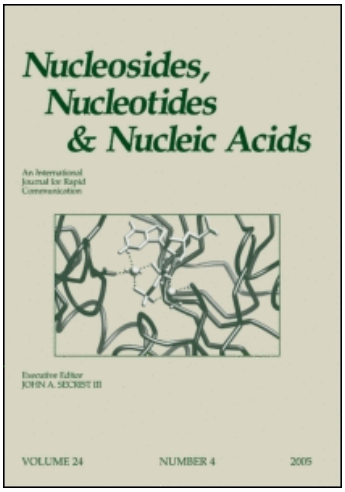

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Synthesis of 2'-Deoxyformycin B and 2'-Deoxyoxoformycin B

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SYNTHESIS OF 2'-DEOXYFORMYCIN B AND 2'-DEOXYOXOFORMYCIN B

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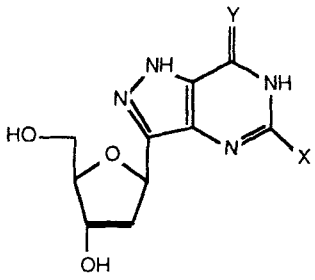
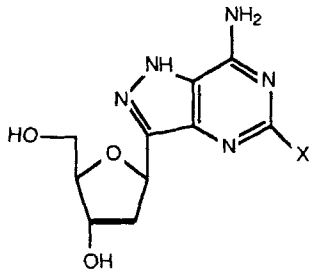
3-β-D-Ribofuranosylpyrazolo[4,3-*d*]pyrimidines (formycins)[1] modified in the heteroaromatic moiety are of biological interest as analogues of adenosine and guanosine, and have been the objects of intensive synthetic chemical effort by several groups.[2-9] 2'-Deoxynucleosides[2c,2d,7b,13] and other analogues of the formycins modified in the sugar moiety[10-12] are also of potential interest, but have been less extensively studied. Examples of the 2'-deoxyribonucleoside type known to date include the 2'-deoxy-6-thioguanosine analogue **1**, the 2'-deoxyadenosine (dAdo) analogue **2** (2'-deoxyformycin A),[10,13] and the 2-chloro-2'-deoxyadenosine analogue **3**.[7b] Compound **2** was found to be 10-15 times more potent than 2'-deoxyadenosine as an inhibitor of the growth of S49 cells, a murine lymphoma line of T-cell origin.[13] Activity depended on 5'-phosphorylation, since mutants lacking the enzymes adenosine kinase (AK) and deoxycytidine kinase (dCK) were insensitive to the drug. Furthermore, activity was comparable in the presence and absence of an AK inhibitor, suggesting that **2**, unlike dAdo, may be a poor substrate for adenosine deaminase. That 5'-phosphorylation of **2** was mediated by AK rather than dCK was indicated by the fact that mutants lacking only dCK retained sensitivity. This contrasted with the behavior of dAdo, which is known to be a substrate for both AK and dCK.[14]



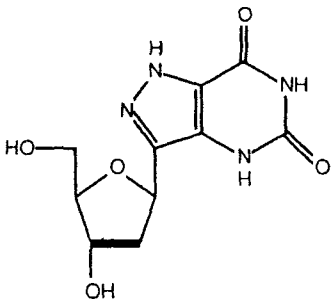
1 : X = NH_2, Y = S

4 : X = H, Y = O



2 : X = H

3 : X = Cl



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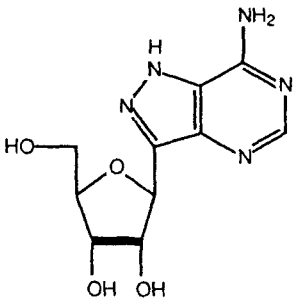

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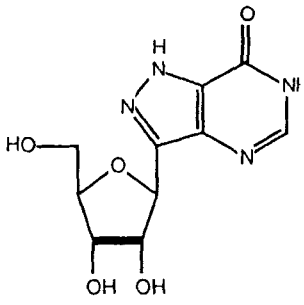

