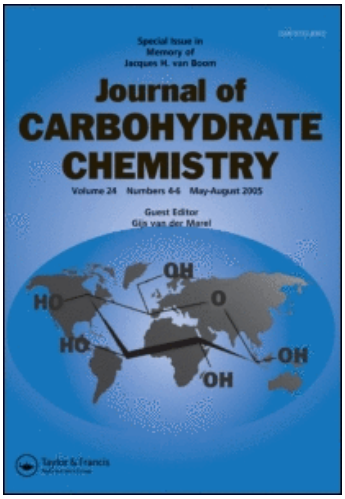

Journal of Carbohydrate Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713617200

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To cite this Article Sun, Xue-Long , Kai, Toshitsugu , Sato, Noriko , Takayanagi, Hiroaki and Furuhata, Kimio(1999) 'Synthesis of 4-Oxo and 4-Hydroxyimino-4-Deoxy-Kdn2En Derivatives', Journal of Carbohydrate Chemistry, 18: 9, 1131 — 1138
To link to this Article: DOI: 10.1080/07328309908544060
URL: http://dx.doi.org/10.1080/07328309908544060

J. CARBOHYDRATE CHEMISTRY, 18(9), 1131–1138 (1999)

SYNTHESIS OF 4-OXO AND 4-HYDROXYIMINO-4-DEOXY-Kdn2en DERIVATIVES

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Received May 6, 1999 - Final Form September 23, 1999

ABSTRACT

2,6-Anhydro-3-deoxy-D-*manno*-non-2-en-4-ulosonic acid (4-oxo-Kdn2en, **2**) was synthesized from methyl 2,6-anhydro-3-deoxy-D-*glycero*-D-*galacto*-non-2-enonate (Kdn-2en methyl ester, **7**) by the oxidation with manganese dioxide in dry acetone in good yield. 4-Hydroxyimino-Kdn2en derivatives (**3**), and 4-carbethoxymethylene-Kdn2en derivatives (**4**) were synthesized from the peracetylated methyl ester of 4-oxo-Kdn2en by oximation and Wittig reaction in good yield, respectively.

INTRODUCTION

2,3-Unsaturated sialic acid analogues with structural modifications at C-4 are particular candidates for studies of structure-activity relationships on developing sialidase inhibitors.[1] For example, approval is being requested to market 4-guanidino-4-deoxy-Neu-5Ac2en, developed as a potent inhibitor of the influenza sialidase, as a new medicine to treat the flu by halting viral replication in human tissues.[2,3] 2,3-Dehydro-2-deoxy-Kdn (Kdn2en, **1**), which is not isolated from nature yet, had been found to be a strong inhibitor of the Kdnase.[4,5] We had established a facile synthesis of Kdn on a large scale,[6] and described the synthesis of C-4 nitrogen-modified Kdn derivatives.[7] In our continuing efforts to define the role of the group at C-4 of Kdn2en analogues for Kdnase binding and inhibition, and to

gain further insight into the interaction of Kdn2en with sialidase from viral and microbial sources, we report herein the synthesis of 2,6-anhydro-3-deoxy-D-*manno*-non-2-en-4-ulosonic acid (4-oxo-Kdn2en, **2**), 4-hydroxyimino-Kdn2en derivatives (**3**), and 4-carbethoxymethylene-Kdn2en derivatives (**4**). The introduction of a doubly bonded functionality at the C-4 positon of Kdn2en was expected to increase its interaction with the enzyme *via* hydrogen bonding.

