Towards Structurally Responsive Synthetic Macrocyclic Receptors for Acetylcholine

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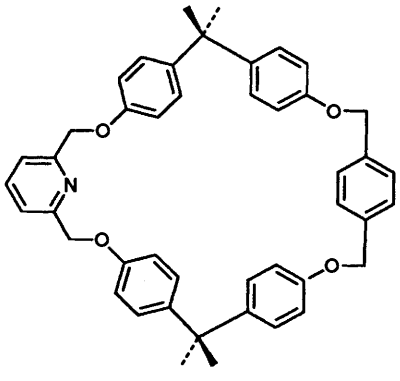
The synthesis and complexation behaviour of structurally related neutral and ionic cyclophanes is reported. A neutral pyridino-cyclophane selectively mediates transport of acetylcholine while ionic receptors incorporating both a pyridine moiety and a carboxyphenyl group form stronger complexes in aqueous THF as revealed by ^{1}H NMR spectroscopy. These ionic receptors form 1:1 complexes with the substrate.

Acetylcholine is an important neurotransmitter.[1] In the crystal structure of its esterase the binding site is lined with electron-rich aromatic residues.[2] It is now believed that the acetylcholine head group may be bound by stabilising 'NC–H $\cdots$ π' interactions between the aromatic π electrons and the $\overset{+}{N}Me_3$ hydrogen-bond donor.[3] The general assumption prior to this structural analysis was that the $\overset{+}{N}Me_3$ head group would be bound by anionic sites (*e.g.*, Glu residues) within the enzyme. This had inspired the synthesis of a series of functionalised cyclophanes incorporating, for example, anionic tartaric acid moieties.[4,5] Cyclophanes incorporating maleate sub-units have also been prepared wherein the only possible binding interaction in water involves charge solvation of the acetylcholine guest by aryloxy groups in the host molecule.[6]

With this in mind we have prepared the simple cyclophanes **1** and **2** which incorporate 'bis-phenol A' flanking groups. Such rigid sub-units have been used quite often in cyclophane design.[7,8] Both a neutral ligand, **1** and an ionic, **2** (anionic or zwitterionic), cyclophane were chosen. The former may probe the need for electrostatic *vs.* π-donor charge interactions in binding 'onium ions and in the latter case, not only did simple molecular modelling experiments suggest that acetylcholine could be included, but also the pyridyl group offered a potential site for acyl transfer, leading to ester hydrolysis.

Synthesis.—The synthesis of **1** and **2** was undertaken in a stepwise manner from the mono-protected tetrahydropyranyl ether, **4**. Reaction of **4** with α,α'-dibromo-*p*-xylene in refluxing ethanol in the presence of K_2CO_3 yielded the bis-ether **5a** (75%) which was deprotected in quantitative yield (HCl, MeOH–$CHCl_3$) to give the bis-phenol **5b**. In an analogous fashion, reaction of 2,6-bis(bromomethyl)pyridine with **4**, yielded **6a** from which **6b** was readily obtained. Reaction of the bis-phenol **5b** with 2,6-bis(bromomethyl)pyridine (EtOH, K_2CO_3) yielded the desired cycle **1** in a reasonable yield (47%), while **2a** was prepared under similar conditions by reaction of **6b** with methyl 2,6-bis(bromomethyl)benzoate, **7**, in a yield of 31%. Attempts to improve the yield of these macrocyclisation reactions by using Cs_2CO_3 in place of K_2CO_3 did not succeed. For example, the preparation of **2a**, using Cs_2CO_3 instead of K_2CO_3 reduced the isolated yield from 31% to 2%. Base hydrolysis of **2a** (0.1 mol dm^{-3} NaOH, 7 days reflux, 90% aqueous ethanol) yielded the sodium salt **2c** which was soluble in tetrahydrofuran. The salt was also soluble in chloroform but was not stable in solution over a period of hours. Acidification of **2a** (HCl, aqueous THF) yielded the monohydrochloride salt from which the zwitterion **2b** was prepared by reaction with propylene oxide. The different protonated species **2b**, **2c** and **2d** were distinguished by their IR spectra: for **2b** a broad carboxylate stretch at 1650 cm^{-1} and a pyridinium NH stretch at 3435 cm^{-1} were discerned while the hydrochloride salt **2d** gave a typical acid carbonyl stretch at 1719 cm^{-1}.

