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ARTICLE *in* CHEMICAL COMMUNICATIONS · AUGUST 2013

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Cite this: *Chem. Commun.*, 2013, **49**, 8504

Received 26th June 2013,
Accepted 1st August 2013

DOI: 10.1039/c3cc44784g

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Synthesis of cyclic peptide hemicryptophanes: enantioselective recognition of a chiral zwitterionic guest†

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The synthesis of the first members of a new class of cyclic peptide-containing hemicryptophanes is described. Synthesis was achieved through attachment of veratryl groups to the L-tyrosine side chains of a cyclic hexapeptide, c(YG)$_3$, followed by intramolecular cyclodehydration to generate the CTV unit. The diastereomeric *P*- and *M*-hemicryptophanes were generated in a 2 : 1 ratio and were separated by chromatography. The enantioselective binding properties of the hemicryptophanes were investigated by complexation with carnitine. Both isomers were found to have significant selectivity for binding (*R*)-carnitine.

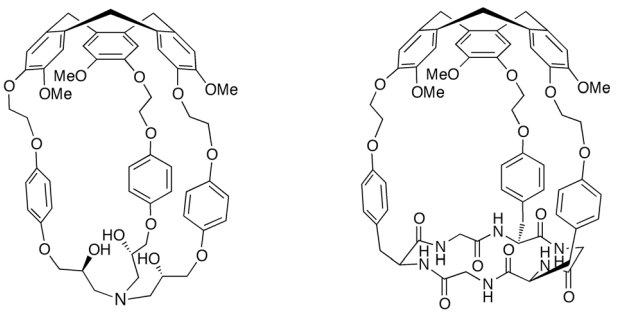

Fig. 1 Chiral hemicryptophanes.

The use of molecular containers as supramolecular host molecules that mimic enzymes and receptors has received significant recent interest.[1–3] Cryptophane hosts, which are comprised of two cyclotriveratrylene (CTV) units, have been investigated for their binding properties towards neutral and charged guests,[3] and for potential applications in chiral recognition.[3,4] Hemicryptophanes are related molecular containers that consist of a single CTV moiety connected to a capping moiety of C_3-symmetry by linkers whose nature and length can be varied (*e.g.*, **1**, Fig. 1).[5–9] The dissymmetry of the hemicryptophanes leads to heteroditopic host molecules that can encapsulate two different guest molecules,[6a] or bifunctional molecules.[6b,c] The heteroditopicity of hemicryptophanes has been exploited for catalytic[7] and recognition properties.[6,8,9]

Functionalised cyclic peptide scaffolds have been investigated as host molecules capable of selective binding of anions and cations,[1,10,11] yet their incorporation into heteroditopic hosts is rare.[11] We report the synthesis of a new class of hemicryptophane that incorporates a CTV unit connected to a cyclic hexapeptide scaffold (*e.g.* **2**, Fig. 1). The cyclic peptide-hemicryptophane design incorporates a cyclic hexapeptide such that the three-fold symmetry matches that of the CTV unit. Three tyrosine residues provide both hydrophobic 'walls' to the cavity and functional groups to link to the CTV unit. Glycine was chosen for the alternate residues to facilitate the synthesis of the cyclic peptide and minimise steric bulk within the cage.

The linear hexapeptide was prepared by standard microwave-assisted Fmoc solid phase peptide synthesis. Cleavage of the hexapeptide from the resin with 5% TFA in CH_3CN : H_2O (4 : 1) gave the unprotected linear peptide [L-Tyr-Gly]$_3$ **4** in 85% yield. Cyclisation of **4** generated the cyclic peptide **6** in quantitative yield after 1 h. Cyclisation of the unprotected linear peptide **4** proceeded much faster than that of the corresponding protected peptide **3** and without the formation of byproducts and poor solubility that plagued purification of **6** generated from **3**.

