Tetrahedron Vol. 44, No 23, pp 7001 to 7006, 1988
Printed in Great Britain

0040-4020/88 $3.00 + 00

Sulfone of the Antibiotic, Nebularine: Synthesis and Conversion to Novel Analogues of Nebularine

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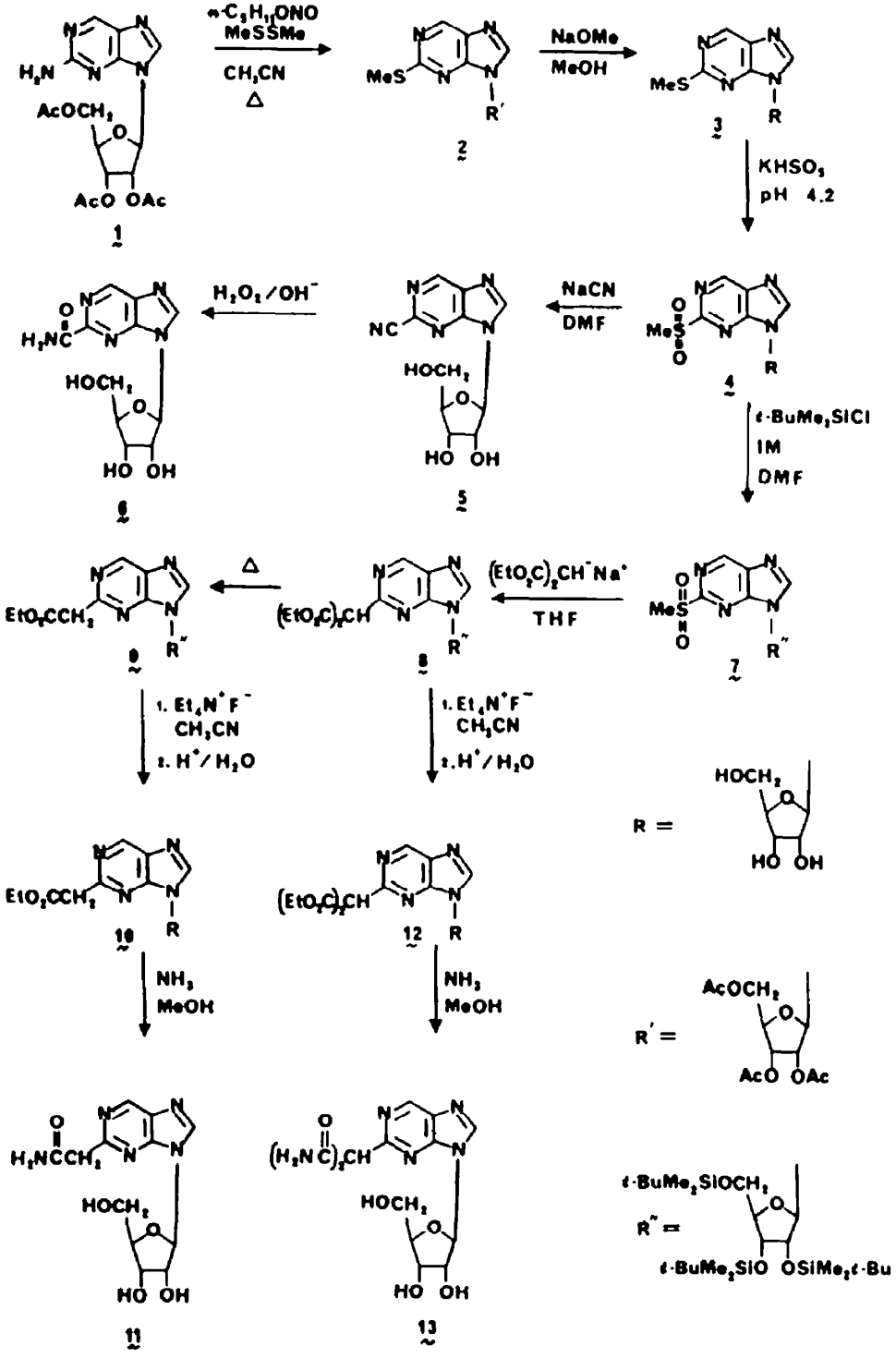
(*Received in USA 26 August 1988*)

