins, immunophilins and various co-chaperones.[15] Chaperoning of the GR-polypeptide is crucial to fix the receptor in a conformation with high affinity for the ligand (general for NRs). GC-binding facilitates dissociation of the complex and transport of the activated receptor into the nucleus by exposure of a nuclear localization signal (NLS). The NLS is specifically recognized by transport proteins and the NLS receptor importin-α. Together with karyopherin β (see section **5.5.4**) importin-α facilitates interaction with the nuclear-pore import machinery.[119] In the nucleus the activated dimeric GR can bind to glucocorticoid response elements (GREs). Via the GR´s transactivation domain and various co-activator proteins the complex couples to the transcriptional machinery, enhancing transcription of e.g. metabolism related gene products like phosphoenolpyruvat-carboxykinase (PEPCK) and tyrosin-amiotransferase (TAT).[120] Binding to negative GREs leads to repression of gene expression of e.g. gene products related to negative feedback of steroid hormone biosynthesis. The activated monomeric GR can also interact with other transcription factors like nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) and activator protein 1 (AP-1), thus affecting transcription of pro-inflammatory gene products like the tumor necrosis factor (TNF), cyclooxygenase 2 (COX-2) and the interleukins. Hereby binding of the activated GR to these transcription factors can either disrupt binding to the DNA or render the interaction with the transcription machinery ineffective (tethering). Most of the GCs anti-inflammatory and immunosuppressive actions can be explained with this transrepression mechanism.[15] Furthermore it is thought that the activated GR modulates histone acetylation and deacetylation, thus regulating gene expression on the level of chromatin structure.[117]

Androgen receptor signaling initiated by either testosterone (T) or the activated analog dihydrotestosterone (DHT) takes a similar course. Testosterone enters a cell via diffusion, is then reduced by 5α-reductase to DHT and binds to the cytosolic AR leading to dissociation of the dormant AR-complex and shuttling of the activated AR to the nucleus (Scheme 11). After AR dimerization, DNA binding on androgen responsive elements (AREs) occurs, enhancing transcription of androgen sensitive genes like the prostate-specific antigen (PSA) and probasin.[121, 122]

The PR is the cognate cellular target of the progestins e.g. the endogenous steroid progesterone (P_4). The PR is expressed in two isoforms, the PRA and the 164 aa longer PRB, exhibiting clearly distinct physiological functions. The two receptor subtypes are known to be differentially and tissue-specifically expressed, especially under pathological conditions like breast cancer. PRA and PRB seem to bind different progesterone response elements on the DNA and differ greatly in the set of co-activators and co-repressors they can recruit. Furthermore, PRA can inhibit the action of PRB and other members of the nuclear receptor family by formation of hetero dimeric NR-complexes.[123] The mechanism of PR action follows widely the general pattern of nuclear receptor activation described above with the exception that the dormant PRA resides in the nucleus and PRB is distributed between nucleus and cytosol.[118, 123, 124] Target genes of the PR cover a broad variety of cellular functions, for instance genes regulating transcription and cell differentiation, membrane- and signaling proteins, steroid, fatty acid, nucleotide and amino acid metabolism.[125]

4.3.4 Non-genomic actions of steroid hormones

As outlined in section **4.3.3**, genomic actions of steroid hormones initiated by inducing transcriptional activity of the cognate nuclear receptors are characterized by their delayed occurrence due to slow response of the transcription/translation machinery (time scale of hours). In contrast, various effects on cell physiology have been observed to occur a very short time after treatment with steroid hormones (seconds to minutes). Obviously the steroid hormone stimulus is coupled in some cases to other cellular signaling pathways relaying on second messenger production (cAMP, $InsP_3$, DAG, Ca^{2+} etc.).[12, 13]

In the case of AR- and PR-signaling it was found that in analogy to ER-signaling a small portion (<5 %) of the corresponding NR current is recruited to the cell membrane. There the nuclear receptors can be activated by steroid hormone binding and communicate with various proteins involved in signal transduction.[123] Translocation of the NRs to the cell membrane is facilitated by palmitoylation at a cysteine residue found in the LBD. Levin et al.[126] found that binding of oligomeric Hsp27 is crucial for palmitoylation at Cys^{447} of the ER_α by a to date unidentified palmitoyl transferase (PAT). Palmitoylation of the ER_α facilitates interaction with caveolin-1 and thus translocation to lipid rafts on caveolae in the peripheral membrane. This mechanism was shown to operate similarly for the PR and AR and requires a highly conserved 9-aa motif found in the LBD of the sex-hormone receptors.[127] Interestingly, this motif can also be found in the GR and the TR but not in the MR. The membrane-bound sex hormone receptors act as mediators of rapid sex hormone signaling by interacting with G-

protein α- and βγ-subunits, phosphoinositide 3 kinase (PI3K) and tyrosine kinase c-Src, further activating downstream signaling cascades.[128, 129]

It should be noted that S-palmitoylation of the NRs is considered a dynamic process, since ligand binding induces enhanced de-palmitoylation of the receptor by an unknown thioesterase. Depalmitoylation then leads to release of the receptor into the cytosol.[130] Likewise, there is mounting evidence that for other steroid hormone NRs like the GR and MR membrane signaling plays an important role in their physiological function.[131, 132]

A high-affinity membrane binding site for GCs has first been identified in neuronal membranes of the amphibian *taricha granulosa*[133] and later in rodent lymphoma cells and human leukemia cells.[134] There is growing evidence that this binding site corresponds to a membrane located classical GR (mGR).[135]

Not all non-genomic effects mediated by steroid hormones are linked to membrane bound classical NRs. It has been shown that proteins released from the dormant NR-complexes upon activation are responsible for triggering different signaling cascades. Examples are the regulation of eNOS or inhibition of arachidonic acid signaling by steroid hormones.[136-138]

Initiation of several signaling pathways has been traced back to binding of steroid hormones to non-classical receptors. Different steroid hormones were shown to operate through a membrane-bound non-classical receptor termed progesterone membrane receptor component 1 (PGMRC1). PGMRC1 possesses a heme binding site which is deemed to be responsible for steroid binding, albeit in a relatively unspecific manner. Actually it is still under discussion if PGRMC1 itself or one of the physiologically associated proteins facilitates steroid binding. The role of the closely related PGMRC2 in cell signaling is even more obscure.[131, 139]

Another progestin binding site was identified on membranes of fish oocytes. The receptor was identified as a G-protein coupled receptor belonging to the family of 7TM-receptors.[140] Later it was shown that the human homologues, termed mPRα, -β and γ play regulatory roles in human myometrium via crosstalk with the PR-B.[141]

Quiet recently protein kinase C was proposed as steroid hormone receptor. It was found that the different PKC-isoforms are activated with high selectivity for various steroid hormones, in particular estradiol an dexamethasone.[142] Non classical receptors for androgens have not been described so far.

Steroid hormones are lipophilic molecules that can easily diffuse into physiological membranes. Thus another mode of non-genomic action is believed to operate via alteration of the physicochemical properties of the cell membrane (fluidity, polarity), in particular at high plasma concentration of steroid hormones.[15, 143] Specific interactions of the steroid and

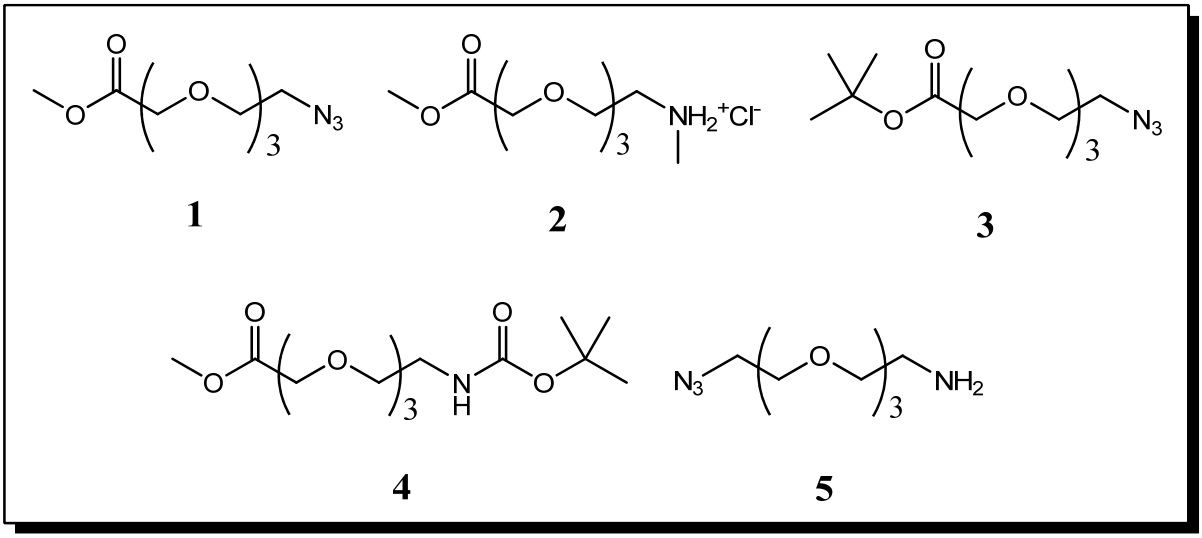
proteins are thought to be not involved. However, some of the effects were observed at low, physiological hormone concentrations and even showed specificity to certain steroids.[143] In case of GCs, changes in membrane fluidity and permeability were described to affect membrane resident proteins, especially ion channels, having an impact on ATP-induced Cl^- segregation, calcium and sodium cycling and mitochondrial proton leak. Lipid peroxidation is also effected, being relevant for inflammatory responses.[15, 144, 145]

5 Results and discussion

5.1 Synthesis of the "fishing"-tools

5.1.1 Synthesis of the ethylene glycol spacers

In order to reduce non-specific protein binding in the pull-down experiments the steroid baits were intended to be coupled with a suitable ethylene glycol spacer. Tanaka et al. have shown that the hydrophilicity of the solid support applied for affinity purification has a direct impact on the amount of non-specifically bound abundant protein e.g. tubulin and actin.[18, 19] Furthermore it was demonstrated that the use of ethylene glycol based linkers enhances hydrophilicity of relatively hydrophobic supports like TOYOPEARL®, thus reducing non-specific binding. The rule of thumb deduced from these studies is, the longer and more hydrophilic the spacer group the less unspecific binding occurs. In search of a compromise between high hydrophilicity and practical synthesis some easily accessible tetraethylene glycol linkers were considered as suitable. For immobilization, the spacer should possess a terminal carboxylic function for coupling to amino functionalized solid supports and an amino function for coupling to the steroidal carboxylic acids (see section **5.1.2**). Furthermore, for the synthesis of the steroid photoaffinity constructs an azido- and amino-functionalized derivative was required.

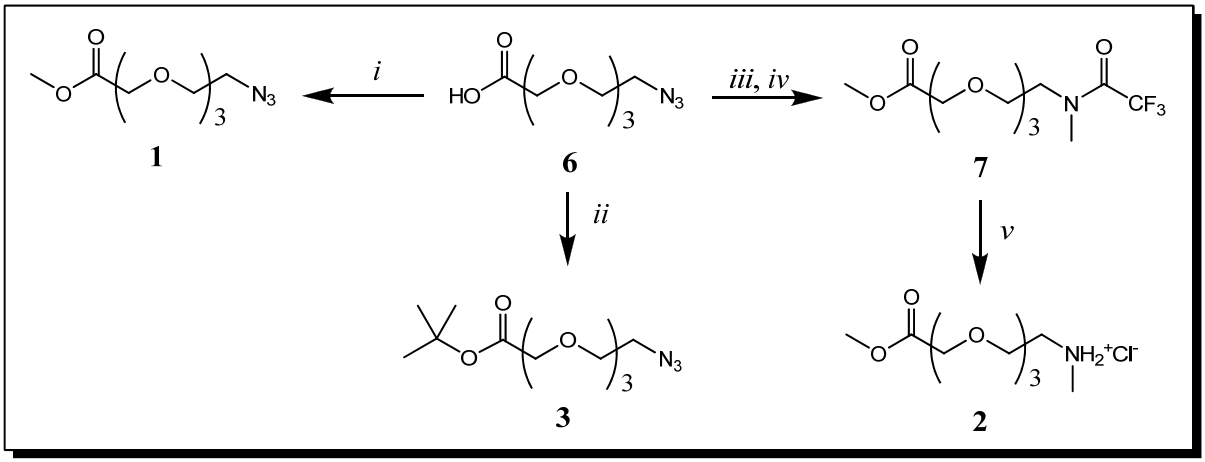


Scheme 12: Ethylene glycol building blocks for the synthesis of steroid-derivatives suitable for affinity purification experiments.

To match the demands of the various experimental procedures used for conjugation of the steroid and allow straight forward buildup of the "fishing"-constructs the tetraethylene glycol building blocks were endowed with appropriate protecting groups (Scheme 12).

Hereby, methyl esters **1** and **2** were found suitable to be conjugated to the base stable steroid 3-CMOs **8**-**11** (see below). The *tert.*-butyl ester **3** was used in the synthesis of the acid stable 17β-carboxamides **22**, **23** and the highly base labile 21-CMOs **24** and **25**. The Linkers **4** and **5** were used for assembly of the photocleavable strained alkyne construct **101** (section **5.1.7**) and the steroid photoaffinity probes (**5.1.4**-**5.1.6**).

Azido-methyl ester **1**[146] was obtained from literature known azido acid **6**[147] by esterification with thionyl chloride in methanol under reflux conditions in 81 % yield (Scheme 13). *N*-methylated amino methyl ester **2** was also obtained from azido acid **6** in a two step sequence. In the initial step, the azide moiety was reduced to the corresponding amine with hydrogen over Pd/C (10%) in dry methanol. The free base was directly converted to the trifluoroacetamide by addition of trifluoroacetic acid methyl ester (Scheme 13). The crude amide was then permethylated with MeI in dry DMF using K_2CO_3 as the base[148] yielding trifluoroacetamide **7** in 78 % yield. The proposed selective cleavage of the trifluoroacetamide moiety in presence of the methyl ester by alkali hydrolysis[148, 149] turned out to be unpractical due to the exceptional base liability of the glycolic acid methyl ester. Accordingly, the trifluoroacetamide moiety was cleaved by acid solvolysis with HCl in dry methanol under reflux conditions, affording the pure amine hydrochloride **2** in quantitative yield (Scheme 13).



Scheme 13: Synthesis of the ethylene glycol building blocks. Reagents and conditions. *i*) MeOH, $SOCl_2$, 0°C-reflux, 3 h, 81 %. *ii*) Boc_2O, DMAP, *t*BuOH, 40 °C, 3 h, 84 %. *iii*) Pd/C (10 %), H_2, MeOH, RT, 3 h, quant. *iv*) TFAcOMe, MeOH then MeI, K_2CO_3, DMF, 110 °C, 10 h, 78 %. *v*) HCl, MeOH, reflux, 36 h, quant.

Azido *tert.*-butyl ester **3**[150] was obtained by esterification of azido carboxylic acid **6** via the Boc_2O/DMAP-method[149] in dry *tert*.-butanol in 84 % yield. *N*-Boc protected amino-methyl ester **4**[148, 151] and azido-amine **5**[152] were prepared according to literature known procedures[149] from **1** and tetraethylene glycol, respectively.

5.1.2 Derivatization of the steroids

Prior to attachment of the ethylene glycol spacers to the steroid derivatives via amide coupling, a carboxylic acid function had to be introduced into the steroid scaffold. For functionalization of position 3 of the steroids scaffold T, P_4, HC and Dex were reacted with CMA to yield the corresponding 3-*O*-(carboxymethyl)oximes (3-CMOs) **8**,[153] **9**,[154] **10**[154] and **11**[155] (Scheme 14).

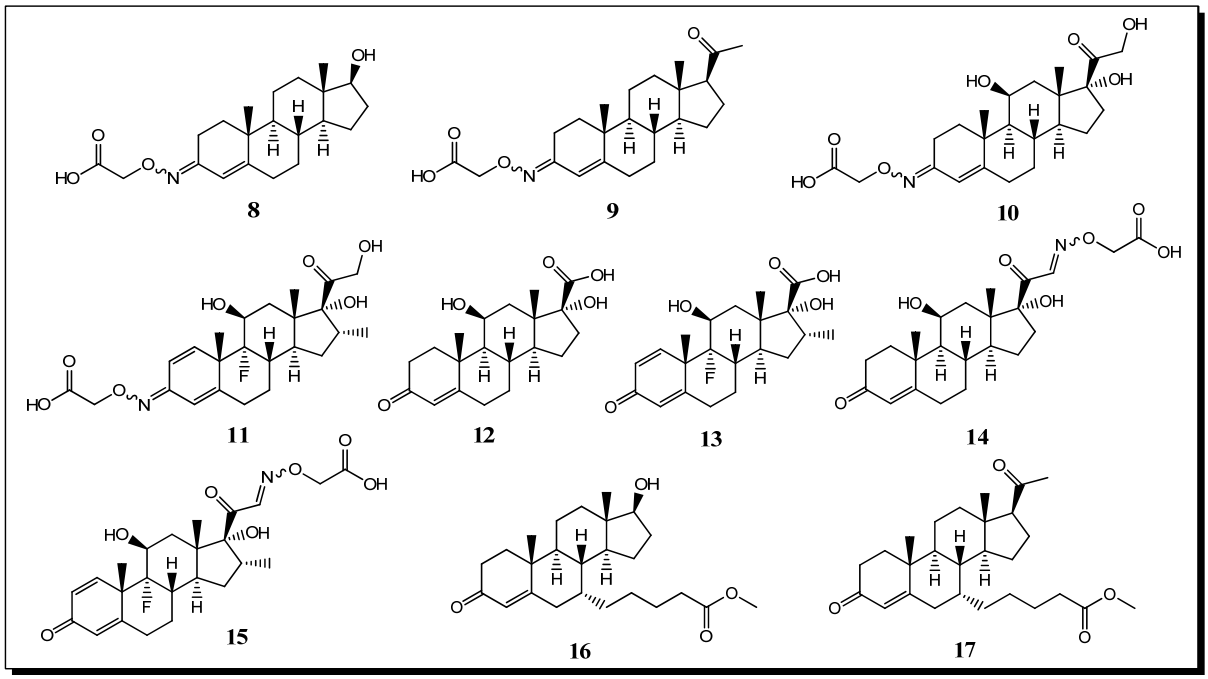
In order to cover different binding modes which might occur in protein-ligand interaction, at least one additional set of steroid derivatives with the spacer attached to a position distinct from the A-ring was synthesized. In a study examining the application of GC-derivatized polymers for affinity chromatographic purification of rat liver GR published by Lustenberger et al., the 17β-carboxyl derivative of dexamethasone **13**, obtained by periodate cleavage of the D-ring α-ketol residue, was proposed as suitable ligand for immobilization.[156] It was found that the free carboxylic acid derivative of dexamethasone **13** itself showed no glucocorticoid activity, but that the activity was restored (at least partially [157]) upon amide bond formation, allowing to place an amide linker at this position. Analogous, treatment of HC led to the formation of the corresponding carboxylic acid **12**.[158]

In another study that correlates side chain modification at position 20 and 21 with glucocorticoid activity, Manz et al.[157] showed that the 21-oximes of HC and Dex display GR-affinity only slightly diminished compared to the parent compounds. Accordingly, the GCs were converted to their corresponding 21-CMOs **14** and **15** by copper(II)-mediated aerial oxidation of the α-ketol moiety[159] followed by regioselective oxime formation with CMA in a one pot manner. Hereby HC-21-CMO **14** and Dex-21-CMO **15** could be obtained in 78 % and 88 % yield, respectively.

Further, the SHs T and P_4 were functionalized with a carboxylic acid methyl ester terminated C5-residue at the 7α-position of the steroid scaffold, affording steroidal carboxylic methyl esters **16** and **17** (Scheme 14). It has been shown previously that attachment of various residues to the 7α-position of T does not hamper its androgen activity.[160, 161] If the same holds true for P_4 regarding progestin activity could not be established so far (see section **5.3**).

The ethylene glycol conjugates of the steroid acids **8-15** were synthesized by HBTU mediated amide coupling with the corresponding free bases of linker **1** and **3** or linker **2** in presence of HOBt and DIPEA in DMF. Optimization of the amide coupling procedure indicated HBTU as the reagent of choice, affording yields superior to those achieved with

PyBop, DIC, DCC or EDC.[162] Hereby, the ethylene glycol conjugates **18**-**25** were obtained in yields of 51-85 % (Scheme 15; Table 1). Ester cleavage of the methyl esters in **18**-**21** was achieved by treatment with 1 M K_2CO_3 in aqueous methanol/THF for one hour at RT furnishing the free acids **26**-**29** in virtually quantitative yield.

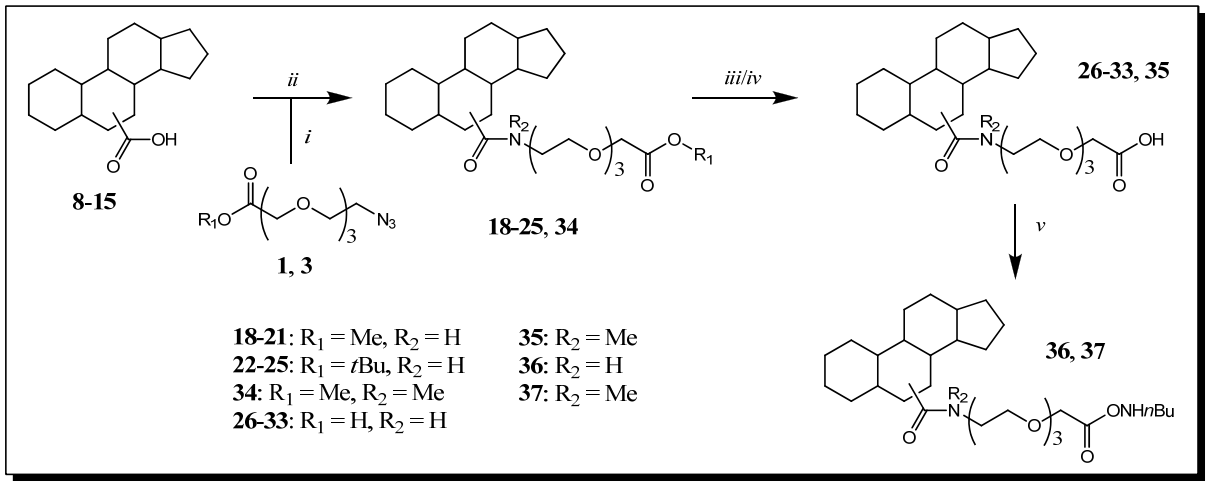


Scheme 14: Steroidal carboxylic acids and methyl esters for attachment to the ethylene glycol and photophore building blocks.

The *tert*.-butyl ester group in **22** and **23** was cleaved with 50 *v*% TFA in DCM in presence of the cation scavenger 1,3-dimethoxybenzene[163] leading to free acids **30** and **31** in nearly quantitative yield. Since *tert*.-butyl ester cleavage in **24** and **25** proceeded not as clean as in **22** and **23**, the free acids **32** and **33** had to be purified by column chromatography and were obtained in 82 % and 86 % yield, respectively.

The free acid **29** proved to be unstable and decomposed with a half-life of approx. one week at ambient temperature. Because initially the dienone oxime-moiety was deemed responsible for increased sensitivity against hydrolysis, Dex-3-CMO **11** was coupled to *N*-methylated linker **2** affording the corresponding amide **34** in 39 % yield. However, like **29** the free acids **35** decomposed within a couple of days, underlining the acid liability of the Dex-3-CMO derivatives. In order to prove the capability of the derivatives **29** and **35** to be employed in the affinity purification experiments they were converted to their corresponding *n*-butyl amides **36** and **37**. HBTU-mediated coupling with *n*-butylamine afforded **36** and **37** in 55 % and 51 % yield, respectively (Scheme 15). Amide **36** was found to be equally stable as its

methyl ester complement **21** and the *N*-methylated derivative **37** showed no sign of decomposition even upon storage for longer time periods.



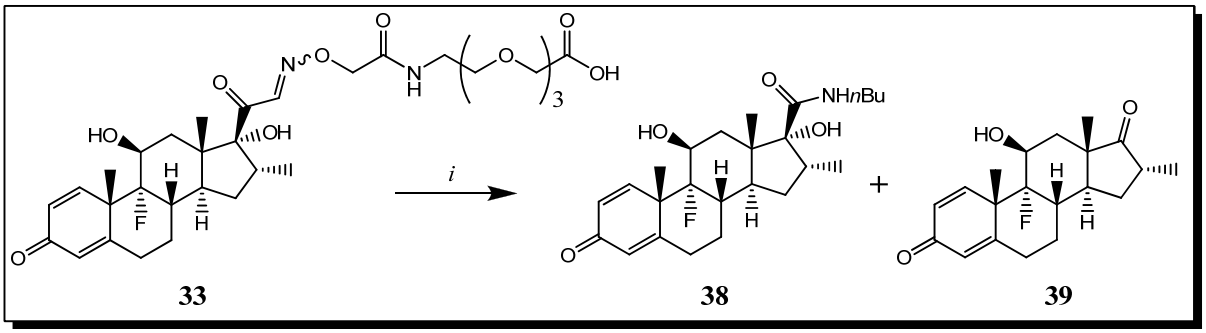
Scheme 15: Synthesis of the ethylene glycol conjugated steroids **18-37**. Reagents and conditions. *i*) **1** or **3**, Pd/C (10%), H_2, MeOH, RT, 3 h. *ii*) Free base from *i*) or **2**, HBTU, HOBt, DIPEA, DMF, 0°C-RT, 12 h. *iii*) 1 M K_2CO_3, MeOH/H_2O, RT, 1 h, quant. *iv*) TFA/DCM 1:1 *v*/*v*, 1,3-dimethoxybenzene, RT, 2 h. *v*) *n*BuNH_2, analogous to *ii*).

Steroid	Spacer	R_1	R_2	Coupling-Product (Yield %)	Free Acid (Yield %)
T-3-CMO **8**	**1**	Me	H	**18** (67)	**26**[a]
P_4-3-CMO **9**	**1**	Me	H	**19** (64)	**27**[a]
HC-3-CMO **10**	**1**	Me	H	**20** (63)	**28**[a]
Dex-3-CMO **11**	**1**	Me	H	**21** (58)	**29**[a]
HC-17β-acid **12**	**3**	*t*Bu	H	**22** (51)	**30**[a]
Dex-17β-acid **13**	**3**	*t*Bu	H	**23** (52)	**31**[a]
HC-21-CMO **14**	**3**	*t*Bu	H	**24** (73)	**32** (82)
Dex-21-CMO **15**	**3**	*t*Bu	H	**25** (85)	**33** (86)
Dex-3-CMO **11**	**2**	Me	Me	**34** (39)	**35**[a]
Dex-3-CMO **29**	-	-	H	**36** (55)	-
Dex-3-CMO **33**	-	-	Me	**37** (51)	-

Table 1: Yields for the coupling reaction of the steroid acids **8-15** with spacers **1-3**. *a*: products were obtained in virtually quantitative yield.

It should be noted that the steroid 21-CMOs **14**, **15**, **24**, **25**, **32** and **33** were found to be prone to elimination, for instance they possessed a half-life of approx. 12 h in aqueous $NaHCO_3$. Degradation proceeds, in analogy to rapid cleavage of phenylglyoxal-aldoxime

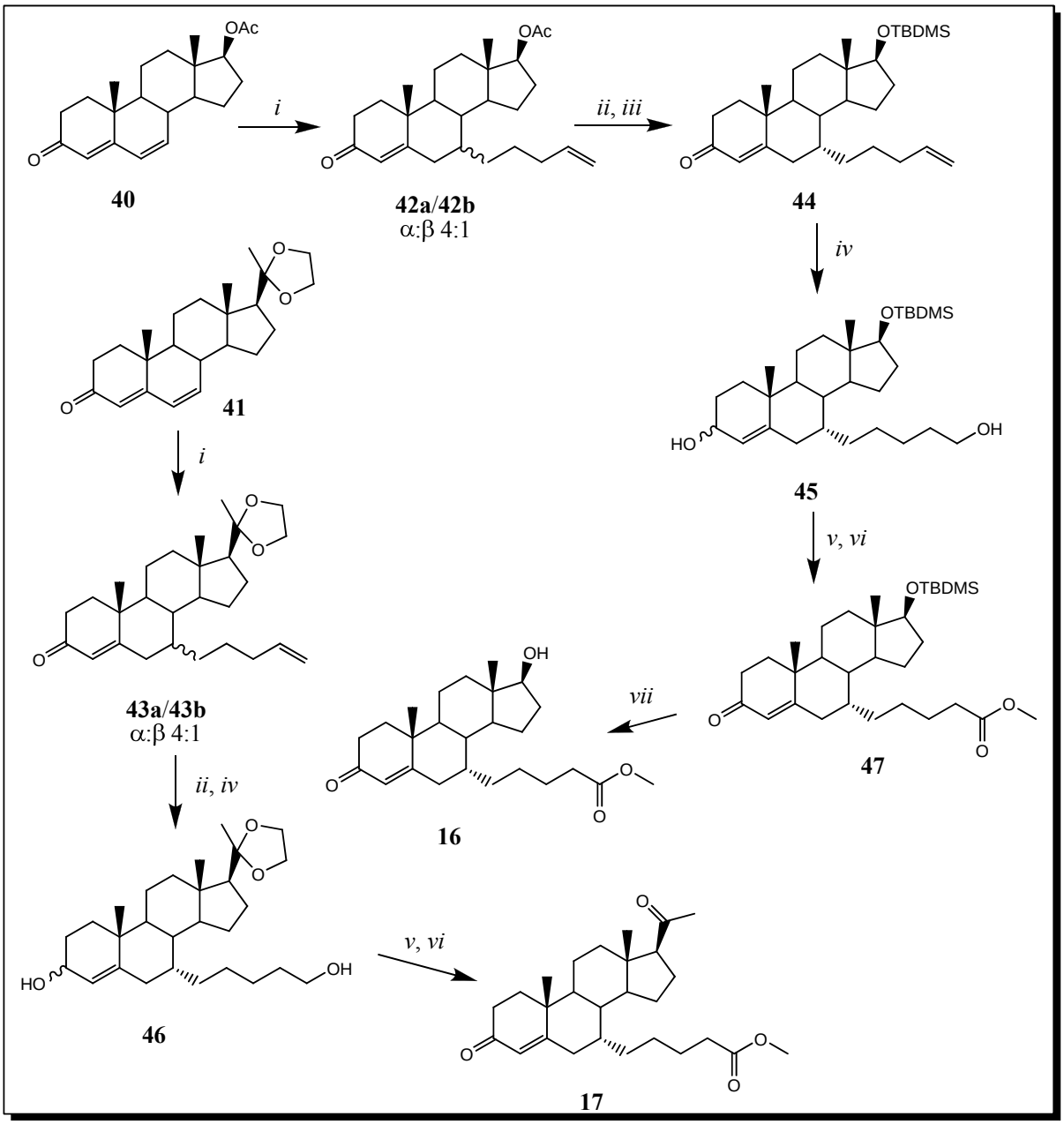
ethers,[164] via elimination of the corresponding alcohol from the oxime and formation of an acyl cyanide species. The acyl cyanide can react further with appropriate nucleophiles to form acids, esters or amides (Scheme 16).



Scheme 16: Decomposition of the GC-21-CMOs in basic media exemplified for Dex derivative **33**. Reagents and conditions: *i*) *n*BuNH$_2$, THF, RT, 12 h, 66 % (**38**), 28 % (**39**).

The assumption was confirmed by treatment of steroid 21-oxime **33** with excess *n*-butylamine in dry THF, leading mainly to formation of the corresponding *n*-butylamide **38** and, to a lesser extent, to the 17-ketone of Dex **39** (Scheme 16).[165] Further evidence for the occurrence of a reactive acyl cyanide intermediate provided the intense odor of hydrogen cyanide emerging from the reaction vessel upon aqueous workup of the reaction mixture.

The synthesis of the SH 7α-derivatives started from the known (17β)-17-(acetyloxy)androsta-4,6-dien-3-one **40**[166] and 20-(1,3-Dioxolan-2-yl)-pregna-4,6-dien-3-one **41**.[167] During the synthesis of dienone **41** the selective dioxolane cleavage in the A-ring with $MgSO_4$ in diethyl ether reported by Zeng et al.[167] could not be reproduced. Instead the A-ring dioxolane was selectively removed by treatment with wet silica gel in DCM following a procedure published by Conia et al.[168] The C5 residue was introduced via Cu(I)CN catalyzed conjugate addition of 1-pentenylmagnesium bromide to the dienone moiety following the published procedure.[169] The corresponding 7-pentenyl derivatized steroids **42** and **43** were obtained as a mixture of the stereoisomeric 7α- and 7β-derivatives **42a**/**42b** and **43a**/**43b** (Scheme 17) in a α:β ratio of approx. 4:1. The corresponding β-isomer could be easily separated by column chromatography furnishing the pure testosterone 7α-derivative **42a**[169] and the progesterone-7α-derivative **43a** in 58 % and 60 % yield, respectively. In the next step, the acetyl ester on position 17 of testosterone derivative **42a** was converted to the corresponding TBDMS-ether as described by Wüst et al.[169] yielding 86 % of silyl ether **43**. Subsequent hydroboration of steroid **44** with 9-BBN in dry THF followed by oxidation with $NaOH/H_2O_2$[169] afforded pure steroid diol **45** in 89 % yield.



Scheme 17: Synthesis of the testosterone and progesterone 7α-derivatives. Reagents and conditions: *i*) Cu(I)CN, 1-pentene-MgBr, THF, -45°C-(-30°C), 4 h. *ii*) column chromatography, 58 % (**42a**)/60 % (**43a**) of α-isomers. *iii*) 1 M NaOH, MeOH, THF, RT, then TBDMSCl, Im, DMF, 0°C-RT, 12 h, 86 %. *iv*) 9-BBN, THF, 0°C-60°C, 1 h, then H_2O, NaOH, H_2O_2, 0°C, 30 min., 89 % (**45**)/93 % (**46**). *v*) DMP, DCM, Py, RT, 1.5 h. *vi*) I_2, KOH, MeOH, 0°C, 0.5 h, then 1 M $NaHSO_4$-soln., then Na_2SO_3, 63 % (**47**)/52 % (**17**). *vii*) 40 % aq. HF, MeCN, 0°C, 1 h, 81 %.

Likewise, progesterone derivative **43a** was converted to the corresponding diol **46** in 93 % yield. Both steroid dioles, **45** and **46**, were then oxidized with Dess-Martin´s periodinane (DMP) to the respective keto-aldehydes[170] and the crude product transformed to the methyl esters **47** and **17** by reacting the steroids with KOH and iodine in dry MeOH.[171] In the case of progesterone derivative **46** the dioxolane group at position 20 of the steroid scaffold was

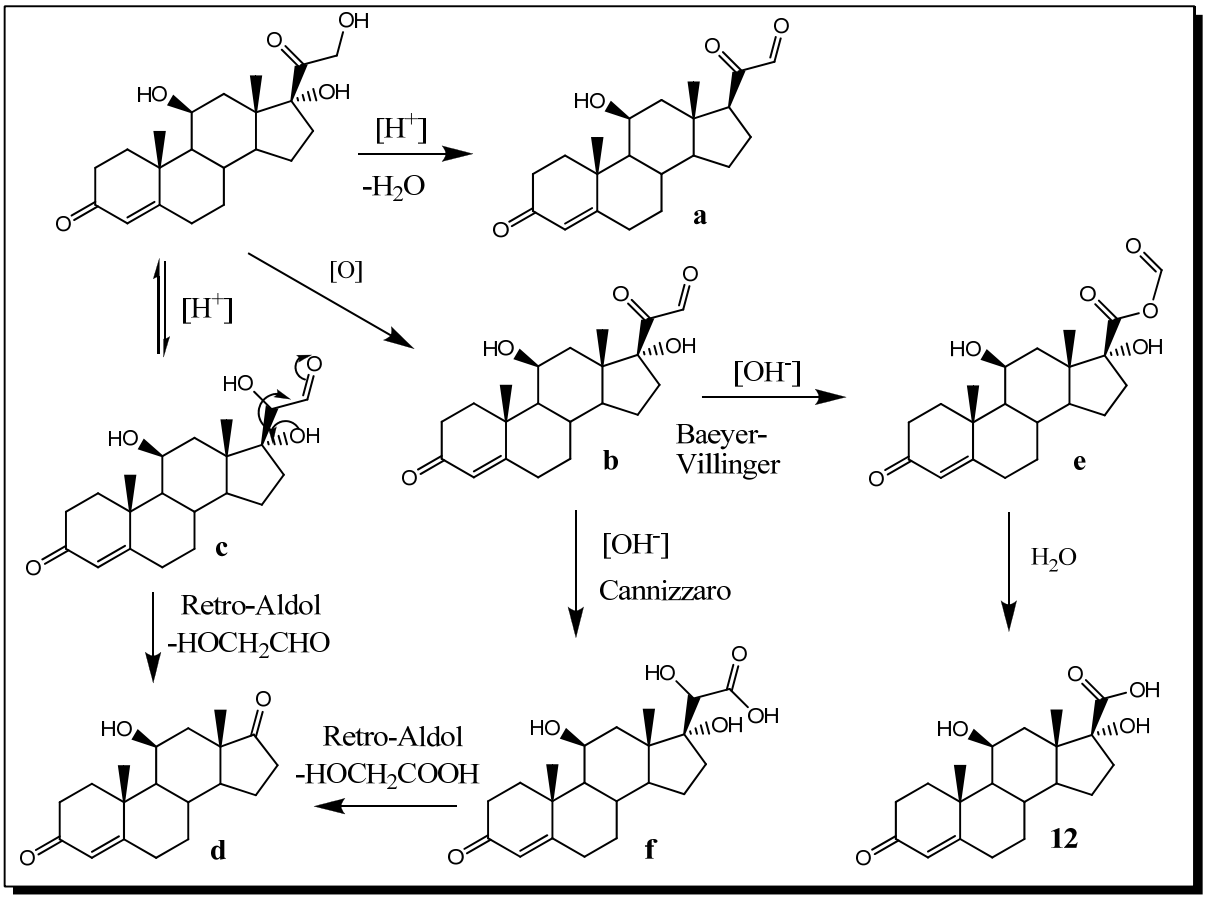
efficiently cleaved during aqueous workup with 1 M $NaHSO_4$-solution within a couple of minutes. The capability of iodine to significantly accelerate acetal cleavage in aqueous media has been described before by Hu et al.[172] Removal of the TBDMS-group from testosterone derivative **47** was accomplished by treatment with 40 % aqueous HF in acetonitrile[169] affording the free alcohol **17** in 81 % yield. It should be mentioned, that other fluoride reagents like TBAF*3H_2O in THF or HF*Py failed to cleave the TBDMS-group on position 17 of the androstane scaffold.

5.1.3 Concerning the stability of the glucocorticoid-oximes

As outlined in section **5.1.2**, the dexamethasone derivatives **29** and **35** were found to be unstable, decomposing rapidly at ambient temperature. As a consequence the corresponding derivatives had to be prepared freshly prior to each of the "fishing"-experiments, making the procedure very laborious. Initially it was believed that intramolecular hydrogen bonding, making the 3-oxime susceptible towards hydrolyses, was responsible for the observed instability.

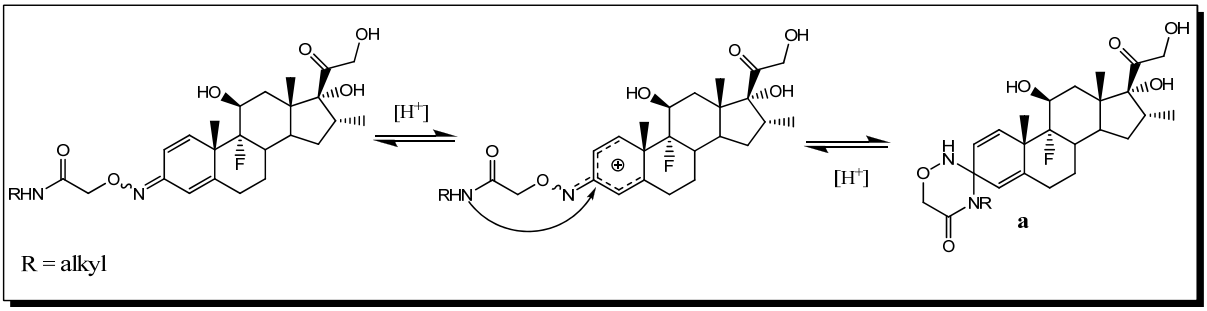
However, when the complex mixture of decomposition products of **29** was chromatographically separated, only one fraction could be clearly identified as the corresponding 21-aldehyde of compound **29**. This observation was in good accordance with a report by Bundgaard and Hansen,[173] who had found that the corticosteroids dihydroxyacetone moiety in the D-ring can degrade via non-oxidative and oxidative pathways. As a non-oxidative degradation product of hydrocortisone they found the 17-deoxy-21-aldehyde **a** (Scheme 18), formed by acid catalyzed ene-diol formation and subsequent dehydration.[174] As a oxidative product the 21-aldehyde **b**, formed by aerial oxidation, was observed. 17-ketone **d** (Scheme 18), most likely formed from hydrocortisone non-oxidatively by α-ketol-enediol-rearrangement/retro-aldol reaction via 21-aldehyde **c** (Scheme 18), was detected as a minor component of the product mixture. Bundgaard and Hansen also observed that oxidative degradation is greatly accelerated by trace metal impurities, in particular copper (see synthesis of **15** and **16**). Similar results for oxidative and non-oxidative degradation of the corticosteroid 17β-sidechain were found in alkaline media.[175, 176] Under these conditions Baeyer-Villinger-type oxidation leading to formic acid anhydride **e** (Scheme 18) and Cannizarro-reaction in keto-aldehyde **b** (Scheme 18) leading to carboxylic acid derivative **f** (Scheme 18) were observed. Indeed, in course of this work it was found that not only the Dex-derivatives **29** and **35** but also the free acid HC derivative **28** decomposed upon storage for longer time periods. These observations and the

identification of the corresponding keto-aldehyde of **29** suggest that degradation (oxidative/non-oxidative) of the dihydroxyacetone moiety of steroidal acids **28** and **29** took place as described above.



Scheme 18: Non-oxidative and oxidative degradation of the dihydroxyacetone-moiety found for the glucocorticoids under moderate acidic (pH 5-6.5) or basic conditions.

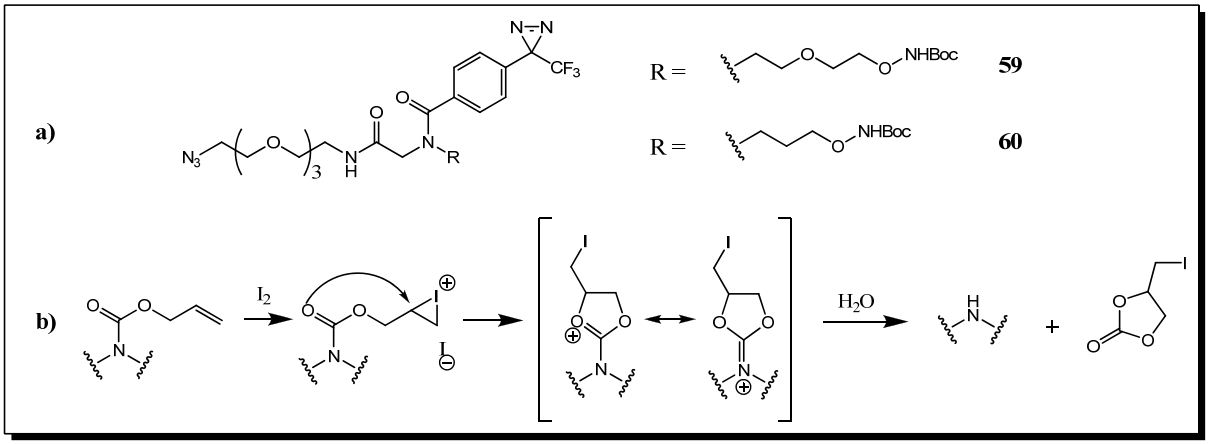
Another pathway for the degradation of glucocorticoids was described to proceed via acid induced dienone-phenol-rearrangement/retro-aldol-reaction. This pathway was observed for the A-ring dienone corticosteroids fluocinonide acetonide[177] and mometasone furoate,[178] among others.[179] Hereby bond rupture between C9 and C10 leads to the corresponding A-ring aromatized secosteroid. However, since the observed bond scission necessitates strong acid and in the case of the Dex-3-CMOs no secosteroid formation could be verified, involvement of this mechanism seems rather unlikely. More plausible appears the cyclization of the CMO-moiety to the corresponding spiro derivative **a** (Scheme 19).[180] Although a corresponding spiro-species could not be isolated so far, this would explain the stability of the neutral *N*-methylated derivatives **34** and **37** and the absence of the corresponding hydrated steroids or hydrolysis products (Dex).



Scheme 19: Proposed mechanism for formation of the oxadiazinanone-spiroaminal **a**.

5.1.4 Synthesis of the aminooxy functionalized photophore building blocks

The photoreactive steroid probes used for the catch-and-release experiments (see section 4.1.6) were designed to bear an aliphatic azide for the capture on the solid support, a suitable photophore like a BP or phenyl(trifluoromethyl)diazirine moiety and an aminooxy residue (this section) or an amino-function (next section) for attachment of the steroidal bait (Scheme 20). In order to simplify the construction of the photoaffinity probes, a synthetic route relying on easily accessible aminooxy building blocks was envisaged. The deprotected aminooxy ethers **59** and **60** can be attached to a variety of ligands bearing a suitable ketone or aldehyde moiety.



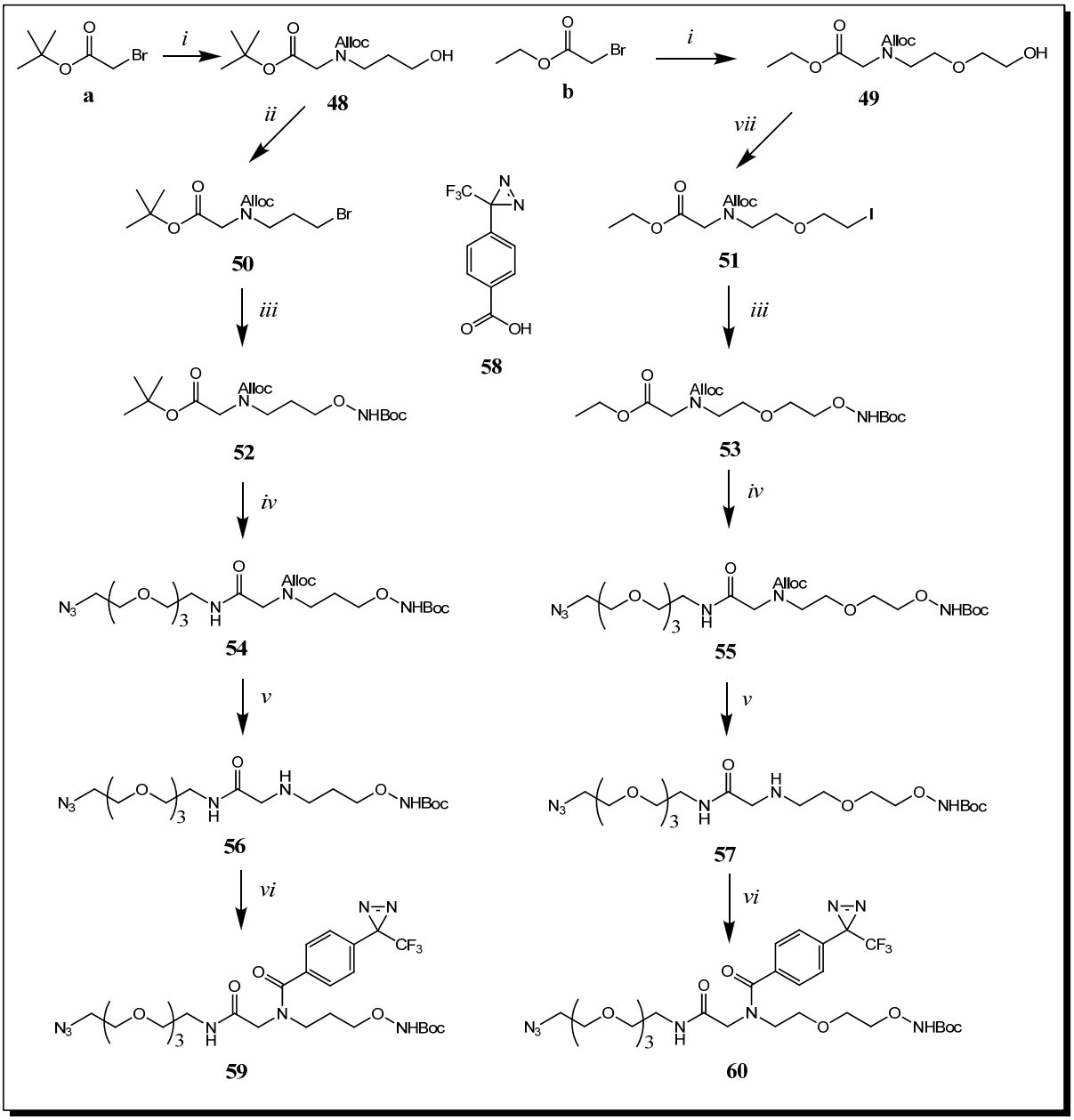
Scheme 20: **a)** Oxy-amine functionalized photophore building block for the coupling to affinity-ligands bearing a ketone or aldehyde functionality. **b)** Mechanism of Alloc-cleavage in **54** and **55**.

The photophore building block is based on a glycine core-unit acting as a trifunctional adapter. Contrary to the synthetic solutions published,[57] this approach increases the variability by allowing attachment of various photophores (e.g. arylazides, ketones) and tags (e.g. fluorescent dyes, biotin etc.). Additionally, in case that the biological activity of the probe is detracted by steric crowding of the ligand[60] the linker length between photophore and aminooxy ether moiety can be varied (see below).

In order to elucidate the impact of spacer length between bait and photophore, two sets of photophore building blocks were synthesized. One exhibits a propylene spacer and the other a 3-oxapentylene spacer. The aa-motif was established in the first step of the synthesis by alkylation of bromoacetic acid *tert*.-butyl ester **a** (Scheme 21) with 3-amino-1-propanol or bromoacetic acid ethyl ester **b** (Scheme 21) with 2-(2-aminoethoxy)ethanol in THF.[181] Alloc-protection of the crude secondary amines yielded the analytically pure protected amino acids **48** and **49** in 86 % and 81 % yield, respectively. Employment of the *tert*.-butyl ester **a** (Scheme 21) proved to be favorable, since alkylation of 3-amino-1-propanol with **b** (Scheme 21) resulted in very low yields (<10 %). In the next step the free hydroxyl function was converted to the corresponding bromide in case of **48**[182] or iodide for **49**[183] employing PPh_3/imidazole and carbon tetrabromide or iodine as electrophile/halogen source. The halogenides **50** and **51** were obtained in 88 % and 92 % yield, respectively. Subsequently, introduction of the aminooxy residue was achieved by nucleophilic substitution with *N*-Boc-hydroxyl amine in dry DCM and DBU as the base.[184] This led to the protected aminooxy ethers **52** and **52** in 61 % and 60 % yield, respectively. The aliphatic azide moiety should be introduced by amide coupling of the corresponding free acids of **52** and **53** with amino azide linker **5**. For this purpose the esters were saponified either with aqueous 3 M KOH-soln. in MeOH/THF 3:1 *v*/*v* in case of *tert*.-butyl ester **52** or aqueous 1 M NaOH-soln. in MeOH/THF 3:1 *v*/*v* for **53**. The crude free acids were directly coupled to linker **5** via DCC technique using HOBt and DIPEA as additives. This furnished diamide **54** in 72 % yield and diamide **55** in 88 % yield.

The best method for removal of the Alloc-group was found to be a iodine-mediated cyclization reaction followed by hydrolysis in aqueous acetonitrile.[185] Remarkably, the reaction proceeded to completion after only 12 h at room temperature. Primary Alloc-protected amines require 48 h under the same reaction conditions. Most likely the increased liability to hydrolyses of the intermediary iminium species is responsible for the observed acceleration (Scheme 20). Thereby the free secondary amines **56** and **57** were obtained in good yields of 88 % and 80 %, respectively.

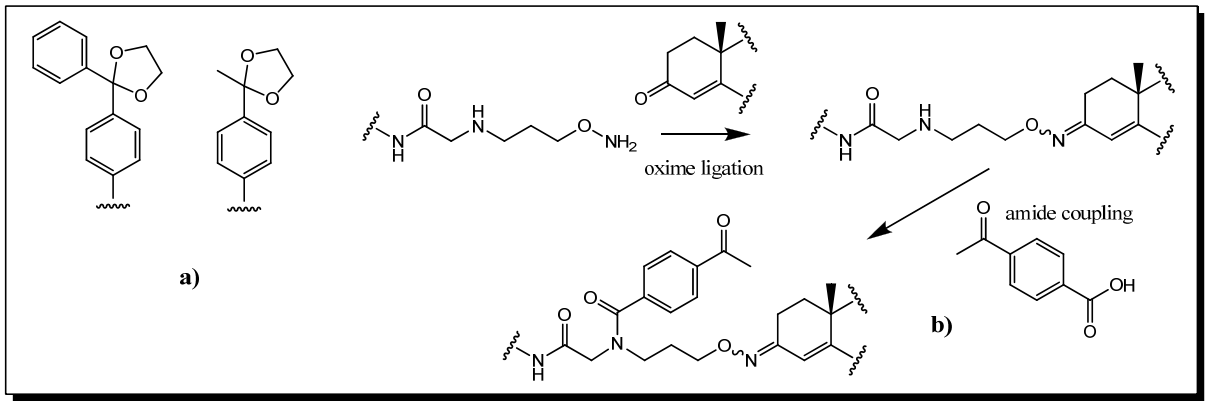
Completion of the photophore building blocks was accomplished by HBTU mediated amide coupling with diazirine **58**[186] adding HOBt and DIPEA in dry DMF. Thus, aminooxy ether **59** and **60** could be obtained in 86 % and 82 % yield, respectively. The *N*-Boc-protected aminooxy ethers **59** and **60** were used to synthesize photoaffinity conjugates of the steroid hormones Dex and HC via oxime ligation reaction (see section 5.1.6)



Scheme 21: Synthesis of the aminooxy photophore building blocks **59** and **60**. Reagents and conditions: *i*) 3-amino-1-propanol/2-(2-aminoethoxy)ethanol, **a**/**b**, THF, 0°C, 4 h, then AllocCl, $NaHCO_3$, H_2O, 0°C-RT, 12 h, 86 % (**48**)/81 % (**49**). *ii*) PPh_3, CBr_4, THF, 0°C-RT, 4 h, 88 %. *iii*) BocNHOH, DBU, DCM, RT, 48 h, 61 % (**52**)/60 % (**53**). *iv*) 3 M KOH/1 M NaOH, MeOH/THF, RT, then **5**, DCC, HOBt, DIPEA, DMF, -20°C-RT, 12 h, 72 % (**54**)/88 % (**55**). *v*) I_2, H_2O, MeCN, RT, 12 h, 88 % (**56**)/80 % (**57**). *vi*) **58**, HBTU, HOBt, DIPEA, DMF, 0°C-RT, 12 h, 86 % (**59**)/82 % (**60**). *vii*) PPh_3, Im, I_2, 0°C, 3 h, 92 %.

A potential drawback of the given reaction sequence is that widely applied ketone-based photophores like benzophenone (BP) and acetophenone (AP) cannot be applied. The ketone functionality present on the linker construct would simply lead to reaction of the linker with itself as soon as the Boc-group is removed. This disadvantage could be circumvented by

using protected ketone photophores (e.g. acetal protected) that can be liberated after oxime ligation with the ligand.



Scheme 22: **a)** Protected ketone photophores orthogonal to the oxime ligation strategy. **b)** Strategy for oxime-ligation between a suitable ligand and suitable amino-aminooxy linker (e.g. linker fragments **56** and **57**) with subsequent introduction of a ketone-based photophore.

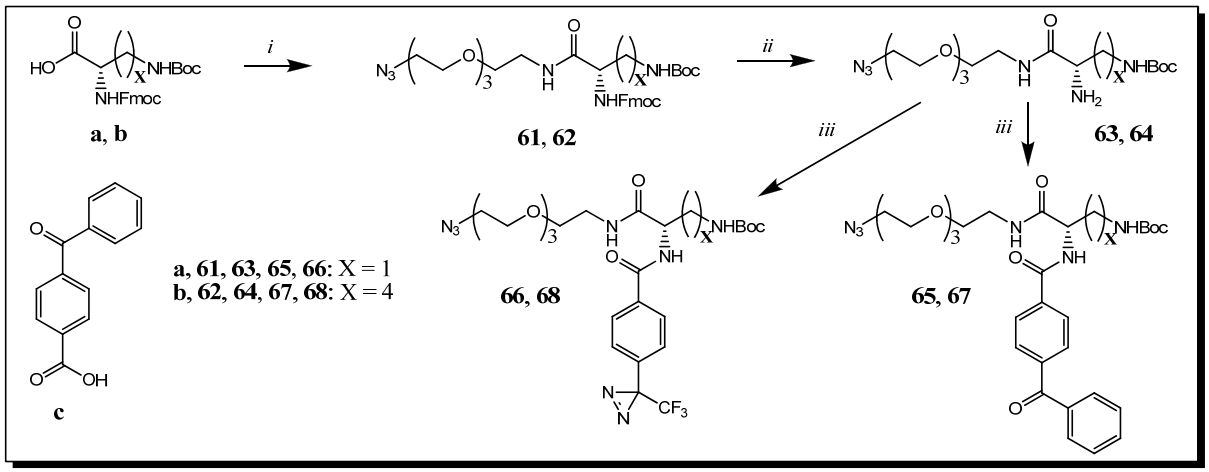
Likewise, it would be feasible to perform the oxime ligation between *N*-Boc-deprotected free amines **56** and **57** and subsequently introduce the ketone-photophore by an appropriate coupling reaction (*N*-alkylation, amide coupling). Naturally, this is only realizable if no nucleophilic amine moiety is present on the ligand (Scheme 22).

5.1.5 Synthesis of the amino-functionalized photophore building blocks

Since the aminooxy photophore building blocks do not allow regioselective oxime formation with a ligand bearing two carbonyl moieties that differ only marginally in their reactivity (e.g. P_4), a second set of photophore building blocks bearing an amino functionality was synthesized. The amino functionalized building blocks facilitate coupling to a ligand exhibiting a carboxylic function, e.g. the steroid CMOs **8**-**15** or the 7α-derivatives **16** and **17**, via standard amide coupling procedures. Hereby, the tri-functional core-unit is provided either by L-β-amino alanine (short spacer) or by L-lysine (long spacer). In analogy to the aminooxy building blocks, the impact of spacer length should be examined.

The synthesis started from the corresponding commercially available α-Fmoc-β-Boc-protected L-amino acid **a** (Scheme 23) and α-Fmoc-ω-Boc-protected L-amino acid **b** (Scheme 23). In the first step the free acids **a** and **b** (Scheme 23) were coupled to amino-azide **5** using DCC, PFP or HOBt and DIPEA in dry THF affording amide **61** and **62** in 64 % and 63 % yield, respectively. The α-deprotected amines **63** and **64** were obtained in virtually quantitative yield by treatment of the Fmoc-derivatives **61** and **62** with 50 *v*% morpholine in dry DMF. Subsequently, the free amines were coupled either to diazirine **58** or 4-benoylbezoic acid **c** (Scheme 23) to furnish the complete *N*-Boc-protected photophore

building blocks **65-68**. Amide coupling with the photophores **58** and **c** (Scheme 23) was achieved with HBTU, HOBt and DIPEA. Thereby, the benzophenone derivatives **65** and **67** were obtained in yields of 90 % and 96 % and the diazirine derivatives **66** and **68** in 92 % and 93 % yield, respectively (Scheme 23).

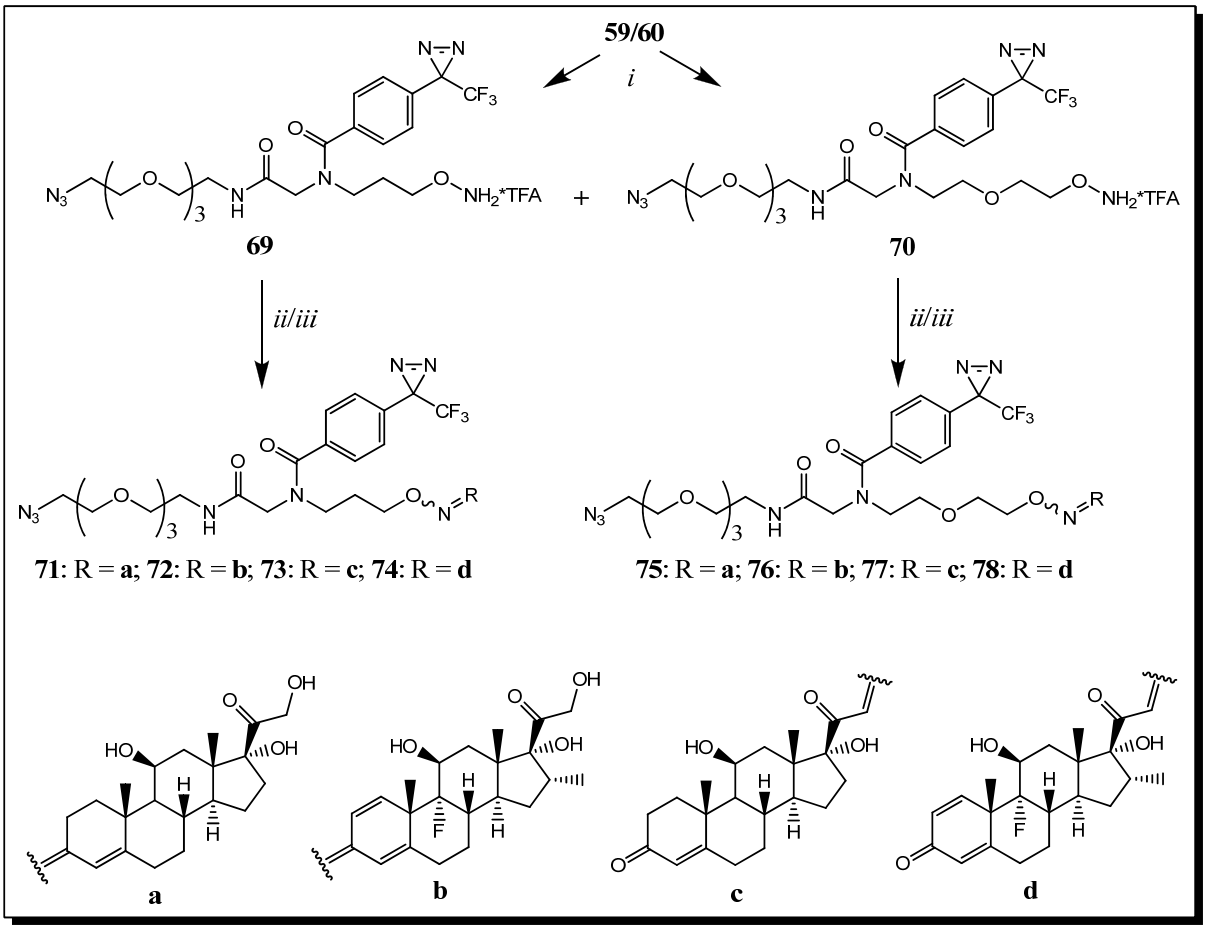


Scheme 23: Synthesis of the amino-functionalized photophore building blocks **65-68**. Reagents and conditions: *i*) DCC, PFP/HOBt, DIPEA, THF, 0°C-RT, 12 h, 64 % (**61**), 63 % (**62**). *ii*) morpholine, DMF, 1:1 *v*/*v*, RT, 1 h, quant. *iii*) **58** or **c**, HBTU, HOBt, DIPEA, DMF, 0°C-RT, 12 h, 90 % (**65**), 92 % (**66**), 96 % (**67**), 93 % (**68**).

5.1.6 Coupling of the steroid ligands with the aminooxy and amino photophore building blocks

Prior to oxime ligation with the steroidal ketones, the linkers **59** and **60** were deprotected with DCM/TFA 4:1 to yield the corresponding crude free aminooxy ethers **69** and **70** (Scheme 24). When linker **69** was used for the oxime ligation reaction with HC and Dex in a mixture of MeOH and acetate buffer at various pH-values (pH 4-8), either the yields were found to be very low (<10 %) or an inseparable product mixture was obtained. Also other solvents or solvent mixtures like EtOH, Py, MeOH/H_2O containing additives like sodium acetate or the nucleophilic catalyst anilinium acetate[187] resulted in low yields (<20 %).

The best results were obtained in dry pyridine containing 5 *v*% of acetic acid at 55°C (Scheme 24, method ***ii***) affording the corresponding 3-oximes **71** and **72** in good yields of 68 % and 71 %, respectively (Scheme 25; Table 3). In analogy to the preparation of **14** and **15**, coupling of linker **69** to the HC- and Dex-21 aldehydes was accomplished by treatment of the respective crude keto aldehyde with the free aminooxy ether in a mixture of MeOH/acetate pH 7 buffer (Scheme 24, method ***iii***) affording the corresponding 21-oximes **73** and **74** in 43 % and 69 % yield, respectively (Scheme 24; Table 3).



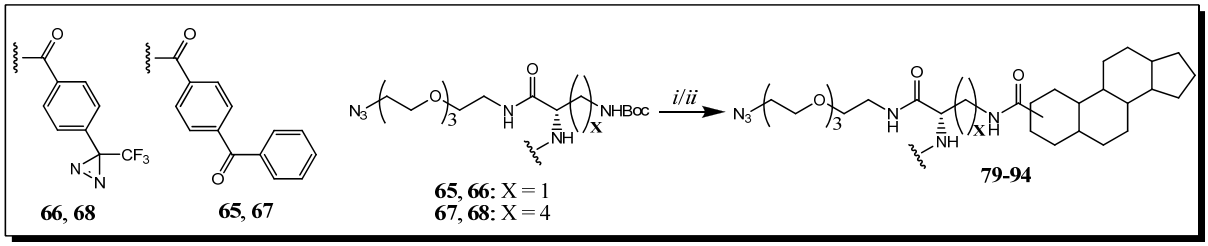
Scheme 24: Oxime ligation of the aminooxy photophores with the steroidal ketones and aldehydes. Reagents and conditions. *i*) TFA/DCM, 1:4 *v*/*v*, RT, 1 h, quant. *ii*) HC/Dex, Py, AcOH, 55°C, 24 h. *iii*) Dex/HC, Cu(II)acetate, O_2, RT, 0.5-1 h, then EDTA, 0.1 M acetate pH 7 buffer, RT, 12 h.

Steroid	Linker	Method	Product (Yield %)
HC	**59 (69)**	***ii***	**71** (68)
Dex	**59 (69)**	***ii***	**72** (71)
HC	**59 (69)**	***iii***	**73** (43)
Dex	**59 (69)**	***iii***	**74** (69)
HC	**60 (70)**	***ii***	**75** (72)
Dex	**60 (70)**	***ii***	**76** (77)
HC	**60 (70)**	***iii***	**77** (48)
Dex	**60 (70)**	***iii***	**78** (64)

Table 2: Yields for oxime ligation between photophore building blocks **67** (**80**), **68** (**81**) and the GCs HC, Dex, HC-21 aldehyde and Dex-21 aldehyde.

Coupling of building block **60**, possessing the longer spacer unit between ligand and photophore, with the respective steroids proceeded analogous to the ligation of **59**,

furnishing the steroid oximes **75**, **76**, **77** and **78** in comparable yields of 48-77 % (see table 3). Naturally, the rules concerning stability of the 21-oximes **32** and **33** also apply for the derivatives **73**, **74**, **77** and **78**.



Scheme 25: Synthesis of the amino photophore-SH conjugates **79-94** from building blocks **65-68** and the steroids **8, 9**, **16** and **17**. Reagents and conditions: *i*) TFA/DCM 1:4 *v*/*v*, RT, 1 h, then **8**/**9**, HBTU, HOBt, DIPEA, DMF, 0°C-RT, 12 h. *ii*) **18**/**19**, 1 M NaOH, MeOH/THF, RT, 1 h, then analogous to *ii*).

Steroid	Linker	Method	Product (Yield in %)
T-3-CMO **8**	**65**	***i***	**79** (82)
P_4-3-CMO **9**	**65**	***i***	**80** (87)
T-3-CMO **8**	**66**	***i***	**81** (76)
P_4-3-CMO **9**	**66**	***i***	**82** (92)
T-3-CMO **8**	**67**	***i***	**83** (78)
P_4-3-CMO **9**	**67**	***i***	**84** (78)
T-3-CMO **8**	**68**	***i***	**85** (96)
P_4-3-CMO **9**	**68**	***i***	**86** (89)
7α-T **16**	**65**	***ii***	**87** (93)
7α-P_4 **17**	**65**	***ii***	**88** (80)
7α-T **16**	**66**	***ii***	**89** (83)
7α-P_4 **17**	**66**	***ii***	**90** (69)
7α-T **16**	**67**	***ii***	**91** (74)
7α-P_4 **17**	**67**	***ii***	**92** (61)
7α-T **16**	**68**	***ii***	**93** (86)
7α-P_4 **17**	**68**	***ii***	**94** (75)

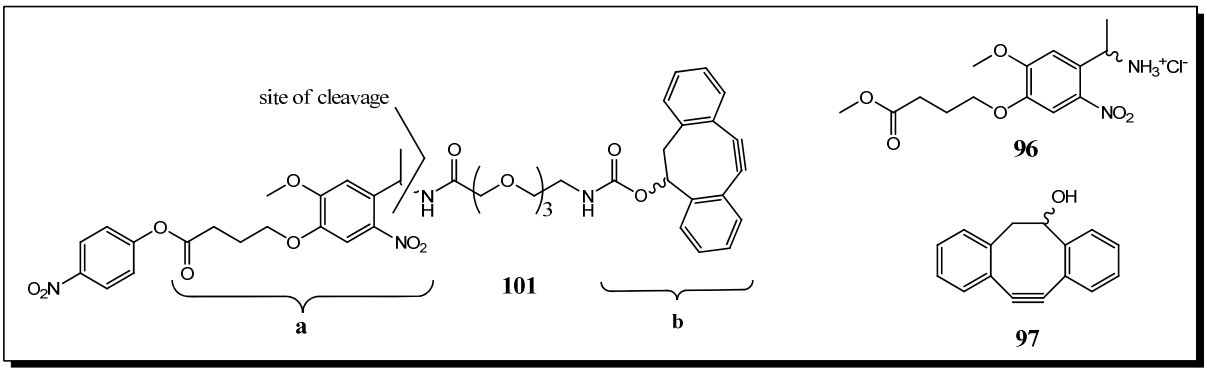
Table 3: Yields of the coupling reactions between the linkers **65-68** and the steroids **8**, **9**, **16** and **17**.

For coupling of the SH-3-CMOs **8** and **9** the *N*-Boc-protected building blocks **65-68** were first deprotected with 20 *v*% TFA in DCM and the crude free amine reacted with the

steroidal carboxylic acids using HBTU, HOBt and DIPEA in dry DMF. Prior to coupling of the 7α-derivatives **16** and **17** the methyl esters were saponified with 1 M aqueous NaOH in MeOH/THF 3:1 (*v*/*v*) and then coupled to the free amines of the linkers **65-68** analogous to the 3-CMOs. As indicated by Table 2, amide coupling of the sex hormones **8**, **9**, **16** and **17** resulted in high average yields of 61-97 % (scheme 14, table 3). Compared to oxime ligation between the aminooxy building blocks **69** and **70** and the GCs the yield obtained by amide-coupling proved to be generally higher.

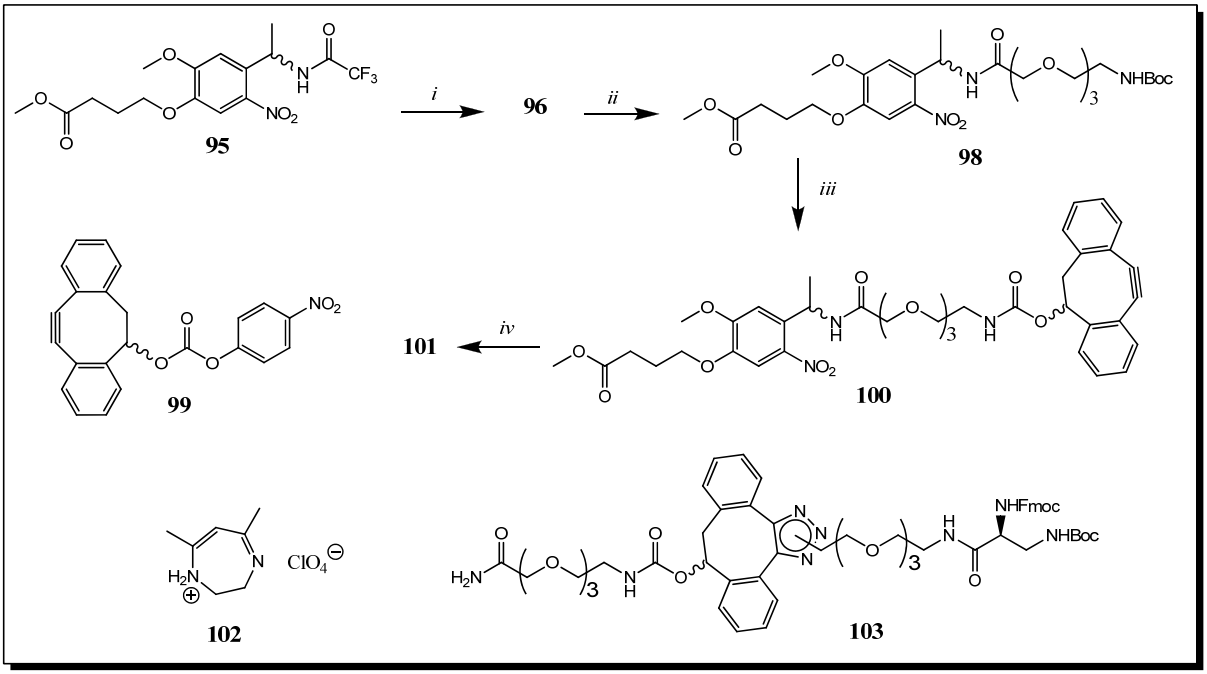
5.1.7 Synthesis of the photocleavable strained alkyne construct

The photocleavable strained alkyne construct **101** (scheme 26) was designed for application in the catch and release experiments with the azide-tagged steroid photoaffinity probes. Capture of azide-tagged biomolecules can be facilitated via strain promoted "click"-reaction and release of the purified proteins by UV-light induced cleavage of the *o*-nitrobenzyl linker. The construct is composed of the *o*-nitrophenyl linker **96** introduced by Holmes[78] and the dibenzocyclooctyne alcohol **97**[188] fused to each other by a tetraethylene glycol spacer (Scheme 26). Octyne **97** can be obtained on a large scale via a very convenient route in seven steps from dibenzosuberenone.[188] Contrary to the literature procedure, TBDMS-protection of the intermediate benzyl alcohol on step five of the synthesis was accomplished by reacting the aromatic with TBDMSCl/Im in DMF instead with TBDMSOTf/lutidine.[188] The yields match the ones reported for the literature procedure and accordingly the more cost-efficient TBDMS/Im standard protocol should be used. Construct **101** can be coupled directly to amino functionalized solid supports via amide bond formation with the *p*NP-active ester residue placed on the Holmes`s linker moiety.



Scheme 26: Photocleavable strained alkyne construct **101** composed of the photolabile *o*-nitrobenzyl linker (**a**) and the strained alkyne (**b**) for azide capture and the linker fragments **96** and **97**.

The synthesis starts from free amine **96** which was obtained directly in 97 % yield from the known nitro-aromatic **95**[78] via solvolysis of the trifluoroacetamide moiety in refluxing dry HCl-methanol solution (Scheme 27). Nitroaromatic **95** can be obtained via a very convenient five step procedure from acetovanillone.[78] In the next step the corresponding free acid of ethylene glycol linker **4**, obtained by saponification using aqueous 1 M K_2CO_3-soln. in methanol, was coupled to the Holmes`s linker fragment **96** using DCC, HOBt in dry THF and DIPEA as the base leading to the *N*-Boc-protected derivative **98** in 91 % yield. For attachment of the cyclooctynol fragment, **97** was first converted to the corresponding *p*NP-carbonate **99**[36] and then coupled to the free base of linker **98** forming the stable carbamate **100** in 90 % yield. In the last two steps the methyl ester was saponified using LiOH in aqueous methanol[37] and the free acid converted to the *p*NP-active ester **101** via DCC technique in moderate 60 % yield.



Scheme 27: Synthesis of the photo cleavable strained alkyne construct **101** and linker fragment **103** released in course of a catch-and-release experiment with linker fragment **61**. Reagents and conditions. *i*) HCl, MeOH, reflux, 36 h, 97 %. *ii*) **4**, 1 M NaOH/MeOH/THF 1:2:1 *v*/*v*/*v*, RT, 1 h, then DCC, HOBt, DIPEA, THF, -20°C-RT, 18 h, 91 %. *iii*) TFA/DCM, 1:4 *v*/*v*, RT, 1 h, then **99**, DIPEA, DMF, RT, 24 h, 90 %. *iv*) LiOH, MeOH/THF/H_2O 1:1:2 *v*/*v*/*v*, RT, 1.5 h, then DCC, *p*NP, THF, -20°C-RT, 14 h, 60 %.

5.2 Catch-and-release experiments

5.2.1 Irradiation conditions and activation of the photophores

Both, activation of the photophores for photoaffinity labeling and cleavage of Holmes`s linker for recovery of proteins was accomplished by irradiation of the samples with a 150 W medium pressure mercury lamp. In order to prevent the azide-tag and the protein samples from taking severe damage by irradiation with light of wavelengths lower than 350 nm, a suitable liquid filter was introduced.

Aliphatic azides are known to absorb light of wavelength of approx. 300 nm.[189] Additionally, in accordance to Holmes et al.[82, 83] it has been found by Behnke and Kutter[190] in previous experiments that protein samples take serious damage upon irradiation without application of a suitable UV-filter. Hence a <350 nm cut off filter solution was applied consisting of a 0.02 *w*% solution of 2,7-dimethyl-3,6-diazacyclohepta-2,6-diene perchlorate **102**[191, 192] (Scheme 27) in 2 M aqueous KNO_3 soln. and the solution placed in a glass casing around the UV-lamp. Diazepinium salt **102** can be easily prepared in one step from acetylacetone and ethylene diamine as described by Schwarzenbacher and Lutz.[193] The thickness of the aqueous filter solution around the lamp was arranged to be between 1.5 and 2 cm (Figure 1).

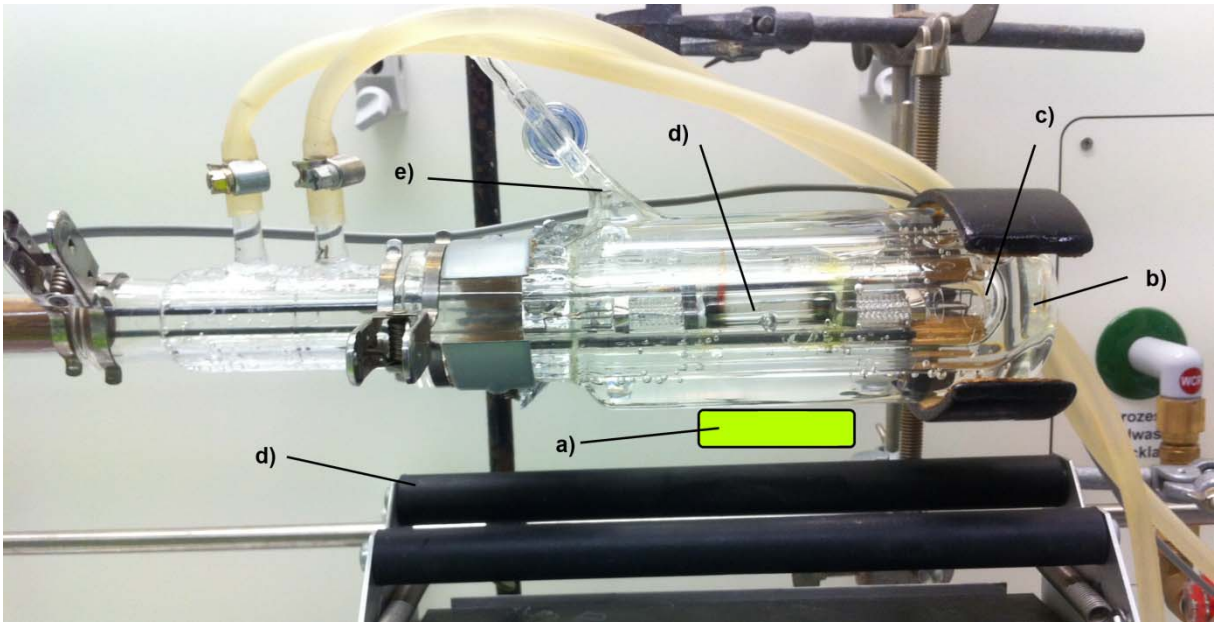


Figure 1: Assembly of the UV-lamp for irradiation of the photoreactive compounds. **a)** Positioning of the sample for irradiation. **b)** Outer glass casing containing the UV-filter solution. **c)** Inner glass casing containing the cooling water. **d)** Tube containing the 150 W medium pressure mercury lamp with the light bulb. **e)** Gas inlet for pressure equalization. **d)** Shaker.

It should be noted that the vessel for the filter solution should not be closed, since gas evolution or thermal extension can occur during irradiation. Further, the filter solution is not infinitely stable to irradiation and turns ember colored after some time. The solution should therefore be replaced every 12 h of irradiation. For the irradiation experiments the samples should be placed in a perpendicular line to the mercury lamb because radiation output is maximal at this angle. Furthermore the samples should be placed as close as possible to the light source in order to optimally exploit the radiation power (Figure 1).

In the preliminary experiments for activation of the diazirine and benzophenone photophores a sample of compound **66** and **65** was dissolved in dioxane/PBS 1:1 *v*/*v* and exposed to the filtered radiation of the 150 W medium pressure mercury lamp. In order to test the stability of the aliphatic azide moiety a sample of compound **61** was prepared in the same manner. For irradiation, the reaction vessels were placed in 2 cm distance to the light source and reacted for 4 h while being shaken. Every 15 min a sample was stippled on a TLC-sheet and after 4 h the sheets were developed in chloroform containing 3 % methanol. For the diazirine photophore in **76** no more progression of the reaction was observed after 15 min of irradiation. The benzophenone photophore in **65** persevered much longer, showing complete decomposition after 60 min of irradiation. Degradation products of the azide **61** could not be detected even after 4 h of irradiation, leading to the conclusion that the azide remains stable when the <350 nm cut-off filter is applied. In stark contrast, the azide **61** shows a half-life of approx. 6 h when irradiated under the same conditions but with a acetone cut-off filter (<310 nm) applied. Thus, use of the <350 cut off filter solution is highly recommended for irradiation of the given probes and the protein samples.

5.2.2 Determination of the catch-and-release kinetics

In order to examine the performance of the catch-and-release approach, active ester **101** was immobilized on TOYOPEARL-AF-650 amino beads (100 µmol/ml -NH_2) in dry MeCN and DIPEA as the base. The rate of azide capture via strain-promoted “click”-reaction was evaluated by adding a twofold excess of photophore precursor **61** to the beads suspended in a mixture of dioxane/PBS. Depletion of **61** from the supernatant was tracked and quantified photometrically. The results show that saturation of the alkyne-functionalized solid support is achieved after 30 min with an effective triazole-loading of 14.7 µmol per ml of TOYOPEARL-suspension. As indicated by the curves in **a**) (Figure 2) azide capture proceeds almost equally fast at RT and 4°C.

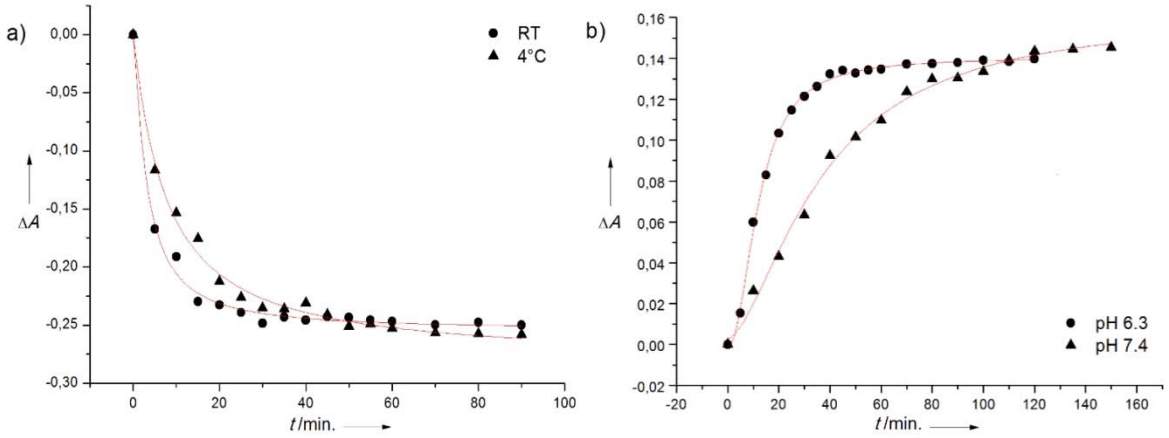


Figure 2: Photometric measurement of the Time-course of the catch-and release procedure. **a)** Capture of the photophore precursor **61** on TOYOPEARL functionalized with the photolabile strained alkyne **101. b)** UV-light 350 nm-induced release of triazole **102** from the solid support.

For examination of *o*-nitrobenzyl linker cleavage and liberation of the linker fragment **103** (Scheme 27) from the solid support, two distinct experiments were conducted. According to the finding that *o*-nitrobenzyl linker cleavage is greatly accelerated in acidic reducing buffer media,[194, 195] one portion of the beads was suspended in a mixture of dioxane and Na-phosphate pH 6.3 buffer containing 10 mM DTT. Another portion was suspended in dioxane/PBS and both samples irradiated with the 150 W medium pressure mercury lamp at RT. Progression of the cleavage reaction was tracked and quantified by photometric measurement of **103** in the supernatant. As seen from curve **b**) (Figure 2), liberation of linker fragment **103** was complete after 2 h in dioxane/PBS. In the acidic reducing buffer, cleavage was significantly accelerated (~3-fold) and complete after 40 min. Quantitative analysis indicated 85% recovery of **103** in 98.2 % purity (HPLC).

5.3. Biological activity tests of the steroid-conjugates

In order to test the glucocorticoid activity of the GC-3-CMOs, 21-CMOs and 17β-carboxamides a test system based on human A549 cells transfected with a GFP-tagged GR construct[196] was established.[1] Upon treatment of the cells with a hormonally active glucocorticoid derivative, translocation of the activated GR from the cytosol into the nucleus could be tracked via fluorescence microscopy. The efficiency of the activation process induced by the sample applied was determined as ratio of nuclear localization versus

[1] Biological activity tests for the GCs were performed by Ms. Yvonne Etzel in the laboratories of Prof. Dr. Gabriele Dodt, Tübingen.

cytosolic localization of the GR. This assay revealed the 17β-amide free acids **30** and **31** to be completely inactive (Figure 3). The steroid 21-CMO free acids **32** and **33** showed significant activity compared to the control sample, but diminished activity (23 and 30 % total, respectively; Figure 3) activity compared to the parent compounds HC and Dex (92 and 93 % total, respectively; Figure 3). Contrary to these findings, Manz et al. reported only slightly diminished activity for the GC-21-oximes.[157] The loss of activity was most likely caused by attachment of the ethylene glycol spacer chain.

Surprisingly, the GC-3-CMO free acids **28** and **29** showed to be almost equally potent (63 and 93 % total, respectively; Figure 3) as HC and Dex (92 and 93 % total, respectively). On the other hand, *N*-methylated derivative **35** was found to be completely inactive.

BSA conjugates of GC-3-CMOs have previously been used to raise glucocorticoid antibodies. These were in turn purified with affinity matrices functionalized with the corresponding GC-3-CMOs.[197, 198] However, these reports did not provide any information about the hormonal activity of the 3-CMOs.

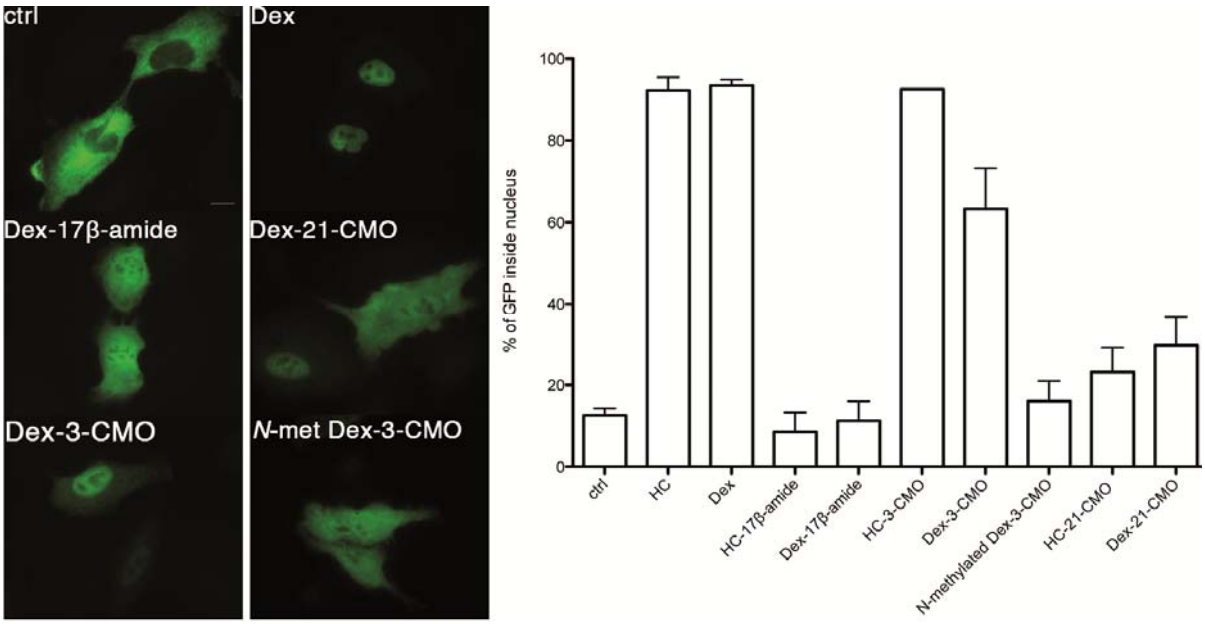


Figure 3: Biological activity assay for the 3-CMO- (**28**, **29**, **35**), 17β-caboxamido- (**30**, **31**) and 21-CMO-derivatives (**32**, **33**) of HC and Dex: GR-GFP transfected A549 cells were grown for 24 h, then incubated for 15 min with 1 µM Dex-derivative or 25 µM HC-derivative. As indicated by the fluorescence images (left) and the quantitative analysis for relative nuclear localization of the GR-GFP (histogram, right), GC-activity is preserved for steroid 3-CMOs (63-93 %) and only moderate for 21-CMOs (23-30 %) compared to HC and Dex (92-93 %) whereas steroid 17β-carboxamides and *N*-methylated Dex-3-CMO completely lack GC-activity. Scale bar: 10 µm.

The given findings can be considered as the first report of the retained glucocorticoid activity of GC-3-CMOs. This implies their superior applicability for affinity purification based methods as compared to the compounds used previously for this purpose.[156] Biological activity of the T-3-CMO **8** and the T-7α-derivative **16** was tested by measuring androgen induced ERK-phosphorylation as observed for T and DHT.[199] The degree of phosphorylation was then correlated to the androgen activity of the samples. In this assay both, the T-3-CMO **8** and 7α-derivative **16** were found to exhibit androgen activity matching the activity of the parent compound T.[2] For progesterone no reliable activity assay could be established so far. Nonetheless P_4-3-CMO conjugates were reported to possess hormonal activity and were used to examine non-genomic effects deployed by progesterone.[200, 201] No such report could be found for P_4-7α-derivatives and thus no statement about their hormonal activity can be made at this point.

5.4 Affinity purification, 1D-, 2D-DIGE-Gel and SILAC-experiments

In order to obtain affinity matrices suitable for affinity purification of PMNL-, PBMC-cell and platelet lysates the SH-3-CMOs **8** and **9**, the SH-7α-derivatives **16** and **17** were immobilized on amino functionalized Sepharose® or poly(methacrylate)-beads (TOYOPEARL®). The obtained protein samples were analyzed via 1D-gel electrophoresis or 2D-DIGE-analysis.[3] The results of the "fishing"-experiments are discussed complementarily below. Detailed information about immobilization procedures, methods for affinity purification and the proteins identified can be found in the PhD thesis of Dr. Felix Behnke.[46] Dr. Carlo Pergola intended to conduct the experiments with the steroid photo-affinity probes and the catch-and-release experiments with the UV-light cleavable strained alkyne construct **101**, but these experimentations are not completed at the present moment. For the "fishing"-experiments with the GCs in A549 cell lysates the ethylene glycol conjugates **28-33** were immobilized on TOYOPEARL® via standard peptide coupling procedures. For the affinity purification experiments in SILAC-derived A549 cell lysates, TOYOPEARL® derivatized with the 3-CMOs **26-29** were employed.[4] The results of the preliminary SILAC experiments are discussed comparatively with the results obtained by Dr. Felix Behnke[46] in his "fishing"-experiments below. It has to be noted that to date no

[2] Biological-activity tests for the SHs were performed by Dr. Carlo Pergola in the laboratories of Prof. Dr. Oliver Werz, Tübingen.
[3] The „Fishing"-experiments with the SHs were conducted by Dr. Felix Behnke in the laboratories of Prof. Dr. Oliver Werz, *Tübingen*.
[4] The „Fishing"-experiments with the GCs and the SILAC-experiments with the SHs and GCs were conducted by Ms. Yvonne Etzel in the laboratories of Prof. Dr. Gabriele Dodt, Tübingen.

further independent experiments in SILAC-derived cell lysates have been conducted. The results are thus considered to give an orientation regarding potential target structures but cannot provide absolute evidence for direct steroid-protein interactions or values for ligand affinities to the found proteins.

5.5 Identified Proteins

5.5.1 HSP27

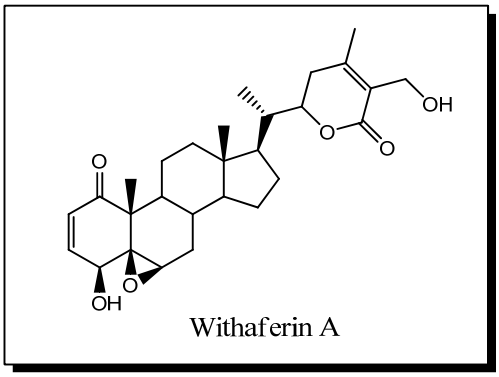
Hsp27 was identified as potential target protein of T and P_4 by 2D-DIGE-experiments with T- and P_4-3-CMOs **8** and **9, 17** and **18** in cell lysates of PBMC-, PMNL-cells and platelets (see Behnke[46]). Western blot analysis revealed that Hsp27 could be isolated from the first two cell lysates by both, P_4 and T with a slight preference for T. Platelets yielded Hsp27 exclusively for T. Later a second band obtained for P_4 distinct from Hsp27 could be identified as its phosphorylated complement p(Ser83)Hsp27. Further it was shown that recombinant Hsp27 could be pulled down selectively with T. P_4 did not seem to bind to Hsp27 but to pHsp27. When levels of Hsp27 and pHsp27 in platelets of male and female donors were determined by western blotting, it was revealed that Hsp27 and pHsp27 were both more abundant in female platelets. As outlined by Behnke[46] Hsp27 is an abundant ubiquitously expressed small heat shock protein. It is involved in stress response, protein folding, chaperone functions and many other cellular events. In fact, Hsp27 is known to associate with members of the nuclear receptor family in vivo e.g. the ER, PR and AR. As noted in section **4.3.4**, Hsp27 plays an important role in nuclear receptor recruitment to the cell membrane. Direct interaction of Hsp27 with steroid hormones has been proposed by Adams et al.[202] In a study they showed that the putative Hsp27 homolog intracellular estrogen binding protein (IEBP) found in new world primates is most likely a steroid hormone binding protein. In their study, Hsp27 disrupts estrogen binding to the ER and hampers transcriptional activity. The facts that IEBP was isolated using an estrogen functionalized affinity column and the existence of a conserved domain responsible for steroid binding in IEBP/Hsp27 supports this hypothesis.

5.5.2 Vimentin

The intermediate filament (IF) protein vimentin (Vim) was identified in the 2D-DIGE-Gel experiments from PBMC lysates selectively for T (see Behnke[46]). Western Blot analysis in PBMC-cell lysates showed a band selectively for T, whereas a western blot of PMNL-lysates

yielded bands for both, T and P_4. The impact of SHs on vimentin function was not examined further since no assay for vimentin activity exists so far.

Behnke[46] previously elucidated, that the intermediary filament protein Vim does not only function as a scaffolding protein but is involved in various cellular processes like signal transduction, intracellular transport and inflammation. Vim functions have been linked to pathological conditions like rheumatism and cancer. Quite recently Vim was proposed as druggable target for cancer therapy.[203] It was found that the steroid lactone withaferin A (Scheme 28) perturbates Vim oligomer assembly by irreversibly binding to vimentin tetramers and effecting Vim phosphorylation. The Vim tetramer provides a suitable binding pocket for interaction with the steroid and Vim-Cys^{328} performs irreversible Michael addition to C3 of the steroid agent.[204]



Scheme 28: Structure of Withaferin A.

The existence of a binding pocket for steroid ligands on Vim-tetramers raises the question if endogenous steroid hormones like the SHs and GCs might interact with Vim. Indeed, Vim could be pulled down from PMNL cell lysates and the specific binding to SHs confirmed by western blot analysis (see Behnke [46]).

5.5.3 Nicotinamide phosphoribosyltransferase (Visfatin)

Visfatin (NAMPT) was obtained as potential target from the pull down experiments in PBMC- and PMNL-cell lysates. Pull down and western blot analysis in PBMC-cell lysates showed a band selective for T and PMNL lysates yielded both, a band for T and P_4 with slight selectivity for T (see Behnke[46]). Pull down of recombinant visfatin confirmed the selectivity for T. In order to evaluate the physiological relevance of the T-visfatin interaction, ERK-phosphorylation, which is known to depend on visfatin[205] and T[199], was examined. Behnke found that a combination of visfatin and DHT results in a higher degree of ERK-phosphorylation compared to DHT or visfatin alone. Visfatin induced AKT phosphorylation was described earlier[205, 206] but could not be confirmed in Behnke`s experiments.

As discussed by Behnke[46] visfatin or NAMPT is a ubiquitously expressed protein deploying various functions. The aspects of visfatin activity range from glycosyltransferase activity over insulin-mimicking function to adipokine activity. However, the exact molecular functions of visfatin are to date only poorly understood. A link to steroid hormone action was not established in the literature so far.

5.5.4 Karyopherin subunit β1

Karyopherin β was identified in 1D-SDS-PAGE experiments via silver staining as potential target for T in PMNL-lysates (see Behnke[46]). Western blot analysis showed that Karyopherin β was fished rather unselectively since it occurs for T, P_4 and matrix control with similar intensity. As illustrated previously by Behnke[46], karyopherin β acts as receptor for proteins bearing a nuclear localization signal, which is recognized and bound by importin subunit α-proteins. After transport of the substrate-importin β-importing α complex through the nuclear pore complex into the nucleus, release of the substrate is facilitated by the small Ras-oncogene related GTPase Ran. RanBP1 is needed for detachment of RanGTP from the importin complex since the importin-RanGTP complex exhibits high stability. RanGAP resides in the cytosol and ensures rapid hydrolysis of GTP by Ran in order to maintain asymmetric distribution of RanGTP (mainly nucleus).[119]

5.5.5 Critical evaluation of the "fishing"-results

As can be seen from the findings discussed above, the identified target proteins are high abundant proteins. A significant selectivity for one of the tested steroid hormones was either absent or not unambiguously determinable. Since karyopherin β was fished completely unselectively and Hsp27 was proposed as steroid hormone binding protein earlier,[202] vimentin and visfatin appear as the most promising target candidates. The physiological relevance to vimentin in inflammatory diseases and cancer and the capability of tetrameric vimentin or provide a binding pocket for steroid agents[203, 204] renders Vim as interesting target structure for further investigations.

Although synergistic enhancement of ERK-phosphorylation by androgen-visfatin could not be confirmed definitely, it is deemed a promising target candidate and experiments for confirmation of androgens-visfatin are currently conducted.

Regarding the SILAC experiments, no clear tendency for specific interaction for either one of the steroids with the target candidates found in the conventional "fishing"-experiments[46] could be confirmed. Either the targets appear as very unselective hits or when selective do not

reach the significance threshold for protein identification. However, several proteins of moderate and low abundance could be identified in the SILAC-LC-MS/MS experiments as very characteristic hits with high selectivity for a single steroid hormone. The fact that these target structures could not be found with conventional 1D- and 2D-Gel-staining analysis shows quite plainly the superior sensitivity and the significant higher yield of potential target structures obtained by SILAC-LC-MS/MS techniques. The most significant SILAC-hits are subject of current investigations conducted in the laboratories of Prof. Dr. Gabriele Dodt and further independent SILAC experiments are planned to be performed in order to validate the putative target structures.

6 Experimental section

6.1 General

All chemicals were purchased from Sigma-Aldrich/Fluka (Boc_2O, AllocCl, tetraethylene glycol, bromoacetic acid methyl ester, bromoacetic acid *tert.*-butyl ester, acetovanillone, dibenzosuberenone, MeI, DMAP, HOBt, PPh_3, CBr_4, DCC, PFP, morpholine), Acros (0.5 M 9-BBN in THF, Cu(I)CN, imidazole), Alfa Aesar (Pd/C (10%), trifluoroacetic acid methyl ester), TCI (HBTU, CMA*0.5 HCl, 2-(2-hydroxyethoxy)ethanolamine, TBDMSCl), Merck (TFA, DIPEA, DBU) Bachem (aminoacids) or Fagron (steroids) and used as received unless noted otherwise. Dry solvents were prepared according to standard methods (DMF and DCM: P_2O_5; DIPEA and Et_3N: CaH_2; THF: sodium/benzophenone; MeOH: Mg), distilled and stored over molecular sieves 3 Å under an atmosphere of nitrogen until used. NMR-spectra were recorded on either a Bruker Avance 400 or a Bruker ACX 250 spectrometer and calibrated on the TMS-peak (0.0 ppm) or solvent-signal peak (^{1}H-$CDCl_3$: 7.26 ppm; ^{13}C-$CDCl_3$: 77.16 ppm; ^{1}H-MeOH-d_4: 3.31 ppm; ^{13}C-MeOH-d_4: 49.00 ppm, ^{1}H-DMSO-d_6: 2.50 ppm; ^{13}C-DMSO-d_6: 39.52 ppm; ^{1}H-benzene-d_6: 7.16 ppm; ^{13}C-benzene-d_6: 128.39 ppm). FT-ICR-MS spectra were recorded on a Bruker Apex II FT-ICR-MS (FAB) spectrometer and FAB-spectra on a Finnigan model TSQ 70. Optical rotations were measured with a Perkin-Elmer Model 341 polarimeter. Melting points were determined with a Büchi Melting Point M-560 and are uncorrected. Elemental analysis was performed on a HEKAtech Euro EA Analyzer. TLC-analysis was performed with Polygram SIL G/UV pre-coated polyester sheets (Macherey-Nagel). Purity of the target compounds was determined by HPLC with either a Thermo Betasil C8 column or a ZORBAX Eclipse XDB-C8 column with a flow rate of 1.5 ml/min. of methanol/KH_2PO_3 pH 2.3 buffer and were higher than 95 % unless noted otherwise. For the irradiation experiments of the photo-labile compounds a 150 W medium pressure mercury lamp from UV-consulting Peschl model TQ 150 was utilized. For elimination of UV-light <350 nm a filter solution (2 cm thickness) composed of 2 M KNO_3-soln. containing 0.02 *w*% 5,7-dimethyl-3,6-dihydro-2H-1,4-diazepinium perchlorate[191, 192] was placed between light source and sample. UV-spectra for photometry were recorded on a Perkin-Elmer Lambda 25 UV-Vis spectrometer using 1 cm quartz cuvettes. Absorbance was measured at 266.8 nm.

2-{2-[2-(2-Azidoethoxy)ethoxy]ethoxy}acetic acid **6** was prepared according to a procedure published by Bong et al.[147] 2-{2-[2-(2-*tert.*-Butoxyaminoethoxy)ethoxy]ethoxy}acetic acid methyl ester **4** was prepared from **1** by hydrogenation over Pd/C (10%) in MeOH and subsequent treatment with Boc_2O/Et_3N as described in[147] with analytical data in accordance

to the literature.[151] 2-{2-[2-(Azidoethoxy)ethoxy]ethoxy}amine **5** was synthesized in three steps according to Schwabacher et al.[152] Dibenzocyclooctynol **97**[188] and the corresponding *p*NP-carbonate **99**,[36] nitroaromatic **95**[78] and 4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzoic acid[186] were all prepared following the published procedures.

6.2 Experimental procedures

Immobilization of **101** *on TOYOPEARL-AF-650-amino*: 1 ml of a TOYOPEARL-AF-650-amino suspension in aq. EtOH was transferred to a 5 ml PP-syringe with inserted PE-frit (35 µm pore size) and washed successively 3 times with H_2O and 5 times with dry MeCN. After re-suspending the beads in 3 ml dry MeCN 21.4 mg of *p*NP-ester **101** (25 µmol) and 13.1 µl DIPEA (75 µmol) were added and the mixture shaken at 1500 rpm and RT for 72 h. Subsequently the beads were washed 5 times with DMSO and re-suspended in 3 ml DMSO. Then 181 mg *p*NP-acetate (1 mmol, 10 eq.) and 175 µl DIPEA were added and the mixture shaken as described for additional 12 h at RT. Afterwards the beads were washed successively 5 times each with DMSO and water. For storage at 4°C the alkyne functionalized beads were suspended in 20 % aqueous ethanol.

Capture of azide **61** *onto the alkyne functionalized solid support:* The alkyne functionalized beads from above were washed 3 times with dioxane/PBS 2:3 *v*/*v* and then re-suspended in 3 ml of the named solvent mixture containing 31,3 mg of azide **61** (50 µmol, 2 eq.). The resulting suspension was shaken at RT and 1500 rpm for 3 h and the reaction progress tracked photometrically. After 30 min no more progression of the reaction was observed. Afterwards the beads were washed 5 times with dioxane/PBS 2:3 *v*/*v*, 3-times with water, re-suspended in 20 % aqueous ethanol and stored at 4°C until used further. Quantitative analysis of the UV-spectra (266.8 nm) indicated an effective loading of the beads with 14.7 µmol of the corresponding triazole.

UV-light induced liberation of the immobilized model compound **103**: The triazole-loaded beads from above were washed 5-times either with dioxane/PBS 2:3 *v*/*v* or dioxane/Na-phosphate pH 6.3 buffer 3:2 *v*/*v* containing 10 mM DTT. Then the beads were re-suspended in 10 ml of one of the named solvents, transferred to a transparent 20 ml PP-tube and subjected to irradiation >350 nm for 4 h at RT while being shaken at 1500 rpm. The reaction was monitored photometrically and after 120 min. and 40 min. no more progression of the cleavage reaction could be observed, respectively. Quantitative analysis of the supernatant (266.8 nm) indicated 85% recovery of triazole **103**.

General Procedure for HBTU-mediated amide coupling **A**: In a round bottom flask equipped with a gas inlet and a stirring bar the corresponding free base or ammonium derivative was dissolved in dry DMF at a concentration of 0.18 M under an atmosphere of nitrogen. After cooling the solution to 0°C HOBt (1.5 eq.), DIPEA (1.5 eq. for free bases; 3 eq. for ammonium derivatives), the free carboxylic acid (1 eq.) and subsequently HBTU (1.5 eq.) were added. Stirring was continued for 2 h at 0°C and at RT for 14 h. Then, the solution was diluted with ethyl acetate, transferred to a separatory funnel, the organic layer separated and washed twice each with 1 M $NaHSO_4$-soln. and sat. $NaHCO_3$-soln. and once with brine. After drying the organic layer with Na_2SO_4 the solvent was removed under reduced pressure and the residue subjected to column chromatography, eluting with the solvent mixtures indicated below.

General procedure for the "one-pot" preparation of steroid 21-oximes **B**: To a stirred 35 mM soln. of the steroid in methanol was added a 11 mM soln. of $Cu(OAc)_2$ (0.45 eq.) in methanol. Then air was bubbled through the solution until TLC indicated complete consumption of the starting material.[159] Subsequently a 0.33 M soln. of EDTA-trisodium salt (0.6 eq.) was added. After stirring for additional 10 min the methanolic soln. was diluted to 1.2 times of its volume with 0.1 M acetate buffer-soln. pH 7 and the corresponding free aminooxy-derivative (1 eq. or 0.5 eq.) was added. The resulting solution was stirred at RT for 14 h. After removal of the remaining methanol under reduced pressure the aqueous phase was acidified with solid $NaHSO_4$, transferred to a separatory funnel and extracted five times with ethyl acetate. The combined organic layers were washed once each with 1 M $NaHSO_4$-soln. and brine, dried over Na_2SO_4 and the solvent evaporated. The residual crude steroid was purified by column chromatography (see below).

General procedure for saponification of EG-methyl esters **C**: To a stirred 0.13 M soln. of the corresponding glycolic acid methyl ester derivative in methanol was added 1 M K_2CO_3 soln. (9 eq.) and the mixture stirred at RT until TLC indicated complete consumption of the starting material. After acidification of the solution with 1 M $NaHSO_4$-soln. the mixture was transferred to a separatory funnel, extracted five times with EA, the combined organic layers dried over Na_2SO_4 and the solvent evaporated leaving the free carboxylic acid in high purity and virtually quantitative yield.

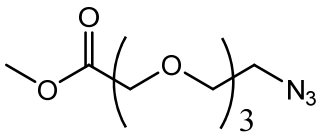
General procedure for conversion of azides to the corresponding free amines **D**: In a round bottom flask equipped with a gas inlet and a stirring bar the azide was dissolved in dry MeOH under an atmosphere of nitrogen at a concentration of 1 mM. Then a spatula tip of Pd/C

(10%) was added and hydrogen bubbled though the solution for three hours. Afterwards the suspension was filtered over celite and the solvent removed under reduced pressure. Unless noted otherwise the crude free amine was used without further purification for the next step of the synthesis.

General procedure for tert.-butyl ester cleavage **E**: In round bottom flask equipped with a gas inlet and a stirring bar a ~0.16 M solution of the corresponding *tert.*-butyl ester in dry DCM was treated with 1,3-dimethoxybenzene (5 eq.) and TFA (20 eq.) under an atmosphere of nitrogen. The mixture was stirred at RT until TLC indicated complete consumption of the starting material (<2 h). After removal of the volatiles in a stream of air the residual free acid was dissolved in sat. $NaHCO_3$-soln. and transferred to a separatory funnel. The aqueous layer was extracted three times with Et_2O, acidified with solid $NaHSO_4$ and extracted five times with EA. The combined organic layers were washed once each with 1 M $NaHSO_4$-soln. and once with brine, dried over Na_2SO_4 and the solvent removed under reduced pressure. The corresponding free acids were generally obtained in high purity but if necessary purified by column chromatography (see below).

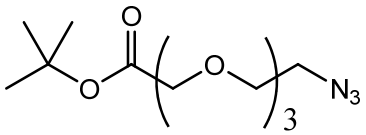
General procedure for deprotection of N-Boc-protected amines and aminooxy ethers **F**: In a round bottom flask equipped with a stirring bar the corresponding *N*-Boc-protected amine or aminooxy ether was dissolved in a mixture of dry DCM/TFA 4:1 *v*/*v* at a concentration of ~0.16 M. The solution was stirred for 1 h at RT and the volatiles then removed in a stream of air. The residual TFA salts were dried in vacuum over night and used for the synthesis of follow-up compound without further purification.

General procedure for oxime-ligation of aminooxy ethers with ketones **G**: In a round bottom flask equipped with a gas inlet and stirring bar the corresponding ketone (2 eq.) was dissolved in dry Py/5 *v*% AcOH at a concentration of ~0.3 M under an atmosphere of nitrogen. To the solution was added the free aminooxy ether and the mixture stirred at 55°C for 24 h. Then the volatiles were removed under reduced pressure, the residue dissolved in EA and transferred to a separatory funnel. The organic layer was washed twice each with 1 M $NaHSO_4$-soln. and sat. $NaHCO_3$-soln. and once with brine. The organic layer was dried over Na_2SO_4, the solvent removed under reduced pressure and the residual crude product subjected to column chromatography yielding the corresponding oxime.



1

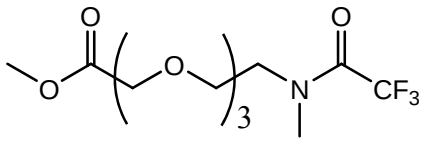
2-{2-[2-(2-Azidoethoxy)ethoxy]ethoxy}acetic acid methyl ester **1**:[146] In a 250 ml round bottom flask equipped with a gas inlet and a stirring bar 7.92 g of *2-{2-[2-(2-azidoethoxy)ethoxy]ethoxy}acetic acid*[147] (34.0 mmol) were dissolved in 100 ml dry MeOH under an atmosphere of nitrogen. The solution was then cooled to 0°C and 3.72 ml thionyl chloride (51.0 mmol, 1.5 eq.) were slowly added to the solution. After refluxing the solution for 3 h the volatiles were removed in a stream of air, the oily residue dissolved in EA and transferred to a separatory funnel. The organic layer was washed twice with small amounts of sat. $NaHCO_3$-soln. and once with brine, dried over Na_2SO_4 and the solvent removed under reduced pressure. The residual viscous oil was subjected to column chromatography eluting with EA/PE (1:1-7:3) yielding 6.8 g of pure title compound **5** (27.5 mmol, 81%) as colorless oil. R_f: 0.36 (PE/EA 2:3). FAB-MS: m/z 270.1 $[M+Na]^+$, 248.1$[M+H]^+$. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 4.15 (s, 2H, OC**H**$_2$COOMe), 3.74-3.12 (m, 10H, EG-chain-C**H**$_2$), 3.72 (s, 3H, C**H**$_3$), 3.36 (t, 2H, J = 5.2 Hz, C**H**$_2$N$_3$). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 170.9 (**C**OOMe), 71.0, 70.8, 70.7, 70.7, 70.1, 68.7 (EG-**C**H$_2$), 51.8 (OMe), 50.7 (**C**H$_2$N$_3$).



3

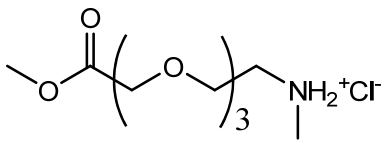
2-{2-[2-(2-Azidoethoxy)ethoxy]ethoxy}acetic acid tert.-butyl ester **3**:[150] In a 100 ml round bottom flask equipped with a gas inlet and a stirring bar 5.0 g of *2-{2-[2-(2-azidoethoxy)ethoxy]ethoxy}acetic acid*[147] (21.4 mmol) were dissolved in 50 ml dry *tert*.-butanol under an atmosphere of nitrogen with warming the solution to 40 °C. Then 9.36 g Boc_2O (42.9 mmol, 2 eq.) were added followed by slow addition of 784 mg DMAP (6.42 mmol, 0.3 eq.; **Caution**: Gas evolution!) and the mixture stirred for 3 h at 40°C. Afterwards the solvent was removed under reduced pressure, the residue dissolved in EA and transferred to a separatory funnel. The organic phase was washed twice with small amounts of 1 M $NaHSO_4$-soln., once with sat. $NaHCO_3$-soln. and once with brine, dried over Na_2SO_4 and the solvent removed under reduced pressure. The residual amber colored oil was subjected to column chromatography eluting with EA/PE (3:2-7:3) yielding 5.2 g of pure title compound **3** (18 mmol, 84%) as colourless oil. FAB-MS: m/z 312.1 $[M+Na]^+$, 290.2 $[M+H]^+$, 234.1 $[M-t\text{Bu}+H]^+$. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 3.98 (s, 2H, OC**H**$_2$COO*t*Bu), 3.69-3.61 (m, 10H,

EG-chain-C**H**$_2$), 3.34 (t, 2H, J = 5.3 Hz, C**H**$_2$N$_3$), 1.43 (s, 9H, *t*Bu). ^{13}C-NMR (100.1 MHz, $CDCl_3$): δ 169.7 (**C**OO*t*Bu), 81.5 (*t*Bu), 70.7, 70.7, 70.0, 69.0 (EG-**C**H$_2$), 50.7 (**C**H$_2$N$_3$), 28.1 (*t*Bu).



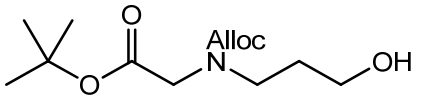
7

N-Methyl-2-{2-[2-(2-trifluoroacetamidoethoxy)ethoxy]ethoxy}acetic acid methyl ester **7**:[148] 4.84 g of *2-{2-[2-(2-azidoethoxy)ethoxy]ethoxy}acetic acid*[147] (20.75 mmol) were reduced to the corresponding free amine following general procedure **D**. The solution of the crude free base in MeOH was then filtered through a pad of celite and 4.94 ml of trifluoroacetic acid ethyl ester (41.5 mmol, 2 eq.) and 5.78 ml of Et_3N (41.5 mmol, 2 eq.) were added and the mixture stirred under an atmosphere of nitrogen until no more free amine could be detected on TLC (24 h). Thereafter the volatiles were removed under reduced pressure and the crude product dissolved in 50 ml dry DMF in a two neck vessel equipped with a gas inlet, a stirring bar and a reflux condenser under an atmosphere of nitrogen. To the solution was added 14.3 g of freshly dried K_2CO_3 (103.75 mmol, 5 eq.). The suspension was then heated to 110 °C and 18.8 ml MeI (291 mmol, 14 eq.) were added in four portions over a period of 10 h. Then the slurry was cooled to RT, diluted with 300 ml EA, filtered, transferred to a separatory funnel, washed twice each with 1 M HCl-soln. and sat. $NaHCO_3$-soln. and once with brine. The organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure. The residual amber colored oil was subjected to column chromatography eluting with PE/EA (4:6-1:3) yielding 5.36 g of pure title compound **7** (16.2 mmol, 78%) as colorless oil. FAB-MS: m/z 354.2 $[M+Na]^+$, 332.2 $[M+H]^+$. FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{12}H_{20}F_3NO_6Na$: 354.1135 found: 354.1135. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 4.12 (s, 2H, OC**H**$_2$COOMe), 3.70 (s, 3H, COOMe), 3.70-3.55 (m, 12H, EG-chain-C**H**$_2$), 3.19-3.16/3.06 (m/s, 3H, NC**H**$_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 170.9 (**C**OOMe), 157.0 (q, J = 36 Hz, **C**OCF$_3$), 116.5 (q, J = 288 Hz, **C**F$_3$), 70.9, 70.7, 70.7, 70.7, 70.6, 70.5, 69.7, 68.6, 68.6 (EG-**C**H$_2$), 52.8 (OMe), 49.5/49.1 (q, J = 2.9 Hz, **C**H$_2$N), 36.6/35.9 (q, J = 3.7 Hz, NMe).



2

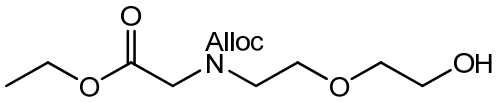
N-Methyl-2-{2-[2-(2-aminoethoxy)ethoxy]ethoxy}acetic acid methyl ester hydrochloride **2**: In a 50 ml round bottom flask equipped with a gas inlet and a stirring bar 500 mg of trifluoroacetamide **7** (1.51 mmol) were dissolved in 10 ml of a 0.36 M HCl soln. in dry MeOH under an atmosphere of nitrogen.[207] The resulting mixture was refluxed for 36 h, cooled to RT and the volatiles removed in a stream of air affording 406 mg of analytically pure title compound **2** (1.49 mmol, 99%). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{10}H_{22}O_5$: 236.1492 found: 236.1492. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 9.25 (s, broad, 2H, N**H**$_2^+$), 4.08 (s, 2H, OC**H**$_2$COOMe), 3.86-3.75 (m, 2H, EG-chain-C**H**$_2$), 3.65 (s, 3H, COOMe), 3.64-3.56 (m, 8H, EG-chain-C**H**$_2$), 3.17-3.08 (m, 2H, EG-chain-C**H**$_2$), 2.67 (s, 3H, NMe). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 170.9 (**C**OOMe), 70.5, 70.2, 70.2, 70.1, 68.3, 65.9 (EG-**C**H$_2$), 51.9 (OMe), 48.6 (**C**H$_2$NHMe), 33.4 (NMe).



48

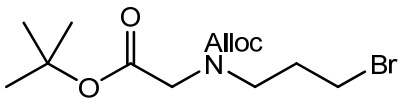
2-[N-(3-Hydroxypropyl)allyloxycarbonylamino]acetic acid tert.-butyl ester **48:** In a 500 ml round bottom flask equipped with a gas inlet and a stirring bar 10 g of bromoacetic acid *tert*.-butyl ester (51.3 mmol) were dissolved in 250 ml dry THF under an atmosphere of nitrogen and the solution cooled to 0°C. Then 19.6 ml propanolamine (256 mmol, 5 eq.) were added dropwise and stirring continued for additional 4 h.[181] After removal of the solvent under reduced pressure the residue was diluted with brine and the mixture stirred for 30 min. The aq. solution was then transferred to a separatory funnel, extracted five times with DCM, the combined organic phases washed twice with brine, dried over Na_2SO_4 and the solvent evaporated to yield 5.44 g of crude 2-(3-hydroxypropyl)aminoacetic acid *tert*.-butyl ester (28.7 mmol, 56%). The crude amino alcohol was subsequently dissolved in 200 ml dioxane/H_2O 1:1, 5.30 g $NaHCO_3$ (63.1 mmol, 2.2 eq.) were added and the solution cooled to 0°C. Then 3.31 ml AllocCl (31.6 mmol, 1.1 eq.) were added dropwise over 30 min, the resulting slurry stirred for 2 h at 0°C and additional 14 h at RT. Thereafter dioxane was evaporated, the aqueous phase transferred to a separatory funnel and extracted three times with EA. The combined organic layers were then washed twice with 1 M $NaHSO_4$-soln., once with sat. $NaHCO_3$ and once with brine, dried over Na_2SO_4 and the solvent evaporated leaving 6.75 g of pure title compound **48** (24.7 mmol, 86%) as colorless oil. An analytical sample was obtained by column chromatography eluting with PE/EA 4.5:5.5. R_f: 0.36 (EA/PE 3:2). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{13}H_{23}NO_5Na$: 296.1468 found: 296.1470. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 5.98-5.81 (m, 1H, **H**C=CH$_2$), 5.34-5.15 (m,

2H, HC=C**H**$_2$), 4.63-4.55 (m, 2H, Allyl-C**H**$_2$), 3.88, 3.82 (s, s, 2H, NC**H**$_2$COO), 3.65, 3.63 (t, t, 2H, J = 5.8 Hz/5.6 Hz, C**H**$_2$OH), 3.46, 3.42 (t, t, 2H, J = 6.1 Hz/6.9 Hz, C**H**$_2$N), 2.83 (s, broad, 1H, O**H**), 1.80-1.72, 1.72-1.64 (m, m, 2H, C**H**$_2$), 1.43 (s, 9H, *t*Bu). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 169.1, 168.8 (**C**OO), 157.1, 156.4 (O**C**ON), 132.8, 132.6 (H**C**=CH_2), 117.9, 117.5 (HC=**C**H_2), 82.0, 81.9 (*t*Bu), 66.6, 66.5 (Alloc-**C**H_2), 59.8, 58.7 (C**H**$_2$OH), 50.3 (NC**H**$_2$COO), 45.5, 45.3 (**C**H_2N), 31.3, 30.7 (**C**H_2), 28.1 (*t*Bu).



