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INHIBITORS OF MMP-1: AN EXAMINATION OF P_1' $C\alpha$ GEM-DISUBSTITUTION IN THE N-CARBOXYALKYLAMINE AND GLUTARAMIDE CARBOXYLATE SERIES.

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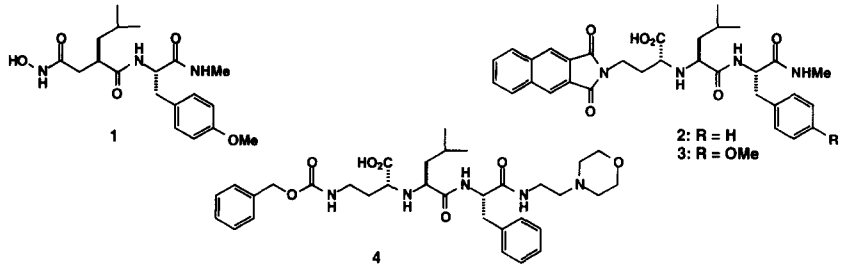
Abstract. Modification of the N-carboxyalkylamine **3** by independent replacement of the P_1' NH group for CH_2 and introduction of P_1' gem-cyclohexyl substitution affords compounds **5** and **6a** which retain appreciable activity against MMP-1 (IC_{50}s = 0.023 μM and 0.09 μM, respectively). The glutaramide **7a** which incorporates both these structural changes also retains potent activity (IC_{50} = 0.038 μM). Copyright © 1996 Elsevier Science Ltd

Introduction. Orally active inhibitors of matrix metalloproteinases (MMP's), particularly interstitial collagenase (MMP-1), are sought as drugs for the treatment of osteoarthritis.[1] Functionality which can ligate the catalytic zinc atom in the active site of the enzyme is essential for inhibition of MMP's. The most widely employed zinc ligand has been the hydroxamate group which, when incorporated onto the appropriate dipeptide equivalent, affords compounds (e.g., the succinamide hydroxamate **1**)[2] having very high intrinsic potency. However, other classes of MMP inhibitors based on alternative zinc ligands remain as attractive targets for drug discovery due to the high rates of clearance which have generally characterized the hydroxamates *in vivo*.



In this paper, we report on our effort to discover potent and novel carboxylate-based inhibitors of MMP-1. Simple substitution of hydroxamate for carboxylate in compounds such as **1** is known to markedly reduce activity against MMP-1. However, potent carboxylate inhibitors of MMP's are known; in particular, a series of N-carboxyalkyl dipeptides, exemplified by **2** and **3**, are potent inhibitors of MMP-1.[3] We found **2** to lack appreciable activity *in vivo* due to poor absorption and pharmacokinetics.[4] Nonetheless, we chose **2** as a starting point for an investigation of carboxylate inhibitors of MMP-1 reasoning that certain structural modifications might improve *in vivo* activity without loss of potency. In particular, replacement of the P_1' NH for CH_2 appeared likely to improve absorption by decreasing the number of hydrogen bond donors.[5,6] Additionally, since the 2,3-naphthalimide group of **2** is susceptible to hydrolysis giving the corresponding carboxynaphthylamide and since

