SYNTHESES OF CARBON-13 AND DEUTERIUM LABELLED L-TRYPTOPHAN, RACEMIC 5-HYDROXYTRYPTOPHAN AND 5-HYDROXYTRYPTAMINE (SEROTONIN)

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SUMMARY

Convenient and efficient syntheses of ^{13}C and ^{2}H sidechain labelled L-tryptophan, racemic 5-hydroxytryptophan and 5-hydroxytryptamine (serotonin) from indole and 5-benzyloxyindole are described.

Keywords: L-Tryptophan, 5-Hydroxytryptophan, 5-Hydroxytryptamine, Serotonin, Carbon-13, Deuterium.

INTRODUCTION

Recent interest concerning the role of 5-hydroxytryptamine (serotonin) in the regulation of physiological processes has stimulated the search for new, sensitive analytical procedures specific for this indoleamine. Development of a mass spectral method applicable to the study of central serotonin-turnover rates required the syntheses of L-tryptophan and the reference compounds 5-hydroxytryptophan and serotonin labelled with ^{13}C and ^{2}H in the aminoethyl sidechain.

Previous syntheses of L-tryptophan-$2^{1},3^{1}-^{3}H_{2}$ (1) and racemic tryptophan and serotonin containing ^{11}C (2), ^{13}C (3) ^{14}C (4), ^{2}H (5) and ^{3}H (1,4b) in the aminoethyl sidechain have been reported. However, these methods were not entirely applicable to preparation of the labelled indoleamines required for this study. Convenient and efficient syntheses of ^{13}C and ^{2}H sidechain labelled (-)-tryptophan, (±)-5-hydroxytryptophan and serotonin are described in this communication.

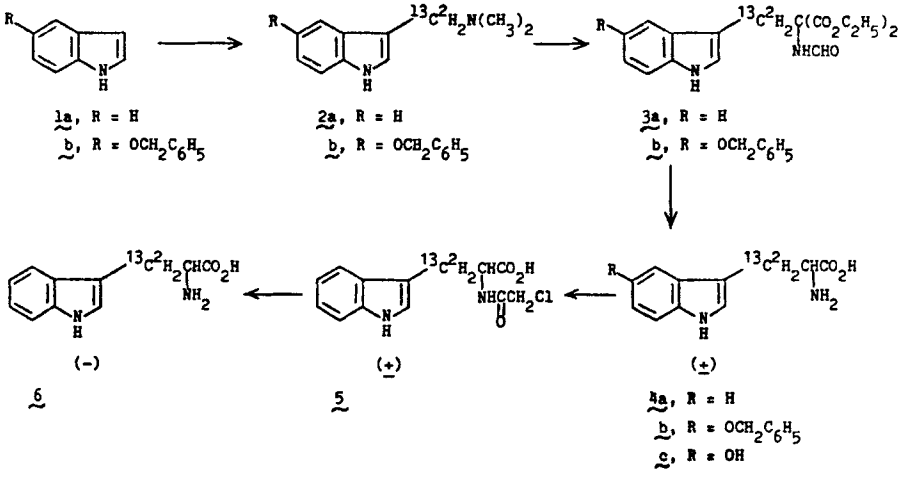
0362-4803/82/030389-10$01.00

Received June 11, 1981
Revised July 31, 1981

RESULTS AND DISCUSSION

Doubly labelled racemic tryptophan, 4a, and 5-hydroxytryptophan, 4c, were synthesized (Scheme I) by modification of the procedure employed by Ek and Witkop (6) for preparation of unlabelled 5-hydroxytryptophan. The ^{13}C and ^{2}H isotopic labels were introduced with labelled paraformaldehyde in the initial Mannich reactions on indole and 5-benzyloxyindole. Resolution of 4a was achieved through an enzymatic hydrolysis of the corresponding N-chloroacetyl derivative 5 with carboxypeptidase A at 38 °C and pH 7.0-7.2 as described (7) for resolution of unlabelled tryptophan. PMR and MS confirmed the complete retention and position of the isotopic labels in 6. Racemic 5-hydroxytryptophan containing only deuterium in the aminoacid sidechain was prepared similarly from $^{2}H_2$-paraformaldehyde by the same route used to synthesize 4c.

