Synthesis of new series of α-cyclodextrin esters as dopamine carrier molecule

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ABSTRACT

A new series of amphiphilic α-cyclodextrins were synthesized by grafting *N*-acylated amino acids [valine, leucine, phenylalanine, methionine, and tryptophan (**3a–e**)] to the primary hydroxyl groups via ester bond formation. The synthetic pathway involves selective hexa-bromination of the primary hydroxyls followed by *per*-substitution with the carboxylate moiety of the N-acetyl residues in the presence of DBU (1,8-diazabicyclo[5,4,0]undec-7-ene). The ability of the synthetic compounds for the extraction of dopamine was studied. The results showed a considerable ability of some of the amphiphilic compounds for the extraction of dopamine into octanol phase from water. To complete the study, the binding affinity of dopamine toward the synthetic host molecules was calculated by using of the molecular docking technique.

1. Introduction

Formation of inclusion complexes is the most important ability of cyclodextrins (CDs) leading to their development as drug carriers,[1] and their utility for the solubilization,[2] encapsulation,[3] and transport of biologically active molecules.[4] Amphiphilic cyclodextrin derivatives are valuable in pharmaceutical applications due to their ability for self-organization in aqueous media. Liposomes,[5] nanoparticles,[6] vesicles[7] and micellar aggregates[8] have been prepared from amphiphilic cyclodextrins to form versatile carrier and delivery systems for hydrophilic and lipophilic drugs. These compounds are produced by grafting various lengths of lipophilic chains on to the primary or secondary hydroxyl groups of the glucopyranose units. Among these derivatizations, grafting of fatty-acids, -alcohols and -amines to the primary hydroxyl group of cyclodextrins are most common.[6] The lipophilic chains of these structures would make an intimate contact with biological membranes.

Furthermore, bilayer vesicles composed of amphiphilic CDs have been shown to bind specific guests to recognize molecular signals.[9,10] This suggests that amphiphilic CDs could be used for the development of biological receptors that may help to understand the complicated mechanism of the molecular recognition by living cells.

In this study, a new series of amphiphilic α-cyclodextrin were synthesized by grafting *N*-acetylated amino acids on to the primary hydroxyl groups via ester bond formation. The new structures are composed of *N*-acylated lipophilic amino acids on the primary hydroxyl groups. The ability of the synthetic compounds for extraction of dopamine (Fig. 1), a hydrophilic bioorganic compound, was then studied.

2. Results and discussion

Our interest in the design and synthesis of amino acid-α-CD derivatives emerges from our previous work in which preparation of amphiphilic peptide β-CD as a phase transfer carrier of glucosamine was reported.[11] The desired structures have been synthesized as esters of the six primary hydroyl groups of α-CD and the carboxyl moiety of *N*-acetylated amino acids: leucine (Leu), valine (Val), phenylalanine (Phe), methionine (Met) and tryptophan (Trp) (Scheme 1).

In this molecular design, lipophilic amino acids were selected for extension of the cavity of α-CD with an external lipophilic structure.

In this molecular design, lipophilic amino acids were selected to extend the cavity of the α-CD to provide additional room for substrate binding. In addition, the amino groups of the appended amino acids were acylated to ensure the overall structure was amphiphilic with a polar end consisting of the secondary hydroxyl groups, and a non-polar end consisting of the amino acid side chains.

The synthetic pathway involves selective hexa-bromination of the hydroxyl groups of α-CD,[12] with *N*-bromosuccinamide (NBS) and triphenylphosphine (PPh₃), then substitution with the carboxylate moiety of the *N*-acetyl amino acids in the presence of DBU

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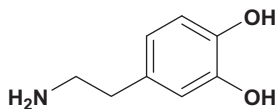*E-mail address:* sadeghianh@mums.ac.ir (H. Sadeghian).

Figure 1. Molecular structure of dopamine.

