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The conversion of pentoses to 3,4-dihydroxyprolines

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Abstract—The synthesis of two naturally-occurring isomers of 3,4-dihydroxyproline is reported. L-2,3-*cis*-3,4-*trans*-3,4-Dihydroxyproline was synthesized from L-arabinose in 10 steps and 31% overall yield. The same series of reactions was employed to convert L-xylose to L-2,3-*trans*-3,4-*trans*-3,4-dihydroxyproline. Orthogonally protected versions of these amino acids were produced on gram scale, en route to the free amino acids, and these will serve as versatile intermediates in peptide synthesis. This synthetic strategy involved *N*α-Fmoc protection and protection of the C3 and C4 secondary alcohols as methoxyethoxymethyl (MEM) ethers.

1. Introduction

3,4-Dihydroxyproline (DHP)[1] contains three stereogenic centers: C2, C3 and C4 and so there are eight possible stereoisomers. Three members of the L-series have been isolated from natural sources (Fig. 1). The L-2,3-cis-3,4-trans isomer (**1**) was isolated from the cell wall of the diatom *Navicula pelliculosa* more than 30 years ago.[2] In 1980, the L-2,3-trans-3,4-trans isomer (**2**) was isolated from the acid hydrolysates of the toxic mushroom *Amanita virosa*[3] and identified in the virotoxin cyclic heptapeptides.[4] In 1994, the L-2,3-trans-3,4-cis isomer **3** was identified as the sixth residue in the repeating decapeptide sequence of Mepf1, an adhesive protein produced by the marine mussel *Mytilus edulis*.[5]

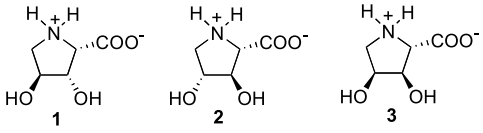

Figure 1. Naturally occurring 3,4-dihydroxyprolines.

There are many syntheses of dihydroxyprolines in the literature.[6] When we began our bid to synthesize the Mefp1 decapeptide,[7] we utilized the approach of Fleet and co-workers[8] to prepare a suitably protected derivative of **3** from D-gulonolactone. Our long term goal, however, was to investigate the role of 3,4-dihydroxyprolines in nature and as such we required a synthesis, which was capable of delivering any of the eight stereoisomers. Most other approaches are limited in this regard to the preparation of only a subset of these target molecules. For example, *syn*-dihydroxylation of a 3,4-dehydroproline can lead only to DHPs with 3,4-cis relative stereochemistry.[9,10] Conversely, the opening of an epoxide has been a useful tool in the synthesis of DHPs with a 3,4-trans relative stereochemistry.[11,12]

Our goal over the past several years has been to develop a synthesis of 3,4-dihydroxyprolines, which should be amenable to producing useful quantities of any stereoisomer, in an efficient and stereochemically predictable manner. We reasoned that the eight stereoisomers of 3,4-dihydroxyproline ought to be accessible from the eight pentose sugars, utilizing Fleet's double displacement chemistry, which we had adopted previously to good effect.[13] The configuration at C3 and C4 would be derived directly from the sugar and that at C2 inverted during the sequence. The overall retrosynthetic analysis is embodied in Table 1.

2. Results and discussion

In our previous endeavors, we utilized *tert*-butyldimethylsilyl (TBDMS) ethers for protection of the secondary alcohols at C3 and C4 (DHP numbering).[14] Thus, we demonstrated the proof of concept by converting D-ribonolactone (**4**) to amino acid building block **7**.[14a] We later reported full experimental details for the application of this chemistry to the synthesis of two other stereoisomers of compound **7**.[14b] We felt compelled to publish this work, given the amount of effort that had been expended.

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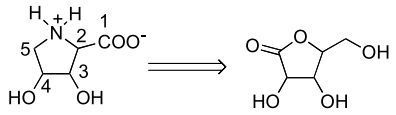
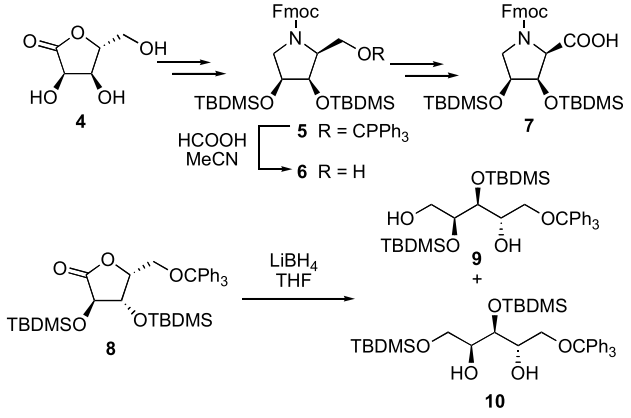
Table 1. Retrosynthetic scheme of 3,4-dihydroxyprolines



DHP isomer	Pentose precursor
L-2,3-cis-3,4-cis-	L-ribose
L-2,3-cis-3,4-trans-	L-arabinose
L-2,3-trans-3,4-cis-	L-lyxose
L-2,3-trans-3,4-trans-	L-xylose
D-2,3-cis-3,4-cis-	D-ribose
D-2,3-cis-3,4-trans-	D-arabinose
D-2,3-trans-3,4-cis-	D-lyxose
D-2,3-trans-3,4-trans-	D-xylose

Unfortunately, the overall efficiency of the synthesis was disappointing and our goal of producing useful quantities of DHP building blocks for peptide synthesis was not realized.

An objective assessment of our previous work (Scheme 1) led us to the conclusion that the problems encountered in the syntheses were directly, or indirectly, associated with the TBDMS protecting group. For example, the reductive opening of the lactone ring in compound **8** was anticipated to give **9** (by analogy to the reduction of two diastereoisomers of **8**). Unfortunately, the major product **10** arose from migration of the silyl group to the primary alcohol.[14b] Another problem in the synthesis involved the chemoselective hydrolysis of the trityl ether from compound **5**; partial removal of one TBDMS group was also observed. The extent of this problem varied from one stereoisomer to another, requiring close monitoring of the reaction progress and fine-tuning of reaction times to optimize the yield of desired products.



