(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 α-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

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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 postoligomerization 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-μmol 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.

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(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 (ϵ 128 L (mmol·cm)$^{-1}$ for **3** and **4**; ϵ 164 L (mmol cm)$^{-1}$ for **6** and **7**) at 260 nm for 1 μmol of 11-mer containing one adducted residue.

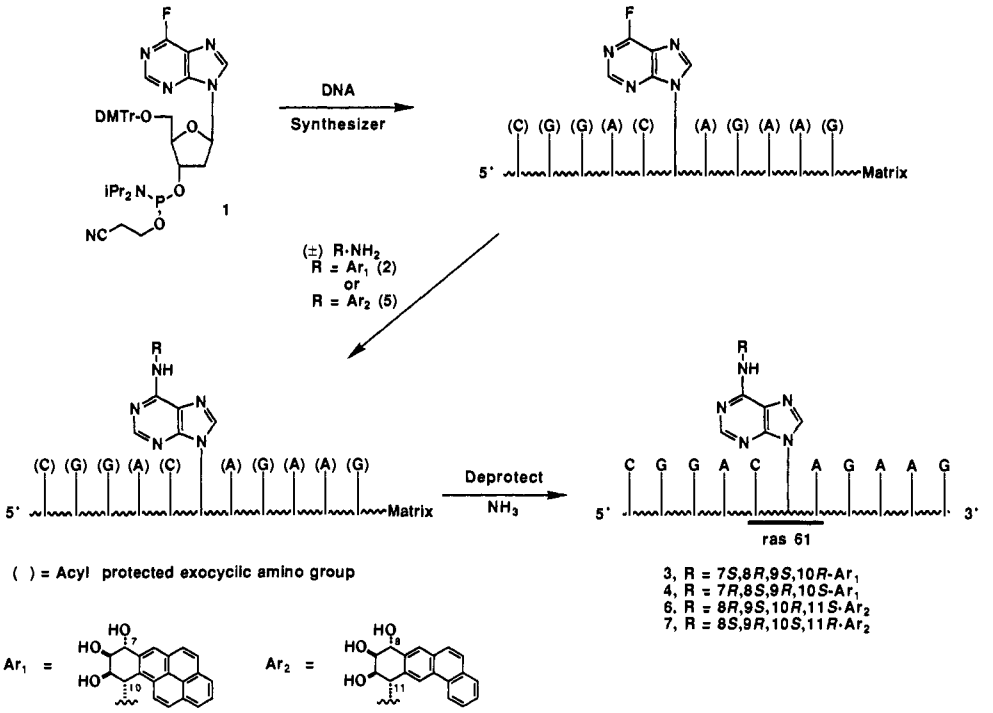
(9) Benz[*a*]anthracene is metabolized to a mixture of bay region (3,4-dihydrodihydroxy 1,2-oxide) and nonbay region (8,9-dihydrodihydroxy 10,11-oxide) epoxides, both reacting mainly with deoxyguanosine; the former is more mutagenic. (a) Lehr, R. E.; Schaefer-Ridder, M.; Jerina, D. M. *Tetrahedron Lett.* **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.

0002-7863/92/1514-5480$03.00/0

Scheme I



confirmed by comparison of the CD spectra of the adducted oligomers to those of the adducted nucleosides.[12]

Room-temperature ^{1}H NMR spectra of the PAH-adducted oligonucleotides were broad. The spectra gradually sharpened as the temperature was raised. The H5 and H6 signals for one cytosine, presumably that adjacent to the adduction site, were particularly broad such that the cytosine H5–H6 cross-peak in the COSY spectrum was difficult to detect, particularly in oligomers **3** and **7**. With **3**, the cross-peak was finally seen when the temperature was raised to 80 °C; with **7** it was seen at 57 °C. The cross-peaks between PAH H9 and H10 in **3** and H10 and H11 in **7** were not detected even at elevated temperature but were visible with isomers **4** and **6**. The broadness of the proton signal on the carbon adjacent to the nitrogen in N^6-adducted adenine nucleosides has been seen by others.[2c,5c] It is noteworthy that the oligomers were not degraded by the high-temperature treatment.

This synthetic approach to adducted oligomers is an attractive adjunct to current routes for preparation of carcinogen-adducted oligonucleotides.[13] It provides adenine N^6 adducts with complete control of regio- and stereochemistry. Reaction of diol epoxides of low reactivity with DNA gives meager, although not necessarily insignficant,[14] yields of adenine adducts, whereas those of high reactivity show poor regiospecificity. Our procedure is economical in its use of the relatively expensive amino triol constituents, the excess of which can readily be recovered. We are now refining the methodology and extending this procedure to other stereoisomers and to other PAHs.

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Supplementary Material Available: Detailed description of synthesis, isolation, and characterization of adducted oligomers **3**, **4**, **6**, and **7**, including UV, CD, and NMR spectra (18 pages). Ordering information is given on any current masthead page.

(12) The absolute configurations of the benz[*a*]anthracene deoxyadenosine adducts were assigned by CD comparisons with other PAH deoxyadenosine adducts (ref 2c,d and Sayer, J. M.; Chadha, A.; Agarwal, S. K.; Yeh, H. J. C.; Yagi, H.; Jerina, D. M. *J. Org. Chem.* **1991**, *56*, 20–29); the isomer with a strong negative band at longer wavelength and strong positive band at shorter wavelength was assigned the *R* configuration at the N-substituted benzylic carbon of the tetrahydro aromatic substituent.

(13) Other approaches include incorporation of an adducted nucleoside into an oligonucleotide: ref 5d and (a) Casale, R.; McLaughlin, L. W. *J. Am. Chem. Soc.* **1990**, *112*, 5264–5271. (b) Stezowski, J. J.; Joos-Guba, G.; Schönwalder, K.-H.; Straub, A.; Glusker, J. P. *J. Biomol. Struct. Dyn.* **1987**, *5*, 615–637. Syntheses involving reaction of a carcinogen with an oligonucleotide: (c) Cosman, M.; Ibanez, V.; Geacintov, N. E.; Harvey, R. G. *Carcinogenesis* **1990**, *11*, 1667–1672. (d) Burnouf, D.; Koehl, P.; Fuchs, R. P. P. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 4147–4151. (e) Benasutti, M.; Ezzedine, D.; Loechler, E. L. *Chem. Res. Toxicol.* **1988**, *1*, 160–168.

(14) Adenine adducts may play a disproportionate role in tumorigenesis. See ref 7 and also DiGiovanni, J.; Romson, J. R.; Linville, D.; Juchau, M. R. *Cancer Lett.* **1979**, *7*, 39–43.