Facile Synthesis of a Diverse Library of Mono-3-substituted β-Cyclodextrin Analogues

K. Kellett, B. M. Duggan, M.K. Gilson*

Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California San Diego, 9500 Gilman Drive, La Jolla, California 92093-0736, USA

Abstract

Substituted cyclodextrins (CDs) have many applications, but synthetic challenges have limited the derivatives that can routinely be accessed. In particular, although there is considerable interest in selective derivatization at the 2- and 3- hydroxyls on the secondary face, since bulky guest molecules are most likely to project through this larger aperture, syntheses of such derivatives have required arduous procedures afforded with poor yields. We address this challenge via synthetic strategies that allow facile creation of diverse libraries of water soluble mono-3-substituted-β-cyclodextrins. The power of these strategies is confirmed through synthesis of twenty water-soluble cyclodextrin analogues with amide, thioureas or urea linkers, using one-pot reactions producing ≥ 55% yields and purifications that do not require chromatography. This work opens new possibilities for the design of selective host molecules for use in supramolecular chemistry, chemical separations, pharmaceutical formulations, and calibration of molecular simulations.

Introduction

Cyclodextrins (CD) are cyclic oligosaccharides, comprising six (α-CD), seven (β-CD) or eight (γ-CD) glucose monomers in the shape of a hollow, truncated cone (Figure 1). Their C6 primary hydroxyls rim the narrower face of the cone, while their C2 and C3 secondary hydroxyls are located on the wider face (Figure 1). The hydroxyls make the exterior of cyclodextrin hydrophilic and contribute to its aqueous solubility, while the internal cavity remains hydrophobic, allowing for noncovalent association with lipophilic and amphiphilic molecules. The cyclodextrins have found practical applications, such as solubilization of small, lipophilic, drug molecules[1,2], and encapsulation of volatile compounds[3,4]. Cyclodextrins also have been adopted as test cases for computational methods of predicting noncovalent binding affinities. In this context, they represent simple models for the association of proteins with drug molecules[5–7], and are being used to test and enhance the accuracy of the potential functions used in molecular simulations[8,9]. Although many experimental cyclodextrin-guest affinity data are available[7,10–12], most are for unmodified cyclodextrins and hence probe only the interactions of guest molecules with the glucose monomers of the native host. Expanding the diversity of chemical interactions is of great interest in the computational chemistry community, because it would open new possibilities for using host-guest binding data to test and improve potential functions Applications in other fields, such as chemical separations and pharmaceutical formulations, are also likely.
Cyclodextrins can be diversified through the addition of functional groups at either the primary or secondary hydroxyls, leading to changes in size, shape and physical properties. For example, although native β-CD is not particularly water-soluble (18 mg/mL), randomly-methylated-β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin have aqueous solubilities of over 500 mg/mL\textsuperscript{13}. Derivatization can also modulate the binding affinities of these hosts for guest molecules that bind in the hydrophobic cavity of CD\textsuperscript{14–17}. When modifying affinity and specificity is the goal, it is preferable to derivatize the secondary hydroxyl groups of the host, rather than the primary ones, as derivatizing the secondary hydroxyls typically has a greater effect on binding affinity\textsuperscript{18,19}. This makes intuitive sense, because bound guest molecules are more likely to protrude through the wider secondary opening, where they can interact with the added substituents, than through the narrower primary opening\textsuperscript{20}. It is also of interest to create mono-substituted derivatives, rather than adding multiple substituents, to avoid crowding of this entryway and/or a combinatorial explosion of variants in the reaction products\textsuperscript{21,22}.

Despite important advances\textsuperscript{23–29}, the selective addition of substituents at the secondary face has long posed challenges in synthesis and purification\textsuperscript{25}. Many modification strategies lead to a complex mixture of products, such as randomly-methylated-β-CD. In addition, modified CDs may aggregate or become insoluble, reducing their effectiveness as host molecules\textsuperscript{30–32}. Furthermore, selective modification of the secondary face of β-CD has involved arduous, low-yielding, reaction procedures, and existing approaches typically require access to purification equipment, such as lyophilizers and centrifuges\textsuperscript{31,33–35}, not available to all groups interested in synthesizing such derivatives. Mechansynthetic methods, which may overcome tedious work ups and time consuming purifications, have still afforded only modest reaction yields\textsuperscript{35}. Thus, the literature has focused mainly on substituting the primary face of β-CD, which is easier to functionalize due to the greater nucleophilicity and reduced steric hindrance of its hydroxyls. However, the resulting products are, arguably, less interesting from the standpoint of generating diversity in binding affinity and specificity.

