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Parallel Solid-Phase Synthesis of Vitronectin Receptor ($\alpha v\beta 3$) Inhibitors

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Abstract—A combinatorial approach for rapid optimization of a vitronectin receptor ($\alpha v\beta 3$) inhibitor lead was accomplished by solid-phase synthesis. Orthogonally bis protected 2,3-diaminopropionic acid was used to immobilize the C-terminus of the molecule. Selective deprotection and functionalization of the α-amino group followed by acyl resorcinol scaffold attachment and N-terminus diversification was used to explore structure–activity relationship (SAR).

Ligation of integrin $\alpha v\beta 3$ within a 3-dimensional dermal collagen matrix prevents apoptosis[1] and promotes melanoma cell growth.[2] Vitronectin receptor antagonists have been shown to inhibit the growth of various solid tumors of human origin.[3] More recently, $\alpha v\beta 3$ has been shown to be involved in liver metastasis.[4] $\alpha v\beta 3$ Has been shown to play a pivotal role in the proliferation and migration of both smooth muscle and vascular endothelial cells, a pathological process leading to restenosis after balloon angioplasty.[5] Various bone diseases involve bone resorption that is mediated by only one known class of cells, the osteoclasts. When activated for resorption, these motile cells intially bind to bone, a process well known to be mediated by $\alpha v\beta 3$.[6] It is also well known that blockade of $\alpha v\beta 3$ with antibodies or RGD containing peptides block osteoclast cell adhesion and bone resorption in vitro.[7] More recently, several RGD peptidomimetics have likewise been shown to inhibit osteoclasts in vitro and in vivo and block bone loss in an ovariectomized rat.[8]

Combinatorial chemistry is becoming an important tool for drug discovery and lead optimization.[9] A combinatorial synthesis requires that at least two components of the molecule be independently variable, so that all combinations of these components can be prepared. Thus, to prepare a combinatorial library of integrin inhibitors with a high degree of potential diversity for rapid SAR generation using solid-phase techniques, it is important to identify a synthesis in which various components can be independently varied. Most of the reported[10] potent integrin inhibitors are RGD mimetics and a subset of these inhibitors use the 2,3-diaminopropionic acid as the C-terminus. While a cyclic or acyclic guanidino moiety is preferred for the N-terminus, other groups such as ureas[11] and amidines[11] have been used as well. The central scaffold, connecting these two pieces, can be varied[12] also. By developing a convenient route to appropriately protected fragments and a mild solid-phase synthesis that incorporates all the components in an independent fashion, it is possible to prepare combinatorial libraries of this important class of integrin inhibitors.

Corbett et al.[13] reported the first solid-phase synthesis of integrin antagonists, however, this synthesis does not provide a means of varying the substitutions on the C-terminus α-amino group of the 2,3-diaminopropionic acid and is limited to the commercially available α-*N*-CBZ-2,3-diaminopropionic acid as the only fragment. A more recent solid-phase synthesis[14] had employed the α-*N*-Alloc-β-*F*moc-L-diaminopropionic acid to incorporate sulfonamide group at the α-position. However, we were interested in a true combinatorial optimization of the N-terminus region and the α-amino group of the 2,3-diaminopropionic acid. The lead chosen for optimization by solid phase parallel synthesis is shown in (Fig. 1).

Chemistry

To facilitate selective functionalization of the α-amino group the 2,3-diaminopropionic acid was orthogonally

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bis protected as β-*N*-dde-α-*N*-*F*moc-L-diaminopropionic acid **1**.[15] It was attached to the Wang resin using HOBT/HBTU as coupling reagents to give **2** and the α-amino group was deprotected to the free amine **3** using 20% piperidine in DMF. The amine **3** was reacted with a number of chloroformates, isocyanates and acids or acid chlorides to give the α-derivatized amine **4** as corresponding carbamates, ureas or amides.[16] The dde group on the β-amino group was deprotected using 2% hydrazine in DMF to give the free amine **5**. The scaffold **6** was synthesized as shown in Scheme 2 and coupled to amine **5** using DIC as the coupling agent to give the *F*moc protected acyl resorcinol[17] derivative **7**. The common intermediate amine **8** was obtained by further deprotection using 20% piperidine in DMF (Scheme 1).

