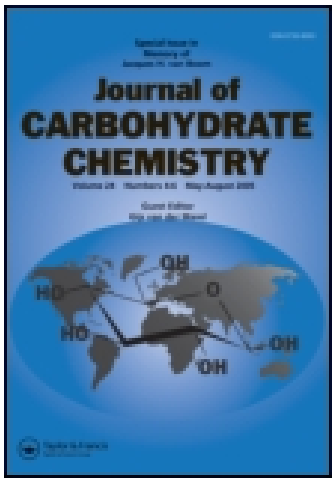


Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/lcar20

THE SYNTHESIS OF C-9 MODIFIED DERIVATIVES OF THE α-METHYL GLYCOSIDE OF KDN METHYL ESTER

Adele K. Norton [a] , Gaik B. Kok [a] & Mark von Itzstein [b]
[a] Department of Medicinal Chemistry , Monash University , 381 Royal Parade, Parkville, Victoria, 3052, Australia
[b] Griffith University (Gold Coast Campus) , Centre for Biomolecular Science and Drug Discovery, 50 Gold Coast Mail Centre, Queensland, 9726, Australia
Published online: 16 Aug 2006.

To cite this article: Adele K. Norton , Gaik B. Kok & Mark von Itzstein (2001) THE SYNTHESIS OF C-9 MODIFIED DERIVATIVES OF THE α-METHYL GLYCOSIDE OF KDN METHYL ESTER, Journal of Carbohydrate Chemistry, 20:2, 227-238, DOI: 10.1081/CAR-100103960

To link to this article: http://dx.doi.org/10.1081/CAR-100103960

J. CARBOHYDRATE CHEMISTRY, 20(2), 227–238 (2001)

THE SYNTHESIS OF C-9 MODIFIED DERIVATIVES OF THE α-METHYL GLYCOSIDE OF KDN METHYL ESTER

Adele K. Norton,[1] Gaik B. Kok,[1] and Mark von Itzstein[1,2,*]

[1]Department of Medicinal Chemistry, Monash University,
381 Royal Parade, Parkville 3052, Victoria, Australia
[2]Centre for Biomolecular Science and Drug Discovery,
Griffith University (Gold Coast Campus),
50 Gold Coast Mail Centre, Queensland 9726, Australia

ABSTRACT

The synthesis of a range of C-9 substituted derivatives of the α-methyl glycoside of KDN methyl ester (2) is reported. 9-Deoxy-9-iodo, 9-deoxy-9-thioacetyl and 9-azido-9-deoxy derivatives (compounds 10, 12 and 13) were all prepared from the corresponding 9-*O*-tosylate 8. Catalytic hydrogenation of 10 and 13 results in reduction to give the 9-deoxy and 9-amino-9-deoxy derivatives 20 and 15, respectively. Further derivatisation of the amine 15 into compounds 17 and 18 was achieved via standard acylation procedures.

INTRODUCTION

Sialic acids are a family of more than 40 different sugars which have a 3-deoxy-2-nonulosonic acid structure.[1] The most commonly occurring sialic acids are *N*-acetylneuraminic acid (Neu5Ac) and *N*-glycolylneuraminic acid (Neu5Gc) with structural diversity arising from substitutions (e.g., *O*-acetylation, *O*-methylation and *O*-sulfonylation) of the various hydroxyl groups of these systems. As terminal

*Corresponding author. E-mail: m.vonitzstein@mailbox.gu.edu.au

carbohydrate units of many glycoproteins and glycolipids, sialic acids take part in a myriad of biological functions including molecular recognition, cell adhesion and inflammation.[1] In 1986, 3-deoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid (KDN, **1**), a unique C-5 deaminated member of the sialic acid family, was discovered from the membrane polysialoglycoproteins (PSGP) of *Salmo gaidneri* (rainbow trout) eggs.[2] Studies have since shown that only sialic acid residues with an acylamino group at C-5 are cleaved by bacterial sialidases.[3] Therefore, it is likely that as a terminal unit of the PSGP chains, KDN protects the membrane from bacterial degradation. Subsequently, the natural presence of KDN has been reported for a wide variety of organisms ranging from bacteria and lower vertebrates to higher vertebrates including mammals,[4–6] suggesting its wide occurrence in nature.

Recently, Li and coworkers reported new sialidases that have unique roles in KDN metabolism. For example, the deaminoneuraminic acid residue-cleaving enzyme, KDNase, specifically hydrolyses the ketosidic linkages of KDN but not *N*-acylneuraminyl linkages and was first isolated from the liver of the loach (*Misgurunus fossilis*).[7] The KDN-cleaving enzyme designated KDNase Sm has been induced and purified from *Sphingobacterium multivorum*.[8] Moreover this protein has been shown[9] to be a retaining enzyme not unlike the sialidases of bacterial and viral origin.

A large amount of work has been directed towards the synthesis of analogues and derivatives of naturally occurring sialic acids both for their use as probes for the study of sialic acid recognising proteins, and for investigating substrate specificities of the enzymes involved in sialic acid metabolism.[10,11] Further study of the biological roles of KDN requires the synthesis of more complex analogues and derivatives of **1**. We report here the synthesis of some C-9 modified analogues of the α-methyl glycoside of KDN methyl ester **2** that may be useful as chemical probes to elucidate the sub-structural requirements for substrate/inhibitor recognition by the enzymes involved in KDN metabolism.

RESULTS AND DISCUSSION

As KDN-bearing glycoconjugates are α-linked, we have chosen the α-methyl glycoside of KDN methyl ester **2** as the key starting material for this study. The synthesis of this compound has previously been reported in the literature. Hence, treatment[12] of the methyl ester of KDN **3**, using Fischer's methyl glycosidation method under controlled conditions (70°C for 15 h), resulted in a mixture of both α and β-methyl glycosides (**2** and **4**) of which the latter was the major product (74%). The α-glycoside **2** was obtained in only 4% yield. Given that there is precedent in the literature for the stereoselective synthesis of the α-methyl glycoside of Neu5Ac methyl ester from the 2-β-chloride, methyl 5-acetamido-4,7,8,9-tetra-*O*-acetyl-2-chloro-2,3,5-trideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate (**5**),[13] we thought it reasonable to explore this methodology for the synthesis of **2**. Accordingly, exposure of the methyl ester of KDN **3** to acetyl chloride gave the 2-β-chloride, methyl 4,5,7,8,9-penta-*O*-acetyl-2-chloro-2,3-dideoxy-D-*glycero*-β-D-

