Polyol Peptidomimetics

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Dedicated to Professor Horst Kessler on the occasion of his 60th birthday

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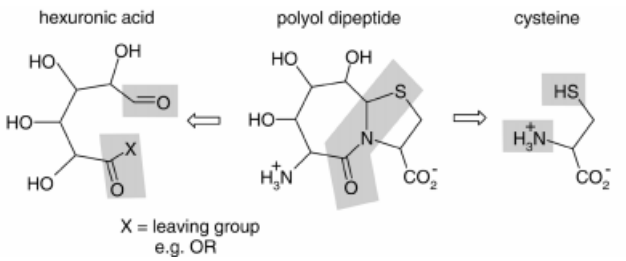
D-Glucurono-3,6-lactone and L-cysteine combine in a highly stereoselective manner to give the 7,5-bicyclic thiazolidinlactam **2**. The α-hydroxy group of the D-glucurono-3,6-lactone was exchanged for an amino function (to give **13**) and, after condensation with L-cysteine methyl ester, the polyol dipeptide **7** was obtained. Peptide couplings proceed without the need to protect the three secondary hydroxy groups of the seven-membered ring. The amino group of **7** was deprotected and selectively elongated to the *pseudo*-tripeptide **16**. The depsipeptide **17** was obtained by condensation of Boc–Ala–OH with the polyol **2**. Elongation at the carboxy terminus yielded **19** and **20**. The bicyclic scaffold populates a well-defined solution conformation; the hydroxy groups mimic the side chains of hydrophilic amino acids and can be further functionalized.

Introduction

Constraining the conformational mobility of peptides can improve their activity and selectivity of receptor binding.[1] The local structural motifs in peptides are efficiently stabilised by short-range cyclisation[2] or by macrocyclisation.[3] Bicyclic dipeptide isosters like BTD (β-turn dipeptide)[4] constrain the torsions ψ_i and ϕ_{i+1} and the side chain orientations of a dipeptide unit. The central amide bond is fixed to the *trans* orientation and βI/βII equilibria[5] are suppressed. Several bicyclic dipeptide isosters have been developed and used in the investigation of the structure-activity relationships of biologically active peptides.[6] The side-chain functionalities of the two amino acids are consumed for assembling the bicyclic ring system, therefore only a few constrained isosters with additional functional groups to mimic peptidic side chains are known.[7] This lack of extra substituents is a general shortcoming of the constrained peptidomimetics based on azabicycloalkanes.[8] Carbohydrates are a source of readily available, stereochemically defined scaffolds for the facile attachment of side chains contained in genetically encoded or other amino acids.[9] Similar concepts are pursued with the so-called sugar-amino acids[10] and other amide-linked oligosaccharides.[11] The strategy summarised in Scheme 1 combines the rigidity of the bicyclic dipeptide isosters with the polyfunctional character of carbohydrates to give a novel type of constrained polyol peptidomimetic.



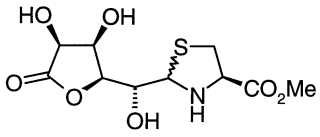
Scheme 1

A hexuronic acid is condensed with cysteine and the amino group is introduced at the α-position of the newly formed amide bond. This bicyclic template can orientate the amino- or carboxy terminal peptide chains and additionally acts as a scaffold which presents extra functionalities. The seven-membered ring contains a *cis* amide bond and therefore populates a well-defined chair conformation. Five stereocentres of the polyol dipeptide shown in Scheme 1 are introduced by the starting materials, the configuration of the newly formed bridgehead centre is controlled by the reaction conditions. We describe the straightforward assembly of highly functionalized azabicycloalkane ring systems which resemble a new class of carbohydrate-peptide hybrids.

Results and Discussion

Synthesis of the Tetrahydroxyoctahydro-5-oxo-thiazolo[3,2-a]azepine Scaffold

Reducing sugars condense with cysteine to give polyhydroxyalkyl thiazolidines; subsequent *N*-acylation stabilizes the new stereocentre against epimerisation or hydrolysis. These carbohydrate derived thiazolidines have been extensively studied.[12] An intramolecular *N*-acylation, as shown in Scheme 1, has never been observed even for activated derivatives like D-glucurono-3,6-lactone, which reacts with the methyl ester of L-cysteine in hot water to give the diastereomeric thiazolidines **1**.[13] To support the aminolysis



1 (1*R*,1*S*)

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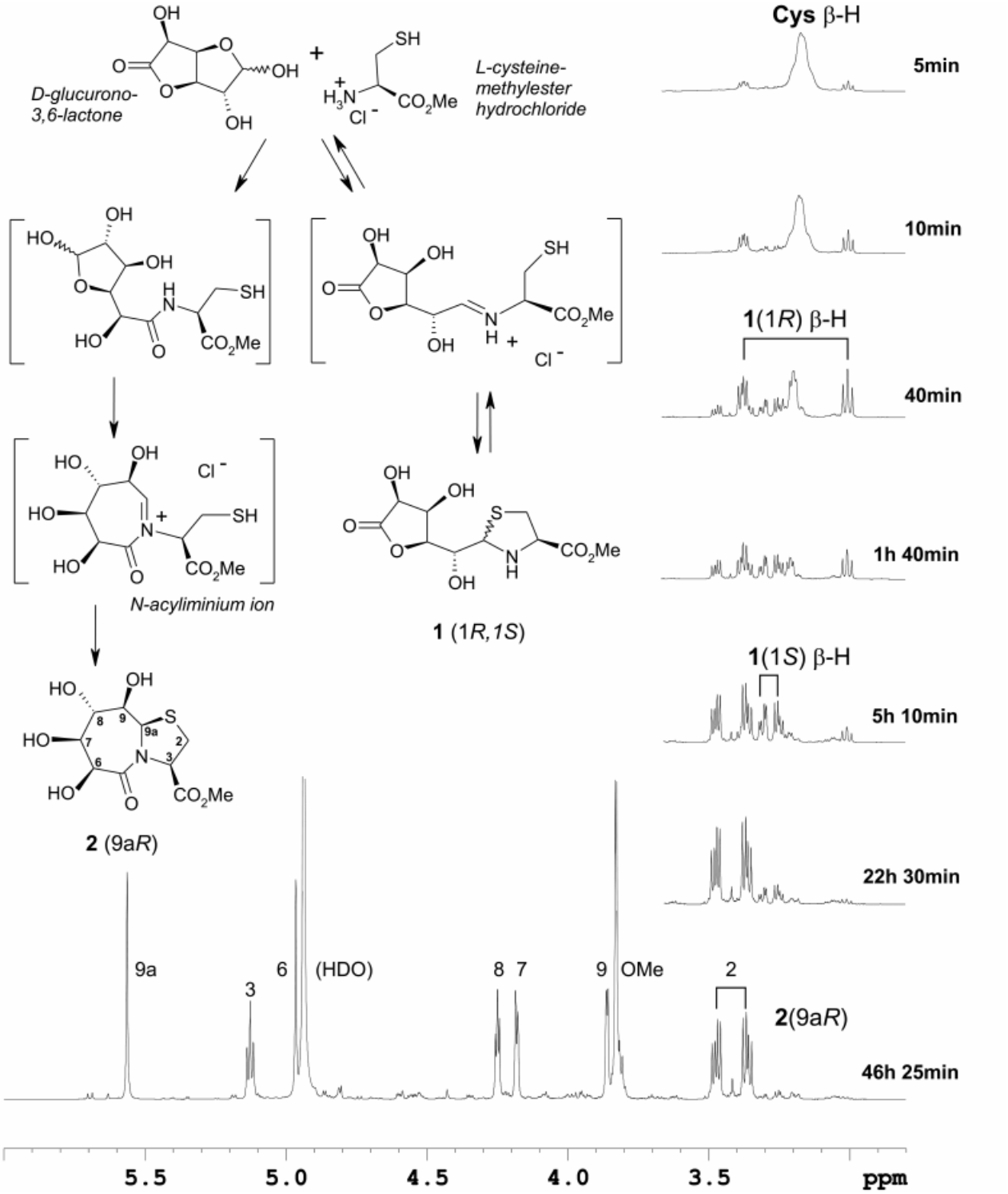
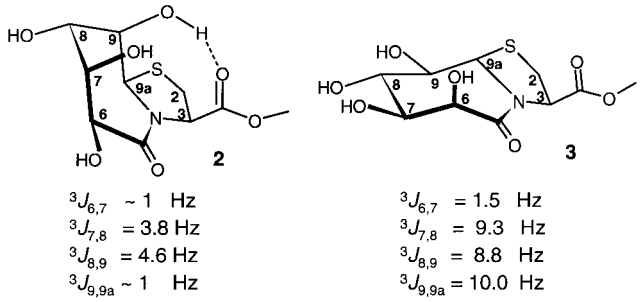


