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Synthesis of photolabile mono- and di-valent α-D-mannoside-6-phosphates as chemically modifying probes for mannose-6-phosphate-receptors

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Abstract

Two photoaffinity probes, disodium 3′-azibutyl α-D-mannopyranoside-6-phosphate and tetrasodium 2-azi-1,10-bis(α-D-mannopyranosyloxy-6-phosphate)decane were synthesized for the regioselective chemical modification of mannose-6-phosphate receptors. Disodium 3′-azibutyl α-D-mannopyranoside-6-phosphate was obtained by chemical α-mannosylation of 3-azibutanol and subsequent 6-phosphorylation of the mannosyl residue. The corresponding divalent ligand, mimicking a high mannose oligosaccharide with two mannose-6-phosphate end groups, was obtained by the same procedure but with 2-azi-1,10-decanediol as the aglyconic alcohol. In preliminary experiments, both photolabile ligands had affinity to cation-independent mannose-6-phosphate receptors from bovine testes.

Keywords: Mannose-6-phosphate-receptor; Photoaffinity labelling; Diazirines; Spacer-modified oligosaccharide; Glycosylation

1. Introduction

D-Mannose-6-phosphate (Man-6-P) receptors (MPRs) are membrane-associated glycoproteins that participate in the intracellular trafficking of acid hydrolases [1–3]. Three species of MPRs have been isolated and purified to apparent homogeneity. These include a membrane-associated, cation-independent form (≅ 300 kDa), a soluble

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cation-independent species (≅ 205 kDa) derived form the 300 kDa species, and a cation-dependent form (≅ 41–46 kDa). Each of the cation-independent MPRs contains two ligand-binding pockets per molecule; the cation-dependent MPR contains one ligand-binding pocket per molecule of receptor. The affinity between the receptors and their ligands varies with the number and position of Man-6-P residues associated with *N*-linked oligosaccharides. The presence of two or more terminal, nonreducing Man-6-P end groups on *N*-linked oligosaccharides of ligands markedly enhances the binding affinity of the ligands for MPRs [3,4]. This phenomenon is attributed to a "clustering effect" observed by Tomada et al. for the binding of Man-6-P containing ligands to the cation-independent MPR in rabbit alveolar macrophages [5]. Additionally, recent information suggests that the nature of the *N*-linked oligosaccharides associated with the MPRs may also influence ligand binding [6].

Ligand binding by MPRs probably requires an extended recognition area on the receptor. A relatively simple method to probe such an area is the use of specific photoaffinity reagents containing structural elements of natural ligands, which are recognized by the receptor. Such photoaffinity analogues require (i) that the photolabile group be as close to the Man-6-P recognition site as possible, (ii) that the photolabile group be sterically unobtrusive, and (iii) that the photolabile group be activated by ultraviolet light at a wavelength that minimizes changes to the native protein. To our knowledge, only a single photoaffinity labeling study has been conducted with MPRs. An ^{125}I labeled aromatic azide coupled to a (1 → 6)-α-D-manno-pentasaccharide containing Man-6-P at the nonreducing terminus was irradiated at 275 nm in the presence of detergent-extracted, affinity-purified, cation-independent MPR [7]. An ^{125}I labeled product was obtained, but the regioselectivity of the chemical modification was not demonstrated.

Mild and regioselective chemical modification of several types of carbohydrate binding proteins by sugar derivates carrying diazirines has been demonstrated [8–11]. The diazirine group is usually attached to a short aliphatic hydrocarbon chain at the anomeric center, or to a spacer connecting different monosaccharides, thereby mimicking di- or tri-antennary oligosaccharides as natural ligands [12,13]. Our experience with photolabile glycosides as monovalent ligands and spacer-modified oligosaccharides has led us to prepare two photolabile Man-6-P derivatives as potential photoaffinity probes for MPRs. The synthesis and properties of these compounds are reported herein.

2. Results and discussion

Synthesis of a monovalent ligand.—Man-6-P and simple α-glycosides of Man-6-P are ligands for MPRs with K_i values approximating 40 μM. An easy approach to the preparation of photoaffinity ligands is the α-mannosylation of an aglyconic alcohol with a diazirine ring, followed by 6-phosphorylation of the mannoside. 3-Azibutanol [14] was α-mannosylated in a silver trifluoromethanesulfonate [15] promoted reaction as described by Bock et al. [16], yielding 3′-azibutyl 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranoside (**1**) (Scheme 1). *O*-Debenzoylation of **1** resulted in a quantitative yield of

