(see Figure 1). The circular dichroism spectra of the FTOs (not shown), as well as their exhibition of discrete, cooperative melting transitions[19] (Table I), indicate that they indeed exist as B-form duplex DNA in solution. Although each of the duplex FTOs bears *two* modified dC residues at adjacent positions, the extent of destabilization caused by the presence of the tethers is modest (Table I). In only one case, 5′-d(GCAAG**2f**TTGC), was duplex stability significantly compromised, likely as the result of charge repulsion between the proximal carboxylate groups of the tether. In most common applications, those in which only one tether would be present in a duplex FTO, the destabilizing effect of the tether alone would be negligible. We believe that the central location of the tether attachment point in these FTOs may make them especially suitable for studies involving attachment of DNA-interactive ligands.[20]

The FTO synthetic technology reported here represents a general, convergent approach to the introduction of tethered functionality into DNA. As demonstrated here, a single phosphoramidite monomer can be used to install a wide variety of tethers, since the tether length and appended functionality are determined solely by the amine employed in the final deprotection step. In this preliminary study, four nonnative functional groups have been tethered to a specific locus in DNA: an aliphatic amine, an alcohol, a thiol (protected as the mixed disulfide),[21] and a carboxylic acid. This base-modification approach leaves the 5′ and 3′ ends free for enzymatic manipulation, a necessity for many molecular biological applications. We expect that this methodology will have significant applicability in the investigation of protein–DNA interactions, the creation of novel, conformationally locked DNA structures, and gene-targeted drug delivery.

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Supplementary Material Available: Detailed procedures for the synthesis, purification, and digestion of modified oligodeoxynucleotides, FPLC analysis of modified oligodeoxynucleotides, and HPLC analysis of enzymatic digestion products (13 pages). Ordering information is given on any current masthead page.

(19) Experimental conditions for the T_m measurements were as follows: samples with initial O.D.$_{260}$ of 0.4 A.U. were prepared in 1 M NaCl, 10 mM KH_2PO_4 pH 7.0. T_m's (±0.1 °C) were obtained from first and second derivative plots of absorbance vs. temperature curves. Data were collected on a Perkin-Elmer Lambda 3B spectrophotometer equipped with an immersible temperature probe and digital temperature controller interfaced to an IBM-XT personal computer using ASYST (version 1.53) data collection software.

(20) Telser et al.[15] have reported that a duplex FTO containing a single N^4-(3-aminopropyl)-dC residue is weakly destabilized. Attachment of a pyrenebutyrate moiety to the tether, however, causes a dramatic perturbation of duplex structure. This result may be interpreted as evidence that the pyrenebutyrate interacts strongly (perhaps by intercalation) with the DNA molecule to which it is tethered.

(21) The mixed-disulfide FTO 5′-d(GCAAG**2g**TTGC)-3′ can be quantitatively reduced to the free thiol form by overnight treatment with dithiothreitol (10 mM) at 55 °C in TE buffer (10 mM Tris, 1 mM EDTA, pH 8.0).

The Synthesis of the First Perfluorocryptand

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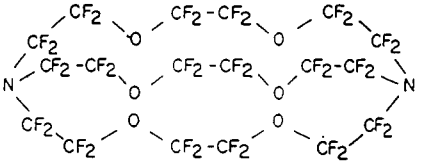
Summary: Using carefully controlled reactions of elemental fluorine, we have prepared and characterized the first perfluorocryptand [222], specifically perfluoro-4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane. This is a very stable, inert, high boiling clear oil.

In 1985, Lagow and co-workers prepared the first perfluoro crown ethers.[1] The compounds perfluoro 12-crown-5, perfluoro 15-crown-5, and perfluoro 18-crown-6, were prepared in good yield using controlled direct fluorination elemental fluorine reactions. Cryptands were prepared by Lehn and co-workers[2] and have been studied extensively by Lehn and others. As is well-known, these are a very useful class of ligands which make very stable complexes with numerous metal cations.

We report in this paper the synthesis of the first perfluorocryptand, perfluoro-4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane, which is the perfluorocryptand [222]. This is a very stable, inert, high-boiling clear oil and was obtained in 28% yield by direct fluorination of the starting hydrocarbon cryptand [222].



The substitution of fluorine into cryptand systems is sure to produce some interesting effects. Indeed, the presence of fluorocarbon groups in crown ethers has been shown to increase the rate of ion transport through a polymer membrane.[3] The presence of fluorine in partially fluorinated cyclams has been shown to reduce the basicities of such compounds.[4]

The hydrocarbon compound 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane, (cryptand [222]), was purchased from Aldrich Chemical Co. Fluorine was

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technical grade from Air Products and Chemicals, Inc. All materials were used as received. A 0.9904-g sample of the cryptand was dissolved in 25 mL of methylene chloride and added to 10 g of dry sodium fluoride powder. The mixture was slurried to coat the starting material on sodium fluoride particles. The solvent was removed under vacuum, and the dry material was finely ground and dispersed over the copper turnings in the reactor chamber. The reactor (disk reactor) system has been previously described.[5] The reactor was connected to the fluorination system and purged with helium to remove any air from the system. The reactor was cooled to −100 °C, and the fluorination sequence was begun.

