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

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Keywords: DTPA / Sialyl Lewis^X mimetic / Contrast agents / MRI / Nuclear medicine

The structure of a mimetic of sialyl Lewis^X, namely 3-[2-(α-D-mannopyranosyloxy)phenyl]phenylacetic acid, was coupled to diethylenetriaminepentaacetic acid (DTPA) via a flexible alkyl spacer and the amide linkage. 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.

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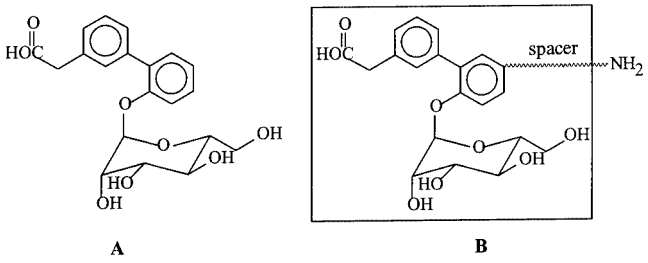
Introduction

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 4−24 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]

Contrary to this, 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 such as 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 super-paramagnetic particles that accelerate the magnetic relaxation process.[9−11] Currently, Gd-DTPA (*Magnevist*) and Gd-DTPA-BMA (*Omniscan*) [DTPA = diethylenetriaminepentaacetic acid; BMA = bis(methyl)amide] are among the most widely used paramagnetic contrast agents in routine MRI examinations around the world. The safety of these agents is based on the very strong chelating capability of DTPA and its derivative toward the paramagnetic but toxic gadolinium ion. In this work, for the purpose of developing novel contrast agents targeted at 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 $^{157}Gd^{3+}$, $^{153}Gd^{3+}$, $^{111}In^{3+}$, etc.



A **B**

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

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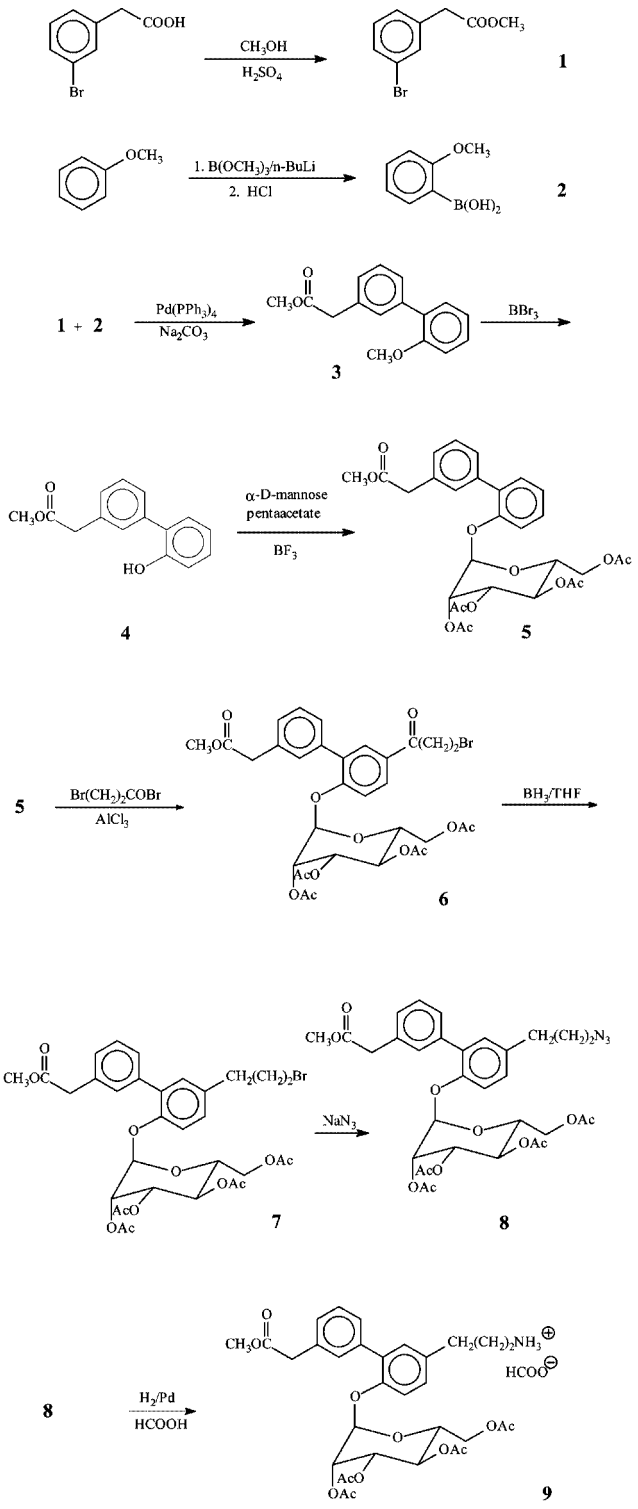
Results and Discussions

