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Generation of a Piperazine-2-carboxamide Library: A Practical Application of the Phenol-Sulfide React and Release Linker.

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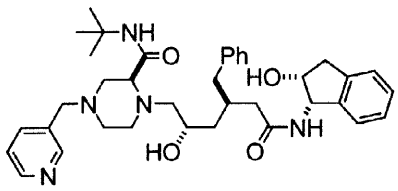
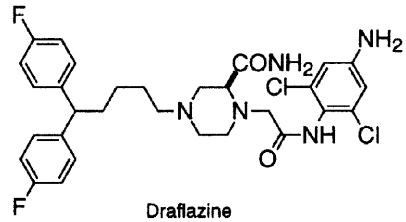
Abstract: *The solid supported synthesis of a library of piperazine-2-carboxamides was accomplished, using a react and release cleavage strategy from a phenol-sulfide linker. Supported Liquid Extraction (SLE) was used effectively to remove cleaving amines from the final products in a high throughput format.* © 1998 Elsevier Science Ltd. All rights reserved.

Methods involving the cleavage of compounds from solid support using diverse cleavage reagents (react and release) are attractive due to the conservation of synthetic operations and the diversity of product structures obtained.[1] However there are numerous practical difficulties in the use of these react and release strategies for the generation of large compound libraries, due to linker activation steps, variable reactivity of cleavage reagents, and impure products arising from incomplete consumption of cleavage reagents. Here we describe some practical solutions to the problems of library generation using react and release methods utilizing Marshall's phenol-sulfide linker.[2]

Piperazine-2-carboxamides are found in a wide variety of pharmacologically active compounds. Two recent examples are the HIV protease inhibitor Indinavir[3] and the cardioprotective agent Draflazine[4], both of which are undergoing clinical evaluation. Because this scaffold contains multiple points for attachment of pendant groups, and its pharmacological relevance piperazine-2-carboxylic acid appeared ideally suited for library generation.



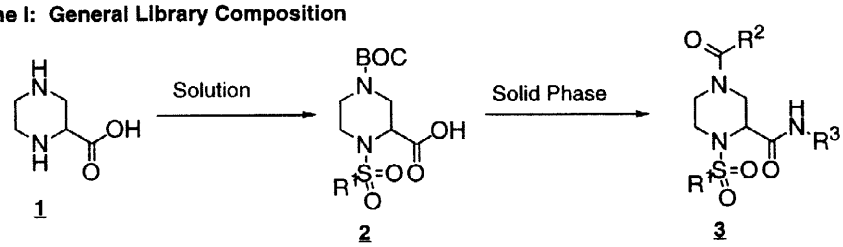
Indinavir



Draflazine

Synthesis of the Scaffold: The plan for the generation of this library was to synthesize, in solution, a set of sulfonamide derivatives of piperazine-2-carboxylic acid **2** and then attach them to solid support for further derivatization. This strategy would allow for the synthesis of 2,000-5,000 compounds from 6-12 sulfonamide scaffolds.

Scheme I: General Library Composition



1 2 3

Selective mono-protection of piperazine-2-carboxylic acid was accomplished by treatment with $(BOC)_2O$ at pH=11.5.[5] A salt free purification of the highly water soluble mono-BOC-piperazine-2-carboxylic acid was

