THE SYNTHESIS OF N^{I}, N^{II}-DIETHYL[6-^{14}C]NORSPERMINE ([^{14}C]CI-1006), A NEW ANTICANCER DRUG

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SUMMARY

N^{I}, N^{II}-Diethylnorspermine, CI-1006, a potential new anticancer drug, was synthesized with a carbon-14 located at the central carbon of the molecule. Condensation of N-(2,4,6-trimethylbenzenesulfonyl)-N-ethyl-propane-1,3-diamine with diethy[2-^{14}C]malonate gave a symmetrical diamide. The resulting protected diamide was reduced and deprotected in one pot to give the product as the tetrahydrobromide salt. A salt conversion gave the desired tetrahydrochloride. A total of 26.9 mCi of material was made with a specific activity of 26.1 mCi/mmol. The overall yield was 38%.

Key Words: N^{I}, N^{II}-Diethyl[6-^{14}C]norspermine, CI-1006, Diethyl[2-^{14}C]malonate, anticancer agent

INTRODUCTION

The role of polyamines in the growth of cancer cells has been under investigation for a number of years.[1] Dialkyl derivatives of natural occurring polyamines in particular exhibit antineoplastic activity against a number of murine and human cell lines both *in vitro* and *in vivo*.[2]

N^{I}, N^{II}-Diethylnorspermine (N-Ethyl-N'-[3-(3-ethylamino-propylamino)-propyl]-propane-1,3-diamine-tetrahydrochloride, CI-1006) has been shown in *in vitro* studies to suppress the polyamine biosynthetic enzymes ornithine decarboxylase and S-adenosylmethionine decarboxylase, induce the

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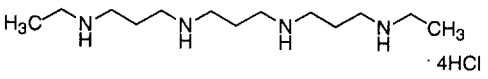
polyamine catabolic enzyme, spermidine/spermine N^1-actyltransferase, deplete polyamine pools, and inhibit cell growth.[3] CI-1006 also exhibited good antitumor activity *in vivo* against MALME-3 M and PANUT-3 human melanoma xenographs.[3] The compound has proceeded to phase 1 clinical studies.

The synthesis of radiolabeled N^1,N^{11}-diethylnorspermine was necessary for studies of the pharmacokinetics and metabolism of the compound. In non-radiolabeled metabolism studies the data suggested that the first step is N^1-deethylation followed by stepwise removal of aminopropyl equivalents.[4] The least desirable place to label the compound would be the terminal N-ethyl groups. The most thorough metabolism investigation may require a multiple labeled compound. The best place to put a single label and keep it intact during metabolism would be in the central propane-1,3-diamine group. We describe here the synthesis of carbon-14 labeled N^1,N^{11}-diethylnorspermine with the carbon-14 located in the middle of this symmetric molecule.

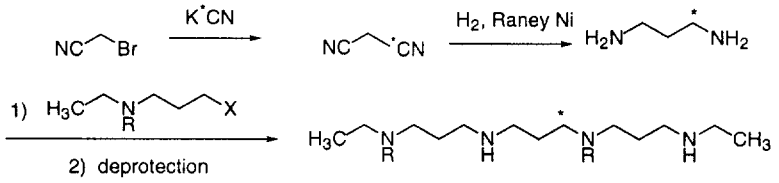


N^1, N^{11}-Diethylnorspermine, CI-1006

RESULTS AND DISCUSSION

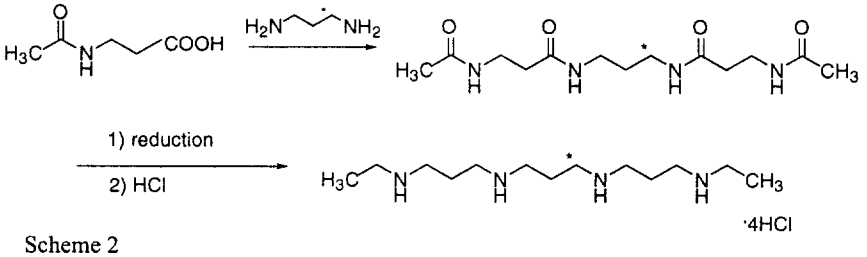
Much work has been done on the synthesis of both symmetrical and unsymmetrical polyamines.[1,2] A retrosynthetic analysis showed several ways to construct N^1,N^{11}-diethylnorspermine, either in a linear fashion or in a convergent manner. Since the target compound was symmetrical, several ways were investigated to build the molecule by addition of two identical fragments to the central propane-1,3-diamine unit. This idea required the formation of the two units to add and also a way to label the propane-1,3-diamine. This could have been done by introduction of the carbon-14 in the form of cyanide to halo-acetonitrile followed by reduction to the diamine. Several different choices existed as to the type of chemistry to use to couple the N-ethyl-diaminopropyl side chains to the central diamine. One choice could have been the N-protected-N-ethyl-3-halopropylamine

