Enantiospecific Synthesis of Polyoxamic Acid from L-Arabinose

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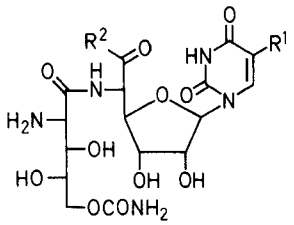
An enantiospecific synthesis of polyoxamic acid, 2-amino-2-deoxy-L-xylonic acid, by phenylthiolate opening of a five-carbon chiral hydroxylated aziridine easily derived from L-arabinose, is reported. The formation of the carboxyl group resulted from a Pummerer reaction.

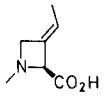
Polyoxins are a class of peptidyl nucleoside antibiotics discovered by Isono and co-workers, who also elucidated their structure.[1] They are excellent agricultural fungicides of wide use which inhibit the synthesis of chitin of a variety of phytopathogenic fungi. Recent studies suggest that polyoxins also inhibit chitin synthetase of *Candida albicans*, a medically important human fungal pathogen.[2] Such results show the usefulness of new synthetic pathways to polyoxins or analogs.

Polyoxins have as a common structural feature a dipeptide comprised of an unusual α-aminoaldonic acid of L-xylo configuration (commonly named polyoxamic acid) connected to one of the several related nucleoside amino acids. A variety of chemical syntheses of polyoxamic acid have been reported over the years,[3] most of them based on carbohydrate templates as starting materials; the stereocontrol in these syntheses however is only partial since in most cases the construction of the molecule results from a chain elongation of a chiral aldehyde. A totally stereocontrolled and efficient access to this compound remains of interest.



R^1 = CO_2H, CH_2OH, Me, H

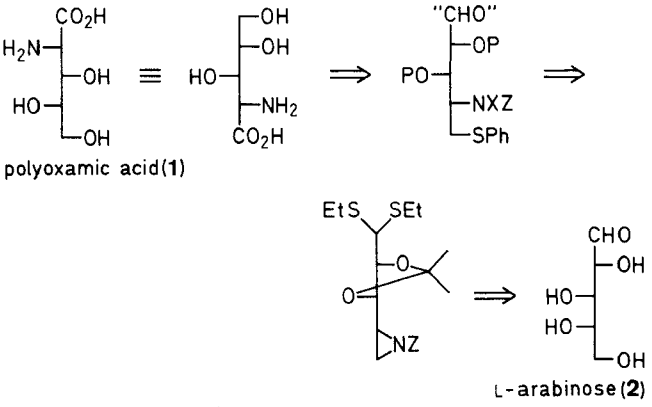
R^2 = OH,



We report here an expedient and enantiospecific synthesis of polyoxamic acid (**1**) and more usefully of a derivative suitably protected for peptide coupling from the easily available pentose, L-arabinose (**2**).

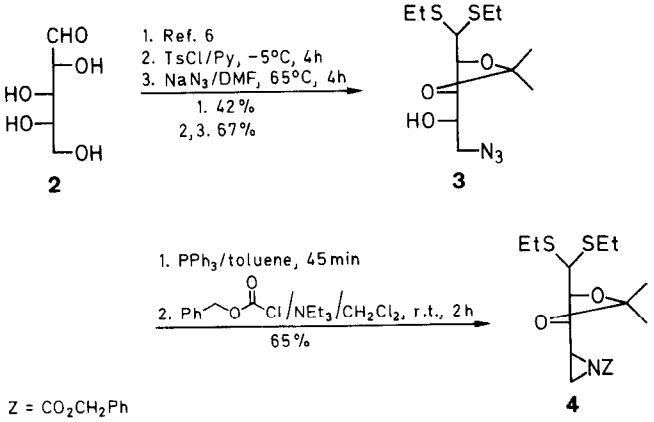
We have recently shown that chiral, functionalized aziridines are useful aminoalkylating intermediates for the synthesis of enantiomerically pure amino derivatives as attested by the ability of various nucleophiles to effect nucleophilic opening of chiral bis-aziridines derived from D-mannitol.[4] In connection with our interest in developing convenient methods for the preparation of functionalized α-amino acids we have studied the possibility of creating the α-amino acid functionality starting from a monosubstituted aziridine.

We have reported in a preliminary note[5] that nucleophilic phenylthiolate opening of a conveniently activated chiral aziridine followed by a Pummerer rearrangement enables the access to the α-amino acid function in a totally stereospecific way. The protected aziridine **4** prepared from L-arabinose constituted a good precursor of polyoxamic acid (**1**) since it possesses the three chiral centers of **1** with the right configuration and must lead to **1** after the reduction of C-1 and the formation of a carboxy group at C-5 (Scheme **1**).



