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The synthesis of deuterium-labeled spermine, N¹-acetylspermine and N¹-acetylspermidine

Vijay Gawandi[‡] and Paul F. Fitzpatrick^{‡,§,*}

[‡]Department of Biochemistry and Biophysics Texas A&M University, College Station TX 77843-2128

[§]Department of Chemistry Texas A&M University, College Station TX 77843-2128

Summary

The synthesis of deuterium labeled spermine, N¹-acetylspermine and N¹-acetylspermidine is reported. 1,1,3,3-²H₄-N¹-Acetylspermine hydrochloride, 1,1,3,3-²H₄-N¹-acetylspermidine hydrochloride and 1,1,3,3,10,10,12,12-²H₈-spermine dihydrochloride were obtained in seven, four and three steps respectively. All the syntheses were carried out by simple protection and deprotection steps from commonly used selective protecting reagents. These deuterium labeled compounds can be used as mechanistic probes of polyamine oxidizing enzymes.

Keywords

deuterium labeling; spermine; N¹-acetylspermine; N¹-acetylspermidine; polyamine oxidase

Introduction

Polyamines such as putrescine, spermidine and spermine are naturally occurring, positively charged compounds found in virtually all living cells.^{1,2} These compounds bind DNA and have been implicated in a number of crucial cellular processes such as cell division, differentiation and membrane function.¹⁻⁴ Inhibition of polyamine synthesis, or their depletion from cells, stops cell growth, suggesting that analogs of these compounds have potential for cancer treatment.⁴⁻⁶ In support of this, several polyamine analogues exhibit antitumor effects.⁷⁻¹⁰

Spermine, N¹-acetylspermine and N¹-acetylspermidine are substrates for polyamine oxidase (PAO), whereas spermine is the substrate for spermine oxidase (SMO).¹¹⁻¹⁴ PAO in the peroxisome oxidizes N¹-acetylspermine to spermidine and 3-acetamidopropanal and N¹-acetylspermidine to putrescine and 3-acetamidopropanal (Fig. 1).¹¹ In contrast, the cytosolic enzyme SMO oxidizes spermine to spermidine and 3-aminopropanol but does not oxidize N¹-acetylspermine or N¹-acetylspermidine.¹²⁻¹⁴ Flavin adenine dinucleotide (FAD) is a noncovalently bound redox cofactor in both PAO and SMO. During the oxidation of N¹-acetylspermine, N¹-acetylspermidine and spermine, FAD is reduced; it is subsequently oxidized by oxygen to generate hydrogen peroxide.¹⁵ Hydrogen peroxide and 3-aminopropanal (obtained as a side product of the enzymatic deacetylation¹⁶ of 3-acetamidopropanal) are cytotoxic.¹⁷⁻¹⁹ 3-Aminopropanal undergoes spontaneous conversion to acrolein which is extremely cytotoxic.¹⁶ It has been proposed that the hydrogen peroxide produced by SMO and PAO may play a role in the progression of apoptosis.²⁰⁻²⁴

*Address correspondence to: Paul F. Fitzpatrick Department of Biochemistry and Biophysics 2128 TAMU College Station, TX 77843-2128 Ph: 979-845-5487 Fax: 979-845-4946 fitzpat@tamu.edu.

Relatively little mechanistic study of animal or human PAO or SMO has been reported. Syntheses of protiated analogues of spermine, N¹-acetylspermine and N¹-acetylspermidine have been reported by several researchers,²⁵⁻²⁷ but the syntheses of deuterated analogues have not. Syntheses of deuterated analogues have now been carried out using simple protection and deprotection steps. These deuterium labeled compounds can be used as mechanistic probes of polyamine oxidizing enzymes.

