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Note

Cyclomaltoheptaose mixed esters of anti-inflammatory drugs and short-chain fatty acids and study of their enzymatic hydrolysis in vitro

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ABSTRACT

In an effort to enhance the drug-loading capacity of cyclomaltoheptaose (β-cyclodextrin, βCD) and to combine the function of anti-inflammatory drugs with short-chain fatty acids (SCFA), ternary esters incorporating seven copies of an anti-inflammatory drug and 14 copies of a SCFA onto a β-cyclodextrin core were designed and prepared. Acetic, propionic, or butyric esters were introduced at secondary OH groups, and ibuprofen, flurbiprofen, or felbinac was attached to primary OH groups through ester bonds. Heptakis[2,3-di-*O*-butanoyl-6-*O*-2-(biphenyl-4-yl)-ethanoyl]-cyclomaltoheptaose was very stable in aqueous and esterase solution. It was hydrolyzed by α-amylase (4 units/mL) with $t_{1/2}$ value of 18 h. The total released amount of biphenyl acetic acid was 38% after 24 h when the esterase was added after the α-amylase hydrolysis. The present results suggest that these nine βCD conjugates may release the anti-inflammatory drug in the colonic contents.

Oral colon-specific drugs are especially valuable in the treatment of colon diseases.[1] Recently, azo-compounds, glycoside, glucuronide, dextran, and polypeptide conjugates have been prepared for oral colon-specific delivery.[2] Conjugates of cyclomaltooligosaccharides (cyclodextrins, CDs) with biphenyl acetic acid,[3,4] prednisolone,[5] and 5-aminosalicylic acid[6] have also been prepared for the same purpose.

Short-chain fatty acids (SCFAs) play an important physiological role in the maintenance of the health and integrity of the colonic epithelium. However, oral administration is difficult because the SCFAs are absorbed or metabolized in the upper intestinal tract. Hirayama et al.[7] prepared a βCD conjugate in an attempt to construct a colon delivery system for SCFAs.

All the CD conjugates reported involved monosubstitution at C-6 of the cyclodextrin core. One aim of this work was to enhance the loading capacity of CDs for drugs by per-modification at the primary and secondary hydroxyl groups. The other was to synthesize anti-inflammatory drugs and SCFAs conjugated CD prodrugs, which may improve therapy of the colon.

These conjugates were prepared by modifying the method of Berberan-Santos et al.[8] The process is shown in Scheme 1. Starting from per(6-deoxy-6-iodo)-βCD,[9] the secondary OH groups were acylated using acyl anhydrides and pyridine in the presence of a catalytic amount of DMAP to give the peracylated derivatives in 23–76.1% yield. Then, treatment of these acylated derivatives with the sodium salt of the selected anti-inflammatory drug in anhydrous DMF yielded the desired βCD conjugates. Difficulties in most of our syntheses arose in the purification of the final products (column chromatography, TLC, and crystallization). Structures of the nine new compounds were confirmed by ^{1}H NMR, FABMS, and elemental analysis.

To gain insight into the stability of the conjugates, the hydrolysis of **12** in aqueous solution at various pHs was analyzed in detail as an example. Figure 1 indicates that **12** was hydrolyzed according to first-order kinetics, and the half-lives of **12** were 25.1, 626.9, and 125.4 days at pH 1.0, 6.8, and 7.4, respectively. The half-lives of **12** are much higher as compared to the monosubstituted β-cyclodextrin biphenylacetic acid conjugate.[4]

Sugar-degrading and ester-hydrolyzing enzymes were applied to gain insight into the hydrolysis mechanism of the conjugates taking **12** as a model. Esterase from porcine liver was used as a model ester-hydrolyzing enzyme. As shown in Figure 2A, neither appreciable hydrolysis of **12** nor release of biphenyl acetic acid was observed for 46 h. These results indicate that **12** is not susceptible to the esterase-catalyzed hydrolysis. *Aspergillus oryzae* α-amylase was chosen as the model of sugar-degrading enzyme.[10,11] As shown in Figure 2B, the conjugate was hydrolyzed by the enzyme according to a first-order kinetics, with a $t_{1/2}$ value of 18 h at enzyme concentration of 4 units/mL. The hydrolysis of the conjugate **12** was slower