(1,8-diazabicyclo[5,4,0]undec-7-ene) (Scheme 1).[13–15] The required N-acetyl amino acids were synthesized via reaction of acetic anhydride with the amino acids. The compounds were prepared in good yields with simple workup to give the expected spectroscopic properties and acceptable elemental analyses. The steric hindrance of valine β-carbon might be a main cause of decrease in synthetic yield of compound **3b**.

Binding behavior of the compounds prepared was studied using dopamine, a natural neurotransmitter, as a guest molecule. The relative binding affinity of **3a–e** toward dopamine was measured using a phase extraction method.[16] The decrease in dopamine concentration of aqueous phase after shaking for 2 h with octanol containing **3a–e** and α-CD, was determined and reported as a fractional extraction (%E). Aqueous solutions of the dopamine (5 mM) (pH 7.4) were separately extracted with octanol (lipid phase), containing **3a–e** or α-CD, (5 mM) and compared with a control consisting octanol alone. The concentration of dopamine was determined by using of triiodide spectrophotometric method.[17] The results in Table 1 show that dopamine was extracted effectively by **3a–d** but no significant extraction was observed for **3e** or α-CD.

The binding affinity of dopamine toward **3a–e** was calculated and reported as estimated binding free energy (ΔG_b). The structures of **3a–e** were modeled via grafting the required residues to the primary hydroxyls of a α-CD crystal structure followed by geometry optimization (MM+ and PM3 methods). In the modeled molecules, residues are aligned above the α-CD ring and the cylindrical structure shape is observed in which the outer part is hydrophobic while the head (α-CD ring) possess the hydrophilic nature. The structure was stabilized by intramolecular hydrogen bonds between amide groups. The binding affinity was estimated in AuoDockTools software using the AUTODOCK4.0 program.[18] A total of 100 docked conformers of dopamine were generated for each of **3a–e**. The detailed analysis of all 100 docked models revealed that all had nearly identical orientations fixed by hydrogen bonding of the amine and hydroxyl groups of dopamine with the acetamide and sugar portions of the host molecules (Fig. 2). The averages of estimated binding free energy of docked molecules (ΔG_b) are outlined in Table 1.

