European Journal of Organic Chemistry

Synthesis of a Sialyl Lewis X Mimetic Conjugated with DTPA, Potential Ligand of New Contrast Agents for Medical Imaging

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Received (will be filled in by the editorial staff)

DTPA / Sialyl Lewis / Contrast agents / MRI / Nuclear Medicine

Via a flexible alkyl spacer and the amide linkage, the structure of a mimetic of sialyl Lewis X, the 3-(2-α-D-mannopyranosyloxyphenyl)phenylacetic acid, was coupled to diethylenetriaminepenta acetic acid (DTPA). The overall yield of the eleven-step synthesis starting from 3-bromophenylacetic acid was 4-8%. This new ligand is expected to target inflammation sites through specific interactions with selectins, the adhesion molecules expressed on the vascular endothelium in pathological conditions. In particular, complexation of the DTPA moiety with gadolinium or radionuclides could produce contrast agents for medical imaging.

Introduction

The specific interaction between cell adhesion proteins and oligosaccharide ligands plays a major role in inflammation by mediating the attraction of leukocytes to the injured area. Selectins, one family of cell adhesion proteins, include three members: E-, P-, and L-selectin. E-selectin is expressed on the activated vascular endothelial cells 4 to 24 h after cytokine-induced transcription; P-selectin is found on platelets and vascular endothelial cells from several minutes to hours following the injury, while L-selectin is continuously expressed on the leukocytes. Sialyl Lewis X (sLe^x), a well known carbohydrate antigen, exists on the surface of leukocytes like neutrophiles and monocytes. It is able to bind to E- or P- selectin expressed on activated endothelial cells. In the early inflammatory response, this binding mediates the rolling of the leukocytes along the activated endothelium of the lumen wall of blood vessels near the injury. Thereafter, L-selectin mediates the interaction of circulating leukocytes with adhered leukocytes.[1-4] The accumulation of leukocytes in the injured area eventually leads to inflammation. Based on this mechanism, research has been devoted to the synthesis of sLe^x mimetics in order to develop effective inhibitors against cell adhesion and, thus, to control inflammatory and other cell-adhesion related diseases.[5-8]

On the contrary, the study of safe, selective and effective molecules aiming at the detection and diagnosis of inflammation has been less significant. Besides nuclear medicine, magnetic resonance imaging (MRI) is one of the most powerful diagnostic modalities in today's medical techniques. It perfectly combines several advantages like safety, high spatial resolution, and multidimensional investigations. The image contrast strongly depends on the relaxation rate of water protons in the tissues and can be enhanced by exogenous compounds, usually paramagnetic compounds or superparamagnetic particles which accelerate the magnetic relaxation process.[9-11] Currently, Gd-DTPA (*Magnevist*) and Gd-DTPA-BMA (*Omniscan*) are among the most widely used paramagnetic contrast agents in routine MRI examinations in the world (DTPA, diethylenetriaminepentaacetic acid; BMA, bis(methyl)amide). The safety of these agents is based on the very strong chelating capability of DTPA and its derivative toward paramagnetic but toxic gadolinium ion. In this work, for the purpose of developing novel contrast agents targeted to inflammation, we have introduced the 3-(2-α-D-mannopyranosyloxyphenyl)phenylacetic acid moiety (**A**, Figure 1), a potent inhibitor of sLe^x-selectin binding,[3] in a DTPA molecule which can subsequently complex paramagnetic and radioactive ions such as Gd^{3+}, $^{111}In^{3+}$, etc.

Results and Discussion

DTPA can be easily converted into the corresponding cyclic bisanhydride. It is therefore convenient to prepare DTPA bisamides by reacting the cyclic anhydride with a twofold amount of the amine.[12] Of special interest, as compared to monovalent ligands for the selectins, divalent or

multivalent ligands have been proved to show greater binding affinity to the selectins.[13] In this work, an amino derivative **B** of sLe^x mimetic **A** (Figure 1) was synthesized starting from 3-bromophenylacetyl acid.

Synthesis of Compounds 1~5. These compounds were synthesized according to the method described in the literature.[3] Reactions and products have been studied by TLC, ^1H and ^{13}C NMR.

(a) Methyl 3-bromophenylacetate (1). This compound was synthesized according to the experimental procedure described in literature.[3]

(b) Boronation, 2-methoxyphenylboronic acid (2). Substituted phenylboronic acid (**2**) was obtained from acid hydrolysis of its methyl ester synthesized by reacting n-butyllithium-treated anisole and trimethylboronate at low temperature. In this reaction, some anisole (~20%) remained unreacted, and a minor byproduct (R_f = 0.29, PE/ AcOEt 8:1; PE = petroleum ether, b.p. 45-60°C), n-butylboronic acid, was obtained at a yield of ~8%. The yield of **2** was 63%. After purification by chromatography, a crystalline product was obtained, which is reported to be an oil in the original reference.[3]

(c) Coupling reaction, methyl 3-(2-methoxyphenyl)phenylacetate (3). A subsequent coupling reaction between **1** and **2** was conducted to obtain a biphenyl derivative **3**. Following the literature,[14] the amounts of catalyst ($Pd(PPh_3)_4$) and base (Na_2CO_3) were chosen as 3.0 mol % and ~200 mol %, respectively, with respect to the bromide. An excess of arylboronic acid (**2**) (20 mol %) was used to ensure a complete conversion of the rather expensive bromide **1**. A small amount of an unknown byproduct with R_f = 0.90 (PE/AcOEt 8:1) was detected in this reaction.

(d) Ether cleavage, methyl 3-(2-hydroxyphenyl)phenylacetate (4). Treated with BBr_3 at a low temperature, the methoxy group was removed from ether **3** to give the phenol **4** at a moderate yield. TLC showed that the increase of the molar ratio of BBr_3 over ether **3** from 3:1[3] to 5:1 resulted in a larger conversion of the material. With the latter ratio, a reaction time of 10 h was found appropriate, whereas shorter (4 h) or longer (18 h) times led to incomplete conversion or increase of byproducts. Separation of the reaction mixture by column chromatography yielded several fractions: phenol **4** (R_f = 0.50, yield: 55 to 70%) and a series of byproducts (R_f = 0.32, 0.09 and 0 respectively, PE/AcOEt 5:1). The byproduct with R_f = 0.32 was confirmed to be 3-(2-hydroxylphenyl)phenylacetic acid (^1H NMR: δ = 3.7 (s, 3H), 6.9-7.7 (m, 8 H)) . It resulted from the cleavage of both ether and ester bonds of **3**.

(e) Glycosylation, methyl 3-[2-(2,3,4,6-O-tetraacetyl-α-D-mannopyranosyloxy)phenyl] phenylacetate (5). BF_3/Et_2O-catalyzed glycosylation of **4** gave one α-mannoside derivative **5** using α-D-mannose pentaacetate as the glycosyl donor. To assure the full conversion, excesses of mannose pentaacetate and catalyst, mole ratio 2:1 and 6:1 respectively as compared to **4**, were used (to be compared to 1:1 and 3.5:1 respectively in the reference[3]). The addition of reactants in

portions was found to improve the yield with respect to the one-time addition. Consistent with the observation reported in the reference,[3] both TLC and column chromatography showed that one of the reactants, α-D-mannose pentaacetate, was always coeluted with the product **5**. Their mole ratio was successfully quantified by comparing the peak area of C-1 H of the sugar ring.