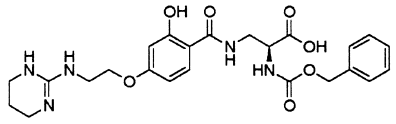
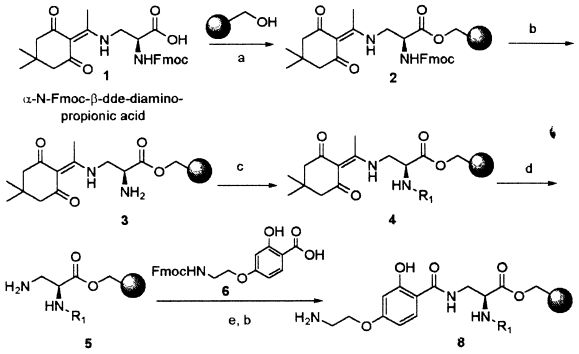
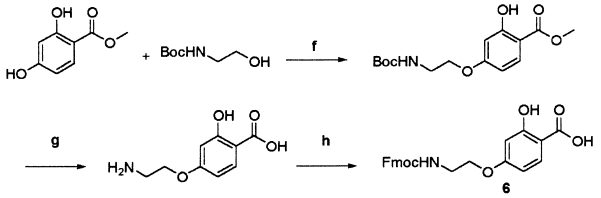


Figure 1.



Scheme 1. Reagents: (a) HOBT, HBTU, DIEA, DMF, rt, 2×4 h; (b) 20% piperidine in DMF; (c) R_1OCOCl, Et_3N, DCM or R_1NCO, DCM or R_1COOH, DIC, DMAP, DMF; (d) 2% hydrazine in DMF; (e) 6, DIC, DMAP, DMF.



Scheme 2. Reagents: (f) DEAD, Ph_3P, THF; 72% (g) KOH, dioxane: water (1:1), 1N HCl; 88% (h) 4M HCl in dioxane, rt, 6 h; Fmoc-Osu, Na_2CO_3, acetone-water (1:1), rt, 18 h; 92%.

The free amine **8** was used for diversification of the N-terminus. As shown in Scheme 3, it was converted to guanidine **9** by reaction with 1,3-bis-Boc-2-methyl-2-thiopseudourea or to the dihydroimidazole derivative **10** using 2-(3,5-dimethylpyrazolyl)-4,5-dihydroimidazole hydrobromide salt or to amidine **11** by reaction with 1-aza-2-methoxy-1-cycloheptene.

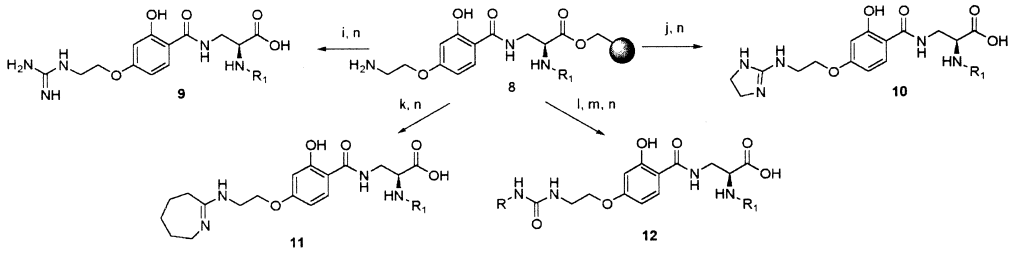
The intermediate amine **8** was also converted to a number of ureas **12** by activation with *p*-nitrophenyl chloroformate followed by treatment with amines. However, the *N*-pyrimidino derivatives **15** and *N*-tetrahydropyrimidino derivatives **16** were obtained by direct coupling with amine intermediate **5** with **13** or **14** (Scheme 4).

Structure–Activity Relationship

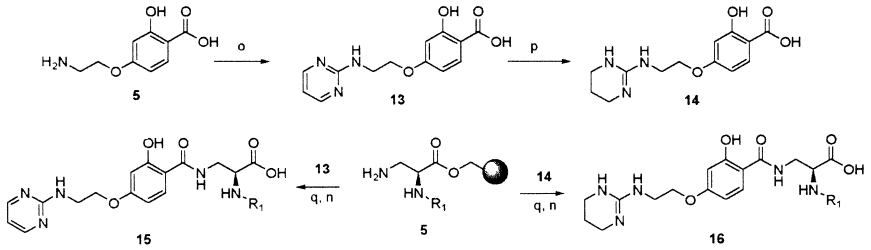
Having established a means of diversifying both the C-terminus amino substitution and the N-terminus, a matrix of 112 carbamate analogues were generated[18] to obtain a rapid SAR. The first array incorporated 14 different carbamates for the C-terminus amino substitution and eight different N-termini. All the analogues were tested in αvβ3 binding assay[19] and results are plotted in Chart A.

