Synthesis of mono- and di-azido-hexopyranoses with N-alkyl and N,N-dialkyl amino groups at C-2 position: Intermediates for accessing new polyaminosugars

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Michael addition reactions of morpholine and benzylamine to methyl 2,3-dideoxy-4,6-O-(phenylmethylene)-3-C-phenylsulfonyl-α-D-erythro-hex-2-enopyranoside furnish gluco-derivatives and. Desulfonation at C-3 followed by synthetic manipulation produces intermediates for generating various new modified monosaccharides carrying N-alkyl and N,N-dialkyl amino groups at the C-2 position of hexopyranosides. This route also has the potential to furnish naturally occurring diaminosugars such as tobrusamine.

The amino groups present in the aminoglycoside antibiotics and polysaccharides play an important role in their biological activities. The most important mechanism of resistance to aminoglycoside antibiotics among resistant bacteria arises from enzymatic N-acetylation, O-phosphorylation, and O-nucleotidylation of specific sites in the antibiotics. To avoid such deactivation processes, several semisynthetic aminoglycoside antibiotics have been designed where either the hydroxyl groups undergoing enzymatic phosphorylation have been removed and/or the amino groups susceptible to acetylation have been masked by acylation or alkylation. The interest in this class of compounds has been renewed with the discoveries that aminoglycoside antibiotics interact with a variety of RNA molecules. Studies on structure-activity relationship have revealed the following important points: (i) changing an amino group to a hydroxyl group of certain aminoglycoside antibiotics eliminates the inhibitory activity. (ii) The overall charge density presented by the aminoglycosides toward the RNA host is likely to be important as aminoglycosides containing four amino groups show very little RNA binding capability while the most active derivatives contain five or six amino groups. (iii) When a hydroxyl group proximal to an amine is removed, higher inhibitory activity is observed, which could be attributed to the increased basicity of an amino group in a deoxygenated analogue.

Tobrosamine (2,6-diamino-2, 3, 6-trIDEOxy-D-ribohexose), pururosamine A (2-amino-2, 3, 4, 6, 7-pentadeoxy-6-methylamino-D-ribo-heptose), pururosamine B (2, 6-diamino-2, 3, 4, 6, 7-pentadeoxy-D-ribo-heptose), pururosamine C (2, 6-diamino-2, 3, 4, 6-tetraDeoxy-D-erythro-hexose), sisosamine (2, 6-diamino-2, 3, 4, 6-tetraDeoxy-D-glycerohex-4-enose), tobrusamine (2, 6-diamo-2, 3, 4, 6-tetraDeoxy-D-arabinohexose) has been isolated from the antibiotic kasugamycin. 3-Deoxy-prumycin,4- (D-alanylamino)-2-amino-2, 3, 4, 6-tetraDeoxy-D-arabinohexose has been synthesized from the antibiotic kasugamycin. 3-Deoxy-prumycin,4- (D-alanylamino) -2-amino-2, 3, 4, 6-tetraDeoxy-D-arabinohexose has been synthesized and its effect on murine macrophages evaluated. Although both mono- and diaminosugars are naturally occurring, sugars containing more than two amino groups are not known in nature. Irrespective of this fact, several triaminosugars and tetraaminosugars have been synthesized to study their biological properties.

It is, therefore, of interest to devise strategies for the synthesis of common intermediates leading to aminosugars in which most if not all the hydroxyl groups
have been replaced by amino groups to see whether they alter activities of the parent antibiotics or any polysaccharide macromolecules.

