Chemistry of Naturally Occurring Polyamines. 9.[1] Synthesis of Spermidine and Spermine Photoaffinity Labeling Reagents

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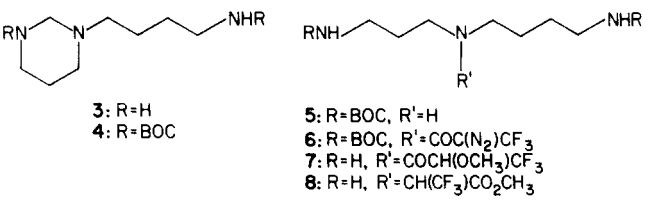
Photosensitive polyamine analogues might provide versatile chemical probes for isolating and identifying putative polyamine receptor sites in both prokaryotic and eucaryotic cells. Efficient syntheses of N^4-(2-diazo-3,3,3-trifluoropropionyl)spermidine (1) and N^4-(2-diazo-3,3,3-trifluoropropionyl)spermine (2) are described. It is shown that the photosensitive functional group undergoes predominantly solvent insertion when irradiated with a medium-pressure mercury lamp in methanol at room temperature. Binding and labeling studies to test the effect of 1 and 2 on several enzymes of polyamine intermediary metabolism are reported.

It is now recognized that the ubiquitous, naturally occurring bases putrescine, spermidine, and spermine[2]—and their conjugates[3]—play important roles in the proliferation, differentiation, and growth cycles of procaryotic and eucaryotic cells. Moreover, disorders of polyamine metabolism, though not yet well understood, have been linked in clinical studies with several pathological conditions and neoplastic states. While basic research has elucidated the step-by-step biosynthesis of polyamines in exquisite detail, much remains to be learned about polyamine intermediary metabolism. The regulation of enzymes such as ornithine decarboxylase, the polyamine acetyltransferases, aminopropyltransferases and transglutaminases are but a few of the problems of interest. Since these proteins bind spermidine and spermine variously as substrates, activators, and/or feedback regulators, we felt much could be learned about their enzymology using the technique of photoaffinity labeling. Photosensitive polyamine analogues would provide versatile chemical probes for isolating and identifying putative polyamine receptor sites. Here we disclose the rational design of two such reagents as well as efficient procedures for synthesizing them.

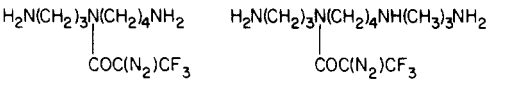
Both the choice of photolabile function and its site of attachment on the polyamine backbone were carefully considered. Of several known light-sensitive diazo reagents, the relatively acid-stable 2-diazo-3,3,3-trifluoropropionyl (DTP) group seemed most promising.[4] DTP esters and thioesters can be prepared by using the corresponding propionyl chloride (DTP-Cl) and are sensitive to both short- (254-nm) and long- (350-nm) wavelength light; moreover, they undergo predominantly the desired insertion reaction and less photochemical Wolff rearrangement than diazoacetates or malonates. The position of attachment of the DTP group to the polyamine was guided by the work of Porter et al.[5] who studied the effect of several modified polyamines on cultured L1210 leukemia cells and found that only N^4-functionalized spermidine derivatives were competitive inhibitors of spermidine uptake by a facilitated transport mechanism.[6] This observation suggested that formation of a DTP amide bond at the internal nitrogen of spermidine and spermine might be tolerated by various polyamine binding enzymes. Thus, structures 1 and 2 were targeted for synthesis by using the methodology we have developed in recent years[7] for site-selective functionalization of naturally occurring polyamines.[8]