The docking results of **3a–e**, showed that the binding free energy of dopamine for all of the host molecules with similar
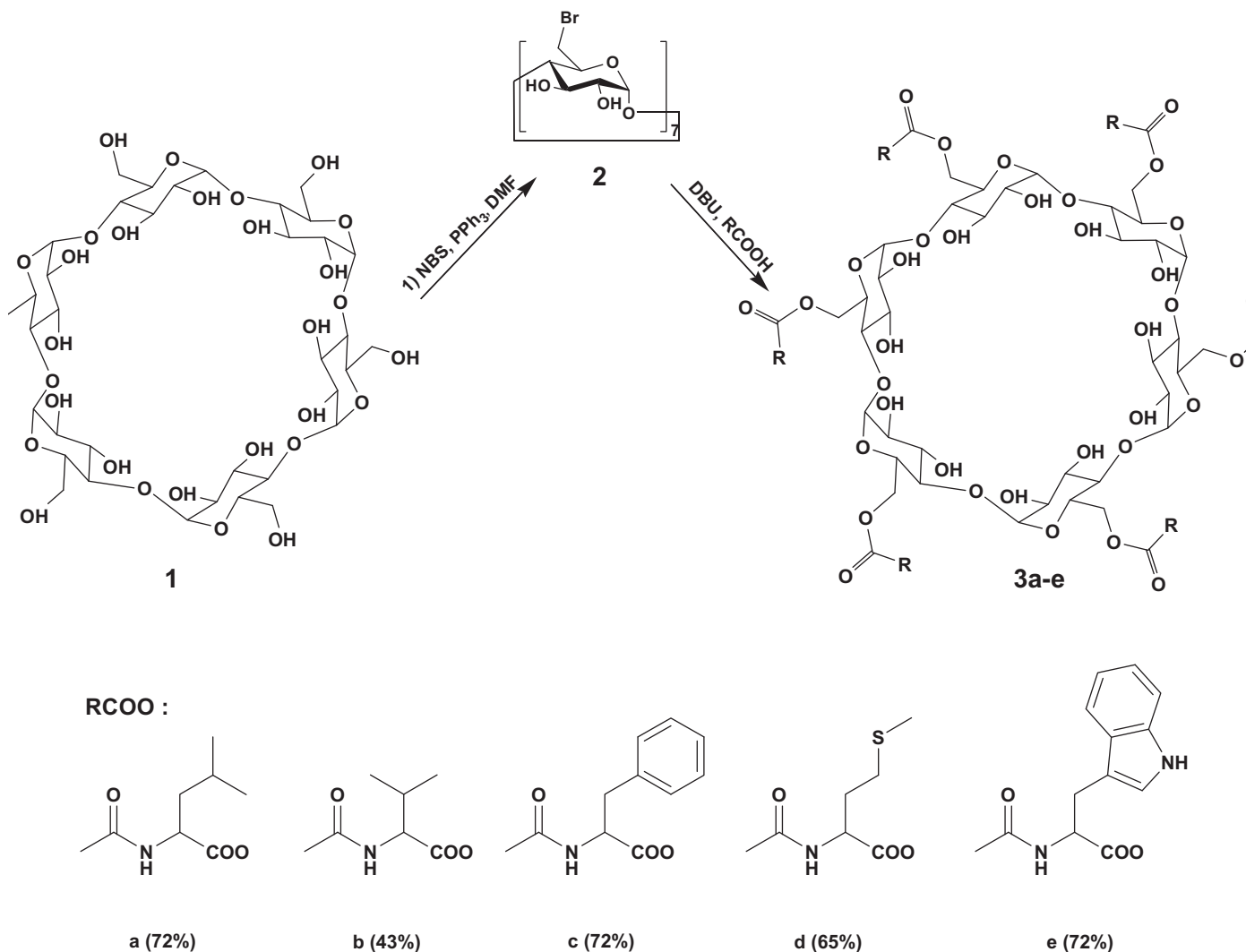
Scheme 1. General procedure for the synthesis of amphiphilic α-CDs (**3a–e**).

Table 1
Extraction content (%E), octanol–water distribution coefficient (log D), and average of estimated free energy of binding (ΔG_b kcal mol^{-1}) for compounds **3a–e**

Compd	$\bar{\Delta}G_b$	%E	Log D
3a	−8.91 ± 0.27	29.8 ± 0.7	1.19 ± 0.03
3b	−8.95 ± 0.33	25.5 ± 0.9	1.06 ± 0.04
3c	−8.81 ± 0.29	31.8 ± 0.5	1.28 ± 0.04
3d	−8.99 ± 0.31	30.7 ± 1.1	1.13 ± 0.05
3e	−8.71 ± 0.35	12.0 ± 0.3	0.91 ± 0.02
α-CD	—	4.3 ± 0.4	−1.8 ± 0.07

orientations are almost equal. This could explain the similarity of **3a–d** extraction contents. The somewhat lower affinity of **3e** is also consistent with the extraction results.

To explore the origin of these effects, the octanol–water distribution coefficient (log D) of the hosts' **3a–e** was measured using a shake flask method (Table 1).[19] It was concluded that the more lipophilic host molecules have a higher capability of extracting dopamine to the octanol phase. The results could rationalize the low extraction ability of **3e**. Self aggregation of tryptophan residue included into the CD cavity or conformational perturbation organized by torsional strains formed via steric hindrance of indol portions of tryptophans in **3e**, could be the two main factors in decrease of octanol extraction ability of dopamine in comparison with the other host molecules. The phase transfer properties of **3a–e** therefore mainly depend on the side chain molecular structure of the host since the host molecules have similar binding affinity toward dopamine; the least lipophilic and bulky derivative (**3e**) transfers the smallest proportion of the guest molecule.

