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Research Article

Synthesis of radio-iodinated melatonergic agents

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Summary

The synthesis of two ^{125}I-labeled trisubstituted aromatic melatonergic agents is reported. *N*-[[2-[^{125}I]iodo-5-methoxyphenyl)-1R, 2R-cyclopropyl]methyl] butanamide was prepared from the corresponding thallium salt and Na ^{125}I in radiochemical yields of 13–45% ($n = 3$). High performance liquid chromatography separation of the two resulting radioiodinated isomers (8:2) from each other and the parent compound required the use of two different columns run in series. Synthesis of *N*-[[2-[^{125}I]iodo-5-fluorophenyl)-1R, 2R-cyclopropyl]-methyl] butanamide was prepared from the corresponding trimethylstannane precursor in radiochemical yields consistently in the range of 60–65% ($n = 6$). Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: melatonergic; radioiodination; thallium trifluoroacetate; trimethylstannane

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Introduction

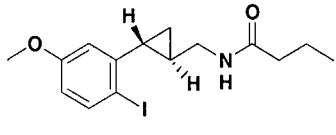
Melatonin, *N*-acetyl-5-methoxytryptamine, is a hormone which is synthesized and secreted primarily by the pineal gland. In mammals, melatonin levels show a cyclical circadian pattern, with the highest levels occurring during the dark period of a circadian light – dark cycle. In humans, administration of melatonin has been used to treat jet-lag-related sleep disturbances thought to be caused by desynchronization of circadian rhythms.[1]

Use of the biologically active, radiolabeled agonist [^{125}I]-2-iodomelatonin, has led to the identification of high affinity melatonin receptors in the central nervous system of a variety of spcies.[2] In humans, [^{125}I]-2-iodomelatonin binding within the hypothalamus is completely localized to the suprachiasmatic nucleus, strongly suggesting that melatonin receptors are located within the human biological clock.[3] Melatonin binding sites have been found outside the CNS and thus melatonin is though to have multiple physiological effects and thus the potential for multi-side effects.[4]

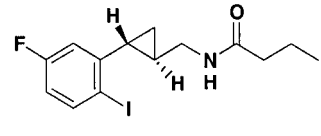
Our research interests have been focused on developing melatonergic agonists which are more selective than melatonin with the goal of producing an agent with fewer side effects and sustained activity.[5] In the course of this work, two iodinated agents (**1a, 2a**) were prepared and found to have high binding affinities (Figure 1). The IC_{50} values for (**1a**) were <0.25 nM for ML_{1A} and 0.3 nM for ML_{1B} while the IC_{50} values for (**2a**) were 38 nM for ML_{1A} and 1.8 nM for ML_{1B}. This manuscript describes the radioiodinated synthesis of these compounds (**1b, 2b**) for use in subsequent binding and *ex vivo* localization studies.

Results and discussion

McKilllop *et al.* reported in the early 1970s the preparation of thallium trifluoroacetate (TTFA), its use as a reagent for effecting electrophilic



(1a)

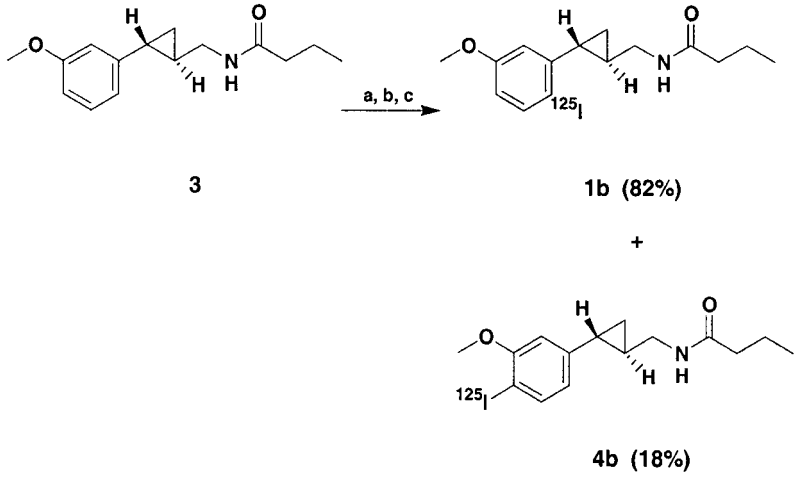


(2a)

