



## Design and synthesis of a new series of amphiphilic peptide- $\beta$ -cyclodextrins as phase transfer carriers for glucosamine

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### ARTICLE INFO

#### Article history:

Received 8 April 2010

Received in revised form 7 June 2010

Accepted 28 June 2010

Available online 3 July 2010

#### Keywords:

Peptide synthesis

DBU

Extraction

Molecular modeling

log *D*

### ABSTRACT

A new series of amphiphilic  $\beta$ -cyclodextrins were designed and synthesized by grafting peptide chains on to all primary hydroxyl groups via ester bond formation. The desired amphiphilic structures have been produced from ester connection between the C-6 of  $\beta$ -cyclodextrin and the carboxyl group of *N*-acetylated residues: H<sub>2</sub>N–Leu–COOH, H<sub>2</sub>N–Leu–Gly–COOH, H<sub>2</sub>N–Leu–Gly–Leu–COOH, and H<sub>2</sub>N–Leu–Gly–Leu–Gly–COOH (**3a–d**). The synthetic pathway involves selective bromination of all primary hydroxyls of  $\beta$ -cyclodextrins and then substitution with the carboxylate moiety of the mentioned *N*-acetyl residues in the presence of DBU (1,8-diazabicyclo[5,4,0]undec-7-ene). The ability of the synthetic compounds for extraction and phase transfer of glucosamine, as a hydrophilic organic compound, was then studied. The results showed a considerable ability of these amphiphilic compounds for extraction and a selective tendency of **3c** for phase transfer of glucosamine.

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## 1. Introduction

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

Furthermore, bilayer vesicles composed of amphiphilic CDs have been shown to bind specific guests to recognize molecular signals.<sup>9,10</sup> 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 and bacteria. While a large variety of amphiphilic CDs were synthesized by modifying with long alkyl chains as hydrophobic substituents, only a few works based on peptide functionalized CDs were reported.<sup>6</sup>

In this study, a new series of amphiphilic  $\beta$ -cyclodextrin derivatives were designed and synthesized by grafting peptide chains on to the primary hydroxyl groups via ester bond formation. The new structures are composed of various lengths of *N*-acylated lipophilic peptide chains on the primary hydroxyl groups. The ability of the synthetic compounds for extraction and phase transfer of glucosamine, a hydrophilic organic compound, was then studied.

## 2. Results and discussion

Our interest in the design and synthesis of  $\beta$ -CD peptide derivatives emerges from the work of Imamura et al. in which preparation of amphiphilic compounds from per-amino- $\beta$ -CD using peptide chemistry was reported.<sup>11</sup> The desired amphiphilic structures have been produced from ester linkage between all the C-6 of  $\beta$ -CD and the carboxyl group of *N*-acetylated residues: H<sub>2</sub>N–Leu–COOH, H<sub>2</sub>N–Leu–Gly–COOH, H<sub>2</sub>N–Leu–Gly–Leu–COOH, and H<sub>2</sub>N–Leu–Gly–Leu–Gly–COOH (Scheme 1).

In this molecular designing, *L*-leucine (Leu), the most lipophilic amino acid, was selected for extension of the cavity of  $\beta$ -CD and

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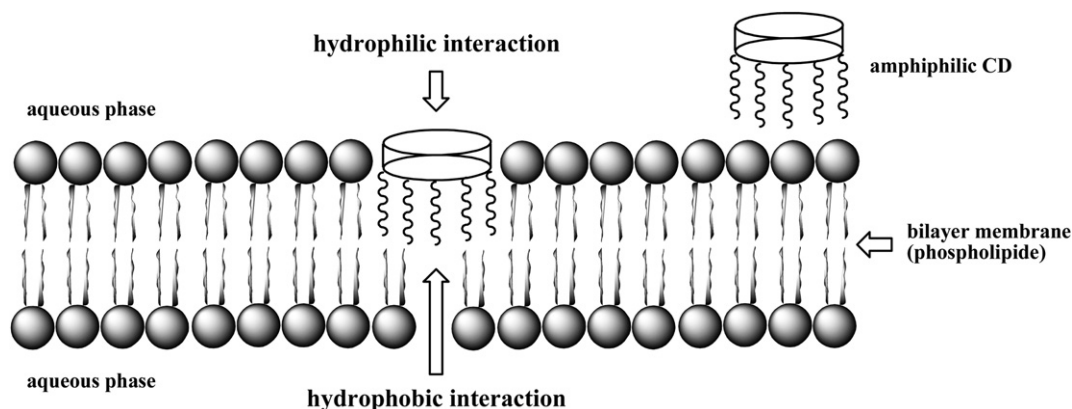
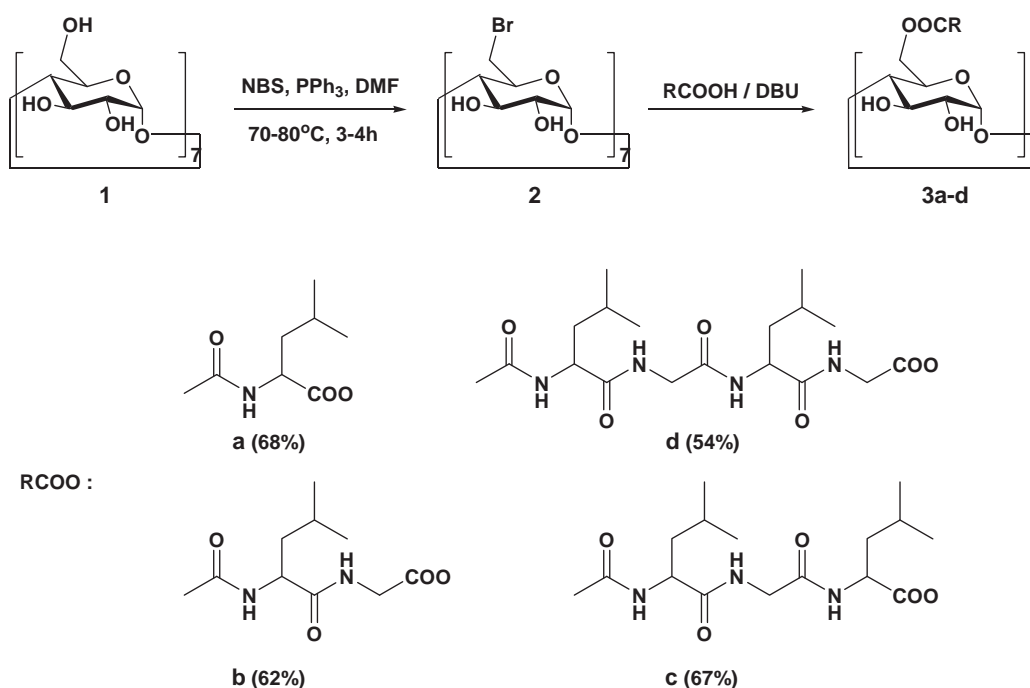


