Pergamon

Bioorganic & Medicinal Chemistry 10 (2002) 3291–3299

BIOORGANIC & MEDICINAL CHEMISTRY

Cocaine Detoxification by Combinatorially Substituted β-Cyclodextrin Libraries

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Received 29 January 2002; accepted 17 April 2002

Abstract—Per-6-substituted- and A,C,E(F)-tri-6-substituted-6-deoxy-β-cyclodextrin (β-CD) libraries were generated using solution-phase combinatorial chemistry techniques starting from the corresponding iodo precursors and different combinations of individual amine nucleophiles. Using a high throughput electrospray mass spectrometry (ESMS) screen to monitor the hydrolysis of cocaine, certain libraries showed the ability to specifically hydrolyze the methyl ester of cocaine, with the most active per-6-substituted β-CD library **I** producing complete hydrolysis in 24 h. The cocaine hydrolytic activity in this series showed structure–activity relationships which appeared to involve specific interaction between the amine side chains and the cocaine molecule. Comparison of the composition of the most active per-6-substiuted β-CD libraries and A,C,E(F)-tri-6-substituted-6-deoxy-β-CD libraries (**I** and **XV**) showed three common side chains (**3**, **4**, and **5**), suggesting that active side chains in the tri-substituted β-CD library might be predicted from evaluation of the more easily prepared per-6-substituted-6-deoxy-β-CD series.

Introduction

Cocaine addiction causes serious medical and social consequences in the United States. Despite intensive efforts for an effective medication, no satisfactory treatment is yet available,[1–3] probably because of the lack of definitive knowledge of the neurochemical basis and specific sites involved in the production and maintenance of cocaine addiction. For instance, a competitive antagonist of cocaine without simultaneously blocking dopamine uptake does not currently exist.[4] An alternate strategy would be to bind and tie up the cocaine by direct complexation in the blood stream such as successfully demonstrated with anti-cocaine antibodies.[5–7] Although such an agent would be depleted stoichiometrically, a potential problem, given the amount of cocaine present under overdose conditions,[8] immunopharmcotherapy for cocaine addiction has been shown to be a viable treatment strategy for cocaine abuse.[9]

Landry and co-workers appear to have been the first to recognize that catalytic antibodies capable of hydrolyzing the benzoic acid ester of cocaine might represent an alternate approach to blunting the effects of cocaine, by intercepting and destroying the molecule before it entered the brain.[10] It should be emphasized that with this approach, a single molecule of catalytic antibody could destroy many cocaine molecules. Landry and co-workers have reported a catalytic antibody with sufficient activity ($k_{cat} = 2.3\ min^{-1}$, $K_m = 220\ \mu M$) for preclinical trials in animal models of cocaine addicition.[11] However, these values of catalytic activity still remain low, and the search for higher activity continues. In addition, the large size (150 kDa) of the antibody protein relative to most small molecules (the M_r of the β-CDs discussed herein are about 100 times less) also requires that a correspondingly large mass of the antibody protein be administered when used as a drug. In addition, the catalytic antibodies which have been reported to date have focused on hydrolysis of the benzoic acid ester of cocaine, even though binding studies suggest that a 50-fold greater reduction in cocaine's activity could be obtained if the methyl ester of cocaine were to be hydrolyzed.[12]

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PII: S0968-0896(02)00205-5

Antibodies are prepared by a combinatorial selection (biologically) of certain variable regions which are folded in such a fashion as to form a pocket where antigen binding occurs,[13] and which show catalytic activity when selected by appropriate transition-state analogues. In the present work, we attempt to mimic the antibody complementary determining region by modifying the primary face of β-CD with structurally variegated side chains to form a pocket for binding the transition state for cocaine hydrolysis.

β-CD, a cyclic α-1,4-linked D-(+)-glucopyranose oligomer containing seven sugars, possesses a central cavity that provides a good binding site for appropriate hydrophobic substrates (Fig. 1). In addition, the modification of hydroxyl groups on the primary (or secondary) face might lead to a functionalized pocket having enzyme-like activity if the substitution at **A**–**G** provides complementary binding to the transition state of a particular reaction, and substituted β-CDs have had a long history as a starting point for enzyme mimics, largely through the work of Breslow and Tabushi (see for example reviews in refs 14–17). The traditional search for substituted β-CD artificial enzymes in the past has involved rational design based on structural information of enzymes' active sites and/or the chemistry involved with the substrates' reactions. Previous work in our group led to a first report of combinatorially prepared per-6-substituted-6-deoxy-β-CD libraries that showed phosphatase-like activity in the presence of Zn^{2+}.[18] We report here an extension of this approach in the search for substituted β-CDs possessing cocaine esterase-like activity. In this study, two combinatorial libraries, per-6-substituted- and A,C,E(F)-tri-6-substituted-6-deoxy-β-CD libraries (**I**–**XIV** and **XV**–**XX**), were prepared by solution-phase combinatorial chemistry techniques and evaluated for their ability to hydrolyze cocaine using an ESMS screen.

