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Application of ReactArray Robotics and Design of Experiments Techniques in Optimisation of Supported Reagent Chemistry

Craig Jamieson,*[,‡] Miles S. Congreve,[†,‡] David F. Emiabata-Smith,[†] Steven V. Ley,[‡] and Jan J. Scicinski[†,‡]

GlaxoSmithKline R&D, Medicines Research Centre, Gunnels Wood Road, Stevenage, SG1 2NY Hertfordshire, UK, and GlaxoSmithKline Cambridge Technology Centre and University Chemical Laboratory, Lensfield Road, Cambridge, CB2 1EW, UK

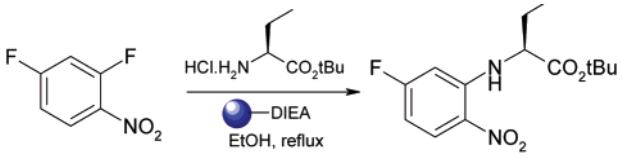
Abstract:

The application of ReactArray automation together with Design of Experiments (DoE) techniques in optimising chemistry involving supported reagents is discussed.

In recent years, the application of supported reagents in chemical library generation has been the subject of unparalled growth, and their utility in synthesis is now beyond question.[1,2] The use of solid-supported reagents maintains the traditional advantages of solid-phase synthesis (e.g., use of excess reagents, ease of separation of byproducts) but in addition offers the added advantage of retaining substrates *in solution*, which greatly simplifies reaction monitoring and analysis of intermediates. Despite the obvious power that the supported-reagent approach offers, there is often a considerable degree of effort required in optimising a particular reaction sequence. To address this issue, we have employed the techniques of Design of Experiments (DoE)[3] coupled with ReactArray[4] automation to enable high-throughput exploration of experimental space.

Recently,[5] we exemplified this approach in optimising an amide-forming reaction mediated by a polymer-supported dicyclohexylcarbodiimide equivalent.[6] The combination of automation and DoE enabled the rapid identification of a general and highly robust set of conditions for this particular transformation. In a separate study, we have also demonstrated the utility of the ReactArray system in the *automated* monitoring of conventional solid-phase synthesis.[7] In this report, we now demonstrate how the DoE/automation approach can be applied as part of a multistep supported-reagent synthesis of some heterocyclic library products.

Table 1. **Variables considered and maximum (+) and minimum (−) levels employed in the design**



	variable	(−)	0	(+)
A	stoichiometry of amino acid (equiv[a])	1	1.5	2
B	stoichiometry of PS-DIEA (equiv[a])	2	3	4
C	reaction time (hours)	14	19	24
D	concentration (volumes)[b]	25	37.5	50

[a] Mole equivalents with respect to 2,4-difluoronitrobenzene. [b] One volume is defined as one gram of substrate in 1 milliliter of solvent.

Table 2. **Experimental plan and results**

run	factor settings A	B	C	D	response yield of **1** (%)
1	0	0	0	0	64.7
2	+	−	+	−	83.3
3	+	+	−	−	92.7
4	0	0	0	0	64.4
5	−	+	+	−	54.5
6	−	−	−	−	41.9
7	−	+	−	+	43.4
8	−	−	+	+	43.8
9	+	+	+	+	89.7
10	+	−	−	+	63.5

The benzodipiperidinone moiety (**4**, Scheme 1) represents the core scaffold of a class of reverse-transcriptase inhibitors.[8] A concise synthesis utilising supported-reagent technology was devised and is illustrated below. We envisaged that S_N-Ar reaction of 2,4-dinitrophenol with a range of amino acid derivatives, followed by deprotection and subsequent nitro reduction, would furnish the cyclised product in a route highly amenable to analogue generation. It was found that a second S_NAr displacement could be carried out using excess sodium methoxide in methanol, enabling the preparation of disubstituted analogues.

However, prior to optimisation, it was found that the aniline (**1**) could only be prepared in low (ca. 40%) yield

[†] GlaxoSmithKline Cambridge Technology Centre.

[‡] University Chemical Laboratory; present address (CJ) GlaxoSmithKline Medicines Research Centre.

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(2) For an example of the use of solid-phase chemistry in scale-up see: Raillard, S. P.; Ji, G.; Mann, A. D.; Baer, T. A. *Org. Process Res. Dev.* **1999**, *3*, 177.

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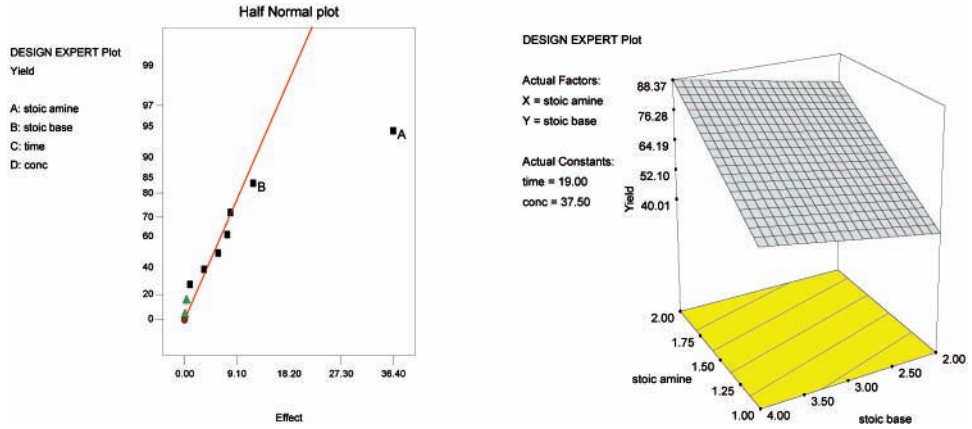