49

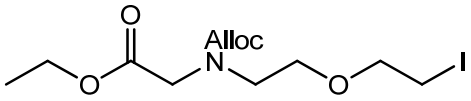
2-[N-(5-Hydroxy-3-oxapentyl)allyloxycarbonylamino]acetic acid ethyl ester **49**: Following the procedure for the preparation of **48**, 10 g bromoacetic acid ethyl ester (59.9 mmol) were reacted with 30 ml 2-(2-aminoethoxy)ethanol (299 mmol, 5 eq.) yielding 5.8 g of the corresponding sec. amino-alcohol (21.1 mmol, 30.3 %) which was subsequently protected using 5.90 g $NaHCO_3$ (72.8 mmol, 2.4 eq.) and 3.87 ml AllocCl (36.4 mmol, 1.2 eq.). Aqueous workup as described above afforded 6.76 g of highly pure title compound **49** (24.6 mmol, 81%) as colorless oil. An analytical sample was obtained by column chromatography eluting with PE/EA 7:3. R_f: 0.57 (EA). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{12}H_{21}NO_6Na$: 298.1261 found: 298.1262. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 5.97-5.80 (m, 1H, **H**C=CH_2), 5.32-5.12 (m, 2H, HC=C**H**$_2$), 4.63-4.54 (m, 2H, Allyl-C**H**$_2$), 4.17 (q, 2H, J = 7.1 Hz, OC**H**$_2$$CH_3$), 4.08, 4.05 (s, s, 2H, NC**H**$_2$COO), 3.70-3.58 (m, 4H, EG-chain-C**H**$_2$), 3.57-3.49 (m, 4H, EG-chain-C**H**$_2$), 2.47, 2.35 (t, t, broad, 1H, J = 5.5Hz/6.0Hz, O**H**), 1.25, 1.25 (t, t, 3H, J = 7.1 Hz/7.2 Hz, OCH_2C**H**$_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 170.3, 170.2 (**C**OO), 156.2, 156.0 (O**C**ON), 132.8, 132.6 (H**C**=CH_2), 117.6, 117.4 (HC=**C**H_2), 72.5, 70.3, 70.2, 66.5, 66.4, 61.7, 61.7, 61.3 (EG-**C**H_2/O O**C**H_2CH_3/Alloc-**C**H_2), 50.5, 50.4 (NC**H**$_2$COO), 48.9, 48.4 (**C**H_2N), 14.3, 14.2 (OCH_2**C**H_3).



50

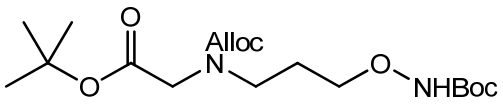
2-[N-(3-Bromopropyl)allyloxycarbonylamino]acetic tert.-butyl ester **50**: In a 100 ml round bottom flask equipped with a gas inlet and a stirring bar 2.5 g of protected alcohol **48** (9.15 mmol) and 4.55 g carbon tetrabromide (13.73 mmol, 1.5 eq.) were dissolved in 25 ml dry THF under an atmosphere of nitrogen. After cooling the solution 0°C 3.72 g PPh_3 (14.2 mmol, 1.55 eq.) dissolved in 25 ml dry THF were slowly added and the mixture stirred for 1 h at 0°C. Thereafter additional 1.52 g CBr_4 (4.58 mmol, 0.5 eq.) and 1.20 g PPh_3 (4.58 mmol,

0.5 eq.) were added and the stirring continued at RT for 3 h.[182] Then the slurry was adsorbed onto silica gel and the solvent evaporated. Column chromatography eluting with PE/EA 8.5:1.5 afforded 2.72 g of pure title compound **50** (8.09 mmol, 88 %) as colorless oil. R_f: 0.66 (PE/EA 3:2). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{13}H_{22}BrNO_4Na$: 358.0624 found: 358.0622. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 5.98-5.81 (m, 1H, **H**C=CH_2), 5.33-5.14 (m, 2H, HC=C**H**$_2$), 4.63-4.54 (m, 2H, Allyl-C**H**$_2$), 3.90, 3.86 (s, s, 2H, NC**H**$_2$COO), 3.49-3.40 (m, 4H, C**H**$_2$Br/C**H**$_2$N), 2.16-2.06 (m, 2H, C**H**$_2$), 1.45, 1.45 (s, 9H, *t*Bu). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 177.3, 168.9 (**C**OO), 156.4, 156.0 (O**C**ON), 132.8, 132.7 (H**C**=CH_2), 117.7, 117.4 (HC=**C**H_2), 82.0, 82.0 (*t*Bu), 66.5, 66.3 (Alloc-**C**H_2), 50.9, 50.6 (NC**H**$_2$COO), 48.2, 47.1 (**C**H_2N), 31.7, 31.5, 31.0, 30.8 (**C**H_2/**C**H_2Br), 28.2, 28.1 (*t*Bu).



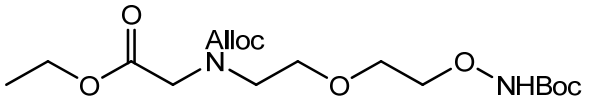
51

2-[N-(5-Iodo-3-oxapentyl)allyloxycarbonylamino]acetic acid ethyl ester **51**: In a 250 ml round bottom flask equipped with a gas inlet and a stirring bar 6.0 g of protected alcohol **49** (21.8 mmol) were dissolved in a mixture of 50 ml dry THF and 30 ml dry MeCN and the solution cooled to 0°C. Then 7.76 g PPh_3 (29.6 mmol, 1.36 eq.) and 1.93 g imidazole (28.3 mmol, 1.3 eq.) were added. Stirring was continued for 5 min, thereafter 7.74 g iodine were slowly added and the mixture stirred for additional 3 h at 0°C.[183] Afterwards the mixture was diluted with 400 ml Et_2O, transferred to a separatory funnel, washed with 20 *w*% Na_2SO_3-soln. until decolorized and twice with water. Then the mixture was adsorbed onto silica gel and subjected to column chromatography. Eluting the product with PE/EA 7:3 yielded 7.74 g of pure title compound **51** (20.1 mmol, 92%) as colorless oil. R_f: 0.57 (PE/EA 3:2). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{12}H_{20}INO_5Na$: 408.0278 found: 408.0280. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 5.97-5.80 (m, 1H, **H**C=CH_2), 5.31-5.12 (m, 2H, HC=C**H**$_2$), 4.62-4.53 (m, 2H, Allyl-C**H**$_2$), 4.16, 4.16 (q, q, 2H, J = 7.2 Hz/7.1 Hz, OC**H**$_2$CH$_3$), 4.11, 4.10 (s, s, 2H, NC**H**$_2$COO), 3.66-3.58 (m, 4H, EG-chain-C**H**$_2$), 3.59-3.50 (m, 2H, EG-chain-C**H**$_2$), 3.19 (t, 2H, J = 6.5 Hz, C**H**$_2$I), 1.24, 1.24 (t, t, 3H, J = 7.1 Hz/7.1 Hz, OCH$_2$C**H**$_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 170.0, 170.0 (**C**OO), 156.1, 155.9 (O**C**ON), 132.8, 132.8 (H**C**=CH_2), 117.6, 117.2 (HC=**C**H_2), 71.6, 71.5, 70.1 (EG-**C**H_2), 66.5, 66.3 (Alloc-**C**H_2), 61.1 (O**C**H_2CH$_3$), 50.4 (NC**H**$_2$COO), 48.6, 48.0 (**C**H_2N), 14.3, 14.3 (CH$_2$**C**H$_3$), 3.0, 2.8 (**C**H_2I).



52

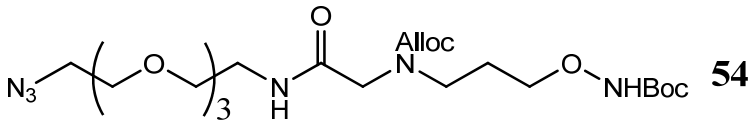
2-{N-[3-(tert.-Butoxyaminooxy)propyl]allyloxycarbonylamino}acetic acid tert.-butyl ester **52**: In a 50 ml round bottom flask equipped with a gas inlet and a stirring bar 2.4 g of bromide **50** (7.14 mmol) were dissolved in 25 ml dry DCM under an atmosphere of nitrogen. To the solution were added 855 mg *N*-Boc-hydroxylamine (10.7 mmol, 1.5 eq.) and 1.6 ml DBU (10.7 mmo, 1.5 eq.) and the mixture stirred at RT for 48 h.[184] Thereafter the solution was diluted with DCM, transferred to a separatory funnel, washed twice with 1 M $NaHSO_4$-soln. and once each with sat. $NaHCO_3$-soln. and brine. The organic layer was then dried over Na_2SO_4 and the solvent removed in under reduced pressure. Purification of the residue via column chromatography eluting with PE/EA 6.5:3.5 afforded 1.7 g of pure title compound **52** (4.38 mmol, 61%) as colorless oil. R_f: 0.48 (PE/EA 3:2). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{18}H_{32}N_2O_7Na$: 411.2102 found: 411.2100. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 7.36, 7.23 (s, s, broad, 1H, NH), 5.98-5.81 (m, 1H, **H**C=CH_2), 5.33-5.13 (m, 2H, HC=C**H**$_2$), 4.63-4.53 (m, 2H, Allyl-C**H**$_2$), 3.92, 3.84 (m, 4H, NC**H**$_2$COO/C**H**$_2$ONHBoc), 3.46-3.40 (m, 2H, C**H**$_2$N), 1.92-1.82 (m, 2H, C**H**$_2$), 1.46, 1.45 (s, s 9H, *t*Bu), 1.44, 1.43 (s/s, 9H, *t*Bu). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 168.1 (**C**OO), 157.0, 156.4, 150.1 (O**C**ON), 133.0, 132.8 (H**C**=CH_2), 117.6, 117.3 (HC=**C**H_2), 81.8, 81.6 (*t*Bu), 74.1, 74.0 (**C**H_2ONHBoc), 66.4, 66.2 (Alloc-**C**H_2), 50.5 (NC**H**$_2$COO), 46.4, 45.6 (NC**H**$_2$COO), 28.3, 28.2, 28.1 (*t*Bu), 27.4, 26.9 (**C**H_2).



53

2-{N-[5-(tert.-Butoxyaminooxy)-3-oxapentyl]allyloxycarbonylamino}acetic acid ethyl ester **53**: Following the procedure for the preparation of **52** 7.5 g of iodide **51** (19.5 mmol) were reacted with 3.89 g N-Boc-hydroxylamine (29.25 mmol, 1.5 eq.) and 4 ml DBU (29.15 mmol, 1.5 eq.) in 75 ml dry DCM. Column chromatography afforded 4.4 g of pure title compound **53** (11.75 mmol, 60%) as colorless oil. R_f: 0.54 (PE/EA 2:3). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{17}H_{30}N_2O_8Na$: 413.1894 found: 413.1895. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 7.50, 7.42 (s, s, 1H, ON**H**Boc), 5.97-5.81 (m, 1H, **H**C=CH_2), 5.32-5.14 (m, 2H, HC=C**H**$_2$), 4.62-4.54 (m, 2H, Allyl-C**H**$_2$), 4.16 (q, 2H, 7.1 Hz, OC**H**$_2$CH$_3$), 4.10, 4.08 (s, s, 2H, NC**H**$_2$COO), 3.98-3.93 (m, 2H, C**H**$_2$ONHBoc), 3.65-3.58 (m, 4H, chain-C**H**$_2$), 3.58-3.50 (m, 2H, chain-C**H**$_2$), 1.25, 1.25 (t, t, 3H, J = 7.1 Hz/7.2 Hz, OCH$_2$C**H**$_3$). ^{13}C-

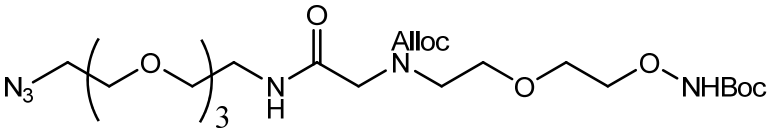
NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 170.1, 170.1 (**C**OO), 157.0, 156.9 (O**C**ON), 156.2, 156.0 (O**C**ON), 132.9, 132.8 (H**C**=CH_2), 117.6, 117.3 (HC=**C**H_2), 81.8, 81.7 (*t*Bu), 75.5, 75.4 (**C**H_2ONHBoc), 70.5, 70.4, 69.0, 68.8 (EG-**C**H_2), 66.5, 66.3 (Alloc-**C**H_2), 61.1 (O**C**H_2CH_3), 50.4/50.2 (NC**H**$_2$COO), 48.5/48.1 (**C**H_2N), 28.3 (*t*Bu), 14.3, 14.3 (OCH_2**C**H_3).



54

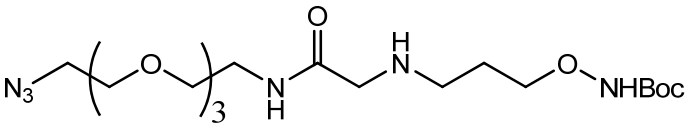
2-{N-[3-(tert.-Butoxyaminooxy)propyl]allyloxycarbonylamino}-N`-(11-azido-3,6,9-trioxaundecyl)acetamide **54**: In a 100 ml round bottom flask equipped with a stirring bar 1.59 g of *tert*.-butyl-ester **52** (4.1 mmol) were dissolved in 25 ml MeOH/THF 3:1. To the solution were added 12.3 ml 3 M KOH-soln. (9 eq.) and the mixture stirred at RT until TLC indicated complete consumption of the starting material (4 h). Then the solution was acidified by addition of 1 M $NaHSO_4$-soln, transferred to a separatory funnel and the aq. phase extracted five times with DCM. The combined organic layers were washed with brine, dried over Na_2SO_4 and the solvent evaporated. The crude acid was then dissolved in 20 ml dry DMF under an atmosphere of nitrogen. After cooling the solution to -20°C 832 mg HOBt (6.15 mmol, 1.5 eq.) and 1.1 g DCC (5.33 mmol, 1.3 eq.) were added and the mixture stirred for 6 h at -20 °C. Thereafter 1.07 g of amino-azide **5** (4.92 mmol, 1.2 eq.) and 1.07 ml DIPEA (6.15 mmol, 1.5 eq.) were added and stirring continued at RT for 14 h. Then the precipitate of DC-urea was filtered off, the solution diluted with EA and transferred to a separatory funnel. The organic phase was washed two times each with 1 M $NaHSO_4$-soln. and sat. $NaHCO_3$-soln., once with brine, dried over Na_2SO_4 and the solvent removed under reduced pressure. The residue was subjected to column chromatography eluting with $CHCl_3$ containing 3% MeOH yielding 1.57 g of pure title compound **54** (2.95 mmol, 72%) as colorless oil. R_f: 0.42 ($CHCl_3$+4%MeOH). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{22}H_{40}N_6O_9Na$: 555.2749 found: 555.2746. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 7.67, 7.56 (s, s, 1H, ON**H**Boc), 6.60 (t, 1H, J = 5.3 Hz, CON**H**), 5.96-5.80 (m, 1H, **H**C=CH_2), 5.33-5.13 (m, 2H, HC=C**H**$_2$), 4.62-4.52 (m, 2H, Allyl-C**H**$_2$), 3.90 (s, 2H, NC**H**$_2$CON), 3.89-3.81 (m, 2H, C**H**$_2$ONHBoc), 3.67-3.55 (m, 10H, EG-chain-C**H**$_2$), 3.54-3.47 (m, 2H, C**H**$_2$), 3.47-3.39 (m, 4H, C**H**$_2$), 3.36 (t, 2H, J = 5.2 Hz, C**H**$_2$N$_3$), 1.90-1.82 (m, 2H, C**H**$_2$), 1.43 (s, 9H, *t*Bu). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 169.2 (**C**ON), 157.0 (O**C**ON), 132.7 (H**C**=CH_2), 117.7 (HC=**C**H_2), 81.5 (*t*Bu), 76.8, 73.8 (**C**H_2ONHBoc), 70.7, 70.7, 70.6, 70.3, 70.1 69.8 (EG-

CH_2), 66.5 (Alloc-**C**H_2), 52.0 (NC**H**$_2$CON), 50.7 (**C**H_2N_3), 46.4, 39.2 (**C**H_2N), 28.3(*t*Bu), 27.0 (**C**H_2).



55

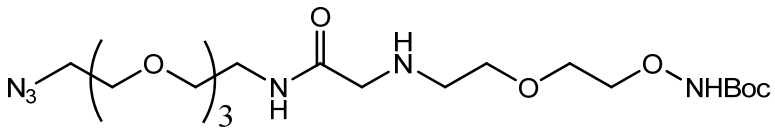
2-{N-[5-(tert.-Butoxyaminooxy)-3-oxapentyl]allyloxycarbonylamino}-N`-(11-azido-3,6,9-trioxaundecyl)acetamide **55**: Following the procedure for the preparation of **54** 3.6 g of ethyl ester **53** (9.61 mmol) were saponified with 1 M NaOH-soln. (3 eq.) and the crude free acid subsequently coupled to linker **5** using 1.91 g HOBt (14.1 mmol, 1.5 eq.), 2.91 g DCC (14.1 mmol, 1.5 eq.), 2.46 ml DIPEA (14.1 mmol, 1.5 eq.) and 2.46 g of azido-amine **5** (11.3 mmol, 1.2 eq.) in 25 ml dry DMF. Purification via column chromatography eluting with $CHCl_3$ containing 3% MeOH yielded 4.65 g of pure title compound **55** (8.27 mmol, 88%) as colorless oil. R_f: 0.42 ($CHCl_3$+3%MeOH). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{23}H_{42}N_6O_{10}Na$: 585.2855 found: 585.2851. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 8.17, 8.00 (s, s, broad, 1H, CON**H**), 7.08, 6.80 (s, s, broad, 1H, CON**H**), 5.96-5.79 (m, 1H, **H**C=CH_2), 5.31-5.12 (m, 2H, HC=C**H**$_2$), 4.61-4.52 (m, 2H, Allyl-C**H**$_2$), 4.01-3.90 (m, 2H, C**H**$_2$), 3.70-3.48 (m, 18H, C**H**$_2$), 3.46-3.39 (m, 2H, C**H**$_2$), 3.35 (t, 2H, J = 5.2 Hz, C**H**$_2N_3$), 1.43 (s, 9H, *t*Bu). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 169.7 (**C**ON), 162.6 (**C**ON), 157.0, 156.2 (O**C**ON), 132.5 (H**C**=CH_2), 117.6 (HC=**C**H_2), 81.4 (*t*Bu), 75.3 (**C**H_2ONHBoc), 70.7, 70.6, 70.3, 70.1, 69.6, 68.7, 68.4 (EG-**C**H_2), 66.5 (Alloc-**C**H_2), 52.7 (NC**H**$_2$CON), 50.7 (**C**H_2N_3), 48.8, 48.2, 39.2 (**C**H_2N), 28.3(*t*Bu).



56

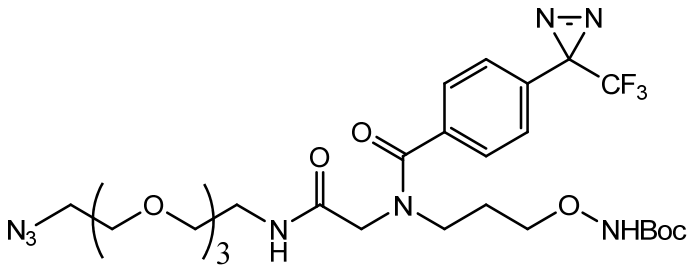
2-{N-[3-(tert.-Butoxyaminooxy)propyl]amino}-N`-(11-azido-3,6,9-trioxaundecyl)acetamide **56**: In a 50 ml round bottom flask equipped with a gas inlet and a stirring bar 1 g of alloc-protected amine **54** (1.88 mmol) was dissolved in 15 ml MeCN under an atmosphere of nitrogen. To the mixture were added 102 µl H_2O (5.62 mmol, 3 eq.) and 1.43 g iodine (5.63 mmol, 3 eq.) and stirring continued until TLC indicated complete consumption of the starting material (<12 h).[185] Then the solution was cooled to 0°C and decolorized by adding 20 *w*% Na_2SO_3-soln., transferred to a separatory funnel and extracted three times with EA. The combined organic layers were washed once each with 20 *w*% Na_2SO_3-soln., water and brine,

dried over Na_2SO_4 and the solvent removed under reduced pressure. Purification via column chromatography eluting with $CHCl_3$ containing 2% MeOH and 1% Et_3N yielded 688 mg of pure title compound **56** (1.53 mmol, 88%) as colorless oil. FT-ICR-MS: m/z $[M+H]^+$ calcd for $C_{18}H_{37}N_6O_7$: 449.2718 found: 449.2719. ^{1}H-NMR (400.1 MHz, DMSO-d_6): δ 9.30 (s, 1H, ON**H**Boc), 7.81 (t, 1H, J = 5.7 Hz, CON**H**), 3.73 (t, 2H, J = 6.3 Hz, C**H**$_2$CONHBoc), 3.62-3.58 (m, 2H, chain-C**H**$_2$), 3.58-3.48 (m, 8H, chain-C**H**$_2$), 3.42 (t, 2H, J = 6.0 Hz, chain-C**H**$_2$), 3.39 (t, 2H, J = 5.0 Hz, chain-C**H**$_2$), 3.05 (s, 2H, NC**H**$_2$CON), 2.51 (t, 2H, J = 6.9 Hz, NHC**H**$_2$), 1.68-1.59 (m, 2H, C**H**$_2$), 1.40 (s, 9H, *t*Bu). ^{13}C-NMR (100.6 MHz, DMSO-d_6): δ 171.1 (**C**ON), 156.1 (O**C**ON), 79.4 (*t*Bu), 73.5 (**C**H$_2$ONHBoc), 69.8, 69.8, 69.7, 69.6, 69.3, 69.1 (EG-**C**H$_2$), 52.2 (**C**H$_2$N), 50.0 (**C**H$_2$N$_3$), 46.1, 38.1 (**C**H$_2$N), 28.0 (*t*Bu), 28.0 (**C**H$_2$).



57

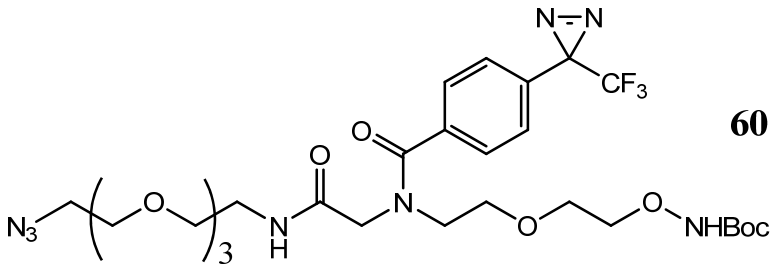
2-{N-[5-(tert.-Butoxyaminooxy)-3-oxapentyl]amino}-N`-(11-azido-3,6,9-trioxaundecyl)acetamide **57**: Following the procedure for the preparation of **56** 4.0 g of alloc-protected amine **63** (7.11 mmol) were reacted with 5.41 g iodine (21.3 mmol) and 384 µl H_2O (21.3 mmol) in 70 ml MeCN. Purification via column chromatography yielded 2.73 g of free base **57** (5.7 mmol, 80%) as colorless oil. FT-ICR-MS: m/z $[M+H]^+$ calcd for $C_{19}H_{39}N_6O_8$: 479.2824 found: 479.2825. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 7.80 (s, 1H, ON**H**Boc), 7.53 (s, broad, 1H, CON**H**), 4.00-3.95 (m, 2H, C**H**$_2$CONHBoc), 3.67-3.56 (m, 12H, chain-C**H**$_2$), 3.55-3.50 (m, 4H, chain-C**H**$_2$), 3.47-3.41 (m, 2H, chain-C**H**$_2$), 3.35 (t, 2H, J = 5.2 Hz, chain-C**H**$_2$), 3.25 (s, 2H, NC**H**$_2$CON), 2.74 (t, 2H, J = 5.0 Hz, NHC**H**$_2$), 2.02 (s, broad, 1H, NH), 1.43 (s, 9H, *t*Bu). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 172.0 (**C**ON), 157.0 (O**C**ON), 81.6 (*t*Bu), 75.5 (**C**H$_2$ONHBoc), 70.7, 70.6, 70.4, 70.3, 70.1, 70.1, 68.7 (EG-**C**H$_2$), 52.4 (**C**H$_2$N), 50.7 (**C**H$_2$N$_3$), 49.2, 38.8 (**C**H$_2$N), 28.3 (*t*Bu).



59

2-{N-[3-(tert.-Butoxyaminooxy)propyl]-[4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzoyl]amido}-N`-(11-azido-3,6,9-trioxaundecyl)acetamide **59**: According to general

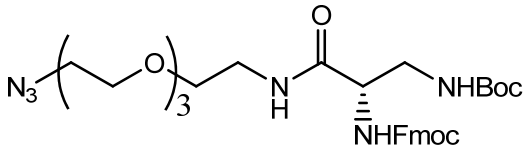
procedure **A** 200 mg of free base **56** (0.446 mmol) were reacted with 90 mg HOBt (0.669 mmol), 117 µl DIPEA (0.669 mmol), 103 mg of diazirine **58** [186] (0.446 mmol, 1 eq.) and 254 mg HBTU (0.669 mol). Column chromatography eluting with $CHCl_3$ containing 2.5% MeOH yielded 283 mg of pure title compound **59** (0.428 mmol, 96 %) as colorless viscous oil. R_f: 0.38 ($CHCl_3$+3%MeOH). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{27}H_{39}F_3N_8O_8Na$: 683.2735 found: 683.2739. ^{1}H-NMR from the mixture of isomers (400.1 MHz, MeOH-d_4): δ 7.61, 7.55 (d, d, 2H, J = 8.2 Hz/8.2 Hz, aryl), 7.35, 7.32 (d, d, 2H, 8.2 Hz/8.1 Hz, aryl), 4.21, 3.97 (s, s, 2H, NC**H**$_2$CON), 3.89, 3.71-3.55 (t, m, 14H, J = 5.9 Hz, C**H**$_2$ONHBoc/chain-C**H**$_2$), 3.50, 3.47-3.33 (t, m, 6H, J = 5.2 Hz, chain-C**H**$_2$), 2.04-1.94, 1.89-1.80 (m, m, 2H, C**H**$_2$), 1.47, 1.44 (s, 9H, *t*Bu). ^{13}C-NMR from the mixture of isomers (100.6 MHz, MeOH-d_4): δ 173.4, 173.3 (**C**ON), 170.7, 170.5 (**C**ON), 159.0 (O**C**ON), 139.1, 138.8, 131.4, 128.7, 127.8 (aryl), 123.4 (q, J = 274 Hz, **C**F_3), 82.0 (*t*Bu), 74.9, 74.2 (**C**H_2ONHBoc), 71.6, 71.6, 71.5, 71.3, 71.2, 71.1, 70.5, 70.4 (EG-**C**H_2), 53.5 (**C**H_2N), 51.7 (**C**H_2N_3), 49.6, 45.8, 40.4 (**C**H_2N), 29.4 (q, J = 40.4 Hz, **C**N_2CF_3), 28.6 (*t*Bu), 28.2, 27.0 (**C**H_2).



60

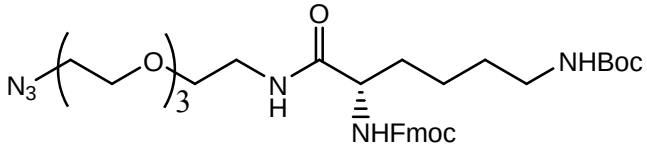
2-{N-[5-(tert.-butoxyaminooxy)-3-oxapentyl]-[4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzoyl]amido}-N`-(11-azido-3,6,9-trioxaundecyl)acetamide **60**: According to general procedure **A** 200 mg of free base **58** (0.418 mmol) were reacted with 85 mg HOBt (0.627 mmol), 109 µl DIPEA (0.627 mmol), 96 mg of diazirine **58** [186] (0.418 mmol, 1 eq.) and 238 mg HBTU (0.627 mol). Column chromatography eluting with $CHCl_3$ containing 2.5 % MeOH yielded 242 g of pure title compound **60** (0.343 mmol, 82%) as colorless viscous oil. R_f: 0.42 ($CHCl_3$+3%MeOH). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{28}H_{41}F_3N_8O_9Na$: 713.2841 found: 713.2846. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 8.25, 8.17 (s, s, broad, 1H, NH), 7.50 (d, 2H, J = 8.3 Hz, aryl), 7.27, 7.01 (s, s, 1H, NH), 7.20-7.08 (m, 2H, aryl), 4.13, 3.96 (s/s, 2H, NC**H**$_2$CON), 3.94-3.88 (m, 2H, C**H**$_2$ONHBoc), 3.78-3.25 (m, 20 H, chain-C**H**$_2$), 3.33-3.25 (m, 2H, chain-C**H**$_2$), 1.38 (s, 9H, *t*Bu). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 171.5 (**C**ON), 168.8, 168.5 (**C**ON), 162.5 (**C**ON), 159.0, 156.9 (**C**ON), 137.2, 130.3, 127.9, 127.6, 126.4 (aryl), 121.9 (q, J = 275 Hz, **C**F_3), 81.3 (*t*Bu), 75.1 (**C**H_2ONHBoc), 70.5, 70.5, 70.3, 70.2, 69.9, 69.8, 68.5, 68.2 (EG-

CH_2), 53.9 (**C**H_2N), 50.5 (**C**H_2N_3), 50.0, 46.4, 39.2 (**C**H_2N), 28.2 (q, J = 40.4 Hz, **C**N_2CF_3), 28.1 (*t*Bu).



61

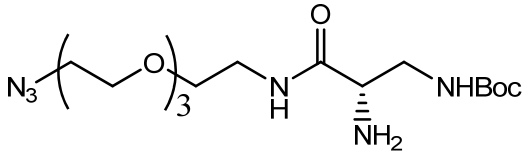
(S)-N-α-(9-Fluorenyl)methoxycarbonyl-N`-β-tert.-butoxycarbonyl-N``-(11-azido-3,6,9-trioxaundecyl)-β-aminoalanylamide **61**: In a 100 ml round bottom flask equipped with a gas inlet and a stirring bar 5 g of α-Fmoc-β-Boc-L-β-aminoalanine (7.98 mmol) were dissolved in 50 ml dry THF under an atmosphere of nitrogen. Then the solution was cooled to 0°C and 1.62 g PFP (8.78 mmol, 1.1 eq.) were added followed by 2.15 g DCC (10.4 mmol, 1.3 eq.) and stirring continued for 6 h at 0°C. Thereafter 1.92 g of azido-amine **5** [152] (8.78 mmol, 1.1 eq.) and 2.08 ml DIPEA (12.0 mmol, 1.5 eq.) were added and the slurry stirred for additional 14 h at RT. After filtration from the DC-urea precipitate the solution was diluted with EA and transferred to a separatory funnel. The organic layer was washed twice each with 1 M $NaHSO_4$-soln. and sat. NaHCO3-soln., once with brine, dried with Na_2SO_4 and the solvent removed under reduced pressure. The residue was purified by column chromatography eluting with Tol/acetone 3:1-7:3 yielding 3.2 g of pure title compound **61** (5.11 mmol, 64%) as colorless resin. R_f: 0.29 (Tol/acetone 3:1). $[\alpha]_D^{20}$= -12.5 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{31}H_{42}N_6O_8Na$: 649.2956 found: 649.2950. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 7.75 (d, 2H, J = 7.5 Hz, Fmoc), 7.62-7.57 (m, 2H, Fmoc), 7.39 (t, 2H, J = 7.5 Hz, Fmoc), 7.30 (dt, 2H, J = 7.5 Hz/0.9 Hz, Fmoc), 6.96 (s, broad, 1H, CON**H**), 6.32 (s, broad, 1H, CON**H**), 5.29 (s, broad, 1H, CON**H**), 4.44-4.24 (m, 2H, Fmoc-C**H**$_2$O/α-C**H**), 4.20 (t, 1H, J = 7.2 Hz, Fmoc-C**H**), 3.65-3.40 (m, 16H, EG-chain-C**H**$_2$/β-C**H**), 3.34 (t, 2H, J = 5.2 Hz, C**H**$_2N_3$), 1.44 (s, 9H, *t*Bu). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 170.2 (**C**ONH), 157.2, 156.6 (O**C**ONH), 143.9, 143.8, 141.4, 127.8, 127.2, 125.2, 120.1 (aryl), 80.1 (*t*Bu), 70.7, 70.7, 70.6, 70.4, 70.0, 69.6 (EG-**C**H_2), 67.3 (Fmoc-**C**H_2O), 56.4 (α-**C**H), 50.7 (**C**H_2N_3), 47.2 (Fmoc-**C**H), 42.9, 39.5 (**C**H_2N), 28.4 (*t*Bu).



62

(S)-N-α-(9-Fluorenyl)methoxycarbonyl-N`-ε-tert.-butoxycarbonyl-N``-(11-azido-3,6,9-trioxaundecyl)lysylamide **62**: In a 100 ml round bottom flask equipped with a gas inlet and a

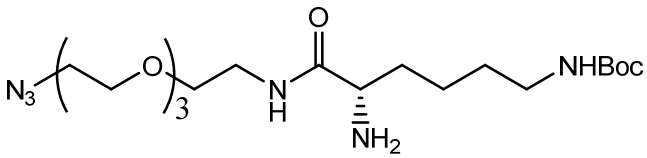
stirring bar 4.47 g α-Fmoc-ε-Boc-L-β-lysine (9.54 mmol) were dissolved in 50 ml dry THF under an atmosphere of nitrogen. The solution was cooled to 0°C and 1.55 g HOBt (11.45 mmol, 1.2 eq.) followed by 2.56 g DCC (12.4 mmol, 1.3 eq.) were added. The mixture was stirred at 0°C for 1 h and another 1 h at RT and again cooled to 0°C. Then 2.29 g of azido-amine **5** [152] (10.49 mmol, 1.1 eq.) were added, the mixture stirred an additional 1 h at 0°C and then at RT for 14 h. After filtration from the precipitated DC-urea the solution was transferred to a separating funnel, washed twice each with 1 M $NaHSO_4$ soln. and sat. $NaHCO_3$ soln. and once with brine and the solvent removed under reduced pressure. The crude amide was subjected to silica gel column chromatography eluting with $CHCl_3$ containing 1.5% MeOH yielding 4.0 g of pure title compound **62** (5.98 mmol, 63%) as colorless resin. R_f: 0.41 ($CHCl_3$+2%MeOH). $[\alpha]_D^{20}$= -2.5° (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{34}H_{48}N_6O_8Na$: 691.3426 found: 691.3422. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 7.75 (d, 2H, J = 7.5 Hz, Fmoc-aryl), 7.59 (d, 2H, J = 7.4 Hz, Fmoc-aryl), 7.39 (t, 2H, J = 7.5 Hz, Fmoc-aryl), 7.30 (t, 2H, J = 7.4 Hz, Fmoc-aryl), 6.67-6.59 (m, 1H, CON**H**), 5.68 (d, broad, 1H, J = 7.0 Hz, O**C**ONH), 4.7 (s, broad, 1H, OCON**H**), 4.47-4.33 (m, 2H, Fmoc-C**H**$_2$O), 4.20 (t, 1H, J = 6.9 Hz, Fmoc-C**H**), 4.24-4.10 (m, 1H, α-C**H**), 3.68-3.40 (m, 14H, EG-chain-C**H**$_2$), 3.35 (t, 2H, J = 4.9 Hz, C**H**$_2$N$_3$), 3.09 (s, broad, 2H, C**H**$_2$NHBoc), 1.91-1.77 (m, 1H, β-C**H**), 1.73-1.60 (m, 1H, β-C**H**), 1.57-1.28 (m, 2H, Lys-C**H**$_2$), 1.43 (s, 9H, *t*Bu). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 171.7 (**C**ONH), 156.2 (O**C**ONH), 143.9, 143.9, 141.3, 127.8, 127.1, 125.1, 120.0 (Fmoc-aryl), 79.2 (*t*Bu), 70.7, 70.6, 70.5, 70.3, 70.0, 69.6, 67.0 (EG-**C**H$_2$), 54.9 (α-CH), 50.7 (**C**H$_2$N$_3$), 47.2 (Fmoc-**C**H), 40.1 (**C**H$_2$N), 39.4 (**C**H$_2$N), 32.6, 29.7 (Lys-**C**H$_2$), 28.5 (*t*Bu), 22.6 (Lys-**C**H$_2$).



63

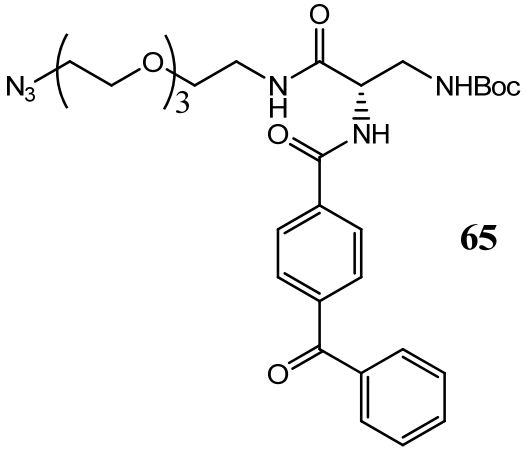
(S)-N-β-tert.-Butoxycarbonyl-N`-(11-azido-3,6,9-trioxaundecyl)-β-aminoalanylamide **63**: In a 50 ml round bottom flask equipped with a stirring bar 3.0 g of protected amide **61** (4.79 mmol) were dissolved in 10 ml dry DMF. Then 10 ml morpholine were added and the mixture stirred at RT for 1 h. After removal of the volatiles under reduced pressure the oily residue was subjected to silica gel column chromatography eluting with $CHCl_3$ containing 2.5% MeOH and 1% Et_3N yielding 1.92 g of the free base **63** (4.74 mmol, 99%) as colorless, viscous oil. $[\alpha]_D^{20}$= -12.1 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+H]^+$ calcd for $C_{16}H_{33}N_6O_6$: 405.2456 found: 405.2454. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 7.64 (m, 1H, CON**H**), 5.21 (s,

broad, 1H, OCON**H**), 3.63-3.54 (m, 10H, EG-chain-C**H**$_2$), 3.50 (t, 2H, J = 5.2 Hz, N_3C**H**$_2$), 3.41-3.29 (m, 7H, EG-chain-C**H**$_2$, α-C**H**, β-C**H**$_2$), 1.65 (s, broad, N**H**$_2$), 1.37 (s, 9H, *t*Bu). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 173.4 (**C**ONH), 156.8 (O**C**ONH), 79.4 (*t*Bu), 70.7, 70.6, 70.3, 70.0, 69.8 (EG-CH_2), 55.6 (α-**C**H), 50.6 (**C**H_2N_3), 44.8, 38.9 (**C**H_2N), 28.3 (*t*Bu).



64

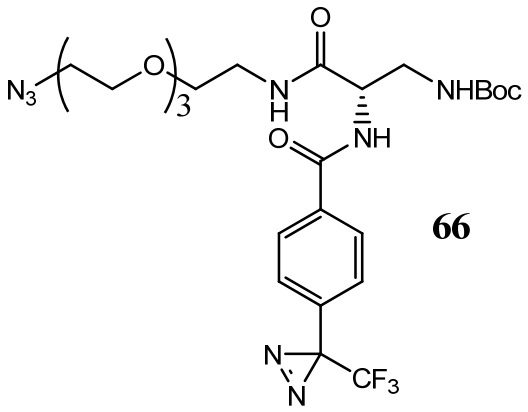
(S)-N-ε-tert.-Butoxycarbonyl-N`-(11-azido-3,6,9-trioxaundecyl)lysylamide **64**: In a 50 ml round bottom flask equipped with a stirring bar 3.8 g of protected linker **62** (5.68 mmol) were dissolved in 10 ml dry DMF. Then 10 ml morpholine were added and the mixture stirred at RT for 1 h. After removal of the volatiles the oily residue was subjected to silica gel column chromatography eluting with $CHCl_3$ containing 2.5% MeOH and 1% Et_3N yielding 2.5 g of the free base **64** (5.6 mmol, 99%) as colorless, viscous oil. R_f: 0.41 ($CHCl_3$+2%MeOH). $[\alpha]_D^{20}$= -6.8 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+H]^+$ calcd for $C_{19}H_{39}N_6O_6$: 447.2926 found: 447.2922. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 7.53-7.45 (m, 1H, CON**H**), 4.65 (s, broad, 1H, OCON**H**), 4.24-4.10 (m, 1H, α-C**H**), 3.67-3.56 (m, 10H, EG-chain-C**H**$_2$), 3.55-3.49 (m, 2H, EG-chain-C**H**$_2$), 3.44-3.32 (m, 4H, EG-chain-C**H**$_2$), 3.29 (dd, 1H, J = 7.9 Hz/4.5 Hz, α-C**H**), 3.12-3.00 (m, broad, 2H, C**H**$_2$NHBoc), 1.84-1.73 (m, 1H, β-C**H**), 1.56-1.27 (m, 7H, Lys-C**H**$_2$, N**H**$_2$, β-C**H**), 1.39 (s, 9H, *t*Bu). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 175.5 (**C**ONH), 156.4 (O**C**ONH), 79.0 (*t*Bu), 70.8, 70.7, 70.3, 70.1, 70.0 (EG-**C**H_2), 55.2 (α-**C**H), 50.7 (**C**H_2N_3), 40.6, 38.8 (**C**H_2N), 34.8, 30.0 (Lys-**C**H_2), 28.5 (*t*Bu), 23.0 (Lys-**C**H_2).



65

(S)-N-α-(4-Benzoyl)benzoyl-N`-β-tert.-butoxycarbonyl-N``-(11-azido-3,6,9-trioxaundecyl)-β-aminoalanylamide **65**: According to the general procedure **A** 1.5 g free base **63** (3.71 mmol) and 923 mg 4-benzoylbenzoic acid (4.08 mmol, 1.1 eq.) were treated with 753 mg HOBt

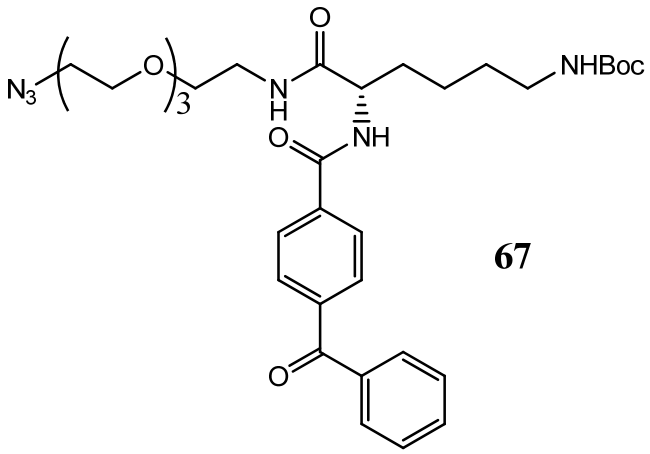
(5.57 mmol), 969 µl DIPEA (5.57 mmol) and 2.11 g HBTU (5.57 mmol). Column chromatography eluting with $CHCl_3$ containing 2% MeOH yielded 2.05 g of pure title compound **65** (3.35 mmol, 90%) as a colorless resin. R_f: 0.34 ($CHCl_3$+2%MeOH). $[\alpha]_D^{20}$= -11.9 (c = 1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{30}H_{40}N_6O_8Na$: 635.2800 found: 635.2802. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 8.24 (d, broad, 2H, J = 8.2 Hz, CON**H**), 7.98 (d, 2H, J = 8.3 Hz, BP-aryl), 7.82 (d, 2H, J = 8.3 Hz, BP-aryl), 7.79-7.74 (m, 2H, BP-aryl), 7.62-7.57 (m, 1H, BP-aryl), 7.50-7.44 (m, 2H, BP-aryl), 7.20-7.12 (m, 1H, CON**H**), 5.53 (s, broad, 1H, OCON**H**), 4.66-4.59 (m, 1H, α-C**H**), 3.67-3.51 (m, 14H, EG-chain-C$\mathbf{H}_2$, β-C$\mathbf{H}_2$), 3.48-3.40 (m, 2H, EG-chain-C$\mathbf{H}_2$), 3.34 (t, 2H, J = 5.0 Hz, $N_3C\mathbf{H}_2$), 1.40 (s, 9H, *t*Bu). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 196.0 (**C**O-BP), 166.9 (**C**ONH), 167.0 (**C**ONH), 158.2 (O**C**ONH), 140.5, 137.1, 136.5, 133.0, 130.1, 128.5, 127.4 (BP-aryl), 81.4 (*t*Bu), 70.7, 70.4, 70.0, 69.6 (EG-**C**H_2), 56.4 (α-**C**H), 50.7 (**C**H_2N_3), 42.4, 39.4 (**C**H_2N), 28.4 (*t*Bu).



66

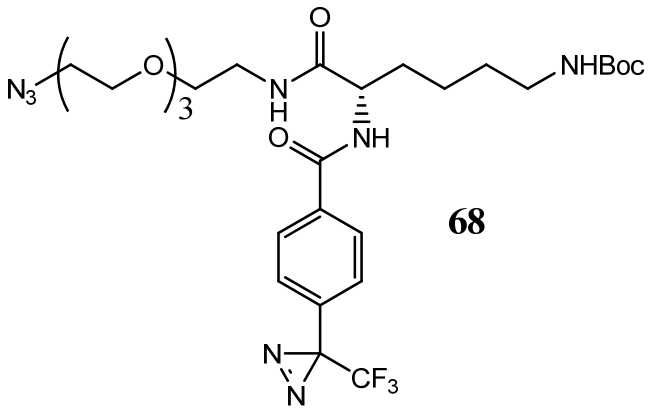
(S)-N-α-{4-[3-(Trifluoromethyl)-3H-diazirin-3-yl]}benzoyl-N`-β-tert.-butoxycarbonyl-N``-(11-azido-3,6,9-trioxaundecyl)-β-aminoalanylamide **66**: According to the general procedure **A** 1.5 g free base **63** (3.71 mmol) and 854 mg 4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzoic acid **58** (3.71 mmol, 1.0 eq.) were treated with 753 mg HOBt (5.57 mmol), 969 µl DIPEA (5.57 mmol) and 2.11 g HBTU (5.57 mmol). Column chromatography eluting with $CHCl_3$ containing 2% MeOH yielded 2.1 g of pure title compound **13** (3.41 mmol, 92%) as colorless resin. R_f: 0.34 ($CHCl_3$+2%MeOH). $[\alpha]_D^{20}$= -16.9 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{25}H_{35}F_3N_8O_7Na$: 639.2473 found: 639.2467. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 8.18 (d, broad, 1H, J = 4.7 Hz, CON**H**), 7.90 (d, 2H, J = 8.4 Hz, aryl), 7.23 (d, 2H, J = 8.3 Hz, aryl), 7.14-7.06 (m, 1H, CON**H**), 5.46 (s, broad, 1H, O**C**ONH), 4.66-4.52 (m, 1H, α-C**H**), 3.68-3.49 (m, 14H, EG-chain-C$\mathbf{H}_2$, β-C$\mathbf{H}_2$), 3.47-3.39 (m, 2H, EG-chain-C$\mathbf{H}_2$), 3.36 (t, 2H, J = 5.0 Hz, C$\mathbf{H}_2N_3$), 1.40 (s, 9H, *t*Bu). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 169.9 (**C**ONH), 166.7 (**C**ONH), 158.2 (O**C**ONH), 134.4, 132.8, 127.9, 126.6 (aryl), 122.0 (q, J = 275 Hz, **C**F_3), 80.5

(*t*Bu), 70.7, 70.7, 70.6, 70.4, 70.1, 69.6 (EG-**C**H_2), 56.4 (α-**C**H), 50.7 (**C**H_2N_3), 42.4, 39.5 (**C**H_2N), 28.4 (q, J = 40 Hz, CN_2CF_3), 28.4 (*t*Bu).



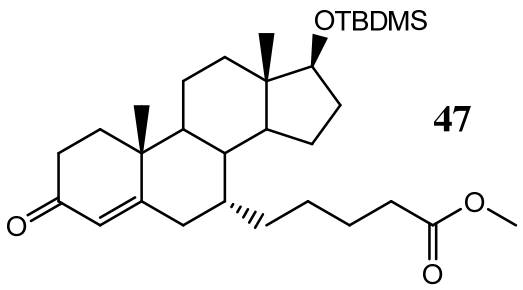
67

(S)-N-α-(4-Benzoyl)benzoyl-N`-β-tert.-butoxycarbonyl-N``-(11-azido-3,6,9-trioxaundecyl)lysylamide **67**: According to the general procedure **A** 1.15 g free base **64** (2.58 mmol) and 640 mg 4-benzoylbenzoic acid (2.83 mmol, 1.1 eq.) were treated with 523 mg HOBt (3.87 mmol), 674 µl DIPEA (3.87 mmol) and 1.47 g HBTU (2.87 mmol). Column chromatography eluting with $CHCl_3$ containing 1.5% MeOH yielded 1.62 g of pure title compound **68** (2.47 mmol, 96%) as colorless resin. R_f: 0.5 ($CHCl_3$+2%MeOH). $[\alpha]_D^{20}$= +7.5° (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{33}H_{46}N_6O_8Na$: 677.3269 found: 677.3275. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 7.94-7.88 (m, 2H, BP-aryl), 7.82-7.73 (m, 4H, BP-aryl), 7.48 (dd, 1H, J = 7.4 Hz/1.2 Hz, BP-aryl), 7.49-7.44 (m, 2H, BP-aryl), 7.34 (d, broad, 2H, J = 8.2 Hz, CON**H**), 6.90 (t, broad, 1H, J = 5.5 Hz, CON**H**), 4.80 (s, broad, 1H, OCONH**H**), 4.68-4.61 (m, 1H, α-C**H**), 3.68-3.58 (m, 10H, EG-chain-C$\mathbf{H}_2$), 3.58-3.52 (m, 2H, EG-chain-C$\mathbf{H}_2$), 3.50-3.42 (m, 2H, EG-chain-C$\mathbf{H}_2$), 3.37 (t, 2H, J = 5.2 Hz, N_3C$\mathbf{H}_2$), 3.25-3.02 (m, 2H, C$\mathbf{H}_2$NHBoc), 1.99-1.88 (m, 1H, β-C**H**), 1.85-1.74 (m, 1H, β-C**H**), 1.58-1.34 (m, 4H, Lys-C$\mathbf{H}_2$), 1.38 (s, 9H, *t*Bu). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 196.0 (**C**O-BP), 171.7 (**C**ONH), 164.4 (**C**ONH), 156.2 (O**C**ONH), 140.3, 137.2, 137.1, 133.0, 130.1, 130.1, 128.5, 127.3 (BP-aryl), 79.1 (*t*Bu), 70.7, 70.7, 70.6, 70.3, 70.1, 69.6 (EG-**C**H_2), 53.6 (α-**C**H), 50.7 (**C**H_2N_3), 40.2, 39.5 (**C**H_2N), 32.7, 29.7 (Lys-**C**H_2), 28.5 (*t*Bu), 22.7 (Lys-**C**H_2).



68

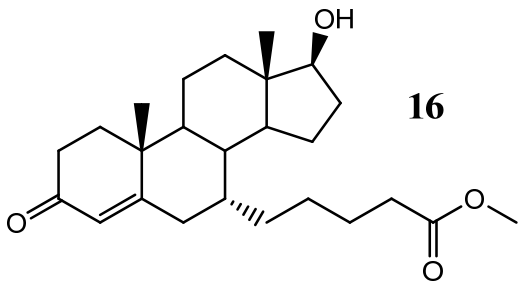
(S)-N-α-{4-[3-(Trifluoromethyl)-3H-diazirin-3-yl]}benzoyl-N`-β-tert.-butoxycarbonyl-N``-(11-azido-3,6,9-trioxaundecyl)lysylamide **68**: According to the general procedure **A** 1.15 g free base **64** (2.58 mmol) and 594 mg of diazirine **58** (2.58 mmol, 1.0 eq.) were treated with 523 mg HOBt (3.87 mmol), 674 µl DIPEA (3.87 mmol) and 1.47 g HBTU (2.87 mmol). Chromatography eluting with $CHCl_3$ containing 1.5% MeOH yielded 1.58 g of pure title compound **68** (2.40 mmol, 93%) as colorless resin. R_f: 0.5 ($CHCl_3$+2%MeOH). $[\alpha]_D^{20}$= +3.6° (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{28}H_{41}F_3N_8O_7Na$: 681.2943 found: 681.2937. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 7.80 (d, 2H, J = 8.5 Hz, aryl), 7.36 (d, broad, 1H, J = 4.7 Hz, CON**H**), 7.16 (d, 2H, J = 8.3 Hz, aryl), 6.90 (t, broad, 1H, J = 5.3 Hz, CON**H**), 4.80 (s, broad, 1H, O**C**ONH), 4.64-4.57 (m, 1H, α-C**H**), 3.67-3.56 (m, 10H, EG-chain-C$\mathbf{H}_2$), 3.56-3.50 (m, 2H, EG-chain-C$\mathbf{H}_2$), 3.47-3.39 (m, 2H, EG-chain-C$\mathbf{H}_2$), 3.36 (t, 2H, J = 5.0 Hz, $N_3C\mathbf{H}_2$), 3.11-3.00 (m, 2H, C$\mathbf{H}_2$NHBoc), 1.95-1.84 (m, 1H, β-C**H**), 1.83-1.71 (m, 1H, β-C**H**), 1.55-1.32 (m, 4H, Lys-C$\mathbf{H}_2$), 1.36 (s, 9H, *t*Bu). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 171.8 (**C**ONH), 166.1 (**C**ONH), 156.2 (O**C**ONH), 135.0, 132.4, 127.8, 126.5, 122.0 (q, J = 275 Hz, **C**F_3), 79.1 (*t*Bu), 70.7, 70.6, 70.3, 70.1, 69.6, 53.6 (α-**C**H), 50.7, 40.1, 39.5, 32.5, 29.7, 28.4 (q, J = 40 Hz, CN_2CF_3), 28.4 (*t*Bu), 23.0.



47

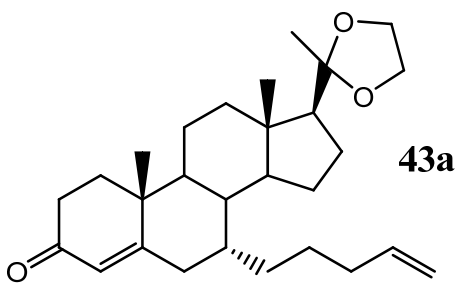
5-[(17β,7α)-17-(tert.-Butyldimethylsiloxy)-3-oxo-androst-4-en-7-yl]-pentanoic acid methyl ester **47** **[154]**: Known steroidal alkene **44** (2.75 g, 5.84 mmol) was converted to the corresponding diol **45** following the procedure published by Wüst et al..[169] Subsequent

purification via column chromatography eluting with PE/EA 2:3 yielded 2.42 g of the steroid **45** (4.93 mmol, 84%) as colorless foam. The diol **45** was then dissolved in 30 ml dry DCM in a 100 ml round bottom flask equipped with a gas inlet and a stirring bar under an atmosphere of nitrogen. To the solution were added 3.58 ml pyridine (44.8 mmol, 9 eq.) and DMP (14.8 mmol, 3 eq.) and the mixture stirred for 90 min at RT.[170] Thereafter Et_2O was added and the white cloudy precipitate filtered off. To the organic phase was then added sat. $NaHCO_3$-soln. containing $Na_2S_2O_3$ and the mixture stirred for 10 min at RT. After transferring the slurry to a separatory funnel the aqueous layer was separated, the organic phase washed two times with sat. $NaHCO_3$-soln. and once each with water and brine. The organic layer was dried over Na_2SO_4 and the solvent removed under reduced pressure yielding 2.38 g of the crude Keto-aldehyde (4.88 mmol, 99%). Judged by TLC the aldehyde was sufficiently pure to be used for the next step without further purification. Accordingly, 2.38 g of the Keto-aldehyde from above (4.88 mmol) were dissolved in 50 ml dry MeOH under an atmosphere of nitrogen. The solution was cooled to 0°C and a solution of 713 mg KOH (12.7 mmol, 2.6 eq.) in 25 ml dry MeOH was added followed by slow addition (10 min) of a solution of 1.61 g iodine (6.34 mmol, 1.3 mmol) in 25 ml dry MeOH.[171] The brownish solution was stirred for additional 30 min at 0°C and then quenched by addition of 1 M $NaHSO_4$-soln. and some solid Na_2SO_3 in order to decolorize the mixture. After transferring to a separating funnel the mixture was extracted three times with EA, the organic layer washed once with 20 *w*% Na_2SO_3-soln., twice with water and once with brine and the solvent removed under reduced pressure. The crude product was then subjected to column chromatography eluting with PE/EA yielding 1.59 g of pure steroid ester **47** (3.07 mmol, 63%) as colorless gum. R_f: 0.31 (PE/EA 4:1). $[\alpha]_D^{20}$= +47.1 (c = 1.0, $CHCl_3$). Anal. calcd for $C_{31}H_{52}O_4$: C, 72.04; H, 10.14; found: C, 71.77; H, 10.14. FAB-MS: m/z 517.4 $(M+H)^+$. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 5.7 (s, 1H, C4-**H**), 3.66 (s, 3H, COOMe), 3.59-3.52 (m, 1H, C17-**H**), 2.44-2.25 (m, 6H, scaffold), 2.08-2.01 (m, 1H, scaffold), 1.95-1.84 (m, 1H, scaffold), 1.78-0.9 (m, 18H, scaffold), 1.19 (s, 3H, C**H**$_3$), 0.87 (s, 9H, TBDMS-*t*Bu), 0.74 (s, 3H, C**H**$_3$), 0.0, 0.0 (s, s, 6H, TBDMS-C**H**$_3$). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 199.4 (**C**3), 174.2 (**C**OOMe), 170.3 (C**5**), 125.9 (**C**4), 81.7 (**C**17), 51.6 (O**C**H$_3$), 47.5 (**C**9), 45.8 (**C**14), 43.3 (**C**13), 39.2 (**C**7), 38.8 (**C**10), 36.8 (**C**1, **C**6, **C**12), 36.3 (**C**8), 36.1 (**C**1, **C**6), 34.2, 34.2 (**C**2, 7α-**C**H$_2$), 30.8 (**C**16), 27.0 (7α-**C**H$_2$), 26.0 (TBDMS-*t*Bu), 25.2, 24.9 (7α-**C**H$_2$), 23.1 (**C**15), 21.1 (**C**11), 18.2, 18.2 (**C**19), 11.3 (**C**18), -4.4, -4.7 (TBDMS-**C**H$_3$).



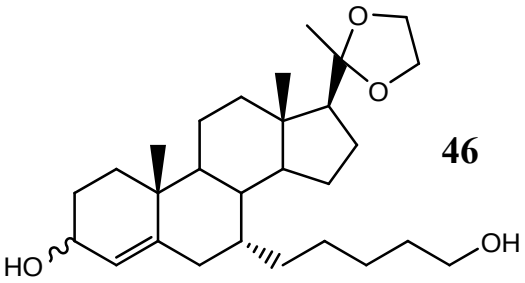
16

5-[(17β,7α)-17-Hydroxy-3-oxo-androst-4-en-7-yl]pentanoic acid methyl ester **16**: In a 50 ml round bottom flask equipped with a stirring bar 1.5 g of the steroid **47** (2.90 mmol) were dissolved in 40 ml MeCN and the solution cooled 0°C. Then 7 ml of 40 % aq. HF were added and the mixture stirred for 1 h at 0°C. After completion of the reaction, the mixture was neutralized by addition of sat. $NaHCO_3$-soln. and transferred to a separatory funnel. The aqueous phase was extracted three times with EA, the combined organic layers washed once each with water and brine, dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The residue was purified by column chromatography eluting with PE/EA 4.5:5.5 yielding 913 mg of title compound **16** (2.35 mmol, 81%) as white solid. An analytical sample of colorless long needles was obtained by crystallization from *n*-hexane/$CHCl_3$. R_f: 0.33 (PE/EA 1:1). $[\alpha]_D^{20}$= +55.1 (C=1, $CHCl_3$). mp: 164.5-165 °C (*n*-hexane/$CHCl_3$). Anal. calcd for $C_{25}H_{38}O_4$: C, 74.59; H, 9.51; found: C, 74.20; H, 9.83. FAB-MS: m/z 403.2 $(M+H)^+$. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 5.7 (s, 1H, C4-**H**), 3.67-3.62 (m, 1H, C17-**H**), 3.66 (s, 3H, COOMe), 2.46-2.22 (m, 6H, scaffold), 2.12-2.01 (m, 1H, scaffold), 1.85-1.79 (m, 1H, scaffold), 1.78-0.97 (m, 18H, scaffold), 1.20 (s, 3H, C**H**$_3$), 0.78 (s, 3H, C**H**$_3$). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 199.3 (**C**3), 174.2 (**C**OOMe), 170.0 (**C**5), 126.0 (**C**4), 81.8 (**C**17), 51.6 (O**C**H_3), 47.3 (**C**9), 46.2 (**C**14), 43.0 (**C**13), 39.1 (**C**7), 38.8 (**C**10), 36.7 (**C**12), 36.4 (**C**1, **C**6), 36.3 (**C**8), 36.1 (**C**1, **C**6), 34.1 (**C**2), 30.4 (**C**16), 27.0, 25.2, 24.9 (7α-**C**H_2), 22.9 (**C**15), 21.0 (**C**11), 18.2 (**C**19), 11.0 (**C**18).



43a

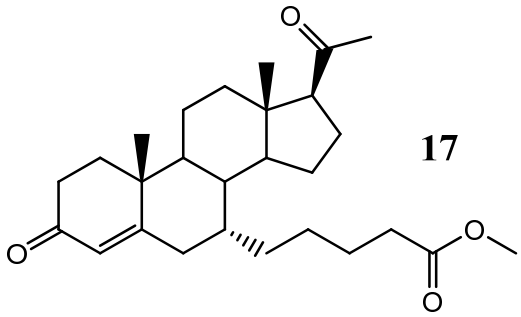
(7α)-20-(1,3-Dioxolan-2-yl)-7-(pent-4-enyl)-pregn-4-en-3-one **43a**: Dienone **41**[167] (8.05 g, 25.8 mmol) was converted to the corresponding 7-pentenyl-derivative following the procedure published by Wüst et al..[169] The crude mixture of the α/β-isomers was separated and purified by column chromatography eluting with PE/EA 4:1. The first fraction yielded 5.3 g of the α-isomer **43a** (12.4 mmol, 60%) as colorless crystals. The second fraction afforded 1.35 g of the β-isomer **43b** (3.16 mmol, 15%) as viscous colorless oil. An analytical sample of colorless crystals of the α-isomer **43a** was obtained by crystallization from *n*-hexane. R_f: 0.41 (**43a**), 0.38 for (**43b**) (PE/EA 3:1). $[\alpha]_D^{20}$ (**43a**) = +70.1 (c=1, $CHCl_3$). mp (**43a**): 127.0 °C (*n*-hexane). Anal. calcd for $C_{28}H_{42}O_3$: C, 78.83; H, 9.92; found: C, 78.97; H, 10.40. FAB-MS: m/z 427.2 $(M+H)^+$. ^{1}H-NMR of **43a** (400.1 MHz, $CDCl_3$): δ 5.82-5.69 (m, 1H, **H**C=CH_2), 5.7 (s, broad, 1H, C4-**H**), 5.02-4.90 (m, 2H, HC=C**H**$_2$), 4.02-3.81 (m, 4H, O(C**H**$_2$)$_2$O), 2.46-2.26 (m, 4H, C2-**H**$_2$, C6-**H**$_2$), 2.09-1.91 (m, 1H, scaffold), 1.84-1.63 (m, 6H, scaffold), 1.62-1.36 (m, 4H, scaffold), 1.31-0.98 (m, 7H, scaffold), 1.28 (s, 3H, C**H**$_3$), 1.18 (s, 3H, C**H**$_3$), 0.79 (s, 3H, C**H**$_3$). ^{13}C-NMR of **43a** (100.6 MHz, $CDCl_3$): δ 199.4 (**C**3), 170.4 (**C**5), 138.8 (H**C**=CH_2), 125.9 (**C**4), 114.6 (HC=**C**H_2), 111.9 (**C**20),65.3, 63.3 ((O**C**H_2)$_2$), 58.3 (**C**17), 51.4 (**C**14), 47.1 (**C**9), 42.0 (**C**13), 39.2 (**C**7), 38.7 (**C**10), 38.5 (**C**12), 36.9 (**C**8), 36.0 (**C**16), 34.2, 34.0 (**C**6, **C**2), 26.9 (7α-**C**H_2), 24.7 (**C**15), 24.7 (**C**21), 23.3, 23.0 (7α-**C**H_2), 21.1 (**C**11), 18.2, 18.2 (**C**19), 12.9 (**C**18).



46

(7α)-20-(1,3-Dioxolan-2-yl)-7-(5-hydroxypentyl)-pregn-4-en-3-ol **46**: Steroidal alkene **43a** (4.91 g, 11.5 mmol) was converted to the corresponding diol following a procedure published by Wüst et al.[169] Subsequent purification by column chromatography eluting with PE/EA 3:1 yielded 4.72 g of the steroid-diol **46** (10.57 mmol, 92%) as colorless foam. R_f: 0.39 (PE/EA 3:7). Anal. calcd for $C_{28}H_{46}O_4$: C, 75.29; H, 10.38; found: C, 75.12; H, 10.52. ESI-MS: m/z 446.4 $(M)^+$. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 5.40, 5.21 (d, s, 1H, J = 4.4 Hz, C4-**H**), 4.20-4.12, 4.05-4.01 (m, m, 1H, C3-**H**), 4.01-3.80 (m, 4H, O(C**H**$_2$)$_2$O), 3.61 (t, 2H, J = 6.7 Hz, C**H**$_2$OH), 2.28-2.17 (m, 1H, scaffold), 2.07-0.93 (m, 28H, scaffold), 1.26 (s, 3H, C**H**$_3$), 1.05, 0.97 (s, 3H, C**H**$_3$), 0.75 (s, 3H, C**H**$_3$). ^{13}C-NMR of the 3-β-hydroxy isomer (100.6 MHz, $CDCl_3$): δ 144.7 (**C**5), 125.6 (**C**4), 112.0 (**C**20), 68.0 (**C**3), 65.2,

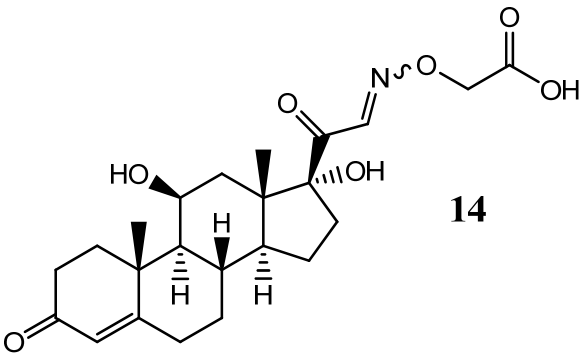
63.3, 63.0 ((O**C**H_2)$_2$, C$\mathbf{H}_2$OH), 58.4 (**C**17), 51.5 (**C**14), 47.4 (**C**9), 42.0 (**C**13), 39.4 (**C**12), 38.6 (**C**7), 37.5 (**C**10), 36.8 (**C**8), 35.8 (**C**1), 35.6 (**C**16), 32.9, 29.6 (**C**6, **C**2), 27.4, 26.2 (7α-**C**H_2), 24.7 (**C**21), 24.3 (**C**15), 23.3, 23.0 (7α-**C**H_2), 21.0 (**C**11), 19.6 (**C**19), 12.9 (**C**18).



17

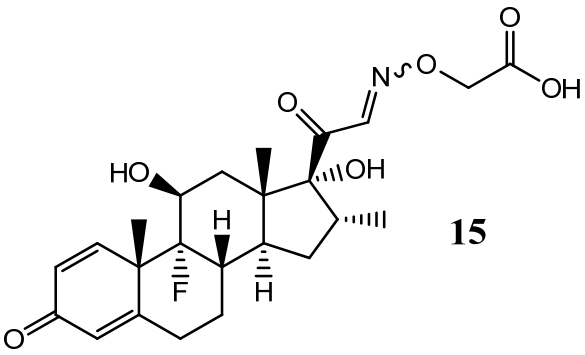
5-[(7α)-3-Oxopregn-4-en-7-yl]pentanoic acid methyl ester **17**: Steroidal alkene **43a** (4.91 g, 11.5 mmol) was converted to the corresponding diol following a procedure published by Wüst et al.[169] Subsequent purification by column chromatography eluting with PE/EA 3:1 yielded 4.72 g of the steroid-diol **46** (10.57 mmol, 92%) as colorless foam. Then diol **46** was further reacted following the procedure given for the preparation of **16** by oxidizing 4.62 g of **46** (10.3 mmol) with 13.1 g DMP (30.9 mmol, 3 eq.) in 50 ml dry DCM containing 7.43 ml dry pyridine (92.7 mmol, 9 eq.).[170] The crude steroid aldehyde (4.51 g, 10.2 mmol, 99%) was obtained as colorless foam and used for the next step without further purification. Analogous to the preparation of **16**, further oxidation to the methyl ester was facilitated by treatment of 4.51 g of the crude keto-aldehyde (10.2 mmol) with 1.49 g KOH (26.5 mmol, 2.6 eq.) and 3.36 g iodine (13.3 mmol, 1.3 eq.).[171] The mixture was then quenched by acidification with 1 M $NaHSO_4$-soln. and stirred for additional 30 min at 0°C leading to efficient cleavage of the dioxolane protecting group. Then the mixture was diluted with water, decolorized by addition of solid Na_2SO_3 and transferred to a separatory funnel. The aqueous layer was extracted five times with EA, the combined organic layers washed once with 20 *w*% Na_2SO_3-soln., water and brine, dried over Na_2SO_4 and the solvent removed under reduced pressure. The residue was subjected to column chromatography eluting with Tol/Acetone 9:1 yielding pure title compound **17** (5.30 mmol, 52%) as colorless amorphous solid. An analytical sample of colorless crystals was obtained by crystallization from *n*-hexane. R_f: 0.24 (Tol/Acetone 9.25:0.75). mp: 91.5 °C (*n*-hexane). $[\alpha]_D^{20}$= +125.8 (c=1.0, $CHCl_3$). Anal. calcd for $C_{27}H_{40}O_4$: C, 75.66; H, 9.41; found: C, 75.32; H, 9.73. FAB-MS: m/z 429.3 $[M+H]^+$. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 5.63 (s, 1H, C4-**H**), 3.58 (s, 3H, COOMe), 2.50-2.43 (m, 1H, C17-**H**), 2.40-1.92 (m, 9H, scaffold), 2.04 (s, 3H, CH_3), 1.70-0.95 (m, 17H, scaffold), 1.12 (s, 3H, C$\mathbf{H}_3$), 0.59 (s, 3H, C$\mathbf{H}_3$). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 209.0 (**C**20), 198.8 (**C**3), 173.9

(**C**OOMe), 169.5 (**C**5), 125.8 (**C**4), 63.5 (**C**17), 51.6 (O**C**H_3), 51.4 (**C**14), 46.9 (**C**9), 43.9 (**C**13), 38.8 (**C**7), 38.5 (**C**10), 38.4 (**C**12), 36.6 (**C**8), 36.5 (**C**16), 35.9 (7α-**C**H_2), 34.0, 33.9 (**C**6, **C**2), 31.4 (**C**21), 26.8, 25.0 (7α-**C**H_2), 24.7 (**C**15), 23.7 (7α-**C**H_2), 22.7 (**C**1), 21.2 (**C**11), 18.2 (**C**19), 13.1 (**C**18).



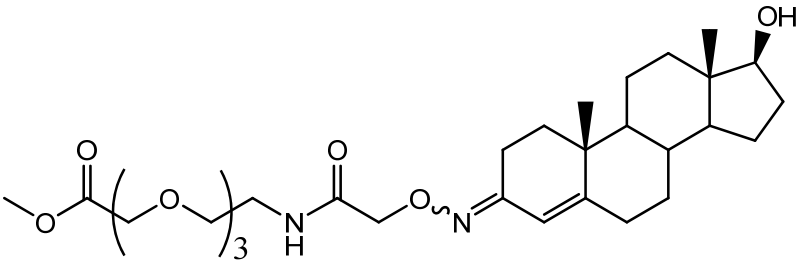
14

2-{[(11β)-3,20-Dioxo-11,17-dihydroxypregn-4-en-21-ylidene]aminooxy}acetic acid **14**: According to the general procedure **B** 1 g HC (2.76 mmol) was converted to the corresponding 21-aldehyde and directly condensed with 302 mg CMA*0.5 HCl (2.76 mmol, 1 eq.). Silica gel column chromatography eluting with $CHCl_3$ containing 4 % MeOH and 1 % HCOOH yielded 942 mg of pure title compound **14** (2.17 mmol, 79 %) as white solid. R_f: 0.24 ($CHCl_3$+2%MeOH+1%HCOOH). $[\alpha]_D^{20}$= +154.3 (c=1.0, DMSO). mp: 172-173°C (decomposition). FAB-MS: m/z: 431.9 $[M-H]^-$. Anal calcd for $C_{23}H_{31}NO_7$: N, 3.23; C, 63.73; H, 7.21; found: N, 3.35; C, 63.66; H, 7.53. ^{1}H-NMR (400.1 MHz, DMSO-d_6): 12.91 (s, broad, 1H, COO**H**) 8.13 (s, 1H, C21-**H**), 5.72 (s, 1H, C17-O**H**) 5.56 (s, 1H, C4-**H**), 4.75 (s, 2H, NOC**H**$_2$), 4.31 (s, 1H, C11-**H**), 4.26 (s, 1H, C11-O**H**), 2.65-2.55 (m, 1H, C16-**H**), 2.52-2.31 (m, 2H, C2-**H**, C6-**H**), 2.24-2.16 (m, 2H, scaffold), 2.12-2.04 (m, 1H, scaffold), 2.00-1.87 (m, 2H, scaffold), 1.84-1.63 (m, 3H, scaffold), 1.54-1.21 (m, 3H, scaffold), 1.36 (s, 3H, C**H**$_3$), 1.07-0.94 (m, 1H, C7-**H**), 0.91-0.85 (m, 1H, C9-**H**), 0.77 (s, 3H, C**H**$_3$). ^{13}C-NMR (100.6 MHz, DMSO-d_6): 198.1 (**C**3), 195.9 (**C**20), 172.3 (**C**5), 170.3 (**C**OOH), 145.9 (**C**21), 121.5 (**C**4), 88.9 (**C**17), 71.1 (NO**C**H_2), 66.4 (**C**11), 55.3 (**C**14), 51.5 (**C**9), 46.4 (**C**13), 39.4, 38.9 (**C**10, **C**12), 34.1, 33.5, 32.7, 31.7, 31.4 (**C**1, **C**2, **C**6, **C**7, **C**16), 31.1 (**C**8), 23.3 (**C**15), 20.5 (**C**19), 17.1 (**C**18).



15

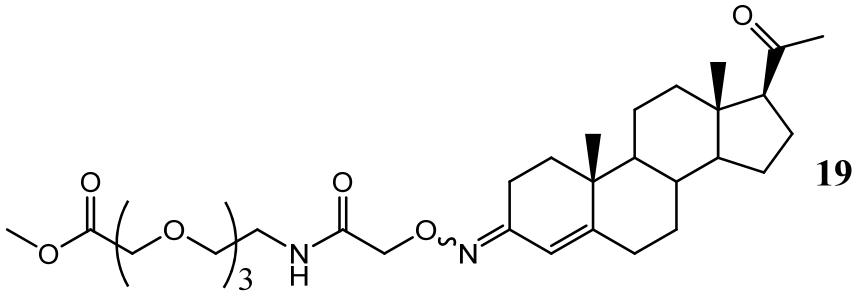
2-{[(11β,16α)-9-Fluoro-3,20-dioxo-11,17-dihydroxy-16-methylpregna-1,4-dien-21-ylidene]aminooxy}acetic acid **15**: According to the general procedure **B** 1g Dex (2.55 mmol) was converted to the corresponding 21-aldehyde and directly condensed with 279 mg CMA*0.5 HCl (2.55 mmol, 1 eq.). Column chromatography eluting with $CHCl_3$ containing 4 % MeOH and 1% formic acid afforded 1.04 g of pure title compound **15** (2.24 mmol, 88 %) as white solid. R_f: 0.22 ($CHCl_3$+3%MeOH/1%HCOOH). mp: 175-176 °C (decomposition). $[\alpha]_D^{20}$= +91.7 (c=1.0, MeOH). Anal calcd for $C_{24}H_{30}NFO_7$: N, 3.02; C, 62.19; H, 6.52. found: N, 2.84; C, 61.93; H, 6.78. FAB-MS: m/z = 461.9 $[M-H]^-$. ^{1}H-NMR (400.1 MHz, DMSO-d_6): δ 12.96 (s, broad, 1H, COO**H**) 8.14 (s, 1H, C21-**H**), 7.27 (d, 1H, J = 10.1 Hz, C1-**H**), 6.22 (dd, 1H, J = 10.1 Hz/1.8 Hz, C2-**H**), 6.01 (s, 1H, C4-**H**), 5.52 (s, 1H, C17-O**H**), 5.35-5.30 (m, 1H, C11-O**H**), 4.76 (s, 2H, NOC**H**$_2$), 4.19-4.11 (m, 1H, C11-**H**), 3.02-2.92 (m, 1H, C16-**H**), 2.69-2.56 (m, 1H, C6-**H**), 2.44- 2.07 (m, 4H, C6-**H**, C8-**H**, C12-**H**, C14-**H**), 1.86-1.77 (m, 1H, C7-**H**), 1.71-1.58 (m, 1H, C15-**H**), 1.48 (s, 3H, C**H**$_3$), 1.43-1.28 (m, 2H, C7-**H**, C12-**H**), 1.14-1.05 (m, 1H, C15-**H**), 0.87 (s, 3H, C**H**$_3$), 0.79 (d, 3H, J = 7.2 Hz, C16-C**H**$_3$). ^{13}C-NMR (100.6 MHz, DMSO-d_6): δ 195.5 (**C**20) 185.3 (**C**3), 170.3 (**C**OOH), 167.0 (**C**5), 152.8 (**C**1), 145.8 (**C**21), 129.0 (**C**2), 124.2 (**C**4), 101.1 (d, J = 174 Hz, **C**9), 90.5 (**C**17), 71.2 (NO**C**H$_2$), 70.6 (d, J = 36.8 Hz, **C**11), 47.9 (d, J = 22.6 Hz, **C**10), 47.5 (**C**13), 43.2 (**C**14), 36.1 (**C**12), 33.9 (**C**16), 33.5 (d, J = 19.4 Hz, **C**8), 31.9, 30.3, 27.3 (**C**15, **C**6, **C**7), 22.9 (d, J = 5.5 Hz, **C**19), 16.8 (**C**18), 15.3 (C16-**C**H$_3$).



18

1-{[(17β)-17-Hydroxyandrost-4-en-3-ylidene]aminooxy}-2-oxo-6,9,12-trioxa-3-azatetradecanoic acid methyl ester **18**: According to the general procedure **A** 100 mg of T-3-

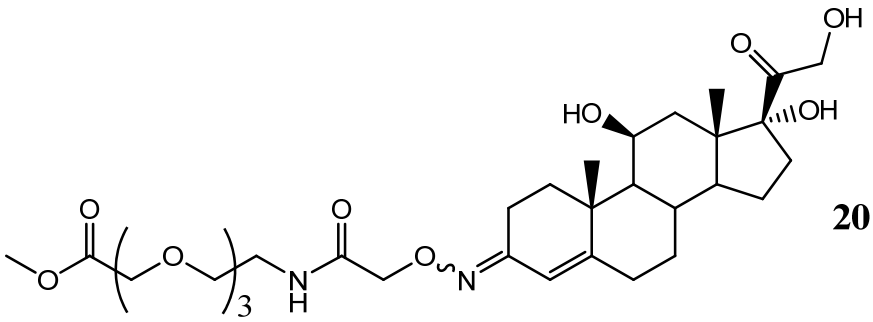
CMO **8** (0.277 mmol) were coupled to 137 mg of freshly hydrogenated linker **1** (0.554 mmol 2 eq.; General procedure **D**) using 56 mg HOBt (0.416 mmol), 73 µl DIPEA (0.416 mmol) and 156 mg HBTU (0.416 mmol). Purification via column chromatography eluting with $CHCl_3$ containing 3% MeOH yielded 105 mg of pure title compound **18** (0.186 mmol, 67%) as colorless foam. R_f: 0.31 ($CHCl_3$+3%MeOH). $[\alpha]_D^{20}$= +71.4 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{30}H_{48}N_2O_8Na$: 587.3303 found: 587.3298. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 6.70-6.59 (m, 1H, CON**H**), 6.32, 5.67 (d, d, 1H, J = 5.3 Hz/1.0 Hz, C4-**H**), 4.43, 4.41 (s, s, 2H, NOC**H**$_2$CONH), 4.08 (s, 2H, OC**H**$_2$COOMe), 3.67 (s, 3H, COOMe), 3.67-3.63 (m, 2H, EG-chain-C**H**$_2$), 3.62-3.58 (m, 2H, EG-chain-C**H**$_2$), 3.58-3.48 (m, 7H, EG-chain-C**H**$_2$, C17-**H**), 3.46-3.40 (m, 2H, EG-chain-C**H**$_2$), 2.97-2.89 (m, 1H, C2-α-**H**), 2.34-1.91 (m, 6H, C2-β-**H**, C6-**H**, scaffold), 1.89-1.67 (m, 3H, scaffold), 1.59-1.15 (m, 7H, scaffold), 1.05, 1.01 (s, s, 3H, C**H**$_3$), 1.03-0.72 (m, 4H, scaffold), 0.71 (s, s, 3H, C**H**$_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 170.7 (**C**O), 170.2 (**C**O), 170.1 (**C**O), 161.2, 158.1, 157.1, 155.2 (**C**3, **C**5), 116.5, 110.5 (**C**4), 81.5, 81.4 (**C**17), 72.7, 72.6 (**C**H$_2$ON), 70.8, 70.6, 70.5, 70.2, 69.8, 69.8, 68.5 (EG-**C**H$_2$), 54.0, 53.8 (**C**9), 51.8 (OMe), 50.6, 50.5 (**C**14), 42.8, 42.7 (**C**13), 39.0 (**C**10), 38.7, 38.7 (**C**12), 38.0 (**C**10), 36.5, 36.4 (**C**2), 36.0(**C**1), 35.7, 35.7 (**C**8), 34.5 (**C**1), 33.0, 32.4, 32.0, 31.6, 30.3, 30.3 (**C**6, **C**7, **C**16), 24.4, 23.3, 20.9, 20.7, 19.5 (**C**11, **C**15), 18.0, 17.7 (**C**19), 11.1, 11.0 (**C**18).



19

1-[(20-Oxo-pregn-4-en-3-ylidene)aminooxy]-2-oxo-6,9,12-trioxa-3-azatetradecanoic acid methyl ester **19**: According to the general procedure **A** 100 mg of progesterone-3-CMO **9** (0.258 mmol) were coupled to 128 mg of freshly hydrogenated linker **1** (0.516 mmol, 2 eq.; General procedure **D**) using 52 mg HOBt (0.387 mmol), 68 µl DIPEA (0.387 mmol) and 147 mg HBTU (0.387 mmol). Purification via column chromatography eluting with $CHCl_3$ containing 3 % MeOH yielded 105 mg of pure title compound **19** (0.186 mmol, 67%) as colorless foam. R_f: 0.31 ($CHCl_3$+3%MeOH). $[\alpha]_D^{20}$= +127.9 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{32}H_{50}N_2O_8Na$: 613.3459 found: 613.3456. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 6.70-6.58 (m, 1H, CON**H**), 6.38, 5.73 (d, d, 1H, J = 1.5

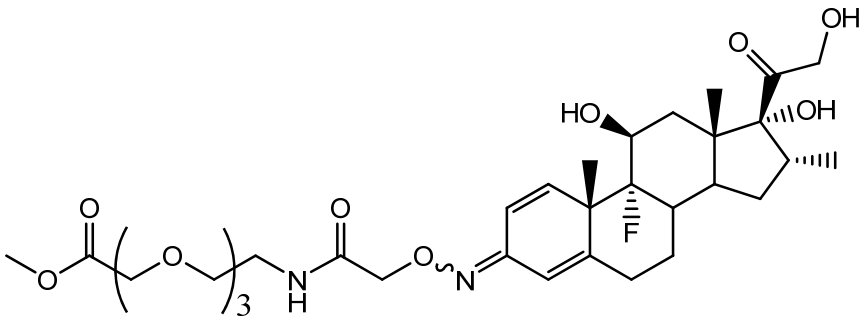
Hz/1.0 Hz, C4-**H**), 4.48, 4.47 (s, s, 2H, NOC**H**$_2$), 4.13 (s, 2H, OC**H**$_2$COOMe), 3.74-3.58 (m, 8H, EG-chain-C**H**$_2$), 3.72 (s, 3H, COOMe), 3.57-3.53 (m, 2H, EG-chain-C**H**$_2$), 3.51-3.45 (m, 2H, EG-chain-C**H**$_2$), 3.03-2.94 (m, 1H, C2-**H**), 2.54-2.46 (m, 1H, C17-**H**), 2.39-1.94 (m, 6H, scaffold), 1.89-1.67 (m, 3H, scaffold), 1.59-1.15 (m, 7H, scaffold), 1.05, 1.01 (s, s, 3H, C**H**$_3$), 1.03-0.72 (m, 4H, scaffold), 0.71 (s, s, 3H, C**H**$_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 209.5, 209.4 (**C**20), 170.9, 170.3, 170.1 (**C**O), 161.1, 158.2, 156.9, 155.2 (**C**3, **C**5), 116.8, 110.7 (**C**4), 72.9, 72.8 (**C**H$_2$ON), 71.0, 70.7, 70.6, 70.3, 70.0, 69.9, 68.7 (EG-**C**H$_2$), 63.7, 63.6 (**C**17), 56.2, 56.2 (**C**14), 53.9, 53.6 (**C**9), 51.9 (OMe), 44.0, 44.0 (**C**13), 39.1 (**C**10), 38.9, 38.8, 38.8 (**C**12, **C**16, **C**H$_2$N), 38.0 (**C**10), 36.1 (**C**2), 35.8, 35.7 (**C**8), 34.7 (**C**2), 33.1, 32.5, 32.5, 32.1 (**C**6, **C**7), 31.6 (**C**21), 24.6, 24.5, 22.9, 21.4, 21.2, 19.7 (**C**1, **C**11, **C**15), 18.1, 17.8 (**C**19), 13.4, 13.4 (**C**18).



20

1-{[(11β)-20-Oxo-11,17,21-trihydroxypregn-4-en-3-ylidene]aminooxy}-2-oxo-6,9,12-trioxa-3-azatetradecanoic acid methyl ester **20**: According to the general procedure **A** 755 mg of hydrocortisol-3-CMO **10** (1.73 mmol) were coupled to 855 mg of freshly hydrogenated linker **1** using (3.46 mmol, 2 eq.; General procedure **D**) 441 mg HOBt (2.26 mmol), 567 µl DIPEA (2.26 mmol) and 1.24 g HBTU (2.26 mmol). Purification via column chromatography eluting with $CHCl_3$ containing 5% MeOH yielded 699 mg of pure title compound **20** (1.09 mmol, 63%) as colorless foam. R_f: 0.2 ($CHCl_3$+4%MeOH). $[\alpha]_D^{20}$= +141.1 (c = 1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{32}H_{50}N_2O_{11}Na$: 661.3307 found: 661.3310. ^{1}H-NMR *E*-isomer (400.1 MHz, $CDCl_3$): δ 6.68 (t, 1H, J = 5.7 Hz, CON**H**), 5.64 (s, 1H, C4-**H**), 4.61 (dd, 1H, J = -19.5 Hz/2.8 Hz, C21-**H**), 4.44 (s, 2H, NOC**H**$_2$CONH), 4.39 (s, broad, 1H, C11-**H**), 4.22 (dd, 1H, J = -19.6 Hz/3.2 Hz, C21-**H**), 4.11 (s, 2H, OC**H**$_2$COOMe), 3.70 (s, 3H, COOMe), 3.69-3.61 (m, 4H, EG-chain-C**H**$_2$), 3.59-3.56 (m, 4H, EG-chain-C**H**$_2$), 3.54-3.49 (m, 2H, EG-chain-C**H**$_2$), 3.47-3.40 (m, 2H, EG-chain-C**H**$_2$), 3.34-3.27 (m, 1H, C11-O**H**), 3.33 (s, broad, 1H, O**H**), 2.97-2.87 (m, 1H, C2-**H**), 2.71-2.60 (m, 1H, C16-**H**), 2.46-2.08 (m, 3H, C2-**H**, C6-**H**, scaffold), 2.07-1.86 (m, 4H, scaffold), 1.84-1.59 (m, 3H, scaffold), 1.58-1.45 (m, 3H, C12-**H**, C16-**H**, scaffold), 1.45-1.25 (m, 1H, C15-**H**), 1.29 (s, 3H, C**H**$_3$), 1.08-0.95

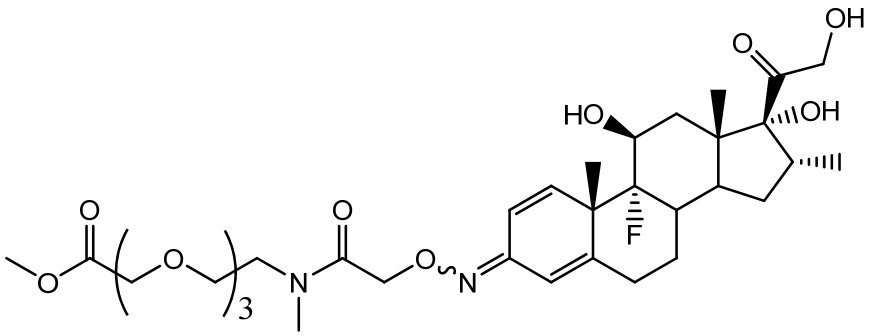
(m, 1H, C7-**H**), 0.93-0.84 (m, 1H, C9-**H**), 0.86 (s, 3H, C**H**$_3$). ^{13}C-NMR *E*-isomer (100.6 MHz, $CDCl_3$): δ 212.4 (**C**20), 171.1 (**C**O), 170.3 (**C**O), 158.3, 158.2 (**C**3, **C**5), 115.0 (**C**4), 88.9 (**C**17), 72.7 (**C**H$_2$ON), 70.9, 70.6, 70.6, 70.3, 69.8, 68.5 (EG-**C**H$_2$), 68.3 (**C**11), 67.3 (**C**21), 56.1 (**C**9), 52.1 , 51.9 (**C**14, OMe), 47.7 (**C**13), 39.8 (**C**H$_2$N, **C**12), 38.7 (**C**10), 38.6 (**C**H$_2$N, **C**12), 34.1, 34.1, 33.1, 31.7 (**C**1, **C**16, **C**6, **C**7), 31.6 (**C**8), 23.8 (**C**15), 21.4 (**C**19), 19.4 (**C**2), 17.5 (**C**18).



21

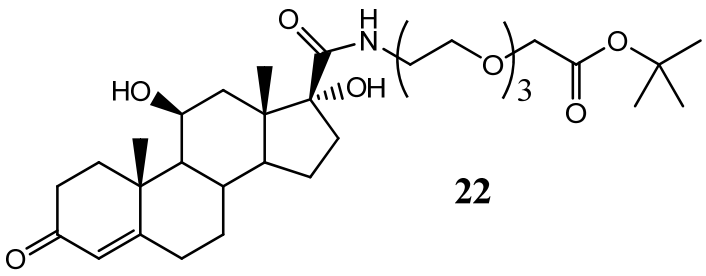
1-{[(11β,16α)-9-Fluoro-20-oxo-11,17,21-trihydroxy-16-methylpregna-1,4-dien-3-ylidene]aminooxy}-2-oxo-6,9,12-trioxa-3-azatetradecanoic acid methyl ester **21:** According to the general procedure **A** 599 mg of Dex-3-CMO **11** (1.27 mmol) were coupled to 628 mg of freshly hydrogenated linker **1** (2.54 mmol, 2 eq.; General procedure **D**) using 258 mg HOBt (1.91 mmol), 333 µl DIPEA (1.91 mmol) and 725 mg HBTU (1.91 mmol). Purification via column chromatography eluting with Tol/acetone 5.5:4.5 yielded 491 mg of pure title compound **21** (0.735 mmol, 58%) as colorless foam. R_f: 0.32 (Tol/acetone 5.5:4.5). $[\alpha]_D^{20}$= +56.2 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{33}H_{49}FN_2O_{11}Na$: 691.3213 found: 691.3215. ^{1}H-NMR from mixture of isomers (400.1 MHz, MeOH-d_4): δ 6.99, 6.21 (dd, dd, 1H, J = 10.3 Hz/1.7 Hz//10.2 Hz/1.7 Hz, C2-**H**), 6.73, 5.94 (s, s, broad, 1H, C4-**H**), 6.68, 6.51 (d, d, 1H, J = 10.3 Hz/10.2 Hz, C1-**H**), 4.60 (d, 1H, J = -19.2 Hz, C21-**H**), 4.50, 4.49 (s, s, 2H, NOC**H**$_2$), 4.26 (d, 1H, J = -19.2 Hz, C21-**H**), 4.26-4.20 (m, 1H, C11-**H**) 4.17 (s, 2H, OC**H**$_2$COOMe), 3.74 (s, 3H, COOMe), 3.71-3.68 (m, 2H, EG-chain-C**H**$_2$), 3.67-3.63 (m, 2H, EG-chain-C**H**$_2$), 3.61 (s, broad, 4H, EG-chain-C**H**$_2$) 3.59-3.54 (m, 2H, EG-chain-C**H**$_2$), 3.50-3.40 (m, 2H, EG-chain-C**H**$_2$), 3.13-3.02 (m, 1H, C16-**H**), 2.72-2.54 (m, 1H, C6-**H**), 2.47- 2.18 (m, 4H, C6-**H**, C8-**H**, C12-**H**, C14-**H**), 1.85-1.66 (m, 2H, C7-**H**, C15-**H**), 1.54-1.39 (m, 2H, C7-**H**, C12-**H**), 1.50 (s, 3H, C**H**$_3$), 1.23-1.15 (m, 1H, C15-**H**), 0.98 (s, 3H, C**H**$_3$), 0.86 (d, 3H, J = 7.3 Hz, C16-C**H**$_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, MeOH-d_4): 212.8 (**C**20), 172.8 (**C**O), 172.5 (**C**O), 159.2, 154.4, 152.3, 152.2 (**C**3, **C**5), 145.7, 141.5 (**C**1), 123.5, 117.3, 117.0, 111.7 (**C**2, **C**4), 101.7, 101.5 (d, d, J = 174 Hz, **C**9), 92.0, 92.0 (**C**17), 79.4, 73.6, 73.6 (**C**H$_2$ON), 72.0, 72.0 (d, d, J = 37.7 Hz/38.0 Hz, **C**11), 71.4, 71.3, 70.6, 69.1,

68.0 (EG-**C**H_2, **C**21), 52.3 (**C**13), 45.2 (**C**14), 39.9 (**C**H_2NH), 37.4, 36.9 (**C**12, **C**16), 35.7, 35.5 (**C**8), 33.4, 32.3, 31.6 (**C**15, **C**6), 28.8, 28.7 (**C**7), 24.7, 24.6 (d, d, J = 30.9 Hz/30.8 Hz, **C**19), 17.5 (**C**18), 15.4 (**C**16-Me).



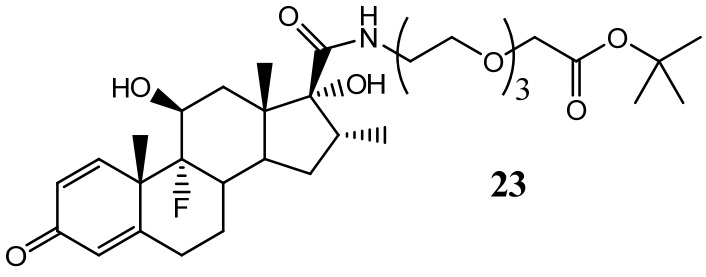
34

1-{[(11β,16α)-9-Fluoro-20-oxo-11,17,21-trihydroxy-16-methylpregna-1,4-dien-3-ylidene]aminooxy}-2-oxo-6,9,12-trioxa-3-aza-3-methyltetradecanoic acid methyl ester **34**: According to the general procedure 377 mg of Dex-3-CMO **11** (0.81 mmol) were coupled to 542 mg of linker **2** (1.62 mmol, 2 eq.) using 165 mg HOBt (1.22 mmol), 212 µl DIPEA (1.22 mmol) and 463 mg HBTU (1.22 mmol). Purification via column chromatography eluting with $CHCl_3$ containing 6% MeOH yielded 214 mg of pure title compound **34** (0.313 mmol, 39%) as colorless foam. R_f: 0.22 ($CHCl_3$+5%MeOH). $[\alpha]_D^{20}$= +80.0 (c = 1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{34}H_{51}FN_2O_{11}Na$: 705.3369 found: 705.3369. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 7.03-6.96, 6.70-6.66 (m, 1H, C2-**H**), 6.54, 6.36 (dd, dd, 1H, J = 10.3 Hz/4.9 Hz//10.1 Hz/4.6 Hz, C1-**H**), 6.22, 6.19, 5.93 (s, s, s, 1H, C4-**H**), 4.75, 4.69 (s, s, 2H, NOC**H**$_2$CONH), 4.58 (dd, 1H, J = -19.8 Hz/4.6 Hz, C21-**H**), 4.31-4.17 (m, 2H, C21-**H**, C11-**H**), 4.13, 4.12 (s, s, 2H, OC**H**$_2$COOMe), 3.71, 3.71 (s, s, 3H, COOMe), 3.70-3.43 (m, 12H, EG-chain-C**H**$_2$), 3.38-3.30 (m, 1H, O**H**), 3.15-3.10, 3.06-2.97, 2.86-2.79 (m, 2H, O**H**), 3.04, 3.02, 2.93, 2.91 (s, s, s, s, 3H, NC**H**$_3$), 2.60-2.41 (m, 1H, scaffold), 2.38-2.06 (m, 5H, scaffold), 1.79-1.13 (m, 5H, scaffold), 1.42 (s, 3H, C**H**$_3$), 0.96 (s, 3H, C**H**$_3$), 0.87 (d, broad, J = 7.11 Hz, C16-C**H**$_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 212.3 (**C**20), 171.1 (**C**O), 169.8 (**C**O), 169.2 (**C**O), 156.0, 155.9, 151.4, 151.3, 150.4, 150.3, 150.2, 150.1 (**C**3, **C**5), 143.4, 143.3, 139.0, 138.9 (**C**1), 123.3, 123.3, 117.1, 117.0, 116.7, 116.6, 111.2, 111.2 (**C**2, **C**4), 100.1, 100.0 (d, d, J = 175 Hz/174 Hz, **C**9), 90.5, 90.4 (**C**17), 71.7 (**C**11), 71.1, 71.0, 70.8, 70.6, 70.4, 69.4, 68.9, 68.6, 67.7 (EG-**C**H_2, **C**H_2ON, **C**21), 51.9 (OMe), 49.0, 48.7 (**C**13), 48.7, 48.2, 47.7 (**C**10, **C**H_2N), 44.2 (**C**14), 36.4, 36.2, 36.2, 36.2, 36.1, 36.0 (**C**12, **C**16, NMe), 34.4, 34.2, 34.1 (**C**8), 32.4, 31.2, 30.6 (**C**15, **C**6), 27.6 (**C**7), 24.0, 23.9 (d, d, J = 28.6 Hz/28.5 Hz, **C**19), 17.1, 17.1 (**C**18), 14.9 (**C**16-Me).



22

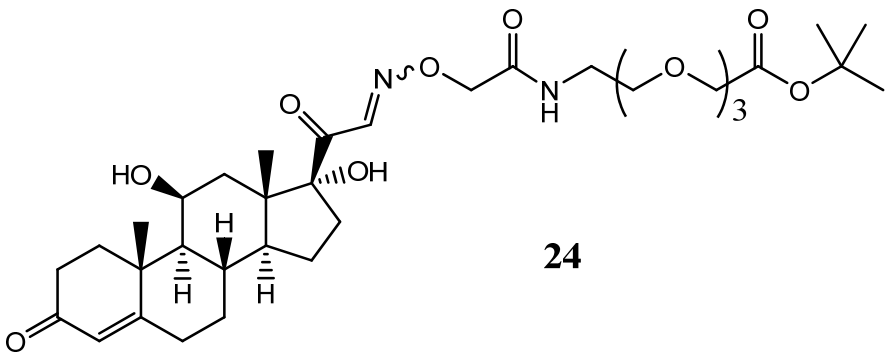
1-[(11β,17α)-3-Oxo-11,17-dihydroxyandrost-4-en-17-carboxamido]-2-oxo-6,9,12-trioxa-3-azatetradecanoic acid tert.-butyl ester **22**: According to the general procedure **A** 100 mg of steroid acid **12** (0.287 mmol) were coupled to 151 mg of freshly hydrogenated linker **3** (0.574 mmol, 2 eq.; General procedure **D**) using 58 mg HOBt (0.43 mmol), 75 µl DIPEA (0.43 mmol) and 163 mg HBTU (0.43 mmol). Purification via column chromatography eluting with $CHCl_3$ containing 4% MeOH yielded 87 mg of pure title compound **22** (0.146 mmol, 51%) as colorless foam. R_f: 0.38 ($CHCl_3$+5%MeOH). $[\alpha]_D^{20}$= +105.7 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{32}H_{51}NO_9Na$: 616.3456 found: 616.3459. ^{1}H-NMR (400.1 MHz, MeOH-d_4): δ 7.60 (t, 1H, J = 5.7 Hz, CON**H**), 5.66 (s, 1H, C4-**H**), 4.42-4.37 (m, 1H, C11-**H**), 4.04 (s, 2H, OC**H**$_2$COO*t*Bu), 3.72-3.61 (m, 8H, EG-chain-C**H**$_2$), 3.60-3.54 (m, 2H, EG-chain-C**H**$_2$), 3.51-3.41 (m, 1H, EG-chain-C**H**$_2$), 3.40-3.33 (m, 1H, EG-chain-C**H**$_2$), 2.84-2.73 (m, 1H, C16-**H**), 2.63-2.43 (m, 2H, C6-**H**, C2-**H**), 2.35-2.19 (m, 3H, C1-**H**, C2-**H**, C6-**H**), 2.10-2.00 (m, 2H, scaffold), 1.97-1.70 (m, 4H, scaffold), 1.65-1.38 (m, 4H, scaffold), 1.48 (s, 12H, *t*Bu, C**H**$_3$), 1.18-1.05 (m, 1H, C7-**H**), 1.03-0.94 (m, 1H, C9-**H**), 0.97 (s, 3H, C**H**$_3$). ^{13}C-NMR (100.6 MHz, MeOH-d_4): 202.4 (**C**3), 176.6 (**C**O), 171.6 (**C**O), 122.5 (**C**4), 86.5 (**C**17), 82.8 (*t*Bu), 71.7, 71.5, 71.4, 71.1, 70.8, 69.7 (EG-**C**H$_2$), 68.8 (**C**11), 57.8 (**C**9), 53.1 (**C**14), 48.3 (**C**13), 40.7 (**C**10), 40.1, 39.9 (**C**H$_2$N, **C**12), 35.8, 34.6, 34.4, 34.0, 33.3 (**C**1, **C**2, **C**6, **C**7, **C**16), 33.0 (**C**8), 28.4 (*t*Bu), 24.8 (**C**15), 21.4 (**C**19), 18.1 (**C**18).



23

1-[(11β,16α,17α)-9-Fluoro-3-oxo-11,17-dihydroxy-16-methylandrosta-1,4-dien-17-carboxamido]-2-oxo-6,9,12-trioxa-3-azatetradecanoic acid tert.-butyl ester **23**: According to the general procedure **A** 530 mg of steroid acid **13** (1.40 mmol) were coupled to 737 mg of freshly hydrogenated linker **3** (2.80 mmol, 2 eq.; General procedure **D**) using 284 mg HOBt

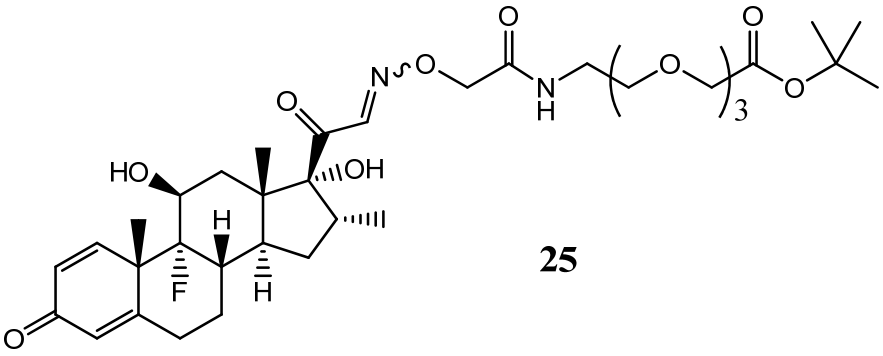
(2.10 mmol), 365 µl DIPEA (2.10 mmol) and 796 mg HBTU (2.10 mmol). Purification via column chromatography eluting with $CHCl_3$ containing 2% MeOH yielded 454 mg of pure title compound **23** (0.728 mmol, 52%) as colorless foam. R_f: 0.43 ($CHCl_3$+5%MeOH). $[\alpha]_D^{20}$= +34.8 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{33}H_{50}FNO_9Na$: 646.3362 found: 646.3359. ^{1}H-NMR (400.1 MHz, MeOH-d_4): δ 7.42 (d, 1H, J = 10.3 Hz, C1-**H**), 6.29 (dd, 1H, J = 10.3 Hz/1.82 Hz, C2-**H**), 6.08 (s, 1H, C4-**H**), 4.28-4.22 (m, 1H, C11-**H**), 4.03 (s, 2H, OC**H**$_2$COO*t*Bu), 3.73-3.61 (m, 8H, EG-chain-C**H**$_2$), 3.60-3.53 (m, 2H, EG-chain-C**H**$_2$), 3.52-3.43 (m, 1H, EG-chain-C**H**$_2$), 3.39-3.32 (m, 1H, EG-chain-C**H**$_2$), 3.17-3.07 (m, 1H, C16-**H**), 2.77-2.67 (m, 1H, C6-**H**), 2.52- 2.35 (m, 2H, C6-**H**, C8-**H**), 2.25-2.14 (m, 2H, C12-**H**, C14-**H**), 1.93-1.83 (m, 1H, C7-**H**), 1.82-1.69 (m, 1H, C15-**H**), 1.59 (s, 3H, C**H**$_3$), 1.65-1.43 (m, 2H, C7-**H**, C12-**H**), 1.47 (s, 9H, *t*Bu), 1.25-1.15 (m, 1H, C15-**H**), 1.08 (s, 3H, C**H**$_3$), 0.90 (d, 3H, J = 7.2 Hz, C16-C**H**$_3$). ^{13}C-NMR (100.1 MHz, MeOH-d_4): δ 188.9 (**C**3), 175.6 (**C**20), 171.5 (**C**OO*t*Bu), 171.0 (**C**5), 156.0 (**C**1), 129.8 (**C**2), 125.1 (**C**4), 102.6 (d, J = 176 Hz, **C**9), 88.1 (**C**17), 82.8 (*t*Bu), 73.0 (d, J = 37.7 Hz, **C**11), 71.6, 71.5, 71.4, 71.0, 70.7, 69.7 (EG-**C**H$_2$), 50.2 (d, J = 22.8 Hz, **C**10), 49.3 (**C**13), 44.7 (**C**14), 39.9 (**C**H$_2$N), 36.7 (**C**12), 36.1 (**C**16), 35.7 (d, J = 19.5 Hz, **C**8), 33.4 (**C**15), 32.2 (C**6**), 28.7 (C**7**), 28.4 (*t*Bu), 23.6 (d, J = 5.7 Hz, **C**19), 17.7 (**C**18), 15.2 (C16-Me).



24

1-{[(11β)-3,20-Dixo-11,17-dihydroxypregn-4-en-21-ylidene]aminooxy}-2-oxo-6,9,12-trioxa-3-azatetradecanoic acid tert.-butyl ester **24**: According to the general procedure **A** 842 mg of HC 21-CMO **14** (1.94 mmol) were coupled to 1.02 g of freshly hydrogenated linker **3** (3.88 mmol, 2 eq.; General procedure **D**) using 507 µl DIPEA (2.91 mmol), 393 mg HOBt (2.91 mmol) and 1.1 g HBTU (2.91 mmol). Column chromatography eluting with Tol/acetone 1:1 yielded 957 mg of pure title compound **24** (1.41 mmol, 73 %) as light yellow foam. R_f: 0.41 (Tol/acetone 1:1). $[\alpha]_D^{20}$= +72.1 (c=1.0, DMSO). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{35}H_{54}N_2O_{11}Na$: 701.3620 found: 701.3618. ^{1}H-NMR (400.1 MHz, $CDCl_3$): 7.84 (s, 1H, C21-**H**), 6.88-6.79 (m, 1H, CON**H**), 5.63 (s, 1H, C4-**H**), 4.81-4.70 (m, 2H, NOC**H**$_2$), 4.42-4.37 (m,

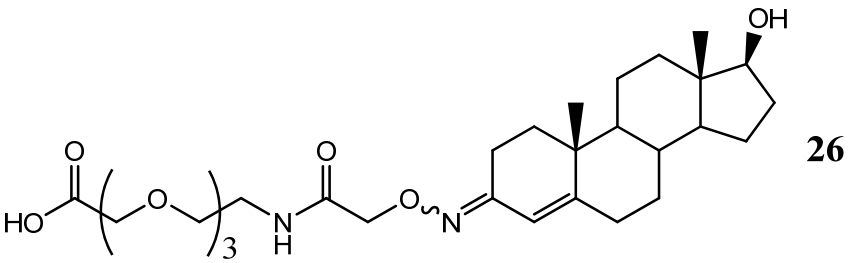
1H, C11-**H**), 3.96 (s, 2H, OC**H**$_2$COO*t*Bu), 3.88 (s, 1H, O**H**), 3.69-3.40 (m, 12H, EG-chain-C**H**$_2$), 2.83-2.74 (m, 1H, C16-**H**), 2.51-2.37 (m, 2H, C2-**H**/C6-**H**), 2.36-2.26 (m, 1H, scaffold), 2.25-2.07 (m, 3H, scaffold), 2.07-1.93 (m, 2H, scaffold), 1.88-1.47 (m, 6H, scaffold), 1.43 (s, 9H, *t*Bu), 1.40 (s, 3H, C**H**$_3$), 1.39-1.25 (m, 1H, C15-**H**), 1.15-1.02 (m, 1H, C7-**H**), 1.03-0.95 (m, 1H, C9-**H**), 0.85 (s, 3H, C**H**$_3$). ^{13}C-NMR *E*-isomer (100.6 MHz, $CDCl_3$): 199.7 (**C**3), 195.0 (**C**20), 172.4 (**C**5), 169.7 (**C**O), 168.7 (**C**O), 149.4 (**C**21), 122.4 (**C**4), 91.6 (**C**17), 81.9 (*t*Bu) 74.2 (**C**H$_2$ON), 70.6, 70.5, 70.5, 69.9, 69.6, 68.9 (EG-**C**H$_2$), 68.2 (**C**11), 55.8 (**C**9), 51.9 (**C**14), 47.3 (**C**13), 40.5, 39.3 (C**H**$_2$N, **C**12), 38.8 (**C**10), 34.9, 33.9, 32.8, 32.8, 32.1 (**C**1, **C**2, **C**6, **C**7, **C**16), 31.6 (**C**8), 28.2 (*t*Bu), 23.9 (**C**15), 20.9 (**C**19), 17.9 (**C**18).



25

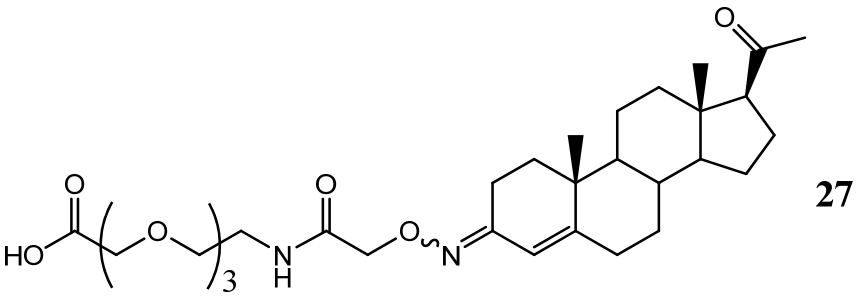
1-{[(11β,16α)-9-Fluoro-3,20-dioxo-11,17-dihydroxy-16-methylpregna-1,4-dien-21-ylidene]aminooxy}-2-oxo-6,9,12-trioxa-3-azatetradecanoic acid tert.-butyl ester **25**: According to the general procedure **A** 904 mg of Dex 21-CMO **15** (1.95 mmol) were coupled to 1.03 of freshly hydrogenated linker **3** (3.9 mmol, 2 eq.; General procedure **D**) using 510 µl DIPEA (2.93 mmol), 395 mg HOBt (2.93 mmol) and 1.11 g HBTU (2.93 mmol). Column chromatography eluting with Tol/acetone 5.5:4.5 yielded 1.17 g of pure title compound **25** (1.65 mmol, 85 %) as light yellow foam. R_f: 0.42 (Tol/acetone 1:1). $[\alpha]_D^{20}$= +79.5 (c=1.0, DMSO). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{36}H_{53}FN_2O_{11}Na$: 731.3526 found: 731.3529. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 7.89 (s, 1H, C21-**H**), 7.21 (d, 1H, J = 10.0 Hz, C1-**H**), 6.85 (t, 1H, J = 5.3 Hz, CON**H**), 6.27 (dd, 1H, J = 10.1 Hz/1.8 Hz, C2-**H**), 6.06 (s, 1H, C4-**H**), 4.68 (s, 2H, NOC**H**$_2$), 4.33-4.25 (m, 1H, C11-**H**), 3.97 (s, 2H, OC**H**$_2$COO*t*Bu), 3.76-3.41 (m, 12H, EG-chain-C**H**$_2$), 3.25 (s, broad, 1H, OH), 3.22-3.11 (m, 1H, C16-**H**), 2.64-2.52 (m, 1H, C6-**H**), 2.41-2.09 (m, 4H, scaffold, C6-**H**, C8-**H**, C12-**H**, C14-**H**), 1.84-1.75 (m, 1H, C7-**H**), 1.72-1.60 (m, 1H, C15-**H**), 1.60-1.47 (m, 2H, C7-**H**, C12-**H**), 1.50 (s, 3H, C**H**$_3$), 1.43 (s, 9H, *t*Bu), 1.28-1.17 (m, 1H, C15-**H**), 0.95 (s, 3H, C**H**$_3$), 0.88 (d, 3H, J = 7.2Hz, C16-C**H**$_3$). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 196.1 (**C**20) 186.7 (**C**3), 169.8 (**C**O), 168.2 (**C**O), 166.6 (**C**5),

152.6 (**C**1), 149.3 (**C**21), 129.7 (**C**2), 125.1 (**C**4), 100.3 (d, J = 175 Hz, **C**9), 92.1 (**C**17), 82.0 (*t*Bu) 74.1 (**C**H_2ON), 72.0 (d, J = 38.3 Hz, **C**11), 70.6, 70.5, 70.4, 69.6, 69.6, 68.9 (EG-**C**H_2), 48.5 (d, J = 22.7 Hz, **C**10), 48.3 (**C**13), 43.7 (**C**14), 38.7 (**C**H_2N), 37.1 (**C**12), 35.1 (**C**16), 34.4 (d, J = 19.4 Hz, **C**8), 32.4, 31.1 (**C**15, **C**6, **C**7), 28.2 (*t*Bu), 27.4 (**C**15, **C**6, **C**7), 23.0 (d, J = 5.5 Hz, **C**19), 17.3 (**C**18), 15.0 (C16-Me).



26

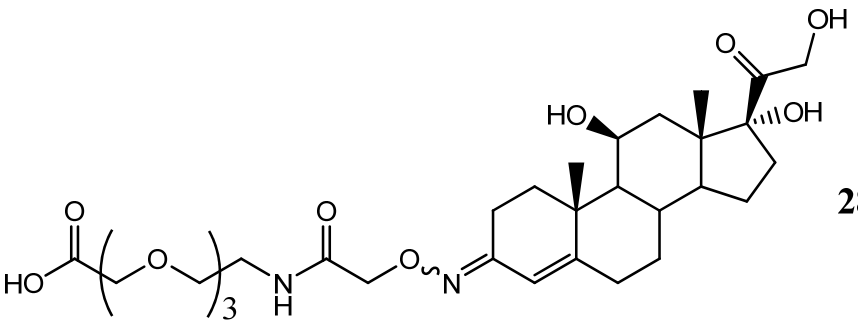
1-{[(17β)-17-Hydroxyandrost-4-en-3-ylidene]aminooxy}-2-oxo-6,9,12-trioxa-3-azatetradecanoic acid **26**: Steroidal free acid **26** from methyl ester **18** according to general procedure **C**. R_f: 0.31 ($CHCl_3$+5%MeOH+TFA). $[\alpha]_D^{20}$= +33.3 (c = 1.0, DMSO). FT-ICR-MS: m/z $[M-H]^-$ calcd for $C_{29}H_{45}N_2O_8N$: 549.3170 found: 549.3168. ^{1}H-NMR from the mixture of isomers (400.1 MHz, MeOH-d_4): δ 6.49, 5.71 (s, s, 1H, C4-**H**), 4.48, 4.46 (s, s, 2H, NOC**H**$_2$CONH), 3.90 (s, 2H, OC**H**$_2$COOH), 3.67-3.44 (m, 13H, EG-chain-C**H**$_2$, C17-**H**), 3.11-3.03, 2.46-2.17 (m, m, 4H, C2-α-**H**, C2-β-**H**, C6-**H**), 2.04-1.77 (m, 4H, scaffold), 1.67-1.24 (m, 7H, scaffold), 1.15, 1.12 (s, s, 3H, C**H**$_3$), 1.14-0.80 (m, 4H, scaffold), 0.78 (s, 3H, C**H**$_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, MeOH-d_4): δ 177.7 (**C**O), 173.6 (**C**O), 173.6 (**C**O), 162.7, 159.8, 158.9, 156.7 (**C**3, **C**5), 117.4, 111.8, (**C**4), 82.3, 82.3 (**C**17), 73.4, 73.3 (**C**H_2ON), 71.6, 71.5, 71.0, 70.7, 70.7, 70.3 (EG-**C**H_2), 55.8, 55.5 (**C**9), 51.9, 51.9 (**C**14), 43.9, 43.9 (**C**13), 40.2 (**C**10), 39.8 (**C**12), 39.2 (**C**10), 37.8, 37.8 (**C**1), 37.4 (**C**2), 37.1, 37.0 (**C**8), 35.8 (**C**2), 34.0, 33.5, 33.4, 33.0, 30.6 (**C**6, **C**7, **C**16), 25.4, 24.3, 22.1, 21.9, 20.5 (**C**11, **C**15), 18.4, 18.2 (**C**19), 11.6 (**C**18).



27

1-[(20-Oxo-pregn-4-en-3-ylidene)aminooxy]-2-oxo-6,9,12-trioxa-3-azatetradecanoic acid **27**: Steroidal carboxylic acid **27** was prepared from methyl ester **19** according to general

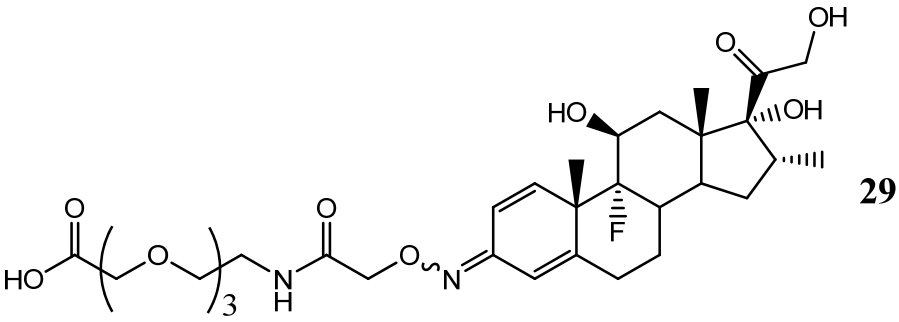
procedure **C**. R_f: 0.31 ($CHCl_3$+5%MeOH+TFA). $[\alpha]_D^{20}$= +90.4 (c=1.0, DMSO). FT-ICR-MS: m/z $[M-H]^-$ calcd for $C_{31}H_{47}N_2O_8$: 575.3327 found: 575.3332. ^{1}H-NMR from the mixture of isomers (400.1 MHz, MeOH-d_4): δ 6.50, 5.72 (s, d, 1H, J = 1.3 Hz, C4-**H**), 4.48, 4.45 (s, s, 2H, NOC**H**$_2$CONH), 3.89 (s, 2H, OC**H**$_2$COOH), 3.68-3.62 (m, 8H, EG-chain-C**H**$_2$), 3.62-3.56 (m, 2H, EG-chain-C**H**$_2$), 3.51-3.46 (m, 2H, EG-chain-C**H**$_2$), 3.13-3.04 (m, 1H, C2-α-**H**), 2.68-2.60 (m, 1H, C17-**H**), 2.48-0.87 (m, 16H, scaffold), 2.12 (s, 3H, C21-C**H**$_3$), 1.15, 1.11 (s, s, 3H, C**H**$_3$), 0.66 (s, 3H, C**H**$_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, MeOH-d_4): δ 212.2, 212.2 (**C**20-CO), 177.6 (**C**O), 173.5 (**C**O), 173.4 (**C**O), 162.3, 159.6, 158.5, 156.6 (**C**3, **C**5), 117.6, 111.9 (**C**4), 73.5, 73.3 (**C**H$_2$ON), 71.5, 71.0, 70.8, 70.7, 70.4 (EG-**C**H$_2$), 64.6, 64.6 (**C**17), 57.4, 57.3 (**C**15), 55.4, 55.1 (**C**9), 45.1, 45.1 (**C**13), 40.1 (**C**10), 39.9, 39.8 (**C**12, **C**16, **C**H$_2$N), 39.1 (**C**10), 37.3 (**C**2), 37.0, 37.0 (**C**8), 35.8 (**C**2), 33.9, 33.8, 33.5, 33.4 (**C**6, **C**7), 31.6 (**C**21), 25.4, 23.7, 22.5, 22.3, 20.5(**C**1, **C**11, **C**15), 18.4, 18.1 (**C**19), 13.7 (**C**18).