Results and Discussion

Although syntheses of a few polyamines, e.g., N¹-acetylspermine and N¹-acetylspermidine, have been reported,²⁵⁻²⁷ preparation of ²H₈-spermine, ²H₄-N¹-acetylspermine and ²H₄-N¹-acetylspermidine have not been described previously. Scheme 1 shows the synthesis of 1,1,3,3-²H₄-N¹-acetylspermine. Commercially available 4-aminopropanol was treated with Boc anhydride to protect the amino group of 4-aminopropanol to yield N-Boc-3-aminopropanol **1**. This compound was then activated by treatment with methane sulfonyl chloride, a hydroxyl group activating reagent, to give N-Boc-3-amino-1-methanesulfonylpropane **2**, which underwent nucleophilic substitution by the amino group of 4-aminobutanol to yield N-(N-Boc-3'-aminopropyl)-4-aminobutanol **3**. The secondary amino group of compound **3** was then protected to yield N-(N-Boc-3'-aminopropyl)-N-Boc-4-aminobutanol **4**. Activation of **4** by methane sulfonyl chloride yielded N-(N-Boc-3'-aminopropyl)-N-Boc-4-amino-1-methanesulfonylbutane **5**, which was coupled with the monoacetyl analogue of 1,1,3,3-²H₄-diaminopropane **6** to yield N¹-acetyl-1,1,3,3-²H₄-N⁹, N¹²-Boc₂-spermine **7**. The method described by Tabor and Tabor²⁷ was used to prepare **6**. Crude 1,1,3,3-²H₄-N¹-acetylspermine hydrochloride was obtained by deprotection of **7** with 4 M hydrochloric acid in dioxane at 25°C. The final compound was then purified by reverse-phase column chromatography using 20% ethanol-water as the mobile phase.

Scheme 2 presents the synthesis of 1,1,3,3-²H₄-N¹-acetylspermidine. The procedure used for the synthesis of 1,1,3,3-²H₄-N¹-acetylspermine is similar to that for 1,1,3,3-²H₄-N¹-acetylspermine.

Scheme 3 shows the synthetic route used to prepare 1,1,3,3,10,10,12,12-²H₈-spermine. 1,4-Dihydroxy butane was activated by treatment with methane sulfonyl chloride in dry pyridine to yield 1,4-dimethanesulfonylbutane **11**. Treatment of **11** with N¹-Boc-1,1,3,3-²H₄-1,3-diaminopropane **12** gave N¹, N¹²-Boc₂-²H₈-spermine **13**, which was deprotected with hydrochloric acid in dioxane to generate 1,1,3,3,10,10,12,12-²H₈-spermine.

Experimental

Materials

3-Aminopropanol, 4-aminobutanol and 1,4-dihydroxybutane were obtained from Aldrich Chemicals. 1,1,3,3-²H₄-1,3-Diaminopropane was obtained from C/D/N Isotopes, Inc. All other reagents were of the highest purity commercially available. ¹H NMR spectra were acquired on an Inova 300 MHz spectrometer.

Synthesis of 1,1,3,3-²H₄-N¹-acetylspermine trihydrochloride

(A) N-Boc-3-aminopropanol (1): To 3-aminopropanol (0.98 g, 13 mmol) in 5 mL of dry acetonitrile was added dropwise 1.1 equivalent (3.12 g, 14.3 mmol) of (Boc)₂O in 2.0 equivalent (1.4 ml, 26 mmol) of triethylamine. After 6 h at 25°C an additional 0.5 equivalent (1.42 g) of (Boc)₂O was added. The reaction was continued overnight until silica gel thin-layer chromatography in hexane-ethylacetate (8:2) showed one major spot. The solvent was evaporated *in vacuo*. The brown residue was treated with 1 M KHSO₄ (60 mL) and ether (100

mL). The solution was extracted with ether (4 × 25 mL), and the combined organic layers were washed with 50 mL each of 1 M KHSO₄ and 1 M NaHCO₃ and twice with a saturated solution of NaCl. The yellowish extract was dried (MgSO₄) and the solvent removed under vacuum. The brown residue was chromatographed on silica using hexane-ethylacetate (8:2) to give 1.63 g (66 %) N-Boc-3-aminopropanol **1**. ¹H NMR (CDCl₃): δ 7.38 (1H, s), 3.72 (2H, m), 1.71 (2H, m), 2.55 (2H, t), 1.32 (9H, s).