Synthesis of the new intermediates (6-10) and of the specific ligand (11)

From this point onward, the synthetic scheme leading to DTPA-B(sLe^x)A **11** is original.

Friedle-Crafts acylation, methyl 3-[2-(2,3,4,6-O-tetraacetyl-α–D–manno-pyranosyloxy)-5-(3-bromopropionyl)phenyl]phenylacetate (**6**). To obtain the bromo ketone **6**, the tetraacetylmannopyranoside **5** was submitted to a Friedel-Crafts reaction using bromopropionyl chloride as acylating agent. Due to the electrophilic nature of the biphenyl group, compound **5** (as well as **3**) can be selectively mono-acylated at the para-position of the mannopyranosyloxy group in the Friedle-Crafts reaction.[4] Tracing this reaction by TLC, demonstrated that increasing the amount of $AlCl_3$ (from mole ratio 10:1 to 15~20:1 with respect to **5**), as well as lengthening the reaction time (from 0.5 to 1~1.5 h), led to a higher conversion. CH_2Cl_2 (m.p.- 97°C) was used as reaction solvent instead of CH_2ClCH_2Cl (m.p. -35°C)[4] to avoid the freezing of the reaction solution during the activation of the acyl group by $AlCl_3$ at a low temperature (-78°C). Further increments of the reaction time at 0°C did not improve the yield but did not induce the formation of byproducts.

Borane reduction, methyl 3-[2-(2,3,4,6-O-tetraacetyl-α–D–mannopyranosyloxy)-5-(3-bromopropyl)phenyl]-phenylacetate (**7**). Bromoketone **6** was reduced to bromide **7** in the presence of BH_3/THF. Here too, the amount of reactant (borane) and the reaction time were found to have a significant effect on the product composition and the yield. Theoretically, 2/3 mole of BH_3 are necessary to reduce C=O into a methylene group[15, 16] but some excess was in fact needed in this reaction. After 2 to 3 hours, a complete conversion of the ketone was achieved and only a small amount of reduced byproducts was obtained. Further ofincreases of the BH_3 amount as well as of the reaction time led to a series of byproducts with a gradually increasing number of reduced ester groups.

NaN_3 substitution, methyl 3-[2-(2,3,4,6-O-tetraacetyl-α–D–mannopyranosyloxy)-5-(3-azidopropyl)phenyl]-phenylacetate (**8**). Bromide **7** was submitted then to a nucleophilic substitution by NaN_3 to give an azide derivative. The yield from **7** to **8** was not very high (54~60%). Unknown byproducts (R_f = 0.40 and 0, PE/AcOEt 3:2) appeared which may be due to side reactions between NaN_3 in excess and some reactive groups such as ester groups in the starting molecule. Considering the similarity in structure of **7** and **8**, a difficult chromatographic separation was expected. The use of an excess of NaN_3 and a long reaction time were thus adopted to guarantee a full conversion of the bromide **7**. IR adsorption occurring at 2100 cm^{-1} confirmed the azide nature of the product.

Reduction, methyl 3-[2-(2,3,4,6-O-tetraacetyl-α–D–mannopyranosyloxy)-5-(3-aminopropyl) phenyl]phenylacetate (**9**). The reduction of azide **8** to amine **9** was carried out by hydrogen in the presence of Pd/C catalyst at room temperature. The CH_3OH-HCOOH mixture was chosen as reaction solvent to protonate the freshly formed amino groups and to prevent attack of the ester bonds by amino groups. Moreover, HCOOH is also able to act as a hydrogen donor and participate in the Pd/C-catalytic reduction through the transfer hydrogenation mechanism.[17] Combined with the ninhydrin test, TLC showed that the hydrogenation of **8** was completed within 4-6 h.

Ester hydrolysis, 3-[2-α–D–mannopyranosyloxy-5-(3-aminopropyl)phenyl]phenylacetic acid (**10**). Treated with concentrated NaOH solution, all ester groups of **9** were removed to give a hydroxyl-exposed α–D–mannoside **10**. A first purification trial run on a silica gel column was not successful due to the very high polarity of the compound. Preparative reversed-phase chromatography was therefore used. Under optimized conditions, efficient separation of **10** from minor organic byproducts was obtained. A small amount of NaCl contaminating the product was removed by chromatography.

Ligand synthesis, amine (**10**)-derived bisamide of diethylenetriaminepentaacetic acid, DTPA-B(SLe^X)A (**11**). Reaction between with bisanhydride of DTPA and two-fold molecules of **10** led to the formation of two amide linkages. Initially, DMF or CH_3CN were chosen as the reaction media but neither worked efficiently because of the poor solubility of **10** in these two solvents. The coupling reaction was thus conducted in water. To limit the hydrolysis of the bisanhydride, a mild pH (< 8.5) was maintained. As shown by reverse phase HPLC, a relatively high yield (~80%) of pure ligand (**11**) was achieved.

Alternative reactions sequence.

Another route to **6** may be conceived by modifying the reaction (Figure 3). Actually, this route was tried (with bromoacetyl bromide) but proved to be unsuccessful. Compound **3** underwent first the Friedel-Crafts reaction to give methyl 3-(2-methoxy-5-bromoacetyl phenyl)phenylacetate **12**. Subsequent BBr_3 cleavage and BF_3-catalyzed mannosylation, gave **14** (the analog of **6**) in poor yields (10%). This is probably due to the existence of the reactive 3-bromoacetyl group in the molecule which may cause side reactions in the presence of a strong Lewis acid like BBr_3 or BF_3.

Alternative amination routes.

(a) Direct ammonolysis of **6**. Considering the high reactivity of 3-bromopropionyl toward amines, direct amination of **6** by 2.0 M (or 19.6 M) ammonia in either CH_3OH or CH_3OH-H_2O has been tried. This reaction, however, gave complex mixtures: as shown by TLC, at least six components were formed. The existence of ketone carbonyl conjugated with the biphenyl unit

obviously increases the complexity of this amination. This shows that reduction of the carbonyl group in **6** has to be performed prior to the amination.

(b) Direct ammonolysis of **7**. Although it is not often of great practical use to prepare a primary amine through direct ammonolysis of a bromide, it might be feasible in the presence of a large excess of ammonia.[18] This strategy could be valuable in the case of a limited availability of the bromide like in the context of a multistep synthesis. Unfortunately, for bromide **7**, the amination carried out in the presence of a 200-500 fold excess of NH_3 in CH_3OH or CH_3OH-H_2O always gives a poor yield (< 15%) of the desired product after column chromatography purification (TLC, R_f = 0.50-0.60, 3:1 CH_3OH/H_2O). The major byproducts contained no amino groups and their structure was not unraveled.

