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STEREOCHEMICAL STUDIES OF ENZYME-CATALYZED ALKYL-TRANSFER REACTIONS. AN NMR METHOD FOR DISTINGUISHING BETWEEN THE TWO PROCHIRAL HYDROGENS AT C-1' OF SPERMIDINE

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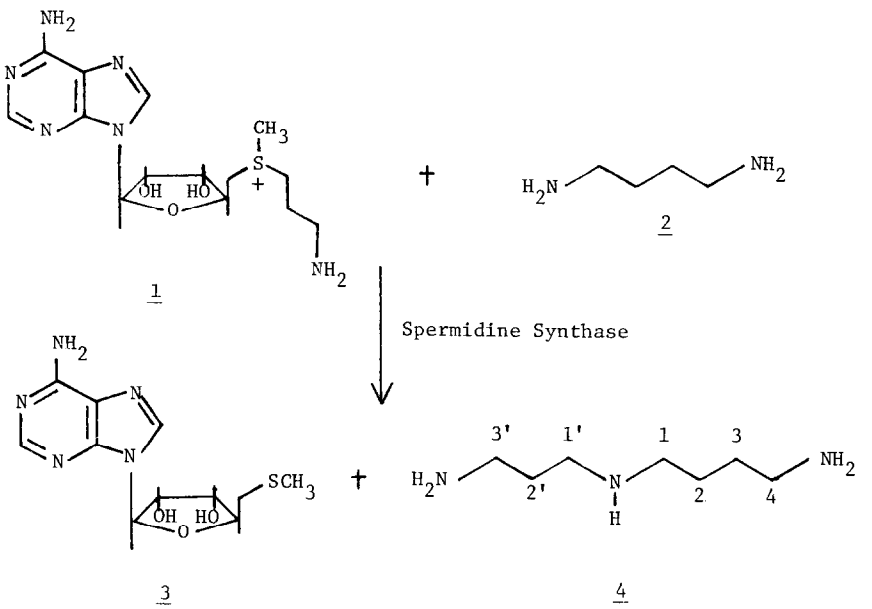
Abstract: A simple NMR method is described that differentiates between the two prochiral hydrogen atoms at C-1' of spermidine, thereby providing a means of distinguishing between a single- and double-displacement mechanism for the spermidine synthase reaction.

The alkyl transfer reactions involved in polyamine biosynthesis are catalyzed by the aminopropyltransferases, spermidine synthase (Scheme 1) and spermine synthase.[2] Such reactions can occur by a single-displacement or a double-displacement mechanism.[3,4] In theory, these two mechanisms can be distinguished by isotope labeling experiments[4] or by steady-state kinetic investigations.[5]

Scheme 1

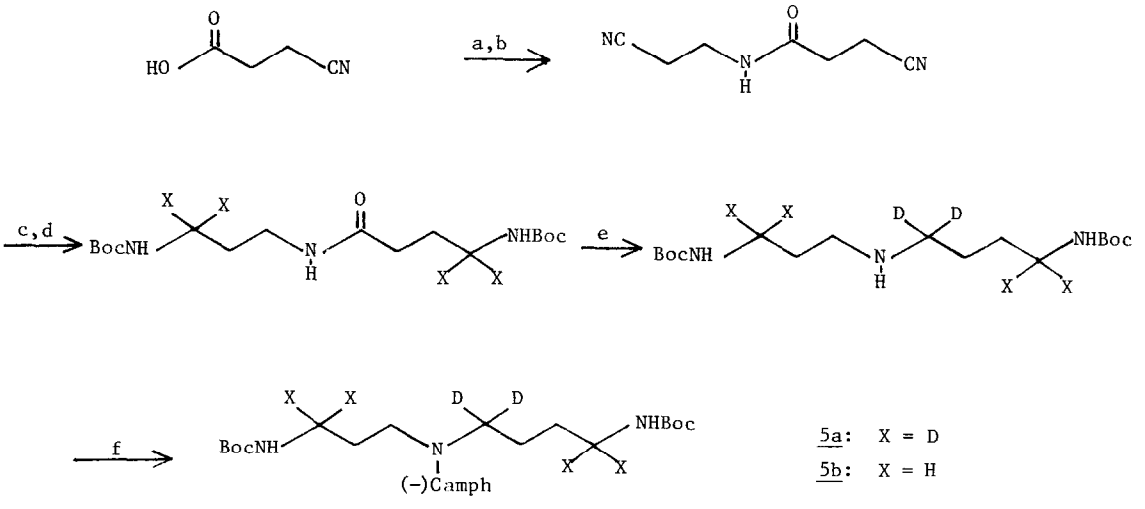


Several years ago we showed that rat prostate spermidine synthase is inhibited by one of the

