SYNTHESIS OF ISOTOPICALLY LABELED *CIS*-(3a*R*)-(-)-2,3,3a,4,5,9b-HEXAHYDRO-3-(N-PROPYL)-1*H*-BENZ[*e*]INDOLE-9-CARBOXAMIDE, A 5-HT_{1A} RECEPTOR AGONIST

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SUMMARY

The title compound is a potent and selective 5-HT_{1A} receptor agonist with clinical potentials for treating anxiety and depression. It has been labeled with stable and radioactive isotopes to support ADME studies in animals and human subjects. Its carboxamide group was labeled with C-14, C-13, or C-13, N-15, and O-18. To provide a radioligand for investigating the receptor binding characteristics of the compound, we also labeled it with tritium in the N-propyl group to obtain the high specific activity of 86.7 Ci/mmol.

Key Words: Synthesis, isotopic labeling, C-13, C-14, N-15, O-18, tritium, aryl cyanation, catalytic reduction

INTRODUCTION

8-Hydroxy-2-(dipropylamino)tetralin (*1*) is a potent serotonin receptor agonist (1,2) which binds selectively to the 5-HT_{1A} receptor in the brain (3). Therapeutically, 5-HT_{1A} receptor agonists are believed to act as anxiolytics which are devoid of the liability of benzodiazepine-like side effects (4). Despite its potency and selectivity, *1* suffers from poor oral bioavailability and short duration of action. As part of an extensive effort to identify a compound with improved pharmacokinetics, SAR investigations into 2-aminotetralin analogs of *1* have led to the synthesis of a series of rigid five/six/six-fused angular tricyclic compounds *2* (5). The title compound (6) is a

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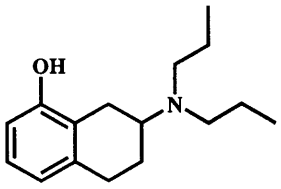
CCC 0362–4803/97/011891–10$17.50

member of this series with excellent receptor selectivity and potency as well as superior pharmacokinetic properties. To facilitate studies on metabolism of this compound and its receptor binding characteristics, we synthesized isotopically labeled versions of the title compound containing radioisotope C-14 or tritium, or stable isotopes C-13, N-15, and O-18.



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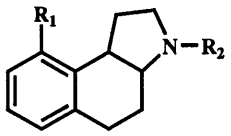

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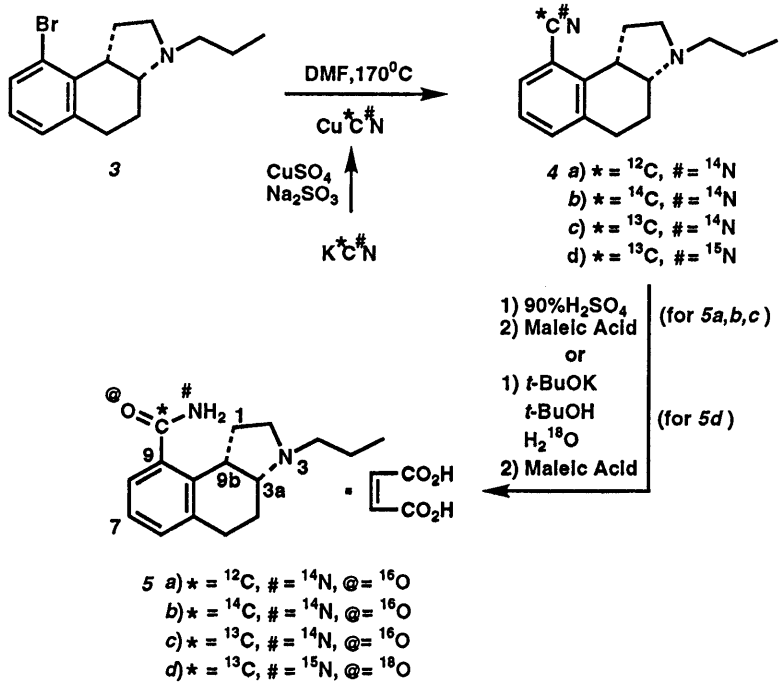
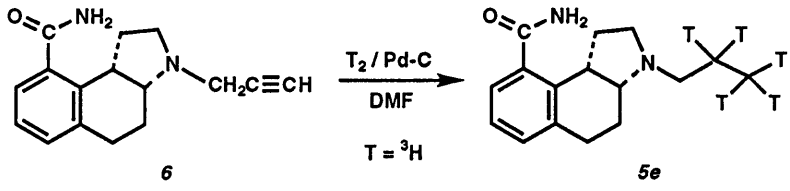
DISCUSSION AND RESULTS

