Deacylation of 4-nitrophenyl acetate by 6^A-(ω-aminoalkyl)amino-6^A-deoxy-β-cyclodextrins †

Kahlee Redman,[a] Bruce L. May,[a] Suzanna D. Kean,[a] Philip Clements,[a] Christopher J. Easton[b] and Stephen F. Lincoln *[a]

[a] *Department of Chemistry, University of Adelaide, Adelaide, SA 5005, Australia. E-mail: Stephen.Lincoln@adelaide.edu.au*

[b] *Research School of Chemistry, Australian National University, Canberra, ACT 0200, Australia*

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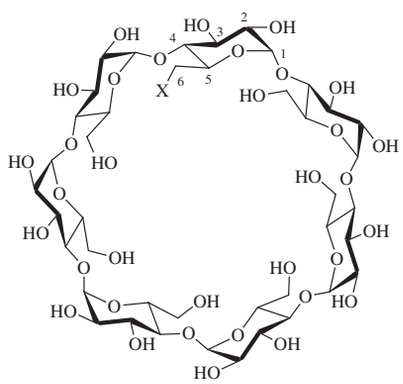
The deacylation of 4-nitrophenyl acetate (*p*NPA) in aqueous solution to give 4-nitrophenolate is significantly accelerated by the 6^A-(ω-aminoalkyl)amino-6^A-deoxy-β-cyclodextrins [βCDNH$(CH_2)_nNH_2$] which are themselves acylated to give predominantly βCDNH$(CH_2)_nNHCOCH_3$. The deacylation is characterised by k_dK = 27.4, 35.5, 24.5 and 16.0 dm^3 mol^{-1} s^{-1} at 298.2 K in aqueous 0.05 mol dm^{-3} borate buffer and I = 0.10 mol dm^{-3} ($NaClO_4$) when n = 2, 3, 4 and 6, respectively, where k_d (s^{-1}) is the rate constant for *p*NPA deacylation through a βCDNH-$(CH_2)_nNH_2$·*p*NPA complex characterised by a stability constant K (dm^3 mol^{-1}). The inhibition of the deacylation by adamantane-1-carboxylate (AC^-) is consistent with a mechanism where AC^- competes with *p*NPA in entering the βCDNH$(CH_2)_nNH_2$ annulus through the formation of a βCDNH$(CH_2)_nNH_2$·AC^- complex. The latter complex has been qualitatively studied by ^{1}H NMR ROESY methods, and its structure and that of βCDNH$(CH_2)_nNH_2$·*p*NPA have also been force-field modelled. The possibility of the operation of an S_N2 mechanism as an alternative explanation for the deacylation data is also considered.

Introduction

Natural and modified cyclodextrins (CDs) possess annuli which accommodate a wide range of guest species in the formation of complexes.[1–6] To some extent, these complexes resemble Michaelis complexes formed between enzymes and substrates and have been studied both as possible enzyme mimics and because of their intrinsic interest as in the classical studies of α- and β-cyclodextrin (αCD and βCD) by Bender and co-workers.[7] Breslow and co-workers have been at the forefront of studies of substantially modified CDs that accelerate reactions of guest species in their complexes by a thousand-fold or so in Michaelis–Menten catalytic cycles.[8] Catalytic studies have also been reported by Tee and co-workers for both natural and modified CDs.[9] A key aspect of such studies is the identification of the mechanistic steps in the catalytic cycle. In this study, our main interest is to identify the mechanistic steps of the reaction acceleration process and the factors determining the effect of the CD modification up to the formation of the first covalent bond between the modified CD and the guest species. This identifies the mode of nucleophilic attack of the modified cyclodextrin on the guest to give a product which may be likened to the intermediate product in an enzymatic cycle prior to its breakdown and regeneration of the active enzyme.

For our study we have chosen the 6^A-(ω-aminoalkyl)amino-6^A-deoxy-β-cyclodextrins (Fig. 1), βCDNH$(CH_2)_nNH_2$ where n is either 2, 3, 4 or 6,[10,11] which we find generate the dominant acylated products βCDNH$(CH_2)_nNHCOCH_3$ and 4-nitrophenolate through reaction with 4-nitrophenyl acetate (*p*NPA). [Deacylation of *p*NPA by βCDNH$(CH_2)_6NH_2$ also yields βCDNH$(CH_2)_6NH_2$ acylated at a secondary hydroxy group as a minor product.] These modified CDs provide an opportunity to study the effect of the flexibility of their diamine substituents on the nucleophilic attack on *p*NPA, and also the influence of protonation of the amine groups on this process.



X = $NH(CH_2)_nNH_2$ where n = 2, 3, 4 or 6

Fig. 1 The 6^A-(ω-aminoalkyl)amino-6^A-deoxy-β-cyclodextrins, βCD-$NH(CH_2)_nNH_2$, studied.

Experimental

Materials and instrumental methods

The preparations of βCDNH$(CH_2)_nNH_2$ were as previously described.[10] They were dried to constant weight and stored over P_2O_5 prior to use, and gave good microanalyses and clean ^{13}C NMR spectra. 4-Nitrophenyl acetate was prepared by standard methods and recrystallised from ethanol. Adamantane-1-carboxylic acid (Aldrich) was used as received. Borate buffer, prepared from $Na_2B_4O_7$·$7H_2O$ (BDH Analar), and HEPES (CalBiochem) were made up as described in the literature.[12] All pH measurements were made with an Orion SA 720 potentiometer and an Orion 8172 Ross Sureflow combination pH electrode. Aqueous solutions were prepared in deionised water, purified with a MilliQ-Reagent system to produce water with a specific resistance of >15 MΩ cm which was boiled to remove CO_2. Thin layer chromatography (TLC) was carried out using Merck Kieselgel 60 F_{254} silica on aluminium sheets. Samples were eluted using a mixture of Pr^iOH–EtOH–H_2O–NH_4OH

† β-Cyclodextrin = cyclomaltoheptaose.

(7:7:5:4). Compounds containing amino groups were detected by dipping the developed plate into a solution of 1% ninhydrin in ethanol and heating the plate. CDs were detected by dipping the developed plate into a solution of 1.5% H_2SO_4 in ethanol and heating the plate. Values of R_f are reported as R_c (retention relative to βCD).

