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Syntheses of ^{15}N-Enriched Polyamines[1)]

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A total synthesis of polyamines was developed using potassium phthalimide as a sole nitrogen source. ^{15}N-Enriched phthalimide was prepared almost quantitatively from ^{15}N-enriched ammonium sulfate by a modified method. Key compounds for the synthesis of ^{15}N-enriched spermidine and spermine were ^{15}N-enriched putrescine (I), *N*-(3-bromopropyl)-phthalimide (II), *N*-(4-bromobutyl)phthalimide (III), and benzylamine, which were easily prepared in high yields from potassium ^{15}N-enriched phthalimide. Spermidine was synthesized by two alternative methods involving an alkylation of monobenzyloxycarbonyl putrescine with II and a stepwise alkylation of benzylamine with II and III in the presence of KF–Celite. The lattter method allowed the preparation of all seven kinds of various ^{15}N-enriched sperm-idines with combinations of the three reagents containing ^{15}N or ^{14}N. Spermine was similarly synthesized by an alkylation of *N,N'*-dibenzylputrescine with II. In these methods, the alkylation using KF–Celite was extremely useful for the synthesis of spermidine and spermine. The present methods were also used to synthesize various other polyamines in high yields.

Keywords——natural polyamine; total synthesis; ^{15}N-enriched ammonium sulfate; potassium ^{15}N-enriched phthalimide; ^{15}N-enriched polyamine; alkylation; KF–Celite

Putrescine, spermidine and spermine are the main polymaines that are widely distributed in living organisms. It is possible on the basis of many observations[2)] that these polyamines participate in the regulation of cell growth, proliferation and differentiation through interactions with a variety of cellular macromolecules, *e.g.* nucleic acids, proteins, lipids, *etc.*, but the mechanisms involved are still unknown. As for the regulation of polyamines, extensive studies[2)] have been made on their metabolism. Recent findings[3)] on the metabolism of spermidine and spermine to putrescine and spermidine, respectively, *via* N^1-acetylation followed by oxidation, clearly showed that the levels of polyamines are diversely controlled by both biosynthetic and biodegradative pathways.

In studies related to the physiological significance of polyamines, ^{15}N-enriched polyamines should be useful, permitting the use of ^{15}N-nuclear magnetic resonance spectroscopy or mass spectrometry. However, no attempt has been made to synthesize such polyamines. The present paper deals with methods for the high-yield synthesis of ^{15}N-enriched putrescine, spermidine and spermine using ammonium sulfate as a ^{15}N-nitrogen source. The methods were also successfully applied to syntheses of other polyamines.

Results and Discussion

It is well known that potassium phthalimide is a generally useful reagent for introducing an amine group into organic compounds. A desirable approach for our purpose was, therefore, to use the reagent as a sole nitrogen source for the total synthesis of polyamines. In setting up the methods, a matter of primary concern was, as in other methods available in