Complexation Studies.—The neutral ionophore **1** was incorporated into a PVC–*o*-nitrophenyloctyl ether based membrane electrode and electrode response studies were undertaken, with various 'onium ion analytes according to standard published procedures.[9] A Nernstian response to acetylcholine was observed (59 mV change in emf per decade change in acetylcholine activity down to 50 μmol dm^{-3} at 298 K), and interference from sodium, calcium and ammonium ions (evaluated using the fixed interference method, at 0.1 mol dm^{-3} concentration) was negligible (Table 1) with the slope being maintained at 59 mV. Slight interference from potassium



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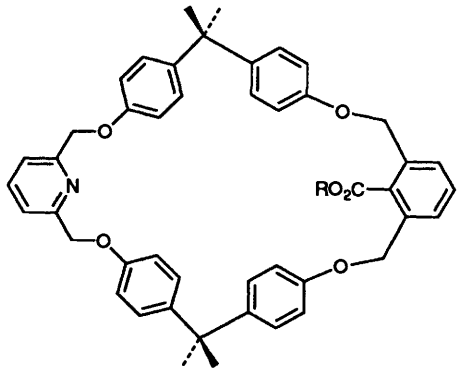
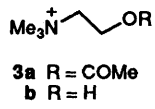
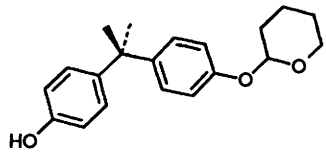


2a R = Me
b R = H
c R = Na
d R = H, hydrochloride salt



3a R = COMe
b R = H



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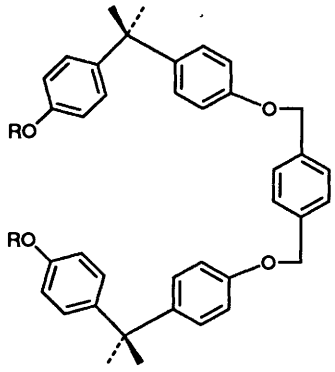
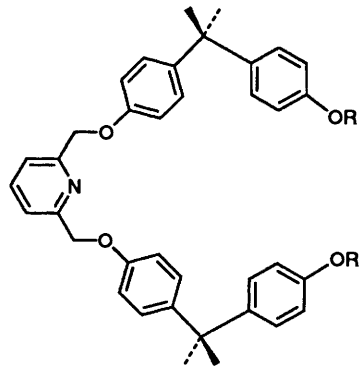


5a R = tetrahydropyran-2-yl
b R = H

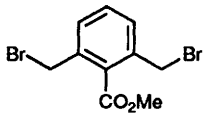


6a R = 2-THP
b R = H

Table 1 Characteristics of membrane electrode incorporating **1**–PVC–*o*NPOE–*p*ClTKB[a]

Interferent ion (0.1 mol dm^{-3})	Electrode slope (mV $decade^{-1}$)	Selectivity coefficient ($-\log K_{ij}^{pot}$)	Limit of detection (mol dm^{-3})
Na^+	59.5	4.2	$10^{-5.2}$
Ca^{2+}	60	4.7	$10^{-5.2}$
K^+	55	3.8	$10^{-4.8}$
NH_4^+	58	4.0	$10^{-5.0}$

[a] Composed of (% by weight): 65% *o*-nitrophenyl octyl ether, 32.8% PVC, 1.2% **1** and 0.4% potassium tetrakis(*p*-chlorophenyl)borate.



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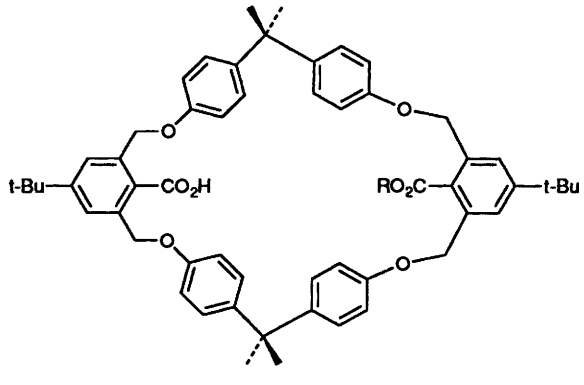
was noted (slope reduced to 55 mV), but pH interference was much more marked and no response to changing acetylcholine activity could be detected in the pH range 2–4. This was not unexpected, as the pyridyl nitrogen was >99% protonated under these conditions leading to coulombic repulsion upon encounter with acetylcholine. In further control experiments, the electrode response to acetylcholine in the absence of ionophore was studied but only a modest slope (42 mV) and a poor limit of detection (10^{-3} mol dm^{-3}) were observed, and the response to both $\overset{+}{N}Me_4$ and $\overset{+}{N}Et_4$ was rather modest (slopes of 39 and 37 mV, respectively) perhaps due to the poorer induced fit of these ions, by the cavitand.

These experiments indicate that the neutral ionophore **1** is capable of mediating the transport of acetylcholine, with some selectivity, across an aqueous–membrane interface. The binding of acetylcholine chloride by **1** was further studied by ^1H NMR spectroscopy ($CDCl_3$). Only a slight line-broadening of the resonances due to the host and guest was observed over a range of stoichiometries, indicative of weak binding in this solvent system.

