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Synthesis of TDP-3-Amino-3,4,6-trideoxy-α-D-*xylo*-hexopyranose—The Immediate Precursor of TDP-α-D-Desosamine

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Abstract—A synthetic pathway producing the title compound starting from methyl α-D-glucose is described. This compound was shown to be a substrate for DesVI, an AdoMet-dependent methyltransferase which catalyzes *N,N*-dimethylation of the title compound to give a biological significant unusual sugar, desosamine.

Aminosugars have been found in a wide variety of natural products.[1] Since an amino group can be protonated, alkylated, or acetylated to alter its capability to form hydrogen-bond(s) and to bear charge, its presence can greatly influence the hydrophobicity and surface properties of the natural product structure as a whole. For example, the amino group of daunosamine (**1**) in doxorubicin (**2**) may be the recognition site for the multidrug transporter P-glycoprotein in drug-resistant tumor cells. Substitution of this basic amino group with a neutral hydroxy group either partially or completely circumvents the resistance due to the reduced transportation by P-glycoprotein.[2] Indeed, aminosugars have been found to play important roles for the biological activities of many macrolide and aminoglycoside antibiotics.[3] A particularly notable example is desosamine (**3**), a 3-*N*,*N*-dimethylamino-3,4,6-trideoxyhexose, which has been found in many macrolide antibiotics, including erythromycin (**4**), methymycin (**5**), pikromycin, and oleandomycin.

It has been shown that des-*N*-methylerythromycin, in which desosamine is mono-demethylated, retains only 5% of the antibacterial activity when compared to erythromycin.[4] Further experiments also indicated that modification of the *N*,*N*-dimethyl group of desosamine resulted in the loss of this drug's antibacterial activity, but enhanced its motor-stimulating effect on gastrointestinal muscle.[5] In fact, the dimethylamino group of desosamine in erythromycin can be oxidized by cytochrome P-450 in liver, affecting the metabolism of other drugs.[6] The basic amino group on erythromycin and other antibiotics of the same type may influence the uptake of these drugs by phagocytes, thus affecting their delivery to the infection sites.[7]

Studies of the biosynthesis of desosamine have established the intermediacy of an amino-sugar, TDP-3-amino-3,4,6-trideoxy-α-D-*xylo*-hexopyranose (**6**), in the pathway.[8] Consequently, *N,N*-dimethylation of **6** has been shown to be the last step in desosamine formation. In an effort to study the biosynthesis of methymycin (**5**)/pikromycin in *Streptomyces venezuellae*,[8c,d] we have found that *N,N*-dimethylation of **6** to make TDP-desosmaine (**7**) is catalyzed by DesVI, which is an *S*-adenosylmethionine (AdoMet) dependent methyltransferase (Scheme 1).[9] This enzyme has been overexpressed and purified. To elucidate the mechanism of DesVI catalyzed *N,N*-dimethylation steps, we have developed an efficient sequence to prepare the substrate **6**. Reported herein is the synthesis of this compound, which has later been converted to TDP-desosamine (**7**) by DesVI in a stepwise manner via a monomethylated intermediate.

As depicted in Scheme 2, the first stage of the synthesis calls for the preparation of the key intermediate **10**. Preparation of this compound was initiated by using a known protocol to remove both C-4 and C-6 hydroxyl groups of glucose to make 4,6-dideoxyhexose **9**.[10]