28

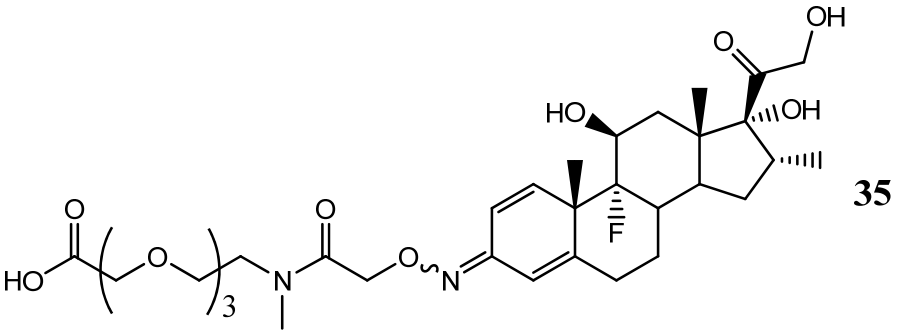
1-{[(11β)-20-Oxo-11,17,21-trihydroxypregn-4-en-3-ylidene]aminooxy}-2-oxo-6,9,12-trioxa-3-azatetradecanoic acid **28**: Steroidal carboxylic acid **28** was prepared from methyl ester **20** according to general procedure **C** in virtually quantitative yield. $[\alpha]_D^{20}$= +83.6 (c=1.0, MeOH). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{31}H_{48}N_2O_{11}Na$: 647.3150 found: 647.3154. HPLC: R_t = 5.504 min. 96.1 %. ^{1}H-NMR *E*-isomer (400.1 MHz, MeOH-d_4): δ 5.66 (s, 1H, C4-**H**), 4.63 (d, 1H, J = -19.2 Hz, C21-**H**), 4.46 (s, 2H, NOC**H**$_2$CONH), 4.40-4.39 (m, 1H, C11-**H**), 4.26 (d, 1H, J = -19.2 Hz, C21-**H**), 4.12 (s, 2H, OC**H**$_2$COOH), 3.73-3.60 (m, 8H, EG-chain-C**H**$_2$), 3.59-3.54 (m, 2H, EG-chain-C**H**$_2$), 3.47-3.42 (m, 2H, EG-chain-C**H**$_2$), 3.07-2.97 (m, 1H, C2-**H**), 2.77-2.66 (m, 1H, C16-**H**), 2.51-2.39 (m, 1H, C16-**H**), 2.38-2.26, 2.23-2.15 (m, 2H, C2-**H**, C6-**H**), 2.12-1.93 (m, 4H, scaffold), 1.84-1.69 (m, 2H, scaffold), 1.67-1.27 (m, 5H, scaffold), 1.36 (s, 3H, C**H**$_3$), 1.12-0.97 (m, 1H, C7-**H**), 0.94-0.84 (m, 1H, C9-**H**), 0.87 (s, 3H, C**H**$_3$). ^{13}C-NMR *E*-isomer (100.6 MHz, MeOH-d_4): δ 213.0 (**C**20), 174.3 (**C**O), 172.7 (**C**O), 160.2, 159.8 (**C**3, **C**5), 115.7 (**C**4), 90.3 (**C**17), 73.4 (**C**H$_2$ON), 71.7, 71.6, 71.6, 71.3, 70.5, 69.3 (EG-**C**H$_2$), 68.8 (**C**11), 67.7 (**C**21), 57.8 (**C**9), 53.6 (**C**14), 48.3 (**C**13), 40.8,

39.9 (**C**12, **C**H_2N), 39.9 (**C**10), 35.1, 34.6, 33.0, 32.8 (**C**1, **C**16, **C**6, **C**7, **C**8), 24.7 (**C**15), 21.9 (**C**19), 20.3 (**C**2), 17.8 (**C**18).



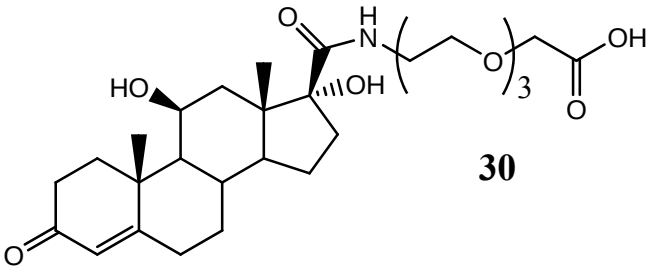
29

1-{[(11β,16α)-9-Fluoro-20-oxo-11,17,21-trihydroxy-16-methylpregna-1,4-dien-3-ylidene]aminooxy}-2-oxo-6,9,12-trioxa-3-azatetradecanoic acid **29**: Steroidal free acid **29** was obtained from methyl ester **21** according to general procedure **C**. Since the free acid **29** decomposes quickly, no reliable NMR- and HPLC-data and values for optical rotation could be obtained. Instead the corresponding stable *n*-butyl amide **36** was fully characterized. It is highly recommended to prepare free acid **29** freshly and use it quickly for the synthesis of follow-up compounds. FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{32}H_{47}N_2O_{11}FNa$: 677.3056 found: 677.3059.



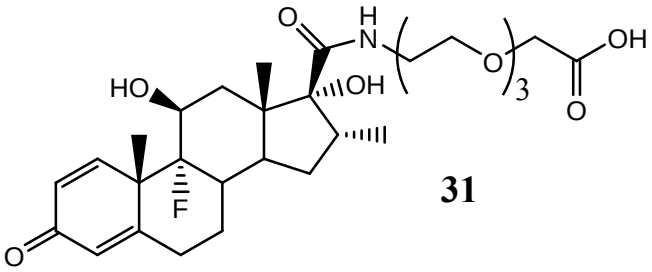
35

1-{[(11β,16α)-9-Fluoro-20-oxo-11,17,21-trihydroxy-16-methylpregna-1,4-dien-3-ylidene]aminooxy}-2-oxo-6,9,12-trioxa-3-aza-3-methyltetradecanoic acid **35**: Free steroidal carboxylic acid **35** was prepared from methyl ester **34** according to general procedure **C**. Since the free acid **35** decomposes quickly no reliable NMR- and HPLC-data and values for optical rotation could be obtained. Instead the corresponding stable *n*-butyl amide **37** was fully characterized. It is highly recommended to prepare free acid **35** freshly and use it quickly for the synthesis of follow-up compounds. FT-ICR-MS: m/z $[M-H]^-$ calcd for $C_{33}H_{48}N_2O_{11}F$: 667.32367 found: 667.32330.



30

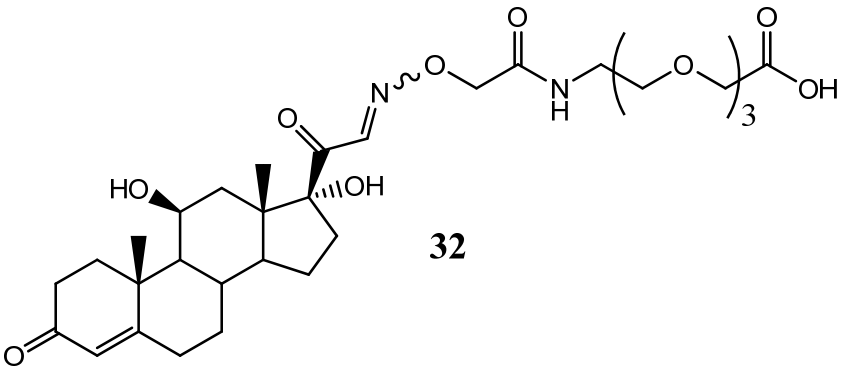
1-[(11β,17α)-3-Oxo-11,17-dihydroxyandrost-4-en-17-carboxamido]-2-oxo-6,9,12-trioxa-3-azatetradecanoic acid **30**: Free steroidal carboxylic acid **30** was prepared according to the general procedure **E** from *tert.*-butyl ester **22** and obtained in quantitative yield. R_f: 0.46 ($CHCl_3$+8%MeOH+1%HCOOH). $[\alpha]_D^{20}$= +68.7 (c=1.0, MeOH). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{28}H_{43}NO_9Na$: 560.2830 found: 560.2827. HPLC: Rt = 4.92 min., 97.7 %. ^{1}H-NMR (400.1 MHz MeOH-d_4): δ 5.66 (s, 1H, C4-**H**), 4.41-4.37 (m, 1H, C11-**H**), 3.91 (d, 1H, J = -15.4 Hz, OC**H**$_2$COO), 3.87 (d, 1H, J = -15.4 Hz, OC**H**$_2$COO), 3.70-3.62 (m, 8H, EG-chain-C**H**$_2$), 3.57-3.54 (t, 2H, J = 5.4 Hz, EG-chain-C**H**$_2$), 3.51-3.43 (m, 1H, EG-chain-C**H**$_2$), 3.42-3.34 (m, 1H, EG-chain-C**H**$_2$), 2.82-2.73 (m, 1H, C16-**H**), 2.62-2.44 (m, 2H, C6-**H**, C2-**H**), 2.36-2.20 (m, 3H, C1-**H**, C2-**H**, C6-**H**), 2.11-1.99 (m, 2H, scaffold), 1.97-1.69 (m, 4H, scaffold), 1.63-1.37 (m, 3H, scaffold), 1.48 (s, 3H, C**H**$_3$), 1.18-1.05 (m, 1H, C7-**H**), 1.04-0.93 (m, 1H, C9-**H**), 0.97 (s, 3H, C**H**$_3$). ^{13}C-NMR (100.6 MHz, MeOH-d_4): 202.5 (**C**3), 177.7 (**C**O), 176.9 (**C**O), 176.7 (**C**O), 122.5 (**C**4), 86.5 (**C**17), 71.6, 71.4, 71.0, 70.9, 70.5 (EG-**C**H$_2$), 68.7 (**C**11), 57.8 (**C**9), 53.0 (**C**14), 48.4 (**C**13), 40.7 (**C**10), 40.0, 39.9 (**C**H$_2$N, **C**12), 35.8 , 34.6, 34.4, 34.0, 33.3 (**C**1, **C**2, **C**6, **C**7, **C**16), 33.1 (**C**8), 24.8 (**C**15), 21.4 (**C**19), 18.1 (**C**18).



31

1-[(11β,16α,17α)-9-Fluoro-3-oxo-11,17-dihydroxy-16-methylandrosta-1,4-dien-17-carboxamido]-2-oxo-6,9,12-trioxa-3-azatetradecanoic acid **31**: Free steroidal carboxylic acid **31** was prepared according to the general procedure **E** from *tert.*-butyl ester **23** and obtained in quantitative yield. R_f: 0.48 ($CHCl_3$+8%MeOH+1%HCOOH). $[\alpha]_D^{20}$= +41.5 (c=1.0, MeOH). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{29}H_{42}FNO_9Na$: 590.2736 found: 590.2738. HPLC: Rt = 5.53 min., 100.0 %. ^{1}H-NMR (400.1 MHz, MeOH-d_4): δ 7.44 (d, 1H, J = 10.1

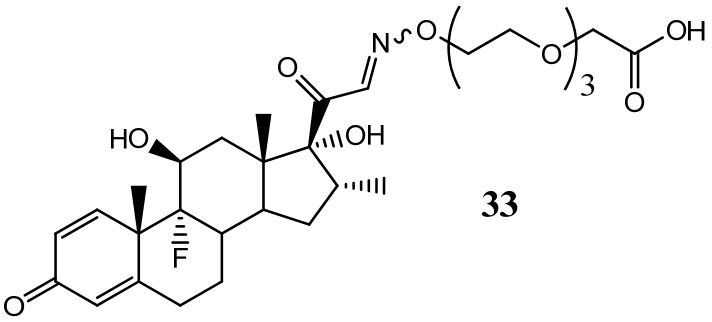
Hz, C1-**H**), 6.29 (dd, 1H, J = 10.1 Hz/1.9 Hz, C2-**H**), 6.08 (s, broad, 1H, C4-**H**), 4.29-4.20 (m, 1H, C11-**H**), 3.90 (s, 2H, OC**H**$_2$COO), 3.71-3.61 (m, 8H, EG-chain-C**H**$_2$), 3.57 (t, 2H, J = 5.4 Hz, EG-chain-C**H**$_2$), 3.53-3.45 (m, 1H, EG-chain-C**H**$_2$), 3.40-3.32 (m, 1H, EG-chain-C**H**$_2$), 3.18-3.07 (m, 1H, C16-**H**), 2.77-2.66 (m, 1H, C6-**H**), 2.53- 2.35 (m, 2H, C6-**H**, C8-**H**), 2.25-2.13 (m, 2H, C12-**H**, C14-**H**), 1.93-1.83 (m, 1H, C7-**H**), 1.81-1.69 (m, 1H, C15-**H**), 1.59 (s, 3H, C**H**$_3$), 1.58-1.45 (m, 2H, C7-**H**, C12-**H**), 1.25-1.16 (m, 1H, C15-**H**), 1.09 (s, 3H, C**H**$_3$), 0.90 (d, 3H, J = 7.2 Hz, C16-C**H**$_3$). ^{13}C-NMR (100.6 MHz, MeOH-d_4): δ 189.1 (**C**3), 175.9 (**C**20), 171.2, 171.2 (**C**3, **C**5), 156.1 (**C**1), 129.8 (**C**2), 125.1 (**C**4), 102.7 (d, J = 176 Hz, **C**9), 88.2 (**C**17), 73.0 (d, J = 37.6 Hz, **C**11), 71.6, 71.4, 71.0, 70.9, 70.9, 70.6 (EG-**C**H$_2$), 50.3 (d, J = 22.7 Hz, **C**10), 49.4 (**C**13), 44.8 (**C**14), 39.9 (**C**H$_2$N), 36.8 (**C**12), 36.2 (**C**16), 35.8 (d, J = 19.4 Hz, **C**8), 33.4 (**C**15), 32.3 (**C**6), 28.7 (**C**7), 23.6 (d, J = 5.7 Hz, **C**19), 17.7 (**C**18), 15.2 (**C**16-Me).



32

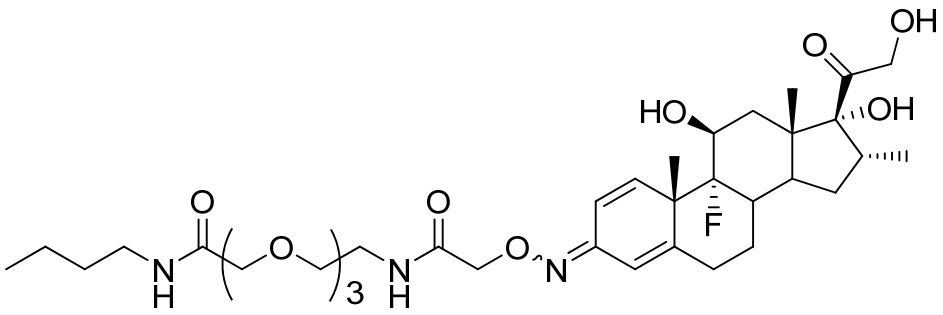
1-{[(11β)-3,20-Dioxo-11,17-dihydroxypregn-4-en-21-ylidene]oxyamino}-2-oxo-6,9,12-trioxa-3-azatetradecanoic acid **32**: Free steroidal carboxylic acid **32** was obtained from *tert.*-butyl ester **24** (397 mg, 0.584 mmol) according to the general procedure **E**. Column chromatography eluting with $CHCl_3$ containing 5.5% MeOH and 1% formic acid yielded 298 mg of pure title compound **32** (0.479 mmol, 82%) as light yellow foam. R_f: 0.35 ($CHCl_3$+6%MeOH+1%HCOOH). $[\alpha]_D^{20}$= +145.9 (c=1.0, MeOH). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{31}H_{46}N_2O_{11}Na$: 645.2994 found: 645.2999. HPLC: Rt = 5.74 min., 96.7%. ^{1}H-NMR (400.1 MHz, $CDCl_3$): 7.84 (s, 1H, C21-**H**), 7.08 (m, 1H, CON**H**), 5.61 (s, 1H, C4-**H**), 4.81-4.70 (m, 2H, NOC**H**$_2$), 4.42 (s, 1H, C11-**H**), 4.14 (s, 2H, OC**H**$_2$COO*t*Bu), 3.78-3.43 (m, 12H, EG-chain-C**H**$_2$), 2.85-2.75 (m, 1H, C16-**H**), 2.53-2.40 (m, 2H, C2-**H**/C6-**H**), 2.39-2.30 (m, 1H, scaffold), 2.27-2.12 (m, 2H, scaffold), 2.08-1.95 (m, 2H, scaffold), 1.91-1.59 (m, 5H, scaffold), 1.59-1.48 (m, 1H, C16-**H**), 1.45-1.28 (m, 1H, C15-**H**), 1.42 (s, 3H, C**H**$_3$), 1.17-1.03 (m, 1H, C7-**H**), 1.03-0.95 (m, 1H, C9-**H**), 0.86 (s, 3H, C**H**$_3$). ^{13}C-NMR (100.6 MHz, $CDCl_3$): 200.1 (**C**3), 196.2 (**C**20), 172.9, 172.3, 168.7 (**C**3, **C**5), 149.4 (**C**21), 122.3 (**C**4), 91.7 (**C**17),

74.2 (**C**H_2ON), 71.3, 70.5, 70.2, 69.8, 69.8, 68.8 (EG-**C**H_2), 68.3 (**C**11), 55.9 (**C**9), 52.0 (**C**14), 47.3 (**C**13), 40.5, 39.3 (C**H**$_2$N, **C**12), 39.1 (**C**10), 34.9, 33.9, 32.8, 32.8, 32.2 (**C**1, **C**2, **C**6, **C**7, **C**16), 31.6 (**C**8), 23.9 (**C**15), 21.0 (**C**19), 17.8 (**C**18).



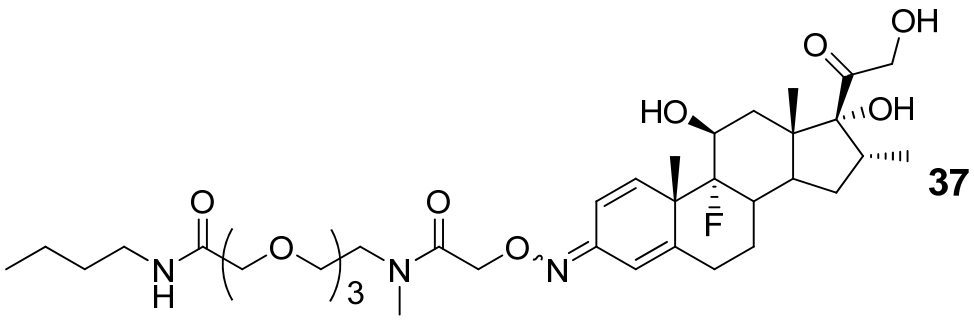
33

1-{[(11β,16α)-9-Fluoro-3,20-dioxo-11,17-dihydroxy-16-methylpregna-1,4-dien-21-ylidene]oxyamino}-2-oxo-6,9,12-trioxa-3-azatetradecanoic acid **33**: Free steroidal carboxylic acid **33** was obtained from *tert.*-butyl ester **25** (226 mg, 0.319 mmol) according to the general procedure **E**. Column chromatography eluting with $CHCl_3$ containing 5.5% MeOH and 1% formic acid afforded 179 mg of pure title compound **33** (0.274 mmol, 86 %) as light yellow foam. R_f: 0.37 ($CHCl_3$+6%MeOH+1%HCOOH). $[\alpha]_D^{20}$= +95.0 (c=1.0, MeOH). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{32}H_{45}FN_2O_{11}Na$: 675.2900 found: 675.2901. HPLC: 6.40 min., 98.1%. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 7.87 (s, 1H, C21-**H**), 7.27 (d, 1H, J = 10.0 Hz, C1-**H**), 7.21-7.17 (m, 1H, CON**H**), 6.30 (d, 1H, J = 10.0 Hz, C2-**H**), 6.09 (s, 1H, C4-**H**), 4.77 (d, 1H, J = -15.6 Hz, NOC**H**$_2$), 4.72 (d, 1H, J = -15.6 Hz, NOC**H**$_2$), 4.29 (d, broad, 1H, J = 10.2 Hz, C11-**H**), 4.13 (s, 2H, OC**H**$_2$COO), 3.76-3.43 (m, 12H, EG-chain-C**H**$_2$), 3.22-3.10 (m, 1H, C16-**H**), 2.66-2.53 (m, 1H, C6-**H**), 2.43- 2.08 (m, 4H, C6-**H**, C8-**H**, C12-**H**, C14-**H**), 1.86-1.77 (m, 1H, C7-**H**), 1.75-1.60 (m, 1H, C15-**H**), 1.60-1.45 (m, 2H, C7-**H**, C12-**H**), 1.52 (s, 3H, C**H**$_3$), 1.28-1.17 (m, 1H, C15-**H**), 0.95 (s, 3H, C**H**$_3$), 0.88 (d, 3H, J = 7.2Hz, C16-C**H**$_3$). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 196.2 (**C**20) 187.2 (**C**3), 172.6 (**C**O), 168.9 (**C**O), 167.5 (**C**5), 153.4 (**C**1), 149.2 (**C**21), 129.5 (**C**2), 125.0 (**C**4), 100.6 (d, J = 175 Hz, **C**9), 92.2 (**C**17), 74.0 (**C**H_2ON), 72.0 (d, J = 38.3 Hz, **C**11), 71.1, 70.4, 70.3, 69.8, 69.7 (EG-**C**H_2), 48.6 (d, J = 22.7 Hz, **C**10), 48.3 (**C**13), 43.8 (**C**14), 39.1 (**C**H_2N), 37.1 (**C**12), 35.1 (**C**16), 34.4 (d, J = 19.4 Hz, **C**8), 32.4, 31.2, 27.5 (**C**15, **C**6, **C**7), 23.0 (d, J = 5.5 Hz, **C**19), 17.2 (**C**18), 15.1 (C16-Me).

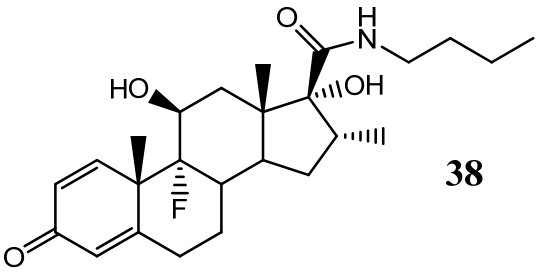


36

1-{[(11β,16α)-9-Fluoro-20-oxo-11,17,21-trihydroxy-16-methylpregna-1,4-dien-3-ylidene]aminooxy}-2-oxo-6,9,12-trioxa-3-azatetradecanoic acid n-butyl-amide **36:** According to the general procedure **A** 98 mg of free acid **29** (0.15 mmol) were coupled to 45 µl *n*-butyl amine (0.45 mmol, 3 eq.) using 31 mg HOBt (0.23 mmol), 40 µl DIPEA (0.23 mmol) and 87 mg HBTU (0.23 mmol). Purification via column chromatography eluting with $CHCl_3$ containing 6 % MeOH yielded 58 mg of pure title compound **36** (81.8 µmol, 55%) as colorless foam. R_f: 0.29 ($CHCl_3$+6%MeOH). $[\alpha]_D^{20}$= +57.8 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{36}H_{56}FN_3O_{10}Na$: 732.3842 found: 732.3847. ^{1}H-NMR from mixture of isomers (400.1 MHz, $CDCl_3$): δ 6.98 (t, broad, 1H, J = 5.4 Hz, CON**H**), 6.90, 6.22 (dd, dd, 1H, J = 10.3 Hz/1.6 Hz//10.1 Hz/1.6 Hz, C2-**H**), 6.78-6.72 (m, 1H, CON**H**), 6.64, 5.95 (s, s, broad, 1H, C4-**H**), 6.59, 6.43 (d, d, 1H, J = 10.3 Hz/10.2 Hz, C1-**H**), 4.59 (d, 1H, J = -19.8 Hz, C21-**H**), 4.50 (s, 2H, NOC**H**$_2$), 4.31-4.25 (m, 1H, C11-**H**), 4.23 (d, 1H, J = -19.8 Hz, C21-**H**), 3.94, 3.94 (s, s, 2H, OC**H**$_2$CON*n*Bu), 3.65-3.57 (m, 8H, EG-chain-C**H**$_2$), 3.57-3.39 (m, 4H, EG-chain-C**H**$_2$), 3.28-3.20 (m, 2H, NHC**H**$_2$), 3.13-2.89 (m, 4H, C16-**H**, O**H**), 2.65-2.47 (m, 1H, **C**6-H), 2.41- 2.09 (m, 4H, C6-**H**, C8-**H**, C12-**H**, C14-**H**), 1.80-1.65 (m, 2H, C7-**H**, C15-**H**), 1.53-1.16 (m, 7H, C7-**H**, C12-**H**, C15-**H**, *n*Bu-C**H**$_2$), 1.46 (s, 3H, C**H**$_3$), 0.98 (s, 3H, C**H**$_3$), 0.93-0.87 (m, 6H, C16-C**H**$_3$, *n*Bu-C**H**$_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 212.3 (**C**20), 170.3, 170.3 (**C**O), 157.3, 152.2, 151.1, 150.9 (**C**3, **C**5), 144.2, 139.8 (**C**1), 122.9, 116.8, 116.1, 110.7 (**C**2, **C**4), 100.3, 100.1 (d, d, J = 174 Hz, **C**9), 90.5 (**C**17), 73.0 (**C**H$_2$ON), 71.3 (**C**11), 71.0 (EG-**C**H$_2$), 71.0 (**C**11), 71.0, 70.6, 70.5, 70.3, 69.9, 67.8 (EG-**C**H$_2$, **C**21), 48.8 (**C**13), 48.1, 48.0 (d, d, J = 22.9 Hz/22.8 Hz, **C**10), 44.2 (**C**14), 38.8, 38.8 (**C**H$_2$N), 36.7 (**C**12), 36.4, 36.3 (**C**16), 34.4, 34.3 (q, J = 19.7 Hz/19.4 Hz, C**8**), 32.4, 31.6, 31.3, 30.7 (**C**15, **C**6, *n*Bu-**C**H$_2$), 27.5 (**C**7), 24.0, 24.0 (d, d, J = 29.5 Hz/29.2 Hz, **C**19), 20.2 (*n*Bu-**C**H$_2$), 17.1 (**C**18), 14.9 (**C**16-Me), 13.9 (*n*Bu-**C**H$_3$).



1-{[(11β,16α)-9-Fluoro-20-oxo-11,17,21-trihydroxy-16-methylpregna-1,4-dien-3-ylidene]aminooxy}-2-oxo-6,9,12-trioxa-3-aza-3-methyltetradecanoic acid n-butyl-amide **37:** According to the general procedure **A** 49 mg of free acid **35** (73 µmol) were coupled with 22 µl *n*-butyl amine (0.22 mmol, 3 eq.) using 15 mg HOBt (0.11 mmol), 19 µl DIPEA (0.11 mmol) and 42 mg HBTU (0.23 mmol). Purification via column chromatography eluting with $CHCl_3$ containing 7 % MeOH yielded 27 mg of pure title compound **26** (37.3 µmol, 51%) as colorless foam. R_f: 0.33 ($CHCl_3$+6%MeOH). $[\alpha]_D^{20}$= +47.3 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{37}H_{58}FN_3O_{10}Na$: 746.3998 found: 746.3991. ^{1}H-NMR from mixture of isomers (400.1 MHz, $CDCl_3$): δ 7.04-6.90, 6.27-6.21 (m, m, 2H, C2-**H**, CON**H**), 6.71, 5.96 (s, s, 1H, C4-**H**), 6.55, 6.53, 6.38, 6.36 (d, d, d, d, 1H, J = 10.3 Hz/10.3 Hz//10.1 Hz/ 10.2 Hz, C1-**H**), 4.85-4.74, 4.71 (m/s, 2H, NOC**H**$_2$), 4.60 (d, 1H, J = -19.9 Hz, C21-**H**), 4.33-4.25 (m, 1H, C11-**H**), 4.24 (d, 1H, J = -19.9 Hz, C21-**H**), 3.96 (s, 2H, OC**H**$_2$CON*n*Bu), 3.67-3.47 (m, 12H, EG-chain-C**H**$_2$), 3.11-2.99 (m, 1H, C16-**H**), 3.29-3.22 (m, 2H, NHC**H**$_2$), 3.06, 3.05, 2.96, 2.94 (s, s, s, s, 3H, NMe), 2.87 (s, broad, 2H, O**H**), 2.61-2.44 (m, 1H, C6-**H**), 2.40-2.09 (m, 4H, scaffold), 1.78-1.66 (m, 2H, scaffold), 1.54-1.17 (m, 10H, scaffold, *n*Bu-C**H**$_2$/C**H**$_3$), 0.99 (s, 3H, C**H**$_3$), 0.94-0.87 (m, 6H, C16-C**H**$_3$/*n*Bu-C**H**$_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 212.4 (**C**20), 170.2 (**C**O), 170.1 (**C**O), 169.7, 169.7 (**C**O), 169.1, 169.1 (**C**O), 156.0, 155.8, 151.4, 151.3, 150.4, 150.4, 150.3, 150.2 (**C**3, **C**5), 143.3, 143.2 (**C**1), 139.0, 138.9 (**C**1), 123.4, 123.4, 117.2, 117.1, 116.7, 116.6, 111.4, 111.3 (**C**2, **C**4), 100.1, 100.0 (d, d, J = 175 Hz, **C**9), 90.6, 90.5, 90.5, 90.5 (**C**17), 72.3, 72.1, 72.0, 72.0, 71.3, 71.1, 71.1, 71.0, 70.7, 70.6, 70.5, 70.4, 70.3, 69.2, 68.8, 68.7, 67.9 (EG-**C**H$_2$, **C**H$_2$ON, **C**11, **C**21), 49.0, 48.8 (**C**13), 48.2, 48.2, 48.0, 47.7 (**C**10), 44.3 (**C**14), 38.8 (**C**H$_2$N), 36.7, 36.5, 36.4, 36.3, 36.2, 36.0, 34.4, 34.3, 34.3, 34.0 (**C**12, **C**16, **C**8, N**C**H$_3$), 32.5, 31.7, 31.2, 30.7 (**C**15, **C**6, *n*Bu**C**H$_2$), 27.7, 27.6 (**C**7), 24.1, 24.1, 24.1, 23.9, 23.8, 23.8 (**C**19), 20.2 (*n*Bu**C**H$_2$), 17.3, 17.2 (**C**18), 15.0 (**C**16-Me), 13.9 (*n*Bu**C**H$_3$).



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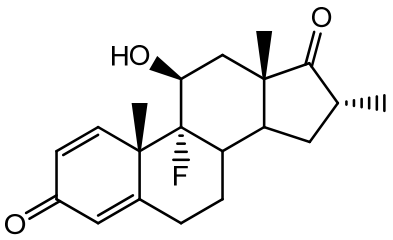
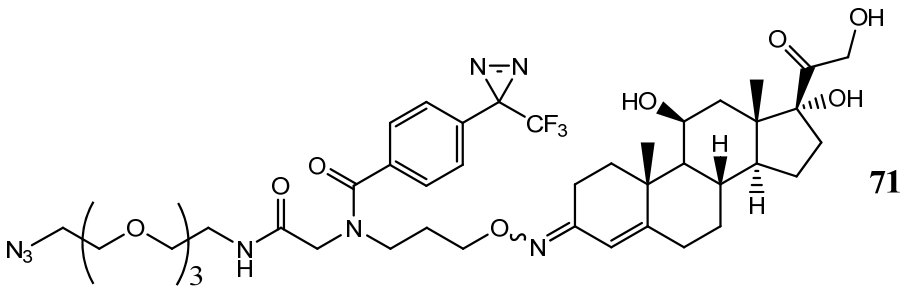


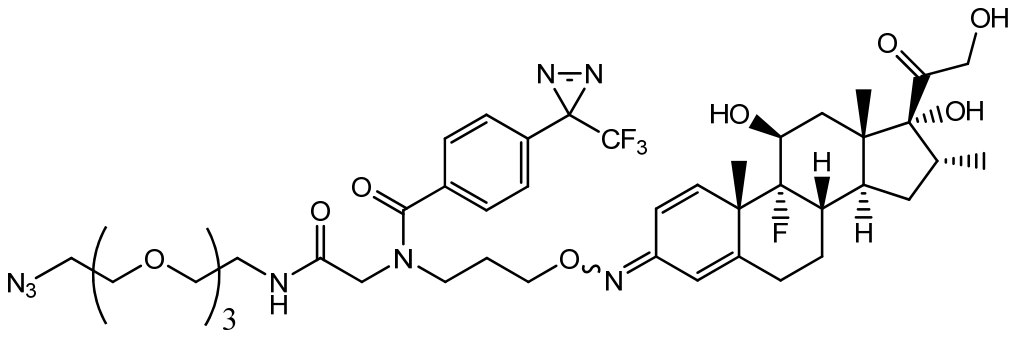
39

(11β,16α,17α)-9-Fluoro-3-oxo-11,17-dihydroxy-16-methylandrosta-1,4-dien-17-carboxylic acid n-butyl amide **38** and (11β,16α)-9-Fluoro-3,17-dioxo-11-hydroxy-16-methylandrosta-1,4-dien **39**: In a 5 ml Schlenk-tube equipped with a stirring bar 50 mg of oxime **25** (76.6 µmol) were dissolved in 1.5 ml THF/*n*-butylamine 2:1 *v/v* under an atmosphere of nitrogen and the solution stirred over night at RT. Then the volatiles were removed under reduced pressure and the residue purified by column chromatography eluting with PE/EA 8.5:1.5-7:3. The first fraction yielded 7 mg of diketone **39** [165] (21.1 µmol), the second fraction 22 mg of amide **38** (50.7 µmol) as colorless foam respectively. Analytical data for **39** was in good accordance to the literature.[165] Analytical data for **38**: R_f: 0.32 (PE/EA 7:3). $[\alpha]_D^{20}$= +38.7 (c=1.0, DMSO). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{25}H_{37}FNO_4Na$: 456.2521 found: 456.2524. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 7.23 (d, 1H, J = 10 Hz, C1-**H**), 6.58 (t, 1H, J = 5.7 Hz, CON**H**), 6.27 (dd, 1H, J = 10.1 Hz/1.7 Hz, C2-**H**), 6.1 (s, 1H, C4-**H**), 4.38-4.29 (m, 1H, **C**11), 3.34-3.10 (m, 3H, C16-**H**, C**H**$_2$N), 2.67-2.55 (m, 1H, C6-**H**), 2.47-2.23 (m, 3H, scaffold), 2.02-2.05 (m, 2H, scaffold), 1.88-1.69 (m, 2H, C7-**H**, C15-**H**), 1.61-1.43 (m, 3H, scaffold, *n*Bu-C**H**$_2$), 1.54 (s, 3H, C19-C**H**$_3$), 1.43-1.28 (m, 3H, scaffold, *n*Bu-C**H**$_2$), 1.27-1.18 (m, 1H, C15-**H**), 1.12 (s, 3H, C**H**$_3$), 0.96-0.90 (m, 3H, *n*Bu-C**H**$_3$), 0.93 (d, 3H, J = 7.4 Hz, C16-Me). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 186.8 (**C**3), 172.6 (**C**O), 166.6 (**C**5), 152.5 (**C**1), 129.9 (**C**2), 125.2 (**C**4), 100.7 (d, J = 176.3 Hz, **C**9), 86.9 (**C**17), 72.3 (d, J = 38.8 Hz, **C**11), 48.5 (d, J = 22.7 Hz, **C**10), 48.1 (**C**13), 43.9 (**C**14), 39.3 (C**H**$_2$N), 36.6 (**C**12), 35.4 (**C**16), 34.5 (d, J = 19.5 Hz, **C**8), 32.4, 32.0, 31.2 (**C**15, **C**6, *n*Bu-C**H**$_2$), 27.5 (**C**7), 23.1 (d, J = 5.7 Hz, **C**19), 20.3 (*n*Bu-C**H**$_2$), 17.5 (**C**18), 14.6 (C16-Me), 13.9 (*n*Bu-C**H**$_3$).



71

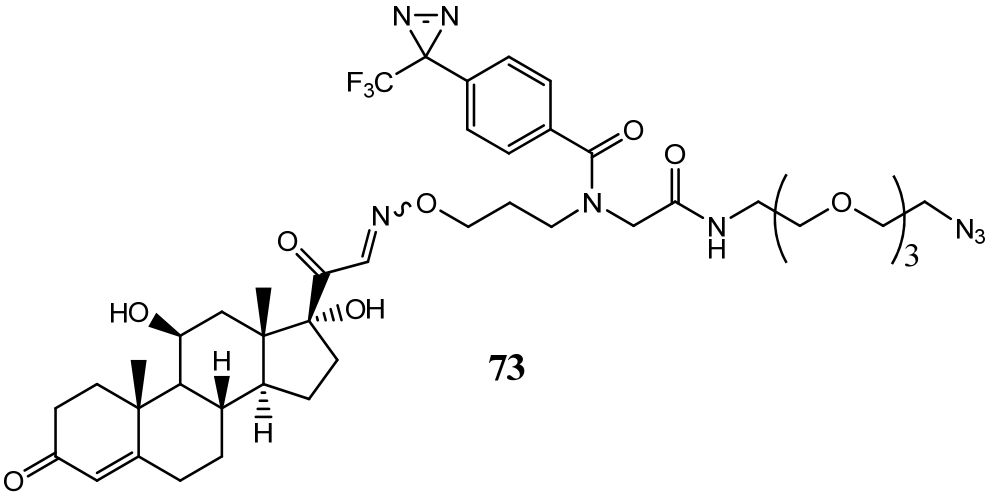
{18-[((11β)-20-Oxo-11,17,21-trihydroxypregn-4-en-3-ylidene)aminooxy]-1-azido-13-oxo-3,6,9-trioxa-12,15-diazaoctadec-15-yl}-4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzamide **71**: According to the general procedure **F** 200 mg of protected oxy-amino ether **59** (0.303 mmol) were deprotected and then condensed with 220 mg HC (0.606 mmol) following the general procedure **G**. Column chromatography eluting with Tol/acetone 3:2 yielded 186 mg of pure title compound **71** (68 %, 0.206 mmol) as colorless foam. R_f: 0.42 ($CHCl_3$+4%MeOH). $[\alpha]_D^{20}$= +85.4 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{43}H_{59}F_3N_8O_{10}Na$: 927.4198 found: 927.4191. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 7.45 (d, 2H, J = 8.2 Hz, aryl), 7.21-7.12 (m, 2H, aryl), 6.91-6.83, 6.69 (m, s, broad, 1H, CON**H**), 5.66, 5.60 (s, s, broad, 1H, CON**H**), 4.60 (d, 1H, J = -19.6 Hz, C21-**H**), 4.43-4.34 (m, 1H, C11-**H**), 4.22 (d, 1H, J = -19.6 Hz, C21-**H**), 4.14- 4.02 (m, 2H, NC**H**$_2$CON), 3.92-3.77 (m, 2H, C**H**$_2$ON), 3.68-3.31 (m, 18H, EG-C**H**$_2$, NC**H**$_2$), 3.08 (s, broad, C11-O**H**), 2.91-2.80, 2.59-2.47 (m, m, 1H, C2-**H**), 2.71-2.59 (m, 1H, C16-**H**), 2.42-2.30 (m, 1H, C2-**H**), 2.20-2.08 (m, 1H, C6-**H**), 2.05-1.82 (m, 7H, scaffold, C**H**$_2$), 1.82-1.72 (m, 1H, scaffold), 1.71-1.61 (m, 1H, scaffold), 1.55-1.31 (m, 6H, C12-**H**, C16-**H**, scaffold, C15-**H**), 1.28, 1.23 (s, s, 3H, C**H**$_3$), 1.07-0.93 (m, 1H, C7-**H**), 0.92-0.82 (m, 1H, C9-**H**), 0.87 (s, 3H, **C**H$_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 212.4 (**C**20), 171.5, 168.7 (**C**O), 156.9, 156.5 (**C**3, **C**5), 137.0, 130.6, 127.5, 126.5 (aryl), 122.0 (q, **C**F_3, J = 275.2 Hz,), 115.5 (**C**4), 88.9 (**C**17), 70.7, 70.6, 70.6, 70.3, 70.0, 69.7 (EG-**C**H_2), 68.4 (**C**11), 67.2 (**C**21), 56.2 (**C**9), 52.1 (**C**14), 50.7 (**C**H_2N_3), 49.3, 48.1 (**C**H_2N), 47.7 (**C**13), 39.8, 39.4 (**C**H_2N, **C**12), 38.6 (**C**10), 34.2, 33.1, 31.7 (**C**16, **C**6, **C**7), 31.6 (**C**8), 28.4 (q, **C**N_2CF_3, J = 40.1 Hz), 28.3, 28.0 (**C**2), 23.9 (**C**15), 21.4 (**C**19), 19.1 (**C**2), 17.6 (**C**18).



72

{18-[((11β,16α)-9-Fluoro-20-oxo-11,17,21-trihydroxy-16-methylpregna-1,4-dien-3-ylidene)aminooxy]-1-azido-13-oxo-3,6,9-trioxa-12,15-diazaoctadec-15-yl}-4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzamide **72**: According to the general procedure **F** 200 mg of protected oxy-amino ether **59** (0.303 mmol) were deprotected and then condensed with 238 mg dexamethasone (0.606 mmol) following the general procedure **G**. Column

chromatography eluting with Tol/acetone 6.25:3.75 yielded 201 mg of pure title compound **72** (71%, 0.215 mmol) as colorless foam. R_f: 0.42 ($CHCl_3$+4%MeOH). $[\alpha]_D^{20}$= +46.8 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{44}H_{58}F_4N_8O_{10}Na$: 957.4104 found: 957.4106. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 7.45 (d, 2H, J = 7.8 Hz, aryl), 7.21-7.10 (m, 2H, aryl), 7.09-7.00, 6.89-6.81, 6.59-6.49, 6.25-6.13 (m, 2H, **C**2-H, **C**ONH), 6.51, 6.36, 5.95, 5.90 (s, s, s, s, 1H, **C**4-H), 6.43, 6.29 (d, d, 1H, J = 10.4 Hz/10.1 Hz, C1-H), 4.55 (d, 1H, J = -19.7 Hz, C21-**H**), 4.32-4.15 (m, 1H, C11-**H**), 4.20 (d, 1H, J = -19.7 Hz, C21-**H**), 4.15-4.01, 3.97-3.87 (m, 2H, **C**H_2ON), 4.08, 3.81 (s, s, 2H, N**C**H_2CONH), 3.66-3.30 (m, 18H, EG-C**H**$_2$, C**H**$_2$N), 3.07-2.42 (m, 5H, C16-**H**, C6-**H**, O**H**), 2.36-2.07 (m, 4H, C6-**H**, C8-**H**, C12-**H**, C14-**H**), 2.06-1.81 (m, 2H, C**H**$_2$), 1.77-1.62 (m, 2H, C7-**H**, C15-**H**), 1.51-1.29 (m, 2H, C7-**H**, C12-**H**), 1.42 (s, 3H, C**H**$_3$), 1.27-1.12 (m, 1H, C15-**H**), 0.93 (s, 3H, C**H**$_3$), 0.84 (d, 3H, J = 7.2 Hz, C16-C**H**$_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 212.1 (**C**20), 171.6, 171.4, 168.7, 168.3 (**C**O), 155.9, 155.8, 150.8 (**C**3, **C**5), 149.5, 149.5 (aryl), 142.9, 138.6 (**C**1), 137.3, 137.0, 130.5, 127.4, 126.6 (aryl), 122.0 (q, **C**F_3, J = 275.1 Hz), 117.2, 116.3, 110.9 (**C**2, **C**4), 100.2, 100.1 (d, d, J = 171.2, 173.2 Hz, **C**9), 90.4 (**C**17), 71.2, 70.8 (**C**11), 70.6, 70.4, 70.2, 69.9, 69.7 (EG-**C**H_2), 67.6 (**C**21), 52.6 (**C**H_2N), 50.7 (**C**H_2N_3), 49.0 (**C**H_2N), 48.6 (**C**13), 48.0 (**C**H_2N), 47.8, 47.7 (d, d, J = 22.9 Hz, 23.1 Hz, **C**10), 44.1 (**C**14), 39.4 (**C**H_2N), 36.5, 36.1 (**C**12, **C**16), 34.4, 34.2 (**C**8), 32.4, 31.1, 30.6, 29.7, 28.4 (q, J = 44.4 Hz, **C**N_2CF_3), 27.8, 27.5, 26.6 (**C**15, **C**6, **C**7, **C**H_2), 24.0 (d, 28 Hz, **C**19), 17.1 (**C**18), 14.9 (**C**16-Me).

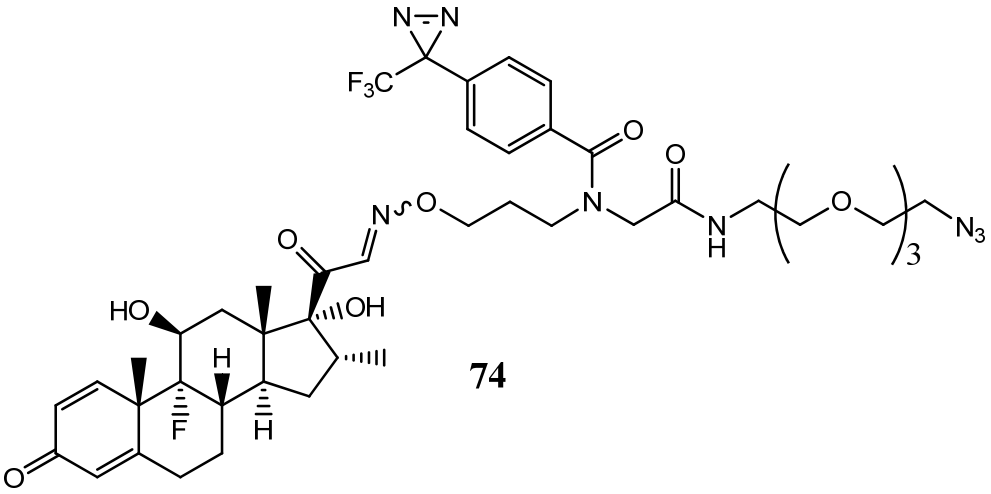


73

{18-[((11β)-3,20-Dioxo-11,17-dihydroxypregn-4-en-21-ylidene)aminooxy]-1-azido-13-oxo-3,6,9-trioxa-12,15-diazaoctadec-15-yl}-4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzamide

73: Previous to oxime ligation, 200 mg *N*-Boc-protected oxy-amino ether **59** (0,303 mmol) were deprotected according to general procedure **F** and then condensed with 220 mg HC

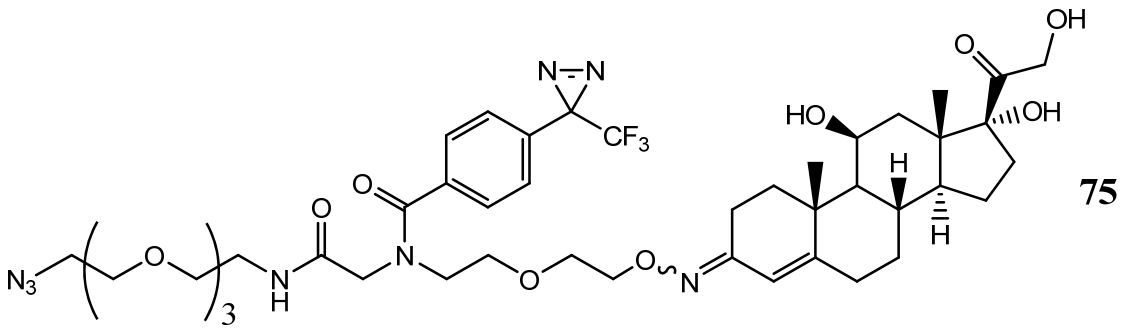
(0.606) following the general procedure **B**. Column chromatography eluting with Tol/acetone 7:3 yielded 118 mg of pure title compound **73** (43 %, 0.130 mmol) as light yellow foam. R_f: 0.43 (Tol/acetone 3:2). $[\alpha]_D^{20}$= +105.0 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{43}H_{57}F_3N_8O_{10}Na$: 925.4042 found: 925.4042. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 7.68, 7.56 (s, s, 1H, C21-**H**), 7.50-7.39 (m, 2H, aryl), 7.25-7.12 (m, 2H, aryl), 6.95-6.83, 6.69-6.59 (m, 1H, CON**H**), 5.63 (s, 1H, C4-**H**), 4.46-3.74 (m, 6H, C11-**H**, NC**H**$_2$CON, C**H**$_2$ON, O**H**), 3.68-3.28 (m, 18H, EG-**C**H_2), 2.87-2.72 (m, 1H, C16-**H**), 2.60-2.09 (m, 6H, C2-**H**, C6-**H**, scaffold), 2.07-1.57 (m, 9H, scaffold, C**H**$_2$), 1.56-1.20 (m, 5H, scaffold, C**H**$_3$), 1.16-0.89 (m, 2H, C7-**H**, C9-**H**), 0.88-0.79 (m, 3H, C**H**$_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 199.7 (**C**3), 196.0 (**C**21), 172.7, 172.6 (**C**O), 171.4 (**C**5), 168.7, 167.9 (**C**O), 148.2, 147.9 (**C**21), 136.9, 131.0, 127.5, 127.3, 126.7 (aryl), 122.2 (**C**4), 121.9 (q, J = 274.7 Hz, **C**F_3), 91.9, 91.5 (**C**17), 73.6, 72.7 (C**H**$_2$ON), 70.6, 70.4, 70.3, 70.2, 69.9, 69.4 (EG-**C**H_2), 67.9 (**C**11), 55.8 (**C**9), 53.1 (N**C**H_2), 52.0 (**C**14), 50.7 (**C**H_2N_3), 49.7, 47.7 (N**C**H_2), 47.3, 47.1 (**C**13) 44.7 (C**H**$_2$N), 40.6, 39.5 (**C**H_2N, **C**12), 39.3 (**C**10), 34.8, 33.9, 32.9, 32.6, 32.2 (**C**1, **C**2, **C**6, **C**7, **C**16), 31.7, 31.5 (**C**8), 28.3 (q, J = 40.8 Hz, **C**N_2CF_3), 28.3, 27.1 (**C**H_2), 24.0 (**C**15), 20.8 (**C**19), 17.7 (**C**18).



74

{18-[((11β,16α)-9-Fluoro-3,20-dioxo-11,17-dihydroxy-16-methylpregna-1,4-dien-21-ylidene)aminooxy]-1-azido-13-oxo-3,6,9-trioxa-12,15-diazaoctadec-15-yl}-4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzamide **74**: Previous to oxime-ligation, 200 mg *N*-Boc-protected oxy-amino ether **59** (0,303 mmol) were deprotected according to general procedure **F** and then condensed with 238 mg Dex (0.606) following the general procedure **B**. Column chromatography eluting with Tol/acetone 7:3 yielded 195 mg of pure title compound **74** (69 %, 0.209 mmol) as light yellow foam. R_f: 0.43 (Tol/acetone 3:2). $[\alpha]_D^{20}$= +107.6 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{44}H_{56}F_4N_8O_{10}Na$: 955.3948 found: 955.3939.

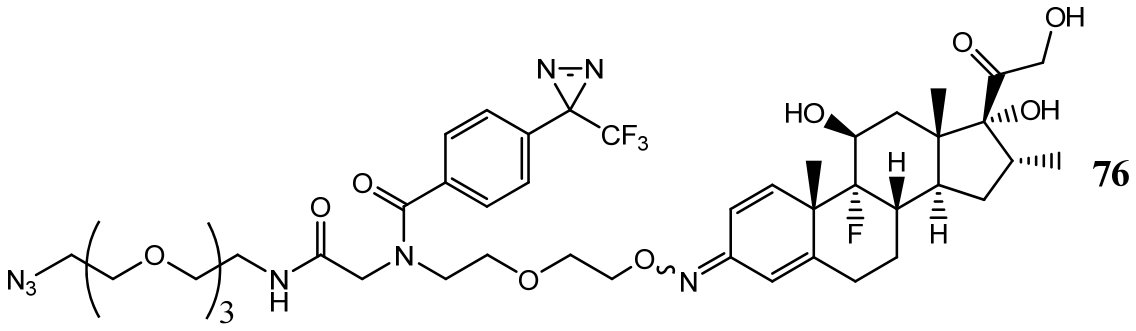
^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 7.73, 7.60 (s, s, 1H, C21-**H**), 7.52-7.37 (m, 2H, aryl), 7.28-7.14/6.97 (m, d, 4H, J = 10.0 Hz, aryl, C1-**H**, **C**OH), 6.25, 6.20 (d, d, 1H, 10.0 Hz, **C**2-H), 6.05 (s, 1H, **C**4-H), 4.40-3.69 (m, 5H, C**H**$_2$ON, C11-**H**, C**H**$_2$), 4.07, 3.84 (s, s, 2H, NC**H**$_2$CON), 3.67-3.29 (m, 18H, EG-C**H**$_2$, C**H**$_2$N), 3.22-3.10 (m, 1H, C16-**H**), 2.79-2.50 (m, 2H, C6-**H**, O**H**), 2.41-2.05 (m, 4H, C6-**H**, C8-**H**, C12-**H**, C14-**H**), 2.03-1.39 (m, 6H, C**H**$_2$, C7-**H**, C15-**H**, C7-**H**, C12-**H**), 1.51, 1.43 (s, s, 3H, C**H**$_3$), 1.27-1.16 (m, 1H, C15-**H**), 0.94, 0.91 (s, s, 3H, C**H**$_3$), 0.86 (d, 3H, J = 7.2 Hz, C16-**C**H$_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 195.9 (**C**20), 186.8 (**C**3), 171.5, 169.0, 168.0 (**C**O), 167.0 (**C**5), 152.8 (**C**1), 148.2, 147.8 (**C**21), 136.8, 130.9 (aryl), 129.5 (**C**2), 127.4, 127.2, 126.7, 126.0 (aryl), 124.9 (**C**4), 121.9 (q, J = 275.1 Hz, **C**F$_3$), 100.6 (d, J = 174.8 Hz, **C**9), 92.2 (**C**17), 73.5, 72.8 (**C**H$_2$ON), 72.0, 71.8 (d, d, J = 38.5 Hz/38.0 Hz, **C**11), 70.5, 70.4, 70.3, 70.1, 69.9, 69.8, 69.5 (EG-**C**H$_2$), 52.9 (**C**H$_2$N), 50.7 (**C**H$_2$N$_3$), 49.3 (**C**H$_2$N), 48.5 (d, J = 22.7 Hz, **C**10), 48.2, 48.1 (**C**13), 47.8, 44.5 (**C**H$_2$N), 43.7 (**C**14), 39.5 (**C**H$_2$N), 37.2 (**C**12), 35.0, 34.7 (**C**16), 34.5 (d, J = 20.7 Hz, **C**8), 32.4, 31.1 (**C**15, **C**6, **C**7), 28.3 (q, J = 40.6 Hz, **C**N$_2$CF$_3$), 28.2 (**C**H$_2$), 27.4, 27.0 (**C**15, **C**6, **C**7), 22.9 (**C**19), 17.0 (**C**18), 15.0 (C16-Me).



{20-[((11β)-20-Oxo-11,17,21-trihydroxypregn-4-en-3-ylidene)aminooxy]-1-azido-13-oxo-3,6,9,18-tetraoxa-12,15-diazaicos-15-yl}-4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzamide

75: According to the general procedure **F** 209 mg of protected oxy-amino ether **60** (0.303 mmol) were deprotected and then condensed with 220 mg hydrocortisone (0.606 mmol) following general procedure **G**. Column chromatography eluting with Tol/acetone 3:2 yielded 204 mg of pure title compound **75** (72 %, 0.218 mmol) as colorless foam. R_f: 0.31 (Tol/acetone 3:2). $[\alpha]_D^{20}$= +86.8 (c=1, CHCl3). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{44}H_{61}F_3N_8O_{11}Na$: 957.4304 found: 957.4298. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 7.58-7.42 (m, 2H, aryl), 7.33-7.23, 7.08-6.96 (m, 1H, CON**H**), 7.21-7.11 (m, 2H, aryl), 6.23, 5.64 (s, s, broad, 1H, C4-**H**), 4.57 (d, 1H, J = -19.6 Hz, C21-**H**), 4.34 (s, broad, 1H, C11-**H**), 4.19 (d, 1H, J = -19.6 Hz, C21-**H**), 4.18-4.06 (m, 2H, C**H**$_2$ON), 3.91, 3.76 (s, s, broad, NC**H**$_2$CON), 3.72-3.25 (m, 22H, EG-C**H**$_2$), 2.91-2.78 (m, 1H, C2-**H**), 2.75-2.52

(m, 2H, O**H**, C16-**H**), 2.43-2.05 (m, 3H, O**H**, C6-**H**, C2-**H**), 2.03-1.82 (m, 4H, scaffold), 1.80-1.55 (m, 3H, scaffold), 1.53-1.65 (m, 7H, C12-**H**, C16-**H**, scaffold, C15-**H**, C$\mathbf{H}_3$), 1.04-0.75 (m, 2H, C7-**H**, C9-**H**), 0.83 (s, 3H, C$\mathbf{H}_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 212.3, 212.3 (**C**20), 171.7, 168.8, 168.5 (**C**O), 160.9, 157.0, 156.7, 153.9 (**C**3/**C**5), 137.0, 130.5, 128.0, 127.6, 126.5 (aryl), 121.9 (q, J = 274.5 Hz, **C**F_3), 115.4, 109.4 (**C**4), 88.9 (**C**17), 72.9, 72.6 (**C**H_2ON), 70.6, 70.5, 70.5, 70.2, 69.9, 69.7, 69.6, 69.2 (EG-**C**H_2), 68.2 (**C**11), 67.2 (**C**21), 56.2, 54.3 (**C**9), 54.3 (**C**H_2N), 52.0, 51.9 (**C**14), 50.6 (**C**H_2N_3), 50.3, 49.6 (**C**H_2N), 47.6, 47.6 (**C**13), 47.3 (**C**H_2N), 39.7, 39.4 (**C**H_2N, **C**12), 39.5, 38.5 (**C**10), 35.6, 34.1, 33.1, 32.1, 31.7 (**C**16, **C**6, **C**7), 31.6, 31.5 (**C**8), 28.3 (q, J = 40.7 Hz, **C**N_2CF_3), 24.5, 23.8, 23.8, 19.3 (**C**15, **C**2), 21.7, 21.4 (**C**19), 17.5 (**C**18).



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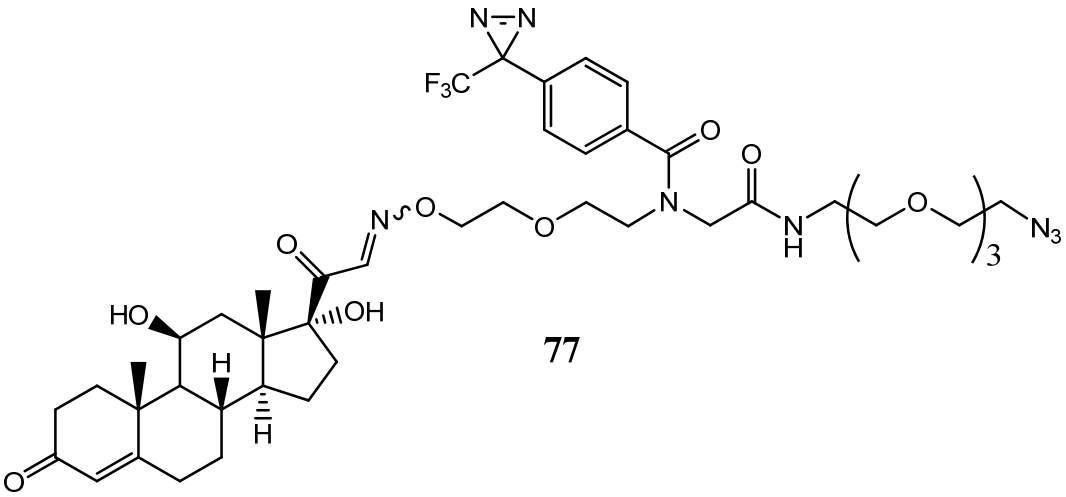
{20-[((11β,16α)-9-Fluoro-20-oxo-11,17,21-trihydroxy-16-methylpregna-1,4-dien-3-ylidene)aminooxy]-1-azido-13-oxo-3,6,9,18-tetraoxa-12,15-diazaicos-15-yl}-4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzamide **76**: According to the general procedure **F** 209 mg of protected oxy-amino ether **60** (0.303 mmol) were deprotected and then condensed with 238 mg Dex (0.606 mmol) following the general procedure **G**. Column chromatography eluting with Tol/acetone 3:2 yielded 225 mg of pure title compound **76** (77 %, 0.233 mmol) as colorless foam. R_f: 0.31 (Tol/acetone 3:2). $[\alpha]_D^{20}$= +57.7 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{45}H_{60}F_4N_8O_{11}Na$: 987.4210 found: 987.4207. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 7.60-7.42 (m, 2H, aryl), 7.23-7.12 (m, 2H, aryl), 7.1-6.81, 6.64-5.86 (m, 4H, CON**H**, C1-**H**, C2-**H**, C4-**H**), 4.55 (d, 1H, J = -19.4 Hz C21-**H**), 4.30-4.06 (m, 5H, C21-**H**, C11-**H**, C$\mathbf{H}_2$ON), 3.90, 3.77 (s, s, 2H, NC$\mathbf{H}_2$CON), 3.74-3.29 (m, 22H, EG-C$\mathbf{H}_2$), 3.09-2.42 (m, 5H, C16-**H**, C6-**H**, O**H**), 2.37-2.03 (m, 4H, C6-**H**, C8-**H**, C12-**H**, C14-**H**), 1.78-1.61 (m, 2H, C7-**H**, C15-**H**), 1.54-1.28 (m, 5H, C7-**H**, C12-**H**, C$\mathbf{H}_3$), 1.28-1.11 (m, 1H, C15-**H**), 0.94 (s, 3H, C$\mathbf{H}_3$), 0.85 (d, 3H, J = 6.9 Hz, C16-C$\mathbf{H}_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 212.2 (**C**20), 171.7, 168.9, 168.6 (**C**O), 155.9, 150.8, 149.6 (**C**3, **C**5), 142.9, 138.6 (**C**1), 137.2, 130.5, 128.0, 127.6, 126.5, 123.4 (**C**2, **C**4), 122.0 (q, J = 275.3 Hz, **C**F_3), 117.2, 116.5, 111.1 (**C**2, **C**4), 100.3, 100.1 (d, d, J = 173.5

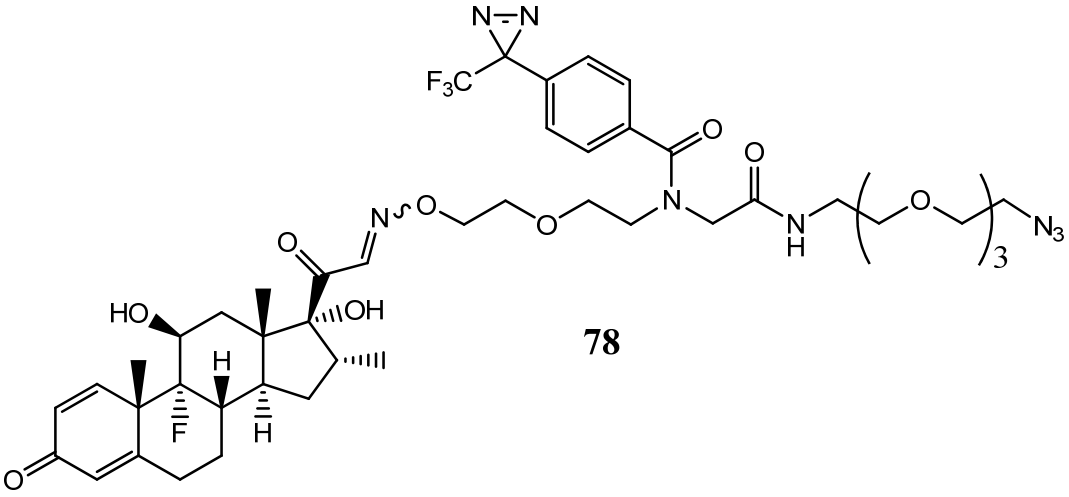
Hz/174.6 Hz, **C**9), 90.4 (**C**17), 73.1 (**C**H_2ON), 71.0, 70.9 (d, d, J = 38.6 Hz/38.6 Hz, **C**11), 70.6, 70.5, 70.2, 70.0, 69.6, 69.2, 68.4 (EG-**C**H_2), 67.7 (**C**21), 54.3 (**C**H_2N), 50.6 (**C**H_2N_3), 50.4, 49.5 (**C**H_2N), 48.6 (**C**13), 47.8, 47.7 (q, J = 22.5 Hz/22.9 Hz, **C**10), 44.1 (**C**14), 39.4 (**C**H_2N), 36.5 (**C**12), 36.1 (**C**16), 34.3, 34.2 (d, d, J = 19.7 Hz/19.3 Hz, **C**8), 32.4, 32.3, 31.1, 30.6 (**C**15, **C**6, **C**7), 28.4 (q, J = 40.4 Hz, **C**N_2CF_3), 27.5 (**C**15, **C**6, **C**7), 24.0, 23.9 (d, d, J = 25.7 Hz/25.5 Hz, **C**19), 17.1 (**C**18), 14.9 (**C**16-Me).



77

{20-[((11β)-3,20-Dioxo-11,17-dihydroxypregn-4-en-21-ylidene)aminooxy]-1-azido-13-oxo-3,6,9,18-tetraoxa-12,15-diazaoctadec-15-yl}-4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzamide **77**: According to the general procedure **F** 209 mg *N*-Boc-protected oxy-amino ether **60** (0.303 mmol) were deprotected and then condensed with 220 mg HC (0.606) following the general procedure **B**. Column chromatography eluting with Tol/acetone 3:2 yielded 160 mg of pure title compound **77** (48 %, 0.144 mmol) as light yellow foam. R_f: 0.27 (Tol/acetone 3:2). $[\alpha]_D^{20}$= +104.6 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{44}H_{59}F_3N_8O_{11}Na$: 955.4147 found: 955.4146. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 7.83, 7.68 (s, s, 1H, C21-**H**), 7.51, 7.45 (d, d, 2H, J = 8.1 Hz/7.8 Hz, aryl), 7.40-7.31, 6.99-6.89 (m, m, 1H, CON**H**), 7.24-7.14 (m, 2H, aryl), 5.65 (s, 1H, C4-**H**), 4.49-4.30 (m, 3H, C11-**H**, CH_2ON), 4.24-4.02 (m, 2H, CH_2), 4.00-3.40 (m, 21H, NCH_2CON, O**H**, EG-CH_2), 3.40-3.32 (m, 2H, EG-CH_2), 2.86-2.70 (m, 1H, C16-**H**), 2.53-2.08 (m, 7H, C2-**H**, C6-**H**, scaffold), 2.06-1.20 (m, 11H, scaffold), 1.40 (s, 3H, CH_3), 1.15-1.00 (m, 1H, C7-**H**), 0.99-0.90 (m, 1H, C9-**H**), 0.84, 0.59 (s, s, 3H, CH_3). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 199.8 (**C**3), 196.2, 195.8 (**C**20), 172.6 (**C**5), 172.0, 168.7, 168.4 (**C**O), 148.2, 147.4 (**C**21), 136.8, 136.7, 131.4, 130.8, 127.9, 127.5, 126.9, 126.6 (aryl), 122.3 (**C**4), 122.0 (q, J = 274.6 Hz, **C**F_3), 91.9, 91.4 (**C**17), 75.2, 74.9 (CH_2ON), 70.7, 70.7, 70.6, 70.3, 70.0, 69.7, 69.3, 68.4 (EG-CH_2), 67.9 (C11-**H**), 56.0 (**C**9), 54.9 (N**C**H_2), 52.0 (**C**14),

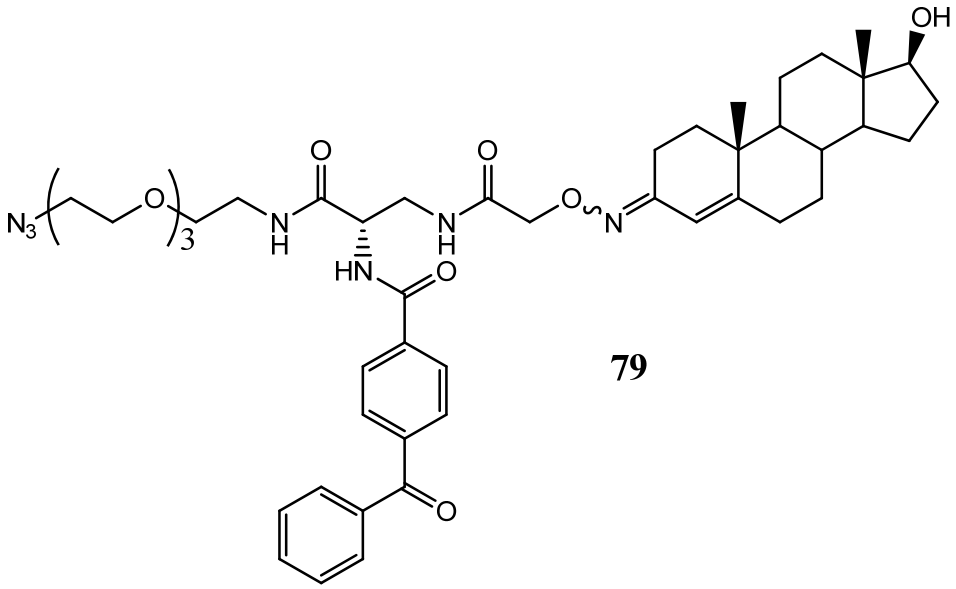
50.7 (**C**H_2N_3), 50.4, 49.9, 48.1 (**C**H_2N), 47.3 (**C**13), 40.8, 40.5 (**C**H_2N), 39.5 (**C**12), 39.3 (**C**10), 35.0, 33.9, 32.9, 32.7, 32.2 (**C**1, **C**2, **C**6, **C**7, **C**16), 31.6 (**C**8), 28.8, 28.4 (q, J = 40.4 Hz, **C**N_2CF_3), 23.8 (**C**15), 21.0 (**C**19), 17.8, 17.5 (**C**18).



78

{20-[((11β,16α)-9-Fluoro-3,20-dioxo-11,17-dihydroxy-16-methylpregna-1,4-dien-21-ylidene)aminooxy]-1-azido-13-oxo-3,6,9,18-tetraoxa-12,15-diazaoctadec-15-yl}-4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzamide **78**: According to the general procedure **F** 209 mg *N*-Boc-protected oxy-amino ether **60** (0.303 mmol) were deprotected and then condensed with 238 mg Dex (0.606) following the general procedure **B**. Column chromatography eluting with Tol/acetone 3:2 yielded 186 mg of pure title compound **78** (64 %, 0.193 mmol) as light yellow foam. R_f: 0.26 (Tol/acetone 3:2). $[\alpha]_D^{20}$= +96.0 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{45}H_{58}F_4N_8O_{11}Na$: 985.4053 found: 985.4046. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 7.80, 7.64 (s, s, 1H, C21-**H**), 7.55-7.45, 7.06-6.98 (m, 1H, CON**H**), 7.52, 7.42 (d, d, 2H, J = 8.1 Hz, aryl), 7.22-7.11 (m, 3H, C1-**H**, aryl), 6.28-6.20 (m, 1H, C2-**H**), 6.04 (s, 1H, C4-**H**), 4.48-4.28 (m, 2H, C$\mathbf{H}_2$ON), 4.25-4.14, 4.11-3.37 (m, 27H NC$\mathbf{H}_2$CON, C11-**H**, O**H**, EG-C$\mathbf{H}_2$), 3.36-3.30 (m, 2H, EG-C$\mathbf{H}_2$), 3.19-3.01 (m 1H, C16-**H**), 2.63-2.44 (m, 2H, C6-**H**, O**H**), 2.37-1.96 (m, 4H, C6-**H**, C8-**H**, C12-**H**, C14-**H**), 1.83-1.72 (m, 1H, C7-**H**), 1.70-1.13 (m, 4H, C7-**H**, C12-**H**, C15-**H**), 1.47 (s, 3H, C$\mathbf{H}_3$), 0.91-0.77, 0.55 (m, s, 6H, C$\mathbf{H}_3$, C16-C$\mathbf{H}_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 195.9, 195.6 (**C**20), 186.72 (**C**3), 172.4, 172.0, 168.7, 168.3 (**C**O), 166.8 (**C**5), 152.7 (**C**1), 148.4, 147.2 (**C**21), 136.6, 136.5, 131.5, 130.8 (aryl), 129.6 (**C**2), 127.9, 127.4, 127.0, 126.6 (aryl), 124.9 (**C**4), 121.9 (q, J = 275.8 Hz, **C**F_3), 100.6 (d, J = 175.3 Hz, **C**9), 92.3, 92.0 (**C**17), 75.4, 74.8 (C$\mathbf{H}_2$ON), 71.7 (d, J = 38.4 Hz, **C**11), 70.6, 70.5, 70.3, 69.9, 69.6, 69.1, 68.2, 68.1 (EG-C$\mathbf{H}_2$), 55.0 (**C**H_2N), 50.7 (**C**H_2N_3), 50.4, 49.9 (**C**H_2N), 48.5, 48.2 (**C**13), 48.3 (**C**H_2N), 43.7, 43.6 (**C**14), 39.5 (**C**H_2N), 37.3, 37.0 (**C**12), 35.0, 34.9 (**C**16), 34.2 (d, J = 19.6

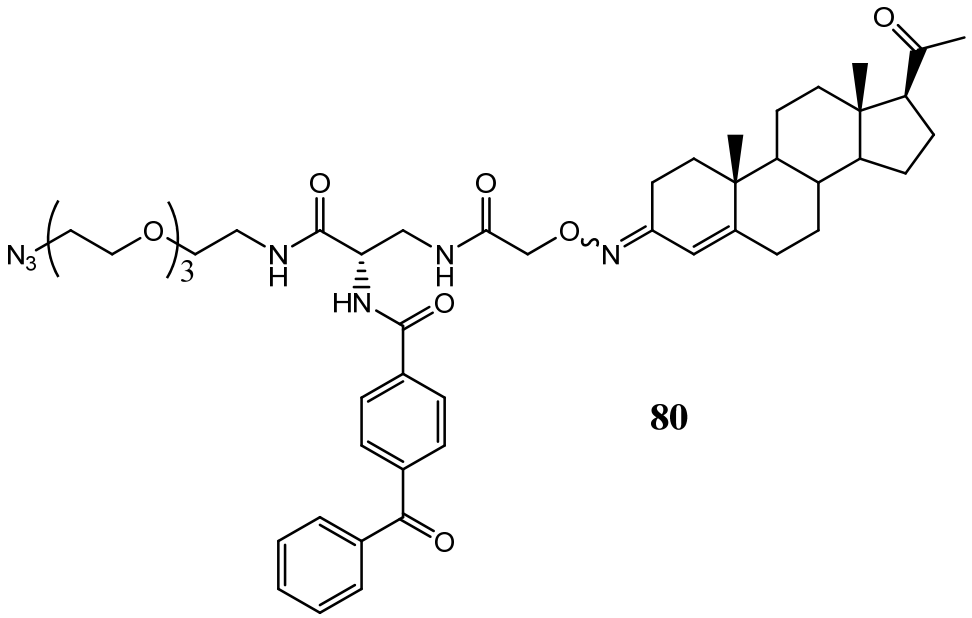
Hz, **C**8), 32.1, 31.1 (**C**15, **C**6, **C**7), 28.3 (q, J = 40.7 Hz, **C**N_2CF_3), 27.4, 23.1 (d, J = 5.6 Hz, **C**19), 16.9, 16.6 (**C**18), 15.0 (C16-Me).



79

N-{(14S)-18-[((17β)-17-Hydroxyandrost-4-en-3-ylidene)aminooxy]-1-azido-13,17-dioxo-3,6,9-trioxa-12,16-diazaoctadec-14-yl}-4-benzoylbenzamide **79**: According to the general procedure **F** 200 mg Boc-protected photophore **65** (0.326 mmol) were converted to the corresponding free ammonium derivative and then reacted with 66 mg HOBt (0.489 mmol), 170 µl DIPEA (0.978 mmol), 177 mg of T-3-CMO **8** (0.489 mmol, 1.5 eq.) and 189 mg HBTU (0.489 mmol) following the general procedure **A**. Column chromatography with $CHCl_3$ containing 3% MeOH yielded 229 mg of triamide **79** (0.267 mmol, 82%) as colorless foam. R_f: 0.33 ($CHCl_3$+3%MeOH). $[\alpha]_D^{20}$= +68.0 (c=1.0, $CHCl_3$). Anal calcd for $C_{46}H_{61}N_7O_9$: N, 11.45; C, 64.54; H, 7.18. found: N, 11.22; C, 64.45; H, 7.32. FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{46}H_{61}N_7O_9Na$: 878.4423 found: 878.4416. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 8.47-8.42, 8.35-8.30 (m, 1H, CON**H**), 7.99-7.93 (m, 2H, BP), 7.85-7.79 (m, 2H, BP), 7.77-7.72 (m, 2H, BP), 7.60-7.54 (m, 1H, BP), 7.49-7.42 (m, 2H, BP), 7.25-7.10 (m, 2H, CON**H**), 6.35, 5.60 (s, s, broad, 1H, C4-**H**), 4.71-4.63 (m, 1H, α-C**H**), 4.55-4.42 (m, 2H, NOC$\mathbf{H}_2$CONH), 3.89-3.79 (m, 1H, β-C**H**), 3.79-3.66 (m, 1H, β-C**H**), 3.63-3.47 (m, 13H, EG-chain-C$\mathbf{H}_2$, C17-**H**), 3.45-3.36 (m, 2H, EG-chain-C$\mathbf{H}_2$), 3.32 (t, 2H, J = 5.2 Hz, $N_3C\mathbf{H}_2$), 3.00-2.92 (m, 1H, C2-**H**), 2.36-1.93 (m, 5H, C2-**H**, C6-**H**, scaffold), 1.87-1.65 (m, 3H, scaffold), 1.60-1.17 (m, 7H, scaffold), 1.08-0.94 (m, 1H, scaffold), 1.05, 1.00 (s, 3H, C$\mathbf{H}_3$), 0.94-0.69 (m, 3H, scaffold), 0.72 (s, 3H, CH_3). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 195.9 (**C**O-BP), 173.3, 173.0 (**C**ON), 168.7, 169.6 (**C**ON), 167.0, 166.9 (**C**ON), 161.7, 158.8, 157.6, 155.8 (**C**3, **C**5), 140.4, 137.0, 136.4, 132.9, 130.1, 130.1,

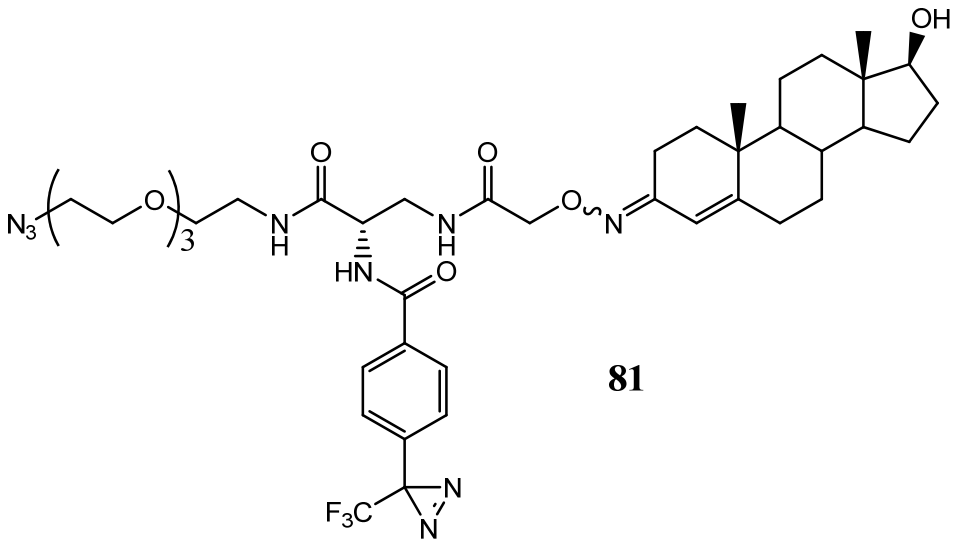
128.5, 127.4 (BP-aryl), 116.3, 110.5 (**C**4) , 81.6, 81.6 (**C**17), 72.5, 72.3 (**C**H_2ON), 70.6, 70.5, 70.3, 70.0, 69.5 (EG-**C**H_2), 56.1, 55.7 (**C**H-α), 54.0, 53.8 (**C**9), 50.6 (**C**H_2N_3), 50.6, 50.5 (**C**14), 42.8, 42.8 (**C**13), 41.5, 41.4 (**C**H_2N), 39.4, 39.4 (**C**12), 39.1, 38.0 (**C**10), 36.5, 36.5 (**C**2), 35.9 (**C**1), 35.7, 35.7 (**C**8), 34.5 (**C**1), 33.0, 32.5, 32.0, 31.7, 30.4 (**C**6, **C**7, **C**16), 24.4, 23.4, 20.9, 20.7, 19.7 (**C**11, **C**15), 18.0, 17.7 (**C**19), 11.1 (**C**18).



80

N-{(14S)-18-[(20-Oxopregn-4-en-3-ylidene)aminooxy]-1-azido-13,17-dioxo-3,6,9-trioxa-12,16-diazaoctadec-14-yl}-4-benzoylbenzamide **80**: According to general procedure **F** 200 mg of Boc-protected photophore **65** (0.326 mmol) were converted to the corresponding free ammonium derivative and then reacted with 66 mg HOBt (0.489 mmol), 170 µl DIPEA (0.978 mmol), 189 mg of P_4-3-CMO **9** (0.489 mmol, 1.5 eq.) and 189 mg HBTU (0.489 mmol) following the general procedure **A**. Column chromatography with $CHCl_3$ containing 3% MeOH yielded 249 mg of triamide **80** (0.282 mmol, 87%) as colorless foam. R_f: 0.36 ($CHCl_3$+3%MeOH). $[\alpha]_D^{20}$= +113.1 (c=1.0, $CHCl_3$). Anal. calcd for $C_{48}H_{63}N_7O_9$: N, 11.12; C, 65.36; H, 7.20. found: N, 10.89; C, 65.52; H, 7.43. FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{48}H_{63}N_7O_9Na$: 904.4580 found: 904.4572. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 8.44, 8.36-8.29 (d, m, 1H, J = 6.0 Hz, CON**H**), 7.99-7.93 (m, 2H, BP), 7.85-7.79 (m, 2H, BP), 7.77-7.72 (m, 2H, BP), 7.60-7.54 (m, 1H, BP),7.49-7.42 (m, 2H, BP), 7.22-7.07 (m, 2H, CON**H**), 6.36, 5.60 (s, s, broad, 1H, C4-**H**), 4.71-4.63 (m, 1H, α-C**H**), 4.55-4.42 (m, 2H, NOC**H**$_2$CONH), 3.91-3.80 (m, 1H, β-C**H**), 3.80-3.66 (m, 1H, β-C**H**), 3.63-3.47 (m, 12H, EG-chain-C**H**$_2$), 3.45-3.36 (m, 2H, EG-chain-C**H**$_2$), 3.32 (t, 2H, J=5.2 Hz, N_3C**H**$_2$), 3.02-2.93, 2.57 (m, s, 1H, C2-**H**), 2.51-2.43 (m, 1H, C17-**H**), 2.36-1.92 (m, 5H, scaffold), 2.07 (s, 3H, C**H**$_3$), 1.89-1.27 (m, 9H, scaffold), 1.27-0.74 (m, 4H, scaffold), 1.04, 1.00 (s, s,

3H, C**H**$_3$), 0.60 (s, 3H, C**H**$_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 209.4, 209.4 (**C**20), 195.8 (**C**O-BP), 173.4, 173.0 (**C**ON), 169.6, 169.6 (**C**ON), 167.0, 166.9 (**C**ON), 161.5, 158.7, 157.3, 155.8 (**C**3, **C**5), 140.4, 137.0, 136.4, 136.3, 132.9, 130.1, 130.1, 128.5, 127.4 (BP-aryl), 116.5, 110.6 (**C**4), 72.5, 72.3 (**C**H_2ON), 70.6, 70.5, 70.3, 70.0, 69.5 (EG-**C**H_2), 63.6, 63.5 (**C**17), 56.3, 56.2, 56.1, 55.8 (**C**14/**C**H-α), 53.7, 53.5 (**C**9), 50.6 (**C**H_2N_3), 44.0, 43.9 (**C**13), 41.5, 41.4 (**C**H_2N), 39.4, 39.4 (**C**16), 39.0 (**C**10), 38.8, 38.7 (**C**12), 37.9 (**C**10), 35.9 (**C**2), 35.7, 35.6 (**C**8), 34.5 (**C**2), 33.0, 32.5, 32.3, 32.0 (**C**6, **C**7), 31.5 (**C**21), 24.4, 22.8, 21.3, 21.1, 19.7 (**C**1, **C**11, **C**15), 17.9, 17.7 (**C**19), 13.3 (**C**18).

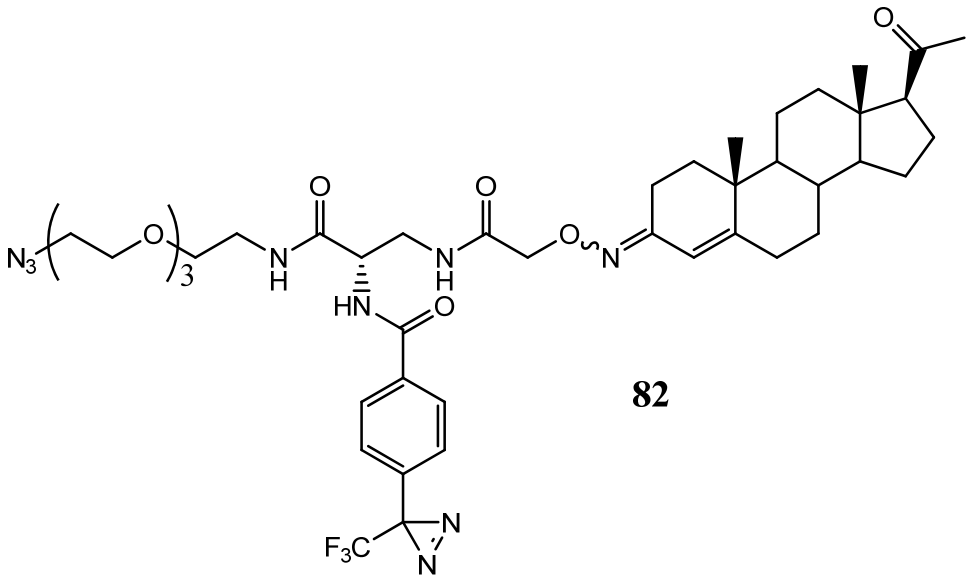


81

N-{(14S)-18-[((17β)-17-Hydroxyandrost-4-en-3-ylidene)aminooxy]-1-azido-13,17-dioxo-3,6,9-trioxa-12,16-diazaoctadec-14-yl}-4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzamide

81: According to general procedure **F** 200 mg of Boc-protected photophore **66** (0.324 mmol) were converted to the corresponding ammonium derivative and then reacted with 66 mg HOBt (0.486 mmol), 169 µl DIPEA (0.972 mmol), 176 mg T-3-CMO **8** (0.486 mmol, 1.5 eq.) and 184 mg HBTU (0.486 mmol) following the general procedure **A**. Column chromatography with $CHCl_3$ containing 4% MeOH yielded 256 mg of triamide **81** (0.298 mmol, 92%) as colorless foam. R_f: 0.33 ($CHCl_3$+4%MeOH). $[\alpha]_D^{20}$= +57.8 (c=1.0, $CHCl_3$). Anal. calcd for $C_{43}H_{58}F_3N_9O_8$: N, 14.23; C, 58.29; H, 6.60. foumd: N, 14.17; C, 58.31; H, 6.87. FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{41}H_{56}F_3N_9O_8Na$: 882.4096 found: 882.4103. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 8.44-8.39, 8.32-8.26 (m, 1H, CON**H**), 7.92-7.85 (m, 2H, aryl), 7.22 (d, broad, J = 8.2 Hz, aryl), 7.15 (t, broad, 1H, CON**H**), 7.13-7.07 (m, 1H, CON**H**), 6.33, 5.57 (s, s, broad, 1H, C4-**H**), 4.65-4.58 (m, 1H, α-C**H**), 4.53-4.40 (m, 2H, NOC**H**$_2$CONH), 3.86-3.64 (m, 2H, β-C**H**), 3.63-3.46 (m, 13H, EG-chain-C**H**$_2$, C17-**H**), 3.43-3.35 (m, 2H, EG-chain-C**H**$_2$), 3.33 (t, 2H, J=5.2 Hz, N_3C**H**$_2$), 2.99-2.90 (m, 1H,

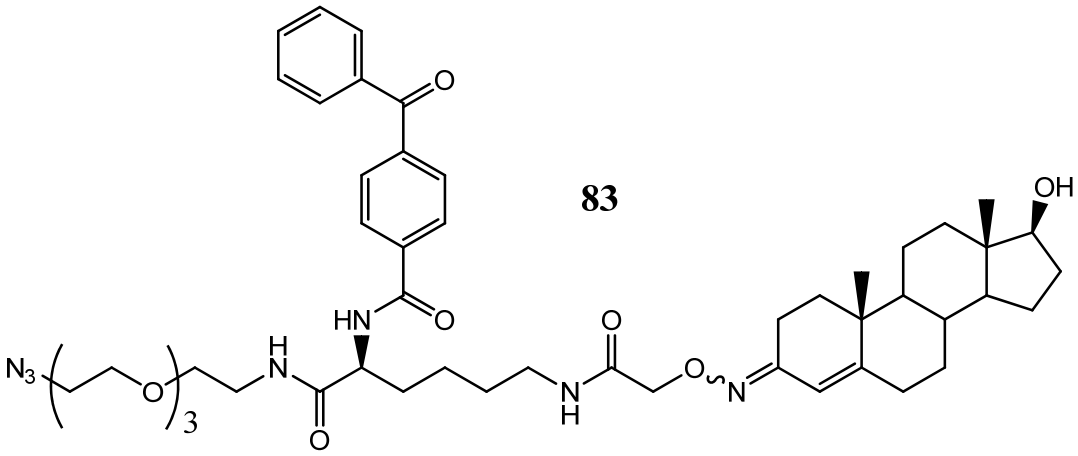
C2-**H**), 2.36-1.93 (m, 5H, scaffold), 1.88-1.68 (m, 3H, scaffold), 1.61-1.16 (m, 7H, scaffold), 1.08-0.71 (m, 4H, scaffold), 1.06, 1.01 (s, s, 3H, C**H**$_3$), 0.73 (s, 3H, C**H**$_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 173.4, 173.1 (**C**ON), 169.6, 169.6 (**C**ON), 166.6, 166.6 (**C**ON), 161.8, 158.8, 157.6, 155.8 (**C**3, **C**5), 134.3, 132.7, 127.9, 126.6 (aryl), 122.0 (q, J = 275 Hz, **C**F_3), 116.3, 110.5 (**C**4), 81.6, 81.6 (**C**17), 72.5, 72.3 (**C**H_2ON), 70.6, 70.6, 70.5, 70.3, 70.0, 69.5 (EG-**C**H_2), 56.2, 55.8 (**C**H-α), 54.0, 53.8 (**C**9), 50.7 (**C**H_2N_3), 50.6, 50.6 (**C**14), 42.9, 42.8 (**C**13), 41.4, 41.4 (**C**H_2N), 39.4, 39.4 (**C**12), 39.1, 38.0 (**C**10), 36.6, 36.5 (**C**2), 36.0 (**C**1), 35.8, 35.7 (**C**8), 34.5 (**C**1), 33.0, 32.0, 31.7, 30.4 (**C**6, **C**7, **C**16), 28.4 (q, J = 40.7 Hz, **C**N_2CF_3), 24.4, 23.4, 21.0, 20.8, 19.7 (**C**11, **C**15), 18.0, 17.7 (**C**19), 11.1, 11.1 (**C**18).



82

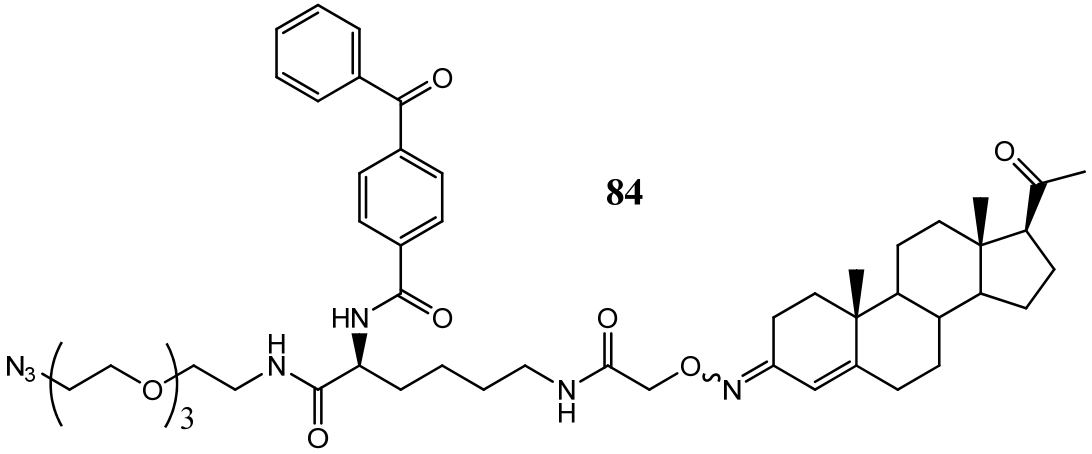
N-{(14S)-18-[(20-Oxopregn-4-en-3-ylidene)aminooxy]-1-azido-13,17-dioxo-3,6,9-trioxa-12,16-diazaoctadec-14-yl}-4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzamide **82**: According to general procedure **F** 200 mg of Boc-protected photophore **66** (0.324 mmol) was converted to the corresponding free ammonium derivative and then reacted with 66 mg HOBt (0.486 mmol), 169 µl DIPEA (0.972 mmol), 188 mg P_4-3-CMO **9** (0.486 mmol, 1.5 eq.) and 184 mg HBTU (0.486 mmol) following the general procedure **A**. Column chromatography with $CHCl_3$ containing 1.5% MeOH yielded 218 mg of triamide **82** (0.246 mmol, 76%) as colorless foam. R_f: 0.44 ($CHCl_3$+2%MeOH). $[\alpha]_D^{20}$= +90.9 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{43}H_{58}F_3N_9O_8Na$: 908.4253 found: 908.4244. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 8.42-8.38, 8.31-8.27 (m, 1H, CON**H**), 7.91-7.84 (m, 2H, aryl), 7.21 (d, broad, J = 8.2 Hz, aryl), 7.16-7.04 (m, 2H, CON**H**), 6.33, 5.59-5.56 (s, m, broad, 1H, C4-**H**), 4.65-4.58 (m, 1H, α-C**H**), 4.52-4.40 (m, 2H, NOC**H**$_2$CONH), 3.86-3.61

(m, 2H, β-C**H**), 3.62-3.45 (m, 12H, EG-chain-C**H**$_2$), 3.43-3.35 (m, 2H, EG-chain-C**H**$_2$), 3.32 (t, 2H, J=5.1 Hz, N_3C**H**$_2$), 2.99-2.91, 2.62 (m, s, 1H, C2-**H**), 2.51-2.44 (m, 1H, C17-**H**), 2.35-1.93 (m, 5H, scaffold), 2.07 (s, 3H, C**H**$_3$), 1.87-0.77 (m, 13H, scaffold), 1.04, 0.99 (s, s, 3H, C**H**$_3$), 0.60 (s, 3H, C**H**$_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 209.3 (**C**20), 173.4, 173.0 (**C**ON), 169.6, 169.6 (**C**ON), 166.6, 166.6 (**C**ON), 161.4, 158.7, 157.3, 155.7 (**C**3, **C**5), 134.3, 132.7, 127.9, 126.6 (aryl), 122.5 (q, J=275 Hz, **C**F$_3$), 116.4, 110.6 (**C**4), 72.5, 72.3 (**C**H$_2$ON), 70.6, 70.6, 70.5, 70.3, 70.0, 69.5 (EG-**C**H$_2$), 63.6, 63.5 (**C**17), 56.3, 56.2, 56.1, 55.9 (**C**14, **C**H-α), 53.7, 53.5 (**C**9), 50.6 (**C**H$_2$N$_3$), 44.0, 43.9 (**C**13), 41.4, 41.3 (**C**H$_2$N), 39.4, 39.3 (**C**16), 39.0 (**C**10), 38.8, 38.7 (**C**12), 37.9 (**C**10), 35.9 (**C**2), 35.7, 35.6 (**C**8), 34.5 (**C**2), 33.0, 32.5, 32.3, 32.0 (**C**6, **C**7), 31.5 (**C**21), 28.4 (q, J = 40.7 Hz, **C**N$_2$CF$_3$), 24.4, 22.8, 21.3, 21.1, 19.6 (**C**1, **C**11, **C**15), 17.9, 17.6 (**C**19), 13.3, 13.3 (**C**18).



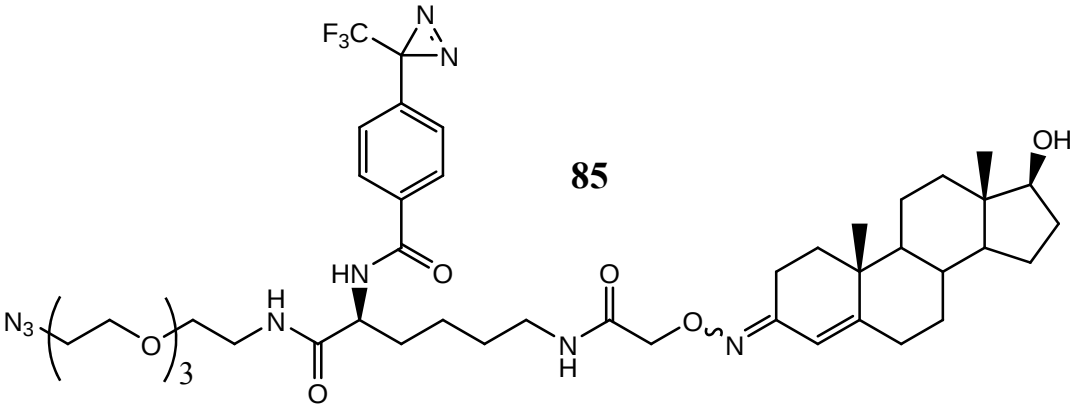
N-{(14S)-21-[((17β)-17-Hydroxyandrost-4-en-3-ylidene)aminooxy]-1-azido-13,20-dioxo-3,6,9-trioxa-12,19-diazahenicos-14-yl}-4-benzoylbenzamide **83**: According to general procedure **F** 200 mg of Boc-protected photophore **67** (0.305 mmol) were converted to the corresponding free ammonium derivative and then reacted with 62 mg HOBt (0.458 mmol), 159 µl DIPEA (0.915 mmol), 166 mg T-3-CMO **8** (0.458 mmol, 1.5 eq.) and 174 mg HBTU (0.458 mmol) following the general procedure **A**. Column chromatography eluting with $CHCl_3$ containing 3% MeOH yielded 213 mg of triamide **83** (0.237 mmol, 78%) as colorless foam. R_f: 0.33 ($CHCl_3$+3%MeOH). $[\alpha]_D^{20}$= +45.4 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{49}H_{67}N_7O_9Na$: 920.4893 found: 920.4895. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 8.00-7.93 (m, 2H, BP), 7.84-7.74 (m, 4H, BP), 7.62-7.56 (m, 1H, BP), 7.50-7.44 (m, 2H, BP), 7.41-7.42 (m, 1H, CON**H**), 6.97-6.91 (m, 1H, CON**H**), 6.41-6.32 (m, 1H, CON**H**), 6.35, 5.70 (s, s, broad, 1H, C4-**H**), 4.64-4.56 (m, 1H, α-C**H**), 4.40, 4.38, 4.33, 4.31 (d, d, d, d, 2H, J = -16.1 Hz/-16.0 Hz, NOC**H**$_2$CONH), 3.67-3.52 (m, 13H, EG-

chain-C**H**$_2$, C17-**H**), 3.49-3.43 (m, 2H, EG-chain-C**H**$_2$), 3.39-3.31 (m, 1H, β-C**H**), 3.36 (t, 2H, J = 5.2 Hz, N_3C**H**$_2$), 3.30-3.20 (m, 1H, β-C**H**), 2.99-2.91 (m, 1H, C2-**H**), 2.38-1.70 (m, 10H, scaffold, Lys-C**H**$_2$), 1.64-1.18 (m, 11H, scaffold, Lys-C**H**$_2$), 1.11-0.71 (m, 4H, scaffold), 1.08, 1.04 (s, s, 3H, C**H**$_3$), 0.75 (s, 3H, C**H**$_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 196.0 (**C**O-BP), 171.7, 170.8, 170.6, 166.5 (**C**ON), 161.7, 158.5, 157.5, 155.6 (**C**3, **C**5), 140.3, 137.2, 137.1, 132.9, 130.2, 130.1, 128.5, 127.4 (BP-aryl), 116.5, 110.5 (**C**4), 81.7, 81.6 (C17-**H**), 72.8, 72.6 (**C**H_2ON), 70.7, 70.7, 70.6, 70.4, 70.1 (EG-**C**H_2), 54.1, 53.8, 53.7 (**C**H-α/**C**9), 50.7 (**C**H_2N_3), 50.6, 50.6 (**C**14), 42.9, 42.9 (**C**13), 39.5 (**C**12), 39.2 (**C**10), 38.2 (Lys-**C**H_2), 38.1 (**C**10), 36.6, 36.5 (**C**2), 36.1 (**C**1), 35.8, 35.8 (**C**8), 34.6 (**C**1), 33.1, 32.5, 32.1, 32.0, 31.7, 30.5, 29.4 (**C**6, **C**7, **C**16, Lys-C**H**$_2$), 24.5, 23.4, 22.5, 21.0, 20.8, 19.7(**C**11, **C**15, Lys-C**H**$_2$), 18.1, 17.8 (**C**19), 11.1 (**C**18).



N-{(14S)-21-[(20-Oxopregn-4-en-3-ylidene)aminooxy]-1-azido-13,20-dioxo-3,6,9-trioxa-12,19-diazahenicos-14-yl}-4-benzoylbenzamide **84**: According to general procedure **F** 200 mg of Boc-protected photophore **78** (0.305 mmol) were converted to the corresponding free ammonium derivative and reacted with 62 mg HOBt (0.458 mmol), 159 µl DIPEA (0.915 mmol), 177 mg P_4-3-CMO **9** (0.458 mmol, 1.5 eq.) and 174 mg HBTU (0.458 mmol) following the general procedure **A**. Column chromatography eluting with $CHCl_3$ containing 2.5% MeOH yielded 221 mg of triamide **84** (0.239 mmol, 78%) as colorless foam. R_f: 0.36 ($CHCl_3$+3%MeOH). $[\alpha]_D^{20}$= +69.7 (c=1.0, $CHCl_3$). Anal. calcd for $C_{51}H_{69}N_7O_9$: N, 10.61; C, 66.28; H, 7.53. found: N, 10.28; C, 66.05; H, 7.63. FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{51}H_{69}N_7O_9Na$: 946.5049 found: 946.5046. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 7.96-7.91 (m, 2H, BP), 7.79-7.70 (m, 4H, BP), 7.58-7.53 (m, 1H, BP), 7.48-7.41 (m, 3H, BP/CON**H**), 6.93 (t, broad, 1H, J = 5.4 Hz, CON**H**), 6.42-6.34 (m, 1H, CON**H**), 6.33, 5.67 (s, s, broad, 1H, C4-**H**), 4.62-4.53 (m, 1H, α-C**H**), 4.39, 4.38, 4.32, 4.30 (d, d, d, d,

2H, J = -15.9 Hz/-15.9 Hz, NOC**H**$_2$CONH), 3.65-3.48 (m, 12H, EG-chain-C**H**$_2$), 3.46-3.38 (m, 2H, EG-chain-C**H**$_2$), 3.33 (t, 2H, J = 5.2 Hz, N_3C**H**$_2$), 3.36-3.17 (m, 2H, β-C**H**), 2.98-2.89 (m, 1H, C2-**H**), 2.52-2.41 (m, 1H, C17-**H**), 2.35-0.77 (m, 24H, scaffold/Lys-**C**H$_2$), 2.06 (s, 3H, C**H**$_3$), 1.04, 0.99 (s, s, 3H, C**H**$_3$), 0.59 (s, 3H, C**H**$_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 209.4, 209.4 (**C**20), 195.9 (**C**O-BP), 171.6, 170.6, 170.4, 166.4 (**C**ON), 161.2, 158.3, 157.0, 155.4 (**C**3, **C**5), 140.1, 137.1, 137.0, 132.9, 130.0, 129.9, 128.4, 127.3 (BP-aryl), 116.5, 110.5 (**C**4), 72.7, 72.5 (**C**H$_2$ON), 70.6, 70.6, 70.5, 70.3, 70.0, 69.6 (EG-**C**H$_2$), 63.5, 63.5 (**C**17), 56.1, 56.0 (**C**14), 53.7, 53.4 (**C**H-α, **C**9), 50.6 (**C**H$_2$N$_3$), 43.9, 43.9 (**C**13), 39.4 (**C**16), 38.9 (**C**10), 38.7, 38.7 (Lys-**C**H$_2$), 38.2, 38.1 (**C**12), 37.9 (**C**10), 36.0 (**C**2), 35.6, 35.6 (**C**8), 34.5 (**C**2), 33.0, 32.4, 32.3, 32.0, 31.8 (**C**6, **C**7, Lys-**C**H$_2$), 31.5 (**C**21), 29.3, 24.4, 24.4, 22.8, 22.5, 21.3, 21.1, 19.6 (**C**1, **C**11, **C**15, Lys-**C**H$_2$), 17.9 (**C**19), 13.3 (**C**18).

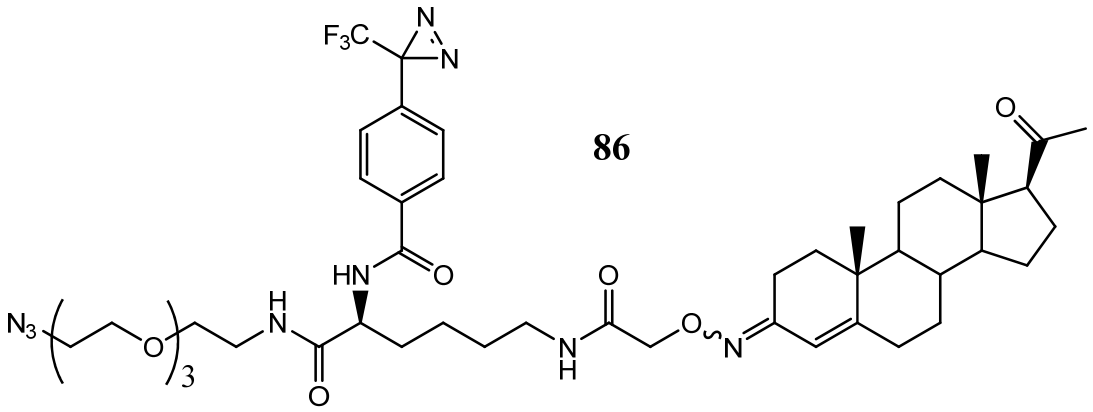


85

N-{(14S)-21-[((17β)-17-Hydroxyandrost-4-en-3-ylidene)aminooxy]-1-azido-13,20-dioxo-3,6,9-trioxa-12,19-diazahenicos-14-yl}-4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzamide

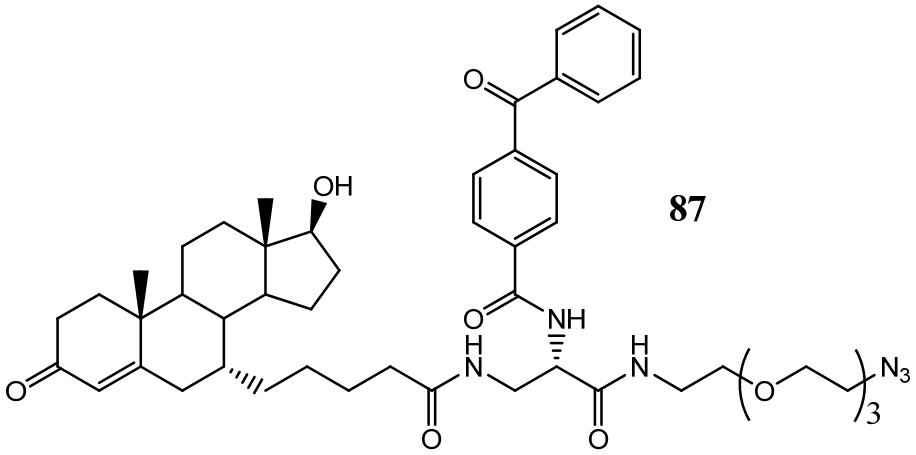
85: According to general procedure **F** 200 mg of Boc-protected photophore **68** (0.304 mmol) were converted to the corresponding free ammonium derivative and then reacted with 62 mg HOBt (0.456 mmol), 159 µl DIPEA (0.912 mmol), 165 mg T-3-CMO **8** (0.456 mmol, 1.5 eq.) and 173 mg HBTU (0.456 mmol) following the general procedure **A**. Column chromatography with $CHCl_3$ containing 3.5 % MeOH yielded 262 mg of triamide **85** (0.290 mmol, 96%) as colorless foam. R_f: 0.26 ($CHCl_3$+3.5% MeOH). $[\alpha]_D^{20}$= +50.8 (c=1.0, $CHCl_3$). Anal. calcd for $C_{44}H_{62}F_3N_9O_8$: N, 13.98; C, 58.59; H, 6.93. found: N, 13.61; C, 58.50; H, 7.24. FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{44}H_{62}F_3N_9O_8Na$: 924.4566 found: 924.4471. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 7.87-7.82 (m, 2H, aryl), 7.45 (d, 1H, J = 7.5 Hz, CON**H**), 7.17-7.13 (m, 2H, aryl), 6.93 (t, 1H, J = 5.5 Hz, CON**H**), 6.40-6.32 (m, 1H, CON**H**), 6.31, 5.66 (s, s, broad, 1H, C4-**H**), 4.57-4.48 (m, 1H, α-C**H**), 4.40, 4.38,

4.33, 4.31 (d, d, d, d, 2H, J = -16.2 Hz/-15.9 Hz, NOC**H**$_2$CONH), 3.64-3.52 (m, 11H, EG-chain-C**H**$_2$/ C17-**H**), 3.52-3.47 (m, 2H, EG-chain-C**H**$_2$), 3.46-3.15 (m, 4H, EG-chain-C**H**$_2$, β-C**H**), 3.33 (t, 2H, J = 5.2 Hz, N_3C**H**$_2$), 2.96-2.87 (m, 1H, C2-**H**), 2.42-1.67 (m, 11H, scaffold), 1.60-1.15 (m, 12H, scaffold), 1.05, 1.01 (s, s, 3H, C**H**$_3$), 1.04-0.68 (m, 4H, scaffold), 0.71 (s, 3H, C**H**$_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 171.7, 170.6, 170.4, 166.1 (**C**ON), 161.6, 158.4, 157.4, 155.5 (**C**3, **C**5), 134.9, 132.3, 127.8, 126.4 (aryl), 121.9 (q, J = 275 Hz, **C**F_3), 116.4, 110.4 (**C**4), 81.5, 81.4 (**C**17), 72.7, 72.5 (**C**H_2ON), 70.6, 70.6, 70.5, 70.3, 70.0, 69.5 (EG-**C**H_2), 54.0, 53.8, 53.6 (**C**9, **C**H-α), 50.6 (**C**H_2N_3), 50.6, 50.5 (**C**14), 42.8, 42.8 (**C**13), 39.4 (**C**12), 39.1 (**C**10), 38.2, 38.2 (Lys-**C**H_2), 38.0 (**C**10), 36.5, 36.5 (**C**2), 36.0 (**C**1), 35.8, 35.7 (**C**8), 34.5 (**C**1), 33.0, 32.5, 32.0, 31.8, 31.7, 31.6, 30.3, 29.3, 29.3 (**C**6, **C**7, **C**16, Lys-**C**H_2), 28.3 (q, J = 40.7 Hz, **C**N_2CF_3), 24.4, 23.3, 22.5, 20.9, 20.7, 19.6 (**C**11, **C**15, Lys-**C**H_2), 18.0, 17.7 (**C**19), 11.1, 11.1 (**C**18).



N-{(14S)-21-[(20-Oxopregn-4-en-3-ylidene)aminooxy]-1-azido-13,20-dioxo-3,6,9-trioxa-12,19-diazahenicos-14-yl}-4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzamide **86**: According to general procedure **F** 200 mg of Boc-protected photophore **68** (0.304 mmol) were converted to their corresponding free ammonium derivative and then reacted with 62 mg HOBt (0.456 mmol), 159 µl DIPEA (0.912 mmol), 176 mg P_4-3-CMO **9** (0.456 mmol, 1.5 eq.) and 173 mg HBTU (0.456 mmol) following the general procedure **A**. Column chromatography with $CHCl_3$ containing 2 % MeOH yielded 251 mg of triamide **86** (0.270 mmol, 89%) as colorless foam. R_f: 0.13 ($CHCl_3$+1.5% MeOH). $[\alpha]_D^{20}$= +71.2 (c=1.0, $CHCl_3$). Anal. calcd for $C_{46}H_{64}F_3N_9O_8$: N, 13.58; C, 59.53; H, 6.95. found: N, 13.19; C, 59.77; H, 7.28. FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{46}H_{64}F_3N_9O_8Na$: 950.4722 found: 950.4726. ^{1}H-NMR from the mixture of isomers (400.1 MHz, $CDCl_3$): δ 7.88-7.79 (m, 2H, aryl), 7.45 (d, 1H, J = 7.2 Hz, CON**H**), 7.17-7.10 (m, 2H, aryl), 6.89 (s, broad, 1H, CON**H**), 6.41-6.29 (m, 1H, CON**H**), 6.31, 5.66 (s, s, broad, 1H, C4-**H**), 4.57-4.48 (m, 1H, α-C**H**), 4.39, 4.38, 4.32, 4.30 (d, d, d, d,

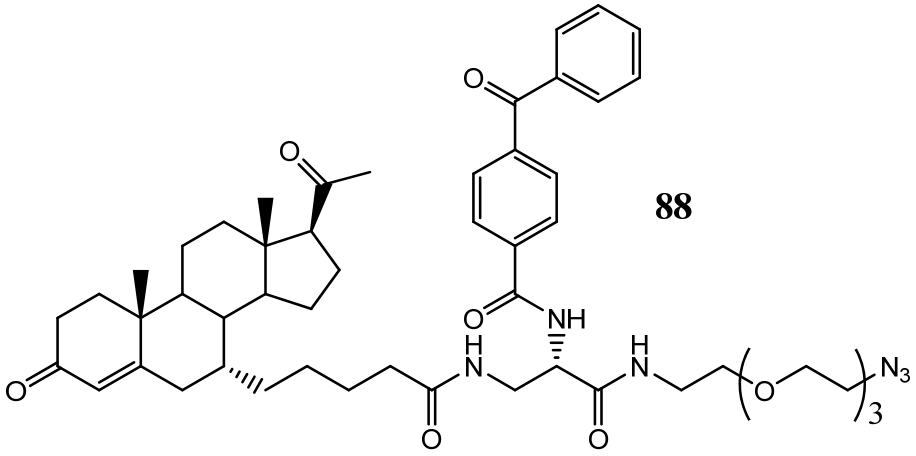
2H, J = -16.1 Hz/-15.9 Hz, NOC**H**$_2$CONH), 3.65-3.45 (m, 12H, EG-chain-C**H**$_2$), 3.44-3.15 (m, 6H, EG-chain-C**H**$_2$/β-C**H**), 2.97-2.84 (m, 1H, C2-**H**), 2.51-2.41 (m, 1H, C17-**H**), 2.34-0.78 (m, 24H, scaffold), 1.03, 0.99 (s, s, 3H, C**H**$_3$), 0.58 (s, 3H, C**H**$_3$). ^{13}C-NMR from the mixture of isomers (100.6 MHz, $CDCl_3$): δ 209.4, 209.3 (**C**20), 171.7, 170.6, 170.4, 166.1 (**C**ON), 161.2, 158.2, 157.0, 155.3 (**C**3, **C**5), 134.9, 132.3, 127.8, 126.3 (aryl), 121.0 (q, J = 275 Hz, **C**F_3), 116.5, 110.5 (**C**4), 72.7, 72.5 (**C**H_2ON), 70.6, 70.6, 70.5, 70.2, 70.0 (EG-**C**H_2), 63.5, 63.5 (**C**17), 56.1, 56.0 (**C**14), 53.7, 53.7, 53.5 (**C**H-α, **C**9), 50.6 (**C**H_2N_3), 43.9, 43.9 (**C**13), 39.3 (**C**16), 38.9 (**C**10), 38.7, 38.7 (Lys-**C**H_2), 38.2, 38.1 (**C**12), 37.9 (**C**10), 36.0 (**C**2), 35.6, 35.6 (**C**8), 34.5 (**C**2), 32.9, 32.4, 32.3, 32.0, 31.7 (**C**6, **C**7, Lys-**C**H_2), 31.4 (**C**21), 29.3, 28.3 (q, J = 40.6 Hz, **C**N_2CF_3), 24.4, 24.3, 22.8, 22.5, 21.3, 21.1, 19.5 (**C**1, **C**11, **C**15, Lys-**C**H_2), 17.9, 17.6 (**C**19), 13.1 (**C**18).



87

N-{(14S)-1-Azido-21-[(17β,7α)-17-hydroxy-3-oxoandrost-4-en-7-yl]-13,17-dioxo-3,6,9-trioxa-12,16-diazahenicos-14-yl}-4-benzoylbenzamide **87**: According to general procedure **G** 100 mg of steroid methyl ester **16** (0.257 mmol) were saponified and directly coupled to 173 mg of the free ammonium derivative of photophore **65** (0.283 mmol; general procedure **F**) using 52 mg HOBt (0.385 mmol), 134 µl DIPEA (0.771 mmol) and 146 mg HBTU (0.385 mmol). Column chromatography eluting with $CHCl_3$ containing 4.5% MeOH yielded 221 mg of triamide **87** (0.239 mmol, 93%) as colorless foam. R_f: 0.35 ($CHCl_3$+5% MeOH). $[\alpha]_D^{20}$= +7.1 (c=1.0, $CHCl_3$). Anal. calcd for $C_{49}H_{66}N_6O_9$: N, 9.52; C, 66.64; H, 7.53. found: N, 9.19; C, 66.14; H, 7.88. FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{49}H_{66}N_6O_9Na$: 905.4784 found: 905.4791. HPLC: R_t: 8.004 min., 99.3%. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 8.58 (d, 1H, J = 5.9 Hz, CON**H**), 7.98-7.93 (m, 2H, BP), 7.80-7.76 (m, 2H, BP), 7.74-7.69 (m, 2H, BP), 7.57-7.52 (m, 1H, BP), 7.46-7.40 (m, 2H, BP), 7.33-7.25 (m, 1H, CON**H**), 7.12 (t, broad, 1H, J = 6.1 Hz, CON**H**), 5.60 (s, broad, 1H, C4-**H**), 4.64-4.57 (m, 1H, α-C**H**), 3.76-3.61 (m, 2H, β-C**H**$_2$), 3.60-3.44 (m, 13H, EG-chain-C**H**$_2$, C17-**H**), 3.44-3.33 (m, 2H, EG-chain-C**H**$_2$), 3.29 (t,

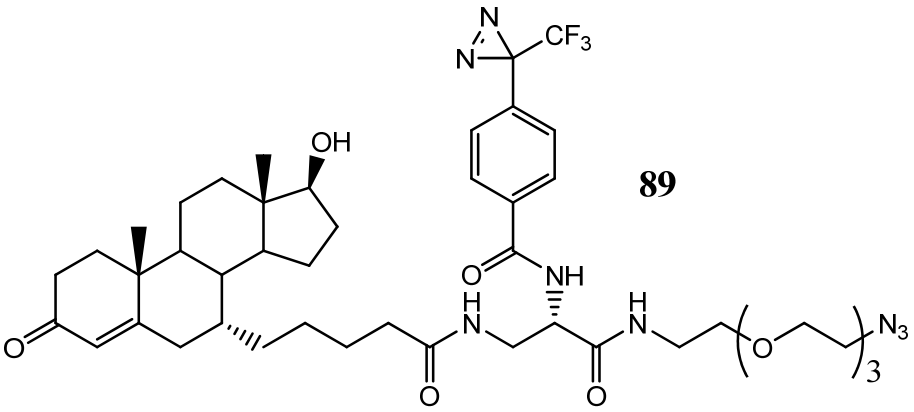
2H, J = 5.2 Hz, $N_3C\mathbf{H}_2$), 2.55 (s, broad, 1H, O**H**), 2.38-2.07 (m, 6H, scaffold), 2.01-1.88 (m, 2H, scaffold), 1.80-1.71 (m, 1H, scaffold), 1.69-1.53 (m, 4H, scaffold), 1.53-1.23 (m, 6H, scaffold), 1.23-0.87 (m, 7H, scaffold), 1.11 (s, 3H, C**H**$_3$), 0.70 (s, 3H, CH_3). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 199.2 (**C**3), 195.8 (**C**O-BP), 176.0 (**C**ON), 170.2, 169.8 (**C**5, **C**ON), 166.8 (**C**ON), 140.2, 136.9, 136.3, 132.9, 130.0, 130.0, 128.4, 127.3(BP-aryl), 125.5 (**C**4), 81.3 (**C**17), 70.5, 70.5, 70.4, 70.1, 69.9, 69.4 (EG-**C**H_2), 56.4 (**C**H-α), 50.5 (**C**H_2N_3), 47.2 (**C**9), 45.9 (**C**14), 42.8 (**C**13), 41.8 (**C**H_2N), 39.2 (7α-**C**H_2), 38.9 (**C**7), 38.6 (**C**10), 36.5 (**C**12), 36.2 (**C**1, **C**6), 36.1 (**C**8), 35.9 (**C**1, **C**6), 33.9 (**C**2), 30.1 (**C**16), 26.9, 25.8, 24.8 (7α-**C**H_2), 22.7 (**C**15), 20.8 (**C**11), 18.0 (**C**19), 10.9 (**C**18).



