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Design and synthesis of novel χ^2-constrained phenylalanine, naphthylalanine, and tryptophan analogues and their use in biologically active melanotropin peptides

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Abstract—A series of novel hydrophobic, bulky χ^2-constrained phenylalanine, naphthylalanine, and tryptophan derivatives was designed and synthesized. The key steps involved asymmetric hydrogenations of α-enamides using Burk's DuPHOS-based Rh(I) catalysts to give high enantiomerically pure α-amino acid derivatives. The subsequent Suzuki cross couplings of the amino acid analogues with boronic acid derivatives afforded these aromatic substituted amino acids in high yields and with high enantioselectivity. The incorporation of these novel χ^2-constrained amino acids into peptides and peptidomimetics provides fruitful information in the development of peptide and peptidomimetic ligands of melanotropins and an understanding of the interactions between ligands and receptors/acceptors.

1. Introduction

In the event of molecular recognition, the peptide backbone serves as a scaffold for the three-dimensional structures when interacting with their receptors/acceptors. The side-chain moieties of amino acids involved directly in the interaction are critical for biological activity and selectivity between receptors/acceptors or sub-types of receptors and ligands. Their three dimensional topography (e.g. their χ^1, χ^2, etc. torsional angles (Fig. 1, see the definition of χ angles in Ref. 1)) and stereoelectronic properties provide the critical complementary shape and chemical properties that favors efficient molecular recognition. The χ angles, in conjunction with the backbone angles, define the position of side-chain functional groups in χ space and thus must be regarded as of key importance in understanding the mode of action of peptides and peptidomimetics. A better understanding of χ space and how to manipulate it for a better understanding of structure–activity relationship and can definitely provide fruitful information in the development of peptide and peptidomimetic ligands.[2–4] As a result, an array of amino acids with well-defined complementary χ-characteristics is clearly of considerable importance. In recent years, design and synthesis of conformationally

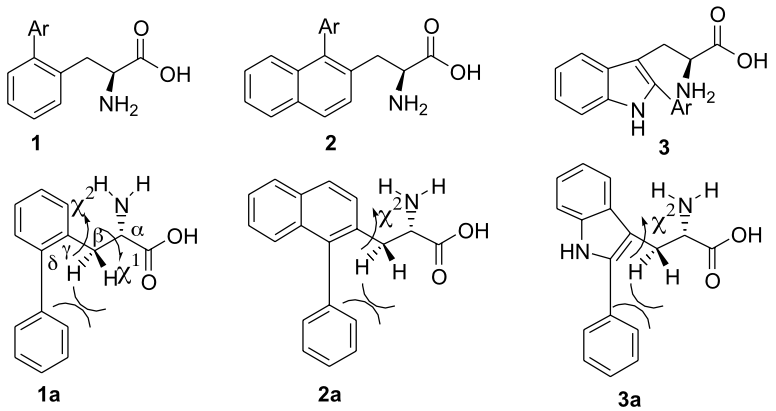

Figure 1. Novel χ^2 constrained *o*-substituted-aryl phenylalanines, naphthylalanines, and tryptophans.

Keywords: asymmetric hydrogenation; DuPHOS; constrained amino acids; phenylalanine; tryptophan; naphthylalanine.

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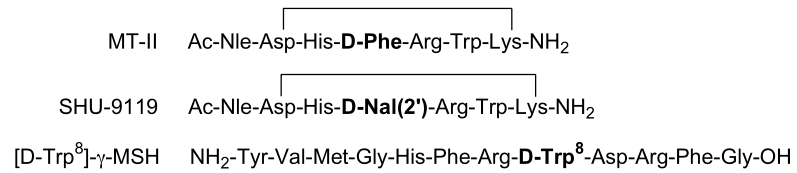
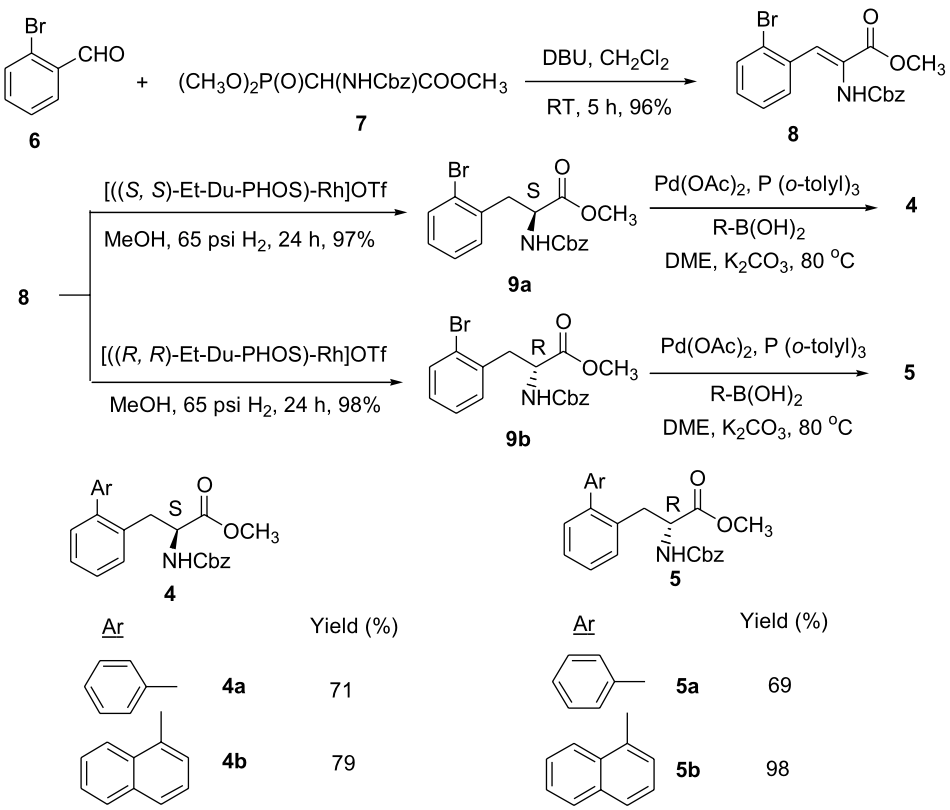


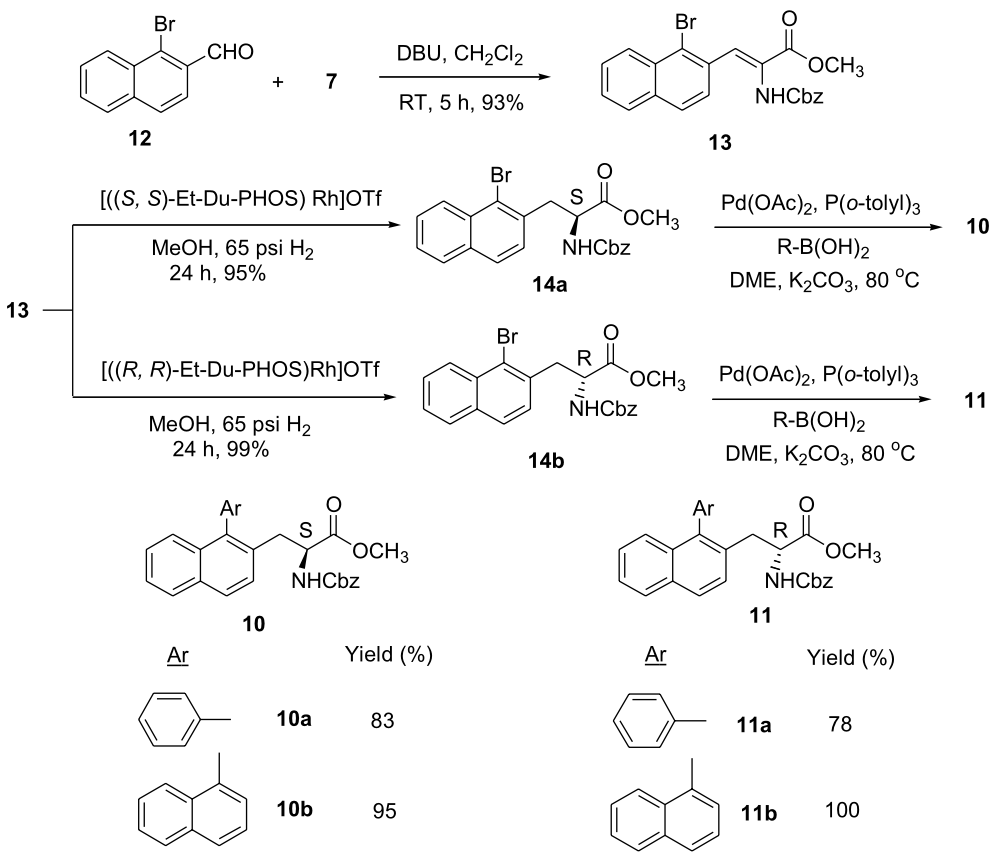
Figure 2. Structures of potent and selective MSH peptides.

