

## Kemp's Triacid Scaffolding for Synthesis of Combinatorial Nonpeptide Uncoded Libraries

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**Abstract:** Synthesis of a differentially protected molecular scaffold for nonpeptide combinatorial libraries is described. Solid phase synthesis of model compounds and nonpeptide combinatorial library as well as the structure elucidation in the absence of coding is disclosed.

Molecular scaffolding and generation of structural diversity involving the scaffold is one of the recent advances in combinatorial chemistry<sup>1</sup> and has recently found application in the development of nonpeptidic mimetics recognized by the endocrine receptor<sup>2,3</sup>. An appropriate selection of the molecular scaffold can enable a search of defined conformational space. Further, when the chosen scaffold itself is involved in the expression of biological activity, the structural diversity of relatively small molecules may be explored by combinatorial chemistry techniques. Another utilization of molecular scaffold is to expose different structural elements or known pharmacophores to enzyme or receptor in a discontinuous epitope fashion.

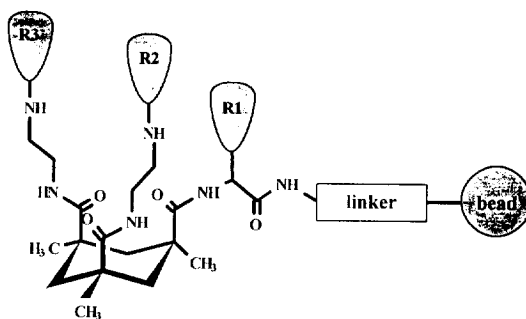
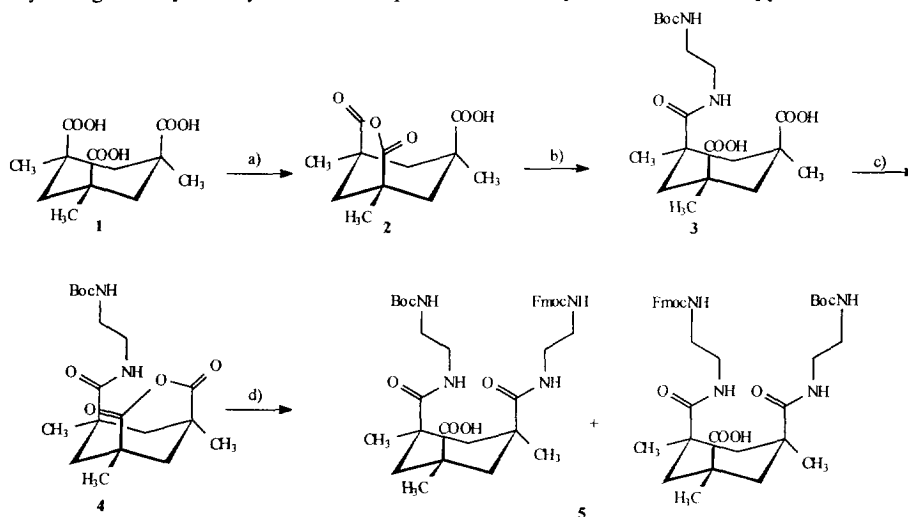


Fig 1 Kemp's triacid scaffold non-peptidic uncoded combinatorial library

Kemp's acid is known in supramolecular chemistry as a useful building block/precursor for the preparation of synthetic receptors<sup>4,5</sup>. Our application of this interesting molecule was to reverse its function to produce ligand molecules which bind to real biological receptors. Taking advantage of the

triaxial orientation of the three carboxyls and "discontinuous" functional group presentation, this triacid was chosen as one of scaffolds for our nonpeptidic libraries. Here, we report the synthesis of this molecular scaffold, the solid phase synthesis of the corresponding chemical library using the one-bead-one-structure concept<sup>6</sup> and structure elucidation using mass spectroscopy (MS). The synthesis of this scaffold is outlined in Scheme 1 where commercially available 1,3,5-trimethyl-1,3,5-cyclohexanetricarboxylic acid (Kemp's triacid) was transformed into the corresponding *cis,cis* anhydride acid **2** using the Dean-Stark dehydration procedure under argon.<sup>5</sup> Acid anhydride **2** was opened by nucleophilic attack by mono *tert*-butyloxycarbonyl ethylenediamine<sup>7</sup> yielding the amide diacid **3**. Due to the instability of the Boc protecting group, the Dean-Stark dehydration method is not applicable for this amide diacid.<sup>8</sup> Alternatively, a mild preparation of the amide anhydride **4** commonly used in peptide chemistry using the dicyclohexylcarbodiimide procedure in methylene chloride was applied.



