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Synthesis and characterization of water-soluble hydroxybutenyl cyclomaltooligosaccharides (cyclodextrins)

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Abstract

We have examined the synthesis of hydroxybutenyl cyclomaltooligosaccharides (cyclodextrins) and the ability of these cyclodextrin ethers to form guest–host complexes with guest molecules. The hydroxybutenyl cyclodextrin ethers were prepared by a base-catalyzed reaction of 3,4-epoxy-1-butene with the parent cyclodextrins in an aqueous medium. Reaction byproducts were removed by nanofiltration before the hydroxybutenyl cyclodextrins were isolated by co-evaporation of water–EtOH. Hydroxybutenyl cyclodextrins containing no unsubstituted parent cyclodextrin typically have a degree of substitution of 2–4 and a molar substitution of 4–7. These hydroxybutenyl cyclodextrins are randomly substituted, amorphous solids. The hydroxybutenyl cyclodextrin ethers were found to be highly water soluble. Complexes of HBen-β-CD with glibenclamide and ibuprofen were prepared and isolated. In both cases, the guest content of the complexes was large, and a significant increase in the solubility of the free drug was observed. Dissolution of the complexes in pH 1.4 water was very rapid, and significant increases in the solubility of the free drugs were observed. Significantly, after reaching equilibrium concentration, a decrease in the drug concentration over time was not observed.

Keywords: Hydroxybutenyl cyclomaltooligosaccharide; Hydroxybutenyl cyclodextrin; Cyclodextrin ether; Guest–host complex; Glibenclamide; Ibuprofen

1. Introduction

Cyclomaltooligosaccharides (cyclodextrins, CDs), are cyclic oligosaccharides comprised of α-1,4-linked glucose monomers. The readily available CDs contain six, seven, or eight glucose monomers, and they are commonly referred to as α-CD, β-CD, and γ-CD, respectively. Of these, β-CD is currently the most widely available and CD of lowest cost. Relative to β-CD, α-CD has a smaller cavity (5.3 versus 6.6 Å) and higher water solubility (14.5 versus 1.9 g/mL at 25 °C). Similarly, γ-CD has a larger (8.4 Å) and more flexible cavity, and a higher water solubility (23.2 g/mL at 25 °C).[1]

The utility of CDs arises from their unique cyclic structure, which, topologically, may be viewed as a torus in which the exterior is relatively hydrophilic and the interior is relatively lipophilic.[2] Because of this unique structure, the CD can act as a host and form inclusion complexes with a variety of guest molecules, including pharmaceutically active molecules. Not surprisingly, the size of the cavity will influence complex formation, with the cavity of β-CD being optimal for complexation of many of the relevant pharmaceutical molecules. In general, upon complexation with a pharmaceutical, the CD will increase the solubility of a sparingly water-soluble drug, thereby increasing its bioavailability.[3] In addition to increased solubilization of the pharmaceutically active compound, the CD can also act as a taste-masking agent, can provide stabilization for an otherwise unstable pharmaceutical, alleviate local toxicity effects of the pharmaceutical, and can

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provide for increased absorption of the pharmacologically active guest.[3]

All three of the unmodified CDs are known to be parenterally unsafe.[3] Specifically, unmodified β-CD has been shown to cause renal and liver damage after parenteral administration.[4] Because of the lack of enzymes specific to β-CD, it is thought that the cyclodextrin molecules remain intact after parenteral administration and hence accumulate in the renal tissue. Crystallization of the β-CD or its complexes leads to the observed necrotic damage. Hence, the use of unmodified CD in a clinical setting is limited to oral or topical formulations.

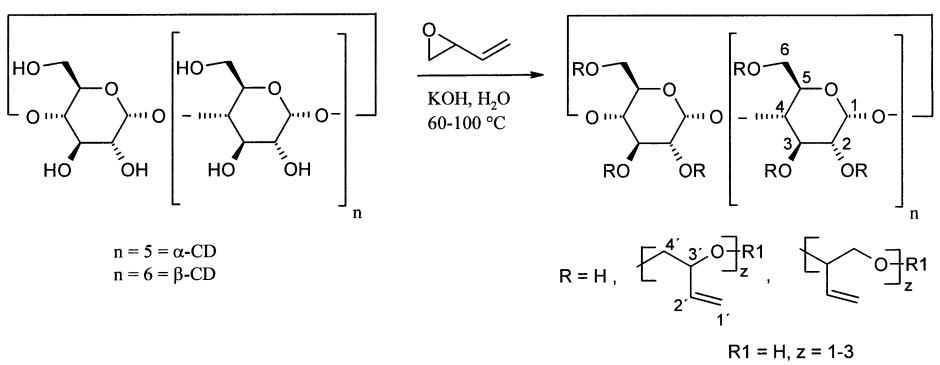
In view of its desirable cavity size and availability, it is most unfortunate that β-CD has low-water solubility and is parenterally unsafe. In order to both improve the safety profile and to increase water solubility over that of β-CD, a number of cyclodextrin derivatives have been examined. These include neutral,[5] anionic,[6] cationic,[7] and zwitterionic[8] cyclodextrin derivatives. In general, the preferred degree of substitution (DS) is typically lower than DS 7. It should be noted that at less than full substitution, there would be a distribution of substituted cyclodextrin molecules in the reaction product. At a low DS, some of the CD molecules potentially will have no substituents. The reported DS will reflect the average value of this distribution.

In this context, we present the synthesis of the previously unknown hydroxybutenyl-β-cyclodextrin (HBen-β-CD) and hydroxybutenyl-α-cyclodextrin (HBen-α-CD). Our investigations indicate that HBen-β-CD and HBen-α-CD will form complexes with and increase the solubility of a variety of pharmaceutically active molecules.

2. Results and discussion

Preparation of hydroxybutenyl cyclodextrins.—In designing a hydroxybutenyl cyclodextrin that could potentially serve as a drug-delivery vehicle, we felt that it was important that the DS should be high enough to insure that there was no unsubstituted cyclodextrin present in the reaction product. This approach eliminates the need for subsequent purification to remove unsubstituted cyclodextrin that could raise toxicity concerns. As a first approximation, we felt that a DS greater than approximately 2.0 or a DS/glucose unit (DS/Glu) of 0.29 was a realistic target. It is important to note that ring opening of 3,4-epoxy-1-butene can generate either a 1° or 2° hydroxy group, which in turn can react with 3,4-epoxy-1-butene. If chain extension does occur by subsequent reaction of the butenyl substitutent with the epoxide, the molar substitution (MS, the total number of substituents attached to the cyclodextrin) will be greater than the DS. In terms of regioselectivity, we targeted a randomly substituted cyclodextrin ether, which we felt we could obtain by using a relatively high concentration of base as the catalyst.[9] Besides eliminating the additional constraint of control of regioselectivity, a random arrangement of substituents decreases the possibility of crystallization and the associated toxicity concerns.

In practice, reaction of β-CD with 8.7 equivalents of 3,4-epoxy-1-butene in the presence of 0.25 equivalents of KOH at 100 °C for 1.4 h, followed by cooling to 55 °C, at which point the reaction was terminated by adjustment of the pH to 7.0 (3.6 h total reaction time), proceeded smoothly providing the expected HBen-β-CD (Scheme 1). Duplicate experiments under these conditions (Table 1, entries 1 and 2) gave HBen-β-CD having a DS of 3.40 and 3.16 (DS/Glu = 0.49 and 0.45) with a molar substitution of 5.55 and 5.72. Analysis of these samples by MALDI-TOFMS (vide infra) revealed that these samples contained no unsubstituted β-CD and that the distribution of substituents was random. When the reaction time at 100 °C was increased marginally to 1.5 h, but with a reduction of the total reaction time to 3.0 h, both the DS and the MS were observed to decrease slightly to 3.05 and 5.25, respectively (Table 1, entry 3). Interestingly, when both the number of equivalents of base (0.125) and the reaction time (0.5 h at 100 °C, 2.0 h total reaction time) were decreased, the DS was essentially unchanged from the



Scheme 1.

