# Self-Assembling Systems of the Amphiphilic Cationic Per-6-amino-β-cyclodextrin 2,3-Di-O-alkyl Ethers

Hélène Parrot-Lopez,[1] Chang-Chun Ling,[2] Ping Zhang,[3] Adam Baszkin,[4] Geneviève Albrecht,[4] Colette de Rango,[2] and Anthony W. Coleman*[,3]

*Faculté de Pharmacie, Université de Paris V 75006 Paris, France*
*Centre Pharmaceutique, Université de Paris XI 92290 Châtenay-Malabry, France*

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The cyclodextrins (CD), well documented for their ability to form inclusion complexes with a wide range of guest molecules,[5] are cyclic oligosaccharides possessing a "pseudo face to face" symmetry arising from the presence of two hydrophilic faces surrounding a hydrophobic cavity (Figure 1).

Lipophilic substitution at only one face leads to the formation of amphiphilic molecules possessing a rigid two-dimensional polar adhesion surface. Modification of the primary face by attachment of thio- or aminoalkyl groups has allowed the preparation of Langmuir layers[6] and lyotropic liquid crystals.[7] Hydrophobic esterification at the secondary face allows the formation of mixed vesicles with phospholipids.[8] Molecular modeling analysis[9] of such molecules suggests a "rigid" cylinder of area ~250 Å$^2$ and the length for a $C_{12}$ alkyl chain of ~23 Å, a geometry conductive to the formation of bilayer systems.[10] We report the synthesis of per-6-amino-2,3-di-*O*-alkyl-β-cyclodextrin hydrochlorides (**6a**, hexyl; **6b**, dodecyl). These compounds form stable Langmuir layers and in tetrahydrofuran solution are present as large vesicles. Variable-temperature NMR studies show clear differentiation between chain mobilities. The retention of inclusion capacity is demonstrated by the complexation of *N*-acetylphenylalanine by **6a** in chloroform-*d*. The synthetic route to **6a** and **6b** is shown in Figure 2.

Per-6-bromo-β-cyclodextrin (**2**) is prepared by a modification of the Defaye[11] procedure; reaction of **2** with $NaN_3$ gives per-6-azido-β-CD (**3**), in 93% yield. Preliminary X-ray crystallographic studies show that in contrast to β-CD and mono-6-azido-β-CD,[12] which pack in a monomeric herringbone structure, **3** forms face to face hydrogen-bonded dimers,[13] while **2** represents a novel dimeric structural type.[14] The key stage, alkylation at O2 and O3, proceeds in 58% yield by treatment with NaH and the relevant

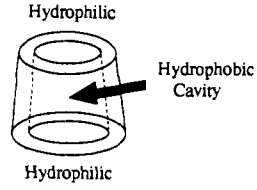


**Figure 1.**

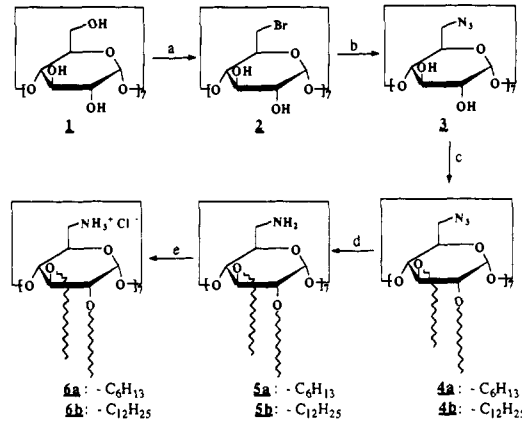


**Figure 2.** Synthetic route to per-6-amino-2,3-di-*O*-alkyl-β-cyclodextrin hydrochloride salts (**6a**, hexyl; **6b**, dodecyl).

