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Synthesis of Tic-D-Phe Ψ[CH_2–CH_2] isostere and its use in the development of melanocortin receptor agonists

Xinrong Tian,* Xuemei Chen,† Lixian Gan, Jeffery C. Hayes, Adrian G. Switzer, Mark G. Solinsky, Frank H. Ebetino, John A. Wos, Beth B. Pinney, Julie A. Farmer, Doreen Crossdoersen and Russell J. Sheldon

Procter & Gamble Pharmaceuticals, Health Care Research Center, 8700 Mason Montgomery Rd., Mason, OH 45040, USA

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Abstract—The first synthesis of Tic-D-Phe Ψ[CH_2–CH_2] isostere is described, which features diastereoselective alkylation of the tricyclic lactam **14**. The use of this novel dipeptide isostere in the development of melanocortin agonists has been demonstrated by the synthesis of peptidomimetic **7** and non-peptidic ligand **27**. Both compounds displayed significant binding and agonist potency at the MC4R.

The melanocortin receptors (MCRs) belong to a family of 7-transmembrane G-protein-coupled receptors and five subtypes have been identified and cloned (MC1R-MC5R). These receptors are activated by the peptide ligands: α,β,γ-melanocyte stimulating hormones (MSH) and adrenocorticotropin (ACTH), which are derived from a common precursor protein, proopiomelanocortin (POMC), by post translational cleavage.[1] A common structural feature present in all endogenous ligands (MSH and ACTH) of the MCRs is the His-Phe-Arg-Trp sequence, which has been identified as the minimal peptide fragment necessary for activating the receptors.[2] Further structural modifications toward α-MSH showed that inverting Phe7 to the D-configuration resulted in an analog, NDP-MSH, with enhanced potency and enzymatic stability.[3] The tetrapeptide His-D-Phe-Arg-Trp[4] and tripeptide D-Phe-Arg-Trp[5] have been thus employed as intriguing templates for SAR studies and for MCR ligand design. A key approach within our research program centered on replacement of each amino acid residue of tetrapeptide His-D-Phe-Arg-Trp and led to the identification of a number of structurally diverse analogs displaying significant binding and agonist activity at MC4R as well as moderate selectivity relative to MC1R, such as Ac-Tyr-D-Phe-Arg-2-Nal-$NHCH_3$ (**1**) and Tic-D-Phe-Arg-2-Nal-$NHCH_3$ (**2**) (Figs. 1 and 2).

A major drawback to the use of peptides in therapeutic applications is the susceptibility to rapid degradation in vivo by numerous peptidases. One well-established strategy to enhance metabolic stability of peptides to proteolytic enzymes is to substitute a peptide amide bond with a non-hydrolyzable amide surrogate.[6] In the course of our efforts to develop peptidomimetic MCR agonists, we have explored the replacement of two adjacent amino acids of tetrapeptide leads derived from His-D-Phe-Arg-Tyr with a variety of Ψ[CH_2–CH_2] dipeptide isosteres. We anticipated that systemic substitution of each amide bond with a CH_2–CH_2 unit would provide insight into the relative importance of each amide bond with regard to geometrical, electrostatic, and hydrogen bond properties toward the binding and functional potency of the tetrapeptide. This type of information, in turn, could be incorporated into the design of peptidomimetics with significantly reduced peptidic characters by removing those amide bonds that are not necessary for biological activity. Our initial results obtained with replacement of the amide bond between Tyr and D-Phe of tetrapeptide **1** were quite encouraging. For instance, the corresponding 'carba' analog of **1** (**3**) not only displayed comparable affinity and agonist potency (EC_{50}) at MC4R but also was 7-fold more selective for MC4R relative to MC3R, as compared to the

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* Corresponding author. Tel.: +1 513 622 1444; fax: +1 513 622 1195; e-mail: tian.x@pg.com
† Present address: Department of Chemistry, University of Minnesota, Minneapolis, MN 55455, USA.

