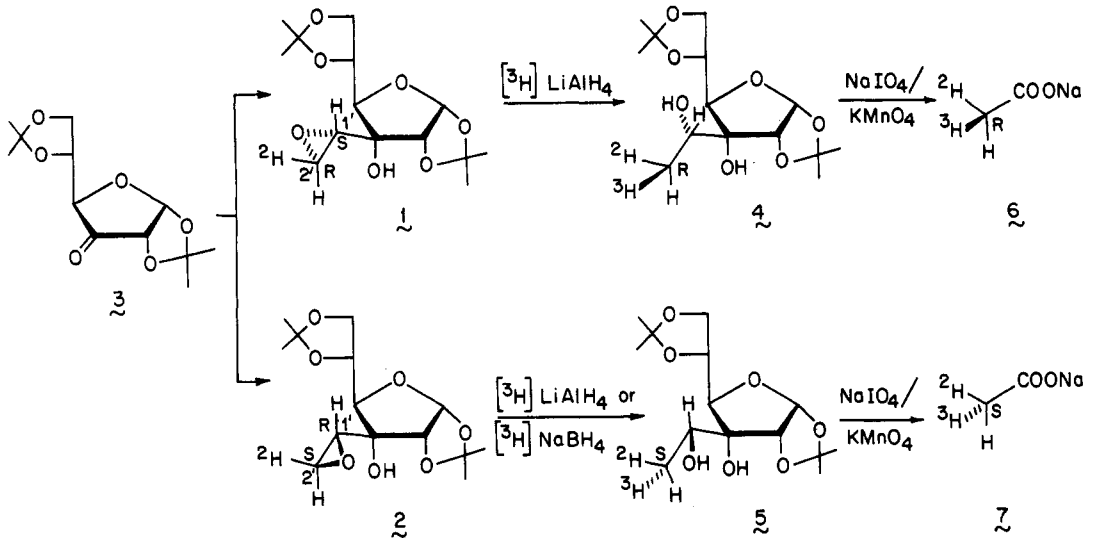
Scheme I



during the slow epoxide ring-opening reaction, the chiral purity of the resulting [2-^{2}H,^{3}H]acetic acid is excellent.

In view of the low radiochemical yield and the high cost of tritiated $LiAlH_4$, we explored the use of the less expensive [^{3}H]$NaBH_4$ for the epoxide ring opening. Reduction of *S* epoxide **2** with [^{3}H]$NaBH_4$ in Me_2SO at 50 °C[11] gave **5** in 10.6% radiochemical yield, together with a more polar compound containing 3% of the tritium. Chromatographic separation and permanganate/periodate oxidation of **5** produced acetic acid **7** (F = 21.8 corresponding to 97% ee *S* isomer) in 86% yield. Similarly, the (1′*R*,2′*R*)-[2′-^{2}H$_1$]epoxide[5] produced acetic acid **6** (F = 76.5, 91% ee *R* isomer) in 10.5% overall radiochemical yield.

Experimental Section

(a) Reduction with [^{3}H]$LiAlH_4$. To a solution of *R* epoxide **1** (80 mg, 0.265 mmol, 99% ^{2}H) in 1.5 mL of dry THF was added $LiAlH_4$ (1 mg) at 0 °C under an argon atmosphere. After stirring for 10 min, a suspension of [^{3}H]$LiAlH_4$ (2.4 mg, 11.1 mCi) in 0.5 mL of dry THF was added, and the mixture was stirred for 1.5 h at room temperature. $LiAlH_4$ (10.1 mg, 0.265 mmol) was then added and stirring was continued for 1 h at room temperature. The mixture was diluted with ether and excess reagent was decomposed with water. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo to leave a residue (175 μCi), which was purified by preparative layer chromatography (silica gel, *n*-hexane–ether, 7:3) to give the (*R*)-methyl glycol **4** (76.5 mg, 146 μCi). To 68.9 mg (131.4 μCi) of the latter in 112 mL of water was added 40 mg of K_2CO_3 and 28 mL of oxidation mixture (584 mg (2.73 mmol) of $NaIO_4$ and 11.1 mg (0.07 mmol) of $KMnO_4$). After stirring for 16 h at room temperature, 1 mL of concentrated H_2SO_4 was added, and the mixture was subjected to steam distillation. Neutralization of the distillate with 0.1 N NaOH and evaporation to dryness gave sodium (*R*)-[2-^{2}H,^{3}H]-acetate **6** (96.8 μCi).