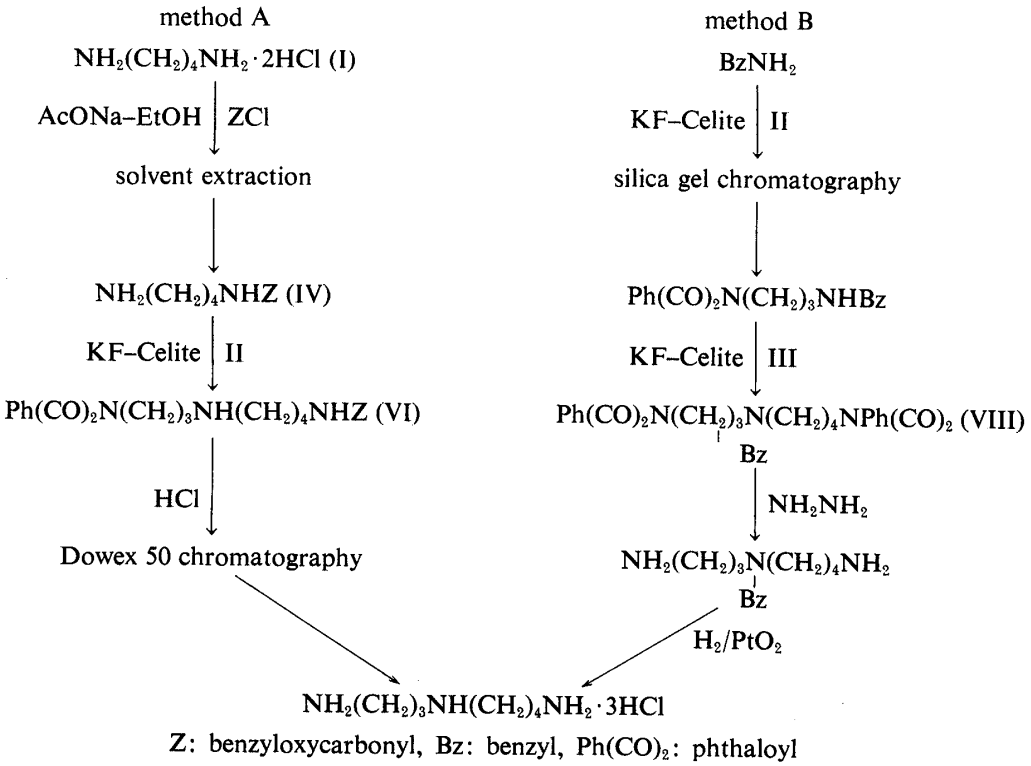
synthetic polyamine chemistry,[4] how to attain a high yield, by limiting the inevitable loss that occurred during alkylation of the amine group due to the lack of a genuine sitespecific reaction. After preliminary experiments, we finally established the procedures described below, using neutral alkylation with KF–Celite[5] for the synthesis of spermidine and spermine.

This work began with the preparation of ^{15}N-enriched phthalimide from ^{15}N-enriched ammonium sulfate, which is not excessively expensive. The popular synthetic method for phthalimide consists of the reaction of phthalic anhydride with excess amounts of ammonium hydroxide or ammonium carbonate.[6a] To save ^{15}N-ammonia in this case, we adopted an alternative method using mono-ammonium phthalate which was prepared by introducing ammonia gas evolved from ammonium sulfate under alkaline conditions into an ethanol solution of a slight excess of phthalic acid. By this method we could obtain ^{15}N-enriched phthalimide in almost quantitative yield. Potassium ^{15}N-enriched phthalimide was prepared in the usual way.[6b]

The key compounds for the present synthesis of spermidine and spermine were putrescine dihydrochloride (I) and *N*-(3-bromopropyl)-phthalimide (II) as an aminopropyl donor. ^{15}N-Enriched I was easily prepared by the reaction of 1,4-dibromobutane with the calculated amount of potassium ^{15}N-enriched phthalimide in *N,N*-dimethylformamide (DMF), followed by acid hydrolysis and cation exchange chromatography. We avoided an alternative method using hydrazine for removal of the protecting phthalic group on account of a possible loss of volatile putrescine and its inadequate transfer to an organic solvent such as chloroform. ^{15}N-Enriched II was prepared by the reaction of potassium ^{15}N-enriched phthalimide with a large excess of 1,3-dibromopropane in DMF.

Synthesis of ^{15}N-Enriched Spermidine

Two methods were developed for the synthesis. Method A involved an alkylation of *N*-(benzyloxycarbonyl)putrescine (IV) with II followed by removal of the protecting groups and



