Towards Lipophilic Derivatives of S-Adenosyl-L-methionine

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Two different strategies for the synthesis of S-Adenosyl-L-methionine liphophilic derivatives have been attempted. The first chemical direct modification performed on S-adenosyl-L-methionine is described.

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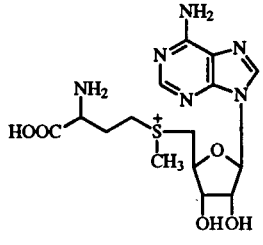
S-Adenosyl-L-methionine is a naturally occurring compound which participates in many crucial biochemical processes [1]. S-Adenosyl-L-methionine is a precursor of polyamine synthesis and the methyl donor of most transmethylation reactions. Moreover, S-adenosyl-L-methionine metabolism is necessary for the folate cycle as well as for the synthesis of homocysteine, cysteine and glutathione [2].



S-Adenosyl-L-methionine

Patients with chronic liver disease have an acquired deficiency in S-adenosyl-L-methionine synthesis and metabolism, and this alteration might complicate and maintain the clinical syndrome [3]. This finding has led to the proposal that S-adenosyl-L-methionine is a possible therapeutic agent for the treatment of certain liver diseases [4,5].

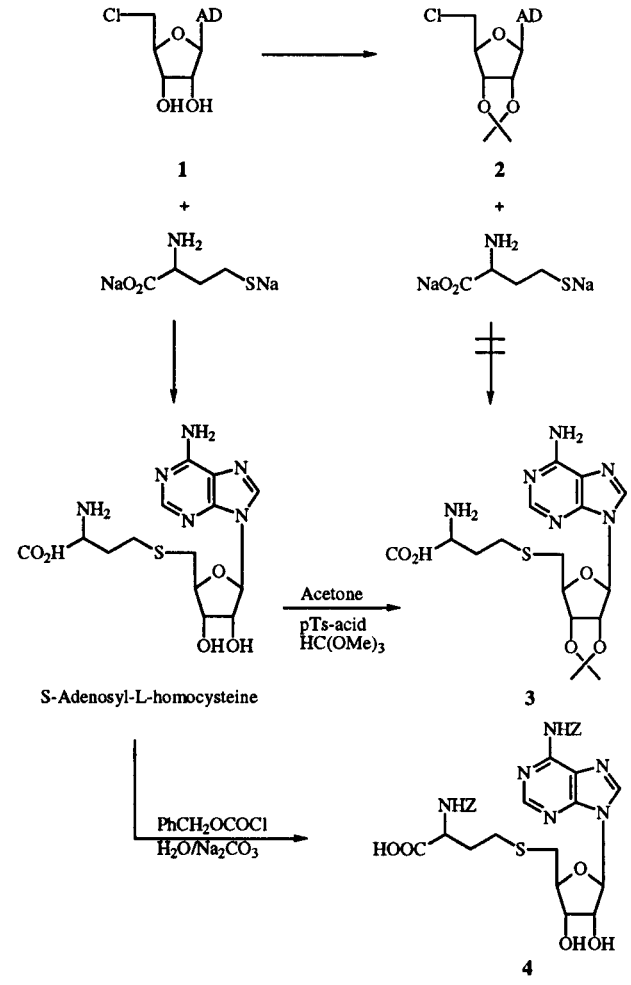
For the present, S-adenosyl-L-methionine is clinically used in hepatitis, cirrhosis and alcoholic hepatopathy, because it is able to increase the intracellular glutathione levels [6]. Moreover, neuronal transmethylation reactions might be of interest in psychiatric pathology, and S-adenosyl-L-methione is currently being evaluated as a new therapeutic agent in depression [7]. Due to its high polarity, one of the problems found when S-adenosyl-L-methionine is administered to patients, is the low absorption by the cell membranes. Therefore, from a therapeutic point of view, an important goal would be to transform the polar groups of S-adenosyl-L-methionine into other more lipophilic groups which could facilitate absorption by cells and at the same time would be easy to hydrolyze.

In this paper we describe one of the first enhanced lipophilicity S-adenosyl-L-methionine derivatives which has been obtained by the first chemical direct modifications performed on S-adenosyl-L-methionine. The results are compared to those obtained by using traditional methods for synthesis of S-adenosyl-L-methionine [8,9].

One can think of preparing S-adenosyl-L-methionine derivatives by previous modifications of its precursors or by direct reaction of S-adenosyl-L-methionine.

We first tried the reaction of **2** with the appropriate amino acid, (Scheme 1) to give S-adenosyl-L-homocysteine derivative **3**. Unfortunately, in all the attempts made, the acetonide was destroyed during the workup procedures. The synthesis of the 2',3'-isopropylidene derivative of S-adenosyl-L-homocysteine **3** [10] was finally accomplished by reaction of S-adenosyl-L-homocysteine [11] with acetone using *p*-toluenesulphonic acid and methyl orthoformate yielding **3** in reasonable yield (65%).

