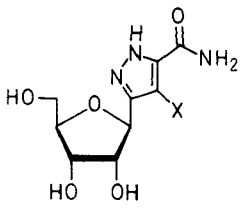
A Convenient Synthesis of 4-Deoxypyrazofurin

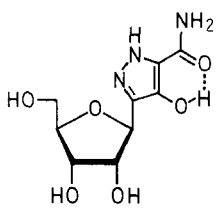
Daryl R. Sauer, Stewart W. Schneller*
Department of Chemistry, University of South Florida, Tampa, FL 33620-5250, USA

A preparation of 3-β-D-ribofuranosyl-1*H*-pyrazole-5-carboxamide (4-deoxypyrazofurin, **3**) is reported in nine steps (in an overall yield of 21%) beginning with 2,5-anhydro-3,4,6-tri-*O*-benzoyl-D-allonitrile (**6**) and proceeding via a 1,3-dipolar cycloaddition reaction between methyl propiolate and 2,5-anhydro-3,4,6-tri-*O*-benzyl-1-deoxy-1-diazo-D-allitol (**4**).

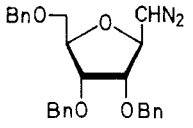
The antitumor[1] and antiviral[2] properties of pyrazofurin (**1**)[3] require its intracellular conversion to the 5'-monophosphate[1,4] whose formation may be dependent on the ability of **1** to adopt the adenosine-like structure **2** through intramolecular hydrogen bonding.[5] In order to produce an analogue that could be used to evaluate the significance of **2** in the biological properties of **1**, a synthesis of 4-deoxypyrazofurin (**3**) was necessary. A review of the literature revealed two syntheses of **3**,[6,7] both of which were inconvenient for the goals of this laboratory. Thus, a new route to **3** was sought and is reported herein in a nine-step sequence (Scheme) that proceeds with an overall yield of 21% via a 1,3-dipolar cycloaddition reaction of the diazoribofuranose derivative **4** with methyl propiolate (**5**).



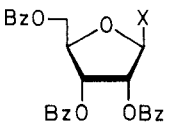
1 X = OH
3 X = H



2



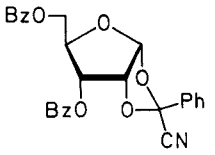
4



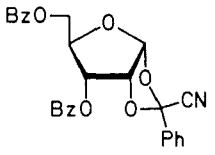
6 X = CN
7 X = OAc

Compound **4** has been reported[8] from 2,5-anhydro-3,4,6-tri-*O*-benzoyl-D-allonitrile (**6**), which, in turn, is generally prepared[9] by treating 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**7**) first with hydrogen bromide followed by mercuric cyanide. For our purposes, this method to **6** was found to be laborious and time consuming and the removal of the mercury salts from the crude product often incomplete, resulting in only moderate yields of **6** (50–60%). On the other hand, reaction of **7** with trimethylsilyl cyanide in the presence of tin chloride[10] smoothly resulted in an 80% yield of crystalline **6**.

Two notable observations were made during our trimethylsilyl cyanide/tin chloride preparation[10] of **6** from **7**. First, not surprisingly, it was found that a 2-*O*-ester group (for example, benzoyl) was necessary for the stereospecific introduction of the cyano group. Secondly, at low temperature, four compounds were isolated from the reaction including **6**, **7**, and the intermediate cyanoketals **8** and **9**, which could be converted to **6** when subjected to the trimethylsilyl cyanide tin chloride conditions. The structures for **8** and **9** were substantiated by ^{13}C-NMR spectral analysis that showed the appearance of (i) nitrile carbons (δ = 116.3 and 117.1) different from the nitrile carbon of **6** (δ = 115.83) and (ii) carbons (δ = 100.40, 101.86, 104.60, and 105.65) attributable to the anomeric carbon and the cyano bearing carbon for each intermediate.



8

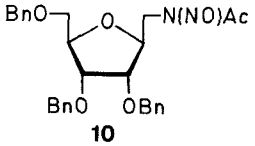


9

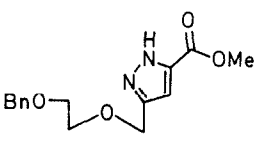
With **6** available, its conversion to the diazo derivative **4** via **10**[8] began with reduction to 1-amino-2,5-anhydro-3,4,6-tri-*O*-benzoyl-1-deoxy-D-allitol (**11**, Scheme) using sodium trifluoroacetoxyborohydride,[11] which avoided reductive cleavage of the benzoyl groups whose presence simplified product isolation. Compound **11** was then converted to 1-acetamido-2,5-anhydro-3,4,6-tri-*O*-benzoyl-1-deoxy-D-allitol (**12**) upon treatment with acetic anhydride/pyridine.

