Table II. Local Anesthetic Potency and Toxicity

Compd	Corneal anesthesia[a] Duration min ± SE	Corneal anesthesia[a] Relative activity	Sciatic nerve block *in vitro*[b] Depression of action potential, % ± SE	Sciatic nerve block *in vitro*[b] Relative activity	LD_{50},[c] iv mg/kg	LD_{50},[c] iv Relative toxicity
(*R*)-(+)-1	15.4 ± 1.8	1.0	96 ± 3	1.0	8	1.0
(*S*)-(−)-1	8.4 ± 0.9	0.5	44 ± 1	0.5	20	0.4
(*R*)-(+)-2	14.2 ± 0.9	0.9	87 ± 3	0.9	11	0.7
(*S*)-(−)-2	1.8 ± 0.6	0.1	50 ± 4	0.5	19	0.4
(*R*)-(+)-Bupivacaine	14.6 ± 1.8	1.0	87 ± 6	0.9	6	1.3
(*S*)-(−)-Bupivacaine	11.5 ± 1.5	0.7	48 ± 7	0.5	10	0.8

[a]Rabbit, 0.25%, 0.25 ml applied for 0.5 min, $N = 6$. [b]Frog, 0.1 m*M*, equilibrium block, $N = 4$. [c]Mice.

scribed for the (+) enantiomer,[7] mp 192–193.5° (from EtOH–H_2O), $[\alpha]^{20}D$ −105.1° (*c* 1.02, 95% EtOH). *Anal.* ($C_{10}H_{10}ClNO_5$) C, H, N.

Resolution of (±)-*N*-Butyl-3-hydroxypiperidine (3). To a solution of 26.1 g (0.083 mole) of (+)-4-chlorotartranilic acid[7] in warm 95% EtOH (175 ml) (±)-**3** (25 g, 0.166 mole) was added. The mixt was kept at room temp for 24 hr, and the formed salt was collected. Two recrystns from 95% EtOH and seeding with crystals from a previous experiment afforded 13.0 g of product, mp 151–153°, $[\alpha]^{20}D$ +63.5° (*c* 1.02, H_2O).

The optically active salt (5.8 g) was decomposed with 5 *N* NaOH, and the amino alcohol was extracted with Et_2O. After drying (Na_2SO_4) and evaporation of the solvent, 2.0 g of (+)-**3** was obtained, $[\alpha]^{20}D$ +4.0° (*c* 1.15, EtOH).

Treatment of (±)-**3** with (−)-4-chlorotartranilic acid yielded similarly the enantiomeric salt, mp 151.5–153.5°, $[\alpha]^{20}D$ −63.7° (*c* 1.10, H_2O). Decomposition of the salt yielded (−)-**3**, $[\alpha]^{20}D$ −4.3° (*c* 1.38, EtOH).

Resolution of (±)-3-Hydroxypiperidine. (±)-3-Hydroxypiperidine (5.0 g, 0.05 mole) was treated with (−)-4-chlorotartranilic acid (6.5 g, 0.025 mole) in 95% EtOH as described for (±)-**3**. The salt obtained was recrystd twice from 95% EtOH affording 4.2 g of product, mp 152–153°, $[\alpha]^{20}D$ −77.6° (*c* 0.75, H_2O). The salt was stirred in a mixture of MeOH–PhMe (7:3) containing equivalent amounts of K_2CO_3. After filtrn and evapn, (*S*)-(−)-3-hydroxypiperidine was obtd, $[\alpha]^{20}D$ −8.0° (*c* 1.32, MeOH), lit.[8] $[\alpha]^{20}D$ −7.5° (*c* 2.0, MeOH).

(*S*)-(+)-*N*-Butyl-3-hydroxypiperidine [(*S*)-(+)-3]. (*S*)-(−)-3-Hydroxypiperidine (0.5 g, 5 mmoles), BuI (0.5 g, 6 mmoles), and K_2CO_3 (0.7 g, 5 mmoles) were mixed in BuOH (5 ml) and stirred at 80° for 5 hr. After filtration, evapn, and trituration with Et_2O, followed by filtration and evapn, 0.42 g (54%) of (*S*)-(+)-**3** was obtd, $[\alpha]^{20}D$ +4.2° (*c* 1.0, EtOH). Ir and tlc data were identical with those shown by (+)-**3** prepd by resoln of (±)-**3**.

