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SOLID-PHASE SYNTHESIS OF A *N*-CARBOXYALKYL TRIPEPTIDE COMBINATORIAL LIBRARY

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Abstract: A combinatorial library of *N*-carboxyalkyl tripeptides was prepared to generate new leads against metalloproteinases. Using the base labile TentaGel S HMB resin, an Fmoc strategy was employed to yield 100 mixtures of 200 compounds each of the general structure **5**. A synthetic protocol combining both mix and split and indexed combinatorial strategies was used, and selected inhibition data against MMP-3 is reported. © 1997 Elsevier Science Ltd.

Since the first report of the synthesis of small molecules on solid support,[1-3] there has been considerable interest in applying this technology to the drug discovery and development process.[4-10] *N*-carboxyalkyl peptides are known inhibitors of a variety of metalloproteinases, and inhibitors of angiotensin converting enzyme (ACE) of this type have been extremely successful in the treatment of hypertension.[11,12] We have sought to use combinatorial strategies to synthesize mixtures of broad-based metalloproteinase inhibitors. We have purposely chosen mostly natural amino acids and their analogs as subunits to allow for the highest probability of enzymatic recognition (Table 1). In addition, in order to provide a fair amount of structural information before deconvolutions and a manageable mixture complexity, we have chosen to use both mix and split and indexed[13-15] combinatorial techniques (Figure 1).

Figure 1. Mix and Split plus Indexed Strategy

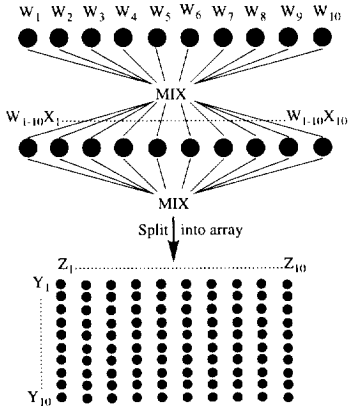


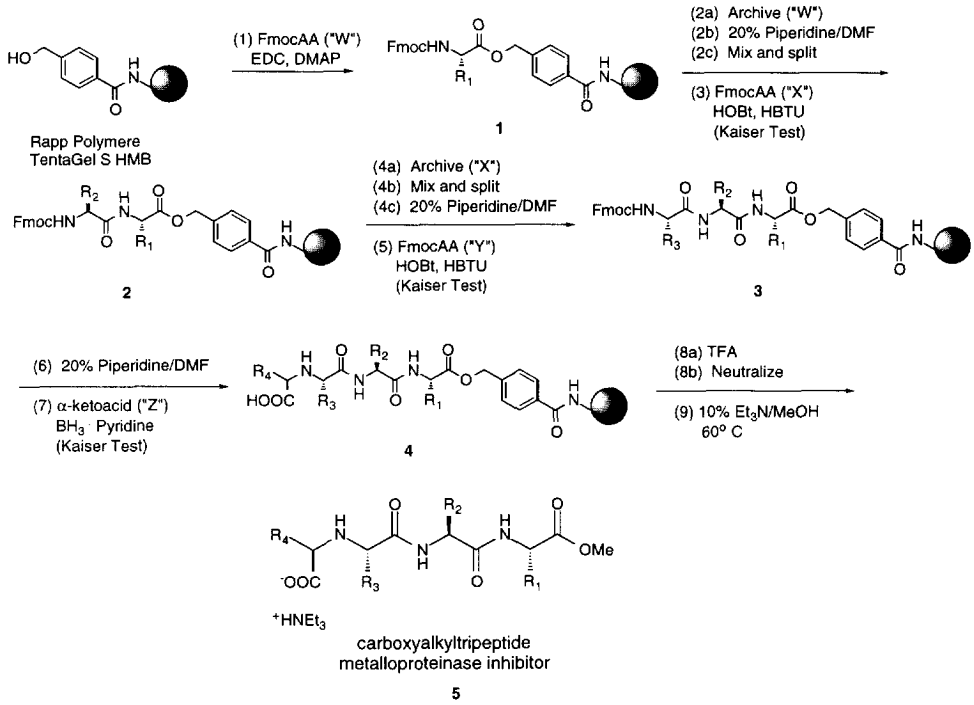
Table 1. Subunits for *N*-Carboxyalkyl Combinatorial Library

