very facile. Estimated electron-transfer distances (R_p's)[2] are about the same for the two proteins (3.3 Å for plastocyanin; 3.8 Å for azurin), suggesting that the hydrophobic bpy ligands of Ru^{2+*} can penetrate the three residues (Met-13, Met-44, Phe-114)[13] that isolate the Cu-(His-117) unit from water molecules as well as hydrophilic redox agents.[1d]

The PCu^I/Ru^{2+*} system represents an unambiguous case of electron-transfer quenching of an electronically excited metal complex by a metalloprotein.[14] This type of system offers great advantages in studying the kinetics of very rapid electron-transfer reactions, owing to the relative ease of in situ preparation of the required oxidants and reductants. In future work we will attempt to attach certain photoredox agents directly to selected binding sites on PCu^I surfaces, with the goal of investigating the kinetics of intramolecular electron transfer between copper and relatively remote (>5-Å site-to-site distances) acceptors and donors.

Acknowledgment. We thank Drs. R. A. Marcus and N. Sutin for helpful discussions. Postdoctoral fellowships from NIH (1978-1980; V.R.L.) and NSERC (1980-1982; A.M.E.) are acknowledged. Support for this research was provided by NIH Grant AM19038 (aided by BRSG RR07003).

Registry No. $Ru(bpy)_3^{2+}$, 15158-62-0; Cu, 7440-50-8.

(14) Quenching of Ru^{2+*} by ferricytochrome *c* has been reported previously (Sutin, N. *Adv. Chem. Ser.* **1977**, *162*, 156). However, in this case a significant contribution to the quenching rate may come from electronic energy transfer (McLendon, G.; Lum, V. R.; English, A. M.; Gray, H. B., unpublished results).

Studies of Enzyme Stereochemistry. Elucidation of the Stereochemistry of *S*-Adenosylmethionine Formation by Yeast Methionine Adenosyltransferase

Ronald J. Parry* and Akwasi Minta

Department of Chemistry, Rice University
Houston, Texas 77001

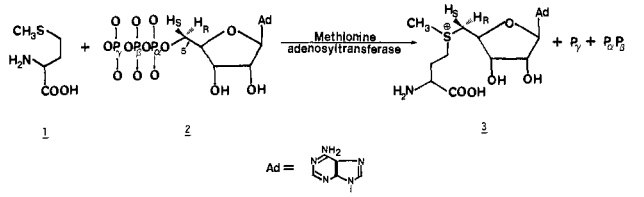
Received October 13, 1981

S-Adenosylmethionine (SAM, **3**), the principal agent for biological transmethylations, is biosynthesized from methionine (**1**) and adenosine triphosphate (ATP, **2**) via a reaction catalyzed by the enzyme methionine adenosyltransferase (Scheme I). This enzyme has been isolated in purified form from a number of sources, and it has been subjected to considerable mechanistic scrutiny.[1] These studies allow one to formulate two plausible reaction paths for the generation of SAM from ATP and methionine (Scheme II). The first pathway (Scheme IIA), which is consistent with all the available evidence,[1] assumes that ATP and methionine bind to the enzyme in such a way that a single displacement takes places at C-5′ of ATP to give SAM and enzyme-bound tripolyphosphate. This reaction path would presumably lead to overall inversion of configuration at C-5′ of the resulting SAM. The second pathway (Scheme IIB) would proceed by displacement of tripolyphosphate from C-5′ of ATP by a nucleophilic group present at the active site of the enzyme. A second displacement at C-5′ would then result in the transfer of the adenosyl group to the sulfur atom of methionine. Since two displacements occur in the second pathway, overall retention of configuration could be expected at C-5′ of SAM. The double displacement mechanism for SAM formation appears to be a less likely possibility than the single displacement mechanism due to the failure of attempts to detect an adenosyl-enzyme intermediate.[1] We would now like to report the results of stereochemical studies that also support the single displacement mechanism.

