1

2 **Polymer Journal, Note**

3

4 **Facile preparation of cyclodextrin-grafted chitosans and**

5 **their conversion into nanoparticles for an anticancer drug**

6 **delivery system**

7 Hironori Izawa,* Keisuke Yamamoto, Satoshi Yoshihashi, Shinsuke Ifuku, Minoru Morimoto,

8 Hiroyuki Saimoto*

9 Graduate School of Engineering, Tottori University, 4-101 Koyama-Minami, Tottori 680-

10 8550, Japan.

11

12 *Correspondence to: Hironori Izawa and Hiroyuki Saimoto.

13 Postal Address: Department of Science and Biotechnology, Graduate School of Engineering,

14 Tottori University, 4-101 Koyama-Minami, Tottori 680-8550, Japan.

15 HI, Phone: +81-857-31-5813. Fax: +81-857-31-5813. E-mail: h-izawa@chem.tottori-u.ac.jp

16 HS, Phone: +81-857-31-5694. Fax: +81-857-31-5813. E-mail: saimoto@chem.tottori-u.ac.jp

17

20
21

INTRODUCTION

Chitosan (CS) is a natural polysaccharide, composed of β-D-glucosamine (60% <) and β-D-*N*-acetylglucosamine linking through a β-(1→4) linkage, obtained by chitin deacetylation.[1] CS is one of the most commercially important biocompatible polymers from an environmental or biomedical viewpoint.[2] Because of CS's biocompatibility and the high reactivity of amino groups, considerable research efforts have been directed toward developing safe and efficient chitosan-based materials as biomaterials.[1-3]

Cyclodextrin (CyD) is a host molecule that forms inclusion complexes with a variety of guests.[4] It has thus been widely used as an excipient to improve the physicochemical and pharmaceutical properties of drug molecules. The grafting of CyD onto CS can result in an increase in the extent of complexation ability, sorption, and controlled-release properties due to CyD's inclusion properties.[5] We previously reported interesting abilities of a β-CyD−grafted chitosan (β-CyD-g-CS) that formed supramolecular aggregates with insulin[6] and cholesterol[7]. Although CyD-g-CS is scientifically fascinating and potentially applicable as a biomaterial, its synthesis is not easy because multiple steps and cumbersome isolations are necessary to prepare a CyD derivative bearing one reactive group such as carboxylic acid or aldehyde.[5-8] A facile method with shorter steps and an easier procedure would accelerate the further application and functionalization of CyD-g-CS. Besides, both CS [9-12] and CyD derivatives[13-15] are known as candidate materials for anticancer drug delivery systems. Thereby, CyD-g-CSs, which are conjugates of both candidates, and their derivatives would provide improved anticancer drug delivery systems showing enhanced binding of a drug as well as improved therapeutic efficacy.

Herein we investigated facile preparation of α-, β-, and γ-CyD-g-CSs by carboxymethylation of the CyDs without purification and a subsequent dehydration-

condensation reaction to CS (Scheme 1). Furthermore, doxorubicin (Dox)-capturing nanoparticles composed of CyD-g-CS and sodium triphosphate (TPP) were prepared.

EXPERIMENTAL PROCEDURES

Materials

Chitosan (CS) (M_n estimated by GPC analysis with pullulan standards was 64 kDa *(M_W/M_n=2.0)). The degree of deacetylation as estimated by elemental analysis was 76.5%) was supplied by Koyo Chemical CO., Ltd. (Tottori, Japan). α-, β-, and γ-CyDs and sodium triphosphate (TPP) were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Chloroacetic acid, 2-morpholinoethanesulfonic acid (MES), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC), and N-hydroxysuccinimide (NHS) were purchased from Tokyo Chemical Industry CO., Ltd. (Tokyo, Japan). Other reagents were obtained in commercial grade and used without further purification.

