A Convenient Preparation of *S*-Adenosylhomocysteine and its Analogues

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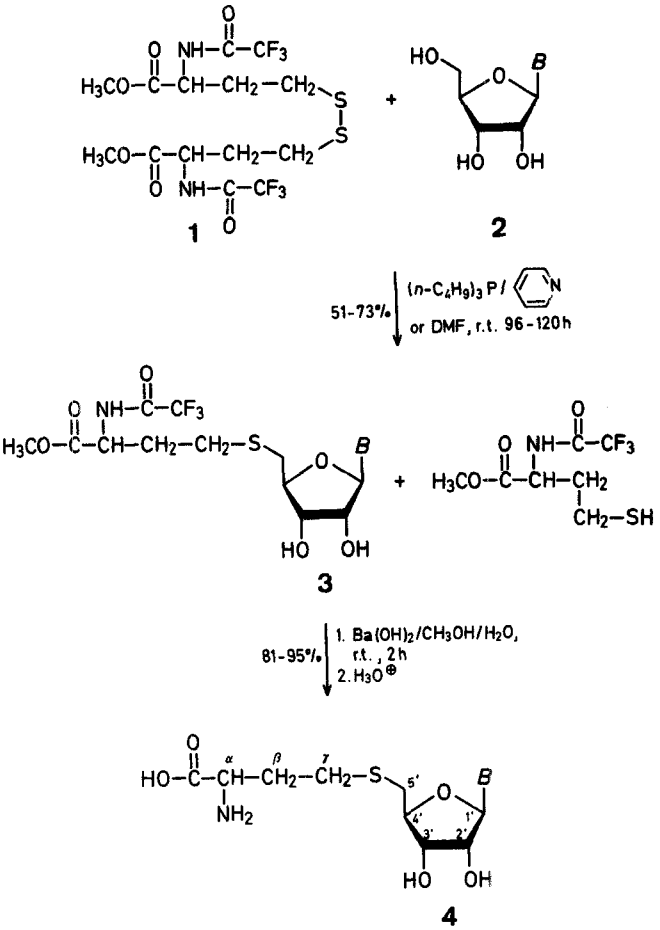
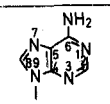
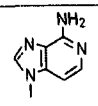
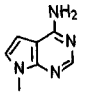
N,N-Bis[trifluoroacetyl]-L-homocystine dimethyl ester (**1**) was condensed with adenosine (**2a**), 3-deazaadenosine (**2b**), and 7-deazaadenosine (**2c**) in the presence of tri-*n*-butylphosphine in pyridine or dimethylformamide to give the *N*-trifluoroacetyl-*S*-adenosyl-homocysteine derivatives **3a**, **3b** and **3c**. Subsequent hydrolysis of **3a**, **3b** and **3c** with aqueous barium hydroxide afforded *S*-adenosylhomocysteine (**4a**), *S*-3-deazaadenosylhomocysteine (**4b**), and *S*-7-deazaadenosylhomocysteine (**4c**) in good yields.

S-Adenosylhomocysteine and many of its structural analogues are potent inhibitors of methyltransferase enzymes[1]. Two procedures have been used for the synthesis of these compounds[2-8]. The shorter route[4-8] is based on the replacement of the 5'-hydroxy group of the nucleoside by chlorine followed by the condensation of the resulting 5'-chloro-5'-deoxy derivative with L-homocysteine sodium salt. The approach presented here involves condensation of the suitably protected L-homocystine with an unprotected nucleoside in the presence of trialkyl phosphine[9,10].

L-Homocystine was converted into its *N,N*-bis[trifluoroacetyl] dimethylester **1** in 77% yield by successive reactions with dimethyl sulfite and trifluoroacetic anhydride[11]. Compound **1** was condensed with the unprotected nucleoside **2a**, **2b**, or **2c** in the presence of tri-*n*-butyl- or triethylphosphine in pyridine or *N,N*-dimethylformamide at room temperature for 48–120 hours. The resulting *N*-trifluoroacetyl-*S*-adenosylhomocysteine derivatives **3a–c** were sufficiently lipophilic to be readily isolated by column chromatography on silica gel in yields of 51–73% (Table).