Figure 1. Structures (1a) and (2a)

aromatic thallation, and a facile synthesis of aromatic iodides by treatment of the resulting arylthallium ditrifluoroacetates with aqueous potassium iodide.[6] A critical feature of this synthesis is that the entering iodine always replaces thallium at the same position on the aromatic ring.[7] Both the nature of the substituent on the aromatic ring, as well as the temperature of the reaction, have significant impact on the ultimate position of thallation (and therefore iodination).[7] Ortho substitution occurs when substituents on the aromatic ring are capable of chelation (e.g. CO_2H, CO_2R) with the thallium reagent, while para substitution results when such capabilities are absent and the substituent activates the ring to electrophilic substitution.[8] *p*-Methoxy substituted aromatics are a special case in that although the substituent is capable of chelating with TFAA, it also activates the ring towards electrophilic substitution.[7] Model studies with anisole resulted in complete thallation of the aromatic ring within 1 min at room temperature with a 93:7 selectivity for the para:ortho position.[7]

Our original synthesis of (**1a**) involved the electrophillic aromatic thallation of (**3**) via thallium (III) triacetate in carbon tetrachloride followed by treatment with a solution of I_2/CCl_4 to yield a mixture of the para and ortho substituted iodo-derivatives (9:1).[5] These results encouraged us to utilize this method to introduce radio-iodine (Scheme 1). The obvious benefit of this approach was the utilization

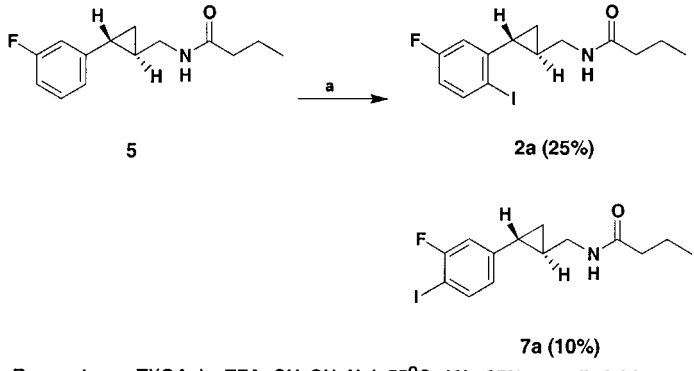


Scheme 1. Synthesis of ^{125}I-*N*-[[2-[^{125}I]iodo-5-methoxyphenyl)-1R, 2R-cyclopropyl-1-yl]methyl] butanamide, (1b)

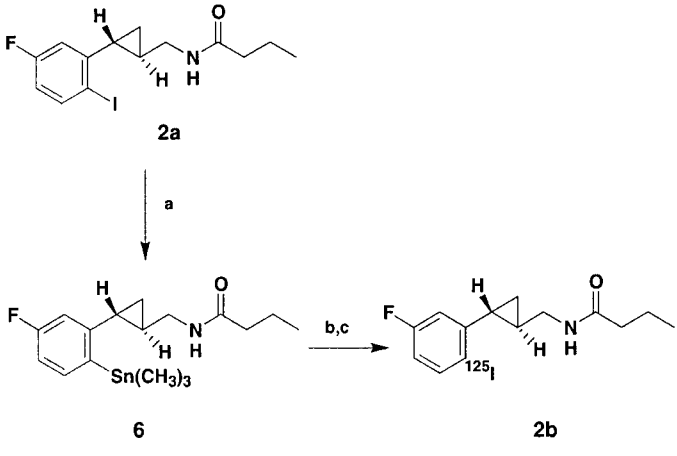
of a readily available intermediate (**3**); however, with this approach we realized the need to develop an high performance liquid chromatography (HPLC) system capable of separating both the precursor (**3**) and the undesired radio-iodinated compound (**4b**) from (**1b**). The development of the HPLC purification method suitable for purification of the desired radio-iodinated product proved more difficult than was originally anticipated. Use of either a Zorbax C8 or C18 analytical column ($4.6 \times 250\,mm^2$) with a mobile phase of 35% CH_3CN and 65% H2O provided excellent separation of precursor (**3**) from both of the radio-iodinated compounds (**1b, 4b**), but was unable to resolve the two resulting ^{125}I products. Use of a Zorbax cyano analytical column ($4.6 \times 250\,mm^2$) with the same mobile phase provided baseline separation of the two radio-iodinated products but poor separation of the desired product (**1b**) from the precursor (**3**). Successful baseline separation of all of the reaction components was achieved by the utilization of these two columns in series with the C8 column (first) and cyano column (second) and a mobile phase of 35% CH_3CN and 65% H_2O. In this system the precursor (**3**) had a retention time of approximately 37 min, the desired isomer (**1b**) had a retention time of approximately 101 min and the undesired isomer (**4b**) had a retention time of approximately 106 min. Several preparations of (**1b**) were performed with radiochemical yields ranging from 13 to 45%. The specific activity of (**1b**) was > 1600 Ci/mmol, the exact value could not be determined since the mass of (**1b**) associated with the radioactive material was below our limit of detection.

Thallation of deactivated haloaromatics, as in the case of (**5**), often require extended reflux times to form the thallium salt and usually result in lower thallation yields of the para position.[6] In our original synthesis of (**2a**) from (**5**) via the thallium intermediate and a 12 h reflux, we obtained (**2a**) in only a 25% yield. (Scheme 2).

