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# Design and scalable synthesis of new chiral selectors. Part 1: Synthesis and characterization of a new constrained cyclopeptide from unnatural bulky amino acids

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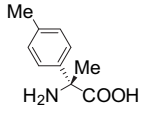
ABSTRACT

We describe the conception, synthesis, and characterization of a novel cyclopeptide designed for chiral recognition. The asymmetric units are built from an unnatural amino acid in the series of α-aryl-α-methyl glycine. Modifications of standard methods of peptide synthesis are described in order to improve yields and purities when applied to hindered amino acids. First set of experiments about host–guest ability of the obtained cyclopeptide is disclosed.



## 1. Introduction

Asymmetric recognition by means of chiral receptors remains a topic of increasing interest, with opportunities in various domains ranging from supramolecular chemistry to selective extraction.[1] Up to now, the main artificial receptors used for such a purpose are found mostly in molecular classes, such as crown-ethers, cyclodextrins, various cryptands, and calixarenes.[2] Beyond numerous successful approaches in the field, the major drawback inherent to these classes of receptors is the lack of molecular diversity due to tedious and difficult modifications of those structures. An interesting development of new families of chiral receptors is the conception of 'tailor-made' structures that could be either easily modified by chemical transformations or synthesized according to a requested property. In the latter case, we consider cyclopeptides as powerful tools,[3] since they can be prepared from a large variety of available chiral amino acids, which can be coupled to give an infinite series of sequences. Having in hands a powerful method for the synthesis of chiral quaternary amino acids,[4] we embarked on a preliminary study for the construction of such cyclopeptides. Choosing hindered quaternary precursors relied on the expected limitation of molecular flexibility, a behavior generally not compatible with an efficient chiral recognition. Indeed, the main questions to be addressed in this context are the re-examination of coupling procedures for such hindered precursors, as well as the cyclisation step. It is well documented that coupling hindered amino acids, especially when the stereogenic carbon center is quaternary, is not an obvious question.[5] Fig. 1 shows the chosen amino acid 2-methyl-2-*p*-tolylglycine (MPG) from which we decided to obtain cyclopeptides.



(*R*)-MPG

**Fig. 1.** Chiral quaternary amino acid (*R*)-MPG.

## 2. Results and discussion

### 2.1. Synthesis of enantiomerically pure MPG

The overall process relies on the synthesis of the corresponding hydantoin, which is then resolved using the AS3PC (Auto-Seeded Programmed Polythermic Preferential Crystallization)[4] procedure and hydrolyzed (see Scheme 1).

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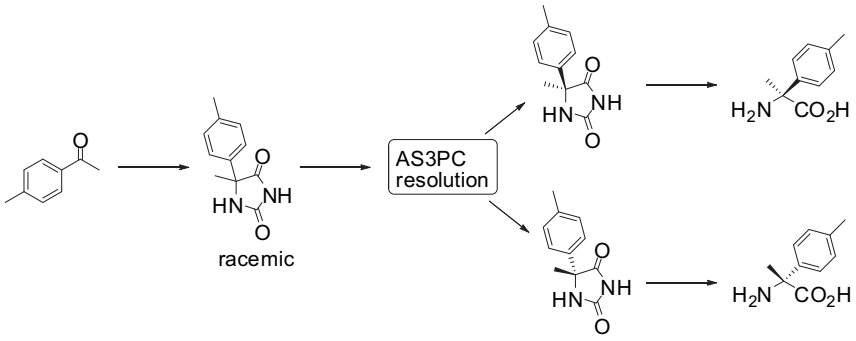
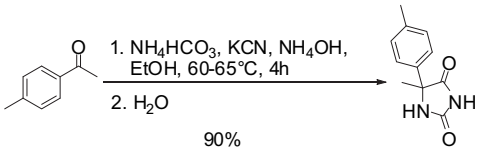



**Scheme 1.** Overall process for the preparation of the enantiomerically pure amino acid.

The first step consists in a typical Bücherer–Berg procedure. Hydantoins are thus produced in good yields (ca. 90%), using reaction conditions given in Scheme 2.




**Scheme 2.** Bücherer–Berg procedure.

The resolution process (AS3PC) has already been described elsewhere.[6] Both enantiomers of the hydantoin are readily obtained in ee superior to 99% after crystallization.

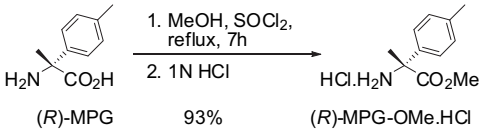
The final step consists of hydrolysis of hydantoins giving the amino acids. This reaction was not fully described in previous studies, and therefore required an optimization work. We finally proceeded by basic hydrolysis in presence of sodium hydroxide: after 3 days at reflux, conversion was quantitative, and the amino acid was recovered by acidic treatment. Chiral HPLC analysis (Chirobiotic T, eluting with $H_2O$/EtOH 90/10) confirmed that, as expected for quaternary amino acids, no racemization occurred at this stage. Nevertheless, most of our samples were contaminated by various amounts of NaCl, which could not be easily removed by recrystallization. As further functional modifications, such as esterifications and N-protections, which are obviously necessary for peptide coupling, are not affected by the presence of salt, we carried out those protection steps on the amino acid/NaCl mixture. The protected amino acid could then be separated from NaCl by a simple extraction in an organic solvent.

### 2.2. Determination of absolute configuration of α-aryl-β-methyl glycines

A careful examination of literature data showed us that previously published values in this series were not consistent, in sense that opposite optical rotations were reported for the same enantiomer. We were thus obliged to settle this point by univocal methods, including determination of absolute configurations by X-ray analysis, either by anomal diffraction (MPBrG, 2-methyl-2-(*p*-bromophenyl)glycine) or co-crystallization with chiral units of cyclodextrins (MPG), as well as chemical correlations: both MPG and MPBrG present a (−)-*R* relationship. The complete study of the rotation sign/absolute configuration relationship in this series was published elsewhere.[7]

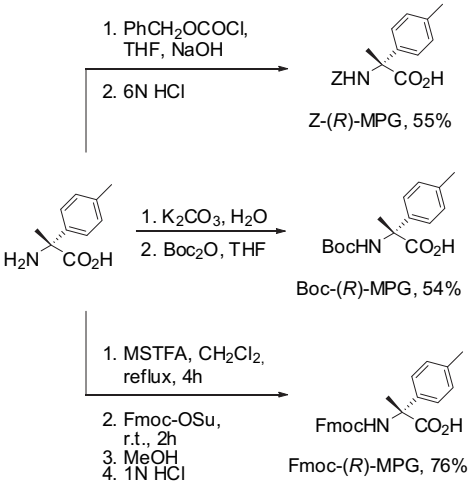
### 2.3. Protection of quaternary amino acids

C-Terminal protections were realized as methyl esters, using the thionyl chloride method (Scheme 3). High yields were obtained, comparable for these hindered amino acids with those of their proteinogenic congeners. When contaminated by sodium chloride (vide supra), the esters hydrochlorides were purified using treatment with calcium carbonate, thus releasing the free ester, which could then be extracted with an organic solvent and converted back to the hydrochloride.[8] Overall yields were, respectively, 93 and 79% for MPG-OMe·HCl and MPBrG-OMe·HCl.




**Scheme 3.** Synthesis of methyl ester hydrochloride.

MPG was *N*-protected as Z,[9] BOC,[10] and FMOC[11] derivatives, with fairly good yields (ca. 55–75%) in regards with the bulkiness of the starting amino acids (Scheme 4).



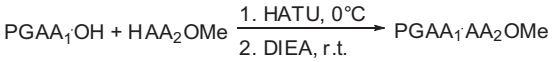

**Scheme 4.** Synthesis of *N*-protected amino acids.

### 2.4. Synthesis of dipeptides

Most of the standard methods for peptide coupling are devoted to proteinogenic units that are less hindered structures in which the chiral center is tertiary. Anyway, it has been showed that these reactions are strongly dependent on the nature of the coupling agent, with the best results being usually observed when using HATU.[12,13] We therefore realized our first experiments with HATU and related coupling agents. After optimization of reaction conditions (duration time, temperature), we were delighted to obtain

rather good yields in protected dipeptides, even for quaternary/quaternary couplings (Table 1).

