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Fully automated multi-step solution phase synthesis using polymer supported reagents: preparation of histone deacetylase inhibitors

Emma Vickerstaffe,[a] Brian H. Warrington,[a] Mark Ladlow*[a] and Steven V. Ley*[b]

[a] *GlaxoSmithKline Cambridge Technology Centre, University of Cambridge, Lensfield Road, Cambridge, UK CB2 1EW. E-mail: Mark.2.Ladlow@gsk.com; Fax: +44 (0) 1223 331532; Tel: +44 (0) 1223 336349*

[b] *Department of Chemistry, University of Cambridge, Lensfield Road, Cambridge, UK CB2 1EW. E-mail: svl1000@cam.ac.uk; Fax: +44 (0) 1223 336442; Tel: +44 (0) 1223 336398*

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The first fully automated multi-step polymer assisted solution phase (PASP) synthesis is described. An array of histone deacetylase (HDAc) inhibitors was prepared by an unattended 4–5 step sequence incorporating in-line 'catch and release' purification.

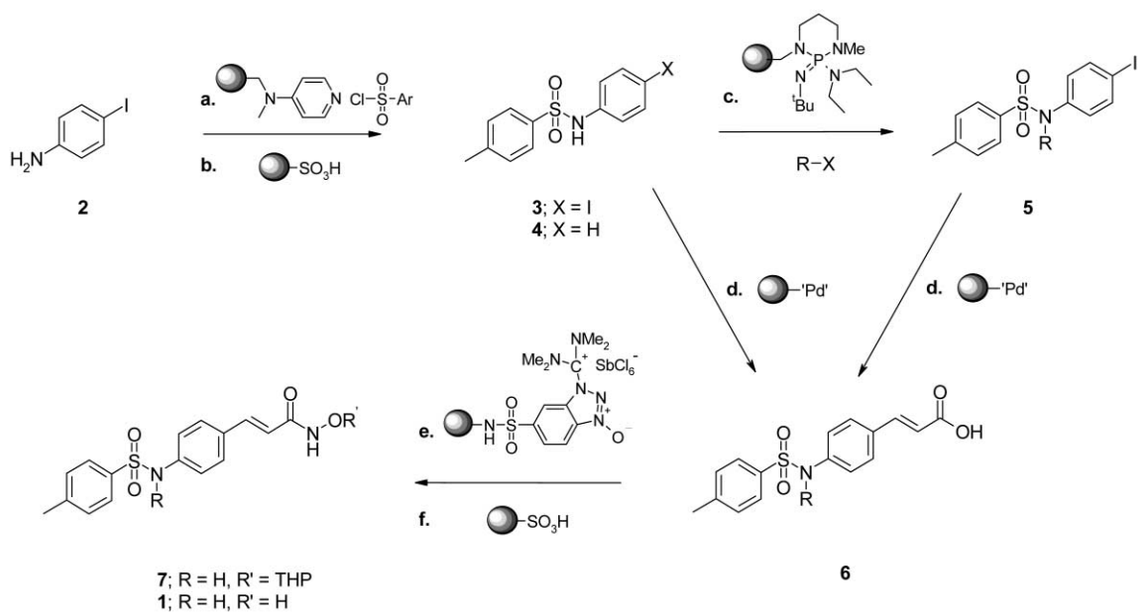
In recent years, the high-throughput screening of synthetic compound libraries has emerged as a key strategy within the pharmaceutical industry as a means of identifying new starting points for medicinal chemistry programs.[1] This in turn has created an increased demand for novel compounds, and has led to the development of strategies to simplify, expedite and automate the process of organic synthesis.[2]

Solid phase organic synthesis is currently widely used to implement high-throughput chemistry in an attempt to meet these demands.[3] However, this approach often suffers from unacceptably long development times, together with the inability to separate by-products prior to cleavage from the resin. This has led to a re-evaluation of solution phase approaches with the objective of introducing strategies and methods that lead to higher throughput. In particular, the use of polymer supported reagents and scavenger resins in library synthesis has been widely adopted.[4] In this way, the inherent advantages of both solid and solution phase methods, namely simplified work up, the use of reagent excesses to drive reactions to completion, and facile reaction monitoring in real time using conventional analytical methods, are exploited.