**Results and Discussion**

Aminosugars are normally synthesized by reacting sugar derived epoxides, tosylates, halides or ketones with azides, phthalimides or amines although several other minor methods are also reported. Nucleophilic addition (Michael) to double bonds activated by electron withdrawing groups as part of carbohydrates could, in theory, serve as a useful methodology for the functionalisation of monosaccharides. Several examples of Michael addition of nitrogen nucleophiles including amines to hex-2-enose and 3-nitro-hex-2-enopyranosides have been reported. 2,3-Dideoxyhex-2-en-4-ulopyranosides, for example have been reacted with azide, partially protected amino acids and benzylamine to generate several new classes of aminosugars. The major drawbacks of using 4-uloses as starting materials are that a) the products will always be 2, 3-dideoxy derivatives and b) the stereochemical outcome of the reduction of the carbonyl group (C-4) is dependent on the orientation of the C-2 substituent. Such limitations made this route less attractive for synthetic work. The studies on the addition of nitrogen nucleophiles to 3-nitro-hex-2-enopyranosides, however, have remained limited mostly to gluco- and galacto-pyranoses and have been used for the preparation of few diamino- and triaminosugars.

Aminosugars 1-7 (Figure 1) represent a class of deoxysugars where C-3 positions are devoid of any hydroxyl groups. We have recently reported that methyl 2,3-dideoxy-3-C-phenylsulfonyl-α-D-hex-2-enopyranoside, synthesized from the epoxide, reacted with primary and secondary amines in a diastereoselective fashion. Such C-N bond formation in Michael fashion at C-2 of the vinyl sulfone-modified carbohydrates exclusively produced the thermodynamically more stable C-N equatorial aminosugars with primary amines. Secondary amines, on the other hand afforded a mixture of gluco- (major) and manno-(minor) products with 9. The major isomers were separated by crystallization. The products 10 (X = morpholinyl) and 11 (X = benzylamino) obtained from the addition reactions of morpholine and benzylamine respectively to 9, can be desulfonated easily to generate compounds 12 (X = morpholinyl) and 13 (X = benzylamino) with methylene groups at C-3 positions (Scheme I). Thus the core structure present in aminosugars 1-7 (CH2-CH-N) can be generated using the sequence of reactions mentioned above. This methodology has been used in the synthesis of D-lividosamine (2-amino-2,3-dideoxy-D-glucose) and its analogues. The same strategy can be utilised for the synthesis of 6-azido, 4-azido, and 4,6-diazido intermediates with 2-N-benzylamino and 2-N-morpholino at C-2 position in equatorial configuration to afford a series of new deoxypolyaminosugars.

**Synthesis of methyl 4-O-acetyl-6-azido-2-N-morpholino-2,3,6-trideoxy-α-D-ribohexopyranoside** 18a and methyl 2-N-(acetyl benzylamino)-4-O-acetyl-6-azido-2,3,6-trideoxy-α-D-ribohexopyranoside 18b.
Methyl 2,3-dideoxy-2-N-morpholino-4, 6-O(phenylmethylene)-α-D-ribo-hexopyranoside 12 (X = morpholinyl) obtained through known sequence of reactions\textsuperscript{18,19} was debenzyldenated with 0.1 N HCl in methanol. The resulting 4,6-diol 15a was selectively tosylated at 6-position by treatment with tosyl chloride in pyridine to afford 16a. The 6-tosylate of 16a was then displaced by azido group using LiN\textsubscript{3} in DMF at 110-20°C to produce 17a. The 4-hydroxyl group of 17a was acetylated to afford methyl 4-O-acetyl-6-azido-2-N-morpholino-2,3,6-trideoxy-α-D-ribo-hexopyranoside 18a (Scheme II). In order to mask the NH function of 13 (X = benzylamino) from electrophilic reagents, 13 was reacted with acetic anhydride in pyridine to obtain 2-N-(acetylbenzylamino)-2,3-dideoxy-4,6-O(phenylmethylene)-α-D-ribo-hexopyranoside 14 (Scheme I). The same reaction sequence discussed for morpholino derivative was then repeated with 14 to afford 18b (Scheme II).