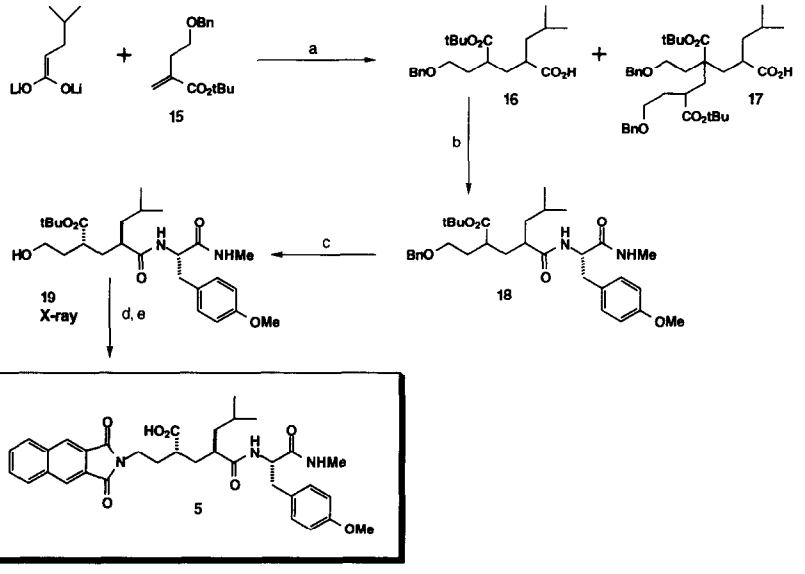
the mechanism of this hydrolysis is thought to involve autocatalysis by the P_1' NH,[2] replacement of this NH group by CH_2 offered the potential for increased stability. The other structural modification we chose to explore was P_1' C_α gem-disubstitution which would hinder enzymatic hydrolysis of the P_1'-P_2' amide bond should this play a role in metabolism.[6] In the preceding paper, we examined the effect of P_1' C_α gem-disubstitution on the inhibition of MMP-1 by a series of succinamide hydroxamates.[7] While this substitution uniformly reduced potency against collagenase, P_1' C_α gem-cyclohexyl substitution was significantly less deleterious to activity than other P_1' C_α gem-disubstitutions, including gem-cyclopentyl. Thus, we were particularly interested in exploring P_1' gem-cyclohexyl substitution, both in the N-carboxyalkylamine series and the related glutaramides (P_1' NH replaced by CH_2). Newly available X-ray crystallographic information (in particular, the crystal structure of the N-carboxylalkylamine **4** bound to 19 kDa collagenase),[8] in conjunction with molecular modeling studies, gave support to P_1' C_α gem-cyclohexyl substitution being an acceptable structural modification in both carboxylate series.

Chemistry. The glutaramide **5** in which the P_1' NH of **3** is replaced by CH_2 was prepared as shown in Scheme 1. The C-C bond-forming step, involving conjugate addition of the lithiodianion of 4-methylvaleric acid to *tert*-butyl 1-(2-benzyloxyethyl)acrylate (**15**)[9], gave **16** (a mixture of racemic diastereomers) as well as the unwanted bis-Michael adduct **17**. Separation of the components of this rather complex mixture was not attempted. However, after coupling with H-L-Tyr(Me)NHMe, chromatographic separation of **18** (four diastereomers) from the products arising from **17** could be achieved and, after hydrogenolysis of the O-benzyl group, partial chromatographic separation of the four diastereomeric alcohols was possible. Fortunately, the crystalline alcohol **19** eluted ahead of the other three diastereomers and workable quantities of this intermediate could be readily obtained in a pure state. An X-ray crystallographic analysis of **19** established the 2*R*, 4*S* stereochemistry corresponding to that of **2** and **3**. Final conversion of **19** to **5** proceeded uneventfully as shown.

The diastereomeric P_1' C_α gem-cyclohexyl glutaramides **7a,b** and the gem-cyclopentyl glutaramides **8a,b** were prepared by routes closely analogous to that in Scheme 1. In the case of gem-cyclopentyl, the conjugate addition of the lithiodianion of cyclopentanecarboxylic acid to **15** was quite efficient (78%). In contrast, the reaction of the lithiodianion of cyclohexanecarboxylic acid with **15** gave unacceptable yields of the desired conjugate addition product. However, by using the dilithio dienolate of cyclohexenecarboxylic acid, a 78% yield was obtained of **20** (a mixture of 2 diastereomers), the product of α–attack by the dienolate (Scheme 2). Following coupling to H-Tyr(Me)NHMe, hydrogenation of the cyclohexene double bond was achieved concurrently with hydrogenolysis of the O-benzyl ether. In both the gem-cyclohexyl and gem-cyclopentyl instances, separation of the C-4 diastereomers was achieved just before final cleavage of the *tert*-butyl ester. The stereochemistry at C-4 in compounds **7a,b** and **8a,b** was not assigned spectroscopically but could be inferred from the activity against collagenase (vide infra).

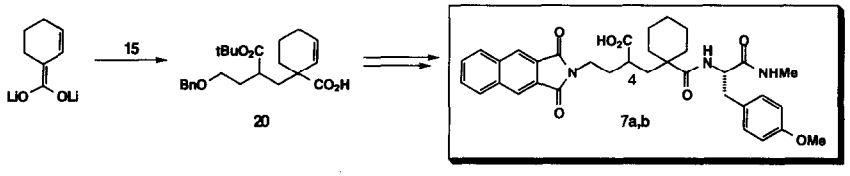
The substituted and unsubstituted phthalimide derivatives **9-11** were prepared similarly to **6a,b** by substituting 2,3-naphthalimide with the appropriate imide in the Mitsunobu step. Compound **12**, bearing only a methyl group at P_1, was prepared analogously starting with the conjugate addition of cyclohexenecarboxylic acid dienolate to *tert*-butyl methacrylate. Compounds **13** and **14** were obtained by coupling of **20** to benzylamine and N-(2-aminoethyl)morpholine respectively, followed by hydrogenation (with accompanying hydrogenolysis of the benzyl ether), Mitsunobu reaction with 2,3-naphthalimide and *tert*-butyl ester cleavage.

