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Investigation of the active turn geometry for the labour delaying activity of indolizidinone and azapeptide modulators of the prostaglandin $F_{2\alpha}$ receptor†

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On pursuing molecules that delay labour, so-called tocolytics, the prostaglandin $F_{2\alpha}$ receptor (FP) was targeted, because of its role in the stimulation of uterine contractions leading to birth and preterm birth. Previously, both the indolizidinone PDC-113.824 (**5**) and the aza-glycinyl-proline analog **6** were shown to delay labour in mice by modulating the FP function, likely by an allosteric mechanism, which features biased signalling. The crystal structure and computational analyses of the indolizidin-2-one amino acid and aza-glycinyl-proline components of **5** and **6** in model peptides have shown them to adopt a geometry that mimics ideal type I and II′ β-turns. To elucidate the precise turn geometry for receptor recognition, analogs **1–4** have now been synthesized: macrocycle and pyrroloazepinone mimics **1** and **2** to mimic type I, and glycinyl-proline and D-alaninyl-proline analogs **3** and **4** to favour type II′ β-turn geometry. Notably, transannular cyclization of peptide macrocycle **13** has provided diastereoselectively pyrroloazepinone **15** by a novel route that provides effective access to mimics **1** and **2** by way of a common intermediate. Among the four analogs, none exhibited efficacy nor potency on par with **5** and **6**; however, D-alaninyl-proline analog **4** proved superior to the other analogs in reducing $PGF_{2\alpha}$-induced myometrial contractions and inhibiting FP modulation of cell ruffling, a response dependent on the $G_{\alpha12}$/RhoA/ROCK signaling pathway. Furthermore Gly-Pro analog **3** potentiated the effect of $PGF_{2\alpha}$ on Gαq mediated ERK1/2 activation. Evidence that **4** adopted turn geometry was obtained by conformational analysis using NMR spectroscopy to characterize respectively the influence of solvent and temperature on the chemical shifts of the amide NH protons. Although mimicry of the type II′ geometry by **3**, **4**, **5** and **6** may favour activity, distortion from ideal geometry by the indolizidinone and aza-glycinyl residues of the latter appears to enhance their biological effects.

Introduction

A major determinant of neonatal mortality and morbidity, preterm birth, defined as childbirth before 37 weeks of gestation, can have long-term adverse health consequences.[1–3] Notably, >10% of all babies are born too soon, accounting for ~15 million preterm babies worldwide every year, among which ~1 million die each year due to complications at birth.[1] Contemporary drugs which delay labour,[4–7] so-called tocolytics, have had limited success, such that the incidence of preterm birth has increased over the past thirty years. Beyond concerns for the wellbeing of premature infants, the socio-economic considerations of preterm birth include costs for hospitalization, rehabilitation and special schooling, which have increased paradoxically due to improved survival rates. In 2005, the costs associated with preterm birth in the US was estimated to be >$26 billion, making preterm birth the highest per patient cost of any medical disorder.[8,9]

On pursuing a novel approach to delayed labour, we have targeted the prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) receptor (FP), because of the major role played by this G protein-coupled receptor (GPCR) in the regulation of uterine activity and the molecular

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mechanisms of human labour and parturition.[10] The expression of FP augments markedly during term and especially preterm parturition.[11,12] Moreover, the FP knockout mouse never goes into labour.[13]

In targeting FP, we have created two series of modulators, which do not compete with the native orthosteric ligand $PGF_{2\alpha}$ for FP binding, but appear to bind to a remote allosteric site and influence receptor signalling by altering receptor conformation.[14,15] For example, indolizidinone analogs typified by PDC113.824 (**5**, Fig. 1) exhibit no activity on FP in the absence of $PGF_{2\alpha}$; however, in the presence of this native ligand, they enhance coupling to Gαq resulting in increased PKC-dependent ERK1/2 signalling, and simultaneously inhibit $G_{\alpha 12}$ coupling and RhoA/ROCK-dependent cytoskeletal rearrangement.[14] In addition, **5** inhibited myometrial contractions. In pregnant mice, indolizidinone **5** delayed respectively lipopolysaccharide- and $PGF_{2\alpha}$-induced delivery at average times of 28 and 42 h.[14] A second series of FP modulators was prepared by replacement of the indolizidinone moiety of **5** with an aza-amino acyl-proline residue, as typified by aza-glycinyl-proline analog **6**, which exhibited similar effects on myometrium contraction and biased signalling as **5**.[15] In this azapeptide series, the side-chain of the aza-residue appears to influence the biased signalling of FP.[15] For example, an aza-propargylglycinyl-proline analog **7** exhibited comparable effects on myometrial contractions and $ERK_{1/2}$ signalling as **6**, albeit with reduced influence on RhoA/ROCK signalling. Such findings illustrate that FP signalling can be modulated by these allosteric ligands to favour specific modalities.[14–16]

Underlying their similar mechanism of action may be a common conformation adopted by (3*S*,6*S*,9*S*)-indolizidin-2-one **5** and azaGly-Pro analog **6**. In the crystal structure of (3*S*,6*S*,9*S*)-indolizidinone *N*-(Boc)amino methyl ester **8**, the dihedral angles of the backbone atoms constrained inside the heterocycle ($\psi = -176°$ and $\phi = -78°$) resembled the values of the central residues in an ideal type II′ β-turn ($\psi = -120°$ and $\phi = -80°$, Table 1).[17] In tetrapeptide models, conformational analysis using the Monte Carlo/stochastic dynamics simulation with AMBER* parameters in water as implicitly represented by the GB/SA solvation model indicated however that the (3*S*,6*S*,9*S*)-indolizidin-2-one amino acid residue was more effective as a reverse turn than as a β-turn mimic.[18] Confor-

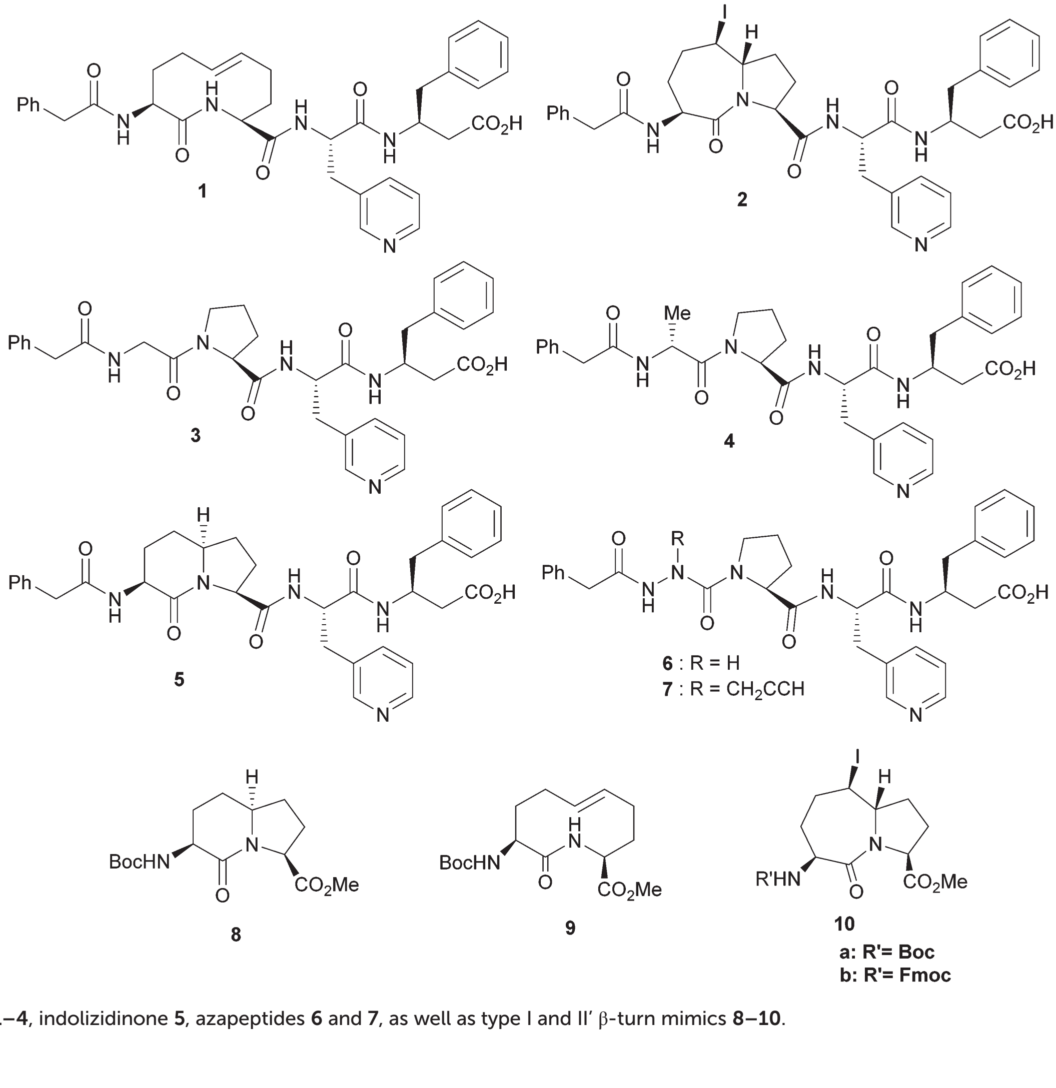


Fig. 1 Targets **1–4**, indolizidinone **5**, azapeptides **6** and **7**, as well as type I and II′ β-turn mimics **8–10**.