3′-azibutyl α-D-mannopyranoside (**2**). Compound **2** was sequentially 6-*O*-tritylated, per-*O*-acetylated, and then detritylated to yield 3′-azibutyl 2,3,4-tri-*O*-acetyl-α-D-mannopyranoside (**5**). Normally, phosphorylation of a suitably blocked mannoside is carried out with an activated phosphodiester. In the present studies, the normal procedure of removing blocking groups by hydrogenolysis could not be utilized because of the sensitive nature of the diazirine residues. Therefore, phosphorylation of **5** was achieved with β-cyanoethyl phosphate [17]. The resulting compound, sodium 3′-azibutyl 2,3,4-tri-*O*-acetyl-6-*O*-(β-cyanoethylphosphoryl)-α-D-mannopyranoside (**6**), yielded after deblocking disodium 3′-azibutyl α-D-mannopyranoside-6-phosphate (**7**) as a photolabile ligand for MPRs.

Synthesis of a divalent ligand.—The affinity of chemically synthesized ligands for receptors can be enhanced by designing means to cluster the recognition features of ligandes [5]. Oligosaccharides carrying Man-6-P residues are recognized by the MPRs provided that the receptors contain several subsites for noncovalent binding interaction. For example, chemically synthesized oligosaccharides that contain more than one Man-6-P end group exhibit enhanced affinities for the MPRs [4].

The application of the principle of spacer-modified oligosaccharides allows mimicking of naturally occurring polydentate oligosaccharides. Monosaccharide units, not essential for binding, are replaced by an acyclic flexible spacer [12,13]. The spacer maintains the recognition residue(s) at an appropriate distance and at the same time can serve as a sterically unobtrusive carrier of photolabile groups for affinity labelling [8–11].

The structure of a chemically synthesized high affinity oligosaccharide recognized by MPRs is presented in Fig. 1. The two Man-6-P end groups are linked by a line of covalent bonds (bold line), which represents a portion of naturally occurring *N*-linked oligosaccharides. This oligosaccharide exhibits an approximate 100-fold increase in affinity over that found for Man-6-P [4]. The shortest link between two Man-6-P groups comprises ten atoms, not including the two anomeric oxygens. A spacer-modified oligosaccharide with two α-linked Man-6-P end groups and a diazirine group attached to the spacer can be superimposed on the outlined oligosaccharide structure. A potential high affinity photoreactive derivate of this structure is relatively easily prepared and can be used to probe the ligand binding pockets present on the MPRs. Furthermore, depending on the position of insertion of the diazirinogroup on the aliphatic spacer, this methodology permits regioselectively modifying different portions of the binding pocket(s) of each MPR.

As an initial approach, a 2-azi-1,10-bis(α-D-mannopyranosyloxy-6-phosphate)decane (**14**) was synthesized by glycosylating 2-azi-1,10-decanediol [12], the spacer containing the diazirine group. The spacer consists of ten atoms in line, not including the glycosidic oxygens of the Man-6-P end groups, and is of the same length as the Man-6-P connecting covalent bonds (bold line) in the manno-pentasaccharide structure depicted in Fig. 1. Phosphorylation was carried out with β-cyanoethyl phosphate using the partially blocked spacer-modified oligosaccharide 2-azi-1,10-bis(2′,3′,4′-tri-*O*-acetyl-α-D-mannopyranosyloxy)decane (**12**). Removal of the blocking groups yielded the divalent spacer-modified oligosaccharide **14**. Preliminary experiments have revealed that compounds **7** and **14** do not differ greatly in binding capacity to CI-MPR from that of free

Man-6-P [18]. The unexpected absence of any clustering effect in the divalent ligand is surprising and is the subject of further investigation.

3. Experimental

General methods.—All reaction products were detected by TLC on Silica Gel 60 F_{254} (E. Merck) by the quenching of fluorescence and/or by charring with H_2SO_4. Flash chromatography [19] was performed on ICN-silica 32–63 (ICN Biomedicals). HPLC was performed with an LKB HPLC controller, two pumps, a variable wavelength monitor, and a Shimadzu C-R2AX integrator. Melting points were measured with a Büchi apparatus and are uncorrected. Optical rotations were obtained with a Schmidt and Haensch Polartronic I polarimeter. Ultra violet spectra and extinction coefficients were recorded with a Zeiss PMQ II spectrometer. Ionic compounds are hygroscopic. Water content was determined by rigorous drying over P_2O_5 in high vacuum. ^{1}H NMR spectra were recorded at 400.13 MHz (Bruker AM 400) with either internal $(Me_3)_4Si$ or disodium 2,2-dimethyl-2-silapentane-5-sulfonate. ^{13}C NMR were recorded at 100.62 MHz (Bruker AM 400) with internal $(Me_3)_4Si$ or acetone. ^{31}P NMR were recorded at 81.015 MHz (Bruker WH 200) with aq 85% H_3PO_4 as the external reference. The ^{13}C and ^{31}P spectra were ^{1}H-decoupled.