The carboxamide function in the title compound ***5a*** can be derived from a corresponding nitrile, which in turn is obtainable through a variety of cyanation processes to substitute an aryl halide with cyanide. Potassium [^{14}C]cyanide is a readily available and inexpensive source for carbon-14. We therefore elected to focus our effort on effecting a cyanation with K^{14}CN on the previously reported bromo analog ***3*** (6) of ***5a***. Attempts to carry out the displacement in the presence of either Pd(0) or Pd(II) catalyst at 100 °C (7,8) in hexamethylphosphoramide (HMPA) were not successful. Addition of potassium iodide in an attempt to generate aryl iodide *in situ* to facilitate the substitution reaction afforded only traces of the desired product. An alternative method described by Friedman and Schecter (9) for preparing aryl nitriles from aryl halides using copper catalysis did prove effective for our purposes, albeit under rigorous conditions. As shown in Scheme 1, Cu^{14}CN was prepared from K^{14}CN according to known procedures (10,11). The bromo compound ***3*** on treatment with Cu^{14}CN at 170 °C in dimethylformamide (DMF) produced the ^{14}C-labeled nitrile ***4b***. This compound was hydrolized in 90% H_2SO_4 to gave the corresponding amide, isolated as its maleate salt ***5b*** with specific activity (SA) of 19.3 μCi/mg

Scheme 1



Scheme 2



(7.2 mCi/mmol) and radiochemical purity (RCP) in excess of 99% by HPLC and TLC. An analogous cyanation reaction carried out with K^{13}CN instead of K^{14}CN led to ***4c*** labeled with C-13 in the nitrile function. Subsequent hydrolysis of the nitrile followed by salt formation afforded the C-13 labeled ***5c***. To provide another stable isotope labeled version with greater mass differential, we also synthesized ***5d*** labeled with C-13, N-15, and O-18 in the carboxamide group by performing the cyanation reaction with K^{13}C^{15}N instead of K^{14}CN. A modified procedure of Hall and Gisler (12)

for introduction of oxygen-18 into the amide function, using a refluxing mixture of potassium *t*-butoxide and [^{18}O]water in anhydrous *t*-butanol, converted ***4d*** to ***5c*** with isotope enrichment of 99%, 99%, and 97% for C-13, N-15, and O-18, respectively.

To provide a radioligand for studying the receptor binding characteristics of ***5a***, we also prepared high SA tritium labeled ***5e*** (Scheme 2). A previously reported analog of ***5a***, compound ***6*** with a propargyl side chain (13), was used as the precursor for tritium labeling. Catalytic reduction of the propargyl group with 10% Pd-C catalyst in the presence of nominally carrier-free tritium gas introduced tritium into the *N*-propyl moiety of the tritiated product ***5e***, as determined by ^{3}H-NMR analysis. Purification of the crude product by means of preparative HPLC afforded ***5e*** with SA of 328 mCi/mg (86.7 Ci/mmol), and RCP of 99% by HPLC and 96% by TLC. The high SA of ***5e*** necessitated its storage as a 0.5 mCi/mL solution in methanol at -70^{0}C.

The product SA and distribution of tritium, found to be 4%:39%:57% at the *N*-propyl carbon atoms alpha, beta, and gamma, respectively, to the nitrogen atom in *5e*, indicated that the tritium gas effecting the reduction was less than carrier-free, probably resulting from exchanges between the tritium gas and labile proton sources. The higher tritium content at the gamma carbon in comparison to the beta position suggested that the acetylenic proton in ***6*** underwent exchange with tritium gas under the reaction conditions prior to reduction of the carbon-carbon triple bond. A small amount of tritium incorporation into the alpha position may be attributable to allylic proton-tritium exchange, or to double bond migration on the palladium catalyst.

