Pergamon

Tetrahedron 58 (2002) 9049–9054

TETRAHEDRON

Synthesis of 3-deoxy-2-ulosonic acid KDO and 4-*epi*-KDN, a highly efficient approach of 3-C homologation by propargylation and oxidation

Lian-Sheng Li and Yu-Lin Wu*

State Key Laboratory of Bio-organic and Natural Products Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, 354 Fenglin Road, Shanghai 200032, People's Republic of China

Received 6 June 2002; revised 9 August 2002; accepted 5 September 2002

Abstract—Through the introduction of a pyruvate segment by asymmetric propargylation and oxidation of terminal alkynes, both KDO and 4-*epi*-KDN have been concisely synthesized in the furanose form from readily available sugars in high overall yields. © 2002 Elsevier Science Ltd. All rights reserved.

Higher 3-deoxy-2-ulosonic acids, such as 3-deoxy-D-manno-2-octulosonic acid (KDO, **1**) and 3-deoxy-D-glycero-D-galacto-2-ulosonic acid (KDN, **2**), are involved in a number of biological processes. KDO is an essential constituent of the outer-cell membrane lipopolysaccharide (LPS) of Gram-negative bacteria, and incorporation of KDO is likely to be a key step in the growth of these bacteria.[1] KDN is a deaminated sialic acid first isolated in 1986 by Inoue and co-workers[2] from the membrane polysialoglycoproteins of rainbow trout eggs. It has been shown that KDN is likely to be responsible for protection of the egg membrane from attacks by bacterial sialidases.[3] A more recent discovery shows that free KDN occurs at elevated levels in human fetal cord red blood cells and ovarian cancer cells. Thus it can be an early warning signal of disease and a marker for detecting recurrence of disease.[4] Consequently, more and more attention has been paid to the synthesis of these ulosonic acids and their analogs[5,6] in efforts to develop potential biologically interesting agents. One of the challenging problems in the synthesis of such ulosonic acids is the introduction of the α-keto acid moiety. A straightforward and efficient way is asymmetric reaction of a proper aldehyde with a pyruvate equivalent followed by conversion of the adduct to the α-keto acid ester, which to some extent is similar to the biosynthesis. Herewith we would like to describe such a general strategy for the synthesis of a range of 3-deoxy-2-ulosonic acids based on the asymmetric propargylation of sugar aldehydes[7] developed earlier by us and a subsequent oxidation of the terminal alkynes[8] (Fig. 1).

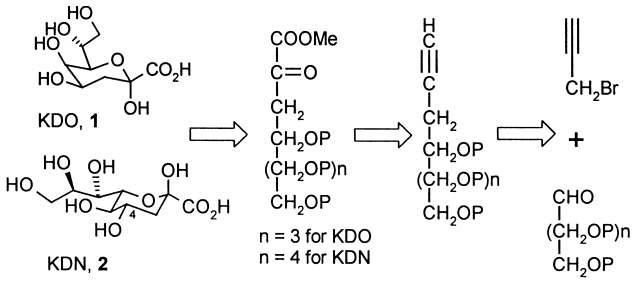


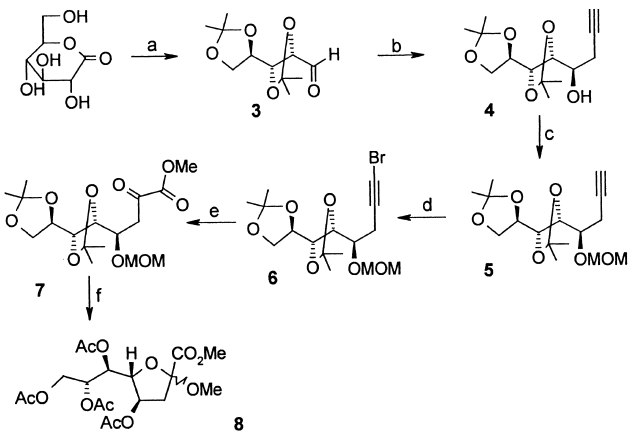
Figure 1. Retrosynthetic analysis of KDO and KDN.

Our synthetic approach to KDO (**1**) started with the readily available D-glucono-α-lactone-derived aldehyde **3**[9] (Scheme 1). Subsequent coupling with 3-bromopropyne according to the previously described procedure gave the desired *erythro* (*anti*) adduct **4**[7d] in good yield (83%). After protection of the homopropargylic alcohol as a MOM ether, compound **5** was converted to the corresponding bromoalkyne **6** (86% yield) by stirring with *N*-bromosuccinimide and silver nitrate at room temperature. Subsequent oxidation of compound **6** with $KMnO_4$ afforded the key intermediate α-keto acid ester **7** in 87% yield. It has been shown that methanolic HCl is a good reagent for deprotection and cyclization in the synthesis of nonulosonic acid.[10] Therefore, compound **7** was treated with conc. HCl in methanol at room temperature for 36 h. The generated methyl glycoside of KDO was then treated with acetic anhydride in pyridine to produce a ca. 4:1 mixture of anomeric acetates **8**. The major isomer was carefully isolated and fully characterized. The structure of compound **8** was then unambiguously assigned to the furanose form by comparison with the data[5e] of known corresponding pyranose form compounds, and especially the chemical shifts of C_3-protons (2.63 and 2.34) also confirmed[5r] the furanose form of compound **8**.

Keywords: carbohydrates; ulosonic acid; KDO; KDN; propargylation; oxidation.

