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'Bis-ornithine' (2,2-bis(aminopropyl)glycine): a new tetravalent template for assembling different functional peptides

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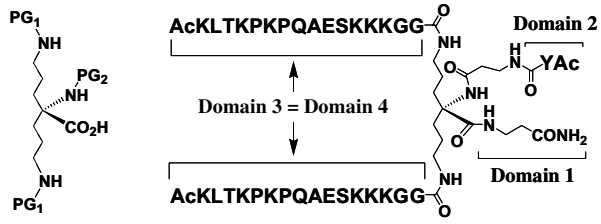
Abstract—Synthesis of bis-ornithine, a new $C^{\alpha,\alpha}$-disubstituted α-amino acid bearing orthogonally protected α and δ amine groups is reported. Bis-ornithine (bisOrn) and dipeptides containing bis-ornithine have been synthesized in solution up to multigram scale. As a first example, one of these compounds Boc-βAla-bisOrn(Alloc)$_2$-OH has been attached to a solid support and used as template for the synthesis of a symmetrical assemblage of peptides.

1. Introduction

Proteins are composed of sub-domains that may be involved in distinct functions such as catalytic transformation, cell regulation and/or intracellular trafficking. Some may also serve as three-dimensional scaffold to support other domains. The identification of the domains and their associated functions has been mainly based on molecular dissections and mutagenesis experiments. The activities detected with some protein fragments have demonstrated that (i) the full sequence of the parent protein is not a prerequisite and (ii) some domains are not pre-folded prior to their association with their ligand. An assemblage of peptides (AP) into a minimal structure that displays—like proteins—multiple functions represents a challenging target for chemists.

The initial step of our approach to build peptide assemblages rests on the design of a small highly functionalized template where the spatial distribution does not prevent the freedom of each domain. The tetravalent template 'bis-ornithine' or 2,2-bis(aminopropyl)-glycine was selected on the basis of the conformational properties of 2,2-bis(*n*-propyl)glycine,[1] where the alkyl side chains and the backbone adopt a fully extended conformation. The introduction of two orthogonal protections on the α and δ amino groups of bis-ornithine leads to the construction of APs, with a C_2 symmetry (Scheme 1). Indeed, domains 3 and 4, which are linked to the δ amino groups will contain the same peptidic sequence



Bis-ornithine (bisOrn) **Target assemblage of peptides (AP)**

Scheme 1.

Keywords: Peptide template; α,α-Disubstituted α-amino acid.
Abbreviations: Ac, acetyl; Alloc, allyloxycarbonyl; bisOrn, bis-ornithine; Boc, *tert*-butyloxycarbonyl; Cbz, benzyloxycarbonyl; 2-BrCbz, 2-bromobenzyloxycarbonyl; 2-ClCbz, 2-chloro benzyloxycarbonyl; DIEA, diisopropylethylamine; DMAP, *N*,*N*-dimethylaminopyridine; DMF, *N*,*N*-dimethylformamide; Fmoc, fluorenylmethyloxycarbonyl; HBTU, *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate-*N*-oxide; HOAt, 1-hydroxy-7-azabenzotriazole; HPLC, high performance liquid chromatography; IBCF, isobutylchloroformate; MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight; MBHA-PS, 4-methylbenzhydrylamine polystyrene; NMM, *N*-methylmorpholine; NMR, nuclear magnetic resonance; PG, protecting group; PyAOP, 7-azabenzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate; PyBOP, benzotriazol-1-yloxytris(pyrrolidino)phosphonium hexafluorophosphate; Tfa, trifluoroacetamide; TFFH, tetramethylfluoroformamidinium hexafluorophosphate; THF, tetrahydrofuran; TLC, thin layer chromatography.

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and a total of three different sequences can be incorporated in the AP. This template will find interesting applications, for example, for ligand dimerization, with domains 1 and 2 remaining available for the introduction of a reporter group (fluorophore or radioisotope) and/or an affinity tag (biotin or poly-histidine).

In the present work, we report an efficient synthesis of the achiral quaternary amino acid, bis-ornithine, which can be achieved on a multigram scale. The preparation in solution of various dipeptides containing bis-ornithine or precursors with protecting groups that are compatible with solid-phase peptide synthesis is also presented. As a proof of concept, one of these dipeptides has been used in the elaboration of an AP by stepwise solid-phase synthesis. The target AP contains a peptidic ligand in domains 3 and 4, a tyrosine for radioactive labeling in domain 2 and a β-alanine in domain 1 (Scheme 1).