Z: benzyloxycarbonyl, Bz: benzyl, $Ph(CO)_2$: phthaloyl

Chart 1. Synthesis of Spermidine

purification of spermidine with a cation exchange column (Chart 1). The problem in this reaction was the low yield (approximately 40%) of IV, which is needed in a larger amount than II in order to reduce production of the *N,N*-bis-alkylated by-product. However, we chose IV as a phthalimidopropyl group acceptor for the following reasons: (1) almost pure IV could be easily separated by a simple extraction from a reaction mixture containing unreacted putrescine and *N,N'*-bis(benzyloxycarbonyl)putrescine (V), which are inevitably present in these attempts to mono-derivatize symmetrical diamines, (2) putrescine and V in the reaction mixture were mostly recovered as I by a cation exchange chromatography after appropriate treatments as described in Experimental, and (3) the yield of IV under the conditions described was comparable to those reported for mono-trifluoroacetylated or *tert*-butyloxycarbonylated diamines.[7] Two equivalents of IV thus obtained was smoothly alkylated with an equivalent of II in acetonitrile under reflux for a few hours in the presence of KF–Celite to give *N*-(3-phthalimidopropyl)-*N'*-(benzyloxycarbonyl)putrescine (VI) and a trace of *N,N*-bis(3-phthalimidopropyl)-*N'*-(benzyloxycarbonyl)putrescine (VII) together with IV.[8] After acid hydrolysis of the reaction mixture, spermidine and putrescine were chromatographed on a Dowex 50 column. We obtained recrystallized ^{15}N-enriched spermidine trihydrochloride in 70% yield from ^{15}N-enriched II, and the combined recovery of spermidine trihydrochloride and I was approximately 90% from ^{15}N-enriched IV.

Method B[9] involved a successive alkylation of benzylamime, which is easily prepared from potassium phthalimide and benzylchloride, with II or similarly prepared *N*-(4-bromobutyl)phthalimide (III) using KF–Celite (Chart 1). The first alkylation with either one of the two produced a relatively low amount of *N,N*-bis(phthalimidoalkyl)benzylamine relative to *N*-(phthalimidoalkyl)benzylamine, and the bis-derivative and unreacted benzylamine were removed by silica gel column chromatography using a solvent system of benzene and acetone. The purified *N*-(phthalimidoalkyl)benzylamine was re-alkylated similarly with the other *N*-(bromoalkyl)phthalimide (II or III) to give *N*-(3-phthalimidopropyl)-*N*-(4-phthalimidobutyl)benzylamine (VIII). After removal of the protecting phthalic group with hydrazine, the resulting N^4-benzylspermidine was hydrogenolyzed to spermidine. With various combinations of the three reagents containing ^{15}N or ^{14}N, method B thus allowed the preparation of all seven kinds of spermidines variously ^{15}N-enriched at N^1, N^4, and N^8, some of which were, of course, also prepared by method A (Table I). In addition, both method A and method B allow the synthesis of other naturally occurring triamines such as *sym*-norspermidine, *sym*-homospermidine, aminopropylcadaverine, *etc.*

Synthesis of ^{15}N-Enriched Spermine

A selective method was developed for the synthesis as outlined in Chart 2. The first step, involving preparation of *N,N'*-bis(benzyl)putrescine (X), proceeded almost quantitatively without isolation of the intermediate Schiff base of putrescine and benzaldehyde, which was reduced with $NaBH_4$. Although the crude ether extract of the reaction mixture showed a few ultraviolet (UV)-absorbing trace bands on thin layer chromatography (TLC), the sample of X was subjected without further purification to the next alkylation step with II. The alkylation of the secondary amine groups was completed after refluxing for 20 h in acetonitrile in the presence of KF–Celite. The resulting bis-alkylated spermine precursor (XI), when contaminated with the starting compounds and mono-alkylated by-product (XII), was easily purified by silica gel column chromatography with stepwise elution (increasing concentrations of acetone in benzene). This chromatographic system also permitted the recovery of pure starting compounds as well as pure XII. As described above in method B for spermidine, the purified XI was first deprotected by hydrazine treatment followed by catalytic reduction to spermine, which was recrystallized as terahydrochloride. By this method we could obtain pure ^{15}N-enriched spermine tetrahydrochloride in 78% yield from ^{15}N-enriched I.