Abstract: 2-Methylsulfonylnebularine has been synthesized from 2-aminonebularine (isoadenosine or 2-aminopurine ribonucleoside) by a radical deamination-thioalkylation followed by sulfur oxidation. This sulfone is potentially an intermediate for the synthesis of many new 2-substituted analogues of nebularine as the nucleofugic methylsulfonyl group can be displaced by a variety of nucleophiles. Representative examples include 2-cyano-, 2-carboxamido-, 2-acetamido-, and 2-bis (carboxamido)methyl- nebularine. Structures of the intermediate and products were confirmed by spectroscopic data, particularly high-field NMR and FAB HRMS data.

While many syntheses have been reported for analogues of the natural purine nucleosides, adenosine and guanosine (for a few representative examples see references 1-10), relatively few compounds related to nebularine are known.[11-13] Nebularine [9-(β -D-ribofuranosyl)purine] has been isolated from microorganisms *S. yokosukaensis*[14], fungi *Clitocyba nebularis batsch*[15], and *Microbispora sp. SDC 1779*.[16] It is an antibiotic with strong competitive inhibitory properties for the enzyme, adenosine deaminase.[17] Additionally, it has been studied as an anticancer agent.[15,18] Analogues of nebularine could potentially be of considerable biological importance. For example, 2-aminonebularine has been found to be an inhibitor of a number of purine metabolizing enzymes.[19-21] Also, 2-methylnebularine has shown activity as a presynaptic inhibitor of acetylcholine release,[22] and the corresponding 2-trifluoromethylpurine nucleoside has proven to be active against sarcomas and neoplasts.[23,24] Our interest in the synthesis of unusual purine nucleosides as potential antiviral agents has led us to explore general approaches to functionalized nebularine systems. This paper reports on the preparation of the new intermediate 4, a purine sulfone system, and its conversion to some novel congeners of nebularine.

Synthesis of the purine 2-sulfone 4, required the development of a procedure that would allow gram scale preparation of this intermediate. The starting compound was protected 2-aminopurine nucleoside 1, which, when treated with n-pentyl nitrite (or t-butyl nitrite) and dimethyl disulfide in refluxing acetonitrile under nitrogen, gave a 37 % yield of 2-methylmercaptonebularine (2). The reaction appears to be a radical deamination-thioalkylation and is inhibited by molecular oxygen. Deprotection of 2 followed by selective oxidation of the sulfur with potassium hydrogen persulfate (oxone) in buffered aqueous

solution,[25] gave the sulfone 4 in about 80% yield after chromatographic purification (Scheme 1). The pH of this reaction must be maintained between about 4 and 6. Below this pH, the nucleoside decomposes slowly *via* glycosidic bond cleavage, while at higher pHs, decomposition of the oxone reagent impairs the oxidation reaction. It should be mentioned that oxone exhibits much greater selectivity and efficiency in this reaction than potassium permanganate.



Scheme 1

The nucleofugic sulfonyl group in 4 is susceptible to displacement by selected nucleophiles. Thus, reaction of 4 with sodium cyanide in DMF at 0 °C for 15 h afforded the novel 2-cyanonebularine 5 in 40% yield after purification. If this reaction is viewed as a dark $S_{RN}1$ reaction [26,27], then the failure of previous displacements of halogens at this position [12] may be explained by a more favorable reduction potential for the sulfone compared to the halogenated systems. The cyano functionality at the 2-position in 5 can be hydrolyzed to give the carboxamide 6, another new nucleoside. The structures of both the cyanide and the carboxamide were established by UV, FTIR, FAB HRMS, and high-field NMR data.

The generality of the utility of the sulfone as a precursor can be illustrated by the synthesis of other analogues of nebularine. For example, when the silyl protected sulfone 7 was treated with the anion of diethylmalonate, the corresponding malonate derivative 8 was isolated. If the reaction is run at room temperature, it could be stopped at the malonate stage. However, raising the temperature to that of refluxing tetrahydrofuran, results in decarboxylation to produce the ethylacetate derivative 9. The conversion of 8 to 9 may be mechanistically explained through a base-catalyzed retro-Claisen reaction.