constrained amino acids has been a very active area.[2–4] In general, the side-chain conformation can be controlled by introducing an alkyl/aryl group at the β position or on the aromatic rings of aromatic amino acid residues. These kinds of modifications often do not perturb the backbone conformation significantly, and still allow the peptide backbone and side-chain some degree of flexibility, which often is necessary and sometimes even crucial for peptide and peptidomimetic activity. As one of the leading groups in the area, we have designed and synthesized many novel χ-constrained amino acids, mainly focusing on introducing alkyl and aryl groups at the β-position of amino acids.[2,5,6] Incorporation of these unnatural amino acids in strategic positions of biologically active peptides and peptidomimetics can enhance the potency and selectivity significantly.[2,3,6–8] However, introducing an alkyl or aryl group on the aromatic ring of an aromatic amino acid residue, particularly in the *ortho* position which can significantly restrict its conformation in χ^2 space, has been much less explored.[2a,8–11] The χ^2 torsional angle can be efficiently restricted by the interaction between the aryl moiety and the β-hydrogens of amino side-chain in the *o*-substituted phenylalanine, naphthylalanine, and tryptophan derivatives **1**, **2** and **3**, respectively (Fig. 1).

In the melanocyte stimulating hormone (MSH) peptides, the core sequence and pharmacophore of these peptide ligands contains three aromatic amino acids His, Phe, and Trp.[3,12] By modification of these amino acids, we have found that Phe (preferably D-Phe) in α-MSH peptides and D-Trp in γ-MSH peptides play critical roles in activity and selectivity.[3,12] For example, the substitution of D-Phe in MT-II with D-Nal (naphthylalanine) (2′) led to a potent and selective antagonist ligand, SHU-9119, for the melanocortin MC4 receptor,[13] whereas substitution of the Trp residue in γ-MSH with D-Trp generated a potent and selective MC3R agonist (Fig. 2).[14] Aiming at further enhancing activity and selectivity and better understanding the relationship of peptide ligands and their receptors, we have proposed to use χ^2-constrained, hydrophobic and bulky aromatic-substituted phenylalanines **1**, naphthylalanines **2**, and tryptophans **3** to substitute D-Phe in MT-II or D-Nal (2′) in SHU-9119 and D-Trp in [D-Trp^8]-γ-MSH. In addition, these amino acids can provide a large lipophilic surface for binding to receptors, and for crossing membrane barriers (e.g. blood brain barriers (BBB) and intestinal mucosa) and stability to metabolic degradation, which provides an opportunity to address three issues simultaneously. Herein we would like to report an efficient approach to the asymmetric synthesis



Scheme 1. Synthesis of *o*-substituted phenylalanine derivatives.



Scheme 2. Synthesis of *o*-substituted nathphylalanine derivatives.

of these unusual amino acids.[15a] Two key steps are involved in this approach: (1) the asymmetric hydrogenation of α-enamides to generate functional α amino acids in high optical purity and (2) Suzuki-type cross couplings of the resulting α amino acid derivatives with boronic acids (Schemes 1 and 2). Recently, we used a similar method to synthesize novel 5-aryl tryptophan derivatives.[15b]

2. Results and discussion

2.1. Synthesis of *o*-aryl substituted phenylalanines 4 and 5

Commercially available 2-bromobenzylaldehyde **6** was used as the starting material for the synthesis of the *o*-aryl substituted phenylalanines **4** and **5** (Scheme 1). The dehydroamino acid derivative **8** was furnished with *Z*-configuration as a major product (*Z*/*E*>95/5) in 96% yield through the Horner–Emmons olefination of aldehyde **6** with phosphonate $(MeO)_2P(O)CH(NHCbz)COOMe$ **7**.[16] Compound **7** was easily synthesized in three steps and on a large scale (up to 100 g) following literature procedures.[17] Asymmetric hydrogenations of the dehydroamino ester **8** gave optically pure α-amino acid derivatives. Burk's 1,2-bis ((2*S*,5*S*)/(2*R*,5*R*)-2,5-diethylphospholano)benzene (cyclooctadiene) rhodium(I) trifluoromethane sulfonate ((*S*,*S*)/(*R*,*R*) [Et-DuPHOS-Rh] OTf) catalyst was employed for the asymmetric hydrogenations since it gives a single enantiomer (>97% ee) in high yields (>96%) and high efficiency (at a ratio of catalyst to substrate up to 1/2500).[18,19] It should be mentioned that the catalysts are commercially available.[20] Furthermore, both *Z* and *E* dehydroamino acids using this type of catalysts gave one single isomer.[19] In this case, we separated the two isomers by column chromatography. The isolated (*Z*)-dehydroamino acid ester **8** used for asymmetric hydrogenations gave a higher ee than that of (*E*) isomer. The (*S*,*S*) catalyst afforded the amino acid derivative **9a** with an absolute *S* configuration, based on the selectivity of the (*S*,*S*)-Et-DuPHOS ligand, in high yield and high ee (>96%).[19] The (*R*) amino acid **9b** was also obtained using (*R*,*R*)-Et-DuPHOS as the ligand in high yield and high ee as well. The α-amino group in **9** was protected by Cbz, which can be readily removed by Pd catalyzed hydrogenation to give a free amine. The resulting amino group can be reprotected as N^{α}-Boc (*tert*-butoxycarbonyl) or N^{α}-Fmoc (9-fluorenylmethoxycarbonyl) for the solid-phase peptide synthesis.

The optically pure *o*-bromophenylalanines **9a**,**b** were used as intermediates for the introducing aromatic moieties at *ortho*-positions. Suzuki cross couplings were employed with phenyl and naphthyl boronic acids to give amino acid derivatives **4** and **5** in 69–98% yields. We have tried several Suzuki cross coupling reaction conditions and found the following reaction conditions to give the best yields without any racemization: in situ formation of catalyst Pd (0) by reaction with 5 mol% $Pd(OAc)_2$ and 10 mol% tri(*o*-tolyl)phosphine, 1.5 equiv. boronic acid and 2.0 equiv. Na_2CO_3 in a mixture of ethylene glycol dimethyl ether (DME) and H_2O at 80°C. Compared with the formation of less hindered products,[15b] a longer reaction time (12 h) was