However, whereas automation of solid phase chemistry is already well established, to the best of our knowledge *full* automation of multi-step solution phase synthesis has not been reported.[5] Nevertheless, polymer assisted solution phase (PASP) methods are intrinsically well suited to automation, in that they typically utilise an iterative series of incubation and filtration steps. Therefore, fully automated PASP synthesis should be achievable. In particular, when the multi-step solution phase synthesis can be performed in a single solvent, full automation is feasible using existing robotic platforms.

Here, we describe the first fully automated multi-step PASP synthesis of an array of histone deacetylase inhibitors (HDAc), based upon the reported lead structure **1**.[6] HDAc inhibitors play a key biological role in chromatin remodelling and in the regulation of gene transcription.[7] As such, they have considerable potential as the basis for new therapeutic approaches in the treatment of cancer,[8] and as novel anti-protozoal[9] and anti-viral agents.[10]

Initially, we devised a PASP synthesis leading to the hydroxamic acid **1** based upon the reported solution phase synthesis but incorporating changes to facilitate automation (Scheme 1). The synthesis was first developed and performed



Scheme 1 *Reagents and conditions:* a) DMF, 40 °C, 1 h, ×2; b) DMF, rt, 2 h; c) DMF, RX, rt, 18 h; d) acrylic acid, tributylamine, DMF, 90 °C, 18 h; e) DIPEA, NH_2OTHP, DMF, 50 °C, 18 h; f) MeOH, DMF, rt, 3 h.

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manually, and utilised immobilised reagents for each step chosen such that the entire sequence could be performed in a single solvent (DMF), thereby removing the additional complexity associated with the need for solvent interchanges. In addition, to avoid the need for conventional chromatographic purification of intermediates, in-line purifications were incorporated utilising a combination of scavenger resins and a 'catch and release' strategy.[11]

The new synthesis also benefits from other advantages associated with the use of polymer supported reagents. For example, when sulfonylation of the aniline **2** is performed conventionally in solution in the presence of a sulfonyl chloride, a mixture of mono- and bis-sulfonamides is typically obtained. Therefore, this transformation is usually performed with a sufficient excess of the sulfonyl chloride to yield the bis-sulfonamide exclusively which is subsequently hydrolysed to the mono-sulfonamide in a separate step. However, we quickly established that the mono-sulfonamide **3** could be prepared directly by treating the aniline **2** with the sulfonyl transfer reagent derived from the immobilisation of an appropriate aryl sulfonyl chloride on polymer-supported dialkylamino-pyridine[12] in DMF. Any unreacted aniline was then readily removed following incubation with the sulfonic acid ion-exchange resin Amberlyst H-15 to afford **3** in high purity (>98% by LC-MS[13]) and acceptable yield (66%).[14]

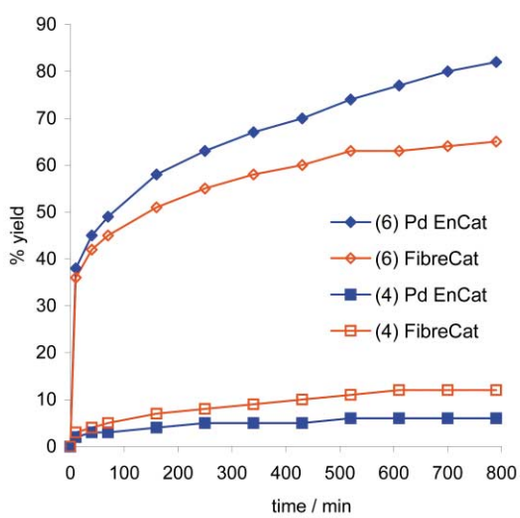


Fig. 1 Reactions profiles for the Heck olefination of **3** in the presence of immobilised palladium catalysts[16,17] leading to **4** and **6**.

