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The chemoenzymatic synthesis of 9-substituted 3,9-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acids

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Abstract

A series of C-6 functionalised mannosides has been synthesised. Subsequent exposure of 6-azido-6-deoxy-D-mannopyranose and 6-bromo-6-deoxy-D-mannopyranose to Neu5Ac aldolase and sodium pyruvate provided the corresponding 9-azido- and 9-bromo-3,9-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid, respectively. © 1998 Elsevier Science Ltd.

Keywords: Neu5Ac aldolase; Enzyme; Sialic acid; Kdn; Mannose

1. Introduction

The sialic acid, 3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (Kdn, **1**), was first isolated in 1986 from the polysialoglycoprotein of rainbow trout eggs [1]. Since then, several Kdn glycoconjugates have been reported to occur in various living organisms ranging from bacteria to mammals [2]. Enzymes, in particular Kdn-cleaving sialidases, that recognise and metabolise Kdn-containing conjugates have been identified over the past few years [3–5]. This widespread interest has led not only to the enzymatic synthesis of Kdn (**1**) from D-mannose (**2**) using *N*-acetylneuraminate pyruvate lyase (Neu5Ac aldolase, EC 4.1.3.3) [6], but also the synthesis of Kdn glycoconjugates [7–9].

As part of our investigations into the biochemistry of a Kdn-cleaving sialidase [10] and the synthesis of Kdn-glycoconjugates, we required a readily available source of 9-substituted-9-deoxy-Kdn derivatives. Our interest in the substrate specificity of Neu5Ac aldolase [11], an enzyme used extensively in the synthesis of modified sialic acids [11–20], provided us with a good entry into the synthesis of the target Kdn derivatives from C-6 modified D-mannoses.

2. Results and discussion

Preparation of substrates.—We required a convenient synthesis of 6-substituted-6-deoxy-D-mannose substrates. In principle, the manipulation of either mannopyranoses or mannofuranoses provide reasonable entry points into functionalised mannoses for use in the chemoenzymatic synthesis of novel Kdn derivatives.

Our initial approach utilised the 6-*O*-*p*-toluenesulfonyl-D-mannopyranose (**3**) for displacement chemistry. Thus, selective *p*-toluenesulfonylation

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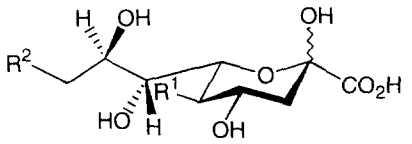
[21–23] of D-mannose (**2**) afforded the tosylate **3** in reasonable isolated yield (66%). A more complete characterisation of **3** was achieved by peracetylation [24] of the product using acetic anhydride and 4-dimethylaminopyridine in pyridine, which gave an anomeric mixture of the known and partially characterised [21] tetraacetate **4** in good overall yield (84%). Our attempts to displace the 6-*p*-toluenesulfonate **3** with lithium azide in *N*,*N*-dimethylformamide at 50–60 °C provided 6-azido-6-deoxy-D-mannopyranose (**5**) in moderate yield (< 60%). This simple, two-step sequence for the preparation of the desired 6-azide **5** is a significant improvement over a previously reported synthesis which involved a seven-step procedure [25]. Treatment of the tetra-*O*-acetate derivative **4**, under identical conditions, with lithium azide provided the corresponding per-*O*-acetylated 6-azido-6-deoxy pyranose **6** in 65% yield. Similarly, although in better yield, treatment of **4** with lithium bromide afforded the novel 6-bromo-tetra-*O*-acetyl derivative **7** in 83% yield.

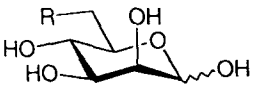
Our interest in the possible modification of C-5 on mannose prompted us to also investigate the use of the known [26] mannofuranoside **8** as a suitable alternative starting material for the preparation of 6-substituted D-mannoses. Thus, *O*-isopropylidenation and methyl glycosidation of D-mannose (**2**) using known methodology [26] provided the mannofuranoside **8** in good overall yield. Selective tosylation of **8** afforded the known [27] 6-*p*-toluenesulfonate **9** in 65% yield.

Treatment of **9** with excess lithium azide in *N*,*N*-dimethylformamide at 50–60 °C gave the novel 6-azido sugar **10** in near-quantitative yield (98%). The synthesis of the 6-bromofuranoside **11** was also readily achieved from **9**. Thus, when an *N*,*N*-dimethylformamide solution of **9** was exposed to lithium bromide at 45–55 °C, the desired compound **11** was isolated in excellent yield (98%).