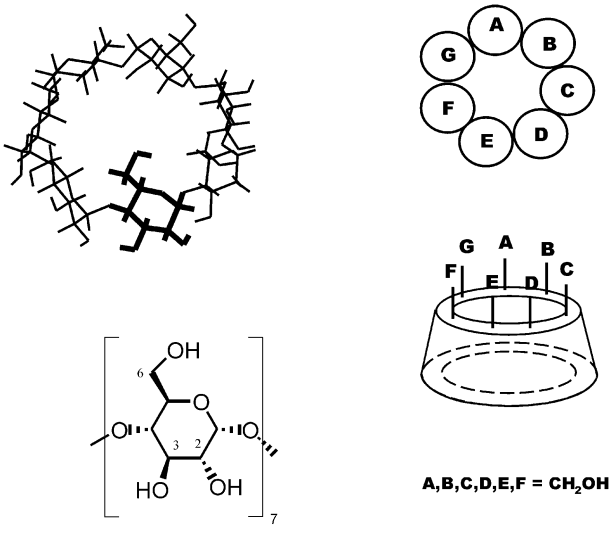


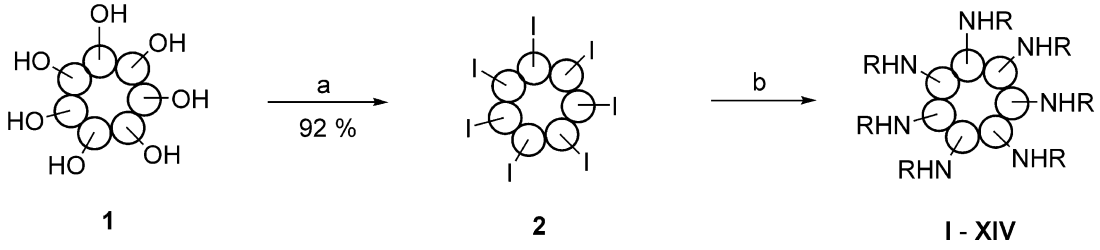
Figure 1. β-CD structure and nomenclature.

Results and Discussion

The β-CD scaffold provides a unique opportunity to apply solution-phase chemistry and still separate the desired library products from the reagents used in the synthesis. This is because the highly polar β-CD derivatives do not have good solubility in organic solvents such as ethyl acetate, acetone, and ether. Thus, common solution phase chemical transformations can be carried out in aprotic solvents such as DMF and DMSO, which are compatible with many organic reactions, and the product β-CD derivatives then isolated by precipitation and washing with non-polar solvents. It will be noted that such a procedure is in many ways similar to solid-phase synthesis, where the β-CD scaffold is acting as a miniature solid-state particle.

Synthesis and characterization of per-6-substituted-6-deoxy-β-CD libraries

Prior to library synthesis, the least active nucleophile, imidazole, was used to optimize reaction conditions and workup procedures. Per-6-iodo-6-deoxy-β-CD (**2**) was prepared[19] followed by nucleophilic displacement of iodine with imidazole (10 mol excess relative to each iodine) in DMF at 80 °C for 24 h, similar to chemistry used by Thatcher et al. (Scheme 1).[20] After removal of the DMF under reduced pressure, crude per-6-imidazolyl-β-CD was obtained by precipitation with ethyl acetate. This product was filtered and washed with ethyl acetate and then sonicated in ethyl acetate for 10 min to give a fine powder, which was further stirred for an h and filtered to give the final product (ESMS = 1485.7, 743.3, 496.1 *m/z* for the 1+, 2+, and 3+ [per-6-imidazolyl-6-deoxy-β-CD + nH]$^{n+}$ ions)[21] after through drying in vacuo. Per-6-substituted-6-deoxy-β-CD libraries syntheses were achieved using the same reaction conditions and purification procedures as for the model reactions. Excess amines (10-fold each reactant) were used in an attempt to get a statistical distribution of amines on the primary face. Fourteen β-CD libraries (**I**–**XIV**),



Scheme 1. Synthesis of per-6-substituted-6-deoxy-β-CD libraries: (a) I_2, Ph_3P in DMF; (b) excess RNH_2 in DMF, then precipitation with ethyl acetate.

totalling approximately 30,000 compounds ($3^7 \times 14$), were prepared using three amines at a time selected from the 19 amines given in Figure 2.

Each of these libraries was characterized using ESMS. For example, the ESMS of library **I** is shown with the theoretically calculated spectrum in Figure 3. The doubly charged ion envelope of library **I** (showing the greatest cocaine hydrolytic activity) was similar to that calculated for the theoretical distribution of side chains, assuming an equal distribution of amines on the primary face. As may be seen, the observed doubly charged ion envelope (Fig. 3b) extends somewhat beyond that for the theoretical distribution (Fig. 3a). These mass shifts were attributed to non-covalent ion adducts with excess amine reagents used in the reactions. Such non-covalent adducts with β-CD have been previously reported by Cunniff and Vouros.[22]

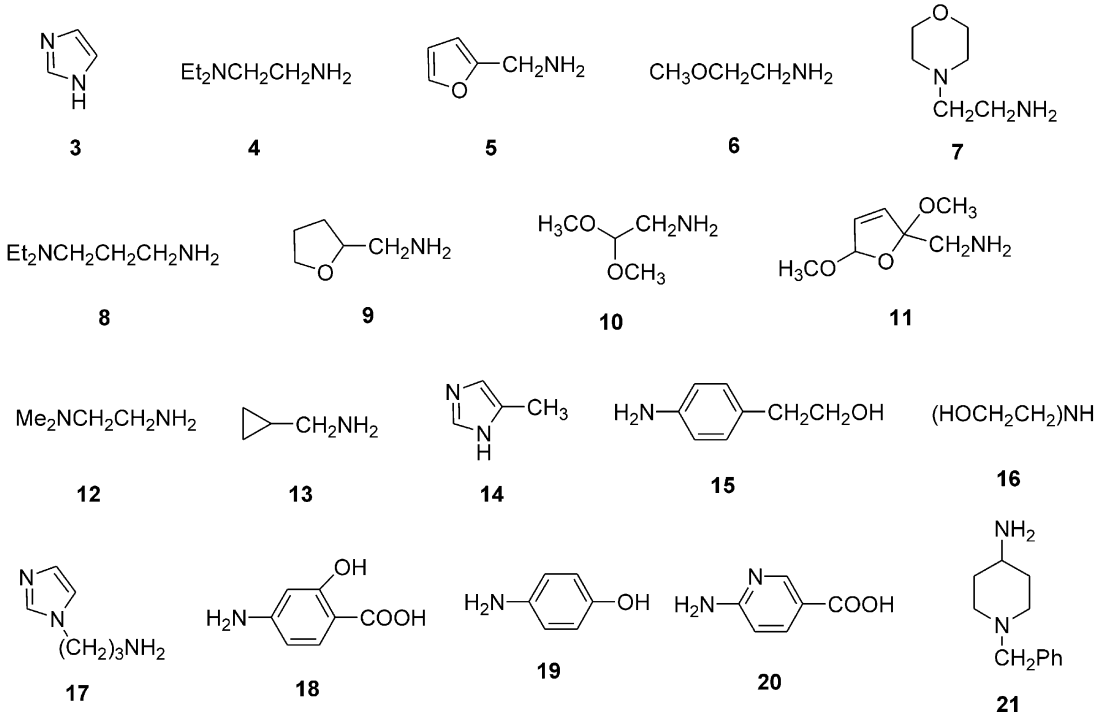


Figure 2. Amine side chains used in library syntheses.

