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Syntheses and properties of the major hydroxy metabolites in humans of blonanserin AD-5423, a novel antipsychotic agent

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Abstract—Two major metabolites in humans of blonanserin, 2-(4-ethyl-1-piperazinyl)-4-(4-fluorophenyl)-5,6,7,8,9,10-hexahydrocycloocta-[b]pyridine (code name AD-5423), were synthesized. The first, 7-hydroxylated AD-5423, was synthesized through a four-step process starting from 4-fluorobenzoylacetonitrile (**1**), and the second, 8-hydroxylated AD-5423, a nine-step process also from **1**. The optical resolution, structures, and receptor binding properties of the metabolites were documented.

1. Introduction

Blonanserin (AD-5423), having dopamine D_2 and serotonin 5-HT_2 receptor antagonist properties, is a novel antipsychotic agent now under clinical development.[1–3] Some antipsychotics often produce oversedative and/or autonomic side effects, probably because of their adrenaline-α_1 receptor antagonist properties. The potent serotonin 5-HT_2 antagonism, together with weak adrenaline-α_1 and virtually no dopamine D_1 antagonist properties suggests that blonanserin is expected to offer an advantageous clinical profile.[1] In pharmacokinetic studies, many metabolites (deethyl form, N-oxide form, etc.) of the agent were detected and identified in animals.[4] However, hydroxy forms were not identified and four hydroxy isomers were estimated in the in vitro metabolism study using rat liver microsomes (unpublished). In the mass balance study using ^{14}C-labeled compound in humans, 7- and 8-hydroxylated AD-5423 were suggested as major metabolites. We describe preparation of enantiomerically pure 7- and 8-hydroxy AD-5423 derivatives to confirm chemical structures of metabolites and for additional biological studies.

2. Chemistry

7-Hydroxylated AD-5423 (**6**) was synthesized in a four-step process as shown in Scheme 1. First, the starting material, 1,5-cyclooctanonedione (**2**)[5] was synthesized from the commercially available 1,5-cyclooctanediol by PCC oxidation. Then, 4-fluorobenzoylacetonitrile (**1**)[6] was condensed to the compound **2** by heating with PPA to give the compound **3**, which provides a basic framework of the target.[7] The compound **3** was converted to the triflate **4** by CF_3SO_2Cl, followed by $NaBH_4$ reduction to yield the alcohol **5**. Finally, condensation of **5** with N-ethylpiperazine gave the 7-hydroxylated AD-5423 (**6**).[8]

8-Hydroxylated AD-5423 (**16**) was synthesized in a nine-step process as shown in Scheme 2. First, condensation of **1** with cyclooctanone **7** through the above described method gave the compound **8**, which was converted to the corresponding 2-chloro derivative **9** by treatment with $POCl_3$.[1] The compound **9** was then converted to the N-oxide **10** with *m*-CPBA, which underwent

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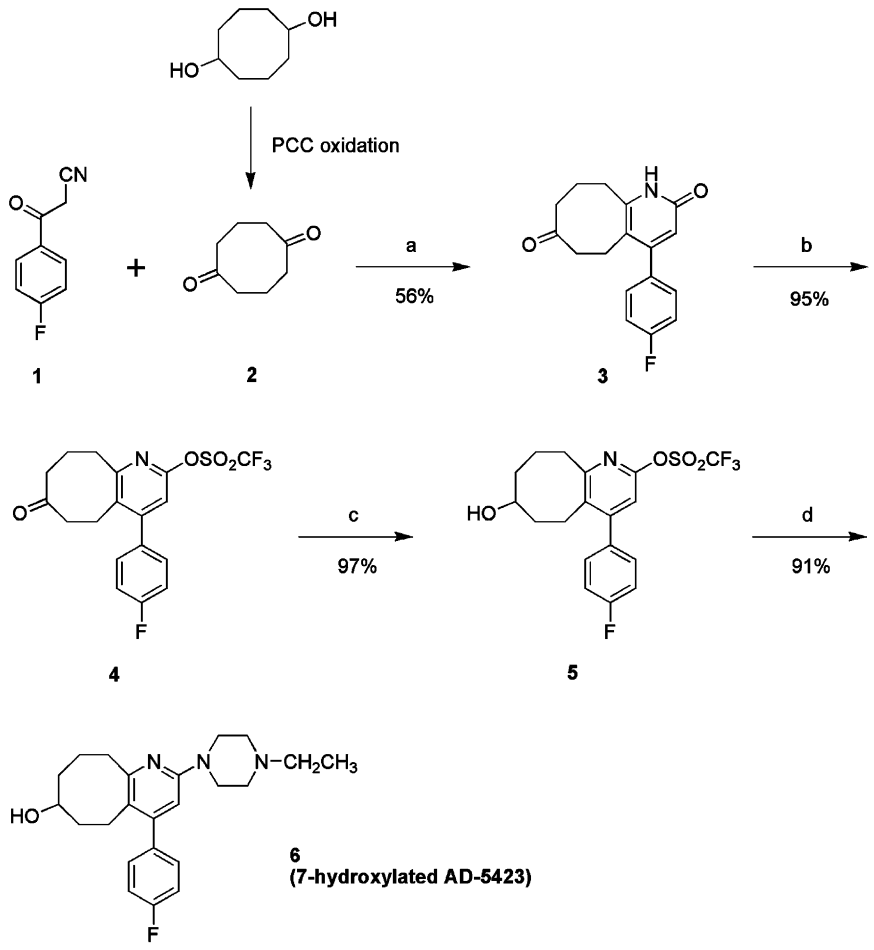