In principle, the cyclophanes **1** and **2** may take up two low-energy conformations depending upon the relative orientation of the pyridyl and phenyl/carboxyphenyl groups. For example with **2**, in the conformation with the pyridyl nitrogen and carboxy groups convergent and more-or-less coplanar, the molecule adopts a 'cupped arrangement' suitable for substrate inclusion. Alternatively, the groups may diverge with the nitrogen lone-pair and carboxy group oriented inwards but above and below the plane of the four flanking aryloxy groups. This conformation is not suitable for substrate inclusion. This analysis has been confirmed in the case of **8**, for which a crystal



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structure has been reported of the ethanol solvate wherein the carboxy groups converge,[8,10] although a conformation of lower energy (by 17.5 kJ mol^{-1}) (with divergent carboxy groups in a centrosymmetric structure) was pinpointed by molecular mechanics calculations.

The sodium salt of the anionic macrocycle was examined in

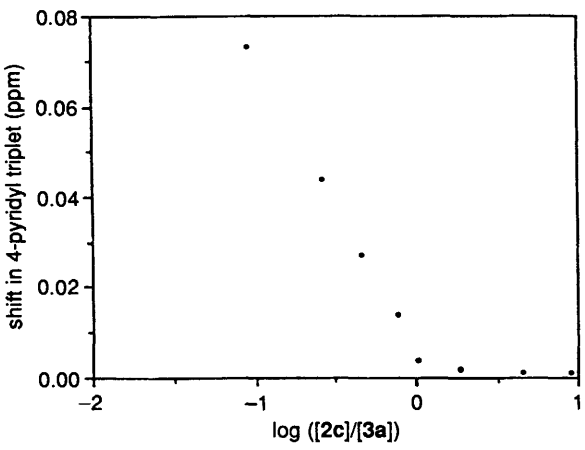

Fig. 1 Variation in the ^{1}H NMR shift in the 4-pyridyl proton resonance in **2c** with addition of acetylcholine, **3a** (293 K; 98% [$^2H_8$]THF–2% D_2O)

deuterio–THF (2% D_2O to solubilise) by ^{1}H NMR spectroscopy over the temperature range 223–313 K. All of the ligand resonances were relatively unchanged over this range of temperature. The major change in the spectrum was that due to the solvent (HOD) which changed from a sharp resonance at 3.15 ppm, at 313 K, broadened considerably around 273 K (*ca.* 3.5 ppm) and between 253 K and 223 K was a sharp resonance at 3.68 ppm. These changes are probably related to the degree of hydrogen bonding to the ligand, but no definite conclusions can be reached about the precise ligand hydration state.

Incremental addition of acetylcholine to the anion **2c** (in 98% [$^2H_8$]THF–2% D_2O) was monitored by ^{1}H NMR spectroscopy at 293 K and many of the host protons shifted to higher frequency. For example the shift of the 4-pyridyl proton (resonating as a triplet) moved by 0.08 ppm and broadened and when plotted against the ratio of host : acetylcholine this clearly indicated that a 1:1 complex had formed (Fig. 1) After 0.5 equivalents of acetylcholine had been added the sample was cooled to 273 K and two distinct resonances for the 4-pyridyl proton were discerned. At 293 K, considerable exchange broadening ($\omega_{\frac{1}{2}} \approx 15$ Hz) of the proton signals (400 MHz) due to both free and bound acetylcholine was discerned, indicating that these species were in relatively slow exchange on the NMR timescale at this temperature. Solubility problems limited the amount of acetylcholine which could be added.

Addition of choline trifluoroacetate (**3b**; *n.b.* choline is the product of hydrolysis of acetylcholine) to **2c** in [$^2H_8$]THF–D_2O (98 : 2) resulted in shifts and broadened signals for choline were observed up to a stoichiometry of 10:1 at 200 MHz, consistent with fast exchange between free and bound guest at 293 K. None of these broadened resonances corresponded to those observed in the complex between acetylcholine and **2c**. Indeed addition of a tenfold excess of choline trifluoroacetate to the 1:1 [**2c**·acetylcholine] complex led to the formation of the exchange-broadened choline complex with release of unbound acetylcholine into solution.

Complexation of the zwitterion, **2b**, with acetylcholine was also monitored by ^{1}H NMR spectroscopy under parallel conditions. Again a plot of the change in the shift of the *para* pyridinium proton *versus* added substrate concentration indicated that a 1:1 complex had formed, but in this case, only one set of exchange-broadened resonances for acetylcholine was observed over the range 0.1–5 : 1 guest–host. At a 1 : 2 guest–host stoichiometry, a variable temperature run showed that at 233 K signals due to the host in the free and bound state could be discerned. For example one of the $ArCH_2O$ signals (integrating to four protons in total) appeared as two broadened singlets at 5.45 and 5.10 ppm. At the same time the 4-pyridyl proton also split and resonated as two singlets at 7.66 and 7.58 ppm (233 K). A coalescence of these two signals was observed to occur at around 273 K.

The resonance due to the pyridinium N–H was evident throughout these studies with the zwitterion **2b**, resonating as a broad singlet at *ca.* 11 ppm at 298 K which sharpened upon lowering the temperature to −50 °C (+11.3 ppm). The lack of H/D exchange (with the 2% D_2O co-solvent) of this pyridinium proton is consistent with the macrocycle adopting a conformation in which the N–H proton is directed inside the cavity of the macrocycle in a relatively hydrophobic environment. This behaviour is consistent with the host adopting a conformation in which the pyridyl and carboxyaryl groups converge, although it does not prove it.