Heck olefination of **3** with acrylic acid to afford **6** (R = H) was investigated using a variety of immobilised palladium catalysts to facilitate product work-up and these reactions were profiled using the Reactarray™ SK233 automated reaction sampling system[15] (Fig. 1). In this way, we determined that the competing dehalogenation pathway leading to **4** was minimised using microencapsulated $Pd(OAc)_2$ (Pd EnCat™)[16] as the source of palladium and tributylamine as base. At this stage, although it was possible to remove the palladium catalyst by simple filtration, a number of contaminants remained in solution. Bearing in mind the subsequent synthetic steps, in particular the need to activate the acid **6** prior to hydroxamate formation, the presence of tributylamine was acceptable. Further, the presence of any unreacted acrylic acid could be minimised by limiting the amount introduced at the Heck olefination stage. However, the dehalogenated contaminant **4** was not removed by filtration of the reaction mixture. A much better work-up procedure, therefore, was to utilise the carboxylic acid functionality present in the desired product **6** in an in-line 'catch and release' purification step. This was made even more attractive by the possibility of effecting concomitant activation of the acid functionality. This was achieved by incubation of the supernatant from the Heck reaction with 2-(1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluoroantimonate on polystyrene (PS-HBTU)[18] resin to generate the resin-bound activated HOBt ester of **6** directly without the need for any other additional reagents. This resin was then washed free of contaminants before treatment with a solution of *O*-THP hydroxylamine to release the hydroxamate **7** into solution. Exposure of **7** to Amberlyst H-15 effected final *O*-deprotection of the tetrahydrofuranyl ether to afford the desired hydroxamic acid **1**. Although **1** was obtained directly in good purity (87% by LC-MS), on this occasion further purification was performed by HPLC to afford the hydroxamic acid **1** in 33% overall yield (purity >99% by LC-MS).[19]

At this stage we recognised that an extra point of diversity could be incorporated into the synthesis by *N*-alkylation of the intermediate sulfonamide **3** prior to Heck olefination to afford **5**. In practise, this could be reliably achieved in the presence of the polymer-supported Schwesinger base PS-BEMP[20] to afford **5** (R = Me) in 90% yield and >98% purity by LC-MS. The use of an excess of less volatile alkylating agents is also acceptable because any residual alkylating agent is effectively scavenged in the following Heck reaction by conversion to the quaternary ammonium salt of tributylamine at 90 °C in DMF.

Our attention next turned to full automation of the array synthesis. A number of factors influenced our choice of robotic

