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The use of cellulose (chromatography paper) as a cheap, versatile and non-covalent support for organic molecules during multi-step synthesis

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Cellulose chromatography paper provides a novel non-covalent support for synthesis and *in-situ* purification of multi-dimensional arrays.

The recent use of support technologies for the production of libraries and focussed arrays of compounds for biological evaluation has become commonplace and features a wide range of chemistries.[1] Primarily, these technologies rely upon the covalent attachment of the substrate to a matrix. Many different types of matrices have been used, from three-dimensional supports such as polystyrene beads[1] to planar supports such as glass[2] and cellulose sheets.[3] Many of these sophisticated supports are expensive, present specific handling issues and can require elaborate techniques for the attachment of the substrate, for the derivation of the support surface prior to attachment and for deconvolution. Often only very small quantities (sub-milligram) of the final products are produced.

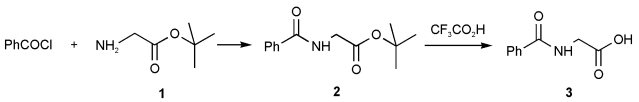
Recently, Williams[4] has described the use of thin layer chromatography for reaction optimisation of a piperazine array. This is the only instance of which we are aware, where the substrate is not *covalently* attached to a support which might be used for the production of arrays. We describe here the use of cellulose (Whatman 17CHR Chromatography Paper) as a very *cheap* and *versatile* support where the substrates are *not* covalently attached but instead simply absorbed onto the cellulose. This stands in contrast to previous reports where the substrate is covalently attached to derivatised cellulose.[3] Importantly, even when the substrates are only absorbed on the support, sequential chemical transformations are still feasible. The compounds can be purified whilst still absorbed on the support and the final products can be prepared in milligram quantities. In addition, the format provides significant advantages in handling and in reaction monitoring over conventional supports where the substrates are covalently bound.

All the chemistry described here was carried out on 10 × 10 × 0.9 mm square shaped pieces (a 'tile') of the cellulose chromatography paper, cut from the commercially available sheets using scissors.

Substrates and reagents can be loaded onto the paper simply by application as neat liquids or solutions with a Gilson pipette. Loading the reagent mixture as a single application to the centre of a cellulose square was found to give complete and even distribution.

Experimental techniques were optimised for a model reaction sequence conducted on 0.04 mmol scale, where typically 6mg of substrate are loaded onto a tile in 40 μl of liquid. (Scheme 1).

Benzoyl chloride was first loaded neat onto the cellulose support. *tert*-Butyl glycinate **1** was then pre-mixed with an excess of triethylamine and similarly loaded. Although **1** was an oil, it was found that pre-mixing it with triethylamine simplified the loading procedure by reducing its viscosity. Multiple repeats of the reaction were conducted on different tiles; tiles could then be sacrificed for analysis at intermediate points by extraction with boiling ethyl acetate and the resulting solutions analysed by LC/MS.† In this manner, formation of impure amide **2** was observed by LC/MS.



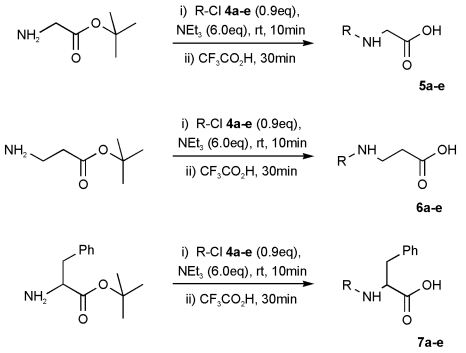
Scheme 1

An important component of this system is the ability to remove unreacted starting materials from the tile by washing with acidic or basic aqueous solutions. Un-ionised organic substrates tend to remain associated with the paper when it is washed with aqueous solutions and conversely, ionisable organic substrates or inorganic water soluble salts are removed by exposure to an aqueous environment. This forms the basis of an *in-situ* purification method for impure reaction products absorbed on the support. Amide **2** was purified by washing the support briefly with both aqueous base (to remove unreacted benzoyl chloride) and aqueous acid (to remove excess amine **1**). The support was then dried by irradiating in a microwave oven. Amide **2** remained non-covalently associated with the cellulose paper in good purity, as observed by LC/MS and ^{1}H-NMR analysis of material extracted from a sacrificial tile.

Conversion of **2** to carboxylic acid **3** was achieved by subsequent brief treatment of the dried support with neat trifluoroacetic acid. This led to slight swelling of the support (increasing with time) but no observed leaching of substrate. Following drying under a nitrogen stream, final extraction gave **3** in high purity by LC/MS and ^{1}H-NMR (68% yield from **1**).

A 3 × 5 array of 15 *N*-substituted amino acids was prepared using the cellulose support technology in order to test the generality of the method and to determine ease of handling when synthesising multiple samples (Table 1). Chemistry (Scheme 2) was analogous to the previous two step preparation of **3** from *tert*-butyl glycinate **1**, and utilised similar scale and experimental procedures. Included were reactions of amines with acid chlorides, sulfonyl chlorides and chloroformates.‡ Each sequence was conducted on a separate tile. Whilst the yields (over two steps) of final products obtained varied from moderate to good, the purities were generally excellent,§ indicating the efficiency of the washing procedures.