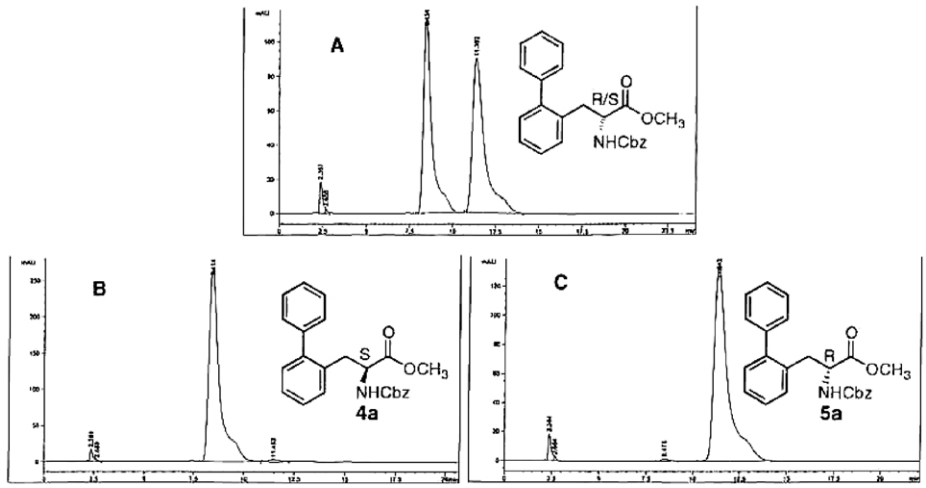


Figure 3. Typical HPLC chromatograms.[21] Panel A: a mixture of **4a**-(*S*) and **5a**-(*R*); panel B: **4a**-(*S*) enantiomer; panel C: **5a**-(*R*) enantiomer.

required for the formation of the *o*-substituted, sterically hindered products. The enantiomeric purity was determined by chiral HPLC analysis and no racemizations were observed during the cross couplings (Fig. 3).[21]

2.2. Synthesis of *o*-aryl substituted naphthylalanines 10 and 11

With success for the synthesis of *o*-aromatic substituted phenylalanine analogues **4** and **5**, we utilized a similar strategy for the preparation of *o*-aromatic substituted naphthylalanine derivatives **10** and **11** (Scheme 2). The isolated (*Z*) α-enamide **13** was obtained as the major product (*Z*/*E*>95/5) by condensation of 1-bromo-2-naphthylaldehyde **12** with **7** in the presence of DBU in CH_2Cl_2 in 93% yield. Asymmetric hydrogenation of α-enamide **13** in the presence of 5 mol% catalyst, Rh(I)-(*S*,*S*)-Et-DuPHOS or Rh(I)-(*R*,*R*)-Et-DuPHOS, 65 psi of H_2, 24 h in methanol gave (*S*) or (*R*) N_α-Cbz-*o*-bromo-naphthylalanine methyl esters **14a**,**b** in 95–99% yield and >98% ee.[22] The *o*-bromonaphthylalanines **14a**,**b** were reacted with phenyl and naphthyl boronic acids through Suzuki cross coupling reactions to give the desired compounds **10** and **11**. Initially, we employed the same coupling reaction conditions as used before in good yields for the phenylboronic acid-based reactions. An incomplete reaction was observed for the coupling with a bigger naphthylboronic acid. However, using 5 mol% $Pd(PPh_3)_4$ instead of $Pd(OAc)_2$ and P(*o*-tolyl)$_3$, 1.5 equiv. boronic acid, and 2.0 equiv. Na_2CO_3, in benzene–water, at 80°C for 36 h, afforded a complete reaction in high yields (>95%).

These *o*-aryl substituted phenylalanines and naphthylalanines were designed to restrict the χ^2 torsional angels. Indeed the interactions between nathphyl groups and β-hydrogenations were observed **4b**, **5b**, **10b**, and **11b**. We found two diastereomers existed in **4b**, **5b**, **10b**, and **11b** by ^{1}H NMR. ^{1}H NMR showed two groups of proton signals. About 1:1 and 1:2 ratios of diastereomers in **4b** and **5b** and in **10b** and **11b**, respectively, were observed during the Suzuki couplings based on ^{1}H NMR integrations. Although they are diastereomers, they could not be separated by silica gel chromatography. Their structures are presumably assigned as shown (**5b** and **11b** not shown) in Fig. 4. However, the absolute stereochemistry was not determined. Meanwhile, we also investigated how the size of N_α-amino protecting groups affect the outcome of diastereoselectivity during the Suzuki-cross couplings. Three different protecting groups, CH_3CO, Cbz, and Boc, were chosen for the study using **14a** with 1-naphthylboronic acid as an example (Scheme 3). Surprisingly, it was found that the smaller protecting group CH_3CO gave the better diastereoselectivity with a ratio of 1:3 of the two isomers in these three cases. In contrast, the compounds with the bigger protecting groups Cbz or Boc had almost the same diastereoselectivity with a ratio of about 1:2 of the isomers based on ^{1}H NMR integrations. We did not make efforts to separate them at this stage since we believe that the individual isomer-derived peptides can be separated after incorporation into peptides. Meanwhile, detailed conformational studies of these interesting amino acids currently are being investigated via X-ray crystal structures, NMR, and computer modeling and calculations. It also is realized that these conformationally

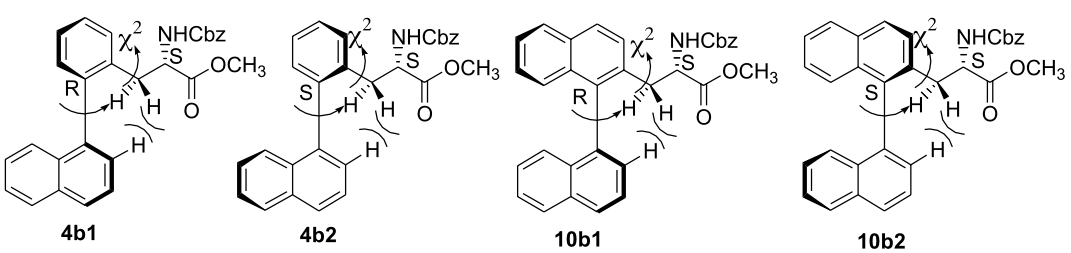
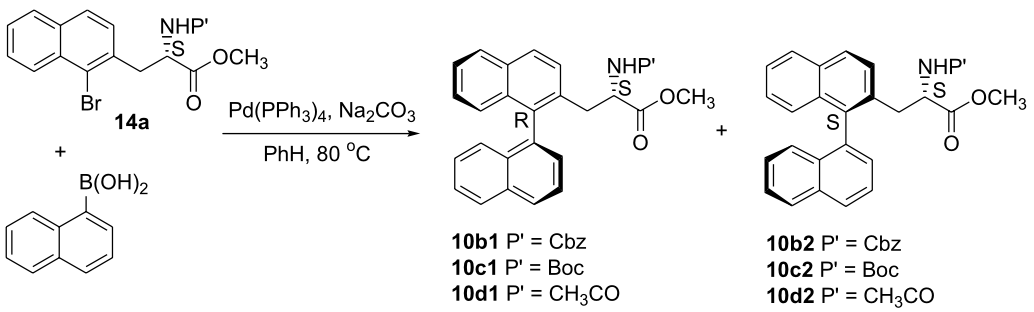
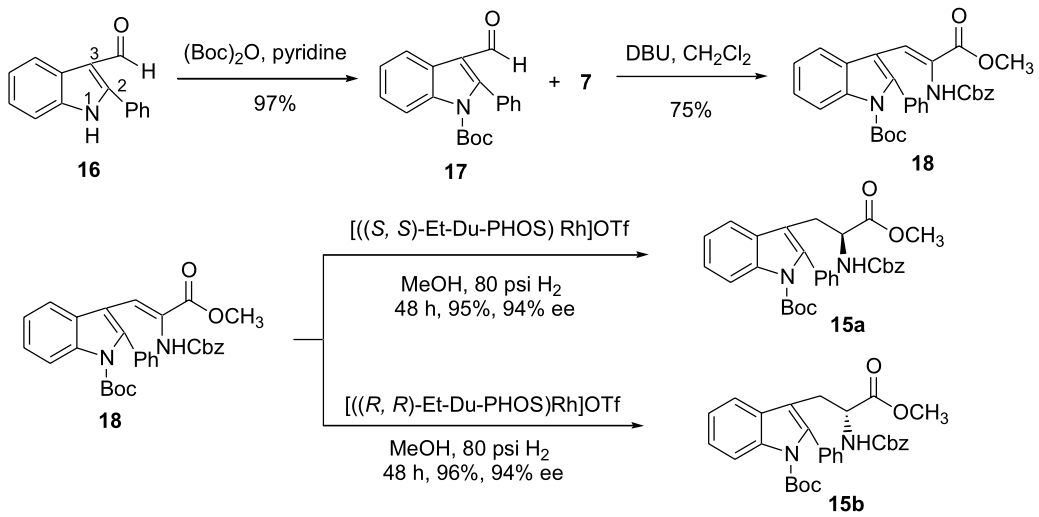


Figure 4.



