SYNTHESIS AND CYTOTOXIC ACTIVITY OF DIOSGENYL SAPONIN ANALOGUES

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science

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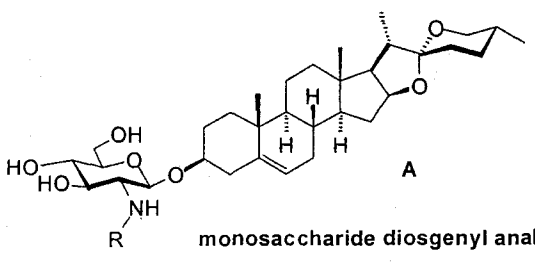
ABSTRACT

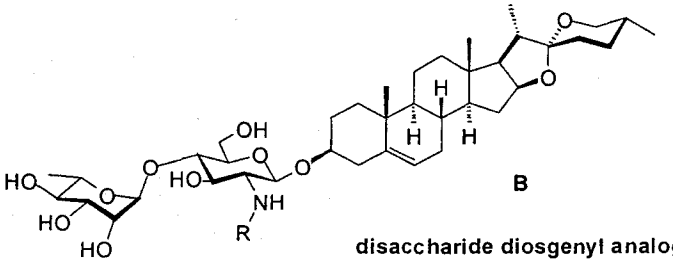
Saponins are naturally occurring glycosides that are widely distributed in the plant kingdom and lower marine organisms. Saponins consist of a carbohydrate chain attached to either a steroid or triterpene aglycone. Diosgenyl saponins are among the most abundant steroidal saponins and have attracted much attention for a host of varying biological activities. In recent years, diosgenyl saponins have demonstrated anticancer activity in a variety of cancer cell lines. The antitumour effect of diosgenyl saponins is shown to be through apoptosis.

Structural complexity and limited accessibility have led to a poor understanding of the mechanism of diosgenyl saponin anticancer activity. The position of attachment of moieties on the aglycone and the variety of the side chains are all factors that contribute to the complexity of a structure activity relationship study. Monosaccharide diosgenyl analogues bearing a 2-amino-2-deoxy-β-D-glucopyranose containing a variety of substituents replacing the α-L-rhamnose residue have been designed and synthesized. This rhamnose residue is usually found at the 2-*O*-position of the inner glucose moiety for most diosgenyl saponins. Moderate cytotoxic activity is found for most analogues against neuroblastoma SK-N-SH cells, breast cancer MCF-7 cells, and cervical cancer HeLa cells. Mechanistically, diosgenyl saponin analogue **A** with R = Bz was able to induce cell cycle arrest at G_1 phase in SK-N-SH cells, while, in contrast, induced cell cycle arrest at G_2 phase in MCF-7 cells. Disaccharide diosgenyl analogues **B** bearing an α-L-rhamnopyranosyl moiety attached to the 4-OH of the 2-amino-2-deoxy-β-D-glucopyranose residue containing a variety of substituents at the amino group have also been synthesized. The biological investigation of disaccharide analogues **B** is still underway.



A

monosaccharide diosgenyl analogues



B

disaccharide diosgenyl analogues

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ABBREVIATIONS

Ac	acetyl
AcHN-L-Ala-OH	*N*-acetyl-L-alanine
Ac_2O	acetic anhydride
Anal	analytical
approx	approximate
Ar	aryl
br	broad
brs	broad singlet
Bz	benzoyl
BzCl	benzoyl chloride
calcd	calculated
2-CdA	cladribine
Cl_3CCN	trichloroacetonitrile
CoA	coenzyme A
COSY	correlated spectroscopy
d	doublet
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCM	dichloromethane
dd	double doublet
DDMP	2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one
DIC	N,N'-diisopropylcarbodiimide
Dios	diosgenin
DIPEA	diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	*N*,*N*-dimethylformamide
ESI	electrospray ionization
EtOAc	ethyl acetate
FG	functional group
$GHNO_3$	guanidinium nitrate

HBTU	2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate
HOAc	acetic acid
HR	high resolution
Hz	hertz
IC_{50}	inhibitory concentration (concentration that inhibits cell growth by 50%)
J	coupling constant
LDL	low density lipoprotein
LR	low resolution
MALDI	matrix assisted laser desorption ionization
Me	methyl
MeOH	methanol
mg	milligram
MHz	megahertz
min	minutes
mol	moles
mmol	millimoles
MS	mass spectroscopy
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
Na_2SO_4	sodium sulphate
$NaHCO_3$	sodium bicarbonate
NaOMe	sodium methoxide
NMR	nuclear magnetic resonance
OSC	organic sulphur compound
py	pyridine
q	quartet
Rha	rhamnose
rt	room temperature
s	singlet

SAR	structure activity relationship
SD	standard deviation
t	triplet
TLC	thin layer chromatography
TMS	tetramethylsilane
TMSOTf	trimethylsilyl trifluoromethanesulfonate
Troc	2,2,2-trichloroethoxycarbonyl

1 INTRODUCTION AND OBJECTIVES

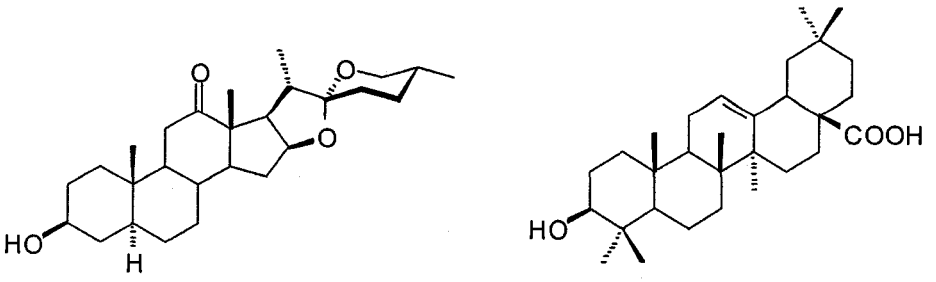
1.1 Saponins

1.1.1 General saponin features

Saponins are naturally occurring glycosides that are widely distributed in the plant kingdom and lower marine organisms.[1] In recent years, saponins have attracted much attention for a host of different biological activities. Saponins are often referred to as natural detergents with the name derived from the soapwort plant *Saponaria*, the root of which was historically used to form a soap-like foam. Saponins are also classified as phytochemicals and can be found in reasonably high number in numerous food and beverage plants.[2] Some sources of substantial amounts of saponins include yucca, soybeans, garlic, onions, yams, asparagus, quillaja, tea, etc.[3,4] There is also evidence that the active constituents in numerous traditional oriental medicines and commercially available herbs on the market, such as ginseng, licorice, horse chestnut and red clover are saponins.[4,5] The most common commercial source of steroid saponins is from *Yucca schidigera,* while, *Quillaja saponaria* produces a wide variety of extensively studied triterpenoid saponins.[6]

1.1.2 Structure and classification of saponins

Saponins consist of either C-27 or C-30 polycyclic aglycones attached to carbohydrate based side chains. The aglycone part, which is also called a sapogenin, is either a steroid (C-27) such as hecogenin[7] or a triterpenoid (C-30) such as oleanolic acid found in oleanane saponins[8] (Figure 1). The aglycone may contain one or more unsaturated C-C bonds. The foaming ability of saponins is caused by the combination of the hydrophobic sapogenin and the hydrophilic carbohydrate part. The carbohydrate component consists of one or more sugar moieties containing glucose, galactose, xylose, arabinose, rhamnose, or glucuronic acid.[6]



hecogenin oleanolic acid

Figure 1. Structure of typical sapogenins

Monodesmoside saponins are saponins that have one carbohydrate moiety attached to the aglycone, while saponins with two sugar moieties attached to the aglycone at different positions, for example, at C-3 and C-26 like in methyl protodioscin, are called bidesmoside saponins[6] (Figure 2).

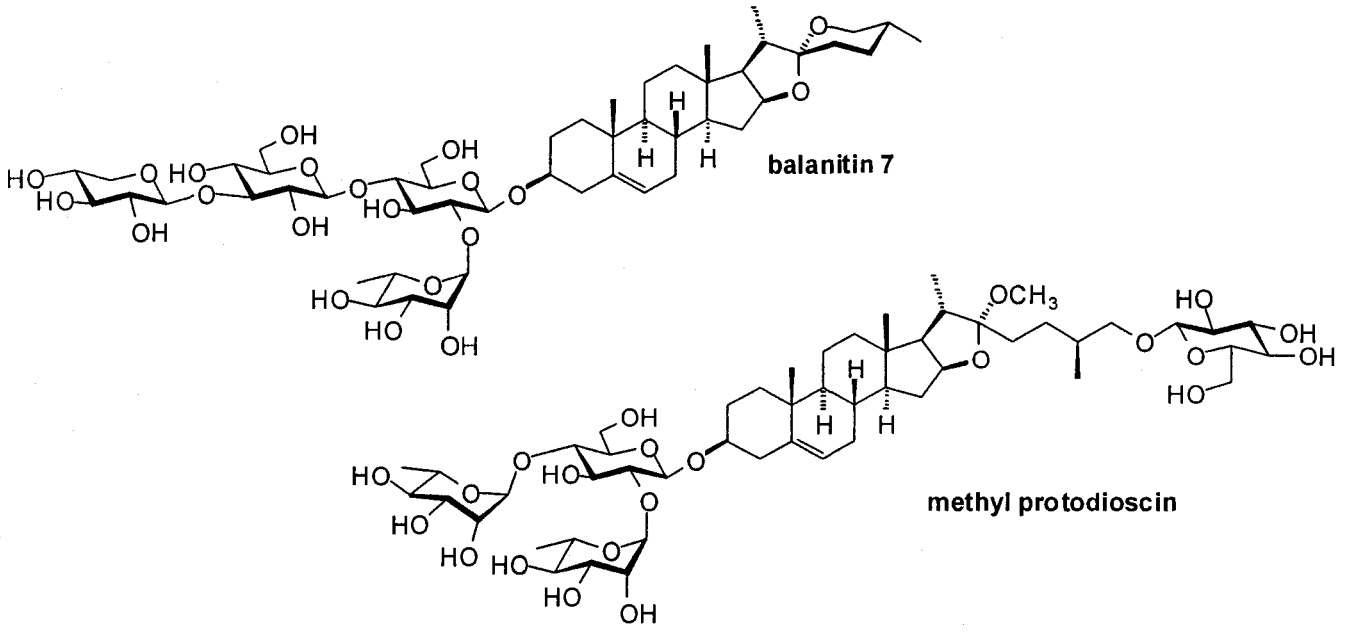


Figure 2. Monodesmoside balanitin 7 and bidesmoside methyl protodioscin

The variability of the aglycone structure, the position of sugar moieties on the aglycone and the variety of side chains are all factors that contribute to the complexity of saponins. Thus, the functionality of saponins has motivated substantial interest in these molecules.

As a result of the diversity of structures, steroidal saponins are usually divided into three classes, spirostan, furostan, and cholestan saponins, which are, interestingly, biosynthetically interrelated.[4]

1.1.3 Biological properties of saponins

Various biological activities have been shown by saponins throughout recent years. Commonly, in plants, saponins are found in tissues that are most vulnerable to fungal or bacterial attack, or insect predation.[6,9] Therefore, one of their roles is to act as a chemical barrier or shield in the plant defence system. Some of these saponins act as a means of controlling fungus while others act as a defence against insect attack.[9] Furthermore, recent research has shown evidence that saponins have potential as a natural insecticide against caterpillars *Spodoptera littoralis* and aphids *Acyrthosiphon pisum*, as various saponins have shown growth inhibition against these tested insects.[10] Saponins have also been shown to control bacteria in soil.[11]

Numerous studies have demonstrated that saponins are shown to exhibit various effects on cholesterol metabolism. Decreased intestinal absorption of cholesterol has been exhibited by some saponins[12] while also reducing levels of harmful LDL cholesterol selectively in the serum of test subjects including humans.[13,14] The immunostimulatory effects of saponins have been noted in several publications in recent years. Quillaja saponins have been shown to boost antibody production[15] as well as induce specific cytotoxic T-lymphocyte responses.[16] Antiviral activity has been observed in saponins purified from *Maesa lanceolata*[17] as maesa saponins are capable of deactivating certain viruses.[18] Furthermore, saponins have strong antifungal activity against many types of fungi. Saponins isolated from *Kalopanax pinctus* and the lower leaves of *Asparagus officinalis* have shown high toxicity to fungi through a possible membrane sterol interaction.[19,20] Interestingly, it has been found that some saponins have antioxidant or reductive properties. The *Fabaceae* species has given a group of soyasaponins with a distinct aglycone component capable of scavenging free radicals.[21] Attached at C-23, the 2,3-dihydro-2,5-dihydroxy-6-methyl-4*H*-pyran-4-one (DDMP) moiety reduces superoxides

to less harmful intermediates therefore reducing cell damage by free radicals.[21] One of the most significant findings in recent years has been the cytotoxic activity of saponins on numerous cancer cell lines, which will be discussed in further detail in this study.

1.2 Chemotherapeutic biological background

1.2.1 The cell cycle

The cell cycle is the series of events that take place in the cell leading to its replication. Immediately after a cell has successfully divided, an intermediate phase called G_1 is initiated.[22] This gap phase provides additional time for the cell to grow. During the G_1 phase, the cell undergoes an overview of the cellular environment to ensure that the conditions are appropriate to support DNA replication. Cells in the G_1 phase that have received inhibitory signals from other cells or are exposed to extracellular conditions that inhibit cell proliferation accumulate in a prolonged non-dividing state called G_0. After the cell has established the appropriate environment for DNA replication and has reached the correct size, it will enter the next phase called the S phase. Synthesis, or S phase, is the phase in which DNA is replicated in chromosomes. This phase allows for the replication of each cell created by cell division to have identical genetic make-up. Once the process of DNA replication has been complete, the cell with duplicated chromosomes enters the G_2 phase. The G_2 phase, similar to G_1, is a gap phase occurring between the end of DNA replication in S phase and before cell division occurs. G_2 is the final checkpoint to ensure that DNA and other intracellular components have been properly duplicated. Regulatory control by important cellular signals and cell growth also occur at this stage before it is split into two independent cells during mitosis, the M phase[22,23] (Figure 3).

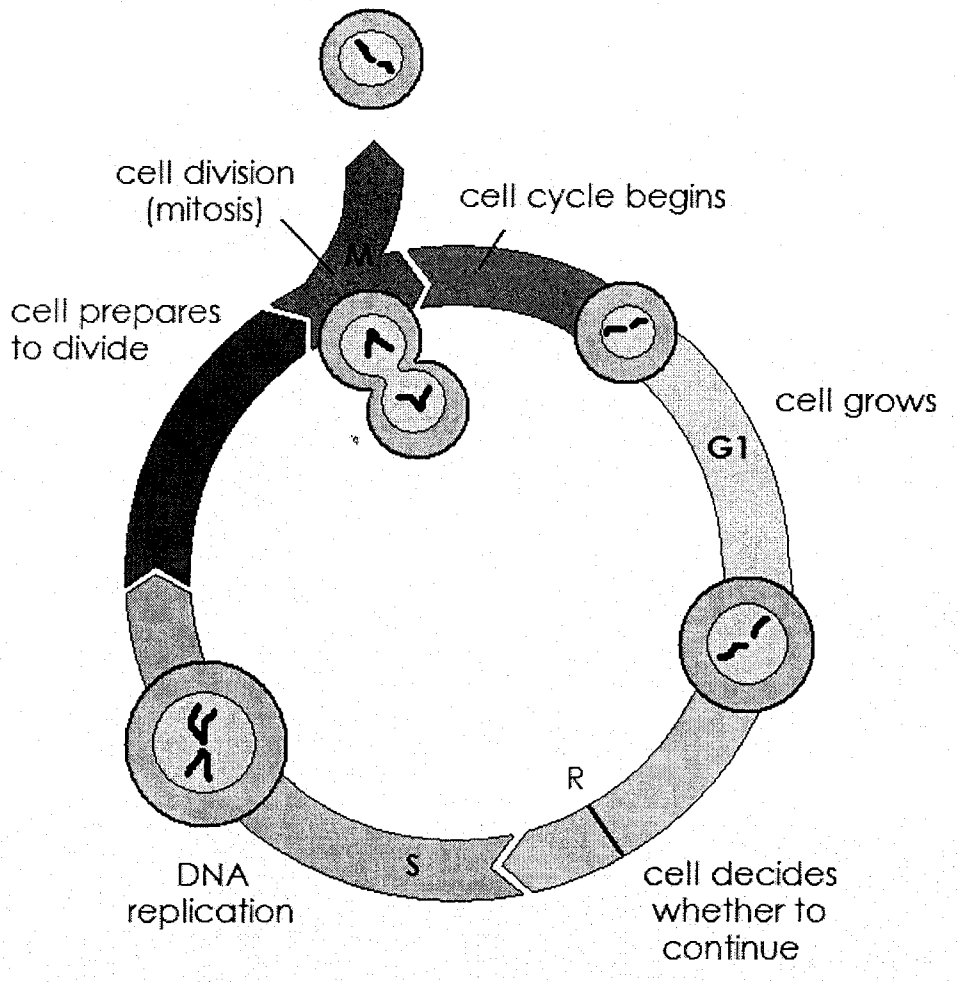


Figure 3. The cell cycle

1.2.2 Apoptosis

Cell proliferation and subsequently cell death are executed in a steady continuous process by an array of multiple signalling pathways. For most of the constituents of the cell, growth is a steady, continuous process, interrupted only briefly at the M phase, when the nucleus, and, consequently, the cell divide in two. Any deregulation or mutation of cell cycle components may halt cell death and cause the cells to multiply uncontrollably resulting in tumour formation.[24] Nearly all cancers are caused by abnormalities in the genetic material of transformed cells.[25] Genetic abnormalities found in cancer activate genes which give cells new properties such as protection against programmed cell death.[26] Thus, interference in the apoptotic capability of a cell leads to the development of

cancer in many cases.[26,27] However, when a cytotoxic agent is introduced into the process, cell proliferation is interrupted and programmed cell death or apoptosis occurs.[27] A portion of the complex apoptosis process is shown in Figure 4. Consequently, cell cycle checkpoints and apoptosis play an important part in cancer prevention.[23] For example, deregulation of the cells' p53 protein, an apoptotic tumour suppressor, results in impaired apoptosis activity and, as a consequence, the possible formation of tumours.[28,29] When DNA has sustained damage, the p53 gene accumulates to give the cell time to repair by preventing cell replication by cell cycle arrest. It can hold the cell in this phase long enough to fix damage and thus continue the cell cycle. However, if damage is extensive and irreparable, p53 will initiate apoptosis.[30,31] The process of apoptosis is controlled by a diverse range of cell signals and pathways but only activation by caspases causes cell death.[32-35] Caspases are cysteine proteases essential in the role of apoptosis.[32] There are two major signalling pathways in apoptosis, with mitochondrial regulation the core of the intrinsic pathway and death receptor activation involved in the extrinsic pathway.[36] Apoptosis in this pathway is controlled by anti-apoptotic Bcl-2 proteins and pro-apoptotic Bax and Bak expressions.[34,37,38] When a deregulation of mitochondria occur, cytochrome *c* is released which in turn activates caspase-9.[36] This intrinsic pathway is the most observed pathway with diosgenyl saponins. Caspase-8 is activated in the direct signal transduction pathway or death-receptor extrinsic pathway, of which, along with caspase-9, in the mitochondrial regulation pathway, both converge at caspase-3 activation.[39,40]

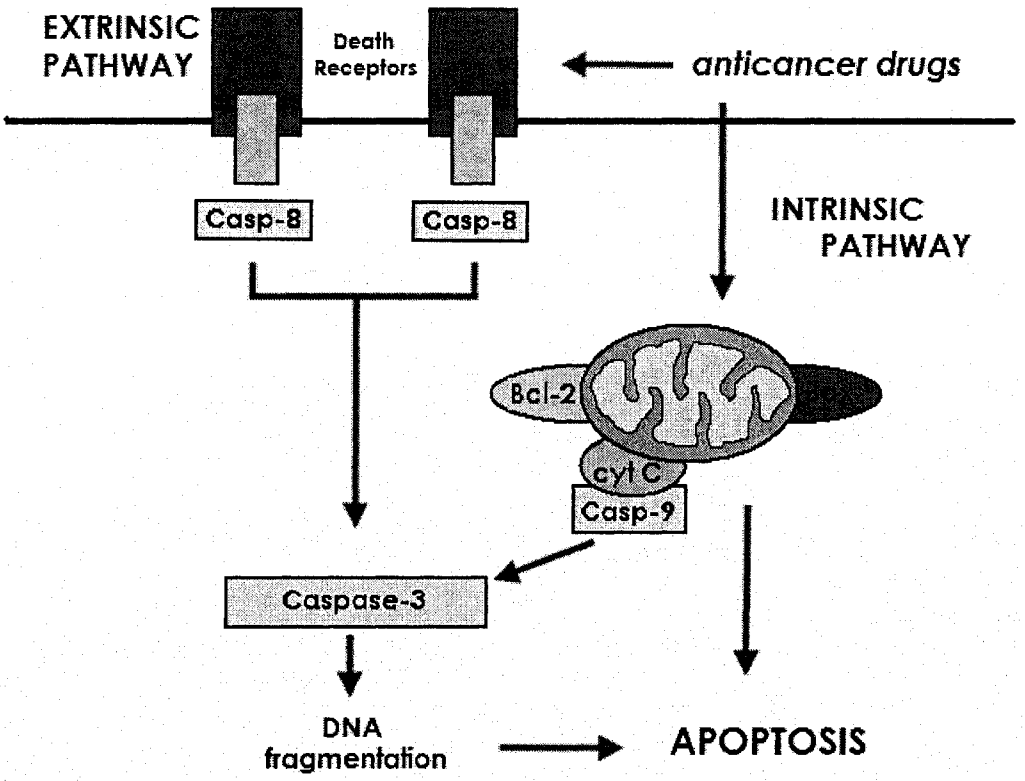


Figure 4. Apoptotic pathways

1.3 Diosgenyl saponins

1.3.1 The aglycone - diosgenin

Of particular interest for anti-cancer studies are the spirostan saponins, and more specifically, diosgenyl saponins. Recently, studies on diosgenyl saponins have shown growth inhibition by induction of apoptosis in cancer cells.[41-43] Diosgenin, (25R)-5-spirosten-3β-ol (Figure 5), is the steroidal sapogenin of diosgenyl saponins.

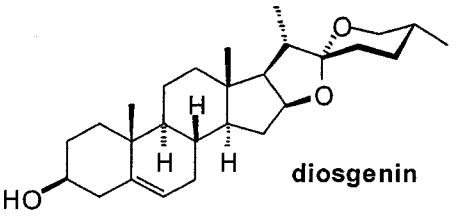


Figure 5. Structure of diosgenin

Diosgenin is found in several plant species including *Trigonella foenum graecum*, commonly called fenugreek[44] and the *Dioscorea* species referred to as yams.[45] It is obtained by hydrolysis of the glycosidic steroidal saponin form in other species. Since being isolated from the Mexican yam *Dioscorea mexicana*, diosgenin is now an essential compound in the pharmaceutical industry as a natural source of steroidal hormones.[46] The raw material is used for the production of contraceptive steroid drugs such as progesterone, and anti-inflammatory steroids, such as cortisone.[47] Diosgenin is reported to have hypocholesterolemic effects by decreasing cholesterol in serum LDL and elevating cholesterol in the HDL fraction.[48-50] Additionally, diosgenin has been investigated for possible hypolipidemic and antioxidative effects by upregulating the expression of antioxidative enzymes.[51]

1.3.2 Anticancer effects of diosgenin

In recent years, multiple preclinical studies have demonstrated the anticancer effects of diosgenin. Diosgenin has been shown to inhibit the proliferation of HT-29 human colon cancer cells and induce apoptotic activity via the intrinsic pathway by modulation of caspase-3 and Bcl-2 expressions *in vitro*.[52] The antiproliferative activity of diosgenin was also demonstrated by suppressing fatty acid synthase expression in HER2-overexpressing breast cancer cells[53] and by suppressing 3-hydroxy-3-methylglutaryl CoA reductase expression in HCT-116 human colon carcinoma cells.[54] Diosgenin induces cell cycle arrest and apoptosis in human leukemia K562 cells with the disruption of Ca^{2+} homeostasis.[55] Varying apoptotic mechanisms have been observed as induction through G_2/M cell cycle arrest and p53-independent p21 up-regulation occurred in HeLa cells.[56]

The proapoptotic mechanism of diosgenin also involved the activation of caspase-3 in HeLa cells.[57,58] The antiproliferative effects of diosgenin were demonstrated through the p53-dependent apoptotic mechanism in melanoma M4Beu cells and laryngocarcinoma HEp-2 cells.[59] In addition, diosgenin arrested myelogenous leukemia KBM-5 cells at the sub-G_1 phase of the cell cycle.[60] Other investigators have reported diosgenin to initiate cell cycle arrest in G_1 phase and apoptosis in the human osteosarcoma 1547 cell line by increasing the tumour suppressing p53 expression.[61] Further, its proapoptotic activity was found to be greater than that of two structurally similar plant steroids, hecogenin (Figure 1) and tigogenin.[62]

1.3.3 Structure function activity of diosgenin

The ability of diosgenin to alter cell cycle arrest and induce apoptosis has accordingly inspired considerable interest for structure function studies. Trouillas *et al.* have compared eight plant steroids with structural similarities to diosgenin for their biological activity against human 1547 osteosarcoma cells.[63] In the study, they chose to investigate the apoptotic activity, proliferation rate, and cell cycle distribution on human 1547 osteosarcoma cells. The other objective was to establish structural criteria as related to molecular activity. The structural characteristics focused on were: the 5,6-double bond, the configuration at C-25, and the role of the osidic bond.

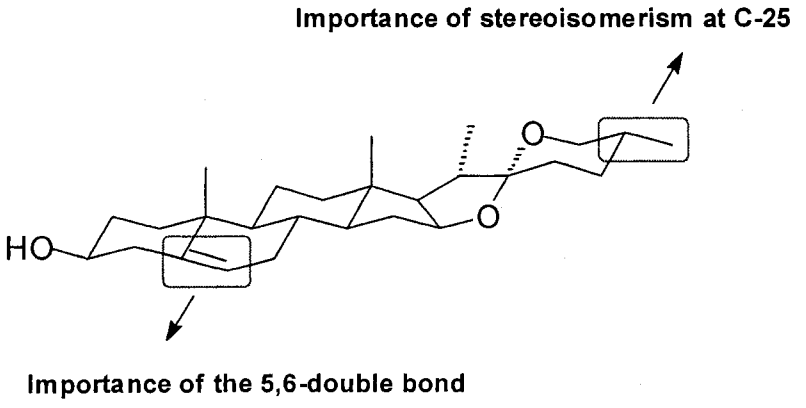


Figure 6. Structure function attributes of diosgenin

The key results of the study demonstrate that biological activity is attributed to the presence of the 5,6-double bond and the *S* stereochemistry at C-25 on the androst-5-en-3-ol skeleton (Figure 6).[63] These two important structural features are present in diosgenin and their presence is essential for compounds to exhibit strong apoptosis and cell cycle arrest.

1.3.4 Properties of diosgenyl saponins

Diosgenyl saponins are the most abundant steroidal saponins in nature. These saponins have been shown to exhibit a wide variety of biological activities including cardiovascular,[64] antifungal,[65] and antibacterial activities.[66] A common property of several diosgenyl saponins is their ability to inhibit the growth of cancer cells.[61,67,68] The structural diversity of diosgenyl saponins lies in the sugar moiety. The structure of diosgenyl saponins is mostly based on diosgenyl β-D-glucopyranoside, commonly called trillin[69] (Figure 7).

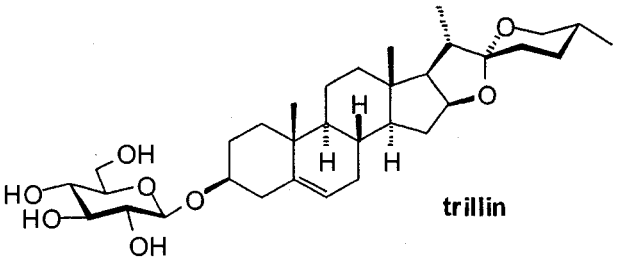


Figure 7. Structure of trillin

Having a single glucose sugar attached via the 3-*O*-position on the aglycone diosgenin, it is the simplest diosgenyl saponin. Some of the more common saponins belonging to this group are ophiopogonin C', dioscin, and polyphyllin D (Figure 8).

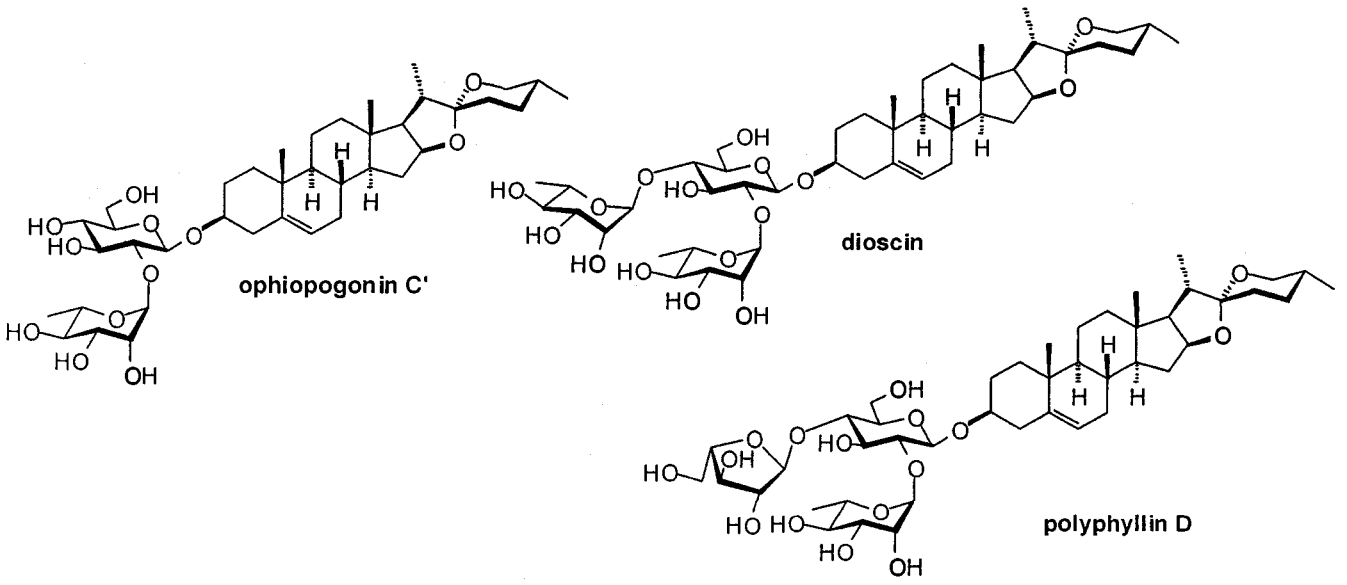


Figure 8. Diosgenyl saponins

The structure of most diosgenyl saponins usually has an L-rhamnopyranose moiety attached to the 2-*O*-position and another sugar moiety at the 3-*O*- or 4-*O*-position. In one study, trillin has been shown to have no significant cytotoxic activity (IC_{50} > 20 μM) against HL-60 human leukemia cells, however, when an α-L-rhamnopyranosyl sugar is attached at C-2 of the glucopyranosyl moiety, ophiopogonin C' is formed, and significant cytotoxic activity appears (IC_{50} = 1.8 μM).[70] Polyphyllin D, isolated from the *Paris polyphylla* species, has been shown to promote some cardiovascular and cytotoxic activities (IC_{50} = 0.5-7.0 μM).[71-75] Polyphyllin D has been shown to be cytotoxic and induce apoptosis in the hepatocellular carcinoma HepG2 (IC_{50} = 7.0 μM), and the drug resistance R-HepG2 cell lines (IC_{50} = 5.0 μM).[41] Mechanistically, it was found that polyphyllin D initiated apoptotic activity via mitochondrial fragmentation.[74] In MCF-7 breast cancer cells, polyphyllin D inhibited growth with an IC_{50} value of 5.0 μM. It induced a down-regulation of the anti-apoptotic Bcl-2 expression and activated the caspase-9 expression suggesting that polyphyllin D elicits apoptosis through mitochondria dysfunction in these cells.[75]

1.3.5 Dioscin

Another one of the most promising diosgenyl saponins has been dioscin. Dioscin exists widely in the plant kingdom and has been a major component in many traditional Chinese herb medicines. Structurally, dioscin starts with a β-D-glucopyranosyl unit attached at the 3-OH of diosgenin and extends with two α-L-rhamnopyranosyl residues at the 2-*O*- and 4-*O*-positions of the glucose moiety (Figure 8). Dioscin has been shown to be one of the most potent diosgenyl saponins with a range of cytotoxicity (IC_{50} = 0.5-3.8 μM) in various cancer cell lines.[68] Cai *et al.* have demonstrated the induction of apoptosis in the sub-G_1 phase in HeLa cells by dioscin.[76] Low activity of caspase-8 indicated that the death-receptor pathway was not activated, whereas high activity of caspase-9 was shown suggesting involvement of mitochondria. In addition, a down-regulation of the anti-apoptotic Bcl-2 expression was detected further confirming the mitochondrial pathway. The intrinsic mitochondrial pathway was also revealed by dioscin action on human myeloblast leukemia HL-60 cells.[41] The activation of caspase-9 and, subsequently, caspase-3 with a deregulation of the Bcl-2 protein confirms this pathway.

1.3.6 Structure activity relationships of dioscin

Recently, some structure-activity studies have been presented with dioscin. In one study,[77] all eight mono-methylated dioscin derivatives were synthesized and their cytotoxicity was observed. The tumour inhibitory activities of all eight were tested against lymphocytic leukemia P388 and alveolar basal epithelial A549 cell lines and it was observed that the inhibitory activity was retained when the 6-OH of the glucopyranosyl moiety and the 4-OH of the rhamnopyranose attached at the 4-*O*-position on the glucopyranosyl moiety were methylated.[77] The results show that the other six of the eight hydroxyls on dioscin might be key for antitumour activity. As a result, 6'-*N*-acyl-and 6'-*O*-acyl-dioscin derivatives were prepared in hopes of improving the antitumour activity of dioscin.[78,79] Interestingly, results from both studies indicate that all analogues were largely inactive. The importance of the 1,2-*trans* β-glycosidic bond in dioscin is proven by Miyashita *et al.* as they have prepared both α- and β-chacotriosyl glycosides and compared their activity.[80] The results show that

in cytotoxicity tests with A549 and HepG2 cell lines, the β-glycosides show superior growth inhibition over their α-glycoside counterparts.

1.4 Objectives of the present work

Structural similarity is a problem that arises from the extraction of saponins in one specific plant species.[4] The distinct morphology of saponins calls for tedious and exhaustive techniques for isolation, structure clarification, and biological testing. As a result, many saponin extracts from herbs have been used directly, without pharmacological assessment, to treat various human diseases. Therefore, isolation of a substantial amount of a single pure saponin would be of considerable interest. Chemical synthesis of diosgenyl saponin analogues would provide a realistic way to obtain homogeneous saponins. The variability of the aglycone structure, the position of attachment of moieties on the aglycone and the variety of the side chains are all factors that contribute to the complexity of a structure activity relationship study (SAR).[68] In this study, it is intended to synthesize a catalogue of diosgenyl saponin analogues to study their cytotoxic effects against cancer cells. In addition, the mechanism of their anticancer activity will be investigated.

1.4.1 Monosaccharide diosgenyl saponin analogues

There has been evidence that less complex diosgenyl saponins like ophiopogonin C'[70] and analogues synthesized by Myszka *et al.*[81] have proven to exhibit cytotoxic activity. The main structural features for activity include a β-linked carbohydrate moiety at C-3 of the steroidal aglycone and modification at C-2 of the sugar residue. Myszka *et al.*[81] reported the apoptosis-inducing property in B cell chronic leukemia cells by synthetic diosgenyl 2-amino-2-deoxy-β-D-glucopyranoside hydrochloride and one urea derivative in combination with cladribine (2-CdA). These findings, along with the strong cytotoxicity of ophiopogonin C',[70] indicate that diosgenyl saponin analogues carrying only one or two sugar residues might show good antitumour potential. However, limited studies into the structure activity relationship have been carried forward. Therefore, one aspect of the

thesis is to synthesize simpler diosgenyl saponin analogues (Figure 9) for biological evaluation and structure activity relationship studies. In addition, one dimeric diosgenyl saponin analogue was prepared.

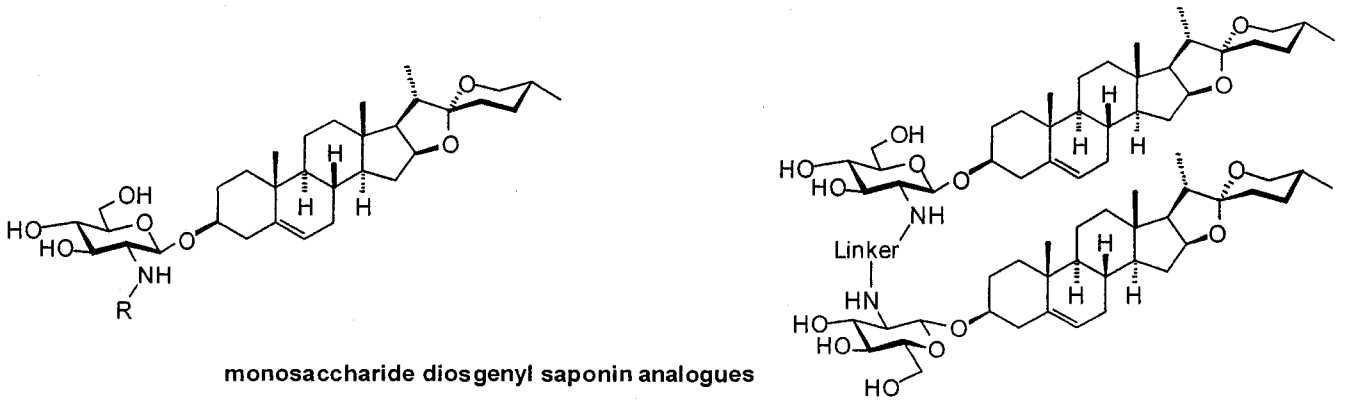


Figure 9. Monosaccharide diosgenyl saponin analogues

1.4.2 Disaccharide diosgenyl saponin analogues

Diosgenyl saponins isolated from various extracts of plants have showed cytotoxicity against a variety of cancer cell lines.[61,67,68,76] Activity of diosgenyl saponins has been shown to increase when the 4-OH of the glucopyranosyl moiety is rhamnosylated as shown by the activity of dioscin.[70] Thus, a set of disaccharide diosgenyl saponin analogues designed from more structurally complicated molecules like dioscin were designed for anticancer testing and structure activity relationship studies (Figure 10).

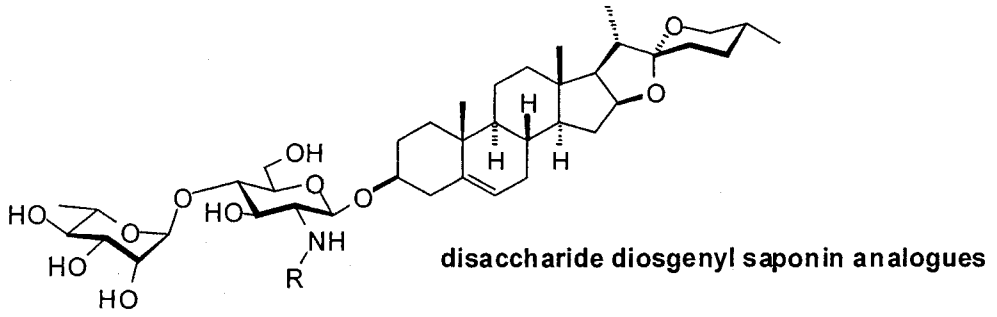


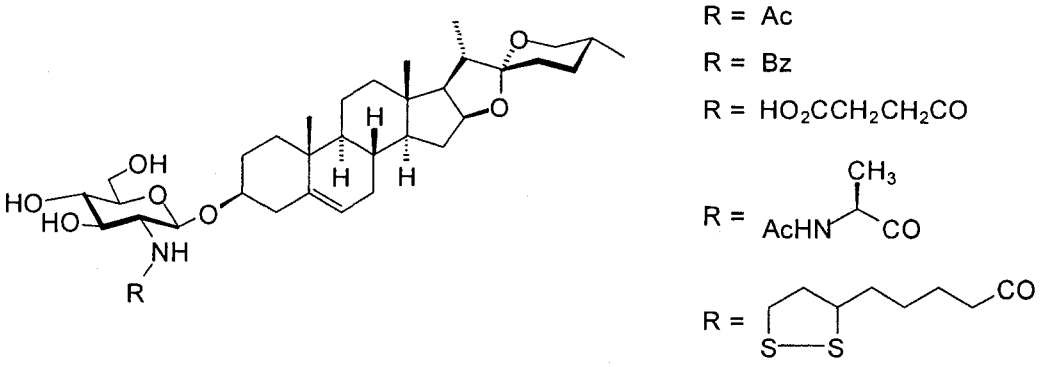
Figure 10. Disaccharide diosgenyl saponin analogues

2 RESULTS AND DISCUSSION

2.1 Monomeric diosgenyl saponin analogues

2.1.1 Design of monomeric diosgenyl saponin analogues

As mentioned, diosgenyl saponins have shown growth inhibition against a variety of cancer cell lines.[61,67,68,76] Since rhamnose attachment at C-2 of the inner sugar moiety results in increased activity, modification at C-2 is a viable option for synthesis of diosgenyl saponin analogues. Thus, a synthetic strategy towards simpler structural analogues was initiated (Scheme 1). A selection of functional groups were incorporated: an acetyl group, benzoyl providing an aromatic moiety, succinic anhydride as an acid functionality, alanine as an amino acid residue, and lipoic acid as a disulphide.

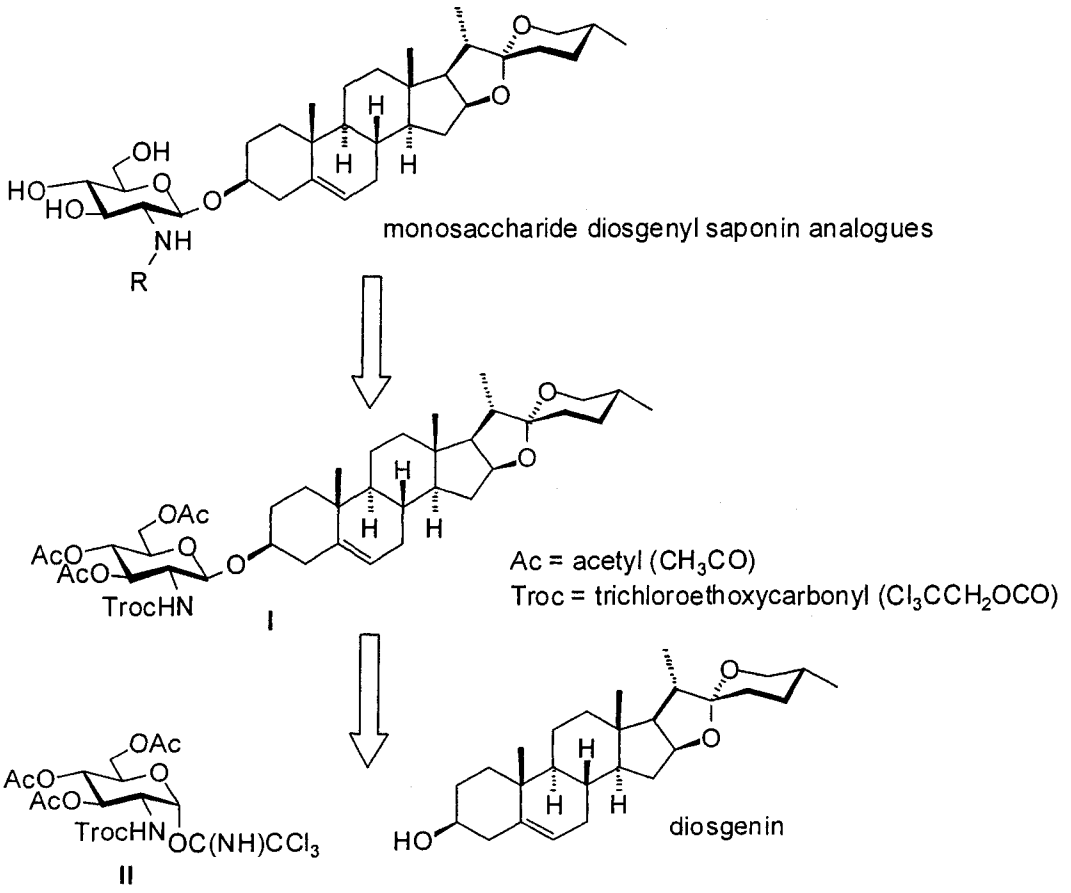


Scheme 1. Monomeric diosgenyl saponin analogues

2.1.2 Synthetic strategy for monomeric diosgenyl saponin analogues

A strategy of employing the efficient 2,2,2-trichloroethoxycarbonyl (Troc) amine protection group for 2-deoxy sugars was implemented. The reactive nucleophilic amine was *N*-protected by the Troc group which is part of an orthogonal set of protecting groups that can be cleaved under reductive β-elimination conditions.[82] This establishes the crucial *N*-Troc group which possesses effective participating group properties for the formation of

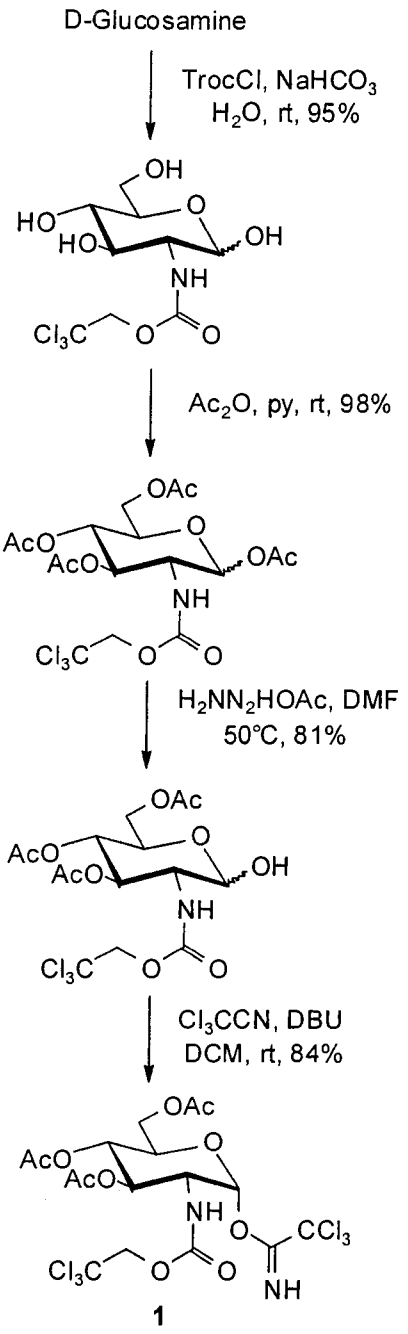
stereospecific β-glycosides.[83,84] Therefore, the monosaccharide diosgenyl saponin analogue structure with a variety of R groups was chosen as the target molecule for the synthesis of diosgenyl saponin analogues (Scheme 2). Acylating agents were chosen for coupling to the deprotected amino group on intermediate **I**. Accordingly, the aglycone chosen was commercially available diosgenin and the carbohydrate moiety is the suitably protected monosaccharide **II**[84] connected via a β-glycosidic linkage.



Scheme 2. Retrosynthetic analysis for monomeric diosgenyl saponin analogues

2.1.3 Synthesis of the glycosyl trichloroacetimidate donor 1

According to known literature procedures, protected glycosyl imidate **1**[84] was prepared as the glycosylation donor according to Scheme 3.

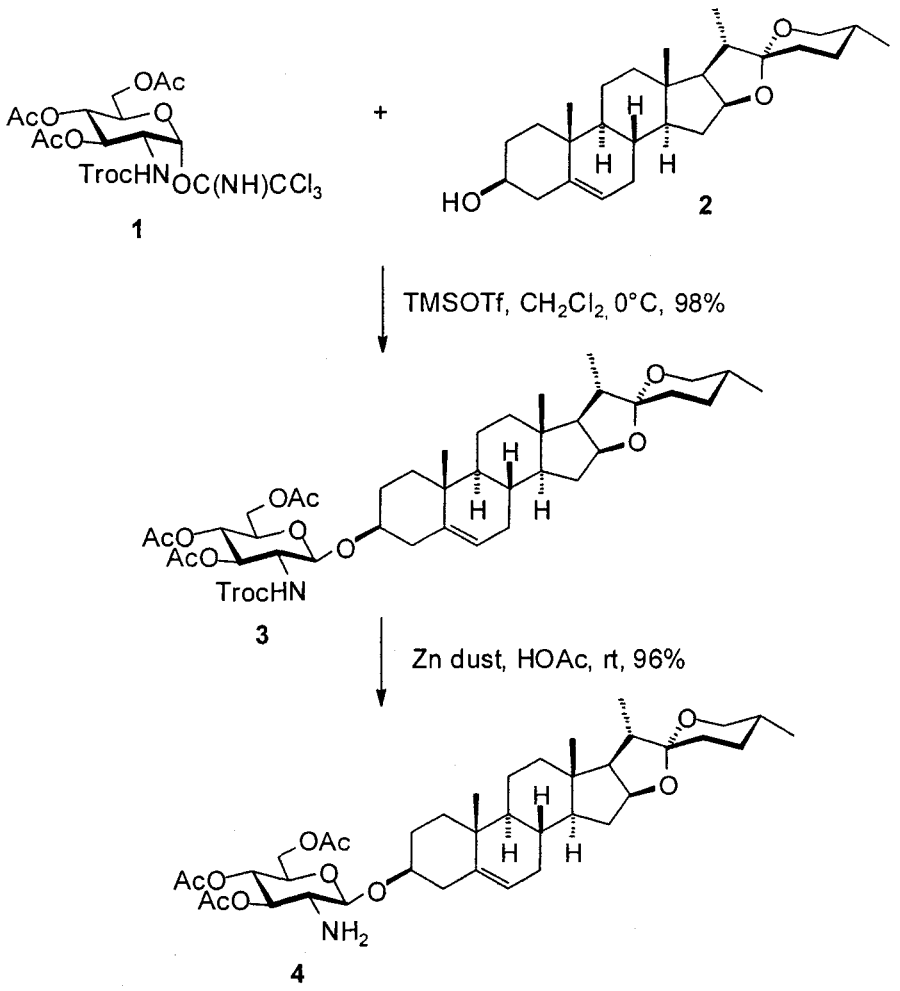


Scheme 3. Synthesis of the glycosyl imidate

2.1.4 Synthesis of β-glycoside 4

The glycosylation with 2-*N*-Troc group donors usually affords 1,2-*trans*-glycosides stereoselectively by virtue of neighbouring group participation.[83,84] Synthesis of β-glycoside formation in saponins has been demonstrated efficiently by Deng *et al.*[85]

Therefore, glycosylation was achieved between **1** and diosgenin **2** (Scheme 4) under promotion by the catalyst trimethylsilyl trifluoromethanesulfonate (TMSOTf) in dichloromethane (DCM) at 0°C to afford exclusively β-glycoside **3** in 98% yield.

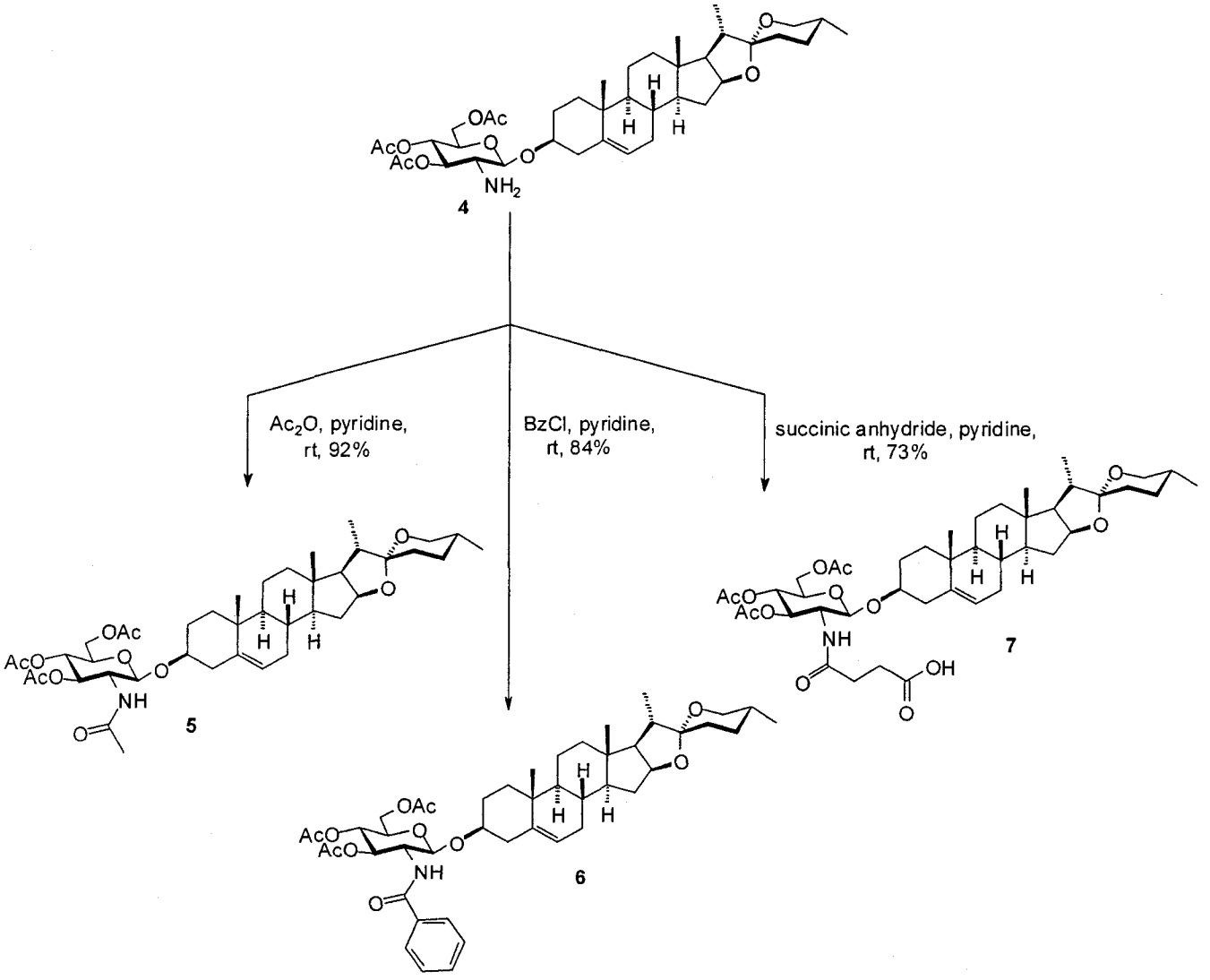


Scheme 4. Glycosylation with the steroidal acceptor and synthesis of amine intermediate

The ^{1}H NMR data of **3** (δ 4.80, d, J = 8.0 Hz)[84] confirmed the existence of the 1,2-*trans* β-linkage by the coupling constant of the anomeric proton. Compound **3** is then treated with zinc in acetic acid to provide free amine **4** in 96% yield as the main intermediate for subsequent amide bond synthesis.

2.1.5 Synthesis of the diosgenyl monosaccharide analogue series

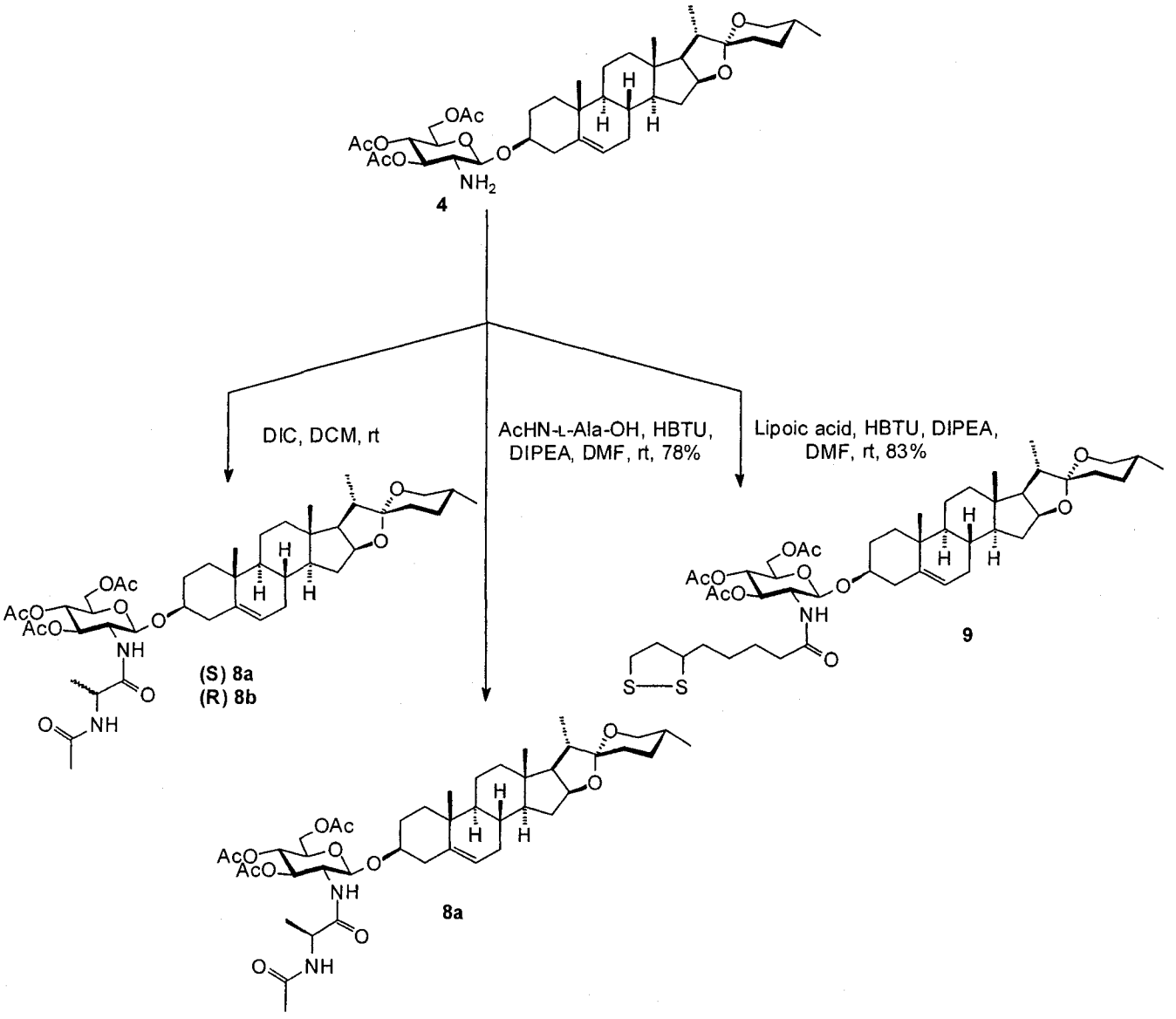
Treatment of **4** with acetic anhydride (Ac_2O) in pyridine gave **5** in 92%, with benzoyl chloride (BzCl) in pyridine gave **6** in 84% yield, and with succinic anhydride in pyridine gave **7** in 73% yield (Scheme 5).



