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Synthesis and enantioseparation of atropisomers of serotonin dimer

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ABSTRACT

A preparative scale synthesis of a dimer of serotonin (5-HT) is described. DHBT or 5,5′-dihydroxy-4,4′-bitryptamine was prepared in good yields using methanol in the presence of copper(II) chloride and air at room temperature. Exclusion of air resulted in no DHBT. Neither 5-HT hydrochloride nor N-BOC-protected 5-HT yielded DHBT under identical reaction conditions, evidence that the primary amino group of 5-HT plays a critical role in the dimerization reaction. The atropisomers of DHBT can be resolved by chiral capillary electrophoresis.

5-Hydroxytryptamine (5-HT, serotonin, Fig. 1a) occurs naturally in central and peripheral nervous systems, where it plays a key role in the regulation of body temperature, appetite, alcoholism, drug abuse, sleep, and emotional states, as well as in cognition including memory and learning.[1–4] Previous studies have indicated that 5-HT forms higher order structures in a faulty oxidative environment that could deplete serotonin levels and cause certain types of mental disorders including depression,[5] schizophrenia,[6] and Alzheimer's disease.[7,8] Studies on 5-HT oxidation products have suggested that 5-HT dimer, 5,5′-dihydroxy-4,4′-bitryptamine (DHBT, Fig. 1b) is potentially neurotoxic and contributes to neurodegenerative diseases.[9–12] Following an early report by Eriksen's group on a non-enzymatic oxidation of 5-HT by using inorganic $CuSO_4$ oxidant,[12] Wrona and Dryhurst proposed an electrochemical oxidative pathway of 5-HT to DHBT by coupling of phenoxy radicals.[13] They further reported that DHBT is the major nitrogen-centered radical to induce the generation of C(4)–C(4′) and N(1)–C(4′) dimers in the course of this initial autoxidation product of 5-HT at physiological pH and temperature.[5,9] Jones and his colleagues studied the formation of DHBT induced by low concentration of Cu^{2+} ion (1 mmol/L). They showed that DHBT had only a minor toxic effect on the neuronal cell model PC12, suggesting that reactive oxygen species are primarily responsible for the observed toxic effect.[11] Heuther et al. showed that 5-HT is oxidized to DHBT in the presence of peroxidase and hydrogen peroxide.[14] The major product was isolated and analyzed by mass spectrometry.

Apart from the foregoing studies on the formation and neurotoxicity of DHBT, no further work has appeared on preparative scale synthesis of DHBT. We report here the convenient synthesis of DHBT using copper(II) chloride in methanol in the presence of air. We find that both air (molecular oxygen) and the primary amino function of 5-HT are essential for good yields. As expected, DHBT is found to exhibit atropisomerism. The atropisomers are resolved by chiral capillary electrophoresis.

DHBT was obtained by the approach shown in Scheme 1. In situ ^1H NMR spectroscopy studies were used to monitor the formation of dimer. The optimum amount of copper(II) chloride and added base were determined in a similar manner reported by Jones et al.[11]

No significant difference between 0.625 and 1.25 equiv of Cu^{2+} on yield of DHBT was observed, but 1.8 and 2.5 equiv resulted in significantly lower NMR yields. Thus preparative scale synthesis of DHBT was accomplished in the presence of 1.25 molar equivalents of copper(II) chloride dihydrate in methanol, which provided the highest isolated yield. The respective DHBT

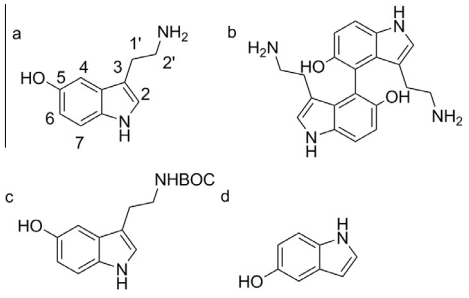

Figure 1. (a). Schematic of 5-hydroxytryptamine (serotonin) showing numbering scheme used in this work; (b) DHBT; (c) Boc-protected 5-HT; (d) 5-hydroxyindole.

