Journal of Medicinal Chemistry

ARTICLE

pubs.acs.org/jmc

Targeting the Prostaglandin F2α Receptor for Preventing Preterm Labor with Azapeptide Tocolytics

Carine B. Bourguet,[†] Eugénie Goupil,[¶,‡] Danaë Tassy,[§] Xin Hou,[∥] Eryk Thouin,[†] Felix Polyak,[†] Terence E. Hébert,[‡] Audrey Claing,[§] Stéphane A. Laporte,[¶,‡] Sylvain Chemtob,[‡,∥] and William D. Lubell*[,†]

[¶]Department of Medicine, McGill University Health Center Research Institute, Montréal H3A 1A1, Québec, Canada

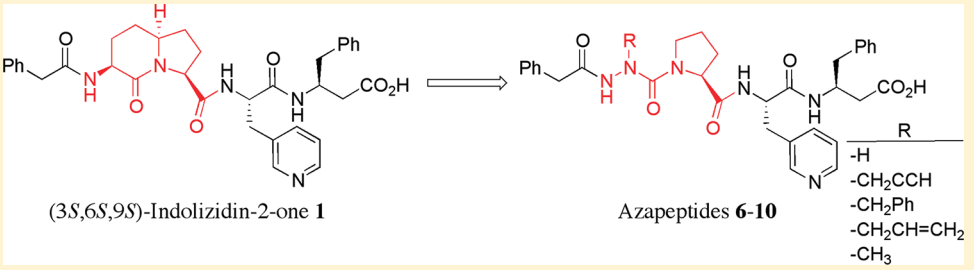
[‡]Department of Pharmacology and Therapeutics, McGill University, Montréal H3G 1Y6, Québec, Canada

[§]Département de Pharmacologie and [†]Département de Chimie, Université de Montréal, Montréal H3C 3J7, Québec, Canada

[∥]Centre Hospitalier Universitaire Sainte-Justine Research Center, Montréal H3T 1C5, Québec, Canada

Supporting Information

ABSTRACT:



The prostaglandin-F2α (PGF2α) receptor (FP) was targeted to develop tocolytic agents for inhibiting preterm labor. Azabicycloalkane and azapeptide mimics **2**−**10** were synthesized based on the (3*S*,6*S*,9*S*)-indolizidin-2-one amino acid analogue PDC113.824 (**1**), which was shown to modulate FP by a biased allosteric mechanism, involving both Gαq- and Gα12-mediated signaling pathways, and exhibited significant tocolytic activity delaying preterm labor in a mouse model (Goupil; *J. Biol. Chem.* **2010**, *285*, 25624−25636). Although changes in azabicycloalkane stereochemistry and ring size caused loss of activity, replacement of the indolizidin-2-one amino acid with azaGly-Pro and azaPhe-Pro gave azapeptides **6** and **8**, which reduced PGF2α-induced myometrial contractions, potentiated the effect of PGF2α on Gαq-mediated ERK1/2 activation, and inhibited FP modulation of cell ruffling, a response dependent on the Gα12/RhoA/ROCK signaling pathway. Revealing complementarities of azabicycloalkane and azapeptide mimics, novel probes, and efficient tocolytic agents were made to study allosteric modulation of the FP receptor.

INTRODUCTION

Preterm births (<37 weeks gestational age) contribute significantly to mortality and morbidity in obstetric practice in developed countries, where rates of preterm delivery vary between 5% and 13%.[1] In spite of a better understanding of risk factors related to and mechanisms underlying preterm labor, as well as medical interventions to reduce its occurrence,[2] the rate of preterm birth has risen in most industrialized countries, increasing in the U.S. from 9.5% in 1981 to 12.7% in 2005.[3] Preterm birth has major socioeconomic implications with associated hospital stays among the most expensive diagnoses for all children:[4] in 2001, they represented 47% of the costs ($5.8 billion) for all infant hospitalizations and 27% for all pediatric stays. Infant mortality rates are also 15-fold and 75-fold higher for preterm and very preterm (<32 weeks) delivery relative to term birth.[4] In addition to morbidity and disability,[5] preterm birth and low birth weight account for neurodevelopmental disorders,[6] respiratory and gastrointestinal complications,[7] and lifelong chronic conditions such as hypertension and dyslipidemia.[8]

Preterm deliveries are associated with various epidemiological and clinical risk factors; however, >45% are due to spontaneous contractions, for which causes are usually unknown.[1,2] The most effective method for reducing morbidity and mortality related to preterm birth has been early inhibition of the initiation of uterine contractions using labor-suppressing drugs (tocolytics).[9] Tocolytic drugs are used to prolong pregnancy in women with acute risk of preterm birth, caused mainly by active preterm labor and less commonly by ruptured membranes. Classes of medication for tocolysis include β_2-adrenergic agonists (ritodrine and terbutaline), calcium channel blockers (i.e., nifedipine), prostaglandin synthetase inhibitors (i.e., indomethacin), an oxytocin (OT) receptor antagonist (atosiban), and magnesium sulfate (Figure 1).[10−18] In clinical practice, some of these agents have delayed delivery up to 48 h; however, adverse effects have generally limited the utility of contemporary tocolytics.[10−18] FP has emerged as a complementary target for designing tocolytic agents

Received: May 13, 2011
Published: July 20, 2011

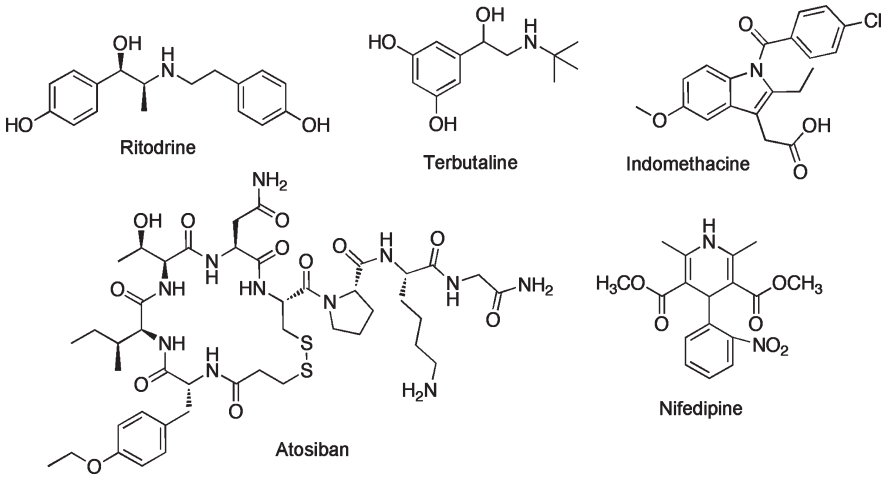


Figure 1. Contemporary tocolytic drugs.

for two particular reasons: (1) its expression increases at onset of labor[19−21] (the OT receptor is not involved in the initial stages of preterm parturition);[22] (2) FP knockout mice never go into labor.[23]

In search of novel tocolytic agents, we have designed peptides and mimics that modulate FP by an allosteric mechanism of action.[24] Allosteric modulators bind at sites spatially distinct from the endogenous orthosteric ligands and may exhibit reduced side effects, due to their potential for increased selectivity without disruption of normal rhythms of endogenous ligand release.[25,26] Short peptide leads were developed based on the second extracellular loop of the FP receptor, synthesized, and examined in *in vitro*, *ex vivo*, and *in vivo* assays. For example, the D-peptide PDC113 (ilghrdyk, small letters refer to D-amino acids; IC_{50} = 340 nM) inhibited >80% (100 μM) of the vasomotor response induced by PGF2α in porcine retinal microvascular contraction assays, without antagonist effects against other known vasoconstrictors.[27] Replacement of D-arginine[5] by D-citrulline[5] yielded PDC31 (ilghxdyk, x = citrulline), which exhibited improved efficacy (>85%) and potency (IC_{50} = 13 nM),[28] effectively diminishing myometrial contractions in mouse, bovine, and ovine animal models.[29] In the mouse model, PDC31 delayed delivery for a mean time of 48 h (at 0.8 mg/day). PDC113 and PDC31 both exhibited noncompetitive selective inhibition of FP in the presence of PGF2α.

Although peptide drugs have advantages, including high potency, efficacy,[30] and negligible toxicity,[31] their use may be limited because of poor pharmacokinetic profiles, including rapid degradation and low bioavailability, requiring intravenous administration. Contingent on structural complexity, peptide manufacture may entail relatively high production costs.[32] Conversion from peptide to small molecule formats was thus pursued to improve pharmacokinetic properties without sacrificing efficacy. These studies led to the synthesis of the peptide mimic PDC113.824 (**1**) (Figure 2), which caused decreases in basal and PGF2α-induced uterine contractions.[24] In pregnant mice, **1** delayed LPS- and PGF2α-induced delivery with mean times of 28 and 42 h, respectively.[24] Furthermore, the mechanism of action for **1** was demonstrated to be allosteric and contingent on PGF2α binding,[24] which resulted in increased ERK1/2 activity by way of biased activation of $G\alpha_q$, and inhibition of Rho-dependent actin remodeling and myometrial cell contraction by way of inhibition of $G\alpha_{12}$. These findings provided a chemical framework for further tocolytic drug design.

We report now two approaches, which have been pursued to study the influences of configuration, conformation, and structure on the biological activity of (3*S*,6*S*,9*S*)-indolizidin-2-one **1**. In the first, the indolizidin-2-one amino acid (I^2aa) residue of **1** was replaced by alternative azabicyclo[X.Y.0]alkanone amino acids to study the influence of ring size and stereochemistry on biological activity. In the second, aza-aminoacyl-L-proline dipeptides were employed as surrogates of the I^2aa residue. As detailed below, although alternative azabicyclo[X.Y.0]alkanone mimics proved to be less efficacious, the application of aza-aminoacyl-L-proline analogues provided a novel means for modulating FP function. Conveniently synthesized in a few steps, the azapeptide analogues represent a new generation of allosteric ligands, some of which have exhibited effective tocolytic effects on mouse myometrial contractions and bias signaling similar to **1** and others which exhibited distinct patterns of FP modulation.

RESULTS AND DISCUSSION

Chemistry. In earlier studies, a modest 2-fold reduction in potency was observed on replacement of L-pyridylalanine in **1** by L-citrulline.[28] Moreover, in the X-ray crystal structures of different protected azabicyclo[X.Y.0]alkanones, the heterocycle ring size was shown to influence significantly the peptide backbone geometry.[33−37] For example, the fused 6,5- and 5,6-ring components of the (3*S*,6*S*,9*S*)-I^2aa and (2*S*,6*R*,8*S*)-indolizidin-9-one (I^9aa) systems mimicked different aspects of the central residues of an ideal type II′ β-turn.[33,37] The enantiomeric (3*R*,6*R*,9*R*)-I^2aa residue folded in a type II β-turn conformation.[33] In studies of the configuration and conformation of **1**, the (3*S*,6*S*,9*S*)-I^2aa residue was thus replaced by its enantiomeric (3*R*,6*R*,9*R*)-I^2aa counterpart in mimic **2**, as well as by (2*S*,6*R*,8*S*)-I^9aa (**4**) and (2*S*,6*R*,8*S*)-quinolizidinone amino acids (Qaa, **5**, Figure 2).[33,36,37] In addition to these studies of the ring system, an enantiomeric analogue (**3**) was prepared to examine the relationship between stereochemistry and activity.

Indolizidin-2-one **1** and peptide mimics **2**−**5** all were synthesized on solid support using a Boc strategy on oxime resin as illustrated for the former in Scheme 1.[38] *N*-Boc-(3-pyridyl)-alanine [or *N*-(Boc) citrulline] was coupled to the resin using

dx.doi.org/10.1021/jm200608k |*J. Med. Chem.* 2011, 54, 6085–6097

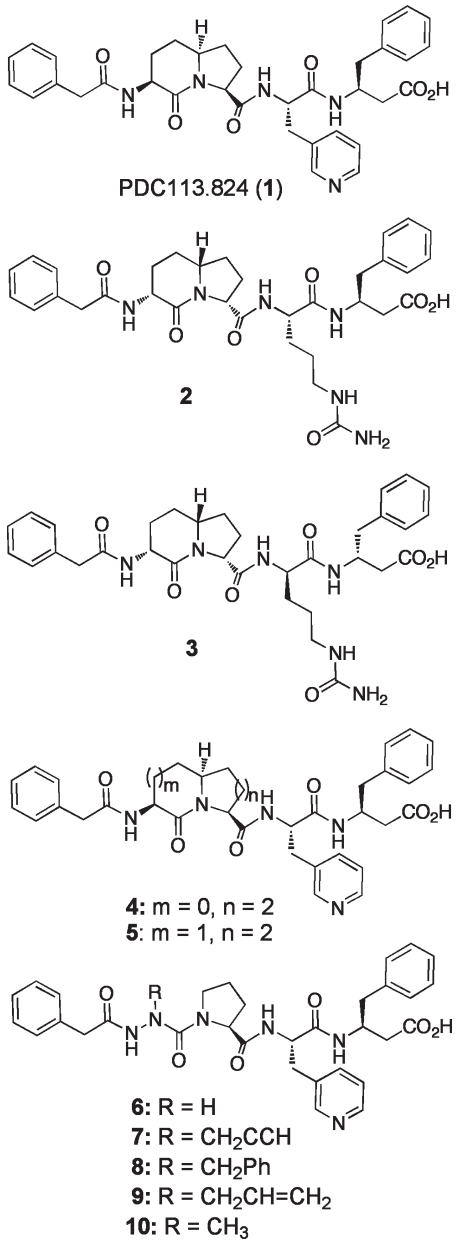
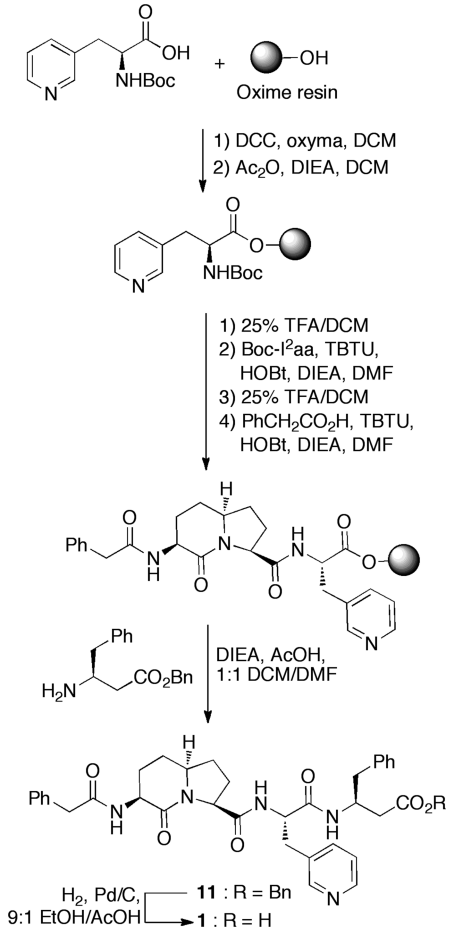