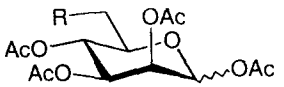
Deprotection of these C-6 derivatised mannofuranosides was readily achieved under aqueous acidic conditions. Thus, exposure of **10** to a mixture of



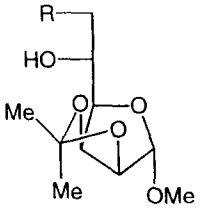
1 R^1 = OH, R^2 = OH
13 R^1 = OH, R^2 = N_3
14 R^1 = OH, R^2 = Br



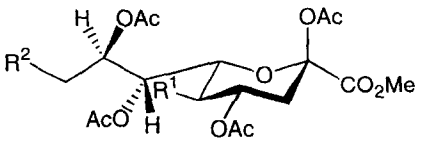
2 R = OH
3 R = OTs
5 R = N_3
12 R = Br



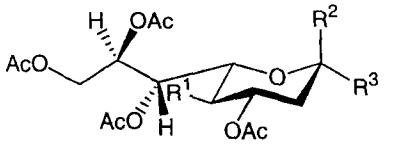
4 R = OTs
6 R = N_3
7 R = Br



8 R = OH
9 R = OTs
10 R = N_3
11 R = Br



15 R^1 = OAc, R^2 = N_3
16 R^1 = OAc, R^2 = Br



17 R^1 = NHAc, R^2 = CO_2Me, R^3 = OAc
18 R^1 = NHAc, R^2 = OAc, R^3 = CO_2Me

Scheme 1.

trifluoroacetic acid and water (1:1) at 40–50 °C gave the 6-azido-6-deoxy-D-mannopyranose (**5**). In a similar manner, the 6-bromo derivative **12** was prepared from furanoside **11**. Peracetylation of the pyranoses **5** and **12** provided **6** and **7**, respectively. The peracetates were spectroscopically indistinguishable from the same compounds that were synthesised from the mannopyranose starting material described above Scheme 1.

Enzymatic synthesis of 9-modified Kdn derivatives.—Exposure of the 6-azido pyranose **5** to Neu5Ac aldolase in excess sodium pyruvate using membrane-enclosed enzyme catalysis [28] at pH 7.5 gave the corresponding 9-azido-3,9-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (9-azido-9-deoxy-Kdn, **13**). After a reaction time of 96 h, 89% of the starting pyranose (by ^{1}H NMR) was consumed. In a similar manner was prepared 9-bromo-3,9-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (9-bromo-9-deoxy-Kdn, **14**) from the 6-bromo-6-deoxy pyranose **12** with a conversion of 76% after 96 h.

Full characterisation of the novel nonulosonic acids was achieved by methyl esterification and peracetylation. Thus, treatment of 9-azido-9-deoxy-Kdn (**13**) with Dowex (H^+) resin in methanol [29], followed by peracetylation with acetic anhydride in pyridine [30] in a one-pot, two-step reaction, gave the pentaacetate methyl ester **15** as the β anomer. Similarly, the β pentaacetate methyl ester **16** was prepared from the 9-bromo-9-deoxy-Kdn (**14**). Both **15** and **16** were fully assigned by examination of their ^{1}H NMR spectra, and by comparison of the chemical shifts of H-4, H-7 and H-8 with the corresponding α and β anomers of the per-*O*-acetylated Neu5Ac methyl esters **17** and **18** as reported by Marra and Sinaÿ [30].

We thought that it might also be interesting to investigate the possibility of using the *p*-toluenesulfonate **3** as a substrate for Neu5Ac aldolase. The resultant product would be a C-9-activated Kdn derivative that provides scope for further chemistry. Interestingly, our preliminary investigation of this chemistry appeared to suggest that when the 6-*p*-toluenesulfonate **3** was exposed to the Neu5Ac aldolase, no product was formed. Moreover, a loss of enzyme activity was observed. Work is currently in progress to further investigate this observation.

In conclusion, we have successfully prepared 9-azido-9-deoxy- and 9-bromo-9-deoxy-Kdn from the appropriately modified D-mannose using a chemoenzymatic method. These compounds will provide useful biological probes to study Kdn-recognising proteins.

3. Experimental

General methods.—Melting points were determined on a Gallenkamp MFB-595 apparatus and are uncorrected. Optical rotations were measured with a JASCO DIP-370 polarimeter. Infrared spectra were recorded using a Hitachi 270-30 grating spectrophotometer. Low resolution (LRFAB) and high resolution (HRFAB) fast-atom bombardment mass spectra (FABMS) were obtained on a Jeol JMX DX-300 double-focusing instrument. ^{1}H and ^{13}C spectra were recorded using a Bruker AM 300 MHz instrument working, respectively, at 300 MHz and 75.5 MHz. Where (*) is indicated, assignment is tentative. ^{13}C assignments of the mannopyranoses and nonulosonic acids are in reference to reported ^{13}C spectra of D-mannose [31] and ammonium salt of Kdn [6], respectively. All column chromatography was performed on Merck Kieselgel 60 (230–400 mesh) silica gel. Microanalyses were performed by Chemical and Micro Analytical Services Pty., Belmont, Victoria.