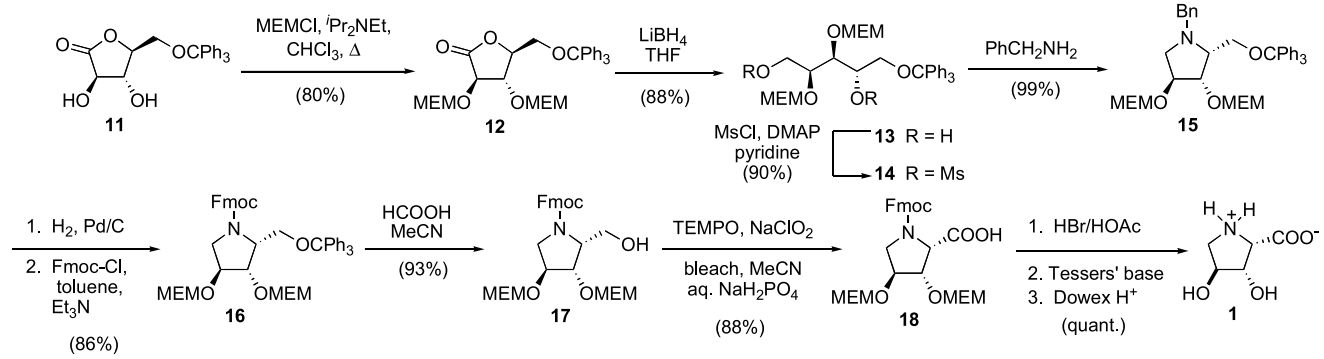
Scheme 1. Important issues from previous work.

We proposed that substitution of the MEM (methoxyethoxymethyl) group for the TBDMS group might improve the viability of the synthesis. Indeed, the use of the MEM protecting group has been described previously for trans-4-hydroxyproline building blocks during the synthesis of collagen mimetics.[15]

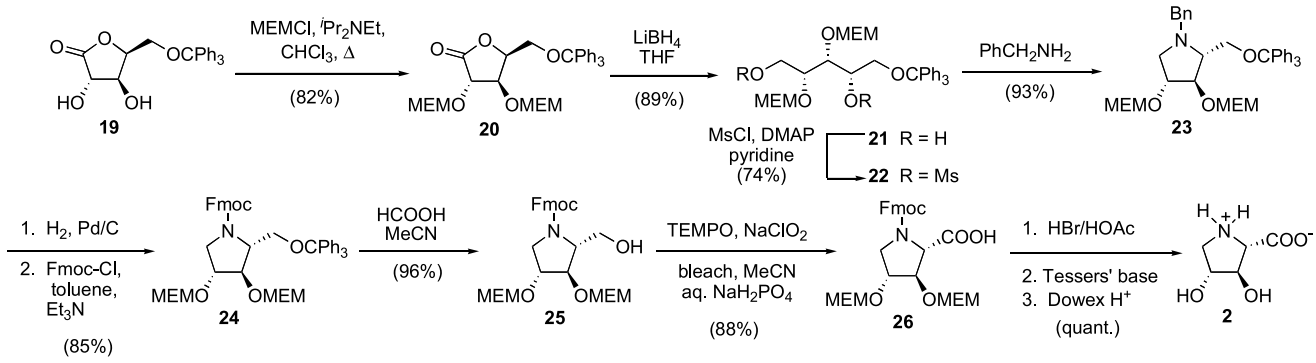
Herein, we report the synthesis of two isomers of 3,4-dihydroxyproline in this manner, on reasonable scale and with greatly improved overall yields. While our original aim was to produce building blocks for peptide synthesis, we are becoming increasingly aware of the need for the free amino acids as authentic samples for comparison in amino acid analyses where the occurrence of 3,4-dihydroxyprolines is suspected in peptides and proteins.[16] Thus, we also describe conditions for producing the free amino acids.

The conversion of compound **11** (derived from L-arabinose) to 2,3-*cis*-3,4-*trans*-3,4-dihydroxy-L-proline (**1**) is summarized in Scheme 2. In our 2002 paper,[14b] we described some of the trials and tribulations associated with producing the 5-*O*-triphenylmethyl ethers typified by **11**. Some further observations and recommendations are given in the Section 4. Standard conditions for the protection of alcohols as MEM ethers (MEMCl, $^{i}Pr_2NEt$, CH_2Cl_2, rt)[17] were ineffective for the conversion of **11** to **12**. Optimized conditions involved heating at reflux in chloroform with 5 equiv of alkylating agent. A drawback of the MEM protecting group, which is perhaps not widely appreciated, is the complexity that it introduces into ^{1}H NMR spectra. Each MEM group introduces a singlet at ~3 ppm; in addition there are three $-CH_2-$ groups, which give rise to six signals, since the two protons of each $-CH_2-$ unit are diastereotopic. ^{1}H NMR spectra of intermediates in Schemes 2 and 3 were thus difficult to fully assign. However, ^{13}C NMR spectra were less complex and provided evidence for the identity and purity of compounds.

Reduction of the fully protected lactone **12** gave diol **13**, which was readily converted to the bis-mesylate **14**. Cyclization to form the pyrrolidine **15** gave an essentially quantitative yield if the benzylamine was distilled immediately prior to the reaction. Benzylamine, which had been distilled in recent weeks and stored over KOH



Scheme 2. Synthesis of L-2,3-*cis*-3,4-*trans*-3,4-dihydroxyproline.