Similarly, reduction of 100 mg of *S* epoxide **2** with [^{3}H]$LiAlH_4$ (13.9 mCi) gave 88 μCi of (*S*)-methyl glycol **5**, which was oxidized to produce 58.9 μCi sodium (*S*)-[2-^{2}H,^{3}H]acetate **7**.

(b) Reduction with [^{3}H]$NaBH_4$. To a solution of *S* epoxide **2** (75.8 mg, 0.25 mmol) in dry Me_2SO (0.66 mL) was added $NaBH_4$ (0.2 mg) under an argon atmosphere, and the mixture was stirred for 20 min at 50 °C. [^{3}H]$NaBH_4$ (12.5 mCi, 1.4 mg, specific activity 341 mCi/mmol) was then added. After stirring for 24 h at 50 °C, excess $NaBH_4$ (16.9 mg, 0.447 mmol) was added and stirring was continued for 6 h. The reaction mixture was diluted with ether (40 mL), washed four times with brine, dried over $MgSO_4$, and concentrated in vacuo to a residue, which was purified by preparative layer chromatography (silica gel, *n*-hexane/ether, 1:1, three developments) to give (*S*)-methyl glycol (29.0 mg, 1.32 mCi, 10.6% radiochemical yield) and a more polar compound (36.1 mg, 0.41 mCi). Oxidation of the glycol gave sodium (*S*)-[2-^{2}H,^{3}H] acetate **7** (1.13 mCi, 85.8% radiochemcial yield, F = 21.8).

Under identical conditions, reduction of (1′*R*,2′*R*)-[2-^{2}H]epoxide[5] (0.25 mmol) with [^{3}H]$NaBH_4$ (12.5 mCi) gave (*R*)-methyl glycol (32.6 mg, 1.52 mCi, 12.2% radiochemical yield), which was oxidized to produce sodium (*R*)-[2-^{2}H,^{3}H]acetate **6** (1.31 mCi) of F = 76.5 in 86.2% yield.

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Registry No. **1**, 89103-63-9; **2**, 82977-95-5; **3**, 2847-00-9; **4**, 89032-30-4; **5**, 89103-64-0; **6**, 62678-94-8; **7**, 62678-90-4.

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An Improved Synthesis of *S*-Adenosylhomocysteine and Related Compounds

Kondareddiar Ramalingam and Ronald W. Woodard*

College of Pharmacy, The University of Michigan, Ann Arbor, Michigan 48109

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In our study on the structural requirements of the active site of the enzyme 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, we had the occasion to prepare several *S*-adenosyl-L-homocysteine (SAH) analogues. ACC synthase is the pyridoxal phosphate requiring enzyme that

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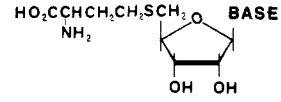
Table I. *S*-Adenosylhomocysteine and Related Analogues Prepared by the Condensation of the Sodium Salt of Homocysteine and 5′-Chloro-5′-deoxynucleosides[7]



base	homocysteine	mp, °C dec	lit. mp, °C dec	% yield based on 5′-chloro-5′-deoxynucleoside	$[\alpha]^{23}_D$ (*c* 1, 0.2 N HCl), deg
adenine	L	210–212	212[4]	81	+37.5[a]
adenine	D	208–210	*b*	79	+10.7
adenine	D,L	206–208	206[4]	83	+24.5
N^6-methyladenine	L	208–210	208–210[9]	70	+36.4
N^6,N^6-dimethyladenine[c]	L	228–230	70[9]	45	+37.9
cytidine	L	184–186	184–186[8]	65	+79.5[d]