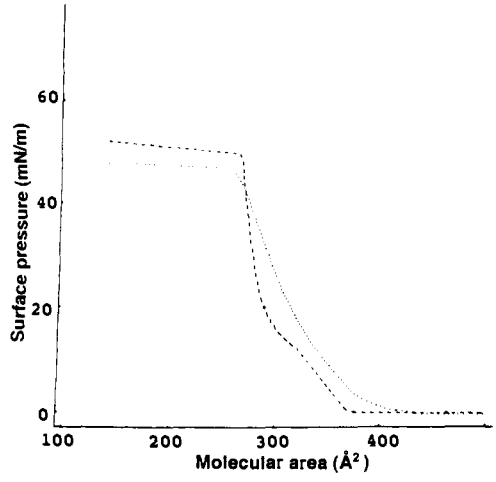


**Figure 3.** Compression isotherms of compounds **6a** (···) and **6b** (- - -) at the air/water interface.

bromoalkane (**4a**, **4b**). Reduction to the peramine by $PPh_3$/$NH_3$·$H_2O$ followed by treatment with aqueous HCl yields the title ammonium derivatives.[15]

Deposition of **6a** and **6b** on a Langmuir film balance gives stable monolayers with collapse pressures of 46 and 50 mN m$^{-1}$, respectively.[16] The pressure–area isotherms, shown in Figure 3, extrapolate to molecular areas of 260 (**6a**) and 266 Å$^2$ (**6b**) at collapse pressure, in agreement with the calculated molecular area

(1) Laboratoire de Chimie Organique 2, Faculté de Pharmacie, Université de Paris V, 75006 Paris, France.
(2) CNRS UPR 180, Laboratoire de Biophysique, Centre Pharmaceutique, Université de Paris XI, 92290 Châtenay-Malabry, France.
(3) CNRS SDI 6233, Laboratoire de Chimie Organique, Centre Pharmaceutique, Université de Paris XI, 92290 Châtenay-Malabry, France.
(4) CNRS URA 1218, Laboratoire de Physico-chimie des Surfaces, Centre Pharmaceutique, Université de Paris XI, 92290 Châtenay-Malabry, France.
(5) Szejtli, J. *Cyclodextrin Technology*; Kluwer Academic Publishers: Dordrecht, The Netherlands, 1988.
(6) (a) Kawabata, Y.; Matsumoto, M.; Tanaka, M.; Takahashi, H.; Irinatsu, Y.; Tamura, S.; Tagaki, W.; Nakahara, H.; Fukuda, K. *Chem. Lett.* **1986**, 1933. (b) Kawabata, Y.; Matsumoto, M.; Nakamura, T.; Tanaka, M.; Manda, E.; Takahashi, H.; Tamura, S.; Tagaki, W.; Nakahara, H.; Fukuda, K. *Thin Solid Films* **1988**, *159*, 353.
(7) Coleman, A. W.; Djedaini, F.; Perly, B. *Proceedings of the 5th International Symposium on Cyclodextrins*; Editions de Santé: Paris, 1990; p 328.
(8) Zhang, P.; Ling, C. C.; Coleman, A. W.; Parrot-Lopez, H.; Galons, H. *Tetrahedron Lett.* **1991**, *32*, 2769.
(9) Sybyl 5.10, Molecular Graphics Package, Tripos, St. Louis, MO.
(10) Mahendra, K. J. *Introduction to Biological Membranes*; John Wiley & Sons: New York, 1988.
(11) Gadelle, A.; Defaye, J. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 78.
(12) (a) β-CD: $P2_1$, $a$ = 15.123 Å, $b$ = 10.306 Å, $c$ = 20.898 Å, $\beta$ = 109.76°. Lindner, K.; Saenger, W. *Carbohydr. Res.* **1982**, *99*, 103. (b) Mono-6-azido-βCD: $P2_1$, $a$ = 15.044 Å, $b$ = 10.274 Å, $c$ = 20.846 Å, $\beta$ = 109.89°. Charpin, P.; Nicolis, I.; De Rango, C.; Coleman, A. W. *Acta Crystallogr.* **1990**, *46*, C169.
(13) Per-6-azido-β-CD: $P2_1$, $a$ = 15.539 Å, $b$ = 31.527 Å, $c$ = 15.557 Å, $\beta$ = 100.94°.
(14) Per-6-bromo-β-CD: De Rango, C.; Nicolis, I.; Villain, F. Private communication.

