A Model Route Toward the Synthesis of Conformationally Constrained Polyhydroxylated Dipeptides from Natural Carbohydrates

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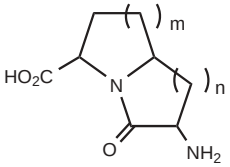
Received 6 October 2003

Abstract: Enantiopure 6,7-diacetoxy-3-*t*-butoxycarbonylamino-azabicyclo[3.3.0]octan-2-one-8-carboxylic acid **14** (pyrrolizidinone amino acid) was synthesized in 14 steps and 5.8% overall yield from tri-*O*-benzyl-D-arabinose **5** through the formyl *C*-iminosugar **9** as a key intermediate.

Key words: integrins, peptidomimetics, pyrrolizidinone amino acids, reverse-turn, RGD

The field of peptidomimetics[1] has gained an enormous importance in recent years, particularly with the emergence of conformationally constrained systems that mimic certain structural features and therapeutic effects of natural peptides.[2] A special class of rigidified peptidomimetics is constituted of the azabicyclo[X.Y.0]alkanone amino acids[2b,3] **1** (Figure 1), i. e. fused bicyclic dipeptides that simulate the bioactive conformation of the β-turn sites.[4] The stereocontrol[3,5] at the chiral carbon backbone and ring-fusion center, the side chain attachment,[6,7b] and the ring size,[5,8] are issues, which have been widely addressed. These molecules have been used not only to mimic a dipeptide motif[2b,4c,6b] but also as a scaffold featuring an amine and a carboxylate handles suitable for the linkage with an important pharmacophoric tripeptide, namely the Arg–Gly–Asp (RGD) sequence,[9] which is implicated in several biological events including angiogenesis and osteoporosis.[10]

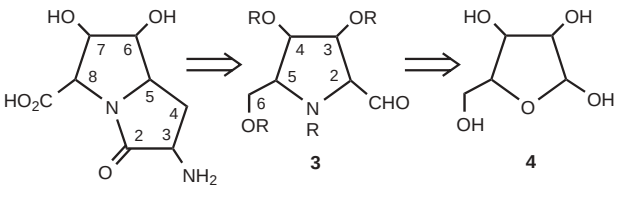


1

Figure 1 Azabicyclo[X.Y.0]alkanone amino acids

Our interest in this field was stimulated by the growing demand of efficient and versatile methodologies for the stereocontrolled introduction of side chain functionalities in **1**. In particular, the functionalization with hydroxyl groups[11] of the carboxyl bearing ring should allow further synthetic modification since these functionalities may serve as anchoring points for side chain moieties implicated in ligand-receptor interaction.[6,7] Actually, it has been demonstrated that the three dimensional structure and the stereoelectronic properties of side chain groups of amino acids are critical for biological activity and for selective ligand-receptor interaction.[7,10b] Therefore, diastereomeric hydroxylated peptidomimetics may feature different conformational arrangements required in molecular recognition. An easy access to a collection of hydroxylated dipeptidic scaffolds derived from **1** may provide useful tools to probe and elucidate the structure-activity relationships of these peptide surrogates in different molecular events in which natural peptides are implicated. Moreover, since molecules containing a specific pharmacophoric peptide sequence have been proved to display a tumor-homing ability,[12] hydroxylated derivatives of peptidomimetics **1** can be used as linkers between the peptide-binding motif coupled to the amine and carboxylate appendages and specific drugs anchored on the hydroxyl handles. This application opens opportunities of markedly improving the delivery and selectivity of drugs[12] in several diseases, such as inflammation and cancer, where integrins are implicated.

A convenient route was envisaged (Scheme 1) to enantiopure 6,7-dihydroxylated azabicyclo[3.3.0]octanone amino acids **2** (pyrrolizidinone amino acids) from polyhydroxylated formyl pyrrolidines **3**, so-called formyl *C*-iminosugars, which in turn have been made readily available from recent work in our laboratory[13] by thiazole-based aminohomologation of pentofuranoses **4**. In this way one would take advantage of the hydroxyl groups and their stereochemistry already in place in compound **3** and would exploit the formyl group for the construction of the second functionalized pyrrolidine ring.