Conclusions

The neutral ionophore **1** potentially has an electron-rich aromatic binding pocket for acetylcholine. Although binding of acetyl choline by **1** was too weak to be characterised unambiguously by ^{1}H NMR spectroscopy in deuteriochloroform, the macrocycle did mediate the transport of the substrate across an aqueous/PVC-based membrane and could be used therefore as an ionophore in a PVC membrane-based potentiometric ion-selective electrode. Electrode response studies suggested that there was little or no interference from sodium, calcium and ammonion ions, and a Nernstian response to acetylcholine was observed, down to 10 μmol dm^{-3}.

The anionic cyclophane **2c** formed a 1:1 complex with acetylcholine in 98 : 2 [$^2H_8$]THF–D_2O. The observation of free and bound signals for acetylcholine (albeit significantly exchange broadened at 293 K) is indicative of a relatively strong complex ($\log K \geqslant 4$). No evidence for formation of choline was found following binding of the ester acetylcholine.

The zwitterionic receptor **2b** also formed a 1:1 complex with acetylcholine, and at 293 K appeared to bind the guest less strongly than the anion **2c**.

Experimental

Fabrication of the membrane and electrode response studies were carried out as described in ref. 9. 2,6-Bis(bromomethyl)-pyridine and methyl 2,6-bis(bromomethyl)benzoate were prepared according to literature methods.[11,12] ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC250, a Varian VXR400S or a Varian Gemini 200 instrument. Infrared spectra were recorded as thin films using a Perkin-Elmer 1720X FTIR spectrometer. Mass spectra were recorded using a VG7070E spectrometer operating in desorption chemical ionisation (dci) or fast-atom bombardment (fab) ionisation modes.

O-Tetrahydropyran-2-yl-4,4′-isopropylidenediphenol, (**4**).—4,4′-Isopropylidenediphenol (bis-phenol-A, 45.24 g, 0.20 mol) was dissolved in ether (300 cm^3) and the solution cooled to 0 °C in an ice bath. Concentrated HCl was added (10 drops) followed by 3,4-dihydro-2*H*-pyran (18 cm^3; 0.20 mol). The solution was stirred at 0 °C for 40 min and then allowed to warm to room temperature and stirred for a further 2 h. The flask was stoppered and left to stand at room temperature overnight. The solution was washed with 0.1 mol dm^{-3} $KHCO_3$ (3 × 50 cm^3), dried over magnesium sulfate and filtered and the ether removed from the filtrate at reduced pressure to give a very viscous, clear, colourless oil. This was purified by flash column chromatography (silica gel; 1:4 ethyl acetate–hexane) to give the monoether as a colourless oil (26.2 g, 42%). R_f = 0.21 (silica gel; 1:4 ethyl acetate–hexane) (Found: C, 76.8; H, 7.8. $C_{20}H_{24}O_3$ requires C, 76.9; H, 7.69%); *m/z* 85, 102, 135, 213

and 228 (M^+); δ_H($CDCl_3$; fully assigned using COSY NMR experiment 7.14–7.07 (4 H, m), 6.94 (2 H, d, J = 8.2 Hz, part of aromatic AA'BB'), 6.70 (2 H, d, J = 8.0 Hz, part of aromatic AA'BB'), 5.39 (1 H, t, OCHO), 5.09 (1 H, br s, OH), 3.94 (1 H, m, OC*H*H'CH_2), 3.61 (1 H, m, OCH*H*'CH_2), 2.00 (1 H, m, aliphatic CH_2), 1.85 (2 H, m, aliphatic CH_2), 1.70–1.57 (3 H, m, aliphatic CH_2) and 1.62 (6 H, s, CCH_3); δ_C($CDCl_3$; 100 MHz) 154.8 and 153.3 (aromatic C–OR), 144.1 and 143.1 [aromatic *C*–C(CH_3)$_2$], 127.9 and 127.6 (aromatic C–H), 115.8 and 114.7 (aromatic C–H), 96.5 (OCHO), 62.2 (O*C*H_2C), 41.7 [Ar_2*C*(CH_3)$_2$], 31.0 (C*C*H_3), 30.4 (O_2CH*C*H_2), 25.2 (OCH_2*C*H_2) and 18.9 (aliphatic CH_2); ν_{max}/ cm^{-1} 3369 (O–H), 2963 (C–H), 2871 (C–H), 1610 (aromatic C–C), 1510 and 1236.