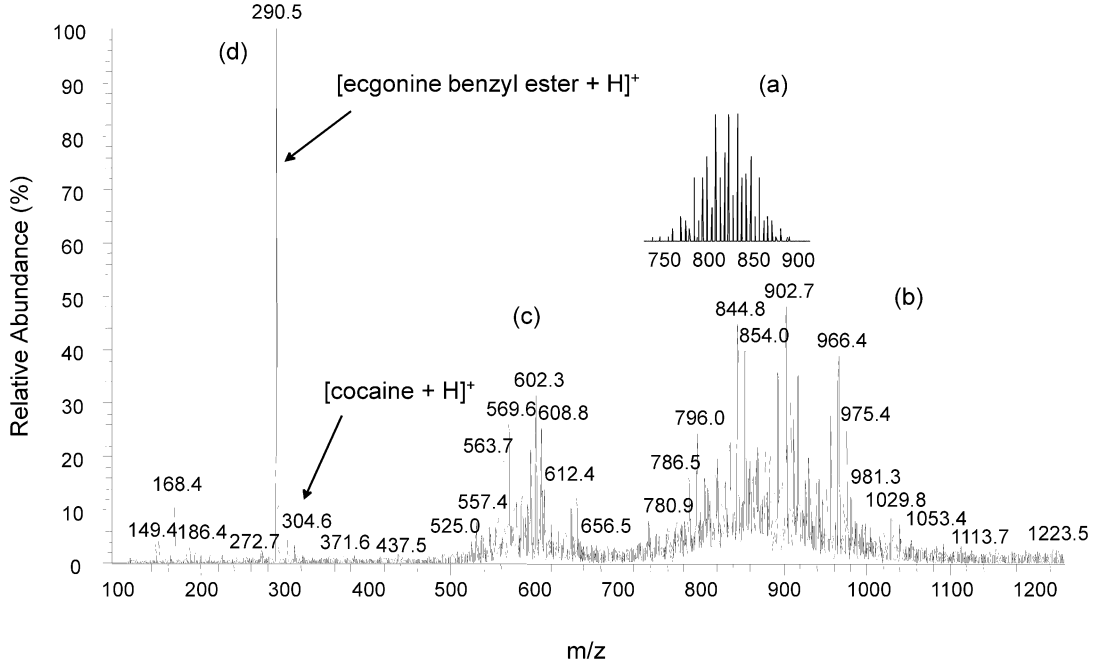


Figure 3. ESMS of per-6-substituted-6-deoxy-β-CD library **I**. Library was incubated with cocaine in ammonium acetate buffer (pH 7.4) for 24 h: (a) theoretically calculated spectrum; (b) doubly charged ions; (c) triply charged ions; (d) ion from cocaine hydrolysis.

Evaluation of cocaine hydrolytic activity for per-6-substituted-6-deoxy-β-CD libraries

Each of the 14 libraries were examined for the ability to hydrolyze cocaine against appropriate controls using the 304 → 290 and 304 → 200 $[M+H]^+$ ions for defining the hydrolysis of the benzoate and methyl esters, respectively (304, 290, and 200 in positive ESMS spectrum correspond to cocaine, benzoylecgonine, and ecgonine methyl ester). The signal intensity of these peaks is a function of the extent of cocaine hydrolysis and could be used as an indication of the ability of the library to hydrolyze cocaine. Thus, each library was incubated with cocaine in NH_4OAc buffer (pH 7.4) for 24 h, and the hydrolysis of the cocaine was monitored using positive ion ESMS. The relative activity of the library was defined by the relationship: [(ion 290 + ion 200) / (ion 290 + ion 200 + ion 304)] × 100%. Although this can only be considered quantitative in a crude sense, the results are consistent, and replicate measurements show reproducibility within ten percent (Table 1).

As shown in Table 1 and Figure 3d, none of the libraries tested showed hydrolysis of the cocaine benzoate ester. Library **I** was the most active for the hydrolysis of cocaine methyl ester, producing 95% hydrolysis of the cocaine methyl ester after 24 h, while the side chain amines alone used in the synthesis of library **I** showed 29% hydrolysis under identical conditions. Furthermore, ESMS analysis immediately after mixing cocaine and library **I** gave a value of 18% conversion (data not shown), suggesting relatively low hydrolysis due to the electrospray ionization process itself. Other libraries accelerated, to varying degrees, the hydrolysis of cocaine methyl ester. Control experiments with buffer and β-CD showed little or no hydrolysis (2 and 4%, respectively).

