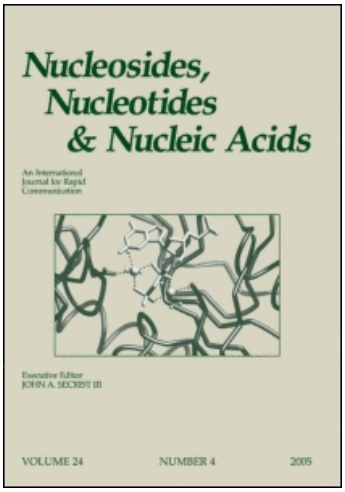

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Margherita Gavagnin[a]; Guido Sodano[b]
[a] Istituto per la Chimica di Molecole di Interesse Biologico, Arco Felice, (NA), Italy [b] Istituto di Chimica, Università della Basilicata, Potenza, Italy

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CONVERSION OF METHYLTHIOADENOSINE INTO ITS NATURALLY OCCURRING 3'-ISOMER

Margherita Gavagnin[&] and Guido Sodano[§]

[&]Istituto per la Chimica di Molecole di Interesse Biologico, CNR
Via Toiano 6, 80072 Arco Felice (NA) Italy

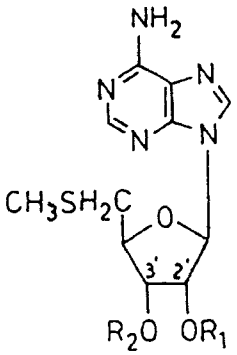
[§]Istituto di Chimica, Università della Basilicata, Via N. Sauro
85100 Potenza Italy

ABSTRACT. Methylthioadenosine (MTA) has been converted into its 3' naturally occurring isomer (xylosyl-MTA) via protection at 2'-OH, oxidation, reduction and deprotection.

5'-Deoxy-5'-methylthioadenosine (1; MTA) is a naturally occurring sulfur nucleoside ubiquitously distributed in nature, endowed with antiproliferative activity[1-4]. It derives from S-adenosylmethionine (AdoMet) metabolism through several metabolic pathways[1-4].

Recently the first naturally occurring analog of MTA, 9-[5'-deoxy--5'-(methylthio)-ß-D-xylofuranosyl]adenine (2; xylosyl-MTA), has been isolated from the Mediterranean nudibranch Doris verrucosa[5]. In connection with studies[6] on the biosynthesis and metabolic fate of xylosyl-MTA (2) we needed labelled 2 and therefore we devised a short synthesis of the target molecule starting from the commercially available MTA (1). A more elaborated synthesis of 2 has been already reported[7].

Treatment of MTA with tert-butyl-dimethylsilylchloride (TBDMS-Cl) in the conditions used for the protection of other nucleosides[8] afforded the 2'-protected derivative 3 as the major product (45%) along with minor amounts of the 3'- (4; 26%) and 2',3'- (5; 5%) derivatives. The position of the protecting group in 3 and 4 was inferred by the low field shift of the appropriate proton in the ^{1}H-NMR spectra and confirmed by the subsequent transformation of 3 into 2 (see below).



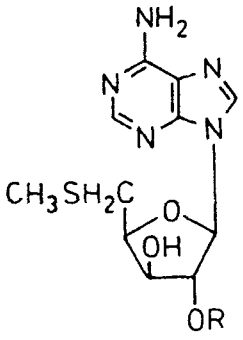
1; $R_1=R_2=H$

3; R_1=TBDMS, R_2=H

4; R_1=H, R_2=TBDMS

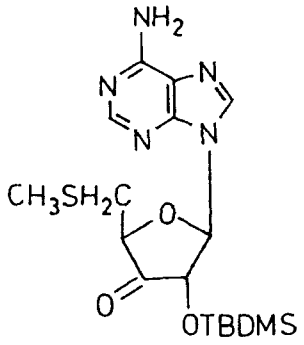
5; $R_1=R_2$=TBDMS

7; R_1=TBDMS, $R_2=CH_2SCH_3$



2; R=H

8; R=TBDMS



6

Attempted oxidation of 3 using $CrO_3/Py/Ac_2O$ complex, a reagent of choice for the oxidation of TBDMS-protected nucleodides[9], resulted only in untractable material probably due to the presence of the sensitive sulfur moiety. Thus 3 was oxidized to the desired ketone 6 using $DMSO/Ac_2O$[10,11]. In this reaction the best yields (35%) were obtained when the oxidation was carried out on small amounts of 3 (0.1 mmoles or less); when 0.2-0.4 mmoles of 3 were used, the yields lowered to 15-20%. $DMSO/Ac_2O$ promoted oxidation of 3 resulted also in the formation of the 3'-O-methylthiomethylether (7) in variable amounts (2-23%), depending on the reaction time and on the DMSO batch used.