**Table 1**
Synthesis of dipeptides




| Entry | Protecting group | $AA_1$ | $AA_2$ | Time | Solvent | Yield (%) |
|---|---|---|---|---|---|---|
| 1 | Boc | MPG | Gly | 24 h | $CH_2Cl_2$ | 92 |
| 2 | Boc | Gly | MPG | 24 h | $CH_2Cl_2$ | 82 |
| 3 | Z | Gly | MPG | 36 h | $CH_2Cl_2$ | 93 |
| 4 | Boc | MPG | MPG | 8 days | THF | 61 |
| 5 | Boc | MPG | β-Ala | 4days | THF | 85 |
| 6 | Z | β-Ala | MPG | 2 days | THF | 82 |
| 7 | Boc | MPG | Sar | 4 days | $CH_2Cl_2$ | 53 |
| 8 | Boc | MPG | Aib | 4 days | $CH_2Cl_2$ | 58 |
| 9 | Boc | Aib | MPG | 4 days | $CH_2Cl_2$ | 59 |
| 10 | Boc | MPG | Ac5c | 4 days | THF | 94 |
| 11 | Boc | Ac5c | MPG | 4 days | THF | 91 |

Most of these dipeptides were also prepared using ionic liquids as solvents, with at least equal efficiency.[14,15]

Very high yields were obtained in the construction of dipeptides incorporating a glycine residue either in C- or N-terminal position (entries 1–3). We also verified that less expensive coupling methods (acid chloride generated by oxalyl chloride, BOP, CMPI, and DCC/HOBt) were efficient for those couplings, with nevertheless inferior yields. The homocoupling of two MPG-units was also realized in a fairly good yield, at the cost of a longer reaction time (entry 4). Finally, a series of achiral non-proteinogenic amino acids (Fig. 2) were efficiently coupled with MPG at either C- or N-terminal position using similar procedure, with HATU as coupling agent (entries 5–11).

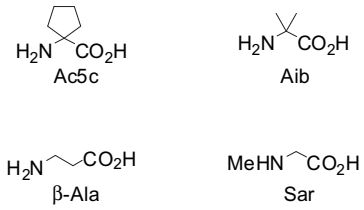



**Fig. 2.** Achiral amino acids.

X-ray analysis of *Z*-Gly-(*R*)-MPG-OMe has already been published elsewhere,[14] and the corresponding ORTEP is presented in the following figure (Fig. 3). In the crystalline state, this dipeptide presents an intramolecular hydrogen bond that stabilizes the structure via a five-membered pseudo cycle. This is a rather common behavior for peptides containing quaternary amino acids.

For the synthesis of dipeptides, one can observe that HATU is generally the best coupling agent, as was previously published in literature. Results with Ac5c are particularly attractive, while Sar and Aib gave moderate yields.

## 2.5. Synthesis of the linear octapeptide

In order to have a good compromise between the size of the ring and the ease of synthesis, we decided to prepare the cyclo-octapeptide *cyclo*(Gly-(*R*)-MPG)$_4$ or cGM (Fig. 4). The use of glycine as 'spacer' between MPG-units ensures very high yields at each coupling step, and the linear octapeptide precursor of cGM can be

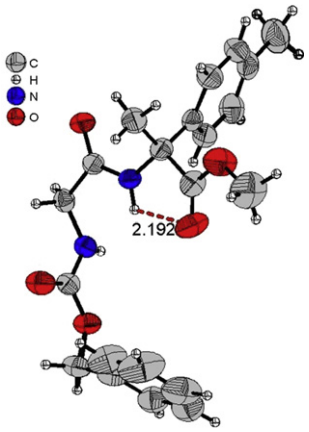



**Fig. 3.** ORTEP of *Z*-Gly-(*R*)-MPGOMe. All non-hydrogen atoms are represented by their displacement ellipsoids drawn at the 50% probability level. Supplementary crystallographic data for CCDC 292584 are available free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, United Kingdom; fax: +44 1223 336033 or deposit@ccdc.cam.ac.uk).

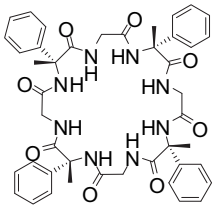
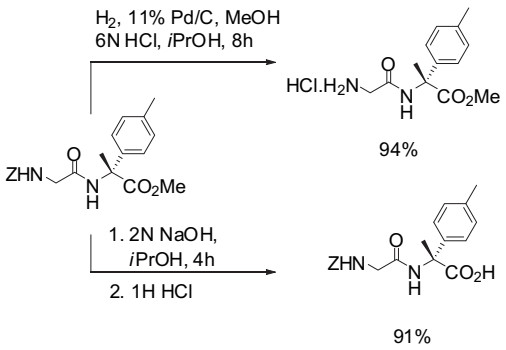


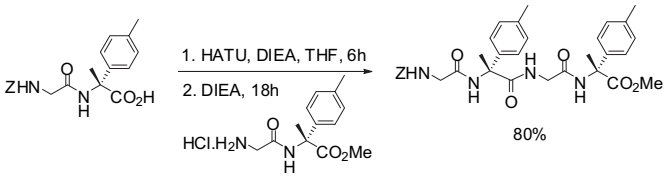
**Fig. 4.** Targeted *cyclo*(Gly-(*R*)-MPG)$_4$, (cGM).

obtained by convergent synthesis from Gly-MPG dipeptide via the tetrapeptide as sole intermediate.

First, N- and C-protections were removed on separate samples, as showed in Scheme 5. Presence of HCl solution during the Z deprotection avoids intramolecular cyclisation of the dipeptide ester into diketopiperazine, which is a usual by-product in this chemistry. Having these two compounds in hands in high yields, we engaged them in the coupling reaction leading to the corresponding tetrapeptide (Scheme 6). We used very similar conditions than that described for dipeptide synthesis (vide supra). Worthy of note is that pre-activation of the acid function with HATU is required for obtaining good yields. Indeed, absence of this pre-activation step, giving in situ the corresponding activated ester, led to the formation of by-products (which will be discussed in part 2.6).




**Scheme 5.** Deprotection of terminal functions of *Z*-Gly-(*R*)-MPG-OMe.




**Scheme 6.** Synthesis of tetrapeptide *Z*-(Gly-(*R*)-MPG)$_2$-OMe.

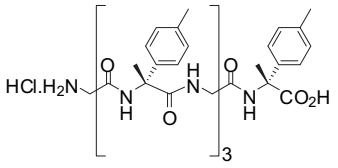
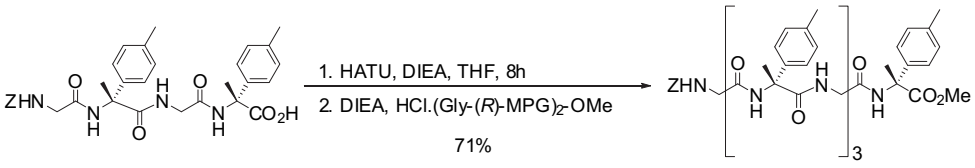


**Fig. 5.** HCl,H-(Gly-(*R*)-MPG)$_4$-OH.

The tetrapeptide was then deprotected either on the N- or the C-terminal function, as showed above, with respectively quantitative and 85% yields. Coupling of those two deprotected tetrapeptides gave the required linear protected octapeptide (Scheme 7). In this particular coupling, we also used a pre-activation step as for the construction of the tetrapeptide. In addition, we observed that conversion was not complete after 18 h. Two successive additions of HATU and DIEA were required for completing the reaction. Following this procedure, a 71% yield was obtained after purification. As for the dipeptides, we also prepared tetra- and octapeptides in ionic liquids, with similar performance.[14]

peptides was rather disappointing since, apart from a very small amount (c.a. 4%) of the expected cyclopeptide, the major obtained products were identified by LC/MS as derivatives of the linear octapeptide (Scheme 8). The main impurity (65%) was identified as the *N*-guanidine derivative (P1 in Scheme 8), along with ca. 23% of another guanidine compound in which the carboxyl moiety was substituted as a diethylamide residue (P2 in Scheme 8). The guanidine substitution results of the nucleophilic attack of the free amino function onto HATU, while the carboxyl transformation into amide is assumed to come from reaction with DMF used as solvent because of limited solubility in other molecular solvents. Similar