Synthesis of methyl 4-azido-2-N-morpholino-6-O-trityl-2,3,4-trideoxy-α-D-xylo-hexopyranoside 21a and methyl 2-N-(acetyl benzylamino)-4-azido-6-O-trityl-2,3,4-trideoxy-α-D-xylo-hexopyranoside 21b. The 4,6-diol 15a was selectively protected at the 6-hydroxy position with triyl chloride in anhyd. pyridine to afford 6-O-trityl derivative 19a. The 4-hydroxy was then mesylated using methansulfonyl chloride at 0°C. The mesylated derivative 20a on treatment with LiN\textsubscript{3} in DMF at 110-20°C, afforded methyl 4-azido-2-N-morpholino-6-O-trityl-2,3,4-trideoxy-α-D-xylo-hexopyranoside 21a, with inversion of configuration at C-4 (Scheme III). Synthesis of methyl 2-N-(acetylbenzylamino)-4-azido-6-O-trityl-2,3,4-trideoxy-α-D-xylo-hexopyranoside 21b was attempted following the same reaction sequences as reported for 21a. Although the intermediate 20b was synthesized with reasonable purity, the final product 21b could not be obtained in pure form.

Synthesis of methyl 4, 6-diazido-2-N-morpholino-2,3,4,6-tetrahydroxy-α-D-xylo-hexopyranoside 23a and methyl 2-N-(acetyl benzylamino)-4, 6-diazido-2,3,4,6-tetrahydroxy-α-D-xylo-hexopyranoside 23b. The 4,6-diol 15a was mesylated using methansulfonyl chloride at 0°C. The dimesylated derivative 22a was treated with LiN\textsubscript{3} in DMF at 110-20°C to afford methyl 4,6-diazido-2-N-morpholino-2,3,4,6-tetrahydroxy-α-D-xylo-hexopyranoside 23a (Scheme IV). Methyl 2-N-(acetyl benzylamino)-4,6-diazido-2,3,4,6-tetrahydroxy-α-D-xylo-
hexopyranoside 23b was synthesized following the same reaction sequence from 15b (Scheme IV).

The H-1 protons of compounds 16a, 18a, 20a, 21a, and 22a appeared in the $^1$H spectra as doublets ($J_{1,2} = 3.0-4.0$ Hz) in the region of 4.75-4.88 ppm as expected\(^{(19)}\). Similarly in $^{13}$C NMR spectra of these compounds C-1 appeared in the region 96.8-98.1 ppm. In the mass spectra, compounds 16a, 18a, 20a, 21a, 22a, and 23a showed peaks corresponding to M$, M^-\text{-OCH}_3$, or $M^-\text{-N}_3$ fragments. The presence of IR band in the region of 2102-2100 cm$^{-1}$ in compounds 18a, 21a, and 23a indicates the presence of azido groups in these compounds. The H-1 peaks of N-acetyl benzylamino compounds 18b and 23b could not be assigned because they always overlapped with the peaks arising of other ring protons. It should be noted that in this series most of the compounds were always contaminated with minor unknown impurities. Compound 23b, however gave the correct elemental analysis. In the mass spectra, 18b and 23b showed peaks corresponding to M$, M^-\text{-OCH}_3$, or $M^-\text{-N}_3$ fragments. The presence of IR band at 2120 cm$^{-1}$ in compounds 18b and 23b indicated the presence of azido groups in these compounds.

Conclusion

We have, for the first time synthesized intermediates for generating various new synthetic polyaminosugars with N-alkyl and N,N-dialkyl amino groups at the C-2 position of the hexopyranosides. It will also be possible to access the naturally occurring diaminosugars through this route because we have already established\(^{(19)}\) the protocols for debenzylations of benzylamino derivatives closely related to 18b, 21b or 23b; direct reaction of ammonia with vinyl sulfone-modified carbohydrates\(^{(19)}\), such as 9 followed by the synthetic manipulations presented here should also generate naturally occurring aminosugars. The stability of the acetylated benzylamino derivatives to the reaction conditions described here should be studied in detail before embarking on the synthesis of aminosugars containing secondary amino groups. Work is currently in progress to synthesize aminosugars 1-7 and their analogues using this powerful strategy of addition of amines to vinyl sulfone-modified carbohydrates.