Scheme 1



To block the polar groups of S-adenosyl-L-homocysteine, we allowed it to react with benzyloxycarbonyl chloride (Scheme 1) and obtained compound **4** where two benzyloxycarbonyl carbamates were formed.

Finally, the last step in this synthesis involves the *S*-methylation of S-adenosyl-L-homocysteine derivatives. Two methods were attempted; methyl iodide in formic acid and methyl *p*-toluenesulfonate used as the reagent and as the solvent [12]. Unfortunately, both carbamates and the isopropylidene group were removed and only S-adenosyl-L-methionine and decomposition products were isolated.

Thus, we decided to use S-adenosyl-L-methionine as the starting material. To our knowledge, this has not been done yet in the literature possibly due to low stability of S-adenosyl-L-methionine and to difficulties in the isolation steps.

First, we prepared **5** (Scheme 2), the methyl ester of S-adenosyl-L-methionine, by treatment with dimethyl sulfite in methanol saturated with hydrogen chloride. The two amino groups were then transformed into the corresponding trifluoroamides with trifluoroacetic anhydride yielding S-adenosyl-L-methionine derivative **6** in a reasonable isolated yield (60%).

We accomplished the protection of the 2',3'-hydroxyl groups at this stage by treatment with acetone and methyl orthoformate yielding **7** also in reasonable yield (48%) (Scheme 2). The structures of compounds **5**, **6** and **7**, which are obtained as a mixture of two diastereomers due to the chirality of the sulfur atom, were established from their analytical and spectroscopic data which are collected in Table 1.

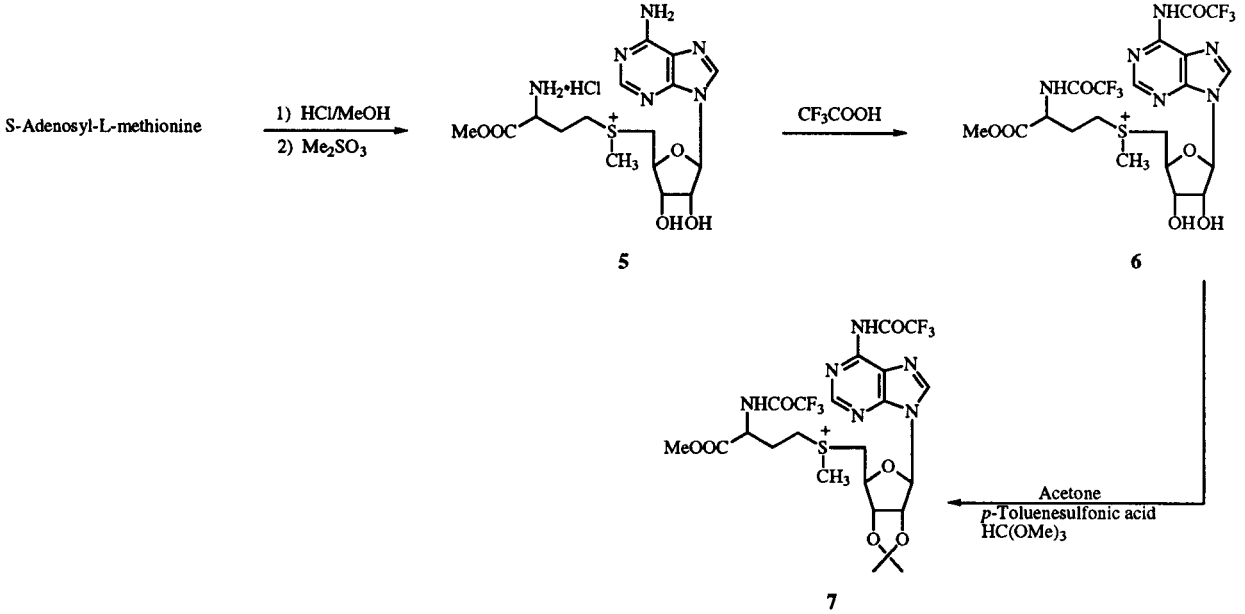
In the ^1H nmr spectra some signals of the pair of diastereomers appeared clearly differentiated. In all cases there are two values for the chemical shift of the SCH_3 and $COOCH_3$ protons, and only in compound **6** differences in almost all the signals in the spectrum can be observed (Table 1).

In this paper chemical synthesis of new S-adenosyl-L-methionine lipophilic derivatives have been worked out using an easy direct chemical modification of S-adenosyl-L-methionine, which can lead to the enhanced potential of S-adenosyl-L-methionine as a chemotherapeutic agent.