substrates, decarboxylated S-adenosylmethionine (dcAdoMet, 1) at concentrations above 40 μM, giving rise to non-linear double-reciprocal plots (1/V vs. 1/S).[6] This substrate inhibition makes interpretation of steady-state kinetic data difficult. However, a recent paper has reported steady-state kinetic studies of the reaction catalyzed by spermidine synthase isolated from E. coli,[7] and the conclusion reached by the authors was that the E. coli reaction occurs by a double-displacement ("ping-pong") mechanism. We have recently synthesized S-adenosyl-1, 8-diamino-3-thiooctane, as a multisubstrate adduct inhibitor of spermidine synthase from rat prostate.[8] The potent and specific inhibitor activity of this compound supports a single displacement mechanism. In this paper we report an unambiguous NMR method that clearly distinguishes between the two prochiral hydrogen atoms at C-1' of spermidine, thereby providing a non-kinetic approach for studying the question of single- vs. double-displacement mechanism in enzyme-catalyzed aminopropyl transfer.

We have synthesized spermidines 5a-d, selectively deuterated at strategic methylene groups adjacent to nitrogen, and the ^{1}H NMR spectra (500 MHz) of these compounds were obtained. Hexadeutero-dibocspermidine camphanamide 5a was synthesized as shown in Scheme 2. As shown in Figure 1a, four multiplets, slightly overlapping, are present between 3.30 and 3.62 ppm due to the C-1' hydrogens. These result from geminal and vicinal coupling as well as long range coupling from the C-3' deuterium. The presence of four multiplets rather than two results from the hindered rotation about the camphanamide bond.[9] A small signal at 3.12 ppm is due to residual protium at C-3' and C-4; a full four-proton multiplet is present at this chemical shift in the corresponding spectra of 5b-d. It is clear that at this field strength the diastereotopic C-1' hydrogens can be distinguished from each other even without the addition of Europium shift reagent.[10,11]

Scheme 2



5a: X = D
5b: X = H

5

Reagents: a, isobutylchloroformate; b, 3-aminopropionitrile; c, X_2/PtO_2; d, Boc_2O
e, $NaBD_3O_2CCF_3$; f, (-)camphanoyl chloride.