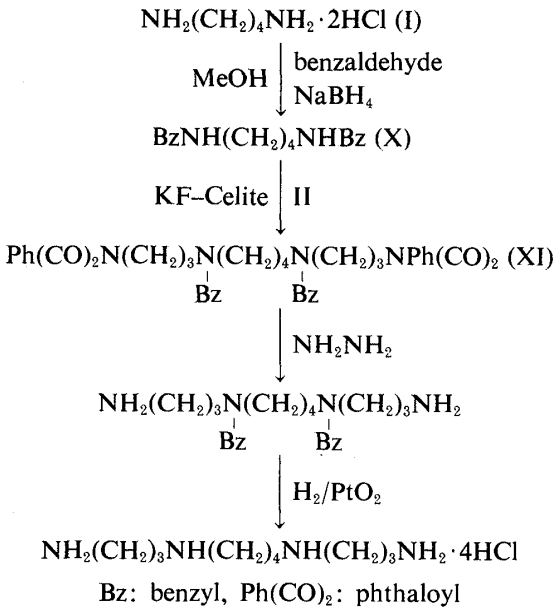


Chart 2. Synthesis of Spermine

Furthermore, this method with a slight modification was applied for the preparation of both symmetrical and unsymmetrical tetraamines (Table II), *e.g.* 1,13-diamino-4,9-diazatridecane, 1,14-diamino-5,10-diazatetradecane, and the natural products thermospermine and canavalmine. The symmetrical tetraamines were prepared by the combination of *N,N'*-dibenzyl-1,3-diaminopropane (IX) or X with III for canavalmine or 1,14-diamino-5,10-diazatetradecane, respectively. For unsymmetrical tetraamines, IX or X was mono-alkylated with II or III. The product was purified by silica gel chromatography, then the second alkylation was carried out with the other substituted phthalimide (III or II). After deprotection, the former gave thermospermine and the latter 1,13-diamino-4,9-diazatridecane.

In conclusion, all the methods described above are useful not only for the synthesis of ^{15}N-enriched polyamines but also for general syntheses of polyamines, offering high yields and wide applicability.

Experimental

Materials——^{15}N-Enriched ammonium sulfate (99% atom% ^{15}N, purity >95%) was obtained from CEA, France. All organic solvents and reagents were of analytical reagent grade. *o*-Phthalic acid, benzaldehyde, anhydrous magnesium sulfate, and DMF were purchased from Wako Pure Chemical Industries, Ltd. (Osaka), and 1,3-dibromopropane, 1,4-dibromobutane, benzyloxycarbonyl chloride, sodium borohydride, and acetonitrile were from Nakarai Chemical, Ltd. (Kyoto). Benzyl chloride, hydrazine hydrate (*ca.* 100%), and triethylamine were purchased from Tokyo Kasei, Ltd. (Tokyo), and platinic oxide from Kawaken Fine Chemicals, Ltd. (Tokyo). Potassium fluoride and Celite (535, a product of Johns Manville) were obtained from Wako.

For column chromatography, a cation exchange resin (Dowex 50W-X8, 200—400 mesh, Dow Chemical Co.), and a silica gel (Wako gel C-300, Wako) were used. For TLC, pre-coated silica gel plates (Silica gel 60 F-254, E. Merck) were used. Developing solvents for di- and polyamines were *n*-BuOH–AcOH–pyridine–H_2O (3:3:2:1) and *n*-BuOH–AcOH–pyridine–HCHO (3:3:2:1) as described in a previous report.[10]

Potassium ^{15}N-Enriched Phthalimide——A solution of $(^{15}NH_4)_2SO_4$ (14.52 g, 0.11 mol) dissolved in 12 ml of H_2O was placed in a 50 ml Pyrex three-necked flask tightly fitted with a dropping funnel and two connecting glass tubes. Then 19 ml of 21.4 N NaOH solution was added dropwise to the stirred solution under a continuous N_2 stream at room temperature; after the end of the addition, the temperature was gradually elevated to 135 °C. This procedure resulted in controlled generation of $^{15}NH_3$ gas, which was passed into a solution of *o*-phthalic acid (40 g, 0.24 mol) dissolved in 400 ml of hot EtOH[11] with an increasing precipitate of ammonium phthalate. The resulting ammonium phthalate, after removal of the solvent, was transferred to a 200 ml flask fitted with a top-open three-way joint

coupled to a condenser connected to an aspirator. The flask was then heated to 260 °C in a sand bath for 2 h under mild aspiration, and allowed to cool. This procedure effectively removed H_2O and subliming material. The resulting ^{15}N-enriched phthalimide was recrystallized from EtOH; yield, 31.27 g (97.5%). mp 235 °C (uncorr.).

