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HEXADEOXYCYCLOHEPTAAMYLOSE–PYRIDOXAMINE, AN ARTIFICIAL TRANSAMINASE WITH A "DEEPER" BINDING POCKET

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ABSTRACT

Cycloheptaamylose was converted in four steps into a monosulfonylated, hexadeoxy derivative that, on treatment with pyridoxaminethiol, provided an artificial transaminase with activity similar to that found in the nondeoxygenated analogue. Characterization of the intermediate hexadeoxycycloheptaamylose was facilitated by the use of field-desorption mass spectrometry, which was uniquely able to distinguish between penta-, hexa-, and hepta-deoxycycloheptaamylose. A detailed description of experimental methods for the first time makes this modified binding-site available for general use in the design of enzyme mimics.

INTRODUCTION

The utility of cycloamyloses (cyclodextrins) in the modeling of enzyme-catalyzed reactions involving hydrophobic binding has been reported with increasing frequency[1]. Our own work has shown, among other things, that the complexation in aqueous solution between cycloamylose derivatives and appropriate substrates can lead to rate accelerations and/or enhanced selectivity in acyl transfer[2], transamination[3], aromatic chlorination[4], phosphate hydrolysis[5], and cycloaddition reactions[6].

We became interested for two reasons in the preparation of derivatives of cycloheptaamylose (**1**) in which all unsubstituted hydroxyl groups on the primary face were deoxygenated to methyl groups. Previous studies have shown that, as expected, maximal rate accelerations are obtained when substrates are bound more strongly as the reaction proceeds towards the transition state[2b]; by effectively making one face of the binding site more hydrophobic, differences in rates and product distributions might be expected. In addition, modeling schemes in which a reactive species is generated near the primary face would be expected to experience com-

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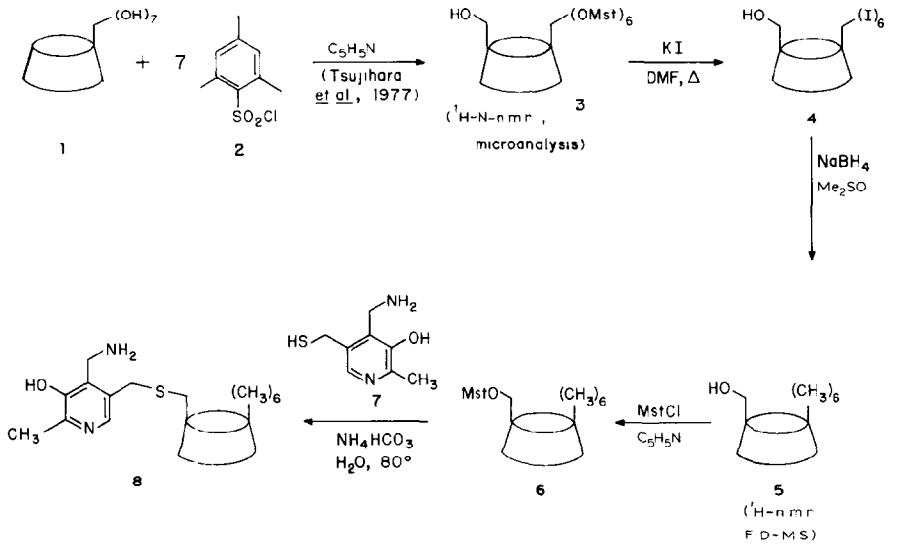
peting reactions by both the bound substrate and the adjoining hydroxymethyl groups. While this problem could probably be overcome by protection of the hydroxyl groups, such substitution generates a "floor"[7] and inhibits entry of the substrate *via* that face of the cycloheptaamylose torus; the deoxygenation method avoids this problem.

Mono-[8,9], di-[9], tetra-[9], and hepta-deoxy-[10] cycloheptaamylose have all been previously reported, but the hexadeoxy derivative has not been described. Therefore, we now report the preparation and use of a monosulfonated, hexadeoxy derivative (**6**) of cycloheptaamylose that can readily be adapted to the preparation of a wide variety of catalysts incorporating this unique binding-site.

RESULTS AND DISCUSSIONS

Reaction of cycloheptaamylose (**1**) with 8.8 equivalents of mesitylenesulfonyl chloride (**2**) in pyridine has been reported to provide the hexakis(6-*O*-mesitylsulfonyl)cycloamylose (**3**) as the major product[11]. In our hands, this compound is obtained, but the major product is clearly the symmetrically substituted heptakis(6-*O*-mesitylsulfonyl)cycloamylose, as evidenced by the very simple ^{1}H- and ^{13}C-n.m.r.-spectral patterns observed. While this provides a disappointingly low yield of the desired product, the fact that it can be made on a relatively large scale in one step still makes this route attractive as compared with multistep methods.

