New Cyclic Aspartic Acid and *N*-Glucosyl Asparagine Mimetics

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Dedicated to Professor Miha Tišler on the occasion of his 80th birthday

Abstract: Enantiopure *tert*-butyl 4-oxopipecolic esters were converted into cyclic analogues of bishomoaspartic acid and homoglutamic acid by Horner–Wadsworth–Emmons olefination and hydrogenation. Condensation of the side chain carboxylic group with anomeric glucosylamine and suitable protection of the α-amino acid moiety afforded the corresponding *N*-glucosyl asparagine or glutamine mimetics as useful building blocks in glycopeptidomimetic synthesis.

Key words: glycoamino acids, piperidines, stereoselectivity

4-Hydroxy- and 4-oxopipecolic acids (Figure 1) are unusual amino acids that occur occasionally in nature. For example, they are present in some depsipeptide antibiotics belonging to the virginiamycin and actinomycin families.[1]

These cyclic compounds represent valuable precursors of conformationally constrained amino acids and peptidomimetics, since a variety of functionalized side chains can be introduced on C-4 making use of the hydroxy or carbonyl moiety.[2]

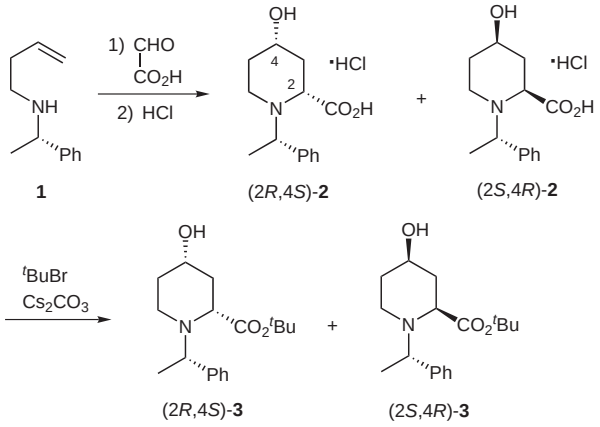
Cyclic amino acids such as pipecolic acid are able to stabilize or promote turn conformations when incorporated into polypeptides.[3] Therefore, substituted pipecolic acids can favorably expose side chain groups on the outermost surface of the peptide, promoting the interaction with specific receptors.

4-hydroxypipecolic acid 4-oxopipecolic acid

Figure 1

Glycoproteins play a key role in many important biological processes. The pendant carbohydrates which range from monosaccharides to large complex glycans, can strongly affect protein properties including folding, proteolytic stability and recognition processes.[4]

In this context, we are interested in synthesizing new cyclic analogues of *N*-glycoasparagine to be used in the preparation of glycopeptides of biological interest. Herein, we describe the synthesis of N^{α},C^{α}-protected 4-(carboxymethyl)pipecolic acids starting from the 4-oxo-derivatives. Furthermore, the (2*S*,4*R*) and (2*R*,4*S*) stereoisomers of the cyclic amino acids, that can also be considered as rigid mimetics of aspartic and glutamic acids, were successfully glycosylated with per-*O*-acetylated glucosamine to generate the corresponding N^4-β-D-glucopyranosyl asparagine analogues.



Scheme 1

4-Hydroxypipecolic acid derivatives **2** were prepared in two steps from enantiopure homoallylic amine **1** following a slight modification of Beaulieu's procedure (Scheme 1).[5] The crude strongly acidic mixture of **2** was made alkaline with cesium carbonate and treated with *tert*-butylbromide to obtain a diastereomeric mixture (2:3) of *tert*-butyl pipecolic esters **3**, which were easily separable by chromatography on silica gel. The absolute configuration of C-2 and C-4 stereocenters was established by comparison of ^{1}H NMR spectra of diastereomers **3** prepared by hydrolysis and *tert*-butyl esterification of the corresponding separated bicyclic lactones.[5]

The reported process allows a rapid synthesis of *tert*-butyl *cis*-4-hydroxypipecolic ester on multigram scale and is particularly convenient when both enantiomers are required.