Scheme 5. Coupling of amine with various acylating reagents

The coupling reaction between **4** and *N*-acetyl-L-alanine (AcHN-L-Ala-OH) resulted in almost complete racemization of the alanine residue when *N,N'*-diisopropylcarbodiimide (DIC) is used as the condensation agent (Scheme 6). The so-formed diastereomeric

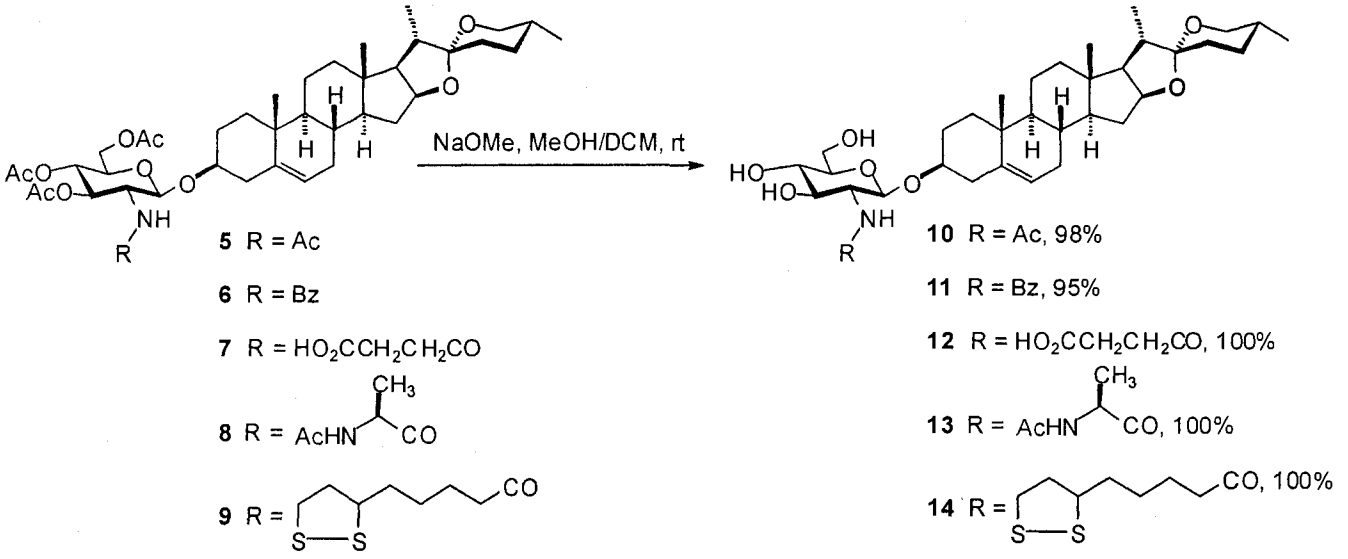
mixture **8a/8b** is chromatographically inseparable, but readily recognized on its ^{1}H NMR spectrum (δ 4.84, d, J = 8.5 Hz for the anomeric proton of the sugar residue in L-alanine-bearing isomer; δ 4.90, d, J = 8.5 Hz for the anomeric proton of the sugar residue in D-alanine-bearing isomer).



Scheme 6. Coupling of the amine with *N*-acetyl-L-alanine and (±)-α-lipoic acid

This racemization can be largely suppressed (<5%) when 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HBTU) is employed as the activating

agent. As a result, **4** was coupled to AcHN-L-Ala-OH by HBTU with *N,N*-diisopropylethylamine (DIPEA) in dry DMF to give **8a** in 78% yield. Under the same conditions, (±)-α-lipoic acid was coupled to **4** to give **9** in 83% yield. The final removal of the acetyl groups in **5-9** by treatment with sodium methoxide in dichloromethane-methanol affords the corresponding diosgenyl saponin analogues **10-14**, respectively (Scheme 7). The structures of compounds **10-14** have been confirmed by ^{1}H NMR, ^{13}C NMR, and MS spectral data.



Scheme 7. Deprotection to target monomeric diosgenyl saponin analogues

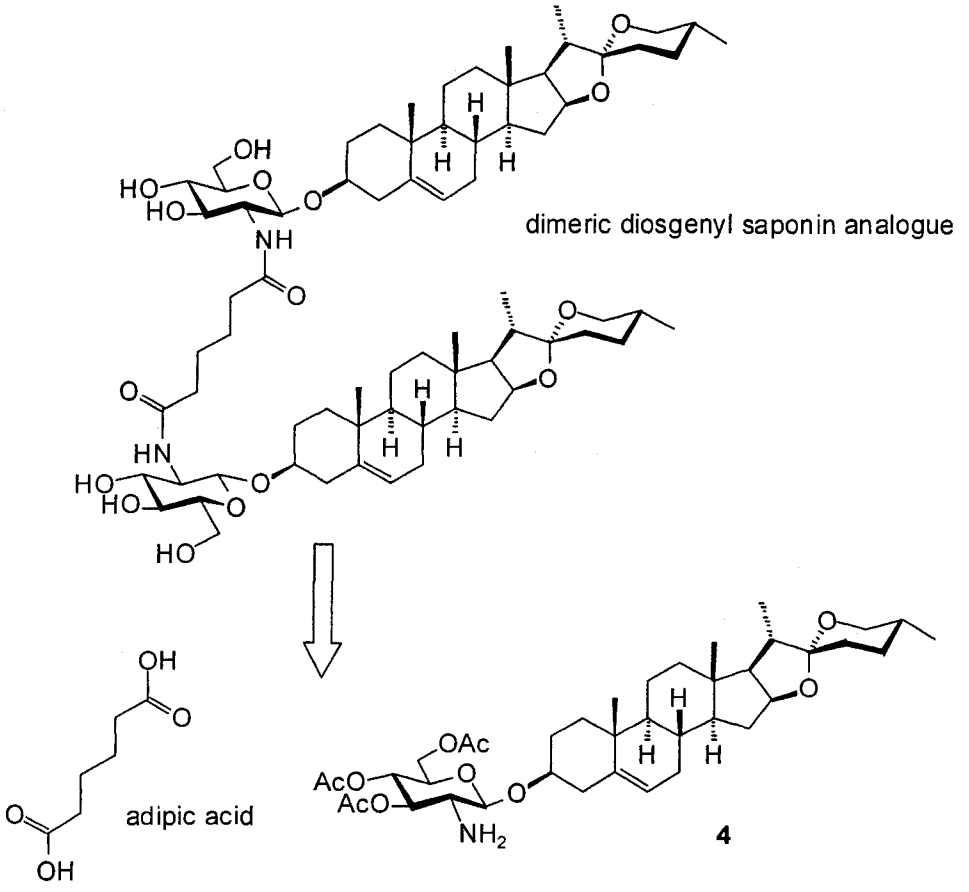
2.2 Dimeric diosgenyl saponin analogue

2.2.1 Design of dimeric diosgenyl saponin analogue

Many biological systems interact through multiple, simultaneous molecular contacts, and, in the recent past the design of multivalent ligands and inhibitors has become a focus of inquiry in molecular biochemistry and drug discovery.[86] As a result, one dimeric diosgenyl saponin analogue was prepared in hopes of improving the antitumour activity of the monomeric analogue.

2.2.2 Synthetic strategy for dimeric diosgenyl saponin analogue

The strategy employed for the dimeric diosgenyl saponin analogue (Scheme 8) was to use the key synthesized amine intermediate **4** connected via a linker molecule. Thus, the dimer consists of two copies of the intermediate **4** linked via adipic acid.

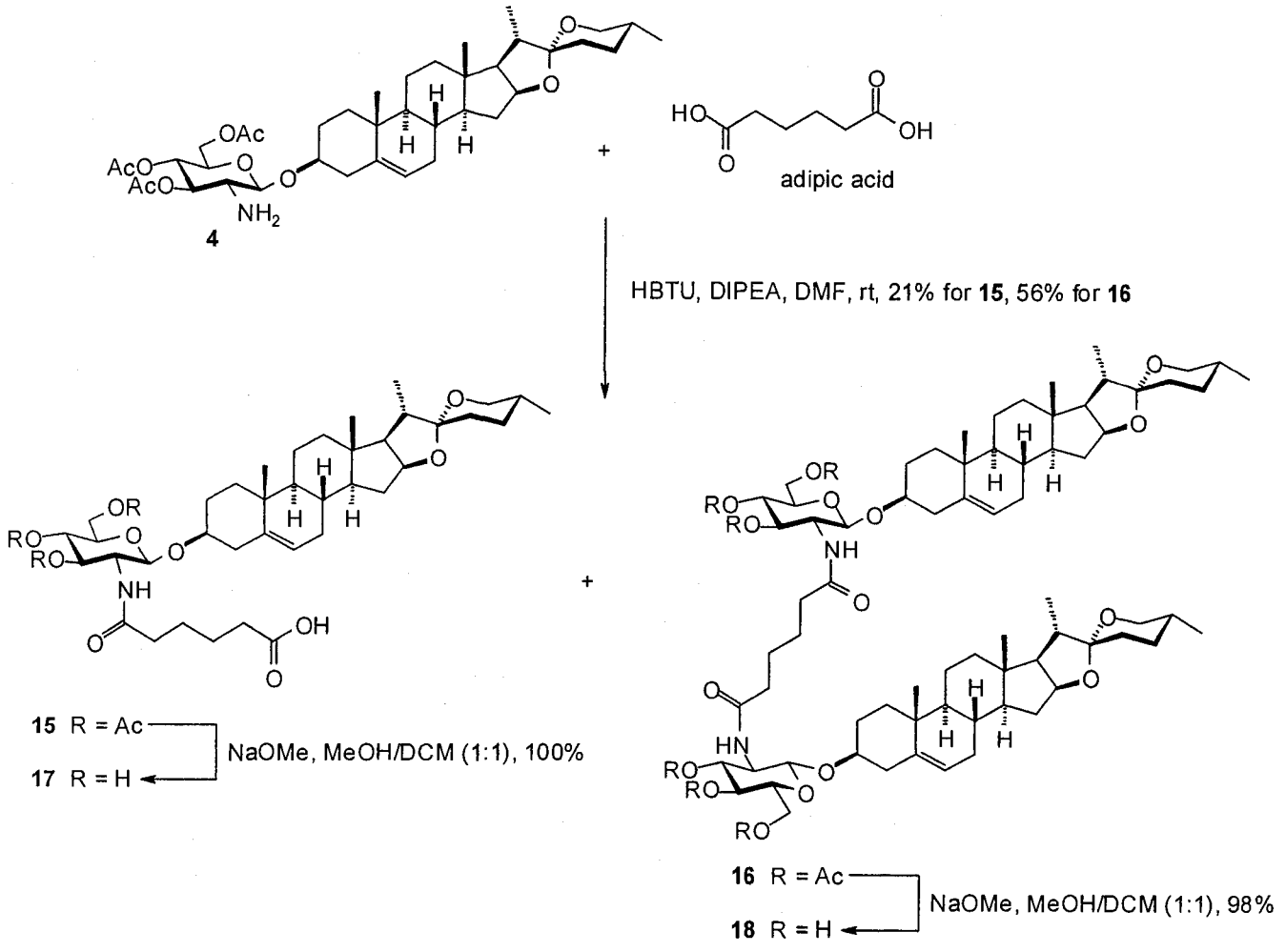


Scheme 8. Retrosynthetic analysis for dimeric diosgenyl saponin analogue

2.2.3 Synthesis of dimeric diosgenyl saponin analogue

The reaction of two equivalents of amine **4** with one equivalent of the dicarboxylic acid, adipic acid, in the presence of HBTU as the activating agent, gives the desired dimer **16** in 56% yield, together with 21% of the monomeric product **15**. Compounds **15** and **16** are treated with sodium methoxide to give saponin analogues **17**, in quantitative yield, and **18**,

in 98% yield (Scheme 9).



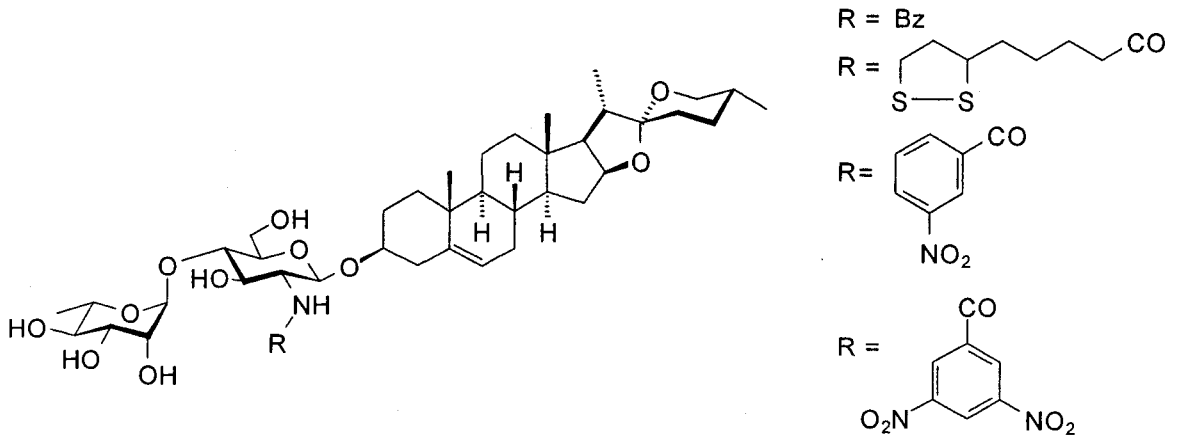
Scheme 9. Synthesis of diosgenyl saponin dimer

2.3 Disaccharide diosgenyl saponin analogues

2.3.1 Design of disaccharide diosgenyl saponin analogues

Dioscin, isolated from various extracts of plants, has shown cytotoxicity against multiple cancer cell lines.[41,76,77] Structurally, β-D-glucopyranose is attached to the 3-OH group of the aglycone diosgenin, and α-L-rhamnopyranose is attached to the 2-*O*- and 4-*O*-positions. Encouraged by the synthesis of our monosaccharide saponin analogues and

previous SAR studies[70], a set of disaccharide diosgenyl saponin analogues with a dioscin-like backbone were chosen as target molecules for the synthesis (Scheme 10). A variety of acylating reagents were selected to replace the α-L-rhamnose found at the 2-*O*-position on dioscin and various other diosgenyl saponins. Benzoyl and lipoic acid functionalities were chosen for direct comparison in biological activity to the monosaccharide analogues. In addition, two aromatic nitro groups were integrated as they are involved in redox processes causing oxidative stress in cells, leading to apoptosis or programmed cell death[87].

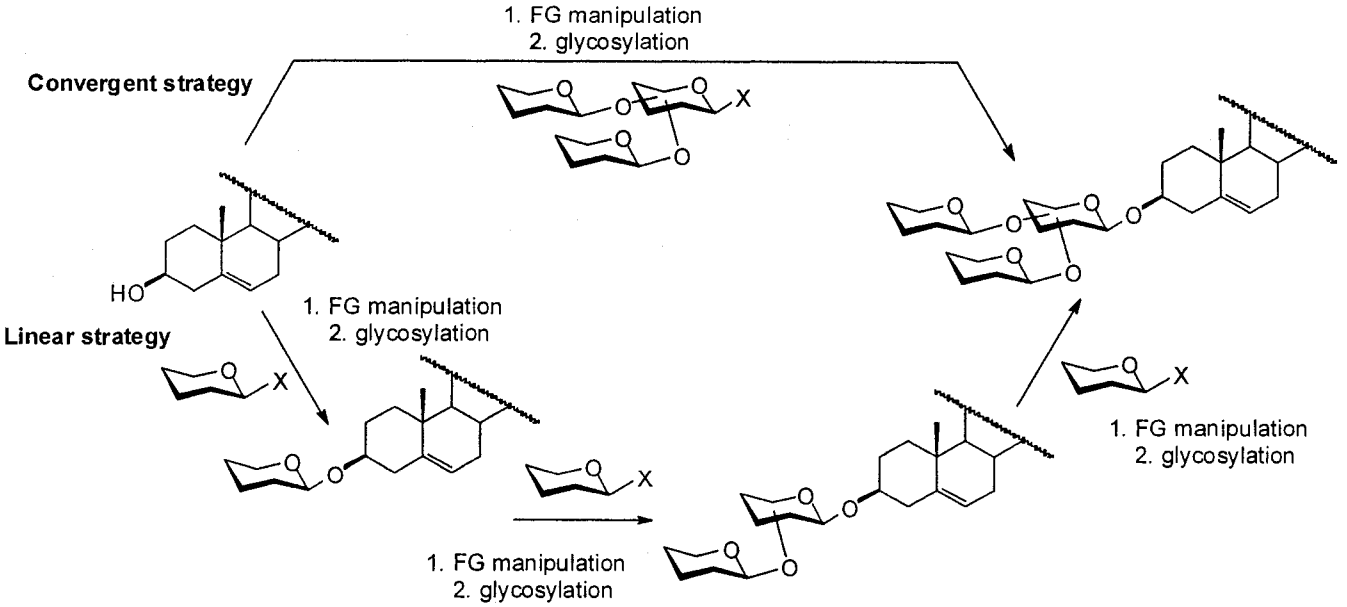


Scheme 10. Disaccharide diosgenyl saponin analogues

2.3.2 Synthetic strategies for disaccharide diosgenyl saponin analogues

From a strategic standpoint, two differing reaction pathways are evident with oligosaccharide saponin synthesis (Scheme 11).[88] Fabrication of a separate sugar moiety followed by subsequent glycosylation with an aglycone presents a convergent synthetic strategy. This strategy is advantageous as the sugar moiety can be manipulated without the aglycone present. Whereas, attaching sugar moieties sequentially to a glycoside complex can be perceived as synthesis in a linear fashion. The second strategy generally benefits stereospecific and high yielding glycosidic bond formations. Both strategies have thus been explored as alternate pathways in this work to provide a complete illustration of

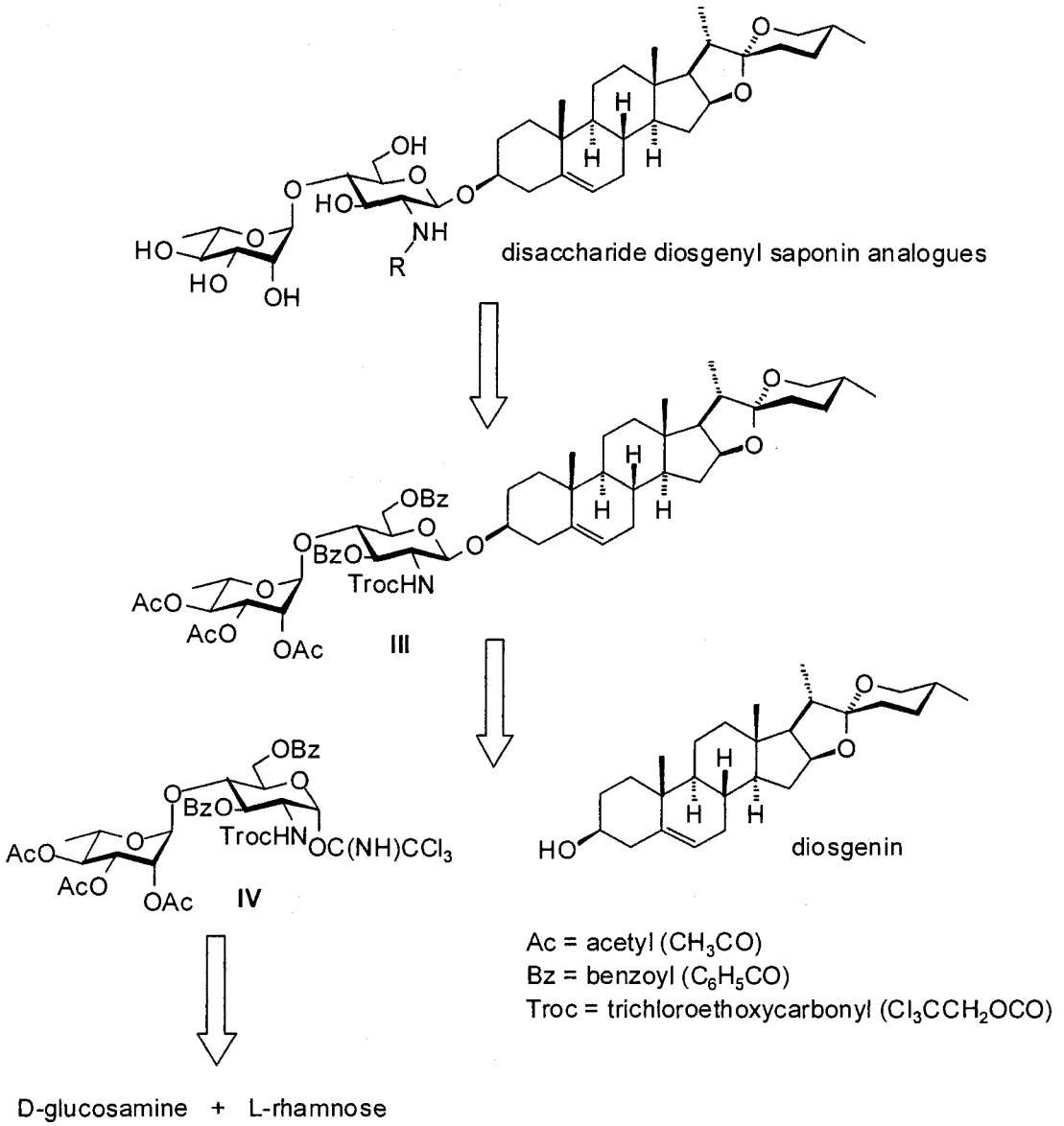
saponin analogue synthesis.



Scheme 11. Two main strategies for saponin synthesis

2.3.3 Convergent synthesis strategy for disaccharide diosgenyl analogues

Following a convergent type strategy, disaccharide diosgenyl saponin analogues with a variety of R groups were chosen as target molecules for the synthesis (Scheme 12). Acylating agents were chosen for coupling to the deprotected amino group on intermediate **III**, while the aglycone is diosgenin. The carbohydrate moiety is a disaccharide, **IV**, composed of two sugar residues: the 2-amino-2-deoxy-D-glucopyranose connected via a β-glycosidic linkage to the aglycone and an L-rhamnopyranose moiety connected via an α-linkage at the 4-O-position.

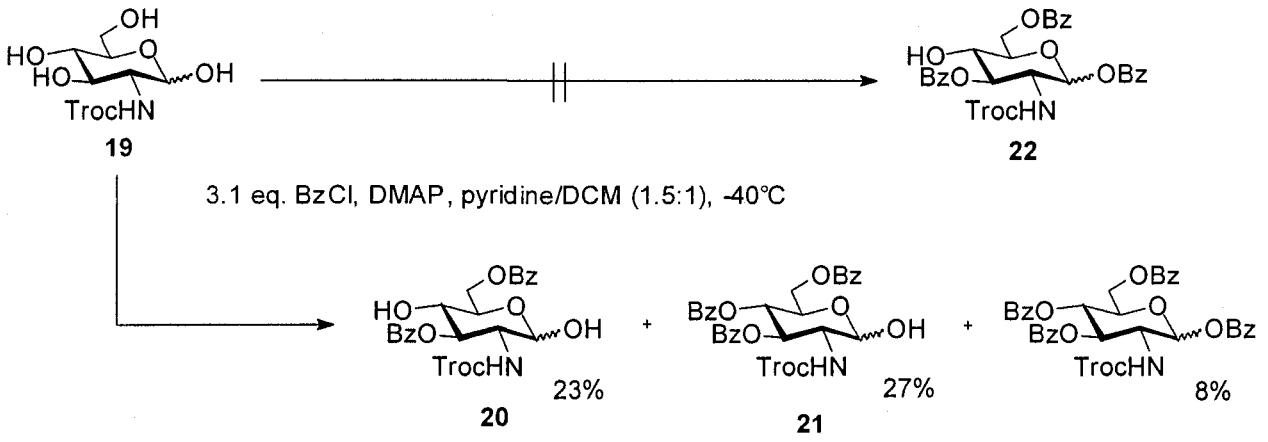


Scheme 12. Retrosynthetic analysis for disaccharide diosgenyl saponin analogues

2.3.3.1 Selective benzoylation of glucosamine derivative 19

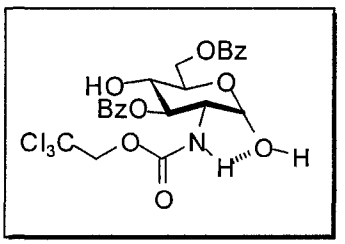
The key sugar of the disaccharide is the 2-amino-2-deoxy-glucopyranose, protected at 2-NH and rhamnosylated at 4-OH. The relative reactivity of hydroxyl groups in D-glucopyranose was estimated to be 6-OH>>3-OH≈1-OH>2-OH>4-OH.[89] We hoped that similar reactivity could be observed in glucosamine derivative **19**[90] so that selective benzoylation might lead to the formation of 1,3,6-tri-*O*-benzoylated **22** as the major

product. With its 4-OH free, **22** could serve as the proper glycosylation acceptor for the preparation of disaccharide intermediate **IV** (Scheme 12). Therefore, the known 2-deoxy-glucopyranosyl derivative **19**[90] was treated with 3.1 equivalents of benzoyl chloride (BzCl) and catalytic dimethylaminopyridine (DMAP) at -40 °C in a pyridine-dichloromethane mixture (1.5 : 1). The 3,6-di-*O*-benzoylated product **20** was obtained in 23% yield, the 3,4,6-tri-*O*-benzoylated product **21** in 27% yield, and the 1,3,4,6-tetra-*O*-benzoylated product in 8% yield, but no observed formation of desired **22** was detected (Scheme 13).



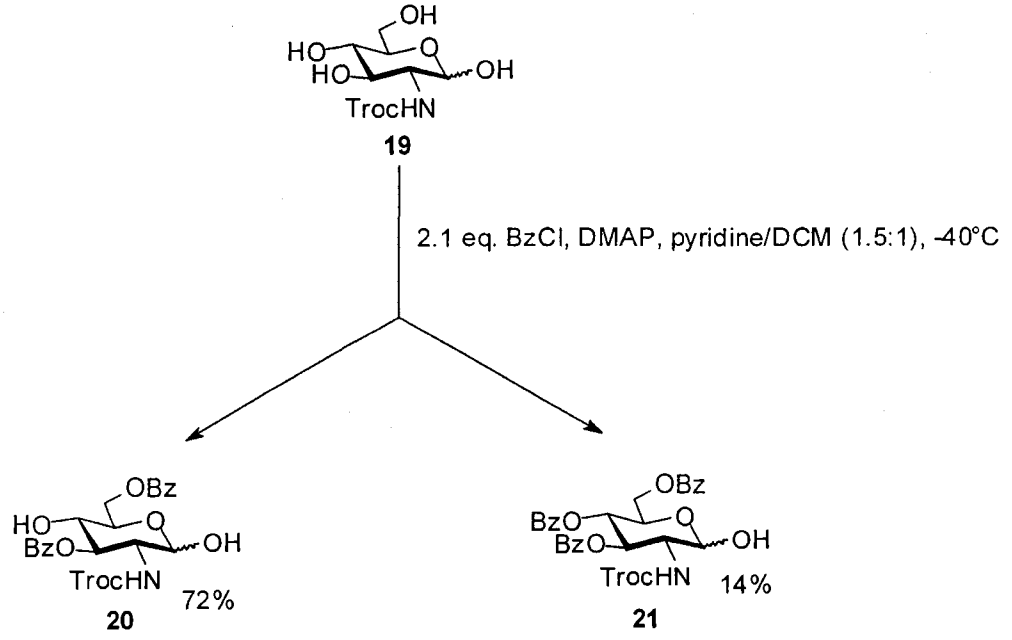
Scheme 13. Benzoylation of glucosamine derivative

As 1,3,6-tri-*O*-benzoylated **22** was not formed, Scheme 14 shows a possible conformation which explains 4-OH benzoylation over 1-OH. The anomeric hydroxyl group stabilizes the sugar in a 5-member ring formation. Intramolecular hydrogen bonding from the anomeric hydroxyl and the N-H group form this system. The hydrogen bonding decreases electron density on the oxygen and as a result nucleophilicity decreases. Further investigation is needed to provide evidence for the existence of such intramolecular hydrogen bonding.



Scheme 14. Possible intramolecular hydrogen bonding interaction in derivative **20**

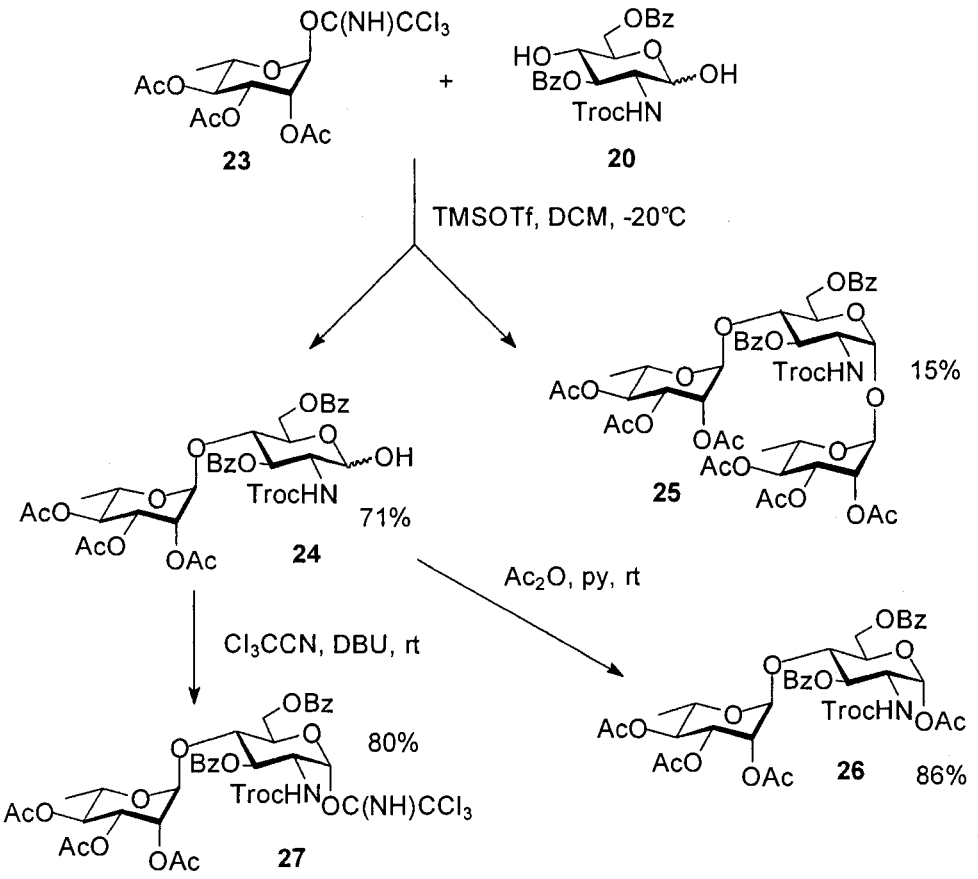
Since the observed reactivity of glucosamine derivative **19** was shown to be 6-OH>>3-OH>4-OH>1-OH, it was decided that compound **20** could be used as the acceptor. Therefore, glucosamine derivative **19** was treated with 2.1 equivalents of benzoyl chloride and catalytic DMAP at -40 °C in a pyridine-dichloromethane mixture (1.5 : 1) to yield the desired 3,6-di-*O*-benzoylated product **20** in 72% yield and 3,4,6-tri-*O*-benzoylated **21** in 14% yield as mostly the α isomers (Scheme 15). The ^{1}H NMR spectrum of **21** shows a signal at δ = 5.72 (dd, J = 9.5, 10.0 Hz) demonstrating the existence of 4-OH benzoylation as this signal is not present in the ^{1}H NMR spectrum of di-*O* -benzoylated **20**.



Scheme 15. Selective benzoylation of glucosamine derivative

2.3.3.2 Synthesis of disaccharide glycosyl imidate 27

Glycosylation of the readily available rhamnopyranosyl donor **23**[91] with **20** in the presence of TMSOTf as the catalyst at -20 °C in dichloromethane gave the desired 4-*O*-rhamnosylated disaccharide **24** in 71% yield (Scheme 16). The rhamnose connection was ascertained by ^{1}H NMR data as a broad singlet is observed at δ = 5.36 ppm. Observation of a di-rhamnopyranosyl trisaccharide **25** from a crude ^{1}H NMR spectrum was observed in approximately 15% yield but an analytically pure product was not obtained.



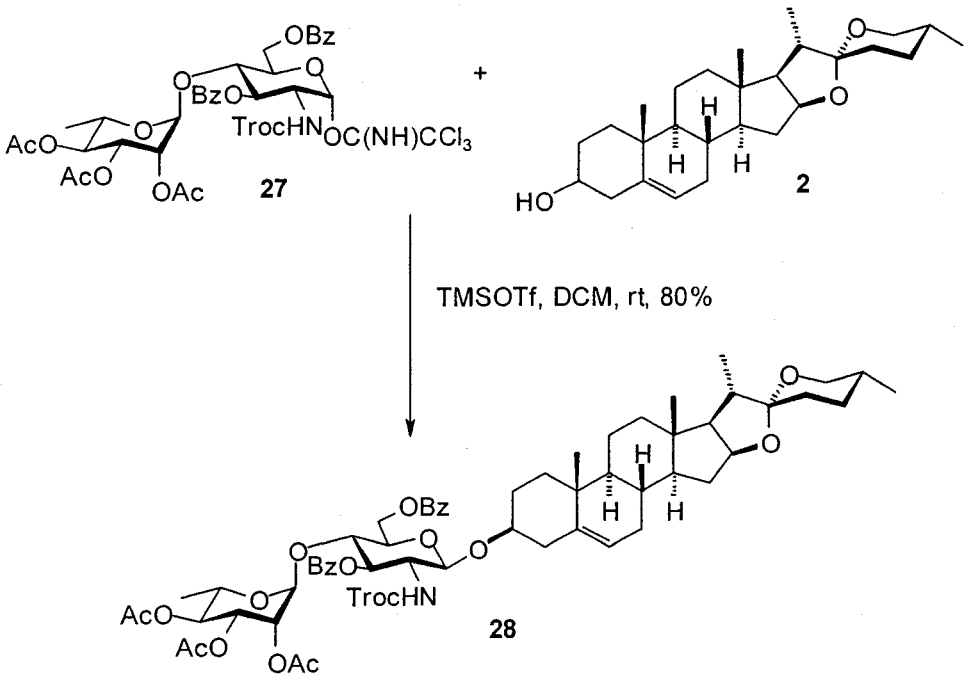
Scheme 16. Synthesis of disaccharide

Compound **24** was readily transformed into acetate derivative **26** by acetic anhydride in pyridine. The coupling constant of the ^{1}H NMR spectrum (δ 6.24, d, J = 3.5 Hz) confirmed that the anomeric position was acetylated, and that this glycosylation occurred at 4-OH.

Imidate formation was achieved within 20 min by addition of trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as base to provide the disaccharide trichloroacetimidate donor **27** in 80% yield. Longer reaction times in this reaction lead to decreasing yield as additional spots on TLC were observed. To further confirm that the anomeric position was indeed modified, ^{1}H NMR data of the anomeric proton of **27** (δ 6.42, d, J = 3.5 Hz) confirmed the existence of the α-imidate.

2.3.3.3 Glycosylation of disaccharide moiety 27 with the aglycone

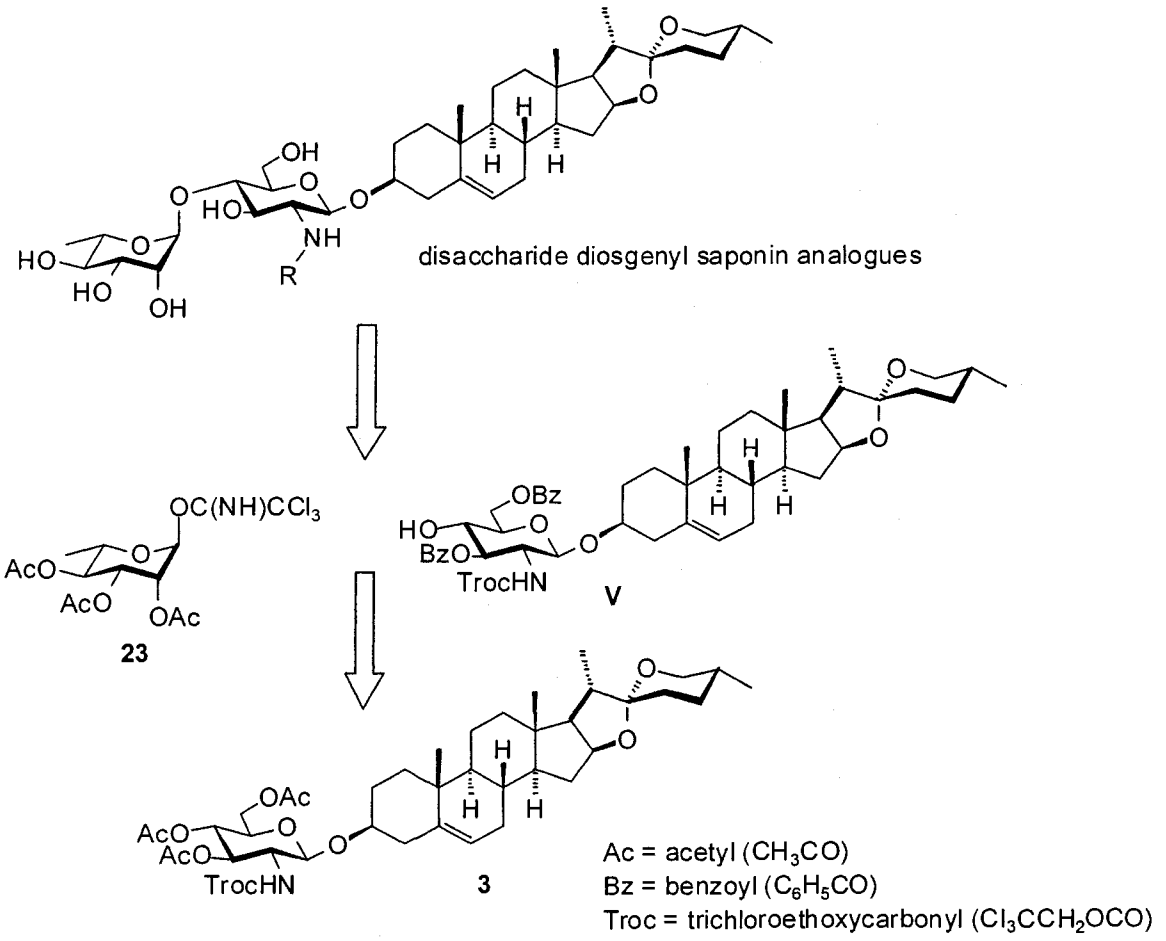
Glycosylation of disaccharide donor **27** with diosgenin in the presence of TMSOTf as the catalyst in dichloromethane at room temperature gave the desired steroidal glycoside **28** in 80% yield (Scheme 17). The signals of two anomeric carbons were found at 98.89 and 99.88 ppm in its ^{13}C NMR spectrum, while the presence of the 1,2-trans β-glycosidic linkage was verified by the observation of a ^{1}H doublet at δ = 4.77 (J = 8.0 Hz) in the ^{1}H NMR spectrum.



Scheme 17. Glycosylation of disaccharide donor

Although the yield was good for this convergent synthetic strategy, product **20** in Scheme 15 and product **24** in Scheme 16 exist as α/β mixtures and therefore have presented challenges in chromatographic separations. The alternate linear pathway was thus investigated, as intermediate **3** from the previous monomeric diosgenyl saponin analogue synthesis was readily available.

2.3.4 Linear synthesis strategy of disaccharide diosgenyl saponin analogues



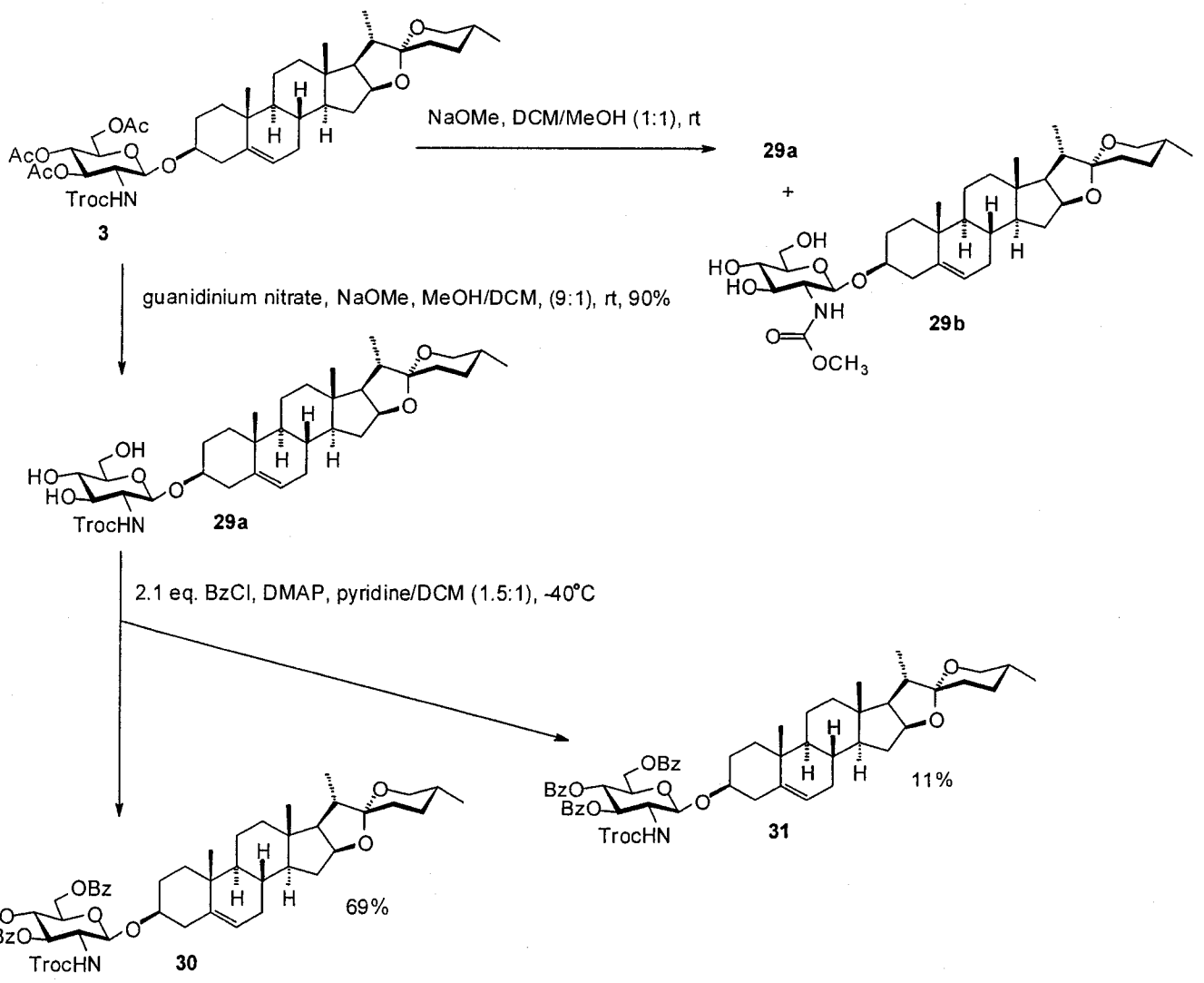
Scheme 18. Linear synthesis strategy for disaccharide analogues

The observed reactivity of glucosamine derivative **19** was shown to be 6-OH>>3-OH>4-OH>1-OH and a 3,6-di-benzoylated product **20** was shown to be readily formed.
As an alternative pathway for synthesizing disaccharide diosgenyl saponin analogues, the

linear tactic was chosen to reach the target molecules (Scheme 18). Suitably protected rhamnose donor **23** was attached to the partially protected acceptor **V** made from the previously synthesized intermediate **3**.

2.3.4.1 Synthesis of diosgenyl glycosyl acceptor 30

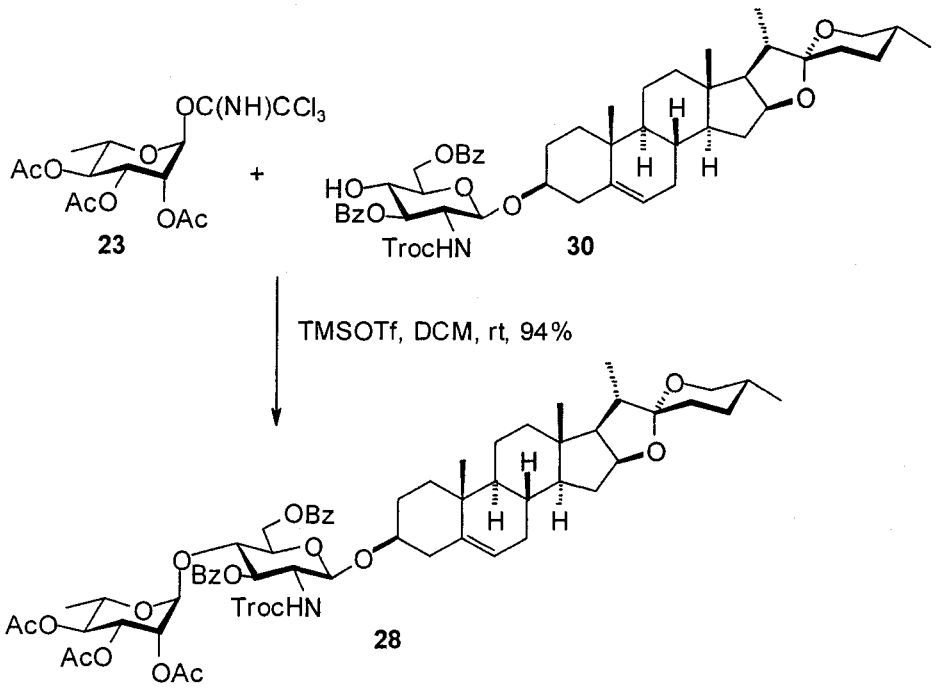
The previously synthesized diosgenyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside **3** is the intermediate for the synthesis of the diosgenyl glycosyl acceptor (Scheme 19). Initially, intermediate **3** was treated with sodium methoxide (NaOMe) in a methanol-dichloromethane mixture for subsequent acetyl group removal. However, selective *O*-deacetylation leaving the *N*-Troc group intact was unsuccessful as additional spots on TLC were observed. The ^{1}H NMR data of the crude product suggested that the corresponding methyl carbamate **29b** was formed as the side product. This carbamate was eventually formed as the major product when the reaction was extended long enough. Further investigation led to the guanidine/guanidinium nitrate method[92] for mild and selective *O*-deactylation which leaves the *N*-Troc group intact. Thus, intermediate **3** was treated with a stock solution composed of guanidinium nitrate and NaOMe in a methanol-dichloromethane mixture (9 : 1) affording the deacetylated glucopyranoside **29a** in 90% yield. Selective benzoylation of **29a** was achieved with 2.1 equivalents of BzCl and catalytic DMAP at -40 °C in a pyridine-dichloromethane mixture (1.5 : 1) to give the 3,6-di-*O*-benzoylated **30** in 69% yield. The tri-*O*-benzoylated product **31** was formed in approximately 11% yield, but an analytically pure product was not obtained. Precautionary action of freeze drying **29a** in dioxane had to be implemented to remove all methanol from the previous reaction step as methyl benzoate is readily formed. Consumption of benzoyl chloride by residual methanol interfered with the portion size of benzoyl chloride added in this selective reaction. Thus, the reaction was carefully monitored by TLC and the quantity of benzoyl chloride was adjusted accordingly. Additionally, the 3-*O*- and 6-*O*-sites of benzoylation were proven with ^{1}H-^{1}H COSY 2D NMR spectroscopy, as both the 3-*O*- and 4-*O*-benzoylated protons can give a double doublet splitting pattern in the ^{1}H NMR spectrum.



Scheme 19. Synthesis of the diosgenyl glycosyl acceptor

2.3.4.2 Glycosylation of diosgenyl glycosyl acceptor 30

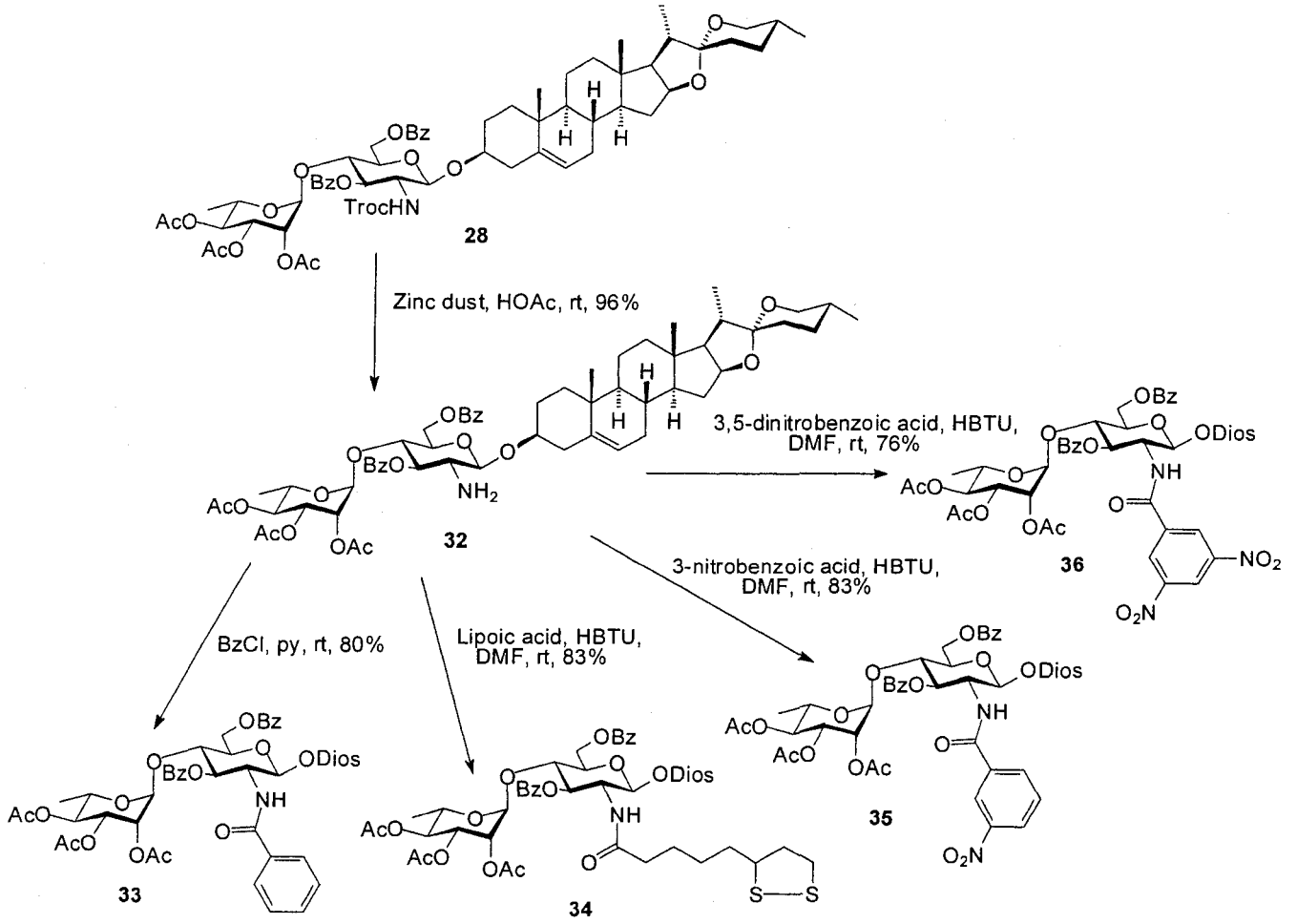
Glycosylation of rhamnopyranosyl donor **23** with acceptor **30** in the presence of TMSOTf as the catalyst in dichloromethane at room temperature gave the desired steroidal glycoside **28** in 94% yield (Scheme 20). The ^{1}H NMR and ^{13}C NMR spectra of the samples of **28** obtained from both synthetic pathways were indistinguishable indicating that the products of the two sequences were identical.



Scheme 20. Glycosylation of diosgenyl glycosyl acceptor

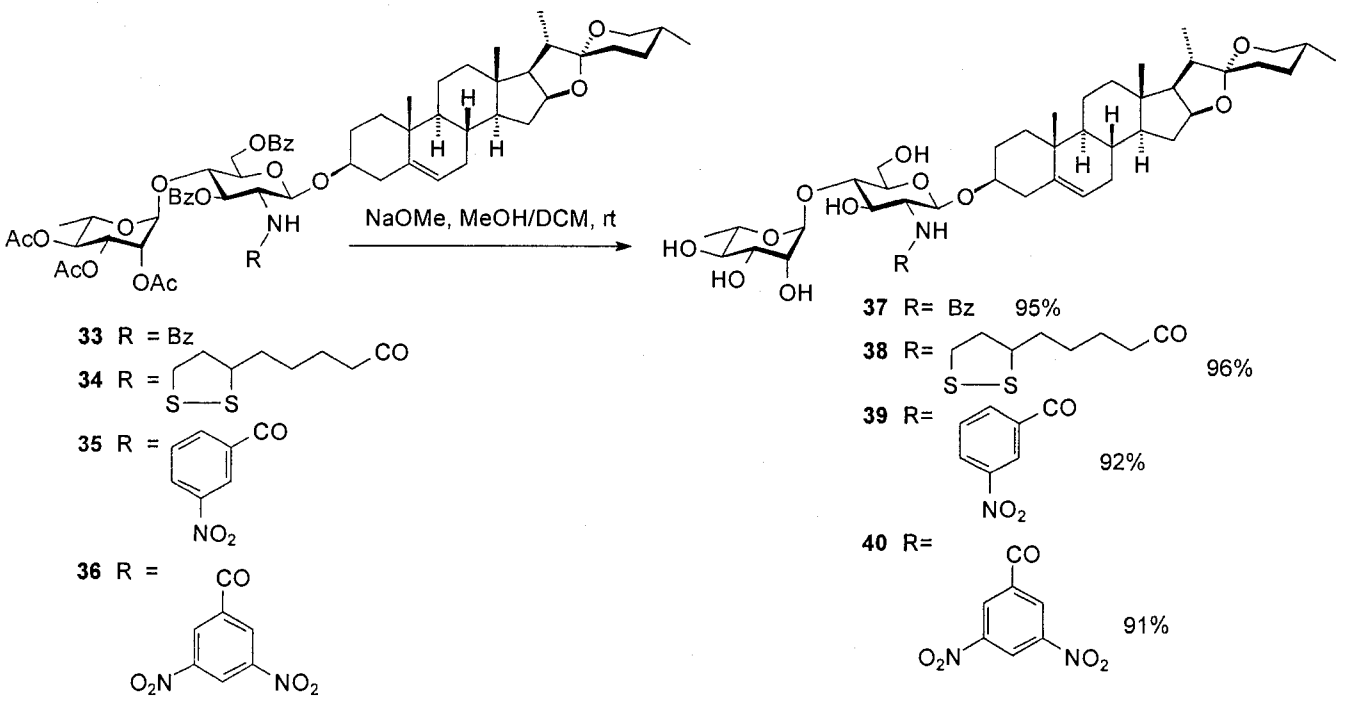
2.3.5 Synthesis of the disaccharide diosgenyl analogue series

Arriving at the same diosgenyl disaccharide intermediate **28** through both pathways, a variety of functional groups can now be added for structure activity relationship studies. Removal of the amino protecting Troc group on the 2-deoxy-2-aminosugar moiety of **28** was achieved under β-elimination conditions by treatment with zinc dust in acetic acid to afford **32** in 96% yield. Benzoyl chloride was coupled to **32** in pyridine at room temperature to give **33** in 80% yield. Lipoic acid was coupled to **32** by HBTU with catalytic DIPEA in dry DMF to give **34** in 83% yield. Compound **32** was treated with 3-nitrobenzoic acid in the presence of HBTU and catalytic DIPEA in dry DMF to yield **35** in 83% yield and in the same way 3,5-dinitrobenzoic acid was added to **32** under similar conditions to yield **36** in 76% yield (Scheme 21).



Scheme 21. Coupling of diosgenyl disaccharide with various acylating reagents

Finally, compounds **33**-**36** were subjected to deprotection with NaOMe in a methanol-dichloromethane mixture (2 : 1) to afford target analogues **37**-**40** in excellent yield (Scheme 22).



Scheme 22. Deprotection to target diosgenyl disaccharide analogues

2.4 Biological evaluation

All diosgenyl saponin analogues are new compounds with the exception of compound **10**. The synthesis of compound **10** was reported earlier,[81] but no biological activity has yet been described for this compound. The cytotoxicity of monosaccharide analogues **10-14**, **17** and **18** have been tested against several human cancer cell lines by Dr. Mary Lynn Tassotto and Dr. John Th'ng at the Thunder Bay Regional Health Sciences Centre and the results are described below (Table 1). Cell lines included in testing are SK-N-SH neuroblastoma, MCF-7 breast cancer, and HeLa cervical cancer cell lines. The rapid assay employed was the MTT method that measures cell viability, according to the method described by Carmichael *et al.*[93]

Compound	*Cancer cell line*		
	SK-N-SH	MCF-7	HeLa
10	11.6 ± 0.88	18.3 ± 0.63	21.5 ± 1.06
11	14.9 ± 1.62	19.4 ± 0.35	18.0 ± 1.41
12	14.1 ± 1.37	22.0 ± 1.41	13.9 ± 0.88
13	15.3 ± 0.42	11.0 ± 0.35	20.0 ± 1.27
14	4.8 ± 0.28	6.9 ± 0.60	7.3 ± 0.56
17	16.3 ± 0.56	11.3 ± 0.42	>20
18	19.5 ± 0.98	19.2 ± 0.56	>20
Doxorubicin		0.06	

Table 1. In vitro cytotoxic activity (IC_{50}, μM) of diosgenyl saponin analogues

The inhibitory concentration (IC_{50}) is the concentration that inhibits the growth of cells by 50% when compared to untreated cells. The IC_{50} results represent the average IC_{50} ± SD value obtained from a minimum of n = 2 independent MTT assays for each compound. As a control, the MCF-7 cells were treated with the chemotherapeutic agent Doxorubicin.