Figure 1. Schematic presentation of amphiphilic  $\beta$ -CD situated in bilayer membrane.



Scheme 1. General procedure for the synthesis of amphiphilic  $\beta$ -CDs (**3a–d**).

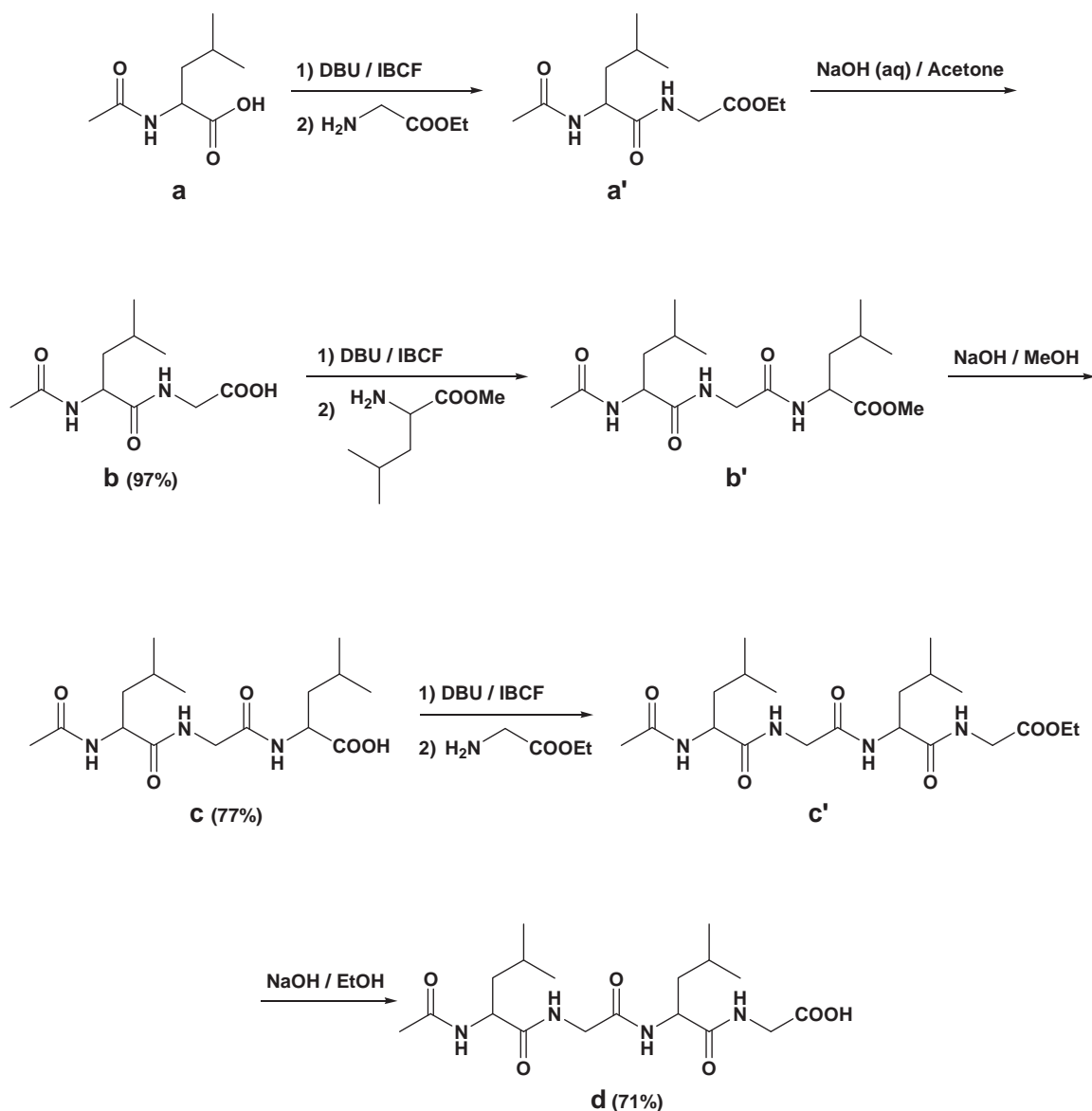
organizing an external lipophilic structure. Due to the inhibiting effect of pH on the amphiphilic property of the compounds, the *N*-acetyl form of the aforementioned residues was used. To prevent steric hindrance adjust to leucine, Gly was selected as the preferred connector of the two residues.