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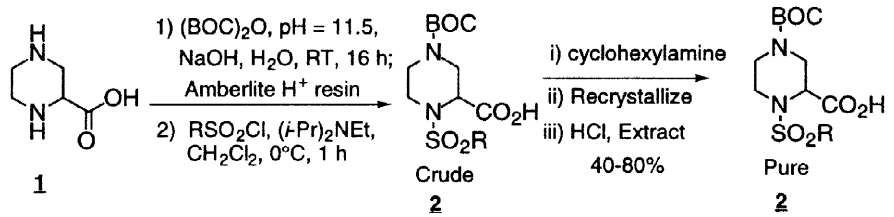
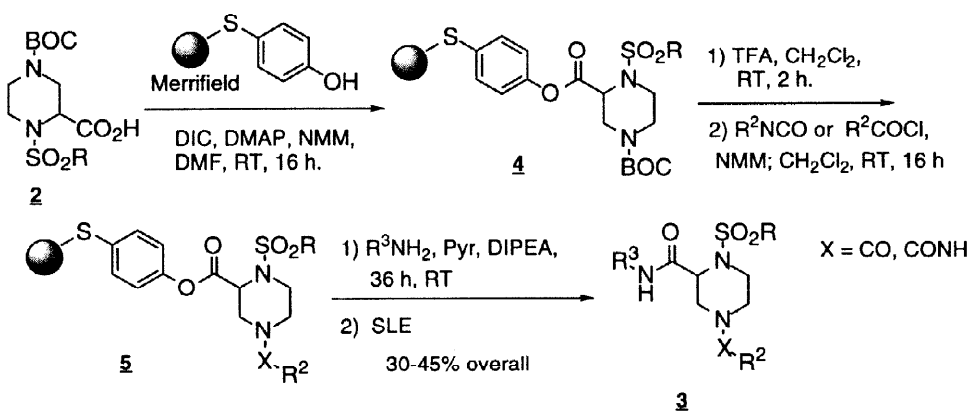
accomplished by acidification with ion exchange resin, filtration, and lyophilization. The BOC-piperazine-2-carboxylic acid was then converted to sulfonamides **2** by addition of the appropriate sulfonyl chloride in the presence of DIPEA. Crystallization of the sulfonamides as their cyclohexylamine salts provided pure **2** without the need for chromatography.[6]

Scheme II: Synthesis of Scaffold



Solid Phase Steps: Marshall's phenol-sulfide linker[2] was used to attach the sulfonamides **2** to Merrifield resin.[7,8] The piperazine-2-carboxylic acid was attached to the resin with DIC and DMAP to provide intermediates **4** in good yield.[9] A qualitative $FeCl_3$-pyridine test was devised for use on this resin to detect for residual phenol.[10] The test provides a deep purple color in the presence of phenols, which is detectable at phenol loadings >0.08 mmol/g.

Scheme III: Library Synthesis



Removal of the BOC protecting group with TFA, followed by addition of various isocyanates or acid chlorides afforded intermediates **5**. These two reactions could be effectively monitored by single bead IR for the presence or absence of the appropriate carbonyl stretching frequencies. We also found that cleavage of the intermediates could be very cleanly accomplished using NH_3 as a 0.5 M solution in dioxane. Analysis of the resulting primary amides could then be performed by HPLC and NMR.

Cleavage and Purification: The previously reported procedures for cleavage from the resin involved oxidation of the sulfide linker to the sulfoxide, to activate the linker, followed by cleavage with an amine nucleophile to form amide products of type **3**.[2] In our hands hydrolysis from the resin to give the carboxylic acid was often observed when this method was used. Alternatively, we found that cleavage could be effectively performed without any prior activation of the linker by direct treatment with amines.[9] However in DMF this lead to unacceptably long reaction times (5-7 days). Therefore a study of cleavage rates using *n*-butyl amine in different solvents was undertaken (see below). We found pyridine to be the solvent of choice for cleavage from the phenol-sulfide linker providing 80%

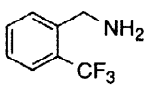
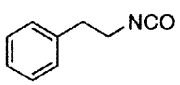
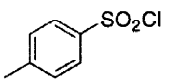
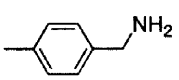
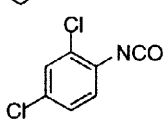
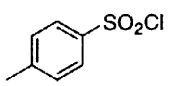
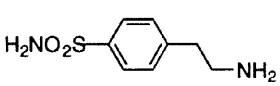
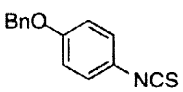
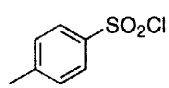
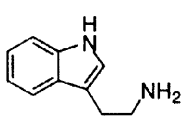
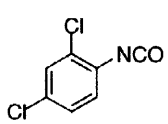
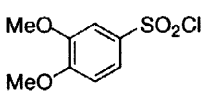
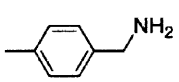
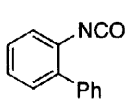
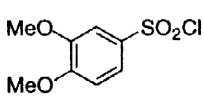
cleavage in 24 h. Pyridine also offered the additional advantage of excellent solubility and resin swelling properties.

