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Synthesis of peptide dendrimers based on a β-cyclodextrin core with guest binding ability☆

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Abstract—The synthesis of three first-order dendrimers based on a β-cyclodextrin core containing fourteen Val, Phe and Val-Phe residues is described. The guest binding ability of the tetradecavalent peptidyl β-cyclodextrin derivative has been tested by calorimetric titration and the thermodynamic parameters for the complex formation with adamantanecarboxylic acid were obtained.

Peptide dendrimers are highly branched structures usually consisting of (1) a core or template bearing several branching points responsible for the spatial architecture and the dendrimeric character and (2) multiple copies of bioactive peptides attached onto the core.[1] Examples of those kind of compounds are the so called Multiple Antigen Peptides (MAP) developed in order to amplify synthetic peptide immunogens.[1,2] Multivalent peptide systems have been used as well in protein de novo design based on the template-assembled synthetic proteins (TASP) concept.[1,3]

Cyclodextrins[4] (CD) derivatives bearing peptides may be useful as carriers for transporting drugs to biological targets containing specific peptide receptors.[5,6] Thus, the peptide bio-recognizability together with the CD host–guest complexation properties would provide a potential site-specific drug carrier system. In addition, the well-defined torus-shaped structure of CDs[4] provides a versatile scaffold for the construction of branched structures of bioactive molecules such as peptides. Therefore, CDs are suitable templates for the application of the MAP concept as a means to increase the peptide-receptor binding and hence improve the site specificity of the drug delivery system.

We have been working on a similar concept but using carbohydrates as biological markers. We reported the synthesis of a variety of per-substituted β-CD derivatives branched and/or hyperbranched with *O*-, *S*-glycosides and glycopyranosylamines having enhanced affinity with carbohydrate-specific receptor proteins and with inclusion complexation abilities.[7]

The majority of the the reported CDs with grafted amino acids are monosubstituted derivatives in which the amino acid[8–10] or the peptide[6,8,11–14] have been bound either directly or via a spacer. Peptidyl-cyclodextrins consisting in a neuropeptide derivative bound to two CD rings have been also reported.[6] The synthesis of per-substituted CDs with phenylalanine and cysteine has been described[15] and also symmetrically-substituted CDs with the C-terminal peptide amide of gastrin have been also reported.[14,16]

Here we describe the synthesis of four first-order amino acid/peptide dendrimers based on a β-CD core following a convergent approach. The synthesis was carried out by first constructing the amino acid or peptide dendrons, followed by their coupling to the key template heptakis 6-deoxi-6-iodo-β-CD derivative[17] (**1**). The branching unit 3,3′-iminobispropylamine was converted into the bis(carbobenzyloxy) derivative **2** by regioselective reaction with benzylic alcohol, carbonyl diimidazole and KOH (Scheme 1). Compound **2** was then transformed into the *N*-chloroacetylated derivative **3** by a three-steps synthesis as reported elsewhere.[7c] We used the amino acid derivatives of H-Val-OMe (**10**) and