Figure 2. PDC113.824 (**1**), azabicycloalkanone mimics **2**−**5**, and azapeptides **6**−**10**.

dicyclohexylcarbodiimide (DCC) and oxyma (EACNOx) in the presence of DIEA in dichloromethane.[39] After resin capping with acetic anhydride, the Boc group was removed with TFA in dichloromethane and the amine was free-based by washing the resin with DIEA in dichloromethane. The appropriate azabicyclo-[X.Y.0]alkanone amino acid [(3*S*,6*S*,9*S*)- and (3*R*,6*R*,9*R*)-I^{2}aa, (2*S*,6*R*,8*S*)-I^{9}aa, and (2*S*,6*R*,10*S*)-Qaa] was coupled using a mixture of TBTU/HOBt/DIEA in DMF for 3 h. Subsequent Boc group removal with TFA and coupling of phenylacetic acid under similar conditions as mentioned above gave the *N*-terminal peptide linked to the resin, which was cleaved with (*S*)-*β*-homophenylalanine benzyl ester in the presence of DIEA and acetic acid in dichloromethane to give the respective mimics as their corresponding esters. Benzyl ester cleavage was accomplished using hydrogen and palladium-on-carbon in a 9:1 ethanol/acetic acid

Scheme 1. Solid-Phase Synthesis of 1



mixture to furnish the acids, which were purified by preparative reverse-phase HPLC (Table 1).

A solution-phase method was developed for making **1** on a larger scale (Scheme 2). *β*-Homophenylalanine benzyl ester hydrochloride **12** was obtained from Boc-*β*-homophenylalanine benzyl ester by removal of the Boc group using TFA in DCM, counterion exchange by evaporation from aqueous HCl, and freeze-drying. *N*-(Boc)pyridylalanine was coupled to ester **12** using TBTU, HOBt, and DIEA in dichloromethane to provide protected dipeptide **13** in 91% yield after chromatography. By use of a similar protocol, the Boc group was removed with TFA and converted to amine hydrochloride **14**, which was coupled with Boc-I^{2}aa to furnish amide **15** in 84% yield after chromatography. Removal of the Boc group from **15** and acylation of the resulting amine **16** with phenylacetyl chloride and DIEA in EtOAc gave benzyl ester **11**, which was converted to **1** by hydrogenation as described above.

Azapeptides, in which the α-carbon of an amino acid residue is replaced by nitrogen, have been observed to adopt turn conformations in computational models, as well as X-ray crystallographic and spectroscopic analyses.[40−43] For example, the central *i* + 1 and *i* + 2 residues of a type I *β*-turn have been observed in X-ray structures of Boc-azaAla-Pro-NH-*i*-Pr and Cbz-azaAsn(Me)-Pro-NH-*i*-Pr.[40] A complementary relationship between azabicycloalkane amino acid and azapeptide mimics was observed, when the Gly^{33}-Pro^{34} moiety of the antagonist

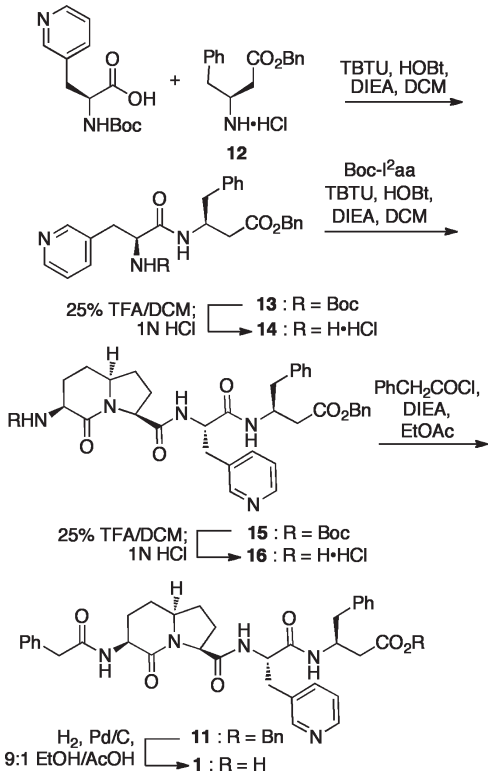
dx.doi.org/10.1021/jm200608k |*J. Med. Chem.* 2011, 54, 6085–6097

Table 1. HPLC Results of PDC113.824 (1) and Mimics 2−10

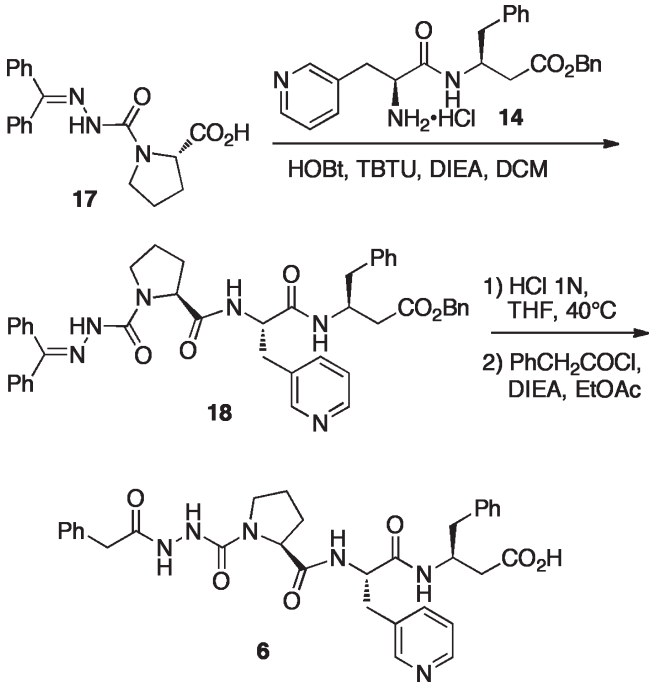
entry	compd	retention time (system)[a]	purity (%)	retention time (system)[b]	purity (%)
1	**1**	9.77 (1)	98.6	12.18 (3)	95.5
2	**2**	7.83 (2)	97.3	10.88 (4)	95.6
3	**3**	7.77 (2)	98.4	10.23 (4)	96.7
4	**4**	5.59 (1)	99.8	9.72 (4)	95.7
5	**5**	4.92 (1)	99.8	10.94 (4)	97.2
6	**6**	6.88 (1)	99.0	8.76 (3)	97.1
7	**7**	7.96 (1)	98.9	11.86 (3)	98.5
8	**8**	8.84 (1)	97.4	13.99 (3)	98.9
9	**9**	8.20 (1)	95.8	12.68 (3)	98.7
10	**10**	7.77 (1)	98.6	11.58 (3)	99.0

[a] Retention times (min) and purity were assessed using system 1 or 2, eluting with a gradient of aqueous 0.1% formic acid (FA) in acetonitrile with 0.1% FA. See General Methods of Experimental Section. [b] Retention times (min) and purity were assessed using system 3 or 4, eluting with a gradient of aqueous 0.1% formic acid (FA) in methanol with 0.1% FA. See General Methods of Experimental Section.

Scheme 2. Solution-Phase Synthesis of 1



Scheme 3. Synthesis of AzaGly-Pro Mimic 6

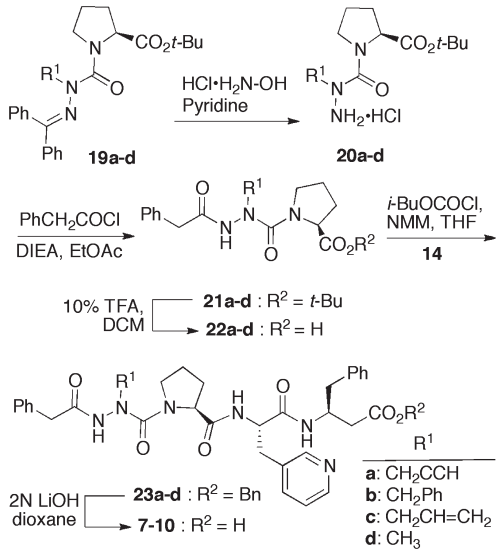


[D^{31},P^{34},F^{35}]calcitonin gene-related peptide$_{27-37}$ was replaced by azaGly^{33}-Pro^{34} and I^{2}aa^{33,34}, giving respectively 10- and 7-fold increases in potency.[44] Considering that computational analysis of *N*-acetyl-aza-glycinyl-L-alanine *N*′-methylamide had previously suggested a preference for a type II′ β-turn conformation, the azaGly-Pro and I^{2}aa residues were hypothesized to mimic similar secondary structures.[44] Their similar conformational preferences, as well as the fact that azaGly-Pro could be synthesized more expediently than the I^{2}aa dipeptide, evoked the preparation of aza-amino acylproline analogues **6**−**10**, with expectations to induce an active turn conformation, due to electronic restrictions from the semicarbazide moiety.[45,46] Having developed submonomer approaches for synthesizing azapeptides by alkylation of protected aza-glycine residues,[46,47] opportunity was provided for exploring the effects of alkyl substituent (side chains) on the aza-residue in the structure−activity studies.

The azaGly-Pro dipeptide **17** was synthesized as previously described by acylation of proline using the activated methylidene carbazate prepared from reacting benzophenone hydrazone with *p*-nitrophenyl chloroformate.[45] Hydrazone protected azaGly-Pro **17** was then coupled to pyridinylalaninyl-β-homophenylalanine benzyl ester **14** using TBTU, HOBt, and DIEA to give the protected aza-tetrapeptide **18** in 70% yield after chromatography. Solvolysis with 1 N HCl in THF converted semicarbazone ester **18** to its amino acid counterpart, which reacted with phenylacetyl chloride to give AzaGly-Pro mimic **6**, albeit in 20% overall yield after purification by preparative HPLC (Scheme 3).

Four additional azapeptides **7**−**10** were prepared by a common route that featured alkylation of benzhydrylidene aza-glycinyl

Scheme 4. Synthesis of Azapeptide Mimics 7−10



proline *tert*-butyl ester as previously described, using a set of alkyl halides: propargyl bromide, benzyl bromide, allyl iodide, and iodomethane (Scheme 4).[46] Four alkyl groups were selected to probe the impact of aliphatic and aromatic side chains on the efficacy of the FP ligands. Selective removal of the benzhydrylidene protection from **19a**−**d** without *tert*-butyl ester cleavage was accomplished using hydroxylamine hydrochloride in pyridine.[46] Acylation with phenyl acetyl chloride and *tert*-butyl ester removal with trifluoroacetic acid gave the *N*-terminal dipeptides **22a**−**d**, which were subsequently activated as mixed anhydrides using isobutyl chloroformate and coupled to pyridinylalaninyl-β-homophenylalanine benzyl ester **14** to give the desired benzyl ester protected azapeptides **23a**−**d** in 40−55% yields. To avoid reduction of olefin side chains, ester hydrolysis was performed with lithium hydroxide in dioxane to give the azapeptide mimics **7**−**10** in 66−85% yields.

Biological Effects of Azapeptide Mimics. *Effect of Indolizidinone **1** and Azapeptides **6**−**10** on Myometrial Contraction.* Previously, **1** was shown to significantly reduce the strength and duration of both PGF2α-induced and spontaneous contractions in a dose-dependent manner using myometrium obtained from spontaneous postpartum mice.[24] The influences of mimics **2**−**10** on PGF2α-induced myometrial contractions were thus examined to evaluate their potential to serve as tocolytics. Subsequently, active analogues were further examined in order to dissect their putative mechanisms of action.

In spite of the activity of (3*S*,6*S*,9*S*)-indolizidin-2-one **1**, none of the related azabicyclo[X.Y.0]alkanone amino acid analogues **2**−**5** reduced PGF2α-induced myometrial contractions.[48,49] On the other hand, azapeptides **6**−**10** exhibited varying degrees of activity in the myometrial contraction assay, contingent on the nature of the aza-amino acid side chain. In particular, the aza-glycine, aza-propargylglycine, and aza-phenylalanine analogues **6**−**8** all reduced PGF2α-induced myometrial contractions with similar efficacy as indolizidin-2-one **1** (Figure 3A). The aza-allylglycine and aza-alanine analogues **9** and **10** were relatively unable to reduce the strength or duration of PGF2α-induced myometrial contractions. Traces for the active analogues **1** and aza-phenylalanine mimic **8** are juxtaposed against that of the inactive aza-alanine analogue **10** to indicate the manner by which the different analogues influence the myometrium (Figure 3B).