Preparation of 6-O-p-toluenesulfonyl-D-mannopyranose (**3**) *and 1, 2, 3, 4 - tetra - O - acetyl - 6 - O - p - toluenesulfonyl - D - mannopyranose* (**4**).—The title compounds were prepared according to literature methods [21]: Compound **3**. $[\alpha]_D^{27}$ +24.4° (*c* 1.05, MeOH); IR (KBr): ν_{max} 3460 (br, OH), 1354, 1188, 1172, 1092 cm^{-1}; ^{1}H NMR (deuterium oxide): δ 2.40 (s, 3 H, CH_3), 3.50 (t, 0.3 H, $J_{4,3} = J_{4,5}$ 9.5 Hz, H-4β), 3.56 (t, 0.7 H, $J_{4,3} = J_{4,5}$ 9.3 Hz, H-4α), 3.70–4.48 (m, 5 H, H-2, H-3, H-5, H-6a, H-6b), 4.76 (s, 0.3 H, H-1β), 5.05 (s, 0.7 H, H-1α), 7.46 (d, 2 H, *J* 8.0 Hz, Ar–H-3, Ar–H-5), 7.81 (d, 2 H, *J* 8.0 Hz, Ar–H-2, Ar–H-6); ^{13}C NMR (deuterium oxide, α anomer only): δ 23.4 (Ar–CH_3), 69.0 (C-4), 72.4 (C-6), 72.5 (C-3*), 72.8 (C-2*), 73.2 (C-5), 96.8 (C-1), 130.4 (Ar–C-3, Ar–C-5), 132.8 (Ar–C-2, Ar–C-6), 133.2 (Ar–C-4), 149.1 (Ar–C-1); LRFAB mass spectrum: 352 [$(M + H_2O)^+$, 42%], 335 [$(M + 1)^+$, 21], 317 (89), 230 (99), 228 (100), 155 (65). Compound **4**. mp 49–51 °C; $[\alpha]_D^{26}$ +50.0° (*c* 0.86, $CHCl_3$); IR (KBr): ν_{max} 3000, 1756 (C=O), 1370, 1220, 1188, 1174, 1146 cm^{-1}; ^{1}H NMR spectroscopic data was in good agreement with that previously published [21]; ^{13}C NMR ($CDCl_3$, α anomer only): δ 20.5 ($4 \times OC(O)CH_3$), 21.5 (Ar–CH_3), 65.6 (C-4), 67.8 (C-6), 68.2 (C-3*), 68.5 (C-2*), 70.3 (C-5), 90.2 (C-1), 128.0 (Ar–C-3, Ar–C-5), 129.8 (Ar–C-2, Ar–C-6), 132.5 (Ar–C-4), 145.0 (Ar–C-1), 167.9, 169.4, 169.6, 169.8 ($4 \times OC(O)CH_3$); LRFAB mass spectrum: 443 {[$M - OC(O)CH_3$]$^+$, 71%} 289 (32), 281 (39), 229 (33),

185 (100). Anal. Calcd for $C_{21}H_{26}O_{12}S$: C, 50.20; H, 5.22; S, 6.38. Found: C, 50.10; H, 5.27; S, 6.29.

1, 2, 3, 4 - Tetra - O - acetyl - 6 - azido - 6 - deoxy - D - mannopyranose (**6**).—To a solution of **4** (400 mg, 0.8 mmol) in *N,N*-dimethylformamide (6.8 mL) was added lithium azide (156 mg, 3.2 mmol) under nitrogen. The mixture was stirred at 50–60 °C for 24 h, concentrated, and chromatographed (1:2 EtOAc–hexane) to give **6** (193 mg, 65%) as a colourless syrup having a ratio of α to β anomer (by ^{1}H NMR) of 21:4, respectively: $[\alpha]_D^{25}$ +70.6° (*c* 0.68, $CHCl_3$); IR (KBr): ν_{max} 2968, 2104 (N_3), 1754 (C=O), 1436, 1368, 1218, 1082 cm^{-1}; ^{1}H NMR ($CDCl_3$, α anomer only): δ 1.96, 2.02, 2.12, 2.13 (s, 12 H, 4 × OC(O)CH_3), 3.27 (dd, 1 H, $J_{6a,5}$ 5.6, $J_{6a,6b}$ 13.5 Hz, H-6a), 3.36 (dd, 1 H, $J_{6b,5}$ 2.9 Hz, H-6b), 3.93–4.04 (m, 1 H, H-5), 5.15–5.30 (m, 3 H, H-2, H-3, H-4), 6.05 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1); ^{13}C NMR ($CDCl_3$, α anomer only): δ 20.8 (4 × OC(O)CH_3), 51.1 (C-6), 66.9 (C-4), 68.6 (C-3*), 68.9 (C-2*), 72.1 (C-5), 90.7 (C-1), 168.2, 169.8, 169.9, 170.1 (4 × OC(O)CH_3); LRFAB mass spectrum: 314 {[M − OC(O)CH_3]$^+$, 100%}, 244 (85), 202 (36), 184 (71), 142 (100), 139 (100). Anal. Calcd for $C_{14}H_{19}N_3O_9$: C, 45.04; H, 5.13; N, 11.26. Found: C, 45.14; H, 5.13; N, 10.96.

