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Synthesis and Biological Evaluation of Two Analogues of (S)-α-Methyl-3-carboxyphenylalanine

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Abstract: Two analogues **2**, **3** of (S)-α-methyl-3-carboxylphenylalanine **1** were synthesized to test their activity for metabotropic glutamate receptors (mGluRs). Both compounds **2** and **3** were inactive as antagonist for mGluRs, but **2** showed weak agonistic activity for GluR6 in contrast to that reported by Kemp and co-workers.

Metabotropic glutamate receptors (mGluRs) have been recently described as a growing family that contains at least eight subtypes[1]. These receptors fall into three subgroups according to their sequence homology, signal transduction mechanism and agonist selectivity. The group I of mGluRs includes mGluR1 and mGluR5 which are potently activated by quisqualate resulting in an increase in phosphoinositide hydrolysis. In contrast, group II including mGluR2 and mGluR3 and group III that contains mGluR4, mGluR6, mGluR7 and mGluR8, are negatively linked to adenylyl cyclase. However, group II and III can be distinguished by their marked agonist-selectivity. The former effectively interacts with (2S,1'S,2'S)-2-(carboxycyclopropyl)glycine (L-CCG-I) and LY354740 as that reported[2] very recently, whereas the latter potently interacts with (S)-2-amino-4-phosphonobutyric acid (L-AP4)[1]. The lack of selective antagonists for these subtypes and/or subgroups has led to the intensive studies in the past three years[1-7]. In their review[1a], Watkins and Collingride mentioned that (S)-α-methyl-3-carboxyphenylalanine **1** ((S)-αM3CPA) was a novel antagonist for mGluRs. Kemp and co-workers reported that this compound has potent antagonist action and the IC_{50} is about 1 μM to antagonize the effect of 10 μM L-AP4 in cortical slices[8]. These reports promoted us to synthesize two analogues as illustrated in Scheme 1 based on the structure of (S)-αM3CPA. One analogue is (S)-α-methyl-3-phosphonophenylalanine **2** whose selectivity for subtypes still remains ambiguous[2l]. While in another analogue **3**, a six-membered ring was introduced to freeze the rotation of Cα-Cβ bond. It was expected that these modifications might improve the isoform-selectivity or activity of **1**.

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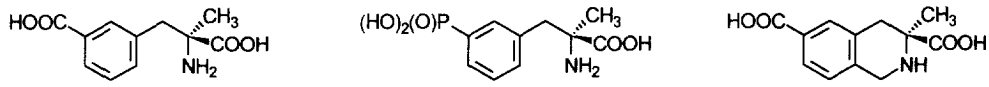
Scheme 1



1 2 3

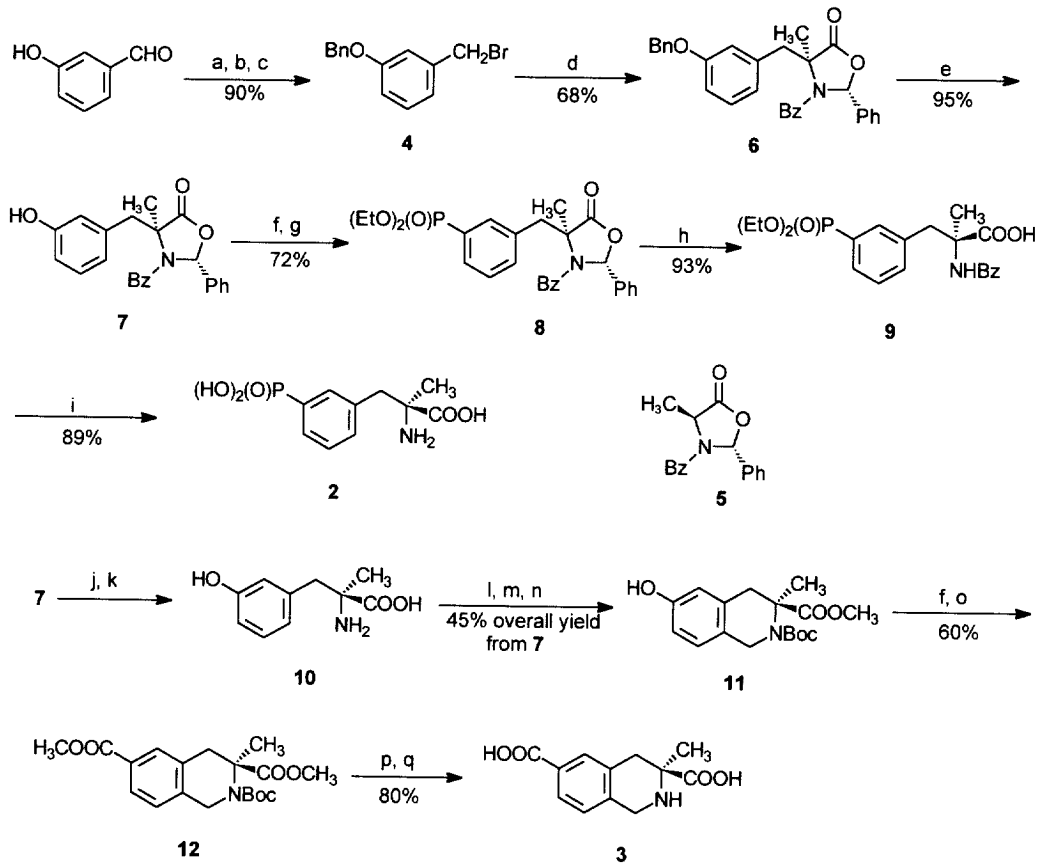
Chemical synthesis. Our synthesis for both analogues was shown in Scheme 2. The key steps included the self-regeneration of stereocenter strategy developed by Seebach and co-workers[9], and palladium-catalyzed carbonylation and phosphonation. After reduction of 3-hydroxybenzaldehyde to 3-hydroxybenzyl alcohol, the 3-hydroxy group was protected with benzyl ether and the benzyl alcohol was treated with tribromophosphine to produce **4** in 90% overall yield. The coupling of **4** with the lithium salt of *trans*-2-phenyloxazolidinone **5**[10,11] processed in a highly diastereoselective manner to afford **6** in 68% yield. This compound was deprotected by Pd/C-catalyzed hydrogenation to give **7**, which would serve as the same intermediate for preparing **2** and **3**.