At this point, it became apparent that the benzoyl protecting groups of **12**, which were needed on **7** (particularly at C-1) for the stereospecific synthesis of **6**, would not survive the later basic conditions needed to form **4** from **10**. Thus, **12** was first reacted with sodium methoxide to remove the benzoyl groups and this was followed by reprotection with benzyl chloride at 15°C to give **13**. It was critical that the temperature of the benzylation reaction not exceed 15°C since higher temperatures resulted in *N*-benzylation of the amide functionality.

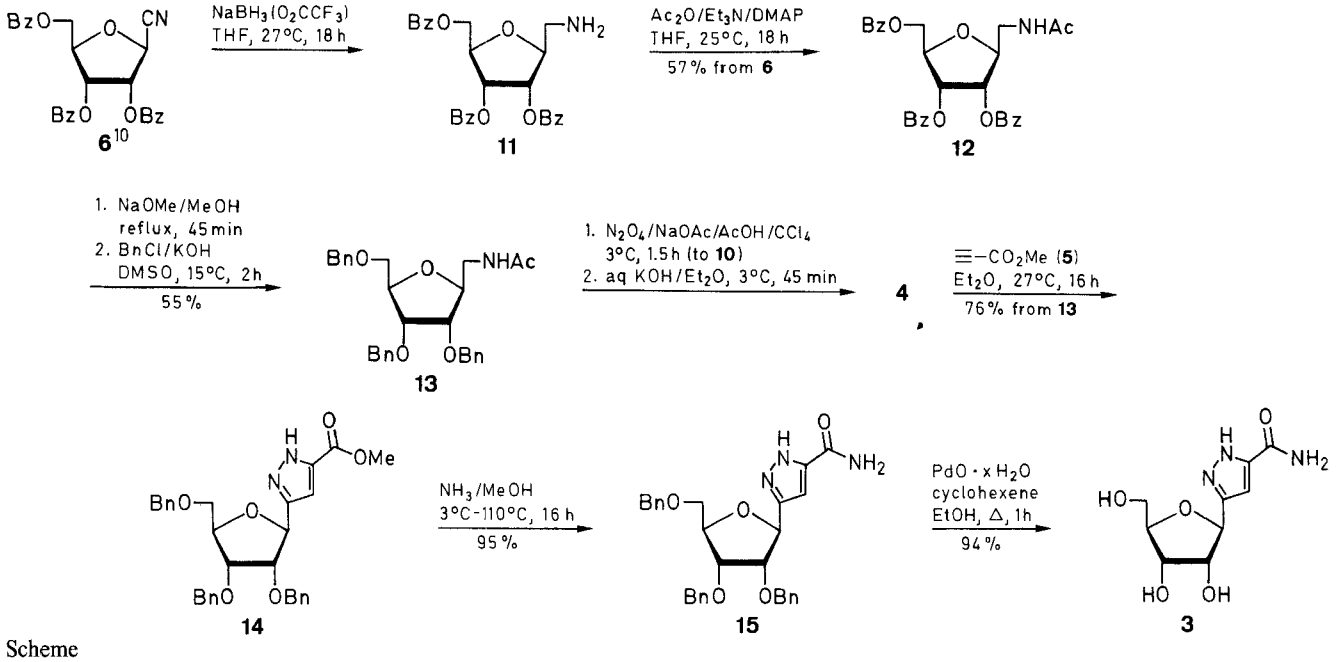
Treatment of **13** with nitrogen dioxide/acetic acid at 3°C easily provided the *N*-nitrosamide **10**,[8] which was treated directly with a well stirred mixture of aqueous potassium hyroxide/diethyl ether to generate the diazo dipole **4**. Reaction of **4** with methyl propiolate resulted in the pyrazole nucleoside **14** as the only detectable regioisomer. This structural assignment was based on the ^{1}H- and ^{13}C-NMR analysis in which the ^{1}H shift of 6.61 ppm observed for the pyrazole proton and the ^{13}C resonance for the unsubstituted pyrazole carbon at 104.94 ppm correlated well with the data for **16** reported recently from this laboratory.[12]



10



16



Scheme

Exposure of **14** to methanolic ammonia at 110 °C resulted in the formation of amide **15**. Deprotection of **15** by transfer hydrogenation with palladium(II) oxide hydrate/cyclohexene resulted in the formation of the desired 4-deoxypyrazofurin (**3**).