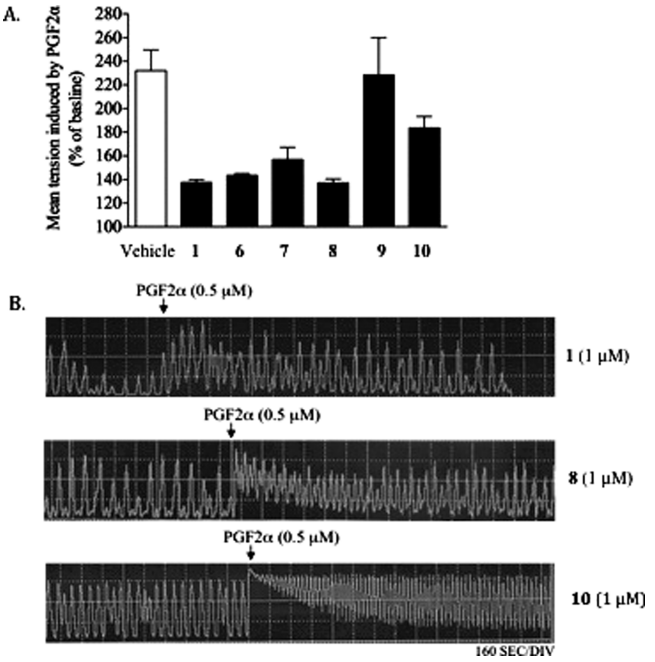


Figure 3. Effects of **1** and azapeptides **6**−**10** on mean tension induced by PGF2α. At the beginning of each experiment, mean tension of spontaneous myometrial contractions was considered as the basal response. (A) Changes in the mean tension (*g*) were expressed as percent of the initial response (% baseline). Indolizidin-2-one **1** or azapeptide (**6**−**10**) were given 15 min before PGF2α. Values are the mean ± SEM of three to five experiments for each compound: $p < 0.05$ compared to all values without asterisks. (B) Typical traces of contractions: **1** and azapeptides **8** and **10**.

Effect of Azapeptides on PGF2α-Stimulated Signaling Pathways. Previously, (3*S*,6*S*,9*S*)-indolizidin-2-one **1** was shown to affect the binding of PGF2α on HEK-293 cells stably expressing FP (FP cells) and PGF2α-dependent signaling.[24] Pretreatment of FP cells with **1** followed by addition of PGF2α facilitated Gαq-mediated signaling via PKC/ERK1/2 and inhibited signaling by way of the Gα12-mediated RhoA/ROCK pathway, impairing actin reorganization and membrane ruffling.[24] Aza-analogues **6**−**8** were thus examined for their potential to regulate both of these signaling pathways.

Treatment of FP cells with the azapeptides **6**−**8** (1 μM, 30 min) in the absence of PGF2α had no effect on ERK1/2 activation or membrane ruffling (data not shown). On the other hand, pretreatment of FP cells with an azapeptide (**6**−**8**) followed by PGF2α significantly potentiated ERK1/2 signaling relative to cells stimulated with PGF2α alone (vehicle, Figure 4). The effects of azapeptides **6**−**8** were similar to that of indolizidin-2-one **1** (1 μM, Figure 4A).

Stimulation with PGF2α (1 μM, 30 min) caused membrane ruffle formation in 65.7 ± 1.6% of FP cells (Figure 5). The aza-Gly and aza-Phe analogues **6** and **8** both were as effective as (3*S*,6*S*,9*S*)-indolizidin-2-one **1** in inhibiting FP-induced cell ruffling (**6**, 37.5 ± 2.5%; **8**, 37.4 ± 5.9%; **1**, 40.3 ± 1.5%). Although the aza-propargylglycine analogue **7** reduced PGF2α-mediated cell ruffling, this effect was less significant (56.7 ± 1.4% ruffling cells).

Azapeptides **6**−**8** were as effective as **1** in sensitizing Gαq/PKC/ERK1/2 signaling. However, they exhibited variable

dx.doi.org/10.1021/jm200608k |*J. Med. Chem.* 2011, 54, 6085–6097

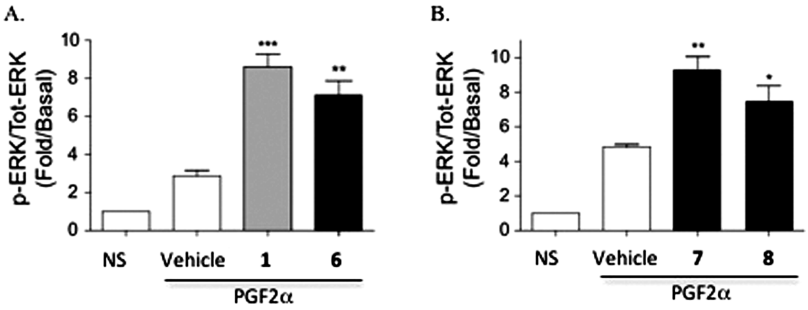


Figure 4. Effects of (3*S*,6*S*,9*S*)-indolizidin-2-one **1** and azapeptides **6**−**8** on PGF2α-mediated ERK1/2 activation. Effects are shown for **1** and **6** (A) and for **7** and **8** (B) on MAPK activation induced by PGF2α. FP expressing cells were starved and pretreated with 1 μM **1** or azapeptide (**6**−**8**) for 30 min and then stimulated with 0.1 μM (A) or 1 μM (B) PGF2α for 5 min as described.[24] Signals were quantified by densitometry and plotted compared to the not stimulated (NS) condition. Results are representative of three (A) and four (B) independent experiments: ∗, $p < 0.1$; ∗∗, $p < 0.01$; ∗∗∗, $p < 0.001$; one-way ANOVA compared to vehicle.

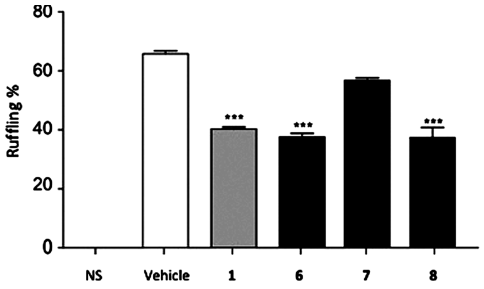


Figure 5. Effects of (3*S*,6*S*,9*S*)-indolizidin-2-one **1** and azapeptides **6**−**8** on cell ruffling. FP expressing cells seeded onto coverslips were pretreated with **1** or azapeptide (**6**−**8**) (1 μM, 30 min) and then stimulated with PGF2α (1 μM, 30 min) as previously described.[24] Cells were then fixed with paraformaldehyde (4%), stained with Alexa Fluor 488 phalloidin, and mounted onto coverslips using GelTol medium. Numbers of cells exhibiting circular ruffling for each condition were counted. Results are the mean ± SEM of three independent experiments where more than 100 cells were counted. ∗∗∗, $p < 0.001$ are values compared to the control unstimulated conditions.

efficacies in inhibiting the Gα12/RhoA/ROCK signaling cascade and cell ruffling. Considering their activity on the two different pathways, azapeptides **6**−**8** may modulate the conformation of FP receptor with subtle differences, toggling the allosteric effects into distinct patterns.

CONCLUSION

In pursuit of novel modulators of FP as potential tocolytic agents for delaying preterm labor, the series of azabicycloalkane and azapeptide mimics **2**−**10** was synthesized based on the structure of the allosteric ligand (3*S*,6*S*,9*S*)-indolizidin-2-one **1**. Although changes in the stereochemistry of the parent ligand and in the ring size of its azabicyclo[X.Y.0]alkanone amino acid component abolished inhibitory activity on myometrial contractions, substitution of the constrained dipeptide surrogate with certain aza-amino acylproline residues caused retention of the effects on PGF2α-induced contractions and cell signaling pathways. In particular, similar to (3*S*,6*S*,9*S*)-indolizidin-2-one **1**, aza-Gly mimic **6** and aza-Phe mimic **8** reduced myometrial contractions and exhibited biased signaling, potentiating the effects of PGF2α on ERK1/2 activation, a response dependent on the Gαq/PKC pathway, and inhibiting its influence on cell ruffling, which is dependent on the Gα12-mediated RhoA/ROCK signaling cascade. A third azapeptide **7**, possessing an aza-propargylglycine residue, exhibited comparable effects on myometrial contractions and ERK1/2 signaling, albeit with reduced influence on RhoA/ROCK signaling. In contrast, azapeptides **9** and **10**, bearing aza-allylglycine and aza-alanine residues, did not show activity in the contraction assay.

The results of this study provide more insight with respect to the complementarities between azabicycloalkane and azapeptide constrained dipeptide mimics, as well as begin to reveal the structural requirements for modulation of FP. As previously observed in analogues of calcitonin gene-related peptide,[44] the similar conformational preferences of the (3*S*,6*S*,9*S*)-indolizidin-2-one amino acid and the azaGly-Pro dipeptide surrogates have been validated by the comparable activity of azabicycloalkane **1** and azapeptide **6**. In contrast, the loss of activity employing (3*R*,6*R*,9*R*)-indolizidin-2-one, indolizidin-9-one, and quinolizidinone amino acid moieties in mimics **2**−**5**, the last two which possess pipecolate components instead of proline, indicates the stringent need for a precise turn geometry. The addition of side chains to the aza-residue in mimics **7**−**10** has provided a set of analogues exhibiting varying degrees of biological activity, which may likely be due to both the effects on ligand conformation and interactions with the receptor caused by the new substituent. Modulation of ligand activity with the composition of the aza-residue side chain is intriguing because the submonomer synthesis of azapeptides offers effective access to a diverse range of side chains,[46,47] which may exhibit a spectrum of biological effects. With respect to the latter, the subtle difference in behavior of the aza-propargylglycine analogue **7** on Gα12-mediated RhoA/ROCK signaling, relative to **1** and azapeptides **6** and **8**, suggests that molecular probes may be designed to have selective effects on G protein signaling. By employing the power of submonomer synthesis to prepare a larger spectrum of azapeptide mimics, we are focused on using these probes to investigate the chemical biology of FP in pursuit of fundamental knowledge of the factors that govern its allosteric modulation toward the development of a novel class of tocolytic agents for preventing preterm labor.

EXPERIMENTAL SECTION

Chemistry. General Methods. Unless otherwise noted, reagents were obtained from commercial sources and used without further purification, and reactions were performed under an argon atmosphere using dry solvents transferred by syringe. Anhydrous solvents (THF,

CH_2Cl_2, and CH_3OH) were obtained by passage through solvent filtration systems (GlassContour, Irvine, CA). DIEA was distilled over ninhydrin and CaH_2. Final reaction mixture solutions were dried over $MgSO_4$ or Na_2SO_4. Column chromatography was carried out on 230−400 mesh silica gel, and TLC was on glass-backed silica plates. Specific rotations $[\alpha]_D$ were measured at 20 °C at the specified concentrations (c in g/100 mL) using a 1 dm cell length on a Perkin-Elmer polarimeter 341 and the general formula: $[\alpha]_D^{20} = (100\alpha)/(dc)$. Purity of all final products was determined by LC−MS and/or analytical HPLC to be ≥95%. HPLC purity of compounds was measured with a reverse phase HPLC (Phenomenex Gemini C18 column, 4.6 mm × 150 mm, 3 μm, 214 nm) with two different solvent systems. In system 1, the gradient of elution was from 40% to 90% A/B over 20 min at a flow rate of 0.4 mL/min, in which solvent A was aqueous 0.1% formic acid (FA) and solvent B was acetonitrile with 0.1% FA. In system 2, the gradient of elution was from 20% to 90% A/B over 20 min at a flow rate of 0.4 mL/min, where solvent A was aqueous 0.1% FA and solvent B was acetonitrile with 0.1% FA. In system 3, the gradient of elution was from 40% to 90% A/B over 20 min at a flow rate of 0.4 mL/min, where solvent A was aqueous 0.1% FA and solvent B was methanol with 0.1% FA. In system 4, compounds were eluted using a gradient of elution from 60% to 90% A/B over 20 min at a flow rate of 0.4 mL/min, where solvent A was aqueous 0.1% FA and solvent B was methanol with 0.1% FA. Accurate mass measurements were performed on a LC-MSD-TOF instrument from Agilent technologies in positive electrospray mode for high-resolution mass spectrometry (HRMS) at the Université de Montréal Mass Spectrometry facility. Either protonated molecular ions $[M + H]^+$ or sodium adducts $[M + Na]^+$ were used for empirical formula confirmation. ^{1}H NMR spectra were measured in $CDCl_3$, CD_3OD, or DMSO-d_6 at 400 or 700 MHz and referenced to $CHCl_3$ (7.26 ppm), CH_3OD (3.31 ppm), or DMSO (2.50 ppm). ^{13}C NMR spectra were measured in $CDCl_3$, CD_3OD, or DMSO-d_6 at 100 MHz and respectively referenced to $CDCl_3$ (77.0 ppm), CD_3OD (49.0 ppm), or DMSO (39.52 ppm). Coupling constant J values were measured in hertz (Hz) and chemical shift values in parts per million (ppm).