Table 1
Summary of reaction conditions for the preparation of hydroxybutenyl cyclodextrins

Entry	Parent CD	KOH [a]	Epoxide [a]	Reaction time [b] (h)	Reaction temperature (°C) [c]	Yield (%) [d]	% Diol prior to nanofiltration [e]	% Diol after nanofiltration [e]	DS [f]	MS [f]	MS [g]
1	β	0.25	8.7	3.6	100 (1.4 h)	78	4.90	0.06	3.40	5.55	5.96
2	β	0.25	8.7	3.6	100 (1.4 h)	69	4.69	0.04	3.16	5.72	5.84
3	β	0.25	8.7	3.0	100 (1.5 h)	87	2.72	0.10	3.05	5.25	5.56
4	β	0.125	8.7	2.0	100 (0.5 h)	84	8.30 [h]	0.13	3.15	4.91	6.24
5	β	0.06	8.7	2.5	100 (0.5 h)	82	ND [h,i]	0.58	1.27	2.47	3.29
6	β	0.02	8.7	2.5	100 (0.5 h)	53	ND [h,i]	0.70	0.50	1.41	1.06
7	β	0.125	8.7	3.6	80 (1.4 h)	77	11.9 [h]	0.07	2.69	4.54	5.59
8	β	0.25	8.7	5.4	80 (4)	81	6.75	0.06	3.16	5.53	5.91
9	β	0.25	8.0	24	60 (24)	73	2.20	0.02	2.75	5.12	5.90
10	α	0.27	8.6	3.0	100 (1.5 h)	88	8.42	0.03	3.10	4.96	5.46
11	α	0.27	8.6	3.0	100 (1.5 h)	61	3.32	0.02	3.20	5.03	5.39
12	α	0.16	8.6	3.0	100 (1.5 h)	70	3.92	0.01	2.77	4.42	4.89

[a] Molar equivalents based on cyclodextrin.
[b] Total time held at the reaction temperature plus the time to cool to ca. 55 °C where the pH of the reaction mixture was adjusted to 7.0.
[c] Maximum reaction temperature and the time held at that temperature.
[d] Based on moles of isolated hydroxybutenyl cyclodextrin after nanofiltration.
[e] Per cent 3,4-dihydroxy-1-butene as determined by gas chromatography.
[f] DS, degree of substitution; MS, molar substitution. Determined by ^{1}H NMR spectroscopy.
[g] Determined by MALDI-TOFMS using CH_2Cl_2–HFIP as the solvent.
[h] This sample was not extracted prior to nanofiltration.
[i] ND, not determined.

previous examples, but the MS was observed to drop to 4.91 (Table 1, cf. entry 4 and entries 1–3). When the number of equivalents of base was decreased further to 0.06 with a slight increase in the total reaction time, both the DS (1.27) and the MS (2.47) significantly decreased (Table 1, entry 5). Unlike the prior examples, unreacted β-CD was observed by MALDI-TOFMS (1.5%). Decreasing the number of equivalents of base even further to 0.02 under otherwise similar reaction conditions further decreased both the DS and the MS to 0.5 and 1.41, respectively (Table 1, entry 6). Unlike prior experiments, significant crystallization occurred when this reaction mixture was cooled to room temperature. These solids were removed by filtration prior to purification. Analysis by MALDI-TOFMS revealed a very high concentration of unsubstituted β-CD (13.8%), and the distribution of molecular weights was skewed toward lower substituted β-CD in the reaction product. Collectively, these examples suggest that reaction of β-CD with 3,4-epoxy-1-butene occurs relatively rapidly at 100 °C and that decreasing the number of equivalents of base leads to a decrease in both the DS and MS. However, the more rapid decrease in MS relative to the DS suggests that chain extension can be diminished at lower concentrations of base.

As one would expect, temperature was also found to have an effect on the reaction time and the observed DS. In the presence of 0.125 equivalents of base, decreasing the temperature to 80 °C provided HBen-β-CD with a DS of 2.69 and an MS of 4.54 (Table 1, cf. entry 7 and entries 1–4). Increasing the reaction time at 80 °C increased the DS and MS to 3.16 and 5.53, respectively, which is comparable to that obtained at 100 °C at shorter reaction times (Table 1, cf. entry 8 and entries 1, 2). At 60 °C (Table 1, entry 9), approximately 24 h is required to achieve a DS of 2.75 and an MS of 5.12. These observations are significant in that a lower temperature allows one to avoid the use of an autoclave while still obtaining essentially the same product.

As one would expect, the observations that temperature, reaction time, and concentration of base influenced the DS and MS obtained for HBen-β-CD also held for HBen-α-CD. Reaction of α-CD with 8.6 equivalents of 3,4-epoxy-1-butene in the presence of 0.27 equivalents of KOH at 100 °C for 1.5 h (3 h total reaction time) provided HBen-α-CD with a DS of 3.10 and an MS of 4.96. A duplicate experiment provided HBen-α-CD with a DS of 3.20 and an MS of 5.03. It is interesting to note that although the total DS for HBen-α-CD is very similar to that obtained for HBen-β-CD (Table 1, cf. entries 10, 11 to entries 1, 2), the average DS/Glu for HBen-α-CD (0.51 and 0.53) is slightly higher than that obtained for HBen-β-CD (0.48 and 0.45). Finally, as was observed in the case of HBen-β-CD, decreasing the concentration of base from 0.27 equivalents to 0.16 gave a drop in both the DS and MS for the HBen-α-CD. As before, the MS dropped more than the DS, indicating less chain extension at lower concentration of base.

Purification of hydroxybutenyl cyclodextrins.—Reaction of α-CD or β-CD with 3,4-epoxy-1-butene in an aqueous medium can potentially generate as byproducts 3,4-dihydroxy-1-butene and butene glycol oligomers. A desirable form of purification to minimize or remove these byproducts would be crystallization, but because of the very high water solubility of hydroxybutenyl cyclodextrin (vide infra), we did not find it practical to isolate hydroxybutenyl cyclodextrin by this means. Hence, we initially attempted to remove these byproducts by multiple extractions with low-molecular weight esters such as ethyl acetate or isopropyl acetate. Fig. 1 provides a stacked presentation of three HPLC experiments. The spectra shown in Fig. 1 were collected at 210 nm. At this wavelength, only the reaction byproducts are detected. Fig. 1(a) is the spectrum of the byproducts in the crude reaction product before extraction, Fig. 1(b) is the spectrum of the byproducts removed by extraction with EtOAc, and Fig. 1(c) is the spectrum of the byproducts in HBen-β-CD after extraction with EtOAc. As can be seen, all of the butene glycol oligomers were removed by extraction, and 2.7 wt% of 3,4-dihydroxy-1-butene was found to remain in the isolated HBen-β-CD. However, even with multiple extractions, we found it very difficult to lower the content of 3,4-dihydroxy-1-butene to less than about 3 wt% (Table 1). Moreover, the 3,4-dihydroxy-1-butene content in the isolated hydroxybutenyl cyclodextrins tended to be highly variable (2–9 wt%). Although we did not develop direct proof, we have attributed the difficulty and variability in removing 3,4-dihydroxy-1-butene from the hydroxybutenyl cyclodextrins by extraction to complexation of the 3,4-dihydroxy-1-butene by the hydroxybutenyl cyclodextrins.

In view of the difficulty in removing 3,4-dihydroxy-1-butene by extraction, we investigated nanofiltration as a means of purification. Nanofiltration is a purification technique that is similar to ultrafiltration except that a lower molecular weight cut-off (MWCO) membrane is used (generally less than 1000). Nanofiltration is attractive in this case in that water, which is the reaction solvent, can be used to wash the lower molecular-weight components through a membrane with the permeate, while the higher molecular-weight cyclodextrin derivative cannot pass through the membrane and is held as the retentate. Additionally, the aqueous solution can be significantly concentrated by nanofiltration, reducing the volume of water that must be removed at a later stage. The key to nanofiltration is selection of a membrane that will retain the desirable component and allow the lower molecular-weight component to pass while maintaining a reasonable flux. In this regard, we

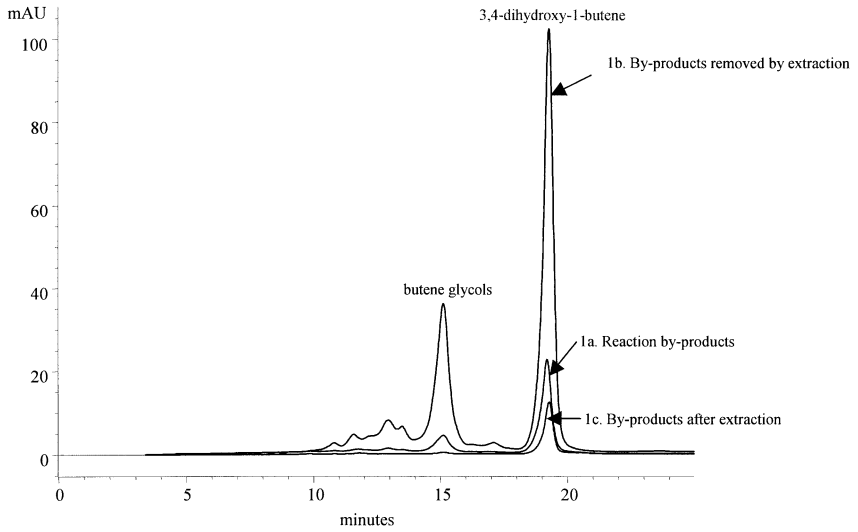


Fig. 1. HPLC analysis of the extraction of a HBen-β-CD reaction mixture with EtOAc. (a) Spectrum of the byproducts in the reaction product mixture prior to extraction; (b) spectrum of the byproducts extracted from the reaction mixture; and (c) spectrum of the byproducts in the HBen-β-CD after extraction.

found that cellulose acetate membranes with a 500 or 1000 MWCO were effective with the hydroxybutenyl cyclodextrins. The 1000 MWCO membrane provided a higher flux but did allow some of the cyclodextrin to pass. Hence, the 500 MWCO cellulose acetate membrane was found to offer the best combination of flux and retention of the hydroxybutenyl cyclodextrins. To illustrate, an 8.3 wt% aqueous solution of HBen-β-CD, which contained 11.9 wt% of 3,4-dihydroxy-1-butene, was concentrated by nanofiltration filtration through a 500 MWCO cellulose acetate membrane. The retentate was concentrated to approximately 25 wt% HBen-β-CD, then diluted to the original volume, which constitutes one exchange. Fig. 2 provides the wt% 3,4-dihydroxy-1-butene as a function of exchanges. After five exchanges, the concentration of 3,4-dihydroxy-1-butene was reduced from 11.9 to 0.07 wt%.