Experimental Section

Methyl 4-O-acetyl-6-azido-2-N-morpholino-2,3,6-trideoxy-$\alpha$-D-ribo-hexopyranoside 18a. A solution of 12 (0.31 g, 0.94 mmole) in 0.1N HCl (18 mL), and methanol (9 mL), was heated under reflux for 3 hr. The reaction mixture was concentrated to dryness under reduced pressure to afford a syrup 15a. The residual moisture was co-evaporated with anhyd. methanol. p-Toluenesulfonyl chloride (0.18 g, 0.94 mmole) in anhyd. pyridine (5 mL) was added to a solution of 15a in anhyd. pyridine (10 mL) at 0°C and the mixture was stirred at ambient temperature for 12 hr. The reaction mixture was poured into satd. aq. NaHCO$_3$ (60 mL) and the solution was extracted with dichloromethane (3 x 20 mL). Organic layers were pooled together, dried over anhyd. Na$_2$SO$_4$, filtered and the filtrate was concentrated under reduced pressure to a syrupy residue. The syrup was purified over silica gel (75% ethyl acetate-pet. ether) to afford a white solid of methyl 2,3-dideoxy-2-N-morpholino-6-O-tosyl-$\alpha$-D-ribo-hexopyranoside 16a, yield 0.35 g (93%); m.p. 156-157°C; [$\alpha$]$^D_{D}^{28}$ +33.4° (c 0.415, MeOH); $^1$H NMR: δ 1.79 (m, 1H), 2.11 (m, 1H), 2.37-2.96 (m, 5H), 2.45 (s, 3H), 3.13 (s, 3H), 3.55-3.63 (m, 3H), 4.20 (m, 1H), 4.39 (dd, $J_1$=3.9, 11.3 Hz, 1H), 4.75 (d, $J_1$=3.4 Hz, 1H), 7.37 (m, 2H), 7.82 (m, 2H); $^{13}$C NMR (DMSO-
ether) to afford a colourless solid of methyl 2,3-dideoxy-4-O-mesyl-2-N-morpholino-6-O-trityl-a-D-ribohexopyranoside. The syrup was purified over silica gel (3 x 15 mL). Organic layers were pooled together, dried over anhyd. Na2SO4, filtered and concentrated to dryness. The crude residue was purified over silica gel (60% ethyl acetate-pet ether) to afford a yellowish syrupy residue, yield, 0.11 g (56% in 3 steps from 15a); IR (CHCl3): 2102 cm-1; [α]22 D: +17.5° (c 1.005, CHCl3); 1H NMR: δ 1.81 (m, 1H), 2.06 (s, 3H), 2.21 (m, 1H), 3.31 (d, J = 4.0 Hz, 2H), 3.45 (s, 3H), 3.71 (m, 4H), 3.86 (m, 1H), 4.72 (m, 1H), 4.79 (d, J = 3.0 Hz, 1H); 13C NMR: δ 20.3, 25.8 (CH3), 50.1 (CH3), 51.1 (CH2), 54.2, 61.9, 66.6 (CH2), 68.4, 69.1, 98.0, 169.1. MS: FAB+ m/z (%) 314 (M+), 329 (M+-CH3, 6), 283 (M+-OCH3, 9), 272 (M+-N2S, 6).

