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Asymmetric Synthesis of Unsaturated and Bis-Hydroxylated (*S,S*)-2,7-Diaminosuberic Acid Derivatives.

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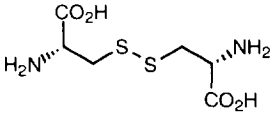
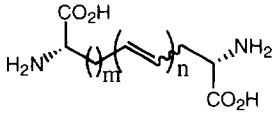
Abstract: Stereoselective syntheses of cystine substitutes with all-carbon C_4-bridges are described. The disulfide unit between the two alanine moieties in cystine has been replaced by a C_2-unit. When the C_2-unit is olefinic, conformationally constrained *cis*- and *trans*-unsaturated analogues result. Vicinal dihydroxy analogues were formed when the C_2-unit was a vicinal 1,2-ethylenediol derivative. *N*-Fmoc protection of the new C_4-bridged bis(amino acids) is described. © 1997 Elsevier Science Ltd.

The disulfide bridged proteinogenic amino acid (*R*)-cystine (**A**, Fig.1) constitutes an essential part of a great number of peptides and proteins. Cystine may function as a scaffolding unit for cross-linking peptide chains or for locking peptides into cyclic structures by disulfide formation. Both functions may be essential for the spacing of the pharmacophoric region and its interaction with the receptor. In our program for the preparation of isosteric analogues of cystine, the latter is regarded as a dimeric amino acid composed of two glycine units which are connected at the glycine α-position by the -CH_2SSCH_2- bridge. We have mainly confined our efforts towards the construction of C_4-bridges to replace the four-atom -CH_2SSCH_2- bridge in cystine.[1-3]

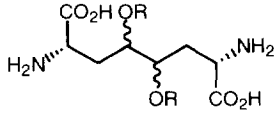
Because of the potential medical importance of such structures when included in small bioactive peptidic structures, we have for some time been involved in the synthesis of cystine analogues, and in particular we and others have been interested in finding substitutes for cystine in immunoactive peptides.[4,5]



(**A**)



(**B**)



(**C**)

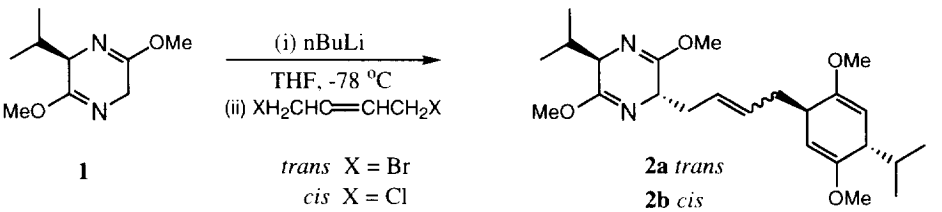
Scheme 1

In the simplest case for isosteric substitution the -CH_2S-SCH_2- bridge between the two glycine α-carbons in (*R*)-cystine can be replaced by the all carbon -$(CH_2)_4$- bridge as in (*S,S*)-2,7-diaminosuberic acid

(**B**, m = 3, n = 0, Scheme 1).[6] The disulfide linkage in cystine, however, confers conformational constraints on cystine compared with the higher conformational freedom of (*S,S*)-2,7-diaminosuberic acid. In the first part of this report we have compensated for the conformational constraints from the disulfide unit by insertion of unsaturation in the new all-carbon bridge; the *trans*- and *cis*-butene isomers **7a** and **7b** (Scheme 4) were prepared. In the second part of this report we describe stereoselective syntheses of bis(α,α′-amino acids) where the saturated C_4-bridge carries two vicinal hydroxy groups on the central carbon atoms of the bridge, *ie*. a vicinal ethylenediol unit has been substituted for the disulfide unit in cystine; the hydroxy amino acids were isolated as the acetonides **9a**, **9b** and **9c** (Scheme 5) for subsequent *N*-protection and inclusion in peptides. The bridged amino acids were prepared in enantiomerically pure forms, including all stereoisomers of the bis-hydroxy acetonide derivatives **9**.

Several chiral auxiliaries are available for amino acid constructions.[7] We have used extensively the Schöllkopf "bislactim ether" chiral auxiliary in bridge forming reactions which involve alkylation of lithiated (*2R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine.[8]

Several stereoselective syntheses of *C*-bridged amino acids have recently been described; the C_1-analogue (**B**, m = n = 0; Scheme 1),[9] the C_2-analogue[5], the C_3- and bridge-substituted C_3-analogues,[10] the C_4-analogue[4a,6] and the C_5-analogue[11].