1, 2, 3, 4 - Tetra - O - acetyl - 6 - bromo - 6 - deoxy - D - mannopyranose (**7**).—To a solution of **4** (400 mg, 0.8 mmol) in *N,N*-dimethylformamide (6.7 mL) was added lithium bromide (277 mg, 3.2 mmol) under nitrogen. The mixture was stirred at 50–60 °C for 24 h and concentrated. Column chromatography (1:2 EtOAc–hexane) gave **7** (271 mg, 83%) with a ratio of α:β anomers (by ^{1}H NMR) of 17:3, respectively: $[\alpha]_D^{25}$ +60.8° (*c* 0.71, $CHCl_3$); IR (KBr): ν_{max} 1754 (C=O), 1372, 1220, 1144, 1052 cm^{-1}; ^{1}H NMR ($CDCl_3$, α anomer only): δ 1.94, 2.01 (s, 6 H, 2 × OC(O)CH_3), 2.10 (s, 6 H, 2 × OC(O)CH_3), 3.36 (dd, 1 H, $J_{6a,5}$ 5.2, $J_{6a,6b}$ 11.3 Hz, H-6a), 3.43 (dd, 1 H, $J_{6b,5}$ 2.9 Hz, H-6b), 3.92–4.04 (m, 1 H, H-5), 5.15–5.30 (m, 3 H, H-2, H-3, H-4), 5.99 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1); ^{13}C NMR ($CDCl_3$, α anomer only): δ 20.9 (4 × OC(O)CH_3), 31.0 (C-6), 68.5 (C-4*), 68.7 (C-3*), 68.9 (C-2*), 72.2 (C-5), 90.8 (C-1), 168.2, 169.7, 169.9, 170.1 (4 × OC(O)CH_3); LRFAB mass spectrum: 353 {[^{81}BrM − OC(O)CH_3]$^+$, 62%}, 351 {[^{79}BrM − OC(O)CH_3]$^+$, 62%}, 251 (38), 249 (38), 223 (32), 231 (32), 191 (94), 189 (100). Anal. Calcd for $C_{14}H_{19}BrO_9 \cdot 0.5H_2O$: C, 40.02; H 4.8. Found: C, 40.31; H, 5.06.

Preparation of methyl 2,3-O-isopropylidene-α-D-mannofuranoside (**8**).—The title compound was prepared according to the literature [26]: $[\alpha]_D^{25}$ +57.9° (*c* 2.82, $CHCl_3$), lit. $[\alpha]_D^{24}$ +65.1° (*c* 0.99, $CHCl_3$) [32]; IR (NaCl): ν_{max} 3468 (br, OH), 2966, 1374, 1270, 1208, 1162, 1020, 962 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.31, 1.46 (s, 6 H, 2 × CH_3), 2.93 (s, 2 H, 2 × OH), 3.29 (s, 3 H, OCH_3), 3.69 (dd, 1 H, $J_{6a,5}$ 5.9, $J_{6a,6b}$ 11.5 Hz, H-6a), 3.85 (dd, 1 H, $J_{6b,5}$ 3.3 Hz, H-6b), 3.90 (dd, 1 H, $J_{4,3}$ 3.7, $J_{4,5}$ 8.3 Hz, H-4), 4.00 (ddd, 1 H, H-5), 4.55 (d, 1 H, $J_{2,3}$ 5.9 Hz, H-2), 4.84 (dd, 1 H, H-3), 4.88 (s, 1 H, H-1); ^{13}C NMR ($CDCl_3$): δ 24.7, 25.9 (2 × CH_3), 54.4 (OCH_3), 64.4 (C-6), 70.0 (C-5), 79.2 (C-4), 80.0 (C-3), 84.7 (C-2), 107.1 (C-1), 112.6 ($C(CH_3)_2$); LRFAB mass spectrum: 235 [(M + 1)$^+$, 100%], 219 (43), 203 (42), 145 (100), 127 (52), 115 (55); HRFAB mass spectrum: $C_{10}H_{19}O_6$ requires 235.11816; found: 235.11814.