The oxidation of alcohols **3** with the *N*-methylmorpholine *N*-oxide–tetrapropylammonium perruthenate (NMO–TPAP) system[6,7] smoothly afforded the corresponding protected 4-oxopipecolic acids **4** in high yields. Ketones **4**

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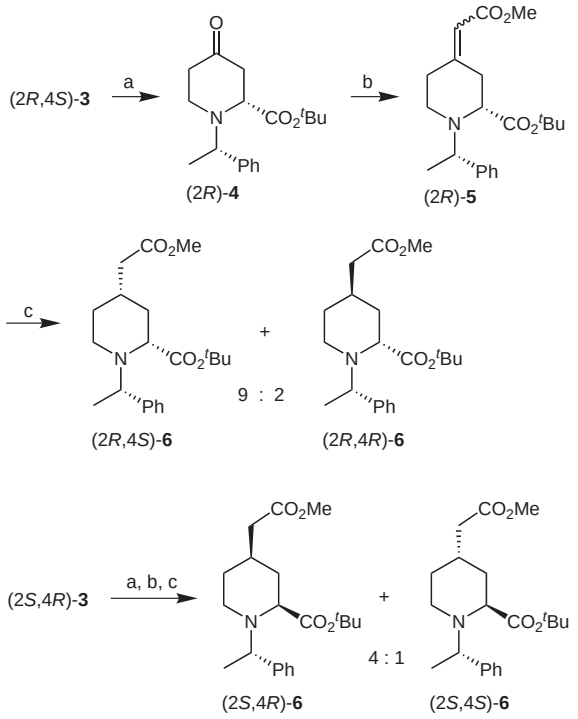
reacted with the sodium salt of methyl(dimethoxyphosphoryl)acetate to give 1.6:1 mixtures of α,β-unsaturated esters **5**, which were directly hydrogenated. Selective hydrogenation of the C–C double bond in the presence of the *N*-benzyl moiety catalyzed by PtO_2 afforded the separable *cis*- and *trans*-4-(2-methoxy-2-oxoethyl)pipecolic esters **6** in ca 4:1 ratio and 80% overall yield based on ketones **4** (Scheme 2). Analogously to hydrogenation of related 4-alkylidene pipecolic ester derivatives,[2g,j] hydrogen adds preferentially on the olefin face opposite to the C-2 substituent, favoring the formation of the *cis* stereoisomers.



Scheme 2 *Reagents and conditions*: (a) NMO, TPAP, 4 Å MS, CH_2Cl_2, 91–92%; (b) $(MeO)_2P(O)CH_2CO_2Me$, NaH, THF; (c) H_2, PtO_2, EtOAc, 80–83%.

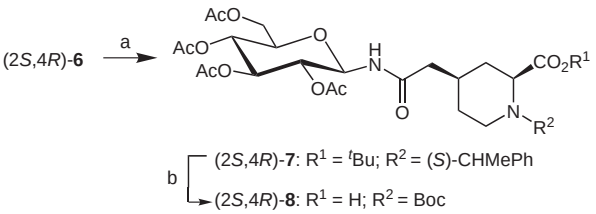
The relative configuration of *cis*- and *trans*-2,4-disubstituted piperidines **6** was determined through analysis of their ^1H NMR[8] spectra.[2a,j,9] In particular, the proton 2-H (δ = 3.27 and 2.79 ppm) in the major isomers displays coupling constants of ca 11 Hz and 2.8 Hz to 3-H_{ax} and 3-H_{eq}, respectively, suggesting a diaxial relationship between 2-H and 3-H_{ax}. The proton 4-H (δ = ca 1.8 and 1.6 ppm) has coupling constants of ca 12 Hz to both 3-H_{ax} and 5-H_{ax}, consistent with an axial orientation.

In the minor isomers, the proton 2-H (δ = 3.88 and 3.20 ppm) displays small coupling constants (≤ 5.4 Hz) to the 3-H protons in accord with an equatorial orientation. The proton 4-H (δ = 1.9 ppm) has coupling constants of nearly 12 Hz to 5-H_{ax}, consistent with an axial orientation.