88

N-{(14S)-1-Azido-21-[(7α)-20,3-dioxopregn-4-en-7-yl]-13,17-dioxo-3,6,9-trioxa-12,16-diazahenicos-14-yl}-4-benzoylbenzamide **88**: According to general procedure **G** 150 mg of steroid methyl ester **17** (0.350 mmol) were saponified and directly coupled to 235 mg of the free ammonium derivative of photophore **65** (0.385 mmol; general procedure **F**) using 71 mg HOBt (0.525 mmol), 183 µl DIPEA (1.05 mmol) and 199 mg HBTU (0.525 mmol). Column chromatography with $CHCl_3$ containing 3.5% MeOH yielded 254 mg of triamide **88** (0.279 mmol, 80%) as colorless foam. R_f: 0.36 ($CHCl_3$+4% MeOH). $[\alpha]_D^{20}$= +34.3 (c = 1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{51}H_{68}N_6O_9Na$: 931.4940 found: 931.4936. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 8.58 (d, 1H, J = 5.8 Hz, CON**H**), 7.99-7.93 (m, 2H, BP), 7.82-7.77 (m, 2H, BP), 7.75-7.70 (m, 2H, BP), 7.58-7.53 (m, 1H, BP), 7.47-7.41 (m, 2H, BP), 7.29-7.20 (m, 1H, CON**H**), 7.04 (t, 1H, J = 6.1 Hz, CON**H**), 5.62 (s, broad, 1H, C4-**H**), 4.63-4.57 (m, 1H, α-C**H**), 3.77-3.62 (m, 2H, β-C$\mathbf{H}_2$), 3.61-3.44 (m, 12H, EG-chain-C$\mathbf{H}_2$), 3.44-3.35 (m, 2H, EG-chain-C$\mathbf{H}_2$), 3.30 (t, 2H, J = 5.2 Hz, $N_3C\mathbf{H}_2$), 2.49-2.41 (m, 1H, C17-**H**), 2.40-1.90 (m, 8H, scaffold), 2.05 (s, 3H, C**H**$_3$), 1.70-0.94 (m, 16H, scaffold), 1.12 (s, 3H, C**H**$_3$), 0.58 (s, 3H,

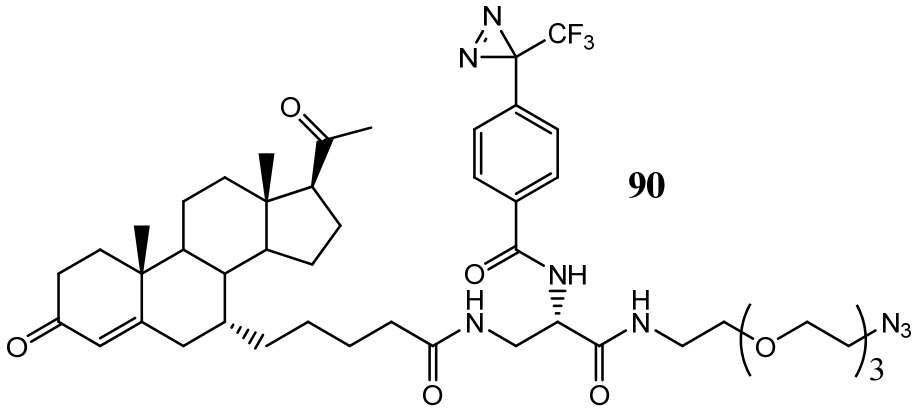
CH_3). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 209.1 (**C**20), 199.1 (**C**3), 195.8 (**C**O-BP), 176.0 (**C**ON), 169.8, 169.8 (**C**5, **C**ON), 166.9 (**C**ON), 161.7, 140.3, 136.9, 136.3, 132.9, 130.0, 130.0, 128.4, 127.3 (BP-aryl), 125.7 (**C**4), 70.6, 70.5, 70.5, 70.1, 69.9, 69.4 (EG-**C**H_2), 63.4 (**C**17), 56.4 (**C**H-α), 51.6 (**C**14), 50.6 (**C**H_2N_3), 46.9 (**C**9), 43.9 (**C**13), 41.9 (**C**H_2N), 39.2(**C**H_2N), 38.8 (**C**7), 38.6 (**C**10), 38.3 (**C**12), 36.7 (**C**8), 36.5, 36.3 (**C**6, 7α-**C**H_2), 35.9 (7α-**C**H_2), 33.9 (**C**2), 31.4 (**C**21), 26.9, 25.8 (7α-**C**H_2), 24.9 (**C**15), 23.7 (7α-**C**H_2), 22.7 (**C**1), 21.2 (**C**11), 18.0 (**C**19), 13.1 (**C**18).



89

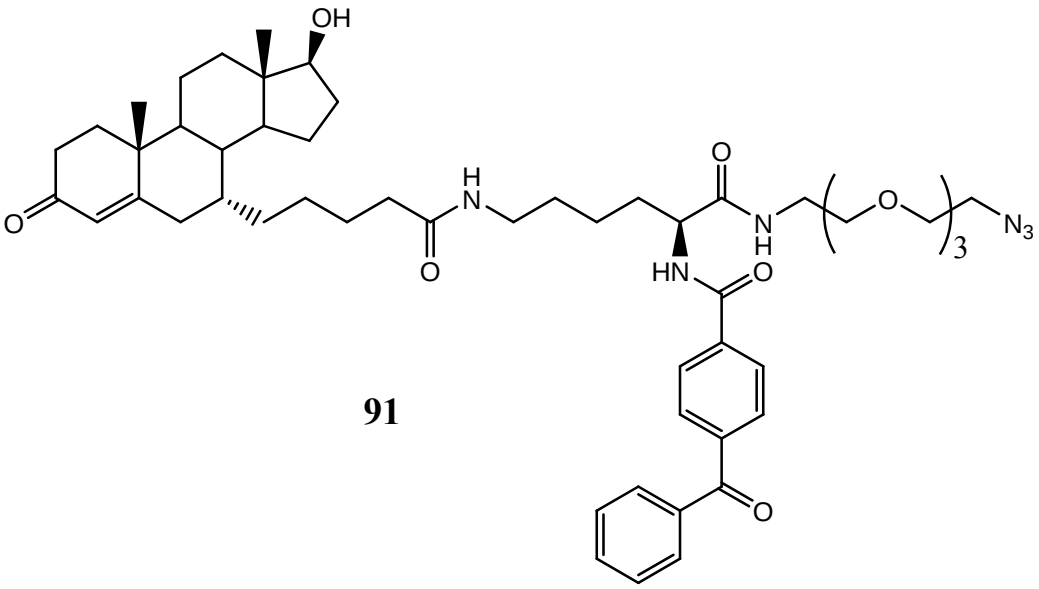
N-{(14S)-1-Azido-21-[(17β,7α)-17-hydroxy-3-oxoandrost-4-en-7-yl]-13,17-dioxo-3,6,9-trioxa-12,16-diazahenicos-14-yl}-4-[3-(trifluoromethyl)-3H-diazirin-3-yl]benzamide **89**: According to general procedure **G** 100 mg of steroid methyl ester **16** (0.257 mmol) were saponified and directly coupled to the corresponding free ammonium derivative of photophore **66** (235 mg, 0.385 mmol; general procedure **F**) using 52 mg HOBt (0.385 mmol), 134 µl DIPEA (0.771 mmol) and 146 mg HBTU (0.385 mmol). Column chromatography eluting with $CHCl_3$ containing 3% MeOH yielded 214 mg of triamide **89** (0.214 mmol, 83%) as colorless foam. R_f: 0.30 ($CHCl_3$+3% MeOH). $[\alpha]_D^{20}$= +5.5 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{44}H_{61}F_3N_8O_8Na$: 909.4457 found: 909.4448. HPLC: R_t: 8.966 min., 98.1%. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 8.54 (d, 1H, J = 5.9 Hz, CON**H**), 7.91-7.87 (m, 2H, aryl), 7.23 (t, 1H, J = 5.9 Hz, CON**H**), 7.21 (d, 2H, J = 8.2 Hz, aryl), 7.04 (t, 1H, J = 6.2 Hz, CON**H**), 5.62 (s, broad, 1H, C4-**H**), 4.60-4.54 (m, 1H, α-C**H**), 3.74-3.44 (m, 15H, EG-chain-C**H**$_2$, C17-**H**, β-C**H**$_2$), 3.43-3.35 (m, 2H, EG-chain-C**H**$_2$), 3.32 (t, 2H, J = 5.1 Hz, N_3C**H**$_2$), 2.50-2.08 (m, 7H, scaffold, O**H**), 2.05-1.93 (m, 2H, scaffold), 1.82-1.74 (m, 1H, scaffold), 1.73-0.91 (m. 18H, scaffold), 1.53-1.23 (m, 6H, scaffold), 1.15 (s, 3H, C**H**$_3$), 0.73 (s, 3H, CH_3). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 199.3 (**C**3), 176.1 (**C**ON), 170.2, 169.9 (**C**5, **C**ON), 166.6 (**C**ON), 134.3, 132.6, 127.9, 126.6 (aryl), 125.6 (**C**4), 121.9 (q, J = 275 Hz, **C**F_3) 81.5 (**C**17), 70.6, 70.5, 70.1, 70.0, 69.9, 69.5 (EG-**C**H_2), 56.5 (**C**H-α), 50.6 (**C**H_2N_3), 47.3 (**C**9),

46.0 (**C**14), 42.9 (**C**13), 41.9 (**C**H_2N), 39.2 (7α-**C**H_2), 39.0 (**C**7), 38.7 (**C**10), 36.6 (**C**12), 36.3 (**C**1, **C**6), 36.3 (**C**8), 36.0 (**C**1, **C**6), 34.0 (**C**2), 30.2 (**C**16), 28.4 (q, J = 40.8 Hz, **C**N_2CF_3), 26.9, 25.8, 24.8 (7α-**C**H_2), 22.8 (**C**15), 20.9 (**C**11), 18.1 (**C**19), 11.0 (**C**18).



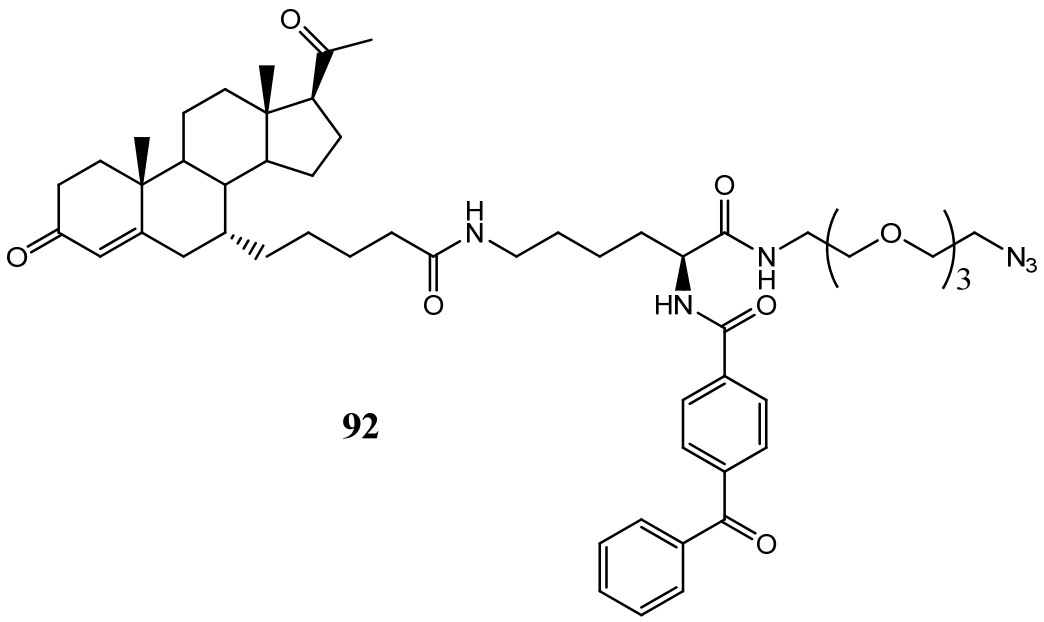
90

N-{(14S)-1-Azido-21-[(7α)-20,3-dioxopregn-4-en-7-yl]-13,17-dioxo-3,6,9-trioxa-12,16-diazahenicos-14-yl}-4-[3-(trifluoromethyl)-3H-diazirin-3-yl]-benzamide **90**: According to general procedure **G** 150 mg of steroid methyl ester **17** (0.350 mmol) were saponified and directly coupled to the corresponding ammonium derivative of photophore **66** (237 mg, 0.385 mmol; General procedure **F**) using 71 mg HOBt (0.525 mmol), 183 µl DIPEA (1.05 mmol) and 199 mg HBTU (0.525 mmol) following general procedure **A**. Column chromatography eluting with $CHCl_3$ containing 3.5% MeOH yielded 222 mg of triamide **90** (0.243 mmol, 69%) as colorless foam. R_f: 0.37 ($CHCl_3$+4% MeOH). $[\alpha]_D^{20}$= +32.0 (c=1.0, $CHCl_3$). Anal. calcd for $C_{46}H_{63}F_3N_8O_8$: N, 12.27; C, 60.51; H, 6.95. found: N, 11.84; C, 60.37; H, 7.09. FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{46}H_{63}F_3N_8O_8Na$: 935.4613 found: 935.4620. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 8.56 (d, 1H, J = 5.8 Hz, CON**H**), 7.88-7.84 (m, 2H, aryl), 7.24-7.16 (m, 3H, aryl/CON**H**), 7.09 (t, 1H, J = 6.1 Hz, CON**H**), 5.60 (s, broad, 1H, C4-**H**), 4.58-4.52 (m, 1H, α-C**H**), 3.71-3.60 (m, 2H, β-C$\mathbf{H}_2$), 3.60-3.41 (m, 12H, EG-chain-C$\mathbf{H}_2$), 3.41-3.32 (m, 2H, EG-chain-C$\mathbf{H}_2$), 3.30 (t, 2H, J = 5.2 Hz, N_3C$\mathbf{H}_2$), 2.50-2.43 (m, 1H, C17-**H**), 2.40-2.07 (m, 7H, scaffold), 2.05 (s, 3H, C$\mathbf{H}_3$), 2.02-1.91 (m, 2H, scaffold), 1.69-0.94 (m, 17H, scaffold), 1.12 (s, 3H, C$\mathbf{H}_3$), 0.59 (s, 3H, CH_3). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 209.2 (**C**20), 199.1 (**C**3), 176.1 (**C**ON), 169.9, 169.8 (**C**5, **C**ON), 166.5 (**C**ON), 134.2, 132.5, 127.8, 126.5 (aryl), 125.6 (**C**4), 121.8 (q, J = 275 Hz, **C**F_3), 70.5, 70.5, 70.4, 70.1, 69.9, 69.4 (EG-**C**H_2), 63.4 (**C**17), 56.5 (**C**H-α), 51.6 (**C**14), 50.6 (**C**H_2N_3), 46.9 (**C**9), 43.9 (**C**13), 41.8 (**C**H_2N), 39.1 (**C**H_2N), 38.8 (**C**7), 38.5 (**C**10), 38.4 (**C**12), 36.6 (**C**8), 36.5, 36.2 (**C**6, 7α-**C**H_2), 35.9 (7α-**C**H_2), 33.9 (**C**2), 31.4 (**C**21), 28.3 (q, J = 40.6 Hz, **C**N_2CF_3), 26.9, 25.8 (7α-**C**H_2), 24.9 (**C**15), 23.7 (7α-**C**H_2), 22.7 (**C**1), 21.2 (**C**11), 17.9 (**C**19), 13.1 (**C**18).



91

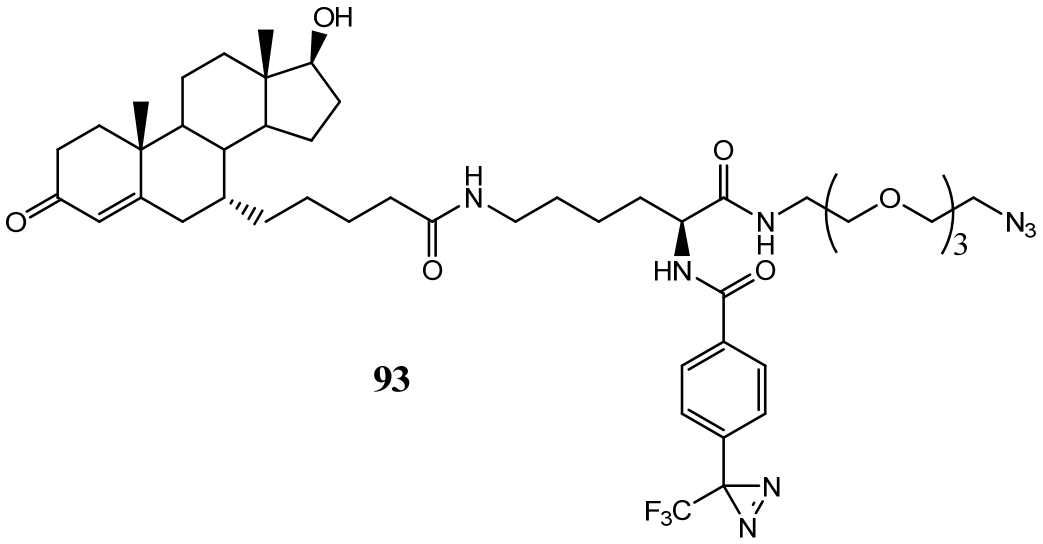
N-{(14S)-1-Azido-24-[(17β,7α)-17-hydroxy-3-oxoandrost-4-en-7-yl]-13,20-dioxo-3,6,9-trioxa-12,19-diazatetracos-14-yl}-4-benzoylbenzamide **91**: According to general procedure **G** 100 mg of steroid methyl ester **16** (0.257 mmol) were saponified and directly coupled to the corresponding free ammonium derivative of photophore **67** (185 mg, 0.283 mmol; General procedure **F**) using 52 mg HOBt (0.385 mmol), 134 µl DIPEA (0.771 mmol) and 146 mg HBTU (0.385 mmol) following general procedure **F**. Column chromatography eluting with $CHCl_3$ containing 4.5% MeOH yielded 176 mg of triamide **91** (0.190 mmol, 74%) as colorless foam. R_f: 0.35 ($CHCl_3$+5% MeOH). $[\alpha]_D^{20}$= +25.1 (c = 1.0, $CHCl_3$). Anal. calcd for $C_{52}H_{72}N_6O_7$: N, 9.08; C, 67.51; H, 7.84. found: N, 8.95; C, 67.04; H, 7.88. FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{52}H_{72}N_6O_9Na$: 947.5253 found: 947.5246. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 7.96-7.91 (m, 2H, BP), 7.79-7.70 (m, 4H, BP), 7.59-7.51 (m, 2H, BP, CON**H**), 7.48-7.41 (m, 2H, BP), 7.06 (t, 1H, J = 5.4 Hz, CON**H**), 6.12 (t, 1H, J = 5.5 Hz, CON**H**), 5.63 (s, 1H, C4-**H**), 4.65-4.57 (m, 1H, α-C**H**), 3.66-3.49 (m, 13H, EG-chain-C**H**$_2$, C17-**H**), 3.48-3.36 (m, 2H, EG-chain-C**H**$_2$), 3.33 (t, 2H, J = 5.0 Hz, N_3C**H**$_2$), 3.24-3.13 (m, 2H, β-C**H**$_2$), 2.54 (s, broad, 1H, O**H**), 2.41-2.29 (m, 2H, scaffold), 2.29-2.17 (m, 2H, scaffold), 2.15-1.73 (m, 7H, scaffold), 1.72-0.90 (m, 22H, scaffold), 1.14 (s, 3H, C**H**$_3$), 0.72 (s, 3H, CH_3). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 199.3 (**C**3), 195.9 (**C**O-BP), 173.3 (**C**ON), 171.7, 170.5 (**C**5, **C**ON), 166.5 (**C**ON), 140.2, 137.1, 137.0, 132.9, 130.0, 129.9, 128.4, 127.3 (BP-aryl), 125.6 (**C**4), 81.4 (**C**17), 70.6, 70.6, 70.5, 70.2, 70.0, 69.5 (EG-**C**H$_2$), 53.6 (**C**H-α), 50.6 (**C**H$_2$N$_3$), 47.2 (**C**9), 46.0 (**C**14), 42.9 (**C**13), 39.3 (7α-**C**H$_2$), 39.0 (**C**7), 38.8 (**C**H$_2$N), 38.7 (**C**10), 36.6, 36.6 (**C**12, **C**H$_2$N), 36.2 (**C**1, **C**6), 36.2 (**C**8), 35.9 (**C**1, **C**6), 34.0 (**C**2), 32.2 (Lys-**C**H$_2$), 30.2 (**C**16), 29.2 (Lys-**C**H$_2$), 26.9, 25.8, 24.8 (7α-**C**H$_2$), 22.8, 22.7 (**C**15, Lys-**C**H$_2$), 20.9 (**C**11), 18.1 (**C**19), 11.0 (**C**18).



92

N-{(14S)-1-Azido-24-[(7α)-20,3-dioxopregn-4-en-7-yl]-13,20-dioxo-3,6,9-trioxa-12,19-diazatetracos-14-yl}-4-benzoylbenzamide **92**: According to general procedure **G** 150 mg of steroid methyl ester **17** (0.350 mmol) were saponified and then coupled directly to the corresponding free ammonium derivative of photophore **67** (252 mg, 0.385 mmol; General procedure **F**) using 71 mg HOBt (0.525 mmol), 183 µl DIPEA (1.05 mmol) and 199 mg HBTU (0.525 mmol) following general procedure **A**. Column chromatography eluting with $CHCl_3$ containing 3% MeOH yielded 203 mg of triamide **92** (0.213 mmol, 61%) as colorless foam. R_f: 0.33 ($CHCl_3$+4% MeOH). $[\alpha]_D^{20}$= +42.5 (c = 1.0, $CHCl_3$). Anal calcd for $C_{54}H_{74}N_6O_9$: N, 8.84; C, 68.19; H, 7.84. found: N, 8.74; C, 68.19; H, 7.92. FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{54}H_{74}N_6O_9Na$: 973.5409 found: 973.5417. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 7.96-7.89 (m, 2H, BP), 7.78-7.68 (m, 4H, BP), 7.58-7.51 (m, 2H, BP/CON**H**), 7.47-7.39 (m, 2H, BP), 7.02 (t, broad, 1H, J = 5.4 Hz, CON**H**), 6.14 (t, 1H, J = 5.5 Hz, CON**H**), 5.62 (s, 1H, C4-**H**), 4.63-4.55 (m, 1H, α-C**H**), 3.62-3.48 (m, 12H, EG-chain-C$\mathbf{H}_2$), 3.45-3.36 (m, 2H, EG-chain-C$\mathbf{H}_2$), 3.31 (t, 2H, J = 5.0 Hz, $N_3C\mathbf{H}_2$), 3.24-3.11 (m, 2H, β-C$\mathbf{H}_2$), 2.50-2.42 (m, 1H, C17-**H**), 2.40-2.18 (m, 4H, scaffold), 2.15-1.75 (m, 7H, scaffold), 2.04 (s, 3H, CH_3) 1.69-0.95 (m, 21H, scaffold), 1.12 (s, 3H, C$\mathbf{H}_3$), 0.58 (s, 3H, CH_3). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 209.2 (**C**20), 199.1 (**C**3), 195.8 (**C**O-BP), 173.2 (**C**ON), 171.7, 170.1 (**C**5, **C**ON), 166.4 (**C**ON), 162.5, 140.1, 137.1, 136.9, 132.9, 130.0, 129.9, 128.4, 127.3 (BP-aryl), 125.7 (**C**4), 70.6, 70.6, 70.5, 70.2, 69.9, 69.5 (EG-**C**H_2), 63.4 (**C**17), 53.6 (**C**H-α), 51.5 (**C**14), 50.6 (**C**H_2N_3), 46.9 (**C**9), 43.9 (**C**13), 39.3 (7α-**C**H_2), 38.8 (**C**7), 38.7 (**C**H_2N), 38.6 (**C**10), 38.3 (**C**12), 36.6 (**C**8), 36.6, 36.4 (**C**6, 7α-**C**H_2), 35.9 (7α-**C**H_2), 34.0 (**C**2), 32.0

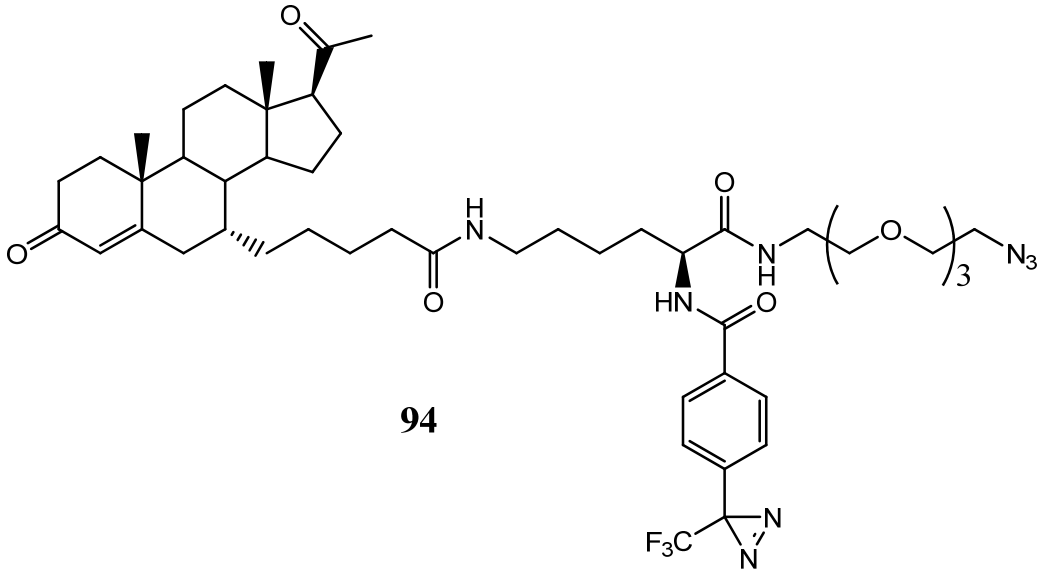
(Lys-**C**H_2), 31.4 (**C**21), 29.2 (Lys-**C**H_2), 26.9, 25.8 (7α-**C**H_2), 24.8 (**C**15), 23.7 (7α-**C**H_2), 22.7, 22.6 (**C**1, Lys-**C**H_2), 21.2 (**C**11) 18.0 (**C**19), 13.1 (**C**18).



93

N-{(14S)-1-Azido-24-[(17β,7α)-17-hydroxy-3-oxoandrost-4-en-7-yl]-13,20-dioxo-3,6,9-trioxa-12,19-diazatetracos-14-yl}-4-[3-(trifluoromethyl)-3H-diazirin-3-yl]-benzamide **93**: According to general procedure **G** 100 mg of steroid methyl ester **16** (0.257 mmol) were saponified and then directly coupled to the corresponding free ammonium derivative of photophore **68** (186 mg 0.283 mmol; General procedure **F**) using 52 mg HOBt (0.385 mmol), 134 µl DIPEA (0.771 mmol) and 146 mg HBTU (0.385 mmol) following general procedure **A**. Column chromatography eluting with $CHCl_3$ containing 3% MeOH yielded 206 mg of triamide **93** (0.222 mmol, 86%) as colorless foam. R_f: 0.30 ($CHCl_3$+3% MeOH). $[\alpha]_D^{20}$= +23.4 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{47}H_{67}F_3N_8O_8Na$: 951.4926 found: 951.4931. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 7.86-7.81 (m, 2H, aryl), 7.54 (d, 1H, J = 7.7 Hz, CON**H**), 7.14 (d, 2H, J = 8.2 Hz, aryl), 7.07 (t, 1H, J = 5.5 Hz, CON**H**), 6.15 (t, 1H, J = 5.7 Hz, CON**H**), 5.61 (s, 1H, C4-**H**), 4.59-4.52 (m, 1H, α-C**H**), 3.64-3.52 (m, 11H, EG-chain-C$\mathbf{H}_2$, C17-**H**), 3.49 (t, 2H, J = 5.3 Hz, EG-chain-C$\mathbf{H}_2$) 3.45-3.35 (m, 2H, EG-chain-C$\mathbf{H}_2$), 3.32 (t, 2H, J = 5.0 Hz, N_3C$\mathbf{H}_2$), 3.22-3.08 (m, 2H, ε-C$\mathbf{H}_2$), 2.55 (s, broad, 1H, O**H**), 2.41-2.29 (m, 2H, scaffold), 2.29-2.17 (m, 2H, scaffold), 2.11-1.91 (m, 4H, scaffold), 1.90-1.72 (m, 3H, scaffold), 1.72-1.29 (m, 13H, scaffold), 1.29-0.90 (m, 8H, scaffold), 1.14 (s, 3H, C$\mathbf{H}_3$), 0.72 (s, 3H, C$\mathbf{H}_3$). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 199.3 (**C**3), 173.3 (**C**ON), 171.8, 170.5 (**C**5, **C**ON), 166.1 (**C**ON), 134.9, 132.3, 127.8, 126.3 (aryl), 125.6 (**C**4), 121.9 (q, J = 274 Hz, **C**F_3), 81.4 (**C**17), 70.6, 70.5, 70.5, 70.2, 69.9, 69.5 (EG-**C**H_2), 53.6 (**C**H-α), 50.6 (**C**H_2N_3), 47.2 (**C**9), 46.0 (**C**14), 42.8 (**C**13), 39.3 (7α-**C**H_2), 39.0 (**C**7), 38.7 (**C**10), 36.6, 36.5 (**C**12, **C**H_2N), 36.2 (**C**1, **C**6), 36.2 (**C**8), 35.9 (**C**1, **C**6), 34.0 (**C**2), 32.0 (**C**16), 30.1 (**C**16),

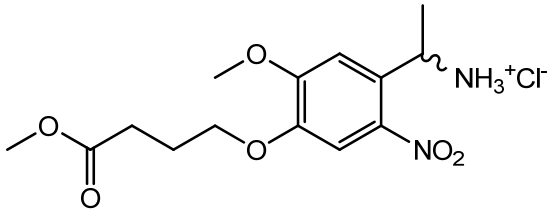
29.2 (Lys-**C**H_2), 28.3 (q, J = 40.5 Hz, **C**N_2CF_3), 26.9, 25.8, 24.8 (7α-**C**H_2), 22.8, 22.7 (**C**15, Lys-**C**H_2), 20.9 (**C**11), 18.0 (**C**19), 10.9 (**C**18).



94

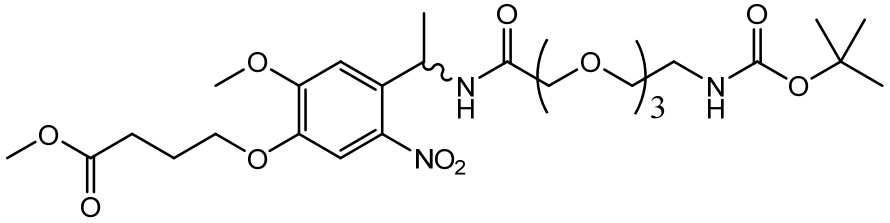
N-{(14S)-1-Azido-24-[(7α)-20,3-dioxopregn-4-en-7-yl]-13,20-dioxo-3,6,9-trioxa-12,19-diazatetracos-14-yl}-4-benzoylbenzamide **94**: According to general procedure **G** 150 mg of steroid methyl ester **17** (0.350 mmol) were saponified and directly coupled to the corresponding free ammonium derivative of photophore **68** (254 mg, 0.385 mmol; General procedure **F**) using 71 mg HOBt (0.525 mmol), 183 µl DIPEA (1.05 mmol) and 199 mg HBTU (0.525 mmol) following general procedure **A**. Column chromatography eluting with $CHCl_3$ containing 3.5% MeOH yielded 248 mg of triamide **94** (0.260 mmol, 74%) as colorless foam. R_f: 0.35 ($CHCl_3$+4% MeOH). $[\alpha]_D^{20}$= +33.2 (c = 1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{49}H_{69}F_3N_8O_8Na$: 977.5083 found: 977.5080. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 7.83-7.79 (m, 2H, aryl), 7.57 (d, 1H, J = 7.5 Hz, CON**H**), 7.13-7.09 (m, 2H, aryl), 7.06 (t, 1H, J = 5.5 Hz, CON**H**), 6.20 (t, 1H, J = 5.8 Hz, CON**H**), 5.59 (s, 1H, C4-**H**), 4.58-4.52 (m, 1H, α-C**H**), 3.61-3.43 (m, 12H, EG-chain-C$\mathbf{H}_2$), 3.43-3.31 (m, 2H, EG-chain-C$\mathbf{H}_2$), 3.29 (t, 2H, J = 5.2 Hz, N_3C$\mathbf{H}_2$), 3.20-3.06 (m, 2H, ε-C$\mathbf{H}_2$), 2.48-2.41 (m, 1H, C17-**H**), 2.38-2.27 (m, 2H, scaffold), 2,26-2.15 (m, 2H, scaffold), 2.13-1.91 (m, 5H, scaffold), 2.03 (s, 3H, CH_3) 1.89-1.71 (m, 2H, scaffold), 1.67-0.91 (m, 21H, scaffold), 1.10 (s, 3H, C$\mathbf{H}_3$), 0.57 (s, 3H, CH_3). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 209.2 (**C**20), 199.1 (**C**3), 173.2 (**C**ON), 171.7, 170.1 (**C**5, **C**ON), 166.1 (**C**ON), 134.9, 132.2, 127.8, 126.2, 126.2 (aryl), 125.6 (**C**4), 121.8 (q, J = 275 Hz, **C**F_3), 70.5, 70.5, 70.4, 70.1, 69.9, 69.4 (EG-**C**H_2), 63.4 (**C**17), 53.6 (**C**H-α), 51.5 (**C**14), 50.5 (**C**H_2N_3), 46.8 (**C**9), 43.9 (**C**13), 39.2 (7α-**C**H_2), 38.7 (**C**7), 38.6(**C**10), 38.5 (**C**H_2N), 38.3 (**C**12), 36.5, 36.5 (**C**6, 7α-**C**H_2, **C**8), 35.8 (7α-**C**H_2), 33.9 (**C**2), 31.8 (Lys-**C**H_2),

31.4 (**C**21), 29.1 (Lys-**C**H_2), 28.2 (q, J = 40.6 Hz, **C**N_2CF_3), 26.9, 25.8 (7α-**C**H_2), 24.8 (**C**15), 23.7 (7α-**C**H_2), 22.6, 22.6 (**C**1, Lys-**C**H_2), 21.1 (**C**11), 17.9 (**C**19), 13.0 (**C**18).



96

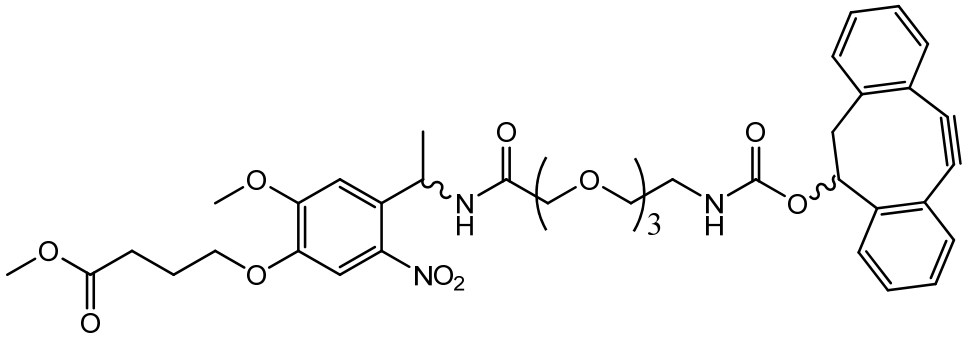
4-[2-Methoxy-5-nitro-4-(1-aminoethyl)phenoxy]butanoicacid methyl ester hydrochloride **96**: In a 250 ml round bottom flask equipped with a gas inlet and a stirring bar 8.2 g of trifluoroacetamide **95** [78] (20.1 mmol) were dissolved in 100 ml of a 0.36 M soln. of HCl in dry MeOH under an atmosphere of nitrogen.[207] The resulting mixture was refluxed for 36 h, cooled to RT and the volatiles removed in a stream of air. The residual white solid was triturated with diethyl ether, filtered and crystallized from ethanol to yield 6.77 g of pure title compound **96** (19.4 mmol, 97 %) as wooly colorless crystals. mp: 191.5°C (EtOH). FAB-MS: m/z 313 $[M\text{-}Cl^-]^+$, 296 $[M\text{-}NH_3\text{-}Cl^-]^+$. Anal. calcd for $C_{14}H_{21}ClN_2O_6$: N, 8.03; C, 48.21; H, 6.07; found: N, 7.87; C, 48.25; H, 6.21. ^{1}H-NMR (400.1 MHz, MeOH-d_4): δ 7.68 (s, 1H, aryl), 7.32 (s, 1H, aryl), 5.13 (q, 1H, J = 6.8 Hz, C**H**NH_4Cl), 4.14 (t, 2H, J = 6.1 Hz, aryl-OC**H**$_2$), 4.02 (s, 3H, C**H**$_3$), 3.68 (s, 3H, C**H**$_3$), 2.55 (t, 2H, J = 7.3 Hz, C**H**$_2$COOMe), 2.16-2.08 (m, 2H, C**H**$_2$), 1.73 (d, 3H, C**H**$_3$CHNH_4Cl). ^{13}C-NMR (100.6 MHz, MeOH-d_4): δ 175.3 (**C**OOMe), 155.6, 149.8, 142.6, 128.2, 110.8, 110.6 (aryl), 69.6 (aryl-O**C**H_2), 57.3 (aryl-O**C**H_3), 52.2 (COO**C**H_3), 47.6 (**C**HN), 31.2 (Bu-**C**H_2), 25.5 (Bu-**C**H_2), 20.0 (NCH**C**H_3).



98

4-[4-(14-tert.-Butoxycarbonylamino-4-oxo-6,9,12-trioxa-3-azatetradec-2-yl)-2-methoxy-5-nitrophenoxy]butanoic acid methyl ester **98**: In a 50 ml round bottom flask equipped with a stirring bar 922 mg of methyl ester **4** (2.87 mmol) were saponified according to the general procedure **C**, the crude free acid dissolved in 15 ml dry THF and transferred to a vessel equipped with a gas inlet and a stirring bar. Then 504 mg HOBt (3.73 mmol, 1.3 eq.) were added, and the solution cooled to -20°C. Subsequently 770 mg DCC (3.73 mmol, 1.3 eq.) were added and the solution stirred for 6 h at -20°C. Thereafter 1 g of nitroaromatic **96** (2.87 mmol, 1 eq.) was added together with 1.5 ml DIPEA (8.61 mmol, 3 eq.) and the mixture

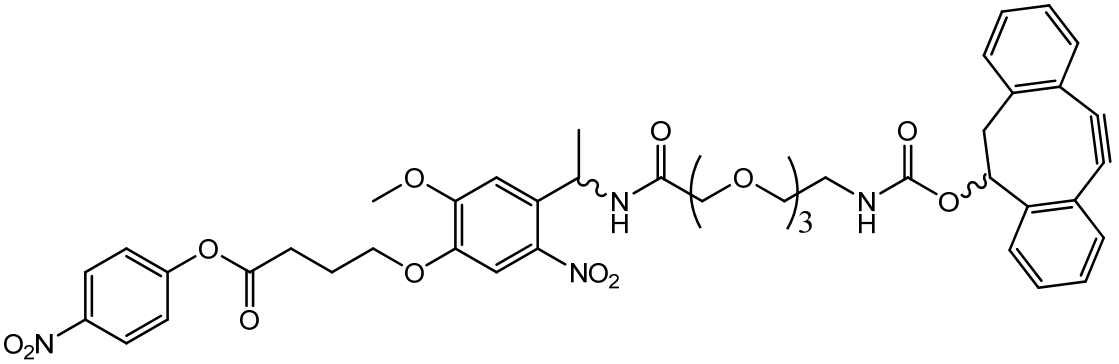
stirred for additional 14 h at RT. Then the precipitate of DC-urea was filtered off, the mixture transferred to a separatory funnel and diluted with EA. The organic layer was washed twice with 1 M $NaHSO_4$-soln. and once each wit sat. $NaHCO_3$-soln. and brine, dried over Na_2SO_4 and the solvent removed under reduced pressure. The residual viscous oil was subjected to column chromatography eluting with $CHCl_3$ containing 1% MeOH yielding 1.52 g of pure title compound **98** (2.60 mmol, 91 %) as pale yellow resin. R_f: 0.32 ($CHCl_3$+1% MeOH). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{27}H_{43}N_3O_{12}Na$: 624.2739 found: 624.2743. ^{1}H-NMR (400.1 MHz, $CDCl_3$): δ 7.63-7.57 (m, 1H, CON**H**), 7.52 (s, 1H, aryl), 6.96 (s, 1H, aryl), 5.65-5.56 (m, 1H, aryl-C**H**N), 4.96 (s, broad, 1H, OCON**H**), 4.06 (t, 2H, J = 6.2 Hz, aryl-OC**H**$_2$), 4.01 (d, 1H, J = -15.8 Hz, OC**H**$_2$CON), 3.89 (s, 3H, C**H**$_3$), 3.89 (d, 1H, J = -15.8 Hz, OC**H**$_2$CON), 3.74-3.58 (m, 8 H, EG-chain-C**H**$_2$), 3.66 (s, 3H, C**H**$_3$), 3.48 (t, 2H, J = 5.2 Hz, EG-chain-C**H**$_2$), 3.27-3.21 (m, 2H, C**H**$_2$NHBoc), 2.51 (t, 2H, J = 7.2 Hz, C**H**$_2$COOMe), 2.18-2.10 (m, 2H, C**H**$_2$), 1.53 (d, 3H, J = 6.9 Hz, C**H**$_3$CHN), 1.40 (s, 9H, *t*Bu). ^{13}C-NMR (100.6 MHz, $CDCl_3$): δ 173.4 (**C**OOMe), 169.3 (**C**ON), 156.0 (O**C**ONH), 153.9, 147.0, 140.6, 134.3, 110.4, 109.9 (aryl), 79.3 (*t*Bu), 71.1, 70.6, 70.4, 70.4, 70.3, 70.2 (EG-**C**H$_2$), 68.3 (aryl-O**C**H$_2$), 56.4 (aryl-O**C**H$_3$), 51.7 (COO**C**H$_3$), 46.1 (**C**HN), 40.4 (**C**H$_2$NHBoc), 30.4 (Bu-**C**H$_2$), 28.5 (*t*Bu), 24.3 (Bu-**C**H$_2$), 21.4 (aryl-NCH**C**H$_3$).



100

4-{4-[14-(11,12-Didehydro-5,6-dihydrodibenzo[a,e]cycloocten-5-oxycarbonylamino)-4-oxo-6,9,12-trioxa-3-azatetradec-2-yl)-2-methoxy-5-nitrophenoxy)butanoic acid methyl ester **100**: In a 50 ml round bottom flask equipped with a stirring bar 1.45 g of nitroaromatic **98** (2.41 mmol) were deprotected according to general procedure **F**. The corresponding ammonium derivative was then dissolved in 10 ml dry DMF in a round bottom flask equipped with a gas inlet and a stirring bar under an atmosphere of nitrogen. To the solution were added 1.26 ml DIPEA (7.23 mmol, 3 eq.) and 929 mg of activated carbonate **99** [36] (2.41 mmol, 1 eq.) and the mixture stirred at RT for 24 h. Thereafter the shiny yellow solution was diluted with EA, transferred to a separating funnel, washed two times with 1 M $NaHSO_4$-soln., three times with 5% Na_2CO_3-soln. and once with brine, dried over Na_2SO_4 and the solvent removed under

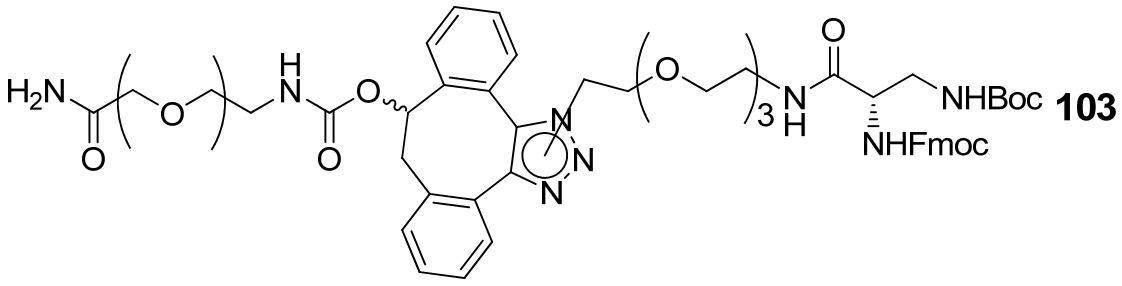
reduced pressure. The residual resin was subjected to column chromatography eluting with $CHCl_3$ containing 1.5 % MeOH yielding 1.62 g of pure title compound **100** (2.17 mmol, 90%) as pale yellow foam. R_f: 0.43 ($CHCl_3$+2% MeOH). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{39}H_{45}N_3O_{12}Na$: 770.2896 found: 770.2894. ^{1}H-NMR from the mixture of diastereomers (400.1 MHz, Benzol-d_6): δ 7.77 (t, 1H, J = 8.2 Hz, CON**H**), 7.71-7.62 (m, 1H, aryl), 7.34 (s, 1H, aryl), 7.30-7.11 (m, 4H, aryl), 7.08-6.91 (m, 4H, aryl), 6.41-6.34, 5.83-5.74 (m, m, 1H, Bn-C**H**O), 6.35, 5.91 (s, s, broad, 1H, OCON**H**), 6.05-5.94 (m, 1H, Bn-C**H**N), 4.10-2.71 (m, 22H), 3.90 (dd, 1H, J = -15.7 Hz/7.3 Hz, Bn-C**H**$_2$), 2.95 (dd, 1H, J = -14.9 Hz/3.8 Hz, Bn-C**H**$_2$), 2.29-2.22 (m, 2H, C**H**$_2$COOMe), 1.88-1.79 (m, 2H, C**H**$_2$), 1.51, 1.56-1.37 (d, m, 3H, J = 6.6 Hz, C**H**$_3$CHN). ^{13}C-NMR from the mixture of diastereomers (100.6 MHz, Benzol-d_6): δ 172.8 (**C**OOMe), 169.3 (**C**ON), 155.7 (O**C**ONH), 154.1, 153.0, 151.6, 147.5, 141.4, 134.6, 130.4, 128.1, 127.9, 127.3, 127.2, 127.0, 126.5, 126.2, 124.3, 124.2, 121.9, 113.6, 110.8, 110.6, 109.6 (aryl), 77.8, 77.1 (aryl-**C**HO), 70.9, 70.5, 70.1, 70.1, 70.0 (EG-**C**H$_2$), 67.8 (aryl-O**C**H$_2$), 55.8, 55.8 (aryl-O**C**H$_3$), 51.1 (COO**C**H$_3$), 46.8, 46.2 (**C**HN, aryl-**C**H$_2$), 41.1 (**C**H$_2$NHBoc), 30.4 (Bu-**C**H$_2$), 24.6 (Bu-**C**H$_2$), 21.4 (aryl-NCH**C**H$_3$).



101

4-{4-[14-(11,12-Didehydro-5,6-dihydrodibenzo[a,e]cycloocten-5-oxycarbonylamino)-4-oxo-6,9,12-trioxa-3-azatetradec-2-yl)-2-methoxy-5-nitrophenoxy)butanoic acid 4-nitophenyl ester **101**: In an 25 ml round bottom flask 1.54 g of nitroaromatic **100** (2.06 mmol) were dissolved in 4.5 ml THF/MeOH 1:1. To the solution were added 184 mg LiOH*H_2O (4.39 mmol, 2.1 eq.) dissolved in 4.5 ml water and the mixture stirred for 90 min at RT.[36] Then the solution was acidified by adding 1 M $NaHSO_4$-soln. and transferred to a separatory funnel. The aq. layer was extracted five times with DCM, the organic layer dried over Na_2SO_4 and the solvent evaporated under reduced pressure. Subsequently, in a 25 ml round bottom flask equipped with a gas inlet and a stirring bar the crude acid was dissolved in 10 ml dry THF under an atmosphere of nitrogen. To the solution were added 315 mg *p*-nitrophenol (2.27 mmol, 1.1 eq.) and after cooling to -20°C 638 mg DCC (3.09 mmol, 1.5 eq.). The mixture was then

stirred for 6 h at -20°C and additional 14 h at RT. After filtration of the precipitated DC-urea the solution was diluted with EA, transferred to a separating funnel, washed twice with 1N-$NaHSO_4$-soln. and once each with sat. $NaHCO_3$ and brine. After removal of the solvent under reduced pressure, the yellow residue was subjected to column chromatography eluting with Tol/acetone 7:3 yielding 1.05 g of pure title compound **101** (1.23 mmol, 60%) as pale yellow foam. R_f: 0.34 (Tol/acetone). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{44}H_{46}N_4O_{14}Na$: 877.2903 found: 877.2905. ^{1}H-NMR from the mixture of diastereomers (400.1 MHz, Benzol-d_6): δ 7.84-7.75 (m, 3H, *p*NP, CON**H**), 7.66-7.55 (m, 1H, aryl), 7.38 (s, 1H, aryl), 7.28-7.09 (m, 4H, aryl), 7.06-6.89 (m, 4H, aryl), 6.79-6.73 (m, 2H, *p*NP) 6.38-6.33, 5.75-5.66 (m, m, 1H, Bn-C**H**O), 6.30, 5.85 (s, s, broad, 1H, OCON**H**), 6.03-5.93 (m, 1H, Bn-C**H**N), 4.08-2.70 (m, 19H), 3.88 (dd, 1H, J = -15.8 Hz/5.1 Hz, Bn-C**H**$_2$), 2.92 (dd, 1H, J = -14.9 Hz/3.8 Hz, Bn-C**H**$_2$), 2.41 (t, 2H, J = 7.1 Hz, C**H**$_2$COOMe), 1.94-1.80 (m, 2H, C**H**$_2$), 1.50, 1.56-1.34 (d/m, 3H, J = 6.6 Hz, C**H**$_3$CHN). ^{13}C-NMR from the mixture of diastereomers (100.6 MHz, Benzol-d_6): δ 170.2 (**C**OOMe), 169.4 (**C**ON), 155.8, 155.5, 154.1 (O**C**ONH/aryl), 153.0, 151.6, 147.4, 145.5, 141.4, 135.1, 130.4, 128.1, 127.9, 127.4, 127.3, 126.6, 126.3, 125.1, 124.3, 124.2, 122.4, 121.9, 113.6, 110.7, 110.6, 109.7 (aryl), 77.8, 77.2 (aryl-**C**HO), 70.9, 70.8, 70.6, 70.5, 70.1, 70.1 (EG-**C**H_2), 67.7 (aryl-O**C**H_2), 55.8 (aryl-O**C**H_3), 46.7, 46.1 (**C**HN, aryl-**C**H_2), 41.2 (**C**H_2NHBoc), 31.0 (Bu-**C**H_2), 24.5 (Bu-**C**H_2), 21.5 (aryl-NCH**C**H_3).



103

{12-[[[8,9-dihydro-1-[2-[2-[2-[[3-(tert.-butoxycarbonyl)amino-2-[(9-fluorenyl)methoxycarbonyl]aminopropanamido]ethoxy]ethoxy]ethoxy]ethyl]-1H-dibenzo[3,4:7,8]cycloocta[1,2-d]triazol-9-yl]oxy]carbonyl]amino}-3,6,10-trioxadodecanoic acid amide **and** *{12-[[[8,9-dihydro-3-[2-[2-[2-[[3-(tert.-butoxycarbonyl)amino-2-[(9-fluorenyl)methoxycarbonyl]aminopropanamido]ethoxy]ethoxy]ethoxy]ethyl]-1H-dibenzo[3,4:7,8]cycloocta[1,2-d]triazol-9-yl]oxy]carbonyl]amino}-3,6,10-trioxadodecanoic acid amide* **103**: Triazole-loaded TOYOPEARL® was irradiated according to the procedure described above and the remaining solid-support filtered off. Then dioxane was removed from the supernatant under reduced pressure and the aqueous residue transferred to a separatory funnel. The aq. layer was extracted five times with DCM, the combined organic layers dried

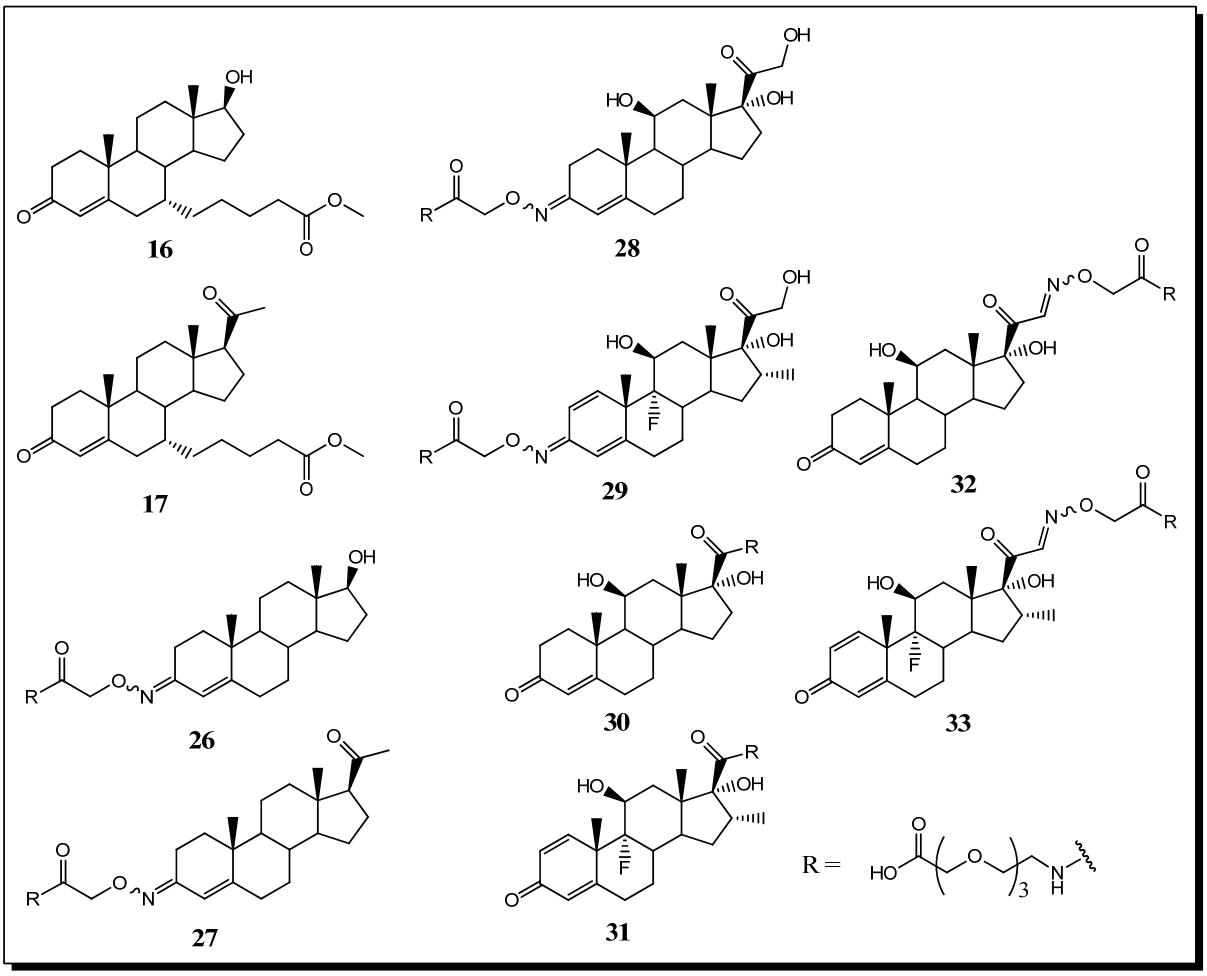
over Na_2SO_4 and the solvent removed under reduced pressure leaving **103** as colorless foam in analytical purity. R_f: 0.34 ($CHCl_3$+6%MeOH). $[\alpha]_D^{20}$= -5.3 (c=1.0, $CHCl_3$). FT-ICR-MS: m/z $[M+Na]^+$ calcd for $C_{56}H_{70}N_8O_{14}Na$: 1101.4904 found: 1101.4900. HPLC: 8.65 min., 98.2%. ^{1}H-NMR from the mixture of regioisomers/diastereomers (400.1 MHz, $CDCl_3$): δ 7.74 (d, 2H, J = 7.6 Hz, Fmoc), 7.62-7.46 (m, 4H, Fmoc, aryl), 7.45-7.34 (m, 2H, aryl), 7.38 (t, 2H, J = 7.4 Hz, Fmoc), 7.33-6.99 (m, 7H, Fmoc, aryl, CON**H**), 6.37 (s, broad, 1H, CON**H**), 6.25-6.15 (m, 1H, CON**H**), 6.06, 5.98-5.91 (m, 1H, CONH), 5.71-5.62, 5.60-5.49 (m, 1H, OCON**H**), 4.76-4.67, 4.63-4.41 (m, 2H, Bn-C**H**O, α-C**H**), 4.41-4.24 (m, 3H, Fmoc-OC**H**$_2$, C**H**$_2$), 4.19 (t, 1H, J = 7.2 Hz, Fmoc-C**H**), 4.05-3.84 (m, 4H, OC**H**$_2$CONH$_2$, Bn-C**H**$_2$), 3.74-3.17/3.01 (m, t, 28H, J = 12.1 Hz, EG-C**H**$_2$,β-C**H**$_2$), 1.42 (s, 9H, *t*Bu). ^{13}C-NMR from the mixture of regioisomers/diastereomers (100.6 MHz, $CDCl_3$): δ 173.4, 170.3 (**C**ONH), 157.2, 156.7, 156.0, 155.4 (O**C**ONH), 147.9, 146.3 (aryl), 143.9, 143.9, 141.4 (Fmoc), 137.3, 135.8, 135.6, 134.6, 134.4, 133.6, 132.8, 132.2, 131.5, 130.3, 129.9, 129.6, 129.4, 129.1, 128.9, 128.7, 128.6 (aryl), 127.8 (Fmoc), 127.7, 127.3 (aryl), 127.2 (Fmoc), 126.8, 126.5, 125.3 (Fmoc), 124.9, 124.5 (aryl), 120.1 (Fmoc), 79.9 (*t*Bu), 71.7, 71.1, 71.0, 70.7, 70.6, 70.4, 70.4, 70.3, 70.2, 70.2, 70.0, 69.7, 69.2 (EG-**C**H$_2$), 67.3 (Fmoc-OC**H**$_2$), 56.1 (α-**C**H), 48.6, 48.3, 48.2 (**C**H$_2$N), 47.2 (Fmoc-**C**H), 43.3, 42.9, 40.9, 39.4, 37,9, 37.3 (**C**H$_2$N), 28.4 (*t*Bu).

7 Summary

Aim of the given work was to synthesize hormonally active derivatives of hydrocortisone, dexamethasone, testosterone and progesterone for immobilization on solid supports. The obtained affinity matrices ought to be used for the affinity purification of steroid binding proteins from various protein sources. Further a strategy for selective photoaffinity labeling of steroid-binding proteins and catch-and-release of the cross linked proteins ought to be developed. Ultimately this should lead to the identification of novel steroid hormone targets, responsible for some of the non-genomic effects deployed by this class of agents.

For the affinity purification of different protein sources two distinct sets of steroid conjugates were synthesized. One of these sets was utilized for immobilization on amino functionalized solid supports, the other for photoaffinity labeling of target proteins in homogenous phase. The steroidal carboxylic acids for immobilization were the 3-*O*-(carboxymethyl)oximes (3-CMOs) and the steroid 7α-yl pentanoic acids of testosterone (T) and progesterone (P_4), respectively. The 3-CMOs were prepared according to literature procedures. The T- and P_4-7α-derivatives **16** and **17** (Scheme 28) were prepared starting from the respective Δ4,6-didehydrosteroids in an overall yield of 23 % (5 steps) and 29 % (3 steps), respectively. Further, the corresponding 17β-carboxylic acids and the 3- and 21-*O*-(carboxymethyl)oximes of hydrocortisone (HC) and dexamethasone (Dex) were prepared. The HC and Dex 17β-carboxylic acids and the 3-CMOs were obtained according to the literature procedures. HC- and Dex-21-CMO was obtained in a one-pot procedure via oxidation-oxime ligation in 78 % and 88 % yield, respectively. In the initial affinity purification experiments the T- and P_4-3-CMOs and 7α carboxylic acids were directly coupled to amino functionalized Sepharose® beads or TOYOPEARL® via standard amide coupling procedures. The obtained affinity matrices were then employed in the affinity purification of PBMC-, PMNL-cell and platelet lysates. In order to reduce non-specific binding in the subsequent experiments, the corresponding glucocorticoid 17β-caboxylic acids, 3- and 21-*O*-(carboxymethyl)oximes were conjugated to an amino functionalized carboxy-terminated tetraethylene glycol linker via peptide coupling. Thereby the steroid-ethylene glycol conjugates **26-33** (Scheme 29) were obtained in overall yields of 51-73 % (2 steps each). In a test system based on nuclear receptor translocation of a GFP-GR chimera ,the glucocorticoid 3-CMOs **28** and **29** proved to be highly active, the 21-CMOs **32** and **33** moderately active and the 17β-carboxamides **30** and **31** were found to be inactive. The carboxylic acid derivatives **16** and T-3-CMO were found to possess high androgen activity in an assay based on androgen induced ERK-phosphorylation.

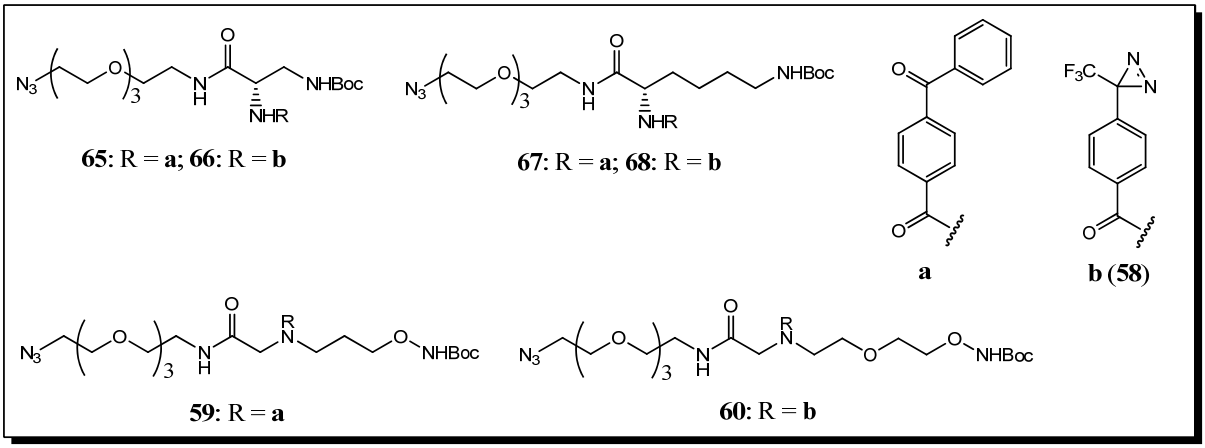
The progestin activity of **17** and P_4-3-CMO could not be established so far. The ethylene glycol conjugates were immobilized on TOYOPEARL® like described above and employed in affinity purification of SILAC-derived human lung cancer A549 cell lysates. LC-MS/MS analysis of the obtained protein samples was performed at the Proteome Center Tübingen. In order to enhance the sample quality regarding protein amount and specificity, a second series of steroid conjugates bearing an azide tag and a photo reactive moiety for photoaffinity labeling of steroid-binding proteins was prepared.



Scheme 29: Steroidal carboxylic acids for immobilization on amino functionalized solid supports and subsequent utilization in affinity purification experiments.

To this end, four building blocks bearing either an amine moiety for attachment of bioactive ligands via amide coupling or an aminooxy function for oxime ligation with ligands possessing a suitable carbonyl function were prepared. The synthesis of the amino series started from α-Fmoc-β-Boc-L-2-aminoalanine and α-Fmoc-ω-Boc-L-lysine and proceeded via amide coupling with 2-{2-[2-(azidoethoxy)ethoxy]ethoxy}amine, Fmoc-deprotection and amide coupling with the photophores 4-benzoylbenzoic acid or 4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzoic acid furnishing the protected building blocks **65-68** (Scheme 30) in

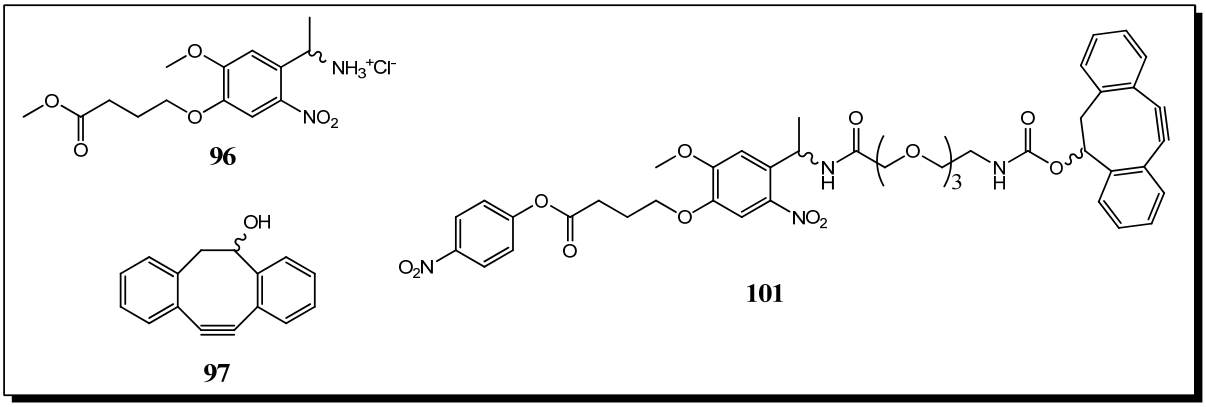
overall yields of 58-61 % (3 steps each). Synthesis of the aminooxy series started with the respective *N*-(3-hydroxypropyl)glycine ester and *N*-(5-hydroxy-3-oxapentyl)glycine ester via Alloc-protection, substitution of the alcohol with bromide or iodide, nucleophilic substitution with *N*-BocNHOH/DBU, saponification of the ester, amide formation with 2-{2-[2-(azidoethoxy)ethoxy]ethoxy}amine, Alloc-deprotection and amide coupling with the photophore 4-(3-(trifluoromethyl)-3H-diazirin-3-yl)benzoic acid furnishing the protected building blocks **59** and **60** (Scheme 30) in overall yields of 25 % and 26 % (6 steps each), respectively. A small library of 16 sex-hormone photoaffinity probes was obtained via HBTU mediated amide coupling of the Boc-deprotected amine-series building blocks **65**-**68** (Scheme 30) with the T- and P_4-3-CMOs and the 7α-pentanoic acid esters **16** and **17** (Scheme 29).



Scheme 30: Building blocks for synthesis of the steroid photoaffinity probes

Thereby the photoreactive steroid probes were obtained in 61-96 % yield. Another small library of 8 glucocorticoid-3- and 21-CMO photoaffinity probes was obtained by oxime ligation of the Boc-deprotected oxyamine-series building blocks **59** and **60** (Scheme 29) from HC, Dex, HC-21-aldehyde and Dex-21-aldehyde, respectively. The corresponding conjugates were thereby obtained in 43-78 % yield. The obtained photoreactive steroidal probes were intended to be employed for labeling of target proteins in homogenous phase. To facilitate isolation, purification and recovery of the azide tagged proteins, a cleavable linker system based on the photolabile *o*-nitrobenzyl linker **95** (Scheme 31) and the strained dibenzocyclooctyne derivative **97** (Scheme 31) was synthesized. Hereby, the strained alkyne facilitates the capture of azide tagged biomolecules onto solid supports via strain-promoted 1,3-dipolar addition reaction rendering a stable triazole. After stringent washing of the beads the covalently bound target proteins can be liberated by irradiation with UV-light of 365 nm wavelength. For the synthesis of the construct **101** (Scheme 31) the two functional fragments were fused to each other by a tetraethylene glycol linker and the methyl ester transformed to

the activated *p*NP-ester furnishing the photo-labile strained alkyne construct in 48 % overall yield (4 steps). The construct was then immobilized on TOYOPEARL®-AF-650 amino beads. Via photometric measurements it could be shown, that the beads functionalized with photo-labile strained alkyne are capable of effectively capturing azide functionalized molecules from solution (reaction complete after 30 min). Afterwards the corresponding triazole could be liberated via irradiation with UV-light of 365 nm wavelength (85 % recovery rate after 40 min). Thus, the linker construct in combination with the photoreactive steroidal probes allows for purification of biomolecules in a two-step process involving irradiation and the strain promoted "Click"-reaction. This obviates the addition of any chemical reagent in course of the affinity purification experiment.



Scheme 31: Photocleavable linker **96** and strained alkyne **97** for build-up of the construct **101** utilized in catch-and-release of azide tagged biomolecules.

The conventional "fishing"-experiments with the sex-hormones yielded Hsp27, vimentin, visfatin and karyopherin β as potential target structures of testosterone and progesterone. Preliminary SILAC-experiments could not provide any additional information about the selectivity of the protein-steroid interaction. However the SILAC data revealed a series of promising target candidates which are subject of current investigations. The photo affinity experiments are not completed at the present moment. Experiments with the photo affinity probes using the catch-and-release strategy developed in course of this work are currently conducted by Dr. Carlo Pergola in the laboratories of Prof. Dr. Werz, Jena.

8 Zusammenfassung und Erklärung

Die Zielsetzung der vorliegenden Arbeit war die Entwicklung und Verbesserung Affinitäts-Chromatographie basierter Methoden für die Bestimmung der zellulären Zielstrukturen endogener und synthetischer Steroidhormone. Steroidhormone sind mit die wichtigsten Regulatoren physiologischer Prozesse in Säugetieren. Darüber hinaus sind Wirkstoffe basierend auf Steroidhormonen für die Behandlung verschiedener Krankheiten, welche im Zusammenhang mit der Deregulierung des Hormonhaushaltes, der Immunabwehr oder entzündlicher Prozesse stehen, unersetzlich. Bei der Behandlung mit Wirkstoffen welche hormonelle Aktivität aufweisen kommt es häufig zu Nebenwirkungen unterschiedlicher Schweregrade da diese Wirkstoffe in das physiologische Hormongleichgewicht des Patienten eingreifen. Obgleich die Wirkmechanismen der Steroidhormone betreffend der Regulation von Gen Transkription und Expression vermittelt durch den jeweiligen spezifischen nuklearen Rezeptor sehr genau untersucht und verstanden wurden, sind die Effekte herbeigeführt durch Wechselwirkung mit anderen zelluläre Zielstrukturen bisher nur unzureichend aufgeklärt. Einer der hauptsächlichen Gründe hierfür ist die Nicht-Kenntnis über die Identität dieser Zielstrukturen. In der jüngeren Vergangenheit wurde ein erheblicher wissenschaftlicher Aufwand betrieben die Zielstrukturen verschiedener pharmazeutischer Wirkstoffe als auch kleiner endogener Moleküle aufzufinden. Häufig angewandt werden hierbei Reinigungsmethoden basierend auf der Affinität des untersuchten Agens zu seinem natürlichen Substrat, welche die Isolierung der betreffenden Zielstrukturen, meist Proteine, ermöglicht. Die Untersuchung und Identifizierung der erhaltenen Proteine geschieht hierbei durch Gel-Elektrophorese, Antikörper Reaktionen und in zunehmenden Maße durch massenspektrometrische Verfahren. Da für endogene als auch synthetische Steroidhormone bisher noch keine systematische Studie betreffend der Aufklärung ihrer zellulären Zielstrukturen berichtet wurde, hat man sich an dieser Stelle zum Ziel gesetzt mittels Affinitäts-basierter Reinigungsmethoden und der Anwendung moderner massenspektrometrischen Messtechniken den Grundriss des „Drug-Interactome“ der Steroidhormone zu zeichnen. Zu eben diesem Zweck wurde eine Serie von Steroidderivaten abgeleitet von den Geschlechtshormonen Testosteron und Progesteron sowie den Glucocorticoiden Hydrokortison und Dexamethason synthetisiert, welche sich für die Anbindung an Amino-funktionalisierte polymere Harze mittels standardisierter Peptid-chemischer Methoden eignen. Einführung einer Carboxyl-Funktion in das Steroidgerüst der Stammverbindungen wurde hierbei durch Oxim-Bildung mit *O*-(Caroxymethyl)oxyamin an

den Positionen 3 und 21, durch oxidativen Abbau der C17 Seitenkette zum entsprechenden 17-Carboxylat oder durch anknüpfen eines Pentansäure-Restes an Position 7α des Steroidgerüstes bewerkstelligt. In den ersten Experimenten wurden die so erhaltenen Steroid-Karbonsäuren auf Amino-funktionalisierter Sepharose® oder TOYOPEARL® immobilisiert und die Affinitätsmatrizes zur Reinigung verschiedener Zell-Lysate eingesetzt. Um in vorangeschrittenen Versuchen die Menge an unspezifisch gebundenen Proteinen zu reduzieren, wurde eine weitere Serie von Steroidderivaten hergestellt, welche vor der Immobilisierung mit einen entsprechenden Amino-/Carboxyl-funktionalisierten Tetraethylenglykol-Linker konjugiert wurden. Die erhaltenen Derivate wurden dann für die Affinitäts-Reinigung natürlicher als auch Isotopenmarkierter (SILAC) Zell-Lysate eingesetzt. Um die Probenqualität in Hinblick auf Verteilung und Spezifität der isolierten Proteine weiter zu verbessern, wurde zusätzlich eine Reihe von Foto-reaktiven Steroidderivaten hergestellt. Diese Derivate sollten zur kovalenten Konjugation der Zielstrukturen unter physiologischen Bedingungen in homogener Phase dienen. Die selektive Anreicherung der kovalent Verknüpften Biomoleküle sollte dabei über einen Azid-Rest an den reaktiven Steroid-Derivaten ermöglicht werden. Dieser kann chemoselektiv und ohne Zugabe zusätzlicher Reagenzien in einer spannungsvermittelten Huisgen 1,3-Cycloadditions Reaktion zwischen dem Cyclooktin-Derivat und dem Azid in ein stabiles Triazol überführt und auf diese Weise auf einem entsprechend derivatisierten polymeren Harz eingefangen werden. Die Abtrennung nicht kovalent gebundener Proteine ist dabei leicht zu bewerkstelligen. Um die eingefangenen Proteine wieder von der polymeren Matrix abzuspalten bedarf es eines spaltbaren Linker-Moleküls. In der vorliegenden Arbeit wurde hierfür ein Foto-sensitiver Linker auf *o*-Nitrobenzyl-Basis verwendet. Die Untersuchungen zu den Foto-reaktiven Steroidderivaten und der Isolierung/Identifizierung möglicher Zielstrukturen dauern allerdings derweil noch an. In den konventionellen „Fishing"-Experimenten durchgeführt von Herrn Dr. Felix Behnke ergaben sich das Chaperon Heat Shock Protein 27 (Hsp27), das Strukturprotein Vimentin, Visfatin und das mit der nuklearen Import-Maschinerie assoziierte Karyopherin β als mögliche Zielstrukturen der Geschlechtshormone. Eine eindeutige Spezifität für eines der beiden untersuchten Geschlechtshormone konnte durch Gel-Elektrophorese/Western-Blot Analyse für Hsp27,Vimentin und Visfatin, nicht aber für Karyopherin β nachgewiesen werden. Anhand der durchgeführten SILAC-Experimente konnten betreffend der genannten Target-Kandidaten keine eindeutigen Schlüsse gezogen werden. Stattdessen konnten den SILAC-Datensätzen für die Geschlechtshormone als auch für die Glucocorticoide eine Reihe sehr aussichtreicher Kandidaten mit hoher Spezifität für eines der Steroidhormone

entnommen werden. Die Validierung dieser möglichen Zielstrukturen ist Gegenstand andauernder Untersuchungen.

Ich versichere, dass ich die von mir vorgelegte Dissertation selbstständig angefertigt, die benutzten Quellen und Hilfsmittel vollständig angegeben und diejenigen Stellen der Arbeit, einschließlich Tabellen und Abbildungen, die anderen Werken im Wortlaut oder dem Sinn nach entnommen sind in jedem Einzelfall als Entlehnung kenntlich gemacht habe; dass diese Dissertation noch keiner anderen Fakultät oder Universität zur Prüfung vorgelegen hat; dass sie, abgesehen von den unten angegebenen Teilpublikationen, noch nicht veröffentlich worden ist sowie, dass ich eine solche Veröffentlichung vor Abschluss des Promotionsverfahrens nicht vornehmen werde.

Die Bestimmungen der Promotionsordnung der Mathematisch-Naturwissenschaftlichen Fakultät der Universität Tübingen sind mir bekannt. Die von mir vorgelegte Dissertation ist von Prof. Dr. Thomas Ziegler betreut worden.

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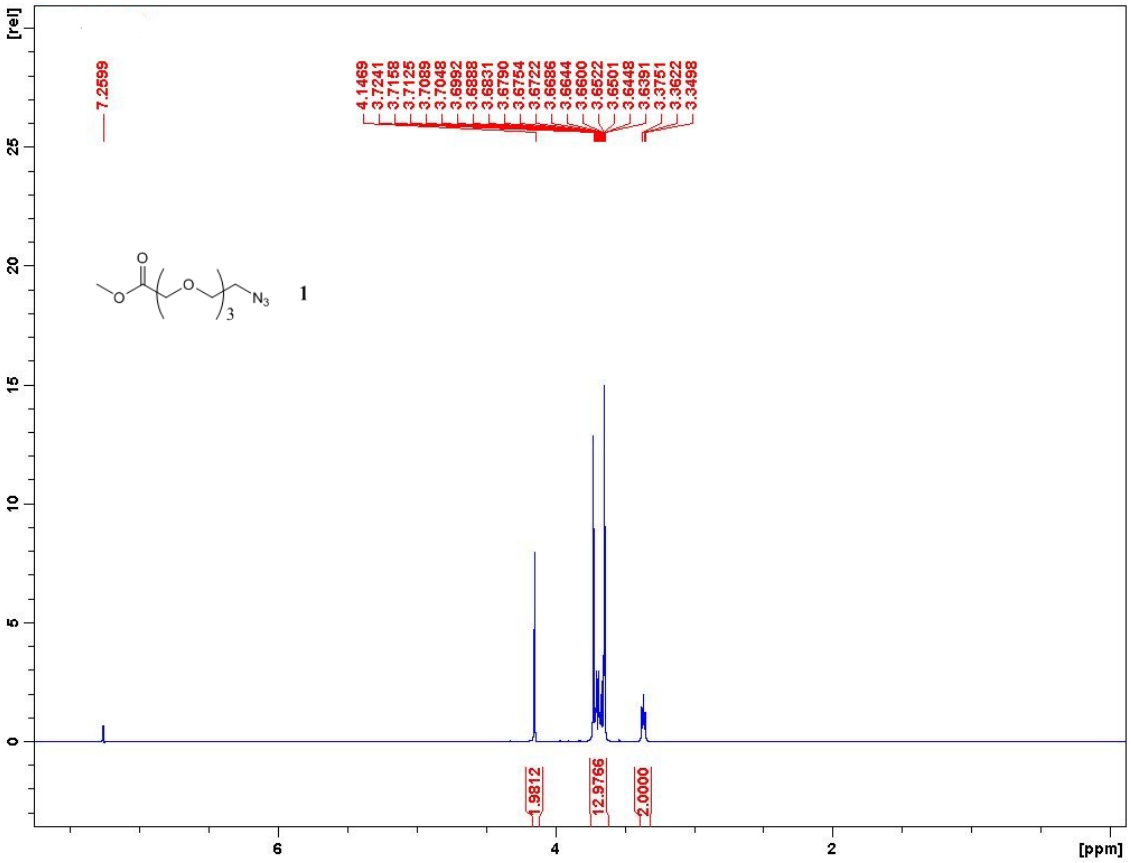
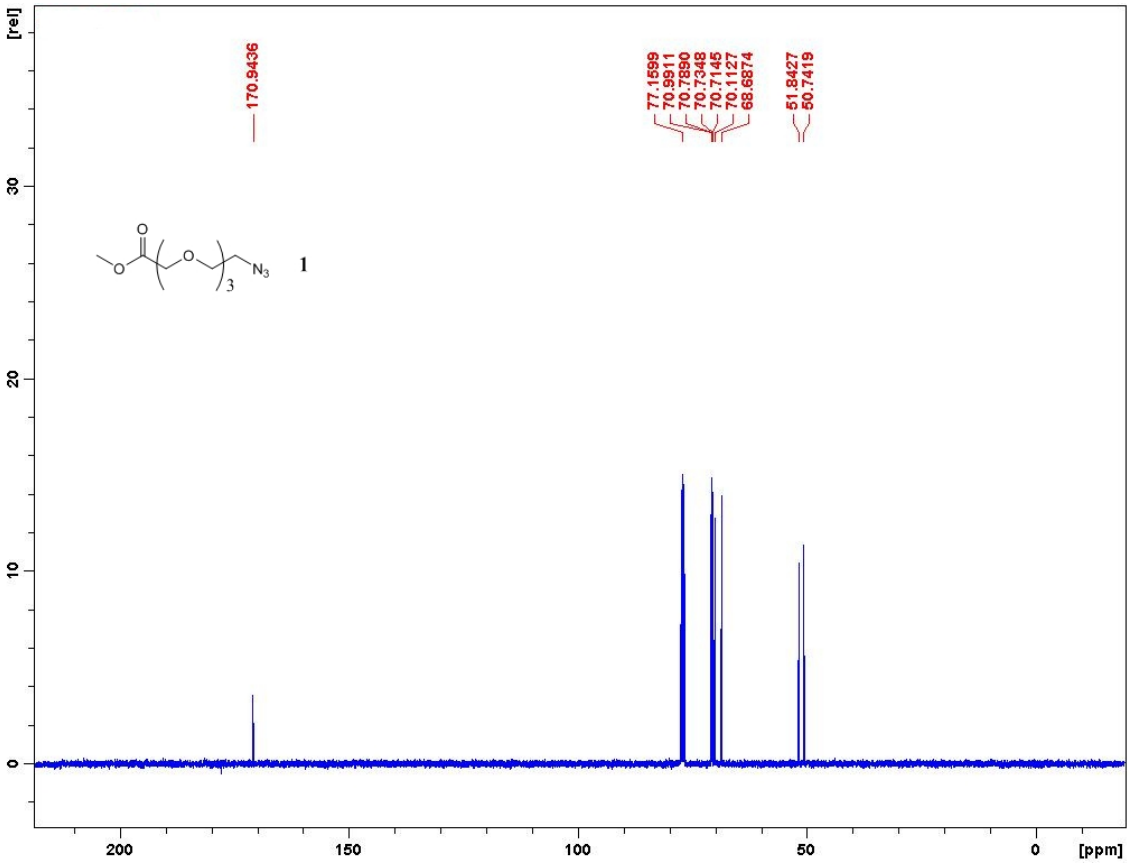
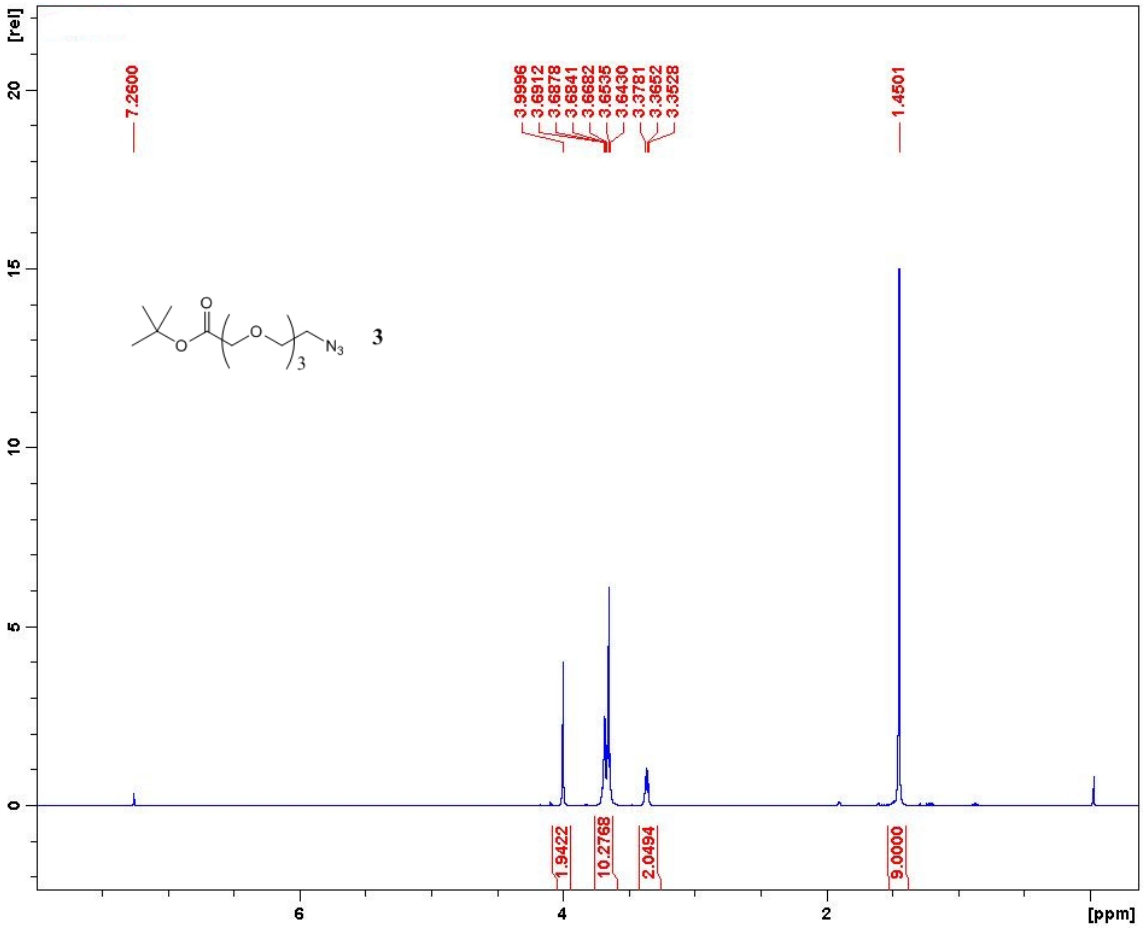
10. NMR-Spectra



