2nd International Electronic Conference on Synthetic Organic Chemistry (ECSOC-2), http://www.mdpi.org/ecsoc/, September 1-30, 1998

[b0004]

Automated Synthesis of Small, Organic Therapeutics

Owen B. Wallace

Combinatorial Drug Discovery, Bristol-Myers Squibb Pharmaceutical Research Institute,

5 Research Parkway, Wallingford, CT 06492. USA

Introduction

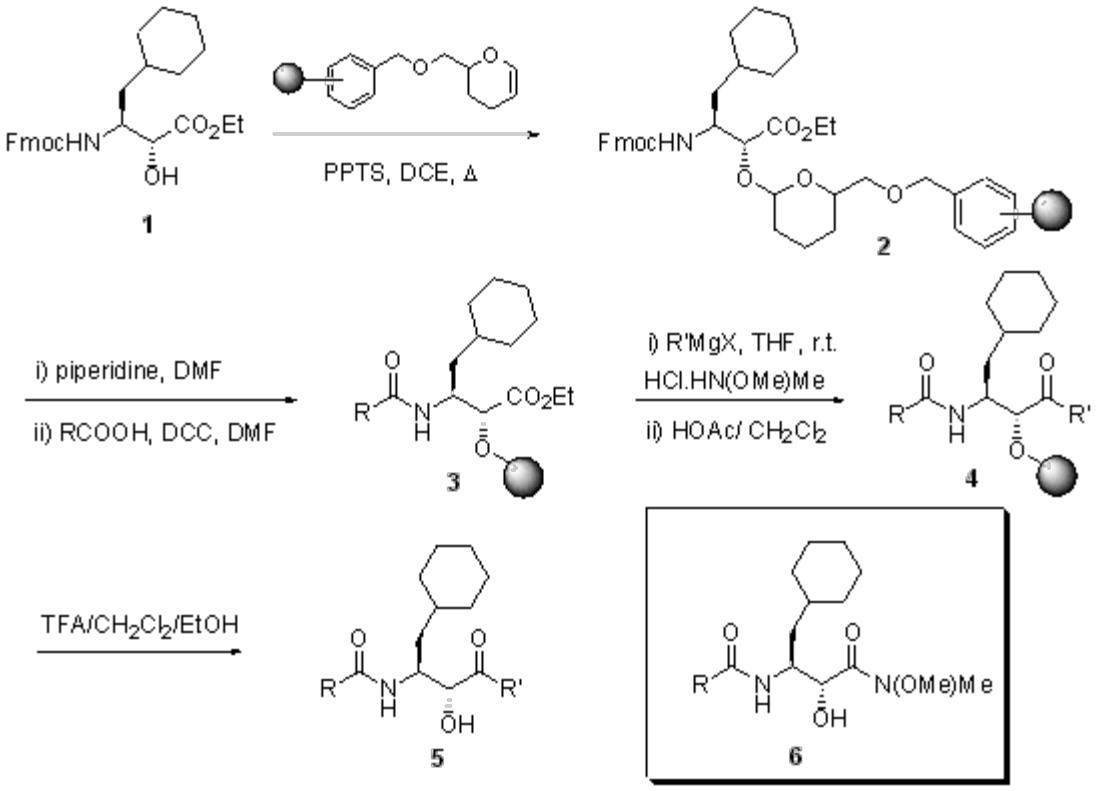
In the drug discovery process, chemists are charged with the task of synthesising compounds with the ultimate aim of producing a clinical candidate. With the aid of combinatorial chemistry, it is hoped that the number and diversity of compounds available for screening will increase, the time taken to optimise a lead will decrease and ultimately the time from the initial high-throughput screen to selection of a clinical candidate will be minimised.

In our combinatorial chemistry effort, all compounds are prepared in a single compound per well format and are tested in solution. The advantages of this approach are that it is compatible with all screening methods, and no deconvolution is necessary once an active compound is identified. Before submission to either therapeutic biology or high throughput screening (HTS), each compound is characterised by HPLC for purity and MS for identity. Finally, the mass of each compound is obtained. This not only allows for the calculation of a yield, but also helps further downstream in that quantitative HTS data can now be obtained.

The following reviews some of our recent developments in solid-phase chemistry in addition to providing an overview of our current automation techniques.

