Selectively Monomodified Cyclodextrins. Synthetic Strategies

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Monomodifications of cyclodextrins to give selectively 2-, 3-, or 6-substituted product is a challenging task because of the number of hydroxyl groups that can potentially react with the incoming reagent. The principles and the methods involved in manipulations of the differences in the chemistry of these hydroxyl groups to control the outcome of an electrophilic reaction with them to produce monoalkylated (ether-linkaged) cyclodextrin derivatives are discussed and illustrated.

Cyclodextrins, the torus-shaped cyclic oligomers of α-D-glucopyranose,[1] have have gained prominence in recent years in diverse fields such as artificial enzymes,[2−5] drug delivery systems,[6] and chiral separation media.[7] Severe constraints, such as availability of limited functional groups for useful chemical processes and the rigid shape and sizes, of these "structural and functional straight jackets"[8] had initially stymied the progress in these fields. However, valiant efforts by several distinguished groups [9−11] have ameliorated this situation to the extent that now cyclodextrins with a variety of shapes, sizes, and functional groups are available.[12] Our efforts in this field have been directed toward conversion of hydroxyl groups of cyclodextrins to other useful functional groups.[13−15]

Among several possible types of modifications[12] (mono-, di-, tri-, and per-), monomodified cyclodextrins have been particularly notable in the exciting field of artificial enzymes.[16] These monomodifications can conceivably be achieved by a reaction of the hydroxyl group on an electrophilic reagent. However, the large number of hydroxyl groups at three different positions of cyclodextrins do not easily lend themselves to modification at a single desired place. Although selective monofunctionalization at a desired (2-, 3-, or 6-) position is a challenging task, the differences in the chemistry among these sites can be exploited to yield a preponderance of a particular product.

Among the linkages (e.g., sulfide,[17,18] amine,[19] ester,[11] ether,[20] etc.) that are available for attachment of functional groups to cyclodextrins, ether bonds are perhaps the most desirable. They are less susceptible to degradation by oxidation or hydrolysis and can conceivably be obtained by a single nucleophilic reaction of a hydroxyl group of cyclodextrin on an appropriate electrophile. The principles and the methods involved in manipulation of the differences in the chemistry of the hydroxyl groups to control the outcome of an electrophilic reaction with cyclodextrin to produce monoalkylated (ether linkaged) cyclodextrin derivatives are illustrated in this paper. 4-Methylamino-3-nitrobenzyl chloride is used as the electrophilic reagent in this investigation because the resulting cyclodextrin derivative, as described in the literature,[14] is an important intermediate in the synthesis of artificial redox enzymes. The synthesis of these artificial redox enzymes using the cyclodextrin derivatives reported here are the subject of a future paper.

Results and Discussion

Selective Monoalkylation at the Primary Side of α-Cyclodextrin. It has been noted that the hydroxyl groups at the primary side of cyclodextrin are the most basic and very often most nucleophilic.[1] Thus, normal reactivity of the hydroxyl groups dictates that these should be alkylated in the presence of an appropriate electrophile in a weakly basic solvent. As described below, although this is found to be true in the case of α-cyclodextrin, it does not apply to *β*-cyclodextrin.

A simple method to selectively connect an alkyl group (4-methylamino-3-nitrobenzyl group) to the primary side of α-cyclodextrin (**1**) consists of (Scheme 1) a reaction of compound **1** with 4-methylamino-3-nitrobenzyl chloride **2** in 2,6-lutidine to give 6-*O*-(4-methylamino-3-nitrobenzyl)-α-cyclodextrin (**3**). During the reaction, a reddish

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(16) (a) D'Souza, V. T.; Bender, M. L. *Acc. Chem. Res.* **1987**, *20*, 146−152. (b) Breslow, R. *Acc. Chem. Res.* **1995**, *28*, 146. (c) Breslow, R., Dong, S. *Chem. Rev.* **1998**, *98*, 1997.
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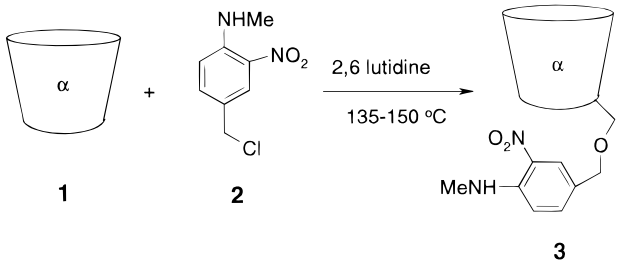
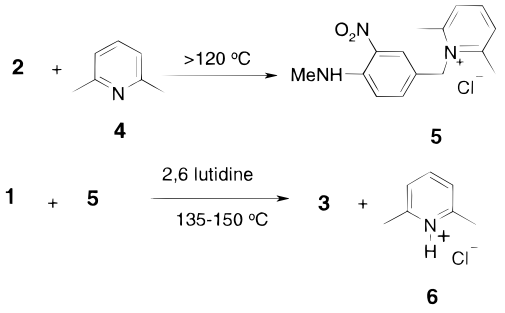
Scheme 1. A simple method for connecting an alkyl group to the primary side of α-cyclodextrin.



1 2 3

Scheme 2. Reaction mechanism for the formation of 6-*O*-(4-methylamino-3-nitrobenzyl)-α-cyclodextrin (5).



intermediate is first generated, which finally converts to lutidinium hydrochloride (**6**) as a white precipitate. To determine the identity of this intermediate, **2** was reacted with 2,6-lutidine (**4**) at 120 °C to give the reddish compound. This compound is highly soluble in water and gives a white precipitate instantly with silver nitrate, indicative of the presence of free chloride ions. In a separate reaction, compound **2** is reacted with **4** at 120 °C in the presence of **1** to form the reddish intermediate, which reacts further to give **3** only when the temperature is raised to 130 °C. The formation of **6** in the reaction mixture is confirmed by comparison of the TLC with an authentic sample. These observations are consistent with literature precedence[21] and support a reaction mechanism in which **2** reacts with lutidine to give a lutidinium salt **5**, which subsequently alkylates the cyclodextrin (Scheme 2).

