at C-12 and C-13, respectively, were determined by ^{2}H NMR.

DL-[2-^{2}H]Arginine Hydrochloride (7d). Feeding a total of 200 mg (0.95 mmol) of **7d** mixed with 13.72 μCi of DL-[1-^{14}C]arginine to two 200-mL production broths yielded 210 mg of pure **1d** (44% recovery), 7.2 × 10^6 dpm/mmol (27% total incorporation), with 22.7% ^{2}H enrichment at C-12.

(2*RS*,3*R*)-[3-^{2}H]Arginine Hydrochloride (7e). Feeding a total of 90 mg (0.42 mmol) of **7e** and 2.36 μCi of DL-[1-^{14}C]arginine to two 200-mL production broths afforded 190 mg of pure **1e** (36% recovery), 1.23 × 10^6 dpm/mmol (31.0% total incorporation), with 11% ^{2}H enrichment at C-12.

(2*RS*,3*S*)-[3-^{2}H]Arginine Hydrochloride (7f). Two 200-mL production broths were fed a total of 65 mg (0.31 mmol) of **7f** mixed with 4.45 μCi of DL-[1-^{14}C]arginine. Workup yielded 180 mg of pure **1f** (36% recovery), 2.4 × 10^6 dpm/mmol (32.0% total incorporation), with 8.2% ^{2}H enrichment at C-13.

δ-*N*-[^{13}CH$_3$]Methyl-L-Arginine Hydrochloride (15a). A 200-mL fermentation was fed 50 mg (0.22 mmol) of **13a** mixed with 1.13 μCi of DL-[*guanidino*-^{14}C]arginine. Workup yielded 260 mg of pure **1g** (53% recovery), 9.95 × 10^6 dpm/mmol (42% total incorporation). The ^{13}C NMR spectrum was identical with that of natural abundance blasticidin S.

Acknowledgment. Dr. Y. Miyazaki, Kaken Chemical Co., Ltd., Japan, is thanked for providing a culture of *S. griseochromogenes* and recipes for seed and production media. Dr. H. Saito of the Kaken Chemical Co., and Dr. Donald Borders, Lederle Laboratories, Pearl River, New York, are thanked for samples of blasticidin S. This work was supported by Public Health Research Grant GM 23110 to S.J.G. Dr. V. A. Palaniswamy is thanked for helpful discussions and technical assistance. Rodger Kohnert is thanked for obtaining the high-field NMR spectra on a Bruker AM 400 spectrometer purchased in part through grants from the National Science Foundation (CHE-8216190) and from the M. J. Murdock Charitable Trust to Oregon State University.

Supplementary Material Available: General details, culture conditions, isolation of blasticidin S, bioassay of blasticidin S, and spectra of blasticidin S (from DL-[3-^{13}C,2-^{15}N]arginine, DL-[2,3,3-^{2}H$_3$]arginine, DL-[2-^{2}H]arginine, DL-[3,3-^{2}H$_2$]arginine, (2*RS*,3*R*)-[3-^{2}H]arginine, and (2*RS*,3*S*)-[3-^{2}H]arginine feedings) (9 pages). Ordering information is given on any current masthead page.

Synthesis of Chirally Deuteriated (*S*-Adenosyl-*S*-methylsulfonio)propylamines and Spermidines. Elucidation of the Stereochemical Course of Putrescine Aminopropyltransferase (Spermidine Synthase)

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Abstract: The stereochemical course of enzyme-catalyzed aminopropyl transfer has been investigated. The stereospecific synthesis of chirally deuteriated (*S*-adenosyl-*S*-methylsulfonio)propylamines and several chirally deuteriated spermidine (−)-camphanamide derivatives has allowed the elucidation of aminopropyltransferase stereochemistry by using ^{1}H NMR techniques. Putrescine aminopropyltransferase (spermidine synthase) isolated from *Escherichia coli* catalyzed the synthesis of chirally deuteriated spermidines from 1,4-diaminobutane (putrescine) and chirally deuteriated (*S*-adenosyl-*S*-methylsulfonio)propylamines. Derivatization of the biosynthetic spermidines to 1,8-bis(Boc)spermidine (−)-camphanamides and comparison of their ^{1}H NMR spectra with those of the synthetic standards permits determination of the absolute configuration of the biosynthetic products. The results show that the reaction catalyzed by *E. coli* spermidine synthase proceeds with inversion of configuration at the methylene carbon undergoing nucleophilic attack by putrescine. These data support a single-displacement mechanism proceeding via a ternary complex of enzyme and both substrates.

The polyamines putrescine, spermidine, **2a**, and spermine, **2b**, are an important series of biomolecules and are universally distributed in Nature. In vivo experimentation has shown that an increase in polyamine biosynthesis is closely associated with cell proliferation.[2] The higher polyamines **2a** and **2b** are biosynthesized by enzymatic transfer of an aminopropyl group from (*S*-adenosyl-*S*-methylsulfonio)propylamine, **1**, (decarboxylated *S*-adenosylmethionine, dcAdoMet) to putrescine or to the primary amine on the four-carbon arm of **2a**, respectively[3] (Figure 1). As part of our ongoing investigation of enzymatic alkyl transfer reactions[4,5] we have examined the biosynthesis of **2a**. The alkyl transfer involved in the biosynthesis of spermidine is catalyzed by putrescine aminopropyltransferase (PAPT, EC 2.5.1.16), often referred to as spermidine synthase.[3] As shown in Figure 1, this reaction could occur by either a single-displacement (path A) or by a double-displacement (path B) mechanism.[6-8] In principle these two mechanisms can be distinguished by isotope-labeling experiments[6-8] or by steady-state kinetic investigations.[9]

Several years ago we showed that rat prostate spermidine synthase is inhibited by one of its substrates, (*S*-adenosyl-*S*-

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(1) (a) Rensselaer Polytechnic Institute. (b) Oregon State University.
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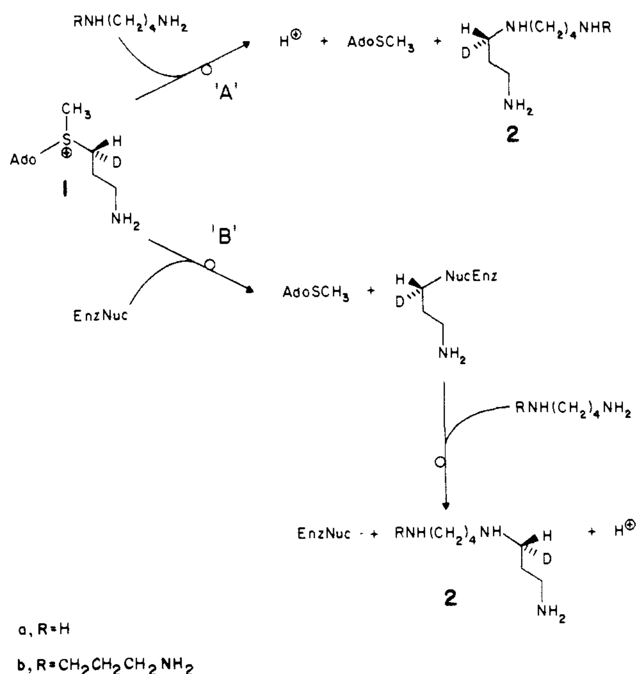

Figure 1. Single-displacement (A) vs double-displacement (B) paths for enzyme-catalyzed aminopropyl transfer reactions.

methylsulfonio)propylamine (**1**), at concentrations above 40 μM, giving rise to nonlinear double-reciprocal plots ($1/V$ vs $1/S$).[10] Despite this difficulty, a steady-state kinetic study of the reaction catalyzed by spermidine synthase isolated from *Escherichia coli* has been reported.[11] On the basis of a computer-assisted analysis of their kinetic data, the authors concluded that the *E. coli* reaction proceeds via a double-displacement mechanism. While the work described in this paper was in progress, a stereochemical investigation of the *E. coli* reaction in vivo was described in the literature. This study relied on a computer-assisted analysis of the ^{1}H NMR coupling patterns of a mixture of hexahydropyrimidine derivatives of spermidines derived from a mixture of chirally deuteriated methionines.[12] This analysis led the authors to conclude that the reaction occurs by a single-displacement mechanism. These conflicting conclusions as well as the complexity of the analyses used in the NMR investigations underscore the value of the simple, unambiguous method outlined below.

In an earlier paper we demonstrated an unambiguous method for distinguishing between the prochiral protons at the C-3 (C-1′) position of **2a**.[13] In addition to the analytical method, an outline of a stereochemical investigation of the spermidine synthase reaction was presented. The strategy of the experiment discussed in this report (Figure 2) was as follows: A chirally deuteriated (*S*-adenosyl-*S*-methylsulfonio)propylamine would be incubated with [1,4-$^2H_4$]putrescine in the presence of the enzyme. The resulting spermidine would then be isolated and converted to the bis(Boc) camphanamide **14**. Comparison of the high-field ^{1}H NMR spectrum of this derivative with those of the chiral standards[13] would yield the configuration of the center in question.

A single-displacement mechanism would involve one inversion of configuration in the conversion of **1** to **2**. A double-displacement mechanism would require two inversions, which would yield a net retention of configuration. This paper discusses the synthesis of chirally deuteriated **1** and **14** and their use in the elucidation of the stereochemical course of the spermidine synthase reaction.