(15) $^{13}$C NMR ($CDCl_3$, 50 MHz, J. Mod.) for **4a**: 98.05 (C-1), 79.56–79.81 (C-2,3,4), 71.05 (C-5), 51.84 (C-6), hexyl chains O2 and O3 74.13, 71.85 (C-1′) 31.91, 31.66 (C-2′), 30.34, 30.05 (C-3′), 25.76, 25.54 (C-4′), 22.58, 22.54 (C-5′), 13.86 (C-6′). $^{13}$C NMR **4b**: 98.00 (C-1), 79.90–79.91 (C-2,3,4), 71.15 (C-5), 51.50 (C-6), dodecyl chains O2 and O3 74.35, 72.00 (C-1′), 29.41–31.93 (C-2′–C-9′), 26.03, 26.34 (C-10′), 22.66 (C-11′), 14.03 (C-12′). $^{13}$C NMR **6a**: 98.71 (C-1), 78.91–79.29 (C-2,3,4), 68.73 (C-5), 41.70 (C-6), hexyl chains O2 and O3 73.76, 71.75 (C-1′), 32.02, 31.84 (C-2′), 30.34, 30.11 (C-3′), 25.76 (C-4′), 22.69 (C-5′), 13.97, 14.03 (C-6′). $^{13}$C NMR **6b**: 98.68 (C-1), 78.95–79.33 (C-2,3,4), 68.77 (C-5), 41.79 (C-6), dodecyl chains O2 and O3 73.87, 71.82 (C-1′), 29.45–31.95 (C-1′–9′), 26.27, 26.18 (C-10′), 22.67 (C-11′), 14.00, 14.08 (C-12′).
(16) Langmuir type film balance (Lauda, Germany), deposition of 1.1 × 10$^{16}$ molecules in tetrahydrofuran at the surface (20 °C); deposition followed by 5 min of equilibration, compression rate 3 cm min$^{-1}$, supported on triply distilled water.

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(250 Å$^2$). Molecular modeling demonstrates that the alkyl chains may align along the molecular axis with only a slight increase in diameter and without adverse van der Waals interactions.[9] Observed $A_0$ values are 351 (**6a**) and 366 Å$^2$ (**6b**). The compression curve of **6b** is typical for passage from a liquid expanded state, through a liquid compressed state (below 18 mN m$^{-1}$ pressure), to a solid compact state. For **6a**, the curve is typical of a system retaining liquid character even at high pressure.

The $^1$H NMR spectra of **6a** and **6b** in tetrahydrofuran-$d_8$ are broad at 25 °C but considerably sharpened at 50 °C, behavior consistent with aggregation. This is confirmed by light-scattering experiments,[17] showing the presence for **6b** of relatively monodisperse vesicles of 350-nm apparent diameter. Variable-temperature COSY (pyridine-$d_5$) of **4a** shows diastereotropic behavior at 95 °C for the C-1′ protons of one alkyl chain (O2). $^{13}$C NMR ($CDCl_3$) data show the inequivalence of the chain C-1 carbons ($\Delta\delta$ = 2.28 ppm **4a**, 2.35 ppm **4b**, 2.01 ppm **6a**, and 2.05 ppm **6b**). For the hexyl derivatives, inequivalence is observed for the C-2′ and C-3′ carbons. Both ammonium compounds show nonequivalence for the terminal methyl carbons.

$^1$H NMR spectroscopy shows **6a** to complex *N*-acetylphenylalanine in $CDCl_3$, with the $\alpha$-CH proton shifted upfield by 0.1 ppm and the phenyl protons broadened. The C-1′ protons of the O2 alkyl chain shift from 3.18 to 2.85 ppm, consistent with complexation occurring at the lower edge of the cyclodextrin cavity.

We are currently investigating the capacity of these molecules to complex molecules in mixed self-assembling systems.

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(17) Coulter Nano-Sizer, measurements carried out at a concentration of 10 g L$^{-1}$.

## A Postoligomerization Synthesis of Oligodeoxynucleotides Containing Polycyclic Aromatic Hydrocarbon Adducts at the N$^6$ Position of Deoxyadenosine

Seong Jin Kim, Michael P. Stone, Constance M. Harris,* and Thomas M. Harris*

*Chemistry Department and Center in Molecular Toxicology, Vanderbilt University Nashville, Tennessee 37235*

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The role of polycyclic aromatic hydrocarbons (PAHs) in the etiology of cancer is the subject of intense investigation.[1] To assess the relative importance of various DNA adducts,[2] we have undertaken the preparation of structurally defined PAH-adducted oligodeoxynucleotides. We report herein the use of a post-oligomerization strategy[3] involving the reaction of appropriate amines[4] with a matrix-bound oligonucleotide containing a halonucleoside to prepare oligodeoxynucleotides bearing PAH adducts at the N$^6$ position of adenine.

