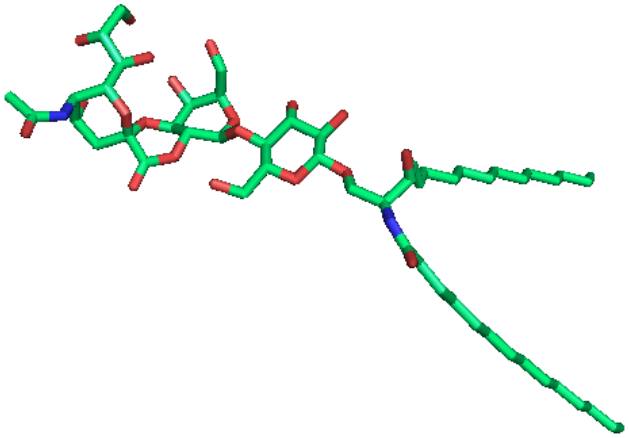
Università degli Studi di Firenze

Dipartimento di Chimica Organica "U. Schiff"

"Immunotherapy of Tumours: Synthesis of a Mimetic of GM-3 Ganglioside Antigen"



Dottorato di Ricerca in Scienze Chimiche

XXI Ciclo

Carlotta Lunghi

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Dott.ssa Carlotta Lunghi

Supervisore

Prof.ssa Cristina Nativi

Coordinatore del Dottorato

Prof. Gianni Cardini

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Introduction

Significant changes in glycoconjugates composition and metabolism are present in almost all human cancers. With the advent of monoclonal antibodies (mAb) technology, many glycoconjugates have been connected with malignant transformations. Indeed a great number of mAbs, showing a bond affinity with a particular tumor cell, have been identified as directed against a specific carbohydrate antigen present on the surface of that cell. These epitopes, known as tumour associated antigens (TAAs) have been successfully used as marker of the tumour progression, and include carbohydrates expressed on normal tissues, but which are accumulated in high density on the surface of neoplastic cells. Developing an effective therapeutic vaccine able to forestall cancer, or to treat patients with an advanced tumor, is a challenging target in oncology. A cancer vaccine is an active immunotherapy and typically consists of an antigen (TAA or TSA) and other components, as adjuvants, able to further stimulate the immune system, very important when the antigen is a carbohydrate antigen, generally poorly immunogenic.

Melanoma is a malignant tumor affecting pigment cells (melanocytes) of the skin and mucous membranes, that are subjected to a unrestrained growth. Among skin cancers, this is as rare as dangerous, having the worst prognosis. Despite many years of intensive laboratory and clinical research, the only effective therapy is the surgical resection of the tumor. In the last years oncologists have demonstrated that metastatic melanoma can respond to non-specific immunotherapy (interferon or interleukin-2), even if it supplies only modest survival benefit. The external membrane of melanoma cells is characterized by the presence of carbohydrate antigens belonging to glycosphingolipids family. They are present in both melanoma and normal cells (melanocytes), but act as efficient immunogens only when they are placed on the surface of tumor cells. This implies that they are able to elicit an immune response that normal GSLs cannot. This is the case of the over-expressed gangliosides GM3, which undergoes an *in vivo* lactonization on melanoma cell surface, giving the corresponding lactone, also found in melanomas as a minor component. This lactonization is likely promoted by the lower pH environment of tumor cells and, possibly, by a different conformation of GM3 ganglioside induced as a result of localised high density. A following

comparative immunization experiment with GM3 and GM3 lactone indicated that the latter was about ten times more immunogenic than its precursor GM3. However, under physiological conditions, the equilibrium amount of the lactone is below the recognition threshold. Hydrolitically stable and structurally similar lactone analogues are thus aimed. A few years ago two hydrolytically stable analogues of GM3 lactone, namely a GM3 lactam and a GM3 ether, were prepared, but to date they have not been used for anticancer therapy. Here we report the conformational analysis and the synthesis of a thioether-bridged mimetic of GM3 lactone, which is a conformational analogue of the parent lactone with a mannose-derived residue replacing the sialic acid portion. We aimed indeed to synthesize a mimetic of GM3 lactone, not only hydrolytically stable, but also structurally simpler than the native compound. This would allow some advantages for the development of a therapeutic melanoma vaccine. In this work, apart from the synthesis of the mimetic, we report first biological results concerning the immunization of mice and the development of suitable glycoconjugates for the vaccine formulation and monoclonal antibodies production.

1. Immunotherapy of melanoma

1.1 Glyconjugates and cancer

The term "glycocalyx" commonly indicates the whole glycoproteins and glycolipids located on the surface of eukaryotic cells. These glycoconjugates play an important role as mediator of both physiological and pathological complex cellular events. In mammalian cells, glycoconjugates are composed by nine different monosaccharide building blocks that can be linked to each other through glycosidic linkages in a vast amount of combinations. They are assembled on their lipidic or proteic platforms by specific enzymes, like glycosiltransferases and glycosidases, and can be built in so many different combinations such that they own a myriad of information fundamental for the life of the cell.

In the past decades, a large amount of knowledge has been collected on this area, and glycobiologists have discovered the role played by a large part of these glycoconjugates. The classification in glycolipids and glycoproteins is only a macro-sectioning. Indeed, glycoproteins can be divided in two other main categories depending on which aminoacid the last carbohydrate of the oligosaccharidic portion is linked to. In particular there are N-glycans, where the saccharidic chain is linked to an asparagine, and O-glycans, where the saccharidic chain is linked to a serine or a threonine. Within these broad categories, there can be precise structural differences corresponding to the large diversity of functions among glycoproteins. Both N-glycans and O-glycans have an important role in the regulation and maintenance of cell functions. They can have a structural function, be component of collagene or protective agents and lubricants of organs; they can also play a crucial role in cell adhesion, in the maintenance of the immunological defence mechanisms, and they can also act as hormones or enzymes.[1]

Glycolipids too can be sub-classified according to their hydrophobic moiety. They are amphiphatic compounds in which the sugar moiety is bound through a glycosidic linkage to a lipidic portion such as an acylglycerol, a sphingoid, a prenyl phosphate, or a ceramide. This last type of glycolipids is called glycosphingolipids (GSLs). They are ubiquitous components of all mammalian cells and are implicated in many cellular events[2]. As a matter of fact, they are considered receptors for microorganisms and their toxins, modulators of cell growth and

differentiation (including apoptosis), and responsible for cellular connection to matrix. The most part of GSLs, including gangliosides, are synthesized from glucosylceramide (GlcCer) by a specific enzyme, namely ceramide glucosyltransferase (GlcT-1, UGCG). In the same way galactosylceramide (GalCer) is formed by the enzyme ceramide galactosyltransferase (CGT).

The importance of glycoconjugates is especially evident during cell differentiation processes. Indeed cell surface oligosaccharides are strictly controlled during development and differentiation, and, frequently, are typical of different cell types. Significant changes in glycoconjugates composition and metabolism are present in almost all human cancers. The aberrant glycosylation in cancerous cells was at first suggested by the discovery that fucose-containing glycolipids accumulate in human adenocarcinoma[3]. Even if, in the last decades, biologists have accumulated a lot of knowledge on this area, the significance of this abnormal glycosylation in cancer has not been completely understood yet.

The search for carbohydrate cancer antigens has become easier since 1975, when Georges Köhler and César Milstein discovered how to make antibody-producer cells immortal, through the fusion with cancer cells; this finding provided inputs for the massive production of antibodies. Since the advent of monoclonal antibodies (mAb) technology, many glycoconjugates have been connected with malignant transformations. Indeed a great number of mAbs, showing a bond affinity with a particular tumor cell, have been identified as directed against a specific carbohydrate antigen present on the surface of that cell.

The portion of the glycoconjugate that is recognised by the mAb is generally composed by 2-5 monosaccharides, here-hence the term "carbohydrate antigen". The aberrant glycosylation processes, peculiar of tumors, result from either the incomplete synthesis of certain glycoconjugates (with subsequent accumulation of precursor) or the (neo)synthesis of glycoconjugates that are absent, or present only in very low concentration, in healthy cells. Glycosyltransferases, as sialyltransferases and fucosyltransferases, are generally over-expressed in tumour tissues. The increased activity of these enzymes leads to an accumulation of certain terminal glycans, as sialyl Lewis (sLe^x), sialyl Tn (sTn), Globo H, Lewis y (Le^y), and plysialic acid (PSA), which are found in malignant tissues of pancreas, breast, lung, colon, and prostate.[4] For an antigen expressed only in tumor cells, glycobiologists refer to "tumor specific antigen" (TSA), and for an antigen present both in malignant and in normal cells, they use the term "tumor associated antigen" (TAA). TAAs occur more frequently than real TSAs. In any case there is an high evidence that the immune

system can recognize tumor antigens (both specific and associated) and potentially destroy malignant cells. Starting from this belief, glycobiologists have increased their attention on immunotherapy, that is the modulation of the immune system to achieve a prophylactic and/or therapeutic cancer vaccine.

1.2 Anticancer vaccines

For many years, scientists believed that the immune system was effective only in fighting infectious diseases caused by bacteria and viruses. The first suggestion of the immune system capability to fight cancer was indicated by W. B. Coley, a researcher and surgeon of the Memorial Sloan-Kettering Institute, in 1888.[5] He demonstrated that he could decrease the growth, and in some cases heal, a few advanced cancers with injections of a mixed culture of streptococcal and staphylococcal bacteria (Coley's toxin). The discovery came while he was examining the files of all the inpatients in the NY Hospital affected by bone cancer. In fact he identified a patient who, despite a bad prognosis, came out of the hospital in an apparently good health after having suffered, during his stay in hospital, from two attacks of erysipelas, a skin infection caused by bacteria *Streptococcus* pyrogens. This finding led him to try the treating of a cancer patient by injecting a particular virulent bacterial culture. The patient who had received the culture developed a serious erysipelas with high fever, but within few days the tumors on his tonsils and neck had completely disappeared.[6] The use of live bacteria was potentially very dangerous for the patients, so Coley tried to improve his method by using a mix of toxins, called then Coley's toxin.

Initial progresses on immunotherapy were very slow, above all because of the little knowledge scientists of that time had on the area of immunity and cancer. Other reasons were the explosive expansion of chemical synthesis and the advent of chemotherapy agents. The attention on the possibility of developing a therapeutic cancer vaccine came out again during the mid '70s, when the extreme growth in the number of chemotherapeutics reached a plateau and it became clear that chemotherapy was not able to induce the same kind of responses in the most common tumor types. In addition, in the last years, treatment approaches using chemotherapy and radiotherapy have enhanced the host toxicity, increasing the attention on tumor-targeted therapies, including immunotherapy.

Today, immunologists would describe Coley's approach to cancer therapy as non

specific: indeed tried to stimulate and reinforce the entirety of the patient's immune system, instead of selectively "waken" the right components of the immune system, most able to fight cancer, by a selective immunization with specific antigens. Immunotherapy is now considered a fourth modality of fighting cancer: many years of research have indeed at last produced the first successful examples, such as certain cytokines, interferon and some antibodies. An example of passive immunotherapy, currently used in the treatment of cancer, is the chimeric antibody rituximab (trade names are Rituxan, MabThera and Reditux). It is a useful therapy of B cell non-Hodgkin's lymphoma and B-cell leukemias, both alone and in combination with chemotherapy.[7] Another example is the humanized monoclonal antibody trastuzumab (with the trade name Herceptin), that acts on the HER2/neu (erbB2) receptor and is useful against certain breast cancers.[8] Many other monoclonal antibodies are now undergoing to clinical trials for different types of tumors.

Developing an effective therapeutic vaccine able to forestall cancer, or to treat patients with an advanced tumor, is much more difficult. A cancer vaccine is an active immunotherapy and typically consists of an antigen (TAA or TSA) and other components, as adjuvants, able to further stimulate the immune system, very important when the antigen is a carbohydrate antigen, that is generally poorly immunogenic.

1.3 Melanoma

Melanoma is a malignant tumor that affects pigment cells (melanocytes) of the skin and mucous membranes, that are subjected to a unrestrained growth. Among skin cancers, this is as rare as dangerous, having the worst prognosis. Despite many years of intensive laboratory and clinical research, the only effictive therapy is the surgical resection of the tumor. In the last years oncologists have demonstrated that metastatic melanoma can respond to immunotherapy by using interferon or interleukin-2 (IL-2). However both interferon and IL-2 are interesting, but non-specific immunotherapeutic agents, and they elicit only a modest survival benefit. The discovery of tumor associated and/or specific antigens present on melanoma cell surface, to be used as therapeutic vaccines, remains a challenging purpose.

The surface of melanoma cells is characterized by the presence of carbohydrate antigens belonging to GSLs family. They are present in both melanoma and normal cells

(melanocytes), but act as efficient immunogens only when they are placed on the surface of tumor cells. This implies that they are able to elicit an immune response that normal GSLs cannot. This is the case of the over-expressed gangliosides GM3 and GD3, that undergo a conformational change, probably because of their presence at high density on melanoma cell surface. Although the function of most gangliosides has not been yet completely revealed, it is known that some of them are involved in cell-micro-environment interaction processes, in angiogenesis, and in metastatic growth regulation. This last function has been demonstrated on MEB4 melanoma cells pharmacologically depleted of gangliosides (prevalently GM3). Indeed cells treated with a specific glucosylceramide synthase inhibitor reveal a conspicuous reduction in their ability to form tumors, and a great reduction in their metastatic potential.[9] Recently gangliosides have also been associated with the tumor-induced immunosuppression processes.[10]

Several studies on ganglioside expression in a group of melanomas, using thin layer chromatography and mAb techniques, have identified GM3, GD3, GM2, and GD2 as the most abundant gangliosides in this neoplastic tissue.[11] Despite GM2 is the most immunogenic one and it is present in most melanomas, it represents only a small percentage of the totality of gangliosides. However natural antibodies, raised against it, have been found on 5% of patients with melanoma.[12] GD3 is highly expressed, but is poorly immunogenic. GM3 is the most abundant in melanoma cells,[13] and, despite its poor immunogenicity, it is a tumor-associated antigen in mice, hamster and humans. An additional factor that makes it the most interesting as immunotherapeutic target, is the recent discovery that chemotherapy-resistant cancer cells have higher levels of this ganglioside.[14] Moreover it is over-expressed in melanoma cells with metastatic potential.[15][16] GM3 ganglioside undergoes an *in vivo* lactonization on melanoma cell surface, giving the corresponding lactone **1** (Figure 1), also found in melanomas as a minor component.[17] This lactonization is likely promoted by the lower pH environment of tumor cells[18] and, possibly by a different conformation of GM3 ganglioside induced as a result of localised high density.[19]

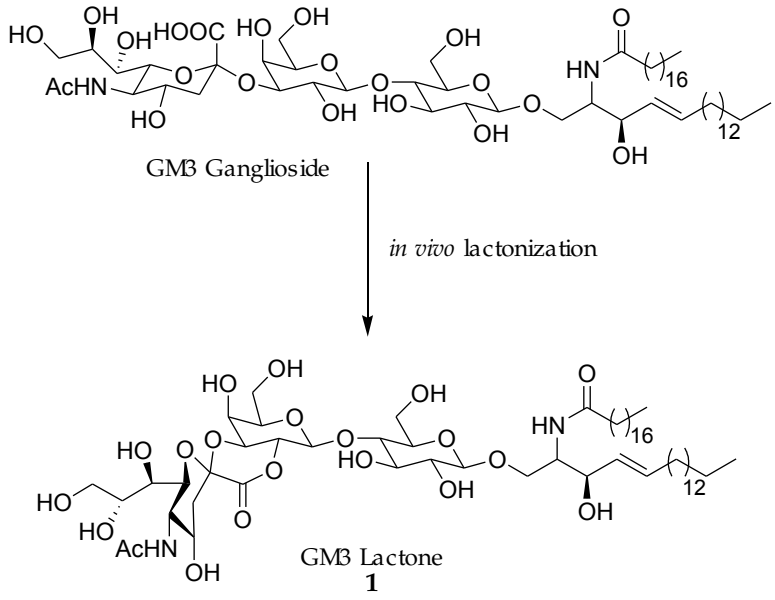


Figure 1

Induction of anti-GM3 antibodies can trigger the immune system against melanoma cells. Therefore, therapeutic use of a GM3-based vaccine in patients with melanoma is an attracting challenge. Indeed, it has been demonstrated that the monoclonal antibody M2590, obtained by immunization of mice with syngenic melanoma B16 cells, recognizes a high density of GM3, as well as GM3 lactone **1**. On the other hand the antibody does not react with a low density of GM3, present on healthy cells. A following comparative immunization experiment with GM3 and GM3 lactone has indicated that the latter is about ten times more immunogenic than its precursor GM3,[17] however, under physiological conditions, the equilibrium amount of the lactone is below the recognition threshold. Hydrolitically stable and structurally similar lactone analogues are thus aimed. A few years ago two hydrolytically stable analogues of GM3 lactone, namely a GM3 lactam[20][21] and a GM3 ether,[22][23] were prepared. (Figure 2).

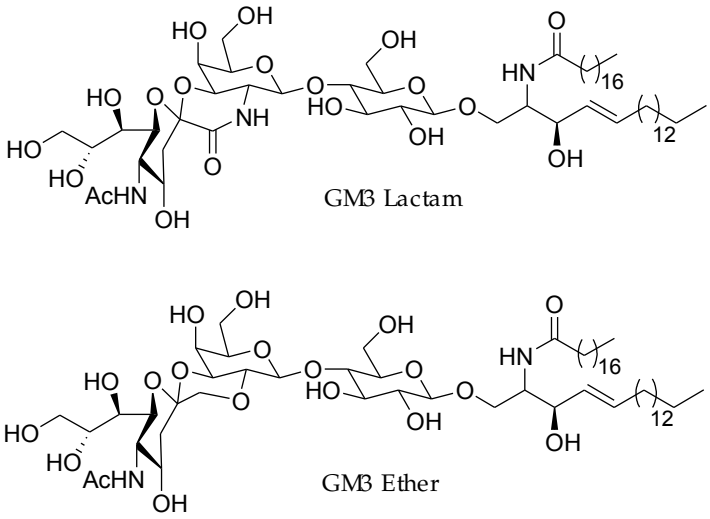


Figure 2

The replacement of the lactone moiety with the lactam guarantees a structure quite similar to the original. Immunization[24] experiments conducted on mice with synthetic GM3 lactam-BSA (bovine serum albumine) gave rise of some hybridomas producing antibodies directed to both GM3 lactam and the native lactone, confirming that the saccharidic conformations are very similar (as predicted by computational calculations).[20] More recent is the Tietze's synthesis of GM3 ether, where the lactone ring is replaced by an ether moiety. The ether analogue is structurally very close to the lactone itself, despite of the substitution of a sp^2 with a sp^3 center. In their paper, the authors emphasize the increased stability of GM3 ether to hydrolytic processes when compared to the previously reported GM3 lactam, underlining that the latter reveals a marked sensitivity to proteases. While Tietze and coauthors have reported the total synthesis of their GM3 analogue, no data of biological tests have been yet reported.

In both cases, the lactam and the ether analogues have, to date, not been used for anticancer therapy. The synthesis of mimetics of GM3 lactone that are resistant to hydrolysis remains a challenging target for the development of a therapeutic melanoma vaccine.

2. Mimetics of GM3 lactone

In the last years we have focused our attention in the development of a mimetic of GM3 lactone stable to hydrolysis. The idea was the total synthesis of the thioether analogue of GM3 lactone, namely GM3 thioether **2** (Figure 3).

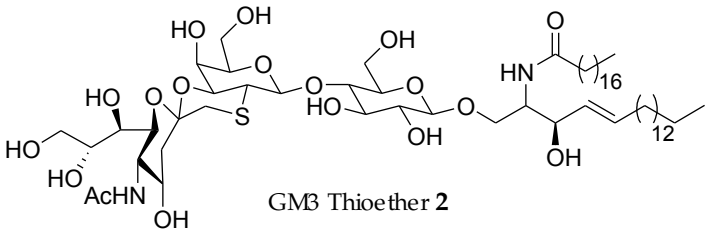
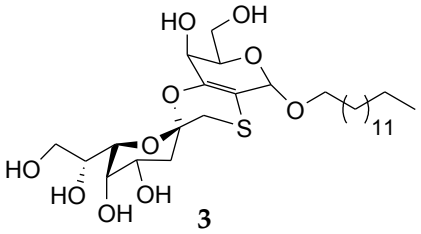


Figure 3

Thioethers usually show a high level of stability toward hydrolysis catalyzed either by acids or bases, and they are therefore expected to be stable under physiological conditions. An increased stability toward hydrolysis does not guarantee that the GM3 lactone analogue **2** maintains the same conformational features of its "mould". This is the reason we performed DFT studies to demonstrate the three-dimensional similarity of **2** towards GM3 lactone **1**, before starting synthesis.

Meanwhile, we aimed to synthesize a mimetic of GM3 lactone, not only hydrolytically stable, but also structurally simpler than native compound. This, indeed, would have been an advantage in view of the development of a therapeutic melanoma vaccine. The glycosidic portion of both GM3 lactone **1** and in its analogue **2** is characterized by the presence of a N-acetyl neuraminic acid (sialic acid) residue. This monosaccharide has a peculiar reactivity, and it is not easily chemically manipulated. The substitution of the sialic acid residue with another sugar would lead to a simplification, in terms of number of reactions, and an improvement, in terms of yields, of the synthetic pathway.

The mimetic **3** we planned to synthesize is the result of a rational design, based on theoretical calculations (Figure 4).



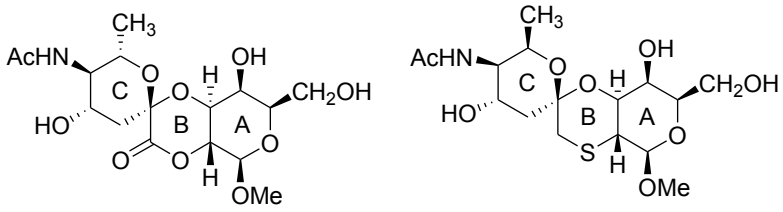
3

Figure 4

As depicted in Figure 4, the sialic acid residue of **3** was replaced by a mannose-derivative.

2.1 Conformational analyses and retrosynthesis of GM3 thioether 2

Magnusson et al.[20] suggested that a boat-like conformation of the lactone ring was preferred by GM_3 lactone. We first performed theoretical calculations on the simplified tricyclic moiety of GM3 lactone **4** and on its thioether analogue **5** by using a DFT approach at the B3LYP/6-31G* level in order to evaluate the effects on the conformational behavior of the substitution of a lactone with a thioether function (Figure 5).

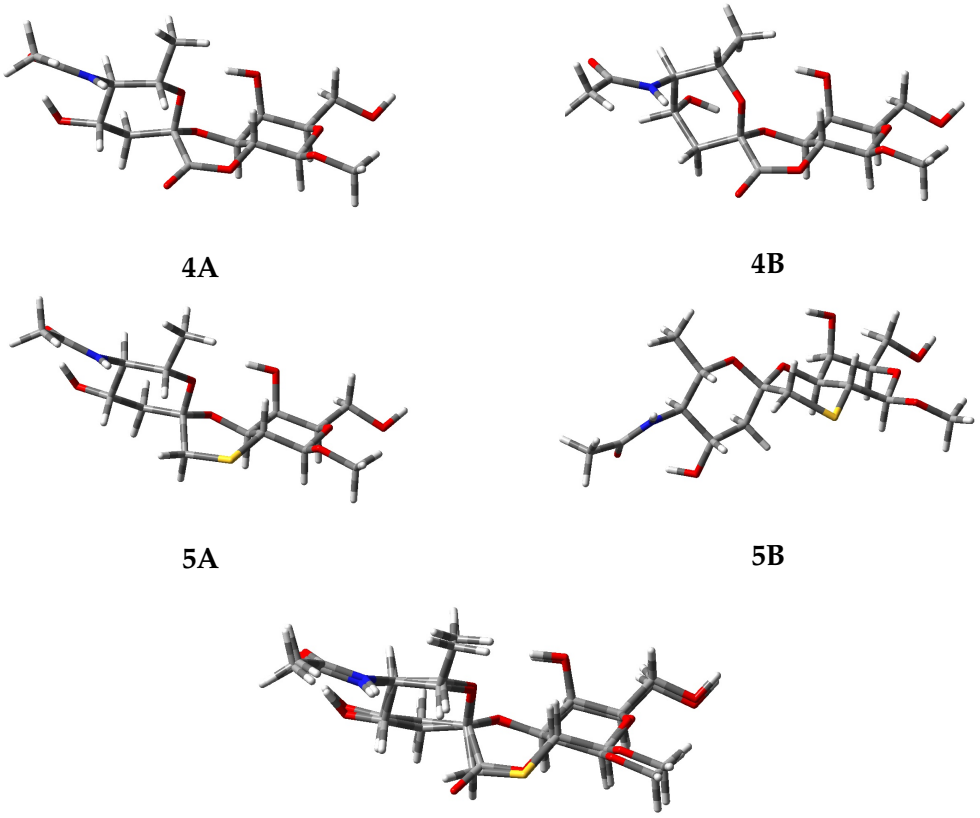


4 **5**

Figure 5

The chair-like conformation of the lactone ring of **4** did not represent an energy minimum, as in every case the twisted-boat conformation **4A** was obtained from optimizations starting either from the chair or from the boat conformation. The behavior of the analogue **5** was slightly different; in this case also the chair conformation of ring B represented an energy minimum so that four geometries of the tricyclic system could be

located. The twisted-boat conformer 5**A** was the most stable one and accounted for about 95% of the overall population suggesting a high analogy with the compound **4**. All the above considerations, however, were based on *in vacuum* optimized conformations. Thus, the energies of all the computed conformers were recalculated by using a continuum solvent model, C-PCM, to simulate the effect of water. Actually, the effects of solvation on **4** were negligible, as **4A** remained the only populated conformation. Conversely, the chair conformation **5B** became even slightly more stable than **5A** with a balanced equilibrium twisted-boat/chair 40:60. However, in the case of the thioether analogue **5**, calculations put in evidence the existence of an equilibrium between the more stable chair **5B**, and the less stable twisted-boat **5A**, since the energy difference between these conformations was less than 2 kcal/mol (Figure 6).



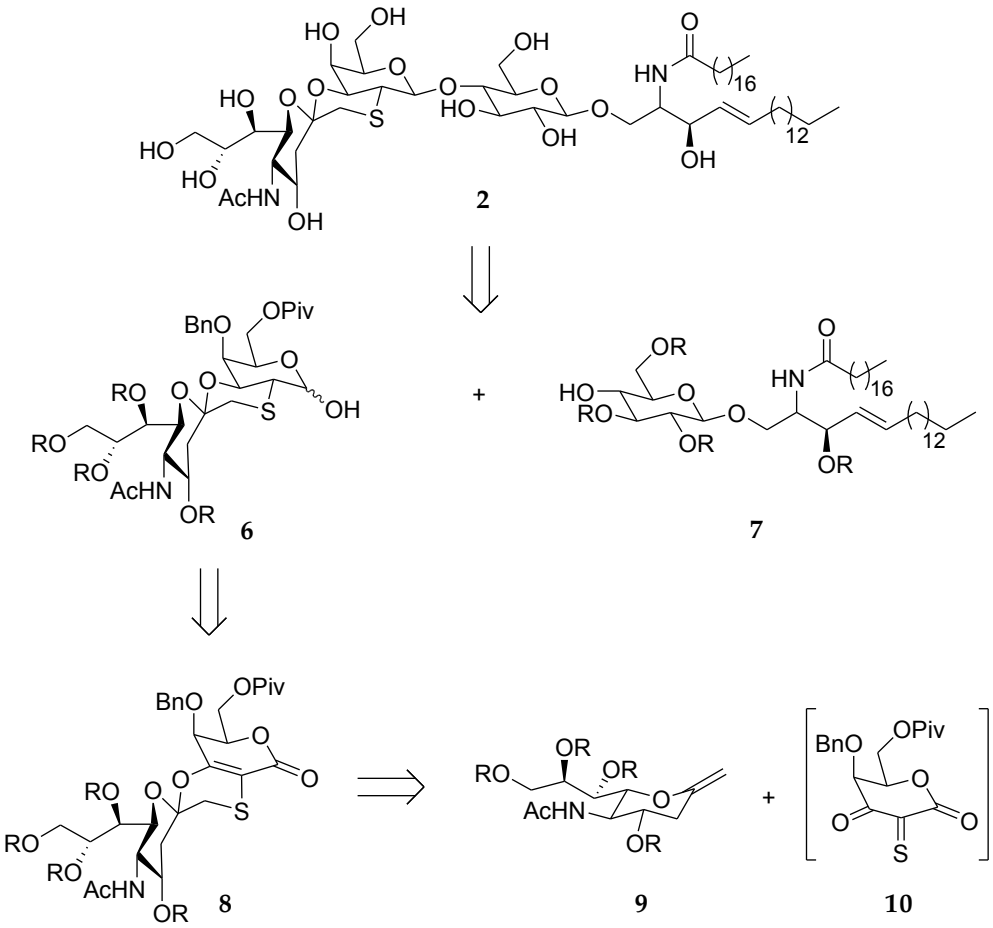
4A **4B**

5A **5B**

4A vs **5A**

Figure 6

The retrosynthetic approach of this compound is shown in Scheme 1.



Scheme 1

The final step of the synthesis is a glycosylation reaction involving glucosyl-ceramide **7** and the tricyclic core **6**. This step would rely on one of the several glycosylation methodologies[25][26][27] reported in the literature. The main target of the proposed scheme is the stereoselective synthesis of the spiro tricyclic core **8**, that can be achieved through an unconventional strategy, "via" a hetero Diels-Alder reaction between the α-thiono-β-keto-δ-lactone **10**[28] and the "sialic acid-derived"-dienophile **9**. Compounds **9** and **10** can be obtained starting, respectively, from N-acetyl neuraminic acid and D-galactal.

Inverse electron demand hetero Diels-Alder reactions between electron-rich dienophiles and α,α'-dioxothiones have been extensively studied by our group.[29][30] The first attempts to synthesize GM3 thioether **2** are reported in chapter 4.

2.2 Conformational analyses and retrosynthetic approach for GM3 lactone mimetic, 3

The spiro glycoside **3** is a simplified thioether-bridged mimetic of GM3 lactone **1**, and therefore a conformational analogue of GM3 thioether **2**, with a mannose-derived residue replacing the sialic acid portion and an alkyl chain instead of the glucosylceramide (Figure 7).

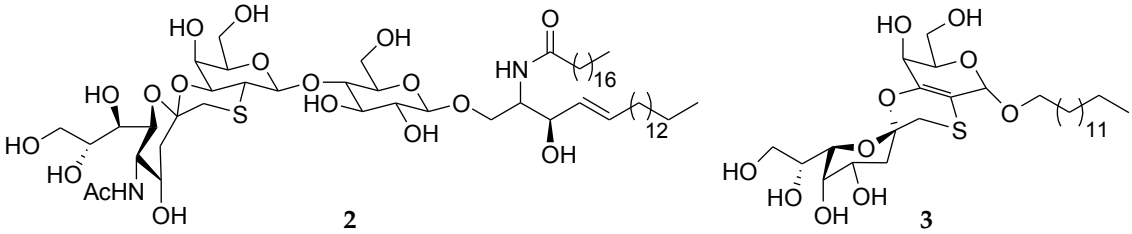
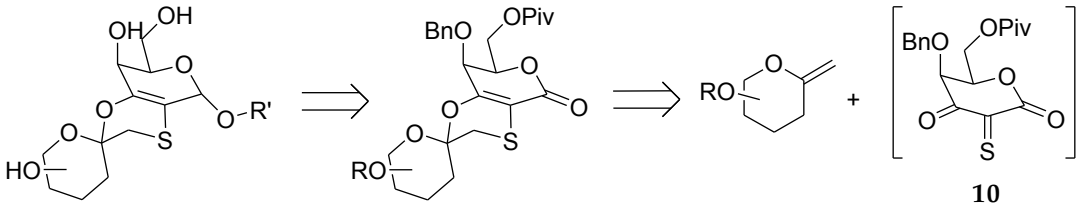


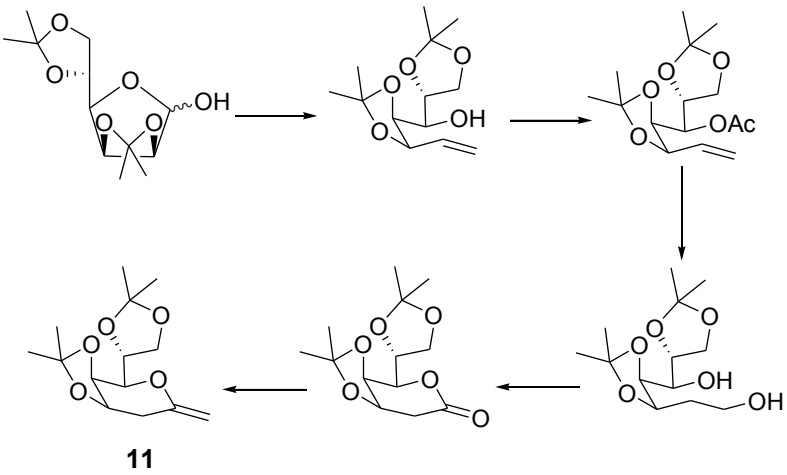
Figure 7

The retrosynthetic approach is similar to those described for the achievement of GM3 thioether **3** and capitalizes in the [4+2] cycloaddition between the α-thiono-β-keto-δ-lactone **10** and a "sialic acid-mimetic" dienophile for the achievement of the spiro unit (Scheme 2).



Scheme 2

This approach was inspired by the synthesis of a mannose-derived 3-deoxy-exoenitol reported by Sïnay et al[31] (Scheme 3).



Scheme 3

The final exoenitol **11** is an unnatural carbohydrate characterized by the presence of a deoxy group and a diol chain, and therefore with good similarities with the sialic acid-derived exoenitol **9**. Starting from this point, we decided to perform conformational analyses on the cycloaddition adducts that would be obtained by reacting the α-thiono-β-keto-δ-lactone **10** with three different unnatural carbohydrates, namely the exoenitol **11** and other two exoenitols (**12** and **13**). The two former ones would be easily obtained following Sïnay's synthetic route (Scheme 2), starting from D-galactose (**12**) or D-gulose (**13**). In Figure 8 are reported the final exoenitols.

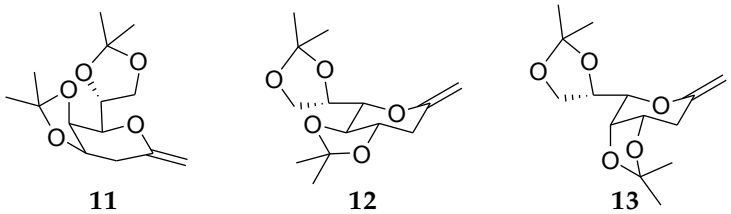
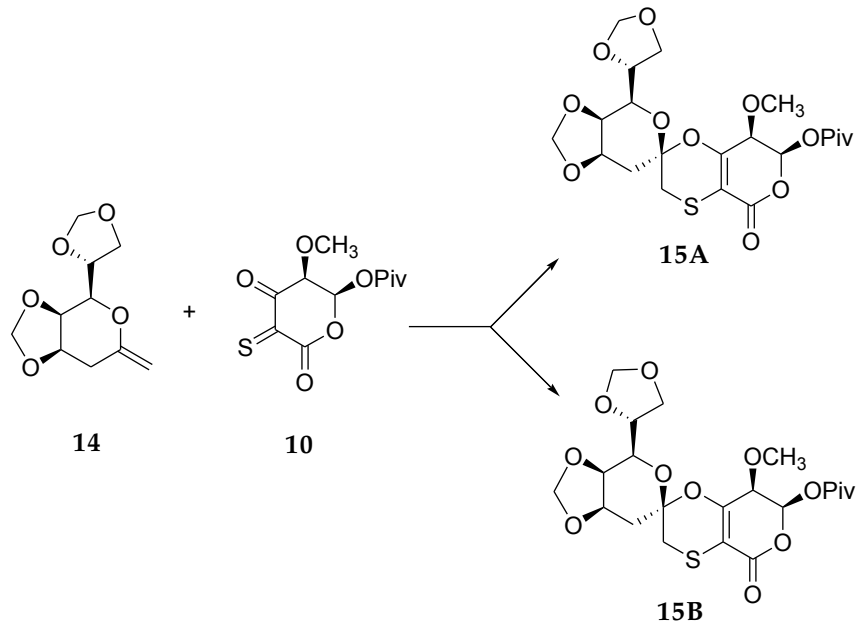


Figure 8

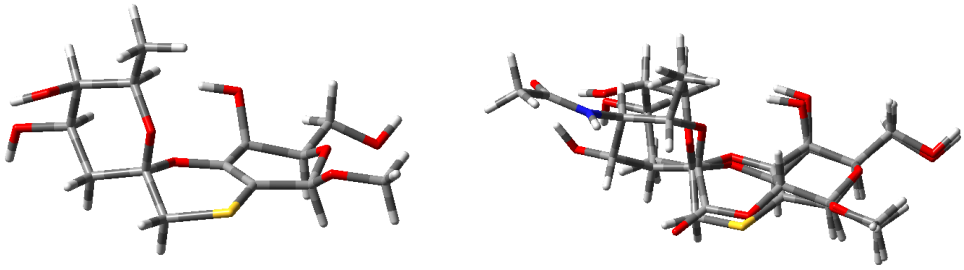
Theoretical calculations were performed in order to choose the carbohydrate that would have conducted to the best cycloadduct in terms of structural similarity with GM3 lactone **2**, between **11**, **12** and **13**. The first aim was the evaluation of the relative stability of adducts coming from the hetero Diels-Alder reaction between exoglycals (**11**, **12** and **13**) and

the α-thiono-β-keto-δ-lactone **10**. To run calculations exoglycals were simplified by replacing the isopropylidene groups with a methylene moiety. In Scheme 4 is reported the cycloaddiction reaction between **10** and the simplified mannose-derived enolether **14**, since the calculations showed the dienophile **11** to be the best candidate for the cycloaddition reaction. Whereas, both the use of galactose-derived **12** and gulose-derived **13** would have conducted to cycloadducts structurally quite different from the tricyclic core of GM3 lactone.



Scheme 4

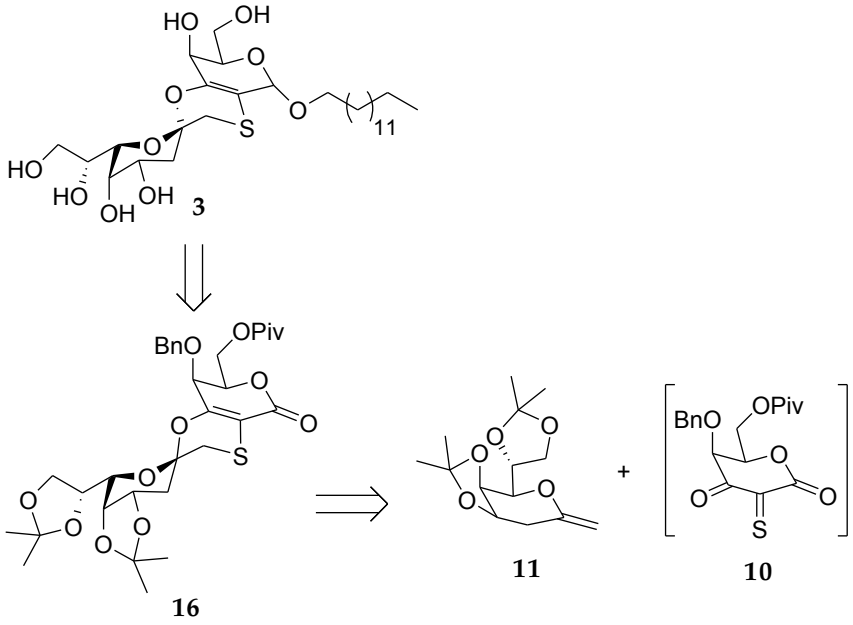
Three cycloadducts were possible for this reaction, but only **15A1** had a stability higher than the other two, specifically more than 5 kcal/mol . The conformation **15A1** was largely preferred both in vacuum and in water and showed a complete geometrical agreement in comparison with the preferred conformation of GM3 lactone **4A**. The conformation **15A1** showed indeed a very good superimposition of rings A and B and a good superimposition of ring C as well as of the triolic chain, represented by the methyl group, which occupied the same position indicated by the red arrow (Figure 9).



15A1 **15A1** vs **4A**

Figure 9

In conclusion, molecular modeling showed that cycloaddiction between the α-thiono-β-keto-δ-lactone **10** and enolether **11** would have lead to the cycloadduct **15A**, with a preferred geometry proxying those of GM3 lactone. Retrosynthetic approach for mimetic **2** is described in Scheme 5.