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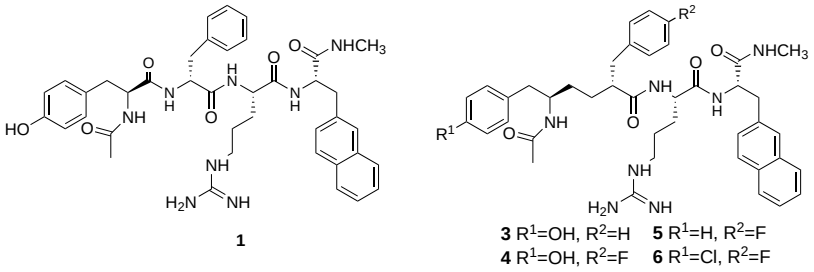


Figure 1. Tetrapeptide **1** and 'carba' analogs.

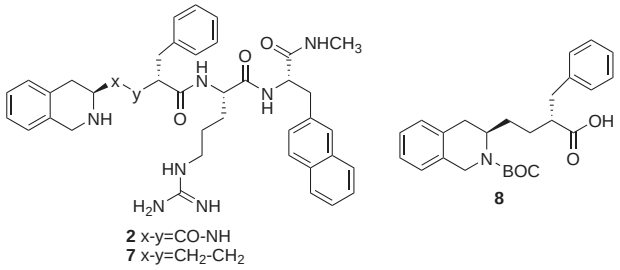


Figure 2. Tetrapeptide **2** and the 'carba' target analog.

parent peptide (Table 1, Figs. 1 and 2). This SAR finding promoted us to investigate additional pseudo-tetrapeptides derived by using other D-Phe-containing $\Psi[CH_2–CH_2]$ dipeptide isosteres and some of them are listed in Figure 1. It is interesting to find that replacement of the phenyl ring of D-Phe side chain of 'carba' analog **3** with 4-fluorophenyl group led to a potent and selective MC4R agonist (**4**) which had a K_i of 5 nM, and was >4400-fold and >1200-fold selective for MC4R over MC1R and MC3R (Table 1), respectively. Removal of 4-hydroxyl group from the side chain of Tyr residue or replacement of 4-hydroxyphenyl with 4-chlorophenyl of **4** resulted in analogs with K_i values of 37 nM (**5**) and 214 nM (**6**), respectively, at MC4R and good selectivity for MC4R over MC1R and MC3R. The D-Phe-containing $\Psi[CH_2–CH_2]$ isosteres discussed above were efficiently synthesized by means of the alkylation of 5-substituted δ-lactam.[8] These data provided preliminary evidence that $CH_2–CH_2$ unit is a viable surrogate for the amide bond between two amino acids at the left side (N-terminus) of the tetrapeptide leads.