Measurements

Nuclear magnetic resonance (NMR) spectra were acquired on a JNM-ECP500 (JEOL, Tokyo, Japan). Infrared (IR) spectra of the samples were recorded with a Spectrum 65 (Perkin-Elmer Japan Co., Ltd., Tokyo, Japan) equipped with an ATR attachment. MALDI–TOF mass spectra were measured using an Autoflex-T2 (Bruker Japan, Kanagawa, Japan) using 2,5-dihydroxybenzoic acid as a matrix. To prepare matrix solution, 2,5-dihydroxybenzoic acid (1.0 mg) was dissolved in acetonitrile (50 μL), to which 0.1% trifluoroacetic acid aqueous solution (50 μL) was added. The matrix solution (0.5 μL) and sample solution (0.5 μL; 1 mg/mL) were mixed and dried naturally on a sample plate to form a matrix crystal for the analysis. Elemental analysis data were recorded on a Perkin Elmer 2400 II CHNS/O (Perkin-Elmer Japan Co., Ltd., Japan). Dynamic light scattering (DLS) was performed with an ELSZ-1000 zeta potential and particle size analyzer (Otsuka Electronics Co., Ltd., Osaka, Japan).

1

Preparation of the CyD-g-CS

Typically, β-CyD (0.50 g, 0.44 mmol) and sodium hydroxide (0.47 g, 12 mmol) were added to water (5.0 mL) and stirred for 10 min at 50 ^{o}C. To which, 16.2% chloroacetic acid solution (0.26 g, 0.44 mmol) was added dropwise. After the solution was continuously stirred at 50 ^{o}C for 24 h, it was cooled to room temperature. The solution was then adjusted to pH 7 by adding 1.0 M HCl aqueous solution. The solution was poured into a large volume of acetone. The crude product containing a carboxymethylated β-CyD (CM-β-CyD) was collected by filtration, followed by drying under reduced pressure. The CM-β-CyD content in the crude product was estimated to be 0.088 mmol by ^{1}H NMR analysis. CS (0.13 g, 0.88 mmol) and the crude product were dissolved in 0.1 M MES aqueous solution (10 mL), to which EDC (34 mg, 0.18 mmol) and NHS (11 mg, 0.088 mmol) were added. The reaction mixture was stirred overnight at room temperature. After the addition of triethylamine (1 mL), the reaction mixture was poured into a large volume of ethanol. The precipitated product was collected by centrifugation, followed by washing with ethanol. The precipitate was purified by dialysis with a visking tube (Mw cutoff: 15 kDa) in a large volume of water. Yield, 17% (0.20 g). ^{1}H NMR (500 MHz, CD$_3$COOD-D$_2$O, DSS, 70 ^{o}C) δ = 3.13 (-C*H*(NH$_2$)- (chitosan, H-2)), 3.21-3.42 (sugar protons (CyD)), 3.48-3.94 (sugar protons (chitosan, CyD)), 3.97-4.12 (CyD), 4.55 (-C*H*(OH)- (*N*-acetylglucosamine and CyD-bearing units, H-1)), 4.79 (C*H*(OH)- (glucosamine units, H-1)), 4.99 (-C*H*(OH)- (CyD, H-1)), 5.20 (-C*H*(OH)- (CyD CM-glucose unit, H-1)), 5.30 (-C*H*(OH)- (CyD CM-glucose unit, H-1)). ^{13}C NMR (125 MHz, CD$_3$COOD-D$_2$O, DSS). δ = 22.17 (chitosan, -NHCOC*H$_3$*), 55.76 (chitosan, C-2), 58.81 (chitosan, C-6), 60.40 (CyD, C-6), 61.02 (CM-CyD, C-6'), 70.40 (chitosan, C-3), 70.62 (-*C*H$_2$CONH-, CM-CyD), 72.01 (CyD, C-2), 72.37 (CyD, C-3), 73.29 (CyD, C-5), 75.05 (chitosan, C-5), 78.50 (chitosan, C-4), 81.33 (CyD, C-4), 97,79 (chitosan, C1), 102.01 (CyD, C-1), 174.14 (-CH$_2$*C*ONH- (CM-CyD)), 174.41 (NH*C*OCH$_3$ (chitosan)). IR（cm^{-1}, neat）3371, 2923, 2894,

1   1647, 1548, 1416, 1381, 1313, 1246, 1153, 1061, 1024, 944, 896. Elemental analysis calcd.

