Tetrahedron Letters 53 (2012) 5791–5795

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

6-Hydroxymethyltriazolyl-6-deoxy-β-cyclodextrin: a highly water soluble and structurally well-defined β-cyclodextrin click cluster

Dong-Hwan Kim [†], Jae Gyu Jang [†], Hoa Thi Le, Jin Young Kim, Choon Woo Lim [*], Tae Woo Kim [*]

Graduate School of East–West Medical Science, Kyung Hee University, Gyeonggi-do 449-701, Republic of Korea

ARTICLE INFO

Article history:
Received 14 May 2012
Revised 8 August 2012
Accepted 17 August 2012
Available online 24 August 2012

Keywords:
Cyclodextrin
Copper(I)-catalyzed azide–alkyne cycloaddition
Cyclodextrin click cluster
Cyclodextrin host guest chemistry

ABSTRACT

The structural, physical, and biological properties of heptakis{6-(4-hydroxymethyl-1*H*-[1,2,3]triazol-1-yl)-6-deoxy}-β-cyclodextrin (**HTβCD**) were investigated by a variety of methods, including NMR, UV/vis, circular dichroism spectroscopy, computer modeling, turbidity testing, K_a measurements, and the MTT assay. The experimental results suggest that **HTβCD** is structurally well-defined, highly water-soluble, and has low cytotoxicity. These advantages of **HTβCD** versus β-CD indicate that β-cyclodextrin click clusters may function both as host molecules and as potential, alternative excipients to β-CD.

β-Cyclodextrin (β-CD) is a cyclic oligosaccharide that consists of seven glucose units. Several members of β-CD are used industrially in pharmaceutical and allied applications.[1] The ability to use the hydrophobic cavity of β-CD to encapsulate bioactive molecules in water has drawn tremendous interest from the pharmaceutical industry because encapsulation improves the stability and bioavailability of drug molecules.[2] β-CD itself is the most commonly used CD although it is the least soluble (solubility in water at 20 °C, 1.85 g/100 mL). When parenterally administered, β-CD is not metabolized but accumulates in the kidneys as insoluble cholesterol complexes, resulting in severe nephrotoxicity.[3] To overcome these disadvantages of β-CD, chemically modified β-CD derivatives have been prepared with a view to extending the physicochemical properties and inclusion capacity of parent β-CD.

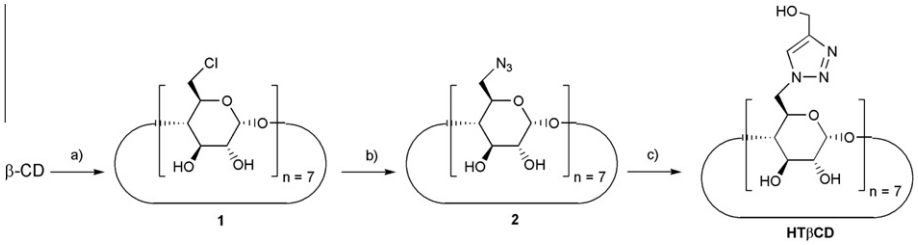
For instance 2-hydroxypropyl-β-cyclodextrins (HPβCDs), which are chemically modified β-CDs, have been shown to have better aqueous solubility[4] and a better pharmacokinetic profile for intravenous administration of lipophiles than β-CD.[5] However, HPβCDs are not single components; they are mixtures of compounds with different substitution degrees (e.g. the average degree of substitution of commercially available HPβCD is 0.6–0.8 units of 2-hydroxypropyl per glucose unit). This heterogeneity in conventional, modified β-CD derivatives makes it difficult for researchers to precisely investigate the inclusion phenomena at the molecular level. It is therefore a challenging task to synthesize novel β-CD derivatives that are well-defined, single component molecules with high aqueous solubility.

Several synthetic approaches to use copper(I)-catalyzed azide–alkyne cycloaddition[6] (described as the 'click' reaction in this paper) to generate cyclodextrin click clusters (CCCs) have been explored.[25] CCCs have unique characteristics, namely well-defined structures, precise molecular weights, and multivalent functionalization sites. Researchers have utilized CCCs as polymers,[7] nucleic acid carriers,[8] sensors,[9] glycoconjugates,[10] and magnetic resonance imaging probes.[11] These previous studies have focused on the development of new applications, but the systematic studies on CCC's physical and biological properties are rare. If you consider the industrial needs to develop new chemically modified β-CDs in the field of drug, food, and cosmetic formulation,[26] the synthesis and characterization of CCCs as potential, alternative excipients to β-CD are really challenging. Before pharmaceutical studies on new chemically modified CCCs, the basic properties of the CCCs should be investigated by diverse techniques such as NMR, UV/vis, circular dichroism, turbidity testing, binding constant studies, and cell viability assay.