New Solid-Phase Chemistry

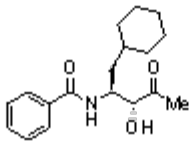
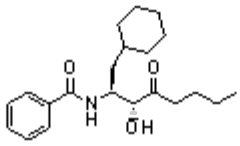
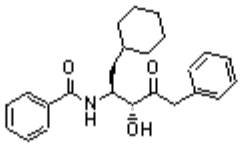
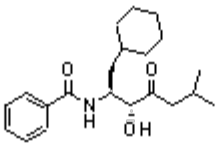
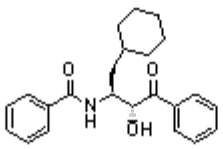
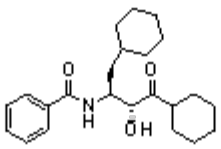
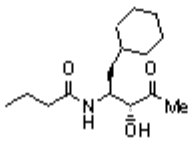
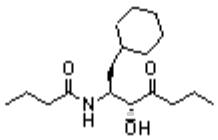
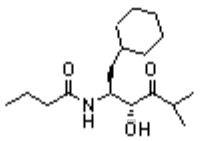
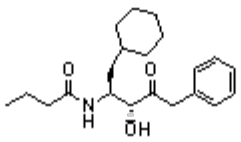
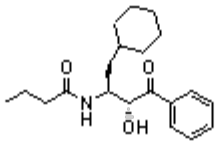
The cyclohexyl norstatine template has proven to be a useful pharmacophore for aspartyl protease inhibitors. a-Hydroxyketones based on the norstatine backbone, which were prepared in solution, have been shown to be potent inhibitors of renin (1) and have found use in the treatment of ocular disorders (2). We have developed a one-pot, solid-phase synthesis of a-hydroxyketones from their corresponding ethyl esters (3)



Scheme 1

The synthesis of the cyclohexyl norstatine transition state element proceeded in 30% overall yield from Boc-Phe and require just one column chromatography (4). The template was attached to the solid-support via Ellman's DHP linker using catalytic PPTS in 1,2-dichloroethane (DCE) (5). Following deprotection, the nitrogen could be acylated with a variety of acids (DIC, DMAP, DMF) to afford resin-bound ester **3**. Treatment of ester **3** with excess Grignard reagent (R'MgX) and *N*,*O*-dimethylhydroxylamine hydrochloride afforded ketone **4**, via the intermediate *N*-methoxy-*N*-methylamide **6**. Cleavage from the resin gave the desired ketone **5** in good purity and yield. The reaction was particularly successful with non-hindered primary Grignard reagents, and generally yielded products of excellent purity (>80%). Secondary Grignard reagents afforded lower yields of ketone (entries 6, 9), as did hindered primary nucleophiles (entry 4).

Table 1

Entry	RMgX	Product	Crude Purity	Yield
1	MeMgCl		86.0%	52%
2	*n*-BuMgCl		85.6%	59%
3	$PhCH_2MgCl$		85.7%	68%
4	*i*-BuMgCl		35.0%	31%
5	PhMgBr		69.9%	54%
6	$C_6H_{11}MgCl$		22.5%	14%
7	MeMgCl		83.1%	67%
8	*n*-PrMgCl		68.5%	49%
9	*i*-PrMgCl		26.6%	13%
10	$PhCH_2MgCl$		91.9%	65%
11	PhMgBr		70.0%	39%

It was found that the addition of excess Grignard reagent (20 equivalents) was necessary to drive the reaction to completion. For example, if only 6 equivalents of PhCH2MgCl and 2 equivalents of HCl.HN(OMe)Me were

employed, a 2:1 mixture of the desired ketone and the corresponding *N*-methoxy-*N*-methylamide was obtained after cleavage from the resin. A noteworthy characteristic of the solid-phase conversion of esters to ketones was its temperature independence. Similar yields and purities were obtained at reaction temperatures of either -15 ^{o}C or 23 ^{o}C.

