furnished a mixture of products which was applied on silica gel column. Elution with a 30% ethyl acetate–hexane mixture furnished 1,8,10,11,11-pentachloro-3-*exo*-hydroxytetracyclo-[6.2.1.$0^{2,7}$.$0^{4,10}$]undec-5-en-9-one (7) (2.49 g, 55%): mp >270 °C; IR (KBr) 3514, 2926, 1788, 1292, 1246, 1105, 1082, 835 cm^{-1}; ^{1}H NMR (CD_3CN) δ 6.49 (1 H, d, J = 7 Hz), 6.11 (1 H, d, J = 7 Hz), 3.9 (1 H, s), 3.84 (1 H, d, J = 2 Hz), 3.58 (1 H, m), 3.22 (2 H, m); ^{13}C NMR (CD_3CN): δ 193.38, 138.89, 129.06, 93.05, 89.0, 86.77, 82.21, 80.73, 63.95, 50.47, 48.87. Anal. Calcd for $C_{11}H_7O_2Cl_5$: C, 38.16; H, 2.03; Cl, 50.54. Found: C, 38.12; H, 2.04; Cl, 50.5.

8-Chloro-3-*exo*-hydroxytetracyclo[6.2.1.$0^{2,7}$.$0^{4,10}$]undec-5-en-9-one (8), 3,5-Dichloropentacyclo[5.4.0.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecane-2,8-*exo*-diol (9), and 3,5,11-Trichloropentacyclo-[5.4.0.$0^{2,6}$.$0^{3,10}$.$0^{5,9}$]undecane-2,8-*exo*-diol (10). The reaction was conducted according to the general procedure using 4 mmol of 7 (1.4 g) and 12 equiv of SmI_2. After workup, the reaction mixture was loaded on a silica gel column. Elution with 20% ethyl acetate–hexane mixture furnished three products; 8 (25 mg, 3%), 9 (0.1 g, 10%), and 10 (0.34 g, 30%).

Data for 8: mp 175 °C; IR (KBr) 3292, 2966, 1738, 1234, 1211, 1186, 1157, 1053, 918 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.79 (1 H, s), 2.16 (1 H, d, J = 19.8 Hz), 2.59–2.41 (1 H, m), 3.04 (1 H, d, J = 7.9 Hz), 3.2–3.5 (4 H, m), 4.05 (1 H, s), 5.73 (1 H, t, J = 7.8 Hz), 6.18 (1 H, t, J = 7.8 Hz); ^{13}C NMR ($CDCl_3$) δ 218.13, 133.59, 126.39, 80.41, 73.55, 66.62, 51.19, 50.68, 47.37, 40.98, 33.55. Anal. Calcd for $C_{11}H_{11}O_2Cl$: C, 62.84; H, 5.27; Cl, 16.64. Found: C, 62.23; H, 5.29; Cl, 16.55.

Data for 9: mp 202 °C; IR (KBr) 3422, 2970, 1350, 1302, 1269, 1232, 1074, 1031, 906 cm^{-1}; ^{1}H NMR (CD_3CN) δ 1.42 (1 H, ddd, J = 12.9, 4.0, 2.9 Hz), 1.57 (1 H, d, J = 12.9 Hz), 2.33 (3 H, m), 2.55 (3 H, m), 2.74 (1 H, td J = 6.4, 1.7 Hz) 4.07 (1 H, m), 4.41 (1 H, d, J = 2.85 Hz), 5.95 (1 H, s); ^{13}C NMR (CD_3CN) δ 86.05, 74.66, 72.53, 70.76, 59.89, 54.02, 49.77, 47.7, 41.21, 37.83, 22.79. Anal. Calcd for $C_{11}H_{12}O_2Cl_2$: C, 53.65; H, 4.91; Cl, 28.42. Found: C, 53.83; H, 4.97; Cl, 28.38.

Data for 10: mp 145 °C; IR (KBr) 3354, 2976, 1414, 1306, 1271, 1047, 908 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.69–1.82 (2 H, m), 2.06 (1 H, d, J = 8.0 Hz), 2.66 (3 H, m), 2.8 (2 H, s), 2.9 (1 H, m), 3.29 (1 H, m), 4.34 (1 H, d, J = 8 Hz), 4.41 (1 H, s); ^{13}C NMR ($CDCl_3$) δ 87.48, 77.20, 75.05, 74.82, 71.32, 58.90, 52.24, 47.93, 42.07, 38.02, 23.59. Anal. Calcd for $C_{11}H_{11}O_2Cl_3$: C, 47.14; H, 3.95; Cl, 37.46. Found: C, 46.98; H, 4.06; Cl, 37.38.