As a model scaffold for CCCs, we designed **HTβCD** (heptakis{6-(4-hydroxymethyl-1*H*-[1,2,3]triazol-1-yl)-6-deoxy}-β-cyclodextrin)[12] and prepared it using a microwave-assisted, protection-free, click reaction, and Diaion™ HP-20 chromatography (Scheme 1). Reaction of azido-cyclodextrin **2** with propargyl alcohol (1.5 equiv per N_3) under the optimized condition ($CuSO_4$/THPTA (tris(3-hydroxypropyltriazolylmethyl)amine) (1:5), THF/phosphate buffer (0.1 mM, pH 7.0), microwave) followed by chromatography on

* Corresponding authors.
E-mail addresses: choonwoo.lim@khu.ac.kr (C.W. Lim), tw1275@khu.ac.kr (T.W. Kim).
† These authors contributed equally to this work.

0040-4039/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tetlet.2012.08.073



Scheme 1. Reactions and reagents. (a) Methanesulfonyl chloride, DMF, 65 °C; (b) NaN_3, DMSO, MW; (c) propargyl alcohol, $CuSO_4$/THPTA, sodium ascorbate, THF/phosphate buffer (0.1 mM, pH 7.0), MW. *Abbrev.*: THPTA = tris(3-hydroxypropyltriazolylmethyl)amine. MW = microwave irradiation.

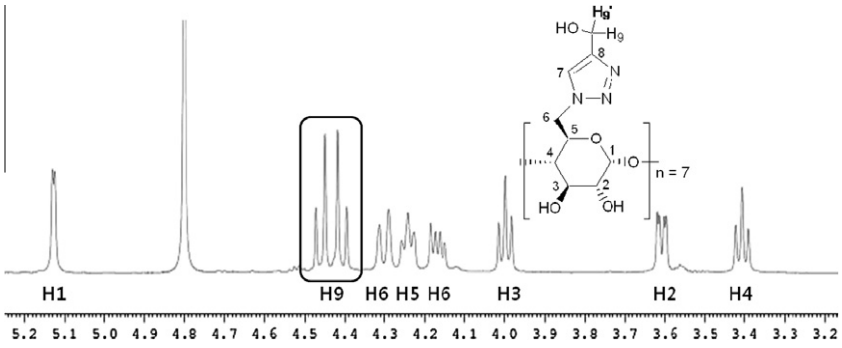


Figure 1. Partial ^{1}H NMR spectrum (600 MHz) of **HTβCD** in D_2O.

Diaion™ HP-20 afforded the desired product at a moderate yield (52%).[13] Copper ligands are often employed both to enhance the rate of the reaction and to protect Cu(I) from oxidation in the presence of adventurous oxygen.[6a,14] THPTA developed by the Finn group has proven to be invaluable for bioconjugation in water.[15] Diaion™ HP-20 is a kind of porous-type synthetic adsorbent that is made up of a styrene and divinyl benzene copolymer.[16] Because of its large porous size, Diaion™ HP-20 is suitable for the purification of relatively larger small-molecules (MW $\geqslant$1500 Da). Diaion™ HP-20 has been successfully utilized for CD derivative purification.[17]

Complete triazole formation was evident by the presence of axial symmetry in the NMR spectra of **HTβCD**. Only a single doublet for all H1 protons appeared in the ^{1}H NMR spectra (δ = 5.12 ppm, J_{H1-H2} = 3.2 Hz), and the corresponding C1 atoms also appeared as one singlet (δ = 103.2 ppm) in the H1 decoupled ^{13}C NMR spectrum.[18] All protons and carbons were fully assigned by HH COSY (homonuclear correlation spectroscopy) and HSQC (heteronuclear single quantum coherence) (Supplementary data A).