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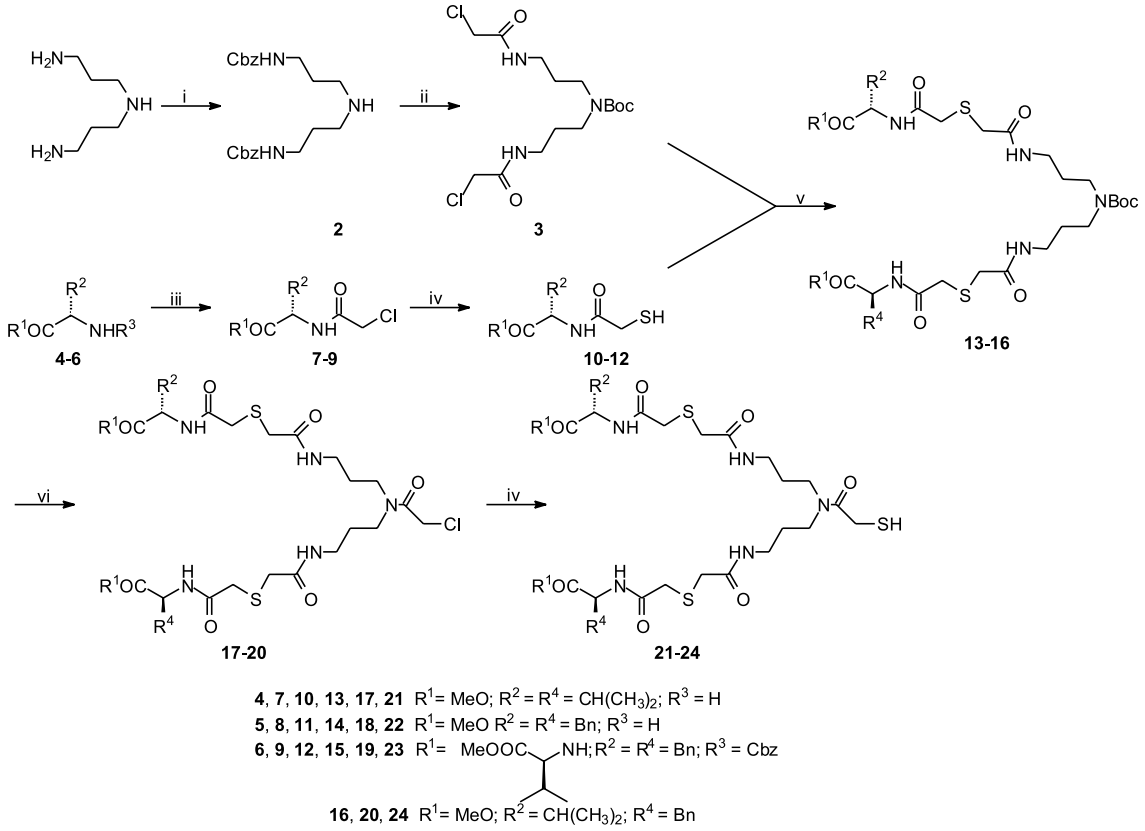
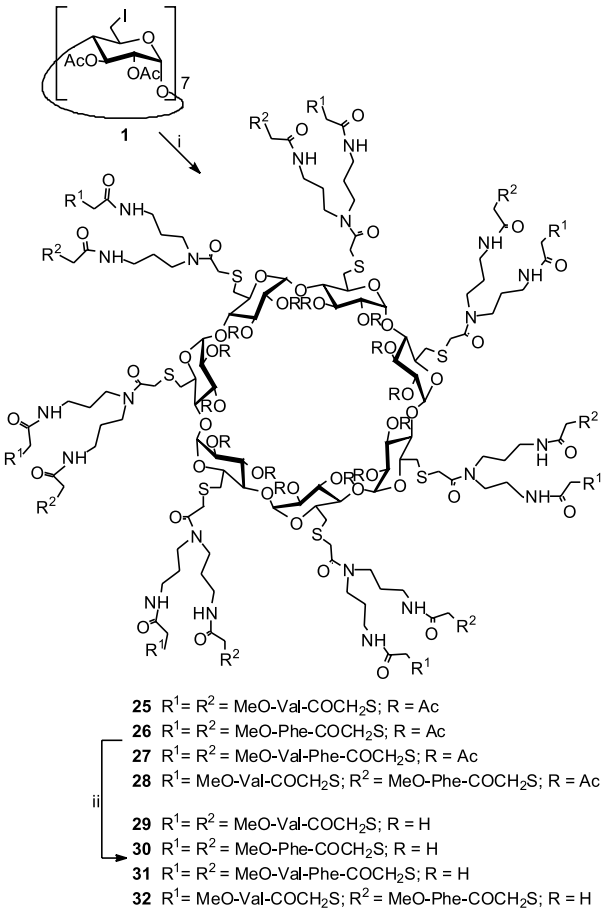


