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Stereocontrolled transformation of nitrohexofuranoses into cyclopentylamines via 2-oxabicyclo[2.2.1]heptanes. Part VI: Synthesis and incorporation of the novel polyhydroxylated 5-aminocyclopent-1-enecarboxylic acids into peptides

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ABSTRACT

The first total synthesis of enantiopure methyl (3a*R*,4*S*,5*S*,6*R*,6a*S*)-4-benzyloxycarbonylamino-6-hydroxy-2,2-dimethyl-tetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carboxylate has been carried out according to our recent novel strategy for the transformation of nitrohexofuranoses into cyclopentylamines. This approach is based on an intramolecular cyclization that leads to 2-oxabicyclo[2.2.1]heptane derivatives. E1cb elimination of the methoxy substituent was observed when attempting to incorporate these β-amino acid into peptides. As a result, the synthesis and incorporation of the first polyhydroxylated 5-aminocyclopent-1-enecarboxylic acid into peptides were developed.

1. Introduction

Peptides play a crucial role in biology. However, their use as therapeutic agents suffers from their poor metabolic stability and transport properties[1] together with their limited structural diversity, which is related to the limited number of secondary structures and backbones resulting from the small number of proteinogenic amino acids.[2] Accordingly, a great deal of work has been carried out in recent years which aimed at the synthesis of unnatural amino acids. This represents the first step for the introduction of elements of diversity for the development of peptidomimetics with high levels of diversity and the capability to generate highly ordered structures that may act as new drugs and overcome the pharmacological limitations of proteins.[3]

Cyclic β-amino acids, in particular cyclopentyl β-amino acids, have become a hot topic in the synthetic and medicinal chemistry as a result of their present interest as stabilizers of peptide conformations—a property that has been related to the high tendency of their homopolymers to fold in rigid secondary structures in short peptide sequences.[4] Accordingly, several procedures for the synthesis of these systems have been developed. However, the panel of known cyclopentane β-amino acids is limited, probably due to the lack of efficient methods for the stereocontrolled preparation of their polysubstituted derivatives, which remains a challenge for synthetic chemists.[5]

We are currently interested in the development of practical stereocontrolled syntheses of biologically and pharmacologically relevant polysubstituted carba- and aza-sugar derivatives from nitrosugars.[6] In this context, we have developed a stereocontrolled route to polyhydroxylated cyclopentyl β-amino acids with the aim of opening up opportunities to access lipophililic or hydrophilic β-peptides, with the hydroxy substituents either protected or unprotected. This approach allowed us to synthesize the first polyhydroxylated *trans*-2-aminocyclopentanecarboxylic acid[6b] from D-glucose and the first polyhydroxylated *cis*-2-aminocyclopentanecarboxylic acid[6f] from L-idose. Herein, we report the application of this synthetic strategy for the preparation of the polysubstituted cyclopentane β-amino acid **1**, a structurally complex cyclopentane-based β-amino acid of interest in order to obtain new insights into β-peptides.

2. Results and discussion

The preparation of amino acid **1** from D-glucose was initially attempted according to the synthetic plan depicted in Scheme 1, which involved the early introduction of a methoxy substituent at C-3. Treatment of compound **5**, obtained from diacetone-D-glucose **4** following a known procedure,[7] with TBDPSCl and imidazole yielded derivative **6**[8] due to selective protection of the hydroxy group at C-6 (Scheme 2). Benzylation of the hydroxyl group at C-5 of compound **6** with sodium hyride and benzyl bromide gave intermediate **7** in 81% yield. Subsequent deprotection of the hydroxy group at C-6 of this compound by treatment with TBAF[9] was followed by a second reaction sequence which aimed at replacing the

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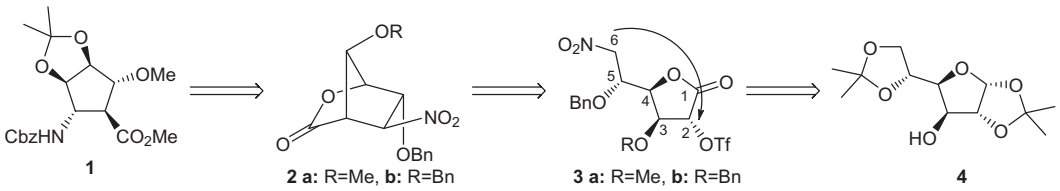
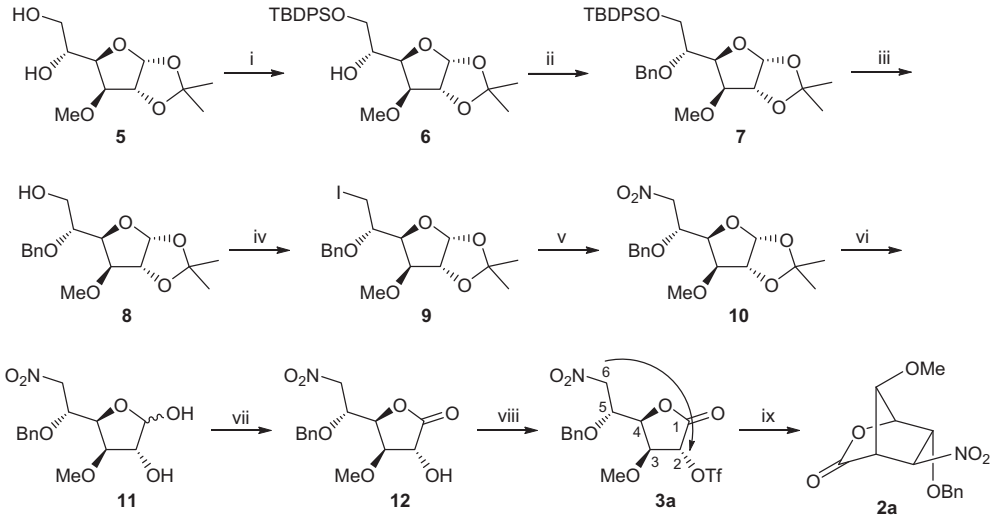


Scheme 1. Synthetic plan for the preparation of amino acid **1** from D-glucose.