Scheme 1



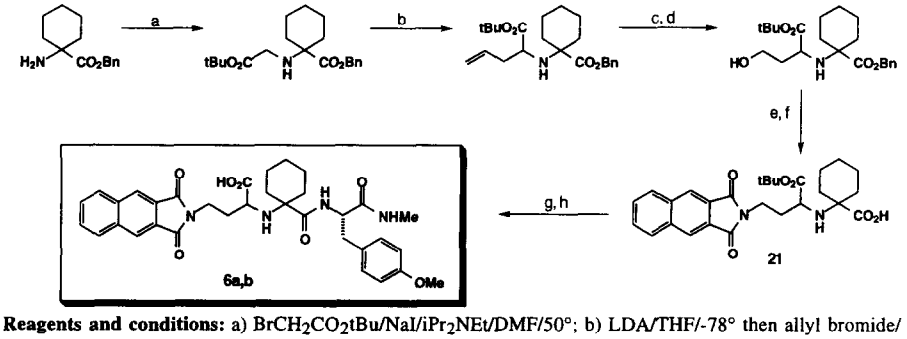
Reagents and conditions: a) THF/-78° (form dianion with 2.2 equiv. LDA); b) H-Tyr(Me)NHMe·HCl/ DEC/HOBT/Et_3N/CH_2Cl_2; c) H_2/Pd(OH)$_2$(C)/AcOH; d) n'imide/PPh_3/DEAD/THF; e) TFA

Scheme 2



The diastereomeric P_1' C_α gem-cyclohexyl N-carboxyalkylamine analogues **6a,b** were prepared in a straightforward manner as shown in Scheme 3. Separation of the diastereomers was carried out by chromatography immediately prior to cleavage of the *tert*-butyl ester.

Scheme 3



Reagents and conditions: a) $BrCH_2CO_2tBu$/NaI/iPr_2NEt/DMF/50°; b) LDA/THF/-78° then allyl bromide/ -78° to 0°; c) $NaIO_4$/cat.OsO_4/dioxane/H_2O; d) $NaBH_4$/MeOH; e) n'imide/PPh_3/DEAD/THF; f) H_2/10%Pd(C)/AcOH;g) H-Tyr(Me)NHMe·HCl/DEC/HOBT/Et_3N/CH_2Cl_2; h) TFA

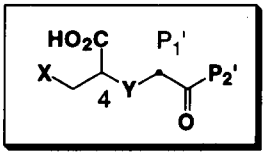
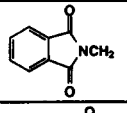
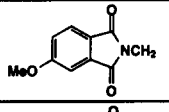
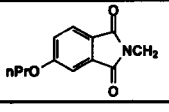
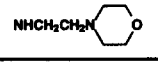


Table 1

Cmpd	X	Y	P_1'	P_2'	IC_{50} (μM)[12] MMP-1[a]
2[b]	N'imide-CH_2[c]	NH		PheNHMe	0.034 (n = 2)
3	N'imide-CH_2	NH		Tyr(Me)NHMe	0.023 (0.0031; 3)
5[d]	N'imide-CH_2	CH_2		Tyr(Me)NHMe	0.023 (0.014; 3)
6a[e]	N'imide-CH_2	NH		Tyr(Me)NHMe	0.09 (0.039; 4)
6b[e]	N'imide-CH_2	NH		Tyr(Me)NHMe	0.97 (0.46; 4)
7a[e]	N'imide-CH_2	CH_2		Tyr(Me)NHMe	0.038 (0.0065; 4)
7b[e]	N'imide-CH_2	CH_2		Tyr(Me)NHMe	0.14 (0.018; 4)
8a[e]	N'imide-CH_2	CH_2		Tyr(Me)NHMe	0.26 (0.12; 4)
8b[e]	N'imide-CH_2	CH_2		Tyr(Me)NHMe	0.94 (0.35; 4)
9[f]		CH_2		Tyr(Me)NHMe	3.2 (1.3; 3)
10[f]		CH_2		Tyr(Me)NHMe	0.57 (0.14; 3)
11[f]		CH_2		Tyr(Me)NHMe	0.14 (0.72; 3)
12[f]	H	CH_2		Tyr(Me)NHMe	>30 (n = 3)
13[g]	N'imide-CH_2	CH_2		$NHCH_2Ph$	3.0 (1.0; 3)
14[g]	N'imide-CH_2	CH_2			12 (5.3; 3)

a) Avg. IC_{50}s: μM (s.d.; n); b) R stereochemistry at C-4; c) N'imide = 2,3-naphthalimide; d) S stereochemistry at C-4; e) Single diastereomer, unassigned stereochemistry at C-4; f) 1:1 mixture of diastereomers; g) Racemic.