Accordingly, *cis* stereochemistry was assigned to (2*R*,4*S*)-**6** and (2*S*,4*R*)-**6** and *trans* to (2*R*,4*R*)-**6** and (2*S*,4*S*)-**6**, assuming a chair-like conformation for the piperidine ring. The preference of the *trans* diastereomer for the conformation with the axial C-2 carboxyl group and the equatorial C-4 substituent has been previously observed on related *trans*-4-substituted pipecolic ester derivatives in solution.[2j]

The relative configuration of (2*R*,4*R*)-**6** was unambiguously confirmed by single crystal X-ray structure analysis that showed the chair conformation of the piperidine ring with the axially oriented *tert*-butyl ester and the equatorially oriented 2-methoxy-2-oxoethyl moiety in the solid state.[10]

Orthogonally protected amino acids **6** can be regarded as conformationally constrained mimetics of Asp or Glu, and the side chain carboxylic group can be used to insert a carbohydrate unit to obtain a glycoamino acid mimetic.



Scheme 3 *Reagents and conditions*: (a) (i) NaOH (1 M); (ii) 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine, CDMT, NMM, THF, 63%; (b) (i) H_2, 10% Pd/C, MeOH; (ii) TFA; (iii) Boc_2O, DIPEA, MeOH, 34%.

The *cis*-4-(2-methoxy-2-oxoethyl)pipecolic esters (2*S*,4*R*)-**6** and (2*R*,4*S*)-**6** were hydrolyzed under basic conditions and the obtained salts were coupled with 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine[11] in the presence of 2-chloro-4,6-dimethoxy-1,3,5-triazine (CDMT) and 4-methylmorpholine (NMM) to yield (2*S*,4*R*)-**7**[12] and (2*R*,4*S*)-**7**, respectively, in 63–69% yields (Scheme 3).

Hydrogenolysis of the *N*-benzyl moiety followed by hydrolysis of the *tert*-butyl ester with trifluoroacetic acid (TFA) afforded the glycoamino acids unprotected at the N- and C-terminus. Finally, reaction with di-*tert*-butyldicarbonate (Boc_2O) in the presence of diisopropyl(ethyl)amine (DIPEA) provided the *N*-Boc glycoamino acids (2*S*,4*R*)-**8**[13] and (2*R*,4*S*)-**8** suitably protected for peptide synthesis (Scheme 3).

In conclusion, the synthesis of new cyclic mimetics of Asp and Glu has been accomplished starting from enantiopure *tert*-butyl 4-oxopipecolic esters. The side chain carboxylic group was coupled with glucopyranosylamine to obtain conformationally restricted mimetics of *N*-glucosyl-Asn and *N*-glucosyl-Gln. Standard protecting group manipulations gave a building block suitable for peptide synthesis.

The present work further demonstrates the value of 4-oxo- and 4-hydroxypipecolic acid as precursors of conformationally constrained amino acids. The possibility of these cyclic amino acids to induce turn conformations when inserted in peptides suggests the use of this class of amino acids for the synthesis of biologically active peptides – work that is currently in progress in our laboratories.

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(7) For examples of oxidation of 4-hydroxypipecolic acid derivatives with other oxidants, see refs. 2c, 2g and 2j.