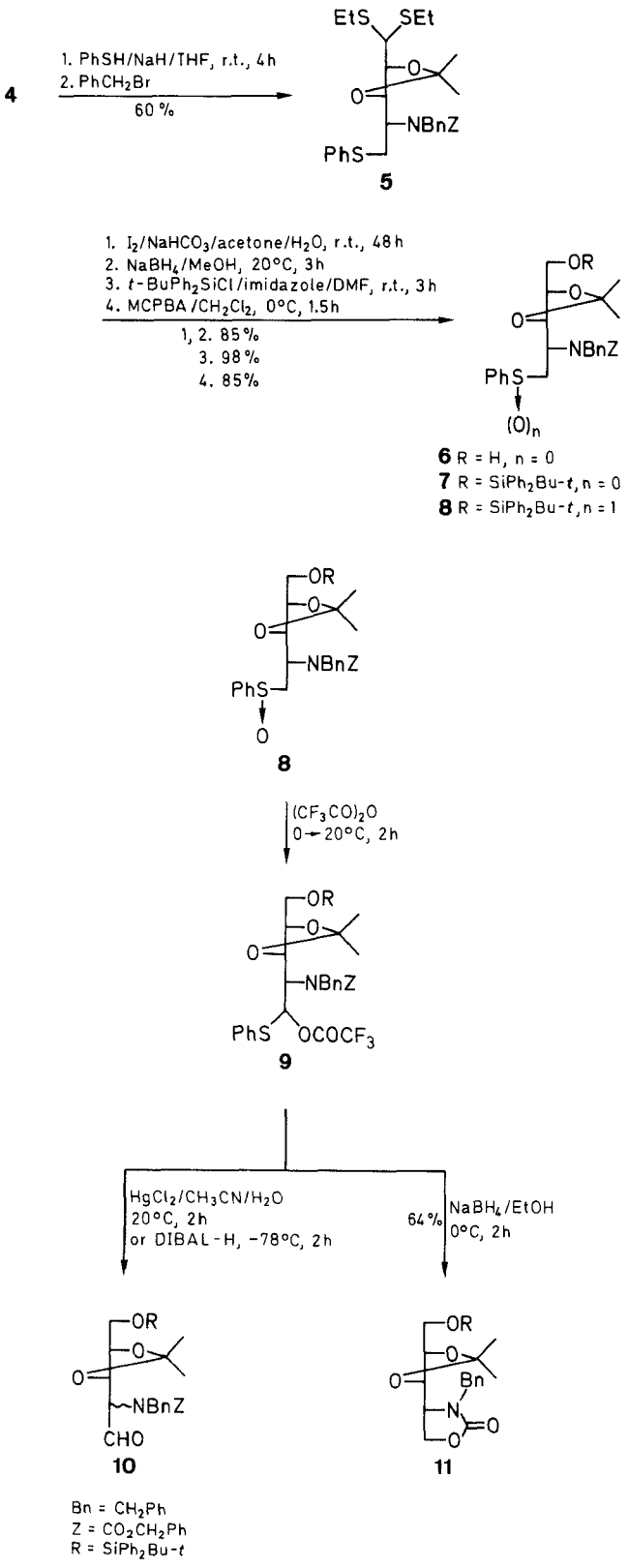
Scheme **1**

The synthesis of aziridine **4** is presented in Scheme **2**. The aziridine **4** was obtained in 18% overall yield starting from L-arabinose (**2**) by introduction of nitrogen with configuration inversion at C-4 of the starting pentose. L-Arabinose (**2**) was converted into the azido alcohol **3** by standard methods.[6] Reductive ring closure of **3** by triphenylphosphine led to the NH aziridine in quantitative yield, which was protected as its *N*-benzyloxycarbonyl derivative **4**.



Scheme **2**

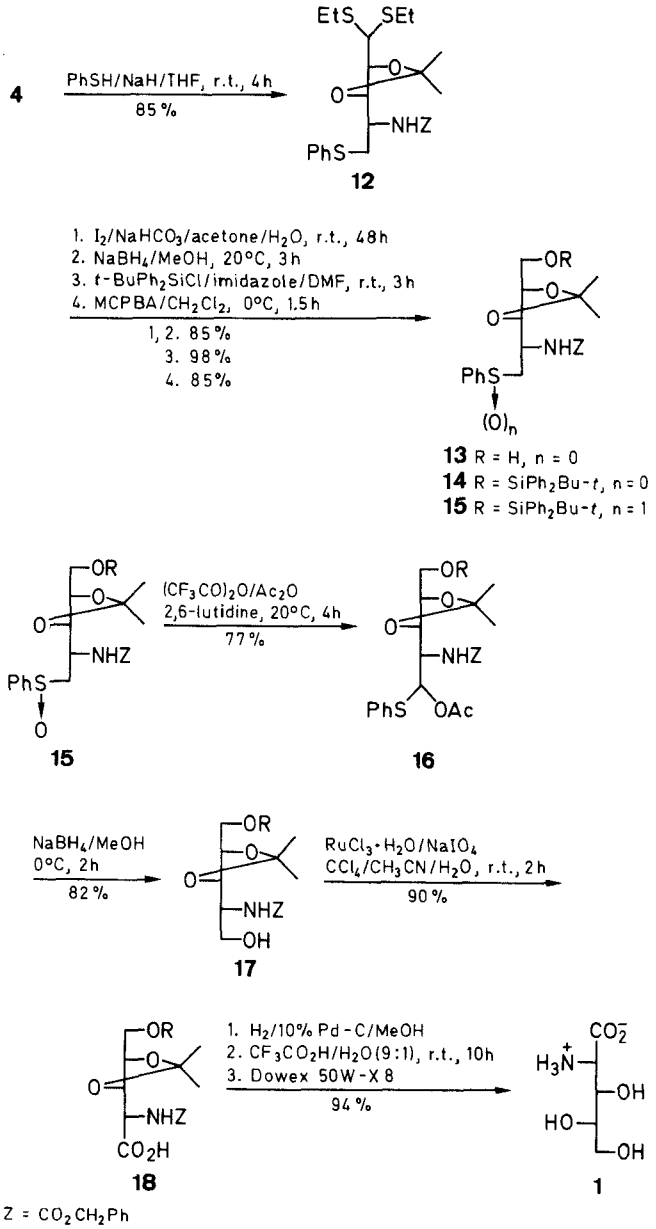
Phenylthiolate opening of the aziridine ring of **4**, the key step of our synthesis, occurs in 85% yield. Iodine promoted thioacetal hydrolysis followed by sodium borohydride reduction at C-1 and silylation of the resulting alcohol was effected afterwards, yielding a protected sulfide, which was oxidized in turn by 3-chloroperoxybenzoic acid (MCPBA) as depicted in Scheme **3**.



Scheme 3

We anticipated that a fully nitrogen substituted sulfoxide such as **8** in which the nitrogen lone pair cannot act as internal nucleophile during the rearrangement could be a suitable precursor for a mild trifluoroacetic anhydride mediated Pummerer reaction.[7]

Reaction of trifluoroacetic anhydride with **8** (Scheme **3**) led at 0 °C to the corresponding trifluoracetoxy sulfide **9** as a sole diastereoisomer, but conversion of **9** into aldehyde **10** either by hydrolysis in the presence of mercuric chloride or by reduction with diisobutylaluminum hydride (DIBAL-H) at −78 °C occurred with appreciable epimerization at the α-position of aldehyde **10**. Complete reduction of **9** using sodium borohydride should prevent such epimerization,[8] but in our case, this resulted not in the formation of the desired α-amino alcohol, but in the formation of the *N*-benzyl cyclic carbamate **11**. Unfortunately under reaction conditions required for basic hydrolysis of **11** (sodium carbonate, methanol, 60 °C), partial desilylation occurred.