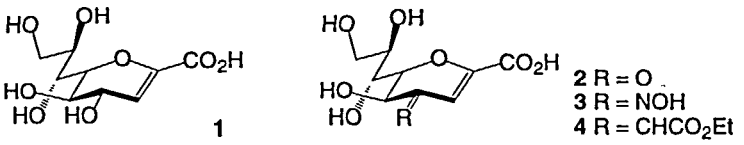


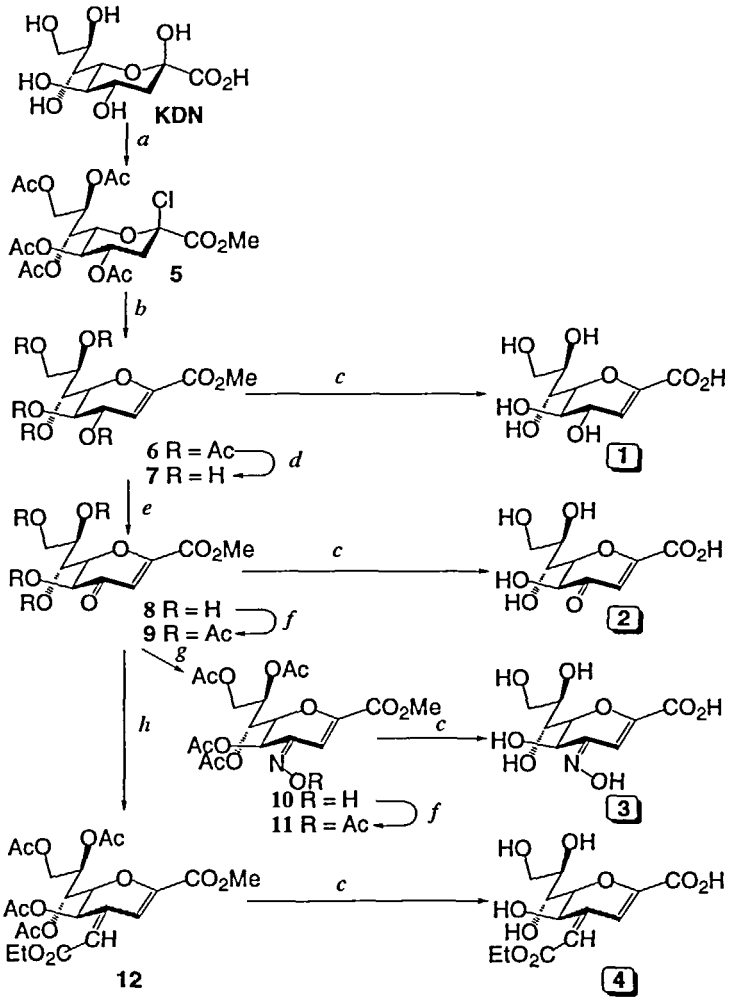
Chart 1

RESULTS AND DISCUSSION

We selected methyl 4,5,7,8,9-penta-*O*-acetyl-2,6-anhydro-2-chloro-2,3-dideoxy-D-*glycero*-D-*galacto*-2-nonulopyranosonate (**5**)[8] as starting material, which whendissolved in pyridine and stirred for 1 h at 50 °C gave the crude elimination product, methyl 4,5,7,8,9-penta-*O*-acetyl-2,6-anhydro-3-deoxy-D-*glycero*-D-*galacto*-non-2-enonate (**6**) in 97% isolated yield after silica gel chromatography. The ^{1}H NMR spectrum of **6** indicated the product was more than 98% pure and suitable for the following chemical transformation. Deacetylation of crude **6** with sodium methoxide in methanol afforded methyl 2,6-anhydro-3-deoxy-D-*glycero*-D-*galacto*-non-2-enonate (**7**). Oxidation of **7** with manganese dioxide in dry acetone gave, after purification using silica gel column chromatography, the 4-oxo-Kdn2en derivative **8** in 71% yield. The ^{1}H NMR spectrum of **8** showed a singlet at 6.07 ppm for H-3 in contrast to the doublet at 5.92 ppm ($J_{3,4}$ = 2.7 Hz) shown by **7**. The presence of a carbonyl carbon atom (196.41 ppm) in ^{13}C NMR spectrum, and the carbonyl absorption at 1685 cm^{-1} in the IR spectrum of **8** were also consistent with the assigned enone structure. Acetylation of **8** with acetic anhydride in pyridine at room temperature followed by purification of the product with silica gel column chromatography, gave per-*O*-acetyl-4-deoxy-4-oxo-Kdn2en methyl ester (**9**) in 83% yield, which had similar physiochemical characteristics to those of **8**. Deprotection of the ester group of **8** with LiOH in MeOH - H_2O afforded 4-oxo-Kdn2en (**2**) in 92% yield. Oximation of **9** by treatment with hydroxylamine hydrochloride in pyridine gave a single 4-oxime derivative **10** in 91% yield. The structure of **10** was confirmed from its ^{1}H and ^{13}C NMR spectra. In the ^{1}H NMR spectrum of **10**, the signal due to H-3 shifted to downfield (7.04 ppm, singlet), the signal due to 4-C=NOH appeared at 8.25 ppm (br.s), in the ^{13}C NMR spectrum, and C-4 appeared at