The deacylation of *p*NPA was followed spectrophotometrically. In a typical hydrolysis run, 2 cm^3 of a solution of $\beta CDNH(CH_2)_nNH_2$ in the concentration range (5.36–99.3) × 10^{-5} mol dm^{-3} buffered in borate buffer (0.05 mol dm^{-3}) was allowed to attain the reaction temperature of 298.2 K over 30 min in a quartz 1 cm pathlength cell at 298.2 K in the thermostatted cell block of a Varian Cary 2200 spectrophotometer. A 50 μm^3 aliquot of 2.0×10^{-4} mol dm^{-3} *p*NPA in acetonitrile was then added with a micropipette and rapidly mixed to make the reaction solution 5.0×10^{-6} mol dm^{-3} in *p*NPA, and the increase of absorbance at 400 nm was recorded digitally for at least 7 reaction half-lives as *p*NPA deacylated to 4-nitrophenolate. The observed first order deacylation rate constant, k_{obs}, was determined by fitting the 3000 digital points to a first order rate equation by conventional methods. The rate constant, k_0, for *p*NPA deacylation was similarly determined in the presence of buffer alone. Deacylation rates were also determined in the presence of $NH_2(CH_2)_nNH_2$ ($n = 2$, 3 and 6) and in the presence of $\beta CDNH(CH_2)_nNH_2$ and adamantane-1-carboxylate (AC^-). All rate constants were determined in triplicate at least.

The ^1H (300 MHz) and ^{13}C (74.57 MHz) NMR spectra of $\beta CDNH(CH_2)_nNHCOCH_3$ were run on a Varian Gemini 300 spectrometer, and ^1H ROESY (mixing time of 0.35 s)[13] 600 MHz spectra of the $\beta CDNH(CH_2)_6NH_2{\cdot}AC^-$ complex were run on a Varian Inova 600 spectrometer. The spectral assignments listed below are given according to the numbering in Fig. 1 and the alphabetical labelling of the methylene groups in the diaminoalkyl substituent from (a) for that adjacent to the secondary amino group. The 600 MHz ^1H NMR spectrum of a solution 0.1 mol dm^{-3} in $\beta CDNH(CH_2)_6NH_2$ and AC^- in D_2O at pH ≥ 11, where the dominant species is the [$\beta CDNH(CH_2)_6NH_2{\cdot}AC^-$] complex, is characterised by: δ_H 4.65 (m, 7H, H1), 3.81 (t, *J* 10.2 Hz, 1H, $H5^A$), 3.5–3.8 (m, 25H, H3, H5, H6), 3.2–3.4 (m, 13H, H2, H4), 3.06 (t, *J* 10.2 Hz, 1H, $H4^A$), 2.92 (d, *J* 14.0 Hz, 1H, $H6^A$), 2.58 (dd, *J* 14.0, 10.2 Hz, 1H, $H6^{A\prime}$), 2.42 (m, 2H, hexyl Hf), 2.23 (dt, *J* 5.4, 10.8 Hz, 1H, hexyl Ha), 2.14 (m 1H, hexyl Ha′), 1.99 (br s, 3H, AC^- H3), 1.76 (br s, 6H, AC^- H2), 1.69 (br d, *J* 10.8 Hz, 3H, AC^- H4), 1.45 (br d, *J* 10.8 Hz, 3H, AC^- H4′), 1.0–1.4 (m, 8H, hexyl Hb–e). In the ^1H ROESY spectrum, δ_H 1.45 (AC^- H4′) shows cross-peaks with 1.69 (AC^- H4), 1.99 (AC^- H2), 3.53 (H5); 3.7 (H3), 1.69 (AC^- H4) shows cross-peaks with 1.45 (AC^- H4′), 1.99 (AC^- H2), 3.53 (H5), 3.7 (H3); 1.76 (AC^- H2) shows cross-peaks with 1.99 (AC^- H2); 3.53 (H5), 3.7 (H3); 1.99 (AC^- H3) shows cross-peaks with 1.45 (AC^- H4′), 1.69 (AC^- H4), 1.76 (AC^- H2), 3.53 (H5), 3.7 (H3); 2.23 (hexyl Ha) shows cross-peaks with 2.42 (hexyl Hf), 2.92 ($H6^A$), 3.81 ($H5^A$); 2.42 (hexyl Hf) shows cross-peaks with 2.23 (hexyl Ha), 3.81 ($H5^A$); 2.58 ($H6^{A\prime}$) shows cross-peaks with 2.92 ($H6^A$), 3.06 ($H4^A$); 2.92 ($H6^A$) shows cross-peaks with 2.58 ($H6^{A\prime}$), 3.81 ($H5^A$); 3.53 (H5) shows cross-peaks with 1.45 (AC^- H4′), 1.69 (AC^- H4), 1.76 (AC^- H2), 1.99 (AC^- H3); 3.7 (H3) shows cross-peaks with 1.45 (AC^- H4′), 1.69 (AC^- H4), 1.76 (AC^- Hb), 1.99 (AC^- H3); 3.81 ($H5^A$) shows cross-peaks with 2.23 (hexyl Ha), 2.42 (hexyl Hf), 2.92 ($H6^A$).

Molecular modelling[14] was carried out using a Silicon Graphics Iris Indigo X2 400 Unix workstation. Computational results were obtained using the force-field programme CVFF incorporating the 6–12 ε function with geometric averages for the heteronuclear interactions. Energy minimisations were performed with the Discover programme, using a steepest descents algorithm until the root mean square of the residuals (RMS) derived was <10, whereafter a conjugate gradients algorithm was used until RMS < 1 and the global minimisation was obtained using a quasi-Newton–Raphson algorithm. Several local energy minima were found before the global minimum was reached. Graphical displays were printed through the Insight II molecular modelling programme.

Acylation reactions

To aid the identification of the products of deacylation of *p*NPA accelerated by $\beta CDNH(CH_2)_nNH_2$, it was necessary to prepare the most probable products as described below. The primary aim of these preparations was to use the products as aids to identification of the products obtained in the kinetic studies under aqueous conditions. Hence, the preparations were not optimised for yield.

Acylation of 6^A-(2-aminoethyl)amino-6^A-deoxy-β-cyclodextrin

A mixture of $\beta CDNH(CH_2)_2NH_2$ (0.100 g, 0.085×10^{-3} mol) and *p*NPA (0.015 g, 0.082×10^{-3} mol) in 1-methyl-2-pyrrolidone (NMP, 2 cm^3) was stirred at room temperature for 18 hours. TLC showed the presence of βCD and a new spot ($R_c = 1.06$). The yellow solution was diluted with 1 mol dm^{-3} HCl (30 cm^3) and washed with dichloromethane (5 × 20 cm^3). The water was evaporated under reduced pressure and the residue was solidified by addition of ethanol and collected by vacuum filtration. The crude product was dissolved in water (5 cm^3) and loaded onto a column of BioRex 70 (NH_4^+ form). The column was washed with water and fractions (8 cm^3) were taken. Fractions containing $\beta CDNH(CH_2)_2NHCOCH_3$ were combined and dried to give a white powder (0.021 g, 21%); $R_c = 1.06$; Electrospray-MS 1219 (M^+) (Found C, 40.43; H, 6.38; N, 1.86. Calculated for $\beta CDNH(CH_2)_2NHCOCH_3{\cdot}HCl{\cdot}6H_2O$ ($C_{46}H_{91}ClN_2O_{41}$) C, 40.52; H, 6.72; N, 2.05%); $\delta_H(D_2O)$ 5.07 (s, 7H, H1), 3.8–4.0 (m, 26H, H3, H5, H6), 3.5–3.7 (m, 13H, H2, H4), 3.43 (t, *J* 9.3 Hz, 1H, $H4^A$), 3.31 (m, 2H, CH_2NCOCH_3), 3.06 (d, *J* 11.7 Hz, 1H, $H6^A$), 2.77 (m, 3H, $H6^{A\prime}$, CH_2NH), 1.99 (s, 3H, methyl); $\delta_C(D_2O)$ 177.05 (C=O), 104.57, 104.21 (C1), 86.32 ($C4^A$), 83.90, 83.56 (C4), 75.84, 74.83, 74.60 (C2, C3, C5), 73.11 ($C5^A$), 63.04 (C6), 51.64 ($C6^A$), 50.14 (ethyl Ca), 41.26 (ethyl Cb), 24.63 (methyl).