Tentative assignment of the regiochemistry of the product is made by an examination of the carbohydrate region in the ^{13}C NMR spectrum (Supporting Information, spectrum 1). Five intense peaks in the carbohydrate region, upon a comparison with the spectrum of α-cyclodextrin and literature precedence, are assigned to unmodified glucose units at 101.87 (C1), 82.02 (C4), 73.21 (C3), 72.03 (C2 and C5), and 59.90 (C6) ppm. The three less intensive peaks of the modified glucose unit, which are indicative of the substitution pattern, are observed at 68.56, 71.18, and 82.31 ppm. The first signal is 8.66 ppm downfield from the chemical shift of C6 of the unmodified glucose unit. The other two are 0.85 ppm upfield and 0.29 ppm downfield from the signals of C5 and C4, respectively, of unmodified glucose units. These data suggest that **3** is modified at the 6-position of α-cyclodextrin since the chemical shifts of its C_α and C_γ are expected to move downfield by about 10 and 1 ppm, respectively, and C_β is expected to move upfield by about 2 ppm when an alcohol is alkylated.[22] They further suggest that these three signals belong to C6′, C5′, and C4′ respectively.

Selective Monoalkylation at the Primary Side of *β*-Cyclodextrin. As often is the case with well laid out strategies for modification of cyclodextrins, the successful method described above for alkylation of the primary side α-cyclodextrin cannot be applied for a similar transformation of *β*-cyclodextrin. Attempts to alkylate the primary side of *β*-cyclodextrin (**7**) by following the above approach were unsuccessful because **7** is not very soluble in lutidine, which makes the reaction extremely slow. TLC of the reaction mixture indicates that more than one hydroxyl group is modified, which can be attributed to the reaction of **2** with monomodified product that remains in solution. Pyridine is known to dissolve *β*-cyclodextrin and direct its modification to the primary side in sulfonation and silylation reactions.[15c] However, in pyridine, compound **2** reacts with the solvent to form pyridinium salt, which does not alkylate **7** even after an overnight reflux (bp, 110 °C). The reaction was carried out in a mixture of solvents containing DMF and 2,6-lutidine (1:1 v/v) in which cyclodextrin is very soluble. However, upon workup, this reaction produced a mixture of the desired compound **8** and its regioisomer **9** (Scheme 3). The formation of these two products can be rationalized by assuming that **2** forms a complex with **6** in such a way that the chloromethyl group of **2** is oriented toward the secondary side of the host as shown in Scheme 3. Thus, whereas this complex leads to the formation of **9**, the normal reactivity of cyclodextrin leads to the formation of **8**. It was anticipated that the separation of these isomers would be very difficult, and therefore an indirect approach via protection of the secondary side was considered a reasonable choice.[23]

Initially, heptakis(2,3-di-*O*-methyl)-*β*-cyclodextrin (**10**), a cyclodextrin derivative whose secondary side is methylated, was selected for further attachment of the desired group to the primary side. The synthesis **10** is described in the literature, and methyl ethers are expected to be stable under our reaction conditions. Compound **2** successfully reacts with **10** (Scheme 4) under conditions similar to those discussed for reaction with α-cyclodextrin to give heptakis(2,3-di-*O*-methyl)-6-*O*-(4-methylamino-3-nitrobenzyl)-*β*-cyclodextrin **11**. Since the secondary side of **10** is occupied with methoxy groups, the modification must take place on its primary side. An analysis of the ^{13}C spectrum further supports this suggestion (Supporting Information, spectrum 2). The three less intense peaks due to the modified glucose unit are at 68.64, 70.45, and 79.69 ppm. The first one is downfield shifted by 8.56 ppm from the C6, and the second one is upfield shifted by 1.41 ppm from the C5 of the unmodified glucose units. These data indicate that the modification has taken place at the 6-position, The antiphase appearance of the signal at 68.64 ppm in APT-NMR (Supporting Information, spectrum 3) suggests that this signal belongs to the methylene group at the 6-position of the modified glucose unit.

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(22) These data are obtained by manipulating the chemical shift parameters listed in a text book by Sillvertein et al. and are confirmed with assignments of ^{13}C NMR spectra of compounds **58** and **59**. Silverstein, R.; Bassler, G. C.; Morrill, T. C. *Spectral Identification of Organic Compounds*, 5th ed.; John Wiley & Sons: New Yoek, 1991; p 236.

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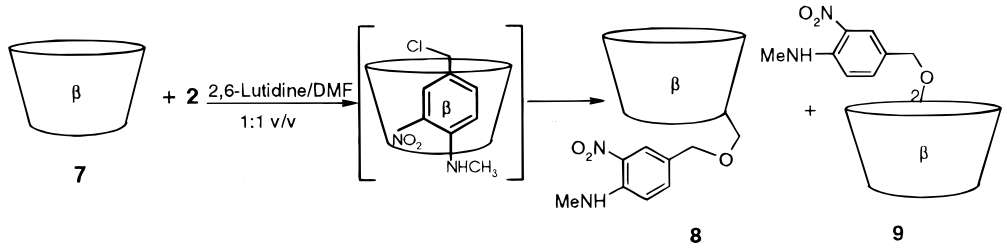
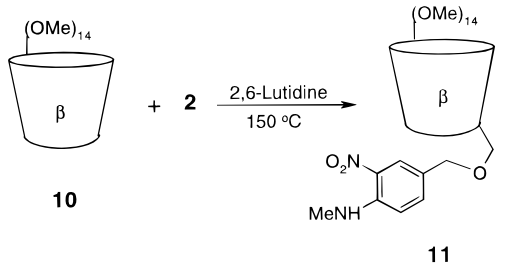
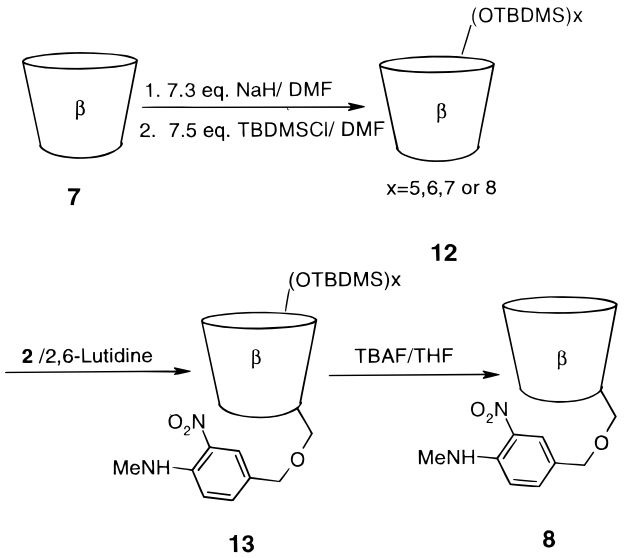
Scheme 3. Reaction of the same electrophile 2 with *β*-cyclodextrin.