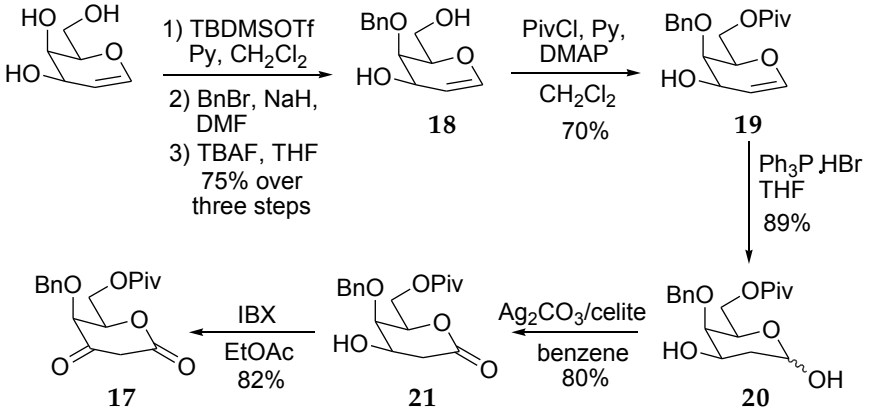
Scheme 5

3. Towards the synthesis of GM3 thioether 2

The main goal in the Scheme 1 (chapter 2) is the stereoselective synthesis of the spirotricyclic core **8** through the hetero Diels-Alder reaction between the α-thiono-β-keto-δ-lactone **10** and the "sialic acid-derived"-dienophile **9**. Diene **10** can be obtained *in situ* during the cycloaddiction reaction from the parent β-keto-δ-lactone **17**, by treatment with phtalimidesulphenyl chloride and following treatment of the corresponding sulfenyl derivative with a weak base (see below).

3.1 Synthesis of the β-keto-δ-lactone 17

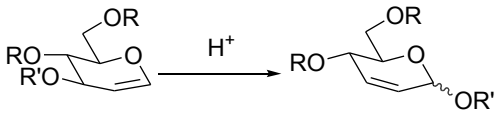
The synthesis of the β-keto-δ-lactone **17** was planned in our group and recently optimized with reference to a several grams scale production, starting from D-galactal. The synthetic pathway is shown in Scheme 6[28] and consists of a sequence of protection deprotection reactions to achieve the 4-benzyl-6-pivaloyl-D-galactal **19**, that afterward is transformed into the correspondent 2-deoxy-galactose derivative **20** and in the final β-keto-δ-lactone **17**, through two selective oxidations of the anomeric and C-3 positions, by using respectively Fetizon's reagent[32] and IBX.[33]



Scheme 6

With respect to the synthesis reported by Bartolozzi and al.[28] we optimized the

synthesis of compound **18**, that is thereafter achieved through the same reactions, but with only a final purification by column chromatography on silica gel. In addition, we modified the hydration of the double bond of the glycal **19**, that was before accomplished by using tetrabutylammonium hydrogensulfate ($TBAHSO_4$) in THF with a 65% yield. Instead, by using $Ph_3P{\cdot}HBr$,[34] we increased the yield thanks to the prevention of Ferrier rearrangement,[35] that generally competes with the addition of water to the glycal, and consists of the acid catalysed isomerization of the double bond with the exit of the C-3 substituent and its incoming to the anomeric position (Scheme 7).

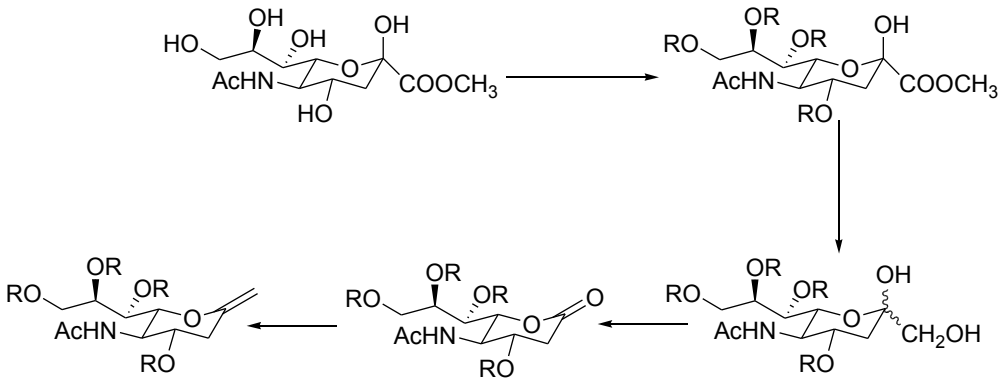


Scheme 7

We tried to perform the final oxidation following the Swern conditions reported by Bartolozzi et al. by using high amount of the starting lactone **21**, but unfortunately we realized that it was not possible without having a dramatic downfall in the yield. Moreover, this reaction was a difficult step because the β-keto-δ-lactone **17** was very sensitive to chromatographic purification conditions and therefore we needed a "cleaner" reaction, being compelled to use the crude for the following cycloaddition. Among various possibilities, we chose IBX as oxidant and we carried out the reaction in EtOAc at 80°C, affording the final compound **17** in a 82%, after a simple filtration of the reaction mixture through a pad of celite, as the only product excepting a little amount of benzoic acid.

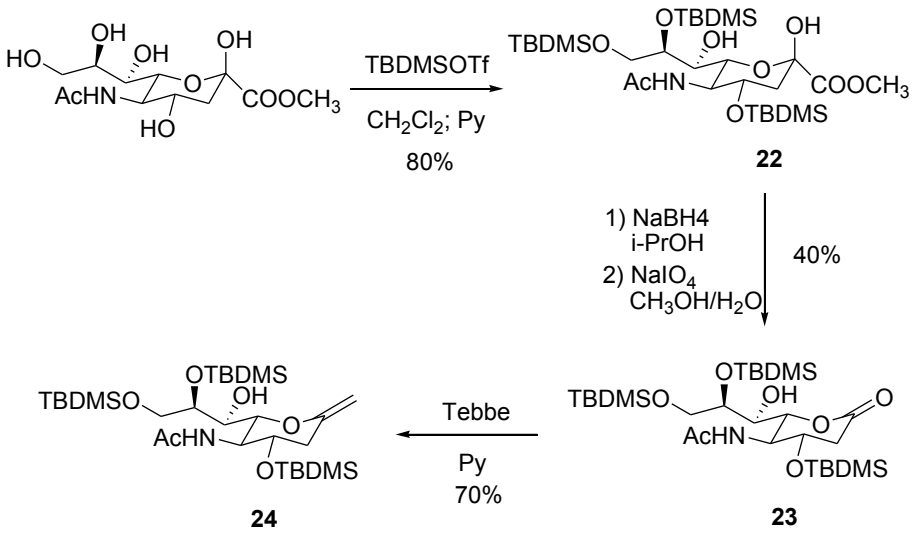
3.2 Synthesis of the "sialic acid-derived" exoenitol

For the preparation of the "sialic acid-derived" exoenitol, a synthetic pathway was devised starting from the commercially available N-acetyl neuraminic acid methyl ester. The proposed synthetic pathway consists of the convenient protection of the sugar hydroxyl groups (except the anomeric position), the reduction of methyl ester, the transformation of the correspondent diol into the lactone moiety, and the final olefination (Scheme 8).



Scheme 8

The initial idea concerned the use of *tert*-butyldimethylsilyl as protective group and its introduction in the form of trifluoromethanesulfonate. Silylethers, indeed, can be easily inserted and removed under mild and selective conditions, by using organic fluorinated salts, as TBAF (tetrabutylammonium fluoride). The synthetic route is summarized in scheme 9.

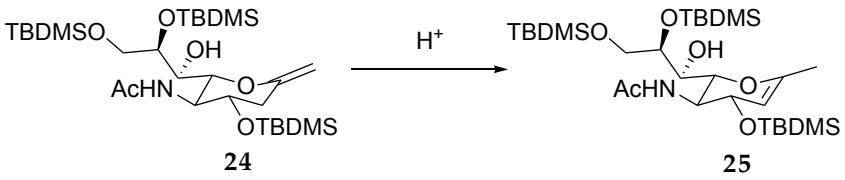


Scheme 9

The methyl ester of sialic acid was protected as silylether by using *tert*-butyldimethylsilyl trifluoromethanesulfonate (TBDMSOTf) in order to afford the trisilylated **22** in a 80% yield. Both the anomeric hydroxyl and those in position seven did not react with TBDMSOTf, the first because of its scarce reactivity, the second probably because of the steric

hindrance of the silyl groups nearby. The methyl ester of compound **22** was then reduced with $NaBH_4$ in *i*-PrOH, thanks to the presence of the free anomeric hydroxyl, and the obtained 1,3-diol was subsequently treated with $NaIO_4$ in a mixture of CH_3OH and H_2O to afford the lactone **23**, with a 40% yield after two-steps. The low yield was due to side reactions occurring to the diol, in equilibrium with its open form, during the second step. Compound **23** was then transformed into the corresponding enolether **24** by using Tebbe reagent in pyridine, with a good yield.

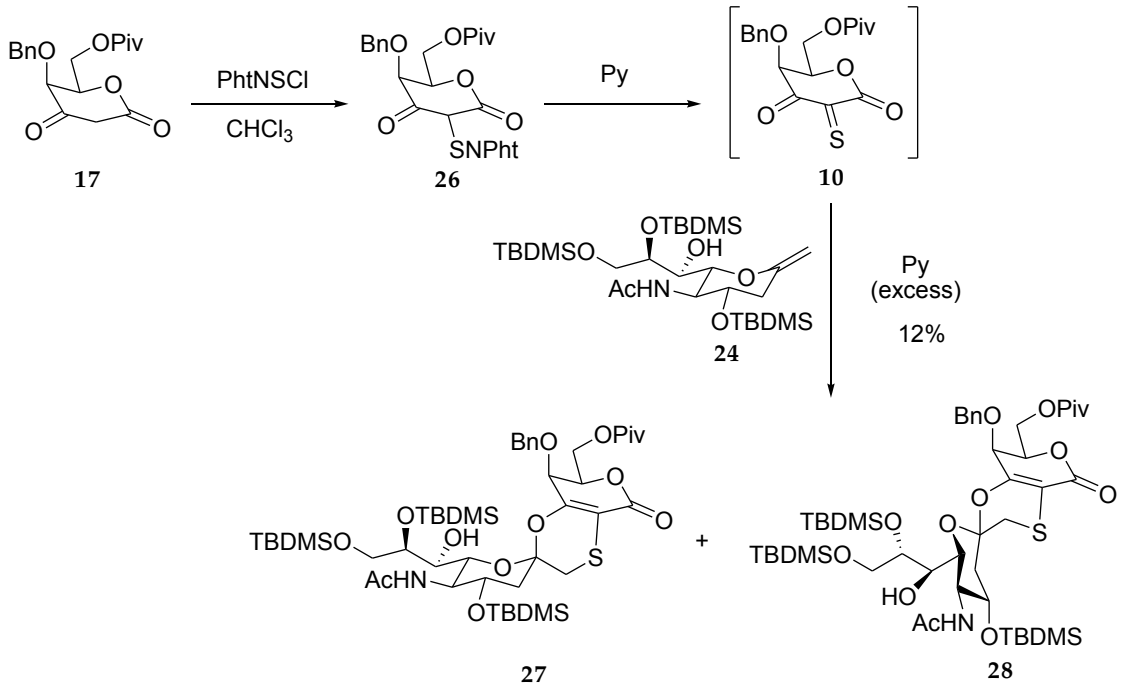
Compound **24** is the first example of a sialic acid-derived enolether. The presence of the deoxy-component makes **24** not highly stable; it can indeed easily isomerize to the thermodinamically favoured internal olefine **25**, mainly with acids, despite their weakness (Scheme 10).



24 **25**

Scheme 10

Straight after its synthesis, the dienophile **24** was reacted with the α-thiono-β-keto-δ-lactone **10**, derived from β-keto-δ-lactone **17**, following the Diels-Alder reaction condition optimized by our group. Compound **17** was treated with phtalimidosulfenyl chloride (PhtNSCl), by employing standard sulfenylating conditions, in order to afford the phtalimidosulfenyl derivative **26**, which underwent to the heterodiene **10** after the addition of the dienophile **24** in Py. Two [4+2] cycloadducts diastereoisomers (**27** and **28**) were formed during the reaction, with a 2.5:1 ratio (Scheme 11).



Scheme 11

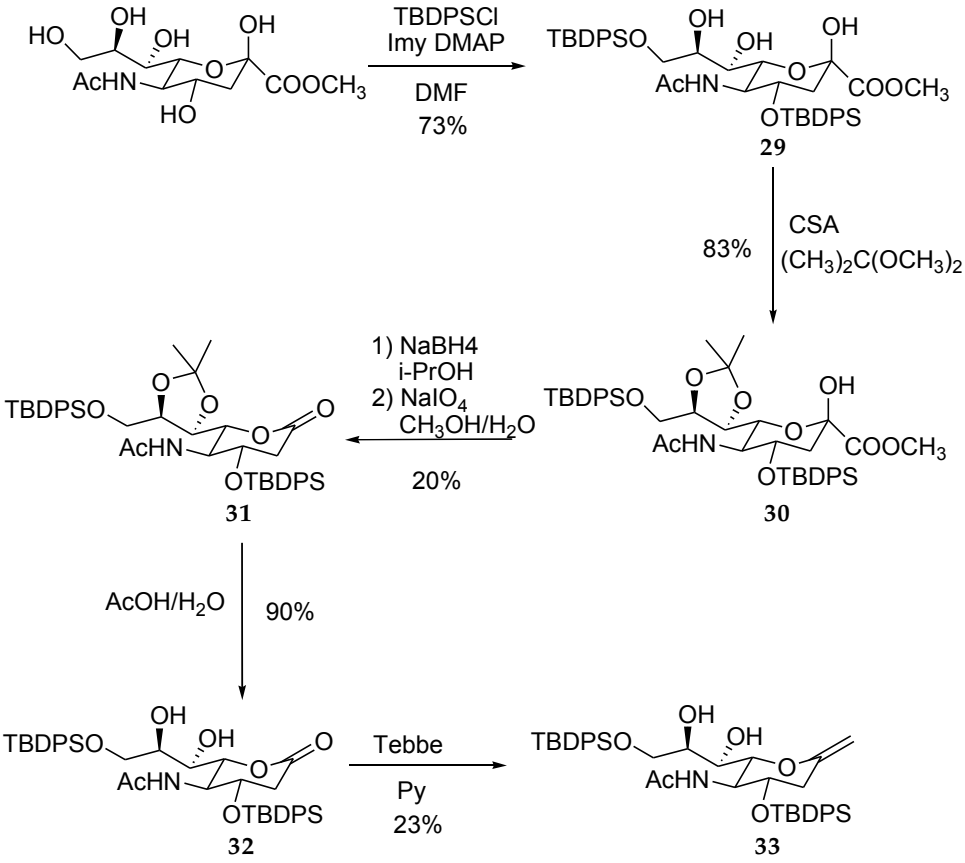
The results demonstrated the feasibility of the hetero Diels-Alder reaction involving a sialic acid-derived enolether as dienophile, but unfortunately, despite a good control of the diastereoselectivity, the overall yield was not good (only 12% for the diastereoisomeric mixture).

Molecular modeling calculations identified that a possible cause of the low yield was the high steric hindrance of the silyl groups of the enolether **24** side chain in the C-8 and, particularly, C-7 positions. These groups could prevent the correct approach between diene and dienophile during the Diels-Alder reaction.

Since these remarks, we tried to maintain the same synthetic approach, but with different protective groups for the C-7 and C-8 hydroxyls, that could be removed before the final cycloaddiction reaction.

Starting again from the methyl ester of the sialic acid, the hydroxyl groups in positions four and nine were selectively protected as silylethers by using *tert*-butyl(chloro)diphenylsilane (TBDPSCl), a less reactive chemical agent than the previously reported TBDSOTf, affording **29** in a 73% yield. The 1,2-diol of compound **29** side chain was then protected as isopropyliden, by using 2,2-dimethoxypropane and camphorsulfonic acid (CSA), affording **30** in a 83% yield. The following two reactions were the same used for the

achievement of the enolether **24**: the reduction of the methyl ester with $NaBH_4$ and the formation of the lactone with $NaIO_4$. The hydoxyls in positions seven and eight of the side chain of the lactone **31** were then deprotected by using a mixture of AcOH and H_2O to afford **32** in a 90% yield. The final olefination with Tebbe reagent afforded the enolether **33** only in a 23% yield, as shown in scheme 12.



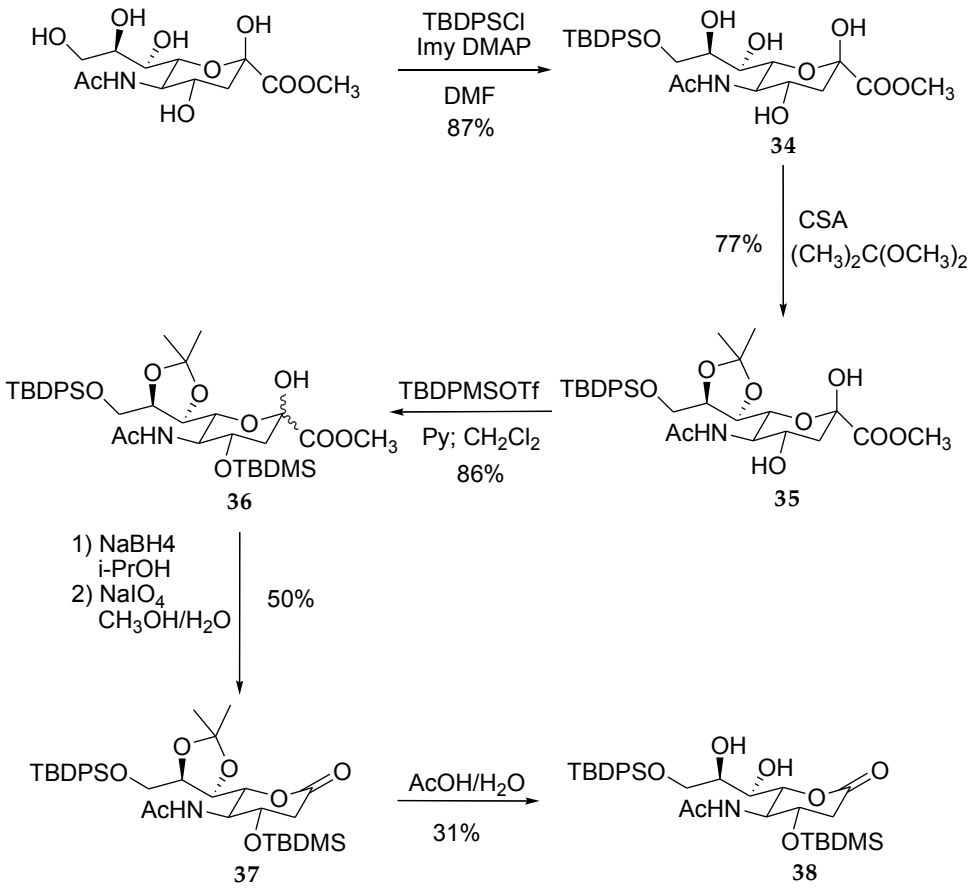
Scheme 12

The freshly prepared enolether **33** underwent Diels-Alder reaction, using the same conditions reported for compound **24**, but in this case the reaction afforded only large amount of unidentified products, likely derived from the decomposition of the starting material (enolether **33** and heterodiene **10**).

The ^1H NMR spectrum of compound **33** showed a 0.2 ppm shift for the sialic acid deoxy-protons signals, if compared to those of compound **24**, suggesting that they were probably deshielded by one of the phenyl groups of *tert*-butyldiphenylsilyl. This indicated that the *tert*-butyldiphenylsilyl group would be too close to the double bond, preventing

completely the correct approach between diene and dienophile during the Diels-Alder reaction.

Since this considerations, we tried to replace the *tert*-butyldiphenylsilyl group with the less hindering *tert*-butyldimethylsilyl, adopting the following synthetic scheme.



Scheme 13

Starting from the methyl ester of the sialic acid, the hydroxyl groups in positions nine was selectively protected as silylethers by using tert-butyl(chloro)diphenylsilane (TBDPSCl), affording **34** in a 87% yield. Hydroxyl groups in position seven and eight of the side chain were then protected as reported for compound **29**, by using 2,2-dimethoxypropane and canphorsulfonic acid (CSA), affording **35** in good yield. The hydroxyl group in position four of **35** was then protected as silylether with TBDMSOTf and Py, to afford **36** in a 86% yield. Compound **36** was then transformed, in a two-step 50% yield, to the corresponding lactone **37**, by using the same procedure previously described. Attempts in deprotecting the 1,2 diol

of **37** with $AcOH/H_2O$, resulted in a low yield (32%), probably because of the partial deprotection of the C-4 hydroxyl caused by the reaction conditions.

These unsatisfying results, together with the early good results obtained in the achievement of the mimetic of GM3 lactone **3**, induced us to drop the total synthesis of GM3 thioether **2** in order to put our efforts into the development of the structurally simplified mimetic of GM3 lactone.

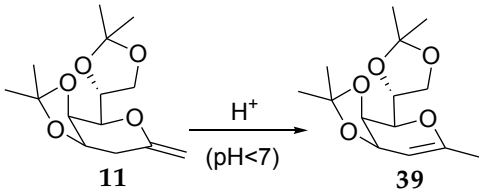
4. Synthesis of mimetic 3 of GM3 lactone

Taking into consideration the good results suggested by conformational analyses, we planned to synthesize the simplified mimetic **3**, in which the mannose-derived portion replaces the sialic acid of the GM3 lactone. The main characteristics of compound **3** in comparison to GM3 lactone is the presence of a di-hydroxyl chain instead of the tri-hydroxyl chain, and a hydroxyl group instead of the N-acetyl moiety. Moreover, **3** is characterized by an unsaturation which replaces the trans fusion between the ring A and B of GM3 lactone.

In order to perform biological tests for the production of monoclonal antibodies, we decided to replace the glucosylceramide portion with a long C14 alkyl chain that we deemed better for the loading into liposomes. Liposomes, indeed, are well known immunogenic carriers for biological molecules.[36]

4.1 Synthesis of the spirotricyclic core of mimetic 3

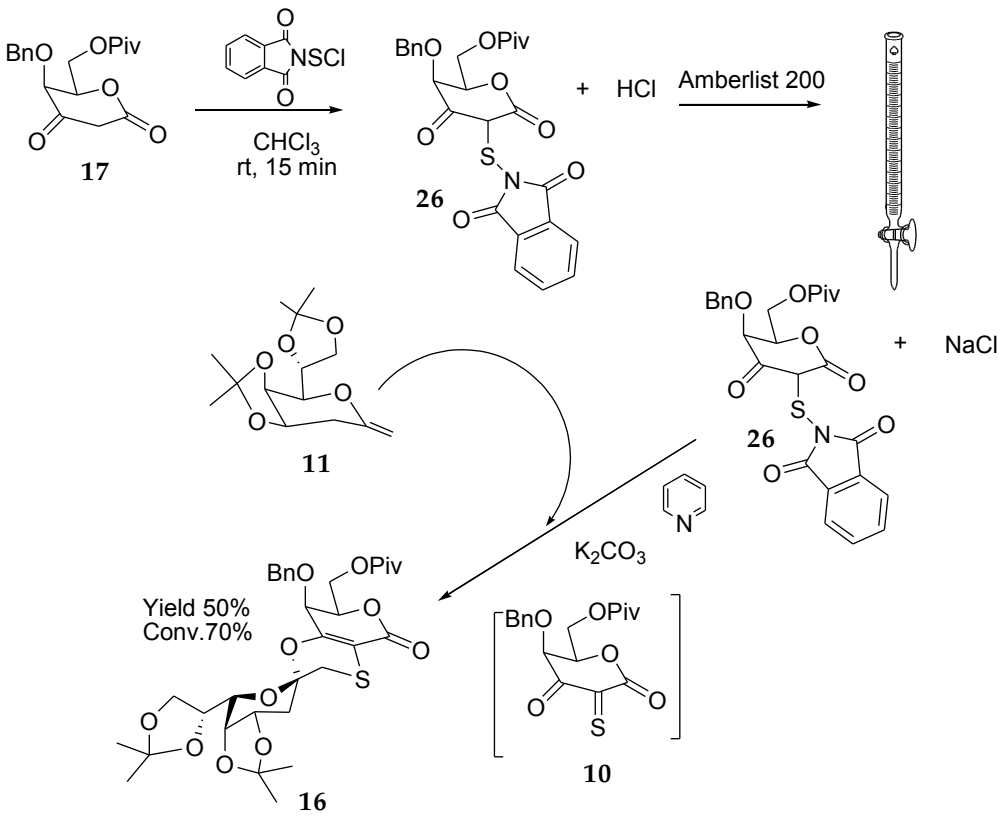
For the achievement of compound **3**, the key reaction is the stereoselective synthesis of the spirotricyclic core **16** (see Scheme 5). This compound can be prepared through an inverse electron demand hetero Diels-Alder reaction between the α-thiono-β-keto-δ-lactone **10** and the "sialic acid-mimetic" dienophile **11**. Diene **10** was obtained *in situ* during the cycloaddiction reaction from the parent β-keto-δ-lactone **17**, (as previously reported, see Chapter 2). The deoxy-exoglycal **11** is highly sensible to even mild acidic conditions, and easily undergoes the transformation into the corresponding 1,2-glycal **39** (Scheme 14).



Scheme 14

During the cycloaddition reaction there are two possible sources of acidity: the 2-

iodobenzoic acid, which is present in the crude derived from the oxidation with IBX, and the hydrochloridric acid developed in the treatment of the β-keto-δ-lactone **17** with phtalimidosulfenyl chloride. In order to overcome acidity-related problems, we decided to filter the crude phtalimidosulfenyl derivative **26** through an ion exchange resin, in order to replace H^+ of the solution with Na^+ of the resin. The so obtained suspension was then slowly added to the enolether **11** in the presence of pyridine and potassium carbonate, affording the desired cycloadduct **16**, as a single diastereoisomer in a 50% overall yield (Scheme 15).



Scheme 15

The structure of the spiro centre of **16** was assigned by X-ray crystal structure determination[37] confirming the results obtained through theoretical calculations described in Chapter 2 (Figure 10).

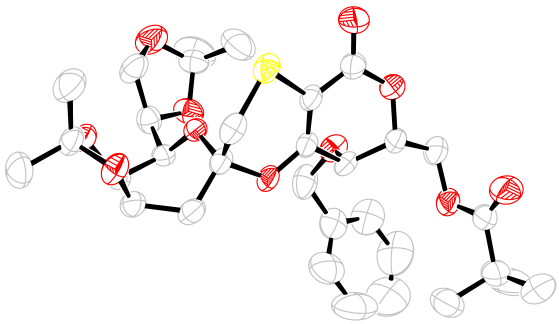
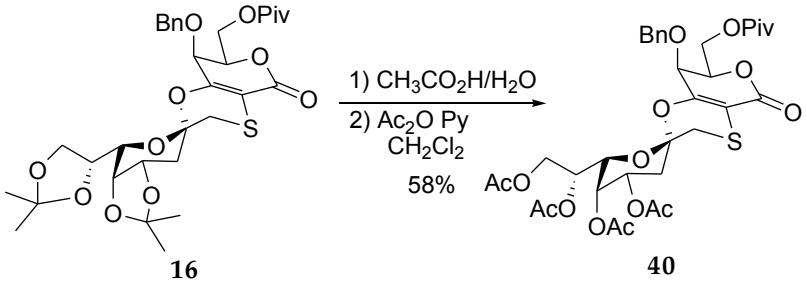


Figure 10

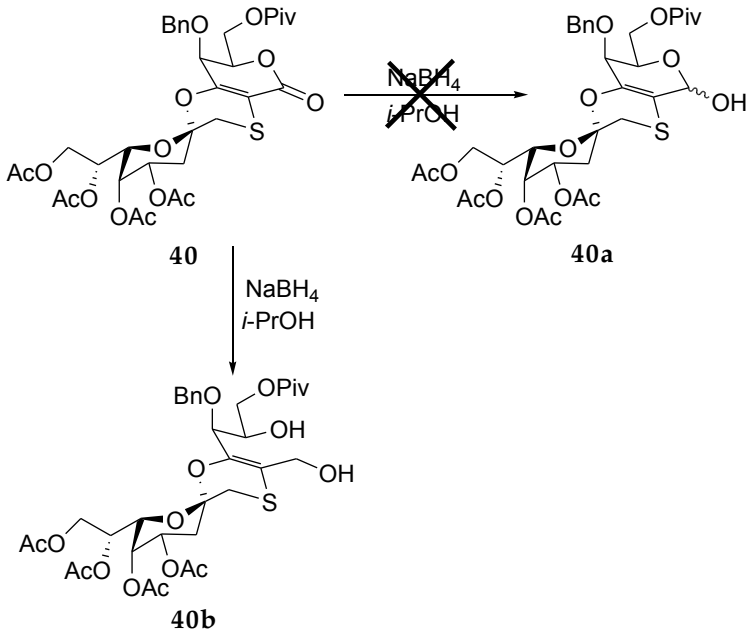
4.2 Achievement of benzyl-mimetic 45

Once obtained the correct cycloadduct, the following reactions for the achievement of mimetic **3** were the reduction of the lactone to a hemiacetalic moiety and the glycosylation with tetradecanol. Isopropylidenes were not compatible with the acidic conditions occurring during the glycosylation reaction, since they easily undergo hydrolization in mild acidic conditions. Cycloadduct **16** was then transformed into the acetyl derivative **40**, through the deprotection of isopropylidenes with a 70:30 mixture of acetic acid and H_2O and the following acetylation under standard conditions, in a 58% yield, after two steps (Scheme 16).



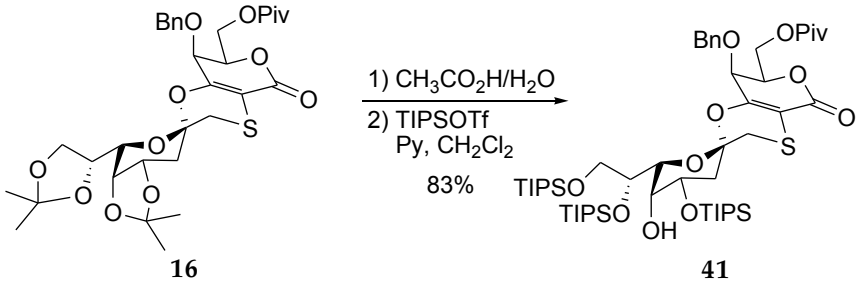
Scheme 16

Compound **40** was then treated with sodium borohydride in order to reduce the lactone moiety and to afford the corresponding hemiacetal. Unfortunately the reduction did not afforded at the hemiacetal **40a**, but gave the diol **40b** (Scheme 17).



Scheme 17

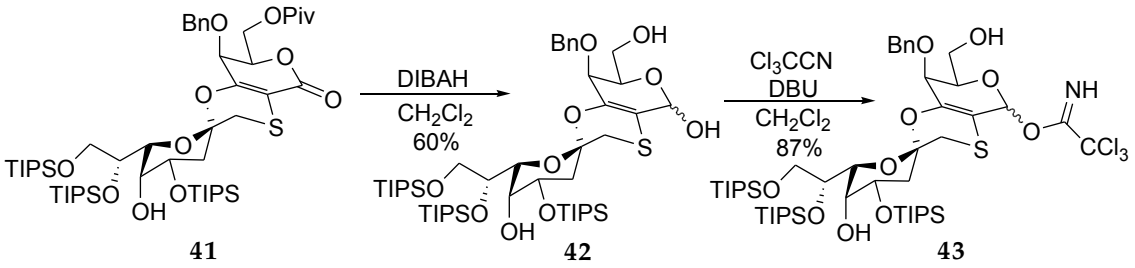
Other reducing agents, as diisobutylaluminum hydride (DIBAH), were not compatible with acetyls: we thus decided to protect the hydroxyls groups of the cycloadduct **16** as sylil ethers, as shown in Scheme 18.



Scheme 18

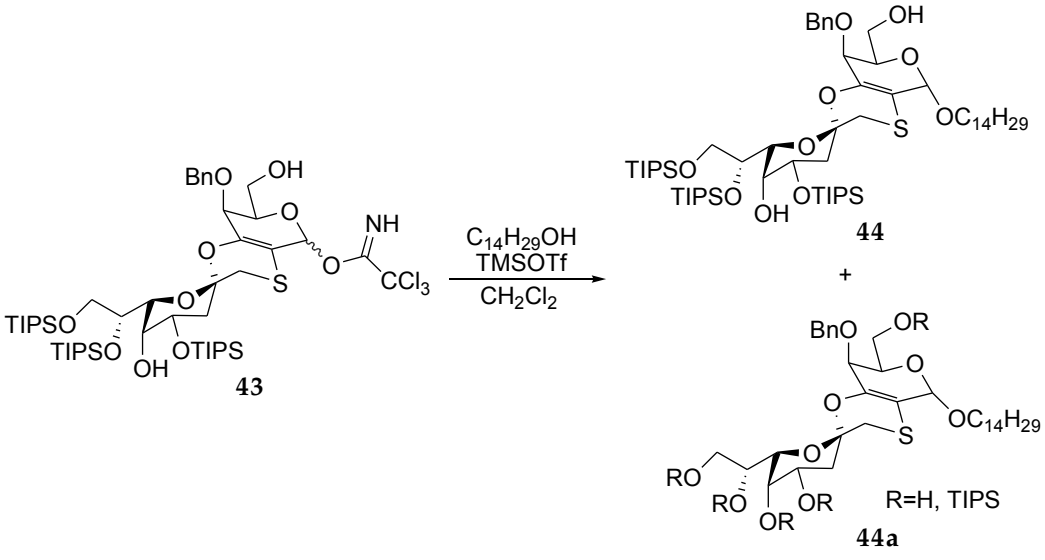
Compound **16** was deprotected with acetic acid/H_2O and reacted with triisopropylsilyl trifluoromethanesulfonate (TIPSOTf) in pyridine and CH_2Cl_2, affording **41** in a 83% yield after two steps. The hydroxyl group in axial position did not reacted, probably due to steric hindrance.

The lactone **41** was then reduced to the hemiacetal **42** with DIBAH in CH_2Cl_2 (60% yield) and treated with trichloroacetonitrile and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in CH_2Cl_2, affording **43** in a 87% yield (Scheme 19). During the reduction the pivaloyl group reacted with DIBAH giving the deprotected hydroxyl group in position six of the galactose residue.



Scheme 19

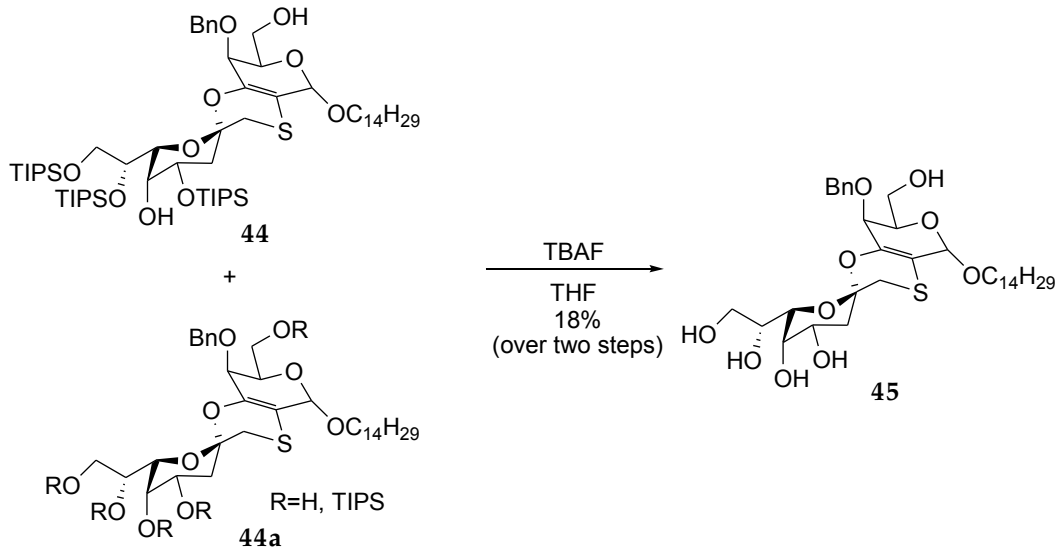
The trichloroacetimidate derivative **43** was submitted to Lewis acid-promoted Schmidt's glycosylation reaction in the presence of an excess of tetradecanol. The reaction gave a mixture of two different products: the expected alkyl-glycoside **44** and another alkyl-glycoside, **44a**, obtained after a migration of triisopropylsilyl groups (Scheme 20).



Scheme 20

The mixture of glycosides **44** and **44a**, was then reacted with TBAF in THF affording

the unprotected pseudo-α-glycoside **45** in a 18% yield after two steps (Scheme 21). The stereochemistry at C_a was determined through NMR analyses.

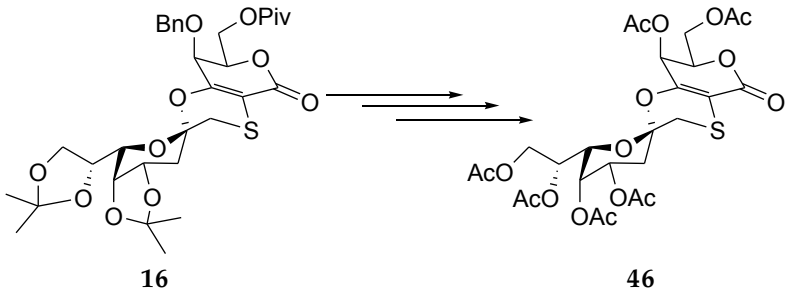


Scheme 21

Although the yield was calculated over two steps, it was quite low. This was mainly due to the migration of triisopropylsilyl groups occurred during the glycosylation, leading up to a loss of partially deprotected glycosides during the purification process. It was clear that silylethers were not compatible with the reaction conditions used. Consequently, we moved back to return to acetyls as protecting groups, since, as reported by Tietze and co-authors bis(2-methoxyethoxy)aluminum hydride (Red-Al®) can successfully be used to reduce an acetyl-protected lactone to hemiacetal in high yields. Red-Al® is indeed a mild reductive agent, compatible with esters and particularly with acetyls.

Attempts to obtain the reductive removal of the benzyl group in compound **45**, by using standard palladium catalyzed hydrogenation conditions, conducted to a complete transformation of the starting material into a complex mixture of unidentified side products.

Therefore, we decided to change the sequence of reactions in order to obtain the peracetylated lactone **46**, which was subsequently reduced with Red-Al® (Scheme 22).

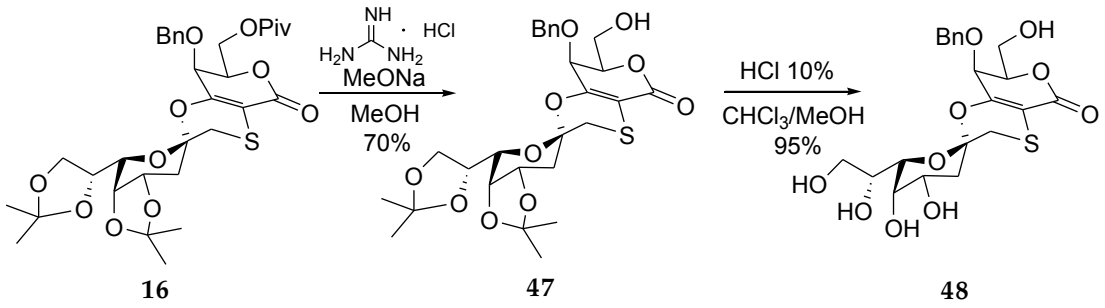


16 **46**

Scheme 22

4.3 Synthesis of mimetic 3

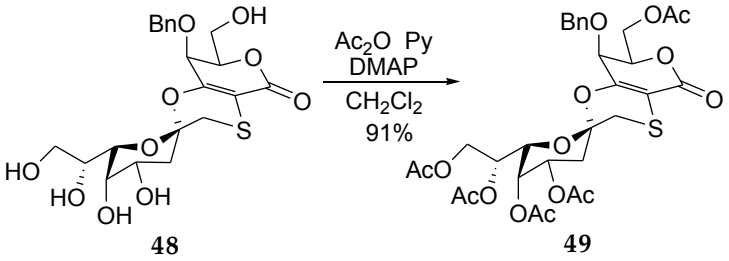
Cycloadduct **16** was treated with a freshly activated solution of guanidine, prepared by stirring guanidine hydrochloride and sodium methoxide (MeONa) in methanol for 30 minutes, to afford **47** in a 70% yield. Isopropylidens of **47** were then removed with a 10% solution of HCl in methanol, affording **48** in 95% yield (Scheme 23).