Figure 1. NMR kinetics of equimolar amounts of D-glucurono-3,6-lactone and the hydrochloride of L-cysteine methyl ester in the solvent mixture water/pyridine (9:1); expansions are shown for the β-protons between 3.0 and 3.6 ppm; the diastereomeric thiazolidines **1** dominate after 40 min (**1**-1*R*) and 5 h (**1**-1*S*); the formation of the bicyclic **2** presumably proceeds via the irreversible amide bond formation followed by *N*-acyliminium cyclization and addition of the thiol group;[14] the final ^{1}H-NMR spectrum after 2 days (bottom) shows mainly **2**; due to the small coupling constants $^3J_{6,7}$ and $^3J_{9,9a}$, the protons 6-H and 9a-H appear as singlets

of the lactone, the condensation of D-glucurono-3,6-lactone and L-cysteine methyl ester was carried out in pyridine. The thiazolidines forms quantitatively within 30 min, but again no amide bond was obtained. The aminolysis of the lactone, which does not occur in pyridine alone, proceeds smoothly in a solvent mixture of water/pyridine (9:1) and the bicyclic scaffold **2** (Figure 1) forms slowly with an excellent diastereoselectivity. The thiazolidines **1** are in equilibrium with the starting materials[14] and the annulation probably proceeds with irreversible amide bond formation[15] fol-

lowed by an *N*-acyliminium ion cyclization and addition of the thiol group.[16] When performed on a preparative scale, crystalline **2** was obtained in nearly quantitative yield.

Lower water/pyridine ratios also lower the diastereoselectivity of the condensation and the thiazolidinlactam **3** (Scheme 2) is additionally observed. In water/pyridine (1:1), the yield of **3** rises to 20%. The configuration at C-9a was unequivocally assigned by X-ray structural analysis. Higher pyridine contents lead to lower overall yields and hydrolysis products like D-glucuronic acid and thiohemiacetals dominate.



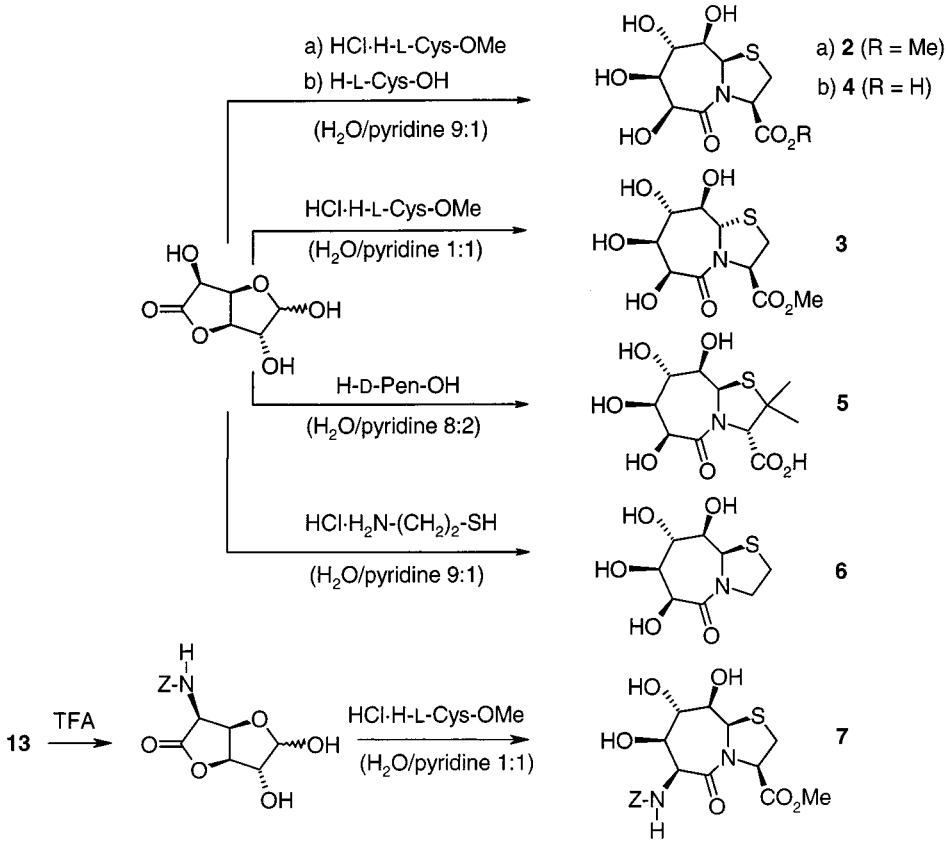
Scheme 2

Thiazolidinlactams exhibit a higher chemical stability than thiazolidines.[17] Even in boiling water, **2** and **3** resist an epimerisation of C-9a via open-chain structures. They are also stable at pH 1, hydrolysis being observed only above pH 9. Treatment with 3% H_2O_2 quantitatively forms the sulfones while thiazolidines decompose under these conditions.

Ring Conformations

The three-bond coupling constants of **2** in water (Scheme 2) are in accordance with the torsion angles of the crystal structure[18] and conformational averaging in solution is therefore excluded.

The seven-membered ring of **2** is found exclusively in the chair conformation. O-6 occupies an equatorial position and O-7, O-8, and O-9 are found in axial positions. 9-O*H* forms a hydrogen bond with the carbonyl oxygen of the ester group and holds the exocyclic torsion (N–C3–CO–O) in a preferred conformation. This hydrogen bond is maintained in solution since all derivatives of **2** exhibit a large three-bond $^3J_{9,9\text{-OH}}$ coupling constant and a distinct shielding of the 9-O*H* NMR resonance in [D_6]DMSO. The conformation of the seven-membered ring is inverted in **3**, where O-7, O-8, and O-9 occupy equatorial positions and O-6 occupies an axial position. Large coupling constants were measured for $^3J_{7,8}$, $^3J_{8,9}$, and $^3J_{9,9a}$ in water and correspond to a *trans* alignment of the corresponding protons (Scheme 2). The shape of the scaffold is determined by the configuration at C-9a. Various vicinal amino thiols can be employed for the formation of the bicyclic scaffold. In the cases studied (Scheme 3), the substitution pattern of the amino thiol had no influence on the formation of the thi-



Scheme 3

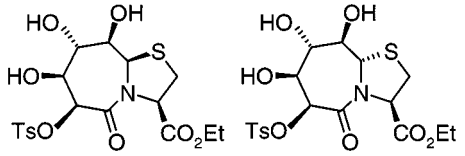
azolidinlactam or on the conformation of the seven-membered ring.