Scheme 4

These difficulties were overcome by carrying out the Pummerer rearrangement at room temperature in an acetic anhydride trifluoroacetic anhydride mixture[9] (Scheme **4**) starting from *N*-benzyloxycarbonyl sulfoxide **15**. Under these conditions no secondary reaction resulting from the nucleophilic attack of the sulfonium by the nitrogen lone pair was ever noticed. Compound **15** was converted cleanly into a diastereoisomeric mixture of acetoxy sulfides **16**, which could be converted into the selectively protected polyoxamic acid **18** in 59% yield from **15** without any epimerization. Since the borohydride reduction of a *N*-benzyloxycarbonyl-α-aminoaldehyde into the corresponding alcohol is well documented[10] and does not lead to a cyclic carbamate, conversion of **16** to **18** was performed by complete sodium borohydride reduction into alcohol **17** followed by ruthenium trichloride catalyzed oxidation of **17** into **18**. Each step of the synthesis occurred with complete stereochemical control, notably the conversion of the acetoxy sulfide **16** to the alcohol **17** as evidenced by ^{1}H- and ^{13}C-NMR spectra of the intermediates.

Unprotected polyoxamic acid (**1**) resulted from the hydrogenolytic removal of the benzyloxycarbonyl protecting group of **18** followed by aqueous trifluoracetic acid hydrolysis. After purification by ion exchange chromatography, its specific rotation compares well with previously reported results (see experimental).

Enantiomerically pure polyoxamic acid (**1**) was thus obtained in 32% overall yield starting from aziridine **4**.

^{1}H-NMR spectra were recorded on 250 MHz Bruker spectrometer or on 90 MHz EM 390 Varian spectrometer. ^{13}C-NMR spectra were recorded on 250 MHz Bruker spectrometer. TMS was used as the standard for NMR spectra. Mass spectra were recorded on Riber 10-10 spectrometer. Specific rotation were measured for λ = 589 nm at 20°C with a Perkin-Elmer 241 polarimeter. IR spectra were measured with a Perkin-Elmer 783 spectrophotometer.

5-Azido-5-deoxy-2,3-*O*-(1-methylethylidene)-L-arabinose Diethyl Dithioacetal (3):

2,3-O-(1-Methylethylidene)-L-arabinose Diethyl Dithioacetal: This is prepared in three steps from L-arabinose (**2**) by a method reported earlier;[6] $[\alpha]_D^{20}$ −94,1° (*c* = 2.07, MeOH) [Lit.[6] $[\alpha]_D^{20}$ −93.8° (*c* = 4.17, MeOH)].

2,3-O-(1-Methylethylidene)-5-O-1-tolylsulfonyl-L-arabinose Diethyl Dithioacetal: To a stirred solution of the above diol (11.5 g, 38 mmol) in pyridine (30 mL), TsCl (7.37 g, 38 mmol) dissolved in pyridine (30 mL) is added dropwise at −5°C and stirring is continued for 4 h at −5°C. The mixture is diluted with Et_2O (60 mL) and hydrolysed with 6N HCl (130 mL). The organic layer is washed with sat. $NaHCO_3$ solution (60 mL) and brine (60 mL). The organic phase is dried ($MgSO_4$), concentrated and subjected to flash column chromatography (silica gel, 30% EtOAc in cyclohexane) to afford the corresponding primary tosylate contaminated with ~15% of the 4-*O*-tosyl isomer as a colorless oil (13.11 g) which is reacted further without purification.

3: To a stirred solution of the above tosylate (13.11 g) in DMF (25 mL), NaN_3 (5.2 g, 80 mmol) is added and stirring is continued for 3 h at 65°C. After evaporation of the solvent, water (40 mL) is added to the residue which is then extracted with CH_2Cl_2 (3 × 40 mL). The extract is dried ($MgSO_4$) concentrated and subjected to flash column chromatography (silica gel, 15% EtOAc in cyclohexane) to afford pure **3** as a colorless oil; yield: 8.35 g (67%); $[\alpha]_D^{20}$ −64.7° (*c* = 1.34, CH_2Cl_2).

$C_{12}H_{23}N_3O_3S_2$	calc.	C 44.84	H 7.21	N 13.07
(321.5)	found	44.93	7.27	13.01

^{1}H-NMR ($CDCl_3$/250 MHz): δ = 1.20 (t, 6H, 2 × CH_2CH_3), 1.35, 1.45 [2s, 6H, $C(CH_3)_2$], 2.71 (m, 4H, 2 × CH_2CH_3), 3.46 (ABX, 1H, $J_{5,5'}$ = 13 Hz, $J_{4,5}$ = 3 Hz, H-5), 3.58 (ABX, 1H, $J_{4,5'}$ = 6.5 Hz, H-5'), 3.77 (m, 1H, H-4), 3.98 (d, 1H, $J_{1,2}$ = 4 Hz, H-1), 4.05 (m, 1H, H-3), 4.27 (dd, 1H, $J_{2,3}$ = 7 Hz, H-2),

4,5-(Benzyloxycarbonyl)imino-4,5-dideoxy-2,3-*O*-(1-methylethylidene)-D-xylose Diethyl Dithioacetal (4):

4,5-Dideoxy-4,5-imino-2,3-O-(1-methylethylidene)-D-xylose Diethyl Dithioacetal:

To a stirred solution of PPh_3 (1.07 g, 4.1 mmol) in toluene (14 mL), azido alcohol **3** (1.32 g, 4.1 mmol) in toluene (14 mL) is added dropwise under Ar. The solution is stirred at 40°C until N_2 evolution has ceased (30 min), and then warmed to 100°C for 1 h. The toluene is evaporated at reduced pressure and Ph_3PO precipitates as a white powder upon addition of Et_2O (10 mL). Ph_3PO is filtered and the Et_2O evaporated to afford crude *N*-unsubstituted aziridine as a syrup (1.25 g) containing about 20% w/w of Ph_3PO (^{1}H-NMR evaluation), which is protected without further purification.