Scheme 3.



Scheme 4. Synthesis of 2-phenyl-substituted tryptophan analogues.

constrained amino acids bearing fluorophores will be very useful in structure–activity studies of peptides and receptors.

2.3. Synthesis of 2-phenyl substituted tryptophans 15

2-Phenyl-substituted tryptophans **15a**,**b** were synthesized from 3-formyl-2-phenylindole **16**[23] using a similar route as used for the synthesis of the phenylalanine and naphthylalanine analogues (Scheme 4). The indole amino group in aldehyde **16** was protected as Boc using $(Boc)_2O$ (di-*t*-butyldicarbonate) in pyridine in 97% yield. Low yields were obtained in CH_2Cl_2, THF, or CH_3CN because of the poor solubility of compound **16**. Horner–Emmons olefination of aldehyde **17** with phosphonate $(MeO)_2$-P(O)CH(NHCbz)COOMe **7** gave the dehydroamino acid **18** with *Z*-configuration as a major product (*Z*/*E*>95/5) in 75% yield. The amino group was protected by Cbz (benzyloxycarbonyl), which was orthogonal to the Boc protected amino group in the indole ring of compound **18**. The dehydroamino ester **18** underwent asymmetric hydrogenations at 80 psi for 48 h to give α-amino acid derivatives **15a**,**b** using ((*S*, *S*)/(*R*,*R*) [Et-DuPHOS-Rh]) OTf as catalysts in good enantioselectivity (ca. 94% ee)[24] and in high yields (>95%). Initially we tried the reaction under the conditions we used earlier at pressures of 65 psi for 24 h. However, an incomplete reaction occurred presumably due to the steric effect of the substrate **18**. The enantioselectivity (ca. 94% ee) of the asymmetric hydrogenations was also a little lower than we observed in earlier cases.

2.4. Preliminary biological results

These χ^2 constrained amino acids were designed for the restriction of the conformations of biologically active peptides to improve activity and selectivity. As a result, in our preliminary biological study, we incorporated two of these amino acids (e.g. D and L *o*-phenyl phenylalanines) into the peptide MT-II (Fig. 2) with substitutions for D-Phe. The two MT-II analogues **19a**,**b** were synthesized on the solid-phase using N^{α}-Boc chemistry (Fig. 5).[25,26] Their preliminary biological results are summarized in Table 1. Overall, their binding affinities (IC_{50}) for three hMCRs (human melanocortin receptors) were lower than those of lead compound MT-II (Table 1). However, both compounds **19a**,**b** showed high affinity in the nM range and better selectivities for the hMCR3. MT-II had poor selectivity for hMCRs (Table 1) in spite of its high potency. There was no selectivity between hMCR3 and hMCR4, and a little selectivity at a ratio of 4 between hMCR5 and hMCR3 for MT-II. Compound **19a** had better selectivity at a ratio of 11 between hMCR4 and hMCR3 and 29 between hMCR5 and

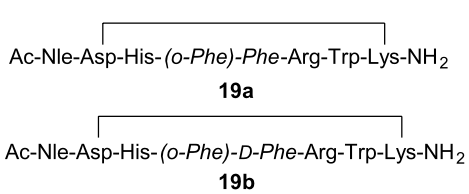


Figure 5. Structures of MT-II analogues.

Table 1. Binding affinities of MT-II analogues at hMCRs

Compounds	hMCR3 (IC_{50}, nM)	hMCR4 (IC_{50}, nM)	hMCR5 (IC_{50}, nM)	Selectivity (hMCR4/hMCR3)	Selectivity (hMCR5/hMCR3)
19a	71.0	760	2040	11	29
19b	28.0	1870	170	66	6
MT-II	4.4	3.7	16.3	1.2	4

Human melanocortin receptors.

hMCR3. Higher selectivity for hMCR3 over hMCR4 and hMCR5 also were observed for analogue **19b** at a ratio of 66 and 6, respectively. Recently we have synthesized large amounts of the other χ^2 amino acids and incorporated them into MT-II using solid-phase Boc chemistry. Their biological studies are under investigation.

3. Conclusions

An efficient method has been developed for the synthesis of novel aromatic-substituted χ^2-constrained phenylalanine, naphthylalanine, and tryptophan derivatives. These amino acids were synthesized through asymmetric hydrogenations using Burk's DuPHOS-based catalysts with high ee (>94%), followed by Suzuki cross couplings also in high yields. Two MT-II analogues **19a**,**b** with incorporation of D and L *o*-phenyl phenylalanines at D-Phe position were synthesized and the preliminary biological study showed that they had higher selectivity for hMCR3 with good activity. The biological evaluation and structure–biological activity relationship studies of the peptides are currently under extensive investigation.

4. Experimental

4.1. General

^1H and ^{13}C NMR were performed on a Varian Unity-300 and Brukers AM-250 and DRX-500 and 600 spectrometers using TMS and $CDCl_3$ as internal standards. High Resolution Mass Spectra (HRMS) were recorded on a JEOL HX110A instrument from University of Arizona Mass Spectroscopy Laboratory. Optical rotations were measured on a JASCO-1020 polarimeter. Melting points (mp) are uncorrected and were obtained on a Thomas-Hoover apparatus in open capillaries. Commercially available starting materials and reagents were purchased from Aldrich and used as received. THF was distilled from Na and benzophenone; methylene chloride (CH_2Cl_2) was distilled from CaH_2; HPLC grade methanol was used for hydrogenations. Column chromatography was performed using silica gel (230–400 mesh) from EM science. Thin layer chromatography (TLC) was performed on 13181 silica gel-based sheet with fluorescent indicator from Kodak. Unless otherwise stated, yields refer to isolated yields of products of greater than 95% purity as estimated by ^1H and ^{13}C NMR spectroscopy. All new compounds were characterized by ^1H, ^{13}C, and HRMS or elemental analyses.

4.1.1. Methyl (*Z*)-2-(benzyloxycarbonyl)amino-3-(*o*-bromophenyl)acrylate 8. To a solution of $(MeO)_2$-CH(NHCbz)COOMe **7** (2.73 g, 8.25 mmol) in 20 mL of dry methylene chloride was added DBU (1.2 mL, 7.88 mmol) slowly under an argon atmosphere with stirring. After ca. 10 min, *o*-bromobenzaldehyde **5** (1.39 g, 7.5 mmol) was added slowly into the above mixture. After 5 h, the solvent was evaporated, the residue was dissolved in 150 mL of ethyl acetate. The organic solution was washed with 1N HCl (2×30 mL) and brine (30 mL), dried over $MgSO_4$ and evaporated. The crude product was purified by flash column chromatography, eluting with ethyl acetate, hexanes and methylene chloride (1/5/1) to give a white solid (2.81 g, 96%). Mp 73–75°C, ^1H NMR (300 MHz, $CDCl_3$) δ 7.13–7.61 (10H, m), 6.44 (1H, s), 5.04 (2H, s), 3.85 (3H, s); ^{13}C NMR δ (75 MHz, $CDCl_3$) δ 165.4, 153.4, 135.9, 134.7, 133.1, 130.3, 129.6, 128.7, 128.6, 128.5, 128.4, 127.4, 126.1, 124.7, 67.7, 53.1; HRMS (FAB) calcd for $C_{18}H_{17}BrNO_4$ 390.0341 (Br 79), 392.0323 (Br 81); found 390.0340 (Br 79), 392.0330 (Br 81).

