6^A-*O*-[(4-Biphenylyl)acetyl]-α-, -*β*-, and -*γ*-cyclodextrins and 6^A-Deoxy-6^A-[[(4-biphenylyl)acetyl]amino]-α-, -*β*-, and -*γ*-cyclodextrins: Potential Prodrugs for Colon-Specific Delivery

Kaneto Uekama,* Kunihiro Minami, and Fumitoshi Hirayama

Faculty of Pharmaceutical Sciences, Kumamoto University, 5-1 Oe-honmachi, Kumamoto 862, Japan

Received March 3, 1997[⊗]

Cyclodextrins (CyDs) are known to be fermented to small saccharides by colonic microflora, whereas they are only slightly hydrolyzable and thus are not easily absorbed in the stomach and small intestine. This property of CyDs is particularly useful for colon-specific delivery of drugs. In this study, an antiinflammatory 4-biphenylylacetic acid (BPAA) was selectively conjugated onto one of the primary hydroxyl groups of α-, *β*-, and *γ*-CyDs through an ester or amide linkage, 6^A-*O*-[(4-biphenylyl)acetyl]-α-, -*β*-, and -*γ*-CyDs (**1**−**3**) and 6^A-deoxy-6^A-[[(4-biphenylyl)acetyl]amino]-α-, -*β*-, and -*γ*-CyDs (**4**−**6**). In rat cecal and colonic contents (10%, w/v), **1** and **3** released more than 95% of BPAA within 1−2 h, and **2** released about 50% of the drug within 12 h. The amide prodrugs, **4**−**6**, did not release BPAA in the cecal contents, but gave BPAA/maltose or BPAA/triose conjugates linked through an amide bond. On the other hand, these prodrugs were found to be stable in the contents of rat stomachs and small intestines, in intestinal or liver homogenates, and in rat blood. The serum levels of BPAA increased about 3 h after oral administration of **1** and **3** to rats, accompanying a marked increase in the serum levels, whereas **2** and **4**−**6** resulted in little increase of the serum levels. These facts suggest that BPAA is released after the ring opening of CyDs followed by the ester hydrolysis, and the BPAA activation takes place site-specifically in the cecum and colon. Therefore, the present CyD prodrug approach provides a versatile means of constructing a novel colon-specific drug delivery system.

Introduction

The potential use of natural cyclodextrins (CyDs) and their synthetic derivatives has been extensively studied in order to improve certain properties of drugs such as solubility, stability and bioavailability.[1] CyD complexes are in equilibrium with guest and host molecules in water, the degree of the dissociation being dependent on the magnitude of the stability constant.[2] This property of CyD complexes is a desirable quality, because the complex dissociates to free CyD and drug at the absorption site, and only the drug in free form enters into systemic circulation. However, such an equilibrium is disadvantageous when drug targeting is to be attempted because the complex dissociates before it reaches the organs or tissues to which it is to be delivered. One of the method to circumvent the dissociation is to bind a drug covalently to CyDs.

A number of CyD derivatives have been synthesized for many purposes such as the construction of sophisticated enzyme models and reagents or catalysts for regio- and stereoselective synthesis or chiral recognition.[3] CyD derivatives coupled to drugs such as enkephalin,[4] doxorubicin,[5] methicillin,[6] ibuprofen,[7] and nucleobase[8] were recently synthesized for modification of biological potencies. However, potential applications of drug/CyD derivatives in medical and pharmaceutical fields are rather scarce, because CyD derivatizations usually cause a decrease in biological activity of drugs.[5,9] CyDs are known to be barely capable of being hydrolyzed and only slightly absorbed in passage through the stomach and small intestine; however they are fermented into small saccharides by colonic microflora and thus absorbed as small saccharides in the large intestine.[10] Most bacteroid strains isolated from the human colon are capable of degrading CyDs, as evidenced by their ability to grow on CyDs using them as the sole carbon source and by the stimulation of cyclodextranase activity by exposure to CyDs.[11] This biodegradation property of CyDs may be useful as a colon-targeting carrier, and thus CyD prodrugs may serve as a source of site-specific delivery of drugs to colon. In a previous paper,[12] we reported that the ester-type prodrug, rather than the amide-type prodrug, of the antiinflammatory drug, 4-biphenylylacetic acid (BPAA), with *β*-CyD may serve as a colon-targeting prodrug, on the basis of the preliminary *in vitro* hydrolysis experiment. In this study, we prepared six BPAA prodrugs coupled to α-, *β*-, and *γ*-CyDs through an ester or amide linkage, 6^A-*O*-[(4-biphenylyl)acetyl]-α-, -*β*-, and -*γ*-CyDs (**1**−**3**) and 6^A-deoxy-6^A-[[(4-biphenylyl)acetyl]amino]-α-, -*β*-, and -*γ*-CyDs (**4**−**6**), and their *in vitro* drug release and *in vivo* oral absorption behavior in the rat model were investigated.

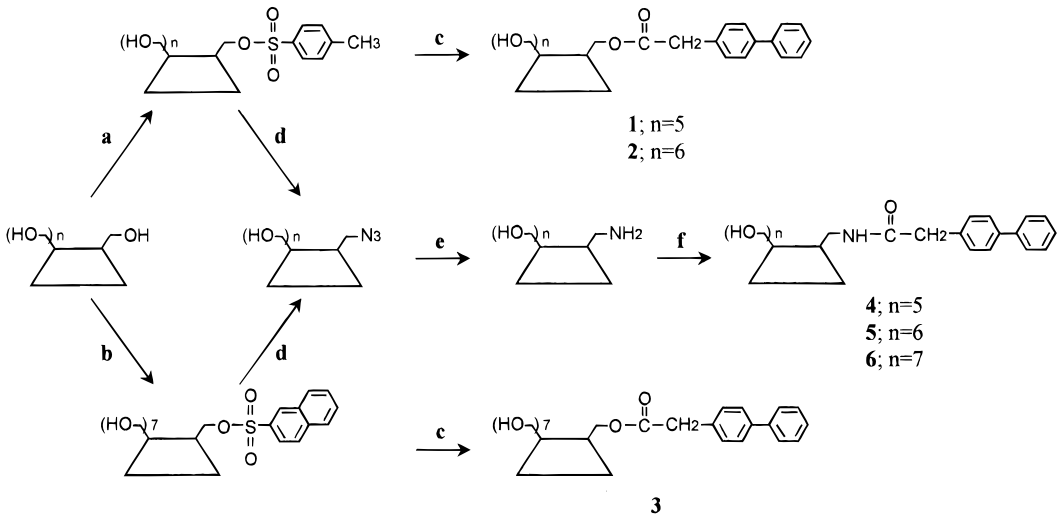
Results and Discussion

Chemistry. 1, **2**, and **3** were prepared in two steps according to the method of Coates *et al.* (Scheme 1).[13] One of the primary alcohol of α- and *β*-CyDs was tosylated using *p*-toluenesulfonyl chloride and that of *γ*-CyD was sulfonylated using 2-naphthalenesulfonyl chloride in pyridine. The sulfonyl groups of CyDs were then replaced by BPAA in DMF. **1** and **3** were purified by ion-exchange chromatography and then lyophilized. **2** was purified by preparative thin layer chromatography, because it was less soluble in water/methanol and ester hydrolysis occurred during evaporation of a large amount of DMF used as an eluent of the chromatogra-

[⊗] Abstract published in *Advance ACS Abstracts,* July 15, 1997.