To insure ourselves that the butene glycol oligomers produced under our typical reaction conditions were sufficiently small to pass through the membrane, we repeated the reaction shown in Scheme 1 but with omission of the cyclodextrin. At the completion of the reaction, water was removed in vacuo. Analysis of the reaction mixture by GC–MS revealed that the product was a mixture of 47% 3,4-dihydroxy-1-butene, 44% butene glycol dimer, and 9% butene glycol trimer. None of the higher oligomers were observed. The mixture of 3,4-dihydroxy-1-butene and butene glycol oligomers was then separated by vacuum distillation (1.8 mmHg). Analysis of the fraction collected at 122–138 °C by GC–MS and by NMR spectroscopy revealed that this fraction was comprised of a mixture of all possible butene glycol dimers (M_W 158) and trimers (M_W 228). Analysis of the small amount of material that distilled at higher temperatures and of the pot residue showed increasing amounts of butene diol trimers; higher molecular-weight oligomers could not be detected. Even if trace amounts of the tetramer or pentamer of butene glycol were present, their molecular weights are sufficiently small (298 and 368) to allow them to easily pass through a 500 MWCO membrane. To further confirm that the butene glycol oligomers can be removed by nanofiltration through a 500 MWCO membrane, a sample of HBen-β-CD containing 0.07 wt% 3,4-dihydroxy-1-butene was spiked with 10 wt% of the isolated butene glycol oligomers (Fig. 3). This spiked sample was then subjected to nanofiltration (five water exchanges) using a 500 MWCO cellulose acetate membrane. Both the permeate and the recovered HBen-β-CD were analyzed by NMR spectroscopy and mass spectrometry. The recovered HBen-β-CD did not contain any detectable amounts of the butene glycols, while the spectrum of the material recovered from the

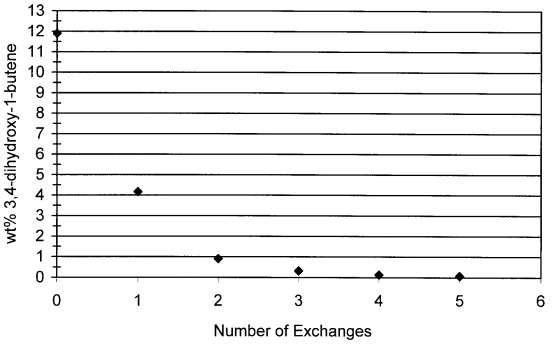


Fig. 2. Nanofiltration of an HBen-β-CD reaction mixture to remove 3,4-dihydroxy-1-butene and butene glycols byproducts.

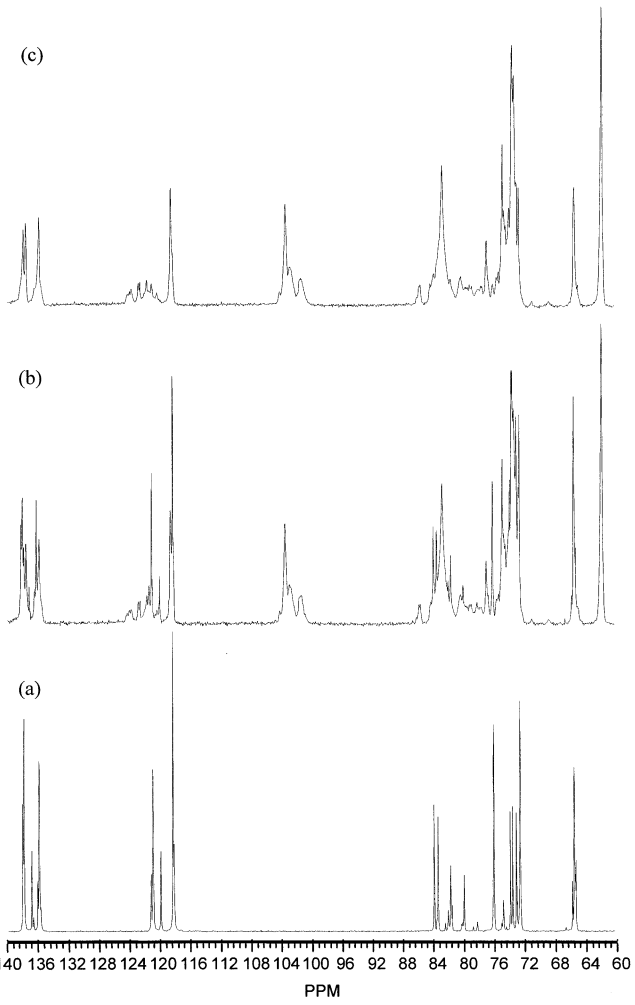


Fig. 3. ^{13}C NMR spectra of (a) butene glycol oligomers that was used to spike a HBen-β-CD sample; (b) HBen-β-CD spiked with 10 wt% of butene glycol oligomers; and (c) HBen-β-CD after nanofiltration of the butene glycol spiked HBen-β-CD.

permeate was identical to that of the material used to spike the sample. Additionally, we examined the nanofiltration permeates of hydroxybutenyl cyclodextrin samples that had not been subjected to extraction. The spectra of these samples were consistent with that of 3,4-dihydroxy-1-butene and butene glycol oligomers. Hence, nanofiltration is an efficient means for direct purification and concentration of hydroxybutenyl cyclodextrin reaction mixtures without any intervening purification steps.

After removal of the byproducts, we isolated the hydroxybutenyl cyclodextrins by first concentrating under vacuum to remove a significant amount of the water. The paste/solid was then taken up in EtOH, and the remaining water was removed by co-evaporation. In general, we found it necessary to repeat the co-evaporation two to three times. After drying at room temperature under vacuum (1 mmHg), we obtained the hydroxybutenyl cyclodextrins as white, amorphous solid.

Characterization of hydroxybutenyl cyclodextrin.—As we noted earlier, our intention was to prepare randomly substituted, amorphous hydroxybutenyl cyclodextrins containing none of the parent CD. In this regard, Fig. 4 provides a typical MALDI-TOFMS spectrum for HBen-β-CD (Table 1, entry 2). The peaks appear as doublets because of the presence of both Na and K ions. No peaks could be detected at a mass of 1157 $[M + Na]^+$ or 1173 $[M + K]^+$, indicating the absence of unsubstituted β-CD. The bell shaped molecular-weight distribution indicates that the distribution of substituents is random. As indicated in Fig. 4, the number of 3-hydroxy-1-butenyl substituents ranged from 2 to 10. In contrast, Fig. 5 gives the MALDI-TOFMS spectrum of a HBen-β-CD having insufficient DS and MS (Table 1, entry 5). As can be seen, this sample contained some parent β-CD.

Although other groups have reported DS values for similar systems based on MALDI-TOFMS,[10] one should note that these masses correspond to MS, not DS. That is, MALDI-TOFMS cannot distinguish a CD substituted with two 3-hydroxy-1-butenyl monomers from a CD substituted with one 3-hydroxy-1-butenyl dimer. Additionally, we have found that MALDI-TOFMS often does not provide the same MS obtained by other techniques. For example, comparison of the MS determined by ^1H NMR (vide infra) and by MALDI-TOFMS (Table 1) reveals that in nearly every case, the MS by MALDI-TOFMS is higher than that obtained by ^1H NMR. All of the MALDI-TOFMS data presented in Table 1 were collected using CH_2Cl_2–HFIP as the solvent for the matrix and hydroxybutenyl cyclodextrins. One sample (entry 7) was selected, and five spectra were collected on five different days using two solvent systems, acetonitrile–water or CH_2Cl_2–HFIP. With acetonitrile–water as the solvent system, the average MS was 5.56 ± 0.15, while with CH_2Cl_2–HFIP, the average MS was 5.20 ± 0.04. The MS determined by ^1H NMR spectroscopy for this sample was 4.54 ± 0.02. Because of the observed difference in MS between the two different solvent systems, and uncertainty about complete ionization and response of the sample in MALDI-TOFMS, we have elected to use NMR spectroscopy (vide infra) as the tool for quantitative determination of the MS.