Preparation of methyl 2,3-O-isopropylidene-6-O-p-toluenesulfonyl-α-D-mannofuranoside (**9**).—The title compound was prepared according to the literature [27]: mp 96–98 °C, lit. 97–98 °C [27]; $[\alpha]_D^{27}$ +47.3° (*c* 1.18, $CHCl_3$); IR (KBr): ν_{max} 3528 (br, OH), 3008 (Ar), 2932, 1356, 1174, 1106, 1038 and 1012 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.29, 1.43 (s, 6 H, CH_3), 2.45 (s, 3 H, Ar–CH_3), 2.73 (s, br, 1 H, OH), 3.27 (s, 3 H, OCH_3), 3.90 (dd, 1 H, $J_{4,3}$ 3.8, $J_{4,5}$ 7.8 Hz, H-4), 4.12–4.22 (m, 2 H, H-5, H-6a), 4.32 (dd, 1 H, $J_{6b,5}$ 2.7, $J_{6b,6a}$ 9.9 Hz, H-6b), 4.55 (d, 1 H, $J_{2,3}$ 5.9 Hz, H-2), 4.79 (dd, 1 H, H-3), 4.87 (s, 1 H, H-1), 7.35 (d, 2 H, *J* 8.3 Hz, Ar–H-3, Ar–H-5), 7.82 (d, 2 H, *J* 8.3 Hz, Ar–H-2, Ar–H-6); ^{13}C NMR ($CDCl_3$): δ 21.5 (Ar–CH_3), 24.5, 25.8 (2 × CH_3), 54.5 (OCH_3), 67.9 (C-5), 71.6 (C-6), 78.1 (C-4), 79.6 (C-3), 84.6 (C-2), 107.0 (C-1), 112.7 [$C(CH_3)_2$], 127.9 (Ar–C-3, Ar–C-5), 129.8 (Ar–C-2, Ar–C-6), 132.7 (Ar–C-4), 144.9 (Ar–C-1); LRFAB mass spectrum: 389 [(M + 1)$^+$, 24%], 373 (47), 358 (41), 357 (35), 186 (42), 185 (100), 154 (100), 138 (63); HRFAB mass spectrum: $C_{17}H_{25}O_8S$ requires 389.12701; found 389.12820.

Methyl 6-azido-6-deoxy-2,3-O-isopropylidene-α-D-mannofuranoside (**10**).—To a solution of the 6-*O*-*p*-toluenesulfonyl **9** (156 mg, 0.4 mmol) in *N,N*-dimethylformamide (3 mL) was added lithium azide (79 mg, 1.6 mmol) under nitrogen. The resultant mixture was stirred at 50–60 °C for 19 h, concentrated and chromatographed (1:2 EtOAc–hexane) to give **10** (101.9 mg, 98%) as a colourless syrup: $[\alpha]_D^{25}$ +52.2° (*c* 2.4, $CHCl_3$); IR (NaCl): ν_{max} 3500 (br, OH), 2948, 2104 (N_3) cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.32, 1.46 (2 × s, 6 H, 2 × CH_3), 2.75 (s, br, 1 H, OH), 3.30 (s, 3 H, OCH_3), 3.46 (dd, 1 H, $J_{6a,5}$ 6.2, $J_{6a,6b}$ 12.7 Hz, H-6a), 3.57 (dd, 1 H, $J_{6b,5}$ 3.0 Hz,

H-6b), 3.89 (dd, 1 H, $J_{4,3}$ 3.9, $J_{4,5}$ 8.5 Hz, H-4), 4.14 (ddd, 1 H, H-5), 4.57 (d, 1 H, $J_{2,3}$ 5.9 Hz, H-2), 4.82 (dd, 1 H, H-3), 4.89 (s, 1 H, H-1); ^{13}C NMR ($CDCl_3$): δ 24.6, 25.9 ($2 \times CH_3$), 54.4 (C-6), 54.6 (OCH_3), 69.5 (C-5), 79.4 (C-4), 79.8 (C-3), 84.8 (C-2), 107.1 (C-1), 112.8 ($C(CH_3)_2$); LRFAB mass spectrum: 260 [$(M + 1)^+$, 31%], 258 (31), 244 (100), 234 (69), 200 (30), 171 (23), 142 (52). Anal. Calcd for $C_{10}H_{17}N_3O_5$: C, 46.29; H, 6.61; N, 15.98. Found: C, 46.33; H, 6.61; N, 16.21; ^{13}C NMR assignments were confirmed by a ^{1}H–^{13}C HMQC experiment.

