Stereochemistry of Spermidine Synthase

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The enzyme spermidine synthase[1] catalyzes the formation of the polyamine spermidine (*N*-(3-aminopropyl)-1,4-diaminobutane, **1**] from 1,4-diaminobutane (putrescine) and decarboxylated adenosylmethionine (**2**) (cf. eq 1, Ad = adenosyl).[2] Spermidine

$$H_2NCH_2CH_2CH_2CH_2NH_2 + MeS^+(Ad)CH_2CH_2CH_2NH_2$$
$$\mathbf{2}$$
$$\rightarrow H_2N\overset{4}{C}H_2\overset{3}{C}H_2\overset{2}{C}H_2\overset{1}{C}H_2NH\overset{1'}{C}H_2\overset{2'}{C}H_2\overset{3'}{C}H_2NH_2 + MeSAd + H^+ \quad (1)$$
$$\mathbf{1}$$

and certain other polyamines bind selectively to nucleic acids and modify their properties.[3] The biosynthesis of polyamines is therefore of fundamental importance to the cell. The primary function of spermidine synthase is to assist the transfer of the aminopropyl group of **2** to one of the nitrogen atoms of 1,4-diaminobutane. We will provide evidence that the cryptic stereochemistry[4] of this reaction, at the reacting methylene group of **2**, is inversion of configuration. Hence, the reaction of eq 1 is probably an enzyme-mediated S_N2 substitution[5] (cf. Scheme I).

In principle, the stereochemistry of spermidine synthase could be elucidated by having this enzyme convert [4-^{2}H(or ^{3}H)]-methionine of known configuration at C-4 into [1′-^{2}H(or ^{3}H)]-*N*-(3-aminopropyl)-1,4-diaminobutane, the absolute stereochemistry of which could be determined somehow. However, our approach has been to use *Escherichia coli* to convert methionine, labeled with deuterium at both C-3 and C-4 and of known relative configuration, into spermidine labeled at C-1 and C-2 of its aminopropyl group.

Studies of the reaction of spermidine with ≥2 mol equiv of ethanal in deuteriochloroform at room temperature showed that rapid formation of the hexahydropyrimidine (**3**) was followed by reaction of the remaining primary amino function to give the imine hexahydropyrimidine (**4**) (cf. Scheme IIa).[6] Compound **4** exhibits a 400-MHz ^{1}H NMR spectrum, which by first-order analysis yields chemical shifts and coupling constants for all protons in

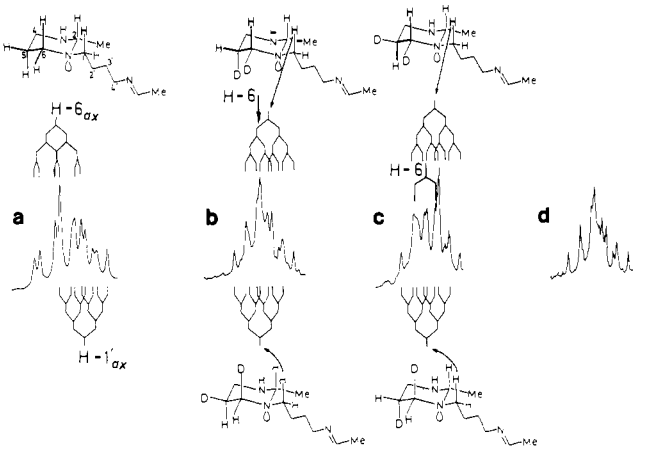


Figure 1. Portions (ca. δ 2.3) of the 400-MHz NMR spectra (scale: 1 mm = 4.5 Hz) of (a) unlabeled imine hexahydropyrimidine (**4**); (b) dideuterated imine hexahydropyrimidine (cf. Scheme IIb) from (2*R*,3*R*,4*S*)/(2*R*,3*S*,4*R*)/(2*S*,3*R*,4*S*)/(2*S*,3*S*,4*R*)-[3,4-$^2H_2$]methionine; (c) dideuterated imine hexahydropyrimidine (cf. Scheme IIc) from (2*R*,3*R*,4*R*)/(2*R*,3*S*,4*S*)/(2*S*,3*R*,4*R*)/(2*S*,3*S*,4*S*)-[3,4-$^2H_2$]methionine; (d) synthetic dideuterated imine hexahydropyrimidine (cf. Scheme III).