*3'-Azibutyl 2,3,4,6-tetra-*O*-benzoyl-α-*D*-mannopyranoside (1).*—Tetramethylurea (1.74 mL, 13.0 mmol), 3-azibutanol [14] (1.30 g, 13.0 mmol) and silver trifluoromethanesulfonate (3.34 g, 13.0 mmol) were dissolved in CH_2Cl_2 (30 mL) and stirred under argon over molecular sieves (4 Å, 1.0 g) for 1 h at 25°C, then cooled to −20°C. A solution of tetra-*O*-benzoyl-α-D-mannopyranosyl bromide [20] (7.8 g, 11.8 mmol) in dry CH_2Cl_2 (60 mL) was added dropwise. The mixture was stirred at −20°C for 4 h, then left overnight to reach room temperature, and filtered through Celite. The filtrate was poured into ice-cold water and washed sequentially with satd aq $NaHCO_3$, water, HCl (1 M), water, satd aq $NaHCO_3$ and water. The resulting solution was dried over Na_2SO_4 and concentrated. Flash-column chromatography (5:1 cyclohexane–EtOAc) of the residue gave a colorless glass **1** (6.17 g, 77%); $[\alpha]_D$ −113°(*c* 2.0, $CHCl_3$); R_f 0.27 (5:1 cyclohexane–EtOAc); λ_{max} 348 nm (ϵ 58 cm^{-1} M^{-1}). ^{1}H NMR ($CHCl_3$): δ 8.20–7.80 and 7.70–7.20 (m, 20 H, 4 Bz), 6.12 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 5.95 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-3), 5.71 (dd, 1 H, $J_{1,2}$ 1.8 Hz, H-2), 5.08 (d, 1 H, H-1), 4.72 (dd, 1 H, $J_{5,6a}$ 4.5, $J_{6a,6b}$ 11.5 Hz, H-6a), 4.53 (m, 2 H, H-5, H-6b), 3.73 and 3.48 (2 dt, 2 H, $J_{1'a,1'b}$ 10.2, $J_{1',2'}$ 6.0 Hz, H-1'), 1.80 (m, 2 H, H-2'), 1.14 (s, 3 H, H-4'). Anal. Calcd for $C_{38}H_{34}N_2O_{10}$: C, 67.25; H, 5.05; N, 4.13. Found: C, 67.20; H, 5.29; N, 4.08.

*3'-Azibutyl α-*D*-mannopyranoside (2).*—Methanolic NaOMe (1.0 M, 3 mL) was added at room temperature to a solution of **1** (5.00 g, 7.4 mmol) in dry MeOH (150 mL). The reaction was complete after 2 h, as established by TLC; R_f 0.21 (27:2:1 EtOAc–MeOH–H_2O). The solution was filtered through silica gel with MeOH. The filtrate was concentrated under reduced pressure to yield **2** (1.93 g, 100%); $[\alpha]_D$ +67° (*c* 0.5, MeOH); λ_{max} 347 nm (ϵ 72 cm^{-1} M^{-1}). ^{1}H NMR (CD_3OD): δ 4.73 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 3.60 and 3.32 (2 dt, 2 H, $J_{1'a,1'b}$ 10.4, $J_{1',2'}$ 6.0 Hz, H-1'), 1.61 (m, 2 H, H-2'),

1.05 (s, 3 H, H-4′). Anal. Cald for $C_{10}H_{18}N_2O_6$: C, 45.80; H, 6.92; N, 10.68. Found: C, 45.42; H, 7.03; N, 10.02.