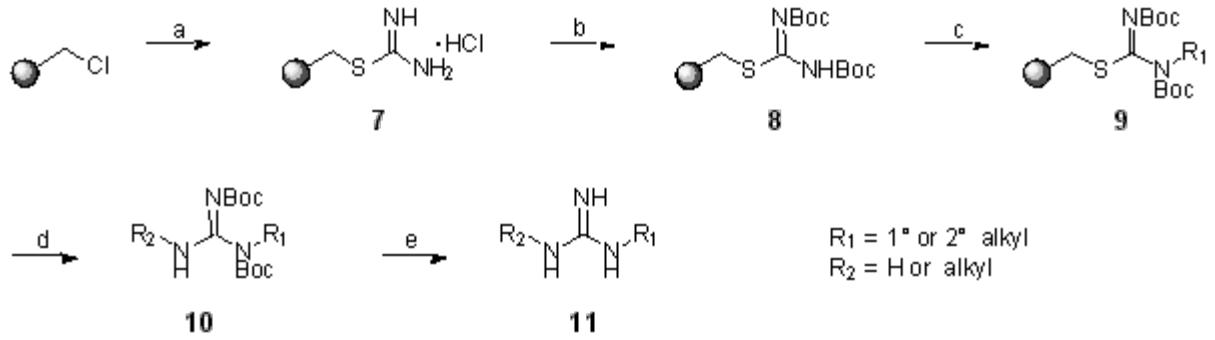
It was found that the reaction was much less successful with organolithium reagents than with Grignard reagents. For example, treatment of a mixture of resin-bound ester **3** (R = C3H7) and HCl.HN(OMe)Me with MeLi, followed by cleavage from the resin resulted in a multi-component mixture, with only a trace (9%) of desired ketone.

In summary, we have developed a one-pot procedure for the solid phase synthesis of ketones from the corresponding esters. The procedure can be conveniently carried out at room temperature and generally affords ketones of high purity when unhindered, primary Grignard reagents are employed.

Solid-Phase Synthesis of Guanidines.

Guanidines are found ubiquitously both in nature and in rationally designed drugs (6). Although there have been several reports of guanidine synthesis on solid-support (7), they often involve the use of a resin-bound amine or azide, thus limiting the scope of the procedures. We have developed a solid-phase synthesis of *N,N'*-substituted guanidines which uses a *N,N'*-bis(t-butoxycarbonyl)thiopseudourea as the masked guanidine scaffold (8). The procedure allows for the preparation of both *N*-mono-alkylated guanidines in addition to *N,N'*-bisalkyalted guanidines in high yield and purity.

Scheme 2

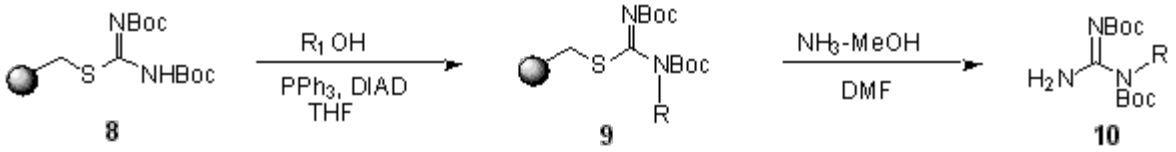


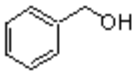
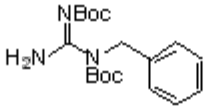
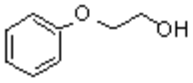
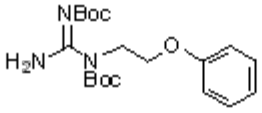
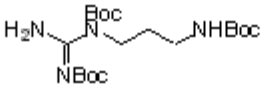
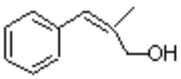
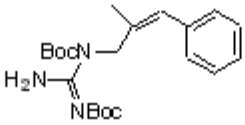
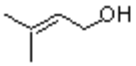
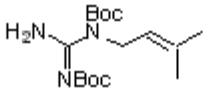
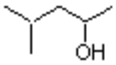
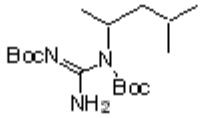
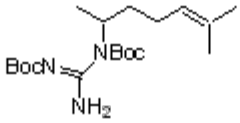
Reagents: (a) thiourea, DMF, 75°C, 20 h; (b) Boc_2O, *i*-Pr_2EtN, CH_2Cl_2, 40 h; (c) R_1-OH, PPh_3, DIAD, THF, 20 h; (d) NH_3, MeOH, DMF, 20 h, rt. or R_2NH_2, DMF, 80°C, 20 h; (e) TFA, CH_2Cl_2.