Hemicryptophanes are commonly assembled by appending three linkers to a preformed CTV unit and attaching the linkers to a central 'cap';[5a,6b,7b,8b] however, such an approach was considered implausible to link a CTV to a cyclic peptide. A template approach was therefore chosen in which the three veratryl units were attached to the cyclic peptide 'cap', with intramolecular cyclodehydration generating the CTV unit as the final step. Accordingly, the cyclic hexapeptide **6** was triply alkylated with bromoethyl vanillyl alcohol **7**[5b,12] to generate hemicryptophane precursor **8** in 55% yield. The CTV unit was generated by treatment of **8** with formic acid under high dilution conditions, providing hemicryptophane **2** in good yield (64%) as a mixture of two diastereomers (Scheme 1). The *P*- and *M*-isomers at the CTV unit were formed in a 2 : 1 ratio, and were

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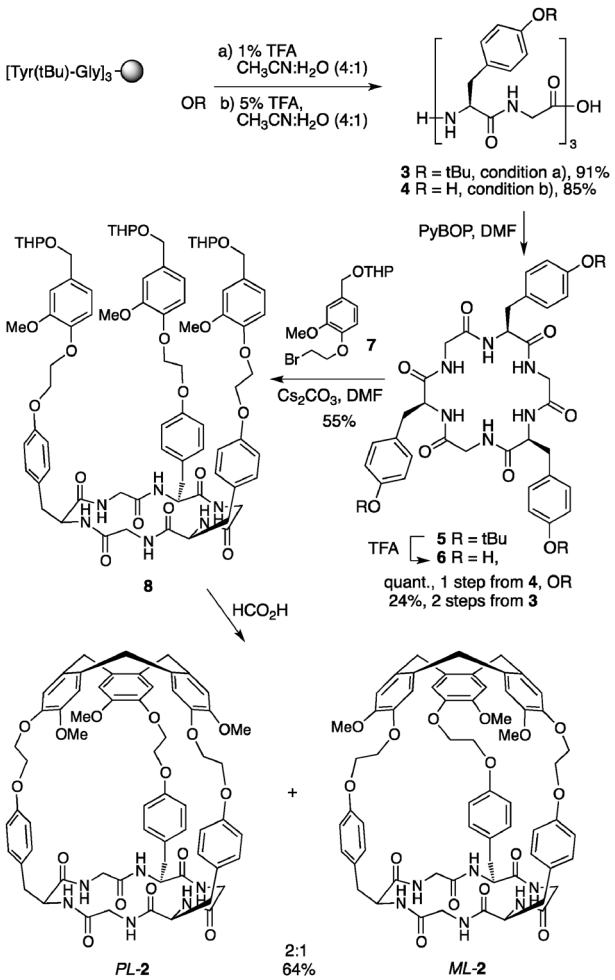
† Electronic supplementary information (ESI) available: Synthetic procedures, NMR and mass spectra of *PL*-**2** and *ML*-**2** and complexes, NMR titration data for K_a determinations. See DOI: 10.1039/c3cc44784g



Scheme 1 Synthesis of hemicryptophanes *PL*-**2** and *ML*-**2**.

readily separated by column chromatography. The inherent chirality of the cyclic peptide presumably induces a modest degree of selectivity for one configuration of the CTV unit.

The absolute configuration of the CTV units of the hemicryptophane isomers was determined by CD spectroscopy. The spectrum of the major isomer exhibits a characteristic negative–positive bisignate curve from 230–250 nm corresponding to the *P*-configuration (ESI,† Fig. S10).[13] The minor isomer shows a similar but inverted signal, thus allowing the assignment the *M*-configuration to the minor diastereomer.

The ^{1}H NMR spectra of hemicryptophanes *PL*-**2** and *ML*-**2** indicate that both molecules exhibit C_3-symmetry, with only one set of resonances for each of the three tyrosyl, glycyl, veratryl and linker units. ESI-MS experiments demonstrated the formation of host–guest complexes with simple cations such as sodium, cesium, ammonium and tetrabutyl ammonium, as well as with zwitterions taurine and lysine (see ESI,† Fig. S2 and S4–S9).