2.4.1 Inhibition effect, anticancer activity, and SAR

The structure activity relationship studies on diosgenyl saponins have been hampered by the availability of homogeneous saponins. The success of the chemical syntheses in this thesis presents an opportunity to investigate the SAR of these molecules. The most significant finding is that modification at the 2-*N*-position of the glycosyl moiety alters growth inhibition and shows some insight into the mechanism of antitumour activity. The inhibitory activity was observed to vary from 4.8 μM to greater than 20 μM for the monosaccharide analogues against all three cell lines. The compound that exhibits the greatest cytotoxicity was analogue **14** with a lipoic acid residue attached showing an IC_{50} value ranging from 4.8-7.3 μM against all three cell lines. Lipoic acid is an antioxidant that can be readily reduced to its open chain dihydrolipoic acid[94] and has been reported to exhibit antiproliferative action against WM35 and A350 melanoma cells.[95] The disulfide bond and redox property of compound **14** in relation to the anticancer action is worth further investigation. The dimeric analogue **18** shows the lowest cytotoxicity with an $IC_{50} \geq$ 19.2 μM and is noticeably less active then its monomer **17** ($IC_{50} \geq$ 11.3 μM) against all

three cell lines. The HeLa cervical cancer cells seem to be the most resistant to all the compounds with the exception of compound **12**. Compounds **13** and **17** are most active against MCF-7 breast cancer cells when compared to the other two cell lines. Compound **17** having an additional C_2H_4 in the side chain relative to **12**, is more active in MCF-7 cells (IC_{50} = 22.0 μM for **12** and IC_{50} = 11.3 μM for **17**) but, interestingly, their activity is reversed against HeLa cells (IC_{50} = 13.9 μM for **12** and IC_{50} > 20.0 μM for **17**). The detailed antitumour mechanism of the action of diosgenyl saponins still remains unclear. However, some insight has been detailed in the study herein. Compound **11** induces cell cycle arrest at G_1 phase in SK-N-SH neuroblastoma cells while in MCF-7 breast cancer cells it induces cell cycle arrest at G_2 phase (data not shown). The cell cycle arrest at different phases suggests cell death involves a different action mechanism against cancer cell lines from different tissue origins.

3 CONCLUSION

Saponins are natural occurring glycosides that are widely distributed in the plant kingdom and lower marine organisms. Diosgenyl saponins are among the most abundant steroidal saponins and possess a variety of biological activities, the most significant being the demonstration of anticancer activity through apoptosis in a variety of cancer cell lines. The chemical synthesis presented provides homogeneous diosgenyl saponins affording opportunities for understanding them and their anticancer mechanism of action. A set of mono- and disaccharide diosgenyl saponin analogues have thus been synthesized and the biological activity of the monosaccharide analogues has been tested.

Key structural features achieved for the monosaccharide analogue synthesis include the 1,2-*trans*-β-linkage at C-3 and the modification at C-2 of the glucosyl unit. A glycosyl donor possessing a neighbouring participating Troc group was used for the construction of a 1,2-*trans*-β-glycosidic linkage, the natural linkage in diosgenyl saponins, in a stereocontrolled manner. A TMSOTf glycosylation method has been successfully applied, utilizing the imidate protocol, which required only a catalytic amount of the Lewis acid for promotion under mild conditions. Various acyl substituents were successfully introduced at the 2-deoxy-2-amino of the sugar moiety, and, additionally one dimer was synthesized. The monosaccharide analogues **10-14**, **17** and **18** synthesized herein were indeed biologically active showing moderate cytotoxicity for most compounds. The most active being analogue **14** with a lipoic acid residue attached showing a IC_{50} value ranging from 4.8-7.3 μM. Different mechanisms of action were also exhibited as diosgenyl saponin analogue **11** was able to induce cell cycle arrest at G_1 phase in SK-N-SH cells, while, inducing cell cycle arrest at G_2 phase in MCF-7 cells.

A convergent strategy was employed for the synthesis of the disaccharide diosgenyl saponin analogues. Starting with glucosamine derivative **19**, it was found during selective benzoylation that the reactivity of the derivative was 6-OH>>3-OH>4-OH>1-OH. As a result, di-*O*-benzoylated **20** was synthesized as the glycosyl acceptor for the glycosylation of the α-L-rhamnose imidate **23** at the key 4-*O*-position using the TMSOTf protocol. This

was followed by subsequent glycosylation with diosgenin using the same TMSOTf method to yield intermediate **28**. Difficulties in chromatographic separation led to the more straightforward linear synthesis, in which the previously synthesized **3** was deprotected and then di-*O*-benzoylated to produce **30**. With 4-OH open, glycosylation with α-L-rhamnose moiety **23** was achieved to yield intermediate **28**. The amino group on **28** was deprotected and four different acylating agents were attached. Benzoyl and lipoic acid residues were attached for comparison to the monosaccharide analogues and two aromatic nitro groups were incorporated. The biological activity of the disaccharide diosgenyl saponin analogues is still under investigation.

In conclusion, the chemical syntheses presented have provided a better understanding of the structure activity relationships of diosgenyl saponins. The successful synthesis of new diosgenyl saponins and the development of the method provided can be used to easily modify or build a saponin library. With promising results from some of the compounds, it is of hope that the information presented will lead to the discovery of a clinically useful synthetic diosgenyl saponin.

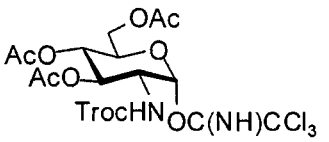
4 EXPERIMENTAL

4.1 General methods

All air and moisture sensitive reactions were performed under nitrogen atmosphere. All commercial reagents were used as supplied. Pyridine was distilled over potassium hydroxide, dichloromethane was distilled over calcium hydride, and methanol was distilled from magnesium turnings and a catalytic amount of iodine. Anhydrous *N,N*-dimethylformamide (DMF) was purchased from Aldrich. ACS grade solvents were purchased from Fisher and used for chromatography without distillation. TLC was performed on Silica Gel 60Å F_{254} (thickness 250 μm) purchased from Silicycle Inc., Canada, with detection by fluorescence (254 nm) and by dipping into 15% H_2SO_4 solution and/or Mostaine reagent [ammonium molybdate $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (20 g) and Cerium (IV) sulfate $Ce(SO_4)_2\cdot xH_2O$ (0.4 g) in 10% H_2SO_4 solution (400 ml)] followed by charring at ~120 °C. Flash column chromatography was performed on Silica Gel 60 (40-63 μm) also purchased from Silicycle Inc., Canada. ^1H and ^{13}C NMR spectra were recorded on a Varian Unity Inova 500 MHz spectrometer. Tetramethylsilane (TMS, δ 0.00 ppm) or solvent peaks were used as internal standards for ^1H and ^{13}C NMR spectra. The chemical shifts were given in ppm and coupling constants in Hz indicated to a resolution of ± 0.5 Hz. Multiplicity of proton signals is indicated as follows: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), m (multiplet), br (broad), and approx (approximate). For spectroscopic assignments, glucopyranosyl carbon atoms are designated with 1^I, 2^I, 3^I..., rhamnopyranosyl carbon atoms are designated with 1^{II}, 2^{II}, 3^{II} ..., and diosgenyl carbon atoms are designated with 1', 2', 3' ..., etc. Optical rotations were measured with a Perkin Elmer 241 Polarimeter at room temperature (20-22 °C) and a Perkin Elmer 343 Polarimeter at 22 °C. Low resolution electrospray ionization (ESI) mass spectra were obtained from a Biflex-IV MALDI linear/reflector instrument at the University of Manitoba, Canada, and high resolution (HR) ESI mass spectra were measured on the Applied Biosystems Mariner Biospectrometry Workstation at the University of Alberta, Canada. Elemental analyses were carried out on a CEC (SCP) 240-XA Analyzer instrument by Lakehead University Instrumentation Laboratory (LUIL).

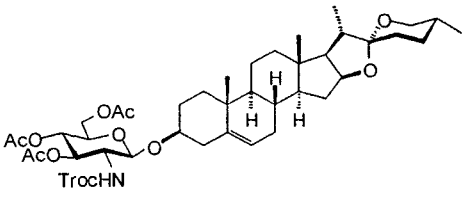
4.2 Synthetic procedures and structure characteristics

3,4,6-Tri-O-acetyl -2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-O-(α-D-glucopyranosyl) trichloroacetimidate (**1**):



According to literature procedures,[84] to a solution of 3,4,6-tri-*O*-acetyl -2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α/β-D-glucopyranose (5.52 g, 11.48 mmol) in anhydrous CH_2Cl_2 (20 mL), trichloroacetonitrile (CCl_3CN, 2.3 mL, 22.97 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 0.17 mL, 1.15 mmol) were added drop-wise. The solution was stirred for 1 h at room temperature, the solvent was removed *in vacuo*, and the residue was purified with flash column chromatography (hexane/EtOAc/Et_3N, 2:1:0.01) to afford compound **1** (6.03 g, 84%) as a white solid.

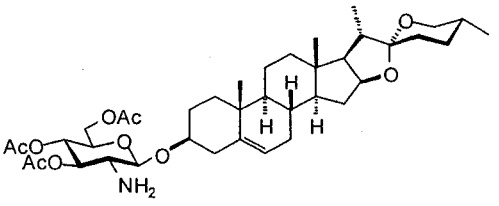
Diosgenyl 3,4,6-tri-O-acetyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino) -β-D-glucopyranoside (**3**):



A suspension of **1** (5.48 g, 8.77 mmol), diosgenin **2** (3.63 g, 8.64 mmol), and activated molecular sieves (4 Å, 2.0 g) in anhydrous CH_2Cl_2 (50 mL) was stirred at room temperature for 15 min then cooled to 0°C under N_2 atmosphere. A solution of trimethylsilyl trifluoromethanesulfonate (TMSOTf, 8.0 mL, 0.02 M in dry CH_2Cl_2) was added drop-wise. The mixture was stirred for 30 min, quenched by Et_3N (0.1 mL), and then filtered through Celite. The filtrate was concentrated *in vacuo* with the resulting

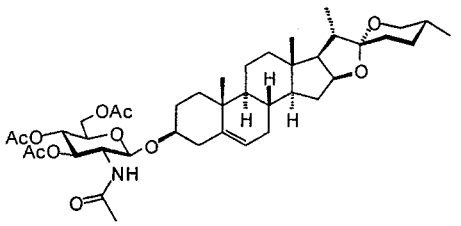
residue purified by flash column chromatography (CH_2Cl_2/MeOH, 100:1) to give compound **3** (7.53 g, 98%) as a white foam. R_f 0.36 (hexane/acetone, 1.5:1); $[\alpha]_D^{22}$ -34.72 (*c* 0.71, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.78 (s, 3H, CH_3), 0.80 (d, 3H, *J* = 6.0 Hz, CH_3), 0.98 (d, 3H, *J* = 7.0 Hz, CH_3), 0.99 (s, 3H, CH_3), 2.03 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 3.37 (dd, 1H, *J* = 11.0, 11.0 Hz, H-26'a), 3.46-3.55 (m, 3H, H-26'b, H-3', H-2), 3.72 (m, 1H, H-5), 4.09-4.15 (m, 1H, H-6a), 4.28 (dd, 1H, *J* = 12.0, 4.5 Hz, H-6b), 4.41 (approx. q, 1H, *J* ≈ 7.5 Hz, H-16'), 4.68 (d, 1H, *J* = 11.5 Hz, Troc-Ha), 4.79 (d, 1H, *J* = 11.5 Hz, Troc-Hb), 4.82 (d, 1H, *J* = 8.5, H-1), 5.05 (dd, 1H, *J* = 9.5, 10.0 Hz, H-4), 5.31 (m, 1H, H-6'), 5.38 (dd, 1H, *J* = 9.5, 10.5 Hz, H-3), 5.48 (d, 1H, *J* = 9.0 Hz, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.35, 14.68, 16.42, 17.29, 19.51, 20.81, 20.87, 20.95, 28.91, 29.54, 30.41, 31.49, 31.96, 32.18, 36.96, 37.24, 38.86, 39.86, 40.38, 41.72, 50.14, 56.60, 60.60, 62.18, 62.38, 66.96, 69.05, 71.70, 71.92, 74.50, 79.96, 80.93, 95.65, 99.35, 109.45, 122.02, 140.39, 154.17, 163.84, 169.71, 170.84, 170.94; ESI-HRMS *m/z*: $[M + Na]^+$ Calcd for $C_{42}H_{60}NO_{12}Cl_3Na$: 898.3073, found: 898.3072.

Diosgenyl 3,4,6-tri-O-acetyl-2-amino-2-deoxy-β-D-glucopyranoside (**4**):



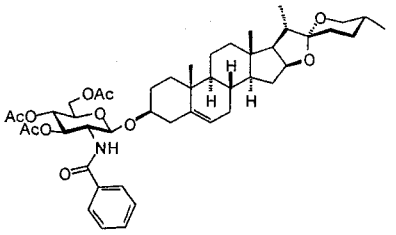
To a solution of **3** (1.65 g, 1.88 mmol) in acetic acid (40 ml), zinc dust (4.0 g) was added and the mixture was stirred at room temperature for 16 h. The mixture was filtered and the solid was thoroughly washed with CH_2Cl_2 (100 mL). The filtrate was concentrated *in vacuo* and the residue was re-dissolved with CH_2Cl_2 (400 mL). The solution was washed with saturated $NaHCO_3$ solution (50 mL), dried with Na_2SO_4, and concentrated under vacuum to give a white solid **4** (1.27 g, 96%) which was used without further purification in the next step.

Diosgenyl 3,4,6-tri-O-acetyl-2-acetamido-2-deoxy-β-D-glucopyranoside (**5**):



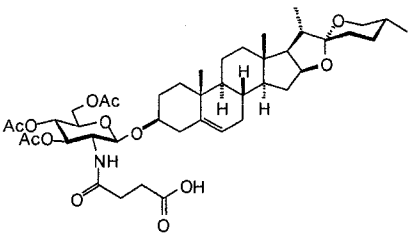
To a cooled solution (ice water bath) of **4** (97 mg, 0.14 mmol) in pyridine (3.2 mL), acetic anhydride (2.5 mL) was added. The mixture was stirred at room temperature for 4 h. The solution was diluted with CH_2Cl_2 (150 mL) and washed successively with cold HCl solution (4 N, 100 mL), and saturated $NaHCO_3$ solution (50 mL x 2). The organic layer was dried over anhydrous $NaSO_4$ and concentrated under vacuum. The residue was subjected to flash column chromatography (hexane/CH_2Cl_2/ethyl acetate, 3:3:7) to give the pure compound **5** (94 mg, 92%) as a white solid. R_f 0.40 (CH_2Cl_2/MeOH, 16:1); $[\alpha]_D^{22}$ -61.0 (*c* 0.36 $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.79 (s, 3H, CH_3), 0.79 (d, 3H, *J* = 6.5 Hz, CH_3), 0.87 (d, 3H, *J* = 7.0 Hz, CH_3), 0.89 (s, 3H, CH_3), 1.96 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 3.38 (dd, 1H, *J* = 11.0, 11.0 Hz, H-26'a), 3.48 (dd, 1H, *J* = 11.0, 4.5 Hz, H-26'b), 3.50 (m, 1H), 3.69-3.78 (m, 2H), 4.10 (dd, 1H, *J* = 12.0, 2.0 Hz, H-6a), 4.26 (dd, 1H, *J* = 12.0, 5.0 Hz, H-6b), 4.40 (approx. q, 1H, *J* ≈ 8.0 Hz, H-16'), 4.85 (d, 1H, *J* = 8.0 Hz, H-1), 5.05 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4), 5.34 (m, 1H, H-6'), 5.38 (dd, 1H, *J* = 9.5, 9.5 Hz, H-3), 5.46 (d, 1H, *J* = 8.5 Hz, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.34, 14.74, 16.49, 17.35, 19.59, 21.02, 22.87, 23.64, 29.00, 29.64, 29.92, 30.50, 31.59, 31.80, 32.04, 32.23, 37.06, 37.35, 39.04, 39.94, 40.47, 41.81, 50.23, 55.71, 56.68, 62.27, 62.48, 67.07, 69.02, 71.84, 72.35, 79.81, 81.02, 99.45, 109.53, 122.10, 140.57, 169.10, 170.50, 170.80, 171.10; ESI-HRMS m/z: $[M + Na]^+$ Calcd for $C_{41}H_{61}NO_{11}Na$: 766.4137, found: 766.4140.

Diosgenyl 3,4,6-tri-O-acetyl-2-benzamido-2-deoxy-β-D-glucopyranoside (**6**):



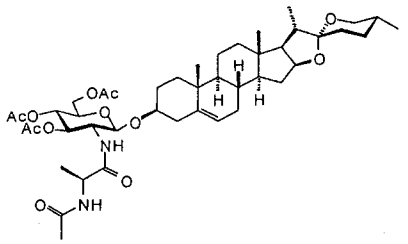
To a cooled solution (ice water bath) of **4** (160 mg, 0.23 mmol) in pyridine (2.0 mL), benzoyl chloride (0.03 mL, 0.26 mmol) was added. The mixture was stirred at room temperature for 4 h. The solution was diluted with EtOAc (150 mL), washed with cold HCl solution (4 N, 50 mL) and saturated $NaHCO_3$ solution (50 mL x 3). The organic layer was dried over anhydrous $NaSO_4$ and concentrated under vacuum. The residue was subjected to flash column chromatography (hexane/CH_2Cl_2/ethyl acetate) 3:3:1.5 to give the pure compound **6** (155 mg, 84%) as a white solid. R_f 0.56 (CH_2Cl_2/MeOH, 25:1); $[\alpha]_D^{22}$ -34.7 (*c* 0.36 $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.76 (s, 3H, CH_3), 0.78 (d, 3H, *J* = 5.5 Hz, CH_3), 0.95 (s, 3H, CH_3), 0.96 (d, 3H, *J* = 7.5 Hz, CH_3), 1.99 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 2.09 (s, 3H, CH_3CO), 3.37 (dd, 1H, *J* = 11.0, 11.0 Hz, H-26'a), 3.46 (dd, 1H, *J* = 11.0, 3.5 Hz, H-26'b), 3.50 (m, 1H, H-2), 3.76-3.78 (m, 1H, H-5), 3.98 (m, 1H, H-3), 4.11 (brd, 1H, *J* = 11.5 Hz, H-6a), 4.30 (dd, 1H, *J* = 12.0, 4.7 Hz, H-6b), 4.39 (approx. q, 1H, *J* ≈ 7.5 Hz, H-16'), 4.95 (d, 1H, *J* = 8.0 Hz, H-1), 5.12 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4), 5.25 (m, 1H, H-6'), 5.52 (dd, 1H, *J* = 9.5, 9.5 Hz, H-3), 6.23 (d, 1H, *J* = 9.0 Hz, NH), 7.40-7.51 (m, 3H, 3 Ar-H), 7.70 (m, 2H, 2 Ar-H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.71, 16.45, 17.33, 19.53, 20.91, 21.01, 28.97, 29.62, 30.47, 31.55, 32.00, 32.22, 37.00, 37.34, 38.99, 39.91, 40.43, 41.77, 50.19, 55.84, 56.64, 62.24, 62.53, 67.03, 68.69, 71.94, 72.43, 79.95, 80.98, 99.75, 109.49, 121.96, 127.02, 128.90, 131.90, 134.54, 140.56, 167.91, 169.69, 170.98, 171.16; ESI-HRMS *m/z*: $[M + Na]^+$ Calcd for $C_{46}H_{63}NO_{11}Na$: 828.4293, found: 828.4289.

Diosgenyl 3,4,6-tri-O-acetyl-2-(3-carboxylpropanamido)-2-deoxy-β-D-glucopyranoside (**7**):



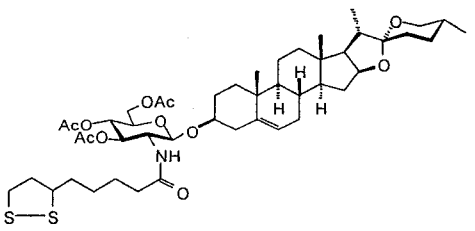
To a cooled solution (ice water bath) of **4** (193 mg, 0.27 mmol) in pyridine (3.0 mL), succinic anhydride (33 mg, 0.33 mmol) was added. The mixture was stirred at room temperature for 4 h. The solvent was removed *in vacuo* and the residue was diluted with CH_2Cl_2 (150 mL) and washed with ice water (20 mL x 2). The organic layer was dried over anhydrous $NaSO_4$ and concentrated under vacuum. The residue was subjected to flash column chromatography (CH_2Cl_2/MeOH) 25:1 to give the pure compound **7** (141 mg, 73%) as a white solid. R_f 0.43 (CH_2Cl_2/MeOH, 12:1); $[\alpha]_D^{22}$ -58.8 (*c* 0.11 $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.78 (s, 3H, CH_3), 0.79 (d, 3H, *J* = 7.0 Hz, CH_3), 0.95 (d, 3H, *J* = 7.5 Hz, CH_3), 1.00 (s, 3H, CH_3), 2.01 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 2.46 (t, 2H, *J* = 7.5 Hz, CH_2CO), 2.70 (m, 2H, CH_2CO), 3.37 (dd, 1H, *J* = 11.0, 11.0 Hz, H-26'a), 3.45 (dd, 1H, *J* = 11.0, 5.0 Hz, H-26'b), 3.70-3.77 (m, 2H, H-5, H-3'), 4.10 (dd, 1H, *J* = 12.5, 2.5 Hz H-6a), 4.26 (dd, 1H, *J* = 12.5, 5.5 Hz, H-6b), 4.39 (approx. q, 1H, *J* ≈ 8.0 Hz, H-16'), 4.85 (d, 1H, *J* = 8.0 Hz, H-1), 5.02 (dd, 1H, *J* = 10.0, 10.0 Hz, H-4), 5.32 (m, 1H, H-6'), 5.37 (dd, 1H, *J* = 10.0, 10.0 Hz, H-3), 6.03 (d, 1H, *J* = 8.5 Hz, NH); ESI-HRMS *m/z*: $[M + Na]^+$ Calcd for $C_{43}H_{63}NO_{13}Na$: 824.4192, found: 824.4193.

Diosgenyl 3,4,6-tri-O-acetyl-2-[(S)-2-acetamido-propanamido]-2-deoxy-β-D-glucopyranoside (**8a**):



To a solution of **4** (300 mg, 0.43 mmol) in dry DMF (5.0 mL), *N*-acetyl-L-alanine (56 mg, 0.43 mmol), diisopropylethylamine (DIPEA, 0.15 mL, 0.86 mmol), and 2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HBTU, 163 mg, 0.43 mmol) were added and the mixture was stirred for 16 h. The solvent was removed *in vacuo* and the residue was dissolved with CH_2Cl_2 (150 mL) and washed with H_2O (10 mL x 2). The organic layer was dried over anhydrous $NaSO_4$ and concentrated under vacuum. The residue was subjected to flash column chromatography (CH_2Cl_2/MeOH, 25:1) to give compound **8a** (272 mg, 78%) as a white solid. R_f 0.36 (CH_2Cl_2/MeOH, 25:1); $[\alpha]_D^{22}$ -53.2 (*c* 0.64, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.81 (s, 3H, CH_3), 0.82 (d, 3H, *J* = 6.0 Hz, CH_3), 0.98 (d, 3H, *J* = 7.0 Hz, CH_3), 1.02 (s, 3H, CH_3), 1.99 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 2.04 (s, 3H, CH_3CO), 2.10 (s, 3H, CH_3CO), 3.37 (m, 1H, H-26'a), 3.48-3.51 (m, 2H, H-26'b, H-2), 3.74-3.83 (m, 2H, H-3', H-5), 4.12 (dd, 1H, *J* = 12.0, 2.0 Hz, H-6a), 4.29 (m, 1H, *J* = 12.0, 4.5 Hz, H-6b), 4.29 (m, 1H, C*H*NHAc), 4.43 (approx. q, 1H, *J* ≈ 7.5 Hz, H-16'), 4.80 (d, 1H, *J* = 8.5 Hz, H-1), 4.91 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4), 5.20 (dd, 1H, *J* = 10.5, 10.5 Hz, H-3), 5.26 (m, 1H, H-6'), 7.62 (d, 1H, *J* = 7.5 Hz, NH), 7.87 (d, 1H, *J* = 8.5 Hz, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.74, 16.50, 17.35, 18.27, 19.59, 20.90, 21.01, 23.40, 29.01, 29.67, 30.51, 31.60, 32.05, 32.27, 37.04, 37.34, 39.03, 39.95, 40.47, 41.82, 49.15, 50.26, 55.32, 56.69, 62.30, 62.52, 67.07, 69.10, 71.81, 72.31, 79.88, 81.00, 98.15, 108.16, 120.68, 139.30, 168.34, 169.00, 169.60, 169.61, 171.54; ESI-HRMS *m/z*: $[M + Na]^+$ Calcd for $C_{44}H_{66}N_2O_{12}Na$: 837.4508, found: 837.4506.

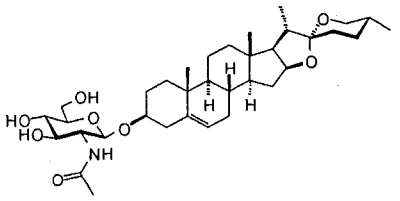
Diosgenyl 3,4,6-tri-O-acetyl-2-deoxy-2-{5-[(R/S)-1,2-dithiolan-3-yl]-pentanamido}-β-D-glucopyranoside (**9**):



To a solution of **4** (150 mg, 0.21 mmol) in dry DMF (5.0 mL), (±)-α-lipoic acid (44 mg, 0.21

mmol), DIPEA (0.075 mL, 0.43 mmol), and HBTU (82 mg, 0.21 mmol) were added and the mixture was stirred for 16 h. The solvent was removed *in vacuo* and the residue was dissolved with CH_2Cl_2 (150 mL) and washed with H_2O (10 mL x 2). The organic layer was dried over anhydrous $NaSO_4$ and concentrated under vacuum. The residue was subjected to flash column chromatography (hexane/EtOAc/MeOH, 1:1:0.025) to give compound **9** (158 mg, 83%) as a white solid. R_f 0.32 (hexane/EtOAc/MeOH, 1:1:0.025); $[\alpha]_D^{22}$ -175.0 (*c* 0.03 $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 0.78 (s, 3H, CH_3), 0.79 (d, 3H, *J* = 6.5 Hz, CH_3), 0.96 (d, 3H, *J* = 7.0 Hz, CH_3), 0.99 (s, 3H, CH_3), 2.01 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 2.23 (m, 1H, CH*H*CO), 2.45 (m, 1H, C*H*HCO), 3.08-3.20 (m, 2H, CH_2S), 3.17 (m, 1H), 3.37 (dd, 1H, *J* = 10.5, 10.5 Hz, H-26'a), 3.45-3.56 (m, 3H, H-26'b, H-2, CHS), 3.65 (m, 1H, H-3') 3.70 (m, 1H, H-5), 4.09 (dd, 1H, *J* = 12.0, 2.5 Hz, H-6a), 4.26 (dd, 1H, *J* = 12.0, 5.0 Hz, H-6b), 4.40 (approx. q, 1H, *J* ≈ 7.5 Hz, H-16'), 4.89 (two sets of dd, each 0.5H, *J* = 8.0 Hz, H-1), 5.03 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4), 5.33 (m, 1H, H-6'), 5.41 (two sets of dd, each 0.5H, *J* = 10.0, 10.0 Hz, H-3), 5.48 (two sets of d, each 0.5H, *J* = 8.5 Hz, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.72, 16.49, 17.34, 19.59, 20.88, 20.96, 25.42, 25.47, 28.98, 29.64, 30.48, 31.59, 32.04, 32.28, 34.78, 34.83, 36.67, 37.04, 37.32, 38.65, 39.11, 39.92, 40.45, 41.79, 50.21, 55.73, 56.07, 56.58, 56.66, 62.26, 62.49, 67.04, 69.08, 71.81, 72.27, 79.72, 79.95, 80.99, 99.32, 109.50, 122.11, 140.54, 169.71, 170.98, 170.99, 172.98; ESI-HRMS *m/z*: $[M + Na]^+$ Calcd for $C_{47}H_{71}NO_{11}S_2Na$: 912.4360, found: 912.4358.

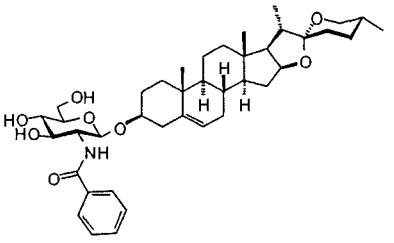
Diosgenyl 2-acetamido-2-deoxy-β-D-glucopyranoside (**10**):



Compound **5** (100 mg, 0.13 mmol) was dissolved in MeOH/CH_2Cl_2 (2:1, 9 mL) and then NaOMe solution in methanol (0.6 mL) was added until pH 10. After stirring for 4 h at room temperature, the mixture was neutralized by adding weak acidic ion-exchange resin

(Amberlite IRC-50, H^+ form). The resin was filtered and the filtrate concentrated *in vacuo*. The residue was applied to a Sephadex LH-20 gel filtration column ($MeOH/CH_2Cl_2$, 2:1) to yield compound **10** (81 mg, 98%) as a white solid. R_f 0.21 (CH_2Cl_2/MeOH, 12:1); $[\alpha]_D^{22}$ -66.6 (*c* 0.26, $CH_3OH/CHCl_3$, 1:1); lit.[81] $[\alpha]_D^{22}$ -75 (*c* 0.5, $CH_3OH/CHCl_3$, 1:1); ^1H NMR (500 MHz, C_5D_5N plus two drops of CD_3OD): δ 0.73 (d, 3H, *J* = 6.0 Hz, CH_3), 0.84 (s, 3H, CH_3), 0.96 (s, 3H, CH_3), 1.18 (d, 3H, *J* = 7.0 Hz, CH_3), 2.18 (s, 3H, CH_3CO), 3.50 (dd, 1H, *J* = 10.5, 10.5 Hz, H-26'a), 3.60 (dd, 1H, *J* =10.5, 3.5 Hz, H-26'b), 3.83-3.88 (m, 1H, H-3'), 3.91-3.94 (m, 1H, H-5), 4.14 (m, 1H, H-2), 4.30 (dd, 1H, *J* = 12.0, 6.0 Hz, H-6a), 4.37-4.44 (m, 2H, H-3, H-4), 4.50 (dd, 1H, *J* = 10.0, 2.0 Hz, H-6b), 4.55 (approx. q, 1H, *J* = 7.0 Hz, H-16'), 5.21 (d, 1H, *J* = 7.5 Hz, H-1), 5.35 (m, 1H, H-6'), 8.89 (d, 1H, *J* = 8.0 Hz, NH); ^{13}C NMR (125 MHz, C_5D_5N plus two drops of CD_3OD): δ 15.54, 16.86, 17.82, 19.92, 21.57, 24.08, 29.73, 30.64, 31.07, 32.12, 32.28, 32.66, 32.74, 37.50, 37.91, 40.00, 40.34, 40.93, 42.43, 50.71, 57.13, 58.49, 63.13, 63.34, 67.33, 72.81, 76.76, 78.98, 78.99, 81.57, 101.27, 109.74, 122.20, 141.44, 171.01; ESI-MS *m/z*: $[M + H]^+$ Calcd for $C_{35}H_{56}NO_8$: 618.83, found: 618.64. The NMR spectral data are in general agreement with the reported data[81] which were measured in DMSO-d_6.

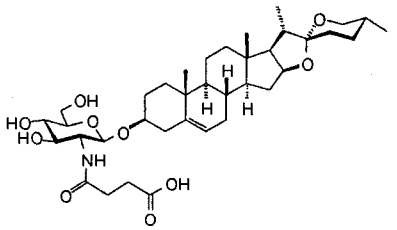
Diosgenyl 2-benzamido-2-deoxy-β-D-glucopyranoside (**11**):



In a similar way as described for the preparation of **10**, compound **6** (71 mg, 0.09 mmol) was treated with sodium methoxide in $MeOH/CH_2Cl_2$ (2:1) to give **11** (57 mg, 95%) as a white solid. R_f 0.18 (CH_2Cl_2/MeOH, 12:1); $[\alpha]_D^{22}$ -28.0 (*c* 0.16, $CHCl_3$/MeOH, 1:1); ^1H NMR (500 MHz, C_5D_5N): δ 0.69 (d, 3H, *J* = 5.5 Hz, CH_3), 0.82 (s, 3H, CH_3), 0.83 (s, 3H, CH_3), 1.14 (d, 3H, *J* = 6.0 Hz, CH_3), 2.43 (m, 1H), 2.69 (m, 1H), 3.50 (dd, 1H, *J* = 10.5, 10.5 Hz, H-26'a), 3.59 (dd, 1H, *J* = 10.5, 3.0 Hz, H-26'b), 3.90 (m, 1H, H-3'), 4.06 (m, 1H, H-5),

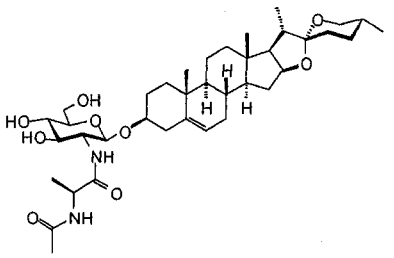
4.32 (m, 1H, H-6a), 4.43 (m, 1H, H-2), 4.54 (approx. q, 1H, J = 7.5, 7.5 Hz, H-16'), 4.60 (brd, 1H, J = 11.0 Hz, H-6b), 4.68-4.80 (m, 2H, H-3, H-4), 5.22 (m, 1H, H-6'), 5.56 (d, 1H, J = 8.5 Hz, H-1), 6.58 (s, 1H, O-H), 7.37-7.44 (m, 5H, 3 Ar-H, 2 OH), 8.39 (d, 2H, J = 6.5 Hz, 2 Ar-H), 9.44 (d, 1H, J = 8.0 Hz, NH); ^{13}C NMR (125 MHz, C_5D_5N): δ 15.51, 16.80, 17.80, 19.80, 21.51, 29.71, 30.47, 30.64, 31.05, 32.04, 32.25, 32.68, 37.43, 37.87, 40.09, 40.28, 40.89, 42.40, 50.64, 57.07, 59.47, 63.31, 67.31, 73.10, 76.30, 79.10, 79.17, 81.54, 101.16, 109.71, 122.19, 128.51, 129.06, 131.67, 137.32, 141.26, 141.28, 168.94; ESI-MS *m/z*: $[M + H]^+$ Calcd for $C_{40}H_{58}NO_8$: 680.41, found: 680.69.

Diosgenyl 2-(3-carboxylpropanamido)-2-deoxy-β-D-glucopyranoside (**12**):



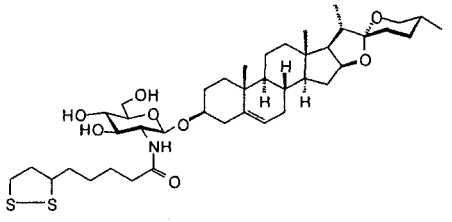
In a similar way as described for the preparation of **10**, Compound **7** (66 mg, 0.08 mmol) was treated with sodium methoxide in MeOH/CH_2Cl_2 (2:1) to give **12** (55 mg, quantitatively) as a white solid. R_f 0.28 ($CHCl_3$/MeOH/H_2O, 6:4:0.5); $[\alpha]_D^{22}$ -29.4 (*c* 0.14 $CHCl_3$/MeOH, 1:1); ^{1}H NMR (500 MHz, CD_3OD): δ 0.69 (s, 3H, CH_3), 0.70 (d, 3H, J = 5.5 Hz, CH_3), 0.86 (d, 3H, J = 7.0 Hz, CH_3), 0.93 (s, 3H, CH_3), 2.40 (m, 4H), 3.14-3.26 (m, 3H), 3.32-3.50 (m, 3H), 3.54-3.60 (m, 1H), 3.74-3.82 (m, 1H), 4.29 (approx. q, 1H, J = 7.5 Hz, H-16'), 4.46 (d, 1H, J = 8.0 Hz, H-1), 4.63-4.68 (m, 1H), 5.27 (m, 1H, H-6'), 8.89 (d, 1H, J = 9.5 Hz, NH); ^{13}C NMR (125 MHz, CD_3OD): δ 15.90, 16.96, 17.69, 20.04, 22.16, 30.07, 30.83, 31.63, 32.61, 32.96, 32.97, 33.10, 33.36, 33.58, 38.15, 38.68, 40.18, 41.10, 41.60, 43.08, 51.84, 57.97, 57.98, 63.00, 63.92, 68.03, 72.15, 76.20, 78.05, 80.42, 82.38, 101.28, 110.74, 122.80, 142.09, 175.93, 178.86; ESI-MS *m/z*: $[M + Na]^+$ Calcd for $C_{37}H_{57}NO_{10}Na$: 698.85, found: 698.86.

Diosgenyl 2-[(S)-2-acetamido-propanamido]-2-deoxy-β-D-glucopyranoside (**13**):



In a similar way as described for the preparation of **10**, compound **8a** (168 mg, 0.21 mmol) was treated with sodium methoxide in MeOH/CH_2Cl_2 (2:1) to give **13** (141 mg, quantitatively) as a white solid. R_f 0.21 (CH_2Cl_2/MeOH, 8:1); $[\alpha]_D^{22}$ -44.8 (*c* 0.36, $CHCl_3$/MeOH, 1:1); ^{1}H NMR (500 MHz, $CDCl_3/CD_3OD$, 1:1): δ 0.71 (s, 3H, CH_3), 0.72 (d, 3H, *J* = 5.0 Hz, CH_3), 0.88 (d, 3H, *J* = 7.0 Hz, CH_3), 0.92 (s, 3H, CH_3), 1.29 (d, 3H, *J* = 7.0 Hz, CH_3), 1.91 (s, 3H, CH_3CO), 3.21 (m, 1H), 3.26 (m, 1H), 3.30 (m, 1H), 3.38 (m, 1H), 3.40-3.51 (m, 1H), 3.64 (dd, 1 H, *J* = 12.0, 5.0 Hz, H-6a), 3.78 (dd, 1 H, *J* = 12.0, 2.5 Hz, H-6b), 4.24 (q, 1H, *J* = 7.0 Hz, C*H*NHAc), 4.33 (approx. q, 1H, *J* = 7.5 Hz, H-16'), 4.47 (d, 1H, *J* = 8.0 Hz, H-1), 5.25 (m, 1H, H-6'); ^{13}C NMR (125 MHz, $CDCl_3/CD_3OD$, 1:1): δ 15.63, 17.56, 18.27, 18.95, 20.59, 22.29, 23.66, 30.10, 30.89, 31.65, 32.71, 32.92, 33.10, 33.50, 38.27, 38.65, 40.21, 41.17, 41.75, 43.11, 50.73, 51.63, 57.98, 58.58, 63.22, 63.53, 68.33, 72.33, 75.40, 77.57, 80.53, 82.53, 100.65, 111.14, 123.11, 141.95, 173.20, 175.20; ESI-MS *m/z*: $[M + Na]^+$ Calcd for $C_{38}H_{60}N_2O_9Na$: 711.42, found: 711.57.

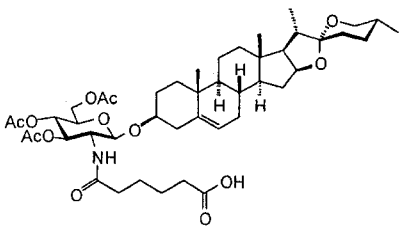
Diosgenyl 2-deoxy-2-{5-[(R/S)-(1,2-dithiolan-3-yl)]-pentamido}-β-D-glucopyranoside (**14**):

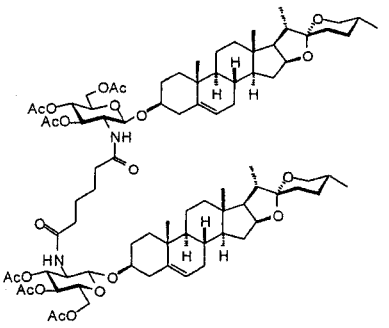


In a similar way as described for the preparation of **10**, compound **9** (139 mg, 0.16 mmol) was treated with sodium methoxide in MeOH/CH_2Cl_2 (2:1) to give **14** (119 mg,

quantitatively) as a white solid. R_f 0.24 (CH_2Cl_2/MeOH, 12:1); $[\alpha]_D^{22}$ -44.8 (*c* 0.21 $CHCl_3$/MeOH, 1:1); ^{1}H NMR (500 MHz, C_5D_5N): δ 0.70 (d, 3H, *J* = 5.5 Hz, CH_3), 0.85 (s, 3H, CH_3), 0.99 (s, 3H, CH_3), 1.15 (d, 3H, *J* = 7.0 Hz, CH_3), 2.22 (m, 1H), 2.48 (t, 2H, *J* = 7.0 Hz), 2.54 (m, 1H), 2.72 (m, 1H), 2.97-3.08 (m, 2H, CH_3S), 3.47-3.53 (m, 2H, H-26'a, CHS), 3.60 (dd, 1H, *J* = 12.0, 3.5 Hz, H-26'b), 3.87-3.94 (m, 1H, H-3'), 4.00 (m, 1H, H-5), 4.25 (dd, 1 H, *J* = 9.0, 9.0 Hz, H-4), 4.40 (dd, 1 H, *J* = 12.0, 5.5 Hz, H-6a), 4.50-4.59 (m, 4H, H-2, H-3, H-6b, H-16'), 5.32 (two sets of d, each 0.5H, *J* = 8.0 Hz, H-1), 5.36 (m, 1H, H-6'), 8.82 (d, 1H, *J* = 7.5 Hz, NH); ^{13}C NMR (125 MHz, C_5D_5N): δ 15.51, 16.83, 17.79, 19.99, 21.57, 26.54, 29.74, 30.68, 31.06, 32.14, 32.26, 32.66, 32.76, 35.46, 37.28, 37.52, 37.89, 39.12, 40.14, 40.32, 40.86, 40.92, 42.42, 50.70, 57.12, 57.28, 58.45, 63.20, 63.33, 67.31, 72.99, 76.79, 78.89, 79.02, 81.54, 101.22, 109.72, 122.25, 141.45, 173.74; ESI-MS *m/z*: $[M + H]^+$ Calcd for $C_{41}H_{66}NO_8S_2$: 764.42, found: 764.70.

Diosgenyl 3,4,6-tri-O-acetyl-2-(5-carboxylpentamido)-2-deoxy-β-D-glucopyranoside (**15**) and *N,N'-bis(diosgenyl 3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosid-2-yl) hexandiamide* (**16**):



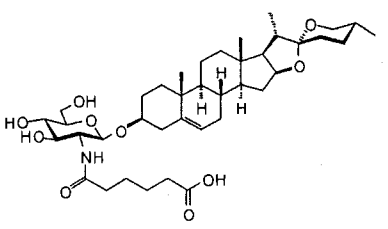


In a similar way as described for the preparation of **8a**, compound **4** (130 mg, 0.19 mmol) was treated with adipic acid (12 mg, 0.085 mmol), DIPEA (0.064 mL, 0.37 mmol), and HBTU (70 mg, 0.19 mmol) in dry DMF (5.0 mL) to the monomeric product **15** (15 mg, 21%) and the dimeric product **16** (71 mg, 56%). For **15**: R_f 0.24 (hexane/EtOAc/MeOH, 1:1:0.1); $[\alpha]_D^{22}$ -36.8 (*c* 0.25, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.78 (s, 3H, CH_3), 0.79 (d, 3H, *J* = 7.0 Hz, CH_3), 0.96 (d, 3H, *J* = 7.0 Hz, CH_3), 0.99 (s, 3H, CH_3), 2.01 (s, 6H, 2-

CH_3CO), 2.07 (s, 3H, CH_3CO), 2.07 (s, 3H, CH_3CO), 2.29 (m, 2H), 2.34 (m, 2H), 3.37 (dd, 1 H, J = 11.0, 11.0 Hz, H-26'a), 3.46-3.52 (m, 2H, H-26'b, H-2), 3.72-3.77 (m, 2H, H-5, H-3'), 4.10 (dd, 1H, J = 12.0, 1.5 Hz, H-6a), 4.28 (dd, 1H, J = 12.0, 4.5 Hz, H-6b), 4.41 (approx. q, 1H, $J \approx 7.5$ Hz, H-16'), 4.85 (d, 1H, J = 8.5 Hz, H-1), 5.03 (dd, 1H, J = 9.5, 10.0 Hz, H-4), 5.31 (m, 1H, H-6'), 5.38 (dd, 1H, J = 9.5, 10.5 Hz, H-3), 5.86 (d, 1H, J = 9.0 Hz, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.73, 16.50, 17.35, 19.58, 20.88, 20.96, 21.02, 21.03, 24.30, 24.99, 28.98, 29.63, 29.91, 30.47, 31.56, 31.57, 32.03, 32.26, 36.36, 37.03, 37.33, 39.05, 39.93, 40.45, 41.79, 50.21, 55.37, 56.67, 62.24, 62.53, 67.05, 69.14, 71.75, 72.50, 79.76, 81.00, 99.44, 109.54, 122.03, 140.57, 169.73, 171.00, 171.22, 173.12, 178.16; ESI-MS *m/z*: $[M + H]^+$ Calcd for $C_{45}H_{68}NO_{13}$: 830.47, found: 830.71.

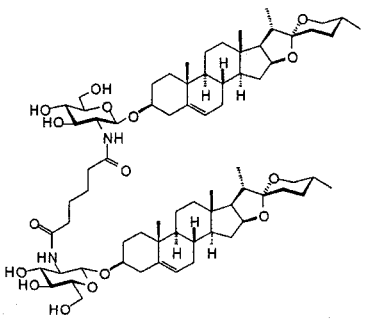
For **16**: R_f 0.29 (hexane/EtOAc/MeOH, 1:1:0.1); $[\alpha]_D^{22}$ -61.7 (*c* 0.29, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.78 (s, 3H, CH_3), 0.80 (d, 3H, J = 7.0 Hz, CH_3), 0.97 (d, 3H, J = 7.0 Hz, CH_3), 0.99 (s, 3H, CH_3), 2.02 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 2.08 (s, 3H, CH_3CO), 2.19 (m, 2H), 3.37 (dd, 1H, J = 11.0, 11.0 Hz, H-26'a), 3.48 (dd, 1H, J = 11.0, 4.0 Hz, H-26'b), 3.50 (m, 1H, H-2), 3.73-3.81 (m, 2H, H-5, H-3'), 4.10 (dd, 1H, J = 12.0, 2.5 Hz, H-6a), 4.28 (dd, 1H, J = 12.0, 5.0 Hz, H-6b), 4.41 (approx. q, 1H, $J \approx 7.5$ Hz, H-16'), 4.90 (d, 1H, J = 8.5 Hz, H-1), 5.06 (dd, 1H, J = 9.5, 10.0 Hz, H-4), 5.29 (m, 1H, H-6'), 5.45 (dd, 1H, J = 9.5, 10.5 Hz, H-3), 6.13 (d, 1H, J = 8.5 Hz, NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ 14.74, 16.51, 17.36, 19.61, 20.91, 21.03, 21.10, 24.31, 28.99, 29.61, 29.91, 30.50, 31.58, 31.59, 32.04, 32.28, 36.06, 37.06, 37.35, 39.05, 39.92, 40.46, 41.80, 50.23, 55.34, 56.67, 62.26, 62.49, 67.06, 69.25, 71.72, 72.76, 79.59, 80.98, 99.33, 109.52, 121.97, 140.69, 169.70, 170.98, 171.17, 173.01; ESI-HRMS *m/z*: $[M + Na]^+$ Calcd for $C_{84}H_{124}N_2O_{22}Na$: 1535.8538, found: 1535.8533.

Diosgenyl 2-(5-carboxylpentamido)-2-deoxy-β-D-glucopyranoside (**17**):



In a similar way as described for the preparation of **10**, compound **15** (26 mg, 0.03 mmol) was converted to **17** (22 mg, quantitatively) as a white solid. R_f 0.20 (CH_2Cl_2/MeOH, 12:1); $[\alpha]_D^{22}$ -14.0 (*c* 0.14, $CHCl_3$/MeOH, 1:1); ^{1}H NMR (500 MHz, C_5D_5N): δ 0.68 (d, 3H, *J* = 5.0 Hz, CH_3), 0.81 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 1.13 (d, 3H, *J* = 7.0 Hz, CH_3), 2.52 (m, 5H), 2.70 (m, 1H), 3.50 (dd, 1H, *J* = 10.5, 10.5 Hz, H-26'a), 3.58 (dd, 1H, *J* = 10.5, 3.5 Hz, H-26'b), 3.88 (m, 1H, H-3'), 3.97 (m, 1H, H-5), 4.21 (dd, 1H, *J* = 9.0, 9.0 Hz, H-4), 4.36 (dd, 1H, *J* = 12.0, 5.5 Hz, H-6a), 4.43-4.58 (m, 4H, H-2, H-3, H-6b, H-16'), 5.25 (d, 1H, *J* = 7.5 Hz, H-1), 5.33 (m, 1H, H-6'), 8.82 (d, 1H, *J* = 8.0 Hz, NH); ^{13}C NMR (125 MHz, C_5D_5N): δ 15.30, 16.62, 17.59, 19.73, 21.34, 25.60, 26.39, 29.51, 30.49, 30.85, 31.87, 32.05, 32.43, 32.50, 34.92, 37.18, 37.28, 37.69, 39.80, 40.11, 40.70, 42.31, 50.49, 56.89, 58.28, 63.11, 64.74, 67.11, 72.83, 76.70, 78.74, 78.83, 81.34, 101.04, 109.52, 121.94, 141.29, 173.64, 176.06; ESI-MS *m/z*: $[M + H]^+$ Calcd for $C_{39}H_{62}NO_{10}$: 704.92, found: 704.71.

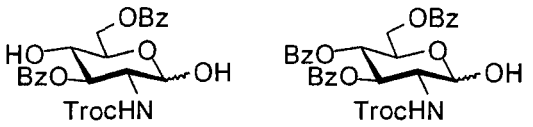
N,N'-Bis(diosgenyl 2-deoxy-β-D-glucopyranosid-2-yl) hexandiamide (**18**):



In a similar way as described for the preparation of **10**, compound **16** (27 mg, 0.02 mmol) was converted to **18** (22 mg, 98%) as a white solid. R_f 0.35 (CH_2Cl_2/MeOH, 5:1); $[\alpha]_D^{22}$ -39.2 (*c* 0.14, $CHCl_3$/MeOH, 1:1); ^{1}H NMR (500 MHz, C_5D_5N): δ 0.68 (d, 3H, *J* = 5.5 Hz, CH_3), 0.86 (s, 3H, CH_3), 0.95 (s, 3H, CH_3), 1.15 (d, 3H, *J* = 7.0 Hz, CH_3), 2.49 (m, 1H), 2.55 (m, 1H), 2.68 (m, 1H), 3.50 (dd, 1H, *J* = 10.5, 10.5 Hz, H-26'a), 3.59 (dd, 1H, *J* = 10.5, 2.5 Hz, H-26'b), 3.89 (m, 1H, H-3'), 3.95 (m, 1H, H-5), 4.21 (dd, 1H, *J* = 9.0, 9.0 Hz, H-4), 4.36 (dd, 1H, *J* = 12.0, 5.5 Hz, H-6a), 4.45 (dd, 1H, *J* = 10.0, 9.0 Hz, H-3), 4.52-4.58 (m, 3H, H-2, H-6b, H-16'), 5.25 (d, 1H, *J* = 8.5 Hz, H-1), 5.32 (m, 1H, H-6'), 8.75 (d, 1H, *J* = 9.0 Hz, NH); ^{13}C NMR (125 MHz, C_5D_5N): δ 15.52, 16.89, 17.80, 19.96, 21.58, 26.20,

29.73, 30.69, 31.06, 32.12, 32.28, 32.69, 32.76, 37.20, 37.51, 37.89, 39.92, 40.33, 40.94, 42.44, 50.71, 57.12, 58.36, 63.28, 63.34, 67.32, 72.93, 76.95, 78.80, 79.01, 81.56, 101.16, 109.73, 122.21, 141.41, 174.09; ESI-HRMS *m/z*: $[M + Na]^+$ Calcd for $C_{72}H_{112}N_2O_{16}Na$: 1283.7904, found: 1283.7903.

3,6-Di-O-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α/β-D-glucopyranose (**20**) and *3,4,6-Tri-O-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α/β-D-glucopyranose* (**21**):

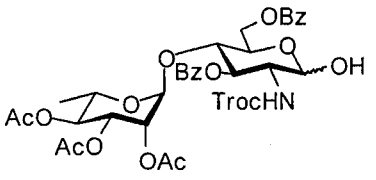


To a solution of **19** (3.0 g, 8.46 mmol) and 4-dimethylaminopyridine (DMAP, 103 mg, 0.10 mmol) in anhydrous pyridine (9 mL) and dry CH_2Cl_2 (6 mL), benzoyl chloride (2.06 mL, 17.77 mmol) in dry CH_2Cl_2 (8 mL) was added drop-wise under N_2 atmosphere at -40 °C. The reaction was carefully monitored by TLC every fifteen minutes. After being stirred at room temperature for 2 h, the mixture was quenched with MeOH. The solution was then diluted with CH_2Cl_2 (200 mL) and washed with cold HCl solution (4 N, 30 mL) and saturated $NaHCO_3$ solution (30 mL). The organic layer was dried over anhydrous $NaSO_4$ and concentrated under vacuum. The residue was subjected to flash column chromatography (hexane/EtOAc, 1.5:1) to yield compound **20** (3.43 g, 72%) as a white foam and **21** (666 mg, 14%) both as anomeric mixtures (α/β, 8:1). For **20α**: R_f 0.26 (hexane/EtOAc, 1.5:1); $[\alpha]_D^{22}$ 0 (*c* 0.50 $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.40 (brs, 1H, OH), 3.69 (brs, 1H, OH), 3.87 (dd, 1H, *J* = 10.0, 9.5 Hz, H-4), 4.21 (m, 1H, H-2), 4.34 (m, 1H, H-5), 4.41 (d, 1H, *J* = 12.0 Hz, Troc-Ha), 4.66 (dd, 1H, *J* = 12.0, 2.0 Hz, H-6a), 4.74 (dd, 1H, *J* = 12.0, 4.0 Hz, H-6b), 4.76 (d, 1H, *J* = 12.0 Hz, Troc-Hb), 5.40 (d, 1H, *J* = 3.5 Hz, H-1), 5.65 (dd, 1H, *J* = 10.5, 9.5 Hz, H-3), 5.81 (d, 1H, *J* = 9.0 Hz, NH), 7.44 (m, 4H, 4 Ar-H), 7.57 (m, 2H, 2 Ar-H), 8.04 (m, 4H, 4 Ar-H); ^{13}C NMR (125 MHz, $CHCl_3$): δ 54.06, 63.58, 69.63, 70.80, 74.44, 74.68, 92.44, 95.45, 128.68, 129.13, 129.80, 130.08, 130.31, 133.57, 133.91, 154.43, 167.23, 168.07; Anal. Calcd for $C_{23}H_{22}Cl_3NO_9$: C, 49.09; H, 3.94;

N, 2.49. Found: C, 49.68; H, 3.84; N, 2.23.

For **21α**: R_f 0.48 (hexane/EtOAc, 1.5:1); $[\alpha]_D^{22}$ +26.4 (*c* 0.50 $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 3.92 (brs, 1H, OH), 4.33 (m, 1H, H-2), 4.41 (dd, 1H, *J* = 12.5, 4.0 Hz, H-6a), 4.47 (d, 1H, *J* = 12.0 Hz, Troc-Ha), 4.63-4.69 (m, 2H, H-5, H-6b), 4.70 (d, 1H, *J* = 12.0 Hz, Troc-Hb), 5.47 (s, 1H, H-1), 5.70 (d, 1H, *J* = 10.0 Hz, NH), 5.72 (dd, 1H, *J* = 9.5, 10.0 Hz, H-4), 5.90 (dd, 1H, *J* = 10.5, 10.0 Hz, H-3), 7.26-7.57 (m, 10H, 10 Ar-H), 7.92 (m, 3H, 3 Ar-H), 8.05 (m, 2H, 2 Ar-H); ^{13}C NMR (125 MHz, $CHCl_3$): δ 54.73, 63.00, 68.40, 69.49, 71.41, 74.54, 92.31, 95.37, 128.60, 128.63, 128.97, 129.03, 129.71, 130.01, 130.15, 133.43, 133.64, 154.43, 165.42, 166.63, 166.87; Anal. Calcd for $C_{30}H_{26}Cl_3NO_{10}$: C, 54.03; H, 3.93; N, 2.10. Found: C, 54.03; H, 3.92; N, 1.89.

2,3,4-Tri-O-acetyl-α-L-rhamnopyranosyl-(1→4)-3,6-di-O-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-α/β-D-glucopyranose (**24**):

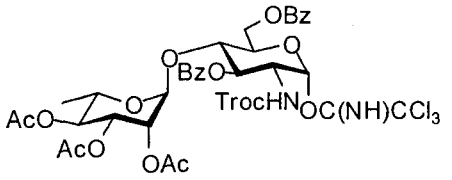


A suspension of **20** (5.0 g, 8.88 mmol), **23** (3.88 g, 8.88 mmol), and activated molecular sieves (4 Å, 3.0 g) in anhydrous CH_2Cl_2 (15 mL) was stirred under N_2 atmosphere at room temperature for 30 min, then cooled to -20 °C. A solution of trimethylsilyl trifluoromethanesulfonate (TMSOTf, 5.5 mL, 0.02 M in dry CH_2Cl_2) was added drop-wise. The mixture was stirred for 30 min, quenched by saturated $NaHCO_3$ solution (10 mL), extracted with CH_2Cl_2 (3 x 20 mL) and dried over $NaSO_4$.The filtrate was concentrated *in vacuo* with the resulting residue purified by flash column chromatography (hexane/EtOAc, 1.5:1) to give compound **24** (5.26 g, 71%) as a white foam. R_f 0.29 (hexane/EtOAc, 1.5:1); $[\alpha]_D^{22}$ +19.8 (*c* 0.50, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 0.68 (d, 3H, *J* = 6.0 Hz, CH_3), 1.89 (s, 3H, CH_3CO), 1.96 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 3.70-3.74 (m, 2H, H-5^{II}, OH), 4.15 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4^{I}), 4.19 (m, 1H, H-2^{I}), 4.40 (d, 1H, *J* = 12.0 Hz, Troc-Ha), 4.41 (m, 1H, H-5^{I}), 4.48 (dd, 1H, *J* = 12.5, 3.0 Hz, H-6^{I}a), 4.65 (d, 1H, *J* = 12.0

Hz, Troc-Hb), 4.87-4.94 (m, 3H, H-4^{II}, H-1^{I}, H-6^{I}b), 5.13-5.18 (m, 2H, H-2^{II}, H-3^{II}), 5.36 (brs, 1H, H-1^{II}), 5.75 (dd, 1H, *J* = 11.0, 10.5 Hz, H-3^{I}), 5.78 (d, 1H, *J* = 10.0 Hz, NH), 7.40-7.62 (m, 6H, 6 Ar-H), 8.08 (m, 4H, 4 Ar-H); ^{13}C NMR (125 MHz, $CHCl_3$): δ 17.04, 20.99, 54.91, 62.74, 67.60, 68.95, 69.41, 70.16, 70.52, 72.12, 74.47, 75.91, 92.10, 95.31, 98.36, 128.54, 128.70, 129.58, 130.03, 130.35, 133.46, 133.63, 154.70, 166.29, 166.59, 170.05, 170.32, 170.58; Anal. Calcd for $C_{35}H_{38}Cl_3NO_{16}$: C, 50.34; H, 4.59; N, 1.68. Found: C, 50.31; H, 4.55; N, 1.52.

