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SYNTHESIS OF HEPTA-ARUBUTIN-BRANCHED β-CYCLODEXTRINS AT THEIR PRIMARY SIDES *VIA* CLICK REACTION

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Abstract – This paper describes the synthesis of β-cyclodextrin (CD) based arubutin (4-hydroxyphenyl β-glucopyanoside) conjugates as a model for drug delivery molecules. We introduced three variants of hepta-arubutin-branched CDs at their primary side. Of these, two incorporated oligo(ethylene)chains within their spacers to demonstrate the effect of varying spatial characteristics between the β-glucopyanoside moieties. Due to the sterically hindered position on the primary side of β-CD, we employed a highly efficient click chemistry reaction.

CDs demonstrate a significant potential for forming complexes with drug molecules.[1,2] Carbohydrate-branched CDs are anticipated to be pivotal in drug delivery systems owing to their ability to recognize specific lectins on cell surfaces, attributed to their branched-carbohydrate ligand(s). Several carbohydrate-branched CDs have been reported by various research groups.[3] For the effective use of CD-based carbohydrate conjugates in drug delivery, we identified two essential technologies. The first entails a robust method for attaching carbohydrate moieties to CD molecules. The second involves an effective approach to enhance the drug inclusion capability of CDs, ensuring targeted drug delivery to cells.

Our previous work documented the synthesis of various mono- or di-carbohydrate-branched β-CDs at their primary sides, containing glucose, galactose, or *N*-acetylglucosamine derivatives.[4-7] Among these, arubutin, a commercially available natural product, proved to be a synthetic asset in designing carbohydrate-branched CD models, namely arubutin-branched CDs. This is because arubutin possesses a glycosidic bond that streamlines the synthetic steps and an acidic phenol hydroxy group that can be differentiated from other carbohydrate hydroxy groups. Our custom β-CDs included a benzene ring

derived from arubutin, located in the spacers between the β-glucoside moiety and the CD molecule. This benzene ring facilitated the delivery of the anticancer agent, doxorubicin, by leveraging the stacking effect between the benzene and doxorubicin's aromatic rings. Moreover, our findings showed that arubutin-branched β-CDs acted as enzymatic transglycosylation acceptors, producing *N*-linked oligosaccharide-branched β-CDs through glycosylation of their β-glucosyl moieties.[8] Figure 1 illustrates our previous research findings.