* Corresponding author. Fax: +86-21-64166128; e-mail: ylwu@pub.sioc.ac.cn

PII: S0040-4020(02)01129-8



Scheme 1. *Reagents and conditions*: (a) Lit[9]; (b) 3-bromopropyne, DMF–Et_2O, Zn, 83%; (c) MOMCl, $^iPr_2NEt$, 0°C to rt, 88%; (d) NBS, $AgNO_3$, acetone, rt, 86%; (e) $KMnO_4$, $NaHCO_3$, $MgSO_4$, MeOH/H_2O (1:1), 0°C, 87%; (f) conc. HCl, anhydrous MeOH, rt, 2 days, then Ac_2O, Py., DMAP, CH_2Cl_2, rt, 75% (two steps) (anomeric mixture about 4:1).

Considering that the furanose isomers of *N*-acetyl neuraminic acid (Neu5Ac) exhibit remarkable inhibitory activity comparable to the pyranose analogue,[11] the KDO isomer in furanose form we obtained is expected to have potent biological activity.

Our interest in modified ulosonic acids then led us to explore application of this methodology to the synthesis of 4-*epi*-KDN. Starting from the known 2,3:5,6-di-*O*-isopropylidene mannitol[12] **9a** (Scheme 2), selective protection of the primary alcohol as a *t*-butyldimethylsilyl ether and treatment with MOMCl followed by removal of the silyl group by $^nBu_4NF$ gave the desired primary alcohol **9d** (82% overall yield for three steps). Swern oxidation of **9d** followed by coupling with 3-bromopropyne produced the desired *erythro* (*anti*) adduct **10** in 77% yield (two steps). Protection of the hydroxy in **10** as a *t*-butyldimethylsilyl ether afforded **12**, which was converted to the corresponding α-keto ester **14** by bromination and oxidation reactions (58% overall yield for three steps). Transformation of α-keto ester **14** to the corresponding 4-*epi*-KDN as furanose form **15** was carried out smoothly as described above. Then we briefly examined a sequence for epimerization of the hydroxy group at C4 as an route to KDN itself. Unfortunately, a Mitsunobo inversion[13] of configuration at C4 by treatment of **10** with either benzoic acid or 3,5-dinitrobenzoic acid in the presence of triphenylphosphine and diethyl azodicarboxylate gave no desired benzoyl derivative **11**, probably due to the steric hindrance caused by the substituent at C5.

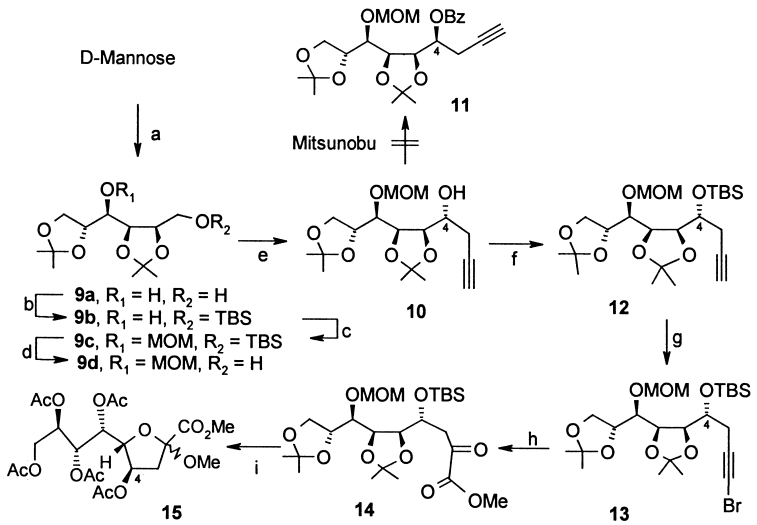
In conclusion, based on the asymmetric propargylation and oxidation of terminal alkynes, both KDO and 4-*epi*-KDN in the furanose form have been synthesized from readily available sugar aldehydes in 41% and 35% overall yield, respectively. Further studies on the synthesis of other 3-deoxy-2-ulosonic acids using this strategy are underway in this laboratory and will be reported in due time.

1. Experimental

1.1. General considerations

IR spectra were recorded on Perkin–Elmer 983 or Shimadzu IR-440 spectrometers. ^1H and ^{13}C NMR were recorded in $CDCl_3$ on an AMX-300, DPX-300 or DRX-400 spectrometers with TMS as the internal standard. Mass spectra were taken on a HP5973N or HP5989A instrument. Optical rotations were measured on a Perkin–Elmer 241 MC polarimeter. Elemental analyses were carried out at the Microanalytic Laboratory of Shanghai Institute of Organic Chemistry. Flash column chromatography was performed on silica gel H (10–40 μm) with petroleum ether/ethyl acetate or ethyl acetate/ethanol as eluent.

1.1.1. 1,2,3-Trideoxy-5,6:7,8-di-*O*-isopropylidene-D-manno-1-yno-octitol (4).[7d] To a vigorously stirred solution of aldehyde **3**[9] (2.5 g, 10.8 mmol) and 3-bromopropyne (1.9 g, 15.8 mmol) in anhydrous DMF–Et_2O (1:1, 40 mL)



Scheme 2. *Reagents and Conditions*: (a) (i) Conc. H_2SO_4, acetone, 82%, (ii) $LiAlH_4$, THF, 92%; (b) TBSCl, DMF, Im., rt, 96%; (c) MOMCl, $^iPr_2NEt$, CH_2Cl_2, rt, 92%; (d) $^nBu_4NF$, THF, 93%; (e) (i) oxalyl chloride, DMSO, Et_3N; (ii) 3-bromopropyne, Zn, DMF–Et_2O, 77%; (f) TBSCl, DMF, Im., rt, 84%; (g) NBS, $AgNO_3$, acetone, 87%; (h) $KMnO_4$, $NaHCO_3$, $MgSO_4$, CH_3OH/H_2O (1:1), 0°C, 79%; (i) conc. HCl, anhydrous CH_3OH, rt, then Ac_2O, Py., DMAP, CH_2Cl_2, rt, 78% (two steps).

