

Synthesis of a [1,4]dioxane-2,5-dione based-peptidomimetic scaffold

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Dedicated to Jim Coxon on the occasion of his 65th birthday

Abstract

(2*S*,3*S*)-3-[*N*-Cbz-L-Leucyl]amino-2-hydroxy-4-phenylbutanoic acid **5** dimerised on treatment with EDCI and HOBT to give the symmetrical [1,4]dioxane-2,5-dione-based peptidomimetic **6**.

Keywords: Peptidomimetic scaffold, synthesis, dimer, cyclisation

Introduction

An endless array of organic compounds exist that exhibit an equally endless array of biological activities. These compounds function by simply presenting a well-defined topology to a complementary receptor or enzyme in spite of a diversity of molecular structure and biological mechanism of action. It therefore follows that new classes of bioactives would result from simply generating new molecular topologies. One way to achieve this objective is to tether known bioactive molecules onto an appropriate molecular scaffold to generate a new, overall molecular topology. The choice of scaffold needs to allow the ready chemical attachment of a range of simple molecules that possess a diversity of structure and function, perhaps in a combinatorial fashion. The result of such a union is a compound whose overall topology is controlled by the nature and combination of its constituents.

This idea has been used to generate important bioactives that contain a core scaffold and a range of attached ligands that can number anywhere from a few (e.g. protease inhibitors that possess a core isosteric unit to which are attached enzyme binding domains)¹ to many (e.g. a dendrimer).² Such hybrid compounds provide leads for important biological probes and potential therapeutics with improved potency, stability and in many cases, vastly different biological properties and mechanisms of action relative to their separate components. There is, therefore, a clear need for new and general molecular scaffolds. In this paper we present a simple example of this idea that allows a peptide, or conceivably another suitable functionalized molecule, to be

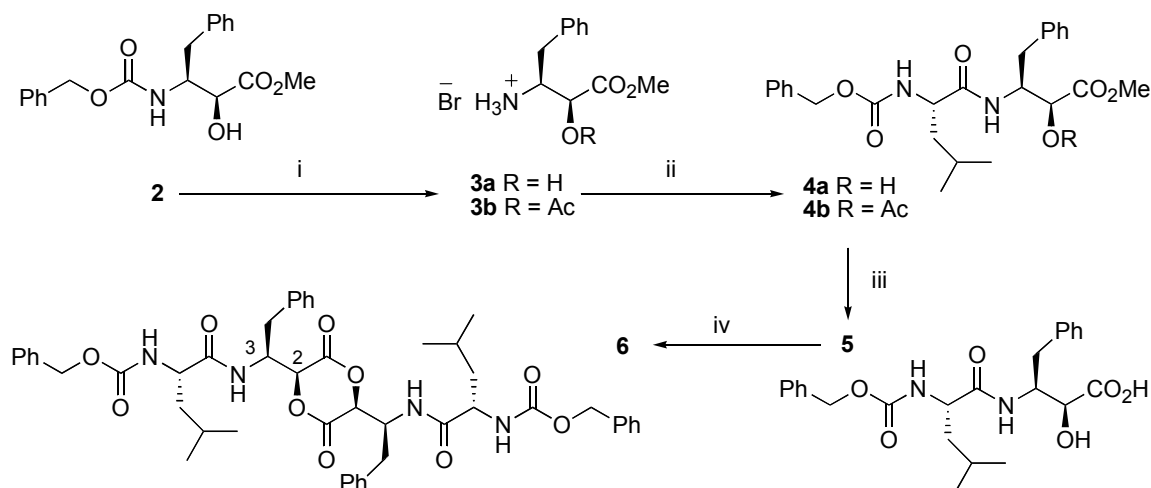
attached at two sites on a heterocyclic scaffold (see compound **6**). The compound presented here is simple to prepare as a crystalline solid, and demonstrates this principle. Closely related compounds have been reported as inhibitors of Calpains,³ an over activity of which is associated with a number of important human diseases.⁴