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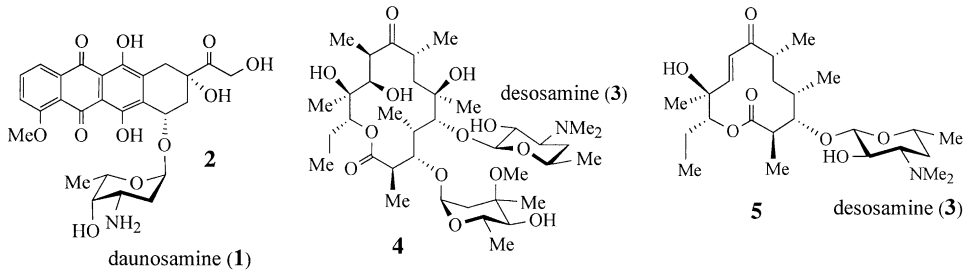
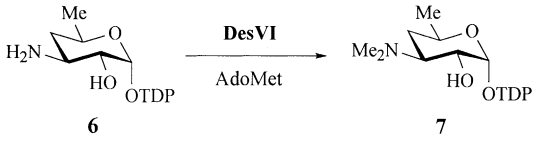
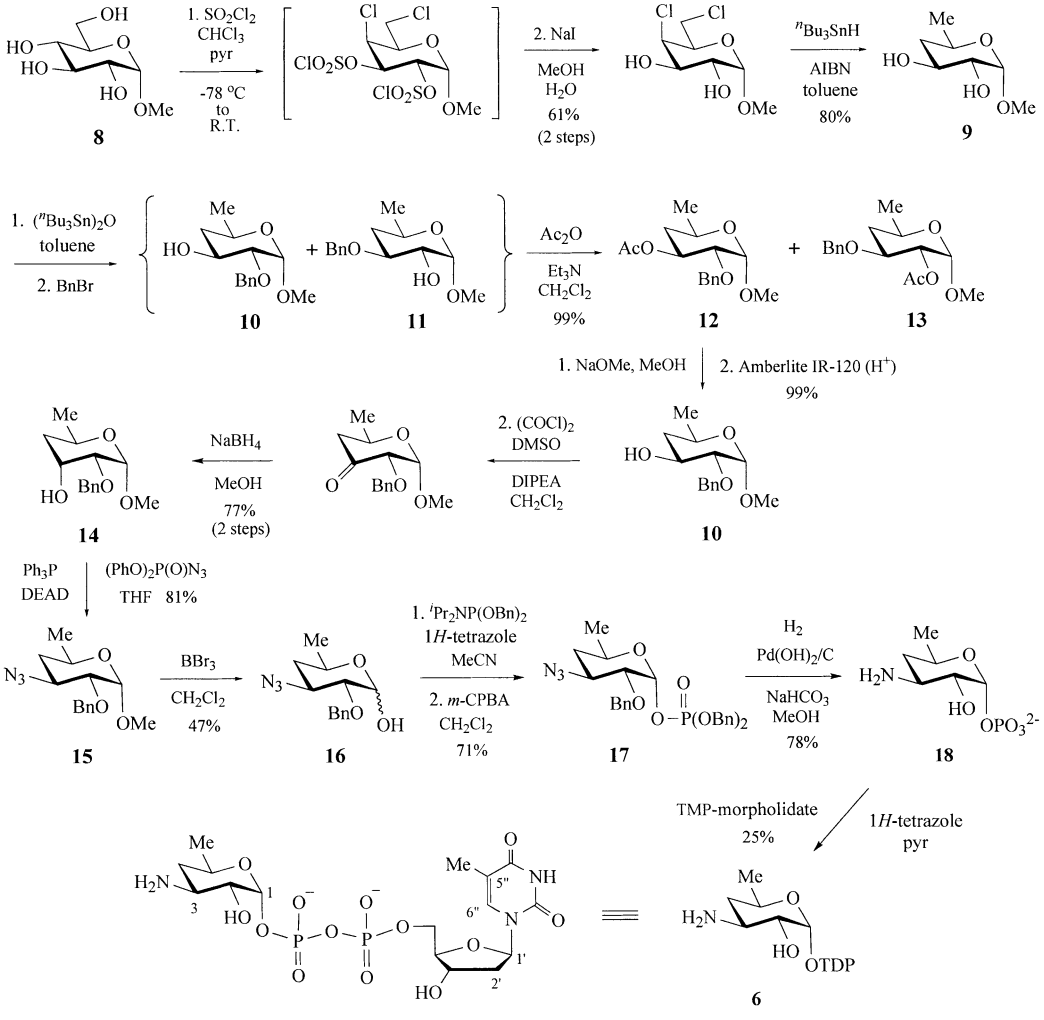


