Homochiral synthesis of an aza analogue of *S*-adenosyl-L-methionine (AdoMet) and its binding to the *E. coli* methionine repressor protein (MetJ)

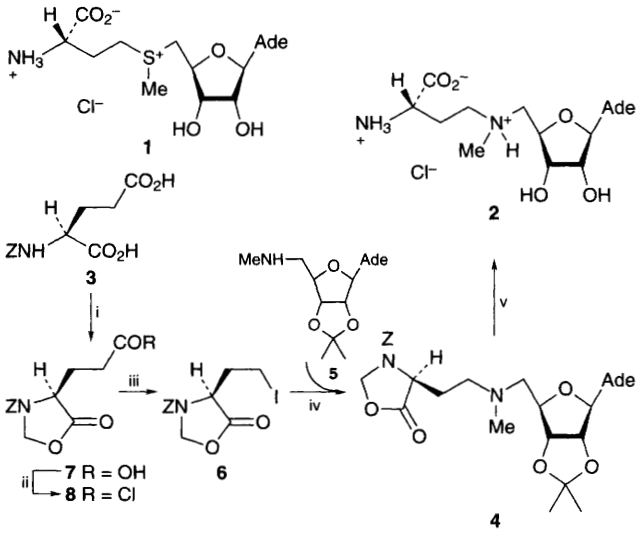
Mark J. Thompson,[a] Abdelaziz Mekhalfia,[a] David L. Jakeman,[a] Simon E. V. Phillips,[b] Kathryn Phillips,[c] Jonathan Porter[b] and G. Michael Blackburn*[a]

[a] *Department of Chemistry, University of Sheffield, Brook Hill, Sheffield, UK S3 7HF*
[b] *Department of Biochemistry and Molecular Biology, University of Leeds, Leeds, UK LS2 9JT*
[c] *MRC Virology Unit, Institute for Virology, Glasgow, UK G11 5JT*

A synthesis of 5′-{*N*-[(*S*)-3-amino-3-carboxypropyl]-methylamino}-5′-deoxyadenosine from D-adenosine and (*S*)-glutamic acid is described; this product, AzaAdoMet 2, has a pK_a of 7.10 for the tertiary amino group and so acts as a charge-switchable analogue of AdoMet 1, a key component of polyamine biosynthesis; the binding of both 1 and 2 to the *E. coli* methionine repressor protein is investigated by X-ray crystallography.

(*S*)-Adenosyl-L-methionine (AdoMet) **1** is well known as an essential co-factor involved in many biological processes. In particular, it has a central role as a biological methylating agent and in the ethylene–polyamine cycle.[1] We describe here a homochiral synthesis of the aza-desthio analogue of **1**, AzaAdoMet **2**. This analogue has previously been prepared as a mixture of epimers[2] that can inhibit *E.coli* tRNA uracil-5-methyltransferase[3] and *Eco*RI DNA methylase.[4] However, these earlier syntheses[2] have failed to control chirality at the amino acid centre, so all existing biological work has necessarily used a mixture of epimers. We therefore planned a homochiral synthesis based on D-adenosine and (*S*)-glutamic acid **3**.

The protected product **4** was assembled from the two key intermediates; **5**, derived from D-adenosine, and **6**, derived from (*S*)-glutamic acid. 2′,3′-*O*,*O*-(1-Methylethylidene)-D-adenosine[5] was converted into the 5′-*O*-tosylate by treatment with toluene-*p*-sulfonyl chloride in dry pyridine,[6] which was stirred in liquid methylamine to give the amine intermediate **5**.[7] The synthesis of **6** is summarised in Scheme 1. (*S*)-Glutamic acid **3** was protected[8] as the oxazolidinone **7**, which was treated with oxalyl chloride to afford the corresponding acid chloride **8**. This was converted into the alkyl iodide **6** *via* a Barton-type[9] decarboxylative iodination using 2-iodo-1,1,1-trifluoroethane as iodine donor.[10]† The key intermediates **5** and **6** were then heated together in acetonitrile solution in the presence of *N*,*N*-diisopropylethylamine to give **4**. Attempts to remove the benzyloxycarbonyl group from **4** by catalytic hydrogenation with 10% Pd–C proved unsuccessful; reactions were incomplete after several days and a mixture of products was detected by TLC. Catalytic transfer hydrogenation[11] also afforded mixtures of products. However, treatment of **4** with BF_3–EtSH complex[12] effected complete removal of all protecting groups. The final product **2**‡ was purified on Dowex 50WX4-400 ion-exchange resin.[2a]