(Scheme 1). Experience by Beylin[5] indicated that it was not a clean reaction with bis-alkylation occurring. To overcome this the central diamine would need protection too.



Scheme 1

Another choice for the side chain could have been N-acetyl-β-alanine. This could have been coupled with the labeled diamine to give a tetraamide. Reduction would have given the title compound (see Scheme 2). Several reactions were run on the coupling of the β-alanine with the diamine both through the acid chloride and with the coupling reagents dicyclohexylcarbodiimide and carbonyldiimidizole. These reactions did not give clean products based on ^{1}H-NMR.

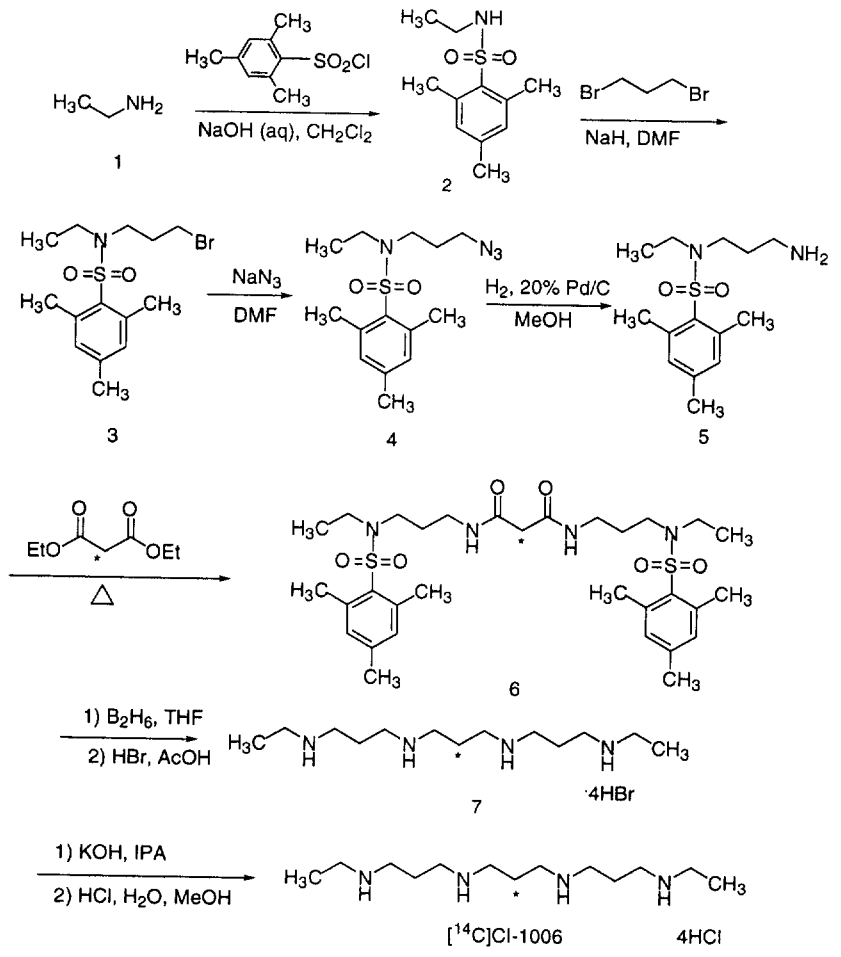


Scheme 2

In investigations going on simultaneoulsy, efforts were made to couple an N-protected N-ethyl-3-amino-propylamine with a carbon-14 malonic acid derivative. Then reduction and deprotection gave the desired compound (see Scheme 3). Bergeron *et al.*[6] used this approach to make tetraethylspermine by reacting N,N-diethyl-1,3-propanediamine with succinyl chloride followed by reduction of the corresponding amide with $LiAlH_4$. This method was cleaner than the method of scheme 2 and used a commercially available carbon-14 starting material.