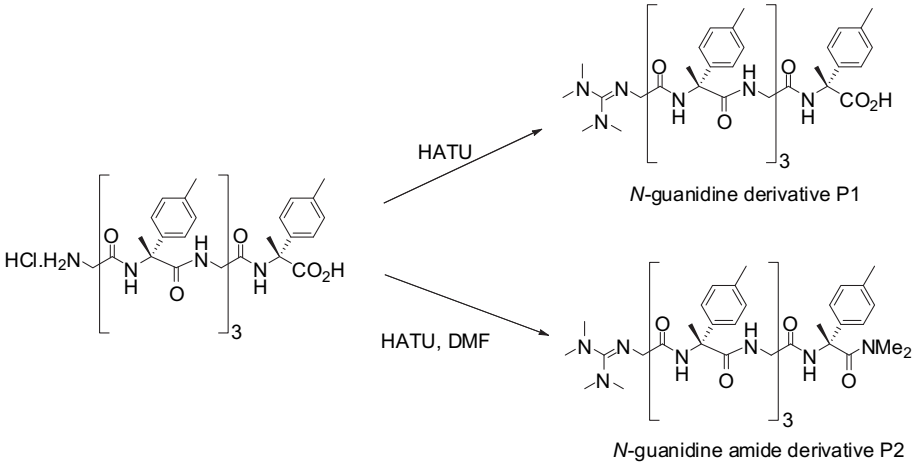

**Scheme 7.** Synthesis of linear octapeptide *Z*-(Gly-(*R*)-MPG)$_4$-OMe.

## 2.6. Synthesis of the cyclooctapeptide

Linear octapeptide was first deprotected by saponification on its C-terminal position with 81% yield. The acid thus obtained was submitted to hydrogenolysis in order to remove the Z protection (86% yield). In this protocol hydrochloric acid should be added only at the end on the deprotection step in order to prevent re-esterification by methanol. The required free octapeptide hydrochloride was thus obtained with 70% overall yield (Fig. 5).

Cyclisation of such linear peptides is not always a simple question. Our first series of experiments using the conditions previously optimized for the synthesis of the linear MPG containing by-products were also observed during the synthesis of tetra- and octapeptides but could be avoided by pre-activation of the free acid by HATU before adding the free amino moiety (vide supra). While not frequently encountered, such side reactions have already been described in literature, and seem to be favored for hindered structures.[16]

Consequently, uronium salts like HATU do not seem appropriate for such cyclisations where no pre-activation of the acid function can be performed. Some data from literature recommend the use of phosphonium salts as coupling agents in such situation.[12] Therefore, we turned to examination of BOP-mediated couplings. A first set of experiments carried out on small samples (ca. 80 mg) at high




**Scheme 8.** Main by-products occurring during the cyclisation step.

dilution (ca. $1.5\times10^{-4}$ M) showed a complete conversion after 5 days at room temperature. After chromatography, cGM was obtained in 51% yield. The reaction was repeated at a larger scale (700 mg), with similar result. Interestingly, a control experiment at much higher concentration ($10^{-2}$ M) showed that high dilution can be avoided. Also we showed that this step could as the precedent, be realized into ionic liquids.[14]

### 2.7. Structural analysis of the cyclooctapeptide monohydrate

Single crystals of sGM could be obtained by slow evaporation of a solution of cGM in a dichloromethane/methanol mixture and were studied by X-ray analysis (Figs. 6 and 7).

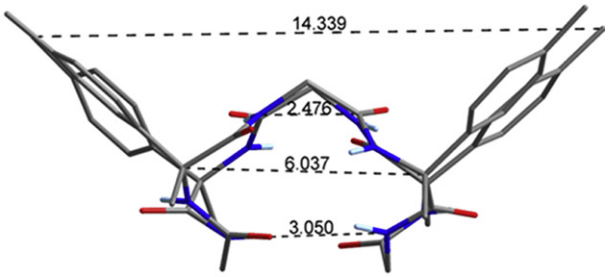



**Fig. 6.** Side view of cGM. Deduced from X-ray structure. Supplementary crystallographic data for CCDC 768235 are available free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, United Kingdom; fax: +44 1223 336033 or deposit@ccdc.cam.ac.uk).

**Table 2**
Hydrogen bonds distances and angles

| D–H···A | d(D–H) | d(H···A) | d(D···A) | <(DHA) |
|---|---|---|---|---|
| Intermolecular H bonds | | | | |
| OW–H(2O)···O(5) | 0.874(3) | 1.9651(16) | 2.838(3) | 178.8(4) |
| OW–H(1O)···O(1)#1 | 1.119(3) | 1.7723(19) | 2.714(4) | 138.45(16) |
| Intramolecular H-bonds | | | | |
| N(1)–H(1)···O(6) | 0.86 | 2.03 | 2.859(3) | 161.4 |
| N(2)–H(2A)···O(1) | 0.86 | 2.62 | 3.310(3) | 137.8 |
| N(2)–H(2A)···OW#2 | 0.86 | 2.52 | 3.338(4) | 158.6 |
| N(3)–H(3A)···O(8) | 0.86 | 2.22 | 3.022(3) | 155.1 |
| N(4)–H(4)···O(3) | 0.86 | 2.24 | 2.969(2) | 143 |
| N(5)–H(5A)···O(2) | 0.86 | 2.13 | 2.895(2) | 148.1 |
| N(6)–H(6A)···O(5) | 0.86 | 2.15 | 2.901(2) | 146.2 |
| N(7)–H(7)···O(4) | 0.86 | 2.08 | 2.907(2) | 161.4 |
| N(8)–H(8)···O(7) | 0.86 | 2.26 | 3.004(2) | 144.5 |

Symmetry transformations used to generate equivalent atoms: #1: $x-1, y, z+1$, #2: $x+1, y, z-1$.

### 2.8. Preliminary studies of recognition ability of cGM

A preliminary study of molecular recognition ability of cGM was performed by means of crystallization of host–guest complexes and gas chromatography. Several attempts of crystallization of host–guest complexes between cGM and guest molecules (listed in Supplementary data section) were carried out in various solvents or mixtures of solvent. The crystals were prepared by slow evaporation of solutions containing cGM and guest molecules with various molar ratios ranging from 1/1 to 1/4. Nevertheless, only crystallization of pure cGM monohydrate was observed. The poor

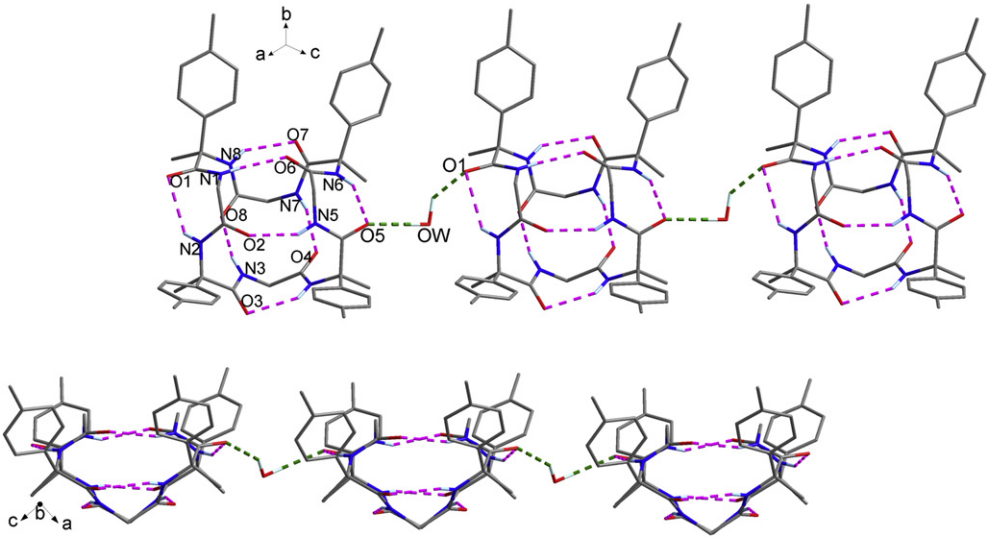



**Fig. 7.** Strong molecular interaction along [101] axis and projection along [010] axis: In dashed pink lines the intramolecular H bonds. In dashed green lines the intermolecular H bonds.

The macrocycle is in the shape of a 'W' as illustrated by Fig. 6. The conformation results from an extensive intramolecular H-bond network between N–H and carbonyls (Fig. 7). These intramolecular H-bonds are listed in Table 2 and provide a genuine rigidity of the macrocycle. Intermolecular bonds involve the water molecule.