At less than full substitution, a non-regiospecific reaction of a CD with 3,4-epoxy-1-butene leads to the formation of many isomers. As an example, a HBen-β-CD with a DS of 3 and an MS of 4 can be comprised of 20 regioisomers provided that ring opening occurs only at C-4 of 3,4-epoxy-1-butene. Analysis of a derivative of this type is further complicated by the loss of ring symmetry of the parent β-CD, and the formation of new stereogenic centers in the side-chain sub-

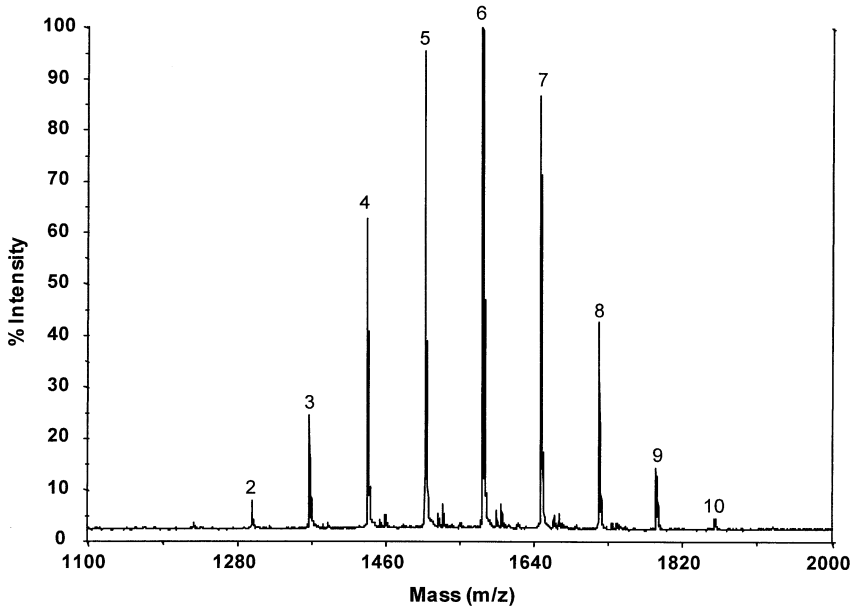


Fig. 4. MALDI-TOFMS spectrum of a HBen-β-CD from which an MS of 5.84 was determined.

stituents. The number of potential isomers increases if ring opening can occur at either C-3 and C-4 of 3,4-epoxy-1-butene. Obviously, complete assignment of each resonance of a HBen-β-CD of this type by NMR spectroscopy would be extremely difficult, if not impossible.

Despite this complexity, NMR spectroscopy can be used to obtain some very useful structural information. Fig. 6 provides the ^{1}H and ^{13}C NMR spectra of a typical HBen-β-CD having a DS of 2.69 and an MS of 4.54. With the exception of H-1 of the anhydroglucose ring, and the olefinic protons of the side chain substituents, all of the ^{1}H NMR resonances appear between 4.5 and 3.4 ppm (Figs. 6 and 7). Three distinct resonances, which can be assigned to C-1 of the anhydroglucose rings, are centered at 102 ppm in the ^{13}C NMR spectrum, and these resonances correlate to the proton resonances centered at 5.08 ppm. Additionally, several resonances between 118 and 125 ppm were observed in the ^{13}C NMR spectra, which we have assigned to the terminal C-1′ olefinic carbons of the side-chain substituents. These carbon resonances correlate to the complex set of overlapping resonances between 5.16 and 5.6 ppm in the ^{1}H NMR spectra. Likewise, the three resonances centered at 137 ppm in the ^{13}C NMR spectra can be assigned to the C-2′ olefinic carbons of the side-chain substituents, and these resonances correlate to the two broad resonances at 5.78 and 5.88 ppm in the ^{1}H NMR spectra.

Assignment of the ^{1}H NMR resonances at 5.78 and 5.88 ppm were made on the basis of the series of spectra shown in Fig. 8, which were obtained on samples from the experiment described above for the preparation of the butene glycol oligomers. These spectra show the evolution of the H-2 olefin ^{1}H resonances on progressing from 3,4-dihydroxy-1-butene to a mixture of butene glycol dimer and trimer. Based on these model compounds, we have assigned the resonances at 5.88 ppm to H-2′ olefinic protons adjacent to a carbon bearing a hydroxy group and the resonances at 5.78 to the H-2′ olefinic protons adjacent to a carbon bearing an ether. Hence, the olefinic protons between 5.7 and 6.0 can be used to determine the MS and the DS. The MS and DS of hydroxybutenyl cyclodextrins can be determined by integration of the ^{1}H NMR spectra using Eqs. (1) and (2);

$$\mathrm{MS} = ((\text{H-2}'_{\mathrm{OH}} + \text{H-2}'_{\mathrm{ether}})/(\text{All H} - ((\text{H-2}'_{\mathrm{OH}} + \text{H-2}'_{\mathrm{ether}}) \times 6))/7) \times \text{number Glc's} \quad (1)$$

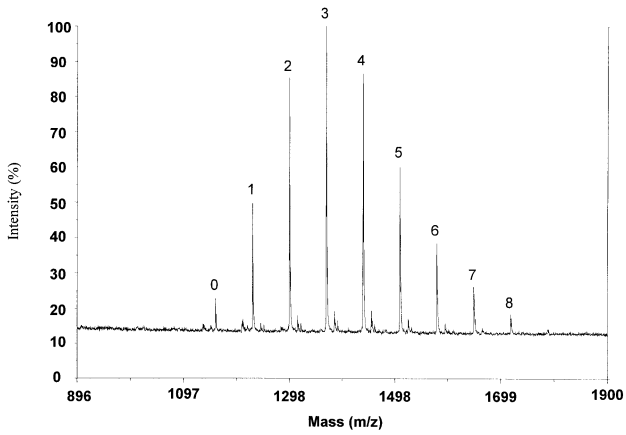


Fig. 5. MALDI-TOFMS spectrum of a HBen-β-CD containing unsubstituted β-CD.

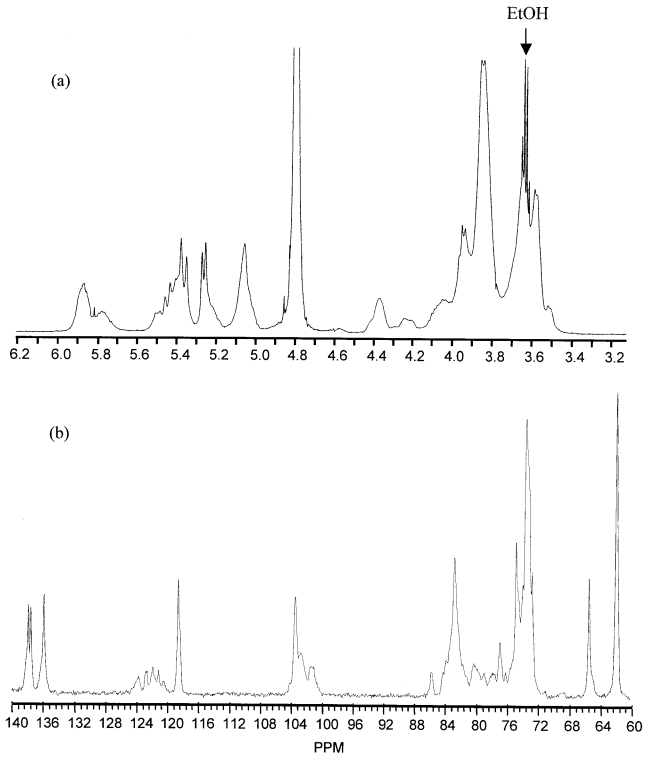


Fig. 6. (a) ^{1}H NMR spectrum and (b) ^{13}C NMR spectrum of a HBen-β-CD having a DS of 2.69 and an MS of 4.54.

$$DS = (H\text{-}2'_{OH}/(H\text{-}2'_{ether} + H\text{-}2'_{OH})) \times MS \quad (2)$$

where H-$2'_{OH}$ is the proton resonance at 5.88 ppm, H-$2'_{ether}$ is the proton resonance at 5.78 ppm, and 'All H' means integration of all of the proton resonances found between 3 and 6 ppm, excluding the resonance from HOD. Because the resonances at 5.88 and 5.78 ppm are broad and partially overlap, we used a curve-fitting routine to determine the integration of these resonances.

Regarding the 1D ^{13}C NMR spectra of hydroxybutenyl cyclodextrins, the severe spectral overlap in the region between 72 and 86 ppm does not allow any comment about selectivity between 1 and 2° hydroxyls of the CD or about selectivity between C-3 and C-4 in the ring opening of 3,4-epoxy-1-butene. However, because of the good resolution of the C-$2'_{OH}$ resonances, and the C-$2'_{ether}$ resonances in a quantitative ^{13}C NMR experiment, the resonances can be directly integrated. The DS can then be calculated by:

$$DS = (C\text{-}2'_{OH}/(C\text{-}2'_{ether} + C\text{-}2'_{OH})) \times MS \quad (3)$$

Regarding the morphology of the hydroxybutenyl cyclodextrins, Fig. 9 provides a typical DSC spectrum of HBen-β-CD. This second scan–heating curve shows only an endotherm at 80 °C that corresponds to the *T*g of the HBen-β-CD. Melting of the HBen-β-CD was not observed. This would indicate that this HBen-β-CD is indeed an amorphous solid.