#	W and X and Y	Z (α-ketoacid analogs)
1	FmocLeu	Ala
2	FmocNleu	Val
3	FmocPhe	Leu
4	FmochPhe	Phe
5	FmocTrp(Boc)	hPhe
6	FmocGlu(O^{t}Bu)	Biphenylgly
7	FmocLys(Boc)	Glu
8	FmocArg(Pmc)	Tyr
9	FmocThr(Trt)	*n*-Hexylgly
10	FmocGln(Trt)	Trp

Chemistry

The synthesis of the library was accomplished by the 9-step sequence illustrated in Scheme 1. Beginning with a base cleavable resin[16] available from Rapp Polymere (TentaGel S HMB), we attached each "W" subunit (4 equiv) to the resin (8.0 g, 0.2 mmol/g loading) via an ester linkage using 4 equiv of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 2 equiv of *N,N*-dimethylaminopyridine (DMAP) in dry 1:1 tetrahydrofuran (THF):dichloromethane (DCM).[17] After washing and suspending the resin in an isopycnic solution of 30% dimethylformamide (DMF)/dichloroethane (DCE), approximately 0.5 g of each subunit was separated by volume for archiving. The Fmoc group was then removed by stirring in a solution of 20% fresh piperidine in dry DMF. After washing with DMF, all of the remaining resin was combined in a 1.0 L round-bottom flask, suspended in a solution of 30% DMF/DCE, mixed via an air-driven paddle for 2 h, then split out into 10 equal portions containing about 7.5 g each.

Scheme 1



The "X" subunit (4 equiv) was built onto the resin via an amide linkage using 5 equiv of 2-(1H-benzotriazole-1-yl)-1,1,3,3,-tetramethyluronium hexaflurophosphate (HBTU), 4 equiv of *N*-hydroxybenzotriazole (HOBt), and 4 equiv. diisopropylethylamine (DIEA) in dry DMF.[18] After washing with DMF, a few resin beads from each reaction tube were removed to test for the presence of free amine. Kaiser test[19] results on these ten samples were negative (positive control turned blue/purple). As before, approximately 0.5 g of each subunit resin was separated and archived. Again, the resin was then combined, mixed, and split out, but into 100 equal portions this time (~0.7 g) to provide the two-dimentional, structually indexed matrix (Figure 1). Fmoc deprotection with 20% piperidine/DMF followed by reaction of the "Y" subunits and HBTU, HOBt, DIEA with the resin produced the "W-X-Y" tripeptide.

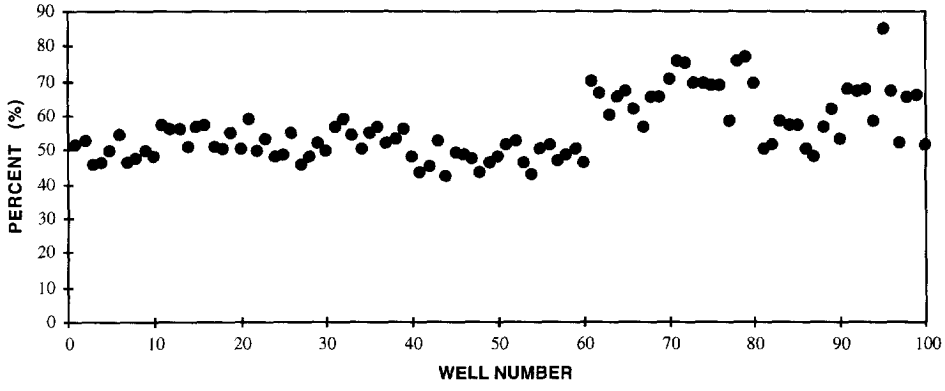
The "Z" subunit (10 equiv) was built onto the resin via the reductive alkylation of the "W-X-Y" tripeptide using 20 equiv of 8.0 M borane–pyridine complex as the reducing agent with mixing for 16–24 h in 1% acetic acid/DMF.[20–22] Kaiser tests were performed on about half the samples (columns Z2, Z3, Z5, Z6, Z10, and five other single wells chosen at random). Results indicated that only Z6 did not couple efficiently, most likely due to steric hindrance caused by the biphenyl group. The Z6 resins were subjected to the reductive alkylation conditions again with 2-ketobiphenylacetic acid, and the subsequent Kaiser tests indicated the absence of any remaining free amines.