The synthetic pathway involves selective bromination of all primary hydroxyl groups of  $\beta$ -CD<sup>12</sup>, with the *N*-bromosuccinamide (NBS) and triphenylphosphine (PPh<sub>3</sub>), then substitution with the carboxylate moiety of the mentioned *N*-acetyl residues in the presence of DBU (1,8-Diazabicyclo[5,4,0]undec-7-ene).<sup>13</sup> Peptide chains were synthesized via reaction of isobutyl carbonate of the *N*-acetyl residue with methyl or ethyl ester of the desired amino acid<sup>14</sup> and finally *C*-terminal ester hydrolysis (Scheme 2). The carbonate was formed in situ via reaction of the DBU-functionalized carboxyl group of residue with isobutyl chloroformate (IBCF).<sup>14</sup> In comparison with triethylamine,<sup>15</sup> using DBU led to an increase in peptide yield with less by-product formation. Reaction of IBCF with *N*-acetyl leucine (**a**) in the presence of 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) produces a corresponding carbonate. In situ condensation of the carbonate with glycine ethyl ester produces the compound (**a'**). Alkali hydrolysis of **a'** lead to formation of *N*-acetyl

peptide **b**. Reaction of Isobutyl chloroformate (IBCF) with **b** in the presence of DBU afford to formation of the related carbonate. Directly reaction of the carbonate with leucine methyl ester produces the compound **b'**. Alkali hydrolysis of **b'** led to formation of *N*-acetyl peptide **c**. *N*-acetyl tetrapeptide **d** was synthesized from **c** according to the procedure reported for **b**. It was found that yield of ester hydrolysis increased when NaOH(aq)/acetone, NaOH/MeOH, and NaOH/EtOH were used for di, tri, and tetrapeptide, respectively (Scheme 2). On the basis of the mentioned synthetic pathway, four amphiphilic compounds, **3a–d**, were synthesized (Scheme 1).

Binding behavior was studied using glucosamine as a guest molecule. The relative binding affinity of **3a–d** toward glucosamine was measured using a phase extraction method.<sup>16</sup> The decrease in concentration of aqueous glucosamine after shaking for 12 h with a lipid phase (octanol) containing **3a–d** and  $\beta$ -CD was determined and reported as extraction content (%E).

Aqueous solutions of the glucosamine (5 mM) (pH 7.4) were separately extracted with octanol (lipid phase), containing **3a–d** and  $\beta$ -CD, (5 mM) and compared with the control. The glucosamine was extracted effectively and without selectivity by **3a–d** but no significant extraction was observed for  $\beta$ -CD (Table 1). If it be

**Table 1**

Extraction and phase transferring percentage of glucosamine (%E and %T, respectively) by compounds **3a–d**

Case	%E	%T
<b>3a</b>	24.4±0.9	1.62±0.3
<b>3b</b>	23.8±2.2	1.50±0.2
<b>3c</b>	28.6±1.8	3.49±0.3
<b>3d</b>	26.3±1.2	1.63±0.4
<b>CD</b>	0.40±0.2	0.74±0.1
<b>Ctrl.</b>	0.21±0.1	0.43±0.1

assumed that glucosamine (Glu) equivalently (1:1) interacts with the host molecules, the equilibrium constant of extraction could be defined as the binding constant ( $K_b$ ):

$$K_b = \frac{[\text{host} - \text{Glu}]}{([\text{Glu}][\text{host}])} \quad \text{host} + \text{Glu} \rightleftharpoons \text{host} - \text{Glu} \quad (1)$$

By considering the insolubility of glucosamine in octanol and the very low water solubility of the host–Glu complex, it was assumed that:

$$[\text{host} - \text{Glu}] = [\text{Glu}]_{\text{aq}}(\text{before extraction}) - [\text{Glu}]_{\text{aq}}(\text{after extraction})$$

and

$$\begin{aligned} [\text{Glu}] &= [\text{Glu}]_{\text{total}} - [\text{host} - \text{Glu}] \text{ and } [\text{host}] \\ &= [\text{host}]_{\text{total}} - [\text{host} - \text{Glu}] \end{aligned}$$

The observed glucosamine binding constants ( $K_b$ ) of **3a–d** are outlined in (Table 2).

**Table 2**

Octanol/water distribution coefficient (log  $D$ ), experimental free energy of binding ( $\Delta G_b^\circ$ ), binding constant ( $K_b$ ) and average of estimated free energy of binding ( $\Delta G_b$ ) for compounds **3a–d**

Compd	$\Delta G_b$	$\Delta G_b^\circ$	$K_b$	log $D$
<b>3a</b>	-5.81±0.13	-2.62	84.2	1.17±0.07
<b>3b</b>	-6.17±0.22	-2.60	80.8	1.05±0.04
<b>3c</b>	-5.55±0.26	-2.79	110.8	1.57±0.05
<b>3d</b>	-6.11±0.29	-2.70	95.6	1.11±0.09

In the other experiment, phase transfer of glucosamine by **3a–d** and  $\beta$ -CD was determined. In this work the tendency of each compound for glucosamine transferring between two aqueous

phases, separated by octanol, was measured. Two stirred aqueous phases (pH 7.4), in which one of them (primary phase) contained glucosamine (25 mM), were connected through octanol, containing test compound (**3a–d** and  $\beta$ -CD; 5 mM), without any solvent diffusion (Fig. 2). After 24 h the glucosamine concentration of the intact aqueous phase (secondary phase), was determined. The ratio of glucosamine concentration of the primary and secondary phases was recorded as phase transferring extent (%T).