Potassium ^{15}N-enriched phthalimide was prepared according to the literature[6b] using ^{15}N-enriched phthalimide (64.11 g). The yield was 68.79 g (85 4%). The remaining potassium phthalimide in the filtrate was recovered as phthalimide by acidification with dil. HCl (6.83 g, 10.2%).

^{15}N-Enriched Putrescine Dihydrochloride (I)[12]——A mixture of potassium ^{15}N-enriched phthalimide (24.2 g, 0.13 mol) and 1,4-dibromobutane (14.0 g, 65 mmol) in 110 ml of DMF was stirred at 90 °C for 3 h, and after removal of about 80 ml of DMF *in vacuo*, the residual suspension was poured over 500 ml of ice-cold H_2O. The resulting precipitate was collected, and hydrolyzed with 220 ml of an equimolar mixture of acetic acid and conc. HCl in a sealed glass tube at 120 °C for 3 d. The reaction mixture was allowed to cool, and precipitated phthalic acid was removed by filtration. The filtrate was concentrated to dryness, and the residue was dissolved in about 100 ml of H_2O. After removal of insoluble phthalic acid, the solution was applied to a Dowex 50 (H^+) column (150 ml). The first eluate with 120 ml of 0.5 N HCl and 100 ml of 1 N HCl contained a trace ninhydrin-positive by-product and was discarded, and the next eluate with 1000 ml of 2 N HCl and 200 ml of 4 N HCl was collected. After evaporation, the residue was subjected to a repeated recrystallization from aqueous EtOH and Et_2O to give ^{15}N-enriched I in a yield of 86.8% (9.2 g). *Anal.* Calcd for $C_4H_{14}Cl_2{}^{15}N_2$: C, 29.46; H, 8.65; ^{15}N, 18.40. Found: C, 29.52; H, 8.64; ^{15}N, 18.38.

^{15}N-Enriched *N*-(3-Bromopropyl)phthalimide (II)[13]——A mixture of potassium ^{15}N-enriched phthalimide (5.59 g, 30 mmol) and 1,3-dibromopropane (60.57 g, 300 mmol) in 50 ml of DMF was stirred at 60 °C for 2 h, then most of the DMF and 1,3-dibromopropane was evaporated off *in vacuo*. The resulting residue was extracted with $CHCl_3$ and H_2O. Removal of $CHCl_3$ left a white solid, which was recrystallized from EtOH to give crystalline ^{15}N-enriched II, mp 70.8 °C (uncorr.), yield 92.3% (7.45 g). *Anal.* Calcd for $C_{11}H_{10}Br_1{}^{15}N_1O_2$: C, 49.09; H, 3.75; ^{15}N, 5.58. Found: C, 49.22; H, 3.70; ^{15}N, 5.50.

N-(4-Bromobutyl)phthalimide (III) was similarly prepared using 1,4-dibromobutane. mp 78.2 °C (uncorr.) for ^{15}N-enriched III. *Anal.* Calcd for $C_{12}H_{12}Br_1{}^{15}N_1O_2$: C, 50.90; H, 4.27; ^{15}N, 5.30. Found: C, 50.82; H, 4.30; ^{15}N, 5.25.

^{15}N-Enriched *N*-(Benzyloxycarbonyl)putrescine (IV)——A solution of ^{15}N-enriched I (7.34 g, 45 mmol) in 55 ml of 2 N AcONa and 180 ml of 99% EtOH was cooled to <0 °C on ice/NaCl. Benzyloxycarbonyl chloride (6.5 ml, 45 mmol) and then 6.75 ml of 4 N NaOH solution were added to the vigorously stirred solution. A precipitate of NaCl appeared during 1 h. The reaction mixture was stirred at 4 °C for 20 h. The solvent was then evaporated off *in vacuo* below 50 °C, and 100 ml of 3 N AcOH was added to the residue. The mixture was extracted with $CHCl_3$ (100 ml × 2). The $CHCl_3$ extract was again washed with 50 ml of 3 N AcOH. Practically pure *N*,*N'*-bis-(benzyloxycarbonyl)putrescine (V) was obtained in this $CHCl_3$ extract. The combined AcOH solution was then made alkaline by the addition of 150 ml of 4 N NH_4OH, and extracted with $CHCl_3$ (100 ml × 3). Unreacted putrescine (TLC, *n*-BuOH–AcOH–H_2O = 3 : 2 : 2, *Rf* 0.40) and an unidentified ninhydrin-positive compound (*Rf* 0.55) stayed in the aqueous phase. Practically pure ^{15}N-enriched IV (*Rf* 0.80) was obtained as an oil from the resulting $CHCl_3$ extract in a yield of 36.7% (3.7 g). The oil, without further purification, was used for the next alkylation. The oil had to be handled under an N_2 atmosphere, since IV quickly absorbed CO_2 from air to form a salt insoluble in CH_3CN, the solvent for the next reaction.