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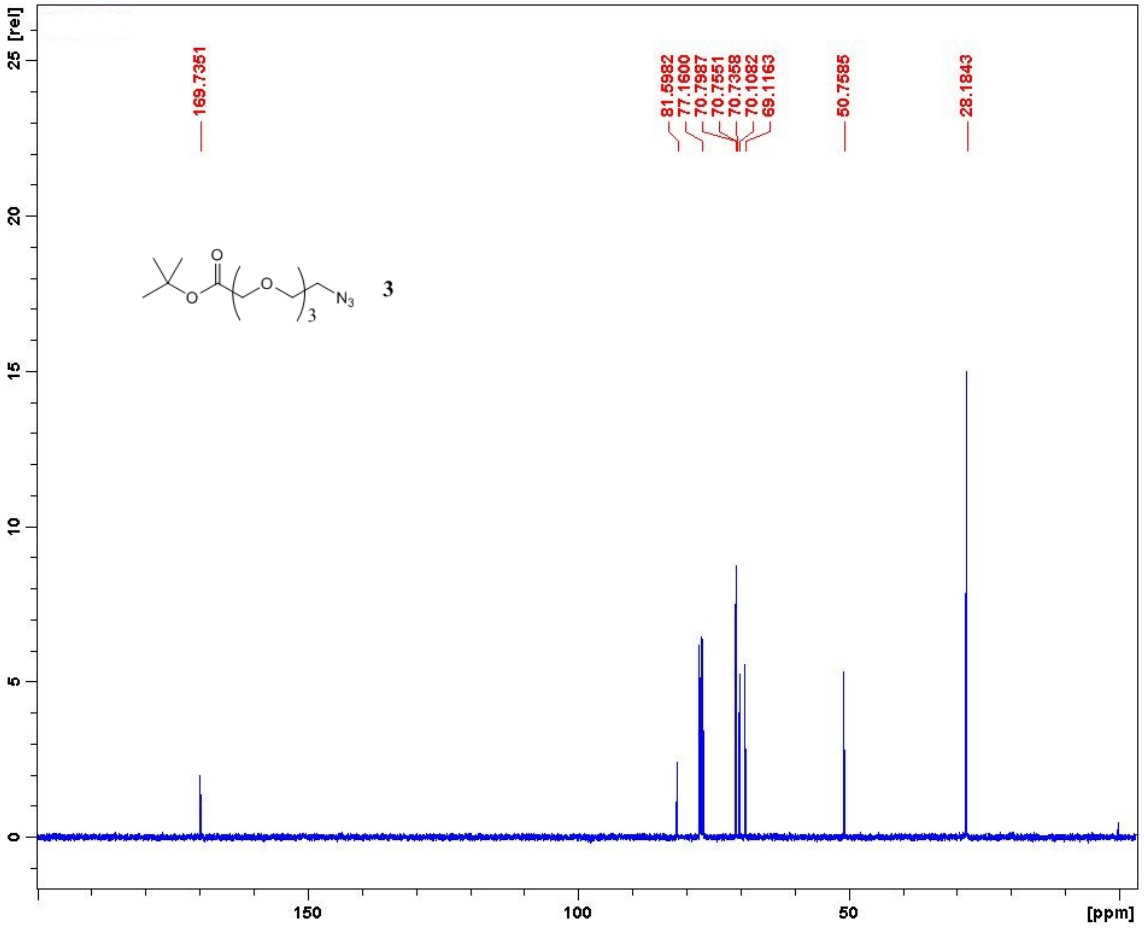
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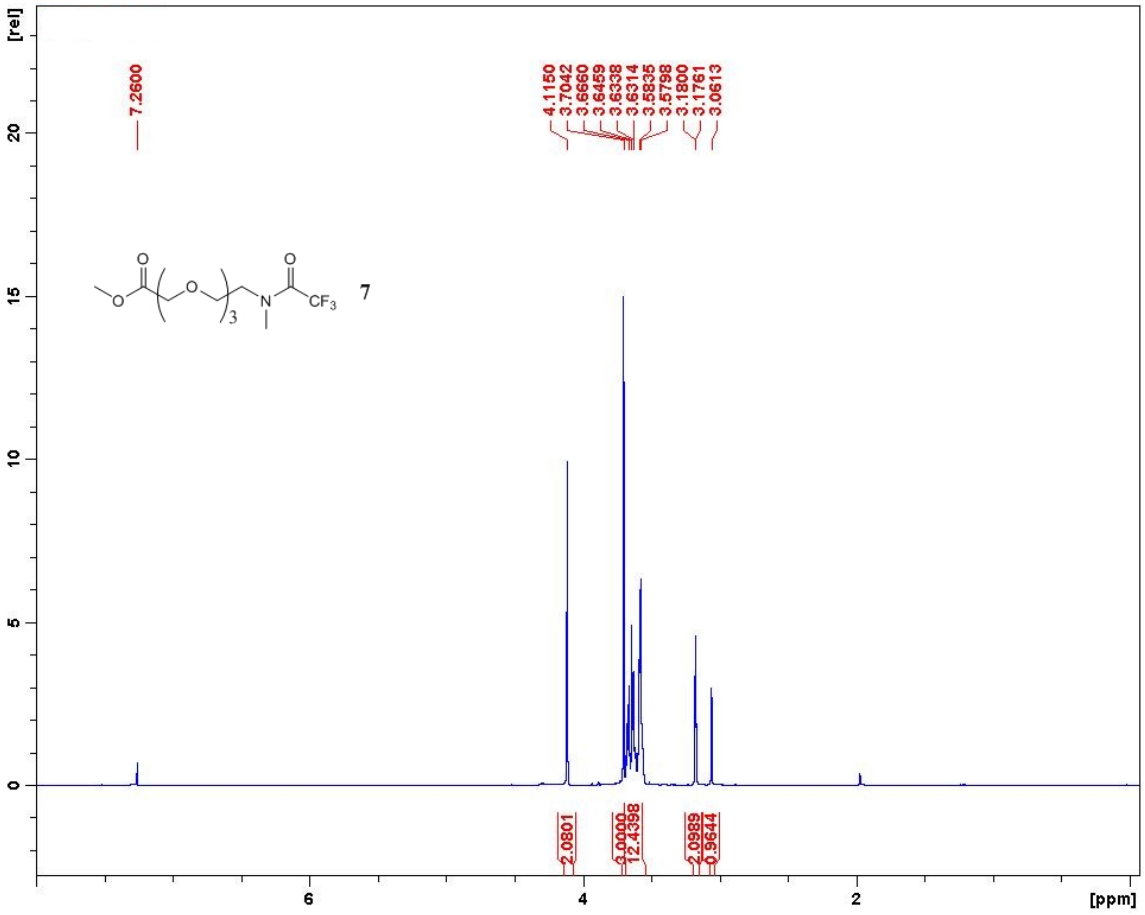
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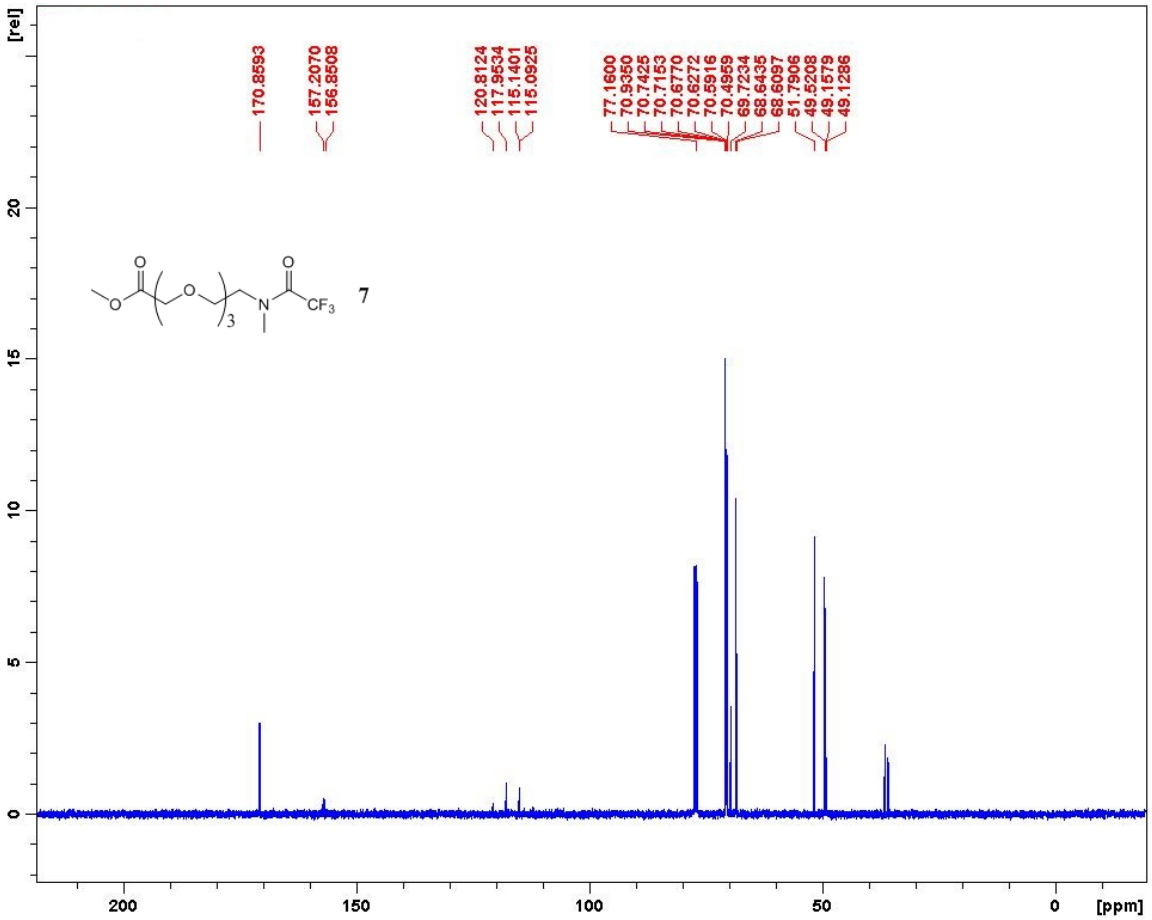
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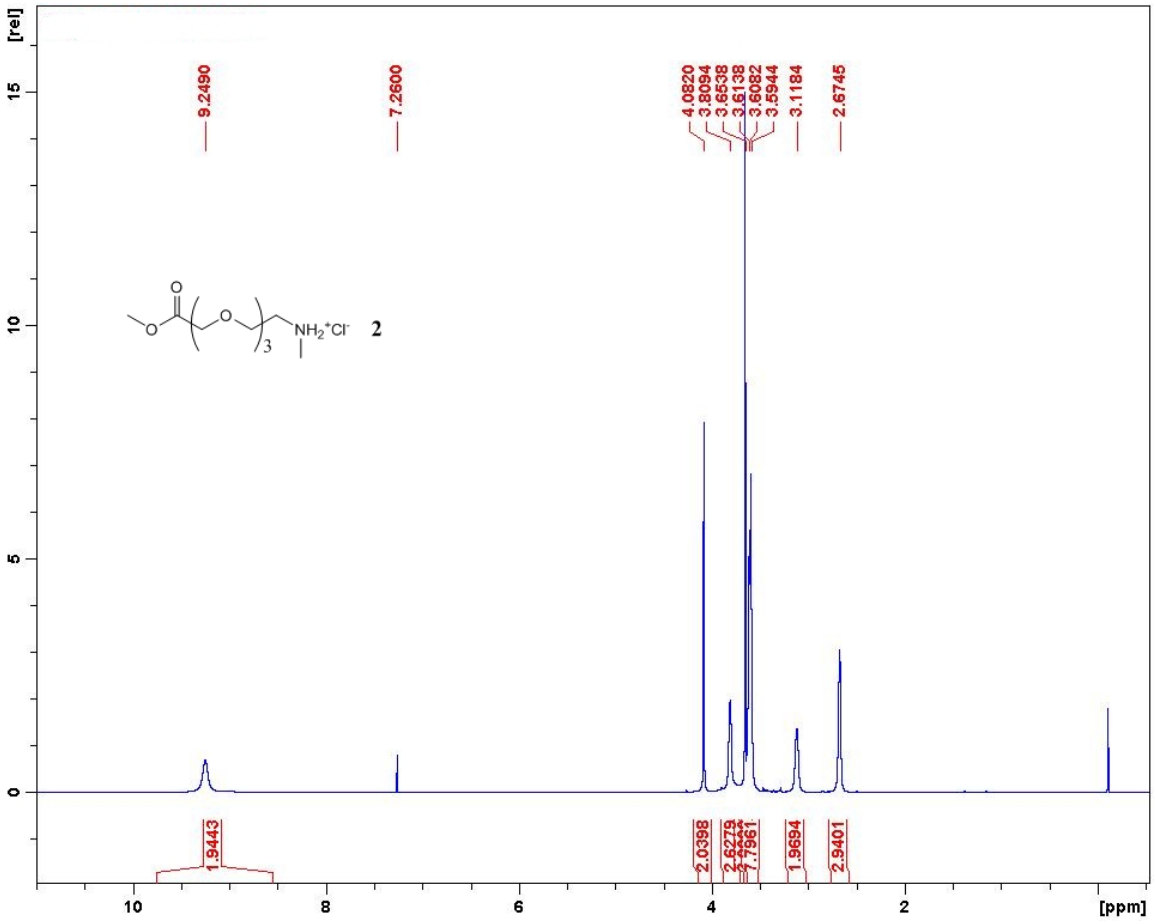
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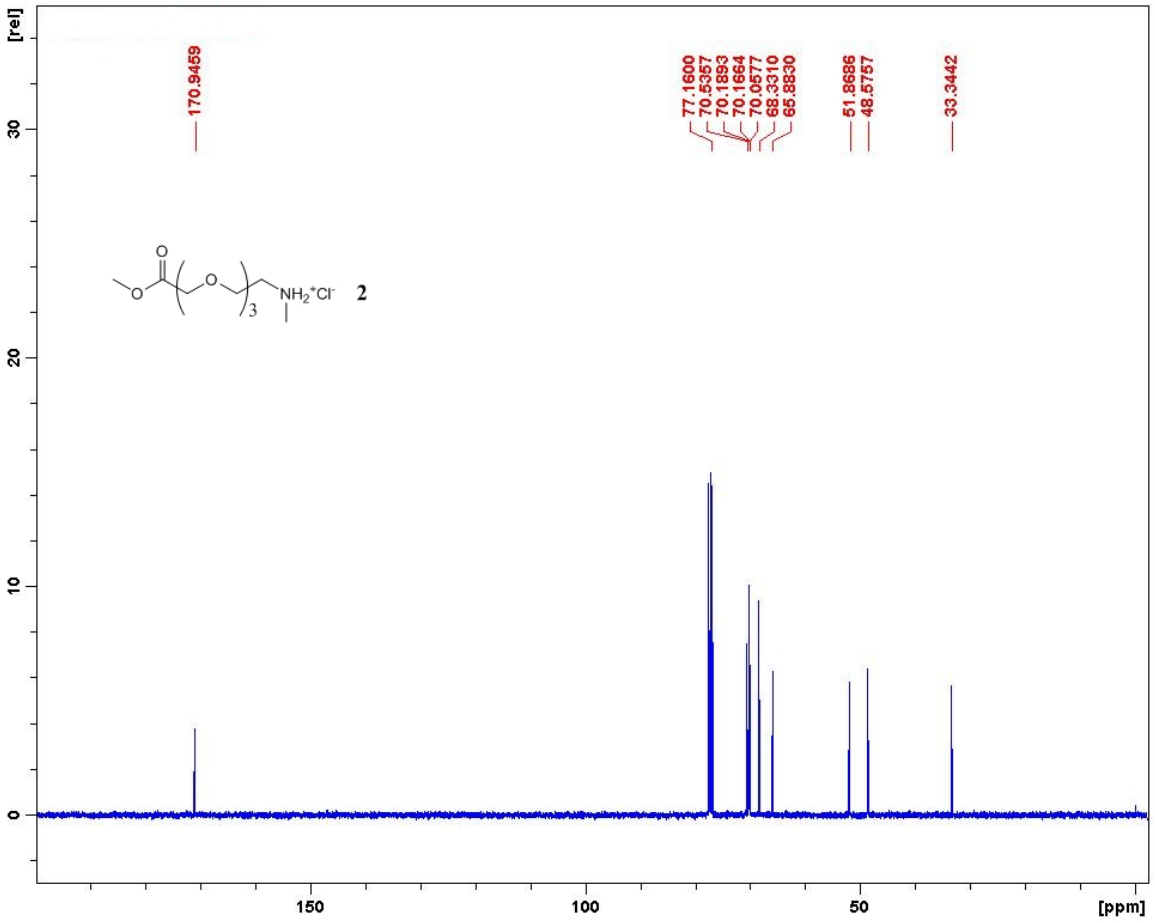
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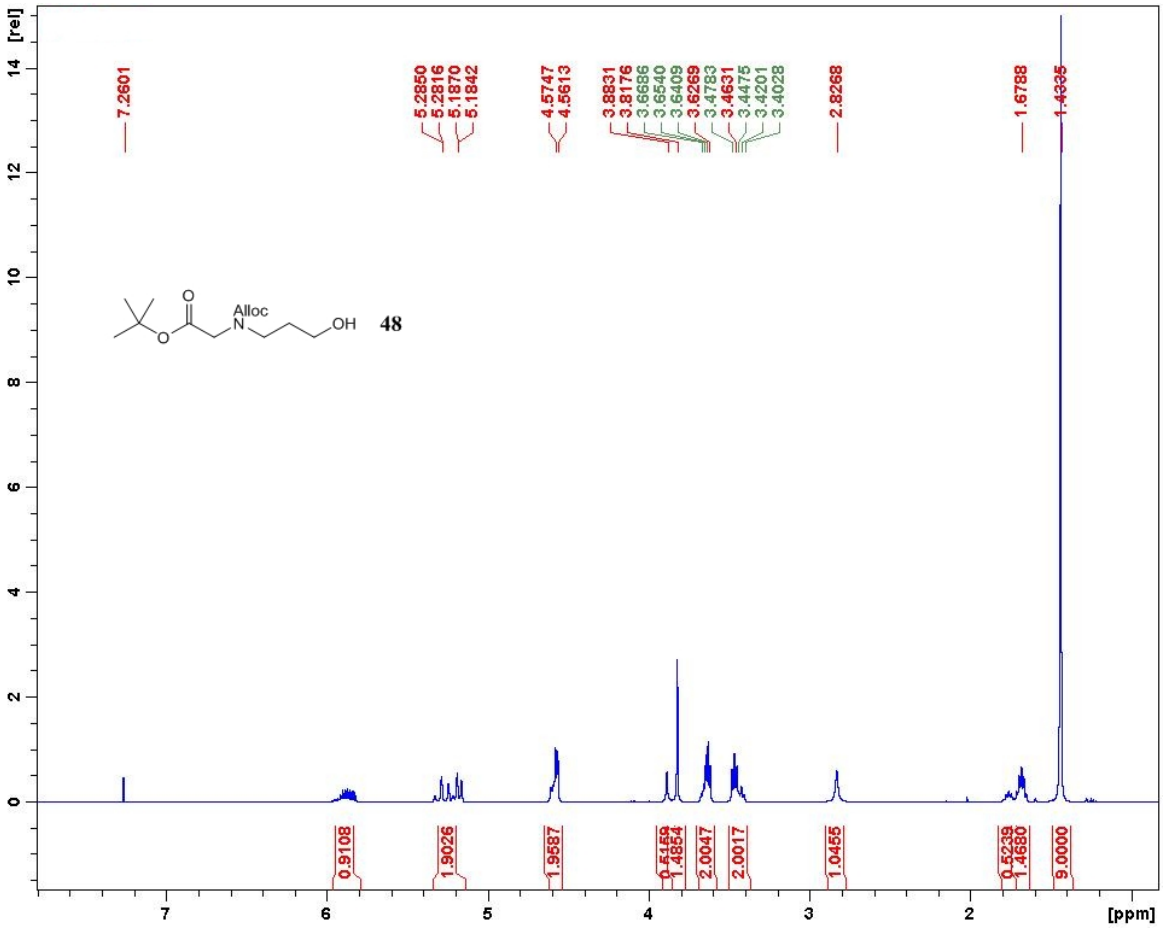
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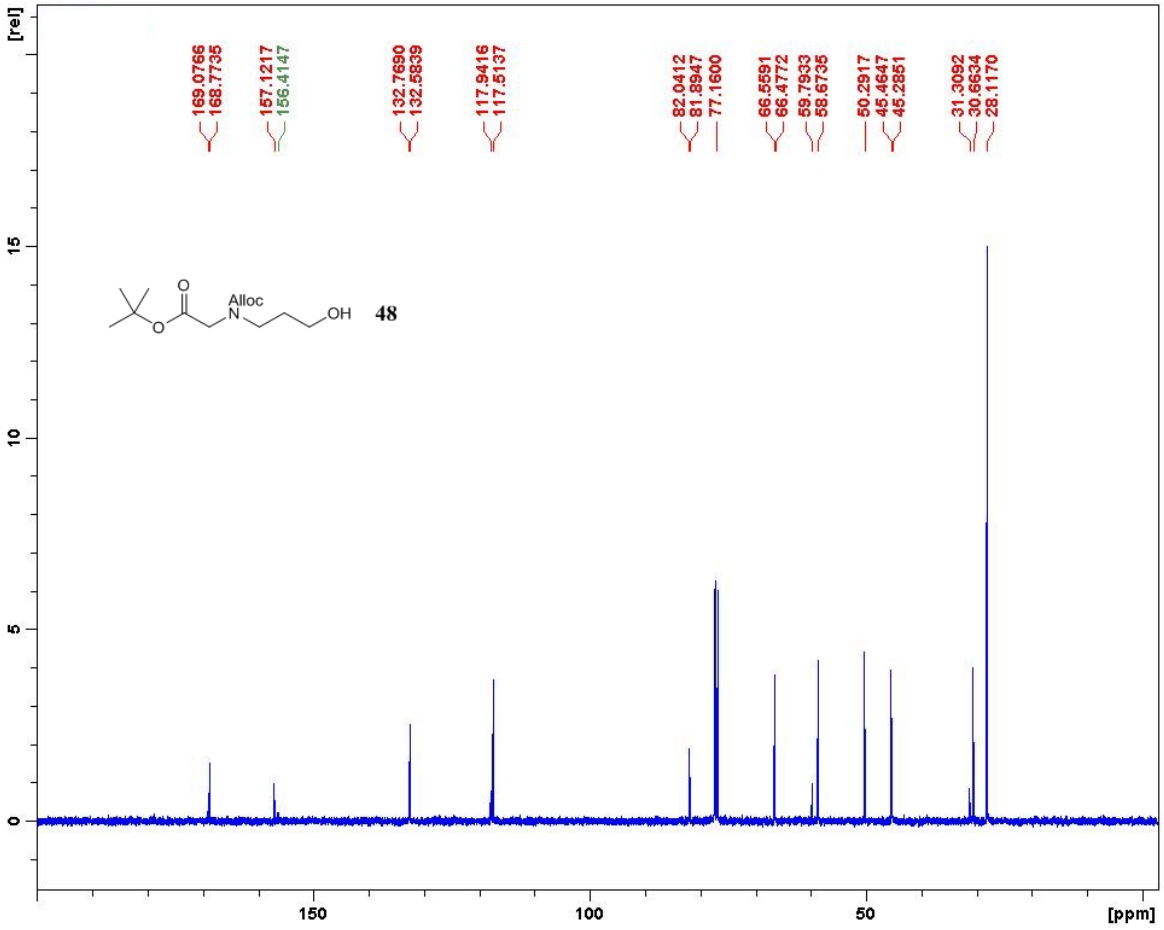
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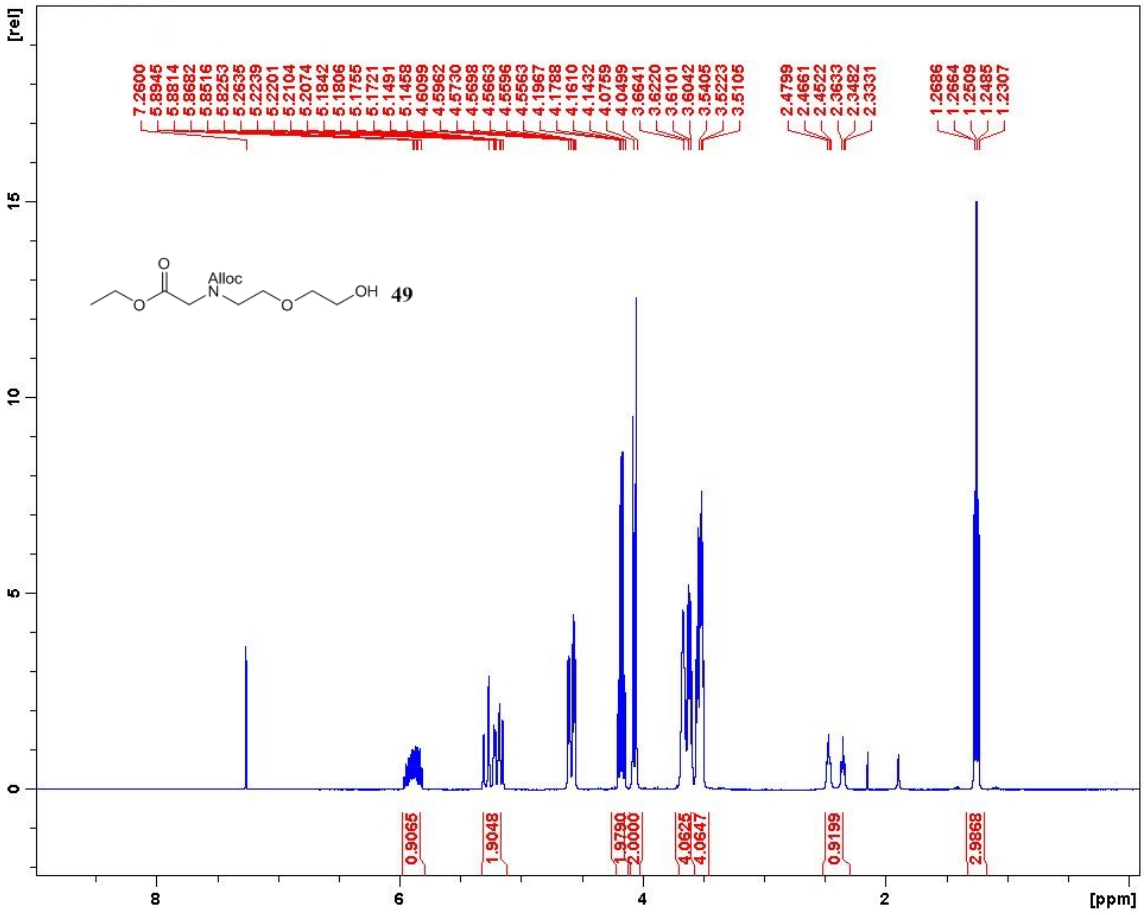
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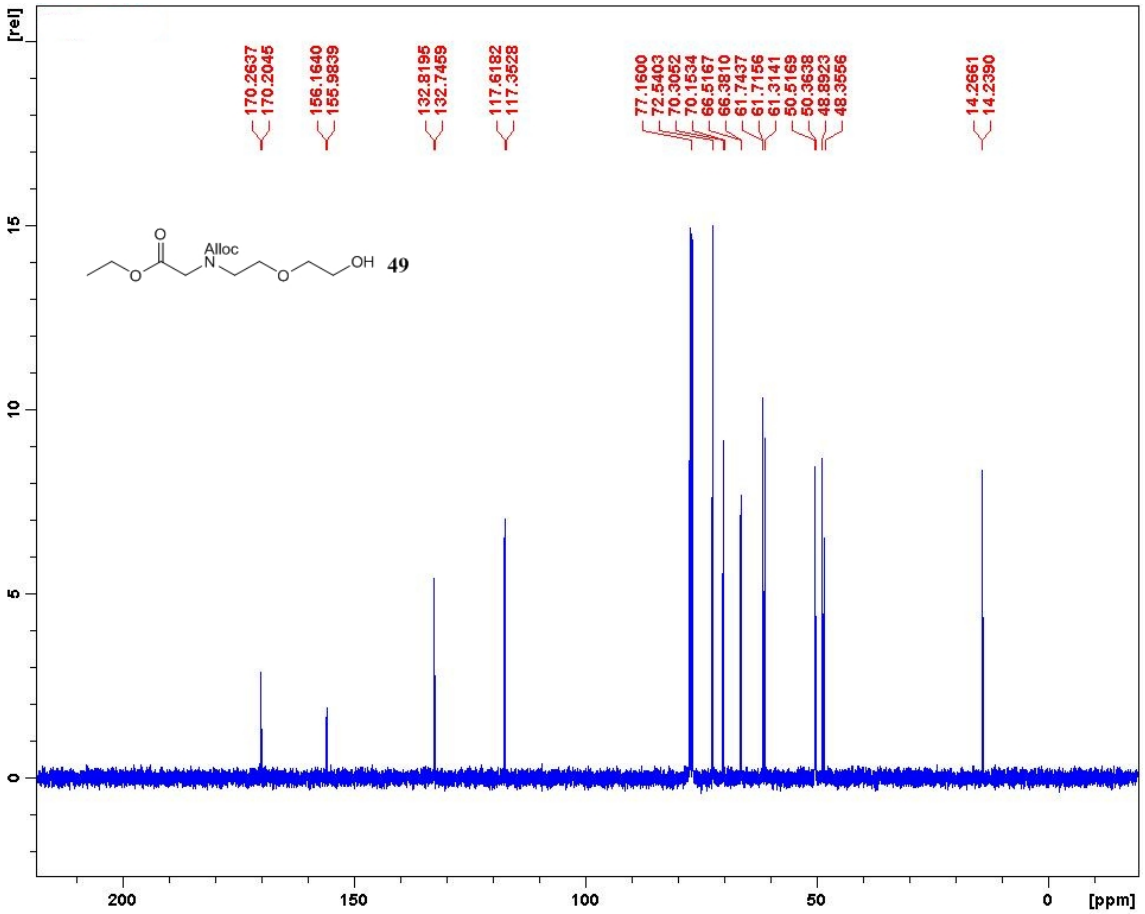
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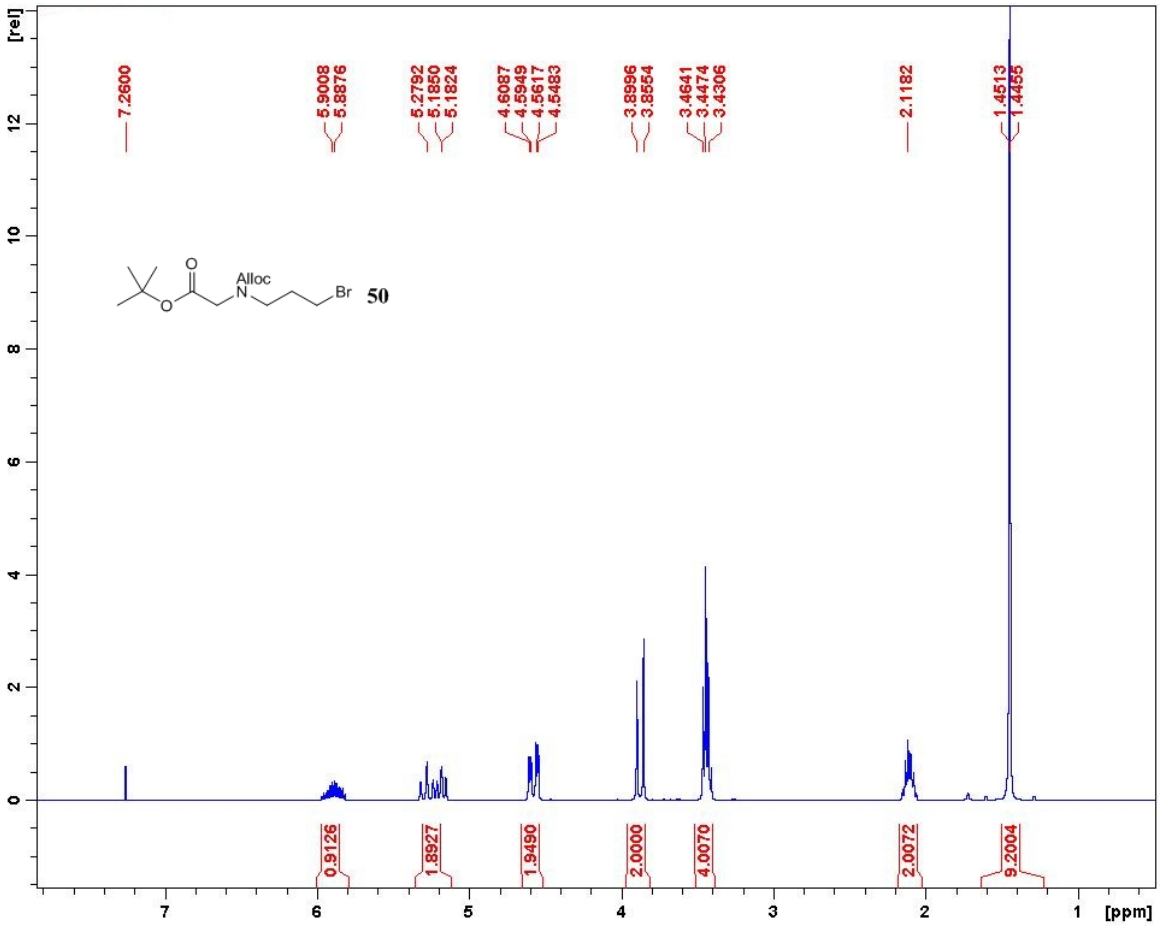
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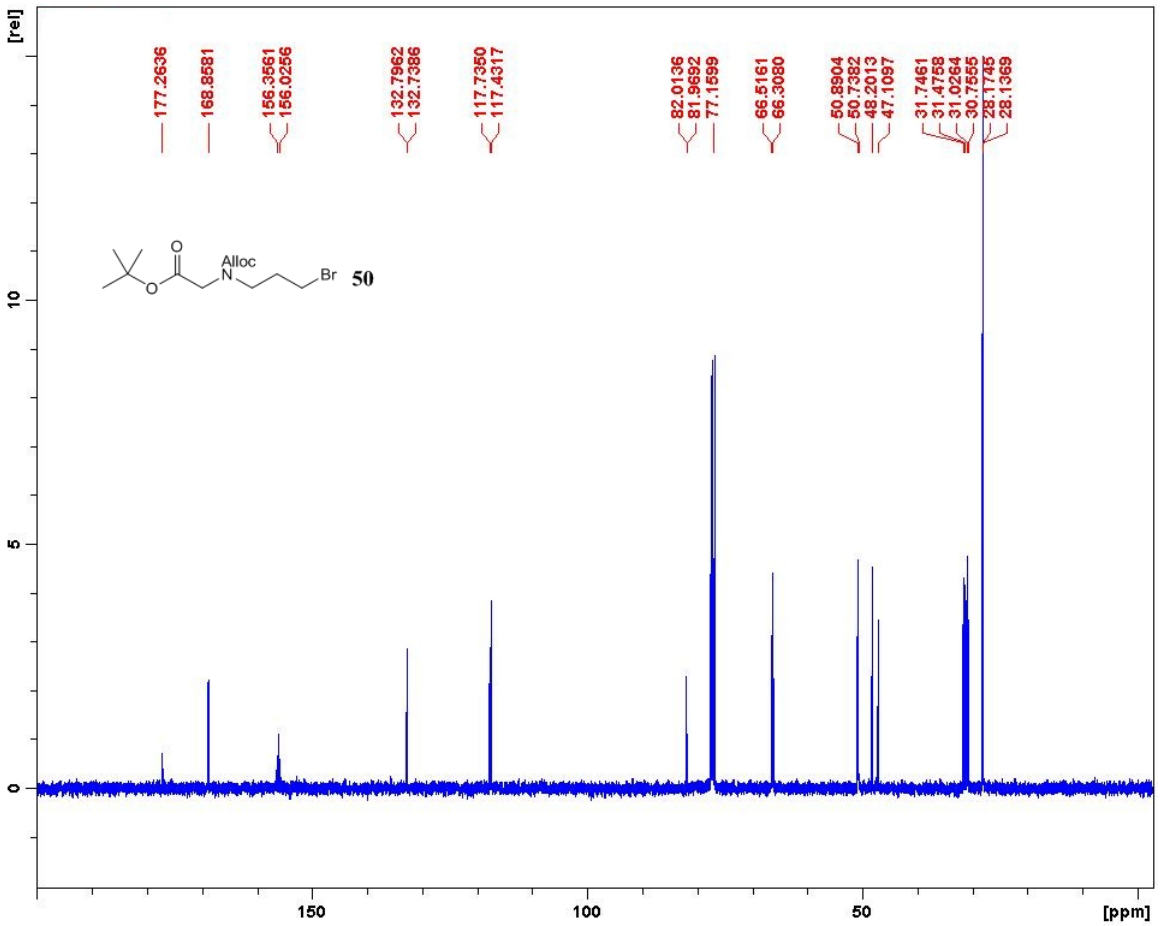
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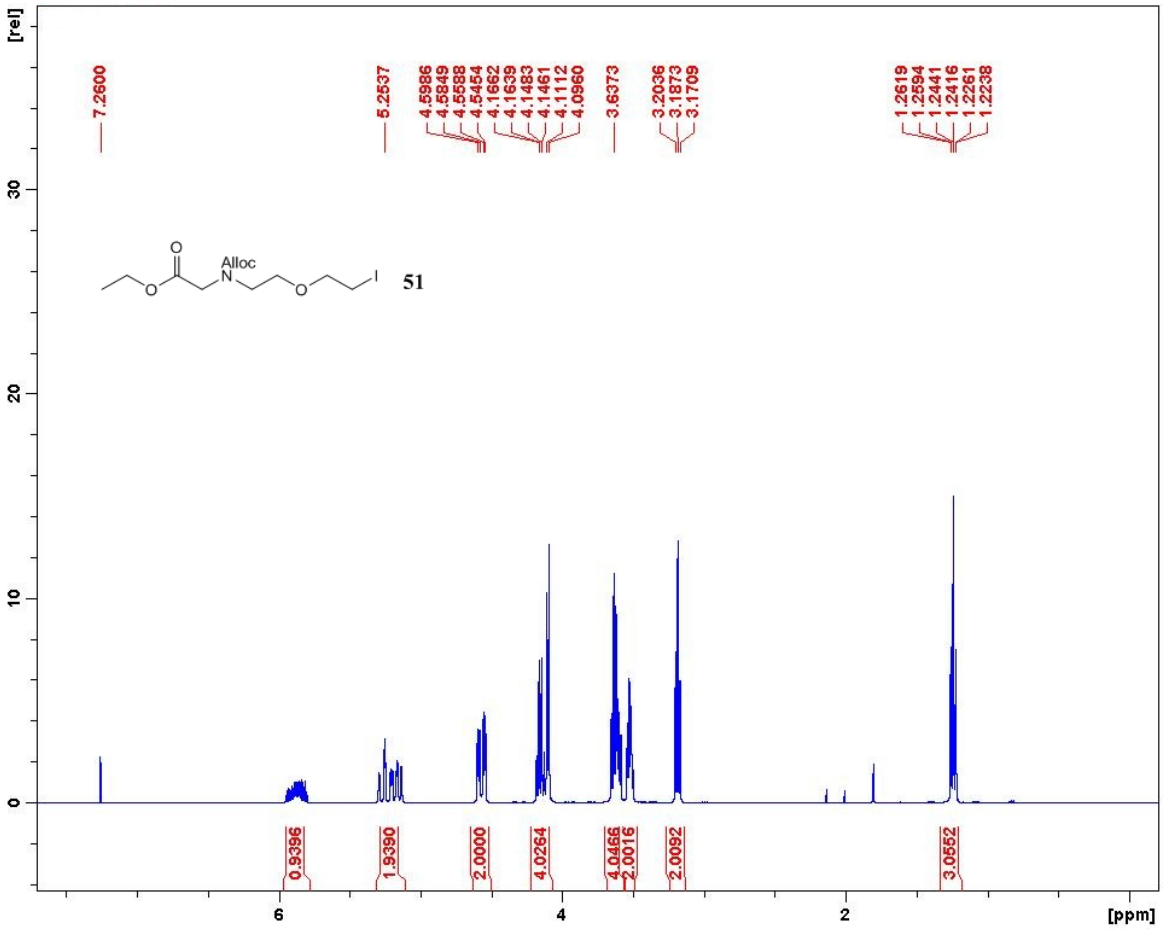
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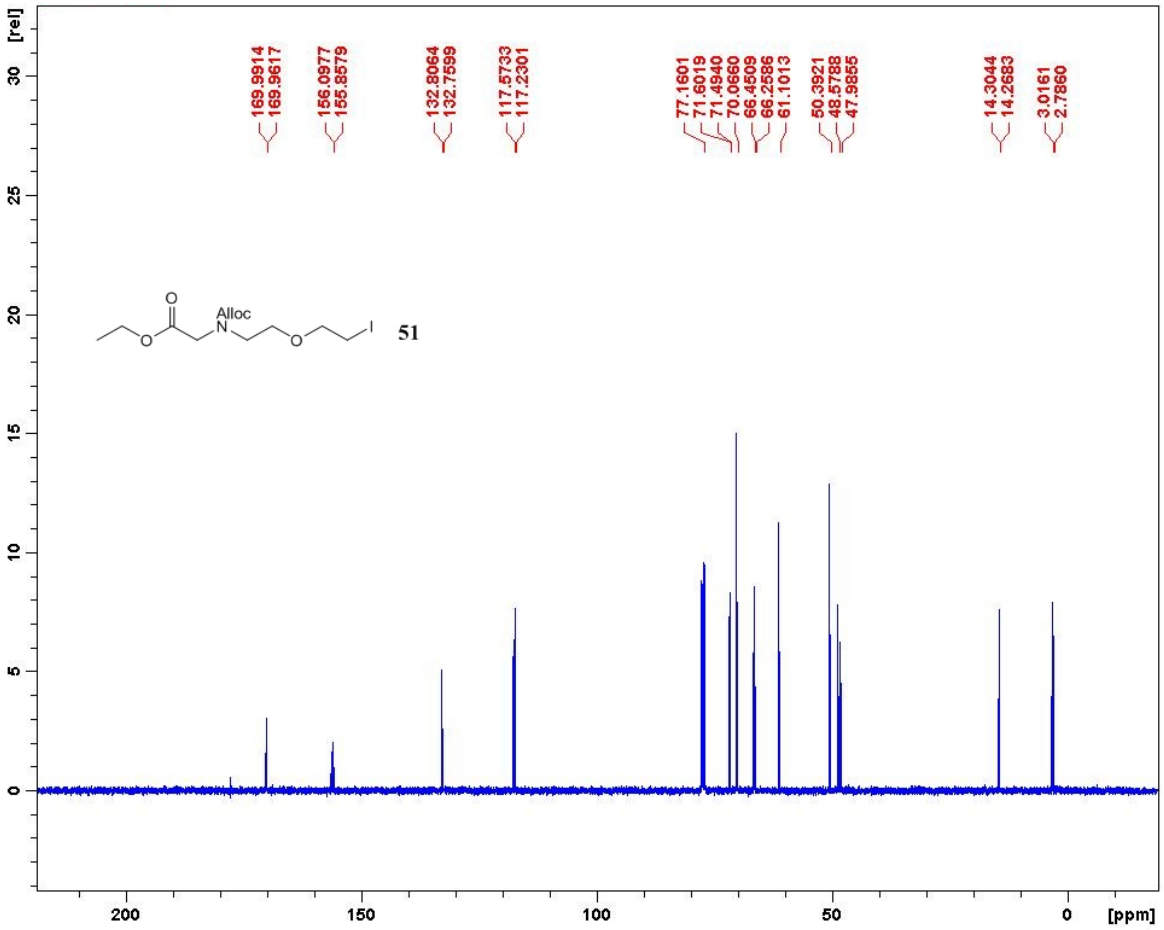
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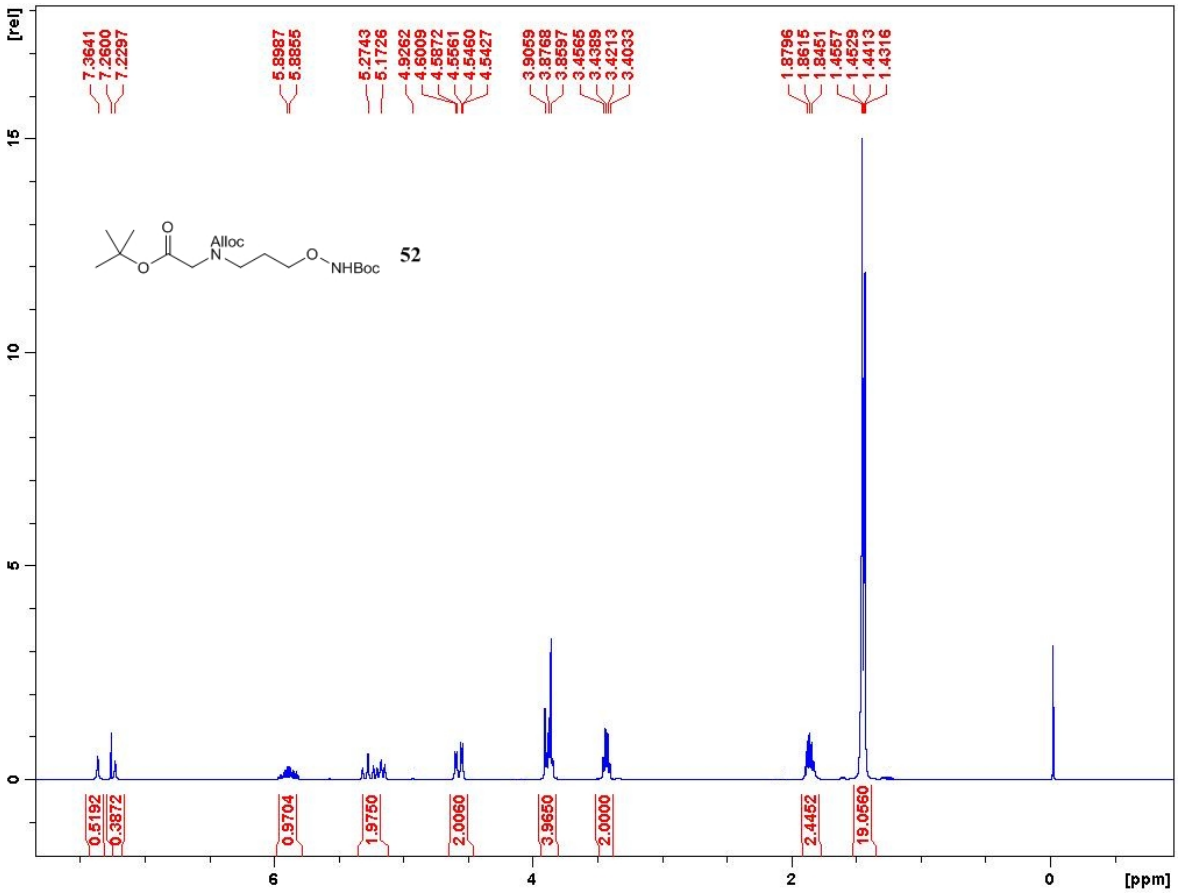
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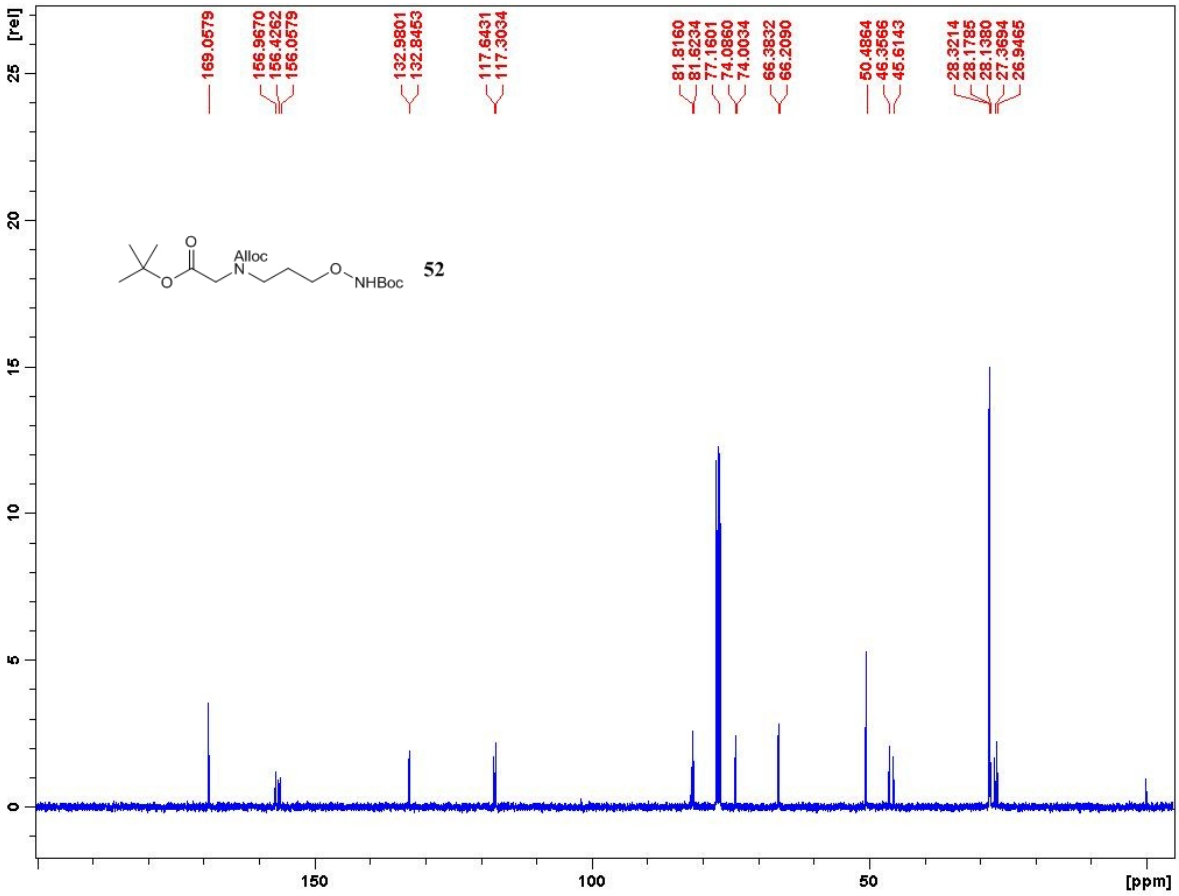
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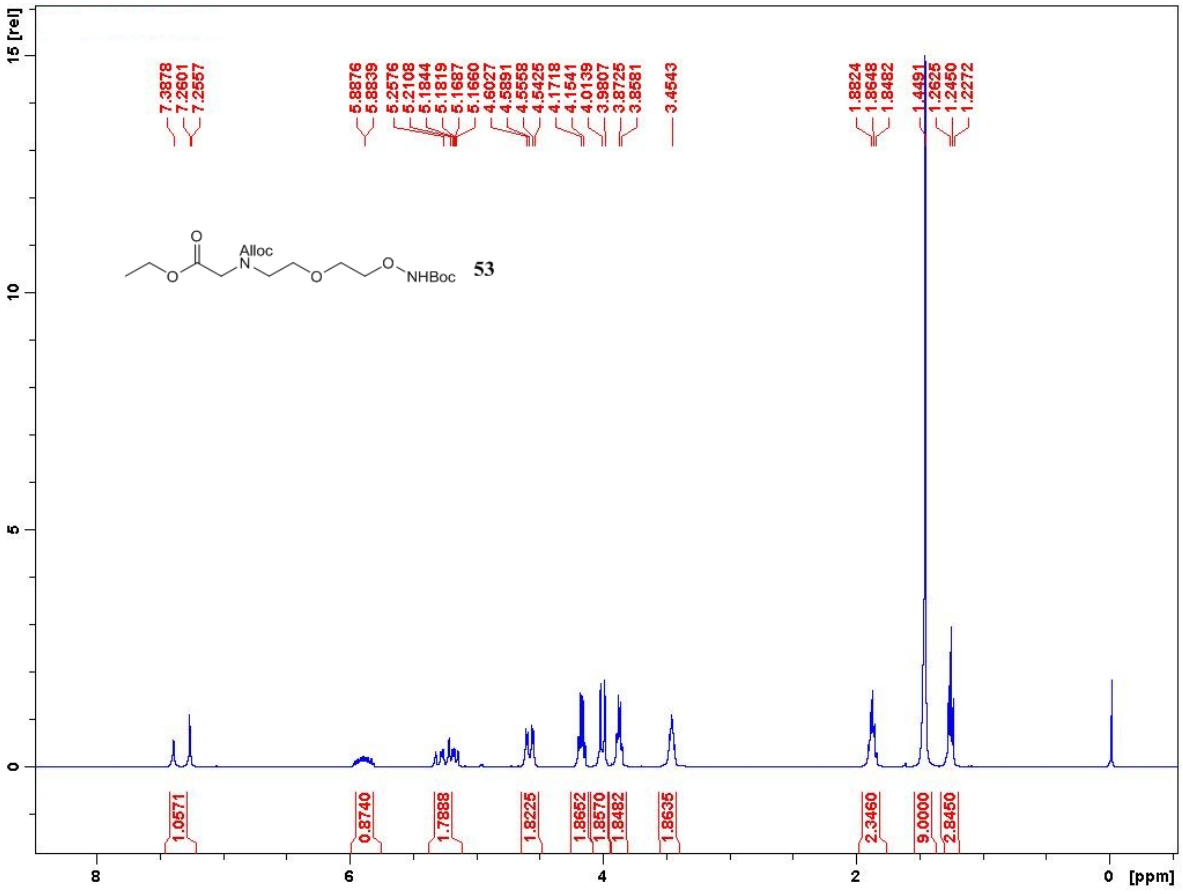
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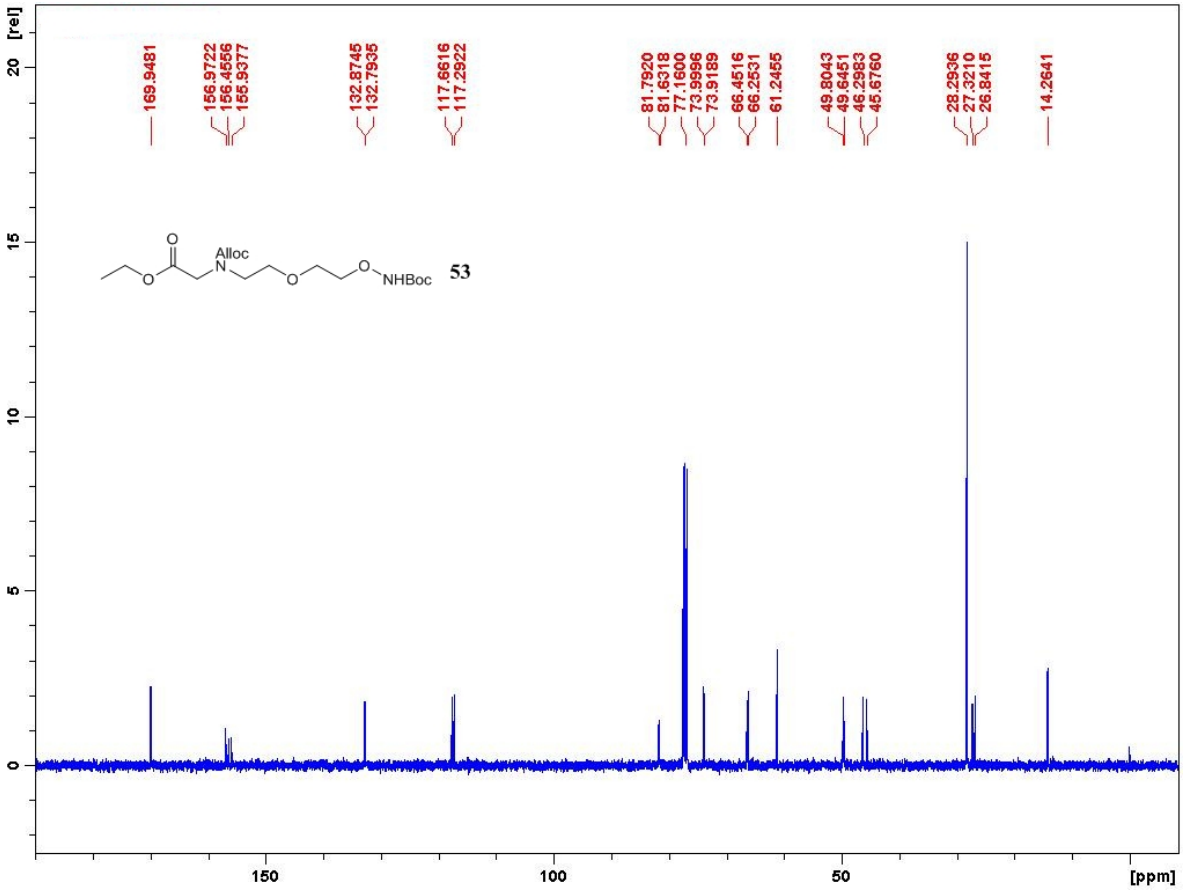
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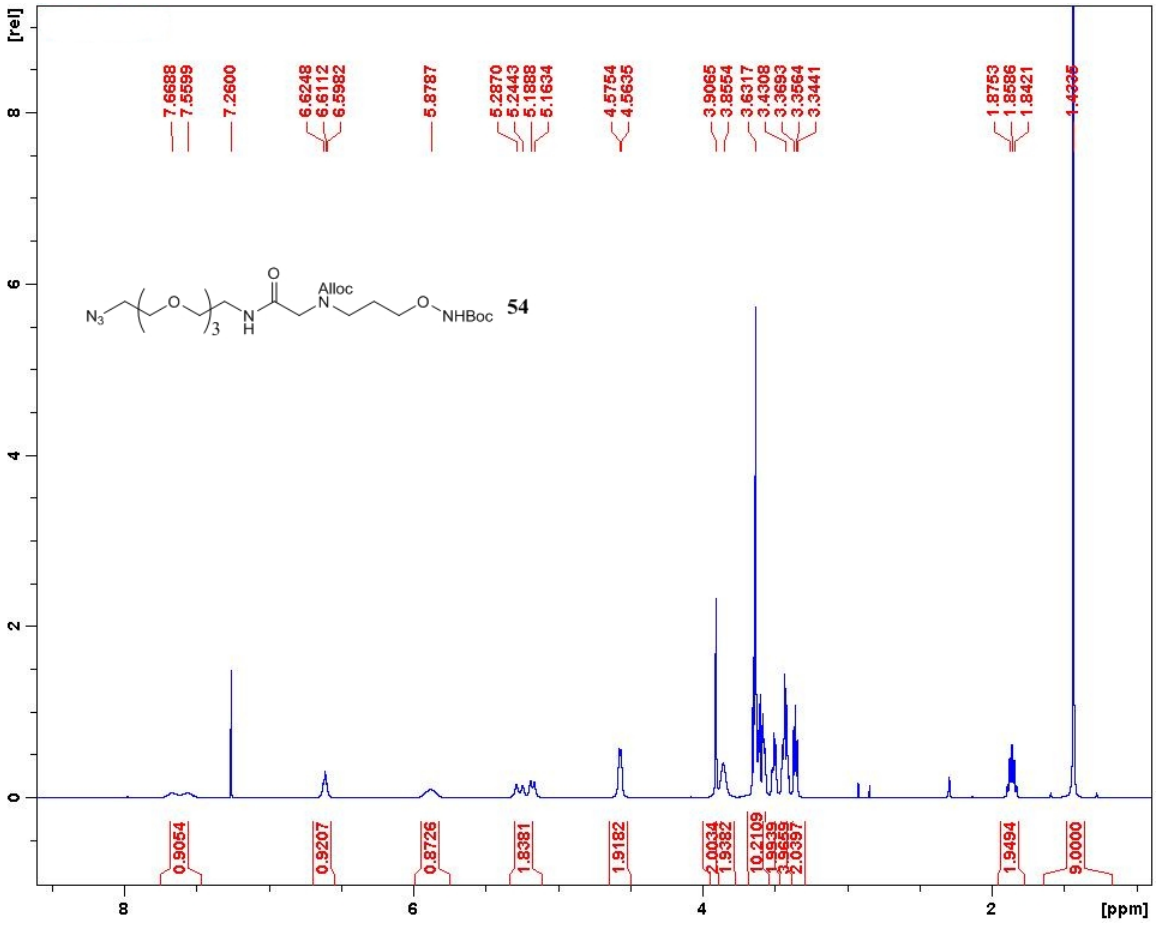
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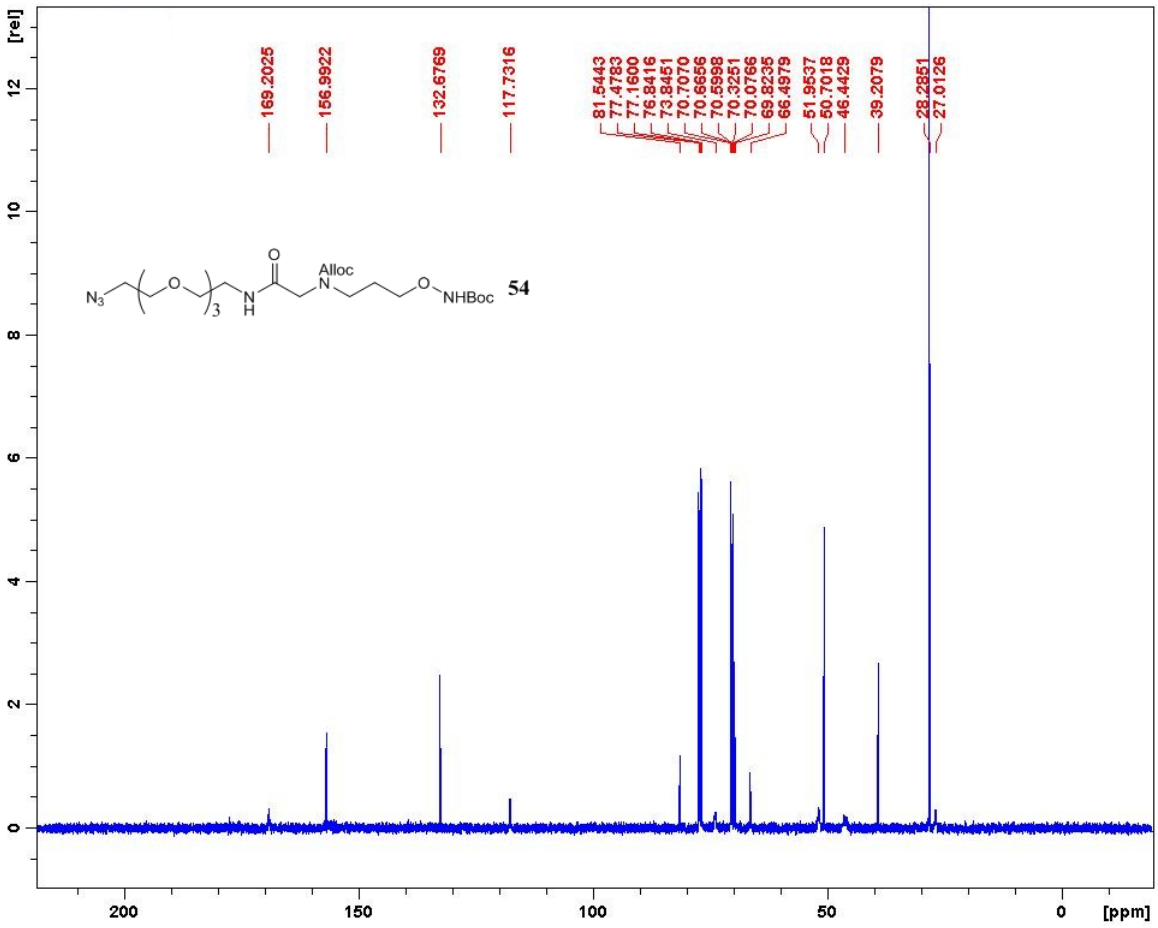
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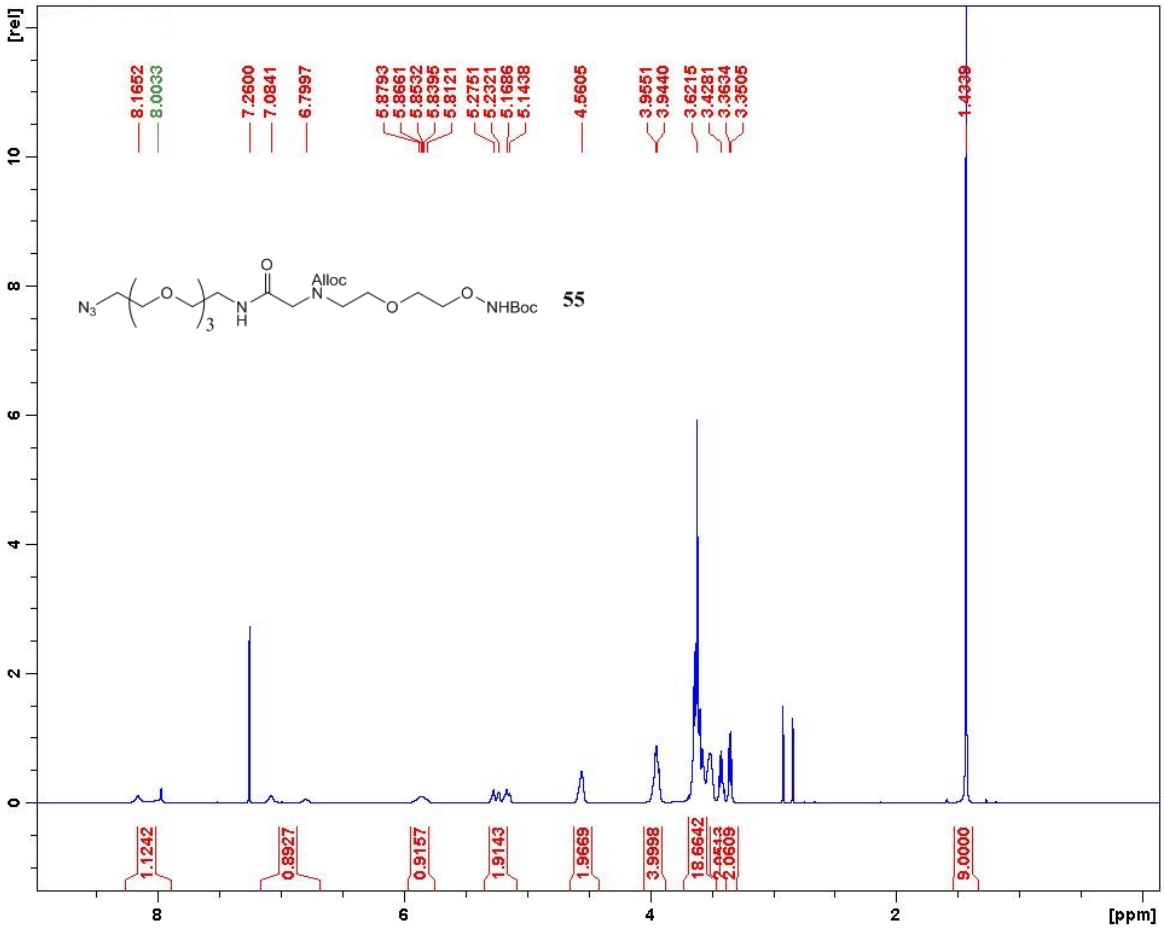
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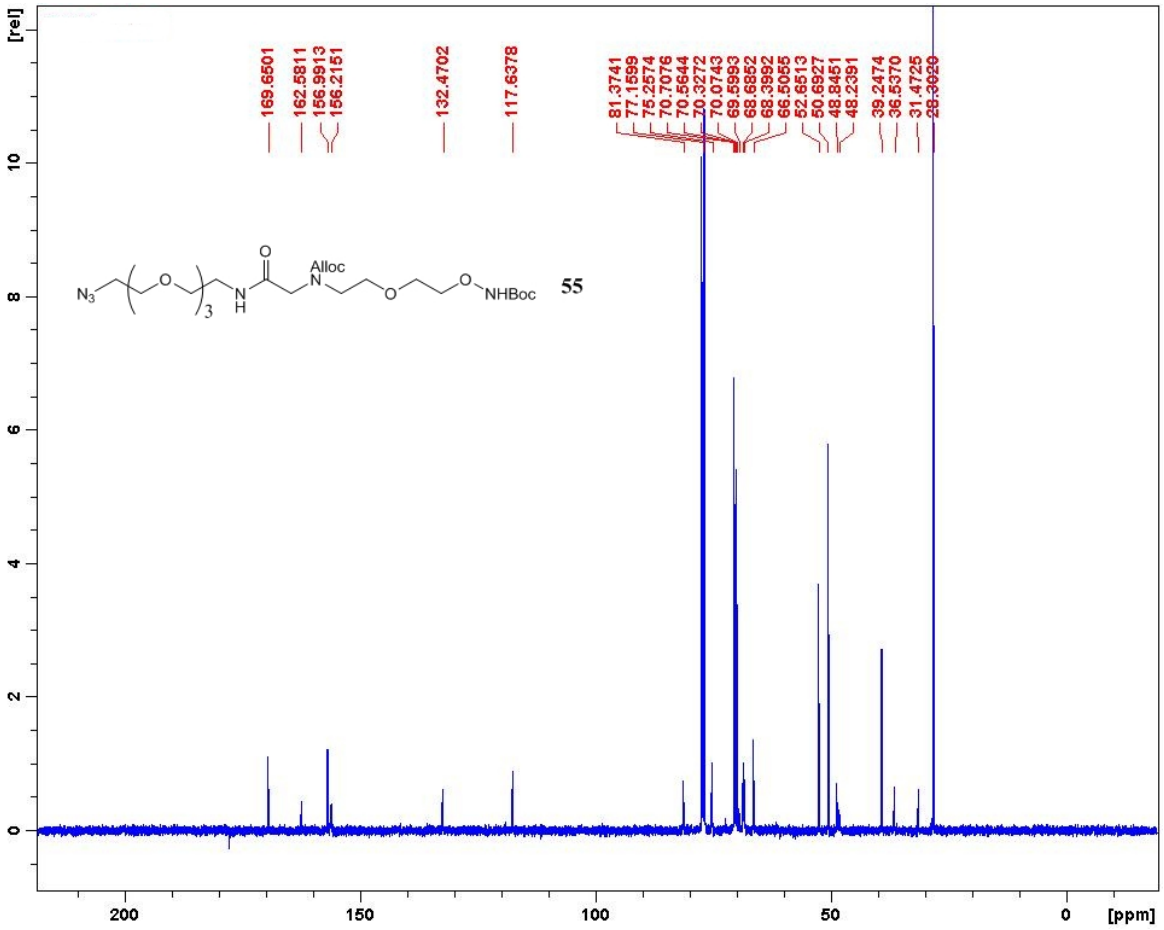
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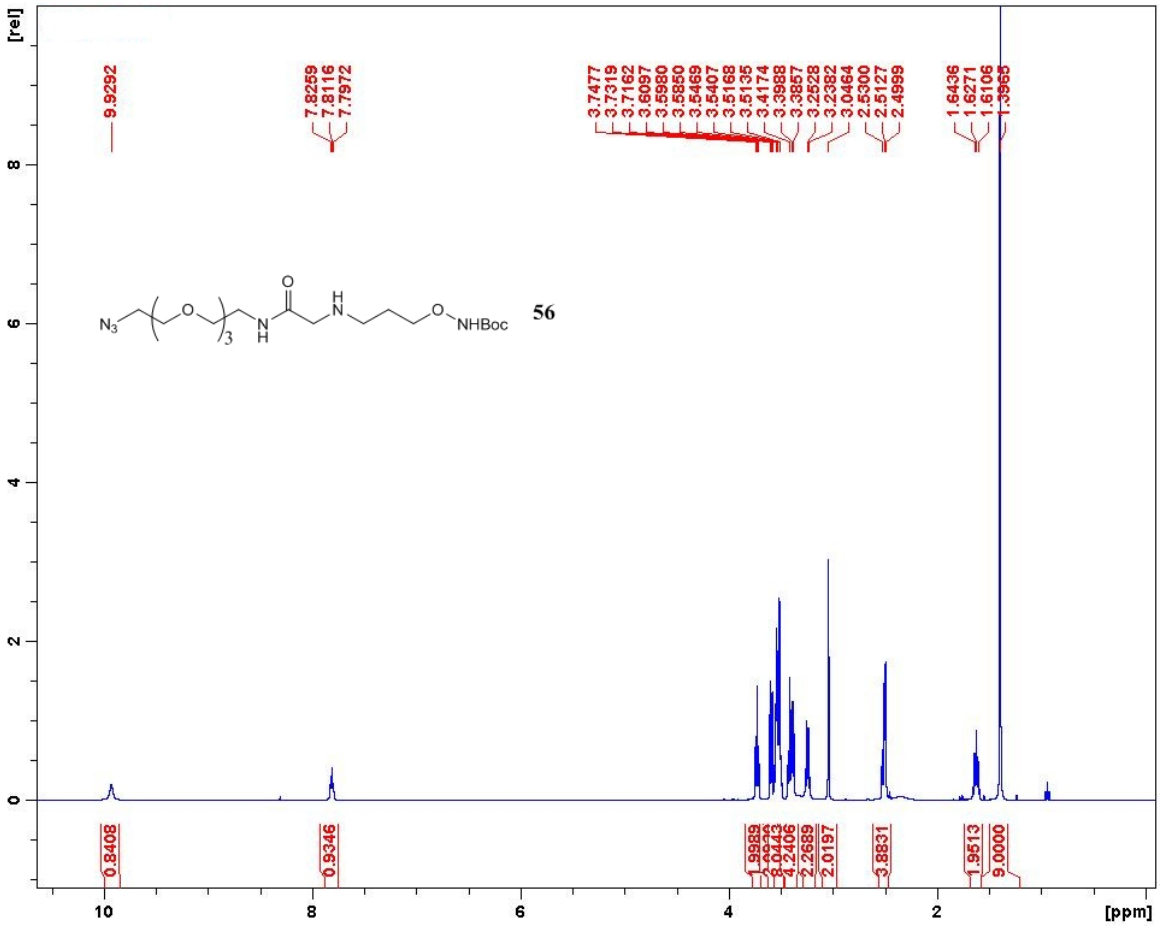
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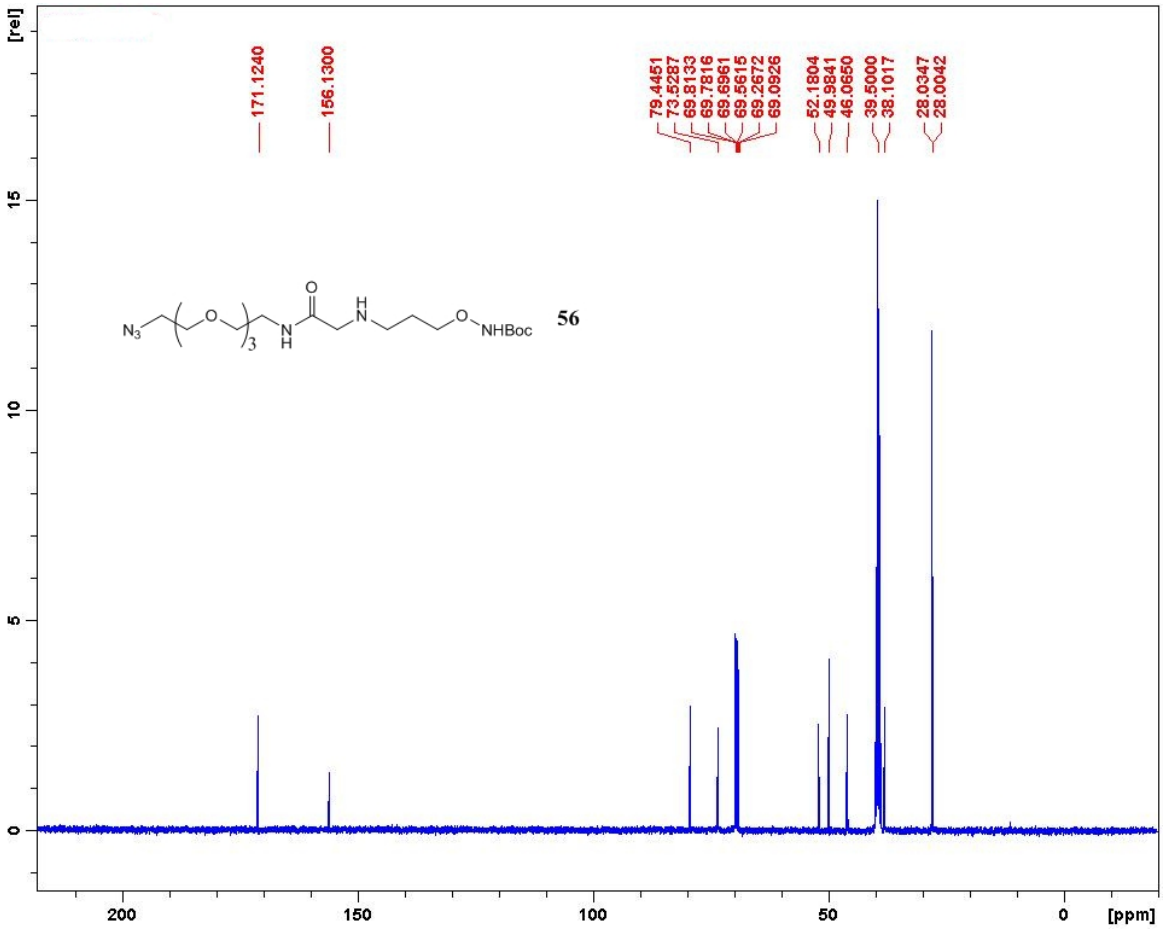
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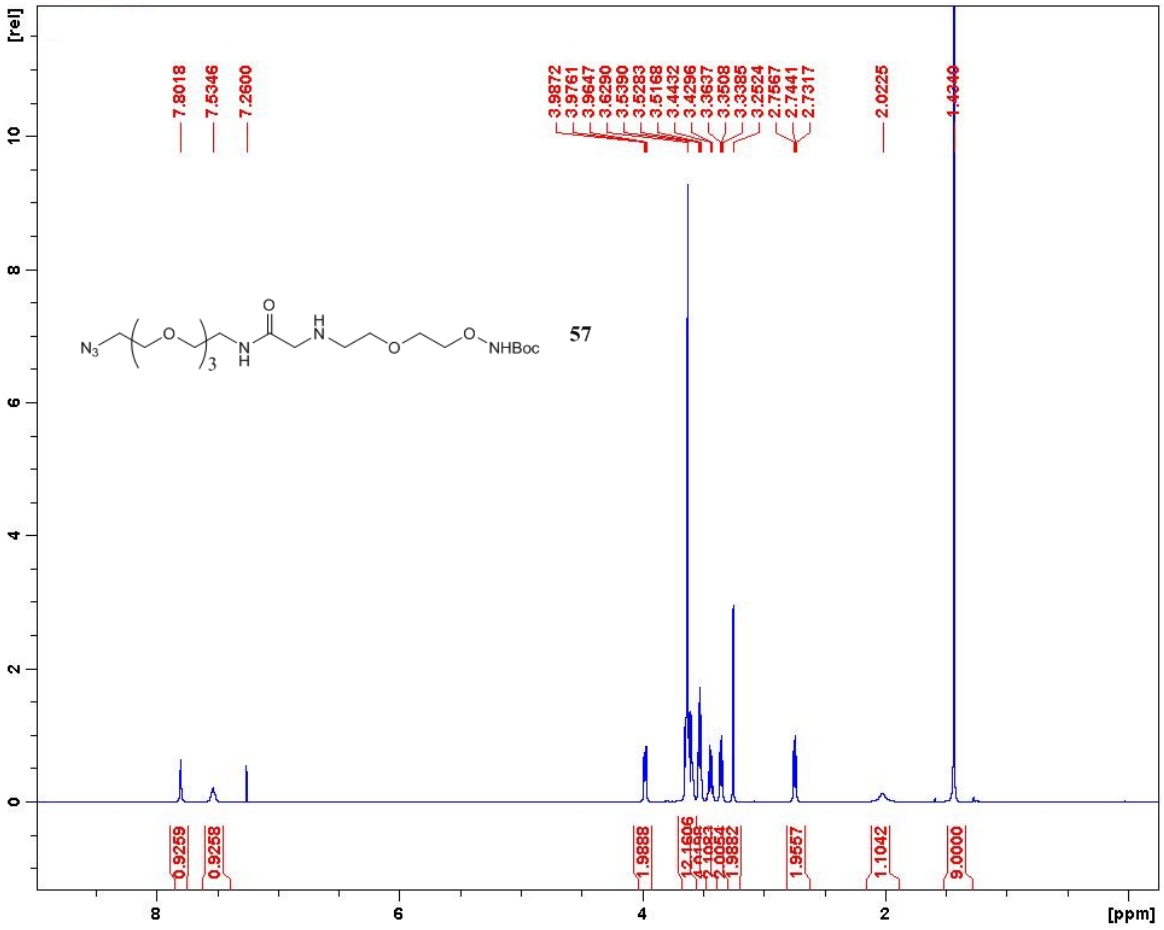
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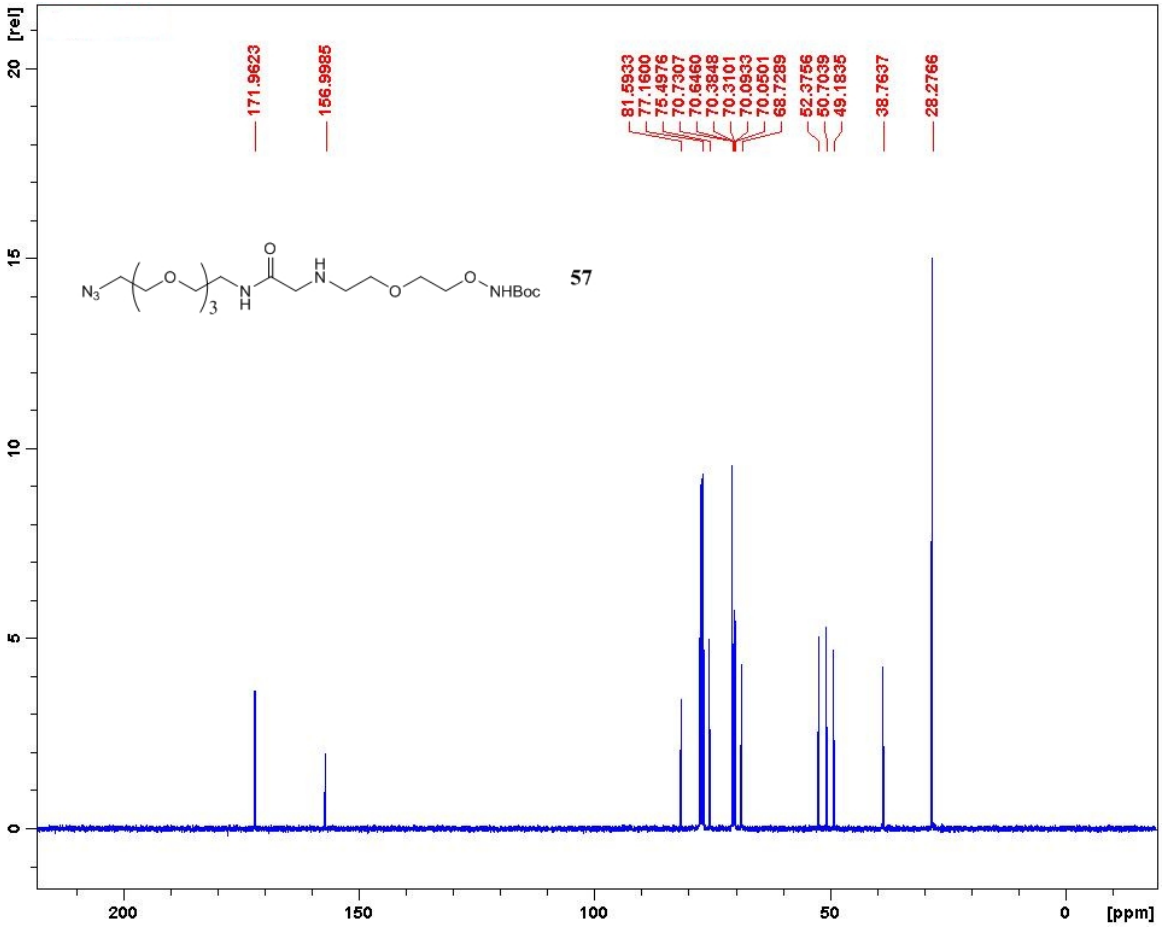
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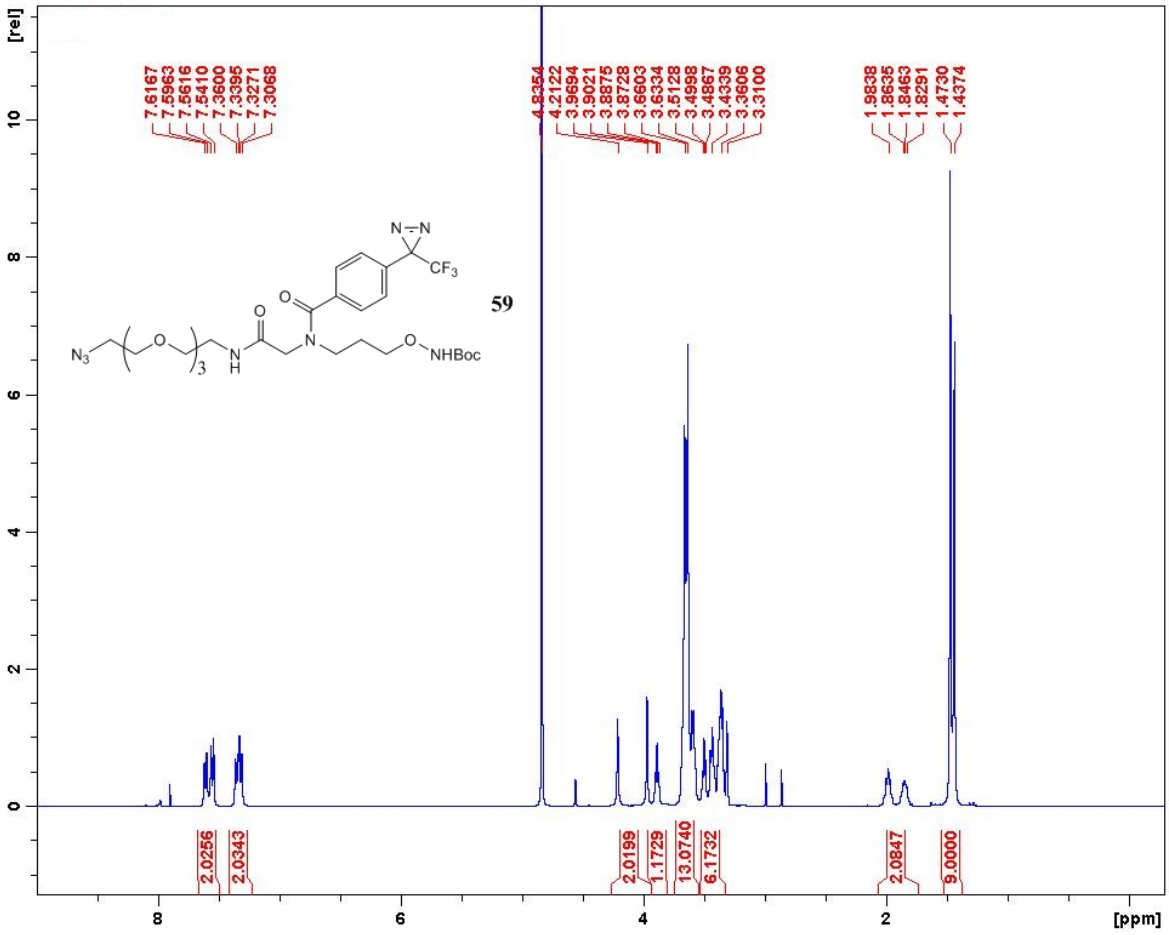
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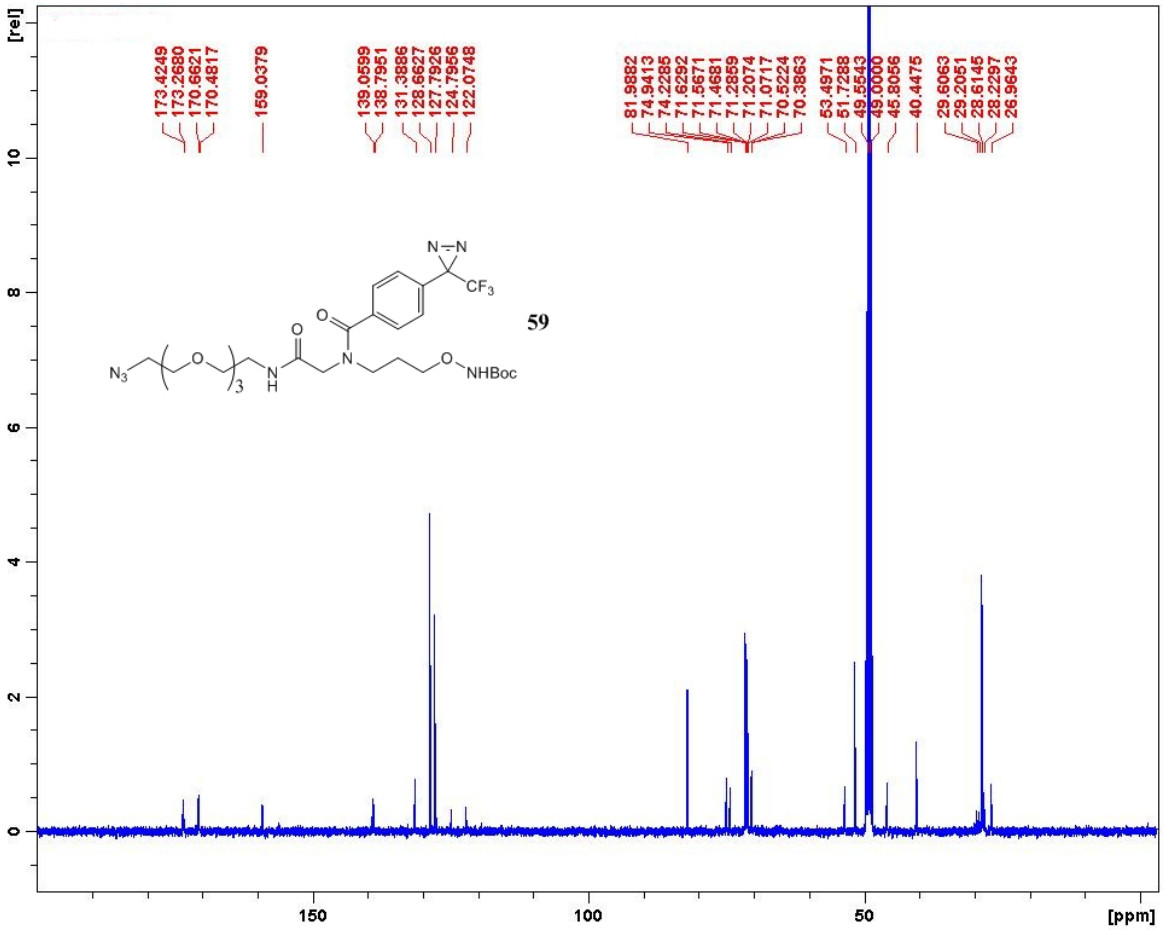
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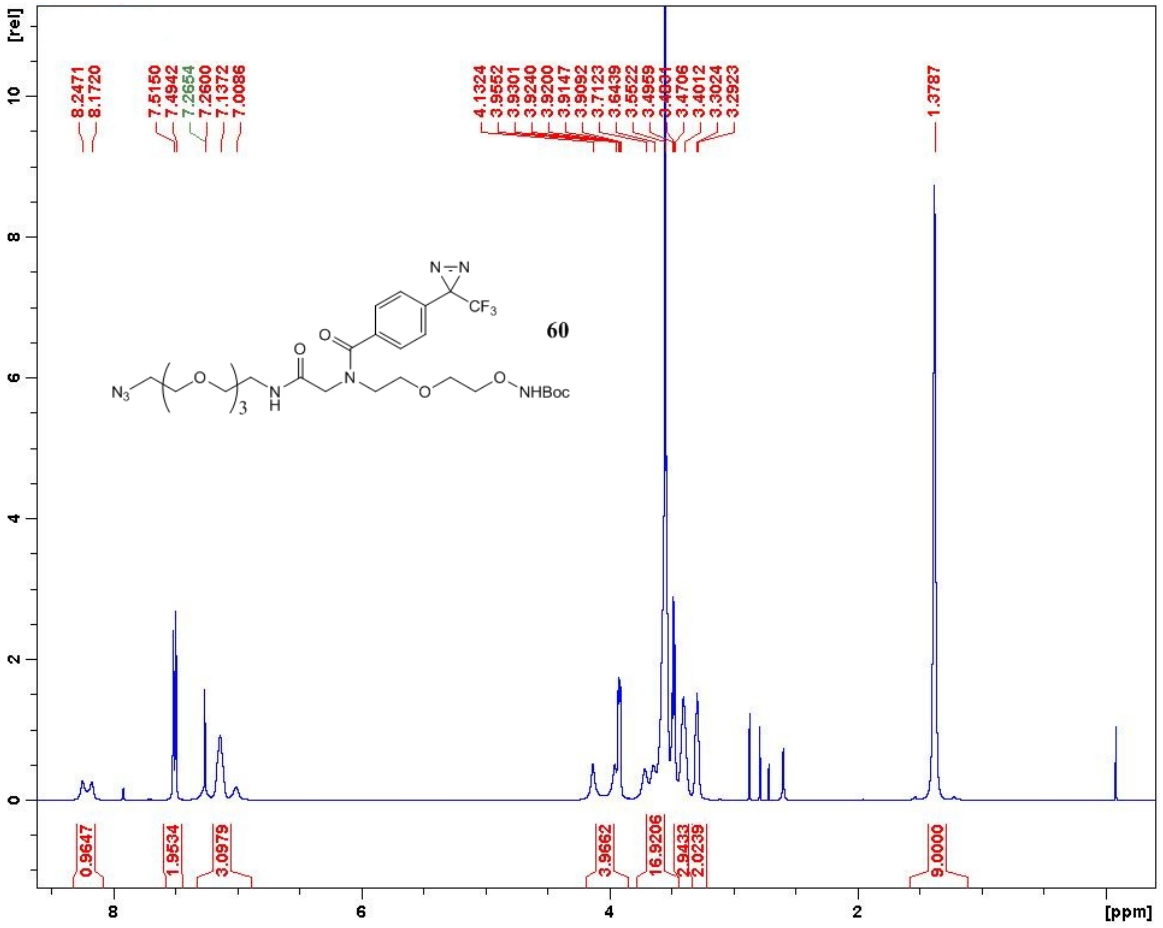
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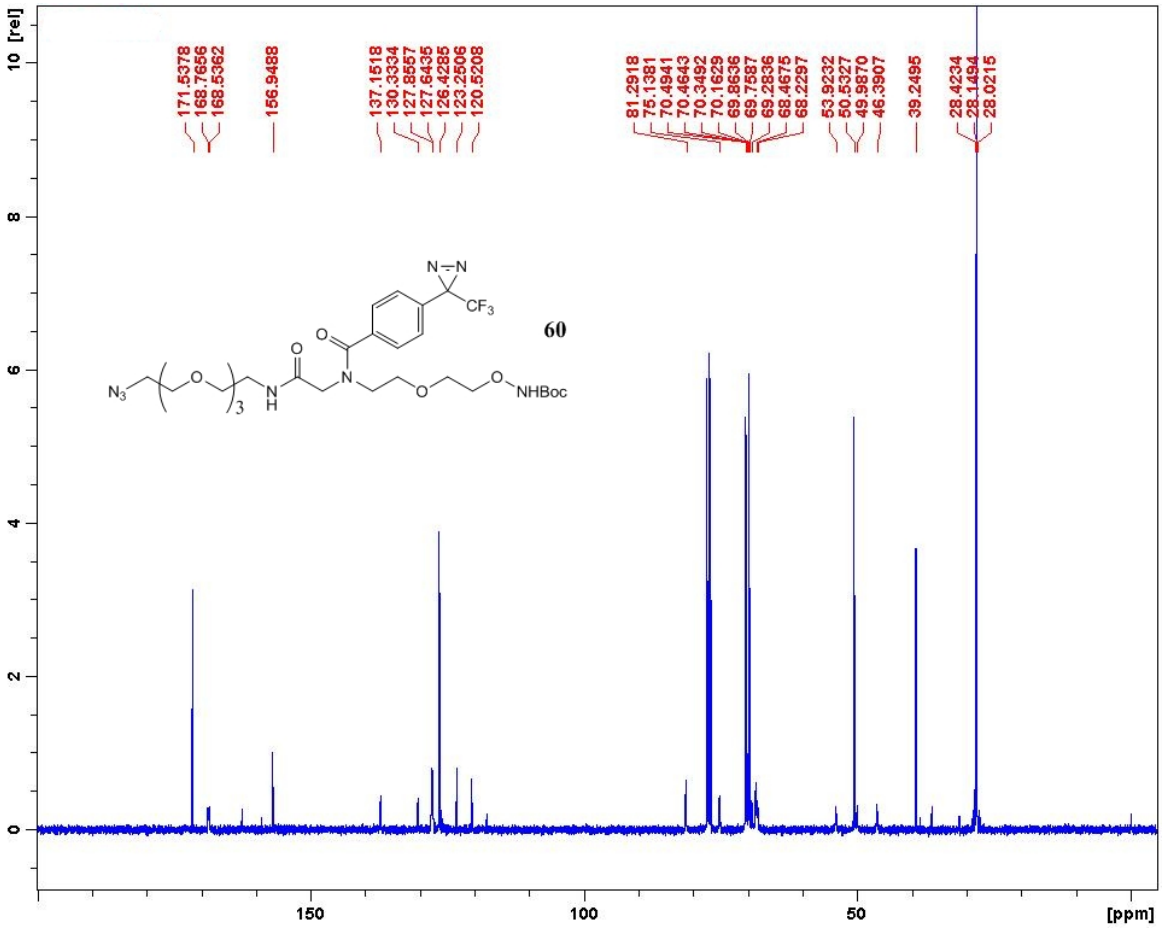
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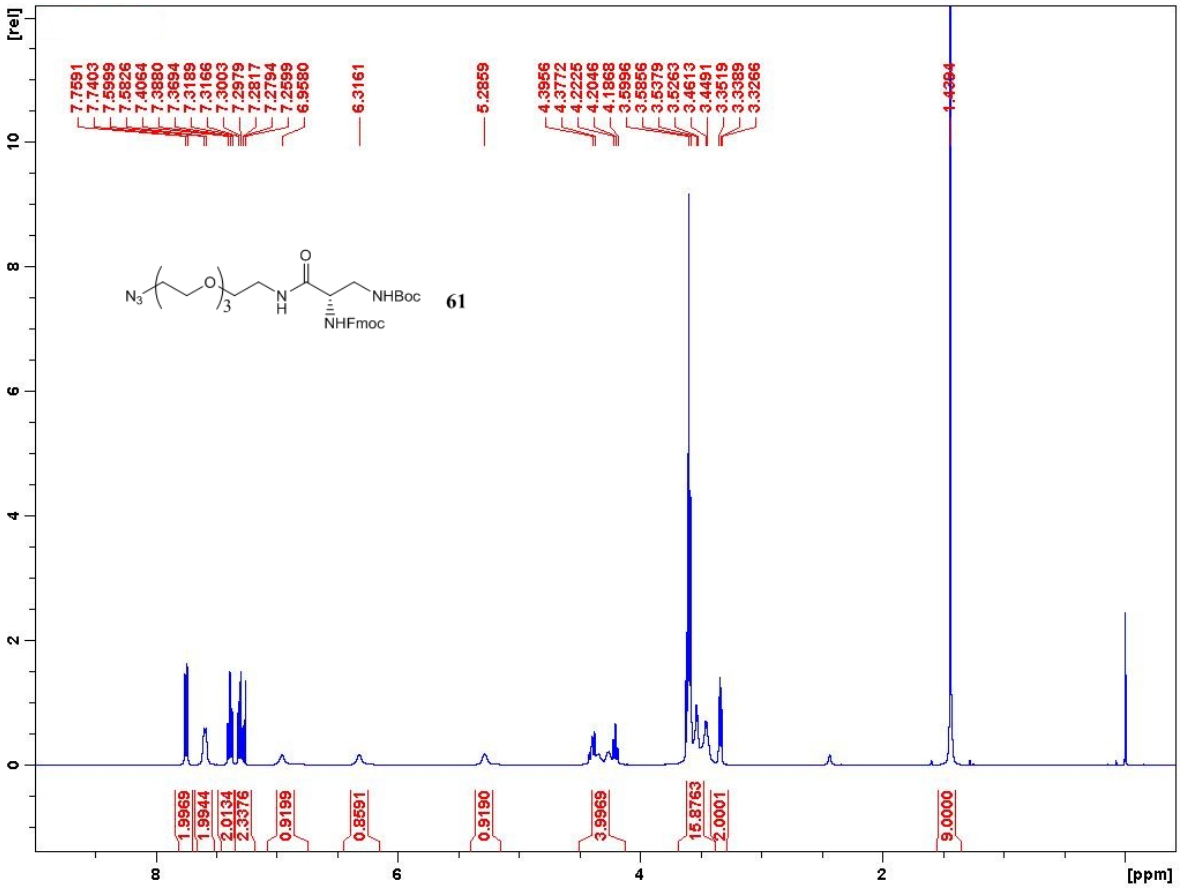
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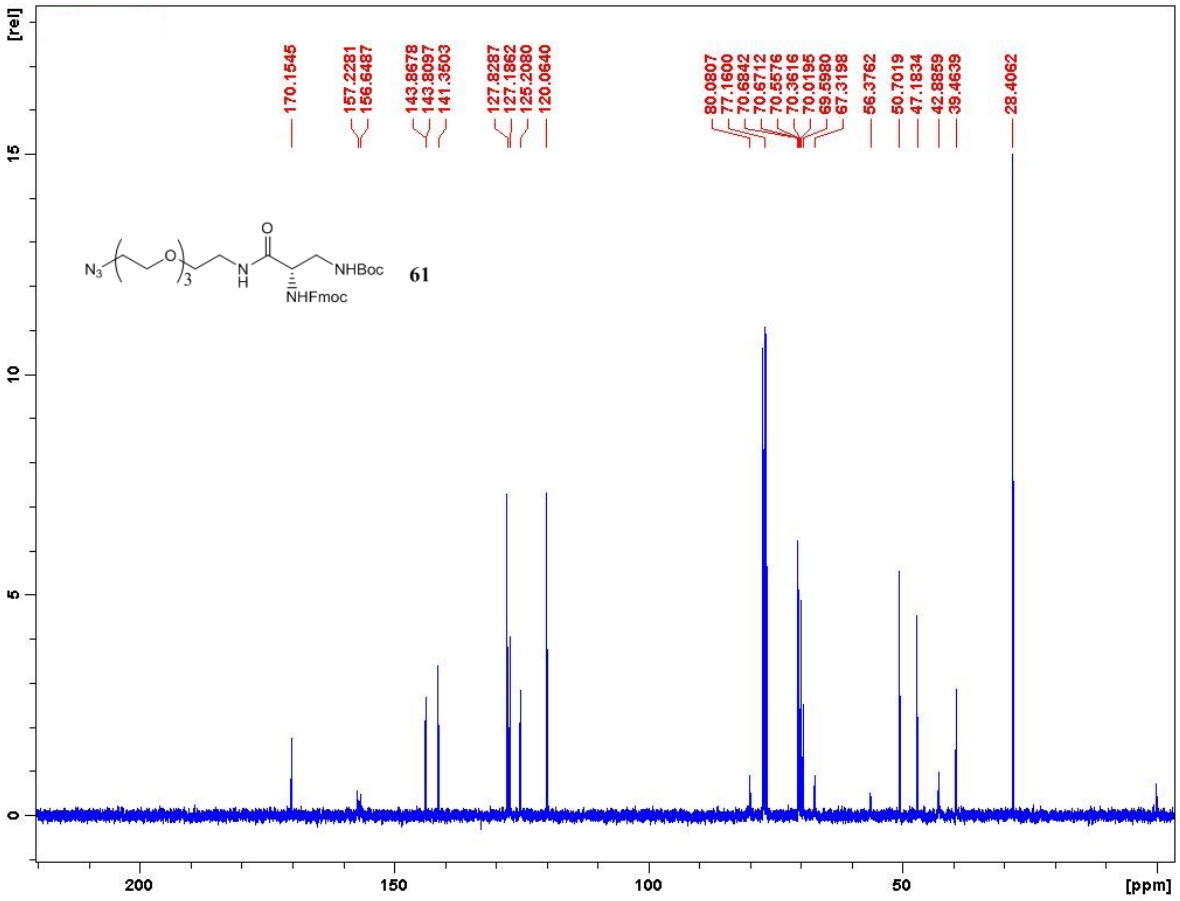
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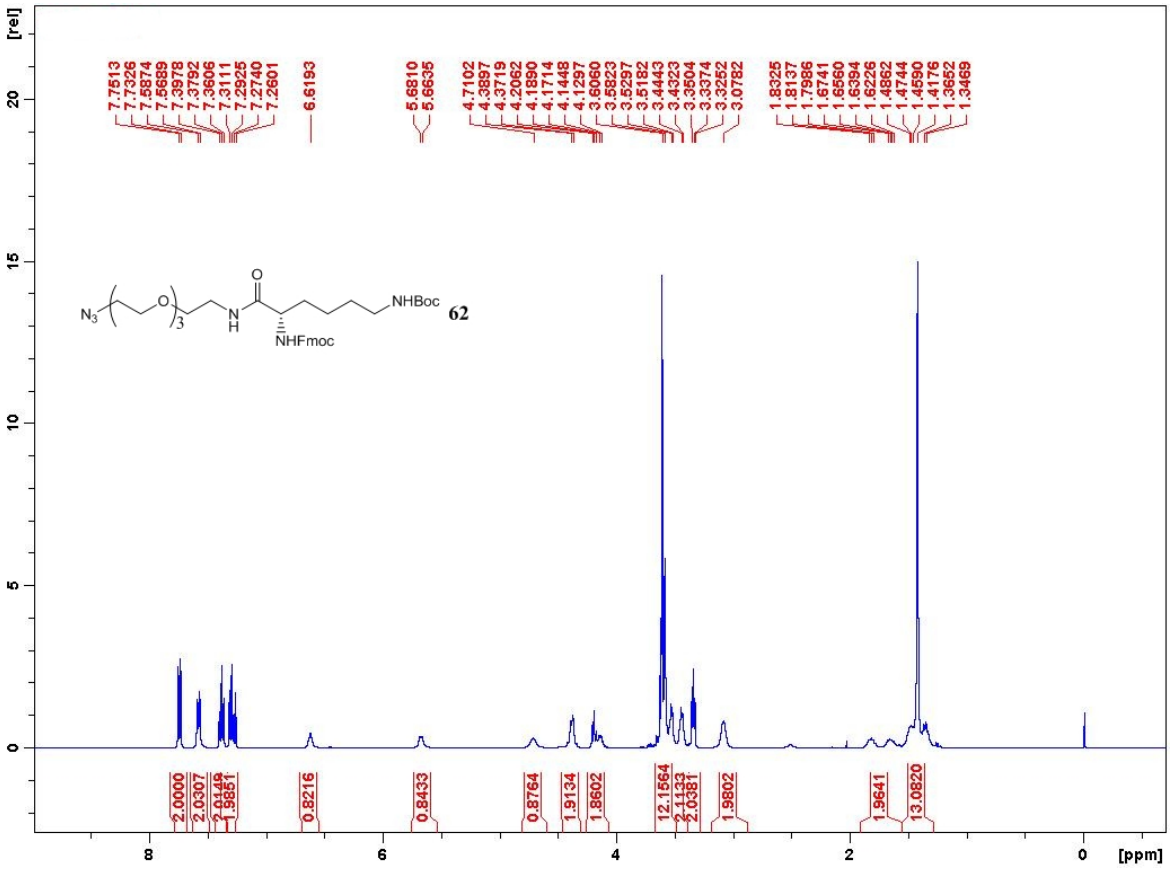
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5.6635
4.7102
4.3897
4.3719
4.2062
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4.1714
4.1448
4.1297
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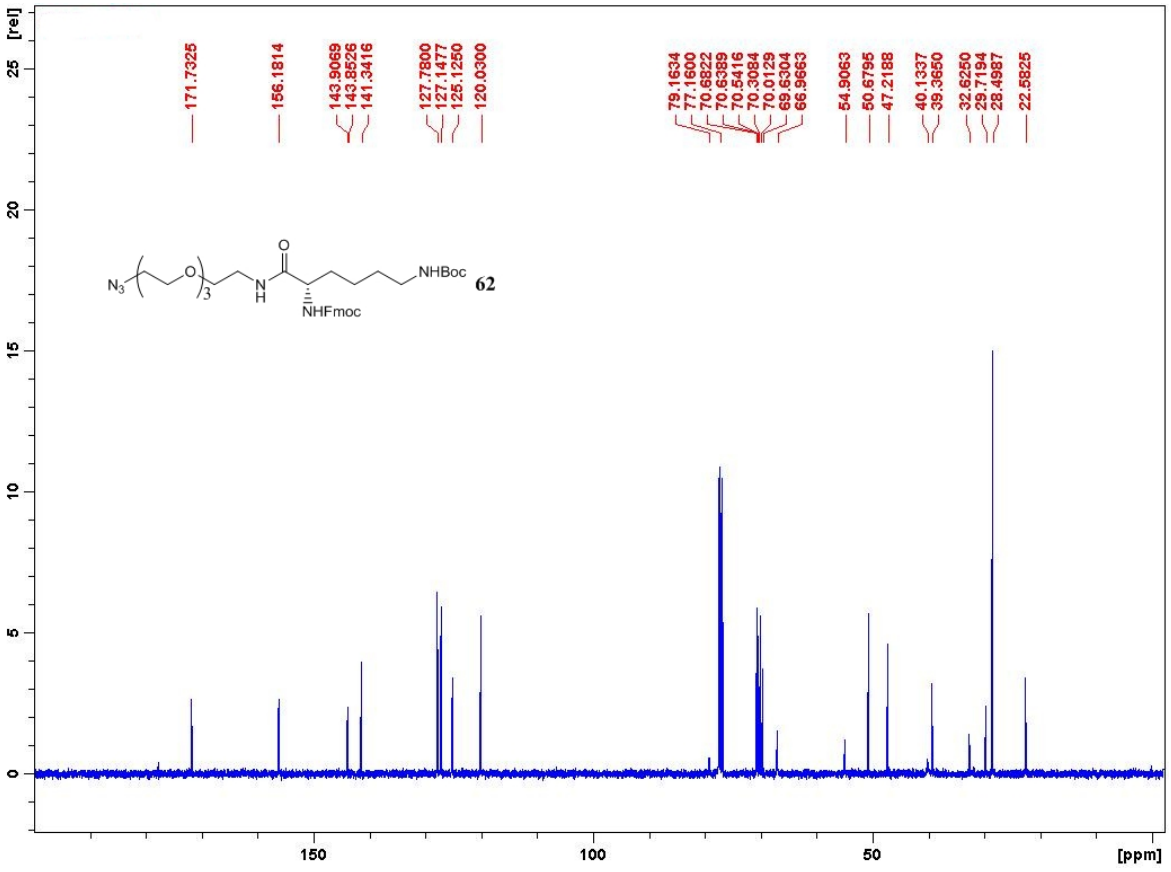
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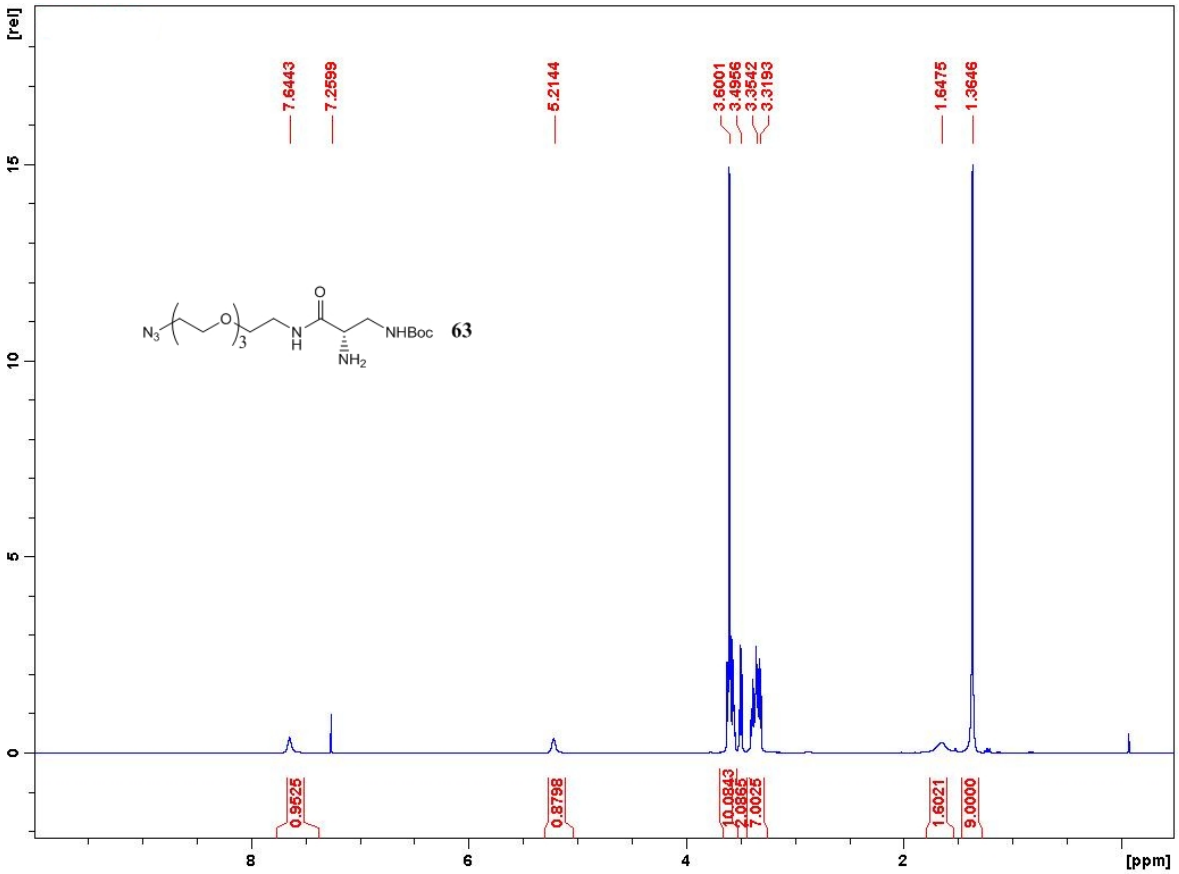
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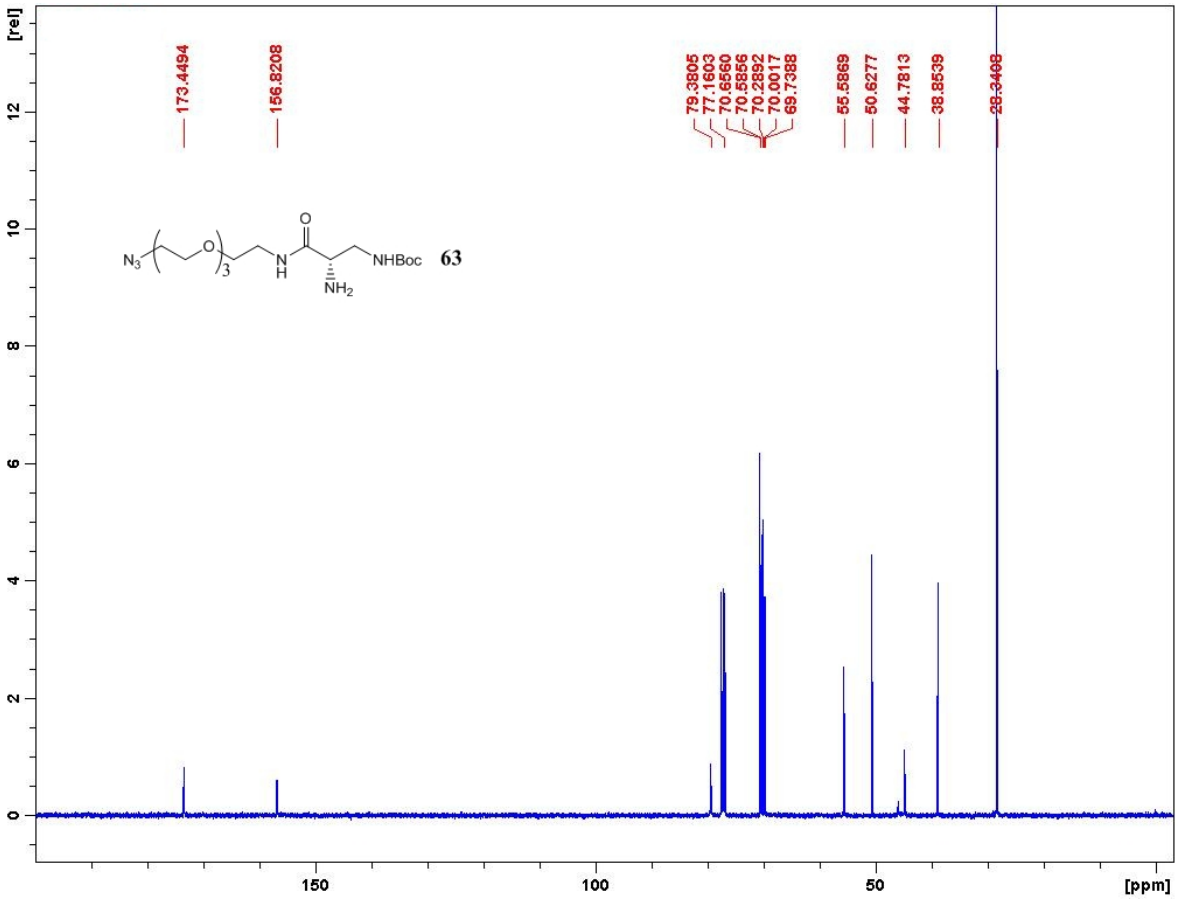
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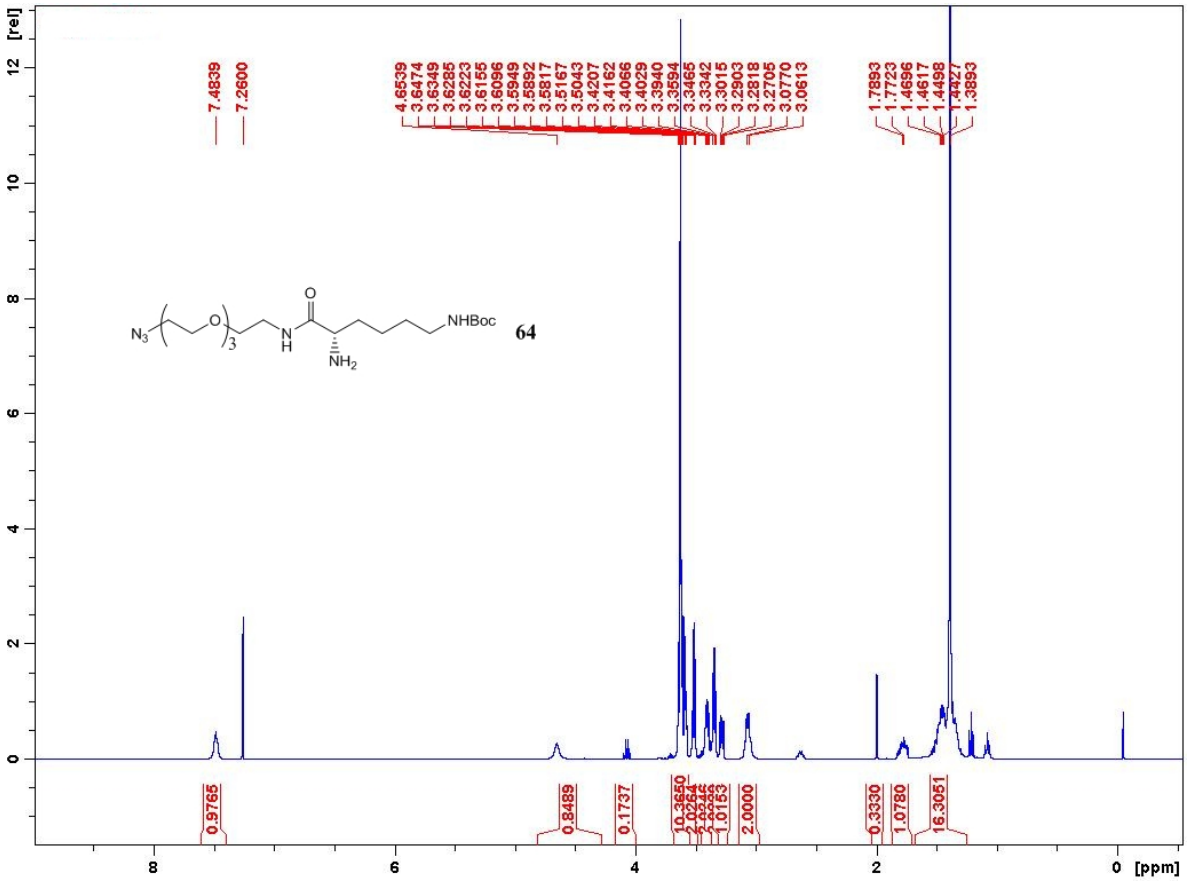
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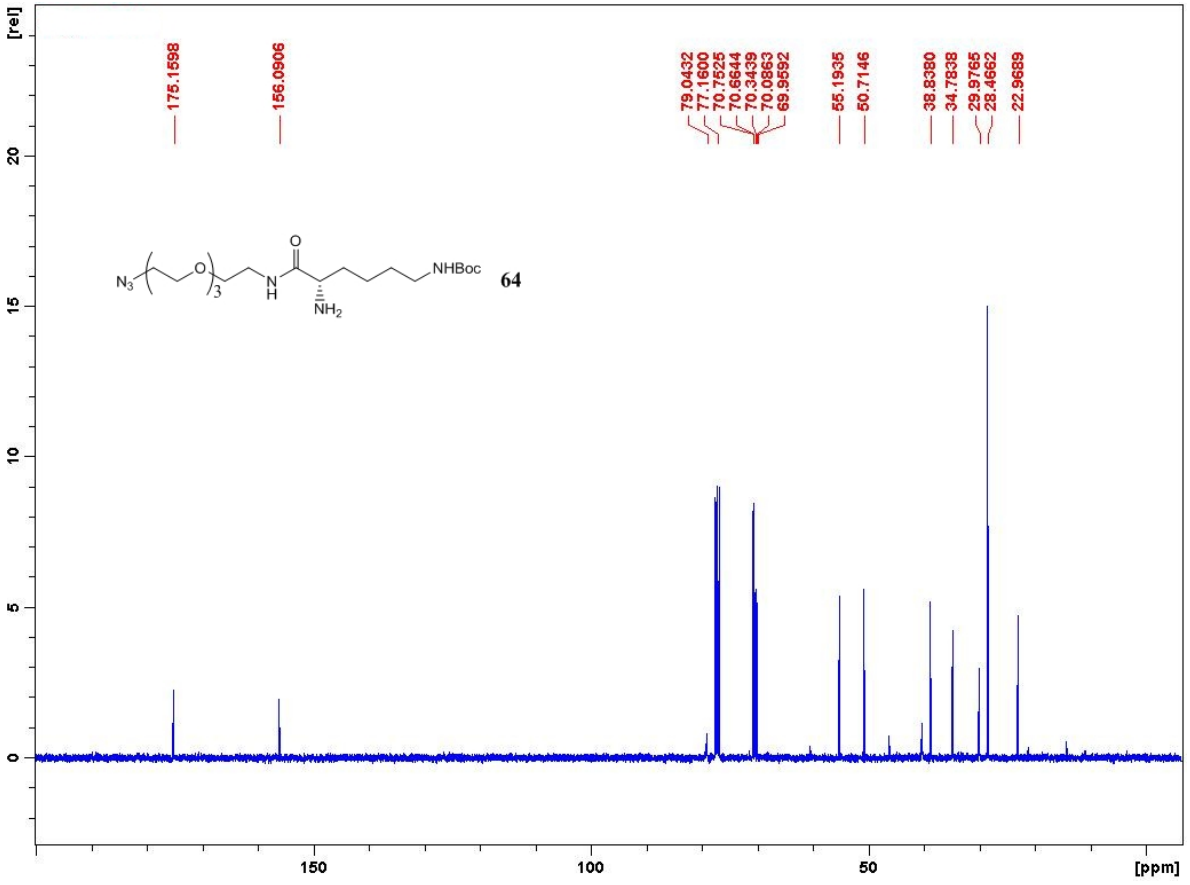
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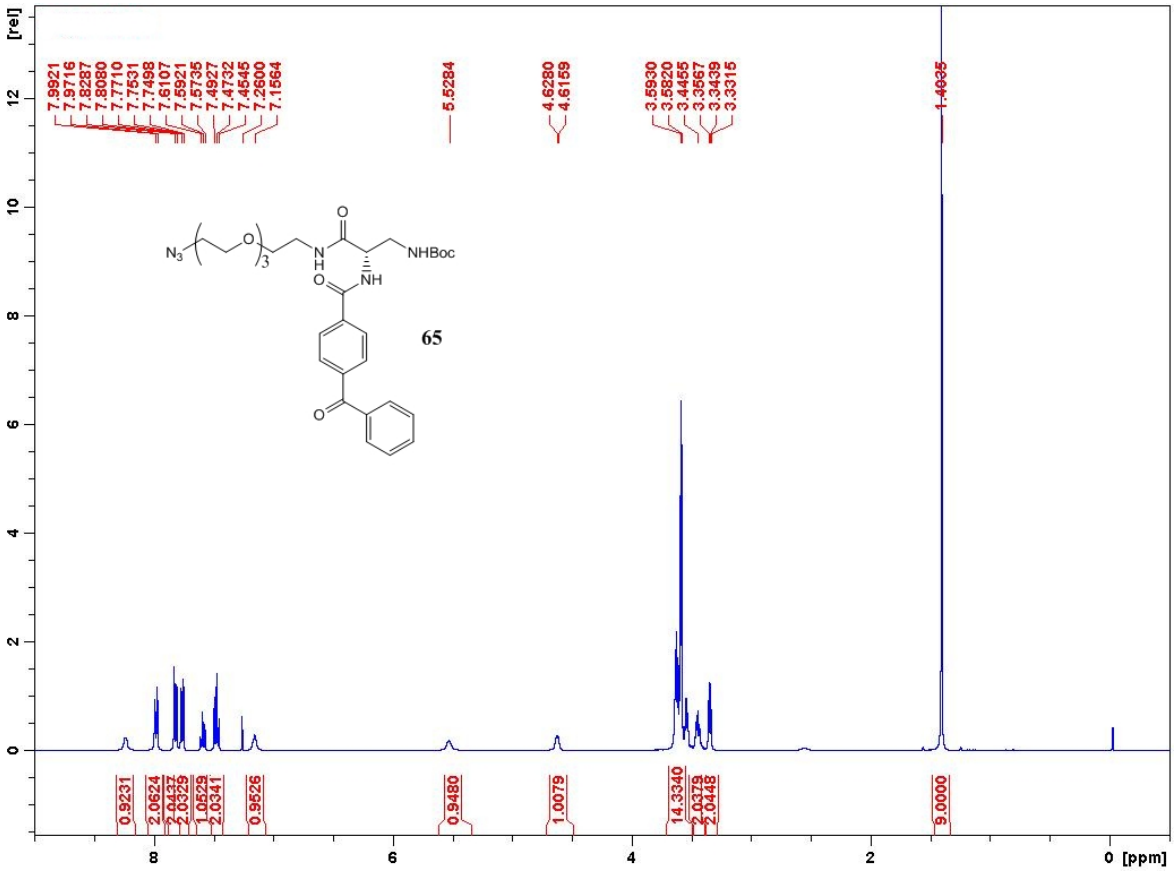
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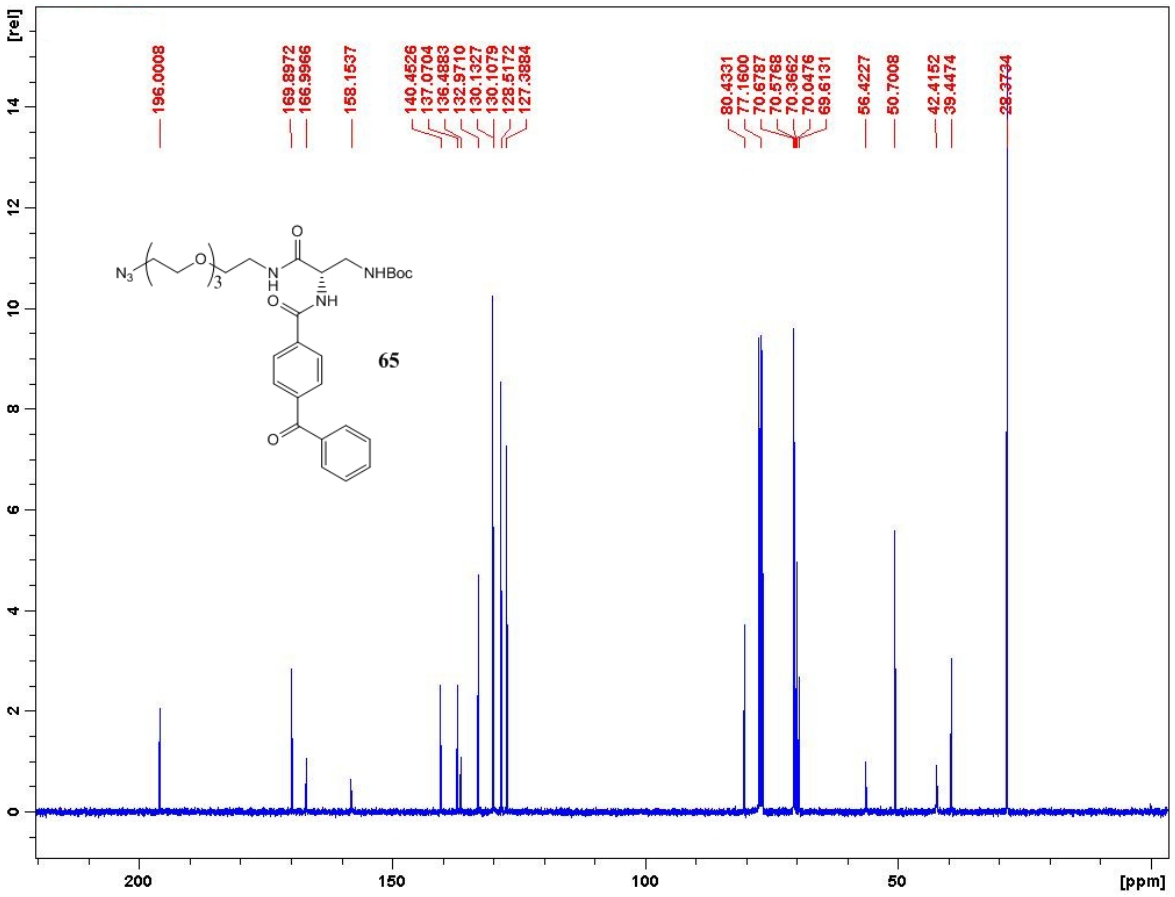
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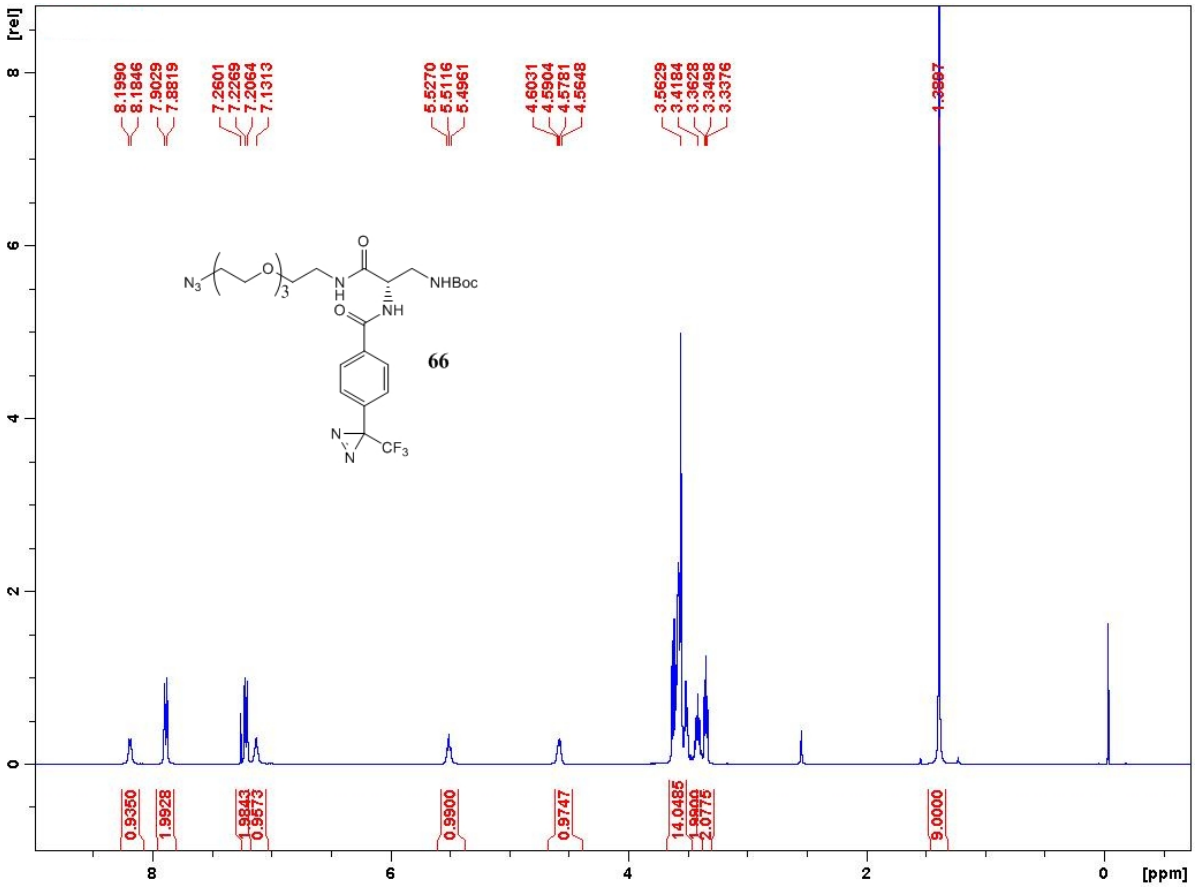
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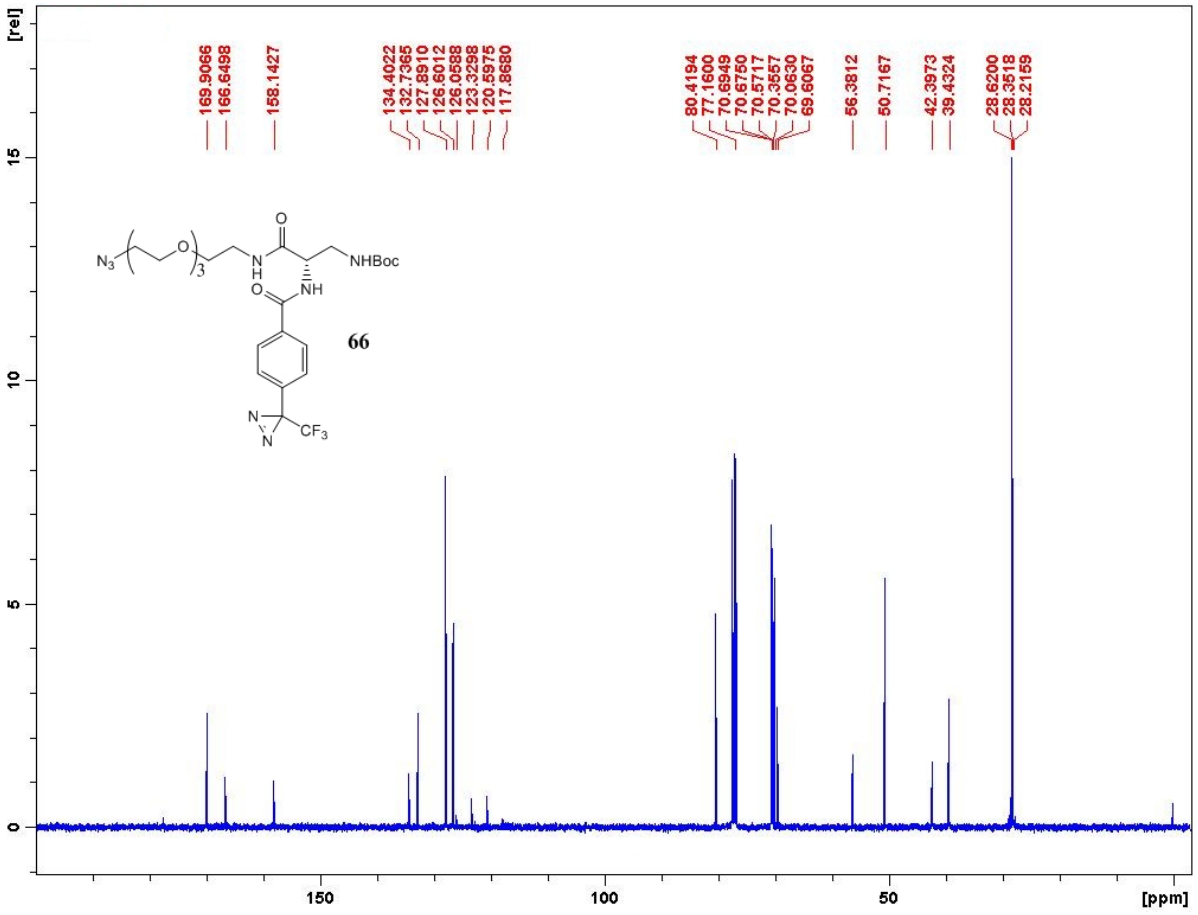