After transformation of **7** to the corresponding triflate, the palladium-catalyzed phosphonation[12] was employed to produce arylphosphonate **8**. Next, treatment of **8** with sodium hydroxide gave acid **9**, which was refluxed with 6 *N* HCl to afford crude **2** as its hydrochloride salt. This salt was loaded onto an ion-exchange column (Dowex 50WX2-200) to produce **2**[13] in 89% yield. To prepare the cyclic analogue **3**, the compound **7** was deprotected to yield amino acid **10**. Initially, we tried the condensation of **10** under the standard Pictet-Spengler reaction conditions (5% HCl, 37% formaldehyde, 90 °C)[14]. Although the desired product was detected by ^{1}H NMR, it was always contaminated with by-products that were difficult to remove after subsequent transformations. After some experiments, we found that the best result could be obtained by using the condition (0.05 *N* HCl, 37% formaldehyde, 90 °C, 45 min.) reported by Ornstein and co-workers[15]. The generated cyclization product was esterified and then *N*-protected to afford **11** in 45% overall yield (from **7**). Transformation of **11** to its triflate followed by palladium-catalyzed carbonylation[16] under standard conditions gave ester **12**. Hydrolysis of two esters in **12** with sodium hydroxide gave diacid, which was dissolved in anhydrous methylene chloride and then gaseous hydrochloride was introduced to remove Boc group. The resulting salt was treated with propylene oxide and then purified by using reverse-phase column to afford **3** in 80% overall yield.

Biological Results. Chinese hamster ovary (CHO) cells stably expressing mGluR1a, mGluR2, mGluR5a, mGluR6 and chimeric mGluR3/1a receptors were prepared and cultured as described previously[18]. The baby hamster kidney (BHK) cell line expressing mGluR4a was a generous gift from Zymogenetics Inc. (Seattle). All cell lines were cultured in 96-well plates and passaged every 6-7 days and used for measurements of phosphoinositide (PI) hydrolysis or cAMP formation. For measurements of PI hydrolysis, cells expressing mGluR1a or mGluR5a were cultured in 96-well plates and then labeled overnight with 0.75 μCi/mL of [^{3}H]myo-inositol (Amersham). CHO cells expressing mGluR2, mGluR4a, or mGluR6 were cultured in 96-well plates. Measurements of PI hydrolysis and of forskolin-induced cAMP formation were performed as described previously.[19,20] When tested at these six subtypes as an antagonist, both compounds **2** and **3** were found to exhibit no activity. Also, when tested as

an agonist at each of these subtypes, **2** was found to have relatively weak activity at mGluR6 (at 300 μM, a percentage of the maximum agonist activity is 27%). These results were quite contrast to that reported in a brief communication by Kemp and co-workers in which **2** had potent antagonist properties on the inhibition of forskolin-stimulated cAMP formation by L-CCG-I (IC_{50} = 52.8 nM) and L-AP4 (IC_{50} = 18.8 nM) in adult rat cortical slices.[21] The similar discrepancy between cells and tissue was observed in the experiment using the compound **1**.[5]

Scheme 2: Self-regeneration of stereocenter method to two analogues



Reagents and Conditions: a. $NaBH_4$, MeOH, H_2O, 0 °C. b. BnBr, NaOH, MeOH, r.t. c. PBr_3, toluene, 100 °C, 10 min. d. 1.0 eq. **5**, 1.1 eq. LHMDS, THF, -78 °C, 45 min; then 1.1 eq **4**, -78 °C (1 h) to r.t. (overnight). e. H_2, Pd/C, EtOH, r.t. f. Tf_2O, Et_3N, DMAP, CH_2Cl_2, -78 °C to r.t. g. $HP(O)(OEt)_2$, $Pd(PPh_3)_4$, Et_3N, sealed tube, 110 °C, 4 h. h. 2 *N* NaOH, MeOH, r.t. 0.5 h. i. 6 *N* HCl, sealed tube, 100 °C, 48 h. then Dowex-50WX2-200. j. 2 *N* NaOH, MeOH, r.t. k. 6 *N* HCl, sealed tube, 30 h. l. 37% HCHO, 0.05 *N* HCl, 90 °C, 45 min. m. HCl/MeOH, reflux. n. $(Boc)_2O$, *i*-Pr_2NEt, CH_2Cl_2, r.t. o. CO, MeOH, $Pd(PPh_3)_4$, dppp, Et_3N, DMSO, 70 °C, 12 h. p. 2 *N* NaOH, MeOH, H_2O, 80 °C. q. HCl, CH_2Cl_2, r.t. then propylene oxide.

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