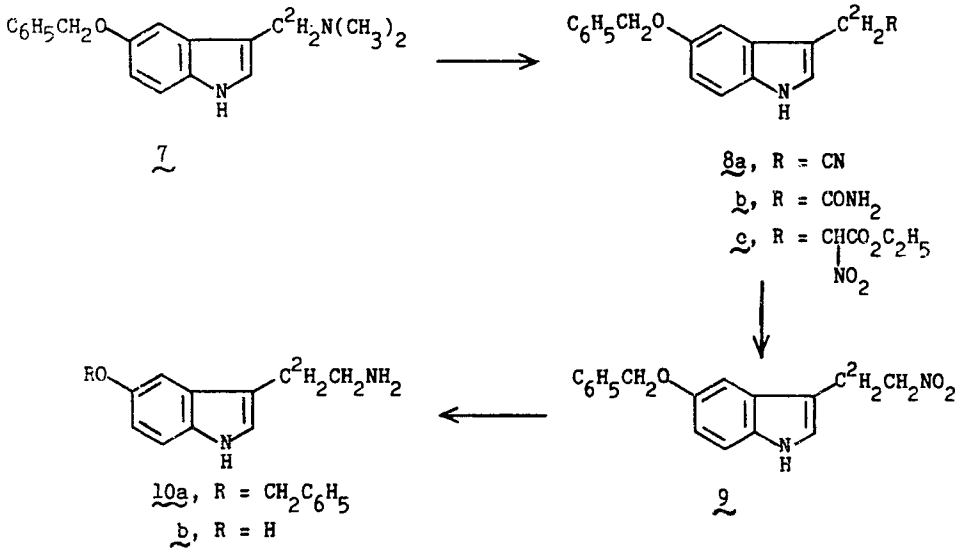
Scheme I



Initially it was planned to prepare the sidechain labelled derivatives of serotonin, e.g. 10b, by reduction of a nitrile 8a or amide 8b intermediate since this method was successful for synthesis of the tetradeuterated (5a) and unlabelled (6) amines. However, preparation of a mixture of nitrile 8a and amide 8b from 7 in ethanol-water by the method of Ek and Witkop (6) led to substantial exchange of deuterium as determined by PMR. Although deuterated nitrile 8a could be prepared under anhydrous conditions in DMF, reduction to the amine 10a with $LiAlH_4$ or catalytically in ethanol saturated with ammonia again resulted in loss of deuterium.

The desired ^{2}H and ^{13}C, ^{2}H labelled serotonin derivatives were obtained conveniently through the synthetic sequence outlined in Scheme 2. Reaction of ethyl nitroacetate with 7 afforded the nitro ester 8c which was not isolated. Basic hydrolysis of 8c followed by decarboxylation gave 9 directly in an overall isolated yield of 45% from 7. $LiAlH_4$ reduction of 9 to the amine 10a and removal of the benzyl protective group under catalytic hydrogenation conditions afforded 10b in an overall yield of 18% from 7. Serotonin containing both ^{13}C and ^{2}H in the aminoethyl sidechain was prepared similarly from 2b in 11% overall yield. Complete retention and position of the isotopic labels in these compounds were confirmed by PMR and MS.

Scheme 2



EXPERIMENTAL

Melting points were determined on a Thomas-Hoover capillary melting point apparatus using open capillaries and are uncorrected. ^{1}H-NMR spectra were recorded for all intermediates and final products on a Varian EM-90 instrument using tetramethylsilane as an internal standard and are consistent with assigned structures. Mass spectra data were acquired with an MS902 instrument. Optical rotations were determined with a Perkin-Elmer polarimeter, Model 141. TLC's were performed on Analtech fluorescent silica gel plates and spots detected by UV, exposure to iodine vapor or with ninhydrin spray for the aminoacids. Paraformaldehyde -^{13}C, $^{2}H_2$ (90 atom % ^{13}C, 98 atom % ^{2}H) and paraformaldehyde -$^{2}H_2$ (98 atom % ^{2}H) were supplied by Merck Sharp &

Dohme Canada Limited, Montreal, Canada. Carboxypeptidase A used in the resolution of labelled racemic tryptophan was obtained from Millipore Corporation, Freehold, New Jersey.