Preliminary attempts to prepare radio-iodinated (**2b**) via thallium chemistry were unsuccessful and resulted in the formation of an unidentified radiolabeled compound. We then proceeded to prepare (**2b**) through the trimethylstannyl precursor (**6**) prepared from the corresponding iodo compound (**2a**) (Scheme 3). HPLC purification of (**2b**) was straightforward as compared to (**1b**), and was achieved on a single Zorbax C8 analytical column ($4.6 \times 250\,mm^2$) with a mobile phase of 35% CH_3CN and 65% H_2O. In this system, the desiodo compound elutes at approximately 19 min, while the desired radiolabeled product (**2b**) elutes at approximately 43 min. In this reaction, in contrast to the



Scheme 2. Synthesis of *N*-[[2-iodo-5-fluorophenyl)-1R, 2R-cyclopropyl-1-yl]-methyl] butanamide (2a) via thallium chemistry



Scheme 3. Synthesis of ^{125}I *N*-[[2-[^{125}I]iodo-5-fluorophenyl)-1R, 2R-cyclopropyl-1-yl]methyl] butanamide (2b)

chemistry used in the methoxy series, none of the undesired radiolabeled product is prepared. Numerous preparations of (**2b**) were made with radiochemical yields of 60–65% ($n = 6$). The specific activity of (**2b**) was > 1600 Ci/mmol, the exact value could not be determined since the mass of (**2b**) associated with the radioactive material was below our limit of detection.

Materials and methods

Previously prepared non-radioactive proprietary precursors and final products were prepared according to published procedures.[5] Na ^{125}I was obtained from both Nordion International Inc. of Kanata, Ontario, and DuPont/NEN of N. Billerica, MA. All other reagents were obtained from Aldrich Chemical Company of Milwaukee, WI and were either ACS grade of the highest quality material commercially available. HPLC purification and analysis was performed on a Rainin Dynamax HPLC system consisting of two SD-200 pumps, a Rainin UV-I detector and an *INUS* γ-RAM radioactive flow-through detector. A Biodex Medical Systems Atomlab^{TM} 100 Dose Calibrator was used for radio-activity measurements.

High performance liquid chromatography

Method A. In this method samples are loaded onto a Zorbax C8 column ($4.6 \times 250\,mm^2$) with a mobile phase of 60% CH_3CN and 40% H_2O at a flow rate of 1.5 ml/min. The UV-1 detector was set at 220 nm.

Method B. This method utilizes two analytical HPLC columns used in series, namely a Zorbax C8 column ($4.6 \times 250\,mm^2$) followed by a Zorbax cyano column ($4.6 \times 250\,mm^2$). The distance between the two columns was kept to an absolute minimum (less than 3 cm). The mobile phase consisted of 35% CH_3CN and 65% H_2O and a flow rate of 1 ml/min resulting in a column pressure of 3.27 kpsi. The UV-1 detector was set at 220 nm.

Method C. In this method samples are loaded onto a Zorbax C8 column ($4.6 \times 250\,mm^2$) with a mobile phase of 35% CH_3CN and 65% H_2O at a flow rate of 1 ml/min. The UV-1 detector was set at 220 nm.

Experimental

*N-[2-(5-Fluoro-2-trimethylstannylphenyl)-1R, 2R-cyclopropylmethyl] butanamide (**6**)*

To a rapidly stirred degassed solution of *N*-[2-(iodo-5-flurophenyl)-1R, 2R-cyclopropylmethyl] butanamide (**2a**) (50.0 mg, 0.14 mmol) in Et_3N (2 ml) was added a mixture of $Pd(OAc)_2$ (2.5 mg, 0.011 mmol) and

$Pd(Ph_3P)_4$ (7 mg, 0.006 mmol). Hexamethylditin (92 mg, 0.28 mmol) was then added in one portion, followed by heating to reflux for 2 h. The reaction mixture was then cooled to room temperature, filtered through celite and the filter cake washed well with Et_2O. The filtrate was concentrated and further purified by flash chromatography (silica gel, 20% EtOAc/hexane increasing to 50% EtOAc/hexane) to afford the desired compound (**6**) (30 mg, 53% yield). MS (ESI), $(M–H)^-$ 398.2 (Sn 120); ^1H NMR ($CDCl_3$) δ 7.31 (t, 1 H, $J = 7.0$ Hz), 6.81–6.88 (m, 1 H), 6.50–6.59 (m, 1 H), 5.55 (s, br, 1 H), 3.10–3.41 (m, 2 H), 2.05 (t, 2 H, $J = 7.2$ Hz), 1.58–1.80 (m, 3 H), 1.33–1.39 (m, 1 H), 0.92 (t, 3 H, $J = 7.5$ Hz), 0.83–0.91 (m, 2 H), 0.31 (s, 6 H). The product was also analyzed by HPLC (Method A). In this system the starting compound (**2a**) has a retention time of approximately 5.1 min, the corresponding desiodo compound has a retention time of approximately 3.7 min, while the desired trimethylstannane (**6**) has a retention time of approximately 9.8 min.