(c) Gabriel synthesis (Figure 4). Bromide **7** was reacted with freshly prepared phthalimide potassium salt (obtained from phthalimide and anhydrous K_2CO_3) in DMF at 95°C for 2 h to give methyl 3-[2-α-(O-2,3,4,6-tetraacetylmannopyranosyloxy)-5-(3-phthalimidylpropyl) phenyl]phenyl-acetate **15** (R_f = 0.45, 1:1 PE/AcOEt) at a 56% yield, which was purified by chromatography with 1:1 PE/AcOEt as the eluant. The appearance of δ 7.6-8.1 (4 H) signal in the ^{1}H NMR spectrum confirmed the expected structure. Due to the existence of acid-sensitive glycoside linkage in the molecule, the phthalic group cannot be removed by hydrazine and HCl or just by concentrated HCl, which are two most common deprotecting agents. The hydrolysis in strongly alkalic conditions thus appears to be the only way to obtain the corresponding amine. Experiments showed that the phthalic group was hardly removed even after refluxing the imide with 10% aqueous NaOH for 22 h.

Alternative synthetic strategy. An attempt was also made to synthesize another amine intermediate, (2-α-D-mannopyranosyloxy-5-aminophenyl)phenylacetic acid, using p-nitroanisole instead of anisol itself as the starting compound. However, the compound expected (2-methoxy-5-nitrophenylboronic acid) was not present in the reaction mixture after the boronation step. The mixture was purified by silica gel chromatography (8:1 PE/AcOEt) and the fractions were analyzed by ^{1}H NMR. It was found that, aside from unreacted 4-nitroanisole, the mixture contained fractions which all revealed the presence of n-butyl group. Side reactions in which the nitro group is attacked by the strongly nucleophilic n-butyl anion are thus likely. This intermediate could not be obtained either by the nitration of **2** because the separation of the p-nitro derivative from the o-nitro one was unsuccessful. Finally, a strategy leading to the synthesis of a compound analogous to **11** but containing a $(CH_2)_2$ linker instead of a $(CH_2)_3$ one was successfully carried out until the BH_3/THF reduction step. This scheme was momentarily abandoned for the benefit of another one affording the system with the longer linker taking into account the strong basicity of NaN_3 which could lead to the unsuited á-elimination in which the newly formed C=C bond could be substantially stabilized owing to its conjugation with a biphenyl system.

Conclusion

In summary, we have described a successful and original synthesis of a new ligand of inorganic cations, conjugated to a sialyl Lewis X mimetic. This molecule can be complexed with stable paramagnetic or radioactive ions to produce specific contrast agents for medical imaging aiming at the targeting of the inflammation sites.

Experimental Section

General. All solvents and reagents were commercially available and were used without further purification unless specified otherwise. 3-bromo-phenylacetic acid (98%), anisole, trimethyl boronate (99%), boron trifluoride etherate, tetrakis(triphenylphosphine) palladium(0), sulfuric acid, acetic anhydride, pyridine, and phthalimide were obtained from Acros (Geel, Belgium); borane-tetrahydrofuran complex (1.0 M), sodium carbonate, boron tribromide (99+%), potassium carbonate, sodium hydroxide, bromopropionyl chloride, bromoacetyl bromide, formic acid (95-97%), 2.0 M ammonium in methanol, 2.5 M n-butyllithium in hexane (determined as 2.13 M before use), 10wt% Pd/C catalyst, gadolinium chloride hexahydrate (99.9%), silica gel (Merck, grade 60, 70-230 mesh, 60 Å), pre-coated TLC plates SIL G-25 UV_{254} (silica gel on glass, 5×20 cm), and 3A molecular sieve (4-8 mesh) were purchased from Aldrich (Bornem, Belgium); anhydrous aluminium chloride (granule), sodium azide, and diethylenetriaminepentaacetic acid (DTPA, 99+%) were from Fluka (Bornem, Belgium); α-D-mannose pentaacetate, ninhydrin, and arsenazo III were from Sigma (Bornem, Belgium). Most reactions were monitored by thin layer chromatography and spots could generally be visualized under UV light as well as by iodine vapor. Melting points (uncorrected) were determined on a Büchi-512 melting point apparatus. IR spectra were recorded on a Perkin-Elmer FTIR 1760X spectrometer (PerkinElmer Instruments, Zaventem, Belgium,). ^{1}H and ^{13}C NMR spectra were recorded at 7.04 T on a Bruker AMX-300 spectrometer (Bruker, Karlsruhe, Germany). Mass spectra were obtained on a VG AUTOSPEC 6F mass spectrometer (VG Analytical, Manchester, UK). Reverse-phase HPLC with Waters 600 controller and Waters 996 photodiode array detector was performed on a Novapak C18 column (i.d. 4.56 mm × 150 mm) for analytical use or Novapak C18 PrepLC Cartridge column (i.d. 25 mm × 100 mm) for the preparative scale (Waters, Milford, USA). A gradient of 15:85 to 50:50 (S1/S2, v/v) solvent was run over 20 min at a flow rate of 0.7 mL/min for analysis or 9.0 mL/min for preparation (solvent S1, 5% aqueous CH_3CN with 3 mM HCl; solvent S2, 95% aqueous CH_3CN with 3 mM HCl. The addition of such a small amount of HCl has the purpose of decreasing the adsorption of samples on the column materials). UV adsorption of the effluent was monitored at 254 nm. Size exclusion chromatography on Sephadex G-15 (Sigma) is used for desalt and purification of some synthesized compounds.

The synthesis of intermediates **1-5** were carried out according to the methods reported in the literature[3] and modified. To improve the purity of these intermediates, every reaction step from 1 to 5 was closely monitored and investigated by TLC. All these products were characterized by NMR and TLC, while some byproducts were also identified. For chromatographic separations, AcOEt-petroleum ether (PE, b.p. 45-60°C) was used instead of AcOEt-hexane. This avoided the use of large quantities of more expensive n-hexane without any loss of performance.

Methyl 3-bromophenylacetate (1). Briefly, 3-bromoacetylphenylacetic acid (10.0 g, 46.5 mmol) and methanol (80 mL) were refluxed overnight in the presence of several drops of sulfuric acid to give the ester **1**. After neutralization of the catalyst (sulfuric acid) by $NaHCO_3$ and saturation with NaCl, a pure product was obtained at a 92% yield (9.80 g). Further purification by chromatography as proposed in the reference was not necessary. TLC (PE/AcOEt 8:1): R_f = 0.67 (ester) as compared to the acid with R_f = 0.96. ^{1}H NMR ($CDCl_3$): δ = 3.6 (s, 2 H, CH_2C=O), 3.7 (s, 3 H, OCH_3), 7.2-7.5 (m, 4 H, aromatic H). ^{13}C NMR ($CDCl_3$), δ = 40.9 (CH_2Ph), 52.5 (OCH_3), 122.8 (aromatic C), 128.2, 130.3, 130.5, 132.6, 136.3, 171.6 (C=O).