The zwitterionic L-cysteine yields the free acid **4**. The stereocentre C-3 is inverted in **5**, which is obtained from the condensation of D-penicillamine and D-glucurono-3,6-lactone. The hydrochloride of cysteamine yields **6** without a substituent at C-3.

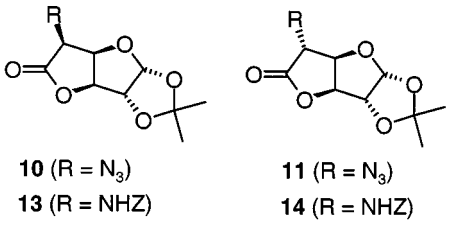
The chirality at C-3 (D-Pen or L-Cys) determines the orientation of the carboxy terminus, and the chirality of the substituent in the α-position of the uronic acid determines the orientation of the amino terminus. From the eight possible stereoisomers (3*R*/3*S*, 6*R*/6*S*, and 9a*R*/9a*S*), the bicyclic dipeptide **7**, with C-3 and C-9a in the *R*-configuration and C-6 in the *S*-configuration, resembles two L-amino acids with constrained torsions ψ_i (170°), ω_i (180°), and ϕ_{i+1} (≈ –90°) and preferred orientations for the torsions ϕ_i (≈ –130°) and ψ_{i+1} (–160°). Steric factors (β-branching) restrict the rotation about ϕ_i, the hydrogen bond between 9-O*H* and the carboxy terminus restricts the torsion ψ_{i+1}.

Formation of the α-Amino Group and Peptide Couplings

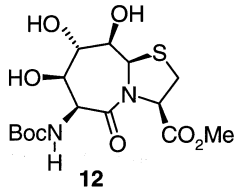
Since the α-hydroxy groups of esters can be functionalized selectively,[19] we attempted to introduce the amino terminus by this strategy. The 6-*O*-tosylates **8** and **9** form without the need for protection of the residual hydroxy groups. However, both tosyl groups could not be exchanged for nitrogen nucleophiles.



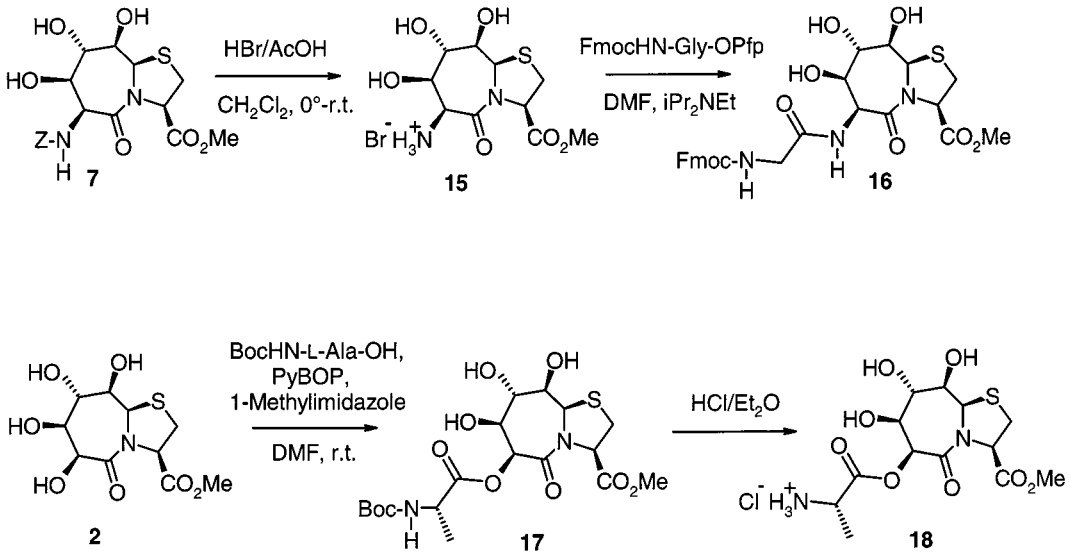
Presumably, the stiff seven-membered ring forbids a nucleophilic attack at O-6. Therefore, the amino group was introduced by known procedures at C-5 of D-glucurono-3,6-lactone before the condensation with cysteine. D-Glucurono-3,6-lactone selectively forms an acetonide[20] which was converted into the protected amino acid in either the *gluco*- (**10**) or the *ido*- (**11**) configuration.[21]



The acetonide in **10** was cleaved with TFA and the condensation with L-cysteine methyl ester gave the Boc-protected dipeptide **12**, as described in our preliminary publication.[18] The condensation was accompanied by elimination reactions and therefore yields were only moderate.



Such side reactions are avoided when the azido group was reduced and protected as a carbamate before the condensation with the vicinal amino thiol. The benzyloxycarbonyl protecting group (*Z*) in **13** and **14** allowed for the acidic cleavage of the acetonide. The orthogonally protected polyol dipeptide **7** forms from the reaction of the 1,2-deprotected **13** with the hydrochloride of the L-cysteine methyl ester in 53% yield (Scheme 3). The amino group of **7** was removed with HBr/AcOH and the salt **15** was coupled to Fmoc–Gly–OPfp in DMF in the presence of Hünig's base to yield **16** (Scheme 4).

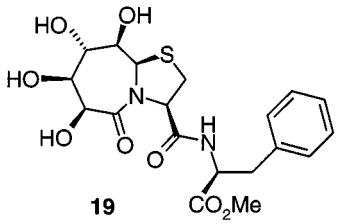


Scheme 4

Despite the strong activation by the pentafluorophenyl ester, no *O*-acylation of O-7, O-8, or O-9 was observed during the synthesis of **16**. Solubility problems of the amphiphilic **16** lead to a reduction of the isolated yield (46%).

The α-hydroxy groups of **2** can be selectively acylated and depsipeptides are accessible by this strategy: Boc–Ala–OH was activated by (benzotriazol-1-yl)oxytris(pyrrolidino)-phosphonium hexafluorophosphate (PyBOP) in DMF and coupled to the O-6 of **2** yielding **17** (95%). The addition of 1-methylimidazole proved to be essential for quantitative reaction. Deprotection of the Boc group to give **18** proceeds smoothly in 1 M HCl in diethyl ether.

C-terminal modifications were also performed: the carboxylic group in **4** was activated with 2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline (EEDQ) and condensed with HCl·H–Phe–OMe to give the *pseudo*-tripeptide **19** (44%).