Compound 9 was elaborated by reaction of its deprotected form (i.e. compound 10) with methanolic ammonia to give the novel carboxamide 11. Similarly, the diethylmalonyl derivative 8 was converted through its deprotected form to the bis-carboxamide 13.

In summary, the novel 2-methylsulfonylnebularine, synthesized from 2-aminonebularine by a radical alkylthiolation followed by oxidation, is an important intermediate for the synthesis of some new analogues of nebularine. This approach to novel congeners of nebularine may have considerable generality as a wide variety of nucleophiles could conceivably be utilized to displace the nucleofugic group of the nebularine sulfone intermediate.

Experimental Section

Irradiations were accomplished using a Rayonet photochemical reactor. The melting points provided are uncorrected and were taken on a Thomas-Hoover melting point apparatus fitted with a microscope. Nuclear magnetic resonance spectra using tetramethylsilane or chloroform as internal standards were recorded on JEOL Model FX90Q and Bruker Model WM360 pulse Fourier transform spectrometers. Mass spectra were obtained on a Hewlett-Packard 5985 GC/MS system or a VG Analytical Model ZAB-HF instrument with high-resolution FAB capability. The ultraviolet spectra were recorded on Varian Cary Model 219 or Gilford Response spectrophotometers. Infrared spectra were recorded on a Mattson FTIR instrument.

2-Amino-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (1) was prepared from guanosine in 50% overall yield as previously described.[12]

2-Methylmercapto-9-(2,3,5-tri-O-acetyl-β-D-ribofuranosyl)purine (2). A solution consisting of 1.630 g (4.14 mmol) of 1, 50 mL of acetonitrile, 1.1 mL (12.3 mmol) of dimethyldisulfide, and 2.6 mL (22 mmol) of n-pentyl nitrite was purged with nitrogen. The solution was then heated to reflux under N_2 with protection from light for 12 h. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography on silica gel (elution with ether) to give 0.663 g (1.56 mmol, 37%) of 2 as a yellow oil; ^{1}H NMR ($CDCl_3$) δ 2.15-2.04 (m, 9 H), 2.65 (s, 3 H), 4.39 (m, 3 H), 6.14 (d, 1 H), 8.93 (s, 1 H),

9.25 (s, 1 H); UV(EtOH) λ_{max} 258, 295nm; mass spectrum m/z (relative intensity) 424 (M^+, 7.4), 166 (M^+-sugar, 15.9). The physical data for 2 was identical to that reported previously for the same compound prepared by a different method.[28]

2-Methylsulfonyl-9-β-D-riboferanosylpurine (4). A solution consisting of 1.080 g (2.54 mmol) of 2, 0.480 g (8.90 mmol) of sodium methoxide, and 40 mL of methanol was stirred for 1 h. Ammonium chloride (0.600 g, 10.9 mmol) was then added, and the solution stirred at room temperature for an additional hour. The solvent was removed under reduced pressure, and the residue was purified by flash chromatography on silica gel (9:1 chloroform/methanol) to give 0.674 g (2.22 mmol, 81%) of 3 as a yellow oil: ^{1}H NMR (Me_2SO-d_6) δ 2.59 (s, 3 H), 3.62 (m, 2 H), 3.93 (m, 1 H), 4.16 (m, 1 H), 4.37 (m, 1 H), 4.52 (m, 1 H), 5.23 (m, 1 H), 5.55 (m, 1 H), 5.97 (d, 1 H), 8.68 (s, 1 H), 9.01 (s, 1 H); UV(EtOH) λ_{max} 258, 295nm.