Further work investigated a three step reaction sequence using the cellulose support technology (Scheme 3). Using protocols analogous to those already described, bis-amide **8** was isolated in 33% overall yield. This result demonstrates that the



Scheme 2

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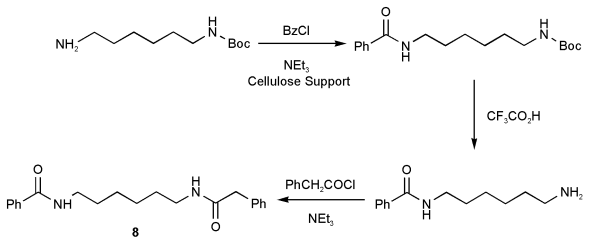
Table 1 Yields and products from the array

R	Product	Isolated yield (%)
PhCO **a**	**5a**	49
	6a	38
	7a	61
$PhOCH_2CO$ **b**	**5b**	34
	6b	21
	7b	40
BnOCO **c**	**5c**	8
	6c	11
	7c	29
$PhCH_2CO$ **d**	**5d**	32
	6d	27
	7d	69
$PhSO_2$ **e**	**5e**	46
	6e	45
	7e	64

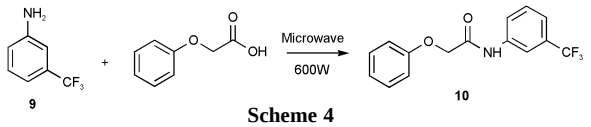
method may be applied to the preparation of arrays possessing multiple points of diversity, thus greatly increasing its utility.



Scheme 3

Microwave assisted chemistry was also investigated on the support for reactions that do not occur under ambient conditions. The reaction studied was the difficult amide formation between unactivated phenoxyacetic acid and electron deficient aniline **9** (Scheme 4).

Working on 0.04 mmol scale, a melt was formed between phenoxyacetic acid and a tenfold excess of aniline **9** (a liquid at room temperature). This was applied to the centre of a cellulose square as described previously. Loading was even and near saturation point. The loaded tile 'set firm' on cooling and was placed in a glass 20 ml scintillation vial. Microwave irradiation (600 W, 30 min) gave partial conversion to amide **10**. Purification by a similar support washing procedure as described previously and product extraction into hot ethyl acetate gave amide **10**, pure by LC/MS and NMR, in 12% yield. The same reaction on the same scale conducted without the support gave similar results.¶ Despite the low yield this experiment establishes the principle of microwave assisted



Scheme 4

chemistry on a cellulose support, opening up a much wider range of potential chemistry.

There are numerous potential applications of this work including a plethora of different chemistries. Further applications could also include the use of ionic liquids, other washing protocols (*e.g.* an aqueous solution of bisulfite for the removal of aldehydes) and altering the properties of the cellulose by derivatisation (*e.g.* with fluorinated alkyl chains). Each of these applications might open this technology to a much broader range of reactions.

Notes and references

† The possibility of monitoring reactions by sampling a *ca*. 1 mm^2 square from the corner of a tile was investigated. Analysis did not always correlate with whole tile analysis, presumably due to spacial concentration differences. The sacrificial tile may also be analysed by the use of TLC spray reagents. For example, incomplete consumption of primary amines gives a positive result with iodoplatinic acid.

‡ Loading levels and techniques were found to be critical. Whilst exceeding the absorbent capacity of the paper causes material losses, underloading gives sub-optimal substrate–reagent contact thus lowering chemical yields. Solvent volume should also be kept to a minimum as chromatographic separation of reagents across the support can occur leading to spacial concentration differences. Particularly volatile solvents such as dichloromethane or diethyl ether should be avoided for reagent loading because uniform reagent penetration into the cellulose is seldom achieved before solvent evaporation. Solid reagents can be warmed and applied as melts. Premixing reagents before application was found to give best contact, when possible without premature reaction.

§ All final compounds were characterised by LC/MS and ^{1}H-NMR.

¶ However, when the support-free reaction was scaled up to ~1 mmol, amide **10** was isolated in 60% yield after conventional aqueous workup and silica gel chromatography. This scale dependency is common in microwave chemistry.[5] From these and other studies, the cellulose appears only to act as an absorbent support; the rates of reaction appear independent of whether they are carried out in solution or on the support.

Representative experimental procedure: benzoyl chloride (6.1 mg) was loaded onto a 10 mm^2 square of Whatman 17CHR Chromatography Paper by a single addition to the centre of the 'tile' using a Gilson pipette. A solution of *tert*-butyl glycinate **1** (6.3 mg in 40 μl of triethylamine) was similarly loaded. After 10 min, the 'tile' was vigorously shaken sequentially with saturated aqueous $NaHCO_3$ (5 ml, 30 s), water (5 ml, 5 min), 0.5 M HCl (5 ml, 30 s) and water (5 ml, 5 min). The support was dried by heating in a microwave oven (600 W, 10 min). The 'tile' was then immersed in trifluoroacetic acid (0.25 ml) for 30 min, removed and dried under a nitrogen stream. Final product was extracted from the support by hot ethyl acetate, which was blown down under nitrogen to give **3** as white crystals (5.3 mg, 68%).

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