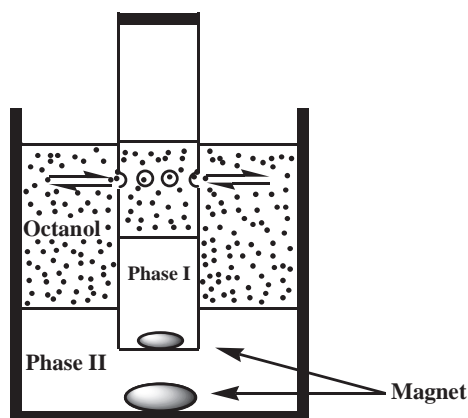


Figure 2. Schematic presentation of the apparatus used for phase transfer experiment.

Among the tested compounds, a modest phase transfer was observed for **3c**, while the experiments with **3a,b**, and **d** showed worse results (Table 1). Phase transfer of glucosamine by  $\beta$ -CD was significantly lower.

To explore the origin of these effects, the octanol/water distribution coefficient ( $\log D$ ) of **3a–d** was measured using shake flask method (Table 2).<sup>17</sup> By considering the  $\log D$  values, the phase transfer results of **3a–d** could be rationalized. It was concluded that more lipophilic host molecule (**3c**;  $\log D$  1.57) has a higher capability for transferring glucosamine in determined period time.

To complete the study, the binding affinity of glucosamine toward **3a–d** was calculated and reported as estimated binding free energy ( $\Delta G_b$ ). In this section, the 3D structures of **3a–d** were modeled via grafting the desired residues on all primary hydroxyls of  $\beta$ -CD crystal structure followed by geometry optimization (PM3 methods). In the modeled molecules, residues are aligned above the  $\beta$ -CD ring and the cylindrical structure shape is observed in which the outer part is hydrophobic while the head ( $\beta$ -CD ring) possess the hydrophilic nature. The structure was stabilized by cross intermolecular hydrogen bonds of amide groups. The binding affinity was estimated in Auo-DockTools software using autodock4.0 program.<sup>18</sup> 100 docked conformers of glucosamine were generated in ADT software for each of **3a–d**. The detailed assessment of all 100 docked models revealed as followings: 100% of docking results had nearly identical orientations in the  $\beta$ -CD ring with average  $\Delta G_b$  of  $-5.81$  kcal/mol for **3a**; while for **3b**, 68% of docking results had nearly identical orientations in the  $\beta$ -CD ring and 32% in the pocket formed by the residues with average  $\Delta G_b$  of  $-6.13$  and  $-6.25$  kcal/mol, respectively; for **3c**, 58% of docking results had nearly identical orientations in  $\beta$ -CD ring and 42% in the mentioned pocket with average  $\Delta G_b$  of  $-5.62$  and  $-5.45$  kcal/mol, respectively, and finally for **3d**, 69% of docking results had nearly identical orientations in  $\beta$ -CD ring and 31% in the mentioned pocket with average  $\Delta G_b$  of  $-6.14$  and  $-6.08$  kcal/mol, respectively (Fig. 3) (Table 2). Due to the orientation mentioned above, the docked conformers displayed hydrogen bonds with hydroxyl and amides of

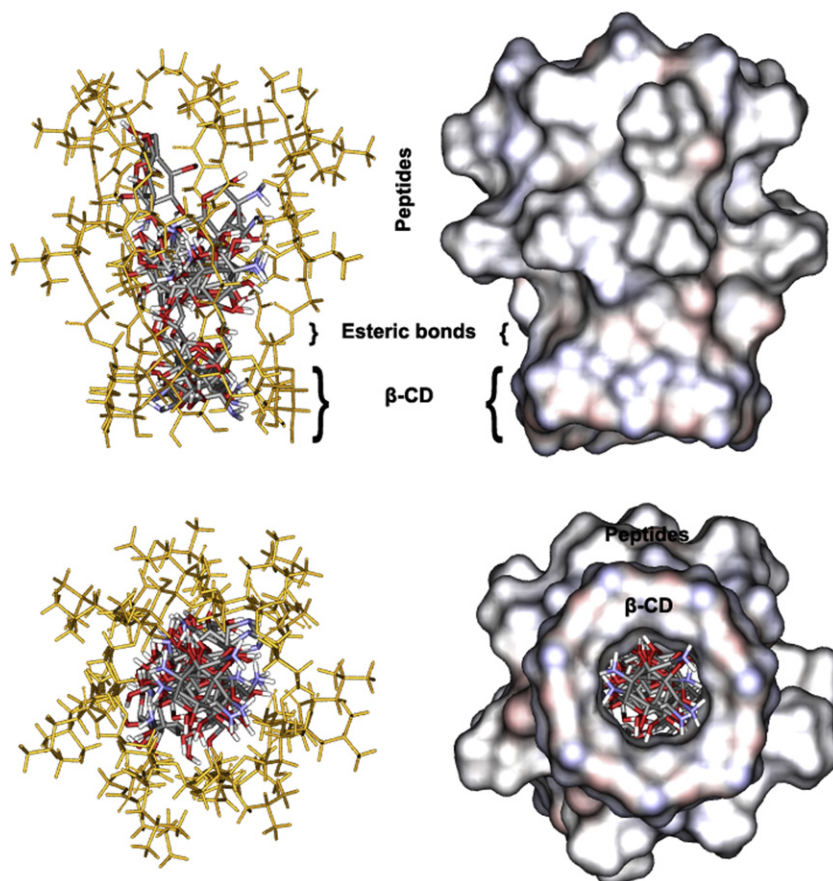


Figure 3. All of the docked models of glucosamine in the cavity of **3d**. The below figure represents the cross view of docked models in **3d** cavity.

the sugars and residues in **3a–d**. Considering  $\Delta G_b^0 = -RT \ln K_b$  and Eq. (1), the high similarity between experimental and theoretical results ( $\Delta G_b^0$  and  $\Delta G_b$ , respectively) was anticipated.