Preparation of the Enantiomers of 1 and 2. The enantiomers of 1 and 2 were prepared from (+)-**3** and (−)-**3** and the appropriate isocyanate by the method described for the racemates.[1] Physical data for the optical isomers are given in Table I. When equivalent amounts of the appropriate pair of enantiomers were mixed and recrystd from ligroin, the observed mp were identical with, and did not depress, the mp of the racemic compounds. The ir spectra were also identical.

Acknowledgment. The enantiomers of bupivacaine were made available through the courtesy of Dr. G. Åberg, AB Bofors Nobel-Pharma, Mölndal, Sweden.

References

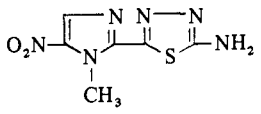
(1) J. L. G. Nilsson, H. Sievertsson, R. Dahlbom, and B. Åkerman, *J. Med. Chem.*, 14, 710 (1971) (paper 4).
(2) B. Åkerman, H. Persson, and C. Tegnér, *Acta. Pharmacol. Toxicol.*, **25**, 233 (1967).
(3) R. Adler, G. Adler, and G. Åberg, *Svensk Tandläkare Tidskrift*, **62**, 501 (1969).
(4) F. P. Luduena, *Annu. Rev. Pharmacol.*, 9, 503 (1969).
(5) H. Schönenberger, K. D. Fuchsberger, A. Petter, and R. Brinkman, *Pharm. Acta Helv.*, **42**, 163 (1967).
(6) B. Åkerman, G. Camougis, and R. Sandberg, *Eur. J. Pharmacol.*, 8, 337 (1969).
(7) T. A. Montzka, T. L. Pindell, and J. D. Matiskella, *J. Org. Chem.*, **33**, 3993 (1968).
(8) C. C. Deane and T. D. Inch, *Chem. Commun.*, 813 (1969).
(9) B. F. Tullar, *J. Med. Chem.*, 14, 891 (1971).
(10) F. P. Luduena, J. O. Hoppe, and J. K. Borland, *J. Pharmacol. Exp. Ther.*, **123**, 269 (1958).
(11) R. Dahlbom and R. Mollberg, *Acta Chem. Scand.*, 17, 1182 (1963).
(12) R. Dahlbom and L. E. Österberg, *Acta Chem. Scand.*, 9, 1553 (1955).

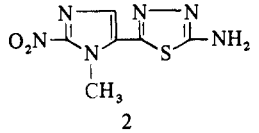
Nitroheterocyclic Antimicrobial Agents. 1-Methyl-2-nitro-5-imidazolyl Derivatives

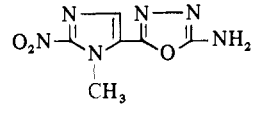
Goro Asato* and Gerald Berkelhammer

Chemical Research and Development Laboratories, Agricultural Division, American Cyanamid Company, Princeton, New Jersey.
Received March 29, 1972

Since the discovery of the broad-spectrum antimicrobial activity of 2-amino-5-(1-methyl-5-nitro-2-imidazolyl)-1,3,4-thiadiazole (**1**),[1] a comparison of its activity with that of the corresponding 2-nitro isomer (**2**) was of interest, especially in view of the high antiprotozoal activities reported for both 5-nitro- and 2-nitroimidazoles.[2,3] In this report, the synthesis and antimicrobial activity of **2** and its amino-oxadiazole analog (**3**) are described.