There were several possible choices also for N-protecting groups, such as t-BOC, benzyl, and arylsulfonyl groups.[1, 2, 3, 7] We chose the 2,4,6-trimethylphenylsulfonyl group because of its

chromophore, potential to produce solid intermediates and its literature presidence.[2,7] Serendipitously, the deprotection of this group with 30% hydrobromic acid[8] in acetic acid proved to save a step late in the synthesis.



Scheme 3

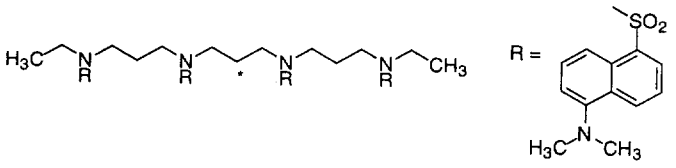
The bromopropylamine derivative 3 was made from ethylamine in two steps in an 85% yield.[2] A mixture of 3 and sodium azide in dimethylformamide for 22 h gave a 90% yield of 4. Catalytic hydrogenation with 20% palladium on charcoal gave a 52% yield of 5.

Attempts were made with unlabeled compound to use malonyl dichloride in a method similar to that of Bergeron *et al.*[6] With triethylamine as base only traces of product were isolated from a very complex mixture. Dimethylaminopyridine as base gave only slightly better results. The reaction worked best without base, just malonyl dichloride and 5 in dichloromethane gave a 57% yield of unlabeled 6. Next we tried coupling 5 with malonic acid using dicyclohexylcarbodiimide or carbonyldiimidazole. Both methods gave the desired product in a complex mixture. Diethyl malonate and 5 with triethyl aluminum in toluene gave an unidentified side product. Diethyl malonate and neat 5 , at 180°C gave yields of 6 in the 70-75% range for unlabeled experiments. The yield increased substantially by venting the reaction vial to remove the ethanol formed. Diethyl [2-^{14}C]malonate and 5 gave a 92% yield of 6.

Attempted reduction of the amide carbonyls using $LiAlH_4$ in refluxing THF for one hour gave only starting material. Using the method of Pratt and Sutherland,[9] reduction of 6 with refluxing diborane-tetrahydrofuran complex went cleanly in one hour. The work-up of the reaction with 6 *M* HCl gave a mixture of products, likely from various stages of deprotection. Prolonged heating did not afford complete deprotection. When this mixture was heated with 30% HBr in AcOH, unlabeled 7 was obtained in a 79% yield. The work-up of the diborane reaction was altered by first addition of a small amount of water to destroy excess diborane, removal of the solvent *in* vacuo, then treating the residue with 30% HBr in AcOH for 16 h gave 7 as a crystalline solid in a 93 % yield. The hydrobromide salt 7 was converted to the free base and the hydrochloric salt, [^{14}C]CI-1006, was isolated by crystallization from 3 *M* hydrochloric acid in methanol. The yield was 46% with a substantial amount of product still in the mother liquor. The purity of the isolated material was 98.6% as determined by HPLC of the dansyl chloride derivative. The specific activity was 26.1 mCi/mmol. The overall yield from Diethyl [2-^{14}C]malonate was 38%.

The chemical and radiochemical purity of the compound was determined by HPLC analysis of its 5-dimethylamino-1-naphthalenesulfonyl (dansyl) deriviative. The tetradansyl deriviative was the

expected product according to N. Seiler.[10] This was confirmed by mass spectral analysis of the deriviative.



EXPERIMENTAL

General. Diethyl [2-^{14}C]malonate, at a specific activity of 53 mCi/mmol, was purchased from Amersham Corporation. 2,4,6-Trimethylbenzenesulfonyl chloride, 1,3-dibromopropane, and 70 % ethylamine were purchased from Aldrich Chemical Company, Milwaukee, Wisconsin. Diethyl malonate was purchased from Kodak , Rochester, New York. N-(3-Bromo-propyl)-N-ethyl-2,4,6-trimethyl-benzenesulfonamide(3) was made from ethylamine in two steps by the method of Bergeron *et al.*[2]

Liquid scintillation counting was performed with a Packard Tri-Carb 4530 liquid scintillation counter using Beckman Ready-solve MP liquid scintillation cocktail. Thin layer chromatography (TLC) was done with E. Merck silica gel (0.25 mm) or Whatman KC18F reverse phase glass plates (0.2 mm). The plates were analyzed for radiochemical purity (RCP) using a Berthold LB-2832 automatic TLC-linear analyzer. High performance liquid chromatography (HPLC) was performed using a Waters Associates model 600 solvent delivery system with a model 996 UV detector and an IN/US β-RAM radioactivity flow detector. The R_f or t_R values of all labeled compounds matched those of the authentic unlabeled standards. ^{1}H-NMR spectra were run on a Varian XL-200 (200 MHz) spectrometer. Chemical shifts were reported in δ units downfield from tetramethylsilane.