under nitrogen, was slowly added activated zinc dust (1.5 g, 21.6 mmol) in portions. The resulting refluxing mixture was cooled to room temperature; stirring was continued until TLC showed the completion of the reaction. Then, the mixture was poured into saturated aqueous NH_4Cl, extracted with Et_2O (50 mL×3). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel to afford **4** (2.43 g, 83%). $[\alpha]_D$=+11.3 (*c*, 1.02, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.22–3.72 (6H, m, OC*H*), 2.6–2.4 (2H, m, CH_2), 2.05 (1H, t, *J*=2.4 Hz, ≡–*H*), 1.46 (3H, s, CH_3), 1.42 (3H, s, CH_3), 1.38 (6H, s, CH_3). EIMS (*m*/*z*, %): 255 (M^+−Me, 100.0), 173 (19.1), 143 (63.1), 101 (51.6), 59 (96.2), 43 (90.3). IR (film): 3463, 3290, 2989, 2121, 1382, 1216, 1071, 846, 645 cm^{-1}.

1.1.2. 4-*O*-Methoxymethyl-1,2,3-trideoxy-5,6:7,8-di-*O*-isopropylidene-D-manno-1-yno-octitol (5). To a solution of **4** (2.4 g, 8.9 mmol) in anhydrous CH_2Cl_2 (30 mL) at 0°C under nitrogen, were added MOMCl (2.8 mL, 35.6 mmol) and ^{i}Pr$_2$NEt (6.6 mL, 39.2 mmol) dropwise. After stirring for 30 h at room temperature, the reaction mixture was diluted with Et_2O, washed with water, saturated aqueous NH_4Cl and brine, then dried over $MgSO_4$. After concentration under reduced pressure, the residue was purified by column chromatography on silica gel (PE/EA=10:1) to afford compound **5** (2.24 g, 88%). $[\alpha]_D$=+10.5 (*c*, 1.0, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.84 (1H, d, *J*=6.8 Hz, OC*H*HO), 4.76 (1H, d, *J*=6.8 Hz, OCH*H*O), 4.20–3.7 (6H, m, OC*H*), 3.44 (3H, s, OCH_3), 2.60 (2H, m, CH_2), 2.03 (1H, t, *J*=2.0 Hz, ≡–*H*), 1.44 (3H, s, CH_3), 1.41 (6H, s, CH_3), 1.36 (3H, s, CH_3). EIMS (*m*/*z*, %): 299 (M^+−Me, 28.9), 255 (3.7), 143 (60.6), 101 (27.5), 45 (100.0), 43 (57.0). IR (film): 3278, 2994, 2119, 1377, 1156, 1073, 643 cm^{-1}.

1.1.3. 4-*O*-Methoxymethyl-1-bromo-1,2,3-trideoxy-5,6:7,8-di-*O*-isopropylidene-D-manno-1-yno-octitol (6). To a solution of **5** (1.57 g, 5 mmol) in acetone (20 mL) under nitrogen, were added $AgNO_3$ (340 mg, 2 mmol) and NBS (0.90 g, 7.5 mmol). After stirring at room temperature for 10 h, the reaction mixture was diluted with Et_2O, and then filtered through Celite. The filtrate was concentrated under reduced pressure; the residue was dissolved in Et_2O, and then washed with H_2O, and brine, dried over $MgSO_4$. The solvent was evaporated under reduced pressure, and the residue was purified by column chromatography on silica gel to afford **6** (1.69 g, 86%). $[\alpha]_D$=+11.6 (*c*, 1.1, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.79 (1H, d, *J*=6.9 Hz, OC*H*HO), 4.75 (1H, d, *J*=6.9 Hz, OCH*H*O), 4.2–3.80 (6H, m, OC*H*), 3.43 (3H, s, OCH_3), 2.65 (1H, dd, *J*=17.3, 5.5 Hz, C*H*H), 2.58 (1H, dd, *J*=17.3, 6.0 Hz, CH*H*), 1.43 (3H, s, CH_3), 1.39 (6H, s, CH_3), 1.36 (3H, s, CH_3). EIMS (*m*/*z*, %): 379 (M^+−Me, 28.6), 377 (M^+−Me, 28.7), 217 (10.3), 143 (52.8), 101 (28.0), 85 (15.4), 45 (100.0). IR (film): 1988, 2973, 1456, 1381, 1215, 1154, 1069, 1045, 848 cm^{-1}. Elemental analysis for $C_{16}H_{25}O_6Br$ Calcd: C 48.85, H 6.36; Found: C 49.03, H 6.32.

1.1.4. Methyl (4-*O*-methoxymethyl-5,6:7,8-di-*O*-isopropylidene-D-manno-3-deoxy-2-keto)octonate (7). To a vigorously stirred solution of **6** (786 mg, 2 mmol) in MeOH (30 mL) at 0°C, was added a solution of $NaHCO_3$ (100 mg, 1.2 mmol) and $MgSO_4$ (480 mg, 4 mmol) in H_2O (30 mL), followed by addition of solid $KMnO_4$ (620 mg, 4 mmol) in portions. The mixture was stirred for 1.5 h at 0°C, then poured into 30 mL of ice water and extracted with EtOAc (50 mL×4). The combined organic phase was washed with H_2O and brine, then dried over $MgSO_4$. The solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel to give **7** (650 mg, 87%). $[\alpha]_D$=+29.5 (*c*, 1.3, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.73 (1H, d, *J*=6.8 Hz, OC*H*HO), 4.69 (1H, d, *J*=6.8 Hz, OCH*H*O), 4.36 (1H, m, OC*H*), 4.10 (4H, m, OC*H*), 3.97 (1H, dd, *J*=7.3, 4.4 Hz, OC*H*), 3.90 (3H, s, OCH_3), 3.88 (1H, m), 3.34 (3H, s, OCH_3), 3.28 (1H, dd, *J*=17.1, 7.7 Hz, CH*H*), 3.12 (1H, dd, *J*=17.1, 4.9 Hz, C*H*H), 1.44 (3H, s, CH_3), 1.40 (3H, s, CH_3), 1.38 (3H, s, CH_3), 1.35 (3H, s, CH_3). ^{13}CNMR (75 MHz, $CDCl_3$): δ 191.2, 161.1, 110.0, 109.7, 97.0, 82.3, 78.5, 77.1, 73.5, 67.7, 55.8, 52.8, 41.8, 27.2, 26.6, 26.4, 25.1. EIMS (*m*/*z*, %): 361 (M^+−Me, 33.1), 257 (21.4), 199 (26.0), 143 (66.6), 59 (24.6), 45 (100.0). IR (film): 2989, 2938, 2898, 1757, 1733, 1382, 1253, 1215, 1070, 1039, 847 cm^{-1}. Elemental analysis for $C_{17}H_{28}O_9$ Calcd: C 54.26, H 7.45; Found: C 54.24, H 7.51.