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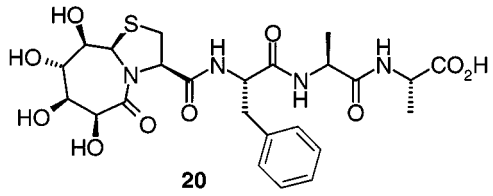
Condensation with D-glucurono-3,6-lactone in water/pyridine (9:1) is not restricted to amino acids but may be useful for the amino terminal modification of peptides. As an example, the TFA salt of H–Cys–Phe–Ala–Ala–OH was condensed with D-glucurono-3,6-lactone to give the modified analogue **20**. By this strategy, derivatives of D-glucurono-3,6-lactone can be used for the amino terminal blocking of peptides.



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Conclusion

Compound **7** is the first bicyclic peptidomimetic derived from a carbohydrate precursor. This polyol dipeptide was synthesised in a tandem reaction from commercially available vicinal amino thiols and easily accessible derivatives of D-glucurono-3,6-lactone. The 7,5-bicyclic polyol thiazolidinlactams described here mimic hydroxy amino acids or offer the possibility of further derivatisation of the azabicycloalkane backbone. Three backbone torsions of a dipeptide are constrained by the bicyclus structure (ψ_i, ω_i, and ϕ_{i+1}), the exocyclic torsion ϕ_i is restricted by the β-branched side chain (O-7) and the carboxy terminal torsion ψ_{i+1} is constrained by a hydrogen bond.

Experimental Section

General Remarks: Solvents were purified in the usual manner. Melting points are not corrected. Thin-layer chromatography (TLC): plastic sheets, silica gel F_{254} (layer thickness 0.2 mm). Optical rotations: 1-dm cell at 20 °C. NMR spectra were acquired at 600 MHz (^{1}H) and 300 K. Spin systems were assigned from DQF-COSY spectra, coupling constants were quantified after resolution enhancement by Lorentz-to-Gauss transformation of the resonance signals. The stereochemistry of the ring systems was determined with compensated ROESY spectra. Carbon assignments were taken from HMQC and HMBC spectra. MALDI-MS: positive mode, 2,5-dihydroxybenzoic acid (DHB) matrix; FAB-MS: positive mode, 3-nitrobenzyl alcohol (NBOH)/NaI matrix. EI-MS: 70 eV, 215 °C.

Compound 2: D-Glucurono-3,6-lactone (1.0 g, 5.7 mmol) and the hydrochloride of L-cysteine methyl ester (0.98 g, 5.7 mmol) were dissolved in 15 mL water/pyridine (9:1) and stirred for 3 days at room temp. The solvent was removed in vacuo and the colourless foam was dissolved in water where crystals of **2** formed slowly (1.63 g, 98%). TLC (ethyl acetate): R_f = 0.38; mp = 196 °C. – $[\alpha]_D^{20}$ –55.5 (c = 1.07, H_2O). – ^{1}H NMR ([D$_6$]DMSO): δ = 5.56 (d, $^3J_{8\text{-OH},8}$ = 3.9 Hz, 1 H, 8-OH), 5.42 (s, 1 H, 9a-H), 5.29 (d, $^3J_{7\text{-OH},7}$ = 5.4 Hz, 1 H, 7-OH), 4.73 (t, 1 H, 3-H), 4.68 (d, $^3J_{6,6\text{-OH}}$ = 6.6 Hz, 1 H, 6-H), 4.45 (d, $^3J_{6\text{-OH},6}$ = 6.6 Hz, 1 H, 6-OH), 4.25 (d, $^3J_{9\text{-OH},9}$ = 11.0 Hz, 1 H, 9-OH), 3.81 (dd, $^3J_{8,8\text{-OH}}$ = 3.9 Hz, $^3J_{8,9}$ = 3.8 Hz, 1 H, 8-H), 3.78 (t, 1 H, 7-H), 3.63 (s, 3 H, OCH_3), 3.52 (dd, $^3J_{9,9\text{-OH}}$ = 11.1 Hz, $^3J_{9,8}$ = 3.8 Hz, 1 H, 9-H), 3.26–3.29 (m, 2 H, 2-H). – ^{13}C NMR: δ = 170.67 (5-C), 170.46 (CO$_2$CH$_3$), 77.00 (9-C), 76.13 (7-C), 70.99 (8-C), 69.28 (6-C), 63.90 (3-C), 61.07 (9a-C), 52.19 (OCH$_3$), 31.42 (2-C). – EI-MS: m/z 293 [M]$^+$. – $C_{10}H_{15}NO_7S$ (293.3): calcd. C 40.95, H 5.15, N 4.78; found C 41.38, H 5.26, N 4.62.

Compound 3: D-Glucurono-3,6-lactone (10.1 g, 57.2 mmol) was dissolved in 100 mL H_2O/pyridine (1:1) and the hydrochloride of the L-cysteine methyl ester (9.82 g, 57.2 mmol) was added. The solution was stirred for 3 days at room temp. The reaction mixture was absorbed on silica gel 60. Compound **3** was separated from **2** by flash chromatography (ethyl acetate/MeOH, 10:1) and crystallised as colourless needles (3.43 g, 21%). TLC (ethyl acetate/MeOH, 10:1) R_f = 0.48; mp = 190.5 °C. – $[\alpha]_D^{20}$ = –178.6 (c = 0.87, H_2O). – ^{1}H NMR (D_2O): δ = 5.66 (d, $^3J_{9a,9}$ = 9.8 Hz, 1 H, 9a-H), 5.18 (d, $^3J_{3,2}$ = 6.8 Hz, 1 H, 3-H), 4.50 (s, 1 H, 6-H), 3.88 (t, $^3J_{9,9a/8}$ 9 Hz, 1 H, 9-H), 3.80 (s, 3 H, OCH_3), 3.74 (d, $^3J_{7,8}$ = 9.4 Hz, 1 H, 7-H), 3.45 (m, 1 H, 8-H), 3.40 (dd, $^2J_{gem}$ = 12.9 Hz, $^3J_{2proR,3}$ = 7.4 Hz, 1 H, 2-H^{proR}), 3.19 (dd, $^2J_{gem}$ = 12.9 Hz, $^3J_{2proS,3}$ < 1 Hz, 1 H, 2-H^{proS}). – ^{13}C NMR: δ = 174.54 (CO$_2$Me), 173.70 (5-C), 79.55 (6-C), 77.86 (8-C), 75.65 (9-C), 73.91 (7-C), 67.42 (3-C), 66.82 (9a-C), 56.31 (OCH$_3$), 31.22 (2-C). – MALDI-MS: m/z = 315.9 [MNa]$^+$. – $C_{10}H_{15}NO_7S$ (293.3): calcd. C 40.95, H 5.15, N 4.78; found C 40.98, H 5.09, N 4.48.

Compound 4: D-Glucurono-3,6-lactone (10.2 g, 58 mmol) and L-cysteine (7.0 g, 58 mmol) were dissolved in 150 mL water/pyridine (9:1) and refluxed for 2 h. The solvent was removed in vacuo and twice coevaporated with toluene. The yellowish foam was purified by flash chromatography ($CHCl_3$/MeOH, 3:1) and crystals of **4** formed slowly (14.5 g, 85%). TLC ($CHCl_3$/MeOH, 3:1): R_f = 0.4. – ^{1}H NMR ([D$_4$]methanol): δ = 5.54 (s, 1 H, 9a-H), 4.92 (t, 1 H, 3-H), 4.83 (s, 1 H, 6-H), 4.05 (t, 1 H, 8-H), 3.99 (d, $^3J_{7,8}$ = 4.3 Hz, 1 H, 7-H), 3.69 (d, $^3J_{9,8}$ = 3.4 Hz, 1 H, 9-H), 3.44 (dd, $^3J_{2,3}$ =

7.0 Hz, $^2J_{gem}$ = 11.3 Hz, 1 H, 2-H), 3.32 (dd, $^3J_{2,3}$ = 7.4 Hz, $^2J_{gem}$ = 11.3 Hz, 1 H, 2-H′). – ^{13}C NMR: δ = 174.34 (*C*O$_2$ H), 172.62 (5-C), 77.92 (9-C), 77.49 (7-C), 72.49 (8-C), 71.06 (6-C), 66.00 (3-C), 62.70 (9a-C), 33.00 (2-C). – FAB-MS (positive mode, glycerol matrix): *m*/*z* = 280 [MH]$^+$.

