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SYNTHESIS OF A RADIOLABELED CHARGED MELATONIN RECEPTOR LIGAND

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SUMMARY

The synthesis of the first permanently charged melatonin receptor ligand: N-[2-(2-trimethyl-ammoniumethyleneoxy-7-methoxynaphthyl)ethyl]propionamide iodide (**2a**, TMEPI) and its carbon-14 analog (**2b**, ^{14}C-TMEPI) are described. The ligands can serve as tools to elucidate the mechanism of melatonin receptor regulation. Both compounds **2a** and **2b** are synthesized in 8 steps. The radiochemical yield and purity of **2b** are 99.6 and 99.8% respectively, with specific radioactivity of 7.6 mCi/mmol.

Key Words: melatonin, internalization, receptor regulation, pineal hormone, carbon-14

INTRODUCTION

The pineal hormone melatonin **1**, is involved in circadian rhythm, retinal physiology, seasonal breeding and cardiovascular regulation (1-3). Melatonin also appears to be involved in a number of pathological conditions, and there is strong evidence for its use in pathologies associated with disorders in circadian rhythm. Melatonin was shown to alleviate jet lag (4), to induce sleep (5), and to advance the sleep rhythm of subjects with delayed sleep phase syndrome (6).

Elucidating the mechanisms underlying melatonin receptor regulation is critical to our understanding of how melatonin functions. Melatonin receptors are highly regulated according to diurnal rhythm and following melatonin exposure. For example, the density and affinity of melatonin receptors decrease during the night and following melatonin exposure (1, 8-9). Critical to the normal functioning of melatonin within the body may be its ability to "turn off" or desensitize its receptors. One component of desensitization, that is, internalization, may be one of the mechanisms by which melatonin receptors become refractory to melatonin. Internalization is the process by which receptors are sequestered and removed from the membrane surface following agonist exposure. This form of desensitization is utilized by many other G-protein-coupled receptors including muscarinic cholinoceptors (10) and beta-adrenoceptors (11). Whether or not melatonin receptors internalize following melatonin exposure has not been determined due to the unavailability of specific probes, that is, charged melatonin receptor ligands that are unable to penetrate membranes and bind only to surface melatonin receptors. Recently, we reported the development of the first permanently charged melatonin receptor ligand N-[2-(2-trimethylammoniumethyleneoxy-7-methoxynaphthyl)ethyl]propionamide iodide (**2a**, TMEPI)

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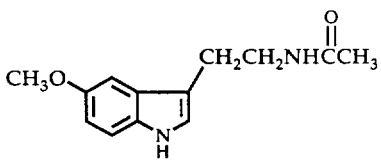
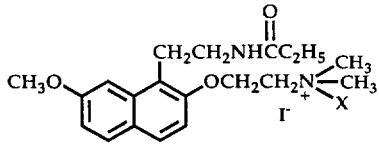
which displayed nanomolar affinity for the melatonin receptors (12). The charged ligand has potential in determining whether internalization of melatonin receptor occurs following melatonin exposure and use of this ligand would greatly enhance our understanding of melatonin's action at the receptor level. In the previous synthesis of compound **2a** (TMEPI), the 2-position side chain was synthesized based on the alkylation of the corresponding 2-naphthol derivative with iodoethanol. However, the yield was low (33%). Recently, we have worked out a shorter and more efficient synthesis of compound **2a** (TMEPI). Here we report in details the synthesis of compound **2a** (TMEPI) and its C-14 analog **2b** (^{14}C-TMEPI) by this new approach.