As expected, the hydroxybutenyl cyclodextrins were found to be highly soluble in water and in other solvents. The solubilities of representative HBen-β-CD and HBen-α-CD in water, ethanol–water mixtures, and propylene glycol are given in Table 2. As anticipated, the CD derivatives exhibited excellent solubility in all three solvent systems. Only a slight difference in solubility is seen between the two HBen-β-CDs, and only in one solvent (96% ethanol). In this instance, the HBen-β-CD with the higher MS of 5.72 exhibited the better solubility in 96% ethanol. In water and in 15% aqueous ethanol, HBen-α-CD exhibited slightly higher solubility than HBen-β-CD. Relative to the parent cyclodextrin, the hydroxybutenyl cyclodextrins exhibited greatly improved solubility in water.

Guest–host complexes.—We have examined the solubility of a variety of guest molecules in water as a function of hydroxybutenyl cyclodextrin concentration. As an illustration, Fig. 10 shows the increase in the solubilities of glibenclamide and ibuprofen with increasing concentrations of HBen-β-CD (DS = 3.16, MS = 5.72). A binding constant, K_c, of 136 M^{-1} was calculated from the slope of this solubility isotherm.[11] Hydroxybutenyl-α-CD, having a very similar DS and MS (DS = 3.20, MS = 5.03), exhibited a higher binding constant ($K_c = 240$ M^{-1}), indicating that this HBen-α-CD binds more effectively with glibenclamide. For comparative purposes, hydroxypropyl-β-CD, with a reported DS of 2.7, had a binding constant of 62 M^{-1}. With ibuprofen, the HBen-β-CD had a higher binding constant ($K_c = 520$ M^{-1}) than HBen-α-CD ($K_c = 20$ M^{-1}); the K_c for hydroxypropyl-β-CD was 376 M^{-1}. As these examples illustrate, the size of the CD cavity and the type of substituent impacts binding of these guest molecules.

Encouraged by the increase in solubility of the two illustrative guest molecules, we prepared solid complexes of the HBen-β-CD (DS = 3.16, MS = 5.72) with both glibenclamide and ibuprofen (Table 3). Ibuprofen formed a 1:1 host–guest complex, while the larger glibenclamide formed a 1:2 host–guest complex. In both cases, the guest content in the complexes was high (14.4 and 12.9 wt%, respectively). In both cases, the relative increase in solubility of the free drug at pH 1.4 was quite large. For example, the solubility of ibuprofen was increased from < 0.05 mg/mL to 1.92 mg/mL.

Finally, Fig. 11 provides the dissolution rates of ibuprofen and glibenclamide at pH 1.4. In both cases, dissolution was very rapid. With ibuprofen, a very small burst effect was observed with a concentration of ca. 2.0 mg/mL being achieved within 2–5 min, and an equilibrium concentration being achieved in approximately 20 min. In the case of glibenclamide, the burst effect was even smaller, with equilibrium concentration being reached within 10 min. In both cases it is worth noting that after reaching the equilibrium concentra-

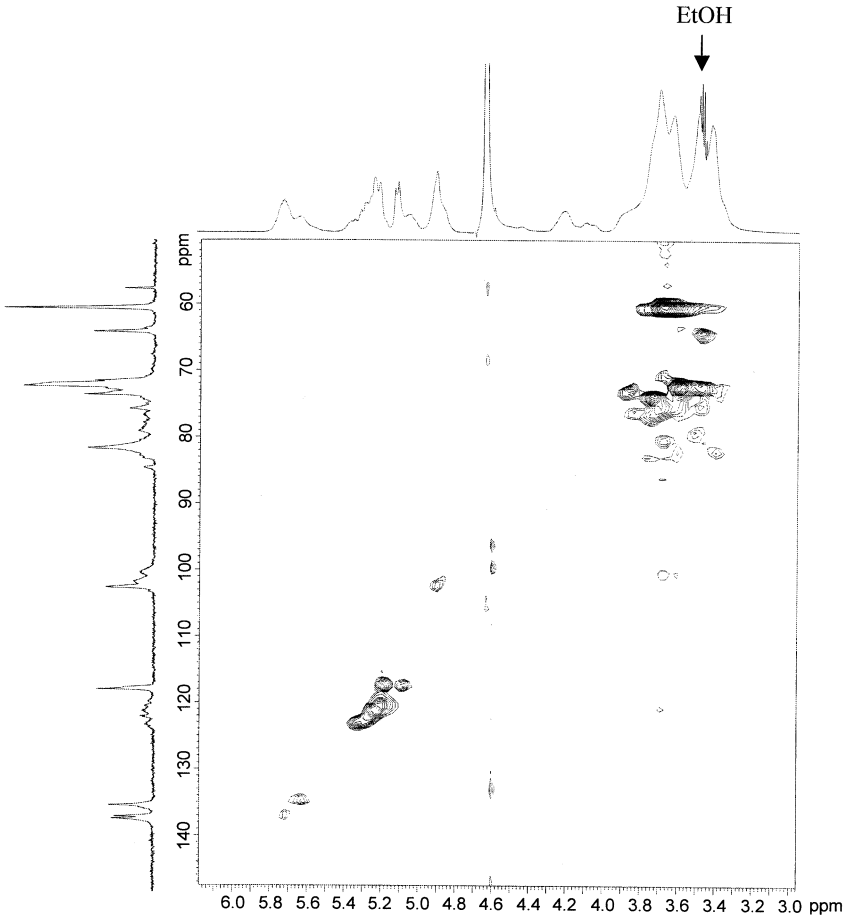


Fig. 7. HSQC 2D NMR spectrum of a HBen-β-CD having a DS of 2.69 and an MS of 4.54.

tion, a decrease in drug concentration over time was not observed. This observation indicates that the solubilized drug does not crystallize or precipitate from solution.

3. Conclusions

Reaction of cyclodextrins with 3,4-epoxy-1-butene in water provides highly water-soluble hydroxybutenyl cyclodextrin ethers. Reaction temperature, concentration of base, and reaction time are three significant variables that influence the MS and DS values that are obtained. Reaction byproducts can be removed, and the concentration of the hydroxybutenyl cyclodextrins in the reaction medium can be increased by nanofiltration. The hydroxybutenyl cyclodextrins can be isolated by co-evaporation of EtOH–water. The DS and the MS can be quickly determined by ^{1}H NMR spectroscopy. Typically, hydroxybutenyl cyclodextrins, which contain no unsubstituted parent cyclodextrin, have a DS of 2–4 and an MS of 4–7. These hydroxybutenyl cyclodextrins are randomly substituted, amorphous solids, which exhibit good solubility in water and in aqueous EtOH. As demonstrated with glibenclamide and ibuprofen, the hydroxybutenyl cyclodextrins readily form complexes with a variety of molecules. Complexation results in a significant increase in the aqueous solubilities of guest molecules. The dissolution rates of the complexes in pH 1.4 water were very high, and significant increases in the solubility of the guest molecules were observed. Significantly, after reaching equilibrium concentration, a decrease in the guest concentration over time was not observed. Additional details regarding complexation of different guest molecules by hydroxybutenyl cyclodextrins and toxicity studies on hydroxybutenyl cyclodextrins will be reported in due course.

4. Experimental

General methods.—The parent cyclodextrins were obtained from Wacker Chemie GmbH and were used as received. The 3,4-hydroxy-1-butene is a product of Eastman Chemical Company and is also available from Aldrich Chemical Co.

High-pressure liquid chromatography was carried out using a Hewlett–Packard 1100 liquid chro-