N-(3-Azido-propyl)-N-ethyl-2,4,6-trimethyl-benzenesulfonamide (4). A mixture of crude 3[2] (66.16 g, 191 mmole) and sodium azide (18.65 g, 287 mmole) in dimethylformamide (200 mL) was stirred at room temperature for 22 h. The solvent was removed by rotary evaporation at 0.5 torr. The residue was partitioned between water and Et_2O. The aqueous layer was extracted with Et_2O

(3 x 100 mL). The combined Et_2O layers were dried (Na_2SO_4) and evaporated *in vacuo* to give 53.6 g of crude 4 (90% yield). TLC: R_f = 0.38; silica gel, hexane:EtOAc 2:1.

N-(3-Amino-propyl)-N-ethyl-2,4,6-trimethyl-benzenesulfonamide (5). A mixture of 4 (53.3 g, 172 mmol) and 20% palladium on charcoal (2 g) in methanol (500 mL) was shaken under hydrogen (50 psi). The reaction was vented and recharged with hydrogen six times over the first 4 h. After 19.5 h the reaction was filtered through Celite and the methanol was evaporated *in vacuo.* An EtOAc solution of the resulting oil was extracted with 1 *M* HCl (2 x 500 mL). The aqueous extract was washed with EtOAc (2 x 250 mL), made basic with 50% NaOH and extracted with EtOAc (3 x 150 mL). The combined EtOAc solution was washed with brine, dried (Na_2SO_4) and evaporated *in vacuo* to give 21.31 g of 5 (34% yield for 3 steps from 2). A 5.5 g portion of 5 was further purified to remove two trace impurities by flash chromatography on silica gel eluting initially with CH_2Cl_2 :CH_3OH (19:1) then CH_2Cl_2 :CH_3OH (4:1) to give 4.04 g of 5 as a colorless oil (73% recovery). ^{1}H-NMR ($CDCl_3$) δ 1.03(t, 3H, $NCH_2C\underline{H}_3$), 1.64(m, 2H, $NCH_2C\underline{H}_2CH_2NH_2$), 1.67(s, 2H, NH_2), 2.28(s, 3H, p-CH_3), 2.59(s, 6H, o-CH_3), 2.62(t, 2H, $NCH_2CH_2C\underline{H}_2NH_2$), 3.23(m, 4H, $NC\underline{H}_2CH_2CH_2NH_2$, $NC\underline{H}_2CH_3$), 6.92(s, 2H, Ph).

N,N'-Bis-{3-[ethyl-(2,4,6-trimethyl-benzenesulfonyl)-amino]-propyl}-[2-^{14}C]malonamide (6). To a 4 mL vial was added 5 (1.502 g, 5.28 mmol) and unlabeled diethyl malonate (210 mg, 1.31 mmole). Diethyl [2-^{14}C] malonate (213 mg, 70. mCi) was added to the vial with the aid of a little Et_2O. The Et_2O was removed with a stream of nitrogen first at room temperature then at 110 °C. The vial was capped and heated at 180 °C. After 5 h the reaction was cooled and a nitrogen stream was used to remove any EtOH produced. The reaction was recapped and heated at 180 °C for an additional 11 h. The cooled reaction mixture was dissolved in CH_2Cl_2 :CH_3OH (19:1) and applied to a flash chromatography column on silica gel (3.5 x 33 cm) eluting with CH_2Cl_2 :CH_3OH (19:1). A second column of the same size was used to isolate additional pure 6 to give a combined total of 1.536 g of 6 (92% yield). TLC: R_f = 0.23, RCP > 97 % silica gel, CH_2Cl_2 :CH_3OH (19:1).