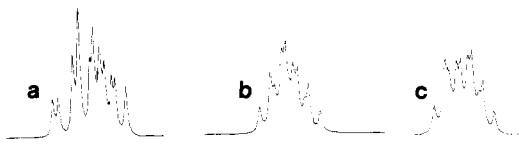
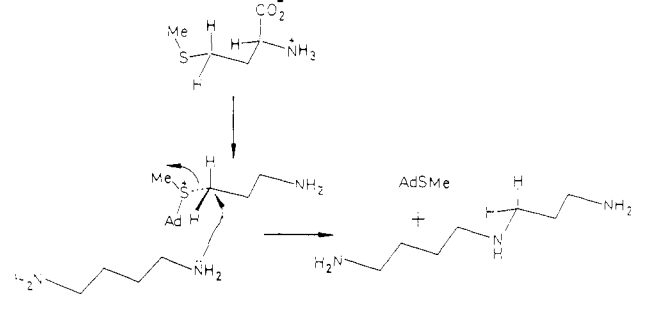


Figure 2. Spectra simulated with a SIMEQ-II program (Varian). (a) This spectrum was obtained by using the following parameters for H-6_{ax} and H-$1'_{ax}$ in compound **4**: H-6_{ax}, δ 495.0 Hz (arbitrary setting), *J* values 3.28, 11.75, and 11.75 Hz; H-$1'_{ax}$, δ 480.0 Hz, *J* values 6.0, 8.19, and 12.0 Hz (line width 2.0 Hz). (b) This spectrum was obtained by summation of two octets for H-$1'_{ax}$ at δ 480.0 and 486.0 Hz (*J* values as in a, line width 2.0 Hz) with a component for H-6_{ax} obtained by using δ 485.0 Hz and J_{vic} = 3.28 Hz; the effect of deuterium was approximated (the program used could not cope with spin^1 nuclei) by superimposing a line width of 8 Hz on the doublet for H-6_{ax}, which converted it to a broad singlet. (c) This spectrum was obtained in a manner similar to b by using identical parameters for the octets and δ 485.0 Hz, J_{vic} = 11.75 Hz, and line width 5 Hz for H-6_{ax}.

Scheme I. S_N2 Mechanism for the Reaction between 1,4-Diaminobutane and Decarboxylated Adenosylmethionine Catalyzed by Spermidine Synthase



4, with the exception of H-5_{eq}, 2 × H-2′ and 2 × H-3′. These data and analyses of spectra for model compounds[6] show that the preferred conformation of compound **4** is a chair with equatorial substituents (i.e. as shown). That the N-substituent extends away from the chair is indicated by the identity of chemical shifts and coupling constants for all ring protons and H-$1'_{ax}$ and H-$1'_{eq}$ in **3** and **4**. The proton H-6_{ax} shows (cf. Figure 1a) a sextet at δ 2.33 (J_{gem} = 12 Hz, J_{vic} = 3, 12 Hz) overlapping an octet for H-$1'_{ax}$ at δ 2.28. A computer-assisted simulation of the resulting pattern is shown in Figure 2a. Hence, the relative configuration of deuterium atoms in a specimen of [1′,2′-$^2H_2$]spermidine can be determined after its reaction with ethanal by analyzing the resonance for H-6_{ax}. This will show a vicinal H–H coupling constant of either 3 or 12 Hz, depending on the relative configuration of

(1) aminopropyl transferase, E.C. 2.5.1.16; isolation from *Escherichia coli*: Bowman, W. H.; Tabor, C. W.; Tabor, H. *J. Biol. Chem.* **1972**, *248*, 2480.

(2) Labeling studies: (a) Tabor, H.; Rosenthal, S. M.; Tabor, C. W. *J. Biol. Chem.* **1958**, *233*, 907. (b) Greene, R. C. *J. Am. Chem. Soc.* **1957**, *79*, 3929. (c) Billington, D. C.; Golding, B. T.; Nassereddin, I. K. *J. Chem. Soc., Chem. Commun.* **1980**, 90.