2-4	a	b	c
B			

The structures of the protected *S*-adenosylhomocysteine derivatives were established by their ^{1}H-N.M.R. spectra which contained signals corresponding to both the amino acid and nucleoside moieties (Table). The presence of signals at $\delta = 5.0-5.5$ ppm which were assigned to 2'-hydroxy and 3'-hydroxy groups indicated that the substitution had occurred at the 5'-position of the nucleosides. The use of protecting groups for the 2',3'-*cis*-diol system of a nucleoside, which would involve extra steps and moreover could interfere with the selective deprotection of the amino acid moiety, was therefore unnecessary. The other protecting groups were removed from **3a–c** with 0.25 molar barium hydroxide in aqueous methanol during 2 hour at room temperature[12] to give good yields of products. The resulting *S*-adenosylhomocysteine (**4a**), *S*-3-deazaadenosylhomocysteine (**4b**), and *S*-7-deazaadenosylhomocysteine (**4c**) were purified

Table. *S*-Adenosylhomocysteine Derivatives **3** and **4** prepared

Product No.	Reaction time [h]	Yield [%]	m.p. [°C]	Molecular formula[a] or Lit. m.p. [°C]	^{1}H-N.M.R. (250 MHz, DMSO-d_6/TMS)[b] δ [ppm]
3a	96	51	indef.	$C_{17}H_{21}F_3N_6O_6S$ (494.5)	2.02 (m, 2H, H-β); 2.60 (m, 2H, H-γ); 2.89 (m, 2H, H-5′,5″); 3.35 (s, 3H, OCH_3); 4.02 (m, 1H, H-4′); 4.14 (m, 1H, H-α); 4.47 (m, 1H, H-3′); 4.76 (m, 1H, H-2′); 5.32 (d, 1H, J = 4.97 Hz, 3′-OH); 5.50 (d, 1H, J = 6.0 Hz, 2′-OH); 5.89 (d, 1H, J = 5.76 Hz, H-1′); 7.29 (br.s, 2H, NH_2); 8.15 (s, 1H, H-2); 8.34 (s, 1H, H-8); 9.82 (br.s, 1H, $NHCOCF_3$)
3b	120	73	indef.	$C_{18}H_{22}F_3N_5O_6S$ (493.4)	2.16 (m, 2H, H-β); 2.62 (m, 2H, H-γ); 2.90 (m, 2H, H-5′,5″); 3.66 (s, 3H, OCH_3); 4.10 (m, 2H, H-α + H-4′); 4.45 (m, 2H, H-2′ + H-3′); 5.81 (d, 1H, J = 6.05 Hz, H-1′); 6.64 (br.s, 2H, NH_2); 6.91 (d, 1H, J = 5.98 Hz, H-3); 7.69 (d, 1H, J = 5.98 Hz, H-2); 8.34 (s, 1H, H-8); 9.87 (d, 1H, J = 7.5 Hz, $NHCOCF_3$)
3c	96	62	indef.	$C_{18}H_{22}F_3N_5O_6S$ (493.4)	2.05 (m, 2H, H-β); 2.65 (m, 2H, H-γ); 2.85 (m, 2H, H-5′,5″); 3.65 (s, 3H, OCH_3); 3.96 (m,1H, H-4′); 4.07 (m, 1H, H-α); 4.45 (m, 2H, H-2′ + H-3′); 5.25 (d, 1H, J = 4.98 Hz, 3′-OH); 5.37 (d, 1H, J = 5.83 Hz, 2′-OH); 6.06 (d, 1H, J = 5.80 Hz, H-1′); 6.64 (d, 1H, J = 3.70 Hz, H-7); 7.04 (br.s, 2H, NH_2); 7.31 (d, 1H, J = 3.70 Hz, H-8); 8.06 (s, 1H, H-2); 9.85 (d, 1H, J = 7.5 Hz, $NHCOCF_3$)
4a	2	81	210–212°	212°[4]	1.90 (m, 2H, H-β); 2.62 (t, 2H, J = 7.5 Hz, H-γ); 2.86 (m, 2H, H-5′,5″); 3.33 (m, 1H, H-α); 4.02 (m, 1H, H-4′); 4.13 (m, 1H, H-3′); 4.69 (m, 1H, H-2′); 5.70 (br.s, 2H, 2′-OH + 3′-OH); 5.86 (d, 1H, J = 5.48 Hz, H-1′); 7.30 (br.s, 2H, NH_2); 8.19 (s, 1H, H-2); 8.32 (s, 1H, H-8)
4b	2	95	indef.[3]	indef.[3]	2.11 (m, 2H, H-β); 2.63 (m, 2H, H-γ); 2.85 (m, 2H, H-5′,5″); 3.35 (m, 1H, H-α); 3.68 (m, 1H, H-4′); 4.49 (m, 2H, H-2′ + H-3′); 5.76 (d, 1H, J = 1.93 Hz, H-1′); 6.19 (br.s, 2H, NH_2); 6.84 (d, 1H, J = 5.65 Hz, H-3); 7.69 (d, 1H, J = 5.36 Hz, H-2); 8.32 (s, 1H, H-8)
4c	2	85	262–264°	178–180°[4]	2.10 (m, 2H, H-β); 2.81 (t, 2H, J = 7.32 Hz, H-γ); 2.95 (m, 2H, H-5′,5″); 4.25 (m, 1H, H-4′); 4.35 (t, 1H, J = 3.89 Hz, H-α); 4.75 (m, 2H, H-2′ + H-3′); 6.19 (d, 1H, J = 6.03 Hz, H-1′); 6.64 (d, 1H, J = 3.73 Hz, H-7); 7.36 (d, 1H, J = 3.73 Hz, H-8); 8.11 (s, 1H, H-2)[c]