Table 1 Ideal type I and II′ β-turn dihedral angles and comparisons with turn mimics

	φ_1	ψ_1	φ_2	ψ_2	Ref.
(3*S*,6*S*,9*S*)-Indolizidin-2-one **5**		−176	−78		17
N-Boc-aza-alaninyl-proline-*N*′-iso-propylamide	−66.7	−17.7	−58.1	−24.7	21
Macrocyclic lactam **9**	−82	−20	−107	−18	22
Azabicyclo[5.3.0]alkan-2-one amino ester **10**		−63.5	−46.6		23
N-Pivaloyl-D-alaniny1-proline-*N*′-isopropylamide	60	−140	−89	9	24
Ideal type I β-turn	−60	−30	−90	0	25
Ideal type II′ β-turn	60	−120	−80	0	25

mational analysis of Ac-aza-Gly-L-Ala-NHMe predicted the type I β-turn as an energy minimum that was 10.5 kJ mol^{-1} lower in energy than its type II′ β-turn conformer.[19] Moreover, X-ray analyses of aza-dipeptide models (Boc-aza-Ala-Pro-NHi-Pr, Cbz-aza-Asp(Et)-Pro-NHi-Pr and Cbz-aza-Asn(Me)-Pro-NHi-Pr) showed the aza-Xaa-Pro dipeptide to adopt the central $i + 1$ and $i + 2$ residues of a type I β-turn.[20,21]

To differentiate whether type I or II′ β-turn conformations were responsible for the activity of **5** and **6**, we have now synthesized and examined the biological activity of analogs **1–4** in which the respective indolizidinone or the aza-Gly-Pro moiety was replaced by other residues known to adopt such ideal turns (Fig. 1). For example, both 10-member unsaturated macrocyclic lactam **9** and azabicyclo[5.3.0]alkan-2-one amino acid **10** have been shown by X-ray crystallography to adopt type I β-turn conformations (Table 1).[22,23,26] On the other hand, to induce type II′ conformers, Gly-Pro and D-Ala-Pro were employed, because these sequences have been shown to prefer the central position of this β-turn type.[24,27] By studying analogs **1–4** for their potential to reduce $PGF_{2\alpha}$-induced myometrial contractions, to potentiate the effect of $PGF_{2\alpha}$ on Gαq-mediated $ERK_{1/2}$ activation and to inhibit FP modulation of cell ruffling, we sought to gain key insights into the conformational requirements for the mechanisms contributing to FP-dependent physiological and patho-physiological responses.

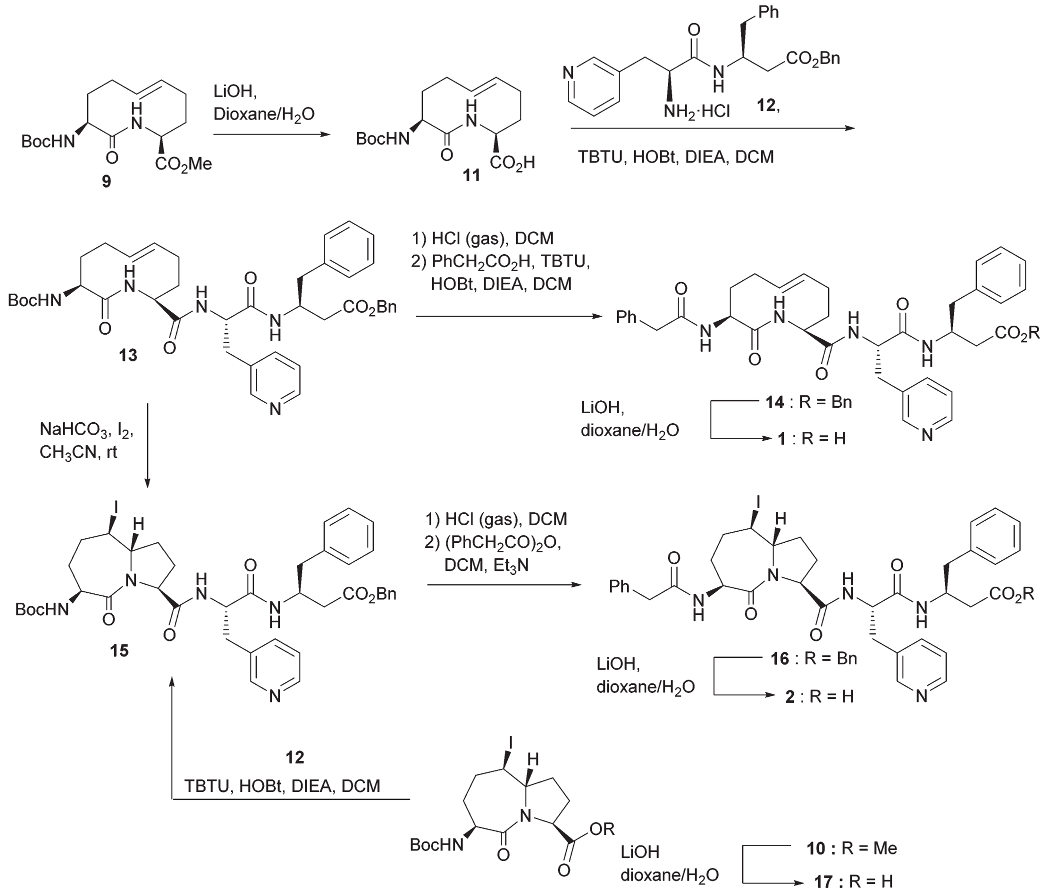
Results and discussion

The importance of the turn geometry of **5** and **6** for their similar biological activity was investigated by replacement of their respective 3-amino indolizidin-2-one 9-carboxylate and aza-glycinyl-proline residues with alternative dipeptide analogs known to adopt type I and II′ geometry. In particular, 10-membered macrocyclic lactam **9** and azabicyclo[5.3.0]alkan-2-one amino acid **10** were employed to favour type I β-turn geometry, and Gly-Pro and D-Ala-Pro were employed as type II′ inducers. Instead of preparing both macrocycle and bicycle independently prior to their insertion into the analogs, transannular cyclization was employed to convert the macrocyclic peptide into its bicyclic counterpart. Although transannular cyclizations of *N*-protected macrocyclic lactam esters have been used to make various azabicyclo[X.Y.0]alkanone amino acid analogs,[23,28] successful application of this method within a larger peptide framework has not been previously reported.

Computational, X-ray and NMR analyses of 10-membered macrocyclic lactam **9** have previously shown that the dipeptide constrained in the ring prefers to adopt φ, ψ, and ω torsional angles similar to that of the central residues of an ideal type I β-turn (Table 1).[22] Macrocycle **9** was synthesized by ring-closing metathesis of methyl *N*-(Boc)homoallylglycinyl-homoallylglycinate using the first generation of Grubb's catalyst.[23] The linear dipeptide was assembled by the coupling of selectively protected homoallylglycine derivatives, which were synthesized by a route featuring the copper-catalyzed cross-coupling reaction of methyl *N*-(Boc)iodoalaninate to allyl chloride.[29] Acid **11** was obtained from hydrolysis of methyl ester **9** with lithium hydroxide in 1 : 1 dioxane/water.

Acid **9** was coupled to pyridinylalaninyl-β-homophenylalanine benzyl ester **12** using TBTU, HOBt, and DIEA to give protected peptide **13** in 74% yield after chromatography.[15] Removal of the Boc protection was effectively accomplished in 97% yield by bubbling HCl gas into a solution of **13** in CH_2Cl_2. Alternative methods for the Boc cleavage, such as 2 N HCl in dioxane and 50% TFA in CH_2Cl_2, gave side products and relatively lower yields. The resulting hydrochloride salt was coupled to phenyl acetic acid using TBTU, HOBt and DIEA in dichloromethane to afford phenylacetamide **14** in 70% yield. Hydrolysis of benzyl ester with 2 N LiOH in dioxane gave acid **1** in >95% purity after purification by preparative reverse-phase HPLC (Scheme 1).

Previously, X-ray analyses of *N*-(Boc)- and *N*-(Fmoc)amino pyrroloazepinone esters **10a** and **10b** indicated that the dihedral angles within the bicycle were similar to those of the central residues of an ideal type I β-turn (Table 1).[23] Pyrroloazepinones **10a** and **10b** were respectively synthesized by diastereoselective transannular cyclizations of macrocyclic lactam **9** and its Fmoc counterpart using iodine in THF.[23,28] Instead of preparing ester **10a** for subsequent introduction into a constrained analog by peptide coupling chemistry, a new strategy featuring transannular cyclization within the peptide framework was examined to introduce the azabicyclo-[5.3.0]alkan-2-one into peptide mimic **2**. Macrocyclic peptide **13** was treated with iodine in the presence of excess sodium bicarbonate for 1 h (Scheme 1). Transannular cyclization of macrocycle **13** provided pyrroloazepinone **15** as a single diastereomer in 94% yield. To confirm the stereochemistry of heterocycle **15**, bicycle **10** was prepared according to a literature protocol from macrocycle **9**,[30] and hydrolysed with 2 N LiOH in dioxane. The resulting acid **17** was coupled to pyridinylalaninyl-β-homophenylalanine benzyl ester **12** using TBTU, HOBt, and DIEA to provide protected peptide **15**, which exhibited identical chemical shift and coupling constant *J* values as those of material prepared from transannular cyclization on peptide **13**. The removal of the Boc group of **15** with HCl gas as described above gave the hydrochloride salt, which was directly coupled to phenyl acetic anhydride to give **16**. To mini-



Scheme 1 Synthesis of 10-membered macrocyclic mimic **1** and azabicyclo[5.3.0]alkan-2-one mimic **2**.

mize side products and simplify purification in the installation of the phenylacetyl group, phenyl acetic anhydride was employed with triethylamine in CH_2Cl_2. Phenylacetamide **16** was isolated in 73% yield after purification by chromatography on silica gel. Finally, ester hydrolysis as described above provided acid **2**, which was purified to >95% purity by preparative reverse-phase HPLC.