[a] lit.[8] $[\alpha]^{30}_D$ +38.1° (*c* 1, 0.2 N HCl). [b] Previously reported by Borchardt et al.,[5] but no melting point was reported. [c] NMR (D_2O) δ 8.2 and 7.94 (2 s, 2 H, C_2-H, C_8-H), 6.0 (d, 1 H, C_1'-H), 4.8 (m, 2 H, C_2'-H, C_3'-H), 4.50 (m, 1 H, C_4'-H), 3.94 (t, 1 H, Cα-H), 3.18 (s, 6 H, $-N(CH_3)_2$), 2.92–2.60 (m, 4 H, C_5'-H_2, Cγ-H_2, 2.3 (m, 2 H, Cβ-H_2). Anal. Calcd for $C_{16}H_{24}N_6O_5S$: C, 46.59; H, 5.86. Found: C, 46.41; H. 5.79. [d] lit.[8] $[\alpha]^{30}_D$ +56.7° (*c* 0.6, H_2O), present study $[\alpha]^{23}_D$ +55.66° (*c* 0.6, H_2O).

converts *S*-adenosyl-L-methionine (SAM) to ACC in the rate-limiting step in the biosynthesis of the plant hormone ethylene.[1-3]

S-Adenosyl-L-homocysteine was first synthesized by Baddiley and Jamieson[4] in a four-step synthesis involving the displacement of the 5′-tosylate function of 2′,3′-*O*-isopropylidene-5′-*O*-(*p*-toluylsulfonyl)adenosine with the sodium salt of L-homocysteine followed by deprotection of the 2′,3′-hydroxyl groups of the nucleoside. Borchardt and co-workers[5] have reported an improved method for the synthesis of SAH analogues that utilizes the intermediate 5′-chloro-5′-deoxynucleoside to avoid protection–deprotection and the general problem of instability of the 5′-tosylate derivatives.

Using the procedure of Borchardt et al.[5] we were, however, unable to prepare N^6,N^6-dimethyladenosyl-L-homocysteine,[6] hence we sought an alternative methodology. This report records a potentially general synthesis of SAH analogues from the appropriate 5′-chloro-5′-deoxynucleosides (prepared in high yield from the corresponding nucleosides via the previously reported thionyl chloride and hexamethylphosphoramide method[7]) and the sodium salt of D-, L-, and D,L-homocysteine (prepared from the appropriate homocystine via sodium in liquid ammonia) in water.

We believe that this method has several advantages over previously described methods. The yields of SAH analogues are consistently higher (5–30%) and the initial products are purer than those obtained from condensation of 5′-chloro-5′-deoxynucleosides and *S*-benzyl-L-homocysteine or L-homocystine under conventional reaction conditions. Most important, however, is the fact that by using this method we have been able to prepare in good yield N^6,N^6-dimethyladenosyl-L-homocysteine, which we were unable to prepare by using the previously published procedures.

Experimental Section

Melting points were obtained on a Mel-Temp apparatus and are uncorrected. Elemental analyses were conducted by M-H-W Laboratories, Phoenix, AZ. The NMR data were consistent with the assigned structures. NMR data were recorded on a Varian Associates Model EM-360 NMR spectrometer in D_2O using DSS as an internal standard. $[\alpha]_D$ data were recorded on a Perkin-Elmer Model 141 polarimeter.

The compounds used in this study, adenosine, N^6-methyladenosine, N^6,N^6-dimethyladenosine, D-homocystine, L-homocystine, and D,L-homocystine, were all commercially available from Sigma Chemical Co.