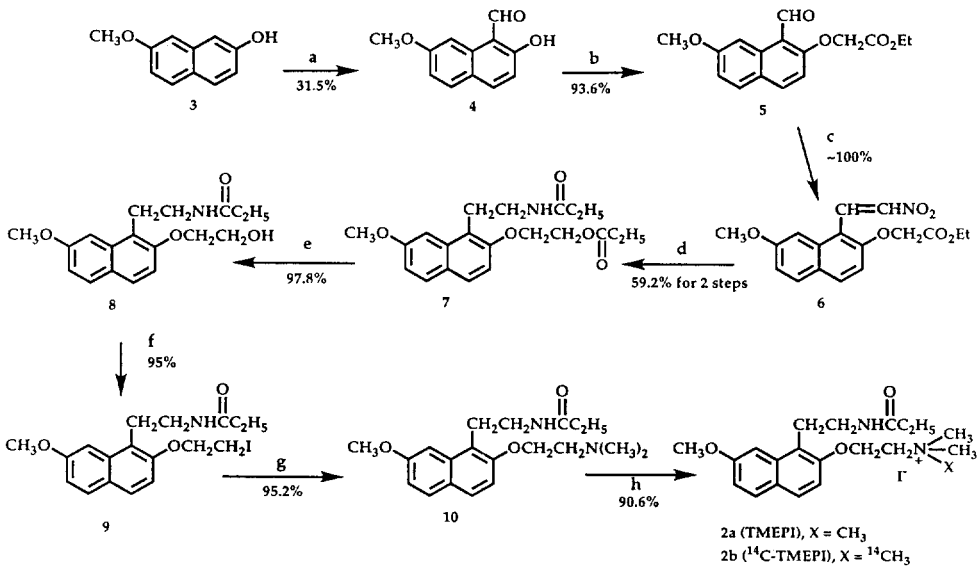
Melatonin 1



2a (TMEPI), X = CH_3
2b (^{14}C-TMEPI), X = $^{14}CH_3$

RESULT AND DISCUSSION

The synthesis of TMEPI and it C-14 analog are outlined in scheme 1. 7-Methoxy-2-naphthol **3** was used as the starting material. Selective formylation at the 2-position (Reimer-Tiemann reaction) of compound **3** yielded aldehyde **4** (31.5 %). Reaction of **4** with ethyl bromoacetate in the presence of K_2CO_3 afforded ester **5** (93.6%) which was then condensed with nitromethane,



Scheme 1. Reagents and conditions: (a) $CHCl_3$,NaOH; (b) $BrCH_2CO_2Et$, K_2CO_3, acetone, reflux; (c) CH_3NO_2, NH_4OAc, reflux, 3 h; (d) 1. $LiAlH_4$/THF, 2. C_2H_5COCl, Et_3N, DMAP; (e) K_2CO_3, CH_3OH-H_2O (9:1); (f) PPh_3/I_2; (g) $(CH_3)_2NH$, K_2CO_3; (h) CH_3I or $^{14}CH_3I$, acetone.

yielding nitroalkene **6** (~100%). Reduction of compound **6** with LiAlH4 followed by acylation with propionyl chloride gave the diacylated compound **7** (59.2% from **6**). Selective hydrolysis of ester functional group in compound **7** was accomplished by treatment with K_2CO_3 in MeOH-H_2O (9:1), furnishing alcohol **8** (97.8%). Compound **8** was converted into the final compound **2** (**a,b**) by the same reaction sequence as reported previously (12): Iodination of alcohol **8** with I_2/PPh_3 (95%), treatment of iodide **9** with dimethylamine (95.2%) and reaction of amine **10** with CH_3I or $^{14}CH_3I$ (90.6%).

EXPERIMENTAL

2-Hydroxy-7-methoxy-naphthaldehyde (**4**)

7-Methoxy-2-naphthol **3** (3.5 g, 20 mmol) was added to 40 mL of aqueous solution of NaOH (6.4 g, 160 mmol). The mixture was heated at 65-70°C and 3.6 mL (44.8 mmol) of chloroform was added in three portions at an intervals of 15 min. After the completion of the addition, the reaction mixture was heated to 100°C and stirred for 1 hour. The mixture was cooled to room temperature and acidified with dilute sulfuric acid and extracted with CH_2Cl_2 (3 x 50 mL). The extracts were combined, dried (Na_2SO_4) and concentrated. The residue was purified by silica gel chromatography using CH_2Cl_2/petroleum ether (3:1) as eluent, yielding compound **4** (1.28 g, 31.5 %). m.p: 128-129°C; ^1H NMR (300 MHz, $CDCl_3$) δ 3.93 (s, 3H, OCH_3), 6.94 (d, 1H, J = 9.0 Hz, ArH), 7.04 (dd, 1H, J = 2.4, 9.0 Hz, ArH), 7.62 (d, 1H, J = 2.4 Hz, ArH), 7.67 (d, 1H, J = 9.0 Hz, ArH), 7.86 (d, 1H, J = 9.0 Hz, ArH), 10.71 (s, 1H, CHO), 13.14 (s, 1H, OH). Analysis calculated for $C_{12}H_{10}O_3$: C, 71.28; H, 4.98. Found: C, 71.12; H, 5.02.