(8) (2*R*,4*S*)-**6**: $[\alpha]_D^{24}$ 6.45 (*c* = 0.93, $CHCl_3$). ^{1}H NMR (400 MHz): δ = 7.49–7.52 (m, 2 H, Ph), 7.28–7.34 (m, 2 H, Ph), 7.19–7.24 (m, 1 H, Ph), 4.01 (q, *J* = 6.9 Hz, 1 H, C*H*Me), 3.65 (s, 3 H, OCH_3), 3.27 (dd, *J* = 2.8, 10.9 Hz, 1 H, 2-H), 2.44 (dt, *J* = 3.5, 11.4 Hz, 1 H, 6-H), 2.22 (d, *J* = 7.0 Hz, 2 H, CH_2CO_2Me), 2.15 (dt, *J* = 2.5, 11.6 Hz, 1 H, 6-H), 1.92 (dm, *J* = 12.3 Hz, 1 H, 3-H), 1.76–1.88 (m, 1 H, 4-H), 1.43–1.57 (m, 2 H, 3-H, 5-H), 1.48 (s, 9 H, *t*-Bu), 1.32 (d, *J* = 6.9 Hz, 3 H, $CHCH_3$), 1.12 (dq, *J* = 3.8, 12.0 Hz, 1 H, 5-H). ^{13}C NMR (100 MHz): δ = 173.1 (s, CO), 172.9 (s, CO), 143.4 (s, Ph), 127.9 (d, 2 × C, Ph), 127.8 (d, 2 × C, Ph), 126.6 (d, Ph), 80.9 (s, *t*-Bu), 64.3 (d, C-2), 57.3 (d, *C*HMe), 51.5 (q, OCH_3), 43.2 (t, C-6), 40.7 (t, CH_2CO_2Me), 36.2 (t, C-3), 32.6 (d, C-4), 31.6 (t, C-5), 28.0 (q, 3 × C, *t*-Bu), 9.0 (q, $CHCH_3$).
(2*R*,4*R*)-**6**: $[\alpha]_D^{22}$ –0.55 (*c* = 1.02, $CHCl_3$). ^{1}H NMR (400 MHz): δ = 7.25–7.38 (m, 4 H, Ph), 7.17–7.23 (m, 1 H, Ph), 4.00 (q, *J* = 6.7 Hz, 1 H, C*H*Me), 3.86–3.91 (m, 1 H, 2-H), 3.65 (s, 3 H, OCH_3), 2.86 (dt, *J* = 2.3, 12.1 Hz, 1 H, 6-H), 2.45 (dm, *J* = 11.7 Hz, 1 H, 6-H), 2.11–2.27 (m, 3 H, CH_2CO_2Me, 3-H), 1.86–1.98 (m, 1 H, 4-H), 1.46–1.56 (m, 2 H, 3-H, 5-H), 1.51 (s, 9 H, *t*-Bu), 1.25 (d, *J* = 6.7 Hz, 3 H, $CHCH_3$), 1.11 (dq, *J* = 4.5, 12.4 Hz, 1 H, 5-H). ^{13}C NMR (100 MHz): δ = 173.0 (s, CO), 172.9 (s, CO), 147.2 (s, Ph), 128.2 (d, 2 × C, Ph), 127.0 (d, 2 × C, Ph), 126.6 (d, Ph), 80.6 (s, *t*-Bu), 61.7 (d, *C*HMe), 57.1 (d, C-2), 51.4 (q, OCH_3), 45.5 (t, C-6), 41.2 (t, CH_2CO_2Me), 34.9 (t, C-3), 32.1 (t, C-5), 29.6 (d, C-4), 28.3 (q, 3 × C, *t*-Bu), 22.0 (q, $CHCH_3$).

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(10) The X-ray CIF file for this structure has been deposited at the Cambridge Crystallographic Data Centre and allocated with the deposition number CCDC 607916. Copies of the data can be obtained free of charge from CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (e-mail: deposit@ccdc.cam.ac.uk; internet://www.ccdc.cam.ac.uk).

