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Solid-phase approach to the synthesis of cyclen scaffolds from cyclotetrapeptides

Maria C. Alcaro, Marco Orfei, Mario Chelli, Mauro Ginanneschi and Anna M. Papini*

Dipartimento di Chimica Organica 'Ugo Schiff' and CNR-ICCOM, Polo Scientifico, via della Lastruccia 13, I-50019 Sesto Fiorentino (FI), Italy

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Abstract—Cyclen derivatives, as coordinating ligands, have recovered considerable interest for the development of diagnostic and therapeutic drugs, mimicking the binding site of metalloproteins. We demonstrate that the on-resin reduction of head-to-tail cyclotetrapeptides, anchored to a solid support by the side-chain of a trifunctional amino acid, is an efficient synthetic strategy. The proposed approach leads to the cyclen scaffold still bound to the resin, ready for further decorations.

The development of new coordinating ligands is of straightforward actuality for the study and the synthesis of diagnostic and therapeutic drugs mimicking the binding site of metalloproteins. Tetraazamacrocyclic compounds, as 1,4,8,11-tetraazacyclotetradecane (cyclam) and 1,4,7,10-tetraazacyclododecane (cyclen) derivatives, have found wide application in medicinal chemistry.[1] In particular, cyclen derivatives have recovered considerable interest in the development of MRI contrast agents,[2] and as radionuclides vehicles.[3]

Cyclen is the key intermediate for the preparation of more complex compounds,[4] such as bis-tetraazamacrocycles, polyazamacrocycles, and *N*-alkylated cyclens. Different condensation strategies for the synthesis of cyclen have been described, but the traditional approach reported by Richman and Atkins (involving *N*,*N*′,*N*″-tritosyldiethylenetriamine and *N*,*O*,*O*′,-tritosyldiethanolamine),[5] with its variants,[6] remains the most convenient. In order to avoid tosylate chemistry, different protection of the aza-compounds were considered, either envisaging condensation of tricyclic intermediates,[7] or tetracyclic precursors.[8]

The solid-phase synthesis introduced by Bruce Merrifield in 1963,[9] was not only strategic for peptide chemistry, but it has also revolutionized organic chemistry. The advantages introduced by this technique are now recognized worldwide. Every year a huge number of publications is dedicated to organic reactions performed in solid-phase.[10]

A convenient retro-synthetic pathway to tetraazamacrocycles can be envisaged considering cyclen and cyclam derivatives as pseudopeptides. In fact, reduction of the amide bond of head-to-tail cyclotetrapeptides containing four α-amino acids can lead to cyclen derivatives, while cyclams[11] can be obtained by cyclotetrapeptides containing α- and β-amino acids in alternate positions. Moreover, the cyclen core derived from cyclotetrapeptides is a possible scaffold for metal complexation with increased chelating properties, thanks to the side-chains of suitable amino acids introduced in the precursor peptide sequence. Thus, a solid-phase approach to cyclen analogs starting from cyclotetrapeptides, is proposed in this paper.

We previously described the synthesis of cyclo(His-Gly-His-Gly) by on-resin cyclization by a side-chain anchoring strategy.[12] The cyclotetrapeptide was synthesized anchoring the imidazole group of the protected amino acid Fmoc-His-OAl (**1**) to a trityl-type resin

Abbreviations: Al, allyl; DCM, dichloromethane; DIPEA, *N*,*N*-diisopropylethylamine; DMF, *N*,*N*-dimethylformamide; ESI MS, electrospray ionization mass spectrometry; Fmoc, 9-fluorenylmethoxycarbonyl; NMM, *N*-methylmorpholine; RP-HPLC, reverse phase-high pressure liquid chromatography; SPPS, solid-phase peptide synthesis; TBTU, 1-[bis(dimethylamino)methylene]-1*H*-benzotriazolium-1-tetrafluoroborate-3-oxide; TFA, trifluoroacetic acid; THF, tetrahydrofurane; Trt, trityl.

Keywords: macrocycles; cyclen; solid-phase synthesis; cyclization; cyclotetrapeptide.

* Corresponding author. Tel.: +39 0554573561; fax: +39 0554573485; e-mail: annamaria.papini@unifi.it