(B) N-Boc-3-amino-1-methanesulfonypropane (2): The Boc-protected aminopropanol (0.82 g, 4.7 mmol) was added to 1.2 equivalents (0.65 g, 5.64 mmol) of methane sulfonyl chloride in 2 mL anhydrous pyridine. After 2 h at 25 °C, the reaction mixture was treated with 1 M NaHCO₃ (30 mL) and ether (70 mL). The solution was extracted with ether (3 × 25 mL), and the combined organic layers were washed with a NaCl solution (2 × 50 mL). A brownish crude product was obtained after evaporation of solvent. Flash silica gel column chromatography using hexane - ethyl acetate (8:2) as solvent yielded 0.79 g (62 %) N-Boc-3-amino-1-methanesulfonypropane **2**. ¹H NMR (CDCl₃): δ (ppm) 2.35 (s, 3H), 4.21 (t, 2H), 1.72 (m, 2H), 2.81 (t, 2H), 1.35 (s, 9H).

(C) N-(N-Boc-3'-aminopropyl)-4-aminobutanol (3): Compound **2** (0.79 g, 3.1 mmol) was added to 1.0 equivalent (0.28 g) of 4-aminobutanol in 5 mL dry dichloromethane. After 5 h at 50 °C, the reaction mixture was cooled, washed with saturated Na₂CO₃ and extracted with ether. Flash silica gel chromatography using hexane-ethyl acetate (9:1) as solvent gave 0.51 g (66 %) N-(N-Boc-3'-aminopropyl)-4-aminobutanol **3**. ¹H NMR (CDCl₃): δ 7.40 (s, 1H), 2.55 (t, 2H), 1.75 (m, 2H), 2.60 (m, 2H), 2.55 (m, 2H), 1.81 (m, 2H), 1.72 (m, 2H), 3.75 (m, 2H), 1.35 (s, 9H).

(D) N-(N-Boc-3'-aminopropyl)-N-Boc-4-aminobutanol (4): Compound **3** (0.51 g, 2.07 mmol) was added to 1.0 equivalent (0.45 g) of (Boc)₂O in 2.0 equivalent (0.6 mL, 4.14 mmol) of triethylamine in 10 mL of dry acetonitrile at 25 °C; the reaction was continued overnight. Pure N-(N-Boc-3'-aminopropyl)-N-Boc-4-aminobutanol **4** (0.325 g, 86 %) was obtained following the procedure described for **1**. ¹H NMR (CDCl₃): δ 2.60-2.66 (m, 6H), 1.78-1.82 (m, 6H), 3.72 (m, 2H), 1.40 (s, 18H).

(E) N-(N-Boc-3'-aminopropyl)-N-Boc-4-amino-1-methanesulfonybutane (5): The reaction of **4** (0.33 g, 0.94 mmol) with 1.2 equivalent (0.13 g) methane sulfonyl chloride as described above for the preparation of **2** yielded 0.33 g (85 %) N-(N-Boc-3'-aminopropyl)-N-Boc-4-amino-1-methanesulfonybutane **5**. ¹H NMR (CDCl₃): δ 2.40 (s, 3H), 2.82-2.86 (m, 6H), 1.68-1.75 (m, 6H), 4.23 (m, 2H), 1.39 (s, 18H).

(F) N¹-Acetyl-1,1,3,3-²H₄-1,3-diaminopropane (6): The synthesis of **6** was adapted from the procedure of Tabor and Tabor.²⁷ 1,1,3,3-²H₄-1,3-Diaminopropane (0.6 g, 7.69 mmol) was added to 45 mL of cooled glacial acetic acid with magnetic stirring. The mixture was heated to 55-60 °C. A mixture of 0.36 mL of acetic anhydride (0.5 equivalent) in 10 mL of glacial acetic acid was added dropwise with stirring during a 1 h period. The mixture was stored at 25°C overnight and then evaporated to dryness. The residue was dissolved in hot water, cooled and adjusted to acidic pH with 6 N HCl. After evaporation to dryness in vacuo, the resultant solid was extracted twice with 50 mL 2-propanol, and the insoluble residue (unreacted 1,3-diaminopropane hydrochloride) was discarded. The combined filtrates were concentrated and cooled to -10 °C. The crystals that formed were collected by filtration and recrystallized from 100 mL of hot 2-propanol. The yield of **6** was 0.48 g (73 %). ¹H NMR (CDCl₃): δ 2.05 (s, 3H), 1.65 (s, 2H).