EXPERIMENTAL

General Procedures

Radioactivity determinations were performed with a Wallac Model 1410 liquid scintillation spectrometer using the external standard method. Ultima Gold® (Packard) was used as the liquid scintillation cocktail. TLC analysis was performed with 2.5 x 10 cm glass plates precoated with a 250 micron layer of silica gel GF (Analtech, Neward, Delaware). The developed zones were visualized by exposure to iodine vapor. TLC radiochromatograms were obtained with a Bioscan System 200

Imaging Scanner. Integrated peak ratios were used to determine radiochemical purity. ^{1}H and ^{13}C-NMR spectra were obtained in deuterated chloroform with tetramethylsilane (TMS) as the internal standard, on a Bruker AM 300 spectrometer. The operating frequency was 300 MHz for ^{1}H and 75.5 MHz for ^{13}C. The ^{3}H-NMR spectra were obtained in perdeuterated methanol with TMS, on an IBM AF-300 spectrometer at 320 MHz. Both spectrometers used 5 mm glass sample tubes. The radioactive sample was placed in a teflon tube (Wilmad Glass) which fit inside the 5 mm glass sample tube providing secondary containment for spill prevention.

HPLC analyses were carried out with a Spectra Physics Model 8700 or a Model 8800 solvent delivery system, using a Supelcosil DB-18, 5μ, 4.6 mm ID x 250 mm column with a mobile phase of 65:35:0.2 v/v methanol:water:triethylamine, pumped isocratically at 1.5 mL/min. All solvents were HPLC grade. UV detection of the column effluent was done with an LDC/Milton Roy Spectromonitor D variable wavelength detector set at 254 nm. Peak integration used IN/US Beta-Ram software version 1.62. Integrated peak ratios were used at the stated wavelength to determine the chemical purity. Radiochemical purity was determined from integrated radiochromatograms using a Radiomatic FLO-ONE Beta radioactivity flow detector with version CR7b.070 software. A 3:1 v/v pre-detection mixing of FLO-Scint II™ (Packard) and column effluent was used for the measurement.

cis-(3a*R*-)-(-)-2,3,3a,4,5,9b-Hexahydro-9-[^{13}C]cyano-3-(n-propyl)-1*H*-benz[e]indole (***4c***)

A solution of 139 mg of sodium sulfite (1.1 mmol) in 1.5 mL of water was added dropwise with stirring at room temperature to a solution of 132 mg of K^{13}CN (2.0 mmol, Cambridge Isotope Laboratories) in 2 mL of water. To the stirred mixture was added, dropwise in 5 minutes, a solution of 524 mg of cupric sulfate pentahydrate crystals (2.1 mmol) in 3 mL of water. The white precipitates which formed were allowed to settle while the mixture was cooled in an ice bath. The supernate was removed by pipette, and the solids were washed twice with 5 mL of water, followed by two 5 mL portions of acetone, and dried under vacuum at room temperature to give 162 mg of Cu^{13}CN, 89.4% yield.

A mixture of the above $Cu^{13}CN$ (1.78 mmol), 524 mg of ***3*** (1.78 mmol), 3 mL of DMF and 0.2 mL of pyridine was heated to 170°C under nitrogen for 18 h. The resulting straw colored solution was cooled to room temperature, and 4 mL of NaCN solution (25 g NaCN in 75 mL water) was added. The mixture was stirred at 60°C for 20 min, and partitioned with 10 mL of water and 20 mL of ether. The aqueous layer was extracted with 20 mL of ether. The combined ether layers were washed with 10 mL of water, followed by 20 mL of brine, and dried over anhydrous sodium sulfate. After removal of solvent, the crude residue was chromatographed on a column of 60 g of silica gel packed in and eluted with 75:25 v/v pentane:ether at 14 mL/fraction/4 min. Fractions containing the desired product only were pooled and concentrated to give 341 mg of ***4c*** as a yellow solid, 79.4% yield, ^{1}H-NMR (300 MHz, $CDCl_3$, TMS) δ, 0.938 (t, 3H, J=7.3 Hz, $C\underline{H}_3$), 1.39-1.64 (m, 4H, H-1, $CH_2C\underline{H}_2CH_3$), 1.93-2.01 (m, 1H, H-4), 2.17-2.26 (m, 2H, H-5), 2.46-2.54 (m, 1H, H-4), 2.59-2.93 (m, 4H, H-2, $C\underline{H}_2CH_2CH_3$), 3.11 (t, 1H, J=8.06 hz, H-3a), 3.69 (dt, 1H, J=8.95, 8.96 Hz, H-9b), 7.15 (dd, 1H, J=7.5, 7.5 Hz, H-7), 7.29 (d, 1H, J=7.6 Hz, H-6), 7.46 (dd, 1H, J=6.5 Hz, J^{13}_{C-H}=6.47 Hz, H-8). This material was used without further purification in the next step. There was also recovered 78 mg of unreacted ***3*** (14.9%).