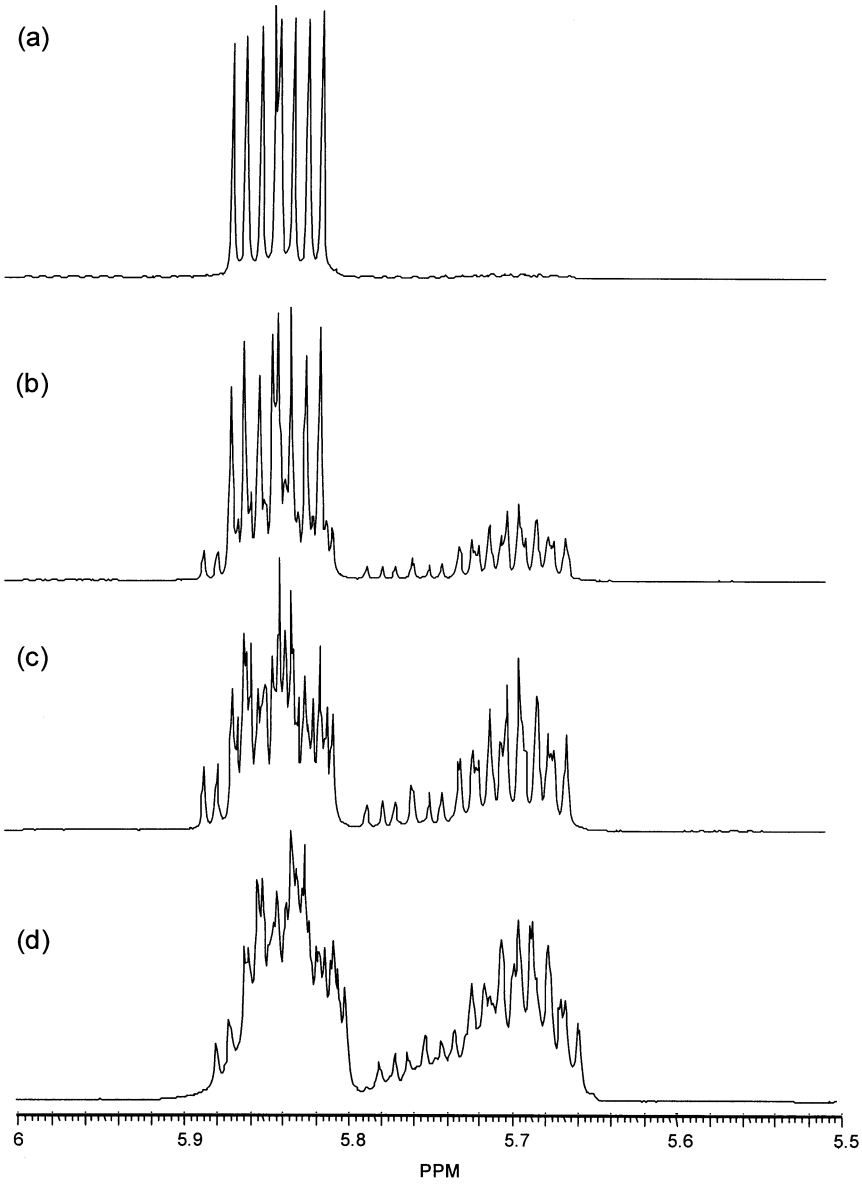


Fig. 8. ^{1}H NMR spectrum of the H-2 protons for selected fractions obtained by fractional distillation of the reaction product of base-catalyzed reaction of 3,4-epoxy-1-butene in water in the absence of CD.

matograph with an integrated pump, auto sampler, and diode array detector (UV at 210 nm, 16 nm bandwidth, 8 nm slit). A Shodex Asahipak GS-220 HQ analytical column (300 × 7.6 mm, 6 μ) with a Shodex Asahipak GS-2G 7B guard column (50 × 7.6 mm) was employed. The sample injection size was 20 μL, and the mobile phase was 35:65 MeCN–water at a flow rate of 0.6 mL/min.

Gas chromatography was conducted using a Hewlett–Packard 5890 II gas chromatograph with a 7673 auto sampler. The column was a 30 m × 0.32 mm × 1 μm DB-5 column and the carrier gas was helium. The temperature of the injector was 250 °C and the injection size was 1 μL. Samples were prepared by adding 100 mg of sample to 1 mL of pyridine containing dimethyl adipate as an internal standard. The initial temperature of the oven was held at 40 °C for 2 min before heating at 20°/min to 260 °C at which point it was held for 5 min.

NMR spectra were collected at ambient probe temperature using a JEOL model Eclipse + 600 NMR spectrometer. The sample (10 mg) was dissolved in 0.5 mL D_2O and added to a 5-mm OD NMR tubes. Chemical shifts for the ^{1}H and ^{13}C NMR spectra were referenced to added protonated dimethyl sulfoxide at 2.5 and 39.44 ppm, respectively. The gradient-enhanced HSQC 2D NMR spectrum was collected and processed with the following parameters: rows, 100 scans; 512 complex points; and 15 ppm spectral width centered at 5 ppm; and columns: 128 complex points zero-filled

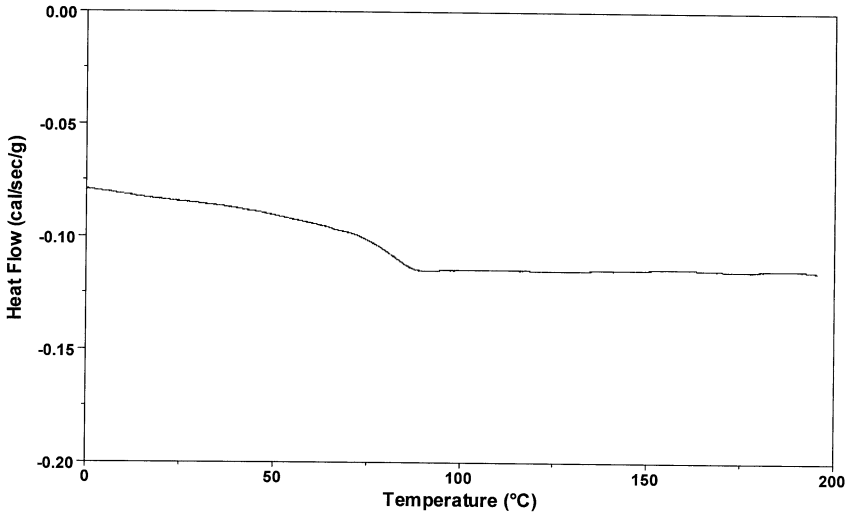


Fig. 9. DSC 2nd scan heating curve for a HBen-β-CD having a DS of 3.16 and an MS of 5.72.

Table 2
Solubility of selected hydroxybutenyl cyclodextrins in different solvents

Solvent	HBen-β-CD DS = 3.16 MS = 5.53 (%)	HBen-β-CD DS = 3.16 MS = 5.72 (%)	HBen-α-CD DS = 3.10 MS = 4.96 (%)	HBen-α-CD DS = 3.2 MS = 5.03 (%)
Water	≥50	≥50	≥60	≥60
15% EtOH	≥50	≥50	≥60	≥60
30% EtOH	≥50	≥50	≥50	≥50
60% EtOH	≥50	≥50	≥50	≥50
96% EtOH	≥40	≥50	≥50	≥50
Propylene glycol	≥40	≥40	ND	ND

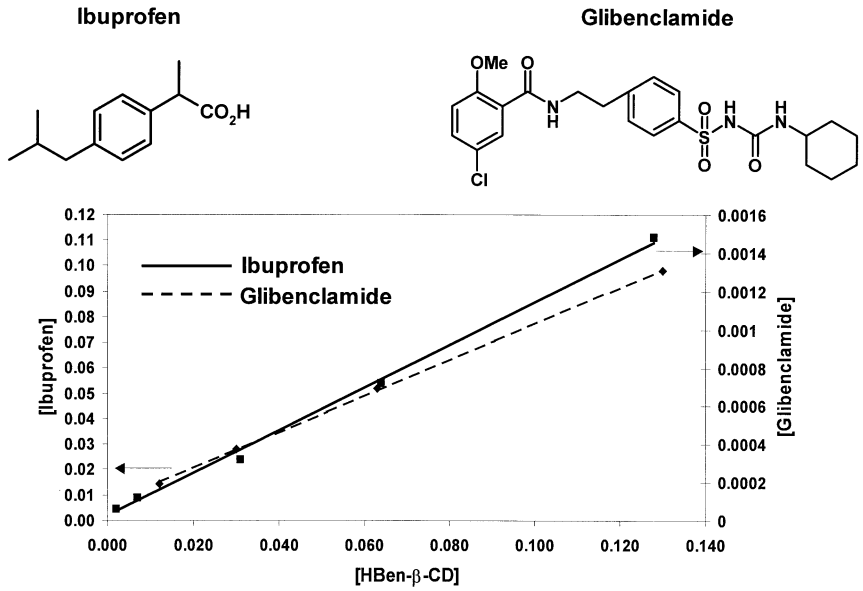


Fig. 10. The solubility of ibuprofen and glibenclamide in H_2O as a function of HBen-β-CD (DS = 3.16, MS = 5.72) concentration.

Table 3
Physical properties for ibuprofen–HBen-β-CD and glibenclamide–HBen-β-CD complexes

Host molecule	HBen-β-CD (DS = 3.16, MS = 5.72)	HBen-β-CD (DS = 3.16, MS = 5.72)
Guest molecule	ibuprofen	glibenclamide
Molar ratio (guest–host)	1:1	1:2
Guest content (w/w%)	14.4	12.9
pH of 20% (w/v) aqueous solution	7.1	7.4
Characteristics of a 20% (w/v) aqueous solution of the complexes	pale-yellow, clear	pale-yellow, clear
Solubility of guest molecule in pH 1.4 HCl solution (1.5% w/v)	1.92 mg/mL	0.17 mg/mL
Solubility of the free drug in pH 1.4 HCl solution	<0.05 mg/mL	<0.01 mg/mL

twice; and 200 ppm spectral width centered at 100 ppm. Spectral deconvolution was accomplished with the software routines provided in the JEOL DELTA version 3.1 software package.

Nanofiltration was carried out using a 200-mL Amicon ultrafiltration cell that was obtained from Millipore Corporation. The membrane was a 63.5 mm flat sheet cellulose acetate membrane (500 or 1000 molecular weight cut-off). Typically, an 8–10 wt% solution of hydroxybutenyl cyclodextrin was concentrated at 70 psi N_2 to approximately 25 wt% solids, which was considered to be one solvent exchange.