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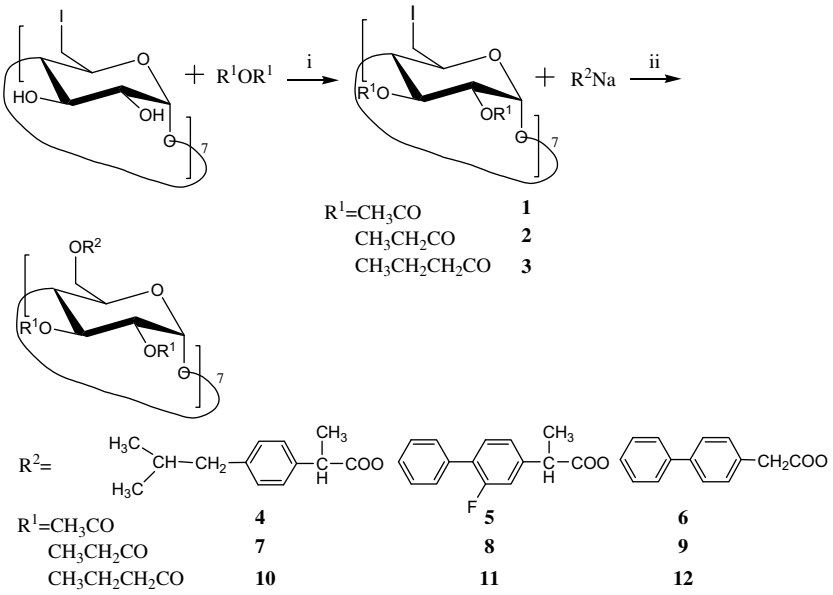

Scheme 1. Sythesis of cyclomaltoheptaose mixed esters of anti-inflammatory drugs and short-chain fatty acids. Reagents and conditions: (i) DMAP, pyridine, rt, 48 h; (ii) DMF, 100 °C, 96 h.

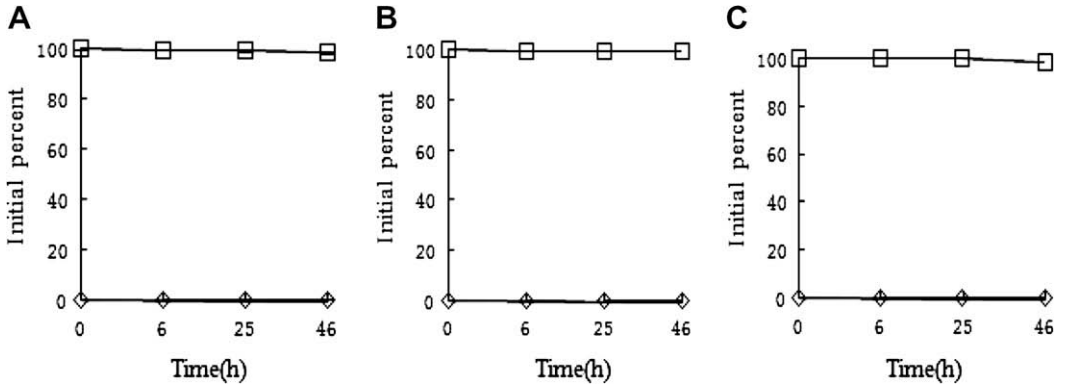

Figure 1. Time courses of disappearance of the conjugate IV_3 (□, 8.0×10^{-5} M) and appearance of biphenyl acetic acid (△) in the aqueous solutions at 37 °C: (A) hydrochloride acid solution (0.1 M); (B) phosphate buffers (pH 6.8, $I = 0.2$); (C) phosphate buffers (pH 7.4, $I = 0.2$).