^{1}H-NMR ($CDCl_3$, 250 MHz): δ = 1.25 (t, 6H, 2 × CH_2CH_3), 1.42, 1.43 [2s, 6H, $C(CH_3)_2$], 1.65 (d, 1H, $J_{4,5}$ = 3.5 Hz, *trans* H-5), 1.80 (d, 1H, $J_{4,5}$ = 6 Hz, *cis* H-5), 2.20 (m, 1H, $J_{3,4}$ = 6 Hz, H-4), 2.75 (m, 4H, 2 × CH_2CH_3), 3.85–3.93 (m, 2H, H-1, H-3), 4.18 (dd, 1H, $J_{1,2}$ = 5.5 Hz, $J_{2,3}$ = 7.5 Hz, H-2).

4: To a stirred solution of the NH aziridine obtained above (1.25 g) and Et_3N (0.75 mL, 5.4 mmol) in CH_2Cl_2 (4 mL) at 0°C, is added dropwise benzylchlorocarbonate (0.77 mL, 5.4 mmol) under Ar. After stirring for 2 h at r.t., Et_2O (6 mL) is added and the precipitated solids are filtered. The supernatant layer is concentrated under reduced pressure and the residue subjected to flash column chromatography (silica gel, 10% EtOAc in cyclohexane) to give aziridine **4** as a colorless oil; yield from **3**: 1.10 g (65%); $[\alpha]_D^{20}$ 6.0° (*c* = 0.67, CH_2Cl_2).

$C_{20}H_{29}NO_4S_2$	calc.	C 58.36	H 7.10	N 3.40
(411.6)ag1found		58.17	7.24	3.33

IR (neat): ν = 2980, 2920, 1725, 1450, 1380, 1290, 1210, 880, 750, 700 cm^{-1}.

^{1}H-NMR ($CDCl_3$, 250 MHz): δ = 1.20, 1.21 (2t, 6H, 2 × CH_2CH_3), 1.37, 1.40 (2s, 6H, $C(CH_3)_2$], 2.37 (d, 1H, $J_{4,5}$ = 3 Hz, *trans* H-5), 2.40 (d, 1H, $J_{4,5}$ = 6.5 Hz *cis* H-5), 2.62–2.80 (m, 5H, 2 × CH_2CH_3, H-4), 3.88 (d, 1H, $J_{1,2}$ = 6 Hz, H-1), 4.13 (dd, 1H, $J_{2,3}$ = 7 Hz, $J_{3,4}$ 4 Hz, H-3), 4.31 (dd, 1H, $J_{1,2}$ = 6 Hz, H-2), 5.10 (s, 2H, CH_2Ph), 7.31–7.37 (m, 5H_{arom}).

^{13}C-NMR ($CDCl_3$, 250 MHz): δ = 14.2, 14.3 (CH_2CH_3), 25.0, 25.2 (CH_2CH_3), 26.9, 27.2 [$C(CH_3)_2$], 29.3 (C-5), 38.5 (C-4), 52.9 (C-1), 68.2 (CH_2Ph), 77.5, 81.7 (C-2, C-3), 110.3 [$C(CH_3)_2$], 128.0, 128.2, 128.4, 135.7 (C_{arom}), 163.0 (C=O).

4-Benzyl(benzyloxycarbonyl)amino-4,5-dideoxy-2,3-*O*-(1-methylethylidene)-5-phenylthio-D-xylose Diethyl Dithioacetal (5):

To a suspension of NaH (121.4 g, 5.1 mmol) in THF (8 mL), thiophenol (0.52 mL, 5.1 mmol) is added dropwise under Ar at 0°C. The mixture is warmed to r.t., stirred 30 min and aziridine **4** (1.3 g, 3.2 mmol) in THF (16 mL) is added dropwise. After stirring for 4 h benzyl bromide (0.6 mL, 5.1 mmol) is added, the temperature is then raised to 40°C and stirring is continued for 2 h at this temperature. The mixture is quenched by addition of H_2O (10 mL), THF is evaporated and the residue extracted with Et_2O (2 × 15 mL). The organic phase is washed with brine (20 mL) dried ($MgSO_4$) and evaporated. Flash column chromatography (silica gel, 5% EtOAc in cyclohexane) gives pure **5** as a colorless oil; yield: 1.13 g (60%); $[\alpha]_D^{20}$ +12° (*c* = 1.9, CH_2Cl_2).

$C_{33}H_{41}NO_4S_3$	calc.	C 64.78	H 6.75	N 2.29
(611.9)	found	64.59	6.85	2.34

^{1}H-NMR ($CDCl_3$, 90 MHz): δ = 1.1 (t, 6H, 2×CH_2CH_3), 1.3 [s, 6H, $C(CH_3)_2$], 2.6 (m, 4H, 2×CH_2CH_3), 3–3.2 (m, 2H, H-5, H-5′), 3.8 (m, 1H, H-1), 4.1 (m, 1H, H-2), 4.4–4.8 (m, 4H, H-3, H-4, NCH_2Ph), 5.1 (s, 2H, OCH_2Ph), 7.1–7.3 (m, 15H_{arom}).

^{13}C-NMR ($CDCl_3$, 250 MHz): δ = 14.3, 14.4 (CH_2CH_3), 25.0, 25.3 (CH_2CH_3), 26.6, 27.1 [$C(CH_3)_2$], 35.0 (C-5), 48.3 (NCH_2-Ph), 52.1 (C-1), 54.6 (C-4), 67.5 (OCH_2Ph), 79.5, 80.8 (C-2, C-3), 109.3 [$C(CH_3)_2$], 126.4, 126.8, 127.9, 128.3, 128.9, 129.8, 135.3, 136.0, 139.1 (C_{arom}), 156.8 (C=O).