A solution consisting of 0.798 g (2.63 mmol) of 3 and 2.420 g (3.94 mmol) of oxone in 30 mL of acetate buffer (pH 4.2) was stirred for 12 h at 0 °C. After adjusting the pH to 7.0 with 2 N NaOH, the solvent was removed under reduced pressure. Methanol (50 mL) was then added and the insoluble material was filtered. The filtrate was dried ($MgSO_4$), and the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel (2% triethylamine/chloroform followed by 9:1 chloroform/methanol) to give 0.655 g (1.95 mmol, 74%) of 4 as a hygroscopic white solid: ^{13}C NMR (Me_2SO-d_6) δ 57.9, 67.1, 70.6, 76.0, 82.8, 84.6, 132.3, 145.3, 145.6, 148.0, 155.7; ^{1}H NMR (Me_2SO-d_6) δ 3.28 (s, 3 H), 3.63 (m, 2 H), 3.90 (m, 1 H), 4.18 (m, 1 H), 4.35 (m, 1 H), 4.52 (m, 1 H), 5.25 (m, 1 H), 5.62 (m, 1 H), 6.03 (d, 1 H), 8.75 (s, 1 H), 9.11 (s, 1 H); UV(EtOH) λ_{max} 265 nm; FAB HRMS 331.0719 (M^++H), calculated for $C_{11}H_{14}N_4O_6S$ 331.0712 (M^++H).

2-Cyano-9-β-D-riboferanosylpurine (5). A solution consisting of 4 (0.271 g, 0.807 mmol), sodium cyanide (0.060 g, 1.2 mmol), and DMF (10 mL) was stirred at 0°C for 15 h. The solvent was removed under reduced pressure, and the residue purified by flash chromatography on silica gel (9:1 chloroform/methanol) to give 0.084 g (0.16 mmol, 40%) of 5 as a white hygroscopic solid: ^{1}H NMR (Me_2SO-d_6) δ 3.68 (m, 2 H), 3.99 (m, 1 H), 4.03 (m, 1 H), 4.55 (m, 1 H), 5.08 (t, 1 H), 5.25 (d, 1 H), 5.59 (d, 1 H), 6.06 (d, 1 H), 9.15 (s, 1 H), 9.38 (s, 1 H); ^{13}C NMR (Me_2SO-d_6) δ 61.1, 70.2, 74.3, 86.0, 88.3, 116.9, 136.0, 136.3, 148.6, 149.4, 151.1, UV(EtOH) λ_{max} 269nm (ε = 9,550); FTIR(KBr) 2248 cm^{-1}; FAB HRMS 278.0900 (M^++H), calculated for $C_{11}H_{11}N_5O_4$ 278.0889 (M^++H).

2-Carboxamido-9-β-D-riboferanosylpurine (6). To a solution consisting of 0.285 g (1.09 mmol) of 5, 30 mL of ethanol, and 1 mL of 2N NaOH was added 0.4 mL of 30% aqueous hydrogen peroxide. After t.l.c. showed no evidence of starting material (1 h), a small amount of $FeSO_4$ was added until no evidence of peroxides was observed. The insoluble material was filtered and the filtrate removed under reduced pressure. The residue was purified by reversed-phase HPLC on Amberlite XAD-4 resin (2.5% ethanol/water) to give 0.111 g (0.370 mmol, 34%) of 6 as white crystals: mp 152°C (decomp.); ^{1}H NMR (Me_2SO-d_6) δ 3.67 (m, 2 H), 4.00 (m, 1 H), 4.24 (m, 1 H), 4.70 (m, 1 H), 5.05 (t, 1 H), 5.21 (d, 1 H), 5.51 (d, 1 H), 6.13 (d, 1 H), 7.71 (bs, 1 H), 8.21 (bs, 1 H), 8.97 (s, 1 H), 9.27 (s, 1 H); ^{13}C NMR (Me_2SO-d_6) δ 61.3, 70.4, 74.0, 86.1, 87.7, 134.8, 147.4, 147.9, 151.7, 152.3, 164.5; UV(EtOH) λ_{max} 287 (ε = 11,000); FTIR(KBr) 1601, 1688 cm^{-1}; FAB HRMS 296.1018 (M^++H), calculated for $C_{11}H_{13}N_5O_5$ 296.0995 (M^++H).