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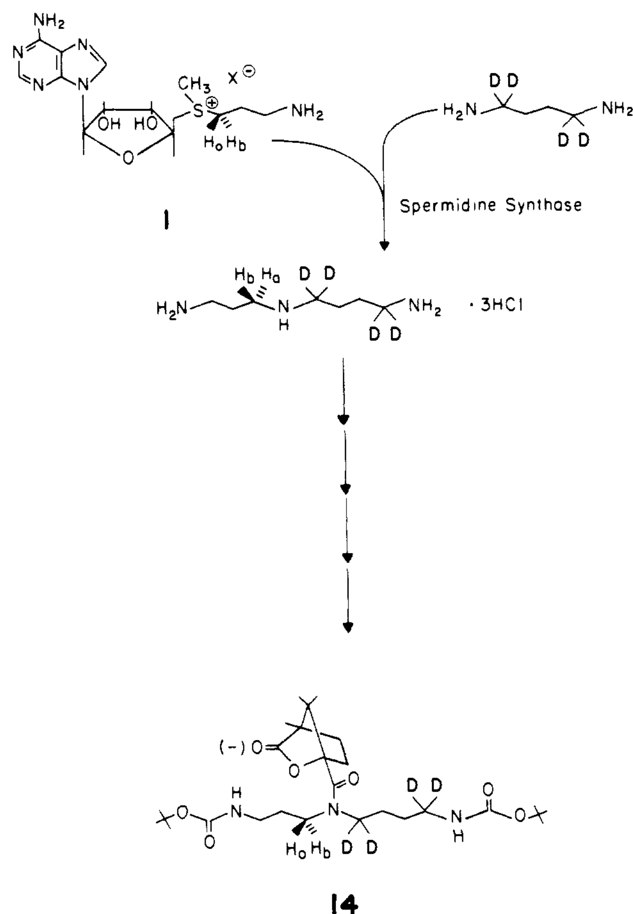

Figure 2. Experimental approach employed in this research.

Preliminary reports of this work have been presented.[14]

Results

The synthesis of chirally deuteriated (*S*-adenosyl-*S*-methylsulfonio)propylamine (**1**) can be envisioned via enzyme-catalyzed reactions or via nonenzymatic organic chemistry. Initially, a biosynthetic route was investigated in which *S*-adenosylmethionine (AdoMet) synthetase and AdoMet decarboxylase were utilized to prepare **1** from methionine.[15] Since Chang and Walsh[16] have described methods for the stereospecific synthesis of [4-^{2}H]methionine, successful application of the biosynthetic approach would lead to the desired chirally deuteriated substrates, **1**, for use in the proposed stereochemical studies. Unfortunately, the biosynthetic route, although effective for the preparation of small amounts of radiolabeled materials,[17] was unable to produce the larger quantities of substrates required for this research. Therefore, the synthetic route outlined in Scheme I was investigated. The synthesis of N^1-phthaloyl-N^3-tosyl-1,3-diaminopropane, **6**, from γ-aminobutyric acid (GABA, **3**) was accomplished via *N*-phthaloyl-GABA, **4**, which could be converted via a modified Curtius rearrangement[18] to the unsymmetrically protected diamine **5**. Transposition of the phthaloyl protecting group was necessary in order to yield a suitably protected tosylamide, **6**, which would not undergo intramolecular displacement of the tosylate[19] on formation of **7**. The conversion of **6** to **7** was

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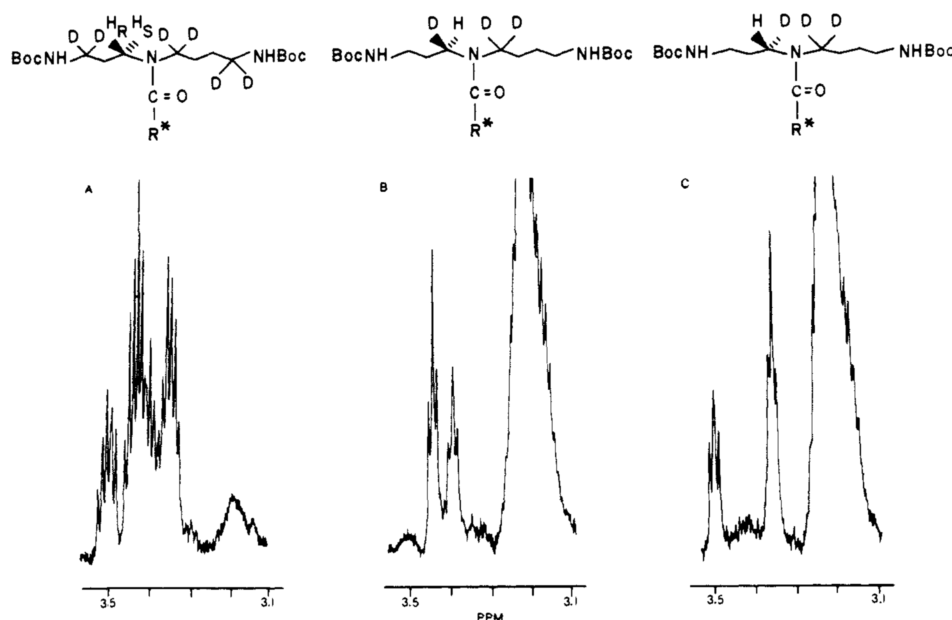

Figure 3. ^{1}H NMR spectra (500 MHz) of **16** (A), **14b** (B), and **14c** (C).

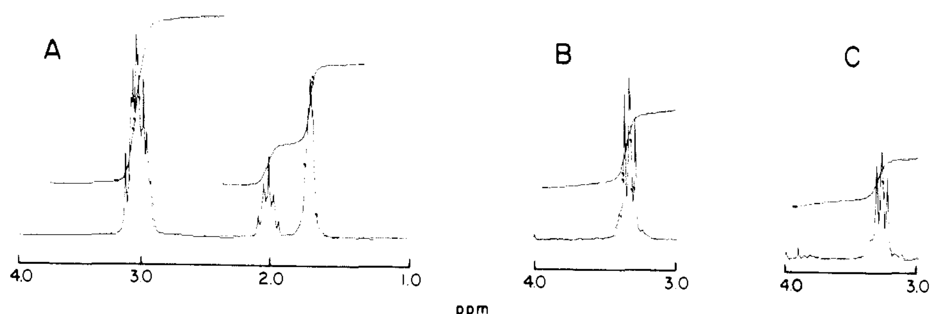

Figure 4. ^{1}H NMR spectra (200 MHz) of commercial spermidine (A), biosynthetic [$^2H_5$]spermidine hydrochloride derived from **1b** (B), and biosynthetic [$^2H_5$]spermidine hydrochloride derived from **1c** (C).

effected by the method of White[20] and was shown to proceed with predominant inversion of configuration at the methylene carbon of interest,[21] although some loss of stereochemical integrity was observed in this step (see Discussion). Coupling of **7** with the nucleoside thiolate, generated in situ from **8**,[22] led to the fully protected nucleoside 5′-thioether[23] from which the phthaloyl and isopropylidene blocking groups could be removed to yield decarboxylated *S*-adenosylhomocysteine, **9**. Methylation at sulfur was effected with CH_3I under acidic conditions to yield the target substrate, **1**, racemic at sulfur. The entire sequence, optimized for yield with achiral GABA, **3a**, was repeated with (4*R*)-[4-^{2}H]- and (4*S*)-[4-^{2}H]GABA,[24] **3b** and **3c**, respectively, as starting materials in order to obtain (3*R*)-[3-^{2}H]- and (3*S*)-[3-^{2}H]dc-AdoMet, **1b** and **1c**. These compounds were used as substrates for elucidating the stereochemical course of the reaction catalyzed by PAPT.

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(22) Coward, J. K.; Anderson, G. L.; Tang, K.-C. *Methods Enzymol.* **1983**, *94*, 286–294.
(23) Formation of the nucleoside 5′-thioether by reaction of **7** and the thiolate derived from **8** is assumed to proceed with inversion of configuration at the methylene of interest based on stereochemical studies of the reaction of **7** with acetate.[21]
(24) Santaniello, E.; Kienle, M. G.; Manzochi, A.; Bosisio, E. *J. Chem. Soc., Perkin Trans. 1* **1979**, 1677–1679. The common starting material, γ-aminobutyric acid (**3**), can be obtained chiral at the methylene of interest by enzyme-catalyzed decarboxylation of 2(*S*)-glutamic acid in D_2O, or of 2(*S*)-[2-^{2}H]glutamic acid in H_2O to give **3b** and **3c**, respectively. A small sample (36 mg) of **3b** and **3c** was converted to the methyl 4-((−)-camphanoylamido)butanoate, and the absolute configuration at C-4 was confirmed by using the NMR shift reagent Eu(dpm)$_3$ according to the method of Santaniello et al.