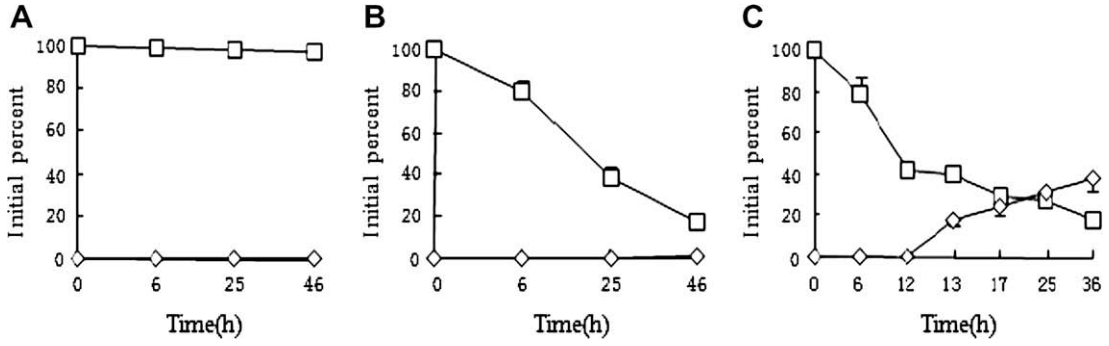

Figure 2. Time courses of disappearance of the conjugate IV_3 (□, 8.0×10^{-5} M) and appearance of biphenyl acetic acid (△) in the presence of enzymes at 37 °C: (A) with the α-amylase (4 units/mL) in acetate buffer (pH 5.5); (B) with the carboxylic esterase (40 units/mL) in HEPES buffer (pH 7.4); (C) the esterase (40 units/mL) was added 12 h after the α-amylase (4 units/mL) hydrolysis. Key: (a) addition of the α-amylase; (b) addition of the esterase. Each point represents the mean ± SD of three experiments.

than β-cyclodextrin ($t_{1/2}$ = 2.5 h calculated under the same condition[7,12]). Figure 2C shows the hydrolysis behavior of **12** when the carboxylic esterase was added 12 h after the α-amylase-catalyzed hydrolysis. α-Amylase hydrolyzed **12** to small saccharide conjugates,[12,13] while no release of biphenyl acetic acid was observed (Fig. 2C). However, biphenyl acetic acid was rapidly released when the esterase was added after the α-amylase treatment (38% after 24 h of the esterase treatment). These results indicate that the ester linkage of the small saccharide conjugates is easily cleaved by the esterase. Moreover, colon transit time may last up to 78 h,[14] which

is likely to increase the time available for biphenyl acetic acid release from **12**. These studies suggest that both sugar-degrading and ester-hydrolyzing enzymes are required for the release of biphenyl acetic acid from its βCD conjugate, which is in agreement with the results of Hirayama et al.[7]

On another hand, the release of biphenyl acetic acid from **12** was slower than that of butyric acid from the monosubstituted butyric acid–βCD ester conjugate (55% *n*-butyric acid was released in the same conditions).[7] It is probable that the esterase hydrolyzed some butyric acid esters at a secondary position of **12**. However, butyric acid does not exhibit properties for direct detection,[15] and it was not quantitatively detected by HPLC in our experiments. Other possible explanation for the observed drug-release discrepancy could not be excluded. For example, the aqueous solubility of the persubstituted βCD ester conjugate **12** is lower than that of the monosubstituted *n*-butyric acid-βCD ester conjugate, affecting the drug release from the conjugate.

1. Experimental

1.1. General methods

βCD was purchased from Sinopharm Chemical Reagent Co. (China). Ibuprofen and Felbinac were purchased from Hubei Biocause Pharmaceutical Co., Ltd (China). Flurbiprofen was purchased from Shanghai Sunve Pharmaceutical Co., Ltd (China). *A. oryzae* α-amylase (EC 3.2.1.1, MW 53,000) and esterase (EC 3.1.1.1, MW 168,000) were bought from Sigma Chemicals Co. (St. Louis, MO, USA). Other chemicals and solvents were of analytical grade, and deionized, doubly distilled water was used throughout the study. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ACF-500 instrument using TMS as an internal standard. Elemental analysis was performed with an Elementar Vario EL III equipment. FAB mass spectra were recorded on a PE ESI-TOF mass spectrometer. Melting points were determined on an X4 micromelting point apparatus, and were uncorrected. Silica gel (200–300 mesh) for chromatography and Silica Gel GF_{254} for TLC were purchased from Qingdao Haiyang Chemical Co., Ltd. [Editor's note: required optical rotations were not provided due to shortage of products].