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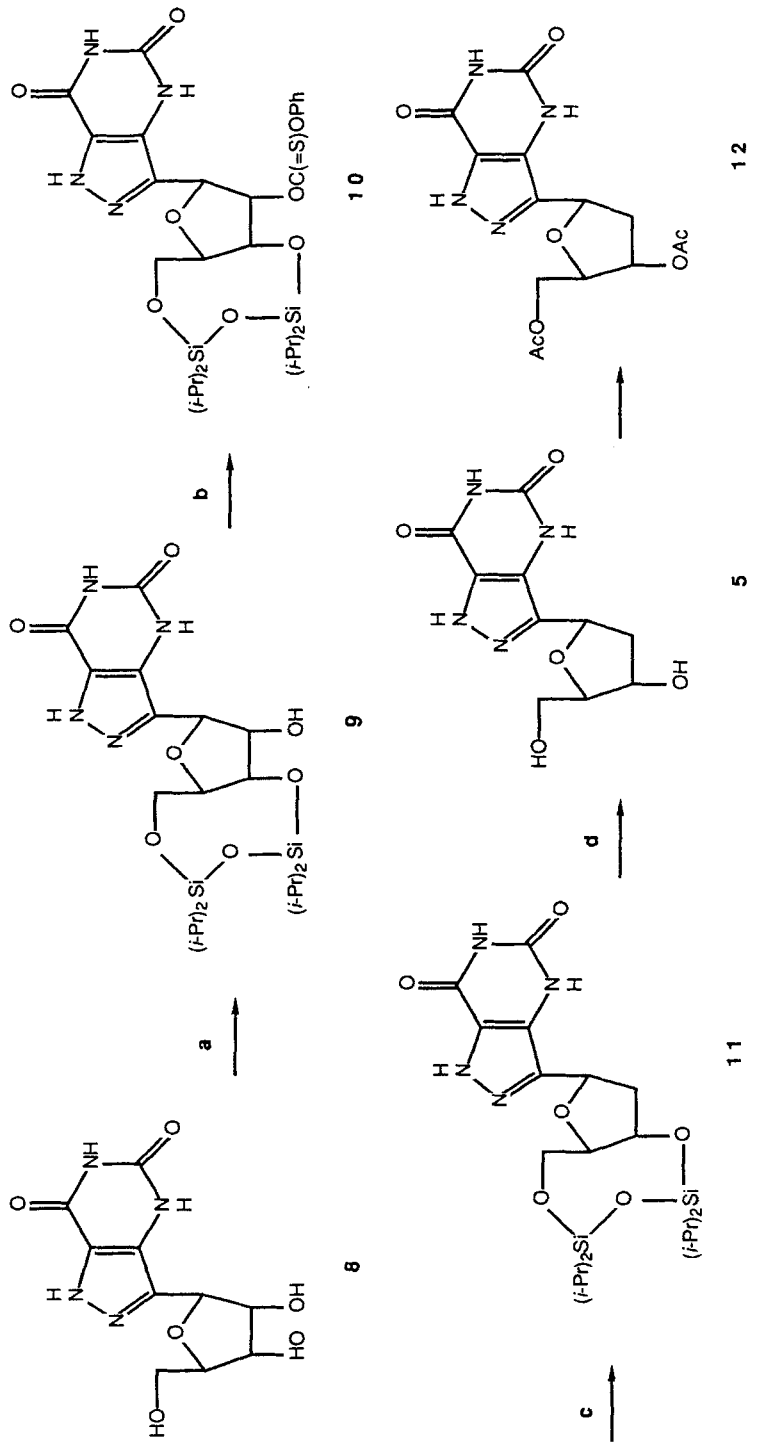
This Note reports the first synthesis of the heretofore undescribed 2'-deoxyformycins **4** and **5**, which may be viewed as C-nucleoside analogues of 2'-deoxyinosine and 2'-deoxyxanthosine, respectively. These compounds also represent potential intermediates for the synthesis of other 2'-deoxyformycins of biological interest. Deamination of **2** proceeded cleanly on incubation with calf intestinal adenosine deaminase at room temperature for 2 days, giving 3-(β-D-ribofuranosyl)-pyrazolo[4,3-*d*]pyrimidin-7(1*H*,6*H*)-one (2'-deoxyformycin B, **4**) in 80% yield. For the synthesis of **5**, incubation of 7-amino-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine (formycin A, **6**) with the same enzyme afforded 3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidin-7(1*H*,6*H*)-one (formycin B, **7**),[15] which was then oxidized with bromine essentially as described by Ugarkar and coworkers[7b] to obtain 3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine-5,7(1*H*,4*H*,6*H*)-dione (oxoformycin B, **8**) in overall yields of 55-65% based on formycin A. Further transformation of **8** to **5** (Scheme 1) followed the general approach[16] used previously in this laboratory to obtain **2**.[13] Treatment of **8** with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane in pyridine afforded the 3',5'-