Scheme 4. Alkylation of the primary side with 2 using secondary-side-protected *β*-cyclodextrin.



Scheme 5. Strategy for monoalkylation of the primary side of *β*-cyclodextrin.



Because methoxy groups of **11** cannot be easily converted to hydroxyl groups to give the desired compound, the protection at the secondary side of *β*-cyclodextrin with removable groups was pursued. Available procedures for the protection of the secondary side of *β*-cyclodextrin involve the protection of its primary side first and then its secondary side with a different group, followed by the removal of the protecting group at its primary side.[20] Because this procedure is tedious, a new approach to protect its secondary side directly from cyclodextrin using TBDMS groups was investigated. The precedence for a direct reaction of the hydroxyl groups at the secondary side with an electrophile comes from the methylation of the secondary side of cyclodextrin with NaH in anhydrous DMF.[24] The TBDMS group has an advantage because it can be removed easily after appropriate modification on the primary side.

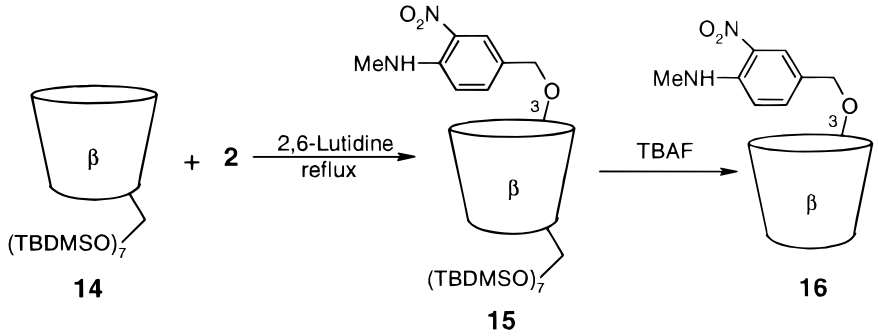
As shown in Scheme 5, compound **7** is stirred with NaH, and TBDMS chloride is added to the gel that is formed. Simultaneously, the temperature is raised to 90 °C in the first 2 h and then kept at 90 °C until the reaction is completed. This addition sequence is used to give maximal selectivity at the beginning of the reaction at low temperature, and high temperature later is used to push the reaction further. TLC of the crude material **12** suggests that it is a mixture of products with varying degrees of substitution. A strong peak centered at 74.00 ppm and a very weak absorption at 61.8 ppm in its ^{13}C NMR spectrum (Supporting Information, spectrum 4) indicate that the silylation reaction has predominantly taken place on the secondary side. The integration ratio between signals at 4.82−4.72 ppm (anomeric protons) and 1.00−0.68 ppm (*tert*-butyl groups) in its ^{1}H NMR spectrum (Supporting Information, spectrum 5) indicates that an average of 6.4 silyl groups are attached to a cyclodextrin molecule. Attempts to improve this situation to obtain a homogeneous product by manipulation of reagent ratios, solvents, and other reaction conditions failed.

Although this is not an ideal situation, it can be rationalized that the complete silylation on the secondary side is unfavorable presumably because the steric hindrance of bulky *tert*-butyldimethylsilyl groups increases as modification continues. If this assumption is correct, then it stands to reason that any further electrophilic reactions on the secondary side would be inhibited and this would enhance the possibility of our original goal of selective modification on the primary side. This assumption is supported if **12** reacts with the electrophile **2** to give exclusively 6-substituted product because, as described earlier, native *β*-cyclodextrin does not do so with this electrophile. When compound **12** is first reacted with **2** and then deprotected with tetrabutylammonium fluoride (TBAF) without the isolation[24] of the intermediate **13**, the desired compound **8** is obtained as the major product (23.8% after appropriate workup of the reaction). A small amount of unreacted **7**, and its higher substituted derivatives obtained in the crude product are separated by Sephadex.

Tentative assignment of regiochemistry of the product is made by an analysis of the ^{13}C spectrum (Supporting Information, spectrum 6). As described earlier, the six intense signals in the carbohydrate region at 101.85, 81.44, 73.00, 72.25, 71.99, and 59.83 ppm are assigned to C1, C4, C3, C5, C2, and C6 of the unmodified glucose units, respectively. Four less intense peaks at 102.35,

(24) In a separate experiment, the intermediate **13** was isolated by rotary evaporation of the reaction mixture to remove most of the lutidine, poured into ice water, washed with water, and dried in the vacuum oven overnight at 90 °C. The ^{1}H and ^{13}C NMR of the crude product indicate that the 4-methylamino-3-nitrobenzyl group is attached to the primary side of the cyclodextrin. Since this is a mixture of varying degrees of silylation on the secondary side of cyclodextrin as indicated by TLC, a complete characterization of this product is not possible. This intermediate, when refluxed with 8 equiv of TBAF in THF overnight, gives the same final product **8** after appropriate workup.