1.2. Heptakis(2,3-di-*O*-acetyl-6-deoxy-6-iodo)cyclomaltoheptaose (1)

The preparation was analogous to that described by Baer et al.,[16] except that the purification stage was omitted. To a soln of per-6-deoxy-iodo-β-cyclodextrin[10] (1.91 g, 1.0 mmol) in 11 mL of dry pyridine were added 9 mL of acetic anhydride and 13.0 mg (0.11 mmol) of DMAP. The reaction mixture was stirred at room temperature under N_2 for 48 h. The reaction mixture was poured into 80 mL of ice and water. The insoluble material was filtered and crystallized from MeOH. Compound **1** (1.89 g, 76.1%) was obtained as a white solid; mp 179–180 °C [dec]; lit.[16] 180 °C; $[\alpha]_D$ +82.5 (*c* 1, $CHCl_3$).

1.3. Heptakis(2,3-di-*O*-propanoyl-6-deoxy-6-iodo)cyclomaltoheptaose (2)

Similar experiments as for **1** carried out with propionic anhydride afforded **2** as an amorphous solid (2.23 g, 58.5%); mp 140–142 °C (MeOH); ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.32–5.36 (t, 7H, *J* 8.58 Hz, H-3), 5.17 (d, 7H, *J* 3.2 Hz, H-1), 4.84–4.86 (m, 7H, H-2), 3.71–3.82 (m, 14H, H-4, H-5′), 3.60–3.64 (m, 14H, H-6, H-6′), 2.17–2.43 (m, 28H, CH_2, CH_2), 1.08–1.13 (m, 42H, CH_3, CH_3); ^{13}C NMR (500 MHz, $CDCl_3$): δ 173.59, 172.26 (C═O,C═O), 96.35 (C-1), 80.27 (C4), 76.9 (C2), 76.4 (C3), 69.8 (C5), 27.02 (CH_2), 8.56 (CH_3), 7.99 (C-6).

1.4. Heptakis(2,3-di-*O*-butanoyl-6-deoxy-6-iodo)cyclomaltoheptaose (3)

A similar experiment as for **1** was carried out with butyric anhydride. After the reaction was stopped, the reaction mixture was poured into 100 mL of ice and water. The insoluble material was filtered, and was purified by silica gel chromatography with 3:1 petroleum ether–acetone. Compound **3** was obtained as a white solid (0.75 g, 23%); mp 110–112 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.33–5.40 (m, 7H, H-3), 5.19 (m, 7H, *J* 3.9 Hz, H-1), 4.83–4.86 (m, 7H, H-2), 3.71–3.86 (m, 14H, H-4, H-5), 3.60–3.63 (m, 14H, H-6, H-6′), 2.17–2.41 (m, 28H, CH_2, CH_2), 1.61–1.66 (m, 28H, CH_2, CH_2), 0.92–0.99 (m, 42H, CH_3, CH_3); ^{13}C NMR (500 MHz, $CDCl_3$): δ 172.73, 171.32 (C═O,C═O), 96.23 (C1), 80.30 (C4), 76.96 (C2), 76.71 (C3), 69.76 (C5), 35.42 (CH_2), 17.80 (CH_2), 13.24 (CH_3), 8.18 (C6); FABMS: calcd for $C_{98}H_{151}I_7NO_{42}$ $[M+NH_4]^+$: 2902.3, found: *m/z* 2902.4.

1.5. General procedure for the preparation of heptakis(2,3-di-*O*-acetyl-6-*O*-acyl)cyclomaltoheptaose derivatives of ibuprofen, flurbiprofen, and felbinac (4–6)

A mixture of **1** (0.24 mmol) and the respective sodium salts of ibuprofen, flurbiprofen, or felbinac (3.08 mmol) in 15 mL anhyd DMF was heated for 96 h at 100 °C. After cooling to room temperature, the reaction mixture was poured into 80 mL of ice and water. The insoluble material was filtered, and was purified twice by silica gel chromatography (1:1 petroleum ether–EtOAc or 3:1 ether–acetone or 1.5:1 petroleum ether–EtOAc).