Scheme 1 *Reagents and conditions*: i, paraformaldehyde, *p*-TsOH (cat.), benzene, Dean–Stark, reflux, 7 h, (61%); ii, $(COCl)_2$ (1.1 equiv.), CH_2Cl_2, DMF (cat.), room temp., 1 h, (94%); iii, *N*-hydroxypyridine-2-thione sodium salt, CF_3CH_2I (5 equiv.), DMAP (cat.), CH_2Cl_2, reflux, irradiate, 1 h, (39–63%); iv, Pr^i_2NEt, MeCN, 55 °C, 2 d, (51%); v, $BF_3.Et_2O$, EtSH, CH_2Cl_2, room temp., 24 h, (33–69%)

Compound **2** was dissolved in 10% D_2O in H_2O (500 mm^3)§, and acetone (2 mm^3) was added as the NMR internal reference (δ 2.214). Spectra were recorded on a Bruker AMX 500 spectrometer at 298 K. The pK_a of the tertiary amino group was determined from a plot of the chemical shift of the *N*-methyl group *vs* pH. The data were fitted using ULTRAFIT[13] to give a value of pK_a = 7.10 ± 0.07. As a result, AzaAdoMet may be employed in either the unprotonated, uncharged form or in its cationic, protonated form by controlling the pH.

The MetJ repressor protein, the product of the *metJ* gene, is a stable dimer of two identical subunits of molecular mass 11,996 Da. It is responsible for the repression of its own gene and of those coding for enzymes involved in methionine and AdoMet synthesis.[14] The protein dimer binds two molecules of the co-repressor AdoMet and this elevates over 100-fold the affinity of the protein for the operator DNA sequence.[15] AzaAdoMet is isosteric with AdoMet[16] and shows a pH-dependent affinity for the repressor protein in the presence of DNA. At pH 7.4, where the unprotonated form dominates, binding to the MetJ protein was found to be very weak. However, at pH 5.5, when AzaAdoMet exists predominantly as the protonated form, binding to the protein is 10 times stronger than for AdoMet itself at the same pH.[16] This result supports the hypothesis that the net positive charge on the co-repressor is an essential part of the mechanism of action of the complex.[15a]

Crystals of the binary complex between the *E. coli* MetJ repressor protein and AzaAdoMet were grown from a solution of PEG 600 by vapour diffusion methods.[17] They were isomorphous with the crystals of the MetJ–AdoMet complex (space group $P2_1$; Z = 2; cell dimensions a = 35.91, b = 63.08, c = 44.20 Å, α = γ = 90.0, β = 102.7°).[18] Data to 2.0 Å resolution were recorded from crystals containing the co-repressor analogue AzaAdoMet and were processed with XDS,[19] then merged and scaled using programs from the CCP4 program suite[20]¶ A model of the MetJ–AzaAdoMet complex was built using the molecular graphics program FRODO[21] and refined in TNT[22] using all data on the resolution range 2.0–2.3 Å‖.

The structure of AzaAdoMet bound to the protein confirms the (*S*)- configuration of the amino acid. For both the natural