cis-(3a*R*)-(-)-2,3,3a,4,5,9b-Hexahydro-3-(n-propyl)-1*H*-benz[*e*]indole-9-[^{13}C]carboxamide (*Z*)-2-Butenedioate (1:1) (***5c***)

A mixture of 300 mg of ***4c*** (1.24 mmol) and 4 mL of 90% sulfuric acid was warmed under nitrogen at 65°C, to become a dark yellow solution after ~1 h. The progress of the reaction was monitored by TLC (EtOAc extract of basified reaction mixture on silica gel eluted with 95:5 v/v acetone:methanol). After 12 h at 65°C, the mixture was diluted with 15 mL of water and extracted with 20 mL of ether (discarded). The aqueous layer was cooled in an ice bath and basified with 30 mL 6N NaOH, and extracted with 25 mL of EtOAc twice. The combined extracts were washed with brine and dried over anhydrous sodium sulfate. Removal of solvent afforded 295 mg of crude product which was chromatographed on a 60 g column of silica gel packed in and eluted with 1.5% v/v methanol in acetone at 3 mL/min. The eluent was collected

in 12 mL fractions, and fractions containing product only were pooled and concentrated to afford 245 mg of the free base of ***5c***. TLC showed presence of a single component (silica gel, 95:5 v/v acetone:methanol, R_f=0.3); ^{1}H-NMR (300 MHz, $CDCl_3$, TMS) δ, 0.93 (t, 3H, J=7.36 Hz, C$\underline{H}_3$), 1.40-1.61 (m, 4H, H-1, $CH_2C\underline{H}_2CH_3$), 1.86-1.94 (m, 1H, H-4), 2.15-2.29 (m, 2H, H-5), 2.36-2.55 (m, 2H, H-2, 4), 2.65-2.91 (m, 3H, H-2, $C\underline{H}_2CH_2CH_3$), 3.06 (t, 1H, J=7.71 Hz, H-3a), 3.92 (dt, 1H, J=9.1, 9.13 Hz, H-9b), 5.79 (bs, 2H, NH_2), 7.09 (dd, 1H, J=7.36, 7.40 Hz, H-7), 7.17 (d, 1H, J=6.6 Hz, H-6), 7.24 (ddd, 1H, ^{3}J=7.8 Hz, $^3J_{C\text{-}H}$=4.2 Hz, ^{4}J=1.1 Hz, H-8); ^{13}C-NMR (75.5 MHz, $CDCl_3$, TMS, 7 mg free base of ***5c***, and 28 mg free base of ***5a***, ~ 20% w/w C-13 enrichment) ppm, 12.15 ($CH_2CH_2\underline{C}H_3$), 21.85 ($CH_2\underline{C}H_2CH_3$), 26.45, 26.96, 34.39 (C-1, 4, 5), 38.31 (C-9b), 52.82 (C-2), 56.67 ($\underline{C}H_2CH_2CH_3$), 62.08 (C-3a), 124.68, 125.03 (C-6, 7), 130.38 (C-8), 135.28 (C-9a), 138.94, 139.77 (C-5a, 9), 173.16 (carboxy-C, ^{13}C enriched). The free amine was dissolved in 1.5 mL of warm methanol with 94 mg of maleic acid. The solution was cooled to room temperature and 2 mL of ethyl acetate was added. The mixture was cooled in an ice bath and a seed crystal of ***5a*** effected copious precipitation. An additional 1 mL of ethyl acetate was added with stirring. The crystals were filtered and washed with 2 mL of ethyl acetate and dried to afford 275 mg of ***5c***, 84.4% yield, homogeneous by HPLC.