*3′-Azibutyl 2,3,4-tri-*O*-acetyl-6-*O*-triphenylmethyl-α-*D*-mannopyranoside (4).*—Chlorotriphenylmethane (2.30 g, 8.4 mmol) was added dropwise at 0°C to a solution of **2** (1.70 g, 6.5 mmol) in pyridine (20 mL). After 30 min, the temperature was allowed to rise to room temperature and the solution stirred overnight. TLC (27:2:1 EtOAc–MeOH–H_2O) then revealed that **2** had reacted quantitatively to yield a single product (R_f 0.73). Acetic anhydride (10 mL) was added, and the solution was stored at room temperature for 12 h, then poured onto crushed ice (100 mL), stirred vigorously for 30 min, and extracted with CH_2Cl_2. The combined extracts were washed with satd aq $NaHCO_3$ and water, dried over $MgSO_4$ and concentrated. Flash-column chromatography (3:1 cyclohexane–EtOAc) of the residue yielded **4** (3.20 g, 78%); $[\alpha]_D$ +56° (*c* 0.5, $CHCl_3$). R_f 0.25 (3:1 cyclohexane–EtOAc); λ_{max} 347 nm (ϵ 64 $cm^{-1} \cdot M^{-1}$). ^1H NMR ($CDCl_3$): δ 7.50–7.20 (m, 15 H, 3 Ph), 5.34 (dd, 1 H, $J_{2,3}$ 3.2 Hz, $J_{3,4}$ 9.8 Hz, H-3), 5.27 (t, 1 H, $J_{4,5}$ 9.8 Hz, H-4), 5.25 (dd, 1 H, $J_{1,2}$ 1.8 Hz, H-2), 4.85 (d, 1 H, H-1), 3.99 (m, 1 H, H-5), 3.72 and 3.41 (2 dt, 2 H, $J_{1'a,1'b}$ 10.2, $J_{1',2'}$ 6.5 Hz, H-1′), 3.23–3.15 (m, 2 H, H-6), 2.20, 2.13 and 1.95 (3 s, each 3 H, 3 Ac), 1.72 (m, 2 H, H-2′), 1.10 (s, 3 H, H-4′). Anal. Calcd for $C_{35}H_{38}N_2O_9$: C, 66.65; H, 6.07; N, 4.44. Found: C, 66.45; H, 6.20; N, 4.30.

*3′-Azibutyl 2,3,4-tri-*O*-acetyl-α-*D*-mannopyranoside (5).*—To a well-stirred solution of **4** (3.10 g, 4.9 mmol) and NaI (2.24 g, 14.7 mmol) in MeCN (62 mL) was added under argon at 0°C chlorotrimethylsilane (1.9 mL, 14.7 mmol) [21]. When TLC indicated the absence of **4** (10 min), ice-cold water (120 mL) was added to the mixture, which was stirred for 15 min, then filtered, and extracted with $CHCl_3$. The combined extracts were washed sequentially with aq satd $Na_2S_2O_3$, aq $NaHCO_3$, and water, dried over $MgSO_4$ and concentrated. The resulting syrup was subjected to flash-column chromatography to yield **5** as a syrup (1.81 g, 95%); $[\alpha]_D$ +127° (*c* 0.5, $CHCl_3$); R_f 0.33 (2:1 cyclohexane–EtOAc); λ_{max} 347 nm (ϵ 41 cm^{-1} M^{-1}). ^1H NMR ($CDCl_3$): δ 5.43 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 9.8 Hz, H-3), 5.28 (dd, 1 H, $J_{1,2}$ 1.8 Hz, H-2), 5.25 (t, 1 H, $J_{4,5}$ 9.8 Hz, H-4), 4.80 (d, 1 H, H-1), 3.87–3.61 (m, 3 H, H-5, 2 H-6), 3.58 and 3.34 (2 dt, 2 H, $J_{1'a,1'b}$ 10.2, $J_{1',2'}$ 6.0 Hz, H-1′), 2.46 (s, 1 H, OH), 2.17, 2.10 and 2.03 (3 s, each 3 H, 3 Ac), 1.70 (m, 2 H, H-2′), 1.10 (s, 3 H, H-4′). Anal. Calcd for $C_{16}H_{24}N_2O_9$: C, 49.48; H, 6.22; N, 7.21. Found: C, 49.33; H, 6.20; N, 7.44.

*Sodium 3′-azibutyl 2,3,4-tri-*O*-acetyl-6-*O*-(β-cyanoethylphosphoryl)-α-*D*-mannopyranoside (6).*—A solution of 2-cyanoethyl phosphate in pyridine (2.20 mL, 2.20 mmol), prepared according to the procedure of Tener [17], was added to a solution of **5** (475 mg, 1.10 mmol) in pyridine (5 mL). The reaction mixture was concentrated to a thick syrup, the residue dissolved in pyridine (10 mL) and again concentrated. This process was repeated three more times, the resulting oily residue was dissolved in pyridine (5 mL), and *N,N′*-dicyclohexyl carbodiimide (1.51 g, 7.32 mmol) was added. After 5 days at room temperature, water was added and the mixture stirred for an additional 7 h. The urea derivative was filtered off and washed with small quantities of MeOH. The supernatant and washings were combined, concentrated, and the residue was flash-column chromatographed (55:10:1:1 EtOAc–MeOH–H_2O–pyridine) to a crude glass. A solution of the solid in MeOH was passed through a Dowex 50 W X8