Figure 1. **Exploration of factor effects in the synthesis of aniline (1, R = Et).**

Scheme 1. **Preparation of a benzodipiperidone scaffold using supported reagents**

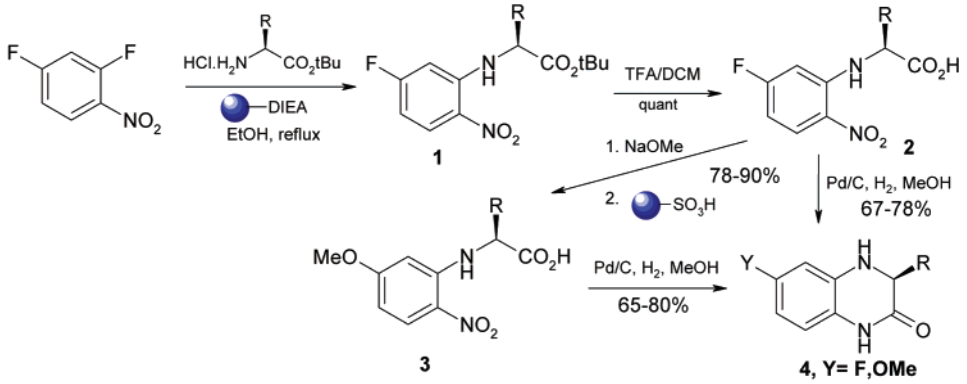

Table 3. **Analogues prepared using the optimised S_NAr chemistry**

amino acid	purity of S_NAr adduct (%)	yield (%)
isoleucine	95	90
phenylalanine	96	86
proline	98	92
phenylglycine	96	81
aminobutyric acid	98	96

and required chromatographic purification to remove unreacted 2,4-difluoronitrobenzene, thus minimising throughput. Therefore, we sought to optimise this conversion using a two-level half-fractional factorial design coupled with ReactArray instrumentation. From consideration of the variables we reasoned to influence this conversion, an experimental design was constructed to examine four factors using eight experiments and two centre points (Table 1). Exploratory experiments were undertaken to establish appropriate levels of each factor for use in the design.

Use of ReactArray automation facilitated both reagent addition and sampling/analysis tasks to be achieved without any user intervention. It was discovered that any resin sampled by the instrument could be expeditiously collected at the bottom of each sample vial by quenching the sample with acetonitrile and inserting a short delay prior to each HPLC injection, thus avoiding accumulation of fines in the injector port.[9]

The experiments were conducted in a random order as delineated in Table 2. Yields were determined by HPLC using 4,4′-di-*tert*-butylbiphenyl as an internal standard in each case.

Analysis of the experimental data was carried out using Design Expert 5 software[10] and is depicted in Figure 1. The most significant factor influencing the yield of the reaction was observed to be the stoichiometry of amino acid derivative employed. In addition, the amount of polymer-supported base utilised influenced the yield of aniline, with reaction time and concentration being considered as less significant. Further analysis of the data indicated that optimum conversions would be achieved when 2 equiv of amino acid and 4 equiv of polymer-supported base were employed, with time and concentration then set at operationally convenient levels (18 h reaction time and 37.5 vols dilution). No further improvement in yield was obtained when using more than 2 equiv of amino acid or 4 equiv of supported base.

Gratifyingly, when the reaction was repeated using the optimised conditions, the desired nitroaniline could be isolated in 91% yield and in greater than 98% purity (as determined by ^{1}H NMR and HPLC analysis). Excess amino

(9) Reaction vessels were manually charged with the appropriate resin systems and reagents added using the ReactArray as solutions in the required solvents. Each reaction was sampled (25 μL) at a given time interval and quenched with acetonitrile (600 μL). The resulting solution was allowed to stand for 2 min before an aliquot (25 μL) was automatically analysed by the instrument using an Agilent 1100 HPLC system and a Supelcosil ABZ+ Plus column (3.3 cm, 4.6 mm diameter) using the following method: eluent A water, 0.1% TFA; eluent B, acetonitrile 95%, water 5%, TFA 0.05%. Gradient: 10−95% eluent B in eluent A (1 mL/min) over 8 min. Detection: UV diode array (215, 254, 386 nm).

(10) Design Expert software is copyright of the Stat-Ease Corporation, Minneapolis, MN, U.S.A.

acid derivative could be efficiently scavenged by the addition of a macroporous sulfonic acid-based resin such as Amberlyst A15.

Application of the optimised conditions with a range of amino acid derivatives, indicated that the process was robust enough for use in array synthesis. Each analogue was obtained in good-to-excellent yields and importantly in high purity, thus obviating the need for chromatographic purification (Table 3). Subsequent deprotection, methoxide S_NAr displacement, and reductive cyclisation was found to occur smoothly without the need for further optimisation, allowing the generation of a number of benzodipiperidinone analogues.

In summary, we have demonstrated how the combination of Design of Experiments and ReactArray automation can facilitate the rapid exploration of experimental space. Such an approach facilitates the development of chemistry involving supported reagents which is of utility in both library synthesis and for larger-scale applications.[2]

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