DTPA can be easily converted into the corresponding cyclic bis(anhydride). It is therefore convenient to prepare DTPA bis(amides) by treating 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 proven 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-bromophenylacetic acid.



Synthesis of Compounds 1−5

These compounds were synthesized according to the method described in the literature.[3] Reactions and products were studied by TLC and ^{1}H and ^{13}C NMR.

Methyl (3-Bromophenyl)acetate (1)

This compound was synthesized according to the experimental procedure described in the literature.[3]

Boronation, (2-Methoxyphenyl)boronic Acid (2)

The substituted phenylboronic acid **2** was obtained from acid hydrolysis of its methyl ester synthesized by allowing *n*-butyllithium-treated anisole and trimethylboronate to react at low temperature. In this reaction, some anisole (ca. 20%) remained unchanged, and a minor by-product, *n*-butylboronic acid [R_f = 0.29; PE/EtOAc, 8:1 (PE = petroleum ether); b.p. 45−60 °C], was obtained at a yield of ca. 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 literature.[3]

Coupling Reaction, Methyl [3-(2-Methoxyphenyl)phenyl]-acetate (3)

A subsequent coupling reaction between **1** and **2** was conducted to obtain the biphenyl derivative **3**. According to the literature,[14] the amounts of catalyst [$Pd(PPh_3)_4$] and base (Na_2CO_3) were chosen as 3.0 mol % and ca. 200 mol %, respectively, in relation 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 by-product with R_f = 0.90 (PE/EtOAc, 8:1) was detected in this reaction.

Ether Cleavage, Methyl [3-(2-Hydroxyphenyl)phenyl]-acetate (4)

The methoxy group was removed from ether **3** by reaction with BBr_3 at a low temperature to give phenol **4** in moderate yield. TLC showed that increasing 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 in by-products. Separation of the reaction mixture by column chromatography yielded several fractions: phenol **4** (R_f = 0.50, yield 55−70%) and a series of by-products (R_f = 0.32, 0.09, and 0; PE/EtOAc, 5:1). The by-product with R_f = 0.32 was confirmed to be 3-(2-hydroxyphenyl)-phenylacetic acid [^{1}H NMR: δ = 3.7 (s, 3 H), 6.9−7.7 (m,

8 H)]. It resulted from the cleavage of both ether and ester bonds of **3**.

Glycosylation, Methyl {3-[2-(2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyloxy)phenyl]phenyl}acetate (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, higher mol ratios of mannose pentaacetate and catalyst (up to 2:1 and 6:1, respectively, as compared to **4**) were used. This is in contrast to the 1:1 and 3.5:1, respectively, used in the literature.[3] The addition of reactants in portions was found to improve the yield when compared 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 co-eluted with the product **5**. Their mole ratio was successfully quantified by comparing the peak areas 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 (Figure 2).

Friedel−Crafts Acylation, Methyl {3-[5-(3-Bromopropionyl)-2-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyloxy)phenyl]-phenyl}acetate (6)

In order to obtain the bromo ketone **6**, the tetra-acetylmannopyranoside **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**) could be selectively monoacylated at the *para* position of the mannopyranosyloxy group by a Friedel−Crafts reaction.[4] Monitoring this reaction by TLC, we demonstrated that a higher conversion was obtained by increasing the amount of $AlCl_3$ (from mole ratio 10:1 to 15−20:1 with respect to **5**), as well as by lengthening the reaction time (from 0.5 to 1−1.5 h). To avoid freezing of the reaction solution during the activation of the acyl group by $AlCl_3$ at a low temperature (−78 °C), we used CH_2Cl_2 (m.p. −97 °C) as the reaction solvent instead of CH_2ClCH_2Cl (m.p. −35 °C).[4] Further increments of the reaction time at 0 °C did not improve the yield, but it did retard the formation of by-products.