Methyl 4-azido-2-N-morpholino-2,3,4-trideoxy-6-O-trityl-α-D-xylo-hexopyranoside 21a. Compound 12 (0.40 g, 1.194 mmole) was deprotected to a syrupy compound following the procedure for the synthesis of 18a. A solution of 15a and trityl chloride (0.66 g, 2.39 mmole) in anhyd. pyridine (20 mL) was stirred overnight at ambient temperature. The reaction mixture was poured into satd. aq. NaHCO3 solution (50 mL) and the aq. layer was extracted with dichloromethane (3 x 20 mL). Organic layers were pooled together, dried over anhyd. Na2SO4, filtered and the filtrate was concentrated under reduced pressure to a syrupy mass. The syrup was purified over silica gel (80% ethyl acetate-pet ether) to afford 19a (0.51 g, 87%). Methanesulfonyl chloride (0.38 mL, 4.91 mmole) in anhyd. pyridine (10 mL) was added dropwise to a solution of 19a (0.48 g, 0.981 mmole) in anhyd. pyridine (25 mL) at 0°C and the reaction mixture was left at +4°C overnight. After usual work-up as described for the synthesis of 19a the crude solid was purified over silica gel (65% ethyl acetate-pet ether) to afford a colourless solid of methyl 2,3-dideoxy-4-O-mesyl-2-N-morpholino-6-O-trityl-α-D-ribohexopyranoside 20a, yield 0.46 g (76% in 3 steps from 12); m.p. 188°C (decomp); [α]20 D: +72.6° (c 0.173, CHCl3); 1H NMR: δ 2.06 (m, 1H), 2.41-2.79 (m, 6H), 2.59 (s, 3H), 3.19 (dd, J = 4.9, 10.2 Hz, 1H), 3.41 (d, J = 2 Hz, 1H), 3.47 (s, 3H), 3.74 (m, 5H), 4.70 (m, 1H), 4.88 (d, J = 3.0 Hz, 1H), 7.25-7.49 (m, 15H); 13C NMR: δ 28.7 (CH3), 37.9, 50.5 (CH3), 54.6, 62.1 (CH2), 62.5, 67.0 (CH2), 68.8, 74.8, 86.6, 97.8, 127.1, 127.7, 128.7, 143.4; MS: FAB+ m/z (%) 567 (M+, 7), 552 (M+-CH3, 5), 536 (M+-OCH3, 1). LiN3 (0.23 g, 4.76 mmole) was added to a solution of 20a (0.27 g, 0.476 mmole) in DMF (10 mL) and the mixture was heated at 120°C for 12 hr. The reaction mixture was worked-up as described for the synthesis of 18a and the crude product was purified over silica gel (45% ethyl acetate-pet ether) to afford a brownish syrupy residue, yield, 0.20 g (82%); IR (CHCl3): 2100 cm-1 (N3); [α]22 D: +17.5° (c 1.005, CHCl3); 1H NMR: δ 2.08 (m, 2H), 2.52 (m, 2H), 2.70 (m, 3H), 3.18 (m, 1H), 3.35-3.55 (m, 1H), 3.38 (s, 3H), 3.71-3.94 (m, 6H), 4.77 (d, J = 3.0 Hz, 1H), 7.21-7.49 (m, 15H); 13C NMR: δ 24.5 (CH2), 49.9 (CH3), 54.7, 57.6, 59.2, 62.8 (CH2), 64.6 (CH3), 68.1, 87.2, 95.6, 127.0, 127.7, 128.4, 143.6; MS: FAB+ m/z (%) 514 (M+, 1), 472 (M+-N2S, 5).

Methyl 4, 6-diazido-2-N-morpholino-2, 3, 4, 6-tetrahydroxy-α-D-xylohexopyranoside 23a. Compound 12 (0.54 g, 1.61 mmole) was deprotected to a syrupy compound following the procedure for the synthesis of 18a. Methanesulfonyl chloride (1.24 mL, 16.11 mmole) in anhyd. pyridine (15 mL) was added dropwise to a solution of 15a in anhyd. pyridine (30 mL) at 0°C and the solution was left at +4°C overnight. The reaction mixture was worked-up as described for the synthesis of 21a and the crude residue was purified over silica gel (ethyl acetate) to afford a colourless syrup 22a, yield 0.37 g (57% in 2 steps from 12); [α]22 D: +103.7° (c 0.644, CHCl3); 1H NMR: δ 2.12 (q, J = 12.0 Hz, 1H), 2.32-2.77 (m, 6H), 3.09 (s, 3H), 3.10 (s, 3H), 3.44 (s, 3H), 3.71 (m, 4H), 3.93 (m, 1H), 4.40 (m, 2H), 4.69 (m, 1H), 4.80 (d, J = 2.0 Hz, 1H); 13C NMR: δ 27.4 (CH3), 37.6, 39.0, 50.5 (CH2), 55.2, 62.2, 66.6 (CH2), 67.8, 69.2 (CH2), 73.2, 98.1; MS: FAB+ m/z (%) 403 (M+), 14), 388 (M+-CH3, 8), 372 (M+-OCH3, 11), 308 (M+-SO2CH3, 40); Anal.: Caled. for C13H15N4O5S2: C, 38.01; H, 6.42; N, 3.83. Found: C, 38.69; H, 6.24; N, 3.47%. LiN3 (0.16 g, 3.22 mmole) was added to a solution of 22a (0.37 g, 0.918 mmole) in DMF (10 mL) and the mixture was heated over-
night at 120°C. The mixture was worked-up as described for the synthesis of 18a and the crude residue was purified over silica gel (40% ethyl acetate-pet. ether) to afford yellowish syrupy 23a, yield 0.202 g (62%). IR (CHCl₃): 2120 cm⁻¹ (N₃); [α]D²⁰⁺: -7.2° (c 0.202, CHCl₃); ¹H NMR: δ 2.10 (m, 2H), 2.66-2.84 (m, 3H), 3.21 (dd, 3H), 3.81 (m, 1H), 3.92 (m, 1H), 4.83 (d, 1H), 4.32 (d, J = 2.9 Hz, 1H); ¹³C NMR: δ 24.8 (CH₂), 50.1 (CH₃), 51.5 (CH₂), 54.7, 57.7, 58.0, 66.5 (CH₂), 67.9, 98.0. MS: FAB⁺ LR m/z (%): 297 (M⁺, 38), 282 (M⁺-CH₃, 8), 266 (M⁺-OCH₃, 12), 255 (M⁺-N₃, 13).