2,2,10,10-*Tetramethyl*-1^4,11^4-*di*(*tetrahydropyran*-2-*yloxy*)-4,8-*dioxa*-1,3(1,4),6(1,4),9(1,4),11-*pentabenzenaundecaphane* (**5a**).—α,α'-Dibromo-*p*-xylene (0.81 g, 3.1 mmol) and the tetrahydropyranyl ether, **4** (1.90 g, 6.1 mmol) were dissolved in hot ethanol (50 cm^3) and potassium carbonate (1.87 g, 14 mmol) was added. The stirred suspension was heated at reflux for 18 h and then filtered while hot. The white solid residue was partitioned between $CHCl_3$ and water (40 cm^3 each). The chloroform layer was washed with 0.01 mol dm^{-3} HCl (15 cm^3) and then water (2 × 40 cm^3), dried (Na_2SO_4) and filtered. The solvent was removed at reduced pressure and the residue was dried under vacuum to give a white solid, 1.76 g (78%) (Found: C, 79.0; H, 7.25. $C_{48}H_{54}O_6$ requires: C, 79.3%, H, 7.48%); m.p. 120–122 °C; *m*/*z* 85, 135, 213, 331 and 558 (M^+ − 2THP); δ_H($CDCl_3$; 250 MHz) 7.43 (4 H, s, phenyl ring), 7.16–7.09 (8 H, m, part of phenyl ether AA'BB' system), 6.97–6.84 (8 H, m, part of phenyl ether AA'BB' system), 5.37 (2 H, t, J = 7.5 Hz, OCHO), 5.02 (4 H, s, $ArCH_2O$), 3.92 (2 H, m, OC*H*H'), 3.59 (2 H, m, OCH*H*'), 1.98 (2 H, m, aliphatic CH_2), 1.83 (4 H, m, aliphatic CH_2), 1.63 (18 H, s, m, overlapping, CCH_3 and aliphatic CH_2 groups).

2,2,10,10-*Tetramethyl*-1^4,11^4-*di*(*tetrahydropyran*-2-*yloxy*)-4,8-*dioxa*-6^2-*aza*-1,3(1,4),6(1,3),9(1,4),11-*pentabenzenaundecaphane* (**6a**).—Monoprotected bis-phenol-A, **4** (21.16 g, 0.07 mol) and 2,6-bis(bromomethyl)pyridine (9.01 g, 0.034 mol) were dissolved in ethanol (120 cm^3). Potassium carbonate was added and the suspension stirred at reflux for 20 h. On completion of the reaction (absence of starting materials on analytical TLC), the suspension was filtered while hot. The filtrate was left to stand overnight at room temperature. The resulting white precipitate was filtered off, washed on the filter with cold ethanol (3 × 20 cm^3) and dried under vacuum to give a fine white powder (18.37 g, 74%). M.p. 122 °C, R_f = 0.68; (silica gel; 1% methanol in dichloromethane) (silica; 4:1 hexane–ethyl acetate) R_f = 0.30 (Found: C, 77.45; H, 7.35; N, 1.9. $C_{47}H_{53}NO_6$ requires C, 77.6; H, 7.29; N, 1.93%); δ_H($CDCl_3$; 400 MHz) 7.73 (1 H, t, J = 8.0 Hz, B of A_2B in pyridine ring), 7.46 (2 H, d, J = 8 Hz, A of A_2B in pyridine ring), 7.17–7.10 (8 H, m, aromatic CH), 6.97–6.86 (8 H, m, aromatic CH), 5.38 (2 H, t, J = 6.3 Hz, OCHO), 5.17 (4 H, s, $ArCH_2O$), 3.92 (2 H, m, OC*H*H'CH_2), 3.63–3.56 (2 H, m, OCH*H*'CH_2), 2.04–1.94 (2 H, m, aliphatic CH_2), 1.84 (4 H, m, aliphatic CH_2) and 1.70–1.55 (18 H, s, m overlapping, aliphatic CH_2 groups and CH_3); δ_C($CDCl_3$; 100 MHz) 156.9 (aromatic, pyridine ring, *ortho* to N), 156.2 and 154.9 (aromatic C–O), 143.9 and 143.7 [aromatic *C*–C(CH_3)$_2$], 137.6 (aromatic, pyridine ring, *para* to N), 127.8 and 127.6 (aromatic C–H, biphenyl), 120.0 (aromatic, pyridine ring, *meta* to N), 115.7 and 114.0 (aromatic C–H, phenyl ether), 96.4 (OCHO), 70.5 ($ArCH_2O$), 62.1 (OCH_2), 41.7 [Ar_2-*C*(CH_3)$_2$], 31.0 (CH_3), 30.4 (O_2CH*C*H_2), 25.2 (OCH_2*C*H_2) and 18.9 (aliphatic CH_2); ν_{max}/cm^{-1} 2944 (C–H), 2864 (C–H), 1518, 1254, 1190 and 981.