1

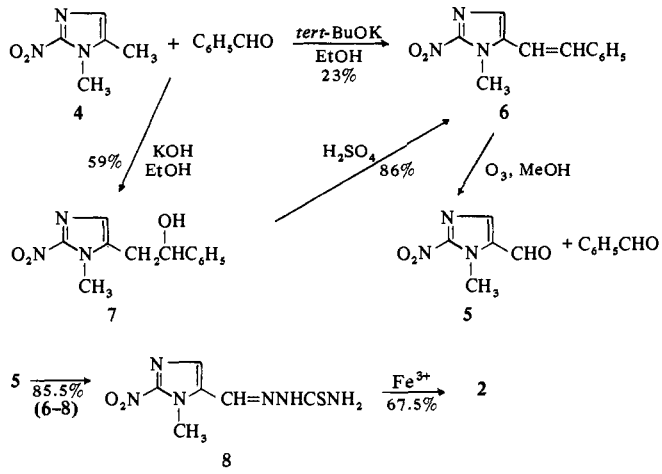

2


3

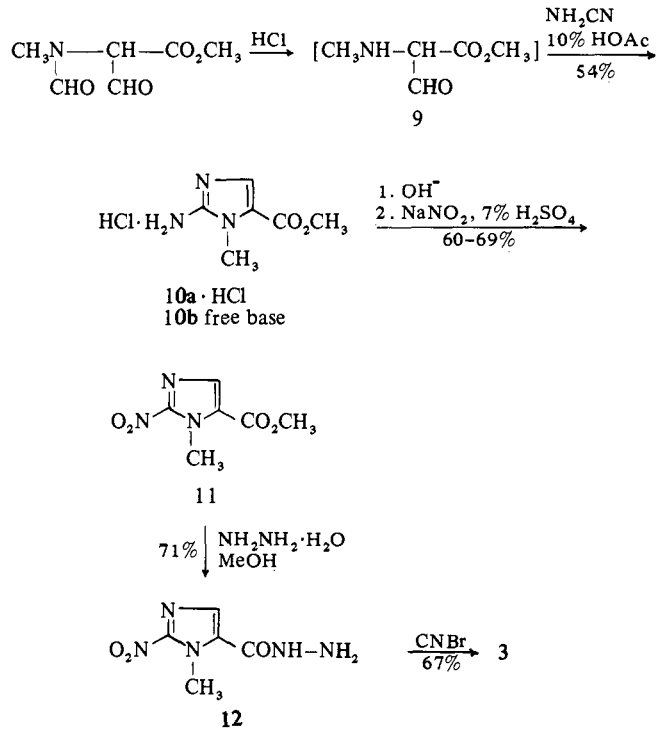
1,5-Dimethyl-2-nitroimidazole (**4**)[3] appeared to be a potential intermediate for 1-methyl-2-nitroimidazole-5-carboxaldehyde (**5**). Probing experiments quickly revealed that oxidation of **4** to **5** with SeO_2 or CrO_3 proceeded only with considerable decomposition and that treatment of **4** with BuONO–HCl did not give the oxime of **5**. Condensation of **4** with benzaldehyde could be accomplished by using an excess of *tert*-BuOK and benzaldehyde to afford **6** in low yield. However, in subsequent experiments, the yield of **6** was improved by using a two-step synthesis† which involved addition of **4** to benzaldehyde in the presence of a catalytic amount of KOH in EtOH to give **7**, which was then dehydrated with H_2SO_4 to give **6**. The benzylidene derivative was easily ozonized to **5**, which was converted to the thiosemicarbazone **8**. The latter was oxidatively cyclized to give **2**.

A second approach was based on the method of imidazole

†We thank Drs. P. Miller and I. Starer of the Organic Chemicals Division (Bound Brook, N. J.) for providing information on this method, which they developed for the 5-nitroimidazole series.



synthesis of Lancini and coworkers.[3] This involved condensation of C-formylated sarcosine ester (**9**) with cyanamide, which proceeded satisfactorily to give 54% of **10**. Compound **10** was converted to the free base and then diazotized in a large excess of $NaNO_2$ with 7% H_2SO_4 in the absence of a catalyst[4] to afford 60-69% yields of the nitroester (**11**). Subsequently, **11** was converted to the hydrazide (**12**), which was allowed to react with CNBr to give **3**.