[a] Satisfactory microanalyses obtained: C ± 0.39, H ± 0.36, N ± 0.19. Exception: **3b**, N −0.6.
[b] 2′-OH, 3′-OH, NH_2 and $NHCOCF_3$ protons were exchangeable with D_2O.
[c] Measured in D_2O.

by recrystallisation from water (**4a**) or by chromatography on Sephadex A-25 and subsequent lyophilisation (**4b**, **4c**). Compounds **4a–c** gave positive tests with ninhydrin and were homogeneous on H.P.L.C.

The method described, which is of potential general applicability is more efficient and convenient than the reported methods. A variety of protecting groups for carboxyl and amino functions of L-homocystine may be used and the protected *S*-adenosylhomocysteine derivatives are easy to purify by column chromatography on silica gel. The overall yields are higher and the use of unprotected nucleoside instead of its 5′-chloro-5′-deoxy derivative is a major advantage especially for modified nucleosides and analogues.

N,N-Bis[trifluoroacetyl]-L-homocystine Dimethyl Ester (1):
This compound is prepared using the reported[11] procedure by successive reactions of L-homocystine with dimethyl sulfite and trifluoroacetic anhydride; yield: 77%; m.p. 89–90°C (methanol/water).

^{1}H-N.M.R. (250 MHz, DMSO-d_6/TMS): δ = 2.25 (m, 2H, H-β); 2.75 (m, 2H, H-γ); 3.70 (s, 3H, OCH_3); 4.50 (m, 1H, H-α); 9.85 ppm (d, 1H, J = 7.5 Hz, NH).

Condensation of Nucleosides 2a–c with N,N-Bis[trifluoroacetyl]-L-homocystine Dimethyl Ester (1); General Procedure:
A solution of the nucleoside **2a**, **2b**, or **2c**, (1 mmol) and *N,N*-bis[trifluoroacetyl]-L-homocystine dimethyl ester (**1**; 3 mmol) in dry pyridine (10 ml) is concentrated under reduced pressure. The process is repeated three times and the residue is dissolved in dry pyridine (5 ml) under argon, then tri-*n*-butylphosphine (1.5 ml, 6 mmol) is added. The mixture is stirred at room temperature and the reaction is monitored by H.P.T.L.C. (silica gel, chloroform/ethanol, 9:1). After 96–120 h, the reaction is quenched with water (15 ml), the mixture is concentrated under reduced pressure, and the traces of pyridine from the residue are azeotropically removed using toluene, the residue is then applied to a column (6 × 2.5 cm) of Kieselgel 60 (Merck, 230–400 mesh). After initial elution with chloroform, the product is eluted with chloroform/ethanol (9:1 for **3a** and **3c**, and 17:3 for **3b**).

The fractions containing **3a**, **3b** or **3c** are combined and concentrated under reduced pressure. Each colourless oily residue is dissolved in small amount of chloroform with the addition of few drops of pyridine and added dropwise to stirred light petroleum (b.p. 30–40°C). The resulting white precipitate is collected by centrifugation and dried in a desiccator (Table).

Deprotection of N-Trifluoroacetyl-S-adenosylhomocysteine Derivatives 3a–c; General Procedure:
A suspension of the *N*-trifluoroacetyl-*S*-adenosylhomocysteine derivative **3a**, **3b** or **3c** (0.5 mmol) in 0.25 molar barium hydroxide in aqueous methanol (1:1, 10 ml) is stirred for 2 h at room temperature, then acidified to pH 6 with 1.0 normal sulfuric acid, centrifuged, and the supernatant is concentrated to dryness. The residue is recrystallised from water (**4a**) or eluted from a column (2.5 × 20 cm) of Sephadex A-25 with 0.01 molar triethylammonium hydrogen carbonate buffer (**4b**, **4c**). The fractions containing the product (UV absorbing, ninhydrin positive) are combined, concentrated, and lyophilised (Table).

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