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

galacto-2-nonulopyranosonate (**6**). Subsequent treatment of the 2-β-chloride (**6**) with methanol at room temperature for one hour led to the formation of **7** in good yield (87%, 2 steps). The α-methyl glycoside **2** was obtained as the only product following de-*O*-acetylation of **7** under standard Zemplen conditions. Both compounds **2** and **7** were found to be identical to known materials.[12]

The 9-deoxy-9-iodo, 9-azido-9-deoxy and 9-deoxy-thioacetyl derivatives of **2** can be accessed via nucleophilic displacement of a tosyl group. Selective tosylation[14] and tritylation[15] of *N*-acetylneuraminic acid derivatives has been previously reported. Selective tosylation at C-9 of the KDN derivative **2** was achieved in high yield (96%) by the reaction with *p*-toluenesulfonyl chloride initially at 0°C (2.5 h) and then at 5°C (20 h) to produce the 9-*O*-tosylate **8**, which was fully characterised as the peracetate **9**. Reaction of **8** with sodium iodide in acetone under reflux proceeded uneventfully to produce iodide **10** in high yield (96%). The appearance of a resonance at δ 15.9 ppm for C-9 in the ^{13}C NMR spectrum is consistent with substitution of an iodo moiety at this position.[16] The iodide **10** was further characterised as the peracetate **11**. Introduction of sulfur at C-9 via the reaction of **8** with potassium thioacetate in acetone proceeded with some concomitant de-*O*-acetylation, as was evident by TLC analysis of the crude reaction mixture. Therefore, for ease of workup, the crude reaction mixture was acetylated and then purified by chromatography which afforded the peracetate **12** in good yield (66%, 2 steps).

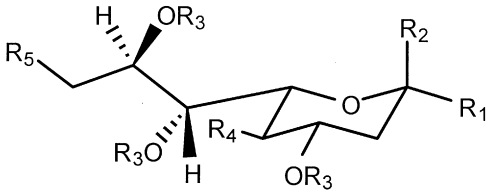
The introduction of a nitrogen functionality at C-9 via the azide was achieved by treatment of the 9-*O*-tosylate **8** with lithium azide in dimethylformamide at 50°C, resulting in the formation of the 9-azide **13** in high yield (91%). An attempt to synthesise this compound directly from the methyl ketoside of KDN methyl ester **2** using well-known Mitsunobu chemistry (TPP, DIAD, $TMSN_3$, DMF) also gave **13**, albeit in moderate yield (54%). This is in comparison to an overall yield of 87% for the two-step procedure via the 9-*O*-tosylate **8**. The 9-azide **13** was further characterised as the peracetate **14**.

The introduction of an azido moiety functionally allowed access to a range of other nitrogen-substituted compounds for structure-activity relationship studies. Hence, hydrogenation of the 9-azide **13** in the presence of 10% palladium on carbon in MeOH produced the 9-amine **15** in quantitative yield. This compound was fully characterised as the peracetate **16** that was then de-*O*-acetylated to produce the 9-acetamide **17** in good yield (72%). Acylation of the amine **15** with benzoyl chloride in the presence of triethylamine provided the 9-benzamido-9-deoxy compound **18** in 68% yield. This compound was further characterised as the peracetate **19**.

Finally, under hydrogenolysis conditions (50 psi H_2, 10% Pd/C), the 9-iodide **10** was converted into the corresponding 9-deoxy compound **20** in quantitative yield. Further characterisation of this compound involved converting it to the peracetate **21**.

In conclusion, we have synthesised a range of novel C-9 substituted derivatives of the methyl ketoside of KDN methyl ester. These systems should be useful as probes for the study of enzymes involved in KDN/sialic acid metabolism.

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016



Compound	R_1	R_2	R_3	R_4	R_5
1	COOH	OH	H	OH	OH
2	OMe	COOMe	H	OH	OH
3	COOMe	OH	H	OH	OH
4	COOMe	OMe	H	OH	OH
5	COOMe	Cl	Ac	NHAc	OAc
6	COOMe	Cl	Ac	OAc	OAc
7	OMe	COOMe	Ac	OAc	OAc
8	OMe	COOMe	H	OH	OTs
9	OMe	COOMe	Ac	OAc	OTs
10	OMe	COOMe	H	OH	I
11	OMe	COOMe	Ac	OAc	I
12	OMe	COOMe	Ac	OAc	SAc
13	OMe	COOMe	H	OH	N_3
14	OMe	COOMe	Ac	OAc	N_3
15	OMe	COOMe	H	OH	NH_2
16	OMe	COOMe	Ac	OAc	NHAc
17	OMe	COOMe	H	OH	NHAc
18	OMe	COOMe	H	OH	NHBz
19	OMe	COOMe	Ac	OAc	NHBz
20	OMe	COOMe	H	OH	H
21	OMe	COOMe	Ac	OAc	H

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

EXPERIMENTAL

General Methods. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) spectra (in δ ppm) were recorded on a Bruker AMX300 spectrometer and were referenced using solvent residues; *J*-values are in hertz (Hz). Both low resolution (LR) and high resolution (HR) fast atom bombardment (FAB) mass spectra were obtained using a JEOL JMS-DX 300 spectrometer. Optical rotations were measured using a JASCO DIP-370 polarimeter with a path length 50 mm; $[\alpha]_D$-values are in units of 10^{-1} deg cm^2 g^{-1}; concentrations are quoted in 10^{-2} deg cm^{-3}. IR spectra were recorded using a Hitachi 270-30 infrared spectrophotometer. Column chromatography was performed on Merck silica gel 60 (0.040–0.063 mm). Reactions were monitored by TLC on Kieselgel 60 F_{254} plate (Merck 5554) and spots were detected under ultraviolet (UV) irradiation and by spraying with a 95% aq. ethanol solution containing 5% H_2SO_4 and charring at ca. 180°C. Microanalyses were performed by the Chemical and Microanalytical Service, Essendon, Victoria. KDN was prepared by aldolase (EC 4.1.3.3) condensation of D-mannose and pyruvate according to the literature.[17] Methanolysis of **1** and treatment of the resulting methyl ester **3** with neat acetyl chloride (rt for 2.5 days) afforded the 2-β-chloride, methyl 4,5,7,8,9-penta-*O*-acetyl-2-chloro-2,3-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate (**6**) (95%) which was used in subsequent step without further purification. Spectral data for 2-β-chloride (**6**) were consistent with the literature.[18]

General Procedure for Acetylation. Approximately 50–100 mg of the compound to be acetylated was dissolved in pyridine (1 mL) and the solution treated with Ac_2O (0.5 mL) and DMAP (ca. 5 mg) at rt for 16 h. The mixture was then concentrated to dryness and the residue chromatographed on silica using the solvent(s) specified.