upfield at 147.66 ppm. The configuration of the oxime was determined as (*E*)-isomer, placing the hydroxy group of the oxime close to H-3, since a NOE was observed at H-3 and OH (1.0%). Furthermore, **10** was also converted into its peracetate **11**.

Deprotection of the acetyl and methyl ester groups of **10** with LiOH in MeOH - H_2O afforded **3** in 91% yield. Next, the Wittig reaction of **9** with (carbethoxymethylene)triphenyl phosphorane in dry diethyl ether was conducted at 40 °C to afford the Wittig product **12** in



Reaction conditions: *a*, ref. 6; *b*, Pyridine, 50 °C; *c*, LiOH, H_2O/MeOH; *d*, NaOMe/MeOH; *e*, MnO_2/acetone; *f*, Ac_2O/Pyridine; *g*, NH_2-OH.HCl/P *h*, Ph_3P=CHCOOEt.

Scheme 1

47% yield. The structure of **12** was confirmed from its ^{1}H NMR spectrum, the signal due to H-3 shifted to upfield (5.57 ppm, dd, $J_{3,10}$ = 0.9 Hz), and the signal due to new olefin proton (H-10) appeared at 7.75 ppm (dd, $J_{3,10}$ = 0.9 Hz). In the ^{13}C NMR spectrum of **12**, C-4 appeared upfield at 141.72 ppm, and a new olefin carbon (C-10) appeared at 106.49 ppm. The configuration of double bond formed in the Wittig reaction was determined as the (*Z*)-isomer, with the exocyclic =C-H close to H-3, based on a NOE at H-3 and H-10 observed (1.2%). Finally, deprotection of the acetyl and methyl ester groups of **12** with LiOH in MeOH - H_2O afforded **4** in 90% yield.

In summary, we have synthesized the 4-oxo-, 4-hydroxyimino- and 4-carbethoxy-methylene-Kdn2en derivatives (**2**, **3**, and **4**) *via* the peracetylated methyl ester of Kdn2en (**6**) as a key intermediate, prepared from glycosyl chloride **5** by a new facile method.

EXPERIMENTAL

General methods. Melting points were measured on a Yamato melting point apparatus without correction. Fast atom bombardment mass spectra (FAB MS) were recorded on a JEOL JMS-DX 300. Optical rotations were measured with a JASCOJIP-4 digital polarimeter (at 23 °C). The ^{1}H NMR spectra were determined with Varian VXR-300 spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C), in the solution state, with tetramethylsilane (TMS) as an internal reference. Thin-layer chromatography (TLC) was performed on Kieselgel 60 F_{254} (Merck) plates, and spots were detected under ultraviolet (UV) irradiation or by spraying with 5% sulfuric acid solution. Column chromatography was conducted on silica gel 60 (70-230 mesh, Merck).