1.1.5. Methyl 4,6,7,8-tetra-*O*-acetyl-2,5-anhydro-3-deoxy-2-methoxyl-D-manno-octofuranos-2-ulosonate (8). To a stirred solution of **7** (165 mg, 0.44 mmol) in anhydrous methanol (10 mL) at 0°C under nitrogen, was added conc. HCl (0.2 mL). After stirring at room temperature for 2 days, the reaction mixture was cooled to 0°C, carefully neutralized with solid $NaHCO_3$. The solvent was evaporated under reduced pressure and the residue was dissolved in anhydrous CH_2Cl_2 (3 mL), followed by addition of Ac_2O (1.2 mL), pyridine (1.4 mL) and a catalytic amount of DMAP. After stirring at room temperature for 24 h, the reaction mixture was concentrated under reduced pressure, and purified by column chromatography on silica gel to afford **8** (140 mg, 75%). $[\alpha]_D$=+34.5 (*c*, 0.9, $CHCl_3$). ^{1}H NMR (300 MHz, C_6D_6): δ 5.75 (1H, dd, *J*=6.1, 3.2 Hz, OC*H*), 5.69 (1H, dt, *J*=2.4, 6.1 Hz, OC*H*), 5.18 (1H, m, OC*H*), 4.59 (2H, m, OC*H*), 4.33 (1H, dd, *J*=12.4, 5.6 Hz, OC*H*), 3.32 (3H, s, OCH_3), 3.31 (3H, s, OCH_3), 2.63 (1H, dd, *J*=14.8, 8.1 Hz, C*H*H), 2.34 (1H, dd, *J*=14.8, 2.7 Hz, CH*H*), 1.72 (3H, s, CH_3COO), 1.68 (3H, s, CH_3COO), 1.67 (3H, s, CH_3COO), 1.58 (3H, s, CH_3COO). ^{13}C NMR (150 MHz, C_6D_6): δ 170.1, 170.0, 169.6, 169.5, 168.1, 106.7, 83.3, 74.1, 71.1, 70.2, 62.1, 51.9, 51.4, 42.7, 20.4, 20.3, 20.1. EIMS (*m*/*z*, %): 403 (M^+−OMe, 15.8), 375 (84.7), 283 (34.6), 153 (85.8), 43 (100.0). IR (film): 2959, 1744 (broad), 1373, 1213, 1104 cm^{-1}. Elemental analysis for $C_{18}H_{26}O_{12}$ Calcd: C 49.77, H 5.99; Found: C 49.91, H 6.04.

1.1.6. 2,3:5,6-Di-*O*-isopropylidene-D-mannitol[12] (9a). To a solution of 2,3:5,6-di-O-isopropylidene-D-mannofuranose (6 g, 23 mmol) in dry THF (100 mL) at 0°C, was slowly added $LiAlH_4$ (965 mg, 25.4 mmol) portion-wise. The mixture was stirred at rt for 2 h. Then the excess $LiAlH_4$ was carefully destroyed by addition of saturated aqueous NH_4Cl, Celite and Et_2O, then stirring was continued for 30 min. Then the suspension was filtered through Celite, washed with Et_2O. The combined filtrate was concentrated under reduced pressure, and then purified by column

chromatography on silica gel (PE/EA=1:2) to give diol **9a** (4.84 g, 81%). $[\alpha]_D=-8.7$ (*c*, 0.6, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 4.40 (1H, dd, *J*=7.4, 1.5 Hz, OC*H*), 4.42 (1H, m, OC*H*), 4.14–4.10 (3H, m, OC*H*), 3.85 (1H, m, OC*H*), 3.60 (1H, t, *J*=6.3 Hz, OC*H*), 3.12 (1H, d, *J*=6.3 Hz, OC*H*), 2.65 (1H, bt, *J*=5.7 Hz, O*H*), 1.52 (3H, s, CH_3), 1.41 (3H, s, CH_3), 1.40 (3H, s, CH_3), 1.36 (3H, s, CH_3). EIMS (*m*/*z*, %): 247 (M^+-Me, 36.5), 205 (88.1), 147 (100.0), 101 (78.0), 43 (74.2). IR (film): 3427, 2987, 1381, 1216, 1068, 851 cm^{-1}.

