Supporting information

Penetratin derivative-based nanocomplexes for enhanced intestinal insulin delivery

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Synthesis

The synthesis procedure was schemed in Fig 1a. Mono-6-deoxy-6-amino-β-cyclodextrin (compound 2, β-CD-NH_2; degree of substitution in one β-CD molecule = 1.0) was prepared by the reported procedure via tosylation, nucleophilic substitution with sodium azide, and reduction with triphenyl phosphine [1]. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.30-3.65 (m, 42 H), 4.41-4.51 (m, 6 H), 4.83, 4.98 (two d, 7 H), 5.64-5.76 (m, 14H); (Fig S1).

Bis-CD-glutamic acid derivative (Compound **3,** 2-(tert-butyl-loxycarbonylamino)-N^1, N^5-bis(6-mono- 6-deoxy-β-cyclodextrin) pentanediamide), was obtained by di-coupling one molecule of N-protected glutamic acid (N-Boc-L-glutamic acid) with two moieties of β-CD-NH_2. Briefly, N-protected amino acid (0.247 g, 1.0 mmol), HOBT (0.270 g, 2.0 mmol), and DCC (0.412 g, 2.0 mmol) were dissolved in DMF (10 ml) and stirred at 25 ℃ for 1 h. β-CD-NH_2 (2.268 g, 2.0 mmol) was added and then stirred for another 48 h at 25 ℃. The insoluble dicyclohexylurea was filtered off and then the remaining solution was precipitated by addition over acetone (100 ml). The crude product was purified on a Sephadex G15 column (60% yield). Compound **3** was obtained by removing the N-protecting Boc group using TFA. Briefly, Bis-CD-glutamic acid derivative (0.248 g, 0.1 mmol) was dissolved in TFA (10 ml) and the mixture was stirred at 25 ℃ for 1 h. The solvent was removed by evaporation under reduced pressure. The residue was dissolved in 1M NaOH and then poured into acetone. The white precipitate was filtered and dried under vacuum (80% yield.). TLC analysis of compound **3** performed on silica plates (1-butonal: ethanol: NH_4OH: Water-4:5:6:3) showed one major spot. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 1.71-2.15 (m, 4 H), 3.30-3.63 (m, 84 H), 4. 45-4.47 (m, 12 H), 4.82 (m, 14 H), 5.64-5.76 (m, 28 H); (Fig S2). MS m/z: 2377.5; (Calcd 2377.8); (Fig S3)

Dicyclohexylcarbodiimide (61.8 mg, 0.3 mmol) was added to a cold solution of appropriate amount of 2-maleimido acetic acid (0.1 mmol) in dry N,N-dimethylformamide (5 ml), and the solution was stirred at 5 ℃ for 30 min. To this solution, compound **3** (238 mg, 0.1 mmol) was added in one portion. After 24 h, the insoluble precipitate of dicyclohexylurea was filtered off and the remaining solution was precipitated by addition over acetone (10 ml), washed several times with acetone and dried under high vacuum to give the compound **4** in 60–70% yield. ^{1}H-NMR (400 MHz, DMSO-d_6) δ: 3.30-3.65 (m, 42 H), 4.35 (s, 2 H), 4.41-4.51 (m, 6 H), 4.83, 4.98 (two d, 7 H), 5.64-5.76 (m, 14H), 7.02 (s, 2H); (Fig S4). MS m/z: 2537.7 for

$[M+Na]^+$; (Calcd 2537.8); (Fig S5).

The PEN peptide was synthesized with an additional cysteine residue at its amino terminus in order to facilitate site-specific conjugation (CRQIKIWFQNRRMKWKK, KaiJie Biopharm Co., Ltd. China). PEN-bis-β-CD conjugate (compound **5**, P-bis-CD) was synthesized with the procedure described below. Compound **4** (100.6 mg, 0.04 mmol) and Cys-PEN (47.0 mg, 0.02 mmol) peptide were dissolved in a 3 ml phosphate buffer solution (pH 5.5), and agitated for 12 h at room temperature for coupling. The solution was precipitated by addition over methanol. P-bis-CD conjugate was purified on a Sephadex G25 column. Yield 90%; MS m/z: 4863.6 for $[M-H]^-$ (Calcd 4863.7) (Fig. S6). LC-MS m/z: 1623.8 for $[1/3M+H]^+$, 1217.4 for $[1/4M+H]^+$, 974.5 for $[1/5M+H]^+$; purity > 95%.