Methyl 4,5,7,8,9-Penta-*O*-acetyl-2,3,5-trideoxy-D-*glycero*-D-*galacto*-non-2-eno-pyranosonate (6). Glycosyl chloride **5**[8] (1.63 g, 3.19 mmol) was dissolved in pyridine (20 mL), and the reaction mixture was stirred for 1 h at 50 °C, then concentrated to dryness. The product was directly purified on silica gel column with *n*-hexane - acetone (4:3) to afford **6** (1.46 g, 97%). It was also crystallized from ethyl acetate - *n*-hexane to give **6** as colorless prisms: mp 129-131 °C. The ^{1}H NMR spectrum was identical to the published spectral data.[8]

Methyl 2,6-Anhydro-3-deoxy-D-*glycero*-D-*galacto*-non-2-enonate (7). To a solution of **6** (990 mg, 2.1 mmol) in methanol (20 mL), 28% sodium methoxide in methanol solution (0.8 mL) was added. The mixture was stirred at room temperature for 30 min, then neutralized to pH 7 with Dowex 50 (H^+), filtered to remove the resin, concentrated to dryness, and was purified by recrystallization from methanol - chloroform (6:1) to yield **7** (532 mg, 96%) as colorless needles: $[\alpha]_D$ +7.8° (*c* 0.35, MeOH); ^{1}H NMR (C_5D_5N) δ 6.41 (d, 1H, $J_{3,4}$ = 2.4 Hz, H-3), 4.42 (dd, 1H, $J_{4,5}$ = 8.5 Hz, H-4), 4.16 (d, 1H, $J_{6,5}$ = 11.2 Hz,

H-6), 3.95-3.70 (m, 4H, H-7,8,9), 3.64 (dd, 1H, $J_{9,9'}$ = 12.2, $J_{8,9'}$ = 5.4 Hz, H-9'), 3.80 (s, 3H, $COOCH_3$); FAB-MS *m/z*: 285 $(M+1)^+$ (*m*-NBA as matrix).

Anal. Calcd for $C_{10}H_{16}O_8$: C, 45.46; H, 6.10. Found: C, 45.44; H, 6.16.

Methyl 2,6-Anhydro-3-deoxy-D-*manno*-non-2-en-4-ulosonate (8). To a solution of **7** (85 mg, 0.32 mmol) in anhydrous acetone (20 mL), manganese(IV) oxide (425 mg) was added. The reaction mixture was stirred at room temperature for 12 h until the starting material was no longer detected by TLC ($CHCl_3$ - MeOH, 6:1). The solution was filtered to remove manganese(IV) oxide, and the filtrate was evaporated to dryness under reduced pressure. The residue was purified by silica gel chromatography ($CHCl_3$ - MeOH, 10:1) to yield **8** (60 mg, 71%) as a colorless syrup: $[\alpha]_D$ +41.9° (*c* 0.17, MeOH); ^1H NMR (acetone-d_6) δ 6.07 (s, 1H, H-3), 4.63 (d, 1H, $J_{4,5}$ = 12.9 Hz, H-5), 3.95 (m, 1H, H-6), 3.97 (m, 1H, H-7), 4.61 (m, 1H, H-8), 3.76 (m, 2H, H-9), 3.88 (s, 3H, $COOCH_3$). ^{13}C NMR (acetone-d_6) δ 162.43 (C-1), 159.84 (C-2), 106.28 (C-3), 196.41 (C-4), 83.43 (C-5), 70.69 (C-6), 70.07 (C-7), 68.41 (C-8), 64.90 (C-9), 53.47 ($COOCH_3$). FAB-MS *m/z* : 283 $(M+1)^+$ (*m*-NBA as matrix).

Anal. Calcd for $C_{10}H_{14}O_8$: C, 45.81; H, 5.38. Found: C, 45.64; H, 5.46.