a: $(i\text{-}Pr)_2Si(Cl)OSi(Cl)(i\text{-}Pr)_2/C_5H_5N$ b: $PhO(C{=}S)Cl/DMAP$ c: $n\text{-}Bu_3SnH$ d: $n\text{-}Bu_4N^+F^-$ d: $Ac_2O/DMAP$

Scheme 1

cyclosilylated derivative **9** (55%), which on further reaction of **9** with phenoxythiocarbonyl chloride in acetonitrile containing *N,N*-dimethylaminopyridine (DMAP) gave the xanthate ester **10** (70%). Reduction of **10** with tri-*n*-butyltin hydride in the presence of α,α'-azobisisobutyronitrile then gave the blocked 2'-deoxynucleoside **11** (85%). Deprotection of **11** with tetra-*n*-butylammonium fluoride in a mixture of toluene and tetrahydrofuran at 70°C proceeded in 87% yield, completing the synthesis of **5** in an overall four-step yield of 28% starting from oxoformycin B (**8**). Treatment of **5** with acetic anhydride in pyridine gave the 3',5'-di-*O*--acetyl derivative **12** (87%).

Proton magnetic resonance spectra of compounds **5** and **9-12** revealed several notable features consistent with their structures. For example, while the signal for the $C_{5'}$ methylene group in the ribosides **9** and **10** occured at δ 4.0-4.1, the corresponding signal for deoxyribosides **5** and **11** was seen as a pair of multiplets at δ 3.7 and δ 3.9. Similarly, there was a difference in the chemical shift of the $C_{1'}$ proton, which appeared at δ 5.1 in the riboside **9** but δ 5.5 in the deoxyribosides **5** and **11**. The 2'-O-phenoxythiocarbonyl group in **10** had a marked deshielding effect on the $C_{1'}$ proton, as evidenced by a displacement in chemical shift from δ 5.1 to δ 6.1. Finally, while the $C_{2'}$, $C_{3'}$, and $C_{4'}$ protons gave rise to a broad multiplet centered at δ 4.3 in the ribosides **9** and **10**, the deoxyribosides **5** and **11** displayed separate multiplets at δ 4.1-4.3 and δ 4.5 for the $C_{4'}$ and $C_{3'}$ protons, and an upfield multiplet at δ 2.3-2.7 for the $C_{2'}$ protons.

The availability of compounds **4** and **5** via the route described here offers opportunities for further semisynthetic modifications in the formycin series.

Experimental

Infrared spectra were obtained with a Perkin-Elmer Model 781 double-beam recording spectrophotometer, ultraviolet spectra with a Varian Model 210 instrument, and 60 MHz proton nmr spectra with a Varian T60 spectrometer using Me_4Si as the reference. TLC was carried out on Whatman MK6F silica gel and Baker 250F silica gel plates containing a fluorescent indicator. Spots were visualized under 254 nm ultraviolet light or with the aid of iodine. Column chromatograpy was performed on Baker 3405 (60-200 mesh) silica gel, Baker "Flash" (40 μm) silica gel, or Whatman DE-52 pre-swollen carboxymethylcellulose. Melting points were measured in Pyrex capillary tubes in a Mel-Temp Apparatus (Cambridge Laboratory Devices, Cambridge, MA) or a Fisher-Johns hot stage apparatus (Fisher, Medford, MA), and are not corrected. Reagent grade solvents were redistilled and routinely stored over 4A molecular sieves. Chemicals were purchased

from Aldrich (Milwaukee, WI), Sigma (St. Louis, MO), and Calbiochem (La Jolla, CA). 2'-Deoxy-formycin A was prepared as previously described.[13]