The ketone 6 was reduced with $NaBH_4$ to the unseparable mixture of epimers 3 and 8 which were deprotected with tetra-n-butylammoniumfluoride in THF[8] affording 2 (30% from 6) along with 1 (16%). The structure of the reduced deprotected compounds was identified by TLC and ^{1}H-NMR comparison with authentic samples[5].

The $NaBH_4$ reduction of 6 also afforded the 3'-protected MTA derivative 4 (17%), probably by isomerization of 3 under the reaction conditions. In fact, when 3 was mixed with $NaBH_4$ in EtOH as in the reduction of 6, a 35% amount of 4 was isolated after 3 hours. Isomerization of 3 in the

EtOH only proceeded very slowly resulting in trace amounts of the isomerized product 4 after 12 hours.

EXPERIMENTAL

General procedure

NMR spectra were recorded on a Bruker WM 500 spectrometer for DMSO-d_6 solutions. Chemical shifts are reported in ppm (δ) from DMSO-d_5 taken as internal standard at δ 2.49. Mass spectra were taken on an AEI MS-30 apparatus. Methylthioadenosine was kindly provided by Dr. M. Porcelli, Department of Biochemistry of Macromolecules, University of Naples.

Reaction of methylthioadenosine (1) with tert-butyl-dimethylsilylchloride.

600 mg of 1 (2 mmol) were dissolved in anydrous pyridine (6 ml) and 900 mg (6 mmol) of tert-butyl-dimethylsylylchloride (TBDMS-Cl) were added and the solution was stirred at r.t. for 48 h. Sat NaCl/H_2O was added and the mixture was extracted with EtOAc. The organic phase was washed with sat NaCl/H_2O, dried over Na_2SO_4 and evaporated. The resulting material was subjected to Si-gel column chromatography (EtOAc) affording, in order of increasing polarity, 5'-deoxy-5'-(methylthio)-2',3'-di-O-TBDMS--adenosine (5; 50 mg; 5%), m.p. 228-230 °C, MS (m/z) found 525.2634 ($C_{23}H_{43}N_5O_3SSi_2$ requires 525.2625), 510, 468 (base peak), 333, 230, 217, 164; ^{1}H-NMR δ 8.39 (s, H-8),8.14 (s, H-2), 7.27 (bs, 6-NH_2), 5.91 (d, H-1', J=7.1 Hz), 5.08 (dd, H-2', J=7.1 and 4.4 Hz), 4.28 (bd, H-3', J=4.4 Hz), 4.03 (m, H-4'), 3.03 (dd, H-5'A, J=13.9 and 7.6 Hz), 2.86 (dd, H-5'B, J=13.9 and 5.6 Hz), 2.07 (s, SCH_3), 0.92 (s, $SiC(CH_3)_3$), 0.68 (s, $SiC(CH_3)_3$), 0.15 (s, $SiCH_3$), 0.12 (s, $SiCH_3$), -0.12 (s, $SiCH_3$), -0.43 (s, $SiCH_3$); 5'-deoxy-5'-(methylthio)-2'-O-TBDMS-adenosine (3; 370mg; 45%); m.p. 114-116 °C; MS (m/z) 411.1783 (M^+; $C_{17}H_{29}N_5O_3SSi$ requires 411.1761), 396, 364, 354 (base peak), 230, 219, 164, 136, 135; ^{1}H-NMR δ 8.36 (s, H-8), 8.15 (s, H-2), 7.33(bs, 6-NH_2) 5.91 (d, H-1', J=5.7 Hz), 5.20 (bd, 3'-OH, J=5.1 Hz), 4.86 (t, H-2', J= 5.4 Hz), 4.12 (m, H-3'), 4.05 (m, H-4'), 2.92 (dd, H-5'A, J=13.9 and 5.9 Hz), 2.83 (dd, H-5'B, J=13.9 and 7.0 Hz), 2.06 (s, SCH_3), 0.72 (s, $SiC(CH_3)_3$), -0.07 (s, $SiCH_3$), -0.20 (s, $SiCH_3$); and 5'-deoxy-5'-(methylthio)-3'-O-TBDMS-adenosine (4; 210 mg; 26%), m.p. 193-195 °C; MS (m/z) 411.1731 (M^+;