1.1.7. 1-*O*-[(*t*-Butyl)dimethylsilyl]-2,3:5,6-di-*O*-isopropylidene-D-mannitol (9b). To a solution of diol **9a** (4.6 g, 17.5 mmol) in anhydrous DMF (30 mL) at 0°C, were added TBSCl (3.2 g, 21 mmol) and imidazole (3.6 g, 53 mmol). The mixture was stirred at room temperature for 4 h, then poured into water (50 mL), extracted with Et_2O (40 mL×4). The combined organic layers were washed with brine, dried over $MgSO_4$, then filtered, concentrated under reduced pressure, the residue was purified by column chromatography on silica gel (PE/EA=4:1) to give **9b** (6.8 g, 96%). $[\alpha]_D=-17.6$ (*c*, 0.6, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 4.38 (1H, d, *J*=7.1 Hz, OC*H*), 4.25 (1H, dt, *J*=3.9, 6.6 Hz, OC*H*), 4.15–3.96 (4H, m, OC*H*, O*H*), 3.84 (1H, dd, *J*=11.0, 3.8 Hz, OC*H*), 3.68 (1H, m, OC*H*), 3.20 (1H, d, *J*=6.0 Hz, OC*H*), 1.50 (3H, s, CH_3), 1.40 (3H, s, CH_3), 1.39 (3H, s, CH_3), 1.36 (3H, s, CH_3), 0.9 (9H, s, ^{t}*Bu*-Si), 0.11 (6H, s, CH_3–Si–CH_3). EIMS (*m*/*z*, %): 361 (M^+-Me, 30.4), 261 (38.7), 185 (62.3), 117 (85.3), 101 (100.0), 59 (52.1). IR (film): 3492, 2935, 1381, 1257, 1215, 1069, 837 cm^{-1}.

1.1.8. 4-*O*-Methyoxymethyl-1-*O*-[(*t*-Butyl)dimethylsilyl]-2,3:5,6-di-*O*-isopropylidene-D-mannitol (9c). To a solution of **9b** (6.6 g, 17.5 mmol) in anhydrous CH_2Cl_2 (30 mL) at 0°C, were added $^i Pr_2NEt$ (9.2 mL, 54.6 mmol) and MOMCl (4.0 mL, 51 mmol). The mixture was stirred overnight at rt, then diluted with Et_2O (100 mL), washed with H_2O, saturated aqueous NH_4Cl, and brine, dried over $MgSO_4$. The solution was concentrated under reduced pressure, and then the residue was purified by column chromatography on silica gel (PE/EA=5:1) to give **9c** (6.8 g, 92%). $[\alpha]_D^{25}=+19.4$ (*c*, 1.93, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 4.83 (2H, s, OCH_2O), 4.21 (1H, m, OC*H*), 4.12 (1H, m, OC*H*), 4.1–4.0 (4H, m, OC*H*), 3.85 (1H, dd, *J*=10.5, 7.0 Hz, OC*H*), 3.72 (1H, dd, *J*=10.5, 4.7 Hz, OC*H*), 3.42 (3H, s, OCH_3), 1.45 (3H, s, CH_3), 1.40 (3H, s, CH_3), 1.35 (3H, s, CH_3), 1.34 (3H, s, CH_3), 0.9 (9H, s, ^{t}*Bu*-Si), 0.1 (6H, s, CH_3–Si–CH_3). EIMS (*m*/*z*, %): 405 (M^+-Me, 9.1), 331 (24.8), 305 (8.4), 185 (65.1), 101 (96.2), 45 (100.0). IR (film): 2956, 2934, 1380, 1253, 1218, 1032, 837 cm^{-1}. Elemental analysis for $C_{20}H_{40}O_7Si$ Calcd: C 57.14, H 9.52; Found: C 57.40, H 9.42.

1.1.9. 4-*O*-Methyoxymethyl-2,3:5,6-di-*O*-isopropylidene-D-mannitol (9d). To a solution of **9c** (6.8 g, 16.2 mmol) in THF (50 mL) at 0°C, was added TBAF (1 M solution in THF, 16 mL). After stirring for 2 h at room temperature, the mixture was diluted with Et_2O, washed with H_2O and brine, dried over $MgSO_4$, and then concentrated under reduced pressure, the residue was purified by column chromatography on silica gel (PE/EA=1:1) to give **9d** (4.5 g, 92%). $[\alpha]_D^{25}=+103.3$ (*c*, 2.1, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 4.87 (1H, d, *J*=6.8 Hz, OCH_2O), 4.74 (1H, d, *J*=6.8 Hz, OCH_2O), 4.25–4.03 (4H, m, OC*H*), 3.94 (1H, t, *J*=7.7 Hz, OC*H*), 3.86 (1H, t, *J*=6.6 Hz, OC*H*), 3.78 (1H, m, OC*H*), 3.66 (1H, dd, *J*=11.1, 6.9 Hz, OC*H*), 3.40 (3H, s, OCH_3), 2.66 (1H, bs, O*H*), 1.48 (3H, s, CH_3), 1.42 (3H, s, CH_3), 1.37 (3H, s, CH_3), 1.35 (3H, s, CH_3). EIMS (*m*/*z*, %): 291 (M^+-Me, 9.6), 275 (30.3), 217 (55.7), 101 (65.8), 45 (100.0). IR (film): 3475, 2987, 1372, 1219, 1159, 1032, 852 cm^{-1}. Elemental analysis for $C_{14}H_{26}O_7$ Calcd: C 54.89, H 8.55; Found: C 55.14, H 8.53.

1.1.10. 7-*O*-Methyoxymethyl-1,2,3-trideoxy-5,6:8,9-di-*O*-isopropylidene-D-*erythro*-D-manno-1-yno-nonitol (10). To a solution of oxalyl chloride (1.90 mL, 18.6 mmol) in anhydrous CH_2Cl_2 (30 mL) stirred at −78°C, was added DMSO (2.9 mL, 41.5 mmol) in CH_2Cl_2 (15 mL). The mixture was stirred at −78°C for 30 min. A solution of compound **9d** (3.8 g, 12.4 mmol) in anhydrous CH_2Cl_2 (15 mL) was added, and the stirring was continued at −78°C for 1 h before NEt_3 (15 mL) was added at the same temperature. After stirring at 0°C for 30 min and then at room temperature for 30 min, water (30 mL) was added. The organic layer was washed with 2 M aqueous HCl, saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$. Removal of the drying agent and the solvent under reduced pressure gave the crude aldehyde, which was used without further purification to the next step.