(Na^+ form) column to yield **6** (445 mg, 74%); $[\alpha]_D$ +52° (*c* 0.7, MeOH); R_f 0.33 (7:2:1 EtOAc–MeOH–H_2O); λ_{max} 348 nm (ϵ 103 cm^{-1} M^{-1}). ^1H NMR (CD_3OD): δ 5.41 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 10.2 Hz, H-3), 5.36 (t, 1 H, $J_{4,5}$ 10.2 Hz, H-4), 5.33 (dd, 1 H, $J_{1,2}$ 1.8 Hz, H-2), 4.96 (d, 1 H, H-1), 4.22 (m, 1 H, H-5), 4.13–3.98 (m, 4 H, O–CH_2–CH_2–CN, 2 H-6), 3.72 and 3.48 (2 dt, 2 H, $J_{1'a,1'b}$ 11.2, $J_{1'2'}$ 6.0 Hz, H-1′), 2.86 (t, 2 H, *J* 6.0 Hz, O–CH_2–CH_2–CN), 2.21, 2.14, 2.04 (3 s, each 3 H, 3 Ac), 1.70–1.80 (m, 2 H, H-2′), 1.09 (s, 3 H, H-4′);^{13}C NMR (CD_3OD): δ 98.8 (C-1), 71.7 (d, $J_{5,P}$ 8.0 Hz, C-5), 65.9 (d, $J_{6,P}$ 5.7 Hz); ^{31}P NMR (CD_3OD): δ 2.1. Anal. Calcd for $C_{19}H_{28}N_3NaO_{12}P$: C, 41.91; H, 5.18; N, 7.72. Found: C, 42.21; H, 5.19; N, 7.53.

*Disodium 3′-azibutyl α-*D*-mannopyranoside-6-phosphate (7).*—Sodium hydroxide (34 mg, 0.14 mmol) was added at room temperature to a solution of **6** (76 mg, 0.14 mmol) in water (1 mL). After 2 h, the mixture was adjusted to pH 8.5 with HCl (0.2 M) and lyophilized. The residue was redissolved in water and purified by HPLC (Hypersil ODS, 80:20 MeOH–H_2O). Lyophilization provided a white powder **7** (40 mg, 70%); mp 175°C (EtOH–H_2O); $[\alpha]_D$ +67° (*c* 0.5, H_2O); R_f 0.44 (6:3:1 *i*Prop–NH_3–H_2O); λ_{max} 347 nm (ϵ 66 $cm^{-1} \cdot M^{-1}$). ^1H NMR (D_2O): δ 4.86 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 4.07 (m, 1 H, H-6a), 4.00 (m, 1 H, H-6b), 3.94 (d, 1 H, $J_{2,3}$ 3.2 Hz, H-2), 3.86 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 3.81 (dd, 1 H, H-3), 3.68 (m, 1 H, H-5), 3.52 and 3.48 (2 dt, $J_{1'a,1'b}$ 11.0, $J_{1',2}$ 6.2 Hz, 2 H-1′), 1.72 (m, 2 H, H-2′), 1.08 (s, 3 H, H-4′); ^{13}C NMR (D_2O): δ 101.0 (C-1), 73.2 (d, $J_{5,P}$ 7.8 Hz, C-5), 64.4 (d, $J_{6,P}$ 5.1 Hz, C-6); ^{31}P NMR (D_2O): δ 2.32. Anal. Calcd for $C_{10}H_{17}N_2Na_2O_9P \cdot H_2O$: C, 29.71; H, 4.70; N, 6.90. Found: C, 29.40; H, 4.70; N, 6.75.