(3) Tabor, C. W.; Tabor, H. *Annu. Rev. Biochem.* **1976**, *45*, 285. Cohen, S. S. *Nature (London)* **1978**, *274*, 210 and references cited therein.

(4) Terminology: Aberhart, D. J.; Lin, H.-J.; Weiller, B. H. *J. Am. Chem. Soc.* **1981**, *103*, 6750.

(5) For mechanistic discussions concerning spermidine synthase see: (a) Zappia, V.; Cacciapuoti, G.; Pontoni, G.; Oliva, A. *J. Biol. Chem.* **1980**, *255*, 7276. (b) Tang, K. C.; Mariuzza, A.; Coward, J. K. *J. Med. Chem.* **1981**, *24*, 1277 and ref 2c.

(6) Golding, B. T.; Nassereddin, I. K., unpublished observations. Compounds **3** and **4** were prepared by dropwise addition of 1 and 2 mol equiv, respectively, of redistilled acetaldehyde to spermidine in HCl-free chloroform; monitoring by ^{1}H NMR spectroscopy showed that each derivative was formed within 5 min at room temperature; compounds **3** and **4** were principally characterized by their ^{1}H NMR spectra (cf. ref 7) and mass spectra [the electron impact spectrum of **3** showed $(M-15)^+$ at *m/z* 156.1494 (35%, calcd for $C_8H_{18}N_3$ 156.1497); its chemical ionization spectrum showed MH^+ at 172 (most intense ion of $m > 40$); for **4** the electron impact spectrum showed $(M-15)^+$ at *m/z* 182.1657 (10% of *m/z* 84, calcd for $C_{10}H_{20}N_3$ 182.1657); the chemical ionization spectrum of **4** showed MH^+ at 198 (63% of base peak at 98)].

(7) **4**: 400-MHz ^{1}H NMR ($CDCl_3$) δ 1.22 (d, *J* = 6 Hz, C-2 Me), 1.35–1.65 (complex multiplets, H-5_{eq}, 2 × H-2′, 2 × H-3′), 1.67 (q of t, J_{gem} = 12 Hz, J_{vic} = 4, 4, 12, 12 Hz, H-5_{ax}), 1.95 (d, *J* = 4.5 Hz, *Me*CH=N), 2.28 (octet, J_{gem} = 13 Hz, J_{vic} = 6, 8.5 Hz, H-$1'_{ax}$), 2.33 (sextet, J_{gem} = 12 Hz, J_{vic} = 3, 12 Hz, H-6_{ax}), 2.63 (m, J_{gem} = 13 Hz, J_{vic} = 8, 6.5 Hz, H-$1'_{eq}$), 2.66 (sextet, J_{gem} = ca. 13 Hz, J_{vic} = 3, ca 12 Hz, H-4_{ax}), 3.04 (d with further splitting, H-4_{eq} and H-6_{eq}), 3.20 (q, *J* = 5.8 Hz, H-2), 3.36 (t, *J* = 6.9 Hz, NCH_2), 7.60 (q, *J* = 4.5 Hz, MeC*H*=N). These assignments are supported by decoupling experiments and computer-assisted simulations of the resonances at δ 2.28, 2.33 (cf. Figure 2a), 2.63, 2.66.