The molecular weight and the molecular-weight range of the hydroxybutenyl cyclodextrins were determined by matrix-assisted laser desorption ionization-time of flight-mass spectrometry (MALDI-TOFMS, PerSeptive Biosystems Voyager Elite DE MALDI/time-of-flight Instrument). The cyclodextrin was dissolved in 7:3 CH_2Cl_2–HFIP or 1:1 MeCN–water at a concentration of 10 mg/mL. An aliquot of 10 μL of the sample solution was added to 90 μL of the matrix solution (methyl 4-hydroxy-α-cyanocinnamate) and spotted (2 μL) on a sample plate for sample introduction. The molar substitution was determined by a weighted average of the components in the sample.[10]

The solubility of hydroxybutenyl cyclodextrins in selected solvents was determined by the addition of a 150-μL portion of the studied solvent into a test tube

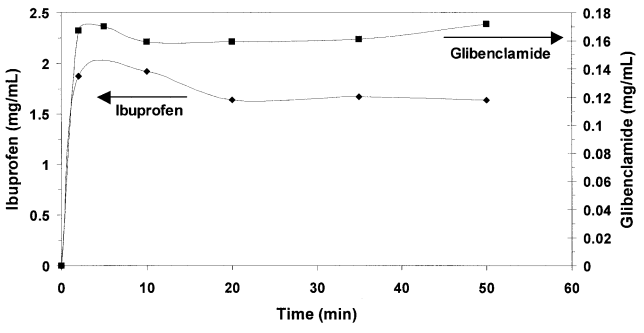


Fig. 11. Concentration of the guest molecule after the addition of the HBen-β-CD host–guest complex to H_2O (pH 1.4).

that contained 300 mg of hydroxybutenyl cyclodextrin. The sample was sonicated for 10 min at 25 °C. Additional 150-μL portions of solvent were added until complete dissolution of the solid was observed.

The solubility isotherms of hydroxybutenyl cyclodextrins and hydroxypropyl-β-CD were determined by adding excess amounts of guest molecules to 5.0-mL solutions of the host cyclodextrin at different concentrations. The suspensions were stirred for 24 h at 25 °C. The undissolved residues were removed by filtration through a 0.22 μM filter. The solutions were diluted with a 1:1 water–EtOH mixture, and concentrations of the guest molecules were determined spectrophotometrically using an HP 8542 diode array spectrophotometer. The apparent binding constants were calculated from the slope of the solubility isotherm.

In vitro dissolution rate of active ingredients from complexes were studied in acidic medium simulating the human stomach. Complexes (300 mg) were added to 20 mL pH 1.4 HCl solutions. The suspensions were stirred, and 1-mL volumes were taken at the prescribed time, after membrane filtration, were diluted with 50% EtOH and the dissolved drug concentration was determined by UV spectrophotometry.

A summary of the reaction conditions for the preparation of hydroxybutenyl cyclodextrins can be found in Table 1.

General procedure: O-*(hydroxy-1-butenyl)-cyclomaltoheptaose* (**1**).—Water (400 mL), β-CD (217 mmol), and KOH (54 mmol) were charged to a glass-lined autoclave. The mixture was heated to 100 °C with stirring at which time the β-CD dissolved. 3,4-Epoxy-1-butene (1.89 mol) was pumped into the reaction vessel at 6.2 mL/min, and the reaction mixture was held at 100 °C for 1.4 h. The reaction mixture was allowed to cool to approximately 50 °C and was neutralized with HCl (3.6 h total reaction time). The reaction mixture was concentrated to a solid/paste, which was then taken up in 435 mL of deionized water. The solution was heated to 70 °C, and 485 mL of EtOAc was added. The mixture was then stirred vigorously for 30 min. The layers were separated, and the extraction was repeated an additional five times before concentrating to a white

solid (270.8 g). Analysis by GC revealed that the crude reaction mixture contained 4.90 wt% 3,4-dihydroxy-1-butene. A portion (4 g) of the crude product was taken up in 50 mL of deionized water, and the solution was concentrated by nanofiltration (75 psi N_2) through a 500 MWCO cellulose acetate membrane to 25–30 wt% solids. The concentrated sample was diluted to the original volume. Five solvent exchanges were completed, and the sample was concentrated to approximately 25 wt% of HBen-β-CD. Most of the remaining water was removed in vacuo, which gave a solid/paste. The remaining water was removed by co-evaporation with EtOH (2 ×), which provided 3.51 g of HBen-β-CD as a white solid. Yield, 78%. ^{1}H NMR indicated a DS of 3.40 and an MS of 5.55. GC: 0.06 wt% 3,4-dihydroxy-1-butene. ^{1}H and ^{13}C NMR: see entry 7. MALDI-TOFMS: *m/z* 1297.4, 1367.6, 1437.6, 1507.7, 1577.7, 1647.7, 1717.7, 1787.7 corresponding to DS 2–9 $[M + Na]^+$, MS = 5.96. Anal. Calcd for $(C_6H_{10}O_5)_7$-$(C_4H_6O)_{5.55}\cdot 3H_2O$: C, 48.85; H, 6.98. Found: C, 49.02; H, 7.32.

O-*(Hydroxy-1-butenyl)-cyclomaltoheptaose* (**2**).—This HBen-β-CD was prepared according to the general procedure of **1**. White solid, yield, 69%. ^{1}H NMR indicated a DS of 3.16 and an MS of 5.72. GC: 0.04 wt% 3,4-dihydroxy-1-butene. ^{1}H and ^{13}C NMR: see entry 7. MALDI-TOFMS: *m/z* 1297.4, 1367.5, 1437.6, 1507.7, 1577.8, 1647.8, 1717.9, 1787.8, 1858.1 corresponding to DS 2–10 $[M + Na]^+$, MS = 5.84. Anal. Calcd for $(C_6H_{10}O_5)_7(C_4H_6O)_{5.72}\cdot 3\ H_2O$: C, 48.99; H, 6.99. Found: C, 48.75; H, 7.24.

O-*(Hydroxy-1-butenyl)-cyclomaltoheptaose* (**3**).—This HBen-β-CD was prepared according to the general procedure of **1**. During the addition of 3,4-epoxy-1-butene, the internal pressure rose from 0.5 to 3 bar. Fifteen min after the completion of the addition of 3,4-epoxy-1-butene, the pressure dropped to 0.5 bar. White solid, yield, 87%. ^{1}H NMR indicated a DS of 3.05 and an MS of 5.25. GC: 0.10 wt% 3,4-dihydroxy-1-butene. ^{1}H and ^{13}C NMR: see entry 7. MALDI-TOFMS: *m/z* 1297.2, 1367.3, 1437.3, 1507.4, 1577.5, 1647.6, 1717.6, 1787.5 corresponding to DS 2–9 $[M + Na]^+$, MS = 5.56. Anal. Calcd for $(C_6H_{10}O_5)_7$-$(C_4H_6O)_{5.25}\cdot 4\ H_2O$: C, 48.03; H, 7.00. Found: C, 47.87; H, 7.22.

O-*(Hydroxy-1-butenyl)-cyclomaltoheptaose* (**4**).—This HBen-β-CD was prepared according to the general procedure of **1**. The crude reaction mixture was concentrated to a white solid, but the solid was not extracted prior to nanofiltration. White solid, yield, 84%. ^{1}H NMR indicated a DS of 3.15 and an MS of 4.91. GC: 0.13 wt% 3,4-dihydroxy-1-butene. ^{1}H and ^{13}C NMR: see entry 7. MALDI-TOFMS: *m/z* 1297.4, 1367.4, 1437.5, 1507.6, 1577.7, 1647.7, 1717.8, 1787.5 corresponding to DS 2–9 $[M + Na]^+$, MS = 5.81. Anal. Calcd for $(C_6H_{10}O_5)_7(C_4H_6O)_{4.91}\cdot 2\ H_2O$: C, 48.85; H, 6.88. Found: C, 48.63; H, 7.29.

O-*(Hydroxy-1-butenyl)-cyclomaltoheptaose* (**5**).—This HBen-β-CD was prepared according to the general procedure of **1**. After neutralizing with HCl, the sample was not concentrated or extracted with EtOAc. Rather, the reaction mixture was simply concentrated using nanofiltration. After four solvent exchanges, the slightly hazy solution was filtered to remove insoluble material. White solid, yield, 82%. ^{1}H NMR indicated a DS of 1.27 and an MS of 2.47. GC: 0.58 wt% 3,4-dihydroxy-1-butene. ^{1}H and ^{13}C NMR: see entry 7. MALDI-TOFMS: *m/z* 1157.6 (1.5%), 1227.5, 1297.6, 1367.6, 1437.7, 1507.7, 1577.8, 1647.8, 1717.8 corresponding to DS 0–8 $[M + Na]^+$, MS = 3.45. Anal. Calcd for $(C_6H_{10}O_5)_7(C_4H_6O)_{2.47}\cdot 6\ H_2O$: C, 43.98; H, 6.89. Found: C, 44.21; H, 7.39.

O-*(Hydroxy-1-butenyl)-cyclomaltoheptaose* (**6**).—This HBen-β-CD was prepared according to the general procedure of **1**. After neutralizing and cooling to rt, solids formed in the solution, which were removed by filtration. The solids were washed with water that was combined with the liquids from the reaction. The liquid sample was concentrated by nanofiltration (5 ×). White solid, yield, 53%. ^{1}H NMR indicated a DS of 0.50 and an MS of 1.41. GC: 0.70 wt% 3,4-dihydroxy-1-butene. ^{1}H and ^{13}C NMR: see entry 7. MALDI-TOFMS: *m/z* 1157.6 (13.8%), 1227.6, 1297.7, 1367.7, 1437.9 corresponding to DS 0–4 $[M + Na]^+$, MS = 1.41. Anal. Calcd for $(C_6H_{10}O_5)_7(C_4H_6O)_{1.41}\cdot 8\ H_2O$: C, 40.97; H, 6.96. Found: C, 41.11; H, 7.23.