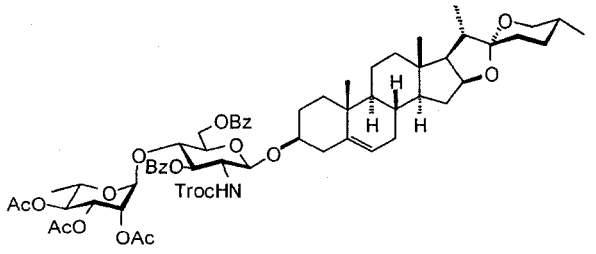
*For structure confirmation that **24** was rhamnosylated at 4-OH, **24** was converted into the acetate **26**.* To a cooled solution (ice water bath) of **24** (78 mg, 0.09 mmol) in pyridine (2.1 mL), acetic anhydride (1.7 mL) was added. The mixture was stirred at room temperature for 4 h. The solution was diluted with CH_2Cl_2 (150 mL) and washed successively with cold HCl solution (4 N, 20 mL), and saturated $NaHCO_3$ solution (20 mL x 3). The organic layer was dried over anhydrous $NaSO_4$ and concentrated under vacuum. The residue was subjected to flash column chromatography (hexane/ethyl acetate, 1.5:1) to give compound **26** (70 mg, 86%) as a white solid. R_f 0.73 (CH_2Cl_2/MeOH, 25:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.67 (d, 3H, *J* = 6.0 Hz, CH_3), 1.91 (s, 3H, CH_3CO), 1.97 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 2.28 (s, 3H, CH_3CO), 3.70 (m, 1H, H-5^{II}), 4.17 (dd, 1H, *J* = 9.5, 10.0 Hz, H-4^{I}), 4.18 (m, 1H, H-2^{I}), 4.33 (m, 1H, H-5^{I}), 4.39 (d, 1H, *J* = 12.0 Hz, Troc-Ha), 4.51 (dd, 1H, *J* = 12.5, 2.5 Hz, H-6^{I}a), 4.67 (d, 1H, *J* = 12.0 Hz, Troc-Hb), 4.75 (m, 1H, H-6^{I}b), 4.89 (dd, 1H, *J* = 10.0, 9.5 Hz, H-4^{II}), 4.90 (brs, 1H, H-1^{II}), 5.15 (m, 3H, H-2^{II}, H-3^{II}, NH) 5.65 (dd, 1H, *J* = 11.0, 10.5 Hz, H-3^{I}), 6.24 (d, 1H, *J* = 3.5 Hz, H-1^{I}), 7.42-7.50 (m, 4H, 4 Ar-H), 7.59 (m, 2H, 2 Ar-H), 8.05 (m, 4H, 4 Ar-H).

2,3,4-Tri-O-acetyl-α-L-rhamnopyranosyl-(1→4)-3,6-di-O-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-O-α-D-glucopyranosyl trichloroacetimidate (**27**):



To a solution of **24** (500 mg, 0.58 mmol) in anhydrous CH_2Cl_2 (3 mL), trichloroacetonitrile (CCl_3CN, 1.0 mL, 9.97 mmol) and cesium carbonate (Cs_2CO_3, 377 mg, 1.16 mmol) were added and stirred for 20 min at room temperature. The solvent was removed *in vacuo* and the residue was purified with flash column chromatography (hexane/EtOAc/Et_3N, 1.5:1:0.05) to afford compound **27** (466 mg, 80%) as a white solid. R_f 0.29 (hexane/EtOAc, 1.5:1); $[\alpha]_D^{22}$ +26.2 (*c* 0.50, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 0.68 (d, 3H, *J* = 6.0 Hz, CH_3), 1.90 (s, 3H, CH_3CO), 1.96 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 3.71 (m, 1H, H-5^{II}), 4.22 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4^{I}), 4.30 (m, 1H, H-5^{I}), 4.42 (m, 1H, H-2^{I}), 4.48 (d, 1H, *J* = 12.0 Hz, Troc-Ha), 4.53 (dd, 1H, *J* = 12.5, 3.5 Hz, H-6^{I}a), 4.63 (d, 1H, *J* = 12.0 Hz, Troc-Hb), 4.79 (dd, 1H, *J* = 12.5, 1.5 Hz, H-6^{I}b), 4.88 (dd, 1H, *J* = 10.0, 10.0 Hz, H-4^{I}), 4.91 (brs, 1H, H-1^{II}), 5.15 (m, 1H, H-2^{II}), 5.17 (dd, 1H, *J* = 10.0, 3.5 Hz, H-3^{II}), 5.22 (d, 1H, *J* = 10.0 Hz, NH), 5.71 (dd, 1H, *J* = 11.0, 9.5 Hz, H-3^{I}), 6.42 (d, 1H, *J* = 3.5 Hz, H-1^{I}), 7.47 (m, 4H, 4 Ar-H), 7.60 (m, 2H, 2 Ar-H), 8.06 (m, 4H, 4 Ar-H), 8.81 (s, 1H, NH); ^{13}C NMR (125 MHz, $CHCl_3$): δ 17.06, 20.88, 20.92, 20.96, 54.46, 62.20, 67.68, 68.58, 70.11, 70.74, 71.66, 71.85, 74.61, 75.89, 90.83, 94.87, 95.09, 99.03, 128.65, 128.69, 129.19, 129.81, 129.99, 130.25, 133.43, 133.92, 154.44, 160.70, 165.94, 166.73, 170.01, 170.20; Anal. Calcd for $C_{37}H_{38}Cl_6N_2O_{16}$: C, 45.37; H, 3.91; N, 2.86. Found: C, 45.12; H, 3.32; N, 2.33.

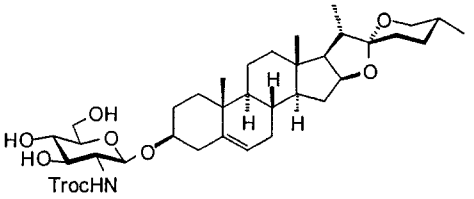
Diosgenyl 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→4)-3,6-di-O-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (**28**):



Method 1: A suspension of **27** (230 mg, 0.22 mmol), diosgenin **2** (95 mg, 0.22 mmol), and activated molecular sieves (4 Å, 2.0 g) in anhydrous CH_2Cl_2 (3 mL) was stirred at room temperature for 15 min under N_2 atmosphere. A solution of trimethylsilyl

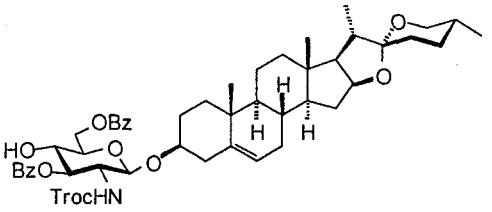
trifluoromethanesulfonate (TMSOTf, 0.45 mL, 0.01 M in dry CH_2Cl_2) was added drop-wise. The mixture was stirred for 15 min, quenched by saturated $NaHCO_3$ solution (10 mL), extracted with CH_2Cl_2 (3 x 10 mL) and dried over $NaSO_4$. The filtrate was concentrated *in vacuo* with the resulting residue purified by flash column chromatography (hexane/EtOAc/MeOH, 5:2:0.05) to give compound **28** (221 mg, 80%) as a white foam. *Method 2*: A suspension of **30** (230 mg, 0.22 mmol), **23** (191 mg, 0.44 mmol), and activated molecular sieves (4 Å, 2.0 g) in anhydrous CH_2Cl_2 (5 mL) was stirred at room temperature for 15 min under N_2 atmosphere. A solution of trimethylsilyl trifluoromethanesulfonate (TMSOTf, 0.44 mL, 0.01 M in dry CH_2Cl_2) was added drop-wise. The mixture was stirred for 30 min, quenched by saturated $NaHCO_3$ solution (5 mL), extracted with CH_2Cl_2 (3 x 20 mL) and dried over $NaSO_4$. The filtrate was concentrated *in vacuo* with the resulting residue purified by flash column chromatography (hexane/EtOAc/MeOH, 5:2:0.05) to give compound **28** (262 mg, 94%) as a white foam. R_f 0.17 (hexane/EtOAc/MeOH, 6:2:0.05); $[\alpha]_D^{22}$ -52.5 (*c* 0.20, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.67 (d, 3H, *J* = 6.0 Hz, CH_3), 0.77 (s, 3H, CH_3), 0.79 (d, 3H, *J* = 6.0 Hz, CH_3), 0.95 (s, 3H, CH_3), 0.98 (d, 3H, *J* = 6.5 Hz, CH_3), 1.91 (s, 3H, CH_3CO), 1.95 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 3.38 (dd, 1H, *J* = 11.5, 10.5 Hz, H-26a), 3.48 (m, 2H, H-26b, H-3), 3.69 (m, 1H, H-5^{II}), 3.75 (dd, 1H, *J* = 9.0, 9.5 Hz, H-2^{I}), 3.86 (m, 1H, H-5^{I}), 4.04 (dd, 1H, *J* = 9.0, 9.5 Hz, H-4^{I}), 4.41 (approx. q, 1H, *J* ≈ 7.5 Hz, H-16), 4.52 (dd, 1H, *J* = 12.0, 5.0 Hz, H-6^{I}a), 4.54 (d, 1H, *J* = 12.0 Hz, Troc-Ha), 4.71 (d, 1H, *J* = 12.0 Hz, Troc-Hb), 4.77 (d, 1H, *J* = 8.0 Hz, H-1^{I}), 4.80 (dd, 1H, *J* = 12.5, 2.5 Hz, H-6^{I}b), 4.87 (brs, 1H, H-1^{II}), 4.88 (dd, 1H, *J* = 10.0, 9.5 Hz, H-4^{II}), 5.12 (m, 1H, H-2^{II}), 5.15 (dd, 1H, *J* = 10.0, 3.5 Hz, H-3^{II}), 5.29 (m, 1H, H-6), 5.60 (dd, 1H, *J* = 10.5, 9.0 Hz, H-3^{I}), 7.45 (m, 4H, 4 Ar-H), 7.58 (m, 2H, 2 Ar-H), 8.05 (m, 4H, 4 Ar-H); ^{13}C NMR (125 MHz, $CHCl_3$): δ 14.74, 16.48, 17.02, 17.35, 19.53, 20.91, 28.98, 29.71, 30.49, 31.56, 32.03, 32.25, 36.96, 37.18, 39.01, 39.95, 40.45, 41.78, 50.16, 56.67, 57.17, 62.25, 62.99, 67.05, 67.66, 68.65, 70.19, 70.73, 73.25, 73.45, 74.49, 80.17, 81.01, 95.50, 98.89, 99.88, 109.51, 121.99, 128.60, 128.67, 129.52, 130.02, 130.22, 133.28, 133.72, 140.49, 154.35, 166.06, 166.39, 170.05, 170.16, 170.26; Anal. Calcd for $C_{62}H_{78}Cl_3NO_{18}$: C, 60.46; H, 6.38; N, 1.14. Found: C, 60.14; H, 6.48; N, 0.89.

Diosgenyl 2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (**29a**):



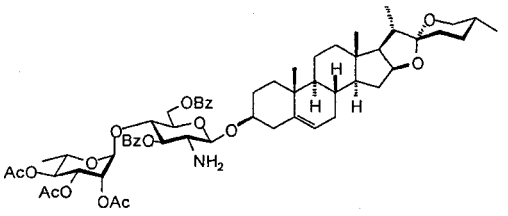
Compound **3** (2.88 g, 3.29 mmol) was dissolved in a stock solution composed of guanidine nitrate (3.72 g, 30.4 mmol) and NaOMe (1.0 M, 6 mL) in MeOH/CH_2Cl_2 (9:1, 300 mL). After stirring for 20 h at room temperature, the mixture was neutralized by adding weak acidic ion-exchange resin (Amberlite IRC-50, H^+ form). The resin was filtered and the filtrate concentrated *in vacuo*. The solid was subjected to flash column chromatography (CH_2Cl_2/MeOH, 12.5:1) to give compound **29a** (2.21 g, 90%) as a white solid. The compound was freeze dried in dioxane to ensure removal of residual methanol which interferes with the subsequent reaction. R_f 0.19 (CH_2Cl_2/MeOH, 14.3:1); $[\alpha]_D^{22}$ -45.0 (*c* 0.5, MeOH); ^{1}H NMR (500 MHz, C_5D_5N): δ 0.70 (d, 3H, *J* = 5.5 Hz, CH_3), 0.84 (s, 3H, CH_3), 0.92 (s, 3H, CH_3), 1.15 (d, 3H, *J* = 7.0 Hz, CH_3), 2.49 (m, 1H), 2.62 (m, 1H), 3.51 (dd, 1H, *J* = 10.0, 11.0 Hz, H-26'a), 3.60 (dd, 1H, *J* = 10.0, 3.5 Hz, H-26'b), 3.85 (m, 1H, H-3'), 4.05 (m, 1H, H-5), 4.30 (dd, 1H, *J* = 9.0, 9.0 Hz, H-4), 4.33 (dd, 1H, *J* = 10.0, 9.0 Hz, H-3), 4.42-4.49 (m, 2H, H-2, H-6a), 4.55 (approx. q, 1H, *J* ≈ 7.5 Hz, H-16'), 4.61 (brd, 1H, *J* = 11.5 Hz, H-6b), 4.95 (d, 1H, *J* = 12.0 Hz, Troc-Ha), 5.17 (d, 1H, *J* = 12.0 Hz, Troc-Hb), 5.18 (m, 1H, H-6'), 5.22 (d, 1H, *J* = 8.0 Hz, H-1), 6.61 (brs, 1H, OH), 7.52 (brs, 1H, OH), 7.59 (brs, 1H, OH), 9.26 (d, 1H, *J* = 9.0 Hz, NH); ^{13}C NMR (125 MHz, C_5D_5N): δ 15.39, 16.69, 17.68, 19.79, 21.42, 29.60, 30.44, 30.94, 31.94, 32.14, 32.53, 37.35, 37.70, 39.79, 40.18, 40.78, 42.30, 50.54, 56.95, 60.17, 63.10, 63.21, 67.20, 67.54, 72.64, 74.94, 76.60, 79.04, 81.42, 97.50, 101.47, 109.61, 121.98, 141.22, 156.20; Anal. Calcd for $C_{36}H_{54}Cl_3NO_9$: C, 57.56; H, 7.25; N, 1.86. Found: C, 56.61; H, 6.41; N, 1.67.

Diosgenyl 3,6-di-O-benzoyl-2-deoxy-2-(2,2,2-trichloroethoxycarbonylamino)-β-D-glucopyranoside (**30**):



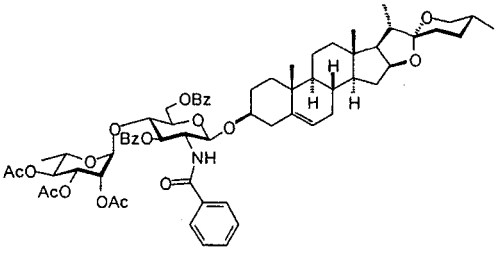
To a solution of **29a** (386 mg, 0.51 mmol) and 4-dimethylaminopyridine (DMAP) (6 mg, 0.05 mmol) in anhydrous pyridine (4 mL) and CH_2Cl_2 (2 mL), benzoyl chloride (0.12 mL, 1.03 mmol) in dry CH_2Cl_2 (2 mL) was added drop-wise under N_2 atmosphere at -40 °C. The reaction was carefully monitored by TLC every fifteen minutes. After being stirred at room temperature for 2 h, the mixture was quenched with MeOH. The solution was then diluted with CH_2Cl_2 (150 mL) and washed with cold HCl solution (4 N, 30 mL) and saturated $NaHCO_3$ solution (30 mL). The organic layer was dried over anhydrous $NaSO_4$ and concentrated under vacuum. The residue was subjected to flash column chromatography (toluene/acetone, 11:1) to give compound **30** (340 mg, 69%) as a white solid. R_f 0.24 (toluene/acetone, 11:1); $[\alpha]_D^{22}$ -33.5 (*c* 0.20 $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 0.78 (s, 3H, CH_3), 0.79 (d, 3H, *J* = 6.5 Hz, CH_3), 0.97 (s, 3H, CH_3), 0.98 (d, 3H, *J* = 8.0 Hz, CH_3), 3.38 (dd, 1H, *J* = 11.0, 11.0 Hz, H-26'a), 3.40 (brs, 1H, OH), 3.48 (dd, 1H, *J* = 11.5, 3.5 Hz, H-26'b), 3.53 (m, 1H, H-3'), 3.75-3.81 (m, 3H, H-2, H-4, H-5), 4.41 (approx. q, 1H, *J* ≈ 7.5 Hz, H-16'), 4.57 (d, 1H, *J* = 12.0 Hz, Troc-Ha), 4.63 (d, 1H, *J* = 12.5, 1.5 Hz, H-6a), 4.71 (d, 1H, *J* = 12.0 Hz, Troc-Ha) 4.72 (m, 1H, H-6b), 4.83 (d, 1H, *J* = 8.5 Hz, H-1), 5.27 (m, 1H, H-6'), 5.40 (d, 1H, *J* = 9.0 Hz, NH), 5.50 (dd, 1H, *J* = 10.0, 8.5 Hz, H-3), 7.38-7.45 (m, 4H, 4 Ar-H), 7.56 (m, 2H, 2 Ar-H), 8.02 (m, 4H, 4 Ar-H); ^{13}C NMR (125 MHz, $CHCl_3$): δ 14.75, 16.49, 17.35, 19.56, 21.00, 28.98, 29.72, 30.49, 31.56, 32.03, 32.25, 36.99, 37.25, 39.03, 39.97, 40.46, 41.78, 50.21, 56.56, 56.68, 62.26, 63.99, 67.05, 70.02, 74.23, 74.48, 75.81, 80.10, 81.00, 95.61, 99.90, 109.53, 121.99, 128.62, 128.68, 129.17, 129.80, 130.10, 130.25, 133.50, 133.85, 140.51, 154.46, 167.21, 167.51; Anal. Calcd for $C_{50}H_{62}Cl_3NO_{11}$: C, 62.60; H, 6.51; N, 1.46. Found: C, 63.01; H, 6.32; N, 1.10.

Diosgenyl 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→4)-2-amino-3,6-di-O-benzoyl-2-deoxy-β-D-glucopyranoside (**32**):



To a solution of **28** (217 mg, 0.17 mmol) in acetic acid (10 ml), zinc dust (2.0 g) was added and the mixture was stirred at room temperature for 20 h. The mixture was filtered and the solid was thoroughly washed with CH_2Cl_2 (100 mL). The filtrate was concentrated *in vacuo* and the residue was re-dissolved with CH_2Cl_2 (200 mL). The solution was washed with saturated $NaHCO_3$ solution (20 mL), dried with Na_2SO_4, and concentrated under vacuum to give a white solid **32** (180 mg, 96%) which was used without further purification in the next step.

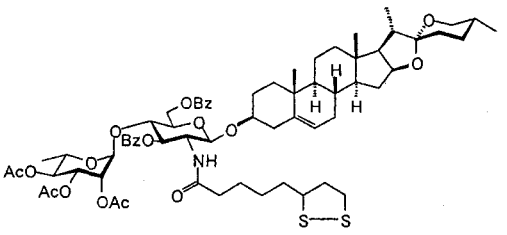
Diosgenyl 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→4)-2-benzamido-3,6-di-O-benzoyl-2-deoxy-β-D-glucopyranoside (**33**):



To a cooled solution (ice water bath) of **32** (85 mg, 0.08 mmol) in pyridine (2.0 mL), benzoyl chloride (14.0 μL, 0.12 mmol) was added drop-wise. The mixture was stirred at room temperature for 4 h. The solution was diluted with CH_2Cl_2 (150 mL), washed with cold HCl solution (4 N, 20 mL) and saturated $NaHCO_3$ solution (20 mL). The organic layer was dried over anhydrous $NaSO_4$ and concentrated under vacuum. The residue was subjected to flash column chromatography (toluene/acetone, 9:1) to give compound **33**

(92 mg, 80%) as a white solid. R_f 0.25 (toluene/acetone, 9:1); $[\alpha]_D^{22}$ -33.9 (*c* 1.0 $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 0.69 (d, 3H, *J* = 6.0 Hz, CH_3), 0.76 (s, 3H, CH_3), 0.79 (d, 3H, *J* = 6.5 Hz, CH_3), 0.92 (s, 3H, CH_3), 0.97 (d, 3H, *J* = 7.0 Hz, CH_3), 1.90 (s, 3H, CH_3CO), 1.95 (s, 3H, CH_3CO), 2.01 (s, 3H, CH_3CO), 3.37 (dd, 1H, *J* = 10.5, 11.5 Hz, H-26a), 3.49 (m, 2H, H-26b, H-3), 3.72 (m, 1H, H-5^{II}), 3.93 (m, 1H, H-5^{I}), 4.14 (dd, 1H, *J* = 9.0, 9.5 Hz, H-4^{I}), 4.27 (dd, 1H, *J* = 9.0, 9.0 Hz, H-2^{I}), 4.40 (approx. q, 1H, *J* ≈ 7.0 Hz, H-16), 4.55 (dd, 1H, *J* = 12.0, 5.0 Hz, H-6^{I}a), 4.83 (dd, 1H, *J* = 12.0, 2.0 Hz, H-6^{I}b), 4.88 (dd, 1H, *J* = 10.0, 10.0 Hz, H-4^{II}), 4.91 (d, 1H, *J* = 8.5 Hz, H-1^{I}), 4.92 (brs, 1H, H-1^{II}), 5.16 (m, 1H, H-2^{II}), 5.17 (m, 1H, H-3^{II}), 5.21 (m, 1H, H-6), 5.71 (dd, 1H, *J* = 8.5, 9.0 Hz, H-3^{I}), 6.24 (d, 1H, *J* = 9.0 Hz, NH), 7.28-7.60 (m, 11H, 11 Ar-H), 8.00 (m, 2H, 2 Ar-H), 8.08 (m, 2H, 2 Ar-H); ^{13}C NMR (125 MHz, $CHCl_3$): δ 14.73, 16.46, 17.02, 17.34, 19.50, 20.91, 28.98, 29.71, 30.48, 31.53, 32.02, 32.22, 36.93, 37.21, 39.14, 39.93, 40.43, 41.77, 50.16, 55.51, 56.65, 62.24, 63.11, 67.03, 67.61, 68.73, 70.17, 70.76, 73.27, 74.02, 76.81, 79.94, 80.99, 98.67, 100.22, 109.49, 128.61, 128.73, 129.53, 130.04, 130.15, 131.65, 133.26, 133.60, 134.65, 140.64, 166.12, 166.92, 168.06, 170.04, 170.18, 170.23; ESI-LRMS *m/z*: found 1182 $[M + Na]^+$; ESI-HRMS *m/z*: $[M + Na]^+$ Calcd for $C_{66}H_{81}NO_{17}Na$: 1182.5396, found: 1182.5394.

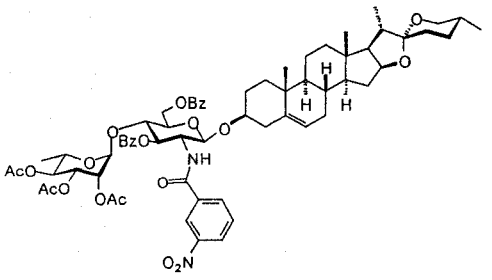
Diosgenyl 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→4)-3,6-di-O-benzoyl-2-deoxy-2-{5-[(R/S)-1,2-dithiolan-3-yl]-pentanamido}-β-D-glucopyranoside (**34**):



To a solution of **32** (95 mg, 0.09 mmol) in dry DMF (5.0 mL), (±)-α-lipoic acid (18 mg, 0.09 mmol), DIPEA (0.03 mL, 0.17 mmol), and HBTU (33 mg, 0.09 mmol) were added and the mixture was stirred for 16 h. The solvent was removed in vacuo and the residue was dissolved with CH_2Cl_2 (150 mL) and washed with H_2O (10 mL x 2). The organic layer was dried over anhydrous $NaSO_4$ and concentrated under vacuum. The residue was subjected

to flash column chromatography (toluene/acetone, 9:1) to give compound **34** (92 mg, 83%) as a white solid and as a diastereomeric mixture (*R*/*S*, 1:1). R_f 0.29 (toluene/acetone, 9:1); $[\alpha]_D^{22}$ -50.7 (*c* 0.33 $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 0.68 (d, 3H, *J* = 6.0 Hz, CH_3), 0.78 (s, 3H, CH_3), 0.79 (d, 3H, *J* = 6.5 Hz, CH_3), 0.95 (s, 3H, CH_3), 0.98 (d, 3H, *J* = 7.0 Hz, CH_3), 1.91 (s, 3H, CH_3CO), 1.95 (s, 3H, CH_3CO), 2.00 (s, 3H, CH_3CO), 3.03-3.15 (m, 2H, CH_2S), 3.31 (m, 1H), 3.37 (dd, 1H, *J* = 11.0, 11.0 Hz, H-26a), 3.47 (m, 2H, H-26b, H-3), 3.69 (m, 1H, H-5^{II}), 3.86 (m, 1H, H-5^{I}), 4.04 (m, 1H, H-2^{I}), 4.05 (dd, 1H, *J* = 9.0, 9.5 Hz, H-4^{I}), 4.42 (approx. q, 1H, *J* ≈ 7.5 Hz, H-16), 4.51 (dd, 1H, *J* = 12.0, 5.0 Hz, H-6^{I}a), 4.78 (two sets of d, each 0.5H, *J* = 8.0 Hz, H-1^{I}), 4.79 (dd, 1H, *J* = 12.0, 2.5 Hz, H-6^{I}b), 4.87 (brs, 1H, H-1^{II}), 4.88 (dd, 1H, *J* = 9.5, 9.5 Hz, H-4^{II}), 5.14 (m, 1H, H-2^{II}), 5.15 (m, 1H, H-3^{II}), 5.28 (m, 1H, H-6), 5.54 (d, 1H, *J* = 9.0 Hz, NH), 5.56 (m, 1H, H-3^{I}), 7.45 (m, 4H, 4 Ar-H), 7.58 (m, 2H, 2 Ar-H), 8.05 (m, 4H, 4 Ar-H); ^{13}C NMR (125 MHz, $CHCl_3$): δ 14.72, 16.47, 17.01, 17.33, 19.53, 20.90, 20.95, 25.35, 28.76, 28.98, 29.71, 30.48, 31.56, 32.03, 32.26, 34.66, 36.64, 36.95, 37.20, 38.57, 39.22, 39.94, 40.29, 40.44, 41.78, 50.17, 54.98, 56.27, 56.67, 62.26, 63.07, 67.04, 67.59, 68.72, 70.15, 70.74, 73.17, 73.89, 76.80, 79.72, 80.99, 98.62, 99.90, 109.49, 121.92, 128.58, 128.72, 129.61, 130.01, 130.18, 133.25, 133.67, 140.58, 166.08, 166.68, 170.02, 170.16, 170.22, 172.96; ESI-LRMS *m/z*: found 1267 $[M + Na]^+$; ESI-HRMS *m/z*: $[M + Na]^+$ Calcd for $C_{67}H_{89}NO_{17}S_2Na$: 1266.5469, found: 1266.5463.

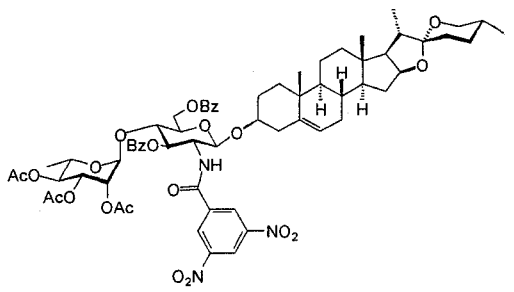
Diosgenyl 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→4)-3,6-di-O-benzoyl-2-deoxy-2-(3-nitrobenzamido)-β-D-glucopyranoside (**35**):



To a solution of **32** (150 mg, 0.14 mmol) in dry DMF (5.0 mL), 3-nitrobenzoic acid (28 mg,

0.16 mmol), DIPEA (0.05 mL, 0.28 mmol), and HBTU (53 mg, 0.14 mmol) were added and the mixture was stirred for 16 h. The solvent was removed *in vacuo* and the residue was dissolved with CH_2Cl_2 (150 mL) and washed with H_2O (10 mL x 2). The organic layer was dried over anhydrous $NaSO_4$ and concentrated under vacuum. The residue was subjected to flash column chromatography (toluene/acetone, 11:1) to give compound **35** (143 mg, 83%) as a pale yellow solid. R_f 0.28 (toluene/acetone, 11:1); $[\alpha]_D^{22}$ -29.8 (*c* 1.0 $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.72 (d, 3H, *J* = 6.5 Hz, CH_3), 0.76 (s, 3H, CH_3), 0.79 (d, 3H, *J* = 6.5 Hz, CH_3), 0.91 (s, 3H, CH_3), 0.97 (d, 3H, *J* = 7.0 Hz, CH_3), 1.90 (s, 3H, CH_3CO), 1.95 (s, 3H, CH_3CO), 2.02 (s, 3H, CH_3CO), 3.37 (dd, 1H, *J* = 11.0, 11.0 Hz, H-26a), 3.47 (m, 1H, H-26b) 3.53 (m, 1H, H-3), 3.73 (m, 1H, H-5^{II}), 3.98 (m, 1H, H-5^{I}), 4.19 (dd, 1H, *J* = 9.0, 9.0 Hz, H-4^{I}), 4.36 (dd, 1H, *J* = 9.0, 9.0 Hz, H-2^{I}), 4.40 (approx. q, 1H, *J* ≈ 7.0 Hz, H-16), 4.55 (dd, 1H, *J* = 12.5, 5.0 Hz, H-6^{I}a), 4.88 (m, 1H, H-6^{I}b), 4.90 (dd, 1H, *J* = 9.5, 10.0 Hz, H-4^{II}), 4.95 (brs, 1H, H-1^{II}), 4.96 (d, 1H, *J* = 8.0 Hz, H-1^{I}), 5.15 (m, 2H, H-2^{II}, H-3^{II}), 5.19 (m, 1H, H-6), 5.71 (dd, 1H, *J* = 9.0, 9.0 Hz, H-3^{I}), 6.69 (d, 1H, *J* = 9.0 Hz, NH), 7.39 (m, 1H, 1 Ar-H), 7.46-7.61 (m, 6H, 6 Ar-H), 7.94 (m, 1H, 1 Ar-H), 8.02 (m, 2H, 2 Ar-H), 8.08 (m, 2H, 2 Ar-H), 8.25 (m, 1H, 1 Ar-H), 8.42 (m, 1H, 1 Ar-H); ^{13}C NMR (125 MHz, $CHCl_3$): δ 14.72, 16.46, 17.05, 17.34, 19.49, 20.92, 20.97, 28.98, 29.75, 30.48, 31.51, 31.56, 32.03, 32.22, 36.90, 37.19, 39.34, 39.93, 40.42, 41.77, 50.16, 55.56, 56.64, 62.23, 63.00, 67.02, 67.64, 68.82, 70.09, 70.66, 73.24, 74.18, 76.32, 79.71, 80.97, 98.53, 99.99, 109.47, 121.92, 122.04, 126.24, 128.64, 128.69, 129.28, 129.98, 130.06, 130.18, 133.15, 133.35, 133.82, 136.13, 140.44, 148.27, 165.63, 166.19, 167.13, 170.01, 170.26, 170.35; ESI-LRMS *m/z*: found 1228 $[M + Na]^+$; ESI-HRMS *m/z*: $[M + Na]^+$ Calcd for $C_{66}H_{80}N_2O_{19}Na$: 1227.5252, found: 1227.5240.

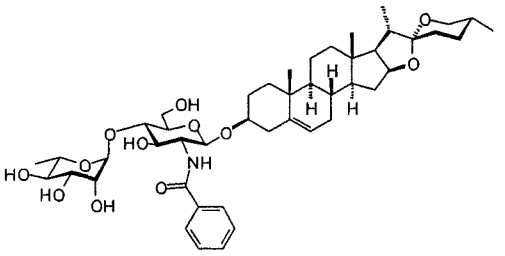
Diosgenyl 2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl-(1→4)-3,6-di-O-benzoyl-2-deoxy-2-(3,5-dinitrobenzamido)-β-D-glucopyranoside (**36**):



To a solution of **32** (150 mg, 0.14 mmol) in dry DMF (5.0 mL), 3,5-dinitrobenzoic acid (34 mg, 0.16 mmol), DIPEA (0.05 mL, 0.28 mmol), and HBTU (53 mg, 0.14 mmol) were added and the mixture was stirred for 18 h. The solvent was removed *in vacuo* and the residue was dissolved with CH_2Cl_2 (150 mL) and washed with H_2O (10 mL x 2). The organic layer was dried over anhydrous $NaSO_4$ and concentrated under vacuum. The residue was subjected to flash column chromatography (toluene/acetone, 11:1) to give compound **36** (143 mg, 83%) as a light orange solid. R_f 0.32 (toluene/acetone, 11:1); $[\alpha]_D^{22}$ -25.9 (*c* 0.5 $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 0.73 (d, 3H, *J* = 6.0 Hz, CH_3), 0.76 (s, 3H, CH_3), 0.78 (d, 3H, *J* = 6.0 Hz, CH_3), 0.91 (s, 3H, CH_3), 0.96 (d, 3H, *J* = 6.5 Hz, CH_3), 1.91 (s, 3H, CH_3CO), 1.97 (s, 3H, CH_3CO), 2.03 (s, 3H, CH_3CO), 3.37 (dd, 1H, *J* = 11.0, 11.0 Hz, H-26a), 3.47 (m, 1H, H-26b), 3.54 (m, 1H, H-3), 3.73 (m, 1H, H-5^{II}), 4.01 (m, 1H, H-5^{I}), 4.20 (dd, 1H, *J* = 9.5, 8.5 Hz, H-4^{I}), 4.40 (approx. q, 1H, *J* ≈ 7.0 Hz, H-16), 4.41 (m, 1H, H-2^{I}), 4.55 (dd, 1H, *J* = 12.5, 5.0 Hz, H-6^{I}a), 4.90 (dd, 1H, *J* = 9.5, 10.0 Hz, H-4^{II}), 4.92 (m, 1H, H-6^{I}b), 4.96 (brs, 1H, H-1^{II}), 4.97 (d, 1H, *J* = 8.0 Hz, H-1^{I}), 5.13 (m, 1H, H-2^{II}), 5.14 (dd, 1H, *J* = 10.0 Hz, 3.0 Hz, H-3^{II}), 5.19 (m, 1H, H-6), 5.67 (dd, 1H, *J* = 9.0, 8.5 Hz, H-3^{I}), 7.01 (d, 1H, *J* = 9.0 Hz, NH), 7.38-7.62 (m, 6H, 6 Ar-H), 8.01-8.10 (m, 4H, 4 Ar-H), 8.80 (d, 2H, *J* = 2.0 Hz, 2 Ar-H), 9.08 (t, 1H, *J* = 2.0 Hz, 1 Ar-H); ^{13}C NMR (125 MHz, $CHCl_3$): δ 14.73, 16.48, 17.08, 17.36, 19.49, 20.90, 20.95, 28.99, 29.79, 30.49, 31.51, 31.57, 32.05, 32.23, 36.90, 37.18, 39.45, 39.93, 40.44, 41.78, 50.17, 55.66, 56.65, 62.25, 62.92, 67.04, 67.71, 68.86, 70.05, 70.60, 73.22, 74.19, 75.99, 79.60, 80.98, 98.44, 99.74, 109.50, 121.32, 122.10, 127.43, 128.70, 128.80, 129.03, 129.88, 130.09, 130.20, 133.46,

134.07, 137.89, 140.27, 148.78, 163.40, 166.29, 167.27, 170.01, 170.35, 170.55; ESI-LRMS *m/z*: found 1273 $[M + Na]^+$; ESI-HRMS *m/z*: $[M + Na]^+$ Calcd for $C_{66}H_{79}N_3O_{21}Na$: 1272.5103, found: 1272.5095.

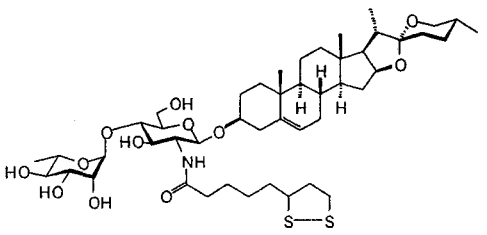
Diosgenyl α-L-rhamnopyranosyl-(1→4)-2-benzamido-2-deoxy-β-D-glucopyranoside (**37**):



Compound **33** (50 mg, 0.04 mmol) was dissolved in $MeOH/CH_2Cl_2$ (2:1, 3 mL) and then NaOMe solution in methanol (1.5 mL) was added until pH 10. After stirring for 24 h at room temperature, the mixture was neutralized by adding weak acidic ion-exchange resin (Amberlite IRC-50, H^+ form). The resin was filtered and the filtrate concentrated in vacuo. The solid was washed thoroughly with CH_2Cl_2 and then applied to a Sephadex LH-20 gel filtration column (MeOH) to yield compound **37** (33 mg, 95%) as a white solid. R_f 0.47 (CH_2Cl_2/MeOH, 5:1); $[\alpha]_D^{22}$ -55.2 (*c* 0.33, MeOH); ^1H NMR (500 MHz, C_5D_5N): δ 0.68 (d, 3H, *J* = 5.0 Hz, CH_3), 0.80 (s, 3H, CH_3), 0.81 (s, 3H, CH_3), 1.13 (d, 3H, *J* = 7.0 Hz, CH_3), 1.68 (d, 3H, *J* = 5.5 Hz, CH_3), 2.39 (m, 1H), 2.68 (m, 1H), 3.49 (dd, 1H, *J* = 10.5, 10.5 Hz, H-26a), 3.58 (m, 1H, H-26b), 3.78 (m, 1H, H-5^I), 3.83 (m, 1H, H-3), 4.13 (dd, 1H, *J* = 12.0, 4.5 Hz, H-6^Ia), 4.26 (brd, 1H, *J* = 11.0 Hz, H-6^Ib), 4.37 (dd, 1H, *J* = 9.5, 9.5 Hz, H-3^I), 4.52 (m, 2H, H-2^I, H-16), 4.57 (dd, 1H, *J* = 9.0, 3.0 Hz, H-3^{II}), 4.63 (dd, 1H, *J* = 9.0 Hz, 9.5 Hz), 4.72 (m, 1H, H-2^{II}), 4.76 (dd, 1H, *J* = 9.5 Hz, 9.5 Hz), 5.02 (m, 1H, H-2^I), 5.54 (d, 1H, *J* = 8.5 Hz, H-1^I), 5.71 (m, 1H, H-6), 5.93 (brs, 1H, H-1^{II}), 7.40 (m, 3H, 3 Ar-H), 8.40 (m, 2H, 2 Ar-H), 9.57 (d, 1H, *J* = 8.0 Hz, NH); ^{13}C NMR (125 MHz, $CHCl_3$): δ 13.38, 16.67, 17.66, 18.83, 19.66, 21.39, 29.57, 30.47, 30.92, 31.90, 32.12, 32.54, 37.28, 37.71, 39.87, 40.14, 40.75, 42.27, 50.52, 55.42, 56.92, 61.75, 63.17, 67.17, 70.74, 72.97, 73.17, 74.07, 74.35, 77.67, 79.05, 79.49, 81.41, 100.65, 103.24, 109.59, 122.11, 128.44, 129.00, 131.64, 137.14, 141.03, 168.84; ESI-LRMS *m/z*: found 848 $[M + Na]^+$; ESI-HRMS *m/z*: $[M + Na]^+$

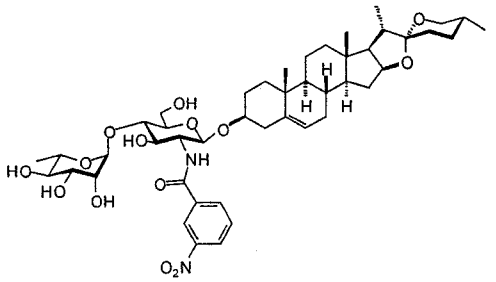
Calcd for $C_{46}H_{67}NO_{12}Na$: 848.4560, found: 848.4552.

Diosgenyl α-L-rhamnopyranosyl-(1→4)-2-deoxy-2-{5-[(R/S)-1,2-dithiolan-3-yl]-pentanamido}-β-D-glucopyranoside (**38**):



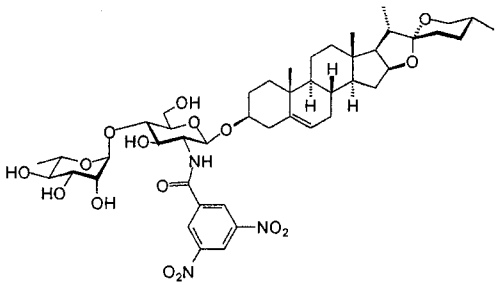
Compound **34** (50 mg, 0.04 mmol) was dissolved in MeOH/CH_2Cl_2 (2:1, 3 mL) and then NaOMe solution in methanol (1.5 mL) was added until pH 10. After stirring for 24 h at room temperature, the mixture was neutralized by adding weak acidic ion-exchange resin (Amberlite IRC-50, H^+ form). The resin was filtered and the filtrate concentrated in vacuo. The solid was washed thoroughly with CH_2Cl_2 and then applied to a silica gel flash chromatography column (DCM/MeOH, 50:1 → 5:1) to yield **38** (46 mg, 96%) as a white solid and as a diasteromeric mixture (*R*/*S*, 1:1). R_f 0.53 (CH_2Cl_2/MeOH, 5:1); $[\alpha]_D^{22}$ -78.0 (*c* 0.10, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 0.76 (d, 3H, *J* = 6.5 Hz, CH_3), 0.77 (s, 3H, CH_3), 0.92 (d, 3H, *J* = 7.0 Hz, CH_3), 0.99 (s, 3H, CH_3), 1.20 (d, 3H, *J* = 6.5 Hz, CH_3), 2.21 (m, 2H), 2.30 (m, 1H), 2.41 (m, 1H), 3.05 (m, 1H, CH_2S), 3.13 (m, 1H, CH_2S), 3.25-3.31 (m, 2H, H-26a, H-2^I), 3.36 (dd, 1H, *J* = 10.5, 10.5 Hz, 1H, H-26b), 3.41 (m, 1H, H-5^I), 3.46 (m, 1H, H-3), 3.49-3.64 (m, 5H, H-6^{I}a, H-3^I, H-4^I, H-6^{I}b, H-4^{II}), 3.75-3.79 (m, 2H, H-2^{II} H-3^{II}), 3.90 (m, 1H, H-5^I), 4.35 (approx. q, 1H, *J* ≈ 7.5 Hz, H-16), 4.54 (two sets of d, each 0.5H, *J* = 8.0 Hz, H-1^I), 4.82 (d, 1H, *J* = 1.5 Hz, H-1^{II}), 5.33 (m, 1H, H-6); ^{13}C NMR (125 MHz, CD_3OD): δ 15.03, 16.92, 17.65, 17.99, 20.09, 22.11, 27.02, 30.02, 30.13, 30.78, 31.58, 32.54, 32.88, 33.32, 40.39, 41.04, 41.46, 41.56, 43.04, 51.74, 57.69, 57.91, 70.76, 72.34, 72.57, 73.85, 74.58, 76.99, 80.21, 82.35, 101.02, 103.10, 110.73, 122.91, 141.98, 176.47; ESI-LRMS *m/z*: found 932 $[M + Na]^+$; ESI-HRMS *m/z*: $[M + Na]^+$ Calcd for $C_{47}H_{75}NO_{19}S_2Na$: 932.4628, found: 932.4619.

Diosgenyl α-L-rhamnopyranosyl-(1→4)-2-deoxy-2-(3-nitrobenzamido)-β-D-glucopyranoside (**39**):



In a similar way as described for the preparation of **38**, compound **35** (50 mg, 0.04 mmol) was converted to **39** (32 mg, 92%) as a white solid. R_f 0.50 (CH_2Cl_2/MeOH, 5:1); $[\alpha]_D^{22}$ -41.8 (*c* 0.50, MeOH); ^{1}H NMR (500 MHz, C_5D_5N): δ 0.75-0.76 (m, 6H, 2 CH_3), 0.91 (d, 3H, *J* = 7.0 Hz, CH_3), 0.92 (s, 3H, CH_3), 1.21 (d, 3H, *J* = 6.5 Hz, CH_3), 3.27 (dd, 1H, *J* = 10.5, 11.5 Hz, H-26a), 3.28 (m, 1H, H-2^I), 3.38 (dd, 1H, *J* = 10.0, 9.5 Hz, 1H, H-26b), 3.39 (m, 1H, H-5^I), 3.49 (m, 1H, H-3), 3.57-3.60 (m, 2H, H-3^I, H-6^{I}a), 3.68 (dd, 1H, *J* = 12.0, 4.0 Hz, H-6^{I}b), 3.75-3.87 (m, 4H, H-4^I, H-2^{II}, H-3^{II}, H-4^{II}), 3.93 (m, 1H, H-5^{II}), 4.34 (approx. q, 1H, *J* ≈ 7.5 Hz, H-16), 4.70 (d, 1H, *J* = 8.5 Hz, H-1^I), 5.24 (m, 1H, H-6), 7.72 (t, 1H, *J* = 8.0 Hz, Ar-H), 8.20 (m, 1H, 1 Ar-H), 8.38 (m, 1H, 1 Ar-H), 8.69 (m, 1H, 1 Ar-H); The H-1^{II} peak is most likely buried under the solvent peak at δ 4.87. ^{13}C NMR (125 MHz, CD_3OD): δ 15.04, 16.91, 17.65, 17.97, 19.92, 22.10, 30.02, 30.80, 31.58, 32.54, 32.86, 33.25, 38.07, 38.57, 40.35, 41.02, 41.54, 43.02, 51.74, 57.90, 58.80, 62.12, 63.86, 67.99, 70.77, 72.36, 72.58, 73.87, 74.46, 77.15, 80.13, 80.63, 82.32, 101.26, 103.13, 110.71, 122.85, 123.42, 127.18, 131.26, 134.56, 138.14, 141.84, 149.79, 168.49; ESI-LRMS *m/z*: found 893 $[M + Na]^+$; ESI-HRMS *m/z*: $[M + Na]^+$ Calcd for $C_{46}H_{66}N_2O_{14}Na$: 893.4411, found: 893.4403.

Diosgenyl α-L-rhamnopyranosyl-(1→4)-2-deoxy-2-(3,5-dinitrobenzamido)-β-D-glucopyranoside (**40**):



In a similar way as described for the preparation of **38**, compound **36** (62 mg, 0.05 mmol) was converted to **40** (42 mg, 91%) as a white solid. R_f 0.55 (CH_2Cl_2/MeOH, 5:1); $[\alpha]_D^{22}$ -58.0 (*c* 0.10, MeOH); ^{1}H NMR (500 MHz, CD_3OD): δ 0.74 (s, 3H, CH_3), 0.75 (d, 3H, *J* = 6.0 Hz, CH_3), 0.90 (d, 3H, *J* = 7.0 Hz, CH_3), 0.92 (s, 3H, CH_3), 1.20 (d, 3H, *J* = 6.0 Hz, CH_3), 3.27 (m, 2H, H-2^I, H-26a), 3.37 (dd, 1H, *J* = 10.0, 9.5 Hz, 1H, H-26b), 3.38 (m, 1H, H-5^I), 3.50 (m, 1H, H-3), 3.57-3.60 (m, 2H, H-3^I, H-6^{I}a), 3.68 (dd, 1H, *J* = 12.0, 4.0 Hz, H-6^{I}b), 3.74-3.87 (m, 4H, H-4^I, H-2^{II}, H-3^{II}, H-4^{II}), 3.92 (m, 1H, H-5^{II}), 4.33 (approx. q, 1H, *J* ≈ 7.5 Hz, H-16), 4.70 (d, 1H, *J* = 8.0 Hz, H-1^I), 5.25 (m, 1H, H-6), 9.04 (d, *J* = 2.0 Hz, 2 Ar-H), 9.11 (t, 1H, *J* = 2.0 Hz, 1 Ar-H); The H-1^{II} peak is most likely buried under the solvent peak at δ 4.87. ^{13}C NMR (125 MHz, CD_3OD): δ 15.03, 16.89, 17.65, 17.96, 19.91, 22.09, 30.01, 30.80, 31.58, 32.54, 32.86, 33.25, 38.07, 38.56, 40.34, 41.02, 41.53, 43.02, 51.74, 57.89, 59.02, 62.09, 63.85, 67.98, 70.77, 72.36, 72.57, 73.85, 74.47, 77.20, 80.03, 80.51, 82.32, 101.04, 103.17, 110.71, 122.19, 122.91, 128.59, 139.53, 141.79, 150.25, 166.09; ESI-LRMS *m/z*: found 938 $[M + Na]^+$; ESI-HRMS *m/z*: $[M + Na]^+$ Calcd for $C_{46}H_{65}N_3O_{16}Na$: 938.4262, found: 938.4256.

4.3 Cell lines, cell culture, and medium

Cell lines used in this study include SK-N-SH (neuroblastoma), MCF-7 (breast cancer), and HeLa (cervical cancer). SK-N-SH and MCF-7 cell lines were supplied by the American Type Culture Collection (Manassas, Virginia), and HeLa cells were obtained from the Lady Davis Institute for Medical Research (Montreal, Quebec). All cell lines were grown in Dulbecco's modified Eagle's medium (Sigma) containing 10% fetal bovine serum (PAA Laboratories) at 37 °C in a 5% CO_2 humidified atmosphere.

4.4 Cell proliferation assay

Measurement of cell proliferation was performed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as described by Carmichael et al.[92] Briefly, 2000 cells were plated out into each well of a 96-wellplate and allowed to adhere. Saponin analogues (dissolved in DMSO (Sigma) and diluted with tissue culture medium) were added at increasing concentrations (0-30 μM, 8 wells per concentration). The cells were incubated in the presence of each saponin analogue for 72 h. MTT reagent (Sigma) dissolved in phosphate-buffered saline was added to each well at a concentration of 0.5 mg/mL, and the cells were incubated for four additional hours. Following this time, the medium containing the MTT reagent was aspirated, and DMSO (100 μL) was added to each well. The absorbance of each well was measured in a microplate reader (PowerWave Xs, Bio-Tek) at a wavelength of 490 nm. The IC_{50} value for each compound tested was determined by plotting concentration vs. percent absorbance obtained in the MTT assay. The IC_{50} results listed in Table 1 represent the average IC_{50} value obtained from multiple MTT assays for each compound.

