Solid Phase Synthesis of Conformationally Constrained Peptidomimetics Based on a 3,6-Disubstituted-1,4-diazepan-2,5-dione Core.

Lucia Raffaella Lampariello[#], Daniela Piras,[§] Manuela Rodriquez and Maurizio Taddei*

Dipartimento di Chimica, Università degli Studi di Sassari, Via Vienna 2, I-07100 Sassari (Italy) [§], Dipartimento di Chimica[#] and Dipartimento Farmaco Chimico Tecnologico, Università degli Studi di Siena, Via A. Moro, 53100 Siena Italy

taddei.m@unisi.it

Supplementary material

Procedure for

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1-Benzyloxy, 3-Benzyl, 6-(*tert*-Butoxycarbonylamino), 1,4-diazepan, 2,5-dione 3. A solution of compound **2** (0.5 g, 1.09 mmol) was dissolved DMF (3 mL) together with DIAD (0.28 g, 1.4 mmol) and PPh_3 (0.42 g, 1.5 mmol) in a pressure tube sealed with a teflon cup. The tube was inserted inside a microwave cavity and irradiated at 100 W for 5 min. The inside temperature rised to 210°C. The tube was cooled to room temperature and submitted to a second cycle of 5 min at 100 W. The solution was cooled to room temperature and dissolved into ethyl acetate (25 mL). The solution was washed with a saturated solution of NH_4Cl, separed and dried over anhydrous Na_2SO_4. The solvent was evaporated and the residue taken up in dry diethyl ether (50 mL). The solid formed was filtered away, the solvent evaporated and the crude purified by column chromatography on silica gel (eluent EtAc). Obtained 0.36 g of compound **3** (75 % yield). The analytical sample was submitted to an additional lyophilization in order to obtain correct microanalysis. M.p. 114-116°C. ^{1}H NMR ($CDCl_3$, 300 MHz) 1.43 (s, 9H) 2.96 (AB part of an ABX system, 2H, J = 12, 7, 7 Hz), 4.01 (m, 1H), 4.23 (AB part of an ABX system, 2H, J = 12, 7, 4 Hz), 4.61 (m, 1H), 5.30 (AB system, 2H, J =10 Hz), 6.2 (bs, 1H), 7.20- 7.60 (m, 10 H), 7.90 (bs, 1H). ^{13}H NMR ($CDCl_3$, 75 MHz) 28.2 (3C), 46.7, 54.8, 59.3, 61.0, 67.3, 80.3, 128.1 (2C), 128. 8(4C), 128.9 (4C), 134.8, 139.6, 155.1, 164.3, 166.1. ES-MS 440.51 (M^++H). Anal. Calcd. for $C_{24}H_{29}N_3O_5$: C, 65.59; H,6.65; N, 9.56. Found: C, 65.43; H,6.67; N, 9.60.

Solid phase synthesis. Compound 8. N-Fmoc-hydroxylamine 2-chlorotrityl resin (0.5 g of a 0.7 mmol/g loaded resin, 0.35 mmol) was swollen in dry CH_2Cl_2 for 4 h and washed with 5 portions of CH_2Cl_2. Piperidine in DMF (25%) was added and the mixture stirred for 1 h. The solution was filtered away and replaced with a fresh solution. After 1 h the solution was eliminated and the resin washed several times with DMF and CH_2Cl_2. NFmoc-PheOH (0.27 g, 0.7 mmol) was dissolved in 3 mL of NMP and this solution added to the resin followed by DMTMM (0.193 g, 0.7 mmol) and DIPEA (0.5 mL). The mixture was stirred for 2 h at room temperature. After washing the resin with NMP and DMF, a sample of the resin was deprotected and submitted to the Kaiser test[1] to control the effective coupling. In the case of a positive test, the resin was disperded in piperidine DMF (25%) and stirred for 2 h. After a positive Kaiser test, NFmoc-Ser-OH (0.229 g, 0.7 mmol) dissolved in NMP (3 mL) was added followed by DMTMM (0.193 g, 0.7 mmol) and DIPEA (0.5 mL). The mixture was stirred for 2 h at room temperature. After washing the resin with NMP and DMF a sample of the resin was submitted to the Kaiser test that resulted negative and to the TCT-alizarine test[2] that resulted positive. The resin was washed several times with NMP, DMF, CH_2Cl_2 and DMF and transferred in a tube. DIAD (0.14 g, 0.7 mmol) and PPh_3 (0.42 g, 1.5 mmol) were added and the tube sealed with a teflon cup and inserted in a microwave cavity. The tube was irradiated at 60 W for 2 min. The inside temperature rised to 210°C. The tube was cooled to room temperature and submitted to two additional cycles of 2 min. each at 60 W followed by cooling down. A sample of the resin was submitted to the TCT-alizarine test that resulted negative. The resin was washed several times with DMF and CH_2Cl_2. The Fmoc group was removed with piperidine in DMF (25%) and the mixture treated with Ac_2O (0.5 mL) in CH_2Cl_2 (3 mL) and DIPEA (0.5 mL). When the Kaiser test was negative, the resin was washed several times with CH_2Cl_2, DMF, DMF-MeOH 1 : 1 and CH_2Cl_2. After drying the resin under vacuum, it was treated with 4 mL of a solution of TFA and TFE in CH_2Cl_2 (10 : 40 : 40) for 6 h. The solution was recovered by filtration and the