3-(Dimethylaminomethyl-^{13}C-$^{2}H_2$-1H-indole (2a). Glacial acetic acid, 25 mL, was added over 30 min with stirring to 25 mL of a 25% aqueous solution of dimethylamine cooled in an ice bath. After adding paraformaldehyde (90 atom % ^{13}C; 98 atom % ^{2}H) (2.53 g, 76.7 mmol), the mixture was allowed to warm to room temperature and indole (9.90 g, 84.5 mmol) and 125 mL ethanol were added. The solution was stirred at reflux for 2 h and then cooled to room temperature over 12 h. Water, 300 mL, was added and after filtering through a pad of diatomaceous earth, the filtrate was basified with 40% sodium hydroxide. The precipitated solid was removed by filtration and dried to give 9.90 g (72.8%) of labelled gramine, 2a, mp 119-122 °C, mass spectrum, m/e 177 (85%), 176 (10%), 175 (3%).

5-Benzyloxy-3-(dimethylaminomethyl-^{13}C-$^{2}H_2$)-1H-indole (2b). To 4.6 mL of a 25% aqueous solution of dimethylamine cooled with an ice bath was added over 10 min, 4.6 mL of glacial acetic acid. After 5 min, paraformaldehyde (90 atom % ^{13}C, 98 atom % ^{2}H) (455 mg, 13.8 mmol) was added and the mixture allowed to warm to room temperature. Ethanol, 23 mL, and 5-benzyloxyindole (3.39 g, 15.2 mmol) were added and the solution stirred at reflux for 2 h and then cooled. Water, 50 mL, was added and after filtering through a pad of diatomaceous earth, product was precipitated by addition of 40% sodium hydroxide. After filtering and drying, 2.05 g (52.4%) of labelled 5-benzyloxygramine, 2b, mp 131-135 °C dec were obtained, mass spectrum, m/e 283 (87%), 282 (13%).

5-Benzyloxy-3-(dimethylaminomethyl-$^{2}H_2$)-1H-indole (7). Deuterated 5-benzyloxygramine, 7, mp 131-138 °C dec, mass spectrum, m/e 282 (98.1%), was prepared from 5-benzyloxyindole and paraformaldehyde -$^{2}H_2$ in 80% yield by the same procedure used to prepare 2b.

Diethyl Formamido-(indol-3-ylmethyl-^{13}C-$^{2}H_2$)malonate (3a). Diethyl formamidomalonate (12.6 g, 62 mmol) was added to a solution of 1.46 g sodium in 110 mL absolute ethanol followed by 3-(dimethylaminomethyl-^{13}C-$^{2}H_2$)-1H-indole, 2a (9.90 g, 55.9 mmol). During addition of 6.94 mL dimethylsulfate at room temperature over 1 h, the reaction

mixture solidified. After addition was complete, the reaction was heated on the steam bath for 2 h, cooled and poured into 600 mL water. Precipitated solid was removed by filtration, washed with water, dried and recrystallized from a toluene-hexane mixture to give 13.5 g (71.8%) of labelled malonate 3a, mp 177-180 °C, homogenous TLC (5% methanol-95% chloroform) R_f = 0.5.

Diethyl Formamido-(5-benzyloxyindol-3-ylmethyl-^{13}C-$^{2}H_2$)malonate (3b). A mixture of 5-benzyloxy-3-(dimethylaminomethyl-^{13}C-$^{2}H_2$)-1H-indole, 2b (2.05 g, 7.23 mmol), diethyl formamidomalonate (1.66 g, 8.17 mmol) and 10 mg of 50% sodium hydride dispersion in mineral oil in 30 mL toluene was stirred at reflux with nitrogen bubbling through the reaction mixture for 15 h. After cooling, the reaction mixture was diluted with ethyl acetate, filtered and diluted further with hexane. Upon cooling, the labelled formamidomalonate 3b crystallized to afford 1.82 g (57.2%) of product, mp 136.0-137.5 °C, homogeneous upon TLC (5% methanol-95% chloroform) R_f = 0.5.

Diethyl Formamido-(5-benzyloxyindol-3-ylmethyl-$^{2}H_2$malonate was prepared in 33% yield by the same procedure used to prepare 3b.