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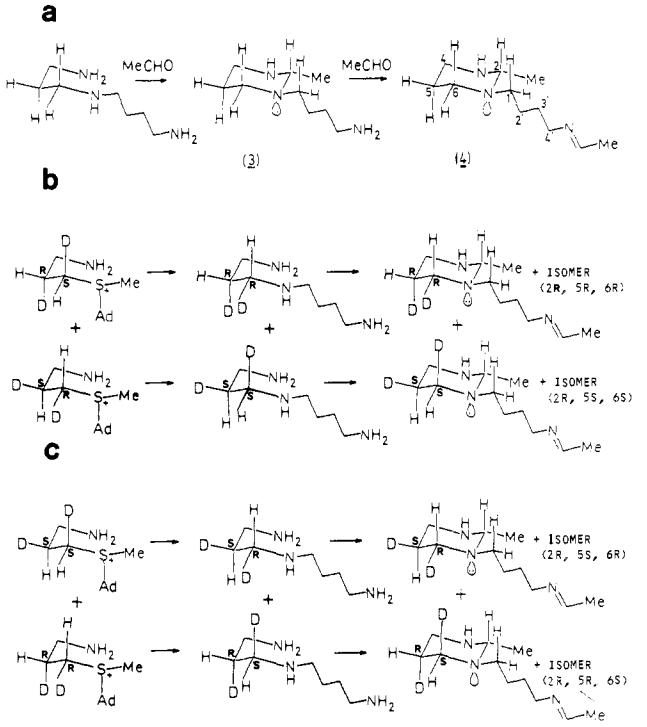
Scheme II. (a) Reaction between Spermidine and Ethanal Leading to Hexahydropyrimidine (3) and Imine Hexahydropyrimidine (4), (b) Dideuterated Imine Hexahydropyrimidine Derived from (2*R*,3*R*,4*S*)/(2*R*,3*S*,4*R*)/(2*S*,3*R*,4*S*)/(2*S*,3*S*,4*R*)-[3,4-$^2H_2$]Methionine, and (c) Dideuterated Imine Hexahydropyrimidine Derived from (2*R*,3*R*,4*R*)/(2*R*,3*S*,4*S*)/(2*S*,3*R*,4*R*)/(2*S*,3*S*,4*S*)-[3,4-$^2H_2$]Methionine

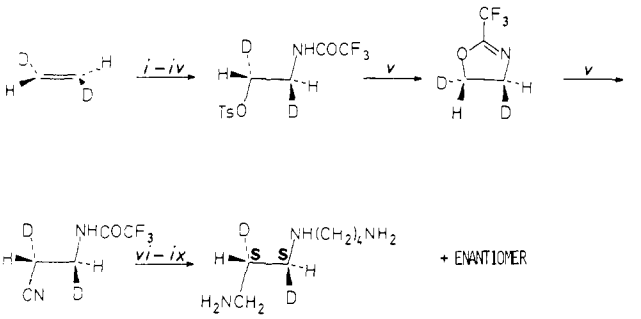


deuteriums at H-5 and H-6 (axial–equatorial or equatorial–equatorial, respectively).

(*Z*)-[1,2-$^2H_2$]ethene was reacted with methanesulfenyl chloride (in CH_2Cl_2, −35 °C) to give a 1:1 mixture of (*R*,*R*)- and (*S*,*S*)-[1,2-$^2H_2$]-1-chloro-2-(methylthio)ethane.[8,9] This mixture was reacted with sodium acetamidomalonate in ethanol[10] (5 h at reflux), and the product was hydrolyzed (hot aqueous HCl[11]) to equal amounts of four of the stereoisomers of [3,4-$^2H_2$]methionine [(2*R*,3*R*,4*S*), (2*R*,3*S*,4*R*), (2*S*,3*R*,4*S*), and (2*S*,3*S*,4*R*)].[10-12] By a similar series of reactions, the other four stereoisomers of [3,4-$^2H_2$]methionine were prepared from (*E*)-[1,2-$^2H_2$]ethene.[8,12] L-[2,3,3-$^2H_3$]methionine[13] was obtained from methionine by pyridoxal/Al(III)-catalyzed exchange[14] in D_2O followed by resolution.[15] With use of the methionine-requiring auxotroph of *Escherichia coli* K_{12}, 630 Hfr_1[16] cells were grown in media supplemented with each of the above samples of labeled methionines (0.05 g dm^{-3} L isomer).[17] Deuterated spermidines were isolated from each medium by the methods described.[18] The 220-MHz ^1H and 61.4-MHz ^2H NMR spectra of deuterated spermidine from [2,3,3-$^2H_3$]methionine [examined as its tris(*N*-phenylamino)thiocarbonyl derivative],[18] showed retention of deuterium originally at C-3 of methionine but substantial (≥90%) loss of that at C-2.[19] The importance of this experiment is that it shows that the conversion of adenosylmethionine into decarboxylated adenosylmethionine catalyzed by adenosylmethionine decarboxylase does not perturb hydrogens (deuteriums) originally at C-3 of methionine. Deuterated spermidine from the mixture of [3,4-$^2H_2$]methionines containing the (2*R*,3*R*,4*S*), (2*R*,3*S*,4*R*), (2*S*,3*R*,4*S*), and (2*S*,3*S*,4*R*) isomers was reacted with ethanal in deuteriochloroform. This reaction was monitored by 400-MHz ^1H NMR spectroscopy, which showed sequential formation of a hexahydropyrimidine and *N*-methylimine. If the reaction of eq 1 proceeds with inversion of configuration, the imine hexahydropyrimidine will be a mixture of (2*S*,5*R*,6*R*)- and (2*S*,5*S*,6*S*)-[5,6-$^2H_2$] isomers and their enantiomers (ca. 25% of each isomer)[20] (cf. Scheme IIb). As expected for this stereochemical course, the resonance for H-6_{ax} from the (2*S*,5*R*,6*R*) isomer and its enantiomer was observed as a broad singlet[21] (δ 2.30) superimposed on resonances for H-$1'_{ax}$ (cf. Figure 1b). A computer-assisted simulation of these resonances is shown in Figure 2b.