Phosphoramidite reagent **1** (Scheme I) was prepared by conversion of the 5′-DMT-6-chloro nucleoside[3] to the 6-fluoro derivative ($Me_3N$, KF, DMF)[5] followed by phosphitylation.[6] Reagent **1** was used in the automated solid-phase synthesis of 5′-CGGA-*CA*A*-GAAG-3′ using the standard 1-$\mu$mol protocol (Applied Biosystems Model 391 synthesizer). This DNA sequence is a segment of the H-ras proto-oncogene; mutations in codon 61 (italics) have been implicated in tumorigenesis.[7] After oligomer assembly *but before deprotection*, the immobilized oligomer was treated with (±)-amino triol **2** (5 mg, $CH_3CONMe_2$, $Et_3N$, 53 °C, 5 days) derived from (±)-*trans*-7,8-dihydroxy-*anti*-9,10-epoxy-7,8,9,10-tetrahydrobenzo[*a*]pyrene (BPDE). Excess amino triol was removed with MeOH. Treatment with concentrated $NH_4OH$ (60 °C, 8 h) and chromatography (Hamilton PRP-1 column, 10 mM ethylenediamine acetate (pH 7.45)/$CH_3CN$, 45 °C) gave two equal, closely eluting peaks exhibiting appropriate UV spectra, designated as **3** and **4** by order of elution, which were further purified by gel chromatography (Bio-Gel P-2, $H_2O$ elution). The combined yield was ~8%.[8] A similar reaction of matrix-bound fluoro oligomer with the less sterically hindered amino triol **5** derived from (±)-*trans*-8,9-dihydroxy-*anti*-10,11-epoxy-8,9,10,11-tetrahydrobenz[*a*]anthracene (BADE),[9] except that the reaction time was limited to 2 days, gave oligomers **6** and **7**. Combined yield was ~27%.

The absolute configurations of the PAH moiety attached to the oligomers were determined by enzymatic degradation to mononucleosides; products were compared by HPLC with authentic samples of known stereochemistry.[4,10,11] The results were

(1) There are numerous books and review articles on this subject. Some of the more recent are the following: *Polycyclic Aromatic Hydrocarbon Carcinogenicity: Structure-Activity Relationships*; Yang, S. K., Silverman, B. D., Eds.; CRC Press, Boca Raton, FL, 1988; Vols. I and II. Harvey, R. G.; Geacintov, N. E. *Acc. Chem. Res.* **1988**, *21*, 66-73. Dipple, A.; Chen, S. C.; Bigger, C. A. H. *Prog. Clin. Biol. Res.* **1990**, *347*, 109-127.

(2) See, for example: (a) Sims, P.; Grover, P. L.; Swaisland, A.; Pal, K.; Hewer, A. *Nature* **1974**, *252*, 326. (b) Straub, K. M.; Meehan, T.; Burlingame, A. L.; Calvin, M. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 5285-5289. (c) Cheng, S. C.; Hilton, B. D.; Roman, J. M.; Dipple, A. *Chem. Res. Toxicol.* **1989**, *2*, 334-340. (d) Jeffrey, A. M.; Grzeskowiak, K.; Weinstein, I. B.; Nakanishi, K.; Roller, P.; Harvey, R. G. *Science* **1979**, *206*, 1309-1311. (e) Jerina, D. M.; Sayer, J. M.; Agarwal, S. K.; Yagi, H.; Levin, W.; Wood, A. W.; Conney, A. H.; Pruess-Schwartz, D.; Baird, W. M.; Pigott, M. A.; Dipple, A. In *Biological Reactive Intermediates III*; Kocsis, J. J., Jollow, D. J., Witmer, C. M., Nelson, J. O., Snyder, R., Eds.; Plenum Press: New York, 1986; pp 11-30. (f) Jerina, D. M.; Chadha, A.; Cheh, A. M.; Schurdak, M. E.; Wood, A. W.; Sayer, J. M. *Adv. Exp. Med. Biol.* **1991**, *283*, 533-553.