(±)-β-^{13}C-β,β-$^{2}H_2$-Tryptophan (4a). Diethyl formamido(indol-3-ylmethyl-^{13}C-$^{2}H_2$)-malonate, 3a (11.64 g, 34.7 mmol) was added to a solution of 15.3 g sodium hydroxide in 155 mL water and the mixture stirred at reflux for 6 h. Concentrated hydrochloric acid, 31 mL, and 6N hydrochloric acid, 10.4 mL, were then added and the mixture stirred at reflux for 90 min. After cooling and filtering through a pad of diatomaceous earth, the pH of the filtrate was adjusted to 5.5 with concentrated ammonium hydroxide. Cooling gave 6.37 g (88.6%) of racemic labelled tryptophan 4a; mp 278-282 °C dec with darkening at 265 °C; homogeneous and identical with authentic tryptophan upon TLC (50% n-butanol-20%-acetic acid-30% water) R_f = 0.8; mass spectrum m/e 207; ^{1}H NMR (CF_3CO_2D) δ 4.6-4.8 (br d, HC-N), 7.1-7.7 (m, indole CH).

(±)-N-(2-Chloroacetyl)-β-^{13}C-β,β-$^{2}H_2$-tryptophan (5). Chloroacetyl chloride (5.05 g, 44.7 mmol) was added over 30 min to a vigorously stirred solution of (+)-β-^{13}C-β,β-$^{2}H_2$-tryptophan, 4a (8.7 g, 42 mmol) in 50.5 mL of 0.83N sodium hydroxide solution. The pH of the reaction was maintained at 10-11 by the simultaneous addition of approximately 12 mL of 5N sodium hydroxide solution. After addition was complete, stirring was continued for 45 min more at ice bath temperature. Ethyl acetate,

125 mL, and 6N sulphuric acid were then added to adjust the pH to 1-2. The organic extract was removed, the aqueous layer re-extracted with fresh ethyl acetate and the ethyl acetate extracts combined and dried over anhydrous sodium sulphate. After filtering and concentrating under reduced pressure, the oily residue was triturated with 75 mL n-butylchloride and cooled to give 7.67 g (64.3%) of the chloroacetamide 5: mp 144.5-147.5 °C dec; homogeneous upon TLC (90-chloroform, 10-methanol, 1-acetic acid) R_f = 0.5; mass spectrum, m/e 283.

β-^{13}C-β,β-$^{2}H_2$-L-Tryptophan (6). To a solution of racemic chloroamide 5 (9.26 g, 32.6 mmol) in 330 mL water and 16.3 mL of 2N lithium hydroxide at 38 °C was added 100 mg of carboxypeptidase A. The pH was maintained at 7.0-7.2 by the addition of dilute lithium hydroxide solution. An additional 33 mg of enzyme was added after 4 h. After stirring at 38 °C for 24 h, the pH was adjusted to 4 by the addition of acetic acid and the reaction filtered through a pad of diatomaceous earth. The filtrate was cooled, acidified to pH 1-2 with 6N sulfuric acid and extracted with ethyl acetate to remove unreacted chloroacetamide. The aqueous xtract was cooled and the pH adjusted to 6 with concentrated ammonium hydroxide to precipitate 1.42 g (42%) of labelled L-tryptophan. Concentration of the mother liquor under reduced pressure afforded an additional 0.76 g (22.5%) of labelled L-tryptophan 6; mp 272-274 °C dec with softening at 260 °C; homogeneous and identical with authentic L-tryptophan upon TLC (50% n-butanol-20% acetic acid-10% water) R_f = 0.8; mass spectrum, m/e 207; ^{1}H NMR (CF_3CO_2D) δ 4.6-4.8 (br d, HC-N), 7.1-7.7 (m, indole CH); $[\alpha]^{27^{\circ}}$ 5890Å -30.3°, 5780Å -31.3°, 5460Å -35.0°, 4360Å -52.3°, 3650Å -61.7° (C = 3, water) (8).

(+)-5-Benzyloxy-β-^{13}C-β,β-$^{2}H_2$-tryptophan (4b). Diethyl formamido-(5-benzyloxy-indol-3-ylmethyl-^{13}C-$^{2}H_2$)malonate, 3b (1.82 g, 4.11 mmol) was added to a solution of 1.82 g sodium hydroxide in 20 mL water and the mixture stirred at reflux for 6 h. Concentrated hydrochloric acid, 3.7 mL, and 6N hydrochloric acid, 0.8 mL, were then added and the mixture stirred at reflux for 1 h. After cooling, concentrated ammonium hydroxide was added to adjust the pH to 5. Cooling gave 1.0 g (78%) of labelled aminoacid 4b, mp 220-235 °C dec with softening at 210 °C.