In order to carry out the proposed ^{1}H NMR analysis of the enzyme reaction products, we required stereospecifically deuteriated spermidines in which the protons of interest in the NMR experiment were made diastereotopic by introduction of a second chiral center into the spermidine derivatives. The synthetic route employed to obtain these derivatives is shown in Scheme II. Stereospecifically deuteriated GABA's, **3a** and **3b**, were again used as starting materials. Protection of the amino group of GABA as a benzyl carbamate, **10**, was followed by conversion of the carboxylic acid moiety to the amide **11**. A modified Hofmann rearrangement using $Pb(OAc)_4$ and involving trapping of the intermediate isocyanate with *t*-BuOH,[25] followed by removal of the benzyl carbamate, led to N^1-Boc-1,3-diaminopropane, **12**. Coupling of this selectively protected diamine with *N*-Boc-GABA,[26] via a mixed anhydride, followed by selective reduction of the amide carbonyl with $NaBD_3(OTfa)$[27] led to the bis(Boc) deuteriated spermidines **13**. Acylation of the central secondary amine of **13** with (−)-camphanoyl chloride gave the camphanamide derivatives **14**, suitable for ^{1}H NMR analysis. In order to make an unequivocal assignment of the NMR signal due to the C-3 methylene of interest, the syntheses of **15** and **16** were effected by the reactions shown in Scheme III.

The hypothesis that diastereotopic protons at the methylene of interest could be differentiated was tested directly. Thus, the ^{1}H NMR spectrum of **15** in methanol-d_4 (not shown) gives a two-proton triplet for the enantiotopic protons of interest, devoid

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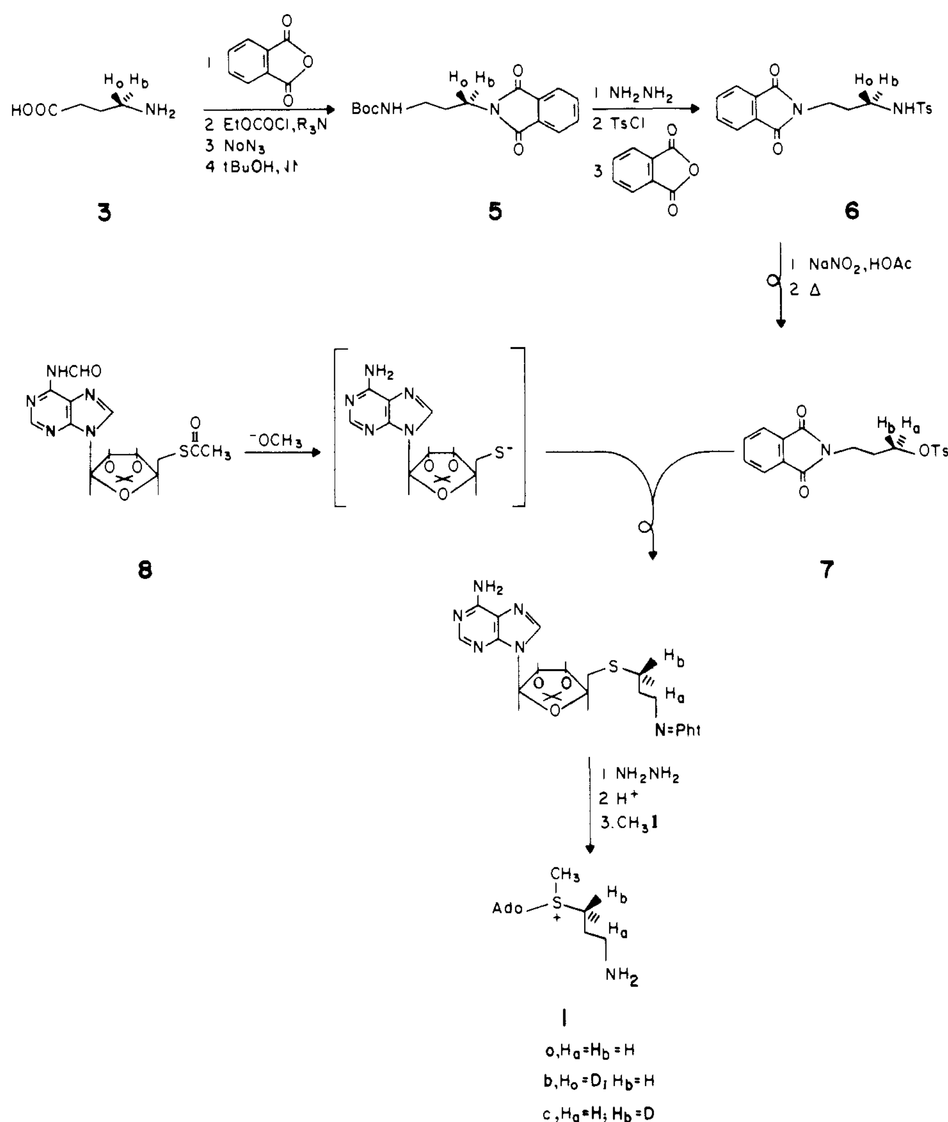
Scheme I



of any stereochemical information. In contrast, the spectrum of **16** shown in Figure 3A reveals a complex multiplet due to the protons of interest (H_R, H_S), now diastereotopic by reason of the neighboring chiral acyl substituent. The complex multiplet presumably arises from the nonequivalence of H_R and H_S, in addition to geminal coupling and hindered rotation about the camphanamide bond. The stereospecific synthesis of **14b** and **14c** (Scheme II) and the subsequent ^1H NMR analysis (Figure 3B,C) demonstrated magnetic nonequivalence of H_S and H_R in **14b** and **14c**, respectively.[13] This result established the basis for a straightforward NMR analysis of stereochemistry in the enzyme-catalyzed formation of spermidine.

The formation of spermidine (**2**) from **1** and putrescine-d_4 in a PAPT-catalyzed reaction (Figure 2) was carried out as described in the Experimental Section. ^1H NMR analysis of the isolated products (Figure 4) shows a simplified pattern at the methylenes adjacent to nitrogen (δ ca. 3.0), reflecting deuterium incorporation at those positions in the biosynthetic material. Conversion of spermidine to the bis(Boc)camphanamide, **14**, was carried out as shown in Scheme IV. Reaction of spermidine with 1 equiv of formaldehyde led to the intermediate hexahydropyrimidine,[28] which was then acylated at the free primary and secondary amine functions, followed by acid-catalyzed opening of the hexahydropyrimidine ring[28] to afford the 1,8-bis(Boc)spermidines. A chiral acyl group was introduced at the central nitrogen by reaction of the bis(Boc)spermidines with (−)-camphanoyl chloride to yield [$^2H_2$]-**14**. This material was identical with that prepared from GABA (**3**) as described above (Scheme II), with the exception of the anticipated changes in the ^1H NMR spectrum arising from differences in the ^2H substitution pattern (Scheme II vs Scheme IV).

Shown in Figure 5 are the relevant portions of four ^1H NMR spectra (500 MHz), two of the derivatized biosynthetic spermidines, prepared as just described (Scheme IV), and two of the stereospecifically synthesized standards, **14b** and **14c** (Scheme II). The chemical synthesis of dcAdoMet (**1**) involves two steps in which an inversion of configuration occurs at the methylene carbon of interest (Scheme I),[21,23] leading to net retention of configuration at that carbon. Thus, (*R*)-GABA (**3b**) leads predominantly to (*R*)-dcAdoMet (**1b**), and (*S*)-GABA (**3c**) to (*S*)-dcAdoMet (**1c**). The two chiral substrates, **1b** and **1c**, were converted to isomeric spermidines in two PAPT-catalyzed reactions, followed by purification and derivatization as described above (Scheme IV). The spectra shown in Figure 5 demonstrate that the *R* substrate, **b**, leads ultimately to the *S* product (Figure 5, part D vs part B), whereas the *S* substrate, **1c**, leads to the *R* product (Figure 5, part C vs part A). This indicates that the PAPT-catalyzed reaction proceeds with inversion of configuration at the electrophilic methylene of **1** involved in the aminopropyl transfer reaction. During the chemical synthesis of **1**, a partial loss of stereochemical integrity at the chiral methylene of interest occurs during the conversion of **6** to **7** (see Discussion). However, chiral purity observed in acetates derived from **7b** (71% *R*-acetate)

(28) McManis, J. S.; Ganem, B. *J. Org. Chem.* **1980**, *45*, 2041–2042.