The use of inherently chiral hemicryptophanes in enantioselective recognition shows much promise, but has yet to widely explored.[9] The presence of fixed asymmetric centres introduces additional chiral recognition elements into the host structure and also avoids difficulties in the separation of enantiomeric hemicryptophanes by chiral HPLC or derivatisation of the hemicryptophanes into diastereomers.[4,13] The binding properties of *PL*-**2** and *ML*-**2** were investigated employing carnitine **9** as the guest molecule. L-Carnitine (*R*-**9**) is involved in fatty acid transport through the formation of fatty acid–carnitine esters, while the unnatural *D*- or (*S*)-isomer has no known biological role.[14] Carnitine **9** was chosen for investigation as it allows exploration of enantioselective binding properties in addition to exploitation of the ditopic nature of the hosts in association with the zwitterionic character of the guest.[6b,c]

Binding studies of the hemicryptophane isomers *PL*-**2** and *ML*-**2** with the (*R*)- and (*S*)-enantiomers of carnitine **9** were undertaken through ^{1}H NMR spectroscopic analysis upon titration in CD_3CN (Fig. 2). Job plot analysis of the titration of (*R*)-carnitine with *PL*-**2** suggested a 1 : 1 binding model (ESI,† Fig. S10). Further evidence for this 1 : 1 ratio was obtained by ESI-MS experiments, which indicated the presence of a 1 : 1 complex ($[PL\text{-}2 + R\text{-}9 + H]^+$; *m*/*z* 1308.57, ESI,† Fig. S5). Association constants (K_a) were determined from plots of $\Delta\delta$ *vs.* [guest **9**] : [host **2**],[15] and ranged from 690–4100 M^{-1} (Fig. 3 and Table 1). In all titration experiments only a single set of peaks were observed indicating fast exchange on the NMR time scale between the free and complexed species. The complexes retain the C_3 symmetry of the host. Significant downfield shifts were observed for both the glycyl and tyrosyl NH protons of the

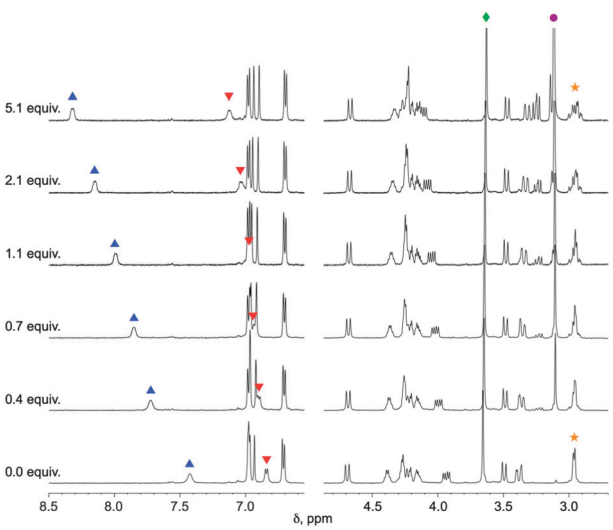


Fig. 2 ^{1}H NMR spectra (500 MHz, 298 K) from the titration of *PL*-**2** with (*R*)-carnitine *R*-**9** in CD_3CN. ▲ Gly-NH; ▼ Tyr-NH; ◆ OMe; ● NMe; ★ Tyr-βHs.

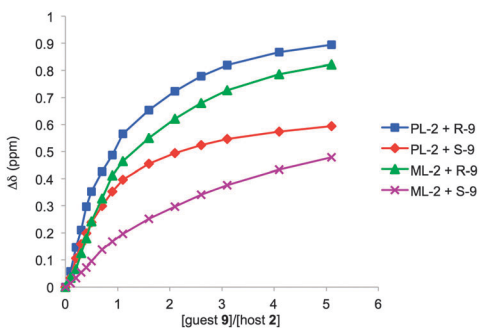


Fig. 3 Binding-induced change in ^{1}H NMR δ of glycyl-NH protons of host **2** as a function of [guest **9**] : [host **2**] ratio.