Docking results of **3a–d** showed that the binding free energy of glucosamine for all of the host molecules is almost equal and the docked models are mainly located in  $\beta$ -CD cavity. This could explain why the extraction contents and  $K_b$  of **3a–d** are similar.

Phase transfer properties of **3a–d** is mainly depend on the external lipophilic character of their cavity while the host molecules have similar binding affinity toward glucosamine, the more lipophilic one (**3c**), would transfer higher quantities of the guest molecule.

### 3. Conclusion

In summary, we have designed and synthesized a new series of hydrophobic peptide  $\beta$ -cyclodextrins as a phase transfer carrier for glucosamine. These types of cage-molecules could be a precursor for designing and synthesis of other similar molecules as carriers for desired biological compounds in the future. Considering the external hydrophobicity and terminal hydrophilicity of these molecular structures, it would be hypothesized, that the natural capacity of these compounds to locate in the bilayer membrane of the cells, act as a channel for transferring special compounds.

## 4. Experimental

### 4.1. Chemicals and instruments

Melting points were recorded on an Electrothermal type 9100 melting point apparatus.  $^1\text{H}$  NMR (500 MHz) and  $^{13}\text{C}$  NMR (125 MHz) were obtained by using a Bruker Avance DRX-500 Fourier transformer spectrometer. Chemical shifts are reported in parts per million ( $\delta$ ) 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 Agilent 8453 spectrophotometer.

### 4.2. Molecular modeling and docking study

**4.2.1. Structure optimization.** 3D structures of compounds **3a–d** were simulated by grafting desired residues on all primary hydroxyl of the X-ray structure of  $\beta$ -CD and minimized under semi-empirical PM3 method (Convergence limit=0.01; Iteration limit=50; rms gradient=0.1 kcal/mol; Fletcher–Reeves optimizer algorithm) in HyperChem7.5.<sup>19</sup>

Crystal structure of  $\beta$ -CD complexed with cyclodextrin-binding protein was retrieved from RCSB Protein Data Bank (PDB entry: 2ZYN).

**4.2.2. Molecular docking.** Automated docking simulation was implemented to dock glucosamine into the cavity of **3a–d** with AutoDockTools 4.0 version 1.5.4<sup>18</sup> using Lamarckian genetic algorithm.<sup>20</sup> This method has been previously shown to produce binding models similar to the experimentally observed models.<sup>14,21</sup> 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 enzyme 3D structure. Partial atomic charges were then assigned to the macromolecule 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 glucosamine structures with lowest-energy. After docking procedure in AD4, docking results were submitted to Accelrys DS Visualizer v2.0.1.7347<sup>22</sup> for further evaluations. The results of docking processing ( $\Delta G_b$ : Estimated Free Energy of Binding) are outlined in (Table 2).

### 4.3. Assessment of extraction and phase transfer ability

The extraction content of the synthetic host molecules and  $\beta$ -CD were assayed by 12 h shaking a 2 mL solution of the glucosamine (5 mM) in water (pH 7.4—phosphate buffer) with 2 mL solution of host molecule (5 mM) in octanol at 25 °C. In the experiment, shaking of glucosamine 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 glucosamine in aqueous phase was measured by using the spectrophotometric method reported by Rondle et al.<sup>23</sup> in which acetylacetone and Ehrlich's reagent used as chromogenic materials. The extracted quantity of glucosamine as same as the Glu–host concentration is equal to:

$$\frac{[\text{Glu}]_{(\text{before extraction})} - [\text{Glu}]_{(\text{after extraction})}}{\text{content}(\%T) = 100 \times [\text{Glu} - \text{host}] / [\text{Glu}]_{\text{total}}}$$

Phase transferring extent (%T) of compounds **3a–d** was determined by using the apparatus shown in (Fig. 2).<sup>24</sup> In Figure 2, phase I (10 mL) is aqueous solution of glucosamine (25 mM) with pH 7.4 (phosphate buffer) and phase II (30 mL) is only aqueous solution of phosphate buffer (pH 7.4). The octanol phase (30 mL) containing the host molecule (5 mM) contacts with both aqueous phases. In control test, the octanol phase had no host compound. After stirring of the aqueous phases (24 h), the glucosamine concentration in phase II was measured according to the Rondle method. The experiment was repeated three times for each host molecules and control. %T equal to:

$$100 \times [\text{Glu}]_{\text{phase II}} / [\text{Glu}]_{\text{total}}$$

**4.3.1. N-Acetyl-L-leucylglycine (b).** Isobutyl chloroformate (1.4 mL, 11 mmol) was added dropwise to a solution of N-acetyl-L-leucine (**a**) (1.73 g, 10 mmol) and 1.6 mL of DBU in 50 mL chloroform while stirring in ice-water. After removing the ice-bath, the reaction mixture was stirred at room temperature for 1 h. Glycine ethyl ester (1.39 g, 10 mmol) was then added and stirring continued for 2 h. The resulting solution was washed with 1 M NaOH (2 × 30 mL), 1 M HCl (2 × 30 mL), and water (1 × 30 mL). The organic layer was dried over anhydrous magnesium sulfate. After removing the solvent under reduced pressure, the residue was recrystallized from hexane to give N-acetyl-L-leucylglycine ethyl ester (**a'**) (1.41 g, 82%).

White crystal, mp: 223–225 °C;  $^1\text{H}$  NMR: ( $\text{CD}_3\text{OCD}_3$ )  $\delta$  0.85–0.93 (m, 6H, 2CH<sub>3</sub>), 1.20 (t,  $J=7$  Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.50–1.70 (m, 3H, CHCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 1.91 (s, 3H, CH<sub>3</sub>-acetyl), 3.90 (d,  $J=6$  Hz, 2H, NCH<sub>2</sub>CO), 4.15 (q,  $J=6$  Hz, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 4.40–4.53 (m, 1H, NCHCO), 7.30 (m, 1H, NH), 7.60 (m, 1H, NH).