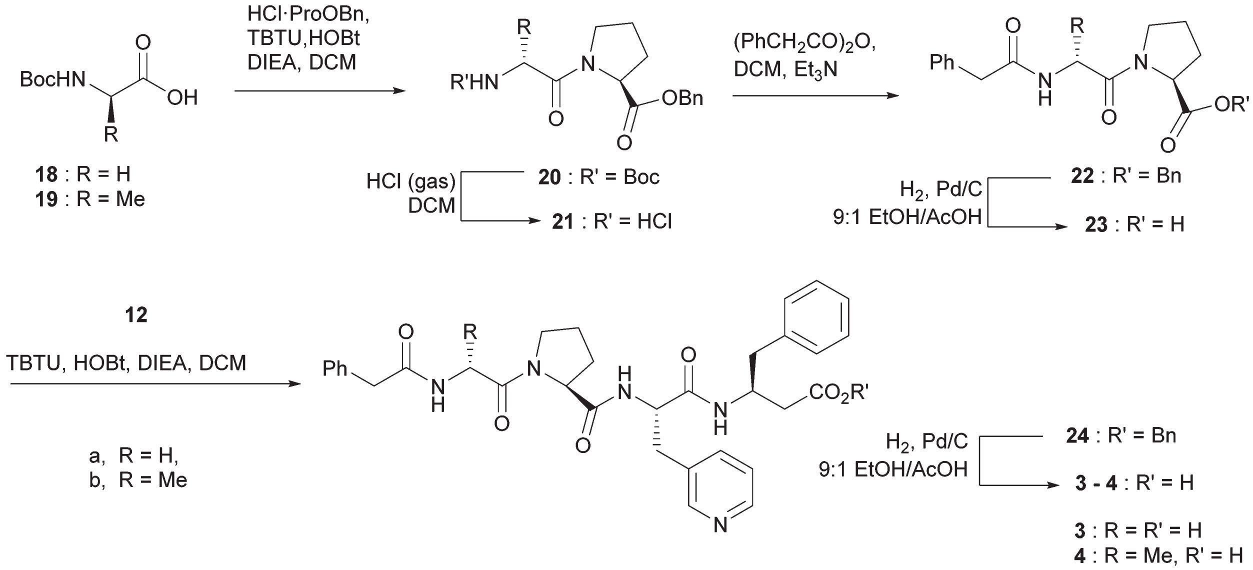
Natural amino acids were employed to favour the type II′ β-turn geometry. Specifically, Gly-Pro and D-Ala-Pro were used, because of their preference to adopt the central positions of the type II′ β-turn in natural peptides.[27] Proline benzyl ester was respectively coupled to *N*-(Boc)glycine **18** and *N*-Boc-D-alanine **19** using TBTU, HOBt, and DIEA to give quantitatively the protected dipeptides **20a** and **20b**, which were observed to exist as prolyl amide isomers in their respective NMR spectra. The removal of the Boc group with HCl gas and phenylacetylation of the respective salts **21** with phenyl acetic anhydride and triethylamine in CH_2Cl_2 afforded respectively phenylacetamides **22a** and **22b** in 86% and 78% yields. The hydrogenolytic removal of the benzyl ester of **22** with palladium-on-carbon as the catalyst in 9 : 1 EtOH : AcOH furnished respectively in 90% and 85% yields acids **23a** and **23b**, which were coupled to pyridinylalaninyl-β-homophenylalanine benzyl ester **12** using TBTU, HOBt, and DIEA to give respectively tetrapeptides **24a** and **24b** in 72% and 81% yields. Finally, hydrogenation using the conditions described above removed the benzyl ester to provide respectively acids **3** and **4** in >95% purity after purification by preparative reverse-phase HPLC (Scheme 2).

Biological activity

Biological effects of azapeptide mimics

*Effect of indolizidinone **5**, azapeptide **6** and analogs **1–4** on myometrial contraction.* As previously reported,[14–16] indolizidinone **5** and azapeptide **6** reduced significantly in a dose-dependent manner the strength and duration of both $PGF_{2\alpha}$-induced and spontaneous contractions of the myometrium obtained from spontaneous post-partum mice. The influences of mimics **1–4** on $PGF_{2\alpha}$-induced myometrial contractions were thus examined to evaluate their inhibitory potential.

In contrast to the activity of indolizidin-2-one **5** and azapeptide **6**, the related mimics **1–4** exhibited no or significantly reduced effects on $PGF_{2\alpha}$-induced myometrial contractions (Fig. 2). The type II′ mimics **3** and **4** exhibited better activity than the type I mimic counterparts **1** and **2**. Among the four analogs, D-Ala-Pro peptide **4** possessed the best activity and at 10 μM exhibited about 60% of the inhibitory activity shown by indolizidin-2-one **5** and aza-peptide **6** in the myometrial contraction assay.



Scheme 2 Synthesis of Gly-Pro and D-Ala-Pro analogs **3** and **4**.

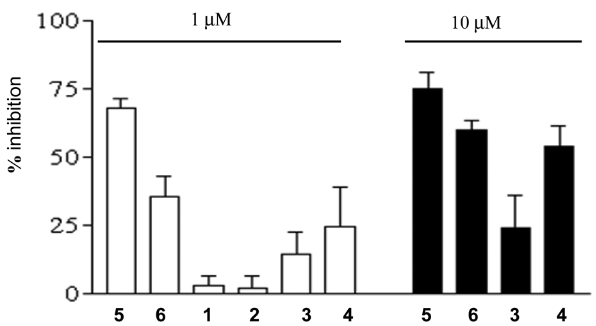


Fig. 2 Effects of indolizidinone **5**, azapeptide **6** and analogs **1–4** on mean tension induced by $PGF_{2\alpha}$. At the beginning of each experiment, mean tension of spontaneous myometrial contractions was considered as the basal response.

Effect of aza-peptides on $PGF_{2\alpha}$-stimulated signaling pathways. Binding of $PGF_{2\alpha}$ on HEK 293 cells stably expressing FP (FP cells) and $PGF_{2\alpha}$-dependent signaling have been shown to be influenced by indolizidinone **5** and azapeptide **6** in ways that facilitated Gαq-mediated signaling *via* PKC/$ERK_{1/2}$ and inhibited signaling by way of the $G_{\alpha12}$-mediated RhoA/ROCK pathway.[14–16] The latter impaired actin reorganization and cell membrane ruffling.[14–16] To gain insight into the importance of modulator conformation on these downstream effects, analogs **1–4** were examined for their potential to regulate both these signaling pathways.

The effects of analogs **1–4** on $PGF_{2\alpha}$-mediated $ERK_{1/2}$ activation was assessed in HEK 293 cells expressing HA-tagged FP. Among the four analogs, compared to untreated conditions (vehicle, DMSO), only Gly-Pro derivative **3** exhibited no effect on $ERK_{1/2}$ activation (not shown), but increased $PGF_{2\alpha}$-mediated phosphorylation of $ERK_{1/2}$ (Fig. 3). The latter effect was more obvious and significant at higher $PGF_{2\alpha}$ concentrations. The increase in efficacy of $PGF_{2\alpha}$-mediated $ERK_{1/2}$ activation was comparable to that produced by azaGly-Pro analog **6**, which was previously shown to potentiate MAPK activity.[15] On the other hand, type I mimics **1** and **2**, and type II′ mimic **4**, all did not show any significant effect on $PGF_{2\alpha}$-mediated activation of $ERK_{1/2}$ relative to agonist-stimulated cells treated with DMSO (Fig. 3).

Stimulation with $PGF_{2\alpha}$ (1 μM, 15 min) caused membrane ruffle formation in ~84% of FP cells (Fig. 4). Azapeptide analogs **1–3** did not affect membrane ruffling; although the effects of analog **4** were not statistically significant, this compound exerted a tendency in reducing $PGF_{2\alpha}$-elicited membrane ruffling, consistent with its efficacy in diminishing $PGF_{2\alpha}$-induced myometrial contraction (Fig. 4).

Conformational analysis of D-alaninyl proline mimic 4 using NMR spectroscopy. Considering that D-alaninyl proline mimic **4** exhibited the best activity in the myometrial contraction assay among the four analogs, further examination of its conformation in solution was performed using NMR spectroscopy. Although conformation in the environment of the membrane bound receptor may vary from that in solution, 5% DMSO-d_6 in chloroform was chosen as the solvent to favour a folded geometry and to facilitate spectroscopic experiments.

Initially, the proton signals of **4** were assigned using two-dimensional COSY and HSQC experiments in 5% DMSO-d_6 in $CDCl_3$. The prolyl major *trans*-isomer (>97%) was then confirmed by observing its characteristic through-space transfer of magnetization between the proline residue δ-protons at 3.33 ppm and both the α-proton (4.30 ppm) and methyl protons (1.26 ppm) of the D-alaninyl residue in the ROESY spectrum in 5% DMSO-d_6 in $CDCl_3$ (Fig. 5, ESI†). The environment of the amide protons of mimic **4** was next evaluated by NMR experiments in which variations of their chemical shift

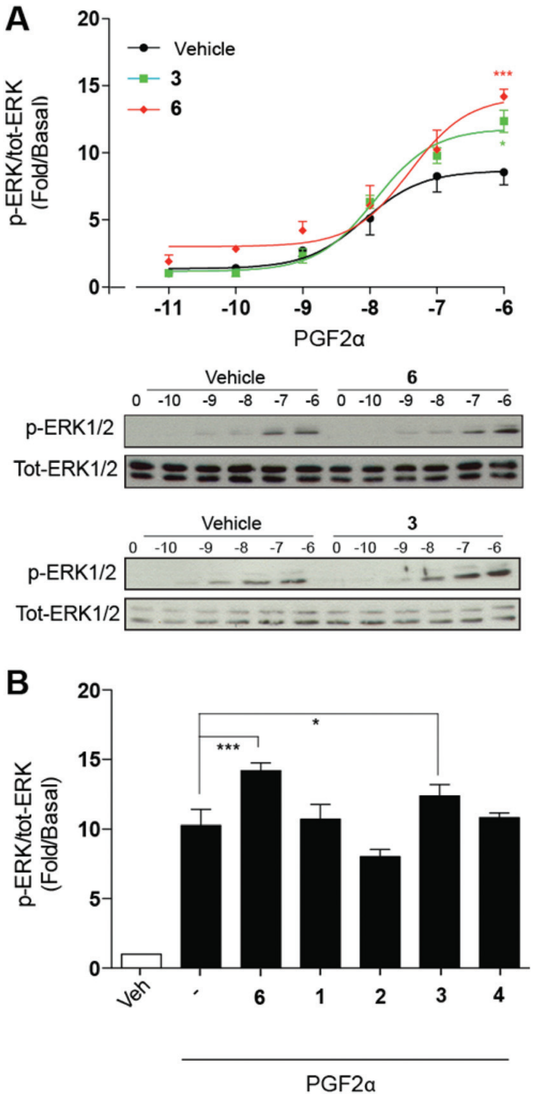


Fig. 3 Effect of compounds **1–4** on $PGF_{2\alpha}$-mediated ERK1/2 activation. (A) HEK 293 cells expressing HA-FP untreated (Vehicle), treated with azapeptide **6** (2 μM, 30 min) or **3** (2 μM, 10 min), then challenged with increased concentrations of $PGF_{2\alpha}$ (2 min). p-ERK signals were quantified by densitometry, normalized to that of t-ERK and plotted in dose–response curves as fold over basal (non-stimulated conditions) (top panel). Representative immuno-blots of ERK activation treated with azapeptide **6** and **3** are shown. Lysates were immunoblotted for phosphorylated ERK (p-ERK) and total ERK (t-ERK) (bottom panels). (B) Densitometry analysis of data from HEK 293 cells either left unstimulated (Vehicle) or challenged with $PGF_{2\alpha}$ (1 μM, 2 min) in the presence of either DMSO (–), azapeptide **6**, and analogs **1–4** (2μM, 30 min). Data are presented as mean ± SEM and results are representative of at least 3 independent experiments. *, $p < 0.05$; ***, $p < 0.001$.

