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Synthetic Approach toward Antibiotic Tunicamycins

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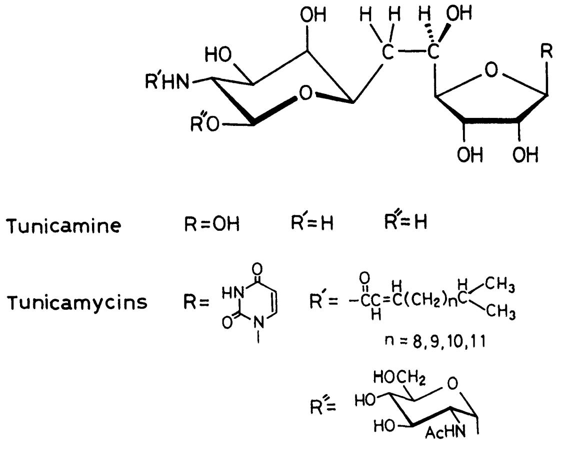
Higher-carbon carbohydrates, undecose, and dodecose derivatives have been synthesized by base-catalyzed addition between nitro sugar and sugar aldehydes. The addition reaction of 3,5-*O*-benzylidene-6,7-dideoxy-1,2-*O*-isopropylidene-7-nitro-α-D-*gluco*-heptofuranose (**3**) with 2,3 : 4,5-di-*O*-isopropylidene-D-arabinose yielded two isomeric dodecose derivatives. The analogous addition of **3** with 2,4-*O*-ethylidene-D-erythrose afforded an undecose derivative.

The nucleoside antibiotics tunicamycins exhibit broad-spectrum antitumor activity.[1] They have a unique common structure, consisting as they do of uracil, fatty acids, and an undecose derivative named tunicamine as the carbohydrate moiety, together with *N*-acetyl-D-glucosamine. The tunicamine is, in turn, bound to the uracil residue, designated as the tunicaminyl uracil[5] (Scheme 1).



Scheme 1.

The higher-carbon carbohydrates, such as undecose and decose derivatives, have been found in other nucleoside antibiotics, anthelmycin (hikizimycin),[2,3] and sinefungin.[4]

Since carbon-chain elongation toward the higher-carbon carbohydrates has not been well established, a synthesis of tunicamine is an attractive challenging target for a carbohydrate chemist. In synthetic chemistry, several methods of preparing higher-carbon sugar have been described in the literature.[6–10] More recently, two undecose derivatives have been prepared by a method involving the condensation of galactose 6-phosphorane with two pentose aldehydes.[11] A hexadecose derivative has been synthesized by the oxidative dimerization of acetylenic sugar, followed by catalytic hydrogenation.[12]

As one of the methods of lengthening a carbon-chain in carbohydrates, the base-catalyzed addition of nitroalkanes with aldehyde has been applied;[13–16] such reactions have been widely used for ascensions of sugar chains.[17–19] However the reagents are restricted to low-molecular-weight nitro compounds, such as nitromethane,[20] nitroethane,[21] and nitroethanol.[22,23] Furthermore, neither the reaction of nitro sugar with aldehyde nor that of nitro sugar with sugar aldehyde has been described in the literature as a synthetic approach to a higher-carbon carbohydrate. Therefore, as a preliminary synthetic study toward the tunicamine, the possibility of the formation of higher-carbon sugar by the addition reaction between nitro sugar and sugar aldehyde has been explored. In the present article we wish to report the development of addition reactions for undecose and dodecose derivatives.

Results and Discussion

The addition of 3,5-*O*-benzylidene-6,7-dideoxy-1,2-*O*-isopropylidene-7-nitro-α-D-*gluco*-heptofuranose (**3**) to 2,3 : 4,5-di-*O*-isopropylidene-D-arabinose[24] (**4**) and 2,4-*O*-ethylidene-D-erythrose[25] (**9**) afforded dodecose (**5** and **6**) and an undecose derivative (**10**) respectively.

As a starting material, the readily accessible 3,5-*O*-benzylidene-1,2-*O*-isopropylidene-α-D-glucofuranose[26] (**1**) was used. The oxidation of **1** by the Pfitzner-Moffatt method[27,28] afforded 3,5-*O*-benzylidene-1,2-*O*-isopropylidene-α-D-*gluco*-1,6-dialdo-1,4-furanose, which was further converted to the 7-nitro compound (**2**) in a 61% yield by adding nitromethane in the presence of sodium methoxide in methanol.