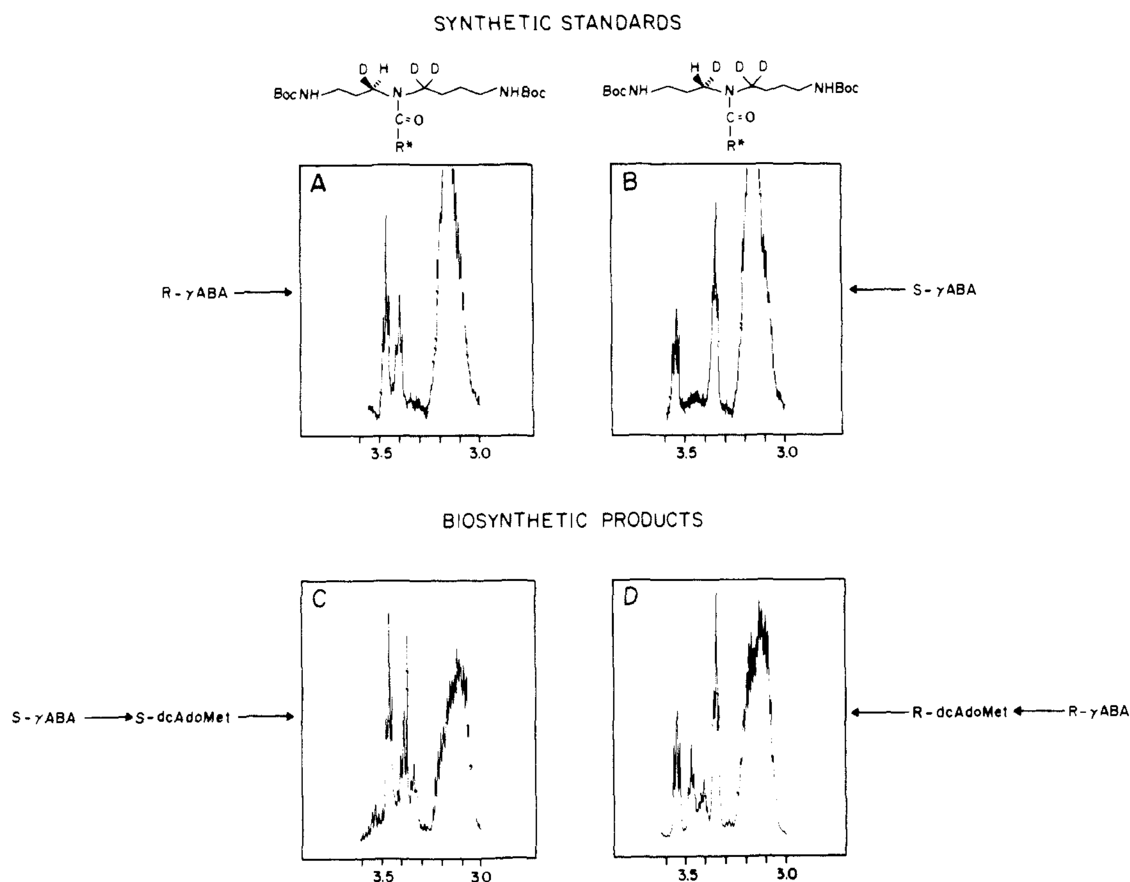
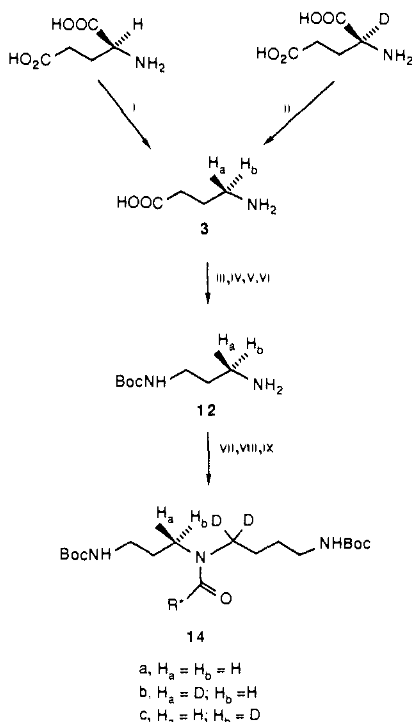


Figure 5. ^{1}H NMR spectra (500 MHz) of chemically synthesized standards, **14b** (A) and **14c** (B), and the biosynthetic spermidine camphanamides derived from **1c** (C) and **1b** (D).

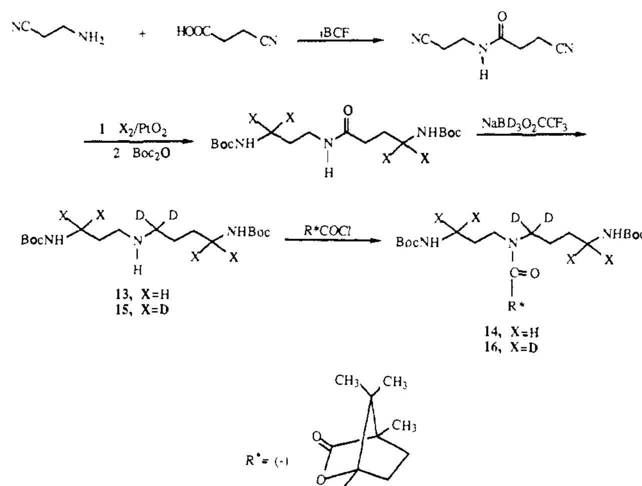
Scheme II



Reagents: i, Glutamate decarboxylase, D_2O; ii, Glutamate decarboxylase, H_2O; iii, CbzCl; iv, $SOCl_2$, NH_3; v, $Pb(OAc)_4$, t-BuOH; vi, H_2/Pd-C; vii, i-BCF, Boc-GABA; viii, $NaBD_3O_2CCF_3$; ix, (-) camphanoyl chloride (R*COCl)

and **7c** (69% *S*-acetate) is maintained in both biosynthetic products (Figure 5D, 69% *S*; Figure 5C, 71% *R*), thereby indicating that the enzyme-catalyzed reaction occurs via a clean single-displacement mechanism.

Scheme III

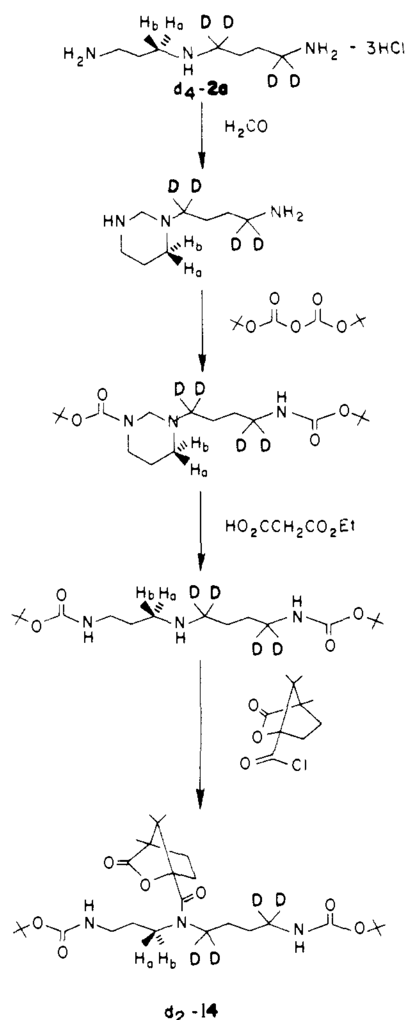


Discussion

Elucidation of the stereochemical course of an enzyme-catalyzed reaction allows for the unambiguous distinction between a single-displacement and a double-displacement mechanism.[6-9] On occasion, the stereochemical approach has resolved conflicts in the literature arising from the interpretation of steady-state kinetics experiments. Thus, in previous work from this laboratory the reaction catalyzed by catechol *O*-methyltransferase (COMT) was shown to proceed with inversion of configuration at the methyl group,[4] thereby providing very strong evidence for a single-displacement mechanism. Prior kinetics investigations of the COMT-catalyzed reaction had suggested either a single-displacement[29] or double-displacement[30] mechanism. Similarly, there

(29) Coward, J. K.; Slisz, E. P.; Wu, F. Y.-H. *Biochemistry* **1973**, *12*, 2291–2297.

Scheme IV



currently exists in the literature conflict on this aspect of the mechanism of the reaction catalyzed by PAPT. The reaction catalyzed by the enzyme isolated from *E. coli* has been investigated by steady-state kinetics methods, and the conclusion has been drawn that the reaction proceeds via an aminopropylated enzyme intermediate, i.e., a double-displacement mechanism.[11] In contrast, studies with PAPT isolated from bovine brain have led to the conclusion that this enzyme catalyzes the reaction without the intervention of an enzyme-bound intermediate, i.e., a single-displacement mechanism.[31] The results described herein unambiguously demonstrate that the reaction catalyzed by *E. coli* PAPT proceeds via a single-displacement mechanism with inversion of configuration at the chiral methylene of **1**. This conclusion is in agreement with that from in vivo biosynthetic studies with diastereomeric mixtures of chirally deuteriated methionine as spermidine precursors in *E. coli*.[12]

The synthesis of the chirally deuteriated substrate, dcAdoMet, **1**, was accomplished by the reactions shown in Scheme I. Most of these reactions do not involve chemistry at the chiral methylene, CH_aH_b. However, two reactions, namely the conversion of **6** to **7**, and coupling of the latter compound to an adenosine 5′-thiolate derived from **8**, do involve the chiral methylene carbon. The rearrangement of the *N*-nitroso derivative of **6** to the tosylate **7** was effected by methods described by White.[20] In that work, the author demonstrated that the rearrangement of a chiral tosylamide to a chiral tosylate could proceed with either inversion or retention of configuration, depending on the reaction conditions. In our work, we sought to optimize the chemical yield for this reaction prior to establishing its stereochemical course. The chiral tosylates, **7b** and **7c**, prepared under our optimal conditions (see the Experimental Section) were then converted to the corresponding acetates and (−)-camphanoates, as described in an accompanying paper.[21] ^{1}H NMR analyses of these esters in the presence of the appropriate lanthanide shift reagent[21] revealed that (a) the rearrangement leads to some loss of stereochemical integrity at the chiral methylene carbon of **7a** and **7b** and (b) the overall reaction **6** → **7** proceeds with predominant (ca. 70%) inversion of configuration at that carbon. The acetolysis of **7** has also been shown to proceed with inversion of configuration at the chiral methylene carbon,[21] thereby ruling out any neighboring-group participation by the *N*-phthaloyl group.[32] By analogy, we have assumed that thiolysis of **7** by the thiolate derived from **8** proceeds with inversion of configuration at the chiral methylene carbon. The fact that two reactions (**6** → **7** and the thiolysis of **7**) proceed with inversion means that the overall synthesis of **1** from **3** (Scheme I) proceeds with retention of configuration at CH_aH_b. Therefore substrate **1b**, derived ultimately from (*R*)-GABA (**3b**) has the *R* configuration at the methylene of interest, and, conversely, substrate **1c**, derived from (*S*)-GABA (**3c**) has the *S* configuration at CH_aH_b.