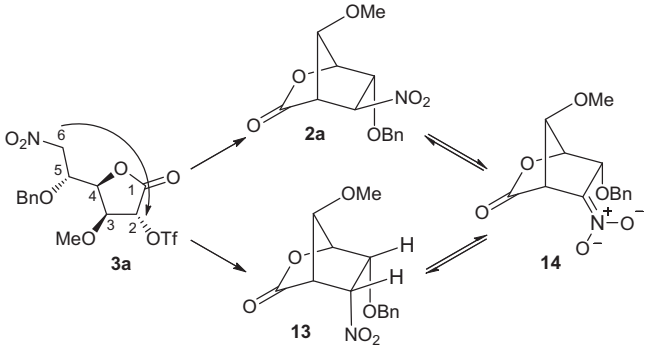


Scheme 2. Reagents and conditions: (i) TBDPSCl, imidazole, DMF, −20 °C, 1 h, 87%; (ii) NaH, BnBr, NBu_4I, DMF, 0 °C→rt, 24 h, 81%; (iii) TBAF, THF, rt, 17 h, 97%; (iv) imidazole, Ph_3P, I_2, toluene, 85 °C, 4 h, 90%; (v) $NaNO_2$, phloroglucinol, DMSO, rt, 96 h, 65%; (vi) TFA/H_2O (1:1), rt, 15 h; (vii) Br_2, $BaCO_3$, dioxane/H_2O (2:1), rt, 15 h (80% from **10**); (viii) Tf_2O, pyridine, CH_2Cl_2, −30 °C, 1 h; (ix) TBAF, THF, rt, 12 h (36% from **12**).

free hydroxy group of the resulting compound **8** with a nitro group. Accordingly, treatment of primary alcohol **8** under Appel[10] conditions provided the iodo derivative **9**, from which our key nitrosugar derivative **10** was easily obtained in 65% yield by displacement of the iodide by nitrite in the presence of phloroglucinol.[11]

Reaction of compound **10** with trifluoroacetic acid and water, followed by anomeric oxidation of the resulting lactol **11** with bromine and barium carbonate, afforded the lactone **12** as a yellowish oil. Treatment of this compound with triflic anhydride in pyridine furnished the desired triflate **3a**, which was maintained under vacuum overnight and then directly reacted with TBAF in THF to promote an intramolecular cyclization that provided the desired bicyclic nitrolactone **2a** in only 36% yield.

The stereochemical outcome of this key step leading to compound **2a** was explained, as for previous similar cases,[6b] with the assumption that both bicyclic compounds **2a** and **13** should be formed from the nitronate of compound **3a** (Scheme 3). However,

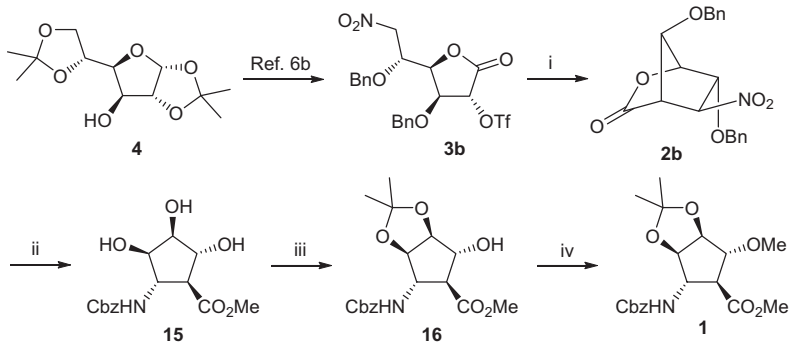


Scheme 3.

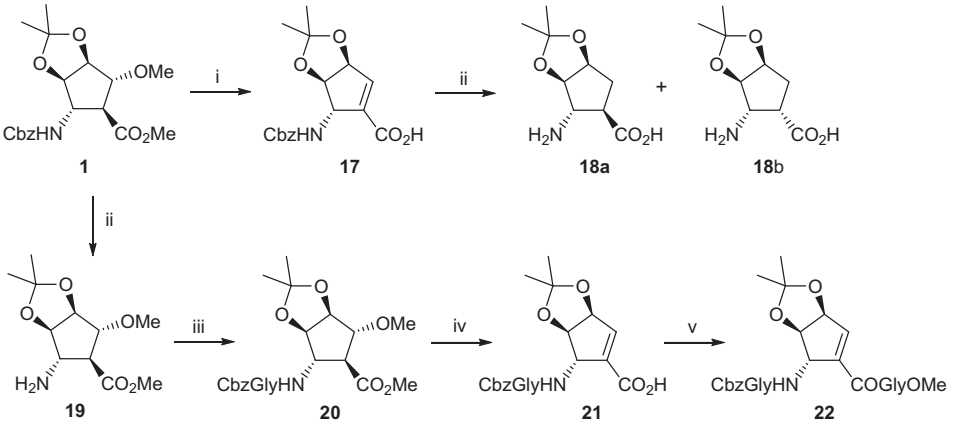
under the reaction conditions, compounds **2a** and **13** should be in equilibrium with their common nitronate **14**, but the thermodynamically more stable compound **2a** should be favoured over compound **13**, in which the nitro and benzyloxy groups are eclipsed. This explains the highly remarkable stereoselectivity of the cyclization of nitronate of **3a**.