4.1.2. (*S*)-N^{α}-Benzyloxycarbonyl-*o*-bromophenylalanine methyl ester 9a. A hydrogenation bottle charged with **8** (1.26 g, 3.23 mmol) in degassed methanol (30 mL), was purged with argon for about 15 min, followed by adding (*S*,*S*) (COD) Et-DuPHOS Rh(I) OTf (4.7 mg, 0.0065 mmol). After 5 vacuum/hydrogen cycles, the reaction bottle was pressurized to an initial pressure of 65 psi. The reaction was allowed to proceed for 24 h. After the evaporation of solvent, the crude product was purified by flash column chromatography, eluting with ethyl acetate, hexanes, and methylene chloride (1/5/1) to afford a white solid (1.213 g, 96%). Mp 81.5–83.5°C; $[\alpha]_D^{26}$=+3.9 (*c* 1.25, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.54 (1H, d, *J*=7.8 Hz), 7.07–7.34 (8H, m), 5.32 (1H, d, *J*=8.1 Hz), 5.06 (2H, s), 4.72 (1H, dd, J_1=8.1 Hz, J_2=13.8 Hz), 3.72 (3H, s), 3.33 (1H, dd, J_1=6.0 Hz, J_2=13.8 Hz), 3.15 (1H, dd, J_1=8.1 Hz, J_2=13.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.2, 155.8, 136.4, 135.9, 133.2, 131.5, 129.0, 128.7, 128.3, 128.2, 127.7, 125.2, 67.1, 54.2, 52.7, 38.7; HRMS (FAB) calcd for $C_{18}H_{19}BrNO_4$ 392.0497 (Br 79), 394.0479 (Br 81); found 392.0486 (Br 79), 394.0485 (Br 81).

4.2. General procedures for palladium-catalyzed Suzuki coupling reactions

A reaction flask fitted with a Teflon valve was charged with a bromoarylalanine derivative, boronic acid (1.5 equiv.), Na_2CO_3 (2.0 equiv.), $Pd(OAc)_2$ (5 mol%), P(*o*-tolyl)$_3$ (10 mol%), DME (6 mL/mmol), degassed water (1 mL/mmol) and then was heated to 80°C for 12 h. The reaction mixture was passed through a short column containing a bottom 1″ layer of silica gel (230–400 mesh) and a top 1″ layer of $NaHCO_3$ using ethyl acetate as eluent. The solvent was removed under reduced pressure with a rotary evaporator. The crude product was purified by flash column chromatography using an appropriate mixture of ethyl acetate and hexanes as eluent.

4.2.1. (*S*)-*N*$^{\alpha}$-Benzyloxycarbonyl-*o*-(phenyl)phenylalanine methyl ester 4a. 71% yield, $[\alpha]_D^{26}=+16.0$ (*c* 1.35, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.18–7.41 (14H, m), 4.98 (2H, dd, $J_1=12.3$ Hz, $J_2=21.6$ Hz), 4.79 (1H, d, $J=11.1$ Hz), 4.41 (1H, dt), 3.56 (3H, s), 3.22 (1H, dd, $J_1=5.7$ Hz, $J_2=13.8$ Hz), 3.00 (1H, dd, $J_1=8.4$ Hz, $J_2=13.8$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.4, 155.6, 142.9, 141.3, 136.4, 133.6, 130.6, 130.1, 129.5, 128.6, 128.5, 128.2, 128.1, 127.7, 127.3, 127.1, 66.9, 54.8, 52.4, 35.3; HRMS (FAB) calcd for $C_{24}H_{24}NO_4$ (M+H) 390.1705; found 390.1710.

4.2.2. (*S*)-*N*$^{\alpha}$-Benzyloxycarbonyl-*o*-(1-naphthyl)phenylalanine methyl ester 4b. 79% yield, two diastereomers (ca. 1:1 ratio); ^1H NMR (300 MHz, $CDCl_3$) δ 7.84–7.89 (2×2H, m), 7.23–7.52 (2×14H, m), 4.86–5.02 (12×2H, m), 4.33–4.47 (2×1H, m), 3.50 (1×3H, s), 3.47 (1×3H, s), 3.03 (1×1H, dd, $J_1=5.1$ Hz, $J_2=14.4$ Hz), 2.88 (1×1H, dd, $J_1=5.1$ Hz, $J_2=14.4$ Hz), 2.75 (1×1H, dd, $J_1=8.7$ Hz, $J_2=14.1$ Hz), 2.59 (1×1H, dd, $J_1=8.7$ Hz, $J_2=14.1$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.4, 155.7, 155.6, 140.8, 140.7, 138.8, 138.5, 136.4, 135.2, 135.0, 133.7, 133.6, 132.3, 132.2, 131.3, 131.2, 129.7, 129.6, 128.6, 128.5, 128.2, 128.1, 128.0, 127.5, 127.3, 127.1, 127.0, 126.5, 126.4, 126.1, 126.0, 125.9, 125.5, 125.4, 66.9, 55.1, 54.7, 52.3, 35.7, 35.6, 29.9; HRMS (FAB) calcd for $C_{28}H_{26}NO_4$ (M+H) 440.1862; found 440.1875.

4.2.3. (*R*)-*N*$^{\alpha}$-Benzyloxycarbonyl-*o*-bromophenylalanine methyl ester 9b. A hydrogenation bottle was charged with **7** (1.20 g, 3.08 mmol) in degassed methanol (30 mL) and then was purged with argon for about 15 min, followed by adding (*S*,*S*) (COD) Et-DuPHOS Rh(I) OTf (4.5 mg, 0.0062 mmol). After 5 vacuum/hydrogen cycles, the reaction bottle was pressurized to an initial pressure of 65 psi. The reaction was allowed to proceed for 24 h. After the evaporation of solvent, the crude produce was purified by flash column chromatography, eluting with ethyl acetate, hexanes, and methylene chloride (1/5/1) to give a white solid (1.19 g, 98%). Mp 82–84°C; $[\alpha]_D^{23}=-5.3$ (*c* 1.40, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.52 (1H, d, $J=8.1$ Hz), 7.05–7.36 (8H, m), 5.43 (1H, d, $J=8.1$ Hz), 5.04 (2H, s), 4.71 (1H, dd, $J_1=8.1$ Hz, $J_2=13.8$ Hz), 3.70 (3H, s), 3.31 (1H, dd, $J_1=6.0$ Hz, $J_2=13.8$ Hz), 3.13 (1H, dd, $J_1=8.1$ Hz, $J_2=13.8$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 155.7, 136.4, 135.9, 133.1, 131.4, 128.9, 128.6, 128.2 (2C), 127.6, 125.1, 67.0, 54.1, 52.6, 38.5; HRMS (FAB) calcd for $C_{18}H_{19}BrNO_4$ (M+H) 392.0497 (Br 79), 394.0479 (Br 81); found 392.0492 (Br 79), 394.0460 (Br 81).

4.2.4. (*R*)-*N*$^{\alpha}$-Benzyloxycarbonyl-*o*-(phenyl)phenylalanine methyl ester 5a. 69% yield, $[\alpha]_D^{26}=-15.9$ (*c* 1.40, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.18–7.41 (14H, m), 4.94–5.02 (3H, m), 4.38–4.45 (1H, m), 3.57 (3H, s), 3.22 (1H, dd, $J_1=5.7$ Hz, $J_2=14.1$ Hz), 3.00 (1H, dd, $J_1=8.1$ Hz, $J_2=14.1$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.4, 155.6, 142.9, 141.3, 136.4, 133.6, 130.6, 130.1, 129.5, 128.6, 128.5, 128.2, 128.1, 127.7, 127.3, 127.2, 66.9, 54.8, 52.4, 35.3; HRMS (FAB) calcd for $C_{24}H_{24}NO_4$ (M+H) 390.1705; found 390.1712.