The synthesis (Schemes II and III) of stereospecifically and regiospecifically deuteriated spermidine derivatives (e.g., **14** and **16**) was required in order to demonstrate the feasibility of the NMR method[13] and for use as standards for comparison with biosynthetic material (Figure 5). Stereospecific synthesis of **14b** and **14c** allowed for the unequivocal assignment of the resonances attributed to H_R and H_S (Figure 3). The resonances of interest appear as pairs of triplets of unequal intensity (Figure 3B,C) rather than as a single one-proton triplet. On the basis of appropriate model studies, this is thought to be due to restricted rotation around the camphanamide bond, leading to an unequal population of two isomers resulting in the observed pair of triplets. Thus, raising the temperature from ambient (ca. 20 °C) to 65 °C results in a coalescence of the pair of singlets (NCH_3) in the spectrum of *N*-methyl-*n*-propylamine (−)-camphanamide (data not shown). The spectra of **14b** and **14c** shown in Figures 3 and 5 were obtained at ambient temperature. However, similar coalescence could not be demonstrated due to the chemical instability of these compounds at elevated temperatures. It is also interesting to note that the excellent separation of the resonances due to H_a and H_b of **14** (Figures 3 and 5) is obtained in the *absence* of an added lanthanide shift reagent. This is in contrast to similar experiments with (−)-camphanamides of **3** and **3b** (ref 24, this work) or (−)-camphanoates derived from **7**[21] where an added shift reagent is required in order to observe the desired separation of resonances.

The enzyme-catalyzed reaction of the chiral substrates, **1b** and **1c**, with tetradeuterioputrescine yielded spermidine-d_5, the structure of which was confirmed by ^{1}H NMR spectroscopy (Figure 4). The two isomers of spermidine-d_5 were converted to **14** by the series of reactions shown in Scheme IV. As noted in the Experimental Section, rigorous purification of each biosynthetic isomer was crucial for its successful conversion to **14**. The presence of impurities carried over from components (e.g., protein, buffer, metals, etc.) present in the enzyme-catalyzed reaction resulted in unacceptably low yields and a multitude of undesired side products.

Analysis of the isomeric bis(Boc)spermidine camphanamides, **14**, by ^{1}H NMR spectroscopy shows clearly that the reaction utilizing (*R*)-dcAdoMet (**1b**) as the aminopropyl donor gives a product with predominant *S* configuration at the chiral methylene (Figure 5D). Conversely, (*S*)-dcAdoMet (**1c**) gives a product with predominant *R* configuration (Figure 5C). As discussed above, the conversion of **6** to **7** results in a partial loss of stereochemical integrity of CH_aH_b. However, the presence of ca. 30% of the other isomer in each product provides an internal standard in the NMR analysis, allowing complete confidence in the assignments made. The results shown in Figure 5, together with knowledge of the chiral purity of **1b** and **1c**, lead to the conclusion that the PAPT-catalyzed proceeds via a single-displacement mechanism,

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(31) Pajula, R.-L.; Raina, A.; Eloranta, T. *Eur. J. Biochem.* **1979**, *101*, 619–626.
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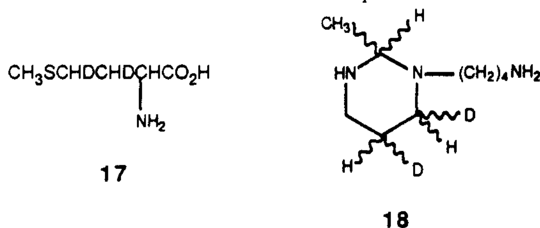
resulting in inversion of configuration at CH_aH_b of **1**.[33]

While this work was in progress, papers by Golding and coworkers[12] reported the results of in vivo stereochemical studies of spermidine biosynthesis in *E. coli*. These authors used a mixture of deuteriated methionines, **17**, and analyzed the ^1H NMR spectra of hexahydropyrimidines, **18**, prepared from the biosynthetic spermidines isolated from *E. coli*. Computer simulation using



chemical shift data and coupling constants obtained from a variety of model spectra were reported. These, together with the spectra of **18** derived from spermidine synthesized in vivo from **17**, provided evidence that the aminopropyl transfer reaction proceeds with inversion of configuration at the electrophilic methylene of interest. However, the spectra reported for **18** and the computer simulation are extremely complex, owing to the presence of diastereomeric mixtures. In addition, the use of an in vivo system to study the stereochemical course of an enzyme-catalyzed reaction introduces the possibility of adventitious racemization at one or more of the chiral centers in **17** or any intermediate on the spermidine biosynthetic pathway.

The conclusion that the PAPT-catalyzed reaction goes via a single-displacement mechanism is in accord with nearly all previously studied methyl[7,8] and phosphoryl[6] transfers. This conclusion also rules out a nonsequential "ping-pong" mechanism previously proposed on the basis of a kinetics analysis of the *E. coli* PAPT reaction[11] and supports the sequential mechanism proposed for the bovine brain enzyme.[31] Finally, conclusion of a single-displacement mechanism via a ternary complex is in accord with the potent and specific inhibition of PAPT by 3-(*S*-adenosylthio)1,8-diaminooctane (AdoDATO).[34]

Experimental Section

General Procedures. All chemicals were of reagent quality and were used without purification unless otherwise noted. Solvents were dried and distilled by standard methods.[35] (4*R*)-[4-^{2}H]- and (4*S*)-[4-^{2}H]-γ-aminobutyric acid (**3b** and **3c**, respectively) were prepared by the method of Santaniello et al.[24] 5′-Deoxy-5′-(acetoxythio)-N^6-formyl-2′,3′-isopropylideneadenosine (**8**) was prepared by a modification of the literature method.[10,22]

***N*-[3-(Tosyloxy)-1-propyl]phthalimide (7), *R*-Derived 7b.** A stirred suspension of **6b** (625 mg, 1.689 mmol) in 12 mL of Ac_2O–HOAc (1:1) was cooled to 0 °C and then treated with excess $NaNO_2$ (1.25 g, 18.1 mmol) in small portions over a 4-h period. The reaction mixture was then stored at 4 °C overnight, after which the reaction was diluted with 100 mL of ice–H_2O and stirred for 5 min, and the resulting crystals were collected on a filter: yield 671 mg (99%); mp 118–119 °C dec (lit.[36] mp 118–119 °C); ^1H NMR ($CDCl_3$) δ 7.86 (6 H, m, Ar), 7.38 (2 H, d, *J* = 8 Hz, Ar), 3.75 (1 H, t, *J* = 6 Hz, CHDN(NO)), 3.64 (2 H, t, *J* = 6 Hz, CH_2NNPht), 2.45 (3 H, s, Ar CH_3), 1.82 (2 H, q, *J* = 6 Hz, CH_2).

The *N*-nitroso compound prepared above (641 mg, 1.51 mmol) and 1.8 g (17 mmol) of Na_2CO_3 were suspended in 50 mL of freshly distilled dioxane, and the reaction mixture was stirred at 70 °C for 20 h. The reaction solvent was then removed in vacuo, and the residue was partitioned between EtOAc and H_2O. The dried organic extract was concentrated in vacuo to give crude product containing both the desired product **7b** and the denitrosated amide **6b**. These were separated by flash chromatography on a silica gel column (4 cm × 22 cm) with $CHCl_3$ containing 0.7% CH_3OH as the eluting solvent. Fractions of 25 mL were collected. The fractions containing the desired product were combined and washed with H_2O, and the dried $CHCl_3$ solution was concentrated in vacuo to give 402 mg (68%) as a glass (lit.[36] mp 147–148 °C): ^1H NMR ($CDCl_3$) δ 7.77 (6 H, m, Ar), 7.35 (2 H, d, *J* = 8 Hz, Ar), 4.09 (1 H, t, *J* = 6 Hz, CHDO), 3.74 (2 H, t, *J* = 7 Hz, CH_2NPht), 2.44 (3 H, s, CH_3), 2.07 (2 H, q, *J* = 7 Hz, CH_2).