4-Benzyl(benzyloxycarbonyl)amino-4,5-dideoxy-2,3-*O*-(1-methylethylidene)-5-phenylthio-D-xylitol (6):

4-Benzyl(benzyloxycarbonyl)amino-4,5-dideoxy,2,3-O-(1-methylethylidene)-5-phenylthio-D-xylose: A solution of the dithioacetal **5** (1.5 g, 2.5 mmol) in acetone (25 mL) and H_2O (3.5 mL) is treated with $NaHCO_3$ (440 mg, 5.3 mmol) and with slow portionwise addition of I_2 (680 mg, 2.7 mmol) at 0 °C under good stirring. The mixture is allowed to warm up to r.t. and stirred overnight. Further portions of I_2 (680 mg) and $NaHCO_3$ (440 mg) are then added at 0 °C and stirring is continued at r.t. for a further 24 h, at which time the excess I_2 is destroyed by adding 1 N $Na_2S_2O_3$ solution (10 mL). After partial evaporation of acetone, the product is extracted into EtOAc (3×30 mL) and the combined extracts are washed with H_2O (30 mL). Drying ($MgSO_4$) followed by concentration gives crude product (1.2 g) which is used for the next step without further purification.

6: To the aldehyde obtained above (1.2 g) in MeOH (25 mL), $NaBH_4$ is added portionwise (270 mg, 7.1 mmol) at 0 °C and stirring is continued for 2 h at 0 °C. After addition of AcOH (1.2 mL) and evaporation of MeOH, H_2O (20 mL) is added and the solution is extracted with Et_2O (40 mL). The organic phase is washed with sat. $NaHCO_3$ solution (20 mL) and brine (20 mL), dried ($MgSO_4$) and concentrated. The residue is subjected to flash column chromatography (silica gel, 30% EtOAc in cyclohexane) to afford pure **6** as an oil; yield from **5**: 1.05 g (85%); $[\alpha]_D^{20}$ + 19.1° (*c* = 0.70, CH_2Cl_2).

$C_{29}H_{33}NO_5S$	calc.	C 68.61	H 6.55	N 2.76
(507.7)	found	68.55	6.23	2.44

IR (neat): ν = 3500, 1700, 1450, 1320, 820, 750, 690 cm^{-1}.

^{1}H-NMR ($CDCl_3$, 90 MHz): δ = 1.3 [s, 6H, $C(CH_3)_2$], 3.1–3.2 (m, 2H, H-5, H-5′), 3.5–3.7 (m, 3H, H-2, H-3, H-4), 4.2–4.4 (m, 2H, H-1, H-1′), 4.6–4.7 (m, 2H, NCH_2Ph), 5.1 (s, 2H, OCH_2Ph), 7.2–7.8 (m, 15H_{arom}).

^{13}C-NMR ($CDCl_3$, 250 MHz): δ = 26.7, 26.9 [$C(CH_3)_2$], 29.7 (C-5), 34.7 (C-1, C-4), 62.2 (NCH_2Ph), 67.7 (OCH_2Ph), 77.8, 78.1 (C-2, C-3), 108.8 [$C(CH_3)_2$], 126.7, 126.9, 127.8, 128.3, 129.0, 130.1, 136.1, 138.8 (C_{arom}), 159.1 (C=O).

4-Benzyl(benzyloxycarbonyl)amino-1-*O*-*tert*-butyldiphenylsilyl-4,5-dideoxy-2,3-*O*-(1-methylethylidene)-5-phenylthio-D-xylitol (7):

Alcohol **6** (400 mg, 0.79 mmol), imidazole (134 mg, 2 mmol) and *tert*-butylchlorodiphenylsilane (0.226 mL, 0.87 mmol) are dissolved in DMF (15 mL) and stirred under Ar at r.t. for 3 h. After evaporation of DMF, the mixture is subjected to flash column chromatography (silica gel, 10% EtOAc in cyclohexane) to give the silyl ether as a colorless oil; yield: 570 mg (97%); $[\alpha]_D^{20}$ 26.2° (*c* = 0.89, CH_2Cl_2).

$C_{45}H_{51}NO_5SiS$	calc.	C 72.45	H 6.89	N 1.87
(746.0)	found	72.39	6.72	1.85

^{1}H-NMR ($CDCl_3$, 90 MHz): δ = 1.2 (s, 9H, *t*-C_4H_9), 1.5 [s, 6H, $C(CH_3)_2$], 3.3–3.4 (m, 2H, H-5, H-5′), 3.7–4.1 (m, 3H, H-2, CH_2OSi), 4.3–4.9 (m, 4H, H-3, H-4, NCH_2Ph), 5.2 (s, 2H, OCH_2Ph), 7.1–7.9 (m, 25H_{arom}).

4-Benzyl(benzyloxycarbonyl)amino-1-*O*-*tert*-butyldiphenylsilyl-4,5-dideoxy-2,3-*O*-(1-methylethylidene)-5-phenylsulfinyl-D-xylitol (8):

To a solution of sulfide **7** (300 mg, 0.40 mmol) in CH_2Cl_2 (12 mL), MCPBA (76 mg, 0.44 mmol) is added at 0 °C. After stirring at 0 °C for 2 h, the mixture is washed with saturated $NaHCO_3$ solution (10 mL) and brine, dried ($MgSO_4$) and evaporated. Flash column chromatography (silica gel, 20% EtOAc in cyclohexane) gives **8** as white crystals (mixture of diastereoisomers); yield: 265 mg (85%).

$C_{45}H_{51}NO_6SiS$	calc.	C 70.92	H 6.74	N 1.84
(762.1)	found	70.85	6.78	1.82

^{1}H-NMR ($CDCl_3$, 90 MHz): δ = 1.2 [s, 9H, *t*-C_4H_9), 1.4 [s, 6H, $C(CH_3)_2$], 3.1–3.2 (m, 2H, H-5, H-5′), 3.5–4.0 (m, 3H, H-4, CH_2OSi), 4.2–4.8 (m, 4H, H-2, H-3, NCH_2Ph), 5.1 (s, 2H, OCH_2Ph), 7.1–7.7 (m, 25H_{arom}).