A suspension of **a'** (1 g, 3.8 mmol) in 6 mL of NaOH 1 N and acetone (4 mL) was stirred at room temperature for 2 h, and after removing acetone, acidified with HCl 6 M. The precipitated crystals were collected and washed with water. The crystals were recrystallized from ethyl acetate and oven dried to give N-Acetyl-L-leucylglycine (**b**) (0.97 g, 97%).

White crystal, mp: 176–178 °C;  $^1\text{H}$  NMR: (DMSO-*d*<sub>6</sub>)  $\delta$  0.85–0.9 (m, 6H, 2CH<sub>3</sub>), 1.4–1.6 (m, 3H, CHCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 1.80 (s, 3H, CH<sub>3</sub>-acetyl), 3.70 (d,  $J=6$  Hz, 2H, NCH<sub>2</sub>CO), 4.30 (q,  $J=6$  Hz, 1H, NCHCO), 8.00 (d,  $J=9$  Hz, 1H, NH), 8.20 (t,  $J=6$  Hz, 1H, NH), one exchangeable proton is missing.

Anal. Calcd for  $C_{10}H_{18}N_2O_3$ : C, 52.17; H, 7.83; N, 12.17. Found: C, 52.34; H, 7.80; N, 12.22.

4.3.2. *N*-Acetyl-*L*-leucylglycyl-*L*-leucine (**c**). Isobutyl chloroformate (1.4 mL, 11 mmol) was added dropwise to a solution of *N*-acetyl-*L*-leucylglycine (**b**) (2.30 g, 10 mmol) and DBU (1.6 mL) in chloroform (50 mL) while stirring in ice-water. After removing the ice-bath, the reaction mixture was stirred at room temperature for 1 h. Leucine methyl ester (1.81 g, 10 mmol) was then added and stirring continued for 3 h. The resulting solution was washed with 1 M NaOH (2×30 mL), 1 M HCl (2×30 mL), and water (1×30 mL). The organic layer was dried over anhydrous magnesium sulfate. After removing the solvent under reduced pressure, the residue was recrystallized from ethyl acetate/hexane to give *N*-acetyl-*L*-leucylglycyl-*L*-leucine methyl ester (**b'**) (1.58 g, 69%).

White crystal, mp: 133–135 °C;  $^1H$  NMR: ( $CD_3OCD_3$ )  $\delta$  0.80–0.97 (m, 12H, 4CH<sub>3</sub>), 1.5–1.7 [m, 6H, 2CHCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>], 1.91 (s, 3H, CH<sub>3</sub>-acetyl), 3.60 (s, 3H, OCH<sub>3</sub>), 3.70–3.90 (m, 2H, NCH<sub>2</sub>CO), 4.20–4.40 (m, 1H, NCHCO), 7.40–7.53 (m, 2H, 2NH), 7.70–7.85 (br m, 1H, NH).

Anal. Calcd for  $C_{17}H_{31}N_3O_5$ : C, 57.12; H, 8.74; N, 11.76. Found: C, 56.49; H, 8.70; N, 11.77.

A suspension of **b'** (0.89 g, 2.5 mmol) in solution of NaOH (2 g) in methanol (30 mL) was stirred at room temperature for 2 h. Then water (10 mL) was added and after removing of methanol, acidified with HCl 6 M. The precipitated crystals were collected and washed with water. The crystals were recrystallized from acetone and oven dried to give *N*-Acetyl-*L*-leucylglycyl-*L*-leucine (**c**) (0.68 g, 77%).

White crystal, mp: 174–175 °C;  $^1H$  NMR: (DMSO-*d*<sub>6</sub>) 0.80–0.87 (m, 12H, 4CH<sub>3</sub>), 1.4–1.48 (m, 4H, 2CHCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 1.54–1.62 (m, 2H, 2CHCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 1.82 (s, 3H, CH<sub>3</sub>-acetyl), 3.62–3.73 (m, 2H, NCH<sub>2</sub>CO), 4.14–4.22 (m, 1H, NCHCO), 4.26–4.32 (m, 1H, NCHCO), 7.94–8.04 (m, 1H, 2NH), 8.05–8.07 (m, 1H, NH), 8.16–8.32 (m, 1H, NH), one exchangeable proton is missing.

Anal. Calcd for  $C_{16}H_{29}N_3O_5$ : C, 55.98; H, 8.45; N, 12.24. Found: C, 55.77; H, 8.49; N, 12.20.

4.3.3. *N*-Acetyl-*L*-leucylglycyl-*L*-leucylglycine (**d**). Isobutyl chloroformate (1.4 mL, 11 mmol) was added dropwise to a solution of *N*-acetyl-*L*-leucylglycyl-*L*-leucine (**c**) (3.43 g, 10 mmol) and of DBU (1.6 mL) in chloroform (50 mL) while stirring in ice-water. After removing the ice-bath, the reaction mixture was stirred at room temperature for 1 h. Glycine ethyl ester (1.81 g, 10 mmol) was then added and stirring continued for 3 h. The resulting solution was washed with 1 M NaOH (2×30 mL), 1 M HCl (2×30 mL), and water (1×30 mL). The organic layer was dried over anhydrous magnesium sulfate. After removing of the solvent under reduced pressure, the residue was recrystallized from hexane to give *N*-acetyl-*L*-leucylglycyl-*L*-leucine methyl ester (**c'**) (2.16 g, 63%).