Results and Discussion

The optically active peptidomimetic scaffold **6** was prepared as detailed in the Scheme below. In the key step, a solution of (2*S*,3*S*)-3-[*N*-Cbz-L-leucinyl]amino-2-hydroxy-4-phenylbutanoic acid **5** in DMF was reacted with a solution of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDCI) and hydroxybenztriazole (HOBT) in dichloromethane containing diisopropylethylamine (DIEA) to give **6** as a stable colorless solid in 74% yield. The starting material **5** was prepared in three steps as shown in the Scheme. The known⁵ α -hydroxy β -amino acid **2** was *N*-deprotected with HBr in acetic acid to give a mixture of **3a** and **3b** (9:1), which was subsequently coupled with *N*-Cbz-L-leucine under standard EDCI conditions to give **4a** and **4b** (9:1). This mixture was finally hydrolysed to give the desired derivative **5** (see step iii) in 69% over two steps. It is interesting to note that the ratio of **3a** to **3b** obtained in step i was dependent upon the concentration of **2** used in the reaction. Product ratios of 4:1, 9:1, 12:1 and 19:1 were obtained for concentrations of 0.26, 0.97, 1.47 and 2.00 M, respectively.

The dimeric nature of **6** was confirmed by a combination of ¹H NMR, ¹³C NMR and mass spectrometry. The two-fold axis of symmetry in the dimer **6** was confirmed by the presence of only twenty peaks in the ¹³C NMR spectrum while high resolution mass spectral data was consistent with the dimeric structural formula [C₄₈H₅₇O₁₀N₄ (M+1)⁺]. In addition, the ¹H NMR of **6** revealed a resonance at 5.49 ppm that was shown to correspond with H2 by a 2D NMR COSY experiment - a key correlation was observed in the COSY experiment between H3 (4.66 ppm) and H2 (5.49 ppm). Importantly, the chemical shift of H2 is consistent with O-acylation⁶ where a similar chemical shift was observed for H2 in **4b** (5.53 ppm), whereas H2 in the corresponding alcohol **4a** resonated further upfield at 4.29 ppm.

In this paper we have presented the synthesis of an interesting and new class of peptidomimetic molecular scaffold whose architecture should be amenable to attaching a wide range of amino acid R groups and thus the presentation of a diverse range of topologies.



Scheme 1. Reagents and conditions: (i) 33% HBr in AcOH, 20 min, 9 (**3a**):1 (**3b**); (ii) *N*-Cbz-*L*-Leu, EDCI, HOBT, DIEA, DMF, DCM, 16 h, 9 (**4a**): 1 (**4b**); (iii) LiOH, MeOH, H₂O, 4h; (iv) EDCO, HOBT, DIEA, DMF, DCM < 16 h, 74%.

Experimental Section

General Procedures. ¹H NMR spectra were obtained on a Varian Inova spectrometer, operating at 500 MHz. Coupling constants (*J*) are quoted in Hz. ¹³C NMR spectra were obtained on a Varian Unity 300 spectrometer, operating at 75 MHz, with a delay (*D*₁) of 1 s. Electron impact (EI) mass spectra were detected on a Kratos MS80 RFA mass spectrometer operating at 4000 V (accelerating potential) and 70 eV (ionisation energy) using a source temperature of 200-250°C.