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6 APPENDIX

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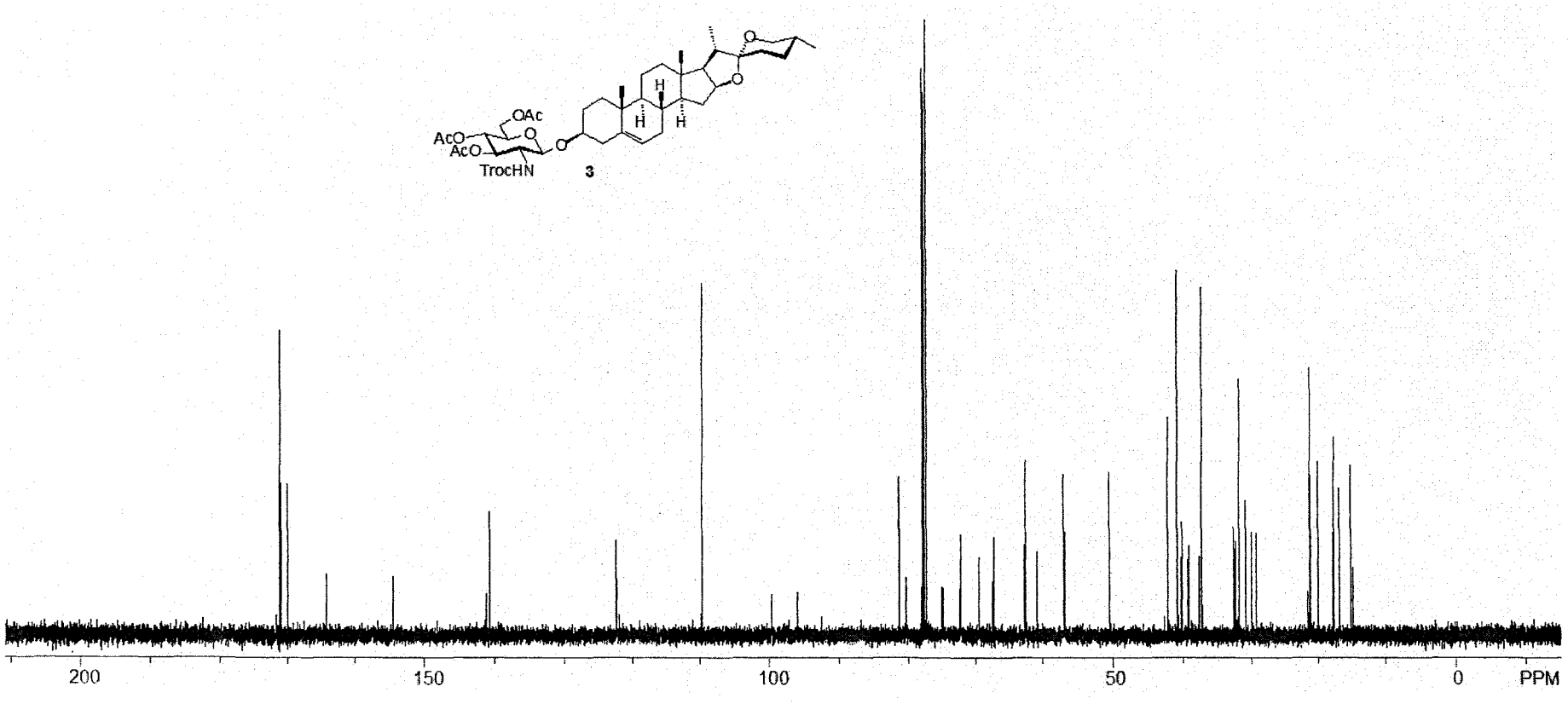
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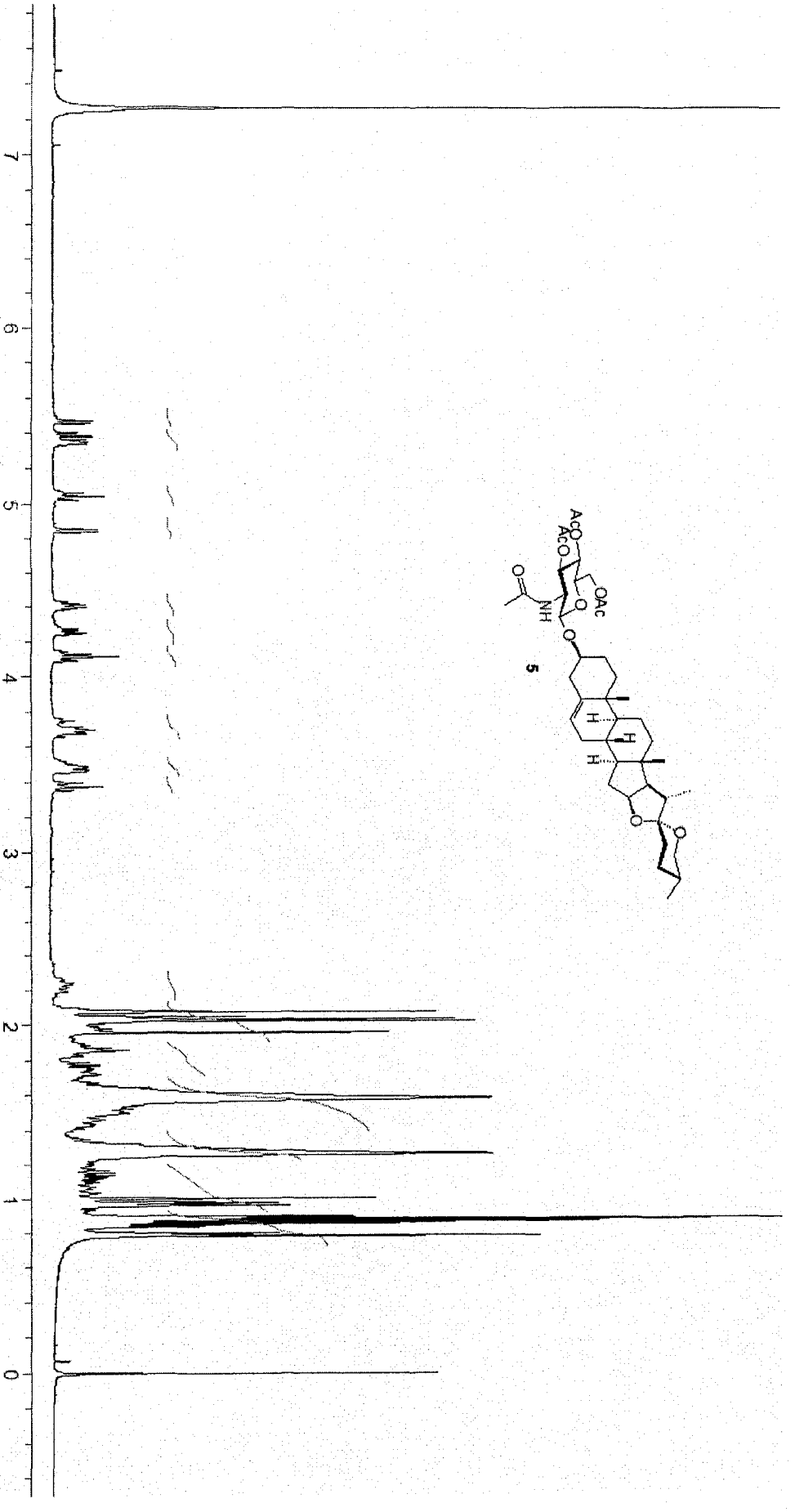
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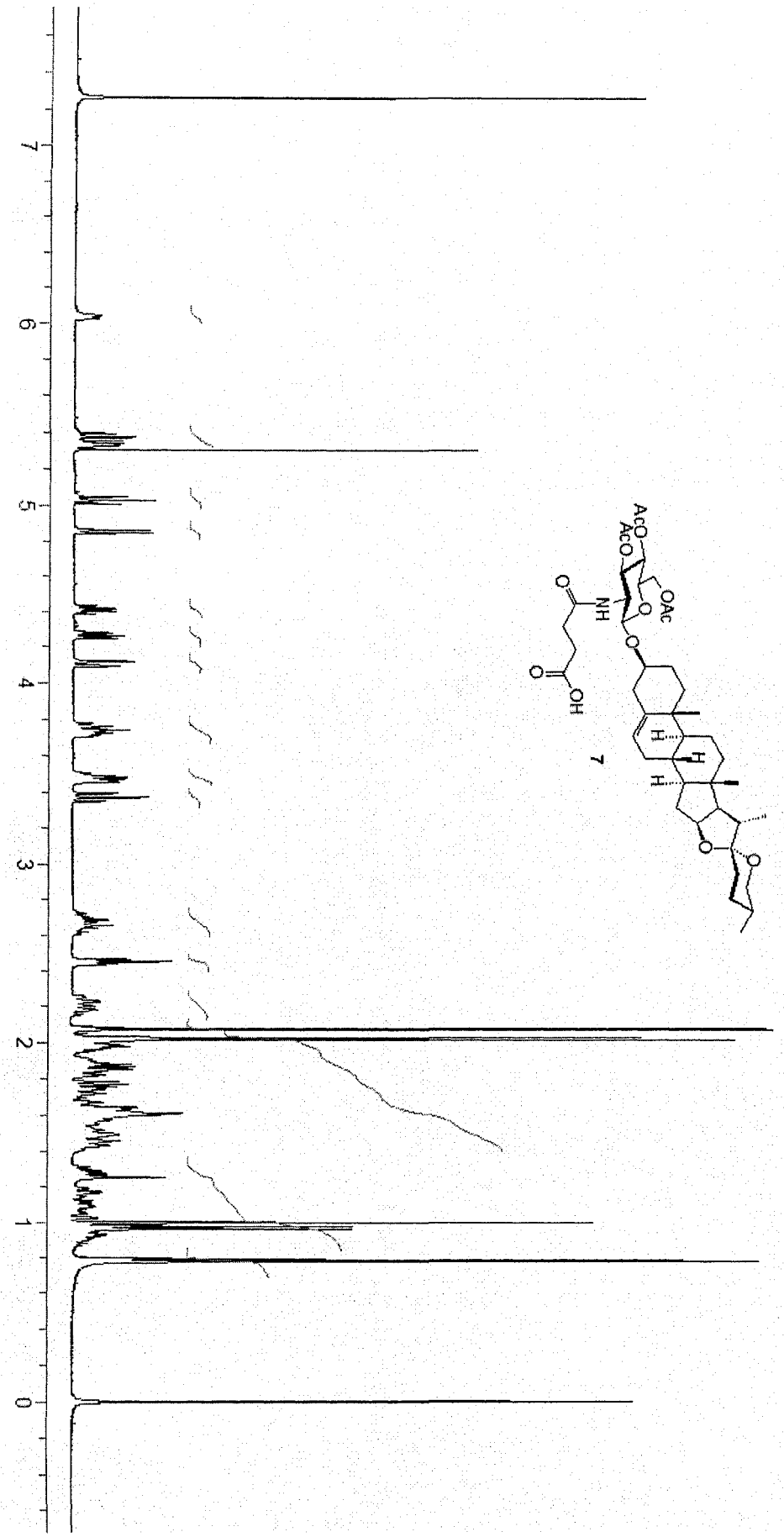
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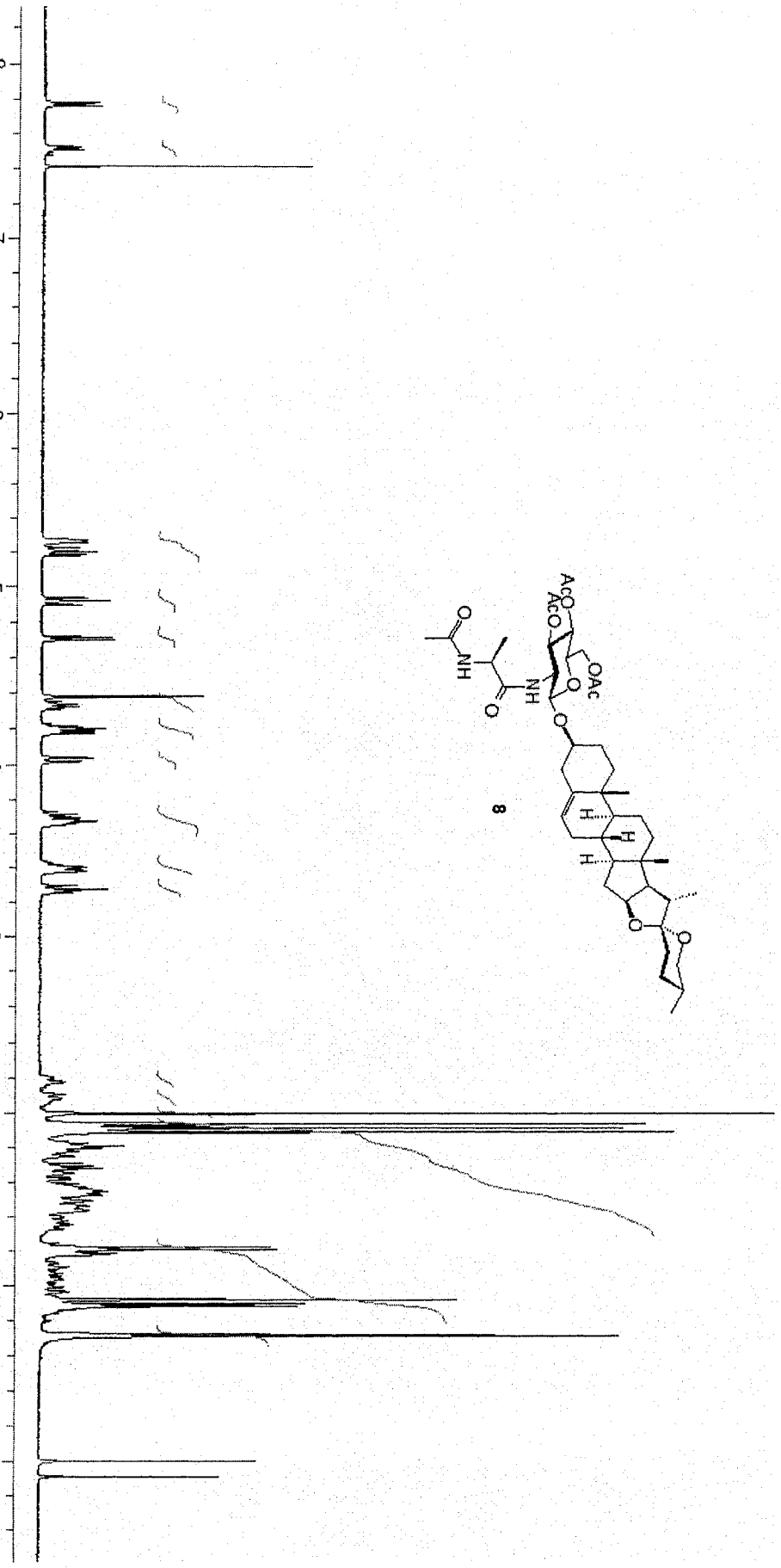
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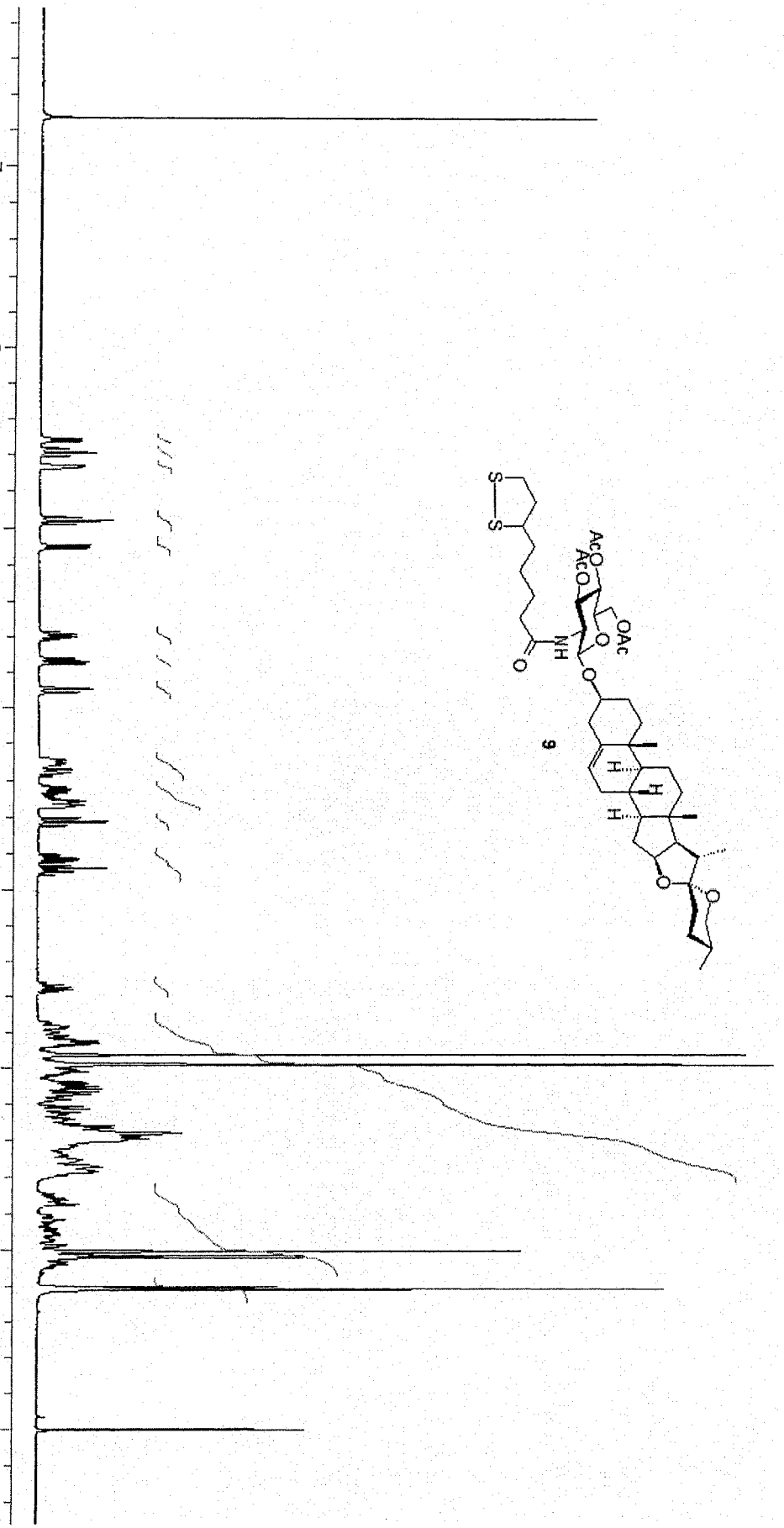
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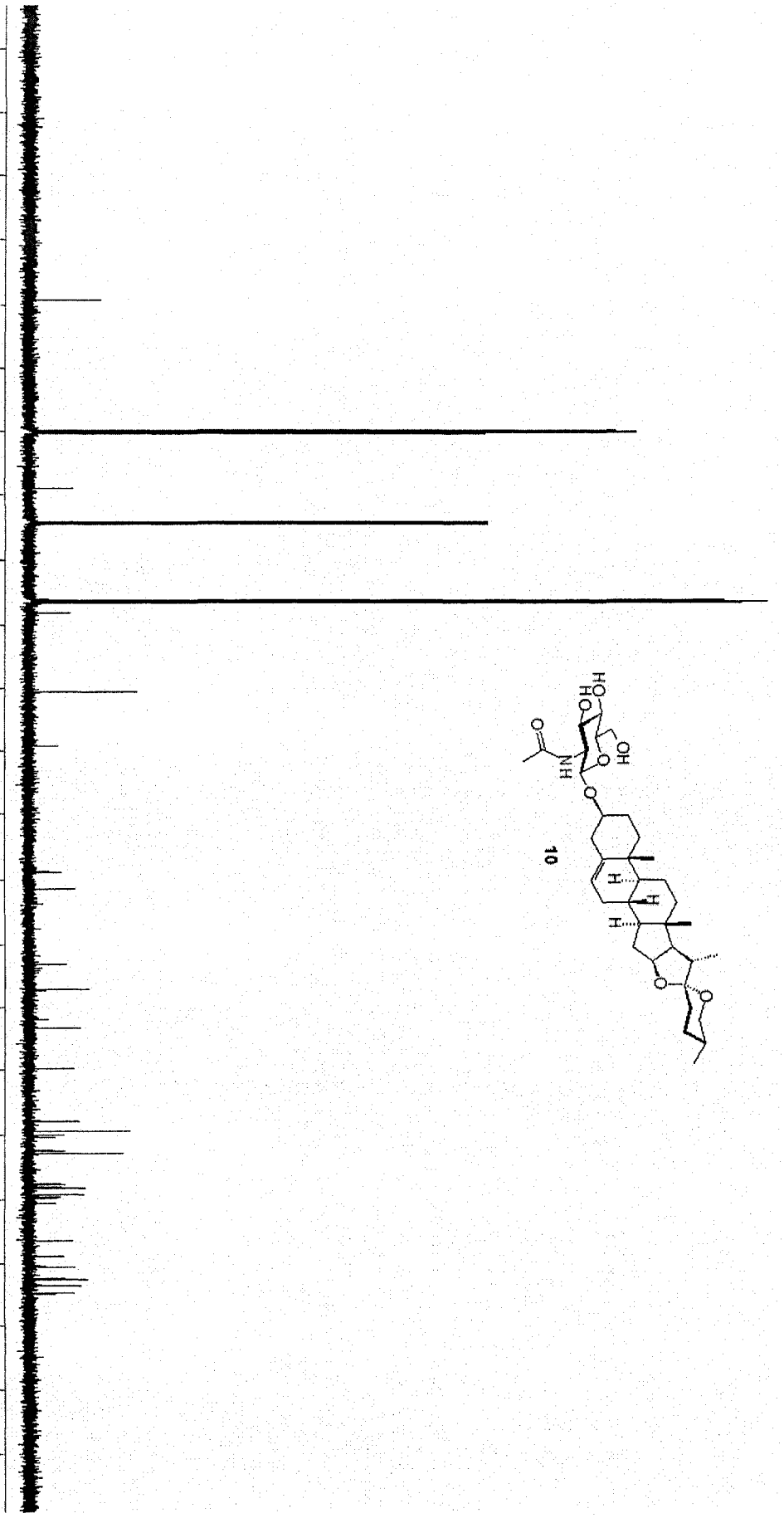
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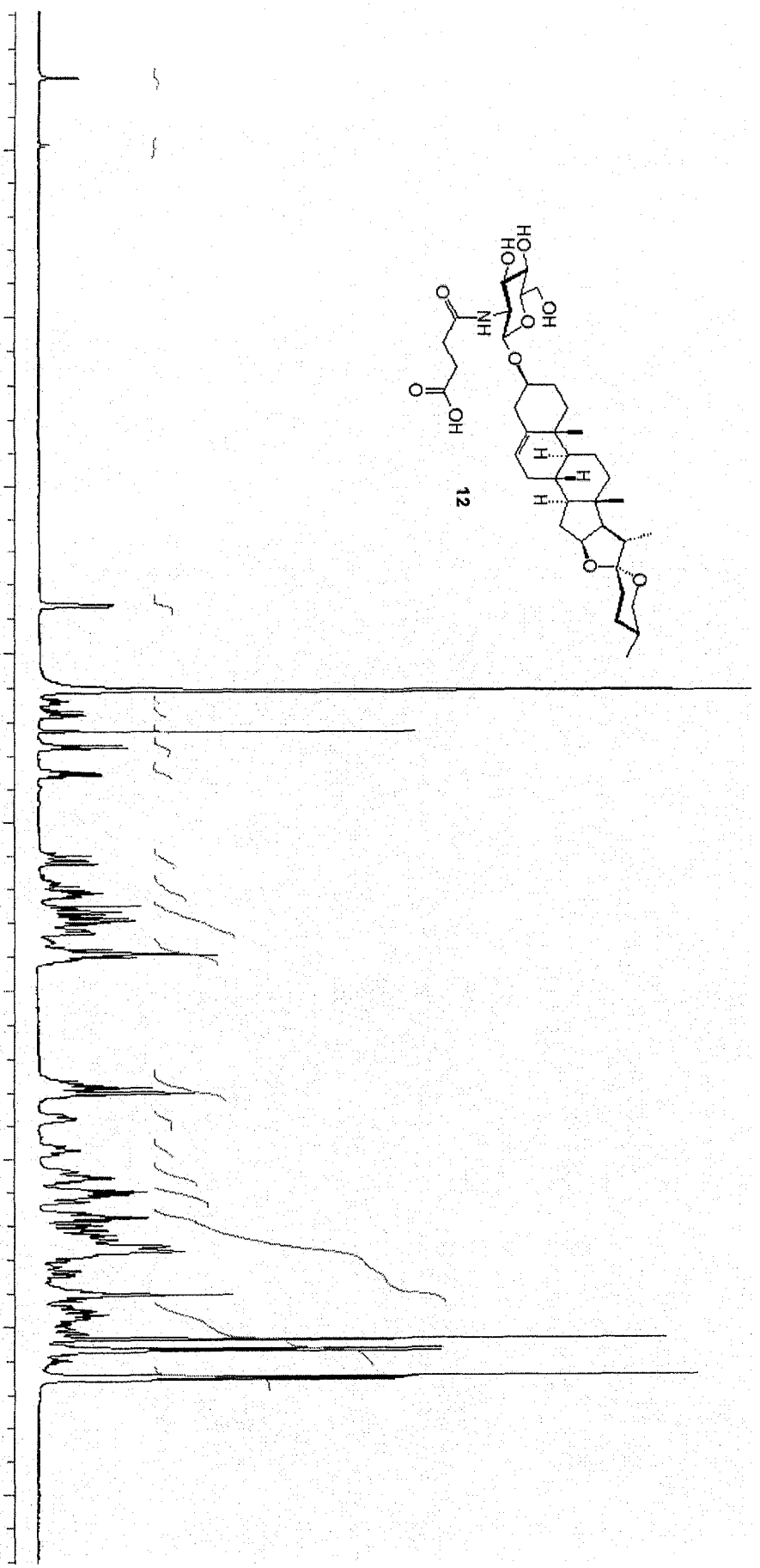
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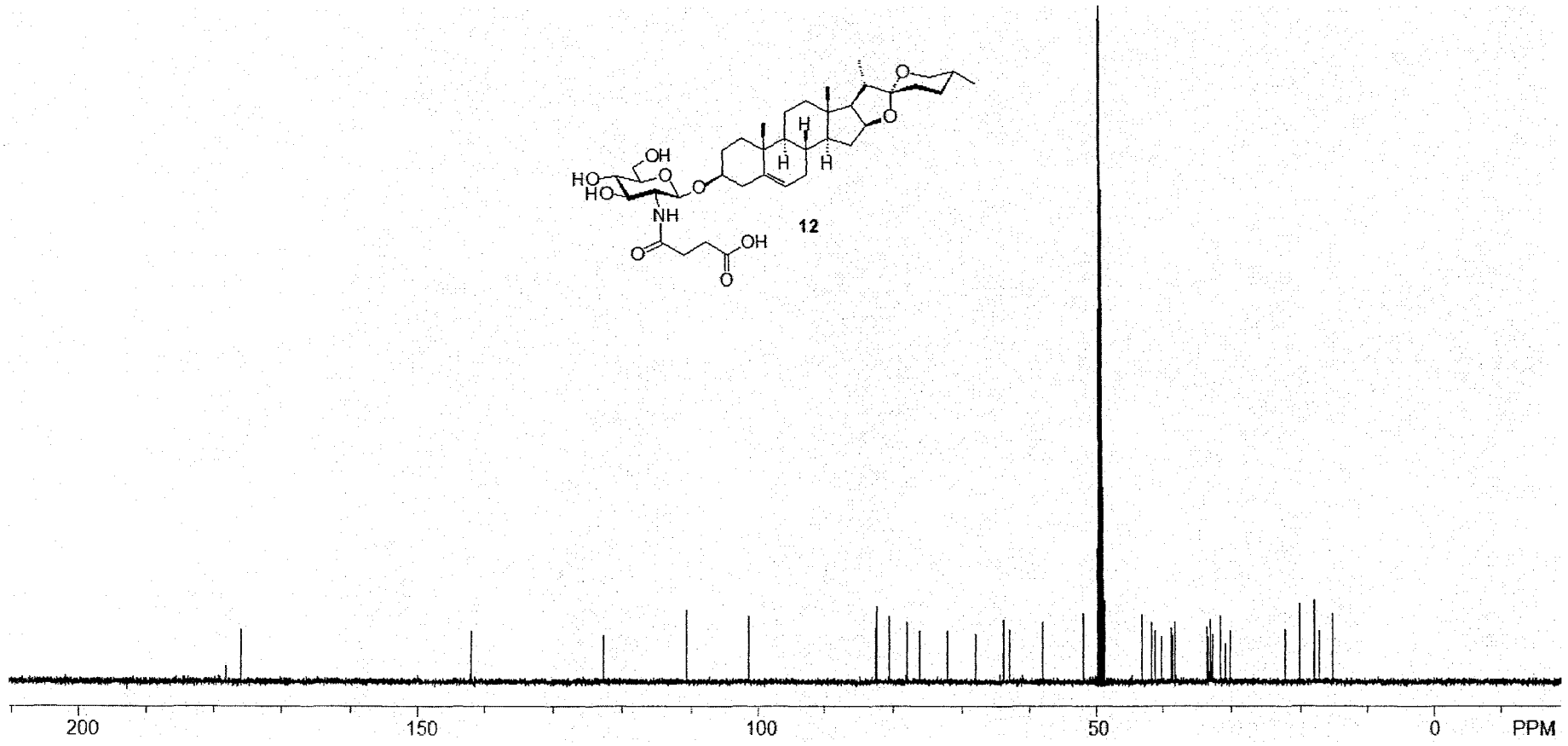
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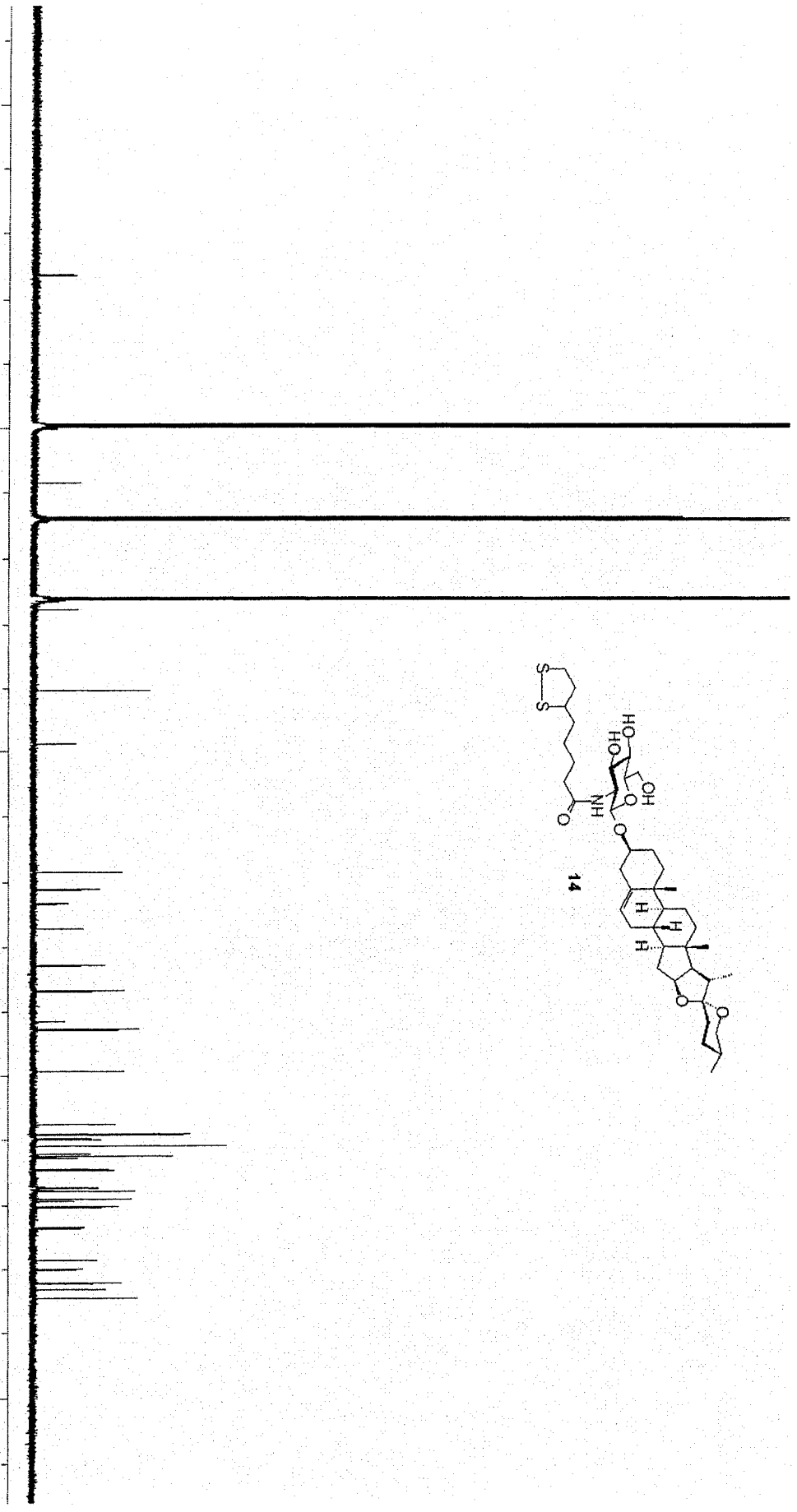

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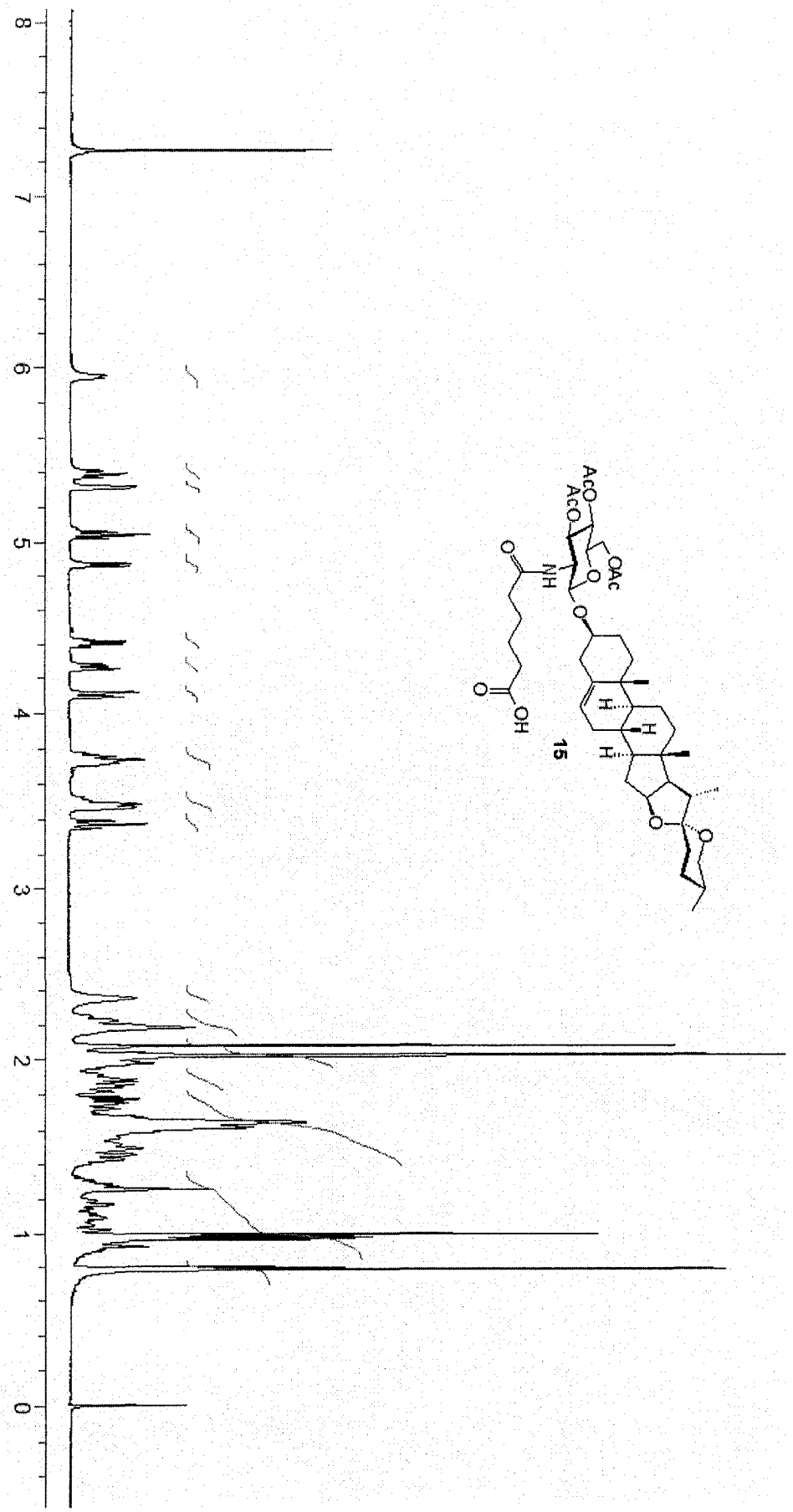
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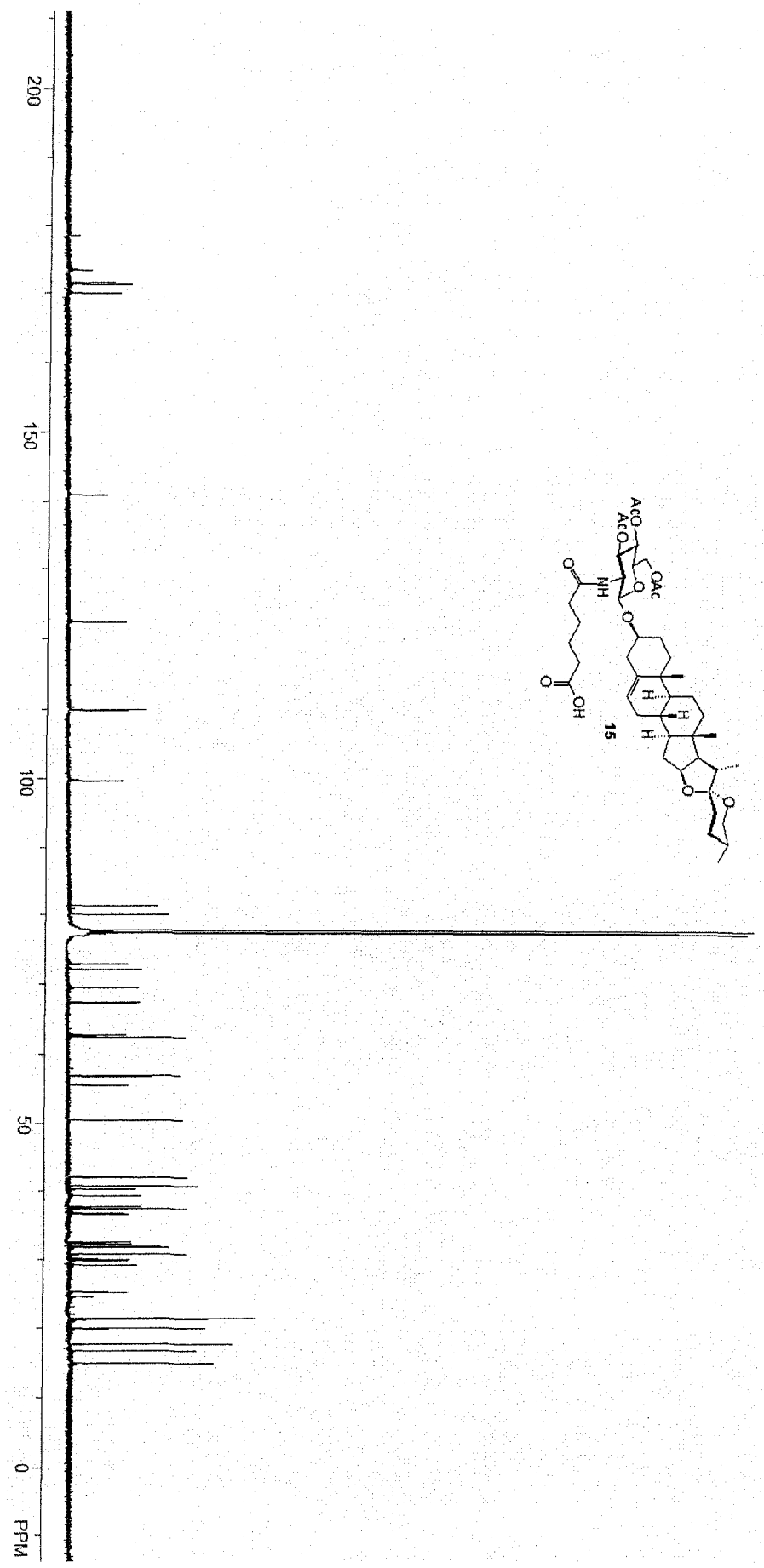
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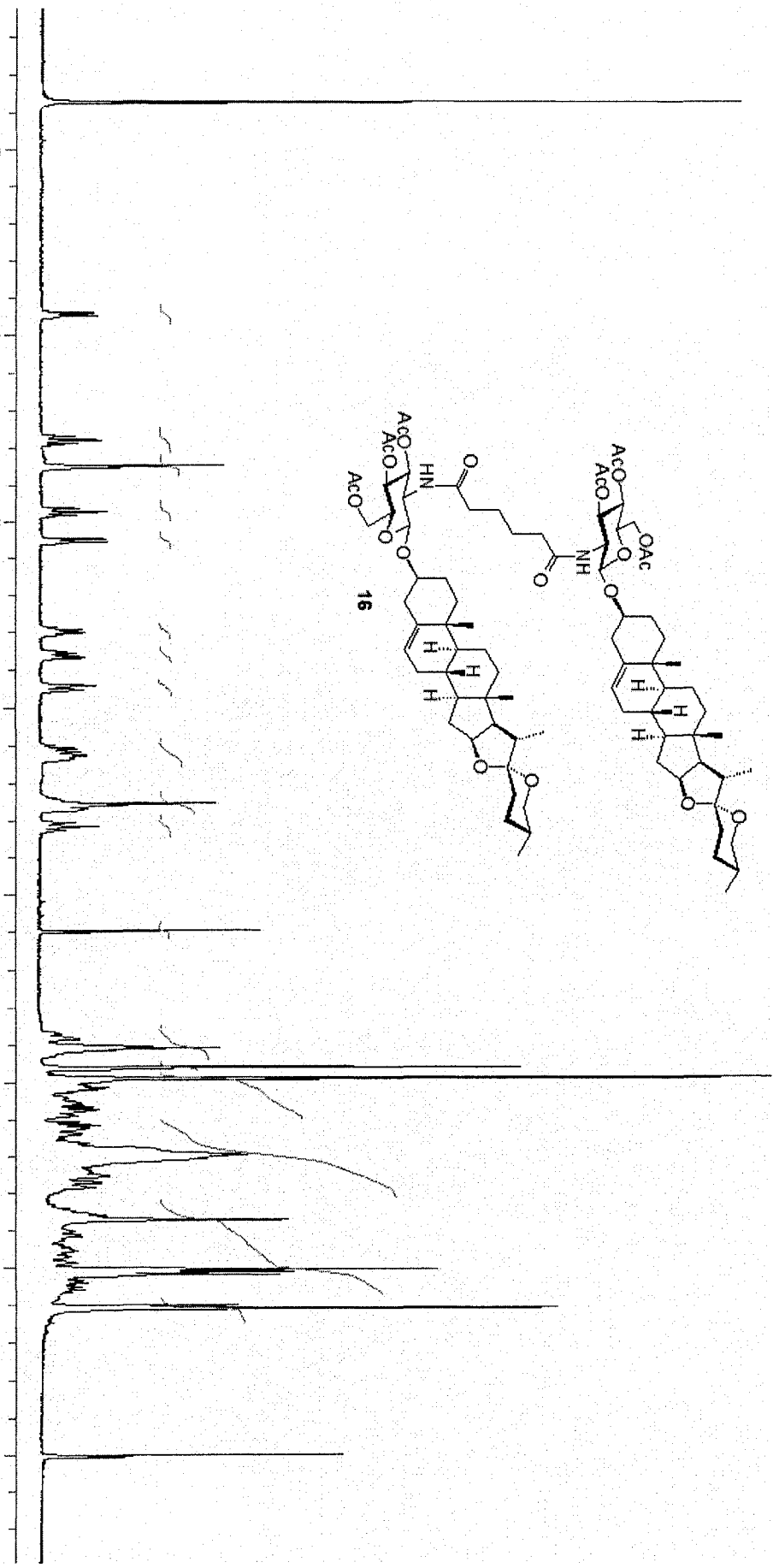
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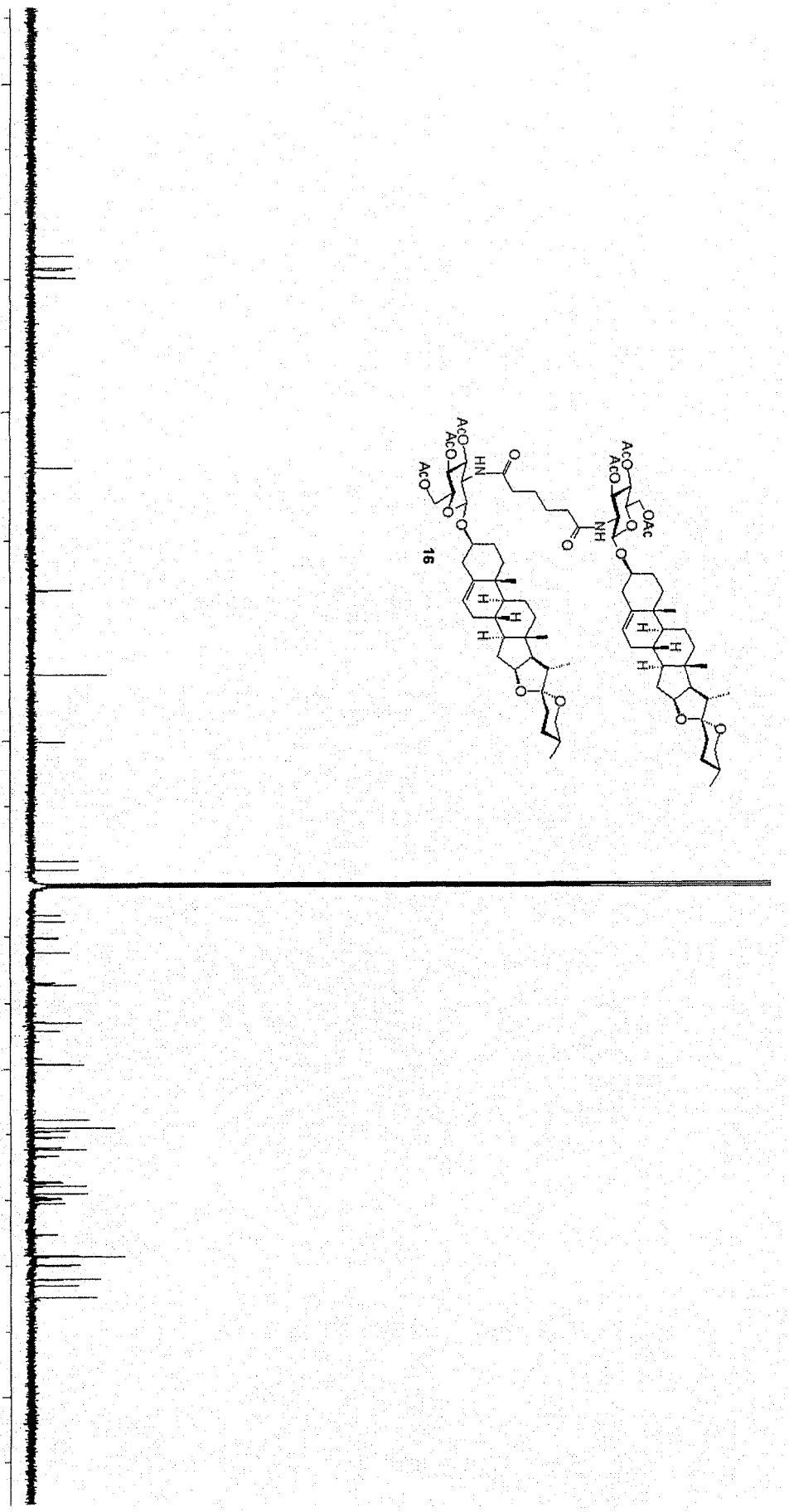
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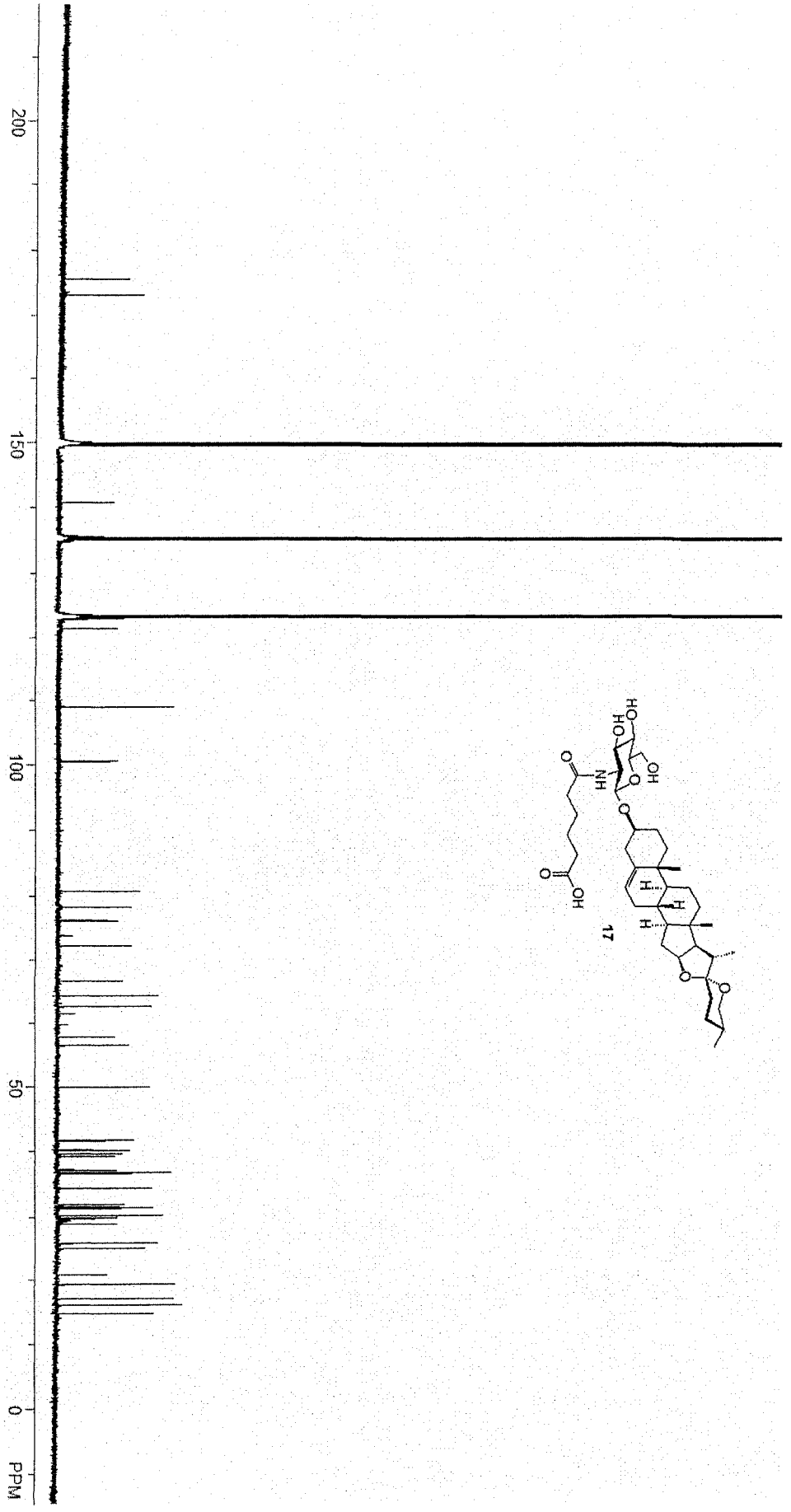
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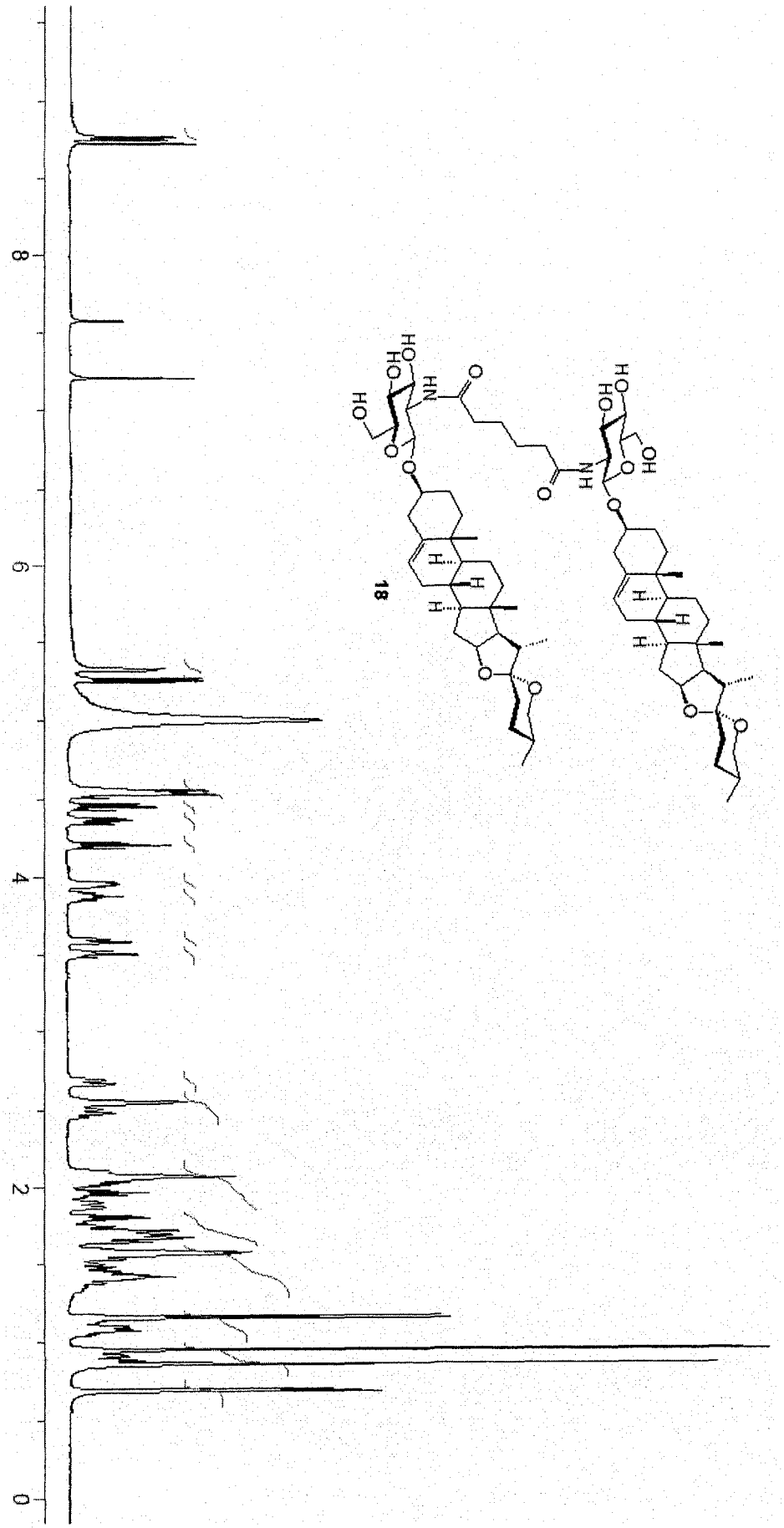
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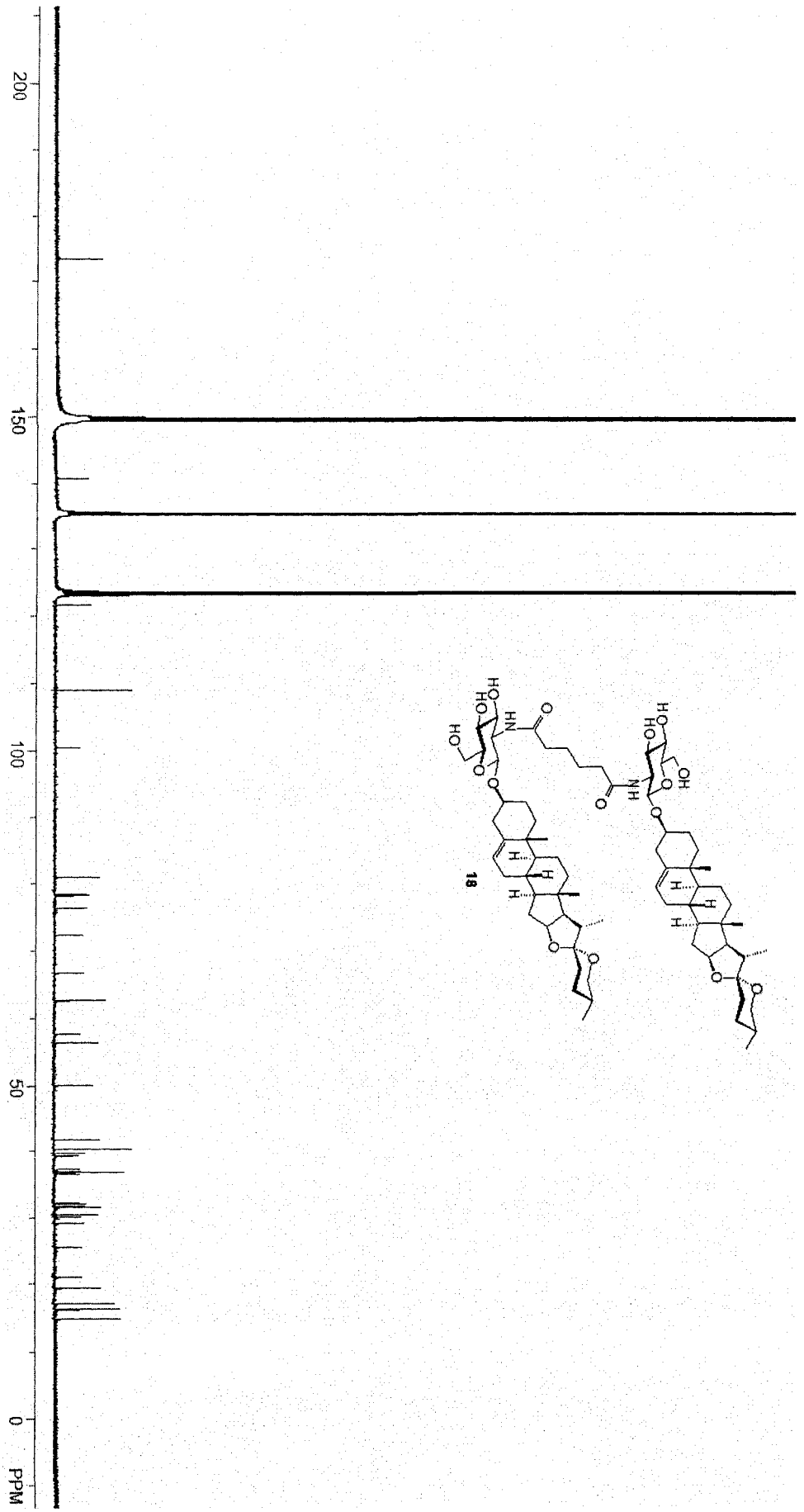