Scheme 3. Synthesis of L-2,3-*trans*-3,4-*trans*-3,4-dihydroxyproline.

gave yields in the order of 60–70%. Due to the MEM ethers, pyrrolidine **15** is much more polar than analogous compounds with other protecting groups at C3 and C4 (acetonide,[13] TBDMS[14]). This facilitated separation of the pyrrolidine from benzylamine-derived byproducts, including benzaldehyde. The benzyl group was removed hydrogenolytically from pyrrolidine **15** and the amine protected as its Fmoc derivative. The trityl ether was removed cleanly in 2 h. The differential in acid lability of the two ethers (trityl vs. MEM) removed all problems associated with the chemoselectivity in this step (vide supra, conversion of **5**→**6**, Scheme 1).

As in our previous work, we intially utilized a two step oxidation of **17**: Swern oxidation to the aldehyde, followed by sodium chlorite oxidation to acid **18**.[14b] The NMR spectra of compound **18** featured very broad peaks, which were doubled up in the ^{13}C NMR. We attributed this to restricted rotation about the carbamate C–N bond. Unfortunately, removal of the protecting groups gave a 3:1 mixture (determined by integration of ^{1}H NMR signals) of two dihydroxyprolines, which appeared to be diastereoisomers. HPLC analysis of compound **18** (from Swern/$NaClO_2$ oxidation of **17**) revealed the same ratio of two similar compounds. Our suspicion was that the α-amino aldehyde derived via Swern oxidation of **17** was undergoing epimerization. This was not observed for the analogous compound bearing TBDMS ethers.[14b] Moreover, others have reported the successful Swern oxidation of similar prolinol compounds.[18]

Ruthenium (III) oxidation of **17** under Sharpless conditions[19] gave acid **18** directly; deprotection yielded a stereoisomerically pure sample of **1**. Our hunch was confirmed, but as we had found earlier,[14a] the yield of this oxidation (36%) was unsatisfactory. A number of recent reports suggested that TEMPO may be the best reagent for this oxidation.[20] Indeed, oxidation of **17** to **18** was achieved in good yield, with no loss of stereochemical integrity.

The deprotection of the amino acid was attempted in a number of ways. Reactions employing TFA/dichloromethane, 95% TFA/H_2O or 1 N HCl in TFA were slow and not clean. The best results were obtained using a solution of HBr in acetic acid. This acid treatment removed the MEM protecting groups. The Fmoc group was removed using Tesser's base[21] and the free amino acid was purified by ion exchange chromatography.

For clarity, the reaction sequence, as applied to the conversion of L-xylose to amino acid building block **26**, is depicted in Scheme 2. The intermediates behaved similarly and yields were comparable.

3. Conclusion

In summary, two isomers of 3,4-dihydroxyproline have been synthesized, via orthogonally protected derivatives. Building blocks **18** and **26** ought to prove useful in peptide synthesis. We believe that the synthetic route should be applicable to the conversion of the appropriate pentose sugar to any of the eight stereoisomers of 3,4-dihydroxyproline. This route has been arrived at via the exploration of several protecting groups and reaction conditions for each step. While the bromine oxidation of the pentose sugars, followed by 5-*O*-trityl ether formation remains a somewhat capricious undertaking, the subsequent steps are highly reproducible. The overall yield for the production of **1** from **11** is 44% (eight steps); this reduces to 31% starting from L-arabinose (10 steps). Likewise, isomer **2** was produced from lactone **19** in 36% yield (eight steps); or 22% yield (ten steps) from L-xylose.

4. Experimental

4.1. General details

All reactions were conducted under a dry nitrogen atmosphere unless otherwise noted. Reagents were obtained from commercial suppliers and used directly with the following exceptions. Tetrahydrofuran was dried over sodium/benzophenone and distilled prior to use. Diisopropylethylamine, triethylamine and pyridine were dried and distilled from CaH_2 and stored over KOH pellets. Acetonitrile and benzylamine were freshly distilled from CaH_2. Methanesulfonyl chloride was best distilled from P_2O_5 immediately prior to use. Flash chromatography was performed using Scharlau 60 silica gel (230–400 mesh) with the indicated solvents. Thin-layer chromatography (TLC) was carried out on precoated silica plates (Merck Kieselgel $60F_{254}$) and compounds were visualized by UV fluorescence or by staining with anisaldehyde or ninhydrin. ^{1}H and ^{13}C NMR spectra were obtained using either a JEOL JNM-GX270W or a Bruker Avance 400 spectrometer. Chemical shifts for spectra in $CDCl_3$ are given in parts per million

(ppm) downfield from tetramethylsilane as internal standard (^{1}H) or relative to residual solvent (^{13}C). Spectra of the free amino acids in D_2O were referenced to DSS as an external standard. High resolution mass spectra were recorded using a VG7070 mass spectrometer operating at nominal accelerating voltage of 70 eV.

4.1.1. 5-*O*-Trityl-L-arabinono-γ-lactone (11). Potassium carbonate (2.26 g, 16.3 mmol, 1.22 equiv) was added in portions, over 2 h, to a solution of L-arabinose (2.00 g, 13.3 mmol, 1.00 equiv) in Milli-Q water (6 mL) at 0 °C. Bromine (0.8 mL, 2.48 g, 15.5 mmol, 1.16 equiv) was then added dropwise, from a dropping funnel (constructed of glass and teflon only) over 2 h. The solution was warmed to rt and left to stir overnight. The solution was still yellow/orange (if it was a not, a couple more drops of bromine were added and the mixture left to stir another 12 h) and the excess bromine was quenched by the addition of neat formic acid (two drops from a Pasteur pipette). Decolorization was not immediate, but was complete within 10 min. The mixture was concentrated on a rotary evaporator, with the water bath at 60 °C. When the volume of the mixture reached ~10 mL, glacial acetic acid (1 mL) was added. Rotary evaporation at 60 °C was continued for at least 2 h and then under high vacuum. The dry residue was suspended in pyridine (25 mL) under N_2. DMAP (325 mg, 2.7 mmol, 0.2 equiv) was added, followed by trityl chloride (4.45 g, 16.0 mmol, 1.2 equiv) and the mixture heated at reflux for 12 h. The brown solution was cooled, diluted with CH_2Cl_2 (300 mL) and washed successively with water (250 mL), 1 M HCl (250 mL), satd aq $NaHCO_3$ (250 mL) and brine (250 mL). The organic layer was filtered through $MgSO_4$ and concentrated. The residue was purified by flash chromatography, eluting with 1:1 EtOAc/hexane to give **11** as a colorless foam (3.71 g, 71%). Data reported elsewhere.[14b]

