Amino Acids (2008) 34: 333–336
DOI 10.1007/s00726-006-0469-9
Printed in The Netherlands

Amino Acids

Synthesis of a versatile constrained analogue of dipeptide DG (Asp-Gly)

Short Communication

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Received October 2, 2006
Accepted October 19, 2006
Published online November 30, 2006;

Summary. The synthesis of an orthogonally protected constrained analogue of dipeptide DG (Asp-Gly) is reported exploiting alkylation of a chiral lactam. The versatility of this analogue was proven by removal of *t*-Boc protecting group, followed by coupling under homogeneous conditions with *t*-Boc-Arg(Z_2)-Gly, to give a conformationally restricted analogue of RGDG tetrapeptide.

Keywords: Amino acids – Mimetics – Lactams – Conformational restriction – Orthogonal protection

Introduction

The properties of biologically active peptides inspired the targeting of these compounds as lead structures for the development of many novel drugs (Loffet, 2002). However, the problems of metabolic stability, bioavailability, and non-selectivity against different receptors and receptor sub-types hampered their utilization as therapeutics. This prompted the development of synthetic strategies leading to biologically stable peptides and converting the native peptides into conformationally restricted analogues, in order to improve their pharmacokinetic properties (Hruby, 2002). A field that is gaining momentum is the synthesis of constrained or rigidified dipeptide units as exemplified by cyclic dipeptide mimetics (Aubé, 2000; Hanessian et al., 1997; Halab et al., 2000; Artale et al., 2003), which allow to fully elucidate the active conformation when they incorporate the critical pharmacophores (side chain groups) needed for biological activity.

Materials and methods

Melting points were obtained on an Electrothermal apparatus IA 9000 and are uncorrected. IR spectra were recorded in $CHCl_3$ on a FTIR Nicolet 20-SX spectrophotometer. Only noteworthy IR absorptions are listed (cm^{-1}). ^1H and ^{13}C NMR spectra were recorded on a Varian Gemini 200 spectrometer at 200 MHz and 50.3 MHz, respectively, using $CDCl_3$ as a solvent unless stated otherwise. Chemical shifts (δ) are reported in ppm relative to TMS and coupling constants (*J*) in Hz. Assignments were aided by decoupling and homonuclear two-dimensional experiments. Optical rotations were measured on a Perkin Elmer 341 polarimeter. The samples were analyzed with a liquid chromatography Agilent Technologies HP1100 equipped with a Zorbax Eclipse XDB-C8 Agilent and Technologies column (flow rate 0.5 ml/min) and equipped with a diode-array UV detector (220 and 254 nm). Acetonitrile and methanol for HPLC were purchased from a commercial supplier. All the samples were prepared by diluting 1 mg in 5 ml of a 1:1 mixture of H_2O and acetonitrile in pure acetonitrile or in pure methanol. The MSD1100 mass detector was utilized under the following conditions: mass range 100–2500 uma, positive scanning, energy of fragmentor 50 V, drying gas flow (nitrogen) 10.0 ml/min, nebulizer pressure 45 psig, drying gas temperature 350 °C, capillary voltage 4500 V. Column chromatography was performed by using silica gel 60 (230–400 mesh). Compound **1** was synthesized according to Galeazzi et al. (2005).

*(3S,4R)-3-t-Butoxycarbonylamino-4-methoxycarbonylpyrrolidin-2-one (**2**)*

A solution of **1** (Galeazzi et al., 2005) (0.39 g, 1.0 mmol) in CH_3CN (5 ml) was treated at rt with cerium ammonium nitrate (CAN) (1.1 g, 2.0 mmol) dissolved in H_2O (5 ml), and the reaction mixture was stirred for 1.5 h at rt. The aqueous layer was extracted with EtOAc (3×25 ml), the organic layers were combined, washed with brine and dried (Na_2SO_4). Removal of the solvent under reduced pressure provided a crude residue, which was purified by silica gel chromatography (cyclohexane-EtOAc, 3:7) to give **2** (0.21 g, 82%) as a white solid. Mp 131–133 °C. IR ($CHCl_3$): 3340, 1733, 1725, 1665 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.40 (s, 9H, *t*-Bu), 3.24–3.65 (m, 3H, H-4 + 2 H-5), 3.75 (s, 3H, OCH_3), 4.31 (dd, $J = 7.3$, $J = 9.2$ Hz, 1H, H-3), 5.44 (d, $J = 7.3$ Hz, 1H, NH), 7.12 (br s, 1H, NH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.2, 31.3, 46.6, 52.4, 55.1, 80.3, 155.4, 172.2, 173.5; $[\alpha]_D$ −36.7 (*c* 0.6, $CHCl_3$); ESI-MS: m/z 258 $[M]^+$, 281 $[M + Na]$. Anal. Calcd for $C_{11}H_{18}N_2O_5$: C, 51.16; H, 7.02; N, 10.85. Found: C, 51.12; H, 6.97; N, 10.79.