S0022-2623(97)00130-1 CCC: $14.00

Scheme 1. Preparation of **1**−**6**[a]



[a] Reagents: (a) *p*-toluenesulfonyl chloride; (b) 2-naphthalenesulfonyl chloride; (c) sodium 4-biphenylylacetic acid; (d) sodium azide; (e) triphenylphosphine + ammonium hydroxide; (f) 4-biphenylylacetic acid + 1,3-dicyclohexylcabodiimide + hydroxysuccinimide.

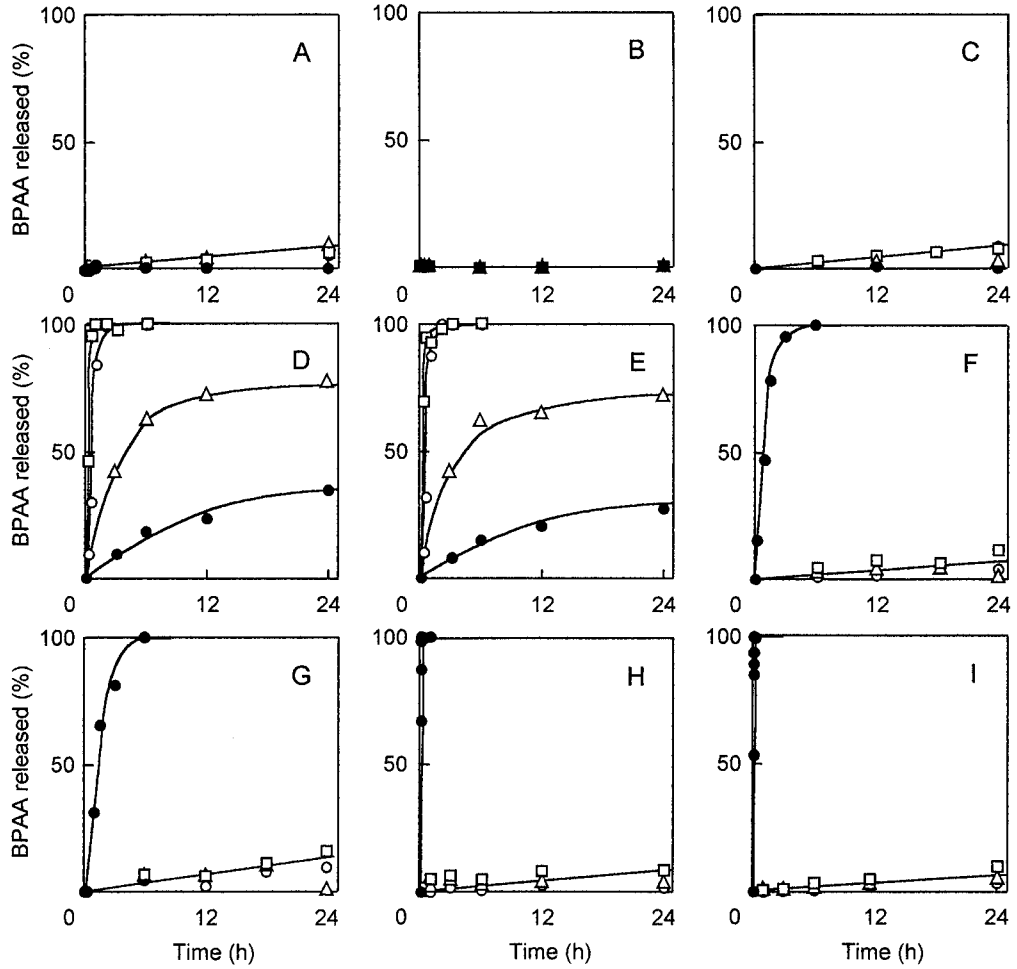


Figure 1. Time courses of BPAA liberation from **1** (○), **2** (△), **3** (□), and ethyl 4-biphenylylacetate (●) (1.0×10^{-5} M) in rat intestinal contents and biological fluids in isotonic buffer/DMF (1.0%, v/v) solution after incubation at 37 °C: panel A, isotonic buffer (pH 4.3 and 7.4); panel B, stomach contents (10% (w/v), pH 4.3); panel C, small intestinal contents (10% (w/v), pH 7.4); panel D, cecal contents (10% (w/v), pH 6.8); panel E, colonic contents (10% (w/v), pH 6.8); panel F, small intestinal homogenates without contents (10% (w/v), pH 6.7); panel G, large intestinal homogenates without contents (10% (w/v), pH 7.0); panel H, liver homogenates (4% (w/v), pH 6.6); panel I, blood (50% (v/v), pH 7.2).

phy. **4**, **5**, and **6** were prepared in four steps according to the method of Belanger *et al.* (Scheme 1).[14] The *p*-toluenesulfonyl-α- and -*β*-CyDs and 2-naphthalenesulfonyl-*γ*-CyD were converted to mono(6-azido-6-deoxy)-CyDs by the reaction with sodium azide in water. These compounds were reduced by treatments with triphenylphosphine in DMF and then with concentrated ammonium hydroxide. The monoamino-CyDs were coupled to the BPAA carboxyl group which was first activated using 1,3-dicyclohexylcarbodiimide-hydroxysuccinimide

in ethyl acetate. The final materials were purified by ion-exchange chromatography. The chemical purity was checked by ^{1}H NMR and FAB mass spectra and by the BPAA liberation after alkaline hydrolysis, confirming that the substitution degree of BPAA is unity.

Hydrolysis of Prodrugs in Rat Biological Media. In aqueous solution, the ester prodrugs, **1**−**3**, were hydrolyzed to BPAA with first-order rate constants of 9.60×10^{-2} h^{-1} ($t_{1/2}$ = 7.2 h, **3**), 8.58×10^{-2} h^{-1} ($t_{1/2}$ = 8.1 h, **2**), 6.36×10^{-2} h^{-1} ($t_{1/2}$ = 10.9 h, **1**), 3.11×10^{-2} h^{-1} ($t_{1/2}$ = 22.3 h, ethyl 4-biphenylylacetate) at pH 9.0 at 37 °C and only small amounts (<10%) within 24 h under the physiological pH condition (pH 7.4). The amide prodrugs, **4**−**6**, were stable under these pH and temperature conditions.