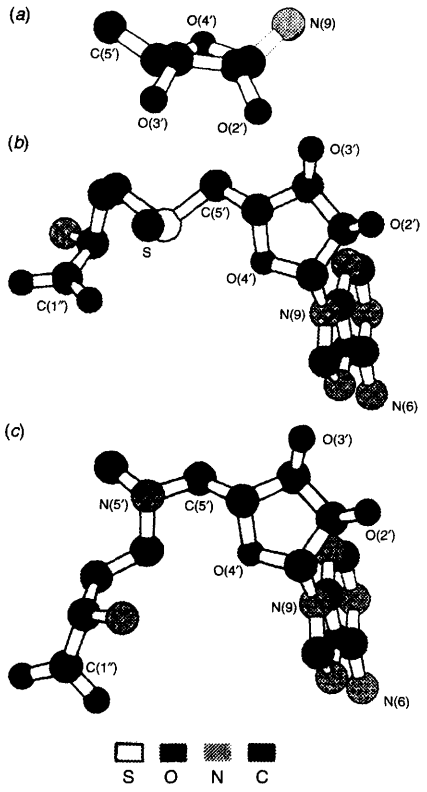


Fig. 1 (*a*) The X-ray crystal structure of the ribofuranose moiety of **2**, showing its O4′-*endo* character. (*b*) The X-ray structure of AdoMet **1** as bound in a crystalline complex with MetJ showing the *syn*-conformation of the glycosylic bond. (*c*) The X-ray structure of AzaAdoMet **2** as bound in a crystalline complex with MetJ with the same orientation for the adenine base as in (*b*) above.

co-repressor, AdoMet, and the isosteric analogue, AzaAdoMet, the purine ring lies in a hydrophobic pocket and is secured by two hydrogen bonds to the protein. The glycosylic bond of the nucleoside adopts the unusual *syn*-conformation with torsion angle χ +76° for AdoMet (+77° for AzaAdoMet). The pucker of the ribose ring is ${}^{0}T_{4}$, with largely O4′-*endo* character (Fig. 1). AdoMet and AzaAdoMet are almost superimposable in these respects.

The ammonium and carboxylate groups of the co-repressor lie at the surface of the protein where they are exposed to solvent. This part of the AdoMet is flexibly bound and the amino acid moieties in AdoMet and its analogue differ in conformation, making hydrogen bonds to different protein amino acid side-chains. This divergence between the structures begins with the exocyclic ribose torsion angle γ [C3′–C4′–C5′–S(N)], which is +17° in AdoMet and +62° in AzaAdoMet, and continues through to the amino acid carboxyl group. This work has established the value of AzaAdoMet as an isosteric, charge-switchable analogue of AdoMet. Its binding to other proteins, *e.g.* M.*Hha*I and M.*Msp*I, is under investigation.

Footnotes

† Benzene could be replaced by toluene in step 1 but with significant reduction in yield of **7**. The yield of pure **6** was variable because of its instability on silica; isolated yields ranged from 39 to 63%. Step 2 was also performed replacing dichloromethane by benzene as solvent, but lower yields were obtained.

‡ *Spectral data* for **2** (free base form): δ_H (D_2O, 400 MHz, *J*/Hz) 8.10 (s, 1 H), 7.98 (s, 1 H), 5.88 (d, 1 H, *J* 5), 4.61 (t, 1 H, *J* 5), 4.25–4.19 (m, 1 H), 4.11 (t, 1 H, *J* 5), 3.60 (dd, 1 H, *J* 5, 7), 2.88–2.76 (m, 2 H), 2.73–2.61 (m, 2 H), 2.27 (s, 3 H), 2.01–1.91 (m, 1 H) and 1.88–1.78 (m, 1 H). δ_C (D_2O, 100 MHz) 174.0, 154.7, 152.0, 147.9, 139.4, 118.1, 87.5, 79.9, 72.6, 71.3, 58.5, 53.8, 53.5, 40.2 and 25.7.

§ The pK_a was corrected to compensate for the D_2O–H_2O mixture.[23]

¶ X-Ray diffraction data collection and refinement statistics for MetJ–AzaAdoMet: d_{min} = 2.3 Å, 7497 independant reflections, multiplicity = 2.1, completeness = 83%, R_{symm} = 5.3% [$R_{symm} = \Sigma_h|I(h) - < I(h) >|/\Sigma_h|I(h)|$].

‖ Summary of structure refinement of MetJ–AzaAdoMet: 1690 protein atoms, 54 water atoms, 52 AdoMet atoms, resolution range = 20–2.3 Å, 7497 reflections, R factor = 16.7% [*R*-factor = $\Sigma_h|F_o(h) - F_c(h)|/\Sigma_h F_o(h)$], Rmsd bonds = 0.019 Å, Rmsd angles = 2.63°.