Scheme 1. Reagents and conditions: (a) PPA; (b) CF_3SO_2Cl, pyridine; (c) $NaBH_4$, MeOH; (d) N-ethylpiperazine.

chlorination using $POCl_3$ and Et_3N to yield the 2,10-dichloro derivative **11**. Elimination of HCl from **11** using DBU and subsequent oxidation of the resultant compound **12** with SeO_2 and HCOOH gave the 8-oxyformyl derivative **13**. Deformylation with HCl followed by reduction of the olefin **14** with H_2 and PtO_2 gave the alcohol **15**. Finally, condensation of **15** with N-ethylpiperazine gave the 8-hydroxylated AD-5423 (**16**) Figure 1.[9]

Chemical structures of the two hydroxylated AD-5423 derivatives, **6** and **16**, were confirmed by mass spectrometry (MS) and nuclear magnetic resonance spectrometry (NMR).[8,9]

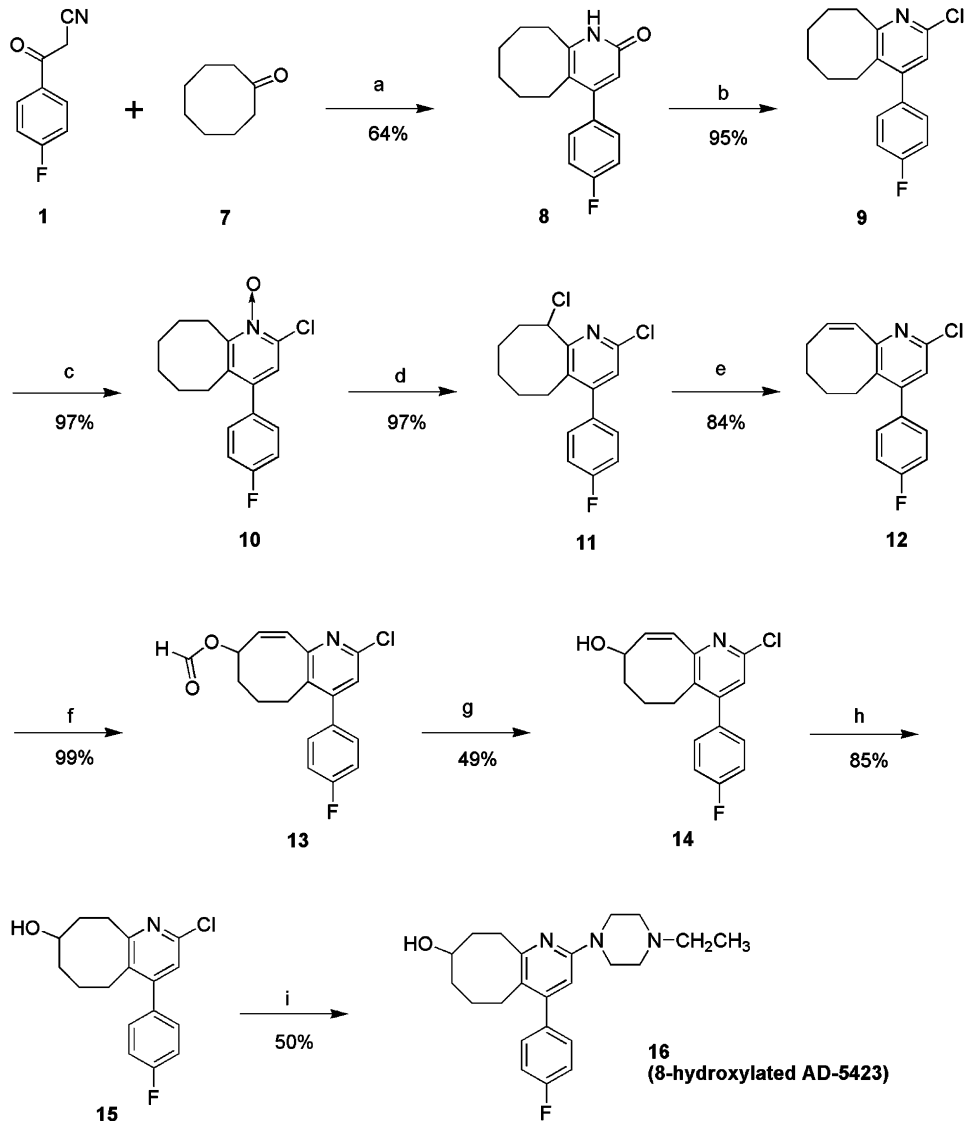
A chiral resolution of the compound **6** (12 g) by preparative HPLC using a CHIRALCEL AD column was then conducted to give the optically pure alcohols, **6a** (*S*-configuration, 4.2 g) and **6b** (*R*-configuration, 5.2 g), with an enantiomeric purity of >98%ee.[10] Similarly, the compound **16** (10 g) was resolved using a CHIRALCEL OJ column to give optically pure **16a** (4.5 g) and **16b** (4.4 g) with >98%ee.[10]