16 **47** **48**

Scheme 23

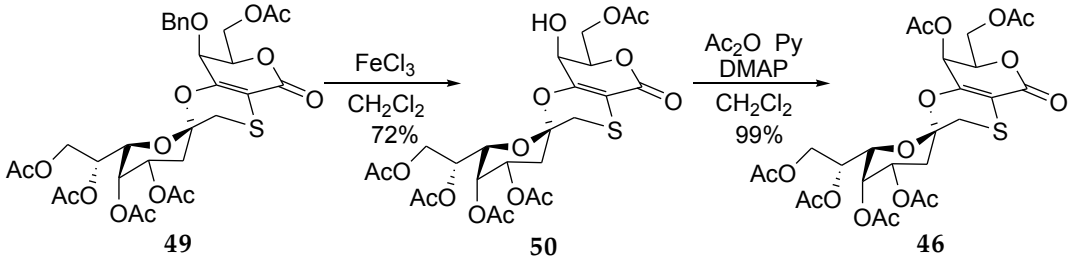
Compound **48** was then acetylated under standard condition to afford **49** in a 91% yield (Scheme 24).



48 **49**

Scheme 24

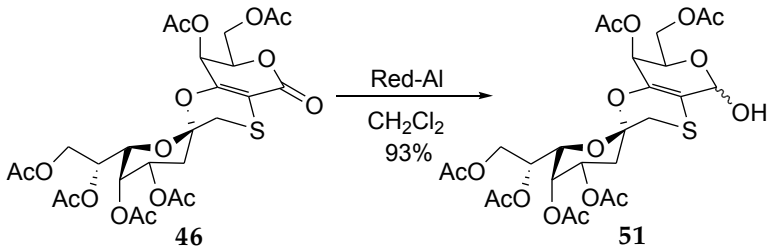
Since the difficulties occurredtrying to remove the benzyl group by using standard palladium catalyzed hydrogenation conditions, we decided to use iron(III) chloride in dry CH_2Cl_2 under argon atmosphere to afford compound **50** in good yield (72%). The peracetylated derivative **46** was then obtained after acetylation, under standard conditions, in a quantitative yield. (Scheme 25).



49 **50** **46**

Scheme 25

The peracetylated compound **46** was then treated with Red-Al® in CH_2Cl_2 at -78°C, affording the hemiacetalic derivative **51** in a 93% yield (Scheme 26).

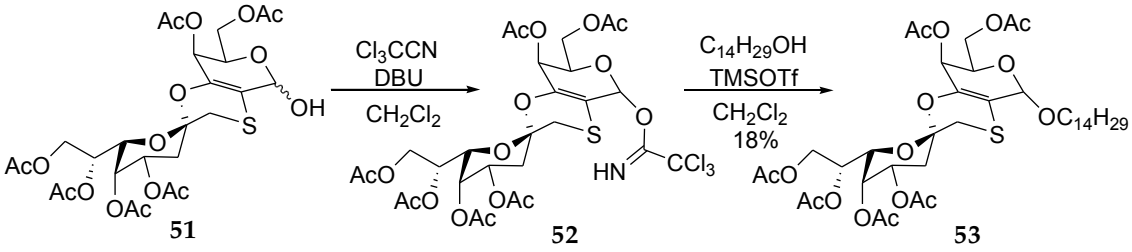


46 **51**

Scheme 26

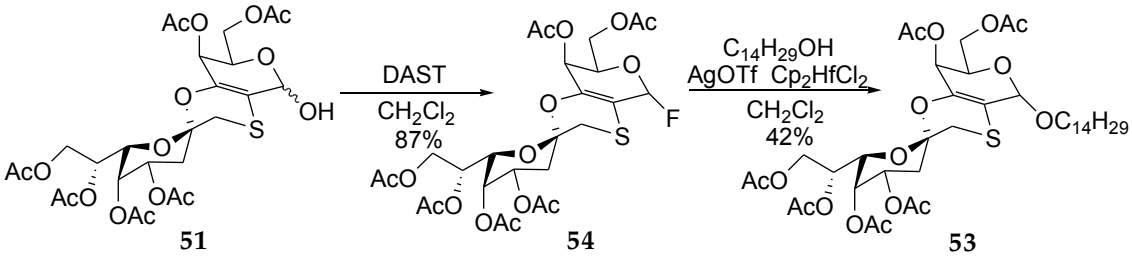
The hemiacetalic mixture **51** was then transformed into the corresponding

trichloroacetimidate derivative **52**, by using trichloroacetonitrile in dry CH_2Cl_2. Unfortunately the reaction, after 1 hour at 0°C, did not conduct to the complete transformation of starting material, giving instead a 7.8:1 mixture of **51** and **52**. This mixture was then submitted to Schmidt's glycosylation reaction in the presence of an excess of tetradecanol and trimethylsilyl trifluoromethanesulfonate (TMSOTf) as Lewis acid, affording the pseudo α-alkyl-glycoside **53** as a single product (Scheme 27).



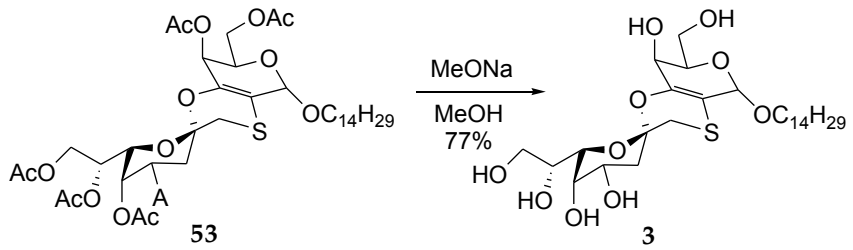
Scheme 27

Compound **53** was obtained in a 18% yield, after two steps, because of the scarce reactivity of the hemiacetal **51** towards the transformation into the corresponding alkyl glycoside "via" trichloroacetimidate derivative. Hemiacetal **51** was therefore activated as the fluorine glycoside **54**, by using (diethylamino)sulfur trifluoride (DAST),[38] and transformed into the corresponding α-alkyl-glycoside **53**, through the treatment with tetradecanol, silver trifluoromethanesulfonate (AgOTf), and hafnocene dichloride (Cp_2HfCl_2), under Ley's glycosylation conditions[39] (Scheme 28).



Scheme 28

Final deacetylation of **53** with MeONa in methanol, gave the thioether **3** in a 77% yield (Scheme 29).



Scheme 29

The stability of compound **3** was observed at different pH values and was monitored through NMR. The daily acquisition of ^{1}H NMR spectra revealed that compound **3** was stable for more than 1 month at room temperature in water (pH 7), and for about 15 days in a pH 5-6 buffer.

4.4 Loading of thioether derivatives 45 and 3 into liposomes

Vectors to deliver and concentrate molecules of biomedical interests at target sites have been extensively investigated and many delivery agents were proposed, including surfactant-based structures,[40] polymers[41] and, recently, nanospheres[42] and nanotubes.[43] However, only liposomes are currently adopted for clinical tests on humans.[44] Liposomes can be also used as adjuvants in vaccine formulations, since it was shown that antibodies coupled to liposomes were more immunogenic than their free-form counterparts.[45][46][47][48]

In order to verify the feasibility of the insertion of GM3 lactone mimetic **3** into liposomes, we firstly decided to use the benzyl derivative **45**. Indeed, the presence of the benzyl group did not alter the correct insertion of the molecule into the lipidic bilayer and, on the other hand, it allowed the quantification of the inserted molecule with respect to lipids, through the NMR analysis. Compound **45** was loaded into two different types of zwitterionic (i.e. globally uncharged) liposomes: the mixed 1:1 ratio of 1,2-dioleoyl-*sn*-glycerophospho-choline and 1,2-dioleyl-*sn*-glycerophospho-ethanolamine (DOPC/DOPE) and the 1,2-dioleoyl-*sn*-glycero-3-phosphocholine. Liposomes were prepared by the extrusion method.[49] Multilamellar vesicles were formed by hydration of a thin layer of mixed lipids and were then submitted to eight cycles of freeze and thaw (liquid nitrogen/water bath at

50°C) to improve homogeneity. Final downsize and conversion to unilamellar vesicles was performed by extrusion through 200 nm polycarbonate membranes.

Quantification of the inserted drug was carried out with high resolution ^{1}H NMR spectroscopy for the mixed DOPC/DOPE liposomes, by integrating the signals of the benzyl group (black arrow) in **45** as a function of the peaks of the terminal CH_3 in the phospholipid chains (white and grey arrows) (Figure 11).

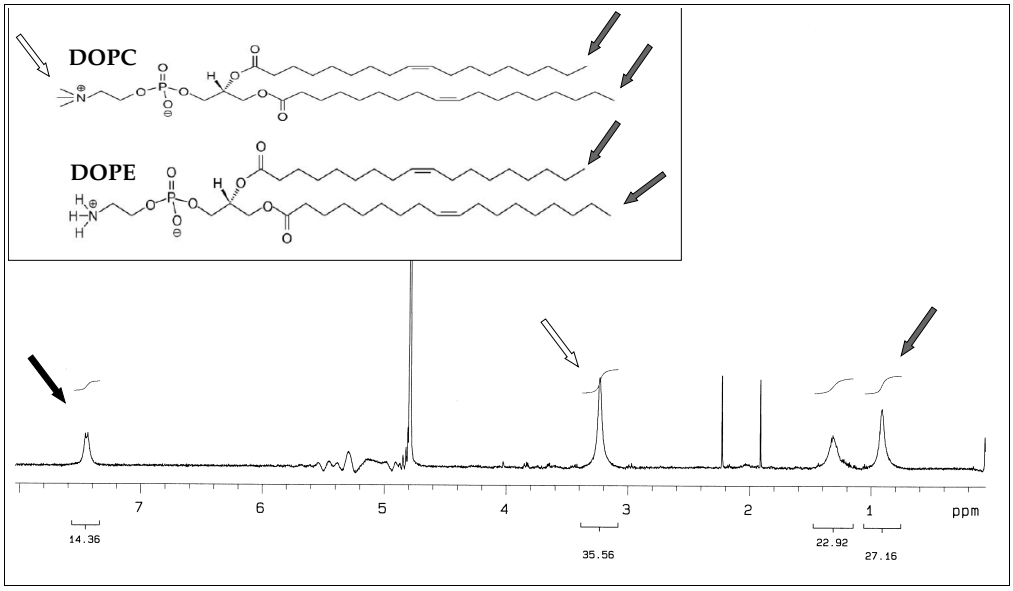


Figure 11

The presence of the benzyl group was essential for the quantification of mimetic **3** inserted, since the aromatic protons resonate downfield from the other protons of the lipids and were easily noticeable. Integrating the signals of the protons of the benzyl of **3** and the methyl groups of the lipids we obtained an about 3:1 ratio of mimetic **3** to respect to the DOPC/DOPE (instead of the theoretical 5:1 ratio) .

The size of plain and loaded liposomes was measured through dynamic light scattering (DLS); pure liposomes showed a mean diameter of 160 ± 20 nm and 190 ± 20 nm, for the DMPC and DOPC/DOPE formulations, respectively. The polydispersity index was fairly low, namely 0.15 for both types of liposomes. Insertion of thioether **45** caused this value to increase in the case of DMPC liposomes (Table 1), while for DOPC/DOPE liposomes variations were very small, approximately in the range of experimental error.

	Mean diameter (nm)	Polydispersion
Pure DMPC liposomes	160 ± 20 nm	0.15
DMPC liposomes + **45** (10:1)	340 ± 20 nm	0.40
DMPC liposomes + **45** (5:1)	380 ± 20 nm	0.45

Table 1

In order to characterize the bilayers structure of liposomes, small-angle X-ray scattering (SAXS) experiments were performed at the ID02 beamline of the European Synchrotron Radiation facility (ESFR) in Grenoble (France). The scattering profile of pure DMPC liposomes showed the characteristic of monolamellar vesicles. Upon thioeter **45** addition, pronounced Bragg peaks were observed in the SAXS profile of DMPC liposome, whose intensity increased progressively with host molecule content. This indicated that correlating among bilayers was established and oligolamellar onion-like structures were formed. SAXS diagrams of DMPC liposomes in the pure form and with different loading of thioether **45** are reported in Figure 12.

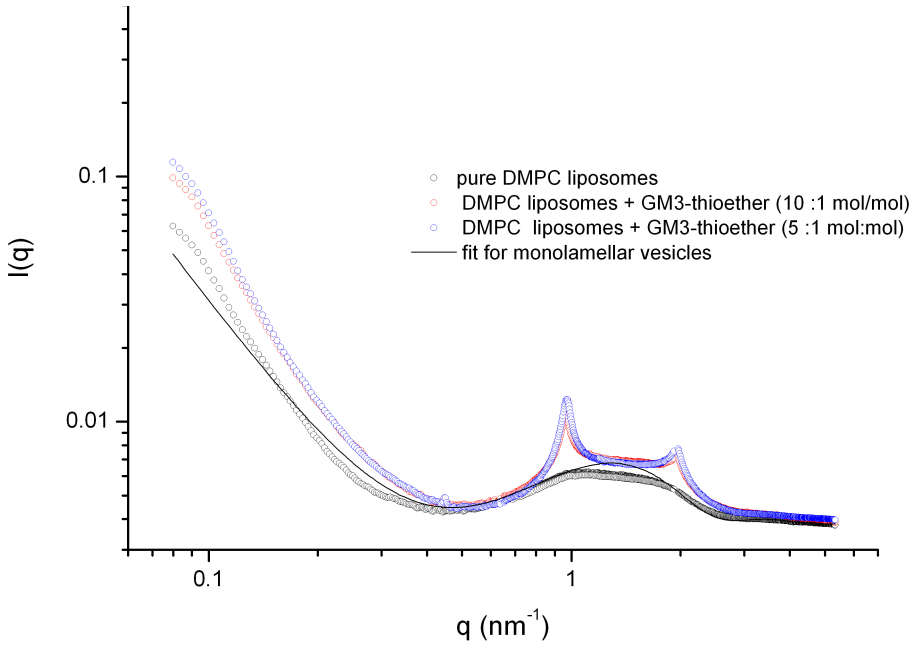


Figure 12

Contrarily to the DMPC case, the SAXS diagrams of pure DOPC/DOPE liposomes

showed that they had the tendency to give oligolamellar structures, as revealed by Bragg peaks. This feature almost disappeared for thioether **45**-loaded liposomes, as shown in Figure 13.

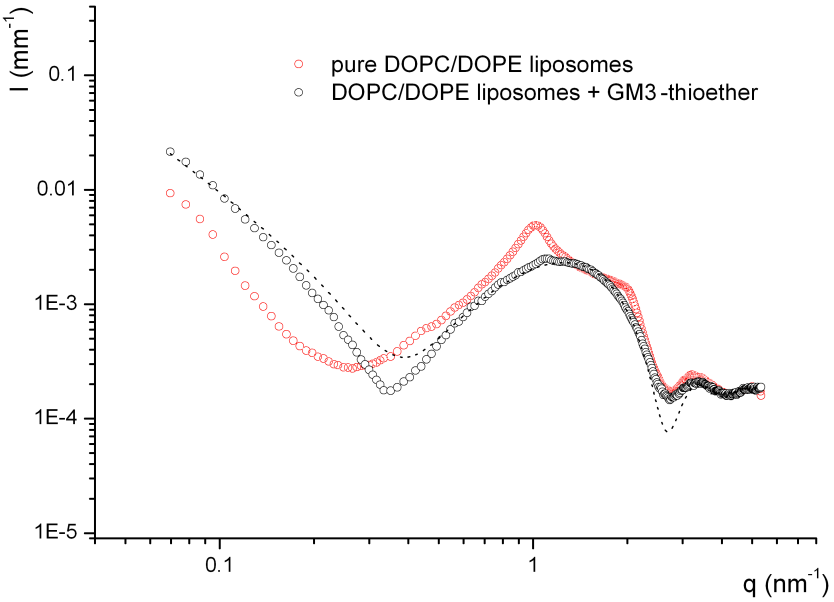


Figure 13

The mean diameter obtained for DOPC/DOPE for both plain and loaded carriers, confirmed that the overall structure of liposomes was not drastically changed by the insertion of thioether **45** and, therefore that DOPC/DOPE vectors were good candidates for drug-delivery purposes.

For compound **3** a deeper investigation was carried out by increasing the number of liposomes formulations. In particular, cationic liposomes were used in addition to the more standard zwitterionic liposomes presented for compound **45**, since it was reported in the literature that cationic lipids posses pronounced immunogenic character.[50][51] In particular, C_{14}TAB (tetradecyl trimethylammonium bromide) and DOTAP (1,2-dioleoyl-3-trimethylammonium-propane, chloride salt) components were added as 25% mol with respect to the zwitterionic phospholipids. The results obtained with synchrotron SAXS on liposomes loaded with compound **3** are currently under investigation by extensive fitting with a dedicated software package (GAP 1.3,[50][51] by Georg Pabst, Austrian Academy of Science, Graz) which allows to simulate the coexistence of mono- and multilamellar

structures in liposome suspensions. However, we observed that in the case zwitterionic liposomes, the obtained SAXS diagrams had the same features described above for the case of compound **45**, while the diagrams of cationic liposomes showed marked differences in the low q region upon loading with compound **3** (see figure 14 as an example). These differences could be ascribed to the polar head layer alone, which means insertion of **3** occurred without major changes in the hydrophobic portion of the liposome bilayer and, on the other hand, that the saccharidic portion of the GM3 mimetic **3** resided on the water layer were it could exert its recognition function.

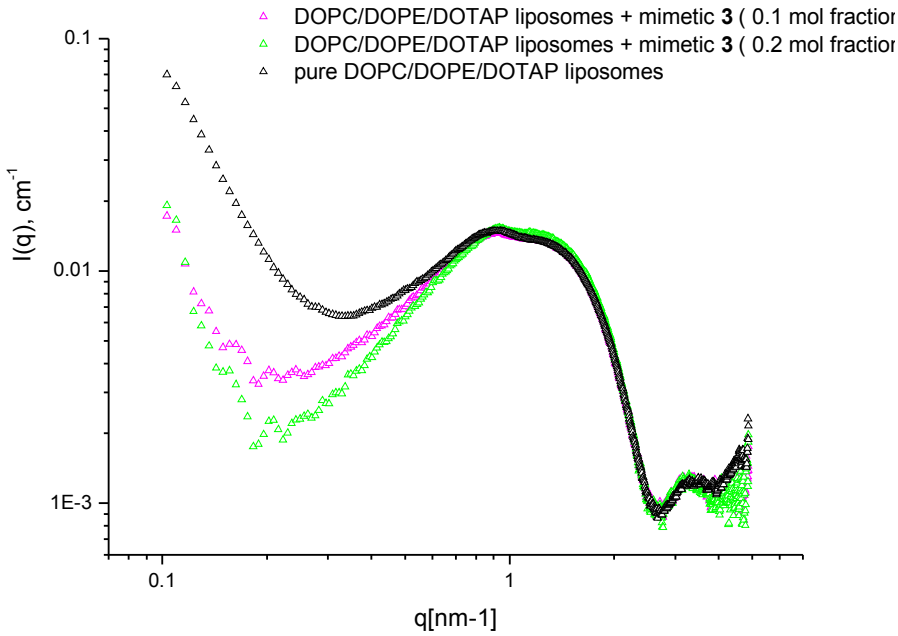


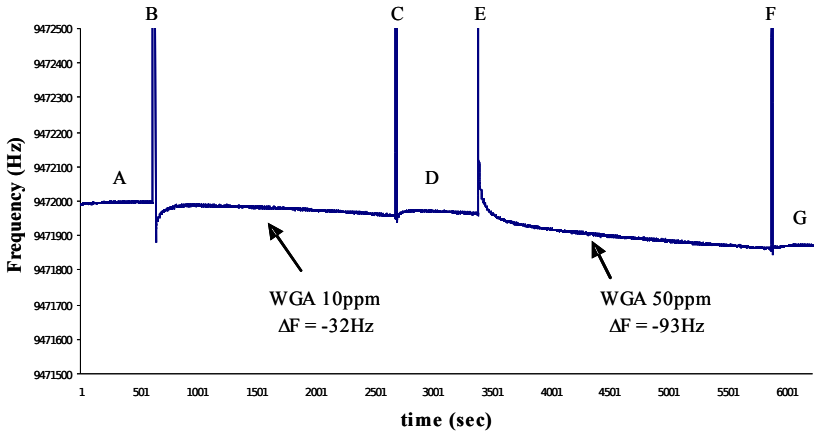
Figure 14

4.5 Binding evaluation of thioether 3 to sialic acid-specific lectins through Quartz Crystal Microbalance (QCM)

Lectins are a class multivalent non-immunoglobulin proteins able to agglutinate cells and/or precipitate complex carbohydrates. They play a central role in a wide range of biological processes, as cell-cell interactions, viral and bacterial pathogenesis, and inflammation. Due to their high sugar selectivity, lectins are routinely used to evaluate and

investigate carbohydrate-protein interactions.[52][53] Wheat Germ Agglutinin (WGA) is a lectin with a marked and selective binding affinity for saccharides as N-acetylglucosamine (GlcNAc) and sialic acid. It was reported by Sato and co-authors that WGA showed a high binding affinity to both GM3 ganglioside and GM3 lactone.[54] In order to confirm the similarity of mimetic **3** to a sialic acid-containing saccharide, we developed a quartz crystal microbalance (QCM) method to assess the binding affinity of mimetic **3** to WGA, by comparing these data with those obtained for GM3 ganglioside. The gold crystal surface, previously treated with 1-dodecane-thiol, was modified through the construction of a monolayer made by the alkyl mimetic **3** or the glycosphingolipid GM3. Two different concentrations of WGA (10 ppm and 50 ppm) were added to the treated sensor surface and the interaction between the sugar and the protein was monitored for 30 minutes.

Figure 15 shows the sensorgram (frequency versus time) recorder during the interaction GM3 ganglioside-WGA. The frequency shifts observed, -32 Hz for WGA 10 ppm and -93 Hz for WGA 50 ppm, were an evidence of the binding between GM3 ganglioside and the protein.

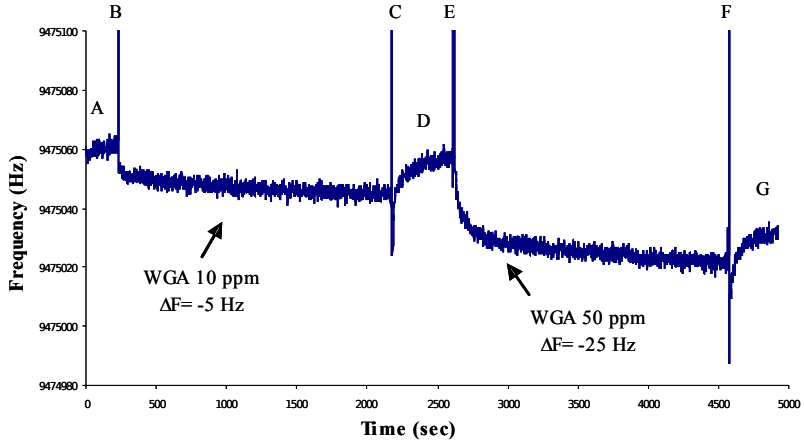


A: baseline in PBS buffer. B: injection of WGA 10 ppm. C: washing with PBS buffer D: baseline in PBS buffer. E: injection of WGA 50 ppm. F: washing with PBS buffer. G: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A and F_G-F_D).

Figure 15

In the same way, WGA (10 ppm and 50 ppm) was added to the gold sensor surface previously modified with a thioether **3** monolayer. The frequency was monitored and recorded as a function of time during the experiment. The frequency shift observed indicated

the binding between thioether **3** and the protein (Figure 16).



A: baseline in PBS buffer. B: injection of WGA 10 ppm. C: washing with PBS buffer D: baseline in PBS buffer. E: injection of WGA 50 ppm. F: washing with PBS buffer. G: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A and F_G-F_D).

Figure 16

Even if the binding observed for mimetic **3** was lower compared to GM3 ganglioside (-5 Hz and -25 Hz for WGA 10 ppm and 50 ppm, respectively), the binding of **3** to WGA was preserved despite the lack of the NHAc residue and the modification of the trihydroxylated side chain. This proved that the thioether **3** was an effective mimetic of a sialic acid-containing saccharide. The results were summarized in Figure 17.

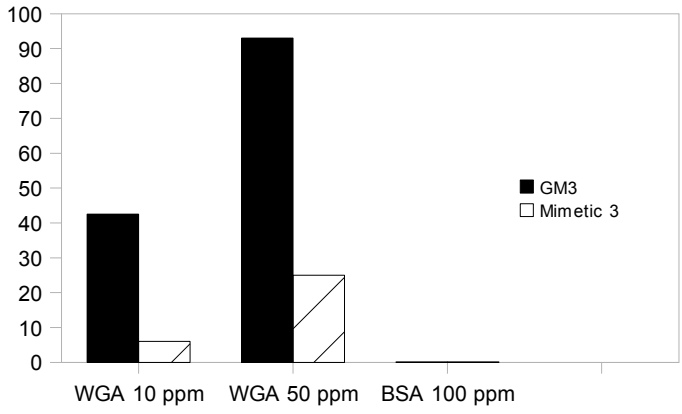
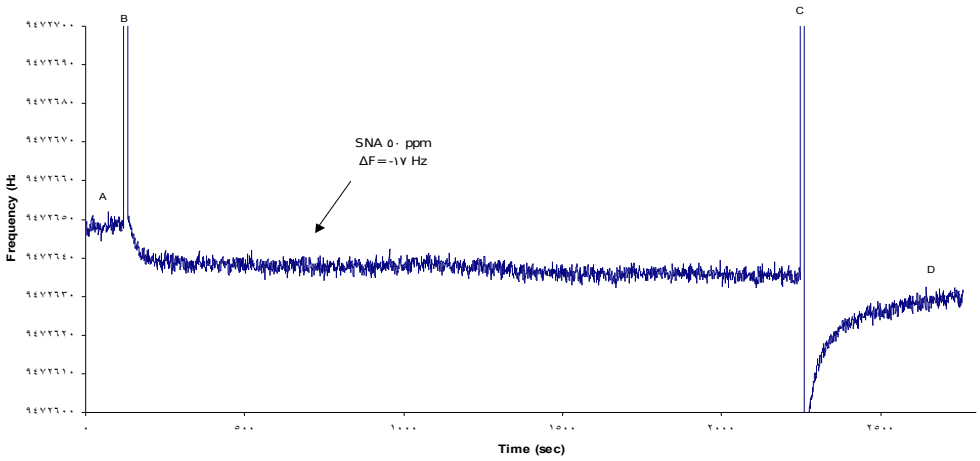


Figure 17

Since the good results obtained with WGA lectin, we decided to perform similar experiments using other two different lectins, namely Sambucus Nigra Agglutinin (SNA) and Maackia Amurensis Agglutinin (MAA). SNA and MAA have been extensively used to detect 2– 6- and 2–3-linked sialic acids in the disaccharide sialic acid-galactose, respectively. [55][56]

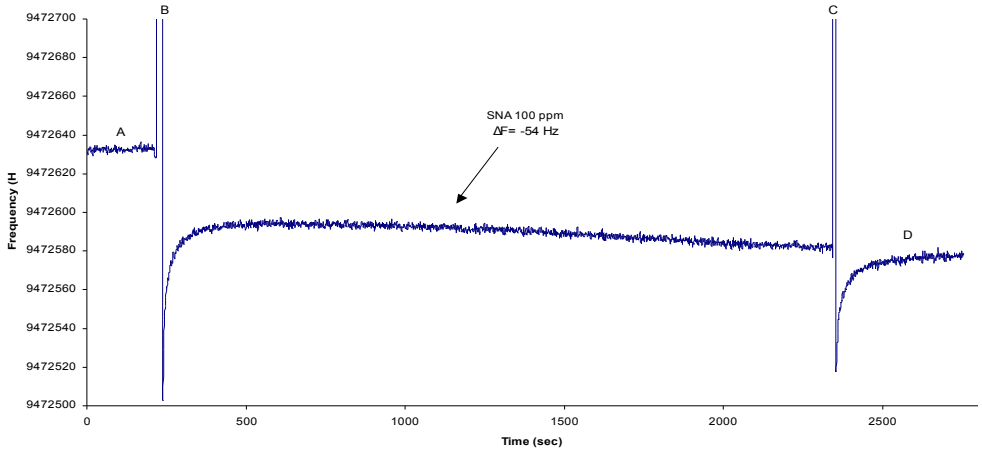
Figure 18 shows the sensorgram (frequency versus time) recorded during the interaction GM3 ganglioside-SNA 50 ppm. The frequency shifts observed, -17 Hz, was an evidence of the binding between GM3 ganglioside and the protein.



Sensorgram recorded during interaction GM3 ganglioside-SNA 50 ppm.
A: baseline in PBS buffer. B: injection of SNA 50 ppm. C: washing with PBS buffer. D: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A).

Figure 18

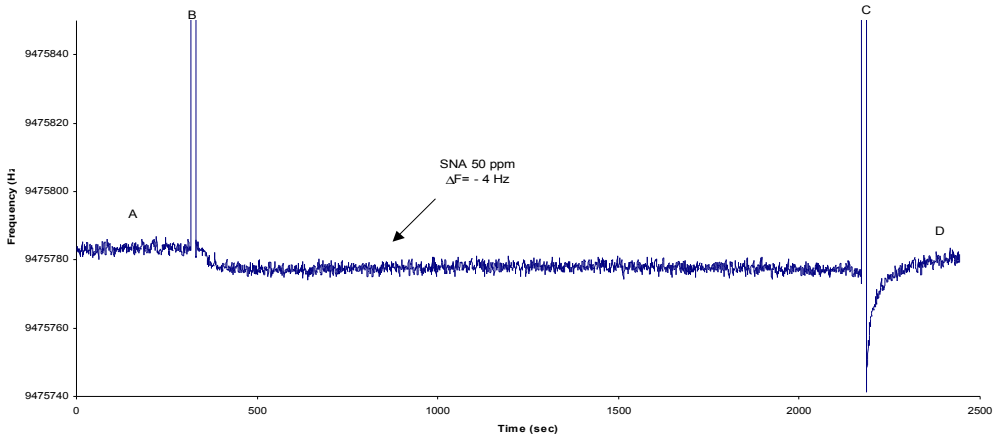
The same experiment was conducted using SNA 100 ppm and the frequency shift observed was greater (- 54 Hz), as shown in the sensorgram in Figure 19.



Sensorgram recorded during interaction GM3 ganglioside-SNA 100 ppm.A: baseline in PBS buffer. B: injection of SNA 100ppm. C: washing with PBS buffer. D: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A).

Figure 19

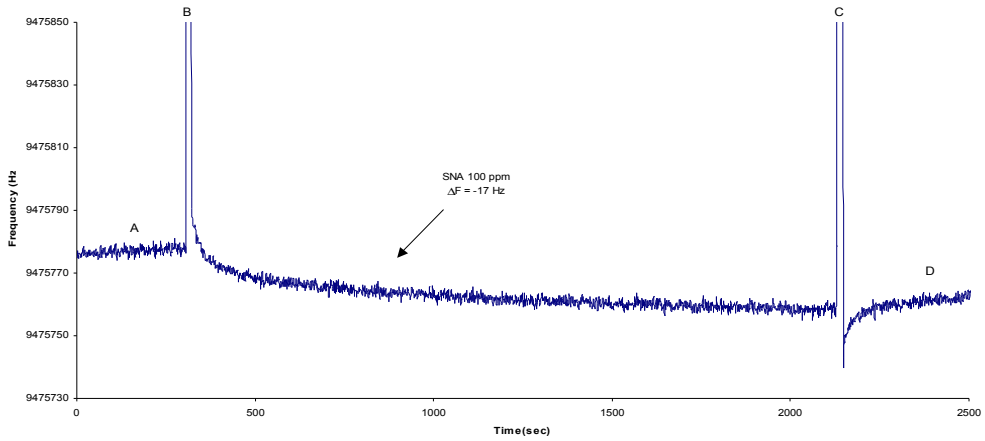
Analogously, SNA (50 ppm and 100 ppm) was added to the gold sensor surface previously modified with a thioether **3** monolayer. The frequency was monitored and recorded as a function of time during the experiment. The frequency shift observed with SNA 50 ppm was – 4 Hz and is shown in Figure 20.



Sensorgram recorded during the interaction between thioeher **3** and SNA (50 ppm). A: baseline in PBS buffer. B: injection of SNA 50 ppm. C: washing with PBS buffer. D: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A).

Figure 20

In the case of SNA 100 ppm the the frequency shift observed was – 17 Hz. Figure 21 shows the sensorgram recorded.



Sensorgram recorded during interaction between **3** and SNA 100 ppm. A: baseline in PBS buffer. B: injection of SNA 100ppm. C: washing with PBS buffer. D: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A).

Figure 21

The histogram reported in Figure 22 summarize the data obtained, by comparing GM3 ganglioside and mimetic **3**.

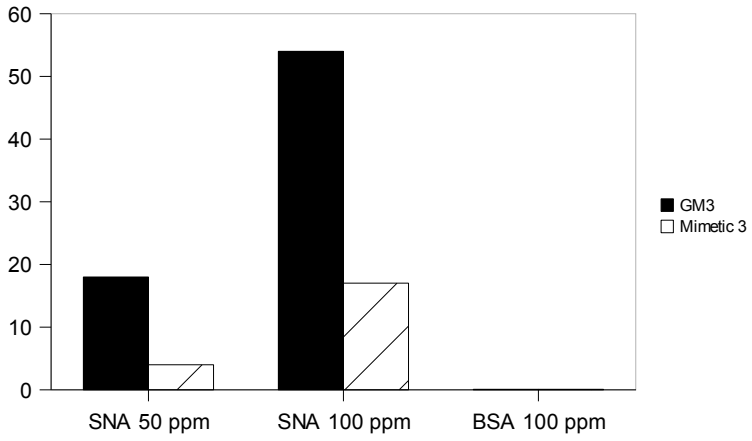
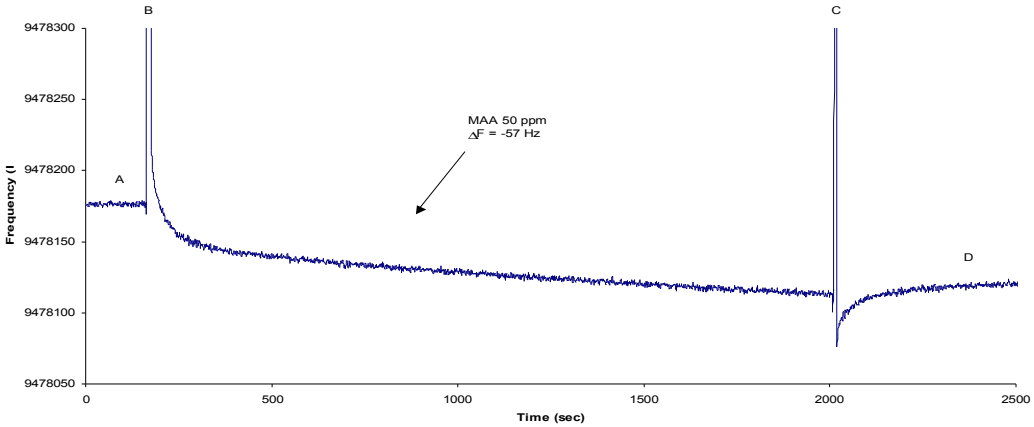


Figure 22

Analogously, these experiments were carried out with MAA (50 ppm and 100 ppm) with gold crystals modified with GM3 ganglioside and mimetic **3**. Figure 23 shows the

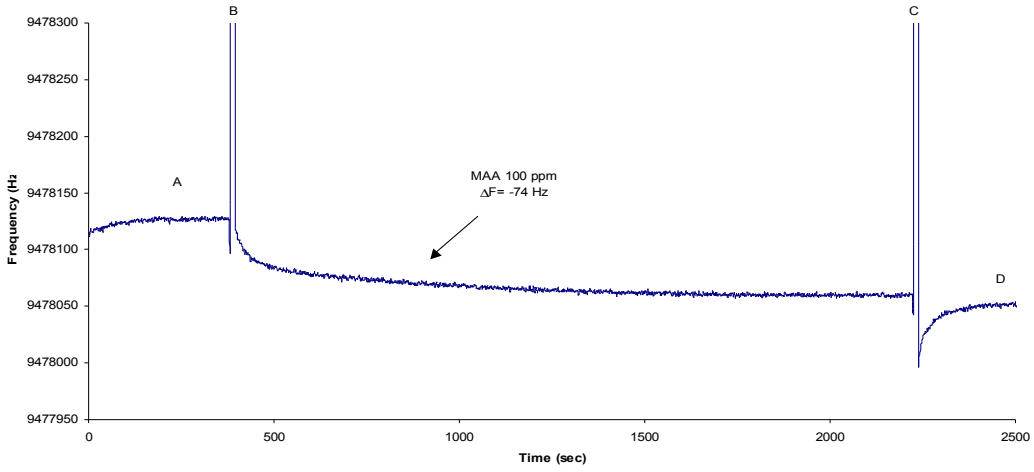
sensorgram (frequency versus time) recorded during the interaction GM3 ganglioside-MAA 50 ppm. The frequency shifts observed, -57 Hz, was an evidence of the binding between GM3 ganglioside and the protein.



Sensorgram recorded during interaction GM3 ganglioside-MAA 50 ppm.
A: baseline in PBS buffer. B: injection of MAA 50ppm. C: washing with PBS buffer. D: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A).

Figure 23

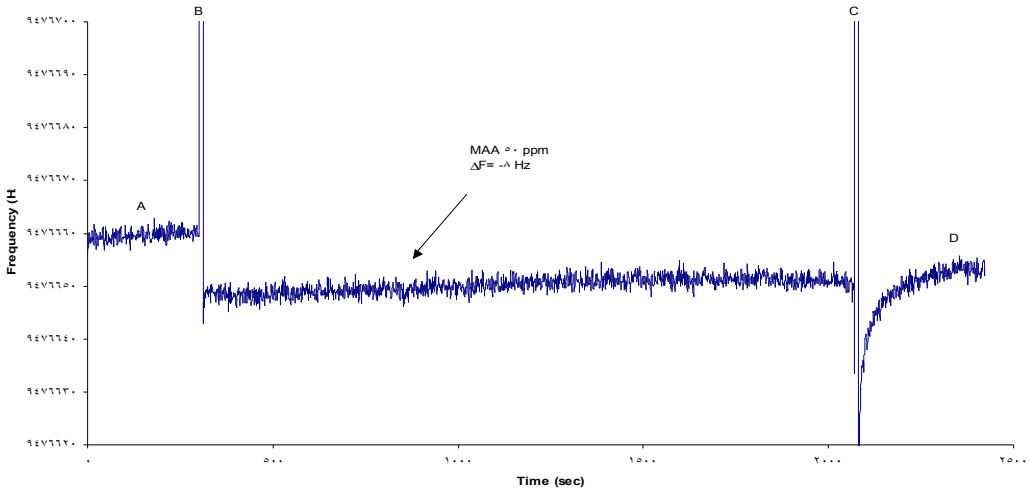
When MAA 100 ppm was added, the frequency shift observer was greater (-74 Hz), as shown in Figure 24.



Sensorgram recorded during interaction GM3 ganglioside-MAA 100 ppm.A: baseline in PBS buffer. B: injection of MAA 100ppm. C: washing with PBS buffer. D: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A).

Figure 24

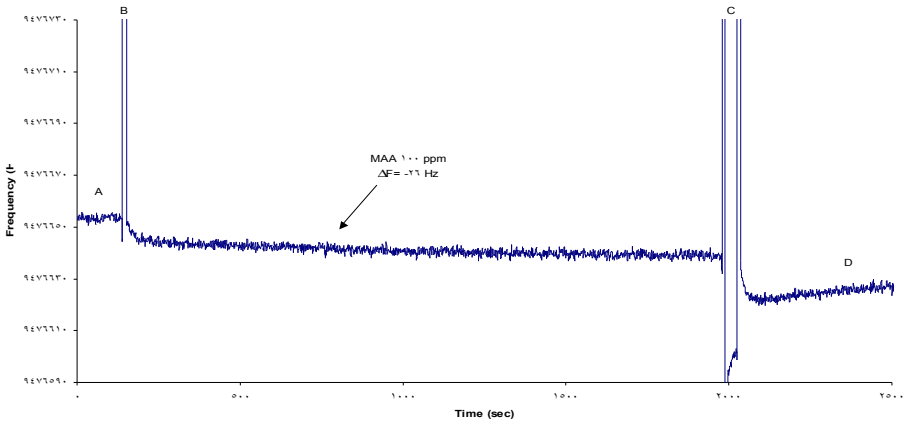
In the same way MAA (50 ppm and 100 ppm) was added to the crystal surface previously modified with a mimetic **3** monolayer. The frequency shift observed for MAA 50 ppm (-8 Hz) is reported in the sensorgram in Figure 25.



Sensorgram recorded during the interaction between thioeher **3** and MAA (50 ppm). A: baseline in PBS buffer. B: injection of MAA 50 ppm. C: washing with PBS buffer. D: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A).

Figure 25

The frequency shift was greater in the case of MAA 100 ppm (- 26 Hz), as shown in the sensorgram in Figure 26.



Sensorgram recorded during the interaction between thioeher **3** and MAA (100 ppm). A: baseline in PBS buffer. B: injection of MAA 100 ppm. C: washing with PBS buffer. D: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A).

Figure 26

The results obtained for both GM3 ganglioside and mimetic **3** are summarized in the histogram in Figure 27.