Methyl (Methyl 3-Deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (2). A solution of the crude 2-β-chloride, methyl 4,5,7,8,9-penta-*O*-acetyl-2-chloro-2,3-dideoxy-D-*glycero*-β-D-*galacto*-2-nonulopyranosonate (**6**) (7.14 g, 13.98 mmol) in anhydrous methanol was stirred at rt for 1 h. The reaction mixture was then concentrated under reduced pressure which gave methyl (methyl 4,5,7,8,9-penta-*O*-acetyl-3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (**7**) as a colourless foam, 6.17 g (87% from **3**). Subsequent Zemplen de-*O*-acetylation of the peracetate **7** (5.96 g, 11.77 mmol) under standard conditions (NaOMe/MeOH) furnished the title compound **2** as a colourless foam, 2.07 g (59%).

7: mp 112–116°C (lit.[12] 115–116°C); ^{1}H NMR ($CDCl_3$) data were consistent with the literature[12]; ^{13}C NMR ($CDCl_3$) δ 20.6, 20.8, 21.0 ($OCOCH_3$ × 5), 37.4 (C-3), 52.5, 52.7 (OCH_3, $COOCH_3$), 62.2 (C-9), 66.7, 67.8, 67.9, 69.3, 71.2 (C-4, C-5, C-6, C-7, C-8), 98.9 (C-2), 167.7, 169.8, 170.1, 170.6 (carbonyls); LRMS *m/z*: 507 (MH^+, 2%), 474 (25), 446 (60), 414 (100); IR (υ_{max}, KBr): 1750, 1736, 1370, 1232, 1066 cm^{-1}.

2: ^{1}H NMR (D_2O) and IR data were consistent with the literature[12]; ^{13}C NMR (D_2O) δ 41.0 (C-3), 54.3 (OCH_3), 56.0 ($COOCH_3$), 65.8 (C-9), 70.7, 71.8, 72.3,

73.6, 76.7 (C-4, C-5, C-6, C-7, C-8), 101.9 (C-2), 172.7 (carbonyl); LRMS *m/z*: 297 (MH^+, 3%), 265 (100), 229 (50), 169 (33), 144 (23).

Methyl (Methyl 3-Deoxy-9-*O*-*p*-toluenesulfonyl-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (8). To a stirred solution of **2** (427 mg, 1.44 mmol) in pyridine (9 mL) at 0°C, was added *p*-TsCl (426 mg, 2.23 mmol). After stirring at the same temperature for 2.5 h, additional *p*-TsCl (82 mg, 0.43 mmol) was added and stirring maintained for a further 20 h at 5°C. The reaction mixture was allowed to warm up to rt before MeOH (9 mL) was added. After 1 h, the volatiles were removed under reduced pressure and the residue purified by chromatography (EtOAc) which provided the 9-*O*-tosylate 8 as a colourless solid (620 mg; 96%): $[\alpha]_D$ −13.2° (*c* 3.25, $CHCl_3$); ^{1}H NMR (D_2O) δ 1.63 (1 H, dd, $J_{3ax,3eq}$ 12.3, $J_{3ax,4}$ 12.9, H-3^{ax}), 2.35 (3 H, s, PhCH_3), 2.52 (1 H, dd, $J_{3eq,4}$ 4.5, H-3^{eq}), 3.10 (3H, s, OCH_3), 3.40 (1 H, dd, $J_{5,4}$ 9.2, $J_{5,6}$ 9.7, H-5), 3.50 (1 H, m, H-4), 3.56 (1 H, pseudo d, H-6), 3.76 (3 H, s, $COOCH_3$),. 3.91 (2 H, m, $J_{8,9'}$ 6.8, H-7, H-8), 4.16 (1 H, dd, $J_{9,9'}$ 10.4, H-9), 4.34 (1 H, pseudo d, H-9′), 7.39–7.75 (4 H, m; Ph); ^{13}C NMR (D_2O) δ 23.4 (Ph-CH_3), 41.2 (C-3), 54.3, 55.9 (OCH_3, $COOCH_3$), 70.7, 71.7, 71.9, 72.2, 76.8 (C-4, C-5, C-6, C-7, C-8), 75.3 (C-9), 102.0 (C-2), 130.5, 132.9, 138.1, 149.2 (aromatic carbons), 172.5 (carbonyl); LRMS *m/z*: 451 (MH^+, 3%), 418 (77), 382 (42), 351 (23), 211 (25), 185 (26), 173 (29) no base peak was observed; IR (υ_{max}, KBr): 3480, 2956, 1742, 1588, 1452, 1356, 1290, 1172, 1094, 974 cm^{-1}.

Methyl (Methyl 4,5,7,8-Tetra-*O*-acetyl-3-deoxy-9-*O*-*p*-toluenesulfonyl-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (9). Acetylation of **8** (30 mg, 0.07 mmol) according to the general conditions gave, after chromatography (EtOAc/Hex, 1:1), 9 as a colourless solid (39 mg; 96%): $[\alpha]_D$ −10.4° (*c* 6.12, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.87 (1 H, dd, $J_{3ax,3eq}$ 13.0, $J_{3ax,4}$ 12.4, H-3^{ax}), 1.99 (6 H, s, OAc × 2), 2.03, 2.09 (each 3 H, s, OAc × 2), 2.44 (3 H, s, PhCH_3), 2.63 (1 H, dd, $J_{3eq,4}$ 4.5, H-3^{eq}), 3.26 (3H, s, OCH_3), 3.81 (3 H, s, $COOCH_3$),. 4.05 (1 H, dd, $J_{9,8}$ 3.9, $J_{9,9'}$ 12.1, H-9), 4.09 (1 H, dd, $J_{6,5}$ 9.8, $J_{6,7}$ 3.0, H-6), 4.32 (1 H, dd, $J_{9',8}$ 2.2, H-9′), 4.80 (1 H, pseudo t, $J_{5,4}$ 9.5, H-5), 4.90 (1 H, m, H-4), 5.37 (2 H, m, $J_{7,8}$ 8.3, H-7, H-8), 7.33–7.77 (4 H, m, Ph); ^{13}C NMR ($CDCl_3$) δ 20.5, 20.7, 20.8, 20.9 ($OCOCH_3$ × 4), 21.6 (PhCH_3), 37.3 (C-3), 52.4, 52.8 (OCH_3, $COOCH_3$), 66.6, 67.4, 67.8, 69.3, 71.3 (C-4, C-5, C-6, C-7, C-8), 67.4 (C-9), 98.9 (C-2), 128.0, 129.8, 132.8, 144.9 (aromatic carbons), 167.6, 169.5, 170.0, 170.6 (carbonyls); LRMS *m/z*: 610 (MH^+, 2%), 559 (29), 527 (100), ; IR (υ_{max}, KBr): 3508, 1748, 1598, 1444, 1368, 1218 cm^{-1}.