2   for $(C_6H_{11}NO_4)_{7.3}(C_8H_{13}NO_5)_{2.6}(C_{50}H_{81}NO_{40})_{1.0} \cdot 3.3H_2O$ C 44.40 H 6.56 N 4.92, found

3   C .44.56 H 6.48 N 4.75.

4

5   **Binding constants between CyD and Dox**

6   The binding constants (M^{-1}) between the CyD or the CyD residue and Dox were estimated

7   from ^{1}H NMR measurements. A Benesi-Hildebrand−type equation[16] was employed:

8
$$\frac{1}{\delta - \delta_0} = \frac{1}{K \cdot a \cdot [CyD]} + \frac{1}{a} \tag{1}$$

9   where δ, δ_0, K, and a are the chemical shift of a signal attributed to an aromatic proton of Dox

10   in the presence and absence of CyD, the binding constant, and a constant, respectively.

11

12   **Preparation of Dox-capturing nanoparticles composed of CyD-g-CS and TPP**

13   Typically, α-, β-, or γ-CyD-g-CS (10 mg) or CS (5.9 mg) was dissolved in 100 mM

14   CH_3COOH aqueous solution (1.0 mL), to which 1.0 mM Dox aqueous solution (1.0 mL) was

15   added. After the solution was stirred for 1 h, 1.0 mM TPP aqueous solution (100 μL) was

16   added and the solution was stirred again for 1 h. The solution containing the nanoparticle was

17   placed in a centrifugation tube equipped with semi-permeable membranes whose molecular-

18   weight cutoff was 3 kDa (Amicon Ultra-0.5, Millipore, Darmstadt, Germany). The tube was

19   centrifuged at 5000 rpm at 25.0 ^{o}C. Absorbance of the filtrate, which had passed through the

20   membrane, at 480 nm was measured to determine Dox concentration by a standard curve. The

21   adsorption of Dox to tubes and membranes was negligible, and the mass balance before and

22   after membrane separation was maintained. Capturing efficiency (%) was defined as follows:

23
$$Capturing\ Efficiency = \frac{[Dox_0] - [Dox]}{[Dox_0]} \times 100 \tag{2}$$

24   where $[Dox]$ and $[Dox_0]$ are the Dox concertation of the filtrates in the presence and absence

25   of the nanoparticle, respectively.

RESULTS AND DISCUSSION

Preparation of CyD-g-CS

Facile preparation of β-CyD-g-CS by means of the carboxymethylation of β-CyD and the dehydration-condensation reaction to CS was investigated. The carboxymethylation of β-CyD was carried out with an equivalent amount of chloroacetic acid. The crude product containing CM-β-CyD and impurities (β-CyD unreacted and NaCl) was obtained by precipitation with a large volume of ethanol. MALDI-TOF mass and ^{1}H NMR analyses of the crude product were performed. Figure 1A shows the MALDI-TOF mass spectrum of the crude product. In the spectrum, significant peaks attributed to β-CyD and CM-β-CyD were observed at 1157 Da (β-CyD + Na$^+$), 1216 Da (CM-β-CyD + Na$^+$), and 1238 Da (CM-β-CyD-Na salt + Na$^+$). Peaks attributable to a di-substituted product (CM$_2$-β-CyD) were slightly observed at 1295 Da and 1318 Da, which were trace amounts compared to CM-β-CyD in HPLC analysis with an ODS column. Figure 1B shows the ^{1}H NMR spectrum of the crude product. In the spectrum, signals attributable to the anomeric protons of the glucose units in β-CyD and CM-β-CyD (**b**), the 6-*O*-carboxymethylglucose unit in CM-β-CyD (**a**), and the 2 and/or 3-*O*-carboxymethylglucose unit in CM-β-CyD (**a'**) were observed at 5.12 ppm, 5.32 ppm, and 5.43 ppm, respectively. The apparent yields estimated from the integrated ratio of the anomeric protons of carboxymethylglucose units (**a** and **a'**) and of glucose units (**b**) were 19.8%. Subsequently, the crude product containing CM-β-CyD was conducted to the grafting reaction onto CS by the dehydration-condensation reaction without further purification, where the molar feed ratio (CM-β-CyD/CS unit) was adjusted to 0.10. Finally, the impurities from both carboxymethylation and the dehydration-condensation reaction were removed by dialysis. The structure of the β-CyD-g-CS was confirmed by ^{1}H NMR analysis (Figure 1C), revealing signals attributed to protons of residual acetyl groups in a CS (**g**), the 2-position of unreacted-