Isolation of ^{15}N-Enriched I from the Fraction Containing V: After removal of $CHCl_3$, V was obtained as a white powder. It was then refluxed in 40 ml of 6 N HCl for 20 h. The residue was once extracted with Et_2O and H_2O, and the aqueous phase was applied to a Dowex 50 column (H^+) (50 ml). Stepwise elution with HCl as described above gave pure ^{15}N-enriched I in a yield of 20.7% (1.52 g).

Isolation of ^{15}N-Enriched I from the Ammoniacal Fraction: The ammoniacal fraction was diluted 7-fold with H_2O and neutralized with AcOH (about 10 ml). This solution was applied to a Dowex 50 column (H^+) (50 ml), which was first washed with 1.5 l of 0.2 N HCl and then eluted with 300 ml of 2 N HCl. Pure ^{15}N-enriched I was recovered in a yield of 28.2% (2.07 g).

Hence, the loss of ^{15}N-enriched I used for the preparation of IV was less than 15%.

^{15}N-Enriched Spermidine Trihydrochloride——Method A: A stirred solution of ^{15}N-enriched IV (3.7 g, 16.5 mmol) and ^{15}N-enriched II (2.15 g, 8 mmol) in 32 ml of CH_3CN was refluxed for 2 h in the presence of KF–Celite (3.2 g) prepared according to the literature.[5] The end of the reaction was confirmed by TLC (disappearance of II; *Rf* 0.8, benzene–acetone = 5 : 1). KF–Celite was then removed by filtration. The filtrate was evaporated to dryness, and 40 ml of 6 N HCl was added to the oily residue. The solution was refluxed for 20 h, then the solvent was removed. The residue was extracted with Et_2O and H_2O, and the aqueous phase was applied to a Dowex 50 (H^+) column (50 ml). Putrescine and spermidine were separated by stepwise elution with 1 N HCl (100 ml), 1.5 N HCl (200 ml), and 3 N HCl (400 ml) using a fraction collector. Most of the putrescine was eluted with 1.5 N HCl and spermidine with 3 N HCl. After removal of the solvent, the residue from each fraction was recrystallized from aqueous EtOH and Et_2O. Repeated recrystallization gave ^{15}N-enriched I (1.47 g, 9.02 mmol) and ^{15}N-enriched spermidine trihydrochloride (1.43 g, 5.55 mmol). The combined recovery, 14.57 mmol, corresponded to 88.3% of the ^{15}N-enriched IV used. *Anal.* Calcd for $C_7H_{22}Cl_3{}^{15}N_3$: C, 32.63; H, 8.61; ^{15}N, 17.47. Found: C, 32.79; H, 8.60; ^{15}N, 17.62.

Method B: ^{15}N-Enriched benzylamine was prepared by the reaction of potassium ^{15}N-enriched phthalimide