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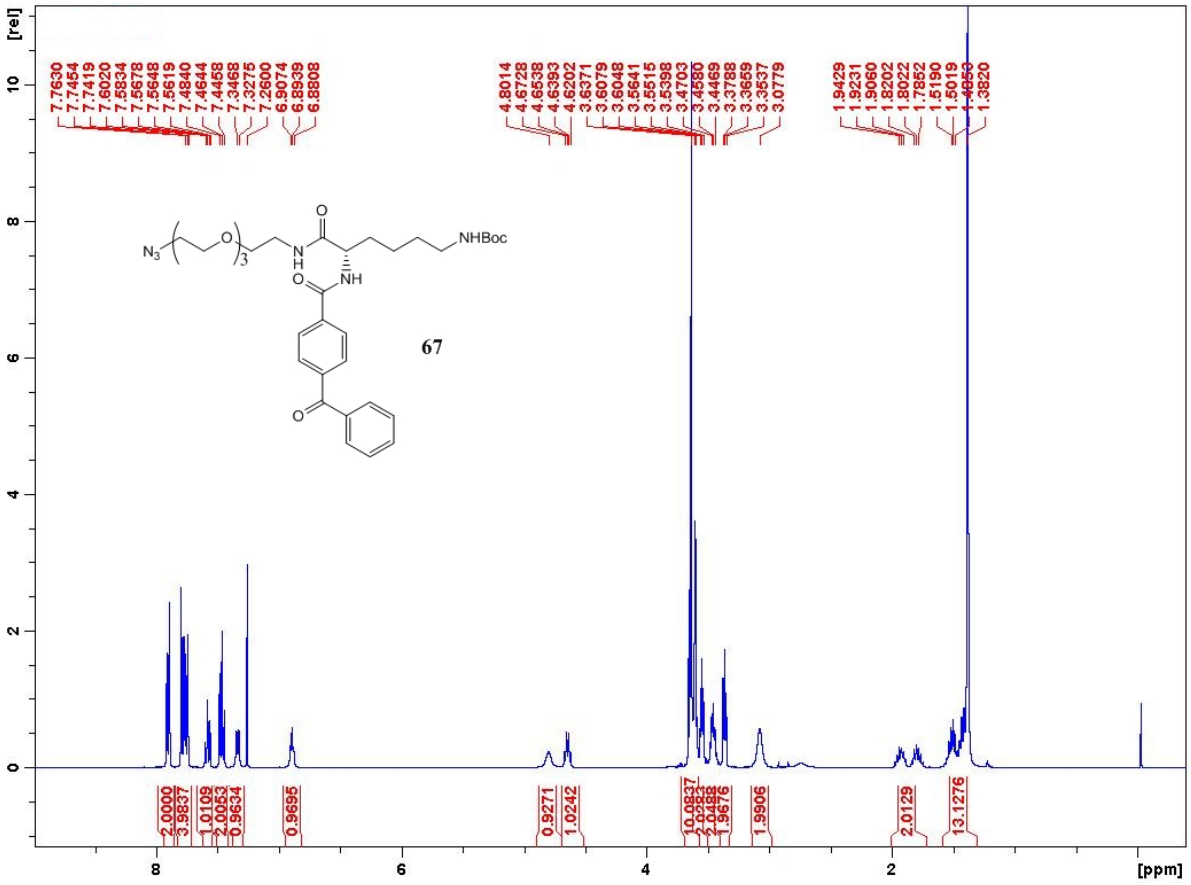
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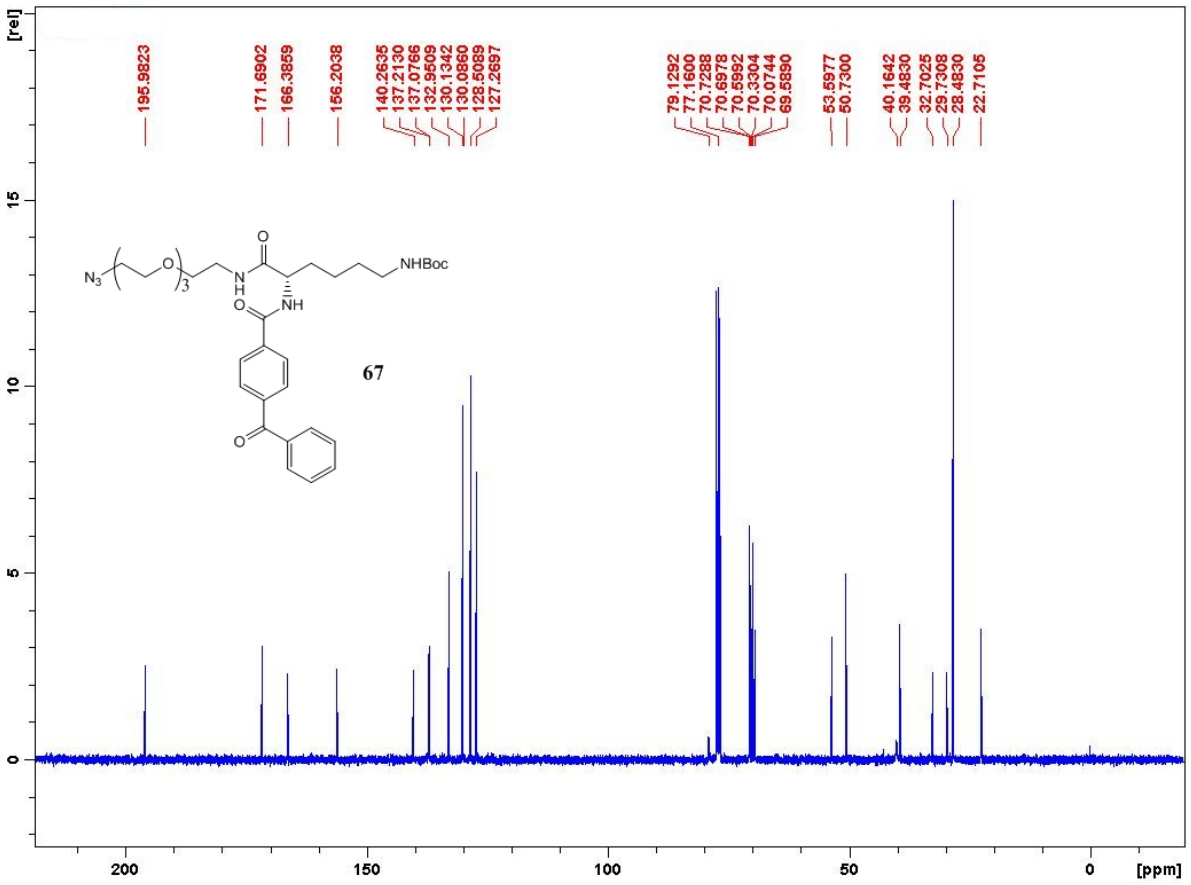
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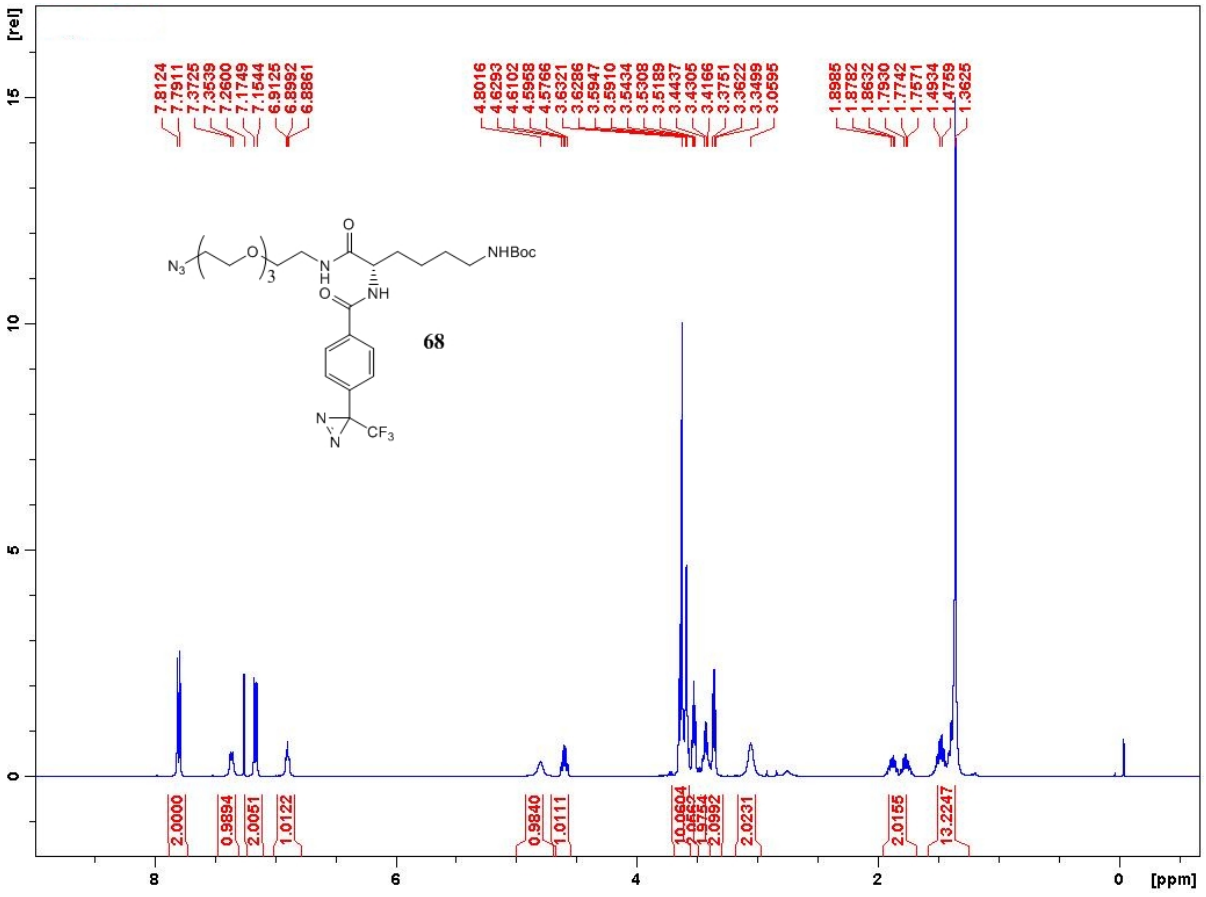
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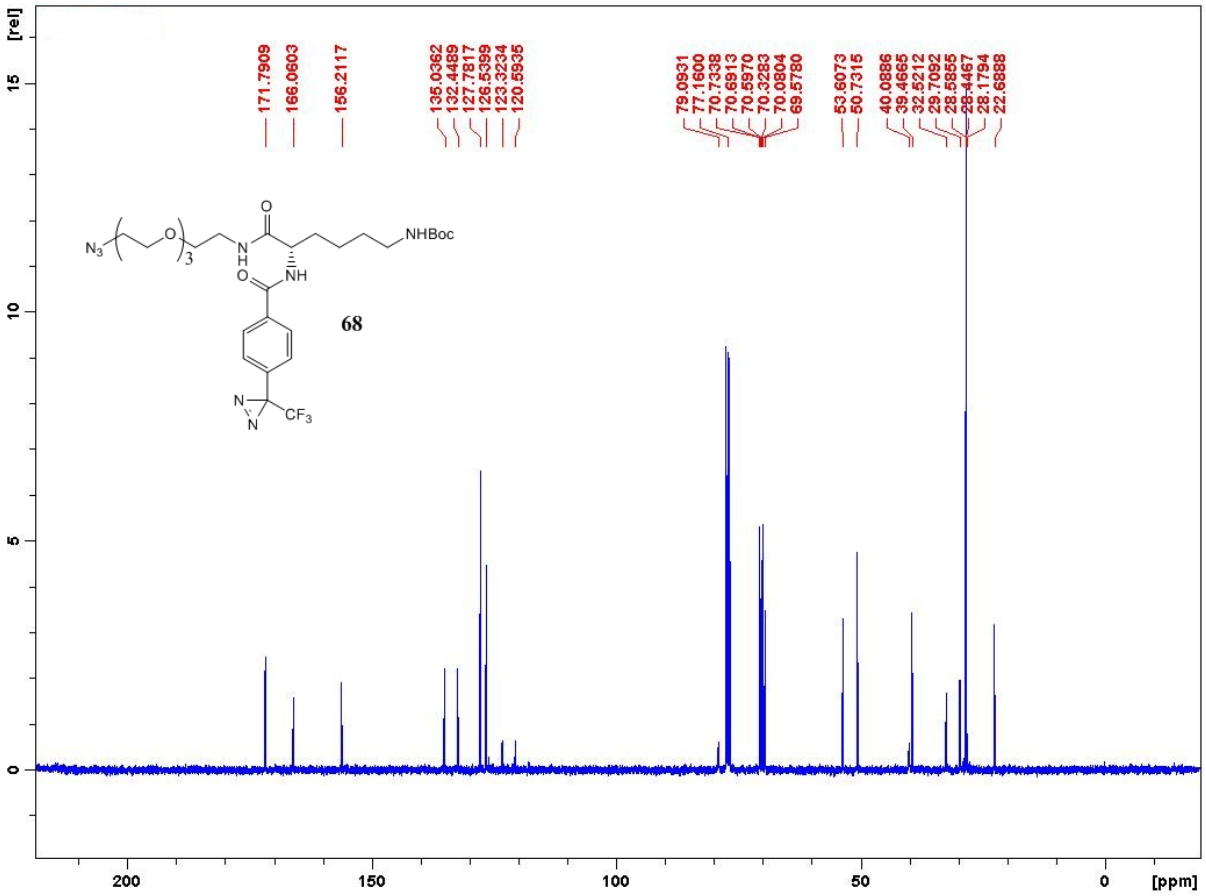
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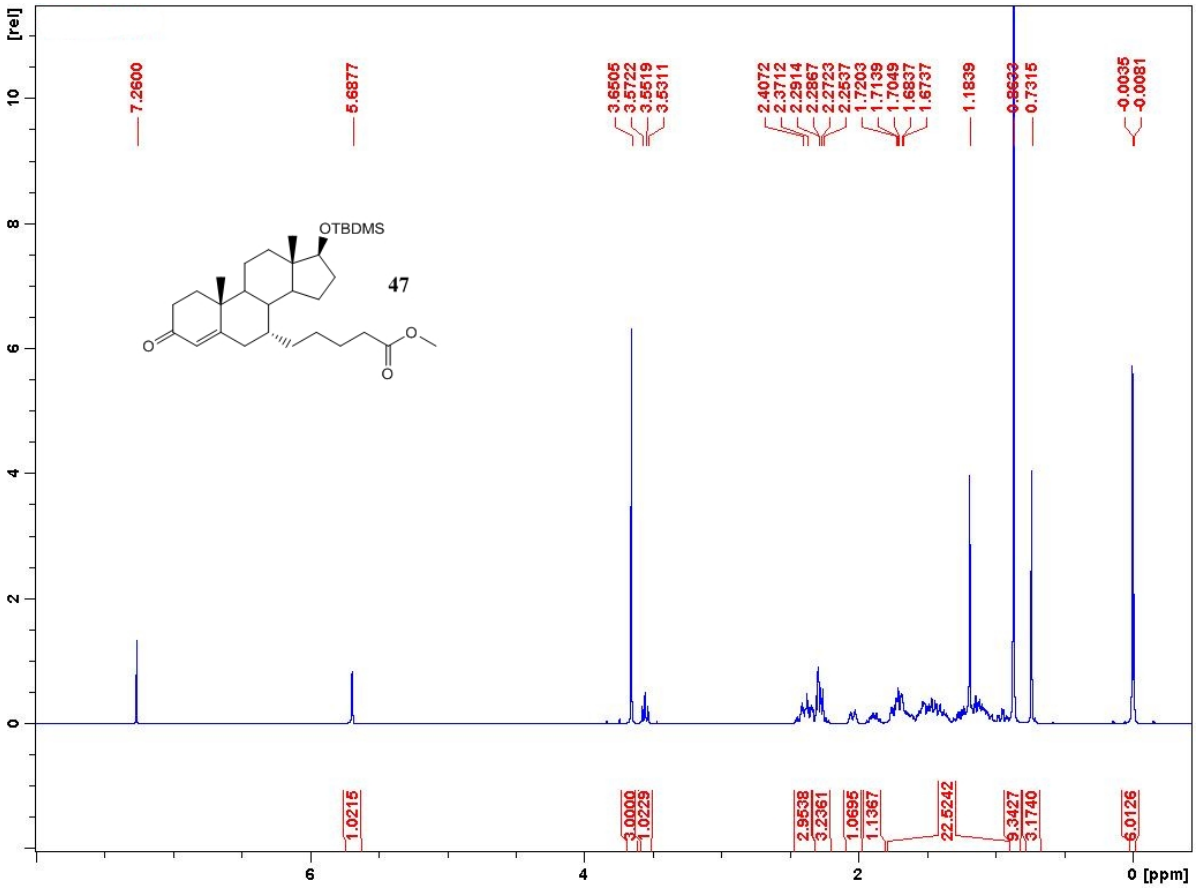
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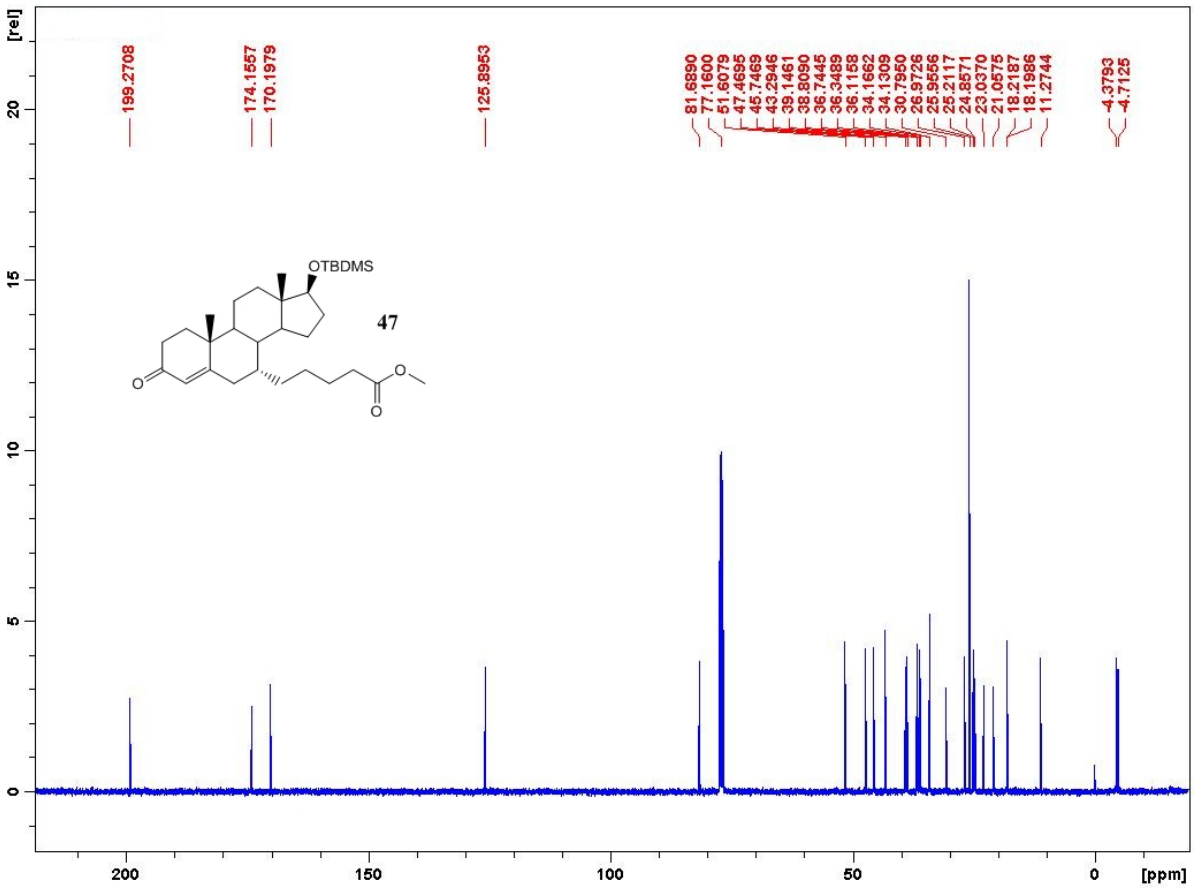
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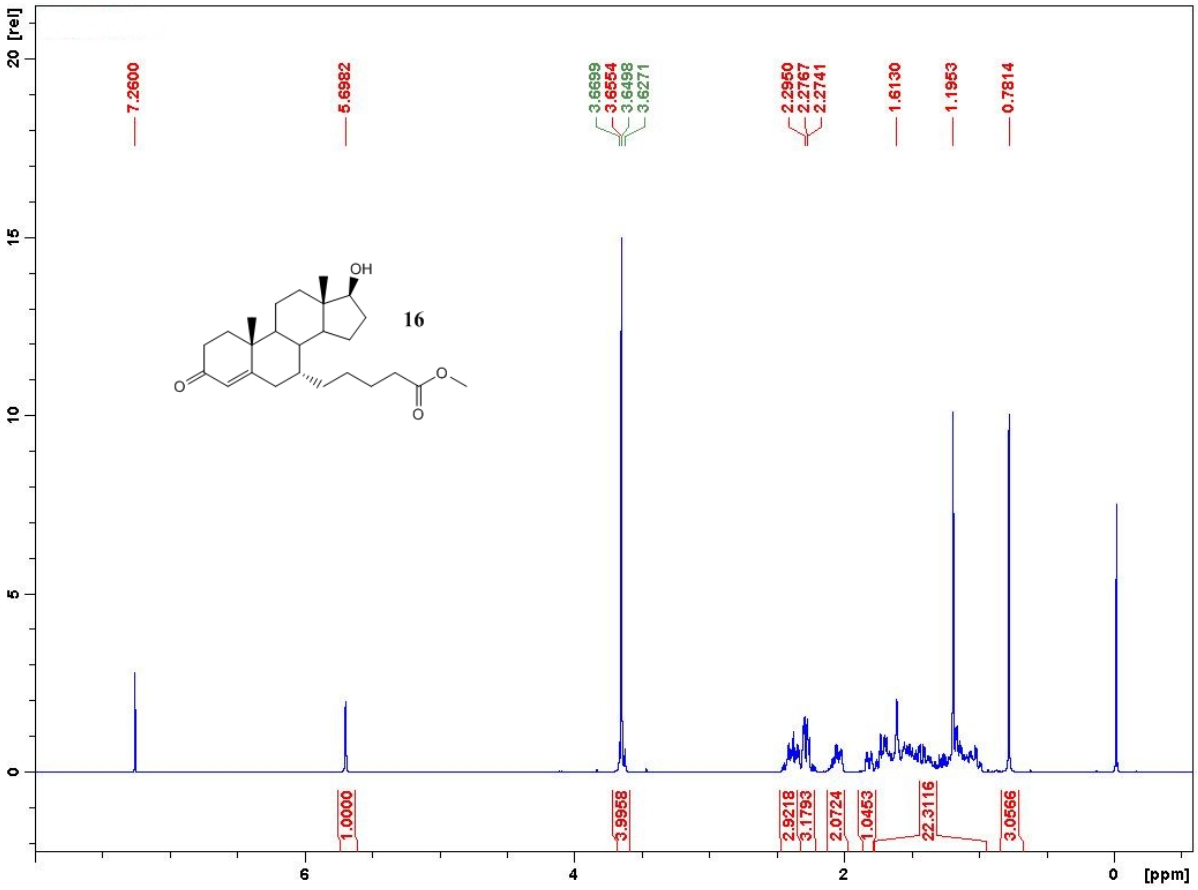
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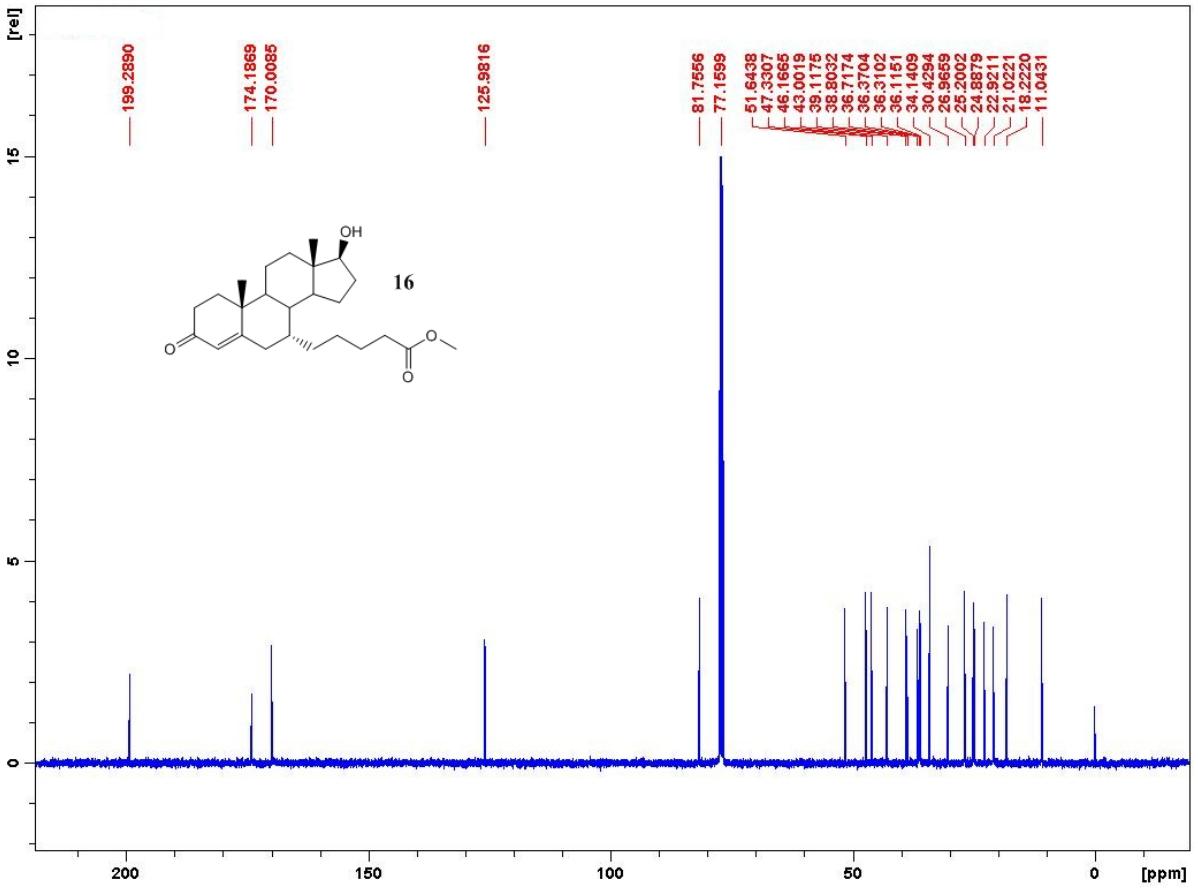
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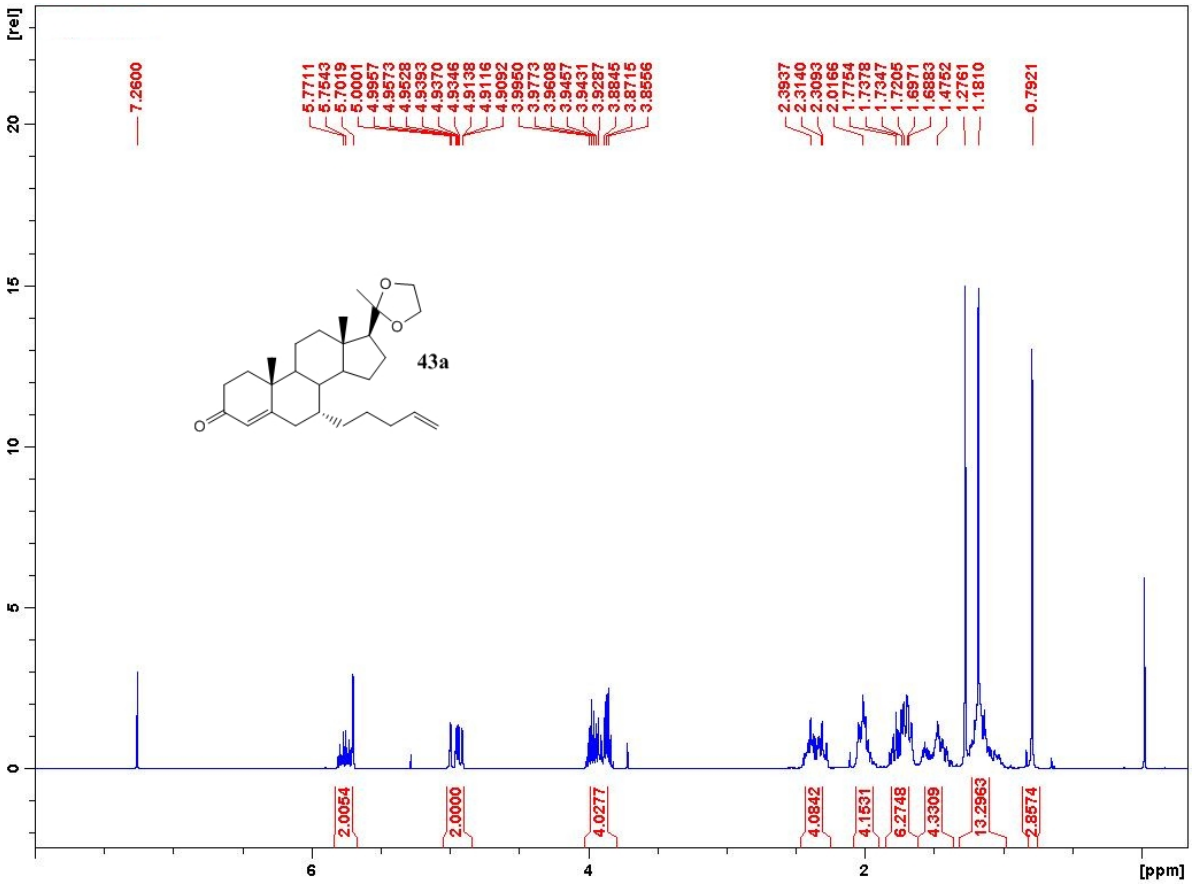
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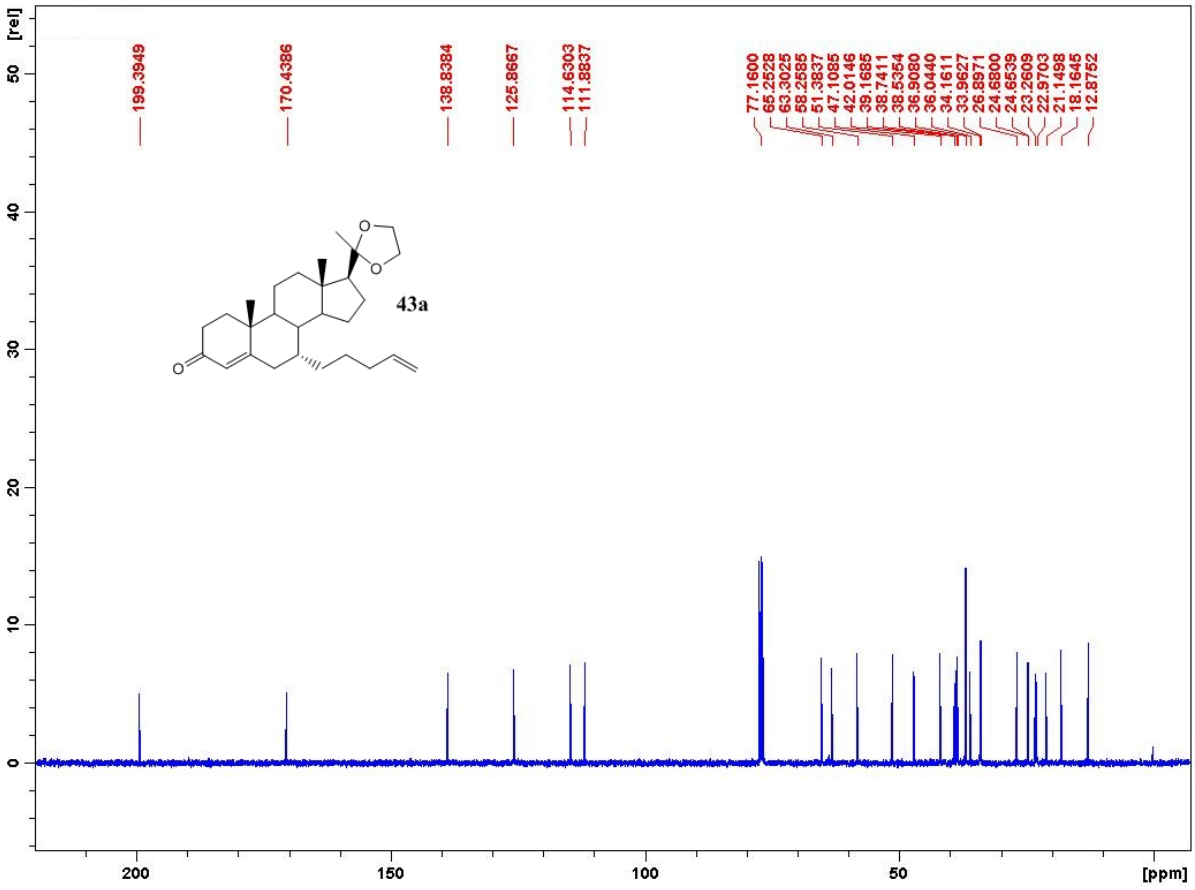
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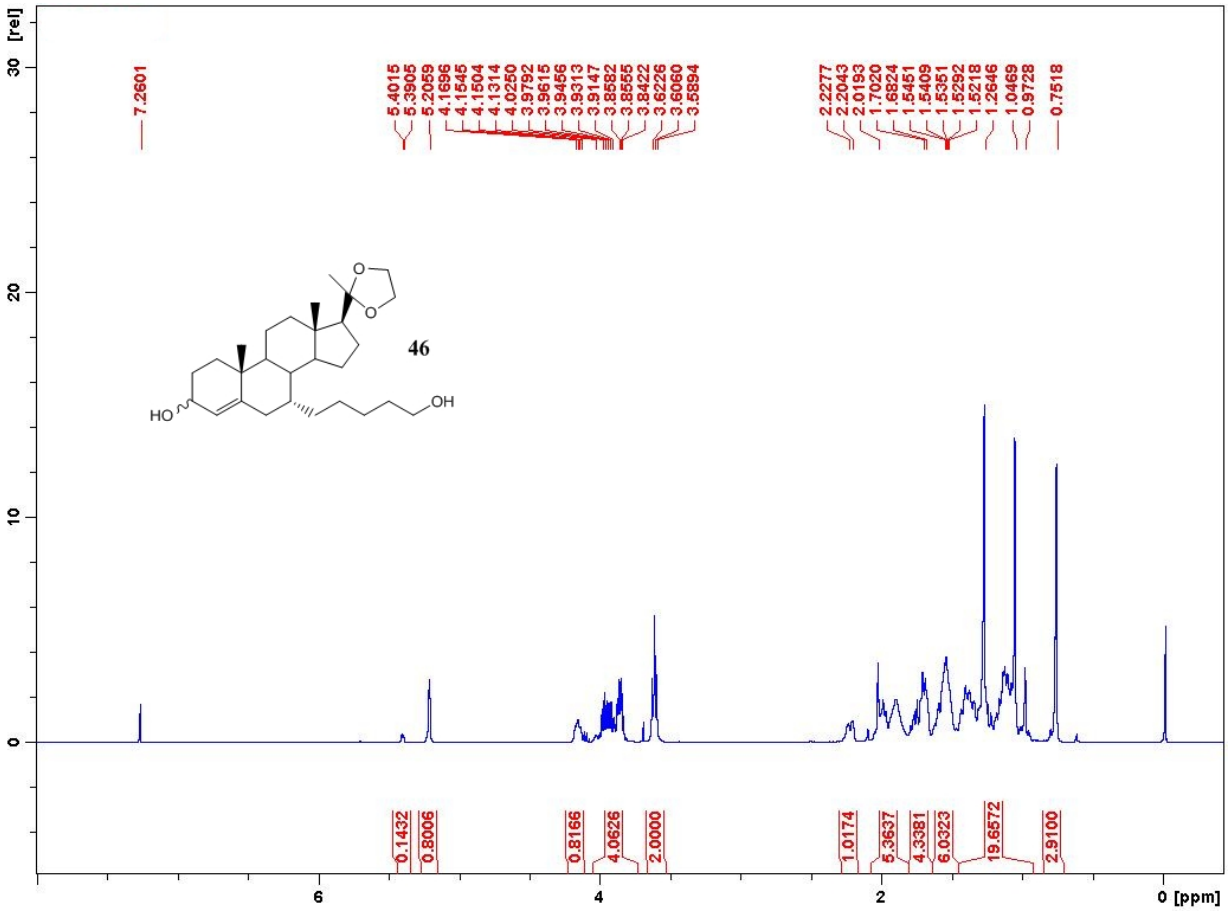
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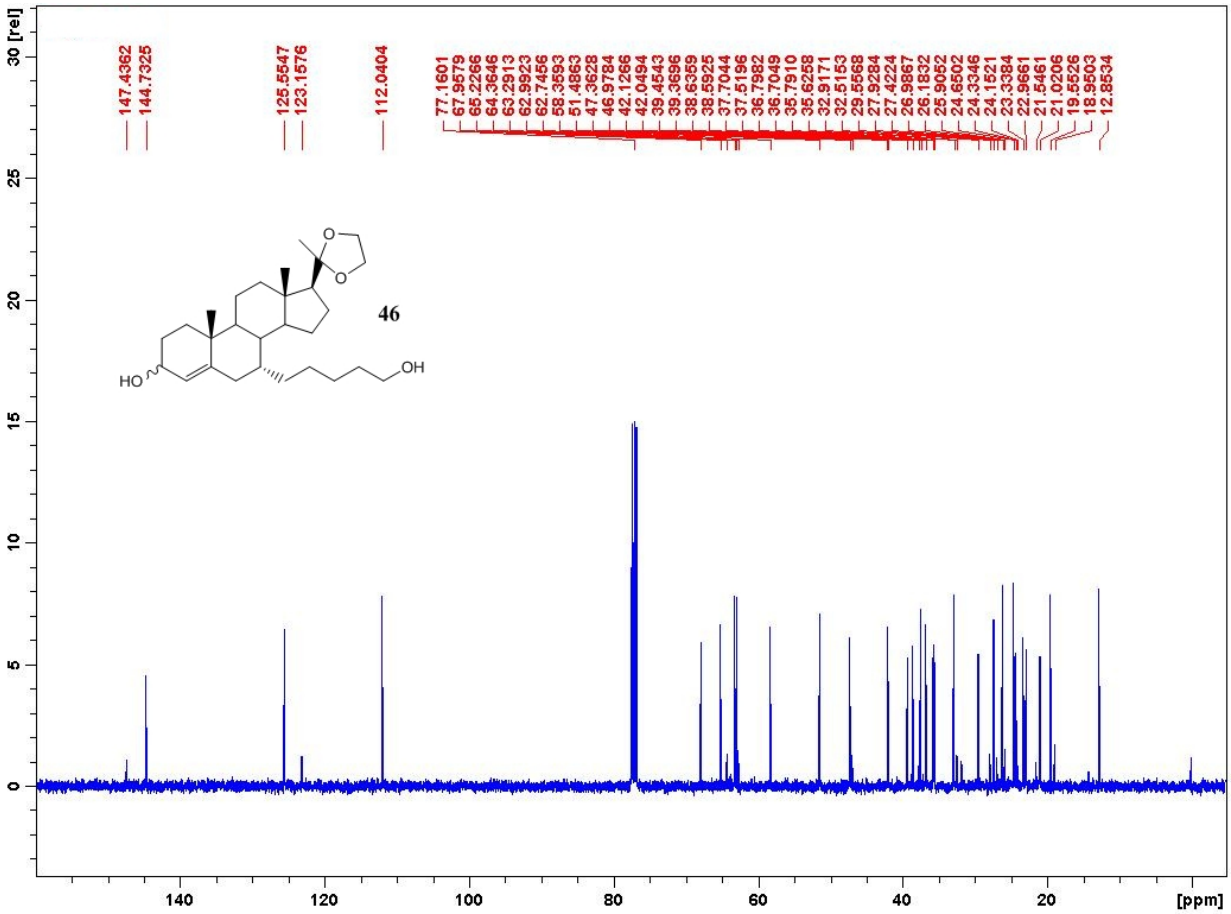
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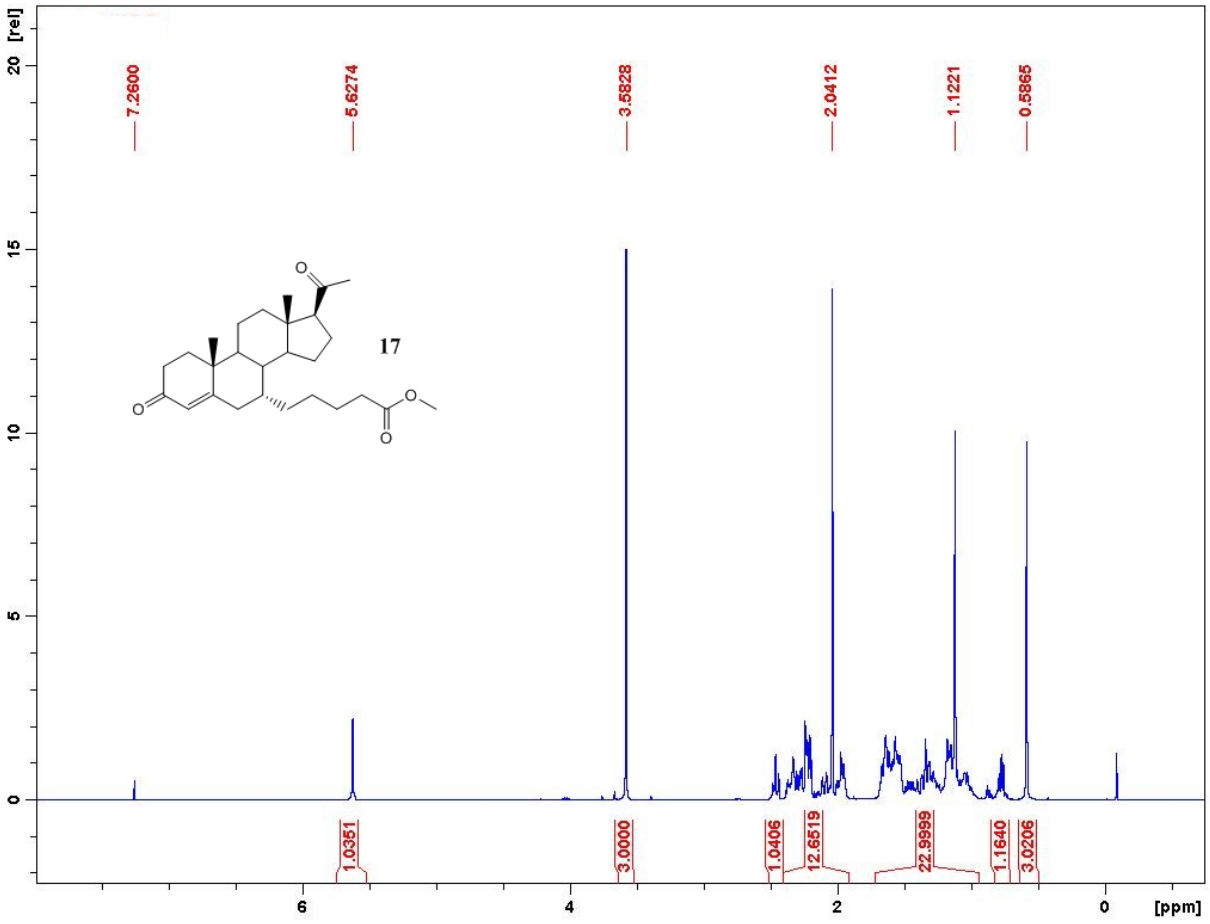
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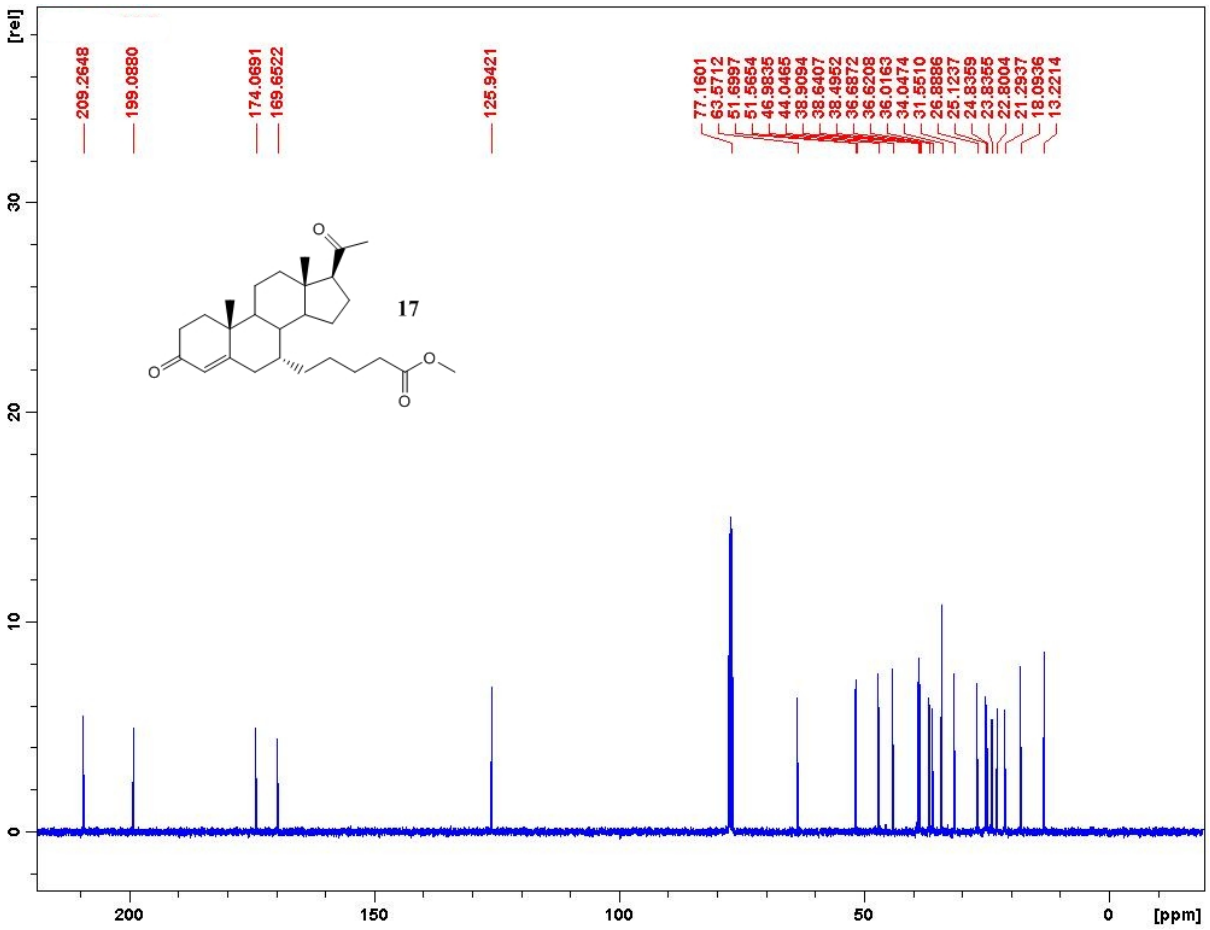
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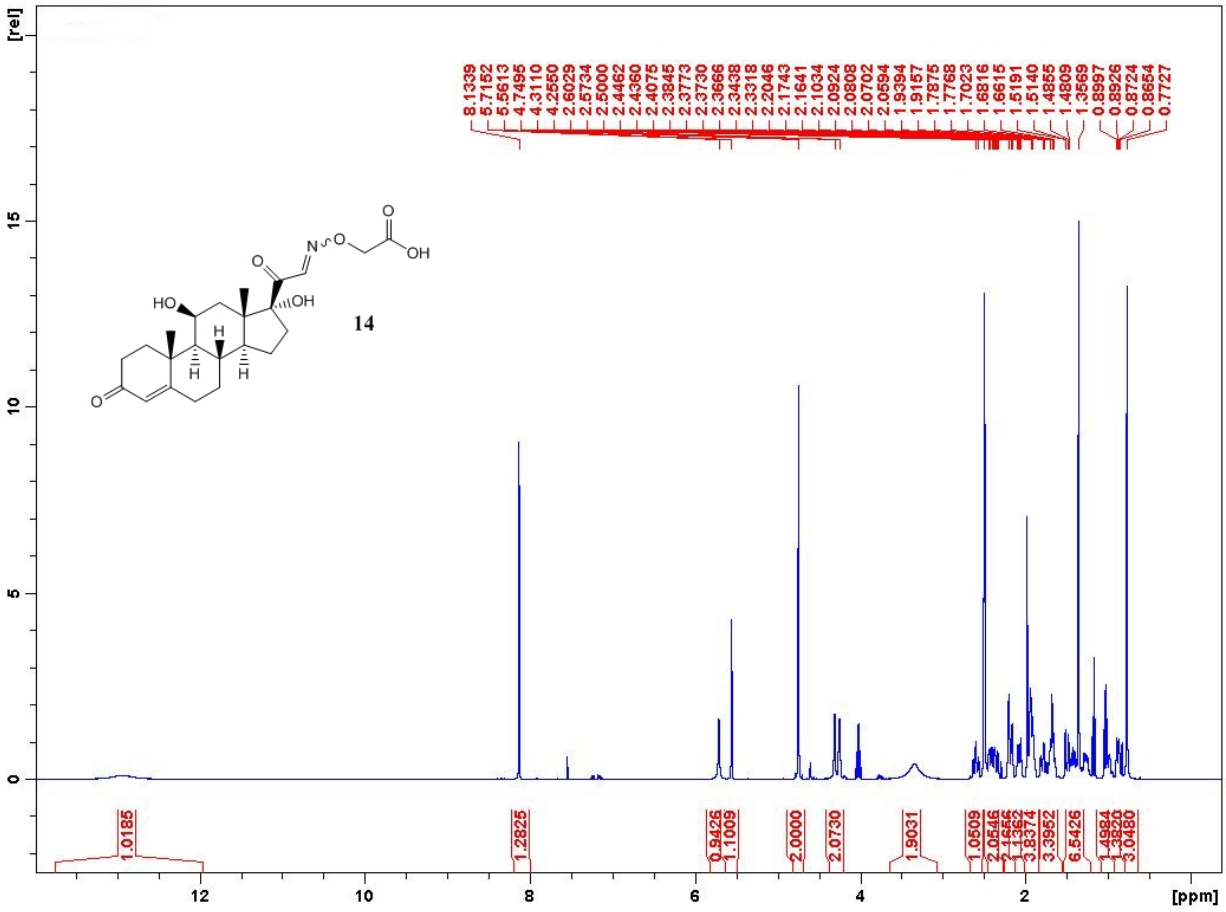
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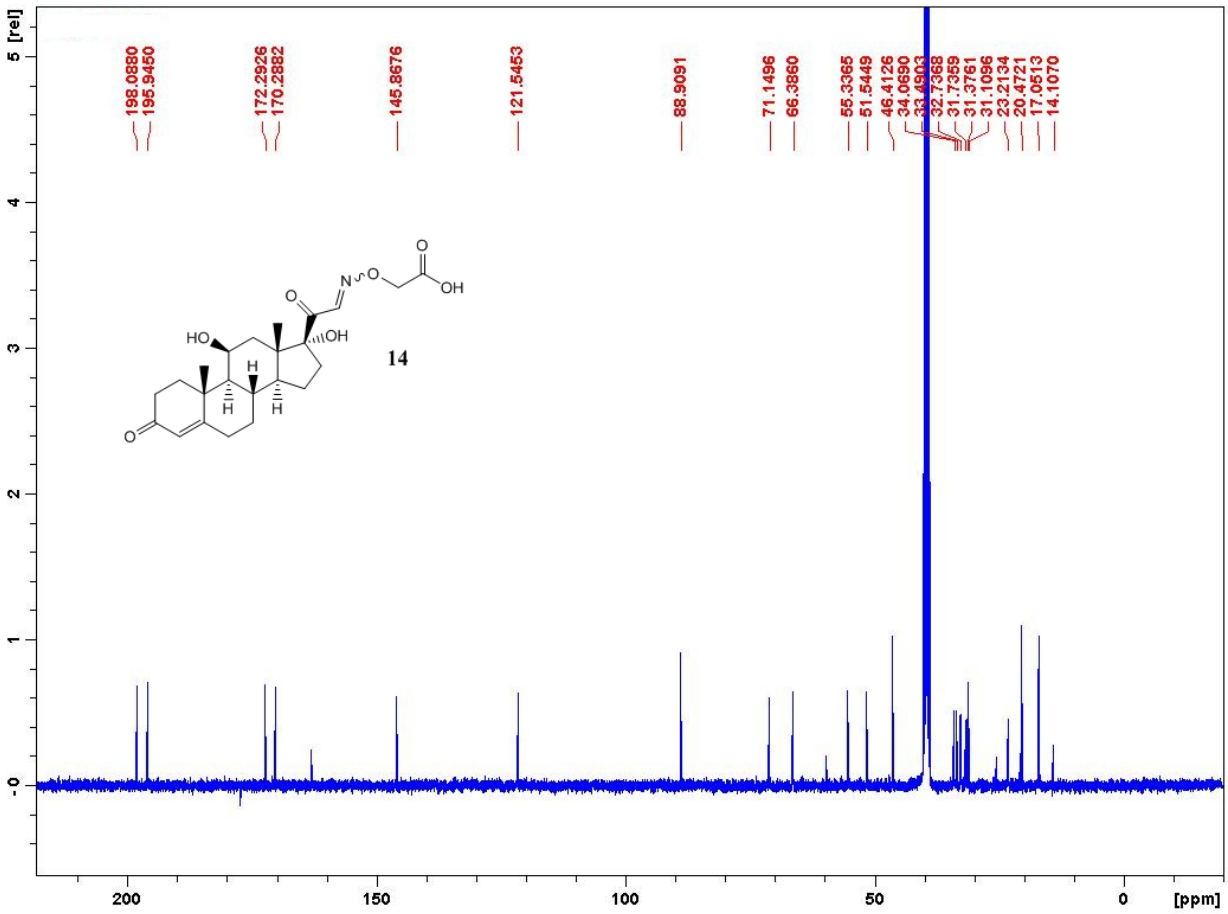
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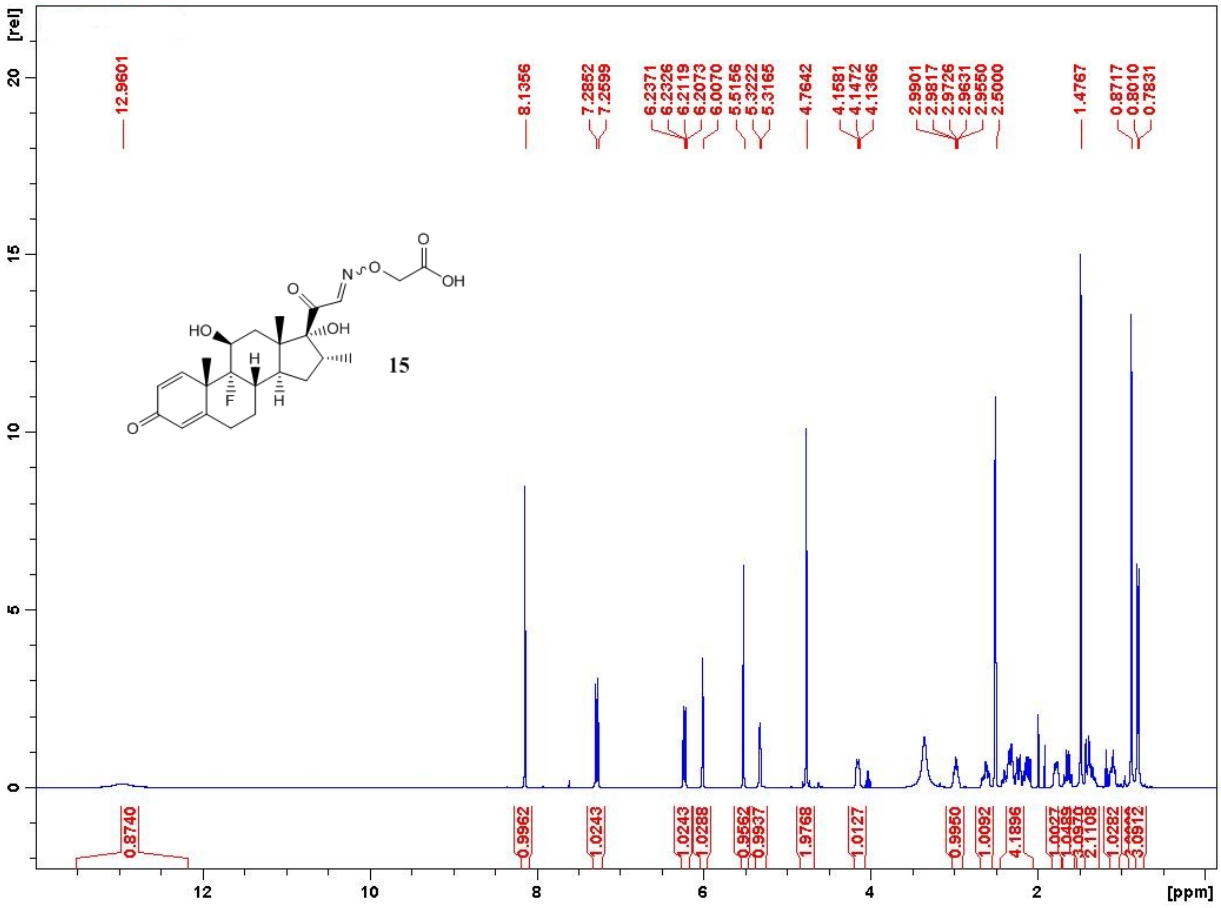
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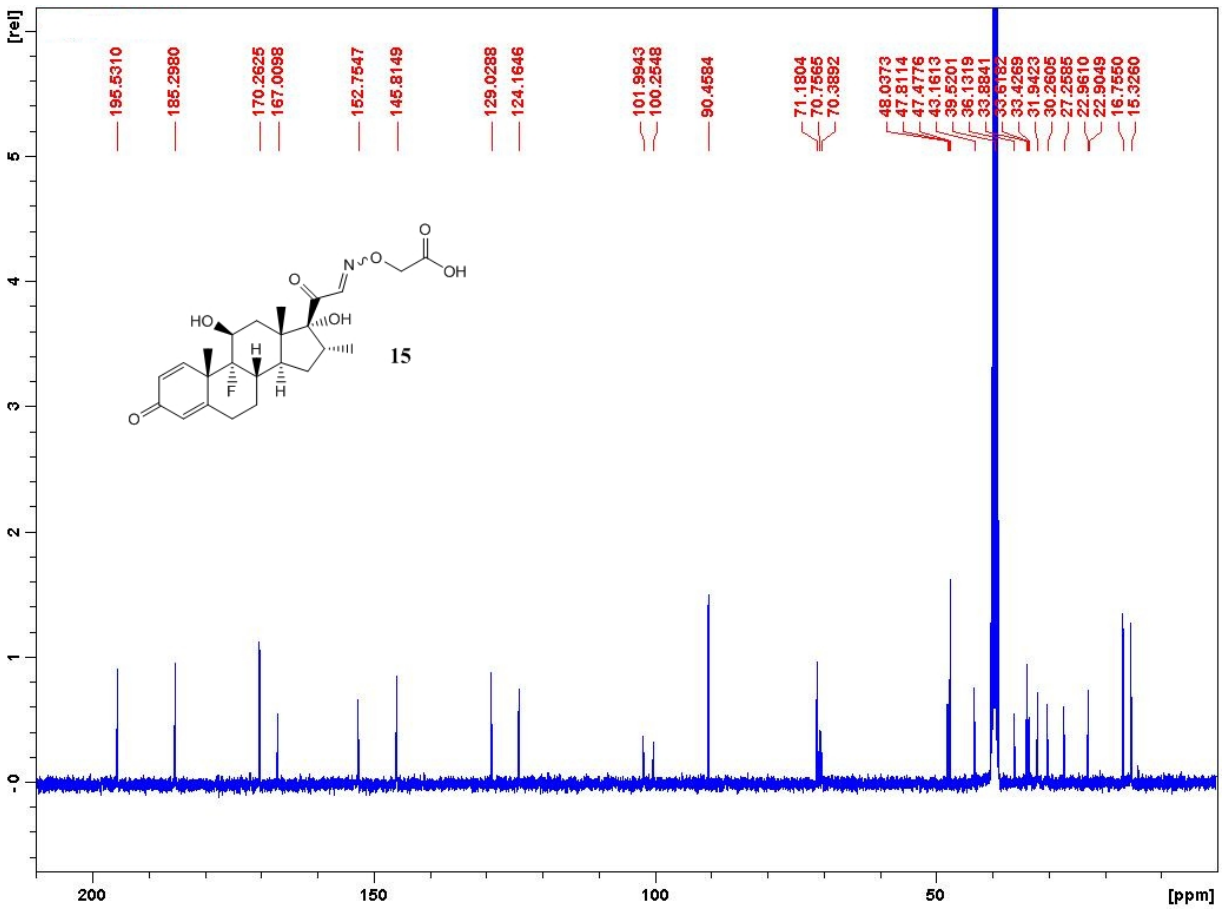
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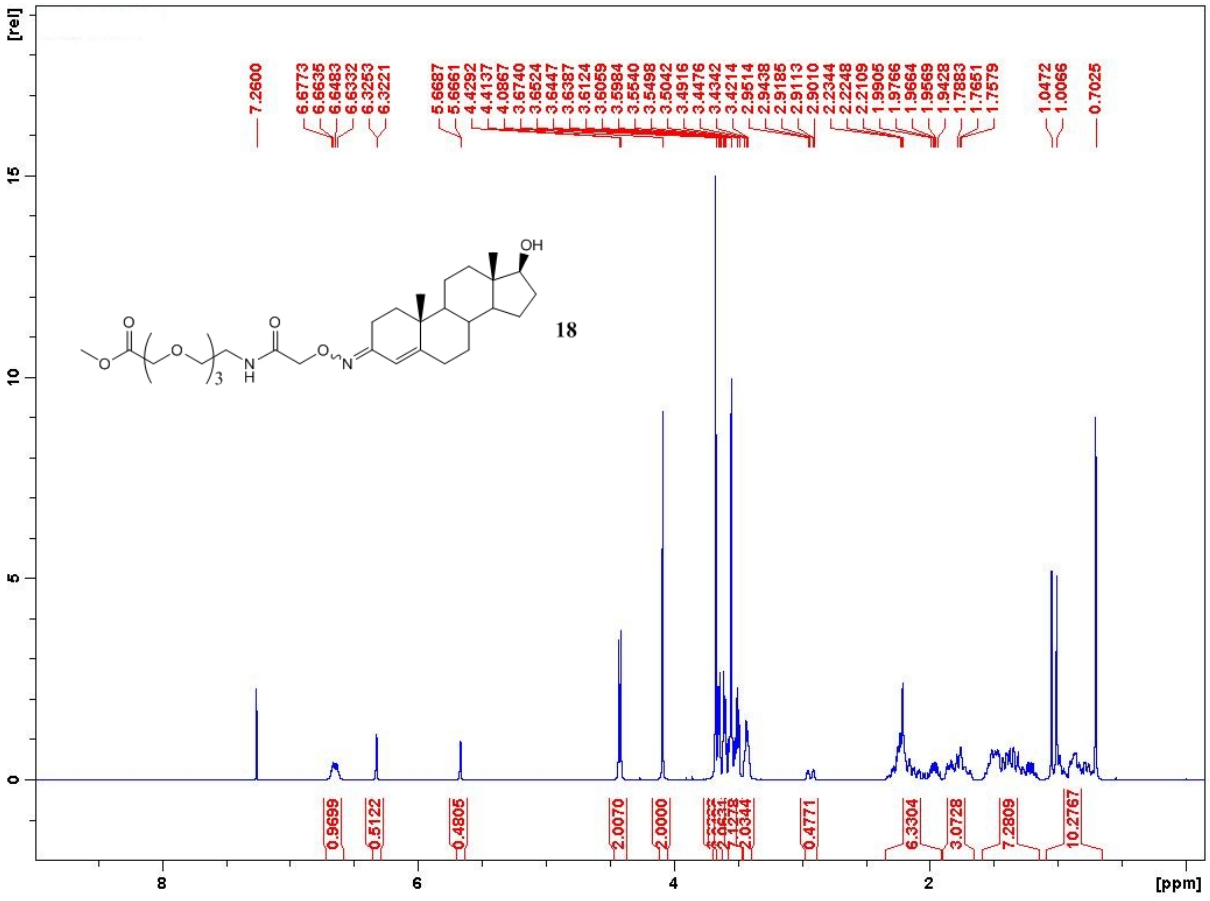
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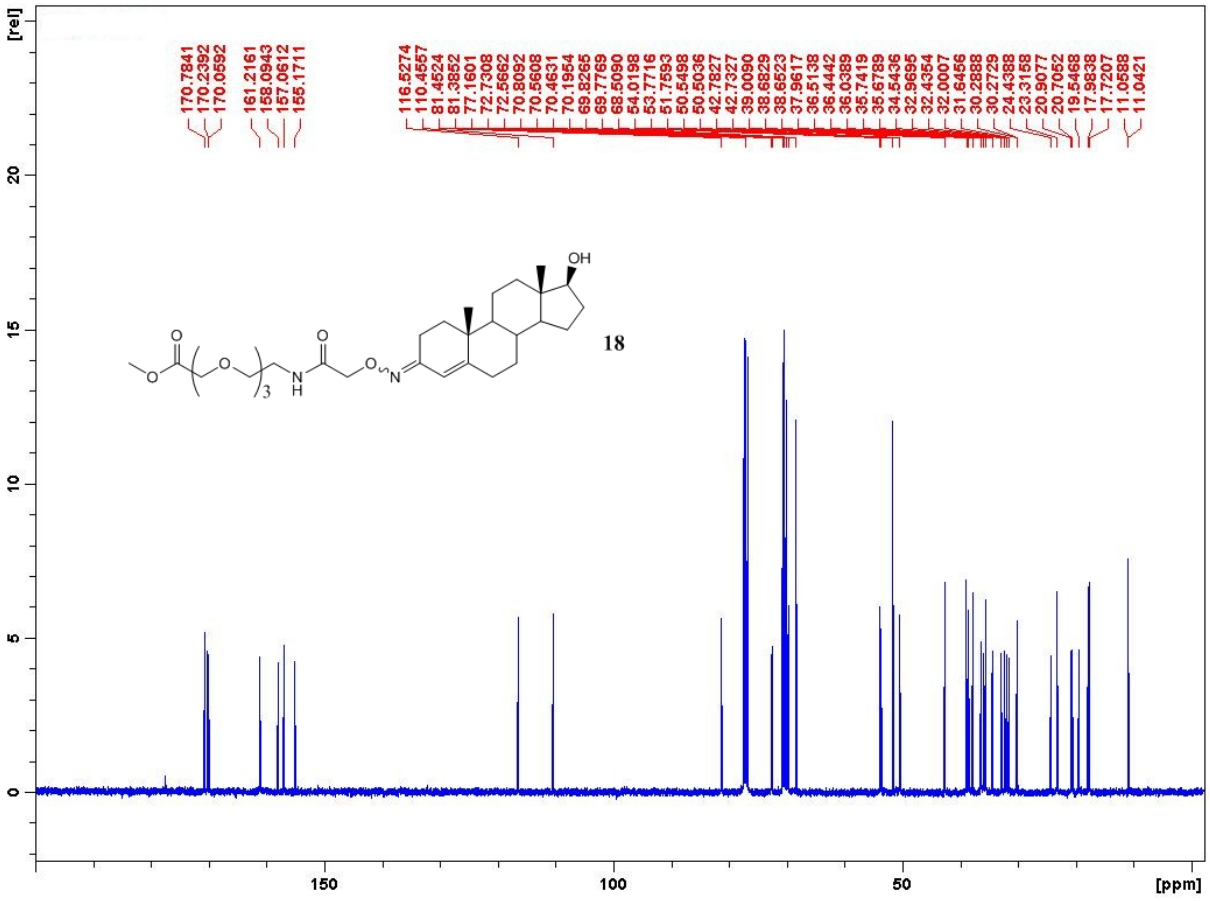
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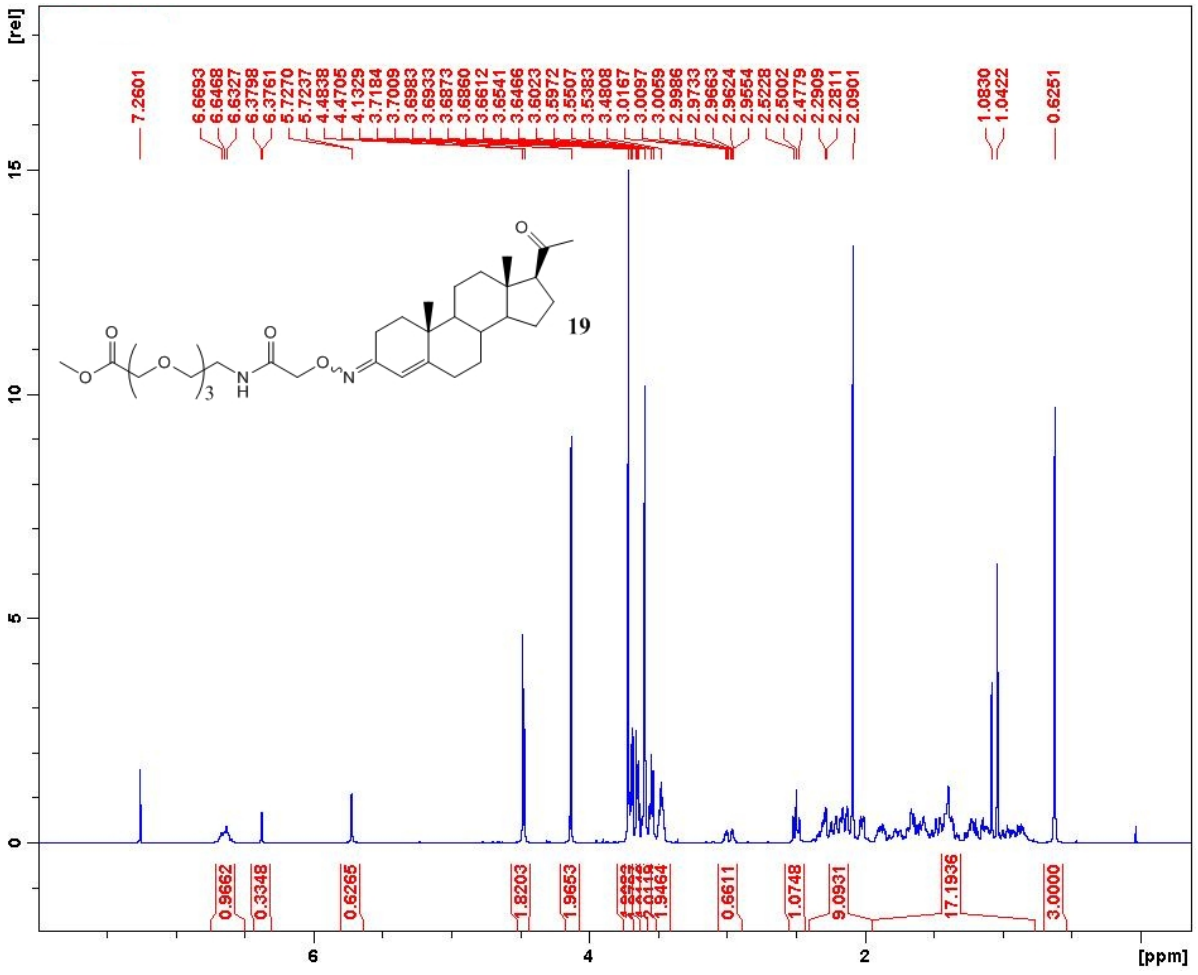
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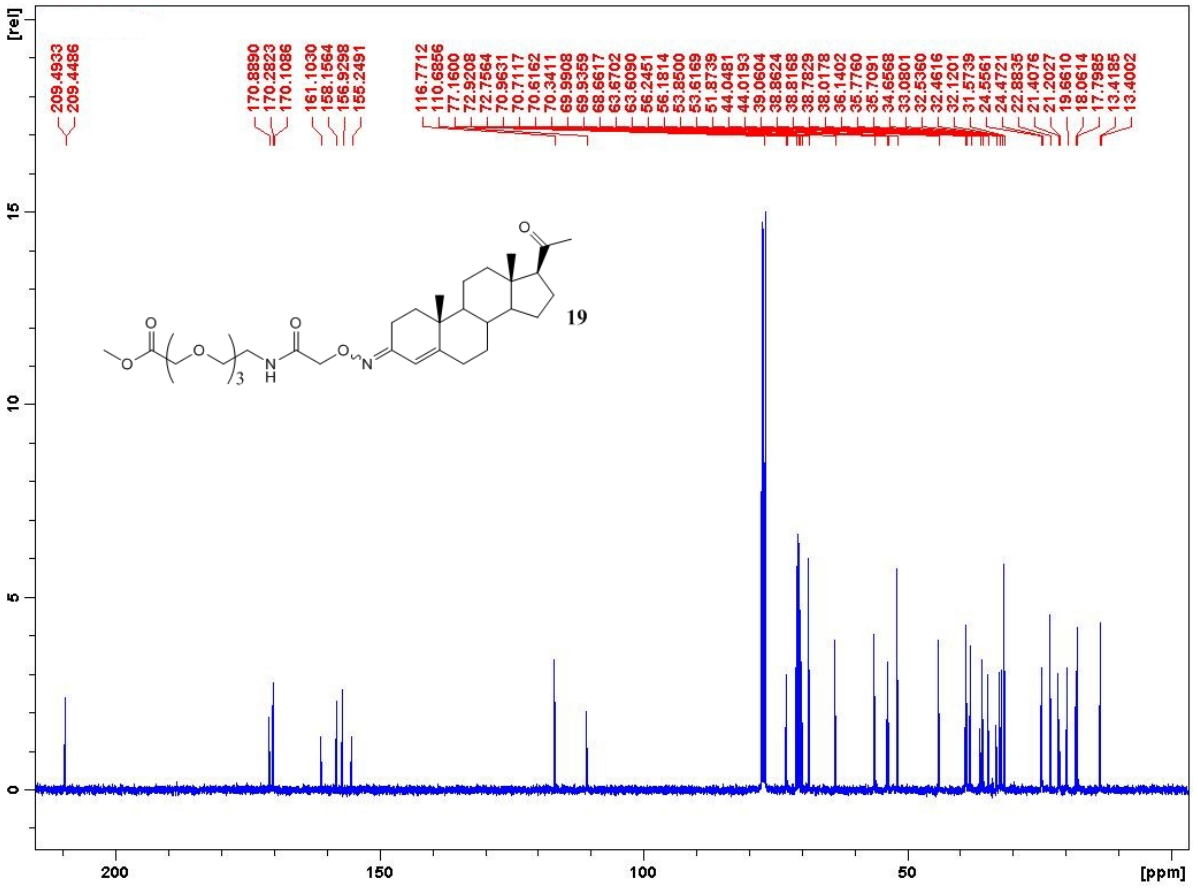
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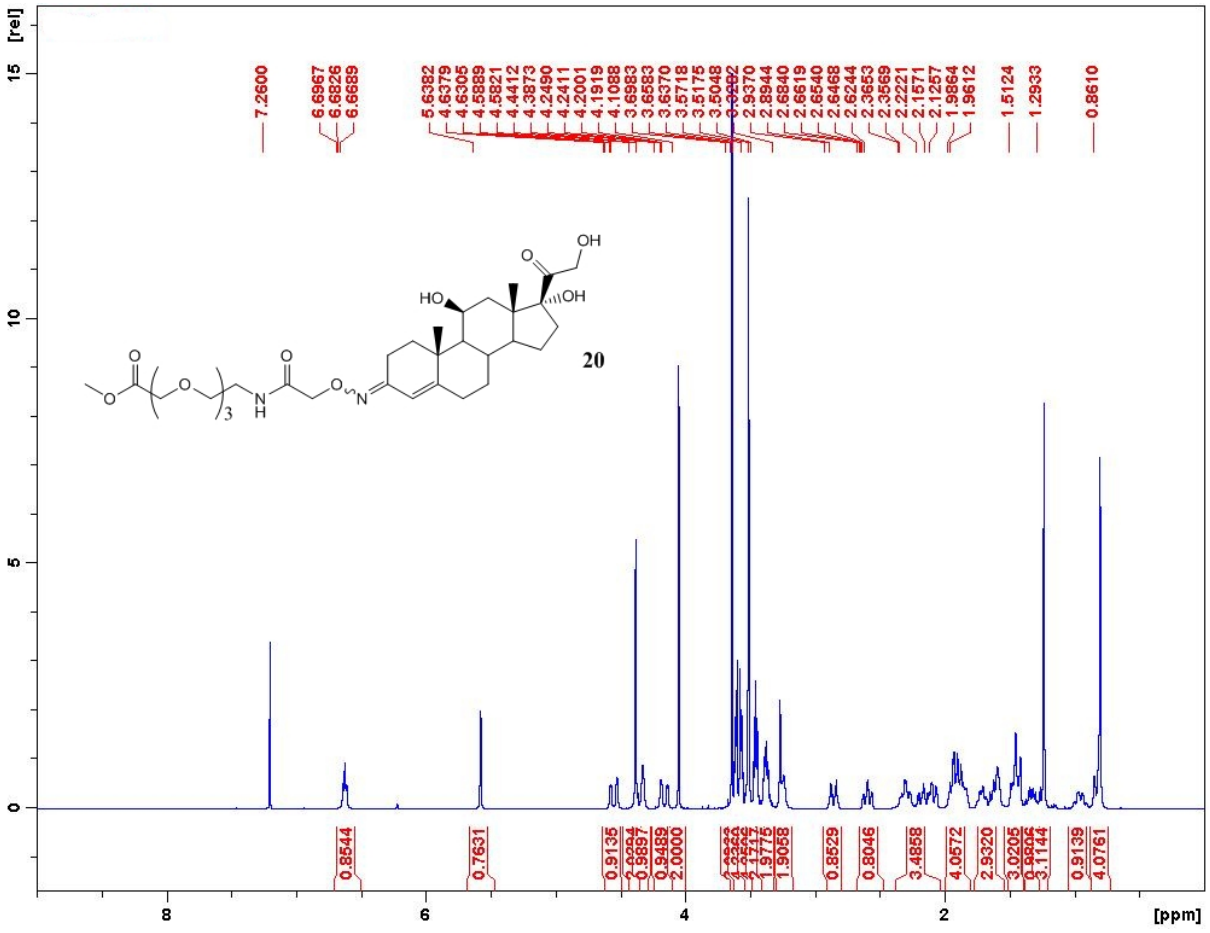
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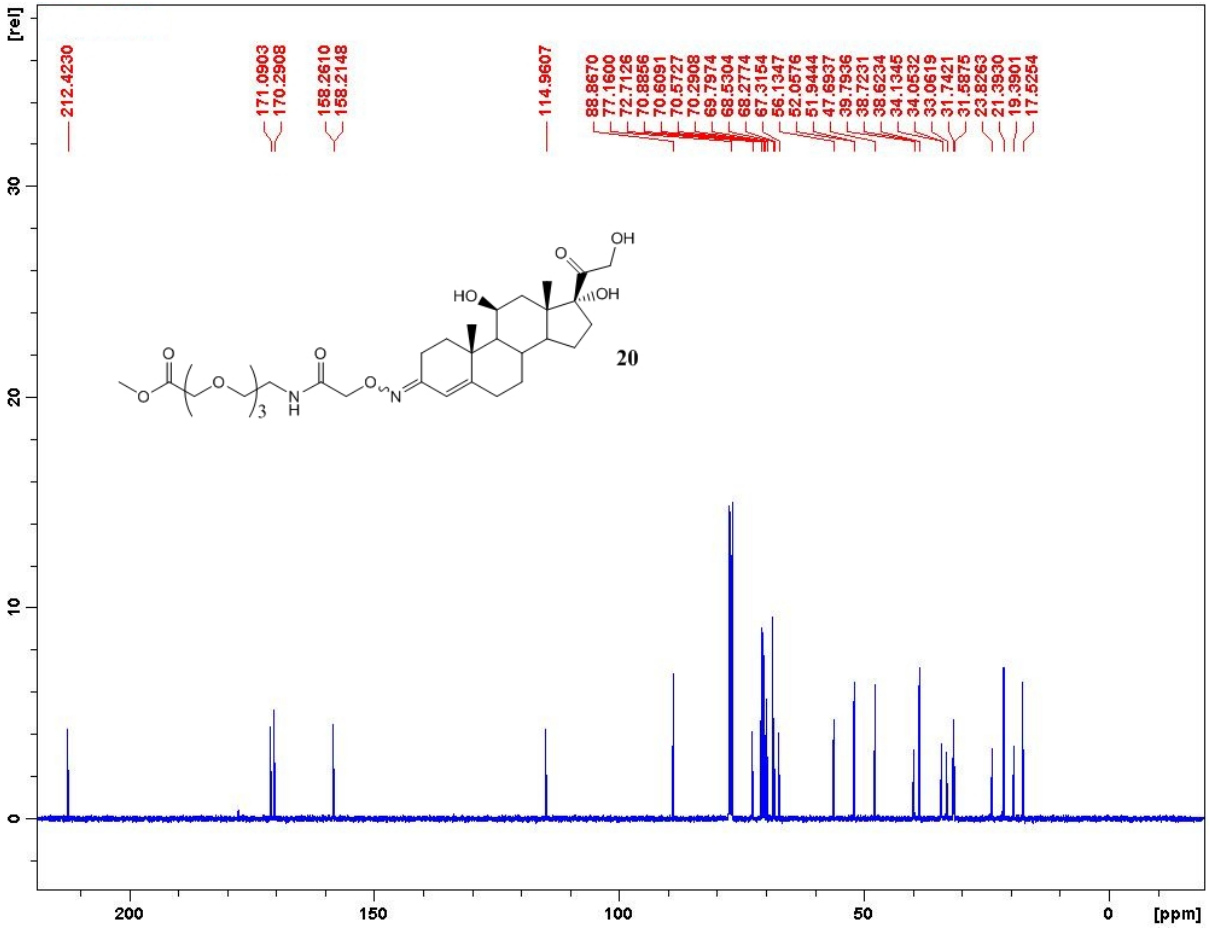
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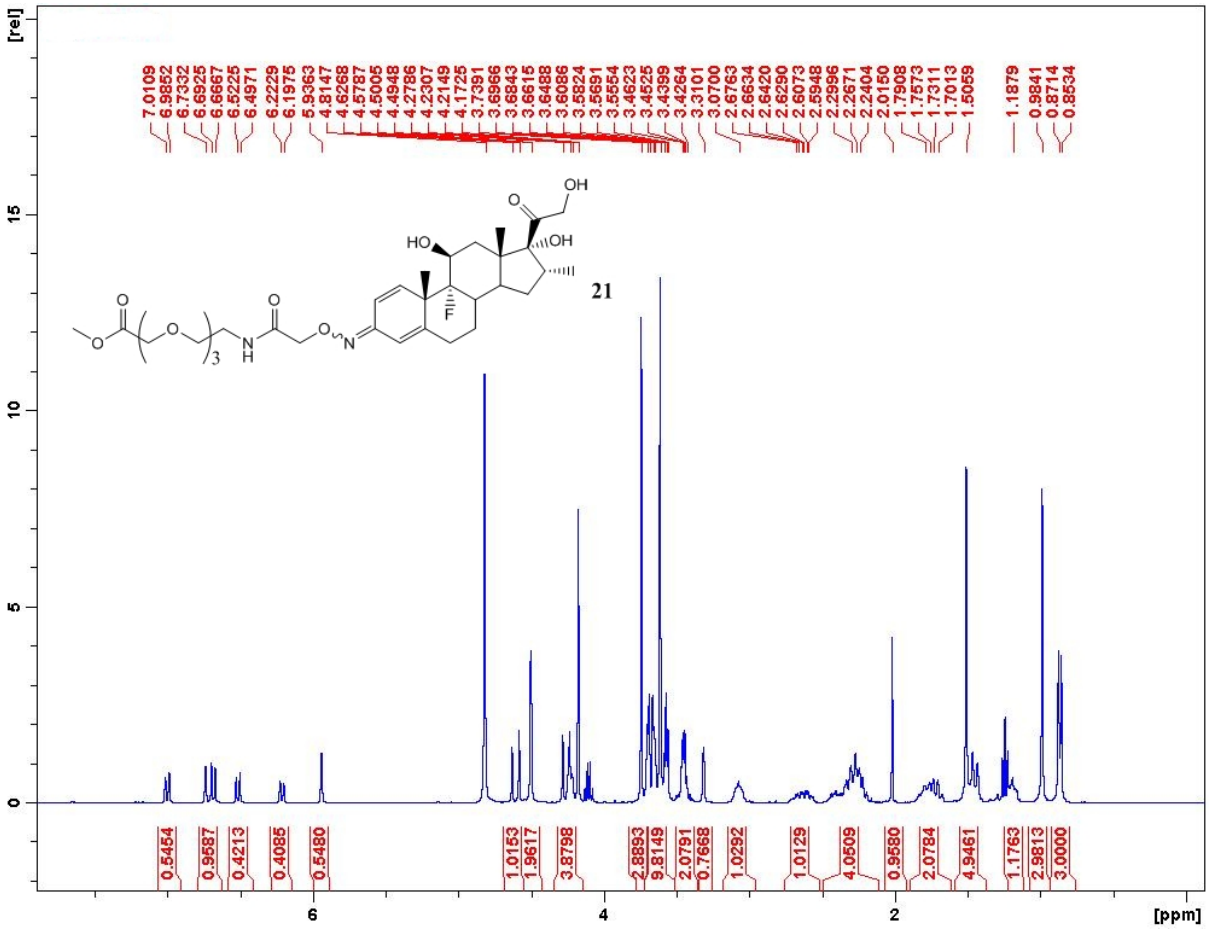
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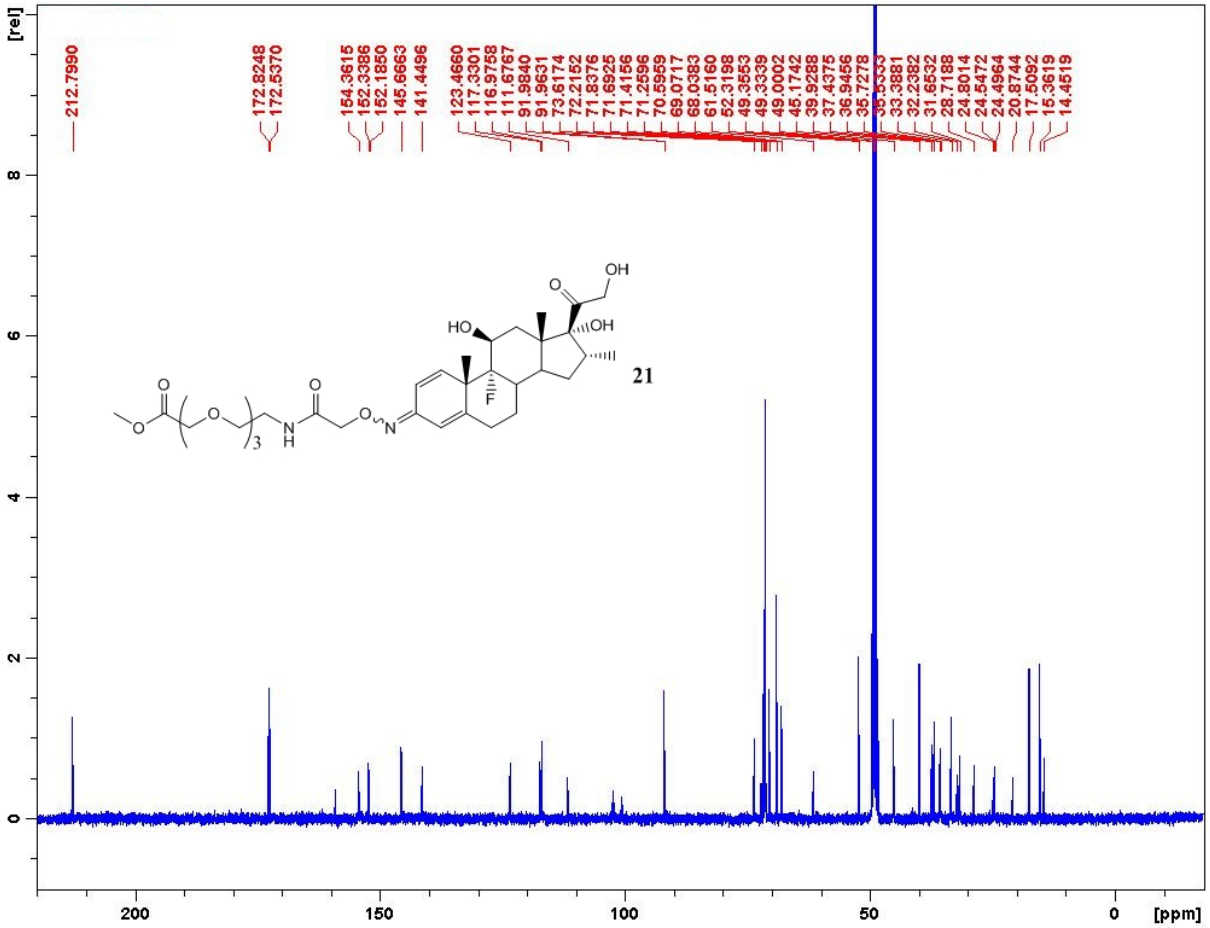
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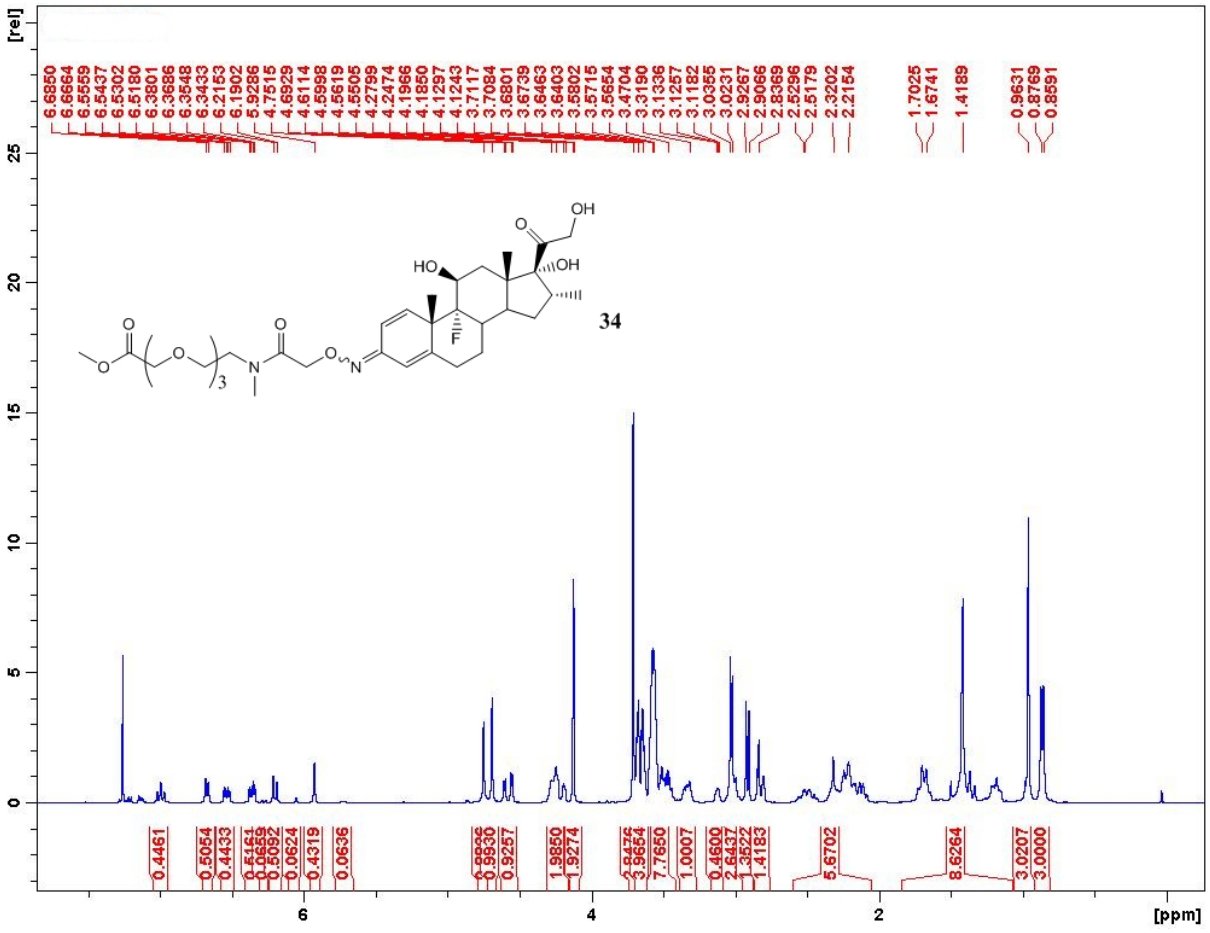
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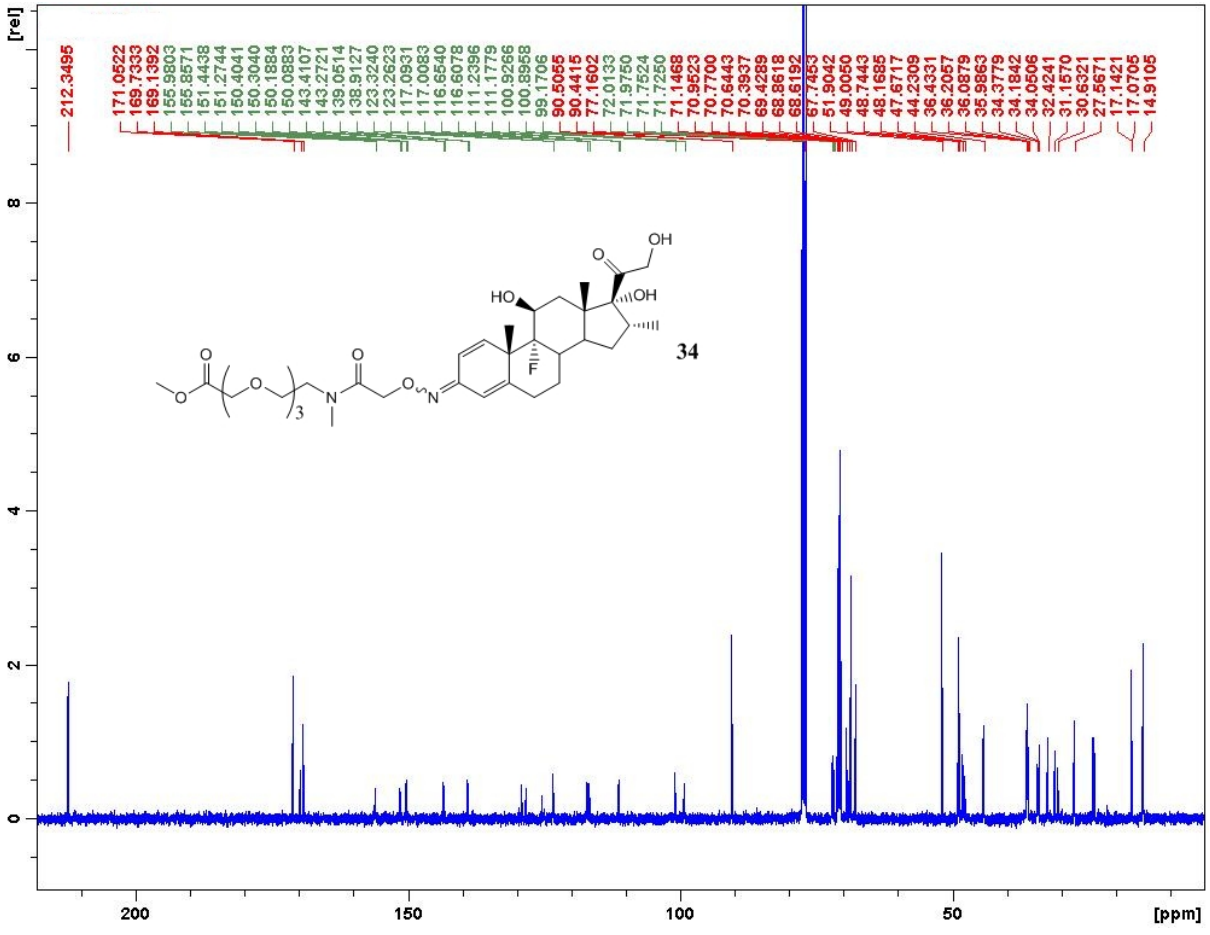
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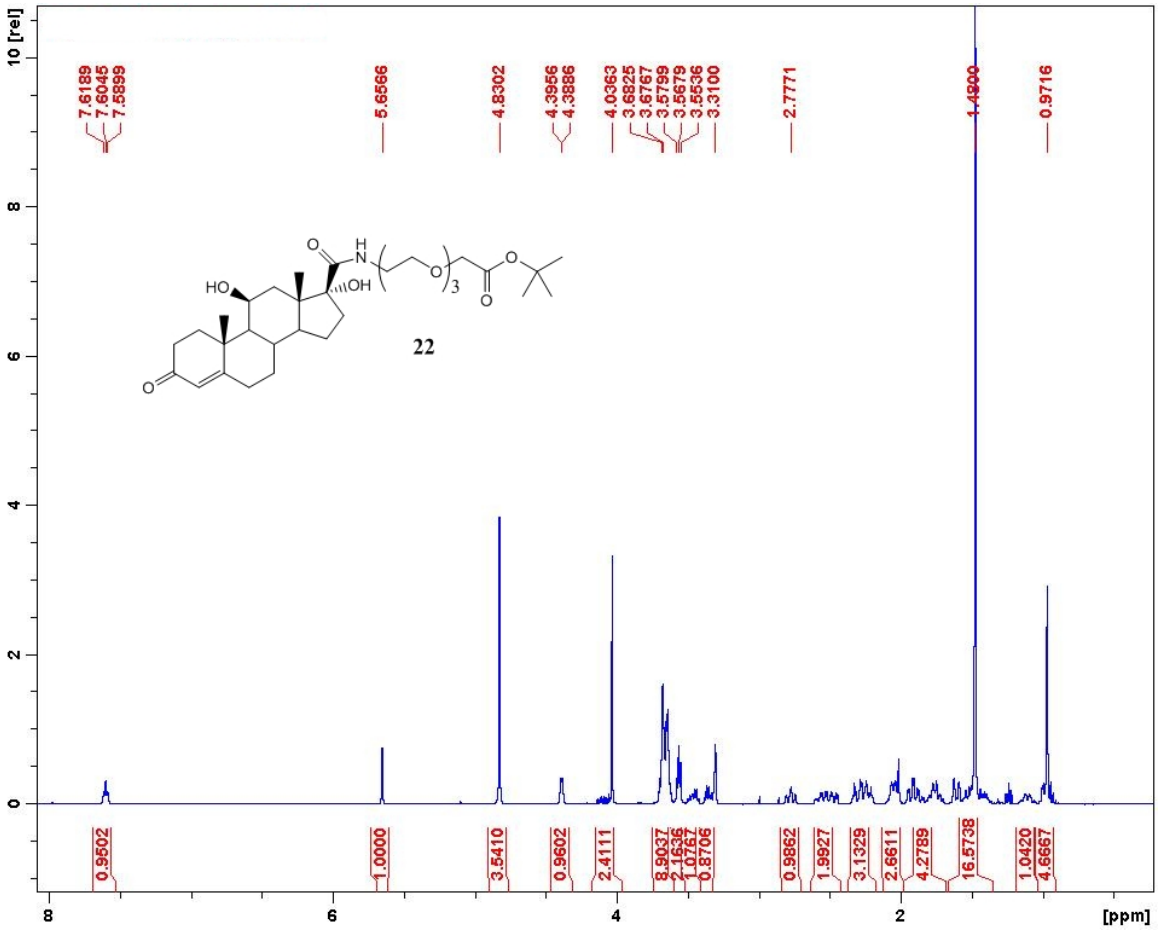
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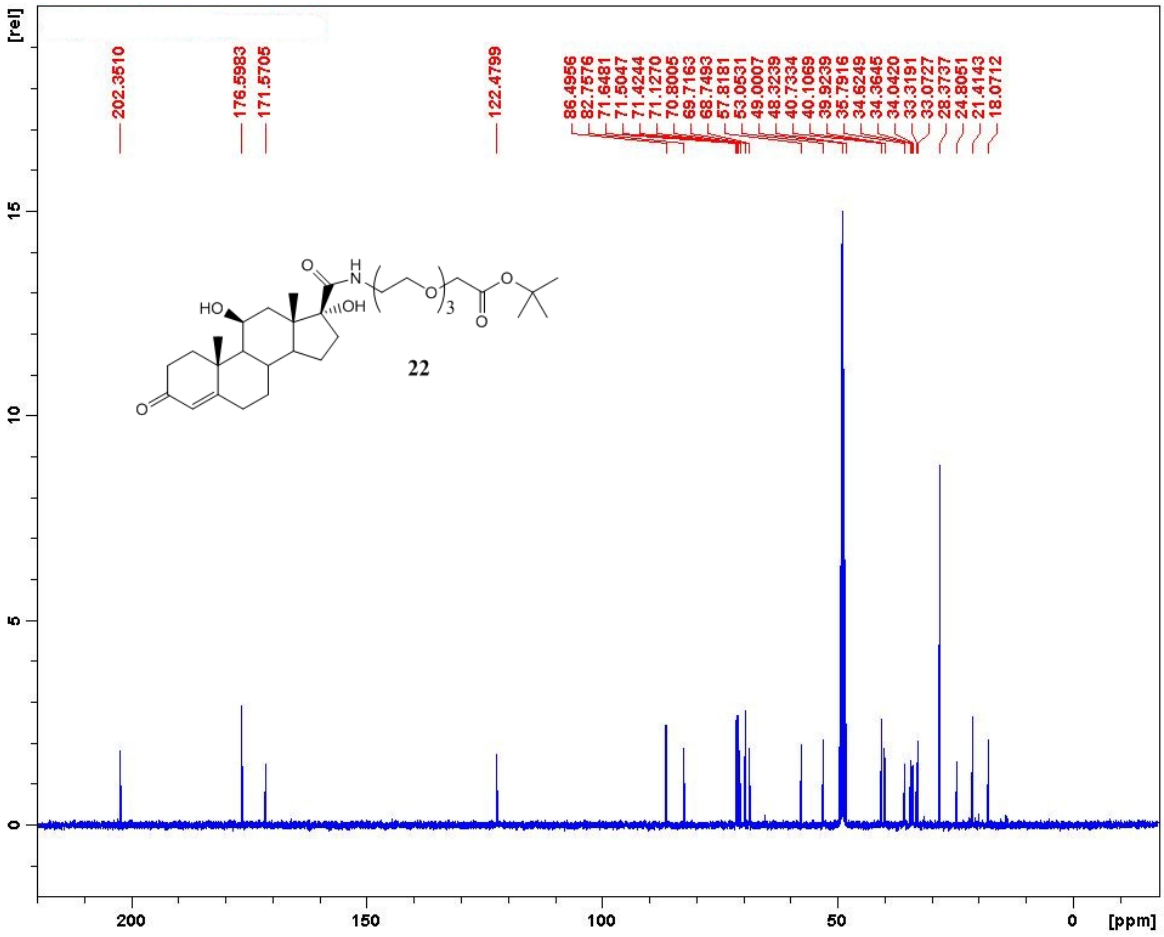
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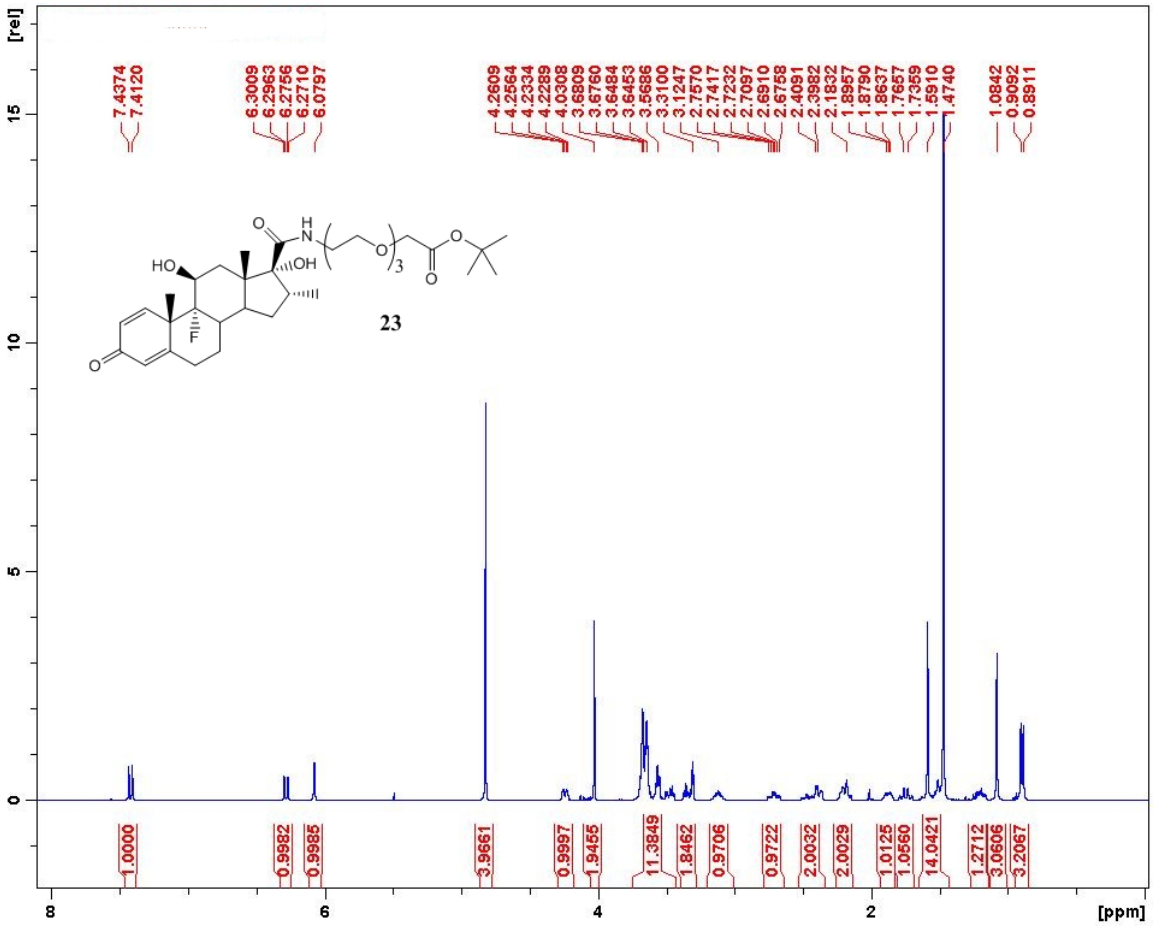
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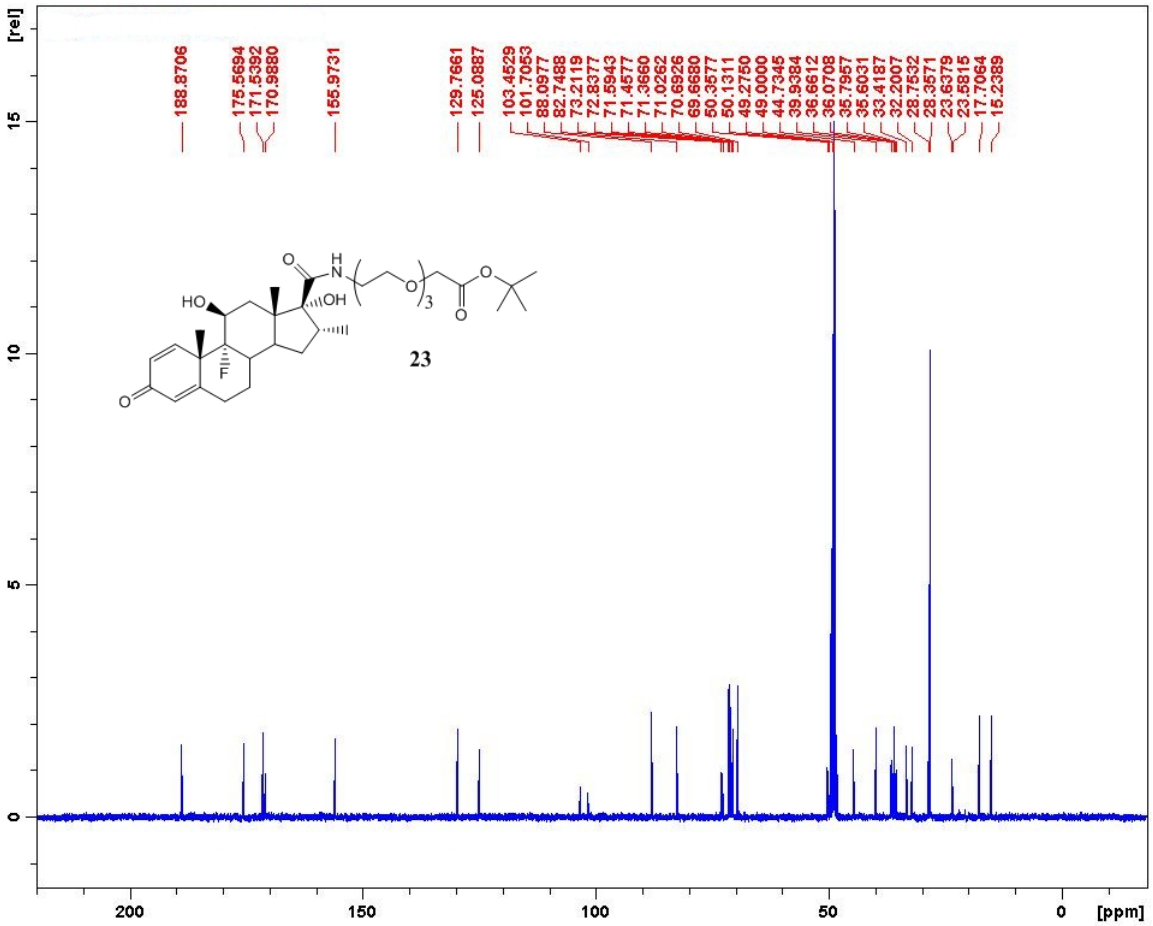
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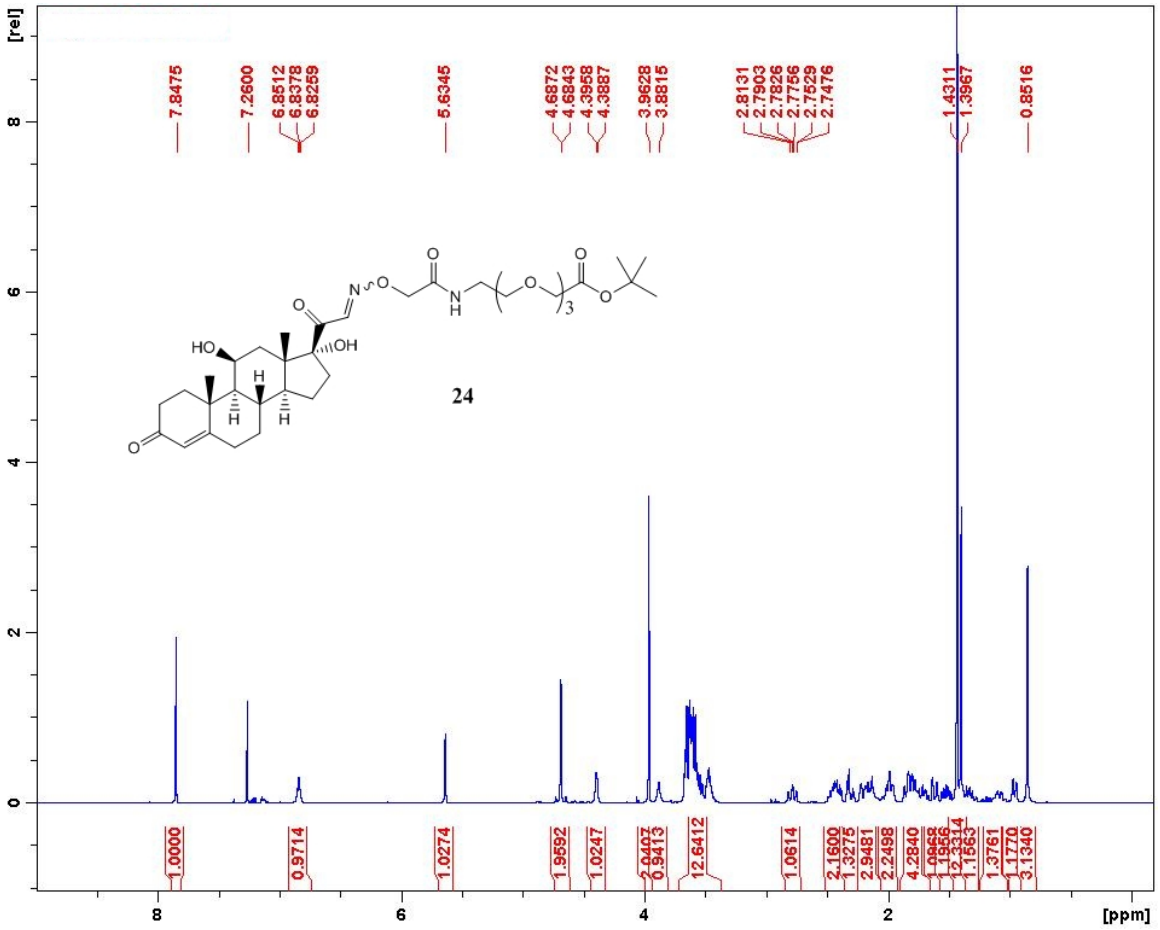
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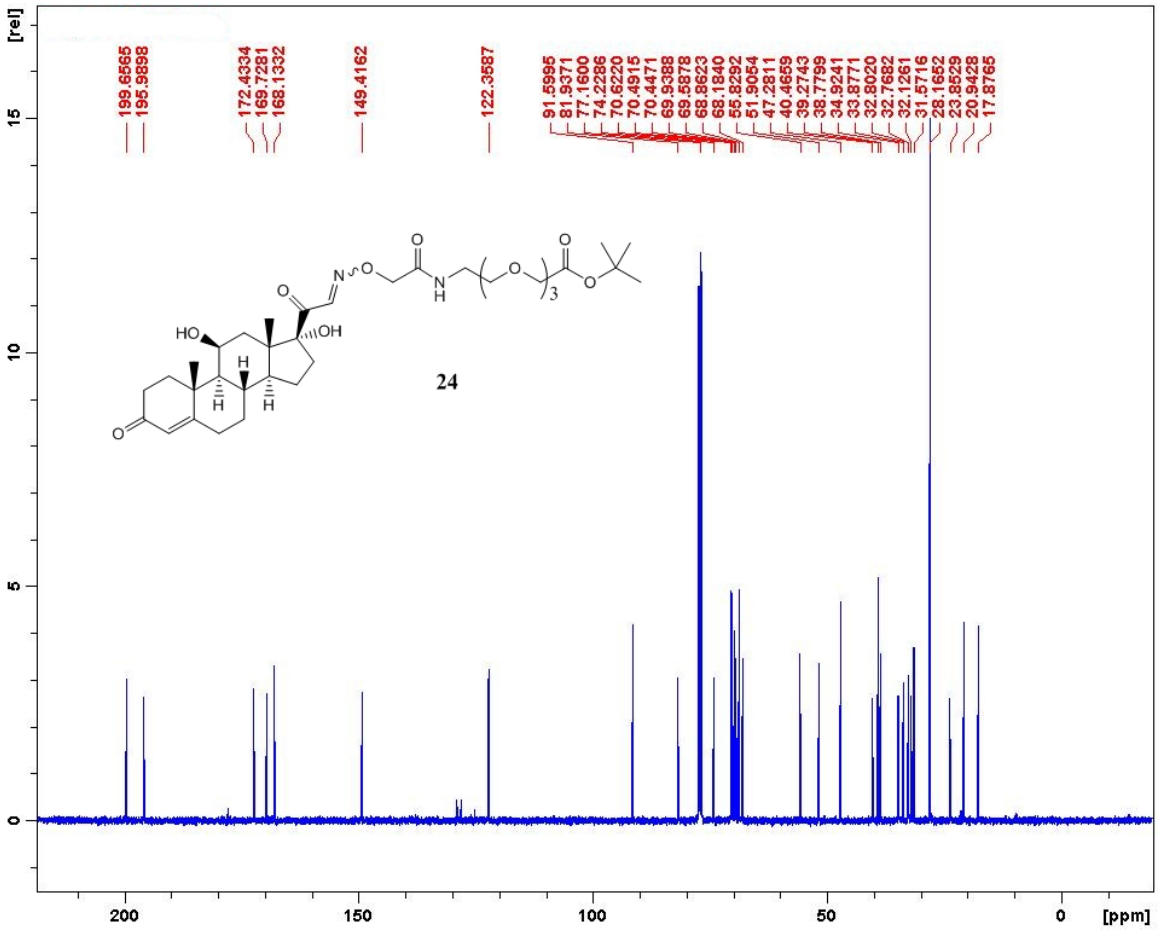
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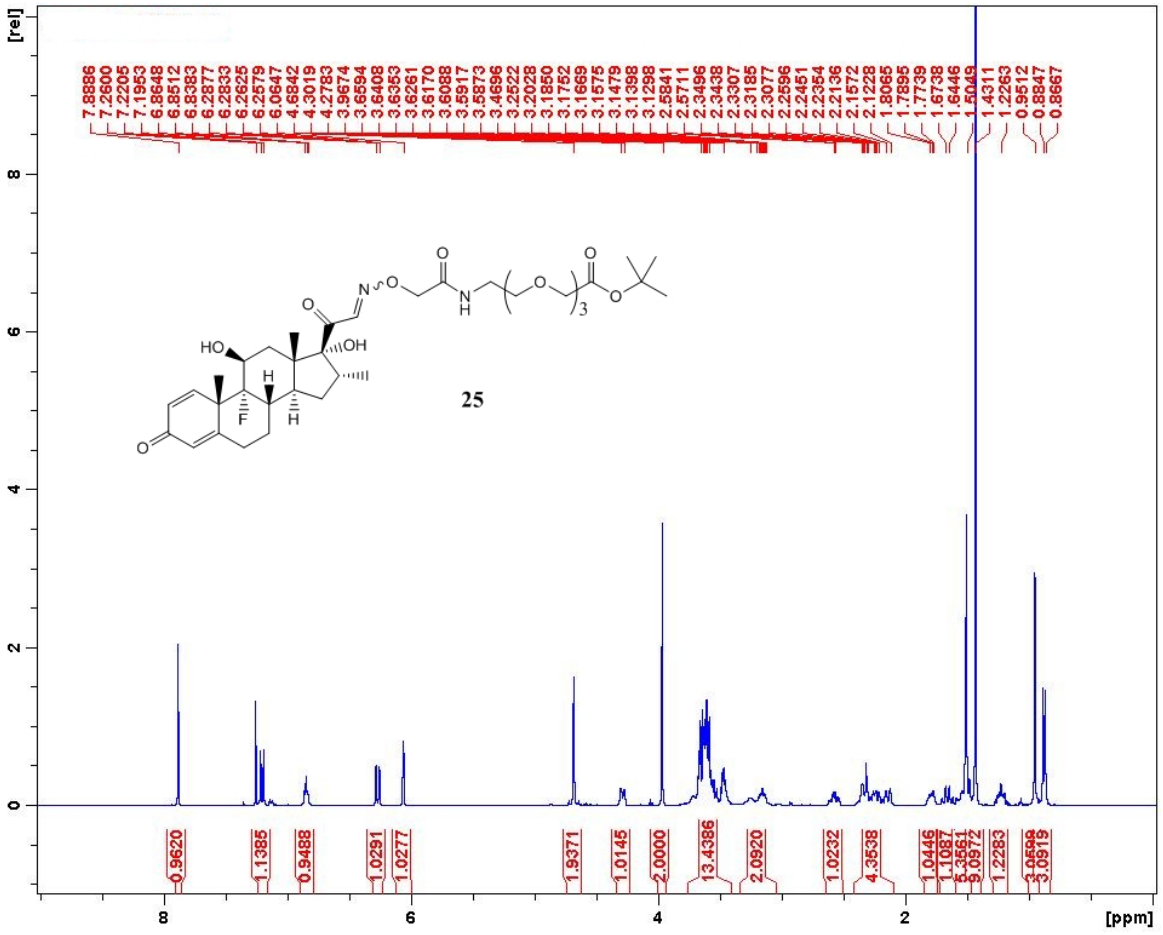
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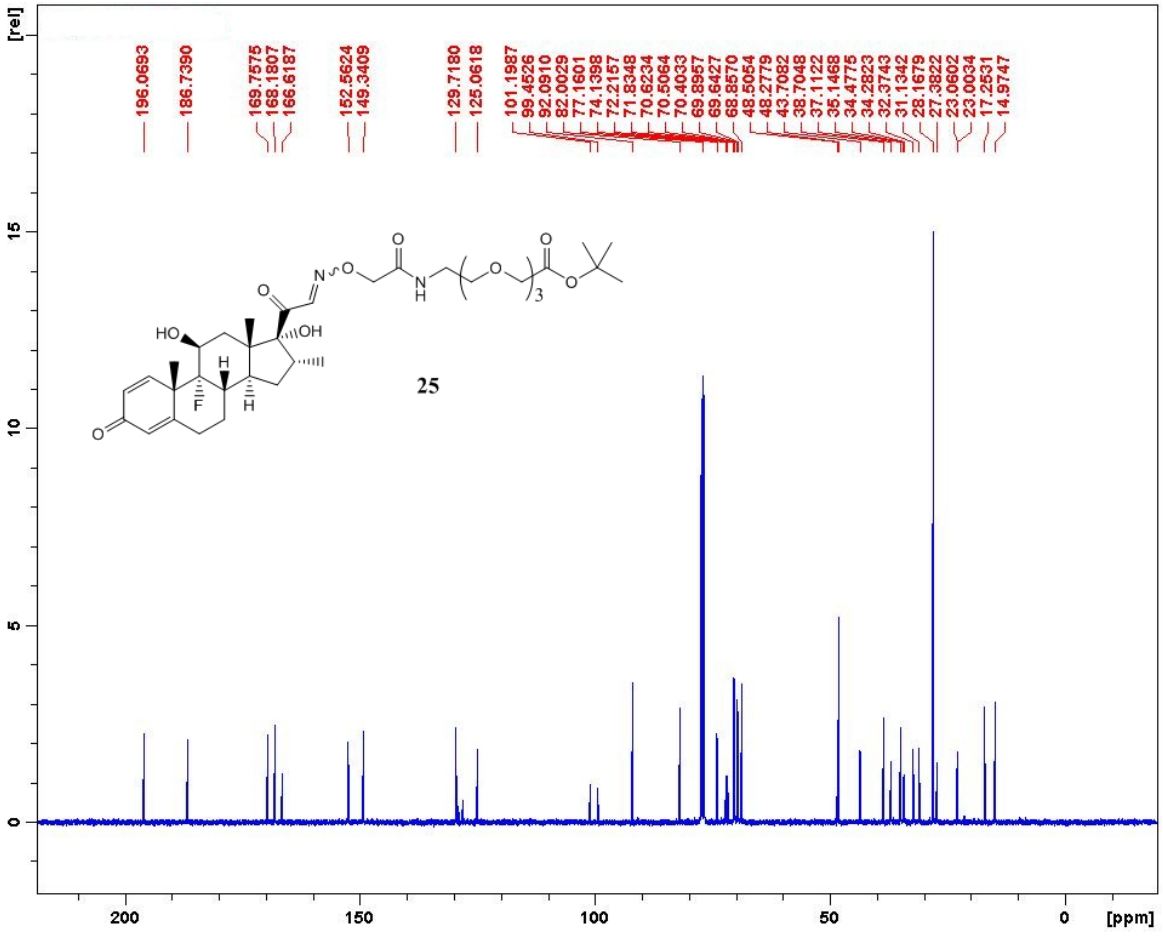
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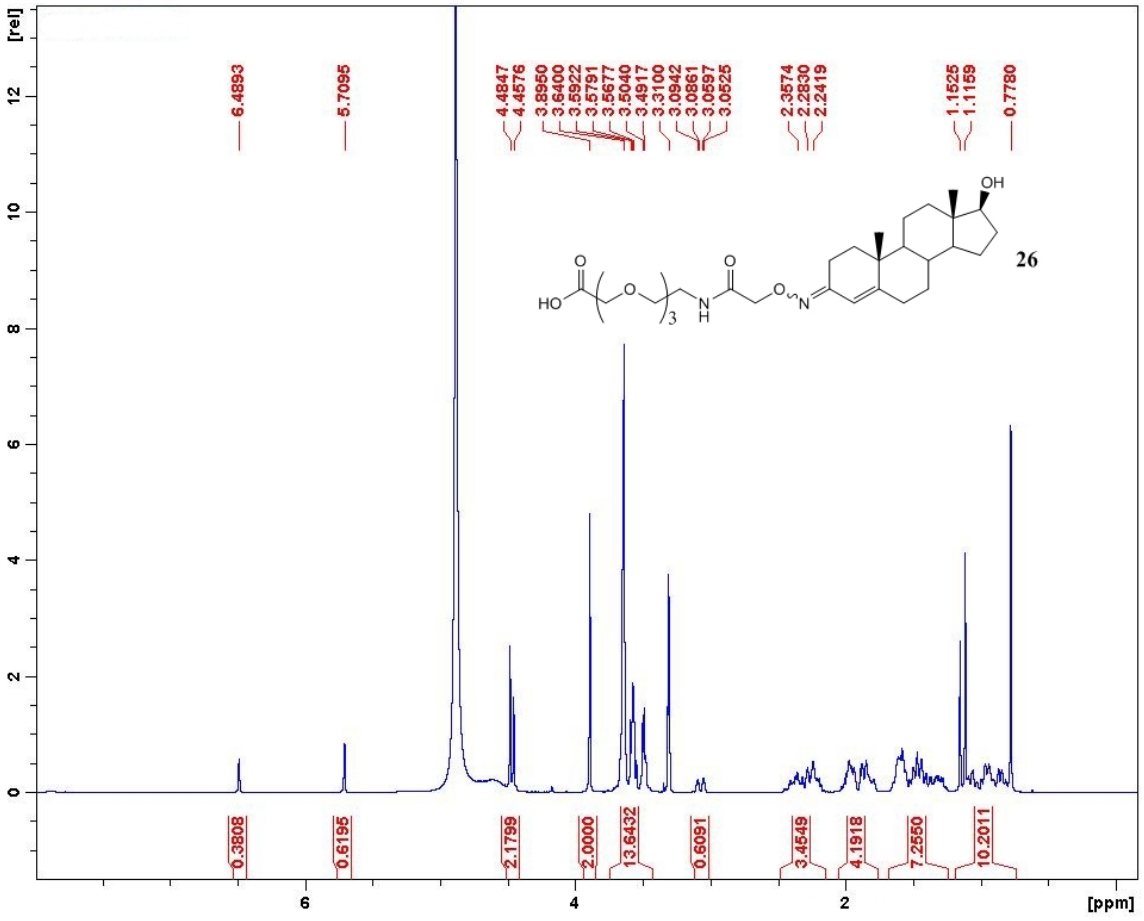
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3.0861
3.0597
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2.3574
2.2830
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1.1159
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O
O
3
N
H
O
N
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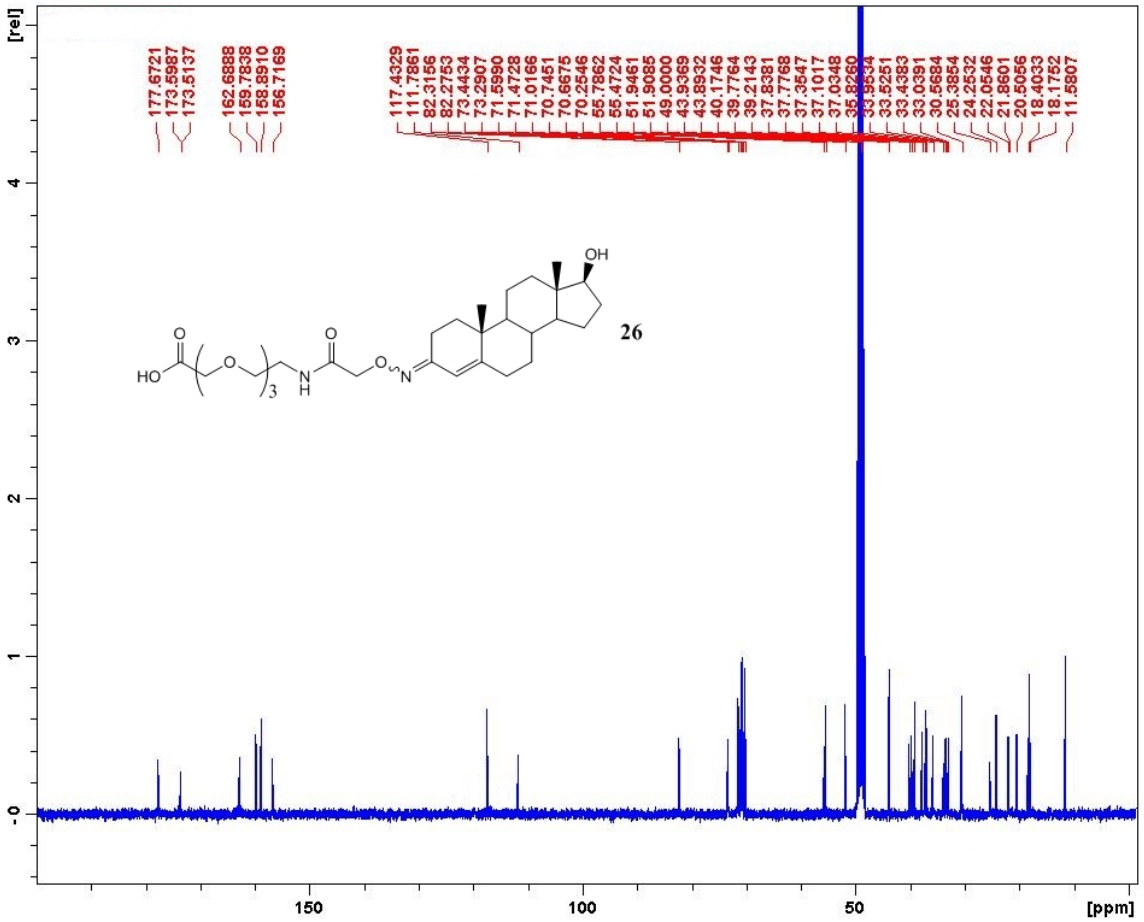
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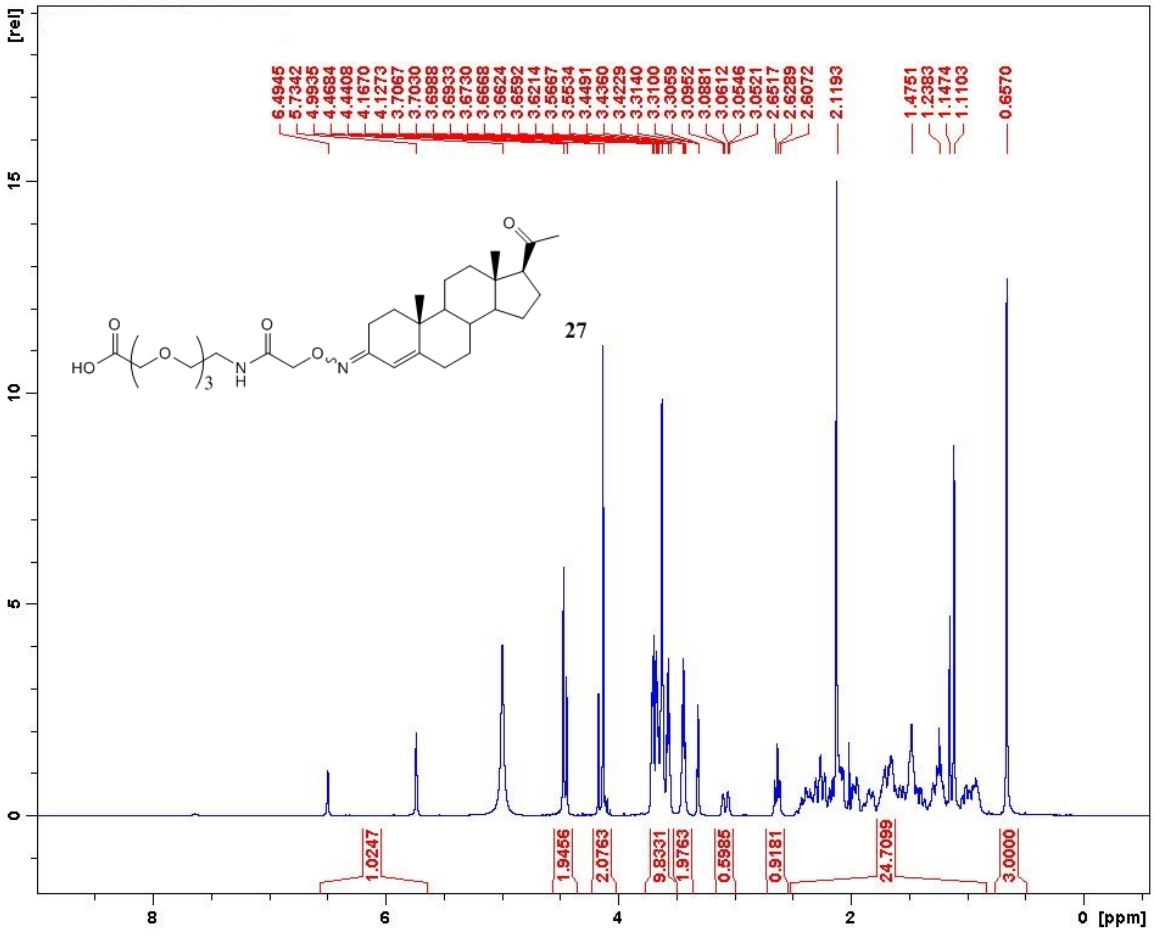