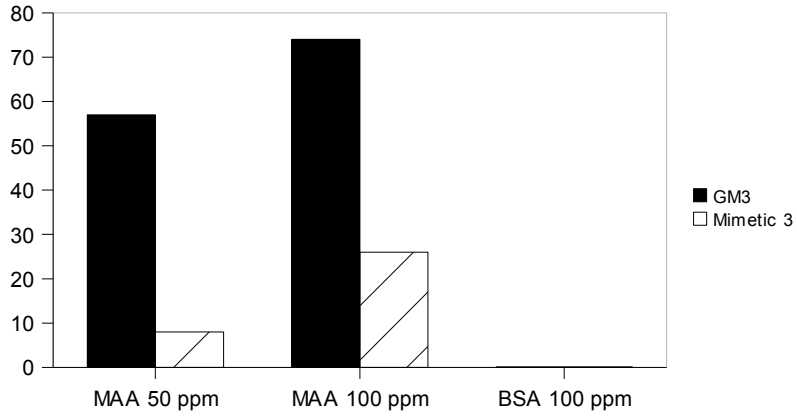


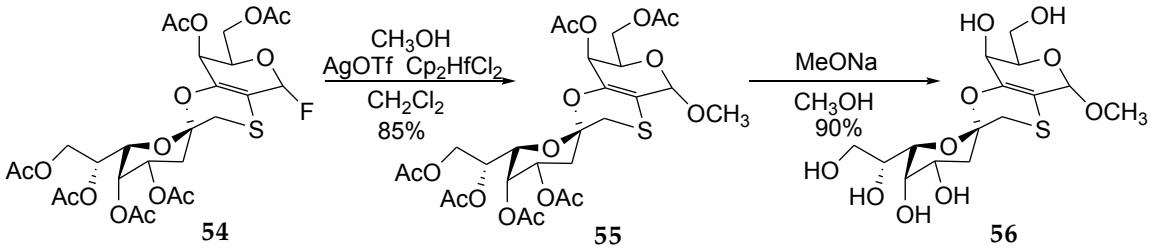
Figure 27

The binding observed between mimetic **3** and MAA was greater than those observed for SNA. This was consistent with the fact that MAA recognises terminal sialic acid (*2-3*) linked to galactose, while SNA recognises preferentially the (*2-6)* linkage. In the mimetic proposed **3**, indeed, the linkage between the galactosidic portion and the sialic acid-mimetic residue is (2-3) type. The experiments performed with SNA and MAA confirmed that thioether **3** was a mimetic of a sialic-containing saccharide and, in particular, with a (2-3) linkage, as in GM3 ganglioside and in GM3 lactone.

In order to quantify the binding between thioether **3** and WGA, SNA, and MAA lectins, we planned to synthesize a water soluble derivative of **3** to be used for microcalorimetric analyses. Isothermal titration calorimetry (ITC) indeed enables the simultaneous determination of all binding parameters, such as stoichiometry, affinity, enthalpy, and entropy, in an environment that is completely label-free, in-solution, and it requires no immobilisation. It has gained wide acceptance in drug discovery and development to fully characterize biomolecular interactions.[57]

We synthesized an O-methyl glycoside in which the saccharidic portion was the same of mimetic **3**. This compound was achieved starting from the fluorine glycoside **54** through the treatment with methanol under Ley's glycosylation conditions, as previously described for compound **53**. The acetylated O-methyl glycoside **55** was obtained in good yield (85%)

and it was subsequently deacetylated under standard conditions, with MeONa in methanol, affording **56** in a 90% yield (Scheme 30)



Scheme 30

Compound **56** was stable and soluble in water. Calorimetric analyses are currently in progress.

4.6 Immunogenicity evaluation of thioether 3

In order to verify if thioether **3** was a real mimetic of GM3 lactone, we first of all managed an indirect ELISA assay. The commercial murine monoclonal antiboby M2590, obtained through immunization of mice with singenic B16 melanoma cells, was indeed able to recognize both GM3 ganglioside and GM3 lactone. We compared the binding of mAb M2590 to GM3 ganglioside to those between mAb M2590 and thioether **3**.

The solution of the monoclonal antibody M2590 was added to the antigen-coated (mimetic **3** and GM3 ganglioside) microtitre well and was allowed to react. After the removal of unbound antibody, the antibody bound to the antigen was detected by adding an enzyme-conjugated secondary antibody, specific to primary antibody. The free secondary antibody was then removed by washing with suitable buffer. The substrate specific to the enzyme was added and allowed to react to develop a coloured product detected by a spectrophotometric plate reader able to measure the absorbance (Figure 28).

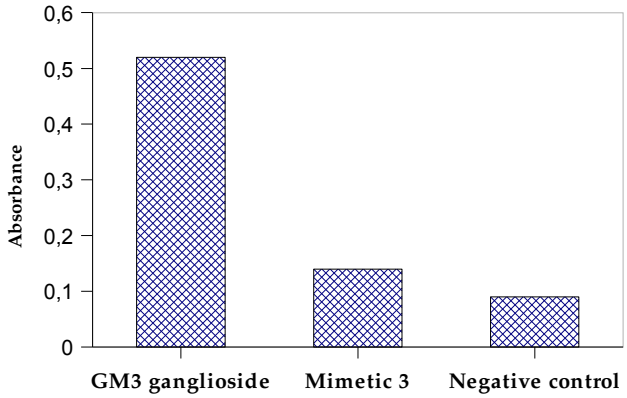


Figure 28

Despite the absorbance value observed for mimetic **3** was sensibly lower than those observed for GM3 ganglioside, it was a good result. Although the recognition antigen–antibody is extremely selective, mimetic **3** is recognized by mAb M2590.

Due to these encouraging results, and since the interaction between **3** and WGA, SNA, and MAA lectins were clearly assessed by QCM, thus proving that **3** was a veritable mimetic of a sialyl-containing saccharide, we envisaged to use thioether **3** as immunogen to raise polyclonal and monoclonal antibodies for a specific recognition of GM3 lactone and GM3 ganglioside. For polyclonal antibodies production three C57 mice were used: the first one was immunized with GM3 ganglioside (0,6 μg/μL), the second one with thioether **3** (0,3 μg/μL), and the last one was not immunized. Salmonella (500 μL) was used as adjuvant. Mice were immunized with 100 μL of these solutions during four weeks. The last immunizations were carried out without salmonella and, after one week, mice were sacrificed. From the blood sample of mice, we obtained the serum which was used for an immunocytochemistry assay on HCT116 colon carcinoma cells. It was known, indeed, that HCT116 colon carcinoma cells are characterized by an over-expression of GM3 ganglioside, similarly to melanoma cells.[58] To confirm this data, we performed an immunocytochemistry assay on these cells using monoclonal antibody M2590. Figure 29 shows the electron microscopy images of HCT116 colon carcinoma cells marked with mAb M2590. The signal was localized in the cellular cytoplasm with exception of nucleus, confirming the presence of specific binding.

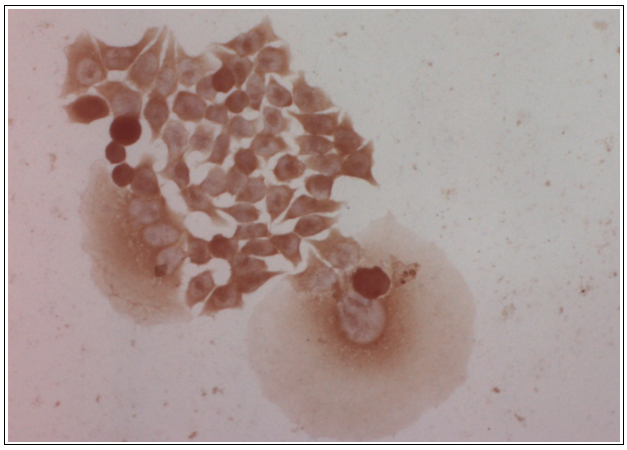


Figure 29

Cells treated with polyclonal anti-GM3-serum and those treated with anti-**3**-serum presented a similar marking to mAb M2590. Indeed, also in these cases nuclei resulted "cleaned", confirming the presence of a specific binding, as shown in the electron microscopy images reported in Figure 30.

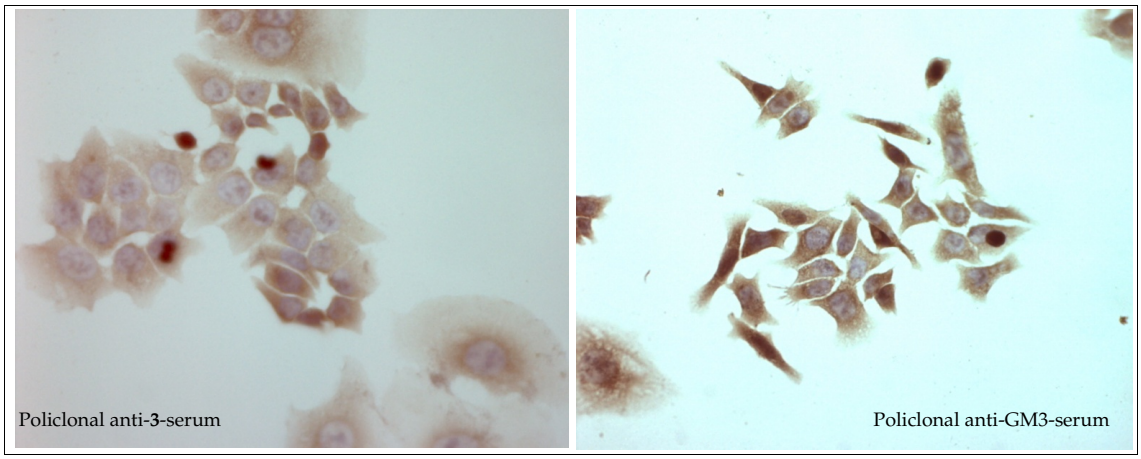


Figure 30

Analogously the immunocytochemistry assay was performed with the serum of the wild tipe mouse, which was used as negative control. The electron microscopy image, in this case, showed an evident and spread marking both in the cytoplasm and in the nuclei (Figure 31), revealing a considerable non-specific binding.

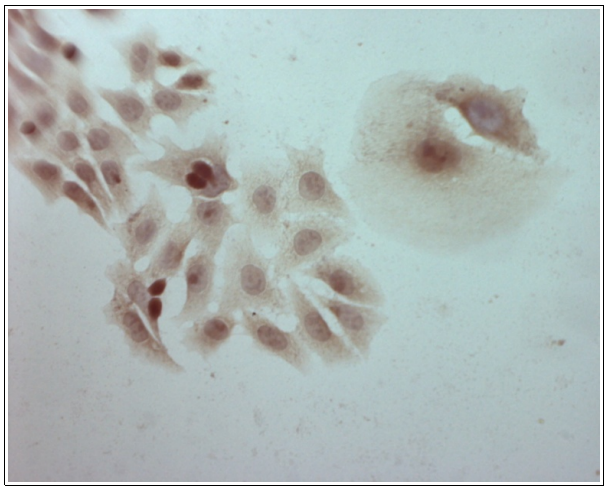
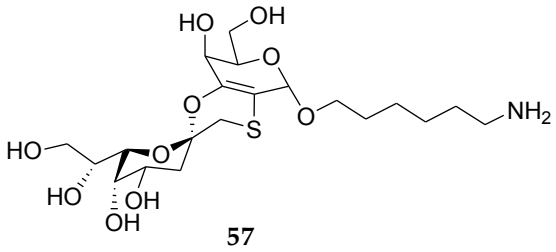


Figure 31

These results clearly revealed that thioether **3** elicited an *in vivo* immunoresponse, and that the antibodies (anti-**3** PAbs) was able to recognize GM3 ganglioside over-expressed by neoplastic cell lines. Since the good results obtained with anti-**3** polyclonal antibodies, and taking into account the known low immunogenicity of saccharidic antigens, we prepared a modified derivative of **3** in which the alkyl chain was replaced by an amino-alkyl chain, in order to link the thioether hapten to a suitable spacer for coupling to carrier proteins (such as KLH or BSA), immunostimulants lipopeptides (such as Pam3CysSer), gold nanoparticles, and dendrimeric multivalent systems (such as PAMAM).

5. Toward the development of a suitable melanoma vaccine

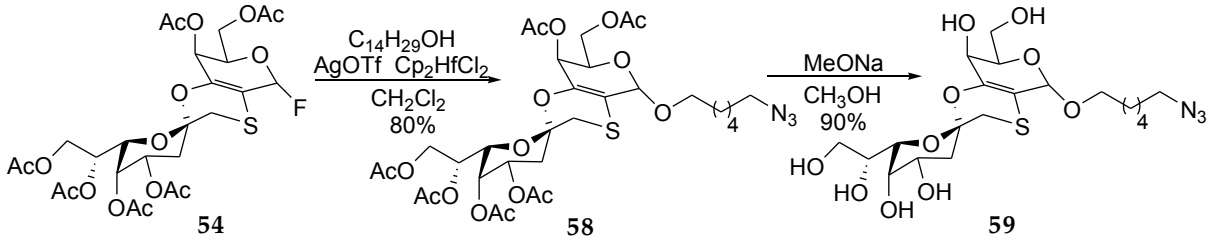
Since the known scarce immunogenicity of carbohydrate-based vaccines, thioether **3** was modified in order to be linked to specific carriers for the immunization of mice and monoclonal antibodies production. For this purpose, the ω-amino derivative **11** (Figure 32) was prepared.



57

Figure 32

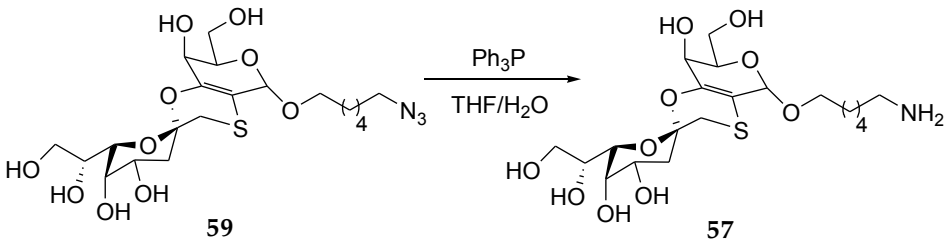
The presence of the amino group, at the end of the alkyl chain allowed the manipulation for multiple purposes. Compound **32** was obtained starting from the fluoro derivative **54**, by the treatment with 6-azido-hexanol under Ley's glycosylation conditions, as previously reported, in a 80% yield. The afforded azido-glycoside **58** was deacetylated under standard condition to give **59** in a 90% yield (Scheme 31).



54 **58** **59**

Scheme 31

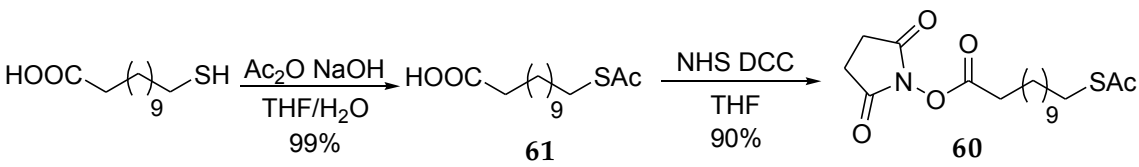
Azide **59** was reduced to amine by triphenylphosphine in THF/H_2O under Staudinger conditions, affording **57**, which was used immediately, without any purification (Scheme 32).



Scheme 32

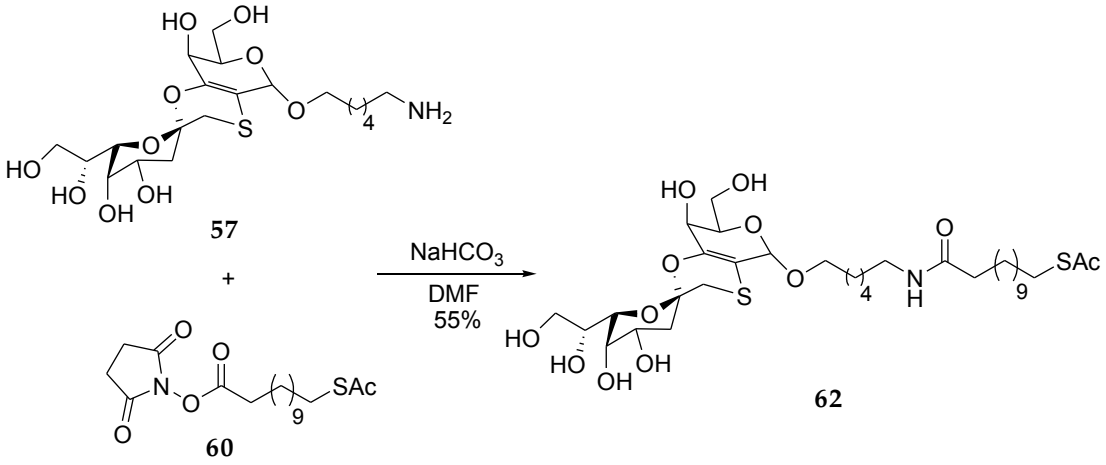
We wanted to use compound **57** for the conjugation to gold nanoparticles. Nano- and microparticles have long been used for the delivery of drugs and are currently being evaluated as vaccine delivery systems.[59] Nanoparticles can elicit potent immune responses, either by direct immunostimulation of antigen presenting cells (APC) or/and by delivering antigen to specific cellular compartments and promoting antigen uptake by appropriate stimulatory cell types. In traditional vaccines, the antigen is usually mixed with the adjuvant to provide a potent immunogenic response. In the case of nanoparticles, the antigen is covalently coupled to the bead and it induces by itself a sufficiently high cellular and humoral responses.

For the linkage to the gold surface of nanoparticles amine **57** was reacted with the activated 12-mercaptododecanoic acid **60** shown in Scheme 33.



Scheme 33

The commercially available 12-mercaptododecanoic acid was reacted with acetic anhydride and NaOH in THF/H_2O to afford the acetylated derivative **61** in a quantitative yield. The acid group of **61** was then activated as succinimide-derivative by reacting with N-hydroxysuccinimide (NHS) and dicyclohexylcarbodiimide (DCC), affording **60** in a 90% yield. By reacting activated acid **60** with amine **57** in DMF and in the presence of $NaHCO_3$ compound **62** was afforded in a 55% yield (Scheme 34).



Scheme 34

Componud **34** will be soon linked to gold nanoparticles and the immunogenicity properties of the conjugate will be evaluate and compared to the results obtained with thioether **3** and salmonella as adjuvant. Nanoparticles can indeed exploit multivalency for an effective and enhanced antigen presentation.

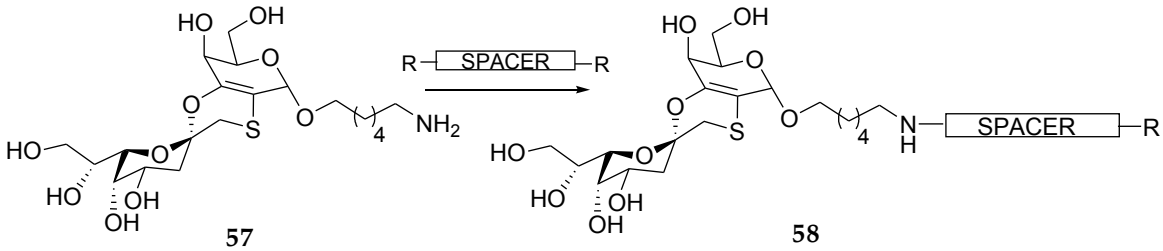
Multivalency is a phenomenon deeply studied during the last years.[60] Indeed it was ascertained that many biological processes use multivalent interactions, such as virus-cell, bacteria-cell, and cell-cell adhesion, and cell-antibodies binding. This is still valid for carbohydrate–protein interactions, which, in physiological settings, often benefit from multivalency, namely multiple copies of the carbohydrate epitope engage with multiple copies of a carbohydrate–binding protein. Multivalency indeed consists of the interaction between two entities which present multiple and complementary binding sites on their surfaces. Polyvalent interactions have many advantages, such as the obtainment of strong binding if compared to small contact-surfaces, the modulation of the biological response as a response of an on-off signal, and the increasing of the strength and the specificity of the binding. Apart from nanoparticles, other methods to exploit multivalency in a vaccine formulation are the linkage of the epitope to a carrier protein, or to a dendrimer.

The most used carrier proteins in vaccine development are Bovine Serum Albumine (BSA) and Keyhole Limpet Hemocyanin (KLH). Proteins can exploit both multivalency - the apten is generally linked to free amines (as in lysine) or mercaptans (as in cystein) - and their own immunogenicity. In particular KLH has many immunostimulatory properties, including the ability to enhance the host's immune response by interacting with T cells, monocytes,

macrophages, and polymorphonuclear lymphocytes.[61]

Dendrimers are branched, synthetic polymers with precise architectures that possess potentialities in a number of biomedical applications.[62] It was reported that the use of dendrimers as adjuvants made it possible to adopt amount of influenza antigen reduced from those necessary to elicit a comparable antigenic response if the antigen was given without the dendrimer.[63]

Amine **57** is a versatile molecule that can be used for the conjugation to both dendrimeric molecules and immonostimulant proteins for vaccine development. The free amine group indeed can be used for the linkage to an appropriate spacer, and then linked to the macromolecule in order to exploit the multivalency and enhance hapten immunogenicity. The schematic synthetic approach is reported in Scheme 35.



Scheme 35

Compound **58** can be useful also for the synthesis of a different glycoconjugate, in which the saccharidic portion is linked to a synthetic lipopeptide. Lipopeptides increase the antigenic response not through multigenicity, but thanks to their similarity to the NH_2-terminus of bacteria lipoproteins, that makes them strongly immunogenic.[64] Examples are N-palmitoyl-S-[2,3-bis(palmitoyloxy)-(2RS)-propyl]-(R)-cysteine (Pam3Cys), N-palmitoyl-S-[2,3-bis(palmitoyloxy)-(2RS)-propyl]-(R)-cysteinyl-(S)-serine ($Pam_3CysSer$) and N-palmitoyl-S-[2,3-bis(palmitoyloxy)-(2RS)-propyl]-(R)-cysteinyl-(S)-seryl-(S)-lysyl-(S)-lysyl-(S)-lysyl-(S)-lysine (Pam3CysSerLys4).

For the development of a suitable melanoma vaccine different carriers and/or adjuvants were considered and synthesised. These glyco-derivatives are currently under biological analysis in order to evaluate their immunologic properties and to choose the best one for the preclinical development of the vaccine.*

* Patent for these moleculus is currently under progress

Conclusions

The main target of this work was the synthesis of a new simple and stable mimetic of the melanoma-associated antigen GM3 ganglioside lactone. We reported the conformational analysis and the synthesis of the thioether-bridged mimetic of GM3 lactone **3**, which is a conformational analogue of the parent lactone with a mannose-derived residue replacing the sialic acid portion (Figure 34).[37]

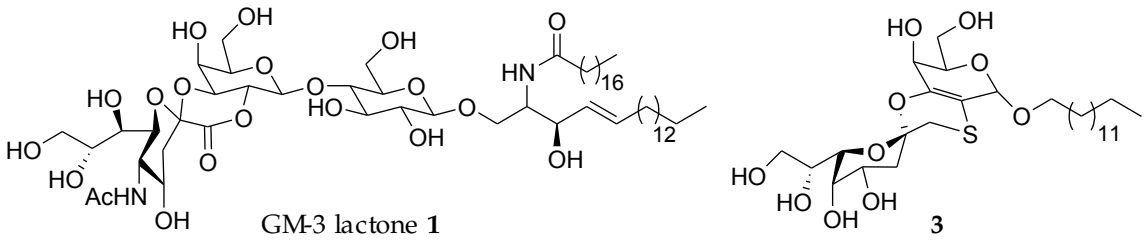


Figure 34

Indeed we synthesized a mimetic of GM3 lactone stable to hydrolysis under physiological conditions and structurally simpler than the native compound. This would allow some advantages for the future development of a therapeutic melanoma vaccine. Compound **3** was, first of all, tested as mimetic of a sialic-acid containing saccharide, by using a quartz crystal microbalance (QCM) method. This analytical method was useful to assess the binding affinity between thioether **3** and three different lectins: WGA, for which a binding evidence to GM3 ganglioside and GM3 lactone was reported,[54] SNA, and MAA, which recognize a (2,6) and (2,3) sialic acid galactose bound, respectively.

The following step was the evaluation of immunogenicity properties of **3**, through the immunization of mice in order to produce polyclonal antibodies which specifically recognized GM3 lactone and GM3 ganglioside on neoplastic cells surface. From blood sample of mice, we obtained the serum which was used for immunocytochemistry assays on HCT116 colon carcinoma cells. It was known, indeed, that HCT116 colon carcinoma cells are characterized by an over-expression of GM3 ganglioside, similarly to melanoma cells.[58]

The results obtained with immunocytochemistry assays clearly indicated that

thioether **3** elicited an *in vivo* immunoresponse, and that the antibodies (anti-**3** pAbs) were able to recognize GM3 ganglioside over-expressed by neoplastic cell lines. Due to the good results obtained with anti-**3** polyclonal antibodies, and taking into account the known low immunogenicity of saccharidic antigens, we prepared suitable glycoconjugates for the vaccine formulation and monoclonal antibodies production. Compund **3** was directly used for the insertion into liposomes. As a matter of fact, liposomes can be used as adjuvants in vaccine formulations, since it was shown that antibodies coupled to liposomes were more immunogenic than their free-form counterparts.[45][46][47][48]

Moreover, in order to obtain monoclonal antibodies and evaluate different glycoconjugates for a vaccine formulation, we synthesized a modified derivative of **3** in which the alkyl chain was replaced by an amino-alkyl chain. The presence of the amino group allowed the linkage of the thioether hapten to a suitable spacer for coupling to carrier proteins[61] (such as KLH or BSA), immunostimulants lipopeptides[64] (such as Pam3CysSer), gold nanoparticles,[59] and dendrimeric multivalent systems[62] (such as PAMAM). These macromolecules are good candidate for vaccine formulation since they exploit their own immunostimulant properties and/or the multivalency.[60]

Experimental Part

Abbreviations

Ac:	Acetyl
Ac_2O:	Acetyc anhydride
AcOH:	Acetic acid
Bn:	Benzyl
CSA:	Camphorsulfonic acid
DCC:	N-Hydroxysuccinimide
DBU:	1,8-Diazabicyclo[5.4.0]undec-7-ene
DMAP:	4-(Dimethylamino)pyridine
DMF:	N,N-dimethylformamide
DMSO:	Dimetilsulfoxyd
Et_2O:	Diethyl ether
Et_3N:	Triethylamine
EtOH:	Ethanol
EtOAc:	Ethyl acetate
i-PrOH:	Isopropanol
IBX:	2-Iodoxybenzoic acid
Imy:	Imidazole
MeOH:	Methanol
NHS:	N-Hydroxysuccinimide
PhtNSCl:	Phthalimidosulfenyl chloride
Piv:	Pivaloyl
PivCl:	Pivaloyl chloride
Py:	Pyridine
TBAF:	Tetrabutylammonium fluoride
TBDMS:	*tert*butyldimethyilsilyl
TBDMSOTf:	*tert*-butyldimethylsilyl trifluoromethanesulfonate
TBDPS:	*tert*-butyldiphenylsilyl
TBDPSCl:	*tert*-butyl(chloro)diphenylsilane
TIPSOTf:	triisopropylsilyl trifluoromethanesulfonate
THF:	Tetrahydrofuran
TMSOTf:	Trimethylsilyl trifluoromethanesulfonate

General Remarks

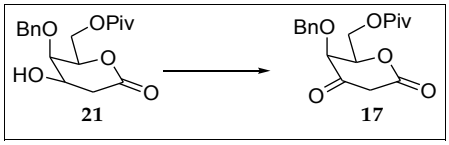
All reaction, when anhydrous conditions were needed, were performed under nitrogen atmosphere in dry solvents. Anhydrous dichloromethane were distilled over lithium aluminum hydride, at the time of using. Anhydrous ethyl acetate were distilled over calcium hydride, at the time of using. Chloroform was washed with water, dried over $CaCl_2$ and kept

over molecular sieves, previously activated for 12 h at 250°C under vaccum. TLC was performed on aluminum-backed silica gel. Unless otherwise noted, detection was achieved by treatment with a solution of vanilline (3 g) and H_2SO_4 (4 mL) in EtOH (250 mL).

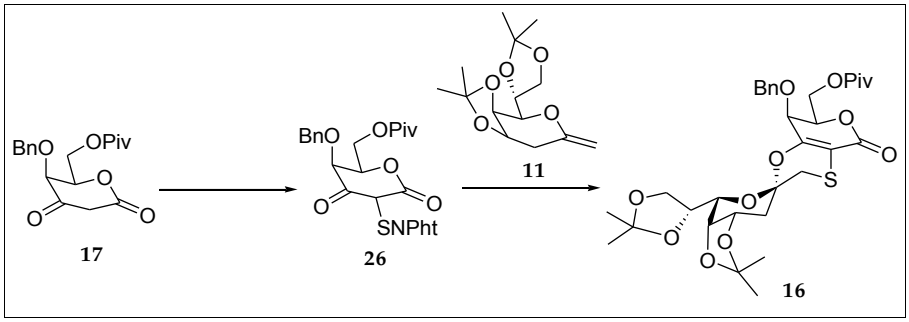
Meting points are uncorrected and were recorded on Melting Point Büchi 510. Optical rotations were determined with a Jasco DIP-370 polarimeter. NMR spectra were recorded on Varian Gemini 200 (1H 200 MHz) and Varian Mercury 400 (1H 400 MHz). Notations s, d, t, q, m, b, a, Ph indicate respectively singlet, doublet, triplet, quadruplet, multiplet, broad, apparent, aromatic. Mass spectra were recorded with QMD 1000 Carlo Erba with direct injection (EI, 70 eV). Intensity at the peaks level is expressed as relative percentage and shown between brackets. ESI-MS spectra were recorded with ESI-TOF, Mariner, Applied Biosystems. Elemental analyses were performed with Elementary Analyzer Perkin–Elmer serie II CHNS/O 2400.

Quartz Crystal Microbalance experiments were performed with quartz crystals (9.5 MHz AT-Cut, 14 mm) with gold evaporated (42.6 mm^2 area) on both sides (International Crystal Manufacturing, USA). The resonance frequency variations caused by changes in mass on the crystal (according to Sauerbrey's equation[65]) was continuously recorded by the quartz crystal analyzer QCA 922 by SEIKO, and read directly by a computer connected to the instrument. Before using, the electrode surface of the quartz crystals was cleaned by dipping it in a boiling solution of H_2O_2 (33%): NH_3 (33%): milliQ water (1:1:5) for 10 minutes, then washed with milliQ water and used immediately. The freshly cleaned crystal was dipped overnight into an unstirred 1mM solution of 1-dodecanethiol at room temperature and in the dark. The crystal was then washed with milliQ water, and treated with a 10 mM solution of 6-mercapto-1-hexanol for 1 h.[66]

Synthesis of compound 16:

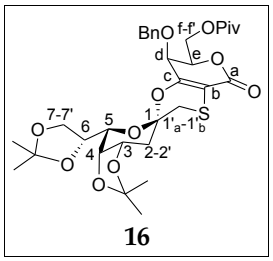


A suspension of lactone **21** (0.908 g, 2.70 mmol) and IBX (5.26 g, 18.6 mmol) in EtOAc (27 mL) was stirred at 80°C for 3h. The reaction mixture was cooled to 0°C, then the white solid obtained was filtered through a pad of Celite. The filtrate was concentrated to dryness to give 1.016 g of a crude mixture of β-keto-δ-lactone **17** (741 mg, 2.21 mmol, 82%, on the basis of NMR spectra), and benzoic acid (275mg, 1.10 mmol).



To an ice-cooled solution of crude ketolactone **17** (4.37 mmol) in dry $CHCl_3$ (5 mL), phthalimidosolfenyl chloride (930 mg, 4.37 mmol) was slowly added; after 5 min the ice bath was removed. The reaction mixture was stirred at rt until the complete disappearance of starting material (TLC analysis, 10 min). The crude solution of the phthalimidosulfenyl derivative formed was passed through a column with a cation-exchanger resin (Amberlite 200) and then the neutralized suspension obtained was added to a suspension of K_2CO_3 (278 mg, 2.02 mmol) and **11** (850 mg, 3.148 mmol) in dry Py (4 mL) at 50°C. The mixture was stirred for 30 min then a second, freshly prepared, suspension of sulfenyl derivative **26** (prepared starting from 3.89 mmol of ketolactone **17** and 3.89 mmol of phtalimidosulfenyl chloride) was added. The reaction mixture was stirred at 50°C for 16 h then diluted with CH_2Cl_2 and filtered. The filtrate was washed with a saturated solution of NH_4Cl (4 x 20 mL)

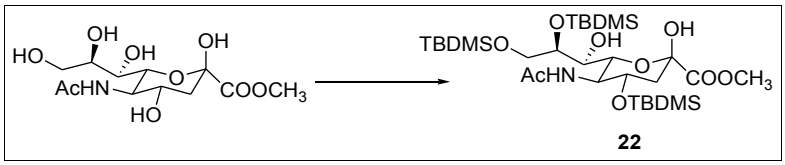
and dried over Na_2SO_4. The solvent was evaporated under vacuum affording a crude which was purified by a flash column chromatography on silica gel (petroleum ether : EtOAc = 2:1) to give unreacted **11** (250 mg, 0.925 mmol) as pale yellow oil, (petroleum ether : EtOAc = 3:2), and **16** (990 mg, 50%) as white solid (petroleum ether : EtOAc = 1:3).



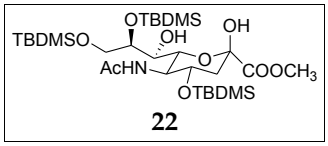
16

^{1}H NMR (400MHz, $CDCl_3$): δ 7.36-7.29 (m, 5H), 4.87-4.85 (A part of an AB system, 1H, $J_{A\text{-}B}$= 11.6 Hz, CH_2Ph), 4.68-4.65 (M part of an AMXY system, 1H, $J_{M\text{-}A}$= 7.6 Hz, $J_{M\text{-}X}$= $J_{M\text{-}Y}$= 2.8 Hz, H_3), 4.56-4.52 (m, 1H, H_e), 4.55-4.52 (B part of an AB system, 1H, $J_{A\text{-}B}$= 11.6 Hz, CH_2Ph), 4.41-4.37 (m, 2H, H_f, H_4), 4.31-4.26 (m, 2H, $H_{f'}$, H_6), 4.068- 4.046 (A part of an ABX system, 1H, $J_{A\text{-}B}$= 8.8 Hz, $J_{A\text{-}X}$= 1.2 Hz, H_7), 4.032- 4.01 (B part of an ABX system, 1H, $J_{A\text{-}B}$= 8.8 Hz, $J_{B\text{-}X}$= 1.2 Hz, $H_{7'}$), 3.90 (d, 1H, $J_{d\text{-}e}$= 2.8 Hz, H_d), 3.84 (dd, 1H, J= 2.0 Hz, J= 6.4 Hz, H_5), 3.33-3.29 (A part of an AB system, 1H, $J_{A\text{-}B}$= 13.2 Hz, $H_{1'a}$), 2.83-2.80 (B part of an AB system, 1H, $J_{A\text{-}B}$= 13.2 Hz, $H_{1'b}$), 2.52-2.47 (X part of an AMXY system, 1H, $J_{X\text{-}A}$= 2.8 Hz, $J_{X\text{-}Y}$= 15.6 Hz, H_2), 2.10-2.06 (Y part of an AMXY system, 1H, $J_{Y\text{-}A}$= 3.2 Hz, $J_{Y\text{-}X}$= 15.6 Hz, $H_{2'}$), 1.46 (s, 3H, $C(CH_3)_2$), 1.35 (s, 3H, $C(CH_3)_2$), 1.31 (s, 3H, $C(CH_3)_2$), 1.28 (s, 3H, $C(CH_3)_2$), 1.17 (s, 9H, $C(CH_3)_3$). **^{13}C NMR** (50MHz, $CDCl_3$): δ 178.1, 162.6, 156.1, 136.9, 128.49 (2C), 128.45 (2C), 128.1, 109.7, 109.1, 102.3, 95.9, 76.3, 73.8, 72.9, 72.1 (2C), 70.2, 70.0, 65.8, 62.1, 38.7, 35.2, 33.4, 27.0, 26.3, 25.9, 25.4, 24.6. **M.p.**: 186-187° C. **$[\alpha]_D^{25}$** + 8.32 (c 0.33, $CHCl_3$). **ESI-MS** 657.3 $[M+Na]^+$, 673.2 $[M+K]^+$.

Synthesis of compound 22:

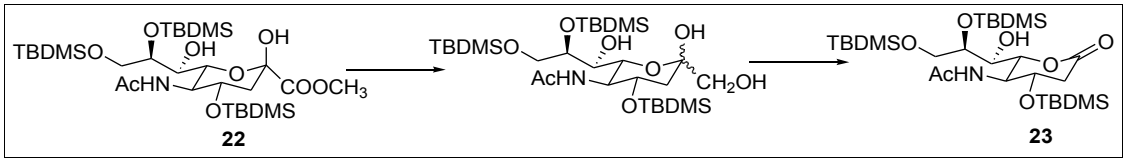


To a suspension of sialic acid methyl ester (1.00 g, 3.093 mmol) in dry CH_2Cl_2 (6 mL) and Py (1.3 mL) TBDMSiOTf (2.56 mL, 11.135 mmol) was slowly added at 0°C. After 10 min the mixture was warmed to rt, stirred for 30 min, diluted with CH_2Cl_2 (50 mL) and washed with a saturated solution of NH_4Cl (2 x 20 mL). The organic phase was dried over Na_2SO_4, filtered and the solvent was removed under reduced pressure. The crude was purified by a flash column chromatography on silica gel (petroleum ether : EtOAc = 6:1) to afford **22** (1.66g, 81%) as a white solid.

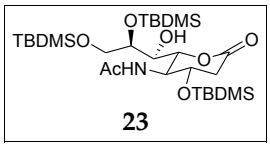


^{1}H NMR (400MHz, C_6D_6): δ 4.79 (d, 1H, $J_{OH\text{-}7}$= 4.8 Hz, OH), 4.43 (d, 1H, $J_{NH\text{-}5}$= 4.0 Hz, NH), 4.25-4.18 (m, 3H, H_5 , H_6, OH), 4.12-4.04 (m, 3H, H_4, H_8, H_{9a}), 3.90 (dd, 1H, $J_{6\text{-}OH}$= 4.8 Hz, $J_{6\text{-}5}$= 9.2 Hz, H_7), 3.81 (d, 1H, $J_{9b\text{-}9a}$ = 8.8 Hz, H_{9b}), 3.15 (s, 3H, CH_3 OCO), 2.29 (ddt, 1H, $J_{3a\text{-}3b}$= 12.6 Hz, $J_{3a\text{-}4}$= 10.6 Hz, $J_{3a\text{-}5}$= 2.0 Hz, H_{3a}), 2.15 (dd, 1H, $J_{3b\text{-}3a}$= 12.6 Hz, $J_{3b\text{-}4}$= 5.2 Hz, H_{3b}), 1.50 (s, 3H, CH_3CONH), 1.04, 0.99, 0.80 (3s, 27H, CH_3 *tert*-Bu), 0.26, 0.19, 0.10, 0.06, -0.07, -0.14 (6s, 18H, CH_3Si). **^{13}C NMR** (400MHz, C_6D_6): δ 171.2, 170.6, 95.0, 72.3, 72.1, 68.0, 67.9, 64.2, 53.9, 52.5, 40.2, 26.0, 25.9, 25.3, 22.4, 18.3, 18.2, 17.5, -4.2, -4.5, -5.0, -5.6, -5.7. **M.p.** = 171-173 °C. **$[\alpha]_D^{25}$**= +25.3 (c 1.25, $CHCl_3$).

Synthesis of compound 23:

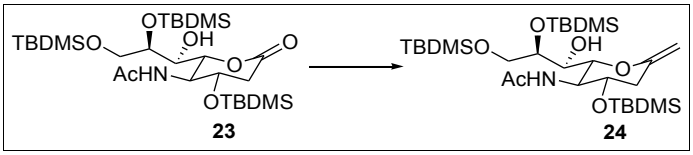


To a suspension of **22** (165 mg, 0.248 mmol) in *i*-PrOH (2.5 mL), cooleed to 0°C, $NaBH_4$ (9 mg, 0.248 mmol) was added. The mixture was stirred for 30 min at 0°C, then AcOH (0.015 mL, 0.248 mmol) was added dropwise. The mixture was warmed to rt, diluted with EtOAc (50 mL) and washed with a saturated solution of $NaHCO_3$ (10 mL) and with brine (10 mL). The organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude obtained (155 mg, 0.243 mmol) was dissolved in MeOH (1.2 mL), and a 1M suspension of $NaIO_4$ (52 mg, 0.243 mmol) in H_2O (0.24 mL) was added. The mixture was warmed to 40°C and stirred for 20 h, then it was diluted with CH_2Cl_2 (30 mL) and washed with H_2O (3 x 5 mL). The organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude was purified by flash column chromatography on silica gel (CH_2Cl_2 : EtOAc : Tol = 30:1:1) affording **23** (70 mg, 0.116 mmol, 40% over two steps) as a glassy solid.