The ^{1}H NMR spectrum of **HTβCD** in D_2O shows the unique spin–spin coupling pattern of the two H9 protons (Fig. 1). If we consider the long distance between the methylene carbon (C9) and the nearest chiral carbon center (C5), the germinal coupling (J = 13.3 Hz) of two diastereomic H9–H9′ experimentally supports the structural rigidity of **HTβCD**. In other words the triazole rings on the crowded primary rim of β-CD are conformationally fixed and the diastereomeric environment around C5 carbon propagates to the H9 protons.

The circular dichroism spectrum of **HTβCD** in water, together with the absorption spectrum, are shown in Figure 2 (Supplementary data B). Because the β-CD backbone is chiral, the excitonic coupling between the individual triazoles borne on β-CD is circular dichroism active. **HTβCD** shows a positive Cotton effect in which the zero crossover point between the peak and the trough corresponds closely to the normal UV λ_{max} **HTβCD** has $\varepsilon_{max} \approx 23{,}000\ M^{-1}\,cm^{-1}$ and $\Delta\varepsilon_{(L-R)}(max) \approx 350\ M^{-1}\,cm^{-1}$. As a precedent, Blanchard-Desce and co-workers reported the circular dichroism of their multichromophoric cyclodextrin derivative

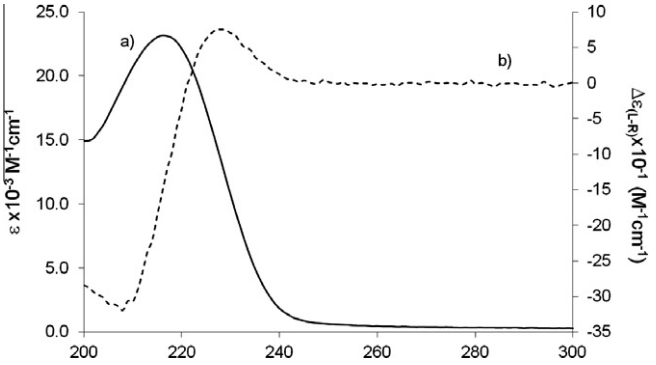


Figure 2. (a) Ultraviolet (solid, 4.0×10^{-5} M) and (b) circular dichroism (dot, 5.0×10^{-4} M) spectra of **HTβCD** in water.

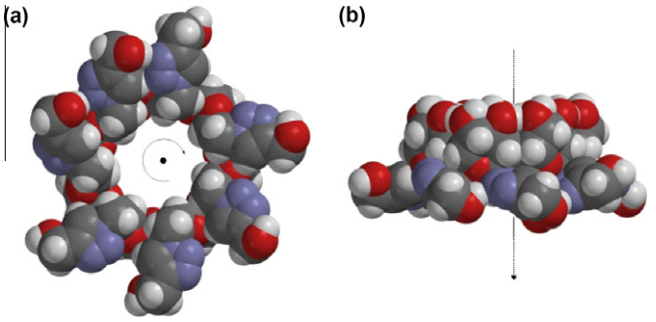


Figure 3. Molecular modeling (DFT/B3LYP calculation with 6-31g(d) basis set in vacuum, GAUSSIAN 09′ rev. 03) of **HTβCD**. (a) Top view and (b) side view.

bearing a pendent chromophore (1-[5-(4-dimethylamino-benzyliden)-4-oxo-2-thioxo-thiazolidin-3-yl]-acetic acid): $\varepsilon_{max} \approx 200{,}000\ M^{-1}\,cm^{-1}$ and $\Delta\varepsilon_{(L-R)}(max) \approx 1000\ M^{-1}\,cm^{-1}$.[19] If we

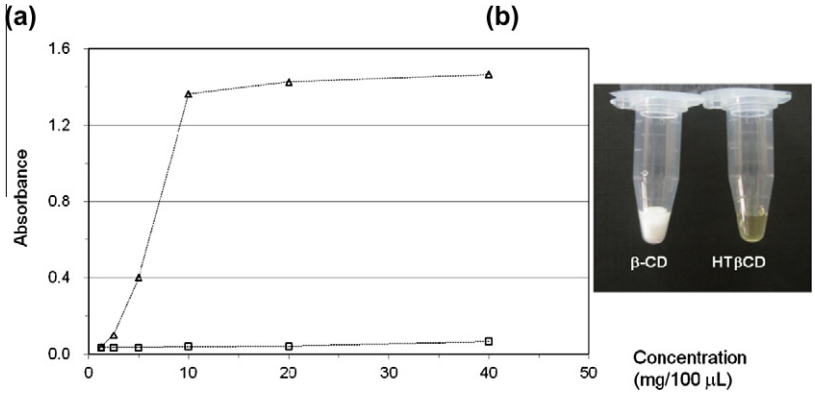


Figure 4. (a) The turbidity changes β-CD (Δ)and **HTβCD** (□) at 595 nm, (b) a photograph of 40 mg of β-CD and 40 mg of **HTβCD** in 100 μL water.