*(3S,4R)-1-Benzyloxycarbonylmethyl-3-t-butoxycarbonylamino-4-methoxycarbonylpyrrolidin-2-one (**3**) (Method A)*

To a suspension of NaH (53 mg, 50% dispersion in oil, 1.1 mmol) in dry THF (7 ml), a solution containing compound **2** (258 mg, 1.0 mmol) was added at 0 °C and the mixture was stirred for another 30 min at rt, after which benzyl bromoacetate (240 mg, 1.05 mmol) dissolved in dry THF (3 ml) was added at rt. After 3 h, the reaction mixture was diluted with H_2O (5 ml), extracted with EtOAc (2 × 10 ml), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel chromatography (cyclohexane-EtOAc, 1:1) to give **3** (288 mg, 71%) as a colorless oil. IR ($CHCl_3$): 1744, 1720, 1711, 1667 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.38 (s, 9H, *t*-Bu), 3.21–3.36 (m, 1H, H-5β), 3.48–3.64 (m, 1H, H-4), 3.69 (s, 3H, OCH_3), 3.78 (dd, $J = 7.1$, $J = 9.6$ Hz, 1H, H-5α), 4.08 (ABq, $J = 17.7$ Hz, 2H, CH_2COOBn), 4.37 (dd, $J = 6.6$, $J = 9.3$ Hz, 1H, H-3), 5.11 (s, 2H, $COOCH_2Ph$), 5.46 (d, $J = 6.6$ Hz, 1H, NH), 7.29 (m, 5 ArH); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.0, 44.3, 44.4, 46.3, 52.2, 55.1, 67.0, 79.9, 128.1, 128.3, 128.4, 134.2, 155.1, 167.7, 170.5, 171.8; $[\alpha]_D$ −20.6 (c 1.7, $CHCl_3$); ESI-MS: m/z 407.3 $[MH]^+$, 429.2 $[M + Na]^+$. Anal. Calcd for $C_{20}H_{26}N_2O_7$: C, 59.10; H, 6.45; N, 6.89. Found: C, 59.03; H, 6.49; N, 6.83.

*(3S,4R)-1-Allyl-3-t-butoxycarbonylamino-4-methoxycarbonylpyrrolidin-2-one (**4**)*

To a suspension of NaH (106 mg, 50% dispersion in oil; 2.2 mmol) in dry THF (7 ml), a solution containing compound **2** (516 mg; 2.0 mmol) was added at 0 °C and the mixture was stirred for 30 min at rt, after which allyl bromide (254 mg; 2.1 mmol) dissolved in dry THF (3 ml) was added at rt. After 3 h, the reaction mixture was diluted with H_2O (5 ml), extracted with EtOAc (2 × 10 ml), dried (Na_2SO_4) and concentrated under reduced pressure. The residue was purified by silica gel chromatography (cyclohexane-EtOAc, 1:1) to give **4** (388 mg, 65%) as a colorless oil. IR ($CHCl_3$): 1741, 1722, 1710, 1667, 908 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.41 (s, 9H, *t*-Bu), 3.17–3.36 (m, 1H, H-5β), 3.38–3.56 (m, 2H, H-4 + H-5α), 3.74 (s, 3H, OCH_3), 3.91 (d, $J = 6.1$ Hz, 2H, NCH_2–$CH = CH_2$), 4.33 (dd, $J = 6.6$, $J = 9.3$ Hz, 1H, H-3), 5.16–5.28 (m, 3H, $CH = CH_2 + NH$), 5.61–5.70 (m, 1H, $CH = CH_2$); ^{13}C NMR (50 MHz, $CDCl_3$) δ 28.2, 44.8, 45.0, 45.7, 52.4, 56.0, 80.2, 118.7, 131.4, 155.2, 169.6, 172.4; $[\alpha]_D$ −19.4 (c 0.8, $CHCl_3$); ESI-MS: m/z 299.2 $[MH]^+$, 321.2 $[M + Na]^+$. Anal. Calcd for $C_{14}H_{22}N_2O_5$: C, 56.36; H, 7.43; N, 9.39. Found: C, 56.31; H, 6.39; N, 9.31.