3-(2-Deoxy-β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidin-7(1*H*,4*H*)-one (2'-deoxyformycin B) (4). Calf intestinal adenosine deaminase (5 mg) was added to a solution of 2'-deoxyformycin A (20 mg, 0.072 mmol) in 0.05 *M* phosphate buffer, pH 6.8 (8 mL), and the solution was left at 37°C for 2 days before being concentrated to dryness under reduced pressure. The residue was applied onto a Dowex 50W-X2(H^+) column (20 x 1 cm), which was washed first with water to remove salt and then with a gradient of 1% to 5% NH_4OH. Fractions containing the product (R_f 0.4; silica gel, 4:1 $CHCl_3$-MeOH) were pooled and evapo-rated. Recrystallization from water afforded a white solid (16 mg, 80% yield), mp. 240-243°C. Anal. ($C_{10}H_{12}N_4O_4$) Calcd.: C, 47.62; H, 4.80; N, 22.21; Found: C, 47.27; H, 4.83; N, 22.10.

3-(β-D-Ribofuranosyl)pyrazolo[4,3-d]pyrimidine-5,7(1*H*,4*H*,6*H*)-dione (8). A suspension of formycin A (**6**) (10 g, 0.037 mol) in 450 mL of 0.05 *M* sodium phosphate buffer, pH 7.4, was stirred gently, and calf intestinal adenosine deaminase (Sigma type II) (150 mg) was added. After 24 h of stirring at room temperature, TLC analysis revealed that deamination was incomplete. The mixture was left to stand for another 120 h, at which time the starting material was no longer detectable. The product crystallized out upon concentration of the mixture to a small volume, and was filtered, washed with cold water, and dried in vacuo at 40°C overnight. Two crops weighing a total of 9 g (90% yield) were obtained, and were shown to be identical with a reference specimen of formycin B (**7**) (Sigma). The best solvent system to resolve **6** and **7** on silica gel plates was obtained by combining $CHCl_3$, MeOH, and 15% NH_4OH in a 3:2:1 ratio (v/v/v), shaking the mixture vigorously, allowing the layers to separate overnight, and using the lower layer to develop the plate. Observed R_f values for adenosine, **6**, and **7** with this system were 0.47, 0.25, and 0.15, respectively.

A vigorously stirred suspension of **7** (4.7 g, 0.018 mol) in 90 mL of water was treated dropwise at ambient temperature with a solution of bromine (5.3 mL) in water (440 mL) over a period of 45 min. After being left to stir for 5 days, the mixture was purged with nitrogen to remove most of the excess bromine, the pH was adjusted to 7 with 1 *N* NaOH, and the product was collected by filtration, washed with water, and dried under reduced pressure to obtain **8** as a colorless solid (3.1 g, 62% yield); mp 284-286°C (lit.[3] 284-286°C); IR (KBr): ν 3080, 1700, 1680 cm^{-1}; UV: λ_{max} (pH 7) 286 nm; λ_{max} (pH 11) 296 nm.

3-[3,5-O-(1,1,3,3-tetraisopropyl-1,3-disiloxanediyl)-β-D-ribofuranosyl]pyrazolo[4,3-*d*]pyrimidine-5,7(1*H*,4*H*,6*H*)-dione (9). 1,3-Dichloro-1,1,3,3-tetraisopropyldisiloxane (2.5 mL, 8 mmol) was added to a stirred suspension of **8** (1.14 g, 4 mmol) in dry pyridine (40 mL) at room temperature. After 24 h, the solvent was evaporated under reduced pressure and the residue was partitioned between EtOAc and water. The organic layer was washed successively with ice-cold 1 *N* HCl (2 x 50 ml), water, saturated $NaHCO_3$, and saturated NaCl. Drying (Na_2SO_4) and evaporation of the solvents left a gum (3 g), which was chromatographed on a silica gel column (35 x 2.5 cm) with 2% MeOH in $CHCl_3$ (500 mL) followed by 3% MeOH in $CHCl_3$ (500 mL) as the eluents. Fractions containing only the desired product (R_f 0.8; silical gel, 4:1 $CHCl_3$-MeOH) were pooled and evaporated, and the residue was recrystallized from MeCN to obtain a white solid (1.15 g, 55% yield), mp 145-148°C; IR (KBr): ν 3420, 3200, 2960, 2880, 1710, 1470, 1040 cm^{-1}; NMR ($CDCl_3$ + CD_3OD): δ 1.05 (m, 28H, i-Pr_2Si), 4.06 (s, 2H, $C_{5'}$-H_2), 4.3 (m, 3H, $C_{2'}$-H, $C_{3'}$-H, $C_{4'}$-H), 5.1 (d, 1H, $C_{1'}$-H). Anal. ($C_{22}H_{38}N_4O_7Si_2$) Calcd.: C, 50.17; H, 7.27; N, 10.63; Si, 10.66. Found: C, 49.89; H, 7.35; N, 10.47; Si, 10.45.