Here, we address the challenge of selectively modifying the secondary face of cyclodextrins by defining principles for the facile synthesis of diverse mono-3-substituted cyclodextrins and illustrating their use in high-yielding, one-pot syntheses which do not require complicated procedures or equipment.

**Results and Discussion**
Reactions designed for primary face modification are typically unsuitable for the less reactive secondary hydroxyls, so our synthetic strategies are optimized for the secondary face. We take mono-3-amino-β-cyclodextrin (3-NH₂-β-CD) as a starting point. Its synthesis is well documented[26,33], its amino group is more reactive than the hydroxyls, and chemo-selective reactions directed towards the amine eliminate the large number of products often produced upon direct functionalization of the hydroxyls. Nonetheless, adoption of this starting material does not resolve critical challenges of reactivity and purification. The synthetic strategies described here thus are based on several key observations and approaches, as follows.

- Bulky coupling agents and reagents, which may work at the primary face, can be ineffective at the sterically congested secondary face. Obtaining high yields requires avoiding steric conflicts by using linear nucleophiles and less hindered coupling agents. Bulky groups can be attached by first adding a short linker to the secondary face and then attaching the bulky group to the linker.

- Having observed poor results for seemingly straightforward linking reactions, we conjectured that some reactants might bind in the CD cavity and prevent reactions from progressing on the rim. This concept motivated the addition of adamantane as an inert guest known to bind β-CD with good affinity, in order to displace the reactant from the cavity; and, indeed, this measure often enabled recalcitrant reactions to proceed smoothly.

- Published procedures for isolating CD derivatives are typically complex, requiring chromatography, lyophilization, and/or centrifugation[35–39]. We wished to use the simpler purification approach of precipitation from DMF with acetone, but our initial attempts failed. Although visible precipitate formed on addition of acetone to the reaction vessel, this passed through the finest grades of filter paper. Experiments varying the precipitation conditions revealed that evaporative removal of DMF to a minimal volume without occurrence of precipitation, followed immediately by precipitation with cold acetone, vacuum filtration, and washing with copious acetone, consistently yielded the pure product without any need for chromatography. This worked for all compounds synthesized in this paper.

The following subsections detail reaction protocols, based on these principles and methods, for facile synthesis of diverse mono-3-substituted CD analogues (Figure 2). The derivatives chosen all represent functional groups that we anticipate will lead to interesting binding properties, through interactions of guest molecules with aromatic, ionic, or hydrogen bonding functionalities.
Figure 2. Library of water-soluble, mono-3-substituted, \(\beta\)-cyclodextrin analogues synthesised here, with final yields as noted.
Amic acids

Amic acid β-CD derivatives are excellent candidates to vary the character of host-guest complexation, because they add both hydrogen bond donor and acceptor groups to the native host. They can be easily synthesized through nucleophilic ring opening of cyclic anhydrides, and the commercial availability of these reagents allows a variety of CD analogues to be synthesized under uniform reaction conditions. Amic acid derivatives 1-5 were synthesized at yields of > 80% (Figure 2) through a one pot strategy that required only stirring at room temperature. This synthesis is based on a primary face derivatization of Onagi et al.[40], but we replaced their purification procedure, which requires lyophilization and centrifugation, with the simple acetone precipitation procedure mentioned above. While problems attributed to steric obstruction have been reported for functionalization of the secondary face with bulky groups[25], the high yields produced in this reaction indicate that steric congestion is not a significant obstacle to the formation of the amic acid derivatives, and led us to the concept that linear nucleophiles are preferred in the context of secondary face modification.