*2-Azi-1,10-bis(2′,3′,4′,6′-tetra-*O*-benzoyl-α-*D*-mannopyranosyloxy)decane (8).*—2-Azi-1,10-decanediol [12] (750 mg, 2.5 mmol), tetramethylurea (0.31 mL, 5.1 mmol) and silver trifluoromethanesulfonate (1.30 g, 5.1 mmol) were dissolved in CH_2Cl_2 (8 mL) and stirred under argon over molecular sieves (4 Å, 0.5 g) for 1 h at 25°C, then cooled to −20°C. A solution of tetra-*O*-benzoyl-α-D-mannopyranosyl bromide [20] (3.36 g, 5.1 mmol) in dry CH_2Cl_2 (20 mL) was added dropwise. The mixture was stirred at −20°C for 4 h, left overnight at room temperature and filtered through Celite. The filtrate was poured into ice-cold water, washed sequentially with satd aq $NaHCO_3$, water, HCl (1 M), water, satd aq $NaHCO_3$, water, dried over Na_2SO_4 and concentrated. Flash-column chromatography (4:1 cyclohexane–EtOAc) of the residue gave a colorless glass **8** (1.90 g, 56%); $[\alpha]_D$ −25° (*c* 1.4, $CHCl_3$); R_f 0.32 (3:1 cyclohexane–EtOAc); λ_{max} 347 nm (ϵ 62 cm^{-1} M^{-1}). ^1H NMR ($CDCl_3$): δ 8.20–7.70 and 7.60–7.20 (m, 40 H, 8 Bz), 6.10 and 6.09 (2 t, 2 H, $J_{3',4'} = J_{4',5'}$ 9.8 Hz, H-4′), 5.93 and 5.90 (2 dd, 2 H, $J_{2',3'}$ 3.2 Hz, H-3′), 5.71 and 5.69 (2 dd, $J_{1',2'}$ 1.8 Hz, 2 H, H-2′), 5.06 and 5.04 (2 d, 2 H, H-1′), 4.71 and 4.68 (2 dd, 2 H, $J_{5',6'a}$ 3.7, $J_{6'a,6'b}$ 12.0 Hz, H-6′a), 4.53–4.30 (m, 4 H, 2 H-5′, 2 H-6′b), 3.82 and 3.58 (2 dt, 2 H, $J_{9,10}$ 6.8, $J_{10a,10b}$ 9.8 Hz, H-10), 3.52 and 3.48 (2 d, 2 H, $J_{1a,1b}$ 11.8 Hz, H-1), 1.67 (m, 2 H, H-9), 1.54 (m, 2 H, H-3), 1.46–1.12 (m, 10 H, 5 CH_2). Anal. Calcd for $C_{78}H_{72}N_2O_{20}$: C, 69.02; H, 5.35; N, 2.06. Found: C, 68.90; H, 5.38; N, 1.98.

*2-Azi-1,10-bis(α-*D*-mannopyranosyloxy)decane (9).*—Methanolic NaOMe (1.0 M, 5 mL) was added to a solution of **8** (1.80 g, 1.33 mmol) in dry MeOH (50 mL) at room temperature. The reaction was complete after 8 h as shown by TLC; R_f 0.58 (7:2:1 EtOAc–MeOH–H_2O). The solution was filtered through silica gel, using MeOH, and

the filtrate was concentrated in vacuo. A colorless glass was obtained (680 mg, 98%); $[\alpha]_D$ +265° (*c* 1.0, H_2O); λ_{max} 347 nm (ϵ 70 cm^{-1} M^{-1}). ^1H NMR (pyridine-d_5): δ 5.35 and 5.32 (2 d, 2 H, $J_{1',2'}$ 1.65 Hz, H-1'), 3.85 (dt, 1 H, $J_{9,10}$ 6.8, $J_{10a,10b}$ 9.8 Hz, H-10a), 3.78 (d, 1 H, $J_{1a,1b}$ 11.8 Hz, H-1a), 3.47 (dt, 1 H, H-10b), 3.42 (1 d, 1 H, H-1b), 1.62–1.53 (m, 2 H, H-9), 1.48–1.39 (m, 2 H, H-3), 1.35–1.10 (m, 10 H, 5 CH_2). Anal. Calcd for $C_{22}H_{40}N_2O_{12}$: C, 50.37; H, 7.68; N, 5.34. Found: C, 50.51; H, 7.62; N, 5.51.

*2-Azi-1,10-bis(2',3',4'-tri-*O*-acetyl-6'-*O*-triphenylmethyl-α-*D*-mannopyranosyloxy)decane (11).*—Chlorotriphenylmethane (730 mg, 2.4 mmol) was added at 0°C to a solution of **9** (480 mg, 0.9 mmol) in pyridine (5 mL). After 30 min, the temperature was allowed to rise to room temperature. The reaction mixture was stirred at this temperature for 2 days. The progress of the reaction was followed by TLC (27:2:1 EtOAc–MeOH–H_2O); **9** reacted quantitatively yielding product **10** (R_f 0.78). Acetic anhydride (2.5 mL) was added, and the solution was stored at room temperature for 12 h. The reaction mixture was then poured onto crushed ice (20 mL), stirred vigorously for 30 min, and extracted with CH_2Cl_2. The combined extracts were washed sequentially with satd aq $NaHCO_3$ and water, dried over $MgSO_4$ and concentrated. Flash-column chromatography (3:2 cyclohexane–EtOAc) of the residue yielded **11** (403 mg, 36%); $[\alpha]_D$ +61° (*c* 1.0, $CHCl_3$); R_f 0.40 (2:1 cyclohexane–EtOAc); λ_{max} 348 nm (ϵ 60 cm^{-1} M^{-1}). ^1H NMR ($CDCl_3$): δ 7.50–7.15 (m, 30 H, 6 Ph), 5.31 and 5.29 (2 t, 2 H, $J_{3',4'} = J_{4',5'}$ 9.8 Hz, H-4'), 5.27 (dd, 2 H, $J_{1',2'}$ 1.7, $J_{2',3'}$ 3.2 Hz, H-2'), 5.23 and 5.21 (2 dd, 2 H, H-3'), 4.82 and 4.81 (2 d, 2 H, H-1'), 3.93 and 3.84 (2 ddd, 2 H, $J_{5',6'a}$ 4.2 Hz, $J_{5',6'b}$ 3.8 Hz, H-5'), 3.78 and 3.49 (2 dt, 2 H, $J_{9,10}$ 6.8, $J_{10a,10b}$ 9.8 Hz, H-10), 3.50 and 3.40 (2 d, 2 H, $J_{1a,1b}$ 11.8 Hz, H-1), 3.17–3.12 (m, 4 H, H-6'), 2.17–1.72 (6 s, each 3 H, 6 Ac), 1.67–1.57 (m, 2 H, H-9), 1.51–1.45 (m, 2 H, H-3), 1.40–1.05 (m, 10 H, 5 CH_2). Anal. Calcd for $C_{72}H_{80}N_2O_{18}$: C, 68.56; H, 6.39; N, 2.22. Found: C, 68.62; H, 6.61; N, 1.97.