Anal. Calcd for $C_{26}H_{34}O_{15}S$: C, 50.48; H, 5.54. Found: C 50.49; H 5.60.

Methyl (Methyl 3,9-Dideoxy-9-iodo-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (10). Sodium iodide (259 mg, 1.73 mmol) was added to a solution of **8** (259 mg, 0.58 mmol) in acetone (25 mL) and the mixture heated under reflux for 2.5 days. After cooling and removal of volatiles under reduced pressure, flash chromatography (EtOAc) gave the 9-iodide **10** as an amorphous mass (255 mg;

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016

96%): $[\alpha]_D$ –10.7° (*c* 6.26, $CHCl_3$); ^1H NMR (D_2O) δ 1.78 (1 H, dd, $J_{3ax,3eq}$ 12.6, $J_{3ax,4}$ 12.4, H-3^{ax}), 2.66 (1 H, dd, $J_{3eq,4}$ 4.6, H-3^{eq}), 3.40 (3H, s, OCH_3), 3.55 (2 H, m, H-4, H-5), 3.69 (3 H, m, H-6, H-7, H-8), 3.83 (2 H, m, H-9, H-9′), 3.92 (3 H, s, $COOCH_3$); ^{13}C NMR (D_2O) δ 15.9 (C-9), 41.0 (C-3), 54.3, 56.0 (OCH_3, $COOCH_3$), 71.7, 71.8, 72.4, 73.4, 76.6 (C-4, C-5, C-6, C-7, C-8), 101.9 (C-2), 172.6 (carbonyl); LRMS *m/z*: 407 (MH^+, 2%), 375 (100), 339 (47); IR (υ_{max}, KBr): 3448, 1734, 1632, 1438, 1290, 1202, 1070, 1036 cm^{-1}.

Methyl (Methyl 4,5,7,8-Tetra-*O*-acetyl-3,9-dideoxy-9-iodo-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (11). Acetylation of **10** (121 mg, 0.30 mmol) according to the general conditions gave, after chromatography (EtOAc/Hex, 1:1), 11 as a colourless solid (148 mg; 86%): $[\alpha]_D$ –10.5° (*c* 7.54, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.91 (1 H, dd, $J_{3ax,3eq}$ 12.6, $J_{3ax,4}$ 12.2, H-3^{ax}), 2.01 (6 H, s, OAc × 2), 2.14, 2.19 (each 3 H, s, OAc × 2), 2.66 (1 H, dd, $J_{3eq,4}$ 4.1, H-3^{eq}), 3.21 (1 H, dd, $J_{9,9'}$ 11.2, $J_{9,8}$ 6.2, H-9), 3.34 (3 H, s, OCH_3), 3.58 (1 H, dd, $J_{9',8}$ 3.5, H-9′), 3.84 (3 H, s, $COOCH_3$), 4.18 (1 H, pseudo d, H-6), 4.86 (1 H, pseudo t, $J_{5,4}$ 9.3, H-5), 4.93 (1 H, m, H-4), 5.16 (1 H, m, $J_{8,7}$ 7.5, H-8), 5.28 (1 H, m, H-7); ^{13}C NMR ($CDCl_3$) δ 3.8 (C-9), 20.6, 20.7, 21.0 ($OCOCH_3$ × 4), 37.3 (C-3), 52.4, 52.8 (OCH_3, $COOCH_3$), 67.9, 69.2, 69.3, 69.6, 71.5 (C-4, C-5, C-6, C-7, C-8), 99.0 (C-2), 167.7, 169.8, 169.9 (carbonyls); LRMS *m/z*: 515 (MH^+ – OAc, 46%), 483 (100), 357 (34), 321 (22); IR (υ_{max}, KBr): 1750, 1436, 1370, 1218, 1144, 1088, 1056 cm^{-1}.

Anal. Calcd for $C_{19}H_{27}O_{12}I$: C, 39.74; H, 4.74. Found: C, 39.85; H 4.75.

Methyl (Methyl 4,5,7,8-Tetra-*O*-acetyl- 3,9-dideoxy-9-thioacetyl-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (12). A mixture of the 9-*O*-tosylate **8** (99 mg, 0.22 mmol), KSAc (30 mg, 0.40 mmol) and acetone (20 mL) was stirred at rt for 36 h. A further portion of KSAc (60 mg; 0.79 mmol) was added and the mixture stirred for 5 days. The mixture was filtered and the filtrate concentrated. The resulting residue was acetylated according to the general conditions. After chromatography (EtOAc/Hex, 1:1), the title compound **12** was obtained as a colourless foam (76 mg; 66%): $[\alpha]_D$ +8.2° (*c* 4.13, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.86 (1 H, dd, $J_{3ax,3eq}$ 12.9, $J_{3ax,4}$ 12.5, H-3^{ax}), 1.96, 1.97, 2.08, 2.10 (each 3 H, s, OAc × 4), 2.28 (3 H, s, $SCOCH_3$), 2.60 (1 H, dd, $J_{3eq,4}$ 4.4, H-3^{eq}), 2.93 (1 H, dd, $J_{9,9'}$ 14.6, $J_{9,8}$ 5.7, H-9), 3.29 (3H, s, OCH_3), 3.42 (1 H, dd, $J_{9',8}$ 3.6, H-9′), 3.79 (3 H, s, $COOCH_3$), 4.14 (1 H, dd, $J_{6,5}$ 10.0, $J_{6,7}$ 2.1, H-6), 4.82 (1 H, pseudo t, $J_{5,4}$ 9.5, H-5), 4.86 (1 H, m, H-4), 5.25 (2 H, m, $J_{7,8}$ 8.8, H-7) 5.40 (1 H, m, H-8); ^{13}C NMR ($CDCl_3$) δ 20.8, 20.9, 21.0, 21.2 ($OCOCH_3$ × 4), 29.9 (C-9), 30.5 ($SCOCH_3$), 37.6 (C-3), 52.7, 52.9 (OCH_3, $COOCH_3$), 68.2, 68.3, 68.4, 69.5, 71.5 (C-4, C-5, C-6, C-7, C-8), 99.1 (C-2), 167.9, 170.0, 170.1, 170.2, 170.3, 194.7 (carbonyls); LRMS *m/z*: 523 (MH^+, 6%), 495 (31), 463 (31), 431 (100), 389 (54); IR (υ_{max}, KBr): 3668, 2964, 1754, 1698, 1436, 1370, 1220, 1108, 1088, 1054 cm^{-1}.