Hydrogen atoms from the water molecules were located with constrains on the H–O and the H–H distances. Nevertheless, the water molecule presents a high thermal agitation factor at room temperature. The environment clearly indicates the existence of a periodic bond chain along <1O1>, as carbonyl functions are oriented toward the water molecule. Fig. 8 illustrates the additional contacts between the macrocycles via Π-stacking.

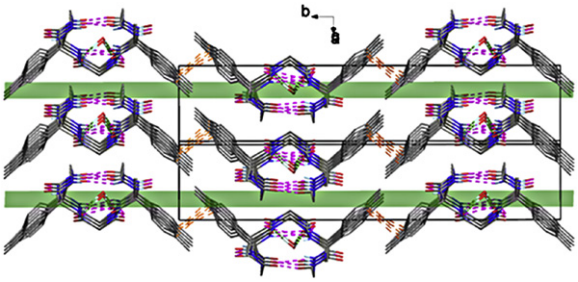



**Fig. 8.** Folded layers propagating in (101) direction with 8.57 Å thickness.

accommodation ability of cGM to host a molecule is likely to result from the rigidity of the cyclopeptide structure due to the presence of numerous intramolecular H-bonds.

The poor molecular flexibility of this cyclopeptide allows no conformational adjustment to any guest molecule tested so far. Methylation of NH groups or substitution of oxygen by sulfur of cGM could enhance the structure flexibility and improve its molecular recognition ability. Modification of the spacer, e.g., cyclopentylglycine could also result in global shape modification beneficial to the crystallization of host–guest complexes.

The chiral recognition ability of cGM was evaluated by gas chromatography. cGM was diluted at 20% in mass in OV 1701 (polysiloxan, 86% methyl, 7% phenyl, 7% cyanopropyl) and coated on a fused silica column (10 m×0.25 μm). The resulting column presented good efficiency above 3000 theoretical plates/meter but showed no enantioselectivity on a panel of racemic mixtures of various chiral compounds. This result could be explained by the poor solubility of the cyclopeptide in the polysiloxan and its high melting point, which are major drawbacks for the preparation of chiral stationary phase. In future work, in order to overcome this problem, the cyclopeptide could be grafted to a polyhydromethylsiloxan to ensure the homogeneity of the chiral stationary phase.

## 3. Conclusion

We have succeeded in the preparation of a novel artificial cyclopeptide based on the bulky quaternary amino acid 2-methyl-2-*p*-tolylglycine for future studies in chiral recognition. X-ray structure of the cyclopeptide shows a rigid shape due to numerous intra- and intermolecular H-bonds, as well as π-stacking. This study is part of our continuous search for new chiral selectors (for part 2 on the use of chiral ionic liquids for enantioselective liquid–liquid extraction, see Ref. 18).

## 4. Experimental section

### 4.1. General procedures

The NMR spectra were recorded on a Bruker AVANCE 300 spectrometer ($^1$H at 300 MHz and $^{13}$C at 75 MHz) in deuterochloroform or deuteromethanol, the chemical shifts are quoted in parts per million in δ-values and the coupling constants are quoted in hertz. Optical rotations were measured with a Perkin–Elmer polarimeter, in a 1 dm length cell. HPLC were performed on an HP series 1100 apparatus (integrator HP 3395, column Capital HPLC LTD, C18-KL5-25091, 25 cm×4.6 mm, 1 mL min$^{-1}$, 20 μL, λ 215 nm).

### 4.2. General procedure for the preparation of aminoesters

Thionyl chloride (3.6 equiv) was added gradually to a solution of amino acid (68 mmol) in distilled methanol (~1.2 mL/mmol) at −15 °C with continuous stirring. After the addition was complete, the cooling bath was removed and the reaction mixture was allowed to warm to room temperature, then refluxed for 7 h. The stirring was continued overnight at room temperature. The solvent was removed under vacuum. Water (1.5 mL/mmol) and $CaCO_3$ (2.3 equiv) were added and the mixture was stirred at room temperature. After 1 h, AcOEt (100 mL) was added and the stirring continued for 1 h. After decantation, the organic layer was washed with water (2×10 mL) and extracted with 1 N HCl (6×10 mL). Water was then removed and the resulting solid was recrystallized in water.

*4.2.1. HCl,H-(R)-MPG-OMe.* Yield 5.1 g (93% starting from 24 mmol of amino acid). Colorless crystals, mp 110 °C; retention time 8.93 min (HPLC, ACN/$H_2O$/TFA: 25/75/0.1); $^1$H NMR (DMSO-$d_6$) 1.79 (s, 3H, C$H_3$), 2.24 (s, 3H, C$H_3$), 3.66 (s, 3H, OC$H_3$), 7.19–7.34 (AA′BB′, 4H, *J*=8.3 Hz); $^{13}$C NMR (MeOD) 21.6, 22.7, 55.0, 63.2, 127.1, 131.5, 134.5, 141.7, 173.0; $[\alpha]_D^{20}$ −19.8 (*c* 0.5, MeOH); $C_{26}H_{34}N_2O_5$ (229.71): calcd C 53.33, H 7.32, N 5.65; found C 53.53, H 7.03, N 5.68.

*4.2.2. HCl,H-(S)-MPBrG-OMe.* Yield 15.8 g (79% starting from 68 mmol of amino acid). Colorless crystals, mp 130 °C; retention time 13.86 min (HPLC, ACN/$H_2O$/TFA: 25/75/0.1); $^1$H NMR ($D_2O$) 1.88 (s, 3H, C$H_3$), 3.80 (s, 3H, OC$H_3$), 7.36–7.66 (AA′BB′, 4H, *J*=8.7 Hz); $^{13}$C NMR ($D_2O$) 21.4, 54.6, 61.7, 124.1, 127.9, 132.8, 134.2, 171.8; $[\alpha]_D^{20}$ +53.8 (*c* 0.5, MeOH); $C_{26}H_{34}N_2O_5$ (294.57): calcd C 38.42, H 4.84, N 4.48; found C 38.03, H 4.87, N 4.14.

### 4.3. Preparation of *Z*-MPG-OH

Benzyl chloroformate (392 μL, 1 equiv) in THF (2.5 mL) and 0.69 mL of 10% NaOH (1 equiv) were added dropwise and simultaneously to a solution of MPG (2.73 mmol) in a mixture of THF (9 mL) and NaOH (1 equiv) in water (9 mL) in an ice bath. After 1 h at 0 °C, the cooling bath was removed and the reaction mixture was stirred overnight at room temperature. THF was removed under vacuum and a solution of 10% NaOH in water was added until pH=13. Aqueous layer was washed with 2×15 mL of dichloromethane and a solution of 6 N HCl was added until pH=2.2. AcOEt (20 mL) was added and the mixture was stirred for 2 h. After decantation, the aqueous layer was extracted with 2×10 mL of AcOEt, and the combined organic layers were washed with water, brine, and dried ($Na_2SO_4$). Solvent removal under vacuum gave *Z*-MPG-OH as a yellow oil (469 mg, 55%), which was used without purification. $^1$H NMR ($CDCl_3$) 1.91 (s, 3H, C$H_3$), 2.25 (s, 3H, C$H_3$), 4.93 (s, 2H, OC$H_2$), 7.01–7.26 (m, 9H, Ar), 9.43 (s, 1H, COO*H*); $^{13}$C NMR 20.8, 22.7, 61.6, 66.3, 125.6, 127.8, 129.0, 134.9, 137.4, 138.2, 154.7, 176.6.