2-Bis(carboxamido)methyl-9-β-D-riboferanosylpurine (13). A solution consisting of 0.958 g (2.85 mmol) of 4, 1.72 g (11.4 mmol) of t-butyldimethylsilyl chloride, 1.55 g (22.8 mmol) of imidazole, and 10 mL of DMF was stirred at 40°C for 24h. The reaction mixture was partitioned between water (100 mL) and ether (40 mL). The organic layer was collected and dried ($MgSO_4$), and the solvent was removed under reduced pressure. The residue was purified by flash chromatography on silica gel (1:1 ether/hexanes) to give 1.37 g (2.04 mmol, 72%) of 7 as a yellow oil: ^{1}H NMR ($CDCl_3$) δ 0.117 to -0.238 (m, 18H), 0.931-0.736 (m, 27 H), 2.90 (s, 3 H), 3.95 (m, 2 H), 4.07 (m, 1 H), 4.30 (m, 1 H), 4.55 (m, 1 H), 6.08 (d, 1 H), 8.63 (s, 1 H), 9.17 (s, 1 H); UV(EtOH) λ_{max} 265.5 nm; mass spectrum m/z (relative intensity) 616 (22.6), 615 (M^+-t-Bu, 48.9).

A solution of 0.650 g (16.3 mmol) of sodium hydride (60%), 3.1 mL (20 mmol) of diethylmalonate, and 10 mL of THF was added dropwise to 90 mL of a THF solution containing 1.37 g (2.04 mmol) of 7. The resulting solution was purged with nitrogen, and stirred for 29 h. After mass spectral evidence indicated the absence of the starting material, 1.300 g (25.00 mmol) of ammonium chloride was added. The solution was stirred for an additional 2 h and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (hexanes followed by 1:1 ether/hexanes) to give a mixture of 0.763 g (1.06 mmol, 50%) of 8 and 0.344 g (0.505 mmol, 25%) of 9. Data for 8: ^{1}H NMR ($CDCl_3$) δ 0.39 to -0.29 (m, 18 H), 0.85-0.69 (m, 27 H), 1.15 (t, 6 H), 3.95 (m, 2 H), 4.27-3.95 (m, 6 H), 4.45 (m, 1 H), 5.02 (s, 1 H), 5.98 (d, 1 H), 8.47 (s, 1 H), 8.99 (s, 1 H); UV(EtOH) λ_{max} 274.5 nm; mass spectrum m/z (relative intensity) 754 (2.7), 696 (21.9), 695 (M^+-t-Bu, 40.9). Data for 9: ^{1}H NMR ($CDCl_3$) δ 0.07 to -0.23 (m, 18 H), 0.88-0.74 (m, 27 H), 1.17 (t, 3 H), 3.65 (m, 2 H), 3.99 (s, 2 H), 4.23-4.15 (m, 4 H), 4.52 (m, 1 H), 6.01 (d, 1 H), 8.44 (s, 1 H), 8.99 (s, 1 H); UV(EtOH) λ_{max} 272.5 nm; mass spectrum m/z (relative intensity) 651 (M^+- CO), 624 (45.8), 623 (M^+ - t-Bu, 100.0).