Deprotection of the "R" groups was accomplished with a cocktail of trifluoroacetic acid (TFA) containing several cation scavengers (2% each of anisole, thioanisole, dithiothreotol, and triisopropylsilane). Vigorous washing followed the TFA deprotection step to insure all traces of TFA were neutralized and removed. Finally, the product was cleaved from the resin as its methyl ester using 15% triethylamine in methanol at 60 °C for 18 h and lyophilized from 80% acetonitrile in water to afford a fluffy, white solid in most cases.

Results and Discussion

Based on an average molecular weight and the mass recovered from each well, we calculated a yield for each mixture and plotted those results in Figure 2. Overall, the average yield for the library was 56%, which indicated >90% yield for each reaction in the nine step sequence. However, one can notice two distinct scatter patterns in Figure 2. Wells #1–60 are clustered very close to 50% (std. dev. = 4.2), while wells 61–100 are more widely dispersed around a higher average yield of about 64% (std. dev. = 8.4). Electrospray ionization mass spectrometry was used to establish the molecular weight distributions contained in each well. Comparison of these datum with the predicted molecular weights revealed that wells #61–100 contained a fraction of compounds whose side-chains were only partially deprotected by TFA (Scheme 1, Step 8a). As a result, increased recoveries were obtained for these wells. Molecules that contained at least two Arg or two Glu subunits were not deprotected by TFA at all. In these cases, the expected protonated molecular ions plus their protecting group (e.g., $[M+Pmc+H]^+$, $[M+OtBu+H]^+$, etc.) were detected. Overall, the mass spectral data indicated that a large majority (>90%) of the predicted molecular ions were observed for this library.

Figure 2. Yield of *N* -Carboxyalkyl combinatorial mixtures



The library has been tested in well over 50 biological assays to date. Among them were assays for connective tissue degradation involving matrix metalloproteinases (MMPs), for which there is considerable known structural-activity relationship data.[23–25] A representative example of the screening data collected from one of these assays is shown in Figure 3. Each well was evaluated for inhibitory activity against MMP-3, and the most active well (Y4,Z9) was deconvoluted[26] to determine the optimal P_2' and P_3' subunits. The results of these deconvolutions are shown in Table 2.

Figure 3. Relative activity vs. MMP-3

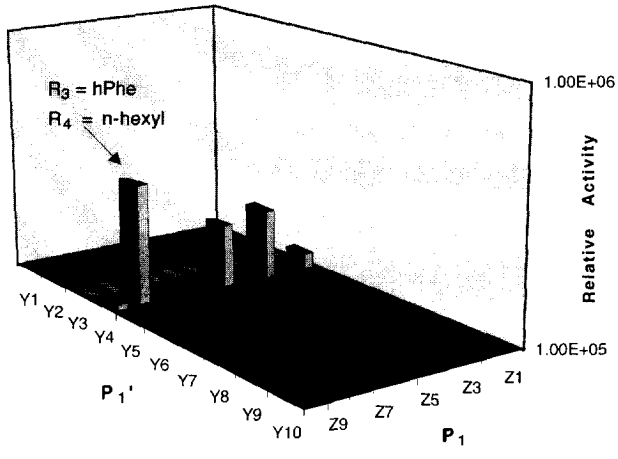
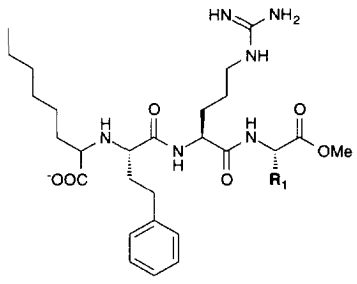


Table 2. Selected results of deconvoluted mixtures, IC_{50} vs. MMP-3



R_1	IC_{50} (μM)
iBu	0.4
nBu	0.9
CH_2CH_2Ph	0.8
CH_2-2-Indole	0.9
$CH_2CH_2CONH_2$	0.9

The results versus MMP-3 confirm known SAR data for this enzyme. Inhibition data versus MMP-3 identified MMP-3's preference for a hydrophobic group at P_1, a phenethyl group at P_1', and the tendency for increased potency with an Arginine at P_2'.

Combinatorial chemistry continues to be a powerful tool for drug discovery and development. By combining both mix and split and indexed techniques in a combinatorial library with a manageable mixture complexity, we have demonstrated that it is possible to obtain useful structural information and *rapidly* find active compounds from testing mixtures.

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