Acylation of 6^A-(3-aminopropyl)amino-6^A-deoxy-β-cyclodextrin

A mixture of $\beta CDNH(CH_2)_3NH_2$ (0.096 g, 0.086×10^{-3} mol) and *p*NPA (0.016 g, 0.088×10^{-3} mol) in NMP (2 cm^3) was stirred at room temperature for 18 hours. TLC showed the presence of βCD and a new spot ($R_c = 1.1$). The yellow solution was diluted with 1 mol dm^{-3} hydrochloric acid (30 cm^3) and washed with dichloromethane (5 × 10 cm^3). The water was evaporated under reduced pressure and the residue was solidified by addition of ethanol and collected by vacuum filtration. The crude product was dissolved in water (5 cm^3) and loaded onto a column of BioRex 70 (NH_4^+ form). The column was washed with water and fractions (8 cm^3) were taken. Fractions 4–14, containing $\beta CDNH(CH_2)_3NHCOCH_3$, were combined and evaporated to dryness to give a white powder (0.048 g, 45%); $R_c = 1.1$; Electrospray-MS 1233 (M^+) (Found C, 41.63; H, 6.81; N, 2.23. Calculated for $\beta CDNH(CH_2)_3NHCOCH_3{\cdot}HCl{\cdot}5H_2O$ ($C_{47}H_{91}ClN_2O_{40}$) C, 41.52; H, 6.74; N, 2.06%); $\delta_H(D_2O$, 25 °C) 5.06 (s, 7H, H1), 3.8–4.0 (m, 26H, H3, H5, H6), 3.5–3.7 (m, 14H, H2, H4), 3.0–3.5 (m, 5H), 2.80 (m, 0.3H), 2.58 (t, *J* 6.9 Hz, 0.6H), 2.00, 1.98 (s, 3H, methyl ratio 2:1), 1.68 (m, 2H, propyl Hb); $\delta_C(D_2O)$ 176.69, 167.46 (C=O), 104.82, 104.33 (C1), 86.57 ($C4^A$), 83.92, 83.52 (C4), 75.94, 74.95, 74.67 (C2, C3, C5), 72.67 ($C5^A$), 65.38, 63.03 (C6), 51.86 ($C6^A$), 48.52 (propyl Ca), 39.95 (propyl Cc), 30.93 (propyl Cb), 24.64 (methyl); $\delta_H(D_2O$, 50 °C) 5.35 (s, 7H, H1), 4.0–4.3 (m, 26H, H3, H5, H6), 3.8–4.0 (m, 13H, H2, H4), 3.68 (t, *J* 8.9 Hz, 1H, $H4^A$), 3.47 (t, *J* 6.9 Hz, 2H, CH_2NAc), 3.30 (d, *J* 11.9 Hz, 1H, $H6^A$), 3.04 (dd, *J* 11.9, 7.1 Hz, 1H, $H6^{A\prime}$), 2.87 (t, *J* 6.9 Hz, 2H, CH_2NH), 2.26 (s, 3H, methyl), 1.94 (br s, 2H, propyl Hb).

Acylation of 6^{A}-(6-aminohexyl)amino-6^{A}-deoxy-β-cyclodextrin

A mixture of βCDNH$(CH_2)_6NH_2$ (0.101 g, 0.082×10^{-3} mol) and *p*NPA (0.014 g, 0.078×10^{-3} mol) in NMP (2 cm^3) was stirred at room temperature for 18 hours. TLC showed the presence of βCD and a new spot ($R_c = 1.2$). The yellow solution was diluted with 1 mol dm^{-3} HCl (30 cm^3) and washed with dichloromethane (5×20 cm^3). The water was evaporated under reduced pressure and the residue was solidified by addition of ethanol and collected by vacuum filtration. The crude product was dissolved in water (5 cm^3) and loaded onto a column of BioRex 70 (NH_4^+ form). The column was washed with water and fractions (8 cm^3) were taken. Fractions containing βCDNH-$(CH_2)_6NHCOCH_3$ were combined and dried to give a white powder (0.018 g, 18%); $R_c = 1.2$; Electrospray-MS 1275 (M^+) (Found C, 42.84; H, 6.64; N, 1.99. Calculated for βCDNH-$(CH_2)_6NHCOCH_3 \cdot HCl \cdot 5H_2O$ ($C_{50}H_{97}ClN_2O_{40}$) C, 42.84; H, 6.97; N, 2.00%); $\delta_H(D_2O)$ 5.07 (s, 7H, H1), 3.5–4.0 (m, 39H, H2, H3, H4, H5, H6), 3.40 (t, *J* 9.0 Hz, 1H, H4^{A}), 3.16 (t, *J* 7.2 Hz, 2H, CH_2NHCOCH$_3$), 3.05 (d, *J* 12.6 Hz, 1H, H6^{A}), 2.76 (m, 1H, H6$^{A'}$), 2.58 (t, *J* 7.2 Hz, 2H, CH_2NH), 1.99 (s, 3H, methyl), 1.2–1.6 (m, 8H, hexyl Hb, hexyl Hc, hexyl Hd, hexyl He); $\delta_C(D_2O)$ 176.51 (C=O), 104.81, 104.73, 104.61, 104.43, 103.36 (C1), 85.72 (C4^{A}), 84.03, 83.89, 83.75, 82.83 (C4), 76.48, 76.04, 75.85, 75.65, 74.79, 74.45 (C2, C3, C5), 71.24 (C5^{A}), 63.04, 62.90 (C6), 50.47 (C6^{A}), 49.20 (hexyl Ca), 42.11 (hexyl Cf), 31.26, 29.92, 28.81, 28.70 (hexyl Cb, hexyl Cc, hexyl Cd, hexyl Ce), 24.76 (methyl).