Similar treatment (cf. Scheme IIc) of deuterated spermidine from the mixture of [3,4-$^2H_2$]methionines containing the

Scheme III. Synthesis of (1′*R*,2′*R*)/(1′*S*,2′*S*)-Spermidine from (*E*)-[$^2H_2$]Ethene[a]



[a] (i) 2 M HOCl/overnight shaking; (ii) excess of concentrated NH_3; (iii) CF_3CO_2Et in MeCN/reflux 20 min; (iv) TsCl in py/overnight at 0 °C; (v) NaCN in Me_2SO/1 week at room temperature; (vi) aqueous LiOH/10 min at room temperature; (vii) $PhCH_2OCONH(CH_2)_3CO_2H$ and DCC in CH_2Cl_2; (viii) Pd/H_2 (Parr); (ix) B_2H_6 in THF/reflux overnight.

(8) Full details: Billington, D. C.; Golding, B. T. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1283.

(9) This is presumed to be a stereospecific anti process. For precedents see: Smit, J. A.; Zefirov, M. S.; Bodrikov, I. V.; Krimer, M. Z. *Acc. Chem. Res.* **1979**, *12*, 282 and references cited therein.

(10) A reaction believed to proceed stereospecifically via an intermediate thiiranium ion and therefore with retention of relative configuration of deuteriums. This contention is supported by kinetic evidence in the literature, [e.g.: Böhme, H.; Sell, K. *Chem. Ber.* **1948**, *81*, 123] examination of the ^1H NMR spectra of derived [$^2H_2$]dehydromethionines (cf. ref 8) and by a competition experiment (equimolar amounts of $MeSCH_2CH_2Cl$ and BuCl + $NaCNHAc(CO_2Et)_2$ in ethanol gave $MeSCH_2CH_2CNHAc(CO_2Et)_2$ as the only detectable product[8]) [N.B. the ratio of rate constants for S_N2 reaction of $MeSCH_2CH_2Cl$ and BuCl with KI/acetone is 1.5:1: Kirner, W. R. *J. Am. Chem. Soc.* **1928**, *50*, 2446. See also ref 9.

(11) Goldsmith, D.; Tishler, M. *J. Am. Chem. Soc.* **1946**, *68*, 144.

(12) There is no detectable cross contamination between the sets of methionine isomers (^1H NMR analyses of the methionines and derived dehydromethionines[8]); their isotopic purity is ≥90% dideuterated species (IR analyses of the [$^2H_2$]ethenes and ^1H NMR analyses of the methionines and derived dehydromethionines).

(13) Containing ca. 14% $^2H_1$, 14% $^2H_2$, and 72% $^2H_3$ species (mass spectral analysis of butyl *N*-trifluoroacetate).