### 4.4. Preparation of Boc-(*R*)-MPG-OH

A mixture of (*R*)-MPG (900 mg, 5.02 mmol) and 714 mg of potassium carbonate (1.03 equiv) in 18 mL of water was stirred for 2 h. A solution of diterbutyl dicarbonate (1.35 g, 1 equiv) in THF (20 mL) was added dropwise at room temperature. The mixture was stirred for 36 h at room temperature then refluxed for 24 h. THF was removed under vacuum and 10% NaOH was added until pH=12. Aqueous layer was washed with 2×10 mL of dichloromethane and an aqueous solution (10%) of citric acid was added until pH=3.2. Aqueous layer was extracted with 4×10 mL of AcOEt. Combined organic layers were washed with 10 mL of water and dried ($Na_2SO_4$). Partial removal of the solvent induced crystallization of Boc-(*R*)-MPG-OH as a white solid (760 mg, 54%). Mp 65 °C (dec); retention time 11.32 min (HPLC, ACN/$H_2O$/TFA: 50/50/0.1); $^1$H NMR (MeOD) 1.38 (s, 9H, C(C$H_3$)$_3$), 1.88 (s, 3H, C$H_3$), 2.30 (s, 3H, C$H_3$), 7.13–7.37 (AA′BB′, 4H, *J*=8.3 Hz); $^{13}$C NMR (MeOD); 21.2, 23.0, 28.2, 61.8, 81.3, 125.6, 126.2, 137.0, 139.2, 156.7, 176.3; $[\alpha]_D^{20}$ −54.0 (*c* 0.5, MeOH); IR (cm$^{-1}$) 1649, 1721. HRMS (ESI): calcd for $C_{15}H_{22}NO_4$ (M+H$^+$) 280.1549; found 280.1555.

### 4.5. Preparation of Fmoc-(*R*)-MPG-OH

*N*-methyl-*N*-trimethylsilyl trifluoroacetamide (1.1 mL, 2 equiv) was added dropwise to a mixture of (*R*)-MPG (532 mg, 2.97 mmol) in 10 mL of freshly distilled $CH_2Cl_2$. The mixture was refluxed for 4 h, and then cooled to room temperature. A solution of *N*-(9-fluorenylmethoxycarbonyloxy)succinimide (1.0 g, 1 equiv) in 8 mL of $CH_2CL_2$ was added dropwise and the mixture was stirred for 2 h. Methanol (5 mL) was then added and the mixture was stirred for 15 min. Solvents were removed and diethylether and a solution of 5% aqueous $K_2CO_3$ in water were added. After decantation, the organic layer was washed with 3×5 mL of 5% aqueous $K_2CO_3$ and a solution of 1 N HCl was added until pH=2. Aqueous layer was extracted with

4×20 mL of AcOEt. Combined organic layers were washed with 2×10 mL of water, dried ($MgSO_4$), and concentrated. Flash chromatography ($CH_2Cl_2$ 100%) gave Fmoc-(*R*)-MPG-OH as a white powder (908 mg, 76%). Mp 110 °C; retention time 15.84 min (HPLC, ACN/$H_2O$/TFA: 60/40/0.1); $^1$H NMR (DMSO-$d_6$) 1.75 (s, 3H, C$H_3$), 2.28 (s, 3H, C$H_3$), 4.22 (m, 3H, C$H_2$/C*H*), 7.08–7.91 (m, 12H, Ar); $^{13}$C NMR ($CDCl_3$); 21.0, 22.8, 47.1, 62.1, 66.7, 119.9, 125.1, 125.9, 127.1, 127.6, 129.2, 137.3, 137.9, 141.3, 143.8, 157.1, 177.9; $[\alpha]_D^{20}$ −33.4 (*c* 0.5, MeOH). HRMS (ESI): calcd for $C_{25}H_{24}NO_4$ (M+$H^+$) 402.1705; found 402.1708.

## 4.6. General procedure for the preparation of dipeptides with HATU or BOP

The *N*-protected amino acid, the amino ester hydrochloride (1.1 equiv), and HATU (1.1 equiv) were introduced in a 10 mL flask under nitrogen. The appropriate freshly distilled solvent (3–6 mL) was then added and the mixture was stirred for 20 min at 0 °C. Diisopropylethylamine (3.3 equiv) was added dropwise and the reaction mixture stirred at room temperature for 2–8 days. The reaction mixture (after concentration and addition of dichloromethane if the reaction solvent was THF) was then washed with 1 M aqueous $NaHCO_3$ (5 mL), 10% aqueous citric acid (2×5 mL), and water (5 mL), dried ($MgSO_4$), and concentrated. Flash chromatography on silica gel gave the desired dipeptide.

*4.6.1. Boc-(R)-MPG-(R)-MPG-OMe.* Yield 100 mg (61%) starting from 100 mg (0.37 mmol) Boc-(*R*)-MPG-OH in 5 mL THF. For flash chromatography: cyclohexane/AcOEt (80/20).[17]

*4.6.2. Boc-(R)-MPG-Gly-OMe.* Yield 171 mg (92%) starting from 147 mg (0.53 mmol) Boc-(*R*)-MPG-OH in 5 mL $CH_2Cl_2$. For flash chromatography: $CH_2Cl_2$/MeOH (97/3).[17]

*4.6.3. Boc-Gly-(R)-MPG-OMe.* Yield 150 mg (82%) starting from 92 mg (0.53 mmol) Boc-Gly-OH in 3 mL $CH_2Cl_2$. Eluant for flash chromatography: $CH_2Cl_2$/MeOH (95/5). White solid; mp 103 °C; retention time 6.18 min (HPLC, ACN/$H_2O$/TFA: 60/40/0.1); $^1$H NMR ($CDCl_3$) 1.37 (s, 9H, C(C$H_3$)$_3$), 1.91 (s, 3H, C$H_3$), 2.24 (s, 3H, C$H_3$), 3.59–3.68 (m, 5H, OMe/C$H_2$), 5.32 (s, 1H, N*H*), 7.05–7.25 (m, 4H), 7.36 (s, 1H, N*H*); $^{13}$C NMR ($CDCl_3$) 21.1, 22.3, 29.8, 44.8, 53.3, 61.9, 80.3, 125.8, 129.5, 137.1, 138.0, 156.3, 168.7, 173.5. HRMS (ESI): calcd for $C_{18}H_{27}N_2O_5$ (M+$H^+$) 351.1920; found 351.1907.

*4.6.4. Z-Gly-(R)-MPG-OMe.* Yield 511 mg (93%) starting from 300 mg (1.43 mmol) *Z*-Gly-OH in 6 mL $CH_2Cl_2$.[17]

*4.6.5. Boc-(R)-MPG-Aib-OMe.* Yield 78 mg (58%) starting from 100 mg (0.36 mmol) Boc-(*R*)-MPG-OH in 3 mL $CH_2Cl_2$.[17]

*4.6.6. Boc-Aib-(R)-MPG-OMe.* Yield 110 mg (59%) starting from 92 mg (0.49 mmol) Boc-Aib-OH in 3 mL $CH_2Cl_2$.[17]

*4.6.7. Boc-(R)-MPG-β-Ala-OMe.* Yield 110 mg (85%) from 100 mg (0.37 mmol) Boc-(*R*)-MPG-OH in 5 mL THF. White solid, mp 68 °C; retention time 7.28 min (HPLC, ACN/$H_2O$/TFA: 60/40/0.1); $^1$H NMR ($CDCl_3$) 1.28 (s, 9H, C(C$H_3$)$_3$), 1.77 (s, 3H, C$H_3$), 2.25 (s, 3H, C$H_3$), 2.36 (t, 2H, C$H_2$, *J*=6.8 Hz), 3.34 (t, 2H, C$H_2$, *J*=6.8 Hz), 3.50 (s, 3H, OC$H_3$), 6.06 (s, 1H, N*H*), 6.26 (s, 1H, N*H*), 7.05–7.20 (m, 4H); $^{13}$C NMR ($CDCl_3$) 21.0, 24.1, 28.2, 33.4, 35.3, 51.7, 61.6, 79.5, 125.6, 129.4, 137.2, 138.9, 154.2, 172.5, 173.7; $[\alpha]_D^{20}$ −22.8 (*c* 0.5, MeOH). $C_{19}H_{28}N_2O_5$ (364.44): calcd C 62.62, H 7.74, N 7.69; found C 62.56, H 7.74, N 7.71.

*4.6.8. Z-β-Ala-(R)-MPG-OMe.* Yield 145 mg (82%) from 100 mg (0.45 mmol) *Z*-β-Ala-OH in 5 mL THF. White solid, mp 127 °C; retention time 6.51 min (HPLC, ACN/$H_2O$/TFA: 60/40/0.1); $^1$H NMR ($CDCl_3$) 1.89 (s, 3H, C$H_3$), 2.21 (s, 3H, C$H_3$), 2.32 (m, 2H, C$H_2$), 3.34 (m, 2H, C$H_2$), 3.57 (s, 3H, OC$H_3$), 4.98 (s, 2H, OC$H_2$), 5.46 (s, 1H, N*H*), 6.81 (s, 1H, N*H*), 7.01–7.24 (m, 9H, Ar); $^{13}$C NMR ($CDCl_3$) 21.0, 22.2, 36.2, 37.2, 53.7, 61.9, 66.6, 125.6, 128.0, 128.5, 129.4, 136.6, 137.1, 137.9, 156.5, 170.5, 173.5; $[\alpha]_D^{20}$ −23.0 (*c* 0.5, MeOH). HRMS (ESI): calcd for $C_{22}H_{27}N_2O_5$ (M+$H^+$) 399.1920; found 399.1918.