Results and discussion

Deacylation kinetics in the presence of βCDNH$(CH_2)_nNH_2$

The deacylation kinetic data are first discussed in terms of the mechanism proposed in Fig. 2 where a rapid preequilibrium between βCDNH$(CH_2)_nNH_2$ (**1**) and *p*NPA (**2**) results in the reactive complexes **3a** and **3b** which lead to the products βCDNH$(CH_2)_nNHCOCH_3$ (**4**) and βCDNH$(CH_2)_nNH_2$ acylated at a secondary hydroxy group (**5**). The *p*NPA deacylation rate varies with pH according to eqn. (1) where k_0 is the rate constant observed at a particular pH in either 0.05 mol dm^{-3} borate or HEPES aqueous buffer at 298.2 K and $I = 0.10$ mol dm^{-3} ($NaClO_4$) over the pH range 6.08–10.03 (Table 1). The observed rate constant for the deacylation of *p*NPA in excess [βCDNH$(CH_2)_nNH_2]_{total}$ at a given pH is k_{obs}, and $k_{obs} - k_0$ is the acceleration of the deacylation of *p*NPA (Table 1). The βCDNH$(CH_2)_nNH_2$ bearing the shortest and longest substituents ($n = 2$ and 6) were selected to determine the form of the deacylation pH dependence which is considered to encompass the characteristics of the βCDNH$(CH_2)_nNH_2$ series. This deacylation is greatly accelerated as pH is increased when $n = 2$ and 6 consistent with βCDNH$(CH_2)_nNH_2$ being the dominant nucleophile such that $k_{obs} - k_0$ varies according to eqns. (2) and (3), where [**3**] refers to either **3a** or **3b** or both depending on their reactivities, to give $k_dK = 27.4 \pm 0.2$ and 16.0 ± 0.2 dm^3 mol^{-1} s^{-1} when $n = 2$ and 6, respectively. (When the $k_{obs} - k_0$ data is fitted to an equation analogous to eqn. (2), but including a term in [βCDNH$(CH_2)_nNH_3^+$], the derived rate constants characterising βCDNH$(CH_2)_nNH_3^+$ are <5% of that characterising βCDNH$(CH_2)_nNH_2$. Accordingly βCDNH$(CH_2)_nNH_3^+$ is judged to be an insignificant participant in the deacylation process.) Thus k_0 and the rate are given by eqns. (1) and (2),

$$k_0 = 5.43 \times 10^{(0.823pH - 12)} \quad (1)$$

$$\text{rate} = (k_{obs} - k_0)[p\text{NPA}]_{total} = k_d[\mathbf{3}] = k_dK[p\text{NPA}][\mathbf{1}] = k_dK[p\text{NPA}][\mathbf{1}]_{total}/(1 + [H^+]/K_a) \quad (2)$$

where $[\mathbf{1}] \approx [\mathbf{1}]_{total}$ because $[\mathbf{1}]_{total} \gg [p\text{NPA}]_{total}$, and $K = [\mathbf{3}]/([p\text{NPA}][\mathbf{1}])$ and K_a is the acid dissociation constant of βCDNH-$(CH_2)_nNH_3^+$. Given eqn. (3), it follows that when $1 \gg K[\mathbf{1}]$,

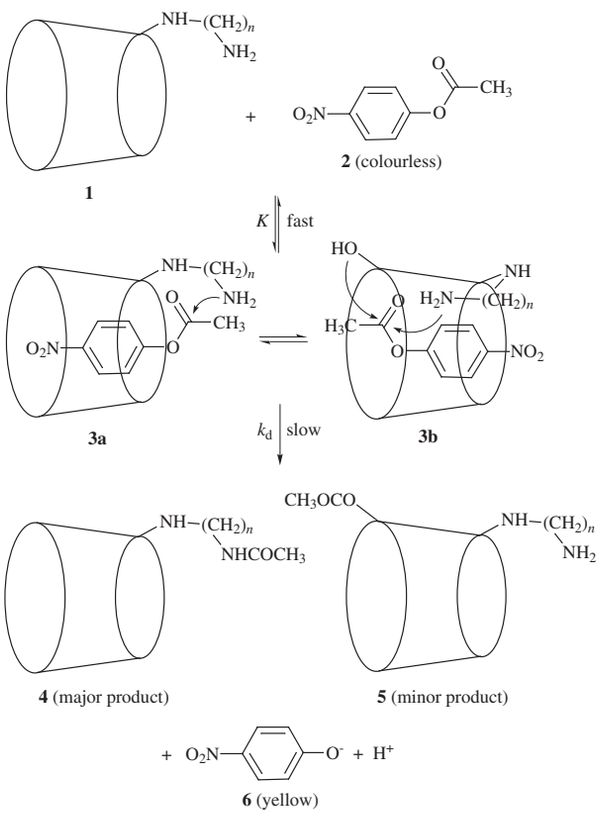


Fig. 2 Scheme for the deacylation of *p*NPA, **2**, by βCDNH-$(CH_2)_nNH_2$, **1**. The truncated cones represent the βCD annulus where the wide face is delineated by 14 secondary hydroxy groups and the narrow face by 6 primary hydroxy groups and the secondary amine group of the (ω-aminoalkyl)amino substituent. Two opposed orientations are shown for *p*NPA in the annulus in the complexes, **3a** and **3b**, where the curved arrows indicate possible directions of nucleophilic attack. The minor product, **5**, was detected when $n = 6$.

Table 1 Variation of the rate constants[a] for the deacylation of *p*NPA in the absence (k_0) and the presence of βCDNH$(CH_2)_nNH_2$ (k_{obs}) in aqueous buffered solutions at 298.2 K and $I = 0.10$ mol dm^{-3} ($NaClO_4$)

	$n = 2$[b]			$n = 6$[c]	
pH	k_0/ 10^{-5} s^{-1}	k_{obs}/ 10^{-3} s^{-1}	pH	k_0/ 10^{-5} s^{-1}	k_{obs}/ 10^{-3} s^{-1}
6.08[d]	0.055	0.13	7.89[e]	1.71	0.083
6.40[d]	0.10	0.19	8.37[e]	4.32	0.25
7.04[d]	0.34	0.40	8.73[e]	8.67	0.58
7.88[d]	1.66	0.91	9.10[e]	17.7	1.13
8.04[e]	2.24	1.48	9.41[e]	28.4	1.86
8.44[d]	4.78	2.88	9.73[e]	57.9	3.63
8.78[e]	9.11	4.61	10.03[e]	97.3	5.91
9.31[e]	24.9	9.79	10.3[e]	187	10.8
9.81[e]	64.1	16.48			
10.03[e]	97.3	19.49			

[a] Each rate constant represents the average of three determinations. The error is < ± 4%. [b] [βCDNH$(CH_2)_2NH_2$] = 8.12×10^{-4} mol dm^{-3}. [c] [βCDNH$(CH_2)_6NH_2$] = 9.77×10^{-4}-mol dm^{-3}. [d] HEPES buffer. [e] Borate buffer.

$$k_{obs} - k_0 = k_dK[p\text{NPA}][\mathbf{1}]/([p\text{NPA}] + [\mathbf{3}]) = k_dK[\mathbf{1}]/(1 + K[\mathbf{1}]) \quad (3)$$