O-*(Hydroxy-1-butenyl)-cyclomaltoheptaose* (**7**).—This HBen-β-CD was prepared according to the general procedure of **1**. The crude reaction mixture was concentrated to a white solid but the solid was not extracted prior to nanofiltration. White solid, yield, 77%. ^{1}H NMR indicated a DS of 2.69 and an MS of 4.54. GC: 0.07 wt% 3,4-dihydroxy-1-butene. ^{1}H NMR (D_2O): δ 5.88 (H-2′ adjacent to a carbon bearing an OH), 5.78 (H-2′ adjacent to a carbon bearing an ether, H), 5.55–5.16 (H-1′), 5.06 (H-1), 4.4–3.4 (H-2, -3, -4, -4′, -5, -6) ppm. ^{13}C NMR (D_2O): δ 136.9 (C-2′), 136.6 (C-2′), 134.9 (C-2′), 122.8 (C-1′), 121.9 (C-1′), 121.7 (C-1′), 120.2 (C-1′), 119.5 (C-1′), 117.7 (C-1′), 102.4 (C-1), 101.7 (C-1), 100.3 (C-1), 84.7–71.7 (C-2, -3, -4, -4′, -5), 64.4 and 60.8 (C6) ppm. MALDI-TOFMS: *m/z* 1297.5, 1367.4, 1437.4, 1507.4, 1577.4, 1647.4, 1717.4, 1787.3 corresponding to DS 2–9 $[M + Na]^+$, MS = 5.65. Anal. Calcd for $(C_6H_{10}O_5)_7(C_4H_6O)_{4.54}\cdot 3\ H_2O$: C, 47.92; H, 6.90. Found: C, 47.79; H, 7.15.

O-*(Hydroxy-1-butenyl)-cyclomaltoheptaose* (**8**).—This HBen-β-CD was prepared according to the general procedure of **1**. White solid, yield, 81%. ^{1}H NMR indicated a DS of 3.16 and an MS of 5.53. GC: 0.06 wt% 3,4-dihydroxy-1-butene. ^{1}H and ^{13}C NMR: see entry 7. MALDI-TOFMS: *m/z* 1297.2, 1367.6, 1437.7,

1507.9, 1578.0, 1648.0, 1718.1, 1788.1 corresponding to DS 2–9 $[M + Na]^+$, MS = 5.91. Anal. Calcd for $(C_6H_{10}O_5)_7(C_4H_6O)_{5.53}\cdot 2\ H_2O$: C, 49.39; H, 6.93. Found: C, 49.60; H, 7.44.

O-*(Hydroxy-1-butenyl)-cyclomaltoheptaose* (***9***).—This HBen-β-CD was prepared according to the general procedure of **1** with the exception that the reaction was conducted in a round bottom flask. White solid, yield, 73%. ^{1}H NMR indicated a DS of 2.75 and an MS of 5.12. GC: 0.02 wt% 3,4-dihydroxy-1-butene. ^{1}H and ^{13}C NMR: see entry 7. MALDI-TOFMS: *m*/*z* 1297.3, 1367.4, 1437.4, 1507.5, 1577.6, 1647.6, 1717.6, 1787.5 corresponding to DS 2–9 $[M + Na]^+$, MS = 5.90. Anal. Calcd for $(C_6H_{10}O_5)_7(C_4H_6O)_{5.12}\cdot 2\ H_2O$: C, 49.62; H, 6.85. Found: C, 49.59; H, 7.15.

O-*(Hydroxy-1-butenyl)-cyclomaltohexaose* (**10**).—This HBen-α-CD was prepared according to the general procedure of **1**. White solid, yield, 88%. ^{1}H NMR indicated a DS of 3.10 and an MS of 4.96. GC: 0.03 wt% 3,4-dihydroxy-1-butene. ^{1}H and ^{13}C NMR: see entry 7. MALDI-TOFMS: *m*/*z* 1135.3, 1205.3, 1275.4, 1345.4, 1415.4, 1485.5, 1555.6, 1625.7 corresponding to DS 2–9 $[M + Na]^+$, MS = 5.46. Anal. Calcd for $(C_6H_{10}O_5)_6(C_4H_6O)_{4.96}$: C, 50.77; H, 6.85. Found: C, 50.59; H, 7.44.

O-(Hydroxy-1-butenyl)-cyclomaltohexaose (**11**).—This HBen-α-CD was prepared according to the general procedure of **1**. White solid, yield, 61%. ^{1}H NMR indicated a DS of 3.20 and an MS of 5.03. GC: 0.02 wt% 3,4-dihydroxy-1-butene. ^{1}H and ^{13}C NMR: see entry 7. MALDI-TOFMS: *m*/*z* 1135.3, 1205.3, 1275.4, 1345.4, 1415.4, 1485.6, 1555.7, 1625.7 corresponding to DS 2–9 $[M + Na]^+$, MS = 5.39. Anal. Calcd for $(C_6H_{10}O_5)_6(C_4H_6O)_{5.03}\cdot 3\ H_2O$: C, 48.85; H, 7.02. Found: C, 49.03; H, 6.98.

O-*(Hydroxy-1-butenyl)-cyclomaltohexaose* (**12**).—This HBen-α-CD was prepared according to the general procedure of **1**. White solid, yield, 70%. ^{1}H NMR indicated a DS of 2.77 and an MS of 4.42. GC: 0.01 wt% 3,4-dihydroxy-1-butene. ^{1}H and ^{13}C NMR: see entry 7. MALDI-TOFMS: *m*/*z* 1135.3, 1205.3, 1275.4, 1345.4, 1415.4, 1485.6, 1555.7, 1625.7 corresponding to DS 2–9 $[M + Na]^+$, MS = 5.39. Anal. Calcd for $(C_6H_{10}O_5)_6(C_4H_6O)_{4.42}\cdot H_2O$: C, 49.55; H, 6.86. Found: C, 49.93; H, 7.31.

1-Butene glycol oligomers.—Water (400 mL), 3,4-epoxy-1-butene (135 g, 1.89 mmol), and KOH (54 mmol) were charged to a glass-lined autoclave. The mixture was heated to 100 °C and held for 1.5 h. The reaction mixture was allowed to cool to approximately 50 °C and was neutralized with HCl (3.0 h total reaction time). The water was removed in vacuo at 45–75 °C and 25 mmHg to afford a viscous yellow liquid mixed with inorganic salts. The salts were removed by filtration resulting in 197.6 g of a yellow liquid. Analysis of this mixture by GC–MS revealed that it was comprised of 3,4-dihydroxy-1-butene (47%), three 1-butene glycol dimers (44%), and 1-butene glycol trimers (9%). The liquid was distilled through a short-path column at 1.8 mmHg, and seven fractions were collected. The fraction collected at 78–80 °C (64.5 g) was analytically pure 3,4-dihydroxy-1-butene (NMR, GC–MS). The fifth fraction collected at 122–138 °C (20.3 g) did not contain any 3,4-dihydroxy-1-butene and was comprised of 75% 1-butene glycol dimers (M_W 158) and 25% trimers (M_W 228). Fractions 2–4 contained decreasing amounts of 3,4-dihydroxy-1-butene and increasing amounts of 1-butene glycol dimers and trimers. The fractions distilling at higher temperatures contained increasing amounts of 1-butene glycol trimers.

1:1 Ibuprofen–HBen-β-CD complex.—HBen-β-CD (48.6 g, 0.031 mol) was dissolved in 550 mL of water containing 2.2 g (0.055 mol) of NaOH at rt under continuous stirring. A pale-yellow, clear solution was obtained. Solid ibuprofen (6.44 g, 0.031 mol) was added to the solution in small portions over a period of 30 min. The guest substance dissolved completely. The pH of the solution (12.2) was adjusted with 85% phosphoric acid to pH 7.0. The clear solution was filtered through a glass filter and freeze-dried. A nearly white powder was obtained (yield, 57.2 g, 98.5%). In order to determine the guest content of the complex, 100 mg of the complex was dissolved in 5mL of water. The clear solution was diluted with 50% EtOH, and the guest molecule content of the complex was measured by UV spectrophotometry.

1:2 Glibenclamide–HBen-β-CD complex.—HBen-β-CD (47.3 g, 0.030 mol) was dissolved in 550 mL of water containing 2.2 g (0.055 mol) of NaOH at rt under continuous stirring. A pale-yellow, clear solution was obtained. Solid glibenclamide (7.51 g, 0.015 mol) was added to the solution in small portions over a 20-min period. The guest molecule dissolved completely in the reaction mixture. The pH of the solution (12.4) was adjusted with 3.5 M phosphoric acid to pH 7.3. The clear solution was filtered through a glass filter and freeze-dried. A nearly white powder was obtained (yield: 57.9 g, 100.1%). The guest content of the complex was determined as above for the ibuprofen complex.

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