***S*-Derived 7c.** The *S* isomer **6c** was converted to the tosylate **7c** exactly as described above for the *R* isomer, except that the intermediate *N*-nitroso derivative (yield 669 mg (99%), mp 117–119 °C dec) rearranged to the tosylate in only 56% isolated yield after flash chromatography. The recovered starting material (118 mg, 18%) was resubjected to the rearrangement conditions to yield an additional 65 mg of pure **7c**: total yield, 400 mg (67%) of a glass; ^1H NMR ($CDCl_3$) identical with the tosylate **7b** derived from **6b**.

3-(*S*-Adenosylthio)-1-propylamine Sulfate (9a). The bromide corresponding to **7a**, *N*-(3-bromopropyl)phthalimide, 1.961 g (7.316 mmol), and **8** (2.875 g, 7.316 mmol) were suspended in 100 mL of dry CH_3OH, and the resulting mixture was purged of oxygen by alternately freezing and thawing (5×) under a dynamic atmosphere of Ar. $NaOCH_3$ (870 mg, 11.1 mmol) was then added to the cold mixture following the final freezing, and, as stirring commenced, the mixture was allowed to warm slowly to ambient temperature. Stirring was continued overnight under a static atmosphere of Ar. At the end of this time solvent was removed in vacuo, and the resulting residue was partitioned between $CHCl_3$ and H_2O. The dried organic layer was concentrated in vacuo to yield crude phthalimidopropylthio nucleoside in quantitative yield. Purification by flash chromatography with *i*-PrOH–EtOAc (1:1) as the eluting solvent yielded 3.404 g (91%) of pure phthalimidopropylthio nucleoside as a glassy foam.

The phthalimide group was removed by reacting the protected nucleoside (3.233 g, 6.36 mmol) in 75 mL of absolute EtOH with 3.14 mL of 2.22 M ethanolic $NH_2NH_2 \cdot H_2O$ (0.882 mmol). The stirred reaction mixture was heated at reflux temperature for 24 h under an atmosphere of N_2. At the end of this time, solvent was removed in vacuo, and the residue was dissolved in 20 mL of H_2O. The solution was adjusted to pH 4.0 with HOAc, and a flocculent precipitate formed as stirring continued for 2 h. The precipitate was removed by filtration through a 0.45-μm filter, and the filtrate was adjusted to pH 12.5 with solid KOH. The alkaline aqueous solution was extracted with $CHCl_3$ (5 × 75 mL), and the combined dried organic extracts were concentrated in vacuo to give 2.097 g (95%) of an oil.

This 2′,3′-isopropylidene derivative of **9a** (2.069 g, 5.47 mmol) was dissolved in 12 mL of 1 N H_2SO_4, and the resulting solution was stirred at ambient temperature for 36 h. At the end of this time the reaction mixture was shell-frozen and lyophilized. The resulting solid residue was exhaustively triturated with Et_2O–EtOH (5:1) to yield a solid, which was dissolved in water and filtered, and the filtrate was lyophilized to give 2.204 g (93%) of **9a** as a glassy foam. This material was identical by chromatographic and spectral analyses with authentic **9a**.[22]

3(*R*)-(*S*-Adenosylthio)-1-[3-^{2}H]propylamine Sulfate (9b). The chiral tosylate **7b** (367 mg, 0.99 mmol), **8** (432 mg, 1.09 mmol), and $NaOCH_3$ (123 mg, 2.20 mmol) were dissolved in 15 mL of dry CH_3OH and allowed to react as described for **7a**. After purification by flash chromatography, 430 mg (85%) of the phthalimidopropylthio nucleoside was obtained. This material (0.84 mmol) was allowed to react with $NH_2NH_2 \cdot H_2O$ (0.88 mmol) as described in the synthesis of **9a** to give 278 mg (87%) of the 2′,3′-isopropylidene derivative of **9b** as an oil. The oil was dissolved in 1.4 mL of 1 N H_2SO_4 to remove the isopropylidene blocking group. Isolation of **9b** as an amorphous white powder was carried out as described above for **9a**: yield 354 mg; ^1H NMR (D_2O, TSP external standard) δ 8.58 (1 H, s, H_8), 8.51 (1 H, s, H_2), 6.19 (1 H, d, H_1'), 4.49 (1 H, m, H_3'), 4.37 (1 H, m, H_4'), 3.09 (4 H, m, H_5' and CH_2NH_2), 2.71 (1 H, m, CHDS), 1.97 (2 H, q, CH_2). The peak for H_2' was obscured by the HOD signal (δ 4.90).

3(*S*)-(*S*-Adenosylthio)-1-[3-^{2}H]propylamine Sulfate (9c). The chiral tosylate **7c** (330 mg, 0.89 mmol), **8** (385 mg, 0.98 mmol), and $NaOCH_3$ (112 mg, 1.958 mmol) were allowed to react as described above for **9b**. Removal of blocking groups as described above gave 318 mg of **9c**: ^1H NMR (D_2O, TSP external standard) identical with the spectrum obtained for **9b**.

3(*R*)-((±)-*S*-Adenosyl-*S*-methylsulfonio)-1-[3-^{2}H]propylamine Perchlorate (1b). (3*R*)-[3-^{2}H]Decarboxylated adenosylhomocysteine, **9b**, (318 mg, 0.734 mmol) was dissolved in 8 mL of HCOOH–CH_3COOH (1:1), and $AgClO_4$ (304 mg, 1.468 mmol) was then added, followed by CH_3I (618 mg, 4.35 mmol). The resulting reaction mixture was protected from light by aluminum foil, and stirred overnight at ambient

(33) In a preliminary report of this work,[14b] a double-displacement mechanism was indicated since the PAPT-catalyzed reaction appeared to proceed with retention of configuration. However, a more complete spectral analysis of synthetic intermediates (Scheme I) revealed that *N*-phthaloyl substituents apparently complex with lanthanide shift reagents, leading to misinterpretation of shift reagent experiments with acetates or (−)-camphanoates derived from **7**.[21]

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temperature. At the end of this time the reaction mixture was diluted with an equal volume of H_2O and filtered, and the filtrate washed with Et_2O (3 × 10 mL). The aqueous layer was filtered through a 0.2-μm filter, shell-frozen, and lyophilized to give **1b** as a glass; yield 0.462 mmol (47% overall yield from **7b**) based on absorbance at 260 nm; HPLC t_R 6.50 min on Whatman SCX, mobile phase 0.5 M NH_4OFm, pH 4.0,[37] flow rate 1.2 mL min^{-1}, >90% purity of **1b** indicated; ^1H NMR (D_2O, pD 1.2) δ 8.58 (2 H, s, H_2 and H_8), 6.25 (1 H, d, H_1'), 4.68 (2 H, overlapping m, H_3' and H_4'), 4.05 (2 H, br, H_5'), 3.53 (1 H, br m, $CHDS^+$), 3.19 (2 H, br, CH_2NH_2), 3.04 (3 H, d, CH_3S^+), 2.28 (2 H, m, CH_2). The peak for H_2' was obscured by the HOD signal (δ 4.95).

3(*S*)-((±)-*S*-Adenosyl-*S*-methylsulfonio)-1-[3-^{2}H]propylamine Perchlorate (1c). (3*S*)-[3-^{2}H]-Decarboxylated adenosylhomocysteine, **9c**, (274 mg, 0.63 mmol) was converted to **1c** by a procedure identical with that described above for the synthesis of **1b**: yield 0.378 mmol (44% overall yield from **7c**) based on absorbance at 260 nm; HPLC t_R 6.46 min on Whatman SCX as described above for **1b**, >90% purity of **1c** indicated; ^1H NMR (D_2O, pD 1.2) idential with the spectrum obtained for **1b**.

***N*^{1},*N*^{8}-Bis(*tert*-butoxycarbonyl)-*N*^{4}-(−)-camphanoyl-[5,5-$^2H_2$]spermidine (14).** Compound **14b** was prepared from 20 mg (0.057 mmol) of **13b** in 1 mL of CCl_4 and 1 mL of pyridine. A solution of 12.5 mg (0.057 mmol) of freshly sublimed (−)-camphanoyl chloride (Fluka) in 1 mL of CCl_4 was added dropwise, and the reaction mixture was allowed to stir overnight at ambient temperature. The desired product was isolated by standard procedures detailed below for the synthesis of **16**: yield, 16 mg (54%). Prior to high-field ^1H NMR experiments, this product was further purified by reverse-phase HPLC (CH_3CN with 1.25% Et_3N–H_2O (86:14)): 500-MHz ^1H NMR ($CDCl_3$) δ 5.15, 4.93, 4.88, 4.68 (2 H, 4 br s, NHCO), 3.38, and 3.43 (1 H, 2 t, CHDN), 3.12 (4 H, m, CH_2NHCO), 2.40 and 2.04–1.40 (10 H, overlapping multiplets, CH_2), 1.44 (18 H, s, $C(CH_3)_3$), 1.16 and 1.08 (6 H, ds, CH_3), 0.96 (3 H, s, CH_3) (see Figure 3B).

Compound **14c** was prepared from 20 mg (0.057 mmol) of **13c** by a procedure identical with that described above for the 3*R* isomer **14b**: yield, 25 mg (81%). Prior to high-field ^1H NMR experiments, this product was further purified by reverse-phase HPLC as described for the *R* isomer: 500-MHz ^1H NMR ($CDCl_3$) identical with spectrum obtained for **14b** except the resonances for CHDN appear at δ 3.32 and 3.52 (1 H, 2 t) (see Figure 3C).