The yield achieved in this intramolecular cyclization of **3a** was substantially lower than the 75% yield previously reported for a similar cyclization of C-3 O-benzylated nitronate **3b** to the bicyclic lactone **2b** (Scheme 1).[6b] This result led us to discard our initial plans for the preparation of our synthetic objective **1** from **3a**, via **2a**, in favour of an alternative route as outlined in Scheme 4. Accordingly, trihydroxylated cyclopentane β-amino acid derivative **15** was prepared from diacetone-D-glucose **4** as previously reported (Scheme 4) and subsequent treatment with 2,2-dimethoxypropane in dry acetone gave **16** as a result of the selective protection of its *cis*-1,2-diol system. Finally, the reaction of this compound with methyl iodide, using silver(I) oxide as a base,[12] resulted in the efficient methylation of its free hydroxy group, a process that allowed our target compound **1** to be obtained. Compound **1** was easily identified from its analytical and spectroscopic properties. Following this approach, compound **1** was obtained in 13% overall yield after a 15-step sequence.

In order to investigate the incorporation of this amino acid derivative **1** into peptides, a freshly isolated sample of this compound was subjected to diverse basic hydrolysis conditions with the aim of obtaining the corresponding carboxylic acid. However, the formation of β-amino acid derivative **17** was observed in all cases (Scheme 5); this constitutes the first reported example of this novel class of cyclopentene based β-amino acid. The formation of **17** probably involved an E1cb elimination of methanol, followed by hydrolysis of the methoxycarbonyl moiety.



Scheme 4. Reagents and conditions: (i) TBAF, THF, rt, 6 h (75%, two steps); (ii) see Ref.[6b] (three steps, 39% from **2b**); (iii) 2,2-DMP, PTSA, $MgSO_4$, acetone, rt, 13 h, 88%; (iv) MeI, Ag_2O, CH_3CN, 50 °C, 60 h, 93%.



Scheme 5. Reagents and conditions: (i) NaOH, THF, rt, 2 h, 90%; (ii) H_2, Pd/C, EtOAc, rt, 2 h; (iii) Cbz-Gly-OH, DIEA, TBTU, CH_2Cl_2, rt, 12 h (88% from **1**); (iv) NaOH, THF, rt, 2 h; (v) H-Gly-OMe·HCl, DIEA, TBTU, CH_2Cl_2, rt, 12 h (65% from **20**).

Compound **17** is an amino acid of interest as a novel scaffold in β-peptide chemistry due to the extra rigidity provided by its endocyclic carbon–carbon double bond. As a result, we decided to study the incorporation of **17** into peptides. Accordingly, we first tried to remove selectively the benzyloxycarbonyl group by catalytic hydrogenation but an inseparable 1:1 mixture of compounds **18a** and **18b** was obtained due to simultaneous hydrogenation of the carbon–carbon double bond. However, satisfactory results were achieved by the early incorporation of a glycine subunit in the *N*-terminus of β-amino acid derivative **1**. Thus, catalytic hydrogenation of **1** provided the expected cyclopentylamine **19**, which upon treatment with benzyloxycarbonylglycine, TBTU and DIEA gave dipeptide **20** in 88% yield for the last two steps. Subsequent treatment of this compound with base led to the expected dipeptide **21**, which was finally reacted with glycine methyl ester hydrochloride under the same coupling conditions as for **19**. This sequence allowed us to obtain the desired tripeptide **22** in 65% yield for the last two steps.

3. Conclusion

In conclusion, we have adapted our strategy for the stereocontrolled synthesis of polyhydroxylated cyclopentane β-amino acids from nitro sugars[6a] and applied it to the preparation of methyl (3a*R*,4*S*,5*S*,6*R*,6a*S*)-4-benzyloxycarbonylamino-6-hydroxy-2,2-dimethyl-tetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carboxylate **1**, a new member of this family of amino acids. Basic hydrolysis of **1** was accompanied by elimination of methanol. This allowed us to access the first reported polyhydroxylated 5-aminocyclopent-1-enecarboxylic acid and to develop a specific protocol for its incorporation into peptides.

Work aiming at the preparation of diverse 5-aminocyclopent-1-enecarboxylic acids as an initial stage in the study of the structural, physical and biological properties of their oligomers is currently in progress. Specifically, we are interested in establishing how the extra rigidity of the cyclopentane ring, due to the presence of a carbon–carbon double bond, can influence the folding properties.

4. Experimental

Melting points were determined using a Kofler Thermogerate apparatus and are uncorrected. Specific rotations were recorded on a JASCO DIP-370 optical polarimeter. Infrared spectra were recorded on a MIDAC Prospect-IR spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Bruker DPX-250 spectrometer. Mass spectra were obtained on a Hewlett–Packard 5988A mass spectrometer. Elemental analyses were obtained from the Elemental Analysis Service at the University of Santiago de Compostela. Thin layer chromatography (TLC) was performed using Merck GF-254 type 60 silica gel and ethyl acetate/hexane mixtures as eluants; the TLC spots were visualized with Hanessian mixture. Column chromatography was carried out using Merck type 9385 silica gel. Compounds **5**, **6**, **14**, **3b**, **2b** and **15** were prepared by following known procedures.

4.1. 6-*O*-*tert*-Butyldiphenylsilyl-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose 6

Compound **5** (6.11 g, 26.08 mmol) was dissolved in dry DMF (55 mL) and cooled to −20 °C. Imidazole (3.55 g, 52.26 mmol) and TBDPSCl (6.8 mL, 26.13 mmol) were added and the mixture