Chart I



3: R=H
4: R=BOC

5: R=BOC, R'=H
6: R=BOC, R'=$COC(N_2)CF_3$
7: R=H, R'=$COCH(OCH_3)CF_3$
8: R=H, R'=$CH(CF_3)CO_2CH_3$



1 **2**

Chart I depicts the synthesis of 1. Cycloadduct 3 of formaldehyde with spermidine[7] was converted to its di-*tert*-butoxycarbonyl (BOC) derivative 4 in 67% yield by using 2-[(*tert*-butoxycarbonyl)oximino]-2-phenylacetonitrile (BOC-ON, Aldrich Chemical Co.). The methylene bridge in 4 could be removed by a Knoevenagel-like reaction (malonic acid, pyridine) to afford the known[8b] N^1,N^8-di-BOC-spermidine 5 (80%) by a shorter, more efficient route. DTP-Cl (2 equiv), prepared from 2,2,2-trifluorodiazoethane and phosgene according to the published procedure,[4] was reacted with 5 (CH_2Cl_2, 2 equiv of Et_3N, room temperature) to furnish 6 nearly quantitatively after flash chromatography. The efficiency of this amidation was gratifying in view of the relatively disappointing yields reported for thioesterifications with DTP-Cl,[4] and may be attributed to the use of Et_3N in place of pyridine or lutidine. Diisopropylamine and *N*-methylaniline afforded the corresponding amides in 61 and 70% yields, respectively, under similar conditions. Deprotection of 6 with neat trifluoroacetic acid (TFA, −190 °C to room temperature) led to the desired spermidine derivative 1 in excellent yield. When stored without solvent, 1 was stable for several months at −20 °C.

Photolysis of 1 in methanol at 254 nm using a medium-pressure mercury lamp for 200 min at room temperature gave exclusively 7, the product of solvent insertion, in 59% yield. No trace of 8, arising from Wolff rearrangement of 1, was detected.

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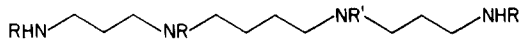
The synthesis of **2** proceeded along similar lines. Direct condensation of spermine **9** with BOC-ON (3 equiv, Et_3N)



9: R, R' = H
10: R = BOC, R' = H
11: R = BOC, R' = $COC(N_2)CF_3$

furnished tri-BOC derivative **10** (46% after chromatography). Acylation with DTP-Cl (88%) and then deprotection of **11** using TFA furnished **2** (75%). When tested as a photoaffinity substrate for spermidine or spermine synthase, **1** behaved as a weak inhibitor.[9] It had no effect on spermidine N^1 acetyltransferase.[10] However, both **1** and **2** deactivated transglutaminase from the slime mold *Physarium polycephalum* when irradiated with UV light.[11] Controls showed that the enzyme alone was stable to irradiation and that no inactivation took place in the absence of light. Preincubation of the enzyme with spermidine or spermine did protect against photodeactivation by the inhibitor. A full account of these bioassays will be reported elsewhere.

Experimental Section

General Section. Dichloromethane and triethylamine were distilled from CaH_2 prior to use. All reactions were conducted under a nitrogen or argon atmosphere. IR spectra were determined on a Perkin-Elmer 681 infrared spectrophotometer. ^1H NMR spectra were recorded on a Bruker WM-300 spectrometer at 300 MHz. Chemical shifts are expressed relative to internal tetramethylsilane $CDCl_3$ or to HOD at 4.8 ppm (D_2O). Mass spectra were obtained on a computerized AEI MS902 instrument using isobutane as reagent gas. Thin-layer chromatography was carried out on Merck precoated silica gel 60F-254 plates. Flash chromatography refers to the technique of Still et al.[12] Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville TN.

N^1,N^8-Bis(*tert*-butoxycarbonyl)-N^1,N^4-methylenespermidine (4). To a solution of N^1,N^4-methylenespermidine (1.75 g, 11.2 mmol)[7] in THF (25 mL) was added triethylamine (3.5 g, 34.6 mmol) and BOC–ON (6.26 g, 25.4 mmol). The resulting solution was stirred at room temperature for 16 h, and then THF was removed in vacuo and the residue taken up in ethyl acetate (100 mL). The ethyl acetate solution was washed with aqueous 5% NaOH (4 × 25 mL) and water (4 × 25 mL) and the organic layer dried ($MgSO_4$) and concentrated. Flash chromatography of the residue using 36:9:5 hexane–ethyl acetate–methanol afforded 2.68 g (67%) of **4** as an oil: ^1H NMR ($CDCl_3$) δ 4.97 (br m, 1 H), 4.03 (s, 2 H), 3.43 (br t, 2 H), 3.10 (br, 2 H), 2.67 (br s, 2 H), 2.38 (t, 2 H), 1.59 (t, 2 H), 1.50 (br s, 4 H), 1.43 (s, 9 H), 1.40 (s, 9 H); IR (film) 3350, 2980, 2920, 1690 cm^{-1}; CIMS (isobutane) 358 (M + 1, 100%), 302 (18%), 256 (14%).