The absolute configurations of **6a** (*S*-configuration) and **16b** (*R*-configuration) were determined by X-ray crystallography of their HBr salt,[11] and the ORTEP diagrams of **6a** and **16b** are shown in Figures 2 and 3, respectively. In addition, the absolute configuration of hydroxy center for each isomer was also confirmed by the improved Mosher method using 2-methyl-2-(1-naphthyl)propionic acid (MαNP acid),[12] a powerful chiral auxiliary Scheme 3.

3. Biological results and discussion

The in vitro metabolism study using human liver microsomes (unpublished) revealed that the main stereoisomers were **6a** of the 7-hydroxy and **16b** of the 8-hydroxy metabolites. These findings indicate that the hydroxyl groups of the two major stereoisomers of human metabolites are situated on the same side of their eight-membered rings.

The four isomers (**6a**, **6b**, **16a**, and **16b**) and AD-5423 were examined for their binding affinity to the human D_{2L} and $5HT_{2A}$ receptors (Table 1). The binding affinity of AD-5423 is >25.7 and >11.9 times higher than those of the pair of **6a** and **6b**, and of **16a** and **16b** isomers, respectively. Therefore, introduction of a hydroxyl group at 7- and 8-positions on the eight-membered ring reduced the affinity to both receptors.



Scheme 2. Reagents and conditions: (a) PPA; (b) $POCl_3$; (c) *m*-CPBA, $CHCl_3$; (d) $POCl_3$, Et_3N, $CHCl_3$; (e) DBU, DMSO; (f) SeO_2, HCOOH, dioxane; (g) HCl, MeOH; (h) H_2, PtO_2, EtOH; (i) N-ethylpiperazine.

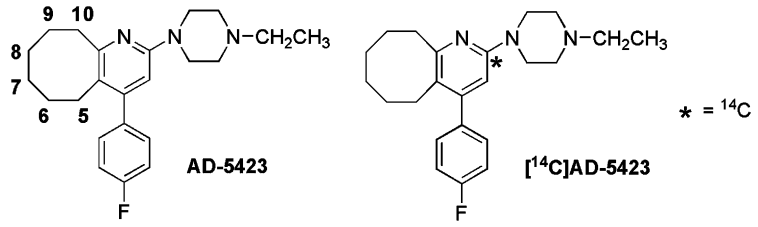


Figure 1. The chemical structure of AD-5423 and [^{14}C]AD-5423.