N-Ethyl-N'-[3-(3-ethylamino-propylamino)-propyl]-[2-^{14}C]propane-1,3-diamine-tetrahydrobromide (7). Diborane (22 mL, 22 mmol, 1 *M* in tetrahydrofuran) was placed in a 100 mL 2-neck flask with a septum and condenser. A solution of 6 (1.53 g, 2.41 mmol) in tetrahydrofuran (20 mL) was added over 10 minutes controlling gas evolution. The reaction was heated at reflux for 3 h. After cooling, water (1.5 mL) was added and the solvent was removed *in vacuo*. To the milky residue was added 30% hydrobromic acid in acetic acid (20 mL). The orange solution was heated at 90 °C. After ten minutes a precipitate formed. After 16 h the reaction was cooled and filtered. The isolated solid was rinsed with acetone until white and air dried to give 1.2785 g of 7 (93% yield).

TLC: R_f = 0.0, RCP = 80.7%, impurities at R_f = 0.84 (12.7%) and 0.20 (6.6%)

C_{18}, CH_3OH:0.5 *M* NaCl (4:1).

N-Ethyl-N'-[3-(3-ethylamino-propylamino)-propyl]-[2-^{14}C]propane-1,3-diamine tetrahydrochloride, [^{14}C]CI-1006. A suspension of 7 (1.278 g, 2.25 mmol) in 2-propanol (20 mL) was heated at 65 °C. Fine ground potassium hydroxide (85%, 780 mg, 11.9 mmol) was added and the reaction was stirred for 3 h at 65 °C. The cooled reaction mixture was filtered and the solid was rinsed with 2-propanol. The filtrate was evaporated *in vacuo* to an oil. The residue was dissolved in methanol (15 mL) and evaporated *in vacuo*. This residue was dissolved in methanol (30 mL) and warmed to 65 °C. To the solution was added 3 M hydrochloric acid (10 mL. 30 mmol) and the reaction was stirred for 45 minutes. After cooling, the precipitate was isolated by filtration, rinsed with methanol, and dried *in vacuo* (70 °C) to give 405 mg of [^{14}C]CI-1006 (46% yield, 26.9 mCi, specific activity 26.1 mCi/mmol). ^{1}H-NMR (D_2O) δ 0.86(t, 6H, $NCH_2C\underline{H}_3$), 1.6-1.8(m, 6H, $NCH_2C\underline{H}_2CH_2N$), 2.76(m, 16H, $NC\underline{H}_2CH_2C\underline{H}_2N$, $NC\underline{H}_2CH_3$). CI+MS (*m/e*) 247 (^{14}C-M^+ + 1), 245 (^{12}C-M^+ + 1).

Analysis of N-Ethyl-N'-[3-(3-ethylamino-propylamino)-propyl]-[2-^{14}C]propane-1,3-diamine tetrahydrochloride, [^{14}C]CI-1006. Derivatization of [^{14}C]CI-1006 for purity determination by HPLC: [^{14}C]CI-1006 (0.5 mg, 0.0013 mmol) and sodium carbonate (120 mg) were dissolved in water (1.5 mL) and were stirred for 15 min. An acetone (0.5 mL) solution of dansyl chloride (containing 5 mg, 0.0185 mmol) was added with stirring. The reaction mixture was heated at 70 °C for 20 min.,

vortexed and cooled to room temperature for 20 min. The dansylated [^{14}C]CI-1006 mixture was transferred to a solid phase extraction column C18 SPE (Baker) which had been prepared by successive rinses of MeOH and 25% aqueous CH_3CN (CH_3CN:H_2O 25:75 v/v). The reaction flask was rinsed with 25% aqueous CH_3CN (2x500 μL) followed by MeOH (2x500 μL). The combined solution was transferred to the solid phase extraction column. The C18 SPE column was rinsed with 25% aqueous CH_3CN (2x2.5 mL). The dansylated [^{14}C]CI-1006 was eluted with MeOH (2x2.5 mL) for the purity test. HPLC retention time: 14 min.; radiochemical purity: 98.60%; chemical purity: 98.03%; HPLC conditions: Column: Beckman Ultrasphere ODS 5μ, 4.6x250 mm; Mobile phase: A=45% aqueous CH_3CN, B=MeOH, initial 100% A run for 5 min., at 5 min., directly change to 100% B to 25 min.; Flow rate: 1.0 mL/min.; UV detection: 254 nm. MS of unlabeled derivative APCI *(m/e)* (M+1) 1177, base peak at 725.

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