Melting points were recorded on a Mel-Temp capillary melting point apparatus and are uncorrected. Combustion analyses were performed by M-H-W Laboratories, Phoenix, AZ. IR spectra were recorded on a Beckman Model FT 1100 spectrophotometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a JEOL FX90Q spectrometer (operated at 90 MHz and 22.5 MHz, respectively) in $CDCl_3$ or DMSO-d_6 referenced to internal TMS. The mass spectral data for **3** was obtained using a VG model 70-250S spectrometer and the optical rotation for **3** was obtained using a Perkin-Elmer 241 polarimeter. Reactions were monitored by thin layer chromatography (TLC) using 0.25 mm. E. Merck Silica gel 60-F_{254} precoated silica gel plates with visualization by irradiation with a Mineralight UVGL-25 lamp or exposure to I_2 vapor. Column chromatography was performed on Aldrich silica gel (230–400 mesh, 60 Å) eluting with the indicated solvent system. Yields refer to chromatographically and spectroscopically (^{1}H- and ^{13}C-NMR) homogeneous materials.

1-Amino-2,5-anhydro-3,4,6-tri-*O*-benzoyl-1-deoxy-D-allitol (11):
A solution of sodium trifluoroacetoxyborohydride (59 mmol) is prepared by adding CF_3CO_2H (6.8 g, 59 mmol) dropwise, under N_2, to an ice bath cooled, stirred suspension of $NaBH_4$ (2.4 g, 63 mmol) in dry THF (10 mL). A solution of **6**[10] (20 g, 42.2 mmol) in dry THF (30 mL) is added dropwise, under N_2, to the reducing agent and the mixture is stirred at 27 °C for 18 h. After this period of time, the reaction is cooled in an ice-H_2O bath and is quenched with H_2O (2 mL). The mixture is then concentrated *in vacuo* and the resulting white paste is partitioned between CH_2Cl_2 (200 mL) and H_2O (200 mL). The organic layer is separated, washed with H_2O (100 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to yield crude **11** as a yellow syrup, which is used directly in the preparation of **12** without further purification.

1-Acetamido-2,5-anhydro-3,4,6-tri-*O*-benzoyl-1-deoxy-D-allitol (12):
Compound **11** is dissolved in dry THF (100 mL) and is treated with Et_3N (5.16 g, 51 mmol), Ac_2O (4.76 g, 46.6 mmol), and 4-dimethylaminopyridine (DMAP, 0.01 g). The mixture is stirred at 25 °C for 18 h. After this period, the reaction is cooled to 0 °C and is quenched with MeOH (1.5 g, 46.8 mmol). The mixture is then concentrated *in vacuo* and the resulting light brown syrup is dissolved in benzene (200 mL). The benzene solution is washed with 1 N HCl (100 mL), sat. aq $NaHCO_3$ (100 mL), sat. aq NaCl (100 mL), dried (Na_2SO_4), filtered, and concentrated *in vacuo* to afford a light yellow syrup. This syrup is purified by silica gel chromatography (hexane/EtOAc, 1:1) to give **12** as a syrup; yield: 12.1 g (57% from **6**). R_f 0.4 (benzene) EtOAc, 1:1).

$C_{29}H_{27}NO_8$	calc.	C 67.30	H 5.26	N 2.71
(517.5)	found	67.12	5.28	2.67

IR (neat): ν = 1653 (amide CO), 1730 (ester CO), 3311 cm^{-1} (NH).

^{1}H-NMR ($CDCl_3$/TMS): δ = 1.85 (s, 3 H, CH_3), 2.0 (m, 2 H), 4.5 (m, 4 H), 4.9–5.2 (m, 2 H), 5.7 (m, 1 H), 7.3–8.1 (m, 15 H_{arom}).

^{13}C-NMR ($CDCl_3$/TMS): δ = 22.9, 40.4, 64.0, 72.3, 72.7, 79.5, 80.6, 128.7, 128.9, 129.2, 133.4, 133.6, 164.7, 165.5, 166.3, 170.0.