2-Methoxyphenylboronic acid (2). n-Butyllithium/THF (45 mL, 2.13 M) was added to a solution of anisole (10.0 g, 92.6 mmol) in anhydrous THF (250 mL) cooled to -78°C. The mixture was warmed to 0°C and stirred for 1 h at this temperature. $B(OCH_3)_3$ (92.6 mmol, 9.63 g) was added. After 10 h reaction at room temperature, the solution was acidified to pH 3 with HCl and stirred for 1 h vigorously. After extraction with ether, the organic phase was dried and the solvent was evaporated. Contrarily to the protocol published in the literature, this crude product is purified by chromatography. After elution of the unreacted anisole by PE, the product was recovered by 1:1 PE/AcOEt. After concentration, colorless crystals of **2** are obtained (yield of 63%, 8.87 g). Melting point: 80-81°C (a clear oil in 95% yield was reported previously[3]). TLC (PE/AcOEt 8:1): R_f = 0.19 (product), 0.77 (anisole, spot visible in I_2 vapor instead of under UV light). ^{1}H NMR ($CDCl_3$): δ = 3.9 (s, 3 H, OCH_3), 6.6 (s, 2 H, $B(OH)_2$, exchangeable with D_2O), 6.9, 7.0, 7.4, 7.9 (4 s, 4 H, aromatic H). ^{13}C NMR ($CDCl_3$), δ = 55.7 (OCH_3), 110.2 (aromatic C), 121.5, 133.1, 137.2, 137.8, 164.8 (MeO-**C**, aromatic C).

Methyl 3-(2-methoxyphenyl)phenylacetate (3). Methyl 3-bromophenylacetate (1.79 g, 7.82 mmol) was dissolved in toluene (12 mL), then $Pd(PPh_3)_4$ (270 mg, 0.23 mmol, 3.0 mol% of the amount of bromide **1**) was added under stirring in nitrogen atmosphere. Ten minutes later, 2-methoxy-phenylboronic acid (1.43 g, 9.41 mmol) in toluene (6 mL) and Na_2CO_3 solution (1.70 g in 6 mL of water, 16.04 mmol) were successively added to the yellow solution. The mixture was

refluxed for 16 h under nitrogen. The black mixture was cooled, neutralized by HCl and extracted with AcOEt (2 x 20 mL). The organic layer was separated, washed with saturated saline (2 x 10 mL), dried ($MgSO_4$) and finally concentrated to dryness to give the crude product. During purification by chromatography, an unknown byproduct (R_f = 0.90, PE/AcOEt 8:1) and the product (R_f = 0.58) were separated by gradient elution using 10:1-8:1 PE/AcOEt. Product **3** was isolated as a clear oil in a yield of 88% (1.76 g). In another preparation, a 96% yield was obtained by decreasing the amount of Pd catalyst to 1.5 mol% of the bromide. ^{1}H NMR ($CDCl_3$): δ = 3.6 (s, 2 H, CH_2), 3.7 (s, 3 H, CH_3OC=O), 3.8 (s, 3H, OCH_3), 6.9-7.0 (m, 2 H, aromatic H), 7.2-7.5 (m, 6 H, aromatic H). ^{13}C NMR ($CDCl_3$), δ = 41.6 (CH_2Ar), 52.3 (CO_2CH_3), 55.8 (CH_3OAr), 111.5 (aromatic C), 121.1, 128.1, 128.5, 128.7, 129.0, 130.6, 130.7, 131.2, 133.9, 139.1, 156.7 (MeO-C, aromatic C), 172.3 (C=O).

Methyl 3-(2-hydroxyphenyl)phenylacetate (4). A solution of **3** (1.30 g, 5.08 mmol) in CH_2Cl_2 (45 mL) was cooled to -78°C under argon. BBr_3 (6.5 g, 25.90 mmol) was added and the reaction was carried out at -10°C for 10 h. Ice-water (100 mL) was added to stop the reaction. After washing with $NaHCO_3$ and saturated saline solution, the organic phase was dried and concentrated to give an oil. Purification by chromatography (3:1 PE/AcOEt) gave 0.68 g of the phenol **4** (yield = 60%). TLC (PE/AcOEt 5:1): R_f = 0.50 (product); 0.84 (material). ^{1}H NMR ($CDCl_3$): δ = 3.6 (s, 2 H, CH_2C=O), 3.7 (s, 3 H, OCH_3), 6.2 (br, 1 H, OH), 6.8-7.0, 7.1-7.3, 7.3-7.5 (m, 8 H, 3:3:2, aromatic H). ^{13}C NMR ($CDCl_3$), δ = 41.3 (CH_2Ar), 52.5 (CO_2CH_3), 116.3 (aromatic C), 121.0, 128.2, 128.3, 128.8, 129.4, 129.5, 130.4, 130.6, 134.9, 138.0, 152.9 (C-OH, aromatic C), 172.5 (C=O).

Methyl 3-[2-(2,3,4,6-O-tetraacetyl-α–D–mannopyranosyloxy)phenyl]phenylacetate (5). α–D–Mannose pentaacetate (17.5 g, 44.9 mmol) and BF_3/ Et_2O (25.84 g, 182.1 mmol) were added in portions to the solution of **4** (7.86 g, 32.48 mmol) in CH_2ClCH_2Cl (150 mL). The reaction was carried out for 24 h at room temperature under nitrogen. After adding ice-water (60 mL) and stirring vigorously for 30 min, the organic phase was separated, dried ($MgSO_4$) and concentrated to give 20.2 g of a clear syrup, which contained 29.44 mmol of mannoside **5** (91% yield) and 8.62 mmol of α–D–mannose pentaacetate as measured by NMR. Complete elimination of the mannose pentaacetate by chromatography proved to be difficult. TLC (PE/AcOEt 2:1): R_f = 0.35 (product), 0.81 (phenol). ^{1}H NMR ($CDCl_3$): δ = 2.2-1.9 (4 s, 12 H, 1:1:1:1, acetyl groups), 3.7 (s, 3 H, OCH_3), 3.8 (s, 2 H, CH_2C=O), 3.9-4.3 (m, 3 H, sugar ring H), 5.2-5.4 (m, 3 H, sugar ring H), 5.5 (d, J = 1.2 Hz, 1 H, sugar C-1 H), = 7.1-7.5 (m, 8 H, aromatic H). ^{13}C NMR ($CDCl_3$), δ = 20.9 (CH_3CO), 41.2 (CH_2Ar), 52.2 (OCH_3), 62.2 (mannose C-2 ~ C-6), 65.9, 68.5, 68.9, 69.6, 96.8 (mannose C-1),

116.4 (aromatic C), 123.9, 128.4, 128.8, 129.0, 130.6, 131.3, 132.3, 134.3, 138.0, 152.7 (C-O, aromatic C), 169.8 (C=O of Ac groups), 169.9, 170.2, 170.8, 172.4 (CO_2CH_3).