$(k_{obs} - k_0)/[\mathbf{1}] \approx (k_{obs} - k_0)/[\mathbf{1}]_{total} \approx k_dK$ consistent with the linear dependence of $k_{obs} - k_0$ on $[\mathbf{1}]_{total}$ at pH 9.10 and 298.2 K seen in Fig. 3 where $n = 2$, 3, 4 and 6. The slopes: $(k_{obs} - k_0)/[\beta\text{CDNH}(CH_2)_nNH_2]_{total}) = 7.03 \pm 0.05$, 3.57 ± 0.02, 1.15 ± 0.04, and 0.90 ± 0.01 dm^3 mol^{-1} s^{-1} for $n = 2$, 3, 4 and 6, respectively. When these values are corrected to reflect the proportions of $[\beta\text{CDNH}(CH_2)_nNH_2]_{total}$ existing as the uncharged and

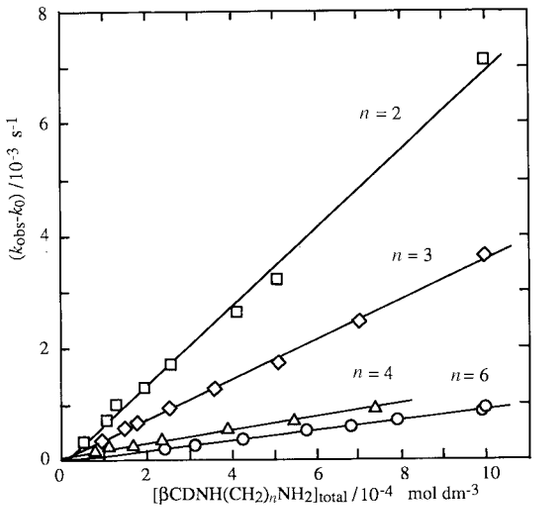


Fig. 3 The variation of $k_{obs} - k_0$ for the deacylation of *p*NPA with $[\beta CDNH(CH_2)_nNH_2]_{total}$ for $n = 2$ (□), 3 (◇), 4 (△) and 6 (○) at 298.2 K in borate buffer at pH 9.10 and $I = 0.10$ mol dm^{-3} ($NaClO_4$). The solid lines represent linear least-square fits of $(k_{obs} - k_0)/[\beta CDNH(CH_2)_nNH_2]_{total} = k_dK$ to the data.

dominant nucleophile at pH 9.10, $\beta CDNH(CH_2)_nNH_2$, $k_dK = 25.5 \pm 0.2$, 35.5 ± 0.1, 24.5 ± 0.8, and 26.4 ± 0.1 dm^3 mol^{-1} s^{-1} when $n = 2$, 3, 4 and 6. The k_dK obtained when $n = 2$ is in reasonable agreement with that obtained from the pH dependency studies. However, $k_dK = 25.0$ dm^3 mol^{-1} s^{-1} is greater than $k_dK = 16.0$ dm^3 mol^{-1} s^{-1} obtained from the pH dependency studies. The reason for this is probably that when $n = 2$ the $\beta CDNH(CH_2)_nNH_2$ constitutes 27.54% of the total at pH 9.10 whereas when $n = 6$ $\beta CDNH(CH_2)_nNH_2$ constitutes 3.40% of the total and as a consequence small errors in pH measurement have a substantial effect on the calculation of k_dK at pH 9.10 in the latter case. Because the pH range employed in the pH dependence studies extends to higher pH values the k_dK derived from them when $n = 6$ is probably more reliable. [At pH 9.10, the percentage of $\beta CDNH(CH_2)_nNH_2$ existing in the un-, mono- and di-protonated states are: 27.54, 72.43 and 0.04; 10.05, 87.54 and 2.41; 4.70, 85.49 and 9.82; and 3.40, 63.35 and 33.25, where $n = 2$, 3, 4 and 6, respectively. The pK_as of $\beta CDNH_2(CH_2)_nNH_3^{2+}$ are: 5.70 and 9.42, 7.39 and 9.90, 8.06 and 10.26, and 8.72 and 10.27 where $n = 2$, 3, 4 and 6, respectively.[10]] The product identification studies discussed below show that the dominant CD product of the deacylation reactions is $\beta CDNH_2(CH_2)_nNHC)CH_3$ (**4**) and that when $n = 6$, ≤ 25% of the CD product could be $\beta CDNH_2(CH_2)_nNH_2$ acylated at a secondary hydroxy group (**5**).

The reported acceleration of *p*NPA deacylation caused by 6^A-amino-6^A-deoxy-β-cyclodextrin[15] ($\beta CDNH_2$, X = NH_2 in Fig. 1), where $k_dK = 7$ dm^3 mol^{-1} s^{-1} (calculated from $k_dK = 6$ dm^3 mol^{-1} s^{-1} reported for pH 10 and p$K_a = 9.20$ for $\beta CDNH_3^+$) is less than those caused by $\beta CDNH(CH_2)_nNH_2$ by ≥ 2. [It is not possible to apportion the increased reactivity of $\beta CDNH(CH_2)_nNH_2$ between k_d and K as these parameters were not separately determined.] Overall, this is consistent with the flexible $-NH(CH_2)_nNH_2$ substituent allowing the primary amine to more effectively make a nucleophilic attack on the carbonyl carbon of *p*NPA. This flexibility allows the $-NH(CH_2)_6NH_2$ substituent to enter the annulus of $\beta CDNH(CH_2)_6NH_2$ and those of its complexes with benzoate, 4-methylbenzoate and (*RS*)-2-phenylpropanoate as shown from ^{1}H NMR and modelling studies,[11] and the modelling studies discussed below are consistent with this occurring in the $\beta CDNH(CH_2)_6NH_2$·*p*NPA complex. [The deacylation of 3-nitrophenyl acetate (*m*NPA) in the presence of $\beta CDNH(CH_2)_6NH_2$ occurs much more slowly than does that of *p*NPA and produces 25% $\beta CDNH(CH_2)_6NHCOCH_3$ and 75% $\beta CDNH_2(CH_2)_nNH_2$ acylated at a secondary hydroxy group at pH 9.10. Acylation at a secondary hydroxy group is also the dominant pathway through which βCD[7] and $\beta CDNH_2$[15] deacylate *m*NPA.]

An alternative explanation of the kinetic data is that deacylation occurs through an S_N2 mechanism such that eqn. (4)

$$\text{rate} = (k_{obs} - k_0)[p\text{NPA}]_{total} = k_2[p\text{NPA}]_{total}[\beta CDNH(CH_2)_nNH_2]_{total}/(1 + [H^+]/K_a) \quad (4)$$

applies and $k_2 = 27.4$, 35.5, 24.5 and 16.0 dm^3 mol^{-1} s^{-1} when $n = 2$, 3, 4 and 6, respectively. This is further discussed under *Deacylation inhibition*.