Compound 5: D-Glucurono-3,6-lactone (1.00 g, 5.1 mmol) and D-penicillamine (0.76 g, 5.1 mmol) were dissolved in 20 mL water/pyridine (8:2) and stirred for 3 days at room temp. The reaction mixture was absorbed on silica gel 60 and after flash chromatographic workup (CHCl$_3$/MeOH, 3:1) **5** was obtained as a yellow solid (1.09 g, 69%). TLC (CHCl$_3$/MeOH, 3:1) R_f = 0.21; mp = 127 °C. – $[\alpha]_D^{20}$ = 61.0 (*c* = 1.0, H$_2$O). – ^{1}H NMR ([D$_6$]DMSO): δ = 5.39 (s, 1 H, 9a-H), 4.50 (s, 1 H, 3-H), 4.49 (s, 1 H, 6-H), 3.89 (m, 1 H, 8-H), 3.84 (m, 1 H, 7-H), 3.55 (bs, 1 H, 9-H), 1.75 (s, 3 H, C*H*$_3$), 1.34 (s, 3 H, C*H*$_3$). – ^{13}C NMR: δ = 172.25 (*C*O$_2$ H), 170.62 (5-C), 76.16 (9-C), 75.56 (7-C), 75.44 (3-C), 69.82 (8-C), 69.78 (6-C), 61.30 (9a-C), 33.39 (*C*H$_3$), 26.08 (*C*H$_3$). – MALDI-MS: *m*/*z* = 328.7 [MNa]$^+$. – C$_{11}$H$_{17}$NO$_7$S (307.3): calcd. C 42.99, H 5.58, N 4.56; found C 42.70, H 6.01, N 4.04.

Compound 6: D-glucurono-3,6-lactone (1.00 g, 5.7 mmol) and the hydrochloride of cysteamine (0.64 g, 5.7 mmol) were dissolved in 50 mL water/pyridine (9:1) and stirred for 3 days at room temp. The reaction mixture was absorbed on silica gel 60 and purified by flash chromatography (MeOH/CHCl$_3$, 1:3). Compound **6** was isolated as a colourless solid (1.10 g, 82%). TLC (MeOH/CHCl$_3$, 1:3) R_f = 0.55; mp = 167 °C. – $[\alpha]_D^{20}$ = –1.12 (*c* = 1.07, DMSO). – ^{1}H NMR ([D$_6$]DMSO): δ = 5.57 (d, $^3J_{8\text{-OH},8}$ = 4.1 Hz, 1 H,8-OH), 5.44 (d, ^3J = 5.9 Hz, 1 H, 7-OH), 5.21 (s, 1 H, 9a-H), 4.61 (d, $^3J_{9\text{-OH},9}$ = 8.5 Hz, 1 H, 9-OH), 4.59 (d, ^3J = 5.6 Hz, 1 H, 6-H), 4.33 (d, ^3J = 5.6 Hz, 1 H,6-OH), 3.80 (m, 2 H, 3-H, 8-H), 3.75 (t, 1 H, 7-H), 3.54 (dd, $^3J_{9,8}$ = 4.1 Hz, $^3J_{9,9\text{-OH}}$ = 8.8 Hz, 1 H, 9-H), 3.21 (m, 1 H, 2-H), 2.89 (m, 1 H, 2-H′). – ^{13}C NMR: δ = 169.58 (5-C), 77.54 (9-C), 75.59 (7-C), 70.49 (8-C), 69.08 (6-C), 59.30 (9a-C), 51.52 (3-C), 28.23 (2-C). – FAB-MS: *m*/*z* = 236 [MH]$^+$, 258 [MNa]$^+$, 471 ([2M]H$^+$).

Compound 7: Acetonide deprotected **13** (2.00 g, 7.4 mmol) and the hydrochloride of L-cysteine methyl ester (1.27 g, 7.4 mmol) were dissolved in 50 mL of water/pyridine (1:1). The reaction mixture was stirred for 7 h at 70 °C and then absorbed on silica gel 60. Flash chromatography (toluene/ethyl acetate, 1:4) yields **7** as a yellow solid (1.69 g, 53%). TLC (toluene/ethyl acetate, 1:4) R_f = 0.18; mp = 81.5 °C. – $[\alpha]_D^{20}$ = –30.8 (*c* = 1.07, DMSO). – ^{1}H NMR ([D$_6$]DMSO): δ = 7.09–7.37 (m, 5 H, Z–P*h*), 7.09 (d, $^3J_{NH,6}$ = 8.8 Hz, 1 H, Z–N*H*), 5.68 (d, $^3J_{8\text{-OH},8}$ = 3.9 Hz, 1 H, 8-OH), 5.48 (s, 1 H, 9a-H), 5.33 (d, $^3J_{7\text{-OH},7}$ = 4.7 Hz, 1 H, 7-OH), 5.03 (s, 2 H, Z–CH$_2$), 4.86 (d, $^3J_{6,NH}$ = 8.6 Hz, 1 H, 6-H), 4.70 (t, $^3J_{3,2}$ = 7.7 Hz, 1 H, 3-H), 4.21 (d, $^3J_{9\text{-OH},9}$ = 11.5 Hz, 1 H, 9-OH), 3.83 (m, 1 H, 8-H), 3.81 (m, 1 H, 7-H), 3.63 (s, 3 H, OC*H*$_3$), 3.57 (dd, $^3J_{9,8}$ = 3.6 Hz, $^3J_{9,9\text{-OH}}$ = 11.5 Hz, 1 H, 9-H), 3.16 (m, 2 H, 2-H). – ^{13}C NMR: δ = 170.65 (*C*O$_2$Me), 167.79 (5-C), 155.72 (O*C*ON), 136.95, 128.30, 127.79, 127.62 (Phe-arom.), 77.23 (9-C), 75.26 (7-C), 70.46 (8-C), 65.49 (*Z*–CH$_2$), 64.07 (3-C), 61.02 (9a-C), 54.28 (6-C), 52.21 (O*C*H$_3$), 31.48 (2-C). – MALDI-MS: *m*/*z* = 448.0 [MNa]$^+$, 464.0 [MK]$^+$. – C$_{18}$H$_{22}$N$_2$O$_8$S (426.4): calcd. C 50.70, H 5.20, N 6.57; found C 51.68, H 5.39, N 6.33.