*Methyl 6-bromo-6-deoxy-2,3-O-isopropylidene-α-*D*-mannofuranoside* (**11**).—To a solution of **9** (741 mg, 1.91 mmol) in *N*,*N*-dimethylformamide (10 mL) was added lithium bromide (690 mg, 7.9 mmol) under nitrogen. The mixture was stirred at 45–55 °C for 24 h, concentrated and chromatographed (1:4 EtOAc–hexane) to give the 6-bromo compound **11** (558 mg, 98%) as a colourless syrup: $[\alpha]_D^{27}$ +62.9° (*c* 1.23, $CHCl_3$); IR (NaCl): ν_{max} 3496 (OH), 2948, 1374, 1290, 1270, 1228 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.33, 1.47 (s, 6 H, $2 \times CH_3$), 2.50 (s, br, 1 H, OH), 3.32 (s, 3 H, OCH_3), 3.64 (dd, 1 H, $J_{6a,5}$ 5.8, $J_{6a,6b}$ 0.6 Hz, H-6a), 3.75 (dd, 1 H, $J_{6b,5}$ 3.3 Hz, H-6b), 3.95 (dd, 1 H, $J_{4,3}$ 3.7, $J_{4,5}$ 8.3 Hz, H-4), 4.11 (ddd, 1 H, H-5), 4.58 (d, 1 H, $J_{2,3}$ 5.9 Hz, H-2), 4.84 (dd, 1 H, H-3), 4.90 (s, 1 H, H-1); ^{13}C NMR ($CDCl_3$): δ 24.7, 26.0 ($2 \times CH_3$), 37.7 (C-6), 54.4 (OCH_3), 68.6 (C-5), 79.7 (C-4), 80.0 (C-3), 84.8 (C-2), 107.0 (C-1), 112.7 ($C(CH_3)_2$); LRFAB mass spectrum: 299 [(^{81}BrM + 1)$^+$, 23%], 297 [(^{79}BrM + 1)$^+$, 41%], 283 (70), 281 (74), 267 (67), 265 (76), 223 (22), 221 (24), 209 (44), 207 (53); HRFAB mass spectrum: $C_{10}H_{18}{}^{79}BrO_5$ requires 297.03375; found 297.03437. Anal. Calcd for $C_{10}H_{17}BrO_5$: C, 40.42; H, 5.77. Found: C, 40.19; H, 5.88.

*Preparation of 6-azido-6-deoxy-*D*-mannopyranose* (**5**).—To the furanoside **10** (500 mg, 1.93 mmol) was added 1:1 TFA–water mixture (26 mL), and the resultant mixture was stirred at 40–50 °C for 5.5 h and concentrated. The residue was dissolved in water and concentrated. This was repeated three times, after which time the residue was dissolved in water and lyophilised to afford a brownish foam. The foam was chromatographed (9:1 EtOAc–MeOH) to give **5** (341 mg, 86%) as a white hygroscopic foam with a ratio of α:β anomers (by ^{1}H NMR) of 3:2, respectively: $[\alpha]_D^{28}$ +30.5° (*c* 1.54, water), lit. $[\alpha]_D^{20}$ +31° (*c* 4.3, water) [25]; IR (KBr): ν_{max} 3400 (br, OH), 2108 (N_3) cm^{-1}; ^{1}H NMR (deuterium oxide): δ 3.37–3.88 (m, 6 H, H-2, H-3, H-4, H-5, H-6a, H-6b), 4.85 (s, 0.4 H, H-1β), 5.11 (s, 0.6 H, H-1α); ^{13}C NMR (deuterium oxide, α anomer only): δ 53.7 (C-6), 70.1 (C-4), 72.6 (C-3), 73.2 (C-2), 73.6 (C-5), 96.7 (C-1); LRFAB mass spectrum: 228 [$(M + Na)^+$, 46%], 223 [$(M + H_2O)^+$, 38%], 206 [$(M + 1)^+$, 79%], 188 (100), 180 (100), 164 (20), 146 (12).

In a similar manner, 6-bromo-6-deoxy-D-mannopyranose (**12**) was prepared from **11** in 80% (329 mg) yield as a colourless hygroscopic foam with a ratio of α:β anomers (by ^{1}H NMR) of 7:3, respectively: $[\alpha]_D^{26}$ +17.1° (*c* 1.87, water); IR (KBr): ν_{max} 3428 (br, OH), 1088, 1044 cm^{-1}; ^{1}H NMR (deuterium oxide): δ 3.48–3.91 (m, 6 H, H-2, H-3, H-4, H-5, H-6a, H-6b), 4.91 (s, 0.3 H, H-1β), 5.14 (s, 0.7 H, H-1α); ^{13}C NMR (deuterium oxide, α anomer only): δ 36.2 (C-6), 71.4 (C-4), 72.6 (C-3), 73.2 (C-2), 73.7 (C-5), 96.9 (C-1); LRFAB mass spectrum: 267 [(^{81}BrM + Na)$^+$, 41%], 265 [(^{79}BrM + Na)$^+$, 48%], 227 (43), 225 (42), 166 (16), 164 (13).