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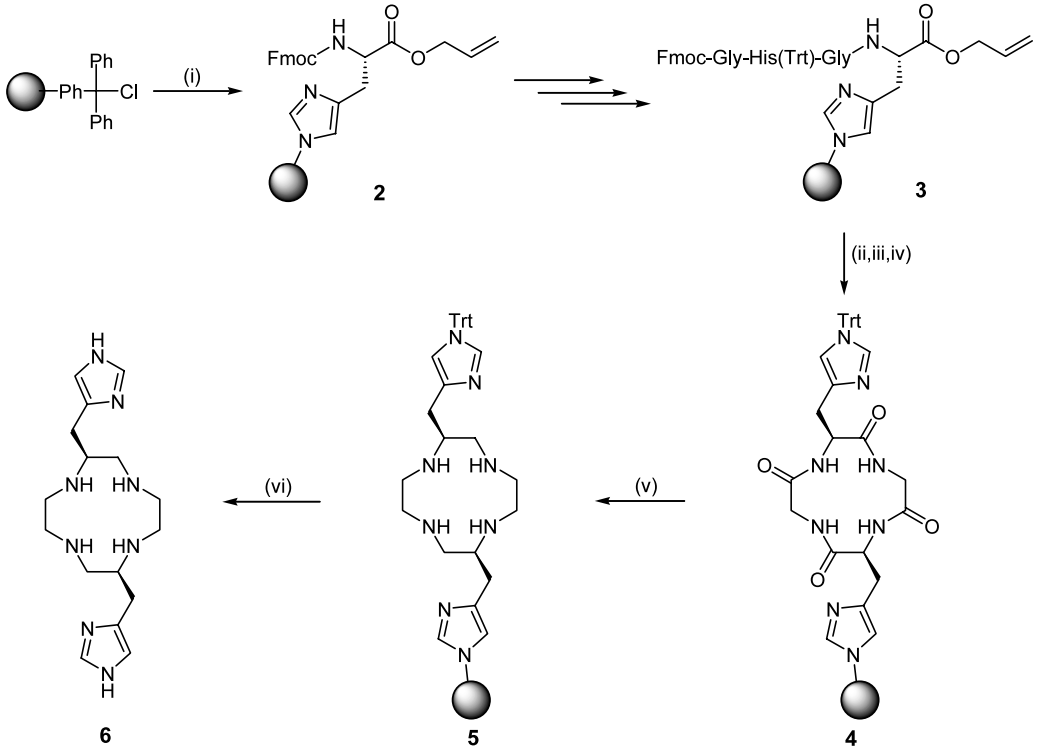
(Scheme 1). The protected linear precursor **3**, anchored to the resin, was synthesized by SPPS using the Fmoc/^{t}Bu/allyl three-dimensional protection scheme.[13] After treatment of the peptide-resin **3** with $PhSiH_3$ (24 equiv.) and $Pd(PPh_3)_4$ (0.25 equiv.) in dry DCM under argon (2×40 min) (allyl removal), and then with 20% piperidine in DMF (Fmoc-deprotection), the linear peptide was obtained with the free amino and carboxyl functions ready for final cyclization. Thus, taking advantage from the pseudodilution phenomenon, the on-resin cyclization was performed with TBTU/DIPEA in 4 h at rt, with no evidence of cyclooligomerization by-products.

The head-to-tail cyclization, by the side-chain anchoring strategy, allowed to obtain the cyclotetrapeptide **4**, an on-resin scaffold suitable for further modifications. Reduction of the amide bonds of cyclo-[His-Gly-His(trityl-resin)-Gly] (**4**) was undertaken by an on-resin procedure,[14] by treatment with a solution of BH_3·THF (40 equiv.) at 65°C for 24 h.† The reaction was quenched with MeOH and the resin treated with piperidine at 65°C for 20 h to disproportionate the borane complex. After cleavage by 95% TFA, the crude (overall yield 72%), was analyzed by LC–ESI MS. The cyclen derivative **6** ($R_t = 8.7$ min; $[M+H]^+ = 333.5$ m/z) was obtained with a 70% reduction yield and purified by semi-preparative RP-HPLC.†

Our synthetic strategy is based on an SPPS standardized protocol. Therefore, it is an easy methodology to obtain new enantiomerically pure cyclen cores mono-, di-, tri-, and tetrasubstituted, characterized by diversities provided by the amino-acids side chains used as starting materials.

In particular the new cyclen analogue scaffold **6**, containing two imidazole groups, possesses increased chelating properties, respect to the usual tetraazamacrocycles, which can be used as coordinating system in metalloproteins characterization. Copper complexation studies of this compound are under investigations in our laboratory.

Moreover, the proposed approach to the scaffold **6**, is a valuable tool for further decorations of the cyclen core.



Scheme 1. *Reagents and conditions*: (i) Fmoc-His-OAl (**1**), DIPEA, 2 h, rt; (ii) $PhSiH_3/Pd(PPh_3)_4$ (96:1), 2×40 min, rt; (iii) 20% piperidine in DMF, 20 min; (iv) TBTU, DIPEA, 4 h, rt; (v) BH_3·THF, 2 h, 65°C; (vi) 95% TFA, 3 h, rt.

† The cyclen derivative (2*S*,8*S*)-2,8-bis(1*H*-imidazol-4-ylmethyl)-1,4,7,10-tetraazacyclododecane **6** was prepared as follows. A solution of 1 M BH_3·THF (40 equiv.) was slowly added to cyclo[His-Gly-His(trityl-resin)-Gly] (**4**) (100 mg, 0.42 mmol/g), the reaction mixture was heated at 65°C for 24 h. The solution was filtered off and the resin washed with MeOH (3×2 min), DMF (2×2 min) and MeOH (2×2 min). The resin was treated with piperidine for 20 h at 65°C and then washed with DMF (3×2 min), MeOH (2×2 min) and DCM (2×2 min). Cleavage was performed with 95% TFA for 3 h at rt, the solution was concentrated and the product precipitated with cold Et_2O. After lyophilization, the crude (10 mg, 72% yield) was purified by semipreparative RP-HPLC on a Phenomenex Jupiter C18 column (10 μm, 250×10 mm) (4 mL/min, 5–30% of 0.1% TFA in MeCN in 20 min). HPLC purification in terms of yield was not optimized. However, the purified product (1.0 mg, 7% overall yield) was analysed by LC-ESI-MS on a ThermoFinnigan LCQ Advantage MS, on a Phenomenex Aqua C18 column (5 μm, 150×2.0 mm), flow 200 μL/min, 5–30% of 0.1% TFA in MeCN in 20 min, $R_t = 8.5$ min. ESI MS (m/z): found 333.5 $[M+H]^+$, calcd 333.2.

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