Sodium Salt of Homocysteine. To homocystine (1.0 g, 3.72 mmol) in 25 mL of liquid ammonia was added sodium in small pieces until a blue color was maintained for 20 min (just sufficient excess to give a blue color). Enough ammonium chloride was added to discharge the blue color and the ammonia was allowed to slowly evaporate. The solid residue was dissolved in absolute ethanol and filtered to remove suspended impurities. Addition of dry ether precipitated the sodium salt of homocysteine. The precipitate was collected and dried in vacuo and stored in a desicator over P_2O_5. Although the salt is rather hygroscopic it may be stored for at least 9 months without decomposition; yield 1.1 g (82.5%). For reprecipitated material: mp, sealed capillary tube, above 300 °C; ^1H NMR (D_2O) δ 2.12 (m, 2 H, $NaSCH_2CH_2$–), 2.58 (m, 2 H, $NaSCH_2$–), 3.45 (t, 1 H, Cα-H). Anal. Calcd for $C_4H_8NNaO_2S\cdot 2H_2O$: C, 24.86; H, 6.26; N, 7.25; S, 16.59. Found: C, 24.85; H, 6.58; N, 7.18; S, 16.33.

General Procedure for the Preparation of *S*-Adenosylhomocysteine and Its Analogues from the 5′-Chloro-5′-deoxynucleoside and the Sodium Salt of Homocysteine. A suspension of 5′-chloro-5′-deoxynucleoside (2.0 mmol), potassium iodide (10 mg), and the sodium salt of L-homocysteine (2.5 mmol) was refluxed in water (7 mL) for 2 h. The reaction mixture became clear, and TLC (Avicel F, 250 μm, $CHCl_3$–EtOH–H_2O, 3:2:0.5) showed the absence of any 5′-chloro-5′-deoxynucleoside. The mixture was allowed to cool and made acidic with 1 N HCl. The solution was then applied directly to a column of Dowex 50-X 4-200 (NH_4^+ form) (125 mL). The column was washed thoroughly with water (200 mL) and then eluted with 1 N NH_4OH. The eluate was evaporated to a small volume in vacuo and lyophilized. The products were recrystallized from appropriate solvents. Yields averaged from 45–80%. All compounds exhibited physical and spectral characteristics in accordance with the published values. (See Table I).

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Registry No. (L)-(SAH) analogue (base = adenine), 979-92-0; (D)-(SAH) analogue (base = adenine), 53276-26-9; (DL)-(SAH) analogue (base = adenine), 58976-18-4; (SAH) analogue (base = N^6-methyladenine), 53228-06-1; (SAH) analogue (base = N^6,N^6-dimethyladenine), 58936-13-3; (SAH) analogue (base = cytidine), 50615-58-2; 5′-chloro-5′-deoxyadenosine, 892-48-8; 5′-chloro-5′-deoxy-N^6-methyladenosine, 19254-36-5; 5′-chloro-5′-deoxy-N^6,N^6-dimethyladenosine, 59987-43-8; 5′-chloro-5′-deoxycytidine, 31652-78-5; L-homocysteine monosodium salt, 82695-92-9; D-homocysteine monosodium salt, 88945-99-7; DL-homocysteine monosodium salt, 28223-71-4; homocystine, 462-10-2.

Solvent Effects on Fluorescence Properties of Sodium Lasalocid (Ionophore X-537A)

Ashim K. Mitra* and Milind M. Narurkar

Department of Pharmaceutics, College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska 68105

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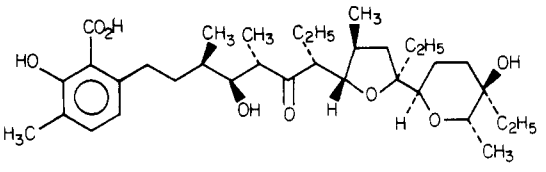
Recent interest in lasalocid has been focused on its ability to transport metal ions and biogenic amines across lipoidal membranes.[1-4] This transporting ability may be utilized to facilitate the in vivo absorption of polar molecules.[5,6] The strength of ion–lasalocid interactions and the physical–chemical properties of the associated species largely determine the rate and extent of ion transport. Fluorescence measurements have been used to obtain a relative measure of the ion–ionophore interactions.[7] Complexation of lasalocid with metal ions leads to an increase or decrease of the fluorescence intensity depending on the quantum yield of the resulting complex.[3] The compound lasalocid (I) belongs to the group of carboxylic



