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Synthesis and Antiplatelet Activity of DMP 757 Analogs

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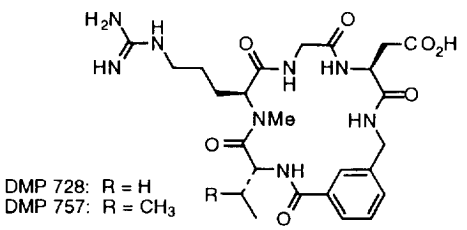
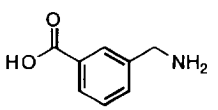
Abstract. A series of novel cyclic peptides related to DMP 757 bearing heterocyclic and otherwise modified linking moieties were prepared by solution-phase methods. Synthetic methods for the preparation of linking groups and cyclic peptides are presented. *In vitro* data for the purpose of QSAR is discussed.

Platelet activation, adhesion, and aggregation are important events in hemostasis and, when poorly controlled, in the pathophysiology of thrombosis.[1] The binding of fibrinogen to the activated form of glycoprotein IIb-IIIa (GP IIb-IIIa) is the obligatory event resulting in platelet aggregation.[2] As was demonstrated in man with the monoclonal antibody ReoPro, antagonism of GP IIb-IIIa represents an attractive therapy for the treatment of arterial thrombosis.[3]

We recently described the discovery of a novel class of template-constrained cyclic peptides represented by DMP 728 and DMP 757 that were potent and selective GP IIb-IIIa receptor antagonists.[4] These compounds contained a highly optimized tetrapeptide framework cyclized through a *m*-(aminomethyl)benzoic acid (Mamb, **1**) linker moiety. Encouraged by the finding of oral bioavailability in DMP 728, we undertook efforts to improve the receptor affinity and *in vivo* profile of this class of compounds through the preparation of novel cyclic peptides containing structural alternatives to **1**.





1

In DMP 728, DMP 757, and other potent compounds of this series, the backbone conformation is highly rigid and is dominated by a Type II' β-turn centered at the D-Abu(D-Val)-N^{α}-methylArg bond, an extended Gly residue and a C_7 turn centered at the Asp residue.[5] The Mamb moiety had been described in the peptide-mimetic literature as a *trans*-dipeptide isostere,[6] and it was thought that this characteristic might have helped to stabilize the extended conformation of the Gly residue. Upon examination of this model, it was reasoned that replacement of the Mamb moiety with heterocyclic analogs wherein the heteroatom is situated between the carboxyl and

aminomethyl groups might increase binding affinity through further rigidization of the peptide backbone *via* intramolecular hydrogen bonding. Regardless of this possibility, we desired to further explore the SAR with respect to the linker moiety.

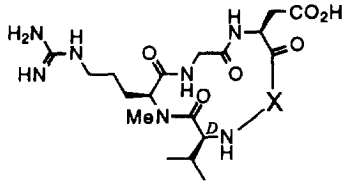
Results and Discussion

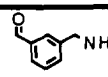
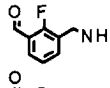
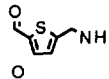
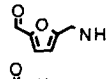
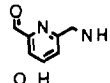
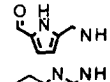
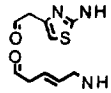
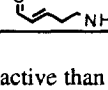
Biology

All compounds were assayed for inhibition of platelet aggregation *in vitro* using human platelet rich plasma (200 μL, 2-3 x 10^8 platelets/mL, n = 1) with ADP (10 μM) as the agonist.[4]

An examination of Table 1 reveals that the closest analog to DMP 757, 2-fluoro analog **2a**, was the most active compound of this series. Since as configured, IC_{50} values for compounds more potent than about 0.02 μM could not be determined using the PRP assay, **2a** was compared to DMP 757 in the fibrinogen binding assay.[7] In this assay, **2a** and DMP 757 had IC_{50} values of 0.0040 and 0.0060 μM, respectively. While the fluoro-substituent had little effect on potency, it indicated that electron withdrawing groups at this position could be tolerated, and that unproductive hydrogen bonding arrangements were not created. Thiophene **2b** was prepared as a bioisostere of phenyl having little propensity for hydrogen bonding. Somewhat surprisingly, it was 5-fold less active than DMP 757. Replacement of the phenyl moiety with heterocycles capable of forming strong hydrogen bonds (**2c-f**) resulted in compounds having reduced activity *in vitro*. The observed 2- to 20-fold loss in activity was likely due to the stabilization of non-optimal turn structures and/or Gly conformations, leading to reduced complementarity of the antagonist with the receptor. The reduced activity of pyridine **2d** also suggested a preference for a lipophilic linking moiety, and the possible presence of a lipophilic binding pocket. In thiazole **2f**, the position of the heterocylic moiety was shifted by one atom. Thiazole **2f** was 20-fold less active than DMP 757, and 4-fold less active than the corresponding *m*-aminophenyl acetate.[8] We next evaluated the *in vitro* activity of alkenes **2g** and **2h**. These acyclic linking groups had the effect of relaxing the conformational constraints imposed by the cyclic linkers, however, they were 10-fold less active than DMP 757, and likely allowed too much conformational flexibility.