Compound 8: The ethyl ester of **2** (2.00 g, 6.5 mmol) was dissolved in 50 mL dry pyridine and cooled to 0 °C. *p*-Toluenesulfonyl chloride (1.36 g, 7.1 mmol) and a catalytic amount of DMAP were then added and the reaction mixture was stirred for 72 h at 4 °C and then poured on ice. The organic phase was separated and the aqueous phase was extracted three times with ethyl acetate. The combined organic phases were dried with MgSO$_4$. The oil was dissolved in hot ethyl acetate (5 mL) from which **8** solidified (2.40 g, 79%). TLC (ethyl acetate) R_f = 0.63; mp = 199 °C. – $[\alpha]_D^{20}$ = –71.0 (*c* = 1.0, acetone). – ^{1}H NMR ([D$_6$]DMSO): δ = 7.81 (d, $^3J_{Ts}$ = 8.2 Hz, 2 H, Ts-arom.), 7.44 (d, $^3J_{Ts}$ = 8.2 Hz, 2 H, Ts-arom.), 5.83 (s, 1 H, 8-OH), 5.73 (s, 1 H, 7-OH), 5.66 (s, 1 H, 6-H), 5.49 (s, 1 H, 9a-H), 4.65 (m, 1 H, 3-H), 4.33 (d, ^3J = 10.9 Hz, 1 H, 9-OH), 4.06 (m, 2 H, C*H*$_2$–CH$_3$), 3.77 (m, 2 H, 7-H, 8-H), 3.54 (m, 1 H, 9-H), 3.29 (m, 2 H, 2-H), 2.40 (s, 3 H, Ts–C*H*$_3$), 1.51 (t, 3 H, CH$_2$–C*H*$_3$). – ^{13}C NMR: δ = 169.58 (*C*O$_2$Et), 163.95 (5-C), 144.78, 133.57, 129.95, 127.58 (Ts-arom.), 78.26 (6-C), 77.08 (7-C), 74.70 (9-C), 70.30 (8-C), 64.40 (3-C), 61.10 (*C*H$_2$–CH$_3$), 60.95 (9a-C), 31.33 (2-C), 21.12 (Ts–CH$_3$), 13.77 (CH$_2$–CH$_3$). – FAB-MS: *m*/*z* = 462 [MH]$^+$, 484 [MNa]$^+$. – C$_{18}$H$_{23}$NO$_9$S$_2$ (641.5): calcd. C 46.85, H 5.02, N 3.03; found C 46.50, H 5.38, N 2.64.

Compound 9: The ethyl ester of **3** (50 mg, 0.16 mmol) was dissolved in dry pyridine (2 mL) and cooled to 0 °C. *p*-Toluenesulfonyl chloride (68 mg, 0.36 mmol) and catalytic amounts of DMAP were added and the reaction mixture was stirred for 72 h at 4 °C and then poured on ice. The aqueous phase was extracted three times with ethyl acetate. The combined organic phases were dried with MgSO$_4$. The oil was dissolved in hot ethyl acetate (5 mL) from which **9** solidified (30 mg, 40%). TLC (ethyl acetate) R_f = 0.72; mp = 199 °C. – ^{1}H NMR ([D$_6$]DMSO): δ = 7.75 (d, $^3J_{Ts}$ = 8.2 Hz, 2 H, Ts-arom.), 7.42 (d, $^3J_{Ts}$ = 8.2 Hz, 2 H, Ts-arom.), 5.53 (d, $^3J_{9a,9}$ = 10.2 Hz, 1 H, 9a-H), 4.87 (dd, $^3J_{3,2}$ = 2.4 Hz, $^3J_{3,2}$ = 6.9 Hz, 1 H, 3-H), 4.35 (s, 1 H, 7-H), 4.29 (d, $^3J_{6,7}$ = 6.5 Hz, 1 H, 6-H), 4.06 (m, 1 H, C*H*$_2$–CH$_3$), 3.73 (t, $^3J_{9,9a/8}$ 9 Hz, 1 H, 9-H), 3.20 (m, 1 H, 2-H), 3.13 (m, 1 H, 8-H), 2.89 (dd, $^2J_{gem}$ = 12.1 Hz, $^3J_{2,3}$ = 2.4 Hz, 1 H, 2-H′), 2.42 (s, 3 H, Ts–C*H*$_3$), 1.27 (t, 3 H, CH$_2$–C*H*$_3$). – ^{13}C NMR: δ = 168.89 (*C*O$_2$Et), 166.99 (5-C), 144.89, 133.46, 129.73, 127.82 (Ts-arom.), 82.60 (7-C), 74.94 (6-C), 73.80 (9-C), 72.26 (8-C), 64.06 (3-C), 63.79 (9a-C), 60.82 (*C*H$_2$–CH$_3$), 28.27 (2-C), 21.07 (Ts–CH$_3$), 13.93 (CH$_2$–CH$_3$).

Bromide Salt 15: Compound **7** (500 mg, 1.2 mmol) was dissolved in dry CH$_2$Cl$_2$ (3 mL) and cooled to 0 °C. HBr/HOAc (2 mL) in CH$_2$Cl$_2$ (2 mL) was added dropwise and the reaction mixture was stirred for 1 h at room temp. The solvent was removed in vacuo to give **15** as a brown solid (340 mg, quant.) which was used without further purification. TLC (toluene/ethyl acetate, 1:4) R_f = 0.02. – ^{1}H NMR ([D$_6$]DMSO): δ = 5.45 (s, 1 H, 9a-H) 4.94 (t, $^3J_{3,2}$ = 6.4 Hz, 1 H, 3-H), 4.7 (s, 1 H, 6-H), 4.01 (m, 2 H, 7-H, 8-H), 3.67 (m, 1 H, 9-H), 3.61 (s, 3 H, OC*H*$_3$), 3.28 (m, 1 H, 2-H), 3.19 (m, 1 H, 2-H′). – ^{13}C NMR δ = 173.41 (*C*O$_2$Me), 167.52 (5-C), 76.30 (9-C), 72.68 (7-C), 71.28 (8-C), 65.68 (3-C), 62.85 (9a-C), 54.90 (6-C), 54.86 (O*C*H$_3$), 32.80 (2-C).

[*N*-(*N*-Fmoc-Glyceryl)] Derivative 16: Compound **15** (500 mg, 1.7 mmol) was dissolved in 10 mL of dry DMF and the pH was adjusted to 8 with Hünig′s base. FmocNH–Gly–OPfp (793 mg, 1.7 mmol) was added and the reaction mixture was stirred for 6 h at room temp. The solvent was removed in vacuo and **16** (452 mg, 46%) was obtained as a beige solid after flash chromatography (CHCl$_3$/MeOH, 9:1). TLC (CHCl$_3$/MeOH, 9:1) R_f = 0.33; mp = 162.5 °C. – $[\alpha]_D^{20}$ = –24.5 (*c* = 1.0, DMSO). – ^{1}H NMR ([D$_6$]DMSO): δ = 8.00 (d, $^3J_{NH,6}$ = 8.1 Hz, 1 H, NH), 7.87, 7.71, 7.40, 7.32 (8 H, Fmoc-arom.), 7.62 (t, 1 H, Fmoc–NH), 5.65 (s, br, 1 H, OH), 5.50 (s, 1 H, 9a-H), 5.41 (s, br, 1 H, OH), 5.18 (d, $^3J_{6,6\text{-OH}}$ = 8.2 Hz, 1 H, 6-H), 4.67 (t, $^3J_{3,2}$ = 7.9 Hz, 1 H, 3-H), 4.4 (m, 1 H, OH), 4.28–4.23 (m, 3 H, Fmoc–C*H*–C*H*$_2$), 3.84 (m, 1 H,

8-H), 3.76 (m, 1 H, 7-H), 3.69 (m, 2 H, α-Gly), 3.63 (m, 4 H, OCH_3, 9-H), 3.17 (m, 2 H, 2-H). – ^{13}C NMR: δ = 170.65 (*C*O_2Me), 168.56 (*C*ONH), 167.65 (5-C), 156.52 (O*C*ON), 143.86, 140.74, 127.63, 127.11, 125.27, 120.09 (Fmoc-arom.), 77.44 (9-C), 75.09 (7-C), 70.83 (8-C), 65.82 (Fmoc-CH_2), 64.16 (3-C), 61.12 (9a-C), 52.20 (O*C*H_3), 52.08 (6-C), 46.64 (Fmoc-CH), 43.59 (α-Gly), 31.52 (2-C). – MALDI-MS: *m*/*z* = 593.3 $[MNa]^+$, 609.3 $[MK]^+$.