2-Ethoxy carbonyl methyleneoxy-7-methoxy-1-naphthaldehyde (**5**)

Compound **4** (1.2 g, 5.94 mmol) was dissolved in 80 mL of acetone. To this solution was added ethyl bromoacetate (1.2 mL, 10.8 mmol) and K_2CO_3 (1.3 g, 9.42 mmol). The reaction mixture was refluxed for 1.5 h and then filtered. The filtrate was concentrated. The residue was purified by silica gel chromotography using petroleum ether/CH_2Cl_2/EtOAc (4:1:1) as eluent, giving, compound **5** (1.6 g, 93.6 %). m.p: 117.5-118.5°C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.28 (t, 3H, J = 7.2 Hz, CH_3), 3.95 (s, 3H, OCH_3), 4.27 (q, 2H, J = 7.2 Hz, $COOCH_2$), 4.84 (s, 2H, OCH_2), 6.93 (d, 1H, J = 9.0 Hz, ArH), 7.07 (dd, 1H, J = 2.4 Hz, 9.0 Hz, ArH), 7.64 (d, 1H, J = 9.0 Hz, ArH), 7.93(d, 1H, J = 9.0 Hz, ArH), 8.82 (d, 1H, J = 2.4 Hz, ArH), 10.97 (s, 1H, CHO). Analysis calculated for $C_{16}H_{16}O_5$: C, 66.66; H, 5.59. Found: C, 66.49; H, 5.50.

1-Nitro-2-(2-ethoxycarbonyl methyleneoxy-7-methoxynaphthyl)ethylene (**6**).

A solution of aldehyde **5** (1.6 g, 5.56 mmol) and ammonium acetate (600 mg, 7.8 mmol) in nitromethane (36 mL) was refluxed for 3 h. After evaporation of the solvent under reduced pressure, CH_2Cl_2 (50 mL) and H_2O (30 mL) were added to the residue. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 30 mL) and the combined organic layers were washed with H_2O (30 mL) and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to furnish nitro compound **6** (1.83 g, ~100 %). m.p: 158.5-159.5°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.35 (t, 3H, J = 7.2 Hz, CH_3), 3.97 (s, 3H, OCH_3), 4.34 (q, 2H, J = 7.2 Hz, $COOCH_2$), 4.83 (s, 2H, OCH_2), 6.95 (d, 1H, J = 9.0 Hz, ArH), 7.09 (dd, 1H, J = 2.1, 9.0 Hz, ArH), 7.36 (d, 1H, J = 2.1 Hz, ArH), 7.69 (d, 1H, J = 9.0 Hz, ArH), 7.84 (d, 1H, J = 9.0 Hz, ArH), 8.48 (d, 1H, J = 13.3 Hz, $CHNO_2$), 8.72 (d, 1H, J = 13.3 Hz, ArCH). Analysis calculated for $C_{17}H_{17}NO_6$: C, 61.63; H, 5.17; N, 4.23. Found: C, 61.73; H, 5.10; N, 4.16.

N-[2-(2-Propionyloxyethyleneoxy-7-methoxynaphthyl)ethyl]propionamide (**7**)

To a stirred suspension of $LiAlH_4$ (1.8 g, 47.4 mmol) in anhydrous THF (100 mL) under nitrogen atmosphere at 0°C was added dropwise a solution of compound **6** (1.83 g, 5.56 mmol) in THF (20 mL). After the addition, the reaction mixture was heated at 40°C for 24 h and then cooled with an ice bath. To the mixture was added in the following order: H_2O (1.8 mL), 15 % NaOH (1.8 mL), EtOAc (50 mL) and H_2O (5.4 mL). The mixture was then stirred at r.t for 15 min and filtered. The filtrate was dried (Na_2SO_4) and concentrated. The residue was dissolved in CH_2Cl_2 (50 mL) and cooled with an ice bath. To this solution were added 4-dimethylamino pyridine (DMAP, 66 mg, 0.56 mmol) and triethylamine (3.2 mL, 23 mmol) followed by propionyl chloride (1.3 mL, 15 mmol). After the addition, the reaction mixture was stirred at r.t overnight. The mixture was then washed with saturated aqueous $NaHCO_3$ (20 mL) and H_2O (20 mL), and dried (Na_2SO_4). After evaporation of the solvent in vacuo, the residue was purified by silica gel chromatography using petroleum ether/EtOAc (1 : 1) as eluent, affording diacylated product **7** (1.2 g, 59.2 % for 2 steps). m.p: 76.5 - 77.5°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (t, 3H, J = 7.5 Hz, CH_3), 1.13 (t, 3H, J = 7.5 Hz, CH_3), 2.12 (q, 2H, J = 7.5 Hz, CH_2CO), 2.37 (q, 2H, J = 7.5 Hz, CH_2CO), 3.24 (t, 2H, J = 6.9 Hz, $ArCH_2$), 3.51 (m, 2H, CH_2NH), 3.94 (s, 3H, OCH_3), 4.29 (t, 2H, J = 4.5 Hz, $ArOCH_2$), 4.50 (t, 2H, J = 4.5 Hz, $COOCH_2$), 5.90 (br s, 1H, NH), 7.01(dd, 1H, J = 2.2, 9.0 Hz, ArH), 7.04 (d, 1H, J = 9.0 Hz, ArH), 7.33 (d, 1H, J = 2.2 Hz, ArH), 7.64 (d, 1H, J = 9.0 Hz, ArH), 7.65 (d, 1H, J = 9.0 Hz, ArH). Analysis calculated for $C_{21}H_{27}NO_5$: C, 67.54; H, 7.29; N, 3.75. Found: C, 67.62; H, 7.24; N, 3.85.