Acknowledgment. One of us (S.C.S.) is thankful to Drs. S. L. Rodgers and J. W. Gilman for their unconditional support. The authors are thankful to Mr. Ngo Q. Vu for making starting materials. Financial support of this study by the Air Force Office of Scientific Research, Directorate of Chemistry & Materials Science, is gratefully acknowledged.

Supplementary Material Available: Tables of atomic positional coordinates, anisotropic thermal parameters, bond lengths, and bond angles for compounds 5 and 6 (16 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

Anodic Amide Oxidations: The Synthesis of Two Spirocyclic L-Pyroglutamide Building Blocks

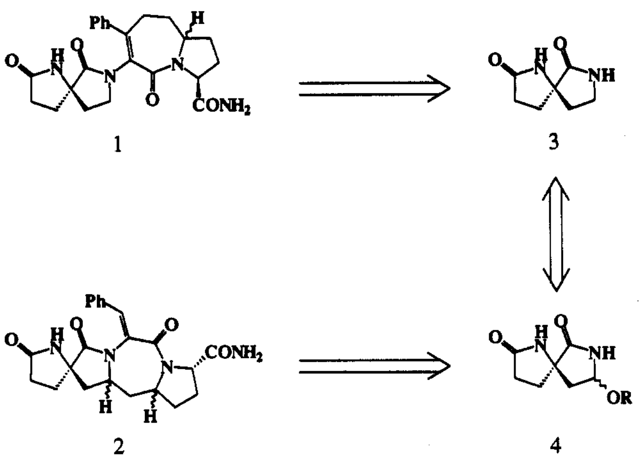
Kevin D. Moeller* and Lawrence D. Rutledge

Department of Chemistry, Washington University, St. Louis, Missouri 63130

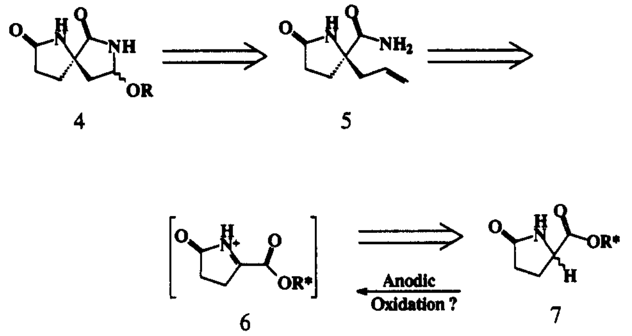
Received July 13, 1992

To date, attempts to use the active-analog approach to computer-assisted drug design in an effort to gain insight into the three-dimensional requirements of thyroliberin (TRH)-receptor binding have utilized active analogs that were both flexible and structurally similar to each other.[1]

Scheme I



Scheme II



These efforts were partially successful in reducing the number of possible TRH conformations that might satisfy the 3-D requirements of the receptor site, but left many questions unanswered. For example, Marshall and Font used the active analog approach to design the conformationally restricted TRH analogs 1 and 2.[2] However, modeling efforts could not differentiate between the potential effectiveness of these analogs or even attempt to determine the stereochemistry best suited for binding at the centers shown as racemic in 1 and 2. With this in mind, a program aimed at synthesizing and testing conformationally restricted TRH analogs was undertaken. It was hoped that the biological data obtained from these analogs would serve to clarify the conformation responsible for TRH binding and lead to the design of new, more active analogs. In order to provide a synthetic solution for 1 and 2, a generally useful method for constructing spirocyclic pyroglutamide analogs was required.[3] We report here the efficient synthesis of spirocyclic building blocks 3 and 4 from pyroglutamic acid (Scheme I).

From the start, it was clear that an asymmetric synthesis of building blocks 3 and 4 was needed in order to control the stereochemistry of the quaternary center relative to the remainder of the TRH analogs. For this reason,

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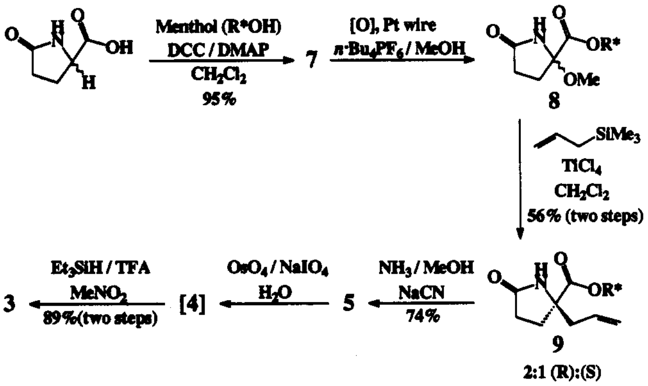
(1) For reviews concerning the active analog approach see: (a) Marshall, G. R.; Barry, C. D.; Bosshard, H. E.; Dammkoehler, R. A.; Dunn, D. A. In *Computer-Assisted Drug Design*; Olson, E. C., Christoffersen, R. E., Eds.; American Chemical Society: Washington DC, 1979; pg 205. (b) Marshall, G. R. *Ann. Rev. Pharmacol. Toxicol.* **1987**, *27*, 193. (c) Marshall, G. R.; Motoc, I. In *Molecular Graphics and Drug Design*; Burgen, A. S. V., Roberts, G. C. K., Tute, M. S., Eds.; Elsevier: Amsterdam, 1986; p 115.

