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Stereoselective Synthesis of (3*S*)- and (3*R*)-3-Hydroxy-3-(2,6-Dimethyl-4-Hydroxyphenyl)Propanoic Acid and its Incorporation into a Cyclic Enkephalin Analogue

Grazyna Weltrowska, Carole Lemieux, Nga N. Chung, Brian C. Wilkes, and Peter W. Schiller

Laboratory of Chemical Biology and Peptide Research, Clinical Research Institute of Montreal, Montreal, Quebec, Canada H2W 1R7

Introduction

2',6'-dimethyl substitution of the Tyr¹ residue of opioid agonist peptides and deletion of the N-terminal amino group have been shown to represent a general structural modification to convert opioid peptide agonists into antagonists [1]. This conversion required the syntheses of opioid peptide analogues containing 3-(2,6-dimethyl-4-hydroxyphenyl)propanoic acid (Dhp) in place of Tyr¹. The cyclic enkephalin analogue Dhp-c[D-Cys-Gly-Phe(*p*NO₂)-D-Cys]NH₂ is a potent μ opioid antagonist and a less potent δ and κ antagonist [2]. An analogue of this peptide with β -methylated Dhp, (3*S*)-3-methyl-3-(2,6-dimethyl-4-hydroxyphenyl)propanoic acid [(3*S*)-Mdp], in place of Dhp showed increased antagonist activity at all three receptors, whereas an analogue containing β -isopropyl-substituted Dhp [(3*R*)-Idp] turned out to be a mixed κ agonist/ μ antagonist [3]. To further investigate the SAR at the β position of Dhp in the cyclic analogue, we examined the effect of substituting β -hydroxylated Dhp on the opioid activity profile. This required the development of stereoselective syntheses of (3*S*)- and (3*R*)-3-hydroxy-3-(2,6-dimethyl-4-hydroxyphenyl)propanoic acid [(3*S*)- and (3*R*)-Hdp].

Results and Discussion

(3*S*)- and (3*R*)-Hdp were synthesized using Evans chemistry [4] (Scheme 1). Aldol reaction between silyl-protected aldehyde **2** and the dibutylboron enolate derived from chloroacetylloxazolidinone **3** or **4** afforded the desired intermediate **5** or **6** in 70% and 75% yield, respectively. The diastereoselectivity ratio for **5** (78:22) and for **6** (82:18) was determined by ¹H NMR spectroscopy. Similar to this moderate diastereomeric excess, limited stereoselectivity had also been observed upon introduction of alkyl substituents in the β -position of Dhp [2]. However, the diastereomers were easily separated by silica gel chromatography, yielding the pure diastereomer **5** or **6**. Dechlorination of **5** or **6** was accomplished by treatment with zinc dust and glacial acetic acid to afford **7** or **8** in 50% yield in both cases. Silylation of the free hydroxyl group in 80% yield for **9** and 85% yield for **10** was followed by removal of the chiral auxiliary in standard manner [5] to give **11** or **12**. Structures were confirmed by ¹H NMR spectroscopy and EI mass spectrometry.

The target peptides (3*S*)- and (3*R*)-Hdp-c[D-Cys-Gly-Phe(*p*NO₂)-D-Cys]NH₂ were prepared by a combination of manual solid-phase and solution techniques. The linear precursor peptide of H-c[D-Cys-Gly-Phe(*p*NO₂)-D-Cys]NH₂ was assembled on a *p*-methylbenzhydrylamine resin, using Boc-protection of the α -amino function and Ac₂O protection of the Cys side chain. After cleavage from the resin by HF/anisole treatment, disulfide bond formation was carried out in MeOH/H₂O using iodine as oxidation agent and the peptide was purified by preparative reversed-phase HPLC. (3*S*)- or (3*R*)-Hdp(TBDMS)₂ (**11**,**12**) was attached to the N-terminal amino group of H-c[D-Cys-Gly-Phe(*p*NO₂)-D-Cys]NH₂ using HBTU as coupling agent. After

removal of the silyl protection by 90% TFA/anisole treatment, the final peptide products were purified by reversed-phase HPLC, and their purity and structural identity were established by TLC, analytical HPLC and FAB-MS.

(3*S*)- and (3*R*)-Hdp-c[D-Cys-Gly-Phe(*p*NO₂)-D-Cys-NH₂] both showed neither agonist nor antagonist activity in the guinea pig ileum and mouse vas deferens assays at concentrations up to 10 μM. In the opioid receptor binding assays, both the (3*S*)-Hdp¹- and the (3*R*)-Hdp¹- analogues showed no μ and κ receptor binding affinity at concentrations up to 10 μM and very weak δ receptor binding affinity with K_i values of 7.65 and 26.3 μM, respectively. This result indicates that introduction of the hydrophilic hydroxyl group at the β-position of Dhp is not tolerated at all three receptors.

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Scheme 1.

(i) TBDMSCl, imidazole, DMF, rt., 95%; (ii) Cl-CH₂-COXc, TEA, Bu₂BOTf, CH₂Cl₂, -78° C to rt., 1,5h, phosphate buffer pH7, 30% H₂O₂, ether, 0°C, 1h; (iii) Zn, AcOH, THF, rt., 15min, 50%; (iv) TBDMSCl, imidazole, DMF, 80%; (v) LiOH, 30% H₂O₂, THF/H₂O, 0°C to rt., 90%.