Figure 1 shows the amounts of BPAA liberated from **1**−**3** and ethyl 4-biphenylylacetate (1.0×10^{-5} M) after the incubations with contents (10%, w/v) of rat intestinal tracts such as stomach, small intestine, cecum, and colon or in rat intestinal homogenates (10%, w/v) without contents, liver homogeneous (4%, w/v), and blood (50%, v/v) at 37 °C. In the contents of stomach and small intestine, the ester prodrugs released BPAA only in small amounts (<10%) which could be accounted for by spontaneous hydrolysis in aqueous solution. On the other hand, in the cecal and colonic contents the ester prodrugs released BPAA significantly in the order of **3** > **1** ≫ **2** > ethyl 4-biphenylyl acetate: 100% release of BPAA from **3** and **1** within 1 and 2 h, respectively, and more than 50% from **2** within 12 h. In the homogenates and blood, **1**−**3** liberated BPAA only in small amounts (<10%) for 24 h, whereas ethyl 4-biphenylylacetate was rapidly hydrolyzed to BPAA because of high esterase activities of the homogenates and blood. The resistance of the prodrugs against the ester hydrolysis in the blood and homogenates may be due to a large steric effect of the bulky CyD moiety. From **4**−**6**, no release of BPAA was observed within 24 h in all intestinal contents and biological media employed. These results indicate that the BPAA activation took place site-specifically in the rat cecal and colonic contents.

To gain insight into the hydrolysis mechanism, the hydrolysate of the α-CyD amide prodrug, **4**, in rat cecal contents was analyzed in detail, because the amide prodrugs released no BPAA in rat cecum and colon contents in spite of the disappearance of the intact prodrugs with time, as will be discussed later. Figure 2 shows HPLC chromatograms 0, 3, 24, 48, and 72 h after incubation of **4** (5.7×10^{-3} M) in the cecal contents (10%, w/v). The peak corresponding to **4** (retention time = 6.5 min) decreased with time, and two peaks appeared in the chromatogram during the incubation, which was detected when a large amount of water (0.1 M acetic acid/methanol, 54:46 (v/v)) was used as an eluent of HPLC. The second fraction (retention time = 7.1 min) increased and decreased with time and converted finally to the third fraction (retention time = 8.4 min). These fractions were analyzed by mass spectroscopy after the separation by HPLC and gave molecular ions $[M + H]^+$ of 536 and 698, respectively. (See the Supporting Information for the mass spectra.) These mass numbers were identical with those of the BPAA conjugates coupled to maltose and triose, respectively, indicating that **4** was hydrolyzed finally to 6^A-deoxy-6^A- or 6^B-deoxy-6^B-[[(4-biphenylyl)acetyl]amino]maltose (**7**). The BPAA/linear oligosaccharide conjugates longer than tetraose could not be detected under the present HPLC conditions.

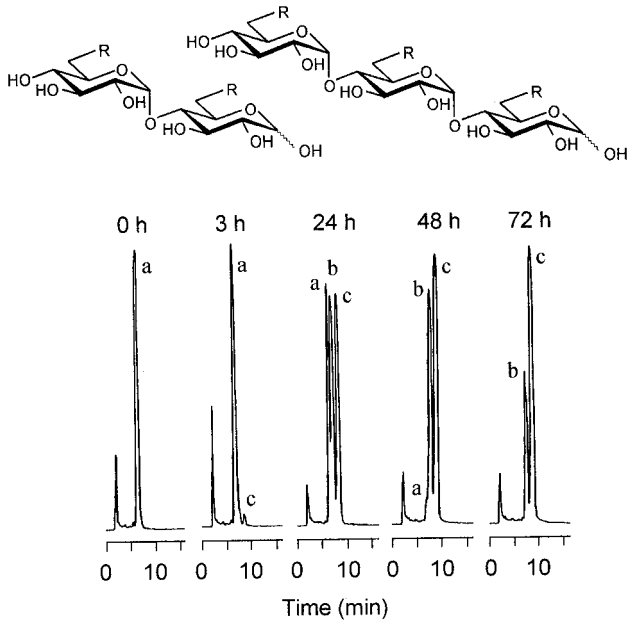


Figure 2. HPLC chromatograms of the hydrolysate after incubation of **4** (5.7×10^{-3} M) in rat cecal contents (10%, w/v) in isotonic buffer/DMF (1.0%, v/v) solution at 37 °C. **4** (a), triose conjugates (b), maltose conjugates (c). Eluent (0.1 M acetic acid/methanol (54:46, v/v)) on a CAPCEll PAK C18 column. One of R groups in structural formula of the maltose and triose conjugates is $NHCOCH_2$Ph-Ph, and the others are OH.

Figure 3 shows time profiles for appearance/disappearance of **1**−**6**, BPAA, **7**, and BPAA/triose conjugate (**8**) in rat cecal contents (10%, w/v). **7** has two positional isomers described above, and **8** has three isomers of 6^A-deoxy-6^A-amino-, 6^B-deoxy-6^B-amino-, and 6^C-deoxy-6^C-[[(4-biphenylyl)acetyl]amino]triose. However, these isomers were determined as a single component, because the quantitative determination of each isomer was difficult under the present HPLC conditions. **3**, **6**, **1**, and **4** disappeared within 1 and 2 h, respectively, in the cecal contents, and about half of **2** and **5** within 1 h. The disappearance rate of the ester prodrugs was almost the same as that of the corresponding amide prodrugs; the disappearance rate constants in the 10% (w/v) rat cecal contents were $2.08(\pm0.02) \times 10^{-2}$ and $1.98(\pm0.01) \times 10^{-2}$ min^{-1} for **1** and **4**, $1.50(\pm0.02) \times 10^{-2}$ and $1.57(\pm0.01) \times 10^{-2}$ min^{-1} for **2** and **5**, and $4.43(\pm0.03) \times 10^{-2}$ and $4.53(\pm0.02) \times 10^{-2}$ min^{-1} for **3** and **6**, respectively. **1** and **3** produced quantitatively BPAA, whereas **4** and **6** formed **7** and **8** without the production of BPAA. The total amount of **7** and **8** was almost the same as that of BPAA produced from **1** and **3**. If the ester hydrolysis precedes the ring opening, the disappearance rate of prodrugs can be different between the ester and amide prodrugs. However, the rate was same between them, although the products were different. These facts indicate that **1**, **3**, **4**, and **6** were firstly subject to the ring opening to give the maltose and triose conjugates directly or indirectly *via* BPAA/longer linear oligosaccharide conjugates which were rapidly hydrolyzed to the small saccharide conjugates. After the fermentation to small saccharide conjugates, they were rapidly hydrolyzed to BPAA, whereas the amide conju-