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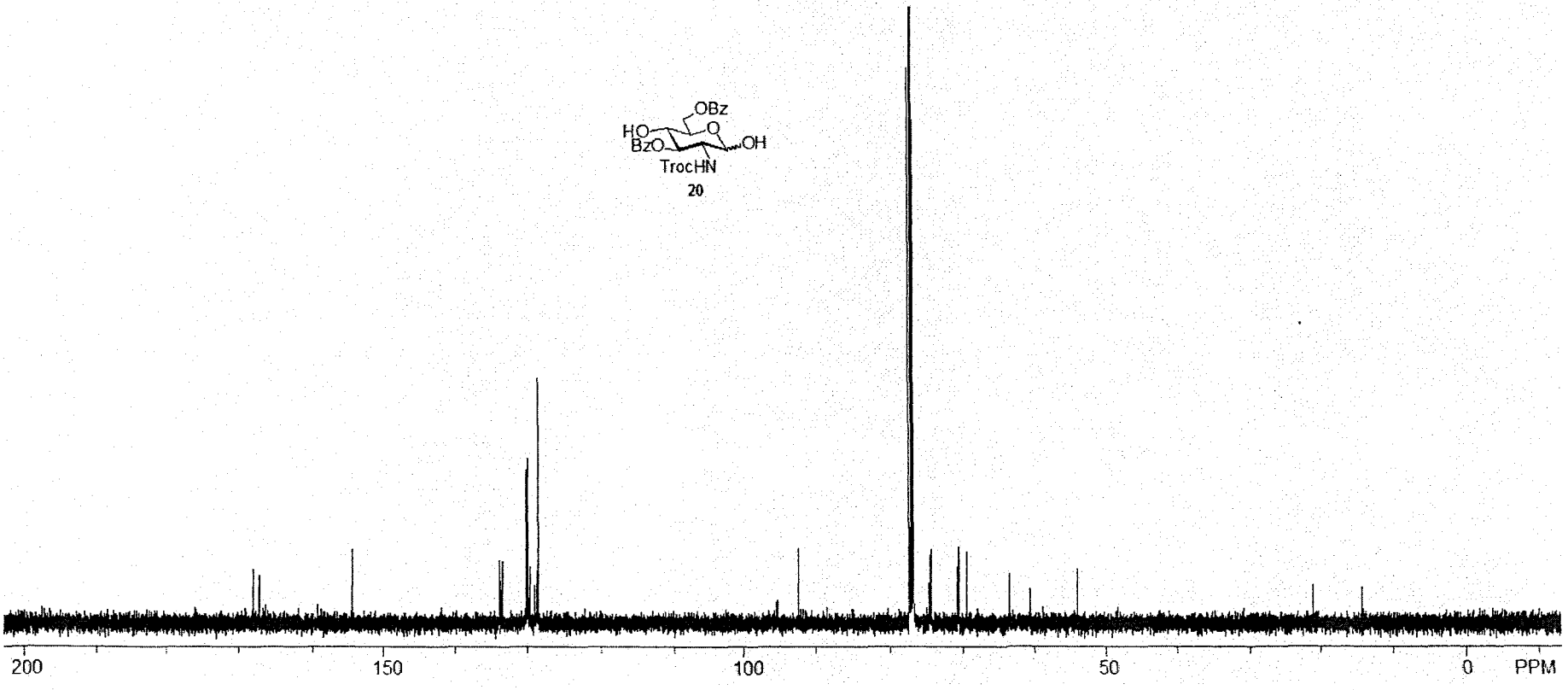
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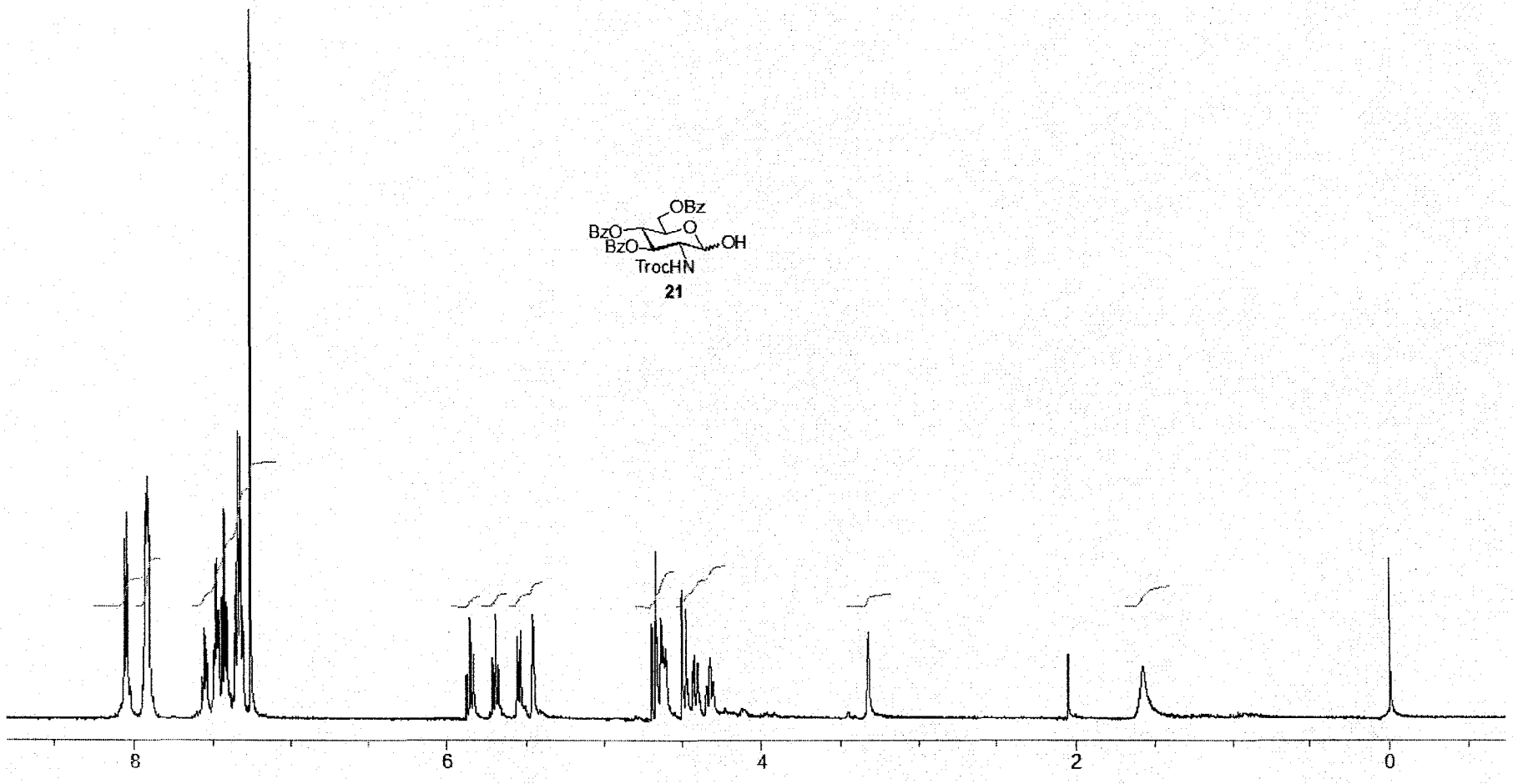
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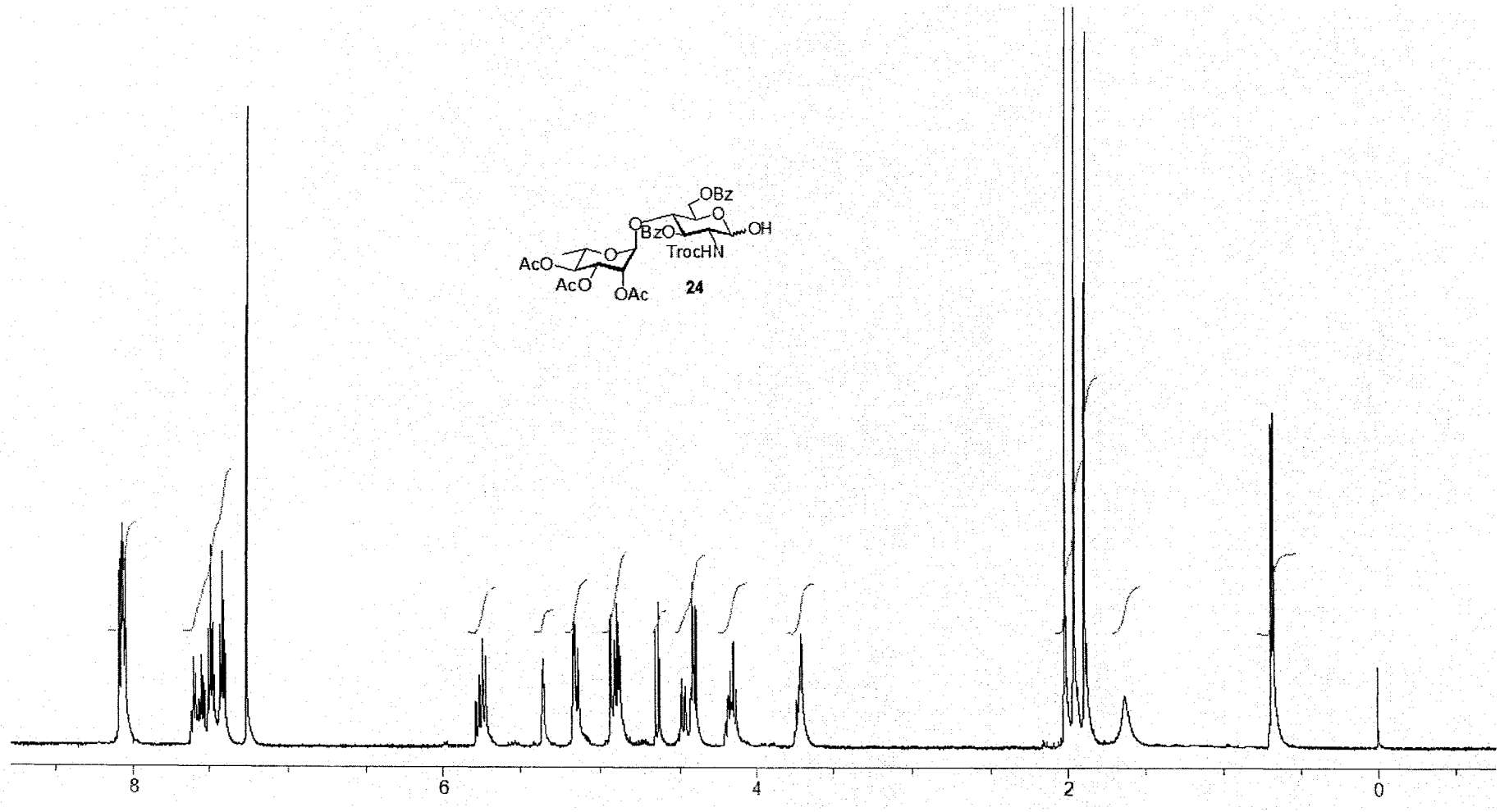
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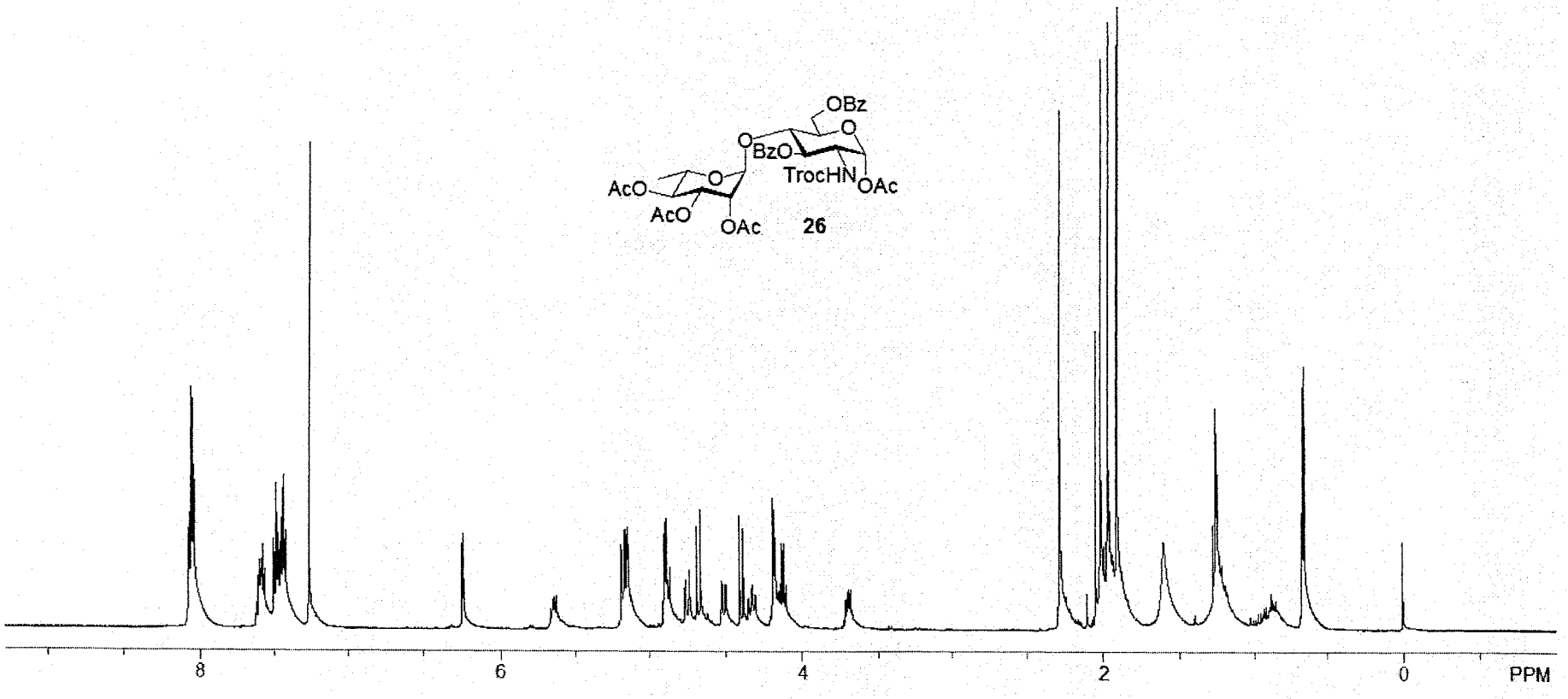
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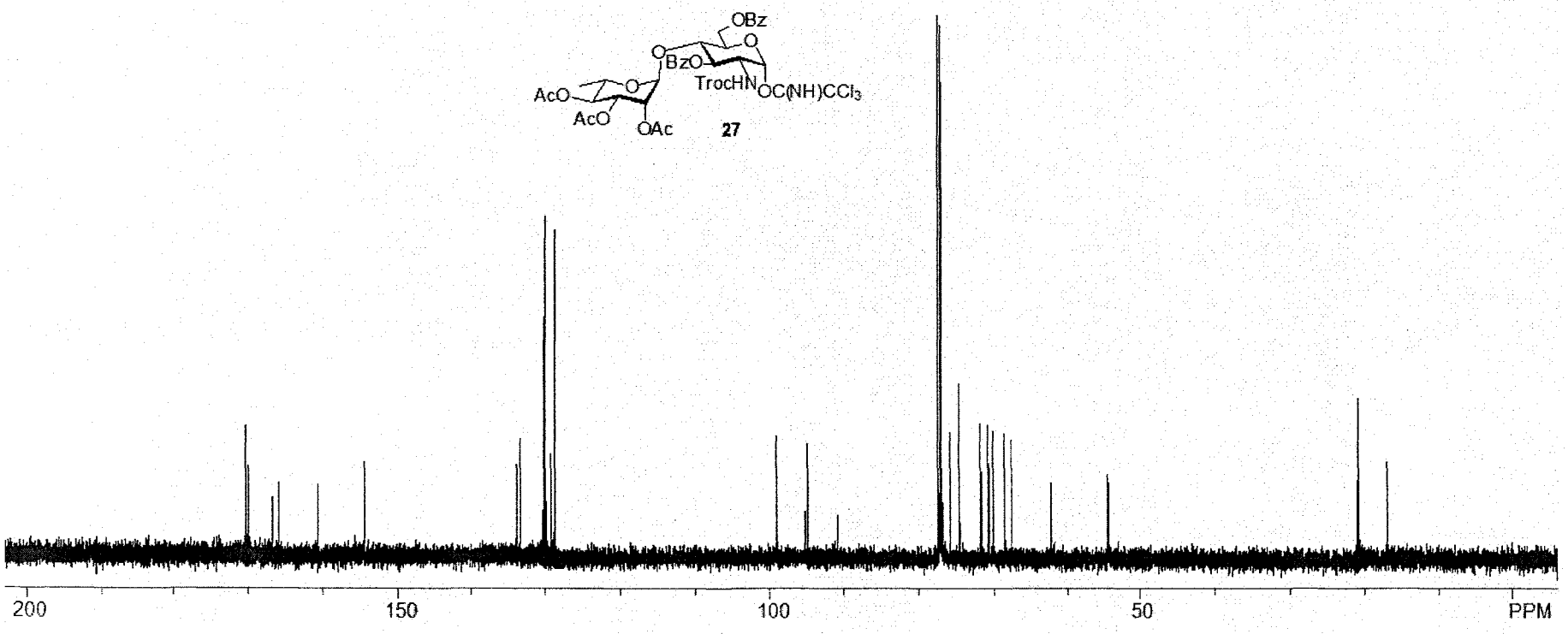
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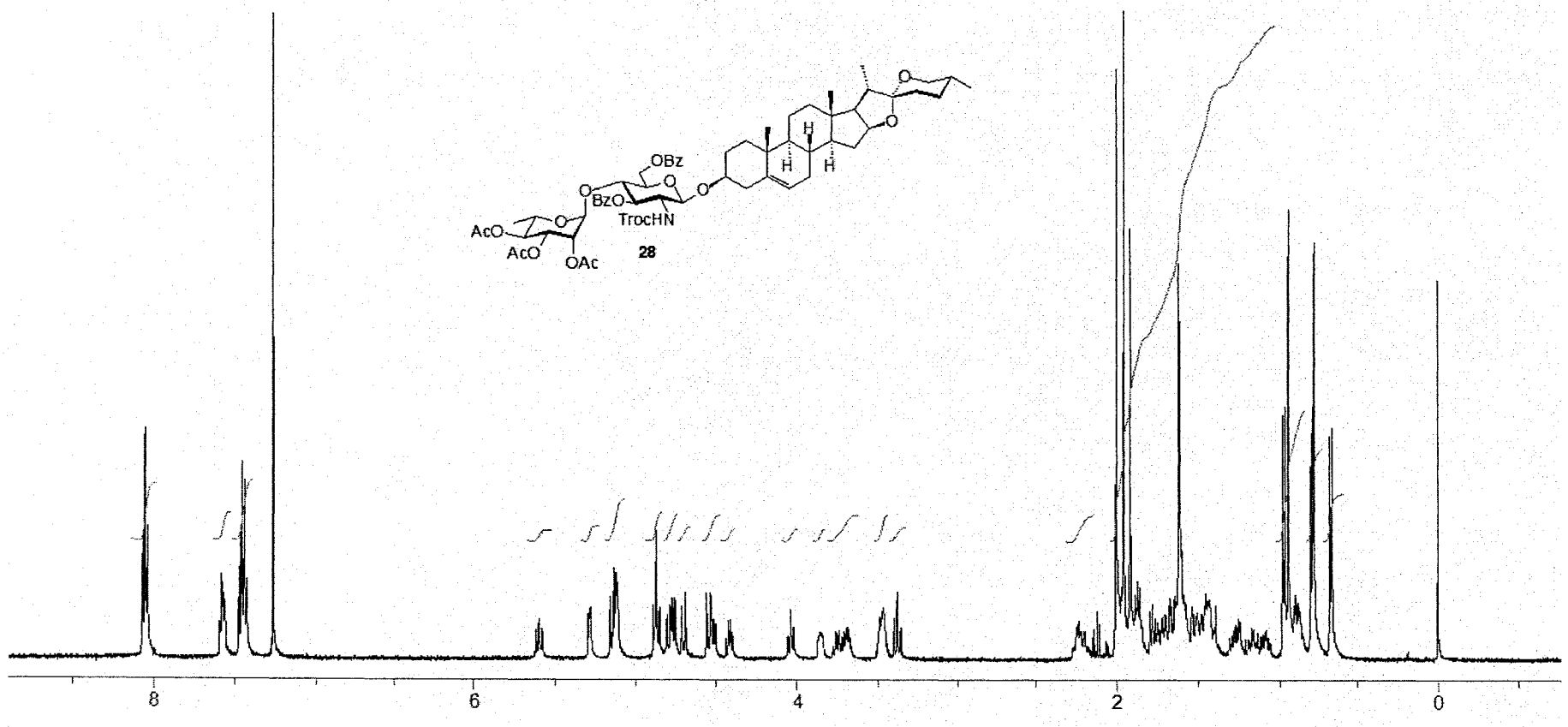
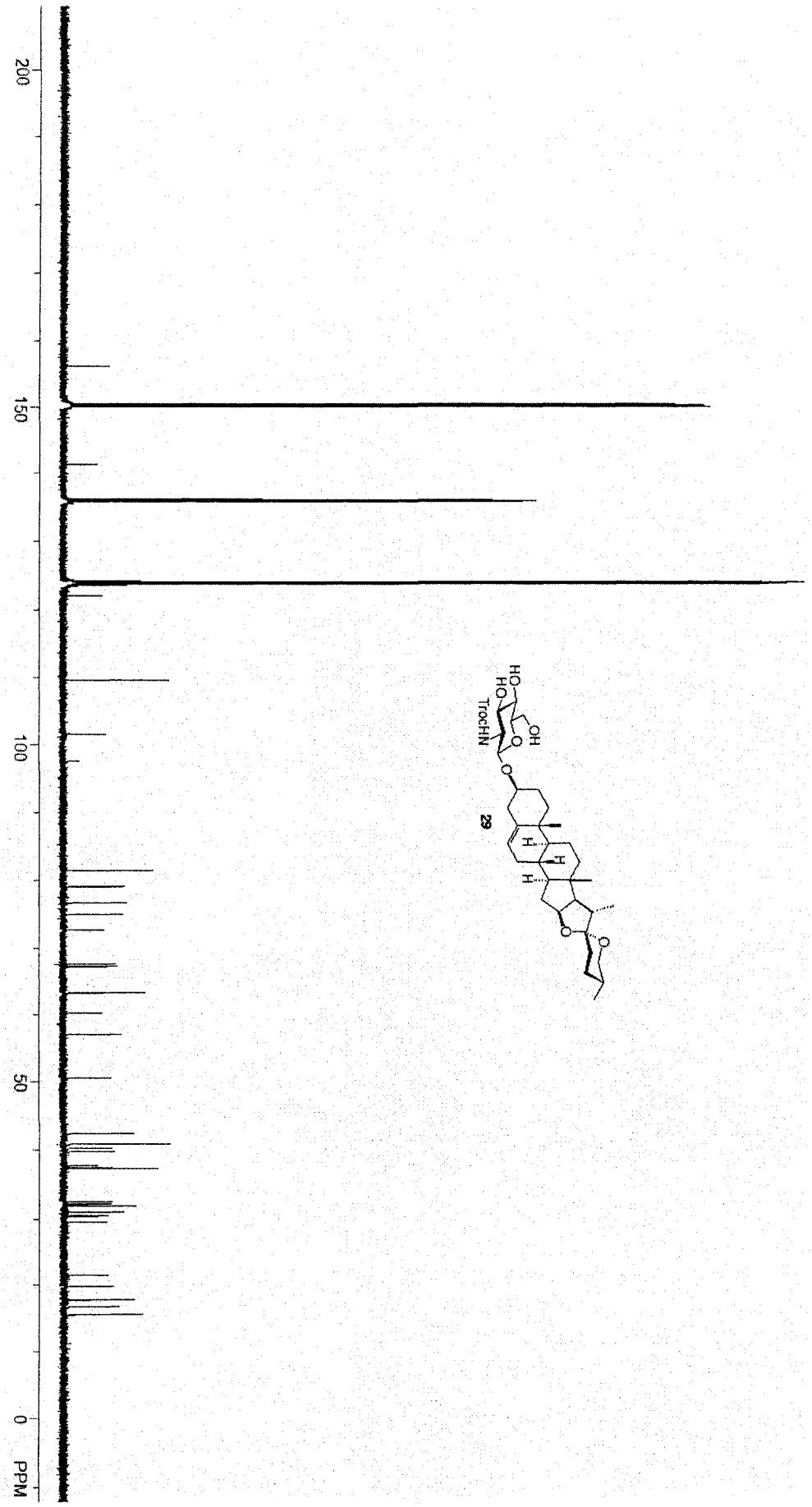
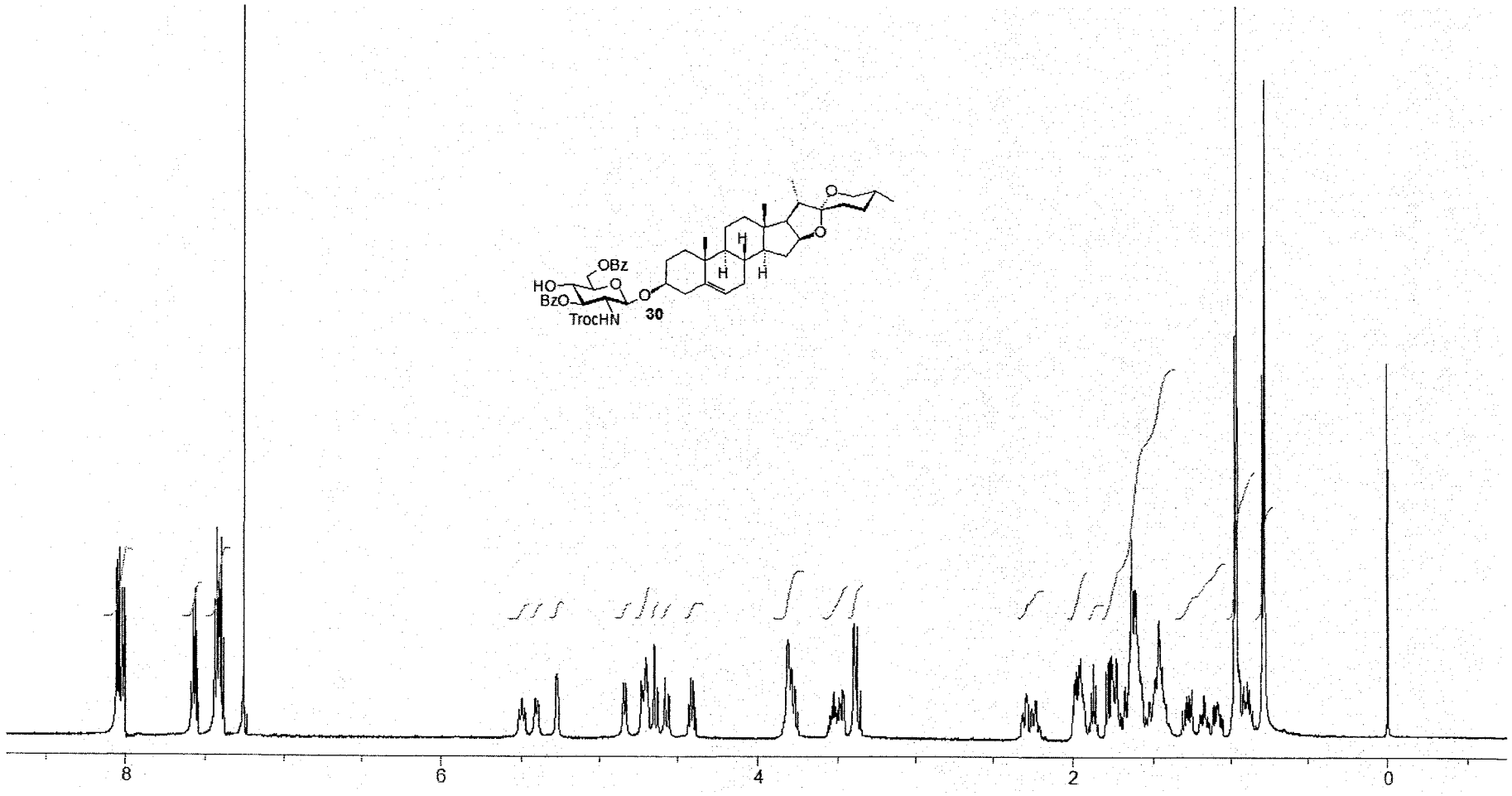
BzO
TrocHN
OC(NH)CCl3
AcO
AcO
OAc
27
200
150
100
50
PPM


OBz
BzO
TrocHN
AcO
AcO
OAc
28
8
6
4
2
0

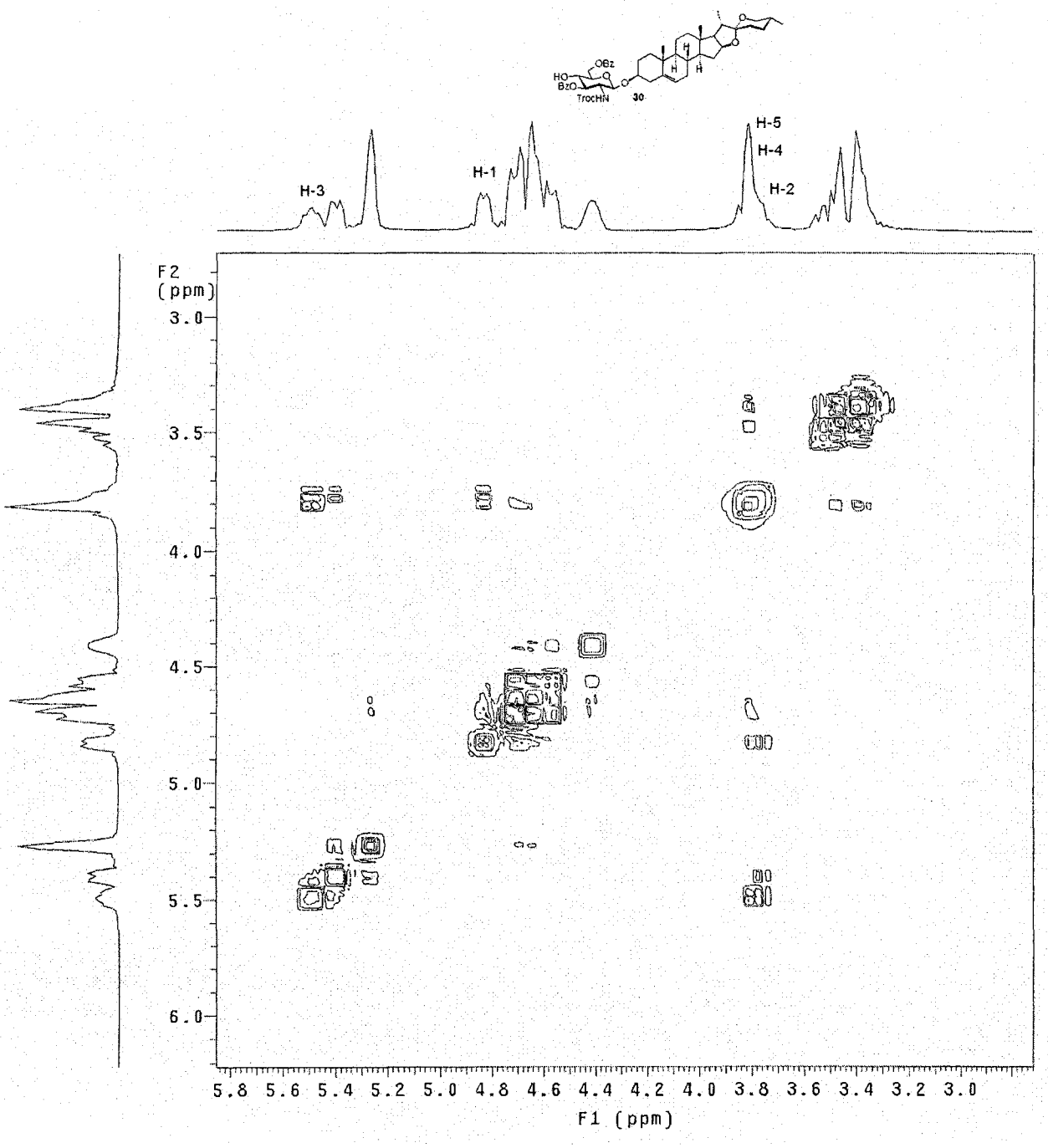
200
150
100
50
0
AcO
AcO
OAc
BzO
TrocHN
OBz
28

0
2
4
6
8
HO
HO
TrocHN
OH
O
O
H
H
H
O
O
O
29


200
150
100
50
0
PPM
HO
HO
TrocHN
OH
O
O
H
H
H
O
O
O
82

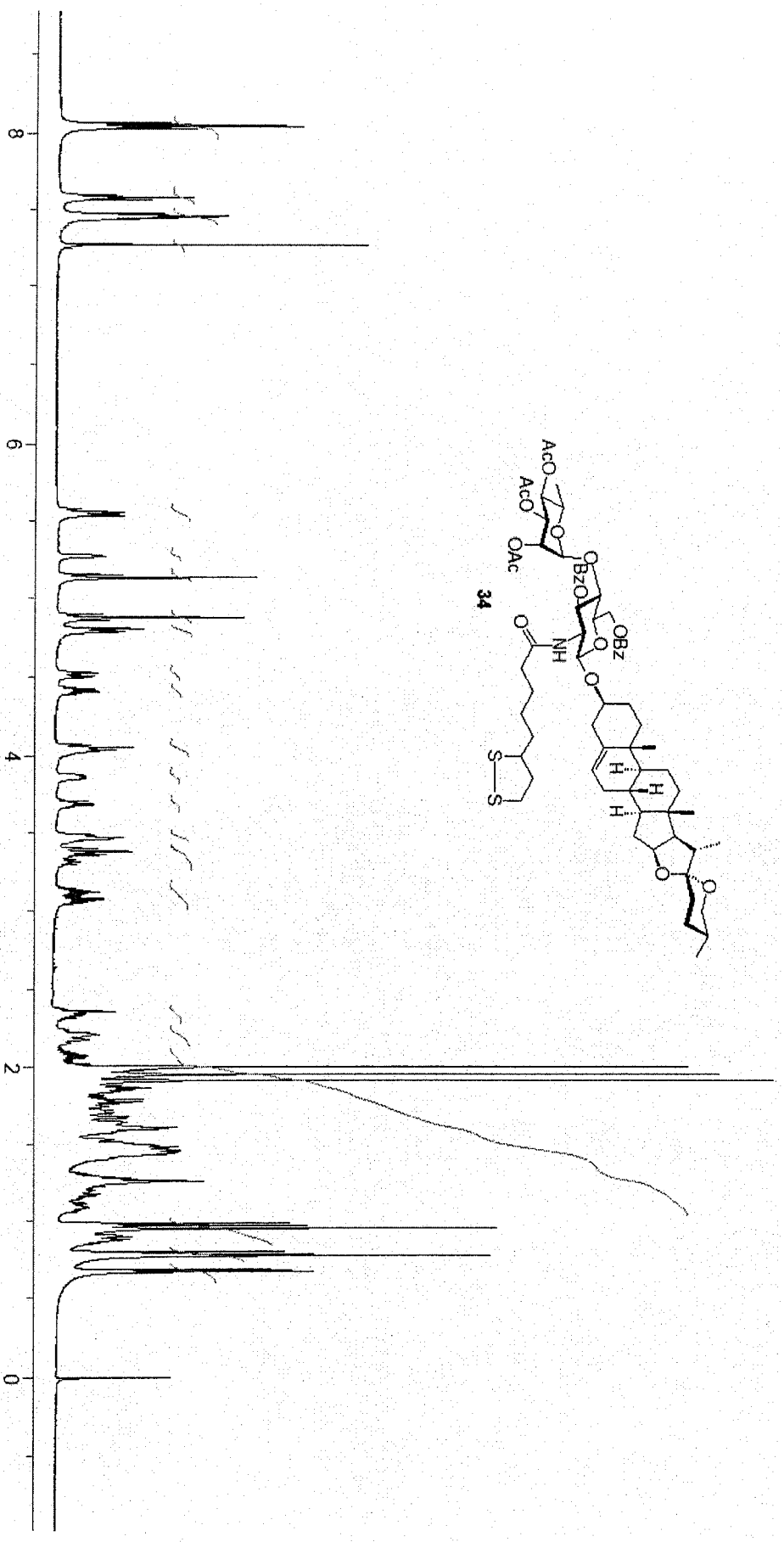

OBz
HO
BzO
TrocHN
30
8
6
4
2
0

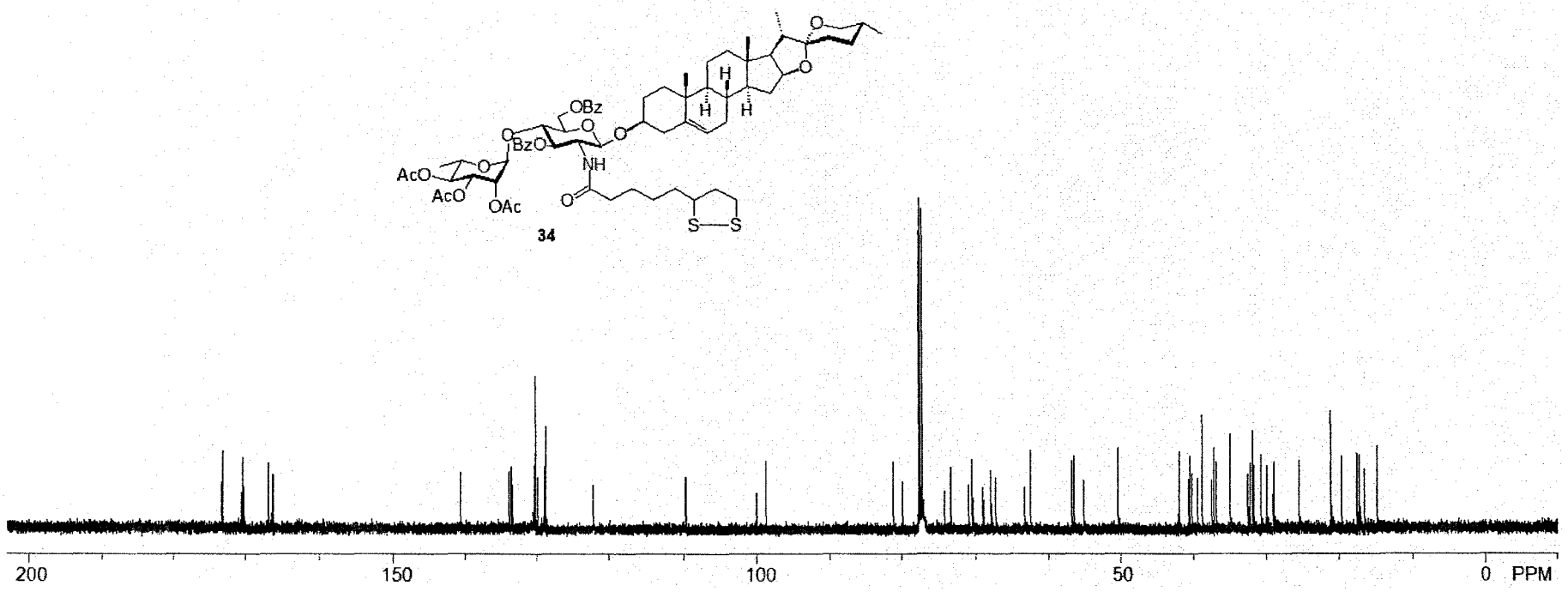
200
150
100
50
0
PPM
HO
BzO
TrocHN
OBz
O
30
H
H
H
O
O


OBz
HO
BzO
TrocHN
30
H-3
H-1
H-5
H-4
H-2
F2 (ppm)
3.0
3.5
4.0
4.5
5.0
5.5
6.0
5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8 3.6 3.4 3.2 3.0
F1 (ppm)

8
6
4
2
0
AcO
AcO
OAc
BzO
OBz
NH
O
H
H
H
33

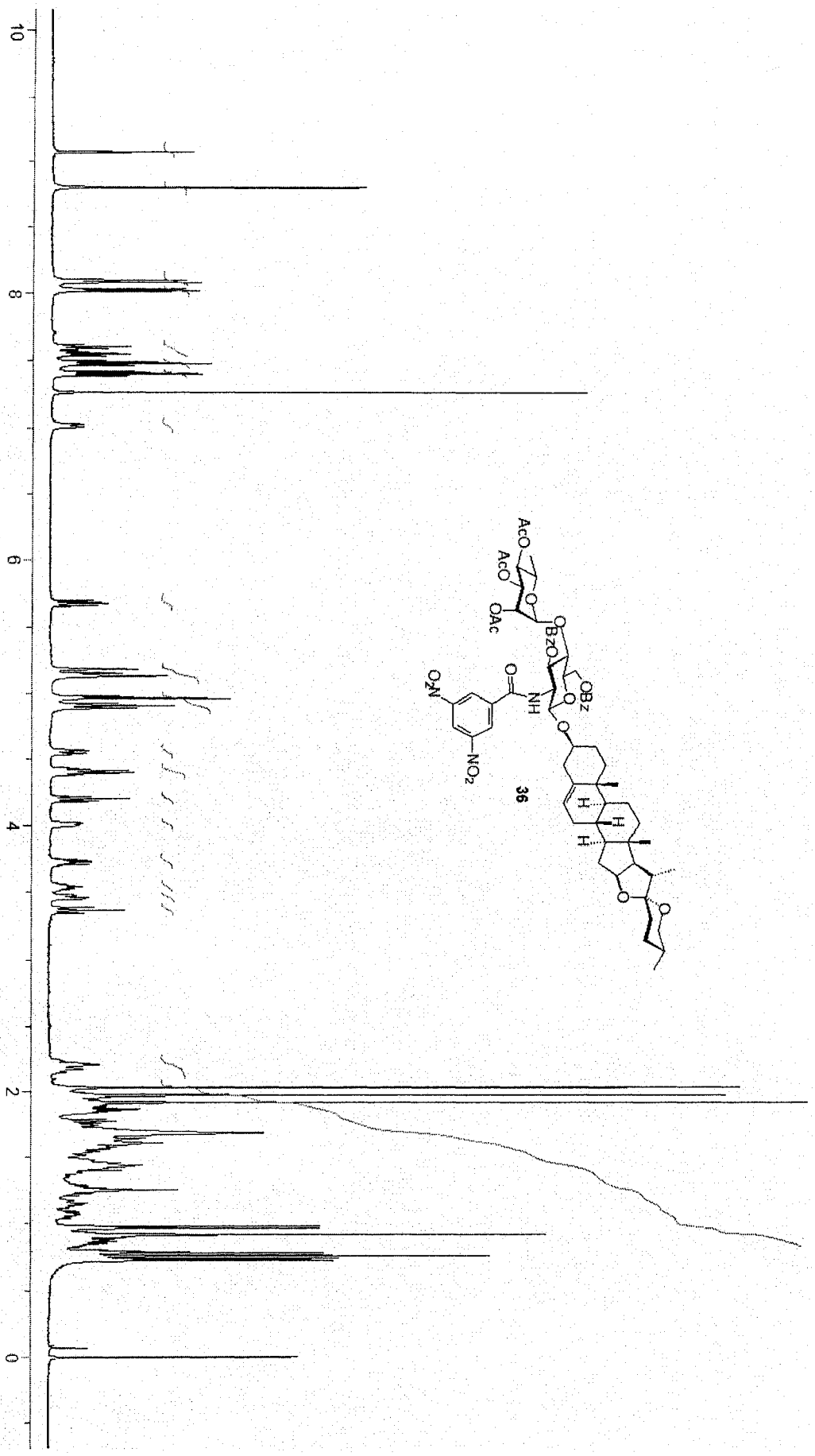
200
150
100
50
0
PPM
AcO
AcO
OAc
BzO
OBz
NH
O
H
H
H
33

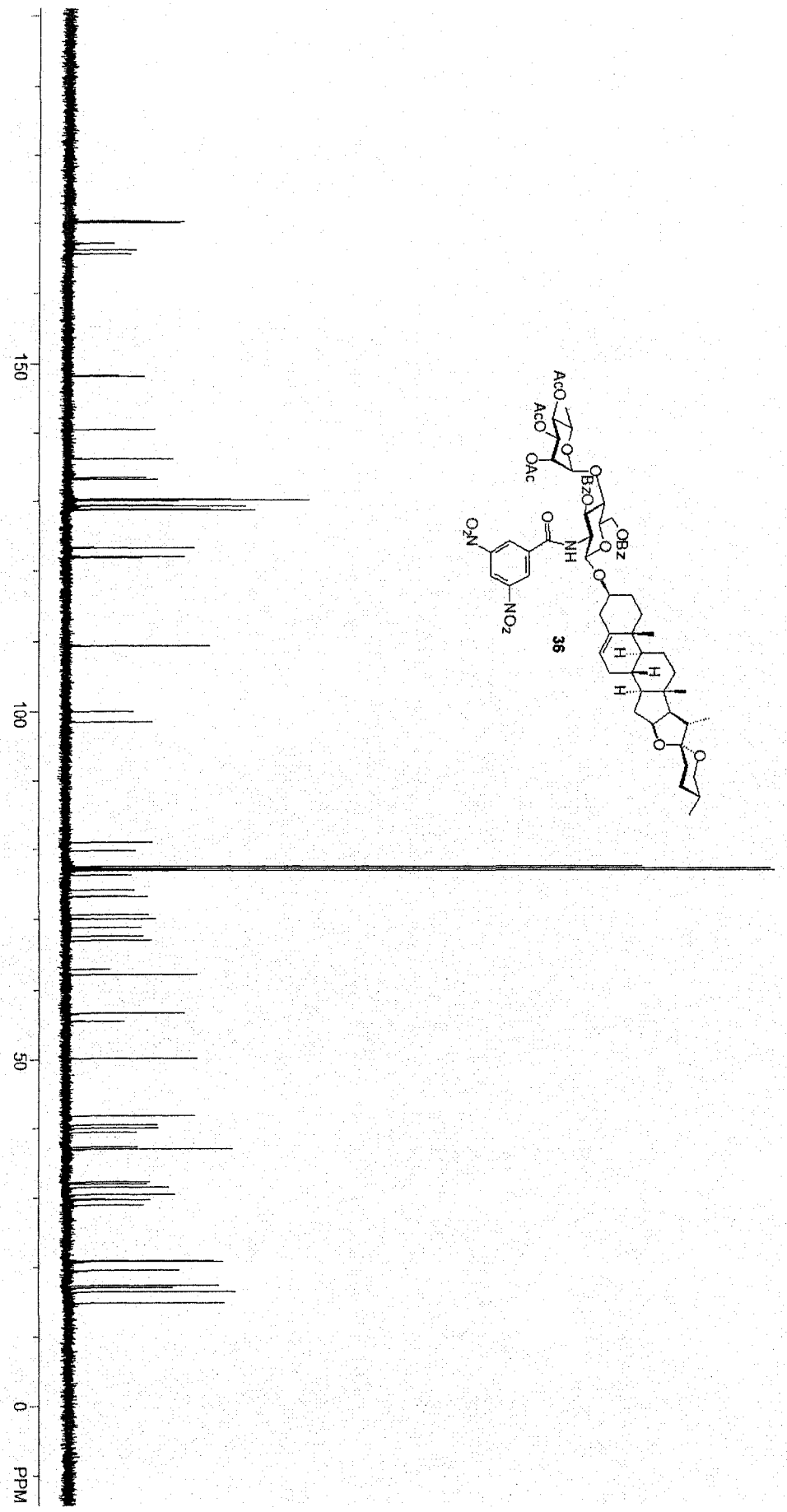

8
6
4
2
0
AcO
AcO
OAc
BzO
OBz
NH
S
S
H
H
H
O
O
O
34


OBz
BzO
AcO
AcO
OAc
NH
H
H
H
O
S—S
34
200
150
100
50
0
PPM

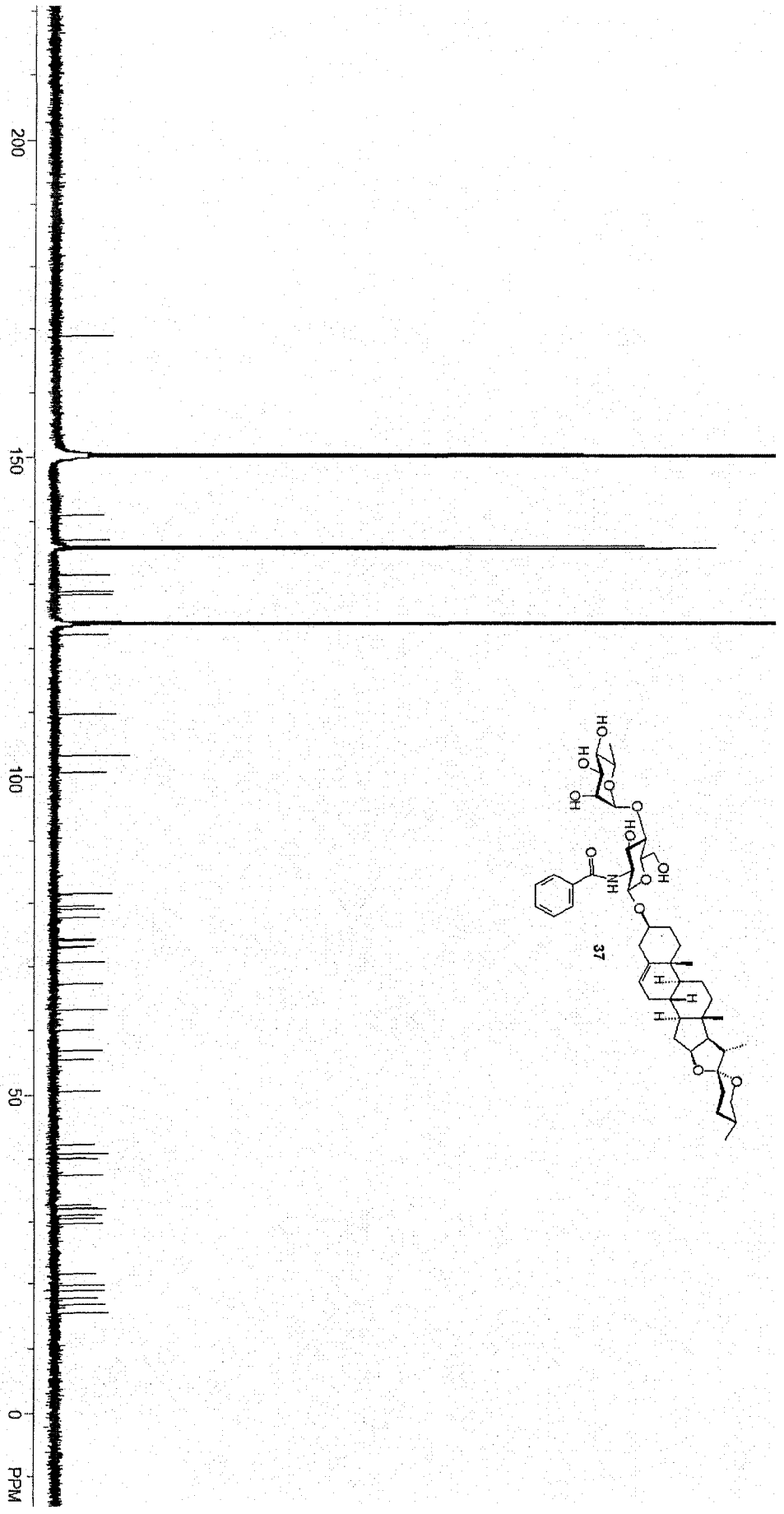
8
6
4
2
0
AcO
AcO
OAc
BzO
OBz
O2N
NH
35

150
100
50
0
PPM
AcO
AcO
OAc
BzO
OBz
NH
O2N
35


10
8
6
4
2
0
AcO
AcO
OAc
BzO
OBz
O
NH
O
O2N
NO2
36


150
100
50
0
PPM
AcO
AcO
OAc
BzO
OBz
O
NH
O2N
NO2
H
H
H
36

10
8
6
4
2
0
HO
HO
OH
HO
O
NH
OH
37
H
H
H
O
O


200
150
100
50
0
PPM
HO
HO
OH
O
HO
OH
O
NH
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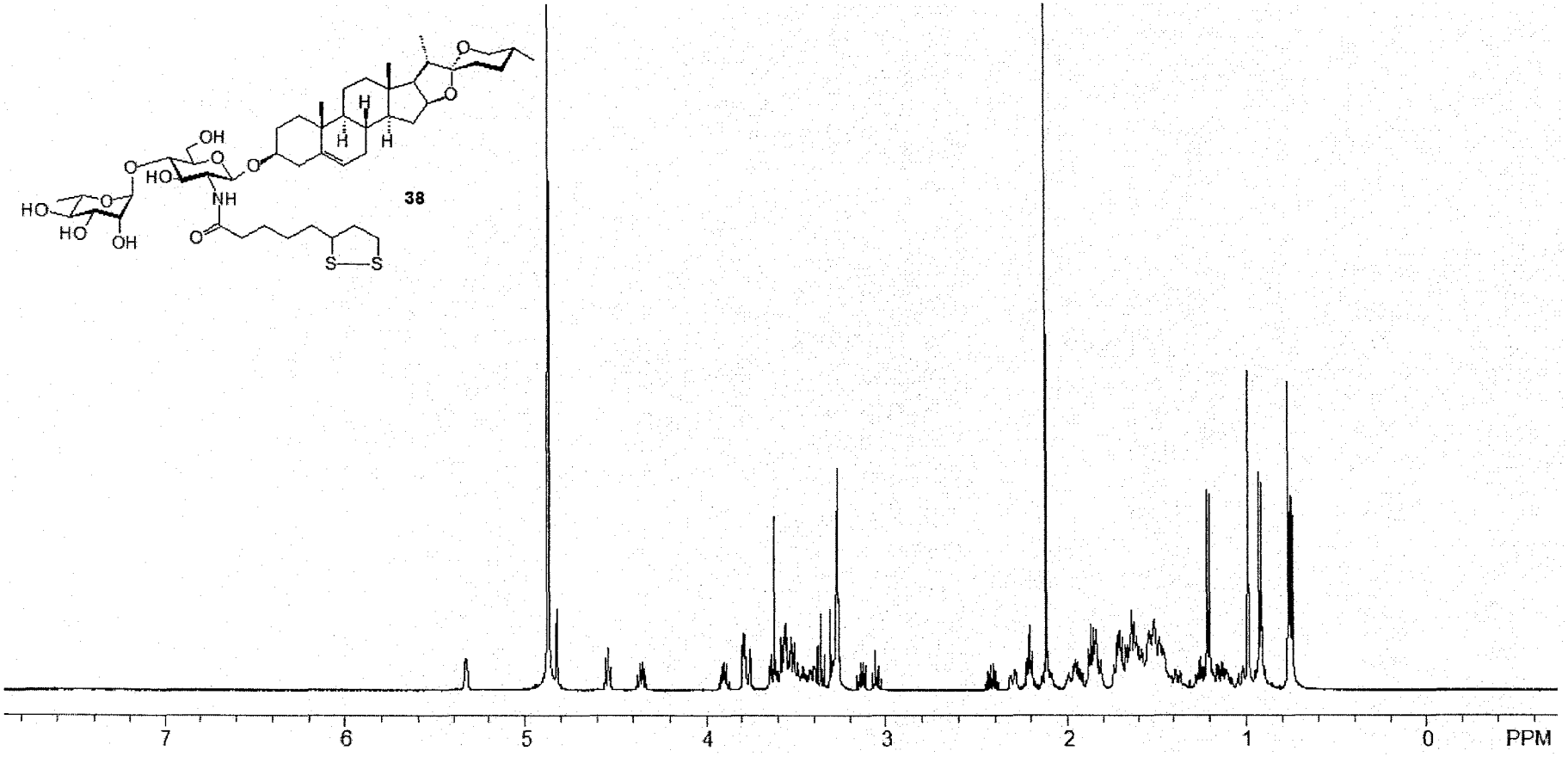
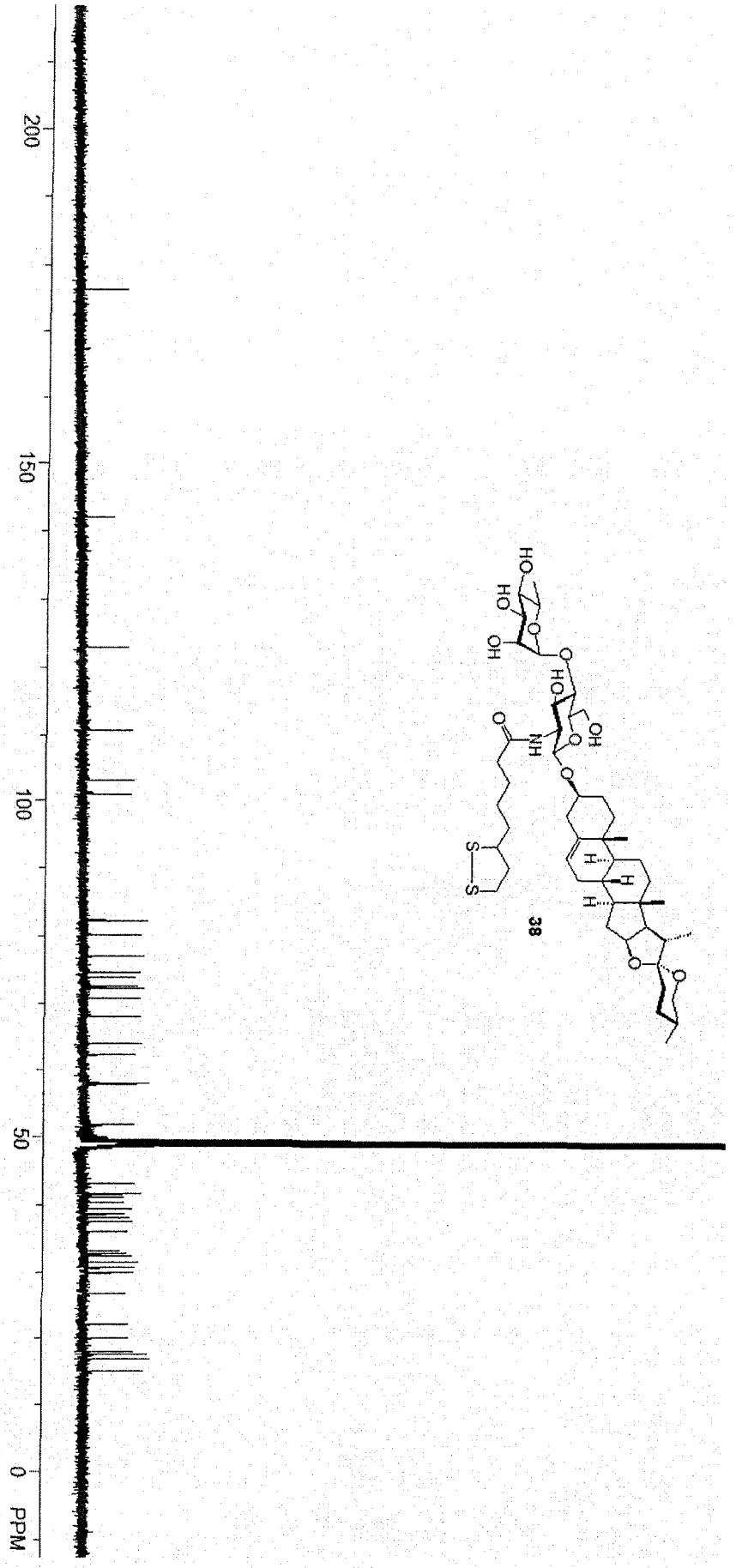
O
H
H
H
O
O
O
37