Methyl 3-[2-(2,3,4,6-O-tetraacetyl-α–D–mannopyranosyloxy)-5-(3-bromopropionyl)-phenyl] phenylacetate (6). Bromopropionyl chloride (3.43 g, 20 mmol) and **5** (8.27 mmol), contaminated with α-D-mannose pentaacetate, were dissolved in CH_2Cl_2 (150 mL) and then cooled to –78°C. Anhydrous $AlCl_3$ (16.6 g) was added and the mixture was stirred in an ice-water bath for 1 h. The reaction was quenched by ice-water (50 mL). The mixture was stirred until the aluminum chloride was completely hydrolyzed (30 min), and then extracted with dichloromethane (50 mL). The organic phase was dried and concentrated to give 7.15 g of a clear syrup containing 7.68 mmol of **6** (yield 93%). This compound could be used in the next step without further purification although it still contained α-D-mannose pentaacetate. TLC (PE/AcOEt 3:2) showed that the starting compound **5** (R_f = 0.48) was entirely consumed (product R_f = 0.33). Pure **6** could be easily separated from the crude product through silica gel chromatography (3:2 PE/AcOEt) at a 93% yield. ^{1}H NMR ($CDCl_3$): δ = 1.9-2.2 (4 s, 12 H, 1:1:1:1, acetyl groups), 3.0 (t, 2 H, O=CC**H**$_2$CH$_2$Br), 3.6 (t, 2 H, CH_2Br), 3.7 (s, 3 H, OCH_3), 3.8 (s, 2 H, O_2CCH_2), 3.9-4.4 (m, 3 H, sugar ring H), 5.2-5.5 (m, 3 H, sugar ring H), 5.6 (d, J = 1.4 Hz, 1 H, sugar C-1 H), 7.2-7.6 (m, 5 H, aromatic H), 7.9-8.1 (m, 2 H, aromatic H). ^{13}C NMR ($CDCl_3$), δ = 21.3; 25.9; 37.9; 41.6; 52.7; 62.5; 66.1; 69.1; 69.6; 70.3; 96.6; 115.8; 124.2; 128.7; 129.4; 130.6; 130.9; 132.0; 133.2; 134.9; 137.2; 139.4; 156.9; 170.3; 171.2; 172.0; 172.8; 173.9; 196.1.

Methyl 3-[2-(2,3,4,6-O-tetraacetyl-α–D–mannopyranosyloxy)-5-(3-bromopropyl)phenyl]-phenylacetate (7). In a nitrogen atmosphere, BH_3/THF reagent (1.0 M, 15 mL) was added in portions to the Friedel-Crafts reaction residue (containing 7.68 mmol of **6** with 4.41 mmol of mannose pentaacetate) dissolved in anhydrous THF (150 mL). In anhydrous conditions, this reaction lasted 3 h at room temperature. After adding methanol (15 mL) to destroy the excess borane, the mixture was concentrated under reduced pressure to afford a clear syrup. The crude product was purified by silica gel chromatography using 3:2 PE/AcOEt as eluant. 3.68 g (5.31 mmol) of pure **7** (R_f = 0.19, 3:2 PE/AcOEt) was obtained. The overall yield of the last two steps was 64% and this reduction step gave a yield of 69% (in several preparations this yield ranged from 69-74%). To monitor the reaction progress, samples obtained from the reaction mixture were analyzed by TLC. ^{1}H NMR ($CDCl_3$): δ = 1.8 (m, 2 H, C**H**$_2$CH$_2$Br), 1.9-2.2 (4 s, 12 H, 1:1:1:1, acetyl groups), 3.4 (t, 2 H, ArC**H**$_2$CH$_2$), 3.5 (t, 2 H, CH_2Br); 3.7 (s, 3 H, OCH_3), 3.8 (s, 2 H, C**H**$_2$CO$_2$), 3.9-4.2 (m, 3 H, sugar ring H), 5.2-5.4 (m, 3 H, sugar ring H), 5.5 (d, J = 1.0 Hz, 1 H, sugar C-1 H), 7.1-7.5 (m, 7 H, aromatic H). ^{13}C NMR ($CDCl_3$), δ = 20.5; 32.9; 34.1; 34.7; 40.4; 52.2; 61.9; 68.8; 70.9; 71.5;

72.5; 97.1; 118.2; 122.0; 126.7; 128.4; 129.6; 130.7; 131.0; 134.8; 135.4; 139.9; 141.2; 157.1; 169.8; 169.9; 170.8; 171.1; 171.9.

Methyl 3-[2-(2,3,4,6-O-tetraacetyl-α–D–mannopyranosyloxy)-5-(3-azidopropyl)phenyl]-phenylacetate (8). The bromide 7 (2.37 g, 3.42 mmol) was dissolved in DMF (40 mL) and heated to 50°C. Sodium azide (1.11 g, 17.1 mmol) was added. The light yellow solution immediately turned orange. The reaction mixture was left at 50°C for 24 h. The insoluble salts were filtered off and the filtrate was concentrated by evaporation under reduced pressure. The residue was dissolved in $CHCl_3$ (100 mL), and washed with cold saline (2 x 15 mL). The organic layer was dried over $MgSO_4$. After evaporation of the solvent, a crude product was obtained as a slightly yellow syrup. Further purification was carried out by silica gel chromatography with 3:2 PE/AcOEt as eluant. The fractions with R_f = 0.17 (PE/AcOEt 3:2) were collected to give 1.22 g of **8** as a syrup. Yield = 54%. IR (film): 2100 cm^{-1}. ^1H NMR ($CDCl_3$): δ = 1.3 (m, 2 H, $\mathbf{CH_2}CH_2N_3$), 1.9-2.2 (4 s, 12 H, 1:1:1:1, acetyl groups), 3.1 (t, 2 H, $ArC\mathbf{H_2}CH_2$), 3.4 (t, 2 H, CH_2N_3), 3.6 (s, 3 H, OCH_3), 3.7 (s, 2 H, $\mathbf{CH_2}CO_2$), 3.8-4.2 (m, 3 H, sugar ring H), 5.1 (m, 3 H, sugar ring H), 5.3 (d, J = 1.2 Hz, 1 H, sugar C-1 H), 6.8-7.4 (m, 7 H, aromatic H). ^{13}C NMR ($CDCl_3$), δ = 20.9; 26.1; 32.1; 40.3; 44.8; 52.3; 61.9; 68.3; 71.0; 71.6; 72.5; 97.1; 117.4; 122.0; 126.3; 126.4; 126.7; 129.6; 130.7; 134.8; 136.3; 141.1; 141.5; 156.1; 169.9; 170.1; 170.5; 171.1;171.9.