cis-(3a*R*)-(-)-2,3,3a,4,5,9b-Hexahydro-3-(n-propyl)-1*H*-benz[*e*]indole-9-[^{13}C, ^{15}N, ^{18}O]carboxamide (*Z*)-2-Butenedioate (1:1) (***5d***)

The C-13 and N-15 labeled nitrile ***4d*** was prepared in 68.4% yield from $Na^{13}C^{15}N$ (MSD Isotopes, K & K Greef Ltd., Croydon, UK) *via* $Cu^{13}C^{15}N$ with the same procedures described above for the preparation of ***4c***. ^{1}H-NMR (400 MHz, $CDCl_3$, TMS) δ, 1.0 (t, 3H, C$\underline{H}_3$), 1.5 (m, 4H, 2'-CH, H-4, H-1), 2.0 (qq, 1H, H-4), 2.25 (m, 2H, $C\underline{H}_2CH_2CH_3$, H-2), 2.5 (m, 1H, H-1), 2.6 (m, 1H, H-5), 2.75 (m, 2H, $C\underline{H}_2CH_2CH_3$, H-3a), 2.9 (m, 1H, H-5), 3.1 (t, 1H, H-2), 3.7 (q, 1H, H-9b), 7.2 (m, 3H, Ar-H). υ (film) 770 (s), 1080 (s), 1180 (s), 1360 (s), 1450 (s), 1550 (m), 2150 (w), 2780 (s), 2860 (m), 2950 (s), 3030 (w) cm^{-1}. MS m/z 242 (M^+; 10%), 213 (100%), 199 (2%), 184 (5%), 169 (2%), 156 (10%) mass units.

A mixture of 1.6 g of ***4d*** (6.6 mmol), 20 mL of *t*-butanol, 13.5 mL of 1M potassium *t*-butoxide in tetrahydrofuran, and 0,264 mL of [^{18}O]water was stirred at ambient temperature for 30 min and then refluxed under nitrogen for 2 h. A further quantity of *t*-butanol (20 mL) and potassium *t*-butoxide (1M; 0.5 mL) was added and the mixture refluxed for another 8 h. The mixture was cooled to room temperature, the solvents removed *in vacuo* and the resulting solids partitioned between distilled water (50 mL) and ethyl acetate (100 mL). The aqueous layer was extracted further with ethyl acetate (3 x 50 mL) and the pooled organic extracts were washed with 20 mL of brine. The organic layer was dried (sodium sulfate) and the solvents removed *in vacuo* to yield an off-white solid. Column chromatography (silica gel, 40 x 150 mm column; 6.5:2.5:1 v/v hexane:acetone:triethylamine, 30 ml fractions) gave 0.437 g of the free base of ***5d*** in fractions 20-24. This material was dissolved in warm methanol (2.7 mL), filtered, and treated with 0.193 g of maleic acid in methanol (3.6 mL). The mixture was cooled to 4°C. Crystals were collected to give 0.308 g of ***5d*** (12.5% yield from ***4d***), mp 211 - 214 °C. [$^{13}C_1$] Calcd for [$^{13}C_1$] C_{12} H_{26} [$^{18}O_1$] O_4 [$^{15}N_1$] N. 0.3 H_2O: C, 62.84; H, 6.98; N, 7.56. Found: C, 62.89; H, 6.92; N, 7.35. ^{1}H-NMR δ (400 MHz, $CDCl_3$, TMS), 0.6 (t, 3H, CH_3), 1.5 (m, 4H, $CH_2CH_2CH_3$, H-4, H-1), 1.9 (qq, 1H, H-4), 2.22 (m, 2H, $CH_2CH_2CH_3$, H-2), 2.4 (m, 1H, H-1), 2.51 (m, 1H, H-5), 2.7 (m, 1H, $CH_2CH_2CH_3$), 2.75 (m, 1H, H-3a), 2.83 (m, 1H, H-5), 3.2 (t, 1H, H-2), 3.9 (q, 1H, H-9b), 7.15 (m, 3H, Ar-H). ^{13}C-NMR (400 MHz, $CDCl_3/CD_3OD$) 173.75 ppm (d,1C, J = 14.26 Hz, carboxy C). υ (Nujol) 720 (w), 870 (w), 1570 (m), 1620 (w) cm^{-1}. MS m/z 262 (M^+; 20%), 233 (100%), 204 (5%) mass units.