3-[3,5-O-(1,1,3,3-Tetraisopropyl-1,3-disiloxanediyl)-2-O-phenoxythiocarbonyl-β-D-ribofuranosyl]pyrazolo[4,3-*d*]pyrimidine-5,7(1*H*,4*H*,6*H*)-dione (10). Phenoxythiocarbonyl chloride (0.52 mL, 2.6 mmol) was added with the aid of syringe to a stirred solution of **9** (1.05 g, 2 mmol) and 4-*N*,*N*-dimethylaminopyridine (1.13 g, 5 mmol) in dry MeCN. Stirring was continued at room temperature for 24 h, the solvent was removed by rotary evaporation, and the crude product was partitioned between EtOAc and water. The solvent was evaporated, and the residue (1.83 g) was chromatographed on a silica gel column (40 x 2.5 cm) with $CHCl_3$ (500 mL) followed by 1% MeOH in $CHCl_3$ (1000 mL) as the eluents. Fractions containing the desired product (R_f 0.27; silica gel, 95:5 $CHCl_3$-MeOH) were pooled and evaporated, and the residue was recrystallized from MeCN to obtain yellowish crystals (0.93 g, 70% yield), mp 148-151°C (foaming at 131-133°C); NMR ($CDCl_3$): δ 1.05 (m, 28H, i-Pr_2Si), 4.1 (s, 2H, $C_{5'}$-H_2), 4.33 (m, 2H, $C_{3'}$-H, $C_{4'}$-H), 5.5 (m, 1H, $C_{2'}$-H), 6.1 (d, 1H, $C_{1'}$-H), 7.3 (m, 5H, phenyl). Anal. ($C_{29}H_{42}N_4SSi_2O_8$) Calcd.: C, 52.54; H, 6.38; N, 8.45; S, 4.83; Si, 8.46. Found: C, 52.21; H, 6.38; N, 8.38; S, 5.16; Si, 8.36.

3-[2'-Deoxy-3',5'-O-(1,1,3,3-tetraisopropyl-1,3-disiloxyanediyl)-β-D-ribofuranosyl]pyrazolo[4,3-*d*]pyrimidine-5,7(1*H*,4*H*,6*H*)-dione (11). α,α'-Azobisisobutyronitrile (100 mg, 0.615 mmol) was added to a solution of **10** (3.31 g, 5 mmol) in anhydrous toluene (150 mL), and the solution was purged with dry nitrogen for 30 min. Tri-*n*-butyltin

hydride (4.5 mL, 17 mmol) was added through a septum with the aid of a syringe, and the reaction mixture was heated at 75°C for 3 h and then left at room temperature overnight. The solvent was evaporated under reduced pressure, and the crude product was chromatographed on a silica gel column (40 x 2.5 cm), which was eluted first with $CHCl_3$ (500 mL) to remove hexa-*n*-butyltin and then with 2% MeOH in $CHCl_3$ (500 mL) and 5% MeOH in $CHCl_3$ (1000 mL). Fractions containing the desired product (R_f 0.54; silica gel, 9:1 $CHCl_3$-MeOH) were pooled and evaporated. Rerystallization from MeCN afforded a white solid (2.16 g, 85% yield), mp 193-195°C; NMR ($CDCl_3$): δ 1.1 (m, 28H, i-Pr_2Si), 2.3-2.7 (m, 1H, $C_{2'}$-H), 3.7 (m, 1H, $C_{5'}$-H_a), 3.9 (m, 1H, $C_{5'}$-H_b), 4.15 (m, 1H, $C_{4'}$-H), 4.53 (d, 1H, $C_{3'}$-H), 5.5 (dd, 1H, $C_{1'}$-H). Anal. ($C_{22}H_{38}N_4Si_2O_6$) Calcd.: C, 51.74; H, 7.50; N, 10.97; Si, 10.99. Found: C, 51.69; H, 7.78; N, 10.73; Si, 10.18.