Conversion into the hexaiodo compound (**4**) was accomplished by using potassium iodide in *N,N*-dimethylformamide (DMF) at 100° in good yield. Reduction with sodium borohydride in dimethyl sulfoxide afforded the desired



hexadeoxy derivative **5**, which could be separated from solvent and the bulk of inorganic salts only by column chromatography. Elemental analysis revealed ~80% of the expected carbon in the sample obtained; inorganic impurities were more easily removed at the next stage. In contrast to the original cycloamylose, dissolution in water is slow, but in methanol fast. ^{1}H-N.m.r. spectroscopy of this compound was not able to establish unequivocally the degree to which deoxygenation had been accomplished, although an average of repetitive integrations of the methyl region indicated 18.2 hydrogen atoms (expected: 18)*. Estimation of the relative amounts of penta-, hexa-, and hepta-deoxy cycloheptaamylose present was accomplished by field-desorption mass spectrometry†, which gave a molecular ion for the Na^+ chelate of **5** at *m/z* 1061 (100% relative intensity). Small signals within ±2 mass units of *m/z* 1045 ([heptadeoxy + Na]$^+$) and 1077 ([pentadeoxy + Na]$^+$) each accounted for <5% of the area under the hexadeoxy signal.

Activation of the remaining primary hydroxyl group was achieved by using a large excess‡ of mesitylenesulfonyl chloride in dry pyridine, which provided a single product (**6**) as determined by t.l.c. and ^{1}H-n.m.r. Displacement of the sulfonate with pyridoxaminethiol[3] (**7**) in water, followed by isolation by ion-exchange chromatography on Sephadex CM-25, provided the hexadeoxycycloheptaamylose–pyridoxamine **8** as a colorless solid. The 300-MHz ^{1}H-n.m.r. spectrum of this derivative fully supports the structure assignment as displacement through sulfur on the primary side.

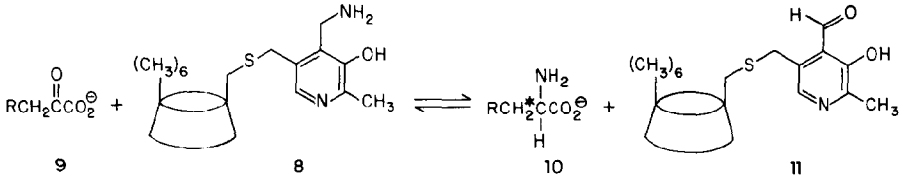
Transamination of keto acids by **8** was examined by the method described previously[3], in which the product amino acids are dansylated and analyzed by h.p.l.c. (Scheme 2). Indolepyruvate was converted into tryptophan, and phenylpyruvate into phenylalanine at rates comparable with those previously observed using the secondary-side cycloheptaamylose–pyridoxamine[12], that is, respectively, about 25- and 18-times as fast as pyruvate was converted into alanine. In addition, enantiomeric excesses of 36% (Phe) and 14% (Trp) of the L enantiomers were obtained. Clearly, participation of the modified binding-site in the transamination reaction does occur in a manner analogous to that observed in the nondeoxygenated series.

In summary, we have prepared a modified binding-site derived from cycloheptaamylose that incorporates increased hydrophobicity in both its binding and

*In our experience, integration of broad multiplet (br m) signals in ^{1}H-n.m.r. spectra of unsymmetrically multisubstituted cycloamyloses has proven too imprecise to be used quantitatively.

†Field-desorption mass spectrometry of cycloheptaamyloses has been used in a previous study (compare ref. 9).

‡Attempted sulfonylation using only a few (1–4) equivalents of mesitylenesulfonyl chloride gave no reaction (t.l.c.). We attribute this to the "inorganic impurity" known to be present, which almost certainly includes silica gel from chromatography of the starting material. Activation of the primary (as opposed to a secondary) hydroxyl group is confirmed by the ^{1}H-n.m.r. spectrum of the final product **8**, in which the chemical shift of the cycloamylose -C*H*$_2$-S-CH$_2$-Ar signal is 2.4 p.p.m. The shift of the methine signal for the analogous secondary-side derivative is known to be at 3.05 p.p.m. (ref. 12), which would, of course, also integrate to one proton.



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solubility properties and eliminates any reactivity from hydroxyl groups on the primary face. An artificial transaminase using this derivative shows activity similar to that already demonstrated in the parent enzymic mimic. Modeling schemes that take advantage of the absence of the unsubstituted primary hydroxyl groups are currently under investigation.