EXPERIMENTAL

Melting points were determined with a Reichert-Jung Thermovar apparatus and are uncorrected. Column chromatography was performed on Merck silica gel 60 (70-230 mesh), Cellulose Avicel (Merck), Resin Dowex-50W and Amberlite IRC-50 (Sigma). The ^1H nmr spectra were obtained at 298 K using tetramethylsilane as the internal standard on Varian-Gemini 200 and Varian XL-300 spectrometers, operating at 200 and 300 MHz, respectively. Elemental analyses were performed by the analytical department at C.N.Q.O. (CSIC).

Scheme 2



5'-Chloro-5'-deoxy-2',3'-*O*-isopropylideneadenosine (**2**).

5'-Chloroadenosine **1** (0.1 g, 0.35 mmole) was added to a mixture of copper sulfate (54 mg) with sulfuric acid (3-4 drops) in acetone (4 ml) and stirred at room temperature overnight. The solids were filtered and the organic layer was neutralized with 6*N* ammonium hydroxide. The solvent was evaporated under

Table 1

^{1}H NMR Data (Chemical shifts (ppm) and coupling constants (Hz)) of S-Adenosyl-L-homocysteine, S-Adenosyl-L-methionine, **3**, **4** and **5-7** in Dimethyl-d_6 Sulfoxide

Compound	H-8	H-2	H-1'	H-2'	H-3'	H-4'	H-5'a	H-5'b	CHα	CH_2β	CH_2γ	S-CH_3	$J_{1',2'}$	$J_{2',3'}$
S-Adenosyl-L-homocysteine [a]	8.34 (s)	8.15 (s)	5.88 (d)	4.71 (dd)	4.17 (dd)	4.08 (m)	2.90 (dd)	2.80 (dd)	3.30 (t)	1.88 (m)	2.62 (t)	—	5.6	5.2
3 [b]	8.35 (s)	8.17 (s)	6.16 (d)	5.50 (dd)	5.02 (dd)	4.62 (m)	2.68 (m)		3.55 (t)	1.97 (m)	2.38 (m)	—	1.5	5.5
4 [c]	8.49 (s)	8.30 (s)	5.89 (d)	4.67 (m)	4.16 (m)	3.90 (m)	2.82 (m)		3.75 (m)	1.81 (m)	2.40 (m)	—	5.5	—
S-Adenosyl-L-methionine [d]	8.37 (s)	8.18 (s)	6.06 (d)	4.76 (dd)	4.50 (m)	4.47 (m)	3.90 (m)		4.15 (t)	2.33 (m)	3.56 (m)	2.92 2.88	4.2	8.4
5 [e]	8.33 (s)	8.14 (s)	5.87 (d)	4.73 (t)	4.46 (m)	4.34 (m)	4.01 (m)		4.15 (m)	2.40 (m)	3.50 (m)	3.00 2.98	5.7	5.4
6 [f]	8.70 8.68	8.48 8.45	6.01 5.99	4.63 (t)	4.50 (m)	4.39 (m)	3.90 (m)		4.27 (t)	2.30 (m)	3.50 (m)	2.96 2.89	1.9	4.8
7 [g]	8.70 (s)	8.50 (s)	6.29 (m)	5.28 (m)	5.1 (m)	4.60 (m)	3.80 (m)		4.40 (m)	2.26 (m)	3.40 (m)	2.93 2.77	—	—

[a] $J_{3',4'}$ = 4.9 Hz, $J_{4',5'a}$ = 5.6 Hz, $J_{4',5'b}$ = 6.7 Hz, $J_{5'a,5'b}$ = -13.8 Hz, $J_{Ha,Hb}$ = 6.3 Hz, $J_{H\gamma,H\beta}$ = 7.5 Hz; [b] 1.50 and 1.25 (*s*, 6H, $C(CH_3)_2$), $J_{3',4'}$ = 2.2 Hz, $J_{H\alpha,H\beta}$ = 6.3 Hz; [c] 7.28 (m, 10H, Ph), 5.00 (s, 2H, CH_2(Z)), 4.96 (*s*, 2H, CH_2(Z)); [d] $J_{H\alpha,H\beta}$ = 7.0 Hz; [e] 3.57 and 3.48 (*s*, 3H, CH_3OCO), [f] 9.90 (d, NH), 9.86 (d, NH), 3.67 and 3.64 (*s*, 3H, CH_3OCO), $J_{H\alpha,H\beta}$ = 4.3 Hz; [g] 9.92 (d, NH), 9.90 (d, NH), 3.60 and 3.57 (s, 3H, CH_3OCO), 1.09 and 1.39 (s, 6H, $C(CH_3)_2$).

reduced pressure, and the pure residue, tested by thin layer chromatography, was recrystallized from water to provide **3** (0.11 g, 95%) mp 256-257° (lit [13] 255-256°).