$C_{17}H_{29}N_5O_3SSi$ requires 411.1761), 396, 364, 354 (base peak), 230, 219, 194, 164, 136, 135; ^{1}H-NMR δ 8.38 (s, H-8), 8.14 (s, H-2), 7.28 (s, 6-NH_2), 5.87 (d, H-1', J=7.0 Hz), 5.41 (d, 2'-OH, J=6.4 Hz), 4.92 (m, H-2'), 4.28 (dd, H-3', J=4.7 and 2.1 Hz), 4.02 (dt, H-4', J=7.4 and 2.1 Hz), 2.90 (dd, H-5'A, J=7.4 and 13.9 Hz), 2.17 (dd, H-5'B, J=7.4 and 13.9 Hz), 2. 05 (s, SCH_3), 0.92 (s, $SiC(CH_3)_3$), 0.14 (s, $SiCH_3$), 0.13 (s, $SiCH_3$).

Oxidation of 3

To a solution of 3 (103 mg; 0.25 mmol) in 2 ml of dry DMSO, 0.2 ml of Ac_2O were added and the mixture was stirred for 15 h at r.t.. The solution was then cooled at 0°C (ice-bath) and made basic with aq $NaHCO_3$. Sat NaCl/H_2O was added and the mixture was extracted with Et_2O. The organic phase was dried over Na_2SO_4, evaporated and chromatographed on a Si-gel column (EtOAc) affording 20 mg (20%) of pure 6, MS: electron impact MS did not afford the molecular ion (m/z 409), nor expected fragmentation ions; further preparations gave the same results. ^{1}H-NMR δ 8.52 (s, H-8), 8.17 (s, H-2), 7.39 (s, 6-NH_2), 6.16 (d, H-1', J=8.4 Hz), 5.44 (d, H-2', J=8.4 Hz), 4.62 (t, H-4', J=5.2 Hz), 2.89 (d, H-5', J= 5.2 Hz), 2.10 (s, SCH_3), 0.67 (s, $SiC(CH_3)_3$), -0.07 (s, $SiCH_3$), -0.26 (s, $SiCH_3$). In other preparations varying amounts (2-23%) of the 3'-O--methylthiomethylether derivative 7 were isolated. 7 exhibited the same chromatographic behaviour as 6 and was isolated after reduction of 6 and Si-gel column purification (EtOAc). 3'-O-methylthiomethylether derivative 7, MS (m/z) 471 (M^+), 456, 414, 410, 364, 308, 230 (base peak), 219, 164, 136; ^{1}H-NMR δ 8.36 (s, H-8), 8.14 (s, H-2), 7.30 (bs, 6-NH_2), 5.93 (d, H-1', J=6.8 Hz), 5.16 (dd, H-2', J=6.8 and 4.7 Hz), 4.83 (ABq, OCH_2S, J=11.4 Hz), 4.31 (dd, H-3', J=4.7 and 1.8 Hz), 4.19 (m, H-4'), 2.98 (dd, H-5'A, J=13.9 and 7.2 Hz), 2.88 (dd, H-5'B, J=13.9 and 6.6 Hz), 2.15 (s, SCH_3), 2.09 (s, SCH_3), 0.69 (s, $SiC(CH_3)_3$), -0.10 (s, $SiCH_3$), -0.35 (s, $SiCH_3$).

Reduction of 6

A solution of 6 (40 mg, 0.10 mmol) in 1 ml abs EtOH was cooled at 0°C (ice bath) and an excess of $NaBH_4$ was added under stirring. After 3 h MeOH

was added. After evaporation of solvents the reaction mixture was partitioned between sat NaCl/H_2O and EtOAc. The organic phase was dried over Na_2SO_4, evaporated and purified on a Si-gel column (EtOAc) giving, in order of increasing polarity, a mixture (20 mg) of 3 and 8 (^{1}H-NMR analysis showed a ratio xylo/ribo 2:1), and 4 (17 mg). The mixture of 3 and 8 was dissolved in THF (1 ml) and stirred with 150 µl of 1M $nBu_4N^+F^-$/THF for 30 min at r.t.. After evaporation of the solvent the material was applied to two semipreparative Si-gel TLC plates ($CHCl_3$/MeOH 8:2). Two UV absorbing bands were eluted with $CHCl_3$/MeOH 8:2 giving 2 (9 mg, 30% from 6) and 1 (5 mg, 16%), identified by TLC and ^{1}H-NMR comparison with authentic samples[5].

$NaBH_4$ catalyzed isomerization of 3 into 4.

20 mg of 3 were dissolved in 1 ml of abs EtOH. The solution was cooled at 0°C and 20 mg of $NaBH_4$ were added. After 3 h the mixture was treated as above, affording 3 (11 mg) and 4 (7 mg).

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