1-Acetamido-2,5-anhydro-3,4,6-tri-*O*-benzyl-1-deoxy-D-allitol (13):
A solution of **12** (2.6 g, 5 mmol) in dry MeOH (20 mL) is treated with a 20% NaOMe/MeOH solution (4.1 g, 15 mmol NaOMe) and the mixture is heated to reflux for 45 min under the protection of a drying tube. Following this, the mixture is cooled to r.t., quenched with conc. HCl (1.49 g, 15 mmol HCl) and concentrated *in vacuo*. The remaining syrup is dissolved in H_2O (50 mL), and the aqueous phase is washed with CH_2Cl_2 (2 × 25 mL). The aqueous layer is concentrated *in vacuo*; the residue is dissolved in absolute EtOH (100 mL), filtered, and concentrated *in vacuo* to afford a yellow syrup. This syrup is dissolved in anhydrous DMSO (10 mL), transferred to a three-neck flask and treated, under N_2, with solid KOH (1 g, 15.1 mmol). The mixture is then cooled to 15 °C and benzyl chloride (2.11 g, 16.7 mmol) is added dropwise with the aid of mechanical stirring. The temperature is maintained at 15 °C for 2 h. After this period, the reaction is poured over ice-H_2O (100 mL) and the mixture stirred for 1 h. The aqueous phase is then extracted with benzene (3 × 75 mL), and the combined organic extracts dried ($MgSO_4$), filtered, and concentrated *in vacuo* to give a colorless syrup. This syrup is purified by silica gel chromatography (benzene/EtOAc, 1:1) to afford of **13** as a white solid; yield: 1.3 g (55%); mp 68 °C (Lit.[8] mp 65–68 °C); R_f 0.2 (benzene/EtOAc, 1:1).

$C_{29}H_{33}NO_5$	calc.	C 73.24	H 6.99	N 2.95
(475.5)	found[13]	73.13	6.97	2.93

IR (KBr): ν = 1658 (amide CO), 3316 cm^{-1} (NH).

^{1}H-NMR ($CDCl_3$/TMS): δ = 1.54 (s, 3 H, CH_3), 3.5–4.0 (m, 8 H, H-1, H-2, H-3, H-4, H-5, and H-6), 4.52 (s, 6 H, 3 × CH_2 of benzyl), 6.20 (br s, 1 H, NH), 7.34 (s, 15 H_{arom}).

^{13}C-NMR ($CDCl_3$/TMS): δ = 22.43, 71.35, 73.41, 73.68, 74.98, 78.61, 79.85, 82.02, 82.45, 129.26, 129.42, 129.80, 129.91, 139.01, 139.12, 171.52.

Methyl 3-(2,3,5-Tri-*O*-benzyl-β-D-ribofuranosyl)-1*H*-pyrazole-5-carboxylate (14):

2,5-Anhydro-3,4,6-tri-O-benzyl-1-deoxy-1-(N-nitrosoacetamido)-D-allitol (**10**): A mixture composed of **13** (1.2 g, 2.5 mmol) is dissolved in a 1:1-mixture of CCl_4/glacial AcOH (20 mL) containing anhydr. NaOAc (1.2 g). This mixture is cooled to 3 °C in an ice-H_2O bath, treated with liquid N_2O_4 (2 mL), and then stirred for 1.5 h at 3 °C. Following this period, the solution is poured over ice-H_2O (120 mL) with subsequent vigorous stirring of the resultant mixture for 0.5 h. The organic layer is then separated and the aqueous layer extracted with CH_2Cl_2 (2 × 25 mL). The combined organic layers are washed with sat. aq $NaHCO_3$ (50 mL), dried ($MgSO_4$), filtered, and the filtrate concentrated *in vacuo* to yield the allitol derivative **10**[8] as a light green syrup;

IR (neat): ν = 1733 (CO), 1500 (NO) cm^{-1}. This syrup, which shows no IR absorption at 3316 cm^{-1} (NH) or 1658 cm^{-1} (CO) to suggest unreacted **13**, is used immediately in the next reaction.

2,5-Anhydro-3,4,6-tri-O-benzyl-1-deoxy-1-diazo-D-allitol (**4**):

The *N*-nitroso amide **10** (assumed to be 2.5 mmol) is dissolved in Et_2O (6 mL) and mixed vigorously with an ice-cold solution of KOH (1.44 g) dissolved in H_2O (3 mL). The mixture is then stirred at 3 °C for 45 min after which the IR spectrum of the ether layer shows the formation of a strong band at 2067 cm^{-1} (CHN_2) with no band at 1500 cm^{-1} (NO) apparent. The mixture is diluted with Et_2O (12 mL) and H_2O (25 mL) and the layers separated. The Et_2O layer is washed with H_2O (10 mL) and dried rapidly by first swirling the ether phase over KOH pellets and followed by decantation into anhydr. $MgSO_4$. After filtration, the golden colored filtrate containing compound **4**,[8] which displays an IR band (neat) at 2067 cm^{-1} (N_2), is used immediately in the next reaction.