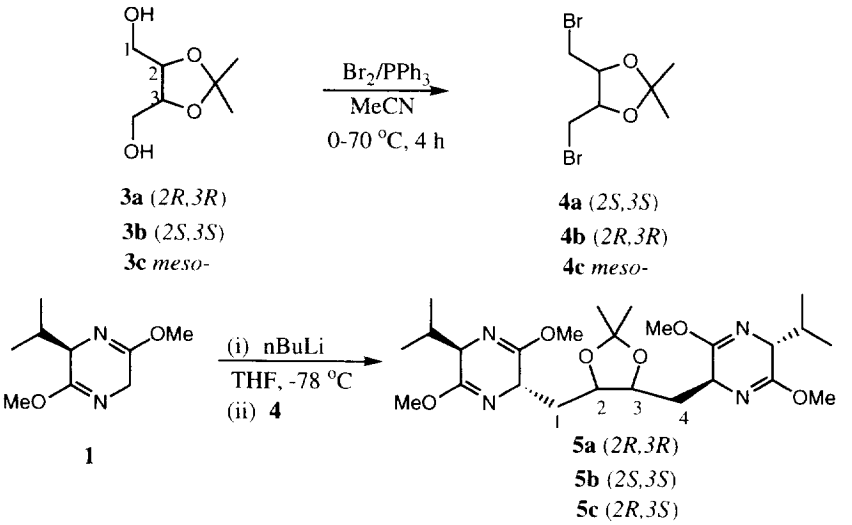


Scheme 2

For the preparation of the *trans*- and *cis*-alkene-bridged amino acids **7a** and **7b** (Scheme 4) by the Schöllkopf methodology, lithiated (*2R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine was alkylated in THF at -78 °C with (*E*)- and (*Z*)-1,4-dibromo- or -dichloro-2-butene. The alkylation with the (*E*)-reagent was characterized by a very high overall diastereoselectivity despite the fact that two alkylation steps are required. In principle two different alkylating agents, *viz*. the original alkylating agent and the intermediate monalkylated alkylating agent, are involved in the formation of the product **2a**; only 3% of the undesired (*2R,5S,2′R,5′R*)-isomer was detected in the crude product. The isolated yield of pure **2a** was 75% with diastereomeric excess (d.e.) >99% after flash chromatography or recrystallization from acetonitrile. The stereoselectivity in this alkylation is better than that we obtained in the preparation of the corresponding saturated C_4-analogue using 1,4-dibromobutane for alkylation.[4a] On the other hand, both the yield and the stereoselectivity were low using (*Z*)-1,4-dichloro-2-butene; the product contained 65% of the desired stereoisomer (**2b**), but the isomers could readily be separated by flash chromatography (d.e.>99%). The main

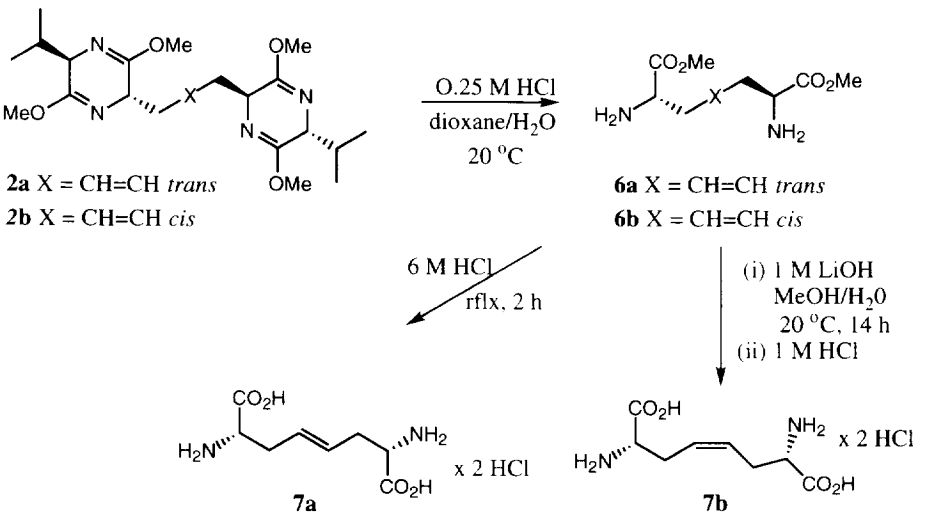
stereochemical information on the homogeneity of a product formed, is based on ^{13}C and ^{1}H NMR spectroscopy. Additional information was based on chromatography (GLC in particular, and TLC).

The substrates for the synthesis of bridged amino acids containing hydroxy functions in the bridge, were the acetonide protected sugar alcohols (*2R,3R*)-2,3-*O*-isopropylidenethreitol (**3a**), (*2SS3S*)-2,3-*O*-



Scheme 3

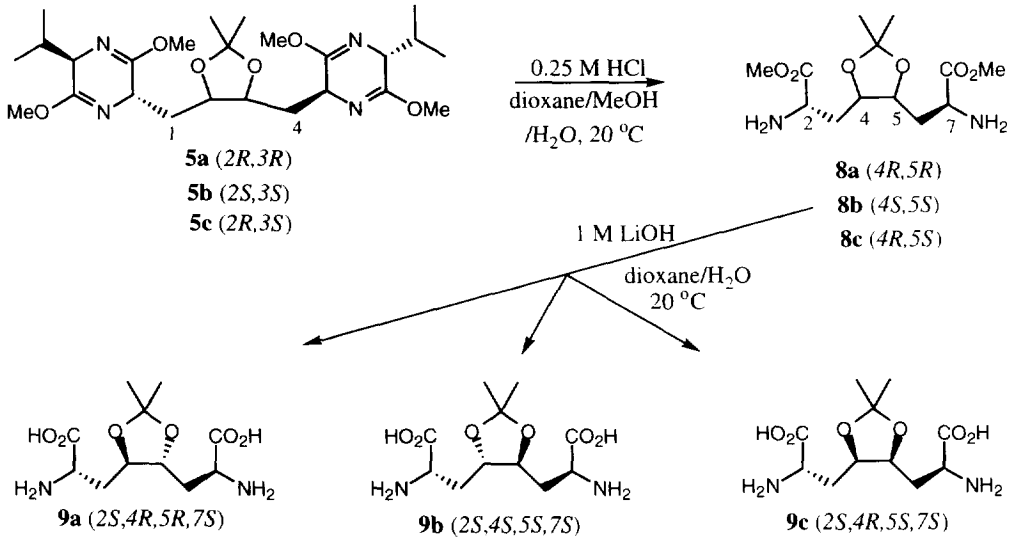
isopropylidenethreitol (**3b**) and the *meso*-form 2,3-*O*-isopropylidenerythritol (**3c**) which were 1,4-dibrominated using bromine together with triphenylphosphine in acetonitrile. The bromides **4** were used for