*4.6.9. Boc-(R)-MPG-sar-OMe.* Yield 69 mg (53%) from 100 mg (0.37 mmol) Boc-(*R*)-MPG-OH in 5 mL $CH_2Cl_2$. Colorless oil; retention time 8.33 min (HPLC, ACN/$H_2O$/TFA: 60/40/0.1); $^1$H NMR ($CDCl_3$) 1.26 (s, 9H, C(C$H_3$)$_3$), 1.89 (s, 3H, C$H_3$), 2.26 (s, 3H, C$H_3$), 2.58 (s, 3H, NC$H_3$,), 3.66–3.85 (m, 5H, C$H_2$/OMe), 6.45 (s, 1H, N*H*), 7.08–7.24 (m, 4H); $^{13}$C NMR ($CDCl_3$) 21.1, 22.4, 28.3, 37.6, 51.7, 52.1, 61.3, 79.0, 126.0, 129.3, 137.3, 137.8, 153.3, 169.6, 173.3; $[\alpha]_D^{20}$ −14.6 (*c* 0.5, MeOH). HRMS (ESI): calcd for $C_{19}H_{29}N_2O_5$ (M+$H^+$) 365.2069; found 365.2076.

*4.6.10. Boc-(R)-MPG-Ac5c-OMe.* Yield 127 mg (94%) from 100 mg (0.37 mmol) Boc-(*R*)-MPG-OH in 5 mL THF. White powder, mp 158 °C; retention time 13.53 min (HPLC, ACN/$H_2O$/TFA: 60/40/0.1); $^1$H NMR ($CDCl_3$) 1.39 (s, 9H, C(C$H_3$)$_3$), 1.61–1.90 (m, 9H, C$H_3$/C$H_2$), 2.06–2.23 (m, 2H, C$H_2$), 2.35 (s, 3H, C$H_3$,), 3.71 (s, 3H, OMe), 5.88 (s, 1H, N*H*), 6.80 (s, 1H, N*H*), 7.16–7.32 (m, 4H); $^{13}$C NMR ($CDCl_3$) 21.0, 24.3, 25.8, 28.3, 36.7, 37.3, 52.3, 62.0, 65.9, 79.8, 125.5, 129.3, 137.2, 138.9, 154.6, 173.0, 174.3; $[\alpha]_D^{20}$ +1.4 (*c* 0.5, MeOH). $C_{22}H_{32}N_2O_5$ (404.50): calcd C 65.32, H 7.97, N 6.93; found C 65.32, H 7.96, N 6.94.

*4.6.11. Boc-Ac5c-(R)-MPG-OMe.* Yield 160 mg (91%) from 100 mg (0.44 mmol) Boc-Ac5c-OH in 4 mL THF. Colorless crystals, mp 144 °C; retention time 13.13 min (HPLC, ACN/$H_2O$/TFA: 60/40/0.1); $^1$H NMR ($CDCl_3$) 1.48 (s, 9H, C(C$H_3$)$_3$), 1.73–1.92 (m, 6H, C$H_3$/C$H_2$), 2.01 (s, 3H, C$H_3$,), 2.22–2.34 (m, 5H, C$H_3$/C$H_2$), 3.69 (s, 3H, OMe), 5.14 (s, 1H, N*H*), 7.15–7.38 (m, 4H), 7.87 (s, 1H, N*H*); $^{13}$C NMR ($CDCl_3$) 20.9, 22.3, 24.3, 28.3, 36.5, 37.0, 52.8, 61.6, 67.0, 80.0, 125.6, 129.2, 137.5, 155.0, 173.2, 173.6; $[\alpha]_D^{20}$ −49.2 (*c* 0.5, MeOH). $C_{22}H_{32}N_2O_5$ (404.50): calcd C 65.32, H 7.97, N 6.93; found C 65.26, H 7.97, N 6.82.

*4.6.12. N-Deprotection of dipeptide Z-Gly-(R)-MPG-OMe.* To a suspension of the *N*-protected dipeptide (6.5 g, 17 mmol) and palladium (10% on charcoal, 1.43 g, 11%) in methanol (70 mL) was added 5–6 N HCl in $^i$PrOH (4.3 mL) at room temperature. The flask was purged with hydrogen and the reaction mixture stirred at room temperature under atmospheric pressure of hydrogen. When the conversion was higher than 99% (HPLC), the reaction mixture was filtered on Celite, solvent was removed and warm AcOEt was added to solubilise the solid. This solution was then poured on diethylether (250 mL) to give a white precipitate. Filtration and drying gave pure HCl,H-Gly-(*R*)-MPG-OMe hydrochloride (4.6 g, 94%). White powder, mp 130 °C (dec); retention time 2.63 min (HPLC, ACN/$H_2O$/TFA: 40/60/0.1); $^1$H NMR (MeOD) 1.81 (s, 3H, C$H_3$), 2.21 (s, 3H, C$H_3$), 3.55 (s, 3H, OC$H_3$), 3.60 (d, 2H, C$H_2$, *J*=5.6 Hz), 7.28–7.56 (AA′BB′, 4H, *J*=7.9 Hz); $^{13}$C NMR (MeOD) 20.7, 23.1, 41.4, 53.0, 63.1, 126.8, 129.9, 137.7, 139.1, 166.4, 173.9; $[\alpha]_D^{20}$ −22.0 (*c* 0.5, MeOD). HRMS (ESI): calcd for $C_{13}H_{19}N_2O_3$ (M+$H^+$) 251.1396; found 251.1411.

*4.6.13. C-Deprotection of dipeptide Z-Gly-(R)-MPG-OMe.* To a cold (0 °C) mixture of the *C*-protected amino acid (1g, 2.6 mmol), water (7 mL), and $^i$PrOH (20 mL) was added 2 N sodium hydroxide in water (2.86 mL, 3 equiv). The reaction mixture was stirred for 4 h at room temperature. After removal of the solvents, water (10 mL) was added and the aqueous solution was washed with diethylether (2×5 mL). HCl (1 N) was added dropwise at 0 °C until pH=2. The white precipitate was filtered and dried in a desiccator containing $P_2O_5$ to give *Z*-Gly-(*R*)-MPG-OH (880 mg, 91%) as a white powder, mp 172 °C; retention time 5.12 min (HPLC, $CH_3CN$/$H_2O$/TFA: 40/60/0.1); $^1$H NMR (DMSO-$d_6$) 1.77 (s, 3H, C$H_3$), 2.27 (s, 3H, C$H_3$), 3.66 (d, 2H, C$H_2$, *J*=6.0 Hz), 5.04 (s, 2H, OC$H_2$), 7.11–7.36 (m, 9H, Ar), 7.57 (t,

1H, N*H*, *J*=6.0 Hz), 8.22 (s, 1H, N*H*); $^{13}$C NMR ($(CD_3)_2O$) 20.8, 23.5, 45.2, 62.6, 66.8, 126.6, 128.5, 129.1, 129.6, 137.9, 138.8, 136.8, 157.5, 169.6, 174.0; $[\alpha]_D^{20}$ −35.0 (*c* 0.5, MeOH). HRMS (ESI): calcd for $C_{20}H_{23}N_2O_5$ ($M+H^+$) 371.1607; found 371.1591.