3-(2-Deoxy-β-Dribofuranosyl)pyrazolo[4,3-*d*]pyrimidine-5,7-(1*H*,4*H*,6*H*)-dione (2'-deoxyoxoformycin B) (5). A solution of 1 *M* tetra-*n*-butyl-ammonium fluoride in THF (30 mL, 30 mmol) was added to a solution of **11** (2.15 g, 4.21 mmol) in anhydrous toluene (125 mL), and the reaction mixture was heated for 1 h at 75°C. The solvents were evaporated under reduced pressure, and the residue was partitioned between Et_2O (250 mL) and water (250 mL). The water layer was evaporated to dryness and final traces of moisture were removed by co-evaporation with EtOH to obtain a gum, which was purified on silica gel column (40 x 2.5 cm) with $CHCl_3$ (500 mL) followed by 5% MeOH in $CHCl_3$ as the eluents. Fractions containing the desired product (R_f 0.2; silica gel, 4:1 $CHCl_3$-MeOH) were pooled and evaporated to dryness. The residue was recrystallized from aqueous MeOH to obtain white crystals (1 g, 87% yield), mp 234-237°C; NMR (CD_3OD): δ 2.3-2.7 (m, 1H, $C_{2'}$-H), 3.7 (m, 1H, $C_{5'}$-H_a), 3.9 (m, 1H, $C_{5'}$-H_b), 4.25 (m, 1H, $C_{4'}$-H), 4.53 (d, 1H, $C_{3'}$-H), 5.5 (dd, 1H, $C_{1'}$-H). Anal. ($C_{10}H_{12}N_4O_5 \cdot 0.33H_2O$) Calcd.: C, 43.79; H, 4.66; N, 20.42. Found: C, 43.91; H, 4.84; N, 20.02.

3-[3,5-Di-O-acetyl-2-deoxy-β-D-ribofuranosyl]pyrazolo[4,3-*d*]pyrimidine-5,7(1*H*,4*H*,6*H*)-dione (12). A mixture of **5** (82 mg, 0.3 mmol), *N*,*N*-dimethylaminopyiridine (60 mg), and acetic anhydride (10 mL) was warmed gently until the solid dissolved. After being left to stand at room temperature overnight, the reaction mixture was evaporated to dryness under reduced pressure. The solid was puried by chromatography on a silica gel column (25 x 1 cm) with 95:5 $CHCl_3$-MeOH as the eluent. Fractions that were homogeneous by TLC (R_f 0.43; silica gel, 9:1 $CHCl_3$-MeOH) were pooled, the solvents were evaporated, and the residue was

recrystallized from water to obtain white crystals (92 mg, 87% yield), mp 248-249°C; IR (KBr): ν 3440, 3180, 1730 (ester C=O), 1680, 1250 cm^{-1}; NMR ($CDCl_3$): δ 2.2 (s, 6H, 3' and 5'-CH_3CO), 2.3-2.5 (m, 1H, $C_{2'}$-H), 2.7 (d, 1H, $C_{5'}$-H_a), 2.8 (d, 1H, $C_{5'}$-H_b), 4.53 (m, 2H, $C_{4'}$-H and $C_{3'}$-H), 5.3 (dd, 1H, $C_{1'}$-H). Anal. ($C_{14}H_{16}N_4O_7$) Calcd.: C, 47.73; H, 4.57; N, 15.90. Found: C, 47.72; H, 4.52; N, 15.54.

Acknowledgments

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