Upon completion of the reaction sequence, the reactor was removed and the contents extracted with $CFCl_2CF_2Cl$. The solvent was removed using a rotovap. The crude product was isolated as ca. 2 mL of cloudy yellowish oil. Purification of the material was performed by preparative gas chromatography using a $^1/_4$ in. × 10 ft stainless steel column packed with 25% OV-101 on Chromosorb A 60-80 mesh. With the column temperature at 150 °C and the helium carrier gas flow at 45 mL/min, the compound was eluted in 5.4 min.

Preparative gas chromatography of the crude material isolated 0.7537 g of a clear colorless oil for a yield of 28%. The compound was identified using ^{19}F NMR, mass spectral analysis, and elemental analysis. The ^{19}F NMR spectrum consisted of three signals at ($CFCl_3$) −81.4 (s), −87.0 (t, $J_{F-F} \sim 1$ Hz), and −88.5 ppm (t, $J_{F-F} \sim 1$ Hz). The $^{13}C\{^{19}F\}$ NMR also shows three peaks at (TMS) 113.5, 115.3, 116.8. The melting point of perfluorocryptand [222] is 32.7 °C. The infrared analysis was performed as a thin film on KBr, showing peaks at 1276 (vs, br), 1229 (vs, br), 1211 (vs, br), 1156 (vs, br), 1131 (vs, br), 1049 (m, sh), 984 (w), 893 (m), 878 (m), 840 (2), 769 (m), 744 (m, sh), 715 (m), 704 (m) cm^{-1}. Elemental analysis was performed by Schwarzkopf Microanalytical Laboratories, Woodside, NY. The elemental analysis agreed well with $C_{18}F_{36}N_2O_6$. Calcd: C, 21.10; F, 66.80; N, 2.74. Found: C, 21.41; F, 66.50; N, 2.39. Electron-impact mass spectral analysis of the compound, performed on a Bell and Howell Model 491 spectrometer at 70 eV, provided little structural information. The molecular ion was observed at m/e 1024 as a small peak (<1% R. A.), allowing confirmation of the expected molecular weight. Electron capture negative ion (ECNI) mass spectrometry combined with collisionally induced dissociation (CID) provided more structural information and contained a large (100%) parent ion at 1024.[6]

An additional mass spectrum was obtained on a Finnigan MAT TSQ70 triple stage quadrupole operated in negative chemical ionization mode at a source pressure of 1.2 Torr of methane and a temperature of 80 °C. The sample was separated with a Varian Model 3400 gas chromatograph with a 15-m BP5 capillary column programmed to 220 °C at 25 °C/min. The compound was injected as a CFC-113 solution and eluted at 100 °C. For CID analyses the sample was coated on a rhenium filament on a direct exposure probe. Argon was used as the collision gas at 1.2 mTorr.

The GC/ECNI spectrum of the compound has a base peak attributed to the molecular ion at m/e 1024. No reagent gas adducts were observed under any conditions investigated, pointing to the high electron affinity of the compound. Ions observed at m/e 986, 908, and 792 are attributed to the losses of F_2, C_2F_4O, and $C_4F_8O_2$, respectively. The other ions at m/e 496, 346, and 296 are the result of further ionization in the source and not major features of the CID spectra. Stepwise or concerted multiple extrusion of neutral perfluoroethylene oxide is observed and produces the ions at m/e 908, 792, and 560. Further loss of C_2F_4O or C_3F_6N fragments yields ions at m/e 428, 444, and 676. CID studies suggest the cyclic structure of the perfluorinated cryptand is preserved as the fragmentation proceeds.

The [222] perfluorocryptand compound is expected to have interesting applications. Aside from the possibility of acting as a perfluoro "host" for certain types of "guest" species, perfluorocryptand [222] has shown potential as a very clean, high mass compound for use as a mass spectral marker material.[6] The compound is expected to be biologically inert (in contrast to the hydrocarbon analogue), and as in the case of the perfluoro crown ether compounds, may be useful in fluorocarbon biological and medical applications where physiologically inert or oxygen carrying fluids are required.

The basicities of the crown systems would be expected to decrease with an increased amount of fluorine substitution in the molecule. This trend is seen in the partially fluorinated cyclams[4] and is continued to the perfluoro crown ethers.[1] The coordination chemistry, organometallic chemistry, and reaction chemistry are being explored in collaboration with Professor Jean-Marie Lehn who provided the original hydrocarbon samples and suggested this project to us many years before we had developed the direct fluorination capability to the extent required to effect this synthesis. In addition, we have underway a collaborative project with Professor Leland C. Clark, Jr., in which we are exploring the physiological and biological properties of the new perfluorocryptand. The physiological applications of perfluorocryptand [222] will be published elsewhere.

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Supplementary Material Available: ^{19}F NMR assignments for perfluorocryptand [222], $^{13}C\{^{19}F\}$ NMR spectrum of perfluorocryptand [222], fluorination conditions for cryptand [222], GC/ECNI spectrum of perfluorocryptand [222], positive ion CI spectrum of perfluorocryptand [222], and CID spectrum of m/e 1024 (6 pages). Ordering information is given on any current masthead page.

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