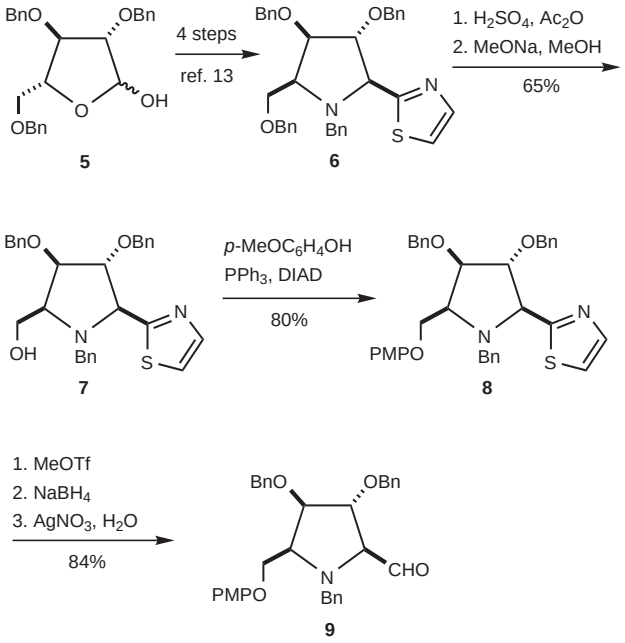


2 **3** **4**

Scheme 1

SYNLETT 2003, No. 15, pp 2345–2348
Advanced online publication: 21.11.2003
DOI: 10.1055/s-2003-43341; Art ID: G26703ST

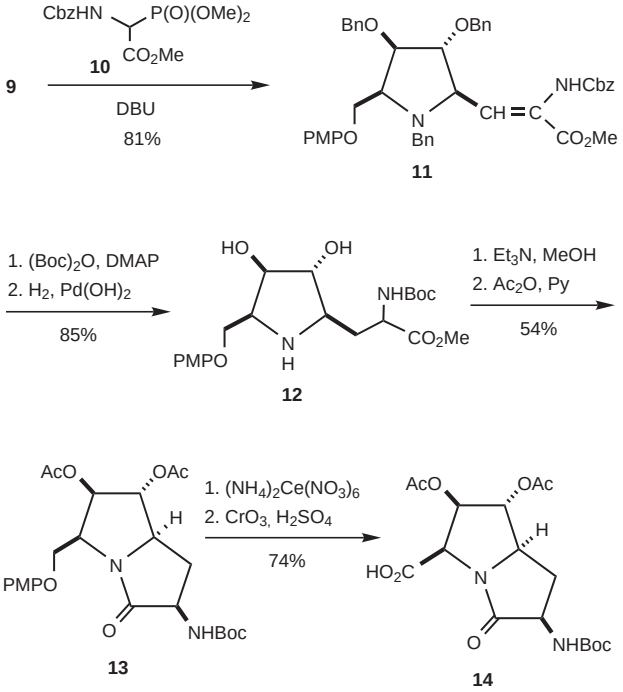
Aiming to obtain the orthogonally protected polyhydroxylated formyl pyrrolidine **9** starting from the known[13] thiazole-masked precursor **6**, the selective removal of the 5-*O*-benzyl group was carried out by acetolysis and the resulting free hydroxyl group of **7**[14] was protected with *p*-methoxyphenol (PMP) via Mitsunobu reaction to give **8**[14] (Scheme 2). Application of silver-based thiazole-to-formyl unmasking protocol[15] (*N*-methylation, reduction, hydrolysis) to **8** afforded the key intermediate **9**[14] in 84% yield.



Scheme 2

The aldehyde **9** was allowed to react with the commercially available phosphonate **10**[16] and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) as a base in a Horner–Emmons olefination reaction to give **11**[17] as a single isomer in 81% yield whose *E* or *Z* configuration was not determined (Scheme 3).