From Chart A it is very clear that cyclic guanidines and guanidine seem to be the best N-terminus groups. While N-terminus ureas are generally less potent, within the ureas there seems to be a substitutent dependence. Among the N-terminus ureas pyridyl ureas or simple benzyl urea were found to be better than substitued benzyl ureas. Among the α-substituents sterically crowded substituent like neopentyl carbamate seems to be more active than smaller methyl or ethyl carbamate analogues.

Similar arrays were generated using ureas and amides as C-terminus α-amino substitution and the same set of N-terminus substituents. Overall it was observed that amides were less potent than carbamates and ureas were less potent than the amides. However the trend within the *N*-substituents was constant with the tetrahydropyrimidine being the best. All the active compounds

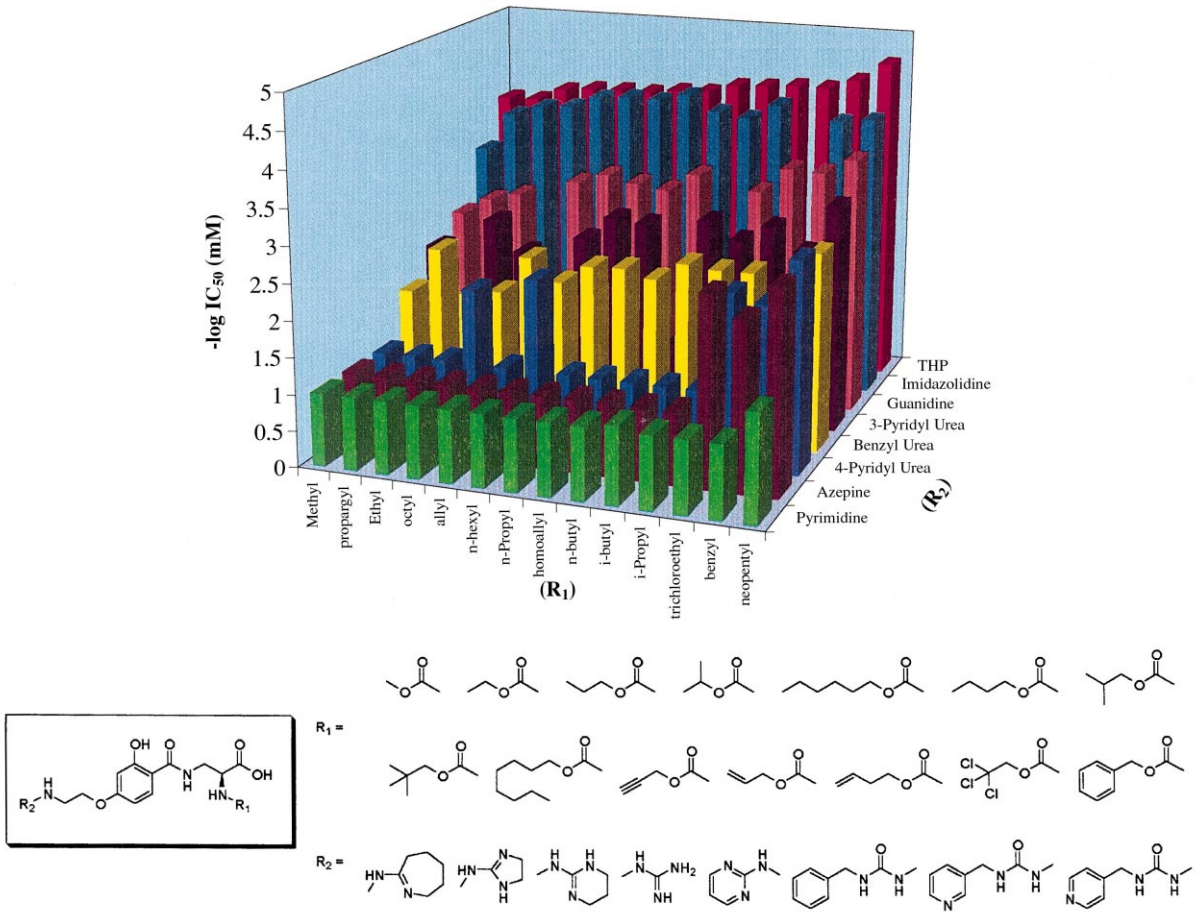


Scheme 3. Reagents: (i) 1,3-bis-Boc-2-methyl-2-thiopsudourea, DIEA, DMF, rt, 18 h; (j) 2-(3,5-dimethylpyrazolyl)-4,5-dihydroimidazole.HBr, DIEA, DMF; (k) 1-aza-2-methoxy-1-cycloheptene, rt, 18 h; (l) *p*-Nitrophenyl chloro-formate, DIEA, DCM:THF (1:1), 0.5 h, rt; (m) RNH_2, Et_3N, DMF, 2 h, rt; (n) 50% TFA in DCM, 0.5 h, rt.



Scheme 4. Reagents: (o) 2-bromopyrimidine, TMSCl, DIEA, dioxane, 2 days, 80 °C; 55% (p) H_2, Pd/C, HCl-HOAc; 80% (q) DIC, DMAP, DMF.

Chart A



showed >100-fold selectivity over the related integrin GPIIb/IIIA in a platelet aggregation assay.[19]

In summary, we have developed a solid-phase method for generating libraries for RGD mimetic class of integrin inhibitors. The method provides a facile means of varying the substitution on the α-amino group as well as the N-terminus groups. The methodology was used for generating lead optimization libraries of a vitronectin inhibitor lead molecule using an acyl resorcinol scaffold. Structure–activity information gathered from these libraries indicates that a carbamate is more potent than a urea which in turn is better than an amide for the α- substitution at the carboxy terminus. Of the lipophilic