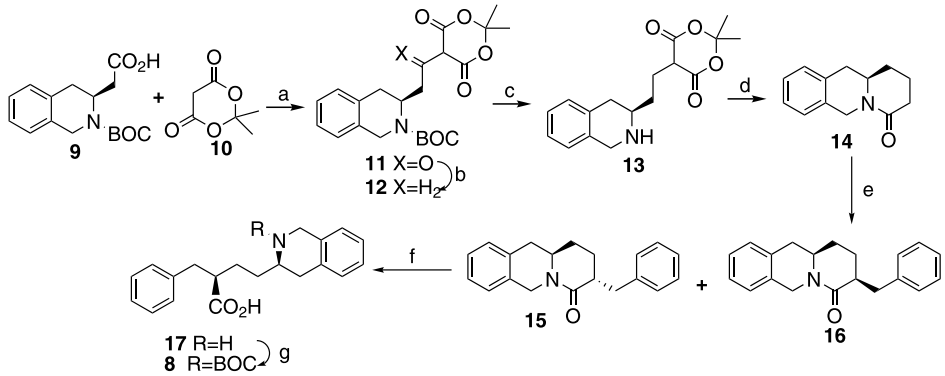
The 'carba' analog **7** became one of our next targets due to the higher affinity and potency at the MC4R displayed by parent peptide **2** as compared to **1** (Fig. 2). While only a few approaches for synthesis of 'carba' $\Psi[CH_2–CH_2]$ dipeptide isosteres have been reported,[8,9] the feasibility of applying these methods to prepare such isosteres that contain a constrained amino acid, such as Tic (a constrained Phe analog), has not been demonstrated. Given the excellent diastereoselectivity associated with the route featuring the alkyation of 5-substituted δ-lactam for the synthesis of 'carba' $\Psi[CH_2–CH_2]$ dipeptide unit,[8] we sought to explore the possibility of extending this approach to synthesis of the Tic-D-Phe $\Psi[CH_2–CH_2]$ dipeptide isostere required for the development of new peptidomimetic MCR agonists. The key question would be whether the alkylation of a constrained tricyclic lactam system (**14**, Scheme 1) in which conformational mobility of the C-5 substituent is highly restricted would proceed with a similar level of 1,4-asymmetric induction to that observed with the flexible 5-substituted δ-lactams.[8]

The precursor for the key tricyclic lactam **14** was prepared by a two-carbon homologation using Meldrum's acid as reported.[8] (*S*)-2-(2-(*tert*-Butoxycarbonyl)-1,2,3,4-tetrahydroisoquinolin-3-yl)acetic acid **9** was coupled with Meldrum's acid to give 3-keto ester **11**, which was then deoxygenated by treatment with sodium borohydride in the presence of acetic acid to afford **12**. Cyclization of **12** to lactam **14** was achieved using a two-step reaction sequence. Following removal of the BOC group of **12** with TFA, the salt of amine **13** was heated at reflux in toluene in the presence of triethylamine to furnish the tricyclic **14** in good yield. With **14** in hand, we then investigated the facial selectivity of the alkylation of this tricyclic lactam to introduce the benzyl group to the carbon next to the carbonyl group. A variety of bases and solvents were examined to define the conditions that would favor the formation of compound **15** possessing the desired D-Phe configuration. In contrast with the case of 5-substituted δ-lactams,[8] a mixture of two diastereomers **15** and **16** was consistently obtained under all conditions examined. The use of NaHMDS in THF/DME (1:1) (0.2 mM) for enolate formation, optimized

Table 1. Binding affinity and agonist potency for **1**–**6**[a]

Compound	MC1R K_i[b] (nM)	MC3R K_i[b] (nM)	MC4R K_i[b] (nM)	MC4R EC_{50} (E_{max}, %)[b] (nM)
1	4520 ± 1257	1727 ± 67	104 ± 10	44 ± 5 (84)
2	2276 ± 677	1632 ± 77	24 ± 2	10 ± 2 (81)
3	8562 ± 4113	24,689 ± 8171	214 ± 28	38 ± 5 (90)
4	22,267 ± 1133	6198 ± 812	5 ± 1	10 ± 3 (91)
5	23,400 ± 0	55,474 ± 13,257	37 ± 6	20 ± 1 (102)
6	12,288 ± 5600	18,016 ± 13,499	214 ± 49	193 ± 45 (100)

[a] The analogs were screened against the human MC1R, MC3R, and MC4R as previously reported.[7]
[b] The data represent means of the at least three experiments ± SEM.