was stirred at room temperature for 1 h. The starting material had been consumed (TLC, EtOAc/hexane 1:1) and the solvent was concentrated in vacuo. The residue was dissolved in EtOAc (30 mL) and washed with H_2O (3 × 50 mL). The organic layer was dried with Na_2SO_4 and concentrated to dryness. The crude product was purified by flash column chromatography (EtOAc/hexane 1:5) to afford compound **6** (10.76 g, 22.76 mmol, 87%) as a yellowish oil. $[\alpha]_D^{22} = -44.6$ (*c* 1.1, $CHCl_3$). ^1H NMR ($CDCl_3$, ppm): 1.07 (s, 9H, 3 × CH_3); 1.31 (s, 3H, CH_3); 1.41 (s, 3H, CH_3); 2.87 (d, 1H, $J_{OH,5}$ = 7.0 Hz, OH); 3.41 (s, 3H, OCH_3); 3.84–3.87 (m, 2H, H-3 + H-5); 3.99–4.14 (m, 2H, H-6a + H-6b); 4.27 (dd, 1H, $J_{4,5}$ = 7.9 Hz, $J_{4,3}$ = 3.0 Hz, H-4); 4.57 (d, 1H, $J_{2,1}$ = 3.6 Hz, H-2); 5.89 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1); 7.34–7.41 (m, 6H, 6 × H-ar); 7.61–7.67 (m, 4H, 4 × H-ar). ^{13}C NMR ($CDCl_3$, ppm): 19.7; 26.0; 26.4; 26.6; 57.7; 65.2; 68.6; 78.9; 81.4; 84.0; 104.9; 111.3; 127.5; 127.6; 129.5; 129.6; 132.6; 132.9; 135.3; 135.4. IR (NaCl, ν_{max}, cm^{-1}): 3559 (br, OH); 1115 (st, Si–O–C). MS (CI, *m*/*z*, %): 474 (8, $[M+H]^+$); 339 (100, $[M-H-{}^tBu-Ph]^+$). Anal. Calcd for $C_{26}H_{36}O_6Si$: C, 66.07; H, 7.68. Found: C, 65.87; H, 7.99.

4.2. 5-*O*-Benzyl-6-*O*-*tert*-butyldiphenylsilyl-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose 7

To a suspension of NaH (1.47 g, 36.69 mmol, 60% dispersion in mineral oil) in dry DMF (120 mL) at 0 °C was added dropwise a solution of compound **6** (11.54 g, 24.46 mmol) in dry DMF (130 mL). When the evolution of hydrogen had ceased, NBu_4I (1.18 g, 3.18 mmol) and BnBr (4.4 mL, 36.69 mmol) were added. The mixture was allowed to warm up to room temperature and then stirred at room temperature for 24 h. The starting material had been consumed (TLC, EtOAc/hexane 1:5); MeOH (14 mL) was added and the mixture was stirred for 1 h. The solvent was partially removed in vacuo, Et_2O was added and the mixture was filtered through a pad of Celite. The solvent was evaporated to dryness and the crude product was purified by flash column chromatography (EtOAc/hexane 1:8) to afford compound **7** (11.15 g, 19.81 mmol, 81%) as a yellow oil: $[\alpha]_D^{24} = -44.4$ (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, ppm): 1.05 (s, 9H, 3 × CH_3); 1.29 (s, 3H, CH_3); 1.53 (s, 3H, CH_3); 3.34 (s, 3H, OCH_3); 3.85–3.94 (m, 3H, H-3 + H-5 + H-6b); 4.06 (dd, 1H, $J_{6a,6b}$ = 13.0 Hz, $J_{6a,5}$ = 3.3 Hz, H-6a); 4.37 (dd, 1H, $J_{4,5}$ = 9.1 Hz, $J_{4,3}$ = 3.0 Hz, H-4); 4.55 (d, 1H, $J_{2,1}$ = 3.6 Hz, H-2); 4.57 (d, 1H, $J_{H,H'}$ = 11.2 Hz, CHPh); 4.86 (d, 1H, $J_{H,H'}$ = 11.2 Hz, CHPh); 4.87 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1); 7.27–7.36 (m, 11H, 11 × H-ar); 7.70–7.77 (m, 4H, 4 × H-ar). ^{13}C NMR ($CDCl_3$, ppm): 19.1; 26.1; 26.5; 26.7; 57.3; 64.1; 72.6; 76.6; 78.1; 80.9; 83.5; 105.1; 111.3; 127.3; 127.5; 127.6; 128.1; 129.4; 129.5; 133.3; 133.4; 135.5; 135.6; 138.6. IR (NaCl, ν_{max}, cm^{-1}): 1105 (st, Si–O–C). MS (CI, *m*/*z*, %): 564 (7, $[M+H]^+$); 91 (100, $[CH_2Ph]^+$). Anal. Calcd for $C_{33}H_{42}O_6Si$: C, 70.43; H, 7.52. Found: C, 70.29; H, 7.69.

4.3. 5-*O*-Benzyl-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose 8

Compound **7** (5.70 g, 10.13 mmol) was dissolved in dry THF (75 mL) and stirred with TBAF (20.3 mL, 20.3 mmol, 1 M solution in THF) at room temperature for 17 h. The starting material had been consumed (TLC, EtOAc/hexane 1:2) and the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (150 mL) and washed with H_2O (3 × 150 mL). The organic layer was dried with Na_2SO_4 and evaporated in vacuo. The crude product was purified by flash column chromatography (EtOAc/hexane 2:3) to afford compound **8** (3.19 g, 9.83 mmol, 97% yield) as a yellowish oil. $[\alpha]_D^{22} = -35.7$ (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, ppm): 1.28 (s, 3H, CH_3); 1.38 (s, 3H, CH_3); 2.83 (br s, 1H, OH); 3.31 (s, 3H, OCH_3); 3.70–3.92 (m, 3H, H-5 + H-6a + H-6b); 3.81 (d, 1H, $J_{3,4}$ = 3.0 Hz, H-3); 4.23 (dd, 1H, $J_{4,5}$ = 8.5 Hz, $J_{4,3}$ = 3.0 Hz, H-4); 4.53 (d, 1H, $J_{H,H'}$ = 11.2 Hz, CHPh); 4.54 (d, 1H, $J_{2,1}$ = 3.9 Hz, H-2); 4.70 (d, 1H, $J_{H,H'}$ = 11.2 Hz, CHPh); 5.84 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1); 7.24–7.34 (m, 5H, 5 × H-ar). ^{13}C NMR ($CDCl_3$, ppm): 25.7; 26.2; 56.8; 61.6; 71.9; 75.5; 78.7; 80.5; 83.0; 104.5; 111.2; 127.3; 127.9; 137.9. IR (NaCl, ν_{max}, cm^{-1}): 3495 (br, OH). MS (CI, *m*/*z*, %): 325 (50, $[M+H]^+$); 91 (100, $[CH_2Ph]^+$). Anal. Calcd for $C_{17}H_{24}O_6$: C, 62.95; H, 7.46. Found: C, 63.06; H, 7.61.