The choice of the strategy for the synthesis of bis-ornithine was dictated by the reactivity of the different sterically hindered intermediates. The two-step Bucherer–Bergs route[2] leading to the bulky hydantoin was therefore a priori discarded. As Fu et al. have reported the reduction of various $C^{\alpha,\alpha}$-disubstituted α-nitro esters,[3] reduction of *tert*-butyl 2,2-bis(cyanoethyl)-2-nitroacetate to give bis-ornithine was first attempted. But, both nitrile groups were efficiently reduced, whereas the nitro group was nearly unaffected, whatever the conditions used. We next planed to use a masked amino group and start the synthesis with an aldimine glycine ester, as described by O'Donnell for alkylations or Michael condensations under solid–liquid phase transfer catalysis.[4]

The synthesis of the starting compound **1** (Scheme 2) was easily achieved by a double Michael addition of acrylonitrile on methyl *N*-parachlorobenzylidene glycinate.[5] The methyl bis-(cyanoethyl)glycinate **2** was readily obtained on 0.5 mol scale after aldimine hydrolysis.[6] Then, this precursor was transformed into the orthogonally protected bis-ornithine derivative **A**, or into dipeptides **B** and **C** containing bis(cyanoethyl)glycine or bis-ornithine, respectively.

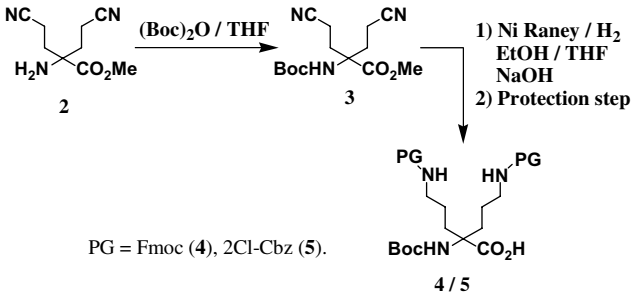
2. Synthesis of 2,2-bis(aminopropyl)glycine, bis-ornithine A

The Boc protecting group was introduced on compound **2** by reacting $(Boc)_2O$ in THF (Scheme 3).[7]

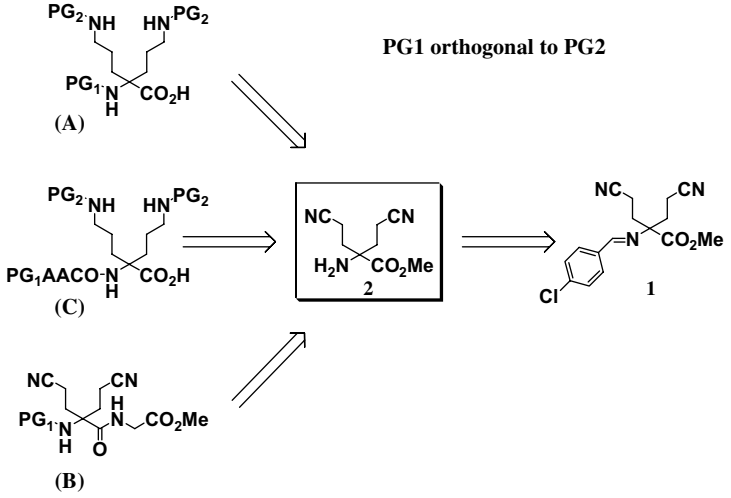
The methyl ester of **3** was saponified and the nitrile groups cleanly reduced in a one-pot procedure.[8] Finally, the δ-amino groups were protected by a Fmoc or 2-chlorobenzyloxycarbonyl (2-ClCbz) group, leading to the protected bis-ornithine derivatives **4** and **5**, respectively.