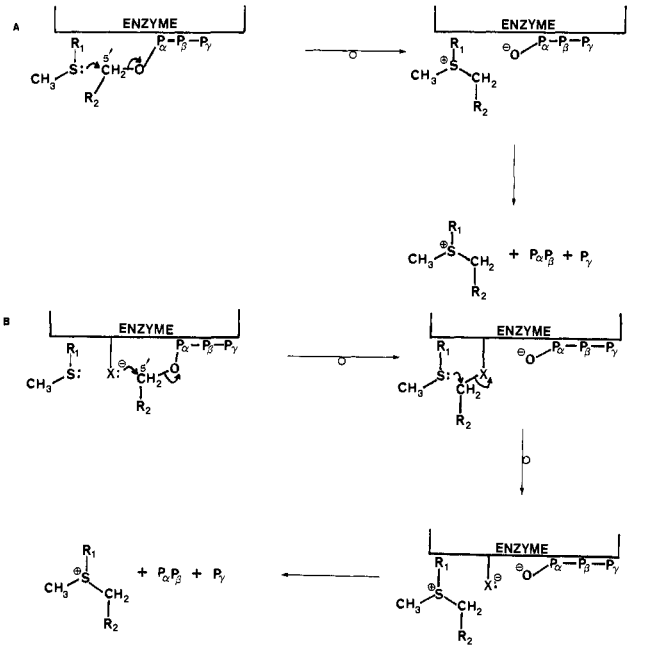
The stereochemistry of *S*-adenosylmethionine formation has been elucidated with the aid of adenosine derivatives that are

(1) Mudd, S. H. *Enzymes, 3rd Ed.* **1973**, *8*, 121-154.

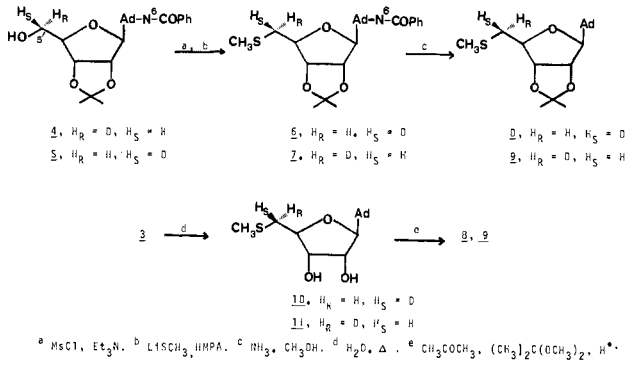
Scheme I



Scheme II



Scheme III



chirally deuterated at C-5′. The initial stages of the investigation were concerned with the synthesis of [5′(*S*)- and [5′(*R*)-$^2H_1$]-2′,3′-*O*-isopropylidene-5′-deoxy-5′-(methylthio)adenosine (**8**, **9**, Scheme III) as NMR reference samples. These reference compounds were synthesized from [5′(*R*)- and [5′(*S*)-$^2H_1$]-N^6-benzoyl-2′,3′-*O*-isopropylideneadenosine (**4**, **5**).[2] The N^6-benzoyl derivatives were converted to the corresponding mesylates and then treated with lithium methylthiolate[3] in HMPA to give [5′(*S*)- and [5′(*R*)-$^2H_1$]-N^6-benzoyl-2′,3′-*O*-isopropylidene-5′-deoxy-5′-(methylthio)adenosine (**6**, **7**) by a displacement reaction that is presumed to occur with inversion of configuration.[4] Removal

(2) (a) Parry, R. J. *J. Chem. Soc., Chem. Commun.* **1978**, 294. (b) Midland, M. M.; Tramontano, A.; Zderic, S. A. *J. Am. Chem. Soc.* **1977**, *99*, 5211. (c) Dupre, M.; Gaudemer, A. *Tetrahedron Lett.* **1978**, 2783.

(3) Kelly, T. R.; Dali, B. B.; Tsang, W. G. *Tetrahedron Lett.* **1977**, 3859.

(4) The use of the N^6-benzoyl derivatives avoids the possibility[5] that the sequence might proceed via the formation of a N^3,5′-cyclonucleoside followed by attack of the methylthiolate anion. If the latter reaction course were to obtain, then the stereochemical result would be retention of configuration at C-5′.

0002-7863/82/1504-0871$01.25/0

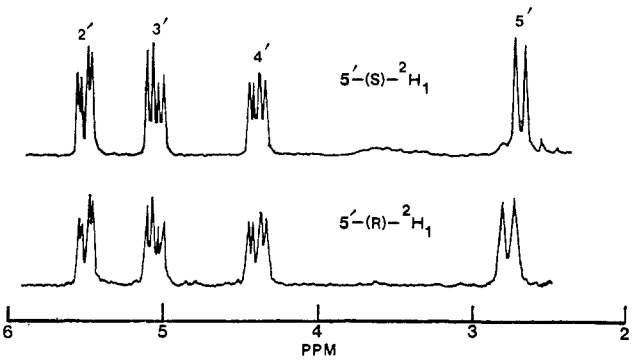


Figure 1. 90-MHz ^1H NMR spectra ($CDCl_3$) of [5'-$^2H_1$]-2',3'-*O*-isopropylidene-5'-deoxy-5'-(methylthio)adenosine.

of the benzoyl protecting groups from **6** and **7** then produced the desired reference compounds **8** and **9**. The 90-MHz proton NMR spectra of these two compounds are shown in Figure 1. It is clear from these spectra that the two diastereomerically deuterated compounds can be readily differentiated.