Scheme 6. Monoalkylation at the 3-position of β-cyclodextrin.



81.88, 71.08, and 68.78 ppm can be attributed to C1′, C4′, C5′, and C6′ of the modified glucose unit, respectively. The downfield shifts of C6′ and C4′ by 8.95 and 0.44 ppm, respectively, and the upfield shift of C5′ by 0.91 ppm suggests that the modification has taken place at the primary side of β-cyclodextrin. The complete NMR assignments for this compound are reported elsewhere.[25]

Selective Monoalkylation at the 3-Position of β-Cyclodextrin. It has been noted that the hydroxyl groups at the 3-position of cyclodextrin are the least accessible for electrophilic attack. The strategy that has been successful in the literature for modification at this position involves the choice of a reagent that binds the cyclodextrin and orients its reactive site toward the hydroxyl group at this position.[26] Discussions in the previous sections suggest that **2** binds to β-cyclodextrin to orient the reactive benzyl chloride group toward the secondary side. Usually hydroxyl groups at 2-positions on the secondary side of cyclodextrin are more reactive and are modified more easily than those at 3-positions.[27−29] However, it is conceivable that the orientation of the guest may change if the nature of the cavity is altered by chemical modification of the native cyclodextrin. The new orientation may direct the reactive site toward the 3-hydroxyl group to alkylate cyclodextrin at that position. Since the cavity of heptkis(*tert*-butyldimethylsilyl)-β-cyclodextrin (**14**) is known[30] to be partly occupied by TBDMS groups, changing its character and binding properties, **14** was reacted with the electrophile **2** to determine the effect of these changes in the reaction product. We discovered that this reaction leads to the alkylation of a hydroxyl group at the 3-position. Thus, 3-*O*-(4-methylamino-3-nitrobenzyl)-β-cyclodextrin (**16**) was synthesized by reacting **14** with **2** in 2,6-lutidine, followed by deprotection with TBAF (Scheme 6), without isolation of the reaction intermediate. The crude product after workup and purification by gel filtration followed by HPLC affords **16** as major product along with a small amount of **9**. The formation of **16** can be explained based on an intramolecular complex between **14** and **5** as illustrated in Figure 1. Compound **2** is expected to react with 2,6-lutedine to form **5** and this is proposed to bind in a way that makes its reactive carbon atom closer to the hydroxyl groups at 3-positions and reacts with one of them to give **16**.

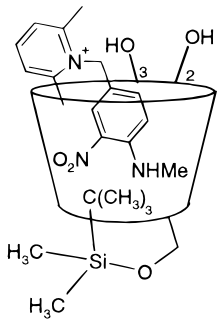


Figure 1. Proposed complex between **14** and **2** that explains the formation of **16**.

A tentative assingment of the regiochemistry of the product is made by an analysis of its ^{13}C spectrum (Supporting Information, spectrum 7). Compound **16** demonstrates a more dispersed carbon spectrum than its regioisomers **9** and **8**. Peaks at 101.64−100.89 and 81.30−80.50 ppm are assigned to C1 and C4 carbons of unmodified glucose units. Peaks between 73.1 and 71.50 ppm are accredited to C2, C3, and C5, and those at 59.65−59.55 are assigned to C6 of unmodified glucose units. The signal at 77.98 ppm is assigned to C3′ of the modified glucose. Both the downfield shift of C3′ and the upfield shift of C4′ suggest that the modification takes place on the hydroxyl group at the 3-position. The complete NMR assignments for this compound are reported elsewhere.[25]

Monoalkylation at the 2-Position of β-Cyclodextrin. The primary principle involved in monoalkylation at the 2-position of cyclodextrins is that these hydroxyl groups are the most acidic and can be preferentially deprotonated by a strong base. The resulting oxyanion is the most nucleophilic species in the resulting reaction mixture and would preferentially react with the available electrophile. This strategy for modification of the secondary side of cyclodextrin has been illustrated[13] using a benzyl chloride derivative as an electrophile. We further illustrate this by using bromomethyl ketone derivative 1′-bromo-4-methylamino-3-nitroacetophenone (**1**).

Monoalkylation of β-cyclodextrin at the 2-position is accomplished by the reaction of **7** with 1′-bromo-4-methylamino-3-nitroacetophenone (**18**). Compound **18** was synthesized by refluxing 4-methylamino-3-nitroacetophenone (**17**)[31] with 1.5 equiv of cupric bromide in a mixture of $CHCl_3$ and ethyl acetate (Scheme 7). 2-*O*-[2-

(25) Forgo, P. Ph.D. Dissertation, University of Missouri-St. Louis, 1999.
(26) (a) Fujita, K.; Nagamura, S.; Imoto, T.; Tahara, T. Koga, T. *J. Am. Chem. Soc.* **1985**, *107*, 3233. (b) Fujita, K.; Tahara, T.; Imoto, T.; Koga, T. *J. Am. Chem. Soc.* **1986**, *108*, 2030.
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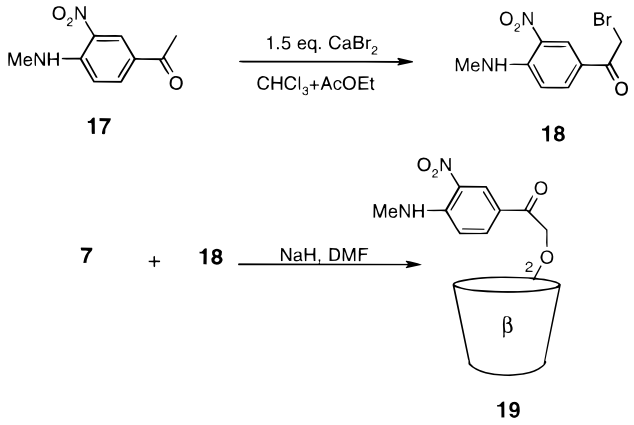
(30) Tabushi, I. *Acc. Chem. Res.* **1982**, *15*, 66.
(31) Levine, H. L.; Kaiser, E. T. *J. Am. Chem. Soc.* **1978**, *100*, 7670.

