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Implementation of a Combinatorial Cleavage and Deprotection Scheme. 1. Synthesis of Phthalhydrazide Libraries.

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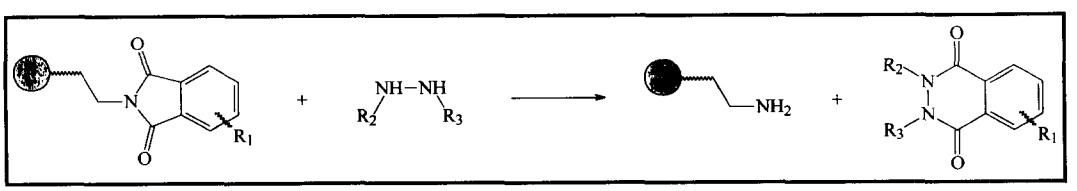
Abstract: Phthalhydrazide libraries are synthesized in solution from substituted hydrazines and phthalimides in several different library formats including single compounds, indexed sub-libraries and a full library. When carried out during solid-phase synthesis, this combinatorial cleavage and deprotection scheme offers the possibility for generating a diverse library of substituted phthalhydrazides.

The generation of molecular diversity using combinatorial chemistry is one of the most active fields of modern organic chemistry. Initially, research was focused on the generation of peptide and nucleotide libraries but more recently - due to the dubious drug-potential of these biopolymers - the generation of non-peptidic small molecule libraries have attracted most of the attention and resources in this field.[1-3]

Recently, some reports on the generation of small molecule libraries in solution appeared, [4-5] but still, the majority of the published work in the area of generating combinatorial small molecule libraries are performed using solid-phase chemistry. The *original reason* for this was the fact that using a solid-phase approach, the *split-mix-recombine method* [6-7] could be used as a way of generating truly vast libraries. Now, when many people are focusing on fewer (or even single compounds) per vial ("sub-library"), the solid-phase methods have retained its popularity - most probably due to its ease of automation of solid-phase chemistry as compared to standard solution organic synthesis.

Therefore, combinatorial libraries are generated comprehensively using solid-phase syntheses which leave the individual members with one specific functional group in common (typically a phenol, a carboxylic acid, an amide, etc.) after deprotection and cleavage from the solid support.[8] Thus, this functionality is required for linking the group of molecules covalently to the solid-support during the course of synthesis. Very recently, two groups independently reported on new linkers which leave no trace of the linking chemistries.[9]

We have for some time been interested in new strategies for the generation of molecular diversity. These include the introduction of a combinatorial cleavage and deprotection scheme *i.e.* a strategy where a number of different compounds are created during the cleavage and/or deprotection step from the solid-support.[10] Using this strategy, large numbers of structurally diverse compounds can be generated

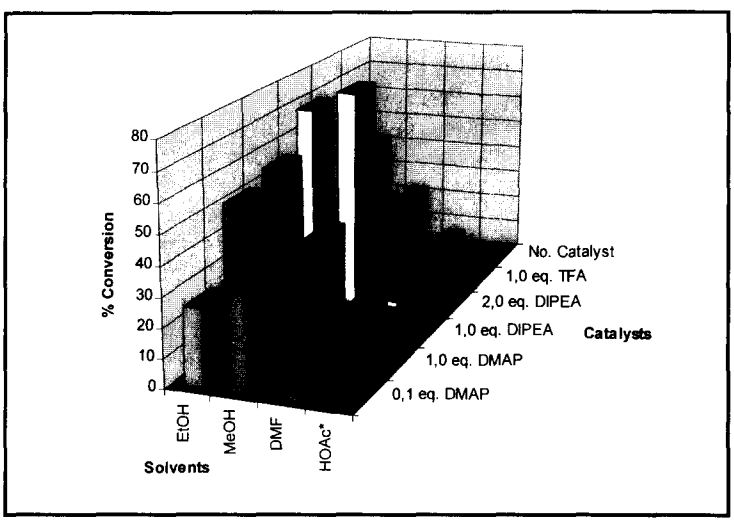


The Ing-Manske procedure for cleavage of the phthalimide linkage by a hydrazine.

Scheme 1

systematically from one common synthon. In this first reported approach the small organic moiety is linked to the solid phase through a phthalimide function. This is a fairly stable linkage which still allows selective cleavage by hydrazines under mild conditions - a reaction known as the *Ing-Manske procedure.*[11] (Scheme 1). The products of these reactions are phthalhydrazides (2,3-dihydro-phthalazine-1,4-diones) which are known to possess a wide range of different biological activities and at the same time comprise a rather unexplored group of potentially new drug leads.[12, 13]

As a model study for the above combinatorial cleavage and deprotection scheme, we performed a systematic study of the reaction between a series of hydrazines (the dinucleophile) and phthalimide. In these experiments, a number of di-aromatic, mono-aromatic and aliphatic hydrazines were investigated as well as a series of different reaction conditions such as variation of solvent, temperature and the addition of a series of possible catalysts. These reaction optimizations were all carried out in a fashion we have termed "*experimental design*" using a 2D-matrix having *e.g.* the solvents in one dimension and the different catalysts in the other dimension. An illustrative example employing 2-hydroxyethylhydrazine as the dinucleophile, 4 different solvents (MeOH, EtOH, DMF and HOAc) and 6 different catalytic conditions (none, DIPEA (1 and 2 eq.), DMAP (0.1 and 1 eq.) and TFA) is shown in Scheme 2. From this and the other experimental design experiments (not shown) it was evident that the ideal reaction conditions were reactions at room temperature, methanol (or ethanol) as the solvent and catalyzing the reaction with 2-3 eq. of diisopropylethylamine (DIPEA).[13] Likewise, we have shown that only aliphatic hydrazines were effective in converting phthalimides to the corresponding phthalhydrazides whereas aromatic hydrazines were either very sluggish or did not react at all under the conditions described.