Table 1. Cocaine hydrolytic activity of per-6-substituted-6-deoxy-β-CD libraries[a]

Library	Amine reactants[b]	290×100/(290+304)%[c]
Blank	None	2
I	**3**, **4**, **5**	95, 93
II	**3**, **6**, **8**	79, 65
III	**3**, **5**, **8**	17, 23
IV	**3**, **5**, **7**	66, 58
V	**3**, **4**, **7**	77, 70
VI	**3**, **7**, **9**	75, 68
VII	**3**, **4**, **10**	70, 60
VIII	**3**, **4**, **9**	76, 71
IX	**6**, **9**, **11**	22, 18
X	**3**, **5**, **12**	60, 54
XI	**3**, **9**, **12**	75, 69
XII	**5**, **12**, **13**	71, 65
XIII	**3**, **12**, **13**	81, 76
XIV	**4**, **5**, **15**	50, 44
I	**3**, **4**, **5**	27, 23[d]
Â-CD[e]	None	4,5
Side chains[f]	None	25, 33

[a]All solutions were 0.1 mg library in 1 mL H_2O (59 μM calculated from average molecular weight of library members) and 5.9 μM substrate. Reactions were run in duplicate.
[b]Amine structures are given in Figure 2.
[c]304 and 290 correspond to cocaine and ecogonine benzyl ester, respectively.
[d]Ecgonine methyl ester (5.9 μM) was used. The number represents 168/(168 + 200) × 100%. 200 and 168 correspond to the substrate and product, respectively.
[e]β-CD is 59 μM.
[f]Amine side chains of library **I** were used alone, each at 138 μM.

The side chains of the most active library **I** were also used to synthesize the corresponding three per-substituted β-CDs which were tested for the ability to hydrolyze cocaine. The per-substituted imidazole and the per-furfuralamine substituted β-CDs showed activity less than the controls, where as, the per-substituted *N,N′*-diethylethylenediamine β-CD showed modest cocaine hydrolytic activity but less than that of library **I** (57% hydrolysis under the same conditions used for the library trials).

Interestingly, when the most active library **I** was employed in the hydrolysis of ecgonine methyl ester, (i.e., cocaine without its benzoate ester), the amount of methyl ester hydrolysis was much less than that seen with cocaine as the substrate. We suggest this reflects cocaine's better fit into the β-CD cavity through intercalation of the aromatic group of the benzoate ester. This would be in agreement with the report by Breslow and co-workers indicating that cocaine binds with β-CD itself with the phenyl ester in the central cavity.[23] It should be noted, however, that they conclude that the cocaine enters from the secondary face of the β-CD, while in the present work cocaine's methyl ester is apparently interacting with substitutents on the primary face of the molecule. As with the previous substituted β-CD hydrolysis of *p*-NPP,[18] structure–activity relationships were also observed for cocaine hydrolysis. Thus, comparison of libraries **I** and **III** showed that a single methylene unit increase in the *N,N*-diethylethylenediamine side chain resulted in a significant decrease in the ability to hydrolyze cocaine (95–20%), whereas replacement of ethyl groups in *N,N*-diethylethylenediamine (library **I**) with methyl groups (library **X**) decreased the hydrolytic activity by 40%. Such structure–activity relationships also suggest that only a portion of the possible 2187 (3^7) per-6-substituted-6-deoxy-β-CDs present in a given library were likely to be responsible for the observed hydrolytic activity.

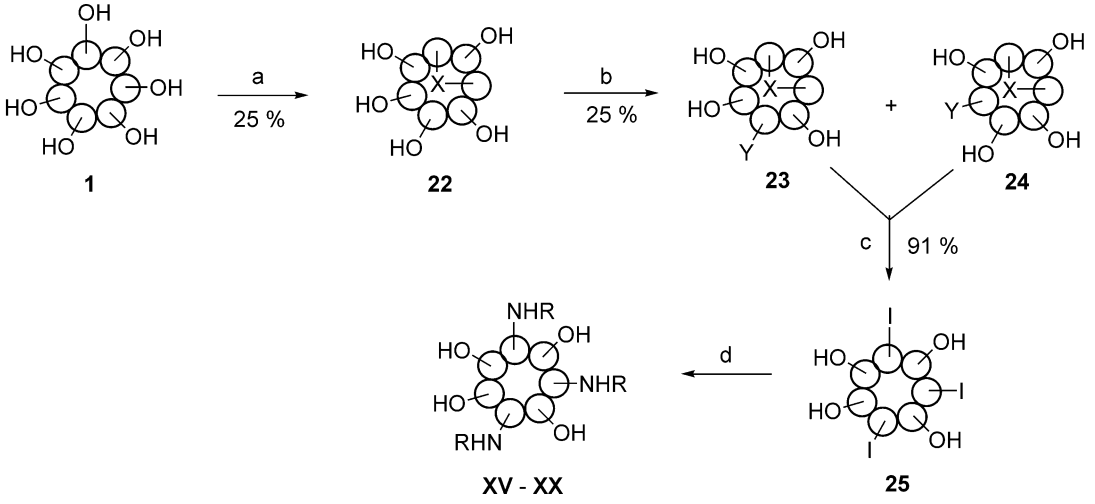
Synthesis and characterization of A,C,E(F)-tri-6-substituted-6-deoxy-β-CD libraries

Compared with per-6-substituted-6-deoxy-β-CDs libraries, less complex A,C,E(F)-tri-6-substituted-6-deoxy-β-CD libraries lend themselves to partial deconvolution through reducing the number of isomers. In addition, the synthesis of A,C,E(F)-tri-6-substituted-6-deoxy-β-CD libraries provides an opportunity to apply subtractive deconvolution techniques (see review in ref 24). Since there are three reactive sites reacting with the amine building block in the A,C,E(F)-tri-6-substituted-6-deoxy-β-CD libraries, no matter how many amine building blocks are used in the synthesis of the parent trisubstituted-β-CD libraries, the maximal number of sub-library components is 27 (3^3).