EXPERIMENTAL

General methods. — Pyridine was stirred over potassium hydroxide for 12 h, boiled under reflux over barium oxide for 3 h, and distilled from barium oxide. DMF was stirred over potassium hydroxide for 12 h and distilled from calcium oxide under diminished pressure. Dimethyl sulfoxide was freshly distilled under diminished pressure prior to use. T.l.c. was performed by using pre-coated silica gel on glass with indicator, thickness 0.2 mm; cycloamylose-containing spots were made visible by spraying with 200:20:10:1 methanol–acetic acid–conc. sulfuric acid–*p*-anisaldehyde and heating on a hot plate. Column chromatography was carried out with "flash-type" silica gel, 230–400 mesh. N.m.r. spectra were obtained with a Bruker 300-MHz spectrophotometer. Mass spectra were obtained with a Varian MAT-711 high resolution spectrophotometer coupled with a 620i computer and a Statos recorder.

Multiple mesitylenesulfonylation of cycloheptaamylose. — The reaction of cycloheptaamylose (**1**, 16.4 g) with 8.8 equivs. of mesitylenesulfonyl chloride (**2**) in dry pyridine was carried out as previously described[11]. Column chromatography followed by analysis of the fractions by careful t.l.c. on silica gel with two solvent-systems (7:3 chloroform–methanol and 7:7:5 2-propanol–ethyl acetate–water) indicated partial resolution of the three major products; these were designated as high- (probably a mixture of closely-eluting products), intermediate- (estimated to be the major product), and low-R_F products. In each case, only those fractions containing uncontaminated product were pooled in order to facilitate characterization.

Analysis of products. — *High* R_F *product.* Evaporation of the appropriate fractions gave the product as a colorless powder (1.3 g): ^{1}H-n.m.r. ($CDCl_3$): δ 2.1–2.8 [br m (see footnote* on p. 135), 5.8 × 9, $ArCH_3$], 3.1–5.2 (br m, 7 × 7, glucose H), 6.7–7.1 (br m, 5.9 × 2, ArH).

Intermediate-R_F *product (***1***-OMst*$_7$*).* Evaporation followed by recrystallization from acetonitrile gave the product as large, colorless flakes (7.2 g); m.p. 190.5–191°; ^{1}H-n.m.r. ($CDCl_3$): δ 2.25 (s, 3, *p*-$ArCH_3$), 2.45 (s, 6, *o*-$ArCH_3$), 3.30

(t, 1, H-4), 3.50 (dd, 1 , H-2), 3.90 (m, 2), 4.10 (dd, 1), 4.35 (d, 1), 4.75 (d, 1, H-1), and 6.85 (s, 2, ArH); ^{13}C-n.m.r. [$(CD_3)_2SO$]: δ 20.3 (*p*-$ArCH_3$), 21.7 (*o*-$ArCH_3$), 67.7 (C-5), 69.4 (C-6), 71.5 (C-3), 71.9 (C-2), 81.1 (C-4), 101.8 (C-1), 130.4, 131.2, 138.7, and 142.8 (Ar).

Anal. Calc. for $C_{105}H_{140}O_{49}S_7$: C, 52.31; H, 5.86; S, 9.31. Found: C, 51.55; H, 5.94; S, 8.84.

Low-R_F product (**3**). Evaporation and recrystallization from acetonitrile gave the product as a colorless powder (1.6 g); m.p. 186.5–187.5°; ^{1}H-n.m.r. ($CDCl_3$–CD_3OD): δ 2.1–2.4 (6 singlets of various sizes, 5.1 × 9, $ArCH_3$), 3.1-4.3 (br m, H-2,3,4,5,6, CH_3OD, and CD_3OH), 4.5–4.8 (cluster of small doublets, 7 × 1), and 6.65–6.8 (4 singlets of various sizes, 5.1 × 2, ArH).

Anal. Calc. for $C_{96}H_{130}O_{47}S_6$: C, 51.74; H, 5.88; S, 8.63. Found: C, 51.66; H, 5.98; S, 8.59.

Hexakis(6-deoxy-6-iodo)cycloheptaamylose (**4**). — A mixture of **3** (1.24 g, 0.56 mmol) and potassium iodide (6.1 g, 37 mmol) in dry DMF (15 mL) was stirred under nitrogen for 3 h, and then the cooled yellow supernatant was added dropwise to cold, stirred water (200 mL). The solid was collected by centrifugation, washed with methanol (2 × 160 mL) and acetone (160 mL), and dried *in vacuo* overnight at 61° to afford a colorless powder (754 mg, 75%): m.p. 203–203.5° dec. with evolution of purple gas; ^{1}H-n.m.r. [$(CD_3)_2SO$]: δ 3–5 (H-1,2,3,4,5,6).

Anal. Calc. for $C_{42}H_{64}O_{29}I_6$: I/C, 1.5. Found: I/C, 1.4.