*9-Azido-3,9-dideoxy-*D*-glycero-*D*-galacto-2-nonulosonic acid (9-azido-9-deoxy-Kdn*, **13**).—To the pyranose **5** (317 mg, 1.54 mmol) was added sodium pyruvate (680 mg, 6.18 mmol), NaN_3 (10.4 mg) and water (6 mL). The pH of the mixture was adjusted to 7.5 with 0.09% NaOH, and the resultant mixture was made up to 9.4 mL with water. To the final mixture was added a dialysis bag containing Neu5Ac aldolase (4.17 U) and bovine serum albumin (8.34 mg) dissolved in water (1 mL). The resultant mixture was stirred at 30–36 °C in a closed system. After 6 days, the crude mixture was acidified to pH 3 with Amberlite IRA-120 (H^+) resin, and the suspension was filtered. The filtrate was applied to an anion-exchange column containing Amberlite IRA-400 (formate) resin (45 mL). The resin was washed with water (400 mL) and eluted with aqueous formic acid [gradient commencing with 0.5 M increasing to 1 M and then 2 M] to afford **13** (355 mg, 86% based on starting material consumed after 6 days) as a white amorphous mass of ~95% purity by ^{1}H NMR. Analysis of the reaction mixture showed 89% conversion after 96 and 91% conversion after 6 days. The product showed a ratio of α to β anomer (by ^{1}H NMR) of 6:94, respectively: IR (KBr): ν_{max} 3422 (br, OH), 2108 (N_3) cm^{-1}; ^{1}H NMR (deuterium oxide): δ 1.59 (t, 0.06 H, $J_{3a,3e} = J_{3a,4}$ 12.6 Hz, H-3aα), 1.76 (t, 0.94 H, $J_{3a,3e} = J_{3a,4}$ 12.8 Hz, H-3aβ), 2.19 (dd, 0.94 H, $J_{3e,4}$ 5.0 Hz, H-3eβ), 2.60 (dd, 0.06 H, $J_{3e,4}$ 4.9 Hz, H-3eα), 3.46 (dd, 1 H, $J_{9a,8}$ 6.3 Hz, $J_{9a,9b}$ 12.9 Hz, H-9a), 3.53 (t, 1 H, $J_{5,4} = J_{5,6}$ 9.6 Hz, H-5), 3.59 (dd, 1 H, $J_{9b,8}$ 2.4 Hz, H-9b), 3.76–3.98 (m, 4 H, H-4, H-6, H-7, H-8); ^{13}C NMR (deuterium oxide): δ 36.3 (C-3), 51.3 (C-9), 65.9 (C-4), 66.3 (C-7), 66.8

(C-5), 67.6 (C-6), 68.7 (C-8); LRFAB mass spectrum: 316 [(M + Na)$^+$, 43%], 279 (30), 237 (53), 207 (100), 185 (100), 131 (100).

In a similar manner was prepared 9-bromo-3,9-dideoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (9-bromo-9-deoxy-Kdn, **14**) from 6-bromo-6-deoxy-D-mannopyranose **12** (319 mg, 1.31 mmol). The reaction mixture was stirred for 96 h to afford 76% conversion (by ^{1}H NMR). The title compound **14** (175 mg, 53% based on starting material consumed) was isolated as a white amorphous mass of 95% purity (by ^{1}H NMR) with the ratio of α:β anomers (by ^{1}H NMR spectroscopy) of 1:99, respectively: ^{1}H NMR (deuterium oxide): δ 1.54 (t, 0.01 H, $J_{3a,3e} = J_{3a,4}$ 11.7 Hz, H-3aα), 1.77 (t, 0.99 H, $J_{3a,3e} = J_{3a,4}$ 13.0 Hz, H-3aβ), 2.24 (dd, 0.99 H, $J_{3e,4}$ 5.1 Hz, H-3eβ), 2.62 (dd, 0.01 H, $J_{3e,4}$ 4.8 Hz, H-3eα), 3.57 (t, 1 H, $J_{5,4} = J_{5,6}$ 9.6 Hz, H-5), 3.72 (dd, 1 H, $J_{9a,8}$ 6.9, $J_{9a,9b}$ 11.1 Hz, H-9a), 3.78 (dd, 1 H, $J_{9b,8}$ 2.0 Hz, H-9b), 3.87–4.02 (m, 4 H, H-4, H-6, H-7, H-8); ^{13}C NMR (deuterium oxide): δ 40.7 (C-3*), 41.9 (C-9*), 71.4 (C-4), 71.6 (C-7), 71.7 (C-5), 72.9 (C-6), 74.0 (C-8); LRFAB mass spectrum: 355 [(^{81}BrM + Na)$^+$, 22%], 353 [(^{79}BrM + Na)$^+$, 20%], 313 (11), 311 (12), 223 (35), 221 (32).