***N*-Boc-Alanyl Derivative 17:** Compound **2** (100 mg, 0.34 mmol) was dissolved in 10 mL of dry DMF and Boc–Ala–OH (161 mg, 0.8 mmol) and PyBOP (429 mg, 0.8 mmol) were added. The reaction mixture was stirred for 5 h at room temp. Then, 1-methylimidazole (0.5 mL) was added and stirring was continued for a further 10 h at room temp. The solvent was removed in vacuo and after flash chromatography (ethyl acetate/toluene, 19:1) **17** was obtained as a yellow solid (150 mg, 95%). TLC (ethyl acetate/toluene, 19:1) R_f = 0.15; mp = 87 °C. – $[\alpha]_D^{20}$ = –14.3 (*c* = 1.0, $CHCl_3$). – ^{1}H NMR ([D$_6$]DMSO): δ = 7.30 (d, $^3J_{NH,\ \alpha\text{-Ala}}$ = 7.0 Hz, 1 H, NH), 5.81 (d, $^3J_{8\text{-OH},8}$ = 3.5 Hz, 1 H, 8-OH), 5.68 (d, $^3J_{7\text{-OH},7}$ = 6.2 Hz, 1 H, 7-OH), 5.65 (s, 1 H, 6-H), 5.48 (s, 1 H, 9a-H), 4.71 (t, 1 H, 3-H), 4.46 (d, $^3J_{9\text{-OH},9}$ = 10.9 Hz, 1 H, 9-OH), 4.04 (m, 1 H, Ala-Hα) 3.91 (m, 1 H, 7-H), 3.88 (m, 1 H, 8-H), 3.63 (s, 3 H, OCH_3), 3.60 (m, 1 H, 9-H), 3.30 (m, 2 H, 2-H), 1.34 (m, 12 H, *t*Bu, Ala-Hβ). – ^{13}C NMR δ = 172.58, 170.34 (*C*O, 5-C), 164.93 (5-C), 155.23 (O*C*ON), 79.16 (*C*Me_3), 77.05 (9-C), 73.00 (7-C), 72.10 (6-C), 70.62 (8-C), 63.83 (3-C), 61.00 (9a-C), 52.27 (O*C*H_3), 48.77 (Ala-Cα), 31.39 (2-C), 28.18 (C*Me*$_3$), 17.02 (Ala-Cβ).

Chloride Salt 18: Compound **17** (90 mg, 0.19 mmol) was dissolved in 5 mL of $CHCl_3$/MeOH (19:1) and treated with 1 M Et_2O/HCl (2 mL) for 2 min at room temp. The solvent was removed in vacuo. Pure **17** (70 mg, quant.) was obtained as a colourless solid. – ^{1}H NMR (D_2O): δ = 5.92 (s, 1 H, 6-H), 5.74 (s, 1 H, 9a-H), 5.05 (m, 1 H, 3-H), 4.36 (m, 1 H, Ala-Hα), 4.25 (m, 2 H, 7-H, 8-H), 3.95 (m, 4 H, OCH_3, 9-H), 3.87 (m, 1 H, 2-H), 3.80 (m, 1 H, 2-H′), 1.67 (d, $^3J_{\beta,\alpha}$ = 7.0 Hz, 3 H, Ala-Hβ). – ^{13}C NMR: δ = 173.3, 169.84 (*C*O, 5-C), 78.23 (9-C), 76.95 (6-C), 75.15 (8-C), 72.93 (7-C), 67.43 (3-C), 64.25 (9a-C), 60.42 (O*C*H_3), 56.33 (Ala-Cα), 51.71 (2-C), 34.03 (Ala-Cβ).

***N*-Phenylalanine Derivative 19:** Compound **4** (200 mg, 0.7 mmol) was dissolved in 5 mL of dry CH_2Cl_2. HCl·H–Phe–OMe (156 mg, 0.7 mmol), EEDQ (270 mg, 0.7 mmol) and 0.5 mL NEt_3 were added and the solution was stirred for 40 h at room temp. The solvent was removed in vacuo. Flash chromatographic workup ($CHCl_3$/MeOH, 3:1) yielded **19** (139 mg, 44%) as a pale beige solid. TLC ($CHCl_3$/MeOH, 3:1) R_f = 0.48; mp = 92.4 °C. – $[\alpha]_D^{20}$ = –4.73 (*c* = 0.93, DMSO). – ^{1}H NMR ([D$_6$]DMSO): δ = 8.64 (d, $^3J_{NH,\ H\alpha}$ = 7.3 Hz, 1 H, NH), 7.29–7.20 (m, 5 H, Phe-arom.), 5.52 (d, $^3J_{9\text{-OH},9}$ = 8.8 Hz, 1 H, 9-OH), 5.51 (d, $^3J_{8\text{-OH},8}$ = 4.1 Hz, 1 H, 8-OH), 5.45 (s, 1 H, 9a-H), 5.01 (d, $^3J_{7\text{-OH},7}$ = 5.3 Hz, 1 H, 7-OH), 4.70 (t, 1 H, 3-H), 4.64 (d, $^3J_{6\ H,6\text{-OH}}$ = 6.5 Hz, 1 H, 6-H), 4.44 (m, 1 H, Phe-Hα), 4.37 (d, $^3J_{6OH,6}$ = 6.5 Hz, 1 H, 6-OH), 3.83 (m, 1 H, 8-H), 3.77 (t, 1 H, 7-H), 3.57 (dd, $^3J_{9,8}$ = 3.2 Hz, $^3J_{9,9\text{-OH}}$ = 9.0 Hz, 1 H, 9-H), 3.52 (s, 3 H, OCH_3). – 3.21 (dd, $^3J_{2,3}$ = 7.3 Hz, $^2J_{gem}$ = 11.1 Hz, 1 H, 2-H), 3.10 (dd, $^3J_{2,3}$ = 7.0 Hz, $^3J_{gem}$ = 11.1 Hz, 1 H, 2-H′), 2.96 (dd, $^3J_{\beta,\alpha}$ = 6.8 Hz, $^2J_{gem}$ = 13.8 Hz, 1 H, Phe-Hβ), 3.10 (dd, $^3J_{\beta,\alpha}$ = 7.6 Hz, $^2J_{gem}$ = 13.7 Hz, 1 H, Phe-Hβ′). – ^{13}C NMR: δ = 171.22, 170.79, 170.17 (*C*O_2Me, *C*ONH, 5-C), 136.70, 129.15, 128.26, 126, 62 (Phe-arom.), 76.05 (9-C), 75.83 (7-C), 71.62 (8-C), 69.41 (6-C), 64.86 (3-C), 61.34 (9a-C), 53.85 (Phe-Cα), 51.76 (O*C*H_3), 37.05 (Phe-Cβ), 32.20 (2-C). – MALDI-MS: *m*/*z* = 462.4 $[MNa]^+$, 478.4 $[MK]^+$. – $C_{19}H_{24}N_2O_8S$ (440.5): Calcd.: C, 51.81, H 5.49, N 6.36; found C 50.23, H 5.51, N 5.29.