The dehydration of **2** with acetic anhydride in chloroform, followed by hydrogenation with sodium borohydride, produced the 6-deoxy-7-nitro compound (**3**) in a 47% yield.

When **3** reacted with the arabinose derivative, **4**, in the presence of sodium methoxide, a mixture of two isomeric dodecose derivatives (**5** and **6**) was obtained in a 44% yield after chromatography. Attempts were made to isolate each compound, but only **6** was isolable as crystals. The structure of **6** was established by ^{1}H NMR and IR as 3,5-*O*-benzylidene-6,7-dideoxy-1,2: 9,10 : 11,12-tri-*O*-isopropylidene-7-nitro-α-dodeco-1,4-furanose.

The catalytic hydrogenation of the mixture of **5** and **6** in ethyl acetate in the presence of Raney nickel afforded a crude mixture of reduction products. The subsequent *N*-acetylation of this mixture, followed by chromatography, produced two crystalline *N*-acetylaminodideoxydodecoses (**7** and **8**) in 51 and 24% yields respectively.

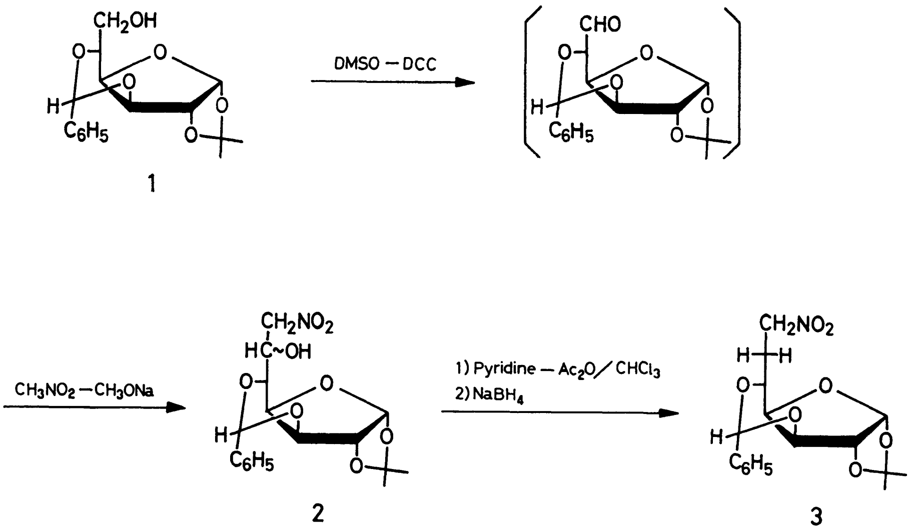
The structures of **7** and **8** were determined by means of the ^{1}H NMR and mass spectra. The ^{1}H NMR spectrum of **7** revealed 18 proton signals at δ 1.2—1.5 attributable to the three *O*-isopropylidene groups, and a sharp singlet of 3-protons at δ 1.85 attributable to the acetamido group. The mass spectrum of **7** yielded the molecular ion peak [M$^+$] at *m/e* 593 and the fragmentation peak [M$^+$—15] at *m/e* 578. The ^{1}H NMR spectrum of **8** showed patterns of the signals similar to those observed for **7**. The mass spectrum of **8** gave the same ion peaks at *m/e* 593 and 578. Concerning the stereochemistry of the two newly introduced chiral centers on C-7 and 8 of the compounds, four diastereomers are theoretically possible, but these configurations have not yet been established.

An analogous addition reaction was carried out between **3** and **9** to give a mixture of the two products. Only the main component (**10**) was isolated as homogeneous crystals in a 32% yield by chromatography. The catalytic hydrogenation of **10** in the presence of Raney nickel, followed by *N*-acetylation, afforded the *N*-acetylaminodideoxyundecose (**11**).

