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CONSTRUCTION OF A FAMILY OF BIPHENYL COMBINATORIAL LIBRARIES: STRUCTURE–ACTIVITY STUDIES UTILIZING LIBRARIES OF MIXTURES†

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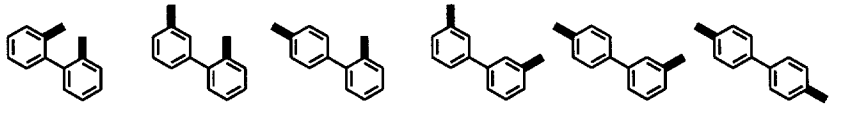
Abstract: A set of biphenyl aminoacid building blocks has been synthesized. These were used to construct partially-peptidic combinatorial libraries as equimolar multi-component samples. Activity of members of this library as vitronectin receptor antagonists is described, together with SAR studies of the most active members. These studies illustrate several important features of combinatorial libraries.

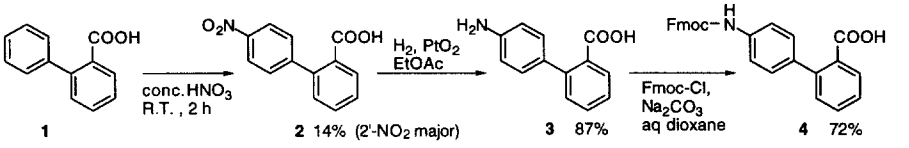
Combinatorial libraries have proven a highly useful tool in the drug discovery process.[1] A critical element in the design of combinatorial libraries is the presentation around a fixed core of groups capable of interaction with a receptor or enzyme target. Particularly useful are lipophilic cores, since lipophilic binding energies have been recognized as a major component of total binding to the target proteins. The value of biphenyl cores for such a presentation was appreciated early, since internal biphenyl units are present in a number of drugs. A global scheme for use of biphenyl cores was explored by workers at Sphinx.[2] For our own studies, we selected a set of biphenyl aminoacids for incorporation as cores in semi-peptidic libraries. This Letter describes synthesis of the biphenyl units, preparation of libraries with these cores, and an SAR study of antagonist activity for the vitronectin receptor.

The set of biphenyl units with a different single substituent on each ring consists of nine isomers. We elected to produce this set of structures as Fmoc-aminoacids for synthesis of combinatorial libraries. For our work, use of the 2,2′-isomer was considered problematic due to cyclization to phenanthridinone. The shapes of the cores are illustrated. We have developed syntheses for six of these cores and homologs of the remaining two.



2,2′ *2,3′ + 3,2′* *2,4′ + 4,2′* *3,3′* *3,4′ + 4,3′* *4,4′*

The 4′-(Fmoc-amino)biphenyl-2-carboxylic acid was prepared starting with the known[3] nitration of acid **1**, providing **2**, together with the 2′-nitro isomer as the major product. Hydrogenation to **3**[4] was facile, as was attachment of the Fmoc-group to yield **4**.



The isomeric 4′-(Fmoc-amino)biphenyl-4-carboxylic acid was prepared in similar fashion from biphenyl-4-carboxylic acid **5**. Again, nitration[5] produced substantial amounts of the 2′-nitro isomer in addition

†Dedicated to the memory of our esteemed consultant Sir Derek H.R. Barton

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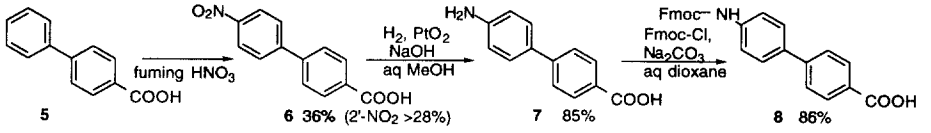
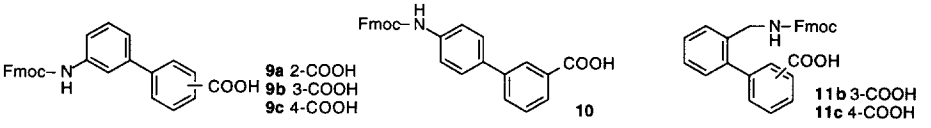
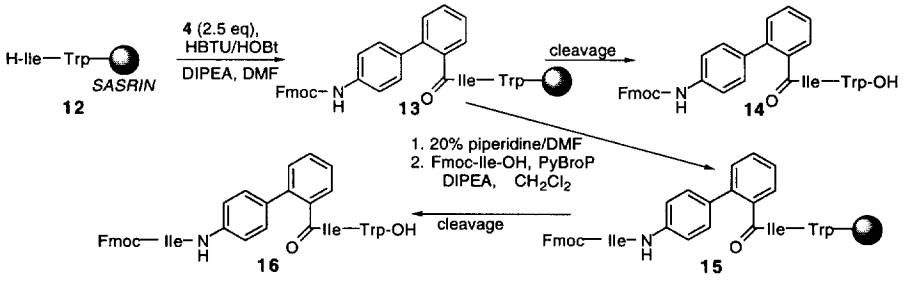
to **6**. For solubility, reduction of **6** was conducted in aqueous methanolic base. The amine **7** was converted to Fmoc-derivative **8**. In spite of the low-yield nitrations, both **4** and **8** could be produced on 20-gram scale for synthesis of libraries.