2-Benzyl(benzyloxycarbonyl)amino-5-*O*-*tert*-butyldiphenylsilyl-2-deoxy-3,4-*O*-(1-methylethylidene)-L-xylose *O*-Trifluoroacetyl *S*-Phenyl Monothioacetal (9):

To a solution of the sulfoxide **8** (500 mg, 0.66 mmol) in CH_2Cl_2 (6 mL) and 2–6 lutidine (76 µL, 0.66 mmol), $(CF_3CO)_2O$ (95 µL, 0.66 mmol) is added under Ar at 0 °C. After stirring for 1 h at 0 °C and 1 h at r.t., the solvent is removed to crude trifluoroacetoxy sulfide **9**, which is unstable and reacted further without purification; yield: 700 mg.

Conversion of Trifluoroacetoxy Sulfide 9 into the Corresponding Aldehyde 10 as a C-2 Epimeric Mixture:

Method A: To the above crude Pummerer reaction product (200 mg, 0.19 mmol) is added a solution of $HgCl_2$ (88 mg, 0.32 mmol) in CH_3CN (1.6 mL) and H_2O (1.6 mL). After stirring for 4 h at r.t., the resultant aldehyde is extracted with Et_2O (3×10 mL). The organic phase is dried ($MgSO_4$) and evaporated to dryness to afford **10** as a syrup as a 60:40 mixture of diastereoisomers as shown by the ^{1}H-NMR ($CDCl_3$, 250 MHz) spectrum of the crude product: δ = 9.21 (s, 0.6H, CHO), 9.36 (s, 0.4H, CHO).

Method B: To the crude Pummerer reaction product (200 mg, 0.19 mmol) in anhydrous toluene (4 mL), DIBAL-H (1.0 M in heptane, 0.372 mL, 0.372 mmol) is added drowpise at − 78 °C. After stirring for 2 h, an aq 3% HCl solution (4 mL) is added at − 78 °C. The mixture is then extracted with EtOAc (3×15 mL), the organic phase is washed with sat. $NaHCO_3$ solution (10 mL), brine, and dried ($MgSO_4$). Evaporation of the solvent yields **10** as a 70:30 mixture of epimers at C-2.

2-Benzylamino-1-*O*-2-*N*-carbonyl-2-deoxy-3,4-*O*-(1-methylethylidene)-5-*O*-*tert*-butyldiphenylsilyl-L-xylitol (11):

To a solution of crude Pummerer reaction product **9** (300 mg, 0.29 mmol) in absolute EtOH (13 mL) at 0 °C, $NaBH_4$ (27 mg, 0.72 mmol) is added portionwise. After stirring for 2 h at 0 °C the reaction is quenched with AcOH (100 µL) and the solvent is evaporated. Then water (20 mL) is added and the product extracted with Et_2O (2×20 mL). The organic phase is dried ($MgSO_4$), concentrated and subjected to flash column chromatography (silica gel, 25% EtOAc in cyclohexane) to afford pure cyclic carbamate **11** as a colorless oil; yield: 101 mg (64%).

^{1}H-NMR ($CDCl_3$, 250 MHz): δ = 1.04 (s, 9H, *t*-C_4H_9), 1.30 [s, 3H, $C(CH_3)_2$], 1.36 [s, 3H, $C(CH_3)_2$], 3.55–3.90 (m, 5H, CH_2OSi, H-1, H-1′, H-2), 4.02–4.34 (m, 4H, H-3, H-4, CH_2Ph), 7.25–7.78 (m, 15H_{arom}).

MS: *m/z* = 563 $(M + 18)^+$.

4-Benzyloxycarbonylamino-4,5-dideoxy-2,3-*O*-(1-methylethylidene)-5-phenylthio-D-xylose Diethyl Dithioacetal (12):

To a suspension of NaH (172.3 g, 7.2 mmol) in THF (10 mL), thiophenol (0.74 mL, 7.2 mmol) is added dropwise, under Ar at 0 °C. After H_2 evolution has ceased, the mixture is warmed to r.t. and stirred for 30 min. Aziridine **4** (1.84 g, 4.5 mmol) in THF (20 mL) is then added dropwise. After stirring for 4 h, the mixture is quenched by addition of H_2O (10 mL), THF evaporated and the residue is extracted with Et_2O (2×20 mL). The organic phase is washed with brine (20 mL), dried ($MgSO_4$) and evaporated. The crude product is subjected to flash column chromatography (silica gel, 5% EtOAc in cyclohexane) to give **12** as a yellow oil; yield: 2 g (85%); $[\alpha]_D^{20}$ + 44.3° (*c* = 1.7, CH_2Cl_2).

$C_{26}H_{35}NO_4S_3$	calc.	C 59.85	H 6.76	N 2.68
(521.7)		59.78	6.58	2.72

^{1}H-NMR ($CDCl_3$, 250 MHz): δ = 1.12, 1.21 (2 t, 6H, 2×CH_2CH_3), 1.36, 1.37 [2 s, 6H, $C(CH_3)_2$], 2.65 (m, 4H, 2×CH_2CH_3), 3.01 (ABX, 1H, J = 8.5 Hz (*gem*), $J_{4,5}$ = 7.5 Hz, H-5), 3.22 (ABX, 1H, $J_{4,5'}$ = 5.5 Hz, H-5'), 3.75 (d, 1H, $J_{1,2}$ = 6.5 Hz, H-1), 3.95 (dd, 1H, $J_{2,3}$ = 7.5 Hz, H-2), 4.18 (m, 1H, H-4), 4.45 (m, 1H, H-3), 5.08–5.17 (m, 3H, NH, CH_2Ph), 7.25–7.5 (m, 10 H_{arom}).

4-Benzyloxycarbonylamino-4,5-dideoxy-2,3-*O*-(1-methylethylidene)-5-phenylthio-D-xylitol (13):

Alcohol **13** is prepared from dithioacetal **12** by the same procedure used to prepare **6** from **5**; **13** is obtained as a colorless oil after flash column chromatography (silica gel, 30% EtOAc in cyclohexane); yield: 85%; $[\alpha]_D^{20}$ +26.4° (c = 1.05, CH_2Cl_2).

$C_{22}H_{27}NO_5S$	calc.	C 63.28	H 6.51	N 3.35
(417.5)	found	63.16	6.42	3.29

IR (neat): ν = 3350 (br), 1700, 1475, 1380, 1250, 1210, 800, 750 cm^{-1}.