Scheme 1 a) xylene, reflux under Ar, 19h, 90% b) mono *tert*-butyloxycarbonyl ethylenediamine, DMF, 6h, RT, 83%, c) DCM, DCCI, 90%, d) mono fluorenylmethylloxycarbonyl ethylenediamine trifluoroacetate, DIEA, DMF, 60%

The amide anhydride **4** was then opened by mono fluorenylmethylloxycarbonyl ethylenediamine trifluoroacetate<sup>9</sup> in the presence of diisopropylethylamine to the corresponding racemic scaffold **5**. The final product was purified by flash chromatography. Since the scaffold's activated carboxyl group is prone to form the cyclic imide **6** in the absence of an amino group (observed while coupling the protected scaffold **5** to the amino terminus of the preceding amino acid),<sup>10</sup> the scaffold was activated in situ. A number of model compounds of the scaffold library have been synthesized on solid phase to demonstrate the feasibility of library synthesis and structure elucidation using mass spectroscopy fragmentation patterns<sup>11</sup> in the absence of coding.

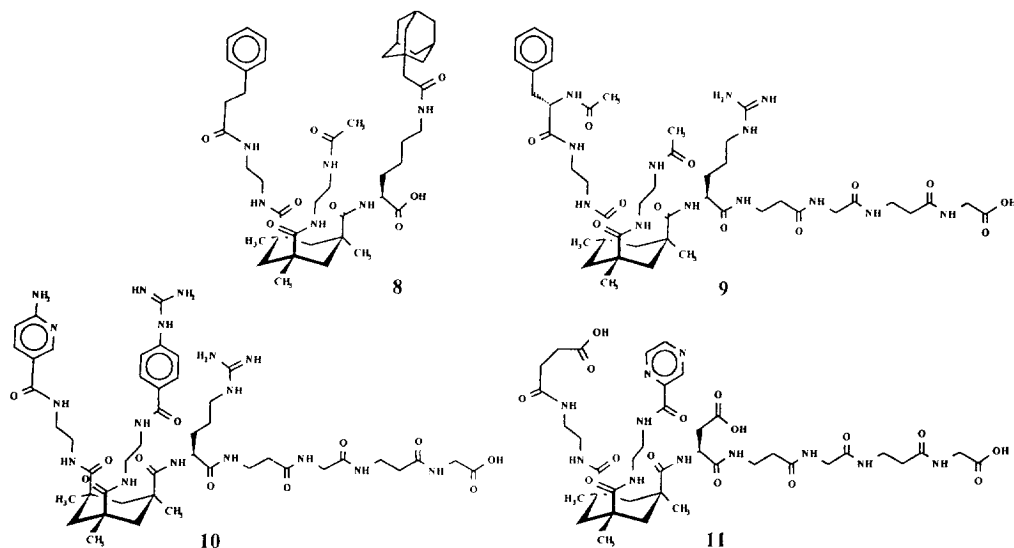


Fig. 2

Some of the synthesized model compounds 8-11 are shown in Fig 2. Evaluation of the model compounds enabled us to choose proper building blocks for the library. Attempts to prepare model compounds containing secondary amines at the randomization position 1 revealed steric hindrance resulting in no coupling of scaffold **5** in the case of N-methylisoleucine and about 50% yield for the compound containing proline. Hence, only primary amino group building blocks were used in the synthesis of the library. Building blocks for the model compounds with a repertoire of structural features have been chosen to reflect various levels of hydrophobicity and charge groups. Furthermore, compound **10** is an example of using a heteroaromatic acid with no protection of the amino group on the pyridine ring. MS studies verified detection of molecules with no net charge by electro spray MS and providing informative fragmentation in a low energy collision cell.