(2) Font, J. Ph.D. Thesis, Washington University in St. Louis, 1986.

(3) Spirocyclic skeletons have been used to add rigidity to proline analogs: (a) Hinds, M. G.; Richards, N. G. J.; Robinson, J. A. *J. Chem. Soc., Chem. Commun.* **1988**, 1447. (b) Ward, P.; Ewan, G. B.; Jordon, C. C.; Ireland, S. J.; Hagan, R. M.; Brown, J. R. *J. Med. Chem.* **1990**, *30*, 1848.

0022-3263/92/1957-6360$03.00/0

Scheme III



building blocks 3 and 4 were envisioned as arising from an asymmetric addition to *N*-acyliminium ion 6 which was in turn envisioned as arising from the anodic oxidation of a chiral pyroglutamate ester 7 (Scheme II).[4,5] In order to quickly test the validity of this approach, (+)-menthol was chosen as the chiral auxiliary.

As outlined in Scheme III, pyroglutamic acid was treated with (+)-menthol, DCC, and DMAP in dichloromethane to afford a 95% isolated yield of the anodic oxidation substrate 7. The amide was oxidized using a platinum wire anode, an undivided cell, tetrabutylammonium hexafluorophosphate as electrolyte, and methanol as solvent to afford the methoxylated amide 8. The crude oxidation product was treated with allyltrimethylsilane and titanium tetrachloride in dichloromethane to afford a 55–65% isolated yield of the desired alkylated product 9 over the two steps. The product was initially isolated as a 2:1 mixture of diastereomers which were then separated by MPLC.[6] The stereochemistry of the major product was assigned after conversion to building block 3.

Several items concerning the anodic amide oxidation reaction warrant further comment. First, the reaction was sensitive to the type of anode employed and benefited from higher current densities. For reactions utilizing the same current flow, proton NMR analysis of the crude reaction product indicated that the use of either a platinum foil (1 cm × 2 cm) or carbon anode in place of the platinum wire anode described above led to a substantial decrease in the cleanliness of the oxidation reaction. Second, both chemical oxidations of 7 using tert-butylhypochlorite[7] and indirect electrochemical oxidations of 7 using sodium chloride as the electrolyte[8] met with failure. In neither case was any of the desired product isolated. Finally, the electrochemical reactions were run on scales ranging from 0.5 to 64 mmol.

The synthesis of building block 4 was completed by treatment of the major alkylation product 9 with ammonia and sodium cyanide in methanol to afford a 74% yield of primary amide 5.[9] The amide was oxidized with a catalytic amount of osmium tetraoxide and sodium periodate in water to afford the key building block 4. Attempts to isolate pure compound 4 met with failure. The material was contaminated with salts and the mass balance was typically greater than 100%. Fortunately, the salts did not interfere with subsequent reactions involving the generation of an *N*-acyliminium ion and the crude material could be utilized without further purification.

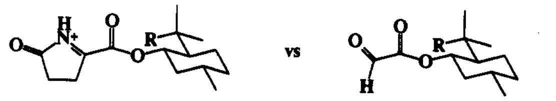


Figure 1.

The synthesis of building block 3, which had been synthesized previously in a 0.15% overall yield,[10] was completed by treating the crude compound 4 with triethylsilane and trifluoroacetic acid in nitromethane solvent (89% over two steps). The use of nitromethane as solvent in this reaction was critical due to the lack of solubility displayed by compound 4 in most solvents compatible with the reduction conditions. The overall yield of building blocks 3 from pyroglutamic acid was 11–12%.

The absolute stereochemistry of building block 3 was assigned by comparison of its CD and ORD spectra with the spectra published previously.[10] This data indicated that the major product formed during the addition of the allylsilane to *N*-acyliminium ion 6 possessed the proper stereochemistry for completing the syntheses of 1 and 2. The formation of this stereoisomer as the major product can be explained by a transition-state model that places *N*-acyliminium ion 6 in an S-cis conformation. Nucleophilic attack would then occur from the sterically less hindered *re* face of the (+)-menthyl ester (Figure 1). This transition-state model is directly analogous to the model used to predict the stereochemical outcome of nucleophilic additions to the isoelectronic glyoxylate ester.[11] In analogy to the glyoxylate esters, we would expect other chiral auxiliaries to be much more effective than (+)-menthol at inducing asymmetry in the present addition reaction. Initial attempts to utilize 8-phenylmenthol as the chiral auxiliary have not been successful. In these cases, oxidation of the phenyl ring interferes with the anodic oxidation of the secondary amide.[12] Current efforts are aimed at identifying a chiral auxiliary that will both increase the selectivity of the addition reaction to *N*-acyliminium ion 6 and be compatible with the anodic oxidation conditions.