Deacylation kinetics in the presence of βCD and $NH_2(CH_2)_nNH_2$

The effects of the components of $\beta CDNH(CH_2)_nNH_2$ on the deacylation of *p*NPA may be assessed to some extent through a direct comparison of the effect of βCD and $NH_2(CH_2)_nNH_2$ on the deacylation of *p*NPA with that of $\beta CDNH(CH_2)_nNH_2$. Thus, at pH 9.10 in borate buffer, $I = 0.10$ mol dm^{-3} ($NaClO_4$) at 298.2 K, $k_{obs} - k_0 = (0.14 \pm 0.01) \times 10^{-3}$ s^{-1} in the presence of excess $[\beta CD] = 1.00 \times 10^{-3}$ mol dm^{-3}, which compares with $k_{obs} - k_0 = (7.03 \pm 0.28) \times 10^{-3}$, $(3.57 \pm 0.14) \times 10^{-3}$, $(1.15 \pm 0.046) \times 10^{-3}$ and $(0.90 \pm 0.04) \times 10^{-3}$ s^{-1} under the same conditions for $\beta CDNH(CH_2)_nNH_2$ where $n = 2$, 3, 4 and 6, respectively ($k_0 = 0.20 \times 10^{-3}$ s^{-1}). This provides an empirical indication of the acceleration of the *p*NPA deacylation caused by the $-NH(CH_2)_nNH_2$ substituent. A similar empirical comparison of the effect of the $-NH(CH_2)_nNH_2$ substituent may be made on the basis of $k_{obs} - k_0 = (0.69 \pm 0.03) \times 10^{-3}$, $(1.15 \pm 0.05) \times 10^{-3}$ and $(1.08 \pm 0.04) \times 10^{-3}$ s^{-1} where $n = 2$, 3 and 6, respectively, for the deacylation of *p*NPA in the presence of excess $NH_2(CH_2)_nNH_2$ ($[NH_2(CH_2)_nNH_2]_{total} = 1.00 \times 10^{-3}$ mol dm^{-3}) under the same conditions as the deacylations discussed above. At pH 9.10 the percentages of these diamines existing as $NH_2(CH_2)_nNH_2$, and $NH_2(CH_2)_nNH_3^+$ are 11.8 and 87.2, 2.0 and 56.3, and 0.1 and 10.3 where $n = 2$, 3 and 6, respectively. [The pK_as of $NH_3(CH_2)_nNH_3^{2+} = 7.16$ and 9.97, 8.97 and 10.56, and 10.04 and 11.01 where $n = 2$, 3 and 6, respectively.[10]] It is assumed that $NH_2(CH_2)_nNH_2$, and possibly $NH_2(CH_2)_nNH_3^+$ also, are nucleophiles for *p*NPA on which basis it is seen that when the relative proportions of $\beta CDNH(CH_2)_nNH_2$ and $NH_2(CH_2)_nNH_2$ and $NH_2(CH_2)_nNH_3^+$ at pH 9.10 are taken into account, the free diamines approach or exceed the nucleophilicities of their $\beta CDNH(CH_2)_nNH_2$ analogues toward *p*NPA.

Acylated product identification

Deacylation of *p*NPA by $\beta CDNH(CH_2)_nNH_2$ gives 4-nitrophenolate and a dominant modified CD identified as $\beta CDNH(CH_2)_nNHCOCH_3$. This was shown by preparing samples of $\beta CDNH(CH_2)_nNHCOCH_3$, where $n = 2$, 3 or 6, by reacting *p*NPA and $\beta CDNH(CH_2)_nNH_2$ in 1-methyl-2-pyrrolidone as discussed under *Experimental*. The $\beta CDNH(CH_2)_nNHCOCH_3$ products gave good analyses, and the substitution site was identified from ^{1}H NMR spectra. The resonances of the methylene group adjacent to the amide nitrogen in $\beta CDNH(CH_2)_nNHCOCH_3$ showed a downfield shift of 0.5–0.6 ppm (see *Experimental*) by comparison with the resonances of the methylene group adjacent to the primary nitrogen in $\beta CDNH(CH_2)_nNH_2$.[10] The methylene group adjacent to the secondary nitrogen and the 6^A proton of the βCD moiety showed no significant change in δ compared with that in $\beta CDNH(CH_2)_nNH_2$.[10] This was taken as evidence that the acylation site was the primary amine in $\beta CDNH(CH_2)_nNH_2$. TLC analyses of $\beta CDNH(CH_2)_nNHCOCH_3$, of a reacted aqueous reaction mixture (buffered at pH 9.10 with borate, where [*p*NPA] approached $[\beta CDNH(CH_2)_nNH_2]_{total}$), $\beta CDNH(CH_2)_nNH_2$, and βCD were run simultaneously. The $\beta CDNH(CH_2)_nNHCOCH_3$ and the major products from the aqueous reaction

mixture had identical R_c values quite different from those for βCDNH$(CH_2)_nNH_2$ and βCD. On this basis the predominant acylated product from the aqueous reaction was identified as βCDNH$(CH_2)_nNHCOCH_3$. In the case of the TLC study of the βCDNH$(CH_2)_6NH_2$ system, a faint spot was observed just ahead of the βCDNH$(CH_2)_6NHCOCH_3$ spot, and was thought to be βCDNH$(CH_2)_nNH_2$ acylated at a secondary hydroxy group, consistent with its identification by ^{1}H NMR spectroscopy as discussed below.

The ^{1}H NMR spectra of the reaction mixture of βCDNH$(CH_2)_6NH_2$ and either *p*NPA or *m*NPA in borate buffer at pH 9.10, freeze dried and redissolved in D_2O, showed the methyl resonance of βCDNH$(CH_2)_nNHCOCH_3$ at 1.99 ppm and that of βCDNH$(CH_2)_nNH_2$ acylated at a secondary hydroxy group at 2.17 ppm in the ratios 25:75 and 75:25, respectively. The latter resonance disappeared when the pH of the solution was raised ≥12 consistent with rapid deacylation of the second product. The assignment of acylation occurring at a secondary hydroxy group is based on a similar assignment for the deacylation of *p*NPA and *m*NPA by βCD where a deprotonated secondary hydroxy group (pK_a ≈12) is thought to be the nucleophile.[7]