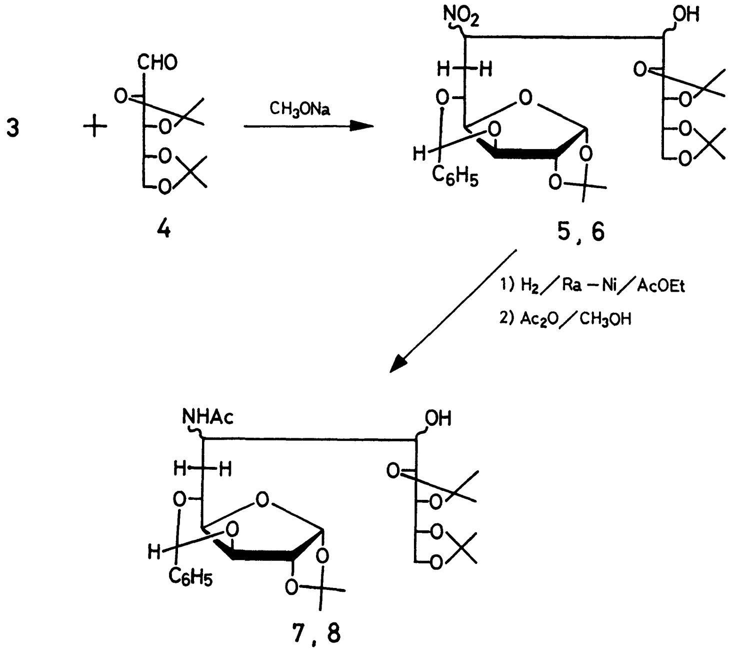
It has been demonstrated by the present study that a higher-carbon carbohydrate is prepared by a base-catalyzed addition between a nitro sugar and a sugar aldehyde. The reaction proceeds quite smoothly and can be used as a general method for the synthesis of higher-carbon complex carbohydrates.

Experimental

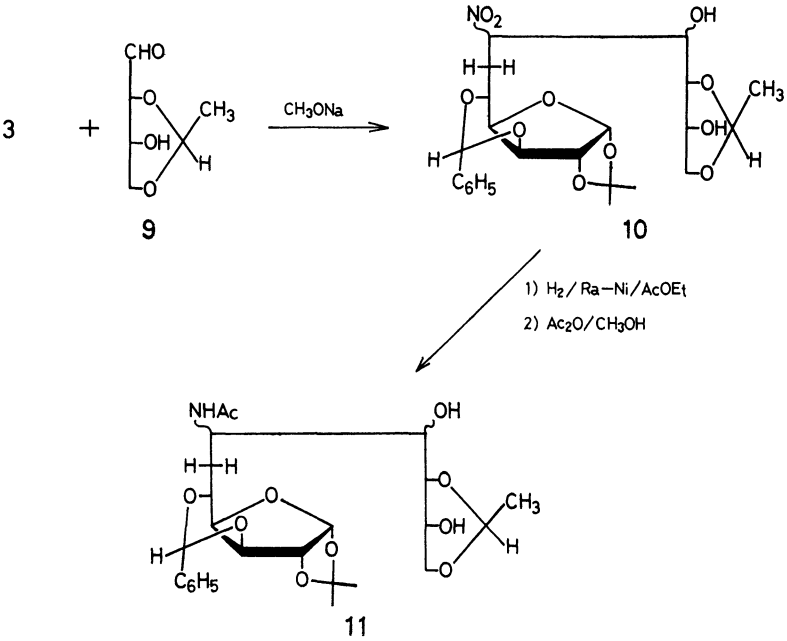
General Methods. The melting points were taken in capillary tubes in a liquid bath and are uncorrected. Solutions were concentrated under reduced pressure below 50 °C. The IR spectra were measured with a Hitachi 225 spectrophotometer and are expressed in reciprocal centimeters. The ^{1}H NMR spectra were obtained on a Varian EM-360A (60 MHz) spectrometer. The chemical shifts are reported as δ values



Scheme 2.



Scheme 3.



Scheme 4.

in parts per million relative to tetramethylsilane as an internal standard. The mass spectra were obtained with a Hitachi RMU-6MG spectrometer. The TLC was performed on precoated silica gel 60 F-254 plaques (Merck, Darmstadt; Art. 5715, 0.25 mm thickness). The silica-gel columns used for chromatography utilized Wako gel C-200 (Wako Pure Chemical Industries, Ltd.).