38
HO
HO
OH
OH
HO
NH
O
S
S
H
H
H
O
O
O
O
7
6
5
4
3
2
1
0
PPM


200
150
100
50
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PPM
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HO
OH
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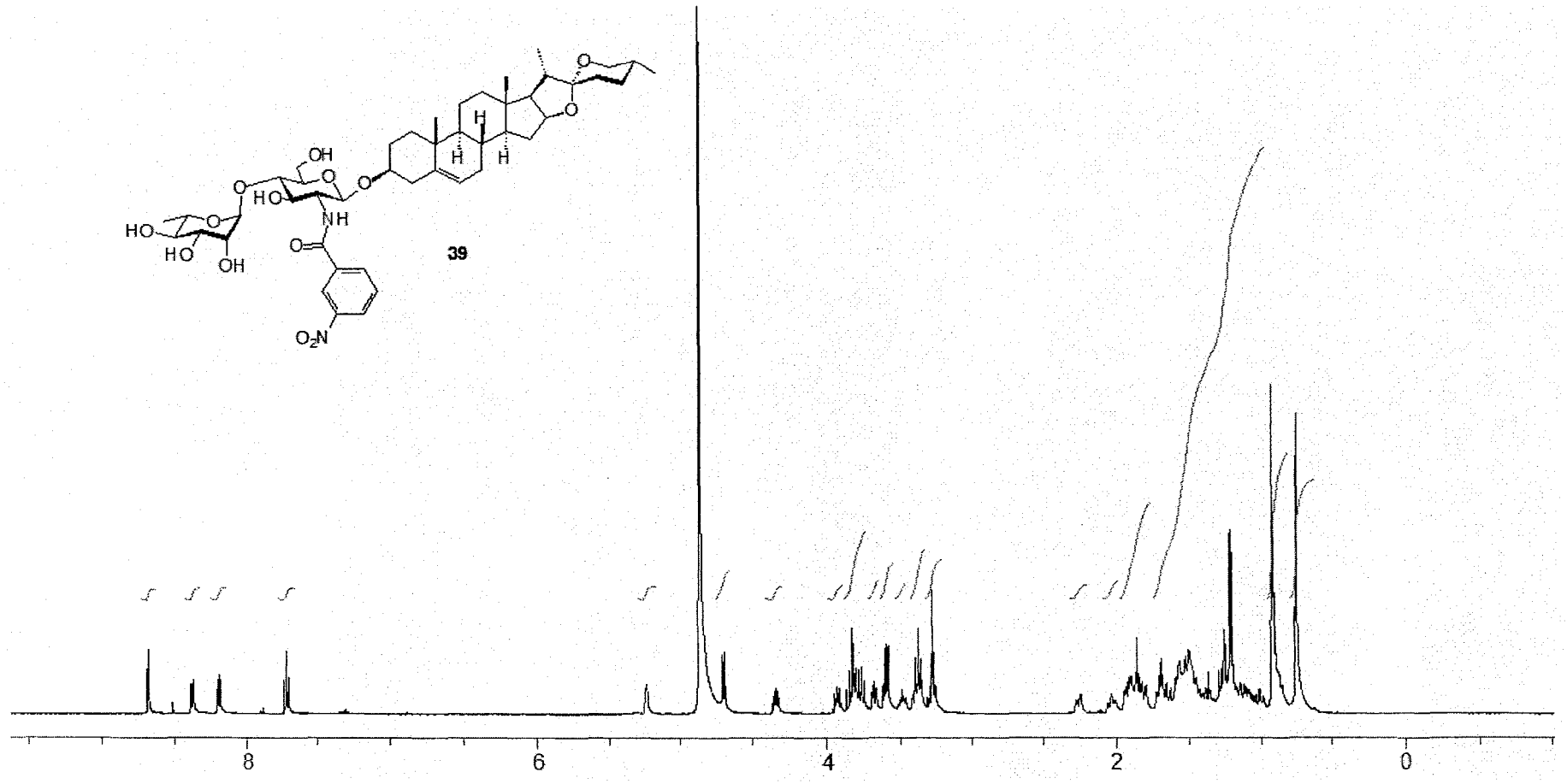
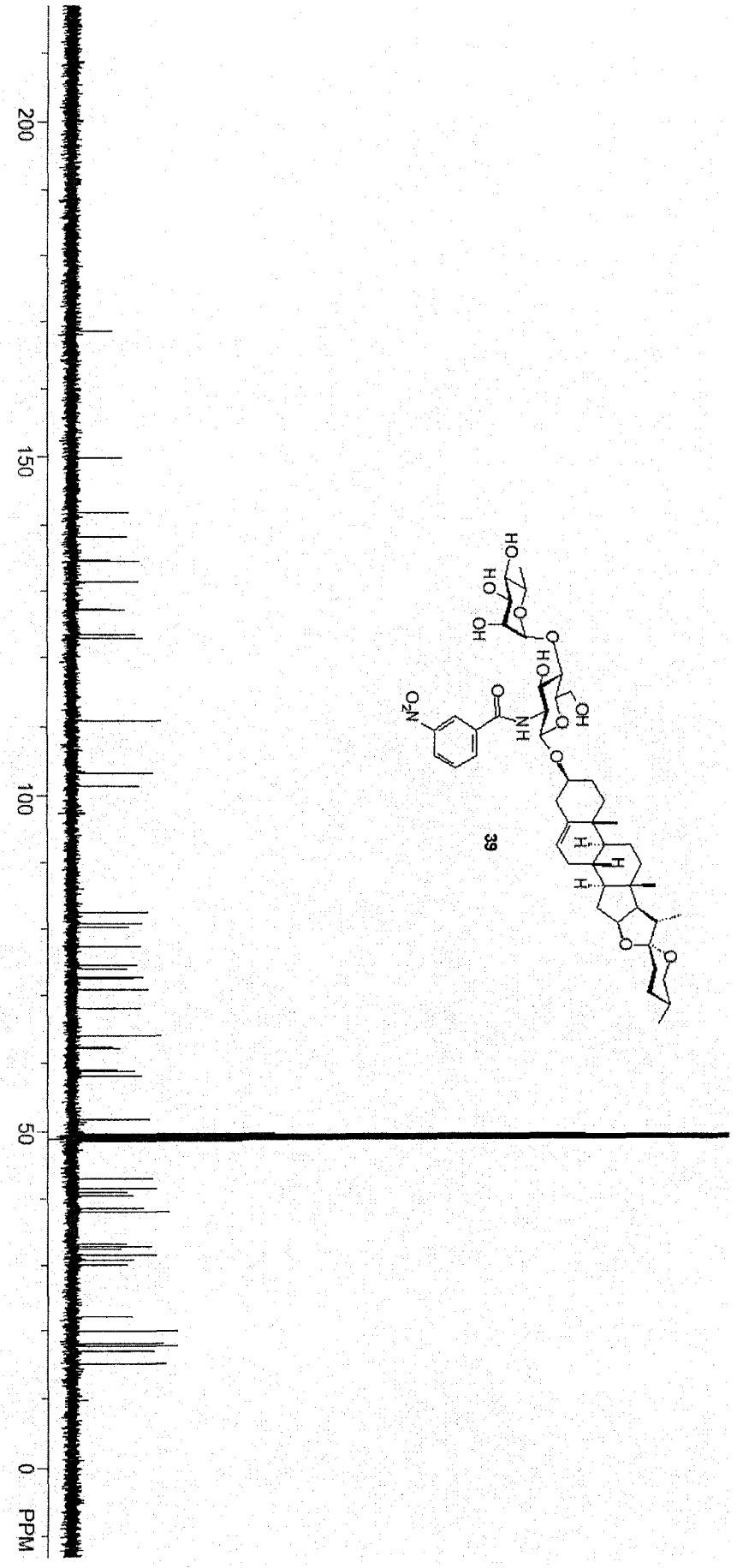
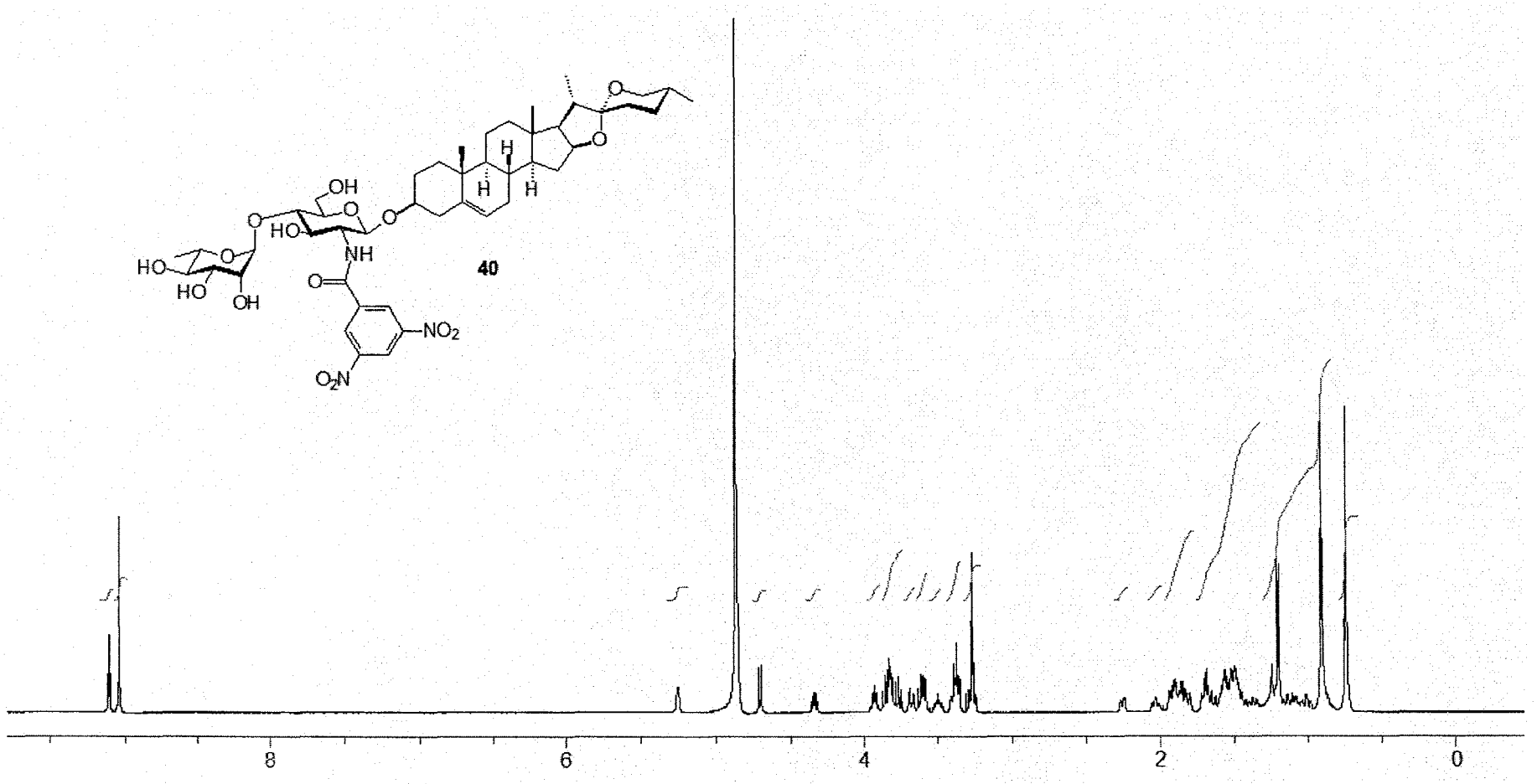
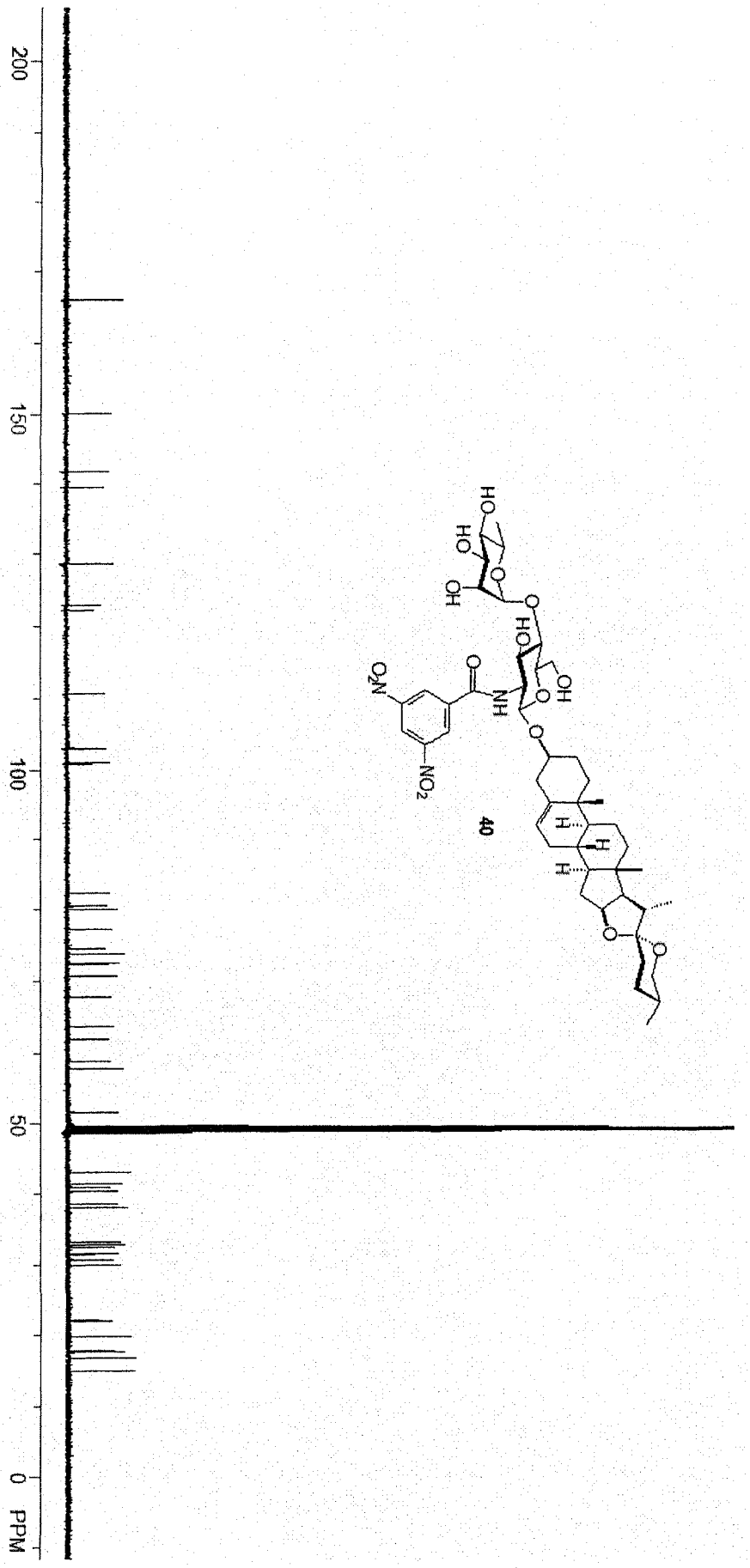
HO
OH
O
NH
O
S
S
H
H
H
O
O
O
38

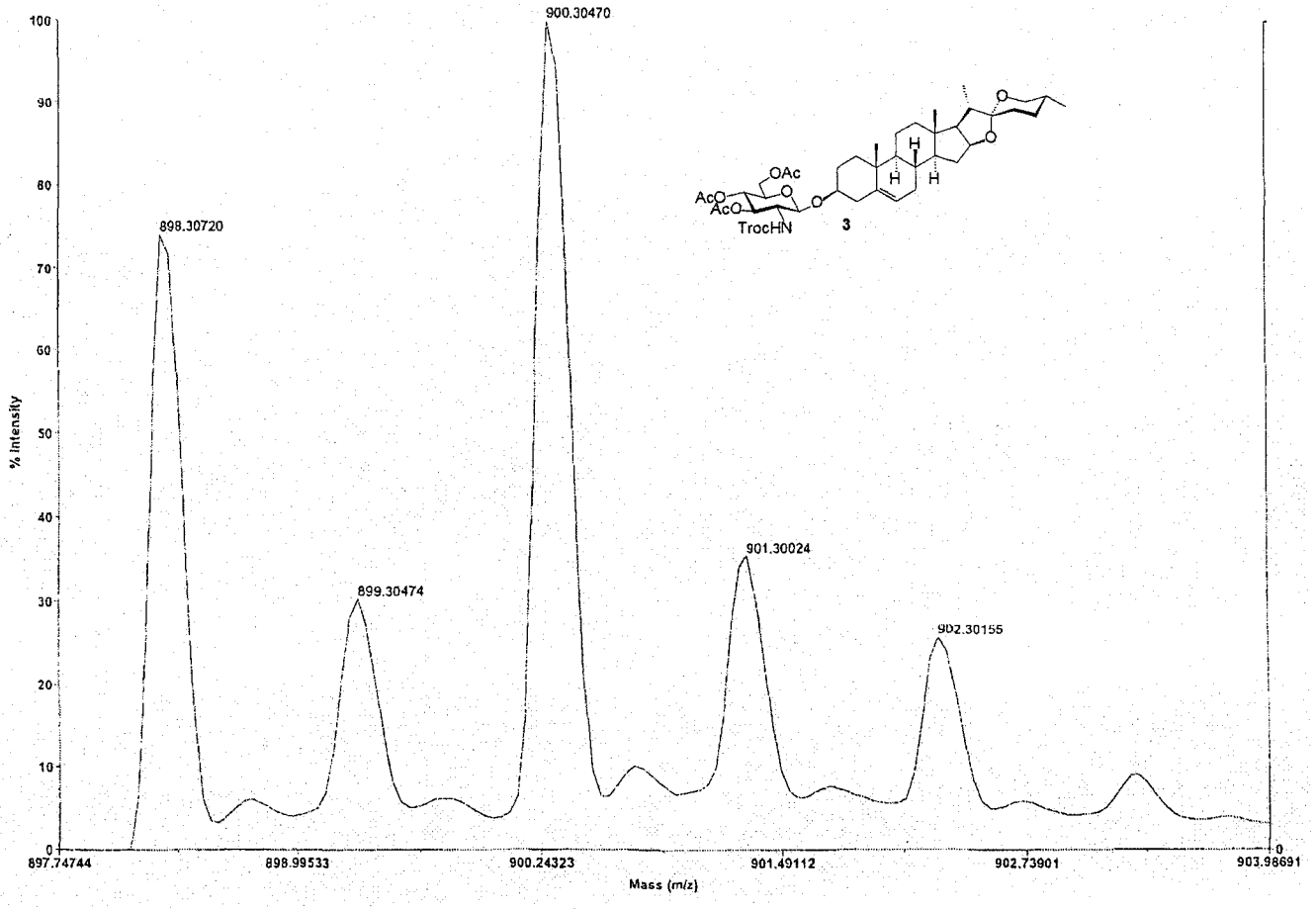

39
8
6
4
2
0


200
150
100
50
0
PPM
HO
HO
HO
OH
O
HO
OH
O
O2N
NH
O
H
H
H
O
O
O
39

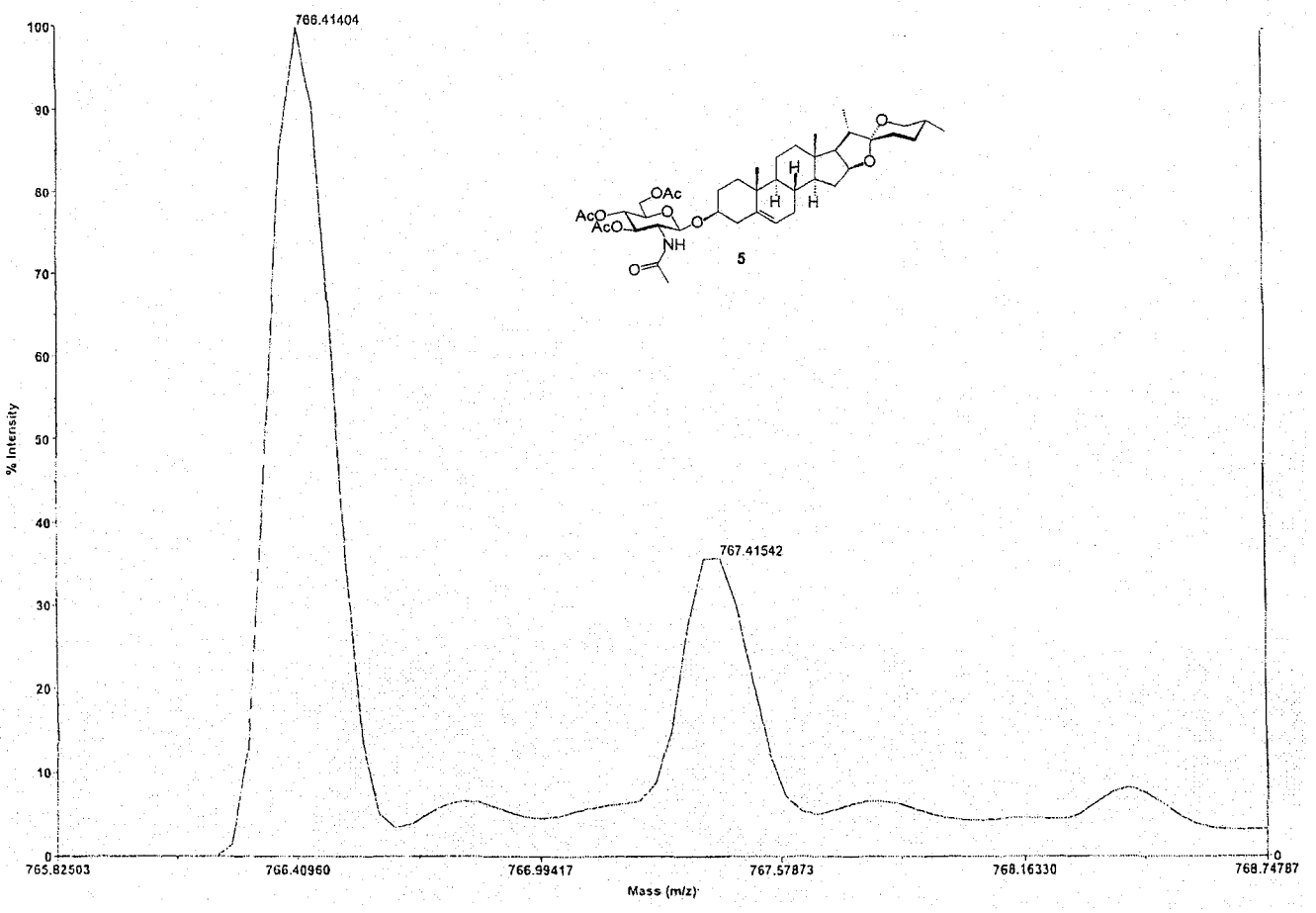

40
HO
HO
OH
O
HO
OH
O
NH
O
NO2
O2N
H
H
H
O
O
8
6
4
2
0


200
150
100
50
0
PPM
40

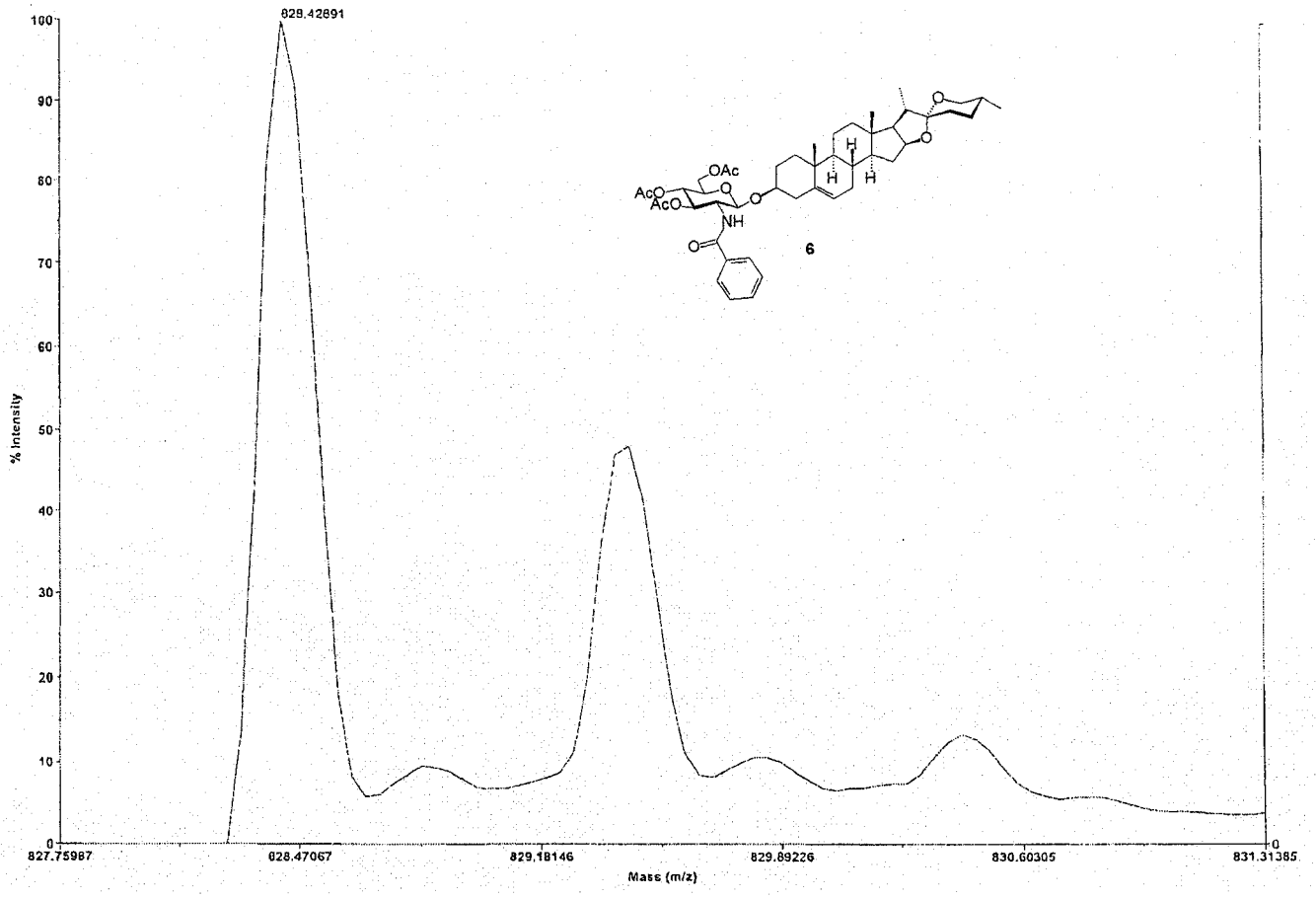
ESI-HRMS Calcd for $C_{42}H_{60}NO_{12}Cl_3Na$ $[M+Na]^+$: 898.3073, found (positive mode): 898.3072.



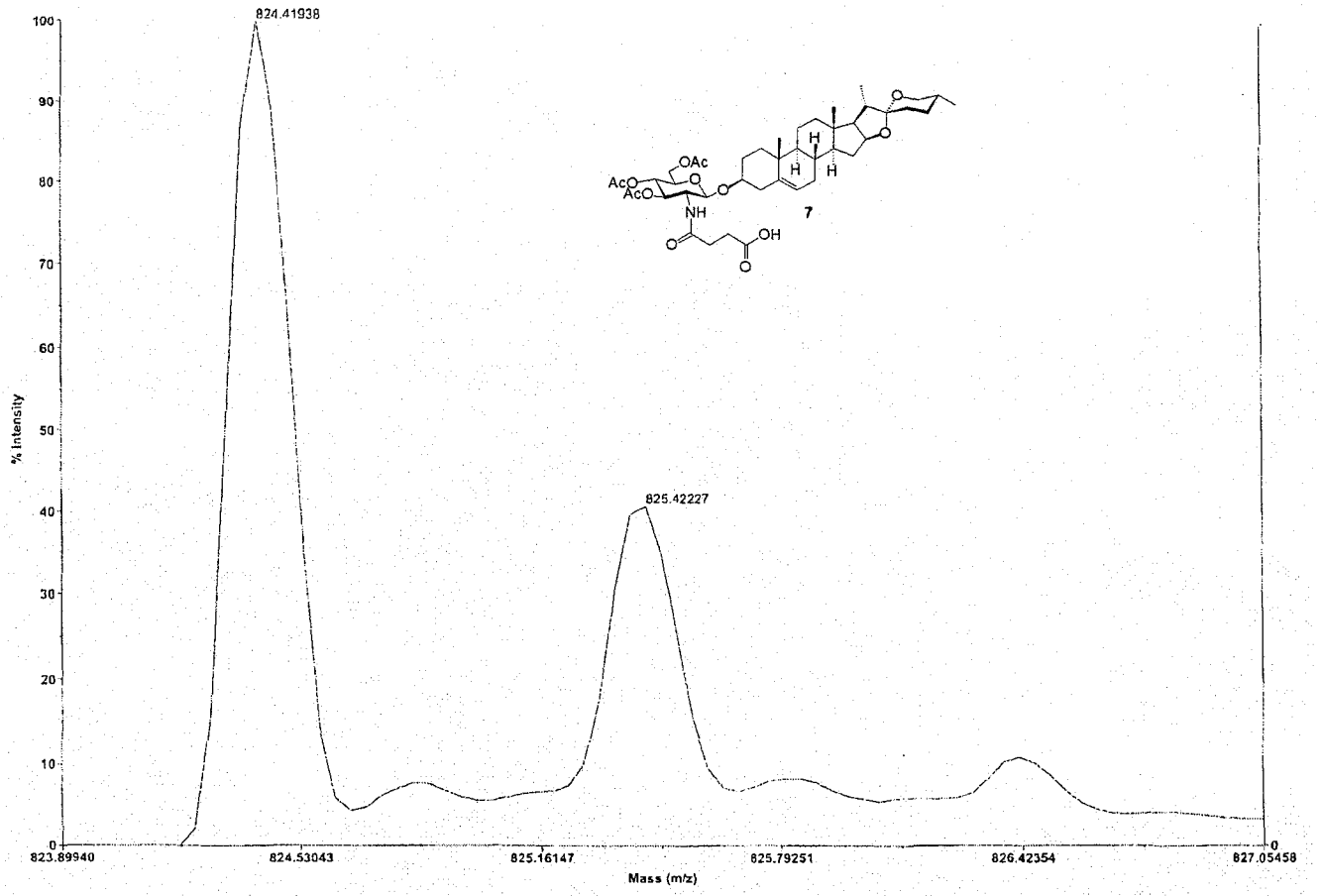
ESI-HRMS Calcd for $C_{41}H_{61}NO_{11}Na$ $[M+Na]^+$: 766.4137, found (positive mode): 766.4140.



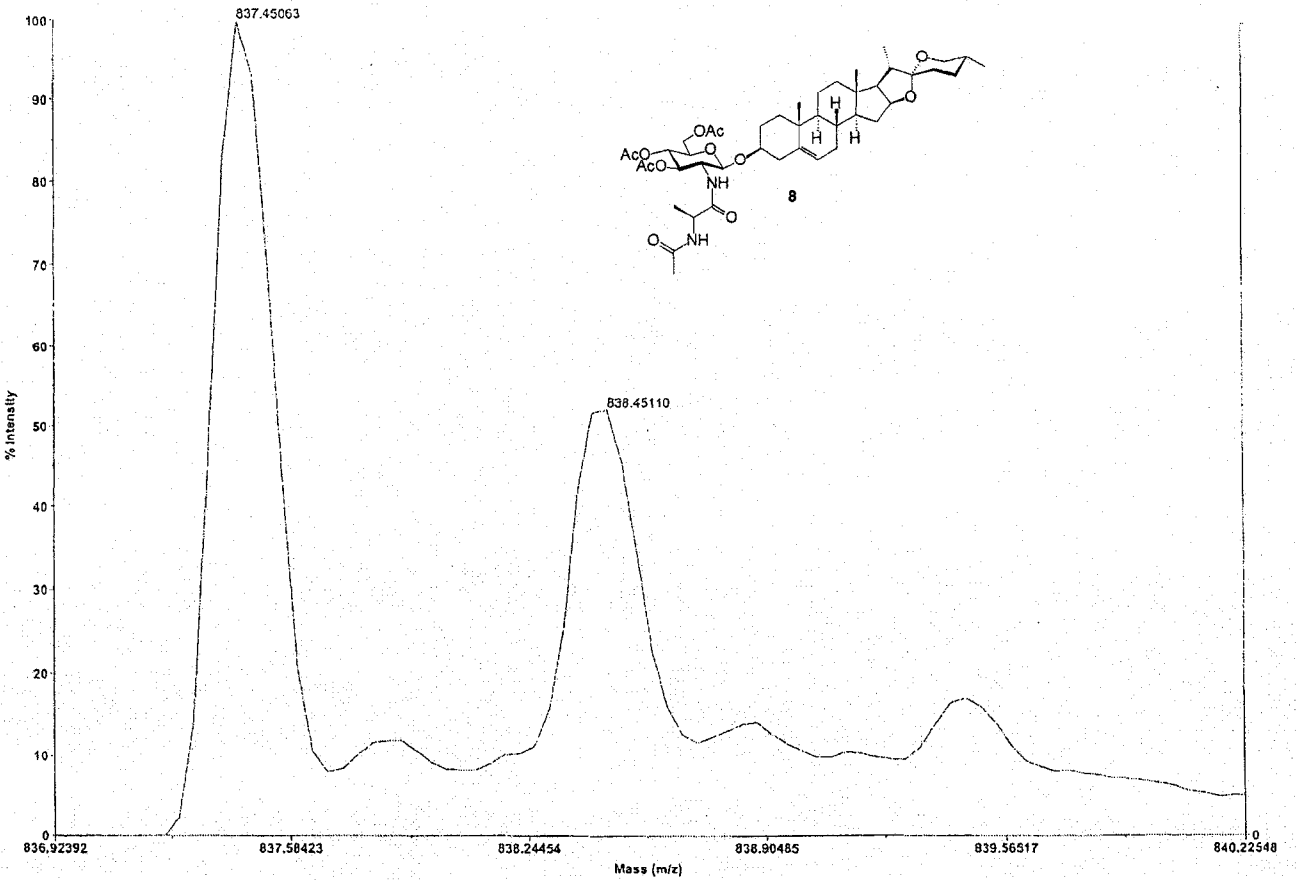
ESI-HRMS Calcd for $C_{46}H_{63}NO_{11}Na$ $[M+Na]^+$: 828.4293, found (positive mode): 828.4289.



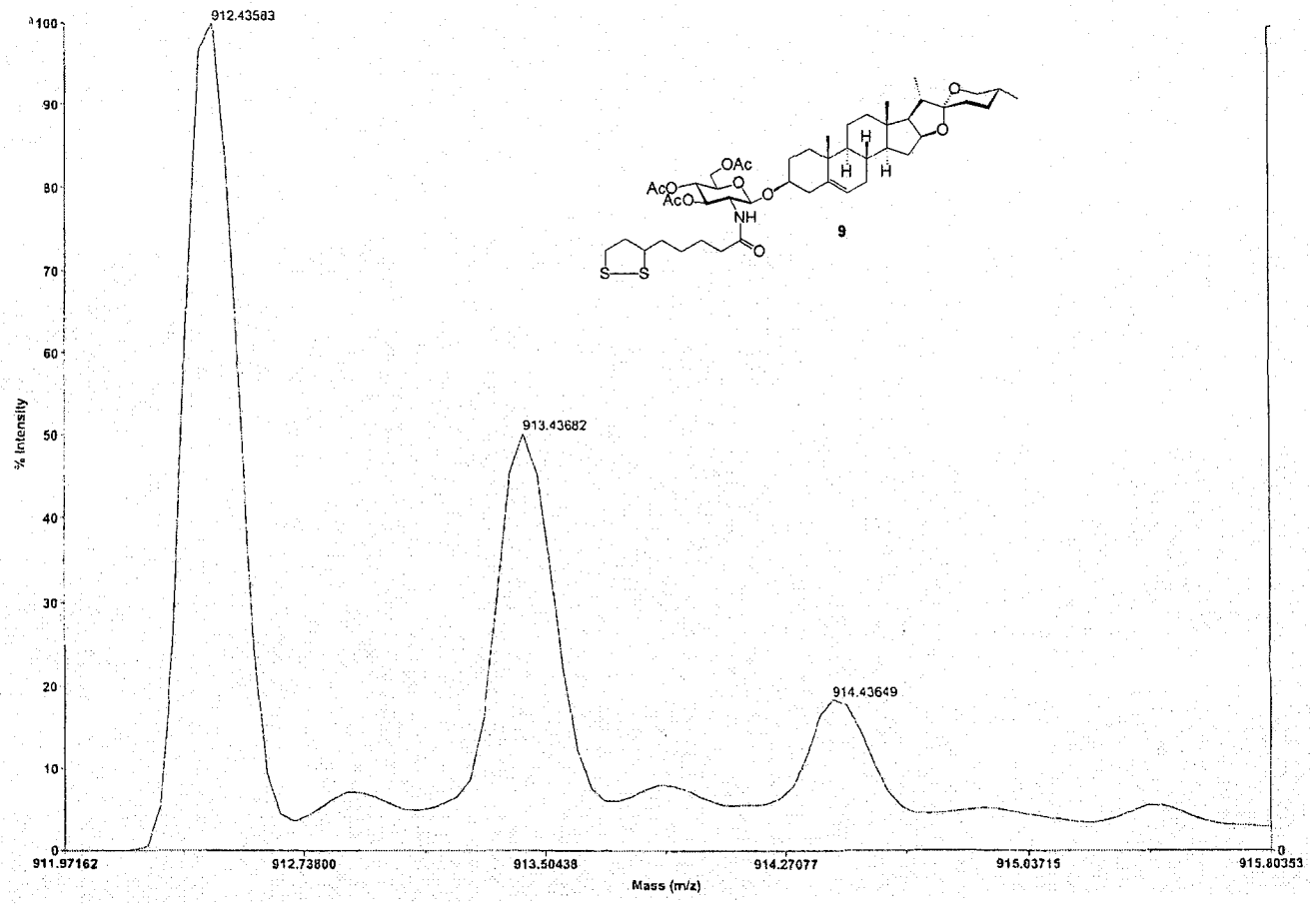
ESI-HRMS Calcd for $C_{43}H_{63}NO_{13}Na$ $[M+Na]^+$: 824.4192, found (positive mode): 824.4193.



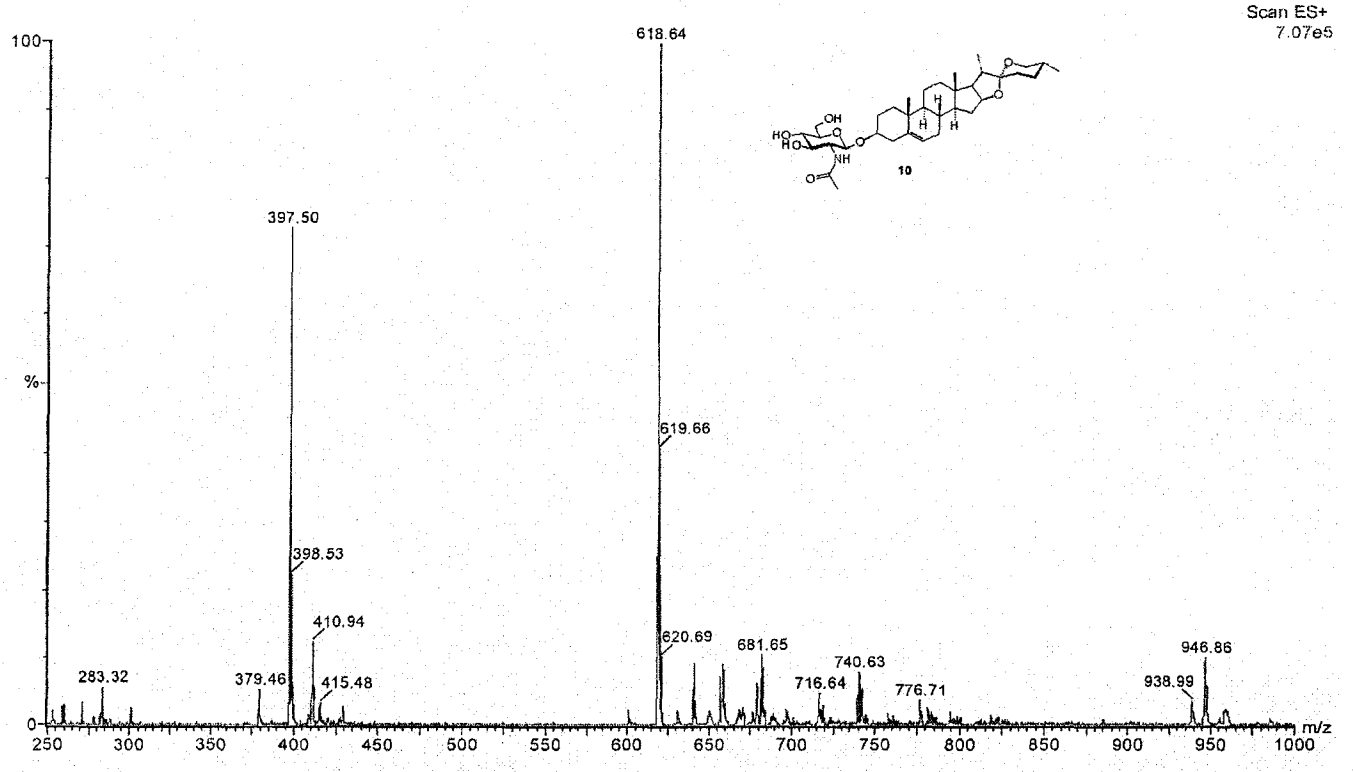
ESI-HRMS Calcd for $C_{44}H_{66}N_2O_{12}Na$ $[M+Na]^+$: 837.4508, found (positive mode): 837.4506.

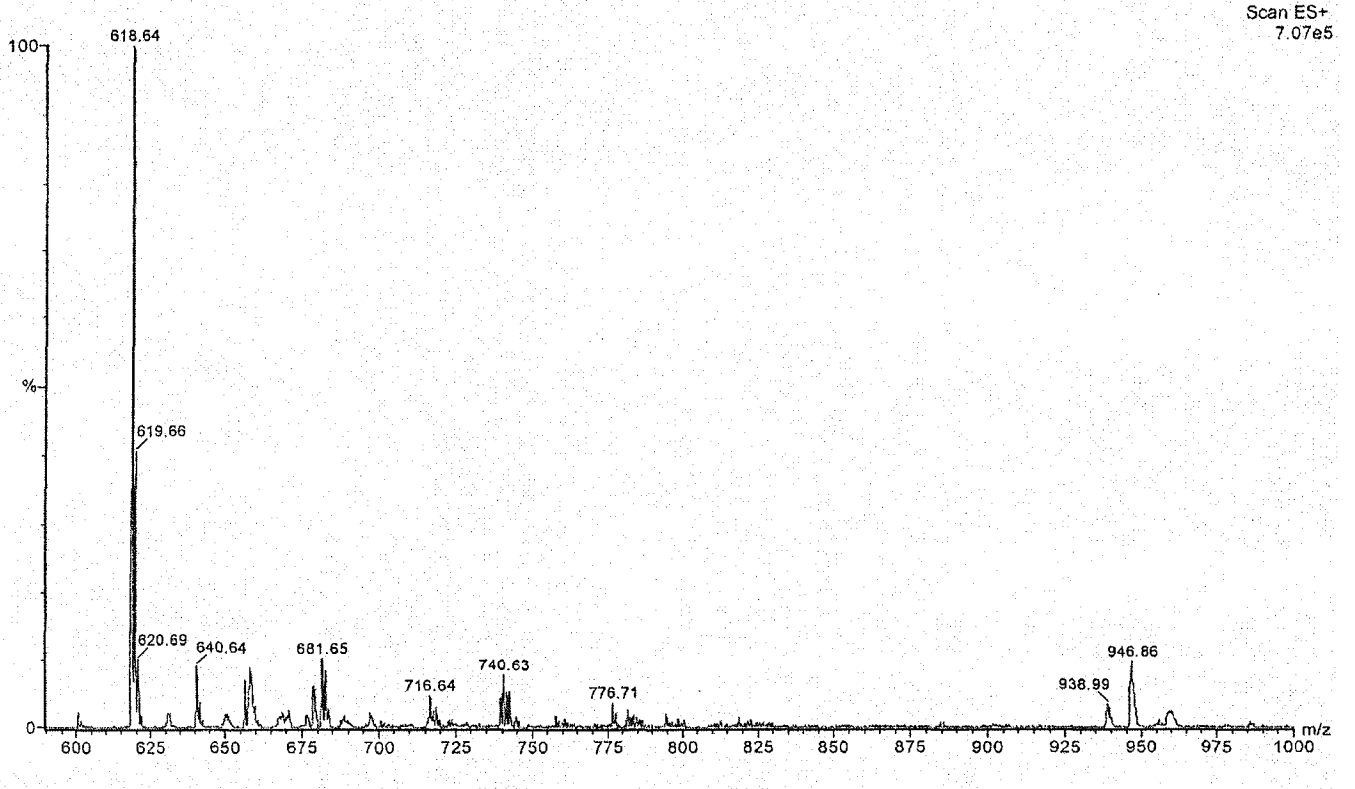


ESI-HRMS Calcd for $C_{47}H_{71}NO_{11}S_2Na$ $[M+Na]^+$: 912.4360, found (positive mode): 912.4358.

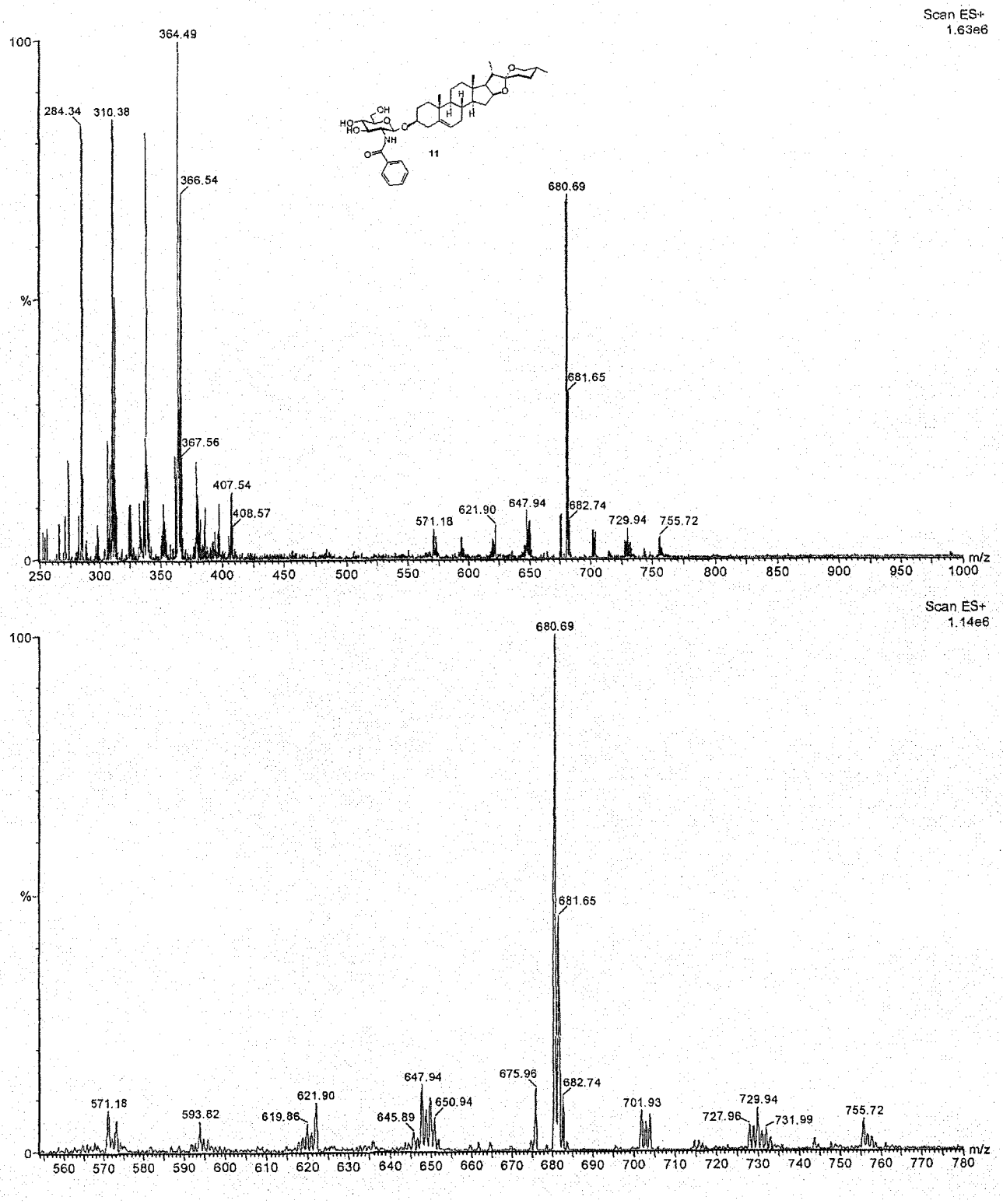


ESI-MS Calcd for $C_{35}H_{55}NO_8$ $[M]^+$: 617.39, found (positive mode): 618.64 $[M+H]^+$.

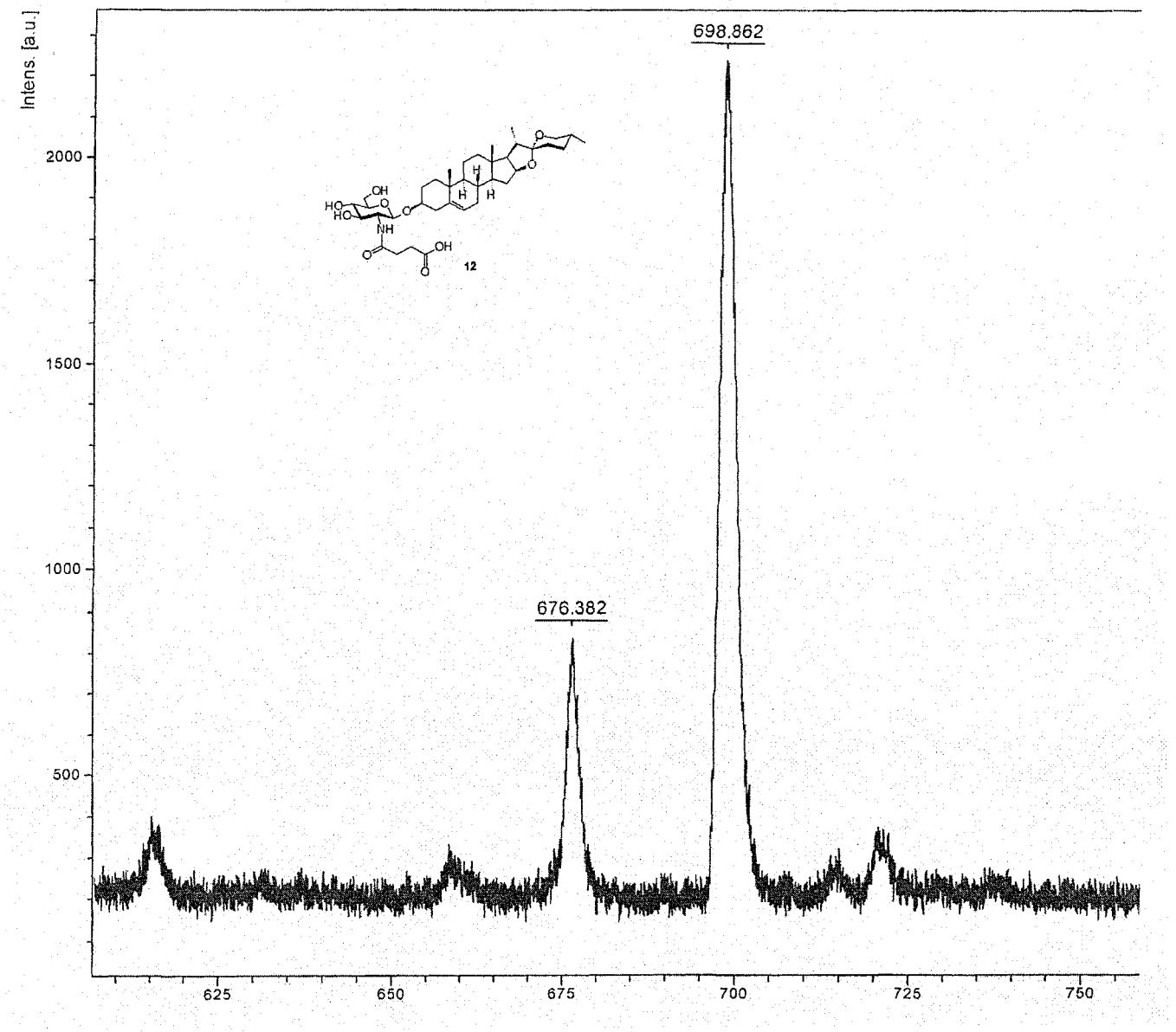




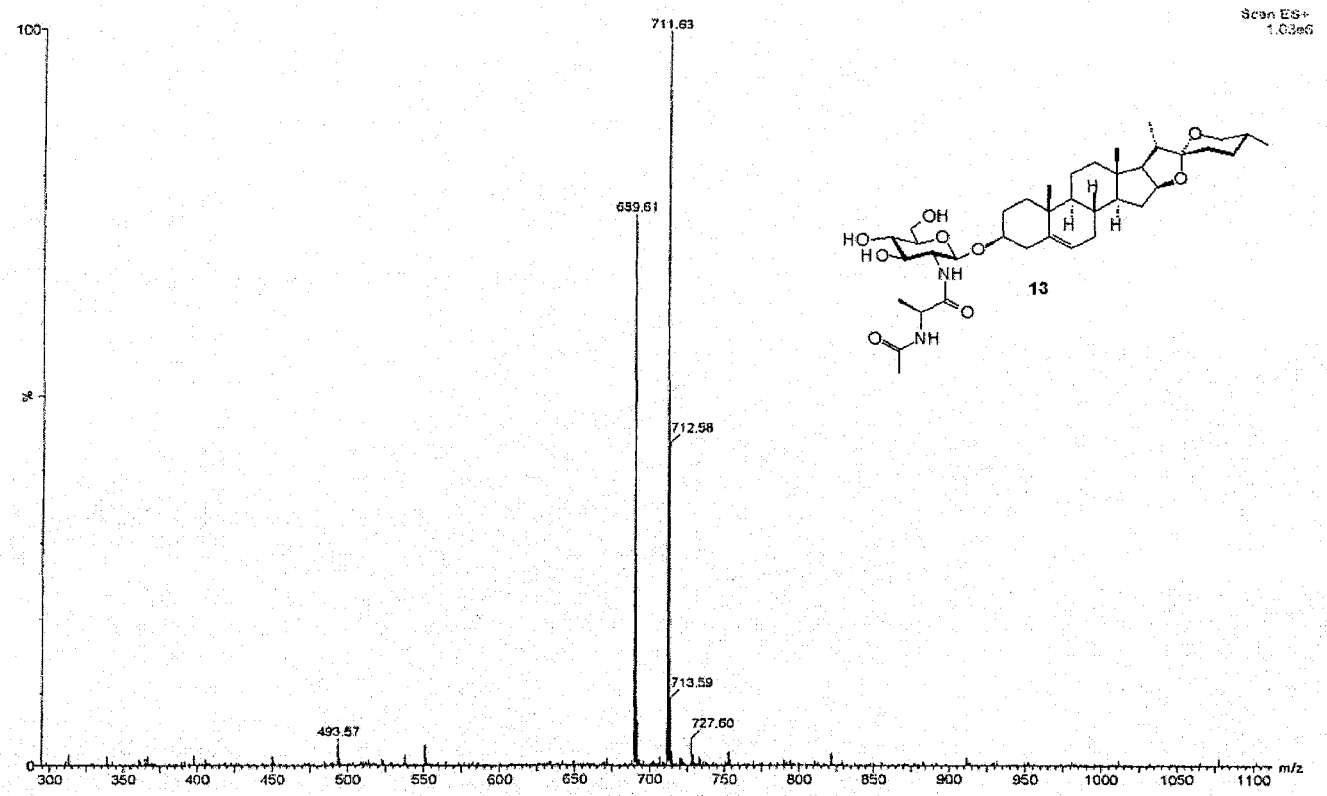
ESI-MS Calcd for $C_{40}H_{57}NO_8$ $[M]^+$: 679.41, found (positive mode): 680.69 $[M+H]^+$.



ESI-MS Calcd for $C_{37}H_{57}NO_{10}$ $[M]^+$: 675.40, found (positive mode): 676.38 $[M+H]^+$, 698.86 $[M+Na]^+$.



ESI-MS Calcd for $C_{38}H_{60}N_2O_9$ $[M]^+$: 688.43, found (positive mode): 689.61 $[M+H]^+$, 711.57 $[M+Na]^+$.



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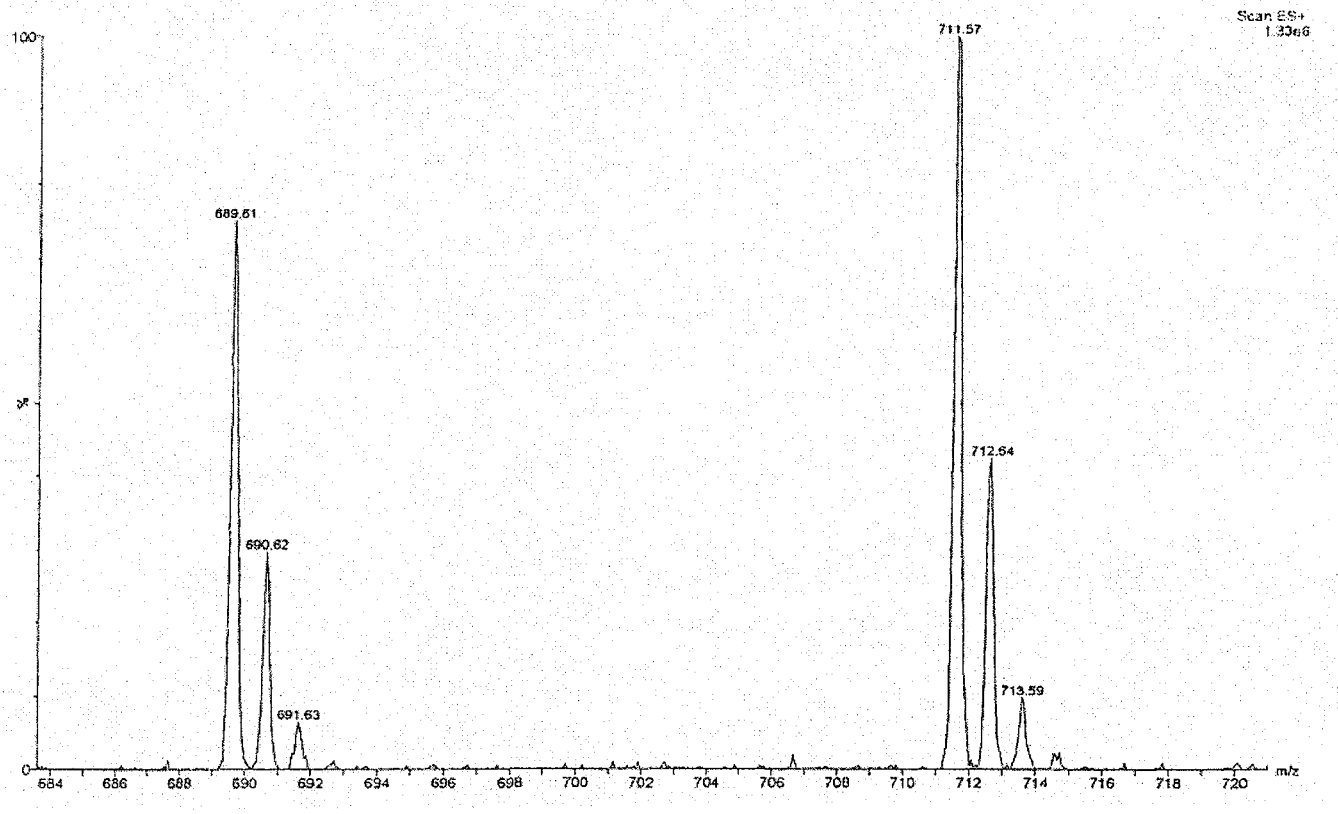
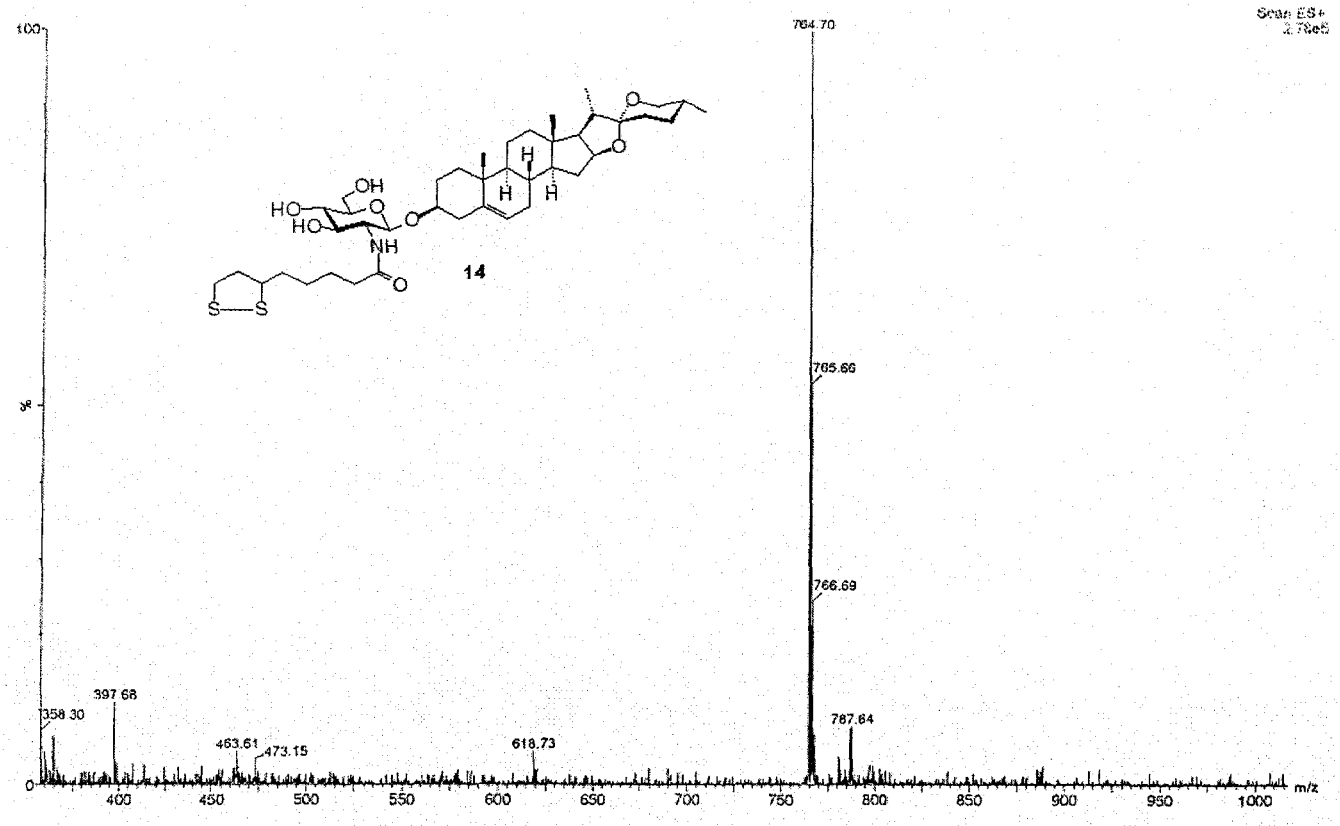
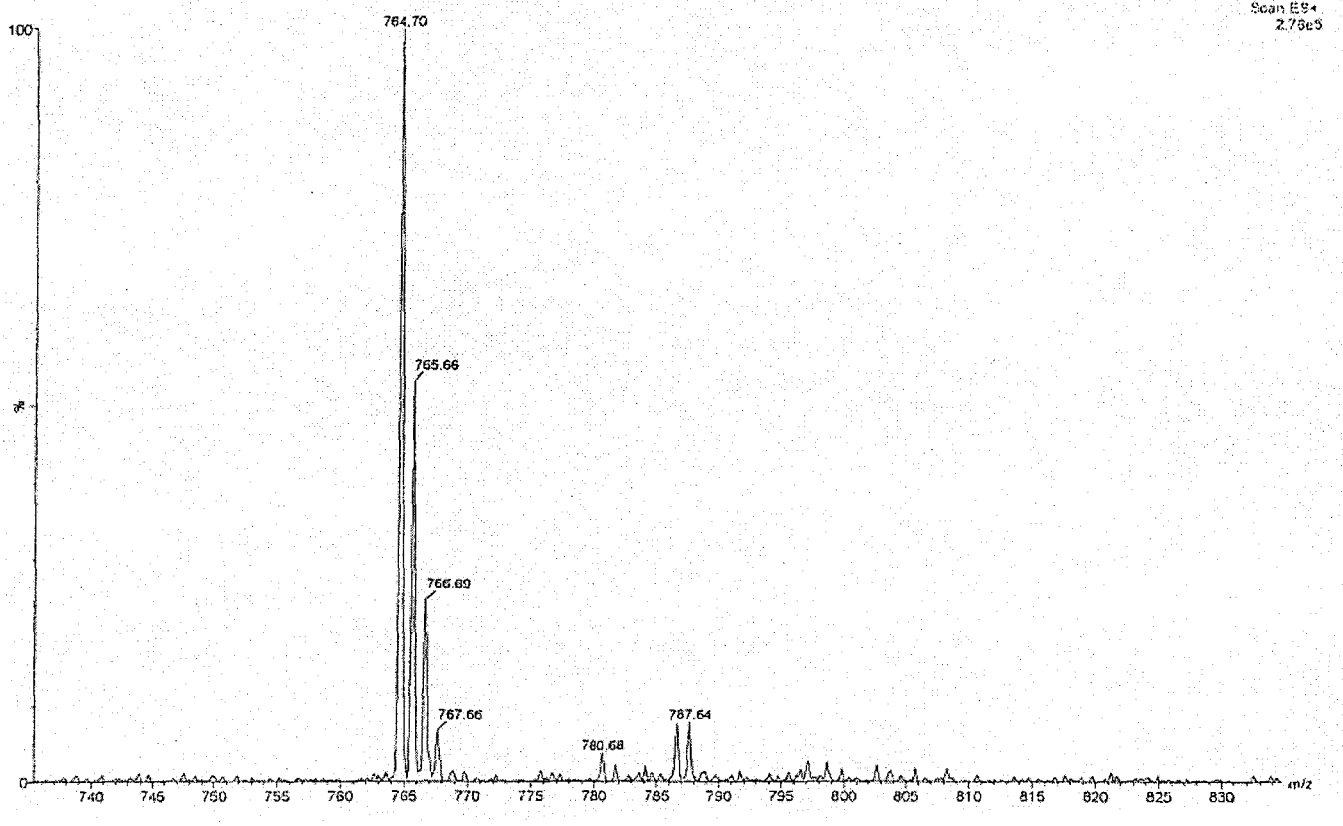
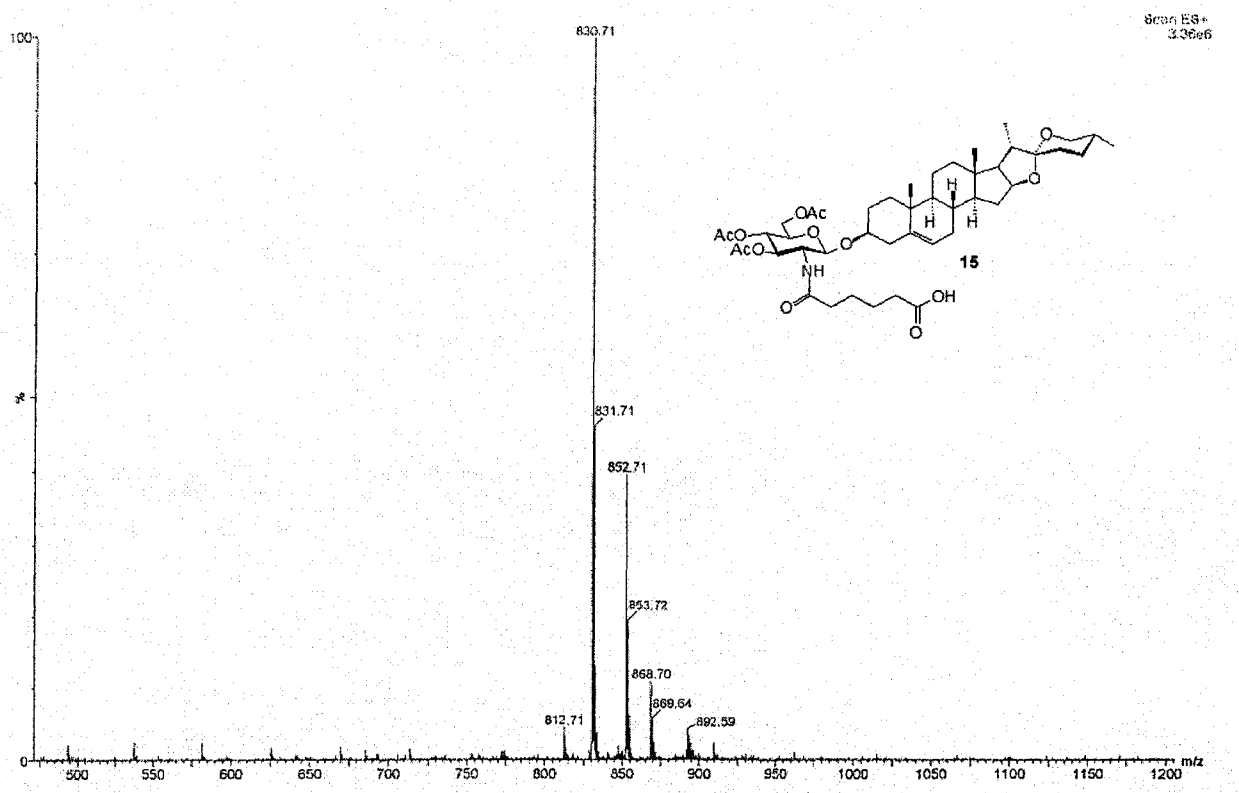


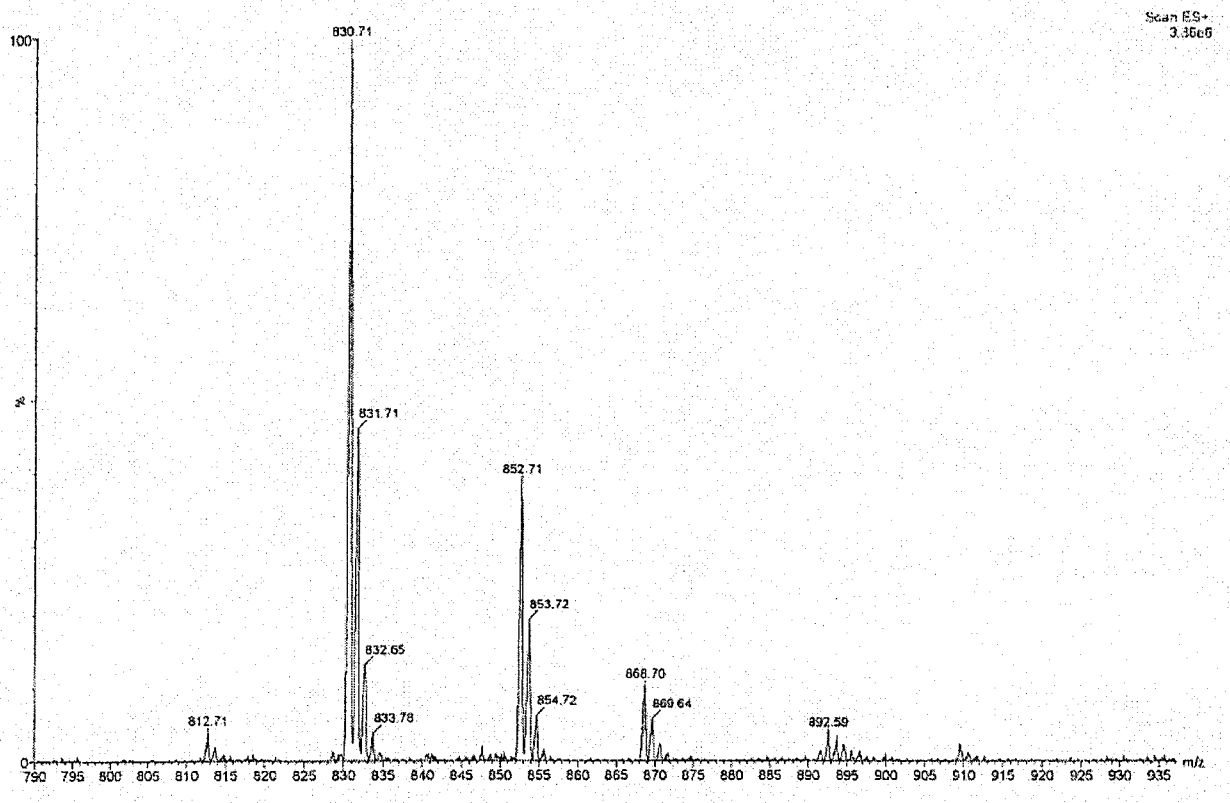
ESI-MS Calcd for $C_{41}H_{65}NO_8S_2$ $[M]^+$: 763.42, found (positive mode): 764.70 $[M+H]^+$.