N-[2-(2-Hydroxyethyleneoxy-7-methoxynaphthyl)ethyl]propionamide (**8**)

Compound **7** (650 mg, 1.74 mmol) was dissolved in 40 mL of a mixture of MeOH - H_2O (9 : 1). To this solution was added K_2CO_3 (780 mg, 5.65 mmol) and the mixture was stirred at r.t for 1.5 h. After removal of the solvent under reduced pressure, CH_2Cl_2 (30 mL) and H_2O (20 mL) were added to the residue. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 30 mL). The combined organic extracts were washed with H_2O (30 mL) and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to give alcohol **8** (540 mg, 97.8 %). m.p: 145.5-146.5°C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.10 (t, 3H, J = 7.5 Hz, CH_3), 2.17 (q, 2H, J = 7.5 Hz, CH_2CO), 3.29 (t, 2H, J = 6.9 Hz, $ArCH_2$), 3.46 (m, 2H, CH_2N), 3.93 (s, 3H, OCH_3), 4.00 (t, 2H, J = 4.2 Hz, CH_2O), 4.16 (t, 2H, J = 4.2 Hz, $ArOCH_2$), 5.92 (br s, 1H, NH), 7.00 (dd, 1H, J = 2.2, 9.0 Hz, ArH), 7.07 (d, 1H, J = 9.0 Hz, ArH), 7.21(d, 1H, J = 2.2 Hz, ArH), 7.65 (d, 1H, J = 9.0 Hz, ArH), 7.68 (d, 1H, J = 9.0 Hz. ArH). Analysis calculated for $C_{18}H_{23}NO_4$: C, 68.12; H, 7.30; N, 4.41. Found: C, 68.15; H, 7.25; N, 4.34.

N-[2-(2-Iodoethyleneoxy-7-methoxynaphthyl)ethyl]propionamide (**9**)

A solution of compound **8** (500 mg, 1.58 mmol) in CH_2Cl_2 (6 mL) was added in one portion to a mixture of PPh_3 (1.52 g, 5.88 mmol), imidazole (400 mg, 5.88 mmol) and iodine (1.5 g, 5.9 mmol) in CH_2Cl_2 (50 mL). The reaction mixture was stirred at r.t for 2.5 h. Saturated aqueous $NaHCO_3$ (30 mL) was added to the reaction mixture and the mixture was stirred for an additional 15 min. The organic layer was then separated and washed with saturated aqueous $Na_2S_2O_3$ (2 x 30 mL) and H_2O (30 mL). The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by silica gel chromatography using petroleum ether/EtOAc (1 : 1) as eluent, yielding pure iodide **9** (640 mg, 95 %). m.p: 125-126°C; ^1H NMR (300 MHz, $CDCl_3$) δ 1.07 (t, 2H, J = 7.5 Hz, CH_3), 2.12 (q, 2H, J = 7.5 Hz, CH_2CO), 3.32 (t, 2H, J = 6.9 Hz, $ArCH_2$), 3.49 (t, 2H, J = 6.3 Hz, CH_2I), 3.56 (m, 2H, CH_2N), 3.95 (s, 3H, OCH_3), 4.37 (t, 2H, J = 6.3 Hz, $ArOCH_2$), 5.78 (br s, 1H, NH), 7.01 (m, 2H, ArH), 7.36 (d, 1H, J = 1.9 Hz, ArH), 7.64 (d, 1H, J = 9.0 Hz, ArH), 7.66 (d, 1H, J = 9.0 Hz, ArH). Analysis calculated for $C_{18}H_{22}NO_3I$: C, 50.60; H, 5.19; N, 3.28. Found: C, 50.66; H, 5.17; N, 3.22.