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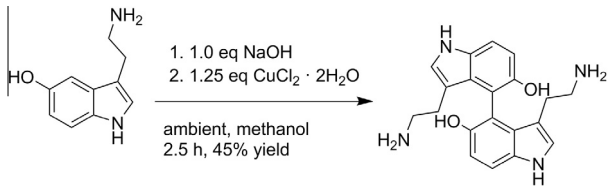

Scheme 1. Cu(II) induced oxidative coupling reaction of 5-HT.

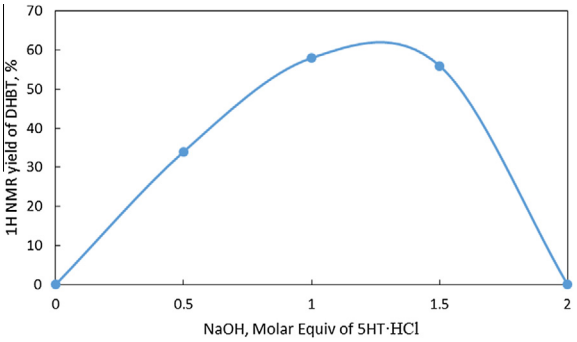

Figure 2. Effect of base added on the conversion of 5-HT to DHBT after 1 h of reaction time as monitored by ^{1}H NMR spectroscopy.

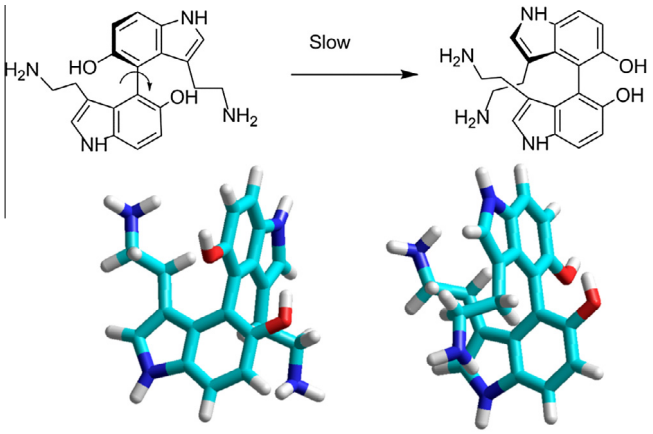

Figure 3. Atropisomers of DHBT attributed to slow rotation about 4-4′ carbon–carbon bond.

dihydrochloride salt was readily purified by Sephadex LH-20 column chromatography (pH 2) in 45% yield.

When addition of base was omitted, no DHBT was isolated. Indeed, addition of one equivalent of base caused an immediate reaction to occur with the formation of a black suspension, and led eventually to the color change of the reaction mixture to reddish. This finding led us to consider that the primary amino group was indispensable for the Cu^{2+}-induced oxidative formation of DHBT. To study this finding systematically, we varied the amount of base to neutralize 5-HT·HCl before adding copper(II) chloride dihydrate (Fig. 2), and then measured the NMR yield after one hour. Addition of 1.0 and 1.5 equiv base gave similar NMR yields, but the highest isolated DHBT yield was obtained when 5-HT·HCl was neutralized with 1.0 equiv of base.

To further investigate the influence of the primary amino function, BOC-protected 5-HT (Fig. 1c) and 5-hydroxyindole (Fig. 1d), which lack the primary amino function, were considered under the same reaction conditions shown in Scheme 1. In situ ^{1}H NMR spectroscopy in methanol-d_4 did not reveal formation of dimer for either substrate, but rather the disappearance of some resonances was observed from an otherwise unchanging spectrum. The results are interpreted as hydrogen/deuterium exchange of H-4 of Boc-protected 5-HT (complete in 1.5 h) and both H-3 and H-4 of 5-hydroxyindole (complete in <5 min and 1 h, respectively) in the presence of Cu^{2+} ion. Exchange did not take place in the absence of Cu^{2+} ion. These results indicate that, while aryl H–D exchange is promoted by Cu^{2+}, the aminoethyl group ($-CH_2CH_2NH_2$) is essential to the Cu^{2+}-induced oxidative coupling of 5-HT under the conditions studied.

Copper-mediated or -catalyzed oxidative couplings have been extensively studied for the synthesis of biaryl compounds. Cupric-amine complexes in particular have been studied as attractive oxidants for the coupling reaction, for instance the oxidative coupling of phenols[15] and naphthols.[16,17] In the oxidative coupling of 5-HT, the primary amino function is critical for achieving high yields of DHBT. The amino group may coordinate to Cu^{2+} to form Cu^{2+}–5-HT complexes, which contributes to the black colored suspension mentioned above, and facilitates the oxidative coupling of two 5-HT molecules within the coordination sphere of Cu^{2+}.