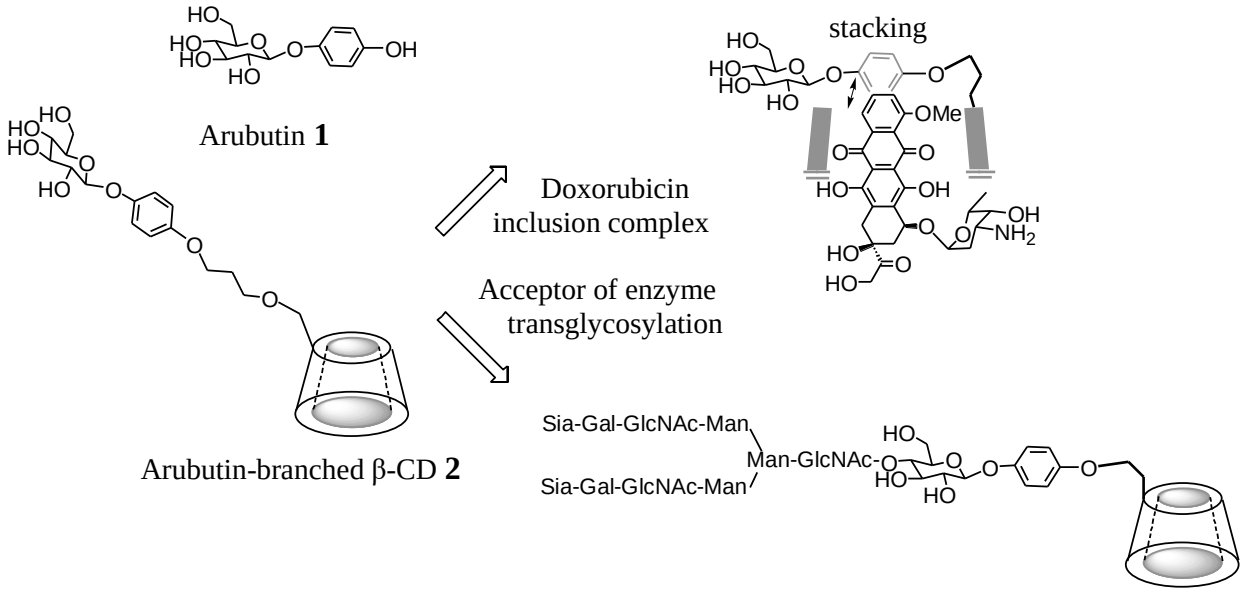


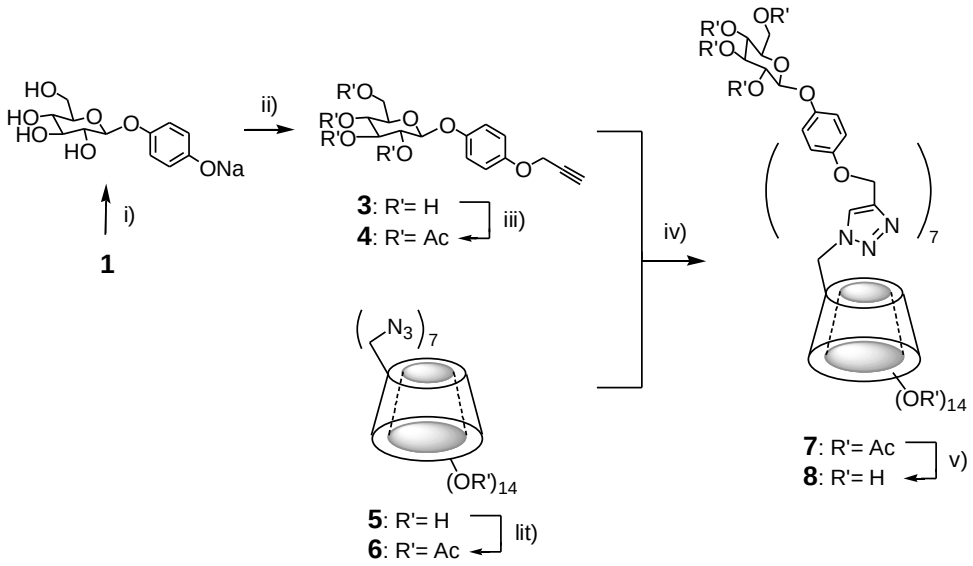
Figure 1. Our representative mono-arubutin branched CD **2** and previous researches

It is noteworthy that many lectins inherently possess multiple carbohydrate-binding sites. While individual carbohydrate-lectin interactions might be weak, their strength is often amplified by the glycocluster effect, which enables multivalent molecular recognitions between lectins and glycoconjugates adorned with multivalent carbohydrate moieties.[9-12] Furthermore, the spatial attributes of the multiple sites of lectins significantly influence multivalent carbohydrate-lectin interactions. This underscores the importance of designing multivalent glycoconjugates, factoring in the spatial relationships among carbohydrate moieties. We subsequently turned our focus to the synthesis and evaluation of heptavalent arubutin-conjugated β-CDs at their primary sides, serving as drug delivery models.

In the present study, we detail the synthesis of three distinct hepta-arubutin-branched β-CDs (**8**, **18** and **20**) at their primary side. Two of these incorporate oligo(ethylene)chains within their spacers, showcasing the impact of varying spatial configurations among β-glucopyanoside units. Given the challenges posed by the sterically constrained position on the primary side of β-CD, we opted for a high-efficiency click

chemistry reaction.[13] The specific reaction steps involved in the synthesis of compounds **8**, **18** and **20** are detailed in the subsequent sections.