^{1}H-NMR ($CDCl_3$, 250 MHz): δ = 1.33, 1.38 [2 s, 6H, $C(CH_3)_2$], 3.03 (ABX, 1H, J = 14 Hz (*gem*), $J_{4,5}$ = 8 Hz, H-5), 3.20 (ABX, J = 14 Hz (*gem*), $J_{4,5'}$ = 7.5 Hz, H-5'), 3.55–3.82 (m, 3H, H-2, CH_2OH), 3.88 (m, 1H, H-4), 4.26 (dd, 1H, $J_{2,3}$ = 8.5 Hz, $J_{3,4}$ = 1 Hz, H-3), 5.09, 5.18 (m, 3H, NH, OCH_2Ph), 7.13–7.42 (m, 10 H_{arom}).

4-Benzyloxycarbonylamino-1-*O*-*tert*-butyldiphenylsilyl-2,3-*O*-(1-methylethylidene)-4,5-dideoxy-5-phenylthio-D-xylitol (14):

Alcohol **13** is silylated to **14** by the same procedure used to prepare **7** from **6**. Flash column chromatography (silica gel, 10% EtOAc in cyclohexane) gives pure silyl ether as a colorless oil; yield: 98%; $[\alpha]_D^{20}$ 11.1° (c = 0.74, CH_2Cl_2).

$C_{38}H_{45}NO_5SiS$	calc.	C 69.58	H 6.91	N 2.13
(655.9)	found	69.67	6.78	2.07

^{1}H-NMR ($CDCl_3$, 250 MHz): δ = 1.14 (s, 9H, t-C_4H_9), 1.48, 1.51 [2 s, 6H, $C(CH_3)_2$], 3.13 (ABX, 1H, J = 14 Hz (*gem*), $J_{4,5}$ = 9 Hz, H-5), 3.36 (ABX, 1H, $J_{4,5'}$ = 5.5 Hz, H-5'), 3.72, 3.97 (m, 3H, H-2, CH_2OSi), 4.06 (m, 1H, H-4), 4.67 (m, 1H, H-3), 5.13 (AB, 1H, J = 12 Hz (*gem*), OCH_2Ph), 5.20 (AB, 1H, OCH_2Ph), 5.42 (d, 1H, $J_{NH,H4}$ = 9.5 Hz, NH), 7.31–7.85 (m, 20 H_{arom}).

4-Benzyloxycarbonylamino-1-*O*-*tert*-butyldiphenylsilyl-4,5-dideoxy-2,3-*O*-(1-methylethylidene)-5-phenylsulfinyl-D-xylitol (15):

Sulfoxide **15** is prepared from sulfide **14** by the same procedure used to prepare **8** from **7**. Compound **15** is obtained as a colorless oil after flash column chromatography, as a mixture of diastereoisomers **15a** and **15b**; yield: 85%.

$C_{38}H_{45}NO_6SiS$	calc.	C 67.93	H 6.75	N 2.08
(671.9)	found	67.82	6.57	1.99

The diastereoisomers **15a** and **15b** could be separated by flash column chromatography (silica gel, 30% EtOAc in cyclohexane).

15a: $[\alpha]_D^{20}$ +34° (c = 0.80, CH_2Cl_2).

^{1}H-NMR ($CDCl_3$, 250 MHz): δ = 1.0 (s, 9H, t-C_4H_9), 1.34–1.37 [2 s, 6H, $C(CH_3)_2$], 3.01 (ABX, 1H, J = 13.5 Hz (*gem*), $J_{4,5}$ = 5.5 Hz, H-5), 3.18 (ABX, 1H, $J_{4,5'}$ = 8.5 Hz, H-5'), 3.71 (m, 2H, CH_2OSi), 3.81 (m, 1H, H-4), 4.10–4.29 (m, 2H, H-2, H-3), 5.02, 5.15 (AB, 2H, J = 12.5 Hz (*gem*), OCH_2Ph), 5.31 (d, 1H, $J_{NH,H-4}$ = 9.5 Hz, NH), 7.28–7.75 (m, 20 H_{arom}).

15b: $[\alpha]_D^{20}$ +53° (c = 0.97, CH_2Cl_2).

^{1}H-NMR ($CDCl_3$, 250 MHz): δ = 1.0 (s, 9H, t-C_4H_9), 1.33, 1.39 [s, 6H, $C(CH_3)_2$], 2.92 (ABX, 1H, J = 13.5 Hz (*gem*), $J_{4,5}$ = 8.5 Hz, H-5), 3.04 (ABX, 1H, $J_{4,5'}$ = 4.5 Hz, H-5'), 3.73–3.91 (m, 3H, CH_2OSi, H-4), 4.19 (m, 1H, H-3), 4.39 (m, 1H, H-2), 5.07, 5.14 (AB, J = 11.5 Hz (*gem*), OCH_2Ph), 5.36 (d, 1H, $J_{NH,H-4}$ = 9.5 Hz, NH), 7.26–7.71 (m, 20 H_{arom}).

2-Benzyloxycarbonylamino-5-*O*-*tert*-butyldiphenylsilyl-2-deoxy-3,4-*O*-(1-methylethylidene)-L-xylose *O*-Acetyl *S*-Phenyl Monothioacetal (16):

A mixture of $(CF_3CO)_2O$ (0.13 mL, 0.89 mmol) and Ac_2O (0.5 mL) is stirred overnight at r.t.. A solution of the sulfoxide **15** (400 mg, 0.59 mmol) in Ac_2O (0.5 mL) and 2,6-lutidine (0.14 mL, 1.18 mmol) is added and stirring is continued for 4 h at r.t. After evaporation to dryness, the crude product is subjected to flash column chromatography (silica gel, 10% EtOAc in cyclohexane) to give monothioacetal **16** as colorless oil; yield: 324 mg (77%). Compound **16** consists of a mixture of the two nonseparable epimers at C-1 in a ratio of 60:40 as shown by ^{1}H-NMR spectrum.

