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Dynamic combinatorial libraries of pseudo-peptide hydrazone macrocycles

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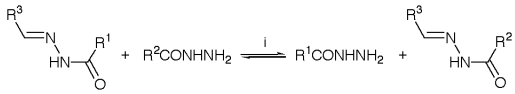
Combinatorial libraries of pseudo-peptide hydrazone macrocycles are formed from amino acid-derived building blocks equipped with hydrazide and aldehyde functionalities; a series of experiments confirm that hydrazone formation is reversible and that the libraries are genuinely dynamic.

Dynamic combinatorial libraries (DCLs) are emerging as interesting systems for the identification of new catalyst, host and guest molecules.[1] In a DCL the connections between building blocks are reversible and in flux: they may be covalent or non-covalent, and are continuously being made and broken. The composition of a DCL is dependent on the environment, in that the addition of a template which selectively binds one member may bias the equilibrium towards that member. Many different reversible reactions have been proposed for the purpose of DCL generation, but none has yet been shown to be ideal.[1–3] Here we report that transimination of hydrazones, a reaction that occurs at room temperature in the presence of a mild acid catalyst (Scheme 1),[4] is a promising candidate for the generation of DCLs. Relative to simple imines (Schiffs bases), hydrazides are expected to have the advantage of greater stability and the potential for peptide-like hydrogen bonding.[5]

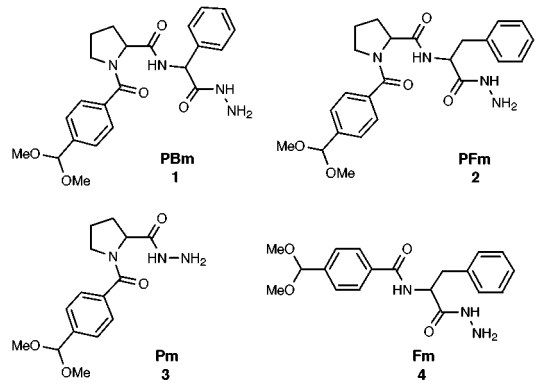
The amino acid-derived building blocks **1**–**4**, each comprising a protected aldehyde moiety and a hydrazide functionality on an amino acid core, were synthesised by standard routes.† Proline features prominently in these monomers because its frequent occurrence in protein β-turns[6] led us to expect that it would give a propensity for the formation of macrocyclic products. Phenylalanine and phenylglycine, components of the other monomers, possess 'chemically inert' side chains, ideal for preliminary investigations.

Cyclisation of the monomers **Fm**, **Pm**, **PFm** and **PBm** is performed by the addition of 50 μl of TFA to a 5 mM solution of monomer in CH_2Cl_2; TFA catalyses both dimethoxy acetal deprotection to the free aldehyde and hydrazone exchange (Scheme 2).‡ The low concentration of monomer directs formation of cyclic oligomers; linear polymers are likely to be formed at higher concentrations. Electrospray mass spectrometry (ES-MS) analysis of reaction solutions of any one of **Pm**, **PFm** or **PBm** reveals the formation of a range of oligomeric macrocyclic species.§ After 2 h there is no evidence of free protected or deprotected linear monomer, and more significantly, no evidence for linear oligomeric products. Under these conditions **Pm** affords cyclic dimer through to cyclic heptamer; cyclic dimer to cyclic nonamer are observed for **PBm**, and finally **PFm** exhibits cyclic dimer through to cyclic hexamer. Monomer **Fm** showed no formation of identifiable oligomeric species; all of the monomer was consumed but the products were largely insoluble and presumably polymeric.

Combinatorial libraries of great diversity are rapidly achieved by the treatment of a solution of two or more monomers with TFA. For example, a solution of **PBm** and **PFm**, when treated with TFA, yields a library of 75 readily



Scheme 1 *Reagents and conditions*: i, Dilute acid.