N^1,N^8-Bis(*tert*-butoxycarbonyl)spermidine (5). To a solution of **4** (0.59 g, 1.67 mmol) in ethanol (15 mL) were added pyridine (0.41 g, 5.23 mmol) and malonic acid (0.71 g, 6.10 mmol), and the mixture was brought to reflux for 2 h. The solution was concentrated in vacuo, water (25 mL) was added, and the aqueous solution was washed with CH_2Cl_2 (3 × 25 mL). The pH was adjusted to 11 with 10% aqueous NaOH, and the aqueous phase was again extracted with CH_2Cl_2 (3 × 25 mL). The final three organic layers were combined, dried ($MgSO_4$), and concentrated to a pale yellow solid: 0.46 g (80%); mp 79–80 °C (reported[8b] gum); ^1H NMR ($CDCl_3$) δ 4.82, 5.16 (br, 2 H), 3.04–3.20 (m, 4 H), 2.50–2.65 (m, 4 H), 1.64 (t, 2 H), 1.52 (m, 4 H), 1.45 (s, 18 H); IR (KBr) 3480, 2980, 2920, 2860, 2800, 1690, 1670, 1520, 1360, 1260, 1170 cm^{-1}; CIMS (isobutane) 346 (M + 1, 100%), 290 (19%); EIMS (70 eV) 216 (19%), 145 (31%), 131 (83%), 57 (99%). Anal. Calcd for $C_{17}H_{35}N_3O_4$: C, 59.13; H, 10.14. Found: C, 59.29; H, 10.07.

N^4-(2-Diazo-3,3,3-trifluoropropionyl)-N^1,N^8-bis(*tert*-butoxycarbonyl)spermidine (6). To a solution of **5** (0.055 g, 0.16 mmol) and triethylamine (0.032 g, 0.22 mmol) in CH_2Cl_2 (1 mL) was added freshly prepared 2-diazo-3,3,3-trifluoropropionyl chloride (0.055 g, 0.32 mmol)[4] in CH_2Cl_2 (1 mL) and the homogeneous mixture stirred at room temperature for 16 h. More CH_2Cl_2 (4 mL) was added and the solution washed with water (3 mL), dried ($MgSO_4$), and concentrated. Silica gel flash chromatography eluting first with CH_2Cl_2 and then with 3:97 CH_3OH–CH_2Cl_2 gave 0.075 g (99%) of **7** as a brown oil: ^1H NMR ($CDCl_3$) δ 4.62, 4.93 (br, 2 H), 3.33 (t, 2 H), 3.24 (t, 2 H), 3.08 (m, 4 H), 1.70 (m, 2 H), 1.45–1.57 (m, 4 H), 1.40 (s, 18 H); IR (film) 3350, 2940, 2980, 2100, 1700, 1650 cm^{-1}; CIMS (isobutane) 382 (32%), 354 (35%), 298 (100%).

N^4-(2-Diazo-3,3,3-trifluoropropionyl)spermidine (1). Trifluoroacetic acid (5 mL) was added to neat **7** (0.26 g, 0.548 mmol) cooled in liquid nitrogen, and the mixture allowed to warm to room temperature and stir for 20 min. The bulk of TFA was removed in vacuo, and last traces of acid were chased with methanol. The residue was dissolved in water (15 mL) and washed with CH_2Cl_2 (5 mL). The aqueous layer was lyophilized and the residue chromatographed (SiO_2, 2:2:1 CH_2Cl_2–CH_3OH–NH_4OH) to afford **8** (0.19 g) as an oil: ^1H NMR (CD_3OD) δ 3.45 (t, 2 H), 3.35 (m, 2 H), 2.93 (m, 4 H), 1.93 (m, 2 H), 1.64 (m, 4 H); IR (film 3400, 3000, 2120, 1680, 1200, 1140 cm^{-1}; CIMS (isobutane) 282 (M + 1, 99%), 254 (77%), 172 (15%).