Scheme 7. Monoalkylation at the 2-position of *β*-cyclodextrin.



(4-Methylamino-3-nitrophenyl)-2-oxyethyl]-*β*-cyclodextrin (**19**) was synthesized by the treatment of *β*-cyclodextrin (**7**) with 1.2 equiv of NaH in dry DMF overnight, followed by the reaction with 1′-bromo-4-methylamino-3-nitroacetophenone. After a regular workup, the reaction product was purified by Sephadex followed by HPLC to yield **19** in 16.5%. Besides regular carbon peaks for unmodified glucose units of *β*-cyclodextrin at 101.74, 81.30, 72.98, 72.34, 71.99, and 59.78 ppm, three less intense peaks at 100.14 (C1′), 82.06 (C4′), and 80.31 (C2′) ppm of modified glucose units are observed (Supporting Information, spectrum 8). Downfield shift of C2′ and upfield shift of C1′ for the modified glucose unit from the corresponding carbons C2 and C1 of unmodified glucose units indicate that substitution is on the hydroxyl group at the 2-position of a *β*-cyclodextrin molecule.

The UV−vis spectra of representative members of these derivatives in aqueous and organic solvents did not exhibit significant differences as shown in spectrum 17 and Table 1 in the Supporting Information. This can be attributed to lack of intermolecular complex formation in these compounds at the concentrations employed in this investigation.

Conclusion

Subtle differences in the chemistry of the hydroxyl groups at the 2-, 3-, and 6-positions of cyclodextrins can be exploited to direct an electrophilic reagent to the desired site. We have thus synthesized derivatives of cyclodextrins in which the same substituent is inserted into each of these positions. A detailed NMR spectral investigation of these compounds will be the subject of a future report.

Experimental Section

General Method. Proton and carbon chemical shifts are reported in ppm (δ) using the residual signal of DMSO-d_6 (2.49 ppm for ^{1}H and 39.50 ppm for ^{13}C) or TMS (0.00 ppm) as the internal standard. TLC was performed on aluminum sheets precoated with 0.2 mm silica 60 F_{254}. The eluent for TLC of cyclodextrin derivatives, unless mentioned otherwise, is 1-butanol/ethanol (95%)/water, 5:4:3 by volume, and the spots were detected first by UV lamp if the product contained a chromophore and then by charring with heat after spraying the plate with 50% methanolic sulfuric acid. Charring is the indication for the presence of cyclodextrin moieties. Gel filtrations were performed on a Sephadex column (length, 125 cm; i.d., 2.8 cm) using deionized water as eluent. HPLC was performed on a Econosphere (C_{18}, 10 μm; length, 250 mm; i.d., 22 mm) column with acetonitrile in water as solvent. α- and *β*-Cyclodextrin were dried overnight in a drying pistol in refluxing 1-butanol under vacuum. DMF was dried over CaH_2 overnight and fractionally distilled before use. 2,6-Lutidine packaged in a sure/seal bottle was purchased from Aldrich and used without further purification. Compounds **14**,[28,32] **2**,[33] **10**,[28] and **17**[34] were synthesized according to literature procedures. All new samples were dried at room temperature under high vacuum for several days before being submitted to elemental analysis.

All of the UV spectra were taken at 25.0 ± 0.1 °C using stock solutions. Solutions were prepared by dissolving **8** (5.0 mg), **16** (5.0 mg),and 4-methylamino-3-nitrobenzyl alcohol (1.0 mg) in 50 mL of water, DMF, and DMSO. The final concentrations were 7.7×10^{-5}, 7.7×10^{-5}, and 1.0×10^{-4} M, respectively.

6-*O*-(4-Methylamino-3-nitrobenzyl)-α-cyclodextrin (3). 4-Methylamino-3-nitrobenzyl chloride (**2**, 0.21 g, 1.04 mmol) was added as a solid to a solution of α-cyclodextrin (**1**, 1.00 g, 1.03 mmol) in 2,6-lutidine (80 mL), and this solution was heated to 150 °C under argon atmosphere for 3.5 h. A major cyclodextrin-containing compound appeared at $R_f = 0.47$ on TLC. Crude product was concentrated to one-third in vacuo, precipitated out in acetone (250 mL), filtered, washed with acetone three times, and dried in a vacuum oven at 45 °C overnight to yield a yellowish powder (1.15 g).The above crude product (200 mg) was dissolved in 8 mL of water, loaded on the Sephadex (G-15) column, and eluted with deionized water at 15 mL/hour. The first orange band (retention volume 560 mL) was collected, concentrated, and dried under vacuum (0.3 mmHg, room temperature) to give compound **3** (70 mg as reddish powder, 34.4%). ^{1}H NMR (300 MHz, DMSO-d_6): (Supporting Information, spectrum 9) δ 8.21−8.11 (m, 1H), 7.99 (s, 1H), 7.52 (d, 1H), 6.98 (d, 1H), 5. 58−5.35, (m, 14H), 4.78 (s, 7H), 4.57−4.33 (m. 8H), 3.86−3.20 (m, 36 H), 2.95 (d, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): (Supporting Information, spectrum 10) δ145.31, 136.60, 130.11, 125.06, 124.98, 114.30, 102.21, 101.87, 82.31, 82.02, 73.31, 72.24, 72.03, 71.08, 70.45, 68.56, 59.91, 29.74. Anal. Calcd for $C_{44}H_{68}N_4O_{32}\cdot 1.5H_2O$: C, 45.40; H, 6.15; N, 2.41. Found: C, 45.37; H, 6.15; N, 2.52.