Anal. Calcd for $C_{21}H_{30}O_{13}S$: C, 48.27; H, 5.79. Found: C, 48.52; H 5.95.

Methyl (Methyl 9-Azido-3,9-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (13).

Procedure A. LiN_3 (0.85 g, 13.25 mmol) was added to a solution of the 9-tosylate **8** (1.99 g, 4.42 mmol) in DMF (50 mL). After stirring at 50°C for 3 days, the reaction mixture was cooled and then concentrated under reduced pressure. The resulting residue was subjected to column chromatography (CH_2Cl_2/acetone, 1:1) which furnished **13** as a colourless amorphous mass (1.30 g; 91%).

Procedure B. A solution of triphenylphosphine (207 mg, 0.79 mmol) in DMF (2 mL) was cooled to 0°C prior to the addition of DIAD (0.16 mL, 0.81 mmol). The temperature was maintained at 0°C for 15 min before azidotrimethylsilane (0.06 mL, 0.45 mmol) was added dropwise. The resulting solution was allowed to warm to rt over 2 h and stirred for 1 h. The solution was then re-cooled to 0°C and a solution of methyl (methyl 3-deoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (**2**) (117 mg, 0.40 mmol) in DMF (4 mL) added. The resulting solution was slowly warmed to rt and stirred at rt for 3 days. The residue obtained after solvent removal was purified by chromatography as described above to give **13** (69 mg; 54%): $[\alpha]_D$ –21.0° (*c* 3.28, $CHCl_3$); ^{1}H NMR (D_2O) δ 1.83 (1 H, dd, $J_{3ax,3eq}$ 12.8, $J_{3ax,4}$ 12.4, H-3^{ax}), 2.70 (1 H, dd, $J_{3eq,4}$ 4.5, H-3^{eq}), 3.44 (3H, s, OCH_3), 3.59 (2 H, m, H-9, H-9′), 3.72 (2 H, m, H-4, H-5), 3.83 (1 H, m, H-7), 3.96 (4 H, m, H-6, $COOCH_3$), 4.10 (1 H, m, H-8); ^{13}C NMR (D_2O) δ 46.2 (C-3), 59.5, 61.1 (OCH_3, $COOCH_3$), 71.9 (C-9), 76.4, 77.0, 77.5, 77.6, 81.8 (C-4, C-5, C-6, C-7, C-8), 107.1 (C-2), 177.8 (carbonyl); LRMS *m/z*: 322 (MH^+, 8%), 290 (100); IR (υ_{max}, KBr): 3460, 2932, 2856, 2104, 1738, 1444, 1382, 1288, 1198, 1066, 1032 cm^{-1}.

Methyl (Methyl 4,5,7,8-Tetra-*O*-acetyl-9-azido-3,9-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (14). Acetylation of the azide **13** (55 mg; 0.17 mmol) according to the general conditions provided, after chromatography (EtOAc/Hex, 1:1), **14** as a colourless amorphous mass (80 mg; 95%): $[\alpha]_D$ –5.0° (*c* 2.09, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.87 (1 H, dd, $J_{3ax,3eq}$ 13.3, $J_{3ax,4}$ 11.4, H-3^{ax}), 1.96, 1.97, 2.09, 2.15 (each 3 H, s, OAc × 4), 2.62 (1 H, dd, $J_{3eq,4}$ 4.0, H-3^{eq}), 3.24 (1 H, dd, $J_{9,9'}$ 13.9, $J_{9,8}$ 4.8, H-9), 3.28 (3H, s, OCH_3), 3.57 (1 H, dd, $J_{9',8}$ 2.2, H-9′), 3.79 (3 H, s, $COOCH_3$), 4.14 (1 H, dd, $J_{6,5}$ 8.8, $J_{6,7}$ 1.3, H-6), 4.83 (1 H, pseudo t, $J_{5,4}$ 9.7, H-5), 4.86 (1 H, m, H-4), 5.32 (2 H, m, $J_{8,7}$ 7.2, H-7, H-8); ^{13}C NMR ($CDCl_3$) δ 20.7, 20.9, 21.1 ($OCOCH_3$ × 4), 37.5 (C-3), 51.0 (C-9), 52.6, 52.9 (OCH_3, $COOCH_3$), 67.6, 68.0, 69.2, 69.3, 71.6 (C-4, C-5, C-6, C-7, C-8), 99.1 (C-2), 167.8, 170 (carbonyls); LRMS *m/z*: 490 (MH^+, 2%), 464 (42), 430 (43), 328 (76), 268 (100); IR (υ_{max}, KBr): 2100, 1744, 1444, 1370, 1282, 1220, 1152, 1114, 1088, 1054, 1002 cm^{-1}.

Anal. Calcd for $C_{19}H_{27}O_{12}N_3$: C, 46.63; H, 5.56; N, 8.59. Found: C, 46.87; H 6.00; N, 8.21.

Methyl (Methyl 9-Amino-3,9-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (15). Treatment of a mixture of the 9-azide **13** (1.14 g, 3.53 mmol), glacial acetic acid (6 drops) and MeOH (40 mL) under hydrogenolysis

conditions (50 psi, 10% Pd/C, 3.5 days) gave, after chromatography (EtOAc/MeOH/H_2O, 25:15:1), the 9-amine **13** as a colourless amorphous mass (1.04 g; 99%): $[\alpha]_D$ –20.8° (*c* 5.72, MeOH); ^{1}H NMR (D_2O) 1.65 (1 H, dd, $J_{3ax,3eq}$ 12.8, $J_{3ax,4}$ 12.4, H-3^{ax}), 2.52 (1 H, dd, $J_{3eq,4}$ 4.6, H-3^{eq}), 2.97 (1 H, dd, $J_{9,9'}$ 13.2, $J_{9,8}$ 9.1, H-9), 3.25 (3 H, s, OCH_3), 3.33 (1 H, dd, $J_{9',8}$ 3.1, H-9′), 3.41 (1 H, pseudo t, $J_{5,4}$ 9.5, $J_{5,6}$ 9.5, H-5), 3.52 (2 H, m, $J_{7,8}$ 6.7, H-7, H-8), 3.65 (1 H, pseudo d, H-6), 3.77 (3 H, s, $COOCH_3$), 3.94 (1 H, m, H-4); ^{13}C NMR (D_2O) δ 41.0 (C-3), 45.2 (C-9), 54.4, 56.0 (OCH_3, $COOCH_3$), 70.9, 71.7, 72.2, 72.5, 76.7 (C-4, C-5, C-6, C-7, C-8), 102.0 (C-2), 175.2 (carbonyl); LRMS *m/z*: 296 (MH^+, 100%), 264 (42), 185 (35); IR (υ_{max}, KBr): 3368, 2948, 1740, 1570, 1412, 1342, 1290, 1198, 1068, 1034, 652 cm^{-1}. HRMS: Calcd for $C_{11}H_{21}NO_8$:[M^+ + 1], 296.1346. Found: 296.1342.