Biological Activity. Compounds **2** and **3** were tested *in vivo* orally against *Salmonella gallinarum* in chicks and *Staphylococcus aureus* (Smith), *Escherichia coli*, and *Trichomonas vaginalis* in mice. Compound **2** was inactive against these organisms. In contrast, **3** saved 5/5 mice at 64 mg/kg and 3/10 at 32 mg/kg (single oral tubing, SOT) in the *E. coli* 311 test. At these levels **1** saved 5/5 and 8/10 mice. Furthermore, **3** was slightly active *vs. Staph. aureus* at 128 mg/kg (SOT 2/5 survival) and *T. vaginalis* at 100 mg/kg, but inactive *vs. S. gallinarum* at 0.025% diet concentration. At these levels **1** is highly effective against the aforementioned infections.

Thus, these 2-nitroimidazoles are less interesting antimicrobial agents than the corresponding 5-nitroimidazoles. As the 2-nitro group is expected to reduce the basicity of the imidazole nitrogen (position 3) to a greater extent than does the 5-nitro group,[5] the electronic effect may be important in decreasing the antimicrobial activity of these isosteric compounds.

Experimental Section‡

Methyl 2-Amino-1-methyl-5-imidazolecarboxylate (10b). A solution of 58.38 g (0.367 mole) of *N*-formyl-*C*-formyl methyl sarcosinate§ in 61 ml (0.734 mole) of concentrated HCl and 400 ml of MeOH was refluxed for 0.5 hr. The solution was evaporated to dryness *in vacuo*, and the residual oil was dissolved in 10% aqueous HOAc and heated on a steam bath for 0.5 hr with 60.3 g (0.735 mole) of NaOAc and 30.8 g (0.734 mole) of NH_2CN. After cooling, the mixture was filtered and the filtrate was acidified to pH 1 with concentrated HCl and evaporated to dryness *in vacuo*. The residue was stirred in MeOH, the mixture was filtered to remove NaCl, and the filtrate was evaporated to dryness to afford crystals and an oil. Addition of Me_2CO and traces of MeOH solubilized the oil and the crystals were collected and washed with Me_2CO–MeOH (trace) to give 39 g (54%) of product (10a): mp 198–202° dec; nmr (D_2O–1% DSS) τ 2.28 (s, 1H, imidazole H), 6.06, 6.26 (2 s, 6H, N-CH_3 and CO_2Me). A sample which was recrystallized from EtOH melted at 199–200° dec. *Anal.* ($C_6H_{10}N_3O_2Cl$) C, H, N, Cl.

The free base was obtained as a precipitate by adding solid K_2CO_3 to 39 g of the salt in 200 ml of H_2O. The amine was collected and washed with H_2O to afford 25.48 g. The analytical sample, mp 175–176.5°, was recrystallized from $CHCl_3$. *Anal.* ($C_6H_9N_3O_2$) C, H, N.

Methyl 1-Methyl-2-nitro-5-imidazolecarboxylate (11). A suspension of 12.05 g (0.0776 mole) of amine in 435 ml of H_2O containing 109 g (1.57 mole) of $NaNO_2$ at 0 to −5° was treated with 545 ml of 7% H_2SO_4 by dropwise addition. After completion of the addition, the cooling bath was removed and stirring was continued for 2 hr. The mixture was extracted with $CHCl_3$ (6 × 100-ml volumes) and the extracts were dried ($MgSO_4$) and evaporated to dryness *in vacuo* to afford 15.75 g of oil. The oil was stirred in 500 ml of Et_2O, and the soluble portion was filtered and evaporated to dryness to give pale yellow crystals, mp 57–64°. After washing with cold H_2O, 8.42 g, mp 63–65° (slightly turbid), remained. The Et_2O-insoluble resin solidified on standing and trituration with $CHCl_3$ gave 1.38 g of an insoluble, yellow solid, mp 186–188° dec; mass spectral analysis indicated this material was probably a mixture with *m/e* 294 and 308. The $CHCl_3$ filtrate was evaporated to dryness and additional nitroester was recovered. This gave 1.1 g of product, mp 61–63.5°, after washing with cold H_2O and a crude yield of 66%. A sample, which was recrystallized twice from Et_2O–hexane, melted at 63–65°. *Anal.* ($C_6H_7N_3O_4$) C, H, N.