Methyl 3-(2,3,5-Tri-O-benzyl-β-D-ribofuranosyl)-1H-pyrazole-5-carboxylate (**14**):

The aforementioned ethereal solution of **4** is added to a solution of methyl propiolate (0.25 g, 3 mmol) in anhydr. Et_2O (10 mL). The mixture is stirred at 27 °C for 16 h after which TLC analysis (hexane/EtOAc, 1:1) indicates that the reaction proceeds to completion (during this time, the solution color changes from golden to light yellow). The mixture is then concentrated *in vacuo* and the residue is purified by column chromatography (hexane/EtOAc, 1:1) to give **14** as a colorless syrup; yield: 1.01 g (76% from **13**); R_f 0.33 (EtOAc/hexane, 75:25).

$C_{31}H_{32}N_2O_6$	calc.	C 70.43	H 6.10	N 5.30
(576.5)	found	70.23	6.12	5.30

IR (neat): ν = 1725 cm^{-1} (ester CO).

^{1}H-NMR ($CDCl_3$/TMS): δ = 3.58–4.6 (m, 14 H, H-2′, H-3′, H-4′, H-5′, 3 × CH_2 of benzyl, and ester CH_3), 5.21 (d, 1 H, J = 3.1 Hz, H-1′), 6.61 (s, 1 H, pyrazole H-4), 7.30 (s, 15 H_{arom}), 13.0 (br s, 1 H, pyrazole NH).

^{13}C-NMR ($CDCl_3$/TMS): δ = 51.95, 68.42, 72.38, 73.41, 76.71, 76.82, 80.50, 81.86, 104.94, 127.91, 128.01, 128.39, 128.50, 128.61, 137.06, 137.33, 137.49, 142.75, 145.78, 162.42.

3-(2,3,5-Tri-*O*-benzyl-β-D-ribofuranosyl)-1*H*-pyrazole-5-carboxamide (15):

A solution of **14** (700 mg, 1.32 mmol) in freshly distilled MeOH (30 mL) is saturated with NH_3 at 3 °C and the resulting mixture is heated in a sealed glass tube at 110 °C for 16 h. Upon cooling, TLC analysis ($CHCl_3$/MeOH, 9:1) indicates that the reaction proceeds to completion. The solution is then concentrated *in vacuo* and the residue is purified by silica gel column chromatography ($CHCl_3$/MeOH, 9:1) to give **15** (650 mg, 95%) as a colorless glass; yield: 650 mg (95%); R_f 0.57 ($CHCl_3$/MeOH, 9:1).

$C_{30}H_{31}N_3O_5$	calc.	C 70.16	H 6.08	N 8.18
(513.6)	found	70.14	6.23	8.14

IR (neat): ν = 1680 (amide CO), 3350 cm^{-1} (NH).

^{1}H-NMR ($CDCl_3$/TMS): δ = 3.54–4.63 (m, 11 H, H-2′, H-3′, H-4′, H-5′, and 3 × CH_2 of benzyl), 5.24 (d, 1 H, J = 3 Hz, H-1′), 6.04 (br d, 2 H, NH_2), 6.57 (s, 1 H, pyrazole H-4), 7.31 (s, 15 H_{arom}), 11.78 (br s, 1 H, pyrazole NH).

^{13}C-NMR ($CDCl_3$/TMS): δ = 68.58, 72.32, 73.57, 76.34, 76.82, 80.18, 81.58, 102.55, 127.85, 127.96, 128.07, 128.39, 128.50, 128.61, 136.95, 137.17, 137.44, 144.65, 146.81, 164.31.

3-β-D-Ribofuranosyl-1*H*-pyrazole-5-carboxamide (4-Deoxypyrazofurin, 3):

A solution of **15** (630 mg, 1.23 mmol) in a 3:1 mixture of abs. EtOH/cyclohexene (20 mL) is treated with PdO · xH_2O (50 mg). The mixture is refluxed for 1 h after which TLC analysis (MeCN/H_2O, 96:4) shows complete loss of starting material. The mixture is then cooled, filtered through a pad of Celite that has been washed with hot EtOH; the Celite pad is then washed with hot EtOH, and the combined filtrates concentrated. The resulting colorless glass is purified by column chromatography using silica gel (MeCN/H_2O, 94:6) to yield **3** as a white amorphous solid; yield: 94% (280 mg); $[\alpha]_D^{20}$ −18.5° (c = 0.87, EtOH) [Lit.[6] $[\alpha]_D^{20}$ −18.8° (c = 0.3, $CHCl_3$)]; R_f 0.29 (MeCN/H_2O, 94:6).