4.4. 5-*O*-Benzyl-6-deoxy-6-iodo-1,2-*O*-isopropylidene-3-*O*-methyl-α-D-glucofuranose 9

Imidazole (1.16 g, 16.98 mmol), Ph_3P (3.19 g, 12.26 mmol) and I_2 (3.13 g, 12.26 mmol) were added to a stirred solution of compound **8** (1.53 g, 4.72 mmol) in toluene (17 mL) and the mixture was stirred at 85 °C for 4 h. After the starting material had been consumed (TLC, EtOAc/hexane 1:2) the reaction mixture was allowed to cool down to room temperature, concentrated in vacuo and then partitioned between CH_2Cl_2 (45 mL) and saturated aq $NaHCO_3$ (45 mL). The aqueous layer was extracted with CH_2Cl_2 (3 × 45 mL) and the resulting organic layer was dried with Na_2SO_4 and evaporated to dryness. The crude product was purified by flash column chromatography (EtOAc/hexane 1:9) to afford compound **9** (1.84 g, 4.25 mmol, 90% yield), which was recrystallized from Et_2O/hexane to give a white solid. Mp 62–64 °C. $[\alpha]_D^{22} = -62.9$ (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, ppm): 1.31 (s, 3H, CH_3); 1.49 (s, 3H, CH_3); 3.36 (s, 3H, OCH_3); 3.45–3.51 (m, 2H, H-5 + H-6b); 3.59 (dd, 1H, $J_{6a,6b}$ = 10.9 Hz, $J_{6a,5}$ = 2.7 Hz, H-6a); 3.82 (d, 1H, $J_{3,4}$ = 3.0 Hz, H-3); 4.13 (dd, 1H, $J_{4,5}$ = 8.5 Hz, $J_{4,3}$ = 3.0 Hz, H-4); 4.46 (d, 1H, $J_{H,H'}$ = 10.9 Hz, CHPh); 4.56 (d, 1H, $J_{2,1}$ = 3.5 Hz, H-2); 4.74 (d, 1H, $J_{H,H'}$ = 10.9 Hz, CHPh); 5.83 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1); 7.31–7.37 (m, 5H, 5 × H-ar). ^{13}C NMR ($CDCl_3$, ppm): 10.7; 26.9; 27.4; 58.0; 72.2; 73.3; 81.7; 81.9; 83.5; 105.3; 112.5; 128.3; 128.5; 128.8; 130.0. IR (NaCl, ν_{max}, cm^{-1}): no characteristic bands MS (CI, *m*/*z*, %): 435 (100, $[M+H]^+$); 91 (20, $[CH_2Ph]^+$). Anal. Calcd for $C_{17}H_{23}IO_5$: C, 47.02; H, 5.34. Found: C, 47.12; H, 5.28.

4.5. 5-*O*-Benzyl-6-deoxy-1,2-*O*-isopropylidene-3-*O*-methyl-6-nitro-α-D-glucofuranose 10

To a solution of compound **9** (2.85 g, 6.56 mmol) in dry DMSO (45 mL) were added phloroglucinol (2.13 g, 13.11 mmol) and $NaNO_2$ (1.04 g, 15.08 mmol). The mixture was stirred at room temperature for 96 h. The starting material had been consumed (TLC, EtOAc/hexane 1:3) and the reaction mixture was partitioned between EtOAc (80 mL) and brine (80 mL). The aqueous layer was extracted with EtOAc (3 × 80 mL) and the combined organic layers were dried with Na_2SO_4 and evaporated to dryness. The crude product was purified by flash column chromatography (EtOAc/hexane 1:2) to afford compound **10** (1.51 g, 4.26 mmol, 65%), which was recrystallized from EtOAc/hexane. Mp 89–91 °C. $[\alpha]_D^{17} = -46.7$ (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, ppm): 1.30 (s, 3H, CH_3); 1.46 (s, 3H, CH_3); 3.37 (s, 3H, OCH_3); 3.80 (d, 1H, $J_{3,4}$ = 3.0 Hz, H-3); 4.17 (dd, 1H, $J_{4,5}$ = 7.6 Hz, $J_{4,3}$ = 3.0 Hz, H-4); 4.52–4.78 (m, 6H, H-2 + H-5 + H-6a + H-6b + CH_2Ph); 5.85 (d, 1H, $J_{1,2}$ = 3.9 Hz, H-1); 7.26–7.32 (m, 5H, 5 × H-Ar). ^{13}C NMR ($CDCl_3$, ppm): 25.9; 26.5; 57.0; 73.2; 73.4; 77.3; 79.0; 80.6; 82.9; 104.7; 111.8; 127.6; 127.8; 128.2; 137.1. IR (NaCl, ν_{max}, cm^{-1}): 1557, 1379 (st, NO_2). MS (CI, *m*/*z*, %): 354 (23, $[M+H]^+$); 91 (100, $[CH_2Ph]^+$). Anal. Calcd for $C_{17}H_{23}NO_7$: C, 57.78; H, 6.56; N, 3.96. Found: C, 57.87; H, 6.57; N, 3.98.