Attempted purification of **1** using CH_2Cl_2–CH_3OH–NH_4OH in 6:3:1 proportion instead of 2:2:1 proportion resulted in extensive decomposition during concentration of the chromatography fractions.

Photolysis of N^4-(2-Diazo-3,3,3-trifluoropropionyl)spermidine. Synthesis of 7. A solution of **1** (0.026 g, 0.09 mmol) in CH_3OH (3 mL) was irradiated at 254 nm with a medium-pressure mercury lamp while monitoring by TLC. After 200 min, chromatography of the concentrated reaction mixture (SiO_2, 2:2:1 CH_2Cl_2–CH_3ON–NH_4OH) afforded **7**: 0.016 g, (59%); ^1H NMR (D_2O) δ 5.11–5.17 (m, 1 H), 3.39–3.83 (m, 4 H), 3.57 (s, 3 H), 3.01–3.13 (m, 4 H), 1.98–2.11 (m, 2 H), 1.71–1.77 (m, 4 H); IR (film) 3400, 3050, 1670, 1200, 1130 cm^{-1}; CIMS (isobutane) 286 (M + 1, 100%), 254 (9%).

N^1,N^8,N^{12}-Tris(*tert*-butoxycarbonyl)spermine (10). Triethylamine (2.0 g, 19.9 mmol) followed by BOC-ON (3.59 g, 14.58 mmol) was added to a solution of spermine (**9**; 0.98 g, 4.87 mmol) in tetrahydrofuran (25 mL) and the resulting solution stirred at room temperature for 14 h. Most of the solvent was removed in vacuo, and the residue was taken up in ethyl acetate (25 mL). The organic phase was washed with 5% aqueous NaOH (4 × 25 mL) and water (4 × 25 mL), dried ($MgSO_4$), and concentrated. Flash chromatography of the residue (SiO_2, 4:3:3 hexane–ethyl acetate–CH_3OH) afforded the undesired tetra-BOC spermine (0.71 g, 24%). Further elution with 3:2 ethyl acetate–CH_3OH gave **10** (1.13 g, 46%) as an oil: ^1H NMR ($CDCl_3$) δ 3.09–3.27 (m, 8 H), 2.60–2.71 (m, 4 H), 1.65 (m, 8 H), 1.47 (s, 9 H), 1.44 (s, 18 H); IR (film) 3350, 2980, 2920, 1700, 1160 cm^{-1}; CIMS (isobutane) 503 (M + 1, 100%), 429 (6%), 329 (6%). Anal. Calcd for $C_{25}H_{50}N_4O_6$: C, 59.71; H, 10.03. Found: C, 59.11; H, 9.99.

N^4-(2-Diazo-3,3,3-trifluoropropionyl)-N^1,N^8,N^{12}-tris(*tert*-butoxycarbonyl)spermine (11). A solution of DTP-Cl (0.47 g, 2.76 mmol) in CH_2Cl_2 (1 mL) was added dropwise to **10** (0.69 g, 1.38 mmol) containing triethylamine (0.28 g, 2.75 mL) in CH_2Cl_2 (11 mL) and the mixture stirred at room temperature for 16 h. More CH_2Cl_2 was added (5 mL), and the organic phase was washed with water (5 mL), dried ($MgSO_4$), and concentrated in vacuo. The crude product was chromatographed (SiO_2, 2:98 CH_3OH–CH_2Cl_2) to afford 0.77 g (88%) of **11** as an oil: ^1H NMR ($CDCl_3$) 2.98–3.30 (m, 12 H), 1.50–1.70 (m, 4 H), 1.46 (m, 4 H), 1.44 (s, 9 H), 1.41 (s, 18 H), IR (film) 3350, 2980, 2920, 2100, 1680, 1650, 1170, 1120 cm^{-1}; CIMS (isobutane) 639 (M + 1, 5%), 611 (8%), 539 (42%), 511 (100%), 455 (57%).