values were measured as a function of the percent of DMSO-d_6 (5 to 100%) added to the $CDCl_3$ solution, as well as a function of temperature (Fig. 6 and 7). Notably, relative to the signals of the other amide protons, the pyridinylalaninyl NH signal exhibited little variation ($\Delta\delta$ = 0.28 ppm) in chemical shift indicative of a solvent shielded proton engaged in a hydrogen

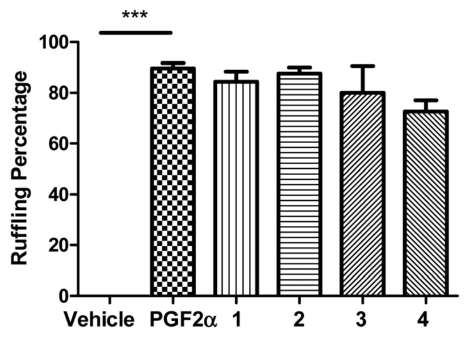


Fig. 4 Effects of analogs **1–4** on cell ruffling. FP expressing cells seeded onto cover-slips were pretreated with analogs **1–4** (1 μM, 30 min) and then stimulated with $PGF_{2\alpha}$ (1 μM, 15 min) as previously described.[14] Cells were then fixed with paraformaldehyde (4%), stained with Phalloidin-Alexa Fluor 488, and mounted onto cover-slips using GelTol media. The numbers of cells exhibiting circular ruffling for each condition were counted. Results are the mean ± SEM of 3 independent experiments where more than 100 cells were counted. ***$P < 0.001$ are values compared to the control unstimulated conditions.

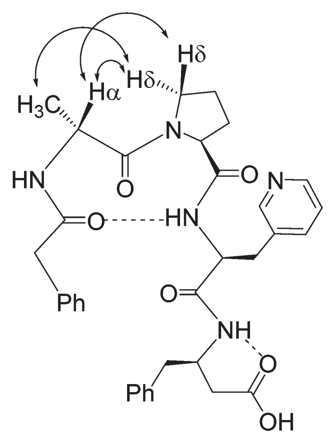


Fig. 5 ROESY correlations for D-alaninyl proline mimic **4**.

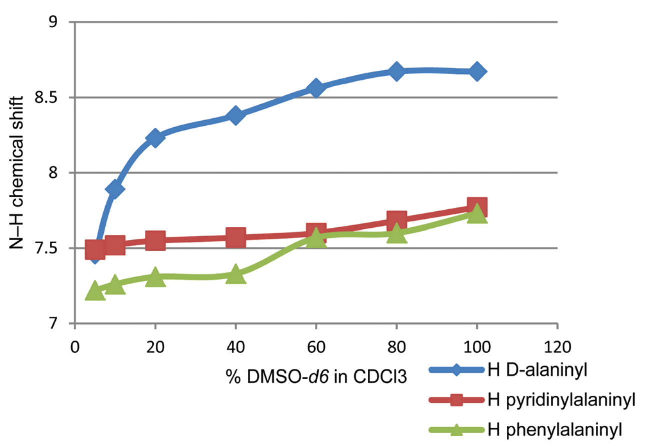


Fig. 6 Variation of amide N–H chemical shift values *versus* the percentage of DMSO-d_6 in DMSO-d_6/$CDCl_3$.

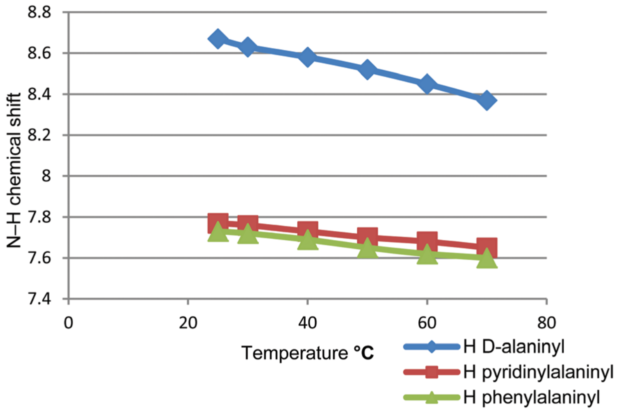


Fig. 7 Variation of amide N–H chemical shift values *versus* temperature °C.

bond. The D-alaninyl NH signal ($\Delta\delta$ = 1.21 ppm) was solvent exposed, and the β-homophenylalaninyl NH signal ($\Delta\delta$ = 0.53 ppm) exhibited an intermediate value. The chemical displacements of the amide protons as a function of increasing temperature from 25 to 70 °C in DMSO-d_6 exhibited a similar pattern. The pyridinylalaninyl NH ($\Delta\delta$ = 0.12 ppm) and D-alaninyl NH ($\Delta\delta$ = 0.30 ppm) signals were respectively the least and most influenced by the change in temperature, indicative of solvent shielded and exposed protons. The chemical shift of the β-homophenylalaninyl NH signal ($\Delta\delta$ = 0.13 ppm) was also solvent shielded, which may be due in part to hydrogen bonding in a six-membered ring with the side chain carboxylate (Fig. 5). In sum, the NMR experiments confirmed that a turn conformation is favoured for D-alaninyl proline mimic **4**.

Conclusions

To study the influence of the β-turn in receptor recognition of FP modulators **5** and **6**, their indolizidin-2-one and aza-glycinyl-proline moieties were replaced with residues previously reported to adopt type I and II′ β-turn conformations. Four analogs were successfully synthesized in high purity and good yields for biological testing. Transannular cyclization on peptide **13** possessing macrocyclic lactam was employed to synthesize azabicyclo[5.3.0]alkan-2-one for the preparation of peptide **2**. Although analogs **1–4** did not exhibit similar potency as the parent indolizidin-2-one and aza-glycinyl-proline derivatives **5** and **6**, type II′ β-turn mimic **4** exhibited statistically significant inhibitory effects on myometrial contraction at 10 μM, and diminished associated RhoA/ROCK-dependent cell ruffling. Moreover, type II′ β-turn mimic **3** was found to potentiate $PGF_{2\alpha}$-mediated $ERK_{1/2}$ activation with similar efficacy as azaGly-Pro analog **6**. Examination of the conformation of **4** by NMR spectroscopy demonstrated solvent shielded and exposed amide NH protons consistent with β-turn geometry. In the light of the activity of mimics **3** and **4**, the biologically active β-turn conformation for indolizidin-2-one and aza-glycinyl-proline derivatives **5** and **6** appears to be similar to a type II′ β-turn; however, a subtle deviation from this geometry appears to be significant for potency in modulating $PGF_{2\alpha}$-induced myometrial contraction, as well as $PGF_{2\alpha}$-mediated $ERK_{1/2}$ activation and associated RhoA/ROCK-dependent cell ruffling.

Experimental

General protocols

Unless otherwise stated, all reactions were run under an argon atmosphere, using distilled solvents, which were transferred using a syringe. Anhydrous solvents (CH_2Cl_2, CH_3OH) were obtained by passage through solvent filtration systems (Glass Contour, Irvine, CA). The final reaction mixture solutions were dried over $MgSO_4$. The reaction progress was monitored by analytical thin-layer chromatography (TLC), using glass-backed plates covered with a 0.25 mm thickness of silica gel. Visualization was accomplished using potassium permanganate reagent, iodine vapours, UV illumination (254 nm), Dragendroff's reagent or ceric ammonium molybdate stain. Flash column chromatography was carried out on 230–400 mesh silica gel.[31] The HPLC analyses of product purity were performed on a reverse phase GunFire™ C18 column 3.5 μm (21 × 50 mm column) using a flow rate of 0.350 mL min^{-1} and a binary solvent system consisting of solvent A, H_2O (0.1% FA) mixed with either solvent B acetonitrile (0.1% FA) or solvent C methanol (0.1% FA); the systems for the gradient of elution were as follows: system 1, 10–50% A in B over 12 min; system 2, 20–70% A in B over 12 min; system 3, 40–90% A in B over 12 min; system 4, 30–70% A in C over 10 min; system 5, 20–90% A in C over 12 min; system 6, 40–90% A in C over 10 min. Low and high resolution mass spectrometric data (ES and FAB) were obtained by the Centre Régional de Spectrométrie de Masse de l'Université de Montréal. ^{1}H NMR (400/500/700 MHz) and ^{13}C NMR (100/125/175 MHz) spectra were recorded in $CDCl_3$ (δ 7.27 and 77), MeOH (δ 3.31 and 49.15) or DMSO (2.50 and 39.52). Chemicals shifts are reported in parts per million; coupling constant J values are reported in hertz. The proton and carbon NMR signals of minor prolyl amide isomers are presented respectively in brackets and parentheses. Specific rotations $[\alpha]_D$ were measured at 20 °C at the specified concentrations (c in g per 100 mL) using a 1 dm cell length on a Perkin-Elmer polarimeter 341 and the general formula: $[\alpha]_D^{20} = (100\alpha)/(dc)$. The HA-tagged prostaglandin $F_{2\alpha}$ receptor construct was made as previously described.[14] MEM, heat-inactivated fetal bovine serum (FBS), L-glutamine and gentamicin were from Invitrogen (Carlsbad, CA). $PGF_{2\alpha}$ was purchased from Cayman. Mouse monoclonal anti-phospho-$ERK_{1/2}$ (T202/Y204) and rabbit polyclonal anti-total $ERK_{1/2}$ were from Cell Signaling Technology (Danvers, MA). Anti-mouse and anti-rabbit HRP-conjugated IgG were from Sigma-Aldrich (St-Louis, MO) and the chemiluminescence lightening (ECL) was from Perkin-Elmer.

