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P_1, P_2'-LINKED MACROCYCLIC AMINE DERIVATIVES AS MATRIX METALLOPROTEINASE INHIBITORS

James J.-W. Duan,* Lihua Chen, Chu-Biao Xue, Zelda R. Wasserman, Karl D. Hardman, Maryanne B. Covington,[1] Robert R. Copeland,[1] Elizabeth C. Arner,[1] and Carl P. Decicco

DuPont Pharmaceuticals Company
Department of Chemical and Physical Sciences
Experimental Station, Wilmington, Delaware 19880-0500, U.S.A.

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Abstract: A novel series of 13- and 14-membered macrocyclic amines was developed by linking the P_1 and P_2' groups. The synthesis entails stereoselective Frater alkylation to install the *anti*-succinate configuration and macrocyclic amination via nucleophilic displacement. This strategy resulted in a new class of conformationally constrained inhibitors that are potent and selective for MMP-8 and 9 over MMP-1 and 3.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent enzymes involved in normal remodeling of the extracellular matrix.[2] They are expressed as inactive zymogens and are activated by numerous proteases. Under normal conditions MMPs are tightly regulated by endogenous inhibitors including tissue inhibitors of metalloproteinases (TIMPs) and α2-macroglobulin. Increased levels of MMPs have been observed in areas of connective tissue remodeling or breakdown in diseases such as osteoarthritis, rheumatoid arthritis, cancer, inflammatory bowel diseases, periodontal disease and corneal ulceration.[3] Small molecule inhibitors of MMPs therefore represent attractive targets of therapeutic importance. In this communication, we disclose a new series of macrocyclic amine derived MMP inhibitors.[4]

Design: Succinate based hydroxamic acids (e.g. BB-16, Figure 1) represent an extensively studied class of MMP inhibitors.[5] In the crystal structure of BB-16/MMP-3 complex,[6a] the peptide backbone of the inhibitor adopts an extended conformation, directing the isobutyl group into the S_1' specificity pocket and the methyl (P_1) and *p*-methoxyphenyl (P_2') groups in the opposite direction. Exploiting this conformational feature, we envisioned the possibility of linking the P_1 and P_2' groups without perturbing the crucial P_1'-S_1' inhibitor/enzyme interactions. The resultant macrocyclic templates may stabilize amides from enzymatic degradation.[7] In 1998, our labs disclosed first examples of macrocyclic MMP inhibitors based on this concept employing macrolactam and paracyclophane templates.[6] More recently, researchers at Abbott published a related series of paracyclophanes.[8,9]

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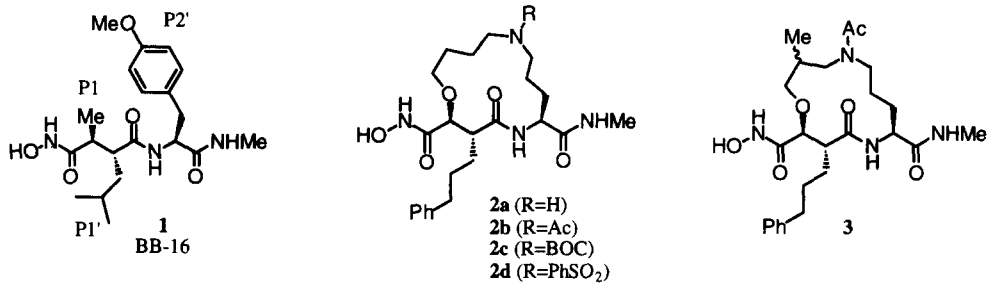
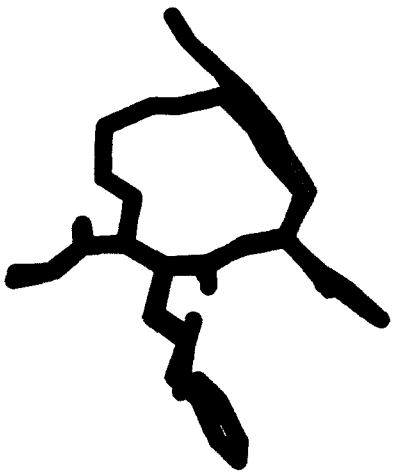
Figure 1



Since the P_1 and P_2' groups extend to solvent and the solvent exposed area is large, we felt that linkers of various sizes and with substitutions should be tolerated and thus designed macrocyclic amines **2** and **3**. The presence of the amino group in the linker provides a point for further derivatization to modify the physical properties of these inhibitors. A computationally-derived model of **2a**/MMP-3 complex (Figure 2) overlays well with the bound conformation of BB-16. The model is also consistent with the design philosophy in that the linker projects into solvent and should therefore tolerate further branching.