groups used, a sterically crowded neopentyl group was found to be preferred over smaller or unbranched alkyl groups. Of the N-terminus groups examined, cyclic guanidines like tetrahydropyrimidine and dihydroimidazole were more potent than guanidine itself. N-terminus ureas were found to be less active than cyclic or acyclic guanidines. N-terminus amidines and pyrimidines were found to be very weak inhibitors. Further study in this area of integrin inhibitors are in progress and will be reported in the future.

Acknowledgements

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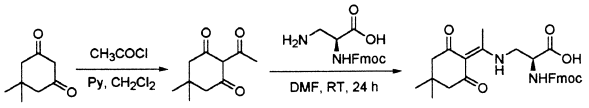
15. Compound **2** is commercially available from Nova-Biochem. However, it was readily prepared from α-*N*-*F*moc-L-diaminopropionic acid as shown below:



16. When the reaction was carried out with some unreactive or sterically hindered chloroformates or isocyanates migration of the dde protecting group to the α-position leading to the β-acylation was observed. This was however overcome by using bulky ddiv following the recent report: Chabra, S. R.; Hothi, B.; Evans, D. J.; White, P. D.; Bycroft, B. W.; Chan, W. C. *Tetrahedon Lett.* **1998**, *39*, 1603.
17. Acyl resorcinol was chosen as the scaffold since the presence of the -OH group ortho to the carbonyl group forms a strong H bond (shown by NMR studies) it may obviate **2** of the hydrating water molecules and improve bioavailability.
18. Isolated yield range 55–88% (5–22 mg) based on loading as determined by the elemental analysis of resin bound amino acid **2**. All the compounds were purified by reverse-phase HPLC and further characterized by LC and MS for >90% purity. LC Conditions: HP 1100, 23 °C, 10μL injected; Column: YMC-ODS-A 4.6×50 5μ Gradient A: 0.05% TFA/Water, B: 0.05% TFA/Acetonitrile; Time 0 & 1 min: 98%A & 2%B; 7 min: 10%A & 90%B; 8 min: 10%A & 90%B; 8.9 min: 98%A & 2%B; Post time 1 min; Flow rate 2.5 mL/min; Detection: 215 and 254 nm, DAD.
19. For details on the binding and selectivity assay format see-Kees, K. L.; Garrick, L. M.; Gopalsamy, A. PCT Int. Appl. WO 99/52879.