The stereo isomers **6b** and **16b** having *R*-configuration at the hydroxy group exhibited a higher affinity than **6a** and **16a**. Concentrations of these metabolites in human plasma are currently investigated in our clinical pharmacokinetic studies, and pharmacological role of the metabolites will be taken into consideration in the course of development of AD-5423.

4. Conclusion

In summary, the synthesis and properties of hydroxy metabolites (**6** and **16**) of blonanserin (AD-5423) in humans have been described. Synthesis of **6** and **16** were achieved in four- and nine-step processes from compound **1**, respectively. Subsequent optical resolution of

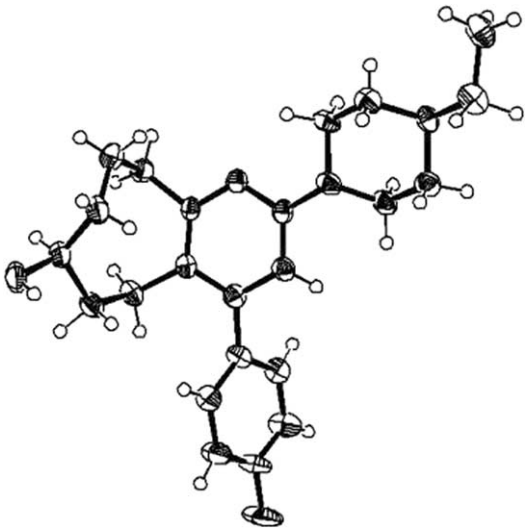


Figure 2. The ORTEP drawing of **6a** with thermal at 50% probabilities.

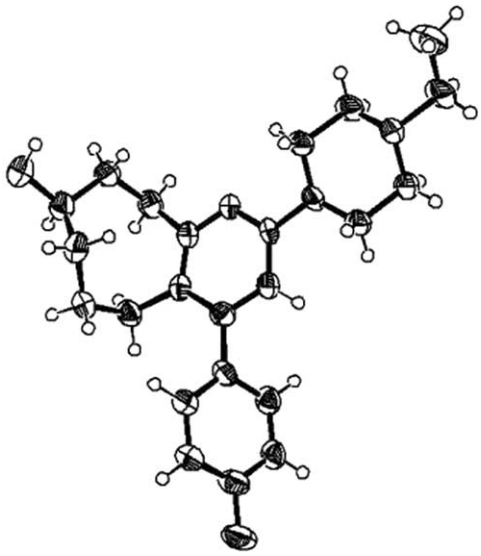
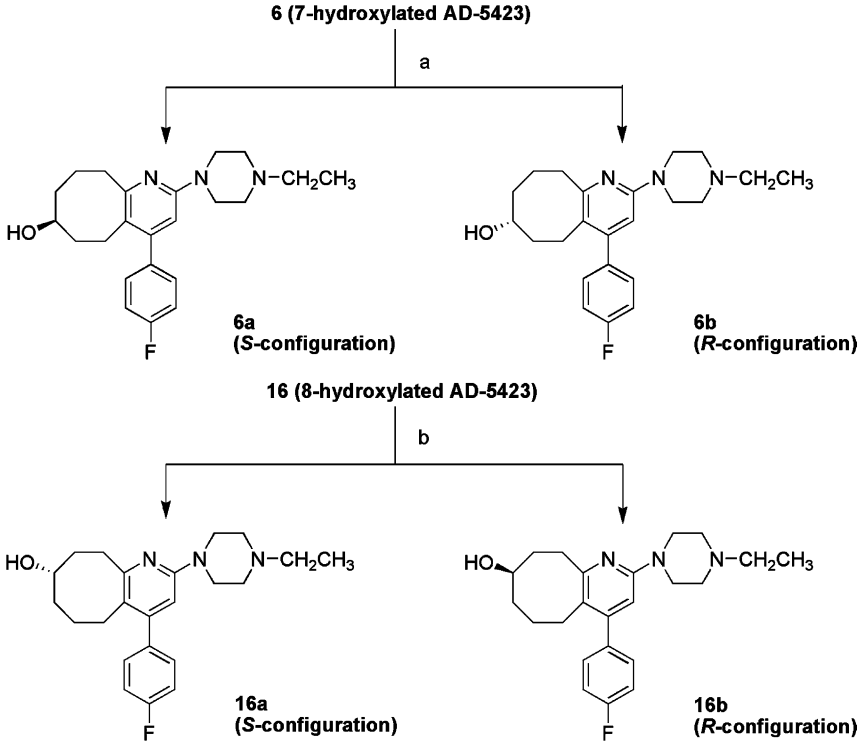


Figure 3. The ORTEP drawing of **16b** with thermal at 50% probabilities.