3. Conclusion

In summary, we have synthesized a new series of hydrophobic esters of α-cyclodextrins as a carrier and lipid solubilizer for dopamine. By considering the external hydrophobicity and terminal hydrophilicity of these molecular structures, this type of cage-molecule could be a precursor for the design and synthesis of other similar molecules as carriers for desired biological compounds.

4. Experimental

4.1. Chemicals and instruments

Melting points were recorded on an Electrothermal type 9100 melting point apparatus. ^{1}H NMR (500 MHz) was obtained by using a Bruker Avance DRX-500 Fourier transformer spectrometer. Chemical shifts are reported in ppm (δ) downfield from tetramethylsilane (TMS). The IR spectra were obtained on a 4300 Shimadzu Spectrometer. Elemental analysis was obtained on a Thermo Finnigan Flash EA microanalyzer. All spectrophotometric measurements were carried out using an Spekol 1500 spectrophotometer.

4.2. Molecular modeling and docking study

4.2.1. Structure optimization

Structures of compounds **3a–e** were simulated by grafting desired residues on all primary hydroxyl of the X-ray structure of α-CD and minimized under molecular mechanic MM+ and semi-empirical PM3 method (convergence limit = 0.01; Iteration limit = 50; RMS gradient = 0.1 kcal mol^{-1}; Fletcher–Reeves optimizer algorithm) in HyperChem7.5.[20]
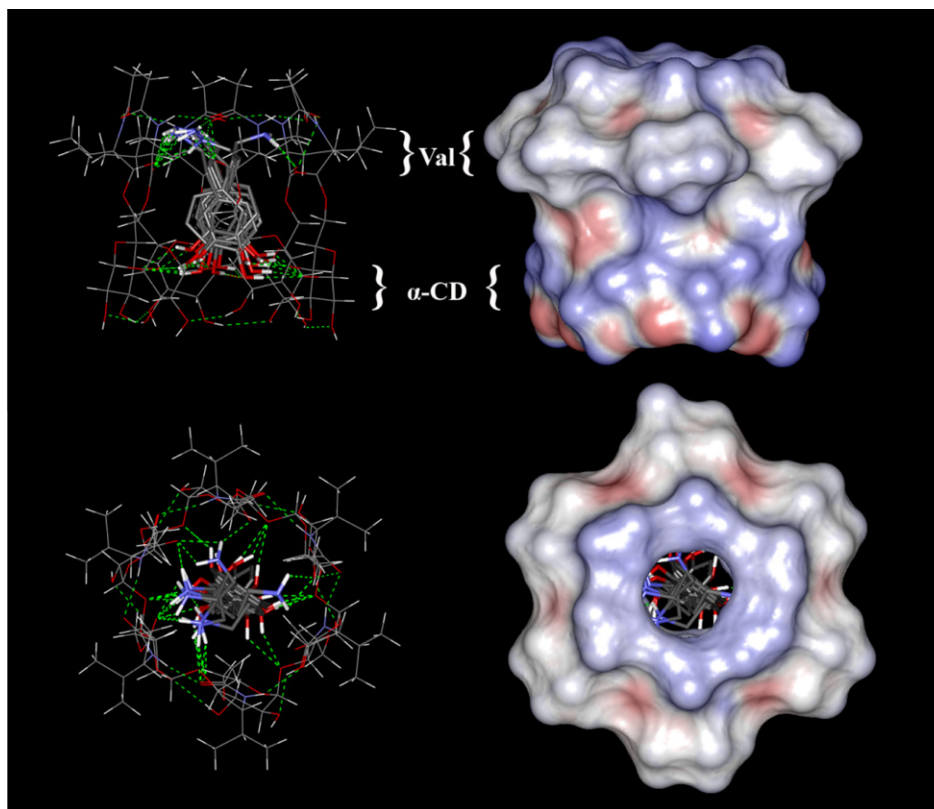

Figure 2. Side (upper) and top (lower) views of docked models of dopamine in the cavity of 3b (Val = valine residue). Hydrogen bonding is shown by dashed green lines. The colors stand as follows: blue = nitrogen; red = oxygen; gray = carbon; white = hydrogen.