Table 1. Inhibition of Platelet Aggregation



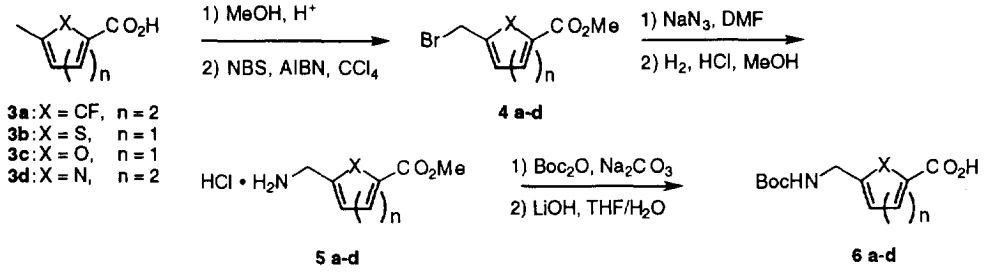
Cmpd	X	hPRP IC_{50} (μM)
DMP 757		0.02
2a		0.02
2b		0.1
2c		0.05
2d		2.0
2e		0.1
2f		0.4
2g		0.2
2h		0.2

Chemistry

The synthesis of the 2-fluoro-Mamb (**6a**), thiophene (**6b**), furan (**6c**), and pyridine (**6d**) linkers were carried out according to Scheme 1. Esterification of the appropriate carboxylic acid (**3**) was followed by benzylic

bromination using NBS to give bromide **4**. Treatment of **4** with NaN_3 in DMF followed by catalytic hydrogenation then gave the amino ester **5**. Protection of the amine as the Boc-derivative was followed by saponification of the ester, affording the linker **6** in a protected form ready for incorporation into the cyclic peptide.

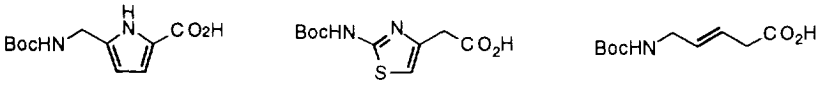
Scheme 1



The pyrrole linker (**6e**) was prepared from methyl 5-formyl-2-pyrrolecarboxylate[9] by conversion to the oxime and subsequent reduction, Boc-protection, and saponification.

The corresponding thiazole linker (**6f**) was prepared by Boc-protection and saponification of methyl 2-amino-4-thiazoleacetate hydrochloride.

The β,γ-unsaturated acid linker (**6g**) was prepared *via* Schmidt reaction of *trans*-β-hydromuconic acid[10] followed by protection of the amino group as the Boc-derivative.

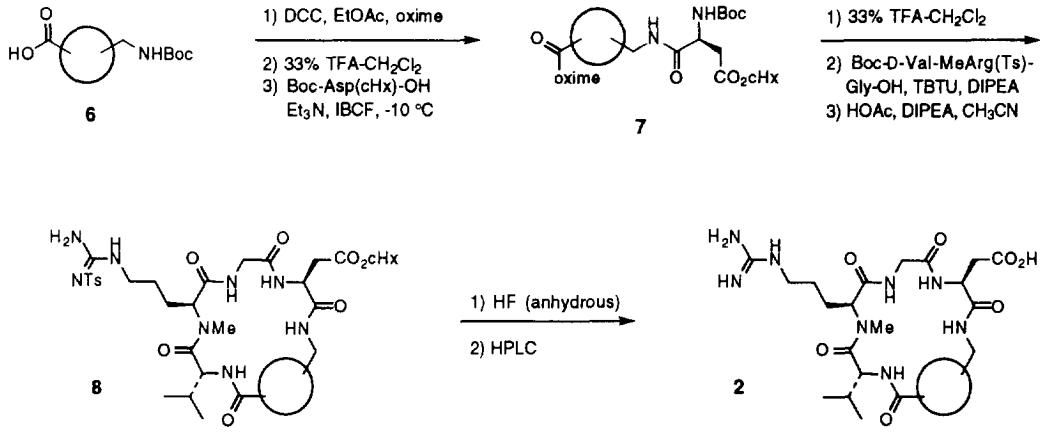


6e **6f** **6g**

The synthesis of the cyclic peptides is illustrated in Scheme 2, and generally follows that described.[4a] The Boc-protected linker (**6**) was condensed with *p*-nitrobenzophenone oxime, deprotected using TFA, and coupled with Boc-Asp(cHx)-OH to give oxime ester **7**. Boc-deprotection, followed by coupling to Boc-D-Val-N^{α}-MeArg(Ts)-Gly-OH, Boc-deprotection, and cyclization in acetonitrile using a Hünig's base-acetic acid buffer then gave the protected cyclic peptide **8**. The choice of cyclization between the D-Val residue and the activated linker was dictated in part by the direction taken by our ongoing analoging program, and the desire to prevent epimerization during cyclization. This protocol worked well except for the preparation of alkene **8g**, in which preliminary couplings of **7g** with Boc-D-Val-N^{α}-MeArg(Ts)-Gly-OH failed due to isomerization of the β,γ-olefin and subsequent side reactions. Protected cyclic peptide **8g** was prepared by coupling **6g** with Boc-D-Val-N^{α}-MeArg(Ts)-Gly-Asp(cHx)-OH, followed by Boc-deprotection, and cyclization as above. Following removal of the tosyl and cyclohexyl protecting groups by treatment with anhydrous HF, or in certain cases using triflic acid,[11] the crude, deprotected product was purified using reverse-phase HPLC (water-acetonitrile gradient containing 0.1% TFA) to afford the final product **2** as the TFA salt. These deprotection methods worked

satisfactorily for all compounds excepting the protected β,γ-unsaturated amide **8g**, which when reacted under the former conditions gave a 2:1 mixture of the desired **2g** and the α,β-unsaturated amide **2h**.

Scheme 2



In conclusion, we prepared a number of DMP 757 analogs using solution phase chemistry, but were unable to improve upon the *in vitro* activity of DMP 757. The linker units themselves may also be of interest for their potential use as *trans*-amide dipeptide bioisosteres or as building blocks within a combinatorial library approach to drug discovery.

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