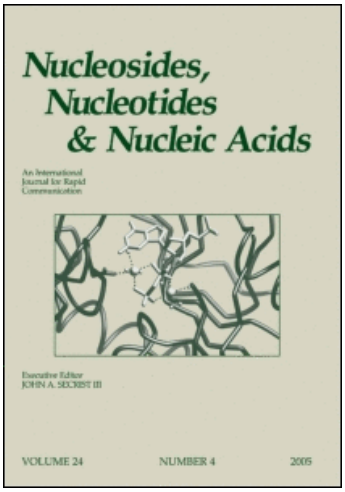

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The Synthesis of 2-Nitro-1-[(2-Hydroxyethoxy)Methyl]Imidazole (Azomycin Acyclonucleoside)

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THE SYNTHESIS OF 2-NITRO-1-[(2-HYDROXYETHOXY)METHYL]IMIDAZOLE (AZOMYCIN ACYCLONUCLEOSIDE)

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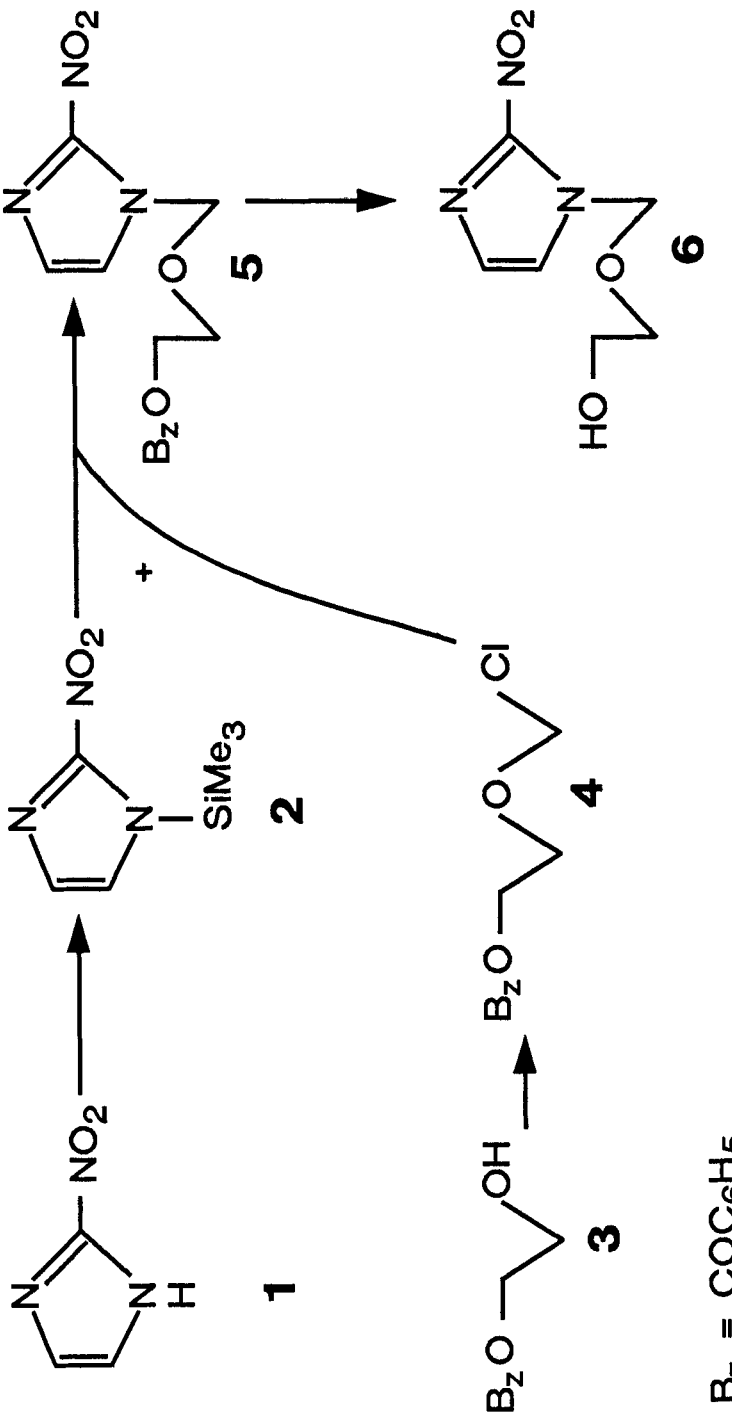
Abstract. The synthesis of 2-nitro-1-[(2-hydroxyethoxy)methyl]imidazole, the acyclonucleoside analogue of antibiotic azomycin (azomycin acyclonucleoside), is accomplished via alkylation of azomycin with 2-benzoyloxyethoxymethylene chloride followed by debenzoylation.