1.5.1. Heptakis[2,3-di-*O*-acetyl-6-*O*-2-(4-isobutylphenyl)propionoyl)]cyclomaltoheptaose (4)

From 0.6 g of **1** and 0.71 g of ibuprofen sodium salt; white solid (0.35 g, 48.2%); mp 110–112 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.18 (d, 14H, *J* 5.7 Hz, Ar), 7.05–7.09 (m, 14H, Ar), 5.14 (d, 7H, *J* 8.0 Hz, H-2), 4.81–4.99, 4.27–4.45 (m, 7H, H-1), 4.66–4.70 (m, 7H, H-5), 4.55–4.57 (m, 7H, H-3), 4.09–4.21 (m, 7H, H-6), 3.84–4.04 (m, 7H, H-6′), 3.74–3.77 (m, 7H, CH), 3.25–3.51 (m, 7H, H-4), 2.42–2.43 (m, 14H, CH_2), 1.98–2.42 (m, 42H, CH_3, CH_3), 1.83–1.85 (m, 7H, CH), 1.48–1.49 (m, 7H, CH_3), 0.88–0.89 (d, 42 H, $(CH_3)_2$); FABMS: calcd for $C_{161}H_{214}NO_{56}$ $[M+NH_4]^+$: 3059.4, found: *m/z* 3059.4. Anal. Calcd for $C_{161}H_{210}O_{56}\cdot 2H_2O$: C, 62.84; H, 7.01. Found: C, 62.45; H, 6.86.

1.5.2. Heptakis[2,3-di-*O*-acetyl-6-*O*-2-(2-fluoro-4-biphenylyl)propionoyl]cyclomaltoheptaose (5)

From 0.6 g of **1** and 0.82 g of flurbiprofen sodium salt; white solid (0.15 g, 21%); mp 114–116 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.51–7.62 (m, 14H, Ar′), 7.26–7.42 (m, 28H, Ar), 7.13–7.21 (m, 14H, Ar, Ar′), 5.15–5.17 (m, 7H, H-2), 4.89–4.94, 4.38–4.42 (m, 7H, H-1), 4.73–4.85 (m, 7H, H-5), 4.43–4.61 (m, 7H, H-3), 4.19–4.31 (m, 7H, H-6), 3.95–4.12 (m, 7H, H-6′), 3.78–3.88 (m, 7H, CH), 3.21–3.41 (m, 7H, H-4), 1.85–2.01 (m, 42H, CH_3, CH_3), 1.55–1.60 (m, 21H, CH_3); FABMS *m/z* calcd for $C_{175}H_{179}F_7NO_{56}$ $[M+NH_4]^+$: 3325.3, found: 3325.0. Anal. Calcd for $C_{175}H_{175}F_7O_{56}\cdot 2H_2O$: C, 62.87; H, 5.40. Found: C, 62.55; H, 5.05.

1.5.3. Heptakis[2,3-di-*O*-acetyl-6-*O*-2-(biphenyl-4-yl)-ethanoyl]cyclomaltoheptaose (6)

From 0.6 g of **1** and 0.72 g of felbinac; white solid (0.20 g, 27%); mp 131–132 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.44–7.52 (m, 28H, Ar′), 7.27–7.37 (m, 35H, Ar), 5.21–5.25 (t, 7H, *J* 8.56 Hz, H-2), 4.94–4.95 (d, 7H, *J* 3.73 Hz, H-1), 4.65–4.70 (m, 14H, H-3, H-5), 4.29–4.32 (m, 7H, H-6), 4.09–4.11 (d, 7H, *J* 9.1 Hz, H-6′), 3.65–3.74 (m, 14H, CH_2), 3.48–3.52 (t, 7H, *J* 8.8 Hz, H-4), 2.00 (s, 21H, CH_3), 1.94 (s, 21H, CH_3); FABMS: calcd for $C_{168}H_{168}NaO_{56}$

$[M+Na]^+$: 3104.0, found: *m/z* 3104.0. Anal. Calcd for $C_{168}H_{168}O_{56}\cdot 2H_2O$: C, 65.69; H, 5.56. Found: C, 64.61; H, 5.20.

1.6. General procedure for the preparation of heptakis(2,3-*O*-propanoyl-6-*O*-acyl)cyclomaltoheptaose derivatives of ibuprofen, flurbiprofen, and felbinac (7–9)

A mixture of 0.22 mmol of **2** and 3.08 mmol of the respective sodium salts of ibuprofen, flurbiprofen, and felbinac in 15 mL anhyd DMF was heated for 96 h at 100 °C. After cooling to room temperature, the reaction mixture was poured into 80 mL of ice and water. The insoluble material was filtered, and was purified by silica gel chromatography (4:1 petroleum ether–EtOAc or 1:1 petroleum ether–EtOAc).