References

1 J. H. Miyazaki and S. F. Yang, *Physiol. Plantarum*, 1987, **69**, 366; F. Schlenk and D. J. Ehninger, *Arch. Biochem. Biophys.*, 1964, **106**, 95.
2 (*a*) G. L. Kenyon and A. A. Minnick, *J. Org. Chem.*, 1988, **53**, 4952; (*b*) M. Davis, N. P. B. Dudman and H. F. White, *Aust. J. Chem.*, 1983, **36**, 1623.
3 D. V. Santi and L. W. Hardy, *Biochemistry*, 1987, **26**, 8599.
4 N. O. Reich and N. Mashhoon, *J. Biol. Chem.*, 1990, **265**, 8966.
5 J. Tomasz, in *Synthetic Procedures in Nucleic Acid Chemistry*, ed. R. S. Tipson and W. W. Zorbach, 1973, vol. 2, pp. 765–769.
6 W. Jahn, *Chem. Ber.*, 1965, **98**, 1705.
7 A. Murayama, B. Jastorff, F. Cramer and H. Hettler, *J. Org. Chem.*, 1971, **36**, 3029.
8 M. Itoh, *Chem. Pharm. Bull.*, 1969, **17**, 1679.
9 D. H. R. Barton, D. Crich and W. B. Motherwell, *Tetrahedron Lett.*, 1983, **24**, 4979.
10 P. E. Eaton and J. Tsanaktsidis, *Tetrahedron Lett.*, 1989, **30**, 6967.
11 A. E. Jackson and R. A. W. Johnstone, *Synthesis*, 1976, 685; A. M. Felix, E. P. Heimer, T. J. Lambros, C. Tzougraki and J. Meienhofer, *J. Org. Chem.*, 1978, **43**, 4194; B. ElAmin, G. M. Anantharamaiah, G. P. Royer and G. E. Means, *J. Org. Chem.*, 1979, **44**, 3442; G. M. Anantharamaiah and K. M. Sivanandaiah, *J. Chem. Soc. Perkin Trans. 1*, 1977, 490.
12 D. S. Bose and D. E. Thurston, *Tetrahedron Lett.*, 1990, **31**, 6903.
13 ULTRAFIT ver 2.11, © 1990–1993 Biosoft, Cambridge, UK CB2 1LR.
14 I. Saint-Girons, C. Parsot, M. M. Zakin, O. Barzu and G. N. Cohen, *CRC Crit. Rev. Biochem.*, 1988, **23**; J. B. Rafferty, W. S. Somers, I. Saint-Girons and S. E. V. Phillips, *Nature* (*London*), 1989, **341**, 705.
15 (*a*) K. Phillips and S. E. V. Phillips, *Structure*, 1994, **2**, 309; (*b*) S. E. V. Phillips, I. Manfield, I. Parsons, B. E. Davidson, J. B. Rafferty, W. Somers, D. S. Margarita, G. N. Cohen, I. Saint-Girons and P. G. Stockley, *Nature* (*London*) 1989, **341**, 711.
16 P. G. Stockley, I. D. Parsons, B. Persson, A. Mekhalfia and G. M. Blackburn, *Nucleic Acids Res.*, 1995, **23**, 211.
17 D. R. Davies and D. M. Segal, *Methods Enzymol.*, 1971, **22**, 266.
18 K. Phillips and S. E. V. Phillips, unpublished results. The coordinates of the MetJ-AzaAdoMet complex will shortly be deposited in the Brookhaven PDB and a full publication describing the structure of this complex and its comparison with those of MetJ– AdoMet and MetJ–sinefungin is in preparation.
19 W. Kabsch, *J. Appl. Cryst.*, 1988, **21**, 67.
20 Collaborative Computing Project Number 4, *Acta Crystallogr.*, 1994, **D50**, 760.
21 T. A. Jones, *J. Appl. Cryst.*, 1978, **11**, 268.
22 D. E. Tronrud and L. F. T. Eyck, *Acta Crystallogr.*, 1994, **D50**, 760.
23 P. K. Glasoe and F. A. Long, *J. Phys. Chem.*, 1960, **64**, 188.

Received, 28th December 1995; Com. 5/08407E