***N*-(Boc)amino-(*E*,3*S*,10*S*)-2-oxo-1,2,3,4,5,8,9,10-octa-hydro-1*H*-azocine-10-carbonyl-(2*S*)-(3-pyridyl)alaninyl-(3*S*)-β-homophenylalanine benzyl ester (13).** A solution of (*E*,2*S*,9*S*)-9-*N*-(Boc)amino-10-oxo-1,2,3,4,7,8,9,10-octahydroazocine-2-carboxylic acid (**11**, prepared according to ref. 26, 195 mg, 0.63 mmol) in 13 mL of CH_2Cl_2 was treated with hydroxybenzotriazole (HOBt, 92 mg, 0.69 mmol) and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU, 221 mg, 0.69 mmol), stirred for 15 min, treated with (2*S*)-(3-pyridyl)alaninyl-(3*S*)-β-homophenylalaninyl benzyl ester hydrochloride (**12**, 286 mg, 0.69 mmol, prepared according to ref. 15) followed by drop-wise addition of DIEA (355 μl, 2.04 mmol), and stirred at room temperature overnight. Evaporation of the volatiles gave a residue, which was purified by flash chromatography on silica gel using 5% isopropanol in $CHCl_3$ as the eluent to furnish the benzyl ester **13** (330 mg, 74% yield) as white powder: R_f = 0.5 (5% isopropanol in $CHCl_3$); mp 170 °C, $[\alpha]_D^{20}$ 8.18 (*c* 1.1, MeOH) ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 1.49 (s, 9H), 1.80–184 (dd, *J* = 5, 14.5 Hz, 1H), 2.02–2.15 (m, 3H), 1.23–1.26 (m, 1H), 2.35–2.46 (m, 4H), 2.73–2.77 (m, 1H), 2.82–2.87 (m, 2H), 3.03–3.08 (m, 1H), 4.31 (s, 1H), 4.41–4.48 (m, 1H), 4.57–4.61 (m, 2H), 5.06–5.08 (d, *J* = 12.5 Hz, 1H), 5.12–5.15 (d, *J* = 12.5 Hz, 1H), 5.22 (s, 1H), 5.36–5.42 (m, 1H), 5.48–5.51 (m, 1H), 6.62 (s, 1H), 6.84–6.86 (d, *J* = 8 Hz, 1H), 6.96 (s, 1H), 7.06–7.08 (d, *J* = 7.5 Hz, 2H), 7.15–7.25 (m, 4H), 7.32–7.39 (m, 5H), 7.46–7.49 (dt, *J* = 2, 7.5 Hz, 1H), 8.35 (d, *J* = 2 Hz, 1H), 8.41–8.42 (dd, *J* = 1.5, 4.5 Hz, 1H), ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 28.3, 29.8, 30.3, 31.8, 35.3, 37.0, 39.7, 47.5, 54.1, 54.23, 54.26, 54.6, 66.4, 81.0, 123.4, 126.6, 128.4, 128.5, 128.6, 129.2, 132.1, 132.2, 135.6, 136.8, 137.2, 148.3, 150.4, 155.5, 169.1, 171.1, 171.5, 173.7. HRMS *m*/*z* calcd for $C_{40}H_{50}N_5O_7$ $[M + H]^+$ 712.3704, found 712.3732.

Phenylacetyl-(*E*,3*S*,10*S*)-2-oxo-1,2,3,4,5,8,9,10-octa-hydro-1*H*-azocine-10-carbonyl-(2*S*)-(3-pyridyl)alaninyl-(3*S*)-β-homophenylalanine benzyl ester (14). Carbamate **13** (100 mg, 0.14 mmol) was dissolved in CH_2Cl_2 (4 mL), cooled to 0 °C, and treated with HCl gas bubbles for 2 h, when TLC showed complete disappearance of the starting material. The volatiles were removed by evaporation and the residue was thrice dissolved in CH_2Cl_2 (5 mL) and evaporated to give the HCl salt. Triethylamine (336 μl, 2.4 mmol) was added to a stirred solution of the resulting hydrochloride salt (160 mg, 0.24 mmol) and phenylacetic anhydride (73 mg, 0.28 mmol) in 5 mL of DCM at room temperature, and the mixture was stirred overnight. Evaporation of the volatiles provided a residue, which was purified by flash chromatography on silica gel using 10% isopropanol in chloroform as the eluent. Evaporation of the collected fractions afforded phenylacetamide **14** (123 mg, 70% yield): R_f = 0.48 (10% isopropanol in chloroform); mp 210 °C; $[\alpha]_D^{20}$ −5 (*c* 1.5, CH_2Cl_2); ^{1}H NMR (500 MHz, DMSO) *δ* 1.51–1.61 (m, 2H), 1.75–1.77 (m, 1H), 1.91–1.97 (m, 2H), 2.07–2.40 (m, 1H), 2.44–2.51 (m, 2H), 2.35–2.40 (m, 1H), 2.44–2.49 (m, 1H), 2.68–2.86 (m, 4H), 3.61–3.64 (d, *J* = 14 Hz, 1H), 3.69–3.72 (d, *J* = 14 Hz, 1H), 4.26–4.30 (m, 2H), 4.36–4.47 (m, 2H), 5.02–5.08 (q, *J* = 12.5, 19.75 Hz, 2H), 5.14–5.19 (m, 1H), 5.59–5.64 (m, 1H), 7.16–7.35 (m, 17H), 7.54–7.56 (d, *J* = 6.5 Hz, 1H), 7.94–7.96 (d, *J* = 9.5 Hz, 1H), 8.09–8.10 (d, *J* = 6.5 Hz, 1H), 8.19–8.21 (d, *J* = 7 Hz, 1H), 8.38 (s, 2H); ^{13}C NMR (125 MHz, DMSO) *δ* 28.0, 30.4, 30.7, 31.9, 35.4, 38.4, 39.7, 40.3, 42.5, 47.8, 52.4, 54.0, 66.1, 123.6, 126.7, 126.8, 128.0, 128.4, 128.5, 128.6, 128.7, 128.8, 129.5, 129.7, 132.6, 133.5, 136.4, 136.7, 136.9, 138.4, 148.0, 150.6, 170.3, 170.9, 171.1, 171.6, 172.0. HRMS *m*/*z* calcd for $C_{43}H_{48}N_5O_6$ $[M + H]^+$ 730.3599, found 730.3616.

Phenylacetyl-(*E*,3*S*,10*S*)-2-oxo-1,2,3,4,5,8,9,10-octa-hydro-1*H*-azocine-10-carbonyl-(2*S*)-(3-pyridinyl)alaninyl-(3*S*)-β-homophenylalanine (1). Benzyl ester **14** (20 mg, 0.027 mmol) was dissolved in 1 mL of dioxane, cooled to 0 °C, and treated with 2 N LiOH (1 mL). The ice bath was removed and the mixture was stirred at room temperature for 1 h, when TLC showed complete disappearance of the starting material. The volatiles were evaporated under reduce pressure. The resulting aqueous volume was acidified to pH 3 using 1 N HCl and extracted 3 times with ethyl acetate (5 mL). The organic layers were combined, washed with brine, dried, filtered, and evaporated. The residue was purified by preparative HPLC on a C18 reverse-phase column. Freeze-drying of the collected fractions gave acid **1** (6 mg, 35%) as white powder; ^{1}H NMR (700 MHz, CD_3OD) *δ* 1.54–1.59 (m, 1H), 1.74–1.76 (m, 1H), 1.91–1.93 (m, 1H), 2.05–2.11 (m, 1H), 2.14–2.15 (m, 1H), 2.32–2.35 (m, 4H), 2.41–2.44 (m, 1H), 2.83–2.87 (m, 3H), 3.01–3.04 (m, 1H), 3.74–3.76 (d, *J* = 14.7 Hz, 1H), 3.78–3.81 (d, *J* = 14.7 Hz, 1H), 4.35 (s, 1H), 4.38–4.41 (m, 1H), 4.45–4.47 (m, 1H), 4.51–4.53 (d, *J* = 11.9 Hz, 1H), 5.31–5.34 (m, 1H), 5.46–5.5 (m, 1H), 7.20–7.38 (m, 11H), 7.64–7.65 (m, 1H), 8.37–8.38 (m, 2H); ^{13}C NMR (175 MHz, CD_3OD) *δ* 27.6, 29.1, 29.3, 31.4, 34.6, 37.3, 38.9, 39.5, 41.9, 53.1, 54.1, 54.3, 123.8, 126.1, 126.6, 127.7, 128.0, 128.3, 128.9, 129.1, 132.3, 133.5, 135.5, 137.8, 137.9, 146.8, 149.2, 170.4, 172.1, 173.1, 173.57, 173.59. HRMS *m*/*z* calcd for $C_{36}H_{41}N_5O_6$ $[M + H]^+$ 640.3129, found 640.3141.