Borane Reduction, Methyl {3-[5-(3-Bromopropyl)-2-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyloxy)-phenyl]phenyl}acetate (7)

Bromo ketone **6** was reduced to bromide **7** in the presence of BH_3/THF. As was previously observed, 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 mol of BH_3 was necessary to reduce the C=O group to a methylene group,[15,16] but some excess was in fact needed in this reaction. After 2−3 h, a complete conversion of the ketone was achieved and only small amounts of reduced by-products were obtained. Further increases in the amount of the BH_3 and the reaction time

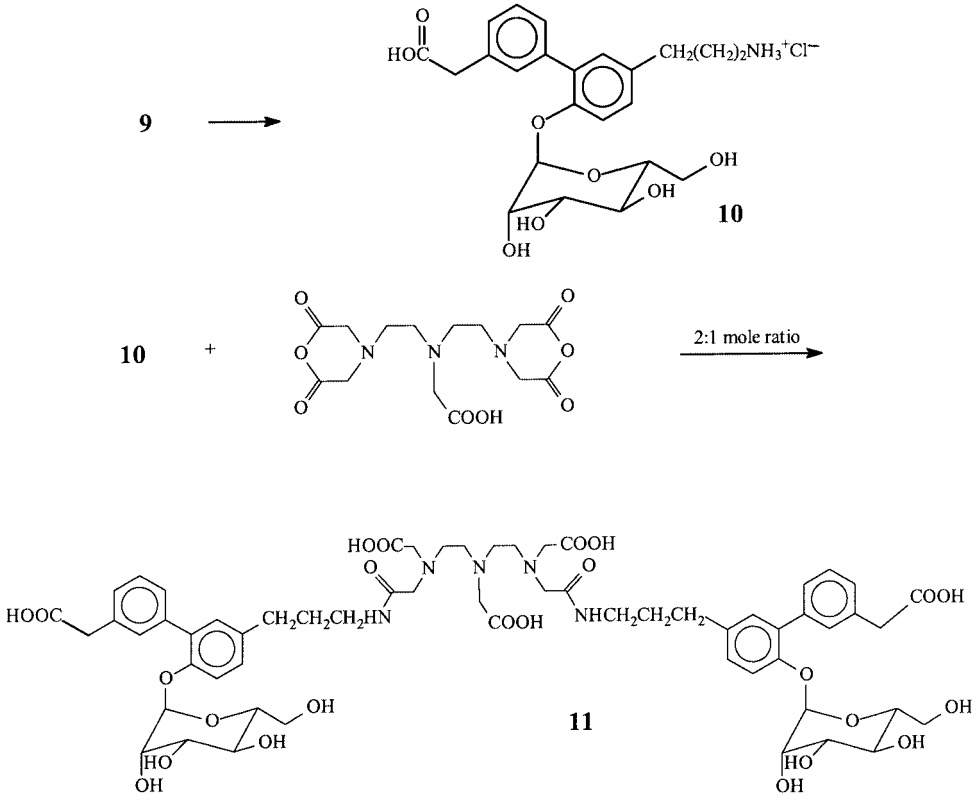


Figure 2. Synthetic route to sLe^X mimetic DTPA conjugate [DTPA-B(SLe^X)A]

led to a series of by-products with an increasing number of hydroxy groups (produced from the ester reduction).

NaN_3 Substitution, Methyl {3-[5-(3-Azidopropyl)-2-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyloxy)phenyl]phenyl} acetate (8)

Bromide **7** was submitted 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 by-products (R_f = 0.40 and 0; PE/EtOAc, 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 excess NaN_3 and a long reaction time were thus adopted to guarantee a full conversion of the bromide **7**. An IR adsorption at 2100 cm^{-1} confirmed the azide nature of the product.

Reduction, Methyl {3-[5-(3-Aminopropyl)-2-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyloxy)phenyl]phenyl}acetate (9)

Reduction of azide **8** to amine **9** was carried out by hydrogen in the presence of Pd/C catalyst at room temperature. A 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 the 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 complete within 4−6 h.

Ester Hydrolysis, {3-[5-(3-Aminopropyl)-2-(α-D-mannopyranosyloxy)phenyl]phenyl}acetic Acid (10)

On treatment with concentrated NaOH solution, all ester groups of **9** were removed to give a hydroxy-exposed α-D-mannoside **10**. An initial 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 by-products was obtained. A small amount of NaCl contaminating the product was removed by chromatography.