(G) N¹-Acetyl-1,1,3,3-(²H₄)-N⁹,N¹²-Boc₂-spermine (7): Compound **5** (0.33 g, 0.84 mmol) was added to 1.3 equivalent (0.13 g, 1.09 mmol) of **6** in 5.0 mL of dry dichloromethane. After

5 h at 50 °C, workup similar to that described for **3** yielded 0.25 g (62 %) of compound **7**. ^1H NMR (CDCl_3): δ 2.11 (s, 3H), 2.85-2.88 (m, 8H), 1.80-1.82 (m, 4H), 1.74-1.76 (m, 4H), 1.40 (s, 18H).

(H) 1,1,3,3- $^2\text{H}_4$ -N 1 -acetylspermine trihydrochloride: Compound **7** (0.22 g, 0.5 mmol) was treated with 10 ml of 4 M HCl in dioxane (5 mL) with stirring at 25°C for 5 h. Most of the solvent was evaporated in vacuo and the sticky residue was then dissolved in distilled water (50 mL) and extracted with ether (3 \times 20 mL). The aqueous layer was then passed through a C18 reverse phase column. The compound was eluted with 20% ethanol-water. Fractions were collected and flushed with N_2 and lyophilized to afford 42 mg (24 %) of 1,1,3,3- $^2\text{H}_4$ -N 1 -acetylspermine trihydrochloride. ^1H NMR (D_2O): δ 1.98 (s, 3H), 3.11 (m, 8H), 2.06 (m, 4H), 1.83 (m, 4H). HRMS ($m + H$) theor: 249.4121, found: 249.4130.

Synthesis of N 1 -acetylspermidine dihydrochloride

(A) N-Boc-4-aminobutanol (8): 4-Aminobutanol (1.02 g, 11.5 mmol) in 5 mL of dry acetonitrile was added to 1.1 equivalent (2.76 g) of $(\text{Boc})_2\text{O}$ in 2.0 equivalent (3.2 ml, 23 mmol) of triethylamine and kept stirring at 25 °C overnight. Further workup and purification as described for **1** yielded 1.81 g (78 %). ^1H NMR (CDCl_3): δ : 2.88 (s, 1H), 3.67 (m, 2H), 1.63 (m, 2H), 1.58 (m, 2H), 2.95 (m, 2H), 1.31 (s, 9H).

(B) N-Boc-4-amino-1-methanesulfonybutane (9): Methane sulfonyl chloride (1.32 g, 11.5 mmol, 1.2 equivalent) in anhydrous pyridine was added dropwise to the Boc-protected aminobutanol **8** (1.81 g, 9.6 mmol). After 2 h at 25 °C, workup and purification as described for **2** gave 1.9 g (69 %). ^1H NMR (CDCl_3): δ 2.37 (s, 3H), 3.81 (m, 2H), 1.69 (m, 2H), 1.60 (m, 2H), 2.97 (t, 2H), 1.35 (s, 9H).

(C) N 1 -Acetyl-1,1,3,3- $^2\text{H}_4$ -N 8 -Boc-spermidine (10): Compound **6** (0.3 g, 1.0 equivalent) in 5.0 mL of dry dichloromethane was added to 2.5 mmol (0.67 g) **9**. After 5 h at 50 °C, workup as described for **3** gave 0.25 g (34 %) of compound **10**. ^1H NMR (CDCl_3): δ 2.105 (s, 3H), 2.88-2.92 (m, 8H), 1.78-1.82 (m, 4H), 1.33 (s, 9H).

(D) 1,1,3,3- $^2\text{H}_4$ -N 1 -acetylspermidine dihydrochloride: Compound **10** (0.146 g, 0.5 mmol) was treated with 10 ml of 4 M HCl in dioxane (5 mL) with stirring at 25 °C for 5 hr. Pure 1,1,3,3- $^2\text{H}_4$ -N 1 -acetylspermidine hydrochloride (50 mg, 38 %) was obtained by following the procedure described for 1,1,3,3- $^2\text{H}_4$ -N 1 -acetylspermine hydrochloride. ^1H NMR (D_2O): δ 1.99 (s, 3H), 3.150 (m, 4H), 2.12 (m, 2H), 1.73 (m, 4H); HRMS ($m + H$) theor: 192.3224, found: 192.3214.