Table I. Solvent Effects on Cleavage Rates

Solvent	Relative Rate	Solvent	Relative Rate
Pyridine	4.9	Dioxane	2.3
Diglyme-Me	3.7	2,6-Lutidine	1.7
Toluene	3.1	CH_2Cl_2	1.0
THF	3.0	DMF	1.0

Additionally, we found that cleavage rates varied widely with the nature and amount of the cleaving amine used. When one equivalent of less nucleophillic amines were used the cleavages were incomplete after 24 h. We decided therefore to use an excess of the amines and remove the remaining amine by high throughput extraction. In this way we could insure high cleavage yields across the entire library within 24 h.

Table II: HPLC Analysis of Partial Library Before and After SLE

Amine	Isocyanate	Sulfonyl Chloride	% Amine Before SLE	% Amine After SLE	Product Purity (HPLC)
			18.0%	<1.0%	78.9%
			19.4%	<1.0%	88.8%
			17.1%	3.0%	73.1%
			21.8%	<1.0%	79.1%
			10.2%	<1.0%	76.8%

* percentages reported are % AUC of total area, measured by UV at 214 nm.

Our group has used a solid supported liquid extraction (SLE) method in a number of solution phase library applications and found it to be an effective method for removal of water soluble impurities in a high throughput format.[11] Varian's Hydromatrix® [12] (diatomaceous earth) was distributed into Polyfiltronics® 96 well plates and treated with 1N HCl. The crude product was then added to the filter aid in CH_2Cl_2. The organic material passed through the Hydromatrix® into a collection plate below, while the amine salts were held up in the solid matrix, resulting in the effective removal of the amine impurities. Table II shows the results of HPLC analysis of some representative products before and after SLE. This purification method has been used to remove both acidic and basic impurities from either solid or solution phase libraries with a high degree of effectiveness.

A screening library composed of 2,816 spatially separated compounds (20 mg/well) was generated using the above described methods (8 sulfonyl chlorides, 16 acylating agents, and 22 amines). Analysis of the library was performed for identity on 12.5% of the components by ESIMS; 91% of the wells showed a base peak consistent with the expected product. LC/ESIMS analysis of the library (2% of the components) demonstrated an average purity of 69% (AUC, 214 nm). This library is currently being screened in a battery of in vitro biological assays.

This work demonstrates the versatility of the phenol-sulfide linker for the generation of large compound libraries. It also demonstrates how high throughput purification methods can be used in conjunction with react and release solid phase techniques to improve the purity of the resulting libraries.

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5. Bigge, C.F.; Hays, S.J.; Novak, P.M., Drummond, J.T.; Johnson, G.; Bobovski, T.P. *Tetrahedron Lett.* **1989**, *30*, 5193.
6. All products were characterized by: ^{1}H-NMR, ^{13}C-NMR, and MS.
7. Resin purchased from Nova Biochem (1.34 meq/g).
8. *The linker was attached using the following procedure:* 4-Hydroxy thiophenol (396 mmol) was added to a 0°C solution of NaOMe (396 mmol) in DMF (400 mL). The cooling bath was then removed and the solution was stirred for 1.0 hr. Merrifield resin (74.6 g, 1.34 mmol/g) was then added to the solution and the resulting mixture was heated to 60°C for 60 h. under N_2. The resin was washed and dried providing 80g of Phenol-sulfide resin. The phenol-sulfide loading was determined by elemental analysis for sulfur to be 98%.
9. Yager, K.; Fantauzzi, P. *Tetrahedron Lett.* **1998**, *39*, 1291-1294.
10. *The test is performed as follows:* Approximately 10 mg of resin is suspended in CH_2Cl_2 (0.5 mL). Pyridine (5 drops) is then added to the resin followed by $FeCl_3$ (10 drops of a 0.5 M soln. in $CHCl_3$). The resin is then washed 5 times with CH_2Cl_2. The presence of a purple color on the resin is indicative of phenol.
11. Charles R. Johnson, Birong Zhang, Pascal Fantauzzi, Michael Hocker, Kraig Yager, Fifth International Symposium, Solid Phase Synthesis & Combinatorial Chemical Libraries, September **1997**, London, UK.
12. Hydromatrix® and ChemElut® are available from Varian Sample Preparation Products, CA.
13. Correspondence regarding Solid Supported Liquid Extraction should be addressed to Charles R. Johnson.