3. Synthesis of N^{α}-protected-bis(cyanoethyl)glycine and dipeptidic derivative B

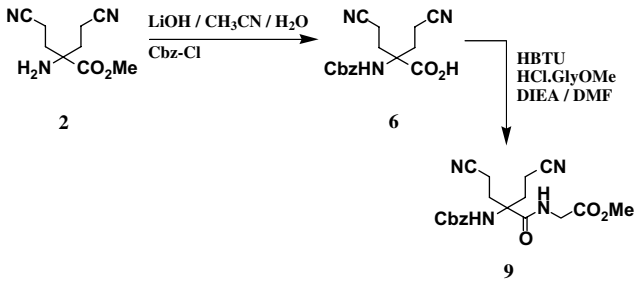
The methyl bis(cyanoethyl)glycinate **2** was submitted to ester hydrolysis by LiOH (Scheme 4). In the same step, the amine was protected by benzyl- or fluorenylchloroformate to give products **6** (N^{α}-Cbz) or **7** (N^{α}-Fmoc)[9] or by $(Boc)_2O$ in the presence of hydroxylamine[10] to give compound **8** (N^{α}-Boc). Noteworthy, compounds **7** and **8** may be directly used as building blocks in solid-phase



Scheme 3.



Scheme 2.



Scheme 4.

peptide synthesis (Fmoc or Boc strategy) with the nitrile functions as precursors of amino groups.

The amino and carboxylic groups of $C^{\alpha,\alpha}$-disubstituted α-amino acids are known to be poorly reactive for amino acids coupling.[11] To check the reactivity of the carboxylic group of bis(cyanoethyl)glycine derivatives, compound **6** was activated by HBTU/DIEA and coupled in solution to glycine methyl ester (Scheme 4). This procedure led to dipeptide **9** in good yields (80%).[12]

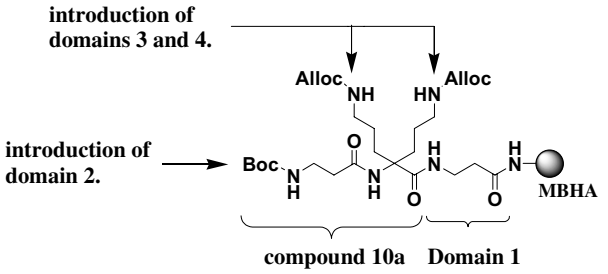
4. Synthesis of dipeptides containing bis-ornithine C

In another series of experiments, we checked the reactivity of the amine function of bis(cyanoethyl)glycine and synthesized in solution various dipeptides. Compound **2** was reacted with the mixed anhydride of Boc- or Cbz-protected amino acids obtained by treatment at 0 °C with isobutyl chloroformate (Scheme 5). Three dipeptides containing either β-alanine (**10**), glycine (**11**) or alanine (**12**) were prepared in good yields using this procedure (60–65%).[13] The nitrile functions were then reduced by H_2/Raney-Ni under basic conditions to give the products with the free amine and carboxylic groups.[8] The δ-amino groups were subsequently protected either by Alloc, 2-ClCbz, Fmoc, Tfa or Boc group, suitable for solid-phase peptide synthesis.

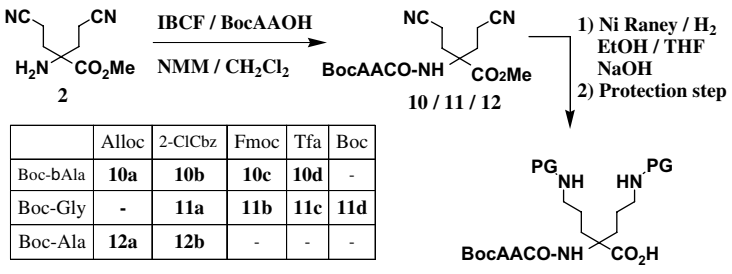
5. Synthesis of an assemblage of peptides AP by solid-phase strategy

As mentioned before, acylation onto the N-terminus of $C^{\alpha,\alpha}$-disubstituted α-amino acids during solid-phase peptide synthesis is known to be very difficult, because of the sterical hindrance of carbon α.[11] Synthesis in solution of the dipeptides containing bis-ornithine was found here to be quite efficient. However complete coupling reactions are required for solid-phase synthesis. For the synthesis of the APs on solid support, we thus decided to directly incorporate a dipeptidic unit (Boc-AA-bisOrn$(PG)_2$-OH) into the elongating peptide to avoid aborted sequences. First, the coupling conditions were optimized by synthesizing on solid-phase model polymers containing a central dipeptidic unit **10a**–**12b**. We compared the activation of the dipeptides by HBTU, TFFH, PyBOP, HATU and PyAOP reagents. In each case, the subsequent activated intermediate was reacted for 12 h at 50 °C with the elongating polymer and double or triple couplings were performed. The best result was obtained using an equimolar mixture of PyAOP and HOAt for activation. In this case no aborted sequence was detected after cleavage from the solid support.