Methyl (Methyl 9-Acetamido-4,5,7,8-tetra-*O*-acetyl-3,9-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (16). Acetylation of the amine **15** (31 mg, 0.11 mmol) according to the general conditions gave, after chromatography (EtOAc), the 9-acetamide **16** as a colourless syrup (36 mg; 68%): $[\alpha]_D$ –17.7° (*c* 3.41, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.91 (1 H, dd, $J_{3ax,3eq}$ 12.9, $J_{3ax,4}$ 11.9, H-3^{ax}), 1.98, 2.01, 2.02, 2.15, 2.17 (each 3 H, s, OAc × 4, $NHCOCH_3$), 2.67 (1 H, dd, $J_{3eq,4}$ 4.4, H-3^{eq}), 2.99 (1 H, dd, $J_{9,8}$ 4.6, $J_{9,9'}$ 9.1, $J_{9,NH}$ 6.0, H-9), 3.33 (3 H, s, OCH_3), 3.82 (3 H, s, $COOCH_3$), 3.94 (1 H, ddd, $J_{9',8}$ 2.9, $J_{9',NH}$ 7.4, H-9′), 4.16 (1 H, dd, $J_{6,5}$ 9.9, $J_{6,7}$ 2.1, H-6), 4.93 (1 H, m, H-4), 4.98 (1 H, pseudo t, $J_{5,4}$ 10.5, H-5), 5.15 (1 H, dd, $J_{7,8}$ 9.5, H-7), 5.25 (1 H, m, H-8), 5.95 (1 H, br t, N*H*$COCH_3$); ^{13}C NMR ($CDCl_3$) δ 20.6, 20.7, 21.0 ($OCOCH_3$ × 4), 23.2 ($NHCOCH_3$), 37.4 (C-3), 38.5 (C-9), 52.4, 52.7 (OCH_3, $COOCH_3$), 67.2, 68.0, 68.3, 69.2, 70.9 (C-4, C-5, C-6, C-7, C-8), 98.8 (C-2), 169.8, 170.2, 171.2 (carbonyls); LRMS *m/z*: 506 (MH^+, 65%), 446 (37), 414 (100), 372 (62), 312 (31); IR (υ_{max}, KBr): 1752, 1678, 1438, 1370, 1220, 1112, 1088, 1052 cm^{-1}.

Anal. Calcd for $C_{21}H_{31}NO_{13}.CHCl_3$: C, 42.32; H, 5.13; N, 2.25. Found: C, 42.32; H, 5.17; N, 2.20.

Methyl (Methyl 9-Acetamido-3,9-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (17). Treatment of a solution of peracetylated 9-acetamide **16** (125 mg; 0.370 mmol) in MeOH under standard Zemplen de-*O*-acetylation conditions (NaOMe, 30 min) followed by conventional workup gave the title compound **17** as a colourless amorphous mass (60 mg; 72%): $[\alpha]_D$ –30.6° (*c* 6.03, MeOH); ^{1}H NMR (D_2O) 1.65 (1 H, dd, $J_{3ax,3eq}$ 12.8, $J_{3ax,4}$ 12.4, H-3^{ax}), 1.94 (3 H, s, $NHCOCH_3$), 2.52 (1 H, dd, $J_{3eq,4}$ 4.6, H-3^{eq}), 3.24 (1 H, dd, $J_{9,9'}$ 14.3, $J_{9,8}$ 7.3, H-9), 3.26 (3 H, s, OCH_3), 3.44 (1 H, dd, $J_{5,4}$ 9.3, $J_{5,6}$ 9.6, H-5), 3.55 (2 H, m, $J_{6,7}$ 0.8, $J_{9',8}$ 2.8, H-6, H-9′), 3.65 (1 H, dd, $J_{7,8}$ 6.0, H-7), 3.68 (1 H, m, H-8), 3.78 (3 H, s, $COOCH_3$), 3.83 (1 H, m, H-4); ^{13}C NMR (D_2O) δ 24.6 ($NHCOCH_3$), 41.0 (C-3), 45.3 (C-9), 54.4, 56.0 (OCH_3, $COOCH_3$), 71.7, 71.8, 72.1, 72.3, 76.7 (C-4, C-5, C-6, C-7, C-8), 102.0 (C-2), 171.3 (carbonyl); LRMS *m/z*: 338 (MH^+, 96%), 306 (100); IR (υ_{max}, KBr): 3348, 2948, 1738, 1638, 1562, 1438, 1374, 1292, 1198, 1072, 984, 880, 782, 650, 454 cm^{-1}. HRMS: Calcd for $C_{13}H_{23}NO_9$:[M^+ + 1], 338.1451. Found: 338.1443.

Methyl (Methyl 9-Benzamido-3,9-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (18). A solution of the 9-amine **15** (91 mg; 0.308 mmol) and freshly distilled Et_3N (1.2 mL) in MeOH (10 mL) was cooled to −70°C prior to the addition of benzoyl chloride (0.5 mL). After 35 min, the solution was warmed to rt and concentrated. Chromatography (EtOAc) gave the title compound **18** (84 mg; 68%): $[\alpha]_D$ –10.4° (*c* 7.08, MeOH); ^{1}H NMR (D_2O) 1.88 (1 H, dd, $J_{3ax,3eq}$ 12.9, $J_{3ax,4}$ 11.9, H-3^{ax}), 2.74 (1 H, dd, $J_{3eq,4}$ 3.5, H-3^{eq}), 2.97 (1 H, dd, $J_{9,8}$ 9.1, $J_{9,9'}$ 13.2, H-9), 3.48 (3 H, s, OCH_3), 3.72 (4 H, m, H-5, H-6, H-9, H-9′), 3.94 (2 H, m, H-7, H-8), 3.99 (3 H, s, $COOCH_3$), 4.17 (1 H, m, H-4), 7.66 – 7.73 (5 H, m, Ph); ^{13}C NMR (D_2O) δ 41.4 (C-3), 46.2 (C-9), 54.7, 56.3 (OCH_3, $COOCH_3$), 72.2, 72.3, 72.6, 72.7, 77.1 (C-4, C-5, C-6, C-7, C-8), 102.3 (C-2), 130.1, 131.8, 135.1, 136.6 (aromatic carbons), 173.0 (carbonyl); LRMS *m/z*: 400 (MH^+, 11%), 367 (79) no base peak was observed; IR (υ_{max}, KBr): 3420, 2948, 1732, 1632, 1576, 1544, 1480, 1450, 1290, 1196, 1070, 984, 780, 710, 656 cm^{-1}. HRMS: Calcd for $C_{18}H_{25}NO_9$:[M^+ + 1], 400.1608. Found: 400.1613.