The crystal structure of α-CD complexed with cyclodextrin-binding protein was retrieved from RCSB Protein Data Bank (PDB entry: 3CK7).

4.2.2. Molecular docking

Automated docking simulation was implemented to dock dopamine into the cavity of **3a**–**e** with AutoDockTools 4.0 version 1.5.4[18] using a Lamarckian genetic algorithm.[21] This method has been previously shown to produce binding models similar to the experimentally observed models.[14,22] The torsion angles of the ligands were identified, hydrogens were added to the macromolecule, bond distances were edited and solvent parameters were added to the structure. Partial atomic charges were then assigned to the host molecules as well as ligands (Gasteiger for the ligands and Kollman for the host molecules).

The regions of interest of the host molecules were defined by considering all of the molecular structure in the grid box. The docking parameter files were generated using Genetic Algorithm and Local Search Parameters (GALS) while number of generations was set to 100. The docked complexes were clustered with a root-mean-square deviation tolerance of 0.5 Å. Autodock generated 100 docked conformers of dopamine structures with lowest-energy. After the docking procedure in AD4, docking results were submitted to Accelrys DS Visualizer v2.0.1.7347[23] for further evaluations. The results of docking processing (ΔG_b (kcal mol^{-1}): estimated free energy of binding) are outlined in (Table 1).

4.3. Assessment of extraction ability

The extraction content of the synthetic host molecules and α-CD were assayed by 2 h shaking a 5 mL solution of the dopamine (5 mM) in water (pH 7.4–phosphate buffer 0.05 M) with 5 mL solution of host molecule (5 mM) in octanol at 25 °C. In the experiment, shaking of dopamine (Dop) solution with intact octanol was set as a control. The experiment was done four times for each of the host molecules and control. The concentration of dopamine in the aqueous phase was measured by using the spectrophotometric method reported by Nour El-Dien et al.[17] in which an I_3^- reagent was used as the chromogenic material. In this step 2 mL of the aqueous phase was mixed with 3 mL phosphate buffer (0.1 M, pH 5) followed by addition of 0.1 mL I_3^- (0.1 M). After shaking, the mixture was allowed to stand for 6 minutes at 23 ± 2 °C. The absorbance of the colored reaction product was measured at 500 nm. The absorbance recorded by applying the same procedure using the intact octanol was set as a control. The extraction content (%E) is equal to :

$$100 \times ([Dop]_{control} - [Dop]_{test})/[Dop]_{control}$$
$$= 100 \times (A_{control} - A_{test})/A_{control}$$

4.4. General procedure for the synthesis of 3a–e

Compound hexakis (6-bromo-6-deoxy)-α-cyclodextrin (**2**) was synthesized according to the method reported in the literature.[12] A mixture of compound **2** (0.50 g, 0.37 mmol), one of the N-acetyl amino acid **a**–**e** (4.0 mmol) and DBU (0.64 mL, 4.2 mmol) in DMF (10 mL) was heated at 70–80 °C for 12 h. After cooling, the reaction mixture was then poured in to a solution of saturated NaCl (40 mL). The precipitated solids were collected, and washed with water and potassium carbonate 5% and then oven dried to give products **3a**–**e**.