(3*S*,6*R*,7*S*,10*S*)-2-Oxo-3-*N*-(Boc)amino-6-iodo-1-azabicyclo-[5.3.0]decane-10-carbonyl-(2*S*)-(3-pyridyl)alaninyl-(3*S*)-β-homophenylalanine benzyl ester (15). Octahydroazocine **13** (150 mg, 0.21 mmol) was dissolved in 2 mL of CH_3CN, treated with $NaHCO_3$ (84 mg, 0.63 mmol) followed by three portions of iodine (total 253 mg, 0.63 mmol) at a rate of one portion every 2 min, stirred for 1 h at room temperature, and treated with 1 M $Na_2S_2O_3$ until the solution became clear. The mixture was concentrated and the aqueous volume was extracted three times with DCM (5 mL). The combined organic phase was washed with brine, dried, filtered, and concentrated under reduced pressure. Purification of the residue by flash chromatography (5% isopropanol in $CHCl_3$) gave iodide **15** (150 mg, 94% yield) as yellow powder: R_f = 0.49 (5% isopropanol in $CHCl_3$); mp 110 °C; $[\alpha]_D^{20}$ −55 (*c* 1.0, MeOH); ^{1}H NMR (700 MHz, $CDCl_3$) *δ* 1.50 (s, 9H), 1.74–1.79 (m, 2H), 1.89–1.93 (m, 1H), 1.97–2.01 (m, 2H), 2.18–2.20 (m, 1H), 2.35–2.38 (m, 1H), 2.54–2.61 (m, 3H), 2.79–2.83 (m, 1H), 2.86–2.89 (m, 1H), 2.96–2.99 (m, 1H), 3.55–3.58 (dd, *J* = 3.5, 14.3 Hz, 1H), 4.06–4.08 (m, 1H), 4.09–4.12 (m, 1H), 4.49–4.50 (d, *J* = 9.8 Hz, 1H), 4.51–4.55 (m, 1H), 4.60–4.62 (m, 1H), 4.77–4.80 (m, 1H), 5.04–5.05 (d, *J* = 11.9 Hz, 1H), 5.08–5.09 (d, *J* = 11.9 Hz, 1H), 5.13 (s. 1H), 7.20–7.27 (m, 6H), 7.30–7.36 (m, 5H), 7.41–7.42

(d, J = 9.8 Hz, 1H), 7.59–7.61 (d, J = 8.4 Hz, 1H), 8.42–8.47 (d, J = 4.5 Hz, 2H), ^{13}C NMR (175 MHz, $CDCl_3$) δ 25.9, 27.9, 28.5, 32.8, 33.7, 34.0, 34.9, 37.8, 40.2, 48.2, 53.9, 56.7, 62.08, 63.96, 66.28, 81.6, 123.5, 126.4, 128.0, 128.30, 128.39, 128.4, 129.5, 134.0, 136.0, 136.2, 138.1, 147.9, 150.1, 156.3, 169.7, 170.4, 170.8, 171.2. HRMS m/z calcd for $C_{40}H_{49}IN_5O_7$ $[M + H]^+$ 838.2671, found 838.2669.

Phenylacetyl-(3*S*,6*R*,7*S*,10*S*)-2-oxo-6-iodo-1-azabicyclo[5.3.0]-decane-10-carbonyl-(2*S*)-(3-pyridyl)alaninyl-(3*S*)-β-homophenyl-alanine benzyl ester (16). As described for the synthesis of **14** above, the Boc group was removed using HCl gas from carbamate **15** (100 mg, 0.12 mmol) in 4 mL of DCM at 0 °C for 2 h to give the HCl salt. The salt (160 mg, 0.21 mmol) was treated with phenylacetic anhydride (66 mg, 0.26 mmol) and triethylamine (294 µl, 2.1 mmol) in 5 mL of dichloromethane to give a residue, which was purified by flash chromatography on silica gel using 8% isopropanol in chloroform to give amide **16** (120 mg, 73%) as yellow powder: R_f = 0.47 (10% isopropanol in chloroform); ^{1}H NMR (700 MHz, CD_3OD) δ 1.48–1.55 (m, 2H), 1.96–1.98 (m, 2H), 2.02–2.12 (m, 2H), 2.39–2.42 (m, 1H), 2.55–2.57 (m, 1H), 2.58–2.59 (m, 1H), 2.61–2.69 (m, 2H), 2.83–2.86 (dd, J = 7, 13.3 Hz, 1H), 2.87–2.90 (dd, J = 7, 13.3 Hz, 1H), 3.01–3.03 (dd, J = 3.5, 14.7 Hz, 1H), 3.58–3.60 (d, J = 14.7 Hz, 1H), 3.73–3.76 (d, J = 14.7 Hz, 1H), 4.14–4.16 (t, J = 6.3 Hz, 1H), 4.33–4.38 (m, 4H), 4.49–4.53 (q, J = 7 Hz, 1H), 5.06–5.07 (d, J = 12.6 Hz, 1H), 5.09–5.11 (d, J = 12.6 Hz, 1H), 7.21–7.23 (m, 2H), 7.25–7.31 (m, 9H), 7.34–7.37 (m, 4H), 7.54–7.56 (d, J = 7.7 Hz, 1H), 8.24 (s, 1H), 8.37–8.38 (d, J = 4.9 Hz, 1H), 8.57 (s, 1H); ^{13}C NMR (175 MHz, CD_3OD) δ 25.2, 26.9, 32.9, 33.0, 33.3, 34.8, 38.1, 39.9, 42.0, 48.1, 55.1, 56.6, 62.0, 63.7, 66.0, 123.7, 126.1, 126.8, 127.7, 128.00, 128.06, 128.09, 128.5, 129.12, 129.13, 134.7, 135.1, 136.6, 137.2, 137.8, 146.8, 146.2, 170.8, 170.9, 172.23, 172.24, 173.0. HRMS m/z calcd for $C_{43}H_{47}IN_5O_6$ $[M + H]^+$ 856.2565, found 856.2573.

Phenylacetyl-(3*S*,6*R*,7*S*,10*S*)-2-oxo-6-iodo-1-azabicyclo[5.3.0]-decane-10-carboxylate-carboxylate-(2*S*)-(3-pyridyl)alaninyl-(3*S*)-β-homophenylalanine (2). As described above for the synthesis of acid **1**, benzyl ester **16** (40 mg, 0.051 mmol) was hydrolyzed with aqueous LiOH in dioxane, and the residue was purified by preparative HPLC on a C18 reverse-phase column to give acid **2** (12 mg, 30%) as a white powder. ^{1}H NMR (700 MHz, CD_3OD) δ 1.50–1.56 (m, 2H), 1.96–1.99 (m, 2H), 2.03–2.13 (m, 2H), 2.41–2.44 (m, 1H), 2.46–2.50 (dd, J = 7, 15.75 Hz, 1H), 2.53–2.57 (dd, J = 7, 15.75 Hz, 1H), 2.58–2.64 (m, 2H), 2.84–2.88 (dd, J = 7.7, 14.35 Hz, 1H), 2.92–2.94 (dd, J = 5.6, 14 Hz, 1H), 3.01–3.04 (dd, J = 4.2, 14.7 Hz, 1H), 3.72–3.74 (d, J = 14.7 Hz, 1H), 4.18–4.19 (t, J = 6.3 Hz, 1H), 4.31–4.34 (m, 2H), 4.35–4.37 (dd, J = 4.2, 11.9 Hz, 1H), 4.42–4.43 (m, 2H), 4.48–4.52 (q, J = 7 Hz, 1H), 7.19–7.23 (m, 2H), 7.26–7.31 (m, 8H), 7.53–7.54 (d, J = 7.7 Hz, 1H), 8.23 (s, 1H), 8.36–7.37 (d, J = 4.9 Hz, 1H), 8.49 (s, 1H); ^{13}C NMR (175 MHz, CD_3OD) δ 25.2, 27.0, 33.0, 33.1, 33.4, 35.0, 38.8, 39.7, 42.0, 48.2, 55.1, 56.6, 62.0, 63.6, 123.6, 126.0, 126.8, 127.9, 128.5, 129.11, 129.13, 134.7, 135.1, 137.3, 138.3, 146.7, 149.2, 167.9, 170.8, 172.1, 172.3, 173.0. HRMS m/z calcd for $C_{36}H_{41}IN_5O_6$ $[M + H]^+$ 766.2096, found 766.2086.

***N*-(Boc)glycinyl-(2*S*)-proline benzyl ester (20a).** A solution of proline benzyl ester (500 mg, 2.07 mmol) in dichloromethane (40 mL) was treated with HOBt (334 mg, 2.48 mmol) and TBTU (700 mg, 2.48 mmol), stirred for 15 min, treated with *N*-(Boc)glycine (**18**, 434 mg, 2.48 mmol), followed by drop-wise addition of DIEA (722 µL, 4.14 mmol), and stirred at room temperature overnight. Evaporation of the volatiles gave a residue, which was purified by flash chromatography on silica gel using 1 : 1 hexanes/EtOAc as the eluent. Evaporation of the collected fractions gave dipeptide **20a** (763 mg, 98% yield) as clear oil: R_f = 0.48 (1 : 1 hexane/EtOAc); $[\alpha]_D^{20}$ −80 (c 1.0, MeOH); ^{1}H NMR (500 MHz, CD_3OD) showed a 2.75 : 1 mixture of prolyl amide isomers δ 1.44 (s, 9H), [1.86 (m, 4H)], 1.96–2.20 (m, 4H), 3.43–3.47 (m, 1H), 3.55–3.59 (m, 1H), [3.63 (m, 1H)], 3.88–4.01 (m, 2H), [4.42 (m, 1H)], 5.12–5.14 (d, J = 12.5 Hz, 1H), 5.16–5.18 (d, J = 12.5 Hz, 2H), 5.45 (s, 1H); 7.31–7.37 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (22.1), 24.6, 28.3, 28.9, (31.3), (42.8), 43.0, 45.8, (46.6), (58.6), 58.9, 66.9, (67.5), 79.5, 128.0, 128.3, 128.5, (128.7), (135.0), 135.7, (155.7), 155.8, 167.4, (167.6), (171.3), 171.6; HRMS m/z calcd for $C_{19}H_{27}N_2O_5$ $[M + H]^+$ 363.1914, found 362.1910.