To a vigorously stirred solution of aldehyde obtained above and bromopropyne (1.8 mL, 27.9 mmol) in 60 mL of anhydrous DMF–Et_2O (1:1) under nitrogen, was slowly added activated zinc dust (1.8 g, 27.6 mmol) in portions. The resulting mixture was stirred for 2 h at room temperature, then poured into saturated aqueous NH_4Cl and extracted with Et_2O (50 mL×3). The combined organic layers were dried over $MgSO_4$, filtered and concentrated. The residue was purified by column chromatography on silica gel (PE/EA=4:1) to afford **10** (3.14 g, 77%). $[\alpha]_D^{25}=6.9$ (*c*, 1.5, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 4.95 (1H, d, *J*=6.6 Hz, OCH_2O), 4.81 (1H, d, *J*=6.6 Hz, OCH_2O), 4.33 (1H, t, *J*=4.1 Hz, OC*H*), 4.23 (1H, m, OC*H*), 4.20 (1H, dd, *J*=8.1, 4.1 Hz), 4.14 (1H, t, *J*=7.1 Hz, OC*H*), 4.07 (1H, dd, *J*=8.4, 6.3 Hz, OC*H*), 4.03 (1H, m, OC*H*), 4.02 (1H, t, *J*=8.1 Hz, OC*H*), 3.44 (3H, s, OCH_3), 3.34 (1H, bd, *J*=5.4 Hz, O*H*), 2.68 (1H, dt, *J*=16.8, 3.0 Hz, –C*H*H–), 2.44 (1H, ddd, *J*=16.8, 6.9, 2.7 Hz, –CH*H*–), 2.06 (1H, t, *J*=2.7 Hz, ≡–*H*), 1.44 (3H, s, CH_3), 1.43 (3H, s, CH_3), 1.36 (3H, s, CH_3), 1.34 (3H, s, CH_3). EIMS (*m*/*z*, %): 329 (M^+-Me, 18.3), 313 (14.7), 255 (34.9), 101 (79.2), 45 (100.0). IR (film): 3449, 3287, 2987, 1371, 1218, 1084, 1033 cm^{-1}. Elemental analysis for $C_{17}H_{27}O_8$ Calcd: C 59.29, H 8.19; Found: C 59.23, H 8.16.

1.1.11. 4-*O*-[(*t*-Butyl)dimethylsilyl]-7-*O*-methyoxymethyl-1,2,3-trideoxy-5,6:8,9-di-*O*-isopropylidene-D-*erythro*-D-manno-1-yno-nonitol (12). This compound was obtained in 84% yield using the procedure as described for **9b**. $[\alpha]_D^{25}=-63.1$ (*c*, 0.7, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 4.98 (1H, d, *J*=6.8 Hz, OCH_2O), 4.70 (1H, d, *J*=6.8 Hz, OCH_2O), 4.45 (1H, dt, *J*=9.0, 3.8 Hz, OC*H*), 4.24 (1H, dd, *J*=9.0, 6.6 Hz, OC*H*), 4.12 (2H, m, OC*H*), 4.03 (3H, m, OC*H*), 3.38 (3H, s, OCH_3), 2.58 (2H, m, CH_2), 2.00 (1H, t, *J*=2.6 Hz, ≡–*H*), 1.47 (3H, s, CH_3), 1.38 (3H,

s, CH_3), 1.35 (3H, s, CH_3), 1.33 (3H, s, CH_3), 0.92 (9H, s, Si*t*Bt), 0.21 (3H, s, SiCH_3), 0.20 (3H, s, SiCH_3). EIMS (*m*/*z*, %): 443 (M^+−Me, 12.0), 369 (4.9), 215 (58.9), 101 (65.1), 73 (94.1), 45 (100.0). IR (film): 3284, 2933, 1370, 1253, 1157, 1086, 1031, 838 cm^{-1}. Elemental analysis for $C_{23}H_{42}O_7Si$ Calcd: C 60.26, H 9.24; Found: C 60.00, H 9.04.

1.1.12. 4-*O*-[(*t*-Butyl)dimethylsilyl]-7-*O*-methyoxymethyl-1-bromo-1,2,3-trideoxy-5,6:8,9-di-*O*-isopropylidene-D-*erythro*-D-manno-1-yno-nonitol (13). This compound was obtained in 87% yield using the procedure as described for **6**. $[\alpha]_D^{25}=-56.0$ (*c*, 0.6, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ 4.98 (1H, d, *J*=6.9 Hz, OCH_2O), 4.69 (1H, d, *J*=6.9 Hz, OCH_2O), 4.43 (1H, dt, *J*=8.4, 3.9 Hz, OC*H*), 4.17 (1H, dd, *J*=8.8, 6.1 Hz, OC*H*), 4.12 (2H, m, OC*H*), 4.02 (3H, m, OC*H*), 3.37 (3H, s, OCH_3), 2.64 (1H, dd, *J*=17.2, 3.6 Hz, C*H*H), 2.55 (1H, dd, *J*=17.1, 4.3 Hz, CH*H*), 1.47 (3H, s, CH_3), 1.38 (3H, s, CH_3), 1.35 (3H, s, CH_3), 1.34 (3H, s, CH_3), 0.91 (9H, s, Si*t*Bt), 0.20 (3H, s, SiCH_3), 0.17 (3H, s, SiCH_3). EIMS (*m*/*z*, %): 523 (M^+−Me, 10.1), 521 (M^+−Me, 9.6), 449 (17.4), 447 (17.6), 333 (10.8), 331 (10.5), 101 (67.2), 73 (81.7), 45 (100.0). IR (film): 2933, 1379, 1253, 1218, 1156, 1033, 838 cm^{-1}. Elemental analysis for $C_{23}H_{41}O_7SiBr$ Calcd: C 51.40, H 7.69; Found: C 51.66, H 7.47.