Ligand Synthesis of the Amine (Compound 10) Derived Bis(amide) of Diethylenetriaminepentaacetic Acid, DTPA-B(SLe^X)A (11)

The reaction between the bis(anhydride) of DTPA and a twofold amount of **10** led to the formation of two amide linkages. Initially, DMF or CH_3CN were chosen as the reaction media, but neither of them 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 bis(anhydride), a mild pH (< 8.5) was maintained. As shown by reversed-phase HPLC, a relatively high yield (ca. 80%) of pure ligand **11** was achieved.

Alternative Reaction Sequences

Another route to **6** may be conceived by modifying the reaction (Figure 3). Actually, this route was attempted (with bromoacetyl bromide) but proved to be unsuccessful. Compound **3** first underwent the Friedel−Crafts reaction to give methyl 3-[5-(bromoacetyl)-2-methoxyphenyl]phenyl acetate (**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

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 was tried. This reaction, however, gave complex mixtures; as shown by TLC, at least six components were formed. The existence of a ketone carbonyl group conjugated with the biphenyl

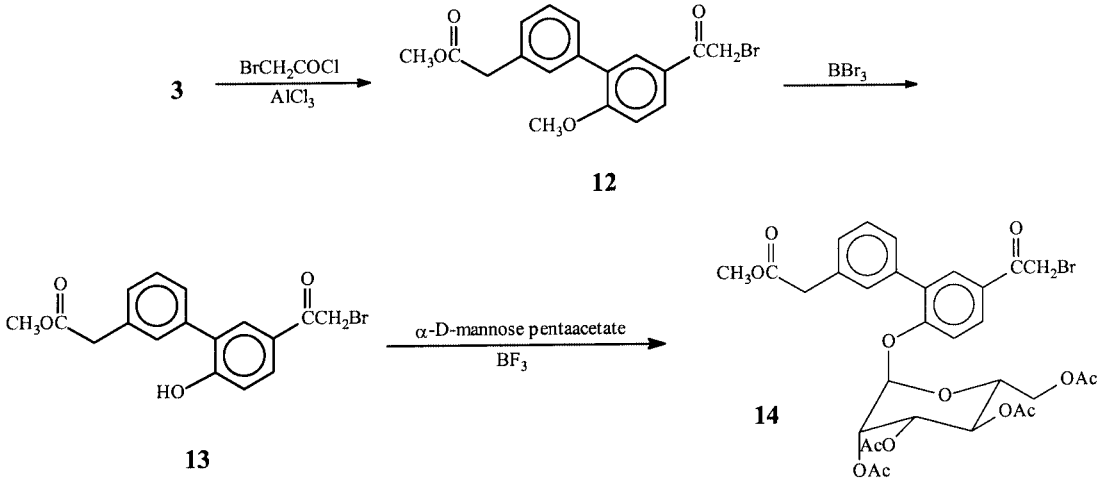


Figure 3. Alternative route to the bromo ketone

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

Direct Ammonolysis of 7

Although the preparation of a primary amine through direct ammonolysis of a bromide is not often of a great practical use, it could 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 gave a poor yield (< 15%) of the desired product after column chromatography purification (TLC; R_f = 0.50−0.60; CH_3OH/H_2O, 3:1). The major by-products contained no amino groups and their structure was not revealed.

Gabriel Synthesis (Figure 4)

Bromide **7** was treated 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-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)-5-(3-phthalimidylpropyl)phenyl]phenylacetate (**15**) (R_f = 0.45; PE/EtOAc, 1:1) in 56% yield, which was purified by chromatography with PE/EtOAc (1:1) as the eluent. The appearance of a signal at δ = 7.6−8.1 (4 H) in the ^{1}H NMR spectrum confirmed the expected structure. Due to the existence of an acid-sensitive glycoside linkage in the molecule, the phthalic group could not be removed by hydrazine and HCl or just by concentrated HCl, which are the two most common deprotecting agents. The hydrolysis in strongly alkaline conditions thus appeared 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, [5-amino-2-(α-D-mannopyranosyloxy)phenyl]-phenylacetic acid, using *p*-nitroanisole instead of anisole 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 (PE/EtOAc, 8:1) and the fractions analyzed by ^{1}H NMR. It was found that, aside from unchanged 4-nitroanisole, all other fractions contained products with *n*-butyl groups. Side reactions in which the nitro group is attacked by the strongly nucleophilic *n*-butyl anion are thus likely. We were also unable to obtain this intermediate 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 $(CH_2)_3$ was successfully carried out up to 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 undesired α-elimination in which the newly formed C=C bond would be substantially stabilized owing to its conjugation with the biphenyl system.