2,2,10,10-*Tetramethyl*-4,8-*dioxa*-6^2-*aza*-1,3(1,4),6(1,3),9(1,4),11-*pentabenzenaundecaphane*-1^4,11^4-*diol* (**6b**).—Compound **6a** (18.25 g, 0.025 mol) was weighed into a round-bottomed flask and 20 cm^3 of methanol were added. Chloroform (15 cm^3) was added to the stirred suspension in small portions until **6a** dissolved. Concentrated HCl was added (30 cm^3), causing phase separation to occur. Small amounts of methanol and $CHCl_3$ were added until the reaction was homogeneous (total volumes: methanol, 60 cm^3; $CHCl_3$, 100 cm^3; HCl, 30 cm^3) and the clear solution was heated overnight at reflux. The reaction was cooled to room temperature after which methanol and $CHCl_3$ were removed on a rotary evaporator, resulting in the formation of a white precipitate. This was basified with saturated aqueous KOH, causing further precipitation. The precipitate was extracted into ether (500 cm^3) and the organic phase was washed with 0.1 mol dm^{-3} KOH (2 × 50 cm^3) followed by water (5 × 50 cm^3), dried (Na_2SO_4) and filtered and the solvent was evaporated off at reduced pressure. The residue was dried under vacuum to leave a white solid, mass 13.47 g (96%). M.p. 69–70 °C, R_f = 0.05 (silica gel; 4:1 hexane–ethyl acetate; *m*/*z* 334, 560 (M^+ + 1) (Found: C, 79.6; H, 6.75; N, 2.4. $C_{37}H_{37}NO_4$ requires C, 79.4; H, 6.62; N, 2.50%); δ_H[$(CD_3)_2CO$] 7.85 (1 H, t, J 7.7, B of A_2B in pyridine ring), 7.49 (2 H, d, J 7.7, A of A_2B in pyridine ring), 7.17 (4 H, d, J = 9.0 Hz, aromatic C–H part of AA'BB' system), 7.06 (4 H, d, J = 8.6 Hz, aromatic C–H, part of AA'BB' system), 6.93 (4 H, d, J = 9.0 Hz, aromatic C–H, part of AA'BB' system), 6.73 (4 H, d, J = 8.6 Hz, aromatic C–H, part of AA'BB' system), 5.17 (4 H, s, $ArCH_2O$), 3.05 (2 H, br s, OH) and 1.60 (12 H, s, CH_3); δ_C[$(CD_3)_2CO$] 158.4 (aromatic, pyridine ring, *ortho* to N), 157.7 and 156.3 (aromatic C–OR), 145.0 (aromatic, phenyl ether, *para* to OH), 143.0 (aromatic, biphenyl, *para* to OH), 138.9 (aromatic, pyridine ring, *para* to N), 128.9 and 128.8 (aromatic C–H, phenyl ether), 121.5 (aromatic, pyridine ring, *meta* to N), 115.8 and 115.3 (aromatic C–H, phenyl ether), 71.7 ($ArCH_2O$), 42.5 [$ArC(CH_3)_2$] and 31.8 (CH_3); ν_{max}/cm^{-1} 3341 (O–H), 2983, (C–H), 1518, 1244, 1190 and 842.

2,2,10,10-*Tetramethyl*-4,8-*dioxa*-1,3(1,4),6(1,4),9(1,4),11-*pentabenzenaundecaphane*-1^4,11^4-diol (**5b**).—This was prepared in 98% yield from the corresponding bis(tetrahydropyranyl) ether by acid hydrolysis using a method identical with that used for the synthesis of **6a**. It was used directly in the next step without further characterisation; ν_{max}/cm^{-1} 3340 (O–H), 2983, 2862, 1515, 1240 and 1190; δ_H[$(CD_3)CO$] 7.9 (2 H, s, OH), 7.3–6.4 (20 H, m, ArH), 5.2 (4 H, s, $ArCH_2O$) and 1.5 (12 H, s, CCH_3).

2,2,10,10-*Tetramethyl*-4,8,12,16-*tetraoxa*-6^2-*aza*-1(1,4),3(1,4),6(1,3),9(1,4),11(1,4),14(1,4)-*hexabenzenacyclohexadecaphane* (**1**).—Diol **5b** (0.34 g, 0.60 mmol) and 2,6-bis(bromomethyl)-pyridine (0.16 g, 0.60 mmol) were dissolved in hot ethanol (70 cm^3). Potassium carbonate (0.34 g, 2.5 mmol) was added and the stirred solution was heated at reflux for 48 h. The hot solution was filtered and the solid residue washed with hot ethanol (3 × 15 cm^3), dried and taken up in H_2O (35 cm^3). This was extracted into ether (4 × 50 cm^3) and then $CHCl_3$ (4 × 50 cm^3). The organic phases were combined, dried (Na_2SO_4) and filtered. The solvent was removed at reduced pressure and the residue dried under vacuum to leave a white, crystalline solid, mass 0.19 g (47%). M.p. 101–103 °C (Found: C, 81.6; H, 6.55; N, 2.01. $C_{45}H_{44}NO_4$ requires C, 81.7; H, 6.51; N, 2.12%); *m*/*z* (EI) 122, 135, 152, 663 (*M* + 1) and 664; *m*/*z* (CI) 652, 662 (M^+), 663 and 664; δ_H($CDCl_3$; 300 MHz) 7.65 (1 H, t, J = 7.8 Hz, B of A_2B, pyridine ring), 7.38 and 7.35 (6 H, d + s, overlapping, A of A_2B in pyridine ring and ArH, phenyl ring), 7.18–7.05, (8 H, m, ArH, part of AA'BB' system), 6.82–6.77 (8 H, m, ArH, part of AA'BB' system), 5.09 (4 H, s, $ArCH_2O$), 4.95 (4 H, s, $ArCH'_2O$) and 1.56 (12 H, s, CCH_3).