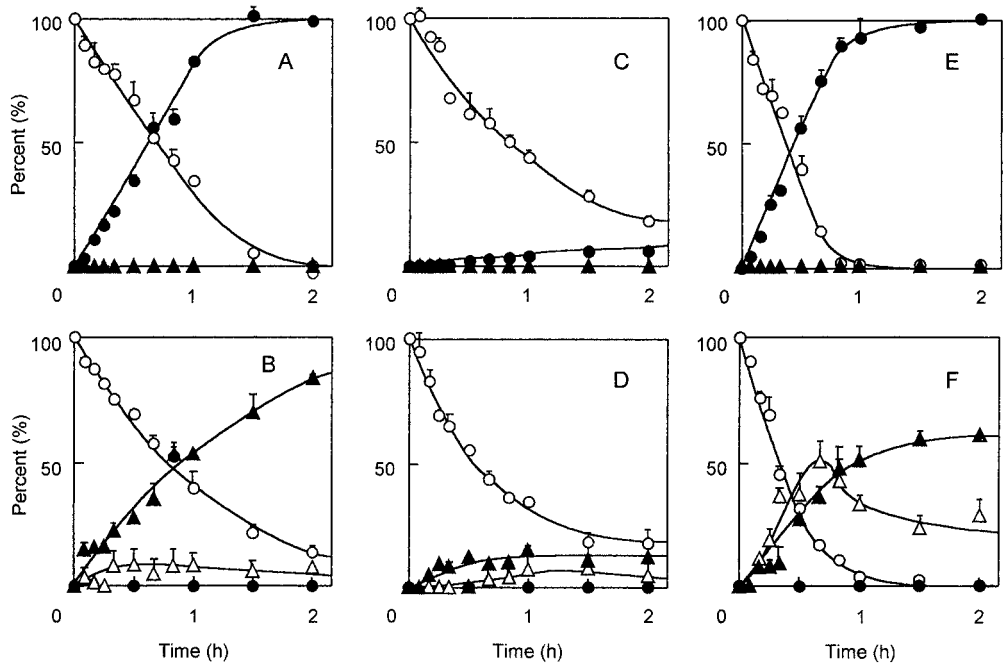


Figure 3. Time courses of disappearance of **1**−**6** (○, 1.0×10^{-5} M) and appearance of BPAA (●) and BPAA conjugates with maltose (▲) and triose (△) in rat cecal contents (10% (w/v), pH 6.7) in isotonic buffer/DMF (1.0%, v/v) solution at 37 °C: panel A, **1**; panel B, **4**; panel C, **2**; panel D, **5**; panel E, **3**; panel F, **6**. Each point represents the mean ± SE of four experiments.

Table 1. Pharmacokinetic Parameters of BPAA after Oral Administrations of **1**, **2**, **3**, BPAA, and β-CyD Complex (Equivalent to 10 mg/kg BPAA) to Rats

system	t_{max}[a] (h)	C_{max}[b] (μg/mL)	MRT[c] (h)	AUC[d] (h·μg/mL)	F[e] (%)
1	9.0 ± 0.0*[f]	15.3 ± 2.5*	9.3 ± 0.5*	119.6 ± 12.0*	64.3 ± 6.4
2	11.8 ± 6.8	1.3 ± 0.4*	11.4 ± 1.5*	10.9 ± 3.3*	5.9 ± 1.8
3	7.8 ± 0.7*	23.6 ± 3.2*	8.8 ± 0.7*	166.1 ± 22.9*	89.3 ± 12.3
BPAA alone	0.5 ± 0.3	6.9 ± 1.2	6.6 ± 0.7	33.6 ± 2.5	18.1 ± 1.3
β-CyD complex	1.5 ± 0.7*	10.5 ± 0.9	5.9 ± 0.2	63.3 ± 1.7*	34.0 ± 0.9

[a] The time required to reach C_{max}. [b] The maximum levels in serum. [c] The mean residence time. [d] The area under the serum BPAA concentration−time curves, determined up to 24 h postadministration. [e] Bioavailability compared with the AUC value of BPAA administered intravenously. [f] An asterisk (*) indicates $p < 0.05$ *versus* BPAA alone.

gates resisted to the hydrolysis and gave **7** as a final product. In rat small intestinal contents (10%, w/v), homogenates (10%, w/v), and blood (50%, v/v), neither appreciable disappearance of the prodrugs nor appearances of BPAA, **7**, and **8** were observed. In the case of **2** and **5**, the ring opening was slower than those of **1**, **3**, **4**, and **6**. This order of the ring-opening rates was different from those expected from acid-catalyzed[15] and most CyD-hydrolyzing enzymatic ring openings,[16] *i.e.*, the larger the cavity size, the faster the rate. Several CyD-hydrolyzing enzymes may take part in the ring opening of the prodrugs by colonic microflora, particularly at the low concentration of the substrates (1.0×10^{-5} M), because it is reported that enzyme preparations from a number of sources show different rates of attack on different CyDs and each CyD-hydrolyzing enzyme has its own preferred CyD substrate.[17] Further, the presence of various biological components such as cholesterol that fit the β-CyD cavity may suppress the ring opening of **2** and **5**, because the inclusion of guests slows down the rate.[18] **2** released BPAA in only small amounts, in spite of the significant disappearance of the prodrugs. The reason for this inconsistency of mass balance is unclear at present. The ring-opening mechanism of **2** and **5** may be different from that of the α- and γ-CyD prodrugs, which should be further studied.

***In Vivo* Absorption Behavior.** Figure 4 shows the plasma levels of BPAA after oral administration of the

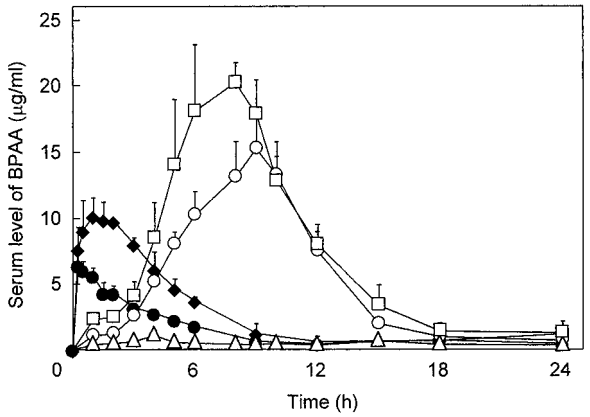


Figure 4. Serum levels of BPAA after oral administration of **1** (○), **2** (△), **3** (□), BPAA (●), or BPAA-β-CyD complex (◆) (equivalent to 10 mg/kg BPAA) to rats. Each point represents the mean ± SE of three experiments.