4.1.2. 5-*O*-Trityl-L-xylono-γ-lactone (19)[22]. By analogy to the procedure in Section 4.1.1, on a scale of 13.3 mmol, affording 5-*O*-trityl-L-xylono-γ-lactone (**19**) as a colorless oil (3.25 g, 63%). R_f 0.19 (1:1 EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.34 (dd, J=11.1, 2.8 Hz, 1H), 3.38 (d, J=6.7 Hz, 1H), 3.64 (dd, J=11.1, 2.8 Hz, 1H), 4.29 (br s, 1H), 4.50 (q, J=7.5 Hz, 1H), 4.57 (dt, J=7.5, 2.8 Hz, 1H), 4.82 (d, J=7.5 Hz, 1H), 7.18–7.36 (m, 9H), 7.50–7.57 (m, 6H); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 60.9, 73.5, 74.2, 78.3, 88.0, 127.3, 128.0, 128.4, 142.8, 175.3; HRMS (FAB^+, NBA, CH_2Cl_2) calcd for $C_{24}H_{22}O_5$ (M^+): 390.146088; obsd: 390.1461.

4.1.3. 2,3-Di-*O*-(methoxyethoxymethyl)-5-*O*-trityl-L-arabinono-γ-lactone (12). MEMCl (4.12 mL, 4.50 g, 36.1 mmol, 5.0 equiv) was added dropwise to a solution of 5-*O*-trityl-L-arabinono-γ-lactone (**11**) (2.82 g, 7.2 mmol, 1.0 equiv) and diisopropylethylamine (6.29 mL, 4.67 g, 36.1 mmol, 5.0 equiv) in AR-grade chloroform (45 mL) at rt under N_2. The mixture was heated at reflux for 11 h, cooled, diluted with chloroform (200 mL), washed with 10% aq citric acid (200 mL), satd aq $NaHCO_3$ (200 mL) and brine (200 mL). The organic layer was filtered through $MgSO_4$ and concentrated. The orange residue was purified by flash chromatography, eluting with 2:1 hexanes/EtOAc to give **12** as a colorless oil (3.89 g, 95%). R_f 0.31 (1:1 EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.26 (dd, J=10.9, 4.6 Hz, 1H), 3.29 (s, 3H), 3.34 (t, J=4.6 Hz, 2H), 3.38 (s, 3H), 3.39–3.61 (m, 5H), 3.71–3.87 (m, 2H), 4.35–4.38 (m, 1H), 4.47 (t, J=7.1 Hz, 1H), 4.58 (d, J=7.3 Hz, 1H), 4.64 (d, J=6.9 Hz, 1H), 4.73 (d, J=5.9 Hz, 1H), 4.89 (d, J=6.9 Hz, 1H), 5.12 (d, J=6.9 Hz, 1H), 7.22–7.32 (m, 9H), 7.43–7.46 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 58.9, 59.0, 62.0, 67.4, 67.7, 71.4, 71.6, 76.4, 77.6, 79.8, 86.8, 95.0, 95.2, 127.1, 127.9, 128.6, 143.3, 172.1; HRMS (EI^+) calcd for $C_{32}H_{38}O_9$ (M^+): 566.25158; obsd: 566.25163.

4.1.4. 2,3-Di-*O*-(methoxyethoxymethyl)-5-*O*-trityl-L-xylono-γ-lactone (20). By analogy to the procedure in Section 4.1.3, on a scale of 10.1 mmol of lactone **19**, affording **20** as a colorless oil (4.70 g, 82%). R_f 0.46 (2:1 EtOAc/hexane); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.29 (s, 3H), 3.35 (s, 3H), 3.26–3.44 (m, 8H), 3.45–3.82 (m, 2H), 4.44 (app. t, J=7.1 Hz, 1H), 4.59–4.70 (m, 3H), 4.87 (d, J=6.6 Hz, 1H), 4.90 (d, J=7.3 Hz, 1H), 5.04 (d, J=6.8 Hz, 1H), 7.20–7.32 (m, 9H), 7.42–7.44 (m, 6H); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 58.9 (2C), 61.0, 67.5, 67.6, 71.4, 71.5, 74.6, 77.9, 78.5, 87.5, 95.0, 95.9, 127.0, 127.7, 128.6, 143.1, 172.5; HRMS (FAB^+, NBA, CH_2Cl_2) calcd for $C_{32}H_{39}O_9$ (MH^+): 567.259408; obsd: 567.259347.

4.1.5. 2,3-Di-*O*-(methoxyethoxymethyl)-5-*O*-trityl-L-arabinitol (13). A solution of $LiBH_4$ (2 M in THF, 6.87 mL, 13.7 mmol, 2.0 equiv) was added dropwise over 1 h to a solution of lactone **12** (3.89 g, 2.20 mmol, 1.0 equiv) in THF (40 mL) at rt under N_2. The mixture was stirred for 1.5 h after the addition was complete, then quenched by the cautious, dropwise addition of satd aq NH_4Cl (5 mL). The mixture was stirred 10 min then partitioned between EtOAc (300 mL) and brine (300 mL). The brine was extracted with a further portion of EtOAc (300 mL). The organic layers were filtered through $MgSO_4$ and concentrated. The residue was purified by flash chromatography, eluting with 95:5 CH_2Cl_2/MeOH, to give **13** as a colorless oil (3.46 g, 88%). R_f 0.15 (95:5 CH_2Cl_2/MeOH); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 3.16 (dd, J=9.7, 5.1 Hz, 1H), 3.33–3.58 (m, 7H), 3.35 (s, 3H), 3.38 (s, 3H), 3.65–3.77 (m, 4H), 3.86 (dd, J=7.6, 2.7 Hz, 1H), 3.92–4.00 (m, 2H), 4.43 (d, J=6.8 Hz, 1H), 4.58 (d, J=6.8 Hz, 1H), 4.70–4.83 (m, 2H), 7.20–7.32 (m, 9H), 7.42–7.48 (m, 6H); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 59.0 (2C), 61.5, 64.4, 67.6, 67.7, 69.8, 71.4, 71.6, 78.5, 80.0, 86.6, 96.2, 96.7, 126.9, 127.7, 128.6, 143.6; HRMS (FAB^+, NBA) calcd for $C_{32}H_{43}O_9$ (MH^+): 571.29163; obsd: 571.29163.