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[1] [1a] R. Hirschmann, *Angew. Chem.* **1991**, *103*, 1305–1330; *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1287–1301. – [1b] J. Gante, *Angew. Chem.* **1994**, *106*, 1780–1802; *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 1699–1712. – [1c] H. Kessler, B. Diefenbach, D. Finsinger, A. Geyer, M. Gurrath, S. L. Goodman, G. Hölzemann, R. Haubner, A. Jonczyk, G. Müller, E. Graf v. Roedern, J. Wermuth, *Letters in Peptide Science* **1995**, *2*, 155–160.

[2] C. Toniolo, *Int. J. Peptide Protein Res.* **1990**, *35*, 287–300.

[3] H. Kessler, *Angew. Chem.* **1982**, *94*, 509–520; *Angew. Chem. Int. Ed. Engl.* **1982**, *21*, 512–523.

[4] U. Nagai, K. Sato, *Tetrahedron Lett.* **1985**, *26*, 647–650; U. Nagai, K. Sato, R. Nakamura, R. Kato, *Tetrahedron* **1993**, *49*, 3577–3592.

[5] [5a] H. Kessler, A. Geyer, H. Matter, M. Köck, *Int. J. Peptide Protein Res.* **1992**, *40*, 25–40. – [5b] K. Gunasekaran, L. Gomathi, C. Ramakrishnan, J. Chandrasekhar, P. Balaram, *J. Mol. Biol.* **1998**, *284*, 1505–1516.

[6] [6a] J. E. Baldwin, C. Hulme, C. J. Schofield, A. J. Edwards, *J. Chem. Soc, Chem. Commun.* **1993**, 935–936. – [6b] N. L. Subasinghe, R. J. Bontms, E. McIntee, R. K. Mishra, R. L. Johnson, *J. Med. Chem.* **1993**, *36*, 2356–2361. – [6c] W. Li, C. E. Hanau, A. d′Avignon, K. D. Moeller, *J. Org. Chem.* **1995**, *60*, 8155–8170. – [6d] M. A. Siddiqui, P. Preville, M. Tarazi, S. E. Warder, P. Eby, E. Gorseth, K. Puumala, J. DiMaio, *Tetrahedron Lett.* **1997**, *38*, 8807–8810. – [6e] F. Cornille, U. Slomczynska, K. L. Smythe, D. D. Beusen, K. D. Moeller, G. R. Marshall, *J. Am. Chem. Soc*. **1995**, *117*, 909–917. – [6f] J. A. Robl, C.-Q. Sun, J. Stevenson, D. E. Ryono, L. M. Simpkins, M. P. Cimarusti, *J. Med. Chem.* **1997**, *40*, 1570–1577. – [6g] P. W. Baures, W. H. Ojala, W. J. Costain, M. C. Ott, A. Pradhan, W. B. Gleason, R. K. Mishra, R. L, Johnson, *J. Med. Chem.* **1997**, *40*, 3594–3600. – [6h] Y. Takeuchi, G. R. Marshall, *J. Am. Chem. Soc.* **1998**, *120*, 5363–5372. – [6i] D. S. Karanewsky, X. Bai, S. D. Linton, J. F. Krebs, J. Wu, B. Pham, K. J. Tomaselli, *Bioorg. Med. Chem. Lett*. **1998**, *8*, 2757–2762.

[7] [7a] F. Polyak, W. D. Lubell, *J. Org. Chem*. **1998**, *63*, 5937–5949. – [7b] C. Gennari, A. Mielgo, D. Potenza, C. Scolastico, U. Piarulli, L. Manzoni, *Eur. J. Org. Chem.* **1999**, 379–388.

[8] H.-G. Lombart, W. D. Lubell, *J. Org. Chem.* **1996**, *61*, 9437–9446.

[9] R. Hirschmann, J. Hynes Jr., M. A. Cichy-Knight, R. D. van Rijn, P. A. Sprengeler, P. G. Spoors, W. C. Shakespeare, S. Pietranico-Cole, J. Barbosa, J. Liu, W. Yao, S. Rohrer, A. B. Smith III, *J. Med. Chem*. **1999**, *41*, 1382–1391.

[10] E. Graf v. Roedern, E. Lohof, G. Hessler, M. Hoffmann, H. Kessler, *J. Am. Chem. Soc.* **1996**, *118*, 10156–10167.

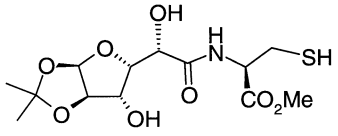
[11] L. Szabo, B. L. Smith, K. D. McReynolds, A. L. Parrill, E. R. Morris, J. Gervay, *J. Org. Chem.* **1998**, *63*, 1074–1078.

[12] [12a] M. P. Schubert, *J. Biol. Chem.* **1939**, *130*, 601–603. – [12b] J. Radomski, A. Temeriusz, *Carbohydr. Res.* **1989**, *187*, 223–227.

[13] Jpn. Kokai Tokkyo Koho JP 81,166,189 (Cl. C07D417/06); *Chem. Abstr.*, *96*: 143262e.

[14] R. Luhowy, F. Meneghini, *J. Am. Chem. Soc*. **1979**, *101*, 420–426.

[15] In a control experiment, the condensation was performed with the 1,2-acetonide of D-glucurono-3,6-lactone and halted at the amide intermediate:



[16] [16a] W. N. Speckamp, H. Hiemstra, *Tetrahedron* **1985**, *41*, 4367–4416. – [16b] Y. S. Lee, D. W. Kang, S. J. Lee, H. Park, *J. Org. Chem.* **1995**, *60*, 7149–7152.
[17] J. E. Baldwin, R. T. Freeman, C. Lowe, C. J. Schofield, E. Lee, *Tetrahedron* **1989**, *45*, 4537–4550.
[18] A. Geyer, D. Bockelmann, K. Weissenbach, H. Fischer, *Tetrahedron Lett*. **1999**, *40*, 477–478.
[19] R. P. Fleming, K. B. Sharpless, *J. Org. Chem.* **1991**, *56*, 2869–2875.
[20] A. Klemer, U. Hofmeister, R. Lemmers, *Carbohydr. Res.* **1979**, *68*, 391–395.
[21] [21a] B. P. Bashyal, H. Chow, L. E. Fellows, G. W. J. Fleet, *Tetrahedron* **1993**, *43*, 415–422. – [21b] T. M. Krülle, B. Davis, H. Ardron, D. D. Long, N. A. Hindle, C. Smith, D. Brown, A. L. Lane, D. J. Watkin, D. G. Marquess, G. W. J. Fleet, *Chem. Commun.* **1996**, 1271–1272.

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