Synthesis of 1,1,3,3,10,10,12,12- $^2\text{H}_8$ -spermine tetrahydrochloride:

(A) 1,4-Dimethanesulfonyl-butane (11): Methane sulfonyl chloride (3.15 g, 27.5 mmol, 2.5 equivalent) in anhydrous pyridine was added to 11 mmol (1.0 g) of 1,4-dihydroxybutane, and the mixture was stirred for 2 h at 25°C. Further steps as described for **2** gave 2.76 g (55 %) of compound **11**. ^1H NMR (CDCl_3): δ 2.40 (s, 6H), 4.16 (t, 4H), 1.99 (m, 4H).

(B) N 1 -Boc-1,1,3,3- $^2\text{H}_4$ -1,3-diaminopropane (12): One equivalent (0.56 g) of $(\text{Boc})_2\text{O}$ in 2.0 equivalent (0.7 ml, 5.12 mmol) of triethylamine was added to 2.56 mmol (0.2 g) of 1,1,3,3- $^2\text{H}_4$ -1,3-diaminopropane in 5 mL of dry acetonitrile while stirring at 25 °C overnight. Workup and purification as described for **1** gave 0.24 g (53 %) of compound **12**. ^1H NMR (CDCl_3): δ 1.30 (s, 9H), 1.70 (s, 2H).

(C) N 1 ,N 12 -Boc-1,1,3,3,10,10,12,12- $^2\text{H}_8$ -spermine (13): Compound **11** (0.172 g, 0.7 mmol) was treated with 2.0 equivalents (0.249 g, 1.4 mmol) of **12** in 4 ml dry dichloromethane at 50

°C for 5 h. The crude product was purified by flash silica gel chromatography using hexane-ethylacetate (9:1) as solvent to afford 0.26g (64 %) of compound **13**. ^1H NMR (CDCl_3): δ 1.35 (s, 18H), 1.74 (s, 4H), 2.82 (m, 4H), 1.85 (m, 4H).

(D) 1,1,3,3,10,10,12,12- $^2\text{H}_8$ -spermine tetrahydrochloride: Compound **13** (0.254 g, 0.62 mmol) was further treated with 10 ml of 4 M hydrochloric acid in 5 mL of dioxane. The pure $^2\text{H}_8$ -spermine tetrahydrochloride was obtained by the procedure described for 1,1,3,3- $^2\text{H}_4$ - N^1 -acetylspermine hydrochloride. The yield of the final product was 60 mg (27 %).

^1H NMR (D_2O): δ 2.61 (m, 4H), 1.62 (m, 4H), 1.51 (m, 4H); HRMS ($m + H$) theor: 211.4113, found: 211.41125.