4.2.5. (*R*)-*N*$^{\alpha}$-Benzyloxycarbonyl-*o*-(1-naphthyl)phenylalanine methyl ester 5b. 98% yield, two diastereomers (ca. 1:1 ratio); ^1H NMR (300 MHz, $CDCl_3$) δ 7.81–7.86 (2×2H, m), 7.20–7.49 (2×14H, m), 4.90–5.05 (2×3H, m), 4.32–4.56 (2×1H, m), 3.44 (1×3H, s), 3.42 (1×3H, s), 3.02 (1×1H, dd, $J_1=5.1$ Hz, $J_2=14.1$ Hz), 2.87 (1×1H, dd, $J_1=5.1$ Hz, $J_2=14.1$ Hz), 2.74 (1×1H, dd, $J_1=8.7$ Hz, $J_2=14.1$ Hz), 2.59 (1×1H, dd, $J_1=8.7$ Hz, $J_2=14.1$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.2, 171.1, 155.6, 155.5, 140.6 (2C), 138.7, 138.4, 136.3, 135.1, 135.0, 133.6, 133.5, 132.2, 132.1, 131.1 (2C), 129.8, 129.6, 128.5, 128.3, 128.1, 128.0, 127.9, 127.4, 127.2, 127.0 (2C), 126.5, 126.4, 126.3, 126.0 (2C), 125.8, 125.4, 125.3, 66.9, 66.7, 54.9, 54.6, 52.3, 52.1, 35.5, 35.4; HRMS (FAB) calcd for $C_{28}H_{26}NO_4$ (M+H) 440.1862; found 440.1861.

4.2.6. Methyl (*Z*)-2-(benzyloxycarbonyl)amino-3-(1-bromonaphthyl)acrylate 13. To a solution of $(MeO)_2$-CH(NHCbz)COOMe **7** (1.98 g, 6.0 mmol) in 15 mL of dry methylene chloride was added DBU (0.76 mL, 5.5 mmol) slowly under an argon atmosphere with stirring. After ca. 10 min, 1-bromo-2-naphthylaldehyde **12** (1.175 g, 5.0 mmol) was added slowly into the above mixture. After 5 h, the solvent was evaporated, and the residue was dissolved in 180 mL of ethyl acetate. The organic solution was washed with 1N HCl (2(35 mL) and brine (35 mL), dried over $MgSO_4$ and evaporated. The crude product was purified by flash column chromatography, eluting with ethyl acetate, hexanes and methylene chloride (1/6/1) to give a white solid (2.05 g, 93%). Mp 127–129°C; ^1H NMR (300 MHz, $CDCl_3$) δ 8.29 (1H, d, $J=8.4$ Hz), 7.74–7.77 (1H, m), 7.49–7.65 (5H, m), 7.15–7.51 (5H, m), 6.63 (1H, brs), 4.95 (2H, s), 3.85 (3H, S); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.5, 153.4, 135.7, 134.2, 133.0, 132.5, 129.4, 128.5 (2C), 128.3, 128.2, 127.8 (2C), 127.6, 127.5, 126.0, 125.6, 125.4; HRMS (FAB) calcd for $C_{22}H_{19}BrNO_4$ (M+H) 440.0497 (Br 79), 442.0480 (Br 81); found 440.0495 (Br 79), 442.0497 (Br 81).

4.2.7. (*S*)-*N*$^{\alpha}$-Benzyloxycarbonyl-1-(1-bromo)-2-naphthylalanine methyl ester 14a. In a similar manner for the preparation of **9a**, using (*S*,*S*) (COD)Et-DuPHOS Rh(I) OTf as a catalyst gave **14a** in 95% yield. Mp 124.5–126°C; $[\alpha]_D^{24}=+0.42$ (*c* 0.90, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.30 (1H, d, $J=8.4$ Hz), 7.79 (1H, d, $J=8.1$ Hz), 7.70 (1H, d, $J=8.1$ Hz), 7.47–7.61 (2H, m), 7.24–7.29 (6H, m), 5.41 (1H, d, $J=8.4$ Hz), 5.03 (2H, s), 4.81 (1H, dd, $J_1=6.6$ Hz, $J_2=8.1$ Hz), 3.70 (3H, s), 3.55 (1H, dd, $J_1=6.3$ Hz, $J_2=13.8$ Hz), 3.42 (1H, $J_1=8.1$ Hz, $J_2=13.8$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.3, 155.8, 136.4, 134.1, 133.8, 132.6, 128.6, 128.3 (2C), 128.2 (2C), 128.0, 127.8 (2C), 126.6, 125.2, 67.1, 54.4, 52.7, 39.7; HRMS (FAB) calcd for $C_{22}H_{21}BrNO_4$ (M+H) 442.0654 (Br 79), 444.0637 (Br 81); found 442.0652 (Br 79), 444.0636 (Br 81).

4.2.8. (*S*)-*N*$^{\alpha}$-Benzyloxycarbonyl-1-(1-phenyl)-2-naphthylalanine methyl ester 10a. 83% yield, $[\alpha]_D^{24}=+14.7$ (*c* 1.10, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.79–7.82 (2H, m), 7.22–7.50 (14H, m), 4.92–5.08 (3H, m), 4.56 (1H, m), 3.62 (3H, s), 3.14 (1H, dd, $J_1=5.4$ Hz, $J_2=14.1$ Hz), 2.91 (1H, dd, $J_1=9.0$ Hz, $J_2=14.1$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 155.7, 139.7, 138.8, 136.4, 133.2, 132.6, 131.4, 130.6, 130.4, 128.7, 128.6, 128.2, 128.0, 127.9, 127.6, 127.4, 126.9,

126.2, 125.7, 66.9, 55.0, 52.5, 36.2; HRMS (FAB) calcd for $C_{28}H_{26}NO_4$ (M+H) 440.1826; found 440.1859.

4.3. Procedures for preparation of compounds 10b–d and 11b through palladium-catalyzed Suzuki coupling reactions

A reaction flask fitted with a Teflon valve was charged with a bromoarylalanine derivative, boronic acid (1.5 equiv.), Na_2CO_3 (2.0 equiv.), $Pd(PPh_3)_4$ (5 mol%), benzene (8 mL/mmol), and degassed water (1 mL/mmol), and then was heated to 80°C for 36 h. The reaction mixture was passed through a short column containing a bottom 1 in. layer of silica gel (230–400 mesh) and a top 1 in. layer of $NaHCO_3$ using ethyl acetate as eluent. The solvent was removed under reduced pressure with a rotary evaporator. The crude product was purified by flash column chromatography using an appropriate mixture of ethyl acetate and hexanes as eluent.

4.3.1. (*S*)-*N*$^{\alpha}$-Benzyloxycarbonyl-1-(1-naphthyl)-2-naphthylalanine methyl ester 10b. 95% yield, two isomers (ca. 1:2 ratio), ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.86–7.96 (3×4H, m), 7.51–7.60 (3×2H, m), 7.35–7.46 (3×3H, m), 7.07–7.26 (3×9H, m), 4.87–5.02 (3×3H, m), 4.51–4.66 (3×1H, m), 3.55, (1×1H, s), 3.53 (2×1H, s), 3.06 (2×1H, dd, J_1=5.1 Hz, J_2=14.1 Hz), 2.96 (1×1H, dd, J_1=5.1 Hz, J_2=14.1 Hz), 2.74 (1×1H, dd, J_1=9.6 Hz, J_2=14.1 Hz), 2.60 (2×1H, dd, J_1=9.6 Hz, J_2=14.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5, 172.1, 155.8, 155.7, 137.5, 137.4, 136.4, 136.1, 133.8 (2C), 133.6, 133.4, 133.0, 132.8 (2C), 132.7, 132.6, 128.6 (2C), 128.4 (2C), 128.3 (2C), 128.2 (2C), 128.1, 128.0, 127.8, 127.74, 127.2, 127.1, 127.0, 126.9, 126.7, 126.6, 126.4, 126.3, 126.2, 126.0, 125.8, 125.6, 67.1, 66.9, 55.0, 54.7, 52.7, 52.4, 36.3, 36.1; HRMS (FAB) calcd for $C_{32}H_{28}NO_4$ (M+H) 490.2018; found 490.2024.