1, **2**, **3**, BPAA, alone, or BPAA/β-CyD complex to rats, and Table 1 summarizes their pharmacokinetic parameters. The BPAA and BPAA/β-CyD complex (molar ratio of 1:1) systems showed a rapid increase and decrease in the serum levels of BPAA, where the complex showed higher serum levels than the BPAA alone. On the other hand, the serum BPAA levels of **1** and **3** increased after a lag time of about 3 h and reached maximum levels after about 9 and 8 h, respectively, accompanying a significant increase in the serum levels.

The lag time phenomenon indicates that BPAA was released and absorbed in the cecum and colon after the administration of the prodrugs, since the time required to reach the colon after oral administration is reported to be about 1.5−3 h in rats.[19] The area under serum BPAA concentration−time profiles of **1** and **3** were 3.6 and 4.9 times larger than that of the BPAA alone. **2** gave little increase in serum levels, because of the slower drug liberation. **4**, **5**, and **6** gave no appearance of BPAA in the serum, reflecting the *in vitro* hydrolysis behavior. **7**, **8**, and intact ester and amide prodrugs were not detected in the serum under the HPLC conditions, suggesting the sugar-substituted BPAA conjugates are poorly absorbed from intestinal tracts probably due to high hydrophilicity.

Conclusion

Colonic drug delivery can be achieved with carriers by making prodrugs that survive passage through stomach and small intestine, but active moiety is released by enzymes specifically produced in colon. A well-known demonstration of this concept is the delivery of 5-aminosalicylic acid by the use of azo-linked prodrugs.[20] Further, Friend *et al*. demonstrated the colon-targeting of steroids by the use of glycoside prodrugs.[21] The present results indicated that the ester type prodrugs of BPAA/CyD, particularly α- and γ-CyDs, released BPAA preferentially in cecal and colonic contents of rats after the fermentation of CyDs to small oligosaccharides, suggesting that CyDs can serve as a new class of site-specific drug carriers. A number of CyD derivatives, where the hydroxyl groups were selectively mono- or multisubstituted by various functional groups such as amine, carboxylic acid, aldehyde, and thiol, have been synthesized mainly for the construction of enzyme models. These derivatives may serve as promoieties for colon-specific targeting prodrugs. Thus, the CyD conjugation approach may provide a versatile means for construction of colon-specific delivery systems for drugs such as steroids, 5-aminosalicylic acid, and anti-cancer agents, some of which are now under investigation.

Experimental Section

CyDs were donated by Japan Maize products Co. Ltd. (Tokyo, Japan), and BPAA and ethyl biphenylylacetate were supplied from Ledrele Japan Co. Ltd. (Saitama, Japan). The inclusion complex of BPAA with β-CyD in a molar ratio of 1:1 was prepared by the kneading method, and the formation of the solid complex was confirmed by powder X-ray diffractometry and differential scanning calorimetry, as reported previously.[22] ^{1}H and ^{13}C NMR spectra were taken on a JEOL GX-400 or A-500 spectrometer (Tokyo, Japan) at 25 °C, using DMSO-d_6 as a solvent. Fast atom bombardment (FAB) mass spectra were recorded by a JEOL JMS-DX 303 mass spectrometer (Tokyo, Japan); **1**−**6** in a negative mode using a matrix of diethanolamine/DMSO and **7** and **8** in a positive mode using glycerol/H_2O. Analytical HPLC was performed on a Hitachi 655A-11 pump with a L-4000 variable wavelength UV detector. Thin layer chromatography (TLC) was conducted using normal-phase plates precoated with silica gel 60 F_{254} obtained from Merck (Darmstadt, Germany) and an eluent of ethyl acetate/2-propanol/ammonium hydroxide/water = 7:7:5:4 (indicator: *p*-anisaldehyde).

6^A-O-[(4-biphenylyl)acetyl]-α-, -β-, and -γ-cyclodextrins (1−3). Monotosyl α-CyD and mononaphthalenesulfonyl-γ-CyD were purified by ion-exchange chromatography (DIAION HP-20, 5 × 20 cm, Mitsubishi Chemical Co., Tokyo, Japan) with methanol/water.[13,23] Monotosyl-β-CyD was recrystallized three times from water. Sodium 4-biphenylylacetate (2 g, 8.54 mmol) was added to the monosulfonylated α- (9.86 g, 8.75 mmol), β- (12.0 g, 9.31 mmol), or γ-CyDs (12.2 g, 8.20 mmol) in DMF (100 mL), and the mixture was stirred at 100 °C for 30 h. The reaction solution was concentrated under reduced pressure, a large amount of acetone (1000 mL) was added, and the precipitate was collected under vacuum. The solid was dissolved in a small amount (about 10 mL) of DMF and the solution added to a large amount (about 100 mL) of acetone. This procedure was repeated several times. **1** and **3** were purified by ion-exchange column chromatography (DIAION HP-20, 5 × 20 cm) eluting with methanol/water with increasing methanol content. The eluates were monitored by TLC, and the prodrugs appeared in the eluates of 20−40% methanol in water. Methanol in the eluate was removed under reduced pressure, and the solution was lyophilized to yield 3.5 g (36%) and 3.1 g (25%) of **1** and **3**, respectively. **2** was purified by preparative thin-layer silica gel chromatography (silica gel 60F, 20 × 20 cm, Merck) with an eluent of acetonitrile/water, 3:7 (v/v). The fraction of TLC containing **2** was collected and suspended in water, and after filtration the solution was lyophilized to yield 7.4 g (62%) of **2**. **1**: mp 255 °C dec; R_f = 0.67; ^{1}H NMR (DMSO-d_6) δ 7.17−7.49 (m, 9H, J = 8 Hz, biphenyl), 5.26−5.40 (m, 12H, CyD 2,3-OH), 4.59−4.66 (d, 6H, CyD 1-H), 4.13−4.38 (m, 5H, CyD 6-OH), 3.45−3.74 (m, 26H, CyD 3,5,6-H, BPAA CH_2), 3.12−3.25 (m, overlaps with HOD, CyD 2,4-H); ^{13}C NMR (DMSO-d_6) δ 171.92 (BPAA C═O), 140.93, 139.72, 134.54, 130.96, 129.84, 128.29, 127.59 (BPAA biphenyl), 40.5 (overlaps with solvent peak, BPAA CH_2), 102.97 (CyD C1), 83.32, 83.14, 82.95 (CyD C4), 74.18 (CyD C3), 74.02 (CyD C2), 73.06 (CyD C5), 69.87, 64.49, 60.87 (CyD C6); FAB mass $[M - H]^-$ m/z 1165 (nominal molecular weight 1166). **2**: mp 258−268 °C dec; R_f = 0.63; ^{1}H NMR (DMSO-d_6) δ 7.34−7.66 (m, 9H, J = 8 Hz, biphenyl), 5.66−5.78 (m, 14H, CyD 2,3-OH), 4.80−4.85 (d, 7H, CyD 1-H), 4.20−4.52 (m, 6H, CyD 6-OH), 3.55−3.87 (m, 30H, CyD 3,5,6-H, BPAA CH_2), 3.30−3.39 (m, overlaps with HOD, CyD 2,4-H); ^{13}C NMR (DMSO-d_6) δ 171.03 (BPAA C═O), 139.94, 138.72, 133.57, 130.00, 128.87, 127.29, 126.61 (BPAA biphenyl), 39.5 (overlaps with solvent peak, BPAA CH_2), 102.37, 101.95 (CyD C1), 82.08, 81.92, 81.60, 81.52, 81.15 (CyD C4), 73.04 (CyD C3), 72.39 (CyD C2), 72.00 (CyD C5), 68.87, 63.54, 59.89 (CyD C6); FAB mass $[M - H]^-$ m/z 1327 (nominal molecular weight 1328). **3**: mp 277−282 °C dec; R_f = 0.53; ^{1}H NMR (DMSO-d_6) δ 7.37−7.66 (m, 9H, J = 8 Hz, biphenyl), 5.76−5.86 (m, 16H, CyD 2,3-OH), 4.84−4.90 (d, 8H, CyD 1-H), 4.19−4.58 (m, 7H, CyD 6-OH), 3.53−3.81 (m, 34H, CyD 3,5,6-H, BPAA CH_2), 3.31−3.44 (m, overlaps with HOD, CyD 2,4-H); ^{13}C NMR (DMSO-d_6) δ 170.96 (BPAA C═O), 139.86, 138.63, 133.51, 129.95, 128.82, 127.23, 126.49 (BPAA biphenyl), 39.4 (overlaps with solvent peak, BPAA CH_2), 102.33, 101.56 (CyD C1), 81.74, 81.37, 81.00, 80.81, 80.72, 80.25 (CyD C4), 72.77 (CyD C3), 72.61, 72.50 (CyD C2), 72.04 (CyD C5), 68.98, 63.51, 59.91 (CyD C6); FAB mass $[M - H]^-$ m/z 1489 (nominal molecular weight 1491).