INTRODUCTION

Synthesis of acyclonucleosides as analogues of naturally occurring ribonucleosides has been the subject of major research investigations since the advent of the chemotherapeutic agent acyclovir (Zovirax), an acyclic analogue of guanosine.[1] The intensity of research interest in this area is obvious from the fact that almost every conceivable acyclic analogue of naturally occurring or biologically active synthetic nucleosides have been prepared during the past decade.[2] The antibiotic azomycin (2-nitroimidazole, **1**) was first isolated[3] from a microbial strain resembling *Nocardia mesenteric* and the corresponding ribonucleoside was subsequently synthesized.[4] Both azomycin and azomycin riboside show interesting biological activities.[5] They preferentially concentrate in hypoxic tumor cells and show cytotoxicity to these cells.[6,7] Because of the potent chemotherapeutic activity exhibited by certain acyclonucleosides, in contrast to their inactive ribonucleoside natural congeners, we pursued the synthesis of 2-nitro-1-[(2-hydroxyethoxy)-methyl]imidazole (azomycin acyclonucleoside (**6**, Scheme I) described in this communication.



Scheme I

RESULTS AND DISCUSSION

Chemistry

The alkylating agent, 2-benzoyloxyethoxymethylene chloride (4) was freshly prepared[8] via chloromethylation of 2-benzoyloxy-1-hydroxyethane[9] (3) with paraformaldehyde and hydrogen chloride in methylene chloride. The crude substrate, 2-nitro-1-(trimethylsilyl)imidazole (2) was prepared by refluxing 1 in hexamethyldisilazane (HMDS) in the presence of a catalytic amount of ammonium sulfate followed by solvent (HMDS) evaporation. Intermediate 2 was condensed with 4 in anhydrous dimethylformamide in the presence of triethylamine under inert atmosphere (argon). Silica gel column chromatography (hexane:methylene chloride, 1:1, v/v) provided 5, which crystallized from benzene-ethyl ether (mp 68-69°C).

Debenzoylation of 5 with methanolic ammonia at ambient temperature yielded (72%) azomycin acyclonucleoside (6) after crystallization from chloroform-petroleum ether: mp 65-66°C.

Biological Activity

Azomycin acyclonucleoside (NSC 628865) has been evaluated at the National Cancer Institute, National Institutes of Health, for its cytotoxicity against a variety of human tumor cell lines using an *in vitro* primary tumor drug screening model.[10,11] The results of calculated percent growth (PG) of various cancer cell lines growing in culture in the presence of NSC 628865 at different $\log_{10}$ concentrations are given in Table 1. NSC 628865 showed moderate effect on cell growth (PG = -0.9) against HOP-18 non-small cell lung cancer at 10^{-4} molar concentration.

Azomycin acyclonucleoside was also evaluated for anti-HIV activity *in vitro* and was found to be inactive at 1.12×10^{-3} - 4.24×10^{-7} molar concentration range.

Table 1. *In vitro* measurement of effect on percent growth of cancer cell lines in the presence of azomycin acyclonucleoside at different concentrations.[a]

	Percent growth at the following $\log_{10}$ concentrations (M)				
cancer cell line	-8.0	-7.0	-6.0	-5.0	-4.0
MOLT-4 leukemia	100.5	106.6	18.8	44.4	39.1
HOP-18 non-small cell lung	101.7	216.0	165.7	91.4	-0.9
HT-29 colon	92.9	92.6	96.9	62.0	90.9
XL-498 L CNS	103.7	94.6	94.1	100.6	55.8

[a]Data from National Cancer Institute Developmental Therapeutic Program *in vitro* testing results.