*(3S,4R)-1-Benzyloxycarbonylmethyl-3-t-butoxycarbonylamino-4-methoxycarbonylpyrrolidin-2-one (**3**) (Method B)*

To a stirred solution of compound **4** (300 mg, 1.0 mmol) in water/acetone (4:1 v/v, 10 ml), acetic acid (8.7 mmol, 0.5 ml) and $KMnO_4$ (0.55 g, 3.5 mmol) were added at 0 °C. After stirring for 1.5 h at rt, the mixture was cooled to 0 °C and a saturated solution of $Na_2S_2O_7$ (5 ml) was added. Acetone was removed under reduced pressure and the solution was then acidified by adding dropwise 6 M HCl until pH 3 was reached. After extraction with ethyl acetate (3 × 15 ml), the organic layer was washed with water (30 ml) and a saturated solution of NaCl, dried (Na_2SO_4) and eventually evaporated under reduced pressure. The residue, dissolved in MeOH (3 ml), was added to a stirred suspension in methanol (4 ml) of Amberlite IRA 900 in the hydroxide form (1.0 g). After 1 h the solvent was filtered off, the polymeric reagent was suspended in cyclohexane (15 ml), benzyl bromide (0.34 g, 2.0 mmol) was added and the mixture was refluxed for 2 h. Then the resin was removed by filtration, washed with methanol (15 ml) and the combined organic phases were evaporated under reduced pressure to give a residue which was purified by silica gel chromatography (cyclohexane-EtOAc, 1:1) affording **3** (280 mg, 69%) as a colorless oil. ESI-MS: m/z 407.3 $[MH]^+$, 429.2 $[M + Na]^+$. Anal. Calcd for $C_{20}H_{26}N_2O_7$: C, 59.10; H, 6.45; N, 6.89. Found: C, 59.02; H, 6.40; N, 6.96.