Subsequent Boc-protection at the enamino nitrogen atom,[8b] hydrogenation of the crude intermediate over $Pd(OH)_2$ removed *O*- and *N*-benzyl protective groups and reduced the carbon-carbon double bond leading to the iminosugar α-amino acid **12** as a mixture of diastereoisomers. Intramolecular amide formation under basic conditions (Et_3N) at 60 °C and acetylation transformed **12** into a mixture of pyrrolizidinone **13**[17] and its C-3 epimer. Each of these bicyclic compounds was isolated in a pure form by medium pressure column chromatography in 54% and 24% yield, respectively. The configuration at the newly formed stereocenter, viz. the C-3, of the major product **13** was assigned by NOE difference experiments. Upon irradiation of the H-5 proton a significant NOE with H-3 and H-7 was observed, thus indicating a *cis*-relationship



Scheme 3

between these protons. The selective removal of the PMP group of **13** by treatment with cerium ammonium nitrate (CAN) led to the corresponding primary alcohol, which when submitted to the Jones oxidation furnished the target 6,7-diacetoxy pyrrolizidinone amino acid **14** (Scheme 3) that was fully characterized as methyl ester.[17] The orthogonal protection of the functional groups of **14** should allow a variety of synthetic elaborations. Hence the use of this constrained dipeptide or its *O*-functionalized derivatives[18] as substrates for the synthesis of cyclopeptides by insertion of pharmacophoric peptides such as RGD (Arg–Gly–Asp) or LDT (Leu–Asp–Thr)[19] now becomes of interest.

In conclusion, an efficient route for the synthesis of a new class of conformationally constrained peptido-mimetics has been developed. This approach should be amenable to the preparation of a collection of polyhydroxylated azabicycloalkanone amino acids of type **1** where molecular diversity is achieved by both stereochemical and ring size variations. Studies are underway in our laboratory using a variety of polyhydroxylated formyl *N*-heterocycles (formyl *C*-iminosugars), which are accessible through the thiazole-based aminohomologation technique.[13]

Acknowledgment

We gratefully acknowledge MIUR (COFIN 2002) for financial support and Interdisciplinary Center for the Study of Inflammation (Ferrara, Italy) for a fellowship to B. R.

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(13) Compound **6** was prepared in gram scale quantities by stereoselective addition of 2-lithiothiazole to the nitrone derived from D-arabinose **5** followed by dehydroxylation of the resulting open-chain hydroxylamine and cyclization by intramolecular nitrogen-carbon bond formation via S_N2 process. See: (a) Dondoni, A.; Perrone, D. *Tetrahedron Lett.* **1999**, *40*, 9375. (b) Dondoni, A.; Giovannini, P.; Perrone, D. *J. Org. Chem.* **2002**, *62*, 7203.