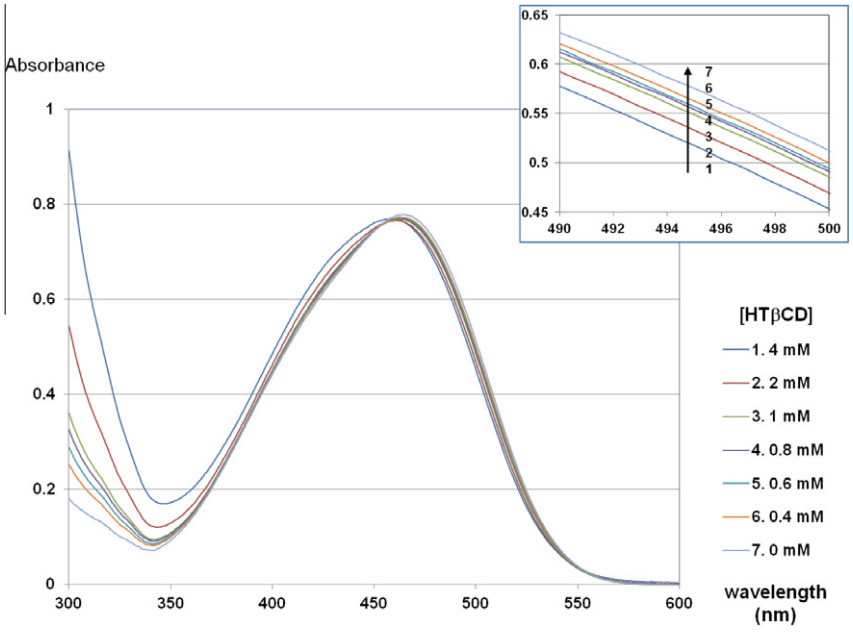


Figure 5. UV–visible absorption spectra of MO at various concentrations of **HTβCD**, upper-right box: the expanded spectra around 495 nm.

consider that the ε_{max} of **HTβCD** is 10 times lower than that of Blanchard-Desce and co-workers' multichromophoric CD, the $\Delta\varepsilon_{(L-R)}$(max) of **HTβCD** is unexpectedly high. The high $\Delta\varepsilon_{(L-R)}$(max) of **HTβCD** may result from the structural rigidity and directionality of the triazole moieties borne on β-CD.

Computer modeling of **HTβCD** provided insight into the details of the molecular structure (Fig. 3). The seven triazole rings have a C_7 symmetrical and directional orientation along the axis that penetrates the cavity (top view). The limited freedom of rotation of the triazole rings keeps them rigid and compact. This interpretation is consistent with the NMR and circular dichroism observations.

The water solubility of **HTβCD** was determined by the turbidity measurement.[20] Compared with the relatively low water solubility of β-CD (solubility in water, 1.85 mg/100 μL), **HTβCD** has higher water solubility and the solution containing **HTβCD** looked clean at the full experimental concentration (Fig. 4 and Supplementary data C). Figure 4 suggests that the minimum water solubility of **HTβCD** is higher than 40 mg/100 μL; at least 20 times higher than that of β-CD. C. Roehri-Stoeckel et al. synthesized 6-deoxy-6-(1-(4,5-dicarboxyl)-l,2,3-triazolyl)-β-CD and reported the enhanced water solubility of it under its neutral (8.5 times) and carboxylate form (35 times) compared with β-CD.[27] If you consider the polarity difference between 4-hyroxymethyl and 4,5-dicarboxyl group, the 6-hydroxymethyltriazolyl modification of β-CD dramatically improved its water solubility.