4.4.1. Hexakis [6-O-(N-acetyl-L-leucyl)]-α-cyclodextrin (3a)

White solid (0.50 g, 72%), mp: 197–198 °C; ¹H NMR: (DMSO-d_6) δ 0.82–0.83 (d, 18H, 6CH₃), 0.86–0.88 (d, 18H, 6CH₃) 1.47–1.52 (m, 12H, 6CHCH₂CH(CH₃)₂), 1.60–1.70 (m, 6H, 6CHCH₂CH(CH₃)₂), 1.84 (s, 18H, 6CH₃-acetyl), 3.32–3.41 (m, 12H, H-2, H-4), 3.85–3.87 (m, 12H, H-3 and H-5), 4.20–4.26 (m, 18H, 6NCHCO and 2H-6), 4.81 (s, 6H, H-1), 5.57–5.73 (m, 12H, OH-2 and OH-3), 8.31–8.33 (m, 6H, 6NH). ¹³C NMR (DMSO-d_6/CDCl₃) δ 21.49, 23.26, 24.65, (CH₃ and CH isobutyl, CH₃ acetyl), 40.48 (CH₂ isobutyl), 50.81 (HNCHCO), 63.69, 69.78, 72.26, 73.19 (C6, C5, C3, and C2), 82.21 (C4), 102.33 (C1), 170.08 (CONH), 172.60 (COO). IR (KBr disc) ν 1738 (C=O ester) and 1682 (C=O amide) cm⁻¹.

Anal. Calcd for C₈₄H₁₃₈N₆O₄₂: C, 52.99; H, 7.25; N, 4.41. Found: C, 52.00; H, 7.10; N, 4.25.

4.4.2. Hexakis [6-O-(N-acetyl-L-valyl)]-α-cyclodextrin (3b)

White solid (0.28 g, 43%), mp: 190–192 °C; ¹H NMR: (DMSO-d_6) δ 0.85–0.90 (d, 36H, 12CH₃), 1.85 (s, 18H, 6CH₃-acetyl), 2.10 (m, 6H, 6CH(CH₃)₂), 3.45–3.75 (m, 12H, H-2, H-4), 3.80–4.00 (m, 12H, H-5 and H-3), 4.10–4.40 (m, 18H, 2H-6 and 6NCHCO), 4.90 (m, 6H, H-1), 5.80–6.00 (m, 12H, OH-2 and OH-3), 8.35 (m, 6H, 6NH). ¹³C NMR (DMSO-d_6/CDCl₃) δ 18.20, 22.73, 30.21, (CH₃ and CH isopropyl, CH₃ acetyl), 57.65 (HNCHCO), 64.47, 69.63, 72.20, 73.37 (C6, C5, C3 and C2), 82.99 (C4), 102.44 (C1), 170.26 (CONH), 171.68 (COO). IR (KBr disc) ν 1738 (C=O ester) and 1682 (C=O amide) cm⁻¹.

Anal. Calcd for C₇₆H₁₂₆N₆O₄₂: C, 51.48; H, 6.93; N, 4.62. Found: C, 50.18; H, 6.71; N, 4.80.

4.4.3. Hexakis [6-O-(N-acetyl-L-phenylalanyl)]-α-cyclodextrin (3c)

White solid (0.56 g, 72%), mp: 162–163 °C; ¹H NMR: (DMSO-d_6) δ 1.80 (s, 18H, 6CH₃-acetyl), 2.80–3.1 (m, 12H, 6CH₂Ph), 3.32–3.50 (m, 12H, H-2, H-4), 3.80–3.92 (m, 12H, H-3 and H-5), 4.21–4.30 (m, 12H, 2H-6), 4.47–4.53 (m, 6H, 6NCHCO), 4.79–4.84 (m, 6H, H-1), 5.50–5.56 (m, 6H, OH-3), 5.62–5.69 (m, 6H, OH-2), 7.22 (s, 30H, ArH), 8.30–8.36 (m, 6H, 6NH). ¹³C NMR (DMSO-d_6) δ 22.63, 36.75 (CH₃ acetyl, CHCH₂Ph), 54.12 (HNCHCO), 64.04, 69.69, 72.21, 73.29 (C6, C5, C3 and C2), 82.15 (C4), 102.38 (C1), 126.93 (C4-Ar), 128.66 (C3, C5-Ar), 129.27 (C2, C4-Ar), 137.87 (C1-Ar), 170.06 (CONH), 171.90 (COO). IR (KBr disc) ν 1738 (C=O ester) and 1682 (C=O amide) cm⁻¹.