This new compound, DTPA-B(Sle^X)A, could represent a brightly sensitive ligand for the non-invasive detection of E-selectin, selectively expressed on the endothelium of the inflamed tissues. The diagnosis of a wide range of pathologies with an inflammatory component (i.e. infection, atherosclerosis, cancer, ischema, etc.) is therefore promising for the clinical medicine.

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-Bromophenyl)acetic acid (98%), anisole, trimethyl boronate (99%), boron trifluoride−diethyl ether, tetrakis(triphenylphosphane)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

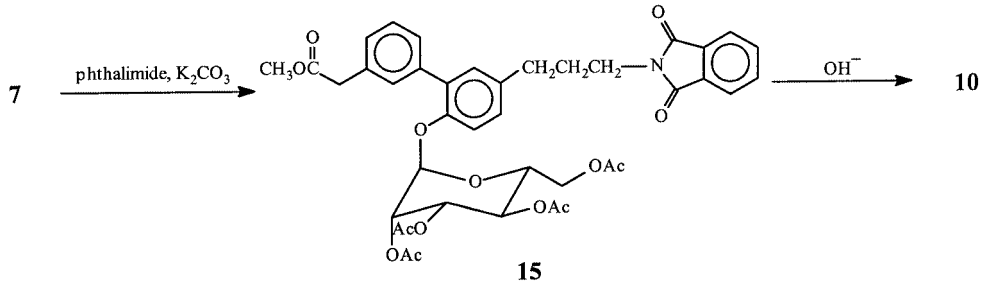


Figure 4. Alternative route to prepare the amine **10** by Gabriel synthesis

(95−97%), 2.0 M ammonia in methanol, 2.5 M *n*-butyllithium in hexane (determined as 2.13 M before use), 10 wt% Pd/C catalyst, 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 3 Å molecular sieves (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 and ninhydrin 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 with a Büchi-512 melting point apparatus. IR spectra were recorded with a Perkin−Elmer FTIR 1760X spectrometer (Perkin−Elmer Instruments, Zaventem, Belgium). ^{1}H and ^{13}C NMR spectra were recorded at 7.04 T with a Bruker AMX-300 spectrometer (Bruker, Karlsruhe, Germany). Mass spectra were obtained with a VG AUTOSPEC 6F mass spectrometer (VG Analytical, Manchester, UK). Reversed-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) was used for desalting and purification of some synthesized compounds. The syntheses of intermediates **1**−**5** were carried out according to the methods reported in the literature[3] and modified procedures. 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 by-products were also identified. For chromatographic separations, EtOAc/petroleum ether (PE, b.p. 45−60 °C) was used instead of EtOAc/hexane. This avoided the use of large quantities of more expensive *n*-hexane without any loss of performance.

Methyl (3-Bromophenyl)acetate (1): Briefly, (3-bromophenyl)acetic acid (10.0 g, 46.5 mmol) and methanol (80 mL) were heated under reflux overnight in the presence of 0.2 mL 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 (yield 9.80 g, 92%). Further purification by chromatography as proposed in the reference was not necessary. TLC (PE/EtOAc, 8:1): R_f = 0.96 (ester) as compared to the acid with R_f = 0.67. ^{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) ppm. ^{13}C NMR ($CDCl_3$), δ = 40.9 (CH_2Ph), 52.5 (OCH_3), 122.8, 128.2, 130.3, 130.5, 132.6, 136.3 (aromatic C), 171.6 (C=O) ppm.

(2-Methoxyphenyl)boronic 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 of reaction at room temperature, the solution was acidified to pH = 3 with HCl and stirred vigorously for 1 h. After extraction with ether, the organic phase was dried and the solvent was evaporated. Contrary to the protocol published in the literature, this crude product was purified by chromatography. After elution of the unchanged anisole by PE, the product was recovered by PE/EtOAc (1:1). After concentration, colorless crystals of **2** were obtained (yield 8.87 g, 63%). M.p. 80−81 °C (a clear oil in 95% yield was previously reported[3]). TLC (PE/EtOAc, 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 (d, q, q, d, J_{34} = 9.1, J_{45} = 3.8, J_{56} = 7.6 Hz, 4 H, 1:1:1:1, aromatic H) ppm. ^{13}C NMR ($CDCl_3$), δ = 55.7 (OCH_3), 110.2, 121.5, 133.1, 137.2, 137.8 (aromatic C), 164.8 (MeO-*C*, aromatic C) ppm.