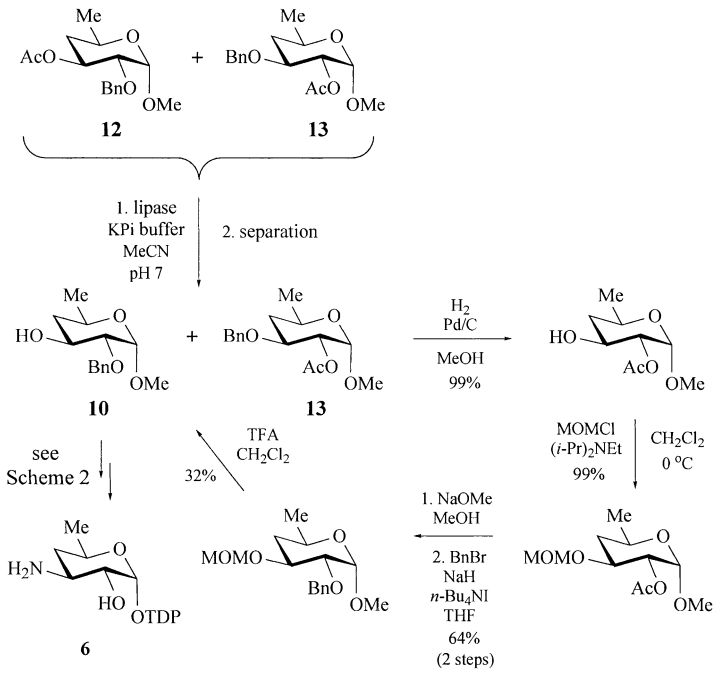
Attempts to carry out this transformation directly on α-D-glucose proved to be problematic. However, much improved yield was achieved when methyl α-D-glucoside (**8**) was used as the starting material. Subsequent benzylation of **9** mediated by bis(tributyltin) oxide[11] led to a 2:1 mixture of monobenzylated **10** and **11**. The regioselectivity for C-2 alkylation is lower than expected and may be a result of C-4 deoxygenation, which renders the C-3 hydroxyl group more nucleophilic and less sterically hindered for alkylation. Since **10** and **11** had nearly identical chromatographic properties, it was difficult to isolate the desired 2-*O*-benzylated **10** in pure form. Acetylation of this mixture, however, provided a pair of regioisomers, **12** and **13**, which could be resolved by repeated chromatography. The desired isomer **12** was collected and subjected to base hydrolysis to give **10**. The configuration of the 3-OH group in **10** was inverted from equatorial to axial in **14** by Swern oxidation and $NaBH_4$ reduction in sequence. The masked amino functionality was introduced into **14** at C-3 under Mitsunobu conditions using diphenylphosphoryl azide as the azide source to afford **15**.[12] Removal of the anomeric methoxyl



Scheme 1.



Scheme 2.



Scheme 3.

group was accomplished by treatment of **15** with BBr_3 at low temperature[13] and the product **16** was benzylphosphorylated to generate compound **17**. The unprotected aminosugar-1-phosphate **18** was obtained in one pot via hydrogenation catalyzed by Dugassa type $Pd(OH)_2$.[14] The final step involved coupling of **18** with TMP-morpholidate in the presence of 1*H*-tetrazole.[15] The crude product was purified using P2 column chromatography (eluted with 25 mM ammonium bicarbonate) and FPLC equipped with a MonoQ column (eluted with a linear gradient of 0–0.5 M ammonium bicarbonate buffer over 15 min) to give **6**, whose structure was confirmed by spectral analyses.[16]