Scheme 3. Reagents and conditions: (a) CHIRALCEL AD column, mobile phase of MeOH/Et_2NH (100:0.1); (b) CHIRALCEL OJ column, mobile phase of hexane/EtOH/Et_2NH (90:10:0.1).

Table 1. Binding affinity to recombinant human D_{2L} and $5HT_{2A}$ receptors

Compds	Configuration of hydroxyl center	hD_{2L} receptor K_i (nM)[a]	$h5HT_{2A}$ receptor K_i (nM)[a]
AD-5423f		0.14 ± 0.002	0.81 ± 0.22
6a	*S*	21.2 ± 1.0	18.5 ± 2.7
6b	*R*	6.20 ± 0.35	14.7 ± 2.4
16a	*S*	11.9 ± 0.3	17.9 ± 0.9
16b	*R*	3.60 ± 0.03	9.67 ± 0.77

[a] Data are expressed as the mean ± SEM of three independent experiments.

6 and **16** were performed by preparative HPLC using a chiral column. Additionally, in an in vitro metabolism study, it was revealed that the main metabolites in humans were **6a** (*S*-configuration) and **16b** (*R*-configuration), whose absolute configuration were determined by X-ray crystallography. All stereoisomers had affinities less than one tenth of the parent AD-5423 to the human D_{2L} and $5HT_{2A}$ receptors. Isomers **6b** and **16b** with *R*-configuration exhibited a higher affinity to the receptors than their respective isomers.