and benzyl chloride according to a slight modification of the reported method.[14] A stirred solution of ^{15}N-enriched benzylamine (1.5 mmol) and ^{15}N-enriched II (1.5 mmol) in 6 ml of CH_3CN was refluxed for 2 h in the presence of KF–Celite (0.6 g). The filtrate, after removal of KF–Celite, was evaporated and the residue was dissolved in benzene (5 ml). This solution was applied to a silica gel column (5 g) equilibrated with benzene. The column was successively eluted with benzene (40 ml, unreacted II), benzene–acetone (20 : 1) (25 ml, *N,N*-bis(phthalimidopropyl)benzylamine, 0.23 mmol), benzene–acetone (20 : 4) (45 ml, *N*-(phthalimidopropyl)benzylamine, 0.76 mmol), and benzene–acetone (20 : 8) (unreacted benzylamine). The resulting *N*-(phthalimidopropyl)benzylamine (0.76 mmol; overall yield from benzylamine, 50.7%) was similarly refluxed for 14 h in the presence of 0.76 mmol of ^{15}N-enriched III, 0.3 g of KF–Celite, and 3 ml of CH_3CN. After similar purification by silica gel (4 g) column chromatography, *N*-(3-phthalimidopropyl)-*N*-(4-phthalimidobutyl)benzylamine (VIII) was obtained in the benzene–acetone (20 : 1) eluate (0.65 mmol; overall yield from benzylamine, 43.3%). A solution of VIII (0.65 mmol) in 6 ml of MeOH containing 100% $NH_2NH_2 \cdot H_2O$ (0.5 ml) was refluxed for 3 h, and evaporated *in vacuo*. The residue was shaken for 30 min with 6 ml of $CHCl_3$ and 6 ml of 4 N NH_4OH, and the ammoniacal phase was again extracted with 6 ml of $CHCl_3$. The combined $CHCl_3$ extract was filtered through a filter paper, and evaporated. The yield of N^4-benzylspermidine thus obtained was 0.58 mmol (overall yield from benzylamine, 38.7%). N^4-Benzylspermidine dissolved in 3 ml of AcOH was then hydrogenolyzed at 60 °C in the presence of PtO_2 catalyst (68 mg).[15] The mixture was stirred until hydrogen absorption ceased (about 1 h), then filtered through a Teflon Millipore membrane. Then 0.2 ml of conc. HCl was added to the filtrate, the mixture was evaporated to dryness, and the residue was recrystallized from aqueous EtOH and Et_2O. Pure ^{15}N-enriched spermidine trihydrochloride was obtained in the overall yield of 30% (0.45 mmol).

Variously ^{15}N-enriched spermidines were similarly prepared by methods A and B with the combinations of reagents summarized in Table I.

Table I. Combination of Reagents for Preparation of Variously ^{15}N-Enriched Spermidine

$N^1H_2(CH_2)_3N^4H(CH_2)_4N^8H_2$			Method A		Method B		
^{15}N	N	N	II,[a]	IV	II,[a]	BzA,	III
N	^{15}N	N	—		II,	BzA,[a]	III
N	N	^{15}N	III,[a]	ZD	II,	BzA,	III[a]
^{15}N	^{15}N	N	III,	ZD[a]	II,[a]	BzA,[a]	III
N	^{15}N	^{15}N	II,	IV[a]	II,	BzA,[a]	III[a]
^{15}N	N	^{15}N	—		II,[a]	BzA,	III[a]
^{15}N	^{15}N	^{15}N	II,[a]	IV[a]	II,[a]	BzA,[a]	III[a]

ZD, *N*-(benzyloxycarbonyl)-1,3-diaminopropane; BzA, benzylamine.
a) ^{15}N-Enriched reagents.

^{15}N-Enriched *N,N'*-Dibenzylputrescine (X)——Freshly distilled benzaldehyde (1.33 ml, 12 mmol) was added to a stirred suspension of ^{15}N-enriched I (0.98 g, 6 mmol) and triethylamine (2.4 ml) in 24 ml of MeOH. The suspension became a clear solution in 15 min at room temp. $MgSO_4$ (1.8 g) was then added, and stirring at room temp. was continued for 1 h. The reaction mixture was cooled to <0 °C on ice–NaCl, and $NaBH_4$ (2.76 g) was carefully added in portions during 1 h, together with additional MeOH (15 ml), to the stirred suspension. Stirring was continued for another 1 h. MeOH was then removed *in vacuo*, and the residue was extracted with Et_2O (100 ml × 2) and H_2O (100 ml). The combined Et_2O extract was thoroughly washed with H_2O (50 ml × 3) and dried, then the Et_2O was removed. The resulting crude oil (1.7 g), although contaminated with traces of UV-absorbing materials, was used as ^{15}N-enriched X (TLC, *n*-BuOH–AcOH–H_2O = 3 : 2 : 2, *Rf* 0.70) for the next reaction (theoretically 1.62 g, 6 mmol). *N,N'*-dibenzyl-1,3-diaminopropane (IX) was similarly prepared.