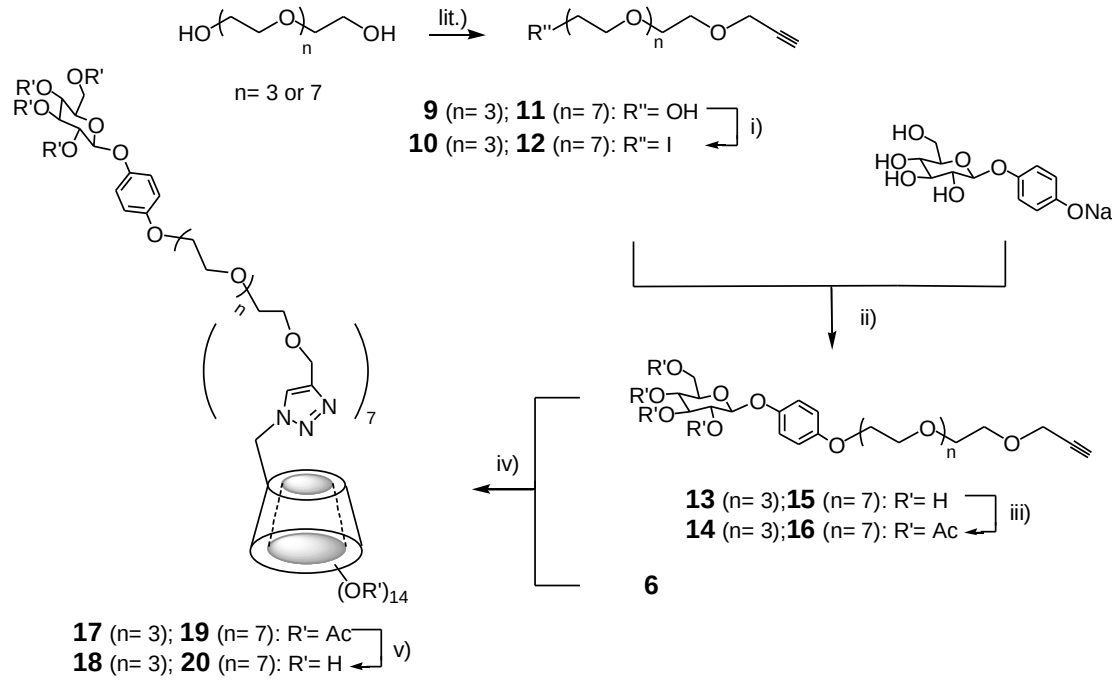
Scheme 1 outlines the synthesis of compound **8**. 2-Propynylated arubutin (**3**) was produced with an 82% yield by reacting dry arubutin sodium salt (pretreated with a 0.5 mol/L sodium hydroxide solution, 1 eq.) with 2-propynyl bromide (1.2 eq.) in *N*,*N*-dimethylformamide at room temperature for 24 h. Acetylation of **3**, using acetic anhydride and pyridine for 24 h at room temperature, resulted in compound **4** with a 91% yield. Based on our previously reported method,[14] the Huisgen cycloaddition of compounds **4** and **6**[15] using sodium ascorbate (0.1 eq.) and copper sulfate (0.12 eq.) in a tetrahydrofuran-water mixture was conducted under microwave irradiation at 18 W, heating the mixture to 70 °C for 40 min. This process resulted in compound **7** with a 93% yield, indicating that all azide groups in **6** efficiently reacted with **4** *via* the click chemistry reaction. Initial attempts at Huisgen cycloaddition using nonacetylated compounds **3** and **5** under similar conditions were unsuccessful, likely due to the poor solubility of **5** in the solvent. Subsequent deacetylation of **7** using catalytic sodium methylate in methanol at room temperature for 16 h yielded a crude product, which was purified by reprecipitation in methanol, ultimately producing the desired compound **8**[16] with an 88% yield.