Scheme 4

alkylation of the lithiated bislactim ether, initially at -78 °C, and finally at ambient temperature. Phosphate buffer, pH 7 was used for quenching the reaction with retention of the acetonide protection. Compound **5a** was isolated in 57% yield (d.e. 96%) after repeated recrystallizations from acetonitrile; analysis (GLC) of the crude reaction product showed 35% of the isomer with the (*R*)-configuration instead of the (*S*)-configuration in the 5-position in one of the bislactim rings. Compound **5b** was obtained in 34% yield (d.e. 94%) after repeated recrystallizations from acetonitrile; analysis of the crude reaction product showed 18% of the (*S,R*)-isomer at stereogenic 5-position in the bislactim ether units. Finally compound **5c** was obtained in 10% yield (d.e. 94%) after flash chromatographic separations. The crude product was almost a 1:1 stereoisomeric mixture of **5c** and its (*S,R*)-isomer in the bislactim ether 5-positions.

The amino acid esters **6** and **8** were obtained after acid catalyzed cleavage of the bislactim ether structures **2** and **5** in dioxane:water 1:1. For the unsaturated compounds **6,** 0.05 M HCl for hydrolysis yielded 85% of the acids **7**. For the acetonides the hydrolytic reaction was carefully controlled using the mole ratio 1:4 between the acetonide **5** and HCl. Under these conditions the acid esters **8** were isolated in high yields (77-98%). The (*E*)-ester **6a** was hydrolyzed to its acid **7a** simply by heating with 6 M HCl, whereas milder conditions were used for the (*Z*)-ester **6b** for conversion to its acid **7b**; 2 M LiOH in aqueous methanol. A recent report describes preparation of the racemic analogue of the *trans*-amino acid **7a** by a Pd-mediated coupling between *cis*- or *trans*-1,4-dichloro-2-butene and lithiated benzophenone imine of glycine ethyl ester.[12]



5a (*2R,3R*)
5b (*2S,3S*)
5c (*2R,3S*)

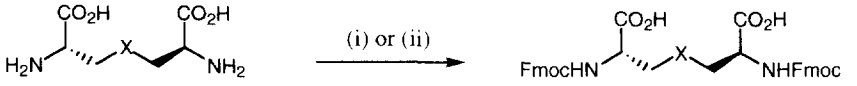
8a (*4R,5R*)
8b (*4S,5S*)
8c (*4R,5S*)

9a (*2S,4R,5R,7S*)
9b (*2S,4S,5S,7S*)
9c (*2S,4R,5S,7S*)

Scheme 5

Hydrolysis of the methyl esters **8** was also effected by using two mole equivalents of lithium hydroxide at ambient temperature. Attempts to isolate the isopropylidene protected amino acids by

neutralization led to partial cleavage of the protecting group. Therefore a one-pot operation was effected at basic pH; the ester was cleaved under mild basic conditions in the usual way by LiOH in dioxane/water. The acids **9** were not isolated, but their solutions were used directly in the transformation to the 2,7-bis(9-fluorenylmethyloxycarbonylamino)-4,5-isopropylidenedioxyoctanedioic acids **11** using 9-fluorenylmethyloxycarbonyl chloride (Fmoc-Cl) for the *N*-acylation. The yields in these reactions were moderate, 34-66% yield of isolated product **11** after flash chromatography. The (*Z*)-4-octenedioic acid **7b** was *N*-Fmoc protected under similar conditions to furnish **10b**. An alternative method was used for the (*E*)-4-octenedioic acid **7b** which was initially silylated by heating its hydrochloride in hexamethyldisilazane (HMDS) and

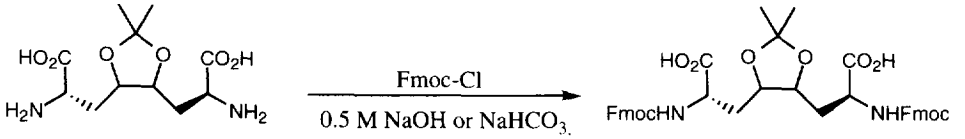


7a X = CH=CH *trans*
7b X = CH=CH *cis*

10a X = CH=CH *trans*
10b X = CH=CH *cis*

(i) **10a** (1) HMDS/TMS-Cl,
(2) Fmoc-Cl, CH_2Cl_2, 20 °C
(3) HCl/H_2O

(ii) **10b** Fmoc-Cl,
Na_2CO_3/dioxane/H_2O
0-20 °C



9a (*4R,5R*)
9b (*4S,5S*)
9c (*4R,5S*)

11a (*4R,5R*)
11b (*4S,5S*)
11c (*4R,5S*)

Scheme 6

trimethylsilyl chloride (TMS-Cl). The persilylated derivative which was formed was soluble in organic solvents. The persilylated product was subsequently dissolved in dichloromethane and treated with Fmoc-Cl, initially at 0 °C, and subsequently at ambient temperature for completion of the acylation to yield the Fmoc-derivative **10a**.

The purified Fmoc-protected amino acids **10** and **11** have all been used in peptide synthesis by the appropriate protocol for solid phase synthesis. This work has been reported in part;[3] a full report including biodata is planned for publication elsewhere.