(2*R*)-*N*-(Boc)alaninyl-(2*S*)-proline benzyl ester (20b). Employing the protocol described for the synthesis of dipeptide **20a**, alaninyl-proline **20b** was prepared from treating proline benzyl ester (500 mg, 2.07 mmol) in dichloromethane (40 mL) with HOBt (334 mg, 2.48 mmol), TBTU (700 mg, 2.48 mmol), *N*-Boc-D-alanine (18, 469 mg, 2.48 mmol), and DIEA (722 µL, 4.14 mmol), and was purified by flash chromatography on silica gel using 40% hexane in EtOAc as the eluent. Evaporation of the collected fractions gave dipeptide **20b** (670 mg, 92% yield) as a yellow oil: R_f = 0.49 (1 : 1 hexane/EtOAc); $[\alpha]_D^{20}$ −25 (c 1.0, MeOH) ^{1}H NMR (500 MHz, CD_3OD) showed a 5 : 1 mixture of prolyl amide isomers, δ [1.16 (d, J = 7 Hz, 3H)], 1.35–1.33 (d, J = 7 Hz, 3H), [1.43 (s, 9H)], 1.46 (s, 9H), 1.98–2.04 (m, 2H), 2.06–2.12 (m, 1H), 2.18–2.24 (m, 1H), 3.50–3.55 (m, 1H), [3.61 (m, 2H)], 3.79–3.84 (m, 1H), [4.30 (m, 3H)], 4.50–4.55 (m, 2H), [4.98 (d, J = 8.5 Hz, 1H)], 5.14–5.16 (d, J = 12.5 Hz, 1H), 5.21–5.23 (d, J = 12.5 Hz, 1H), [5.28 (d, J = 8.5 Hz, 1H)], 5.52–5.54 (d, J = 7 Hz, 1H), 7.33–7.38 (m, 5H); ^{13}C NMR (125 MHz, $CDCl_3$) δ (18.12), 18.73, (22.4), 24.6, (28.33), 28.38, 29.0, (46.6), 46.9, (47.6), 47.9, 59.2, (59.4), 66.8, (67.3), 79.5, 128.0, 128.2, (128.4), 128.5, (128.7), 135.6, 155.0, (155.5), 171.63, 171.69; HRMS m/z calcd for $C_{20}H_{29}N_2O_5$ $[M + H]^+$ 377.2071, found 377.2070.

Phenylacetyl-glycinyl-(2*S*)-proline benzyl ester (22a). As described for the synthesis of **14** above, HCl gas was bubbled through a solution of carbamate **20a** (1 g, 2.76 mmol) in 50 mL of DCM to give a salt. The hydrochloride (796 mg, 2.76 mmol) was treated with phenylacetic anhydride (1.4 g, 5.52 mmol) and triethylamine (3.871 mL, 27.6 mmol), and the residue obtained after evaporation was purified by flash chromatography on silica gel using 7% MeOH in EtOAc to afford phenylacetamide **22a** (866 mg, 86%): R_f = 0.5 (10% MeOH in EtOAc); mp 120 °C; $[\alpha]_D^{20}$ −71 (c 1.0, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) showed a 5.12 : 1 mixture of prolyl amide isomers δ 1.77 (s, 1H), [1.90 (m, 4H)], 1.98–2.04 (m, 2H), 2.17–2.24 (m, 1H), 3.45–3.49 (m, 1H), 3.57–3.64 (m, 3H), 3.96–4.00 (dd, J = 4.18, 18 Hz, 1H), 4.10–4.15 (dd, J = 4.18, 18 Hz, 1H), [4.44 (m, 1H)], 4.55–4.58 (m, 1H), 5.12–5.14 (d, J = 12.5 Hz, 1H), 5.19–5.21 (d, J = 12.5 Hz, 1H), 6.51 (s, 1H), 7.28–7.38 (m, 10H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (22.1), 24.5, 28.9, (31.3), (41.9), 42.1, 43.5, 45.9, (46.6), (58.6), 58.9, 67.0, (67.5), 127.3, 128.1, 128.3, 128.5, (128.6), (128.7), 128.9, 129.4, 134.5, (134.9), 135.4, 166.9, (167.1), (170.9), 171.0, (171.1), 171.5. HRMS m/z calcd for $C_{22}H_{25}N_2O_4$ $[M + H]^+$ 381.1808, found 381.1802.

Phenylacetyl-(2*R*)-alaninyl-(2*S*)-proline benzyl ester (22b). As described above for carbamate **20b** (1 g, 2.65 mmol), the Boc group was removed with HCl to give white powder. The hydrochloride salt (858 mg, 2.65 mmol) was treated with phenylacetic anhydride (1.35 g, 5.31 mmol) and triethylamine (3.72 mL, 26.5 mmol), and the residue after evaporation was purified by flash chromatography on silica gel using 7% MeOH in EtOAc to afford ester **22b** (916 mg, 78% yield) as white powder: R_f = 0.45 (10% MeOH in EtOAc); mp 140 °C; $[\alpha]_D^{20}$ −3.8 (c 1.0, MeOH); ^{1}H NMR (500 MHz, $CDCl_3$) showed a 3 : 1 mixture of prolyl amide isomers δ [1.17 (m, 3H)], 1.29–1.33 (m, 3H), [1.84 (m, 2H)], 1.95–2.03 (m, 2H), 2.14–2.25 (m, 2H), 3.50–3.53 (m, 3H), 3.74–3.77 (m, 1H), 4.49–4.50 (m, 1H), 4.80–4.84 (m, 1H), 5.08–5.09 (m, 2H), 6.69–6.73 (m, 1H), [6.88 (m, 1H)], [7.27 (m, 10H)], 7.28–7.35 (m, 10H). ^{13}C NMR (125 MHz, $CDCl_3$) δ (17.6), 18.2, (22.4), 24.6, 29.0, (31.1), (43.0), 43.5, (46.6), (46.7), 46.8, 46.9, 59.2, (59.4), 66.7, (67.3), 127.1, 128.0, 128.2, (128.3), 128.5, (128.6), 128.7, 129.2, 134.9, (134.96), (135.2), 135.6, 170.1, (170.7), 171.1, 171.5, (172.10), (172.18); HRMS m/z calcd for $C_{23}H_{27}N_2O_4$ $[M + H]^+$ 395.1965, found 395.1960.

Phenylacetyl-glycinyl-(2*S*)-prolyl-(2*S*)-3-pyridinylalaninyl-(3*S*)-β-homophenylalanine benzyl ester (24a). A solution of phenylacetyl-glycinyl-(2*S*)-proline benzyl ester **22a** (100 mg, 0.26 mmol) in anhydrous EtOH (60 mL) and AcOH (6 mL) was treated with palladium-on-carbon (10 wt%, 30 mg) and stirred under 1 atm of hydrogen overnight. The mixture was filtered on Celite™, which was washed with hot MeOH, and the combined filtrate and washings were evaporated and freeze-dried to give acid **23a**. Phenylacetyl-glycinyl-(2*S*)-proline (**23a**, 172 mg, 0.61 mmol) was dissolved in 12 mL of dichloromethane, treated with HOBt (91 mg, 0.67 mmol) and TBTU (216 mg, 0.67 mmol), stirred for 10 min, treated with 3-(pyridyl)alaninyl-β-homophenylalaninyl benzyl ester hydrochloride (**12**, 230 mg, 0.56 mmol), followed by drop-wise addition of DIEA (292 μL, 1.68 mmol), and stirred overnight. Evaporation of the volatiles gave a residue, which was purified by flash chromatography on silica gel using 8% MeOH in EtOAc. Evaporation of the collected fractions afforded peptide **24a** (274 mg, 72%) as yellow oil: R_f = 0.4 (10% MeOH in EtOAc); $[\alpha]_D^{20}$ −23.5 (c 1.0, MeOH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.79–1.87 (m, 2H), 1.90–1.93 (m, 1H), 2.17–2.19 (m, 1H), 2.52–2.54 (d, J = 5.6 Hz, 2H), 2.80–2.94 (m, 3H), 3.14–3.19 (dd, J = 5.2, 14 Hz, 1H), 3.30–3.37 (m, 1H), 3.40–3.45 (m, 1H), 3.67 (s, 2H), 3.76–3.81 (dd, J = 3.6, 17.2 Hz, 1H), 3.99–4.04 (dd, J = 5.6, 17.2 Hz, 1H), 4.41–4.44 (m, 1H), 4.47–4.54 (m, 2H), 5.07–5.10 (d, J = 12 Hz, 1H), 5.13–5.16 (d, J = 12 Hz, 1H), 6.79–6.82 (m, 1H), 6.94–6.95 (d, J = 7.6 Hz, 1H), 7.14–7.15 (m, 3H), 7.22–7.28 (m, 3H), 7.34–7.37 (m, 10H), 7.30 (s, 1H), 7.51–7.54 (d t, J = 2, 2, 8 Hz, 1H), 8.35–8.36 (d, J = 2 Hz, 1H), 8.38–8.39 (dd, J = 1.6, 4.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.7, 27.3, 33.9, 37.1, 39.9, 42.2, 43.4, 46.6, 47.6, 54.3, 60.1, 66.5, 123.7, 126.7, 127.3, 128.3, 128.4, 128.5, 128.6, 128.9, 129.3, 129.4, 133.1, 134.6, 135.6, 137.1, 137.9, 147.6, 150.3, 168.8, 169.6, 170.9, 171.3, 171.7. HRMS m/z calcd for $C_{40}H_{44}N_5O_6$ $[M + H]^+$ 690.3286, found 690.3287.