I

acid ionophores. It is an open-chain compound with one tetrahydrofuran ring, one tetrahydropyranic ring, and a salicylate moiety containing the carboxylate head group.

The molecule assumes an open-chain configuration in a polar environment, but in an apolar environment or upon complexation with metal ions or protonated amines, its configuration is that of a pseudocyclic ring structure characterized by a hydrophobic exterior.[8-11]

Table I. Solvent Effects on Sodium Lasalocid Fluorescence

solvent	ϵ [a]	ϕ^{rel}_f [b]	λ_{max}, [c] nm
CH_3OH	32.63	1.00	405
$CHCl_3$	4.81	1.58	407
C_2H_5OH	24.30	3.63	409
i-C_3H_7OH	18.90	3.88	410
$CH_3CO_2CH_3$	6.68	5.50	412
CH_3CN	37.50	6.25	414
C_6H_6	2.28	8.75	416

[a] Dielectric constants at 25 °C, obtained from: CRC Handbook of Chemistry and Physics, 10th ed., p 62. [b] Quantum yield of lasalocid fluorescence measured relative to its fluorescence in methanol at 25 ± 0.5 °C with λ_{ex} = 310 nm by using a Aminco-Bowman spectrofluorometer and are not corrected for changes in solvent refractive index. Error is ± 5%. [c] Wavelength of maximum fluorescence intensity ± 1 nm.

The aromatic hydroxyl and carboxyl groups collectively constitute a fluorophore exciting at 313 nm and emitting at 420 nm.[12] Large solvent effects have been observed relative to the amplitude of the circular dichroism (CD) spectra of lasalocid.[4] Since fluorescence techniques have been used to obtain information about stoichiometries and association constants of cation–lasalocid interactions, the present studies have been conducted to investigate the solvent effects on fluorescence properties of lasalocid. Solvents of different polarities and hydrogen-bonding capabilities are expected to have strong effects on the conformation and in turn, on the fluorescence properties of the molecule.

The relative fluorescence quantum yield (ϕ^{rel}_f) and the wavelength of the fluorescence maximum (λ_{max}^{fl}) are strongly dependent on the solvent character as shown in Table I. The data clearly suggests that a decrease in hydrogen-bonding ability and/or polarity of the solvent causes an increase in ϕ^{rel}_f and a red shift in the λ_{max}^{fl}. Examining the effects of alcohols, it can be observed that both ϕ^{rel}_f and λ^{fl}_{max} increase in the order of isopropanol > ethanol > methanol. Although, these results along with the fact that acetonitrile > methanol clearly indicate that the hydrogen-bonding ability of the solvent is the prime factor, the observation that benzene > methyl acetate, suggests that solvent polarity, as measured by dielectric constant, has some effect on ϕ^{rel}_f and λ^{fl}_{max}.

Considerable information on the conformation of a molecule can be obtained from the study of its fluorescence properties. The fluorescence process occuring from singlet excited state (S_1) normally competes with other photophysical processes of intersystem crossing and internal conversion to the ground state for deactivation of the excited singlet states. An increase in interaction of lasalocid with the solvent molecules may result in a change in both the excited singlet-state lifetime (τ_f) (eq 1) and the

$$\tau_f = \frac{1}{k_f + k_{st} + k_s} \quad (1)$$

fluorescence quantum yield (ϕ_f). This is clearly suggested by eq 2 and 3, which provide expressions for τ_f and ϕ_f,

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