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(12) A slurry of diester (2*S*,4*R*)-**6** (358 mg, 0.99 mmol) in THF (0.5 mL) was treated dropwise with an aq 1 M NaOH solution (1.6 mL, 1.6 mmol). The mixture was stirred at r.t. for 3 h and concentrated. The residue was dissolved in THF (1.0 mL) and NMM (0.109 mL, 0.99 mmol) and CDMT (174 mg, 0.99 mmol) were added. The reaction mixture was stirred at r.t. for 30 min and then a solution of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosylamine (321 mg, 0.99 mmol) in THF (1.3 mL) was added. The resulting mixture was stirred at r.t. overnight, diluted with EtOAc, and washed sequentially with H_2O and brine. The separated organic layer was dried over Na_2SO_4, filtered, and concentrated. The residue was purified by column chromatography on silica gel (eluent: PE–EtOAc = 2:3) to afford (2*S*,4*R*)-**7** (423 mg, 63%) as a white solid.
(2*S*,4*R*)-**7**: $[\alpha]_D^{27}$ –32.65 (*c* = 0.525, $CHCl_3$). ^{1}H NMR (400 MHz): δ = 7.23–7.34 (m, 3 H, Ph), 7.14–7.19 (m, 2 H, Ph), 6.18 (d, *J* = 9.3 Hz, 1 H, NH), 5.28 (t, *J* = 9.5 Hz, 1 H, 3′-H), 5.19 (t, *J* = 9.4 Hz, 1 H, 1′-H), 5.04 (t, *J* = 9.7 Hz, 1 H, 4′-H), 4.87 (t, *J* = 9.6 Hz, 1 H, 2′-H), 4.29 (dd, *J* = 4.2, 12.5 Hz, 1 H, 6′-H), 4.04 (dd, *J* = 2.0, 12.5 Hz, 1 H, 6′-H), 3.97 (q, *J* = 7.0 Hz, 1 H, C*H*Me), 3.78 (ddd, *J* = 2.0, 4.2, 10.1 Hz, 1 H, 5′-H), 3.00 (dm, *J* = 11.4 Hz, 1 H, 6-H), 2.77 (dd, *J* = 2.9, 11.2 Hz, 1 H, 2-H), 2.07 (A part of an ABX system, *J* = 6.7, 14.6 Hz, 1 H, CH*H*CON), 2.06 (s, 3 H, CH_3CO), 2.02 (s, 3 H, CH_3CO), 2.00 (s, 3 H, CH_3CO), 1.99 (s, 3 H, CH_3CO), 1.96 (B part of an ABX system, *J* = 7.0, 14.6 Hz, 1 H, CH*H*CON), 1.79 (dm, *J* = 12.4 Hz, 1 H, 3-H), 1.71 (tm, *J* = 11.5 Hz, 1 H, 6-H), 1.50–1.60 (m, 2 H, 4-H, 5-H), 1.53 (s, 9 H, *t*-Bu), 1.48 (d, *J* = 7.0 Hz, 3 H, $CHCH_3$), 1.36 (q, *J* = 11.8 Hz, 1 H, 3-H), 1.19–1.30 (m, 1 H, 5-H). ^{13}C NMR (100 MHz): δ = 173.1 (s, CO), 171.6 (s, CO), 171.0 (s, CO), 170.6 (s, CO), 169.8 (s, CO), 169.5 (s, CO), 137.7 (s, Ph), 128.9 (d, 2 × C, Ph), 127.7 (d, 2 × C, Ph), 127.2 (d, Ph), 80.9 (s, *t*-Bu), 78.0 (d, C-1′), 73.5 (d, C-5′), 72.6 (d, C-3′), 70.5 (d, C-2′), 68.1 (d, C-4′), 64.5 (d, C-2), 61.6 (t, C-6′), 59.4 (d, *C*HMe), 43.8 (t, C-6), 43.2 (t, CH_2CON), 36.3 (t, C-3), 32.6 (d, C-4), 31.3 (t, C-5), 28.1 (q, 3 × C, *t*-Bu), 20.7 (q, CH_3CO), 20.6 (q, CH_3CO), 20.5 (q, 2 × C, CH_3CO), 18.5 (q, CH_3CH).

(13) A solution of (2*S*,4*R*)-**7** (105 mg, 0.155 mmol) in MeOH (1.6 mL) and AcOH (18 μL, 0.310 mmol) was hydrogenated in

the presence of 10% Pd/C (9 mg) at r.t. and atmospheric pressure overnight. The reaction mixture was filtered through cotton wool and concentrated. The residue was dissolved in the minimum amount of TFA at 0 °C and the mixture was stirred at r.t. for 2.5 h and then concentrated. The residue was dissolved in anhyd MeOH (217 μL) and treated sequentially with DIPEA (67 μL, 0.386 mmol) and Boc_2O (28.1 mg, 0.129 mmol) at 0 °C. The reaction mixture was stirred at r.t. overnight, and then concentrated. The residue was dissolved in a mixture of EtOAc and CH_2Cl_2 (1:1, 2.8 mL), treated with 5% aq $KHSO_4$ solution (1 mL) and stirred at r.t. for 1 h. After the organic layer was separated, the aqueous layer was extracted with EtOAc (3 × 1.4 mL). The combined organic phases were washed with brine, dried over Na_2SO_4, filtered, and concentrated. The crude product was purified by column chromatography on silica gel (eluent: CH_2Cl_2–MeOH, 10:1) to afford (2*S*,4*R*)-**8** (32.4 mg, 34%) as a white solid. Molecular mass determinations by electrospray ionization mass spectrometry (ESI–MS) was used to identify the N-protected amino acid (2*S*,4*R*)-**8**. MS (ESI): *m*/*z* = 639.3 [M + Na^+].