The synthesis of the reference compounds having been accomplished, attention was turned to the synthesis of [5'(*R*)- and [5'(*S*)-$^2H_1$]ATP. The chirally deuterated N^6-benzoyl derivatives **4** and **5** were deprotected[2] to yield [5'(*R*)- and [5'(*S*)-$^2H_1$]-2',3'-*O*-isopropylideneadenosine, and these two chirally labeled nucleosides were then converted to [5'(*R*)- and [5'(*S*)-$^2H_1$]-adenosine monophosphate (AMP) via their cyanoethylphosphate derivatives.[6] The configuration at C-5' of the adenosine nucleus is unaltered by this reaction sequence since the formation of the intermediate cyanoethylphosphate esters proceeds by attack of the alcoholic hydroxy group of the nucleoside upon an activated phosphate ester generated from cyanoethylphosphate anion and DCC.[7] The two forms of chirally deuterated AMP so obtained were transformed into [5'(*R*)- and [5'(*S*)-$^2H_1$]ATP by reaction of their morpholidate derivatives with tri-*n*-butylammonium pyrophosphate.[6]

The stereochemistry of SAM formation was elucidated by incubating the two chirally deuterated forms of ATP with a partially purified preparation of methionine adenosyltransferase obtained from dried yeast by the method of Chiang and Cantoni.[8] The two samples of chirally deuterated SAM produced by the enzyme were each isolated as their phosphotungstate derivatives.[9] After removal of the phosphotungstate ion,[9] the partially purified samples of SAM were hydrolyzed with boiling water[10] to yield [5'(*S*)- and [5'(*R*)-$^2H_1$]-5'-deoxy-5'-(methylthio)adenosine (**10**, **11**) (Scheme III). Final purification of **10** and **11** was accomplished by conversion[11] to [5'-(*S*)- and [5'(*R*)-$^2H_1$]-2',3'-*O*-isopropylidene-5'-deoxy-5'-(methylthio)adenosine (**8**, **9**) followed by chromatography.

The chirality of the labels in the two enzymatically derived compounds **8** and **9** was determined by comparison of their proton NMR spectra with the NMR spectra of the synthetically prepared reference substances. The results of this comparison were as follows: the SAM derived from [5'(*S*)-$^2H_1$]ATP yielded [5'-(*R*)-$^2H_1$]-2',3'-*O*-isopropylidene-5'-deoxy-5'-(methylthio)adenosine (**9**) while the SAM produced from [5'(*R*)-$^2H_1$]ATP yielded the 5'-(*S*) compound **8**. Therefore, one can conclude that the formation of *S*-adenosylmethionine by yeast methionine adenosyltransferase takes place with *inversion* of configuration at C-5' of ATP. These results support a single displacement mechanism (Scheme IIA) for SAM formation.

Acknowledgment. We would like to thank Professor Frederick B. Rudolph for helpful advice concerning enzyme purification and the National Institutes of Health (GM-26166) and the Robert A. Welch Foundation (C-729) for financial support.

Registry No. 1, 63-68-3; (*R*)-2, 80375-30-0; (*S*)-2, 80408-88-4; 8, 80375-31-1; 9, 80408-89-5; 10, 80375-32-2; 11, 80408-90-8; methionine adenosyltransferase, 9012-52-6.

A Novel and Efficient Entry to (±)-Quadrone†

Steven D. Burke,* Charles William Murtiashaw, Jeffrey O. Saunders, and Meera S. Dike

Department of Chemistry, University of South Carolina Columbia, South Carolina 29208

Received October 23, 1981

Quadrone (**1**), a fungal metabolite from *Aspergillus terreus* reported in 1978, was found to exhibit inhibitory activity in vitro against human epidermoid carcinoma of the nasopharynx (KB) with an ED_{50} of 1.3 μg/mL and in vivo against P338 lymphocytic leukemia in mice.[1] The reported biological activity and the deceptively challenging structural features of this novel sesquiterpene have combined to trigger intensive efforts directed at total synthesis. Two independent entries to quadrone sharing a general strategic theme have recently been reported by Danishefsky[2] and Helquist.[3] We describe herein our efforts directed along an entirely different tactical protocol, culminating in a direct and efficient construction of the quadrone nucleus and a formal total synthesis.