In summary, building blocks 3 and 4 can be efficiently synthesized from pyroglutamic acid in good overall yields. The synthetic route employed takes advantage of anodic electrochemistry's ability to generate *N*-acyliminium ions by oxidizing amides and serves to illustrate the utility of anodic amide oxidations for functionalizing readily available amino acid derived starting materials. Studies aimed at utilizing the chemistry developed here for synthesizing analogs 1 and 2 and using electrochemistry for the synthesis of additional lactam-based peptide building

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(4) For general discussions of anodic amide oxidations see: (a) Shono, T. *Tetrahedron* 1984, *40*, 811. (b) Shono, T.; Matsumura, Y.; Tsubata, K. *Org. Synth.* 1984, *63*, 206 and references therein (Saucy, G., Ed.). (c) Shono, T. In *Topics in Current Chemistry*; Steckhan, E., Ed.; Springer-Verlag: Berlin Heidelberg New York, 1988; Vol. 148, p 131.

(5) For the use of an anodic amide oxidation in the synthesis of a rigid peptide analog, see: Moeller, K. D.; Rothfus, S. L. *Tetrahedron Lett.* 1992, *33*, 2913.

(6) Using a 75% aqueous methanol mobile phase with ODS silica an α of 1.03 was observed, the minor isomer eluting first. Overall yields quoted are based on a recovery of 50% of the desired isomer from the 2:1 mixture.

(7) Three procedures were attempted; see: (a) Poisel, H.; Schmidt, U. *Angew. Chem., Int. Ed. Engl.* 1976, *15*, 294. (b) Baldwin, J. E.; Urban, F. J. *J. Am. Chem. Soc.* 1973, *95*, 2401. (c) Koppel, G. A.; Koehler, G. A. *J. Am. Chem. Soc.* 1973, *95*, 2403.

(8) Ginzel, K.-D.; Brungs, P.; Steckhan, E. *Tetrahedron* 1989, *45*, 1691 and references therein.

(9) For the use of cyanide as a catalyst in the aminolysis of esters, see: Högberg, T.; Ström, P.; Ebner, M.; Rämsby, S. *J. Org. Chem.* 1987, *52*, 2033.

(10) Majer, Z.; Kajtár, M.; Tichý, M.; Bláha, K. *Collect. Czech. Chem. Commun.* 1982, *47*, 950.

(11) For an example, see: Whitesell, J. K.; Lawrence, R. M.; Chen, H. H. *J. Org. Chem.* 1986, *51*, 4779 and references therein.

(12) Under the electrolysis conditions the secondary amide and the phenyl ring were found to oxidize at approximately the same rate. The oxidations of tertiary amides in the presence of phenyl rings are well known. (a) Shono, T.; Matsumura, Y.; Tsubata, K. *J. Am. Chem. Soc.* 1981, *103*, 1172. (b) Moeller, K. D.; Tarazi, S.; Marzabadi, M. R. *Tetrahedron Lett.* 1989, *30*, 1213. (c) Moeller, K. D.; Wang, P. W.; Tarazi, S.; Marzabadi, M. R.; Wong, P. L. *J. Org. Chem.* 1991, *56*, 1058.

blocks are currently underway.

Experimental Section

General. Proton magnetic resonance spectra were recorded using either a Varian Gemini 300, Varian XL-300, Varian XR-500, or Varian Unity 600 spectrometer. Carbon spectra were obtained using the Gemini 300 at 75 MHz. Chemical shifts are reported as parts per million (ppm) downfield from tetramethylsilane in δ units, and coupling constants are given in cycles per second (hertz). Infrared spectra (IR) were obtained using either a Perkin-Elmer 283B or a Mattson Polaris FT-IR spectrophotometer. Low-resolution mass spectral data were obtained on a HP 9500 GC/MS spectrometer. High-resolution mass spectral data were obtained using a VG ZAB-SE MS spectrometer with an 8 keV xenon FAB source. Carbon, hydrogen, and nitrogen analyses were obtained from Oneida Research Services, Inc., Whitesboro, NY.

CD/ORD data was obtained using a Durrum-JASCO Model J-20 circular dichroism recorder and spectropolarimeter.