*Methyl 2,4,5,7,8-penta-*O*-acetyl-9-azido-3,9-dideoxy-*D*-glycero-*β*-*D*-galacto-2-nonulopyranosonate* (**15**).—To a solution of **13** (111 mg, 0.38 mmol) in anhydrous MeOH (2 mL) was added Dowex (H^+) resin (102 mg) [29], and the resultant mixture was stirred at room temperature for 2 days under nitrogen and filtered. The filtrate was concentrated, and the residue was dissolved in water and lyophilised to give a colourless foam. To the foam in KOH–dried pyridine (1.3 mL) was added Ac_2O (1.4 mL) and DMAP (10 mg) at 0 °C under nitrogen. The resultant mixture was stirred at that temperature for 4 h, then at room temperature for 48 h [30]. It was then concentrated and chromatographed (3:7 EtOAc–hexane) to give the title compound **15** (138 mg, 70%) as a colourless foam: mp 40–42 °C; $[\alpha]_D^{28}$ −19.3° (*c* 1.53, $CHCl_3$); IR (KBr): ν_{max} 2968, 2104 (N_3), 1752 (C=O), 1632, 1438, 1372, 1110 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 1.96, 1.97, 2.04, 2.09, 2.13 (s, 15 H, 5 × OC(O)CH_3), 2.56 (dd, 1 H, $J_{3e,4}$ 5.3, $J_{3e,3a}$ 13.5 Hz, H-3e), 3.32 (dd, 1 H, $J_{9a,8}$ 7.2, $J_{9a,9b}$ 13.6 Hz, H-9a), 3.74 (dd, 1 H, $J_{9b,8}$ 2.8 Hz, H-9b), 3.75 (s, 3 H, OCH_3), 4.11 (dd, 1 H, $J_{6,7}$ 2.3, $J_{6,5}$ 10.2 Hz, H-6), 4.87–4.97 (m, 2 H, H-5, H-8), 5.22 (ddd, 1 H, $J_{4,5}$ 9.7, $J_{4,3a}$ 14.9 Hz, H-4), 5.35 (dd, 1 H, $J_{7,8}$ 4.3 Hz, H-7); ^{13}C NMR ($CDCl_3$): δ 20.6 (5 × OC(O)CH_3), 35.6 (C-3), 50.1 (C-9), 53.1 (OCH_3), 67.1 (C-4), 67.6 (C-7), 68.5 (C-5), 71.8 (C-6), 72.3 (C-8), 96.9 (C-2), 166.0 (C-1), 168.2 (OC(O)CH_3), 170.0 (4 × OC(O)CH_3); LRFAB mass spectrum: 458 {[M − OC(O)CH_3]$^+$, 27%}, 328 (40), 268 (100), 226 (48), 208 (86), 155 (53). Anal. Calcd for $C_{20}H_{27}N_3O_{13}$: C, 46.42; H, 5.26; N, 8.12. Found: C, 46.51; H, 5.30; N, 8.15; ^{1}H NMR assignments were confirmed by DQF-COSY experiment.

In a similar manner was prepared methyl 2,4,5,7,8-penta-*O*-acetyl-9-bromo-3,9-dideoxy-D-*glycero*-*β*-D-*galacto*-2-nonulopyranosonate (**16**) from **14** in 32% (28 mg) as a white foam: mp 137–140 °C; $[\alpha]_D^{25}$ −11.2° (*c* 0.50, $CHCl_3$); IR (KBr): ν_{max} 1746 (C=O), 1432, 1370, 1278, 1168, 1114 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.00, 2.01, 2.08, 2.13, 2.17 (s, 15 H, 5 × OC(O)CH_3), 2.60 (dd, 1 H, $J_{3e,4}$ 5.2, $J_{3e,3a}$ 13.5 Hz, H-3e), 3.36 (dd, 1 H, $J_{9a,8}$ 7.4, $J_{9a,9b}$ 11.5 Hz, H-9a), 3.79 (s, 3 H, OCH_3) 3.95 (dd, 1 H, $J_{9b,8}$ 2.5 Hz, H-9b), 4.15 (dd, 1 H, $J_{6,7}$ 2.3, $J_{6,5}$ 10.2 Hz, H-6), 4.96 (t, 1 H, H-5), 5.03–5.08 (m, 1 H, H-8), 5.23 (ddd, 1 H, $J_{4,5}$ 9.6, $J_{4,3a}$ 14.9 Hz, H-4), 5.39 (dd, 1 H, $J_{7,8}$ 4.8 Hz, H-7); ^{13}C NMR ($CDCl_3$): δ 20.7 [5 × OC(O)CH_3], 30.4 (C-3), 35.7 (C-9), 53.2 (OCH_3), 67.3 (C-4), 68.2 (C-7), 68.6 (C-5), 71.7 (C-6), 72.2 (C-8), 97.1 (C-2), 166.1 (C-1), 168.2, 169.7, 169.8, 169.9, 170.0 (5 × OC(O)CH_3); LRFAB mass spectrum: 497 {[^{81}BrM − OC(O)CH_3]$^+$, 9%}, 495 {[^{79}BrM − OC(O)CH_3]$^+$, 8}, 437 (24), 435 (23), 275 (12%), 273 (16), 215 (66). Anal. Calcd for $C_{20}H_{27}BrO_{13}$: C, 43.26; H, 4.90; Br, 14.39. Found C, 43.14; H, 4.81; Br, 14.41; ^{1}H NMR assignments were confirmed by DQF-COSY experiment.

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