1-Methyl-2-nitro-5-imidazolecarboxylic Acid Hydrazide (12). A solution of 9 g (0.0586 mole) of the nitroester in 150 ml of MeOH was cooled to 0° and 4.87 g (0.0973 mole) of $NH_2NH_2 \cdot H_2O$ in 50 ml of MeOH was added. After 1 hr at 0°, the mixture was stirred at room temperature for 4 hr and then evaporated to dryness. The residue was triturated with H_2O, and the yellow-brown solid collected. Recrystallization from MeOH afforded 4.87 g of pale yellow crystals, mp 141–143°. Concentration of the MeOH mother liquor gave an additional 0.57 g, mp 138–144°, while concentration of the initial H_2O wash at room temperature gave 0.95 g, mp 137–141° dec. A sample, which was recrystallized twice from MeOH, melted at 141–144° dec. *Anal.* ($C_5H_7N_5O_3$) C, H, N.

2-Amino-5-(1-methyl-2-nitro-5-imidazolyl)-1,3,4-oxadiazole (3). The hydrazide (3.57 g or 0.019 mole) was dissolved in 75 ml of hot MeOH, and the solution was cooled to near room temperature before 2.25 g (0.0212 mole) of CNBr was added. The mixture was refluxed for 0.5 hr, evaporated to dryness, and the yellow-orange residue was stirred in H_2O and collected to give 2.13 g (67.4%), mp 258–259° dec. Recrystallization from 95% EtOH gave an analytical sample: mp 257–259°; nmr (DMSO-d_6) τ 2.42 (s, 1H, imidazole H), 2.47

‡Melting points were determined on a Thomas-Hoover apparatus and are uncorrected. Ir spectra were taken on a Perkin-Elmer Model 137 spectrophotometer; nmr spectra were taken on a Varian A-60 instrument (Me_4Si). Microanalyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. Where analyses are indicated only by symbols of the elements, analytical results obtained for the elements were within ±0.4% of the theoretical values.

§We thank Dr. M. Martell (Lederle Laboratories) for furnishing the initial sample, which was prepared by the method of Jones.[6]

(broad s, 2H, $-NH_2$), 5.72 (s, 3H, $N-CH_3$) for a crude sample. *Anal.* ($C_6H_6N_6O_3$) C, H, N.

1-Methyl-2-nitro-α-phenyl-5-imidazoleethanol (7). A mixture of 1.7 g (0.012 mole) of 1,5-dimethyl-2-nitroimidazole (**4**)[7] and 1.51 g (0.014 mole) of benzaldehyde in 6.7 g of ethanolic KOH (0.3 g of KOH/25 ml of EtOH) was stirred at room temperature under N_2 atmosphere for 22.5 hr. The mixture was evaporated to dryness *in vacuo* to afford an oil, which was mixed with *ca.* 15 ml of H_2O and extracted with $CHCl_3$ (5 × 10 ml). The extracts were dried ($MgSO_4$) and evaporated to dryness to give an oil. Water was added to the oil, and benzaldehyde was removed by vacuum steam distillation on a rotary evaporator. Ether was added to the residue and an insoluble, yellow solid was obtained. This was collected and washed with Et_2O to afford 1 g of product. Evaporation of the mother liquor gave 1.7 g of semisolid which contained mostly adduct and some **4**. Both fractions were later dehydrated separately to the benzylidene derivative **6**. From an initial run, crude product, mp 111–115°, was recrystallized from Me_2CO–hexane and then from EtOAc to afford crystals melting at 120–121.5°. *Anal.* ($C_{12}H_{13}N_3O_2$) H, N; C: calcd, 58.29; found, 58.89.