glucosamine units in CS (**f**), a methylene in carboxymethyl moiety (**e**), most of the sugar

protons in CS and CyD residues (**sugar protons**), anomeric protons of CS (**d**, **c**), and β-CyD

residues (**a**, **a'**, and **b**). In addition, ^{13}C NMR, IR, and elemental analyses (see the

experimental section) also supported the production of β-CyD-g-CS. The β-CyD content,

which is the molar ratio of the CyD-bearing glucosamine unit in the β-CyD-g-CS, was

estimated from the integrated ratio of anomeric protons of CS (**c** and **d**) and the β-CyD

residues (**a**, **a'**, and **b**) to be 9.6%. This was nearly identical to the value estimated from

elemental analysis (9.2%). This agreed well with the feed ratio. The total yield of β-CyD-g-

CS in the two steps was 16.7%, which was about twice that of our previous route through five

steps from β-CyD.[6] Besides, our previous route requires 2 weeks to prepare β-CyD-g-CS,

whereas the present method requires only 3 days. Furthermore, we demonstrated that the CyD

content in β-CyD-g-CS was controllable by varying the feed ratio (Table 1, Entries 1-3), and

this method was applicable to the preparation of α- and γ-CyD-g-CS (Table 1, Entries 4-6;

Figure S1 and S2). Note that twice the amount of EDC was needed in the case of α-CyD than

in the case of β- or γ-CyD. This is probably due to inhibition by α-CyD, because α-CyD can

include EDC.[4]

Although the condition of caroboxymethylation of CyD and the reaction system for

the dehydration-condensation reaction differed from those for this study, there is a report

concerning the preparation of β-CyD-g-CS through the carboxymethylation of CyD.[8] To

compare that study to the method proposed here, we attempted to prepare β-CyD-g-CS using

those previously reported conditions. β-CyD was carboxymethylated in accordance with the

previous report, in which 5.3 equivalent of chloroacetic acid was used. Although the apparent

yield estimated by ^{1}H NMR analysis was higher (68.4%) than that in this study (Figure S3B),

distinct peaks attributed to di- and tri-substituted products (CM_2-β-CyD and CM_3-β-CyD) are

observed in the MALDI-TOF mass analysis (Figure S3A). In the dehydration-condensation

1   reaction with the crude product containing CM-β-CyD, CM$_2$-β-CyD, and CM$_3$-β-CyD by the

2   EDC/NHS system used in this study, the reaction solution turned into a gel during the reaction

3   due to a crosslinking reaction involving CM$_2$-β-CyD and CM$_3$-β-CyD. The purified product

4   was an insoluble material. These results suggest that the reported method is applicable only to

5   an oligomeric CS, as reported.

6

7   **Binding between Dox and the CyD residues**

8   Binding between Dox and the α-, β-, or γ-CyD residue via a host−guest interaction was

9   investigated by ^{1}H NMR analysis (Figure S4), where we used CyD-g-CSs having CyD

10   contents of ca. 10% and an equivalent amount of Dox toward the CyD residue. In the case of

11   α-CyD-g-CS, the signals attributed to Dox were not changed at all. In the case of β-CyD-g-

12   CS, the signal attributed to an aromatic proton of Dox was slightly shifted. In contrast, in the

13   case of γ-CyD-g-CS some signals were significantly shifted. These results are consistent with

14   the previously reported inclusion behavior of α-, β-, or γ-CyD toward Dox.[14,17] The binding

15   constant (K) due to the host−guest interactions between Dox and the β- or γ-CyD residues was