Scheme 1. Synthesis of protected Tic-D-Phe dipeptide isostere **8**. Reagents and conditions: (a) DMAP, EDCI, CH_2Cl_2, 0 °C to room temperature, 4 h, 94%; (b) $NaBH_4$, acetic acid, CH_2Cl_2, 10h, 95%; (c) TFA, H_2O, CH_2Cl_2; (d) Et_3N, toluene, 120 °C, 1 h, 73% yield over two steps; (e) LDA (1.2 equiv), THF, −78 °C, 1 h; BnBr (1.2 equiv), −78 °C, 4 h, 58%; (f) 4 N HCl, reflux, 24 h; (g) $(BOC)_2O$, Et_3N, CH_3OH, reflux, 4 h, 81% yield over two steps.

conditions reported for the alkylation of 5-substituted lactams,[8] produced a mixture of **15**:**16** in a 1.2:1 ratio in 57% yield. Ultimately, the following procedure was found to be optimal in our hands to give a mixture of **15**:**16** in a 1.6:1 ratio in 58% yield. A solution of **14** was treated with LDA (1.2 equiv) at −78 °C for 1 h and benzyl bromide (1.2 equiv) was added. The reaction mixture was stirred for 4 h at −78 °C before it was quenched with aqueous NH_4Cl solution and extracted with ethyl acetate. The extract was dried over $MgSO_4$, filtered, and concentrated. The residue was then subjected to column chromatography (hexanes/ethyl acetate, 2:1).[10]

The stereochemistry at the newly formed chiral center next to the carbonyl group for diastereomers **15** and **16** was established by the analysis of ^1H, 2D-COSY, and 2D-NOESY NMR data. As revealed by NOESY experiments with **15**, there is a strong NOE between H-5 and H-3α, and between H-5 and H-4α. A strong NOE observed between H-2 and H-3β and between H-2 and H-4β, suggests an *R* configuration at the C-2. For isomer **16**, there is a much stronger NOE effect between H-5 and H-4 α than H-4 β in NOESY spectra run at both 0 °C and −30 °C. The NOESY spectrum acquired at −30 °C clearly shows a strong NOE effect between H-4α and H-2, and a medium NOE between H-4 β and one of two C-1′ protons. These correlation data are suggestive of *S* configuration at the C-2 of **16** (Fig. 3).

The moderate diastereoselectivity achieved with the alkylation of lactam **14** is probably because the high degree of conformational rigidity enjoyed by the central ring precludes the possibility for the C-5 substituent to adopt an axial conformation, which is the key factor contributing to the excellent selectivity seen with the flexible 5-substituted δ-lactams.[8] Diastereomer **15** was subjected to acidic hydrolysis with 4 N HCl at

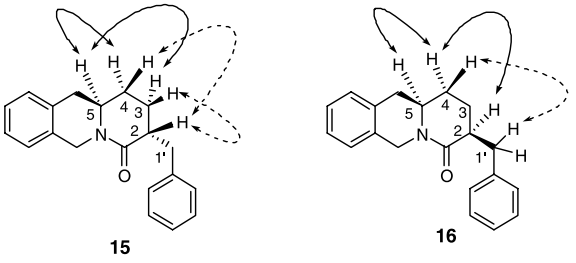


Figure 3. NOESY correlations of isomers **15** and **16**.

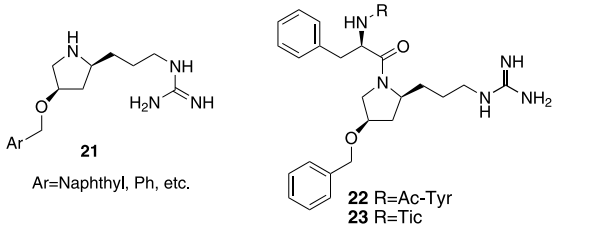
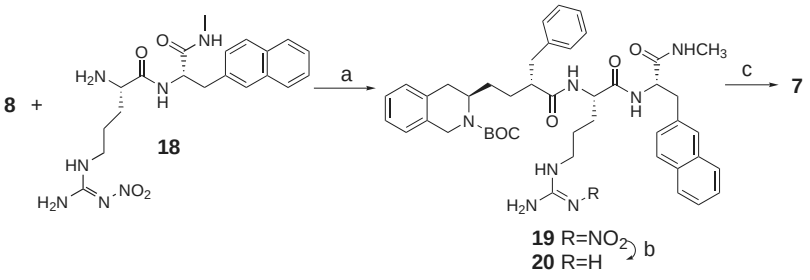


Figure 4. Pyrrolidine-based D-Phe-2-Nal dipeptide mimics and potent peptidomimetic MCR agonists.