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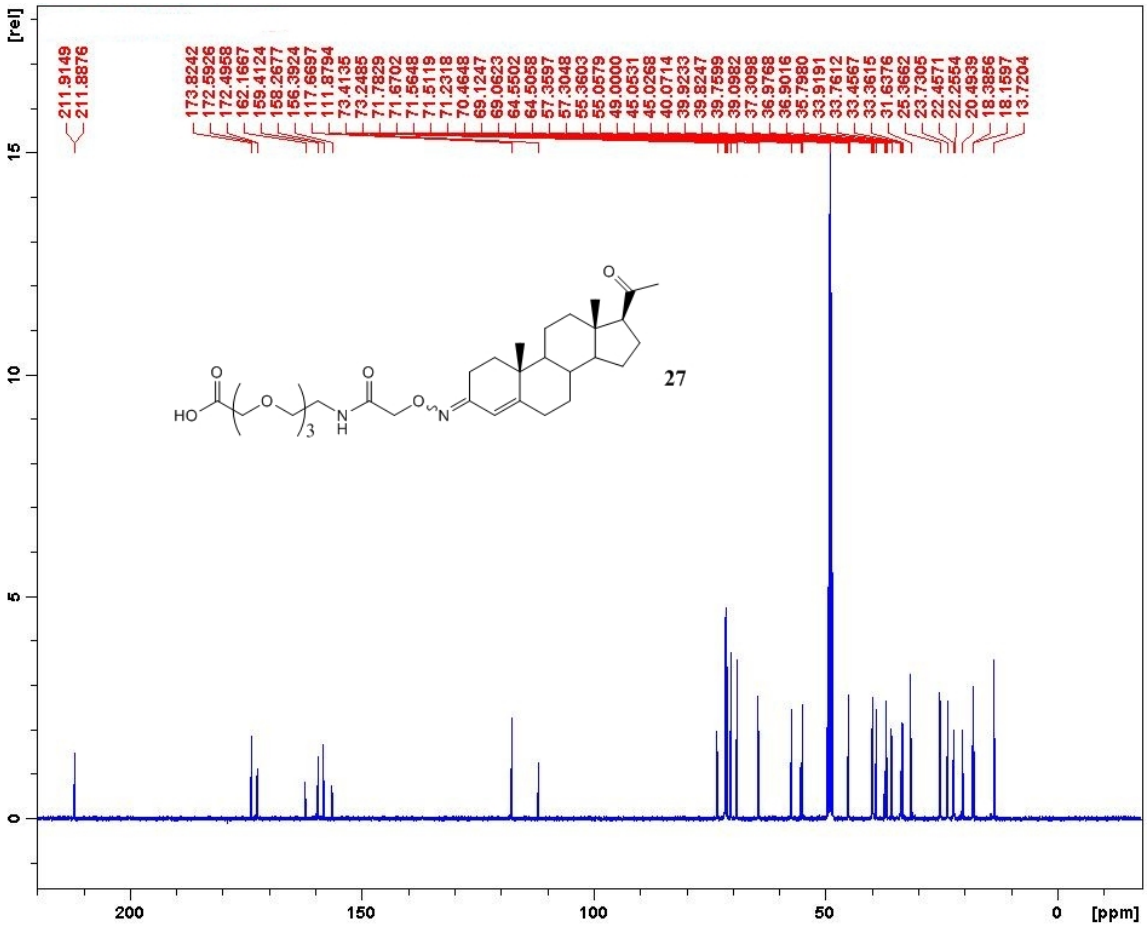
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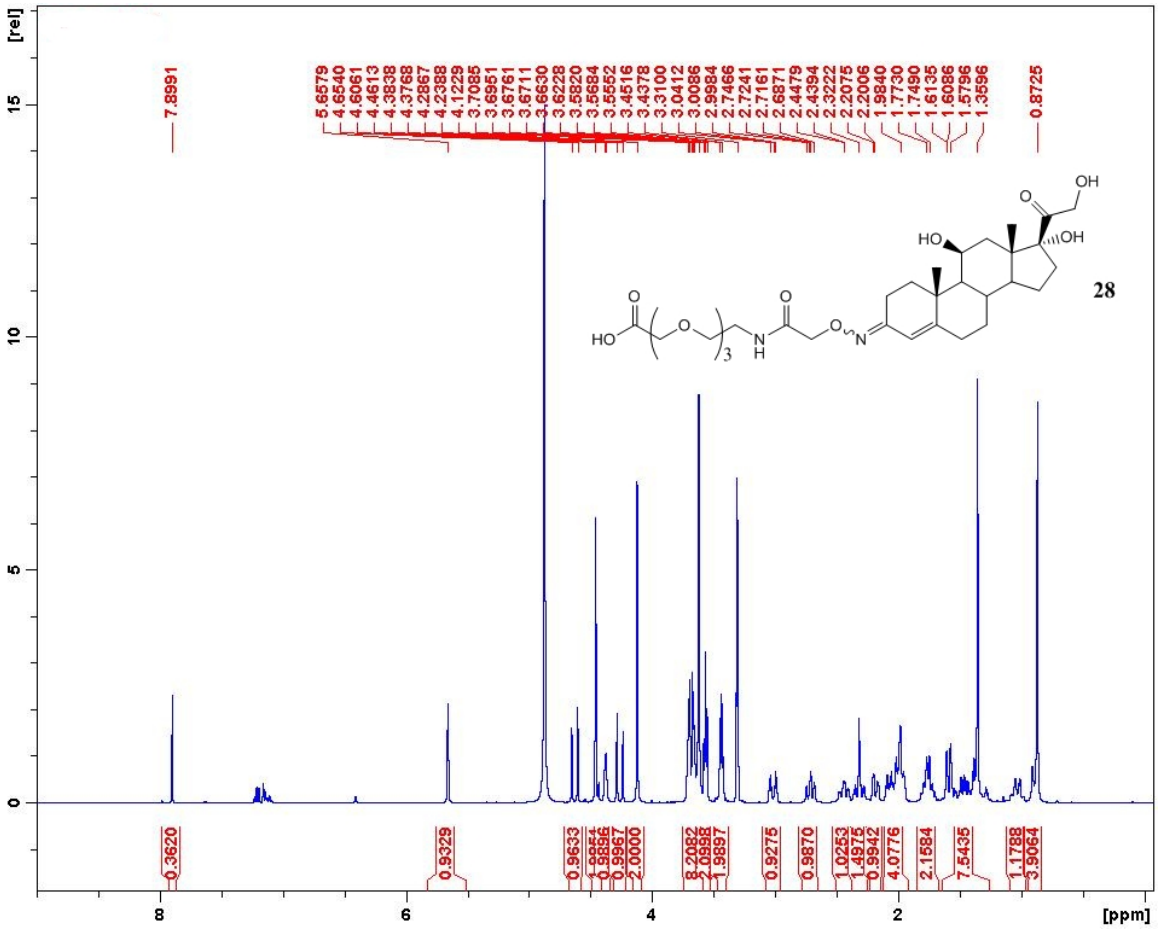
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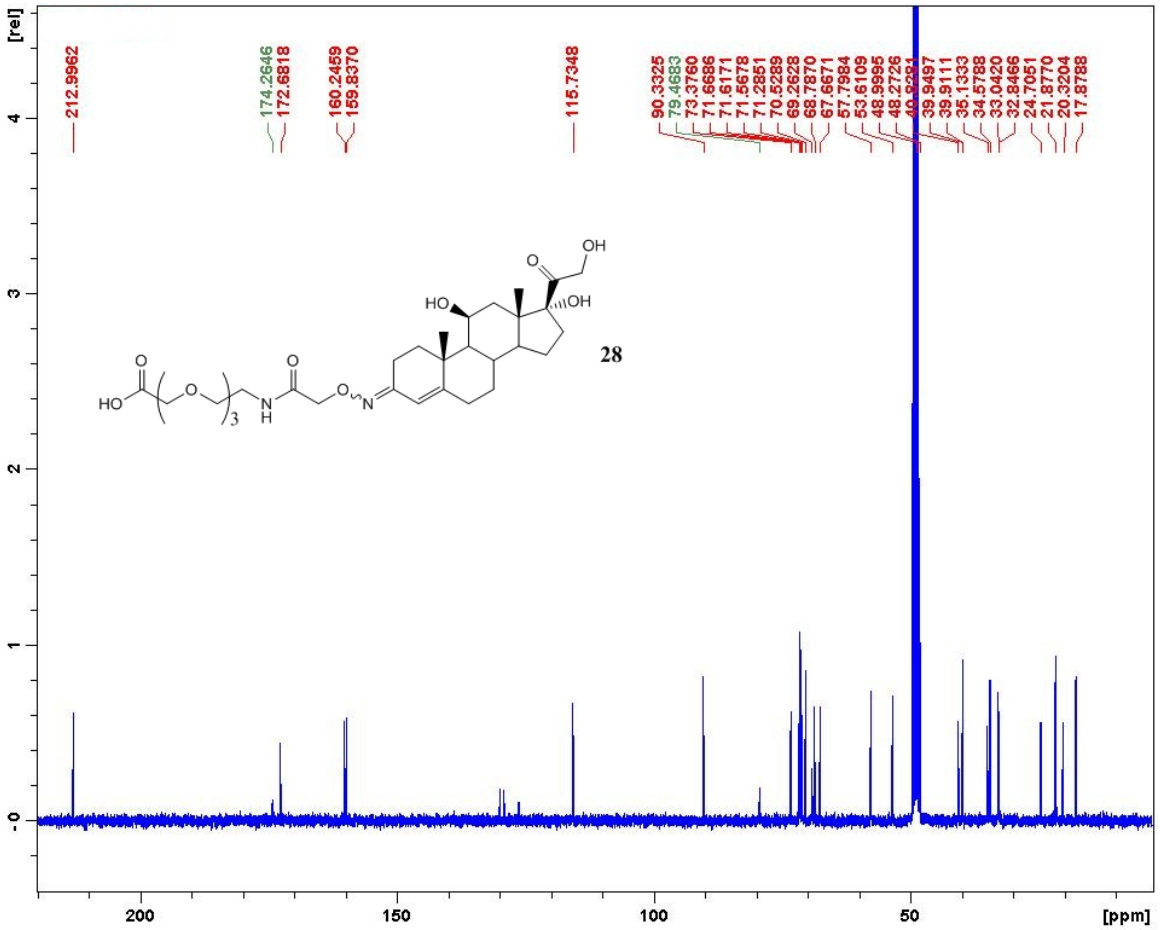
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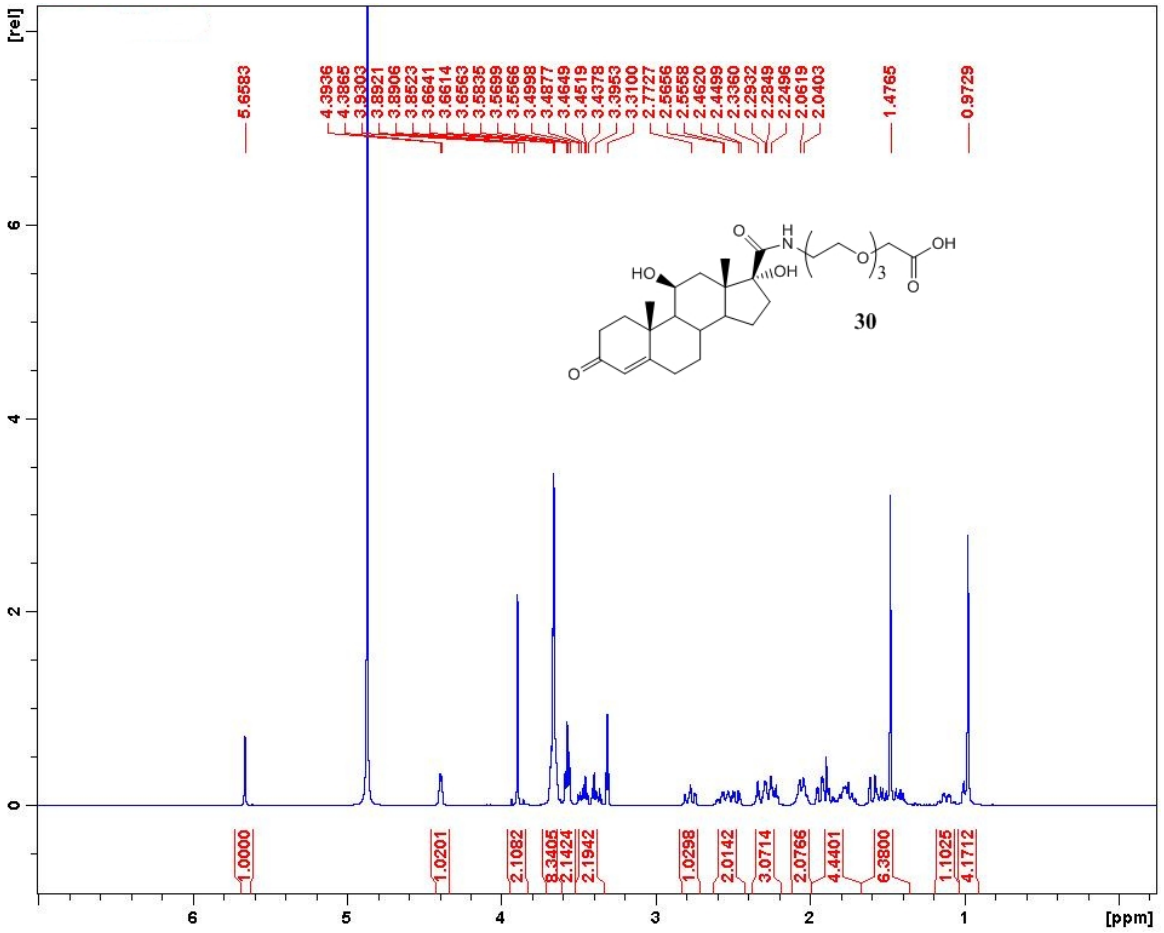
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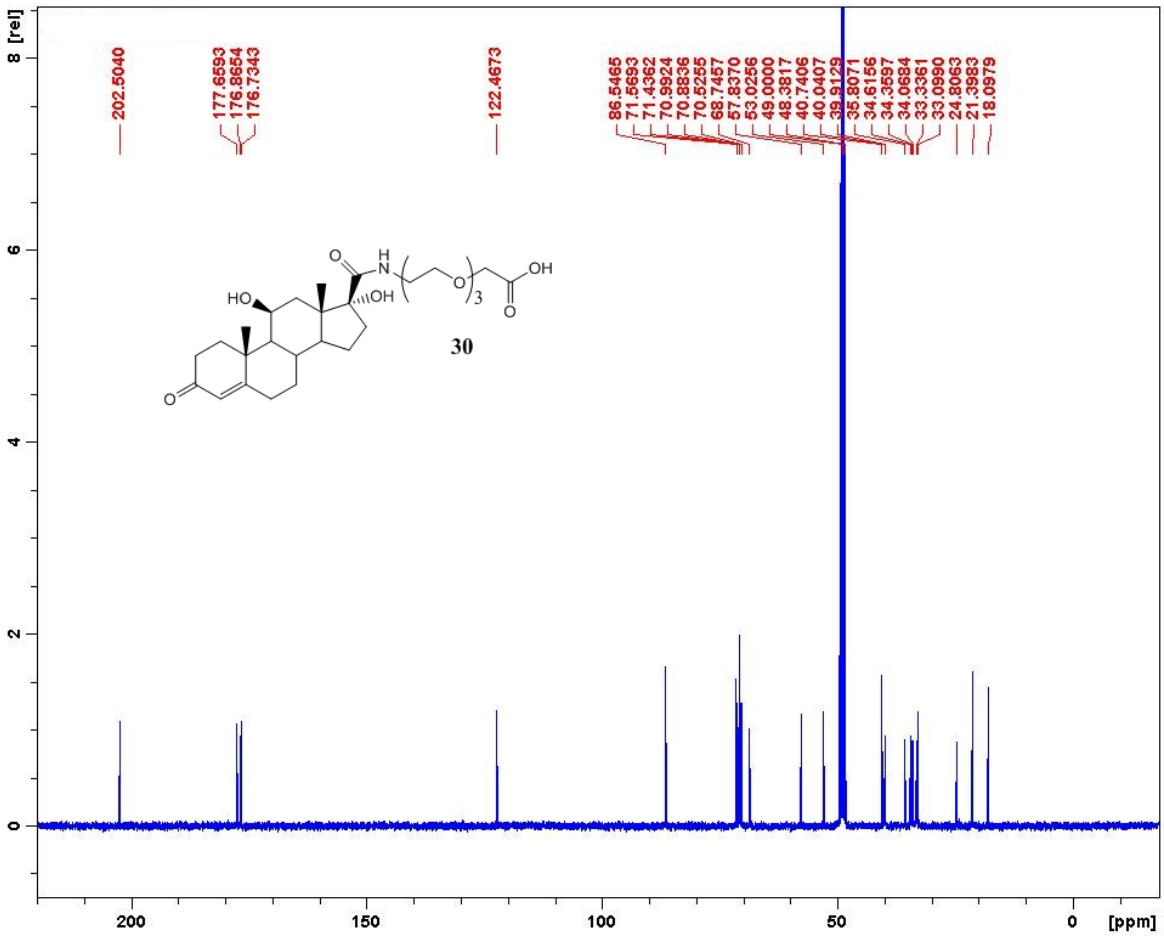
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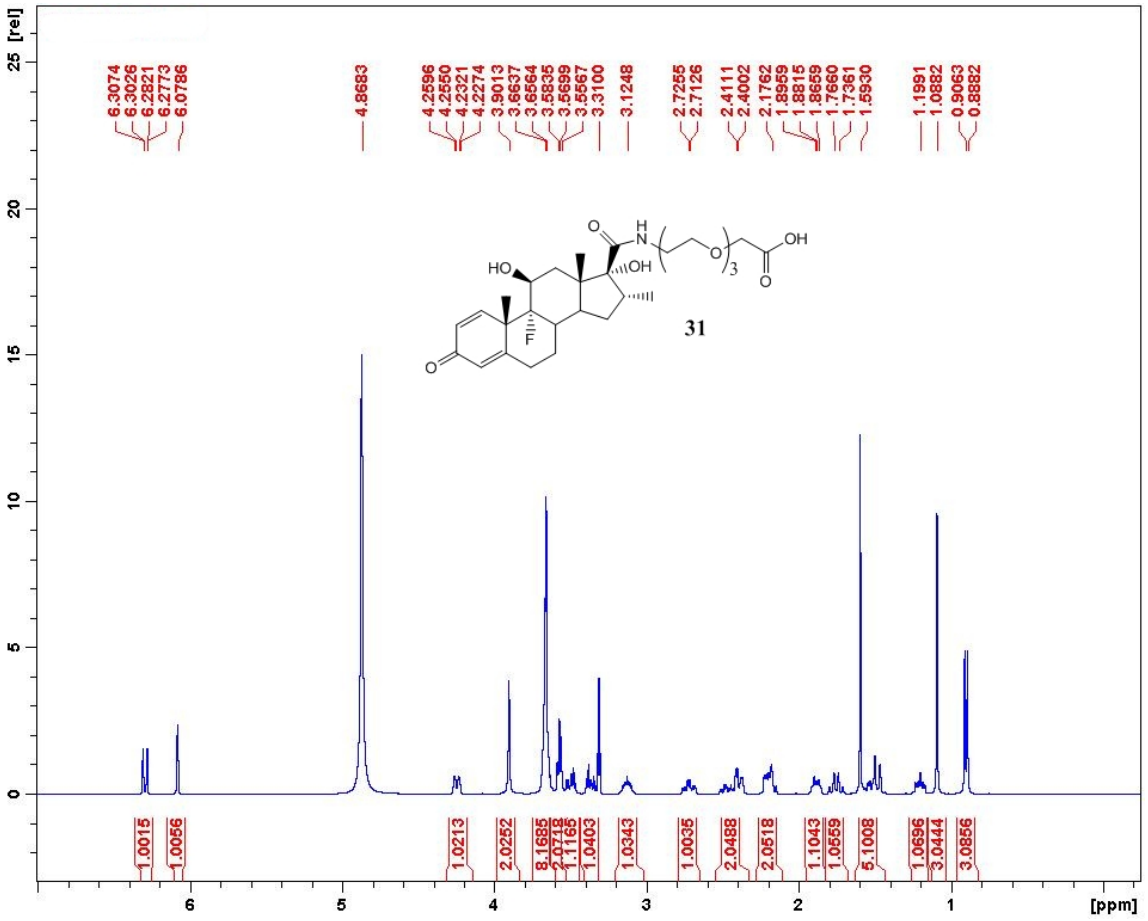
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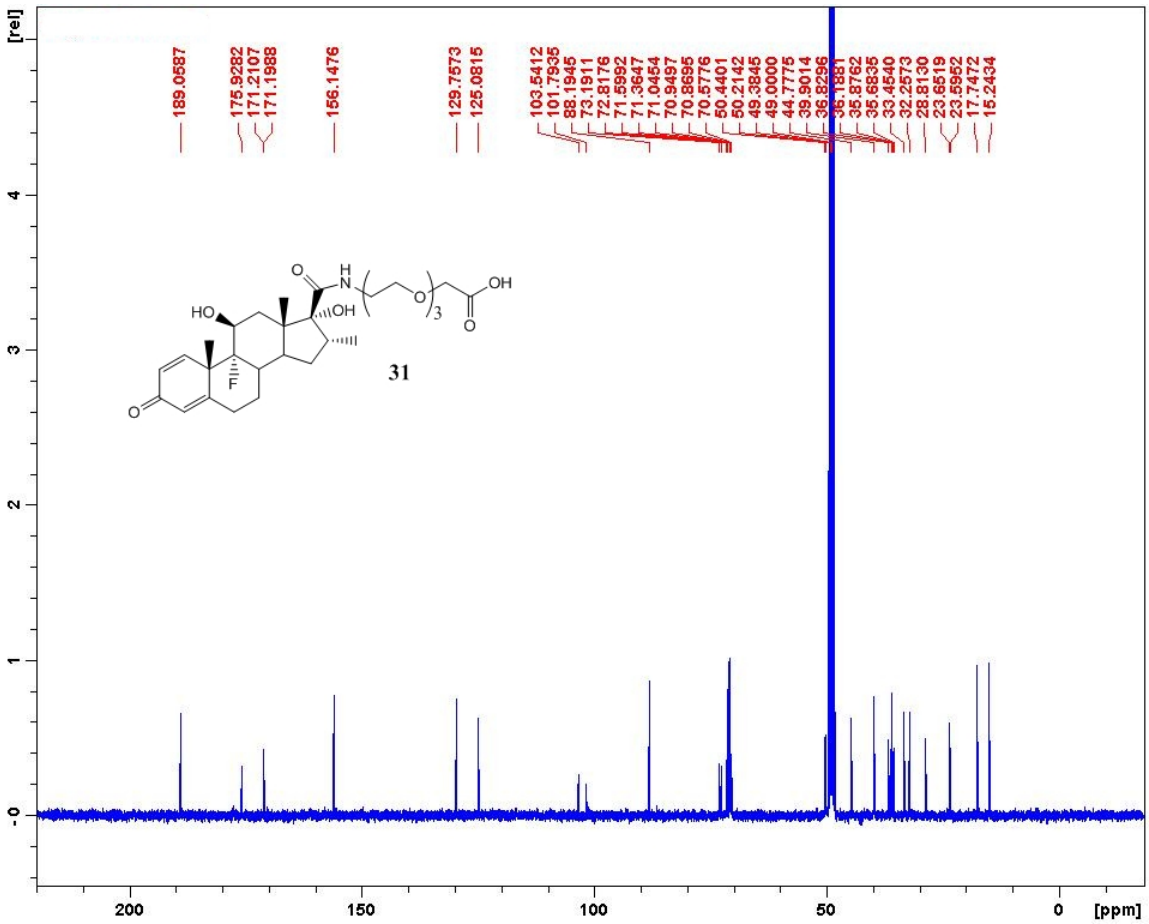
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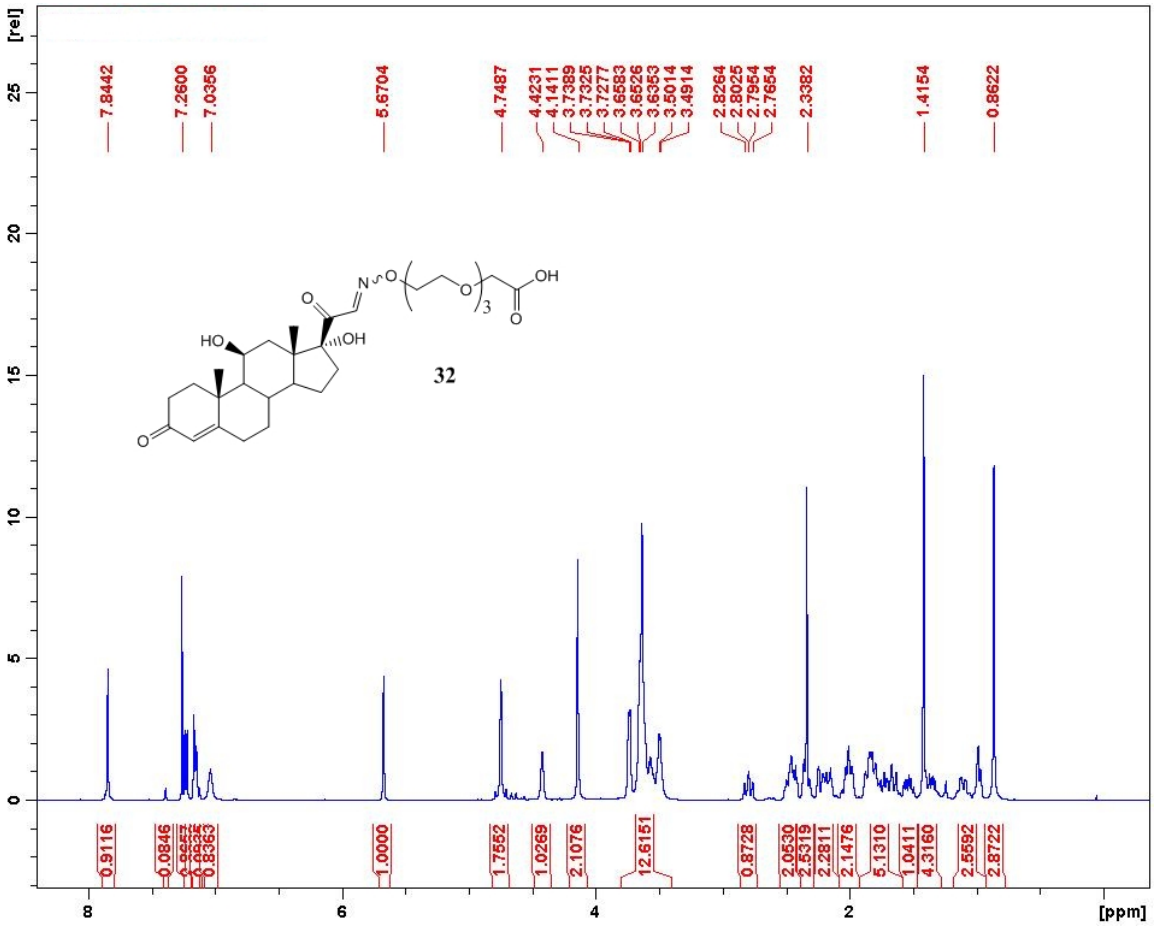
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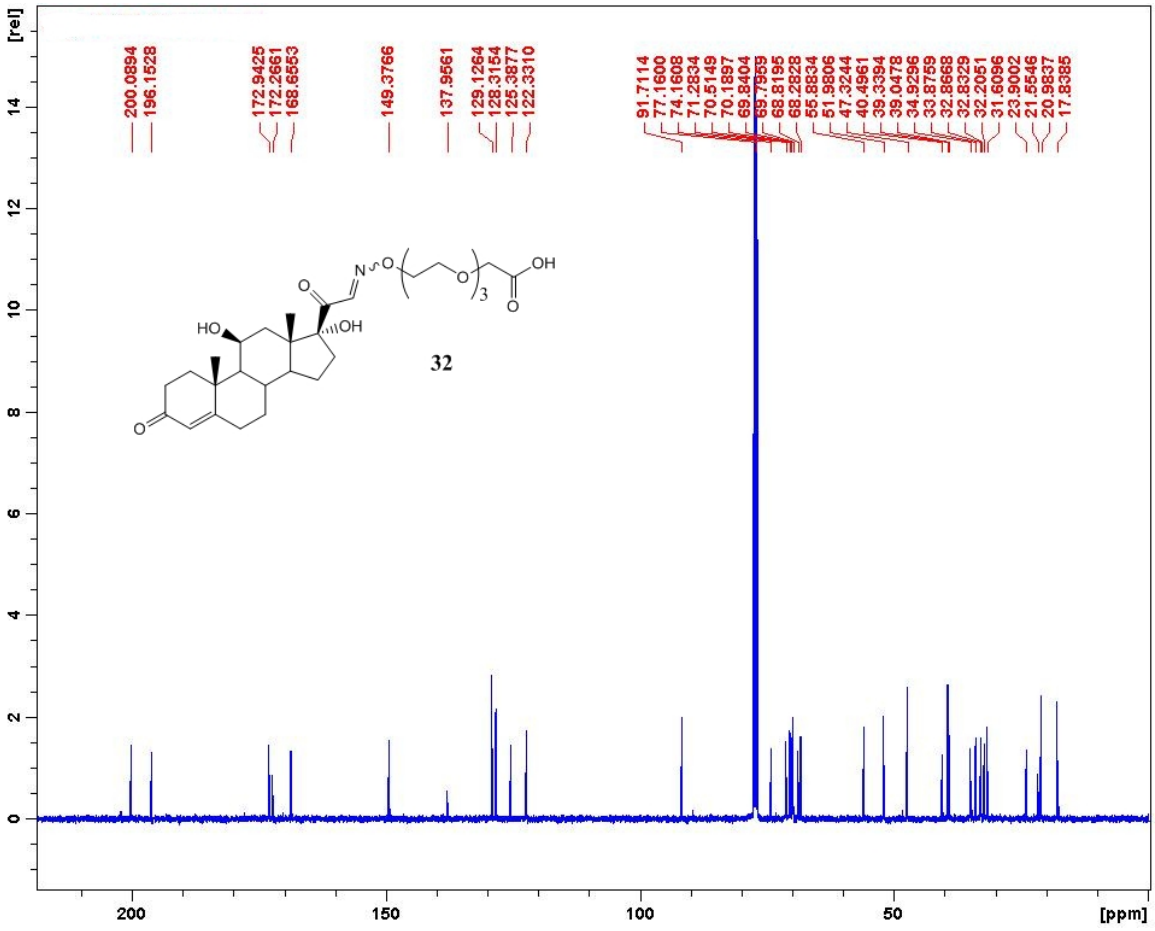
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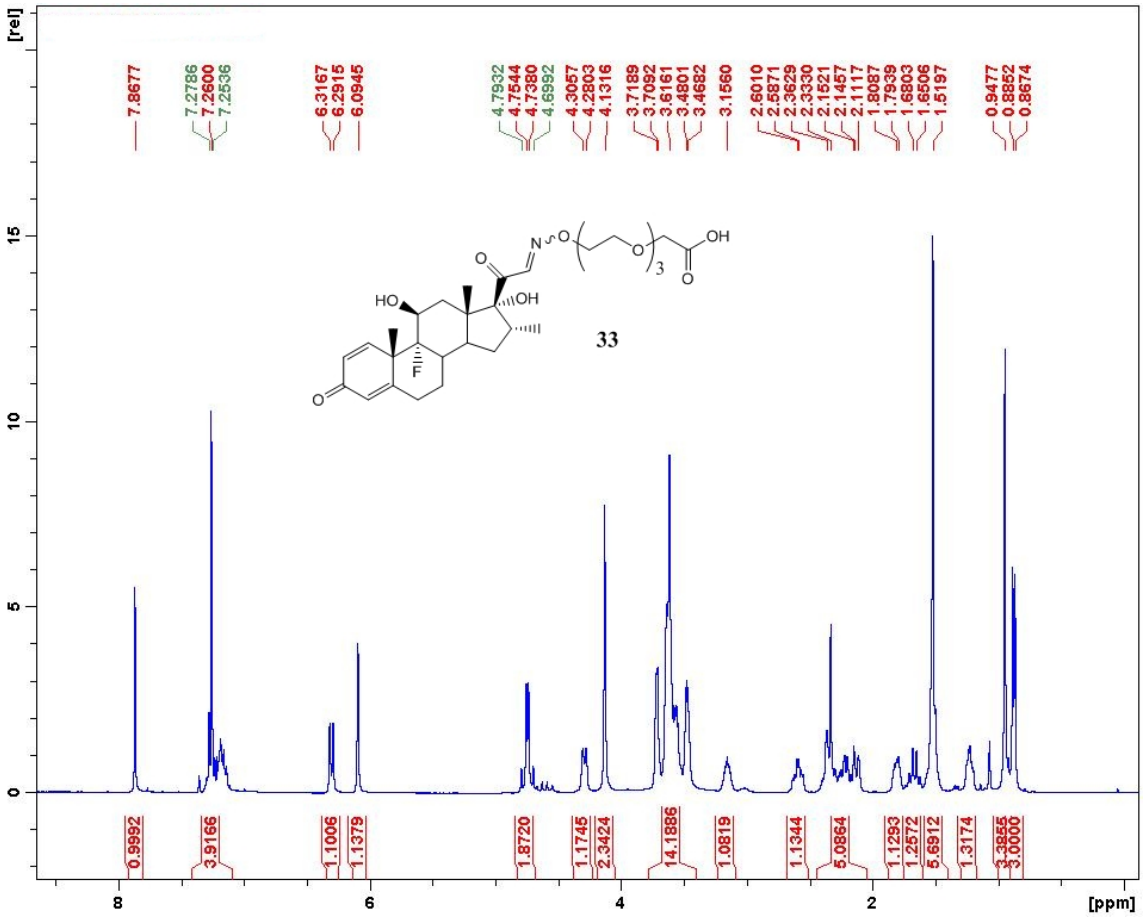
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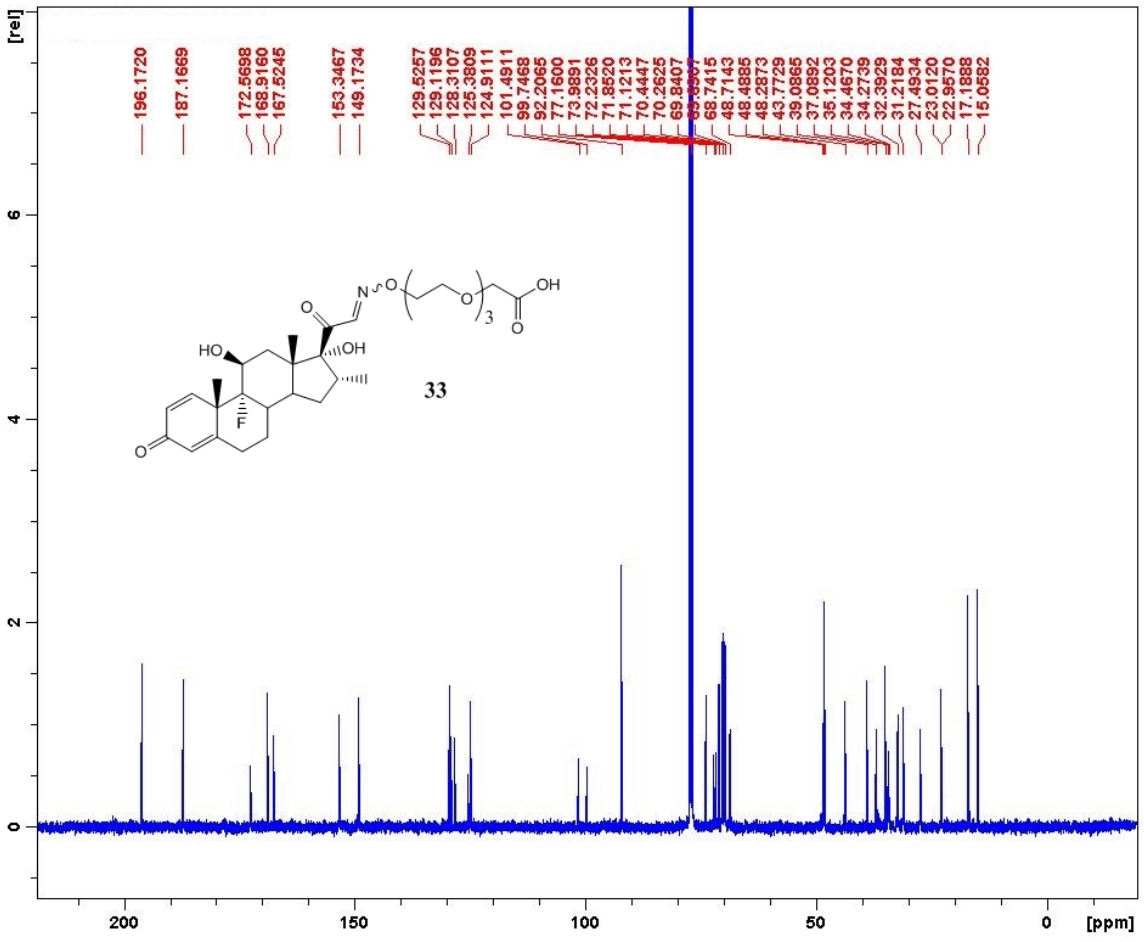
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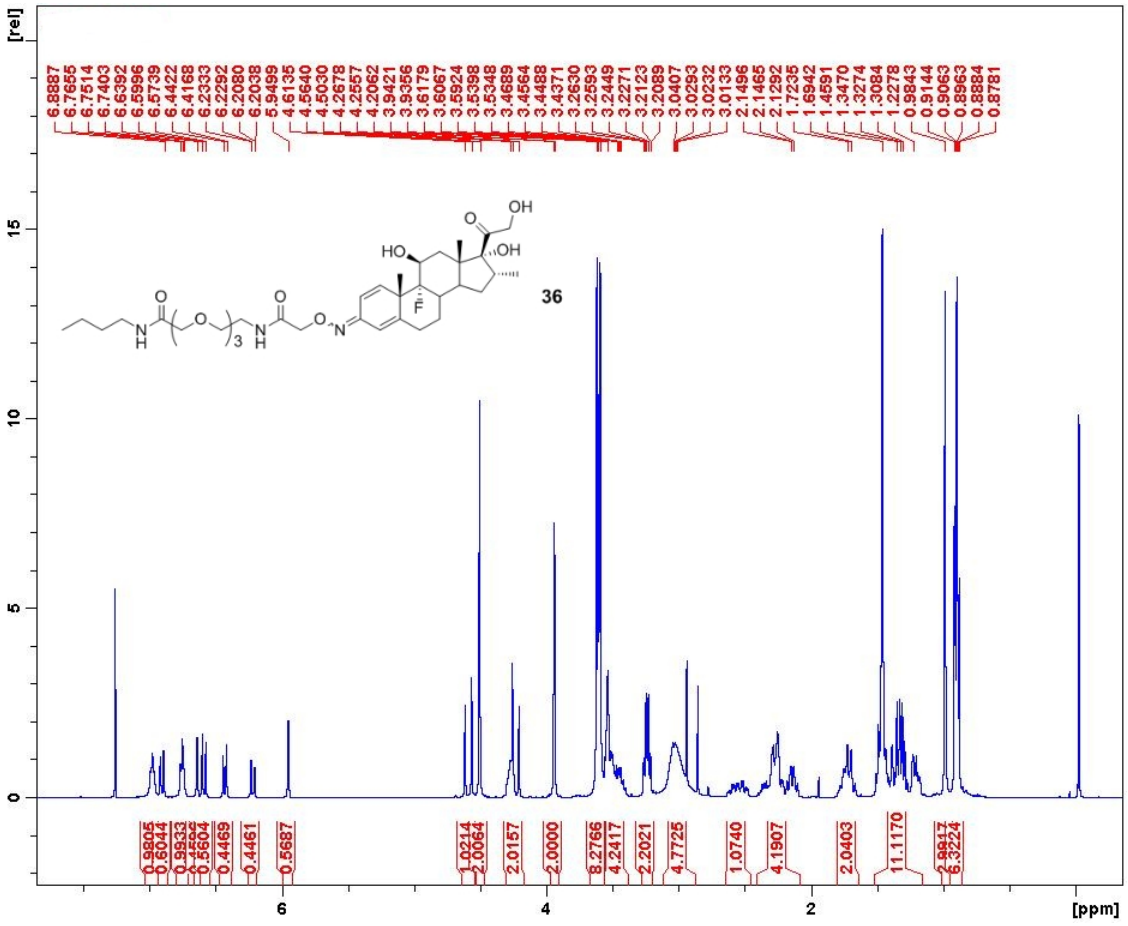
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OH
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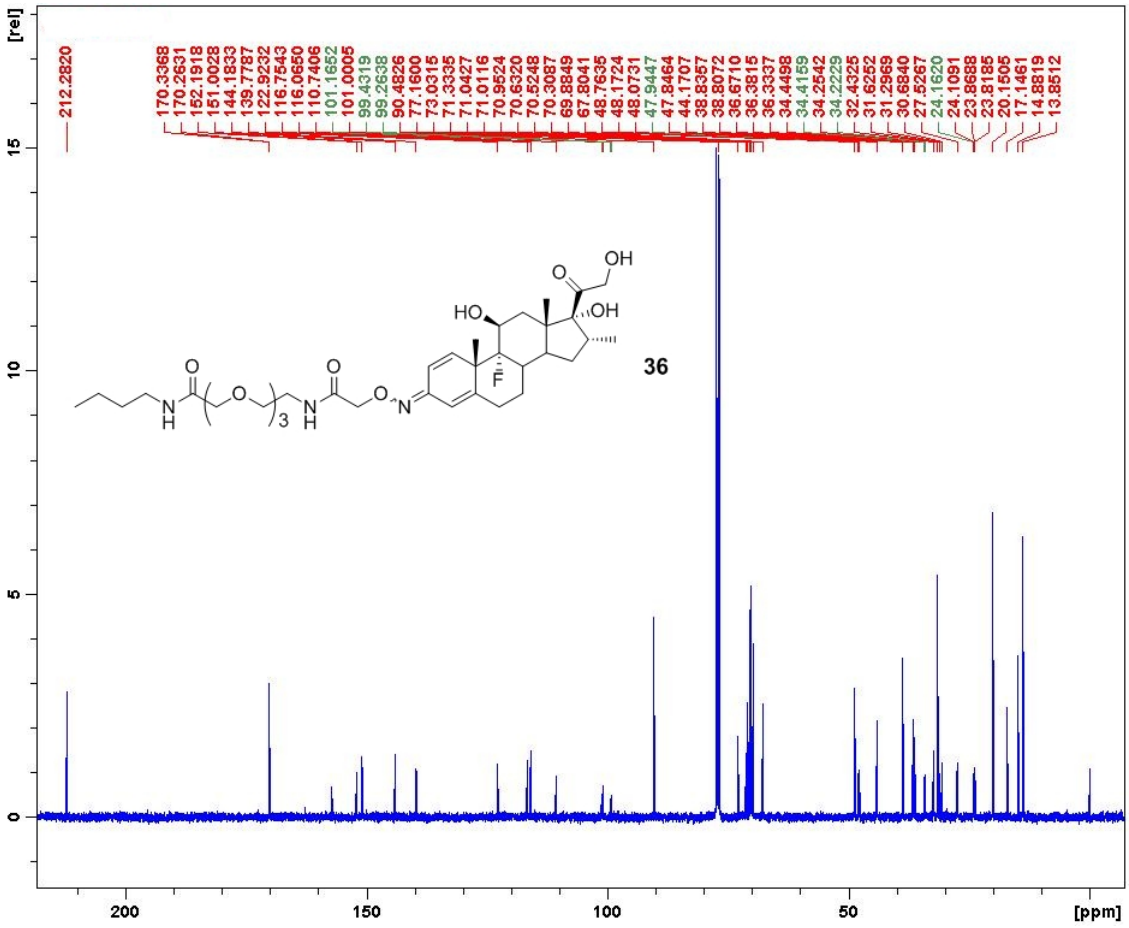
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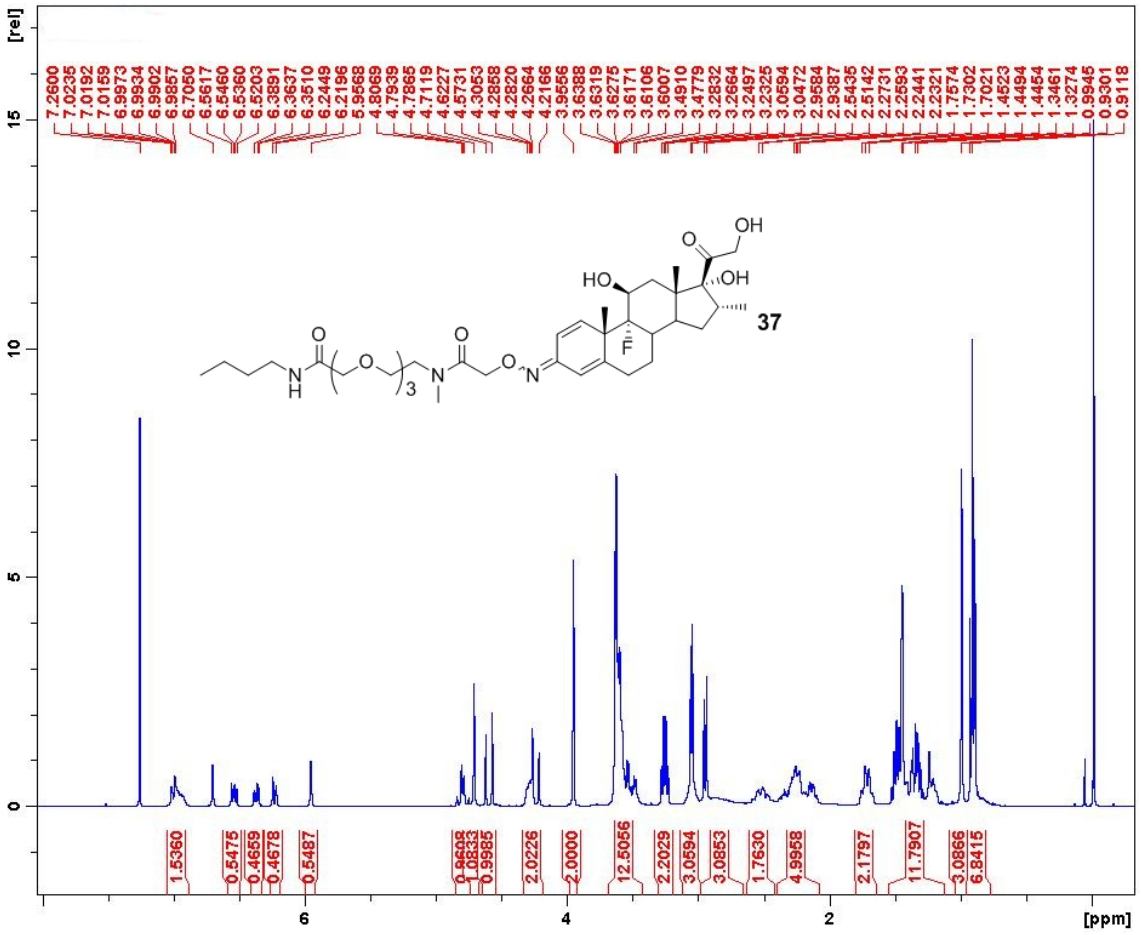
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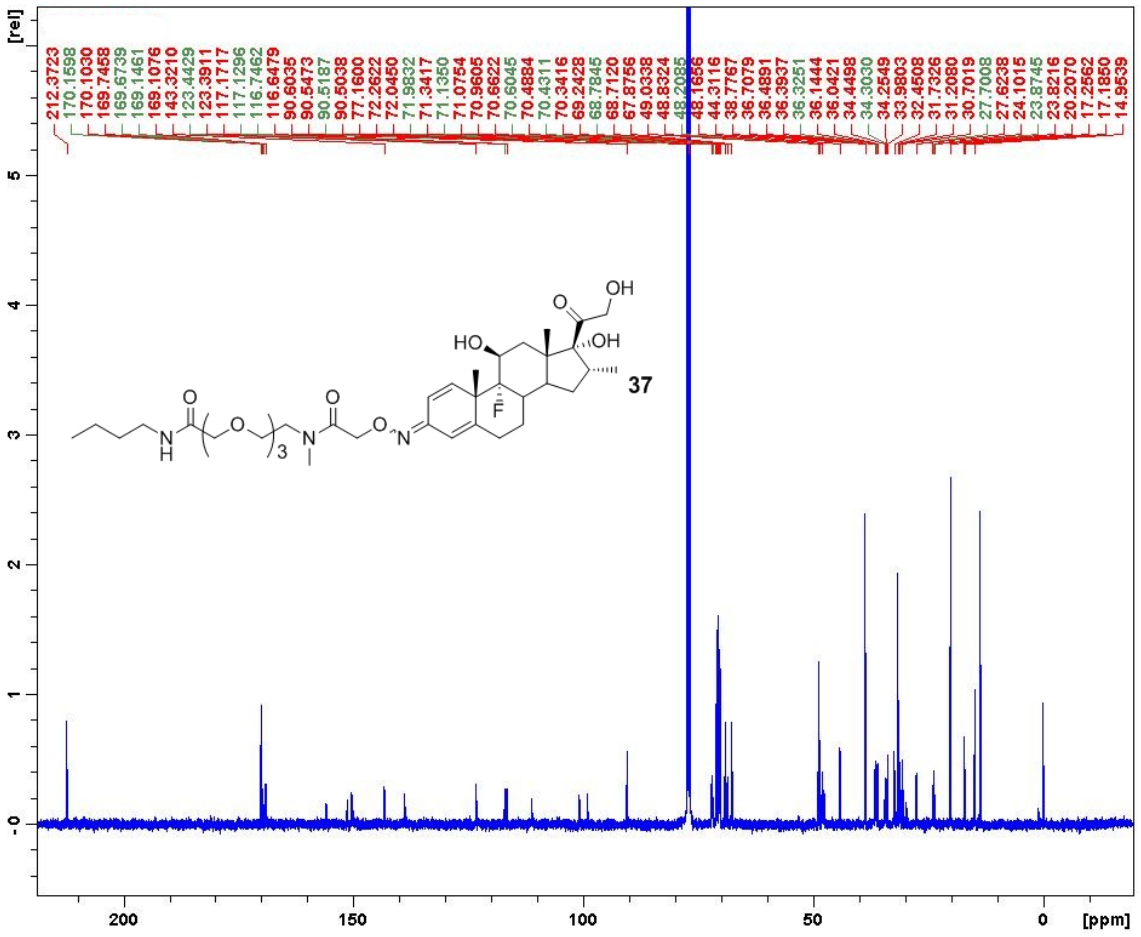
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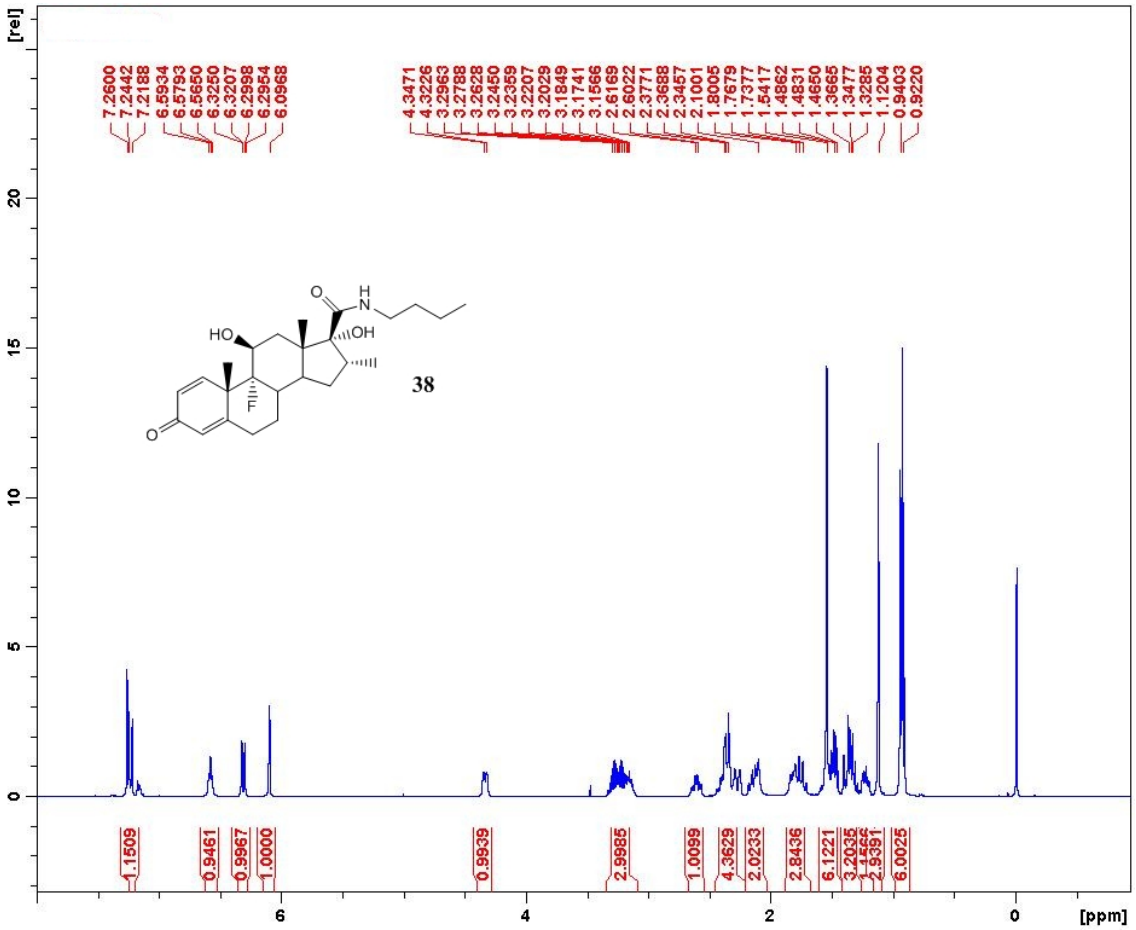
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O
H
N
O
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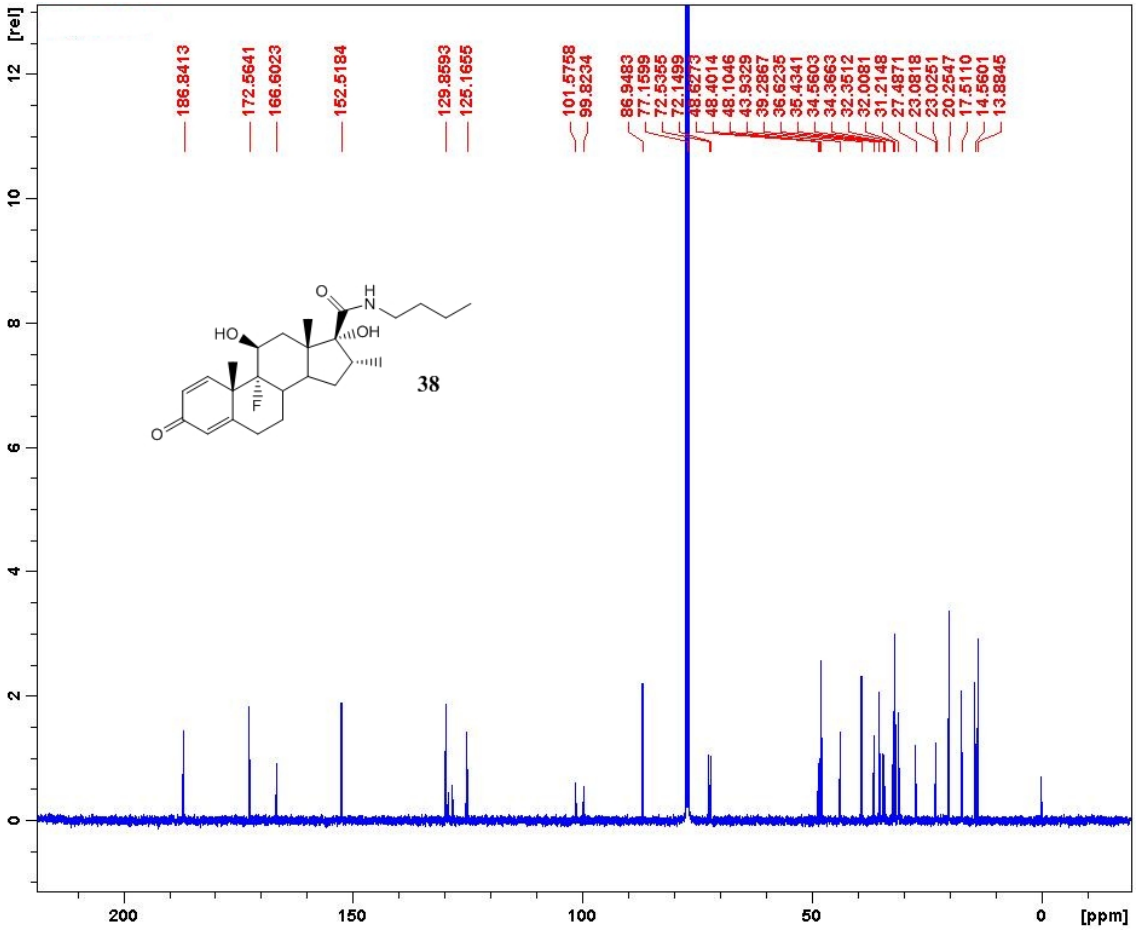
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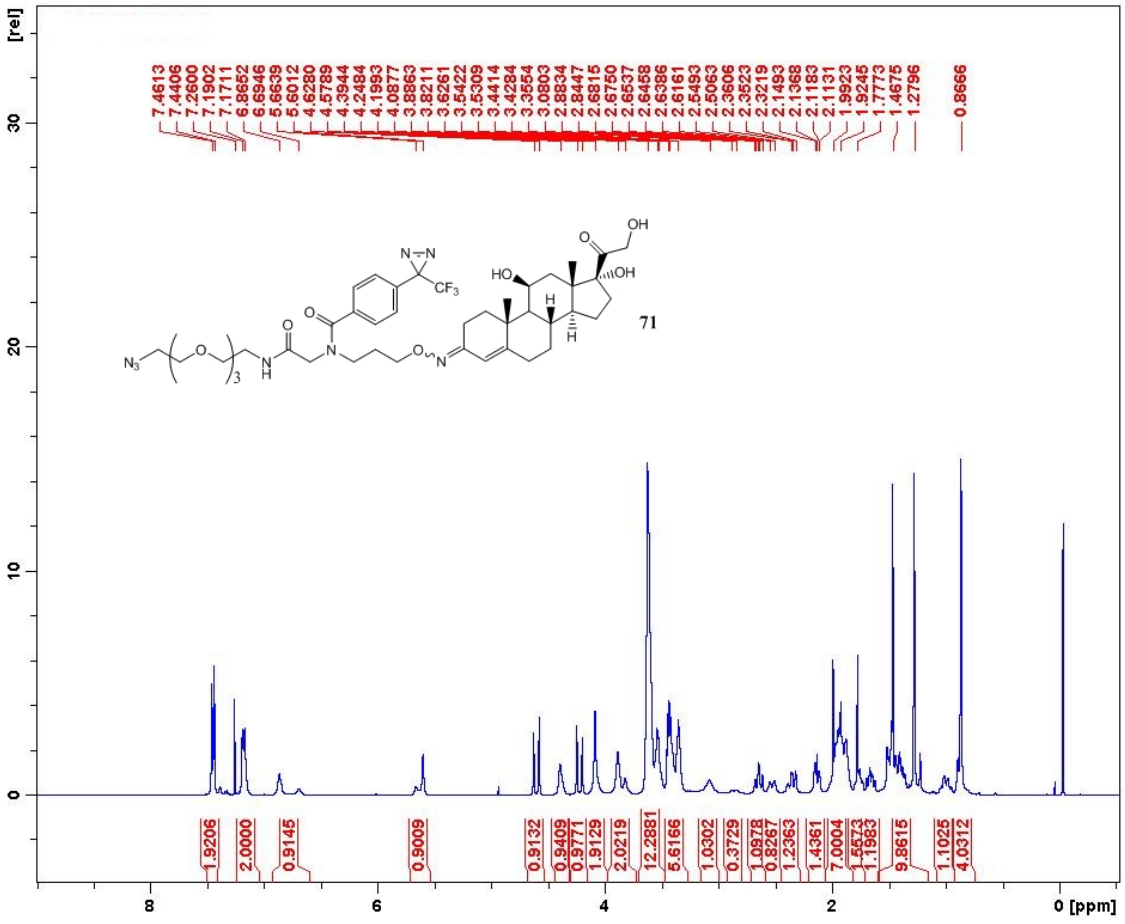
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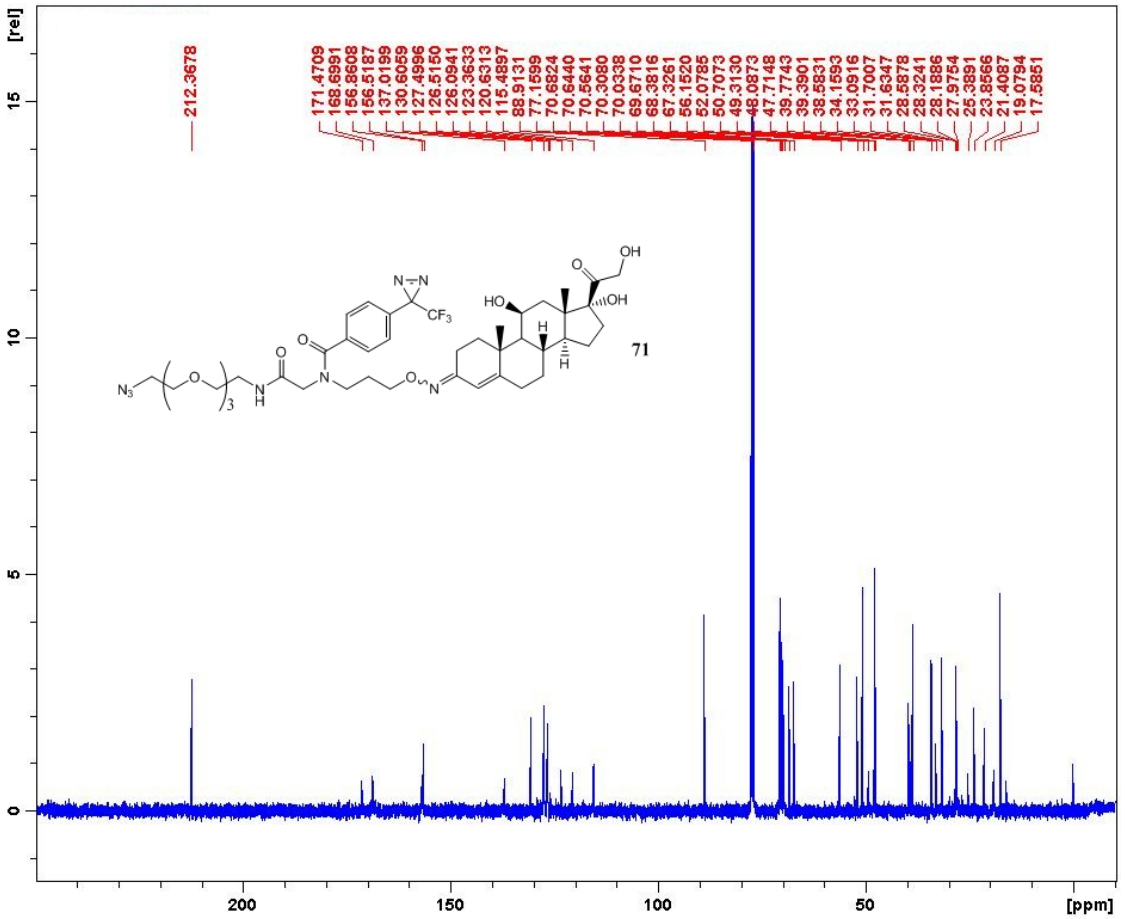
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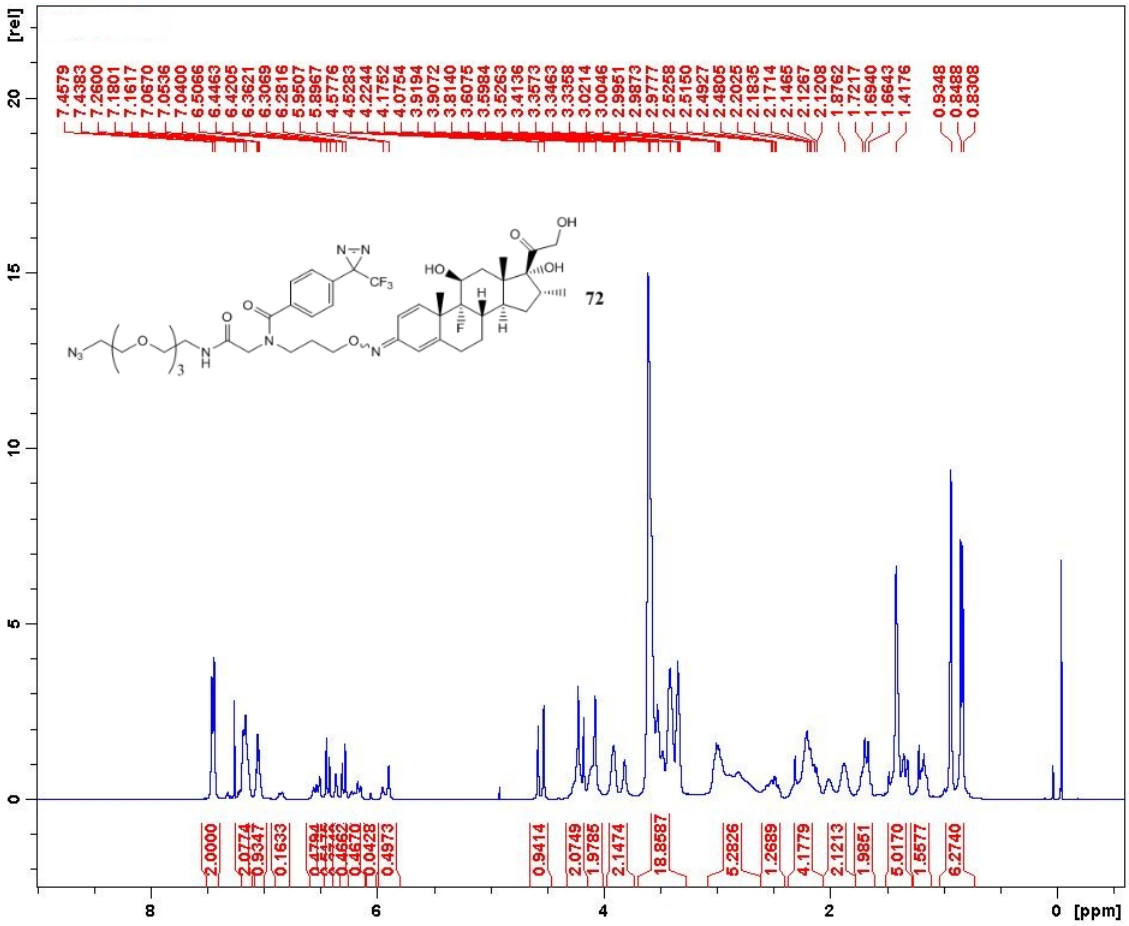
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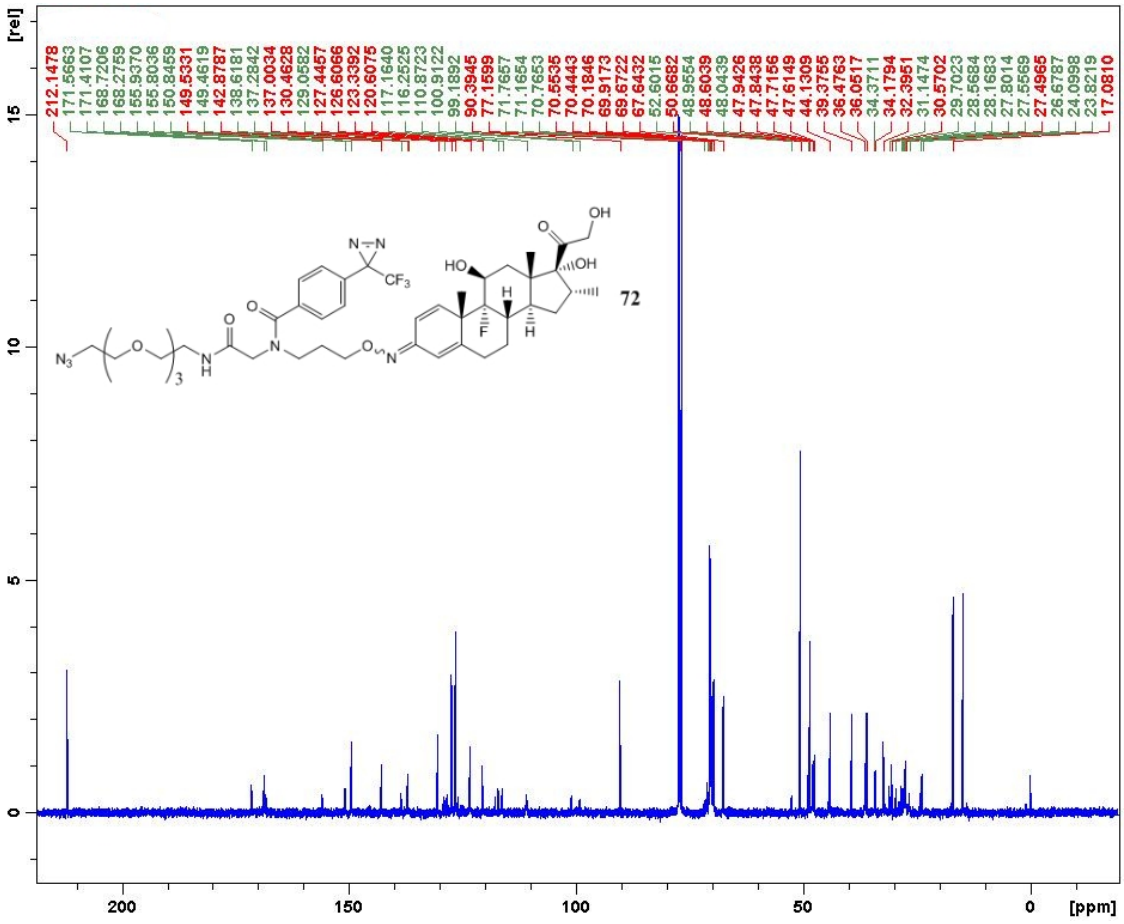
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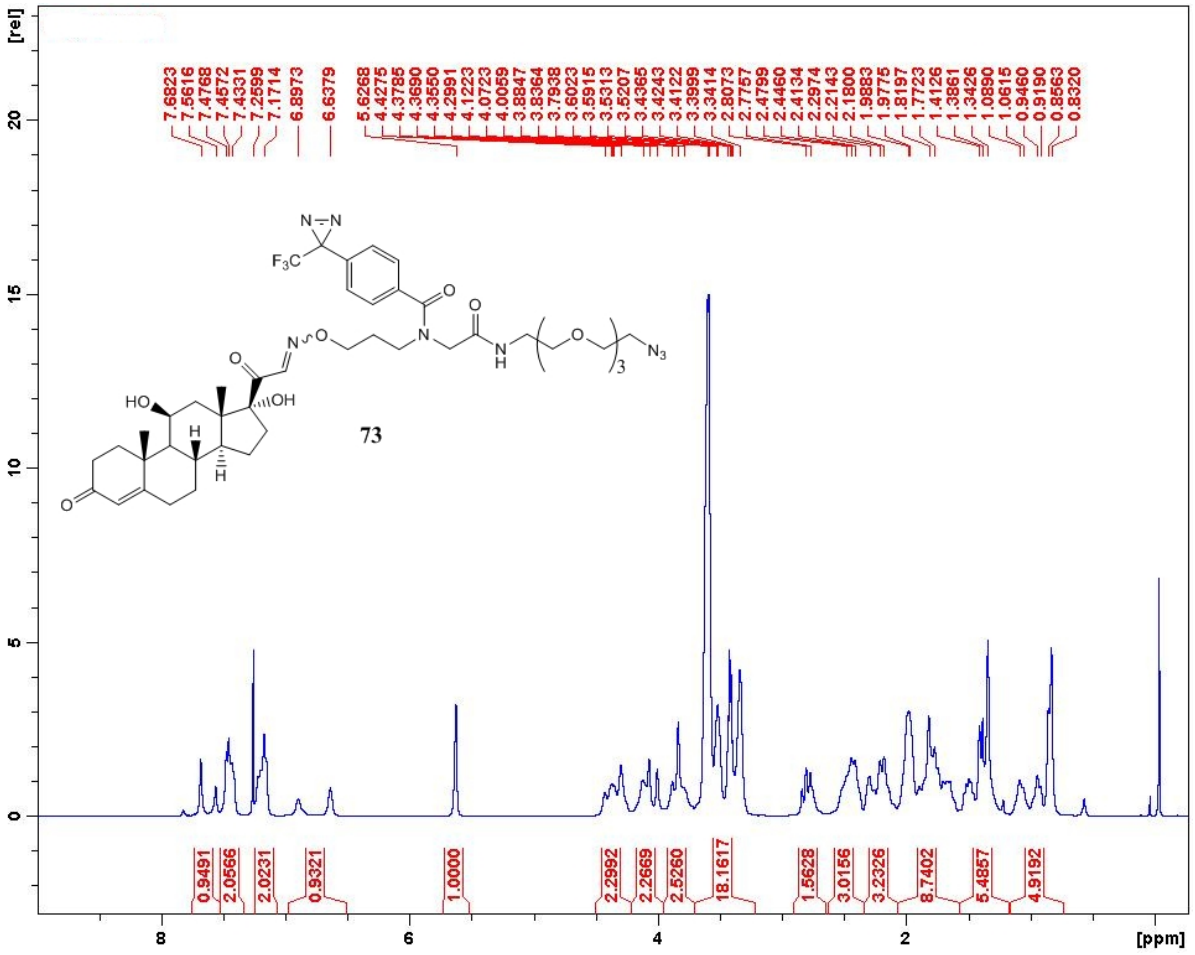
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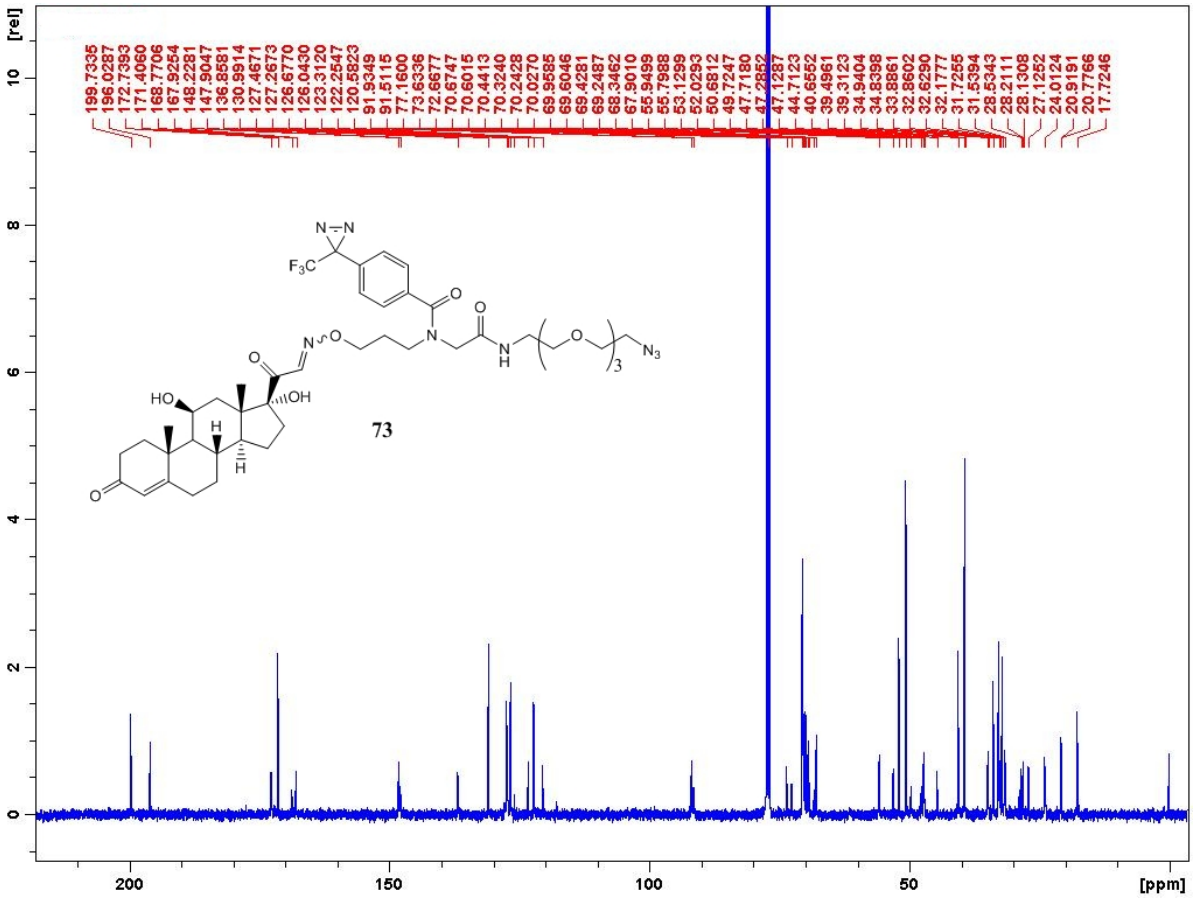
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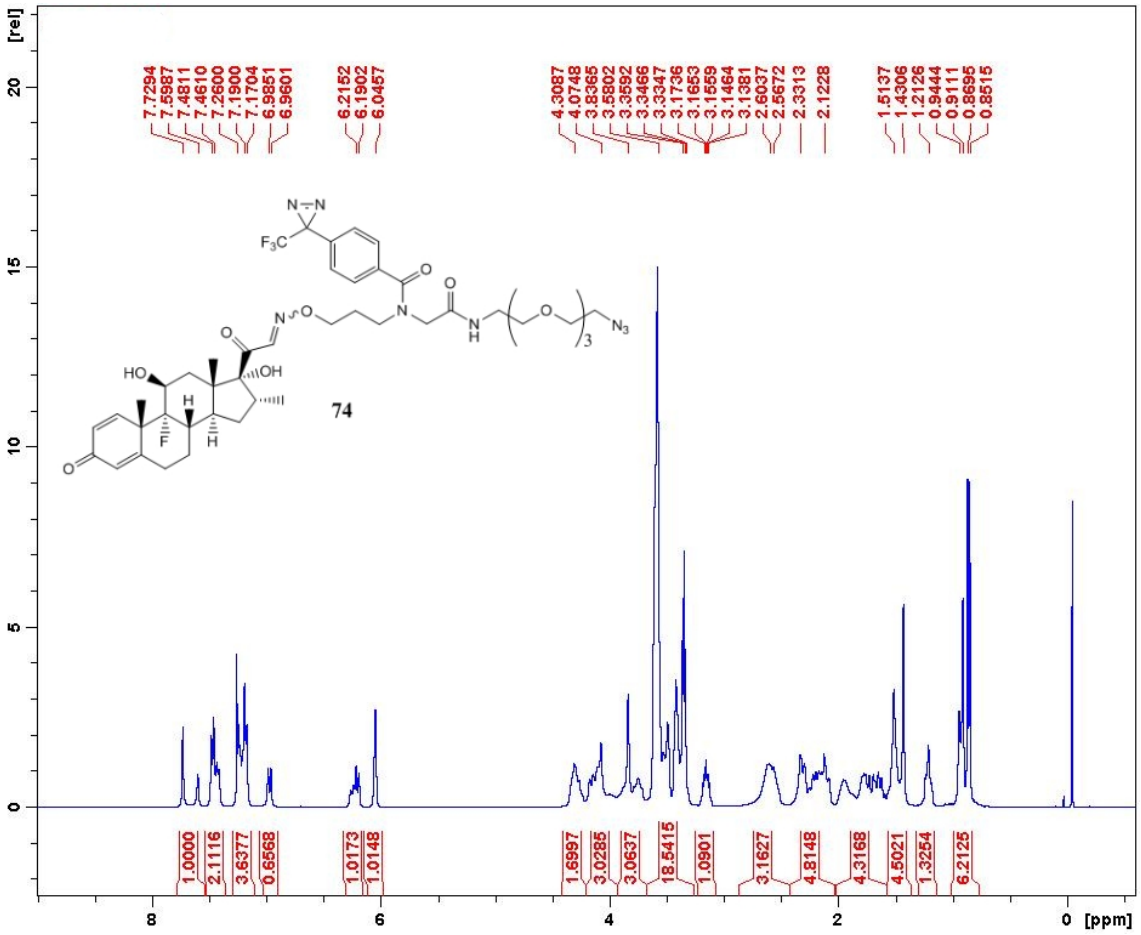
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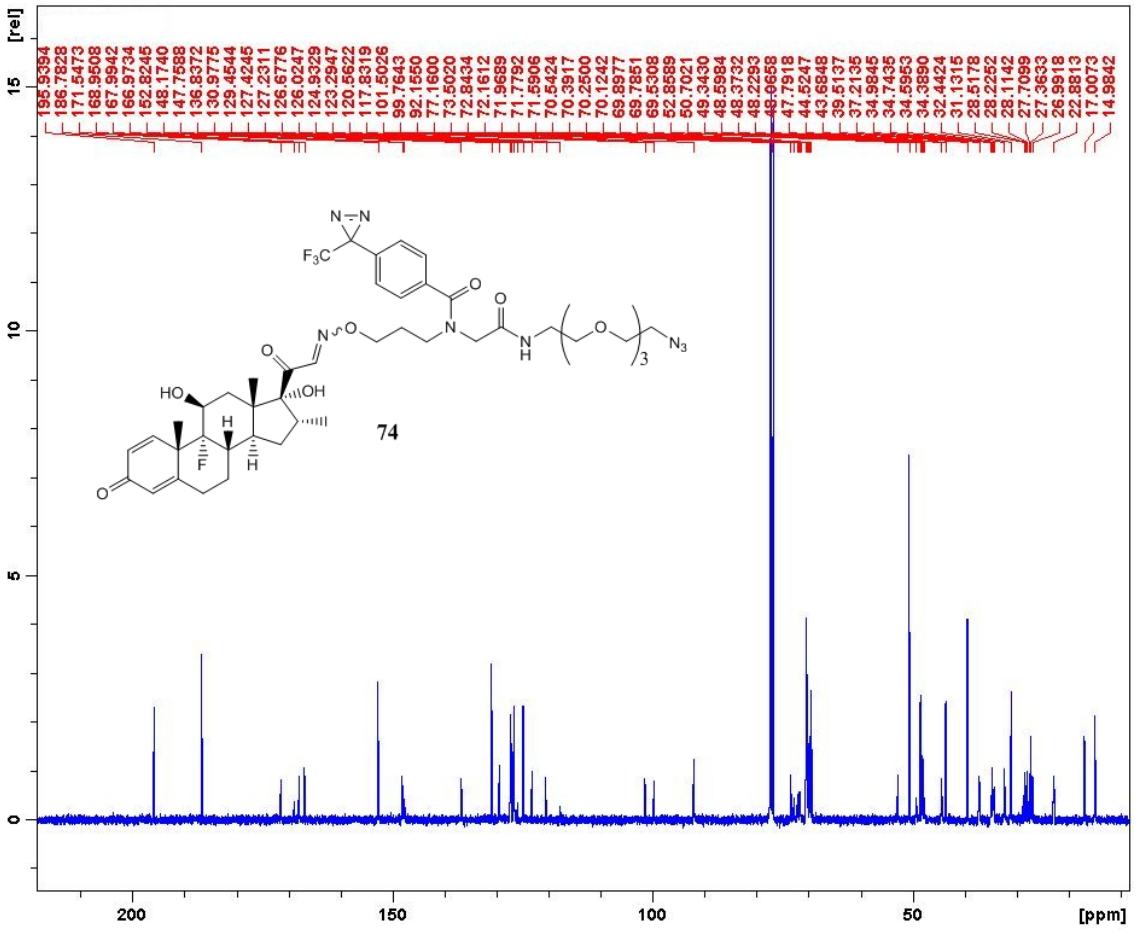
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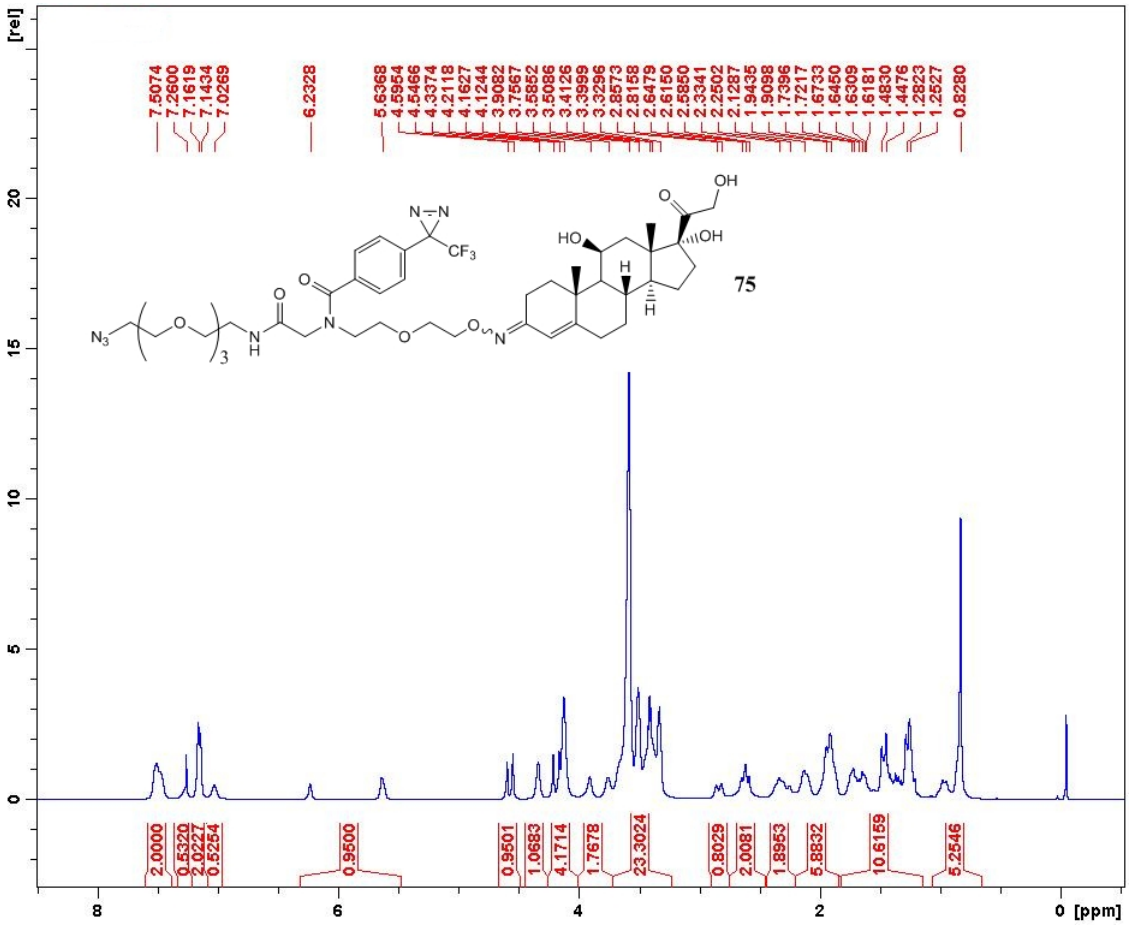
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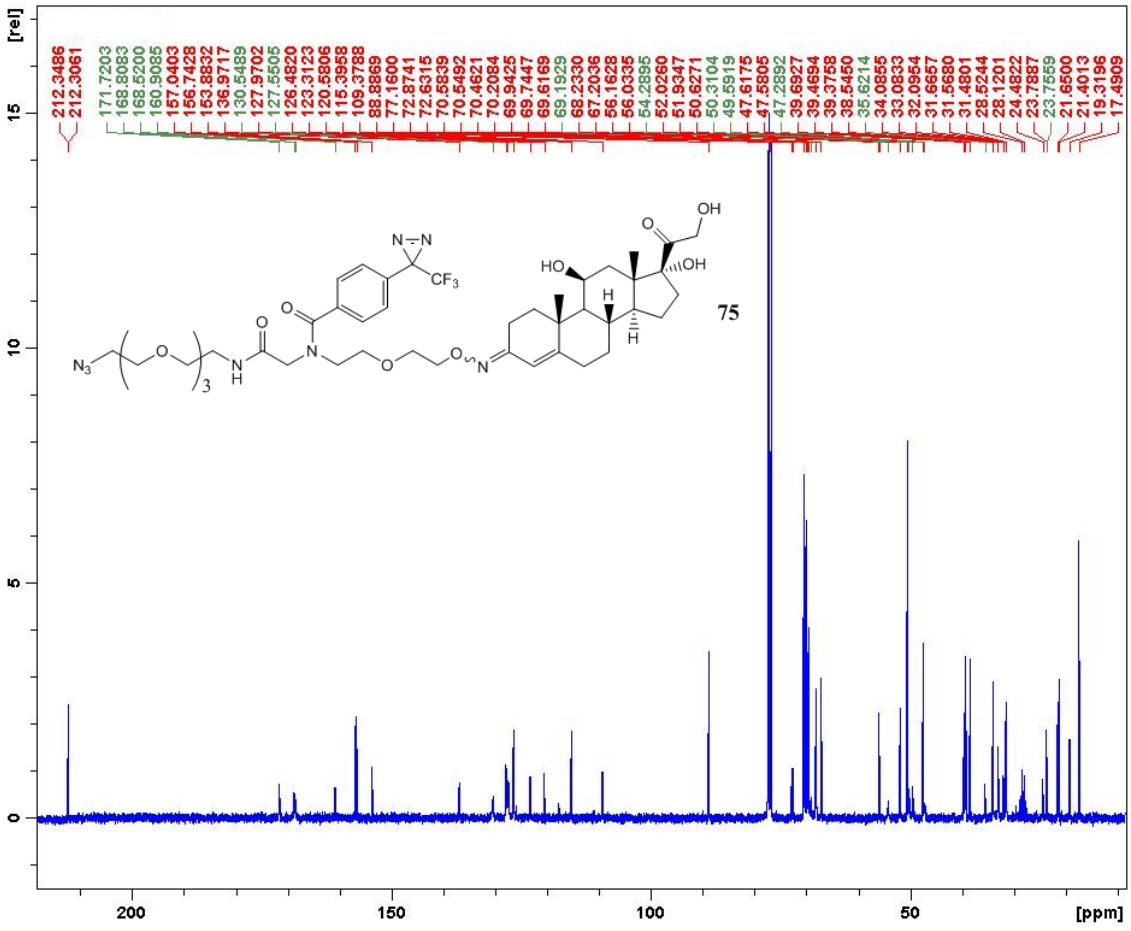
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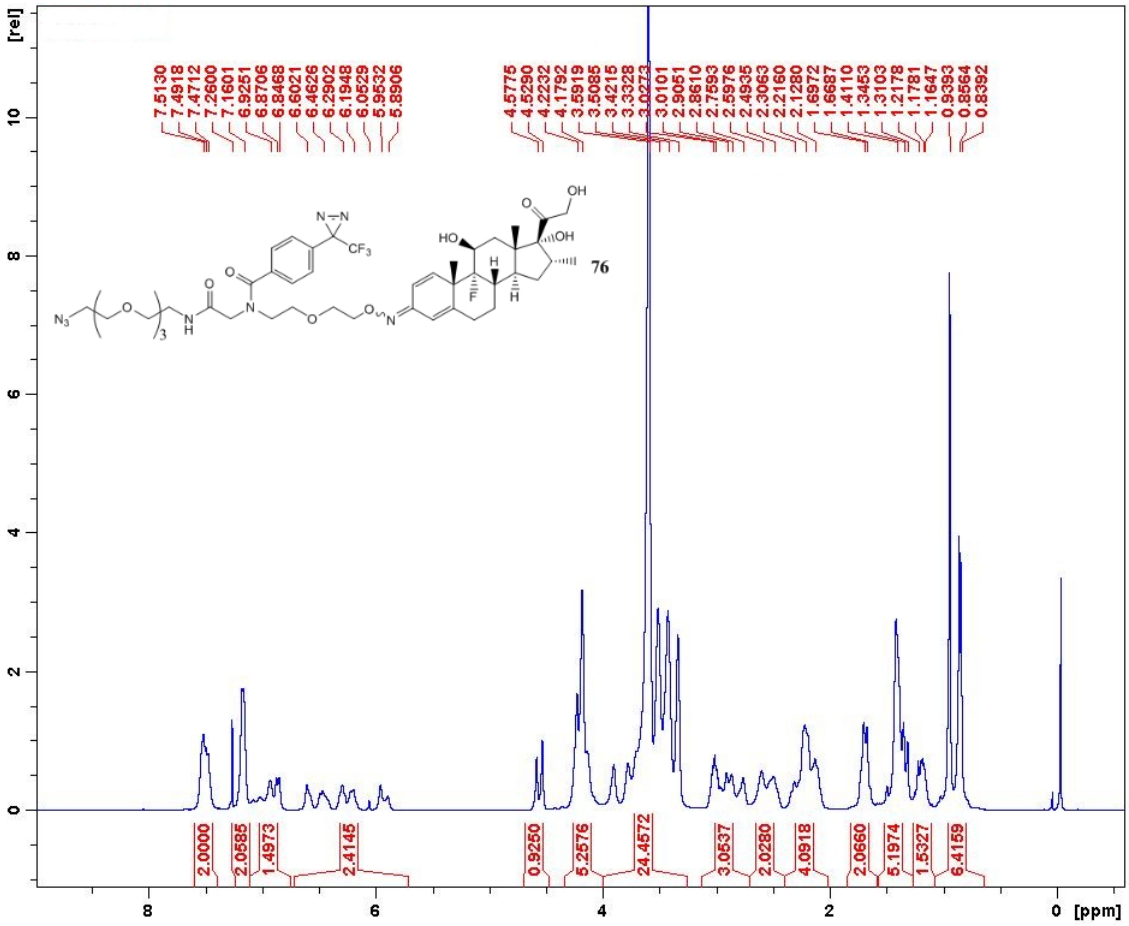
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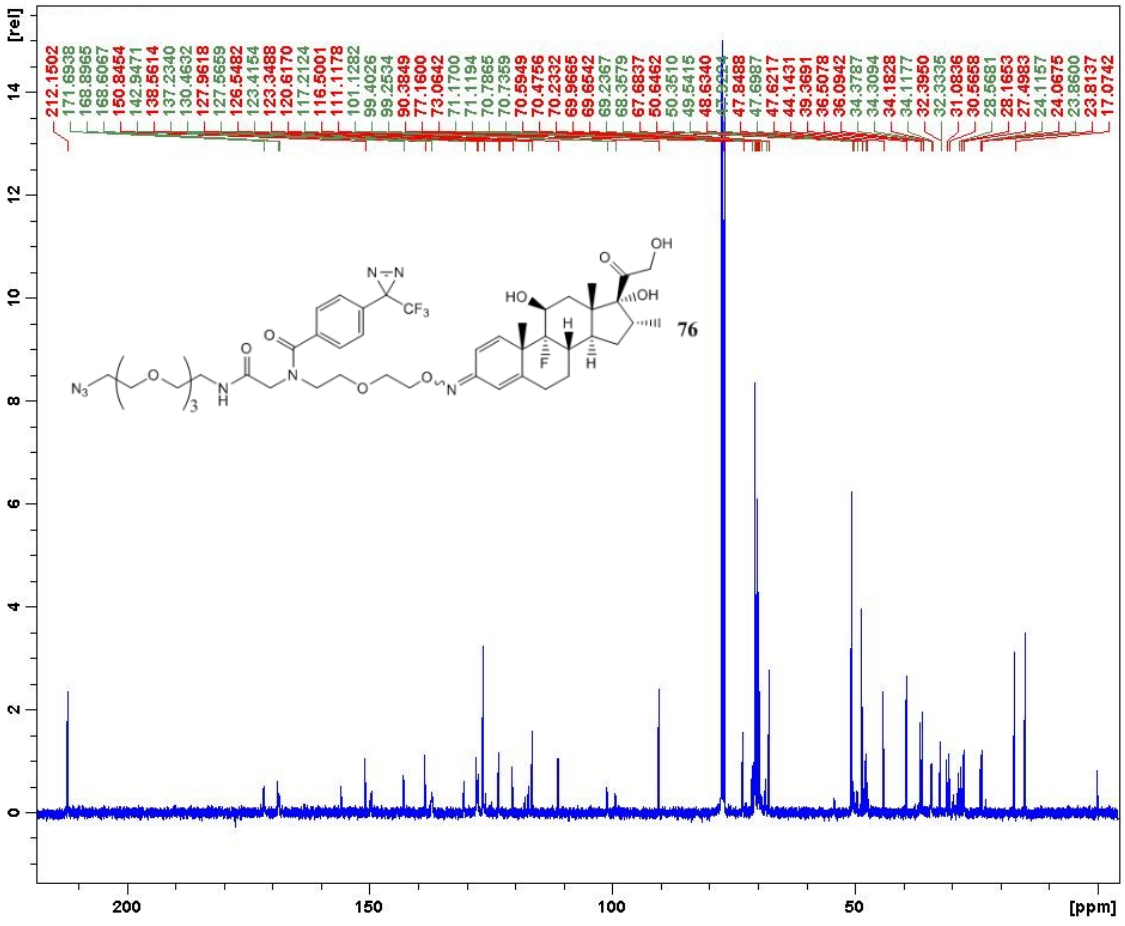
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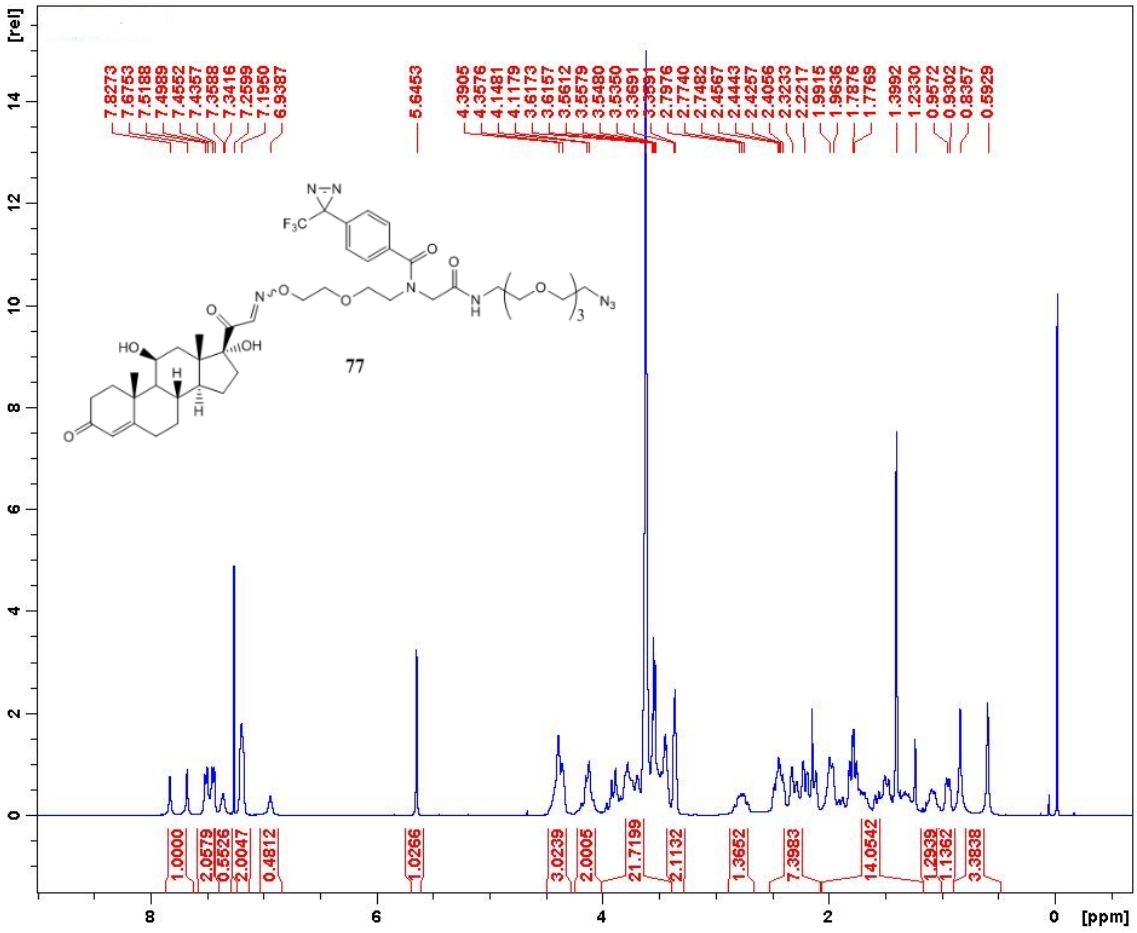
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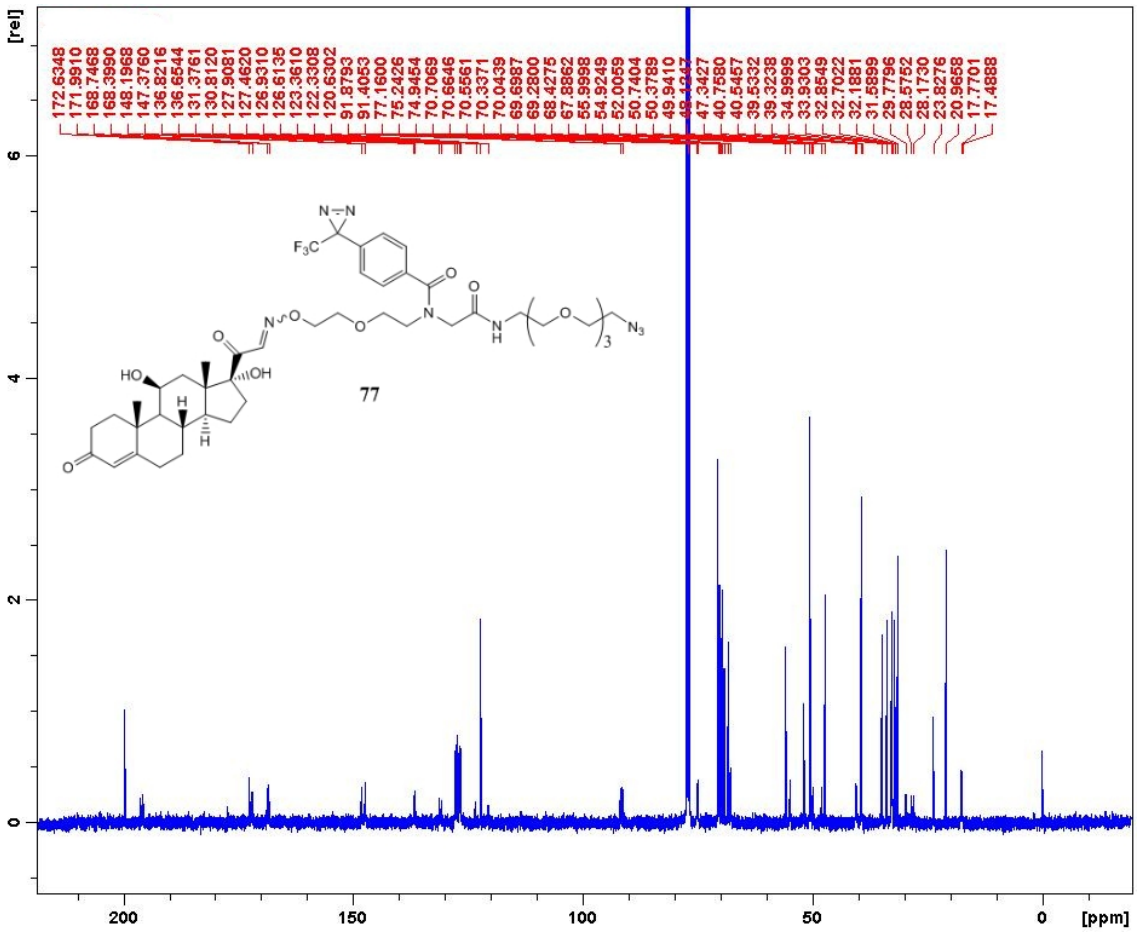
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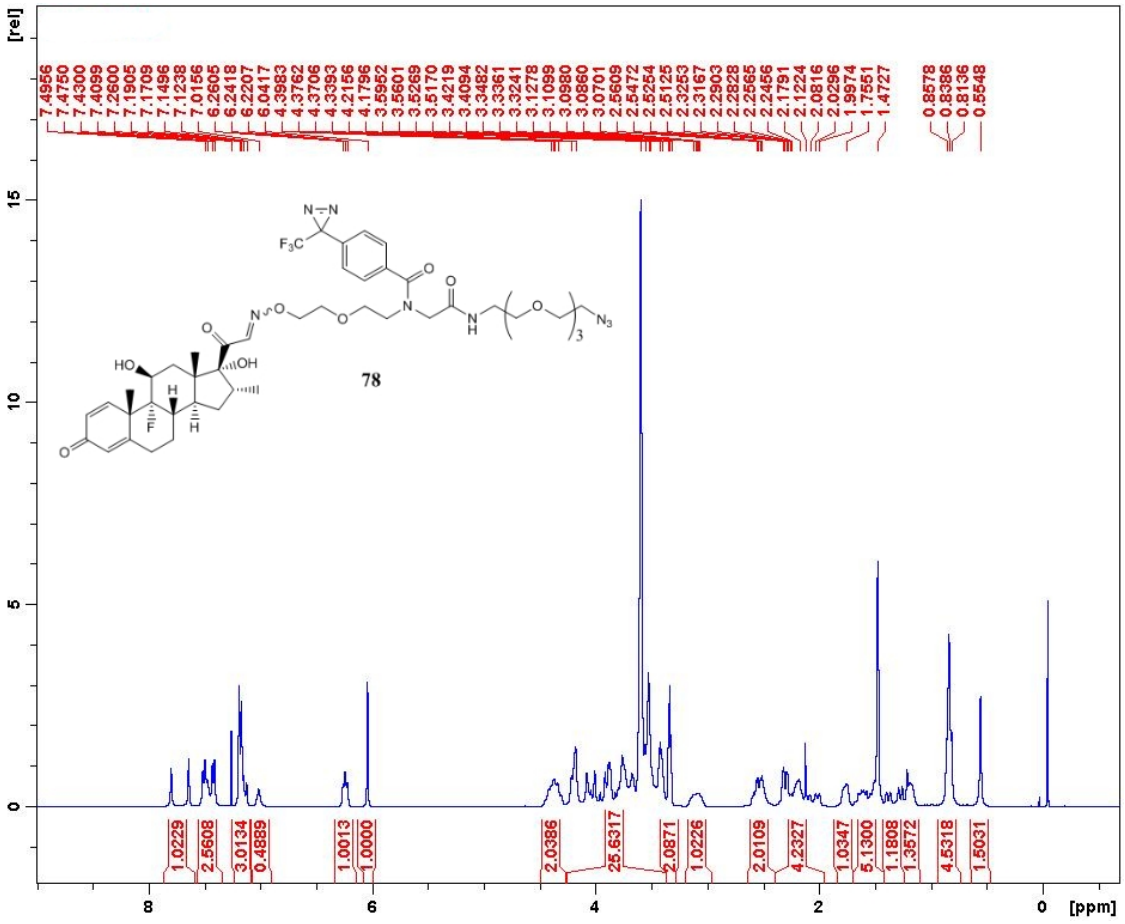
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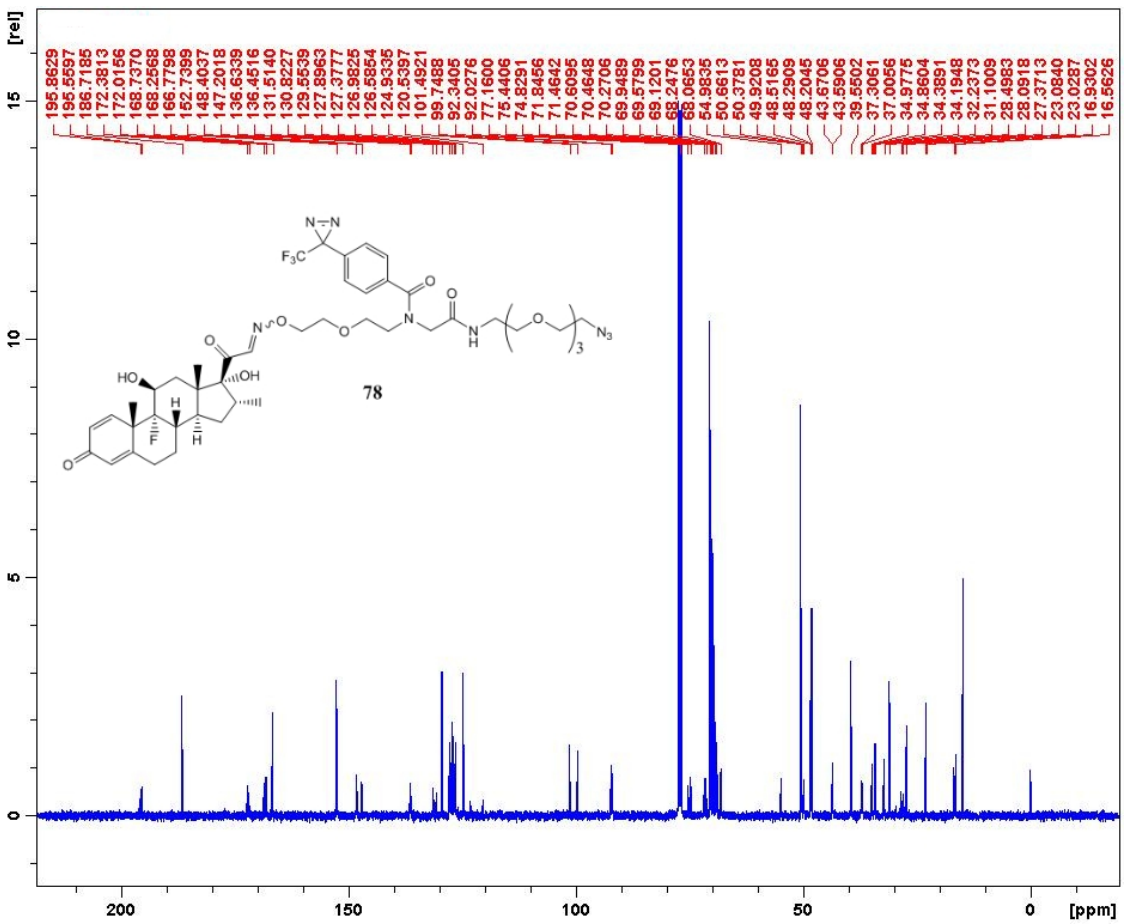
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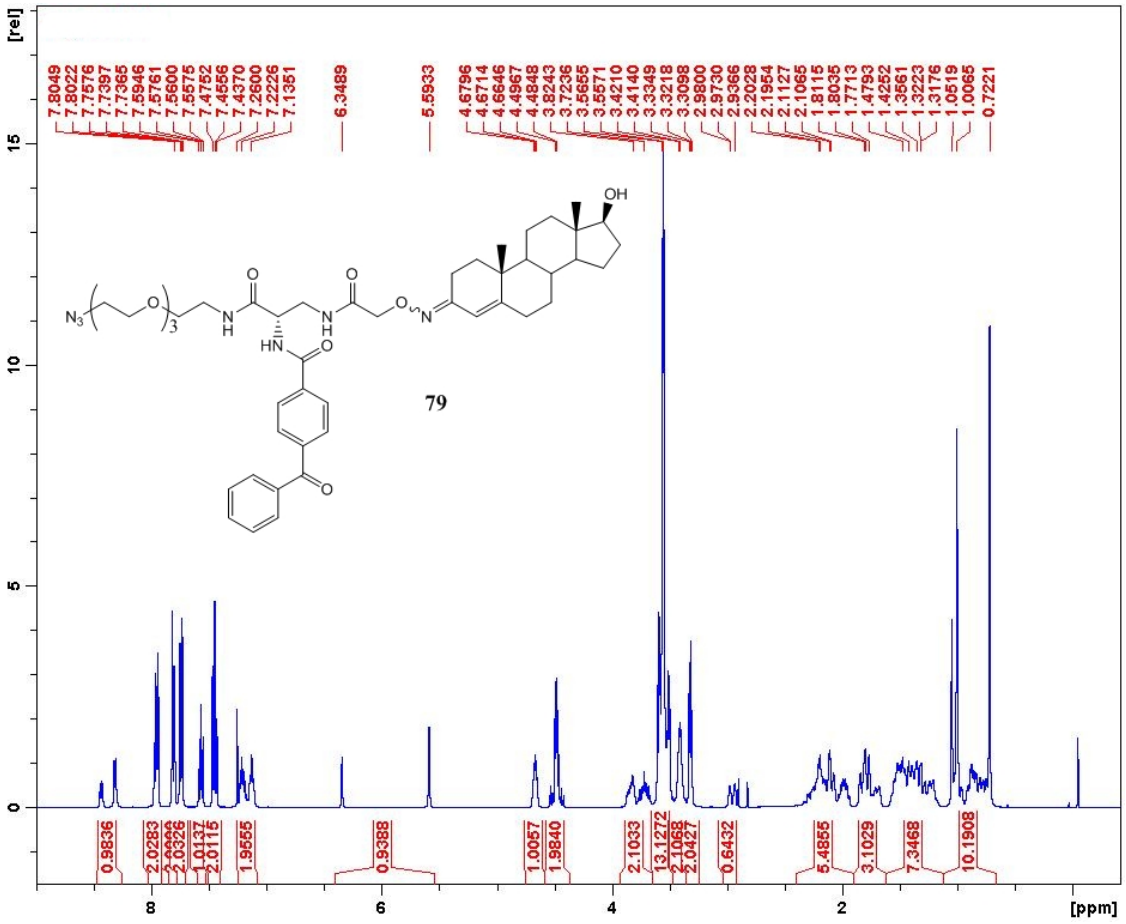
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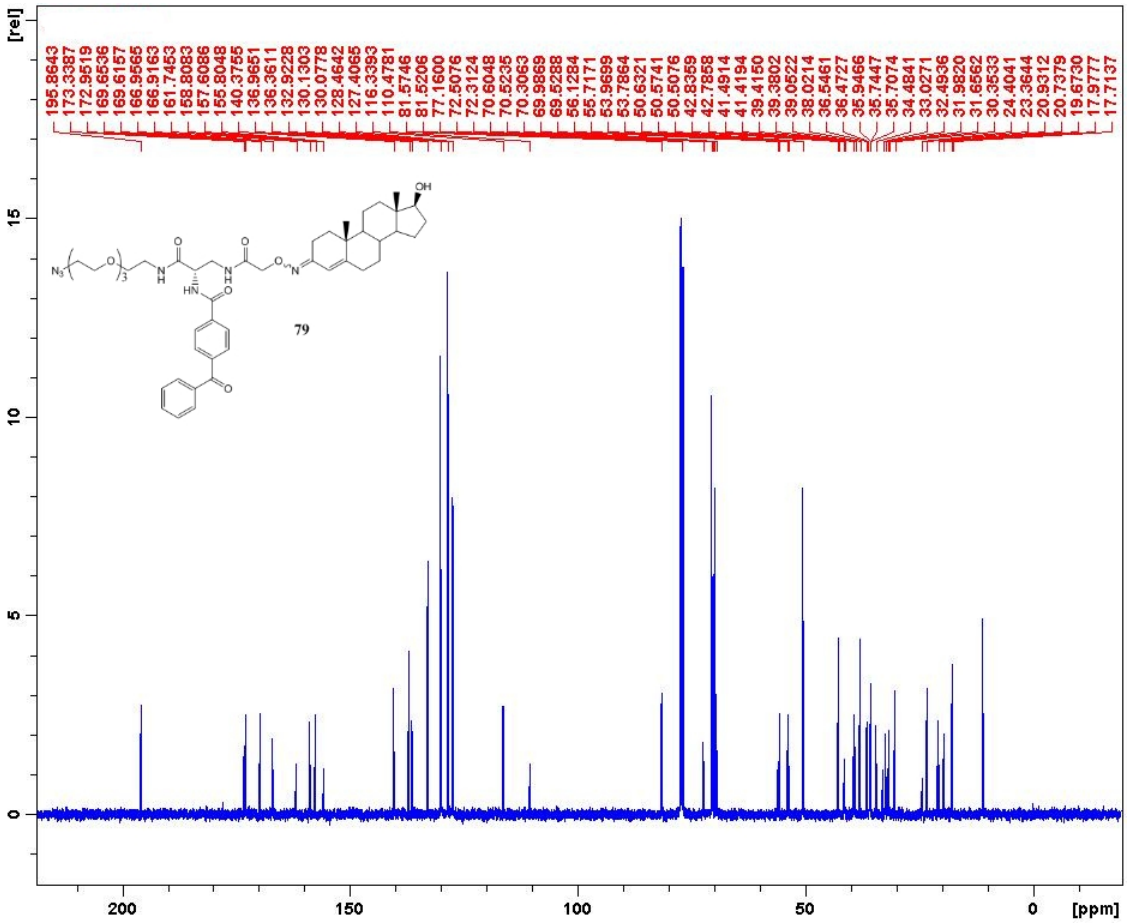
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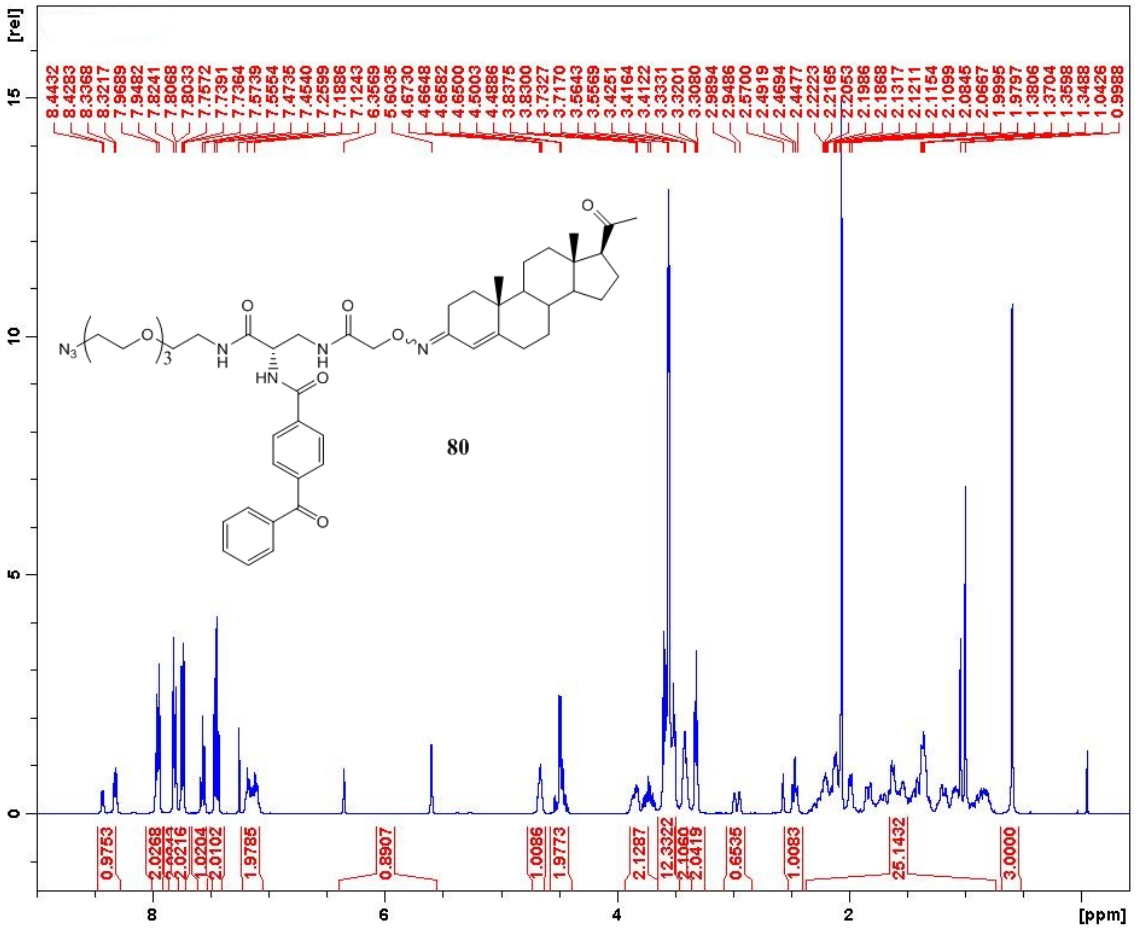
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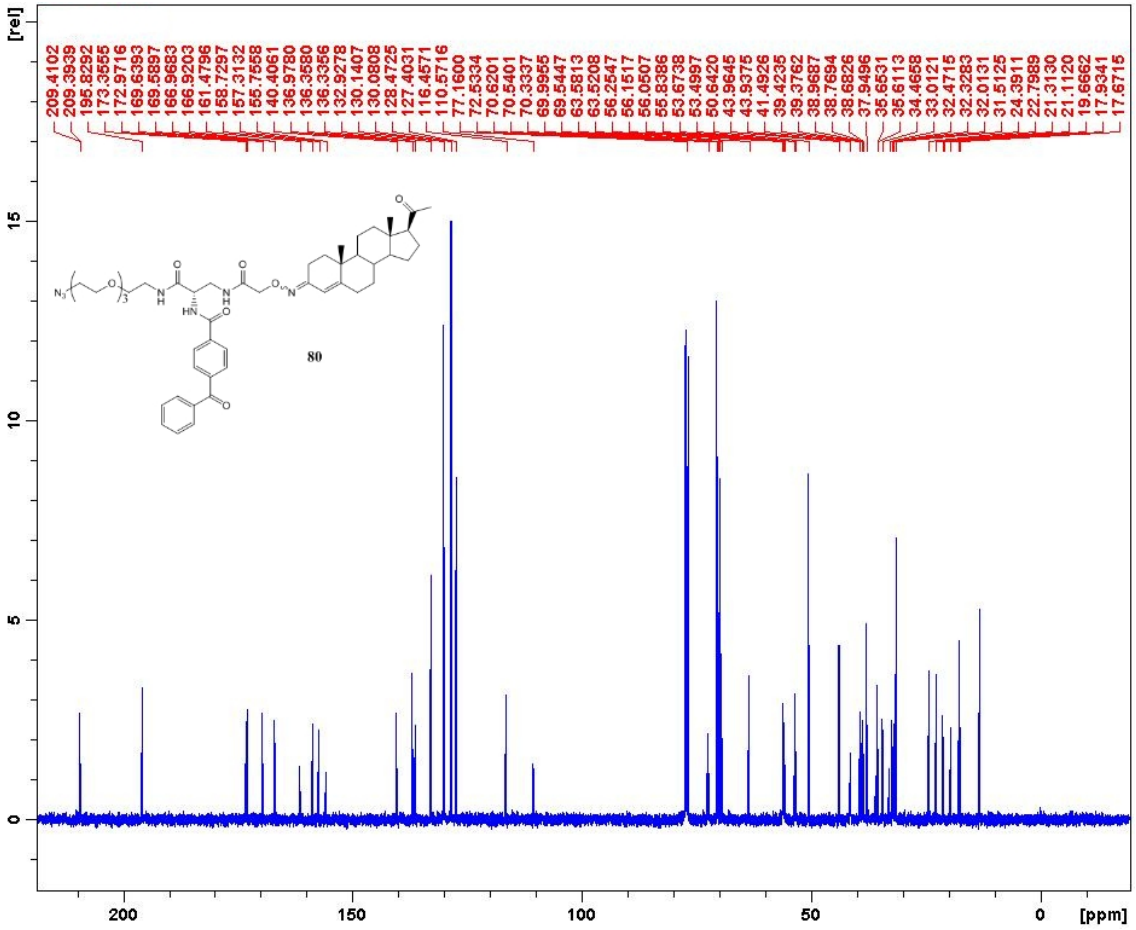
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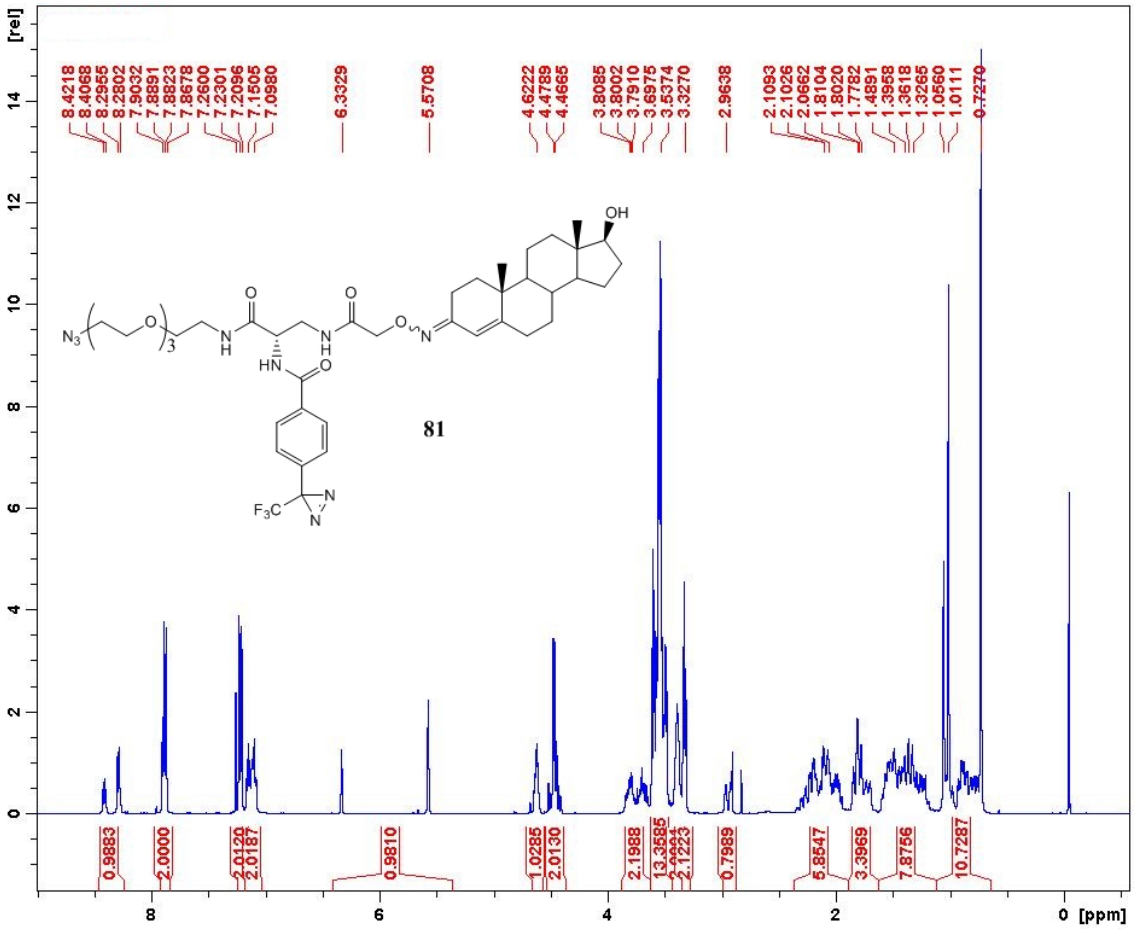
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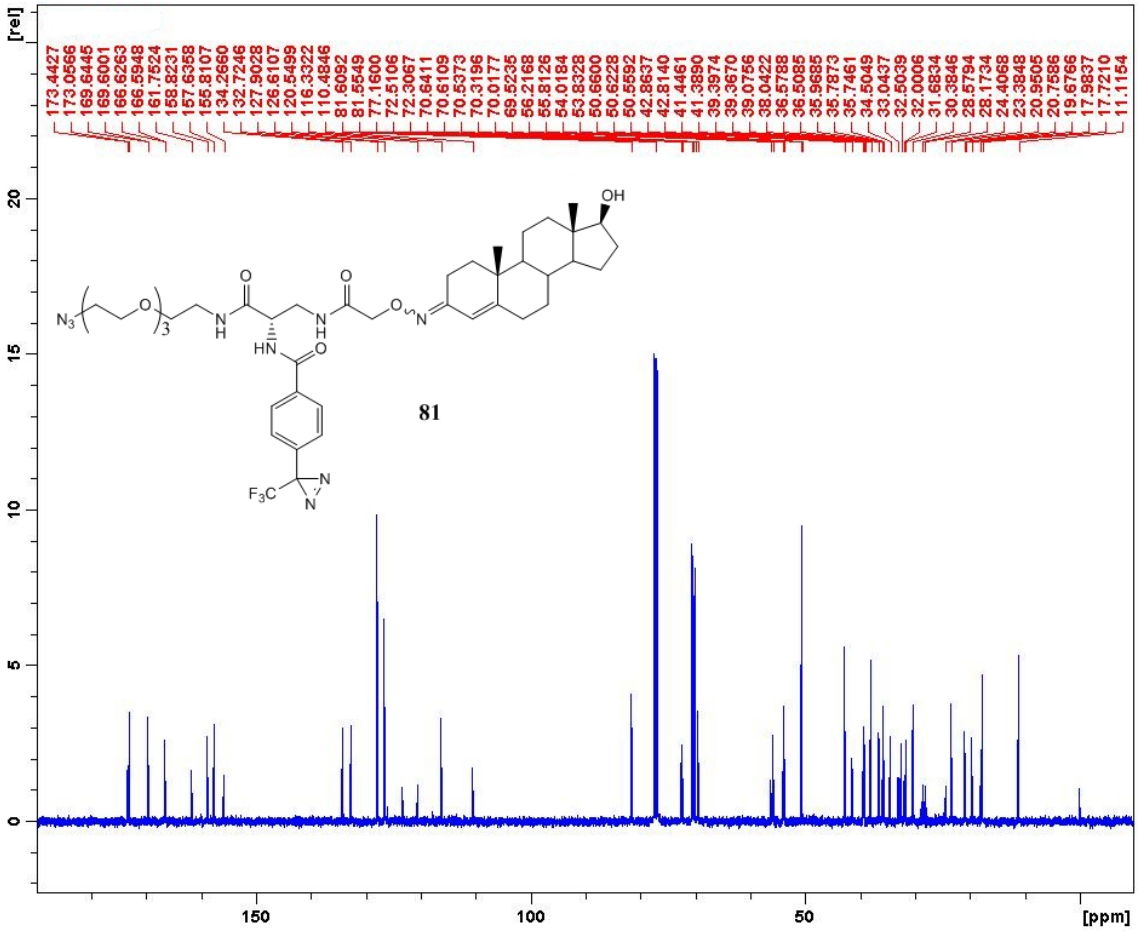
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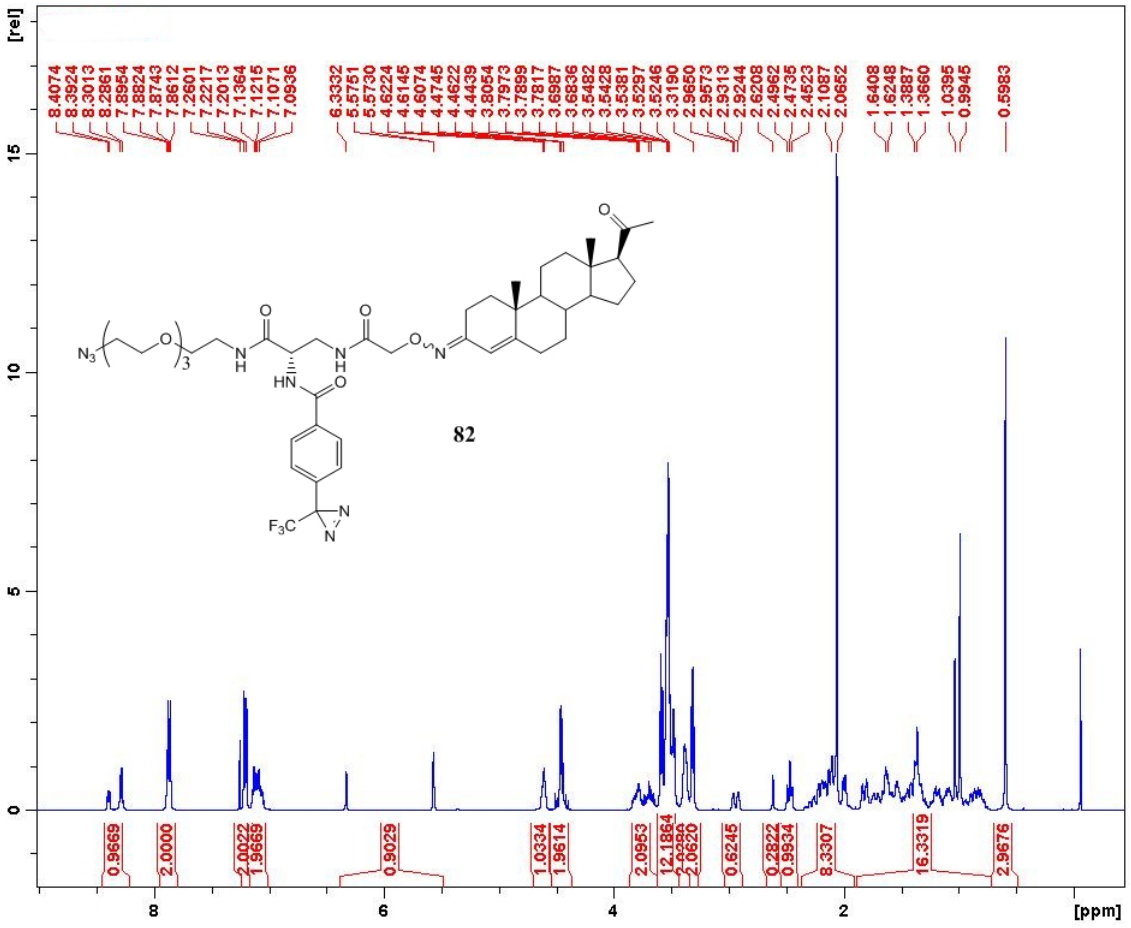
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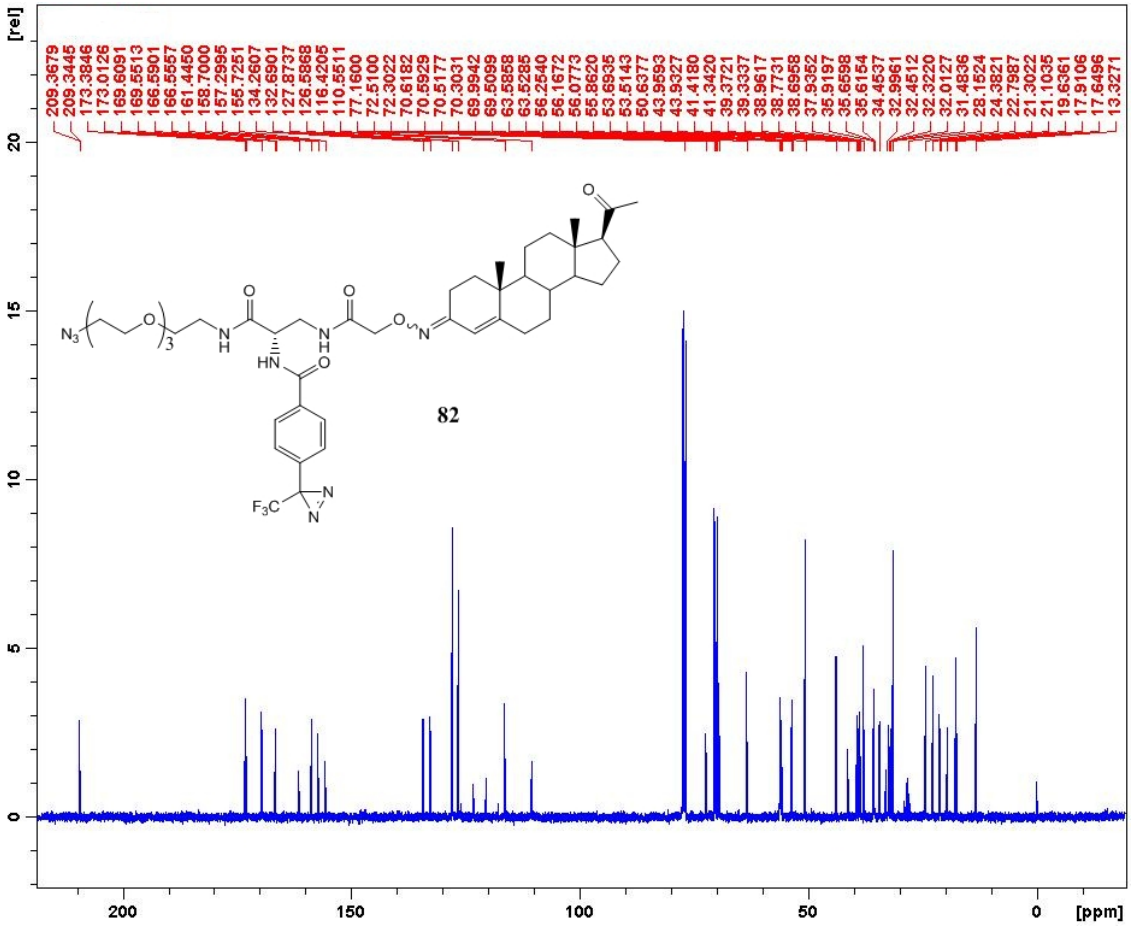