^{15}N-Enriched *N,N'*-Dibenzyl-*N,N'*-bis(3-phthalimidopropyl)putrescine (XI)——A stirred suspension of the crude ^{15}N-enriched X (1.7 g), ^{15}N-enriched II (3.23 g, 12 mmol), and KF–Celite (3.6 g) in 40 ml of CH_3CN was refluxed for 20 h, then allowed to cool to room temp. KF–Celite was removed by filtration and the solvent was evaporated off *in vacuo* to give a crude residue (4.2 g). A slightly turbid solution of the residue dissolved in 35 ml of benzene was then applied to a silica gel column (30 g) previously equilibrated with benzene. The column was successively eluted with 200 ml of benzene (to yield a fraction containing unreacted II) and 160 ml of benzene–acetone (5 : 1) (to yield a fraction containing XI). After removal of the solvent from the latter fraction, practically pure ^{15}N-enriched XI (TLC, benzene–acetone = 5 : 1, *Rf* 0.38) was obtained as an oil (3.9 g, 6 mmol).

In the silica gel column chromatography, *N,N'*-dibenzyl-*N*-(3-phthalimidopropyl)putrescine (XII), when present, was eluted with benzene–acetone (5 : 2), while X was retained on the column. This chromatographic system was successfully applied to the preparation of other symmetrical and unsymmetrical tetraamines (Table II).

TABLE II. Combination of Reagents for Preparation of Various Tetraamines

Tetraamine	Reagents
$NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_3NH_2$ (*sym*-norspermine)	IX, II
$NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_3NH_2$ (spermine)	X, II
$NH_2(CH_2)_3NH(CH_2)_3NH(CH_2)_4NH_2$ (thermospermine)	IX, II, S, III
$NH_2(CH_2)_4NH(CH_2)_3NH(CH_2)_4NH_2$ (canavalmine)	IX, III
$NH_2(CH_2)_3NH(CH_2)_4NH(CH_2)_4NH_2$	X, II, S, III
$NH_2(CH_2)_4NH(CH_2)_4NH(CH_2)_4NH_2$	X, III

S: silica gel chromatography.

^{15}N-Enriched Spermine Tetrahydrochloride——A solution of ^{15}N-enriched XI (3.9 g) and 100% $NH_2NH_2 \cdot H_2O$ (3.6 ml) in 50 ml of MeOH was refluxed for 3 h, then the reaction mixture (containing a curd-like precipitate) was evaporated *in vacuo*. The residue was then shaken for 30 min with 40 ml of $CHCl_3$ and 40 ml of 4 N NH_4OH, and the ammoniacal phase was again extracted with 40 ml of $CHCl_3$. The combined $CHCl_3$ extract was evaporated. The residual oil (2.1 g, 5.4 mmol) was pure ^{15}N-enriched N^4,N^9-dibenzylspermine, showing one band on TLC (*Rf* 0.3, *n*-BuOH–AcOH–H_2O = 3 : 2 : 2). The oil (2.1 g) was dissolved in 25 ml of AcOH and hydrogenolyzed at 60 °C in the presence of PtO_2 catalyst (0.5 g). The mixture was stirred until no more hydrogen was taken up, then filtered through a Teflon Millipore membrane. Conc. HCl (1 ml) was added to the filtrate, the mixture was evaporated to dryness, and the residue was recrystallized from aqueous EtOH. Pure ^{15}N-enriched spermine tetrahydrochloride was obtained in a yield of 78% (1.65 g) from ^{15}N-enriched I. *Anal.* Calcd for $C_{10}H_{30}Cl_4{}^{15}N_4$: C, 34.10; H, 8.59; ^{15}N, 17.04. Found: C, 33.99; H, 8.58; ^{15}N, 17.03.

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References and Notes

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