1-Methyl-2-nitro-5-styrylimidazole (6). The adduct (**7**) (1.18 g or 47.8 mmoles) was added to a stirred mixture of 3.3 ml of HOAc and 1.1 ml of concentrated H_2SO_4 at room temperature. The mixture was heated at 110° for 20 min in an oil bath, cooled, and poured on ice to give a yellow solid which was collected to give 0.943 g (86%), mp 167–170°. A sample, which was recrystallized twice from Me_2CO–95% EtOH, melted at 170.5–171.5°. *Anal.* ($C_{12}H_{11}N_3O_2$) C, H, N.

1-Methyl-2-nitro-5-imidazolecarboxaldehyde Thiosemicarbazone (8). Compound **6** (1.04 g or 4.55 mmoles) was stirred heterogeneously in 40 ml of MeOH at 10° and O_3 was introduced *via* a capillary tube from a generator (Welsbach Corp., 0.081 mole/hr in O_2 stream) until a clear solution was obtained (8 min). After 15 min of additional stirring, 0.431 g (2.27 mmoles) of $Na_2S_2O_5$ in 5 ml of H_2O was added at below 15°, and, after 10 min, 10 ml of H_2O was added, and the mixture was evaporated to near dryness on a rotary evaporator at *ca.* 60–75° to give a yellow paste. An additional 20 ml of H_2O was added and the mixture was concentrated *in vacuo* until the odor of benzaldehyde was not noticeable. Subsequently, 0.42 g (4.62 mole) of thiosemicarbazide was added, and the mixture was heated on a steam bath for 40 min. After cooling, the yellow product was collected and washed with H_2O to afford 0.89 g (85.5%), mp 277° dec. A sample, which was recrystallized from a large volume of Me_2CO–EtOH, melted at 276° dec; ir 1590 cm^{-1} (mineral oil mull). *Anal.* ($C_6H_8N_6SO_2$) H, N, S; C: calcd, 31.58; found, 32.03.

2-Amino-5-(1-methyl-2-nitro-5-imidazolyl)-1,3,4-thiadiazole (2). The thiosemicarbazone (**8**) (1.02 g or 4.47 mmoles) was added to a solution of 3.72 g (17.9 mmoles) of $FeCl_3$[8] in 21 ml of H_2O and *ca.* 2–3 ml of EtOH was added to facilitate wetting of the crystals. After 1.5 hr at 80–85°, the mixture was cooled to room temperature and the yellow-orange crystals were collected and washed with H_2O. The yield was 0.684 g (67.5%), mp 260–261°. A sample was recrystallized from EtOH to give mp 263.5° dec; ir 3450, 3350, 3125, 1660 cm^{-1} (mineral oil mull)–comparable high frequency bands were also present in the spectrum of **1**. *Anal.* ($C_6H_6N_6O_2S$) C, H, N, S.

Acknowledgment. We wish to thank Dr. G. A. Kemp and staff for antibacterial assays, Mr. G. S. Redin and staff (Lederle Laboratories) for their antibacterial assays, and Dr. E. Burden and staff (Lederle Laboratories) for the *T. vaginalis* assays.

References

(1) G. Berkelhammer and G. Asato, *Science*, 162, 1146 (1968).
(2) E. Grunberg, G. Beskid, R. Cleeland, W. F. DeLorenzo, E. Titsworth, H. J. Scholer, R. Richle, and Z. Brener, *Antimicrob. Ag. Chemother.*, 513 (1967).
(3) G. C. Lancini, E. Lazzari, V. Arioli, and P. Bellani, *J. Med. Chem.*, 12, 775 (1969).
(4) L. I. Bagal, M. S. Pevzner, A. N. Frolov, and N. I. Sheludyakora, *Khim. Geterotsikl. Soedin*, 259 (1970).
(5) G. G. Gallo, C. R. Pasqualucci, P. Radaelli, and G. C. Lancini, *J. Org. Chem.*, 29, 862 (1964).
(6) R. G. Jones, *J. Amer. Chem. Soc.*, 71, 644 (1949).
(7) G. C. Lancini, E. Lazzari, and R. Pallanza, *Farmaco Ed. Sci.*, 21, 278 (1966).
(8) W. R. Sherman, *Heterocycl. Compounds*, 7, 595 (1961).