As a first step in the preparation of the trisubstituted series, A,C-disulfonate **22** was prepared according to Tabushi's procedure by a regioselective A,C-capping reagent, benzophenone-3,3′-disulfonyl chloride (Scheme 2).[25] However, in our hands the procedure produced relatively impure disulfonate **22** as measured by HPLC even after repeated flash silica gel chromatography. By subjecting this material to reversed phase chromatography on bulk, homemade, C_{18} silica gel, relatively pure disulfonate (HPLC purity >95%) was obtained in 25% yield. Our strategy was next to regioselectively activate the E or F position, one of the two positions not adjacent to the existing sulfonate, by further sulfonation with a hindered sulfonyl chloride. After trial reactions with several sulfonyl chlorides, 10-camphorsulfonyl chloride was selected as the best reagent to prepare the A,C,E(F)-trisulfonates **23** + **24**. These were subsequently purified by flash, silica gel chromatography followed by reversed phase chromatography to yield 25% of a mixture of the two isomers. FABMS and ESMS of the purified product showed the expected molecular ions as H^+ and Na^+ adducts. NMR data indicated that only one equivalent of 10-camphorsulphonyl chloride had reacted with one 6-hydroxyl group of each A,C-disulfonate. In addition, ESMS spectra indicated that further sulfonation to A,C,D,F- or A,C,E,G-tetrasulfonate did not occur in the presence of five equivalents of 10-camphorsulphonyl chloride relative to A,C-disulfonate. This strongly suggested that the more hindered A,C,D(G)-trisulfonate isomers were unlikely to be produced in the reaction and that the product was the A,C,E(F)-trisulfonates **23** and **24**. Two peaks (2.7, 1.0) were partially resolved in the analytical C_{18} HPLC profile of these trisulfonates, which we attribute to the A,C,E- and A,C,F-trisulfonate isomers (data not shown, structural assignments were not made). Using a procedure similar to that previously reported,[26] the A,C,E(F)-trisulfonates were converted to triiodo-β-CD in 91% yield. It should be noted that where as the A,C,E- and A,C,F-trisulfonates are two different compounds, the corresponding iodo derivatives are identical, that is A,C,E-triiodo-β-CD is equal to A,C,F-triiodo-β-CD.

The library synthesis was carried out by treating the triiodo-β-CD with a variety of amines as shown in Figure 2. Again, 2-methoxyethylamine, expected to be amongst the most reactive building blocks, and imidazole, one of the least reactive building blocks, were used to determine optimal reaction conditions for library synthesis. Model reactions were monitored by ESMS, showing that at least 40 and 72 h were needed for completing the reaction with 2-methoxyethylamine and imidazole, respectively. Therefore, the reaction time for the library synthesis was chosen as 72 h with the other conditions similar to the model reactions. In order to take into account the difference in reactivity, larger equivalents of reactants were also used for the less reactive amines in this particular series. For example, in the synthesis of libraries **XV** and **XVI**, 30 equivalents of imidazole were used relative to 20 equivalents for the aliphatic amines.

Each of the tri-substituted-β-CD libraries was characterized using ESMS. With these smaller libraries, attempts were made to correlate the observed ion peaks in the ESMS spectra with individual library members. The ESMS spectrum of a representative library **XVII** formed from four amine nucleophiles is shown in Figure 4. Theoretically, this library contains 64 compounds having 20 different molecular weights due to the presence of positional isomers. Out of 20 different masses in this library, 17 were found, corresponding to 55 compounds if all positional isomers were present for each detected mass. For instance, the structure of the most abundant peak at *m*/*z* 769.0 was consistent with a β-CD derivative containing one **6** and two **21** side-chain building blocks attached to the β-CD scaffold. Some peaks were tentatively assigned to be non-covalent complexes between library members and the building blocks, such as the peak at *m*/*z* 833.3, a complex between the aforementioned tri-substituted derivative and one imidazole molecule. Certain side products were also indicated by such analyses. For example, the peak at *m*/*z* 673.9 suggested a β-CD derivative containing the **6** and **21** side chains and one 3,6-anhydro glucose unit formed from an intramolecular attack at the iodo group by a hydroxyl group at C-3 of the same glucose unit.



Scheme 2. Synthesis of A,C,E(F)-tri-6-substituted-6-deoxy-β-CD libraries: (a) XCl_2 (X = 3,3″-benzophenone disulfonyl) in pyridine; (b) YCl [Y = (1*S*)-(+)-10-camphorsulfonyl] in pyridine/DMF; (c) KI in DMF; (d) excess RNH_2 in DMF, then precipitation with ethyl acetate.