Amino Acids and Other Amide-Linked Substituents

Biomimetic design is a known approach to developing host molecules for biomedical purposes[25,41]. In one method, a host molecule, serving as a scaffold, is decorated with peptide chains[41,42]. The diverse range of amino acids and their strong binding capabilities make them ideal functionalities for modifying CDs in interesting and potentially biologically relevant ways. Although syntheses of β-CDs substituted with amino acids have been reported, they have focused on primary face substitution or non-selective secondary face substitution[42,43]. Our initial attempts at selective secondary face coupling, with coupling agents HATU, DCC and HBTU, were unsuccessful. However, tetrachloro-N-hydroxyphthalimide tetramethyluronium hexa-fluorophosphate (CITU), a recently developed coupling agent for amide synthesis[44], in combination with N-methylmorpholine, allowed synthesis of the glycine adduct, 6, in 75% yields. Again, the reaction required only stirring at room temperature, partial drying, and acetone precipitation, followed by a simple Boc-deprotection step and purification by precipitation. Coupling of bulkier amino acids directly to 3-NH₂-β-CD remained unsuccessful, though, presumably because of steric hindrance effects, noted above. However, 6 is a good starting point for extension of the peptide chain, as the steric clashes of the polar secondary face are no longer a contributing factor. Accordingly, we have successfully linked Cys and His to the free amino group of 6 (data not shown), leading to β-CD derivatives with Gly-Cys and Gly-His dipeptide substituents.

Scheme 1. Synthesis of mono-3-{(carboxypropionamido-glutathione)-β-cyclodextrin (11), (i) CITU, n-methylmorpholine, DMF, 12h.
The amic acid derivatives described above also represent excellent starting materials for addition of bulky amino acids and peptides to the secondary face. Following the same reaction procedures used for 6, we coupled methionine, lysine, cysteine and β-phenylalanine to 1, producing 7, 8, 9 and 10 at yields ≥ 65%, giving an overall reaction yield of ≥55% from 3-NH₂-β-CD. To further validate this approach, we coupled the tripeptide glutathione to 1, yielding 11 at 58 % yields (Scheme 1). Again, isolation and purification of 11 was achieved without the need for chromatography. Interestingly, NOESY NMR suggests that the pendant arm does not self-associate inside the CD cavity [Figure S34].

We furthermore conjectured that amino acids with bulky side chains could be coupled to the secondary rim if we used β-amino acids, as these would space the bulky side chain an additional methylene group further from the secondary rim. We tested this for β-tyrosine, reacting β-Boc-tyrosine and 3-NH₂-β-CD under the same conditions as to use make 6. This afforded 12 in 65 % yields, after Boc-deprotection. In contrast, the same reaction with the corresponding α-amino acid, tyrosine, proved unsuccessful, presumably because the bulky phenol group sterically hinders reaction with 3-NH₂-β-CD. This result further supports the principle that non-hindered linear nucleophiles are necessary for successful addition directly onto the secondary face. The β-amino acids are appealing substituents because they allow varied side-chains to be positioned particularly close to bound guests.

Carboxylic acids were found to be easily coupled onto NH₂-β-CD under the same conditions as the amino acids. Compounds 13-16 were successfully synthesized in ≥68 % yields. This allows for cyclodextrin to be modified with an even larger variety of functionalities, which are useful both as precursors for further synthesis and to achieve enhanced diversity of the binding data for use in force field optimization. The propargyl (Compound 13) and azide (Compound 14) groups are accessible precursors to further expand the diversity of cyclodextrin analogues through click chemistry reactions on the secondary face of β-CD. The robustness of the method for coupling bulky substituents was confirmed by coupling hemin to NH₂-β-CD via the carboxylate functionality to produce a dark red solid in 71 % yield. Successful synthesis of Compound 15 was confirmed using ESI-MS. However, due to the iron complexed in heme, it is not possible to characterize by NMR; therefore Compound 16 was synthesized using protoporphyrin IX. The synthesis of β-CD conjugated to protoporphyrin IX has been previously published. However, it is on the primary face and involves an arduous synthetic procedure, with unreported final yields[45]. Using the method reported here, yields of 68 % were achieved with a one pot strategy, without any need for chromatographic purification.