Scheme 1. *Reagents and conditions*: (i) DCI, BnOH, KOH, toluene, reflux, 64.7%. (ii) Ref. 7b. (iii) $(ClCH_2CO)_2O$, $NaHCO_3$, CH_3CN, rt, 76% (for **7**), 74% (for **8**), 84% (for **9**). (iv) (a) thiourea, acetone, rt, (b) Na_2SO_3, H_2O, 62% (for **10**), 59% (for **11**), 61% (for **12**), 61% (for **21**), 55% (for **22**), 53% (for **23**), 58% (for **24**). (v) Cs_2CO_3, DMF, rt, 81% (for **13**), 80% (for **14**), 78% (for **15**), 86.8% (for **16**). (vi) (a) TFA, dry CH_2Cl_2, rt, 2–6 h; (b) $(ClCH_2CO)_2O$, DIPEA, CH_3CN, rt, 92% (for **17**), 90% (for **18**), 86% (for **19**), 91% (for **20**).

H-Phe-OMe (**11**) and the peptide derivative of H-Phe-Val-OMe (**12**) as the *N*-mercaptoacetyl derivatives for their coupling at their *N*-terminus with **3**. Compounds **10**–**12** were synthesized from the methyl esters **4**–**6**. The amino acid methyl esters **4** and **5** were treated with chloracetic acid and sodium bicarbonate to obtain the *N*-chloroacetylated amino acids **7** and **8** in 76 and 74% yield, respectively. The peptide methyl ester **6** was subsequently subjected to hydrogenolysis and chloroacetylation to isolate *N*-chloracetyl peptide derivative **9** in 84% yield. Reaction of compounds **7**–**9** with thiourea followed by treatment with sodium sulphite afforded the thiol derivatives **10**–**12** in 58–62% yield. The bis-branched amino acid and peptide derivatives were readily obtained by the reaction of thiols **10**–**12** with **3** in the presence of Cs_2CO_3 in DMF at room temperature. Under these conditions the bis-branched derivatives **13**–**15** were obtained in 81, 80 and 79% yield, respectively. In order to prepare a multivalent diepitopic[2b] model system we also carried out the sequential nucleophilic displacement of the two chloride groups of **3** by the in situ formed cesium thiolate derivatives of **10** and **11**, respectively, obtaining the difunctional bis-branched amino acid **16** in 78% overall yield. Acidolytic removal of the Boc-*N* protecting group in **13**–**16** using TFA in CH_2Cl_2 followed by treatment with chloracetic anhydride in MeOH afforded **17**–**20** in 86–92% yield. Then, the *N*-chloracetyl derivatives **17**–**20** were treated with thiourea and sodium sulfite to give the thiols **21**–**24** (61, 55, 58 and 53%, respectively). The coupling of the dendrons **21**–**24** to the CD core was accomplished by reaction of thiols **21**–**24** with CD template **1** in the presence Cs_2CO_3 at 60°C in dry DMF.[18a] The products were isolated as the corresponding peracetylated derivatives **25**–**28** in 62–72% yield[18b] (Scheme 2). De-*O*-acetylation of **25**–**27** afforded the valine, phenylalanine and Phe-Val-containing 14-mer **29**–**31** in 82, 78–82% yield. Similarly, de-*O*-acetylation of **28** yielded the diepitopic-like tetradeca branched CD **32**, containing Phe and Val residues, in 77% yield.

The amino acid and peptidyl CDs **25**–**32** were characterised by NMR spectroscopic techniques with COSY, HMQC, and HMBC experiments and MALDI-TOF mass spectrometry. The room-temperature ^1H NMR spectra showed a considerable broadening of the signals. This indicates a restricted mobility on the NMR time scale due to the presence of amide bonds in the CD derivatives. However, it is also possible that some conformational isomerism[19a] or ring distortion[19b] may occur as a consecuence of the persubstitution on the CD primary face. When measurements of the NMR data were performed at 80°C, the resolution of the