EXPERIMENTAL

1,4-Bis[(*2R,5S*)-2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl]-(*E*)-2-butene (**2a**). A solution of (*2R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine (5.53 g, 30 mmol) in anhydrous THF at -78°C was lithiated by the addition of a solution of nBuLi in hexane (19.62 ml, 31.0 mmol). The solution was stirred at -78°C for 60 min, a solution of (*E*)-1,4-dibromo-2-butene (3.21 g, 15 mmol) in THF (30 ml) added dropwise with stirring and the resultant mixture allowed to reach ambient temperature overnight. After hydrolysis with phosphate buffer (pH 7), the mixture was extracted with diethyl ether, the organic layer washed with water and brine, dried ($MgSO_4$) and the solvent distilled off. The residual product contained 3 % of the (*2R,5S,2'R,5'R*)-isomer, and was obtained essentially isomer-pure by flash chromatography on silica gel (hexane:ethyl acetate 4:1); yield 4.73 g (75%), d.e. >99%. Found: C, 62.65; H, 8.62; N, 12.91. Calc. for $C_{22}H_{36}N_4O_4$(420): C, 62.86; H, 8.57; N, 13.33.[1] H NMR ($CDCl_3$): δ 0.67(d, 6H), 1.04 (d, 6H), 2.1-2.4 (m, 2H), 2.48 (dd, 4H), 3.67(s, 6H), 3.68 (s, 6H), 3.93 (dd, 2H), 4.03 (dd, 2H), 5.35 (dd, 2H). ^{13}C NMR ($CDCl_3$): δ 16.52, 19.07, 31.61, 37.09, 52.16, 52.23, 55.62, 60.60, 128.50.

1,4- Bis[(*2R,5S*)-2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl]-(*Z*)-2-butene (**2b**).was prepared as above from lithiated (*2R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine and (*Z*)-1,4-dichloro-2-butene in THF. The product contained about 35 % of the undesired stereoisomer with (*R*)-configuration at C-5 in one of the bislactim rings; the latter was removed by flash chromatography on silica gel (hexane:ethyl acetate 9:1); d.e. >99%. ^1H NMR ($CDCl_3$): δ 0.67 (d, 6H), 1.03 (d, 6H), 2.25 (m,2 H), 2.55 (m, 4H), 3.66 (s, 6H), 3.68 (s, 6H), 3.91 (dd, 2 H), 4.07 (m, 2H), 5.38 (dd, 2H). ^{13}C NMR ($CDCl_3$): δ 16.50, 19.01, 31.57, 32.01, 52.22, 52.31, 55.46, 60.67, 127.30, 163.19, 163.66. HRMS: Found for *M* 420.2729. Calc. for $C_{22}H_{36}N_4O_4$: 420.2737.

(*2S,3S*)-1,4-Dibromo-2,3-isopropylidenedioxybutane (**4a**). Bromine (9.92 g, 3.20 ml, 62 mmol) was added to a stirred suspension of triphenylphosphine (16.65 g, 63.55 mmol) in MeCN (150 ml) at 0°C. The mixture was allowed to reach ambient temperature before a solution of (*2R,3R*) 2,3-*O*-isopropylidenethreitol (5.0 g, 31 mmol) in MeCN (75) was added over a period of 5 min. The mixture was stirred at ambient temperature for 1 h, heated at 70°C for 3 h, the solvent distilled off, the residue extracted with diethyl ether, filtered, the filtrate evaporated and the residual dibromide purified by bulb-to-bulb distillation, b.p. 100 °C/0.07 torr; yield 8.93 g (67%). ^1H NMR ($CDCl_3$): δ 1.45(s, 6H), 3.55(m, 4H), 4.14 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 27.38, 32.51, 79.08, 110.50. The product was used for the subsequent alkylation reaction without further purification or characterization.

(*2R,3R*)-1,4-Dibromo-2,3-isopropylidenedioxybutane (**4b**). Compund **4b** was prepared as above from triphenylphosphine (23.8 g, 91 mmol), bromine (14.24 g, 4.53 ml, 89 mmol) and (*2S,3S*)-2,3-*O*-isopropylidenethreitol (7.18 g, 44.32 mmol) in MeCN; b.p. by bulb-to-bulb distillation 100 °C; yield 11.45 g (90%). ^1H NMR ($CDCl_3$): δ 1.45 (s, 6H), 3.55 m, 4H), 4.14 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 27.38, 32.48, 79.08, 110.48. The product was used for the subsequent alkylation reaction without further purification or characterization.

(*2R,3S*)-1,4-Dibromo-2,3-isopropylidenedioxybutane (**4c**). Compund **4c** was prepared as above from triphenylphosphine (13.6 g, 51.9 mmol), bromine (7.93 g, 2.53 ml, 49.6 mmol) and 2,3-*O*-isopropylidenerythreitol (3.82 g, 23.6 mmol) in MeCN; b.p. by bulb-to-bulb distillation 100 °C/0.07 torr; yield 4.52 g (67%). ^{1}H NMR ($CDCl_3$): δ 1.39(s, 3H), 1.50(s, 3H), 3.45(m, 4H), 4.45 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 25.44, 28.02, 29.08, 77.32, 109.54. The product was used for the subsequent alkylation reaction without further purification or characterization.