To investigate the influence of the 6-hydroxymethyltriazolyl moiety on the inclusion phenomenon, we determined binding constants by two different methods: UV/vis spectrophotometry and isothermal titration calorimetry (ITC) measurement. Binding constants of inclusion complexes comprising methyl orange (MO) and β-CD/**HTβCD** were measured by a UV/vis spectrometer following Nakagaki's report (Fig. 5 and Supplementary data D).[21] The binding constant of **HTβCD** to MO (390 M^{-1}) was smaller than that of β-CD to MO (2900 M^{-1}). Because the UV/vis absorbance changes were relatively small ($\Delta A = 0.058$) for the CD/MO system, we decided to double-check the binding constant with ITC. We performed ITC experiments with two reported guests: 1-adamantane carboxylate and decanoate (Supplementary data D). The binding constants between the guests and **HTβCD** (log K 4.2 for 1-adamantanecarboxylate and log K 3.0 for decanoate) were also smaller than those reported for the guests and β-CD (log K 4.60 for 1-adamantanecarboxylate[22] and log K 3.82 for decanoate[23]) (Table 1). The increased water solubility of **HTβCD** may influence the

Table 1
Estimated thermodynamic parameters for guest-**HTβCD** complexation from ITC measurements (T = 303 K, 1:1 binding mode)

Guest[a]	log K	$\Delta H°$ (kJ mol^{-1})	$T\Delta S°$ (kJ mol^{-1})
Adamantane carboxylate	4.2	−15	8.8
Decanoate	3.0	−11	5.8

[a] Countercation: Na^+.

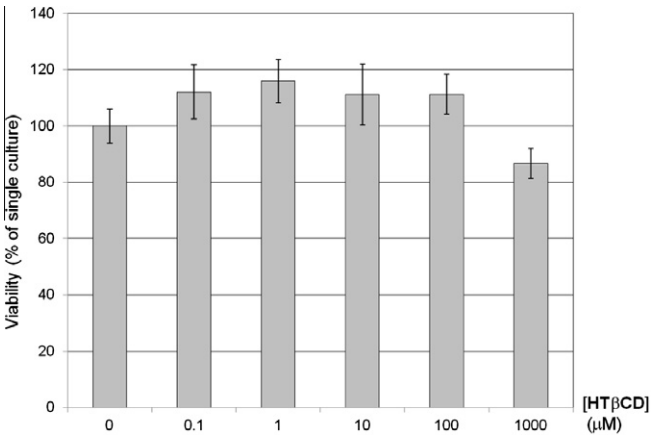


Figure 6. Effect of **HTβCD** on cell viability in Hep-G2 cells. After 24 h of **HTβCD** treatment, the visible absorption of formazan crystals was measured at 570 nm and the cell viability was normalized by the control value ([**HTβCD**] = 0). Results represent the mean (±SEM) of five independent experiments.

solvation of the cyclodextrin cavity, thereby decreasing the K_as. For practical applications, which are currently limited by the low solubility of β-CD, the reduced K_a of **HTβCD** can be compensated for by the enhanced water solubility of **HTβCD**.

The cytotoxicity of a new chemically modified form of β-CD is also of critical importance for future drug delivery and food/cosmetic formulation applications. We measured the effect of the 6-hydroxymethyltriazolyl modification of β-CD on cell viability using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay[24] (Fig. 6 and Supplementary data E). **HTβCD** did not adversely impact Hep-G2 cell viability at concentrations under 100 μM. The cell viability decreased to 86% only at a very high concentration of **HTβCD** (1000 μM) compared with the control.

In conclusion, we synthesized **HTβCD**, a chemically modified β-cyclodextrin through a microwave-assisted, protection-free, copper(I)-catalyzed azide–alkyne cycloaddition reaction, and investigated its properties. **HTβCD** has unique structural and physical characteristics. NMR, circular dichroism, and computer modeling showed that **HTβCD** has a well-defined, rigid structure. In addition the 6-(4-hydroxymethyl-1*H*-[1,2,3]triazol-1-yl) modification of β-CD dramatically improved its water solubility. The binding constants measured by UV/vis and ITC titration revealed that **HTβCD** has a lower K_a than β-CD. An MTT assay revealed that the 6-hydroxymethyltriazolyl modification of β-CD did not adversely affect cell viability for **HTβCD** concentrations of less than 100 μM. These experimental data indicate that **HTβCD** meets the following basic requirements for further applications: (1) facile synthesis, (2) well-defined structure, (3) high water solubility, and (4) low cytotoxicity. Considering these material properties of **HTβCD**, structurally well-defined, highly water-soluble cyclodextrin click clusters appear to have potential as novel excipients in pharmaceutical formulations.