Phenylacetyl-(2*R*)-alaninyl-(2*S*)-prolyl-(2*S*)-3-pyridinylalaninyl-(3*S*)-β-homophenylalanine benzyl ester (24b). **24b** was prepared using the same hydrogenation and coupling procedures as described above for **24a**, employing benzyl ester **22b** (110 mg, 0.28 mmol) and palladium-on-carbon (10 wt%, 30 mg) to give the acid. Acid **23b** (187 mg, 0.61 mmol) was coupled to 3-(pyridyl)alaninyl-β-homophenylalaninyl benzyl ester hydrochloride **12** (230 mg, 0.56 mmol) using HOBt (91 mg, 0.67 mmol), TBTU (216 mg, 0.67 mmol), and DIEA (292 μL, 1.68 mmol). Purification of the residue by flash chromatography on silica gel using 8% MeOH in EtOAc afforded peptide **24b** (320 mg, 81% yield) as white powder: R_f = 0.38 (10% MeOH in EtOAc); $[\alpha]_D^{20}$ −12.4 (c 1.5, MeOH); mp 70 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.32–1.33 (d, J = 7 Hz, 3H), 1.37–1.46 (m, 1H), 1.77–1.83 (m, 1H), 1.92–1.97 (m, 2H), 2.53–2.62 (m, 2H), 2.76–2.81 (m, 1H) 2.85–2.90 (dd, J = 8.5, 13.5 Hz, 1H), 3.00–3.04 (dd, J = 6, 14 Hz, 1H), 3.19–3.23 (dd, J = 6, 14.5 Hz, 1H) 3.37–3.42 (m, 1H), 3.47–3.50 (d, J = 15.5 Hz, 1H), 3.52–3.55 (d, J = 15.5 Hz, 1H), 3.74–3.81 (td, J = 3, 9 Hz, 1H), 4.35–4.40 (m, 1H), 4.42–4.44 (m, 1H), 4.48–4.58 (m, 2H), 5.04–5.06 (d, J = 12.5 Hz, 1H), 5.08–5.11 (d, J = 12.5 Hz, 1H), 6.52–6.53 (d, J = 4.5 Hz, 1H), 7.14–7.28 (m, 12H), 7.30–7.37 (m, 5H), 7.43–7.45 (d, J = 8.5 Hz, 1H), 7.58–7.60 (d, J = 8 Hz, 1H), 8.42–8.43 (d, J = 4.5 Hz, 1H), 8.47 (s, 1H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 16.1, 24.1, 28.7, 33.1, 37.6, 40.3, 42.6, 47.4, 47.9, 48.2, 54.8, 61.0, 66.4, 123.49, 126.6, 127.4, 128.1, 128.3, 128.4, 128.5, 128.9, 129.40, 129.42, 134.1, 134.2, 135.9, 136.8, 137.8, 147.5, 150.4, 170.2, 171.0, 171.1, 172.3, 172.4. HRMS m/z calcd for $C_{41}H_{46}N_5O_6$ $[M + H]^+$ 704.3442, found 704.3446.

Phenylacetyl-glycinyl-(2*S*)-prolyl-(2*S*)-3-pyridinylalaninyl-(3*S*)-β-homophenylalanine (3). **3** was obtained from ester **24a** (100 mg, 0.14 mmol) by hydrogenation with palladium-on-carbon (10 wt%, 17 mg) as described above. Purification by preparative HPLC on a C18 reverse-phase column gave acid **3** (30 mg, 36% yield) as white powder: ^{1}H NMR (700 MHz, CD_3OD) detected a 4.5 : 1 mixture of prolyl amide isomers δ [1.62 (m, 2H)], 1.75–1.85 (m, 2H), 1.89–1.94 (m, 1H), 2.05–2.10 (m, 1H), [2.22 (m, 1H)], 2.44 (s, 2H), 2.85–2.86 (d, J = 7 Hz, 2H), 2.88–2.92 (m, 1H), 3.12–3.15 (m, 1H), 3.53–3.56 (m, 1H), 3.58–3.70 (m, 3H), 4.04 (s, 2H), 4.36–4.38 (m, 1H), 4.41–4.43 (m, 1H), 4.50–4.52 (m, 1H), [4.64 (m, 1H)], 7.20–7.36 (m, 11H), 7.69–7.72 (m, 1H), [8.20 (s, 2H)], 8.35–8.39 (m, 2H); ^{13}C NMR (175 MHz, CD_3OD) δ (21.8), 24.2, 28.8, (31.8), 34.0, (34.7), 37.7, 39.7, (41.1), 41.9, 42.10, (42.15), 46.5, (46.8), 48.2, 54.0, (59.7), 60.4, 123.7, (126.0), 126.1, 126.5, (126.6), (127.9), 128.0, 128.22, (128.23), 128.9, (129.0), (129.11), 129.13, 134.0, 135.1, (135.3), 137.8, (137.9), 138.0, 146.9, 149.4, 168.5, 168.7, 170.5, (170.6), (172.1), 172.7, (172.8), 173.0. HRMS *m*/*z* calcd for $C_{33}H_{38}N_5O_6$ $[M + H]^+$ 600.2816, found 600.2828. RP-HPLC system 1: 97%, R.T. 5.67 min. RP-HPLC system 2: 99%, R.T. 5.89 min.

Phenylacetyl-(2*R*)-alaninyl-(2*S*)-prolyl-(2*S*)-3-pyridinylalaninyl-(3*S*)-β-homophenylalanine (4). **4** was synthesized using the protocol described above for **3**, employing benzyl ester **24b** (119 mg, 0.16 mmol) and palladium-on-carbon (10 wt%, 21 mg). The residue was purified by preparative HPLC on a C18 reverse-phase column to afford **4** (41 mg, 41% yield) as white powder: ^{1}H NMR (700 MHz, CD_3OD) detected a 5 : 1 of mixture of prolyl amide isomers, δ [0.87 (d, *J* = 7 Hz, 3H)], 1.39–1.40 (d, *J* = 7 Hz, 3H), 1.44–1.48 (m, 1H), 1.71–1.74 (m, 1H), [1.78, (m, 1H)], 1.82–1.87 (m, 1H), [1.94 (m, 1H)], 2.05–2.11 (m, 1H), [2.23 (m, 1H)], [2.38 (m, 1H)], 2.44–2.52 (m, 2H), 2.69–2.73 (m, 1H), [2.83 (m, 2H)], 2.88–2.94 (m, 2H), 3.00–3.03 (dd, *J* = 4.5, 14.7 Hz, 1H), [3.10 (dd, *J* = 4.5, 14.7 Hz, 1H)], 3.46–3.49 (m, 1H), 3.55–3.62 (m, 2H), [3.65 (m, 2H)], 3.75–3.78 (m, 1H), 4.36–4.40 (m, 2H), 4.44–4.49 (m, 2H), [4.60 (dd, *J* = 4.9, 10.5 Hz, 1H)], [4.78 (dd, *J* = 2.8, 9.1 Hz, 1H)], [7.08 (m, 1H)], [7.17 (m, 5H)], 7.21–7.27 (m, 6H), 7.31–7.33 (m, 5H), 7.68–7.69 (d, *J* = 8.4 Hz, 1H), [8.29 (d, *J* = 4.1 Hz, 1H)], 8.36–8.38 (m, 2H), [8.46 (m, 2H)]. ^{13}C NMR (175 MHz, CH_3OD) δ 14.9, (15.3), (22.2), 23.8, 28.8, (31.4), 33.0, (34.9), 38.1, (38.4), 39.7, 39.9, 41.5, (41.6), (46.92), 46.99, 47.3, 48.2, (48.3), (54.1), 55.0, (66.1), 60.9, (123.6), 123.7, (126.0), 126.54, (126.56), (127.9), 128.16, (128.19), 128.8, 129.0, 129.1, (133.9), 134.6, 135.2, (135.5), 137.5, (137.8), (138.0), 138.1, 146.7, (146.9), (149.3), 149.4, (170.6), 170.9, (172.0), 172.6, 172.1, 172.7, (172.8), 173.0. HRMS *m*/*z* calcd for $C_{34}H_{40}N_5O_6$ $[M + H]^+$ 614.2973, found 614.2985. RP-HPLC system 1: 99%, R.T. 7.59 min. RP-HPLC system 2: 98%, R.T. 5.40 min.

(3*S*,6*R*,7*S*,10*S*)-2-Oxo-3-*N*-(Boc)amino-6-iodo-1-azabicyclo-[5.3.0]decane-10-carbonyl-(2*S*)-(3-pyridy)lalaninyl-(3*S*)-β-homophenylalanine benzyl ester (15) from ester 10a. Benzyl ester **10a** (20 mg, 0.046 mmol) was dissolved in 1 mL of dioxane, cooled to 0 °C, and treated with 2 N LiOH (2 mL). The ice bath was removed and the mixture was stirred at room temperature for 1 h, when TLC showed complete disappearance of the starting material. R_f = 0.3 (40% hexane in EtOAc). The volatiles were evaporated under reduce pressure. The aqueous volume was acidified to pH 3 using 1 N HCl and extracted 3 times with ethyl acetate (10 mL). The resulting acid **17** was dissolved in 12 mL of dichloromethane treated with HOBt (7 mg, 0.05 mmol) and TBTU (16 mg, 0.05 mmol), stirred for 10 min, treated with 3-(pyridyl)alaninyl-β-homophenylalaninyl benzyl ester hydrochloride **12** (18 mg, 0.055 mmol), followed by dropwise addition of DIEA (22 μL, 0.13 mmol), and stirred overnight. Evaporation of the volatiles gave a residue, which was purified by flash chromatography (5% isopropanol in $CHCl_3$) to give peptide ester **15** (20 mg, 52% yield) as yellow powder: R_f = 0.49 (5% isopropanol in $CHCl_3$); mp 110 °C; $[\alpha]_D^{20}$ −55 (*c* 1.0, MeOH). The spectral data of **15** were identical to those presented above.

Ex vivo myometrial contraction assay

As previously described,[14] immediately after delivery, uteri from mice were dissected to provide myometrial strips (2–3 mm wide and 1–2 cm long), which were suspended in organ baths containing Krebs buffer equilibrated with 21% oxygen at 37 °C with an initial tension. Spontaneous contraction peak, duration, and frequency in the absence or in presence of $PGF_{2\alpha}$ and mimics **1–6** were recorded with a Kent digital polygraph system.

MAP kinase activation

Activation of MAP kinase by $PGF_{2\alpha}$ was measured by conventional western blot methods as previously described.[14] Briefly, HA-FP cells in six-well plates were starved for 30 min and pretreated with 1 μM of each ligand (**1–6**) for 30 min and then challenged with $PGF_{2\alpha}$ (0.1 or 1 μM) for 5 min. Cells were lysed in Laemmli buffer 2× (250 mM Tris-HCl, pH 6.8, 2% SDS, 10% glycerol, 0.01% bromophenol blue). Lysates were migrated on a 10% SDSPAGE gel, transferred to nitrocellulose membrane, and probed using mouse anti-p-ERK1/2 and rabbit anti-total-ERK1/2 antibodies. Signals were quantified by densitometry and statistical tests were performed.[14]

Cell ruffling

As previously described,[14] serum-starved FP cells were plated on coverslips, pretreated or not with each ligand (**1–6**) for 30 min at 37 °C, stimulated with 1 μM $PGF_{2\alpha}$ for 30 min, fixed with 4% paraformaldehyde (PFA), and stained with Alexa Fluor 488 phalloidin. Nine fields (5075 cells per field) per coverslip were quantified to assess circular cellular ruffling.

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