(*2R,3R*)-1,4-Bis[(*2R,5S*)-2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl]-2,3-isopropylidenedioxybutane (**5a**). A solution of nBuLi in hexane (40.0 mmol, 25 ml) was added to a solution of (*2R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine (7.36 g, 40 mmol) in anhydrous THF (150 ml) at -78°C, the result solution stirred at -78°C for 20 min before a solution of (*2S,3S*)-1,4-dibromo-2,3-isopropylidenedioxybutane (3.78 g, 13.1 mmol) in THF (10 ml) was added dropwise. The reaction mixture was stirred at 4°C for 36 h before hydrolysis with phosphate buffer (pH 7). The mixture was extracted with diethyl ether, the organic layer washed with water and brine, dried ($MgSO_4$) and the solution evaporated to dryness. The residual product contained some 35% of the stereoisomer with (*R*)-configuration at C-5 in one of the bislactim rings. The desired stereoisomer was obtained essentially isomerically pure by repeated crystallization from MeCN; yield 3.70 g (57%), d.e. 96%. Found: C, 60.76; H, 8.32; N, 11.36. Calc. for $C_{25}H_{42}N_4O_6$: C, 60.71; H, 8.56; N, 11.33. ^{1}H NMR ($CDCl_3$): δ 0.68 (d, 6H), 1.03 (d, 6H), 1.34 (s, 6H), 1.95-2.15 (m, 4H), 2.24 (m, 2H), 3.65 (s, 6H), 3.67 (s, 6H), 3.94 (m, 2H), 4.04 (m, 2H), 4.11 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 16.91, 19.05, 27.39, 31.73, 38.32, 52.27, 52.36, 52.70, 60.60, 77.89, 108.19, 163.66, 163.70. FAB-MS: *m/z* 495.4 (M^++H, 100).

(*2S,3S*)-1,4-Bis[(*2R,5S*)-2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl]-2,3-isopropylidenedioxybutane (**5b**). A solution of nBuLi in hexane (52 mmol, 32.5 ml) was added to a solution of (*2R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine (9.59 g, 52.1 mmol) in anhydrous THF (180 ml) at -78°C, the mixture stirred at -78°C for 60 min before dropwise addition of a solution of (*2R,3R*)-1,4-dibromo-2,3-isopropylidenedioxybutane (6.0 g, 20.83 mmol) in THF (109 ml). The resultant mixture was allowed to reach ambient temperature, stirred for 36 h before hydrolysis was effected by addition of phosphate buffer (pH 7). The hydrolyzed mixture was extracted with diethyl ether, the organic layer washed with water and brine, dried ($MgSO_4$) and the solvent distilled off. The residual product contained about 18% of the undesired stereoisomer with (*R*)-configuration at C-5 in one of the bislactim rings which was largely removed by repeated crystallization from MeCN; yield 3.43 g (34%), d.e. 94% . Found: C, 60.89; H, 8.42; N, 11.45. Calc. for $C_{25}H_{42}N_4O_6$: C, 60.71; H, 8.56; N, 11.33. ^{1}H NMR ($CDCl_3$): δ 0.70 (d, 6H), 1.03 (d, 6H), 1.44 (s, 6H), 2.15-2.30 (m, 4H), 3.66 (s, 6H), 3.69 (s, 6H), 3.92 (m, 2H), 4.04 (m, 2H), 4.09-4.20 (m, 4H). ^{13}C NMR ($CDCl_3$): δ 16.78, 19.11, 27.67, 31.88, 38.38, 52.37, 52.44, 52.83, 60.84, 77.65, 108.57, 163.15, 163.88. FAB-MS: *m/z* 495.4 (M^++H, 53), 253.2 (40), 197.2 (58), 141.1 (100).

(*2R,3S*)-1,4-Bis[(*2R,5S*)-2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl]-2,3-isopropylidenedioxybutane (**5c**). A solution of nBuLi in hexane (33.0 mmol, 20.6 ml) was added to a solution of (*2R*)-2,5-dihydro-3,6-dimethoxy-2-isopropylpyrazine (6.08 g, 33.0 mmol) in anhydrous THF (120 ml) at -78°C, the mixture stirred at -78°C for 45 min before dropwise addition of a solution of (*2R,3S*)-1,4-dibromo-2,3-isopropylidenedioxybutane (4.52 g, 15.70 mmol) in THF (10 ml). The resultant mixture was allowed to reach

ambient temperature, stirred for 36 h before hydrolysis was effected by addition of phosphate buffer (pH 7). The hydrolyzed mixture was extracted with diethyl ether, the organic layer washed with water and brine, dried ($MgSO_4$) and the solvent distilled off. The residual product contained about 47% of the other diastereomers which were largely removed by by flash chromatography on silica gel (hexane:ethyl acetate 9:1); yield 0.77 g (10%) d.e. 94%. Found: C, 60.78; H, 8.40; N, 11.80. Calc. for $C_{25}H_{42}N_4O_6$:C, 60.71; H, 8.56; N, 11.33. ^{1}H NMR ($CDCl_3$): δ 0.68(m, 6H), 1.03 (m, 6H), 1.32 (s, 3H), 1.45 (s, 3H), 1.30-1.55 (m, 1H), 1.85-2.30 (m, 5H), 3.62 (s, 3H), 3.66 (s, 3H), 3.68 (s, 6H), 3.92 (m, 2H), 4.06-4.26 (m, 3H), 4.45 (m, 1H). ^{13}C NMR ($CDCl_3$): δ 16.62, 16.77, 19.04, 19.08, 26.20, 28.49, 31.71, 31.91, 33.69, 35.07, 52.21, 52.27, 52.32, 52.35, 52.40, 52.98, 60.58, 60.80, 74.31, 74.66, 107.16, 163.03, 163.40, 163.91, 164.30. FAB-MS: *m/z* 495.5 (M^++H).