The procedure is outlined is Scheme 2. Treatment of Merrifield resin with excess thiourea results in the formation of resin bound thiouronium salt **7** (9). Protection of the nitrogens using $(Boc)_2O$ afforded bis(*t*-butoxycarbonyl)thiopseudourea **8** which was now capable of undergoing a Mitsunobu reaction (10) using a variety of primary and secondary alcohols. The mono-*N*-alkylated guanidines were liberated from the resin as the bis(Boc)-protected derivatives **10** by exposure of **9** to excess methanolic NH_3 in DMF (Table 2). The reaction is successful with primary (entries 2,3), secondary (entries 6,7), allylic (entries 4,5) and benzylic alcohols (entry 1) and yields the desired compounds in high crude purity. Deprotection of the Boc groups can be readily achieved by stirring the protected

guanidine with 50% TFA/CH_2Cl_2 overnight.

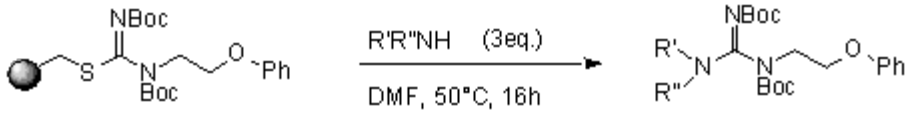
Table 2



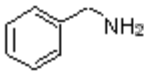
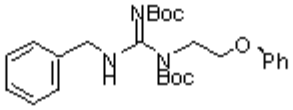
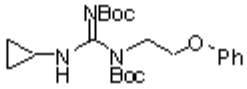
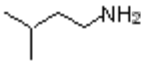
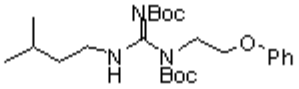
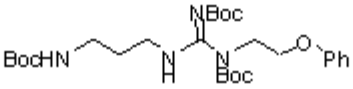
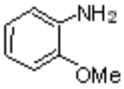
Entry	R_1OH	Product (10)		Yield (%)	HPLC Purity (%)
1			**(10a)**	88	>95
2			**(10b)**	100	>95
3			**(10c)**	88	>95
4			**(10d)**	92	>95
5			**(10e)**	95	100
6			**(10f)**	85	90
7			**(10g)**	85	86

The synthesis of *N,N*-disubstituted guanidines is outlined in Table 3. Resin **9b** was formed by alkylation of resin **8** with 2-phenoxyethanol under Mitsunobu conditions. Reaction of **9b** with a three-fold excess of primary amine at 50 ^oC in DMF cleanly afforded the *N,N*-substituted guanidines **11**. The excess amine can be readily scavenged using isocyanate resin. We observed no reaction of **9** with anilines (entry 6), and secondary amines such as piperidine (entry 5) gave a complex mixture with only a trace of the desired product. It has been reported that reaction of *S*-alkylthiopseudoureas with secondary amines and anilines usually requires the presence of "soft" cationic salts such as silver or mercury salts (11).

Table 3



9b **11**

Entry	R'R"NH	Product (11)		Yield (%)
1			**(11a)**	90[b]
2			**(11b)**	92
3			**(11c)**	92
4			**(11d)**	96
5		trace	**(11e)**	—
6		no rxn	**(11f)**	—

In summary, we have developed an efficient route to the preparation of substituted guanidines on a solid support. The use of alcohols and amines as the sources of diversity allows for the generation of large numbers of substituted guanidines in high purity and yield.

Automation.

Our first generation solid-phase synthesis reactor is illustrated in Figure **1**. The reactor consists of 48 fritted 1.5 mL polypropylene tubes. Each tube is connected to a teflon stopcock and all 48 stopcocks are opened or closed by actuating the handle shown on the right of the reactor. The reactor can be fitted to a circulation bath thus allowing both heated and cooled reaction conditions. A rubber gasket is placed over the reaction vessels, thus affording the capability of carrying out reactions under an inert atmosphere. The first generation reactor is capable of handling up to 0.5 g of resin and is ideal for methodology development. However, is suffers from several drawbacks including high cost of manufacture, heavy weight, large footprint and lack of compatibility with a 96-well screening format.