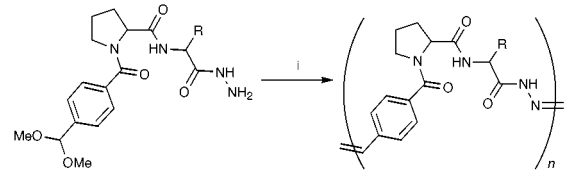


detected macrocyclic species; other products may well be formed in lower concentrations. Importantly, all possible combinations of monomers are detected from cyclic dimer through to cyclic undecamer. Cyclic oligomers of hexamer and above are observed as the doubly-charged species in the range *m/z* 200–2000, and gratifyingly, hexamers, octamers and decamers of odd numbers of each of the two monomers exhibit unique doubly-charged masses, which interdigitate between those signals due to the mono-charged cyclic trimers, tetramers and pentamers respectively (Fig. 1).

The cyclisation of **Pm**, **PFm** and **PBm** in the presence of TFA gives a very diverse macrocyclic library; for example, 14 out of the 15 possible tetramer compositions are detected by ES-MS. In this experiment > 30 product compositions are identified within the sensitivity range of the spectrometer, including dimers, trimers, tetramers and pentamers. These spectra underestimate the number of products formed: doubly charged higher oligomeric species are not observed, perhaps because as the number of possible products increases, their concentration may decrease. This analysis also neglects sequence isomers, which are not detectable by conventional MS.

Mixing experiments performed with monomers **Fm** and **Pm** highlighted the role played by proline in inducing the formation of cyclic species. **Fm** under the cyclisation conditions does not afford any detectable cyclic species, but when **Pm** is introduced mixed cyclic species are observed as well as homo-**Pm** cyclic species. Thus, **Fm** may be incorporated into DCLs but will not stand alone in pseudo-peptide macrocycle formation.

The dynamic reversibility of hydrazone formation is illustrated by two simple experiments. If solutions of macrocycles generated from two different monomers, and containing no free monomer, are mixed, then libraries of the mixed macrocyclic species are observed. The final composition is identical to that observed by mixing the monomers prior to treatment with TFA. It follows that the mixed cyclic species must be the result of



Scheme 2 *Reagents and conditions*: i, TFA, CH_2Cl_2, RT.

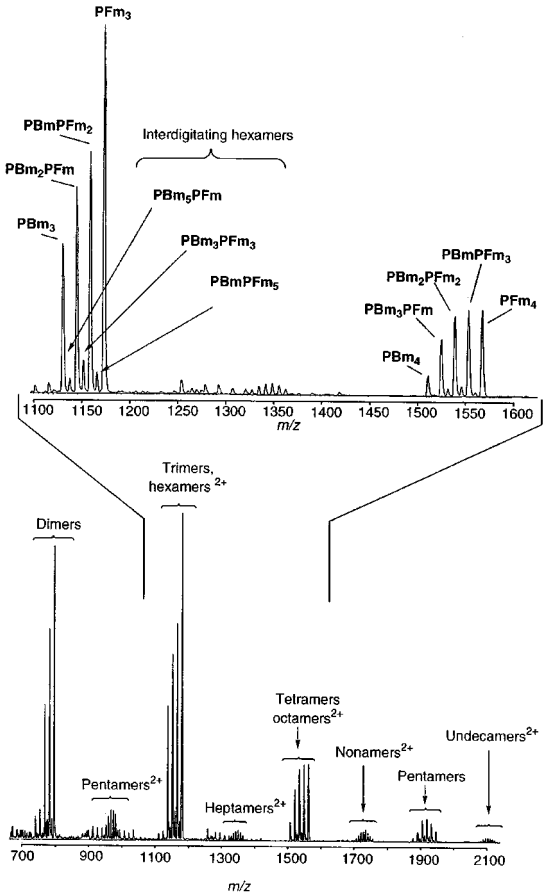


Fig. 1 ES-MS spectrum for the mixed cyclisation of **PBm** and **PFm**.