resin residue washed several times with the cleavage mixture. The solvent was evaporated and the residue treated several times with hexanes that was evaporated to eliminate completely the TFA to give product **8** (67 mg, 66% yield), The analytical sample was obtained after absorption of **8** on a SPE column (poymerically bounded amine) followed by extraction with MeOH / H_20 / TFA 10 / 5 / 0.5 and liophylisation. The analytical sample was submitted to an additional lyophilization in order to obtain correct microanalysis. ^{1}H NMR ($CDCl_3$, 300 MHz) 2.23 (s, 3H) 3.06 (AB part of an ABX system, 2H, J = 12, 7, 7 Hz), 4.11 (m, 1H), 4.28 (AB part of an ABX system, 2H, J = 12, 7, 7 Hz), 4.71 (m, 1H), 7.30 (m, 5 H), 8.90 (bs, 3H). ^{13}H NMR ($CDCl_3$, 75 MHz) 22.7, 48.6, 53.4, 58.9, 60.1, 66.7, 128.1, 128. 8(2C), 139.6, 164.3, 166.1, 169.7. ES-MS 292.43 (M^++H). Anal. Calcd. for $C_{14}H_{17}N_3O_4$: C, 57.72; H,5.88; N, 14.42. Found: C, 57.69; H,5.91; N, 14.46.

Compound **13** was prepared following the above procedure and finally purified by HPLC (C18 Discovery BIO Wide Pore, 15 cm x 4.6mm, 5 mm, 80 : 20 H20 (0.1 % TFA) / MeCN (0.1 % TFA) 17.5 min. ^{1}H NMR (d_6-dmso, 300 MHz) 2.00 (s, 3H), 2.60-3.10 (m, 8H), 3.35 (s, 3H), 3.80 (m, 2H), 4.00 (m, 1H + 1H), 4.20 (m, 2H) 4.80(AB part of an ABX system, 2H, J = 10, 7, 7 Hz), 4.91 (m, 1H), 8.6 (bs, 3H) 9.10 (bs, 1H). ^{13}H NMR ($CDCl_3$, 75 MHz) 22.9, 25.6, 27.8, 29.9, 32.3, 44.3, 47.2, 48.6, 56.4, 58.9, 64.1, 68.7, 160.3, 163.3, 167.7, 169.1, 169.7, 178.2. ES-MS 443.52 (M^++H). Anal. Calcd. for $C_{18}H_{27}N_5O_8$: C, 48.97; H,6.16; N, 15.86. Found: C, 48.90, H,6.17; N, 15.82

Cleavage of resin 12 with SmI_2. Resin **12** (250 mg of the resin with approximatively 0.7 mmol/g loading, 0.175 mmol) was swollen in THF and SmI_2 (7 mL of a blue 0.1 M solution in THF, 0.7 mmol) was added. The mixture stirred at room temperature for 8 h. The resin was filtered off and washed several times with THF and CH_2Cl_2. The solutions were collected and the solvent evaporated. The residue was dissolved in CH_2Cl_2 (10 mL) and mixed with HCl 1 N (1 mL) and a solution of sodium thiosulphate. The mixture was vigorously stirred until it became colorless. The organic layer was separated, washed with brine and dried over Na_2SO_4. The residue was dissolved in CH_2Cl_2 and passed through a silica packed SPE column eluting with CH_2Cl_2. The fractions were collected and evaporated to give **14** (43 mg, 51% yield). An analytical sample was purified by HPLC (C18 Discovery BIO Wide Pore, 15 cm x 4.6mm, 5 mm, 80 : 20 H20 (0.1 % TFA) / MeCN (0.1 % TFA) 12.5 min. The product was submitted to an additional lyophilization in order to obtain correct microanalysis (^{1}H NMR ($CDCl_3$, 300 MHz) 2.02 (s, 3H), 2.60-3.10 (m, 8H), 3.45 (s, 3H), 3.90 (m, 2H), 4.20 (m, 1H + 1H), 4.29 (m, 2H) 4.44 (AB part of an ABX system, 2H, J = 10, 7, 7 Hz), 4.61 (m, 1H), 8.6 (bs, 4H). ^{13}H NMR ($CDCl_3$, 75 MHz) 22.7, 26.6, 28.70, 29.7, 30.2, 44.3, 46.2, 48.6, 55.9, 58.9, 60.1, 66.7, 162.3, 164.3, 165.7, 166.1, 169.7, 174.2. ES-MS 426.52 (M^++H). Anal. Calcd. for $C_{18}H_{27}N_5O_7$: C, 50.82; H,6.40; N, 16.46. Found: C, 50.84, H,6.42; N, 16.43.

[1] Novabiochem catalogue, 2002/2003, Synthesis Notes, White, P:, Dörner, B; Steinauer eds., page 3.4
[2] Novabiochem catalogue, 2002/2003, Synthesis Notes, White, P:, Dörner, B; Steinauer eds., page 2.37