Gravity flow chromatography was accomplished by using E. Merck silica gel 60 (70–230 mesh). MPLC was accomplished with a 51- × 450-mm column packed with Whatman LRP-2 silica (particle size = 37–53 μm). HPLC was accomplished using an SSI system including a Model 222C pump, a Model 500 detector, and Axxiom 727 control and integration software. Analytical HPLC work was performed using a 4.6-mm × 25-cm XPERTEK ODS column from P. J. Cobert. Semipreperative HPLC separations utilized a 9-mm × 50-cm Whatman Magnum 9 ODS-3 column. Reactions were monitored as a function of time by TLC with E. Merck silica gel 60 F_{254} glass plates, E. Merck RP-18 F_{254} glass plates, or analytical HPLC. The solvents used for chromatography were mixed by volume and are reported for each experiment. Capillary GC data were obtained using an HP Model 5890A instrument equipped with a HP 3396A integrator and a HP 20-m ULTRA II (5% phenyl methyl silicone) column.

Preparative electrolyses were conducted using a Model 630 coulometer, a Model 410 potentiostatic controller, and a Model 420A power supply purchased from The Electrosynthesis Company, Inc. The platinum electrodes were also purchased from The Electrosynthesis Company, Inc. Tetrabutylammonium hexafluorophosphate was purchased from Aldrich and used without purification. Anhydrous methanol was purchased from Aldrich in Sure/Seal bottles and used without purification.

Chemical reagents and starting materials were purchased from Aldrich and used without purification unless otherwise noted. Titanium tetrachloride was purchased as a 1 M solution in dichloromethane. Dichloromethane was distilled from calcium hydride before use. Anhydrous nitromethane was purchased from Aldrich in a Sure/Seal bottle and used without purification.

All reactions were run under an inert atmosphere of nitrogen unless specified otherwise.

The purity of all compounds was determined by either C, H, and N analyses or proton and carbon NMR (>90–95%) data.

(2*RS*)-5-Oxoproline, (1*S*,2*R*,5*S*)-5-Methyl-2-(1-methylethyl)cyclohexyl Ester (7). To a mixture of (+)-menthol (15.63 g, 100.0 mmol), *dl*-pyroglutamic acid (16.14 g, 125.0 mmol), and DMAP (3.82 g, 31.3 mmol) in CH_2Cl_2 (50 mL) was added DCC (25.83 g, 125.2 mmol). The mixture was stirred vigorously for 16 h at room temperature. The reaction was judged to be complete by GC (<1% menthol vs ester by area, 170 °C). The solvent was removed on an aspirator-equipped rotory evaporator and then Et_2O (50 mL) added. The mixture was applied to a silica column (75 × 335 mm) and eluted with Et_2O. An overlap fraction containing 1.05 g of 7/menthol mix was concentrated and rechromatographed on a similar column (30 × 250 mm). The fractions containing pure 7 were combined, evaporated to an oil on the rotory evaporator, and then after 4 additional hours on the rotory evaporator, transferred to the freezer. Several alternating treatments with vacuum and cold finally afforded 7 as a white solid (25.45 g, 95%) consisting of a 1:1 mixture of diastereomers as evidenced by GC (170 °C) and NMR: ^{1}H NMR ($CDCl_3$/300 MHz) δ 7.03 (br s, 0.5 H), 7.02 (br s, 0.5 H), 4.74 (tt, 1 H, J = 10.7, 3.7 Hz), 4.24 (dd, 1 H, J = 8.4, 5.2 Hz), 2.55–2.41 (m, 1 H), 2.41–2.33 (m, 2 H), 2.23–2.11 (m, 1 H), 2.04–1.93 (m, 1 H), 1.90–1.77 (m, 1 H), 1.74–1.65 (m, 2 H), 1.58–1.36 (m, 2 H), 1.14–0.80 (m, 3 H), 0.93–0.88 (3 d unresolved, 6 H), 0.75 (d, 3 H, J = 6.9 Hz); ^{13}C NMR ($CDCl_3$/75 MHz) δ 178.2, 171.71, 171.66, 75.62, 75.55, 55.81, 55.62, 46.8, 40.55, 40.61, 34.1, 31.3, 29.45, 29.36, 26.2, 25.0, 23.25, 23.16, 22.0, 20.8, 16.20, 16.06; IR (KBr) 3214, 3107, 2949, 2924, 2868, 1737, 1710, 1456, 1388, 1290, 1232, 1202, 1150, 1109, 1038, 1016, 986, 972 cm^{-1}; MS (PCI) *m/e* (rel intensity) 268 (MH^+, 16), 158 (oxoprolineH$^+$ + C_2H_4, 16), 130 (oxoprolineH$^+$, 100), 129 (4), 85 (6), 84 (MH^+ − menthol − CO, 16), 139 (4), 83 (10); HRMS (PCI) *m/e* calcd for $C_{15}H_{26}NO_3$ (MH^+) 268.19126, found 268.19138. Anal. Calcd for $C_{15}H_{25}NO_3$: C, 67.38; H, 9.42; N, 5.24. Found: C, 67.67; H, 9.59; N, 5.26.