General Protocols for Solid-Phase Synthesis of Mimics. Oxime resin (1 g, 0.45 mmol) was swollen in methanol, filtered, and swollen in dichloromethane (DCM). *N*-(Boc)-(3-pyridyl)alanine (150 mg, 0.56 mmol) [or *N*-(Boc)citruline (154 mg, 0.56 mmol)] diluted in a minimum of DCM was added to the swollen resin mixture, followed by 2-ethyl 2-(hydroxylimino)cyanoacetate (oxyma, 198 mg, 1.4 mmol), dicyclohexylcarbodiimide (DCC, 144 mg, 0.7 mmol), and DIEA (0.24 mL, 1.4 mmol). The resin mixture was shaken for 24 h at room temperature, filtered, and washed with DCM (5 × 10 mL), EtOH (5 × 10 mL), and DCM (5 × 10 mL). Capping of the free sites of the resin was performed using acetic anhydride (0.2 mL, 2.2 mmol) and DIEA (0.175 mL, 1.1 mmol) in dichloromethane for 12 h at room temperature. The capped resin was filtered and washed with DCM (5 × 10 mL), 1:1 *i*-PrOH/DCM (v:v, 1 × 10 mL) and DCM (5 × 10 mL). Removal of the Boc protecting group was performed with 25% TFA in DCM (1 × 2 min and 2 × 15 min). The resin was washed with DCM (5 × 10 mL), 1:1 *i*-PrOH/DCM (v:v, 1 × 10 mL), and DCM (5 × 10 mL). The *N*-(Boc)amino azabicyclo[X.Y.0]alkanone carboxylic acid (prepared according to literature protocols,[33,36,37] 0.6 mmol) was sequentially coupled to the swollen resin using *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU, 193 mg, 0.6 mmol) and hydroxybenzotriazole (HOBt, 81 mg, 0.6 mmol) in the presence of DIEA (0.21 mL, 1.2 mmol) in DMF for 3−5 h. After the coupling reaction, the resin was filtered and washed with DMF (2 × 10 mL), DCM (3× 10 mL), 1:1 *i*-PrOH/DCM (v:v, 1× 10 mL), and DCM (5 × 10 mL). The coupling reaction was monitored by the Kaiser test as well as by LC−MS analysis of product from cleavage of an aliquot of the resin with methoxyethylamine in chloroform. After Boc group removal as discussed above, phenyl acetic acid (82 mg, 0.6 mmol) was coupled to the swollen residue using the TBTU/HOBt protocol described above. Final resin cleavage was performed by treating the resin with the hydrochloride salt of (*S*)-β-homophenylalanine benzyl ester (161 mg, 0.6 mmol), DIEA (104 μL, 0.6 mmol), and AcOH (34 μL, 0.6 mmol) in DCM. After resin cleavage, the crude product was purified on a preparative reverse phase HPLC column (Phenomenex Gemini 5 μm, C18, 250 mm × 21.2 mm) using a solvent gradient from 20% to 80% acetonitrile (containing 0.1% TFA) in water (containing 0.1% TFA) to afford the desired peptide mimic benzyl ester (i.e., **11**, 20−40% yield). Hydrogenation of benzyl ester (0.09 mmol) with hydrogen (1 atm) was performed using palladium-on-activated carbon (10% by wt, 10 mg) in EtOH (15 mL) for 5−8 h. The catalyst was filtered onto Celite and washed with methanol. The filtrate and washings were combined and evaporated to the acid, which was isolated by preparative HPLC (Phenomenex Gemini 5 μm, C18, 250 mm × 21.2 mm) using a gradient from 20% to 80% acetonitrile (containing 0.1% TFA) in water (containing 0.1% TFA) to afford the targeted mimics.

Phenylacetyl-(3*S*,6*S*,9*S*)-I^{2}aa-(2*S*)-(3-pyridyl)alaninyl-(3*S*)-β-homophenylalanine (1). Yield: 5 mg, 2%. ^{1}H NMR (700 MHz, D_2O) δ 1.43−1.54 (m, 2H), 1.65−1.68 (m, 1H), 1.72−1.76 (m, 1H), 2.01−2.12 (m, 4H), 2.36−2.39 (m, 1H), 2.54−2.56 (m, 1H), 2.65−2.68 (dd, J = 9.3, 14 Hz, 1H), 2.87−2.89 (dd, J = 5.2, 14.0 Hz, 1H), 3.02−3.05 (m, 1H), 3.10−3.13 (dd, J = 6.4, 14.5 Hz, 1H), 3.56 (s, 2H), 3.59−3.63 (m, 1H), 4.24 (d, J = 9.2 Hz, 1H), 4.35−4.38 (m, 2H), 4.49 (t, J = 6.4 Hz, 1H), 7.22−7.32 (m, 10H), 7.86 (s, 1H), 8.27 (d, J = 6.9 Hz, 1H), 8.47 (s, 1H), 8.55 (s, 1H). HRMS m/z calcd for $C_{35}H_{40}N_5O_6$ $[M + H]^+$ 626.2973, found 626.2972. Purity was assessed by RP-HPLC system 1: >98%, t_R = 9.77. RP-HPLC system 3: >96%, t_R= 12.18 min.

Phenylacetyl-(3*R*,6*R*,9*R*)-I^{2}aa-(2*S*)-citrullinyl-(3*S*)-β-homophenylalanine (2). Yield: 2.5 mg, 1%. HRMS m/z calcd for $C_{33}H_{43}N_6O_7$ $[M + H]^+$ 635.3188, found 635.3181. RP-HPLC system 1: 97.3%, t_R= 7.83 min. RP-HPLC system 4: 95.6%, t_R= 10.88 min.

Phenylacetyl-(3*R*,6*R*,9*R*)-I^{2}aa-(2*R*)-3-citrullinyl-(3*R*)-β-homophenylalanine (3). Yield: 1.5 mg, 1%. HRMS m/z calcd for $C_{33}H_{43}N_6O_7$ $[M + H]^+$ 635.3188, found 635.3188. RP-HPLC system 1: 98.4%, t_R= 7.86 min. RP-HPLC system 4: 96.7%, t_R= 10.89 min.

Phenylacetyl-(2*S*,6*R*,8*S*)-I^{9}aa-(2*S*)-(3-pyridyl)alaninyl-(3*S*)-β-homophenylalanine (4). Yield: 5 mg, 2%. HRMS m/z calcd for $C_{35}H_{40}N_5O_6$ $[M + H]^+$ 626.2973, found 626.2984. RP-HPLC system 1: 100%, t_R= 5.59 min. RP-HPLC system 3: 95.7%, t_R= 10.23 min.

Phenylacetyl-(3*S*,6*R*,10*S*)-Qaa-(2*S*)-(3-pyridyl)alaninyl-(3*S*)-β-homophenylalanine (5). Yield: 3 mg, 4% yield. HRMS m/z calcd for $C_{36}H_{42}N_5O_6$ $[M + H]^+$ 640.3130, found 640.3135. RP-HPLC system 1: 100%, t_R= 4.92 min. RP-HPLC system 3: 97.2%, t_R= 9.72 min.

***N*-Boc-(2*S*)-(3-pyridyl)alaninyl-(3*S*)-β-homophenylalanine Benzyl Ester (13).** (3*S*)-*N*-(Boc)-β-Homophenylalanine (237 mg, 1 equiv, 0.85 mmol, prepared from Boc-Phe according to the literature procedure[50]) was dissolved in 15 mL of acetonitrile and treated with cesium carbonate (1 equiv, 277 mg) diluted in a minimum volume of water. The mixture was stirred for 1 h, treated dropwise with benzyl bromide (0.15 mL, 1.5 equiv, 1.27 mmol), stirred overnight, and concentrated under reduced pressure. The residue was dissolved in EtOAc and washed successively with 0.1 N HCl, saturated $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated under vacuum. The residue was purified by flash chromatography on silica gel (hexane/EtOAc, 9:1) to afford 253 mg of (3*S*)-*N*-(Boc)-β-homophenylalanine benzyl ester in 81% yield. R_f = 0.37(hexane/EtOAc 4:1); $[\alpha]_D^{25}$ −18° (c 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, 9H), 2.51−2.56 (dd, J = 5.5, 12.1 Hz, 1H), 2.82−2.98 (m, 2H), 4.22 (m, 1H), 5.11 (d, J = 12.3 Hz, 1H), 5.14 (d, J = 12.3 Hz, 1H), 7.15−7.40 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.8, 30.1, 38.1, 40.7, 49.3, 66.9, 79.8, 127.0, 128.8, 128.9, 129.0, 129.8, 136.1, 138.1, 155.5, 171.9. HRMS m/z calcd for $C_{22}H_{27}N_1O_4Na$ $[M + Na]^+$ 392.1832, found 392.1841.

(3*S*)-*N*-(Boc)-β-Homophenylalanine benzyl ester (144 mg, 0.39 mmol) was treated with a solution of 25% TFA in CH_2Cl_2 for 2−3 h at room temperature, when TLC showed complete disappearance of starting material (R_f = 0.92, 10% MeOH in DCM). The reaction mixture was evaporated under reduced pressure. The residue was dissolved and coevaporated three times from dichloromethane and then diluted in 10 mL of 1 N HCl, allowed to stand for 1 h, and freeze-dried to afford (3*S*)-β-homophenylalanine benzyl ester hydrochloride **12** [113 mg (95%)] as a white foam: R_f = 0.38 (10% MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ 95.1° (*c* 0.25, CH_3OH); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (br, 2H), 2.37−2.44 (dd, *J* = 8.8, 16 Hz, 1H), 2.55−2.60 (dd, *J* = 4.4, 16 Hz, 1H), 2.61−2.67 (dd, *J* = 8.4, 13.6 Hz, 1H), 2.76−2.81 (dd, *J* = 5.6, 13.6 Hz, 1H), 3.53 (m, 1H), 5.16 (s, 2H), 7.20−7.39 (m, 10H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 30.1, 42.4, 50.1, 66.7, 126.9, 128.7, 128.9, 129.0, 129.7, 136.2, 138.9, 172.6. HRMS *m*/*z* calcd for $C_{17}H_{20}NO_2$ $[M + H]^+$ 270.1489, found 270.1488.

N-Boc-(2*S*)-(3-pyridyl)alanine (221 mg, 1.2 equiv, 0.83 mmol) was dissolved in 5 mL of CH_2Cl_2, treated with HOBt (1.2 equiv, 112 mg) and TBTU (1.2 equiv, 226 mg), stirred at room temperature for 15 min, treated with a solution of (3*S*)-β-homophenylalanine benzyl ester hydrochloride (**12**, 210 mg, 1 equiv, 0.69 mmol) in 5 mL of CH_2Cl_2 followed by DIEA (0.24 mL, 2 equiv, 1.38 mmol), stirred for 3 h, and evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel using ethyl acetate as eluant to afford 325 mg (91%) of *N*-Boc-(2*S*)-(3-pyridyl)alaninyl-(3*S*)-β-homophenylalanine benzyl ester **13** as a white foam: R_f = 0.52 (10% MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ −17° (*c* 1.55, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (s, 9H), 2.42 (d, *J* = 4.9 Hz, 1H), 2.74 (dd, *J* = 8.0, 10.5 Hz, 1H), 2.88 (m, 2H), 3.05 (dd, *J* = 6.3, 13.9 Hz, 1H), 4.31 (m, 1H), 4.44 (m, 1H), 5.05 (d, *J* = 12.2 Hz, 1H), 5.12 (d, *J* = 12.2 Hz, 1H), 5.20 (d, *J* = 8.1 Hz, 1H), 6.90 (m, 1H), 7.07−7.08 (m, 2H), 7.18−7.23 (m, 5 H), 7.32−7.35 (m, 5 H), 7.57 (d, 1H), 8.41 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.9, 31.3, 36.6, 39.4, 47.1, 61.3, 66.1, 71.5, 123.1, 126.4, 128.0, 128.1, 128.2, 128.9, 135.2, 136.6, 136.9, 147.5, 147.9, 150.0, 169.9, 170.9, 171.0. HRMS *m*/*z* calcd for $C_{30}H_{36}N_3O_5$ $[M + H]^+$ 518.2649, found 518.2667.

(2*S*)-(3-Pyridyl)alaninyl-(3*S*)-β-homophenylalanine Benzyl Ester Hydrochloride (14). *N*-Boc-(2*S*)-(3-Pyridyl)alaninyl-(3*S*)-β-homophenylalanine benzyl ester (**13**, 145 mg, 0.28 mmol) was treated with a solution of 25% TFA in CH_2Cl_2 for 2−3 h at room temperature, when complete disappearance of starting material was observed by TLC (R_f = 0.52, 10% MeOH in DCM). The reaction mixture was evaporated under reduced pressure. The residue was coevaporated three times with dichloromethane and then diluted in 10 mL of 1 N HCl, allowed to stand for 1 h, and freeze-dried to afford 113 mg (96%) of hydrochloride **14** as a fluffy white solid: R_f = 0.16 (10% MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ −60° (*c* 0.55, CH_3OH). ^{1}H NMR (400 MHz, CH_3OD) δ 2.51−2.57 (dd, *J* = 8, 16 Hz, 1H), 2.59−2.65 (dd, *J* = 4.4, 15.6 Hz, 1H), 2.79−2.84 (dd, *J* = 7.6, 13.2 Hz, 1H), 2.96−3.01 (dd, *J* = 6.4, 13.6 Hz, 1H), 3.24−3.28 (dd, *J* = 7.2, 14.4 Hz, 1H), 3.42−3.47 (dd, *J* = 5.6, 18.4 Hz, 1H), 4.35 (t, *J* = 6.4 Hz, 1H), 4.50 (m, 1H), 5.10 (q, *J* = 12.4 Hz, 2H), 7.22−7.32 (m, 8H), 7.35−7.37 (m, 2H), 8.02 (m, 1H), 8.61 (d, *J* = Hz, 1H), 8.78 (m, 2H); ^{13}C NMR (100 MHz, CH_3OD) δ 34.2, 37.6, 40.1, 48.9, 53.4, 65.6, 126.8, 127.2, 128.2, 128.3, 128.6, 128.7, 129.5, 135.5, 136.5, 137.9, 141.0, 142.7, 148.6, 167.0, 171.5. HRMS *m*/*z* calcd for $C_{25}H_{28}N_3O_3$ $[M + H]^+$ 418.2125, found 418.2126.