In evaluating quadrone as a target for total synthesis, attention is quickly drawn to the five contiguous asymmetric centers decorating the quadricyclic framework. At a more subtle level lies the recognition that the centers of asymmetry are shared over the structure such that the four rings have four, three, three, and three chiral centers, respectively. We focused on the quaternary carbon [C(1) in **1**] as the only center of asymmetry common to each of the four rings of the natural product. It was felt that this quaternary center should be established at the outset in a synthetic precursor to quadrone, with surrounding functionality ripe for elaboration. The spiro[4.5]decadienone **2**, readily available in 64% overall yield from 2-methyldimedone isobutyl ether (**3**),[4] fulfilled these requirements as a masked quadrone synthon.

The critical transmutation of the spiro[4.5]decadienone **2** into the tricyclic enedione **5** afforded an interesting study of site-selective reactivity in a polyfunctional molecule (Scheme I). Oxidative cleavage of the trisubstituted olefin linkage in **2** [OsO_4(catalytic), *N*-methylmorpholine *N*-oxide,[5] 1:1 H_2O/acetone; $NaIO_4$ (8 equiv), 1:1 H_2O/THF] proceeded cleanly to give the doubly appended cyclopentenone **4** in 94% yield.[6] Note that in **4a** there are highlighted three potential nucleophilic sites

† This paper is dedicated to Professor Gilbert Stork on the occasion of his 60th birthday.

(1) (a) Ranieri, R. L.; Calton, G. J. *Tetrahedron Lett.* **1978**, 499-502. (b) Calton, G. J.; Ranieri, R. L.; Espenshade, M. A. *J. Antibiot.* **1978**, *31*, 38-42.
(2) (a) Danishefsky, S.; Vaughan, K.; Gadwood, R. C.; Tsuzuki, K. *J. Am. Chem. Soc.* **1980**, *102*, 4262-4263. (b) Danishefsky, S.; Vaughan, K.; Gadwood, R.; Tsuzuki, K. *Ibid.* **1981**, *103*, 4136-4141. (c) Danishefsky, S.; Vaughan, K.; Gadwood, R. C.; Tsuzuki, K. *Tetrahedron Lett.* **1980**, 2625-2628.
(3) Bornack, W. K.; Bhagwat, S. S.; Ponton, J.; Helquist, P. *J. Am. Chem. Soc.* **1981**, *102*, 4646-4648.
(4) For an account of this vinylsilane-mediated spiroannulation, see: Burke, S. D.; Murtiashaw, C. W.; Dike, M. S.; Strickland, S. M. S.; Saunders, J. O. *J. Org. Chem.* **1981**, *46*, 2400-2402.
(5) Van Rheenen, V.; Kelly, R. C.; Cha, D. Y. *Tetrahedron Lett.* **1976**, 1973-1976.
(6) The doubly appended cyclopentenone **4** is presented in three different conformational perspectives (**4a-c**) to focus on each of three possible electrophile-nucleophile pairings.
(5) Jenkins, I. D.; Verheyden, J. P. H.; Moffatt, J. G. *J. Am. Chem. Soc.* **1976**, *98*, 3346. Clark, V. M.; Todd, A. R.; Zussman, J. *J. Chem. Soc.* **1951**, 2952. Jahn, W. *Chem. Ber.* **1965**, *98*, 1705.
(6) Moffatt, J. G. *Methods Enzymol.* **1967**, *12*, 182.
(7) Smith, M.; Moffatt, J. G.; Khorana, H. G. *J. Am. Chem. Soc.* **1958**, *80*, 6204. Gilham, P.; Khorana, H. G. *Ibid.* **1958**, *80*, 6212.
(8) Chiang, P. K.; Cantoni, G. L. *J. Biol. Chem.* **1977**, *252*, 4506.
(9) Schlenk, F.; DePalma, R. E. *J. Biol. Chem.* **1957**, *229*, 1051.
(10) Parks, L. W.; Schlenk, F. *J. Biol. Chem.* **1958**, *230*, 295.
(11) Hampton, A. *J. Am. Chem. Soc.* **1961**, *83*, 3640.

0002-7863/82/1504-0872$01.25/0