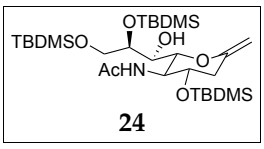


^{1}H NMR (400 MHz, C_6D_6): δ 4.67 (d, 1H, $J_{NH\text{-}4}$= 8.0Hz, NH), 4.46 (dd, 1H, $J_{5\text{-}4}$= 10.0Hz, $J_{5\text{-}6}$= 1.2Hz, H_5), 4.24 (d, 1H, $J_{OH\text{-}4}$= 5.6Hz, OH), 4.10-4.06 (m, 1H, H_7), 3.99-3.92 (m, 2H, H_4 ,H_{8a}), 3.86-3.78 (m, 3H, H_3, $H_{8\ a}$, H_6), 2.80 (dd, 1H, $J_{2a\text{-}2b}$= 16.8Hz, $J_{2a\text{-}3}$= 6.0Hz, H_{2a}), 2.31 (dd, 1H, $J_{2b\text{-}2a}$= 16.8Hz, $J_{2b\text{-}3}$= 7.2Hz, H_{2b}), 1.49 (s, 3H, CH_3), 1.00 (s, 9H, CH_3 *tert*-Bu), 0.96 (s, 9H, CH_3 *tert*-Bu), 0.78 (s, 9H, CH_3 *tert*-Bu), 0.27 (s, 3H, CH_3 Si-Me), 0.18 (s, 3H, CH_3 Si-Me), 0.08 (s, 3H, CH_3 Si-Me), 0.06 (s, 3H, CH_3 Si-Me), -0.18 (s, 3H, CH_3 Si-Me), -0.19 (s, 3H, CH_3 Si-Me). **^{13}C NMR** (50 MHz, C_6D_6): δ 176.3, 173.4, 81.9, 77.5, 74.6, 73.8, 70.8, 58.9, 44.9, 31.6, 31.5, 31.0, 28.1, 24.0, 23.8, 23.2, 1.1 , 1.0, 0.9, 0.5, 0.1, 0.0. **M.p.**= 153-155°C. **$[\alpha]_D^{25}$**= +38.3 (c 1.05, $CHCl_3$).

Synthesis of compound 24:

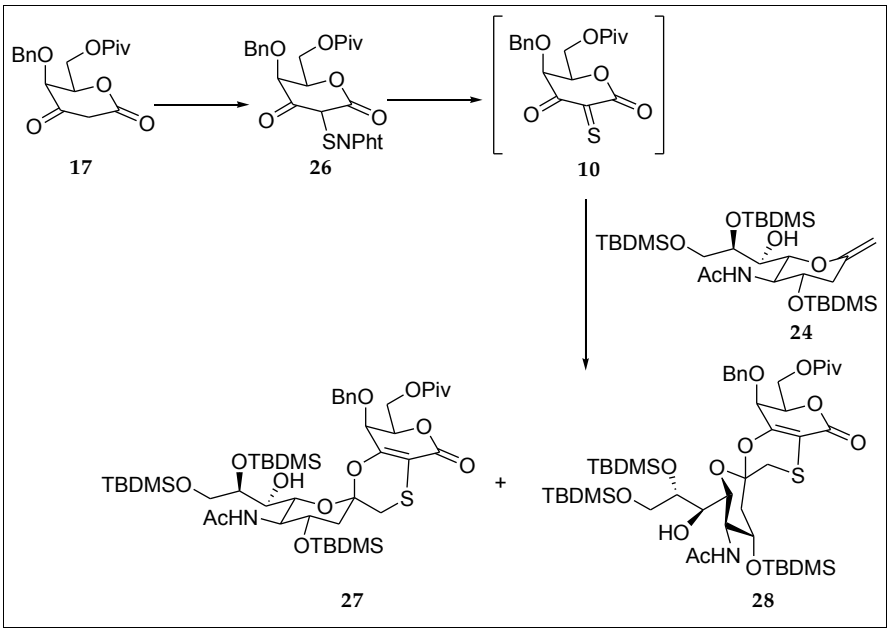


To a stirred solution of **23** (180 mg, 0.297mmol) in dry Py (0.7 mL), cooled to –78°C, Tebbe's reagent (0.5M in toluene, 1.48 mL) was slowly added. The reaction mixture was then warmed to rt and stirred for 30 min. After this time, the mixture was cooled to –40°C and a 0.1 M NaOH acqueous solution (3.5 mL) was added dropwise. The mixture was stirred for 10 min, diluted with Et_2O (30 mL), warmed to rt and filtered through a pad of Celite. The solvent was then removed and the crude obtained was purified by flash column chromatography on silica gel (petroleum ether : EtOAc : Et_3N = 8:1:0.1) to afford **24** (123 mg, 67%) as a colourless oil.



^{1}H NMR (400MHz, C_6D_6): δ 4.90 (d, 1H, $J_{NH\text{-}5}$= 8.8 Hz, NH), 4.64-4.63 (m, 2H, H_{1a}, OH), 4.22 (ddd, 1H, $J_{8\text{-}9a}$= 3.2 Hz, $J_{8\text{-}9b}$= 2.4 Hz, $J_{8\text{-}7}$= 9.2 Hz, H_8), 4.15-4.14 (m, 1H, H_{1b}), 4.11-4.09 (m, 1H, H_5), 4.05 (dd, 1H, $J_{9a\text{-}9b}$= 10.8 Hz, $J_{9a\text{-}8}$= 3.2 Hz, H_{9a}), 3.91 (dd, 1H, $J_{9b\text{-}9a}$= 10.8 Hz, $J_{9b\text{-}8}$= 2.4 Hz, H_{9b}), 3.81-3.77 (m, 1H, H_7), 3.76-3.73 (m, 1H, H_6), 3.63-3.57 (m, 1H, H_4), 2.42 (dd, 1H, $J_{3a\text{-}3b}$= 13.6 Hz, $J_{3a\text{-}4}$= 5.2 Hz, H_{3a}), 2.21-2.14 (m, 1H, H_{3b}), 1.53 (s, 3H, CH_3), 1.02 (s, 9H, CH_3 *tert*-Bu), 0.99 (s, 9H, CH_3 *tert*-Bu), 0.81 (s, 9H, CH_3 *tert*-Bu), 0.25 (s, 3H, CH_3 Si-Me), 0.22 (s, 3H, CH_3 Si-Me), 0.12 (s, 3H, CH_3 Si-Me), 0.09 (s, 3H, CH_3 Si-Me), -0.11 (s, 3H, CH_3 Si-Me), -0.15 (s, 3H, CH_3 Si-Me). **ESI-MS**: 626.4 $[M+Na]^+$.

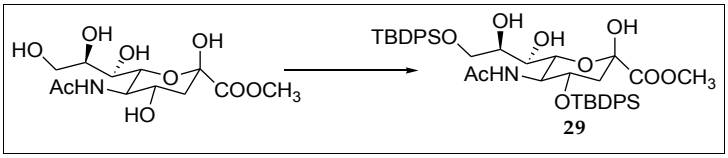
Synthesis of cycloadducts 27 and 28:



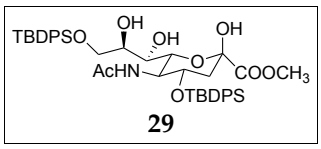
To an ice-cooled solution of crude ketolactone **17** (0.23 mmol) in dry $CHCl_3$ (1.5 mL), phthalimidosolfenyl chloride (70 mg, 0.23 mmol) was slowly added. The mixture was stirred for 30 min and then is added dropwise to a stirred solution of **24** (123 mg, 0.203 mmol) in Py (1.5 mL). The reaction mixture was then cooled to rt, stirred for 16 h, diluted with CH_2Cl_2 (30 mL) and washed with a saturated solution of NH_4Cl (2 x 10 mL). The organic phase was dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography on silica gel (petroleum ether : EtOAc = 4:1) to afford **27**and **28** (30 mg, 12%) as an inseparable mixture of diastereoisomers.

^{1}H NMR (200 MHz, C_6D_6): δ 7.39-7.35 (m, 3H, CH Ph isom. **27** + isom. **28**), 5.01-3.63 (m, 13H, isom. **27** + isom. **28**), 2.96 (d, 1H, $J_{2'a\text{-}2'b}$= 14.0 Hz, $H_{2'a}$ isom. **28**), 2.60 (d, 1H, $J_{2'a\text{-}2'b}$= 13.0 Hz, $H_{2'a}$ isom. **28**), 2.41 (d, 1H, $J_{2'b\text{-}2'a}$= 14.0 Hz, $H_{2'b}$ isom. **27**), 2.26 (d, 1H, $J_{2'b\text{-}2'a}$= 13.0 Hz, $H_{2'b}$ isom. **28**), 1.42 (s, 3H, CH_3CONH isom. **27** + isom. **28**),), 1.02, 0.99, 0.93, 0.89 (4s, 27H, CH_3 *tert*-Bu isom. **27** + isom. **28**), 0.28, 0.22, 0.18, 0.11,-0.15, -0.21 (6s, 18H, CH_3 Si-Me isom. **27** + isom. **28**).

Synthesis of compound 29:

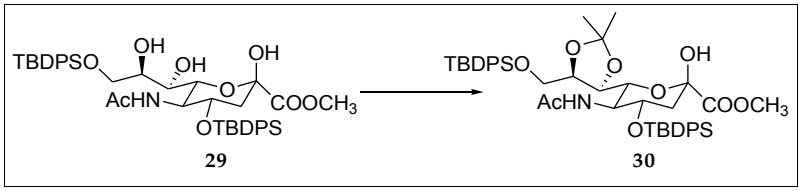


To a solution of sialic acid methyl ester (500 mg, 1.546 mmol) in dry DMF (3 mL) Imy (210 mg, 3.092 mmol) and TBDPSiCl (0.80 mL, 3.092 mL), were slowly added and the mixture was stirred for 20 h at rt. The reaction mixture was then diluted with CH_2Cl_2 (30 mL) and washed with a saturated solution of NH_4Cl (2 x 10 mL). The organic phase was dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography on silica gel (petroleum ether : EtOAc = 2:1) to afford **29** (900 mg, 73%) as a white solid.

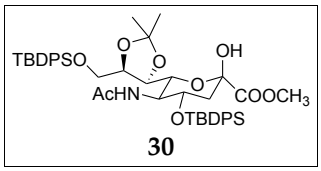


^{1}H NMR (400 MHz, CD_3CN): δ 7.72-7.68 (m, 8H, CH Ph), 7.61-7.39 (m, 12H, CH Ph), 6.23 (d, 1H, $J_{NH\text{-}5}$= 9.2 Hz, NH), 4.54 (bs, 1H, OH *anomeric*), 4.27 (d, 1H, OH), 4.24-4.18 (m, 1H, H_4), 3.95 (ddd, 1H, $J_{5\text{-}NH}$= 9.2 Hz, $J_{5\text{-}4}$= 9.2 Hz, $J_{5\text{-}6}$= 1.2 Hz, H_5), 3.84 (dd, 1H, $J_{6\text{-}7}$= 11.2 Hz, $J_{6\text{-}5}$= 1.2 Hz, H_6), 3.81-3.80 (m, 2H, H_{9a}, H_{9b}), 3.70 (s, 1H, CH_3OCO), 3.65-3.58 (m, 2H, H_7, H_8), 2.88 (d, 1H, OH), 2.05-2.00 (m, 2H, H_{3a}, H_{3b}), 1.74 (s, 3H, CH_3CONH), 1.04 (s, 9H, CH_3 *tert*-Bu), 1.03 (s, 9H, CH_3 *tert*-Bu). **^{13}C NMR** (50 MHz, $CDCl_3$): δ 172.1, 169.8, 135.7, 135.6, 135.4, 135.2, 133.1, 133.0, 132.9, 132.6, 130.1, 129.9, 129.6, 127.9, 127.8, 127.5, 95.0, 71.2, 69.5, 68.5, 67.9, 64.9, 53.8, 53.1, 39.6, 26.9, 26.8, 22.9, 19.4, 19.3. **M.p.**= 108-109 °C. **$[\alpha]_D^{25}$**= -5.3 (c 1.00, $CHCl_3$).

Synthesis of compound 30:

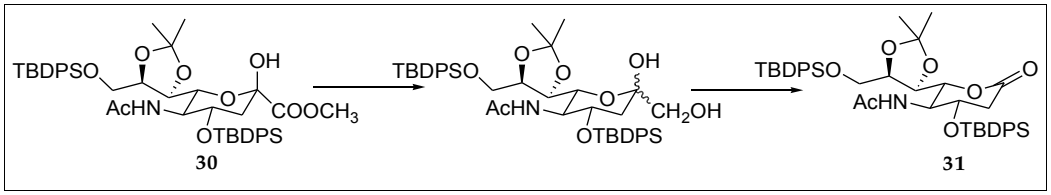


To a solution of **29** (850 mg, 1.06 mmol) in 2,2-dimethoxypropane (10 mL) a catalytic amount of CSA was added and the reaction mixture was stirred at rt for 1 h. The suspension obtained was diluted with acetone (40 mL) and neutralized with Et_3N. The mixture was then diluted with CH_2Cl_2 (50 mL) and washed with H_2O (3 x 10 mL). The organic phase was dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure, affording **30** (895 mg, 85%), without any further purification.

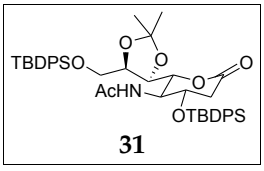


^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71-7.56 (m, 8H, CH Ph), 7.46-7.33 (m, 12H, CH Ph), 4.41 (d, 1H, $J_{NH\text{-}5}$= 8.8 Hz, NH), 4.31-4.24 (m, 1H, H_8), 4.13-4.02 (m, 2H, H_5, H_6), 3.99 (dd, 1H, $J_{4\text{-}3}$= 4.4 Hz, $J_{4\text{-}5}$= 9.6 Hz, H_4), 3.86 (dd, 1H, $J_{9a\text{-}8}$= 5.6 Hz, $J_{9a\text{-}9b}$= 10.0 Hz, H_{9a}), 3.71 (s, 3H, CH_3OCO), 3.70 (dd, 1H, $J_{9b\text{-}8}$= 8.0 Hz, $J_{9b\text{-}9a}$= 10.0 Hz, H_{9b}), 3.65 (m, 1H, H_7), 3.23 (bs, 1H, OH_2), 2.12 (at, 1H, H_{2a}), 2.03 (dd, 1H, $J_{3a\text{-}4}$= 4.4 Hz, $J_{3a\text{-}3b}$= 12.4 Hz, H_{2b}), 1.55 (s, 1H, CH_3CONH), 1.37 (s, 3H, CH_3 *isoprop.*), 1.32 (s, 3H, CH_3 *isoprop.*), 1.03 (s, 9H, CH_3 *tert*-Bu), 0.96 (s, 9H, CH_3 *tert*-Bu). **^{13}C NMR** (50 MHz, $CDCl_3$): δ 169.9, 169.5, 135.7, 135.4, 133.5, 133.2, 133.1, 132.8, 129.9, 129.7, 129.6, 127.7, 127.5, 109.4, 94.4, 76.4, 70.9, 70.9, 68.6, 62.9, 53.2, 53.1, 39.3, 26.9, 26.1, 25.9, 23.5, 19.3, 19.2. **M.p.**= 84-86 °C. **$[\alpha]_D^{25}$**= -15.3 (c 1.20, $CHCl_3$).

Synthesis of compound 31:

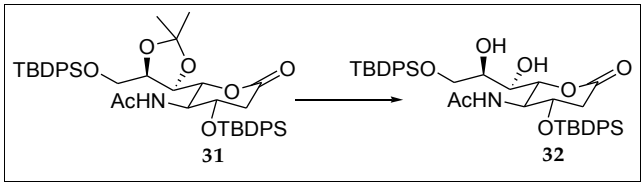


To a suspension of **30** (830 mg, 0.811 mmol) in *i*-PrOH (8 mL), cooled to 0°C, $NaBH_4$ (31 mg, 0.811 mmol) was added and after 5 min the mixture was warmed to rt and stirred for 1 h. The mixture was cooled again to 0°C, and AcOH (0.015 mL, 0.248 mmol) was slowly added, it was warmed to rt, diluted with CH_2Cl_2 (50 mL) and washed with a saturated solution of $NaHCO_3$ (10 mL) and brine (2 x 10 mL). The organic phase was dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The crude obtained (780 mg, 0.787 mmol) was dissolved in MeOH (16 mL), and a 0.5 M suspension of $NaIO_4$ (202 mg, 0.944 mmol) in H_2O (1.6 mL). The mixture was warmed to 40°C and stirred for 20 h. The mixture was diluted with CH_2Cl_2 (60 mL) and washed with H_2O (2 x 10 mL). he organic phase was dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography on silica gel (petroleum ether : EtOAc = 3:1) to afford **31** (114 mg, 20% over two steps) as a white solid.

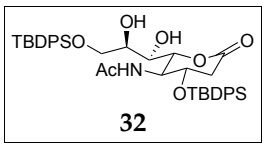


^{1}H NMR (400 MHz, C_6D_6): δ 7.69-7.57 (m, 8H, CH Ph), 7.16-7.10 (m, 12H, CH Ph), 4.51 (d, 1H, $J_{NH\text{-}4}$= 8.8 Hz, NH), 4.48 (d, 1H, $J_{4\text{-}5}$= 1.2 Hz, H_5), 4.46 (ad, 1H, H_7), 4.36 (ad, 1H, H_6), 4.26 (dd, 1H, $J_{8a\text{-}7}$= 8.0 Hz, $J_{8a\text{-}8b}$= 10.0 Hz, H_{8a}), 4.22 (dd, 1H, $J_{8b\text{-}7}$= 5.6 Hz, $J_{8b\text{-}8a}$= 10.0 Hz, H_{8b}), 4.15 (ddd, 1H, $J_{3\text{-}2a}$= 4.8 Hz, $J_{3\text{-}2b}$= 6.4 Hz, $J_{3\text{-}4}$= 5.6 Hz, H_3), 3.94 (ddd, 1H, $J_{4\text{-}NH}$= 8.8 Hz, $J_{4\text{-}3}$= 5.6 Hz, $J_{4\text{-}5}$= 1.2 Hz, H_4), 2.88 (dd, 1H, $J_{2a\text{-}3}$= 4.8 Hz, $J_{2a\text{-}2b}$= 15.6 Hz, H_{2a}), 2.63 (dd, 1H, $J_{2b\text{-}3}$= 6.4 Hz, $J_{2b\text{-}2a}$= 15.6 Hz, H_{2b}), 1.56 (s, 3H, CH_3CONH), 1.29 (s, 3H, CH_3 *isoprop.*), 1.22 (s, 3H, CH_3 *isoprop.*), 1.08 (s, 9H, CH_3 *tert*-Bu), 1.04 (s, 9H, CH_3 *tert*-Bu). **^{13}C NMR** (50 MHz, $CDCl_3$): δ 169.8, 169.2, 135.7, 135.6, 135.3, 133.1, 132.8, 132.7, 130.2, 130.0, 129.7, 127.9, 127.8, 127.6, 109.6, 76.6 , 76.0, 75.7, 68.4, 62.3, 54.6, 38.7, 26.9, 26.8, 26.2, 25.3, 23.2, 19.2. **M.p.**= 74-76 °C. **[α]$_D^{25}$**= +3.7 (c 0.80, $CHCl_3$).

Synthesis of compound **32**:

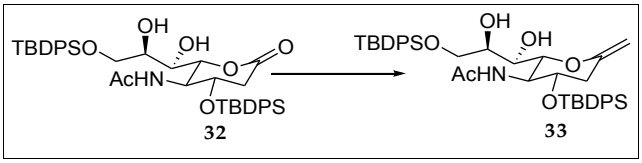


To a solution of **31** (0.114 mg, 0.146 mmol) in glacial AcOH (2.0 mL) H_2O (0.50 mL), was slowly added and the obtained was warmed to 60°C and stirred for 3 h. the solovent was then co-evaporated with toluene, under reduced pressure to afford **32** (96 mg, 90%) as a white solid, which was used without any further purification.

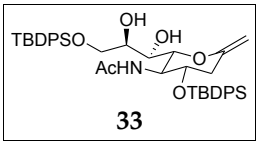


^{1}H NMR (200 MHz, $CDCl_3$): δ 7.72-7.61 (m, 6H, CH Ph), 7.51-7.37 (m, 14H, CH Ph), 5.08 (d, 1H, $J_{NH\text{-}5}$= 4.6 Hz, NH), 4.19-4.06 (m, 3H, H_5, H_7, H_6), 3.96-3.87 (m, 2H, H_{8a}, H_{8b}), 3.81-3.73 (m, 2H, H_3, H_4), 3.44 (d, 1H, OH), 3.06 (dd, 1H, $J_{2a\text{-}3}$= 5.4 Hz, $J_{2a\text{-}2b}$= 17.6 Hz, H_{2a}), 2.75 (dd, 1H, $J_{2b\text{-}3}$= 7.2 Hz, $J_{2b\text{-}2a}$= 18.0 Hz, H_{2b}), 2.37 (s, 1H, OH), 1.63 (s, 3H, CH_3CONH), 1.07 (s, 9H, CH_3 *tert*-Bu), 1.03 (s, 9H, CH_3 *tert*-Bu). **^{13}C NMR** (50 MHz, $CDCl_3$): δ 172.1, 168.7, 135.5, 135.4, 135.3, 132.8, 132.7, 132.5, 132.2, 130.4, 130.2, 129.6, 129.4, 128.1, 128.0, 127.6, 77.0, 69.4 , 68.2, 68.1, 65.3, 52.3, 39.2, 26.9, 26.8, 22.9, 19.4, 19.2.

Synthesis of compound 33:

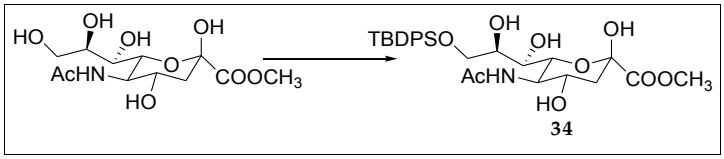


To a stirred solution of **32** (96 mg, 0.136 mmol) in dry Py (0.3 mL), cooled to –78°C, Tebbe's reagent (0.5M in toluene, 0.68 mL) was slowly added. The reaction mixture was then warmed to rt and stirred for 45 min. After this time, the mixture was cooled to –40°C and a 0.1 M NaOH aqueous solution (2 mL) was added dropwise. The mixture was stirred for 10 min, diluted with Et_2O (20 mL), warmed to rt and filtered through a pad of Celite. The solvent was then removed and the crude obtained was purified by flash column chromatography on silica gel (petroleum ether : EtOAc : Et_3N = 3:1:0.1) to afford **33** (22 mg, 23%) as a yellow oil. Compound **33** was used immediately after purification.

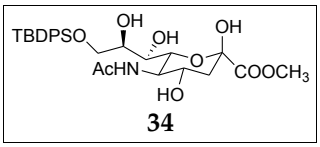


^{1}H NMR (200 MHz, C_6D_6): δ 7.76-7.69 (m, 3H, CH Ph), 7.62-7.52 (m, 3H, CH Ph), 7.21-7.08 (m, 14H, CH Ph), 4.92 (d, 1H, $J_{NH\text{-}5}$= 4.6 Hz, NH), 4.63 (bs, 1H, H_{1a}), 4.39-4.29 (m, 2H, H_8, H_5), 4.12-4.10 (m, 2H, H_{9a}, H_{9b}), 4.04 (bs, 1H, H_{2b}), 3.72-3.62 (m, 2H, H_6, H_7), 3.48-3.44 (m, 1H, H_4), 2.54 (dd, 1H, $J_{3a\text{-}4}$= 5.0 Hz, $J_{3a\text{-}3b}$= 13.2 Hz, H_{3a}), 2.42-2.30 (m, 1H, H_{2b}), 1.25 (s, 3H, CH_3CONH), 1.06 (s, 9H, CH_3 *tert*-Bu), 1.00 (s, 9H, CH_3 *tert*-Bu).

Synthesis of compound 34:

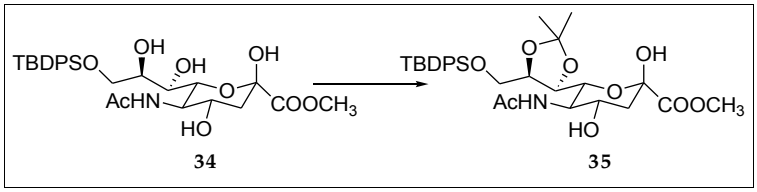


To a solution of sialic acid methyl ester (500 mg, 1.546 mmol) in dry DMF (2.5 mL) Imy (158 mg, 2.32 mmol), TBDPSiCl (0.6 mL, 2.320 mmol), and a catalytic amount of DMAP were slowly added and the mixture was stirred for 24 h at rt. The reaction mixture was then diluted with CH_2Cl_2 (70 mL) and washed with a saturated solution of NH_4Cl (2 x 10 mL). The organic phase was dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography on silica gel (EtOAc : MeOH = 40:1) to afford **34** (735 mg, 87%) as a white solid.

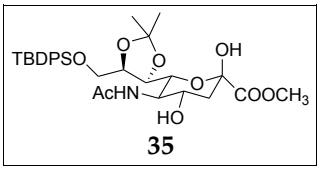


^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65-7.62 (m, 4H, CH Ph); 7.39-7.26 (m, 6H, CH Ph); 6.90 (bd, 1H, NHAc); 5.57 (bd, 1H, OH_2); 4.44 (at, 1H, OH_7); 4.11 (m, 2H, H_4, OH_4); 4.01-3.98 (m, 1H, H_{9a}); 3.93-3-86 (m, 3H, H_{9b}, H_5, H_6); 3.84-3.81 (m, 1H, H_8); 3.71 (s, 3H, CH_3OCO); 3.64 (dd, 1H, $J_{7\text{-}OH}$= 5.6 Hz, $J_{7\text{-}8}$= 8.8 Hz, H_7); 3.33 (bs, 1H, OH_8); 2.25 (dd, 1H, $J_{2a\text{-}3}$= 4.4 Hz, $J_{2a\text{-}2b}$= 12.8 Hz, H_{2a}); 2.06 (at, 1H, $J_{2b\text{-}2a}$= 12.8 Hz, H_{2b}); 1.97 (s, 3H, CH_3 CONH); 1.03 (s, 9H, CH_3 *tert*-Bu). **^{13}C NMR** (50 MHz, $CDCl_3$): δ 173.3; 170.2; 135.4; 135.3; 133.1, 132.8; 129.6; 127.6; 95.4; 71.4, 70.1, 68.0, 66.9; 65.0; 53.4; 53.1; 39.1; 26.9; 22.9; 19.4. **M.p.**= 91-92 °C. **$[\alpha]_D^{25}$**= +26.2 (c 1.09, $CHCl_3$).

Synthesis of compound 35:

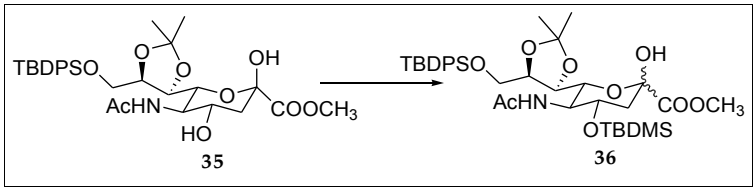


To a solution of **34** (150 mg, 0.275 mmol) in 2,2-dimethoxypropane (5 mL) a catalytic amount of CSA was added and the reaction mixture was stirred at rt for 40 min. After this time Et_3N was added until pH 7. The solvent was then removed under reduced pressure, and the crude obtained was purified by flash column chromatography on silica gel (EtOAc : MeOH = 80:1) to afford **35** (127 mg, 77%), as a white glassy solid.

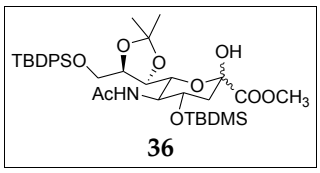


^{1}H NMR (400 MHz, $CDCl_3$): δ 7.69-7.63 (m, 4H, CH Ph); 7.46-7.37 (m, 6H, CH Ph); 5.60 (bd, 1H, NH); 4.38-4.33 (m, 1H, H_8); 4.23 (dd, 1H, $J_{7\text{-}8}$= 6.4 Hz, H_7); 3.96-3.91 (m, 3H, H_5, H_4, H_{9a}); 3.85 (dd, 1H, $J_{9b\text{-}8}$= 7.6 Hz, $J_{9b\text{-}9a}$= 10.4 Hz, H_{9b}); 3.73 (s, 3H, CH_3OCO); 3.60 (bs, 1H, OH_4); 3.47-3.39 (m, 1H, H_6); 2.14-2.10 (m, 2H, H_{3a}, H_{3b}); 1.98 (s, 3H, CH_3 CONH); 1.85 (bs, 1H, OH_2); 1.35 (s, 3H, CH_3 *isoprop.*); 1.31 (s, 3H, CH_3 *isoprop.*); 1.08 (s, 9H, CH_3 *tert*-Bu). **^{13}C NMR** 50 MHz, $CDCl_3$): δ 171.7; 169.9; 135.4 (4 C); 133.1, 132.9 (2 C); 129.8 (2 C); 127.6 (4 C); 109.4; 94.6; 77.0, 74.8, 70.0, 68.5, 62.8; 54.0; 53.2; 38.9; 27.0; 26.1, 26.0; 23.5; 19.4. **M.p.** = 63-65 °C.

Synthesis of compound 36:

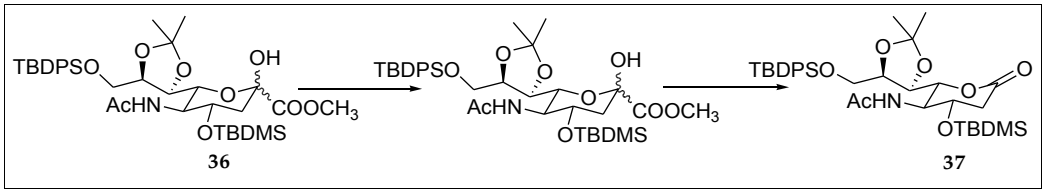


To a stirred solution of **35** (565 mg, 0.940 mmol) in dry CH_2Cl_2 (2 mL), cooled to 0°C, Py (0.36 mL) and TBDMSiOTf (0.238 mL, 1.035 mmol), were slowly added. The reaction mixture was stirred for 20 min, then diluted with CH_2Cl_2, warmed to rt, and washed with a saturated solution of NH_4Cl (2 x 10 mL). The solvent was then removed under reduced pressure, and the crude obtained was purified by flash column chromatography on silica gel (petroleum ether : EtOAc = 2:1) to afford **36** (575 mg, 86%) as a white solid and as an mixture of anomers.

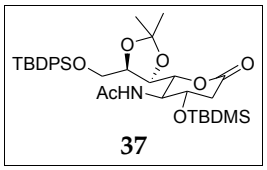


^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71-7.63 (m, 4H, CH Ph anom. α + anom. β); 7.46-7.36 (m, 6H, CH Ph anom. α + anom. β); 5.58 (bs, 1H, NH anom. α); 5.15 (d, 1H, NH anom. β); 4.40-4.32 (m, 1H, H_8 anom. α + anom. β); 4.25-4.22 (m, 1H, H_7 anom. α, H_7 anom. β); 4.12-4.08 (m, 1H, H_4 anom. β); 3.97-3.92 (m, 4H, H_5 anom. α, H_4 anom. α, H_{9a} anom. α, H_{9a} anom. β); 3.86 (dd, 1H, $J_{9b\text{-}8}$= 7.6 Hz, $J_{9b\text{-}9a}$= 11.2 Hz, H_{9b} anom. α); 3.80 (dd, 1H, $J_{9b\text{-}8}$= 7.2 Hz, $J_{9b\text{-}9a}$= 12.0 Hz, H_{9b} anom. α); 3.73 (s, 3H, CH_3OCO anom. α); 3.70 (s, 3H, CH_3OCO anom. β); 3.60-3.58 (m, 1H, H_5 anom. β); 3.52-3.46 (m, 1H, H_6 anom. β); 3.39-3.10 (m, 1H, H_6 anom. α); 2.15-2.01 (m, 2H, H_{3a} anom. α + H_{3b} anom. α); 1.98, 1.95 (2s, 3H, CH_3CONH anom. α + anom. β); 1.97-1.92 (m, 2H, H_{3a} anom. β + H_{3b} anom. β); 1.78 (bs, 1H, OH anom. α + anom. β); 1.37, 1.35, 1.32, 1.29 (4s, 6H, CH_3 isopropilidene anom. α + anom. β); 1.08, 1.07, (2s, 9H, CH_3 *tert*-Bu anom. α + anom. β); 0.85, 0.84, (2s, 9H, CH_3 *tert*-Bu anom. α + anom. β); 0.05, 0.03, 0.02 (3s, 6H, CH_3Si anom. α + anom. β). **^{13}C NMR** (50 MHz, $CDCl_3$): δ 169.2; 168.8; 135.5 (4 C); 133.6 (2 C); 129.6 (2 C); 127.6 (4 C); 109.4; 94.6; 77.1, 74.7, 69.2, 67.3, 63.0; 54.6; 53.1; 39.8; 27.0; 26.1, 25.7; 25.6; 24.0; 19.4, 17.9; -4.2, -4.7. **M.p.**= 89-90 °C.

Synthesis of compound 37:

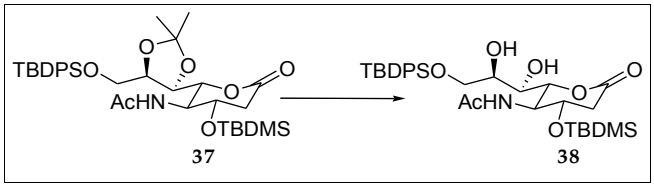


To a suspension of **36** (540 mg, 0.757 mmol) in *i*-PrOH (8 mL), cooleed to 0°C, $NaBH_4$ (29 mg, 0.757 mmol) was added. The mixture was stirred for 15 min at 0°C, then was warmed to rt and stirred for 1 h. After this time, AcOH (0.014 mL, 0.236 mmol) was added dropwise at 0°C. The mixture was then warmed to rt, diluted with EtOAc (50 mL) and washed with a saturated solution of $NaHCO_3$ (2 x 10 mL) and with brine (10 mL). The organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude obtained (532 mg, 0.746 mmol) was dissolved in MeOH (15 mL), and a 1M suspension of $NaIO_4$ (194 mg, 0.908 mmol) in H_2O (1.5 mL) was added. The mixture was warmed to 40°C and stirred for 7 h, then it was diluted with CH_2Cl_2 (60 mL) and washed with H_2O (2 x 10 mL). The organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated under reduced pressure. The crude was purified by flash column chromatography on silica gel (CH_2Cl_2 : EtOAc : Tol = 30:4:1) affording **37** (168 mg, 34% over two steps) as a white glassy solid.

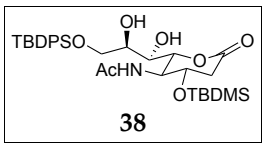


^{1}H NMR (400 MHz, $CDCl_3$): δ 7.68-7.60 (m, 4H, CH Ph); 7.44-7.35 (m, 6H, CH Ph); 5.42 (d, 1H, $J_{NH\text{-}4}$= 8.0 Hz, NH); 4.54 (dd, 1H, $J_{5\text{-}4}$= 8.0 Hz, $J_{5\text{-}6}$= 0.8 Hz, H_5); 4.41-4.33 (m, 2H, H_6, H_{8a}); 4.13-4.03 (m, 3H, H_{8b}, H_3, H_7); 3.81 (dd, 1H, $J_{4\text{-}5}$= 8.0 Hz, $J_{4\text{-}3}$= 8.0 Hz, H_4); 2.81 (dd, 1H, $J_{2a\text{-}3}$= 4.8 Hz, $J_{2a\text{-}2b}$= 16.4 Hz, H_{2a}); 2.50 (dd, 1H, $J_{2b\text{-}3}$= 8.4 Hz, $J_{2b\text{-}2a}$= 16.4 Hz, H_{2b}); 2.00 (s, 3H, CH_3CONH); 1.39 (s, 3H, CH_3 *isoprop.*); 1.32 (s, 3H, CH_3 *isoprop.)*; 1.06 (s, 9H, CH_3 *tert*-Bu); 0.86 (s, 9H, CH_3 *tert*-Bu); 0.07 (s, 3H, CH_3Si); 0.06 (s, 3H, CH_3Si). **^{13}C NMR** (50 MHz, $CDCl_3$): δ 169.9; 169.1; 135.4 (4 C); 132.9 (2 C); 129.7 (2 C); 127.6 (4 C); 109.4; 76.2, 76.0, 75.7, 66.7; 62.4; 54.7; 38.9; 26.9; 26.2; 25.6; 25.2; 23.4; 19.3; 17.8; -4.4, -4.7. **M.p.**= 68-70 °C. **$[\alpha]_D^{25}$**= +34.3 (c 1.20, $CHCl_3$).

Synthesis of compound 38:

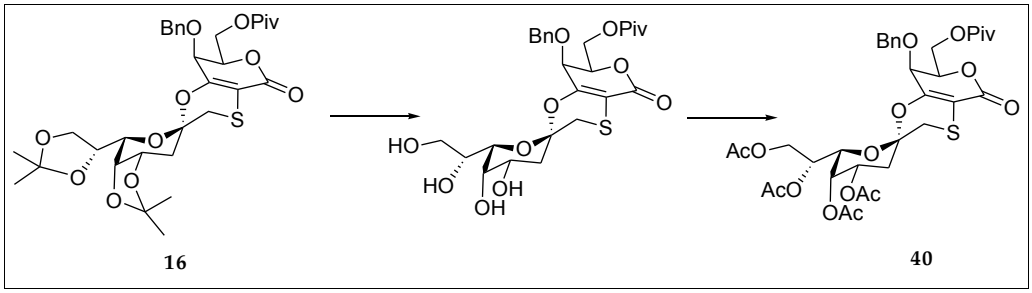


To a solution of **37** (0.150 mg, 0.229 mmol) in glacial AcOH (2.3 mL) H_2O (0.57 mL), was slowly added and the obtained was warmed to 40°C and stirred for 2 h. the solvent was then co-evaporated with toluene under reduced pressure. The crude obtained was purified by flash column chromatography on silica gel (petroleum ether : EtOAc = 1:1) to afford **38** (44 mg, 31%) as a white solid.

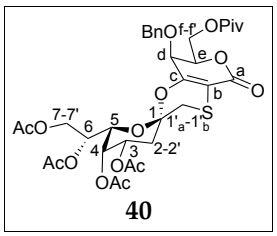


^{1}H NMR (400 MHz, $CDCl_3$): δ 7.66-7.62 (m, 4H, CH Ph); 7.43-7.35 (m, 6H, CH Ph); 6.06 (bd, 1H, NH); 4.50 (d, 1H, $J_{OH\text{-}7}$= 8.8 Hz, OH_7); 4.17 (dd, 1H, $J_{3\text{-}2a}$= 6.0 Hz, $J_{3\text{-}2b}$= 6.8 Hz, H_3); 4.10-4.02 (m, 2H, H_4, H_7); 3.90-3.84 (m, 3H, H_{8a}, H_5, H_6); 3.60 (dd, 1H, $J_{8b\text{-}7}$= 5.2 Hz, $J_{8b\text{-}8a}$= 8.0 Hz, H_{8b}); 2.99 (dd, 1H, $J_{2a\text{-}3}$= 6.0 Hz, $J_{2a\text{-}2b}$= 17.4 Hz, H_{2a}); 2.86 (bs, 1H, OH_8); 2.58 (dd, 1H, $J_{2b\text{-}3}$= 6.8 Hz, $J_{2b\text{-}2a}$= 17.4 Hz, H_{2b}); 1.99 (s, 3H, CH_3CONH); 1.04 (s, 9H, CH_3 *tert*-Bu); 0.89 (s, 9H, CH_3 *tert*-Bu); 0.12 (s, 3H, CH_3Si); 0.11 (s, 3H, CH_3Si). **^{13}C NMR** (50 MHz, $CDCl_3$): δ 172.0; 169.7; 135.5, 135.3, 135.2; 132.8, 132.7, 132.6, 132.3; 130.4, 130.3, 129.6, 129.4; 128.1, 128.0, 127.6; 76.9, 69.5, 68.2, 68.0; 65.3; 52.3; 39.2; 26.9; 26.8; 22.9; 19.4; 19.3. **M.p.**= 74-76 °C. **$[\alpha]_D^{25}$**= +18.9 (c 0.45, $CHCl_3$).