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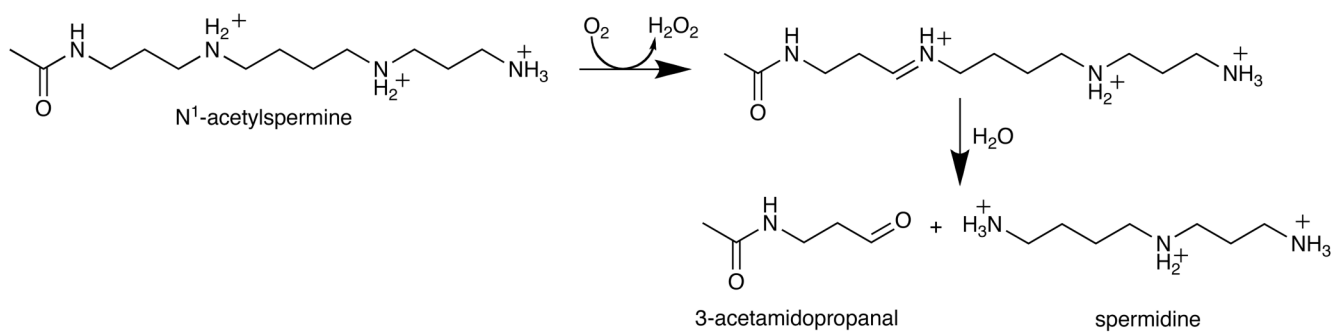
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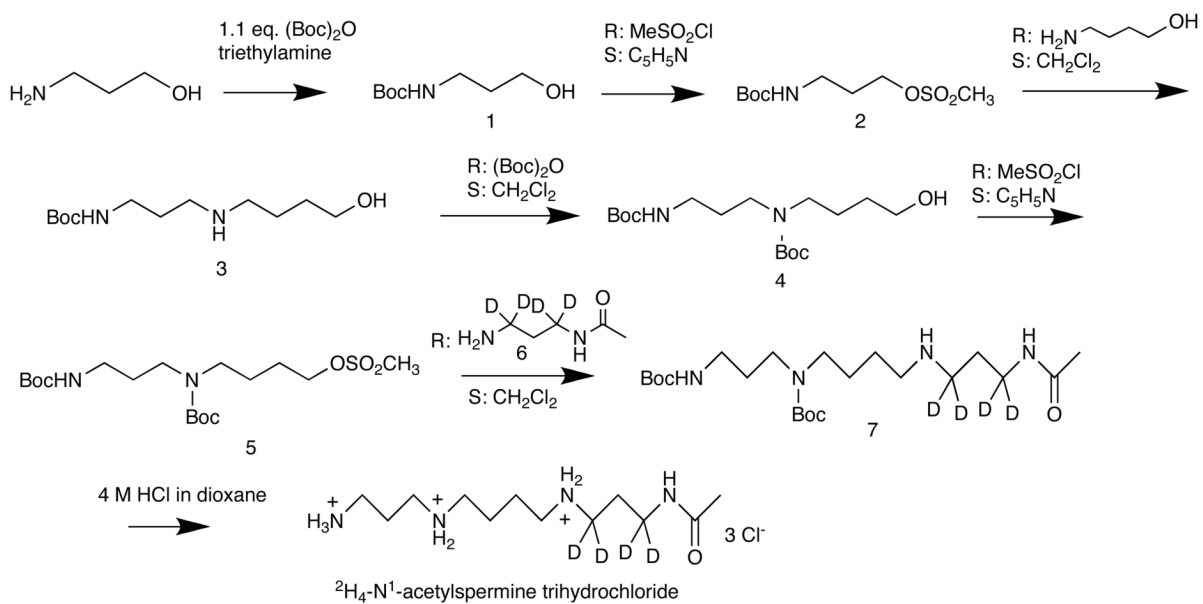
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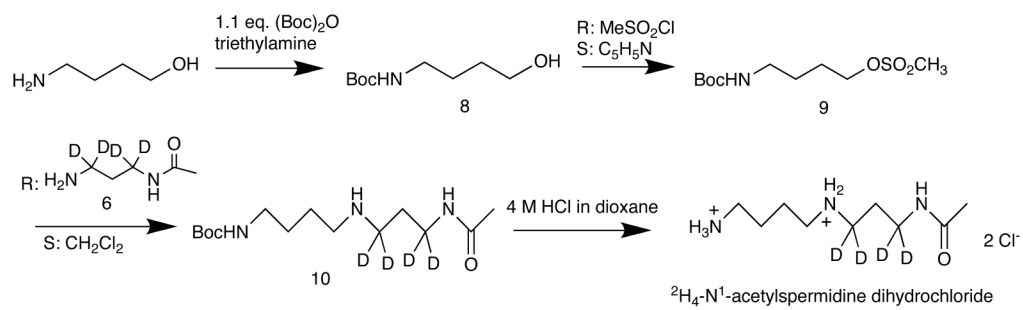
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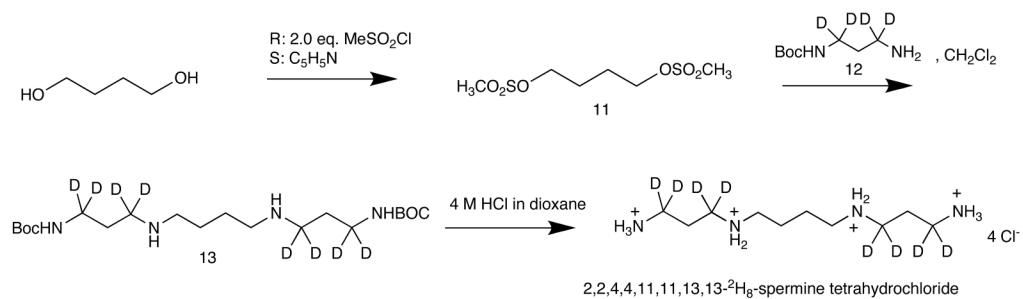
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**Figure 1.**



Scheme 1.

**Scheme 2.**

**Scheme 3.**