Methyl 5,7,8,9-Tetra-*O*-acetyl-2,6-anhydro-3-deoxy-D-*manno*-non-2-en-4-ulosonate (9). To a solution of **8** (60 mg, 0.21 mmol) in pyridine (20 mL), acetic anhydride (10 mL) and a little DMAP was added. The mixture was stirred at room temperature for 12 h, then poured into water (30 mL), and extracted with ethyl acetate (30 mL x 3). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4, concentrated to dryness. The residue was purified by silica gel column chromatography (*n*-hexane - acetone, 5:1) to yield **9** (83 mg, 87%) as a colorless syrup: ^1H NMR ($CDCl_3$) δ 6.29 (s, 1H, H-3), 5.42 (d, 1H, $J_{4,5}$ = 13.2 Hz, H-5), 4.71 (dd, 1H, $J_{6,7}$ = 1.8 Hz, H-6), 5.51 (dd, 1H, $J_{7,8}$ = 7.2 Hz, H-7), 5.44 (ddd, 1H, $J_{8,9'}$ = 8.4, $J_{8,9}$ = 2.7 Hz, H-8), 4.54 (dd, 1H, $J_{9,9'}$ = 12.9 Hz, H-9), 4.21 (dd, 1H, H-9'), 3.89 (s, 3H, $COOCH_3$), 2.17, 2.11, 2.09, 2.06 (each s, 3H, OAc x 4); FAB-MS *m/z*: 431 $(M+1)^+$ (*m*-NBA as matrix).

Anal. Calcd for $C_{18}H_{22}O_{12}$: C, 50.24; H, 5.15. Found: C, 50.18; H, 5.26.

2,6-Anhydro-3-deoxy-D-*manno*-non-2-en-4-ulosonic acid (2). To a solution of **8** (20 mg, 0.07 mmol) in methanol (10 mL), was added LiOH (18 mg, 0.42 mmol, 6 equiv) dissolved in water (2 mL). After stirring for 2 h at room temperature, the reaction mixture was acidified to pH 5.0 by addition cation exchange resins (Dowex 50 (H^+)) at 0 °C. The resins were filtered off and the filtrate was concentrated to dryness in vacuo at 50 °C. Chromatography of the residue on Sephadex LH-20 with methanol afforded product **2** (17 mg, 92%) after lyophilization as an amorphous powder: $[\alpha]_D$ +76.6° (*c* 0.32, MeOH); ^1H NMR (CD_3OD) δ 6.86 (s, 1H, H-3), 5.01 (d, 1H, $J_{5,6}$ = 8.1 Hz, H-5), 4.00 (d, 1H, H-6), 3.66-3.50 (m, 4H, H-7, 8, 9, 9'); FAB-MS *m/z*: 249 $(M+1)^+$ (*m*-NBA as matrix).

Anal. Calcd for $C_9H_{12}O_8$: C, 43.55; H, 4.87. Found: C, 43.48; H, 5.02.

Methyl 5,7,8,9-Tetra-*O*-acetyl-2,6-anhydro-3-deoxy-4-*C*-hydroxyimino-D-*manno*-non-2-en-4-ulosonate (10). To a solution of **9** (50 mg, 0.116 mmol) in pyridine (20 mL), hydroxylamine hydrochloride (41 mg, 0.58 mmol, 5 equiv) was added. The mixture was stirred at room temperature for 7 h. Then the reaction solution was concentrated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane - acetone, 5:1) to yield **10** (45 mg, 86%) as a colorless syrup: ^{1}H NMR ($CDCl_3$) δ 7.04 (s, 1H, H-3), 5.53 (d, 1H, $J_{5,6}$ = 6.6 Hz, H-5), 4.58 (dd, 1H, $J_{6,7}$ = 3.3 Hz, H-6), 5.50 (dd, 1H, $J_{7,8}$ = 6.9 Hz, H-7), 5.34 (ddd, 1H, $J_{8,9'}$ = 5.4, $J_{8,9}$ = 2.7 Hz, H-8), 4.47 (dd, 1H, $J_{9,9'}$ = 12.6 Hz, H-9), 4.19 (dd, 1H, H-9'), 3.86 (s, 3H, $COOCH_3$), 2.11, 2.09, 2.06, 2.04 (s, each 3H, OAc x 4); ^{13}C NMR ($CDCl_3$) δ 170.58, 169.64 x 2, 169.48 ($COCH_3$ x 4), 161.73 (C-1), 145.25 (C-2), 98.37 (C-3), 147.66 (C-4), 64.56 (C-5), 77.28 (C-6), 67.83 (C-7), 69.42 (C-8), 61.49 (C-9), 52.89 ($COOCH_3$), 20.84, 20.70 x 2, 20.42 ($COCH_3$ x 4); FAB-MS *m/z*: 446 $(M+1)^+$ (*m*-NBA as matrix).