*t-Boc-Arg(Z_2)-Gly (**6**)*

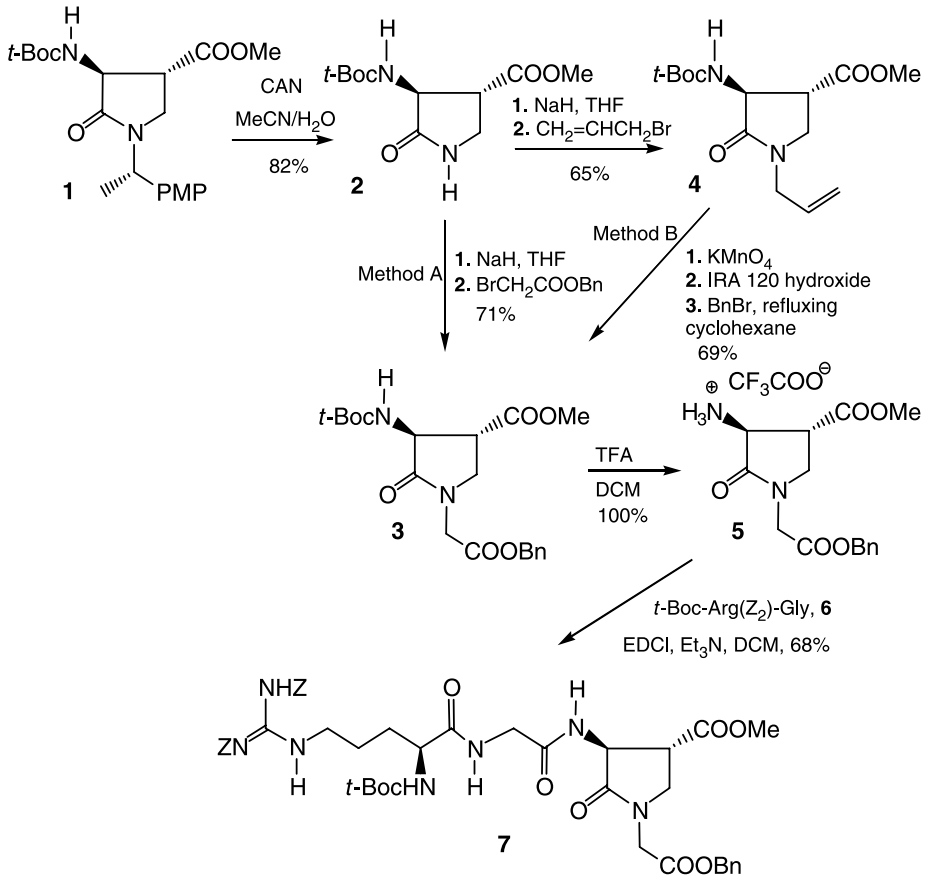
To a solution containing glycine methyl ester hydrochloride (0.25 g; 2.0 mmol) and N_α-*t*-Boc-N_δ,N_ω-di-Z-L-arginine (1.1 g, 2.0 mmol) in DCM (15 ml), EDCl (570 mg, 1.5 mmol) was added and the mixture was stirred for 2 h at rt. Then water (10 ml) was added and the mixture was extracted with EtOAc (3 × 50 ml). After drying (Na_2SO_4) and removal of the solvents under reduced pressure, the residue was purified by silica gel chromatography (EtOAc), to give *t*-Boc-Arg(Z_2)-Gly-OMe (**6**) (0.9 g; 71%) as a white foam. ^1H NMR (200 MHz, $CDCl_3$) δ 1.43 (s, 9H, *t*-Bu), 1.63–1.84 (m, 4H, Arg β- and γ-CH_2), 3.45 (dd, $J = 5.2$, $J = 18.0$ Hz, 1H, CH_2COOMe), 3.64 (s, 3H, OCH_3), 3.77 (dd, $J = 6.2$, $J = 18.0$ Hz, 1H, CH_2COOMe), 3.79–3.91 (m, 1H, Arg α-CH), 4.17–4.37 (m, 2H, Arg δ-CH_2), 5.12 (ABq, $J = 12.4$ Hz, 2H, OCH_2Ph), 5.24 (s, 2H, OCH_2Ph), 5.70 (d, $J = 8.4$, 1H, NH), 7.05 (m, 1H, NH), 7.15–7.43 (m, 10 ArH), 9.36 (br s, 1H, NH), 9.45 (br s, 1H, NH). ^{13}C NMR (50 MHz, $CDCl_3$) δ 24.3, 28.4, 29.2, 40.8, 44.1, 52.0, 53.2, 67.1, 69.0, 79.7, 128.1, 128.2, 128.3, 128.5, 128.6, 128.8, 134.6, 136.4, 155.4, 155.8, 160.9, 163.4, 169.8, 172.2. To a solution containing *t*-Boc-Arg(Z_2)-Gly-OMe (**6**) (0.63 g, 1.0 mmol) in MeOH (5 ml), 1 M NaOH (1.1 ml) was added, the mixture was stirred for 3 h at 0 °C and then extracted with AcOEt (2 × 5 ml). By addition of 0.5 M HCl the pH of the aqueous phase was adjusted to 1. After extraction with AcOEt (2 × 10 ml), drying (Na_2SO_4) and removal of the solvent, *t*-Boc-Arg(Z_2)-Gly **6** was obtained (0.61 g; quantitative yield) as a low melting solid and immediately used for the next reaction without further purification. ^1H NMR (200 MHz, CD_3OD) δ 1.48 (s, 9H, *t*-Bu), 1.58–1.81 (m, 4H, Arg β- and γ-CH_2), 3.21–3.34 (m, 2H, CH_2COOH), 3.73 (m, 1H, Arg α-CH), 3.79 (m, 2H, Arg δ-CH_2), 5.18 (br s, 4H, 2 × OCH_2Ph), 7.28–7.48 (m, 10 ArH).

*(3S,4R)-3-(t-Boc-L-(Z_2)-arginylglycylamino)-1-benzyloxycarbonylmethyl-4-methoxycarbonylpyrrolidin-2-one (**7**)*