**Ureas and Thioureas**

Ureas and thioureas are frequently employed in the construction of supramolecular host systems, to which they can impart strong hydrogen bonding capabilities[46,47]. Syntheses based on urea and thiourea linkages provide access to further chemical diversity, because isothiocyanates and isocyanates are available with a variety of substituents. Here, the nucleophilic addition of such reactants affords a range of urea and thiourea derivatized β-CD analogues, exemplified by 17-20 (Figure 1). These syntheses also offer insight into the determinants of reactivity, as follows.

Initial syntheses of 17 were run at molar ratios of 1:1, 2:1 and 3:1 of isothiocyanate starting material to 3-NH₂-β-CD, keeping all other reaction conditions constant. The reaction proceeded successfully only when the isothiocyanate reactant was present at 3:1 excess and gave yields <35%. We hypothesized that the isothiocyanate was acting as a guest molecule, binding inside the β-CD cavity and hindering progress of
the reaction. To explore this hypothesis, we added a competing guest molecule, adamantane, to block the β-CD cavity. Adamantane is an inert hydrocarbon, known to have a strong affinity for β-CD[12]. This approach was successful, as a reaction mixture 1:1:1 of adamantane, 3-NH₂-β-CD and isothiocyanate yielded 17 at 83% yield. The adamantane was easily removed during the acetone wash purification stage of the workup. Thioureas 18-19 and an analogous urea Compound, 20, were also successfully synthesized at high yield (Figure 2) by this procedure.

**Lack of Aggregation of the Derivatives**

The propensity for aggregation of some modified CDs[48,49] can be problematic. However, for all the compounds reported here, we saw none of the peak-broadening of the ¹H-NMR spectrum that is typically associated with aggregation. The possibility of aggregation can be further examined with diffusion ordered spectroscopy (DOSY) NMR, a technique used to separate NMR signals based on molecular diffusion coefficients. In particular, a molecule that aggregates will yield a lower diffusion coefficient than one of similar molecular weight that remains monomeric. We carried out DOSY studies of β-CD analogues from each series created here, amic acid, amino acid, urea, and thiourea, in the presence of native β-CD as a control and compared these spectra with that of a covalent β-CD dimer (Compound 21, Figure S65), which is larger than any of the derivatives. As shown in Figure 3, the diffusion coefficients of all derivatives studied in this manner fall between those of native β-CD and the covalent dimer, which is as expected if the derivatives remain monomeric in solution.

![Figure 3. A. Diffusion coefficients from DOSY NMR, of a selection of synthesised analogues shown in comparison with unmodified β-CD and a β-CD dimer as controls.](image)

**Conclusions**
We have described simple, high-yield, protocols, requiring only commonly accessible equipment, to synthesize a wide range of β-CD derivatives mono-substituted at the secondary face. Steric hindrance, a key obstacle to functionalizing the secondary face of β-CD, was circumvented by the use of linear nucleophiles and less bulky coupling agents; by using a skinny linker, such as glycine and amic acids, to position bulkier reactants away from the bulk of the host molecule; and by using β-amino acids, instead of α-amino acids, to position bulky side chains slightly further from the secondary rim. Some reactions were also enhanced by inclusion of adamantane, an inert component of the reaction mixture that presumably helps by displacing reactants from the binding cavity. The synthetic approaches described here open new possibilities for the use of modified β-CDs in applications that include testing and improvement of simulation force fields, enhanced pharmaceutical formulations, and novel supramolecular constructs and devices.

Acknowledgements

We thank Profs. Dionicio Siegel and Thomas Hermann for helpful discussions. NMR spectra were collected at the UCSD Skaggs School of Pharmacy and Pharmaceutical Sciences NMR Facility and mass spectrometry was run at the UCSD Molecular Mass Spectrometry Facility. We thank the lab of Prof. Phil Baran (The Scripps Research Institute) for their generous supply of CITU. MKG has an equity interest in and is a cofounder and scientific advisor of VeraChem LLC.

References