1.1.13. Methyl (4-*O*-[(*t*-Butyl)dimethylsilyl]-7-*O*-methyoxymethyl-5,6:8,9-di-*O*-isopropylidene-3-deoxy-D-*erythro*-D-manno-2-keto)nonate (14). This compound was obtained in 79% yield using the procedure as described for **7**. ^1H NMR (300 MHz, $CDCl_3$): δ 4.92 (1H, d, *J*=6.7 Hz, OCH_2O), 4.74 (1H, m), 4.70 (1H, d, *J*=6.7 Hz, OCH_2O), 4.44 (1H, dd, *J*=6.3, 2.0 Hz, OC*H*), 4.13 (3H, s, OC*H*), 4.05 (1H, dd, *J*=8.2, 6.2 Hz, OC*H*), 3.95 (2H, m, OC*H*), 3.85 (3H, s, OCH_3), 3.37 (3H, s, OCH_3), 3.36 (1H, dd, *J*=14.7, 7.2 Hz, CH*H*), 2.93 (1H, dd, *J*=14.7, 5.4 Hz, C*H*H), 1.40 (3H, s, CH_3), 1.38 (3H, s, CH_3), 1.33 (3H, s, CH_3), 1.27 (3H, s, CH_3), 0.86 (9H, s, Si*t*Bt), 0.20 (3H, s, SiCH_3), 0.11 (3H, s, SiCH_3). EIMS (*m*/*z*, %): 505 (M^+−Me, 5.8), 373 (6.1), 245 (34.1), 159 (49.8), 101 (71.0), 45 (100.0). IR (film): 2956, 1760, 1734, 1370, 1257, 1218, 1089, 1068, 1032, 837 cm^{-1}. Elemental analysis for $C_{24}H_{44}O_{10}Si$ Calcd: C 55.38, H 8.53; Found: C 55.43, H 8.37.

1.1.14. Methyl 4,6,7,8,9-penta-*O*-acetyl-2,5-anhydro-3-deoxy-2-methoxyl-D-*erythro*-D-mannofuranos-2-ulosonate (15). This compound was obtained in 75% yield using the procedure as described for **8**. $[\alpha]_D^{25}=26.4$ (*c*, 0.97, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 5.50 (1H, dd, *J*=8.7, 2.5 Hz, OC*H*), 5.32 (1H, dd, *J*=8.0, 2.4 Hz, OC*H*), 5.26 (1H, dt, *J*=6.6, 2.0 Hz, OC*H*), 5.11 (1H, ddd, *J*=8.4, 6.8, 2.8 Hz, OC*H*), 4.28 (1H, dd, *J*=8.0, 1.3 Hz, OC*H*), 4.25 (1H, dd, *J*=12.5, 2.8 Hz, OC*H*), 4.08 (1H, m, OC*H*), 3.83 (3H, s, OCH_3), 3.35 (3H, s, OCH_3), 2.64 (1H, dd, *J*=15.2, 6.5 Hz, C*H*H), 2.45 (1H, dd, *J*=15.2, 2.2 Hz, CH*H*), 2.14 (3H, s, CH_3COO), 2.13 (3H, s, CH_3COO), 2.10 (3H, s, CH_3COO), 2.05 (3H, s, CH_3COO), 2.00 (3H, s, CH_3COO). ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.5, 170.4, 170.0, 169.8, 169.4, 168.8, 107.3, 84.2, 74.3, 69.3, 68.5, 68.3, 62.2, 52.7, 43.0, 21.0, 20.9, 20.8, 20.7, 20.6. EIMS (*m*/*z*, %): 506 (M^+), 475 (4.2), 447 (53.4), 157 (34.6), 43 (100.0). IR (film): 2959, 1744 (broad), 1373, 1213 (broad), 1104, 1060 cm^{-1}. Elemental analysis for $C_{21}H_{30}O_{14}$ Calcd: C 49.80, H 5.93; Found: C 50.07, H 6.17.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (grant No. 29790126, 29872049), the Chinese Academy of Sciences (KJ 95-A1-504), and the State Ministry of Science and Technology (970211006-6, G2000077502). We also express our appreciation to Dr Yikang Wu and Dr Zhu-Jun Yao for their helpful discussion.