Methyl 3-[2-(2,3,4,6-O-tetraacetyl-α–D–mannopyranosyloxy)-5-(3-aminopropyl)phenyl] phenylacetate (9). The Pd/C catalyst (10%, 0.68 g) was suspended in a mixture of formic acid and methanol (30 and 60 mL) and hydrogen was bubbled. This activation process lasted 0.5~1 h. The azide derivative **8** (1.75 g, 2.67 mmol) in methanol (30 mL) was added to the suspension. The reaction was continued for 5 h under H_2 at room temperature. TLC ($AcOEt/CH_3OH$) showed that the azide (R_f = 0.94) was gradually consumed and the amine (as formate, R_f = 0.18) was produced during this period. After this reaction, the solvents were removed and the residue was purified on the silica gel column eluting first with a 3:1 $AcOEt/CH_3OH$ mixture, then with CH_3OH alone. Fractions with R_f = 0.18 (ninhydrin-positive) were collected and gave 1.14 g of the amine with a yield of 63%. ^1H NMR ($CDCl_3$): δ = 1.3 (m, 2 H, $\mathbf{CH_2}CH_2N$), 1.9-2.2 (4 s, 12 H, 1:1:1:1, acetyl groups), 3.1 (t, 2 H, $Ar\mathbf{CH_2}CH_2$,), 3.6 (t, 2 H, CH_2N), 3.7 (s, 3 H, OCH_3), 3.8 (t, 2 H, CH_2CO_2), 3.9-4.3 (m, 3 H), 5.1-5.4 (m, 3 H), 5.5 (d, J = 1.4 Hz, 1 H, sugar C-1 H), 6.1-6.7 (br, 3 H, RNH_3^+, exchangeable with D_2O), 7.0-7.7 (m, 7 H, aromatic H), 8.3 (s, 1 H, HCO- of formate ion). ^{13}C NMR ($CDCl_3$), δ = 20.8; 30.1; 30.6; 40.4; 41.6; 52.2; 61.9; 68.2; 70.9; 71.6; 72.7; 97.0; 117.6; 121.9; 126.5; 129.7; 129.8; 130.2; 131.1; 134.9; 136.3; 140.9; 142.2; 157.1; 169.9; 170.2; 171.1; 171.7.

3-[2-α–D–mannopyranosyloxy-5-(3-aminopropyl)phenyl]phenylacetic acid (10). Four mL of 12.5% NaOH solution were added to 6 mL solution of compound **9** (682 mg, 1.01 mmol) in methanol. The mixture was stirred at room temperature for 24 h, then evaporated after adjustment of the pH to neutrality with HCl. Hot methanol (30 mL) was added to the residue. The insoluble salt was filtered and the filtrate was evaporated. This crude residue was dissolved in distilled water (5 mL) and the pH was adjusted to ~3 with HCl. The purification was conducted by preparative reverse-phase HPLC monitored at 254 nm. The fractions containing the eluted product **10** were concentrated and lyophilized to give 483 mg of a yellowish solid which consisted of HCl salt of **10** and a small amount of salt. From the contents of Na^+ and Cl^- determined by ion selective electrode and classical Cl^- quantification,[19] it could be calculated that this mixture was composed of 0.79 mmol of **10** as HCl salt (382 mg) and 101 mg of NaCl. NaCl was subsequently removed by chromatography on Sephadex G-15. The yield was 78%. Analytical reverse-phase HPLC: retention time 6.20 min. UV: λmax 209, 249 and 280 nm (in H_2O). L-SIMS: m/z = 483 (M^+), 505 (M-H+Na)$^+$ for $C_{23}H_{30}O_8NCl$. ^1H NMR (D_2O): δ = 1.3 (m, 2 H, CH_2CH_2N), 2.9 (t, 2 H, $ArCH_2CH_2$), 3.3 (t, 2 H, CH_2N), 3.6 (s, 2 H, CH_2CO_2), 3.6-4.1 (m, 6 H, sugar ring H), 5.4 (d, J = 1.2 Hz, 1 H, sugar C-1 H), 7.1-7.5 (m, 7 H, aromatic H). L-SIMS: *m/z* = 470 $(M+H)^+$. ^{13}C NMR (D_2O), δ = 30.1; 30.6; 41.3; 41.6; 61.9; 70.8; 71.0; 74.2; 77.3; 77.9; 101.4; 116.7; 120.2; 125.4; 128.7; 129.4; 129.9; 133.9; 134.1; 134.7; 141.1; 142.4; 158.1; 173.1.

DTPA-B(SLe^X)A (11). Diethylenetriaminepentaacetic acid (DTPA) bisanhydride was synthesized according to the literature.[20] DTPA (7.86 g, 20 mmol) was suspended in dry pyridine (10 mL) and acetic anhydride (8 mL). The mixture was stirred for 24 h at 60~65°C under nitrogen. Subsequently, the solid was filtered, washed with anhydrous acetonitrile and ether, and dried in vacuum to give 6.4 g of bisanhydride as a white powder (yield 90%; m.p. 182°C, dec.). Compound **10** (0.793 mmol) was dissolved in 15 mL of distilled water, the pH was adjusted to 8.5, and 142 mg (0.396 mmol) of freshly prepared DTPA bisanhydride were added in portions over 0.5 h at 0-5°C. This reaction was continued for 3 h at room temperature. The pH was maintained at 8.0-8.5 by additions of NaOH. The solution was adjusted to pH 3 with HCl. HPLC analysis showed a yield of 84%. Unreacted DTPA and salts were removed by size exclusion chromatography on Sephadex G-15. Analytical reverse-phase HPLC: retention time 3.58 min (free DTPA, 2.16 min). UV: λmax 252 and 282 nm (in H_2O). L-SIMS: m/z = 1252 $(M+H)^+$ for $C_{60}H_{77}O_{24}N_5$. ^1H NMR (D_2O, pD = 6): δ =, 1.7 (m, 4 H, $ArCH_2CH_2$), 2.8 (t, 4H, $ArCH_2CH_2$), 3.0 (t, 4 H, CH_2NHCO), 3.0-3.4 (m, 12 H, 2 × NCH_2CO_2 and 2 × NCH_2CH_2N), 3.4-4.1 (m, 22 H, 12 sugar ring H and 3 × NCH_2CO and 2 × $ArCH_2CO_2$), 5.4 (d, J = 1.5 Hz, 2 H, sugar C-1 H), 6.9-7.6 (m, 14 H, aromatic H). In ^1H NMR ([D_6]

DMSO), the signal at δ = 7.8 (br, 2 H) confirmed the existance of two CONH functions. ^{13}C NMR (D_2O, pD=6), δ = 41.4 ; 21.5; 29.0; 38.7; 52.7; 52.9; 56.4; 57.9; 60.7; 61.8; 70.8; 74.2; 77.5; 77.9; 101.8; 116.6; 120.3; 125.5; 128.7; 129.7; 130.2; 133.9; 134.0; 134.7; 141.1; 142.5; 158.5; 173.8; 174.3; 175.2; 177.0; 178.2.

Acknowledgments

The authors thank Mrs. Patricia de Francisco for her help in preparing the manuscript. F. Botteman is acknowledged for the perusal of the manuscript and specifically acknowledged by Y. F. for his kind assistance and advice in the laboratory. This work was supported by the ARC Program (contracts number 95/00-194 and 00-05/258) of the French Community of Belgium.