(2*RS*)-2-Prop-2-enyl-5-oxoproline, (1*S*,2*R*,5*S*)-5-Methyl-2-(1-methylethyl)cyclohexyl Ester (9). Compound 7 (1.068 g, 4.00 mmol) and *n*-Bu_4NPF_6 (0.620 g, 1.60 mmol) were dissolved in 16 mL of anhydrous MeOH. The solution was oxidized at a constant current of 26.8 mA until 4.51 F/mol had been passed (1- × 10-mm Pt wire anode, 1- × 15-mm Pt wire cathode). The solvent was removed under reduced pressure, 50 mL of Et_2O added, and the solution filtered. The filtrate was concentrated under reduced pressure at room temperature to an oil. The oil was dissolved in 16 mL of CH_2Cl_2 and cooled to −78 °C, and then $TiCl_4$ (4.0 mL of 1.0 M solution in CH_2Cl_2, 4.0 mmol) and allyltrimethylsilane (2.54 mL, 16.0 mmol) were added. The solution was allowed to warm to room temperature over 16 h and neutralized with an excess of saturated $NaHCO_3$, the layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (2 × 25 mL). The solution was dried with Na_2SO_4, the solvent was removed under reduced pressure at room temperature, and the oil was purified by chromatography (21- × 300-mm, LRP-2, 70% aqueous MeOH, 2 mL/min). The fractions containing **9** were combined, the methanol was removed under reduced pressure, and the solution was extracted with CH_2Cl_2 until no further product was extracted as evidenced by TLC. The combined extracts were dried with Na_2SO_4 and evaporated to an oil under reduced pressure at room temperature, and the oil was treated alternately to vacuum and 0 °C for ca. 1 week until the product solidified. Compound **9** was obtained as a white waxy solid (0.691 g, 56% yield from 7). A 2:1 diastereomer ratio was obtained as evidenced by analytical HPLC (ODS, 75% MeOH), GC (190 °C), and NMR: ^{1}H NMR ($CDCl_3$/300 MHz) δ 6.28 (br s, 1 H), 5.19 (d, 1 H, J = 10.6 Hz), 5.18 (d, 1 H, J = 15.9 Hz), 4.72 (td, 1 H, J = 11.0, 4.4 Hz), 2.69 (dd, 1 H, J = 13.6, 6.8 Hz), 2.46–2.33 (m, 3 H), 2.20–2.04 (m, 1 H), 2.01–1.92 (m, 1 H), 1.89–1.77 (m, 1 H), 1.75–1.65 (m, 2 H), 1.58–1.38 (m, 2 H), 1.14–0.80 (m, 3 H), 0.91 (d, 6 H, J = 6.5 Hz), 0.75 (d, 3 H, J = 7.0 Hz); ^{13}C NMR ($CDCl_3$/75 MHz) δ 176.7, 172.6, 131.04, 130.98, 120.6, 76.0, 65.37, 65.16, 46.8, 43.4, 40.6, 34.1, 31.4, 30.49, 30.36, 29.8, 26.2, 23.1, 22.0, 20.8, 15.9; IR (KBr) 3234, 3110, 2956, 2934, 2995, 2870, 1736, 1709, 1209, 1158, 1056, 1013, 985, 947, 719 cm^{-1}. Anal. Calcd for $C_{18}H_{29}NO_3$: C, 70.32; H, 9.51; N, 4.56. Found: C, 70.06; H, 9.43; N, 4.40.