Acknowledgements

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References and notes

1. Oka, M.; Hino, K. *Drug. Future* **1992**, *17*, 9–11.
2. Hino, K.; Kai, N.; Sakamoto, M.; Murata, T.; Oka, M.; Matsumoto, J. *Abstracts of Papers*, MEDI 11, 200th National Meeting of the American Chemical Society, Washington, DC, April, 1990.
3. Suzuki, K.; Hiyama, Y.; Une, T.; Fujiwara, I. *Anal. Sci.* **2002**, *18*, 1289–1290.
4. Matsuda, M.; Toda, H.; Ishiwata, A.; Mizuki, Y.; Fujii T.; Miyazaki H. *Abstracts of Papers, 140*, 5th International ISSX Meeting, Queensland, Australia, October, 1998.
5. Schreiber, S. L.; Smith, D. B. *J. Org. Chem.* **1989**, *54*, 5994–5996.
6. Ridge, D. N.; Hanifin, J. W.; Harten, L. A.; Johnson, B. D.; Menschik, J.; Nicolau, G.; Sloboda, A. E.; Watts, D. E. *J. Med. Chem.* **1979**, *22*, 1385–1389.
7. Hauser, C. R.; Eby, C. J. *J. Am. Chem. Soc.* **1957**, *79*, 728–731.
8. Spectral data for **6**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.12 (t, J = 7.1 Hz, 3H, CH_3), 1.41 and 1.71 (m, 2H, CH_2), 1.42 (br s, 1H, OH), 1.71 (m, 2H, CH_2), 1.78 and 1.94 (m, 2H, CH_2), 2.46 (q, J = 7.1 Hz, 2H, CH_2), 2.55 (m, 4H, CH_2), 2.55 (m, 1H) and 2.66 (ddd, J = 14.5, 9.9, 4.0 Hz, 1H) (CH_2), 2.85 (m, 2H, CH_2), 3.53 (m, 4H, CH_2), 3.56 (m, 1H, CH), 6.29 (s, 1H, CH), 7.08 (m, 2H, CH), 7.22 (m, 2H, CH) ppm; ^{13}C NMR (400 MHz, $CDCl_3$) δ 11.93, 23.79, 26.61, 35.58, 37.07, 40.25, 45.27, 52.33, 52.57, 72.19, 105.83, 114.77 (d, J = 21.7 Hz), 121.74, 129.72 (d, J = 8.0 Hz), 136.66 (d, J = 3.1 Hz), 150.17, 157.09, 158.43, 161.76 (d, J = 247.1 Hz) ppm; MS (APCI) *m*/*z* (relative intensity): 384 ([MH^+], 100).
9. Spectral data for **16**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.16 (t, J = 7.0 Hz, 3H, CH_3), 1.35 (br s, 1H, OH), 1.46 and 1.53 (m, 2H, CH_2), 1.63 (m, 2H, CH_2), 1.76 and 2.20 (m, 2H, CH_2), 2.53 (q, J = 7.0 Hz, 2H, CH_2), 2.53 (m, 2H, CH_2), 2.61 (m, 4H, CH_2), 2.83 (ddd, J = 13.3, 7.8, 3.8 Hz, 1H) and 2.66 (ddd, J = 13.3, 10.2, 3.5 Hz, 1H) (CH_2), 3.58 (m, 4H, CH_2), 3.58 (m, 1H, CH), 6.30 (s, 1H, CH), 7.07 (m, 2H, CH), 7.18 (m, 2H, CH) ppm; ^{13}C NMR (400 MHz, $CDCl_3$) δ 11.71, 26.78, 27.68, 32.20, 36.18, 39.25, 45.05, 52.34, 52.44, 72.39, 106.01, 114.73 (d, J = 21.5 Hz), 121.75, 129.71 (d, J = 7.5 Hz), 136.68 (d, J = 3.7 Hz), 150.30, 156.80, 158.56, 161.75 (d, J = 245.2 Hz) ppm; MS (APCI) *m*/*z* (relative intensity): 384 ([MH^+], 100).
10. Optical resolution of **6** and **16** were performed at Daicel Chemical Industries, Ltd.
11. Spectral data, for **6a**: mp 194–196 °C (EtOH); $[\alpha]_D^{27}$ −15.1 (*c* 1.0, MeOH); for **6b**: mp 194–196 °C (EtOH); $[\alpha]_D^{27}$ −28.3 (*c* 1.0, MeOH); for **16a**: mp 170–172 °C (MeOH + CH_3CN); $[\alpha]_D^{27}$ +28.4 (*c* 1.0, MeOH); for **16b**: mp 170–172 °C (MeOH + CH_3CN); $[\alpha]_D^{27}$ −28.4 (*c* 1.0, MeOH); for HBr salt of **6a**: mp 172–174 °C (CH_3CN); $[\alpha]_D^{27}$ −15.1 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3326 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (t, J = 7.3 Hz, 3H), 1.23–1.33 (m, 1H), 1.40–1.50 (m, 1H) 1.54–1.67 (m, 3H), 1.76–1.88 (m, 1H), 2.44–2.52 (m, 1H), 2.59 (m, 1H), 2.72–2.85 (m, 2H), 2.95–3.14 (m, 4H), 3.18 (m, 2H), 3.27–3.35 (m, 1H), 3.54 (m, 2H), 4.32 (br s, 1H), 4.40 (m, 2H), 6.54 (s, 1H), 7.25–7.35 (m, 4H), 9.49 (m, 1H) ppm; MS (APCI) *m*/*z* (relative intensity): 384 ([MH^+ of free base], 100). For HBr salt of **16b**: mp 271–273 °C (*i*-PrOH + CH_3CN); $[\alpha]_D^{27}$ +28.4 (*c* 1.0, MeOH); IR (KBr) ν_{max} 3402 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (t, J = 7.3 Hz, 3H), 1.30–1.60 (m, 5H), 2.01 (m, 1H), 2.46–2.54 (m, 2H), 2.73 (m, 1H), 2.91 (m, 1H), 2.96–3.14 (m, 4H), 3.19 (m, 2H), 3.38 (m, 1H), 3.55 (m, 2H), 4.39 (s, 1H), 4.41 (m, 2H), 6.55 (s, 1H), 7.23–7.33 (m, 4H), 9.47 (m, 1H) ppm; MS (APCI) *m*/*z* (relative intensity): 384 ([MH^+ of free base], 100).
12. Harada, N.; Watanabe, M.; Kawahara, S.; Sugio, A.; Kasai, Y.; Ichikawa, A. *Tetrahedron: Asymmetry* **2000**, *11*, 1249–1253.