4.3.2. (*S*)-*N*$^{\alpha}$-*tert*-Butoxycarbonyl-1-(1-naphthyl)-2-naphthylalanine methyl ester 10c. 98% yield, two isomers (ca. 1:2 ratio), ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.87–7.98 (3×4H, m), 7.54–7.64 (3×2H, m), 7.39–7.48 (3×3H, m), 7.08–7.29 (3×4H, m), 4.69 (2×1H, d, J=8.4 Hz), 4.61 (1×1H, d, J=8.4 Hz), 4.55 (1×1H, dd, J_1=8.4 Hz, J_2=13.8 Hz), 4.46 (1×1H, dd, J_1=8.4 Hz, J_2=13.8 Hz), 3.54 (1×3H, s), 3.52 (2×3H, s), 3.02 (2×1H, dd, J_1=4.8 Hz, J_2=13.8 Hz), 2.92 (1×1H, dd, J_1=4.8 Hz, J_2=13.8 Hz), 2.71 (2×1H, dd, J_1=9.0 Hz, J_2=13.8 Hz), 2.58 (1×1H, dd, J_1=9.6 Hz, J_2=13.8 Hz), 1.32 (1×9H, s), 1.30 (2×9H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.9, 172.7, 155.3, 155.2, 137.4, 137.3, 136.5, 136.3, 133.9, 133.6, 133.1, 133.0, 132.9, 132.7, 132.6, 128.7, 128.6, 128.5, 128.4, 128.3, 128.0, 127.8, 127.3, 127.2, 127.0, 126.9, 126.7, 126.6, 126.3, 126.1, 126.0, 125.8, 125.6, 80.0, 79.9, 54.6, 54.3, 52.4, 52.3, 36.3, 36.2, 28.41, 28.36; HRMS (FAB) calcd for $C_{29}H_{29}NO_4$ (M+H) 455.2097; found 455.2088.

4.3.3. (*S*)-*N*$^{\alpha}$-Actyl-1-(1-naphthyl)-2-naphthylalanine methyl ester 10d. 96% yield, two isomers (ca. 1:3 ratio), ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85–7.98 (4×4H, m), 7.37–7.64 (4×5H, m), 7.06–7.30 (4×4H, m), 5.75 (3×1H, d, J=7.8 Hz), 5.59 (1H, d, J=8.1 Hz), 4.80 (1×1H, m), 4.71 (3×1H, m), 3.54 (1×3H, s), 3.52 (3×3H, s), 3.05 (3×1H, dd, J_1=5.7 Hz, J_2=14.1 Hz), 2.95 (1×1H, dd, J_1=5.1 Hz, J_2=14.1 Hz), 2.78 (1×1H, dd, J_1=8.4 Hz, J_2=14.1 Hz), 2.68 (3×1H, dd, J_1=8.4 Hz, J_2=14.1 Hz), 1.69 (1×3H, s), 1.67 (3×3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5 (2C), 169.9, 169.7, 137.2, 136.4, 136.1, 133.9, 133.6, 133.5, 133.4, 133.0, 132.9 (2C), 132.8, 132.6, 132.5, 128.8, 128.7, 128.6, 128.5, 128.4, 128.1, 128.0, 127.8, 127.7, 127.3 (2C), 127.0, 126.9, 126.8, 126.7, 126.6, 126.4, 126.3, 126.2, 126.0 (2C), 125.9, 125.8, 125.7, 125.5, 53.3, 53.2, 52.34, 52.30, 35.9, 35.8, 23.0, 22.8; HRMS (FAB) calcd for $C_{26}H_{24}NO_3$ (M+H) 398.1756; found 398.1750.

4.3.4. (*R*)-*N*$^{\alpha}$-Benzyloxycarbonyl-1-(1-bromo)-2-naphthylalanine methyl ester 14b. In a similar manner to the preparation of **9b**, using (*R*, *R*) (COD)Et-DuPHOS Rh(I) OTf as a catalyst gave **14b** in 99% yield. Mp 125–126.5°C;$[\alpha]_D^{24}$=−0.44 (*c* 1.40, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.30 (1H, d, J=8.4 Hz), 7.80 (1H, d, J=7.2 Hz), 7.71 (1H, d, J=8.4 Hz), 7.48–7.62 (2H, m), 7.24–7.28 (6H, m), 5.40 (1H, d, J=8.4 Hz), 5.03 (2H, s), 4.81 (1H, dd, J_1=6.3 Hz, J_2=8.1 Hz), 3.71 (3H, s), 3.55 (1H, dd, J_1=6.3 Hz, J_2=13.8 Hz), 3.42 (1H, J_1=8.1 Hz, J_2=13.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.3, 155.8, 136.4, 134.1, 133.8, 132.7, 128.6, 128.3 (2C), 128.2 (2C), 128.0, 127.8 (2C), 126.7, 125.2, 67.1, 54.4, 52.8, 39.7; HRMS (FAB) calcd for $C_{22}H_{21}BrNO_4$ (M+H) 442.0654 (Br 79), 444.0637 (Br 81); found 442.0654 (Br 79), 444.0657 (Br 81).

4.3.5. (*R*)-*N*$^{\alpha}$-Benzyloxycarbonyl-1-(1-phenyl)-2-naphthylalanine methyl ester 11a. 78% yield, $[\alpha]_D^{24}$=−13.9 (*c* 0.91, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.79–7.85 (2H, m), 7.21–7.47 (14H, m), 4.92–5.05 (3H, m), 4.55 (1H, m), 3.62 (3H, s), 3.14 (1H, dd, J_1=5.4 Hz, J_2=13.8 Hz), 2.91 (1H, dd, J_1=9.0 Hz, J_2=13.8 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.6, 155.7, 139.7, 138.8, 136.4, 133.2, 132.6, 131.4, 130.6, 130.4, 128.7, 128.6, 128.2, 128.0, 127.9, 127.6, 127.4, 126.9, 126.2, 125.7, 66.9, 55.0, 52.5, 36.2; HRMS (FAB) calcd for $C_{28}H_{26}NO_4$ (M+H) 440.1826; found 440.1857.

4.3.6. (*R*)-*N*$^{\alpha}$-Benzyloxycarbonyl-1-(1-naphthyl)-2-naphthylalanine methyl ester 11b. 100% yield, two isomers (ca. 1:2 ratio), ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.85–7.95 (12H, m), 7.50–7.59 (6H, m), 7.35–7.46 (9H, m), 7.06–.27 (27H, m), 4.89–5.07 (9H, m), 4.51–4.66 (3H, m), 3.53, (1H, s), 3.50 (2H, s), 3.06 (2H, dd, J_1=5.1 Hz, J_2=14.1 Hz), 2.96 (1H, dd, J_1=5.1 Hz, J_2=14.1 Hz), 2.74 (1H, dd, J_1=9.3 Hz, J_2=14.1 Hz), 2.60 (2H, dd, J_1=9.3 Hz, J_2=14.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.5, 172.1, 155.8, 155.7, 137.5, 137.3, 136.4, 136.1, 133.8, 133.7, 133.5, 133.4, 132.9, 132.8 (2C), 132.7, 132.6, 128.6, 128.5, 128.4, 128.3 (2C), 128.2 (2C), 128.1 (2C), 127.9, 127.8, 127.7, 127.3, 127.2, 126.9 (2C), 126.7, 126.6, 126.3, 126.2 (2C), 125.9, 125.8, 125.6, 67.0, 66.9, 54.9, 54.7, 52.5, 52.4, 36.2, 36.1; HRMS (FAB) calcd for $C_{32}H_{28}NO_4$ (M+H) 490.2018; found 490.2027.