4.6. 5-*O*-Benzyl-6-deoxy-3-*O*-methyl-6-nitro-D-glucono-1,4-lactone 12

Compound **10** (2.37 g, 6.73 mmol) was treated with a mixture of TFA/H_2O (1:1, 110 mL) and the mixture was stirred at room tem-

perature for 15 h. The starting material had been consumed (TLC, EtOAc/hexane 1:1). After the solvent was evaporated in vacuo and the residue was coevaporated with toluene (3 × 50 mL) to give the corresponding intermediate **12** as a clear gum, which was used in the next step without further purification. This crude product was dissolved in dioxane/H_2O (2:1, 120 mL). $BaCO_3$ (1.46 g, 7.40 mmol) and then Br_2 (0.87 mL, 16.82 mmol) were added and the reaction mixture was stirred for 15 h at room temperature with the exclusion of light. After the starting material had been consumed (TLC, EtOAc/hexane 3:2), the reaction was quenched with saturated aq $Na_2S_2O_3$ (until the mixture was colourless) and the mixture was then extracted with EtOAc (3 × 200 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by flash column chromatography (EtOAc/hexane 1:1) to afford compound **12** (1.67 g, 5.36 mmol, 80% from **10**) as a yellowish oil: $[\alpha]_D^{25} = +30.8$ (*c* 1.4, $CHCl_3$). ^1H NMR ($CDCl_3$, ppm): 3.45 (s, 3H, OCH_3); 3.87 (br s, 1H, OH); 4.08–4.15 (m, 1H, H-5); 4.54–4.80 (m, 7H, H-2 + H-3 + H-4 + H-6a + H-6b + CH_2Ph); 7.23–7.35 (m, 5H, 5 × H-ar). ^{13}C NMR ($CDCl_3$, ppm): 58.1; 71.2; 74.2; 74.6; 75.8; 78.1; 81.8; 128.0; 128.1; 128.4; 136.4; 174.6. IR (NaCl, ν_{max}, cm^{-1}): 3443 (br, OH); 1793 (st, C=O); 1555, 1380 (st, NO_2). MS (CI, *m*/*z*, %): 312 (8, $[M+H]^+$); 91 (100, $[CH_2Ph]^+$). Anal. Calcd for $C_{14}H_{17}NO_7$: C, 54.02; H, 5.50; N, 4.50. Found: C, 53.86; H, 5.75; N, 4.41.

4.7. (1*S*,4*S*,5*S*,6*R*,7*R*)-6-Benzyloxy-7-methoxy-5-nitro-2-oxabicyclo[2.2.1]heptan-3-one 2a

Compound **12** (0.26 g, 0.84 mmol) was dissolved in dry CH_2Cl_2 (8.5 mL) and the solution was then cooled down to −30 °C under argon. Pyridine (0.2 mL, 2.53 mmol) and Tf_2O (0.22 mL, 1.26 mmol) were added and the mixture was stirred at −30 °C for 1 h. After the starting material had been consumed (TLC, EtOAc/hexane 1:2), the reaction mixture was diluted with CH_2Cl_2 (30 mL), washed with 10% aq HCl (30 mL) and brine (30 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness, affording the corresponding intermediate **3a** as a clear gum, which was used in the next step without further purification after storing it under vacuum overnight. Next, TBAF (0.92 mL, 1 M solution in THF) was added to a solution of the crude product obtained above in THF (8.5 mL) and the resulting mixture was stirred under argon for 12 h. The starting material had been consumed (TLC, EtOAc/hexane 1:2). The solvent was evaporated and the residue was dissolved in CH_2Cl_2 (30 mL). The solution was washed with H_2O (3 × 30 mL) and the organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by flash column chromatography (EtOAc/hexane 1:4) to afford compound **2a** (0.09 g, 0.30 mmol, 36% from **12**) as a yellow oil. $[\alpha]_D^{24} = -8.4$ (*c* 1.2, $CHCl_3$). ^1H NMR ($CDCl_3$, ppm): 3.23 (s, 3H, OCH_3); 3.98–4.00 (m, 1H, H-1); 4.10–4.12 (m, 1H, H-6); 4.48–4.50 (m, 1H, H-7); 4.74–4.77 (m, 1H, H-4); 4.76 (d, 1H, $J_{H,H'}$ = 12.0 Hz, CHPh); 4.84 (d, 1H, $J_{H,H'}$ = 12.0 Hz, CHPh); 4.95–4.98 (m, 1H, H-5); 7.30–7.43 (m, 5H, 5 × H-ar). ^{13}C NMR ($CDCl_3$, ppm): 49.8; 57.5; 72.3; 79.8; 82.0; 83.4; 84.4; 127.8; 128.1; 128.4; 136.5; 169.3. IR (NaCl, ν_{max}, cm^{-1}): 1805 (st, C=O); 1555, 1381 (st, NO_2). MS (CI, *m*/*z*, %): 294 (11, $[M+H]^+$); 91 (100, $[CH_2Ph]^+$). Anal. Calcd for $C_{14}H_{15}NO_6$: C, 57.34; H, 5.16; N, 4.78. Found: C, 57.15; H, 5.18; N, 4.53.

4.8. Methyl (3a*R*,4*S*,5*S*,6*R*,6a*S*)-4-benzyloxycarbonylamino-6-hydroxy-2,2-dimethyl-tetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carboxylate 16