(+)-5-Benzyloxy-β,β-$^{2}H_2$-tryptophan, mp 221-223 °C dec with softening at 210 °C, homogeneous TLC (50% n-butanol, 20%-acetic acid, 30% water) R_f = 0.7, was prepared

in 65% yield by the same procedure used to prepare the ^{13}C, ^{2}H derivative 4b.

(±)-5-Hydroxy-β-^{13}C-β,β-$^{2}H_2$-tryptophan (4c). A mixture of racemic 5-benzyloxy-β-^{13}C-β,β-$^{2}H_2$-tryptophan 4b (1.0 g, 3.2 mmol) and 400 mg of a 10% palladium on carbon catalyst in 15 mL water and 15 mL ethanol was hydrogenated at atmospheric pressure and room temperature for 1 h until one equivalent of hydrogen had been absorbed. After filtering and concentrating under reduced pressure, the residue was recrystallized under nitrogen by dissolving in 6 mL hot water and concentrating to 2 mL. The crystallized product was washed with ethanol and dried to give 300 mg (42%) of labelled 5-hydroxytryptophan 4c; TLC (50%-n-butanol, 20%-acetic acid, 30%-water) major component at R_f 0.6 and identical with an authentic sample of 5-hydroxytryptophan and a weak spot at R_f 0.7 corresponding to unreacted benzyl ether; mass spectrum, m/e strong molecular ion at 223 with very weak benzyl ether molecular ions at 313 and 310; ^{1}H NMR (D_2O) δ 4.1 (d, 1 H, CH-N), 6.8-7.6 (m, 4H, indole CH).

(±)-5-Hydroxy-β,β-$^{2}H_2$-tryptophan was prepared in 51% yield by the same procedure used to prepare 4c; homogeneous and identical with an authentic sample of 5-hydroxytryptophan upon TLC (50%-n-butanol, 20%-acetic acid, 30%-water) R_f 0.6; mass spectrum, m/e 222.

5-Benzyloxy-3-(2-nitro-1,1-$^{2}H_2$-ethyl)-1H-indole (9). A solution of ethyl nitroacetate (1.92 g, 14.4 mmol) and 5-benzyloxy-3-(dimethylaminomethyl-$^{2}H_2$)-1H-indole, 7 (4.06 g, 14.4 mmol) in 160 mL toluene was stirred at reflux for 7 h with nitrogen bubbling through the reaction mixture. After cooling and filtering, toluene was removed under reduced pressure and the residue was redissolved in 68 mL ethanol and added to a solution of sodium hydroxide, 1.52 g, in 10 mL water. This solution was stirred at reflux for 4 h, cooled and acidified to pH 1-2 with concentrated hydrochloric acid. After standing at room temperature for 2 h, ethanol was removed under reduced pressure and the crude product extracted into ethyl ether. The ether extract was washed with water, saturated sodium bicarbonate solution and then water. After drying over anhydrous sodium sulfate, the solvent was removed under reduced pressure and the residue chromatographed over 250 g of silica gel, 40-60 μ mesh (E. Merck). Elution with methylene chloride afforded the deuterated nitro compound 9 (1.94 g, 45.1%): mp 95-97 °C with partial melting and resolidification at 80 °C; homogeneous TLC (methylene

chloride) R_f 0.5; mass spectrum, m/e 298; ^1H NMR ($CDCl_3$) δ 4.5 (s, 2 H, CH_2), 5.1 (s, 2 H, CH_2), 6.8-7.5 (m, 9 H, aromatic CH), 7.9 (br, 1 H, NH).

5-Benzyloxy-3-(2-nitro-1-^{13}C-1,1-$^2H_2$-ethyl)-1H-indole was prepared in 25.3% yield from 2b by the same procedure used to prepare the deuterated derivative 9.