16   estimated from Benesi-Hildebrand plots (Figure 2 and S5). Linear relations, indicating the

17   formation of inclusion complexes with 1:1 stoichiometry, were shown in both β- and γ-CyD-

18   g-CS. The K values for the β- and γ-CyD-g-CS estimated from the slopes and intercepts were

19   6.2 M^{-1} and 171.5 M^{-1}, respectively, which were almost the same as those for β- and γ-CyD

20   (7.2 M^{-1} and 225.1 M^{-1}, respectively). These results indicate that the inclusion ability of the β-

21   or γ-CyD residue is not changed as much as that of β- or γ-CyD.

22

23   **Preparation of Dox-capturing nanoparticles composed of CyD-g-CS and TPP**

24   TPP is a frequently used polyanionic crosslinker for the preparation of a CS nanoparticle. The

25   nanoparticle is used as a drug carrier for Dox,[12] where Dox is captured via the electrostatic

attraction of TPP. Inclusion by CyD residues would enhance adsorption via the host−guest

interaction. Thus, we investigated the preparation of a Dox-capturing nanoparticle with α-, β-,

or γ-CyD-g-CS and TPP. The α-, β-, and γ-CyD-g-CSs were converted into nanoparticles in

the presence of Dox by the addition of TPP solution. The production of nanoparticles was

confirmed by DLS and SEM analyses, in which nanoparticles ca. 100 nm in diameter and

their aggregates (ca. 600 nm) were observed (Figure S6). Figure 3A shows the capturing

efficiency of the nanoparticles for Dox estimated with a centrifugation tube equipped with

semi-permeable membranes. The capturing efficiencies of the nanoparticles prepared with CS,

α-, β-, and γ-CyD-g-CS were 17.0%, 17.1%, 20.2%, and 28.6%, respectively. In the case of γ-

CyD-g-CS forming a moderate inclusion complex with Dox, the capturing efficiency was ca.

1.7-fold higher than that in CS. It is likely that the higher capturing efficiency is attributable

to both the host−guest and electrostatic interactions (Figure 3B). In order to confirm the

contribution of the inclusion, the capturing efficiency in the presence of large amount of

poly(ethylene glycol), that is a macromolecuar guest for γ-CyD[18], was evaluated, in which the

capturing efficiency was decreased to 18.4%. This result indicates the inclusion by γ-CyD-g-

CS enhanced the capturing efficiency for Dox

Conclusion

We have developed a facile method of preparing CyD-g-CS by means of the

carboxymethlation of CyDs and the dehydration-condensation reaction to CS. In this method,

crude product containing ca. 10% of the CM-CyD was subjected to the grafting reaction

without further purification. The product (CyD-g-CS) was finally purified by dialysis. This

method was applicable to all common CyDs (α- , β- , and γ-CyD). Besides, the CyD content

was changeable by the feed ratio (CM-CyD/CS unit). CyD-g-CS was obtained in higher yield

in a shorter period of time compared to our previous report. This facile method makes further

functionalization of the CyD-g-CS accessible. We have found that the inclusion by γ-CyD-g-CS enhanced the capturing efficiency for Dox. Further functionalization of CyD-g-CS for tumor targeting and a triggered release would provide an improved Dox delivery system. This work is now in progress.

Acknowledgments

References

1   Kumar, M. N. V. R., Muzzarelli, R. A. A., Muzzarelli, C., Sashiwa, H. & Domb, A. J. Chitosan Chemistry and Pharmaceutical Perspectives. *Chem. Rev.* **104**, 6017-6084 (2004).

2   Thakur, V. K. & Thakur, M. K. Recent Advances in Graft Copolymerization and Applications of Chitosan: A Review. *Acs. Sustainable Chem. Eng.* **2**, 2637-2652 (2014).

3   El Kadib, A., Bousmina, M. & Brunel, D. Recent Progress in Chitosan Bio-Based Soft Nanomaterials. *J Nanosci Nanotechnol* **14**, 308-331 (2014).