Synthesis of compound 40:

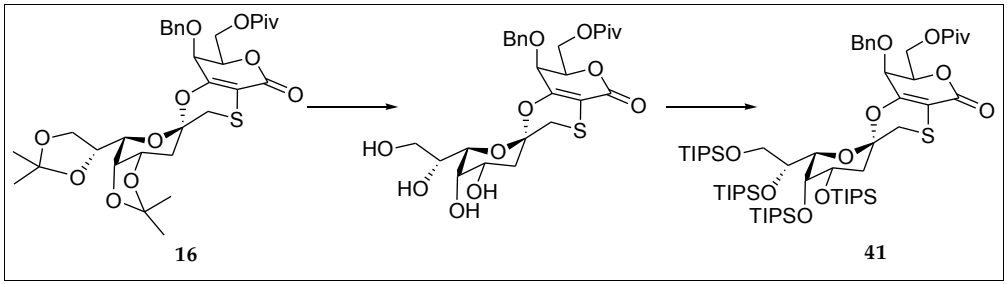


A solution of **16** (84 mg, 0.132 mmol) in 2 mL of CH_3CO_2H : H_2O / 70:30 was stirred at 60°C for 3 h, then toluene (2 mL) was added. The removal of the solvent under vacuum gave a crude which was dissolved in CH_2Cl_2 (3 mL) and treated with pyridine (0.8 mL), acetic anhydride (0.4 mL) and a catalytic amount DMAP. The mixture was stirred for 1 h at rt then diluted with CH_2Cl_2, washed with a saturated solution of NH_4Cl (2 x 10 mL) and dried over Na_2SO_4. The solvent was removed under vacuum and the crude purified by column chromatography on silica gel (petroleum ether : EtOAc = 3:2) to afford **40** (55 mg, 0.076 mmol, 58% over two steps) as pale oil.

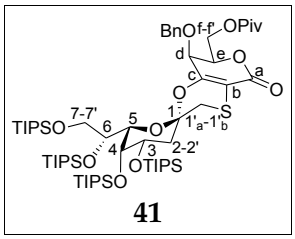


^{1}H NMR (200MHz, $CDCl_3$): δ 7.39-7.30 (m, 5H), 5.43 (m, 2H), 5.098-5.018 (m, 1H), 4.73 (A_2 system, 2H), 4.64 (dt, 1H, *J*= 2.8 Hz, *J*= 6.6 Hz), 4.51-4.31 (m, 3H), 4.15-4.01 (m, 2H), 3.91 (dd, 1H, *J*= 4.4 Hz, *J*= 12.4 Hz), 3.06-3.00 (A part of an AB system, 1H, $J_{A\text{-}B}$= 13.2 Hz) 2.93-2.86 (B part of an AB system, 1H, $J_{A\text{-}B}$= 13.2 Hz), 2.129-2.02 (m, 2H), 2.09 (s, 3H), 2.02 (s, 3H), 1.96 (s, 3H), 1.76 (s, 3H), 1.22 (s, 9H). **^{13}C NMR** (50MHz, $CDCl_3$): δ 177.6, 170.3,169.9, 169.7, 169.3, 161.8, 153.8, 136.0, 128.6, 128.5, 128.3, 103.4, 95.5, 75.76, 75.70, 71.3, 69.2, 67.4, 66.1, 64.1, 61.7, 61.3, 38.8, 33.5, 27.3, 20.8, 20.7, 20.6, 20.3. **$[\alpha]_D^{25}$** +47.9 (c 0.34, $CHCl_3$). **ESI-MS** 745.3 $[M+Na]^+$.

Synthesis of compound **41**:

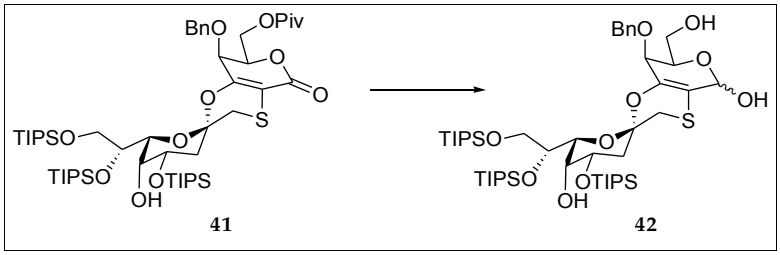


A solution of **16** (684 mg, 1.078 mmol) in 12 mL of a 70:30 mixture of CH_3CO_2H:H_2O was stirred at 60°C for 3 h, then toluene (15 mL) was added. The solvent was removed under vacuum giving a crude that was purified by flash chromatography on silica gel (AcOEt : CH_3OH = 15:1) to afford the unprotected derivative (473 mg, 0.853 mmol) which was dissolved in dry CH_2Cl_2 (1.5 mL) and treated with pyridine (1.5 mL) and TIPSOTf (0.920 mL, 3.408 mmol). The mixture was stirred at 0°C for 25 min, then diluted with CH_2Cl_2 (30 mL), washed with a saturated solution of NH_4Cl (3 x 30 mL), dried over Na_2SO_4, and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel (petroleum ether : EtOAc = 7:1) to afford **41** (460 mg, 0.449 mmol, 83%) as pale yellow oil.

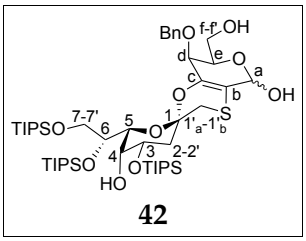


^{1}H NMR (400MHz, CD_3CN): δ 7.37-7.28 (m, 5H, Ph), 4.74-4.71 (A part of an AB system, 1H, $J_{A\text{-}B}$= 11.2 Hz, CH_2Ph), 4.17-4.67 (X part of an ABX, 1H, $J_{e\text{-}d}$= 2.8 Hz, J_{XA}=J_{XB}= 6.0 Hz, H_e), 4.61-4.60 (B part of an AB system, 1H, $J_{A\text{-}B}$= 11.2 Hz, CH_2Ph), 4.38-4.33 (A part of ABX system, 1H, $J_{A\text{-}X}$= 6.0 Hz, $J_{A\text{-}B}$= 11.2 Hz, H_f), 4.34-4.30 (m, 1H, H_3), 4.33-4.28 (B part of an ABX system, 1H, $J_{B\text{-}X}$= 6.0 Hz, $J_{A\text{-}B}$= 11.2 Hz, $H_{f'}$), 4.15 (bs, 1H, H_4), 4.14-4.09 (X part of an ABX system, 1H, $J_{X\text{-}A}$= 2.4 Hz, $J_{X\text{-}B}$=$J_{6\text{-}5}$= 8.0 Hz, H_6), 4.08 (d, 1H, $J_{d\text{-}e}$= 2.8 Hz, H_d), 3.94-3.91 (A part of an ABX, 1H, $J_{A\text{-}X}$ = 2.4 Hz, $J_{A\text{-}B}$= 10.8 Hz, H_7), 3.48 (bd, 1H, $J_{5\text{-}6}$= 8.4 Hz, H_5), 3.38-3.34 (B part of an ABX system, 1H, $J_{B\text{-}X}$ = 8 Hz, $J_{A\text{-}B}$= 10.8 Hz, $H_{7'}$), 3.03 (d, 1H, $J_{OH\text{-}4}$= 2.0 Hz, OH), 2.99-2.95 (A part of an AB system, 1H, $J_{A\text{-}B}$= 13.6 Hz, $H_{1'a}$), 2.94-2.91 (B part of an AB system, 1H, $J_{A\text{-}B}$= 13.6 Hz, $H_{1'b}$), 2.13-2.07 (X part of an AMXY system, 1H, $J_{X\text{-}M}$= 10.8 Hz, $J_{X\text{-}Y}$= 13.2 Hz, H_2), 2.06-2.02 (Y part of an AMXY system, 1H, $J_{Y\text{-}B}$= 5.4 Hz, $J_{Y\text{-}X}$= 12.8 Hz, $H_{2'}$), 1.2 (s, 9H, C(CH_3)$_3$), 1.22-1.06 (m, 54H, Si-CH(CH_3)$_2$), 1.01-0.99 (m, 9H, Si-CH(CH_3)$_2$). **^{13}C NMR** (50MHz, $CDCl_3$): δ 178.3, 162.6, 156.1, 136.9, 128.9 (2C), 128.6 (2C), 127.9, 101.8, 98.3, 76.1, 74.0, 73.4, 73.1, 71.0, 67.8, 67.3, 65.7, 61.8, 36.8, 36.3, 33.5, 27.1 (3C), 18.29 (4C), 18.20 (4C), 18.0 (6C), 17.9 (4C), 12.7 (3C),12.3 (3C), 11.8 (3C). **[α]$_D^{25}$** + 23.12 (c 1.24, $CHCl_3$). **ESI-MS** 1023 $[M+H]^+$, 1045 $[M+Na]^+$.

Synthesis of compound 42:

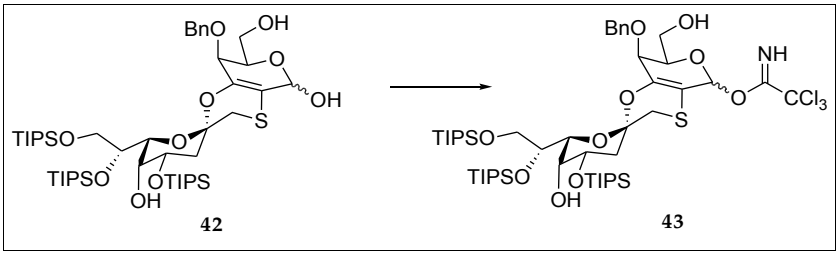


A solution of **41** (770 mg, 0.753 mmol) in dry CH_2Cl_2 (10 mL) was cooled at -40°C and a solution 1.5M in toluene of DIBAl-H (1.75 mL, 2.63 mmol) was added dropwise. The solution was slowly warmed to 10°C and the reaction mixture stirred for 5 h. After this time 0.5 mL of CH_3OH and a 20% aqueous solution of potassium sodium tartrate (20 mL) were added; the mixture was diluted with CH_2Cl_2 (30 mL) and stirred for 20 min at rt. The organic phase was dried over Na_2SO_4 and concentrated under reduced pressure. The crude was purified by column chromatography on silica gel [petroleum ether : EtOAc = 2:1 (+0.1% NEt_3)] to give **42** (424 mg, 0.451 mmol, 60%) as an oily mixture of anomers.



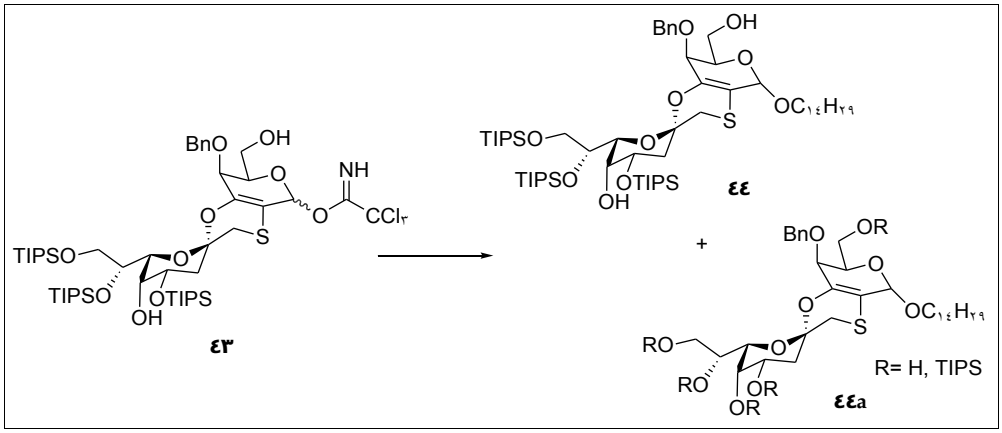
^{1}H NMR (400MHz, $CDCl_3$), selected data: δ 7.34-7.27 (m, 5H+5H), 5.42 (d, *1H*$_{\alpha\text{ -an}}$, *J*= 4.0 Hz), 5.23 (d, 1H$_{\beta\text{ -an}}$, *J*= 10.0 Hz), 4.77-4.74 (A part of an AB system, 1H$_{\alpha\text{ -an}}$, $J_{A\text{-}B}$= 11.6 Hz), 4.63-4.60 (A part of an AB system, 1H$_{\beta\text{ -an}}$, $J_{A\text{-}B}$= 12.0 Hz), 4.53-4.50 (B part of an AB system, 1H$_{\alpha\text{ -an}}$, $J_{A\text{-}B}$= 11.6Hz), 4.13-4.38 (B part of an AB system, 1H$_{\beta\text{ -an}}$, $J_{A\text{-}B}$= 12.0 Hz), 3.04 (bs, 1H, OH), 2.96-2.93 (A part of an AB system, 1H, $J_{A\text{-}B}$= 12.8 Hz), 2.91-2.88 (B part of an AB system, 1H, $J_{A\text{-}B}$= 12.8 Hz). **ESI-MS** 963.4 $[M+Na]^+$.

Synthesis of compound 43:

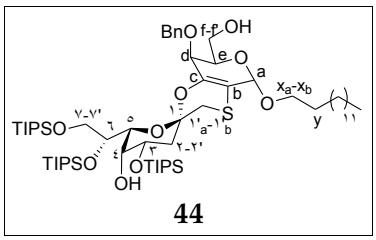


A solution of **42** (100 mg, 0.106 mmol) in dry CH_2Cl_2 (4 mL) was cooled to 0°C and tricloroacetonitrile (254 µL, 1.702 mmol) and DBU (12.5 µL, 0.125 mmol) were added. After 1 h at 0°C the solution was concentrated under vacuum. The residue was dissolved in CH_2Cl_2 filtered through silica gel (eluant: petroleum ether : EtOAc = 6:1). The filtrate was concentrated to dryness to give **43** (100 mg, 87%) which was used without any further purification.

Synthesis of compounds 44 and 44a:



A mixture of **43** (277 mg, 0.255 mmol), tetradecanol (163.0 mg, 0.763mmol) and molecular sieves (4 Å) in dry CH_2Cl_2 (9 mL) was stirred for 20 min at rt. The reaction mixture was cooled to -40°C, treated with 17 µL of triflic acid (0.1M in CH_2Cl_2) and stirred for 10 min at -40°C. Then 51 µL of triflic acid (0.1M solution in CH_2Cl_2) were added in two portions. After 1 h the suspension was neutralized with NEt_3, filtered through a pad of Celite and the filtrate concentrated to dryness. The crude was purified by flash chromatography on silica gel [petroleum ether : EtOAc = 7:1 (+0.1% NEt_3)] to give 130 mg of the α-glycosyl derivative **44** (23 mg, 0.020 mmol, 7.8%) and of an inseparable mixture of partially desilylated glycosyl derivative **44a**. Compound **44** was purified and was totally characterized.

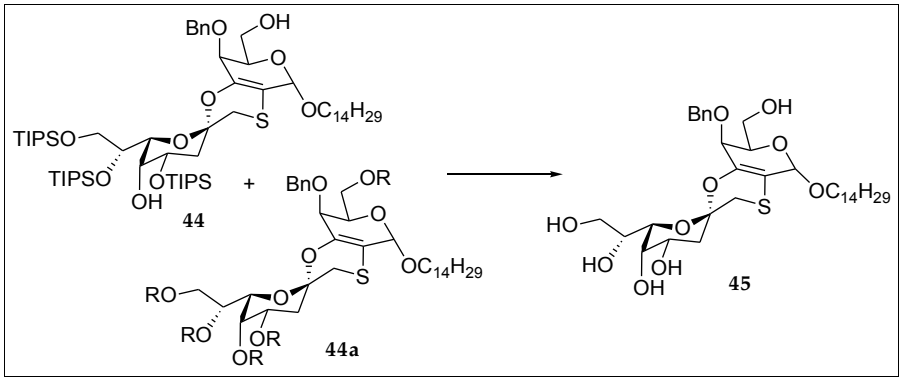


44

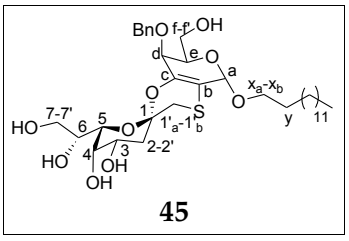
^{1}H NMR (400MHz, CD_3CN): δ 7.34-7.27 (m, 5H, Ph), 4.91 (s, 1H, H_a), 4.73-4.70 (A part of an AB system, 1H, $J_{A\text{-}B}$= 11.2 Hz, CH_2Ph), 4.58-4.55 (B part of an AB system, 1H, $J_{A\text{-}B}$= 11.2 Hz, CH_2Ph), 4.34-4.29 (M part of an AMXY system, 1H, J= 2.8 Hz, J= 6.4 Hz, J= 9.6 Hz, H_3), 4.13-4.07 (m, 3H), 3.97 (A part of an ABX system, 1H, $J_{A\text{-}X}$= 2.0 Hz, $J_{A\text{-}B}$= 10.8 Hz, H_7), 3.74 (d, 1H, $J_{d\text{-}e}$= 2.4 Hz, H_d), 3.72-3.68 (m, 1H, H_{xa}), 3.66-3.64 (at, 2H, J= 6.4 Hz, H_f, $H_{f'}$), 3.53 (bd, 1H, $J_{5\text{-}6}$= 8.4 Hz, H_5), 3.49-3.43 (m, 1H, H_{xb}), 3.39 (B part of an ABX system, 1H, $J_{B\text{-}X}$= 7.6 Hz, $J_{A\text{-}B}$= 10.8 Hz, $H_{7'}$), 2.95-2.92 (A part of an AB system, 1H, $J_{A\text{-}B}$= 13.2 Hz, $H_{1'a}$), 2.91-2.88 (B part of an AB system, 1H, $J_{A\text{-}B}$= 13.2 Hz, $H_{1'b}$), 2.90 (dd, 1H, J= 0.8 Hz, J= 2.4 Hz), 2.76 (t, 1H, J= 6.0 Hz, OH), 2.0-1.98 (m, 2H, H_2, $H_{2'}$), 1.6-1.53 (m, 2H, CH_{2y}), 1.29 (s, 22H, CH_2 chain), 1.1-1.04 (m, 63H, Si-($CH(CH_3)_6)_3$), 0.90 (t, 3H, J= 7.2 Hz, CH_3 chain). **^{13}C NMR** (50MHz, $CDCl_3$): δ 141.3, 137.6, 128.5 (2C), 128.0, 127.9 (2C), 105.9, 96.4, 94.9, 73.9, 73.4, 72.3, 72.1, 71.2,

68.4, 68.1, 67.3, 66.4, 62.2, 36.9, 33.7, 31.9, 29.67 (2C), 29.62 (2C), 29.59 (2C), 29.54, 29.4, 29.3, 26.0, 22.6, 18.3 (4C), 18.2 (4C), 18.05 (4C), 18.01 (6C), 14.0, 12.8 (3C), 12.2 (3C), 11.9 (3C). **ESI-MS** 1159.8 $[M+Na]^+$.

Synthesis of peracetyl derivative 45:

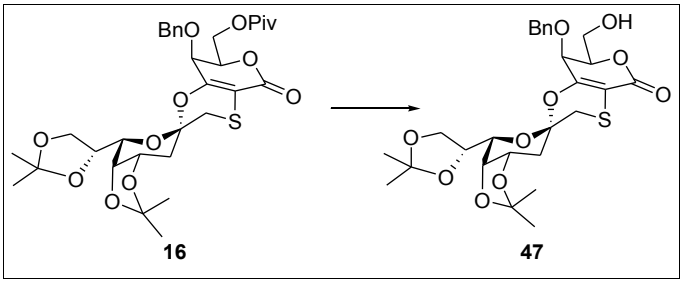


To a solution of **44** and **44a** (123 mg) in THF (3 mL), 110 mg of TBAF (0.345 mmol) were added at rt. The reaction mixture was stirred until no trace of starting material was revealed (TLC analysis). The solution was diluted with EtOAc, washed with a saturated solution of NH_4Cl (2x20mL), dried over Na_2SO_4, and concentrated to dryness. The crude was purified by flash chromatography on silica gel [petroleum ether : EtOAc = 7:1 (+ 0.1% NEt_3)] to afford **45** (30 mg, 0.044 mmol, 18% over two steps) as glassy solid.

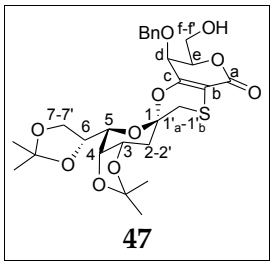


^{1}H NMR (400MHz, $CDCl_3$): δ 77.40-7.31 (m, 5H, Ph), 5.02 (s, 1H, H_a), 4.83-4.80 (A part of an AB system, 1H, $J_{A\text{-}B}$= 10.4 Hz, CH_2Ph), 4.62-4.56 (m, 3H), 4.51-4.46 (m, 1H), 3.94 (m, 1H, H_3), 3.88-3.71 (m, 4H), 3.64-3.57 (m, 2H), 3.28 (bs, 1H, OH), 3.06-3.03 (A part of an AB system, 1H, $J_{A\text{-}B}$= 12.8 Hz, $H_{1'a}$), 2.93-2.90 (B part of an AB system, 1H, $J_{A\text{-}B}$= 12.8 Hz, $H_{1'b}$), 2.93 (bs, 1H, OH), 2.64 (bs, 1H, OH), 2.24 (bs, 1H, OH), 2.04-1.99 (X part of an AMXY system, 1H, $J_{X\text{-}M}$= 5.2 Hz, $J_{X\text{-}Y}$= 12.8 Hz, H_2), 1.9 (bs, 1H, OH), 1.81 (Y part of an AMXY system, 1H, $J_{Y\text{-}M}$= $J_{Y\text{-}X}$= 11.6 Hz, $H_{2'}$), 1.63-1.58 (m, 2H, CH_{2y}), 1.2 (s, 22H, CH_2 chain), 0.87 (t, 3H, J= 8 Hz, CH_3 chain). **^{13}C NMR** (50MHz, $CDCl_3$): δ 39.5, 136.5, 128.9 (2C), 128.8 (2C), 128.7, 108.9, 95.8, 92.5, 76.0, 72.8, 70.1, 68.8, 68.4, 68.2, 66.9, 66.3, 66.1, 61.9, 37.0, 34.0, 31.8, 29.6 (2C), 29.6 (2C), 29.59, 29.50, 29.4, 29.38, 29.33, 26.1, 22.6, 14.1. **[α]$_D^{25}$** +40.3 (c 0.202, $CHCl_3$). **ESI-MS** 691.4 $[M+Na]^+$, 707.3 $[M+K]^+$.

Synthesis of compound 47:

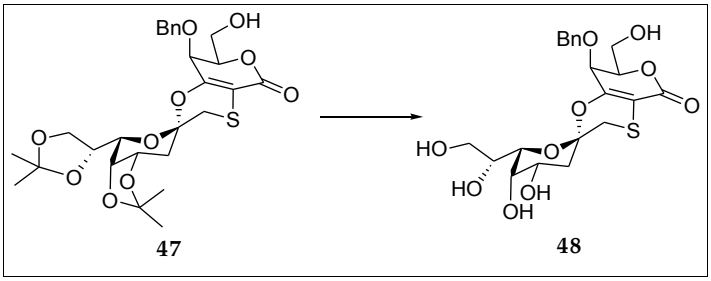


To a solution of guanidine hydrochloride (175 mg, 1.83 mmol) in CH_3OH (3.66 mL), CH_3ONa (82 mg, 1.52 mmol) was added. The suspension was stirred for 30 min at rt, then the white solid obtained was filtered and the filtrate was slowly added to a solution of **16** (388 mg, 0.611 mmol) in a 2:1 mixture of CH_2Cl_2 : MeOH (10 mL). The reaction mixture was stirred overnight then neutralized with a 10% solution of HCl in MeOH and the solvent was removed under vacuum. The crude was purified by flash chromatography on silica gel (petroleum ether : EtOAc = 1:2) to give **47** (233 mg, 70%) as a white solid.



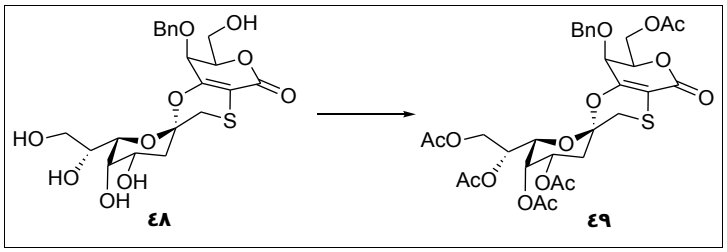
^{1}H NMR (400MHz, $CDCl_3$): δ 7.39-7.28 (m, 5H, Ph), 4.87-4.84 (A part of an AB system, 1H, $J_{A\text{-}B}$= 12Hz, CH_2Ph), 4.67-4.63 (M part of an AMXY system, 1H, $J_{M\text{-}A}$= 7.6 Hz, $J_{M\text{-}X}$= $J_{M\text{-}Y}$= 2.8 Hz, H_3), 4.55-4.52 (B part of an AB system, 1H, $J_{A\text{-}B}$= 12Hz, CH_2Ph), 4.04-4.36 (m, 2H, H_4, H_e), 4.30-4.26 (M part of an A_2M system, 1H, $J_{6\text{-}5}$= 6.8 Hz, $J_{M\text{-}A}$= 5.6 Hz, H_6), 4.03-4.02 (A part of an A_2M system, 2H, $J_{A\text{-}M}$= 5.6 Hz, H_7, $H_{7'}$), 3.94 (d, 1H, $J_{d\text{-}e}$= 2.0 Hz, H_d), 3.940-3.88 (m, 1H, H_f), 3.80 (dd, 1H, $J_{5\text{-}6}$= 6.8 Hz, $J_{5\text{-}4}$= 2.0 Hz, H_5), 3.68 (add, 1H, $J_{f'\text{-}f}$= 11.6 Hz, $J_{f'\text{-}e}$= 5.6 Hz, $H_{f'}$), 3.30-3.27 (A part of an AB system, 1H, $J_{A\text{-}B}$= 13.2 Hz, $H_{1'a}$), 2.83-2.80 (B part of an system, 1H, $J_{A\text{-}B}$= 13.2 Hz, $H_{1'b}$), 2.94-2.44 (X part of an AMXY system, 1H, $J_{X\text{-}Y}$= 16 Hz, $J_{X\text{-}A}$= 3.2 Hz, H_2), 2.3 (bs, 1H, OH), 2.09-2.05 (Y part of an AMXY system, 1H, $J_{Y\text{-}X}$= 16.0 Hz, $J_{Y\text{-}A}$= 3.2 Hz, $H_{2'}$), 1.46 (s, 3H, $C(CH_3)_2$), 1.35 (s, 3H, $C(CH_3)_2$), 1.31 (s, 3H, $C(CH_3)_2$), 1.28 (s, 3H, $C(CH_3)_2$). **^{13}C NMR** (50MHz, $CDCl_3$): δ 163.2, 156.5, 137.0, 128.7 (2C), 128.5 (2C), 128.3, 109.6, 109.2, 102.0, 95.6, 78.9, 73.7, 73.0, 72.1 (2C), 70.3, 69.6, 66.0, 60.8, 35.1, 33.5, 26.4, 25.9, 25.3, 24.6. **M.p.**: 72-75° C. **$[\alpha]_D^{25}$** +1.15 (c 0.695, $CHCl_3$). **ESI-MS** 573.18 $[M+Na]^+$, 589.09 $[M+K]^+$.

Synthesis of compound 48:



To an ice-cooled solution of **47** (450 mg, 0.817 mmol) in a 2:1 mixture of $CHCl_3$:CH_3OH (12 mL), a 10% solution of HCl (1.2 mL) was added. The reaction mixture was stirred overnight at rt, then neutralized with NEt_3. The solvent was removed under reduced pressure and the crude obtained was purified by flash chromatography on silica gel (EtOAc : CH_3OH = 8:1) to give **48** (366 mg, 95%) as a white solid. Compund **15** was fully characterized as peracetyl derivative **49**.

Synthesis of compound 49:



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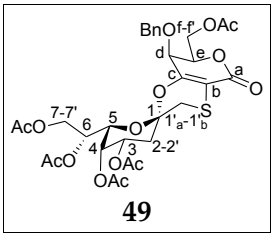
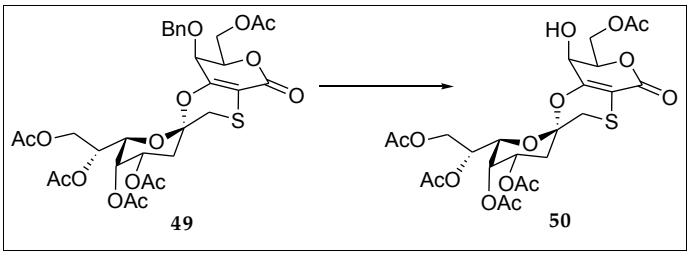
To a stirred solution of **48** (366 mg, 0.778 mmol) in CH_2Cl_2 (3 mL), pyridine (1 mL), acetic anhydride (0.5 mL), and a catalytic amount of DMAP were added. The reaction mixture was stirred at rt overnight, then diluted with CH_2Cl_2 and washed with a saturated solution of NH_4Cl (2 x 15 mL). The organic phase was dried over Na_2SO_4 and concentrated to dryness. The crude product was purified by column chromatography on silica gel (petroleum ether : EtOAc = 1:1) to give **49** (480 mg, 91%) as a white solid.



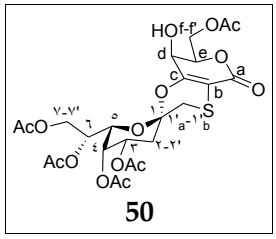
49

^{1}H NMR (400MHz, $CDCl_3$): δ 7.41-7-31 (m, 5H, Ph), 5.41-5.37 (M part of an AMXY system, 1H, $J_{M\text{-}X}$= 12.0 Hz, $J_{M\text{-}Y}$= 5.6 Hz, $J_{M\text{-}A}$= 3.2 Hz, H_3), 5.32 (bs, 1H, H_4), 5.09-5.05 (X part of an ABX system, 1H, $J_{6\text{-}5}$= 7.6 Hz, $J_{X\text{-}A}$= 4.0 Hz, $J_{X\text{-}B}$= 2.0 Hz, H_6), 4.77-4.74 (A part of an AB system, 1H, $J_{A\text{-}B}$= 10.8 Hz, CH_2Ph), 4.72-4.69 (B part of an AB system, 1H, $J_{A\text{-}B}$= 10.8 Hz, CH_2Ph), 4.65-4.61 (X part of an ABX system, 1H, $J_{X\text{-}A}$= $J_{X\text{-}B}$= 6.4 Hz, $J_{e\text{-}d}$= 2.8 Hz, H_e), 4.45-4.40 (A part of an ABX system, 1H, $J_{A\text{-}B}$= 11.6 Hz, $J_{A\text{-}X}$= 6.8 Hz, H_f), 4.41-4.37 (A part of an ABX system, 1H, $J_{A\text{-}B}$= 12.4 Hz, $J_{A\text{-}X}$= 2.4 Hz, H_7), 4.35-4.31 (B part of an ABX system, 1H, $J_{A\text{-}B}$= 11.6 Hz, $J_{B\text{-}X}$= 6.4 Hz, $H_{f'}$), 4.09 (dd, 1H, $J_{d\text{-}e}$= 2.8 Hz, H_d), 4.09-4.06 (dd, 1H, $J_{5\text{-}6}$= 6.8 Hz, $J_{5\text{-}4}$= 1.2 Hz, H_5), 3.96-3.92 (B part of an ABX system, 1H, $J_{A\text{-}B}$= 12.4 Hz, $J_{B\text{-}X}$= 4.4 Hz, $H_{7'}$), 3.03-3.00 (A part of an AB system, 1H, $J_{A\text{-}B}$= 13.2 Hz, $H_{1'a}$), 2.91-2.88 (B part of an AB system, 1H, $J_{A\text{-}B}$= 13.2 Hz, $H_{1'b}$), 2.16-2.05 (m, 2H, H_2, $H_{2'}$), 2.15 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 1.97 (s, 3H, CH_3). **^{13}C NMR** (50MHz, $CDCl_3$): δ 170.1, 169.9, 169.7, 169.5, 169.2, 161.7, 154.0, 135.9, 128.5 (2C), 128.4, 128.3 (2C), 103.0, 95.6, 75.5, 75.3, 70.8, 69.2, 67.3, 65.9, 64.0, 61.6, 61.4, 33.4 (2C), 20.7, 20.67, 20.62 (2C), 20.2. **M.p.**: 78-80° C. **$[\alpha]_D^{25}$** +6.8 (c 0.6, $CHCl_3$). **ESI-MS** 703.2 $[M+Na]^+$, 719.2 $[M+K]^+$.

Synthesis of compound 50:

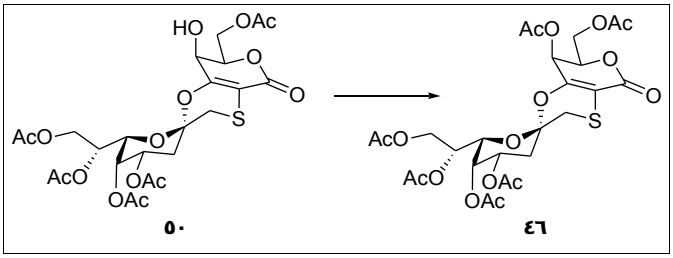


To an ice-cooled solution of **49** (180 mg, 0.264 mmol) in CH_2Cl_2 (15 mL), $FeCl_3$ (428 mg, 2.64 mmol) was added under Ar atmosphere. The reaction mixture was warmed to rt and stirred for 1h, then was diluted with CH_2Cl_2 and washed with water. The organic phase was dried over Na_2SO_4 and concentrated to dryness. The crude was purified by column chromatography on silica gel (CH_2Cl_2 : Acetone = 10:1) to afford **50** (114 mg, 73%) as a glassy solid.

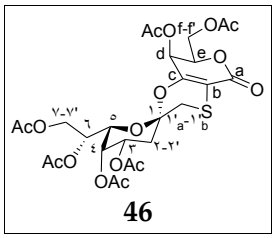


^{1}H NMR (400MHz, $CDCl_3$): δ 5.15-5.46 (M part of AMXY system, 1H, $J_{M\text{-}X}$= $J_{M\text{-}Y}$= 8.0 Hz, $J_{M\text{-}A}$= 2.8 Hz, H_3), 5.42-5.41 (A part of an AMXY system, 1H, $J_{A\text{-}M}$= 2.8 Hz, $J_{4\text{-}5}$= 1.6 Hz, H_4), 5.07-5.03 (X part of an ABX system, 1H, $J_{X\text{-}A}$= $J_{X\text{-}B}$= 3.2 Hz, $J_{6\text{-}5}$= 10.0 Hz, H_6), 4.58-4.51 (m, 2H, H_e, H_f), 4.45 (A part of ABX system, 1H, $J_{A\text{-}B}$= 12.4 Hz, $J_{A\text{-}X}$= 3.2 Hz, H_7), 4.38-4.34 (B part of an ABX system, 1H, $J_{A\text{-}B}$= 10.4 Hz, $J_{B\text{-}X}$= 5.6 Hz, $H_{f'}$), 4.25 (dd, 1H, $J_{5\text{-}6}$= 10.0 Hz, $J_{5\text{-}4}$= 1.6 Hz, H_5), 4.18-4.14 (B part of ABX system, 1H, $J_{A\text{-}B}$= 12.4 Hz, $J_{B\text{-}X}$= 3.2 Hz, $H_{7'}$) 4.14 (d, 1H, $J_{d\text{-}e}$= 3.2 Hz, H_d), 3.08-3.04 (A part of AB system, 1H, $J_{A\text{-}B}$= 13.2 Hz, $H_{1'a}$), 2.97-2.94 (B part of an AB system, 1H, $J_{A\text{-}B}$= 13.2 Hz, $H_{1'b}$), 2.62 (bs, 1H, OH), 2.13 (s, 3H, CH_3), 2.11-2.09 (m, 2H, H_2, $H_{2'}$), 2.09 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.01 (s, 3H, CH_3), 1.98 (s, 3H, CH_3). **^{13}C NMR** (50MHz, $CDCl_3$): δ 70.9, 170.7, 169.9, 169.8, 169.4, 162.2, 155.1, 102.9, 94.5, 76.3, 69.4, 67.5, 66.0, 64.3, 64.2, 61.9, 61.7, 33.9, 33.4, 20.8 (2C), 20.7 (3C). **[α]$_D^{25}$** +79 (c 0.365, $CHCl_3$). **ESI-MS** 613.08 $[M+Na]^+$, 629.0 $[M+K]^+$.

Synthesis of compound 46:



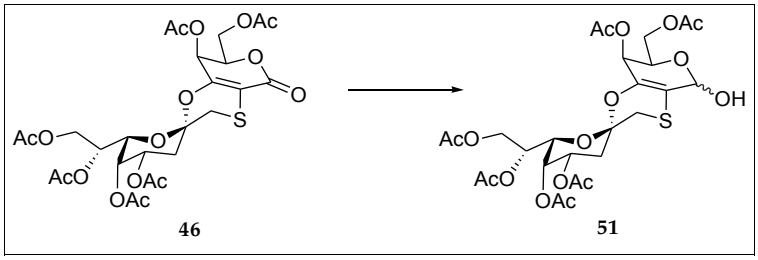
To a stirred solution of **50** (114 mg, 0.193 mmol) in CH_2Cl_2 (3 mL), pyridine (0.5 mL), acetic anhydride (0.25 mL) and a catalytic amount of DMAP were added. The reaction mixture was stirred at rt overnight, then diluted with CH_2Cl_2 and washed with a saturated solution of NH_4Cl (2 x 15 mL). The organic phase was dried over Na_2SO_4 and concentrated to dryness. The crude product was purified on column chromatography on silica gel (CH_2Cl_2 : Acetone = 10:1) to give **46** (120 mg, 99%) as a white solid.



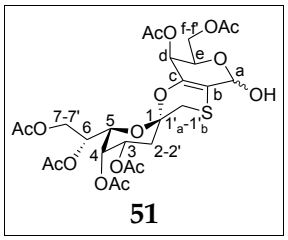
46

^{1}H NMR (400MHz, $CDCl_3$): δ 5.66 (d, 1H, $J_{d\text{-}e}$= 2.8 Hz, H_d), 5.33 (bs, 1H, H_4), 5.32-5.27 (M part of an AMXY system, 1H, $J_{M\text{-}Y}$= 11.6 Hz, $J_{M\text{-}X}$= 5.2 Hz, $J_{M\text{-}A}$= 3.2 Hz, H_3), 5.14-5.09 (X part of an ABX system, 1H, $J_{6\text{-}5}$= 10 Hz, $J_{X\text{-}B}$= 4.8 Hz, $J_{X\text{-}A}$= 2.4 Hz, H_6), 4.82-4.78 (X part of ABX system, 1H, $J_{X\text{-}A}$= $J_{B\text{-}X}$= 6.8 Hz, $J_{e\text{-}d}$= 2.8 Hz, H_e), 4.38-4.34 (A part of an ABX system, 1H, $J_{A\text{-}B}$= 12.4 Hz, $J_{A\text{-}X}$= 2.4 Hz, H_7), 4.34-4.30 (A part of an ABX system, 1H, $J_{A\text{-}B}$= 11.2 Hz, $J_{A\text{-}X}$= 6.4 Hz, H_f), 4.23-4.19 (B part of an ABX system, 1H, $J_{A\text{-}B}$= 11.6 Hz, $J_{B\text{-}X}$= 6.8 Hz, $H_{f'}$), 4.06-4.02 (B part of an ABX system, 1H, $J_{A\text{-}B}$= 12.4 Hz, $J_{B\text{-}X}$= 5.2 Hz, $H_{7'}$), 3.84 (dd, 1H, $J_{5\text{-}6}$= 10.0 Hz, $J_{5\text{-}4}$= 1.2 Hz, H_5), 3.06-3.02 (A part of an AB system, 1H, $J_{A\text{-}B}$= 13.6 Hz, $H_{1'a}$), 2.95-2.92 (B part of an AB system, 1H, $J_{A\text{-}B}$= 13.6 Hz, $H_{1'b}$), 2.16 (s, 3H, CH_3), 2.14-2.03 (m, 2H, H_2, $H_{2'}$), 2.08 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.01 (s, 3H, CH_3), 1.96 (s, 6H, CH_3). **^{13}C NMR** (50MHz, $CDCl_3$): δ 169.9, 169.8, 169.7, 169.3, 169.1, 168.8, 161.1, 151.3, 105.1, 95.3, 74.1, 69.5, 67.0, 65.8, 64.0, 63.3, 62.0, 60.4, 33.3, 33.1, 20.6, 20.5 (3C), 20.4, 20.3. **M.p.**: 174-175 °C. **$[\alpha]_D^{25}$** +25.11 (c 0.36, $CHCl_3$). **ESI-MS** 655.2 $[M+Na]^+$, 671.1 $[M+K]^+$.