White crystal, mp: 140–141 °C;  $^1H$  NMR: (DMSO-*d*<sub>6</sub>, ppm)  $\delta$  0.81 (d,  $J=6.5$  Hz, 6H, 2CH<sub>3</sub>), 0.86 (d,  $J=6.5$  Hz, 6H, 2CH<sub>3</sub>), 1.14 (t,  $J=10$  Hz, 3H, OCH<sub>2</sub>CH<sub>3</sub>), 1.39–1.47 (m,  $J=7.3$  Hz, 4H, 2CHCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 1.55–1.6 (m,  $J=7$  Hz, 2H, 2CHCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 1.82 (s, 3H, CH<sub>3</sub>-acetyl), 3.65–3.79 (m, 4H, 2NCH<sub>2</sub>CO), 4.05 (q,  $J=7.1$  Hz, 2H, OCH<sub>2</sub>CH<sub>3</sub>), 4.16–4.22 (m, 1H, NCHCO), 4.25–4.32 (m, 1H, NCHCO), 7.78 (d,  $J=8.35$  Hz, 1H, NH), 8.03 (dd,  $J=10$ , 5.35 Hz, 1H, NH), 8.19–8.22 (m, 1H, NH), 8.4 (t,  $J=8$  Hz, 1H, NH).

Anal. Calcd for  $C_{20}H_{36}N_4O_6$ : C, 56.06; H, 8.47; N, 13.07. Found: C, 55.79; H, 8.44; N, 12.99.

A suspension of **c'** (1.07 g, 2.5 mmol) in solution of NaOH (2 g) in ethanol (30 mL) was stirred at room temperature for 2 h. Then water (10 mL) was added and after removing of ethanol, acidified with HCl 6 M. The precipitated crystals were collected and washed with water. The crystals were recrystallized from acetone and oven dried to give *N*-Acetyl-*L*-leucylglycyl-*L*-leucine (**d**) (0.76 g, 71%).

White crystal, mp: 170–171 °C;  $^1H$  NMR: (DMSO-*d*<sub>6</sub>, ppm)  $\delta$  0.80–0.87 (m, 12H, 4CH<sub>3</sub>), 1.40–1.48 (m, 4H, 2CHCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>),

1.53–1.62 (m, 2H, 2CHCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 1.82 (s, CH<sub>3</sub>-acetyl), 3.6–3.75 (m, 4H, 2NCH<sub>2</sub>CO), 4.17–4.20 (m, 1H, NCHCO), 4.28–4.32 (m, 1H, NCHCO), 7.74 (d,  $J=8.4$  Hz, 1H, NH), 8.05–8.09 (dd,  $J_1=7.4$  Hz,  $J_2=7.4$  Hz, 1H, NH), 8.1–8.24 (m, 2H, 2NH), 12.5 (br s, 1H, COOH)

Anal. Calcd for  $C_{18}H_{32}N_4O_6$ : C, 53.98; H, 8.05; N, 13.99. Found: C, 53.72; H, 7.99; N, 14.01.

#### 4.4. General procedure for the synthesis of **3a–d**

Compound heptakis (6-bromo-6-deoxy)- $\beta$ -cyclodextrin (**2**) was synthesized according to the method reported in the literature.<sup>12</sup> A mixture of compound **2** (0.50 g, 0.32 mmol), one of the peptides **a–d** (4.0 mmol) and DBU (0.64 mL, 4.2 mmol) in DMF (10 mL) was heated at 70–80 °C for 12–48 h (for **a** and **b** 12 h and for **c** and **d** 48 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–d**.

4.4.1. Heptakis [6-*O*-(*N*-acetyl-*L*-leucyl)]- $\beta$ -cyclodextrin (**3a**). White solid (0.34 g, 68%), mp: 197–198 °C;  $^1H$  NMR: (DMSO-*d*<sub>6</sub>)  $\delta$  0.86 (d,  $J=6$  Hz, 42H, 14CH<sub>3</sub>), 1.45–1.55 (m, 14H, 7CHCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 1.60–1.66 (m, 7H, 7CHCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 1.85 (s, 21H, CH<sub>3</sub>-acetyl), 3.40–3.48 (m, 14H, H-2, H-4), 3.60–3.69 (m, 7H, H-3), 3.80–3.89 (m, 7H, H-5), 4.03–4.10 (m, 7H, 7NCHCO), 4.22–4.28 (m, 14H, 2H-6), 4.80–4.85 (m, 7H, H-1), 5.89–5.96 (m, 14H, OH-2 and OH-3), 8.22–8.24 (m, 7H, 7NH).  $^{13}C$  NMR (DMSO-*d*<sub>6</sub>)  $\delta$  21.56, 22.63, 23.28, 24.91 (CH<sub>3</sub> and CH isobutyl, CH<sub>3</sub> acetyl), 40.39 (CH<sub>2</sub> isobutyl), 50.66 (HNCHCO), 63.63, 69.59, 72.67, 73.18 (C6, C5, C3, and C2), 81.94 (C4), 102.65 (C1), 169.99 (CONH), 172.66 (COO). IR (KBr disc)  $\nu$  1738 (C=O ester) and 1682 (C=O amide)  $cm^{-1}$ .

Anal. Calcd for  $C_{98}H_{161}N_7O_{49}$ : C, 52.99; H, 7.31; N, 4.41. Found: C, 52.70; H, 7.10; N, 4.25.