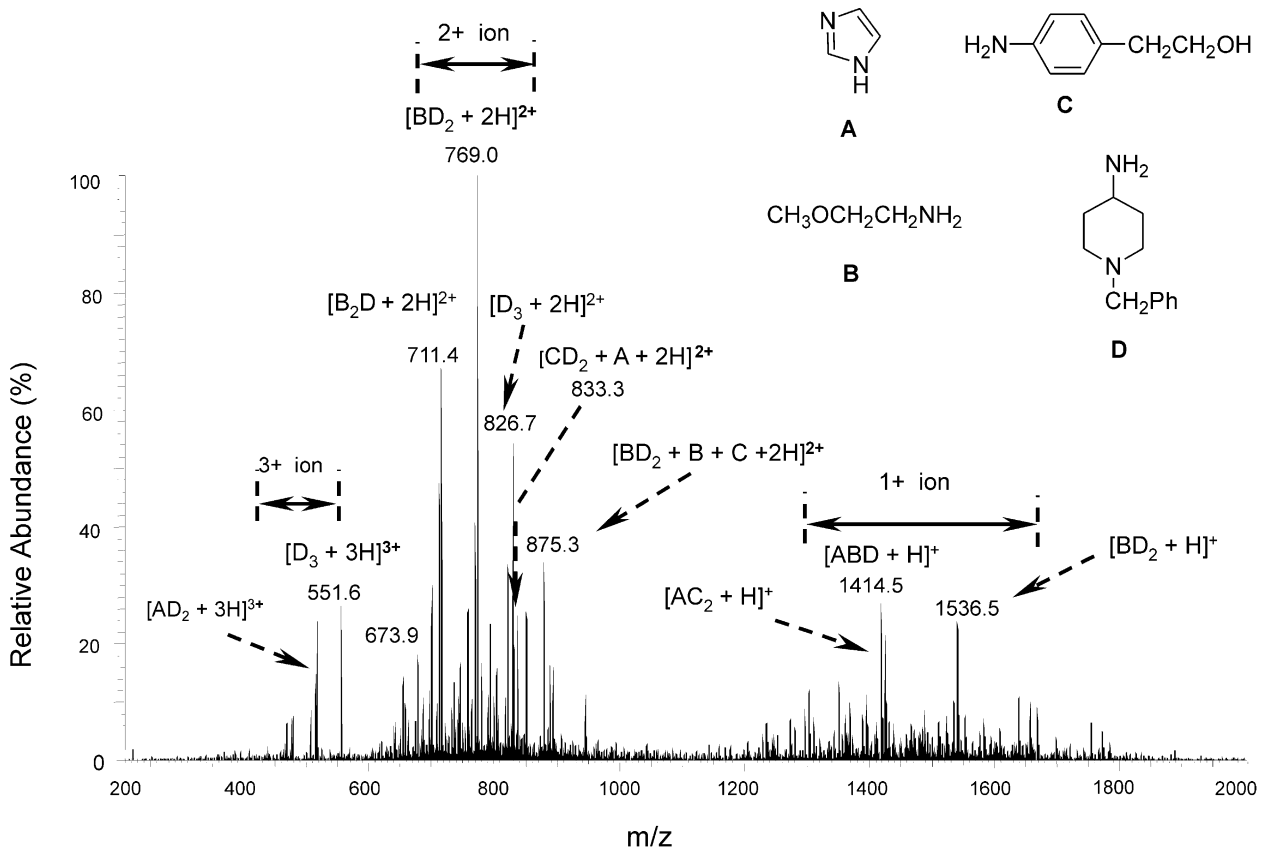


Figure 4. ESMS of A,C,E(F)-tri-6-substituted-6-deoxy-β-CD library **XVII** incubated with cocaine. See text for details.

Evaluation of cocaine hydrolytic activity for A,C,E(F)-tri-6-substituted-6-deoxy-β-CD libraries

Cocaine hydrolytic activity by the tri-substituted β-CD libraries is presented in Figure 5. At pH 8.0, library **XV** showed the best activity, resulting in about 50% hydrolysis of cocaine on overnight incubation. Two other libraries, **XVI** and **XIX**, also showed activity (32 and 41%, respectively) higher than background (12%, buffer only). As a comparison, unmodified β-CD did not show significant activity (9% hydrolysis) for cocaine hydrolysis under identical conditions. The other three libraries (**XVII**, **XVIII**, and **XX**) were essentially inactive relative to controls.

Examination of the composition of the libraries indicates *N*,*N*′-diethylethylenediamine as the common building block for the best two libraries **XV** and **XIX**. Comparison of the side chains used for libraries **XVI** and **XVIII** suggest that the imidazole group contributed in part to the higher activity of library **XVI**. Both of these libraries use 2-methoxyethylamine and 4-(2-aminoethyl)morpholine as building blocks, while library **XVIII** did not contain imidazole. Library **XVI** had imidazole as the only other building block, indicating the importance of imidazole for the activity. On the other hand, inclusion of the imidazole group does not necessarily guarantee the activity for hydrolyzing cocaine, as seen with libraries **XVII** and **XX** and the fact that the per-imidazole derivative was inactive. Both libraries contain imidazole but did not show enhanced activity relative to the control. Nevertheless, a combination of the two building blocks, *N*,*N*′-diethylethylenediamine and imidazole, did render libraries **XX** and **XIX** with the best activity.

Comparison of the composition of the most active per-substituted libraries in Table 1 and the tri-substituted libraries in Figure 5 showed three common side chains (**3**, **4**, and **5**, see Figure 2). This suggests that one might be able to define amine side-chains important for the hydrolytic activity through evaluation of per-6-substituted-6-deoxy-β-CD libraries. This would allow one to more quickly search side chain molecular space by dealing with the more easily synthesized per-6-substituted-6-deoxy-β-CD libraries and then refining their activities on the A,C,E(F)-tri-6-substituted-6-deoxy-β-CD series where there is some degree of synthetic control of the isomers formed.

Conclusions

Per-6-substituted- and A,C,E(F)-tri-6-substituted-6-deoxy-β-CD libraries were synthesized by solution-phase nucleophilic reactions starting from per-6-iodo- and tri-6-iodo-6-deoxy-β-CDs and mixtures of various amine side chains, respectively. The molecular diversity of these libraries was in part verified by ESMS. Significant cocaine hydrolytic activity for the hydrolysis of cocaine methyl ester was observed with several libraries using a convenient ESMS screen. Small structural changes in amine side chains produced significant