(2*R*)-2-Prop-2-enyl-5-oxoprolinamide (5). $NH_{3(g)}$ was bubbled through MeOH (20 mL) at 0 °C until large bubbles began to break the surface, by which time a volume increase of 7 mL was observed. This solution was added to **9** (0.440 g, 1.43 mmol, previously enriched by MPLC to a diastereomer ratio of 8.6:1) and NaCN (0.014 g, 0.286 mmol) and the solution tightly sealed, placed behind a shield, and allowed to warm to room temperature. The reaction was allowed to react for 25 days (typically 14–25 days were required). The solution was evaporated to dryness under reduced pressure, dissolved in MeOH, 5 drops of concd HCl was added, and the solution was again evaporated to dryness. The oil was washed with hexane until only a dry solid remained. The solid residue was purified by HPLC chromatography (ODS-3 Magnum-9, 100% H_2O, 2 mL/min). All fractions containing product were combined and evaporated to dryness under reduced pressure to yield 5 as a tan solid (0.178 g, 74%): ^{1}H NMR (DMSO-d_6/300 MHz) δ 7.85 (s, 1 H), 7.22 (s, 1 H), 7.18 (s, 1 H), 5.72 (ddt, 1 H, J = 17.1, 10.1, 7.1 Hz), 5.12 (d, 1 H, J = 17.1 Hz), 5.10 (d, 1 H, J = 10.0 Hz), 2.46 (A of an ABX, 1 H, J_{AX} = 7.1 Hz, J_{AB} = 13.8 Hz), 2.37 (B of an ABX, J_{BX} = 7.3 Hz, J_{AB} = 13.8 Hz), 2.22–2.08 (m, 3 H), 2.04–1.86 (m, 1 H); ^{13}C NMR (DMSO-d_6/75 MHz) δ 176.7, 176.2, 133.3, 119.5, 65.1, 42.4, 29.8, 29.6; IR (KBr) 3403, 3391, 3166, 1636, 1457, 1436, 1419, 1400, 1373, 1270, 923 cm^{-1}; MS (PCI) *m/e* (rel intensity) 197 (MH^+ + C_2H_4, 9), 169 (MH^+, 60), 152 (MH^+ − NH_3, 7), 127 (MH^+ − C_3H_6, 12), 124 (MH^+ − CO − NH_3, 100), 123 (5); HRMS (EI) *m/e* calcd for $C_5H_7N_2O_2$ (MH^+ − C_3H_6) 127.05075, found 127.05086; calcd for $C_7H_{10}NO$

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($MH^+ - CO - NH_3$) 124.07624, found 124.07713.

(5*R*,8*RS*)-8-Hydroxy-1,7-diazaspiro[4.4]nonane-2,6-dione (4) and (5*R*)-1,7-Diazaspiro[4.4]nonane-2,6-dione (3). Compound **5** (0.130 g, 0.770 mmol) was dissolved in H_2O (5 mL) and OsO_4 (39 μL of a 4% w/v solution in H_2O, 0.006 mmol) added. After 5 min only a very faint brown color had developed. $NaIO_4$ (0.363 g, 1.70 mmol) was then added and the solution stirred for 16 h. The solvent was then removed under reduced pressure by codistillation with *i*-PrOH and the solids extracted with *i*-PrOH. Filtration and removal of the solvent under reduced pressure gave 0.181 g of a hygroscopic solid whose NMR was consistant with the expected product, **4**. The spectral data for the impure product **4** were as follows: ^1H NMR (D_2O/300 MHz) δ 5.17 (dd, 1 H, *J* = 7, 6 Hz), 2.55 (dd, 1 H, *J* = 16, 7 Hz), 2.32 (t, 2 H, *J* = 10 Hz), 2.20–2.06 (m, 1 H), 2.05–1.94 (m, 1 H), 1.87 (dd, 1 H, *J* = 16, 6 Hz); MS (PCI) *m/e* (rel intensity) 153 ($MH^+ - H_2O$, 7), 112 (7), 100 (15), 59 (100); HRMS (EI) *m/e* calcd for $C_7H_8N_2O_2$ ($M^+ - H_2O$) 152.05858, found 152.05533.

To a mixture of the crude product **4** in $MeNO_2$ (5 mL) was added Et_3SiH (246 μL, 1.54 mmol) and TFA (593 μL, 7.70 mmol). After 18 h the solvent was removed under reduced pressure by codistillation with *i*-PrOH. The solid residue was dissolved in water, filtered, and chromatographed (Magnum-9 ODS-3, 100% H_2O, 2 mL/min). The fractions containing product were combined, evaporated to dryness under reduced pressure, dissolved in a minimum of water, filtered, and again evaporated to dryness to produce **3** as a white solid (0.179 g, 89% over the two steps): ^1H NMR (DMSO-d_6/300 MHz) δ 7.96 (s, 1 H), 7.86 (s, 1 H), 3.09–3.24 (m, 2 H), 1.88–2.31 (m, 6 H); ^1H NMR (D_2O/300 MHz) δ (std HOD 4.67 ppm) 3.24 (dd, *J* = 7.9, 5.2 Hz), 2.41–2.32 (m, 2 H), 2.26–2.20 (m, 4 H); ^{13}C NMR (DMSO-d_6/75 MHz) δ 176.7, 176.6, 62.3, 37.1, 34.0, 29.9, 29.7; ^{13}C NMR (D_2O/75 MHz) δ (unstandardized) 189.6, 187.4, 72.8, 46.3, 41.6, 38.0, 37.6; IR (KBr) 3242, 3145, 3068, 2907, 2870, 1716, 1683, 1653, 1360, 1306, 1246, 1054 cm^{-1}; MS (PCI) *m/e* (rel intensity) 155 (MH^+, 100); HRMS (EI) *m/e* calcd for $C_7H_{10}N_2O_2$ 154.07422, found 154.07240; CD λ_{max} = 227.5 nm ($\Delta\epsilon$ = 6.0 M^{-1} cm^{-1}).

Acknowledgment. This work was supported by Washington University, the Biomedical Research Support Program, Division of Research Resources, National Institutes of Health, and the National Institutes of Health (grant no. P01 GM24483-12). L.D.R. thanks the Monsanto Co. for a graduate fellowship. We also gratefully acknowledge the Washington University High-Resolution NMR Facility, partially supported by NIH 1S10R02004, and the Washington University Mass Spectrometry Resource Center, partially supported by NIHRR00954, for their assistance.

Supplementary Material Available: ^1H and ^{13}C NMR spectra for **3**, **5**, **7**, and **9**, as well as a ^1H NMR spectrum for **4** (10 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

A Convenient Method for the Direct Incorporation of 5-Fluoro-2′-deoxycytidine into Oligodeoxynucleotides

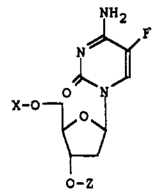
Canio J. Marasco, Jr., and Janice R. Sufrin*

Department of Experimental Therapeutics, Grace Cancer Drug Center, Roswell Park Cancer Institute, Buffalo, New York 14263

Received August 6, 1992

Introduction

To further elucidate the cellular functions governed by DNA methyltransferases we report the direct incorporation of 5-fluoro-2′-deoxycytidine into oligodeoxynucleotides using standard phosphoramidite chemistry. DNA methylation has become an area of great interest due to a demonstrated role in regulating gene expression.[1] In general, methylation of cytidine residues within a specific gene can inhibit transcription of that gene directly by inhibiting the binding of transcription factors or by affecting the surrounding chromatin structure.[2] Central to our interests, DNA methylation has been shown to affect the cellular processes of differentiation and carcinogenesis.[2] Oligodeoxynucleotides (ODNs) containing 5-fluoro-2′-deoxycytidine (FdC, **1A**) have been shown to inhibit bacterial methyltransferases (MTases).[3-5] The incorporation of FdC into ODNs is synthetically problematic and has prompted the development of specialized methodologies to circumvent these difficulties.[3,6,7] Although these different procedures ultimately incorporate FdC into ODNs, we sought a method to directly and simply introduce FdC into any desired ODN via an automated synthesizer.



1A(FdC) where X and Z = H
1B where X = 4,4′-dimethoxytrityl and
Z = $P(OCH_2CH_2CN)(N[CH(CH_3)_2]_2)$

2= CTATATGXGACCTG where X = FdC
3= CTATATGCGACCTG

Figure 1. Overview of structures.

Results and Discussion

Initial studies explored the acid stability of aliphatic amides of the N4 amino group of FdC as a prerequisite for their use as protecting groups during automated ODN synthesis. The isobutyryl protecting group appeared to be stable in a 2% trichloroacetic acid solution (by volume in CH_2Cl_2) for up to 40 min, as evidenced by thin-layer chromatography. However, repeated efforts to amidate the N4 amino group of FdC were only partially successful and clearly indicated that this amine was relatively nonreactive. From these observations, we reasoned correctly that the exocyclic amino group of FdC is sufficiently deactivated by the presence of the 5-fluoro group so that protection of the amino group would not be necessary. Thus, we report the synthesis of ODNs containing FdC which was introduced using standard phosphoramidite chemistry.

Preparation of the desired phosphoramidite of FdC and its incorporation into ODNs was accomplished in the following manner. The 5′-hydroxyl group of FdC was first converted to the trityl ether with 4,4′-dimethoxytrityl chloride in pyridine.[8,9] This intermediate was converted

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* To whom correspondence should be addressed.

[1] Cedar, H. *Cell* **1988**, *53*, 3.
[2] Adams, R. L. P *Biochem. J.* **1990**, *265*, 309.
[3] Osterman, D. G.; DePillis, G. D.; Wu, J. C.; Matsuda, A.; Santi, D. V. *Biochemistry* **1988**, *27*, 5204.
[4] Chen, L.; MacMillan, A. M.; Chang, W.; Ezaz-Nikpay, K.; Lane, W. S.; Verdine, G. L. *Biochemistry* **1991**, *30*, 11018.
[5] Smith, S. S.; Kaplan, B. E.; Sowers, L. C.; Newman, E. M. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 4744.
[6] Schmidt, S.; Pein, C. D.; Fritz, H. J.; Cech, D. *Nucleic Acid Res.* **1992**, *20*, 2421.
[7] MacMillan, A. M.; Chen, L.; Verdine, G. L. *J. Org. Chem.* **1992**, *57*, 2989.

0022-3263/92/1957-6363$03.00/0