The synthesis of the target assemblage of peptides was started by manual coupling of Boc-β-alanine on a MBHA-PS resin (domain 1, Scheme 6). Dipeptide Boc-βAla-bisOrn$(Alloc)_2$-OH **10a** activated by PyAOP/HOAt was then coupled[14] for 12 h at 50 °C (double coupling). After Boc elimination, tyrosine was classically incorporated (Boc-Tyr(2-BrCbz)-OH/HBTU/DIEA) followed by acetylation to give domain 2. The Alloc protecting groups of bis-ornithine side chains were removed.[15] Two glycines serving as spacer and the C-terminal lysine of the selected peptidic sequence constituting domains 3 and 4 (AcKLTKPKPQAESKKK) were incorporated manually to allow a monitoring of the reactions (Kaiser test). Efficient couplings for these residues were obtained using a standard protocol (HBTU/DIEA amino-acid activation). Finally, the rest of the peptide sequence was assembled by stepwise solid-phase synthesis on an ABI 433A peptide synthesizer.



Scheme 6.



	Alloc	2-ClCbz	Fmoc	Tfa	Boc
Boc-bAla	**10a**	**10b**	**10c**	**10d**	-
Boc-Gly	-	**11a**	**11b**	**11c**	**11d**
Boc-Ala	**12a**	**12b**	-	-	-

Scheme 5.

The designed AP (Scheme 1) was cleaved from the resin by treatment with anhydrous HF, purified by HPLC and characterized by MALDI-TOF mass spectrometry.

In conclusion, we have developed a straightforward strategy to prepare the acyclic α-quaternary α-amino acid bis-ornithine. We have demonstrated that dipeptides containing bis(cyanoethyl)glycine can be synthesized in solution by coupling an amino acid on its α-carboxylic or amino group. From these compounds, bis-ornithine containing dipeptides can be obtained as crystalline products in good yields up to the multigram scale. The incorporation of these dipeptides is achievable by solid-phase strategy, leading to new tetravalent templates for the synthesis of peptide assemblages. Various applications in peptide/protein interactions are now under investigation. In addition, linear polymers containing bis-ornithine derivatives have already been employed for drug delivery in culture cells.[14]