***N*-Boc-(3*S*,6*S*,9*S*)-I^{2}aa-(2*S*)-(3-pyridyl)alaninyl-(3*S*)-β-homophenylalanine Benzyl Ester (15).** A solution of Boc-I^{2}aa (75 mg, 1 equiv, 0.25 mmol) in dichloromethane (5 mL) was treated with HOBt (34 mg, 1 equiv) and TBTU (80 mg, 1 equiv). After being stirred for 15 min, the mixture was treated with dipeptide hydrochloride **14** (112 mg, 1 equiv, 0.25 mmol), followed by DIEA (87 μL, 2 equiv, 0.5 mmol), and stirred at room temperature for 6 h. Evaporation of the volatiles gave a residue, which was purified by flash chromatography on silica gel using 5% MeOH in DCM as eluant. Evaporation of the collected fractions afforded 146 mg (0.21 mmol, 84%) of benzyl ester **15** as a pale yellow foam. R_f = 0.42 (10% MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ −41.5° (*c* 0.65, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29−1.44 (m, 3H), 1.47 (s, 9H), 1.81−1.87 (m, 1H), 1.97−2.07 (m, 2H), 2.26−2.32 (m, 2H), 2.52 (d, *J* = 5.6 Hz, 2H), 2.79−2.84 (dd, *J* = 8, 13.6 Hz, 1H), 2.86−2.99 (m, 2H), 3.16−3.21 (dd, *J* = 4.8, 14.4 Hz, 1H), 3.49−3.54 (m, 1H), 4.07−4.13 (q, *J* = 8 Hz, 1H), 4.37 (d, *J* = 8.8 Hz, 1H), 4.47−4.52 (m, 1H), 4.55−4.59 (m, 1H), 5.09 (d, *J* = 12.4 Hz, 1H), 5.12 (d, *J* = 12.4 Hz, 1H), 5.85 (d, *J* = 6.8 Hz, 1H), 7.06 (d, *J* = 8 Hz, 1H), 7.13−7.22 (m, 6 H), 7.26−7.40 (m, 5H), 7.54 (d, *J* = 6,4 Hz, 1H), 7.66 (d, *J* = 8 Hz, 1H), 8.39 (s, 1H), 8.45 (d, *J* = 4.8 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.9, 25.3, 26.9, 28.6, 34.7, 38.0, 40.5, 47.1, 48.2, 54.4, 61.5, 62.3, 66.7, 123.8, 126.9, 127.8, 128.6, 128.7, 128.8, 128.9, 130.3, 130.4, 132.2, 133.6, 136.2, 137.3, 138.1, 148.2, 150.7, 151.4, 154.6, 169.6, 170.1, 171.5, 172.5. HRMS *m*/*z* calcd for $C_{39}H_{48}N_5O_7$ $[M + H]^+$ 698.3548, found 698.3541.

(3*S*,6*S*,9*S*)-I^{2}aa-(2*S*)-(3-Pyridyl)alaninyl-(3*S*)-β-homophenylalanine Benzyl Ester Hydrochloride (16). *N*-Boc-(3*S*,6*S*,9*S*)-I^{2}aa-(2*S*)-(3-pyridyl)alaninyl-(3*S*)-β-homophenylalanine benzyl ester **15** (50 mg, 1 equiv, 72 μmol) was treated with a solution of 25% TFA in CH_2Cl_2 for 2 h at room temperature. Evaporation of the volatiles under reduced pressure followed by coevaporation three more times from dichloromethane gave a residue, which was purified by preparative HPLC using a reverse phase C18 column as described in General Methods. Freeze-drying of the collected fractions gave amino ester **16** (32 mg, 77%) as a fluffy white solid. R_f = 0.2 (10% MeOH/CH_2Cl_2); $[\alpha]_D$ 53° (*c* 0.65, CH_3OH). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.41−1.60 (m, 2H), 1.80−2.10 (m, 5H), 2.30 (m, 1H), 2.55 (m, 2H), 2.79−2.99 (m, 3H), 3.13 (m, 1H), 3.49 (m, 1H), 3.78 (m, 1H), 4.39 (m, 1H), 4.51 (m, 1H), 4.64 (m, 1H), 5.04 (d, *J* = 12.4 Hz, 1H), 5.09 (d, *J* = 12.4 Hz, 1H), 6.91 (m, 2H), 7.08−7.42 (m, 12 H), 7.55 (s, 1H), 7.98 (s, 1H), 8.36 (s, 1H), 8.48 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.3, 28.8, 32.2, 34.7, 37.9, 40.3, 48.1, 54.9, 58.5, 60.0, 66.9, 109.4, 127.0, 128.7, 128.9, 129.0, 129.7, 136.1, 137.9, 138.0, 170.5, 171.4, 171.8. HRMS *m*/*z* calcd for $C_{34}H_{41}N_5O_5$ $[M + H]^+$ 598.3024, found 598.3011.

Phenylacetyl-(3*S*,6*S*,9*S*)-I^{2}aa-(2*S*)-(3-pyridyl)alaninyl-(3*S*)-β-homophenylalanine Benzyl Ester (11). A solution of phenylacetyl chloride (3.2 μL, 1.2 equiv, 24 μmol) was added to amine **16** (14 mg, 1 equiv, 20 μmol) dissolved in 10 mL of dichloromethane followed by DIEA (7 μL, 2equiv, 40 μmol) and stirred at room temperature for 6 h. Evaporation of the volatiles gave a residue, which was purified by preparative HPLC to afford 11.4 mg (80%) of ester **11** as a yellow oil. $[\alpha]_D$ −37° (*c* 0.75, $CHCl_3$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27−1.35 (m, 3H), 1.84 (m, 1H), 1.98−2.05 (m, 2H), 2.25−2.32 (m, 2H), 2.55 (d, *J* = 5.6 Hz, 2H), 2.82−2.95 (m, 3H), 3.13 (dd, *J* = 4.0, 14.4 Hz, 1H), 3.49−3.54 (m, 1H), 3.68 (s, 1H), 4.30−4.38 (m, 2H), 4.46−4.55 (m, 2H), 5.10 (d, *J* = 12 Hz, 1H), 5.13 (d, *J* = 12 Hz, 1H), 7.08−7.39 (m, 18 H), 7.55 (d, *J* = 6.4 Hz, 1H), 7.72 (d, *J* = 7.6 Hz, 1H), 8.38 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.0, 27.5, 27.7, 30.1, 32.5, 37.7, 40.4, 44.0, 47.8, 49.2, 55.2, 58.2, 59.9, 66.9, 127.1, 127.6, 128.7, 128.8, 128.9, 129.0, 129.3, 129.7, 129.8, 135.3, 136.1, 137.9, 170.2, 171.3, 171.7, 171.9. HRMS *m*/*z* calcd for $C_{42}H_{46}N_5O_6$ $[M + H]^+$ 716.3442, found 716.3438.

Benzhydrylidene Aza-glycinyl-(2*S*)-prolyl-(2*S*)-3-pyridinylalaninyl-(3*S*)-β-homophenylalanine Benzyl Ester (18). A solution of benzhydrylidene aza-glycinyl-(2*S*)-proline (**17**, 93 mg, 0.28 mmol) in 10 mL of dichloromethane was treated with HOBt (37 mg, 0.28 mmol) and TBTU (89 mg, 0.28 mmol). After being stirred for 10 min, the mixture was treated with (3-pyridyl)alaninyl-β-homophenylalaninyl benzyl ester hydrochloride (**14**, 125 mg, 0.28 mmol) and DIEA (144 μL, 0.828 mmol) and stirred for 6−8 h. Evaporation of the volatiles gave a residue, which was purified by flash chromatography on silica gel using 5% MeOH in DCM as eluant. Evaporation of the collected fractions afforded benzyl ester **18** (120 mg, 0.19 mmol, 70%) as a yellow oil. R_f = 0.53 (10% MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ −74.4° (*c* 1.25, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$) δ 1.71−1.84 (m, 3H), 1.91−2.03 (m, 1H), 2.44−2.56 (m, 2H), 2.73−2.90 (m, 3H), 3.10−3.19 (m, 3H), 4.39 (m, 1H), 4.47−4.58 (m, 2H), 5.02 (d, J = 12.4 Hz, 1H), 5.06 (d, J = 12.4 Hz, 1H), 7.06−7.20 (m, 6H), 7.23−7.36 (m, 12 H), 7.48−7.56 (m, 6H), 7.77 (s, 1H), 8.28 (s, 1H), 8.31 (d, J = 4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.9, 25.3, 28.6, 34.7, 38.0, 40.5, 48.2, 54.4, 61.5, 66.7, 123.8, 126.9, 127.8, 128.6, 128.7, 128.8, 128.9, 130.3, 130.4, 132.2, 133.6, 136.2, 137.3, 138.1, 148.2, 150.7, 151.4, 154.6, 169.6, 170.1, 171.5, 172.5. HRMS m/z calcd for $C_{44}H_{45}N_6O_5$ [M + H$^+$] 737.3446, found 737.3455.

Phenylacetyl-aza-glycinyl-(2*S*)-prolyl-(2*S*)-3-pyridinylalaninyl-(3*S*)-β-homophenylalanine (6). Benzyl ester **18** (60 mg, 81.5 μmol) in 5 mL of THF was treated with 5 mL of 1 N HCl at 60 °C for 12−24 h, when LC−MS analysis showed total conversion of **18** to aza-glycinylprolinyl-(3-pyridyl)alaninyl-β-homophenylalanine ([M + H]$^+$ = 483). The volatiles were evaporated. The residue was dissolved in 5 mL of dichloromethane, treated with phenylacetyl chloride (11 μL, 81.5 μmol) and DIEA (42.5 μL, 0.25 mmol), stirred for 3−4 h, and evaporated to dryness. Purification was performed by preparative HPLC (Phenomenex Gemini 5 μm, C18, 250 mm × 21.2 mm) using a gradient from 10% to 90% methanol (containing 0.1% TFA) in water (containing 0.1% TFA) to afford 10 mg (21%) of azapeptide **6**. ^{1}H NMR (700 MHz, D_2O) δ 1.51−1.54 (m, 1H), 1.65 (m, 1H), 1.82−1.86 (m, 1H), 2.00−2.05 (m, 1H), 2.28−2.33 (m, 2H), 2.46−2.49 (dd, J = 4.8, 15.8 Hz, 1H), 2.63−2.66 (dd, J = 5.0, 13.9 Hz, 1H), 2.89−2.93 (dd, J = 9.0, 14.4 Hz, 1H), 2.98−3.01 (dd, J = 6.3, 14.5 Hz, 1H), 3.24−3.28 (dd, J = 7.6, 9.3 Hz, 1H), 3.35−3.38 (m, 1H), 3.51 (d, J = 15.1 Hz, 1H), 3.55 (d, J = 15.1 Hz, 1H), 4.11 (dd, J = 4.1, 8.8 Hz, 1H), 4.22 (m, 1H), 4.38 (dd, J = 6.4, 8.9 Hz, 1H), 7.06−7.72 (m, 10 H), 7.74 (t, J = 6.7 Hz, 1H), 8.12 (d, J = 8.0 Hz, 1H), 8.31 (s, 1H), 8.40 (d, J = 5.0 Hz, 1H); ^{13}C NMR (175 MHz, D_2O) δ 26.3, 29.6, 33.7, 38.9, 39.9, 40.0, 46.2, 48.4, 53.6, 60.7, 126.6, 126.9, 127.3, 127.8, 128.4, 128.9, 129.1, 129.2, 129.4, 133.9, 137.4, 137.9, 139.6, 140.4, 147.3, 157.2, 170.3, 174.9, 175.1, 175.2. HRMS m/z calcd for $C_{32}H_{37}N_6O_6$ [M + H]$^+$ 601.2769 found 601.2772. RP-HPLC system 1: 99%, t_R= 6.88 min. RP-HPLC system 3: 97.1%, t_R= 8.76 min.

Aza-propargylglycinylproline *tert*-Butyl Ester (20a). For the synthesis of **20b**, benzhydrylidene aza-propargylglycinyl-proline *tert*-butyl ester **19a** (100 mg, 0.23 mmol) was stirred with hydroxylamine hydrochloride (29 mg, 0.42 mmol) in 20 mL of pyridine overnight at 60 °C. The volatiles were removed by rotary evaporation followed by coevaporation with dichloromethane and ethyl acetate until solidification. Purification by flash chromatography on silica gel using a 1:1 mixture of ethyl acetate in hexane afforded amine **20a** (51.3 mg, 84%) as a pale yellow foam: R_f = 0.39 (hexane/EtOAc 1:1); $[\alpha]_D^{20}$ −48° (c 1.15, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.46 (s, 9H), 1.84−1.99 (m, 3H), 2.13−2.20 (m, 1H), 2.31 (s, 1H), 3.64 (m, 2H), 4.16 (d, J = 17.2 Hz, 1H), 4.24 (d, J = 17.2 Hz, 1H), 4.43 (m, 1H); ^{13}C NMR ($CDCl_3$,100 MHz) δ 23.3, 27.6, 30.1, 42.6, 48.8, 61.5, 73.0, 78.1, 80.3, 159.8, 172.5. HRMS m/z calcd for $C_{13}H_{21}N_3O_3Na$ [M + Na]$^+$ 290.1475, found 290.1488.

Aza-phenylalaninylproline *tert*-Butyl Ester (20b). 20b was prepared from benzhydrylidene aza-phenylalaninylproline *tert*-butyl ester **19b** (50 mg, 0.1 mmol). Purification of the residue by flash chromatography on silica gel using a 1:1 mixture of ethyl acetate in hexane afforded amine **20b** (28 mg, 88%) as a pale yellow foam: R_f = 0.31 (hexane/EtOAc 1:1); $[\alpha]_D^{20}$ −35° (c 0.1, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.44 (s, 9H), 1.83−1.95 (m, 3H), 2.12−2.19 (m, 1H), 3.64−3.67 (m, 2H), 4.46 (m, 1H), 4.59 (d, J = 16 Hz, 1H), 4.61 (d, J = 16 Hz, 1H), 7.28−7.35 (m, 5H); ^{13}C NMR ($CDCl_3$,100 MHz) δ 23.4, 27.6, 30.1, 49.1, 56.1, 61.7, 80.1, 127.2, 127.3, 128.1, 128.3, 136.3, 172.7. HRMS (ESI) m/z calcd for $C_{17}H_{26}N_3O_3$ [M + H]$^+$ 320.1971; found, 320.1969.