The three isomeric 3′-(Fmoc-amino)biphenyl-2-, 3- and 4-carboxylic acids **9a–c**, 4′-(Fmoc-amino)biphenyl-3-carboxylic acid **10**, and the two 2′-aminomethyl homologs **11b** and **11c** were prepared efficiently by multi-step sequences[6] utilizing Suzuki biaryl coupling. In this way, a set of eight core units of fixed geometry was available on large scale for library synthesis.

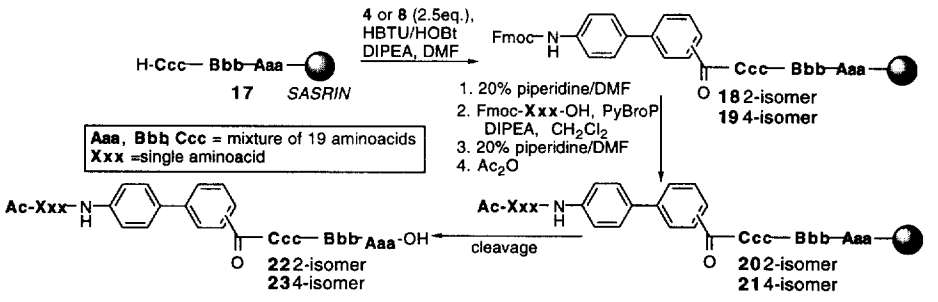


Since these biphenyl units were to be employed in production of library samples as equimolar mixtures, it was critical to demonstrate very clean reaction in pilot examples. For this purpose, we employed the dipeptide SASRIN[7] resin **12**. Attachment of **4** to this resin with HBTU/HOBt[8] provided resin **13**, which upon cleavage with CH_2Cl_2:TFA:H_2O (99:1:0.05) provided **14** in very high purity (HPLC). Removal of the Fmoc from **13** and acylation with Fmoc-isoleucine provided resin **15**, and cleavage of this furnished crude product **33** in very high purity. This acylation step could not be accomplished with HBTU/HOBt and required the stronger coupling agent PyBroP. Conversion of 94% was brought to 100% by a second acylation cycle.



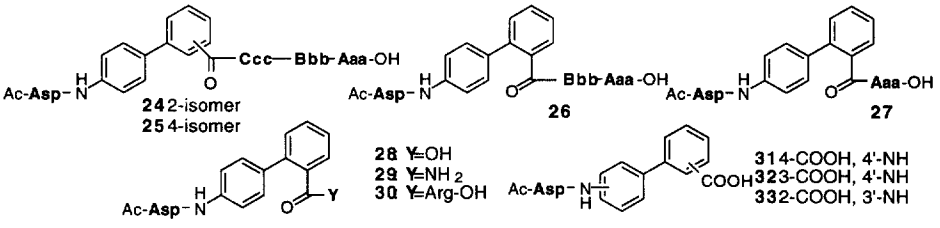
Our first use of these cores in combinatorial libraries employed partially peptidic structures analogous to the all-peptide libraries utilized extensively by Houghten.[9] Based on the successful pilot examples above, synthesis employed SASRIN resin, Fmoc-protection, and HBTU/HOBt coupling. We employed the set of 19 natural aminoacids (cysteine excluded) together with split-and-pool methodology[10] to produce a pool of tripeptide resins **17** comprised of $19 \times 19 \times 19 = 6{,}859$ equimolar components.