Deacylation inhibition

At pH 9.10 in borate buffer at 298.2 K, the acylation of βCDNH$(CH_2)_nNH_2$ is inhibited in the presence of adamantane-1-carboxylate. When $[\beta CDNH(CH_2)_nNH_2]_{total} = 1.00 \times 10^{-3}$ mol dm^{-3}, $k_{obs} - k_0 = (6.33 \pm 0.05) \times 10^{-3}$ s^{-1} which decreases to $(3.14 \pm 0.05) \times 10^{-3}$ s^{-1} when $[AC^-]_{total} = 5.0 \times 10^{-4}$ mol dm^{-3} where $n = 2$. The corresponding values are $(2.73 \pm 0.05) \times 10^{-3}$ s^{-1} and $(1.65 \pm 0.05) \times 10^{-3}$ s^{-1} where $n = 3$, and $(0.69 \pm 0.03) \times 10^{-3}$ s^{-1} and $(0.53 \pm 0.03) \times 10^{-3}$ s^{-1} where $n = 6$. In terms of the mechanism shown in Fig. 2, these decreases in $k_{obs} - k_0$ by 50.4, 39.6 and 23.2% where $n = 2$, 3 and 6, respectively, are consistent with AC^- competing with *p*NPA for complexation by βCDNH$(CH_2)_nNH_2$ and forming a βCDNH$(CH_2)_nNH_2$·AC^- complex. This decreases the proportion of *p*NPA complexed as βCDNH$(CH_2)_nNH_2$·*p*NPA which is the Michaelis-like complex through which *p*NPA deacylation is thought to occur (**3a** and **3b** in Fig. 2). Thus, while inhibition is quantitative for βCDNH$(CH_2)_2NH_2$ which has the shortest substituent, the decreasing effectiveness of the deacylation inhibition by AC^- as *n* increases to 3 and 6 is attributable to AC^- competing less effectively with the simultaneous complexation of *p*NPA and the –NH$(CH_2)_nNH_2$ substituent as the bulk and hydrophobicity of the latter increases with *n*. Evidence for the simultaneous complexation of *p*NPA and the –NH$(CH_2)_nNH_2$ substituent is adduced from reported NMR studies[10] showing the simultaneous complexation of either benzoate, 4-methylbenzoate or (*R*,*S*)-2-phenylpropanoate and the –NH$(CH_2)_nNH_2$ substituent by βCDNH$(CH_2)_nNH_2$, and is consistent with the modelling studies discussed below.

An alternative explanation of the inhibition data is that AC^- is complexed quantitatively in the βCDNH$(CH_2)_nNH_2$ annulus and the ability of the expelled –NH$(CH_2)_nNH_2$ substituent to make a nucleophilic attack on *p*NPA outside the annulus increases with its length so that the decreased deacylation occurring with decreasing βCDNH$(CH_2)_nNH_2$·*p*NPA formation is partially offset by this alternative S_N2 reaction pathway. Should the alternative S_N2 deacylation mechanism operate both in the absence and the presence of AC^- such that βCDNH$(CH_2)_nNH_2$ operates as a sterically hindered diamine the effect of the complexation of AC^- on the magnitude k_2 is not readily predicted. However, it seems unlikely that the quantitative inhibition by AC^- observed for βCDNH$(CH_2)_2NH_2$ would occur.

Adamantane-1-carboxylic acid (pK_a = 6.90 in 50% aqueous ethanol)[16] has a low solubility in aqueous solution which effectively limits aqueous studies to the more soluble AC^-. Because

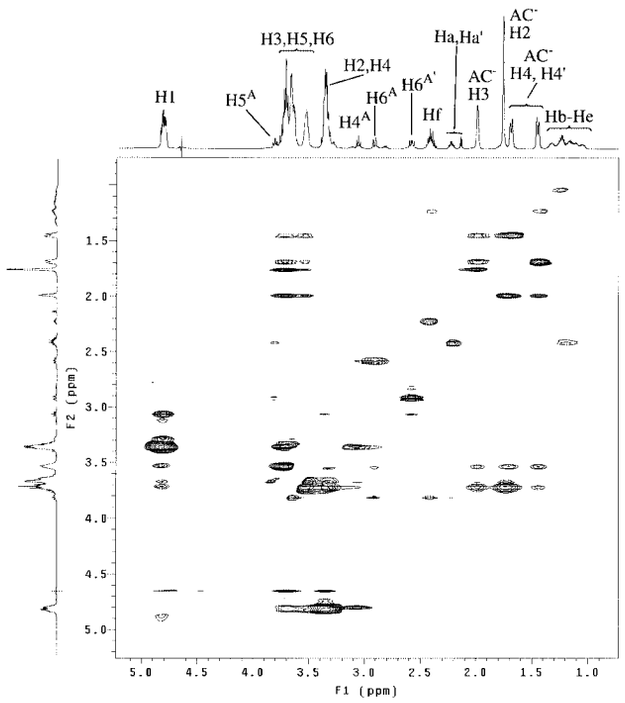


Fig. 4 600 MHz ^{1}H NMR NOESY spectrum of βCDNH$(CH_2)_6NH_2$·AC^- in D_2O at pH ≥ 11.

of the consequently restricted pH range of study, a direct determination of the stability of the βCDNH$(CH_2)_nNH_2$·AC^- complexes by the potentiometric titration methods which we have previously employed for complexation of carboxylic acids and their conjugate bases by βCDNH$(CH_2)_nNH_2$,[11] is precluded. However, calorimetric studies[17] give a stability constant $K = 1.96 \times 10^4$ mol dm^{-3} for the βCD·AC^- complex and it appears from the NMR studies discussed below that similarly stable AC^- complexes are formed by βCDNH$(CH_2)_nNH_2$.

^{1}H NMR studies of the complexation of AC^- by βCDNH$(CH_2)_6NH_2$

The ^{1}H NMR spectrum of an equimolar 0.1 mol dm^{-3} solution of βCDNH$(CH_2)_6NH_2$ and AC^- (Fig. 4 and *Experimental*) shows increased differentiation of the ^{1}H resonances of βCDNH$(CH_2)_6NH_2$ by comparison with those reported[11] in the absence of AC^- consistent with the substantial formation of the βCDNH$(CH_2)_6NH_2$·AC^- complex. [In particular, the fine structure exhibited by the resonances of hexyl Hb–He contrasts with the two broad resonances for hexyl Hb and He, and hexyl Hc and Hd observed in the absence of AC^- where –NH$(CH_2)_6NH_2$ is complexed inside the annulus.[11]] Reported 600 MHz ^{1}H NOESY NMR studies[11] are consistent with strong interaction between the protons (hexyl Ha–Hf) of the four inner methylene groups of the –NH$(CH_2)_6NH_2$ moiety and the H3 and H5 protons on the inside of the βCDNH$(CH_2)_6NH_2$ annulus as shown by strong cross-peaks. These interactions are absent from the NOESY ^{1}H NMR spectrum of βCDNH$(CH_2)_6NH_2$·AC^-. However, significant cross-peaks are observed between Ha and Hf (Table 2 and *Experimental*) consistent with the –NH$(CH_2)_6NH_2$ moiety assuming a coiled configuration outside the annulus adjacent to the primary face as shown in Fig. 5. Strong cross-peaks are observed for the interactions between H3 and H5 and AC^- H2 -H4 (Table 2 and *Experimental*) consistent with AC^- occupying the annulus. While the deacylation of *p*NPA precludes ROESY NMR studies show that its complexes, such studies of the unreactive 4-methylbenzoate and (*R*)- and (*S*)-2-phenylpropanoate complexes of βCDNH$(CH_2)_6NH_2$ all exist with both the guest and the –NH$(CH_2)_6NH_2$ substituent complexed inside the annulus as is also shown through modelling these complexes.[11] Support