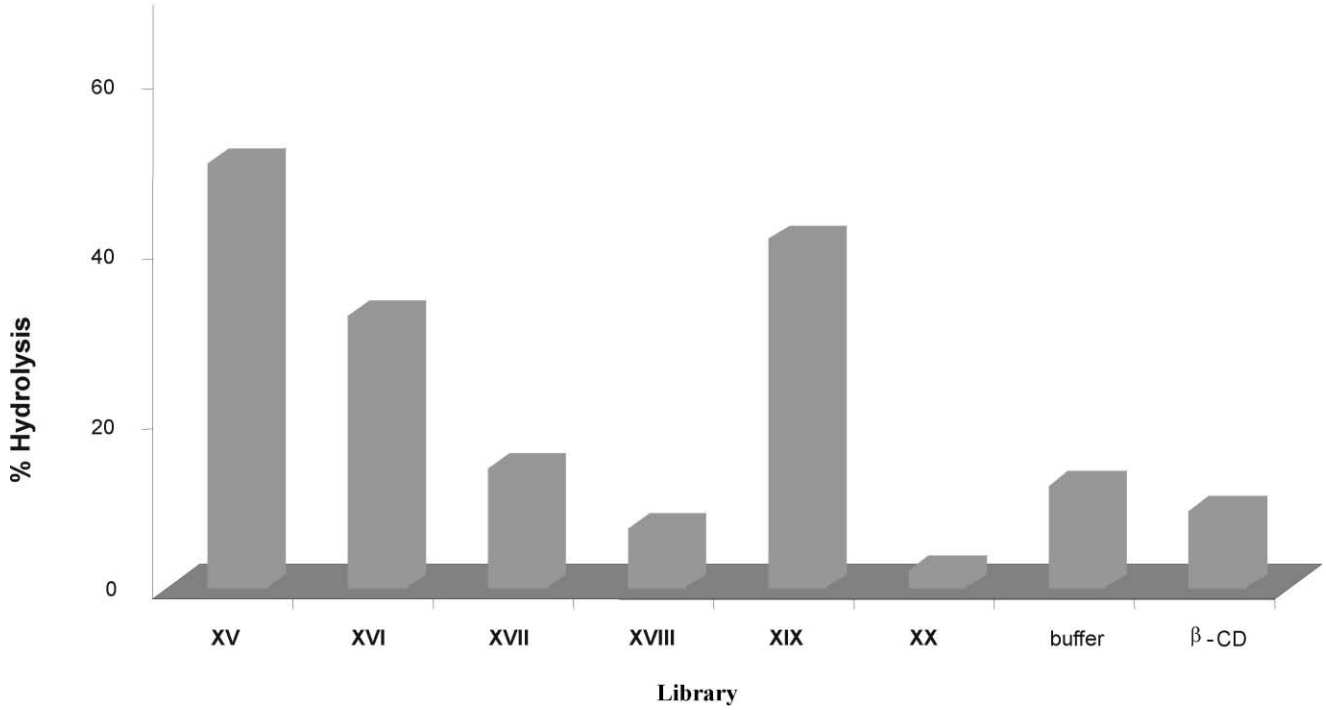


Figure 5. Cocaine hydrolytic activity of A,C,E(F)-tri-6-substituted-6-deoxy-β-CD libraries. See text for details.

changes in this activity, suggesting specific interactions between certain substituted-β-CDs and the cocaine molecule.

In the future, we propose to select for mixtures of per-substituted β-CD isomers having specific catalytic activity using affinity trapping techniques with immobilized transition-state analogues, and to apply what we learn in this series to preparation of selected isomers in the A,C,E(F)-tri-6-substituted-6-deoxy-β-CD series.

Experimental

β-CD containing 12–14% (weight) water was purchased from Wacker Silicones Corp. (Adrian, MI, USA) and was dried at 90 °C over P_2O_5 under vacuum (0.5 mmHg) with dry ice–acetone trap for at least 12 h before use. Anhydrous pyridine and *N,N*-dimethylformamide were obtained in Sure/seal™ bottles from Aldrich Chemical Co. (Milwaukee, WI, USA). All other chemicals used in the synthesis and bioassays were purchased from Aldrich Chemical Co. and Sigma (St. Louis, MO, USA) and were used without further purification unless otherwise noted. Centrifugation was performed on a MicroCentrifuge 235B (Fisher Scientific Company). ^1H and ^{13}C NMR spectra were recorded on a Bruker Avance 300 spectrometer at 300 and 75.5 MHz. Chemical shifts are reported in ppm (δ) using tetramethylsilane (TMS) as the internal reference. ESMS were recorded on a Finnigan LCQ ion trap and FABMS were collected with a Finnigan MAT 90 double-focusing mass spectrometer. pH values were measured on a Corning pH meter 320 with a glass electrode after calibration to standard buffer solutions. Sample incubation was conducted in shallow form shaking bath (Precision). Analytic HPLC was performed using a Nova-Pak C_{18} column (3.9 × 300 mm, 4 μm particle) on a Waters HPLC system with 600E system controller, a Waters 717 autosampler, and a Waters 486 tunable absorbance detector. Preparative HPLC was performed on a Waters 510HPLC pump and a Waters 680 automated gradient controller by using a column (40 × 350 mm) packed with homemade C_{18} silica gel.[27] Thin layer chromatography (TLC) was performed on aluminum-backed silica gel plates (Aldrich 60 F254, 0.2 mm in thickness). TLC plates were visualized by ultraviolet at 254 nm, before being developed with 5% H_2SO_4 in EtOH followed by heating with a heat gun. Flash column chromatography was performed using activated silica gel 60 (particle size 32–63 μm) purchased from Selecto Scientific, GA, USA.

General procedures for the generation of per-6-substituted-6-deoxy-β-CD libraries

To a solution of per-6-iodo-6-deoxy-β-CD (2)[19] (40 mg, 0.021 mmol) in DMF (1 mL) were added three amines (each 1.47 mmol) and the resulting solution was stirred at 80 °C for 24 h. After removal of the DMF under reduced pressure, the crude library was obtained by precipitating the residue with EtOAc, filtering, and washing. This product was sonicated in EtOAc for ten min to give a fine powder, which was further stirred for one h and filtered to give the final product after through drying in vacuo. The libraries were characterized by ESMS. A representative spectrum is shown in Figure 3.