4.1.6. 2,3-Di-*O*-(methoxyethoxymethyl)-5-*O*-trityl-L-xylitol (21). By analogy to the procedure in Section 4.1.5, on a scale of 8.29 mmol of lactone **20**, affording **21** as a colorless oil (4.20 g, 89%). R_f 0.22 (95:5 CH_2Cl_2/MeOH); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 1.80 (br s, 2H), 3.14 (dd, J=9.2, 6.2 Hz, 1H), 3.25 (dd, J=9.2, 6.0 Hz, 1H), 3.32 (s, 3H), 3.35 (s, 3H), 3.42–3.89 (m, 13H), 4.05 (td, J=5.9, 2.6 Hz, 1H), 4.61–4.67 (m, 2H), 4.77 (d, J=7.3 Hz, 1H), 7.17–7.30 (m, 9H), 7.40–7.43 (m, 6H); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 58.9, 59.0, 61.0, 64.4, 67.6, 68.9, 71.5, 77.9, 80.9, 86.6, 96.0, 96.9, 126.9, 127.7, 127.9, 143.6; HRMS (FAB^+, NBA, CH_2Cl_2) calcd for $C_{32}H_{43}O_9$ (MH^+): 571.2907; obsd: 571.2898.

4.1.7. 1,4-Bis-*O*-(methanesulfonyl)-2,3-di-*O*-(methoxyethoxymethyl)-5-*O*-trityl-L-arabinitol (14). *N*,*N*-Dimethylaminopyridine (75 mg, 0.62 mmol, 0.2 equiv) was added to neat methanesulfonyl chloride (0.95 mL, 1.41 g, 12.3 mmol, 4.0 equiv) at 0 °C under N_2. A solution of diol **13** (1.76 g, 3.08 mmol, 1.0 equiv) in pyridine (8, 2 mL rinse) was added dropwise over 40 min. The mixture was gradually warmed to rt and stirred 5 h. The mixture was concentrated and the residue partitioned between chloroform (150 mL) and water (150 mL). The aqueous layer was extracted further with chloroform (2×80 mL). The organic extracts were filtered through $MgSO_4$ and concentrated. The residue was purified by flash chromatography, eluting with 2:1 EtOAc/hexanes, to give **14** as a slightly yellow oil (2.01 g, 90%). R_f 0.26 (2:1 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.02 (s, 3H), 3.11 (s, 2H), 3.34 (s, 3H), 3.36 (s, 3H), 3.36–3.74 (m, 10H), 3.98 (app. q, J=5.0 Hz, 1H), 4.05 (t, J=4.3 Hz, 1H), 4.28 (dd, J=10.5, 5.8 Hz, 1H), 4.34 (dd, J=10.5, 5.2 Hz, 1H), 4.61 (d, J=7.0 Hz, 1H), 4.67–4.75 (m, 3H), 5.00–5.03 (m, 1H), 7.23–7.27 (m, 3H), 7.29–7.34 (m, 6H), 7.41–7.44 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 37.2, 28.8, 58.9, 62.6, 67.6, 68.0 (2C), 71.5 (2C), 75.4, 76.0, 80.9, 87.4, 96.4, 96.7, 127.3, 128.0, 128.6, 143.1; HRMS (FAB$^+$, NBA) calcd for $C_{35}H_{47}O_{13}S_2$ (MH^+): 727.24581; obsd: 727.24494.

4.1.8. 1,4-Bis-*O*-(methanesulfonyl)-2,3-di-*O*-(methoxyethoxymethyl)-5-*O*-trityl-L-xylitol (22). By analogy to the procedure in Section 4.1.7, on a scale of 5.14 mmol of diol **21**, affording **22** (2.776 g, 74%). R_f 0.19 (2:1 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 2.99 (s, 3H), 3.06 (s, 3H), 3.32 (s, 3H), 3.34 (s, 3H), 3.42–3.75 (m, 7H), 3.65 (dd, J=10.0, 4.7 Hz, 1H), 3.68 (app. t, J=4.7 Hz, 1H), 4.37–4.42 (m, 3H), 4.54–4.60 (m, 3H), 4.54–4.60 (m, 3H), 4.75–4.81 (m, 3H), 5.02–5.03 (m, 1H), 7.22–7.35 (m, 9H), 7.41–7.45 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 37.1, 38.8, 58.8, 58.9, 62.8, 67.7, 68.2, 68.4, 71.4, 71.5, 75.3, 75.9, 80.7, 87.3, 96.3, 97.5, 127.3, 128.0, 128.5, 143.0; HRMS (FAB$^+$, NBA) calcd for $C_{35}H_{47}O_{13}S_2$ (MH^+): 727.24581; obsd: 727.243268.