cis-(3a*R*)-(-)-2,3,3a,4,5,9b-Hexahydro-3-(n-propyl)-1*H*-benz[e]indole-9-[^{14}C]carboxamide (*Z*)-2-Butenedioate (1:1) (***5b***)

Carbon-14 labeled nitrile ***4b*** was prepared in 77% radiochemical yield from K^{14}CN (American Radiolabeled Chemicals) *via* Cu^{14}CN with the same procedures described above for the preparation of ***4c***. Hydrolysis of the nitrile in 90% H_2SO_4 followed by conversion of the resulting amide free base to maleic acid salt afforded carbon-14

labeled 5b in overall radiochemical yield of 38.4% based K^{14}CN, SA 19.3 μCi/mg (7.68 mCi/mmol), 99% RCP by HPLC and TLC.

cis-(3a*R*)-(-)-2,3,3a,4,5,9b-Hexahydro-3-(n-[1,2,3-^{3}H]propyl)-1*H*-benz[*e*]indole-9-carboxamide (***5e***)

A solution of 25.4 mg (0.1 mmol) of ***6*** in 1.5 mL of DMF was stirred at room temperature under nominally carrier free tritium gas (14) in the presence of 10 mg of 10% Pd-C catalyst. After 3 h, the pressure had dropped from 629 torr to 481 torr and the gas uptake had stopped. Following recovery of excess tritium gas, the reaction vessel was vented and flushed with nitrogen gas, the reaction mixture was purged of labile tritium by three cycles of alternating addition and vacuum evaporation of methanol. The mixture was then pressure filtered through a 3 mL Supelclean® (Supelco) Si SEP-PAK attached in series to an Acrodisc® (Gelman Science) PTFE membrane filter. The filter rig was rinsed with a total of 20 mL of methanol in portions. The combined filtrate and washings were concentrated at reduced pressure to dryness. Analysis of the residue showed the presence of 10.9 Ci of crude ***5e***. A 50 mCi sample was analyzed by ^{3}H-NMR (14), (320 MHz, CD_3OD, proton decoupled, TMS) δ, 0.91 (d, $J_{T\text{-}T}$=16.3 Hz, $CH_2CH_2C\underline{H}_3$ with adjacent CTH, t, $J_{T\text{-}T}$=15.9 Hz, $CH_2CH_2C\underline{H}_3$ with adjacent CT_2, 56.5% of total tritium by integration), 1.57 (m, $CH_2C\underline{H}_2CH_3$, 39.2% of total tritium), 2.39 (m, $C\underline{H}_2CH_2CH_3$, 4.3% of total tritium).

A portion of this crude material (~310 mCi) was purified by means of preparative HPLC, using a 4.6 mm I.D. x 250 mm Supelcosil LC-18, 5μ, analytical column. The approximately 1 mg sample of crude ***5e*** was applied onto the column as a bolus in a mixture of 200 μL of methanol and 300 μL of mobile phase. The column was eluted isocratically with a mobile phase of 60:40:0.2 v/v methanol:water: triethylamine, at 1.5 mL/min. The eluate was monitored by UV detection at 270 nm. The peak containing the desired product was collected, concentrated under reduced pressure on a rotary evaporator to remove methanol, and the aqueous remainder was lyophilized. The residue (74.6 mCi, 29.5% recovery) was dissolved in 15 mL of methanol, 99% RCP by HPLC, 96% by TLC, SA 328 mCi/mg (84.7 Ci/mmol). This high SA ***5e*** was further diluted with methanol to 0.5 mCi/ml for storage at -70°C.

ACKNOWLEDGEMENT

We thank S.A. Mizsak and A.H. Parton for providing and interpreting NMR and mass spectra. ^{3}H-NMR analysis provided by Dr. P.G. Williams of the NTLF is greatly appreciated.

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14. Tritiation reaction was carried out in collaboration with M. Morimoto at the National Tritium Labeling Facility (NTLF) of Lawrence Berkeley Laboratory, University of California at Berkeley, CA USA.