Scheme 2. *Reagents and conditions*: (i) (a) Cs_2CO_3, DMF, 60°C, 7 days, (b) Ac_2O, Py, DMAP, 40°C, 48 h, 72% (for **25**), 69% (for **26**), 65% (for **27**), 62% (for **28**). (ii) NaOMe, MeOH, 82% (for **29**), 78% (for **30**), 76% (for **31**), 77% (for **32**).

spectra was improved considerably, allowing the assignment of the spectroscopic signals. The ^{1}H NMR spectra show that the ratios of the integrals for the signals of the appended residue protons and for the signals of those belonging to the CD core are in accordance with the structures of the products. The ^{13}C NMR spectra of **25**–**32** show carbon signals at 51.0–51.9 ppm revealing the presence of the methoxycarbonyl groups on the CD torus. ^{13}C NMR spectra of **25**–**32** display only one anomeric carbon signal at 96.3–102.7 ppm. In addition, they show signals at 57.5 and 57.3 ppm for compounds **25** and **29**, at 53.3 ppm for compounds **26** and **30** and at 57.2–58.14 and 53.3–54.0 for compounds **27**, **28**, **31** and **32**, corresponding to the Val and Phe CH α-carbons, respectively.

The inclusion complexation behaviour of the tetradecapeptidyl CD **31** with the guest molecule adamantanecarboxylic acid (AC) was tested at pH 7.4 by using isothermal titration calorimetry (ITC) (Table 1). ITC measurements provide direct determination of n, the stoichiometry, $\Delta H°$, the binding enthalpy change, and K, the affinity constant. From measurements of K, the binding free energy, $\Delta G°$, can be calculated and hence the binding entropy change, $\Delta S°$, determined.[22] In previous studies we have not detected by ITC the formation of inclusion complexes between some naphtalene derivative guests and CDs tetradecabranched with glycoside residues.[7b,c] Thus, the steric congestion on the primary face of CD due to the attachment of fourteen branches at the CD lower rim could lead to a conformational distortion of the CD torus.[19] The distortion, due to the rotation of one or more glucose units around the glycosidic linkages, would alterate the cavity shape, thus preventing guest penetration. In order to investigate whether or not the hyperbranched β-CDs could be used as guest receptors we chose AC that, unlike the naphtalene derivative guests used before,[7c,d] provides a structure with a quasispheric symmetry. In addition, AC forms a very strong 1:1 inclusion complex with β-CD.[20] Stepwise addition of aliquots of AC-containing solution to a solution of **31** led to a decrease in the extent of released heat indicating binding interaction between AC and **31**. From the performed ITC experiments we found that the best fit for the three variables, n, $\Delta H°$, and K was for a 1:2 (AC/**31**) stoichiometry. The complexation of AC with **31** is exothermic and entropy-driven and affords a less stable complex with AC than the native β-CD complex, with a binding constant value, K, of 3.78 M^{-1}.

In summary, we report the synthesis of tetradecavalent amino acid and peptide dendrimers based on a β-CD core. The persubstitution of the primary face of the CD with fourteen peptide residues modifies the CD cavity shape but has the ability to form a complex with AC.

Table 1. Thermodynamics of binding of guest AC with peptidyl CD **31** in H_2O at 25°C and pH 7.4 (10 mM sodium phosphate)

Host	n (AC:host)	$K\times10^{-4}$ (M^{-1})	$-\Delta G°$ (kcal mol^{-1})	$-\Delta H°$ (kcal mol^{-1})	$T\Delta S°$ (kcal mol^{-1})
β-CD[a]	1:1	4.00	–	5.40±0.1	–
β-CD[b]	1:1	2.89±0.86	6.10±0.02	4.50±0.03	1.59±0.03
31	1:2	0.38±0.01	4.89±0.02	1.99±0.03	2.90±0.03

[a] Ref. 20.
[b] Ref. 21.