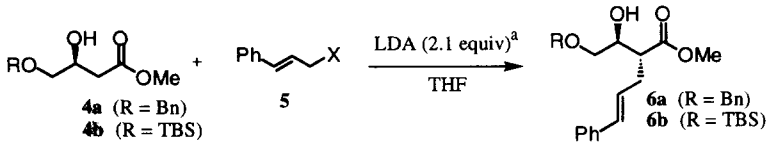
Figure 2



Computer model of **2a** (black) in the active site of MMP-3 overlayed on the X-ray crystal structure of BB-16 (grey). The computer model was generated by placing **2a** in the active site of MMP-3 and relaxing the structure with 20 ps of molecular dynamics computer simulation using the grid-based method of Luty et al.[10] The conformation shown is the average during the final 2 ps of the simulation. Protein not shown for clarity.

Synthesis: To construct the succinate fragment of **2** and **3**, we investigated the Frater alkylation[11] of **4a**[12] and **4b**[13] to install a cinnamyl group with the desired *anti* configuration. Good stereochemical control (8:1, *anti:syn*) was maintained under several sets of conditions (Table 1). Addition of TMEDA had no effect on selectivity, but improved the reaction yield to 80%. Cinnamyl iodide and commercially available cinnamyl bromide gave comparable results. Protecting groups on γ-oxygen (Bn vs TBS) had no effect on the stereochemical outcome. These data support the 6-membered dianion cyclic intermediate proposed by Frater.[11]

Table 1



4a (R = Bn)
4b (R = TBS)

5

6a (R = Bn)
6b (R = TBS)

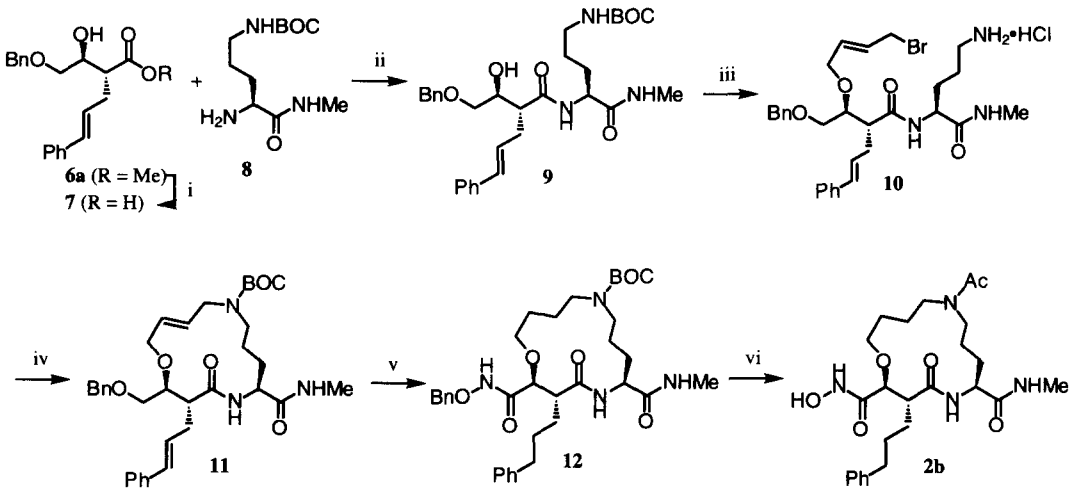
entry	starting material	product	X	yield (%)	ratio (*anti:syn*)[c]
1	**4a**	**6a**	I	62	8:1
2[b]	**4a**	**6a**	I	80	8:1
3[b]	**4a**	**6a**	Br	73	8:1
4	**4b**	**6b**	I	83	7:1

[a] In a typical procedure, **4** was added to freshly prepared LDA at -78 °C. The mixture was stirred at -45 °C for 1 h and -20 °C for 30 min and cooled to -78 °C. Following addition of **5**, the mixture was stirred at -45 °C for 2 h and -20 °C for 1 h. [b] 2 equiv of TMEDA was added immediately following addition of **5**. [c] Ratio of *anti-syn* diastereomers was determined by ^{1}H NMR analysis of the crude mixture.