Aza-allylglycinylproline *tert*-Butyl Ester (20c). 20c was prepared according to the procedure described above from benzhydrylidene aza-allylglycinylproline *tert*-butyl ester **19c** (191.9 mg, 0.43 mmol). Purification of the residue by flash chromatography on silica gel using a 1:1 mixture of ethyl acetate in hexane afforded amine **20c** (54.5 mg, 47%) as a pale yellow foam: R_f = 0.22 (EtOAc); $[\alpha]_D^{20}$ −38.5° (c 2.05, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.44 (s, 9H), 1.79−1.94 (m, 3H), 2.11−2.15 (m, 1H), 3.56−3.62 (m, 2H), 3.73 (br, 2H), 3.95 (d, J = 5.0 Hz, 2H), 4.38−4.41 (dd, J = 4.3, 8.4 Hz, 1H), 5.23−5.27 (m, 2H), 5.81−5.88 (m, 1H); ^{13}C NMR ($CDCl_3$,100 MHz) δ 23.4, 27.6, 30.0, 48.9, 55.4, 61.5, 80.1, 118.6, 132.6, 160.6, 172.6. HRMS m/z calcd for $C_{13}H_{24}N_3O_3$ [M + H]$^+$ 270.1812, found 270.1814.

Aza-alaninyl-(2*S*)-proline *tert*-Butyl Ester (20d). 20d was prepared according to the procedure described above from benzhydrylidene aza-alalinylproline *tert*-butyl ester **19d** (200 mg, 0.51 mmol). Purification of the residue by flash chromatography on silica gel using a 5% methanol in dichloromethane afforded amine **20d** (59.5 mg, 48%) as a yellow oil: R_f = 0.25 (5% MeOH/CH_2Cl_2); $[\alpha]_D^{20}$ −24° (c 3.03, $CHCl_3$). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.43 (s, 9H), 1.80−1.95 (m, 3H), 2.11−2.14 (m, 1H), 3.04 (s, 3H), 3.55 (t, J = 6.8 Hz, 2H), 3.99 (br, 2H), 4.33−4.37 (dd, J = 4.8, 8.0 Hz, 1H); ^{13}C NMR ($CDCl_3$,100 MHz) δ 23.6, 27.6, 29.9, 41.6, 45.6, 48.8, 80.2, 161.5, 172.6. HRMS m/z calcd for $C_{11}H_{22}N_3O_3$ [M + H]$^+$ 244.1656, found 244.1650.

Representative Protocol for Phenylacetylation of Azadipeptide *tert*-Butyl Esters: Phenylacetyl-aza-propargylglycinyl-(2*S*)-proline *tert*-Butyl Ester (21a). Aza-propargylglycinylproline *tert*-butyl ester (**20a**, 60 mg, 0.23 mmol) was dissolved in 10 mL of EtOAc, treated with phenyl acetyl chloride (32 μL, 0.24 mmol) and DIEA (46 μL, 0.26 mmol), stirred 12 h, and washed with 10 mL of 1 N HCl and 10 mL of brine. After evaporation of the volatiles the crude material was purified by chromatography on silica gel using a mixture of ethyl acetate in hexane (1:1) to afford ester **21a** (85.5 mg, 98% yield) as a yellow oil. R_f = 0.32 (hexane/EtOAc 1:1); $[\alpha]_D^{20}$ 47.4° (c 1.9, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, 9H), 1.73−1.90 (m, 3H), 2.05−2.09 (m, 1H), 2.15 (t, J = 2.4 Hz, 1H), 3.33 (m, 2H), 3.61 (s, 2H), 4.20−4.24 (m, 3H), 7.29−7.34 (m, 5H), 7.91 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.2, 28.4, 30.0, 40.0, 42.1, 48.9, 61.8, 73.4, 78.6, 81.7, 127.9, 129.4, 129.6, 134.1, 158.8, 170.0, 172.4. HRMS m/z calcd for $C_{21}H_{27}N_3O_4Na$ [M + Na]$^+$ 408.1894, found 408.1903.

Phenylacetyl-aza-phenylalaninyl-(2*S*)-proline *tert*-Butyl Ester (21b). 21b was obtained from azadipeptide **20b** (50 mg, 0.16 mmol) as described above in 60% yield as a yellow oil. R_f = 0.31 (hexane/EtOAc 1:1); $[\alpha]_D^{25}$ 30.4° (c 1.25, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.47 (s, 9H), 1.76−1.83 (m, 2H), 1.88−1.92 (m, 1H), 2.05−2.16 (m, 1H), 3.35−3.39 (m, 1H), 3.43−3.57 (m, 3H), 4.34 (t, J = 7.2 Hz, 1H), 4.43 (d, J = 14.2 Hz, 1H), 4.71 (d, J = 14.2 Hz, 1H), 7.10−7.13 (m, 2H), 7.17−7.19 (m, 2H), 7.24−7.28 (m, 7H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.7, 27.6, 29.2, 41.4, 48.4, 52.7, 61.1, 80.9, 127.0, 127.3, 128.1, 128.2, 128.6, 128.9, 129.0, 133.2, 135.5, 158.9, 168.9, 171.9. HRMS m/z calcd for $C_{25}H_{32}N_3O_4$ [M + H]$^+$ 438.2387, found 438.2379.

Phenylacetyl-aza-allylglycinyl-(2*S*)-proline *tert*-Butyl Ester (21c). 21c was obtained from azadipeptide **20c** (50 mg, 0.19 mmol) as described above in 90% yield as a yellow oil. R_f = 0.25 (hexane/EtOAc 1:1); $[\alpha]_D^{25}$ 36.6° (c 1.75, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 9H), 1.75−1.92 (m, 3H), 2.09−2.18 (m, 1H), 3.29−3.36 (m, 1H), 3.38−3.47 (m, 1H), 3.55 (s, 2H), 3.84−3.89 (dd, J = 8, 16 Hz, 1H), 3.98−4.03 (dd, J = 8, 16 Hz, 1H), 4.28 (t, J = 7.2 Hz, 1H), 5.01 (dd, J = 1.2, 16.8 Hz, 1H), 5.07 (dd, J = 1.2, 10.0 Hz, 1H), 5.79 (m, 1H), 7.26−7.33 (m, 5H), 7.65 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.5, 28.4, 30.0, 42.3, 49.1, 53.7, 61.7, 81.7, 120.1, 127.9, 129.3, 129.6, 132.7, 134.3, 159.7, 169.9, 172.6. HRMS m/z calcd for $C_{21}H_{29}N_3O_4Na$ [M + Na]$^+$ 410.2050, found 410.2058.

Phenylacetyl-aza-alaninyl-(2*S*)-proline *tert*-Butyl Ester (21d). 21d was obtained from azadipeptide **20d** (59 mg, 0.24 mmol) as described above in 75% yield as a yellow oil. R_f = 0.45 (EtOAc); $[\alpha]_D^{25}$ 27° (c 1.75, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 (s, 9H), 1.74−1.78 (m, 2H), 1.86 (m, 1H), 2.11 (m, 1H), 3.01 (s, 3H), 3.29

(m, 1H), 3.37 (m, 1H), 3.56 (s, 2H), 4.24 (t, J = 6.8 Hz, 1H), 7.28−7.33 (m, 5H), 8.28 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.8, 27.6, 29.2, 38.3, 41.2, 48.4, 60.9, 80.9, 127.0, 128.5, 128.8, 133.7, 159.5, 168.9, 172.0. HRMS m/z calcd for $C_{19}H_{28}N_3O_4$ $[M + H]^+$ 362.2074, found 362.2074.

Representative Protocol for Azapeptide Benzyl Ester Synthesis. Phenylacetyl-aza-propargylglycinyl-(2*S*)-prolyl-(2*S*)-3-pyridylalaninyl-(3*S*)-β-homophenylalanine Benzyl Ester (23a). Phenylacetyl-aza-propargylglycinyl-(2*S*)-proline *tert*-butyl ester (**21a**, 85 mg, 0.22 mmol) was treated with a solution of 9:1 TFA in dichloromethane and stirred for 1 h at room temperature until complete disappearance of starting material. The mixture was then evaporated and coevaporated 3 times with dichloromethane, then dissolved in EtOAc. The organic phase was extracted with saturated $NaHCO_3$, and the basic aqueous phase was acidified with 1 N HCl and then back-extracted with 5 × 10 mL of EtOAc. After evaporation of the volatiles, crude acid **22a** was dissolved in THF (7 mL), cooled to −15 °C, treated sequentially with isobutyl chloroformate (31 μL, 0.242 mmol) and *N*-methylmorpholine (36 μL, 0.33 mmol), stirred for 15 min, and treated with a solution of (3-pyridyl)alaninyl-β-homophenylalanine benzyl ester hydrochloride (**14**, 111 mg, 0.27 mmol) in ethyl acetate (5 mL). After the mixture was stirred at −15 °C for 1 h, the volatiles were removed under reduced pressure and the crude material was purified by chromatography on silica gel using ethyl acetate as eluant to afford benzyl ester **23a** (64 mg, 40% yield) as a pale yellow foam. R_f = 0.42 (10% MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ 14.7° (c 1.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.13−1.28 (m, 1H), 1.61−1.69 (m, 2H), 2.17 (m, 1H), 2.32 (s, 1H), 2.59−2.71 (m, 2H), 2.81−2.84 (m, 2H), 2.85−2.99 (m, 2H), 3.26 (d, J = 12.8 Hz, 1H), 3.36 (t, J = 8 Hz, 1H), 3.64−3.76 (m, 2H), 3.82 (d, J = 17.2 Hz, 1H), 4.24−4.28 (dd, J = 7.2, 10.4 Hz, 1H), 4.41 (d, J = 16.8 Hz, 1H), 4.55 (m, 2H), 5.09 (dd, J = 12.4, 17.6 Hz, 2H), 7.16 (m, 6H), 7.25−7.35 (m, 11H), 7.41 (d, J = 7.6 Hz, 1H), 7.57 (d, J = 8.4 Hz, 1H), 8.42 (s, 2H), 9.98 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.1, 30.0, 30.1, 34.4, 38.9, 40.7, 41.2, 48.5, 50.4, 54.8, 63.4, 66.8, 74.5, 78.3, 123.8, 126.9, 128.0, 128.6, 128.7, 129.4, 129.7, 130.1, 134.2, 134.5, 136.1, 136.3, 136.8, 138.1, 148.1, 150.6, 160.3, 171.0, 171.1, 171.2, 172.1. HRMS m/z calcd for $C_{42}H_{45}N_6O_6$ $[M + H]^+$ 729.3395, found 729.3382.

Phenylacetyl-aza-phenylalaninyl-(2*S*)-prolyl-(2*S*)-3-pyridylalaninyl-(3*S*)-β-homophenylalanine Benzyl Ester (23b). **23b** was obtained from ester **21b** (42 mg, 96 μmol) as described above in 48% yield as a pale yellow foam. R_f = 0.35 (10% MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ 9.3° (c 1.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28 (m, 1H), 1.69−1.74 (m, 2H), 2.19−2.21 (m, 1H), 2.64−2.67 (m, 2H), 2.87−3.01 (m, 3H), 3.07 (m, 1H), 3.22 (d, J = 12.4 Hz, 1H), 3.38 (t, J = 8 Hz, 1H), 3.61 (s, 2H), 3.89 (d, J = 13.6 Hz, 1H), 4.30−4.34 (dd, J = 6.8, 10.2 Hz, 1H), 4.55−4.59 (m, 2H), 5.03−5.14 (m, 3H), 7.12−7.35 (m, 22H), 7.51 (d, J = 7.2 Hz, 1H), 7.60 (d, J = 8.8 Hz, 1H), 8.34 (br, 2H), 8.72 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.4, 29.4, 33.5, 38.3, 40.0, 40.7, 47.7, 49.8, 53.7, 53.9, 62.7, 66.0, 126.0, 127.4, 127.7, 127.8, 127.9, 128.0, 128.1, 128.4, 128.7, 128.8, 128.9, 129.3, 132.9, 135.1, 135.6, 137.6, 160.3, 169.8, 169.9, 170.5, 171.3. HRMS m/z calcd for $C_{46}H_{49}N_6O_6$ $[M + H]^+$ 781.3708, found 781.3727.

Phenylacetyl-aza-allylglycinyl-(2*S*)-prolyl-(2*S*)-3-pyridylalaninyl-(3*S*)-β-homophenylalanine Benzyl Ester (23c). **23c** was obtained from ester **21c** (66 mg, 0.17 mmol) as described above in 55% yield as a yellow foam. R_f = 0.40 (10% MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ −10.6° (c 1.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27 (m, 1H), 1.67−1.74 (m, 2H), 2.16−2.21 (m, 1H), 2.59−2.70 (m, 2H), 2.82−2.96 (m, 3H), 3.02−3.08 (m, 1H), 3.27−3.37 (m, 2H), 3.54−3.59 (dd, J = 8.8, 14.4 Hz, 1H), 3.68 (s, 2H), 4.28−4.32 (m, 2H), 4.54−4.59 (m, 2H), 5.05−5.15 (m, 4H), 5.68−5.77 (m, 1H), 7.19−7.21 (m, 6H), 7.28−7.36 (m, 11H), 7.52 (d, J = 8 Hz, 1H), 7.58 (d, J = 8 Hz, 1H), 8.43 (br, 2H), 8.69 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.1, 30.1, 34.3, 39.0, 40.8, 41.6, 48.5, 50.4, 53.7, 54.7, 63.3, 66.8, 120.7, 126.8, 128.2, 128.5, 128.7, 128.8, 128.9, 129.5, 129.6, 130.0, 132.7, 133.9, 136.4, 138.4, 160.7, 170.6, 170.7, 171.2, 172.1. HRMS m/z calcd for $C_{42}H_{47}N_6O_6$ $[M + H]^+$ 731.5552, found 731.3541.