Scheme 2. Synthesis of peptidomimetic **7**. Reagents and conditions: (a) EDCI, HOBt, NMM, DMF, 24 h, 49%; (b) $BaSO_4$/Pd, acetic acid, CH_3OH, 18 h; (c) TFA, CH_2Cl_2, 4 h, 62% yield over two steps.

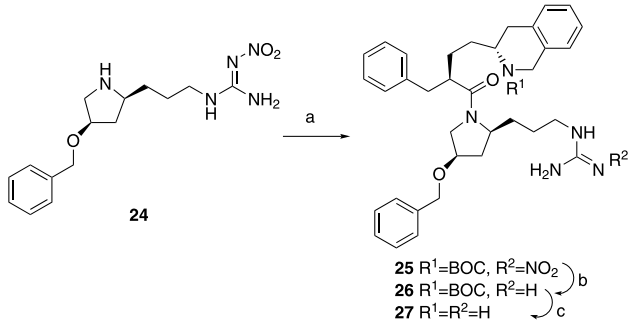
Table 2. Binding affinity and agonist potency for **7**, **22**, **23**, and **27**[a]

Compound	MC1R K_i[b] (nM)	MC3R K_i[b] (nM)	MC4R K_i[b] (nM)	MC4R EC_{50} (E_{max}, %)[b] (nM)
22	9 ± 3	23 ± 9	10 ± 2	8 ± 1 (84)
23	30 ± 8	468 ± 191	13 ± 2	22 ± 5 (89)
7	3992 ± 1322	2411 ± 79	220 ± 14	160 ± 36 (77)
27	71 ± 29	382 ± 53	76 ± 9	142 ± 47 (76)

[a] The analogs were screened against the human MC1R, MC3R, and MC4R as previously reported.[7]
[b] The data represent means of at least three experiments ± SEM.

reflux to afford D-Phe-Tic 'carba' isostere **17** in a quantitative yield, which was subsequently converted to the BOC derivative **8**. Isostere **8** was then applied to synthesis of the desired 'carba' analog **7**, which was accomplished by coupling **8** with Arg(NO_2)-2-Nal-$NHCH_3$ dipeptide (**18**) followed by reductive deprotection of the guanidine moiety and BOC cleavage with TFA to give **7** (Scheme 2).

Development of nonpeptidic MCR agonists by coupling **8** with other dipeptide mimics has also been briefly explored. In addition to the approach of replacing amide bond with a CH_2–CH_2 unit, another strategy we have investigated for developing peptidomimetic melanocortin agonists involves the design of conformationally constrained dipeptide mimetics to substitute two adjacent amino acids of tetrapeptide leads. Our efforts along this line have led to the discovery of pyrrolidine-based Arg-2-Nal, two amino acids at the right side of numerous tetrapeptide leads, dipeptide mimetics as represented by **21**. Appending Xaa-D-Phe dipeptides, which correlate with two amino acids at the left side of tetrapeptide leads, to the Arg-2-Nal dipeptide mimetics resulted in the synthesis of a number of potent MCR agonists, as exemplified by **22** and **23** (Fig. 4 and Table 2, EC_{50} values at MC1R for these two compounds are 8 and 30 nM, respectively).[11] A variety of amino acids are tolerated as the capping groups of the D-Phe reside at the top side chain and the resulting analogs exhibited excellent affinity and agonist potency at MC4R and, in many cases, at MC1R and MC3R as well. The synthetic availability of Tic-D-Phe 'carba' analog **8** using the approach described above has provided an opportunity to expand the utility of these new pyrrolidine dipeptide mimetics to generate non-peptidic melanocortin agonists. Thus, coupling of **8** with guanidine-containing pyrrolidine **24** under EDCI activation afforded **25**. Hydrogenative removal of the nitro group from the guanidine moiety followed by the BOC cleavage with TFA produced **27** (Scheme 3).