N^4-(2-Diazo-3,3,3-trifluoropropionyl)spermine (2). A round-bottom flask containing **11** (0.28 g, 0.44 mmol) was cooled in liquid nitrogen and TFA (8 mL) added. After 5 min, the

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reaction mixture was warmed to room temperature and stirred for 20 min. Most of the TFA was removed in vacuo, and last traces of acid were removed by chasing with CH_3OH on the rotary evaporator. The oily residue was taken up in water, washed with CH_2Cl_2 (10 mL), and lyophilized. Chromatography of the crude product (SiO_2, 2:2:1 CH_2Cl_2–CH_3OH–NH_4OH) gave the desired **2** (0.11 g, 75%) as an oil: ^1H NMR (CD_3OD) δ 3.27, 3.36 (2 t, 4 H), 2.62, 2.69, 2.73, 2.84 (4 t, 8 H), 1.71, 1.78 (2 t, 4 H), 1.35–1.60 (m, 4 H); IR (film) 2950, 2100, 1680, 1640, 1120 cm^{-1}; CIMS (isobutane) 339 (M + 1, 42%), 311 (67%), 203 (32%), 75 (100%).

Attempted purification of **2** using CH_2Cl_2–CH_3OH–NH_4OH in 6:3:1 proportion instead of 2:2:1 proportion resulted in extensive decomposition during concentration of the chromatography fractions.

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Conformational Analysis of 1,4-Cyclohexadienes. A Shallow Boat-Shaped 1,4-Dihydronaphthalene

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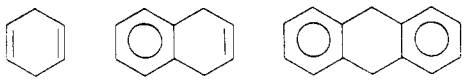
The metal/ammonia reduction of 7,12-dihydropleiadene affords 3,7,12a,12-tetrahydropleiadene which models indicate can exist in one of three different conformations (or in a dynamic equilibrium). Two of these conformers possess "fully-puckered", boat-shaped 1,4-dihydronaphthalene units (**5a** and **5b**) whereas the third (**5c**) incorporates a "flattened" six-membered ring. This latter conformation is the favored one as deduced by a variety of NMR techniques and is also predicted by MM2 force field calculations. The NMR parameters from **5c** are then applied to stereochemical problems associated with a number of previously investigated 1-R-1,4-dihydronaphthalenes (R = CO_2H, Ph, CH_2OH, or $C(CH_3)_2OH$). MM2 results are also presented for the entire sequence. It is concluded that "flattened" boat conformations are expected to be common for many 1-R-1,4-dihydronaphthalenes.

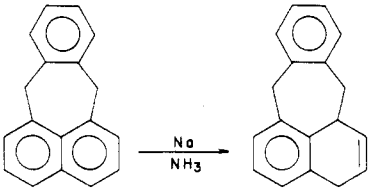
The stereochemistry of cyclohexadienes, which includes 1,4-dihydrobenzenes **1**, 1,4-dihydronaphthalenes **2**, and 9,10-dihydroanthracenes **3**, has attracted considerable attention.[1-8] Various geometries have been suggested for



1 2 3

the 1,4-cyclohexadiene ring in these systems including planar, "normal" boat-shaped and "shallow" boat-shaped. Moreover, boat-shaped conformations also allow for the possibility of boat-to-boat, ring inversion processes. The stereochemistry of these compounds is further complicated by substitution, since substituents can, in principle, prefer either pseudoaxial (pa) or pseudoequatorial (pe) positions.[1]

An especially useful approach to the NMR conformational analysis of these systems has involved studies with compounds of fixed geometries.[1,8] This allows application of NMR data so obtained to structures with unknown (and often flexible) geometry. For this reason we became interested in the metal/ammonia reduction of 7,12-dihydropleiadene (**4**) when we realized that the likely



4 5

product would provide a 1,4-dihydronaphthalene ring which could exist in one or more of three different conformations. However, treatment of **4** with sodium in ammonia according to methods which we had previously developed for naphthalenes,[9] afforded only one product, and variable-temperature NMR suggested a single conformation.

Results and Discussion

Mechanical Dreiding models suggest three different possible conformations for **5**. Two of these structures involve boat-shaped, central rings similar to the parent

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