### 4.7. Preparation of tetrapeptide *Z*-(Gly-(*R*)-MPG)$_2$-OMe

The *N*-protected dipeptide (100 mg, 0.27 mmol) and the coupling reagent HATU (1.1 equiv) were introduced in a 20 mL flask under nitrogen. Freshly distilled THF (10 mL) was then added and the mixture was stirred for 10 min at 0 °C. Diisopropylethylamine (0.104 mL, 2.2 equiv) was then added dropwise. After stirring at room temperature for 6 h, HCl,H-Gly-(*R*)-MPG-OMe (85 mg, 1.1 equiv) and diisopropylethylamine (52 μL, 1.1 equiv) were added and the mixture was stirred at room temperature overnight. Solvent was removed and dichloromethane (10 mL) was added. The organic layer was washed with 1 M aqueous $NaHCO_3$ (2×10 mL), 10% aqueous citric acid (2×10 mL), and water (20 mL), dried ($MgSO_4$), and concentrated. Flash chromatography (AcOEt 100%) gave pure tetrapeptide *Z*-(Gly-(*R*)-MPG)$_2$-OMe as a white solid (130 mg, 80%). Mp 226 °C; retention time 19.04 min (HPLC, ACN/$H_2O$/TFA: 40/60/0.1); $^1$H NMR ($CDCl_3$) 1.92 (s, 3H, C*H*$_3$), 1.98 (s, 3H, C*H*$_3$), 2.23 (s, 3H, C*H*$_3$), 2.31 (s, 3H, C*H*$_3$), 3.64 (s, 3H, OC*H*$_3$), 3.85–3.99 (m, 4H, C*H*$_2$), 4.81 (s, 2H, OC*H*$_2$), 5.67 (t, 1H, N*H*, *J*=4.2 Hz), 6.68 (t, 1H, N*H*, *J*=4.9 Hz), 7.08–7.31 (m, 13H, Ar), 7.71 (s, 1H, N*H*), 8.02 (s, 1H, N*H*); $^{13}$C NMR ($CDCl_3$) 21.0, 21.1, 22.4, 23.4, 44.4, 45.2, 53.1, 62.9, 63.6, 67.8, 126.8, 127.3, 128.8, 129.0, 129.4, 129.9, 130.2, 138.1, 138.6, 138.9, 139.0 (2C), 159.0, 171.0, 171.7, 174.4, 175.3; $[\alpha]_D^{20}$ −38.4 (*c* 0.5, $CH_2Cl_2$); $C_{33}H_{38}N_4O_7$ (602.68): calcd C 65.77, H 6.36, N 9.30; found C 65.75, H 6.62, N 9.03; IR (cm$^{-1}$) 3312, 3064, 3030, 2997, 1725, 1665. Yield=80%.

*4.7.1. N-Deprotection of tetrapeptide Z-(Gly-(R)-MPG)$_2$-OMe.* To a suspension of the protected tetrapeptide (3.72 g, 6.17 mmol) and palladium (10% on charcoal, 521 mg, 11%) in methanol (60 mL) was added 5–6 N HCl in $^i$PrOH (1.6 mL) at room temperature. The flask was purged with hydrogen and the reaction mixture was stirred at room temperature under atmospheric pressure of hydrogen. When the conversion was higher than 99% (HPLC), the reaction mixture was filtered on Celite. Solvent was removed and diethylether (20 mL) was added. Filtration and drying of the precipitate gave pure deprotected tetrapeptide hydrochloride HCl,H-(Gly-(*R*)-MPG)$_2$-OMe as a white powder (3.11 g, quant.). Mp 180 °C (dec); retention time 3.16 min (HPLC, ACN/$H_2O$/TFA: 50/50/0.1); $^1$H NMR (MeOD) 1.84 (s, 3H, C*H*$_3$), 1.92 (s, 3H, C*H*$_3$), 2.27 (s, 3H, C*H*$_3$), 2.30 (s, 3H, C*H*$_3$), 3.61 (s, 3H, OC*H*$_3$), 3.66–3.91 (m, 4H, C*H*$_2$), 7.11–7.15 (m, 4H, Ar), 7.29–7.37 (m, 4H, Ar), 8.06–8.08 (m, 1H, N*H*); $^{13}$C NMR (MeOD) 20.8, 23.4, 25.0, 41.7, 44.2, 53.0, 62.7, 63.9, 126.7, 127.0, 129.8, 130.0, 137.9, 138.5, 138.6, 138.8, 166.9, 170.6, 174.2, 174.8; $[\alpha]_D^{20}$ −30.2 (*c* 0.5, MeOH); $C_{25}H_{33}ClN_4O_5$ (505.01): calcd C, 59.46; H, 6.59; N, 11.09; found: C, 59.25; H, 6.35; N, 10.87.

*4.7.2. C-Deprotection of tetrapeptide Z-(Gly-(R)-MPG)$_2$-OMe.* To a cold (0 °C) mixture of the protected tetrapeptide (3.43 g, 5.7 mmol), water (25 mL), and $^i$PrOH (80 mL) was added 2 N aqueous sodium hydroxide (6.87 mL, 3 equiv). The reaction mixture was stirred for 2 h at room temperature. Solvents were removed and water (30 mL) was added. The aqueous layer was washed with diethylether (2×5 mL). At 0 °C, 1 N HCl was added dropwise until pH=3. White precipitate was filtered and dried ($P_2O_5$) to give deprotected tetrapeptide *Z*-(Gly-(*R*)-MPG)$_2$-OH as a white powder (2.83 g, 85%). Mp 147 °C; retention time 9.93 min (HPLC, ACN/$H_2O$/TFA: 40/60/0.1); $^1$H NMR (MeOD) 1.86 (s, 3H, C*H*$_3$), 1.92 (s, 3H, C*H*$_3$), 2.29 (s, 3H, C*H*$_3$), 2.30 (s, 3H, C*H*$_3$), 3.69–3.87 (m, 4H, C*H*$_2$), 5.10 (s, 2H, OC*H*$_2$), 7.09–7.37 (m, 13H, Ar), 8.06 (s, 1H, N*H*), 8.21 (t, 1H, N*H*, *J*=5.3 Hz), 8.51 (s, 1H, N*H*); $^{13}$C NMR (MeOD) 21.1, 23.6, 24.1, 44.8, 45.4, 63.1, 63.7, 68.0, 127.0, 127.3, 128.9, 129.1, 129.6, 129.9, 130.4, 138.0, 138.5, 138.8, 139.0, 139.1, 159.3, 171.0, 171.8, 175.7, 176.0; $[\alpha]_D^{20}$ −25.0 (*c* 0.5, MeOH). HRMS (ESI): calcd for $C_{32}H_{37}N_4O_7$ ($M+H^+$) 589.2662; found 589.2678.

### 4.8. Preparation of protected octapeptide *Z*-(Gly-(*R*)-MPG)$_4$-OMe

The *N*-protected tetrapeptide (2.57 g, 4.36 mmol) and the coupling reagent HATU (1.1 equiv) were introduced in a 250 mL flask under nitrogen. Freshly distilled THF (100 mL) was then added and the mixture was stirred for 10 min at 0 °C. Diisopropylethylamine (1.67 mL, 2.2 equiv) was then added dropwise. After stirring at room temperature for 8 h, HCl,H-(Gly-(*R*)-MPG)$_2$-OMe (2.42 g, 1.1 equiv) and diisopropylethylamine (0.84 mL, 1.1 equiv) were added and the mixture was stirred overnight at room temperature. HATU (0.5 equiv) and diisopropylethylamine (1.1 equiv) were added and the mixture was stirred at room temperature for 2 days. HATU (0.5 equiv) and diisopropylethylamine (1.1 equiv) were one more time added and the mixture was stirred at room temperature for 3 days. Solvent was removed and dichloromethane (10 mL) was added. Organic layer was washed with 1 M aqueous $NaHCO_3$ (2×30 mL), 10% aqueous citric acid (2×30 mL), and water (30 mL), dried ($MgSO_4$), and concentrated. Flash chromatography (AcOEt/MeOH 99/1) gave pure octapeptide *Z*-(Gly-(*R*)-MPG)$_4$-OMe as a white powder (3.22 g, 71%). Mp 180 °C (dec); retention time 17.07 min (HPLC, ACN/$H_2O$/TFA: 60/40/0.1); $^1$H NMR (DMSO-$d_6$) 1.67 (s, 3H, C*H*$_3$), 1.70 (s, 3H, C*H*$_3$), 1.73 (s, 3H, C*H*$_3$), 1.80 (s, 3H, C*H*$_3$), 2.25–2.28 (m, 12H, C*H*$_3$), 3.51 (s, 3H, OC*H*$_3$), 3.59–3.77 (m, 8H, C*H*$_2$), 5.03 (s, 2H, OC*H*$_2$), 7.08–7.34 (m, 21H, Ar), 7.51 (t, 1H, N*H*, *J*=5.6 Hz), 7.95 (t, 1H, N*H*, *J*=5.6 Hz), 8.07–8.10 (m, 5H, N*H*), 8.35 (s, 1H, N*H*); $^{13}$C NMR (MeOD) 21.0 (4C), 23.5, 24.2, 24.8, 25.0, 44.5, 45.2 (3C), 53.1, 62.9, 63.6, 64.1, 64.2, 67.9, 126.9, 127.1 (2C), 127.4, 128.9, 129.1, 129.5, 129.9, 130.1, 130.2, 130.3, 137.9, 138.1, 138.6, 138.7, 138.8, 138.9, 139.0, 139.1, 159.1, 171.1, 171.7, 171.8, 174.4, 175.4, 175.7, 175.8; $[\alpha]_D^{20}$ −6.0 (*c* 0.5, $CH_2Cl_2$); $C_{57}H_{66}N_8O_{11}$ (1039.18): calcd C 65.88, H 6.40, N 10.78; found C 65.85, H 6.95, N 10.73; IR (cm$^{-1}$) 3305, 3025, 1664.