Scheme 1. Reagents and conditions: i) 0.5 mol/L NaOH solution (1 eq.); ii) 2-propynyl bromide (1.2 eq.)/ DMF, rt, 24 h, 82% yield from **1**; iii) A_2O/ pyridine, rt, 24 h, 91% yield; iv) **4** (8 eq.)-sodium ascorbate (0.1 eq.)-$CuSO_4$ (0.12 eq.)/ THF-H_2O, microwave irradiation (18 W), up to 70 °C, 40 min, 93% yield; v) NaOMe (cat.)/ MeOH, rt, 16 h, 88% yield.

Scheme 2 describes the synthesis route for compounds **18** and **20**. Compound **9** (or **11**) was synthesized from tetraethylene (or octaethylene) glycol using sodium hydride and 2-propynyl bromide in tetrahydrofuran, as detailed in prior literature.[17] Iodination of **9** (or **11**) using triphenylphosphine (3 eq.)

and iodine (3 eq.) in *N*,*N*-dimethylformamide at 40 °C for 24 h produced compound **10** (or **12**) with an 75% (or 85%) yield. Compound **13** (or **15**) was achieved with a 76% (or 61%) yield by reacting compound **10** (or **12**) with arubutin sodium salt (1 eq.) in *N*,*N*-dimethylformamide at room temperature over 24 h. Acetylation of **13** (or **15**), utilizing acetic anhydride and pyridine at room temperature for 24 h, yielded compound **14** (or **16**) with a 95% (or 90%) yield. The Huisgen cycloaddition of compounds **14** (or **16**) and **6**, under the previously described conditions, produced compound **17** (or **19**) with an 94% (or 87%) yield. Deacetylation of **17** (or **19**) using catalytic sodium methylate in methanol at room temperature for 24 h produced a crude product. After purification through reprecipitation in methanol, the desired compound **18**[18] (or **20**[19]) was achieved with a 97% (or 91%) yield.



Scheme 2. Reagents and conditions: i) PPh_3 (3 eq.)-I_2 (3 eq.)/ DMF, 40 °C, 24 h, **10**: 75% (or **12**: 85%) yield; ii) arubutin sodium salt (1 eq.)/ DMF, rt, 24 h, **13**: 76% (or **15**: 61%) yield; iii) A_2O/ pyridine, rt, 24 h, **14**: 95% (or **15**: 90%) yield; iv) **14** (or **16**) (7 eq.)-sodium ascorbate (0.1 eq.)- $CuSO_4$ (0.1 eq.)/ THF-H_2O, microwave irradiation (18 W), up to 70 °C, 40 min, **17**: 94% (or **19**: 87%) yield; v) NaOMe (cat.)/ MeOH, rt, 24 h, **18**: 97% (or **20**: 91%) yield.

In conclusion, we successfully synthesized three variants of hepta-arubutin-branched β-CDs, each differing in spacer lengths between the β-glucopyanoside moieties, *via* a click chemistry reaction. We are currently exploring the potential of these β-CD conjugates as models for drug delivery molecules.

SUPPORTING INFORMATIONS

Supplementary (synthesis of hepta-arubutin-branched β-CD derivatives, ^{1}H and ^{13}C NMR, MS spectra) data associated with this article can be found, in the online version, at URL: https://www.heterocycles.jp/newlibrary/downloads/PDFsi/28034/106/12