4.4.2. Heptakis [6-*O*-(*N*-acetyl-*L*-leucylglycyl)]- $\beta$ -cyclodextrin (**3b**). White solid (0.31 g, 62%), mp: 210–212 °C;  $^1H$  NMR: (DMSO-*d*<sub>6</sub>)  $\delta$  0.84 (d, 42H, 14CH<sub>3</sub>), 1.35–1.50 (m, 14H, 7CHCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 1.53–1.63 (m, 7H, 7CHCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 1.84 (s, 21H, 7CH<sub>3</sub>-acetyl), 3.40–3.46 (m, 14H, H-2, H-4), 3.58–3.64 (m, 7H, H-3), 3.79–3.87 (m, 21H, H-5, and 7NCH<sub>2</sub>CO), 4.19–4.31 (m, 21H, 2H-6, 7NCHCO), 4.80–4.90 (m, 7H, H-1), 5.80–6.00 (m, 14H, OH-2, and OH-3), 7.91–8.00 (m, 7H, 7NH), 8.25–8.39 (m, 7H, 7NH).  $^{13}C$  NMR (DMSO-*d*<sub>6</sub>)  $\delta$  21.97, 22.94, 23.49, 24.62 (CH<sub>3</sub> and CH isobutyl, CH<sub>3</sub> acetyl), 41.03, 41.27 (CH<sub>2</sub> isobutyl, HNCH<sub>2</sub>CO), 51.16 (HNCHCO), 63.84, 69.33, 72.64, 73.28 (C6, C5, C3, and C2), 81.95 (C4), 102.67 (C1), 169.74, 169.92 (CONH), 173.28 (COO). IR (KBr disc)  $\nu$  1731 (C=O ester) and 1680 (C=O amide)  $cm^{-1}$ .

Anal. Calcd for  $C_{112}H_{182}N_{14}O_{56}$ : C, 51.33; H, 7.00; N, 7.48. Found: C, 51.96; H, 7.19; N, 7.81.

4.4.3. Heptakis [6-*O*-(*N*-acetyl-*L*-leucylglycyl-leucyl)]- $\beta$ -cyclodextrin (**3c**). White solid (0.33 g, 67%), mp: 197–199 °C;  $^1H$  NMR: (DMSO-*d*<sub>6</sub>)  $\delta$  0.85 (d,  $J=10$  Hz, 84H, 28CH<sub>3</sub>), 1.43–1.59 (m, 42H, 14CHCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 1.84 (s, 21H, 7CH<sub>3</sub>-acetyl), 3.35–3.38 (m, 14H, H-2 and H-3, H-4), 3.64–3.89 (m, 28H, H-3 and H-5, and 7NHCH<sub>2</sub>CO), 4.1–4.32 (m, 28H, H-6, and 14NCHCO), 4.81–4.87 (m, 7H, H-1), 5.89–6.06 (m, 14H, OH-2, and OH-3), 8.00–8.31 (m, 21H, 21NH).  $^{13}C$  NMR (DMSO-*d*<sub>6</sub>)  $\delta$  21.60, 22.00, 22.09, 22.92, 23.31, 23.44, 24.60 (CH<sub>3</sub> and CH isobutyl, CH<sub>3</sub> acetyl), 40.96, 40.23 (CH<sub>2</sub> isobutyl, HNCH<sub>2</sub>CO covered by DMSO pick), 50.53, 51.74, 52.00 (HNCHCO), 63.66, 69.51, 72.61, 73.26 (C6, C5, C3, and C2), 81.76 (C4), 102.69 (C1), 169.36, 170.14, 172.40 (CONH), 173.03 (COO). (KBr disc)  $\nu$  1733 (C=O ester) and 1681 (C=O amide)  $cm^{-1}$ .

Anal. Calcd for  $C_{154}H_{259}N_{21}O_{63}$ : C, 54.20; H, 7.65; N, 8.26. Found: C, 53.13; H, 7.27; N, 8.15.

4.4.4. **Heptakis [6-O-(N-acetyl-L-leucylglycyl-L-leucylglycyl)]-β-cyclodextrin (3d)**. White solid (0.27 g, 54%), mp: 200–201 °C; <sup>1</sup>H NMR: (DMSO-*d*<sub>6</sub>) δ 0.87 (d, *J*=6.5 Hz, 84H, 28CH<sub>3</sub>), 1.44–1.6 (m, 42H, 14CHCH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub>), 1.84 (s, 21H, 7CH<sub>3</sub>-acetyl), 3.33 (m, 14H, H-2, and H-4), 3.61–3.84 (m, 42H, H-3 and H-5, and 14NCH<sub>2</sub>CO), 4.21–4.32 (m, 28H, 2H-6, and 14NCHCO), 4.85–4.93 (m, 7H, H-1), 5.81–5.96 (m, 14H, OH-2, and OH-3), 7.8–8.3 (m, 28H, 28NH). <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 21.84, 22.09, 22.57, 22.90, 23.42, 23.58, 24.61 (CH<sub>3</sub> and CH isobutyl, CH<sub>3</sub> acetyl), 40.57, 41.00, 41.21, 42.45 (CH<sub>2</sub> isobutyl, HNCH<sub>2</sub>CO), 51.12, 51.85, 52.00 (HNCHCO), 63.66, 69.58, 72.56, 73.33 (C6, C5, C3, and C2), 81.90 (C4), 102.53 (C1), 169.17, 170.05, 170.35, 170.66 (CONH), 173.11 (COO). (KBr disc) ν 1735 (C=O ester) and 1681 (C=O amide) cm<sup>-1</sup>.

Anal. Calcd for C<sub>168</sub>H<sub>280</sub>N<sub>28</sub>O<sub>70</sub>: C, 52.94; H, 7.30; N, 10.29. Found: C, 52.36; H, 7.40; N, 9.96.

#### Acknowledgements

We express our sincere gratitude to Dr. Hossein. Orafaie for reviewing the manuscript. The results described in this paper were part of a M.Sc. student thesis proposal.

#### Supplementary data

Copies of <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra for compounds **b**, **c**, **d**, and **3a–d**. Supplementary data associated with this article can be found in online version at doi:10.1016/j.tet.2010.06.075. These data include MOL files and InChIKeys of the most important compounds described in this article.

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