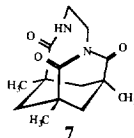
The library itself was synthesized<sup>12</sup> on hydroxy TentaGel using a  $\beta$ -Ala-Gly- $\beta$ -Ala-Gly linker and a set of 30 amino acids randomized in position 1. After attachment of scaffold **5** and deprotection of the Fmoc group, a set of 50 carboxylic acids were coupled in the next randomization step. Deprotection of the scaffold's Boc group was again followed by randomization and attachment of 50 carboxylic acids in the third position. After final deprotection with mixture K, the library was screened against various biological targets. Compounds found as a result of screening were cleaved from the resin by alkaline hydrolysis and analyzed by LC/MS/MS. The fragmentation characteristics were studied using model compounds. These fragmentation patterns enabled us to develop computer software for the automatic determination of the structural identity of compounds contained in the library. Using this software, the structures of hits were determined and verification of this analysis was confirmed by resynthesis. An example of daughter

spectrum of a resynthesized compound (lower trace), matching the parent spectrum of the compound obtained from the library (upper trace) is found in Fig.3.

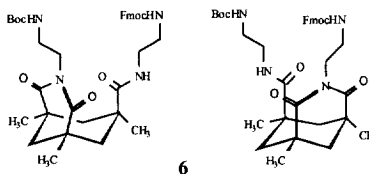
In summary, the synthesis of a differentially protected, conformationally constrained molecular scaffold and subsequent nonpeptide combinatorial library have been described. We have demonstrated characterization of active compounds from the parent bead in the absence of tagging. Lead identification from this combinatorial library applied to the advancement of our cardiovascular program will be described elsewhere.

#### References and Notes:

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- Attempt to dehydrate amide diacid **3** into corresponding amide anhydride **4** by reflux in xylene using Dean-Stark trap under argon led to preparation of the tricyclic byproduct **7**



- Mono fluorenylmethyloxycarbonyl ethylenediamine trifluoroacetate was prepared by acylation of mono tert. butyloxycarbonyl ethylenediamine with 9-fluorenylmethyl succinimide and subsequent deprotection of Boc group
- Formation of cyclic imides **6** have been observed



- Issakova O., Sepetov N., Ma N., Kocis P.: in preparation
- Protocol used for library synthesis: Benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphoniumhexafluoro-phosphate (BOP), Hydroxybenzotriazole (HOBT), DIEA (1:1:1.5) activated Boc and Fmoc amino acids were attached to  $\beta$ Ala-Gly- $\beta$ Ala-Gly-O-TentaGel. After Fmoc and Boc deprotection by 50% piperidine in DMF for 10 min. and TFA/DCM (1:1), 1 hour, resp. scaffold **5** was coupled to amino acids using BOP activation in situ. The Fmoc of scaffold **5** was removed as above and carboxylic acids were coupled as symmetrical anhydrides (DIC, 30 min. preactivation) and DMAP catalysis. The Boc group of scaffold **5** was removed as above and after neutralization (DIEA) the coupling of carboxylic acids was repeated. After side chain deprotection with mixture K (82.5% TFA, 5% p-cresol, 5% thioanisole, 5% water, and 2.5% ethanedithiol) the library was washed with TFA, DCM, DMF, ) 0.1% aq. HCl, and 0.02% aq. HCl.

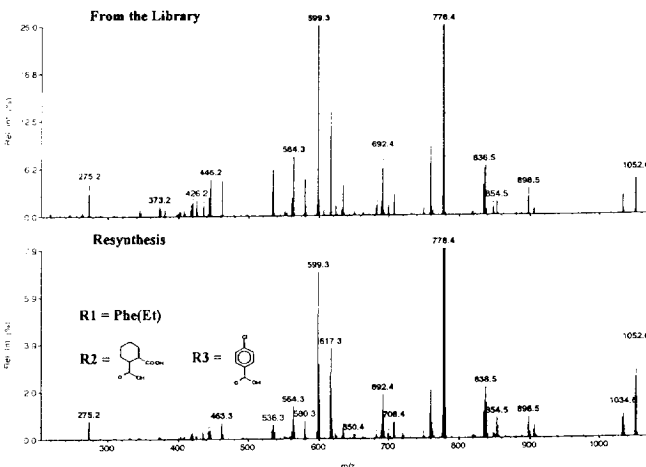


Fig.3 MS spectra of library and resynthesized compound