Methyl 2-N-(acetyl benzylamino) 2,3-dideoxy 4,6-O-(phenylmethylene)-α-D-ribo-hexopyranoside 14. To a solution of methyl 2-N-benzylamino 2,3-dideoxy-4,6-0-(phenylmethylene)-α-D-ribo hexopyranoside 13 (0.25 g, 0.704 mmole) in anhyd. pyridine (10 mL), was added acetic anhydride (0.306 g, 3.22 mmole) and the reaction mixture was stirred at ambient temperature for 8 hr. The reaction mixture was poured into satd. aq. NaHCO₃ (40 mL) and the aq. solution was extracted with dichloromethane (3 x5 mL). Organic layers were pooled together, dried over anhyd. Na₂SO₄, filtered and the filtrate was concentrated under reduced pressure to afford a yellowish syrupy compound methyl 2-N-(acetyl benzylamino)-4,6-0-dimesyl-2,3-dideoxy-α-D-ribo-hexopyranoside methyl 2-N-(acetyl benzylamino)-4,6-O-dimesyl-2,3-dideoxy-α-D-ribo-hexopyranoside 22b. To a solution of 22b (0.15 g, 0.322 mmole) in DMF (5 mL), was added LiN₃ (0.158 g, 3.22 mmole) and the mixture was heated at 100°C for 6 hr. The reaction mixture was worked-up as described for the synthesis of 23a to afford a colourless solid 23b, yield 0.06 g (27% in 3 steps from 14); m.p. 90-92°C; IR (CHCl₃): 2120 cm⁻¹ (N₃); [α]D⁰⁺: +26.9° (c 0.753, CHCl₃); ¹H NMR: δ 1.70-1.90 (m, 1H), 2.09 (s, 3H), 2.12-2.25 (m, 1H), 3.22-3.36 (m, 1H), 3.31 (s, 3H), 3.52 (m, 1H), 3.75 (m, 1H), 3.89 (m, 1H), 4.73 (m, 3H), 5.16-5.27 (m, 1H), 7.15-7.38 (m, 5H); ¹³C NMR: δ 22.1, 26.7 (CH₂), 47.5, 48.5 (CH₂), 51.6 (CH₂), 55.1 58.2, 67.8, 98.3, 125.2, 128.8, 137.7, 172.6. MS: FAB⁺ m/z (%): 356 (M⁺, 4), 328 (M⁺-OCH₃, 100), (M⁺-N₃, 12); Anal.: Calc. for C₁₆H₂₃N₃O₇: C, 53.47; H, 5.89; N, 27.28. Found: C, 52.92; H, 6.07; N, 26.66%.

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