Methyl 3-(2-Methoxyphenyl)phenylacetate (3): Methyl (3-bromophenyl)acetate (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 whilst stirring under nitrogen. After 10 min, (2-methoxyphenyl)boronic 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 heated under reflux for 16 h under nitrogen. The black mixture was cooled, neutralized by HCl and extracted with EtOAc (2 × 20 mL). The organic layer was separated, washed with saturated saline (2 × 10 mL), dried ($MgSO_4$), and finally concentrated to dryness to give the crude product. During purification by chromatography, an unknown by-product (R_f = 0.90; PE/EtOAc, 8:1) together with the expected product (R_f = 0.58) were separated by gradient elution using PE/EtOAc (10:1→8:1). Product **3** was isolated as a clear oil in a yield of 1.76 g (88%). In another preparation, a 96% yield was obtained by decreasing the amount of Pd catalyst to 1.5 mol% relative to 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, 3 H, OCH_3), 6.9−7.0 (m, 2 H, aromatic H), 7.2−7.5 (m, 6 H, aromatic H) ppm. ^{13}C NMR ($CDCl_3$), δ = 41.6 (CH_2Ar), 52.3 (CO_2CH_3), 55.8 (CH_3OAr), 111.5, 121.1, 128.1, 128.5, 128.7, 129.0, 130.6, 130.7, 131.2, 133.9, 139.1 (aromatic C), 156.7 (MeO-C, aromatic C), 172.3 (C=O) ppm.

Methyl [3-(2-Hydroxyphenyl)phenyl]acetate (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-cold 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 (PE/EtOAc, 3:1) gave 0.74 g (60%) of the phenol **4**. TLC (PE/EtOAc, 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) ppm. ^{13}C NMR ($CDCl_3$): δ = 41.3 (CH_2Ar), 52.5 (CO_2CH_3), 116.3, 121.0, 128.2, 128.3, 128.8, 129.4, 129.5, 130.4, 130.6, 134.9, 138.0 (aromatic C), 152.9 (C-OH, aromatic C), 172.5 (C=O) ppm.

Methyl {3-[2-(2,3,4,6-Tetra-*O*-acetyl-α-D-mannopyranosyloxy)-phenyl]phenyl}acetate (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-cold 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 (91%) of mannoside **5** 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/EtOAc, 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) ppm. ^{13}C NMR ($CDCl_3$): δ = 20.9 (CH_3CO), 41.2 (CH_2Ar), 52.2 (OCH_3), 65.9, 68.5, 68.9, 69.6 62.2 (mannose C-2 to C-6), 96.8 (mannose C-1), 116.4, 123.9, 128.4, 128.8, 129.0, 130.6, 131.3, 132.3, 134.3, 138.0 (aromatic C), 152.7 (C-O, aromatic C), 169.8, 169.9, 170.2, 170.8 (C=O of Ac groups), 172.4 ($\mathbf{C}O_2CH_3$) ppm.

Methyl {3-[5-(3-Bromopropionyl)-2-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)phenyl]phenyl}acetate (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-cold 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 (93%) of **6**. This compound could be used in the next step without further purification although it still contained α-D-mannose pentaacetate. TLC (PE/EtOAc, 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 (PE/EtOAc, 3:2) 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, J = 7.4 Hz, 2 H, O=CCH_2CH$_2$Br), 3.6 (t, J = 7.4 Hz, 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) ppm. ^{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 ppm.

Methyl {3-[5-(3-Bromopropyl)-2-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)phenyl]phenyl}acetate (7): Under nitrogen, 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). Under 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 PE/EtOAc (3:2) as eluent. 3.68 g (5.31 mmol) of pure **7** (R_f = 0.19; PE/EtOAc, 3:2) 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, CH_2CH$_2$Br), 1.9−2.2 (4 s, 12 H, 1:1:1:1, acetyl groups), 3.4 (m, 2 H, ArCH_2CH$_2$), 3.5 (t, J = 6.4 Hz, 2 H, CH_2Br); 3.7 (s, 3 H, OCH_3), 3.8 (s, 2 H, CH_2CO_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) ppm. ^{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 ppm.