4.1.9. (2*R*,3*S*,4*S*)-1-Benzyl-3,4-di-*O*-(methoxyethoxymethyl)-2-triphenylmethoxymethyl-pyrrolidine (15). A solution of bis-mesylate **14** (1.80 g, 2.48 mmol) in freshly distilled benzylamine (10 mL) was stirred at 90 °C under N_2 for 4 days. The mixture was cooled and partitioned between chloroform (50 mL) and brine (50 mL). The aqueous layer was extracted further with chloroform (2×50 mL). The combined organic extracts were washed with water (100 mL), filtered through $MgSO_4$ and concentrated. The residue was applied directly to a flash column, eluting with 5:1 hexanes/EtOAc until the benzaldehyde had eluted (R_f 0.63, 1:1 EtOAc/hexanes). The eluant was changed to 1.5:1.0 EtOAc/hexanes to elute pyrrolidine **15** as a yellow oil (1.590 g, 99%). R_f 0.28 (1:1 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 270 MHz) δ 2.31 (dd, J=10.8, 4.4 Hz, 1H), 3.10–3.68 (m, 13H), 3.34 (s, 3H), 3.32 (s, 3H), 3.98 (d, J=13.4 Hz, 1H), 4.06–4.10 (m, 1H), 4.26 (dd, J=5.0, 1.9 Hz, 1H), 4.59 (d, J=7.2 Hz, 1H), 4.68–4.71 (m, 2H), 4.77 (d, J=7.2 Hz, 1H), 7.15–7.30 (m, 14H), 7.41–7.47 (m, 6H); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 57.7, 58.9 (2C), 59.5, 62.2, 65.5, 66.9, 67.0, 71.5, 71.6, 79.7, 81.5, 86.9, 94.4, 95.1, 126.6, 126.8, 127.6, 127.9, 128.5, 128.7, 138.6, 143.9; HRMS (FAB$^+$, NBA) calcd for $C_{39}H_{48}NO_7$ (MH^+): 642.34294; obsd: 642.34294.

4.1.10. (2*R*,3*R*,4*R*)-1-Benzyl-3,4-di-*O*-(methoxyethoxymethyl)-2-triphenylmethoxymethyl-pyrrolidine (23). By analogy to the procedure in 4.1.9, on a scale of 5.47 mmol of bis-mesylate **22**, to give pyrrolidine **23** as a yellow oil (3.23 g, 93%). R_f 0.32 (1:1 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.55 (dd, J=10.8, 4.8 Hz, 1H), 2.80 (dd, J=10.0, 5.7 Hz, 1H), 2.94 (d, J=10.8 Hz, 1H), 3.23 (ddd, J=16.0, 10.0, 5.7 Hz, 2H), 3.31 (s, 3H), 3.32 (s, 3H), 3.36–3.46 (m, 2H), 3.54–3.61 (m, 2H), 4.03 (m, 1H), 4.11 (d, J=9.4 Hz, 1H), 4.58 (d, J=7.2 Hz, 1H), 4.65 (d, J=7.2 Hz, 1H), 4.76 (d, J=2.8 Hz, 2H), 7.12–7.28 (m, 14H), 7.43–7.45 (m, 6H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 57.7, 58.8, 58.9, 59.5, 64.8, 66.8, 67.0, 69.5, 71.5, 71.6, 79.4, 83.4, 86.7, 94.0, 94.3, 126.8, 127.0, 127.7, 128.1, 128.7, 128.8, 138.8, 144.0; HRMS (FAB$^+$, NBA) calcd for $C_{39}H_{48}NO_7$ (MH^+): 642.34294; obsd: 642.34294.

4.1.11. (2*R*,3*S*,4*S*)-1-Fluorenylmethoxycarbonyl-3,4-di-*O*-(methoxyethoxymethyl)-2-triphenylmethoxy-methyl-pyrrolidine (16). Pd/C (10%, 600 mg) was added to a solution of the pyrrolidine **15** (2.43 g, 3.79 mmol) in absolute ethanol (30 mL). The flask was evacuated and then opened up to an atmosphere of H_2 and stirred for 16 h. The mixture was filtered through a pad of Celite, washing well with ethanol. The filtrate was concentrated and evaporated down from toluene. A solution of the pyrrolidine in toluene (15, 4 mL rinse) was added dropwise to a solution of fluorenylmethyl chloroformate (1.08 g, 4.17 mmol, 1.1 equiv) in toluene (8 mL) at 0 °C. Triethylamine (380 μL, 276 mg, 2.73 mmol, 1.1 equiv) was added over 10 min, the mixture warmed to rt and stirred for 2 h. The suspension was filtered through a sintered glass funnel, washing well with toluene. The filtrate was concentrated and the residue purified by flash chromatography, eluting with 1:1 EtOAc/hexanes to give **16** (2.56 g, 87%). R_f 0.27 (1:1 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.22–3.55 (m, 1H), 3.37–3.48 (m, 4H), 3.52–3.62 (m, 4H), 3.71–3.82 (m, 3H), 3.95–4.38 (m, 5H), 4.53–4.86 (m, 5H), 7.17–7.30 (m, 11H), 7.35–7.48 (m, 9H), 7.59 (t, J=7.6 Hz, 1H), 7.71–7.77 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 47.0 and 47.1, 49.8 and 49.9, 57.9 and 58.2, 59.0 (2C), 60.0 and 60.3, 67.3, 71.5, 71.6, 77.2, 78.5 and 79.0, 80.1 and 80.7, 87.1 and 87.2, 95.3 and 95.5, 96.1, 119.9, 124.8, 124.9, 125.0, 126.9, 127.0, 127.5, 127.6, 127.7, 128.6, 128.7, 141.2, 143.8, 143.9, 154.8 and 154.9; HRMS (FAB$^+$, NBA) calcd for $C_{47}H_{52}NO_9$ (MH^+): 774.364208; obsd: 774.362996.