Figure 1

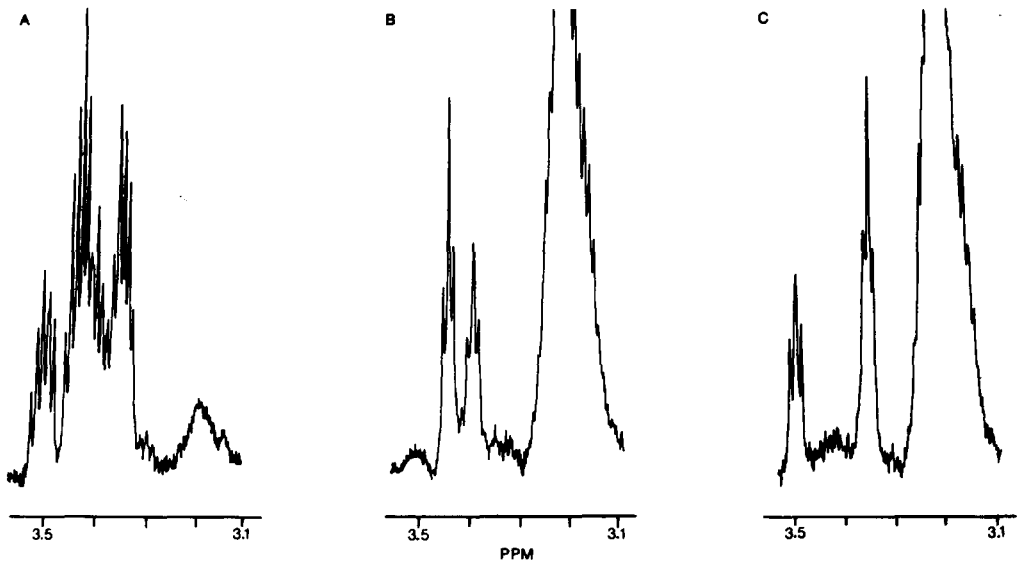
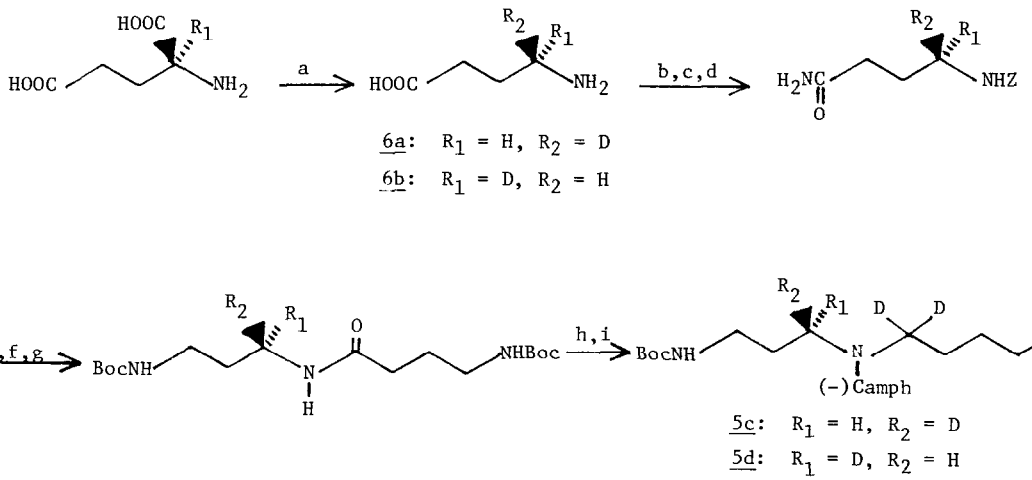


Figure 1. 500 MHz ^{1}H NMR spectra; samples in $CDCl_3$. A. Hexadeutero-dibocspermidine camphanamide 5a; C-4, -1', -3' hydrogen resonances. B. [1-$^{2}H_2$,1'R-^{2}H]-5c. C. [1-$^{2}H_2$, 1'S-^{2}H]-5d.

The pairs of resonances shown in Figure 1a are unequivocally assigned by inspection of the spectra of 5c and 5d, the trideuterodibocspermidine camphanamides chirally labeled, at C-1'.

Scheme 3



Reagents: a, glutamate decarboxylase; b, ZCl; c, $SOCl_2$; d, NH_3; e, $Pb(OAc)_4$/tBuOH; f, H_2/Pd-C; g, isobutylchloroformate/$HOOC(CH_2)_3NHBoc$; h, $NaBD_3O_2CCF_3$; i, (-)camphanoyl chloride.

These were synthesized, as shown in Scheme 3, beginning with [4R-^{2}H]-γ-aminobutyric acid, 6a, and with [4S-^{2}H]-γ-aminobutyric acid, 6b, for 5c and 5d, respectively.[12,13] The spectrum (Figure 1b) of 5c shows a clean pair of triplets at 3.38 and 3.43 ppm, due to the S-hydrogen of the two rotamers, in contrast to the spectrum (Figure 1c) of 5d which shows clean triplets at 3.32 and 3.52 ppm due to the R-hydrogen of the two rotamers.

Methionine chirally labeled at the γ-carbon[14] should be readily converted enzymatically to 1, chiral at the methylene of interest.[15] The spermidine obtained by enzyme-catalyzed decarboxylation and aminopropyl transfer to perdeutero-putrescine (2) will be converted to N,N'-diboc-spermidine, via an intermediate hexahydropyrimidine,[16,17] and then to the camphanamide. The NMR method described in this report should thus be applicable to analysis of biosynthetic chiral spermidine obtained as outlined. Experiments aimed at utilizing this method to answer these stereochemical questions are now in progress.

Acknowledgements

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