(*S,S*)-2,7-Bis(9-fluorenylmethyloxycarbonylamino)-(*E*)-4-octenedioic acid (**10a**). A solution of 1,4-bis[(*2R,5S*)-2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl]-(*E*)-2-butene (4.02 g, 9.9 mmol) in dioxane (40 ml) and HCl (20.0 mmol, 1.67 ml) in water (38 ml) was stirred at ambient temperature for 4 h. The solution was then extracted with diethyl ether, aqueous conc. ammonia added to the aqueous solution until pH 9 and the mixture extracted with chloroform. The dried ($MgSO_4$) organic solution was evaporated and the valine methyl ester removed from the residual material by slow bulb-to-bulb distillation at 0.04 torr/30 °C. The product was dimethyl (*2S,7S*)-2,7-diamino-(*E*)-4-octenedioate (**6a**); yield 1.92 g (84%). ^{1}H NMR ($CDCl_3$): δ 1.70 (s, 4H), 2.20-2.60 (m, 4H), 3.51 (dd, 2H), 3.69 (s, 6H), 5.46 (dd, 2H). ^{13}C NMR ($CDCl_3$): δ 37.99, 52.00, 54.11, 128.84, 175.32.

A solution of **6a** (1.65 g, 7.17 mmol) thus prepared in 6 M HCl (10 ml, 60 mmol) under N_2 was heated under reflux for 2 h, the solution evaporated and the residual material dissolved in water (10 ml). The product (*2S,7S*)-2,7-diamino-(*E*)-4-octenedioic acid dihydrochloride (**7a**) crystallized from the solution on addition of ethanol (100 ml); yield 1.32 g (67%). ^{1}H NMR (D_2O): δ 2.53(dd, 4H), 3.97(dd, 2H), 5.52 (dd, 2H). ^{13}C NMR (D_2O): δ 33.57, 53.07, 128.85, 171.49. FAB-MS: *m/z* 405.3 (11), 203.2 (100), 157.1 (19), 130.1 (7), 93.0 (18), 73.9 (13).

The product **7a** (1.59 g, 5.8 mmol) was added to HMDS (20 ml) and TMSCl (1 ml), the suspension refluxed overnight, the resultant solution evaporated, the residue dissolved in dichloromethane (20 ml), cooled to 0 °C, 9-fluorenylmethyloxycarbonyl chloride (3.10 g, 12 mmol) in dichloromethane (10 ml) added and the solution stirred at 0 °C for 1 h and at ambient temperature overnight. The solution was then evaporated, the residue dissolved in THF and 1 M aqueous HCl added. The resultant solution was stirred for 2 h at ambient temperature, extracted with chloroform and the dried ($MgSO_4$) solution evaporated. The residue was dissolved in dichloromethane and the title compound made to crystallize out from the solution by addition of diethyl ether; yield 2.50 g (67%). Found: C, 70.17; H, 5.58; N, 4.08. Calc. for $C_{38}H_{34}N_2O_8$: C, 70.59; H, 5.26; N, 4.33. ^{1}H NMR (DMSO-d_6): δ 2.2-2.4 (s, 4H), 3.6 (s, 2H), 3.9-4.5 (m, 8H), 5.55 (s, 2H), 7.2-8.1 (m, 16H). ^{13}C NMR (DMSO): δ 33.98, 46.56, 53.93, 65.49, 119.66, 124.81, 126.61, 127.17, 127.98, 140.19, 143.28, 155.43, 172.59. FAB-MS: *m/z* 661.3 (3), 647.4 (M^++H, 3), 191.2 (17), 179.2 (100), 165.1 (18), 78.9 (31).

(*S,S*)-2,7-Bis(9-fluorenylmethyloxycarbonylamino)-(*Z*)-4-octenedioic acid (**10b**). A solution of 1,4-bis[(*2R,5S*)-2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl]-(*Z*)-2-butene (0.23 g, 0.55 mmol) in dioxane

(30 ml) and HCl (40 mmol, 4.80 ml) in water (10 ml) was stirred at ambient temperature for 12 h, the solution extracted with diethyl ether, aqueous conc. ammonia added to the aqueous solution until pH 9 and the mixture extracted with chloroform. The dried ($MgSO_4$) organic solution was evaporated and the valine methyl ester was removed from the residual material by slow bulb-to-bulb distillation at 30 °C/0.02 torr. The residual material was dimethyl (*2S,7S*)-2,7-diamino-(*Z*)-4-octenedioate (**6b**); yield 0.127 g (85%). ^{1}H NMR ($CDCl_3$): δ 1.54 (s, 4H), 2.3-2.6 (m, 4H), 3.52 (dd, 2H), 3.71 (s, 6H), 5.54 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 32.55, 52.02, 54.17, 127.94, 175.67.

The product **6b** (0.108 g, 0.47 mmol) thus obtained was dissolved in methanol (0.7 ml) and a 2 M solution of LiOH in water (1.41 mmol, 0.71ml). The solution was stirred at ambient temperature overnight before acidification by dropwise addition of 1 M HCl when the product (*2S,7S*)-2,7-diamino-(*Z*)-4-octenedioic acid dihydrochloride (**7b**) crystallized out; yield 50 mg (39 %). ^{1}H NMR (D_2O): δ 2.58 (m, 4H), 3.99 (m, 2H), 5.51 (m, 2H). ^{13}C NMR (D_2O): δ 27.59, 52.20, 126.84, 171.10.