The macrocyclic precursor **10** was synthesized from **6a** via selective alkylation of **9** with *trans*-1,4-dibromo-2-butene and NaH (Scheme 1).[14] By design the allylic bromide group in **10** should facilitate the macrocyclization. This crucial intramolecular nucleophilic displacement reaction was investigated under several base/solvent combinations. Hunig's base in acetonitrile proved most effective and provided the 14-membered **11** in 56% yield upon addition of $(BOC)_2O$ at the end of the cyclization. We initially attempted hydrogenation of the two double bonds and hydrogenolysis of the benzyl ether in **11** with $Pd(OH)_2/C$. The desired product was obtained in only 55% yield due to competitive C-O bond cleavage of the crotyl ether moiety. Eventually the yield was improved to 79% with a one-pot protocol by first hydrogenation of the double bonds with Pd/C then hydrogenolysis of the benzyl ether with $Pd(OH)_2/C$. The resultant primary alcohol was oxidized to the acid under Sharpless $RuCl_3/NaIO_4$ conditions[15] and coupled with $BnONH_2$. Compound **12** was used as a common intermediate to prepare **2a~d**.

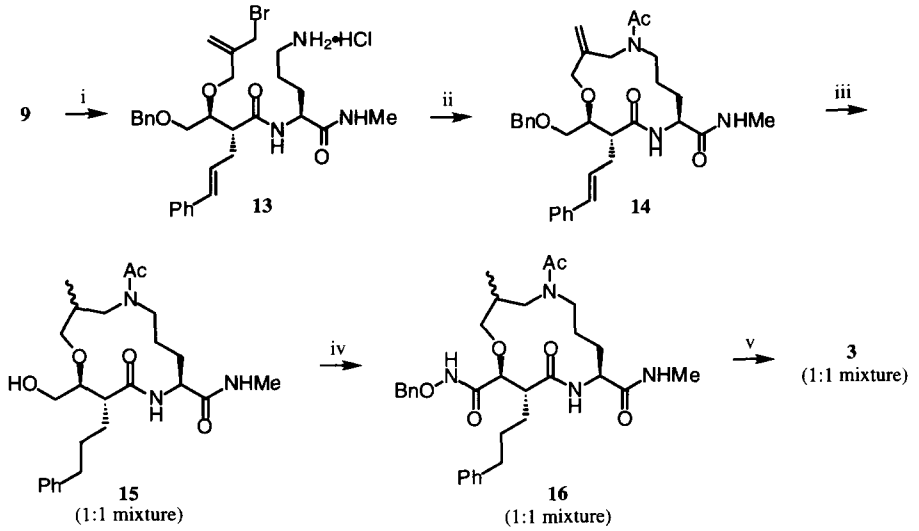
A 13-membered derivative (**3**) was also prepared following an analogous cyclization strategy as shown in Scheme 2. The macrocycle precursor **13** was prepared through alkylation of **9** with 3-bromo-2-bromomethylpropene. The 13-membered cyclization worked best with DBU in DMF. The cyclization product was converted to the acetamide **14** in the same pot by addition of acetic anhydride. The improved cyclization yield (74%) was attributed to the more entropically favored S_N2' displacement mechanism. Conversion of **14** to **15** was effected by stirring with $Pd(OH)_2/C$ under hydrogen. In this case, C-O bond cleavage of the allylic ether moiety was not observed. Oxidation, coupling with $BnONH_2$ and deprotection completed the synthesis of **3**.