Methyl 2,2,10,10-*Tetramethyl*-4,8,12,16-*tetraoxa*-6^2-*aza*-1(1,4),3(1,4),6(1,3),9(1,4),11(1,4),14(1,3)-*hexabenzenacyclohexadecaphane*-14^2-*carboxylate* (**2a**).—Potassium carbonate (0.39 g, 2.83 mmol), methyl 2,6-bis(bromomethyl)benzoate (0.11 g, 0.34 mmol) and compound **6a** (0.19 g, 0.34 mmol) were dissolved in ethanol (150 cm^3) and stirred at reflux for 48 h. The hot solution was filtered and ethanol removed from the filtrate at reduced pressure to leave a yellow solid. This was dried under vacuum and purified by flash column chromatography (silica gel; 4:1 hexane–ethyl acetate as the eluent) to give a white solid, mass 78 mg (31%). When repeated this reaction sometimes gave varying amounts of the ethyl ester as well as the methyl ester. M.p. 209–211 °C (for methyl ester) R_f = 0.24 (silica gel; 4:1 hexane–ethyl acetate); *m/z* (FAB) 108, 135, 334 and 734 (M^+ + 1) (Found: C, 78.35; H, 6.35; N, 1.8. $C_{48}H_{47}NO_6$ requires C, 78.58; H, 6.41; N, 1.91%); δ_H($CDCl_3$; 400 MHz) 7.66 (1 H, t, J = 7.6 Hz, B of A_2B in pyridine ring), 7.41–7.38 (3 H, m, t + d overlapping, A_2A' aromatic protons of benzoate) 7.33 (2 H, d, J = 7.6 Hz, A of A_2B in pyridine ring), 7.08 (4 H, m, part of AA'BB' system, diphenylmethane), 7.01 (4 H, m, part of AA'BB' system, diphenylmethane), 6.81–6.77 (8 H, d + d, overlapping AA'BB' systems of diphenylmethane), 5.18 and 5.19 (8 H, s + s, $ArCH_2O$ and $ArCH'_2O$), 3.42 (3 H, s, OCH_3) and 1.62 and 1.55 (12 H, s + s, CCH_3); δ_C($CDCl_3$; 100 MHz) 168.1 ($ArCO_2$), 157.3 (aromatic C–CH_2O, pyridine ring), 156.4 and 156.3 (aromatic C–O), 143.8 and 143.2 [aromatic *C*–$C(CH_3)_2$], 137.6 (aromatic C–H, pyridine ring, *para* to N), 136.1 (aromatic *C*–CO_2), 131.8 (aromatic *C*–CH_2O, benzoate ring), 129.9 (aromatic C–H, benzoate ring, *para* to ester group), 128.1 (aromatic C–H, benzoate ring, *meta* to ester group), 127.7 and 127.6 (aromatic C–H, diphenylmethane rings, *ortho* to O), 119.8 (aromatic C–H, pyridine ring, *meta* to N), 114.3 and 113.9 (aromatic C–H, diphenylmethane rings, *meta* to O), 70.9 and 68.1 ($ArCH_2O$ and $ArCH'_2O$), 53.4 (OCH_3) and 41.6 [Ar*C*$(CH_3)_2$], 30.8 (CCH_3); ν_{max}/cm^{-1} 2970 (C–H), 2868 (C–H), 1719 (C=O), 1601 (aromatic C–C), 1588 (aromatic C–C) and 1505.

2,2,10,10-*Tetramethyl*-4,8,12,16-*tetraoxa*-6^2-*aza*-1(1,4),3(1,4),6(1,3),9(1,4),11(1,4),14(1,3)-*hexabenzenacyclohexadecaphane*-14^2-*carboxylate* (**2c**).—The ester **2a** (170 mg, 0.23 mmol) was refluxed in 0.1 mol dm^{-3} NaOH in 90% ethanol (50 cm^3) for 2 weeks. The solution was cooled to room temperature and evaporated to dryness at reduced pressure to leave a white solid. This was taken up in 15 cm^3 dichloromethane and quickly washed with water (3 × 10 cm^3), dried (Na_2SO_4) and filtered. The solvent was immediately removed at reduced pressure to leave a white solid, which was dried under vacuum to give the sodium salt of the hydrolysed ester, **2c**, in quantitative yield; *m/z* (DCI) 135, 166, 193, 334, 560, 561, 706 (M^+ + 1) and 707; δ_H([$^2H_8$]THF–2% D_2O) 7.60 (1 H, t, J = 7.8 Hz, B of A_2B in pyridine ring), 7.26 (2 H, d, J = 8 Hz, aromatic C–H, A of A_2B system), 7.12 (2 H, d, J = 7.8 Hz, A of py. A_2B system), 6.93–6.66 (17 H, m, overlapping AA'BB' systems of phenyl ether and B of benzoate A_2B system), 5.20 (4 H, br s, $ArCH_2O$), 5.05 (4 H, s, $ArCH'_2O$) and 1.46 (12 H, s, CCH_3); ν_{max}/cm^{-1} 2962 (C–H), 1685 (CO), 1608 (aromatic C–C), 1581 (aromatic C–C) and 1509.