Compound **15** (0.10 g, 0.31 mmol) was dissolved in dry acetone (3 mL). $MgSO_4$ (0.11 g, 0.91 mmol), 2,2-DMP (4.6 mL, 37.41 mmol) and *p*-TSA (catalytic amount) were added and the mixture was stirred at room temperature for 13 h. After the starting material had been consumed (TLC, CH_2Cl_2/MeOH 15:1), the reaction mixture was concentrated to dryness. The resulting residue was diluted with $CHCl_3$ (15 mL) and washed with brine (3 × 15 mL). The organic layer was dried over Na_2SO_4 and concentrated to dryness. The crude product was purified by flash column chromatography (EtOAc/hexane 1:1) to afford compound **16** (0.10 g, 0.27 mmol, 88% yield) as a colourless oil. $[\alpha]_D^{23} = +70.0$ (*c* 1.0, $CHCl_3$). ^1H NMR ($CDCl_3$, ppm): 1.25 (s, 3H, CH_3); 1.40 (s, 3H, CH_3); 2.96–3.05 (m, 1H, H-1); 3.69 (s, 4H, OCH_3 + OH); 4.39–4.44 (m, 1H, H-5); 4.51–4.53 (m, 1H, H-3); 4.55–4.57 (m, 1H, H-2); 4.66–4.69 (m, 1H, H-4); 5.07 (d, 1H, $J_{H,H'}$ = 12.0 Hz, CHPh); 5.11 (d, 1H, $J_{H,H'}$ = 12.0 Hz, CHPh); 5.56 (m, 1H, NH); 7.28–7.34 (m, 5H, 5 × H-ar). ^{13}C NMR ($CDCl_3$, ppm): 24.4; 26.0; 52.3; 55.8; 58.6; 67.0; 78.1; 84.3; 85.4; 112.1; 128.2; 128.5; 136.1; 155.5; 171.0. IR (NaCl, ν_{max}, cm^{-1}): 3427 (br, NH + OH); 1727 (st, C=O); 1679 (st, N–C=O). MS (CI, *m*/*z*, %): 366 (42, $[M+H]^+$); 350 (77, $[M-CH_3]^+$); 91 (100, $[CH_2Ph]^+$). Anal. Calcd for $C_{18}H_{23}NO_7$: C, 59.17; H, 6.34; N, 3.83. Found: C, 59.24; H, 6.15; N, 4.01.

4.9. Methyl (3a*R*,4*S*,5*S*,6*R*,6a*S*)-4-benzyloxycarbonylamino-6-methoxy-2,2-dimethyl-tetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carboxylate 1

A solution of compound **16** (0.03 g, 0.08 mmol) in CH_3CN (1 mL) was treated with MeI (0.08 mL, 1.31 mmol) and Ag_2O (0.04 g, 0.18 mmol) and the mixture was stirred at 50 °C for 60 h. After the starting material had been consumed (TLC, EtOAc/hexane 1:2) and the mixture was filtered through a pad of Celite and evaporated in vacuo. The crude product was purified by flash column chromatography (EtOAc/hexane 1:2) to afford compound **1** (0.03 g, 0.08 mmol, 93% yield) as a colourless oil. $[\alpha]_D^{17} = -13.6$ (*c* 1.5, $CHCl_3$). ^1H NMR ($CDCl_3$, ppm): 1.26 (s, 3H, CH_3); 1.39 (s, 3H, CH_3); 2.94–2.97 (m, 1H, H-1); 3.40 (s, 3H, OCH_3); 3.71 (s, 3H, OCH_3); 4.18–4.21 (m, 1H, H-2); 4.52–4.61 (m, 3H, H-3 + H-4 + H-5); 5.07 (d, 1H, $J_{H,H'}$ = 12.9 Hz, CHPh); 5.13 (d, 1H, $J_{H,H'}$ = 12.9 Hz, CHPh); 5.28 (br s, 1H, NH); 7.31–7.39 (m, 5H, H-ar). ^{13}C NMR ($CDCl_3$, ppm): 24.0; 25.8; 51.7; 53.6; 57.1; 58.1; 66.3; 82.5; 83.6; 86.3; 112.0; 127.7; 127.8; 128.0; 136.0; 155.1; 170.5. IR (NaCl, ν_{max}, cm^{-1}): 3342 (br, NH); 1742 (st, C=O); 1630 (st, N–C=O). MS (CI, *m*/*z*, %): 380 (74, $[M+H]^+$); 244 (46, $[M-CO_2Bn]^+$); 91 (100, $[CH_2Ph]^+$). Anal. Calcd for $C_{19}H_{25}NO_7$: C, 60.15; H, 6.64; N, 3.69. Found: C, 59.94; H, 6.84; N, 3.60.

4.10. (3a*R*,4*S*,6a*S*,*E*)-4-benzyloxycarbonylamino-2,2-dimethyl-4,6a-dihydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carboxylic acid 17

To a solution of compound **1** (0.03 g, 0.08 mmol) in THF (0.8 mL) was added 1 M aqueous NaOH (0.4 mL) and the mixture was stirred at room temperature for 2 h. After the starting material had been consumed (TLC, EtOAc/hexane 1:3), the mixture was neutralized with DOWEX 50W-X4, filtered and evaporated in vacuo. The crude product was purified by flash column chromatography (EtOAc/hexane 1:1) to afford compound **17** (0.024 g, 0.07 mmol, 90% yield) as a clear oil. $[\alpha]_D^{24} = -12.6$ (*c* 1.8, $CHCl_3$). ^1H NMR ($CDCl_3$, ppm): 1.20 (s, 3H, CH_3); 1.32 (s, 3H, CH_3); 4.65–4.79 (m, 2H, H-3 + H-4); 4.86–4.93 (br s, 1H, H-5); 5.04 (d, 1H, $J_{H,H'}$ = 12.2 Hz, CHPh); 5.15 (d, 1H, $J_{H,H'}$ = 12.2 Hz, CHPh); 5.73 (br s, 1H, NH); 6.60 (s, 1H, H-2); 7.28–7.41 (m, 5H, H-ar); 11.15 (br s, 1H, CO_2H). ^{13}C NMR ($CDCl_3$, ppm): 24.3; 25.6; 52.9; 68.1; 83.5; 84.2; 112.6; 127.9; 128.0; 128.2; 136.2; 137.9; 142.6; 154.8; 169.2. IR (NaCl, ν_{max}, cm^{-1}): 3328 (br, NH); 1725 (st, C=O); 1636 (st, N–C=O). MS (CI, *m*/*z*, %): 334 (15, $[M+H]^+$); 242 (59, $[M-CH_2Ph]^+$); 91 (100, $[CH_2Ph]^+$). Anal. Calcd for $C_{17}H_{19}NO_6$: C, 61.25; H, 5.75; N, 4.20. Found: C, 61.47; H, 5.91; N, 4.10.