$C_{40}H_{47}NO_7SiS$	calc.	C 67.29	H 6.63	N 1.96
(714.0)	found	67.15	6.61	1.91

^{1}H-NMR ($CDCl_3$, 250 MHz): δ = 1.04 and 1.06 (s, 9H, t-C_4H_9), 1.38 [s, 6H, $C(CH_3)_2$], 3.64–3.85 (m, 3H, CH_2OSi, H-2), 4.17 (m, 1H, H-4), 4.41 and 4.60 (m, 1H, H-3), 5.02 and 5.07 (AB, 1H, J = 12.5 Hz and J = 13 Hz, OCH_2Ph), 5.17 and 5.18 (AB, 1H, OCH_2Ph), 5.23–5.32 (m, 1H, NH), 6.15 and 6.23 (d, 1H, $J_{1,2}$ = 7 Hz and J = 7.5 Hz, H-1), 7.18–7.62 (m, 20 H_{arom}).

2-Benzyloxycarbonylamino-5-*O*-*tert*-butyldiphenylsilyl-2-deoxy-3,4-*O*-(1-methylethylidene)-L-xylitol (17):

To a solution of the acetoxy sulfide **16** (320 mg, 0.45 mmol) in MeOH (6 mL), $NaBH_4$ (34 mg, 0.90 mmol) is gradually added at 0°C. After 2 h stirring at r.t. AcOH (0.2 mL) is added, and the MeOH evaporated. H_2O (10 mL) is added to the mixture and the product is extracted with Et_2O (20 mL). The organic phase is washed with sat. $NaHCO_3$ solution (5 mL) and brine, dried ($MgSO_4$), concentrated, and subjected to flash column chromatography (silica gel, 30% EtOAc in cyclohexane) to afford alcohol **17** as colorless oil; yield: 208 mg (82%); $[\alpha]_D^{20}$ −11.3° (c = 0.75, CH_2Cl_2).

$C_{32}H_{41}NO_6Si$	calc.	C 68.17	H 7.33	N 2.48
(563.8)	found	68.13	7.27	2.45

^{1}H-NMR ($CDCl_3$, 250 MHz): δ = 1.08 (s, 9H, t-C_4H_9), 1.39 [s, 6H, $C(CH_3)_2$], 3.63–3.95 (m, 7H, CH_2OSi, CH_2OH, H-2, H-4), 4.27 (m, 1H, H-3), 5.07, 5.14 (AB, 2H, J = 12 Hz, CH_2Ph), 5.40 (d, 1H, $J_{NH,H-2}$ = 8 Hz, NH), 7.24–7.74 (m, 15 H_{arom}).

^{13}C-NMR ($CDCl_3$, 250 MHz): δ = 19.2 [$C(CH_3)_3$], 26.9, 27.0 [$C(CH_3)_3$, $C(CH_3)_2$], 51.4 (C-2), 63.0, 65.1 (CH_2OSi, CH_2OH), 67.1 (OCH_2Ph), 77.8, 78.0 (C-4, C-3), 109.5 [$C(CH_3)_2$], 127.8, 128.1, 128.2, 128.5, 129.7, 129.8, 133.0, 135.7, 136.3 (C_{arom}), 156.7 (C=O).

2-Benzyloxycarbonylamino-5-*O*-*tert*-butyldiphenylsilyl-2-deoxy-3,4-*O*-(1-methylethylidene)-L-xylonic Acid (18):

To a solution of alcohol **17** (200 mg, 0.35 mmol) in CCl_4 (0.65 mL), CH_3CN (0.65 mL) and H_2O (1 mL), $NaIO_4$ (306 mg, 1.43 mmol) and $RuCl_3 \cdot H_2O$ (2 mg, 2.4 mol%) are added and the entire mixture is vigorously stirred for 2 h at r.t. Then CH_2Cl_2 (5 mL) is added and the phases are separated. The aqueous phase is extracted with CH_2Cl_2 (3×10 mL) and the combined organic extracts are dried ($MgSO_4$) and concentrated. Flash column chromatography (silica gel, 8% MeOH in EtOAc) gives pure **18** as white crystals; yield: 182 mg (90%); $[\alpha]_D^{20}$ −21.4° (c = 1.3, CH_2Cl_2).

$C_{32}H_{39}NO_7Si$	calc.	C 66.52	H 6.80	N 2.42
(577.8)	found	66.47	6.74	2.39

^{1}H-NMR ($CDCl_3$, 250 MHz): δ = 1.07 (s, 9H, t-C_4H_9), 1.37, 1.42 [s, 6H, $C(CH_3)_2$], 3.81 (m, 2H, CH_2OSi), 3.95 (m, 1H, H-2), 4.55–4.63 (m, 2H, H-3, H-4), 5.15 (s, 2H, OCH_2Ph), 5.53 (d, 1H, $J_{NH,H-2}$ = 9 Hz, NH), 7.19–7.76 (m, 15 H_{arom}).

2-Amino-2-deoxy-L-xylonic Acid (1):

2-Amino-5-O-tert-butyldiphenylsilyl-2-deoxy-3,4-O-(1-methylethylidene)-L-xylonic Acid: Protected acid **18** (180 mg, 0.31 mmol) is dissolved in MeOH (15 mL) and stirred for 3 h under a H_2 atmosphere with 10% Pd/C (12 mg). The mixture is filtered over Celite and concentrated to afford the *N*-unprotected amino acid as white crystals, which is fully deprotected without further purification; yield: 140 mg (quant).

1: The above amino acid (140 mg, 0.31 mmol) is stirred with 90% CF_3CO_2H/H_2O (5 mL) overnight at r.t. After evaporation of the solvent, the product is purified by ion exchange chromatography on Dowex 50W-X8 (H^+ form). Washing the resin with H_2O, then eluting with 1 M aqueous pyridine followed by lyophilization gives

polyoxamic acid (**1**) as white crystals; yield: 48 mg (94%); mp 170 °C; $[\alpha]_D^{20}$ +3.0°; $[\alpha]_{365}^{20}$ +22° (c = 1.0, H_2O); [Lit.[1] mp 171–173 °C (dec); $[\alpha]_D^{20}$ +2.8° $[\alpha]_{365}^{20}$ +23° (c = 1.0, H_2O)].

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