Anal. Calcd for $C_{18}H_{23}NO_{12}$: C, 48.54; H, 5.21; N, 3.14. Found: C, 48.39; H, 5.32, N, 3.11.

Methyl 5,7,8,9-Tetra-*O*-acetyl-2,6-anhydro-3-deoxy-4-*C*-acetoxyimino-D-*manno*-non-2-en-4-ulosonate (11). To a solution of **10** (65 mg, 0.25 mmol) in pyridine (20 mL), acetic anhydride (10 mL) was added. The mixture was stirred at room temperature for 12 h , then poured into water (30 mL), and extracted with ethyl acetate (30 mL x 3). The combined extracts were washed with brine, dried over anhydrous Na_2SO_4, concentrated to dryness. The residue was purified by silica gel column chromatography (*n*-hexane - acetone, 6:1) to yield **11** (53 mg, 75%) as a colorless syrup: ^{1}H NMR ($CDCl_3$) δ 6.88 (s, 1H, H-3), 5.58 (d, 1H, $J_{5,6}$ = 6.9 Hz, H-5), 4.65 (dd, 1H, $J_{6,7}$ = 3.9 Hz, H-6), 5.46 (dd, 1H, $J_{7,8}$ = 7.5 Hz, H-7), 5.28 (ddd, 1H, $J_{8,9'}$ = 4.8, $J_{8,9}$ = 2.7 Hz, H-8), 4.40 (dd, 1H, $J_{9,9'}$ = 12.9 Hz, H-9), 4.14 (dd, 1H, H-9'), 3.85 (s, 3H, $COOCH_3$), 2.19, 2.09, 2.05, 2.01, 1.99 (each s, 3H, OAc x 5); FAB-MS *m/z*: 488 $(M+1)^+$ (*m*-NBA as matrix).

Anal. Calcd for $C_{20}H_{25}NO_{13}$: C, 49.28; H, 5.17; N, 2.87. Found: C, 49.15; H, 5.22, N, 2.68.

2,6-Anhydro-3-deoxy-4-*C*-hydroxyimino-D-*manno*-non-2-en-4-ulosonic acid (3). To a solution of **10** (30 mg, 0.07 mmol) in methanol (10 mL), was added LiOH. (18 mg, 0.42 mmol, 6 equiv) dissolved in water (2 mL). After stirring for 2 h at room temperature, the reaction mixture was treated as described for the preparation of **2** to give product **3** (17 mg, 91%): $[\alpha]_D$ -73.6° (*c* 0.79, MeOH); ^{1}H NMR (CD_3OD) δ 6.79 (s, 1H, H-3), 4.61 (d, 1H, $J_{5,6}$ = 8.4 Hz, H-5), 4.40 (dd, 1H, $J_{6,7}$ = 1.2 Hz, H-6), 3.91 (m, 1H, H-7), 3.90-3.85 (m, 2H, H-9), 3.78 (m, 1H, H-8); FAB-MS *m/z*: 264 $(M+1)^+$ (*m*-NBA as matrix).

Anal. Calcd for $C_9H_{13}NO_8$: C, 41.07; H, 4.98; N, 5.32. Found: C, 41.01; H, 5.01, N, 5.33.