Acknowledgments

This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2011-0002609). This work was supported by a Grant from the Kyung Hee University in 2011 (KHU-20110242).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2012.08.073.

References and notes

1. Davis, M. E.; Brewster, M. E. *Nat. Rev. Drug Disc.* **2004**, *3*, 1023.
2. Laza-Knoerr, A. L.; Gref, R.; Couvreur, P. *J. Drug Target* **2010**, *18*, 645.
3. *Handbook of Pharmaceutical Excipients*; Kibbe, A. H., Ed., 3rd ed.; American Pharmaceutical Association and Pharmaceutical Press, 2000. pp 165–168.
4. βCDs (molecular weight (1030–1750 Da)) are able to achieve a 50% (w/v) concentration in water.
5. Pitha, J.; Gerloczy, A.; Olivi, A. *J. Pharm. Sci.* **1994**, *83*, 833.
6. (a) Meldal, M.; Tornøe, C. W. *Chem. Rev.* **2008**, *108*, 2952; (b) Bock, V. D.; Hiemstra, H.; van Maarseveen, J. H. *Org. Lett.* **2006**, *8*, 919; (c) Hein, J. E.; Fokin, V. V. *Chem. Soc. Rev.* **2010**, *39*, 1302.
7. Hoogenboom, R.; Moore, B. C.; Schubert, U. S. *Chem. Commun.* **2006**, *14*, 4010.
8. (a) Srinivasachari, S.; Fichter, K. M.; Reineke, T. M. *J. Am. Chem. Soc.* **2008**, *130*, 4618; (b) Méndez-Ardoy, A.; Gómez-García, M.; Ortiz Mellet, C.; Sevillano, N.; Girón, M. D.; Salto, R.; Santoyo-González, F.; García Fernández, J. M. *Org. Biomol. Chem.* **2009**, *7*, 2681; (c) Méndez-Ardoy, A.; Guilloteau, N.; Di Giorgio, C.; Vierling, P.; Santoyo-González, F.; Ortiz Mellet, C.; García Fernández, J. M. *J. Org. Chem.* **2011**, *76*, 5882.
9. (a) David, O.; Maisonneuve, S.; Xie, J. *Tetrahedron Lett.* **2007**, *48*, 6527; (b) Souchon, V.; Maisonneuve, S.; David, O.; Leray, I.; Xie, J.; Valeur, B. *Photochem. Photobiol. Sci.* **2008**, *7*, 1323.
10. (a) Pérez-Balderas, F.; Ortega-Muñoz, M.; Morales-Sanfrutos, J.; Hernández-Mateo, F.; Calvo-Flores, F. G.; Calvo-Asín, J. A.; Isac-García, J.; Santoyo-González, F. *Org. Lett.* **1951**, *2003*, 5; (b) Ortega-Muñoz, M.; Morales-Sanfrutos, J.; Perez-Balderas, F.; Hernandez-Mateo, F'.; Giron-Gonzalez, M. D.; Sevillano-Tripero, N.; Salto-Gonzalez, R.; Santoyo-Gonzalez, F. *Org. Biomol. Chem.* **2007**, *5*, 2291.
11. (a) Bryson, J. M.; Chu, W. J.; Lee, J. H.; Reineke, T. M. *Bioconjugate Chem.* **2008**, *19*, 1505; (b) Song, Y.; Kohlmeir, E. K.; Meade, T. J. *J. Am. Chem. Soc.* **2008**, *130*, 6662.
12. Literature survey shows that Thatcher G. R. J. et al. reported the **HTβCD** synthesis (*Bioorg. Med. Chem.* **2010**, *18*, 809). Their synthesis used the conventional, organic click condition (CuI, DIEA) without a chromatographic purification and the yield was low (15%). They did not report any structural, physical properties of **HTβCD**.