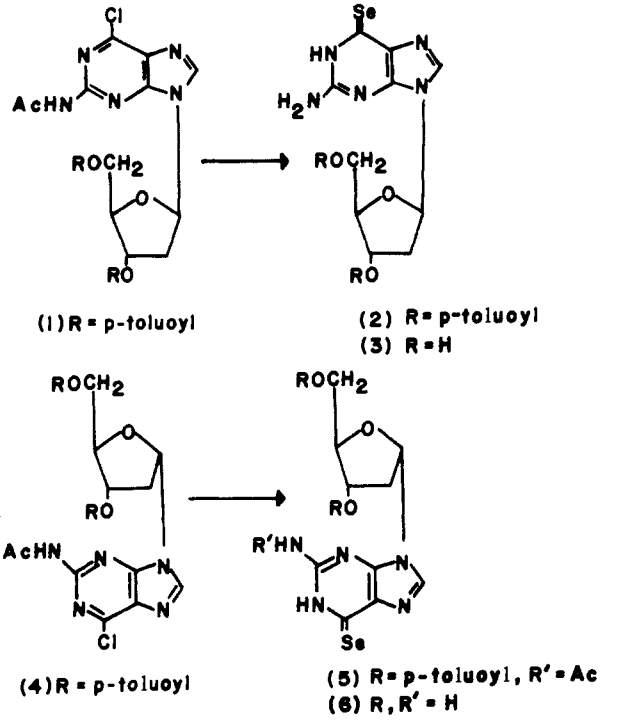
Potential Antitumor Agents. 2. α- and β-2′-Deoxy-6-selenoguanosine and Related Compounds†

Shih-Hsi Chu* and Darrell D. Davidson

Division of Biological and Medical Sciences, Brown University, Providence, Rhode Island 02912. *Received April 6, 1972*

6-Selenoguanine and 6-selenoguanosine were found to have a greater inhibitory effect than either thioguanine or its riboside in Sarcoma 180 ascites cells.[1-3] 6-Selenoguanosine 5′-monophosphate behaves like 6-thioguanosine 5′-monophosphate as a potent competitive inhibitor of guanylate kinase, with inhibition constants of (5.0–7.0) × $10^{-5}M$ and 6.0 × $10^{-5}M$, respectively.[4] These results prompted the synthesis of α- and β-2′-deoxy-6-selenoguanosine (**3** and **6**) for similar biological studies. We now wish to report a convenient two-step synthesis of α- and β-2′-deoxy-6-selenoguanosine (Scheme I) by a modification of the procedure of

Scheme I



Iwamoto, *et al.*[5] The reaction of 2-acetamido-6-chloro-9-(2′-deoxy-3′,5′-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-9*H*-purine (**1**) with alcoholic NaOMe and hydrogen selenide required a long period of 3 days at room temperature because of the low solubility of this compound in MeOH. During this period the *N*-acetyl group was removed, and the partially protected precursor **2** of β-2′-deoxy-6-selenoguanosine (**3**) was obtained in 75% yield. However, the reaction of the α-D anomer **4** required only a short period of time (80 min) to give the protected precursor **5** of α-2′-deoxy-6-selenoguanosine (**6**) in 67% yield.

Treatment of compounds **2** or **5** with methanolic NaOMe gave β-2′-deoxy-6-selenoguanosine (**3**) and α-2′-deoxy-6-selenoguanosine (**6**) in 54 and 70% yields, respectively.

β- and α-2′-deoxy-6-selenoguanisine (**3** and **6**) are unstable in aqueous solution, with the half-life of the 360-nm peak in H_2O at room temperature about 24 hr.

†This work has been supported by Grant T-536 from the American Cancer Society, and Grants 16538-01 and CA 12591-01A1 from the United States Public Health Service.