1.6.1. Heptakis[(2,3-*O*-propanoyl-6-*O*-2-(4-isobutylphenyl)-propionoyl]cyclomaltoheptaose (7)

From 0.6 g of **2** and 0.71 g of ibuprofen sodium salt; white solid (0.12 g, 17%); mp 101–102 °C; ^{1}H NMR (500 MHz, CDCl-d_3): δ 7.16–7.19 (m, 14H, Ar), 7.03–7.09 (m, 14H, Ar), 5.15–5.19 (m, 7H, H-2), 4.81–4.98, 4.27–4.41 (m, 7H, H-1), 4.85–4.86 (m, 7H, H-5), 4.42–4.59 (m, 7H, H-3), 4.16–4.22 (m, 7H, H-6), 3.91–4.02 (m, 7H, H-6′), 3.73–3.77 (m, 7H, CH), 3.20–3.42 (m, 7H, H-4), 2.39–2.42 (m, 14H, CH_2), 2.17–2.39 (m, 28H, CH_2, CH_2), 1.80–1.86 (m, 7H, CH), 1.41–1.47 (m, 7H, CH_3), 1.06–1.13 (m, 42H, CH_3, CH_3), 0.88–0.89 (d, 42 H, $(CH_3)_2$); FABMS: calcd for $C_{175}H_{242}NO_{56}$ $[M+NH_4]^+$: 3353.6, found: *m/z* 3354.5. Anal. Calcd for $C_{175}H_{238}O_{56}$: C, 64.92; H, 7.41. Found: C, 64.60; H, 7.61.

1.6.2. Heptakis[2,3-di-*O*-propanoyl-6-*O*-2-(2-fluoro-4-biphenylyl)-propionoyl]cyclomaltoheptaose (8)

From 0.6 g of **2** and 0.82 g of flurbiprofen sodium salt; white solid (0.20 g, 26%); mp 106–108 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.51–7.62 (m, 14H, Ar′), 7.30–7.49 (m, 28H, Ar), 7.13–7.21 (m, 14H, Ar, Ar′), 5.15–5.19 (m, 7H, H-2), 4.88–4.94, 4.50–4.54 (m, 7H, H-1), 4.79–4.85 (m, 7H, H-5), 4.55–4.63 (m, 7H, H-3), 4.19–4.31 (m, 7H, H-6), 3.92–4.03 (m, 7H, H-6′), 3.79–3.90 (m, 7H, CH), 3.27–3.56 (m, 7H, H-4), 1.82–2.41 (m, 28H, CH_2, CH_2), 1.55–1.60 (m, 21H, CH_3), 0.89–1.1 (m, 42H, CH_3, CH_3); FABMS: calcd for $C_{189}H_{207}F_7NO_{56}$ $[M+NH_4]^+$: 3521.6, found: *m/z* 3521.3. Anal. Calcd for $C_{189}H_{203}F_7O_{56}$: C, 64.79; H, 5.84. Found: C, 64.86; H, 5.82.

1.6.3. Heptakis[2,3-di-*O*-propanoyl-6-*O*-2-(biphenyl-4-yl)-ethanoyl]cyclomaltoheptaose (9)

From 0.6 g of **2** and 0.72 g of felbinac sodium salt**;** white solid (0.15 g, 21%); mp 110–112 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.42–7.49 (m, 28H, Ar′), 7.26–7.34 (m, 35H, Ar), 5.22–5.25 (t, 7H, *J* 9.20 Hz, H-2), 4.94–4.95 (d, 7H, *J* 3.79 Hz, H-1), 4.67–4.94 (m, 14H, H-3, H-5), 4.29–4.32 (m, 7H, H-6), 4.10–4.12 (d, 7H, *J* 8.60 Hz, H-6′), 3.64–3.73 (m, 14H, CH_2), 3.49–3.53 (t, 7H, *J* 9.2 Hz, H-4), 2.10–2.32 (m, 28H, CH_2, CH_2), 1.03–1.08 (m, 21H, CH_3), 1.01–1.02 (m, 21H, CH_3); FABMS: calcd for $C_{182}H_{200}NO_{56}$ $[M+NH_4]^+$: 3295.3, found: *m/z* 3296.2. Anal. Calcd for $C_{182}H_{196}O_{56}\cdot 2H_2O$: C, 65.93; H, 6.08. Found: C, 65.88; H, 5.96.