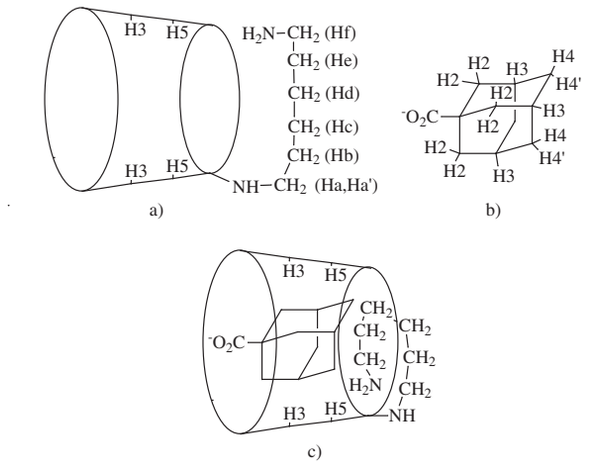


Fig. 5 Representations of: a) βCDNH$(CH_2)_6NH_2$ showing the hydrogen numbering scheme, b) AC^- showing the hydrogen numbering scheme, and c) the βCDNH$(CH_2)_6NH_2$·AC^- complex showing a possible orientation of AC^-.

Table 2 ^{1}H NMR Cross-peaks[a] observed for the adamantane-1-carboxylate complex of [βCDNH$(CH_2)_6NH_2$

	AC^- H2	AC^- H3	AC^- H4	Ha	Hf
H3	++	++	++		
H5	+	+	+		
Ha					+
Hf				+	

[a] The intensity of the cross-peaks increases from + to ++.

for similar structures for the βCDNH$(CH_2)_6NH_2$·*p*NPA complex is adduced from the modelling studies below.

Modelling of the βCDNH$(CH_2)_6NH_2$ complexes

Force field modelling in the gas phase shows the complex with *p*NPA inside the annulus and oriented with its acetate group adjacent to the secondary face of βCDNH$(CH_2)_6NH_2$, where the –NH$(CH_2)_6NH_2$ substituent is also complexed inside the annulus (Fig. 6), to have a globally minimised energy of 971.6 kJ mol^{-1}. The carbonyl carbon of *p*NPA and the nitrogen of the primary amine group are in close proximity (3.65 Å). The analogous complex where the *p*NPA is orientation is reversed has a slightly lower energy of 958.9 kJ mol^{-1}, and the carbonyl carbon of *p*NPA and the nitrogen of the primary amine group are more distant from each other (5.13 Å) However, the small energy difference between the isomeric complexes is consistent with the coexistence of both species. (These complexes correspond to **3a** and **3b**, respectively, in Fig. 2.) The isomers of both complexes where the –NH$(CH_2)_6NH_2$ substituent is outside the annulus are relatively unstable as shown by their higher energies of 1224.8 and 1243.0 kJ mol^{-1}, respectively. Thus, the two lower energy forms of the *p*NPA complex of βCDNH$(CH_2)_6NH_2$ where the –NH$(CH_2)_6NH_2$ substituent is complexed inside the annulus modelled in the gas phase appear plausible representations of the βCDNH$(CH_2)_nNH_2$·*p*NPA ground state Michaelis complexes in solution which lead to transition states whose $\Delta\Delta G^{\ddagger}$ determines the product ratio.[18,19] Modelling of the complexes of βCDNH$(CH_2)_nNH_2$ and *m*NPA yields global energy minimised structures of *m*NPA oriented with its acetate group adjacent to the primary and secondary faces of βCDNH$(CH_2)_6NH_2$ with energies of 941.9 and 874.6 kJ mol^{-1}, respectively.

Modelling also shows the complexation of AC^- by βCDNH$(CH_2)_6NH_2$ to exclude the –NH$(CH_2)_6NH_2$ substituent from the annulus to yield complexes with energies of 1025.5 kJ mol^{-1} where the carboxylate group is oriented towards the secondary face (Fig. 7) and 1111.4 kJ mol^{-1} where the AC^- orientation is reversed. The –NH$(CH_2)_6NH_2$ substituent lies along one side of the rim of the primary face in each case and does not show the close proximity of the methylene groups adjacent to the primary and secondary amine groups that appears to be consistent with the interpretation of the 1H NMR ROESY data. This difference may reflect the influence of hydration on the substituent orientation in aqueous solution.

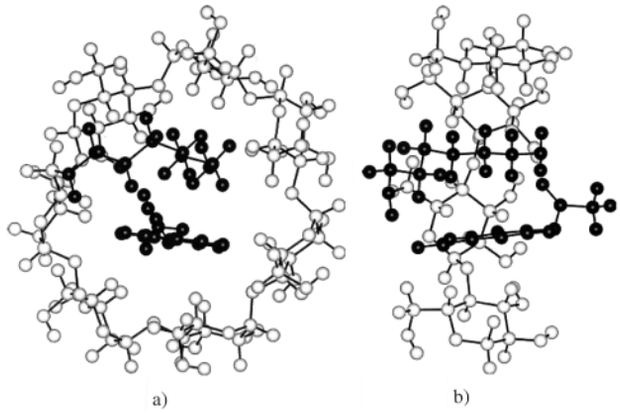


Fig. 6 The global energy minimised structure of βCDNH$(CH_2)_6NH_2$·*p*NPA viewed from a) the primary end of the annulus and b) from the side with three glucopyranose units cut away. The –NH$(CH_2)_6NH_2$ substituent and *p*NPA are shown in dark shading.

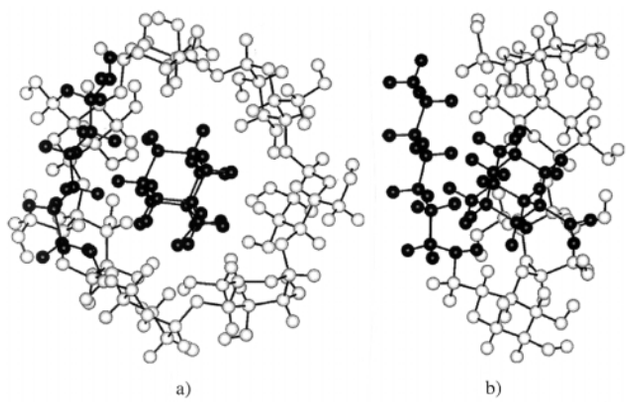


Fig. 7 The global energy minimised structure of βCDNH$(CH_2)_6NH_2$·AC^- viewed from a) the primary end of the annulus and b) from the side with three glucopyranose units cut away. The –NH$(CH_2)_6NH_2$ substituent and AC^- are shown in dark shading.

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