(14) Compound **7**. $[\alpha]_D$ = +22.2 (*c* 1.2, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.74 (d, *J* = 3.3 Hz, 1 H, Th), 7.32–7.14 (m, 16 H, 3 × Ph, Th), 4.68, 4.50 (2 × d, 2 H, *J* = 11.7 Hz, PhC*H*$_2$O), 4.58, 4.46 (2 × d, *J* = 11.7 Hz, 2 H, PhC*H*$_2$O), 4.44 (d, $J_{2,3}$ = 4.8 Hz, 1 H, H-2), 4.27 (dd, $J_{3,4}$ = 4.5 Hz, 1 H, H-3), 4.16 (dd, $J_{4,5}$ = 6.6 Hz, 1 H, H-4), 4.01, 3.82 (2 × d, *J* = 13.7 Hz, 2 H, PhC*H*$_2$N), 3.63 (dd, $J_{5,6}$ = 4.5 Hz, $J_{6,OH}$ = 6.3 Hz, 2 H, 2 × H-6), 3.34 (dt, 1 H, H-5), 2.65 (t, 1 H, OH). Compound **8**. $[\alpha]_D$ = +35.5 (*c* 0.5, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.68 (d, *J* = 3.3 Hz, 1 H, Th), 7.38–7.17, 7.00–6.96 (2 × m, 16 H, 3 × Ph, Th), 6.81–6.72 (m, 4 H, MeO*Ph*), 4.69, 4.54 (2 × d, *J* = 11.8 Hz, 2 H, PhC*H*$_2$O), 4.46 (d, $J_{2,3}$ = 2.5 Hz, 1 H, H-2), 4.37, 4.31 (2 × d, *J* = 11.9 Hz, 2 H, PhC*H*$_2$O), 4.29 (dd, $J_{3,4}$ = 2.3 Hz, 1 H, H-3), 4.17 (dd, $J_{5,6a}$ = 7.1 Hz, $J_{6a,6b}$ = 9.0 Hz, 1 H, H-6a), 4.12 (dd, $J_{4,5}$ = 5.6 Hz, 1 H, H-4), 4.08, 3.99 (2 × d, *J* = 13.6 Hz, 2 H, PhC*H*$_2$N), 3.92 (dd, $J_{5,6b}$ = 5.1 Hz, 1 H, H-6b), 3.77 (s, 3 H, Me), 3.67 (ddd, 1 H, H-5). Compound **9**. $[\alpha]_D$ = +2.5 (*c* 0.6, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.30 (d, *J* = 1.3 Hz, 1 H, CHO), 7.40–7.20, 7.16–7.11 (2 × m, 15 H, 3 × Ph), 6.84 (s, 4 H, MeO*Ph*), 4.59, 4.51 (2 × d, *J* = 11.8 Hz, 2 H, PhC*H*$_2$O), 4.44, 4.28 (2 × d, *J* = 11.7 Hz, 2 H, PhC*H*$_2$O), 4.29 (dd, $J_{5,6a}$ = 7.7 Hz, $J_{6a,6b}$ = 9.3 Hz, 1 H, H-6a), 4.27, 3.76 (2 × d, *J* = 13.2 Hz, 2 H, PhC*H*$_2$N), 4.11 (dd, $J_{2,3}$ = 1.2 Hz, $J_{3,4}$ = 1.5 Hz, 1 H, H-3), 4.10 (dd, $J_{4,5}$ = 4.3 Hz, 1 H, H-4), 4.09 (dd, $J_{5,6b}$ = 5.3 Hz, 1 H, H-6b), 3.78 (s, 3 H, Me), 3.68 (ddd, 1 H, H-5), 3.38 (dd, 1 H, H-2). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 204.7 (C-1), 153.9, 153.0, 115.4, 114.6 (MeO*Ph*), 138.8, 137.5, 137.4, 129.1–127.5 (3 × Ph), 84.2 (C-3), 80.0 (C-4), 76.2 (C-2), 71.8 (Ph*C*H$_2$O), 71.4 (Ph*C*H$_2$O), 67.2 (C-6), 66.2 (C-5), 60.7 (Ph*C*H$_2$N), 55.8 (MeO).