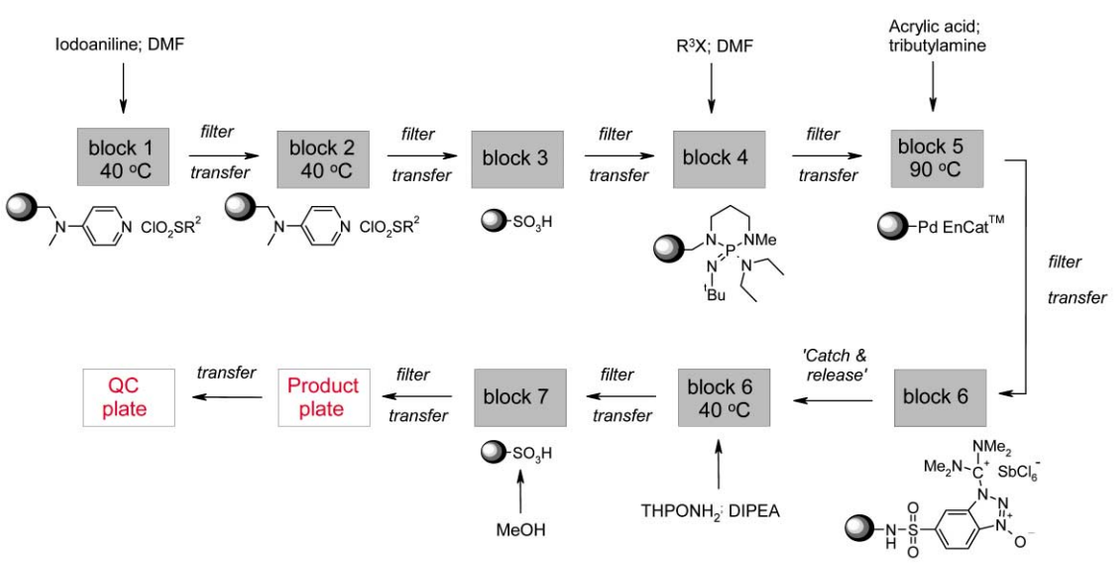
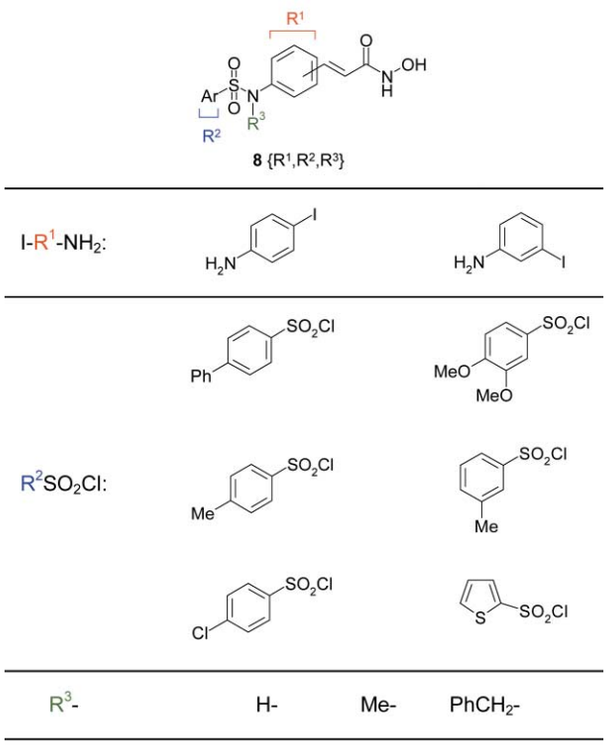


Fig. 2 Flowchart for auto-PASP synthesis of array **8** {R^1, R^2, R^3}.

Table 1 Monomers used to prepare the array of HDAc inhibitors **8**{R^1, R^2, R^3} by auto-PASP synthesis



platform. Firstly, for convenience, we wished to utilise a commercially available robotic platform. Secondly, a suitable robotic synthesiser should be able to: (i) dispense solutions of reagents into reaction vessels in a range of sizes, and (ii) efficiently mix and filter concentrated resin slurries and transfer the supernatant solutions between reaction vessels/blocks. Thirdly, for efficiency, a multi-channel synthesiser was preferred. A top filtration platform with 4 channels and high speed vortexing mixing capability was therefore identified as being most suitable for our purposes.[21]

After preliminary studies to establish that the synthesiser could handle concentrated resin slurries, we developed modular protocols suitable for performing each synthetic transformation. These involved either the addition of reagent solutions to the various resins followed by incubation and vortexing, or the filtration and washing of resins combined with dispensing of the supernatant solutions to the next reaction block. Separate reaction blocks were pre-loaded with all the reagent and scavenger resins required for the complete automated synthesis. Intermediate resin wash steps were performed with the minimum amount of solvent to limit serial dilution of the reaction mixtures during the linear stages of the synthesis. The automated protocols were then combined in an iterative manner to provide the fully automated sequence necessary for the array synthesis shown in Fig. 2.

The automated synthesis was validated by the preparation of the hydroxamic acid **1**, collecting aliquots at each stage and subjecting these to analysis by LC-MS. Reassuringly, the reaction profile obtained was similar to that observed for the corresponding manual synthesis. Importantly, it was possible to run the complete synthesis on the robot without the need for any manual intervention. The process required 3.5 days to run and afforded **1** directly in good purity (76% by LC-MS) and 28% overall yield. With further optimisation of the individual transformations it is anticipated that the overall run time could be reduced even further.