Although synthesis according to the above reaction sequence had provided sufficient quantity of compound **6** for initial enzymatic study, attempts to carry out large-scale preparation were hampered by the tediousness of isolation of multi-grams of **10** via repetitive chromatography. Thus, efforts were directed to develop a more efficient method to separate **10**/**11** or **12**/**13**. Since lipases are known to be able to catalyze chemo-, regio- and/or stereoselective hydrolysis of a variety of esters and/or amides,[17] the feasibility of using a lipase catalyzed regioselective deacylation to achieve differentiation between **12** and **13** was explored. Among three lipases tested, Type I from wheat germ, Type II from porcine pancreas, and Type VII from *Candida rugosa*, only Type I lipase from wheat germ showed selective activity toward **12**. Using the wheat germ lipase, compound **12** could be converted to **10**, while compound **13** remained intact. The optimal selectivity and turnover ratio were achieved using 10% acetonitrile as the cosolvent (in 50 mM potassium phosphate buffer, pH 8.0). Since compounds **10** and **13** are no longer a pair of regioisomers, they can be readily separated by chromatography on silica gel (10–30% ether in hexanes). This procedure had been successfully applied to obtain pure **10** from a mixture of 24 g of **12** and **13** using 5 g of lipase.[18] Since the recovered isomer **13** could be converted to the desired **10** via a recycling route as illustrated in Scheme 3, the combined yield of **10** was greater than 56% based on the total mass of the original mixture of isomers **12** and **13**.

In summary, we have developed a practical method for the preparation of TDP-3-amino-3,4,6-trideoxyhexose **6**, which is the immediate precursor of desosamine (**3**). The synthetic sequence combines chemical and enzymatic reactions to achieve dideoxygenation at C-4 and C-6, and differentiation of the C-2 and C-3 hydroxyl groups. Separation of the structural isomers, **12** and **13**, was facilitated by regioselective hydrolysis catalyzed by lipase. The use of a dialysis bag to confine the lipase during the incubation[18] is worthy of mentioning. Since it not only alleviates the common emulsion problem associated with the chemoenzymatic synthesis, but also enables us to recycle the lipase. Using the synthetic **6** as the substrate, we have unequivocally established the catalytic role of DesVI to be an AdoMet-dependent methyltransferase. The *N*,*N*-dimethylation proceeds via a stepwise manner, and the K_m and k_{cat} values for **6** were determined to be 307.4 μM and 92.0 min^{-1}, respectively, when the concentration of AdoMet was maintained at 3.0 mM.

Acknowledgements

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16. ^{1}H NMR (D_2O) δ 1.24 (3H, d, J = 6.3 Hz, 5-Me), 1.50 (1H, ddd, J = 11.7, 12.3, 12.7 Hz, 4-H_{ax}), 1.93 (3H, s, 5″-Me), 2.27 (1H, ddd, J = 3.0, 4.0, 12.7 Hz, 4-H_{eq}), 2.40 (2H, m, 2′-Hs), 3.59 (1H, ddd, J = 4.0, 10.7, 12.2 Hz, 3-H), 3.74 (1H, ddd, J = 3.4, 2.9, 10.7 Hz, 2-H), 4.20 (3H, m, 4′- and 5′-Hs), 4.29 (1H, ddq, J = 3.0, 11.7, 6.3 Hz, 5-H), 4.62 (1H, dt, J = 3.0, 5.8 Hz, 3′-H), 5.63 (1H, dd, J = 3.4, 7.3 Hz, 1-H), 6.35 (1H, t, J = 6.8 Hz, 1′-H), 7.71 (1H, s, 6″-H); ^{13}C NMR (D_2O) δ 11.6, 19.5, 35.3, 38.5, 48.9, 65.4, 69.5 (d, J = 6.6 Hz), 70.9, 84.9, 85.0, 85.3 (d, J = 10.1 Hz), 95.0 (d, J = 8.0 Hz), 111.7, 137.3, 151.7, 166.5. ^{31}P NMR (D_2O) δ −12.4 (d, J = 20.9 Hz), −10.5 (d, J = 20.9 Hz). High-resolution FABMS calcd for $C_{16}H_{28}N_3O_{13}P_2$ ($M+H^+$) 532.1097, found 532.1098.

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18. The wheat germ lipase (5 g) dissolved in 50 mM potassium phosphate buffer (pH 7.0, 20 mL) was put in a dialysis bag and placed in a solution of **12** and **13** (24.0 g, 81.6 mmol) in the same phosphate buffer (800 mL) containing 10% CH_3CN (80 mL). After gentle stirring for 2–3 days at 4 °C, the reaction solution was extracted with chloroform. The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure. The desired product **10** and the unreacted **13** were then separated by gradient column chromatography on silica gel (10–30% ether/hexanes).