*N-[2-[^{125}I]iodo-5-methoxyphenyl)-1R, 2R-cyclopropylmethyl] butanamide, (**1b**)*

Into a 1 ml Pierce Reacti-Vial™ was added a solution of *N*-[5-methoxyphenyl)-1R, 2R-cyclopropylmethyl]butanamide (**3**) in CH_3CN (10 µl of a 5 mg/ml solution). The vial was placed in a -35° bath (CH_3CN:dry ice) and to this was added a solution of $Tl(O_2CCF_3)_3$ in CF_3CO_2H (30 µl of a 10 mg/ml solution). This solution was allowed to stir for 15 min at -35°C. After 15 min, 10 µl of a carrier solution of Na ^{125}I (370 µCi/µl) was combined with 10 µl of CF_3CO_2H and the solution then transferred to the reaction vial. The reaction mixture was then allowed to stir for 15 min at -35°C, after which time, a solution of sodium metabisulfite (15 µl of a 2 mg/ml solution) was added to quench the reaction. The solution was diluted with H_2O (1 ml) and then passed through a single C-18 Sep-Pak™ cartridge. The cartridge was rinsed with H_2O (10 ml) and then rinsed with CH_3CN (3 ml) to elute the desired compound. The volume of CH_3CN was then reduced via a stream of N_2 to approximately 0.3 ml, and diluted with H_2O to approximate 35%. The crude product was then purified by HPLC (Method B). In this system the precursor (**3**) had a retention time of approximately 37 min, the desired isomer (**1b**) had a retention time of approximately 101 min and the undesired isomer (**4b**) had a retention time of approximately 106 min. Several preparations of (**1b**) were

performed following this procedure with radiochemical yields ranging from 13 to 45%. The specific activity of (**1b**) was >1600 Ci/mmol, the exact value could not be determined since the mass of the radiolabeled product was below our limit of detection.

*N-2-[^{125}I]iodo-5-flurophenyl)-1R, 2R-cyclopropylmethyl] butanamide, (**2b**)*

Into a 1 ml Pierce Reacti-Vial^{TM} was added CF_3CO_2H (10 µl), H_2O (10 µl), a freshly prepared solution of iodogen in absolute EtOH (10 µl of a 1 mg/ml solution), *N*-[5-fluoro-2-trimethylstannylphenyl)-1R,2R-cyclopropylmethyl[butanamide (**6**) (10 µl of a 1 mg/ml solution of **6**) and carrier-free Na ^{125}I (10 µl of a 370 µCi/µl stock solution). The solution was then allowed to stir at room temperature for 1 h after which the reaction was quenched by the addition of sodium metabisulfite (20 µl of a 5 mg/ml solution in water). The reaction mixture was then diluted with mobile phase (30 µl of 35% of aq. CH_3CN) and purified by HPLC (Method C). In this system, the desiodo compound elutes at approximately 19 min, while the desired product (**2b**) elutes at approximately 43 min and the undesired isomer (**7a**) elutes at approximately 48 min. Numerous preparations of (**2b**) were made with radiochemical yields routinely being between 60 and 65% ($n=6$). The specific activity of (**2b**) was >1600 Ci/mmol, the exact value could not be determined since the mass of the radiolabeled product was below our limit of detection.

References

1. Arendt J, Aldhous M, Marks V. *Br Med J* 1986; **292**: 1170.
2. Duncan MJ, Takahashi JS, Dubocovich ML. *Endocrinology* 1988; **122**: 1825–1833.
3. Reppert SM, Weaver DR, Rivkees SA, Stopa EG. *Science* 1988; **242**: 78–81.
4. Song Y, Tam PC, Poon AMS, Brown GM, Pang SF. *J Clin Endocrinol Metab* 1995; **80**: 1560–1565.
5. Keavy DJ, Parker MF, Mattson RJ, Johnson G. *US Patent* # 5,753,709, May 19, 1998.
6. McKillop A, Hunt JD, Zelesko MJ, Fowler JS, Taylor EC, McGillivray G, Kienzle F. *J Am Chem Soc* 1971; **93**: 4841–4844.
7. McKillop A, Hunt JD, Taylor EC. *J Orgonometal Chem* 1970; **24**: 77–88.
8. Taylor EC, Kienzle F, Robey RL, McKillop A, Hunt JD. *J Am Chem Soc* 1971; **93**: 4845–4850.