Phenylacetyl-aza-alaninyl-(2*S*)-prolyl-(2*S*)-3-pyridylalaninyl-(3*S*)-β-homophenylalanine Benzyl Ester (23d). **23d** was obtained from ester **21d** (65 mg, 0.18 mmol) as described above in 40% yield as a pale yellow foam. R_f = 0.32 (10% MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ −19.6° (c 2.8, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27 (m, 1H), 1.67−1.70 (m, 2H), 2.15−2.17 (m, 1H), 2.63−2.71 (m, 2H), 2.81−2.84 (m, 2H), 2.86−2.97 (m, 5H), 3.22−3.32 (m, 2H), 3.67 (s, 2H), 4.25−4.29 (dd, J = 6.8, 10.4 Hz, 1H), 4.52−4.60 (m, 2H), 5.09 (d, J = 12.4 Hz, 1H), 5.13 (d, J = 12.4 Hz, 1H), 7.17−7.19 (m, 6H), 7.27−7.42 (m, 13H), 7.58 (d, J = 8.8 Hz, 1H), 8.35−8.43 (s, 1H), 9.27 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.2, 29.9, 34.4, 39.2, 41.0, 41.4, 48.6, 50.3, 54.8, 63.4, 66.8, 126.8, 128.0, 128.5, 128.7, 128.9, 129.4, 129.5, 130.1, 136.4, 136.9, 138.4, 147.9, 161.4, 170.6, 171.3, 172.4. HRMS m/z calcd for $C_{40}H_{45}N_6O_6$ $[M + H]^+$ 705.3395, found 705.3392.

Phenylacetyl-aza-propargylglycinyl-(2*S*)-prolyl-(2*S*)-3-(pyridyl)-alaninyl-(3*S*)-β-homophenylalanine (7). Phenylacetyl-aza-propargylglycinyl-(2*S*)-prolyl-(2*S*)-3-pyridylalaninyl-(3*S*)-β-homophenylalanine benzyl ester (**23a**, 25 mg, 0.034 mmol) was dissolved in a minimum amount of dioxane, cooled to 0 °C, treated with a 2 N LiOH (5 mL), and stirred for 30 min, and the volatiles were evaporated under reduce pressure. The aqueous residue was acidified to pH 5 using 1 N HCl and extracted with ethyl acetate. The organic extractions were combined, dried with sodium sulfate, and concentrated under vacuum. The residue was purified by preparative HPLC on a C18 reverse-phase column, using methanol in water as eluant. Freeze-drying of the collected fractions gave acid **7** (14.6 mg, 67%) as a pale yellow oil: R_f = 0.24 (10% MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ 9.5° (c 1.05, CH_3OH); ^{1}H NMR (700 MHz, CH_3OD) δ 1.20 (m, 1H), 1.79−1.81 (m, 2H), 2.17 (m, 1H), 2.57 (d, J = 6.9 Hz, 1H), 2.72−2.78 (dd, J = 8.3, 13.6 Hz, 1H), 2.79 (t, J = 2.5 Hz, 1H), 2.81 (m, 1H), 2.98−3.01 (dd, J = 4.9, 13.7 Hz, 1H), 3.06−3.09 (dd, J = 4.1, 14.3 Hz, 1H), 3.16 (m, 1H), 3.47−3.49 (m, 1H), 3.72 (d, J = 14.5 Hz, 1H), 3.76 (d, J = 14.5 Hz, 1H), 3.82−3.89 (m, 1H), 4.22−4.25 (dd, J = 6.9, 10.4 Hz, 1H), 4.37−4.39 (dd, J = 4.0, 11.3 Hz, 1H), 4.47−4.49 (m, 2H), 7.14 (m, 3H), 7.22 (m, 2H), 7.28 (t, J = 7.4 Hz, 1H), 7.34 (m, 3H), 7.42 (m, 2H), 7.53 (d, J = 7.2 Hz, 1H), 8.25 (s, 1H), 8.40 (d, J = 4.8 Hz, 1H), 8.47 (s, 1H); ^{13}C NMR (175 MHz, CH_3OD) δ 25.1, 29.5, 33.6, 38.6, 39.8, 39.9, 47.3, 48.3, 49.8, 54.6, 62.7, 73.7, 77.4, 123.8, 126.0, 127.1, 127.9, 128.5, 129.1, 129.5, 134.4, 134.5, 137.3, 137.9, 146.9, 149.2, 160.0, 170.7, 171.0, 173.1, 173.8. HRMS m/z calcd for $C_{35}H_{39}N_6O_6$ $[M + H]^+$ 639.2926, found 639.2942. RP-HPLC system 1: 98.9%, t_R= 7.96 min. RP-HPLC system 3: 98.5%, t_R= 11.86 min.

Phenylacetyl-aza-phenylalaninyl-(2*S*)-prolyl-(2*S*)-(3-pyridyl)-alaninyl-(3*S*)-β-homophenylalanine (8). **8** was obtained from **23b** (31 mg, 0.039 mmol) as described above in 66% yield: R_f = 0.32 (10% MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ 12.4° (c 2.5, CH_3OH); ^{1}H NMR (700 MHz, CH_3OD) δ 1.16−1.23 (m, 1H), 1.79−1.83 (m, 2H), 2.18−2.20 (m, 1H), 2.61 (m, 2H), 2.79−2.82 (dd, J = 8.7, 13.1 Hz, 1H), 2.86−2.90 (m, 1H), 3.02 (d, J = 10.9 Hz, 1H), 3.12 (d, J = 12.7 Hz, 1H), 3.17−3.21 (dd, J = 9.8, 17.1 Hz, 1H), 3.48 (m, 1H), 3.61 (s, 2H), 3.93 (m, 1H), 4.28−4.31 (dd, J = 6.9, 10.6 Hz, 1H), 4.41 (d, J = 7.9 Hz, 1H), 4.52 (m, 1H), 5.11 (m, 1H), 7.17−7.18 (m, 3H), 7.21 (m, 2H), 7.28−7.37 (m, 11H), 7.59 (s, 1H), 8.31 (s, 1H), 8.42 (s, 1H); ^{13}C NMR (175 MHz, CH_3OD) δ 25.2, 29.4, 29.5, 33.6, 39.8, 40.0, 48.4, 50.0, 54.0, 54.6, 62.9, 126.1, 127.1, 127.5, 128.0, 128.3, 128.5, 129.1, 129.2, 129.5, 134.4, 135.9, 138.1, 161.0, 170.7, 170.8, 173.3. HRMS m/z calcd for $C_{39}H_{43}N_6O_6$ $[M + H]^+$ 691.3238, found 691.3231. RP-HPLC system 1: 97.4%, t_R= 8.84 min. RP-HPLC system 3: 98.9%, t_R= 13.99 min.

Phenylacetyl-aza-allylglycinyl-(2*S*)-prolyl-(2*S*)-(3-pyridyl) alaninyl-(3*S*)-β-homophenylalanine (9). **9** was obtained from **23c** (18 mg, 0.025 mmol) as described above in 72% yield: R_f = 0.28 (10% MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ −54.2° (c 1.2, CH_3OH); ^{1}H NMR

(700 MHz, CH_3OD) δ 1.18 (m, 1H), 1.77−1.82 (m, 2H), 2.17−2.28 (m, 1H), 2.57 (m, 2H), 2.73−2.76 (dd, J = 8.9, 13.4 Hz, 1H), 2.81−2.87 (m, 1H), 2.98−3.02 (dd, J = 3.7, 13.5 Hz, 1H), 3.06−3.08 (d, J = 12.5 Hz, 1H), 3.16−3.20 (q, J = 5.8 Hz, 1H), 3.48 (m, 1H), 3.64 (br, 1H), 3.67 (d, J = 14.4 Hz, 1H), 3.70 (d, J = 14.5 Hz, 1H), 4.24−4.27 (dd, J = 6.9, 10.5 Hz, 1H), 4.38 (d, J = 7.8 Hz, 2H), 4.49 (m, 1H), 5.15 (dd, J = 1.0, 17.2 Hz, 1H), 5.21−5.23 (d, J = 10.2 Hz, 1H), 5.88−5.92 (m, 1H), 7.16 (m, 3H), 7.24−7.28 (m, 3H), 7.32−7.34 (m, 3H), 7.38 (m, 2H), 7.56 (s, 1H), 8.29 (s, 1H), 8.40 (s, 1H); ^{13}C NMR (175 MHz, CH_3OD) δ 25.2, 29.5, 39.9, 40.1, 48.4, 49.9, 53.2, 54.6, 62.7, 118.8, 126.0, 127.1, 127.9, 128.4, 129.1, 129.4, 132.6, 134.4, 138.1, 161.0, 170.7, 170.8, 173.3. HRMS m/z calcd for $C_{35}H_{41}N_6O_6$ $[M + H]^+$ 641.3082, found 641.3086. RP-HPLC system 1: 95.8%, t_R= 8.20 min. RP-HPLC system 3: 98.7%, t_R= 12.68 min.

Phenylacetyl-aza-alaninyl-(2*S*)-prolyl-(2*S*)-(3-pyridyl)alaninyl-(3*S*)-β-homophenylalanine (10). **10** was obtained from ester **23d** (16.7 mg, 0.024 mmol) as described above in 85% yield: R_f = 0.21 (10% MeOH/CH_2Cl_2); $[\alpha]_D^{25}$ −100° (c 1.1, CH_3OH); ^{1}H NMR (700 MHz, CH_3OD) δ 1.13−1.19 (m, 1H), 1.75−1.80 (m, 2H), 2.16−2.18 (m, 1H), 2.60 (m, 2H), 2.72−2.75 (dd, J = 8.9, 13.4 Hz, 1H), 2.80−2.82 (m, 1H), 2.97−2.99 (dd, J = 4.5, 13.6 Hz, 1H), 3.05 (s, 3H), 3.06 (s, 1H), 3.10−3.14 (m, 1H), 3.45 (t, J = 8.7 Hz, 1H), 3.70 (d, J = 14.6 Hz, 1H), 3.71 (d, J = 14.5 Hz, 1H), 4.22−4.25 (dd, J = 6.8, 10.7 Hz, 1H), 4.36−4.39 (dd, J = 3.6, 11.2 Hz, 1H), 4.49 (m, 1H), 7.12−7.18 (m, 3H), 7.20−7.29 (m, 3H), 7.32−7.34 (m, 3H), 7.38−7.41 (m, 2H), 7.57 (s, 1H), 8.25 (s, 1H), 8.48 (s, 1H); ^{13}C NMR (175 MHz, CH_3OD) δ 29.5, 30.8, 33.5, 37.8, 39.9, 40.2, 48.3, 49.8, 54.5, 62.8, 123.8, 126.0, 127.1, 127.9, 128.5, 128.9, 129.0, 129.2, 129.4, 134.4, 134.6, 137.3, 138.0, 146.8, 149.1, 161.1, 170.6, 170.7, 173.3. HRMS m/z calcd for $C_{33}H_{39}N_6O_6$ $[M + H]^+$ 615.2926, found 615.2937. RP-HPLC system 1: 98.6%, t_R= 7.78 min. RP-HPLC system 3: 99%, t_R= 11.58 min.

Myometrial Contraction Assay. Ex vivo myometrial contraction assay was performed as previously described.[24] Briefly, uteri from mice were obtained from animals immediately after delivery. Myometrial strips (2−3 mm wide and 1−2 cm long) were suspended in organ baths containing Krebs buffer equilibrated with 21% oxygen at 37 °C with an initial tension, and peak, duration, and frequency of spontaneous contraction in the absence or in presence of PGF2α and indolizidin-2-one **1** or mimics **2**−**10** were recorded with a Kent digital polygraph system.

MAP Kinase Activation. PGF2α was obtained from Cayman. Mouse monoclonal anti-p-ERK and rabbit polyclonal anti-total ERK antibodies were from Cell Signaling. HEK293 cells stably transfected with HA-FP (HA-FP cells)[24] were employed to measure activation of MAP kinase by PGF2α using conventional Western blot methods. Briefly, HA-FP cells in six-well plates were starved for 30 min and pretreated with 1 μM indolizidin-2-one **1** or an azapeptide (**6**−**8**) for 30 min and then challenged with PGF2α (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% SDS−PAGE 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 as previously described.[24]

Cell Ruffling. Cell ruffling was performed as previously described.[24] Briefly, serum-starved FP cells plated on coverslips were pretreated or not with indolizidin-2-one **1** or azapeptide (**6**−**8**) for 30 min at 37 °C, then stimulated with 1 μM PGF2α for 30 min, fixed with 4% paraformaldehyde (PFA), and stained with Alexa Fluor 488 phalloidin. Nine fields (50−75 cells/field) per coverslip were quantified to assess circular cellular ruffling.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. ^{1}H NMR and ^{13}C NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +1 514 343 7339. Fax: +1 514-343-7586. E-mail: William.Lubell@umontreal.ca.

■ ACKNOWLEDGMENT

The authors thank the Natural Sciences and Engineering Research Council of Canada (NSERC), the Fonds Québécois de la Recherche sur la Nature et les Technologies (FQRNT), and the Canadian Institutes of Health Research (Grant CTP-79848) for financial support. We thank Erin Choi for help with some MAPK assays. We thank Dr. Alexandra Fürtös, Marie-Christine Tang, and Karine Venne of the Université de Montréal Mass Spectrometry Facility for mass spectral analyses and Sylvie Bilodeau and Cedric Malveau of the Regional High-Field NMR Laboratory for aid with NMR spectroscopy. T.E.H. is a Chercheur National of the Fonds de la Recherche en Santé du Québec (FRSQ).

■ ABBREVIATIONS USED

FP, prostaglandin F2α receptor; PGF2α, prostaglandin F2α; OT, oxytocin

■ REFERENCES

(1) Slattery, M. M.; Morrison, J. J. Preterm delivery. *Lancet* **2002**, *360*, 1489–1497.

(2) Goldenberg, R. L.; Culhane, J. F.; Iams, J. D.; Romero, R. Preterm birth 1: epidemiology and causes of preterm birth. *Lancet* **2008**, *371*, 75–84.