Acknowledgements

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18. (a) *Procedure for the synthesis of* ***27***: A mixture of **1** (200 mg, 0.08 mmol), **23** (1.11 g, 1.12 mmol), Cs_2CO_3 (549 mg, 1.68 mmol) in dry DMF (10 mL) was stirred at 60°C under Ar for 7 days. After this time the reaction mixture was filtered and, then was treated with Ac_2O (12 mL), pyridine (8 mL), DMAP (cat.) and stirred for 48 h at 40°C. The reaction crude was poured into ice/H_2O, then aqueous HCl (5%, 100 mL) was added, and the mixture was extracted with EtOAc (2×200 mL). The combined organic phases were washed with aqueous HCl (5%, 100 mL), saturated $NaHCO_3$ (2×100 mL), and then with water (2×100 mL). The organic layer was dried (Na_2SO_4), filtered, and the solvent was evaporated to give a crude product that was purified by flash column chromatography to give **27** (443 mg, 65%) as a solid.
(b) *NMR data for peptidyl CD* ***27***: ^{1}H NMR (300 MHz, $(CD_3)_2SO$, 353 K): $\delta = 8.14$–7.94 (m, 28H, NH), 7.82 (bs, 14H, NH), 7.34 (m, 35H, Ar), 5.32 (dd, 7H, $^3J_{2,3} = 9.3$ Hz, $^3J_{3,4} = 8.2$ Hz, H-3), 5.19 (bs, H-1), 4.81 (dd, 7H, $^3J_{2,3} = 9.3$ Hz, $^3J_{1,2} = 3.3$ Hz, H-2), 4.79 (14H, Hα-Phe), 4.32 (t, 14H, $J = 7.3$ Hz, Hα-Val), 4.25 (d, 7H, $J = 6.5$ Hz, H-5), 4.19 (t, 7H, $^3J = 8.1$ Hz, H-4), 3.74 (d, 42H, $J = 2.4$ Hz, MeO), 3.61 (bd, 7H, $^2J_{6a,6b} = 13.7$ Hz, H-6_a), 3.56 (m, 7H, $^2J_{6a,6b} = 13.7$ Hz, $^3J_{5,6b} = 12.1$ Hz, H-6_b), 3.38 (bs, 28H, Hα-spacer), 3.40 (bs, 28H, CH_2SCH_2), 3.24 (bs, 28H, Hγ-spacer), 3.19 (bd, 14H, $J = 4.85$ Hz, CH_2S), 2.17–2.12 (6H, 2×CH_3CO), 3.16 (bd, 14H, $J = 8.9$ Hz, Hβ-Phe), 2.08 (m, 14H, Hβ-Val), 1.79 (bs, 28H, Hβ-spacer), 1.02 (m, 42H, Hγ-Val). ^{13}C NMR (75.5 MHz, $(CD_3)_2SO$, 353K): $\delta = 171.0$ (CO-Val), 170.7, 170.8 (CO-CD), 169.5, 168.7 (2CON), 167.9–168.0 ($COCH_2S$), 137.0–125.8 (Ar), 96.3 (C-1), 77.7 (C-4), 71.4 (C-2), 69.9 (C-3,5), 57.2 (Cα-Val), 53.5 (Cα-Phe), 51.0 (MeO-Val), 45.4–43.2 (Cα-spacer), 37.5 (Cβ-Phe), 37.1 ($COCH_2S$), 35.2–33.2 (Cγ-spacer), 34.7 (CH_2S), 29.6 (Cβ-Val), 28.2–27.1 (Cβ-spacer), 28.1–26.7 (C-6), 18.9–18.8 (CH_3CO), 18.4–17.8 (2Cγ-Val).
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22. Isothermal titration calorimetry experiments were performed using an MCS isothermal titration calorimeter (ITC) from Microcal, Inc. (Northampton, MA). The calorimeter was calibrated by known heat pulses as recommended by the manufacturer. The observed heat effects were concentration-independent and were identical to the heat signals detected after the saturation is reached. The raw experimental data were presented as the amount of heat produced per second following each injection of guest into the CD derivative solution (corrected for the guest heats of dilution) as a function of time. The amount of heat produced per injection was calculated by integration of the area under individual peaks by the Origin software provided with the instrument. The errors are provided by the software from the best fit of the experimental data to the model of equal and independent sites, and correspond to the standard deviation in the fitting of the curves.