Table 1 Association constants (K_a) of hosts *PL*-**2** and *ML*-**2** with guests (*R*)- and (*S*)-carnitine **9**

Host	Guest	$\Delta\delta_{max}$[a]/ppm	K_a[b]/M^{-1}
PL-**2**	*R*-**9**	−0.8945	$4.1 \pm 0.3 \times 10^3$
PL-**2**	*S*-**9**	−0.5937	$2.7 \pm 0.2 \times 10^3$
ML-**2**	*R*-**9**	−0.7194	$9.1 \pm 0.1 \times 10^2$
ML-**2**	*S*-**9**	−0.4791	$6.9 \pm 0.5 \times 10^2$

[a] δ Gly-NH of host **2**. [b] Determined using WinEQNMR2 (ref. 15).

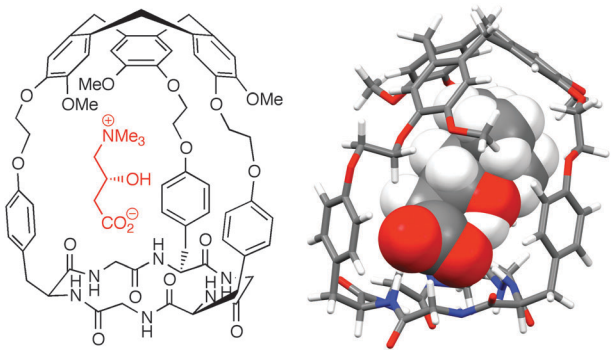


Fig. 4 Modelled *PL*-**2**:*R*-**9** complex.

cyclic peptide scaffold, with these induced shifts indicating encapsulation of the guest within the cavity.[16]

Binding of carnitine within the cavity of *PL*-**2** in acetonitrile was found to be enantioselective with *R*-**9** bound with a 1.5-fold greater K_a than *S*-**9**. Binding of carnitine within the cavity of *ML*-**2** was also found to be enantioselective, with *R*-**9** having a 1.3-fold greater K_a. Although the D-tyrosine-derived hemicryptophanes were not synthesised, association constants for the *PDR*-complex, for example, can be determined from the enantiomeric *MLS*-complex. Accordingly, a 5.9-fold difference in association constants of *R*-**9** with the *PL*-**2** and *PD*-**2** hosts was calculated. In contrast, switching from *PL*-**2** to *ML*-**2** results in a 4.5-fold drop in binding affinity for *R*-**9**. These results suggest that of all four stereoisomers of the hemicryptophane host **2**, the *PL*-**2** host is matched to bind with (*R*)-carnitine *R*-**9**.

Modelling of the inclusion complex was performed with the Gaussian 03 program[17] using a two-layer ONIOM method, where the guest, the three 'walls' comprising the tyrosine side-chain and ethyleneglycol units were computed at the B3LYP/6-31G* level of theory, with the remainder treated with the semiempirical PM3 method. Carnitine binds to the host in a ditopic fashion exploiting hydrogen-bonding interactions between the cyclic peptide moiety and the carnitine carboxylate group, together with cation–π/C–H–π interactions between the CTV unit and the carnitine quaternary ammonium group (Fig. 4). The amide groups are all twisted such that the N–H groups are directed into the cavity to make H-bonds with the carnitine carboxylate. The glycyl NHs, which point 'down' (away from the CTV unit), are significantly more distorted than the 'upwards' pointing tyrosyl NHs, consistent with a greater shift of the glycyl NH protons in the ^{1}H NMR spectrum. No H-bond interactions are observed between the host and the carnitine OH group; instead the OH group is involved in an intramolecular H-bond with the carnitine carboxylate.

In conclusion, we have designed and synthesised a new class of hemicryptophane cage, where the CTV unit is attached to a cyclic peptide scaffold. The efficient synthesis yielded hemicryptophane **2** as a mixture of diastereomers in an overall yield of 30% over 4 steps. The inherent chirality of the L-tyrosine-containing cyclic peptide scaffold induces moderate selectivity for formation of the *P*-configuration of the CTV unit. Host–guest interactions of the hemicryptophanes with the chiral, biologically relevant zwitterion carnitine were investigated, with the hemicryptophanes exhibiting modest chiral discrimination behaviour. The modular design of the cyclic peptide-based hemicryptophane framework should enable the synthesis of a range of analogues of these host molecules, with binding properties tunable through substitution of the amino acid residues.

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