$C_9H_{13}N_3O_5 \cdot 0.5\,MeOH \cdot 0.25\,H_2O$				
(243.1)	calc.	C 43.26	H 5.92	N 15.93
	found[14]	43.24	5.73	15.93

HRMS (EI): m/z calc. for (M^+): 243.0855; found: 243.0856.

IR (KBr): ν = 1670 (amide CO), 3200–3500 cm^{-1} (NH and OH).

^{1}H-NMR (DMSO-d_6/TMS): δ = 3.53–4.80 (m, 9 H, H-1′, H-2′, H-3′, H-4′, H-5′, and 3 × OH), 6.75 (s, 1 H, H-4), 7.5 (br d, 2 H, NH_2), 12.75 (br s, 1 H, pyrazole NH).

^{13}C-NMR (DMSO-d_6/TMS): 61.81, 71.02, 75.68, 76.66, 84.78, 103.47, 143.65, 146.65, 162.42.

MS (EI): m/z (%) = 243 (M^+, 3.25), 225 ($M^+ - H_2O$, 4.4), 140 (heterocycle + CH_2O, 100), which agrees with the literature.[7]

This work was supported by the U.S. Army Medical Research and Development Command (DAMD17-89-C-9092), which is appreciated. It is also to be noted that any opinions, interpretations, conclusions, and recommendations raised herein are those of the authors and are not necessarily endorsed by the U.S. Army.

Received: 29 November 1990; revised: 15 March 1991

(1) Cadman, E.C.; Dix, D.E.; Handschuhmacher, R.E. *Cancer Res.* **1978**, *38*, 682.
(2) De Clercq, E.; Torrence, P.F. *J. Carbohydr. Nucleosides Nucleotides* **1978**, *5*, 187.
Petrie, C.R., III; Revankar, G.R.; Dalley, N.K.; George, R.D.; McKernan, P.A.; Hamill, R.L.; Robins, R.K. *J. Med. Chem.* **1986**, *29*, 268.
(3) Buchanan, J.G., in: *Progress in the Chemistry of Organic Natural Products*, Herz, W.; Grisebach, H.; Kirby, G.W. (eds.), Springer-Verlag, New York, 1983; Vol. 44, pp 270–276.
(4) Gutowski, G.E.; Sweeney, M.J.; DeLong, D.C.; Hamill, R.L.; Gerzon, K.; Dyke, R.W. *Ann. N.Y. Acad. Sci.* **1975**, *255*, 544.
(5) Guranowski, A.; Montgomery, J.A.; Cantoni, G.L.; Chiang, P.K. *Biochemistry* **1981**, *20*, 110.
(6) Vanek, T.; Gut, J. *Collect. Czech. Chem. Commun.* **1982**, *47*, 2004.
(7) Buchanan, J.G.; Saxena, N.K.; Wightman, R.H. *J. Chem. Soc., Perkin Trans. 1* **1984**, 2367.
(8) Acton, E.M.; Ryan, K.J., in: *Nucleic Acid Chemistry, Improved and New Synthetic Procedures, Methods and Techniques*; Townsend, L.B.; Tipson, R.S., (eds.), Wiley: New York, 1978, Part 1, pp 475–480.

(9) Bobek, M.; Farkas, J. *Collect. Czech. Chem. Commun.* **1969**, *34*, 247.
(10) Cook, P.D.; McNamara, D.J. *J. Heterocycl. Chem.* **1986**, *23*, 155.
(11) Umino, N.; Iwakuma, T.; Itoh, N. *Tetrahedron Lett.* **1976**, 2875.
(12) Sauer, D.R.; Schneller, S.W. *J. Org. Chem.* **1990**, *55*, 5535.

(13) Microanalytical data for **13** was obtained since its synthesis was different than that reported in Ref. 8 and an authentic sample of **13** by the latter method[8] was not available.
(14) For the purposes of obtaining the microanalytical data for **3**, MeOH was used to transfer the sample to a small vial for delivery to M-H-W Laboratories and it was not possible to free the sample from all of the MeOH to obtain an analysis without the presence of this solvent.