Synthesis of compound 51:

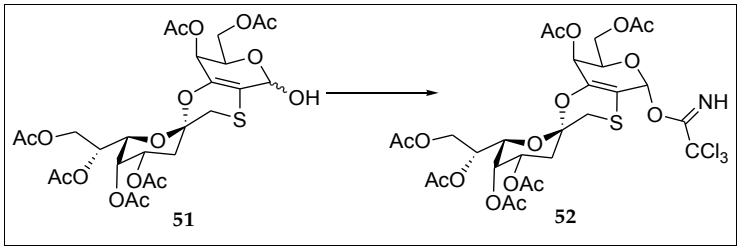


A solution of **46** (123 mg, 0.194 mmol), in dry CH_2Cl_2 (5.8 mL) was cooled to -78°C and a Red/Al solution (990 µL), freshly prepared by addition of ethanol (103 µL) to an ice-cooled solution of 3.4M Red-Al (500 µL) in dry toluene (1 mL), was added dropwise. After 1h at -78°C, 2 drops of CH_3OH and a 20% aqueous solution of potassium and sodium tartrate (20 mL) was added. The mixture was then diluted with CH_2Cl_2 (20 mL) and stirred for 10 min at rt. The organic phase was dried over Na_2SO_4 and concentrated to dryness. The crude was purified by flash column chromatography on silica gel (petroleum ether : EtOAc = 2:3) to afford **51** as an oily mixture of anomers (115 mg, 93%).

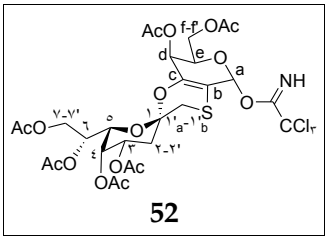


^{1}H NMR (400MHz, $CDCl_3$), selected data: δ 5.43-5.4 (m, 2H), 5.33 (dd, 1H, *J*= 1.4 Hz, *J*= 2.8 Hz), 5.30-5.21 (m, 1H), 4.67-4.59 (td, 1H, *J*= 2.2 Hz, *J*= 6.6 Hz), 3.59 (d, 1H, *J*= 4.0 Hz); **^{13}C NMR** (50MHz, $CDCl_3$): δ 170.8, 170.3, 170.0, 169.5, 169.4 (2C), 137.8, 109.4, 93.2 (C-anom. β), 93.1, 90.1 (C-anom. α), 68.0, 67.4, 67.3, 66.4, 64.4, 642, 62.0, 61.8, 33.8, 33.6, 20.79 (3C), 20.7 (3C). **M.p.**: 90-92 °C. **ESI-MS** 657.1 $[M+Na]^+$, 673.1 $[M+K]^+$.

Synthesis of compound 52:

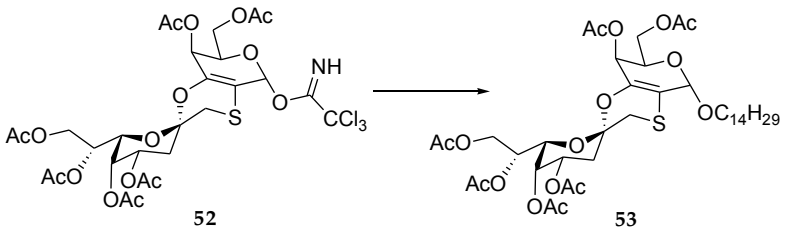


To a solution of **51** (85 mg, 0.134 mmol) in dry CH_2Cl_2 (5 mL), cooled to 0°C, DBU (20 µL, 0.201 mmol) and tricloroacetonitrile (400 µL, 2.68 mmol) were added. After 1 h at 0°C the solution was concentrated to dryness. The residue was dissolved in CH_2Cl_2 and then filtered through a pad of silica gel (petroleum ether : EtOAc = 2:3) to afford 93 mg of a mixture of **51** and **52** in a 7.8:1 ratio. Compound **19** was partially separated from the mixture and totally characterized.

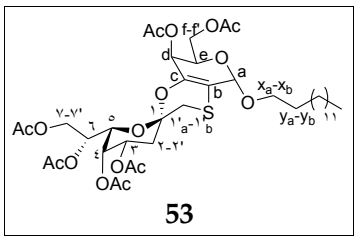


^{1}H NMR (400MHz, $CDCl_3$): δ 7.12 (d, 1H, $J_{NH\text{-}a}$= 7.2 Hz, NH), 5.82 (d, 1H, $J_{a\text{-}NH}$= 7.2 Hz, H_a), 5.46 (d, 1H, $J_{d\text{-}e}$= 2.4 Hz, H_d), 5.36-5.35 (A part of an AMXY system, 1H, $J_{4\text{-}5}$= 1.6 Hz, $J_{A\text{-}M}$= 2.8 Hz, H_4), 5.29-5.24 (m, 1H, H_3), 5.15-5.11 (X part of on ABX system, 1H, $J_{X\text{-}A}$= 2.4 Hz, $J_{X\text{-}B}$= 3.2 Hz, $J_{6\text{-}5}$= 10.0 Hz, H_6), 4.38-4.34 (X part of an ABX system, 1H, $J_{e\text{-}d}$= 2.4 Hz, $J_{X\text{-}A}$= 6.0 Hz, $J_{X\text{-}B}$= 7.6 Hz, H_e), 4.32-4.28 (A part of an ABX system, $J_{A\text{-}X}$= 2.4 Hz, $J_{A\text{-}B}$= 12.4 Hz, H_7), 4.26-4.22 (B part of an AB system, $J_{B\text{-}X}$= 3.0 Hz, $J_{A\text{-}B}$= 13.0 Hz, $H_{7'}$), 4.23-4.19 (A part of an ABX system, 1H, $J_{A\text{-}X}$= 6.0 Hz, $J_{A\text{-}B}$= 11.6 Hz, H_f), 4.11-4.07 (B part of an ABX system, 1H, $J_{B\text{-}X}$= 7.2 Hz, $J_{A\text{-}B}$= 11.6 Hz, $H_{f'}$), 3.96 (dd, 1H, $J_{5\text{-}4}$= 1.2 Hz, $J_{5\text{-}6}$= 10.0 Hz, H_5), 3.06-3.02 (A part of an AB system, 1H, $J_{A\text{-}B}$= 12.8 Hz, $H_{1'a}$), 2.99-2.96 (B part of an AB system, 1H, $J_{A\text{-}B}$= 12.8 Hz, $H_{1'b}$), 2.19 (s, 3H, CH_3), 2.13 (s, 3H, CH_3), 2.11-1.99 (m, 2H, H_2, $H_{2'}$), 2.015 (s, 3H, CH_3), 2.011 (s, 3H, CH_3), 1.98 (s, 3H, CH_3). **^{13}C NMR** (50MHz, $CDCl_3$): δ 170.7, 170.5, 170.1, 170.2, 169.7, 169.6, 161.5, 138.8, 106.7, 98.3, 93.4, 76.2, 69.1, 68.4, 67.4, 66.3, 64.3, 63.8, 61.8, 61.4, 33.7, 33.5, 20.7 (3C), 20.6 (3C). **$[\alpha]_D^{25}$** +28.81 (c 0.2, $CHCl_3$). **ESI-MS** 799.9 $[M+Na]^+$, 816.1 $[M+K]^+$.

Synthesis of compound 53 (method A):

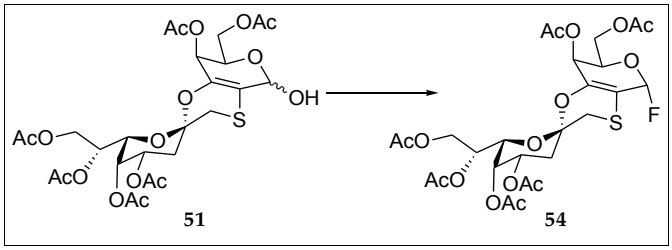


A 7.8:1 ratio solution of **51** and **52** (93 mg) and $C_{14}H_{29}OH$ (50 mg, 0.232 mmol) in dry CH_2Cl_2 (4 mL) was cooled to -30°C and 243 µL of TMSOTf (0.1M in CH_2Cl_2) were added. The reaction mixture was stirred for 30 min at -30°C, then 50 µL of TMSOTf (0.1M in CH_2Cl_2) were added. After 30 min at –30° C the solution was neutralized with NEt_3 and concentrated to dryness. The crude was purified by flash column chromatography on silica gel (petroleum ether : EtOAc = 1:1) to give **53** (20 mg, 18% over two steps) and unreacted **52** (27 mg, 0.042 mmol).

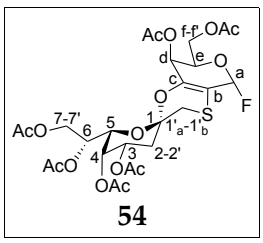


^{1}H NMR (400MHz, $CDCl_3$): δ 5.38 (d, 1H, $J_{d\text{-}e}$= 2.8 Hz, H_d), 5.35-5.34 (A part of an AMXY system, 1H, $J_{4\text{-}5}$= 1.6 Hz, $J_{A\text{-}M}$= 3.2 Hz, H_4), 5.28-5.23 (M part of an AMXY system, 1H, $J_{M\text{-}A}$= 2.8 Hz, $J_{M\text{-}X}$= $J_{M\text{-}Y}$= 8.4 Hz, H_3), 5.14-5.10 (X part of an ABX system, 1H, $J_{X\text{-}A}$= 2.8 Hz, $J_{6\text{-}5}$= 9.6 Hz, H_6), 4.99 (s, 1H, H_a), 4.52-4.48 (X part of an ABX system, 1H, $J_{e\text{-}d}$= 2.4 Hz, $J_{X\text{-}A}$= 5.6 Hz, $J_{X\text{-}B}$= 7.2 Hz, H_e), 4.26-4.22 (A part of an ABX system, 1H, $J_{A\text{-}X}$= 3.6 Hz, $J_{A\text{-}B}$= 12.4 Hz, H_7), 4.22-4.19 (B part of an ABX system, 1H, $J_{B\text{-}X}$= 4.4 Hz, $J_{A\text{-}B}$= 10.0 Hz, $H_{7'}$), 4.20-4.16 (A part of an ABX system, 1H, $J_{A\text{-}X}$= 6.0 Hz, $J_{A\text{-}B}$= 11.6 Hz, H_f), 4.12-4.07 (B part of an ABX system, 1H, $J_{B\text{-}X}$= 7.2 Hz, $J_{A\text{-}B}$= 11.2 Hz, $H_{f'}$), 3.98 (dd, 1H, $J_{5\text{-}4}$= 1.6 Hz, $J_{5\text{-}6}$= 10.0 Hz, H_5), 3.75-3.67 (m, 1H, H_{xa}), 3.53-3.45 (m, 1H, H_{xb}), 3.03-3.00 (A part of an AB system, 1H, $J_{A\text{-}B}$= 12.8 Hz, $H_{1'a}$), 2.98-2.95 (B part of an AB system, 1H, $J_{A\text{-}B}$= 12.8 Hz, $H_{1'b}$), 2.17 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.003 (s, 3H, CH_3), 2.005 (s, 3H, CH_3), 1.98-1.97 (m, 2H, H_2, $H_{2'}$), 1.96 (s, 3H, CH_3), 1.64 (m, 1H, H_{Ya}), 1.36-1.24 (bs, 23H, CH_2), 0.87 (t, 3H, J= 6.8 Hz, CH_3 *chain*). **^{13}C NMR** (50MHz, $CDCl_3$): δ 170.7, 170.1, 170.0, 169.5 (3C), 137.3, 109.1, 95.7, 93.0, 69.0, 68.0, 67.48, 67.47, 66.46, 64.4, 62.0, 61.8, 33.9, 33.7, 29.7, 29.6 (4C), 29.47 (3C), 29.42 (3C), 26.2, 22.7, 20.79 (3C), 20.71 (3C), 14.2. **$[\alpha]_D^{25}$** +27.7 (c 0.155, $CHCl_3$). **ESI-MS** 853.1 $[M+Na]^+$, 869.1 $[M+K]^+$.

Synthesis of compound 54:

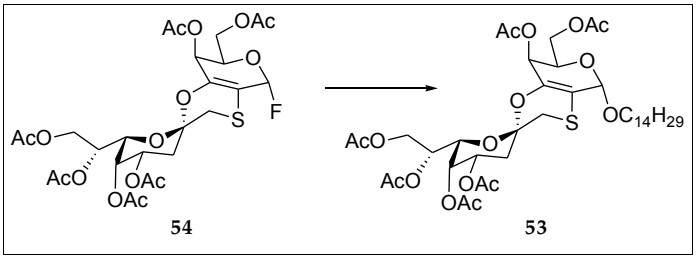


A solution of **51** (100 mg, 0.157 mmol), in dry CH2Cl2 (3.60 mL) was cooled to -40°C and 82 µL of DAST (0.630 mmol) were added. After 30′ at -40°C, 2 drops of CH_3OH and a saturated solution of $NaHCO_3$ (10 mL) were added. Then the mixture was diluted with CH_2Cl_2 (20 mL) and stirred for 10 min at RT. The organic phase was washed with brine (1 x 10 mL), dried over Na_2SO_4 and concentrated to dryness. The crude was purified by flash column chromatography on silica gel (petroleum ether : EtOAc = 2:3) to afford **54** (86 mg, 0.135 mmol, 87%) as glassy solid.



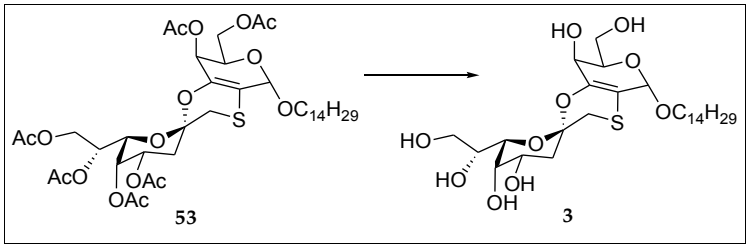
^{1}H NMR (400MHz, $CDCl_3$): δ 5.75 (d, 1H, $J_{a\text{-}F}$= 57.6 Hz, H_a), 5.49-5.47 (dd, 1H, $J_{d\text{-}e}$= 2.4 Hz, $J_{d\text{-}F}$= 3.8 Hz, H_d), 5.36-5.35 (A part of an AMXY system, 1H, $J_{4\text{-}5}$= 1.6 Hz, $J_{A\text{-}M}$= 2.8 Hz, H_4), 5.30-5.24 (M part of an AMXY system, 1H, $J_{M\text{-}A}$= 3.2 Hz, $J_{M\text{-}X}$= 6.8 Hz, $J_{M\text{-}Y}$= 10.4 Hz, H_3), 5.14-5.11 (X part of an ABX system, 1H, $J_{X\text{-}A}$= $J_{X\text{-}B}$= 2.4 Hz, $J_{6\text{-}5}$= 9.6 Hz, H_6), 4.63-4.60 (m, 1H, H_e), 4.32-4.28 (A part of an ABX system, 1H, $J_{A\text{-}X}$= 3.6 Hz, $J_{A\text{-}B}$= 12.4 Hz, H_7), 4.27-4.23 (A part of an ABX system, 1H, $J_{A\text{-}X}$= 6.0 Hz, $J_{A\text{-}B}$= 11.6 Hz, H_f), 4.19-4.16 (B part of an ABX system, 1H, $J_{B\text{-}X}$= 2.4 Hz, $J_{A\text{-}B}$= 12.8 Hz, $H_{7'}$), 4.16-4.12 (B part of an ABX system, 1H, $J_{B\text{-}X}$= 7.2 Hz, $J_{A\text{-}B}$= 11.6 Hz, $H_{f'}$), 3.93 (dd, 1H, $J_{5\text{-}4}$= 1.6 Hz, $J_{5\text{-}6}$= 10.0 Hz, H_5), 3.06-3.02 (A part of an AB system, 1H, $J_{A\text{-}B}$= 13.2 Hz, $H_{1'a}$), 3.01-2.98 (B part of an AB system, 1h, $J_{A\text{-}B}$= 13.2 Hz, $H_{1'b}$), 2.18 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.06-1.99 (m, 2H, H_2, $H_{2'}$), 2.01 (s, 3H, CH_3), 1.98 (s, 3H, CH_3). **^{13}C NMR** (50MHz, $CDCl_3$): δ 170.4, 170.1, 169.9, 169.3 (2C), 169.1, 138.8 (d, 1C, $J_{C\text{-}F}$= 27.4 Hz), 106.8 (d, 1C, $J_{C\text{-}F}$ = 121.2 Hz), 102.8 (d, 1C, J= 218.5 Hz), 93.4, 69.7, 68.2, 67.2, 66.2, 64.2, 63.4, 61.8, 61.06, 33.7, 33.6, 20.7 (6C) . **$[\alpha]_D^{25}$** + 12.7 (c 1.095, CH_2Cl_2) **ESI-MS** 659.1 $[M+Na]^+$, 675.1 $[M+K]^+$.

Synthesis of compound 53 (method B):

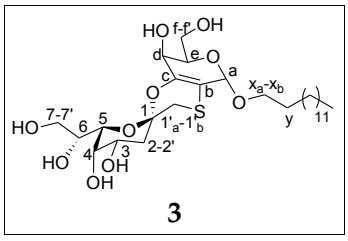


A solution of **54** (86 mg, 0.135 mmol) and $C_{14}H_{29}OH$ (58 mg, 0.270 mmol), in dry CH_2Cl_2 (15.0 mL) was cooled to -40°C and Cp_2HfCl_2 (92 mg, 0.243 mmol) and AgOTf (134 mg, 0.522 mmol) were added. After 40 min at –40° C the solution was neutralized with NEt_3 and filtered through a pad of Celite. The filtrate was washed with a saturated solution of $NaHCO_3$ (1 x 10 mL) and with brine (1 x 10 mL). The organic phase was dried over Na_2SO_4 and concentrated to dryness to give 130mg of crude. The crude was purified by flash column chromatography on silica gel to give **53** (47 mg, 42%), (petroleum ether : EtOAc = 2:1) and 15.6 mg of a secondary product.

Synthesis of thioether 3:

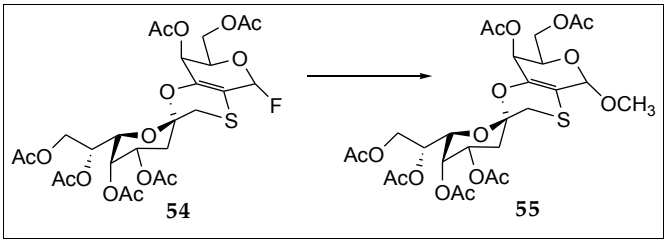


To a stirred solution of **53** (19 mg, 0.022 mmol) in a 1:1 mixture of $CHCl_3$:CH_3OH (1.6 mL), 285 µL of a 0.05 M solution of CH_3ONa in CH_3OH was added. The mixture was stirred for 5 h, then was neutralized with a 10% solution of HCl in CH_3OH. Evaporation of the solvent under vacuum gave a crude that was purified by flash chromatography on silica gel (EtOAc : CH_3OH = 6:1) to afford **3** (10 mg, 77%) as a glassy solid.

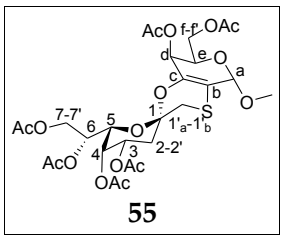


^{1}H NMR (400MHz, CD_3OD): δ 4.95 (s, 1H, H_a), 4.16-4.11 (M part of an AMXY system, 1H, $J_{M\text{-}A}$= 2.8 Hz, $J_{M\text{-}X}$= 7.6 Hz, $J_{M\text{-}Y}$= 9.6 Hz, H_3), 4.08-4.05 (X part of an ABX system, 1H, J= 2.0 Hz, J= 5.2 Hz, J= 6.4 Hz, H_e), 4.017-4.012 (A part of an AMXY system, 1H, $J_{4\text{-}5}$= 0.4 Hz, $J_{A\text{-}M}$= 2.4 Hz, H_4), 3.93 (dd, 1H, $J_{5\text{-}4}$= 0.8 Hz, $J_{5\text{-}6}$= 9.6 Hz, H_5), 3.81-3.60 (m, 7H, H_d, H_6, H_7, $H_{7'}$, H_f, $H_{f'}$,H_{xa}), 3.50-3.44 (m, 1H, H_{xb}), 3.03-3.00 (A part of an AB system, 1H, $J_{A\text{-}B}$= 13.2 Hz, $H_{1'a}$), 2.98-2.95 (B part of an AB system, 1H, $J_{A\text{-}B}$= 13.2 Hz, $H_{1'b}$), 1.93-1.90 (m, 2H, H_2, $H_{2'}$), 1.59-1.54 (m, 2H, CH_{2y}), 1.40-1.25 (m, 23H, CH_2 chain), 0.89 (t, 3H, J= 6.4 Hz, CH_3). **^{13}C NMR** (50MHz, CD_3OD): δ 144.0, 108.7, 98.5, 94.5, 74.7, 73.0, 70.6, 70.2, 68.6, 68.4, 66.7, 65.0, 63.1, 38.4, 35.5, 31.63 (4C), 31.60 (4C), 31.5, 31.3, 28.1, 24.6, 15.3; **$[\alpha]_D^{25}$** +55.58 (c 0.104, CH_3OH). **ESI-MS** 601.2 $[M+Na]^+$, 617.2 $[M+K]^+$.

Synthesis of compound 55:

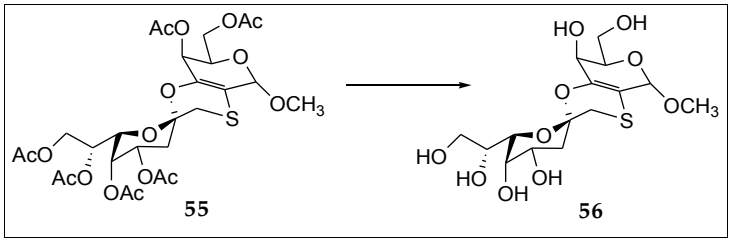


To a mixture of **54** (31 mg, 0.049 mmol), Cp_2HfCl_2 (33 mg, 0.088 mmol) and AgOTf (49 mg, 0.189 mmol) cooled to –40°C, dry CH_2Cl_2 (5 mL) and dry CH_3OH (40 µL, 0.98 mmol) were added. The reaction mixture was stirred at –40°C for 40 minutes, then was neutralized with a solution of Et_3N in CH_2Cl_2, diluted with CH_2Cl_2, warmed to room temperature and filtered through a pad of celite. The filtrate was then washed with a saturated solution of $NaHCO_3$ (1 x 10 mL) and brine (2 x 10 mL). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography on silica gel (petroleum ether : EtOAc = 1 : 1) to afford **55** (27 mg, 0.042 mmol, 85%) as glassy solid.

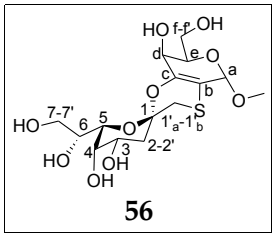


^{1}H NMR (400MHz, $CDCl_3$): δ 5.39 (d, 1H, $J_{d\text{-}e}$= 2.8 Hz, H_d), 5.35-5.34 (dd, 1H, $J_{4\text{-}5}$= 1.2 Hz, $J_{4\text{-}3}$= 2.8 Hz, H_4), 5.28-5.23 (m, 1H, H_3), 5.12 (adt, 1H, $J_{6\text{-}7}$=$J_{6\text{-}7'}$= 3.2 Hz, $J_{6\text{-}5}$= 10.0 Hz, H_6), 4.91 (s, 1H, H_a), 4.50-4.46 (m, 1H, H_e), 4.27-4.24 (X part of an AXY system, 1H, J_{XA} = 3.2 Hz, J_{XY} = 12.4 Hz, H_7), 4.22-4.18 (Y part of an AXY system, 1H, J_{YA}= 3.2 Hz, J_{YX} = 12.4 Hz, $H_{7'}$), 4.21-4.16 (X part of an AXY system, 1H, J_{XA} = 5.6 Hz, J_{XY} = 11.2 Hz, H_f), 4.14-4.10 (Y part of an AXY system, 1H, J_{YA} = 7.6 Hz, J_{YB}= 11.2 Hz, $H_{f'}$), 3.97 (dd, 1H, $J_{5\text{-}4}$ = 1.2 Hz, $J_{5\text{-}6}$ = 10.0 Hz, H_5), 3.44 (s, 3H, OCH_3), 2.98 (A_2 system, 2H, $H_{1'a}$, $H_{1'b}$), 2.17 (s, 3H, CH_3), 2.12 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.00 (s, 3H, CH_3), 1.99-1.98 (m 2H, H_2, $H_{2'}$), 1.96 (s, 3H, CH_3). **^{13}C NMR** (50MHz, $CDCl_3$): δ 170.6, 170.1, 169.9, 169.4, 169.3 (2C), 137.4, 108.5, 96.4, 92.9, 67.8, 67.2, 67.1, 66.2, 64.1, 64.1, 61.8, 61.5, 55.6, 33.5, 33.4, 20.5 (3C), 20.4 (3C). **[α]$_D^{23}$** +29.33 (c 1.27, CH_2Cl_2). **ESI-MS** 671.2 $[M+Na]^+$, 687.1 $[M+K]^+$.

Synthesis of compound 56:

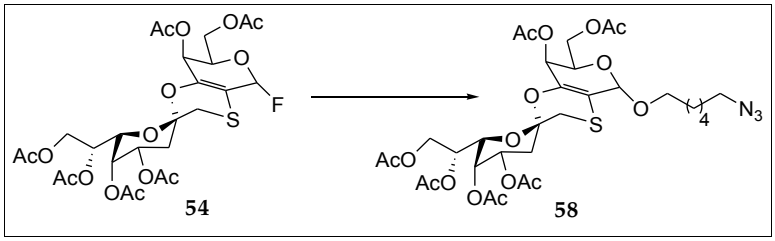


To a solution of **55** (15.5 mg, 0.024 mmol) in CH_3OH (1.5 mL) a 0.1 M solution of $NaOCH_3$ in CH_3OH (0.09 mL, 0.009 mmol) was added. The reaction mixture was stirred at room temperature for 3.5 h, then was diluted with CH_3OH and neutralized with a 10% solution of HCl in CH_3OH. The solvent was removed under reduced pressure and the crude was purified by flash column chromatography on silica gel (EtOAc : CH_3OH = 5 : 1) to afford **56** (8 mg, 0.022 mmol, 90%) as glassy solid.

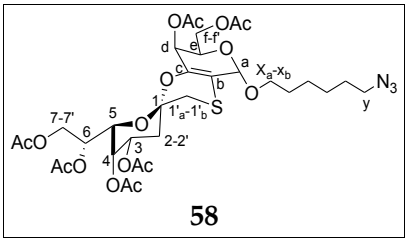


^{1}H NMR (400MHz, CD_3OD): δ 4.87 (s, 1H, H_a), 4.16-4.11 (m, 1H, H_3), 4.06-4.01 (m, 2H, H_e, H_4), 3.93 (dd, 1H, $J_{5\text{-}6}$= 9.6 Hz, $J_{5\text{-}4}$= 0.8 Hz, H_5), 3.77-3.71 (m, 4H, H_d, H_6, H_f, $H_{f'}$), 3.70-3.67 (X part of an MXY system, 1H, $J_{X\text{-}Y}$ = 11.4 Hz, $J_{X\text{-}M}$ = 3.0 Hz, H_7), 3.65-3.61 (Y part of an MXY system, 1H, $J_{Y\text{-}X}$ = 11.4 Hz, $J_{Y\text{-}M}$ = 3.4 Hz, $H_{7'}$), 3.42 (s, 3H, OCH_3), 3.03-3.0 (A part of an AB system, 1H, J_{AB} = 12.6 Hz, $H_{1'a}$), 2.98-2.95 (B part of an AB system, 1H, J_{BA} = 12.6 Hz, $H_{1'b}$), 1.93-1.91 (m, 2H, H_2, $H_{2'}$). **^{13}C NMR** (50MHz, CD_3OD): δ 141.9, 106.1, 97.3, 92.3, 72.5, 70.9, 68.4, 66.4, 66.2, 64.5, 62.7, 61.0, 54.4, 36.1, 33.2. **$[\alpha]_D^{21}$** +62.50 (c 0.4, CH_3OH). **ESI-MS** 419.1 $[M+Na]^+$.

Synthesis of compound 58:

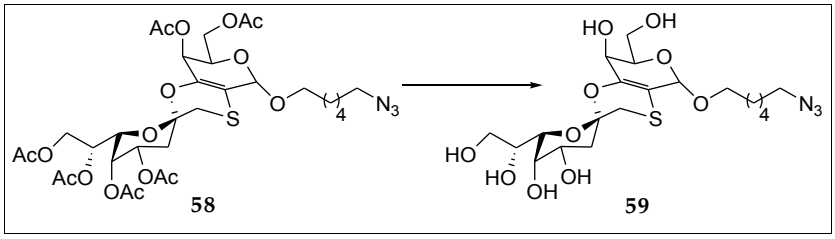


To a mixture of **54** (16 mg, 0.025 mmol), Cp_2HfCl_2 (17 mg, 0.045 mmol), AgOTf (25 mg, 0.097 mmol) and 6-azido-1-hexanol (7 mg, 0.05 mmol) cooled to –40°C, dry CH_2Cl_2 (3 mL) was added. The reaction mixture was stirred at –40°C for 1h, then was neutralized with a solution of Et_3N in CH_2Cl_2, diluted with CH_2Cl_2 (20 mL), warmed to room temperature and filtered through a pad of celite. The filtrate was then washed with a saturated solution of $NaHCO_3$ (1 x 5 mL) and brine (2 x 5 mL). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude was purified by flash column chromatography on silica gel (petroleum ether : EtOAc = 1 : 1) to afford **58** (15 mg, 0.020 mmol, 80%) as glassy solid.

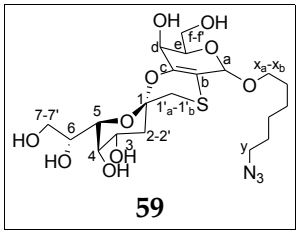


^{1}H NMR (400MHz, $CDCl_3$): δ 5.37 (d, 1H, $J_{d\text{-}e}$= 2.4 Hz, H_d), 5.32 (bs, 1H, H_4), 5.26-5.21 (m, 1H, H_3), 5.12-5.09 (m,1H, H_6), 4.97 (s, 1H, H_a), 4.50-4.46 (m, 1H, H_e), 4.25-4.16 (m, 2H, H_7, $H_{7'}$), 4.18-4.14 (X part of an AXY system, 1H, J_{XA} = 6.4 Hz, J_{XY} = 11.6 Hz, H_f), 4.11-4.06 (Y part of an AXY system, 1H, J_{YA} = 7.6 Hz, J_{YX} = 11.6 Hz, $H_{f'}$), 3.96 (d, 1H, $J_{5\text{-}6}$ = 10.0 Hz, H_5), 3.75-3.69 (m, 1H, H_{xa}), 3.53-3.47 (m 1H, H_{xb}), 3.24 (t, 2H, J = 7.2 Hz, H_y), 3.02-2.98 (A part of an AB system, 1H, J_{AB} = 13.0 Hz, $H_{1'a}$), 2.97-2.94 (B part of an AB system, 1H, J_{BA} = 13.0 Hz, $H_{1'b}$), 2.15 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 2.00 (s, 3H, CH_3), 1.99-1.98 (m 2H, H_2, $H_{2'}$), 1.95 (s, 3H, CH_3), 1.62-1.56 (m, 4H, CH_2CH_2), 1.43-1.36 (m, 4H, CH_2CH_2). **^{13}C NMR** (50MHz, $CDCl_3$): δ 170.8, 170.2, 170.1, 169.6, 169.5 (2C), 137.4, 109.0, 95.7, 93.0, 68.6, 68.0, 67.4, 67.3, 66.4, 64.4 (2C), 62.0, 61.7, 51.3, 33.8, 33.6, 29.3, 28.7, 26.4, 25.7, 20.7 (3C), 20.6 (3C). **$[\alpha]_D^{23}$** +22.31 (c 1.09, CH_2Cl_2). **ESI-MS** 782.2 $[M+Na]^+$, 798.2 $[M+K]^+$.

Synthesis of compound 59:

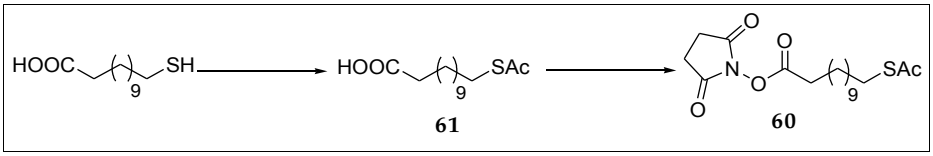


To a solution of **58** (120 mg, 0.158 mmol) in CH_3OH (3.2 mL), $NaOCH_3$ (2 mg, 0.032 mmol) was added. The reaction mixture stirred at rt for 2.5 h, diluted with CH_3OH and neutralized with a 10% solution of HCl in CH_3OH. The solvent was then removed under reduced pressure and the crude obtained was purified by flash column chromatography on silica gel (EtOAc : CH_3OH = 5 : 1) to afford **59** (72 mg, 0.142 mmol, 90%) as glassy solid.

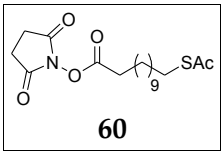


^{1}H NMR (400MHz, CD_3OD): δ 4.97 (s, 1H, H_a), 4.17-4.12 (m, 1H, H_3), 4.09-4.06 (m, 1H, H_e), 4.02 (dd, (dd, 1H, $J_{4\text{-}3}$= 3.2 Hz, $J_{4\text{-}5}$= 1.2 Hz, H_4), 3.94 (dd, 1H, $J_{5\text{-}6}$= 9.6 Hz, $J_{5\text{-}4}$= 1.2 Hz, H_5), 3.84-3.79 (A part of an ABX_2 system, 1H, $J_{A\text{-}B}$ = 10.0 Hz, $J_{A\text{-}X}$ = 6.4 Hz, H_x), 3.77-3.62 (m, 6H, H_d, H_6, H_7, $H_{7'}$, H_f, $H_{f'}$), 3.52-3.47 (B part of an ABX_2 system, 1H, $J_{B\text{-}A}$ = 10.0 Hz, $J_{B\text{-}X}$ = 6.4 Hz, $H_{x'}$), 3.31-3.28 (m, 2H, CH_{2y}), 3.05-3.01 (A part of an AB system, 1H, J_{AB} = 13.0 Hz, $H_{1'a}$), 2.99-2.96 (B part of an AB system, 1H, J_{BA} = 13.0 Hz, $H_{1'b}$), 1.94-1.92 (m, 2H, H_2, $H_{2'}$), 1.64-1.57 (m, 4H, CH_2CH_2), 1.46-1.41 (m, 4H, CH_2CH_2). **^{13}C NMR** (50MHz, CD_3OD): δ 141.6, 106.4, 96.3, 92.3, 72.6, 70.9, 68.5, 67.8, 66.5, 66.3, 64.6, 62.8, 61.1, 51.1, 36.3, 33.4, 29.2, 28.6, 26.3, 25.6. **$[\alpha]_D^{23}$** +51.64 (c 0.25, CH_3OH). **ESI-MS** 530.3 $[M+Na]^+$.

Synthesis of compound 60 :

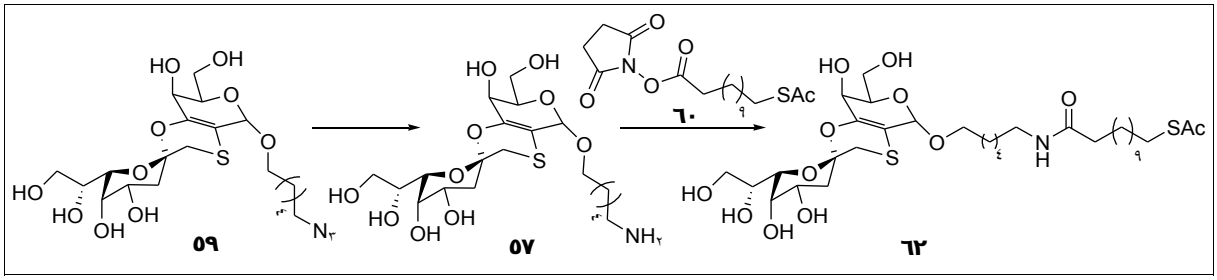


To a solution of 12-mercaptododecanoic acid (290 mg, 1.24 mmol) in dry THF (7 mL), cooled to 0°C, H_2O (7 mL) and NaOH (149 mg, 3.72 mmol) were added. Ac_2O (0.23 mL, 2.48 mmol) was then added and the reaction mixture was warmed to rt and stirred for 4 h. A 10% solution of HCl in CH_3OH was then added to achieve a pH of 4-5. The mixture diluted with Et_2O (30 mL) and the phases were separated. The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude was dissolved in THF (6.5 mL) and NHS (172 mg, 1.49 mmol) was added. The mixture was cooled to 0°C and a solution of DCC (307 mg, 1.49 mmol) in THF (0.5 mL) was added. The mixture was warmed to rt and stirred for 16 h. It was then filtered and the filtate was diluted with Et_2O (30 mL) and washed with H_2O (2 x 10 mL). The organic phase was dried over Na_2SO_4 and the solvent was removed under reduced pressure to afford **60** (414 mg, 1.11 mmol, 90%) as a with solid. The crude was used for the next reaction without any further purification.

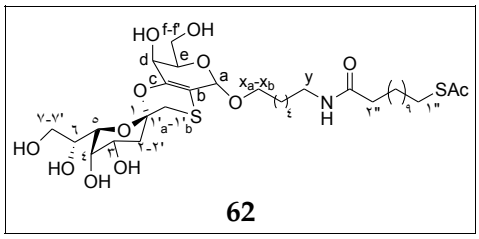


^{1}H NMR (200 MHz, $CDCl_3$): δ 2.86 (t, 2H, *J*= 7.0 Hz, CH_2CO); 2.83 (s, 4H, $COCH_2CH_2CO$); 2.60 (t, 2H, *J*= 7.4 Hz, CH_2S); 2.31 (s, 3H, CH_3); 1.77- 1.26 (m, 18H, CH_2 chain).

Synthesis of compound 62:



To a solution of **59** (31 mg, 0.061 mmol) in a mixture of dry THF (2.4 mL) and dry DMF (0.25 mL), Ph_3P (32 mg, 0.122 mmol) and H_2O (8 µL, 0.008 mmol) were added. The reaction mixture was warmed to 40 °C and stirred for 22 h. The solvent was then removed under reduced pressure and the crude obtained was dissolved in a 2 : 1 mixture of DMF\ H_2O (2.7 mL). $NaHCO_3$ (6 mg, 0.073 mmol) was added and the mixture was cooled to 0°C. A solution of **60** (27 mg, 0.073 mmol) in DMF (0.6 mL) was added dropwise and the reaction mixture was stirred at rt for 22 h. The solvent was then removed under reduced pressure and the crude obtained was purified by flash column chromatography on silica gel (CH_2Cl_2 : CH_3OH = 6 : 1) to afford **62** (25 mg, 55% over two steps) as glassy solid.



62

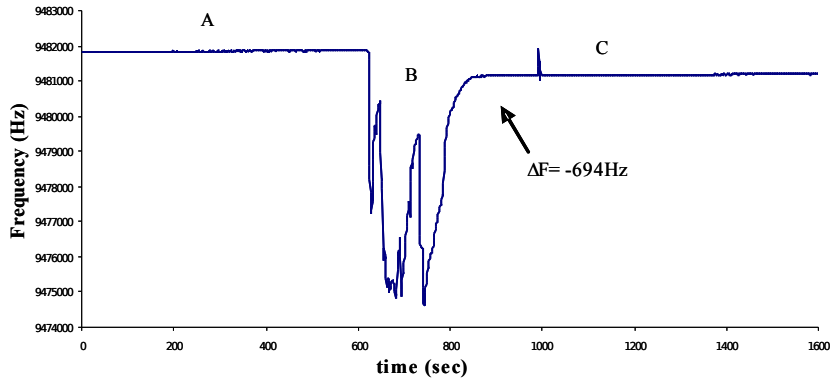
^{1}H NMR (400MHz, CD_3OD):δ 4.96 (s, 1H, H_a), 4.14 (adt, 1H, $J_{3\text{-}4}$= 2.6 Hz, $J_{3\text{-}2}$= $J_{3\text{-}2'}$= *9.6* Hz, H_3), 4.09-4.05 (m, 1H, H_e), 4.02 (dd, 1H, $J_{4\text{-}3}$= 2.6 Hz, $J_{4\text{-}5}$= 0.8 Hz, H_4), 3.94 (dd, 1H, $J_{5\text{-}6}$= 9.6 Hz, $J_{5\text{-}4}$= 0.8 Hz, H_5), 3.82-3.77 (A part of an ABX_2 system, 1H, $J_{A\text{-}B}$ = 9.8 Hz, $J_{A\text{-}X}$ = 6.4 Hz, H_{xa}), 3.76-3.61 (m, 6H, H_d, H_6, H_7, $H_{7'}$, H_f, $H_{f'}$), 3.51-3.45 (B part of an ABX_2 system, 1H, $J_{B\text{-}A}$= 9.6 Hz, $J_{B\text{-}X}$= 6.4 Hz, H_{xb}), 3.16 (t, 2H, *J*= 7.0 Hz, CH_{2y}), 3.04-3.00 (A part of an AB system, 1H, J_{AB} = 13.0 Hz, $H_{1'a}$), 2.98-2.95 (B part of an AB system, 1H, $J_{B\text{-}A}$ = 13.0 Hz, $H_{1'b}$), 2.85 (t, 2H, *J*= 7.0 Hz, $(CH_2)_{2''}$), 2.29 (s, 3H, CH_3), 2.25 (t, 2H, *J*= 7.4 Hz, $(CH_2)_{1''}$), 1.93-1.91 (m, 2H, H_2, $H_{2'}$), 1.59-1.30 (m, 26H, CH_2CH_2). **^{13}C NMR** (50MHz, CD_3OD): δ 196.0, 174.6, 141.6, 106.6, 96.3, 92.3, 72.6, 70.9, 68.4, 67.9, 66.4, 66.3, 64.6, 62.8, 61.0, 39.1, 36.3, 35.9, 33.4, 29.5, 29.3, 29.2, 29.0, 28.6, 28.5, 26.5, 25.9, 25.8. **$[\alpha]_D^{23}$** + 21.16 (c 0.25, CH3OH).