Methyl 5,7,8,9-Tetra-*O*-acetyl-2,6-anhydro-3-deoxy-4-*C*-carbethoxymethylene-D-*manno*-non-2-en-4-ulosonate (12). To a solution of **9** (50 mg, 0.116 mmol) in anhydrous diethyl ether (15 mL), (carbethoxymethylene)triphenylphosphorane (60 mg, 0.174 mmol, 1.5 equiv) was added. The mixture was refluxed for 8 h, then the reaction was stopped, and the solution was concentrated to dryness under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane - acetone, 5:1) to yield **12** (28 mg, 47%) as a colorless syrup: $[\alpha]_D$ +33.3° (*c* 0.14, MeOH); ^{1}H NMR ($CDCl_3$) δ 7.75 (d, 1H, $J_{3,10}$ = 0.9 Hz, H-10), 5.57 (d, 1H, H-3), 5.55 (d, 1H, $J_{4,5}$ = 8.7 Hz, H-5), 4.38 (dd, 1H, $J_{6,7}$ = 2.4 Hz, H-6), 5.48 (dd, 1H, $J_{7,8}$ = 6.6 Hz, H-7), 5.36 (ddd, 1H, $J_{8,9'}$ = 5.7, $J_{8,9}$ = 3.0 Hz, H-8), 4.54 (dd, 1H, $J_{9,9'}$ = 12.6 Hz, H-9), 4.20 (dd, 1H, H-9'), 3.84 (s, 3H, $COOCH_3$), 4.21 (q, 2H, J = 7.5 Hz, CH_2CH_3), 1.30 (t, 3H, CH_2CH_3), 2.16, 2.07, 2.06, 2.05 (each s, 3H, OAc x 4); ^{13}C NMR ($CDCl_3$) δ 170.51, 169.82, 169.67, 169.61, ($COCH_3$ x 4), 165.25 (C-1), 161.63 (C-11), 146.71 (C-2), 141.72 (C-4), 115.27 (C-3), 106.49 (C-10), 77.68 (C-6), 69.77 (C-8), 67.47 (C-7), 65.327 (C-5), 61.69 (C-9), 52.71 ($COOCH_3$), 60.59 (CH_2CH_3), 20.83, 20.75, 20.71, 20.48 ($COCH_3$ x 4), 14.21 (CH_2CH_3); FAB-MS *m/z*: 501 $(M+1)^+$ (*m*-NBA as matrix).

Anal. Calcd for $C_{22}H_{28}O_{13}$: C, 52.80; H, 5.64. Found: C, 52.76; H, 5.72.

2,6-Anhydro-3-deoxy-4-*C*-carbethoxymethylene-D-*manno*-non-2-en-4-ulosonic acid (4). To a solution of **12** (20 mg, 0.04 mmol) in methanol (10 mL), was added LiOH (10 mg, 0.24 mmol, 6 equiv) dissolved in water (1 mL). After stirring for 2 h at room temperature, the reaction mixture was treated as described for the preparation of **2** to give product **4** (12 mg, 90%): $[\alpha]_D$ -27.9° (*c* 0.21, MeOH); ^{1}H NMR (CD_3OD) δ 7.45 (s, 1H, CH), 6.05 (s, 1H, H-3), 4.58 (dd, 1H, $J_{5,6}$ = 10.1, $J_{6,7}$ = 1.8 Hz, H-6), 4.24 (q, 2H, J = 7.5 Hz, CH_2CH_3), 4.22 (d, 1H, H-5), 3.96 (m, 1H, H-7), 3.94 (dd, 1H, $J_{9,9'}$ = 12.4, $J_{8,9'}$ = 1.8 Hz, H-9'), 3.80-3.64 (m, 2H, H-8, 9), 1.30 (t, 3H, CH_2CH_3); FAB-MS *m/z*: 319 $(M+1)^+$ (*m*-NBA as matrix).

Anal. Calcd for $C_{11}H_{14}O_9$: C, 45.52; H, 4.86. Found: C, 45.38; H, 5.01.

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