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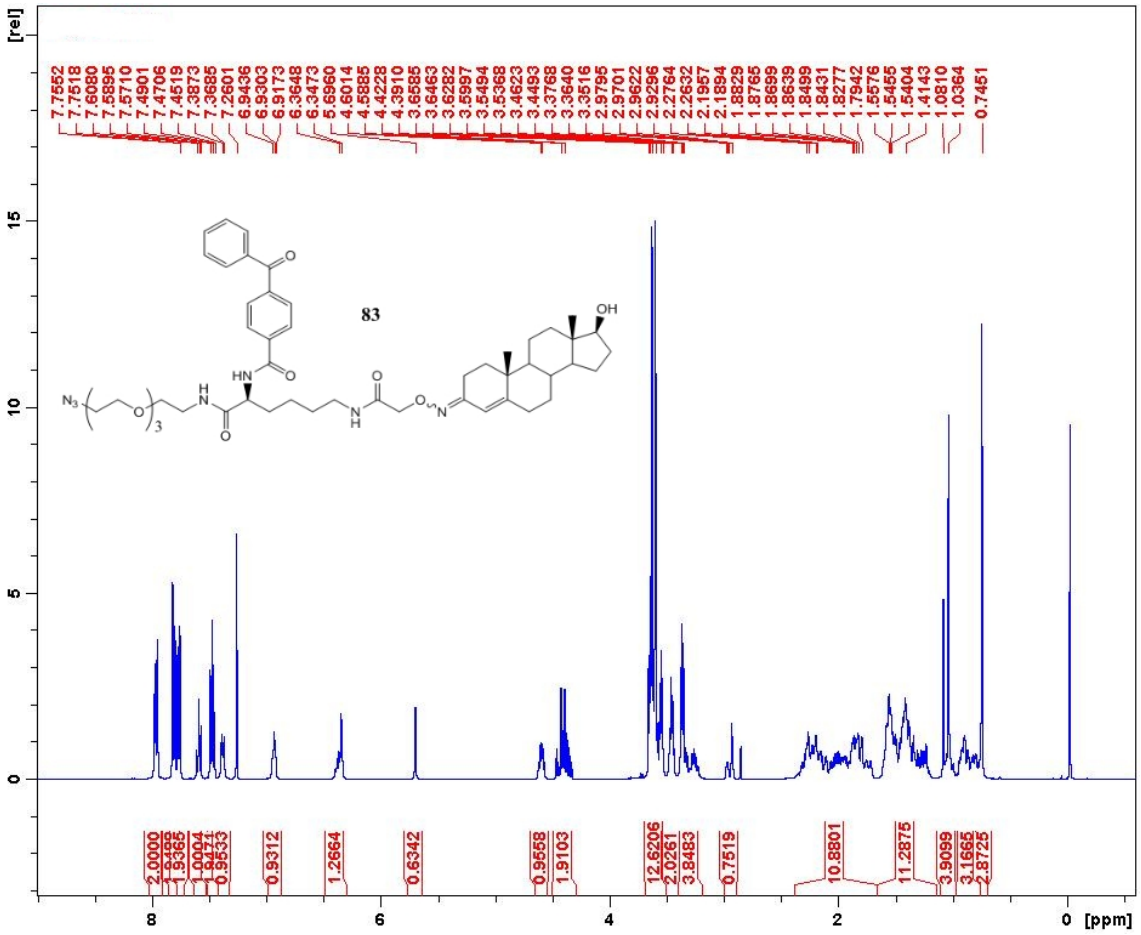
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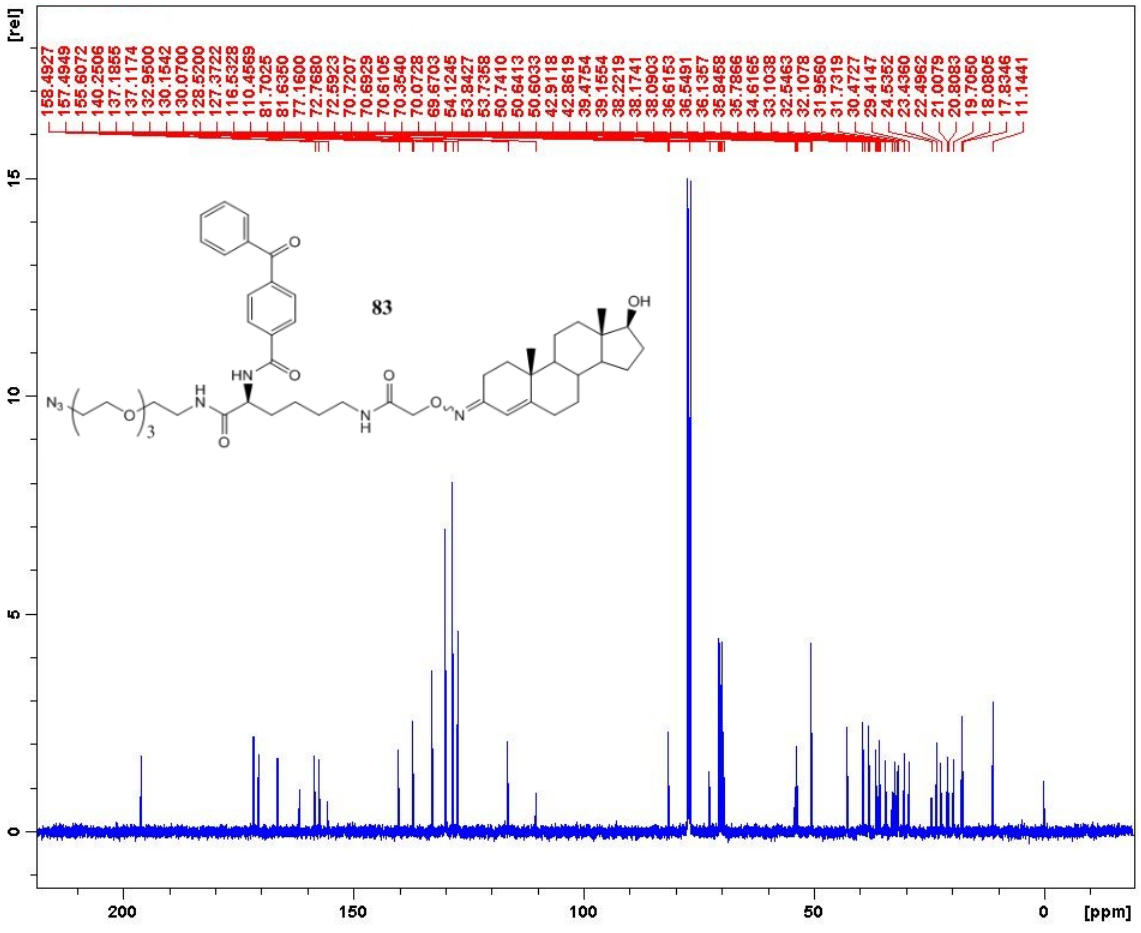
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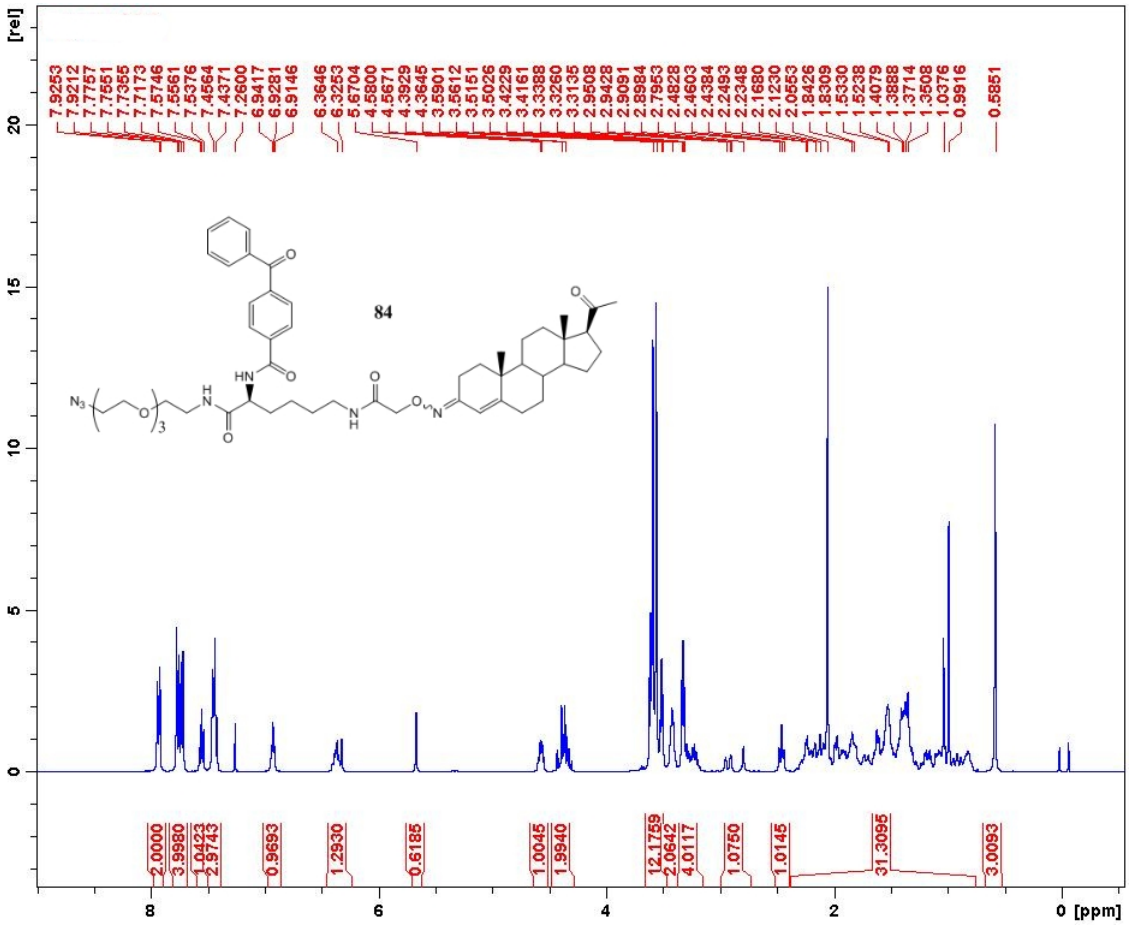
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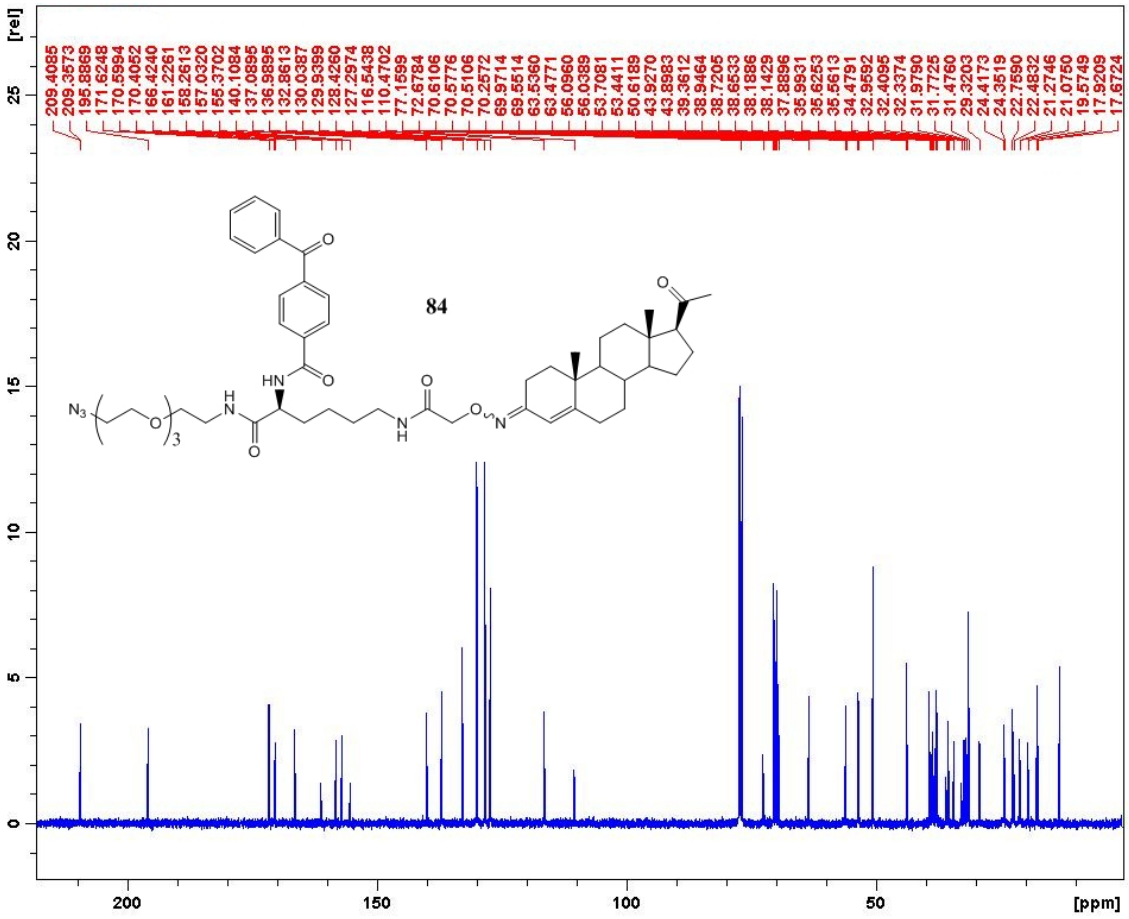
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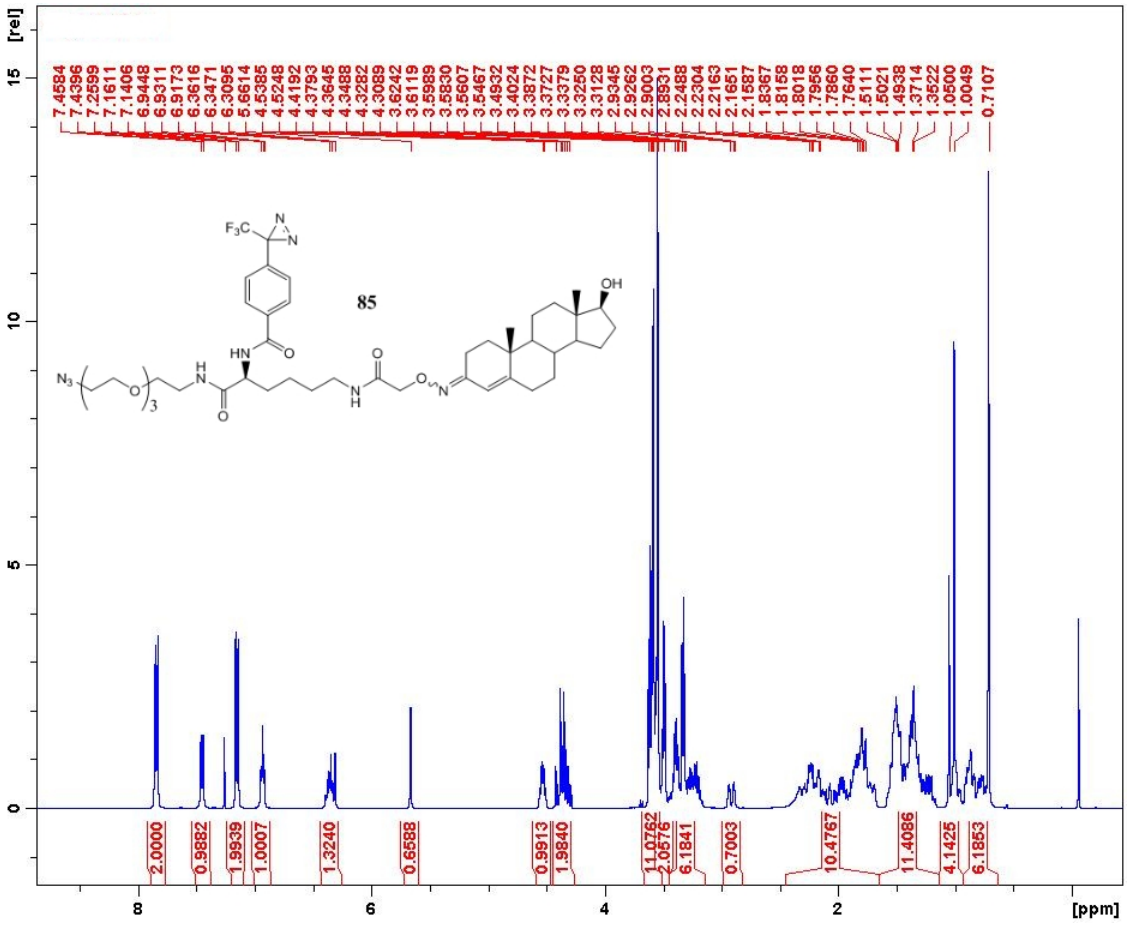
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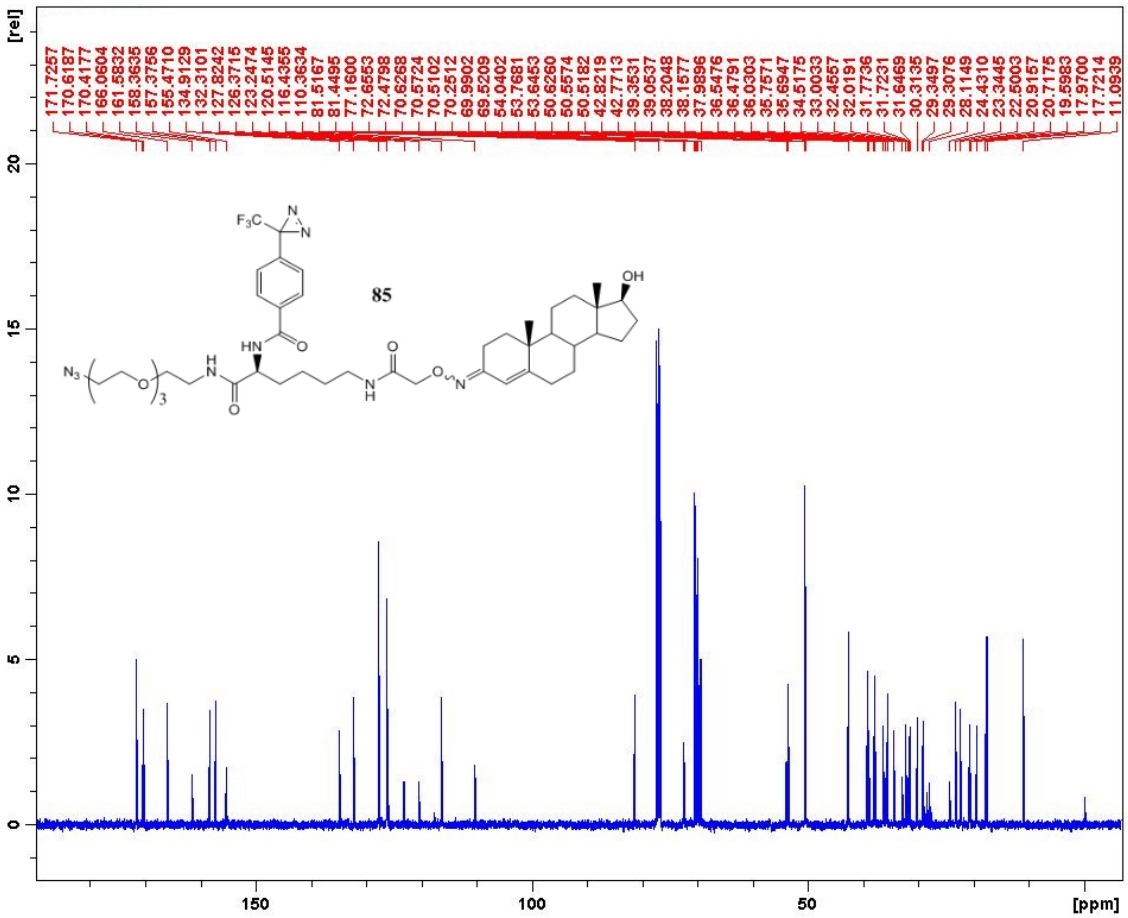
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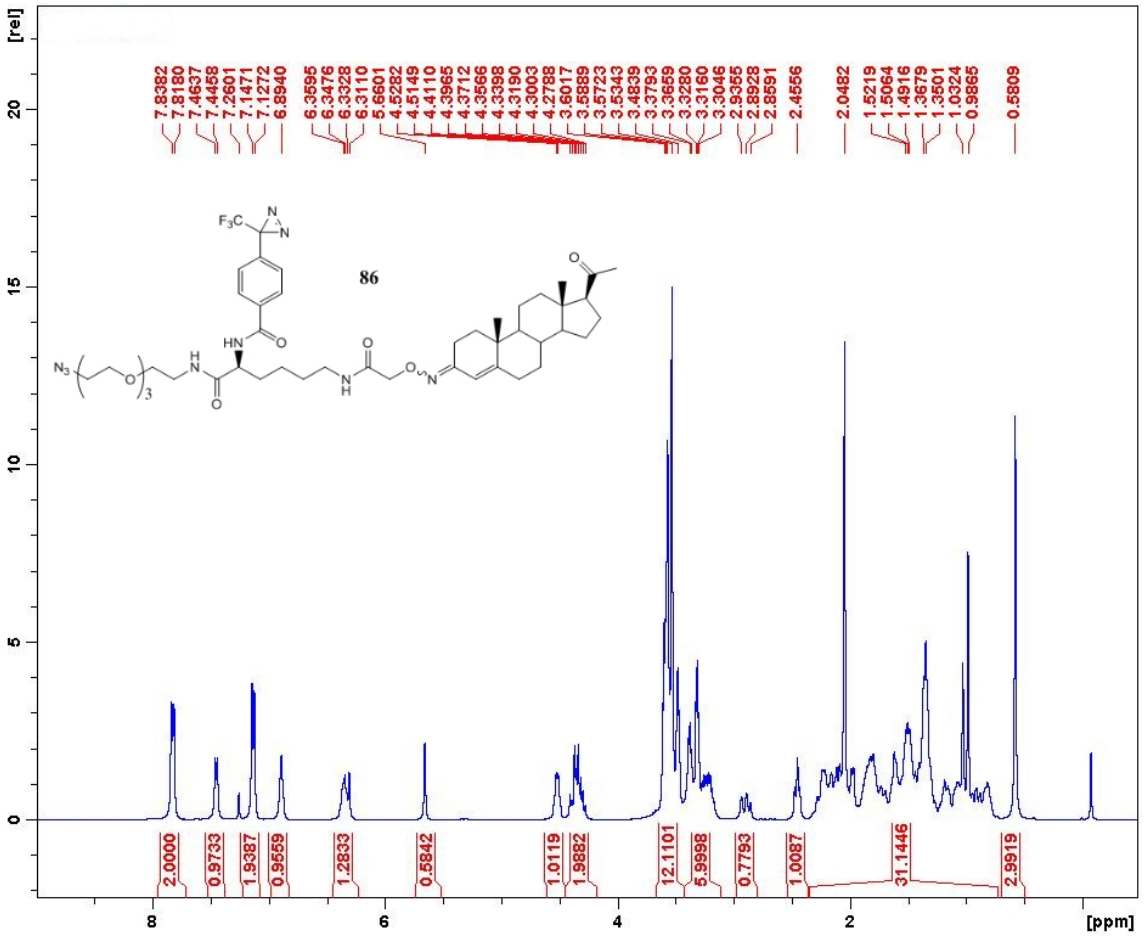
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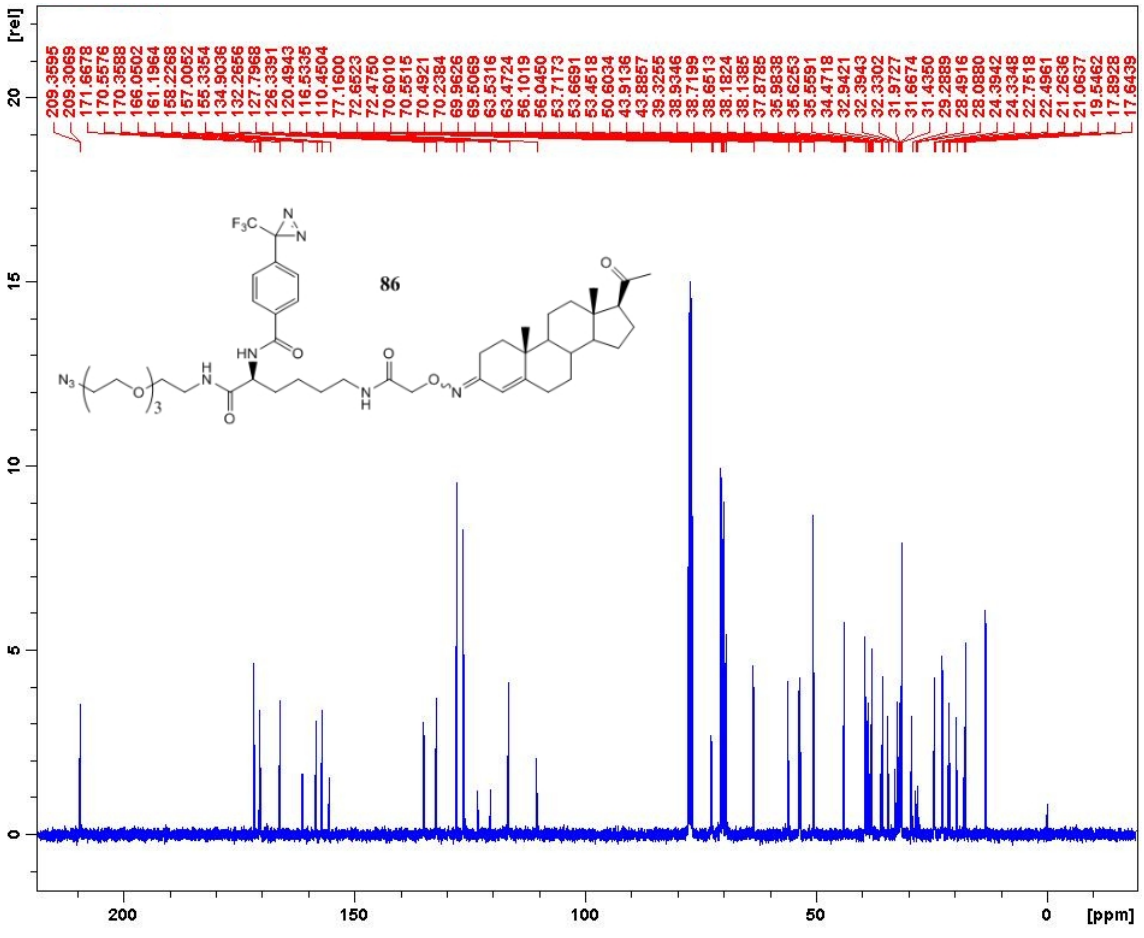
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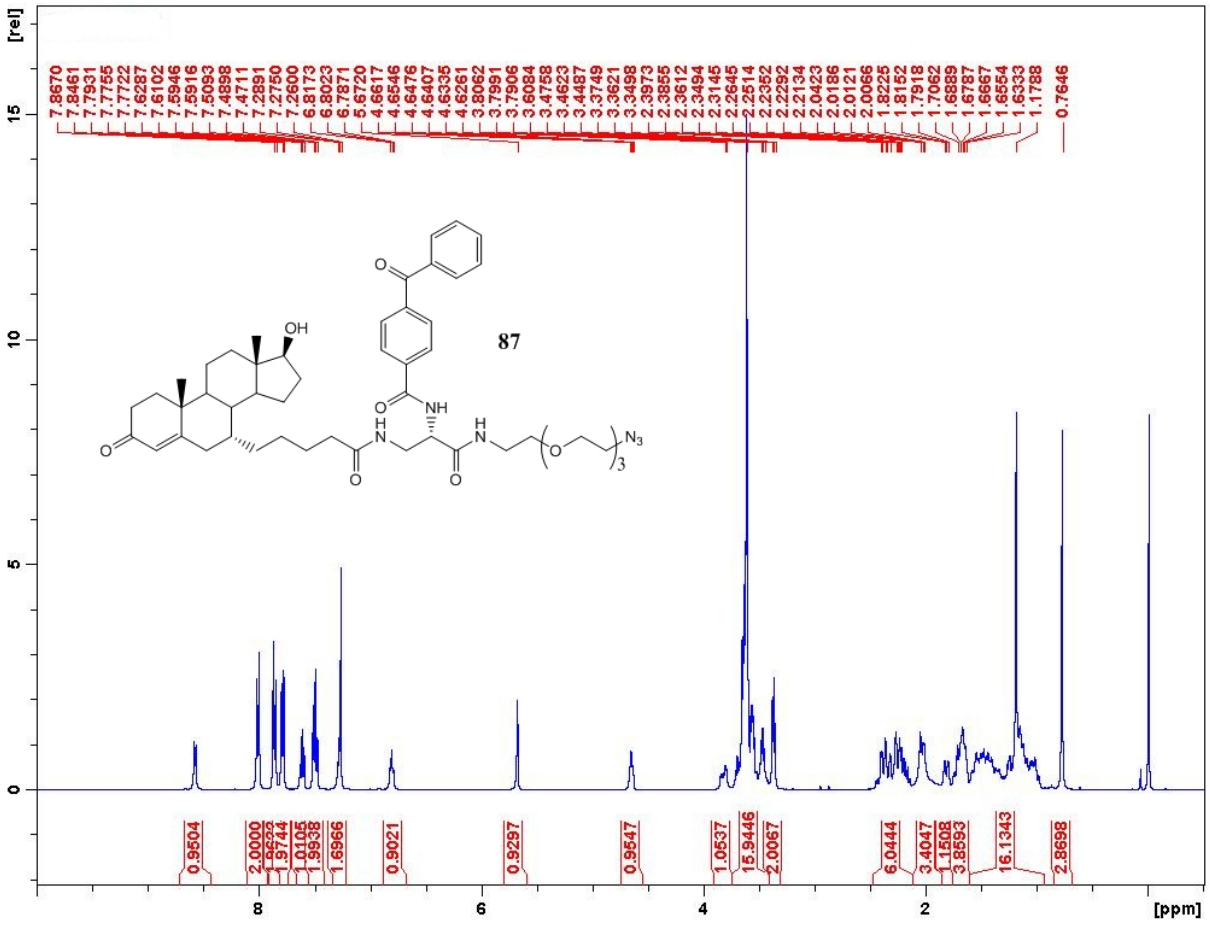
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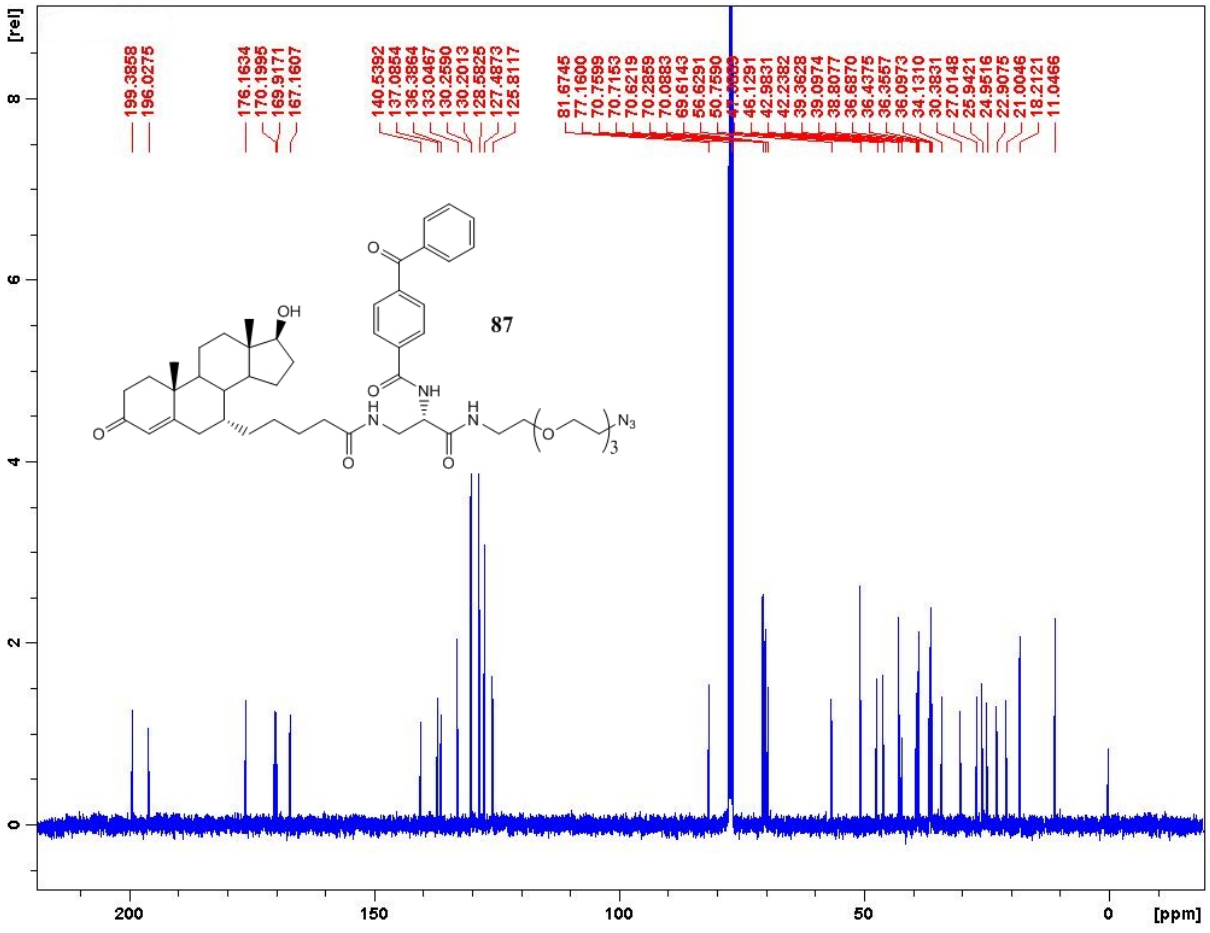
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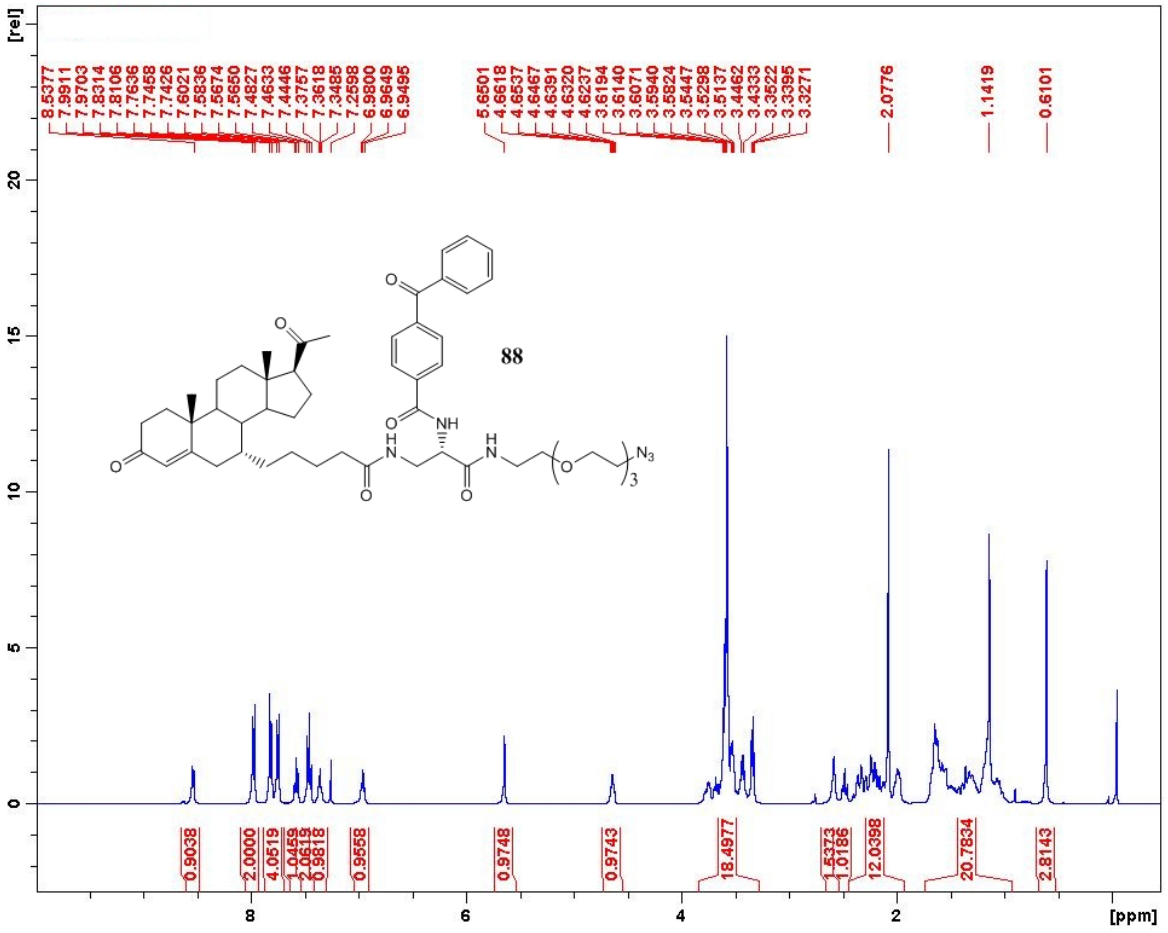
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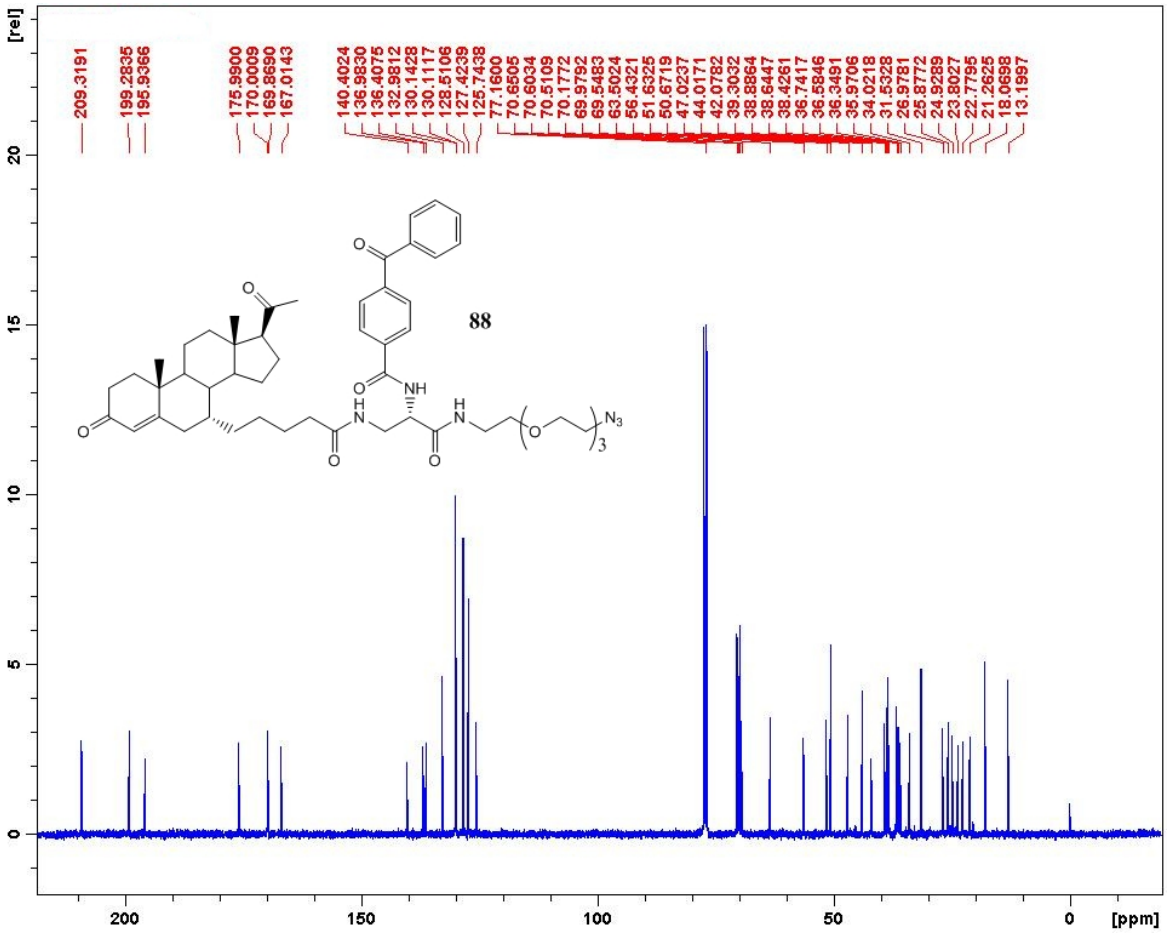
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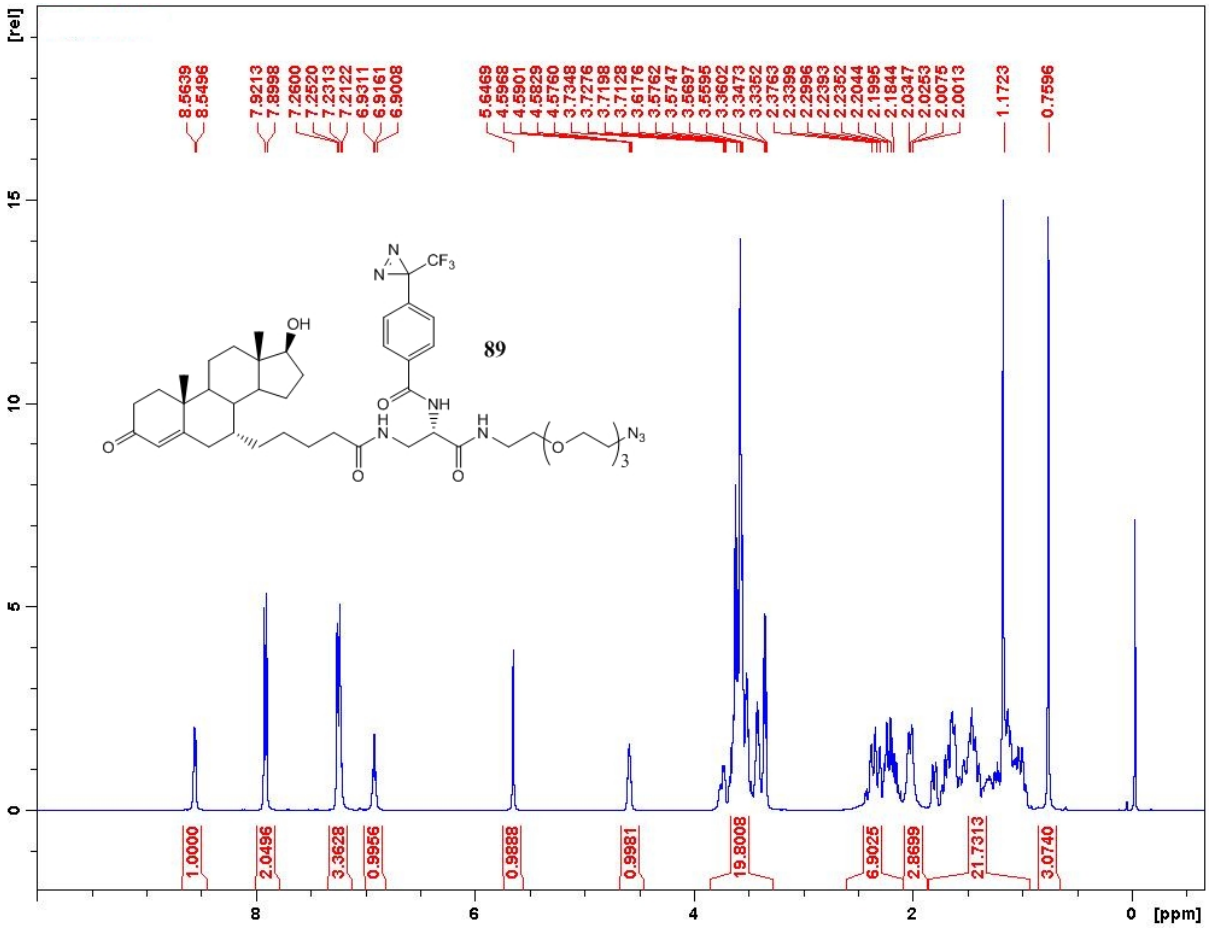
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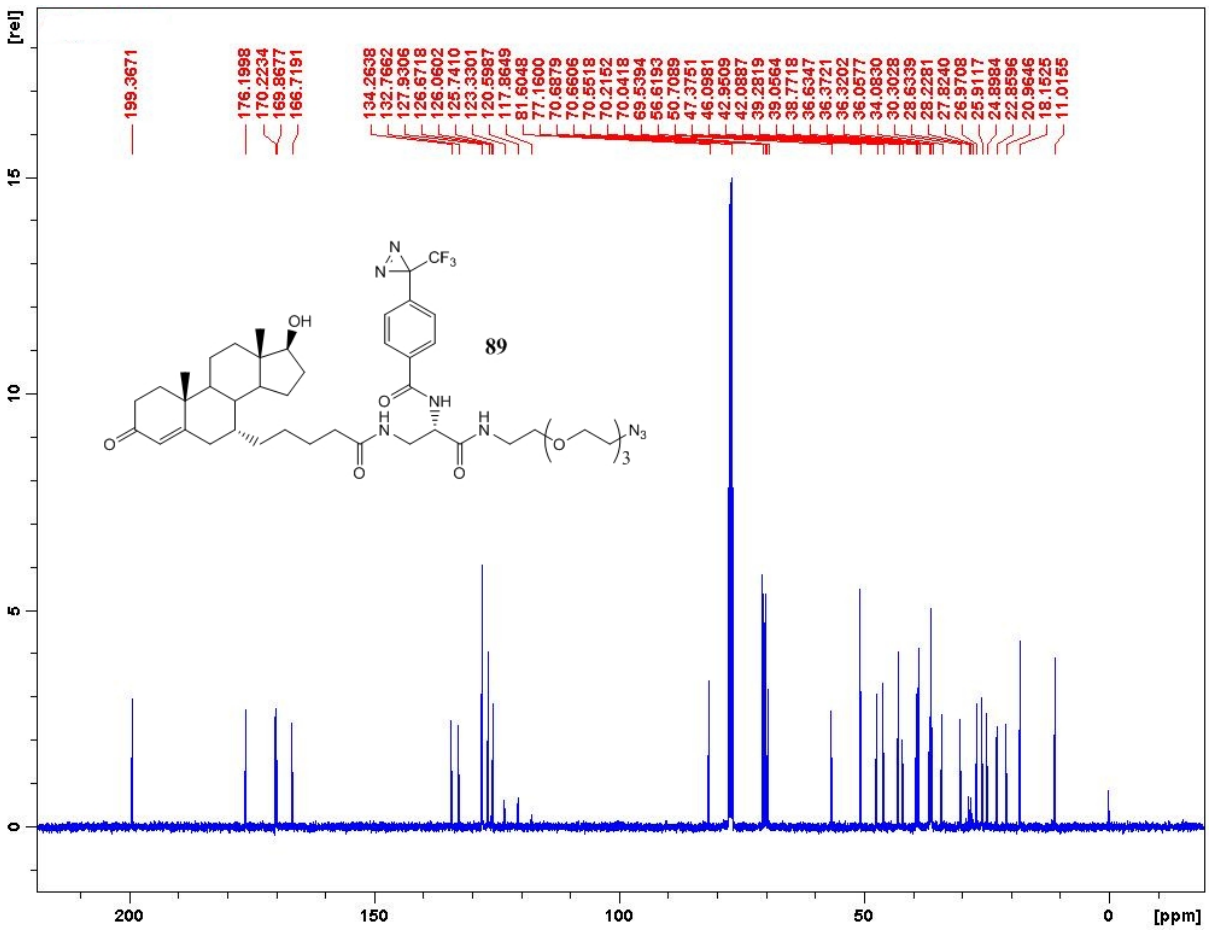
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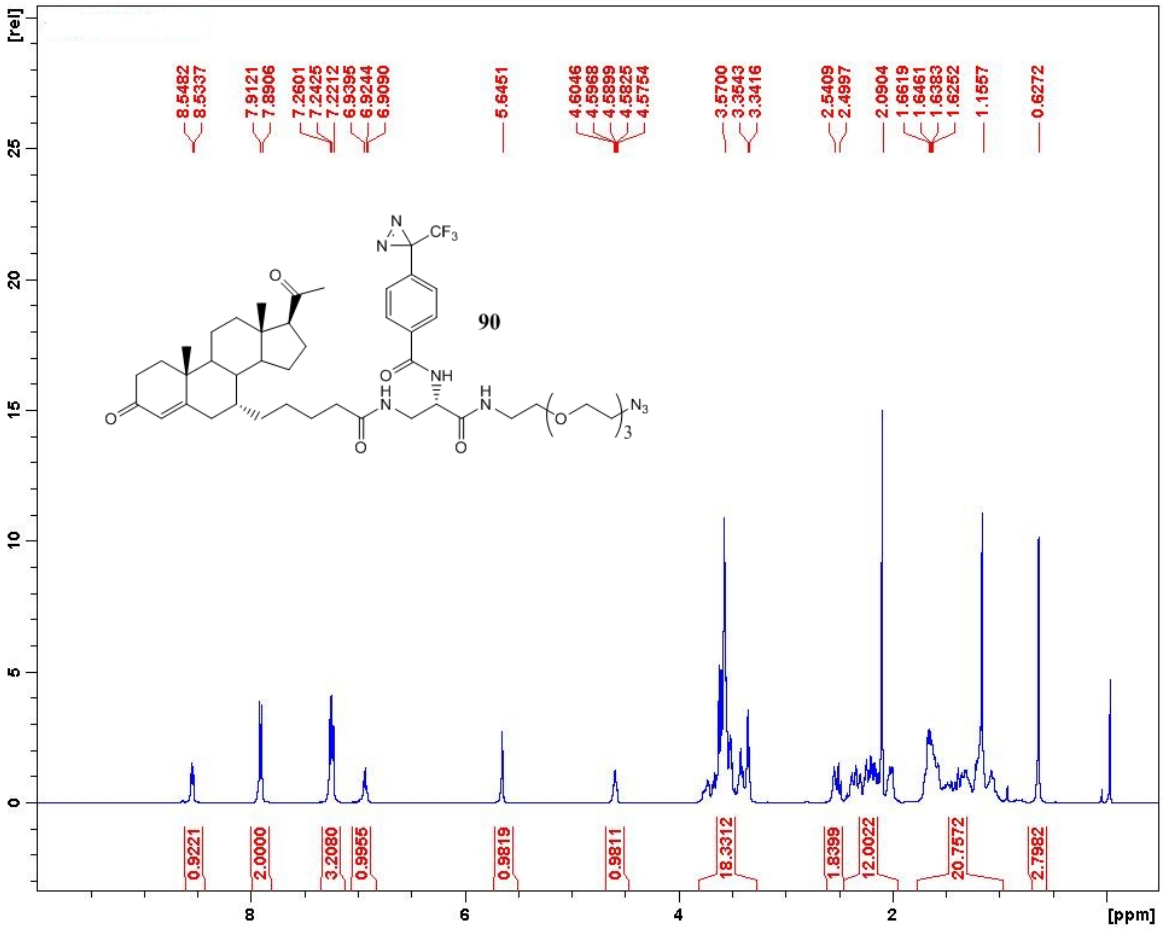
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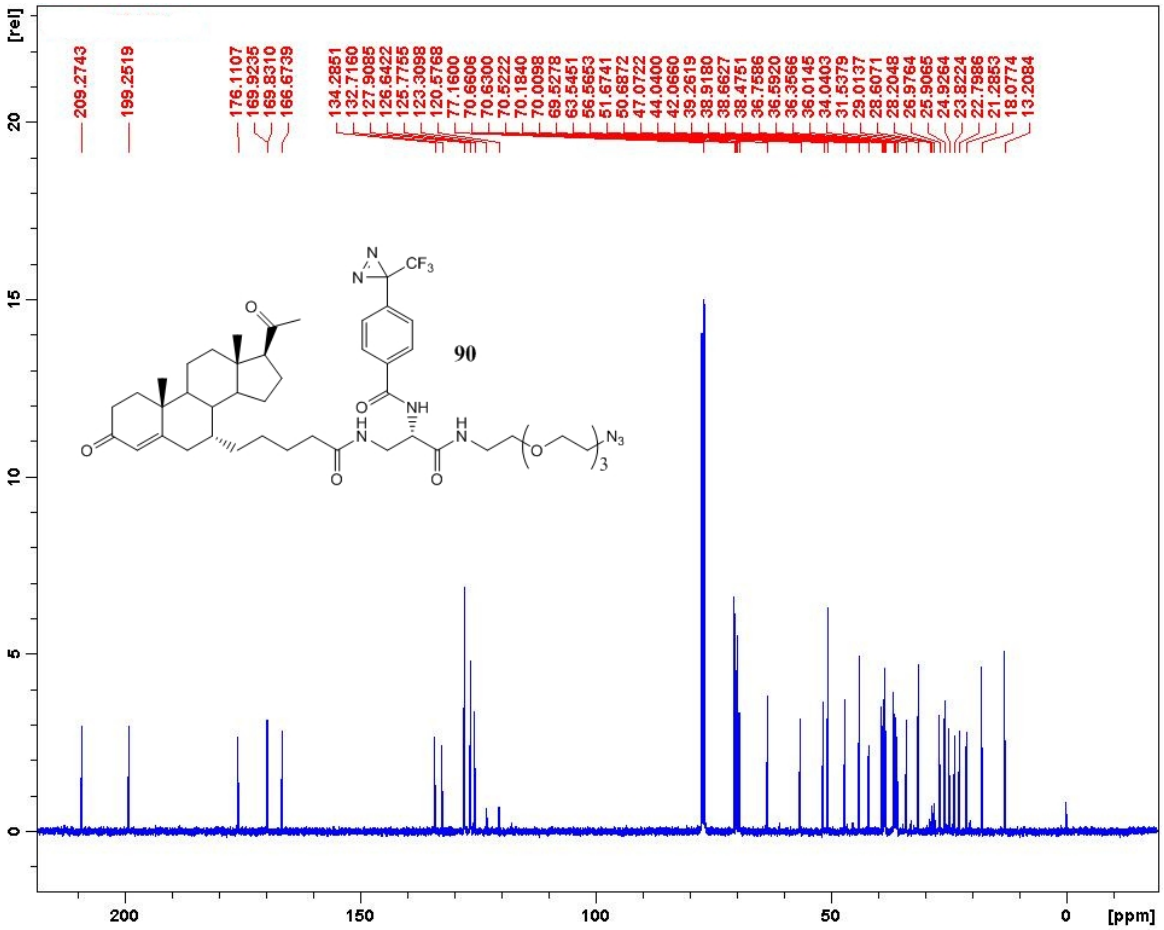
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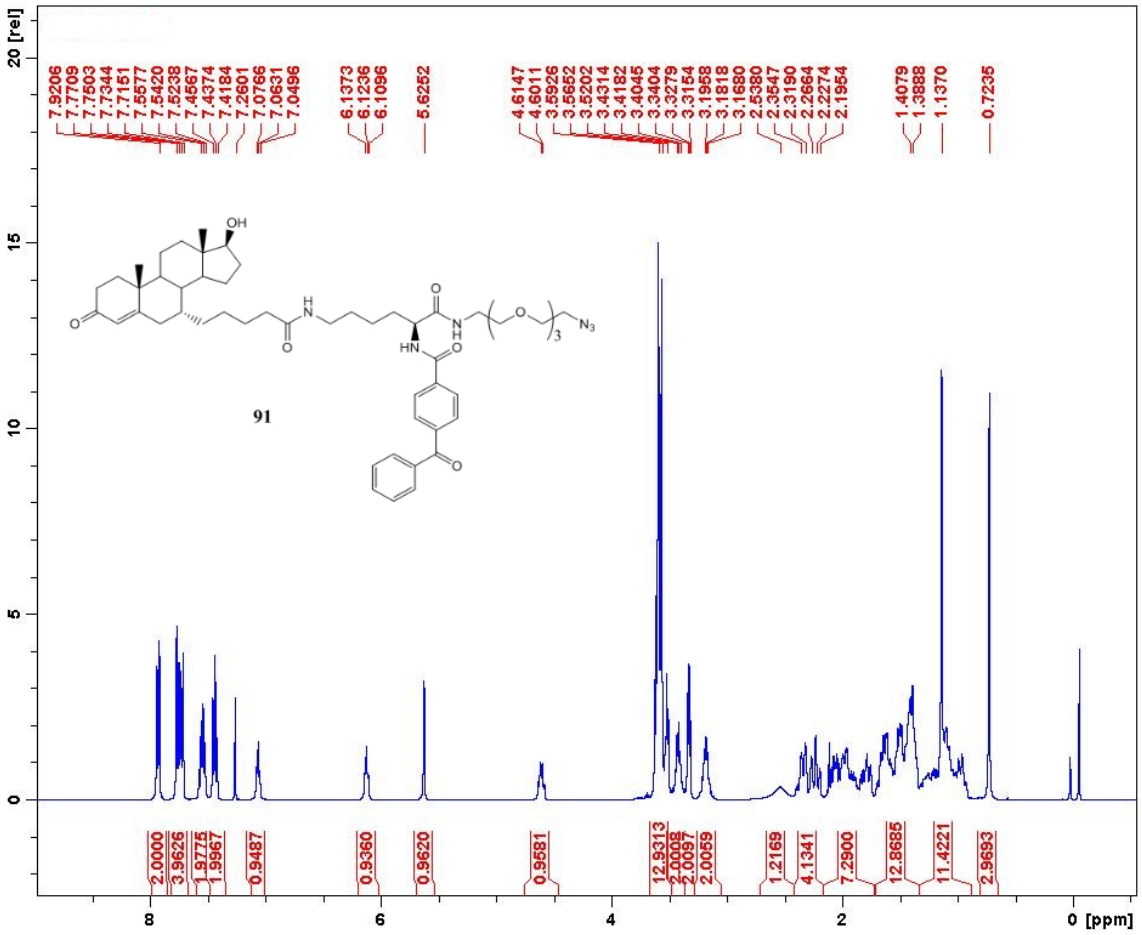
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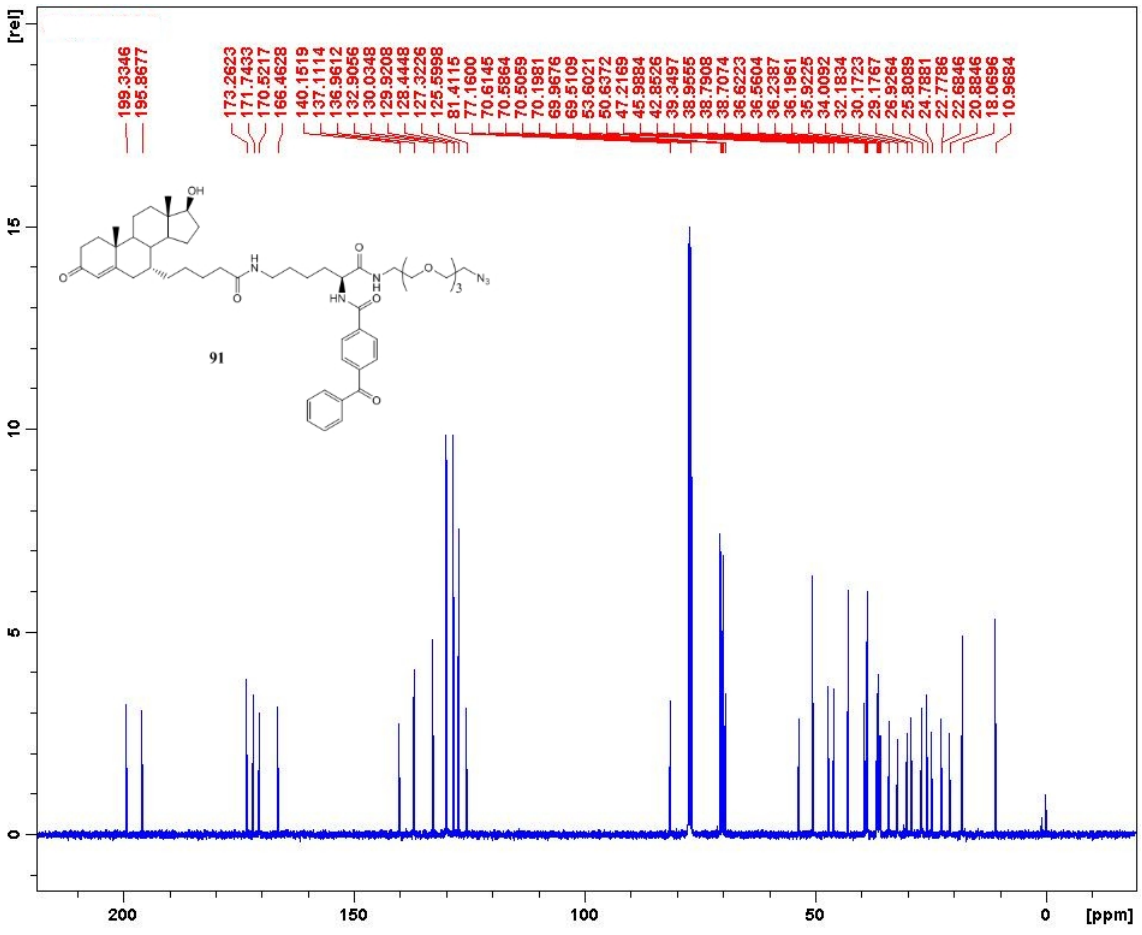
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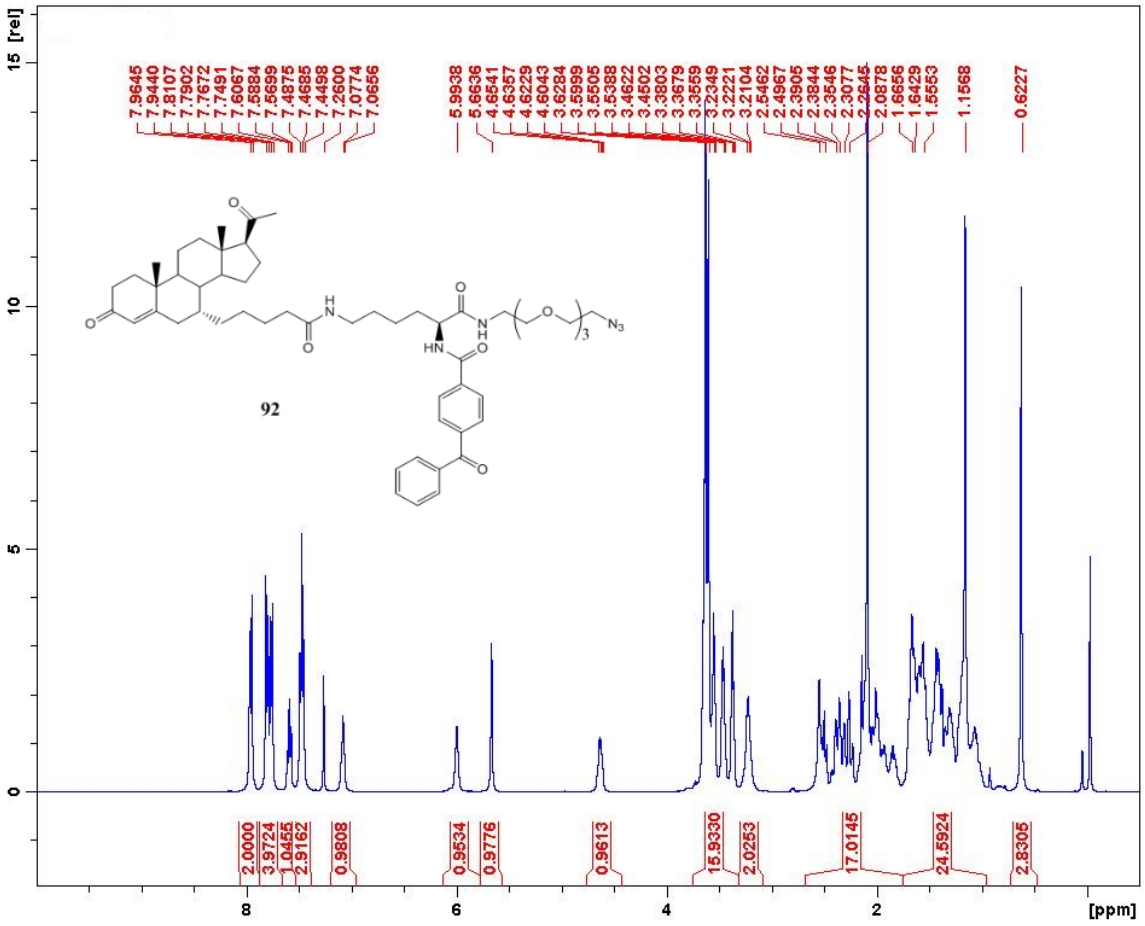
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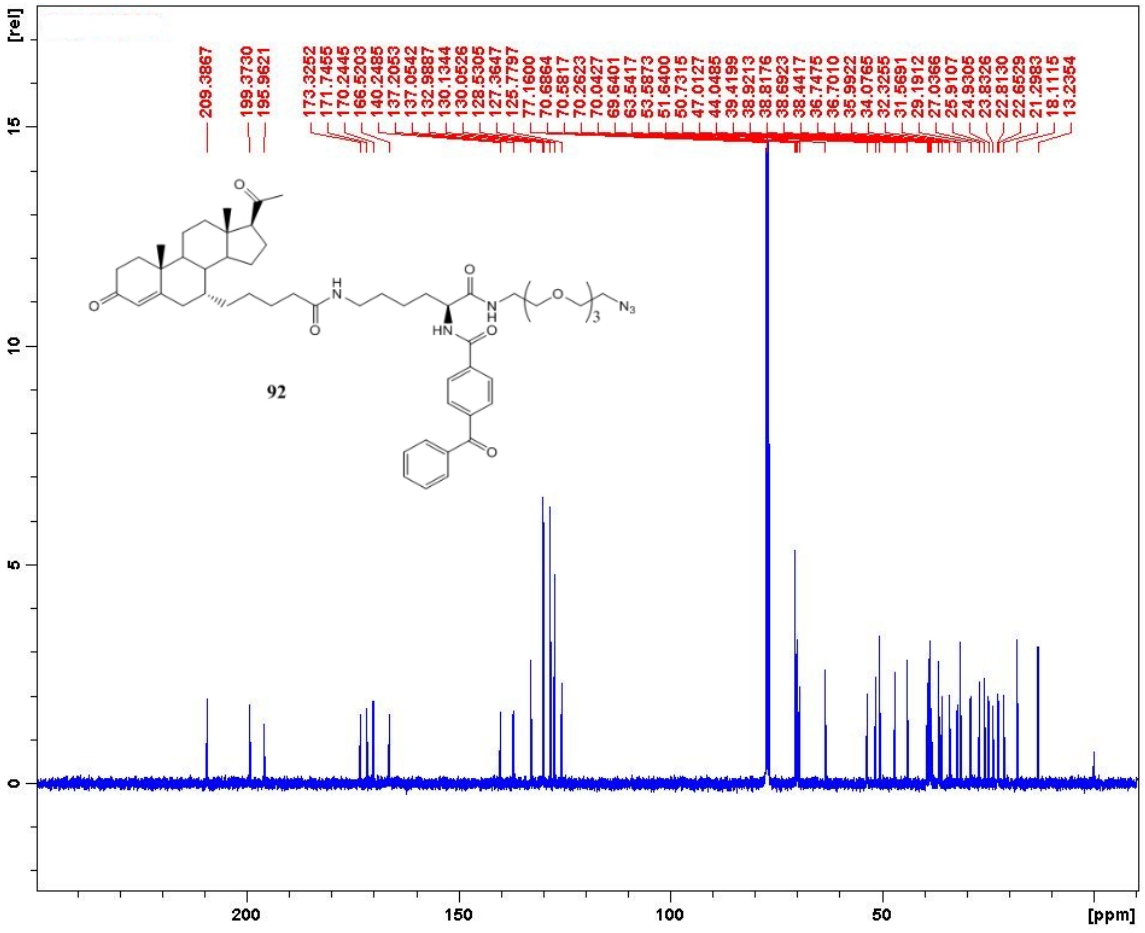
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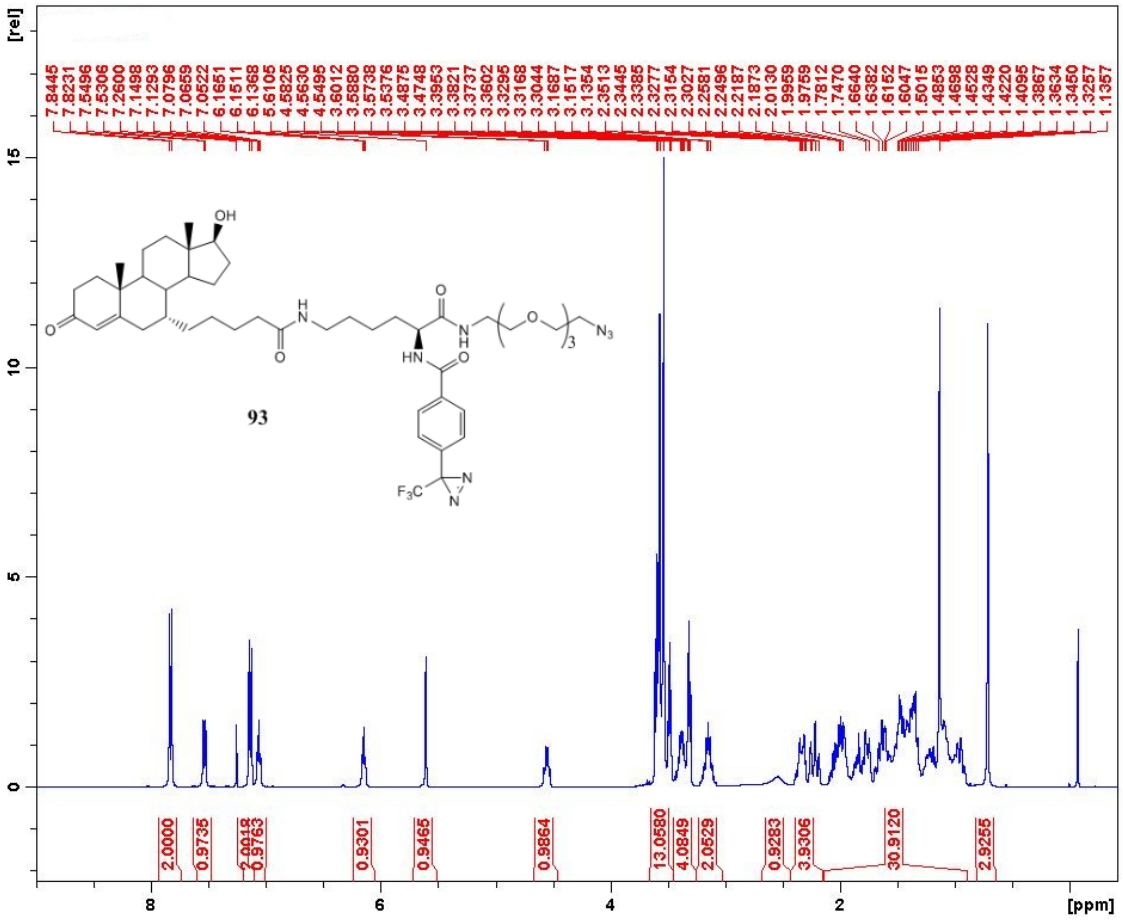
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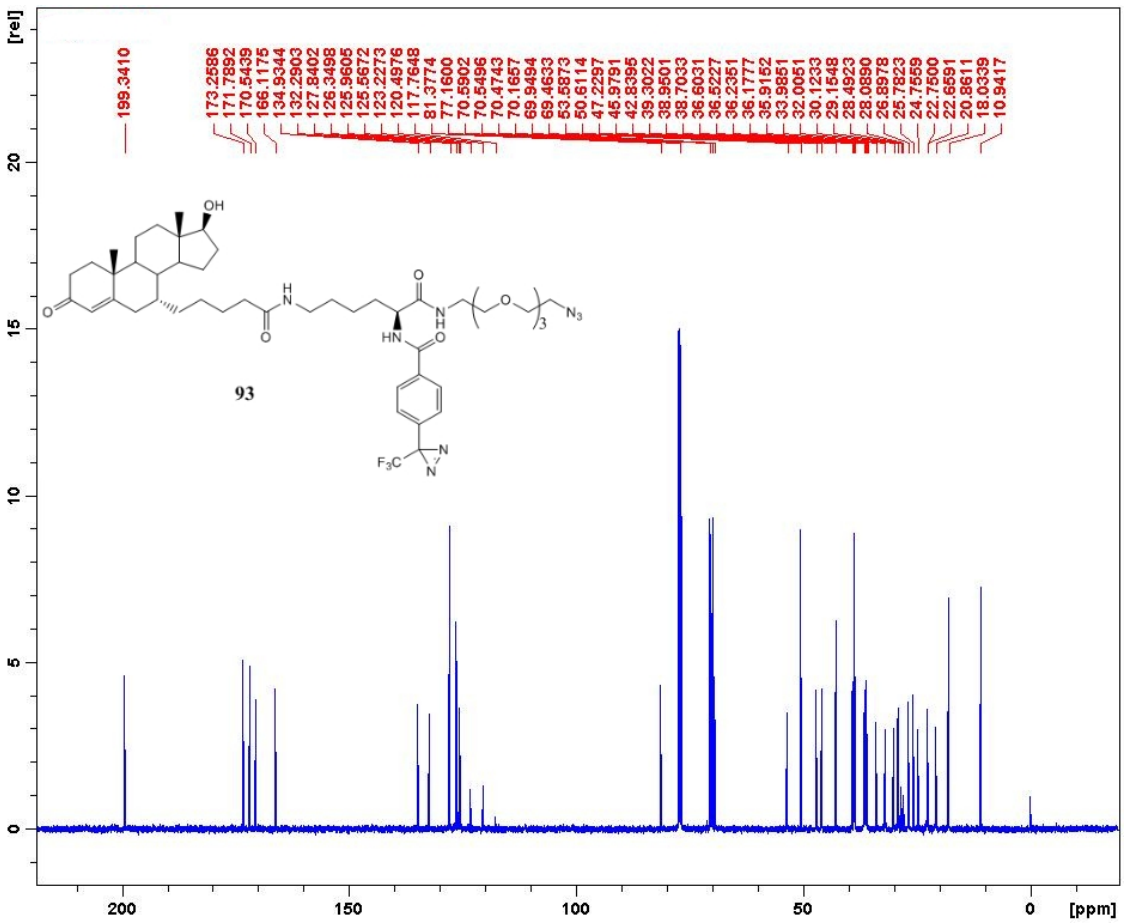
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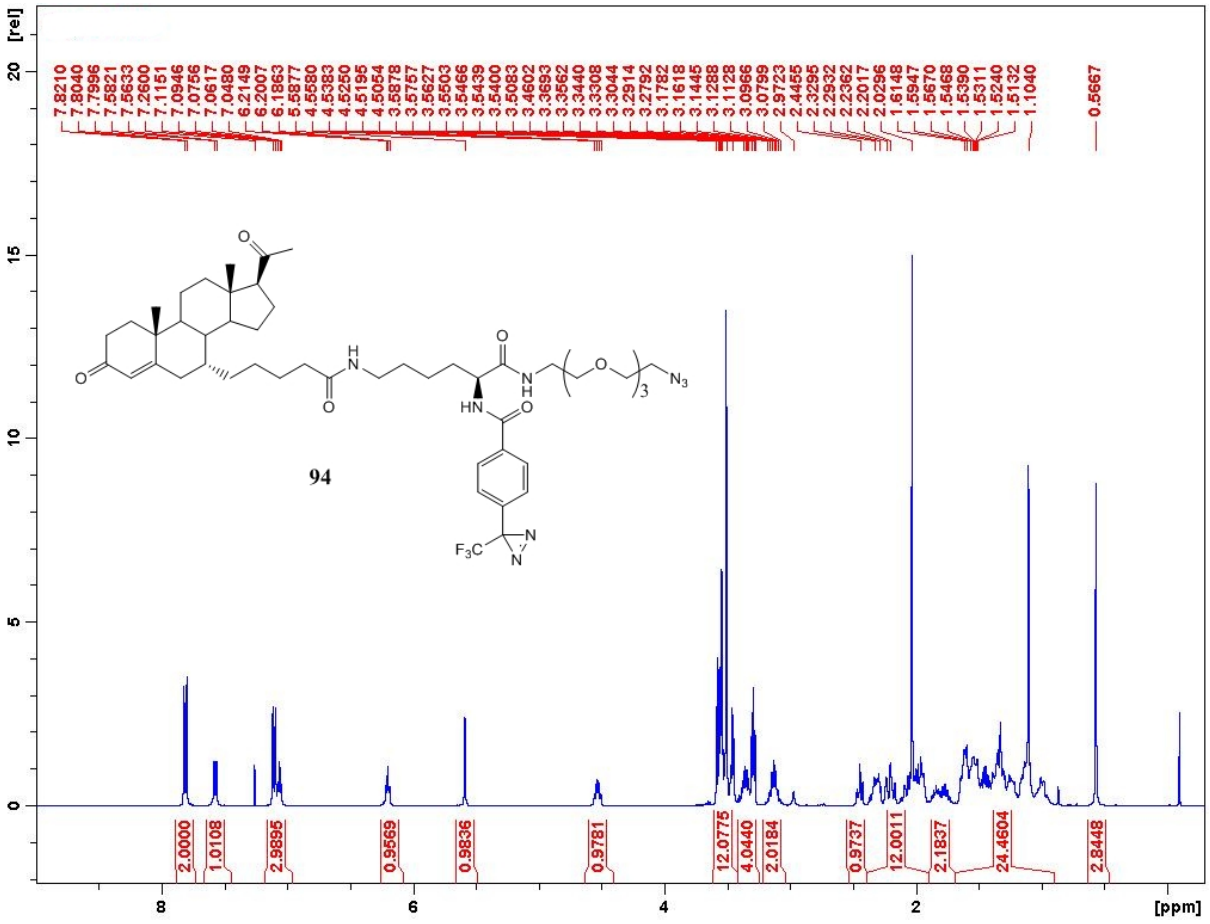
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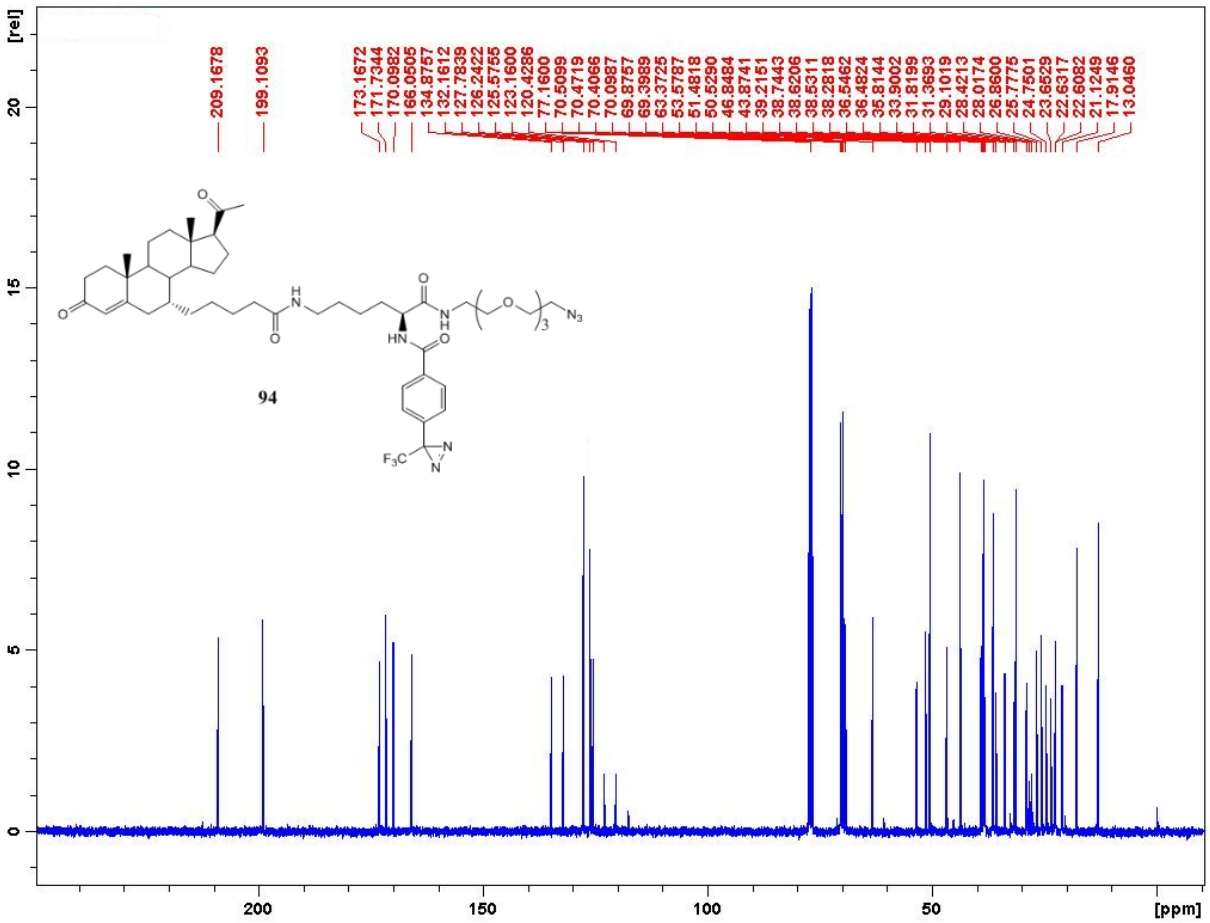
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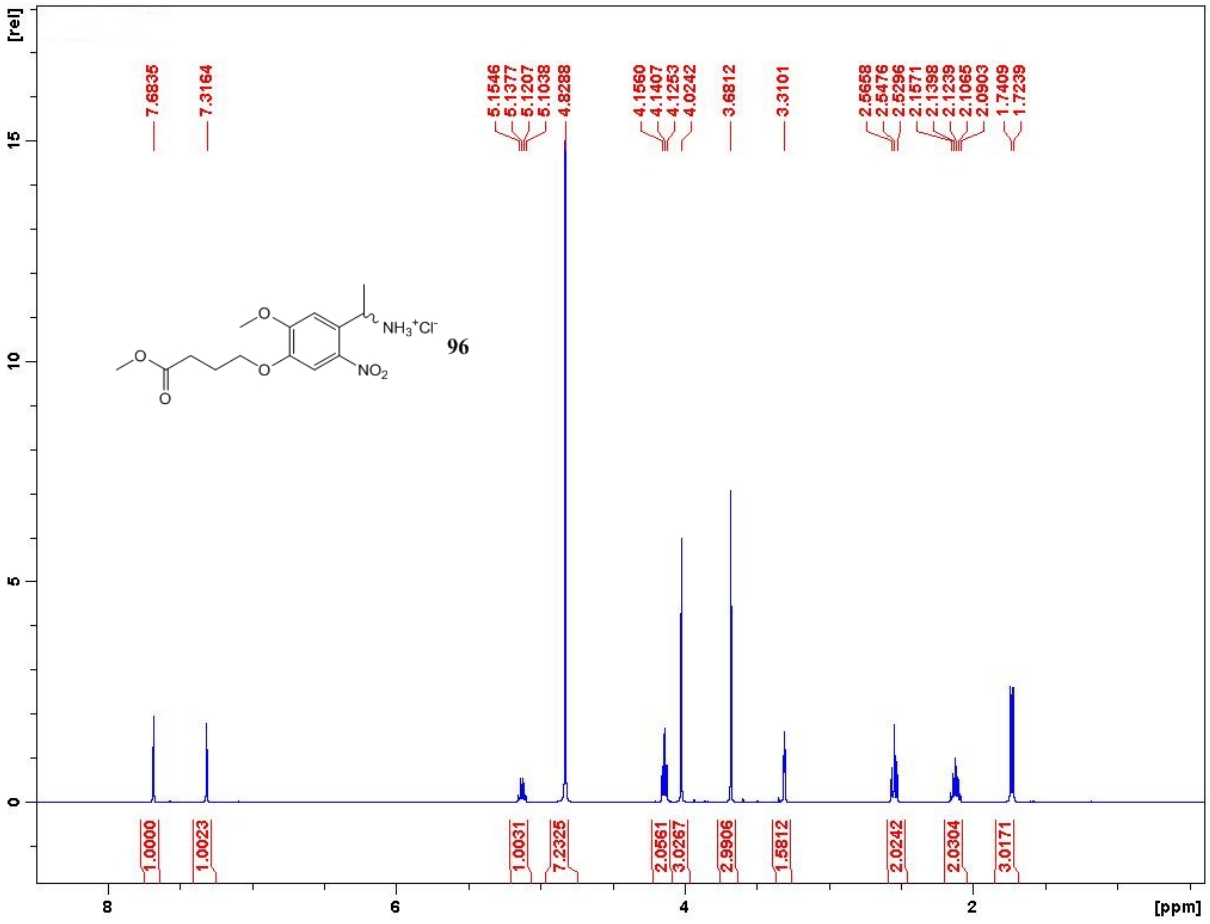
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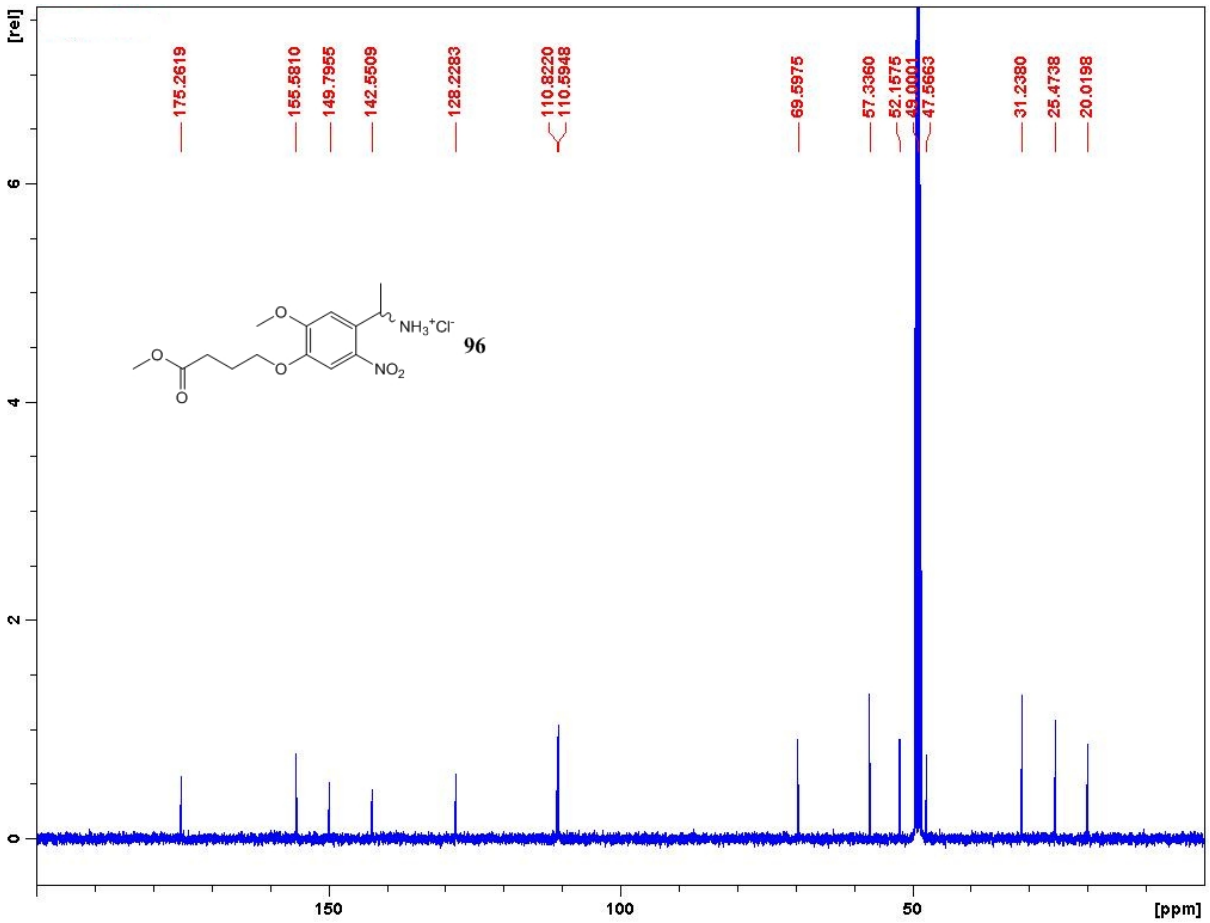
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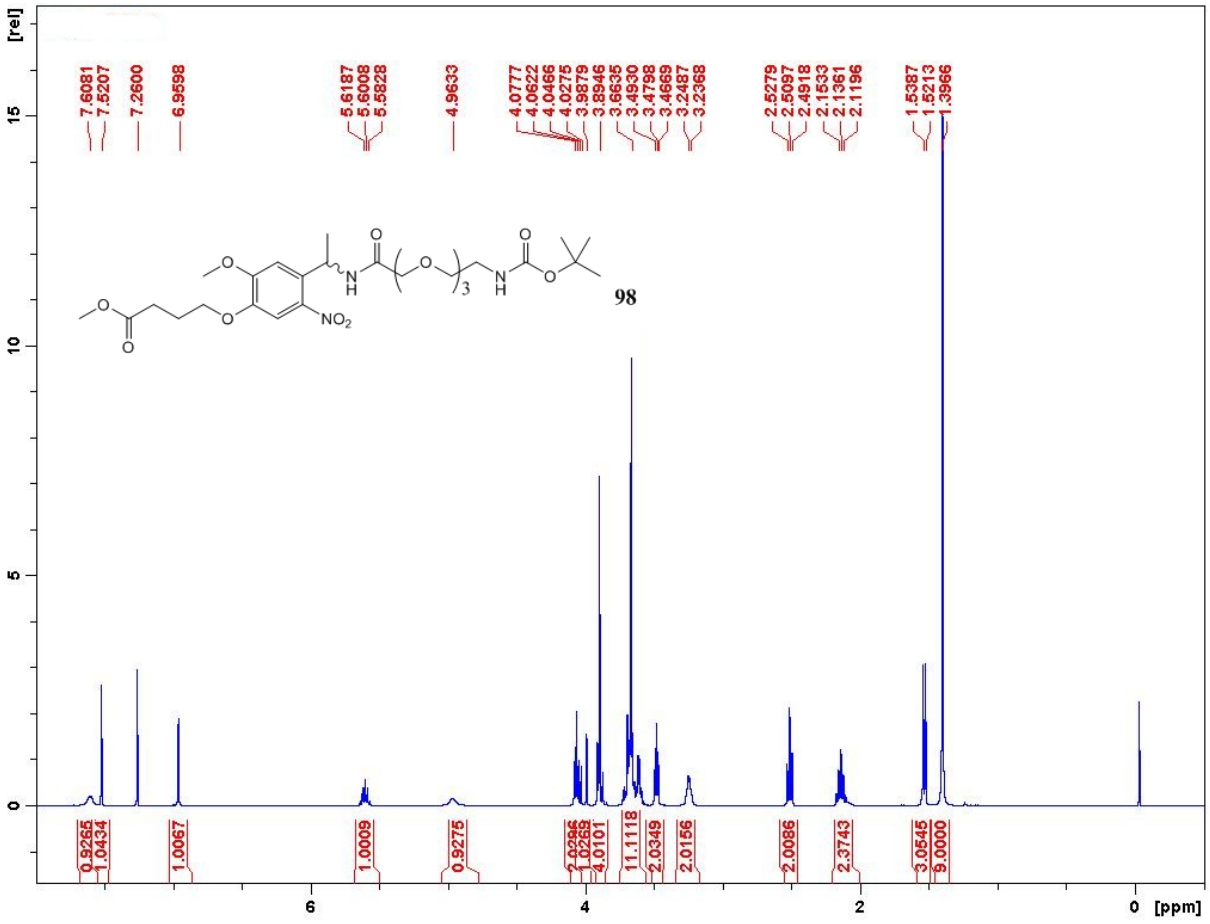
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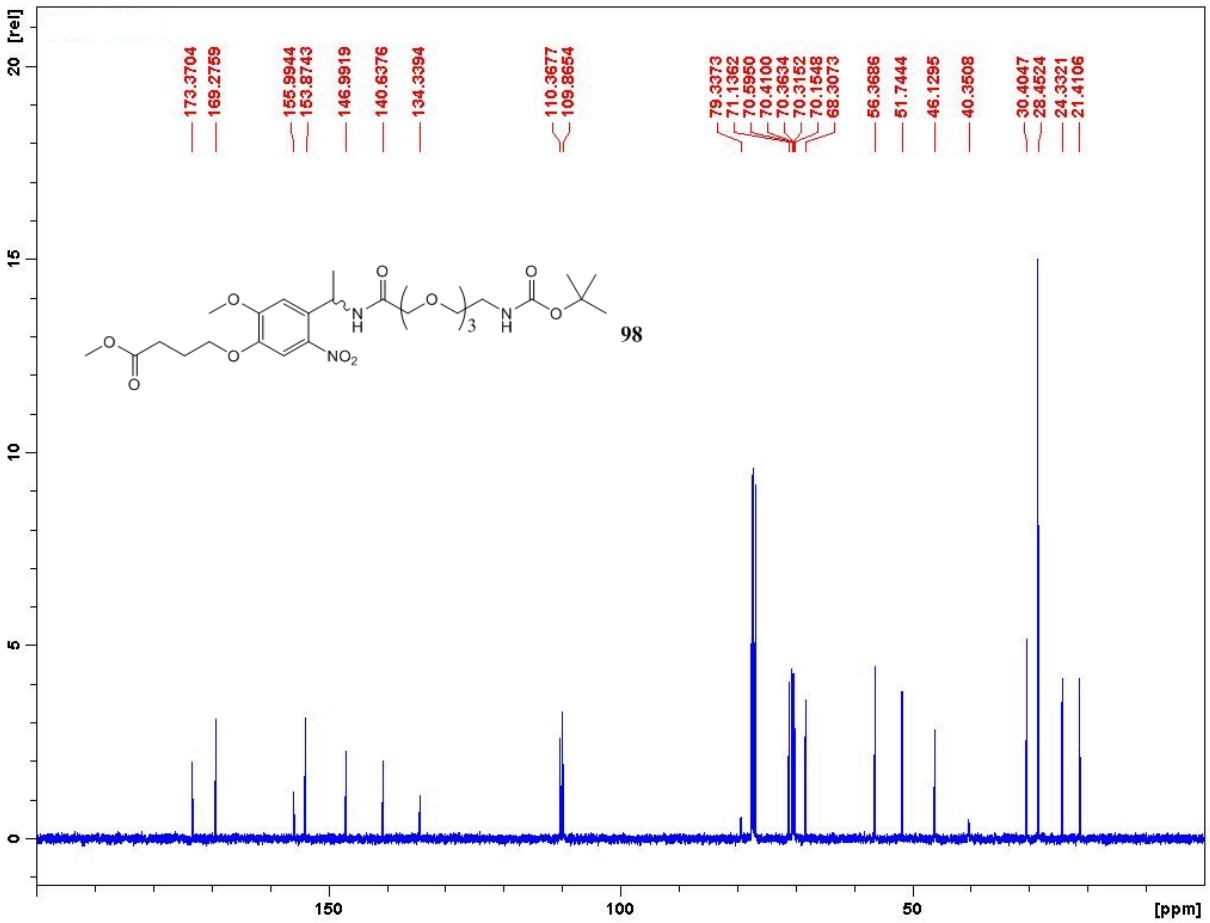
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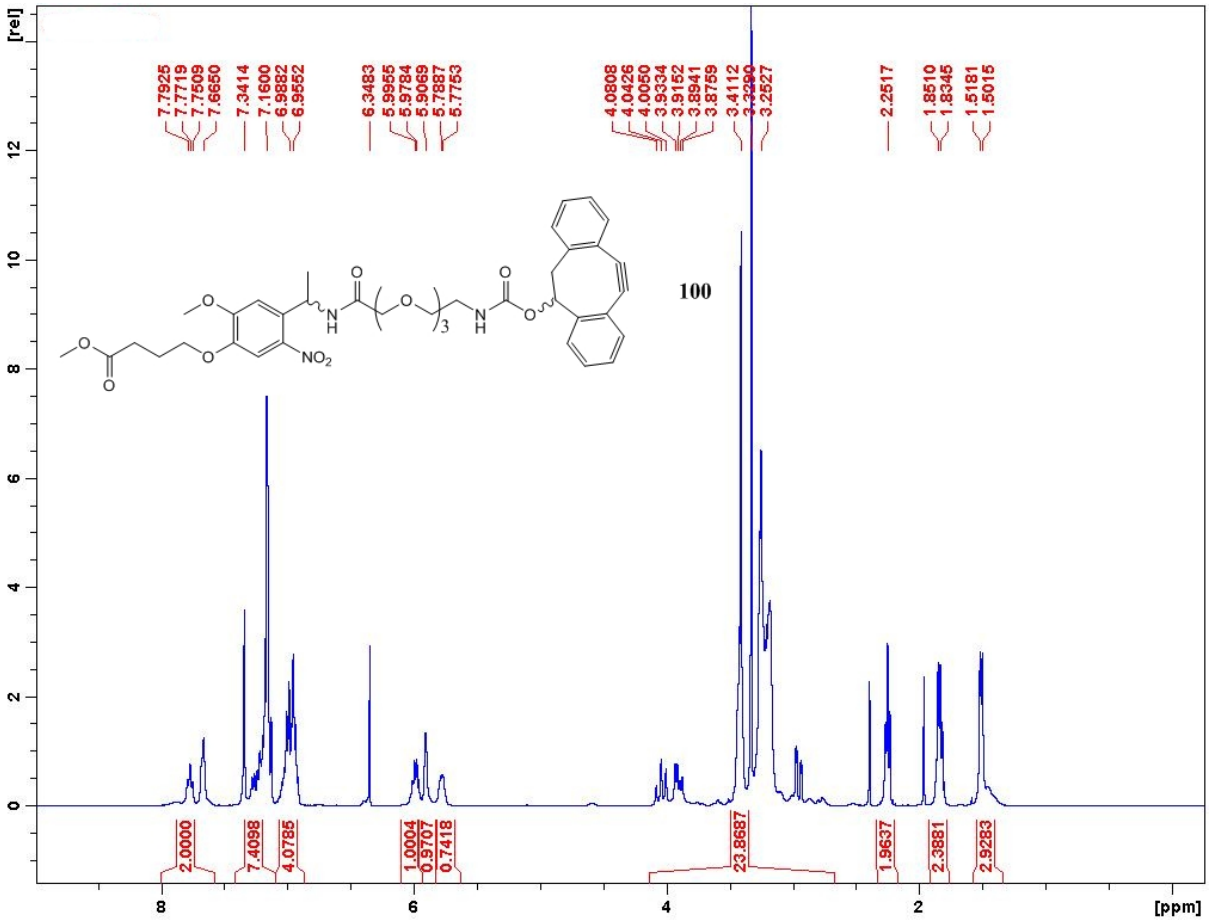
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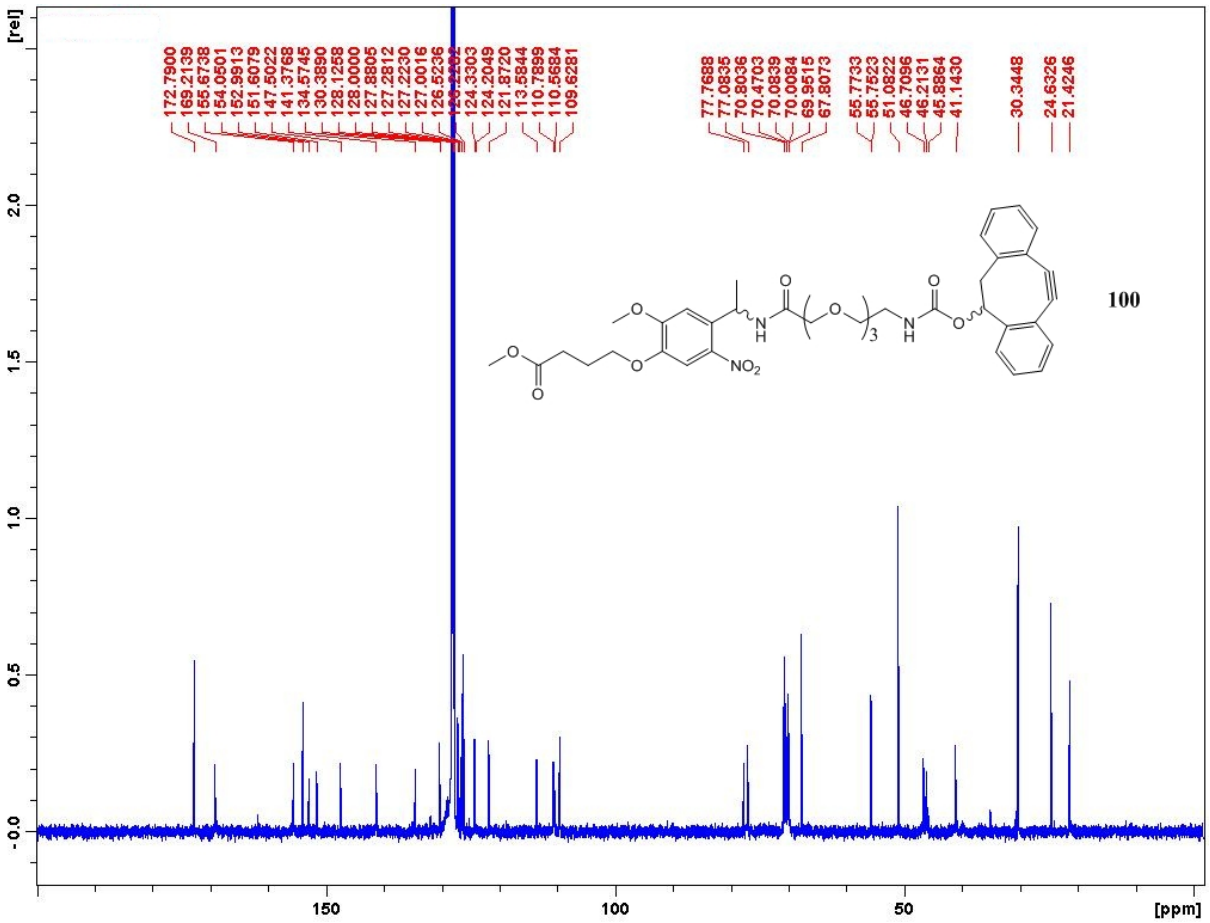
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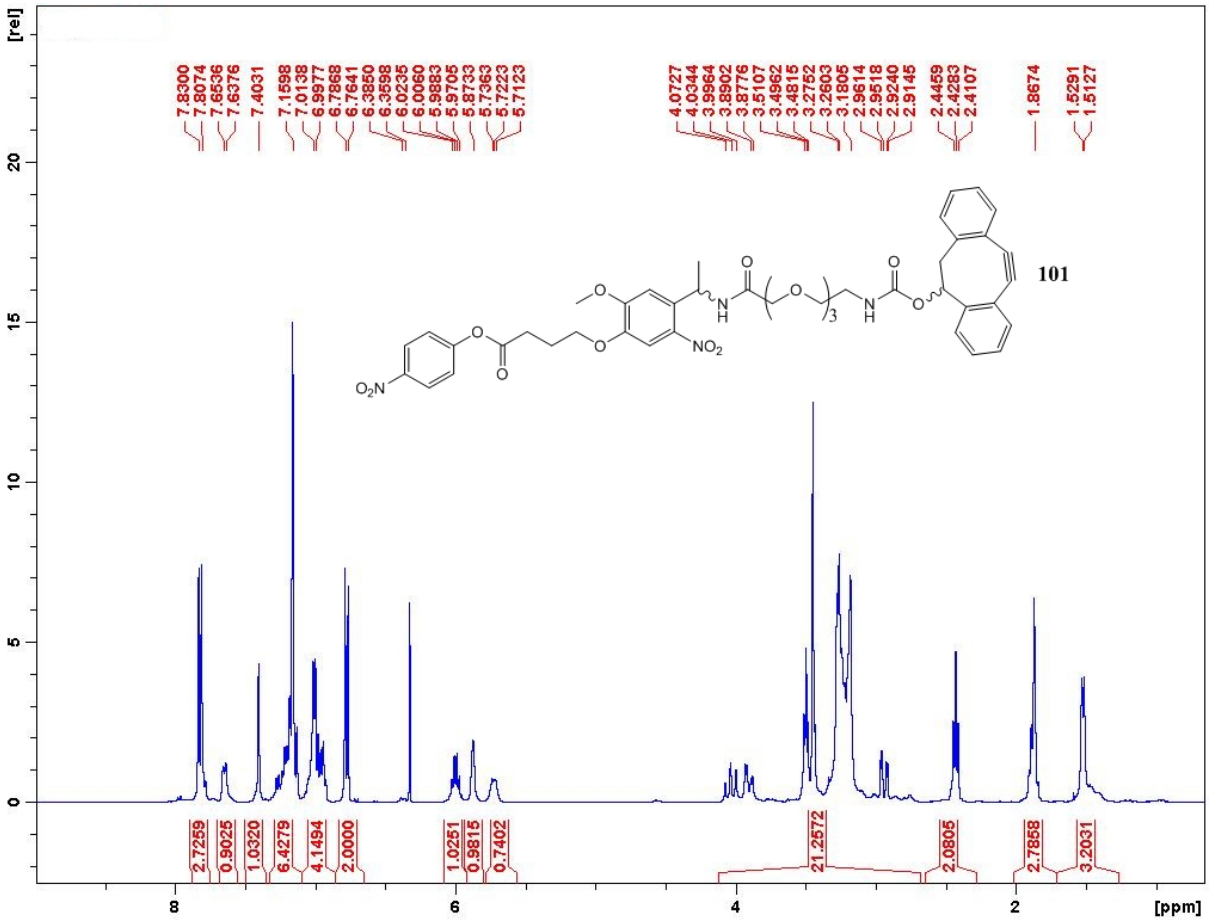
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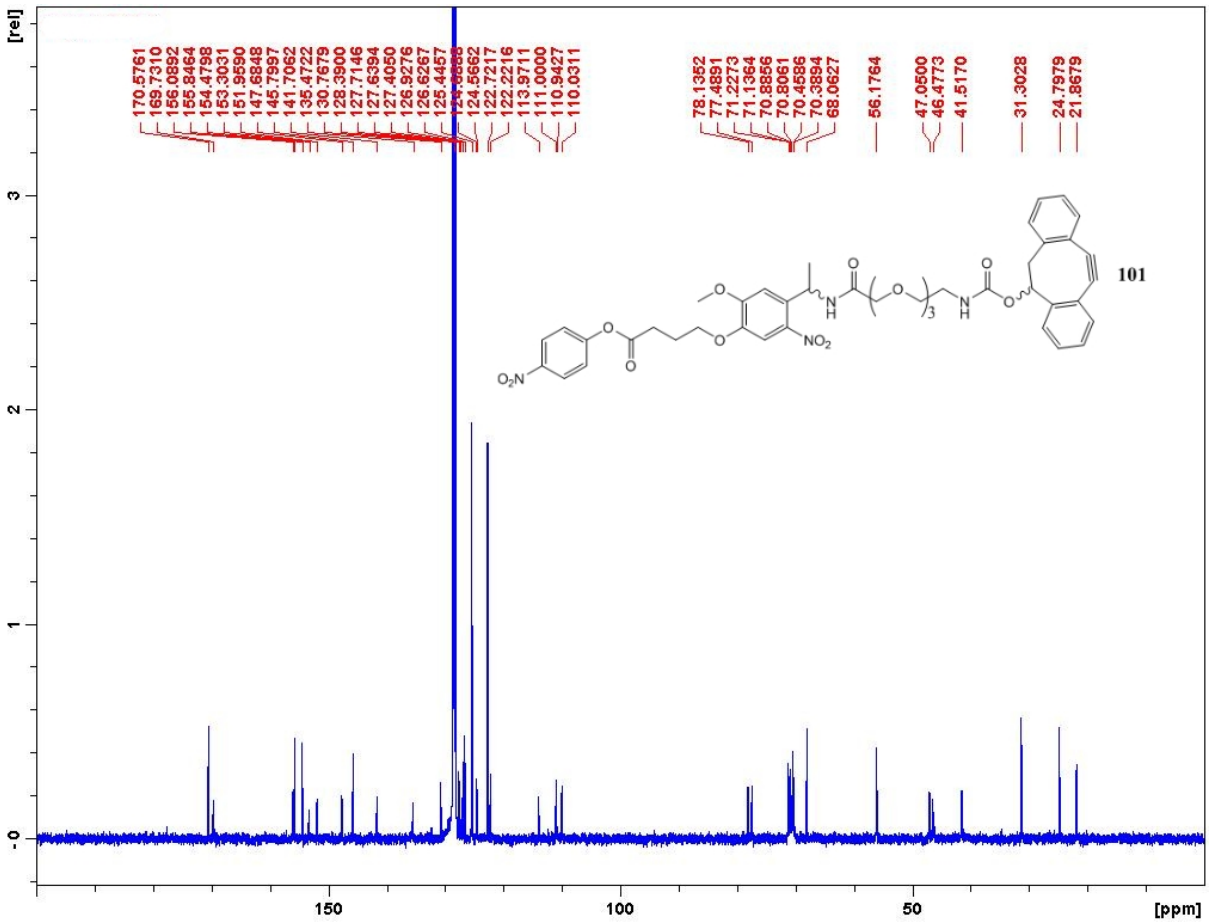
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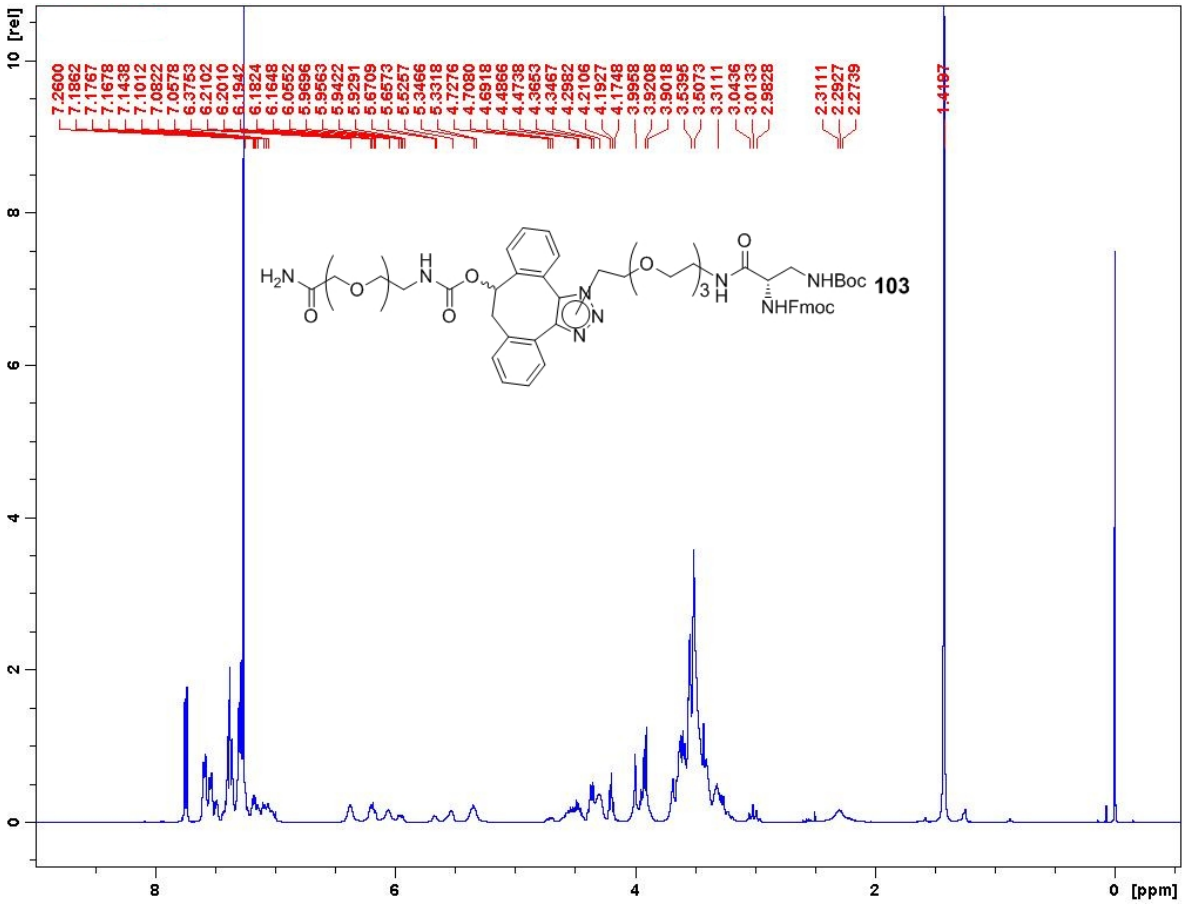
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NHBoc
NHFmoc
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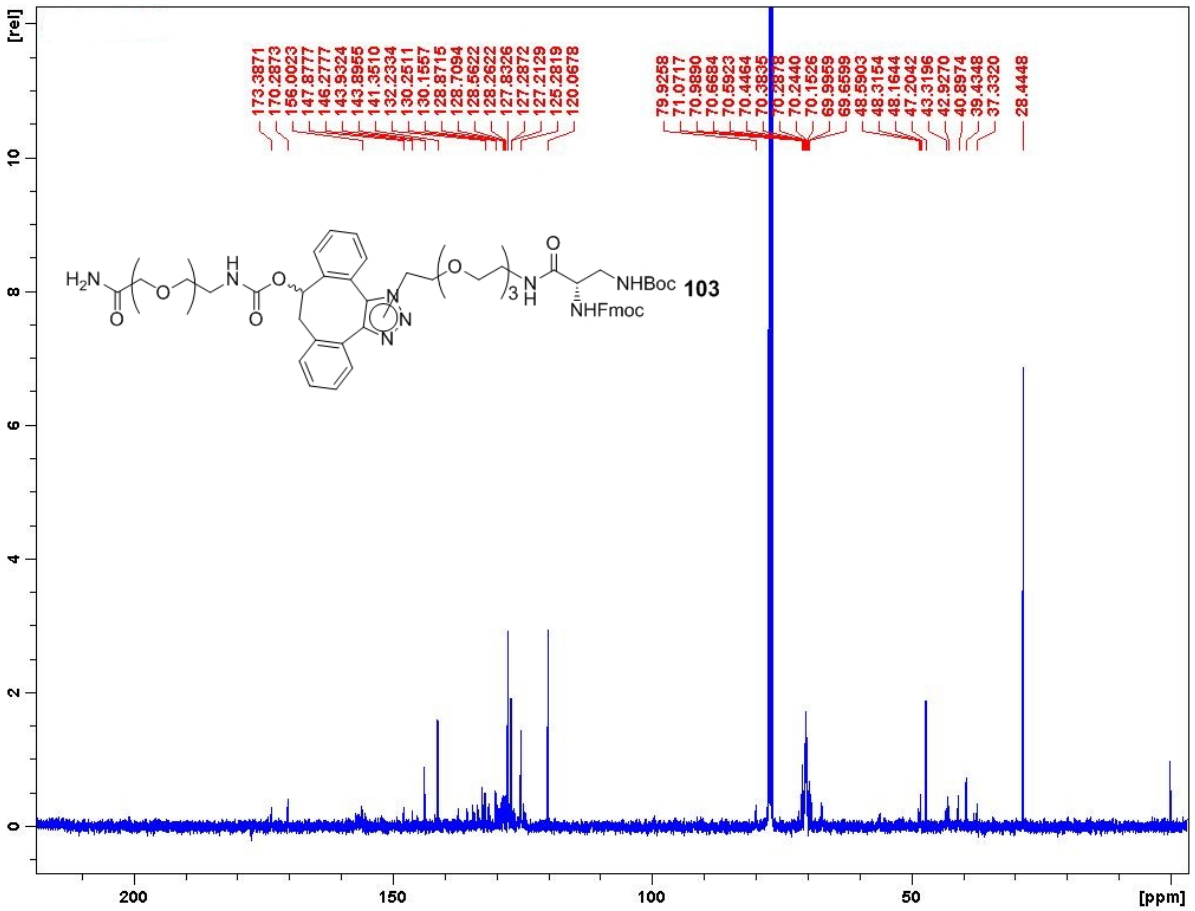
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11 Curriculum Vitae

Name:	Martin Golkowski
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Parents:	Dipl.-Ing. TU Klaus Golkowski, Architect Marianne Golkowski, Teacher
Education:	1987-1991: Elementary School Schöntal-Bieringen 1991-1997: Junior High School Krautheim 1997-2000: Technical High School Öhringen 2000, October: Matriculation for the diploma degree program Chemistry at the University of Tübingen 2002, September: Intermediate diploma degree in chemistry 2003: Specialization in “Synthetic Organic Chemistry” 2006, December: Diploma degree in chemistry 2007, July –2012, February: Ph.D. Student in the research group of Prof. Dr. Thomas Ziegler at the University of Tübingen