9-Fluorenylmethyloxycarbonyl chloride (0.26 g, 1.0 mmol) in a solution in dioxane (1 ml) was added to a solution of the above product **7b** (0.050 g, 0.25 mmol) and Na_2CO_3 (0.21 g, 20.0 mmol) in water (3 ml) and dioxane (2 ml) at 0°C. The mixture was stirred at 0°C for 3 h, at ambient temperature overnight and the mixture shaken with diethyl ether. The aqueous solution was acidified by addition of hydrochloric acid, the solution extracted with chloroform, the chloroform solution washed, dried ($MgSO_4$) and the amino acid crystallized by addition of hexane. The compound was further purified by flash chromatography on silica gel ($CHCl_3$:AcOH 9:1); yield 90.0 mg (56%). ^{1}H NMR (DMSO-d_6): δ 2.12-2.64 (m, 4H), 3.88 (s, 2H), 4.04-4.37 (m, 6H), 5.42 (s, 2H), 7.02 (s, 2H), 7.20-8.00 (m, 16H). ^{13}C NMR (DMSO-d_6): δ 29.77, 46.88, 55.53, 65.66, 120.23, 125.43, 127.23, 127.74, 129.08, 140.84, 144.11, 155.59, 174.10. FAB-MS: *m/z* 676.2 (2), 587.5 (5), 447.4 (10), 419.3 (45), 391.3 (100), 363.3 (38), 261.2 (45), 233.2 (29).

(*2S,4R,5R,7S*)-2,7-Bis(9-fluorenylmethyloxycarbonylamino)-4,5-isopropylidenedioxyoctanedioic acid (**11a**).

A solution of 9.88 mmol HCl (0.82 ml) in water (10 ml) was added to a solution of (*2R,3R*)-1,4-bis[(*2R,5S*)-2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl]-2,3-isopropylidenedioxybutane (1.22 g, 2.47 mmol) in dioxane (10 ml) and methanol (20 ml). The resultant solution was stirred at ambient temperature overnight, the solution evaporated, the residue extracted into water, the water solution shaken with diethyl ether, the pH adjusted to 9 by addition of conc. ammonia and extracted with chloroform. The dried ($MgSO_4$) chloroform solution was evaporated and the valine methyl ester removed from the residual material by slow bulb-to-bulb distillation at 30-40 °C/0.04 Torr. The residual material was dimethyl (*2S,4R,5R,7S*)-2,7-diamino-4,5-isopropylidenedioxyoctanedioate (**8a**); yield 0.67 g (90%). ^{1}H (NMR $CDCl_3$): δ 1.34 s, 6H), 1.64 (br s, 4H), 1.72-1.84 (m, 2H), 1.99-2.08 (m, 2H), 3.65(m, 2H), 3.71 (s, 6H), 3.80 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 27.17, 37.54, 52.04, 52.74, 78.78, 108.99, 175.51.

Aqueous 2 M LiOH (2.1 ml, 4.1 mmol) was added to a solution of the above product **8a** (0.62 g, 2.04 mmol) in dioxane (2.5 ml) and the solution stirred under argon overnight. The acid (*2S,4R,5R,7S*)-2,7-diamino-4,5-isopropylidenedioxyoctanedioic acid (**9a**) was not isolated; the solution was used directly in the subsequent *N*-acylation reaction.

Dioxane (10 ml) and 1 M NaOH (10 ml, 10 mmol) were added to the solution of **9a** at 0 °C and 9-fluorenylmethyloxycarbonyl chloride (2.07 g, 8.0 mmol) added in small portions. The mixture was stirred at 0°C for 5 h, at ambient temperature overnight, extracted with diethyl ether and the aqueous solution acidified

by the addition of $KHSO_4$. The mixture was extracted with chloroform, the dried ($MgSO_4$) organic solution evaporated and excess unreacted Fmoc-Cl removed by silica gel filtration using Me_2CO:MeOH. Evaporation of the filtrate and crystallization of the product from $CHCl_3$:Et_2O gave the title compound; yield 0.49 g (34%). Found: N, 3.81. Calc. for $C_{41}H_{40}N_2O_{10}$: N, 3.89. ^{1}H NMR (DMSO-d_6/D_2O): δ 1.20 (s, 6H), 1.73 (m, 2H), 1.99 (m, 2H), 3.87 (m, 2H), 4.14-4.24 (m, 6H), 7.23-7.82 (m, 16H). ^{13}C NMR (DMSO-d_6): δ 27.78, 36.21, 47.27, 53.75, 66.19, 78.82, 107.89, 120.67, 125.81, 127.76, 128.32, 141.24, 144.39, 156.12, 175.9. FAB-MS: *m/z* 563.2 (2), 179.1 (100).

(*2S,4S,5S,7S*)-2,7-Bis(9-fluorenylmethyloxycarbonylamino)-4,5-isopropylidenedioxyoctanedioic acid (**11b**)
A solution of 20.00 mmol HCl (1.67 ml) in water (40 ml) was added to a solution of (*2S,3S*)-1,4-bis[(*2R,5S*)-2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl]-2,3-isopropylidenedioxybutane (2.5 g, 5.06 mmol) in dioxane (40 ml). The resultant solution was stirred at ambient temperature overnight, the solution evaporated, the residue extracted into water, the water solution shaken with diethyl ether, the pH adjusted to 9 by addition of conc. ammonia and extracted with chloroform. The dried ($MgSO_4$) chloroform solution was evaporated, and the valine methyl ester removed from the residual material by slow bulb-to-bulb distillation at 30-40 °C/0.04 Torr. The residual material was dimethyl (*2S,4S,5S,7S*)-2,7-diamino-4,5-isopropylidenedioxyoctanedioate (**8b**); yield 1.51 g (98%). ^{1}H NMR ($CDCl_3$): δ 1.32 (s, 6H), 1.53 (br s, 4H), 1.60-1.68 (m, 2H), 1.85-1.94 (m, 2H), 3.63 (m, 2H), 3.67 (s, 6H), 3.80 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 27.25, 37.20, 51.96, 52.74, 77.58, 108.80, 176.10

Aqueous 2 M LiOH (4.95 ml, 9.91 mmol) was added to a solution of **8b** (1.51 g, 4.96 mmol) in dioxane (5 ml) and the solution stirred under argon overnight. The acid (*2S,4S,5S,7S*)-2,7-diamino-4,5-isopropylidenedioxyoctanedioic acid (**9b**) was not isolated; the solution was used directly in the subsequent *N*-acylation reaction.