Anal. Calcd for C₁₀₂H₁₂₆N₆O₄₂: C, 58.12; H, 5.98; N, 3.99. Found: C, 57.88; H, 5.87; N, 3.85.

4.4.4. Hexakis [6-O-(N-acetyl-L-metionyl)]-α-cyclodextrin (3d)

White solid (0.48 g, 65%), mp: 145–147 °C; ¹H NMR: (DMSO-d_6) δ 1.76–1.80 (m, 18H, 6CH₃S), 1.85 (m, 30H, 6CH₃-acetyl and 6CHCH₂CH₂SCH₃), 1.93–2.00 (m, 12H, 6CHCH₂CH₂SCH₃) 3.40–3.48 (m, 12H, H-2, H-4), 3.63–3.73 (m, 6H, H-3), 3.75-3.83 (m, 6H, H-5), 3.99-4.07 (m, 6H, 6NCHCO), 4.22–4.28 (m, 12H, 2H-6), 4.81–4.85 (m, 6H, H-1), 5.89–5.96 (m, 12H, OH-2 and OH-3), 8.22–8.24 (m, 6H, 6NH). ¹³C NMR (DMSO-d_6) δ 15.26, 22.63, 36.75 (CH₃S, CH₃ acetyl), 27.62 (CHCH₂CH₂SCH₃), 29.49 (CHCH₂CH₂SCH₃) 53.62 (HNCHCO), 64.06, 69.71, 72.20, 73.38 (C6, C5, C3 and C2), 82.26 (C4), 102.47 (C1), 170.15 (CONH), 172.28 (COO). IR (KBr disc) ν 1737 (C=O ester) and 1680 (C=O amide) cm⁻¹.

Anal. Calcd for C₇₈H₁₂₆N₆O₄₂S₆: C, 46.56; H, 6.27; N, 4.18; S , 9.55 Found: C, 47.10; H, 6.12; N, 4.37; S , 9.09.

4.4.5. Hexakis [6-O-(N-acetyl-L-tryptophyl)]-α-cyclodextrin (3e)

White solid (0.60 g, 72%), mp: 186–187 °C; ¹H NMR: (DMSO-d_6) δ 1.08 (s, 18H, 6CH₃-acetyl), 3.01–3.20 (m, 12H, 6CH₂CH-3-Indolyl), 3.20–3.58 (m, 12H, H-2, H-4), 3.79–3.83 (m, 6H, H-3), 3.90–3.96 (m, 6H, H-5), 4.31–4.40 (m, 12H, 2H-6H), 4.50–4.55 (m, 6H, 6NCHCO), 4.89–4.93 (m, 6H, H-1), 5.60–5.78 (m, 12H, OH-2 and OH-3), 6.95–7.49 (m, 30H, ArH), 8.32–8.41 (m, 6H, 6NH), 10.85 (br s, 6H, NH-Indol). ¹³C NMR (DMSO-d_6) δ 22.89, 27.13 (CH₃

acetyl, CH_2CH-3-Indolyl), 53.82 (HNCHCO), 63.98, 69.78, 72.23, 73.36 (C6, C5, C3 and C2), 82.32 (C4), 102.80 (C1), 110.28 (C3-Indol), 111.88 (C4-Indol), 118.48 (C5-Indol), 118.89 (C6-Indol), 120.89 (C7-Indol), 123.97 (C2-Indol), 128.79 (C9-Indol), 136.33 (C4-Indol), 169.87 (CONH), 171.98 (COO). IR (KBr disc) v 1740 (C=O ester) and 1682 (C=O amide) cm^{-1}.

Anal. Calcd for $C_{108}H_{114}N_{12}O_{42}$: C, 57.60; H, 5.06; N, 7.46. Found: C, 58.23; H, 5.12; N, 7.19.

Acknowledgements

Supplementary data

Supplementary data (copies of ^{1}H NMR and ^{13}C NMR spectra for compounds **3a**–**e**) associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.05.048.

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