EXPERIMENTAL

Melting points (mp) were determined in capillary tubes with a Thomas Hoover (Uni-melt) capillary melting point apparatus and are uncorrected. Thin-layer chromatographic analyses (TLC) were performed with 250 μm thick layers of silica gel G PF-254 coated on glass plates (Analtech, Inc.). Spots on the TLC plates were detected by observation under short-wave UV light or exposure to iodine vapor. Analtech silica gel GF (20 x 20 cm, 1,000 microns thickness) plates were used for preparative chromatography. Where mentioned for silica gel column chromatography, Solvent E was prepared by mixing ethyl acetate, isopropanol, water (7:1:2, v/v) and separating the top (organic) layer. The low-resolution mass spectra (MS) were recorded at 70 eV with a Kratos MS 25 instrument. The proton nuclear magnetic resonance spectra (^{1}H NMR) were obtained at 60 MHz with a Varian 360-L instrument. Samples (30-40 mg) were dissolved in the solvents indicated, and the

resonances are reported downfield (δ) from the internal tetramethylsilane standard. The presence of exchangeable protons was confirmed by treatment with D_2O followed by reintegration of the NMR spectrum. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tennessee.

2-Nitro-1-[(2-benzoyloxyethoxy)methyl]imidazole (5)

A suspension of 2-nitroimidazole (1.09 g, 8.8 mmol) and ammonium sulphate (100 mg) was refluxed in anhydrous HMDS (40 mL) for 8 h to obtain a clear solution. The solvent was evaporated under reduced pressure, the residue was dissolved in anhydrous DMF (20 mL) and triethylamine (3.0 mL) was added. The reaction mixture was stirred and a solution of 2-benzoyloxyethoxymethylene chloride[8] (obtained by passing HCl gas in a solution of 2-benzoyloxy-1-hydroxyethane,[9] 3.5 g and paraformaldehyde, 1.0 g in methylene chloride) in DMF (10 mL) was added. The stirring was continued for 18 h at room temperature under argon. The solvent was evaporated under reduced pressure and co-evaporated with H_2O. The residue was dissolved in $CHCl_3$ and washed with H_2O. The organic phase was dried (Na_2SO_4) and the solvent removed to yield the crude product which was passed through a column packed with silica gel slurry in hexane. The column was eluted with 50% hexane in methylene chloride followed by methylene chloride. The fractions containing pure product were combined and evaporated to yield (1.7 g, 74%) 5. Crystallization (benzene-ether) provided 5 as pale yellow needles: mp 68-69°C; R_f (2% MeOH in $CHCl_3$) 0.3; MS, m/z 292 $(M+H)^+$; ^1H NMR ($CDCl_3$), δ 8.2-7.9 (m, 2H, Ar-H), 7.65-7.35 (m, 4H, Ar-H, H-5), 7.15 (s, 1H, H-4), 5.88 (s, 2H, H-1'), 4.5-4.3 (m, 2H, H-4'), 4.05-3.8 (m, 2H, H-3'). Anal. calcd. for $C_{13}H_{13}N_3O_5$: C, 53.58; H, 4.46; N, 14.43; Found, C, 53.77; H, 4.56, N, 14.38.

2-Nitro-1-[(2-hydroxyethoxy)methyl]imidazole (6)

A solution of 2-nitro-1-[(2-benzoyloxyethoxy)methyl]imidazole (500 mg, 1.7 mmol) in methanol (40 mL) saturated with ammonia at 0°C was kept at room temperature for 2 d. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (chloroform:ethyl acetate, 1:1). The crude product after crystallization with chloroform-petroleum ether

provided (230 mg, 72%) **6**: mp 65-66°C; R_f (solvent E) 0.66; MS: m/z 188 $(M+H)^+$; ^1H NMR ($CDCl_3$), δ 7.4 (s, 1H, H-5), 7.15 (s, 1H, H-4), 5.8 (s, 2H, H-1'), 3.7 (s, 4H, H-3' and H-4'). Anal. calcd. for $C_6H_9N_3O_4$: C, 38.5; H, 4.81; N, 22.46. Found: C, 38.47; H, 4.85; N, 22.43.

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