Synthesis of benzophenone-3,3′-disulfonate-capped-β-CD (A,C-disulfonate-β-CD 22). Using the Tabushi procedure,[25] crude A,C-disulfonate-β-CD was obtained as a white solid by flash column chromatography eluting with *n*-PrOH/EtOAc/H_2O/$NH_3 \cdot H_2O$ = 10/8/5/2. Final

purification of crude A,C-disulfonate-β-CD was accomplished by reversed-phase column chromatography eluting with a water (A)/acetonitrile (B) linear gradient (18–25% B, 0–30 min; 25–28% B, 30–100 min; 28–40% B, 100–110 min; and 40% B, isocratic) at a flow rate of 5.5 mL/min. A,C-disulfonate-β-CD was eluted at about 95 min. The corresponding fraction was collected and concentrated under aspiration. The concentrate was then lyophilized to give a white powder **22** (25%). The purified A,C-disulfonate-β-CD was further subjected to analytical HPLC for purity verification eluting with a water (A)/acetonitrile (B) gradient (5–20% B, 0–20 min; 20–22% B, 20–60 min) at a flow rate of 0.50 mL/min. A,C-disulfonate-β-CD was eluted at 29.5 min with greater than 95% purity. R_f 0.27 (*n*-PrOH/EtOAc/H_2O/$NH_3{\cdot}H_2O$ = 10/8/5/2); ^{1}H NMR (DMSO-d_6) δ 3.30–3.80 (42H), 4.83 (C_1-H, 7H), 4.30–4.62, 5.52–5.88 (C_2, C_3, C_6-OH, 19H), 7.88, 8.20 (aromatic, 8H); FABMS *m*/*z* 1441 ($[M+H]^+$).

Synthesis of A,C,E(F)-trisulfonate-β-CDs 23 + 24. To a solution of dry A,C-disulfonate-β-CD **22** (620 mg, 0.43 mmol) in dry DMF (60 mL) and pyridine (2 mL) was added a solution of (1*S*)-(+)-10-camphorsulfonyl chloride (486 mg, 1.94 mmol) in DMF (5 mL). The solution was stirred under N_2 at room temperature for 5 h. Water was then added to the mixture and the solution was concentrated to about 2 mL under vacuum. The concentrated solution was diluted with about the same volume of water followed by addition of 60 mL of *n*PrOH to produce a white solid. The precipitate was collected by filtration and washed with *n*PrOH and dried. The crude product was first partially purified by flash column chromatography eluting with *n*PrOH/EtOAc/H_2O/$NH_3{\cdot}H_2O$ = 5/3/2/1. The appropriate fractions were combined and concentrated and the product precipitated by addition of *n*PrOH (ca. 50 mL). The partially purified A,C,E(F)-trisulfonate-β-CDs were further purified by reversed-phase column chromatography eluting with a water (A)/acetonitrile (B) gradient (25–35% B, 0–30 min; 35–60% B, 30–120 min) at 5.5 mL/min. A,C,E(F)-trisulfonate-β-CDs were eluted at about 90 min. The fraction was concentrated to about 1 mL and then diluted with the same volume of water. This solution was lyophilized to give a white solid **23 + 24** (178 mg, 25%). R_f 0.27 (*n*PrOH/EtOAc/H_2O/$NH_3{\cdot}H_2O$ = 5/3/2/1); ^{1}H NMR (DMSO-d_6) δ 0.85 (4H), 1.00 (3H), 1.40 (2H), 1.80–2.10 (4H), 2.25 (2H), 3.22–3.80 (42H), 4.83 (C_1-H, 7H), 4.32–4.70, 5.50–6.10 (C_2, C_3, C_6-OH, 19H), 7.88, 8.20 (aromatic, 8H); FABMS *m*/*z* 1655 ($[M+H]^+$) and 1678 ($[M+Na]^+$).

Synthesis of Triiodo-β-CD 25. Using a procedure similar to that of Tabushi,[26] to a finely grounded KI powder (2.69 g, 16.2 mmol) was added to a solution of A,C,E(F)-trisulfonate-β-CDs **23 + 24** (0.60 g, 0.36 mmol) in dry DMF (30 mL). The solution was stirred at 80 °C under N_2 for 2 h. After cooling the insoluble material was removed by filtration, washed with DMF and the filtrate was evaporated to dryness in vacuo at 30 °C. The yellowish residue was dissolved in water (10 mL), and to the aqueous solution tetrachloroethylene (1 mL) was added at 0 °C with vigorous stirring. The precipitate which formed was collected by filtration and dried in vacuo to afford a pale yellowish solid **25** (0.48 g, 91%). R_f value of **25** is very close to that of A,C,E(F)-trisulfonate-β-CDs **23 + 24**, but was essentially non-illuminating under UV and was detected with sulfuric acid and heat. ^{1}H NMR (DMSO-d_6) δ 3.28–3.80 (42H), 4.86 (7H), 4.42–4.73, 5.60–6.00 (19H); ESMS 744 ($[M+H+Na]^{2+}$) and 1487 ($[M+Na]^+$).

General procedures for the generation of A,C,E(F)-tri-6-substituted-6-deoxy-β-CD libraries

To a solution of triiodo-β-CD 25 (44 mg, 0.03 mmol) in DMF (10 mL) were added a mixture of three, four, six, and 10 amines, respectively. For each reaction, 30 equivalents (0.91 mmol) of imidazole, 4-aminophenethyl alcohol, 4-aminosalicyclic acid, 4-aminophenol, and 6-aminonicotinic acid and 20 equivalents (0.6 mmol) of other amines were used. The resulting solution was stirred at 80 °C for 72 h. The solution was concentrated under reduced pressure to about 1 mL and a light yellowish precipitate was obtained by addition of acetonitrile. The solid was filtered, washed, suspended in acetonitrile and sonicated for 5 min. This process was repeated two more times and then the solid was dried in vacuo to afford a light yellowish solid. The libraries were characterized by ESMS. A representative spectrum is shown in Figure 4.

Hydrolysis of cocaine catalyzed by β-CD libraries

In a typical experiment, the library (100 μL, 1 mg in 1 mL H_2O) and cocaine hydrochloride (100 μL, 5.9 μM in H_2O) were mixed thoroughly with NH_4OAc buffer solution (800 μL, pH 7.4, 62.5 mM in H_2O) and the sample was then incubated in a shaking bath at 37 °C for 24 h and finally analyzed by ESMS. The extent of cocaine hydrolysis was calculated by the percentage of the abundance of the product ion peak (290) versus the total abundance of the product and cocaine ion peaks (290 + 304). The formula for the percentage of the cocaine hydrolysis is [ion 290/(ion 290 + ion 304)] × 100% (Table 1 and Fig. 5).

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