References

1. (a) Levin, D. H.; Racker, E. *J. Biol. Chem.* **1959**, *234*, 2532–2539. (b) Unger, F. M. *Adv. Carbohydr. Chem. Biochem.* **1981**, *38*, 323–388.
2. Nadano, D.; Iwasaki, M.; Endo, S.; Kitajima, K.; Inoue, S.; Inoue, Y. *J. Biol. Chem.* **1986**, *261*, 11550–11557.
3. Schreiner, E.; Zbiral, E. *Liebigs Ann. Chem.* **1990**, 581.
4. Inoue, S.; Lin, S.-L.; Chang, T.; Wu, S.-H.; Yao, C.-W.; Chu, T.-Y.; Troy, F. A.; Inoue, Y. *J. Biol. Chem.* **1998**, *273*, 27199–27204.
5. For chemical syntheses of KDO and related compounds, see: (a) Sugisaki, C. H.; Ruland, Y.; Clezio, I. L.; Baltas, M. *Synlett* **2001**, 1905–1908. (b) Sarabia, F.; Lopez-Herrera, F. J. *Tetrahedron Lett.* **2001**, *42*, 8801–8804. (c) Kumaran, G.; Mootoo, D. R. *Tetrahedron Lett.* **2001**, *42*, 3783–3785. (d) Reiner, M.; Schmidt, R. R. *Tetrahedron: Asymmetry* **2000**, *11*, 319–335. (e) Mlynarski, J.; Banaszek, A. *Org. Lett.* **1999**, *1*, 1709–1711. (f) Burke, S. D.; Sametz, G. M. *Org. Lett.* **1999**, *1*, 71–74. (g) Bräuer, N.; Kirschning, A.; Schaumann, E. *Eur. J. Org. Chem.* **1998**, 2729–2732. (h) Mikshiev, Y. M.; Paidak, B. B.; Kornilev, V. I.; Zhdanov, Y. A. *Russ. J. Gen. Chem.* **1998**, *68*, 1168–1169. (i) Schlessinger, R. H.; Pettus, L. H. *J. Org. Chem.* **1998**, *63*, 9089–9094. (j) Jiang, S.; Rycroft, A. D.; Singh, G.; Wang, X.-Z.; Wu, Y.-L. *Tetrahedron Lett.* **1998**, *39*, 3809–3812. (k) Hu, Y.-J.; Huang, X.-D.; Yao, Z.-J.; Wu, Y.-L. *J. Org. Chem.* **1998**, *63*, 2456–2461. (l) Schmidt, R. R.; Betz, R. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 430–431. (m) Danishefsky, S. J.; Pearson, W. H.; Segmuller, B. E. *J. Am. Chem. Soc.* **1985**, *107*, 1280–1285. (n) López-Herrera, F. J.; Sarabia-Garcia, F. *Tetrahedron* **1997**, *53*, 3325–3346. (o) Tsukamoto, H.; Takahashi, T. *Tetrohedron Lett.* **1997**, *38*, 6415–6418. (p) Miynarski, J.; Banaszek, A. *Tetrahedron: Asymmetry* **2000**, *11*, 3737–3746. (q) Kumaran, G.; Mootoo, D. R. *Tetrahedron Lett.* **2001**, *42*, 3783–3785. (r) Gao, J.; Härter, R.; Gordon, D. M.; Whitesides, G. M. *J. Org. Chem.* **1994**, *59*, 3714–3715, and a listing of earlier approaches cited therein. For enzymatic syntheses of KDO and related compounds, see: (s) Kragl, U.; Gödde, A.; Wandrey, C.; Lubin, N.; Augé, C. *J. Chem. Soc., Perkin Trans. 1* **1994**, 119–124. (t) Sugai, T.; Shen, G.-J.; Ichikawa, Y.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 413–421. (u) Augé, C.; Bouxom, B.; Cavayé, B.; Gautheron, C. *Tetrahedron Lett.* **1989**, *30*, 2217–2220. (v) Bednarski, M. D.; Crans, D. C.; DiCosimo, R.; Simon, E. S.; Stein, P. D.; Whitesides, G. M.; Schneider, M. J. *Tetrahedron Lett.* **1988**, *29*, 427–430.
6. For recent chemical syntheses of KDN and related compounds

see: (a) Burke, S. D.; Voight, E. A. *Org. Lett.* **2001**, *3*, 237–240, and a listing of earlier approaches cited therein. Synthesis work not mentioned in (a). (b) Shen, X.; Wu, Y.-L.; Wu, Y. K. *Helv. Chem. Acta* **2000**, *83*, 943–953. (c) Li, C.-J.; Chan, T.-H. *Tetrahedron* **1999**, *55*, 11149–11176. Review for chemoenzymatic syntheses see: (d) Fessner, W.-D.; Walter, C. *Top. Curr. Chem.* **1996**, *184*, 97.

7. (a) Wu, W.-L.; Wu, Y.-L. *Tetrahedron Lett.* **1992**, *33*, 3887–3890. (b) Wu, W.-L.; Wu, Y.-L. *J. Chem. Soc., Perkin Trans. 1* **1992**, 2705–2707. (c) Wu, W.-L.; Wu, Y.-L. *J. Chem. Soc., Chem. Commun.* **1993**, 821–822. (d) Wu, W.-L.; Wu, Y.-L. *J. Chem. Soc., Perkin Trans. 1* **1993**, 3081–3086. (e) Wu, W.-L.; Yao, Z.-J.; Li, Y.-L.; Li, J.-C.; Xia, Y.; Wu, Y.-L. *J. Org. Chem.* **1995**, *60*, 3257–3259.
8. Li, L.-S.; Wu, Y.-L. *Tetrahedron Lett.* **2002**, *43*, 2427–2430.
9. Regeling, H.; de Rouville, E.; Chittenden, G. J. F. *Recl. Trav. Chim. Pays-Bas* **1987**, *106*, 461–464.
10. (a) Banwell, M.; De Savi, C.; Watson, K. *J. Chem. Soc., Perkin Trans. 1* **1998**, 2251–2252. (b) Kang, S. N.; Choi, H.; Kim, J. S.; Youn, J.-H. *Chem. Commun.* **2000**, 227–228.
11. Yamamoto, T.; Kumazawa, H.; Inami, K.; Teshima, T.; Shiba, T. *Tetrahedron Lett.* **1992**, *33*, 5791–5794.
12. Doane, W. M.; Shasha, B. S.; Russell, C. R.; Rist, C. E. *J. Org. Chem.* **1967**, *32*, 1080–1083.
13. (a) Mitsunobu, O. *Synthesis* **1981**, 1–28. (b) Brussani, G.; Ley, S. V.; Wright, J. L.; Williams, D. J. *J. Chem. Soc., Perkin Trans. 1* **1986**, 303–307.