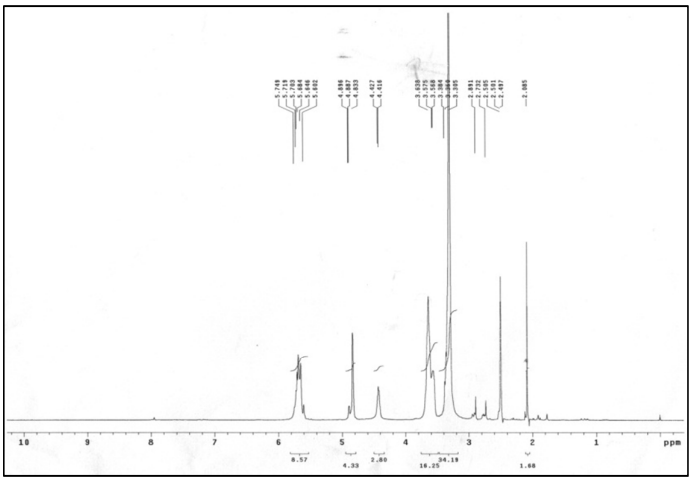
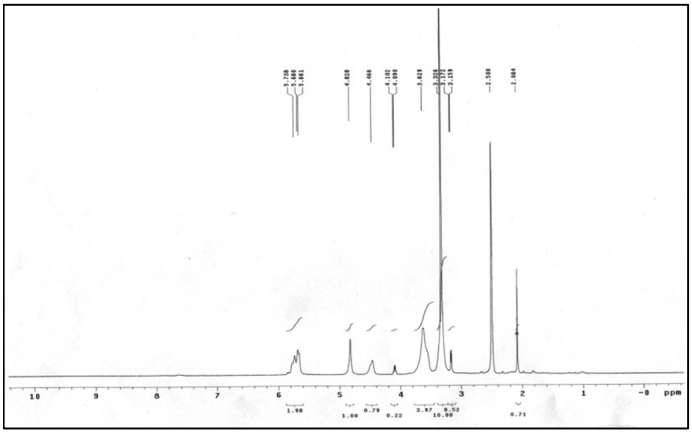