*3,5-O-Benzylidene-7-deoxy-1,2-O-isopropylidene-7-nitro-α-*DL-glycero-D-gluco-*hepto-*1,4*-furanose (2).* Into a solution of 3,5-*O*-benzylidene-1,2-*O*-isopropylidene-α-D-glucofuranose[26] (**1**, 2.0 g) in benzene (8 ml) and dimethyl sulfoxide (16 ml) we stirred dichloroacetic acid (0.4 ml), dicyclohexylcarbodiimide (2.0 g), and pyridine (0.8 ml) under ice cooling. After the mixture was stirred for 5 h at an ambient temperature, a suspension of oxalic acid (2.0 g) in methanol (2.0 ml) was added to the solution. The mixture was then diluted with cold water (20 ml) and extracted with chloroform. The chloroform layer was washed with a $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated. To a solution of the residue in methanol (20 ml) we added nitromethane (6 ml) and 1 M methanolic sodium methoxide (6.5 ml). After standing 1 h, the solution was neutralized with Amberlite IR-120B (H^+) resin and concentrated. The resdiue was chromatographed on a silica-gel column using 1: 10 (v/v) 2-butanone–toluene; the product was then recrystallized from benzene–cyclohexane to give 1.45 g (61%) of **2**; mp 176.5—177.5 °C, $[\alpha]_D^{24}$ −58° (*c* 0.5, chloroform). IR(KBr) 3520 (OH), 1565, 1395 cm^{-1} (NO_2).

Found: C, 55.30; H, 5.74; N, 3.64%. Calcd for $C_{17}H_{21}NO_8$: C, 55.58; H, 5.76; N, 3.81%.

*3,5-O-Benzylidene-6,7-dideoxy-1,2-O-isopropylidene-7-nitro-α-*D-gluco-*hepto-1,4-furanose (3).* Into a suspension of **2** (1.28 g) in chloroform (22 ml) we stirred acetic anhydride (1.56 ml) and pyridine (0.8 ml). After 5 h, the chloroform solution was washed with a $NaHCO_3$ solution, and cold water, dried over Na_2SO_4, and concentrated. $NaBH_4$ (0.8 g) was added to a solution of the residue in ethanol (30 ml). After 1 h, the solution was neutralized with Amberlite IR-120B (H^+) resin and concentrated. The residue was recrystallized from ethanol to give 614 mg (47%) of **3**; mp 154 °C, $[\alpha]_D^{24}$ +89° (*c* 1.0, chloroform). IR(KBr) 1555, 1387 cm^{-1} (NO_2).

Found: C, 58.07; H, 5.97; N, 3.93%. Calcd for $C_{17}H_{21}NO_7$: C, 58.11; H, 6.02; N, 3.99%.

3,5-O-Benzylidene-6,7-dideoxy-1,2: 9,10: 11,12-tri-O-isopropylidene-7-nitro-α-dodeco-1,4-furanose (5 and 6). To a solution of **3** (0.5 g) and 2,3: 4,5-di-*O*-isopropylidene-D-arabinose[24] (**4**, 1.6 g) in methanol (4 ml) and tetrahydrofuran (3 ml) we added 1 M methanolic sodium methoxide (2 ml) under ice cooling. After 3 h at an ambient temperature, the solution was neutralized with Amberlite IR-120B(H^+) resin and concentrated below 30 °C. The residue was chromatographed on a silica-gel column using 20: 1 (v/v) chloroform–ethyl acetate. Fractions homogeneous on TLC (R_f 0.25) in 5: 1 (v/v) chloroform–ethyl acetate gave 75 mg of **6** as crystals; mp 149—150 °C, $[\alpha]_D^{23}$ +38.4° (*c* 0.55, methanol). IR(KBr) 3440 (OH), 1560, 1385 (NO_2), 760, 705 cm^{-1} (C_6H_5).

Found: C, 57.58; H, 6.58; N, 2.47%. Calcd for $C_{28}H_{39}NO_{12}$: C, 57.82; H, 6.76; N, 2.42%.

Compound **5** (R_f 0.34 on TLC) was not obtained as pure crystals. The total yield of the mixture of **5** and **6** was 44% (361 mg).

7-Acetamido-3,5-O-benzylidene-6,7-dideoxy-1,2: 9,10: 11,12-tri-O-isopropylidene-α-dodeco-1,4-furanose (7 and 8). A solution of the mixture of **5** and **6** (75 mg) in ethyl acetate (5 ml) was hydrogenated in the presence of Raney nickel at an initial H_2 pressure of 2.7 kg/cm^2 for 18 h. The catalyst was then filtered off, and the filtrate was concentrated. The *N*-acetylation of the residue with acetic anhydride (0.3 ml) in methanol (3 ml) was followed by purification by column chromatography, using 1: 1 (v/v) chloroform–ethyl acetate, to give 39 mg (51%) of **7** and 18 mg (24%) of **8**.