*2-Azi-1,10-bis(2',3',4'-tri-*O*-acetyl-α-*D*-mannopyranosyloxy)decane (12).*—Chlorotrimethylsilane (0.85 mL, 6.7 mmol) was added to a well stirred mixture containing **11** (1.40 g, 1.1 mmol) and NaI (1.00 g, 6.7 mmol) in MeCN (30 mL) held at 0°C under argon [21]. TLC (1:3 cyclohexane–EtOAc) was used to monitor the progress of the reaction. When **11** was no longer observed (10 min), ice-cold water (60 mL) was added. The mixture was stirred for 15 min, then filtered, and extracted with $CHCl_3$. The combined extracts were washed sequentially with aq satd $Na_2S_2O_3$, aq $NaHCO_3$, and then water, dried over $MgSO_4$, and concentrated. The resulting syrup was subjected to flash-column chromatography to yield **12** as a syrup (650 mg, 75%); $[\alpha]_D$ +44° (*c* 1.0, $CHCl_3$); R_f 0.15 (1:2 cyclohexane–EtOAc); λ_{max} 346 nm (ϵ 63 cm^{-1} M^{-1}). ^1H NMR (pyridine-d_5): δ 5.97 and 5.95 (2 t, 2 H, $J_{3',4'} = J_{4',5'}$ 9.8 Hz, H-4'), 5.77 (dd, 2 H, $J_{1',2'}$ 1.6, $J_{2',3'}$ 3.1 Hz, H-2'), 5.47 and 5.45 (2 dd, 2 H, H-3'), 5.19 and 5.15 (2 d, 2 H, H-1'), 4.32 and 4.22 (2 ddd, 2 H, $J_{5',6'a}$ 3.0, $J_{5',6'b}$ 6.8 Hz, H-5'), 4.20–4.08 (m, 4 H, H-6'), 3.85 (dt, 1 H, $J_{9,10}$ 7.1, $J_{10a,10b}$ 11.7 Hz, H-10a), 3.78 and 3.51 (2 d, 2 H, $J_{1a,1b}$ 11.8 Hz, H-1), 3.48 (dt, 1 H, H-10b), 2.30 (bs, 2 H, OH), 2.08–1.95 (6 s, each 3 H, 6 Ac), 1.62–1.52 (m, 2 H, H-9), 1.51–1.45 (m, 2 H, H-3), 1.35–1.02 (m, 10 H, 5 CH_2). Anal. Calcd for $C_{34}H_{52}N_2O_{18}$: C, 52.57; H, 6.75; N, 3.61. Found: C, 52.24, H, 6.74; N, 3.40.

*Disodium 2-azi-1,10-bis(2',3',4'-tri-*O*-acetyl-6'-*O*-(β-cyanoethylphosphoryl)-α-*D*-mannopyranosyloxy)decane (13).*—A solution of 2-cyanoethyl phosphate in pyridine

