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Synthesis and Evaluation of Azapeptide Prostaglandin $F_{2\alpha}$ Receptor Modulators in Pursuit of Inhibitors of Preterm Labor

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Introduction

The incidence of preterm birth has been steadily increasing over the past thirty years, and presently, preterm delivery accounts for >75% of all neonatal deaths [1]. The prostaglandin $F_{2\alpha}$ receptor (FP) is responsible for initiating labor and preterm labor [2]. In pursuit of drugs to delay uterine contractions and prolong pregnancy, FP has been targeted based on peptide leads using a peptidomimetic approach. For example, the indolizidin-2-one amino acid analogue PDC113.824 (**1**) [3] and aza-phenylalanine analogue **2** (Figure 1) [4], both reduced prostaglandin-$F_{2\alpha}$-induced myometrial contractions by modulating G protein signaling pathways. Studies of structure-activity relationships of aza-amino acyl proline modulators such as **2** have shown that biological activity was contingent on the aza-amino acyl residue side chain [4]. Considering that structural modification of the aza-residue may have consequences on function, we are exploring diversity-oriented synthesis featuring alkylation of the aza-glycinyl-proline residue to introduce different side chains onto the aza-peptide modulators. In this report, we describe the synthesis of aza-*p*-methylphenylalanine analogue **3**.

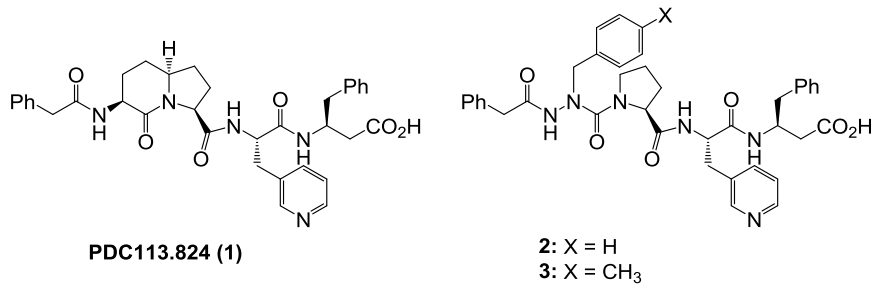


Fig. 1. PDC113.824 and aza-amino acyl proline analogues.

Results and Discussion

In light of the activity of azaPhe analog **2**, a variety of benzyl analogues have been targeted and are being pursued by solution-phase sub-monomer aza-peptide synthesis [4]. The synthesis and alkylation of aza-glycinyl proline *tert*-butyl ester **4** has provided access to azadipeptide building blocks (e.g., **8**), which have been coupled to (2*S*)-(3-pyridyl)alaninyl-(3*S*)-*β*-homophenylalanine benzyl ester hydrochloride **9** to provide the penultimate azapeptide benzyl ester (e.g. **10**, Scheme 1). Aza-glycinyl proline *tert*-butyl ester **4** was synthesized as reported [6] and treated with potassium *tert*-butoxide and 4-methyl benzyl bromide to provide aza-(4-Me)Phe analog **5** in 84% yield [5,6]. Benzhydrylidene removal using hydroxylamine hydrochloride in pyridine [6], acylation of the resulting semicarbazide with phenylacetyl chloride, and *tert*-butyl ester removal in a TFA/DCM solution afforded the *N*-terminal aza-dipeptide **8**. Coupling of aza-dipeptide **8** to dipeptide benzyl ester **9** [4] was performed using *iso*-butyl chloroformate and *N*-methyl morpholine to furnish aza-peptide benzyl ester **10**. Saponification of benzyl ester **10** with LiOH in dioxane gave aza-peptide **3**, which was purified to >98% purity by HPLC using a gradient of 70 to 90% aqueous 0.1% formic acid (FA) in MeOH with 0.1% FA over 20 min at a flow rate of 0.5 mL/min: HRMS m/z calculated for $C_{39}H_{43}N_6O_6$ $[M + H]^+$ 705.3395, found 705.3394.

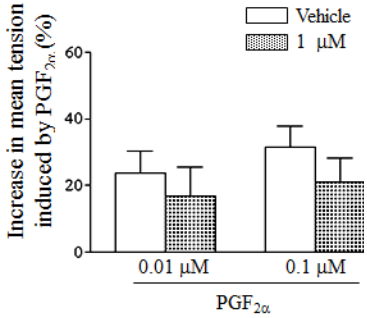
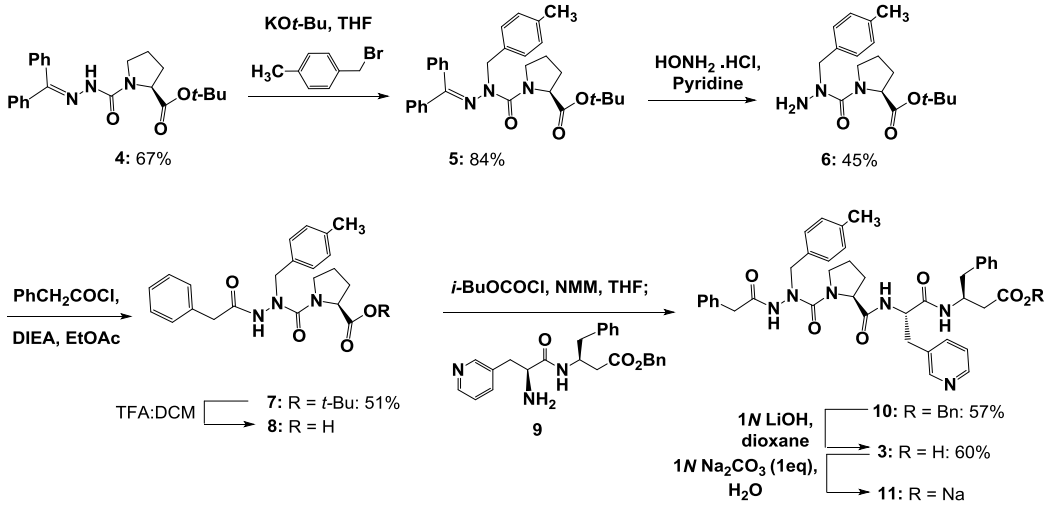


*Fig. 2. Preliminary test of biological activity of sodium salt **11**.*

Due to limited solubility in water, azapeptide **3** was converted to sodium salt **11** by titrating with a 1N solution of Na_2CO_3. In the myometrial contraction assay, in contrast to aza-Phe analogue **2**, sodium salt **11** did not significantly reduce the mean tension of $PGF_{2\alpha}$-induced myometrial contractions.

Conclusion

In conclusion, we have synthesized aza-4-methylphenylalanine peptide **11**, and examined its ability to curb myometrial contractions. The subtle addition of a *para*-methyl substituent onto aza-Phe analogue **2** significantly diminished antagonistic activity, illustrating the consequences of aromatic structure on modulator ability. The synthesis and further examination of other aromatic analogs are ongoing to further probe the structural requirements for modulating FP activity.



*Scheme 1. Synthesis of aza-4-methylphenylalanine analogue **11**.*

Acknowledgments

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