***N*^{1},*N*^{8}-Bis(*tert*-butoxycarbonyl)[1,1,5,5,8,8-$^2H_6$]spermidine (15).** A solution of 1.982 g (20 mmol) of β-cyanopropionic acid[38] in 50 mL of EtOAc was maintained at ca. −25 °C while *N*-methylmorpholine (2.124 g, 21 mmol) and isobutyl chloroformate (2.49 g, 20 mmol) were added sequentially via syringes. The reaction mixture was allowed to stir at ca. −25 °C for 15 min, at which time β-aminopropionitrile (Aldrich) (1.402 g, 200 mmol) was added over a period of 5 min via a syringe. The reaction was maintained at ca. −25 °C for an additional 15 min, the temperature was allowed to rise to 0 °C, and an ice bath was used to maintain that temperature for an additional few hours. The stirred reaction was allowed to warm slowly to ambient temperature as the ice in the bath melted overnight. The precipitated *N*-methylmorpholine hydrochloride was removed by filtration, and the solid was washed with 50 mL of EtOAc. The combined filtrates were washed with H_2O (3 × 65 mL), and the aqueous layer was shell-frozen and lyophilized to give the desired dicyano amide product: yield, 2.374 g (79%). Crystallization from 2-propanol gave 2.11 g (70%) of an analytical sample, mp 73–74 °C. Anal. Calcd for $C_7H_9N_3O$: C, 55.62; H, 6.00; N, 27.80. Found: C, 56.26; H, 5.69; N, 27.41.

The intermediate dicyano amide (604 mg, 4.0 mmol) was dissolved in 10 mL of EtOD, followed by ca. 20% DCl in D_2O (1.5 mL) and 70 mg of PtO_2. The reaction mixture, in a 25-mL round-bottom flask, was attached to a Brown hydrogenation apparatus containing 750 mL of D_2 in the buret. After a 21-h reaction time, 405 mL (18.0 mmol) of D_2 had been consumed, and the reaction was terminated. Filtration of the catalyst through Celite (CH_3OH wash), followed by removal of the solvents in vacuo, gave a quantitative yield of the desired product. The ^1H NMR spectrum of this material showed no absorbances due to the terminal methylenes. The d_4 diamine thus produced was treated immediately with Boc_2O as follows. A solution of the d_4 diamine (900 mg, 3.81 mmol) in 20 mL of dioxane–H_2O (2:1) was cooled to 0 °C, and 4 N NaOH (1.9 mL) was added, followed by 3.201 g (38.05 mmol) of $NaHCO_3$. To the resulting cooled reaction mixture was added, with stirring, 1.924 (8.272 mmol) of Boc_2O via a dropping funnel over a period of 30 min. The reaction mixture was stirred for an additional 2 h at 0 °C, at which time the ice bath was removed and the reaction mixture was allowed to come to ambient temperature as stirring was continued overnight. The solvents were then removed in vacuo, the white solid residue was dissolved in H_2O, and the solution was adjusted to pH ca. 2 with $KHSO_4$. The resulting heterogeneous aqueous system was washed with $CHCl_3$ (3 × 75 mL), and the dried combined organic extracts were concentrated in vacuo to give the desired product: yield, 883 mg (64% overall from the d_4 dicyano amide).

The d_4 bis(boc) diamino amide thus synthesized was reduced to **15** as follows. $NaBD_4$ (451 mg, 10.76 mmol) was dissolved in 40 mL of THF, followed by the dropwise addition of CF_3COOH (1.227 g, 10.76 mmol) and a 10-mL THF wash of the dropping funnel. The resulting solution was allowed to stir at ambient temperature for 15 min, at which time a solution of the d_4 bis(Boc) diamino amide (782 mg, 2.152 mmol) in 25 mL THF was added dropwise over a period of ca. 5 min. The resulting reaction mixture was allowed to stir overnight at ambient temperature. Workup as described for the synthesis of **13a** led to the desired product: yield, 529 7g (69%); ^1H NMR ($CDCl_3$) identical with the spectrum obtained for **13a** except no absorbance observed at δ 3.13 due to the terminal methylenes.

***N*^{1},*N*^{8}-Bis(*tert*-butoxycarbonyl)-*N*^{4}-(−)-camphanoyl[1,1,5,5,8,8-$^2H_6$]spermidine (16).** To a solution of **15** (177 mg, 0.5 mmol) in 1.5 mL of CCl_4 and 1.5 mL of pyridine was added a solution of 108 mg (0.5 mmol) of freshly sublimed (−)-camphanoyl chloride (Fluka), and the resulting reaction mixture was allowed to stir overnight at ambient temperature. The desired product then was isolated by adding $CHCl_3$ (70 mL) to the reaction mixture and washing the organic solution successively with H_2O (15 mL), 0.5 N HCl (2 × 15 mL), H_2O (15 mL), saturated aqueous $NaHCO_3$ (2 × 25 mL), an H_2O (15 mL). The dried organic extract was concentrated by rotary evaporation in vacuo to give an oily residue: yield, 174 mg (65%). Prior to high-field ^1H NMR experiments, this product was further purified by reverse-phase HPLC as described for **14**: 500-MHz ^1H NMR ($CDCl_3$) identical with the spectrum obtained for **14b** and **14c** except that the resonances for CH_2 adjacent to N^4 appear at δ 3.30–3.62 (2 H, four overlapping multiplets) and the resonance at δ 3.12 is greatly diminished due to deuterium substitution (see Figure 3A).

[1,4-$^2H_2$]Putrescine. Succinonitrile (641 mg, 8.0 mmol) was dissolved in 18 mL of EtOH-d_6, and 30% DCl in D_2O (2.75 mL) was then added, followed by 181 mg (0.8 mmol) of PtO_2. The reaction flask was fitted to a Brown hydrogenation apparatus, and the system was flushed three times with argon and finally purged with deuterium. The reservoir was filled with 550 mL of deuterium and refilled once during the ca. 40-h reaction time, during which the theoretical amount of $^2H_2$ was consumed. The catalyst was removed by filtration (Celite), and the filtrate plus washings were concentrated in vacuo to give a brown residue. The residue was dissolved in 150 mL of H_2O and was applied to a Dowex 50 × 8 column (100–200 mesh, H^+ form, 3 × 11 cm). The column was washed with 200 mL of H_2O and then 200 mL of 1 N HCl. The desired product was eluted with 2.3 N HCl, and the eluate was concentrated in vacuo to give 1.073 g (81%) of the crude material. This material was recrystallized from 12 N HCl–EtOH to give 300 mg (23%) of a white microcrystalline material, mp 285 °C dec (authentic putrescine mp 280 °C dec: ^1H NMR (D_2O) δ 1.75 (4 H, s, CH_2CH_2). Integration of the very weak signal at δ 3.04 indicated that $^2H_2$ incorporation to the extent of 93% occurred at the terminal methylene positions.

Conversion of Spermidine to *N*^{1},*N*^{8}-Bis(Boc)-*N*^{4}-(−)-camphanoylspermidine (Scheme IV). Spermidine trihydrochloride (254 mg, 1 mmol) and $KHCO_3$ (300 mg, 3 mmol) were dissolved in 3 mL of H_2O. After the initial evolution of CO_2 subsided, formaldehyde (1 mmol, 76 μL of a 37% aqueous solution) was added, and the reaction solution was stirred at ambient temperature overnight. After this time the pH of the reaction solution was adjusted to ca. 12.5 with 4 N NaOH, and the desired product was extracted into $CHCl_3$ (5 × 10 mL). Concentration of the dried extracts in vacuo led to 117 mg (75%) of the hexahydropyrimidine derivative:[28] ^1H NMR ($CDCl_3$) δ 3.39 (2 H, s, NCH_2N), 2.85, 2.74, 2.60, and 2.28 (8 H, 4 m, CH_2N), 1.63 and 1.49 (9 H, overlapping m, CH_2, NH and NH_2).

This product was converted to the bis(Boc) derivative by dissolving a small portion (9 mg, 5.7 μmol) in 0.3 mL of dioxane–H_2O (2:1) and then adding 63 μL of 1.8 N KOH and 0.3 mL of 1.9 N $KHCO_3$. The reaction mixture was cooled to 0 °C, Boc_2O (0.125 mmol, 347 μL of 0.36 M solution) was added, and stirring was continued overnight at ambient temperature. The reaction then was diluted with 2–3 mL of 1 N NaOH, and the desired product was extracted with $CHCl_3$ (7 × 10 mL). The combined dried organic portions were concentrated in vacuo to give 17 mg (85%) of an oily product: ^1H NMR ($CDCl_3$) δ 4.93 (1 H, br s, NHCO), 4.03 (2 H, s, NCH_2N), 3.41, 3.07, 2.65, and 2.36 (8 H, 4 m, CH_2N), 1.48 (6 H, br m, CH_2), 1.37 (18 H, ds, $C(CH_3)_3$).