7: Mp 126—127 °C, $[\alpha]_D^{23}$ +24.6° (*c* 0.3, methanol), R_f 0.29 on TLC in 1: 2 (v/v) chloroform–ethyl acetate. ^1H NMR ($CDCl_3$) δ 1.2—1.5 (m, 18, $3C(CH_3)_2$), 1.85 (s, 3, NAc), 5.84 (s, 1, benzylidene CH), 5.98 (d, 1, $J_{1,2}$=3 Hz, H-1); mass spectrum *m/e* 593 [M^+], 578 [M^+-15].

Found: C, 60.46; H, 7.27; N, 2.39%. Calcd for $C_{30}H_{43}NO_{11}$: C, 60.70; H, 7.30; N, 2.36%.

8: Mp 188—189.5 °C, $[\alpha]_D^{23}$ +60.8° (*c* 0.67, methanol)

R_f 0.18 on TLC in the same solvent. ^{1}H NMR ($CDCl_3$) δ 1.3—1.5 (m, 18, 3$C(CH_3)_2$), 1.96 (s, 3, NAc), 5.84 (s, 1, benzylidene CH), 6.00 (d, 1, $J_{1,2}$=3 Hz, H-1); mass spectrum *m/e* 593 [M^+], 578 [M^+-15].

Found: C, 60.46; H, 7.21; N, 2.37%. Calcd for $C_{30}H_{43}NO_{11}$: C, 60.70; H, 7.30; N, 2.36%.

*3,5-O-Benzylidene-6,7-dideoxy-9,11-O-ethylidene-1,2-O-isopropylidene-7-nitro-α-D-undeco-1,4-furanose (**10**).* Into a solution of **3** (368 mg) and 2,4-*O*-ethylidene-D-erythrose[25] (**9**, 300 mg) in tetrahydrofuran (3 ml) and methanol (2 ml) we stirred 1 M methanolic sodium methoxide (1.2 ml). After 3 h, the reaction solution was worked up analogously to the preparation of **5** and **6** to give 167 mg (32%) of **10** as crystals; mp 81—83 °C, $[\alpha]_D^{15}$ +33.4° (*c* 0.35, methanol). IR(KBr) 3430 (OH) 1555, 1380 cm^{-1} (NO_2), ^{1}H NMR($CDCl_3$) δ 1.33, 1.50 (2s, 6, $C(CH_3)_2$), 5.60 (s, 1, benzylidene CH), 6.00 (d, 1, $J_{1,2}$=3 Hz, H-1), 7.33 (broad s, 5, C_6H_5).

Found: C, 55.79; H, 6.30; N, 2.88%. Calcd for $C_{23}H_{31}NO_{11}$: C, 55.53; H, 6.28; N, 2.82%.

Two components were detectable on TLC in the crude product, but the other component was not obtained as a pure crystalline product.

*7-Acetamido-3,5-O-benzylidene-6,7-dideoxy-9,11-O-ethylidene-1,2-O-isopropylidene-α-D-undeco-1,4-furanose (**11**).* A solution of **10** (101 mg) in ethyl acetate (3 ml) was hydrogenated and subsequently worked up analogously to the preparation of **7** and **8** to give 48 mg (46%) of **11**; mp 85—86 °C, $[\alpha]_D^{14}$ +8.4° (*c* 0.5, methanol). IR(KBr) 3390 (OH), 1650 cm^{-1} (C=O). ^{1}H NMR ($CDCl_3$) δ 1.27 (d, 3, J=4.5 Hz, ethylidene CH_3), 1.33, 1.50 (2s, 6, $C(CH_3)_2$), 1.73 (s, 3, NAc), 5.62 (s, 1, benzylidene CH), 5.98 (d, 1, $J_{1,2}$=3 Hz, H-1), 7.37 (broad s, 5, C_6H_5).

Found: C, 59.06; H, 6.97; N, 2.97%. Calcd for $C_{25}H_{35}NO_{10}$: C, 58.93; H, 6.92; N, 2.74%.

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