Scheme 3. Synthesis of non-peptidic analog **27**. Reagents and conditions: (a) **8**, EDCI, HOBt, NMM, DMF, 18 h; (b) H_2, Pd/$BaSO_4$, acetic acid (10%), CH_3OH, 12 h, 34% yield over two steps; (c)TFA, CH_2Cl_2, 3 h, ~100%.

The binding affinity at three MCRs and agonist potency for MC4R of **7** and **27** are listed in Table 2. The 'carba' analog **7** possessed a K_i of 220 nM and an EC_{50} of 160 nM at the MC4R with moderate selectivity for the MC4R over the MC1R and MC3R, but it was ~16-fold less potent at the MC4R than the tetrapeptide **2**. Nonpeptidic analog **27**, however, displayed a ~3-fold better affinity than **7** at MC4R and significant affinity at MC1R and MC3R as well and was only 6-fold less potent than the corresponding parent dipeptide **23**. This result is significant in terms of the fact that **27** represents the first analog developed by combining the SAR learning from two different approaches we have explored, replacement of the amide bond between two amino acids at the left side of tetrapeptides and design of conformationally constrained dipeptide mimics for two amino acids at the right side of tetrapeptides. We have previously demonstrated that it is possible to further enhance the potency of 'carba' analogs by introducing substitution to the aromatic ring of D-Phe side chain (**3** vs **4**).

In summary, we have described a synthetic approach to the Tic-D-Phe Ψ[CH_2–CH_2] isostere **8** that features alkylation of a fused tricyclic lactam **14**. Unlike the case of monocyclic 5-substituted δ-lactam, the alkylation of conformationally constrained tricyclic lactam system led to a mixture of two isomers (**15** and **16**) under a variety of conditions, and the major isomer **15** possessed the stereochemistry corresponding to D-Phe configuration. Additionally, the reported approach also allows access to Tic-Phe Ψ[CH_2–CH_2] isostere, although it was formed as the minor product of the alkylation step. The use of protected dipeptide isostere **8** in the development of MCR agonists with reduced peptidic characters has been demonstrated by the synthesis of peptidomimetic **7** and non-peptidic analog **27**.

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10. Spectral and analytic data for **15** and **16**.
Compound **15**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10–7.40 (m, 9H), 5.41 (d, J = 17.2 Hz, 1H), 4.31 (d, J = 17.2 Hz, 1H), 3.72 (m, 1H), 3.51 (m, 1H), 2.95 (dd, J = 15.4, 11.6 Hz, 1H), 2.60–2.80 (m, 3H), 2.04 (m, 1H), 1.60–1.90 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.17 (C═O),140.54, 135.90, 135.50, 130.03, 128.76, 128.18, 127.05, 126.99, 126.85, 126.52, 54.56, 45.79, 44.13, 38.21, 37.95, 29.13, 24.37; MS (CI) *m/z* 292 (M+1); HRMS calcd for ($C_{13}H_{15}NO$+H): 292.1701. Found: 292.1692.
Compound **16**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.10–7.40 (m, 9H), 5.20 (d, J = 17.7 Hz, 1H), 4.43 (d, J = 17.7 Hz, 1H), 3.55 (m, 2H), 2.60–2.90 (m, 2H), 2.17 (m, 1H), 1.84 (m, 1H), 1.40–1.70 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.02 (C═O), 140.48, 134.31, 133.53, 129.59, 128.77, 126.92, 126.82, 126.55, 53.61, 45.63, 43.92, 38.19, 36.64, 26.91, 22.56; MS (CI) *m/z* 292 (M+1); HRMS calcd for ($C_{13}H_{15}NO$+H): 292.1701. Found: 292.1701.
11. Details of this research will be reported elsewhere.