4   Saenger, W. Cyclodextrin Inclusion-Compounds in Research and Industry. *Angew. Chem. Int. Edit.* **19**, 344-362 (1980).

5   Prabaharan, M. & Mano, J. F. Chitosan Derivatives Bearing Cyclodextrin Cavities as Novel Adsorbent Matrices. *Carbohydr. Polym.* **63**, 153-166 (2006).

6   Daimon, Y., Izawa, H., Kawakami, K., Zywicki, P., Sakai, H., Abe, M., Hill, J. & Ariga, K. Media-Dependent Morphology of Supramolecular Aggregates of beta-Cyclodextrin-Grafted Chitosan and Insulin through Multivalent Interactions. *J. Mater. Chem. B* **2**, 1802-1812 (2014).

7   Takechi-Haraya, Y., Tanaka, K., Tsuji, K., Asami, Y., Izawa, H., Shigenaga, A., Otaka, A., Saito, H. & Kawakami, K. Molecular Complex Composed of beta-Cyclodextrin-Grafted Chitosan and pH-Sensitive Amphipathic Peptide for Enhancing Cellular Cholesterol Efflux under Acidic pH. *Bioconjugate Chem.* **26**, 572-581 (2015).

8   Furusaki, E., Ueno, Y., Sakairi, N., Nishi, N. & Tokura, S. Facile Preparation and Inclusion Ability of a Chitosan Derivative Bearing Carboxymethyl-beta-Cyclodextrin. *Carbohydr. Polym.* **29**, 29-34 (1996).

9   Ghaz-Jahanian, M. A., Abbaspour-Aghdam, F., Anarjan, N., Berenjian, A. & Jafarizadeh-Malmiri, H. Application of Chitosan-Based Nanocarriers in Tumor-Targeted Drug Delivery. *Mol. Biotechnol.* **57**, 201-218 (2015).

10   Ta, H. T., Dass, C. R. & Dunstan, D. E. Injectable Chitosan Hydrogels for Localised Cancer Therapy. *J. Control. Release* **126**, 205-216 (2008).

11   Wang, J. J., Zeng, Z. W., Xiao, R. Z., Xie, T. A., Zhou, G. L., Zhan, X. R. & Wang, S. L.Recent Advances of Chitosan Nanoparticles as Drug Carriers. *Int. J. Nanomed.* **6**, 765-774 (2011).

12   Janes, K. A., Fresneau, M. P., Marazuela, A., Fabra, A. & Alonso, M. J. Chitosan Nanoparticles as Delivery Systems for Doxorubicin. *J. Control. Release.* **73**, 255-267 (2001).

13   Anand, R., Ottani, S., Manoli, F., Manet, I. & Monti, S. A Close-Up on Doxorubicin Binding to gamma-Cyclodextrin: an Elucidating Spectroscopic, Photophysical and Conformational study. *Rsc. Adv.* **2**, 2346-2357 (2012).

14   Bekers, O., Kettenesvandenbosch, J. J., Vanhelden, S. P., Seijkens, D., Beijnen, J. H., Bult, A. & Underberg, W. J. M. Inclusion Complex-Formation of Anthracycline Antibiotics with Cyclodextrins - a Proton Nuclear-Magnetic-Resonance and Molecular Modeling Study. *J. Incl. Phenom. Mol.* **11**, 185-193 (1991).

15   Dan, Z. L., Cao, H. Q., He, X. Y., Zeng, L. J., Zou, L. L., Shen, Q. & Zhang, Z. W. Biological Stimuli-Responsive Cyclodextrin-Based Host-Guest Nanosystems for Cancer Therapy. *Int. J. Pharm..* **483**, 63-68 (2015).

16   Agbaria, R. A., Butterfield, M. T. & Warner, I. M. Use of Cyclodextrins and Fluorescence Spectroscopy to Probe the Dual Fluorescence of 9-Anthroic Acid. *J. Phys. Chem.* **100**, 17133-17137 (1996).

17   Husain, N., Ndou, T. T., Delapena, A. M. & Warner, I. M. Complexation of Doxorubicin with beta-Cyclodextrins and Gamma-Cyclodextrins. *Appl. Spectrosc.* **46**, 652-658 (1992).