Scheme 1



i) NaOH, MeOH, H_2O. ii) BOP, *i*-Pr_2NEt, DMF (73% for 2 steps). iii) a. NaH, *trans*-1,4-dibromo-2-butene, DMF, 0 °C (74%). b. HCl, dioxane (80%). iv) *i*-Pr_2NEt, CH_3CN, then $(BOC)_2O$ (56%). v) a. H_2, Pd/C, MeOH, then $Pd(OH)_2/C$ (79%). b. $RuCl_3$, $NaIO_4$, CH_3CN, CCl_4, H_2O. c. $BnONH_2$, BOP-Cl, NMM, THF (67% for 2 steps). vi). a. HCl, dioxane. b. Ac_2O, *i*-Pr_2NEt, CH_2Cl_2 (53% for 2 steps). c. H_2, $Pd/BaSO_4$, MeOH (89%).

Scheme 2



i) a. NaH, 3-bromo-2-bromomethylpropene, DMF, 0 °C (67%). b. HCl, dioxane (100%). ii) DBU, DMF, then Ac_2O (74%). iii) H_2, $Pd(OH)_2/C$, MeOH (85%). iv) a. $RuCl_3$, H_5IO_6, CH_3CN, CCl_4, H_2O (78%). b. $BnONH_2$, BOP, *i*-Pr_2NEt, DMF (26%). v) H_2, $Pd/BaSO_4$, EtOH (100%).

Results and discussion: The compounds of interest were tested in MMP-1, 3, 8 and 9 inhibition assays[6a] and the data are shown in Table 2. As anticipated based on the modeling results, both 13- and 14-membered cyclic amines were potent MMP inhibitors. Substitution on the linker of the 14-membered ring was tolerated as evidenced by the data on **2a~d**. The nature of the substituents affected the potency of these inhibitors by up to ten fold (**2c** vs **2d**). This could be attributed to a change in the low energy conformation distribution of the relatively flexible macrocyclic ring. Compounds **2a** and **2d** represent the most potent inhibitors for MMP-8 and 9, respectively. These macrocyclic inhibitors are in general more potent for MMP-8 and 9 than MMP-1 and 3. The selectivity is likely a combination effect of the macrocyclic template and the long phenpropyl P_1' group. MMP-8 and 9 are known to have deep S_1' pockets. In contrast, MMP-1 has a shallow S_1' pocket and does not accommodate long P_1' groups well.

Since MMP-9 is an important enzyme for angiogenesis process[16] during cancer development, MMP-9 inhibitors could find applications in anticancer treatment. Thus, these macrocyclic compounds provided promising leads for further optimization towards anti-angiogenesis agent.

Table 2

		K_i, nM			
	R	MMP-1	MMP-3	MMP-8	MMP-9
2a	H	496	265	4	21
2b	Ac	248	38	---	6
2c	BOC	1068	373	13	19
2d	$PhSO_2$	110	---	---	2
3	---	440	267	7	13

In summary, we have prepared a series of 13- and 14-membered macrocyclic amines by linking the P_1 and P_2' groups. Intramolecular amination via nucleophilic displacement was used for the cyclizations. This cyclization protocol should also be applicable to the synthesis of other ring sizes. The macrocyclic amine-derived hydroxamic acids are inhibitors of MMP-8 and 9 with low nM affinity and selective over MMP-1 and 3. Furthermore, the conformationally constrained cyclic structure could be more effective in directing spatially well defined substitution groups, thus providing a better template for the design of inhibitors specific for an enzyme in the MMP class.

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12. Compound **4a** was prepared from benzyl (*R*)-(-)-glycidyl ether through the following transformations: (i) *n*-BuLi, $HC(SMe)_3$, HMPA, THF; (ii) NBS, $NaHCO_3$, MeOH, H_2O. For a related reference see: Abood, N. A. *Synth. Commun.* **1993**, *23*, 811.
13. Compound **4b** was prepared from dimethyl (*S*)-(-)-malate through the following sequence: (i) $BH_3{\bullet}SMe_2$, $NaBH_4$, THF; (ii) TBSCl, Et_3N, DMAP, CH_2Cl_2. For a related reference see: Saito, S.; Ishikawa, T.; Kuroda, A.; Koga, K.; Moriwake, T. *Tetrahedron* **1992**, *48*, 4067.
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