5-Benzyloxy-3-(2-amino-1,1-$^2H_2$-ethyl)-1H-indole Hydrogen Oxalate (10a). A solution of 5-benzyloxy-3-(2-nitro-1,1-$^2H_2$-ethyl)-1H-indole, 9, (1.90 g, 6.37 mmol) in 30 mL dry tetrahydrofuran was added over 10 min to an ice-bath cooled and stirred slurry of 700 mg of lithium aluminum hydride in 20 mL dry tetrahydrofuran. After stirring at room temperature overnight, unreacted hydride was decomposed with excess saturated sodium potassium tartrate solution. The insoluble salts were removed by filtration and washed with ethyl acetate. The organic extracts were combined, dried over anhydrous sodium sulfate, filtered and concentrated. The residue was treated with excess oxalic acid in an ethanol-ethyl acetate mixture to give the hydrogen oxalate salt, ethanol solvate of 10a (1.40 g, 6.14%); mp 156-162 °C dec with softening at 124 °C; homogeneous TLC (20% methanol-80% chloroform saturated with concentrated ammonium hydroxide) R_f 0.2; mass spectrum, m/e 268; ^1H NMR (DMSO-d_6) δ 1.0 (t, ethanol CH_3), 3.0 (s, 2 H, CH_2N), 3.4 (q, ethanol CH_2), 5.1 (s, 2 H, CH_2O), 6.8-7.5 (m, aromatic CH), 10.9 (br, 1 H, NH, exchangeable).

5-Benzyloxy-3-(2-amino-1-^{13}C-1,1-$^2H_2$-ethyl)-1H-indole Hydrogen Oxalate was obtained in 65% yield as the ethanol solvate by lithium aluminum hydride reduction of the corresponding nitro intermediate with the procedure used to prepare 10a; mp 158-161 °C dec with softening at 124 °C; homogeneous TLC (20% methanol-80% chloroform saturated with concentrated ammonium hydroxide) R_f 0.2; mass spectrum, m/e 269; ^1H NMR (DMSO-d_6) δ 1.1 (t, ethanol CH_3), 3.1 (d, 2 H, CH_2N), 3.5 (q, ethanol CH_2), 5.2 (s, 2 H, CH_2O), 6.8-7.6 (m, aromatic CH), 10.9 (br, 1 H, NH, exchangeable).

5-Hydroxy-3-(2-amino-1,1-$^2H_2$-ethyl)-1H-indole (Serotonin) Hydrogen Oxalate (10b). A mixture of 5-benzyloxy-3-(2-amino-1,1-$^2H_2$-ethyl)-1H-indole hydrogen oxalate, 10a, (1.32 g, 3.68 mmol) and 700 mg of a 10% palladium on carbon catalyst in 300 mL ethanol and 60 mL methanol was hydrogenated at room temperature and atmospheric pressure for 30 min until one equivalent of hydrogen had been absorbed. After filtering and removing solvents under reduced pressure, the residue was recrystallized from an

ethanol-methanol-ethyl acetate mixture to give deuterated serotonin hydrogen oxalate, 10b (630 mg, 63.8%); mp 193-198 °C dec; homogeneous and identical with an authentic sample of serotonin upon TLC (20% methanol-80% chloroform saturated with conc ammonium hydroxide) R_f 0.1 and (50% n-butanol-20% acetic acid-30% water) R_f 0.7; mass spectrum, m/e 178; ^1H NMR (D_2O) δ 3.2 (s, CH_2), 6.7-7.4 (m, indole CH).

5-Hydroxy-3-(2-amino-1-^{13}C-1,1-$^2H_2$-ethyl)-1H-indole Hydrogen Oxalate was obtained in 69% yield by debenzylation of the corresponding benzyl ether; mp 191-195 °C dec; homogeneous and identical with an authentic sample of serotonin upon TLC (20% methanol-80% chloroform saturated with conc ammonium hydroxide) R_f 0.1 and (50% n-butanol-20% acetic acid-30% water) R_f 0.7, mass spectrum, m/e 179; ^1H NMR (D_2O) δ 3.2 (br d, CH_2), 6.7-7.4 (m, indole CH).

ACKNOWLEDGEMENTS

The author is indebted to J. Murphy and Dr. D. Cochran for providing PMR spectra and to R. Rhodes for the mass spectra.

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8. Found for authentic unlabelled L-tryptophan under the same conditions: $[\alpha]^{27^{\circ}}$ 5890Å -31.0°, 5780Å -32.3, 5460Å -36.7°, 4360Å -54.3°, 3650Å -63.3° (C = 3, water).