Anal. Calcd. for $C_{13}H_{16}N_5O_3Cl$: C, 47.89; H, 4.91; N, 21.49. Found: C, 47.79; H, 5.79; N, 19.50.

S-(5'-Deoxy-2',3'-*O*-isopropylidene-5'-adenosyl)homocysteine (**3**).

To a well stirred suspension of S-adenosyl-L-homocysteine (0.02 g, 0.05 mmole) in acetone (3 ml) *p*-toluenesulfonic acid (2 mg) and ethyl orthoformate (2-3 drops) were added. The mixture was stirred at room temperature overnight and then it was tested by thin layer chromatography. When S-adenosyl-L-homocysteine had disappeared, the solvent was evaporated under reduced pressure and the pale yellow residue was chromatographed on a cellulose Avicel column using ethanol/water 3:2 as the eluent to yield **3** (0.01 g, 65%) mp 162-164°.

Anal. Calcd. for $C_{17}H_{24}N_6O_5S$: C, 48.06; H, 5.65; N, 19.79. Found: C, 47.79; H, 5.79; N, 19.50.

S-(*N*^{6}-Benzyloxycarbonyl-5'-deoxy-5'-adenosyl)-*N*-benzyloxycarbonyl-1-homocysteine (**4**).

S-Adenosyl-L-homocysteine (50 mg, 0.13 mmole) was dissolved in water (5 ml) containing sodium carbonate (42 mg, 0.39 mmole). Benzyl chloroformate (47 mg, 0.27 mmole) was added, and the mixture was stirred for 2 hours, then it was neutralized with hydrochloric acid and the water was liophilized. The solid residue was recrystallized from ethanol/water to yield **4** (72 mg, 85%), mp 250° dec.

Anal. Calcd. for $C_{30}H_{32}N_6O_9S$: C, 55.18; H, 4.90; N, 12.87. Found: C, 54.85; H, 5.10; N, 12.69.

(±)-*S*-(5'-Deoxy-5'-adenosyl)-1-methionine Methyl Ester Acetate (**5**).

S-Adenosyl-L-methionine *p*-toluenesulfonic acid salt (0.2 g, 0.30 mmole) was suspended in methanol (20 ml) which was then saturated with anhydrous hydrogen chloride. The procedure was carried out in an ice-salt bath because the reaction is very exothermic. Dimethyl sulfite (0.3 ml) was added, and the solution was stirred overnight. Methanol and excess of dimethyl sulfite were removed under reduced pressure, and the resultant methyl ester hydrochloride was dried under high vacuum. The methyl ester obtained was pure enough to continue the synthesis, but purification could be accomplished by chromatography on an amberlite column using water and 4*N* acetic acid as eluents to yield **5** (0.14 g, 90%), mp 200° dec.

Anal. Calcd. for $C_{18}H_{28}N_6O_7S$: C, 45.75; H, 5.97; N, 17.79. Found: C, 45.85; H, 6.10; N, 17.69.

(±)-*S*-(*N*^{6}-Trifluoroacetyl-5'-deoxy-5'-adenosyl)-*N*-trifluoroacetyl-1-methionine Methyl Ester Acetate (**6**).

Trifluoroacetic anhydride (8 ml) was added to the flask containing methyl ester **5** obtained from S-adenosyl-L-methionine (0.2 g, 0.35 mmole) by the above procedure without further purification, and the resulting solution was stirred for 2 hours at rt. The excess trifluoroacetic anhydride and trifluoroacetic acid were evaporated with a stream of nitrogen. The solid residue was chromatographed on a silica gel column using acetic acid/butanol/water, 60:25:15 as the eluent to yield **6** (0.13 g, 60%); mp 80-82°.

Anal. Calcd. for $C_{22}H_{26}F_6N_6O_9S$: C, 39.76; H, 3.94; N, 12.65. Found: C, 39.85; H, 4.10; N, 12.69.

(±)-*S*-(*N*^{6}-Trifluoroacetyl-2',3'-isopropilydene-5'-deoxy-5'-adenosyl)-*N*-trifluoroacetyl-1-methionine Methyl Ester *p*-Toluenesulfonate (**7**).

Following the procedure described for compound **3**, the S-adenosyl-L-methionine derivative **6** (0.03 g, 0.05 mmole) reacted with acetone (3 ml), *p*-toluenesulfonic acid (2 mg) and ethyl orthoformate (2-3 drops). The purification step was cellulose column chromatography using dichloromethane as the eluent, to yield **7** (0.02 g, 48%) as a pale yellow oil.

Anal. Calcd. for $C_{30}H_{34}F_6N_6O_{10}S_2$: C, 44.12; H, 4.20; N, 10.29. Found: C, 43.95; H, 4.10; N, 10.59.

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