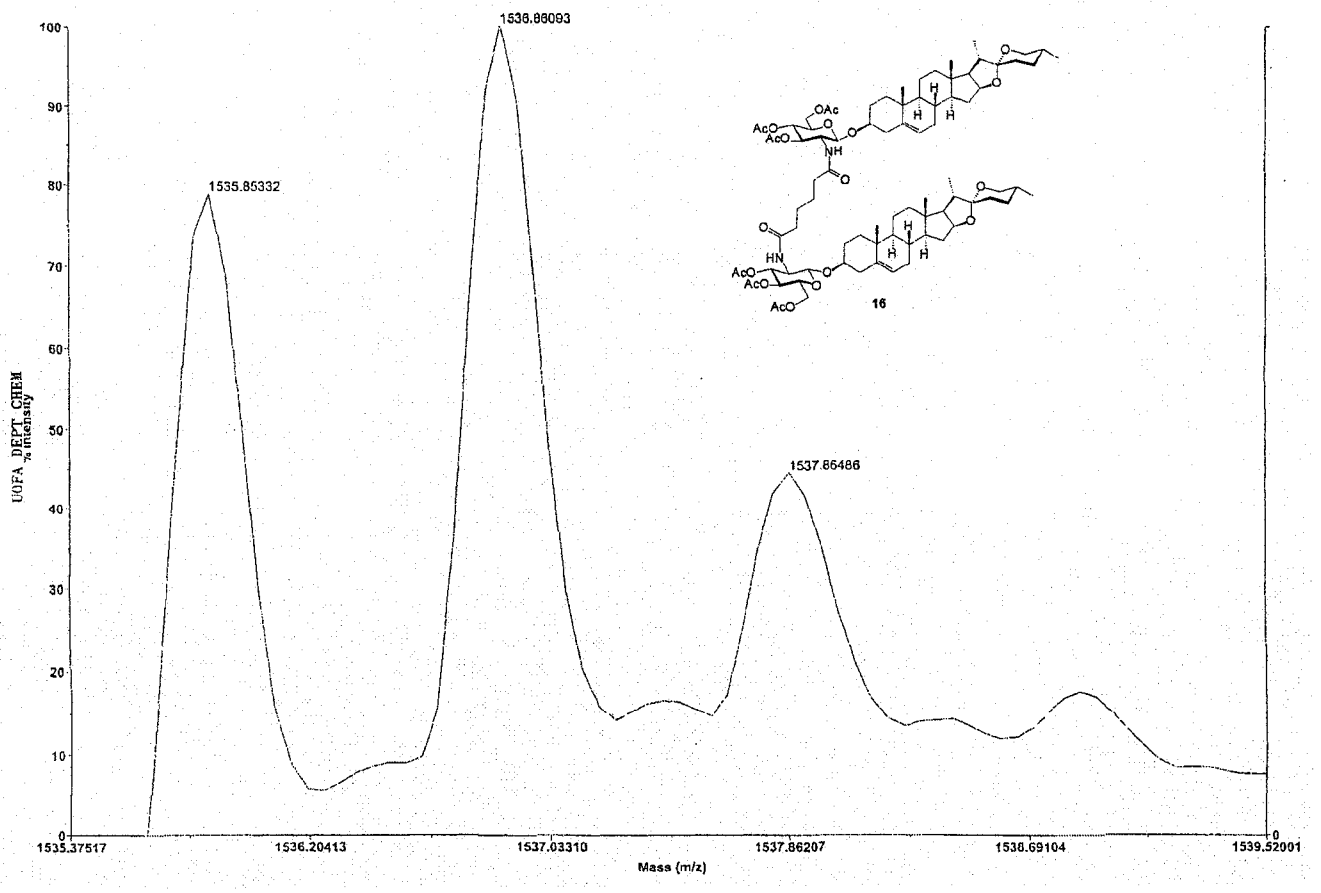


ESI-MS Calcd for $C_{45}H_{67}NO_{13}$ $[M]^+$: 829.46, found (positive mode): 830.71 $[M+H]^+$.

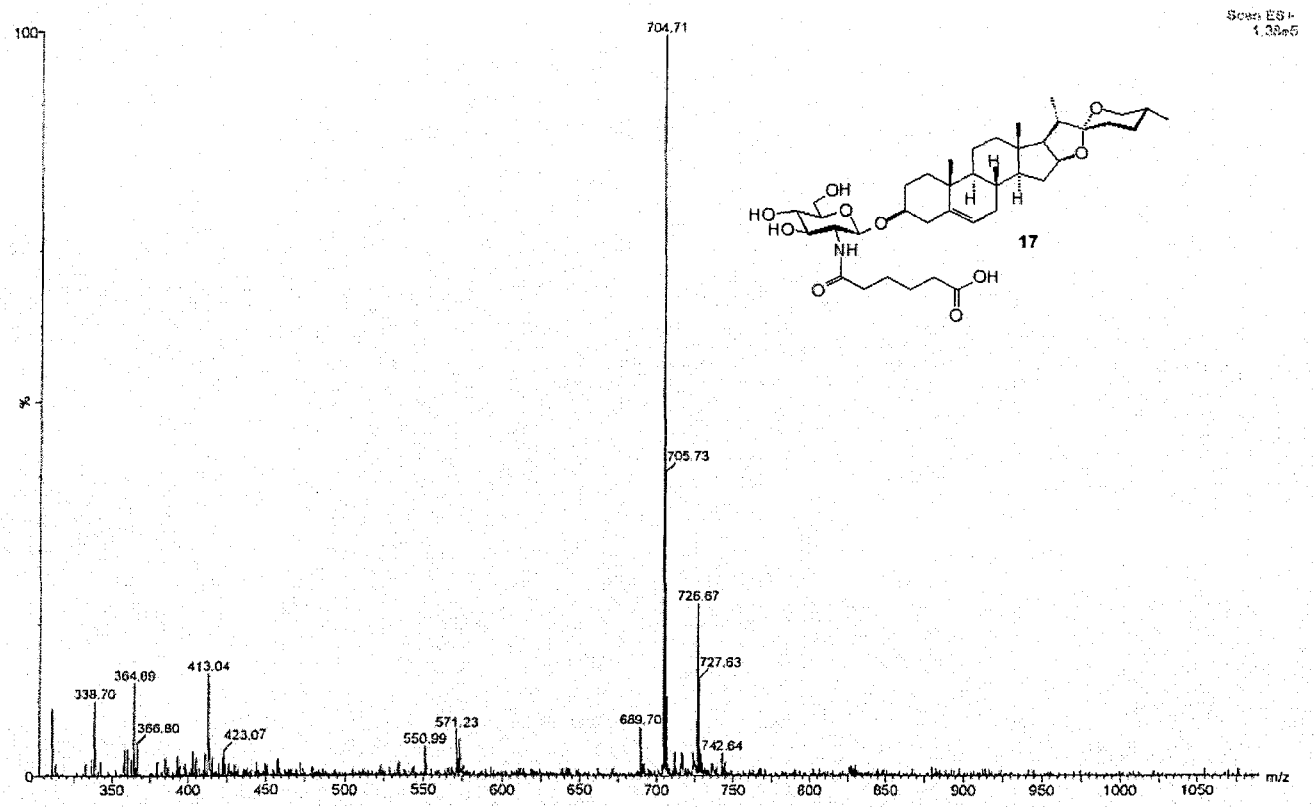


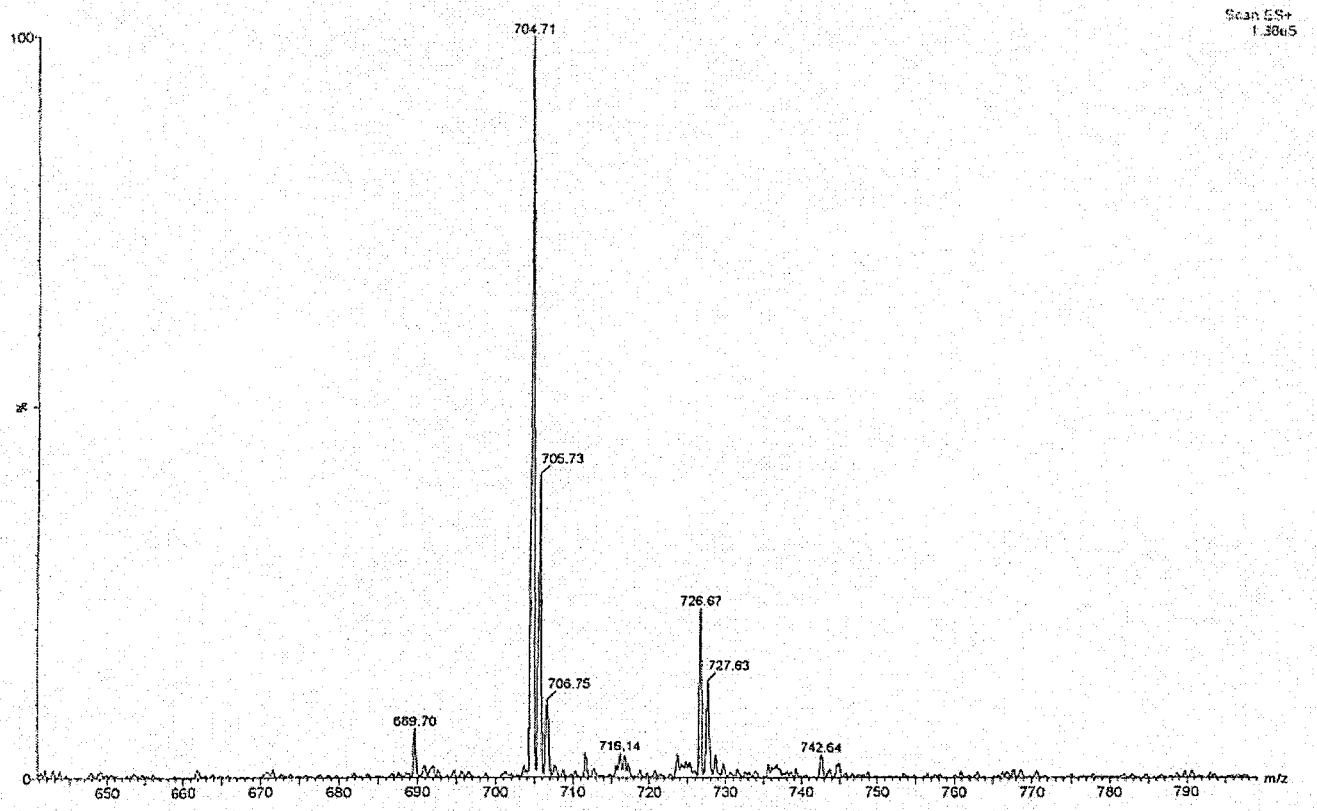


ESI-HRMS Calcd for $C_{84}H_{124}N_2O_{22}Na$ $[M+Na]^+$: 1535.8538, found (positive mode): 1535.8533.

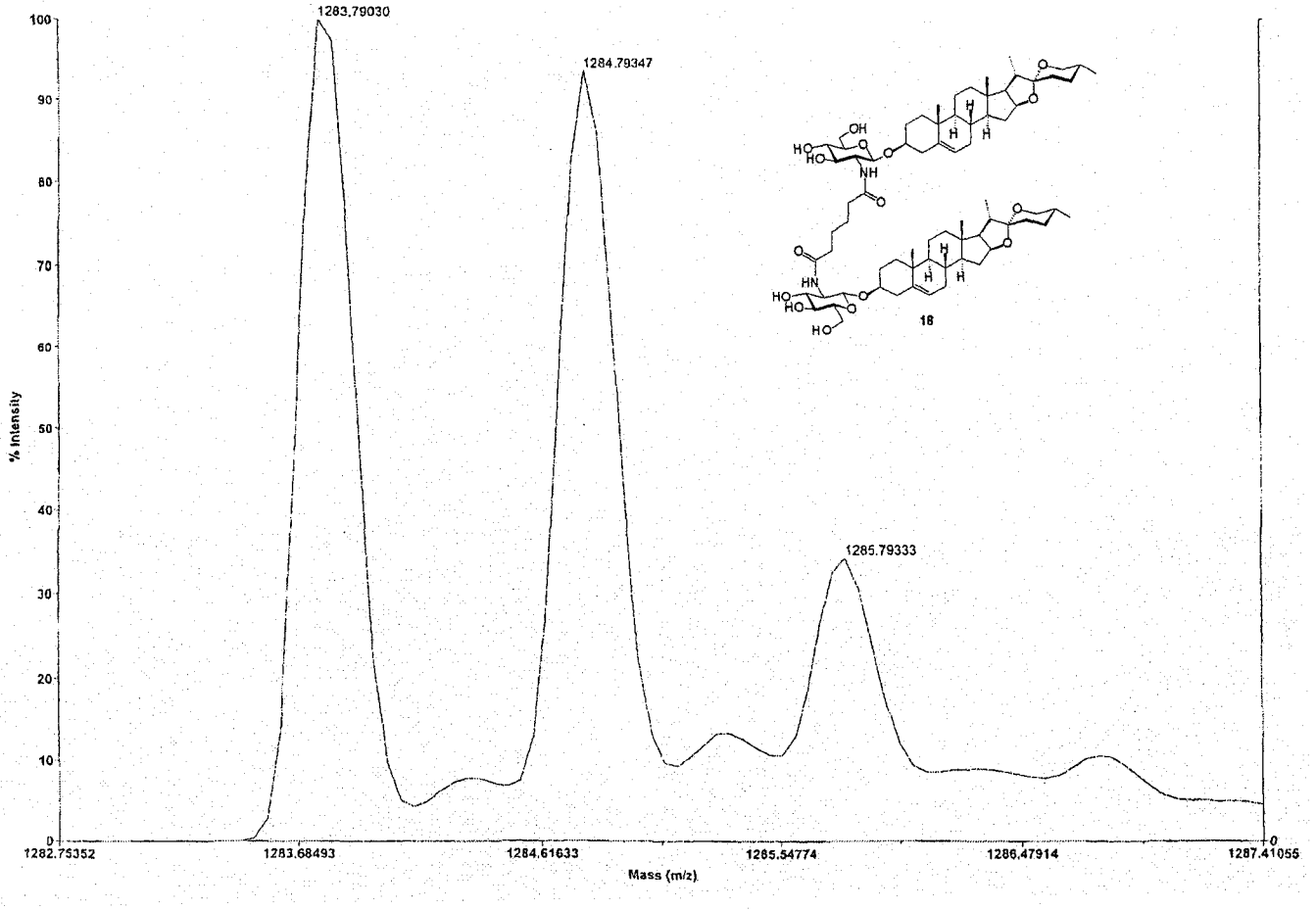


ESI-MS Calcd for $C_{39}H_{61}NO_{10}$ $[M]^+$: 703.43, found (positive mode): 704.71 $[M+H]^+$.

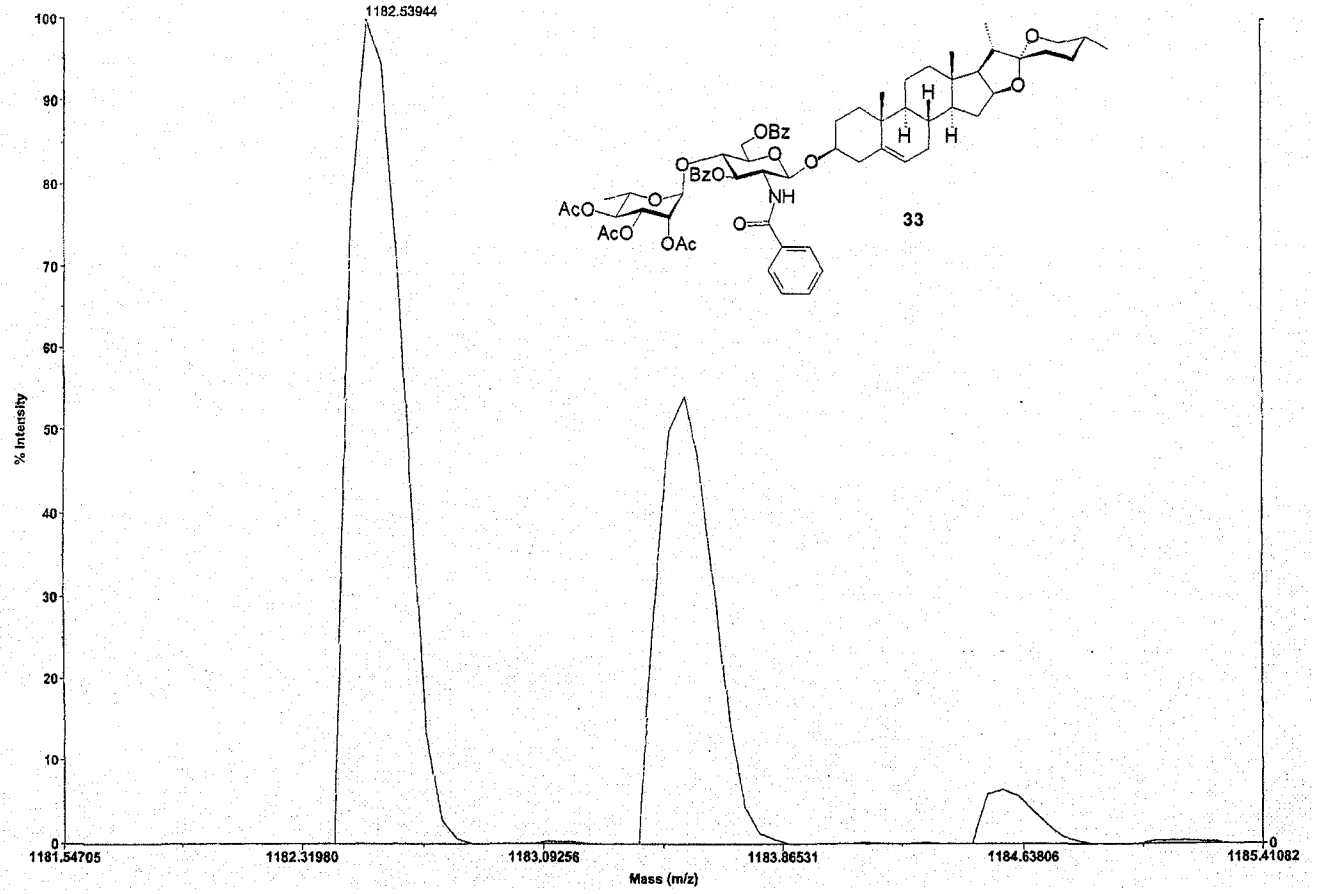




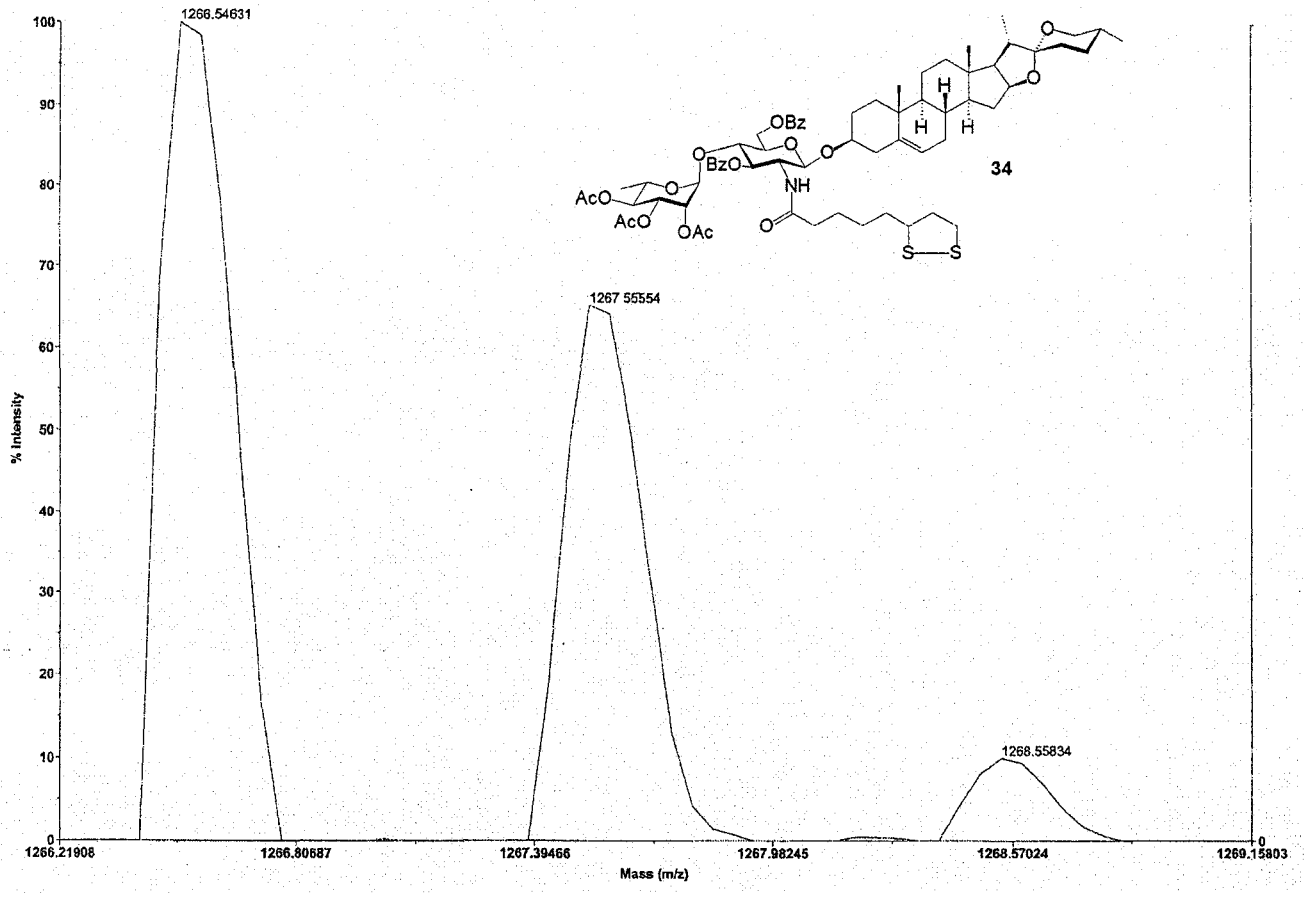
ESI-HRMS Calcd for $C_{72}H_{112}N_2O_{16}Na$ $[M+Na]^+$: 1283.7904, found (positive mode): 1283.7903.



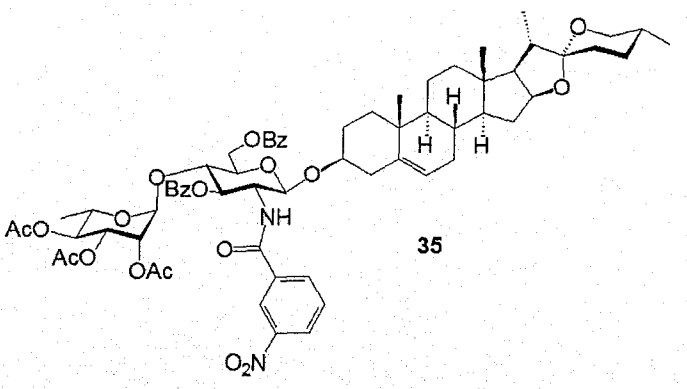
ESI-HRMS Calcd for $C_{66}H_{81}NO_{17}Na$ $[M+Na]^+$: 1182.5396, found (positive mode): 1182.5394.



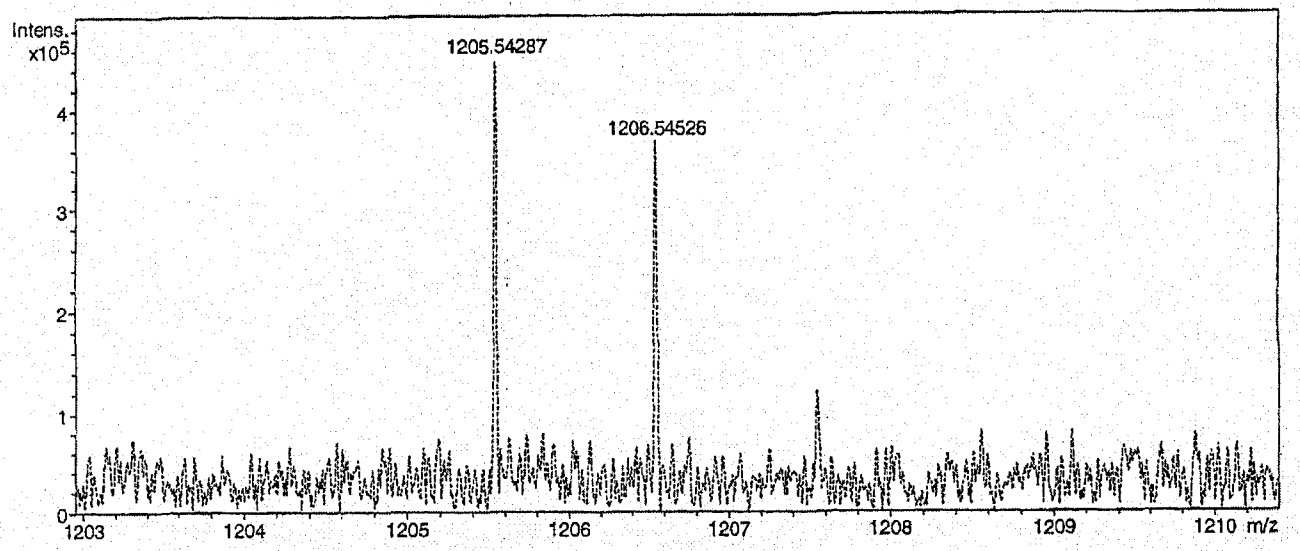
ESI-HRMS Calcd for $C_{67}H_{89}NO_{17}S_2Na$ $[M+Na]^+$: 1266.5464, found (positive mode): 1266.5463.

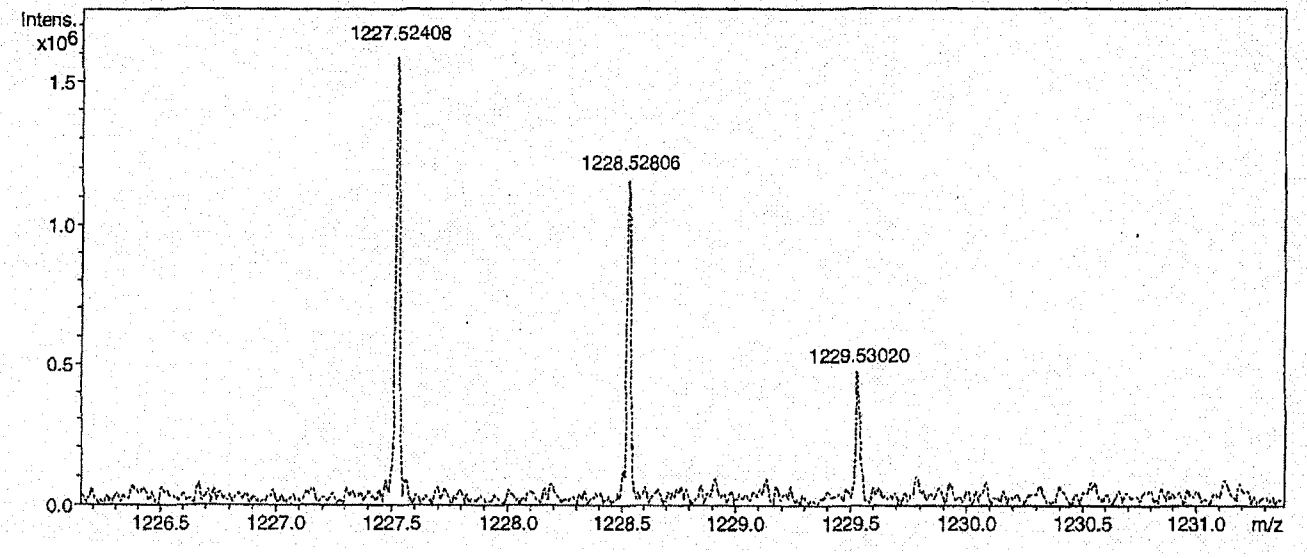


ESI-HRMS Calcd for $C_{66}H_{80}N_2O_{19}$ $[M]^+$: 1204.5355, found (positive mode): 1205.5428 $[M+H]^+$, 1227.5240 $[M+Na]^+$.

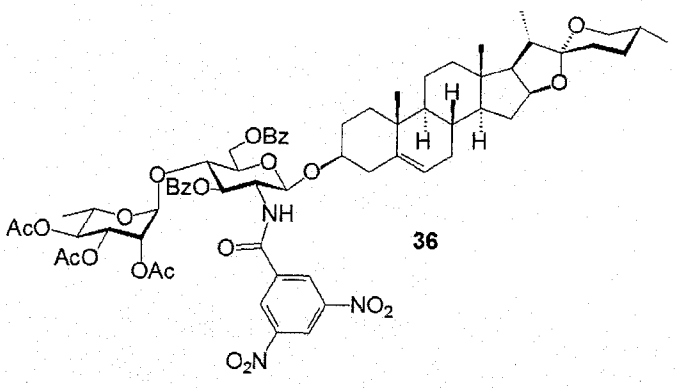


35





ESI-HRMS Calcd for $C_{66}H_{79}N_3O_{21}$ $[M]^+$: 1249.5206, found (positive mode): 1250.5272 $[M+H]^+$, 1272.5095 $[M+Na]^+$.



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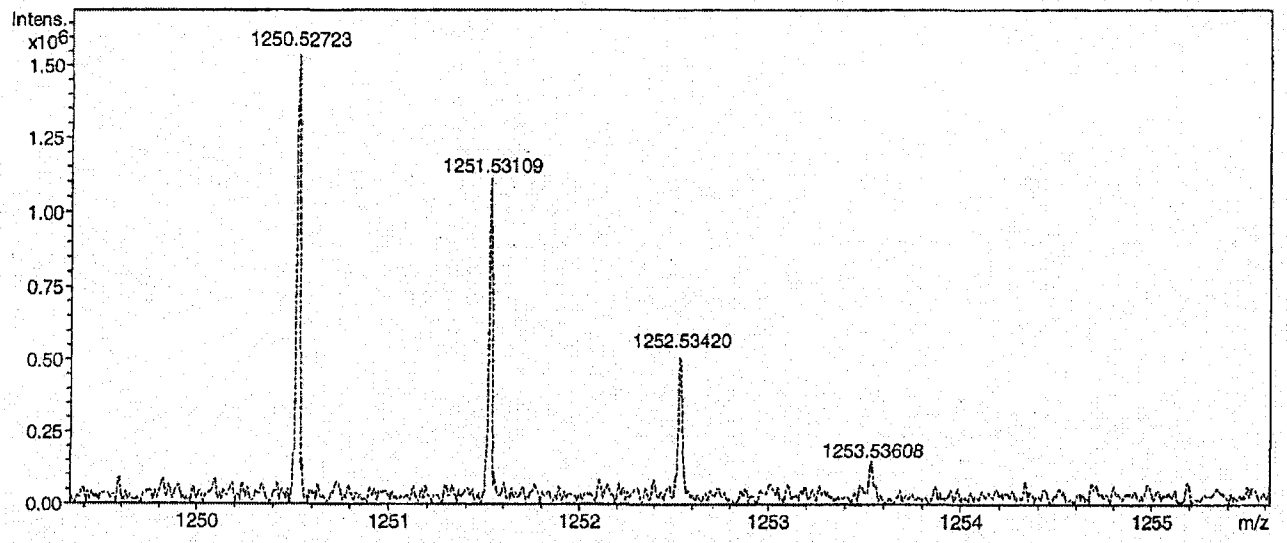
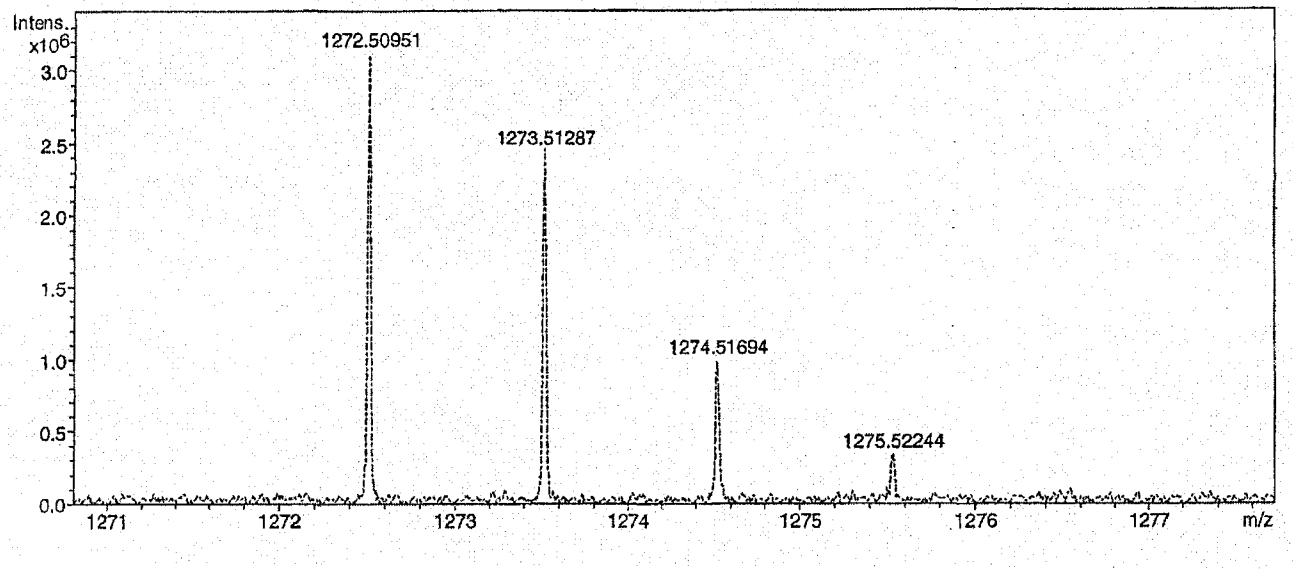
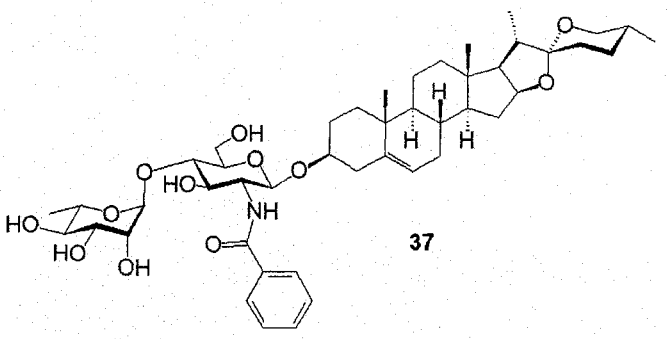




ESI-HRMS Calcd for $C_{46}H_{67}NO_{12}$ $[M]^+$: 825.4663, found (positive mode): 826.4733 $[M+H]^+$, 848.4552 $[M+Na]^+$.



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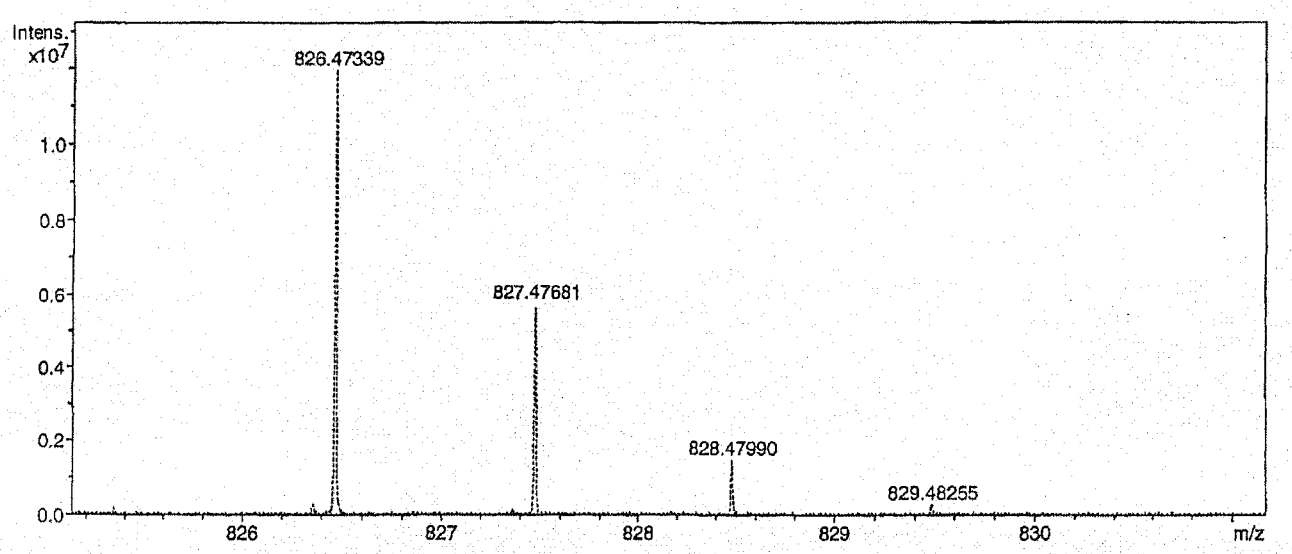
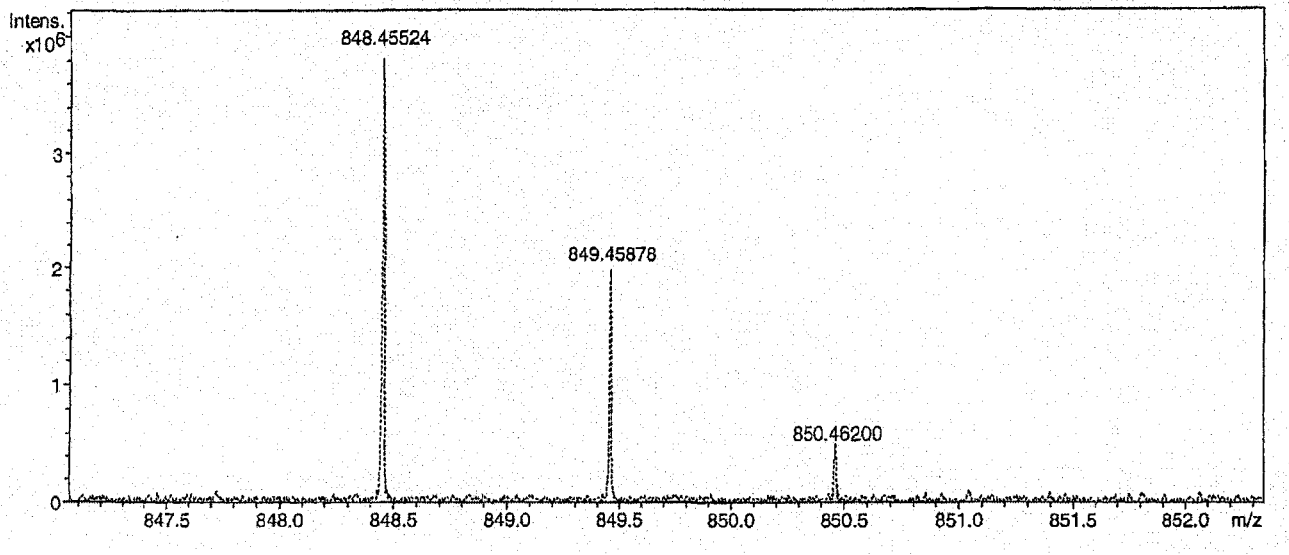
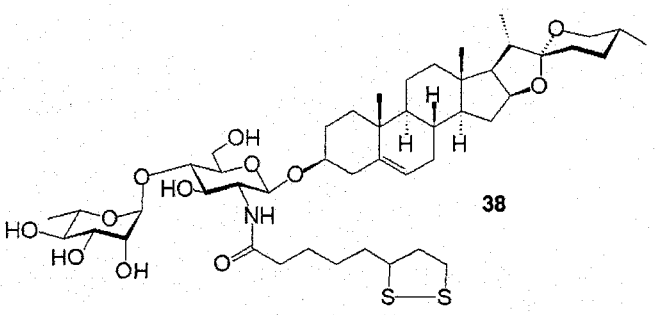




ESI-HRMS Calcd for $C_{47}H_{75}NO_{12}S_2$ $[M]^+$: 909.4730, found (positive mode): 910.4800 $[M+H]^+$, 932.4619 $[M+Na]^+$.



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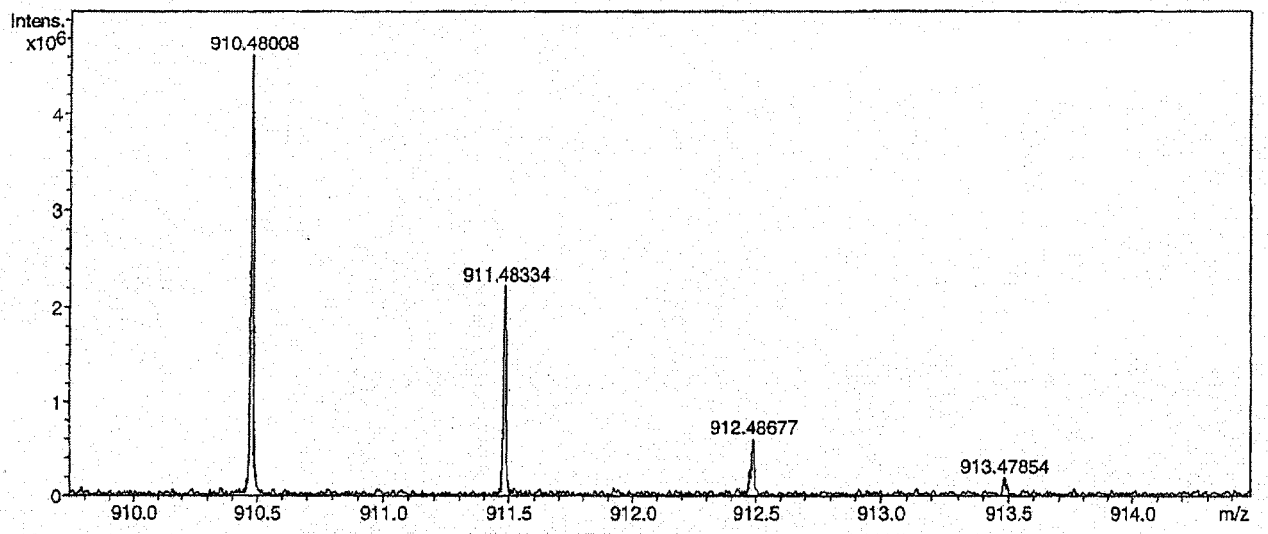
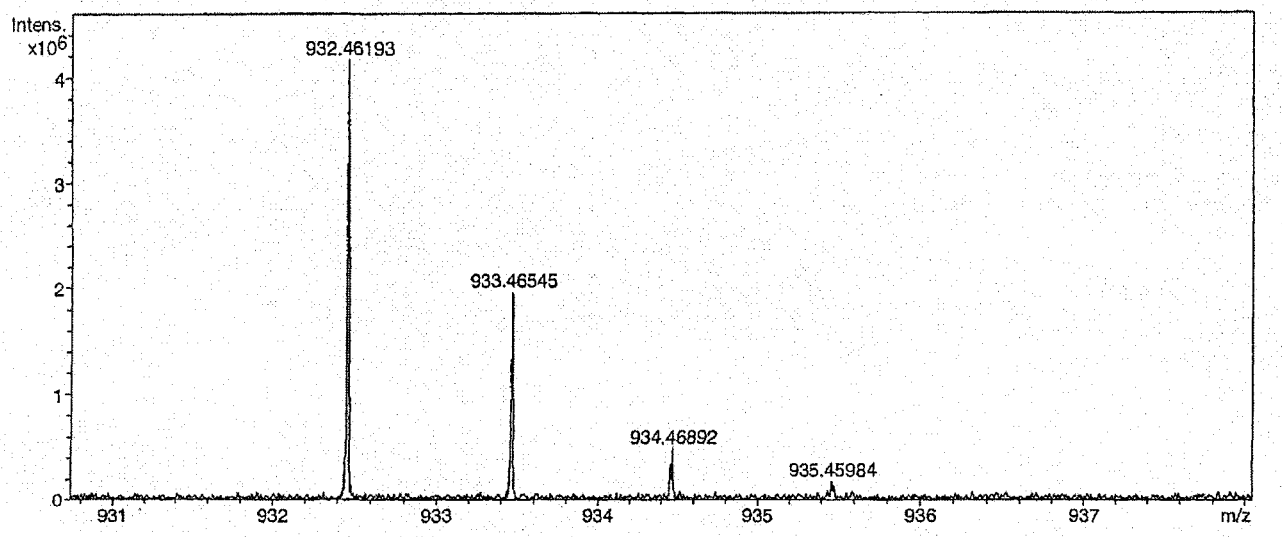
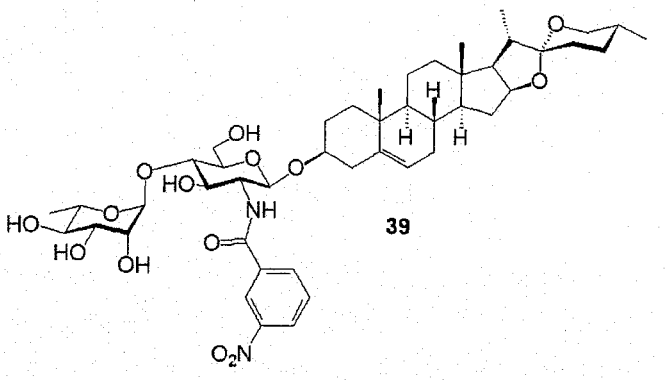




ESI-HRMS Calcd for $C_{46}H_{66}N_2O_{14}$ $[M]^+$: 870.4514, found (positive mode): 871.4583 $[M+H]^+$, 893.4403 $[M+Na]^+$.



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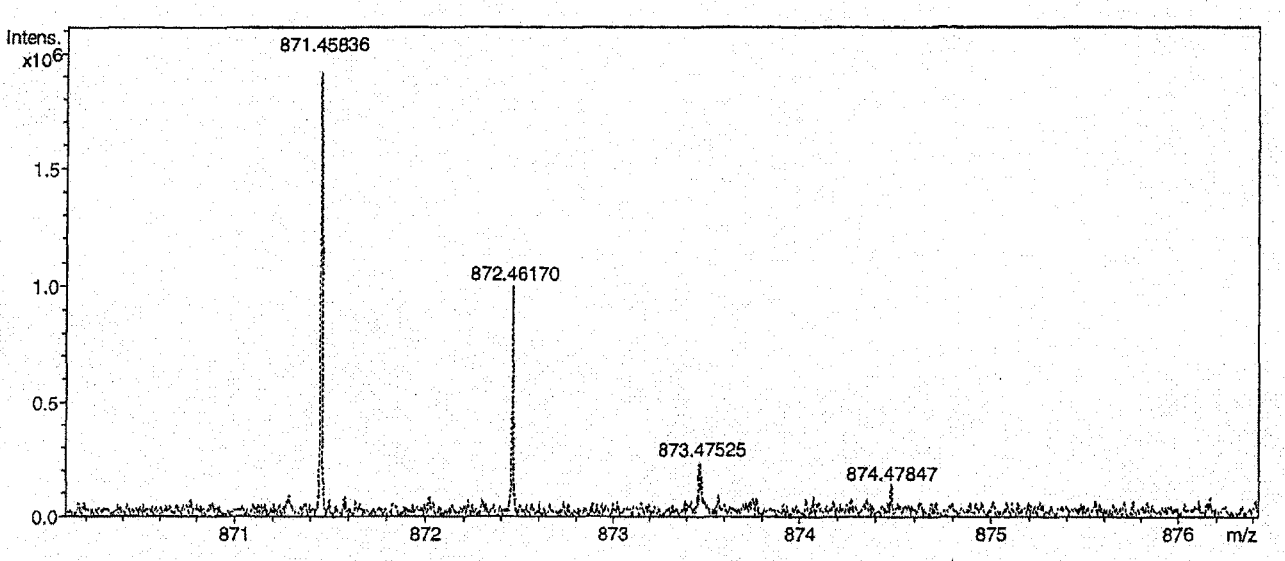
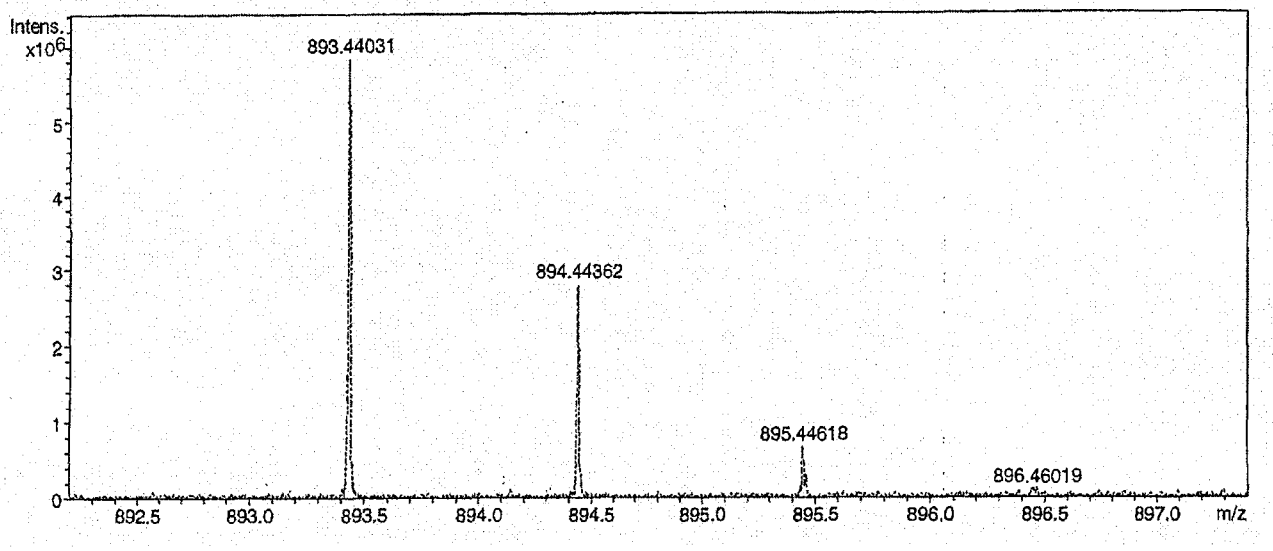
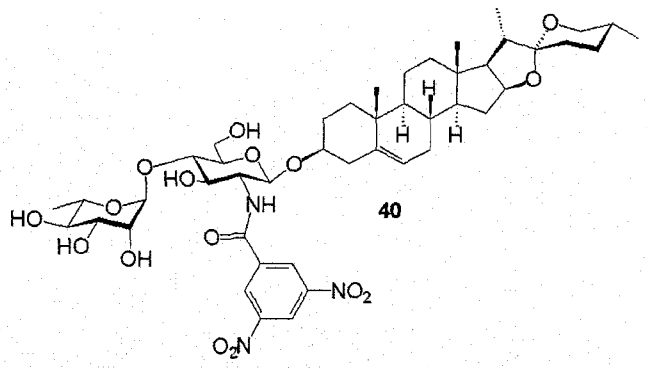




ESI-HRMS Calcd for $C_{46}H_{65}N_3O_{16}$ $[M]^+$: 915.4364, found (positive mode): 916.4436 $[M+H]^+$, 938.4256 $[M+Na]^+$.



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