Binding Evaluation of thioether 3 to WGA lectin

The binding interaction of thioether **3** and WGA (Wheat Germ Agglutinin) was evaluated by using a quartz-crystal microbalance (QCM). These data were then compared to those obtained with the GM3 ganglioside.[54]

Preparation of GM3 ganglioside probe monolayer

A solution of GM_3-ganglioside (50μL of a 10 ng/μL solution) in $CHCl_3$:CH_3OH (4:1) was dropped on the gold crystal surface, previously modified with a monolayer of 1-dodecanethiol, outside the cell. The monolayer formation was monitored by the quartz crystal analyzer QCA 922 by SEIKO.[67] After the evaporation of the organic solvent under nitrogen flow, the electrode was housed in a methacrylate cell and washed with PBS until the frequency was stable.

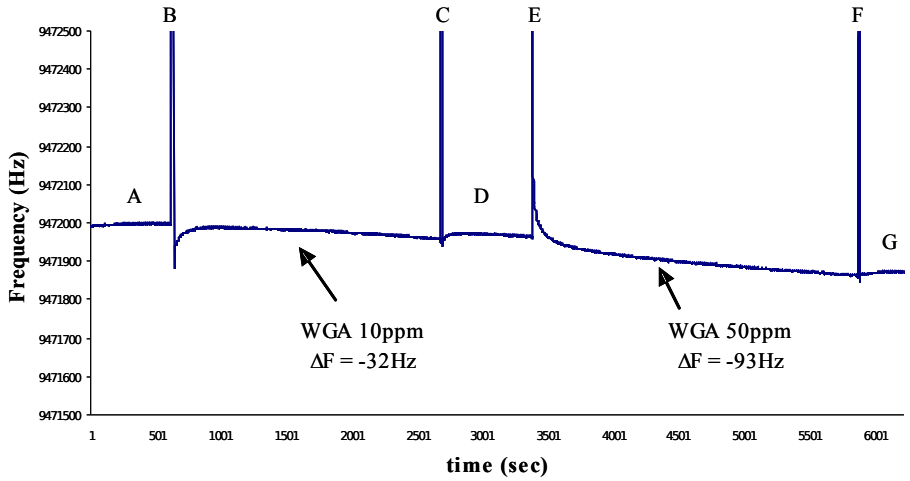


Sensorgram recorded during GM3 ganglioside immobilisation on the gold modified surface of the crystal. A: frequency signal recorded in air. B: frequency signal during GM3-ganglioside dropping. C: frequency signal after organic solvent evaporation.

QCM Measurements

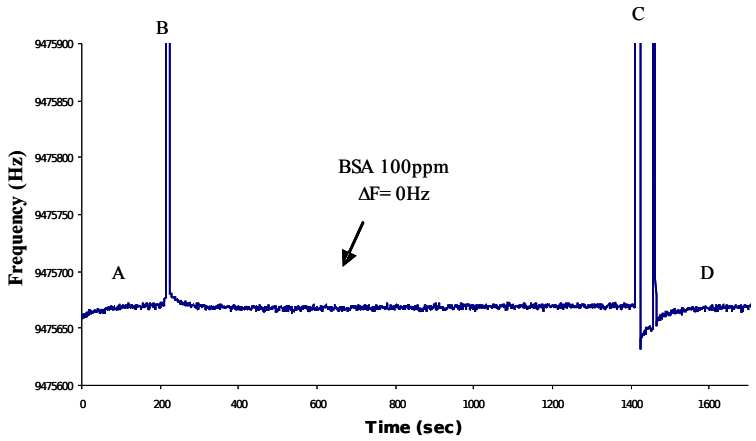
In order to evaluate the molecular recognition with WGA selectin, 200 μL of two different concentrations of WGA were added to the sensor surface previously modified with

the glycosphingolipide GM3 ganglioside monolayer. The interaction was monitored for 30 minutes. The solution was then removed and the surface washed with the PBS buffer to eliminate the unbound proteins. The analytical signal was given by the difference between the frequency recorded after washing with PBS and the frequency displayed before the interaction. This experiment was performed with 10 and 50 ppm of WGA. Figure below shows the sensorgram (frequency versus time) recorded during the experiments. The experiments were performed in triplicate.



Sensorgram recorded during interaction GM3 ganglioside-WGA (10 ppm and 50 ppm). A: baseline in PBS buffer. B: injection of WGA 10ppm. C: washing with PBS buffer. D: baseline in PBS buffer. E: injection of WGA 50 ppm. F: washing with PBS buffer. G: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A and F_G-F_D).

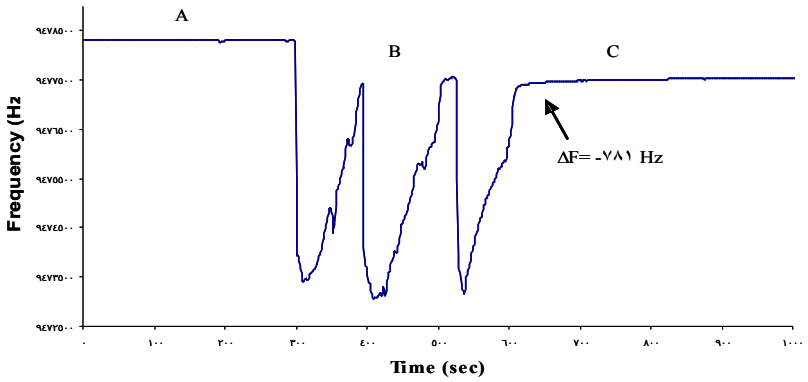
To test the specificity of the surface, 200 µL of a 100 ppm solution of a non specific protein (Bovine Serum Albumine, BSA) was added. The negligible frequency shift demonstrates the specificity of the sensor surface.



Sensorgram recorded during the interaction between GM3-ganglioside and BSA (100 ppm). A: baseline in PBS buffer. B: injection of BSA 100 ppm. C: washing with PBS buffer. D: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A).

Preparation of thioether 3 probe monolayer

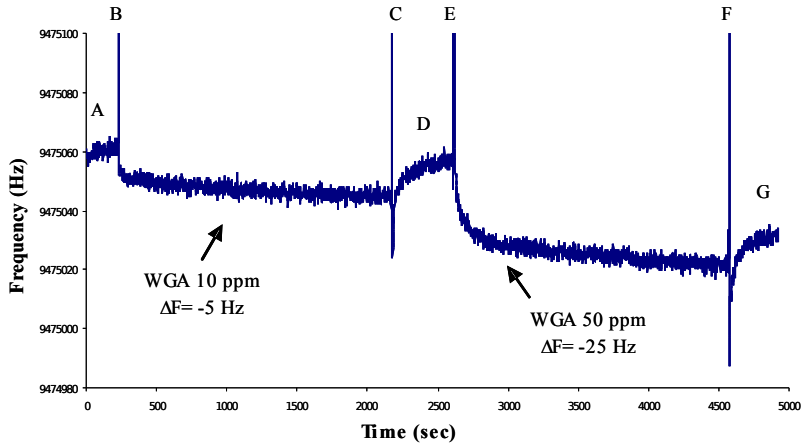
A solution of thioether **3** (50μL of a 10 ng/μL solution) in $CHCl_3$:CH_3OH (4:1) was dropped on the gold crystal surface, outside the cell, previously modified with a monolayer of 1-dodecanethiol. The monolayer formation was monitored by the quartz crystal analyzer QCA 922 by SEIKO. After evaporation of the organic solvent by nitrogen flow, the electrode was housed in a methacrylate cell and washed with buffer until the frequency was stable.



Sensorgram recorded during the immobilisation of thioether **3** on the gold modified surface of the crystal. A: frequency signal recorded in air. B: frequency signal recorded during thioether 2 dropping. C: frequency signal after organic solvent evaporation.

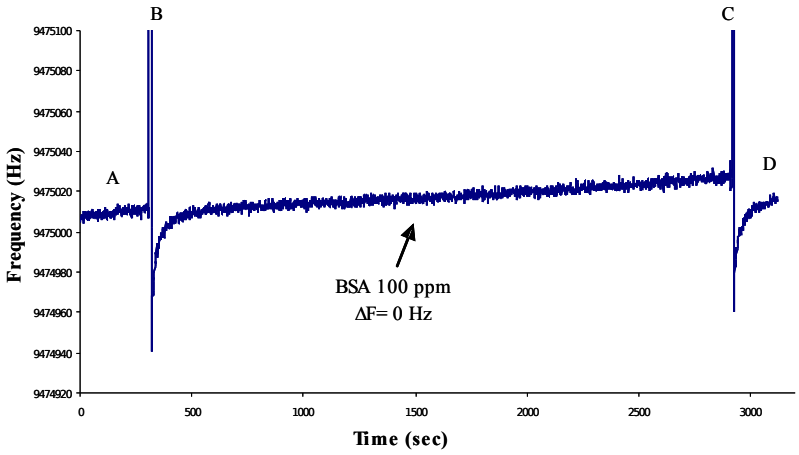
QCM Measurements

In order to evaluate the molecular recognition with WGA selectin, 200 µL of two different concentrations of WGA were added to the sensor surface previously modified with a thioether **3** monolayer. The interaction was monitored for 30 minutes. The solution was then removed and the surface washed with the PBS buffer to eliminate the unbound proteins. The analytical signal was given by the difference between the frequency recorded after washing with PBS and the frequency displayed before the interaction. This experiment was performed with 10 and 50 ppm of WGA. The figure below shows the sensorgram (frequency versus time) recorded during the experiments. The experiments were performed in triplicate.



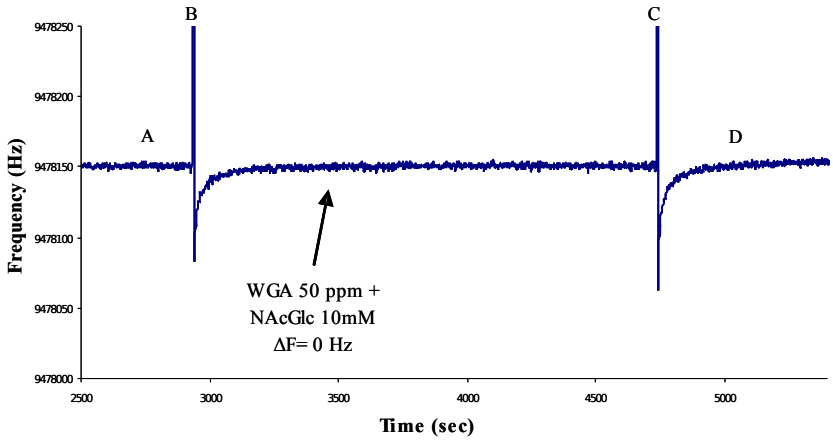
Sensorgram recorded during the interaction between thioether **3** and WGA (10 ppm and 50 ppm). A: baseline in PBS buffer. B: injection of WGA 10ppm. C: washing with PBS buffer. D: baseline in PBS buffer. E: injection of WGA 50ppm. F: washing with PBS buffer. G: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A and F_G-F_D).

To test the specificity of the surface, a 100 ppm solution of BSA was added. The negligible frequency shift demonstrate the specificity of the sensor surface.



Sensorgram recorded during the interaction between thioeher **3** and BSA (100 ppm).A: baseline in PBS buffer. B: injection of BSA 100ppm. C: washing with PBS buffer. D: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A).

The interaction of compound **3** on the surface of the crystal with WGA was inhibited by the presence of N-acetyl-D-glucosamine (GlcNAc). Any decrease of the frequency was observed after the addition of the protein (WGA 50 ppm) in the presence of a 10mM solution of N-acetyl-D- glucosamine in phosphate buffer. This confirmed the selectivity of the interaction.



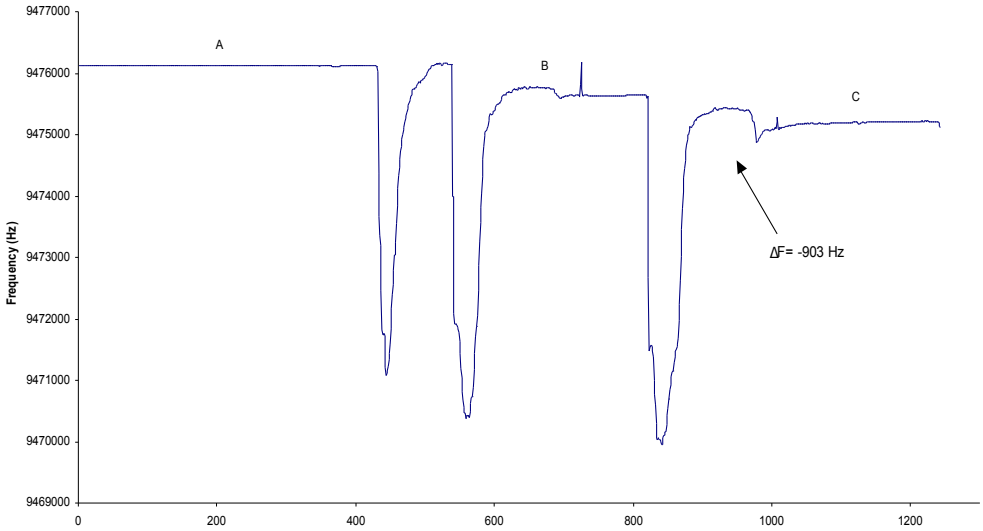
Sensorgram recorded during the interaction between thioeher **3** and WGA (50 ppm) in the presence of GlcNAc 10 mM. A: baseline in PBS buffer. B: injection of a solution of GlcNAc 10 mM and BSA 100 ppm. C: washing with PBS buffer. D: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A).

Binding Evaluation of thioether 3 to SNA lectin

The binding interaction of thioether **3** and SNA (Sambucus Nigra Agglutinin) was evaluated by using a quartz-crystal microbalance (QCM). These data were then compared to those obtained with the GM3 ganglioside.

Preparation of GM3 ganglioside probe monolayer

A solution of GM3 ganglioside (50μL of a 10 ng/μL solution) in $CHCl_3$:CH_3OH (4:1) was dropped on the gold crystal surface, previously modified with a monolayer of 1-dodecanethiol, outside the cell. The monolayer formation was monitored by the quartz crystal analyzer QCA 922 by SEIKO.[67] After the evaporation of the organic solvent under nitrogen flow, the electrode was housed in a methacrylate cell and washed with PBS until the frequency was stable.

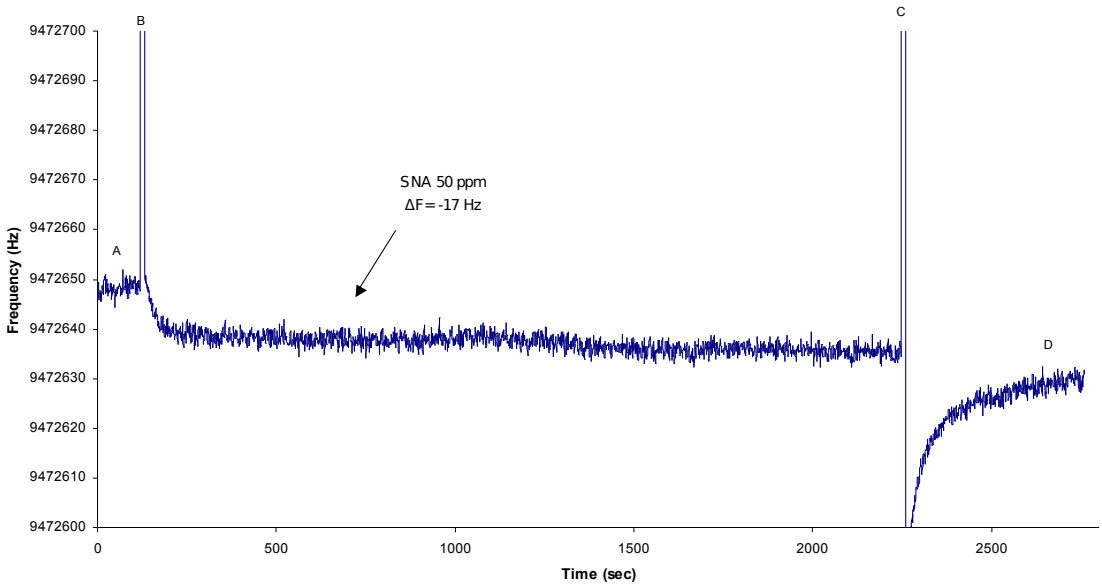


Sensorgram recorded during GM3 ganglioside immobilisation on the gold modified surface of the crystal. A: frequency signal recorded in air. B: frequency signal during GM3-ganglioside dropping. C: frequency signal after organic solvent evaporation.

QCM Measurements

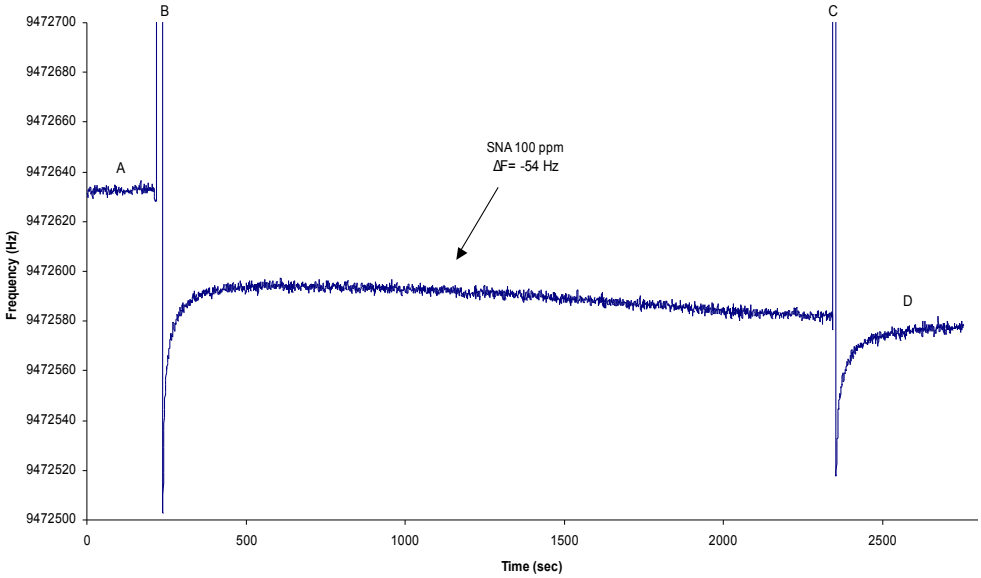
In order to evaluate the molecular recognition with SNA selectin, 200 μL of two different concentrations of SNA were added to the sensor surface previously modified with the glycosphingolipide GM3 ganglioside monolayer. The interaction was monitored for 30

minutes. The solution was then removed and the surface washed with the PBS buffer to eliminate the unbound proteins. The analytical signal was given by the difference between the frequency recorded after washing with PBS and the frequency displayed before the interaction. This experiment was performed with 50 and 100 ppm of SNA. Figure below shows the sensorgram (frequency versus time) recorded during the experiments for SNA 50 ppm. The experiments were performed in triplicate.



Sensorgram recorded during interaction GM3 ganglioside-SNA 50 ppm.
A: baseline in PBS buffer. B: injection of SNA 50ppm. C: washing with PBS buffer. D: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A).

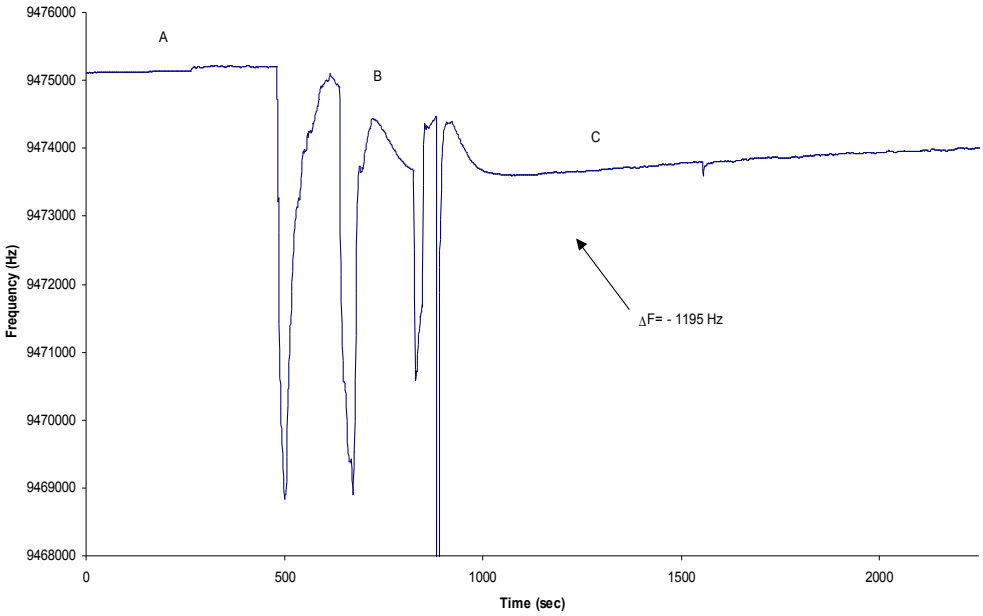
Figure below shows the sensorgram (frequency versus time) recorded during the experiments for SNA 100 ppm.



Sensorgram recorded during interaction GM3 ganglioside-SNA 100 ppm. A: baseline in PBS buffer. B: injection of SNA 100ppm. C: washing with PBS buffer. D: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A).

Preparation of thioether 3 probe monolayer

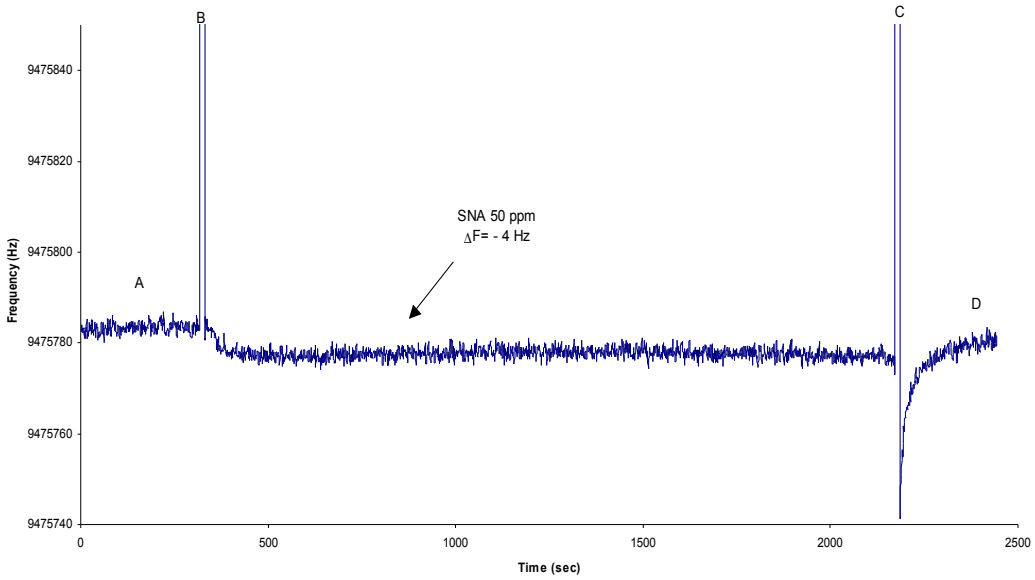
A solution of thioether **3** (50μL of a 10 ng/μL solution) in $CHCl_3$:CH_3OH (4:1) was dropped on the gold crystal surface, outside the cell, previously modified with a monolayer of 1-dodecanethiol. The monolayer formation was monitored by the quartz crystal analyzer QCA 922 by SEIKO.[67] After evaporation of the organic solvent by nitrogen flow, the electrode was housed in a methacrylate cell and washed with buffer until the frequency was stable.



Sensorgram recorded during the immobilisation of thioether **3** on the gold modified surface of the crystal. A: frequency signal recorded in air. B: frequency signal recorded during thioether 2 dropping. C: frequency signal after organic solvent evaporation.

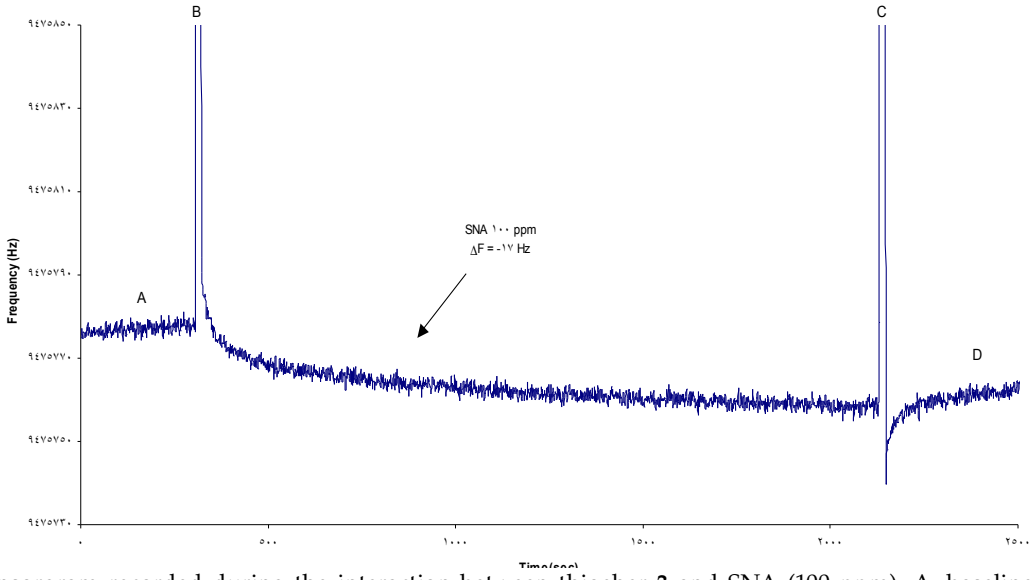
QCM Measurements

In order to evaluate the molecular recognition with SNA selectin, 200 µL of two different concentrations of SNA were added to the sensor surface previously modified with a thioether **3** monolayer. The interaction was monitored for 30 minutes. The solution was then removed and the surface washed with the PBS buffer to eliminate the unbound proteins. The analytical signal was given by the difference between the frequency recorded after washing with PBS and the frequency displayed before the interaction. This experiment was performed with 50 and 100 ppm of SNA. The figure below shows the sensorgram (frequency versus time) recorded during the experiments with SNA 50 ppm. The experiments were performed in triplicate.



Sensorgram recorded during the interaction between thioeher **3** and SNA (50 ppm). A: baseline in PBS buffer. B: injection of SNA 50 ppm. C: washing with PBS buffer. D: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A).

Figure below shows the sensorgram (frequency versus time) recorded during the experiments with SNA 100 ppm.



Sensorgram recorded during the interaction between thioeher **3** and SNA (100 ppm). A: baseline in PBS buffer. B: injection of SNA 100 ppm. C: washing with PBS buffer. D: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A).

Binding Evaluation of thioether 3 to MAA lectin

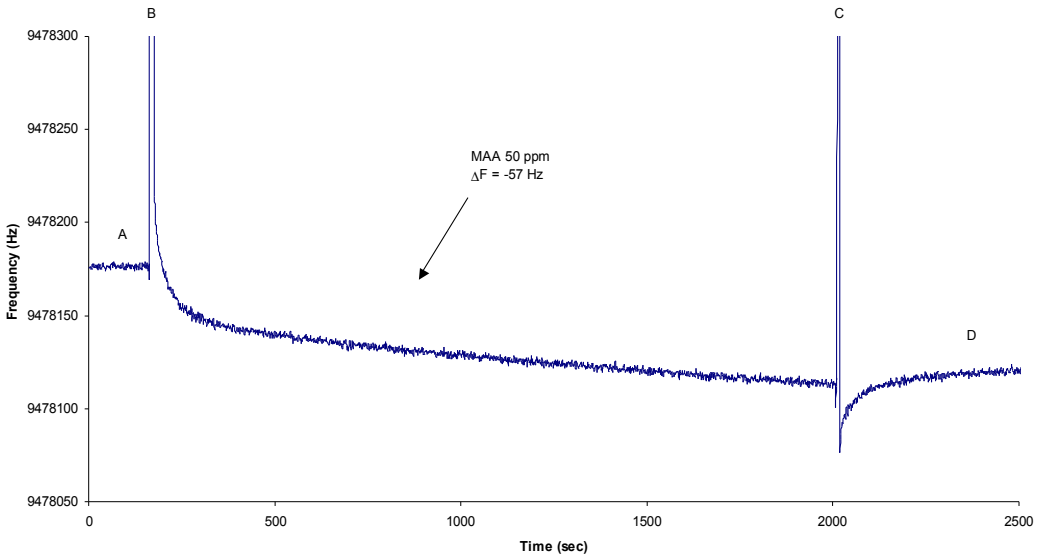
The binding interaction of thioether **3** and MAA (Maackia Amurensis Agglutinin) was evaluated by using a quartz-crystal microbalance (QCM). These data were then compared to those obtained with the GM3 ganglioside.

Preparation of GM3 ganglioside probe monolayer

A solution of GM3 ganglioside (50μL of a 10 ng/μL solution) in $CHCl_3$:CH_3OH (4:1) was dropped on the gold crystal surface, previously modified with a monolayer of 1-dodecanethiol, outside the cell. The monolayer formation was monitored by the quartz crystal analyzer QCA 922 by SEIKO.[67] After the evaporation of the organic solvent under nitrogen flow, the electrode was housed in a methacrylate cell and washed with PBS until the frequency was stable.

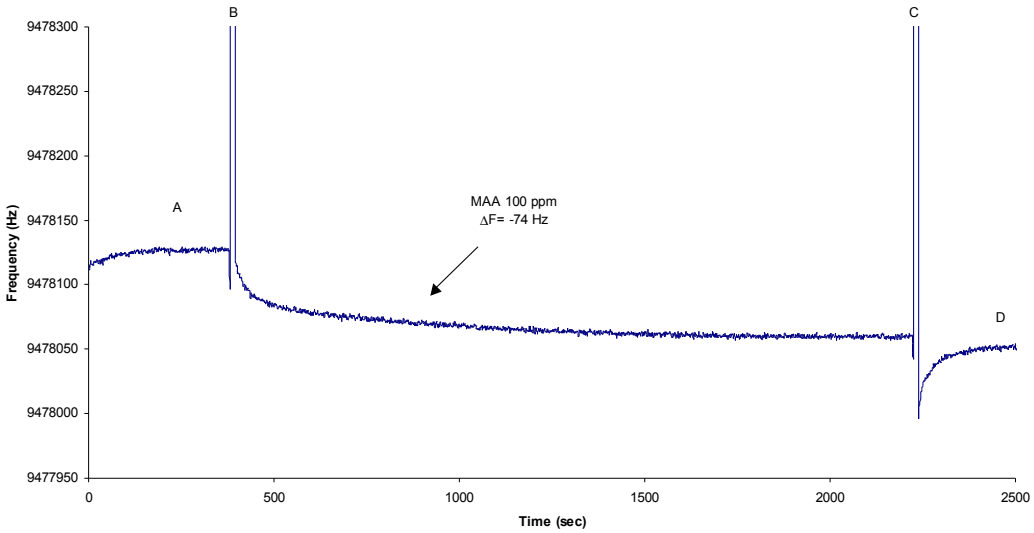
QCM Measurements

In order to evaluate the molecular recognition with MAA selectin, 200 μL of two different concentrations of MAA were added to the sensor surface previously modified with the glycosphingolipide GM3 ganglioside monolayer. The interaction was monitored for 30 minutes. The solution was then removed and the surface washed with the PBS buffer to eliminate the unbound proteins. The analytical signal was given by the difference between the frequency recorded after washing with PBS and the frequency displayed before the interaction. This experiment was performed with 50 ppm and 100 ppm of MAA. Figure below shows the sensorgram (frequency versus time) recorded during the experiments for MAA 50 ppm. The experiments were performed in triplicate.



Sensorgram recorded during interaction GM3 ganglioside-MAA 50 ppm.
A: baseline in PBS buffer. B: injection of MAA 50 ppm. C: washing with PBS buffer. D: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A).

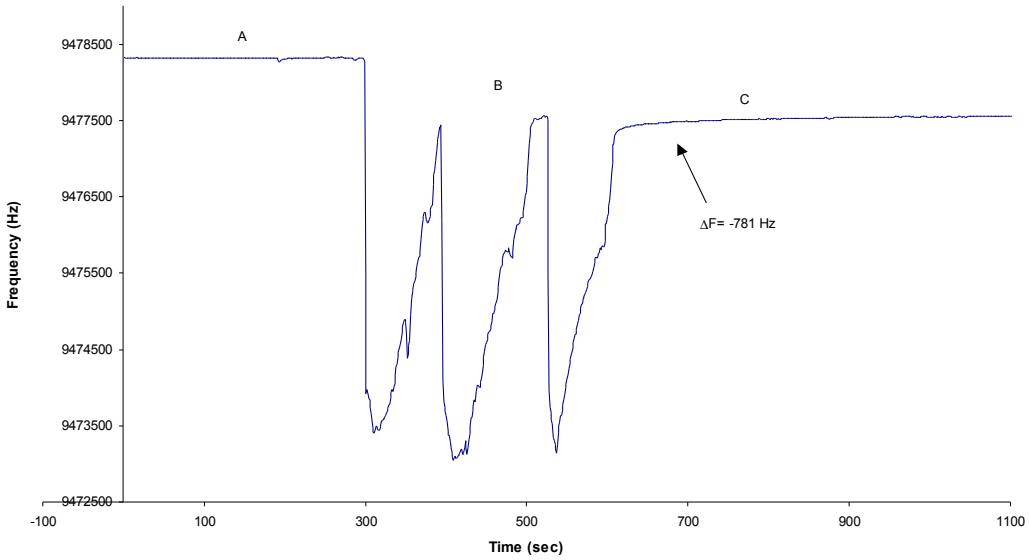
Figure below shows the sensorgram (frequency versus time) recorded during the experiments for MAA 100 ppm.



Sensorgram recorded during interaction GM3 ganglioside-MAA 100 ppm.A: baseline in PBS buffer. B: injection of MAA 100 ppm. C: washing with PBS buffer. D: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A).

Preparation of thioether 3 probe monolayer

A solution of thioether **3** (50µL of a 10 ng/µL solution) in $CHCl_3$:CH_3OH (4:1) was dropped on the gold crystal surface, outside the cell, previously modified with a monolayer of 1-dodecanethiol. The monolayer formation was monitored by the quartz crystal analyzer QCA 922 by SEIKO.[67] After evaporation of the organic solvent by nitrogen flow, the electrode was housed in a methacrylate cell and washed with buffer until the frequency was stable.

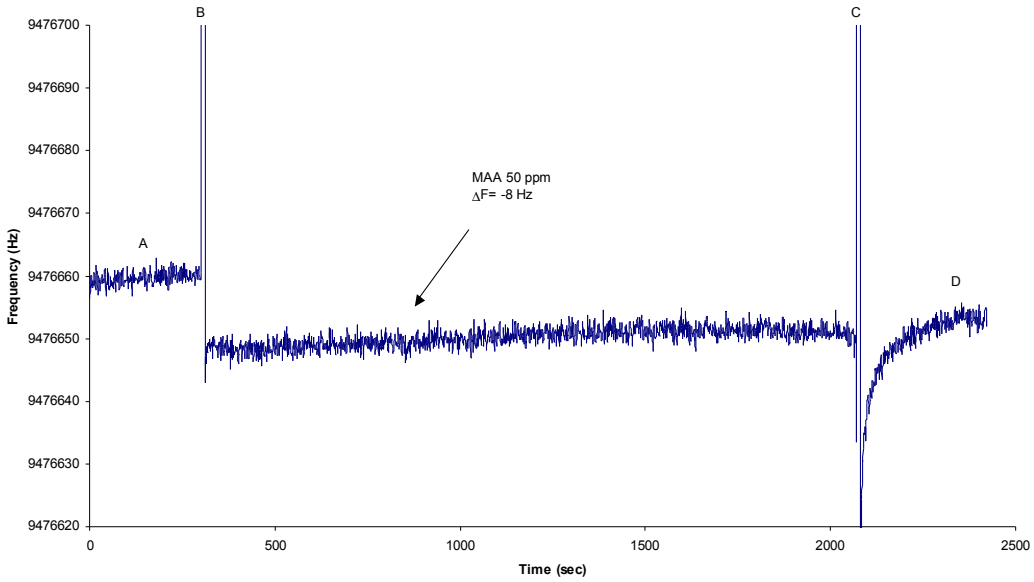


Sensorgram recorded during the immobilisation of thioether **3** on the gold modified surface of the crystal. A: frequency signal recorded in air. B: frequency signal recorded during thioether **3** dropping. C: frequency signal after organic solvent evaporation.

QCM Measurements

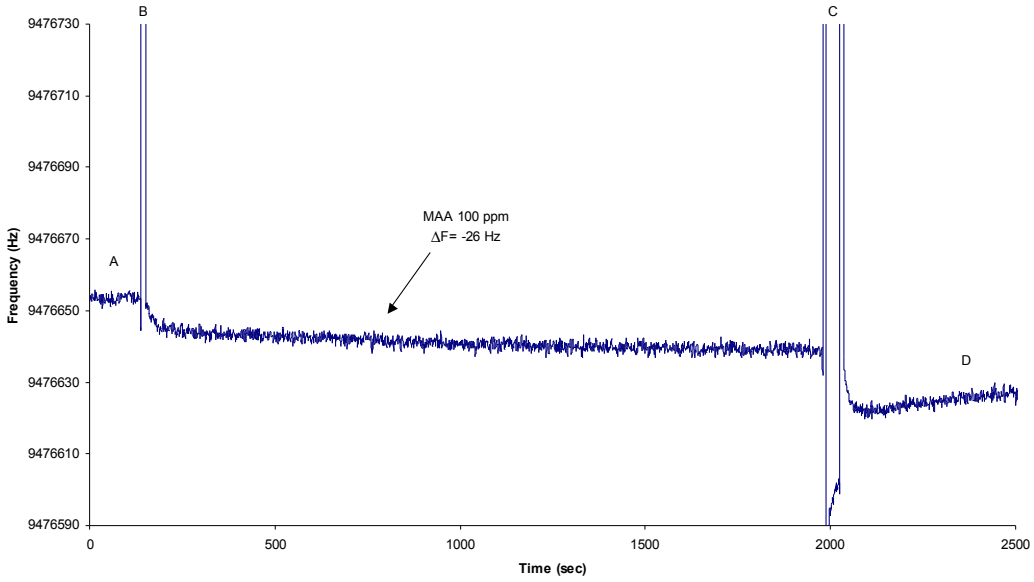
In order to evaluate the molecular recognition with MAA selectin, 200 µL of two different concentrations of MAA were added to the sensor surface previously modified with a thioether **3** monolayer. The interaction was monitored for 30 minutes. The solution was then removed and the surface washed with the PBS buffer to eliminate the unbound proteins. The analytical signal was given by the difference between the frequency recorded after washing with PBS and the frequency displayed before the interaction. This experiment was performed with 50 ppm and 100 ppm of MAA. The figure below shows the sensorgram

(frequency versus time) recorded during the experiments with MAA 50 ppm. The experiments were performed in triplicate.



Sensorgram recorded during the interaction between thioeher **3** and MAA (50 ppm). A: baseline in PBS buffer. B: injection of MAA 50 ppm. C: washing with PBS buffer. D: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A).

Figure below shows the sensorgram (frequency versus time) recorded during the experiments with MAA 100 ppm.



Sensorgram recorded during the interaction between thioeher **3** and MAA (100 ppm). A: baseline in PBS buffer. B: injection of MAA 100 ppm. C: washing with PBS buffer. D: baseline in PBS buffer. Analytical signal was given by ΔF (F_D-F_A).

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