18   Higashi, K., Ideura, S., Waraya, H., Moribe, K. & Yamamoto, K. Incorporation of Salicylic Acid Molecules into the Intermolecular Spaces of gamma-Cyclodextrin-Polypseudorotaxane. *Cryst. Growth. Des.* **9**, 4243-4246 (2009).

1

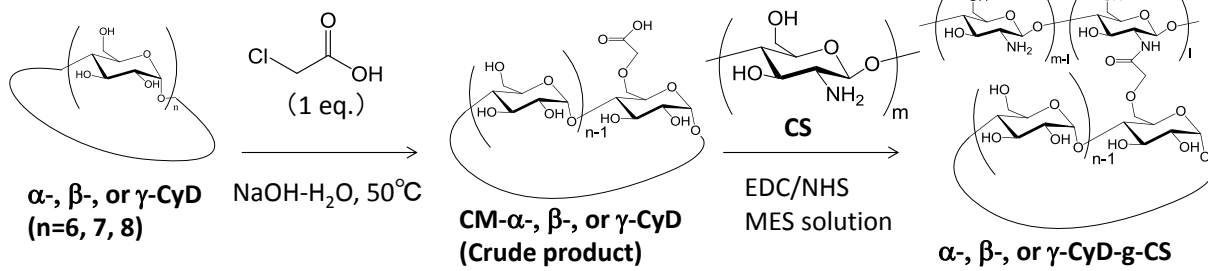**Table 1** Preparation of α-, β-, and γ-CyD-g-CS in various feed ratios

Entry	CyD	CM-CyD[a]/CS unit (mol/mol)	EDC/CM-CyD (mol/mol)	CyD content (%)[a,b]	Total Yield[c] (%)
1	β	0.20	2	18.2	15.3
2	β	0.10	2	9.6	16.5
3	β	0.05	2	4.1	16.3
4	α	0.10	2	3.4	-
5	α	0.10	4	11.4	15.3
6	γ	0.10	2	10.6	16.4

[a] Estimated by ^{1}H NMR analysis. [b] Molar ratio of CyD-bearing glucosamine unit in CyD-g-CS. [c] Estimated as follows: (Yield of CyD-g-CS (g)/Theoretical Yield (g) when CyD is perfectly converted to CyD-g-CS)x100.
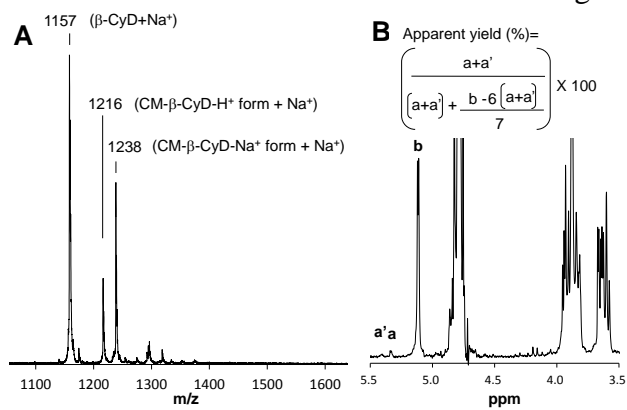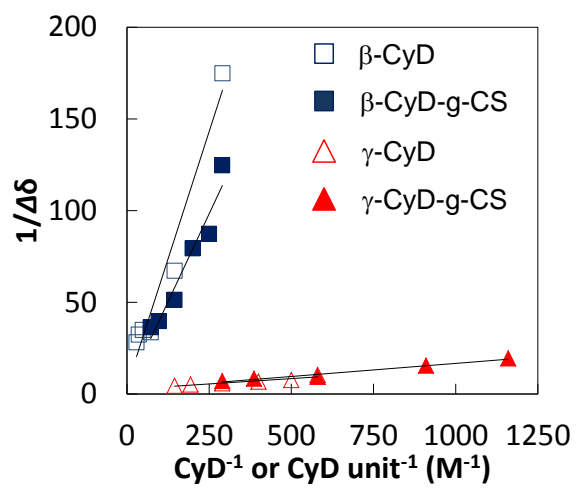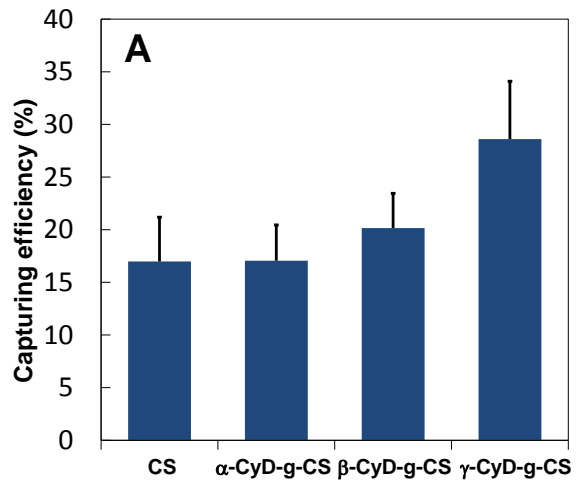
1