The initial two libraries incorporated the biphenyl units **4** and **8**. Attachment to the tripeptide resin produced resins **18** and **19** (conversion verified by ninhydrin test). Following removal of the Fmoc protective groups, the resins were split into 20 portions and each portion acylated with one of the natural Fmoc-aminoacids (one null example). Finally, the Fmoc protective groups were removed and the terminal amines acetylated. Cleavage from the resin and deprotection were accomplished most cleanly in two steps (CH_2Cl_2:TFA:H_2O, 99:1:0.05; then TFA:H_2O, 95:5). This furnished from each library 20 samples of 6,859 equimolar components each.



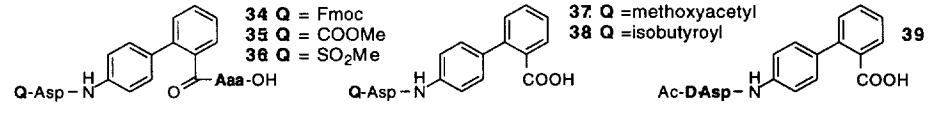
These libraries, together with other libraries, were screened for binding activity at the vitronectin receptor.[11] Only two of the 40 samples showed greater than 50% inhibition at 100 μM (see Table 1). These were the two samples **24** and **25** with N-terminal aspartic acid.[12] The shape of the binding curves was essentially identical to those generated with known antagonists,[11] in spite of the presence of 6,859 components in these two samples.

We began optimization of **24** by step-wise truncation of the structure.[13] Thus, the 361-component mixture **26** was synthesized and possessed potency very similar to that of **24**. A second truncation of the structure produced the 19-component mixture **27**, also of similar potency. Truncation to the single compounds acid **28** and amide **29** produced only a small drop in potency. The C-terminal arginine derivative **30**, which follows the RGD structural motif[14] typical of vitronectin antagonists, was comparable in potency to **28**. In this way, the active 6,859-component lead was reduced to smaller single molecules with retention of activity.



With a set of biphenyl core units available, we examined the three positional isomers **31**–**33** of acid **28**, representing a shift in position of either the amino or carboxy group. These all showed showed potency comparable to that of **28**.

Optimization on the N-terminus was also attempted. Thus an intermediate 19-component resin was converted to the three derivatives **34**–**36**. Similarly, two single-compound analogs of **28**, acids **37** and **38** were prepared. All of these showed greatly reduced antagonist potency. Finally, chirality was examined by use of D-aspartic acid to give enantiomer **39** of **28** with a major loss in potency. Overall, changes in the biphenyl portion of the molecule have little effect on activity, while modification of the N-acetyl-aspartic acid portion greatly reduces activity.



As described above, the synthetic methodology was validated with a set of solid-phase examples. The multi-component library samples were not suitable for rigorous analytical characterization. Consequently, we

were very gratified to observe that all the single compounds (see Table 1), produced by exactly the same methodology, were obtained from the resin in crude purity >90%, as shown by HPLC and NMR analyses.

Table 1. Binding Affinity for the Vitronectin Receptor

Material	24	25	26	27	28	29	30	31
No. components	6,859	6,859	361	19	1	1	1	1
IC_{50}, μM	31	33	67	45	52	96	34	96

Material	32	33	34	35	36	37	38	39
No. components	1	1	19	19	19	1	1	1
IC_{50}, μM	31	46	~1000	>1000	>1000	>500	>500	>500

Since the potency achieved in this series was far below that of many reported antagonists,[15] these lead structures were not explored further. Recently, we have designed libraries specifically targeted for vitronectin antagonism, resulting in a set of highly potent antagonists. In addition, the biphenyl screening libraries described here showed activity as antagonists of a different tumor-associated receptor. There, the initial active mixture was successfully optimized to highly potent single compounds. This work will be reported shortly.

This study illustrates a number of important features of combinatorial libraries: (1) a well-designed library constitutes a long-term asset for screening in multiple assays, (2) testing of a library provides actives accompanied by significant SAR of related structures, and (3) the modular nature of combinatorial library "hits" renders follow-up very facile, although improved activity may not be achieved.

References and Notes

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12. The analogs of **24** and **25** with terminal glutamic acid were much less active (repeat assay).
13. Stepwise truncation was examined prior to iterative optimization (such as employed by Houghten), since a smaller molecule, if active, would be preferred for the drug development process.
14. Samanen, J.; Jonak, Z.; Yuie, T-L. *Current Pharmaceutical Design*, **1997**, *3*, 545.
15. All concentration–response curves were run in duplicate, with at least one repeat pair of assays.