(3.10 mL, 3.10 mmol), prepared according to the procedure of Tener [17], was added to a solution of **12** (600 mg, 0.77 mmol) in pyridine (8 mL). The reaction mixture was concentrated to a thick syrup, the residue dissolved in pyridine (10 mL) and again concentrated. This process was repeated three more times, the resulting oily residue was dissolved in pyridine (8 mL) and *N,N'*-dicyclohexyl carbodiimide (950 mg, 4.60 mmol) added. After 5 days at room temperature, water was added and the mixture stirred for an additional 8 h. The urea derivate was filtered off, washed with small quantities of MeOH and concentrated. The residue was flash-column chromatographed (40:10:1:1 EtOAc–MeOH–H_2O–pyridine) to give a crude solid. The solid was dissolved in MeOH and passed through a Dowex 50 W X8 (Na^+ form) column to yield the corresponding disodium salt of **13** (528 mg, 63%); $[\alpha]_D$ +61° (*c* 0.5, MeOH); R_f 0.25 (7:2:1 EtOAc–MeOH–H_2O); λ_{max} 345 nm (ϵ 65 cm^{-1} M^{-1}). ^1H NMR (CD_3OD): δ 5.26 (t, 2 H, $J_{3',4'} = J_{4',5'}$ 9.8 Hz, H-4'), 5.24 and 5.23 (2 dd, 2 H, $J_{2',3'}$ 3.2 Hz, 2 H-3'), 5.22 and 5.20 (2 dd, 2 H, $J_{1',2'}$ 1.8 Hz, H-2'), 4.83 and 4.81 (2 d, 2 H, H-1'), 4.20–4.05 (m, 8 H, 2 O–CH_2–CH_2–CN, 4 H-6'), 4.03 and 4.05 (2 m, 2 H, H-5'), 3.75 and 3.50 (2 dt, 2 H, $J_{9,10}$ 6.8, $J_{10a,10b}$ 9.8 Hz, H-10), 3.58 and 3.40 (2 d, 2 H, $J_{1a,1b}$ 11.0 Hz, H-1), 2.80 (2 dt, 4 H, *J* 6.3 Hz, O–CH_2–CH_2–CN), 2.20–1.90 (6 s, each 3 H, 6 Ac), 1.70–1.60 (m, 2 H, H-9), 1.51–1.45 (m, 2 H, H-3), 1.45–1.10 (m, 10 H, 5 CH_2); ^{13}C NMR (CD_3OD): δ 99.0 and 98.9 (2 C-1'), 71.9 and 71.6 (2 d, $J_{5',P}$ 9.0 Hz, 2 C-5'), 66.0 and 65.8 (2 d, $J_{6',P}$ 6.2 Hz, 2 C-6'); ^{31}P NMR (CD_3OD): δ 2.22, 2.01. Anal. Calcd for $C_{40}H_{58}N_4Na_2O_{24}P_2$: C, 44.20; H, 5.38; N, 5.15. Found: C, 44.05; H, 5.47; N, 5.09.

*Tetrasodium 2-azi-1,10-bis(α-*D*-mannopyranosyloxy-6-phosphate)decane* ***(14)***.— Sodium hydroxide (37 mg, 0.92 mmol) was added at room temperature to a solution of **13** (80 mg, 0.07 mmol) in water (1 mL). After 2 h, the mixture was titrated to pH 8.5 with HCl (0.2 M) and then lyophilized. The resulting residue was dissolved in water, purified by HPLC (Hypersil ODS, 60:40 MeOH–H_2O) and lyophilized (46 mg, 72%); mp 140–160°C (EtOH–H_2O); $[\alpha]_D$ +79° (*c* 0.5, H_2O); R_f 0.14 (7:3:2 *i*Prop–NH_3–H_2O); λ_{max} 345 nm (ϵ 64 cm^{-1} M^{-1}). ^1H NMR (D_2O): δ 4.87 and 4.76 (2 d, 2 H, $J_{1',2'}$ 1.8 Hz, H-1'), 4.08 and 4.06 (2 m, 2 H, H-6'a), 4.01 and 3.98 (2 m, 2 H, H-6'b), 3.93 and 3.91 (2 dd, 2 H, $J_{2',3'}$ 3.1 Hz, H-2'), 3.85 and 3.83 (2 t, 2 H, $J_{3',4'} = J_{4',5'}$ 9.8 Hz, H-4'), 3.80 and 3.78 (2 dd, H-3'), 3.70 and 3.67 (m, 2 H, H-5'), 3.66–3.44 (m, 4 H, H-1, H-10), 1.67 (m, 2 H, H-9), 1.54 (m, 2 H, H-3), 1.46–1.12 (m, 10 H, 5 CH_2); ^{13}C NMR (D_2O): δ 100.4 and 100.3 (2 C-1'), 70.4 and 71.3 (2 d, $J_{5',P}$ 8.2 Hz, 2 C-5'), 66.7 and 66.4 (2 d, $J_{6',P}$ 4.3 Hz, 2 C-6'); ^{31}P NMR (D_2O): δ 2.44, 2.48. Anal. Calcd for $C_{22}H_{38}N_2Na_4O_{18}P_2 \cdot 7H_2O$: C, 29.40; H, 5.83; N, 3.12. Found: C, 28.99; H, 5.43; N, 2.96.

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