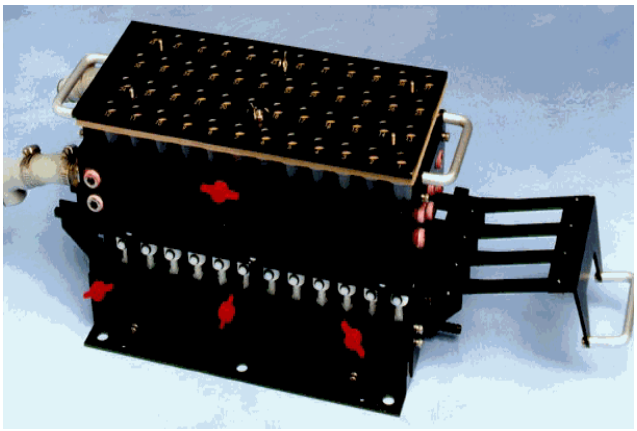


Figure 1. First generation solid-phase synthesis reactor

Our second generation reactor (Figure **2**) addresses these drawbacks. It is relatively inexpensive to manufacture, is lightweight (~6 lbs.), contains a proprietary pinch-valve mechanism instead of the array of teflon stopcocks and its small footprint is compatible with a 96-well plate. Again, the reactor has heating/cooling and inert atmosphere capabilities. Products are collected in custom 2.5 mL polypropylene microtubes.

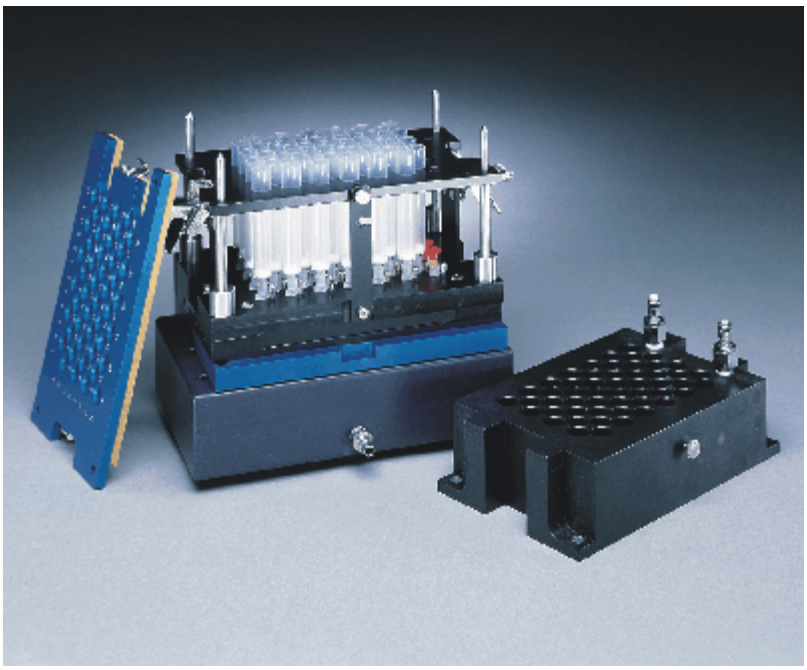


Figure 2. Second generation solid-phase synthesis reactor

For reagent additions, a modified Hamilton MicroLab 2200 liquid handler is used (Figure **3**). The solid-phase synthesis reactors are placed on two indexed orbital shakers. A rack containing the reagent solutions is then placed in the center console. A coaxial probe aspirates from the reagent rack into the reaction vessels. Reagent addition can be carried out under an inert atmosphere and, by using a circulation bath, can be temperature regulated.