4.3.7. 1-*tert*-Butoxycarbonyl-3-formyl-2-phenylindole 17. 3-Formyl-2-phenylindole **16** (1.1 g, 5 mmol) in pyridine (15 mL) was treated with di-*tert*-butyldicarbonate (1.42 g, 6.5 mmol, 1.3 equiv.) at room temperature under argon for 5 h. After removal of solvent under reduced pressure, the

pure product was isolated as a yellowish solid (1.53 g, 97%) by silica gel chromatography with ethyl acetate–hexanes–methylene chloride (1/6/1, v/v/v). Mp 178–179°C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.72 (1H, m), 8.40 (1H, m), 8.22 (1H, m), 7.37–7.52 (7H, m), 1.27 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.3, 150.0, 149.3, 136.4, 131.1, 130.2, 129.4, 128.2, 126.1, 125.6, 124.9, 122.0, 119.8, 115.0, 85.1, 27.5; HRMS (FAB) calcd for $C_{20}H_{20}NO_3$ (M+H) 322.1443; found 322.1446.

4.3.8. Methyl (*Z*)-2-(benzyloxycarbonyl)amino-3-[(1-*t*-butoxycarbonyl-2-phenyl)indole] acrylate 18. To a solution of $(MeO)_2CH(NHCbz)COOMe$ **7** (1.2 g, 3.6 mmol) in 10 mL of dry methylene chloride was added DBU (0.5 mL, 3.3 mmol) slowly under an argon atmosphere with stirring. After ca. 10 min, compound **17** (0.96 g, 3.0 mmol) was added slowly into the above mixture. After 6 h, the solvent was evaporated, and the residue was dissolved in 180 mL of ethyl acetate. The organic solution was washed with 1N HCl (2×30 mL) and brine (30 mL), dried over $MgSO_4$ and evaporated. The crude product was purified by flash column chromatography, eluting with ethyl acetate, hexanes and methylene chloride (1/5/1) to give a pale yellow foam (1.19 g, 75%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.26 (1H, d, J=8.4 Hz), 7.54 (1H, d, J=7.8 Hz), 7.29–7.41 (9H, m), 7.01–7.20 (4H, m), 6.38 (1H, brs), 4.93 (2H, s), 3.76 (3H, s), 1.25 (9H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.4, 153.4, 149.9, 140.1, 136.9, 135.9, 133.4, 130.1, 128.6, 128.4, 128.3 (2C), 128.1 (2C), 126.9, 125.0, 123.4, 122.8, 120.7, 115.7, 80.0, 67.4, 52.7, 27.6; HRMS (FAB) calcd for $C_{31}H_{30}N_2O_6$ (M^+) 526.2104; found 526.2111.

4.3.9. (*S*)-*N*$^{\alpha}$-Benzyloxycarbonyl-1-(1-*t*-butoxycarbonyl-2-phenyl)tryptophan methyl ester 15a. A hydrogenation bottle was charged with **18** (265 mg, 0.5 mmol) in degassed methanol (15 mL), and then was purged with argon for about 15 min, followed by adding (*S*,*S*) (COD) Et-DuPHOS Rh(I) OTf (1 mg, 0.001 mmol). After 5 vacuum/hydrogen cycles, the reaction bottle was pressurized to an initial pressure of 80 psi. The reaction was allowed to proceed for 48 h. After the evaporation of solvent, the crude produce was purified by flash column chromatography, eluting with ethyl acetate and hexanes (1/8) to afford a slightly yellow oil (251 mg, 95%). $[\alpha]_D^{23}$=+19.4 (*c* 1.60, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 (1H, d, J=8.1 Hz), 7.59 (1H, d, J=7.5 Hz), 7.26–7.37 (12H, m), 4.98 (2H, dd, J_1=12.6 Hz, J_2=16.2 Hz), 4.55 (1H, dd, J_1=7.5 Hz, J_2=14.1 Hz), 3.53 (3H, s), 3.15 (1H, dd, J_1=5.4 Hz, J_2=14.1 Hz), 2.99 (1H, dd, J_1=7.5 Hz, J_2=14.1 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.3, 155.6, 150.0, 137.7, 136.5, 136.4, 133.8, 130.0, 129.2, 128.6, 128.3, 128.2, 128.0 (2C), 124.9, 123.0, 118.8, 115.4, 115.0, 83.3, 66.9, 54.0, 52.5, 39.3, 27.6; HRMS (FAB) calcd for $C_{31}H_{32}N_2O_6$ 528.2260; found 528.2266.

4.3.10. (*R*)-*N*$^{\alpha}$-Benzyloxycarbonyl-1-(1-*t*-butoxycarbonyl-2-phenyl)tryptophan methyl ester 15b. In a similar manner to the preparation of **15a**, using (*R*,*R*) (COD) Et-DuPHOS Rh(I) OTf as a catalyst gave **15b** in 96% yield. $[\alpha]_D^{23}$=−19.7 (*c* 1.70, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.25 (1H, d, J=8.1 Hz), 7.59 (1H, d, J=7.5 Hz), 7.26–7.39 (12H, m), 4.99 (2H, dd, J_1=12.3 Hz, J_2=15.0 Hz), 4.55 (1H, dd, J_1=7.5 Hz, J_2=14.4 Hz), 3.53 (3H, s), 3.15 (1H, dd, J_1=6.0 Hz, J_2=14.4 Hz), 2.99 (1H, dd, J_1=7.5 Hz, J_2=14.4 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.3, 155.6, 150.0, 137.7, 136.5, 136.4, 133.8, 130.0, 129.2, 128.6, 128.3, 128.2, 128.0 (2C), 124.9, 123.0, 118.8, 115.4, 115.0, 83.3, 66.9, 54.0, 52.5, 39.3, 27.6; HRMS (FAB) calcd for $C_{31}H_{32}N_2O_6$ 528.2260; found 528.2266.

4.4. Receptor binding assay

Competition binding experiments were performed on whole cells. Transfected HEK293 cell line with hMCRs[27,28] were seeded on 24 well plates, 48 h before assay, 50,000 cells/well. For the assay, medium was removed and the cells were washed twice with a freshly prepared binding buffer containing 100% minimum essential medium with Earle's salt (MEM, GIBCO), 25 mM HEPES (pH7.4), 0.2% bovine serum albumin, 1 mM 1,10-phenanthrolone, 0.5 mg/L leupeptin, 200 mg/L bacitracin. Cells were then incubated with different concentrations of unlabeled peptide and labeled ^{125}I-[Nle^4, D-Phe^7]-α-MSH (Perkin–Elmer Life Science, 100,000 cpm/well, 0.1386 nM) for 40 min at 37°C, the medium was subsequently removed, and each well was washed twice with the assay buffer. The cells are lysed by the addition of 500 μL of 0.1 NaOH and 500 μL of 1% Triton X-100. The lysed cell was transferred to the 12×75 mm^2 glass tubes and counted by Wallac 1470 WIZARD Gamma Counter. Data were analyzed using Graphpad Prism 3.1 (Graphpad Software, San Diego, CA).

4.5. Data analysis

IC_{50} values represent the mean of duplicate experiments performed in triplicate. IC_{50} were determined by fitting the data using a nonlinear regression, one site competition, with the help of Graphpad Prism 3.1 (Graphpad Software, San Diego, CA).

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22. Use the same column and conditions as described in Ref. 21. Essentially one single peak was observed in each case of **14a**-(*S*) and **14b**-(*R*). The retention time: **14a**-(*S*): 15.0 min, **14b**-(*R*): 18.6 min.
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