Hexakis(6-deoxy)cycloheptaamylose (**5**). — To a solution of **4** (754 mg, 0.42 mmol) in dry dimethyl sulfoxide (45 mL) was added sodium borohydride (380 mg, 10 mmol), and the resulting solution was stirred under nitrogen for 19 h at room temperature, and then more sodium borohydride (50 mg, 1.3 mmol) was added, and the reaction was continued for 5 h at 50°. The solvent was removed *in vacuo* (bath temperature 75°) and the residue dissolved in methanol (25 mL). Dropwise addition to stirred ether (400 mL) provided a colorless solid, which was collected by centrifugation and dissolved in methanol (20 mL). Silica gel was added, and the solvent was removed *in vacuo*. The solid was applied to a column (26 × 2.5 cm) of silica gel packed in 1:1 2-propanol–ethyl acetate, and elution was conducted with the same system until the yellow color had been completely removed (~300 mL). Finally, elution with 5:5:2 2-propanol–ethyl acetate–water and evaporation of the cycloamylose-containing fractions provided the product, which was dissolved in the minimal amount of methanol and lyophilized from water to afford a fluffy, colorless solid (179 mg, 41%): R_F 0.76 (7:7:5 ethyl acetate–2-propanol–water), 0.28 (7:2:1 2-propanol–water–concentrated ammonium hydroxide); ^{1}H-n.m.r. [$(CD_3)_2SO$]: δ 1.2 (d, 18.2 [average between initial and D_2O-exchanged spectra], CH_3), 3–4 (m, H-2,3,4,5 and HOD), 4.9 (one large and two small doublets, 7.0, H-1), 5.8 (m, OH); field-desorption mass spectrum *m/z* 1078 (imp., <5%), 1061 (**5**, $[M + Na]^+$, 100%), 1047 (imp., <5%).

Anal. Calc. for $C_{42}H_{70}O_{29}$: C, 48.55. Found: C, 38.40.

Hexakis(6-deoxy)-mono(6-O-mesitylenesulfonyl)cycloheptaamylose (**6**). —

To a solution of hexakis(6-deoxy)cycloheptaamylose (**5**, 40 mg, 39 μmol) in freshly distilled pyridine (12 mL) was added mesitylenesulfonyl chloride (260 mg, 1.2 mmol), and the desiccated mixture was stirred for 14 h at room temperature, after which time t.l.c. with freshly prepared 5:5:2:1 2-propanol–ethyl acetate–water–ammonium hydroxide indicated almost complete disappearance of starting material. Water (~0.5 mL) was added, and after stirring for 30 min, the light-gold solution was evaporated to dryness. The residue was dissolved in methanol and passed through a short column of Bio-Rad AG 3-X4A ion-exchange resin (OH^-) packed in methanol. The cycloamylose-containing eluant was evaporated to low volume, and lyophilization from water afforded a colorless solid (29 mg, 62%): R_F 0.46 (5:5:2:1 2-propanol–ethyl acetate–water–ammonium hydroxide); ^{1}H-n.m.r. (CD_3OD): δ 1.1–1.4 (br m, 24*, aliphatic CH_3), 2.3 (s, 3, *p*-ArCH_3), 2.6 (s, 6, *o*-ArCH_3), 3.0–4.2 (br m, H-2,3,4,5 and CD_2HOD), 4.9 (CD_3OH), and 7.0 (s, 2, ArH).

Hexakis(6-deoxy)mono[6-S-(pyridoxamine-5-methyl)]-mono(6-thio)cycloheptaamylose (**8**). — A mixture of compound **6** (50 mg, 41 μmol), 5-mercaptomethylpyridoxamine dihydrobromide[3] (75 mg, 0.2 mmol; **7**) and ammonium hydrogencarbonate (75 mg, 1 mmol) in water (8 mL; thoroughly oxygen-purged by using nitrogen) was heated at 90° with vigorous stirring under nitrogen. After 3 h, the temperature was lowered to 60° and reaction continued for an additional 12 h. The mixture was added to cold water (100 mL), filtered, and the solution was applied to a 39 × 3 cm column of CM-25 Sephadex. Elution with a linear gradient of 0–0.2M ammonium hydrogencarbonate (5% methanol in each reservoir; 3 L total volume) monitored at 287.5 nm yielded the unreacted thiol (disulfide) at 0.13M and a single major product-peak at 0.04M buffer concentration. The appropriate fractions were pooled, lyophilized, and dissolved in methanol (5 mL, required ~15 min). A small amount of black particulate material was removed by filtration, then lyophilization from water provided the product as a colorless solid (13 mg, 26%): λ_{max} (pH 7.0) 322 nm; ^{1}H-n.m.r. (CD_3OD): δ 1.2 (m, 18, aliphatic CH_3), 2.2 (s, 3, ArCH_3), 2.4 (m, 2, Ar-CH_2-S-CH_2), 3–5 (H-1,2,3,4,5 of glucose residues), and 7.2 (s, 1, ArH).

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*Larger than the expected 18 because of unreacted starting material.

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