*4.8.1. C-Deprotection of octapeptide Z-(Gly-(R)-MPG)$_4$-OMe.* To a cold (0 °C) mixture of the protected octapeptide (300 mg, 0.3 mmol), water (3 mL), and $^i$PrOH (8 mL) was added 2 N aqueous sodium hydroxide (315 μL, 3 equiv). The reaction mixture was stirred for 5 h at room temperature. After removal of the solvents, methanol (10 mL) was added and the solution was stirred for 1 h at room temperature and 5 min at 0 °C. Aqueous citric acid (10%) was added dropwise until pH=4 at 0 °C. Methanol was removed and white precipitate was filtered and dried in a desiccator containing $P_2O_5$ to give deprotected octapeptide *Z*-(Gly-(*R*)-MPG)$_4$-OH as a white powder (240 mg, 81%). Mp 220 °C; retention time 10.69 min (HPLC, ACN/$H_2O$/TFA: 60/40/0.1); $^1$H NMR (DMSO-$d_6$) 1.70 (s, 3H, C*H*$_3$), 1.72 (s, 3H, C*H*$_3$), 1.74 (s, 3H, C*H*$_3$), 1.80 (s, 3H, C*H*$_3$), 2.25–2.27 (m, 12H, C*H*$_3$), 3.59–3.75 (m, 8H, C*H*$_2$), 5.03 (s, 2H, OC*H*$_2$), 7.06–7.33 (m, 21H, Ar), 7.49 (t, 1H, N*H*, *J*=5.6 Hz), 7.94–8.07 (m, 6H, N*H*), 8.34 (s, 1H, N*H*), 12.64 (s, 1H, COO*H*); $^{13}$C NMR (DMSO-$d_6$) 20.7, 23.2, 23.8, 24.3, 24.8, 43.3, 43.7, 44.2, 61.0, 61.7, 61.9, 62.0, 65.7, 126.1, 126.3, 127.8, 127.9, 128.5, 128.6, 128.8, 128.9, 133.5, 136.4, 136.4, 136.5, 137.1, 138.1, 138.8, 138.9, 156.7, 168.2, 168.9, 169.0, 172.6, 172.8, 172.9; 173.6; $[\alpha]_D^{20}$ −20.4 (*c* 0.5, DMSO). HRMS (ESI): calcd for $C_{56}H_{65}N_8O_{11}$ ($M+H^+$) 1025.4773; found 1025.4758.

### 4.9. Preparation of fully deprotected octapeptides HCl,H-(Gly-(*R*)-MPG)$_4$-OH

A suspension of the *N*-protected octapeptide (1.91 g, 1.86 mmol) and palladium (10% on charcoal, 157 mg, 11%) in methanol (50 mL) was purged with hydrogen, then stirred overnight at room temperature under an atmospheric pressure of hydrogen. When reaction was complete (HPLC), 5–6 N HCl in $^i$PrOH (1.2 equiv) was added. The

mixture was filtered on Celite, solvents were removed and warm diethylether (20 mL) was added. Filtration and drying of the white precipitate gave pure deprotected octapeptide hydrochloride HCl,H-(Gly-(*R*)-MPG)$_4$-OH as a white solid (1.49 g, 86%). Mp 260 °C (dec); retention time 4.49 min (HPLC, ACN/$H_2O$/TFA: 50/50/0.1); $^1$H NMR (DMSO-$d_6$) 1.69 (s, 3H, C$H_3$), 1.71 (s, 3H, C$H_3$), 1.76 (s, 3H, C$H_3$), 1.85 (s, 3H, C$H_3$), 2.25 (s, 12H, C$H_3$), 3.75–3.82 (m, 8H, C$H_2$), 7.05–7.36 (m, 16H, Ar), 7.93–8.20 (m, 7H, N*H*), 8.75 (s, 1H, N*H*); $^{13}$C NMR (DMSO-$d_6$) 20.3, 23.1, 23.5, 24.2, 24.3, 42.9, 43.4, 60.6, 61.5, 61.6, 62.0, 125.8, 125.9, 128.3, 128.4, 128.5, 135.9, 136.0, 136.1, 136.5, 137. 7, 138.1, 138.5, 165.9, 168.0, 168.7, 168.8, 171.9, 172.3, 172.4, 173.1; $[\alpha]_D^{20}$ −21.2 (*c* 0.5, DMSO); mass: calcd 891.9, found [MH$^+$] 892.0. HRMS (ESI): calcd for $C_{48}H_{59}N_8O_9$ (M+H$^+$) 891.4405; found 891.4412.

### 4.10. Preparation of cyclooctapeptide *cyclo*(Gly-(*R*)-MPG)$_4$: cGM

In a 10 L reactor, DMF (5 L), the linear peptide (700 mg, 0.75 mmol), and BOP (1.67 g, 5 equiv) were introduced. The mixture was stirred for 10 min at room temperature and diisopropylethylamine (875 μL, 6.6 equiv) was added dropwise. After stirring at room temperature for 2 days, solvent was removed and dichloromethane (20 mL) was added to the residue. Organic layer was washed with 1 M aqueous $NaHCO_3$ (2×40 mL), 10% citric acid (2×10 mL), and water (10 mL), dried ($MgSO_4$), and concentrated. Flash chromatography ($CH_2Cl_2$/MeOH 97/6) and crystallization in a $CH_2Cl_2$/MeOH mixture gave pure cyclooctapeptide *cyclo*(Gly-(*R*)-MPG)$_4$ as colorless crystals (330 mg, 50%). Mp 330 °C (DSC); retention time 22.54 min (HPLC, ACN/$H_2O$/TFA: 50/50/0.1); $^1$H NMR 1.82 (s, 12H, C$H_3$), 2.27 (s, 12H, C$H_3$), 3.61 (dd, 4H, C$H_2$, *J*=16.2, 5.2 Hz), 3.80 (dd, 4H, C$H_2$, *J*=16.2, 6.2 Hz), 7.07–7.29 (m, 16H), 8.17 (t, 4H, N*H*, *J*=5.6 Hz), 8.26 (s, 4H, N*H*); $^{13}$C NMR 20.6, 24.3, 43.6, 61.6, 126.2, 128.6, 136.2, 138.7, 168.8, 173.0; $[\alpha]_D^{20}$ −100 (*c* 0.5, $CH_2Cl_2$), $2C_{48}H_{56}N_8O_8\cdot 1H_2O$ (873,01): calcd C 65.26, H 6.60, N 12.78; found C 65.36, H 6.51, N 12.70; mass: calcd 873.4, found [MH$^+$] 873.7; IR (cm$^{-1}$) 3550, 3325, 1672, 1527; UV $\lambda_{max}$=202 nm.

### 4.11. List of guest compounds for crystallization of host–guest complexes

(±) Phenylethanol, (±) mandelic acid, (±) α-methylbenzylamine, ethanolamine, urea, α-aminoisobutyric acid, phenol, 2,3 butanediol, and (±) 5-ethyl-5-phenylhydantoin.

## Supplementary data

Supplementary crystallographic data for CCDC 292584 and CCDC 768235 are available free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, United Kingdom; fax: +44 1223 336033 or deposit@ccdc.cam.ac.uk).

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