(2*S*,3*S*)-3-[*N*-Cbz-*L*-Leucynyl]amino-2-hydroxybenzenebutanoic acid (5**).** Compound **2**⁵ (500 mg, 1.47 mmol) was dissolved in a solution of HBr in acetic acid (33%, 1.0 ml) and the resulting mixture was stirred at rt for 20 min. The addition of ether (2 mL) caused the amine salt to precipitate. The solvent was drawn off and the resulting residue retained. To the ethereal solution was added petroleum ether (4 mL) to give a further precipitate. The solvent was drawn off and the two batches of solid were combined and dried to give a mixture (397 mg, 12:1 by ¹H NMR) of the amine salts **3a** (86%) and **3b** (6%) as a brown tar. Selected data from the mixture: ¹H NMR **3a** (D₂O) δ 7.19-7.31 (5H, m, ArH), 4.51 (1H, d, *J* = 2.5 Hz, CHCHOH), 4.03 (1H, m, CHCHOH), 3.38 (3H, s, CH₃), 2.89 (2H, m, CH₂Ph). ¹H NMR **3b** (D₂O) δ 7.19-7.31 (5H, m, ArH), 5.41 (1H, d, *J* = 1.7 Hz, CHCHOAc), 4.25 (1H, m, CHCHOAc), 3.41 (3H, s, COOCH₃), 3.00 (2H, m, CH₂Ph), 2.18 (3H, s, OCOCH₃).

To a mixture of **3a** and **3b** (64 mg, 9:1 by ¹H NMR, 0.22 mmol) were added EDCI (47 mg, 0.25 mmol) and HOBT (32 mg, 0.24 mmol). A solution (1.0 M) of *N*-Cbz-*L*-leucine in *N*-methylpyrrolidinone (240 μL, 0.24 mmol), DMF (250 μL) and DIEA (43 μL, 0.25 mmol) were then added and the reaction mixture was stirred overnight at rt under nitrogen. The reaction

mixture was diluted with ethyl acetate (4 mL) and washed with 10% aqueous HCl (3 x 2 mL), saturated NaHCO₃ (3 x 2 mL), brine (2 mL), dried over MgSO₄ and concentrated *in vacuo* to give a mixture (9:1 by ¹H NMR) of **4a** and **4b** (69 mg, 69%) as a white solid. Data from the mixture: [MS (FAB): 499 [(M+1)⁺ **4b**], 457 [(M+1)⁺ **4a**], 414, 307, 210, 154, 136]; mp 138-144 °C; IR (CHCl₃) 3688, 3416, 2961, 1734, 1676, 1601, 1500, 1440 cm⁻¹. ¹H NMR **4a** (CDCl₃) δ 7.14-7.35 (10H, m, ArH), 6.51 (1H, d, J = 8.8 Hz, PheNH), 5.09 (3H, m, CbzCH₂ & CbzNH), 4.51 (1H, ddd, J = 2.9, 7.3, 16.1 Hz, CHCHOH), 4.29 (1H, br s, CHCHOH), 4.11 (1H, m, NHCHCO), 3.59 (3H, s, OCH₃), 2.79 (2H, m, PheCH₂), 1.61 (3H, m, LeuCH & LeuCH_A), 1.39 (1H, m, LeuCH_B), 0.89 (6H, m, 2 x LeuCH₃); ¹³C NMR **4a** (CDCl₃) δ 172.8 (COOMe), 172.0 (NHCHCO), 156.2 (CbzCO), 136.7 (PheArC), 136.0 (CbzArC), 129.3, 128.5, 128.3, 128.3, 128.1, 126.7 (ArCH), 72.0 (CHOH), 67.2 (CbzCH₂), 53.7 (NHCHCO), 53.0 (CHCHOH), 52.5 (COOCH₃), 41.0 (LeuCH₂), 35.4 (PheCH₂), 24.6 (LeuCH), 22.8 (Leu(CH₃)_A), 21.9 (Leu(CH₃)_B). ¹H NMR **4b** (CDCl₃) δ 6.42 (1H, m, PheNH), 5.53 (1H, s, CHOAc), 3.63 (3H, s, COOCH₃), 2.16 (3H, s, OCOCH₃).