The automated PASP protocol was then applied to the preparation of a 36 member combinatorial array of potential HDAc inhibitors **8** {R^1, R^2, R^3} incorporating the monomers shown in Table 1. The robot was pre-loaded with all the resins and reagents necessary and the 180 reactions required were allowed to run unattended over a 4 day period. Finally, the array **8** was subjected to quality control analysis by LC-MS which confirmed that 34 of the 36 desired compounds had been obtained and that these were the major products in each case. In general, the compound purities were between 55–80% and 10–20 mg of crude material was obtained in each case (Fig. 3). These results compared well with those obtained for the automated synthesis of the single hydroxamic acid **1**. Following a single final autopreparative purification step, all compounds were obtained in greater than 95% purity according to LC-MS and ^{1}H NMR analysis.

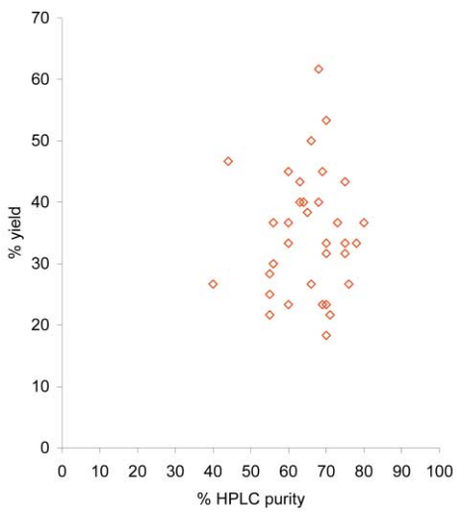


Fig. 3 Yield (%) and HPLC purities (area @ 254 nm) of HDAc array **8**{R^1, R^2, R^3} prepared by auto-PASP synthesis.

Conclusions

In summary, we have demonstrated that immobilised reagents and scavenger resins can be used to perform a series of sequential transformations that may be executed in the same solvent. The transformations required can be automated using a commercially available robotic synthesiser to facilitate the preparation of drug-like motifs. To exemplify this strategy, we have completed the first unattended fully automated PASP synthesis of an array of HDAc inhibitors **8**. Moreover, the synthesis was performed more quickly than the corresponding manual process (3.5 days *vs.* >5 days). Importantly, this study shows for the first time the utility of polymer assisted strategies to facilitate full, unattended automation of solution phase synthesis.

Acknowledgements

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18 A pre-production trial batch of this polymer supported reagent was kindly supplied by Argonaut Technologies Inc. (www.argotech.com).
19 Selected data for **1**: Mp 233–234 °C; HRMS $C_{16}H_{15}NO_4S$ requires $(MH)^+$ 318.0800, found 318.0809; ν_{max} (cm^{-1}): 3255, 1674, 1627, 1322, 1155; δ_H(400 MHz, DMSO-d_6): 12.40 (br s, 1H), 10.5 (br s, 1H), 7.65 (d, 2H, $J = 8$ Hz), 7.51 (d, 2H, $J = 8$ Hz), 7.42 (d, 1H, $J = 16$ Hz), 7.32 (d, 2H, $J = 8$ Hz), 7.08 (d, 2H, $J = 8$ Hz), 6.34 (d, 2H, $J = 16$ Hz), 2.30 (s, 3H); δ_C (100 MHz, DMSO-d_6) 167.9, 143.9, 143.5, 140.0, 136.9, 130.2, 129.9, 129.7, 127.1, 119.5, 118.3, 21.3.
20 2-*tert*-Butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosporine on polystyrene, 2.3 mmol g^{-1} available from Fluka (order No. 20026).
21 The Zinsser Sophas M6 robotic synthesiser (Zinsser Analytic, www.zinsser-analytic.com) was used in this study.