Under aerobic conditions, DHBT could be observed by in situ ^{1}H NMR spectroscopy using either methanol-d_4 or D_2O as solvent. When the reaction was performed under anaerobic condition, no DHBT formed. Oxygen may serve to reoxidize Cu^{1+} to Cu^{2+}, thus

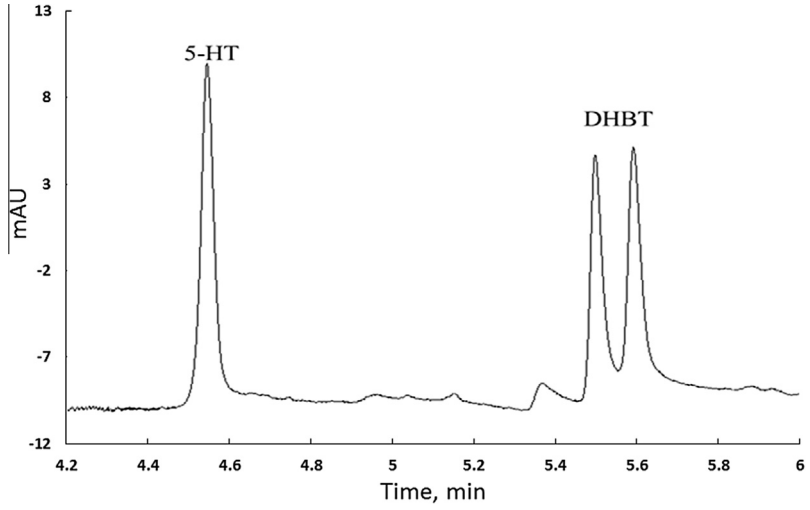

Figure 4. Capillary electrophoresis of 5-HT and DHBT atropisomers. 50.0 μm i.d., bare fused silica capillary, 32.5 cm total length, 24.0 cm to detector; detection 214 nm; injection: 50.0 mBar/1 s; BGE: 80 mM taurodeoxycholate in 20.0 mM tris, pH = 8.0; normal polarity, 10 kV. Sample was injected during the course of the reaction.

allowing for a catalytic cycle.[18,19] However, if the sole role of oxygen were to turnover Cu^{1+} to Cu^{2+}, then some product should form in the absence of oxygen since a stoichiometric amount of Cu^{2+} is used. The fact that no DHBT forms in the absence of oxygen suggests a more complex mechanism involving oxygen.

Many biaryl compounds similar in structure to DHBT are known to exhibit atropisomerism, a type of stereoisomerism, which results from steric hindrance rotation along a bond axis.[20–23] DHBT is predicted as a pair of enantiomeric atropisomers with chirality arising from hindered rotation about the biaryl C4–C4′ bond (Fig. 3). Consistent with this prediction, the chiral separation of DHBT enantiomeric atropisomers was successfully achieved by capillary electrophoresis with sodium taurodeoxycholate (STDC) as chiral selector (Fig. 4). After a systematic study, a buffer system composing of 80 mM STDC in 20.0 mM Tris at pH = 8.0 was adopted to generate nearly baseline resolution for DHBT enantiomeric atropisomers. This technique could be used for monitoring conversion of 5-HT to DHBT during the course of the reaction. The NMR spectrum also supports the existence of atropisomers of DHBT; the four hydrogens of the ethylamino group are diastereotopic by virtue of the chiral axis and reveal distinct, baseline-resolved resonances in the 600 MHz ^{1}H NMR spectrum.

Conclusion

We have demonstrated a preparative procedure for DHBT in methanol induced by Cu^{2+} under oxygen. The coupling condition is simple and the work-up is straightforward. The primary amino function of 5-HT is essential for good yields under the experimental conditions. We hypothesize that Cu^{2+} ion coordinates to a 5-HT through the amino function, and that coupling occurs in the coordination sphere of the metal. DHBT atropisomers are also resolved using chiral capillary electrophoresis.

Supplementary data

Supplementary data (experimental, in situ ^{1}H NMR spectroscopy, mass spectrometry, capillary electrophoresis) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.04.127.

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