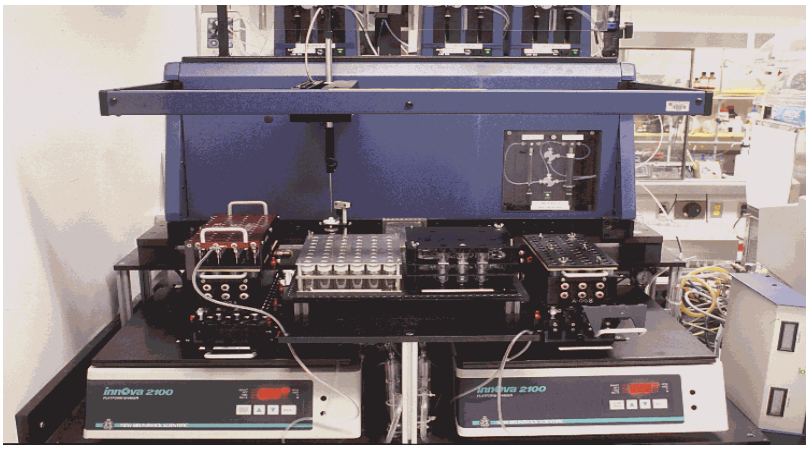


Figure 3. Modified Hamilton MicroLab 2200 liquid handler

Once the synthesis is complete and the compounds have been cleaved from the resin, the compounds are analysed by HPLC and MS. High throughput MS analysis is achieved using a Micromass Platform II mass spectrometer (Figure 4). This instrument is capable of characterising 960 samples per day with minimal operator intervention.

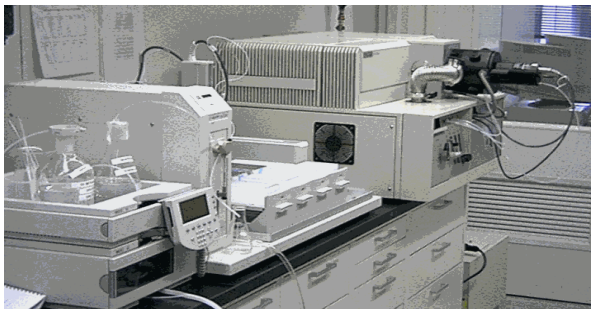


Figure 4. Micromass Platform II mass spectrometer

Finally, before submission for biological testing, all compounds are weighed. This is achieved using a Bohdan Automation workstation. The instrument has a capacity of 30 plates of 96 microtubes and is capable of weighing ~2500 samples per day to within 0.1 mg. Tubes containing compounds which do not meet the purity or MS requirements are removed prior to submission using a Bohdan culling station. Culling and sorting are achieved by moving individual microtubes, not by dissolution with solvent, liquid handling and reconcentration.

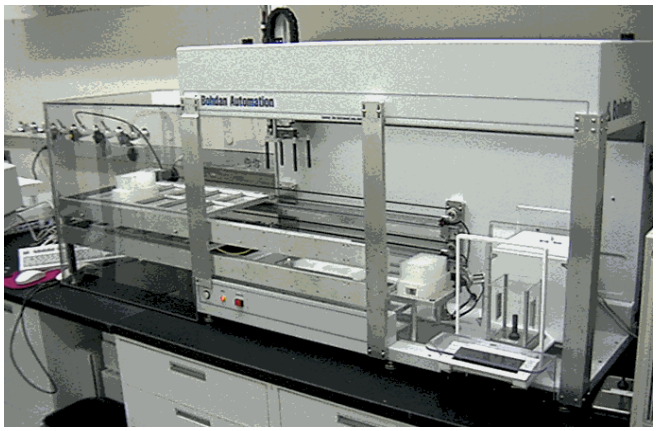


Figure 5. Bohdan weighing station

Experimental:

Solid-Phase Synthesis of Ketones from Esters:

To a stirred mixture of resin-bound ester **3** (150 mg, 0.5 mmol/g) and HCl.HN(OMe)Me (14.6 mg, 2 equiv.) in THF (1.2 mL) at room temperature was added MeMgCl (0.5 mL, 3 M/THF, 20 equiv.) dropwise over 20 minutes. The reaction mixture was stirred at room temperature for 15 hours. The resin was washed with THF to remove unreacted Grignard reagent. It was then washed with DMF/H2O (3:1), CH2Cl2, HOAc/CH2Cl2 (9:1), followed by multiple washings with CH2Cl2 and EtOH. The ketone product was then cleaved from the resin using TFA/CH2Cl2/EtOH (2:2:1, 2 mL).

Solid-Phase Synthesis of Guanidines:

A mixture of Merrifield resin (2.5 g, 2.35 mmol; Cl load of 0.94 mmol/g) and thiourea (0.94 g, 11.8 mmol) in DMF (25 mL) was heated at 75°C for 16 h. The resin was washed successively with DMF (4 x 50 mL), THF (3 x 50 mL), MeOH (3 x 50 mL), and CH_2Cl_2 (3 x 50). The resin was dried under high vacuum for 10 h. The resin and Boc_2O (3.0g, 14.0 mmol) were slurried in CH_2Cl_2 (50 mL) and treated with $(i\text{-Pr})_2EtN$ (4.1 mL, 24 mmol) over 5 min, and were subsequently gently shaken for 40 h. The resin was washed using the sequence of solvents described above and dried under high vacuum for 10 h.