4.11. Methyl (3a*R*,4*S*,5*S*,6*R*,6a*S*)-4-benzyloxycarbonylglycylamino-6-methoxy-2,2-dimethyl-tetrahydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carboxylate 20

To a degassed solution of compound **1** (0.05 g, 0.13 mmol) in EtOAc (1.5 mL) was added 10% Pd/C (0.05 g, 10% w/w) and the mixture was stirred under hydrogen at room temperature for 2 h. After the starting material had been consumed (TLC, EtOAc/hexane 1:3), the mixture was filtered through a pad of Celite and concentrated to dryness. The crude amine **19** was dissolved in CH_2Cl_2 (1 mL) and added to a solution of Cbz-Gly-OH (0.036 g, 0.17 mmol), TBTU (0.055 g, 0.17 mmol) and DIEA (0.07 mL, 0.40 mmol) in CH_2Cl_2 (1 mL) and the mixture was stirred under argon at room temperature for 12 h. After the starting material had been consumed (TLC, CH_2Cl_2/MeOH 9:1), the mixture was diluted with CH_2Cl_2 (5 mL) and washed with 10% aq HCl (10 mL), saturated aq $NaHCO_3$ (10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_4 and concentrated to dryness. The crude product was purified by flash column chromatography (EtOAc/hexane 1:1) to afford compound **20** (0.051 g, 0.116 mmol, 88% from **1**) as a colourless oil. $[\alpha]_D^{15} = -19.2$ (*c* 1.3, $CHCl_3$). ^{1}H NMR ($CDCl_3$, ppm): 1.24 (s, 3H, CH_3); 1.37 (s, 3H, CH_3); 2.90 (br s, 1H, H-1); 3.38 (s, 3H, OCH_3); 3.71 (s, 3H, OCH_3); 3.84 (d, 2H, $J_{H,H'}$ = 5.5 Hz, CH_2-Gly); 4.20–4.25 (m, 1H, H-2); 4.48–4.58 (m, 2H, H-3 + H-4); 4.78–4.88 (m, 1H, H-5); 5.13 (br s, 2H, CH_2Ph); 5.43 (br s, 1H, NH); 6.58 (d, 1H, $J_{NH,5}$ = 7.7 Hz, NH); 7.30–7.42 (m, 5H, H-ar). ^{13}C NMR ($CDCl_3$, ppm): 24.1; 25.7; 44.3; 52.1; 53.5; 56.6; 57.4; 67.0; 82.8; 84.0; 86.7; 112.0; 127.9; 128.1; 128.4; 136.0; 156.5; 168.8; 170.3. IR (NaCl, ν_{max}, cm^{-1}): 3307 (br, NH); 1720 (st, C=O); 1653 (st, N–C=O); 1530 (st, N–C=O). MS (CI, *m*/*z*, %): 437 (60, $[M+H]^+$); 379 (74, $[M-{}^i PrO]^+$); 91 (100, $[CH_2Ph]^+$). Anal. Calcd for $C_{21}H_{28}N_2O_8$: C, 57.79; H, 6.47; N, 6.42. Found: C, 57.60; H, 6.25; N, 6.17.

4.12. Methyl 2-((3a*R*,4*S*,6a*S*,*E*)-4-(2-(benzyloxycarbonylglycylamino)acetamido)-2,2-dimethyl-4,6a-dihydro-3a*H*-cyclopenta[*d*][1,3]dioxole-5-carbamido)acetate 22

Compound **20** (0.051 g, 0.116 mmol) was dissolved in THF (1.2 mL) and treated with 1 M aq NaOH (0.6 mL) at room temperature for 2 h. The starting material had been consumed (TLC, EtOAc/hexane 1:1) and the mixture was neutralized with DOWEX 50W-X4, filtered and evaporated in vacuo. The resulting crude unsaturated carboxylic acid **21** was dissolved in CH_2Cl_2 (2 mL) and reacted with TBTU (0.049 g, 0.152 mmol) and DIEA (0.06 mL, 0.35 mmol) under argon at room temperature. After 15 min H-Gly-OMe-HCl (0.019 g, 0.152 mmol) was added and the mixture stirred for a further 12 h. After complete consumption of the starting material (TLC, EtOAc), the reaction mixture was diluted with CH_2Cl_2 (5 mL) and washed with 10% aq HCl (10 mL), saturated aq $NaHCO_3$ (10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness. The crude product was purified by flash column chromatography (EtOAc) to afford compound **22** (0.035 g, 0.076 mmol, 65% from **20**) as a colourless oil. $[\alpha]_D^{16} = -10.3$ (*c* 1.1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, ppm): 1.32 (s, 3H, CH_3); 1.40 (s, 3H, CH_3); 3.68 (s, 3H, OCH_3); 3.75–3.90 (m, 4H, H-3′ + H-4′ + CH_2-Gly); 4.08–4.26 (m, 2H, CH_2-Gly); 4.56–4.60 (m, 1H, H-5′); 5.09 (br s, 2H, CH_2Ph); 5.15 (br s, 1H, NH); 5.27 (br s, 1H, NH); 5.74 (br s, 1H, NH); 6.71 (s, 1H, H-2′); 7.29–7.41 (m, 5H, H-ar). ^{13}C NMR ($CDCl_3$, ppm): 25.6; 27.3; 41.1; 44.5; 52.4; 58.9; 67.2; 83.1; 84.7; 111.9; 128.0; 128.3; 128.5; 136.0; 138.4; 141.2; 156.7; 163.6; 170.3; 170.4. IR (NaCl, ν_{max}, cm^{-1}): 3310 (br, NH); 1725 (st, C=O); 1664 (st, N–C=O); 1535 (st, N–C=O). MS (CI, *m*/*z*, %): 462 (9, $[M+H]^+$); 296 (10, $[M-CH_2Ph-{}^i PrO_2]^+$); 91 (100, $[CH_2Ph]^+$). Anal. Calcd for $C_{22}H_{27}N_3O_8$: C, 57.26; H, 5.90; N, 9.11. Found: C, 57.49; H, 6.15; N, 9.27.

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