(14) (a) Tenebaum, B. W.; Witherup, T. H.; Abbott, E. H. *Biochim. Biophys. Acta* **1974**, *362*, 308. (b) Billington, D. C.; Golding, B. T.; Kebbell, M. J.; Nassereddin, I. K.; Lockhart, I. M. *J. Labelled Compds. Radiopharm.* **1981**, *18*, 1773.

(15) Method: Wheeler, G. P.; Ingersoll, A. W. *J. Am. Chem. Soc.* **1951**, *73*, 4604.

(16) Purchased from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland.

(17) Comparative experiments in which unlabeled L- (0.05 g dm^{-3}) and racemic (0.1 g dm^{-3}) methionine were added to media showed no appreciable difference in the yields of cells of *E. Coli* and isolated spermidine.

(18) Golding, B. T.; Nassereddin, I. K. *J. Chem. Res., Miniprints* **1981**, *3931*.

(19) The observation that deuterium is lost from C-2 reveals a new feature of the mechanism of adenosylmethionine decarboxylase. For recent studies of this enzyme cf.: Pankaskie, M.; Abdel-Monem, M. M. *J. Med. Chem.* **1980**, *23*, 121.

(20) The NMR spectra of each enantiomer of a pair will be identical, whereas the spectra from the pairs will differ; in the ^1H NMR spectrum of each dideuterated imine hexahydropyrimidine H-5_{ax}, H-5_{eq}, H-6_{ax}, and H-6_{eq} therefore correspond to 0.5 H each, with H-5_{ax} paired with either H-6_{ax} or H-6_{eq}, and H-5_{eq} paired with either H-6_{eq} or H-6_{ax}, depending on the relative configuration of deuteriums in each pair of enantiomers; pairings were confirmed by decoupling experiments.

(21) H-D couplings are ca. one-sixth of corresponding H-H couplings (Brevard, C.; Kintzinger, J. P. In "NMR and the Periodic Table"; Harris, R. K., Mann, B. E., Eds.; Academic Press: New York, 1978; p 110) and so the "theoretical" appearance of this signal is a doublet (J = 3 Hz) of 1:2:3:2:1 pentuplets ($J \sim 2$ Hz).

(2*R*,3*R*,4*R*), (2*R*,3*S*,4*S*), (2*S*,3*R*,4*R*), and (2*S*,3*S*,4*S*) isomers showed a doublet[22] for H-6_{ax} at δ 2.30 ($J \sim 12$ Hz) (cf. Figure 1c). This arises from the (2*S*,5*S*,6*R*)-[5,6-$^2H_2$]imine hexahydropyrimidine and its enantiomer (cf. Scheme IIc). Again, these resonances were satisfactorily reproduced by computer-assisted simulation (cf. Figure 2c).

The signals of H-6_{ax} of dideuterated imine hexahydropyrimidines (cf. Scheme II) derived from dideuterated spermidines were broadened and shifted upfield (ca. 12 Hz) compared to H-6_{ax} in **4**, presumably because of effects from deuterium(s). A further compliction is that these signals overlap those for H-$1'_{ax}$, which appears as two octets separated by 7 Hz in the spectrum of each dideuterated imine hexahydropyrimidine (cf. Figure 1a–c).[23] These complexities necessitated the synthesis of a reference sample of one of the dideuterated imine hexahydropyrimidines. This was achieved from (*E*)-[1,2-$^2H_2$]ethene via (2*S*,3*R*)/(2*R*,3*S*)-3-aminopropionitrile (cf. Scheme III). The key step in this sequence is the conversion of [1,2-$^2H_2$]2-(trifluoroacetamido)ethanol *O*-*p*-toluenesulfonate into [1,2-$^2H_2$]-1-cyano-2-(trifluoroacetamido)-ethane with *retention of configuration* at the reacting deuteriomethylene group.[24] The synthetic [1′,2′-$^2H_2$]spermidine was reacted with ≥2 mol equiv ethanal in deuteriochloroform to give (5*R*,6*R*)-[5,6-$_2H_2$]-**4** + (5*S*,6*S*)-[5,6-$^2H_2$]-**4** and their enantiomers. The 61.4-MHz ^2H NMR spectrum of this mixture showed four broad singlets of equal intensity at δ 1.66 and 3.03, corresponding to the (5*R*,6*R*)-isomer and its enantiomer, and at δ 1.52 and 2.33, for the (5*S*,6*S*)-isomer and its enantiomer. The 400-MHz ^1H NMR spectrum was very similar (peak for peak matching; cf. Figure 1d) to the dideuterioimine hexahydropyrimidine derived from (2*R*,3*R*,4*S*)/(2*R*,3*S*,4*R*)/(2*S*,3*R*,4*S*)/(2*S*,3*S*,4*R*)-[3,4-$^2H_2$]methionines (cf. Scheme II). In particular, the resonance for H-6_{ax} was a broad singlet[21] at δ 2.30, superimposed on resonances from H-$1'_{ax}$ (cf. Figures 1d,b, 2b).