To a solution containing compound **3** (0.4 g, 1.0 mmol) in dry DCM (5 ml), TFA (0.46 g, 4.0 mmol) was added and the mixture was stirred for 4 h at rt. After removal of volatiles under reduced pressure, the residue trifluoroacetate salt **5** was washed with ether (5 ml), dissolved in dry DCM (5 ml), containing Et_3N (0.11 g, 1.0 mmol) and the mixture was stirred at rt for 30 min. Then **6** (0.62 g, 1.0 mmol) was added to the clear solution, followed by EDCl (287 mg, 1.5 mmol) was added and the mixture was stirred at rt for 2 h. Then water (15 ml) was added and the mixture was extracted with EtOAc (3 × 20 ml). After drying (Na_2SO_4) and removal of the solvents under reduced pressure, the residue was purified by silica gel chromatography (EtOAc), to give **7** (0.6 g; 68%) as white amorphous solid. Mp 118–120 °C. ^1H NMR (200 MHz, $CDCl_3$) δ 1.38 (s, 9H, *t*-Bu), 1.45–1.75 (m, 4H, Arg β- and γ-CH_2), 3.08–3.26 (m, 2H, H-5β + H-4), 3.38–3.47 (m, 1H, H-5α), 3.54–3.63 (m, 2H, CH_2CONH), 3.69 (s, 3H, OCH_3), 3.87–3.92 (m, 2H, Arg δ-CH_2), 4.06 (ABq, $J = 17.7$ Hz, 2H, CH_2COOBn), 4.07–4.15 (m, 1H, Arg α-CH), 4.64 (dd, $J = 5.5$, $J = 8.4$ Hz, 1H, H-3), 5.10 (s, 2 × 2H, OCH_2Ph), 5.13 (s, 2H, OCH_2Ph), 5.91 (m, 1H, NH), 7.22–7.38 (m, 15 ArH + 1 NH), 7.77 (m, 1H, NH), 8.05 (d, $J = 6.1$ Hz, 1H, NH); ^{13}C NMR (200 MHz, $CDCl_3$) δ 14.1, 20.9, 24.6, 28.2, 29.3, 40.4, 42.6, 44.4, 46.7, 52.5, 53.9, 60.3, 67.3, 80.1, 127.9, 128.3, 128.4, 128.6, 134.9, 156.2, 167.8, 170.1, 171.1, 171.6, 173.3; $[\alpha]_D$ −17.4 (c 0.72, $CHCl_3$); ESI-MS: m/z 888.3 $[MH]^+$, 910.3 $[M + Na]^+$. Anal. calcd for $C_{44}H_{53}N_7O_{13}$: C, 59.52; H, 6.02; N, 11.04. Found: C, 59.45; H, 5.97; N, 10.96.

Results and discussion

Within a program aimed to obtaining mimetics of oligopeptides, analogues of natural amino acids having conformational constrictions were prepared by using asymmetric



Scheme 1

synthetic strategies (Galeazzi et al., 2005). Then, we considered the lactam **2**, an analogue of aspartic acid in which the conformational restriction arises from the presence of the lactam ring (Kottirsch et al., 1993; Kahn and Eguchi, 2004), as a useful scaffold for obtaining **3**, a constrained mimetic of DG (Asp-Gly) dipeptide (Scheme 1) (Ndungu et al., 2004). Compound **1**, recently prepared in our laboratory in the enantiomerically pure form (Galeazzi et al., 2005), underwent removal of the PMB (*p*-methoxybenzyl) group at the nitrogen atom by using CAN in MeCN–H_2O, to give in good yield the corresponding pyrrolidin-2-one **2**. This product was treated with NaH in THF at 0 °C and the anion was subsequently alkylated with benzyl bromoacetate (Scheme 1, Method A). The reaction proceeded in good yield to give **3** as the sole product, whose 3,4-*trans*-relationship is confirmed by the $J_{3,4}$ value (9.3 Hz). As an alternative pathway to **3**, the mimetic of dipeptide DG (Asp-Gly) in which all the functionalities are protected by orthogonal groups, the lactam **2** was converted into the corresponding 1-allyl derivative **4** in good yield by using allyl bromide as the electrophile. Subsequent reaction of **4** with a potassium permanganate solution (Armaroli et al., 2000) gave the acid that was supported without isolation on IRA 900 in the hydroxide form. This polymeric reagent was treated with benzyl bromide in refluxing cyclohexane and the product **3** was obtained in good yield after washing of the resin with methanol (Scheme 1, Method B).

The compound **3** is a versatile intermediate in that it could be selectively deprotected owing to the presence of orthogonal protecting groups, and we looked for an useful application of a such dipeptide analogue as a replacement for DG in a RGDG sequence (Koivunen et al., 1994, 1995; Humphries et al., 2000; Kao and Liu, 2001, 2002; Kim et al., 2004; Chen et al., 2005, 2006). Thus, with the aim to prepare a conformationally restricted RGDG tetrapeptide suitable to be introduced in place of natural sequences, the *t*-Boc group was removed by treatment with TFA in DCM, to give **5** in quantitative yield. Following the homogeneous phase peptide synthesis methodology, this product was condensed with *t*-Boc-Arg(Z_2)-Gly **6** by using EDCl in DCM, to give eventually **7**, having the full protected RGDG sequence with conformational restrictions (Scheme 1).

In summary, starting from the pyrrolidin-2-one **1**, the preparation of **3**, a novel mimetic of the dipeptide DG was described, useful for modifying peptide conformations, together with a convenient approach to the conformationally restricted RGDG mimetic **7** having all the functionalities protected. Both **3** and **7** could be easily incorporated into more complex structures displaying significant biological activity and work aimed to this goal is currently underway in our laboratory.

Acknowledgements

We wish to acknowledge M.U.R. (Roma, Italy) for funding this research within the framework PRIN 2004, and Dr. Emanuela Crucianelli for technical support.

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