6^A-Deoxy-6^A-[[(4-biphenylyl)acetyl]amino]-α-, -β-, and -γ-CyDs (4−6). Mono(6-deoxy-6-amino)-α-, -β-, and -γ-CyDs were obtained in three steps from the parent CyDs and purified by gel chromatography (CM-Sephadex C-25 (NH_4 form), 2 × 25 cm, Pharmacia Biotech, Sweden) with aqueous NH_4HCO_3 solution.[14,24] To BPAA (0.42 g, 2 mmol)/anhydrous ethyl acetate (20 mL) were added hydroxysuccinimide (0.23 g, 2 mmol) and 1,3-dicyclohexylcarbodiimide (0.41 g, 2 mmol), and the mixture was stirred at room temperature (about 25 °C) for 4 h. After filtration, the solution was dried under reduced pressure. The monoamino-α-, -β-, or -γ-CyD (1.9 g, 2.2 g, 2.6 g, 2 mmol) was added to the residue (activated BPAA ester)/DMF (5 mL), and the mixture was stirred at room temperature for 5 h. The reaction solution was concentrated under reduced pressure, and acetone (50 mL) was added. The precipitate was collected by filtration, washed with acetone, and applied on the DIAION column (eluent: methanol/water, about 1:4 (v/v)). Methanol in the eluates was removed under reduced pressure, and the solution was lyophilized to yield 1.8 g (89%), 2.3 g (100%), and 1.3 g (67%) of **4**, **5**, and **6**, respectively. **4**: mp 249−256 °C dec; R_f = 0.66; ^{1}H NMR (DMSO-d_6) δ 7.92 (br s, 1H, BPAA, NH), 7.33−7.64 (m, 9H, J

= 8 Hz, biphenyl), 5.43−5.57 (m, 12H, CyD 2,3-OH), 4.80−4.86 (d, 6H, CyD 1-H), 4.44−4.54 (m, 5H, CyD 6-OH), 3.59−3.77 (m, 26H, CyD 3,5,6-H, BPAA CH_2), 3.25−3.48 (m, overlaps with HOD, CyD 2,4-H); ^{13}C NMR (DMSO-d_6) δ 170.25 (BPAA C═O), 140.04, 138.19, 135.71, 129.58, 128.82, 127.17, 126.50, 126.38 (BPAA biphenyl), 39.8 (overlaps with solvent peak, BPAA CH_2), 101.89, 101.68 (CyD C1), 83.61, 82.25, 82.03, 81.88 (CyD C4), 73.21 (CyD C3), 73.01 (CyD C2), 72.20, 72.05 (CyD C5), 69.89, 60.06, 59.94, 59.84 (CyD C6); FAB mass $[M - H]^-$ *m/z* 1164 (nominal molecular weight 1165). **5**: mp 258−263 °C dec; $R_f = 0.60$; ^{1}H NMR (DMSO-d_6) δ 7.89 (br s, 1H, BPAA NH), 7.34−7.65 (m, 9H, J = 8 Hz, biphenyl), 5.69−5.82 (m, 14H, CyD 2,3-OH), 4.83 (d, 7H, CyD 1-H), 4.45−4.57 (m, 6H, CyD 6-OH), 3.33−3.64 (m, overlaps with HOD, CyD 2,3,4,5,6-H, BPAA CH_2); ^{13}C NMR (DMSO-d_6) δ 170.31 (BPAA C═O), 140.08, 138.22, 135.71, 129.66, 128.90, 127.24, 126.57, 126.45 (BPAA biphenyl), 39.4 (overlaps with solvent peak, BPAA CH_2), 102.13, 101.98, 101.86 (CyD C1), 83.55, 81.73, 81.67, 81.46, 81.33 (CyD C4), 73.14, 72.99, 72.86 (CyD C3), 72.41 (CyD C2), 72.20, 72.01 (CyD C5), 69.80, 59.93, 59.84 (CyD C6); FAB mass $[M - H]^-$ *m/z* 1325 nominal molecular weight 1327). **6**: mp 279−280 °C dec; $R_f = 0.48$; ^{1}H NMR (DMSO-d_6) δ 7.87 (br s, 1H, BPAA NH), 7.33−7.64 (m, 9H, J = 8 Hz, biphenyl), 5.70−5.85 (m, 14H, CyD 2,3-OH), 4.87−4.91 (d, 7H, CyD 1-H), 4.46−4.67 (m, 7H, CyD 6-OH), 3.45−3.62 (m, 34H, CyD 3,5,6-H, BPAA CH_2), 3.29−3.38 (m, overlaps with HOD, CyD 2,4-H); ^{13}C NMR (DMSO-d_6) δ 170.27 (BPAA C═O), 140.04, 138.22, 135.68, 129.66, 128.87, 127.20, 126.53, 126.41 (BPAA biphenyl), 39.8 (overlaps with solvent peak, BPAA CH_2), 102.37, 101.58 (CyD C1), 83.44, 81.02 (CyD C4), 72.71 (CyD C3), 72.59 (CyD C2), 72.11 (CyD C5), 70.01, 60.00, (CyD C6); FAB mass $[M - H]^-$ *m/z* 1488 (nominal molecular weight 1490).