4.1.12. (2*R*,3*R*,4*R*-1-Fluorenylmethoxycarbonyl-3,4-di-*O*-(methoxyethoxymethyl)-2-triphenyl-methoxymethyl)-pyrrolidine (24). By analogy to the procedure in Section 4.1.11, on a scale of 3.41 mmol of pyrrolidine **23**, to give compound **24** (2.247 g, 85%). R_f 0.28 (1:1 EtOAc/hexanes); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.15 (t, J=8.7 Hz, 1H), 3.27 (t, J=8.7 Hz, 1H), 3.36 (s, 3H), 3.37 and 3.38 (2s, 3H), 3.42–3.62 (m, 7H), 3.70–3.75 (m, 2H), 3.84 (ddd, J=23.0, 11.8, 5.5 Hz, 1H), 4.02–4.33 (m, 5H), 4.45–4.65 (m, 3H), 4.79–4.85 (m, 2H), 7.14–7.29 (m, 11H), 7.36–7.45 (m, 8H), 7.47 (d, J=7.6 Hz, 1H), 7.57 (d, J=7.6 Hz, 1H), 7.75 (d, J=7.6 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 47.1 and

47.2, 51.1 and 51.3, 59.0 (2C), 61.3 and 61.8, 62.8 and 63.1, 67.1, 67.2, 67.5, 71.5, 71.6, 77.2, 78.2 and 79.1, 79.6 and 80.8, 86.6 and 86.7, 94.4, 94.5, 119.9, 124.9, 125.0, 125.2, 127.0, 127.7, 128.6, 141.2, 143.6, 143.8, 144.0, 144.2, 154.8; HRMS (FAB^+, NBA) calcd for $C_{47}H_{52}NO_9$ (MH^+): 774.364208; obsd: 774.362996.

4.1.13. (2*R*,3*S*,4*S*)-1-Fluorenylmethoxycarbonyl-3,4-di-*O*-(methoxyethoxymethyl)-2-hydroxymethyl-pyrrolidine (17). A mixture of formic acid (6.5 mL) and acetonitrile (45 mL) was added to the pyrrolidine **16** (1.637 g, 1.90 mmol) and stirred at rt under N_2 for 2.5 h. The mixture was partitioned between EtOAc (200 mL) and satd aq $NaHCO_3$ (200 mL). The organic layer was washed further with brine (200 mL), filtered through $MgSO_4$, and concentrated. The residue was purified by flash chromatography, eluting with 2% MeOH in EtOAc to give compound **17** (1.049 g, 93%). R_f 0.18 (100% EtOAc); ^1H NMR ($CDCl_3$, 400 MHz) δ 3.38 (s, 3H), 3.40 (s, 3H), 3.54–3.57 (m, 5H), 3.64–3.91 (m, 7H), 4.07–4.10 (m, 1H), 4.19–4.29 (m, 3H), 4.38–4.56 (m, 2H), 4.73–4.85 (m, 4H), 7.32 (t, J=7.5 Hz, 2H), 7.41 (t, J=7.5 Hz, 2H), 7.59 (d, J=7.4 Hz, 2H), 7.77 (d, J=7.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 67.5 MHz) δ 47.2, 50.7, 59.0, 62.1 and 62.3, 67.3, 67.5, 67.7, 71.6, 77.3, 80.6, 94.6, 95.3, 119.9, 124.9, 126.9, 127.6, 141.2, 143.7, 156.3; HRMS (FAB^+, NBA) calcd for $C_{28}H_{38}NO_9$ (MH^+): 532.25466; obsd: 532.25662.

4.1.14. (2*R*,3*R*,4*R*)-1-Fluorenylmethoxycarbonyl-3,4-di-*O*-(methoxyethoxymethyl)-2-hydroxymethyl-pyrrolidine (25). By analogy to the procedure in Section 4.1.13, on a scale of 3.26 mmol of pyrrolidine **24**, to give compound **25** (1.66 g, 96%). R_f 0.19 (100% EtOAc); ^1H NMR ($CDCl_3$, 400 MHz) δ 3.38 (s, 6H), 3.49–3.56 (m, 5H), 3.65–3.89 (m, 7H), 3.97 (d, J=5.8 Hz, 1H), 4.17 (br s, 2H), 4.23 (t, J=7.0 Hz, 1H), 4.32–4.50 (m, 2H), 4.75–4.87 (m, 4H), 7.31 (td, J=7.4, 0.9 Hz, 2H), 7.39 (t, J=7.4 Hz, 2H), 7.59 (dd, J=6.9, 4.2 Hz, 2H), 7.75 (d, J=7.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 47.1, 51.0 and 51.4, 58.9, 61.2 and 63.2, 64.6 and 65.6, 66.9 and 67.1, 67.3, 67.5, 71.5, 78.9, 80.4 and 81.1, 94.4, 94.9 and 95.0, 119.8, 124.9, 125.0, 126.9, 127.6, 141.2, 143.7, 143.8, 154.9 and 156.2; HRMS (FAB^+, NBA) calcd for $C_{28}H_{38}NO_9$ (MH^+): 532.25466; obsd: 532.25514.