Reaction of 4-Methylamino-3-nitrobenzyl Chloride (2) with 2,6-Lutidine. A solution of 4-methylamino-3-nitrobenzyl chloride (**2**, 0.21 g, 1.04 mmol) in 2,6-lutidine (8 mL) was stirred and heated to 120 °C under argon atmosphere overnight to give a reddish precipitate that contained a compound appearing at $R_f = 0.05$ on TLC (ethyl acetate/hexane, 1:1). The precipitate was filtered under vacuum and easily dessolved in water (2 mL), which gave a white precipitate under room temperature instantly with the solution of silver nitrate in ethanol.

Reaction of *β*-Cyclodextrin (7) with 4-Methylamino-3-nitrobenzyl Chloride (2) in Pyridine. A solution of 4-methylamino-3-nitrobenzyl chloride (**2**, 0.177 g, 0.881 mmol) and *β*-cyclodextrin (**7**, 1.00 g, 0.881 mmol) in pyridine (80 mL) was heated to 150 °C under argon atmosphere overnight. TLC of the reaction mixture indicated that the spot of **2** at $R_f >$ 0.95 disappeared, and a red spot at $R_f = 0.05$ appeared. Longer reaction time (5 h) did give a compound that is not UV-visible and chars by heat after the treatment of 50% methanolic H_2SO_4.

Reaction of *β*-Cyclodextrin (7) with 4-Methylamino-3-nitrobenzyl Chloride (2) in 2,6-Lutidine. *β*-Cyclodextrin (**7**, 1.00 g, 0.881 mmol) was added as a solid to a solution of 4-methylamino-3-nitrobenzyl chloride (**2**, 0.177 g, 0.881 mmol) in 2,6-lutidine (80 mL), and the above suspended solution was heated to 150 °C under argon atmosphere overnight. TLC of the reaction solution gave four orange spots at 0.72, 0.64, 0.49, and 0.05; the first three of these charred with heat after the treatment with sulfuric acid, and the last one was not. TLC of the precipitate at the bottom of the reaction flask gave a

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spot that is identical to that of β-cyclodextrin. The reaction solution was cooled to room temperature, filtered, concentrated to one-quarter, and poured into acetone (150 mL) to give an orange precipitate that was filtered, washed with acetone × 3, and dried under vacuum in an oven at 45 °C for 5 h. ^{1}H NMR spectrum (300 MHz, DMSO-d_6) for the crude compound was complicated, and the integration ratio of the signals in the aromatic region to that of anomeric protons was 1:1.

Reaction of β-Cyclodextrin (7) with 4-Methylamino-3-nitrobenzyl Chloride (2) in DMF and 2,6-Lutidine. A solution of 4-methylamino-3-nitrobenzyl chloride (**2**, 0.177 g, 0.881 mmol) and β-cyclodextrin (**7**, 1.00 g, 0.881 mmol) in mixed solvent of DMF (20 mL) and pyridine (20 mL) was heated to 150 °C under argon atmosphere overnight. TLC of the reaction mixture showed three spots at 0.64, 0.55, and 0.49, which were UV-visible and charred with heat after treatment with 50% sulfuric acid, besides cyclodextrin. The reaction mixture was concentrated to one-third by rotary evaporation and poured into acetone (150 mL), and the precipitate was filtered and dried in a vacuum oven at 45 °C to give 1.17 g of an orange powder. A 150 mg portion of the solid was dissolved in 8 mL of distilled water, loaded in a Sephadex column, and eluted with deionized water at 30 mL/h, and the eluent between 560 and 610 mL was collected and concentrated to dryness. TLC of this eluent showed two spots at 0.55 and 0.49.

6-*O*-(4-Methylamino-3-nitrobenzyl)-β-cyclodextrin (8). β-Cyclodextrin (**7**, 5.0 g, 4.41 mmol) was stirred with 7.3 equiv of NaH (1.29 g of 60% dispersion in oil, 31.5 mmol) in dry DMF (250 mL) under argon atmosphere until a gel was formed (48 h). *tert*-Butyldimethylsilyl chloride (4.99 g, 33.1 mmol) in dry DMF (50 mL) was added to the above gel dropwise for 4 h, during which time the temperature was raised from 25 to 90 °C gradually for the initial 2 h and was kept at 90 °C until the reaction was completed. The reaction mixture was cooled to room temperature and approximately two-thirds of the DMF was removed by rotary evaporator. The residue was poured into 500 mL of ice water, and the precipitate was filtered, washed with water, and dried in a vacuum oven at 90 °C overnight to give **12** (6.7 g, 79%).[35] TLC of the crude product, eluted in a mixture of solvents containing AcOEt, EtOH, and H_2O (7:1:1 by volume), gave six peaks at 0.55, 0.50, 0.33, 0.17, 0.07, and 0.00 after the treatment with heat/H_2SO_4. ^{1}H NMR (300 MHz, DMSO-d_6): (Supporting Information, spectrum 5) δ 6.20−5.50 (b, 5H), 4.82−4.72 (s, 7H), 4.60−4.10 (b, 10H), 4.80−3.20 (m, 42H), 1.00−0.68 (2s, 45H), and 0.20−0.03 (d, 18H). ^{13}C NMR (300 MHz, DMSO-d_6): (Supporting Information, spectrum 4) δ 101−102 (b), 83−81(b), 75−74 (b), 73.5−71.2 (b), 60.2−59.0 (b), 26.1 (s), 25.8 (s), 18.8 (s), and −0.42 (d).