(3) Harris, C. M.; Zhou, L.; Strand, E. A.; Harris, T. M. *J. Am. Chem. Soc.* **1991**, *113*, 4328-4329.

(4) Kim, S. J.; Harris, C. M.; Jung, K.-Y.; Koreeda, M.; Harris, T. M. *Tetrahedron Lett.* **1991**, *32*, 6073-6076.

(5) Synthesis of the 6-fluoro nucleoside: (a) Kiburis, J.; Lister, J. H. *J. Chem. Soc. C* **1971**, 3942-3947. (b) Robins, M. J.; Basom, G. L. *Can. J. Chem.* **1973**, *51*, 3161-3169. The fluoro nucleoside has been used to prepare adducts of less functionalized polycyclic aralkyl amines: (c) Lakshman, M.; Lehr, R. E. *Tetrahedron Lett.* **1990**, *31*, 1547-1550. (d) Lakshman, M. K.; Sayer, J. M.; Jerina, D. M. *J. Am. Chem. Soc.* **1991**, *113*, 6589-6594.

(6) Jones, R. In *Oligonucleotide Synthesis, A Practical Approach*; Gait, M. J., Ed.; IRL Press, Washington, DC, 1984; pp 22-34. Sinha, N. D.; Biernat, J.; McManus, J.; Köster, H. *Nucleic Acids Res.* **1984**, *12*, 4539-4556.

(7) (a) Barbacid, M. *Annu. Rev. Biochem.* **1987**, *56*, 779-82. (b) Vousden, K. H.; Bos, J. L.; Marshall, C. J.; Phillips, D. H. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 1222-1226. (c) Dipple, A.; Pigott, M.; Moschel, R. C.; Costantino, N. *Cancer Res.* **1983**, *43*, 4132-4135.

(8) Yields were estimated based on the calculated extinction coefficient ($\epsilon$ 128 L (mmol·cm)$^{-1}$ for **3** and **4**; $\epsilon$ 164 L (mmol cm)$^{-1}$ for **6** and **7**) at 260 nm for 1 $\mu$mol of 11-mer containing one adducted residue.

(9) Benz[*a*]anthracene is metabolized to a mixture of bay region (3,4-dihydrodihydroxy 1,2-oxide) and nonbay region (8,9-dihydrodihydroxy 10,11-oxide) epoxides, both reacting mainly with deoxyguanosine; the former is more mutagenic. (a) Lehr, R. E.; Schaefer-Ridder, M.; Jerina, D. M. *Tetrahedron Lett.* **1977**, 539-542. (b) Carberry, S. E.; Geacintov, N. E.; Harvey, R. G. *Carcinogenesis* **1989**, *10*, 97-103. (c) Boyland, E.; Sims, P. *FEBS Lett.* **1974**, *47*, 30-33. (d) Slaga, T. J.; Huberman, E.; Selkirk, J. K.; Harvey, R. G.; Bracken, W. M. *Cancer Res.* **1978**, *38*, 1699-1704. Cooper, C. S.; Ribeiro, O.; Farmer, P. B.; Hewer, A.; Walsh, C.; Pal, K.; Grover, P. L.; Sims, P. *Chem.-Biol. Interact.* **1980**, *32*, 209-231.

(10) Borowy-Borowski, H.; Lipman, R.; Chowdary, D.; Tomasz, M. *Biochemistry* **1990**, *29*, 2992-2999. In addition to the normal nucleosides, there was a PAH-containing component which eluted faster than the mononucleoside and was tentatively assigned as resulting from partial digestion. Others have observed difficulties in obtaining quantitative digests of DNA containing bulky adducts on adenine (Dipple, A.; Pigott, M. A. *Carcinogenesis* **1987**, *8*, 491-493; Cheh, A. M.; Yagi, H.; Jerina, D. M. *Chem. Res. Toxicol.* **1990**, *3*, 545-550; ref 13b).

(11) The benz[*a*]anthracene deoxyadenosine adducts were prepared by the procedure described in ref 4. Full details of the synthesis will be reported elsewhere.

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