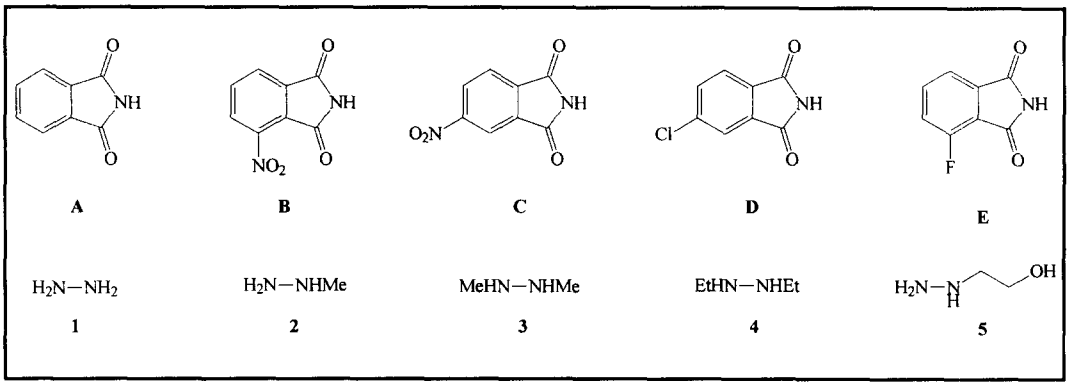
An experimental design optimization of solution reaction between phthalimide and 2-hydroxyethylhydrazine (2 hours reaction time, room temperature, determined by RP-HPLC). HOAc*: For acetic acid, the catalysts were different (1 eq. NaOAc, 2 eq. NaOAc, 1 eq. TFA, 2 eq. TFA and H_2SO_4 [catalytic amount, 2 drops/1 mmole], respectively).

Scheme 2

In order to expand the scope of this reaction, we set up to react 5 different hydrazines with 5 different phthalimides in a 6 x 6 matrix format yielding 25 individual compounds (plus 8 isomers), 10 indexed sub-libraries[5] and 1 full library containing a mixture of all 33 different compounds (Scheme 3). The

reactions were carried out on a 1 mmole scale and in all cases with the 25 individual compounds we obtained a high yield of the expected phthalhydrazides including 8 isomers (from **B2-E2** and **B5-E5**). Likewise, using reversed-phase HPLC (RP-HPLC), we were able to resolve all individual compounds in the indexed libraries whereas the full 33-member library did not allow resolution and identification of individual compounds.

3a



3b

	A	B	C	D	E	
1	A1	B1	C1	D1	E1	[A-E]1
2	A2	B2	C2	D2	E2	[A-E]2
3	A3	B3	C3	D3	E3	[A-E]3
4	A4	B4	C4	D4	E4	[A-E]4
5	A5	B5	C5	D5	E5	[A-E]5
	A[1-5]	B[1-5]	C[1-5]	D[1-5]	E[1-5]	[A-E][1-5]

3a: The 5 different phthalimides (**A-E**) and 5 different hydrazines (**1-5**) tested in the 6 x 6 matrix solution experiment.
3b: The schematic format of the 6 x 6 matrix (IKA-Vibrax-VXR shaker equipped with an Janke & Kunkel VX2 rack)

Scheme 3

Finally, in order to test this approach on solid-phase, we coupled phthalimides **A** and **C** to a chloromethylated polystyrene resin (Merrifield resin) using a known procedure.[14] The resulting resins contained the expected substituted phthalimide structures according to IR-spectroscopy. However, deprotection of phthalides **A** and **C** using hydrazine **5** in EtOH at room temperature was very sluggish although the expected products **A5** and **C5** were obtained after reflux for 16 h. When the deprotection was performed in dichloromethane (DCM) at room temperature, the expected products were obtained in high yields under mild conditions. Likewise, when the resin containing **C** was reacted with hydrazine **1** in 1,2 dichloroethane (DCE) **C1** was obtained in nearly quantitative yield. Reactions performed in DMF did not provide us with any product in the cases tested although the resin was well solvated. This indicates a sluggish reaction in dipolar, aprotic solvents just as observed during the solution experiments.

In summery, we have implemented a synthetic scheme for the generation of diverse libraries of phthalhydrazides both by solid-phase synthesis, by solution synthesis and in several different combinatorial formats (single compounds, indexed libraries and full libraries). Likewise, using this combinatorial cleavage and deprotection method in combination with solid-phase synthesis of structures linked via a phthalimide

linkage, molecular diversity can be introduced in a hitherto unknown fashion. The reactions are very solvent dependent and during the solid-phase synthesis, they are also dependent on the swelling and solvation of the resin. Currently, we are expanding the synthetic schemes for the solid-phase reactions to include more complicated structures and progress will be reported in due course.

Acknowledgments
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