To the mixture of **4a** and **4b** (60 mg, 0.13 mmol, 9:1 by ¹H NMR) at 0 °C was added a solution of 0.25 M lithium hydroxide (1.5 parts) in 2:1 methanol/water. After the reaction mixture was stirred for 0.5-4 h, sufficient 1N aqueous HCl was added to acidify the solution (pH 5 by Universal Indicator) and the methanol was evaporated *in vacuo*. Water was added to the residue and the organics were extracted with ethyl acetate. The combined organic extracts were washed with water, brine, dried over MgSO₄ and concentrated *in vacuo* to the acid **5** (58 mg, qu) as a white solid. ¹H NMR (CD₃OD) δ 7.82 (1H, d, J = 9.3 Hz, CbzNH), 7.33 (5H, m, ArH), 7.10-7.19 (5H, m, ArH), 5.07 (2H, m, CbzCH₂), 4.49 (1H, m, CHCHOH), 4.17 (1H, d, J = 4.4 Hz, CHCHOH), 4.07 (1H, m, NHCHCO), 2.80 (2H, m, PheCH₂), 1.58 (1H, m, LeuCH), 1.33 (2H, m, LeuCH₂), 0.88 (6H, m, 2 x LeuCH₃); ¹³C NMR (CD₃OD) δ 175.8 & 175.2 (CHCONH & COOH), 158.6 (CbzCO), 139.6 (PheArC), 138.4 (CbzArC), 130.7, 129.8, 129.6, 129.3, 129.2, 127.6 (ArCH), 73.9 (CHOH), 68.0 (CbzCH₂), 55.4 (NHCHCO), 54.8 (CHCHOH), 42.5 (LeuCH₂), 36.4 (PheCH₂), 26.1 (LeuCH), 23.6 (Leu(CH₃)_A), 22.2 (Leu(CH₃)_B); [MS (FAB): 465 (M+Na)⁺, 443, 399, 286, 176, 153, 136; HRMS (FAB) calcd for C₂₄H₃₀O₆N₂Na (M+Na)⁺ 465.2002, found 465.1996]; mp 131-134 °C; IR (CHCl₃) 3433, 2963, 1720, 1660, 1510, 1456, 1340, 1217 cm⁻¹.

Dimer 6. To a solution of **5** (14 mg, 32 μmol) in DMF (1 mL) was added EDCI (8 mg, 41 μmol), HOBT (7 mg, 47 μmol), dichloromethane (1 mL) and DIEA (6 μL, 35 μmol). The reaction mixture was stirred overnight at rt before being diluted with dichloromethane (10 mL), washed with 10% aqueous HCl (10 mL), brine (10 mL), dried over MgSO₄, and concentrated *in vacuo* to afford **6** (10 mg, 74%) as a white solid. ¹H NMR (300Mz, DMSO) δ 8.28 (2H, d, J = 8.2 Hz, PheNH), 7.16-7.35 (22H, m, arom & CbzNH), 5.49 (2H, brs, H₂), 5.01 (4H, s, CbzCH₂), 4.66 (2H, m, H₃), 4.05 (2H, m, Leu-αH), 2.81 (4H, m, PheCH₂), 1.49 (2H, m, LeuCH), 1.36 - 1.27 (4H, m, LeuCH₂), 0.81 (12H, m, LeuCH₃); ¹³C NMR (DMSO) δ 172.1 (ring CO), 165.7 (LeuCO), 155.4 (CbzCO), 137.6, 136.7, 128.7, 128.1, 128.0, 127.6, 127.5, 126.1, 77.6 (C₂), 65.2 (CbzCH₂), 52.8 (Leu-αC), 49.9 (C₃), 40.7 (LeuCH₂), 33.8 (PheCH₂), 23.9 (LeuCH), 22.6

(LeuCH₃), 21.3 (LeuCH₃); [MS (ES): 871 (M+Na)⁺, 849 (M+1)⁺, 647, 556, 537, 464, 316, 289, 275, 249; HRMS (ES) calcd for C₄₈H₅₇O₁₀N₄ (M+1)⁺ 849.408, found 849.405]; R_F 0.19 (1:4 ethyl acetate-dichloromethane); IR (CHCl₃) 3628, 3354, 1767, 1722, 1683, 1228 cm⁻¹; [α]_D - 78 ° (c 0.082 acetonitrile).

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