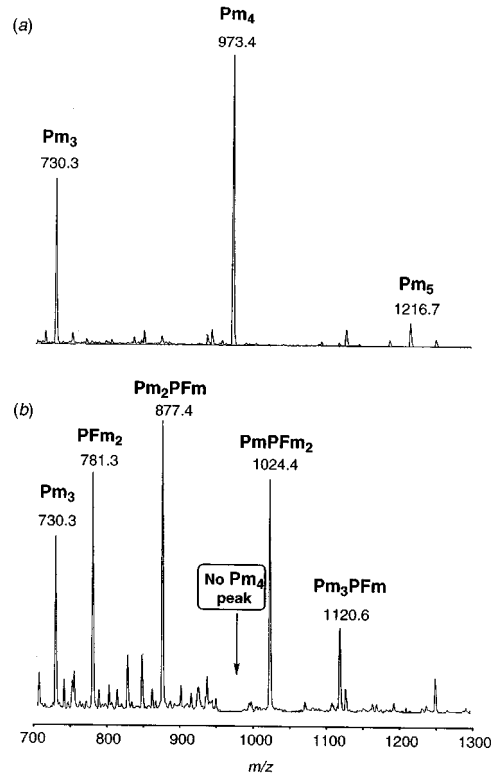


Fig. 2 (*a*) ES-MS spectrum demonstrating cyclisation of **Pm** in the presence of TFA after 2 h. (*b*) ES-MS spectrum of the same reaction mixture recorded 4 h after **PFm** was added. Note the absence of a peak at *m/z* 973 for $\mathbf{Pm}_4$.

homo-oligomer fragmentation and subsequent hydrazone formation. More elegantly, in a second experiment, **Pm** was cyclised in the presence of TFA. To the solution containing preformed macrocyclic **Pm** oligomers was added a 5 mM solution of **PFm**. ES-MS analysis showed the preferential formation of mixed products, with complete loss of *m/z* 973 ($\mathbf{Pm}_4$) and 1216 ($\mathbf{Pm}_5$), present in the solution prior to the addition of **PFm** (Fig. 2). Indeed $\mathbf{Pm_4}$ is the only tetramer not observed in the three monomer library. This preference for mixed species is in marked contrast to the self-sorting of homo-oligomers that we previously observed in relatively rigid alkaloid-derived libraries under transesterification conditions.[3]

The ability to 'switch off' the reaction is of fundamental importance to the success of any DCL. Two separate cyclisation solutions, one of **PFm** and the other of **PBm**, were each treated with 1.5 equiv. of Et_3N relative to the amount of TFA. The solutions were stirred for 15 min before being combined. ES-MS analysis after 2 h showed no formation of mixed cyclic species, demonstrating that in the absence of acid no exchange of hydrazones occurs. Even after 36 h no mixed products are observed. Upon addition of an excess of TFA the exchange reaction is restarted and within a few hours the same distribution of cyclic products is observed as if the reaction had been initiated from a mixture of two protected monomers.

These results demonstrate the applicability of hydrazone chemistry to the generation of DCLs. In conjunction with amino acid-derived building blocks, this chemistry allows for the rapid, and potentially automated, generation of libraries of pseudo-peptide macrocycles which are under thermodynamic control. The mild conditions of hydrazone exchange offer reason to be optimistic that efficient template binding will bias the libraries to receptors with good binding characteristics. The rewards may include a rapid evolution of pseudo-peptide macrocycles as catalysts and host or guest molecules.

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Notes and references

† Monomers were synthesised from carboxybenzaldehyde dimethoxy acetal, Cbz-proline and commercially available amino acid methyl esters using standard peptide chemistry. The notation is derived from the single letter notation for amino acids with **B** assigned to phenylglycine.

‡ The general procedure for cyclisation experiments entailed dissolution of monomers in freshly distilled CH_2Cl_2 and the addition of 50 μl of TFA. All were carried out on a 5–10 mg scale at a concentration of 5 mM. The reactions were stirred at room temperature for 2 h before ES-MS analysis. This deprotection–cyclisation chemistry is compatible with a range of solvents including DMSO and MeCN and aqueous mixtures of these solvents.

§ Electrospray mass spectra were recorded on a Micromass Quattro-LC triple quadrapole apparatus fitted with a *z*-spray electrospray source. The electrospray soucre was heated to 100 °C and the sampling cone voltage (V_c) was 65 V. Samples were prepared by removal of 200 μl of crude reaction mixture and dilution in 200 μl of THF. Samples were introduced into the mass spectrometer source with an LC pump (Shimadzu LC-9A LC pump) at a rate of 4 μl min^{-1} of MeCN–H_2O (1:1). Calibration was performed using protonated horse myoglobin. Scanning was performed from *m/z* 300 to 2100 in 8 s and several scans were summed to obtain the final spectrum which was processed using MassLynx V3.0 software.

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