Scheme 1

α-, β-, or γ-CyD
(n=6, 7, 8)

（1 eq.）

NaOH-H$_2$O, 50°C

CM-α-, β-, or γ-CyD
(Crude product)

CS

EDC/NHS
MES solution

α-, β-, or γ-CyD-g-CS

2
3

Figure 1

1

2

<div align="center">Figure 2</div>

3

4
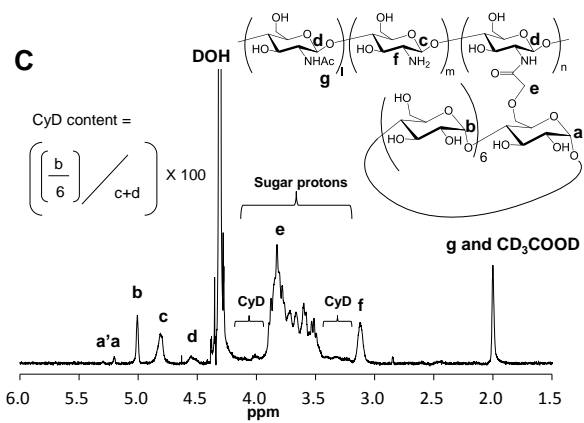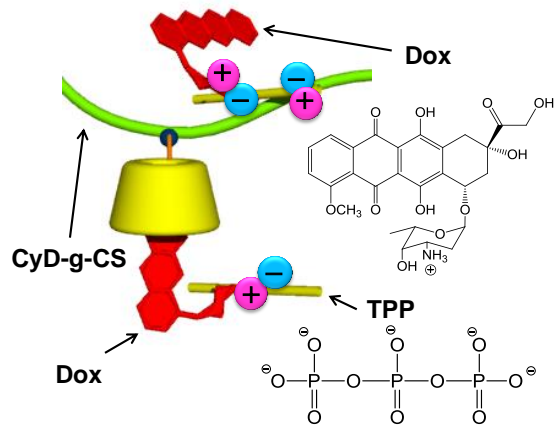
Figure 3

Scheme and Figure Captions

Scheme 1 Facile preparation of α-, β-, or γ-CyD-g-CS.

Figure 1 MALDI-TOF mass (A) and ^{1}H NMR (B) spectra of crude CM-β-CyD and ^{1}H NMR

spectrum of β-CyD-g-CS (C).

Figure 2 Benesi-Hildebrand plots of the β-CyD-Dox system (□), the β-CyD-g-CS-Dox

system (■), the γ-CyD-Dox system (△), and the γ-CyD-g-CS-Dox system (▲).

Figure 3 Capturing efficiencies of the nanoparticles prepared with CS, α-, β-, or γ-CyD-g-CS

for Dox (A) and a graphical image of the binding modes in the nanoparticle prepared with γ-

CyD-g-CS (B).

Table Caption

Table 1 Preparation of α-, β-, and γ-CyD-g-CS in various feed ratios