Captions

Figure 1 : Structure of the sLe^x mimetic described in the literature[3] (A) and of the new amino-containing sLe^x mimetic (B) for derivation of DTPA.

Figure 2 : Synthetic route to sLe^x mimetic-DTPA conjugate (DTPA-B(SLe^X)A).

Figure 3 : Alternative route to the bromo ketone.

Figure 4 : Alternative route to prepare the amine **10** by Gabriel synthesis.

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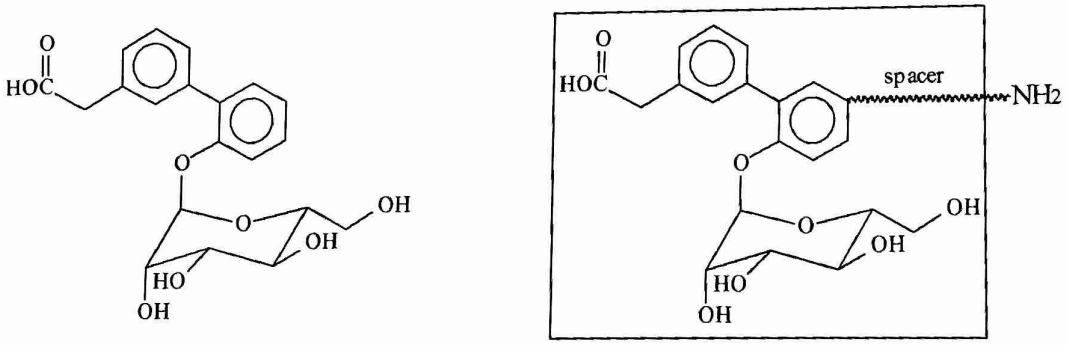
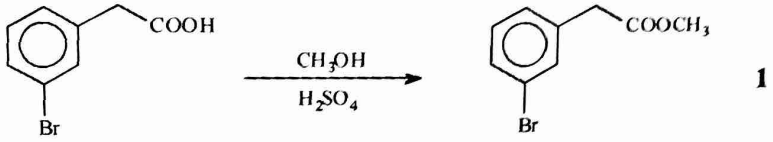
Figures:



A **B**

Figure 1 : Structure of the sLe^x mimetic described in literature[3] (A) and the new amino-containing sLe^x mimetic (B) for derivation of DTPA.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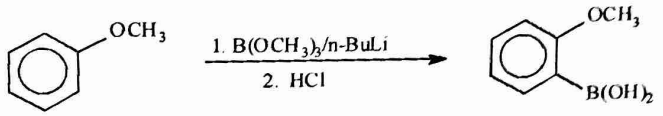
H2SO4
COOCH3
Br

1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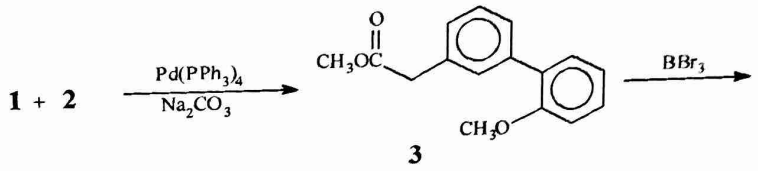
2. HCl
OCH3
B(OH)2

2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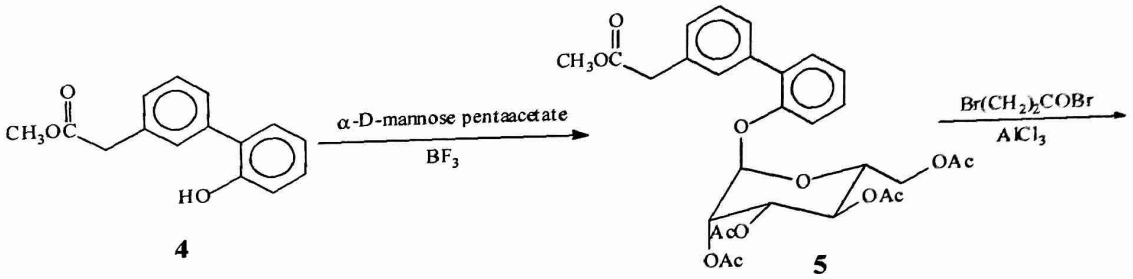
O
CH3OC
CH3O
BBr3

3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AcO
OAc
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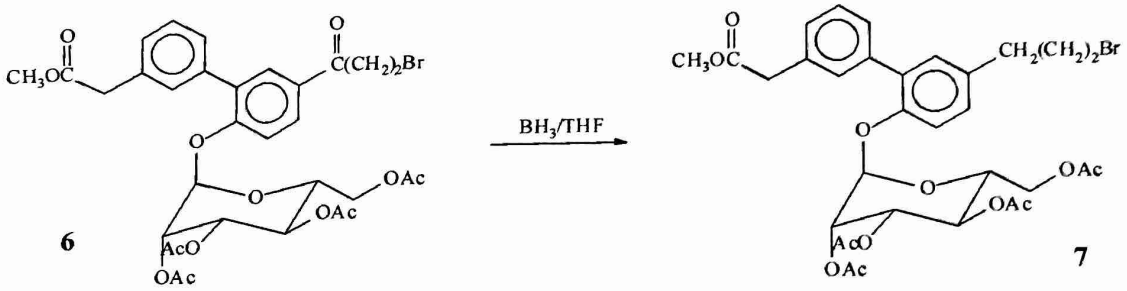
AlCl3

4 5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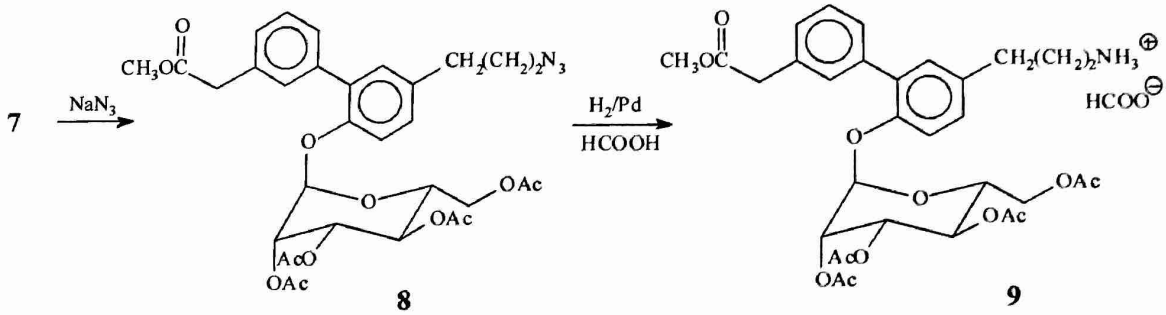
OAc
AcO
OAc
BH3/THF
CH2(CH2)2Br