A recent kinetic study of spermidine synthase from *E. coli* concluded[5a] that a Ping-Pong Bi-Bi mechanism operates, via an intermediate aminopropylated enzyme. If this were correct, the stereochemical course of spermidine synthase should be an overall retention via two inversion steps. The contrasting conclusion from the present study is that spermidine synthase operates by a sequential Bi-Bi mechanism exhibiting the stereochemistry of a classical S_N2 reaction, i.e., inversion.[25] It is therefore analogous to enzymic transmethylation with adenosylmethionine, for which inversion at the sulfonium methyl has been conclusively demonstrated.[26] Recently, transadenosylation of methionine to form adenosylmethionine has also been shown to occur with inversion of configuration at the reacting methylene group.[27]

Acknowledgment. We thank SERC for financial support, Drs. E. Curzon and O. W. Howarth for high-field NMR spectra (Regional NMR Service, University of Warwick), Dr. D. C. Billington for the preparation of dideuterated methionines and Dr. P. Bigler (Universität Bern, Institut für Organische Chemie) for computer-assisted simulations or NMR spectra. A helpful discussion with Professor S. J. Gould, University of Connecticut, is greatfully acknowledged.

(22) This resonance is expected to be a doublet (J = 12 Hz) of 1:1:1 triplets (J = 2 Hz), ignoring vicinal H_{ax}–D_{eq} coupling (cf. ref 21).

(23) This is believed to arise from an isotope effect of deuterium vs. hydrogen transmitted from axial H or D through the nitrogen lone pair (axial) to H-2 (axial) and H-$1'_{ax}$ [N.B. in the spectra of dideuterated samples of **4** H-2 appears as two quartets of similar intensity separated by 7 Hz].

(24) This sequence was established with unlabeled compounds (new compounds gave spectroscopic data and combustion analyses in accord with their assigned structures); 2-(trifluoromethyl)-Δ^2-oxazoline (Tanaka, K.; Shreeve, J. M. *Inorg. Chem.* **1980**, *19*, 2612 and refs cited therein) was isolated from treatment of 2-(trifluoroacetamido)ethanol *O*-*p*-toluenesulphonate with NaCN/Me_2SO or KOH/CH_2Cl_2; exposure of this oxazoline to excess of NaCN in Me_2SO (1 week/room temperature) converts it into 1-cyano-2-(trifluoroacetamido)ethane; we thank Dr. D. J. Robins for experimental details concerning steps viii and ix of Scheme III [cf.: Khan, H. A.; Robins, D. J. *J. Chem. Soc. Chem. Commun.* **1981**, 554]; mass spectral analyses showed deuterated intermediates to contain ≥90% [$^2H_2$] species.

(25) For a comment on the kinetic analysis of ref 5a see footnote 11 of ref 5b.

(26) Floss, H. G.; Mascaro, L.; Tsai, M.-D.; Woodard, R. W. [In "Transmethylation"; Usdin, E., Borchardt, R. T., Creveling, C. R., Eds.; Elsevier: New York, 1979; p 135. Floss, H. G.; Tsai, M.-D. *Adv. Enzymol.* **1979**, *50*, 243. Woodard, R. W.; Mascaro, L.; Hörhammer, R.; Eisenstein, S.; Floss, H. G. *J. Am. Chem. Soc.* **1980**, *102*, 6314. Arigoni, D. *Ciba Foundation Publ.* **1978**, *60*, 243.