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16. Hepta-arubutin-branched CD **8**: ^{1}H NMR (DMSO-d_6): δ 2.09-3.30 (42H, m, Arubutin-2, 3, 4, 5, CD-2, 4), 3.44-3.47 (7H, m, Arubutin-6_a), 3.66-3.70 (14H, m, Arubutin-6, CD-3), 4.00-4.02 (7H, m, CD-5), 4.19-4.21 (7H, m, CD-6_a), 4.33-4.35 (7H, bd, *J* = 12.3 Hz, CD-6_b), 4.54 (OH), 4.70 (7H, d, *J* = 7.5 Hz, Arubutin-1), 4.47-4.82 (14H, m, OCH_2-triazole), 4.98 (OH), 5.04 (OH), 5.08 (7H, bs, CD-1), 5.27 (OH), 5.87 (OH), 6.00 (OH), 6.80 (14H, d, *J* = 9.0 Hz, Ph), 6.90 (14H, d, *J* = 9.0 Hz, Ph), 8.00 (7H, s, CH_2CCHN); ^{13}C NMR (DMSO-d_6): δ 49.4 (CD-5), 60.7 (Arb-6), 61.2 (OCH_2-triazole), 69.6 (CD-6), 69.7 (Arb-4), 71.6 (CD-2), 72.3 (CD-3), 73.2 (Arb-2), 76.5 (Arb-5), 76.9 (Arb-3), 82.5 (CD-4), 101.3 (Arb-1), 101.5 (CD-1), 115.2, 117.4 (Ph), 126.0 (*C*H=CN), 142.5

(CH=*C*N), 151.6, 153.0 (Ph); MALDI-TOF MS: *m/z* calcd for $C_{147}H_{189}N_{21}O_{77}\cdot Na^+$: 3503.14; found 3505.05.

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18. Hepta-arubutin-branched CD **18**:^{1}H NMR (D_2O): δ 3.22 (7H, bs, CD-4), 3.40-4.37 (196H, m, Arubutin-2, 3, 4, 5, CD-2, 5, 6, OCH_2CH_2, OCH_2-triazole), 4.76-4.80 (7H, m, CD-3), 4.84 (7H, d, *J* = 7.6 Hz, Arubutin-1), 4.96 (7H, bs, CD-1), 6.84 (14H, d, *J* = 6.8 Hz, Ph), 7.00 (14H, d, *J* = 4.0 Hz, Ph), 7.87 (7H, s, $CH_2CC\mathit{H}N$); ^{13}C NMR (D_2O): δ 50.1 (CD-5), 60.6 (Arb-6), 63.1 (OCH_2-triazole), 67.8 (CD-6), 69.2 (Arb-4), 69.4-70.0 (OCH_2CH_2), 71.7 (CD-2), 72.5 (CD-3), 73.0 (Arb-2), 75.7 (Arb-5), 76.1 (Arb-3), 82.3 (CD-4), 101.3 (Arb-1), 101.7 (CD-1), 115.8, 118.2 (Ph), 126.6 (*C*H=CN), 143.9 (CH=*C*N), 151.3, 153.8 (Ph); MALDI-TOF MS: *m/z* calcd for $C_{203}H_{301}N_{21}O_{105}\cdot Na^+$: 4735.87; found 4736.10.
19. Hepta-arubutin-branched CD **20**:^{1}H NMR (D_2O): δ 3.18 (7H, bs, CD-4), 3.32-4.32 (308H, m, Arubutin-2, 3, 4, 5, 6, CD-2, 5, 6, OCH_2CH_2, OCH_2-triazole), 4.70-4.71 (7H, m, CD-3), 4.81 (7H, d, *J* = 7.8 Hz, Arubutin-1), 4.95 (7H, bs, CD-1), 6.83 (14H, d, *J* = 6.6 Hz, Ph), 6.96 (14H, d, *J* = 7.2 Hz, Ph), 7.85 (7H, s, $CH_2CC\mathit{H}N$); ^{13}C NMR (D_2O): δ 50.2 (CD-5), 60.6 (Arb-6), 63.1 (OCH_2-triazole), 67.9 (CD-6), 69.2 (Arb-4), 69.5-70.1 (OCH_2CH_2), 71.7 (CD-2), 72.5 (CD-3), 73.1 (Arb-2), 75.7 (Arb-5), 76.1 (Arb-3), 82.3 (CD-4), 101.3 (Arb-1), 101.8 (CD-1), 116.0, 118.2 (Ph), 126.7 (*C*H=CN), 144.0 (CH=*C*N), 151.3, 153.9 (Ph); MALDI-TOF MS: *m/z* calcd for $C_{259}H_{413}N_{21}O_{133}\cdot Na^+$: 5968.61; found 5968.90.