N-[2-(2-Dimethylaminoethyleneoxy-7-methoxynaphthyl)ethyl]propionamide (**10**)

To a solution of dimethylamine in THF (15 mL, 2M, 30 mmol) was added iodide **9** (600 mg, 1.41 mmol) and K_2CO_3 (200 mg, 1.45 mmol). The reaction mixture was stirred ar r.t for 24 h and then concentrated in vacuo. Fifty milliliters of CH_2Cl_2 and H_2O (20 mL) were added to the residue and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (30 mL). The combined organic layers were dried (Na_2SO_4) and the solvent evaporated under reduced pressure.

The solid thus obtained was washed with a mixture of petroleum ether - EtOAc (3 : 1) and then dried in vacuo, affording pure amine **10** (400 mg, 95.2 %). m.p: 108-109.5°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (t, 3H, J = 7.5 Hz, CH_3), 2.10 (q, 2H, J = 7.5 Hz, CH_2CO), 2.36 (s, 6H, $N(CH_3)_2$), 2.79 (t, 2H, J = 5.7 Hz, $CH_2N(CH_3)_2$), 3.28 (t, 2H, J = 6.9 Hz, $ArCH_2$), 3.54 (m, 2H, NCH_2), 3.94 (s, 3H, OCH_3), 4.19 (t, 2H, J = 5.7 Hz, $ArOCH_2$), 6.07 (br s, 1H, NH), 6.99 (dd, 1H, J = 2.3, 9.0 Hz, ArH), 7.08 (d, 1H, J = 9.0 Hz, ArH), 7.29 (d, 1H, J = 2.3 Hz, ArH), 7.64 (d, 1H, J = 9.0 Hz, ArH), 7.65 (d, 1H, J = 9.0 Hz, ArH). Analysis calculated for $C_{20}H_{28}N_2O_3x0.1\ H_2O$: C, 69.38; H, 8.21; N, 8.09. Found: C, 69.22; H, 8.24; N, 8.01.

N-[2-(2-Trimethylammoniumethyleneoxy-7-methoxynaphthyl)ethyl]propionamide iodide (**2a**, TMEPI)

To a solution of compound **10** (100 mg, 0.29 mmol) in acetone (6 mL) was added methyl iodide (0.072 mL, 1.16 mmol). The reaction mixture was stirred at r.t for 24 h and additional methyl iodide (0.072 mL, 1.16 mmol) was added. The mixture was stirred for an additional 24 h during which time the product was precipitated out. Petroleum ether (12 mL) was added to the mixture and it was stirred for another 2 h. The mixture was filtered and the solid was washed with a mixture of petroleum ether - EtOAc (1 : 2) and dried in vacuo, yielding pure compound **2a** (TMEPI) (128 mg, 90.6 %). m.p: 190-192°C; ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.02 (t, 3H, J = 7.5 Hz, CH_3), 2.10 (q, 2H, J = 7.5 Hz, CH_2CO), 3.14 - 3.19 (m, 4H, $ArCH_2$ and CH_2N), 3.88 (m, 2H, CH_2N), 3.93 (s, 2H, OCH_3), 4.58 (br s, $ArOCH_2$), 7.04 (br d, 1H, J = 8.8 Hz, ArH), 7.28 (d, 1H, J = 8.8 Hz, ArH), 7.67 (br s, 1H, ArH), 7.79 (d, 2H, J = 8.8 Hz, ArH), 8.05 (br s, 1H, NH). Analysis calculated for $C_{21}H_{31}N_2O_3I$: C, 51.86; H, 6.42; N, 5.76. Found: C, 51.96; H, 6.41; N, 5.76.

{^{14}C, N-[2-(2-Trimethylammoniumethyleneoxy-7-methoxynaphthyl)ethyl]}propionamide iodide (**2b**, ^{14}C-TMEPI)

To an ice-cooled solution of compound **10** (50 mg, 0.145 mmol) in acetone (1 mL) was added a solution of $^{14}CH_3I$ (1 mCi, 0.018 mmol) in acetone (3 mL). The reaction mixture was stirred at r.t in a sealed round bottom flask. After stirring for 24 h, methyl iodide (0.036 mL, 0.58 mmol) was added. The reaction mixture as stirred overnight and an additional portion of methyl iodide (0.036 mL, 0.58 mmol) was added. The mixture was stirred for another 24 h during which time product was precipitated out. Petroleum ether (10 mL) was added to the mixture and stirred for another 2 h. The mixture was filtered and the solid was washed with a mixture of petroleum ether/EtOAc (1:2) and dried in vacuo, affording **2b** (64 mg, 996 μCi, 99.6% radiochemical yield). The radiochemical purity (99.8%) was determined by TLC analysis (R_f = 0.27, CH_2Cl_2-MeOH, 1:1)

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