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(17) Compound **11**. $[\alpha]_D$ = –8.7 (*c* 0.8, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.34–7.18 (m, 20 H, 4 × Ph), 7.08 (br s, 1 H, NH), 6.81–6.72 (m, 4 H, MeO*Ph*), 6.21 (d, *J* = 8.2 Hz, 1 H, CH=), 5.09, 5.01 (2 × d, *J* = 12.3 Hz, 2 H, PhC*H*$_2$OCO), 4.51, 4.46 (2 × d, *J* = 11.8 Hz, 2 H, PhC*H*$_2$O), 4.45 (s, 2 H, PhC*H*$_2$O), 4.12 (dd, $J_{5,6a}$ = 7.2 Hz, $J_{6a,6b}$ = 9.4 Hz, 1 H, H-6a), 4.04 (dd, $J_{3,4}$ = 3.0 Hz, $J_{4,5}$ = 5.8 Hz, 1 H, H-4), 3.92 (dd, $J_{5,6b}$ = 4.7 Hz, 1 H, H-6b), 3.89, 3.80 (2 × d, *J* = 13.6 Hz, 2 H, PhC*H*$_2$N), 3.89 (dd, $J_{2,3}$ = 4.8 Hz, 1 H, H-3), 3.78, 3.71 (2 × s, 6 H, 2 × Me), 3.56 (dd, 1 H, H-2), 3.44 (ddd, 1 H, H-5). Compound **13**. $[\alpha]_D$ = –45.3 (*c* 0.4, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.86-6.79 (m, 4 H, MeO*Ph*), 5.50 (dd, $J_{6,7}$ = $J_{7,8}$ = 7.7 Hz, 1 H, H-7), 5.19 (dd, $J_{5,6}$ = 7.3 Hz, 1 H, H-6), 4.94 (br d, $J_{3,NH}$ = 5.5 Hz, 1 H, NH), 4.75 (dd, $J_{8,9a}$ = 3.9, $J_{9a,9b}$ = 10.0 Hz, 1 H, H-9a), 4.44 (dd, $J_{3,4a}$ = 6.9 Hz, $J_{3,4b}$ = 12.1 Hz, 1 H, H-3), 4.28 (ddd, $J_{8,9b}$ = 1.0 Hz, 1 H, H-8), 3.87 (dd, 1 H, H-9b), 3.76 (s, 3 H, Me), 3.64 (dd, $J_{4a,5}$ = 5.3 Hz, $J_{4b,5}$ = 10.1 Hz, 1 H,, H-5), 2.96 (ddd, $J_{4a,4b}$ = 11.8 Hz, 1 H, H-4a), 2.10, 2.02 (2 × s, 6 H, 2 × Ac), 1.96 (ddd, 1 H, H-4b), 1.41 (s, 9 H, *t*-Bu). Compound ***epi*-13**. $[\alpha]_D$ = –34.9 (*c* 0.7, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.85–6.78 (m, 4 H, MeO*Ph*), 5.52 (dd, $J_{6,7}$ = 7.8 Hz, $J_{7,8}$ = 7.6 Hz, 1 H, H-7), 5.12 (dd, $J_{5,6}$ = 7.6 Hz, 1 H, H-6), 5.04 (br s, 1 H, NH), 4.69 (dd, $J_{8,9a}$ = 4.6 Hz, $J_{9a,9b}$ = 10.2 Hz, 1 H, H-9a), 4.28 (ddd, $J_{8,9b}$ = 1.6 Hz, 1 H, H-8), 4.05–3.97 (m, 2 H, H-3, H-5), 3.94 (dd, 1 H, H-9b), 3.75 (s, 3 H, Me), 2.48 (ddd, $J_{3,4a}$ = 7.6 Hz, $J_{4a,4b}$ = 13.6, $J_{4a,5}$ = 9.5 Hz, 1 H, H-4a), 2.34 (ddd, $J_{3,4b}$ = 2.2 Hz, $J_{4b,5}$ = 6.8 Hz, 1 H, H-4b), 2.10, 1.99 (2 × s, 6 H, 2 Ac), 1.42 (s, 9 H, *t*-Bu). Compound **14 Methyl Ester**. $[\alpha]_D$ = –63.4 (*c* 0.3, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.61 (dd, $J_{6,7}$ = 9.3 Hz, $J_{7,8}$ = 8.2 Hz, 1 H, H-7), 5.10 (dd, $J_{5,6}$ = 8.4 Hz, 1 H, H-6), 5.08 (br d, $J_{3,NH}$ = 7.0 Hz, 1 H, NH), 4.58 (d, 1 H, H-8), 4.53 (ddd, $J_{3,4a}$ = 6.5 Hz, $J_{3,4b}$ = 12.0 Hz, 1 H, H-3), 3.78 (s, 3 H, Me), 3.69 (ddd, $J_{4a,5}$ = 5.4 Hz, $J_{4b,5}$ = 9.5 Hz, 1 H, H-5), 3.02 (ddd, $J_{4a,4b}$ = 12.5 Hz, 1 H, H-4a), 2.16 (ddd, 1 H, H-4b), 2.09, 2.06 (2 × s, 6 H, 2 × Ac), 1.43 (s, 9 H, *t*-Bu). ^{13}C NMR (100 MHz, $CDCl_3$): δ = 173.4 (Me_3COCO), 173.3 (C-2), 170.4, 169.6 (CH_3CO), 167.8 (CO_2Me), 80.1

(Me_3C), 75.7 (C-7), 75.3 (C-6), 57.7 (C-5), 56.5 (C-8), 54.3 (C-3), 52.8 (MeO), 38.8 (C-4), 28.3 (Me_3C), 20.6 and 20.3 (CH_3CO).

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