Compound **12** (1 g, 0.52 mmol) was added to a solution of **2** in lutidine and refluxed for 8 h under a dry argon atmosphere, cooled to room temperature. An excess amount of tetrabutylammonium fluoride (4 mL, 1 N) was added to the above reaction mixture, which was allowed to react at 80 °C overnight, cooled to 0 °C, and maintained at that temperature for 1 h when the product precipitated out. The product was filtered, and its TLC showed two spots at R_f = 0.61 and 0.49 that were UV-visible. After treatment of the TLC plate with H_2SO_4/heat, these two spots charred, and a new spot appeared at R_f = 0.32 that was consistent with β-cyclodextrin. The crude product was dissolved in 200 mL of deionized water and filtered through a cation exchanger (Amberlite CG-120, sodium form, Sigma company) column (length 25 cm, i.d. 2.8 cm), and the filtrate was concentrated to 32 mL. One-quarter of this solution (8 mL) was loaded onto a Sephadex (G25−100) column and eluted with water at 35 mL/h, and the major orange band that elutes between 580 and 660 mL was collected and concentrated to dryness to yield **8** (R_f = 0.49, 50.3 mg, 23.8% from cyclodextrin). ^{1}H NMR (300 MHz, DMSO-d_6): (Supporting Information, spectrum 11) δ 8.14 (m, 1H), 7.99 (d, 1H), 7.51 (dd, 1H), 6,98 (d, 1H), 5.82−5.58 (m, 14H), 4.80 (d, 7H), 4.55−4.30 (m, 8H), 3.80−3.17 (m, 42H), 2.95 (d, 3H). ^{13}C NMR (75 MHz, DMSO-d_6): (Supporting Information, spectrum 6) δ 145.34, 136.61, 130.15, 125.16, 124.96 114.29, 102.35, 101.85, 81.88, 81.44, 73.00, 72.25, 71.99, 71.08, 70.37, 68.78, 59.83, 29.76. Anal. Calcd for $C_{50}H_{78}N_2O_{37}\cdot 6H_2O$: C, 42.68; H, 6.45; N, 1.99. Found: C, 42.72; H, 6.50; N, 1.87.

Heptakis(2,3-di-*O*-methyl)-6-*O*-(4-methylamino-3-nitrobenzyl)-β-cyclodextrin (11). The solution of heptakis(2,3-di-*O*-methyl)-β-cyclodextrin (**10**,[28] 750 mg, 0.563 mmol) and compound **2**[36] (110 mg, 0.546 mmol) in 2,6-lutidine (30 mL) was refluxed overnight under argon atmosphere. TLC of this reaction mixture showed a new spot on TLC (in AcOEt/CH_3-OH, 1:1 by volume) at R_f = 0.5, which was UV-visible and charred with heat after the treatment of H_2SO_4. The reaction mixture was evaporated under vacuum to dryness to give a yellowish solid (880 mg). The crude product (200 mg) was dissolved in deionized water (9 mL), loaded on a Sephadex (G25−100) column, and eluted with deionized water at 20 mL/h. The first orange band in the Sephadex column (retention volume was 540 mL) was collected, concentrated, loaded on the HPLC column, eluted with 30% acetonitrile in water, and detected with an UV detector at 254 nm. The peak with 21 min retention time was collected, combined, and further purified with HPLC under similar conditions to give **11** (64.91 mg, 35%). ^{1}H NMR (300 MHz, DMSO-d_6): (Supporting Information, spectrum 12) δ 8.17 (q, 1H), 8.00 (d, 1H), 7.50 (dd, 1H), 6.98 (d, 1H), 5.14−5.02 (7H), 4.57−4.43 (m, 6H), 4.43−4.31 (dd, 2H), 34.00−2.97 (m, 84), 2.94 (d, 3H). ^{13}C NMR (DMSO-d_6): (Supporting Information, spectrum 2) δ 145.28, 136.38, 130.14, 125.03, 124.82, 114.24, 97.66, 97.27, 81.69, 81.62, 81.31, 81.19, 81.12, 79.69, 78.93, 78.79, 78.71, 78.66, 78.63, 78.58, 78.47, 71.81, 70.92, 70.45, 68.64, 60.77, 60.58, 60.55, 60.03, 60.00, 57.90, 59.88, 57.77, 57.51, 57.48, 29.72. Anal. Calcd for $C_{64}H_{106}N_2O_{37}\cdot H_2O$: C, 50.79; H, 7.19; N, 1.85. Found: C, 51.03; H, 7.18; N, 1.91.

3-*O*-(4-Methylamino-3-nitrobenzyl)-β-cyclodextrin (16). A lutidine solution (25 mL) of **2** (80.0 mg, 0.40 mmol) was refluxed under argon atmosphere for 4 h when a spot at R_f = 0.80 diminished and new spot at R_f = 0.05 appeared on TLC (in hexane and ethyl acetate (1:1 v/v). The above solution was cooled to 80 °C, compound **14** (1.0 g, 0.52 mmol) was added to it in one portion, and the above solution was refluxed for 4 h when the spot at R_f = 0.05 on TLC disappeared and gave a new spot at R_f = 0.45 (ethyl acetate/ethanol/water, 50:1:1). After the reaction mixture was cooled below 80 °C, an excess amount of TBAF (2 mL, 1.0 N in THF) was added, and the mixture was stirred at 80 °C for 3 h. most of the solvent was removed by rotary evaporation, and the residue was dissolved into 100 mL of water. The aqueous solution was washed with ethyl acetate twice, concentrated to dryness, and further dried under vacuum (0.2 mmHg) for overnight to afford a yellowish solid (730 mg).

The crude product (200 mg) was dissolved in water (7 mL), loaded on a Sephadex (G25−100) column, and eluted with deionized water at the rate of 10 mL/h. Two orange bands were observed in the column. The first, which eluted between 500 and 520 mL, contained cyclodextrin as the major component (R_f = 0.33), a cyclodextrin-containing compound (UV-visible) as minor product (R_f = 0.55), and a cyclodextrin-free compound (R_f = 0.8, UV-visible). The second yellow band, which was collected between 600 and 720 mL, consisted of a major product at R_f = 0.45 and a minor one at R_f = 0.55. The second band was concentrated to dryness and further dried under vacuum (0.2 mmHg) for overnight to afford 40 mg. The sample was further purified on HPLC with 17% aqueous acetonitrile as the solvent, and the peak with retention times between 9 and 10 min was collected, combined, concentrated, and loaded on HPLC for further purification under similar conditions to yield **16** (28.5 mg, 20.0%). ^{1}H NMR (500 MHz, D_2O): (Supporting Information, spectrum 13) δ 8.32 (d, 1H, J = 1.8 Hz), 7.85 (dd, 1H, J = 1.8, 8.8 Hz), 7.21 (d, 1H, J = 8.8 Hz), 5.30−4.92 (m, 9H), 4.12−3.55 (m, 42 H), 3.23 (s, 3H). DEPT (125 MHz, DMSO-d_6): (Supporting Information, spectrum 7) δ 132.37,

(35) Calculated assuming all seven hydroxyl groups are substituted.