Hydrolysis in Rat Gastrointestinal Contents. Male Wistar rats, 400−500 g, were fed a standard diet (CE-2, CLEA Japan Inc., Tokyo). The rats were killed by decapitation, and stomach, small intestine, cecum, and colon were removed. Stomach and other contents were diluted to 20% w/v with isotonic acetate buffer (pH 4.4) and phosphate buffer (pH 7.4), respectively, and the dispersions of contents were filtered through a gauze to remove large particles. The prodrug solution (5.0 mL, 2.0×10^{-5} M in 2.0% v/v DMF/isotonic buffer) was added to the filtrate (5.0 mL) in air-tight vessels and incubated at 37 °C. The pH of incubation solutions was adjusted to 4.4 (stomach) or 7.4 (other contents) by the addition of small amounts of 0.1 M NaOH. No attempt was made to replace the atmosphere with nitrogen, because of the lack of large difference in the hydrolysis rate of the β-CyD prodrugs between aerobic and anaerobic conditions as reported previously.[12] At appropriate intervals, the reaction solutions were determined for BPAA, intact prodrugs, and **7** and **8** by HPLC. BPAA: an aliquot (0.5 mL) of the reaction solution was added to 0.1 M HCl (0.2 mL), and BPAA was extracted with cyclohexane/diethyl ether (3:1, 6.0 mL) containing flurbiprofen as an internal standard (1 μg/mL, 0.5 mL). The organic phase (5.0 mL) was evaporated under reduced pressure and redissolved in methanol (0.1 mL), 0.02 mL of which was subjected to HPLC analysis under the following conditions: a CAPCEll PAK C18 column (6 × 150 mm, Shiseido Co., Tokyo, Japan), a mobile phase of 0.1 M acetic acid/methanol (7:13), a flow rate of 1.5 mL/min, and a detection of 255 nm. Prodrugs (**1**−**6**) and **7** and **8**; an aliquot (0.2 mL) of the reaction solution was ultrafiltered using a membrane filter (Amicon Kit (Tokyo, Japan), a centrifugation of 3500 rpm for 15 min at 4 °C), and the filtrate (0.02 mL) was subjected to HPLC analysis using the same column and the following mobile phases (0.1 M acetic acid/methanol): 54:46 v/v for **1** and **4** (retention time, 10.8 and 6.9 min, respectively), 65:35 for **2** and **5** (7.1 and 7.8 min), 62:38 for **3** and **6** (9.3 and 5.5 min), and 54:46 or 62:38 for **7** and **8** (8.5 and 7 min or 20 and 16 min).

Hydrolysis in Rat Biological Fluids. Rat intestine segments were washed fully with isotonic buffer, cut into small pieces, and homogenized with 5 volume of cold 1.15 w/v KCl using a tissue homogenizer (Physcotron NS-50, Nichion, Tokyo) at 0 °C. The homogenates were filtered through a gauze. The solutions of **1**−**6** (5.0 mL, 2.0×10^{-5} M in 2.0 v/v DMF/pH 7.4 isotonic phosphate buffer) were added to the filtrates (5.0 mL) and incubated at 37 °C. Rat liver (wet weight about 20 g) was washed thoroughly with saline (about 500 mL) and homogenized with 5 volumes of cold 1.15 w/v KCl using a tissue homogenizer (Potter-Elvejem, Corning Glassw) at 0 °C. The homogeates were filtered through gauze, and the filtrate was centrifuged at 9000*g* for 30 min at 4 °C. The supernatant (2.0 mL) was added to the prodrug solutions (8.0 mL, 1.25×10^{-5} M in 1.25 v/v DMF/pH 7.4 isotonic phosphate buffer) containing $MgCl_2$, 101.6 mg/mL, glucose 6-phosphate, 60.8 mg/mL, nicotinamide, 9.16 mg/mL, and nicotinamide−adenine dinucleotide phosphate buffer, 3.35 mg/mL at 37 °C. Rat blood was collected using injection syringes treated with sodium citrate. The prodrug solution (5.0 mL, 2.0×10^{-5} M in 2.0 v/v DMF/pH 7.4 isotonic phosphate buffer) was added to 5.0 mL of blood at 37 °C. At appropriate intervals, the reaction solutions were analyzed for BPAA by HPLC as described above.

***In Vivo* Absorption Studies.** Male Wistar rats weighing about 200 g were fasted for 18 h prior to drug administration, while water was allowed *ad libitum*. BPAA, BPAA/β-CyD complex, and **1**−**6** (equivalent to 10 mg of BPAA/5 mL of water per kg of rat body weight) were orally administered. Blood samples (about 0.6 mL) were taken periodically from the jugular vein, and centrifuged at 10 000 rpm for 5 min. The serum (0.1 mL) was assayed for BPAA, intact prodrugs, and the sugar conjugates by HPLC under the same condition as those described above. The bioavailability parameters were determined using a MULTI program.[25]

Acknowledgment. The authors are grateful to Dr. T. Kondo and Miss Y. Tanaka for their helpful discussion and technical assistance.

Supporting Information Available: ^{1}H and ^{13}C NMR and FAB mass spectra of **1**−**6**, FAB mass spectra of the maltose and triose conjugates with BPAA, and the first-order plots for the hydrolysis of **1**−**3** at pH 9.0, 37 °C (9 pages). Ordering information is given on any current masthead page.

References

(1) (a) Szejtli, J. *Cyclodextrin technology*; Kluwer: Dordrecht, 1988. (b) Loftsson, T.; Brewster, M. E.; Derendorf, H.; Bodor, N. 2-Hydroxypropyl β-cyclodextrin: properties and usage in pharmaceutical formulations. *Pharm. Ztg. Wiss.* **1991**, *4*, 5−10. (c) Duchêne, D., Ed. *New trends in cyclodextrins and derivatives*. Editions de Santé: Paris, 1991. (d) Uekama, K.; Hirayama, F. Improvements of drug properties by cyclodextrins. In *The Practice of Medicinal Chemistry*; Wermuth, C. G., Ed.; Academic Press: London, 1996; pp 793−825.

(2) (a) Bender, M. L.; Komiyama, M. *Cyclodextrin chemistry*; Springer-Verlag: Berlin, 1978. (b) Saenger, W. Cyclodextrin inclusion compounds in research and industry. *Angew. Chem., Int. Ed. Engl.* **1980**, *19*, 344−362.