Figure S1 NMR of spectrum of Compound 2



NMR of spectrum of Compound **3**

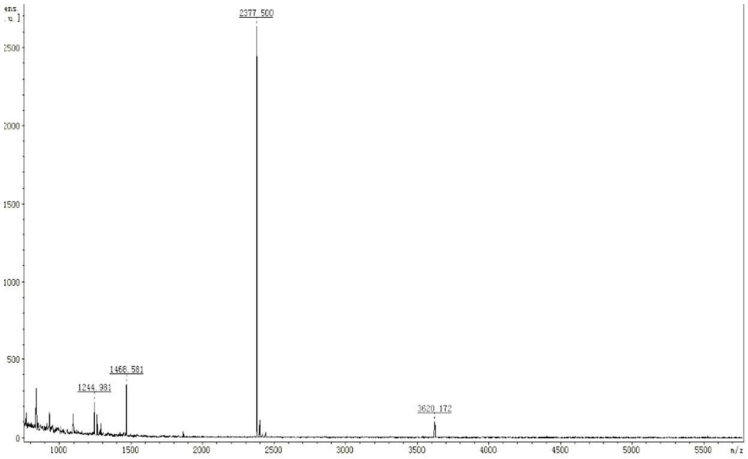


Figure S3 mass spectrum of compound **3**

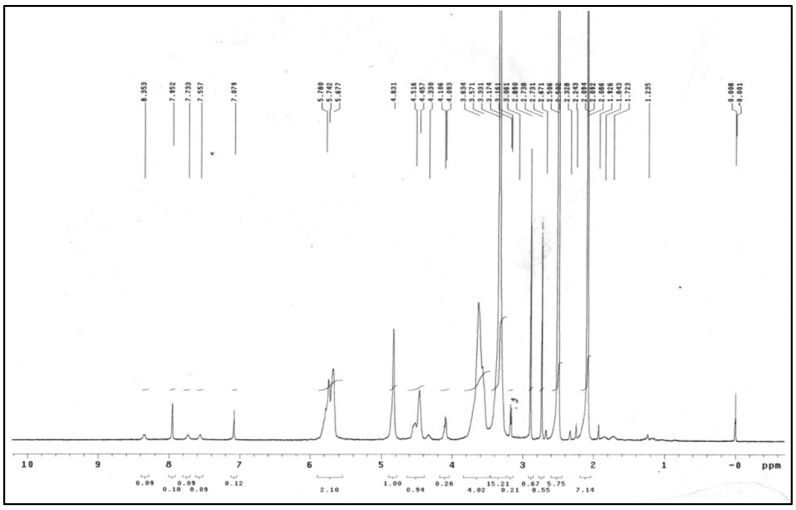


Figure S4 NMR spectrum of Compound **4**

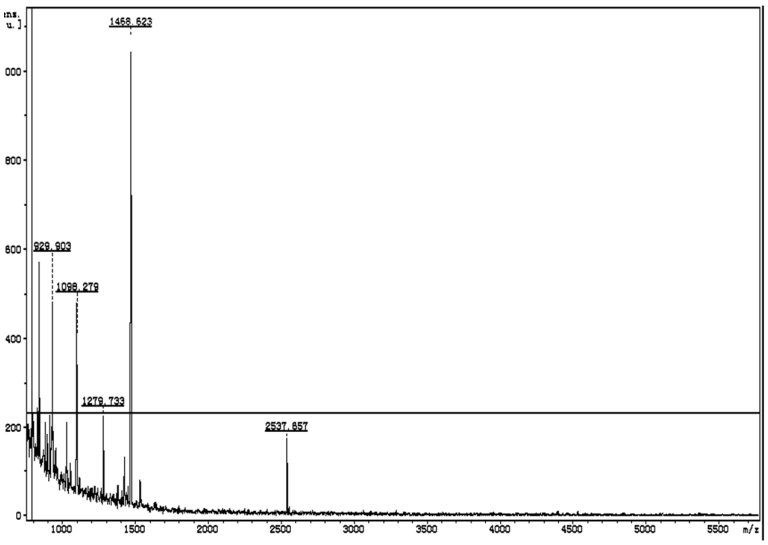


Figure S5. Mass spectrum of compound **4**

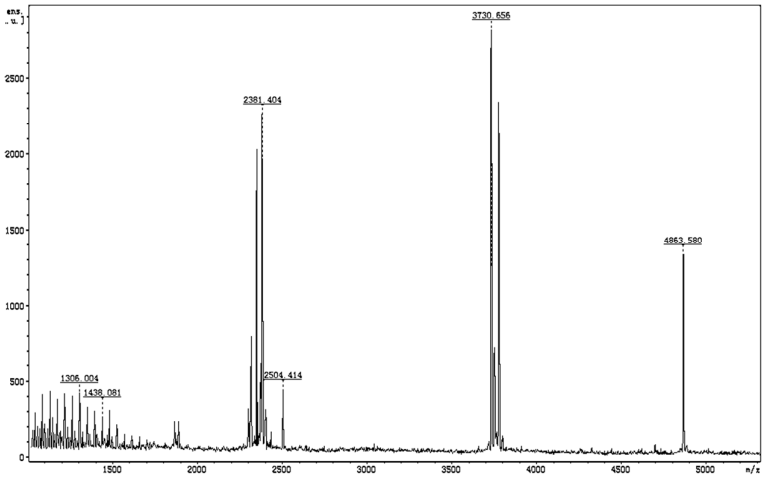


Figure S6. Mass spectrum of compound **5**

References:

1. Jicsinszky, L.; Ivanyi, R. Catalytic transfer hydrogenation of sugar derivatives. *Carbohydr. Polym.* **2001,** *45*, (2), 139-145.