13. 6-Azido-6-deoxy-β-cyclodextrin **2** (30 mg) was dissolved in a 4 mL (1:1, v/v) cosolvent of THF/phosphate buffer (0.1 M, pH 7.0) and propargyl alcohol (1.5 equiv per N_3) was added. $CuSO_4$/THPTA (1:5 ratio) and sodium ascorbate were added to reach the each final concentrations of 1 mM for Cu^{2+} and 5 mM for sodium ascorbate. After a short mixing period, the reaction mixture in a closed vessel was irradiated at 70 W and 130 °C in microwave reactor (CEM Discover, dynamic mode, no cooling air) for 10 min. The reaction progress was roughly checked by TLC (1-propanol/ethyl acetate/water/28% ammonia = 6/1/3/1, R_f = 0.79 for the starting, R_f = 0.35 for the desired product). THF was evaporated in vacuo and the residue was purified by column chromatography on a Diaion™ HP-20 (water/methanol = 100/0 to 60/40). The 80/20 (water/methanol, v/v) fraction afforded the desired product (yield, 52%). Up to ~200 mg of **HTβCD** was successfully obtained by open-vessel MW irradiation at 75 °C for 1 h.
^{1}H NMR (D_2O, 600 MHz): δ 7.89 (s, 7H, *H7*), 5.12 (d, 7H, *J* = 3.2 Hz, *H1*), 4.46 (d, 7H, *J* = 13.3 Hz, *H9*), 4.41 (d, 7H, *J* = 13.3 Hz, *H9*), 4.30 (app d, 7H, *H6*), 4.24 (app t, 7H, *H5*), 4.17 (m, 7H, *H6*), 4.00 (app t, 7H, *H3*), 3.61 (dd, 7H, *J* = 9.8, 3.1 Hz, *H2*), 3.40 (app t, 7H, *H4*); ^{13}C NMR (D_2O, 150 MHz): δ 148.2 (*C8*), 127.2 (*C7*), 103.2 (*C1*), 84.1 (*C4*), 73.8 (*C3*), 73.1 (*C2*), 71.5 (*C5*), 55.8 (*C9*), 51.7 (*C6*); MALDI-TOF (CHCA, positive): calcd 1701.5 for $C_{63}H_{91}N_{21}O_{35}$, observed 1724.2 for $[M+Na]^+$ (relative intensity 100%).
14. Presolski, S. I.; Hong, V.; Cho, S. H.; Finn, M. G. *J. Am. Chem. Soc.* **2010**, *132*, 14570.
15. Hong, V.; Presolski, S. I.; Ma, C.; Finn, M. G. *Angew. Chem., Int. Ed.* **2009**, *48*, 9879.
16. Adachi, T.; Ando, S.; Watanabe, J. *J. Chromatogr. A* **2002**, *944*, 41.
17. (a) Kretschmann, O.; Choi, S. W.; Miyauchi, M.; Tomatsu, I.; Harada, A.; Ritter, H. *Angew. Chem., Int. Ed.* **2006**, *45*, 4361; (b) Hoshino, T.; Masahiko, M.; Kawaguchi, Y.; Yamaguchi, H.; Harada, A. *J. Am. Chem. Soc.* **2000**, *122*, 9876.
18. Ward, S.; Ling, C. C. *Eur. J. Org. Chem.* **2011**, 4853.
19. Rekaï, E. D.; Baudin, J. B.; Jullien, L.; Ledoux, I.; Zyss, J.; Blanchard-Desce, M. *Chem. Eur. J.* **2001**, *7*, 4395.
20. Harada, A.; Kamachi, M. *Macromolecules* **1990**, *23*, 2821.
21. Miyajima, K.; Komatsu, H.; Inoue, K.; Handa, T.; Nakagaki, M. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 6.
22. Zhang, B.; Breslow, R. *J. Am. Chem. Soc.* **1993**, *115*, 9353.
23. Gelb, R. I.; Schwartz, L. M. J. *Inclusion Phenom. Mol. Recognit. Chem.* **1989**, *7*, 465.
24. Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.

25. Boëns, F. B.; Elchinger, P.-H.; Brouillette, F.; Montplaisir, D.; Zerrouki, R.; Lucas, R. *Eur. J. Org. Chem.* in press. doi: http://dx.doi.org/10.1002/ejoc.201200013 (When Cyclodextrins Meet Click Chemistry).
26. *Cyclodextrins in pharmaceutics, cosmetics, and biomedicine: current and future industrial applications*; Bilensoy, E., Ed.; John Wiley & Sons, 2011. Chapters 2, 6, 7, 9.
27. Roehri-Stoeckel, C.; Olivier, D.; Brouillar, R. *Tetrahedron Lett.* **1997**, *38*, 1551.