1 M NaOH (16 ml, 10 mmol) was added to the above solution of **9b** at 0 °C and a solution of 9-fluorenylmethyloxycarbonyl chloride (3.89 g, 15.0 mmol) in dioxane (16 ml) added dropwise. The mixture was stirred at ambient temperature for 90 min, extracted with diethyl ether and the aqueous solution acidified by the addition of $KHSO_4$. The mixture was extracted with chloroform, the dried ($MgSO_4$) organic solution evaporated, and the title compound isolated from the residual material by flash chromatography on silica gel (hexane:AcOEt:AcOH 5:5:1); yield 1.6 g (46%). Found: N, 4.09. Calc. for $C_{41}H_{40}N_2O_{10}$: N, 3.89. ^{1}H NMR (DMSO-d_6/D_2O): δ 1.23 (s, 6H), 1.7-2.0 (m, 4H), 3.62 (m, 2H), 4.02 (m, 2H), 4.1-4.35 (m, 6H), 7.2-7.9 (m, 16H). ^{13}C NMR (DMSO-d_6): δ 27.94, 34.59, 47.39, 52.29, 66.45, 77.49, 109.13, 120.90, 125.91, 127.97, 128.59, 141.44, 144.44, 157.02, 174.75. FAB-MS: *m/z* 743.3 (28), 563.2 (58), 505.2 (72).

(*2S,4R,5S,7S*)-2,7-Bis(9-fluorenylmethyloxycarbonylamino)-4,5-isopropylidenedioxyoctanedioic acid (**11c**).
A solution of 6.0 mmol HCl (0.50 ml) in water (12 ml) was added to a solution of (*2S,3S*)-1,4-bis[(*2S,5R*)-2,5-dihydro-3,6-dimethoxy-2-isopropyl-5-pyrazinyl]-2,3-isopropylidenedioxybutane (0.77 g, 1.55 mmol) in dioxane (12 ml). The resultant solution was stirred at ambient temperature for 5 h, the solution evaporated, the residue extracted into water, the water solution shaken with diethyl ether, the pH adjusted to 9 by addition of conc. ammonia and extracted with chloroform. The dried ($MgSO_4$) chloroform solution was evaporated, and the valine methyl ester removed from the residual material by slow bulb-to-bulb distillation at 30-40 °C/0.04 Torr. The residual material was dimethyl (*2S,4R5S7S*)-2,7-diamino-4,5-isopropylidenedioxy-

octanedioate (**8c**); yield 0.36 g (77%). ^{1}H NMR ($CDCl_3$): δ 1.30 (s, 3H), 1.41 (s, 3H), 1.40-1.48 (m,1H), 1.72 (s, 4H), 1.75-2.00 (m, 3H), 3.60-3.67 (m, 2H), 3.70 (s, 6H), 4.20-4.40 (m, 2H). ^{13}C NMR ($CDCl_3$): δ 25.81, 28.26, 34.69, 34.89, 51.55, 52.00, 52.02, 52.66, 74.26, 75.20, 108.23, 175.43, 176.51.

Aqueous 2 M LiOH (1.19 ml, 2.38 mmol) was added to a solution of the above product **9c** (0.36 g, 1.19 mmol) in dioxane (5 ml) and the solution stirred under argon overnight. The acid (*2S,4R,5S,7S*)-2,7-diamino-4,5-isopropylidenedioxyoctanedioc acid (**9c**) was not isolated; the solution was used directly in the subsequent *N*-acylation reaction.

1 M $NaHCO_3$ (4 ml, 4 mmol) was added to the above solution of **9c** at 0 °C and a solution of 9-fluorenylmethyloxycarbonyl chloride (1.03 g, 4.0 mmol) in dioxane (4 ml) added dropwise. The mixture was stirred at ambient temperature for 60 min, extracted with diethyl ether and the aqueous solution acidified by the addition of $KHSO_4$. The mixture was extracted with chloroform, the dried ($MgSO_4$) organic solution evaporated, and the title compound isolated from the residual material by flash chromatography on silica gel (hexane:AcOEt:AcOH 5:5.1); yield 0.57 g (66%). Found: N, 4.54. Calc. for $C_{41}H_{40}N_2O_{10}$: N, 3.89. ^{1}H NMR (DMSO-d_6, D_2O): δ 1.16 (s, 3H), 1.30 (s, 3H), 1.50-2.05 (m, 4H), 3.80-4.40 (m, 8H), 7.15-8.0 (m, 16H). ^{13}C NMR (DMSO-d_6, D_2O): δ 26.63, 29.05, 31.50, 31.94, 47.43, 51.52, 52.19, 66.50, 74.10, 75.10, 108.36, 120.88, 125.93, 127.98, 128.60, 141.48, 144.39, 144.76, 156.82, 157.12, 174.11, 175.11. FAB-MS: *m/z* 721.7 (M^+), 743.4.

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