(27) Parry, R. J.; Minta, A. *J. Am. Chem. Soc.* **1982**, *104*, 871 [the reverse of this reaction is analogous to Scheme I].

Interconversion of Nitrite and Ammonia: Progress toward a Model for Nitrite Reductase

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Recently, we reported that for the complex [Ru(trpy)(bpy)-NH_3]$^{2+}$ (trpy is 2,2′,2″-terpyridine; bpy is 2,2′-bipyridine), the coordinated ammonia is oxidized rapidly and quantitatively to give the corresponding nitrosyl complex [Ru(trpy)(bpy)NO]$^{3+}$, which is in acid–base equilibrium with the nitro complex [Ru(trpy)-(bpy)NO_2]$^+$ and the two are present in equal amounts at pH 2.34[1] (eq 1).

$$[\underset{\mathbf{1}}{Ru(trpy)(bpy)NH_3}]^{2+} \xrightarrow[+H_2O,\ -5H^+]{-6e^-} [\underset{\mathbf{2}}{Ru(trpy)(bpy)NO}]^{3+} \underset{-H_2O,\ +2H^+}{\overset{+H_2O,\ 2H^+}{\rightleftharpoons}} [\underset{\mathbf{3}}{Ru(trpy)(bpy)NO_2}]^{+} \quad (1)$$

We report here that the reverse reaction, the reduction of coordinated nitrosyl to coordinated ammonia, occurs for a variety of polypyridyl complexes of both ruthenium and osmium and that the mechanism involves a series of facile one-electron-transfer steps, as initially suggested by Armor for the reduction of [Ru-$(NH_3)_5NO$]$^{3+}$.[2] We also report the *catalytic* reduction of nitrite to ammonia, based on the water-soluble metalloporphyrin Fe(II)TPPS (TPPS = *meso*-tetrakis(*p*-sulfonatophenyl)porphine).[3]

In Figure 1 is shown a cyclic voltammogram for [Ru(trpy)-(bpy)NO]$(BF_4)_3$ in aqueous solution buffered at pH 4.68.[4] The first reduction, wave A, at $E_{1/2}$ = 0.19 V vs. SSCE,[4] is a reversible, pH-independent one-electron transfer to an orbital largely π^*(NO) in character (eq 2).[5] The second reduction, wave B (at E_p^c =

$$[\underset{\mathbf{2}}{Ru(trpy)(bpy)NO}]^{3+} \underset{-e^-}{\overset{+e^-}{\rightleftharpoons}} [\underset{\mathbf{4}}{Ru(trpy)(bpy)NO}]^{2+} \quad (2)$$

−0.36 V), is also a pH-independent, one-electron reduction, while the third reduction, wave C (at E_p^c = −0.57 V), is pH dependent. Coulometry[5] past the third wave (E = −0.6 V) results in a quantitative, six-electron (n = 5.9) reduction of the nitrosyl complex **2** to the ammine complex **1** as shown in eq 3.

$$[Ru(trpy)(bpy)NO]^{3+} + 6e^- + 5H^+ \rightarrow [Ru(trpy)(bpy)NH_3]^{2+} + H_2O \quad (3)$$

However, clear evidence for reduced intermediates can be obtained by cyclic voltammetry. Cycling through the first reduction (wave A) and to the onset of the second wave (wave B), results in the appearance of an oxidative wave (wave I) due to an intermediate (I) having E_p^a(I) = +0.38 V. Cycling past both waves A and B to the onset of the third reduction (wave C) results in the loss of the oxidative wave for intermediate (I) and the appearance of an oxidative wave (wave II) for a second intermediate

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(4) Note from eq 1 that at pH 4.68 the dominant form in the nitro–nitrosyl acid–base system is the nitro complex. However, on the time scale of our experiments, the nitrosyl → nitro interconversion is slow and it is possible to observe the electrochemical properties of the nitrosyl complex without interference from the nitro complex.

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