4.1.15. (2*R*,3*S*,4*S*)-1-Fluorenylmethoxycarbonyl-3,4-di-*O*-(methoxyethoxymethoxy)-L-proline (18). Sodium chlorite (78 mg, 0.87 mmol, 2.0 equiv) and TEMPO (4 mg, cat.) were added to a solution of alcohol **17** (229 mg, 0.43 mmol, 1.0 equiv) in a mixture of acetonitrile (0.85 mL) and 0.67 M aq NaH_2PO_4 (0.75 mL). This mixture was heated to 40 °C and bleach (12 μL) added, resulting in a deep rose color. The reaction mixture was checked by TLC and bleach added periodically to maintain the deep rose color, and until the conversion to the acid was complete. The mixture was poured onto ice-water (20 mL) containing Na_2SO_3 (100 mg). This led to immediate decolorizaton; the pH was 5–6 and 2 M HCl (~0.5 mL) was added to give a pH of 2 and considerable precipitation. The mixture was extracted with EtOAc (3×20 mL). The combined extracts were filtered through $MgSO_4$ and concentrated. The residue was purified by flash chromatography, eluting with 10–20% MeOH in CH_2Cl_2 to isolate **18** (207 mg, 88%). R_f 0.36 (9:1 CH_2Cl_2/MeOH); $[\alpha]_D^{20}$ −23.9 (*c* 1.00, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$) 3500–2384 (O–H), 1708 (C=O), 1122 (C–O–C); ^1H NMR ($CDCl_3$, 400 MHz) δ 3.34–3.37 (m, 6H), 3.50–3.81 (m, 10H), 4.14–4.82 (m, 10H), 7.24–7.38 (m, 4H), 7.52–7.57 (m, 2H), 7.67–7.73 (m, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 47.0, 50.0 and 50.8, 58.8, 59.0, 67.2, 67.6 and 67.8, 71.4 and 71.5, 77.2 and 77.8, 79.4 and 80.4, 94.7, 95.3, 119.8, 125.0, 125.1, 126.9, 127.5, 141.1, 143.5, 143.7, 143.9, 154.7 and 155.3, 172.9; HRMS (EI+) calcd for $C_{28}H_{36}NO_{10}$ (MH^+): 546.23392; obsd: 546.23345.

4.1.16. (2*R*,3*R*,4*R*)-1-Fluorenylmethoxycarbonyl-3,4-di-*O*-(methoxyethoxymethoxy)-L-proline (26). By analogy to the procedure in Section 4.1.15 on a scale of 0.63 mmol of alcohol **25**, to afford acid **26** (302 mg, 88%). R_f 0.22 (9:1 CH_2Cl_2/MeOH); $[\alpha]_D^{20}$ −13.6 (*c* 1.04, $CHCl_3$); ν_{max}/cm^{-1} ($CHCl_3$), 3502–2600 (O–H), 1703 (C=O), 1160 (O–C–O); ^1H NMR ($CDCl_3$, 400 MHz) δ 3.31 (s, 6H), 3.30–3.80 (m, 10H), 4.12–4.18 (m, 10H), 7.19–7.66 (m, 8H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 46.9 and 47.0, 50.8, 58.8, 64.3 and 64.9, 67.2, 67.8 and 68.0, 71.4, 71.5, 77.3 and 78.1, 71.5 and 83.0, 94.4, 94.7, 119.8, 125.1, 127.0, 127.6, 141.1, 143.6, 143.7, 144.0, 154.8 and 156.0, 172.9; HRMS (EI^+) calcd for $C_{28}H_{36}NO_{10}$ (MH^+): 546.23392; obsd: 546.23412.

4.1.17. 2,3-*cis*-3,4-*trans*-3,4-Dihydroxy-L-proline (1). A solution of acid **18** (96 mg, 0.18 mmol) in a solution of HBr in glacial acetic acid (33 wt%; 3 mL) was stirred for 18 h at rt. The mixture was concentrated and then dissolved in Tesser's base.[21] The pH was adjusted to about 10 by the addition of a few drops of 2 M aq NaOH. The mixture was stirred for 3 h and then concentrated. The residue was partitioned between water (5 mL) and EtOAc (5 mL). The aqueous layer was extracted further with EtOAc (4×5 mL). The aqueous layer was added to the top of a short column of Dowex H^+ resin and eluted with two column volumes of water. The eluant was changed to 0.5 M NH_4OH and the fractions checked by TLC, staining with ninhydrin. Relevant fractions were lyophilized to give a colorless solid, which was dissolved in water, filtered through a 0.2 μ nylon filter and lyophilized again to give compound **1** (25 mg, quant.). R_f 0.26 (3:3:3:1 ^{n}BuOH, EtOH, NH_3, H_2O); $[\alpha]_D^{20}$ −48.1 (*c* 1.00, H_2O) lit.[2a] $[\alpha]_D^{20}$ −61.2 (*c* 0.5, H_2O) lit.[11] $[\alpha]_D^{27}$ −56 (*c* 0.62, H_2O) lit.[23] $[\alpha]_D^{27}$ −63.2 (*c* 0.5, H_2O) lit.[24] $[\alpha]_D^{27}$ −63.0 (*c* 0.8, H_2O); ^1H NMR (D_2O, 400 MHz) δ 3.16 (d, J=12.8 Hz, 1H), 4.56 (dd, J=12.8, 3.7 Hz, 1H), 4.21 (d, J=4.0 Hz, 1H), 4.27 (d, J=3.7 Hz, 1H), 4.32 (d, J=4.0 Hz, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 50.9, 65.2, 75.0, 75.4, 171.0; HRMS (FAB^+, glycerol) calcd for $C_5H_{10}NO_4$ (MH^+): 148.06098; obsd: 148.06063.

4.1.18. 2,3-*trans*-3,4-*trans*-3,4-Dihydroxy-L-proline (2). By analogy to the procedure in Section 4.1.17, starting with acid **26** (80 mg) and giving rise to **2** (21 mg, quant.). R_f 0.39 (3:3:3:1 ^{n}BuOH, EtOH, NH_3, H_2O); $[\alpha]_D^{20}$ −21.4 (*c* 0.28, H_2O) lit.[11] $[\alpha]_D^{25}$ −19 (*c* 0.4, H_2O) lit.[24] $[\alpha]_D^{22}$ −12.6 (*c* 0.53, H_2O);[22] ^1H NMR (D_2O, 400 MHz) δ 3.36 (d, J=12.4 Hz, 1H), 3.33 (dd, J=12.7, 3.7 Hz, 1H), 3.89 (m, 1H), 4.16 (m, 1H), 4.37 (m, 1H); ^{13}C NMR (D_2O, 100 MHz) δ 50.9, 67.6, 74.1, 78.5, 171.6; HRMS (FAB^+, glycerol) calcd for $C_5H_{10}NO_4$ (MH^+): 148.06098; obsd: 148.06062.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2005.07.072.^{13}C NMR spectra for compounds **1**, **2** and **12–26**.

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