(3) Martin, J. A.; Kochanek, K. D.; Strobino, D. M.; Guyer, B.; MacDorman, M. F. Annual summary of vital statistics: 2003. *Pediatrics* **2005**, *115*, 619–634.

(4) Russell, R. B.; Green, N. S.; Steiner, C. A.; Meikle, S.; Howse, J. L.; Poschman, K.; Dias, T.; Potetz, L.; Davidoff, M. J.; Damus, K.; Petrini, J. R. Cost of hospitalization for preterm and low birth weight infants in the United States. *Pediatrics* **2007**, *120*, E1–E9.

(5) McCormick, M. C. The contribution of low birth-weight to infant-mortality and childhood morbidity. *N. Engl. J. Med.* **1985**, *312*, 82–90.

(6) Hack, M.; Flannery, D. J.; Schluchter, M.; Cartar, L.; Borawski, E.; Klein, N. Outcomes in young adulthood for very-low-birth-weight infants. *N. Engl. J. Med.* **2002**, *346*, 149–157.

(7) Robertson, P. A.; Sniderman, S. H.; Laros, R. K.; Cowan, R.; Heilbron, D.; Goldenberg, R. L.; Iams, J. D.; Creasy, R. K. Neonatal morbidity according to gestational-age and birth-weight from 5 tertiary care centers in the United-States, 1983 through 1986. *Am. J. Obstet. Gynecol.* **1992**, *166*, 1629–1645.

(8) RichEdwards, J. W.; Stampfer, M. J.; Manson, J. E.; Rosner, B.; Hankinson, S. E.; Colditz, G. A.; Willett, W. C.; Hennekens, C. H. Birth weight and risk of cardiovascular disease in a cohort of women followed up since 1976. *Br. Med. J.* **1997**, *315*, 396–400.

(9) Iams, J. D.; Romero, R.; Culhane, J. F.; Goldenberg, R. L. Preterm birth 2: primary, secondary, and tertiary interventions to reduce the morbidity and mortality of preterm birth. *Lancet* **2008**, *371*, 164–175.

(10) Anotayanonth, S.; Subhedar, N. V.; Garner, P.; Neilson, J. P.; Harigopal, S. Betamimetics for inhibiting preterm labour. *Cochrane Database Syst. Rev.* **2004**No. CD004352.

(11) Mittendorf, R.; Covert, R.; Boman, J.; Khoshnood, B.; Lee, K. S.; Siegler, M. Is tocolytic magnesium sulphate associated with increased total paediatric mortality? *Lancet* **1997**, *350*, 1517–1518.

(12) Papatsonis, D.; Flenady, V.; Cole, S.; Liley, H. Oxytocin receptor antagonists for inhibiting preterm labour. *Cochrane Database Syst. Rev.* **2005**No. CD004452.

(13) Goodwin, T. M.; Valenzuela, G.; Silver, H.; Hayashi, R.; Creasy, G. W.; Lane, R. Treatment of preterm labor with the oxytocin antagonist atosiban. *Am. J. Perinatol.* **1996**, *13*, 143–146.

(14) Duchateau, F. X.; Max, A.; Harscoat, S.; Curac, S.; Ricard-Hibon, A.; Mantz, J. Comparison between atosiban and nicardipine in inducing hypotension during in-utero transfers for threatening premature delivery. *Eur. J. Emerg. Med.* **2010**, *17*, 142–145.

(15) King, J. F.; Flenady, V.; Papatsonis, D.; Dekker, G.; Carbonne, B. Calcium channel blockers for inhibiting preterm labour; a systematic review of the evidence and a protocol for administration of nifedipine. *Aust. N. Z. J. Obstet. Gynaecol.* **2003**, *43*, 192–198.

(16) Coomarasamy, A.; Knox, E. M.; Gee, H.; Song, F. J.; Khan, K. S. Effectiveness of nifedipine versus atosiban for tocolysis in preterm labour: a meta-analysis with an indirect comparison of randomised trials. *BJOG* **2003**, *110*, 1045–1049.

(17) Higby, K.; Xenakis, E. M. J.; Pauerstein, C. J.; Harbert, G. M.; Jones, H.; Merkatz, I. R.; Creasy; Woods, J.; Cefalo, R. C.; Gibbs, R. S.; Scott, S.; Queenan, J. T.; Kirschbaum; Nelson, K. Do tocolytic agents stop preterm labor: a critical and comprehensive review of efficacy and safety. *Am. J. Obstet. Gynecol.* **1993**, *168*, 1247–1259.

(18) Haas, D. M.; Imperiale, T. F.; Kirkpatrick, P. R.; Klein, R. W.; Zollinger, T. W.; Golichowski, A. M. Tocolytic Therapy: a meta-analysis and decision analysis. *Obstet. Gynecol.* **2009**, *113*, 585–594.

(19) Marshall, C. The art of induction/augmentation of labor. *J. Obstet. Gynecol. Neonat. Nurs.* **1985**, *14*, 22–8.

(20) Olson, D. M. The role of prostaglandins in the initiation of parturition. *Best Pract. Res., Clin. Obstet. Gynaecol.* **2003**, *17*, 717–730.

(21) Brodt-Eppley, J.; Myatt, L. Prostaglandin receptors in lower segment myometrium during gestation and labor. *Obstet. Gynecol.* **1999**, *93*, 89–93.

(22) Sugimoto, Y.; Segi, E.; Tsuboi, K.; Ichikawa, A.; Narumiya, S. Female Reproduction in Mice Lacking the Prostaglandin F Receptor. Roles of Prostaglandin and Oxytocin Receptors in Parturition. In *Vasopressin and Oxytocin: Molecular, Cellular, and Clinical Advances*; Zingg, H. H., Bourque, C. W., Bichet, D. G., Eds.; Plenum Press: New York, 1998; Vol. 449, pp 317−321.

(23) Sugimoto, Y.; Yamasaki, A.; Segi, E.; Tsuboi, K.; Aze, Y.; Nishimura, T.; Oida, H.; Yoshida, N.; Tanaka, T.; Katsuyama, M.; Hasumoto, K.; Murata, T.; Hirata, M.; Ushikubi, F.; Negishi, M.; Ichikawa, A.; Narumiya, S. Failure of parturition in mice lacking the prostaglandin F receptor. *Science* **1997**, *277*, 681–683.

(24) Goupil, E.; Tassy, D.; Bourguet, C.; Quiniou, C.; Wisehart, V.; Petrin, D.; Le Gouill, C.; Devost, D.; Zingg, H. H.; Bouvier, M.; Saragovi, H. U.; Chemtob, S.; Lubell, W. D.; Claing, A.; Hebert, T. E.; Laporte, S. A. A novel biased allosteric compound inhibitor of parturition selectively impedes the prostaglandin F2 alpha-mediated Rho/ROCK signaling pathway. *J. Biol. Chem.* **2010**, *285*, 25624–25636.

(25) Presland, J. Identifying novel modulators of G protein-coupled receptors via interaction at allosteric sites. *Curr. Opin. Drug Discovery Dev.* **2005**, *8*, 567–576.

(26) Kenakin, T. P. Ligand detection in the allosteric world. *J. Biomol. Screening* **2010**, *15*, 119–130.

(27) Peri, K. G.; Quiniou, C.; Hou, X.; Abran, D.; Varma, D. R.; Lubell, W. D.; Chemtob, S. THG113: a novel selective FP antagonist that delays preterm labor. *Semin. Perinatol.* **2002**, *26*, 389–397.

(28) Peri, K.; Polyak, F.; Lubell, W.; Thouin, E.; Chemtob, S. Peptides and Peptidomimetics Useful for Inhibiting the Avtivity of Prostaglandin F2α Receptor. WO/2003/104266, 2003.

(29) Hirst, J. J.; Parkington, H. C.; Young, I. R.; Palliser, H. K.; Peri, K. G.; Olson, D. M. Delay of preterm birth in sheep by THG113.31, a prostaglandin F2 alpha receptor antagonist. *Am. J. Obstet. Gynecol.* **2005**, *193*, 256–266.

(30) Hummel, G.; Reineke, U.; Reimer, U. Translating peptides into small molecules. *Mol. BioSyst.* **2006**, *2*, 499–508.

(31) Vlieghe, P.; Lisowski, V.; Martinez, J.; Khrestchatisky, M. Synthetic therapeutic peptides: science and market. *Drug Discovery Today* **2010**, *15*, 40–56.

(32) Edwards, C. M. B.; Cohen, M. A.; Bloom, S. R. Peptides as drugs. *QJM* **1999**, *92*, 1–4.

(33) Lombart, H. G.; Lubell, W. D. Rigid dipeptide mimetics: efficient synthesis of enantiopure indolizidinone amino acids. *J. Org. Chem.* **1996**, *61*, 9437–9446.

(34) Halab, L.; Becker, J. A. J.; Darula, Z.; Tourwe, D.; Kieffer, B. L.; Simonin, F.; Lubell, W. D. Probing opioid receptor interactions with azacycloalkane amino acids. Synthesis of a potent and selective ORL1 antagonist. *J. Med. Chem.* **2002**, *45*, 5353–5357.

(35) Polyak, F.; Lubell, W. D. Rigid dipeptide mimics: synthesis of enantiopure 5- and 7-benzyl and 5,7-dibenzyl indolizidinone amino acids via enolization and alkylation of delta-oxo alpha,omega-di-*N*-(9-(9-phenylfluorenyl))amino azelate esters. *J. Org. Chem.* **1998**, *63*, 5937–5949.

(36) Gosselin, F.; Lubell, W. D. Rigid dipeptide surrogates: syntheses of enantiopure quinolizidinone and pyrroloazepinone amino acids from a common diaminodicarboxylate precursor. *J. Org. Chem.* **2000**, *65*, 2163–2171.

(37) Gosselin, F.; Lubell, W. D. An olefination entry for the synthesis of enantiopure alpha,omega-diaminodicarboxylates and azabicyclo X. Y.0 alkane amino acids. *J. Org. Chem.* **1998**, *63*, 7463–7471.

(38) Degrado, W. F.; Kaiser, E. T. Polymer-bound oxime esters as supports for solid-phase peptide synthesis. Preparation of protected peptide-fragments. *J. Org. Chem.* **1980**, *45*, 1295–1300.

(39) Kaiser, E. T.; Mihara, H.; Laforet, G. A.; Kelly, J. W.; Walters, L.; Findeis, M. A.; Sasaki, T. Peptide and protein synthesis by segment synthesis−condensation. *Science* **1989**, *243*, 187–192.

(40) Andre, F.; Boussard, G.; Bayeul, D.; Didierjean, C.; Aubry, A.; Marraud, M. Aza-peptides. 2. X-ray structures of aza-alanine and aza-asparagine-containing peptides. *J. Pept. Res.* **1997**, *49*, 556–562.

(41) Reviewed in the following: Proulx, C.; Sabatino, D.; Hopewell, R.; Spiegel, J.; García-Ramos, Y.; Lubell, W. D. Azapeptides and their therapeutic potential: current issues and new possibilities. *Future Med. Chem.* **2011**, *3*, 1139–1164.

(42) Lee, H. J.; Choi, K. H.; Ahn, I. A.; Ro, S.; Jang, H. G.; Choi, Y. S.; Lee, K. B. The beta-turn preferential solution conformation of a tetrapeptide containing an azaamino acid residue. *J. Mol. Struct.* **2001**, *569*, 43–54.

(43) Thormann, M.; Hofmann, H. J. Conformational properties of azapeptides. *J. Mol. Struct.: THEOCHEM* **1999**, *469*, 63–76.

(44) Boeglin, D.; Hamdan, F. F.; Melendez, R. E.; Cluzeau, J.; Laperriere, A.; Heroux, M.; Bouvier, M.; Lubell, W. D. Calcitonin gene-related peptide analogues with aza and indolizidinone amino acid residues reveal conformational requirements for antagonist activity at the human calcitonin gene-related peptide 1 receptor. *J. Med. Chem.* **2007**, *50*, 1401–1408.

(45) Bourguet, C. B.; Sabatino, D.; Lubell, W. D. Benzophenone semicarbazone protection strategy for synthesis of aza-glycine containing aza-peptides. *Biopolymers* **2008**, *90*, 824–831.

(46) Bourguet, C. B.; Proulx, C.; Klocek, S.; Sabatino, D.; Lubell, W. D. Solution-phase submonomer diversification of aza-dipeptide building blocks and their application in aza-peptide and aza-DKP synthesis. *J. Pept. Sci.* **2010**, *16*, 284–296.

(47) Sabatino, D.; Proulx, C.; Klocek, S.; Bourguet, C. B.; Boeglin, D.; Ong, H.; Lubell, W. D. Exploring side-chain diversity by submonomer solid-phase aza-peptide synthesis. *Org. Lett.* **2009**, *11*, 3650–3653.

(48) Bourguet, C. B.; Hou, X.; Chemtob, S.; Lubell, W. D. Influence of Peptide Mimic Turn Stereochemistry on Allosteric Antagonism at the Prostaglandin F2α Receptor. *Peptides 2006, Proceedings of the 29th European Peptide Symposium 215*; Rolka, K., Rekowski, P., Silberring, J., Eds.; The European Peptide Society, 2006; pp 436−437.

(49) Bourguet, C. B.; Hou, X.; Chemtob, S.; Lubell, W. D. Exploring the Relationship between Turn Geometry and Allosteric Antagonism of Peptide Mimic Ligands for the Prostaglandin F2 Alpha Receptor.

Peptides for Youth, Proceedings of the 20th American Peptide Symposium; Del Valle, S. E., Escher, E., Lubell, W. D., Eds.; Advances in Experimental Medicine and Biology, Vol. 611; Springer Science: New York, 2009; pp 271−273.

(50) Linder, M. R.; Steurer, S.; Podlech, J. (*S*)-3-(*tert*-Butyloxycarbonylamino)-4-phenylbutanoic acid. *Org. Synth.* **2002**, *79*, 154.