2,2,10,10-*Tetramethyl*-4,8,12,16-*tetraoxa*-6^2-*azonia*-1(1,4),-3(1,4),6(1,3),9(1,4),11(1,4),14(1,3)-*hexabenzenacyclohexadecaphane*-14^2-*carboxylate* (**2b**).—Sodium salt **2c** (44 mg, 6.1 × 10^{-5} mol) was stirred in 50:50 H_2O–THF (6 cm^3) to give a turbid solution. 6 mol dm^{-3} HCl (1.5 cm^3) was added, causing precipitation. This suspension was stirred overnight and the precipitate was collected by filtration and washed on the filter with water (4 × 5 cm^3). The white solid residue was allowed to dry on the filter, then washed through with the minimum of THF. The solvent was removed at reduced pressure and the residue dried under vacuum to give the hydrochloride salt, **2d**; δ_H([$^2H_8$]THF–2% D_2O) 7.64 (1 H, t, B of A_2B in pyridine ring), 7.32–7.21 (4 H, m, overlapping A's of A_2B in pyridine ring and benzoate), 7.00–6.61 (17 H, m, overlapping AA'BB's of phenyl ether and t of benzoate), 5.13 (4 H, s, $ArCH_2O$), 5.04 (4 H, br s, $ArCH'_2O$) and 1.26 (12 H, s, CCH_3); ν_{max}/cm^{-1} 2952 (C–H), 2857 (C–H), 1719 (C=O), 1605 (aromatic C–C), 1579 (aromatic C–C) and 1507.

The hydrochloride salt was dissolved in propylene oxide (15 cm^3) and stirred under nitrogen with precipitation occurring after about 15 min. The suspension was allowed to stir under nitrogen for a further 24 h, after which the solvent was removed at reduced pressure and the white solid residue was further dried under vacuum (0.1 mmHg) to give the title compound, 22.4 mg (52% from Na^+ salt); δ_H([$^2H_8$]THF) 7.55 (1 H, t, J = 8 Hz, B of A_2B in pyridine ring), 7.34–7.20 (5 H, m, A of A_2B in pyridine ring and A_2B of benzoate), 6.99–6.95 (8 H, m, part of aromatic AA'BB' systems), 6.73–6.70 (8 H, m, part of aromatic AA'BB' systems), 5.12 and 5.05 (8 H, br s + s $ArCH_2O$) and 1.52 (12 H, s, CCH_3); ν_{max}/cm^{-1} 3435 (N–H), 2976 (C–H), 1650 (CO_2^-) and 1503; *m/z* 87, 135, 334, 560 and 706 (M + 1).

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References

1 D. J. Triggle, *Neurotransmitter–Receptor Interactions*, Academic Press, New York, 1971.
2 J. L. Sussman, M. Harel, F. Frolow, C.Oefner, A. Goldman, L. Toker and I. Silman, *Science*, 1991, **255**, 872.
3 M. A. Petit, T. J. Shepodd, R. E. Barrass and D. A. Dougherty, *J. Am. Chem. Soc.*, 1988, **110**, 6825; S. K. Burley and G. A. Petsko, *FEBS Lett.*, 1986, **203**, 139.
4 M. Dhaenens, L. Lacombe, J.-M. Lehn and J.-P. Vigneron, *J. Chem. Soc., Chem. Commun.*, 1984, 1097.
5 R. Meric, J.-P. Vigneron and J.-M. Lehn, *J. Chem. Soc., Chem. Commun.*, 1993, 129.
6 D. A. Dougherty and D. A. Stauffer, *Science*, 1990, **250**, 1558.
7 K. Saigo, R. J. Lin, M. Kubo, A. Youda and M. Hasegawa, *J. Am. Chem. Soc.*, 1986, **108**, 1976.
8 K. Gloe, H. Stephan, O. Heitzsch, H. Bukowsky, E. Uhlemann, R. Pollex and E. Weber in *Solvent Extraction in Process Industries*, vol. 2, eds. D. H. Logsdail and M. J. Slater, Elsevier, London, 1993, p. 745.
9 P. S. Bates, R. Kataky and D. Parker, *Analyst*, 1994, **119**, 181.
10 K. Gloe, personal communication.
11 W. Baker, K. M. Buggle, J. F. W. McOmie and D. A. M. Watkins, *J. Chem. Soc.*, 1958, 3594.
12 M. Newcombe, S. S. Moore and D. J. Cram, *J. Am. Chem. Soc.*, 1977, **99**, 6405.

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