Results and Discussion. Replacement of the P_1' NH group of **3** with CH_2 to give the glutaramide **5** did not result in a change in potency.[10] Both compounds exhibited IC_{50}s = 0.023 μM (Table 1). The protonated P_1' NH groups of **3** likely makes hydrogen bonds to Ala(182) and Glu(219) of MMP-1 based on the X-ray crystal structure of **4** bound in the active site of the 19 kDa enzyme.[8] Evidently, the free energy gained by removing the hydrophobic CH_2 of **5** from water can compensate for the loss of these hydrogen bonds.[11] Replacement of the P_1' isobutyl of **3** for gem-cyclohexyl to give **6a** or **6b** was associated with decreased potency. However, assuming the more active isomer **6a** to have the same stereochemistry at C-4 as **3**, the loss of activity was only about 4-fold, a loss markedly less than that (80-fold) resulting from the same structural perturbation in the succinamide hydroxamate series.[7] Incorporation of a gem-cyclohexyl ring at P_1' in the glutaramide series was even less detrimental to activity, the glutaramide **7a** (IC_{50} = 0.038 μM) being only about 1.5 times less potent than the P_1' isobutyl glutaramide **5**. We speculate that the increased activity of the glutaramides **7a** and **7b** relative to the N-carboxyalkylamines **6a** and **6b**, respectively, may reflect an ability of the bound glutaramides, being less constrained by hydrogen bonding, to more freely adopt a conformation favorable for projection of the cyclohexyl ring into the S_1' pocket of the enzyme. Paralleling the structure-activity relationships seen in the P_1' C_α disubstituted succinamide hydroxamate series, the P_1' gem-cyclopentyl glutaramides **8a,b** were less potent than the corresponding gem-cyclohexyl analogues (**7a,b**), although the difference in activity (about 7-fold) was not as large as that between the corresponding hydroxamates (about 20-fold).[7]

Compounds **9-14** were prepared to explore structural changes at P_1 and P_2' in the gem-cyclohexyl glutaramide series. The goal here was to retain potency and, at the same time, maximize the potential for achieving oral bioavailability by reducing the molecular weight and the number of hydrogen bond donors. Activity against MMP-1 was very sensitive to the structure of the P_1 imide group as demonstrated by compounds **9-11**; compound **12**, bearing only a methyl group at P_1, was essentially inactive. While the structure-activity relationships at P_1 reflect those seen with the corresponding analogues of **2**,[3] this does not appear to be true for analogues with structural modification at P_2'. Thus, in the gem-cyclohexyl glutaramide series, replacement of the P_2' Tyr(Me)NHMe residue with simple secondary amide groups (e.g., **13**, P_2' = $NHCH_2Ph$) gave poorly active compounds while in the N-carboxyalkylamine series P_2' secondary amide analogues displayed good activity against MMP-1 (IC_{50}'s <0.1 μM).[3]

Conclusion. Significant activity against MMP-1 (IC_{50} < 0.1 μM) is retained following modification of the N-carboxyalkylamine **3** by independent replacement of the P_1' NH group for CH_2 (giving **5**, equipotent to **3**) and introduction of P_1' gem-cyclohexyl substitution (giving **6a**). Furthermore, the compound incorporating both these structural changes (glutaramide **7a**) also retains potent activity and is only slightly less active (1.5 -fold) than **3** or **5**. The latter finding significantly contrasts the 80-fold loss of potency against MMP-1 seen by replacement of P_1' isobutyl for gem-cyclohexyl in the succinamide hydroxamate series.[7] This result underscores the care that must be taken in assuming parallel structure-activity relationships between inhibitors of different structural classes. To our knowledge compounds **6a** and **7a** are the first potent inhibitors of MMP-1 (IC_{50} < 0.1 μM) having gem-disubstitution at P_1'. The inclusion of this structural feature should be considered in designing inhibitors of MMP-1 and other matrix metalloproteinases.

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Footnotes and References

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