Methyl {3-[5-(3-Azidopropyl)-2-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)phenyl]phenyl}acetate (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 kept at 50 °C for 24 h. The insoluble salts were filtered off and the filtrate was concentrated by solvent evaporation under reduced pressure. The residue was dissolved in $CHCl_3$ (100 mL), and washed with cold saline (2 × 15 mL). The organic layer was dried with $MgSO_4$. 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 PE/EtOAc (3:2) as eluent. The fractions with R_f = 0.17 (PE/EtOAc, 3:2) were collected to give 1.22 g (54%) of **8** as a syrup. IR (film): $\tilde{\nu}$ = 2100 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ = 1.3 (m, 2 H, $CH_2CH_2N_3$), 1.9−2.2 (4 s, 12 H, 1:1:1:1, acetyl groups), 3.1 (m, 2 H, ArCH_2CH_2), 3.4 (t, J = 5 Hz, 2 H, CH_2N_3), 3.6 (s, 3 H, OCH_3), 3.7 (s, 2 H, CH_2CO_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) ppm. ^{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 ppm.

Methyl {3-[5-(3-Aminopropyl)-2-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyloxy)phenyl]phenyl}acetate (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 into the solution. 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 (EtOAc/CH_3OH, 8:1) 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 EtOAc/CH_3OH (3:1) mixture, then with CH_3OH alone. Fractions with R_f = 0.18 (ninhydrin-positive) were collected and gave 1.14 g (63%) of the amine. ^{1}H NMR ($CDCl_3$): δ = 1.3 (m, 2 H, CH_2CH$_2$N), 1.9−2.2 (4 s, 12 H, 1:1:1:1, acetyl groups), 3.1 (m, 2 H, ArCH_2CH$_2$,), 3.6 (s, 3 H, OCH_3), 3.7 (t, 2 H, CH_2CO_2), 3.8 (t, 2 H, CH_2N), 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) ppm. ^{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, 169.8, 169.9, 170.2, 171.1, 171.7 ppm.

{3-[5-(3-Aminopropyl)-2-(α-D-mannopyranosyloxy)phenyl]-phenyl}acetic Acid (10): A 12.5% NaOH solution (4 mL) was added to 6 mL of a solution of compound **9** (682 mg, 1.01 mmol) in methanol. The mixture was stirred at room temperature for 24 h, and then concentrated 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 concentarted. This crude residue was dissolved in distilled water (5 mL) and the pH was adjusted to ca. 3 with HCl. The purification was conducted by preparative reversed-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. The NaCl was subsequently removed by chromatography on Sephadex G-15. The yield was 78%. Analytical reversed-phase HPLC: retention time 6.20 min. UV: λ_{max} = 209, 249, and 280 nm (in H_2O). L-SIMS: m/z = 483 [M^+], 505 [M − H + Na]$^+$ for $C_{23}H_{30}ClNO_8$. ^{1}H NMR (D_2O): δ = 1.3 (m, 2 H, CH_2CH_2N), 2.9 (m, 2 H, ArCH_2CH_2), 3.3 (t, J = 8.3 Hz, 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) ppm. 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, 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 ppm.

DTPA-B(SLe^X)A (11): Diethylenetriaminepentaacetic acid (DTPA) bis(anhydride) was synthesized according to a literature procedure.[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 under vacuum to give 6.4 g of bis(anhydride) 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 bis(anhydride) was 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%. Unchanged DTPA and salts were removed by size exclusion chromatography on Sephadex G-15. Analytical reversed-phase HPLC: retention time 3.58 min (free DTPA, 2.16 min). UV: λ_{max} = 252 and 282 nm (in H_2O). L-SIMS: *m/z* = 1252 $[M + H]^+$ for $C_{60}H_{77}N_5O_{24}$. ^1H NMR (D_2O, pD = 6): δ = 1.7 (m, 4 H, $ArCH_2CH_2$), 2.8 (m, 4 H, $ArCH_2CH_2$), 3.0 (m, 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) ppm. In ^1H NMR ([D_6] DMSO), the signal at δ = 7.8 (br, 2 H) confirmed the existence of two CONH functions. ^{13}C NMR (D_2O, pD = 6): δ = 29.0, 31.5, 38.7, 41.4, 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 ppm.

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 (contract numbers 95/00-194 and 00-05/258) of the French Community of Belgium.

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Received May 21, 2002
[O02261]