Methyl (Methyl 4,5,7,8-Tetra-*O*-acetyl-9-benzamido-3,9-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (19). According to the general conditions, acetylation of **18** (84 mg, 0.21 mmol) gave, after chromatography (EtOAc), the title compound **19** as a colourless solid (71 mg; 92%): $[\alpha]_D$ +4.2° (*c* 4.35, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.91 (1 H, dd, $J_{3ax,3eq}$ 12.9, $J_{3ax,4}$ 12.1, H-3^{ax}), 2.01 (6 H, s, OAc × 2), 2.01, 2.15 (each 3 H, s, OAc × 2), 2.69 (1 H, dd, $J_{3eq,4}$ 4.6, H-3^{eq}), 3.12 (1 H, dd, $J_{9,8}$ 8.7, $J_{9,9'}$ 15.0, $J_{9,NH}$ 4.5, H-9), 3.33 (3 H, s, OCH_3), 3.81 (3 H, s, $COOCH_3$), 4.17 (1 H, dd, $J_{6,5}$ 9.7, $J_{6,7}$ 2.2, H-6), 4.24 (1 H, ddd, $J_{9',8}$ 2.9, $J_{9',NH}$ 7.5, H-9′), 4.92 (1 H, m, H-4), 4.98 (1 H, pseudo t, $J_{5,4}$ 9.6, H-5), 5.22 (1 H, dd, $J_{7,8}$ 9.7, H-7), 5.36 (1 H, m, H-8), 6.86 (1 H, br dd, NH), 7.46 – 7.79 (5 H, m, Ph); ^{13}C NMR ($CDCl_3$) δ 20.8, 20.9, 21.0, 21.3 ($OCOCH_3$ × 4), 37.7 (C-3), 39.2 (C-9), 52.6, 52.9 (OCH_3, $COOCH_3$), 67.8, 68.3, 68.6, 69.5, 71.2 (C-4, C-5, C-6, C-7, C-8), 99.1 (C-2), 127.1, 128.7, 131.6, 134.7 (aromatic carbons), 167.6, 167.8, 170.1, 170.7, 171.8 (carbonyls); LRMS *m/z*: 569 (MH^+, 20%), 507 (48), 475 (100); IR (υ_{max}, KBr): 1756, 1636, 1536, 1436, 1372, 1246, 1116, 1054, 990, 702 cm^{-1}.

Anal. Calcd for $C_{26}H_{33}NO_{13}$.1.5 H_2O: C, 52.51; H, 6.11; N, 2.35. Found: C, 52.38; H, 5.85; N, 2.05.

Methyl (Methyl 3,9-Dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (20). A solution of the 9-iodide **10** (23 mg, 0.058 mmol) and glacial acetic acid (2 drops) in MeOH (2 mL) was subjected to hydrogenolysis conditions (50 psi H_2, 10% Pd/C) until complete disappearance of starting material (as monitored by TLC; EtOAc/MeOH, 10:1). The solids were then filtered off and filtrate concentrated. The resulting residue was chromatographed (EtOAc/MeOH, 20:1) which gave the title compound **20** (16 mg; 99%): $[\alpha]_D$ –39.8° (*c* 2.82, $CHCl_3$); ^{1}H NMR (D_2O) δ 1.25 (3 H, d, $J_{9,8}$ 6.3, H-9 × 3), 1.69 (1 H, dd, $J_{3ax,3eq}$ 12.9, $J_{3ax,4}$ 12.4, H-3^{ax}), 2.57 (1 H, dd, $J_{3eq,4}$ 4.6, H-3^{eq}), 3.30 (3H, s, OCH_3), 3.50 (4 H, m, H-5, H-6, H-7, H-8), 3.82 (3 H, s, $COOCH_3$), 3.92 (1 H, m, H-4); ^{13}C NMR (D_2O) δ 25.0 (C-9), 44.4 (C-3), 57.7, 59.4 (OCH_3, $COOCH_3$), 73.4, 75.3, 75.9, 78.1, 80.2

(C-4, C-5, C-6, C-7, C-8), 105.3 (C-2), 176.1 (carbonyl); LRMS *m/z*: 281 (MH^+, 4%), 249 (100); IR (υ_{max}, KBr): 3508, 2944, 1736, 1644, 1448, 1290, 1200, 1078 cm^{-1}.

Methyl (Methyl 4,5,7,8-Tetra-*O*-acetyl-3,9-dideoxy-D-*glycero*-α-D-*galacto*-2-nonulopyranosid)onate (21). Acetylation of **20** (47 mg, 0.17 mmol) according to the general conditions gave, after chromatography (EtOAc/hexane, 1:1) **21** as a colourless solid (64 mg; 86%): $[\alpha]_D$ −17.2° (*c* 5.42, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.20 (3 H, d, $J_{9,8}$ 6.2, H-9 × 3), 1.88 (1 H, dd, $J_{3ax,3eq}$ 12.6, $J_{3ax,4}$ 11.9, H-3^{ax}), 1.99 (6 H, s, OAc × 2), 2.10, 2.11 (each 3 H, s, OAc × 2), 2.65 (1 H, dd, $J_{3eq,4}$ 3.6, H-3^{eq}), 3.31 (3 H, s, OCH_3), 3.81 (3 H, s, $COOCH_3$), 4.15 (1 H, dd, $J_{6,5}$ 8.2, $J_{6,7}$ 1.7, H-6), 4.91 (2 H, m, $J_{4,5}$ 8.3, H-4, H-5), 5.14 (1 H, dd, $J_{7,8}$ 8.7, H-7), 5.27 (1 H, m, H-8); ^{13}C NMR ($CDCl_3$) δ 16.6 (C-9), 20.6, 20.7, 21.4 ($OCOCH_3$ × 4), 37.4 (C-3), 52.3, 52.6 (OCH_3, $COOCH_3$), 66.8, 68.1, 69.3, 70.2, 71.1 (C-4, C-5, C-6, C-7, C-8), 98.8 (C-2), 170.0 (carbonyls); LRMS *m/z*: 449 (MH^+, 4%), 389 (37), 357 (100); IR (υ_{max}, KBr): 1750, 1370, 1236, 1092 cm^{-1}.