1.7. General procedure for the preparation of heptakis(2,3-*O*-butanoyl-6-*O*-acyl)cyclomaltoheptaose derivatives of ibuprofen, flurbiprofen, and felbinac (10–12)

A mixture of 0.21 mmol of **3** and 3.08 mmol of the respective sodium salts of ibuprofen, flurbiprofen, and felbinac in 15 mL anhyd DMF was heated for 96 h at 100 °C. After cooling to room temperature, the reaction mixture was poured into 80 mL ice and water. The insoluble material was filtered and purified by silica gel chromatography with 3:1 petroleum ether–EtOAc, 2:1 petroleum ether–acetone, or 1:1petroleum ether–EtOAc.

1.7.1. Heptakis[(2,3-*O*-butanoyl-6-*O*-2-(4-isobutylphenyl)propionoyl]cyclomaltoheptaose (10)

From 0.6 g of **3** and 0.71 g of ibuprofen sodium salt; white solid (0.12 g, 17%); mp 73–74 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.18–7.22 (m, 14H, Ar), 7.05–7.07 (m, 14H, Ar), 4.84–5.01 (m, 7H, H-2), 4.81–4.98, 4.29–4.41 (m, 7H, H-1), 4.68–4.82 (m, 7H, H-5), 4.47–4.52 (m, 7H, H-3), 4.11–4.25 (m, 7H, H-6), 3.89–4.02 (m, 7H, H-6′), 3.63–3.77 (m, 7H, CH), 3.18–3.52 (m, 7H, H-4), 2.39–2.55 (m, 14H, CH_2), 2.21–2.38 (m, 28H, CH_2, CH_2), 1.84–1.85 (m, 7H, CH), 1.58–1.72 (m, 28H, CH_2, CH_2), 1.40–1.60 (m, 7H, CH_3), 0.89–0.96 (m, 84H, CH_3, CH_3, $(CH_3)_2$); FABMS: calcd for $C_{189}H_{270}NO_{56}$ $[M+NH_4]^+$: 3452.1, found: *m/z* 3452.3. Anal. Calcd for $C_{189}H_{266}O_{56}\cdot 3H_2O$: C, 65.08; H, 7.86. Found: C, 64.98; H, 7.99.

1.7.2. Heptakis[2,3-di-*O*-butanoyl-6-*O*-2-(2-fluoro-4-biphenylyl)-ethanoyl]cyclomaltoheptaose (11)

From 0.6 g of **3** and 0.82 g of flurbiprofen sodium salt**;** white solid (0.30 g, 39.7%); mp 100–102 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.51–7.60 (m, 14H, Ar′), 7.30–7.45 (m, 28H, Ar), 7.09–7.21 (m, 14H, Ar, Ar′), 5.11–5.24 (m, 7H, H-2), 4.89–4.94, 4.40–4.52 (m, 7H, H-1), 4.79–4.88 (m, 7H, H-5), 4.58–4.67 (m, 7H, H-3), 4.21–4.31 (m, 7H, H-6), 3.92–4.10 (m, 7H, H-6′), 3.77–3.90 (m, 7H, CH), 3.31–3.54 (m, 7H, H-4), 1.82–2.38 (m, 28H, CH_2, CH_2), 1.42–1.71 (m, 49H, CH_2, CH_2, CH_3), 0.82–0.92 (m, 42H, CH_3, CH_3); FABMS: calcd for $C_{203}H_{235}F_7NO_{56}$ $[M+NH_4]^+$: 3718.0. Found: *m/z* 3718.2. Anal. Calcd for $C_{203}F_7H_{231}O_{56}$: C, 65.90; H, 6.29. Found: C, 65.68; H, 6.30.