Acknowledgements

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6. To a solution of glycine methyl ester chlorhydrate (31 g, 250 mmol) in dichloromethane (370 mL) were added at room temperature $MgSO_4$ (60 g, 500 mmol), triethylamine (42 mL, 300 mmol) and 4-chlorobenzaldehyde (38.5 g, 275 mmol). The resulting mixture was stirred overnight. Then, $MgSO_4$ was filtered out, the organic layer washed with brine (2×150 mL), dried over $MgSO_4$ and concentrated in vacuo. Crystallization (dichloromethane/pentane) led to a pale yellow solid (87% yield). To the imine (40 g, 190 mmol), dissolved in MeOH (190 mL) K_2CO_3 (5.2 g, 38 mmol) was first added, then acrylonitrile (50 mL, 380 mmol) was added dropwise at 0 °C. The solution was stirred overnight at room temperature. MeOH was evaporated, ether (50 mL) was added and the organic layer was washed with brine (3×50 mL), dried over $MgSO_4$, filtered and the solvent removed in vacuo. The resulting imine was stirred with 1 N HCl (190 mL) in THF (190 mL) at 0 °C for 1 h. The THF was removed, the aqueous layer was washed with ether (2×50 mL) the pH was increased to 9 with Na_2CO_3 (saturated solution in water) and extracted with CH_2Cl_2 (6×200 mL). The organic layers were pooled, dried over $MgSO_4$ and concentrated. Crystallization (AcOEt) led to **2** as a white solid (two steps 52% yield). ^{1}H NMR (400 MHz, MeOD): δ 3.8 (s, 3H); 2.54–2.46 (ddd, $J = 6$, 8.6 Hz, 14.9 Hz, 2H); 2.4–2.32 (ddd, $J = 6.5$, 8.8 Hz, 15.4 Hz, 2H); 2.22–2.14 (ddd, $J = 6.6$, 8.6, 13.9 Hz, 2H); 1.91–1.83 (ddd, $J = 6.3$, 8.8, 14.7 Hz, 2H). ^{13}C NMR (100 MHz, MeOD): δ 173.9, 119.1, 59.5, 53.1, 35.5, 12.2. Anal. Calcd for $C_9H_{13}N_3O_2$: C, 55.37; H, 6.71; N, 21.52. Found: C, 55.35; H, 6.85; N, 21.5.
7. To a solution of **2** (3 g, 15.3 mmol) in THF (20 mL) was added $(Boc)_2O$ (3.7 g, 17 mmol), the mixture was stirred for 8 days at room temperature. Then, water (5 mL) and a catalytic amount of DMAP were introduced, the solution was stirred for 20 min. THF was evaporated, CH_2Cl_2 (50 mL) was added. The organic layer was washed with a saturated solution of NH_4Cl in water (30 mL), dried over $MgSO_4$ and evaporated. The compound **3** was purified on silica gel (CH_2Cl_2/MeOH) (80% yield). ^{1}H NMR (400 MHz, MeOD): δ 3.79 (s, 3H); 2.46–2.33 (m, 6H); 2.27–2.19 (m, 2H); 1.44 (s, 9H). ^{13}C NMR (100 MHz, MeOD): δ 173.4, 156.2, 120.4, 81.2, 62.2, 53.5, 31.5, 28.6, 12.5.
8. In a typical procedure, the dinitrile-ester **3**, **10**, **11**, or **12** (8 mmol) was dissolved in a mixture of absolute ethanol (146 mL) and THF (34 mL). Raney-Nickel (4 g, 34 mmol) as a 50% slurry in water was added together with 2 N NaOH (18 mL). The mixture was stirred under 50 psi hydrogen pressure at room temperature for 8 h. The catalyst was filtered off, pH was increased to 7 (with 1 N HCl) and the solvents were removed in vacuo. The crude product was used without further purification.
9. To a solution of **2** (1.95 g, 10 mmol) in water/CH_3CN (1:1, 50 mL) was added LiOH (420 mg, 10 mmol). After 25 min no remaining ester was detected on TLC. Alkyl chloroformate (30 mmol) was added and pH kept at 9 (with LiOH) during 3 h. Then pH was decreased to 3 (with HCl 1 N) and the organic layer was extracted with ether (30 mL). Compounds **7** and **8** were purified on silica gel (CH_2Cl_2/MeOH/AcOH) (60–65% yield over two steps).
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12. To a solution of **6** (1.26 g, 4 mmol) in DMF (10 mL) were added HBTU (1.52 g, 4 mmol) and DIEA (1.72 mL, 10 mmol), the mixture was stirred for 30 min at room temperature. Then glycine methyl ester chlorhydrate (625 mg, 5 mmol) was added in DMF (10 mL) and the solution was stirred overnight. A saturated solution of NH_4Cl in water was added and the aqueous layer was extracted with ether (6×). The organic layer was dried over $MgSO_4$ and evaporated. Compound **9** was purified on silica gel (AcOEt/cyclohexane) (80% yield).
13. Boc-amino acid or Cbz-amino acid (61.5 mmol) and NMM (7.8 mL, 71 mmol) in CH_2Cl_2 (250 mL) was cooled to 0 °C and stirred during the dropwise addition of isobutyl chloroformate (9.25 mL, 71 mmol) in CH_2Cl_2 (100 mL) over 10 min. The solution was allowed to warm up to room temperature, then **2** (8 g, 41 mmol) in CH_2Cl_2 (50 mL) was added. After 4 h, the solution was washed with a saturated solution of NH_4Cl in water, dried over $MgSO_4$ and evaporated. Compounds **10**, **11** and **12** were purified on silica gel (AcOEt/cyclohexane). Compound **11**: (65% yield) ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.36 (br s, 1H); 5.33 (br s, 1H); 3.91 (s, 3H); 3.75–3.74 (d, $J = 5.8$ Hz, 2H); 2.99–2.92 (dt, $J = 6.6$ Hz, 13.6 Hz, 2H); 2.36–2.24 (m, 4H); 2.2–2.13 (dt, $J = 7.3$, 14.2 Hz, 2H); 1.47 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.8, 169.6, 156.2, 118.4, 80.8, 62.5, 53.9, 45.1, 30.2, 28.2, 12.1. Anal. Calcd for $C_{16}H_{24}N_4O_5$: C, 54.54; H, 6.86; N, 15.9. Found: C, 54.45; H, 6.83; N, 15.83.
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