(3) (a) König, W. A.; Lutz, S.; Wenz, G. Modified cyclodextrins-novel, highly enantioselective stationary phases for gas chromatography. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 979−980. (b) Ueno, A.; Kuwabara, T.; Nakamura, A.; Toda, F. A modified cyclodextrin as a guest responsive colour-change indicator. *Nature* **1992**, *356*, 136−137. (c) Breslow, R. Biomimetic chemistry and artificial enzymes: catalysis by design. *Acc. Chem. Res.* **1995**, *28*, 146−153.

(4) Parrot-Lopez, H.; Djedaini, F.; Perly, B.; Coleman, A. W.; Galons, H.; Miocque, M. An approach to vectorisation of pharmacologically active molecules: the covalent binding of Leu-enkephalin to a modified β-cyclodextrins. *Tetrahedron Lett.* **1990**, *31*, 1999−2002.

(5) Tanaka, H.; Kominato, K.; Yamamoto, R.; Yoshioka, T.; Nishida, H.; Tone, H.; Okamoto, R. Synthesis of doxorubicin-cyclodextrin conjugates. *J. Antibiot.* **1994**, *47*, 1025−1029.

(6) Nedra Karunaratne, D.; Farmer, S.; Hancock, R. E. W. Synthesis of bulky β-lactams for inhibition of cell surface β-lactamase activity. *Bioconjugate Chem.* **1993**, *4*, 434−439.

(7) Coates, J. H.; Easton, C. J.; van Eyk, S. J.; May, B. L.; Singh, P.; Lincoln, S. F. Chiral differentiation in the deacylation of 6^A-O-{2-[4-(2-methylpropyl)phenyl]propanoyl}-β-cyclodextrin. *J. Chem. Soc., Chem. Commun.* **1991**, 759−760.

(8) Djedaini-Pilard, F.; Perly, B.; Dupas, S.; Miocque, M.; Galons, H. Specific interaction and stabilization between host and guest: complexation of ellipticine in a nucleobase functionalized cyclodextrin. *Tetrahedron Lett.* **1993**, *34*, 1145−1148.

(9) Habus, I.; Zhao, Q.; Agrawal, S. Synthesis, hybridization properties nuclease stability, and cellular uptake of the oligonucleotide-amino-β-cyclodextrins and adamantane conjugates. *Bioconjugate Chem*. **1995**, *6*, 327−331.
(10) (a) Andersen, G. H.; Robbins, F. M.; Domingues, F. J.; Moores, R. G.; Long, C. L. The utilization of schardinger dextrins by the rat. *Toxicol. Appl. Pharmacol*. **1983**, *5*, 257−266. (b) Gerlóczy, A.; Fónagy, A.; Keresztes, P.; Perlaky, L.; Szejtli, J. Absorption, distribution, excretion and metabolism of orally administered ^{14}C-β-cyclodextrin in rat. *Arzneim. Forsch. Drug Res*. **1985**, *35*, 1042−4017. (c) Flourié, B.; Molis, C.; Achour, L.; Dupas, H.; Hatat, C.; Rambaud, J. C. Fate of β-cyclodextrin in the human intestine. *J. Nutr*. **1993**, *123*, 676−680.
(11) Antenucci, R.; Palmer, J. K. Enzymatic degradation of α- and β-cyclodextrins by bacteroides of the human colon. *J. Agric. Food Chem*. **1984**, *32*, 1316−1321.
(12) Hirayama, F.; Minami, K.; Uekama, K. *In-vitro* evaluation of biphenylyl acetic acid-β-cyclodextrin conjugates as colon-targeting prodrugs: drug release behaviour in rat biological media. *J. Pharm. Pharmacol*. **1996**, *48*, 27−31.
(13) Coates, J. H.; Easton, C. L.; Fryer, N. L.; Lincoln, S. F. Complementary diastereoselectivity in the synthesis and hydrolysis of acylated cyclodextrins. *Chem. Lett*. **1994**, 1153−1156.
(14) Bellanger, N.; Perly, B. NMR investigations of the conformation of a new cyclodextrin-based amphiphilic transporter for hydrophobic drugs: molecular lollipops. *J. Mol. Struct*. **1992**, *273*, 215−226.
(15) Uekama, K.; Otagiri, M. Cyclodextrins in drug carrier system. *CRC Crit. Rev. Ther. Drug Carrier Syst*. **1978**, *3*, 1−40.
(16) Suetsugu, N.; Koyama, S.; Takeo, K.; Kuge, T. Kinetic studies on the hydrolyses of α-, β-, and γ-cyclodextrins by taka-amylase A. *J. Biochem*. **1974**, *76*, 57−63.
(17) Bender, H. A bacterial glucoamylase degrading cyclodextrins. *Eur. J. Biochem*. **1981**, *115*, 278−291.
(18) Hirayama, F.; Kurihara, M.; Utsuki, T.; Uekama, K. Inhibitory effect of guest molecules on acid-catalyzed ring-opening of β-cyclodextrin. *J. Chem. Soc., Chem. Commun*. **1993**, 1578−1590.
(19) Saffran, M.; Kumar, G. S.; Savariar, C.; Burnham, J. C.; Williams, F.; Neckers, D. C. A new approach to the oral administration of insulin and other peptide drugs. *Science* **1986**, *233*, 1081−1084.
(20) Kopeček, J.; Kopečková, P. N-(2-Hydroxypropyl)methacrylamide copolymers for colon-specific drug delivery. In *Oral colon-specific drug delivery*; Friend, D. R., Ed.; CRC Press: Boca Raton, 1992; pp 189−211.
(21) Friend, D. R. Glycosides in colonic drug delivery. In *Oral colon-specific drug delivery*; Friend, D. R., Ed.; CRC Press: Boca Raton, 1992; pp 153−187.
(22) Arima, H.; Adachi, H.; Irie, T.; Uekama, K.; Pitha, J. Enhancement of the antiinflammatory effect of ethyl 4-biphenylyl acetate in ointment by β-cyclodextrin derivatives: increased absorption and localized activation of the prodrug in rats. *Pharm. Res*. **1990**, *7*, 1152−1156.
(23) Wang, Y.; Ikeda, T.; Ikeda, H.; Ueno, A.; Toda, F. Dansyl-β-cyclodextrins as fluorescent sensors responsive to organic compounds. *Bull. Chem. Soc. Jpn*. **1994**, *67*, 1598−1607.
(24) Croft, A. P.; Bartsch, R. A. Synthesis of chemically modified cyclodextrins. *Tetrahedron* **1983**, *39*, 1417−1474.
(25) Yamaoka, K.; Tanigawara, Y.; Nakagawa, T.; Uno, T. A pharmacokinetic analysis program (MULTI) for microcomputer. *J. Pharmacobio-Dyn*. **1981**, *4*, 879−885.

JM970130R