6 7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HCOO

8 9

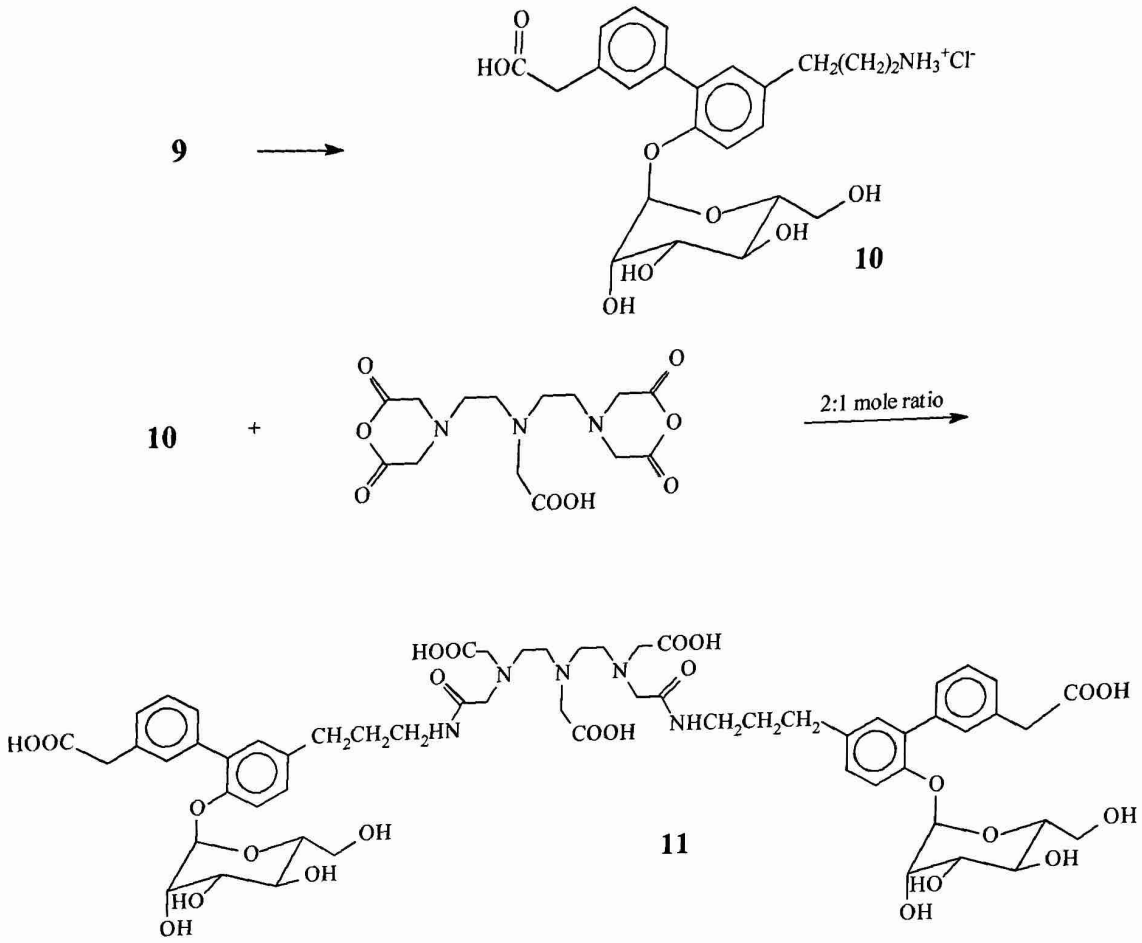

Figure 2 : Synthetic route to sLe^x mimetic-DTPA conjugate (DTPA-B(SLe^X)A).

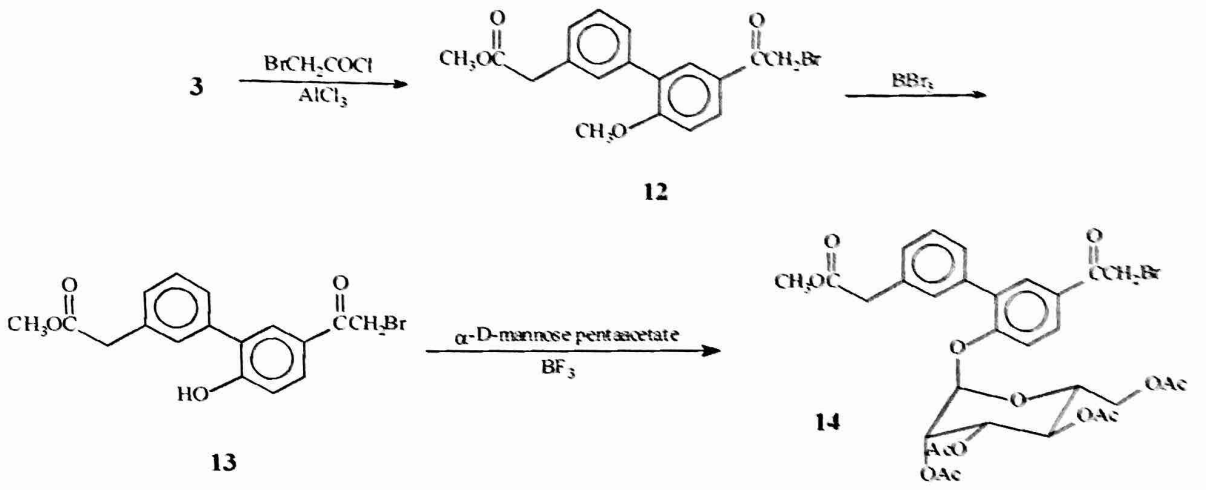

Figure 3: Alternative route to the bromo ketone.

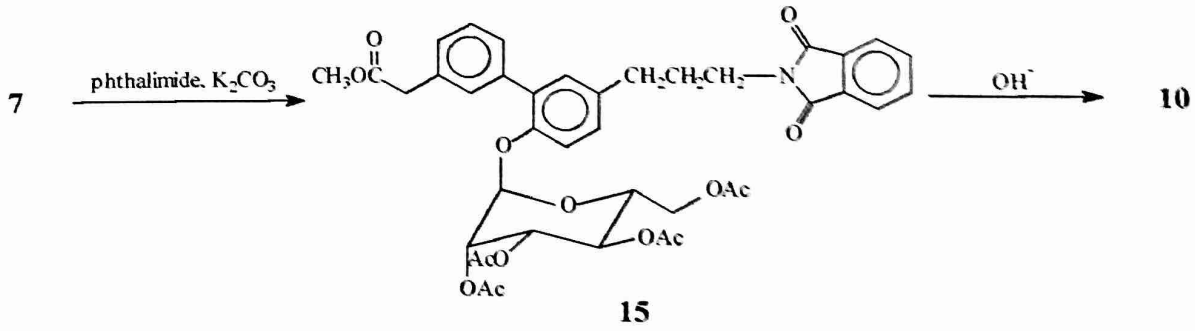

Figure 4 : Alternative route to prepare the amine **10** by Gabriel synthesis.

Graphical Abstract: