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Registry No. 5,5-Dimethyl-1-pyrroline *N*-oxide, 3317-61-1; 4-methyl-4-nitropentanal, 57620-49-2; 5-spirocyclopentyl-1-pyrroline *N*-oxide, 104322-61-4; 4-cyclopentyl-4-nitropentanal, 104322-62-5; nitrocyclopentane, 2562-38-1; 2-propenal, 107-02-8.

trans-2,5-Dimethyl-2,5-bis(3-aminopropyl)-pyrrolidinyl-1-oxy: A *trans*-Diamino Azethoxyl Nitroxide

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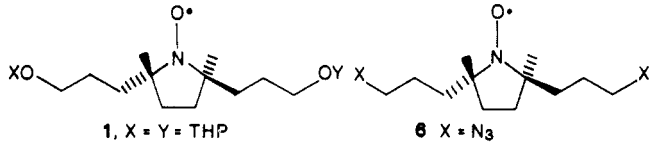
2,5-Disubstituted-2,5-dimethylpyrrolidinyl-1-oxy (azethoxyl[1]) nitroxides differ from most of the other stable nitroxide free radicals used in biophysical spin-labeling studies[2] and under evaluation as magnetic resonance imaging (MRI) contrast-enhancement applications[3] in two important ways. The canted nature of the nitroxide *z* axis with respect to the long molecular axis allows for the detection of restricted motion along this axis using ESR spin-labeling techniques.[4] In MRI applications, the azethoxyl nitroxide substitution pattern allows for the placement of functional groups in the vicinity of the paramagnetic nitroxide moiety. Certain of these groups might improve the resistance of the nitroxide group toward in situ reduction while enhancing the water-relaxing property of the nitroxide moiety. At present, nitroxide reduction seriously limits the use of nitroxides as MRI contrast-enhancing agents.[5] Herein, we describe the synthesis of the title *trans*-diamino azethoxyl nitroxide **7** from azethoxyl diol **3**.[6] The relative stability of **7** and several other nitroxides of novel structure toward reduction by liver homogenate, microsomes, and hepatocytes will be reported elsewhere.



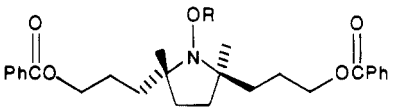
1, X = Y = THP
2, X = THP; Y = H
3, X = Y = H
4, X = Y = SO_2CH_3
5, X = Y = COPh

6 X = N_3
7 X = NH_2
8, X = NH_3^+; $HC_2O_4^-$

In our earlier study[6] diol **3** was obtained in variable yield by acid-catalyzed hydrolysis of a cis–trans mixture of bis(tetrahydropyranyl (THP) ether) **1**.[7] We now find that pure trans bis ether **1** can be obtained by careful chromatography of the mixture. This substance can be hydrolyzed to trans diol **3** consistently in 50–55% yield, accompanied by some starting **1** and mono derivative **2** which may be recycled.[8] More vigorous hydrolysis conditions, however, led to decomposition of the acid-sensitive nitroxide group.

Diol **3** was converted[9] into bis(methanesulfonate) **4**, but attempts to prepare diamine **7** directly from **4** using NH_3 in MeOH or THF in a pressure reactor[10] led to complex mixtures. Therefore, **4** was converted into bis azide **6**[11] which was then allowed to react with triphenylphosphine to give the corresponding bis(phosphinimine).[12] This was then hydrolyzed to the desired bis(amine) **7**, which was isolated and analyzed as the oxalate salt **8**.

The trans geometry of diol **3**, and hence of **7**, was established as follows. Diol **3** was converted into bis(benzoate) **5** which was then hydrogenated catalytically to *N*-hydroxy intermediate **9**. Esterification[1] of **9** with optically active Mosher's reagent[13] gave trifluoro ester **10**, which was shown to be a 1:1 mixture of diastereoisomers by the appearance of the methoxy groups as two singlets (δ 3.491 and 3.513) in the 360-MHz NMR spectrum. If ester **9** had been a cis azethoxyl nitroxide derivative, then it would have been a meso compound and it would have produced **10** as a single stereoisomer. The trans assignment of this series was confirmed by the observation of two singlets (δ −71.767 and −71.809) in a 1:1 ratio for the trifluoromethyl group in the ^{19}F NMR spectrum.



9, R = H
10, R = COC(OCH$_3$)(CF$_3$)Ph

Experimental Section[14]

***trans*-2,5-Dimethyl-2,5-bis[3-(tetrahydropyranyloxy)-propyl]pyrrolidinyl-1-oxy (1).** A cis–trans mixture (1.349 g) of **1** was prepared essentially as described.[6] The mixture was flash chromatographed over silica gel (5 g). Elution with 100 mL of hexane–ether, 3:2, gave 0.5005 g of *trans*-**1** (ESR, CH_2Cl_2, 3 lines, a_N = 14.5 G). Continued elution with this solvent (400 mL) followed by 100 mL of hexane–ether, 1:1, gave 0.638 g of the cis isomer (ESR, CH_2Cl_2, 3 lines, a_N = 14.5 G).

***trans*-2,5-Dimethyl-2,5-bis(3-hydroxypropyl)-pyrrolidinyl-1-oxy (3).** A solution containing 200 mg of **1**, 15 mg of *p*-toluenesulfonic acid monohydrate, 10 mL of MeOH, and 3 drops of water was stirred at 25 °C for 10 h. The reaction was monitored by TLC so as to maximize formation of **3**. Several drops of saturated $NaHCO_3$ were added and the mixture was evaporated to dryness. The residue was dissolved in CH_2Cl_2 and filtered through Celite. Evaporation gave 0.131 g of a mixture of **1**, **2**, and **3** which was flash chromatographed over silica gel (3 g). Elution with ether (65 mL) gave 52 mg of a mixture of **1** and **2**.

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(6) Keana, J. F. W.; Seyedrezai, S. E.; Gaughan, G. *J. Org. Chem.* **1983**, *48*, 2644.
(7) Compounds are racemic; only one enantiomer is shown. Each intermediate that bears a THP ether group is almost certainly a mixture of diastereoisomers owing the additional chiral center present in the THP ether grouping.
(8) Similar hydrolysis conditions applied to the chromatographically slower moving cis isomer of **1** gave the corresponding diol in variable yields, no greater than 10%.
(9) Crossland, R. K.; Servis, K. L. *J. Org. Chem.* **1970**, *35*, 3195.
(10) See: Montgomery, R.; Wiggins, L. F. *J. Chem. Soc.* **1946**, 393.
(11) See: Hankovszky, H. O.; Hideg, K.; Lex, L. *Synthesis* **1981**, 147.
(12) Staudinger, H.; Hauser, E. *Helv. Chim. Acta* **1921**, *4*, 861.
(13) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543.
(14) Melting points were obtained in a Thomas-Hoover apparatus and are uncorrected. Infrared spectra were recorded in $CDCl_3$ on a 3-200 Sargent-Welch spectrometer. NMR spectra were recorded either on a Varian XL-100, Nicolet QE-300, or Nicolet 360-MHz spectrometer in $CDCl_3$. ^{19}F NMR spectra were recorded at 339.7 MHz. ^1H chemical shifts are expressed in δ units with Me_4Si as an internal standard. ESR spectra were recorded on a Varian E-3 spectrometer. Elemental analysis were determined by Mic Anal., Tucson, AZ. All reactions were routinely run under N_2 atmosphere. Solvents were routinely distilled. Flash chromatography used Grade 633, 200–425-mesh 60 A Aldrich Co. silica gel.

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Continued elution with mixtures of ether and EtOAc gave 59 mg (51%) of diol **3** as a pale yellow oil: ESR (CH_2Cl_2) 3 lines, a_N = 14.5 G; MS, m/e 230.176 (calcd for $C_{12}H_{24}NO_3$, 230.175).

The mixture of **1** and **2** was resubjected to hydrolysis, affording an additional 10 mg (8%) of **3**.

***trans*-2,5-Dimethyl-2,5-bis[3-(methanesulfonyloxy)-propyl]pyrrolidinyl-1-oxy (4).** To a solution of **3** (59 mg, 0.257 mmol) in 5 mL of dry CH_2Cl_2 at −20 °C was added Et_3N (0.091 g, 0.90 mmol) followed by methanesulfonyl chloride (71 mg, 0.62 mmol). After 2.5 h, the reaction was allowed to warm to 0 °C and then brine and CH_2Cl_2 were added. The organic layer was washed with brine and dried ($MgSO_4$). This was passed through silica gel (0.7 g) which was flushed with ether–EtOAc. The combined eluent was evaporated to give 96 mg (96%) of **4** as a waxy solid suitable for the next reaction: TLC (EtOAc), 1 spot, R_f 0.7; IR no OH, 1170, 1335 cm^{-1}; ESR (CH_2Cl_2) 3 lines, a_N = 14.5 G. **4** was stable when stored at 0 °C, but slowly decomposed at 25 °C.

***trans*-2,5-Dimethyl-2,5-bis(3-azidopropyl)pyrrolidinyl-1-oxy (6).** A mixture of 170 mg (0.440 mmol) of **4**, 272 mg (4.18 mmol) of NaN_3, 4.4 mL of DMF, and 0.3 mL of water was stirred at 70 °C for 3.5 h and then cooled. The solvent was removed in vacuo and the residue was extracted with CH_2Cl_2. The extract was washed with brine and water and then dried ($MgSO_4$). This was concentrated and then passed through silica gel (0.8 g), giving 86 mg (70%) of **6** as a pale yellow oil suitable for use in the next experiment: IR 2090 cm^{-1}; ESR (CH_2Cl_2) 3 lines, a_N = 14.5 G.

***trans*-2,5-Dimethyl-2,5-bis(3-aminopropyl)pyrrolidinyl-1-oxy (7) and Oxalate Salt 8.** To a solution of **6** (86 mg, 0.307 mmol) in 20 mL of dry ether was added triphenylphosphine (177 mg, 0.676 mmol). An immediate liberation of gas was observed which slowed over 30 min. The mixture was refluxed for 12 h and then the solvent was removed, affording 256 mg of the waxy phosphinimine which resisted attempts at crystallization: IR no azide peak, 1200, 1100 cm^{-1}; ESR (CH_2Cl_2) 3 lines, a_N = 14.5 G. The entire sample was dissolved in 20 mL of ethanol–water, 1:1, and refluxed for 20 h. The solvent was removed in vacuo and the residue was treated with 5 mL of cold water (resulting pH was >10). HCl (2 N) was added to the chilled solution until pH 3–4. The white precipitate that had formed was extracted into ether (50 mL) followed by CH_2Cl_2 (25 mL). The chilled aqueous phase was then basified to pH 10–12 by addition of 0.5 mL of cold 15% NaOH. Brine (4 mL) was added and then the mixture was extracted with CH_2Cl_2. The extract was dried (K_2CO_3) and concentrated to dryness, giving 66 mg (65%) of bis amino nitroxide **7** as a yellow oil: ESR (CH_2Cl_2) 3 lines, a_N = 14.75 G.

To a 40-mg (0.175 mmol) sample of **7** dissolved in 1 mL of CH_2Cl_2 was added dropwise over 5 min a solution of 36.3 mg (0.404 mmol) of dry oxalic acid dissolved in 4 mL of ether. The resulting yellowish precipitate was collected and washed with ether. It was then dissolved in 0.4 mL of water and reprecipitated by addition of cold acetone. The precipitate was washed with ether and dried, giving 61 mg (84%) of oxalate salt **8**, mp 170–175 °C dec. Recyrstallization from EtOH–water, 2:1, gave the analytical specimen: mp 180–182 °C dec; ESR (MeOH–water, 1:1), 3 lines, a_N = 16.00 G. Anal. Calcd for $C_{16}H_{30}N_3O_9$: C, 47.04; H, 7.41; N, 10.29. Found: C, 46.74; H, 7.34; N, 10.12.

***trans*-1-[Methoxy(trifluoromethyl)phenylacetoxy]-2,5-dimethyl-2,5-bis[3-(benzoyloxy)propyl]pyrrolidine (10) and Diesters 5 and 9.** A solution containing diol **3** (21 mg, 0.091 mmol), benzoyl chloride (64 mg, 0.46 mmol), and pyridine (0.8 mL) was stirred at 0 °C for 4 h and then at 25 °C for 6 h. The solvent was removed in vacuo and the residue was dissolved in CH_2Cl_2. This was washed with cold, saturated $NaHCO_3$ and brine and then dried ($MgSO_4$). Removal of the solvent gave 80 mg of residue containing some benzoyl chloride. Preparative TLC (silica gel, elution with hexane–ether, 7:3) gave 38 mg (94%) of pure **5** as a yellow oil: IR 1715 cm^{-1}; ESR (CH_2Cl_2) 3 lines, a_N = 14.5 G. This was dissolved in ether (4 mL) and hydrogenated[1] over 20 mg of 10% Pd/C at 1 atm for 30 min. The mixture was filtered and the solvent was removed. The residue (crude **9**) was dissolved in 1.6 mL of dry CCl_4 containing 0.4 mL of pyridine. To this was added (+)-methoxy(trifluoromethyl)phenylacetyl chloride[13] (65 mg, 0.26 mmol; 0.65 mL of a CCl_4 stock solution) dropwise over 5 min. After 21 h the solvent was removed and the residue was dissolved in CH_2Cl_2. This was washed with chilled 5% HCl, 5% $NaHCO_3$, and brine and then dried ($MgSO_4$). Removal of the solvent gave 41.5 mg of residue that contained some unreacted **9**. Preparative TLC over silica gel (hexane–ether, 1:1) with recovery of the upper (R_f 0.5) band gave 26 mg (65%) of **10** as a colorless oil: IR 1770, 1715 cm^{-1}; 360-MHz ^1H NMR δ 1.160 (s, 6), 1.31–2.16 (m, 12), 3.491 and 3.513 (two s, 1:1, 3) 4.10–4.44 (m, 4), 7.30–7.49 (m, 7), 7.50–7.60 (m, 4), 7.96–8.15 (m, 4); ^{19}F NMR δ −71.767 and −71.809 (two s, 1:1) (from internal hexafluorobenzene taken to be −163 ppm). Anal. Calcd for $C_{36}H_{40}NO_7F$: C, 65.93; H, 6.15; N, 2.14. Found: C, 65.49; H, 6.31; N, 2.17.

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The Synthesis of a Deoxyoligonucleotide Incorporating 5-Iododeoxyuridine

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X-ray scattering methods must be included among the major techniques for studying macromolecular structure, both in solution and in the solid state. In both phases, the presence of a heavy atom label greatly facilitates the elucidation of the geometric and dynamic structure of the molecule. Of the common covalent modifications of nucleic acids, replacement of the thymine methyl group with an iodine atom is one of the most favorable: The iodine atom has a high electron density, yet its presence introduces a minimal perturbation to the molecular structure (e.g., ref 1). Because of the recent successes in utilizing phosphoramidite-based solid-state synthesis for oligodeoxynucleotides, we decided to attempt the synthesis of 5-iodouridine-containing oligonucleotides by the phosphoramidite method.

Several preparations of oligonucleotides incorporating modified deoxyuridine have recently been reported. For example, Metzler, et al. synthesized the 16 nucleotide base pair O_R3 operator containing 5-fluorodeoxyuridine via a phosphotriester method.[2] The preparation of oligomers incorporating 5-bromodeoxyuridine via a mixed phosphotriester–phosphoramidite method has also been detailed by Delort et al.[3] We wish to report the synthesis of a 5-iododeoxyuridine phosphoramidite and its incorporation into a deoxyhexadecanucleotide via phosphoramidite methodology. The oligomer is an analogue of strand 2 of immobile nucleic acid junction J_1,[4,5] and it is expected to be useful for solution and solid-state X-ray scattering studies.

The first step in this synthesis was the preparation of [5′-(4,4′-dimethoxytrityl)-5-iodo-2′-deoxy-3′-uridinyl](*N*,*N*-diisopropylamino)methoxyphosphine (DMTr-5-IdU phosphoramidite, shown in Figure 1) via standard procedures.[6,7] The deoxyoligonucleotide (5′→3′)-CG-IdU-

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