Anal. Calcd for $C_{19}H_{28}O_{12}$: C, 50.89; H, 6.29. Found: C, 50.70; H, 6.30.

ACKNOWLEDGMENTS

This work was supported by an Australian Postgraduate Award to AKN and by the Australian Research Council.

REFERENCES

1. Rosenberg, A., Ed. *Biology of the Sialic Acids*, Plenum Press: New York, 1995.
2. Nadano, D.; Iwasaki, M.; Endo, S.; Kitajima, K.; Inoue, S.; Inoue, Y. A naturally occurring deaminated neuraminic acid, 3-deoxy-D-*glycero*-D-*galacto*-nonulosonic acid (KDN). Its unique occurrence at the nonreducing ends of oligosialyl chains in polysialoglycoprotein of rainbow trout eggs. J. Biol. Chem. **1986**, *261*(25), 11550–11557.
3. Schreiner, E; Zbiral E. Structural variations of *N*-acetylneuraminic acid. 16. A convenient approach to 3-deoxy-D-*glycero*-D-*galacto*-nonulosonic acid (KDN), 5-azido-5-deoxy-KDN and 5-deoxy-KDN, and their 4-methylumbelliferyl 2α-glycosides. Liebigs Ann. Chem. **1990**, 581–586, and references cited therein.
4. Knirel, Y.A.; Shashkov, N.A.; Kochetkov, N.K.; Mamontova, V.A.; Soloveva, T.F. Structure of the capsular polysaccharide of *Klebsiella ozaenae* serotype K4 containing 3-deoxy-D-*glycero*-D-*galacto*-nonulosonic acid. Carbohydr. Res., **1989**, *188*, 145–155.
5. Strecker, G.; Wieruszeski, J.M.; Michalski, J.C.; Alonso, C.; Leroy, Y.; Boilly, B.; Montreuil, J. Primary structure of neutral and acidic oligosaccharide-alditols derived from the jelly coat of the Mexican axolotl. Occurrence of oligosaccharides with fucosyl(α1→3)fucosyl(α1→4)-3-deoxy-D-*glycero*-D-*galacto*-nonulosonic acid and galactosyl(α1→4)[fucosyl(α1→2)]galactosyl(β1→4)-*N*-acetylglucosamine sequences. Eur. J. Biochem., **1992**, *207*(3), 995–1002.

6. Ziak, M.; Qu, B.; Zuo, X.; Zuber, C.; Kanamori, A.; Kitajima, K.; Inoue, S.; Inoue, Y.; Roth, J. Occurrence of poly(α2,8-deaminoneuraminic acid) in mammalian tissues: Widespread and developmentally regulated but highly selective expression on glycoproteins. Proc. Natl. Acad. Sci. USA **1996**, *93*(7), 2759–2763.
7. Li, Y.T.; Yuziuk, J.A.; Li, S.C.; Nematalla, A.; Hasegawa, A.; Kimura, M.; Nakagawa, H. A novel sialidase capable of cleaving 3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (KDN). Arch. Biochem. Biophys., **1994**, *310*, 243–246.
8. Nishino, S.; Kuroyanagi, H.; Terada, T.; Inoue, S.; Inoue, Y.; Troy, F.A. 2nd; Kitajima, K. Induction, localization, and purification of a novel sialidase, deaminoneuraminidase (KDNase), from *Sphingobacterium multivorum*. J. Biol. Chem., **1996**, *27*(6), 2909–2913.
9. See for example: Terada, T.; Kitajima, K.; Inoue, S.; Wilson, J.C.; Norton, A.K.; Kong, D.C.M.; Thomson, R.J.; von Itzstein, M.; Inoue, Y. Catalysis by a new sialidase, deaminoneuraminic acid residue-cleaving enzyme (KDNase Sm), initially forms a less stable α-anomer of 3-deoxy-D-*glycero*-D-*galacto*-nonulosonic acid and is strongly inhibited by the transition state analogue, 2-deoxy-2,3-didehydro-D-*glycero*-D-*galacto*-2-nonulopyranosonic acid, but not by 2-deoxy-2,3-didehydro-*N*-acetylneuraminic acid. J. Biol. Chem, **1997**, *272*(9), 5452–5456.
10. von Itzstein, M.; Thomson, R.J. The synthesis of novel sialic acids as biological probes. In *Topics in Current Chemistry*; Driguez, H.; Thiem, J., Eds.; Springer-Verlag: Berlin, 1997; Vol 186, 119–170.
11. von Itzstein, M.; Thomson, R.J. Sialic acids and sialic acid-recognising proteins: drug discovery targets and potential glycopharmaceuticals. Curr. Med. Chem., **1997**, *4*, 185–210.
12. Nakamura, M.; Takayanagi, H.; Furuhata, K.; Ogura, H. Synthesis and characterization of furanose and pyranose derivatives of 3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (KDN). Chem. Pharm. Bull., **1992**, *40*(4), 879–885.
13. Kononov, L.O.; Magnusson, G. Synthesis of methyl and allyl α-glycosides of *N*-acetylneuraminic acid in the absence of added promoter. Acta Chem. Scand., **1998**, *48*(1), 141–144.
14. Fitz, W.; Rosenthal, P.B.; Wong, C-H. Synthesis and inhibitory properties of a thiomethylmercuric sialic acid with application to the x-ray structure determination of 9-*O*-acetylsialic acid esterase from Influenza C virus. Bioorg. Med. Chem., **1996**, *4*(8), 1349–1353.
15. Akai, S.; Nakagawa, T.; Kajihara, Y.; Sato, K. Selective protecting method for the individual hydroxyl groups of KDN. J. Carbohydr. Chem., **1999**, *18*(6), 639–654.
16. Silverstein, R.M.; Bassler, G.C.; Morrill, T.C., Eds. *Spectrometric Identification of Organic Compounds*, 4th Ed, Wiley: New York, 1981.
17. Augé, C.; Gautheron, C.; David, S.; Malleron, A.; Cavayé, B.; Bouxom, B. Sialyl aldolase in organic synthesis: from the trout egg acid, 3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (KDN), to branched-chain higher ketoses as possible new chirons. Tetrahedron, **1990**, *46*(1), 201–214.
18. Nakamura, M.; Furuhata, K.; Yamasaki, T.; Ogura, H. Studies on sialic acids. XXV. Synthesis of the α- and β-*N*-glycosides of 3-deoxy-D-*glycero*-D-*galacto*-2-nonulosonic acid (KDN). Chem. Pharm. Bull., **1991**, *39*, 3140–3144.

Received October 10, 2000
Accepted November 25, 2000

MARCEL DEKKER, INC.
270 Madison Avenue, New York, New York 10016