To a mixture of resin **8** (250 mg, 0.175 mmol), PPh_3 (175 mg, 0.875 mmol) and 2-phenoxyethanol (110 mL, 0.875 mmol) in dry THF (3 mL) was added DIAD (175 ml, 0.85 mmol). The reaction was gently shaken for 14 h, and the resin was washed as described above and air dried. To a portion (100 mg) of the resin in dry THF (3 ml) was added sat. NH_3-MeOH solution (300 mL, excess). The reaction mixture was shaken for 15 h. The cleaved material was isolated and the resin rinsed with THF (2 x 1 mL); the solvent was removed *in vacuo* to give **10b** (24 mg, 100%, assumed loading of 0.7 mmol/g) with HPLC purity >98%. A second batch of the resin bound phenoxyethylisothiourea (100 mg) was suspended in DMF (2 mL) and was treated with benzylamine (3 eq.) at 50°C for 16h. The resin was

filtered and rinsed with THF (2 x 1 mL) and the solvent was removed *in vacuo*. The product was dissolved in CH_2Cl_2 (3 mL) and the contaminating excess amine was scavenge using 4 fold excess of isocyanate resin at 30°C for 12 h to give **11a** (27 mg, 90%) in >90% purity.

Acknowledgments

The contributions of all the members of the Combinatorial Drug Discovery department are gratefully acknowledged. In particular, I wish to thank Dr. Dharmpal S. Dodd for his work on the solid-phase synthesis of guanidines, and Dr. Harold N. Weller and his team for designing, manufacturing and maintaining the automation equipment.

References:

1 Patel, D.V.; Rielly-Gauvin, K.; Ryono, D.E.; Free, C.A.; Smith, S.A.; Petrillo, E.W., Jr. *J. Med. Chem*. **1993**, *36*, 2431.

2 LaMattina, John L. Eur. Pat. Appl. EP 473337

3 Wallace, O.B. *Tetrahedron Lett*. **1997**, *38*, 4939.

4 a) Matsuda, F; Matsumoto, T.; Ohsaki, M.; Ito, Y.; Terashima, S. *Bull. Chem. Soc. Jpn.* **1992**, *65*, 360

b) Paquet, A. *Can. J. Chem.* **1982**, *60*, 976.

5 Thompson, L.A.; Ellman, J.A. *Tetrahedron Lett.* **1994**, *35*, 9333.

6 (1) (a) "Amidines and guanidines in medicinal chemistry," Greenhill, J. V.; Lue, L. *Prog. Med. Chem.* **1993**, *30*, 203. (b) "The Organic Chemistry of Drug Synthesis," Lednicer, D.; Mitscher, L. A., Eds. Wiley: New York, Vol. I (**1977**) and Vol. II (**1980**).

7 (a) Drewry, D. H.; Gerritz, S. W.; Linn, J. A. *Tetrahedron Lett.* **1997**, *38*, 3377. (b) Kearney, P. C.; Fernandez, M.; Flygare, J. A. *Tetrahedron Lett*. **1998**, *39*, 2663.

8 Dodd, D.S.; Wallace, O.B. *Tetrahedron Lett*. **1998**, *39*, 5701.

9 Obrecht, D.; Abrecht, C.; Grieder, A.; Villalgordo, J. M. *Helv. Chim. Acta* **1997**, *80*, 65. (b) Gayo, L. M.; Suto, M. J. *Tetrahedron Lett.* **1997**, *38*, 211

10 This reaction has been previously carried out in solution. Dodd, D. S.; Kozikowski, A. P. *Tetrahedron Lett.* **1994**, *35*, 977.

11 (a) Kent, D. R.; Cody, W. L.; Doherty, A. M. *Tetrahedron Lett*. **1996**, *37*, 8711. (b) Miel, H.; Rault, S. *Tetrahedron Lett.* **1998**, *39*, 1565.

Comments

During 1-30 September 1998, all comments on this poster should be sent by e-mail to ecsoc@listserv.arizona.edu with b0004 as the message subject of your e-mail. After the conference, please send all the comments and reprints requests to the author.