The bis(Boc) derivative (17 mg, 48 μmol) was deprotected[28] by dissolving it in 1.297 mL of 0.37 M ethanolic ethyl hydrogen malonate.[39]

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To this solution was added 229 μL of 2.09 M ethanolic pyridine, and the resulting solution was stirred under N_2 at ambient temperature for 20 min and then heated at reflux temperature for an additional 1.5 h. The reaction mixture then was concentrated in vacuo, the resulting residue dissolved in $CHCl_3$, and the organic solution was washed with saturated $NaHCO_3$ (containing a few drops of 1.8 N KOH to give pH ca. 12.5). The dried (K_2CO_3) organic layer was concentrated in vacuo to give 16 mg (97%) of an oil: ^{1}H NMR ($CDCl_3$) δ 5.16 (1 H, br s, propyl NHCO), 4.82 (1 H, br s, butyl NHCO), 3.12 (4 H, m, CH_2NHCO), 2.60 (4 H, m, CH_2NHCH_2), 1.44 (6 H, m, CH_2), 1.37 (18 **H**, s, C-$(CH_3)_3$). The NH resonance is obscured by the methylene absorbance. The N^1,N^8-bis(Boc)spermidine thus synthesized (16 mg, 24 μmol) was converted to the N^4-(−)-camphanamide by a procedure similar to that described above for the synthesis of **14** and **16**: ^{1}H NMR ($CDCl_3$) identical with the spectrum obtained for **14b** and **14c** except the resonances for CH_2 adjacent to N^4 appear at δ 3.03–3.58 (4 H, overlapping multiplets).

Enzymology. Spermidine synthase (EC 2.5.1.16) was purified[40] from *E. coli* through ammonium sulfate fractionation (40–60% saturation). Further purification by DEAE-cellulose chromatography did not increase the purity of the enzyme sufficiently (1.5-fold) to warrant the decreased yield (20%). Radiolabeled dcAdoMet-^3H, putrescine-^{14}C, and spermidine-^3H were recovered unchanged after independent incubation with the partially purified enzyme under the experimental conditions described below, thus indicating the absence of contaminating enzymes capable of degrading (e.g., oxidation) these substrates and product. Attempts to further purify the enzyme by 3-*S*-adenosyl 5′-thiopropylamine-Sepharose affinity chromatography as described by Samejima and Yamanoha[41] were unsuccessful.

The incubation mixture contained 150 mM sodium phosphate, pH 8.1, 1.5 mM 1,1,4,4-tetradeuterioputrescine, 1.5 mM of either chirally deuteriated decarboxylated *S*-adenosylmethionine (**1b** or **1c**), and 1 unit of spermidine synthase[42] in a total volume of 135 mL. This mixture was incubated for 4 h at 37 °C, and the reaction was halted by acidification with TCA. Under these conditions 85% of the biologically active decarboxylated *S*-adenosylmethionine (*S* configuration at the sulfonium pole) was converted to spermidine. The precipitated protein was removed from the acidified incubation mixtures by centrifugation, and the clear supernatants were spiked with 0.3 μCi each of [2,9-^{3}**H**$_4$]spermidine trihydrochloride (15.5 Ci/mmol) and [1,4-^{14}C]putrescine dihydrochloride (100 μCi/mmol) as markers. The mixture was applied to a Dowex 50-H$^+$ ion-exchange column (Bio-Rad AG 50W-X8, 100–200 mesh) (1.5 × 4.0 cm), and the column was washed with 2.3 N HCl (ca. 60 mL) until all of the putrescine-^{14}C (98%) was eluted from the column. No spermidine-^3H was detected in the 2.3 N HCl eluant. The spermidine was then eluted with 3.3 N HCl, and the sample was concentrated in vacuo to yield a residue containing 95% of the applied ^{3}H marker.

The chirally labeled spermidine-d_5 was further purified by paper electrophoresis followed by Dowex 50-H$^+$ chromatography. The crude spermidine-d_5 (ca. 60 μmol) was dissolved in 0.5 mL of water and applied as a strip to the center of four pieces of Whatman 3MM chromatography paper (100 × 12.5 cm). The papers were electrophoresed at 2500 V for 1 h and 20 min with 0.25 M pyridinium acetate, pH 4.0, as buffer. The papers were thoroughly dried, and the amines were visualized by spraying the edges with ninhydrin. The band corresponding to spermidine was cut out, and the spermidine was eluted by descending chromatography with 0.1 N HCl. The eluate was evaporated to dryness in vacuo, and the residue dissolved in water and rechromatographed on a Dowex 50-H$^+$ column as described previously. The chirally deuteriated spermidine-d_5 recovered contained 89% of the ^{3}H marker (ca. 53 μmol of spermidine) and was demonstrated to be free from ninhydrin-positive contamination by TLC (silica gel, *n*-BuOH–HOAc–pyridine–H_2O, 3:3:2:1) and electrophoretic analysis (cellulose TLC, 0.25 M pyridinium acetate, pH 4.0, 300 V for 20 min): ^{1}H NMR (D_2O) see Figure 4.

Conversion of Enzymatically Derived [3,5,5,8,8-^{2}H$_5$]Spermidine (d_4-2) to N^1,N^8-Bis(Boc)-N^4-(−)-camphanoyl[3,5,5,8,8-^{2}H$_5$]spermidine (Scheme IV). Chirally deuteriated spermidine-d_5 was converted to the bis(Boc) camphanoyl derivative by a procedure similar to that described above for the unlabeled spermidine. To assure stoichiometric addition of formaldehyde to the labeled spermidine in the formation of the hexahydropyrimidine ring, the following reaction was carried out in an NMR tube and the progress monitored spectroscopically. Thus, chirally deuteriated spermidine-d_5 (ca. 53 μmol), derived enzymatically from **1b**, isolated and purified as described above, was transferred to an NMR tube in 0.5 mL of D_2O, the solution was adjusted to pH 11 with Na_2CO_3 (80 mg), and 20 μL of 10% TSP in D_2O was added as standard. Aliquots of 1 N formaldehyde in D_2O were added until the formation of the hexahydropyrimidine ring was complete as determined by the shift of the NMR resonance due to CH_2CHD from δ 1.82 (2 H, m) for spermidine-d_5 to δ 1.48 for the hexahydropyrimidine derivative, and the appearance of a sharp singlet at δ 3.40 for the methylene bridge between the two nitrogens of the ring.

The reaction mixture was transferred to a flask in a total volume of 2.31 mL of D_2O to which 2.3 mL of dioxane and 0.4 mL of 1 N NaOH were added. The mixture was cooled to 0 °C, treated with Boc_2O (0.40 mmol, 0.1 mL of 0.57 M solution), and stirred overnight at ambient temperature. The chirally labeled hexahydropyrimidine-d_5 product was isolated as an oil by standard procedures as described above for the corresponding unlabeled hexahydropyrimidine: ^{1}H NMR ($CDCl_3$) δ 5.02 (1 H, br, NHCO), 4.04 (2 H, m, NCH_2N), 3.42 (2 H, m, CH_2N), 2.64 (1 H, s, CHD), 1.76 (6 H, m, CH_2), 1.38 (18 **H**, d, $C(CH_3)_3$).

The d_5 bis(Boc) derivative was dissolved in 1.14 mL of 0.37 M ethanolic ethyl hydrogen malonate to which 0.202 mL of 2.09 M ethanolic pyridine was added. The reaction and workup were continued as described above for the unlabeled material to yield the chirally labeled bis(Boc)spermidine-d_5 as an oil: ^{1}H NMR ($CDCl_3$) δ 5.20 (1 **H**, br, propyl NHCO), 4.84 (1 H, br, butyl NHCO), 3.18 (2 H, q, CH_2NHCO), 2.60 (1 H, t, CHD), 1.70–1.40 (6 H, overlapping m, CH_2), 1.36 (18 H, d, $C(CH_3)_3$). The N^4H resonance was obscured by the methylene absorbance.

The N^1,N^8-bis(Boc)spermidine-d_5 was dissolved in 1.0 mL of THF and cooled to 0 °C. Diisopropylethylamine (36.5 μL, 210 μmol) and freshly sublimed (−)-camphanoyl chloride (40.8 mg, 189 μmol) were added, and the mixture was stirred overnight at ambient temperature. The N^1,N^8-bis(Boc)-N_4-(−)-camphanoyl[3,5,5,8,8-^{2}H$_5$]spermidine product was isolated by the standard procedure as detailed above for **16**. The overall recovery of ^{3}H marker through the above reaction sequence was 80%. Prior to high-field ^{1}H NMR experiments, this product was further purified by HPLC (Brownlee Silica Sphere 5 column, EtOAc–$CHCl_3$–isooctane, 35:15:35): 500-MHz ^{1}H NMR ($CDCl_3$) see Figure 5D.

Chirally deuteriated spermidine-d_5 derived enzymatically from **1c** was converted to the N^1,N^8-bis(Boc)-N^4-(−)-camphanoyl[3,5,5,8,8-^{2}H$_5$]spermidine and purified by HPLC prior to high-field ^{1}**H** NMR experiments as described above for spermidine-d_5 derived from **1b**: 500-MHz ^{1}H NMR ($CDCl_3$) see Figure 5C.

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Supplementary Material Available: Detailed description of the synthesis of **6** from **3** (Scheme I) and **13** from **3** (Scheme II), and procedures used in the NMR shift reagent studies (18 pages). Ordering information is given on any current masthead page.

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