(36) Rong, D.; D'Souza, V. T. *Tetrahedron Lett.* **1990**, *31*, 4275−4278.

130.85, 124.46, 101.64, 101.57, 100.89, 81.37, 81.32, 81.26, 81.17, 80.93, 80.72, 77.98, 73.05, 73.01, 72.89, 72.80, 72.75, 72.48, 72.29, 72.19, 72.10, 72.03, 71.96, 71.78, 71.75, 59.65, 59.55, 29.96. ^{13}C NMR (125 MHz, D_2O): δ 149.05, 141.59, 132.37, 130.85, 124.46, 116.68, 104.93, 104.45, 104.31, 104.25, 103.85, 84.00, 83.87, 83.81, 83.55, 83.44, 83.37, 78.33, 76.91, 76.23, 75.83, 75.70, 75.39, 75.35, 75.29, 74.62, 74.54, 74.38, 74.30, 74.20, 74.08, 73.99, 62.11, 61.98, 31.50. Anal. Calcd for $C_{50}H_{78}N_2O_{37}\cdot 3H_2O$: C, 44.38; H, 6.26; N, 2.07. Found: C, 44.43; H, 6.18; N, 2.08.

2-*O*-[2-(4-Methylamino-3-nitrophenyl)-2-oxy-ethyl]-β-cyclodextrin (19). 4-Methylamino-3-nitroacetophenone (**17**, 0.80 g, 4.14 mmol) was dissolved in a mixture of solvents containing freshly distilled ethyl acetate (70 mL) and chloroform (70 mL) and refluxed with cupric bromide ($CuBr_2$, 1.39 g, 6.21 mmol) for 1.5 h. A new spot appeared at $R_f = 0.45$ on TLC (in CH_2Cl_2) besides starting material at $R_f = 0.33$. After the solution was cooled to room temperature, the solid was removed by filtration, and the filtrate was concentrated to dryness by rotary evaporation and purified on chromatotron (silica gel) with pure methylene chloride as an eluent to yield 1′-bromo-4-methylamino-3-nitroacetophenone ($R_f = 0.45$, 0.520 g, 46.0%). ^{1}H NMR (300 MHz, $CDCl_3$): (Supporting Information, spectrum 14) δ 8.82 (1H, d, $J = 2.0$), 8.48 (1H), 8.09 (1H, dd, $J = 2.0$, 9.0), 6.90 (1H, d, $J = 9.0$), 4.36 (2H, s), 3.09 (3H, d, $J = 4.2$). MS (ES, low resolution): calcd for $C_9H_9Br_1N_4O_3$ 273, found 273.

β-Cyclodextrin (**2**) (0.50 g, 0.44 mmol) was stirred with NaH (21.1 mg, 0.530 mmol, 60% in oil) in DMF (30 mL) under dry argon overnight. The above solution was transferred by cannula into a solution of 1′-bromo-4-methylamino-3-nitroacetophenone (163.8 mg, 0.600 mmol) in dry DMF, and the mixture was further stirred under dry argon until it became neutral (3 h). Most of the solvent was then removed under vacuum, and the residue was poured into the solution of acetone (100 mL) to precipitate the crude product. The precipitate was filtered, washed thoroughly with acetone, and dried under vacuum (0.2 mmHg) for 18 h to afford 640 mg of orange powder. The solid (180 mg) was dissolved in water (6 mL) and eluted through Sephadex (G25−100) with deionized water at 15 mL/h. The first yellow band (80 mL from 560 to 640 mL retention volume) was collected, concentrated by rotaty evaporation, and dried under vacuum (0.2 mmHg) for 10 h to afford 30 mg (26.3%) of **19**. ^{1}H NMR (300 MHz, DMSO-d_6): (Supporting Information, spectrum 15) δ 8.79 (b, 1H), 8.62 (s, 1H), 8.04 (d, 1H, $J = 8.0$ Hz), 7.12 (d, 1H, $J = 9.0$ Hz), 6.02−5.59 (b, 14H), 5.34−5.09 (m, 2H), 4.88 (d, 6H, $J = 2.7$ Hz), 4.72 (s, 1H), 4.67−4.31 (b, 7H), 3.99−3.23 (m, 76H, some from water), 3.06 (d, 3H, $J = 4.8$ Hz). ^{13}C NMR (75 MHz, DMSO-d_6): (Supporting Information, spectrum 16) δ 192.46, 147.61, 133.66, 130.00, 126.68, 120.70, 114.05, 101.74, 100.14, 82.06, 81.30, 80.31, 73.65, 72.98, 72.34, 71.99, 59.78, 29.70. The sample was further purified by HPLC with 9% aqueous acetonitrile, and the band with 15 min retention time was collected, combined, concentrated to dryness, and further dried under vacuum to yield 18.8 mg (16.5%). Anal. Calcd for $C_{51}H_{78}N_2O_{38}\cdot 6H_2O$: C, 43.78; H, 6.20; N, 2.00. Found: C, 43.76; H, 5.90; N, 1.97.

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Supporting Information Available: ^{1}H and ^{13}C NMR and UV sprectra discussed in the paper. This material is available free of charge via the Internet at http://pubs.acs.org.

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