1.7.3. Heptakis[2,3-di-*O*-butanoyl-6-*O*-2-(biphenyl-4-yl)-ethanoyl]cyclomaltoheptaose (12)

From 0.6 g of **3** and 0.72 g of felbinac sodium salt**;** white solid (0.13 g, 17%); mp 110–112 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.43–7.45 (m, 28H, Ar′), 7.26–7.33 (m, 35H, Ar), 5.23–5.26 (m, 7H, H-2), 4.97–4.98 (m, 7H, H-1), 4.66–4.71 (m, 14H, H-3, H-5), 4.32–4.34 (m, 7H, H-6), 4.10–4.12 (d, 7H, *J* 8.55 *Hz*, H-6′), 3.66–3.72 (m, 14H, CH_2), 3.50–3.53 (t, 7H, *J* 8.4 Hz, H-4), 2.01–2.30 (m, 28H, CH_2, CH_2), 1.531.62 (m, 28H, CH_2, CH_2), 0.90–0.96 (m, 21H, CH_3), 0.86–0.89 (m, 21H, CH_3); FABMS: calcd for $C_{196}H_{228}NO_{56}$ $[M+NH_4]^+$: 3493.9, found: *m/z* 3493.5; Anal. Calcd for $C_{196}H_{224}O_{56}\cdot H_2O$: C, 67.38; H, 6.52. Found: C, 67.37; H, 6.29.

1.8. Analytical methods

The HPLC system that consisted of LC-10ADvp pumps, a DGU-14A online degasser, an SPD-10ADvp detector, a CTO-10AS vp column oven, and a Class-VP 6.12 software was purchased from Shimadzu. Normal-phase HPLC conditions for the determination of **12** were as follows: mobile phase 93.5:6.5 CH_2Cl_2–MeCN; flow rate 1.0 mL/min, and detection of wavelength 253 nm. The injection vol was 20 μL, and the relative retention time was found to be about 10.0 min at 25 °C. This method was evaluated through intra-day and inter-day analysis for precision and accuracy. All the precisions were less than 2.55%, and all the recoveries were evaluated to be 90.75–97.15%.

HPLC conditions for the determination of biphenyl acetic acid were as follows: mobile phase 70:30:0.1 MeOH–water–phosphoric acid; flow rate 1.0 mL/min, and detection of wavelength 253 nm. The injection vol was 20 μL, and the relative retention time was found to be about 10.0 min at 25 °C.

1.9. Stability studies in aqueous solution

The hydrolysis was performed at an initial concentration of the conjugate **12** (8.0×10^{-5} M) in HCl soln (0.1 M) and phosphate buffers (pH 6.8, 7.4, $I = 0.2$) at 37 °C. At fixed intervals, an aliquot (0.1 mL) was withdrawn and mixed with 4 vol of cold CH_2Cl_2 at

−20 °C immediately. For the aliquots with pH 6.8 and 7.4, 0.1 mL of HCl (0.1 M) at −20 °C was added to acidify the samples before extraction with CH_2Cl_2. The organic phase was analyzed for the conjugate and biphenyl acetic acid by HPLC.

1.10. Hydrolysis studies with α-amylase and esterase

The α-amylase-catalyzed hydrolysis of the conjugate was carried out in 0.2 M acetate buffer (pH 5.5) containing 0.01 M $CaCl_2$ at 37 °C. The concentrations of *A. oryzae* α-amylase and the conjugate were 4 units/mL and 2.0×10^{-5} M, respectively. At appropriate intervals, an aliquot (0.1 mL) of the reaction solution was acidified by the addition of 0.1 mL HCl (1.0 M), and was shaken with CH_2Cl_2 (0.4 mL) for 10 min. After centrifugation (8000 rpm, 5 min), the organic phase was analyzed for the residual conjugate and biphenyl acetic acid by HPLC.

The esterase-catalyzed hydrolysis of the conjugate was carried out in 0.1 M HEPES/NaOH buffer (pH 7.4) at 37 °C. The concentrations of carboxylic esterase from porcine liver (EC 3.1.1.1), MW 168,000, Sigma, St. Louis, MO) and the conjugate were 40 units/mL and 8.0×10^{-5} M, respectively. The analytical procedure for the conjugate and biphenyl acetic acid was the same as that already described.

1.11. Hydrolysis studies combining α-amylase and esterase

The α-amylase-catalyzed hydrolysis of the conjugate was conducted under the conditions as above. In brief, the concentrations of *A. oryzae* α-amylase and the conjugate were 4.0 units/mL and 8.0×10^{-5} M, respectively, in pH 5.5 acetate buffer containing 0.01 M $CaCl_2$ at 37 °C. After 12 h, the pH of the soln was changed to 7.4 by the addition of 0.1 M NaOH, and then esterase from porcine liver was added to the soln to start the esterase-catalyzed hydrolysis. The concentration of the esterase was 39 units/mL.

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