A solution consisting of 1.34 g (2.01 mmol) of 8, 50 mL of acetonitrile, and 12 mL of 0.5 M tetraethylammonium fluoride (acetonitrile) was stirred for 2 h after which time 0.540 g (10.0 mmol) ammonium chloride was added. The resulting solution was stirred for an additional 2 h. The solvent was removed under reduced pressure and the residue purified by flash chromatography on silica gel (9:1 chloroform/methanol) to give 0.586 g (1.73 mmol, 86%) of 12 as a yellow oil; ^{1}H NMR (Me_2SO-d_6) δ 1.20 (t, 6 H), 3.62 (m, 2 H), 4.24-4.04 (m, 6 H), 4.32 (m, 1 H), 5.18 (t, 1 H), 5.26 (m, 2 H), 5.51 (d, 1 H), 5.97 (d, 1 H), 8.87 (s, 1 H), 9.19 (s, 1 H); UV(EtOH) λ_{max} 274.0 nm. A solution containing 0.379 g (1.12 mmol) of 12 in 50 mL of methanol was purged with ammonia at 0°C and stored in the freezer for 6 days. The solvent was then removed under reduced pressure and the residue purified by reversed-phase HPLC on Amberlite XAD-4 resin (2.5% ethanol/water) to give 229 mg (0.651 mmol, 58%) of 13 as a pale yellow hygroscopic solid; ^{1}H NMR (Me_2SO-d_6) δ 3.43 (m, 2 H), 3.51 (m, 1 H), 4.38 (m, 1 H), 4.68 (m, 1 H), 4.78 (s, 1 H), 4.98 (t, 1 H), 5.23 (d, 1 H), 5.50 (d, 1 H), 6.03 (d, 1 H), 7.29 (bs, 2 H), 7.59 (bs, 2 H), 8.62 (s, 1 H), 9.15 (s, 1 H); ^{13}C NMR (Me_2SO-d_6) δ 62.2, 63.7, 71.3, 74.4, 86.6, 87.6, 133.4, 146.2, 148.8, 152.4, 159.6, 169.4; UV(EtOH) λ_{max} 266.5 nm (ϵ = 7090); IR (KBr pellet) 1687 cm^{-1}; FAB HRMS 353.1217 (M^++H), calculated for $C_{13}H_{16}N_6O_6$ 353.1209 (M^++H).

2-Acetamido-9-(β-D-ribofuranosyl)purine (11). A solution consisting of 0.560 g (14.0 mmol) of sodium hydride (60%), 2.50 mL (16.4 mmol) of diethylmalonate, and 15 mL of THF was added dropwise to 40 mL of a THF solution containing 1.100 g (1.64 mmol) of 7. The resultant solution was purged with nitrogen and stirred for 48 h at reflux. After nmr evidence indicated the absence of intermediate 8, ammonium chloride (0.810 g, 15.0 mmol) was added. The solution was stirred for an additional 2 h and the solvent was removed under reduced pressure. The residue was purified by flash chromatography (hexanes followed by 1:1 ether/hexanes) to give 0.803 g (1.18 mmol, 72%) of 9 as a light yellow oil.

Deprotection of 9 (0.803 g, 1.18 mmol) was carried out as described previously for the deprotection of 8 to give 0.301 g (0.956 mmol, 81%) of 10 as a light-yellow oil; ^{1}H NMR (Me_2SO-d_6) δ 1.25 (t, 3 H), 3.62 (m, 2 H), 4.28-4.10 (m, 6 H), 4.35 (m, 1 H), 5.15 (t, 1 H), 5.32 (d, 1 H), 5.55 (d, 1 H), 6.05 (d, 1 H), 8.71 (s, 1 H), 9.02 (s, 1 H); UV(EtOH) λ_{max} 272.0 nm. The formation of 11 from 10 (0.204 g, 0.603 mmol) was carried out as described previously for the formation of 13 to afford 0.160 g (0.514 mmol, 85%) of 11 as white crystals; mp. 175-176°C; ^{1}H NMR (Me_2SO-d_6) δ 3.65 (m, 2 H), 3.81 (s, 2 H), 3.95 (m, 1 H), 4.16 (m, 1 H), 4.60 (m, 1 H), 5.08 (t, 1 H), 5.21 (d, 1 H), 5.49 (d, 1 H), 6.03 (d, 1 H), 7.41 (bs, 1 H), 7.48 (bs, 1 H), 8.78 (s, 1 H), 9.11 (s, 1 H); ^{13}C NMR (Me_2SO-d-$_6$) δ 46.2, 61.5, 70.6, 73.7, 87.1, 89.0, 132.5, 145.1, 148.2, 151.6, 159.7, 170.5; UV(EtOH) λ_{max} 266.5 (ϵ = 7730); IR (KBr pellet) 1664 cm^{-1}; FAB HRMS 310.1132 (M^++H), calculated for $C_{12}H_{15}N_5O_5$ 310.1151 (M^++H).

Acknowledgment. We thank the U.S. Army Medical Research and Development Command for support of this research. The National Science Foundation provided funds towards the purchase of the high-field NMR spectrometer used in this work.

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