Pergamon

Tetrahedron Letters 40 (1999) 1405–1408

TETRAHEDRON LETTERS

Split-Split. A Multiple Synthesiser Approach to Efficient Automated Parallel Synthesis

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Received 4 November 1998; accepted 8 December 1998

Abstract: A multiple synthesiser approach to high efficiency, high throughput synthesis is described. It involves eliminating duplication in multi-step synthesis by establishing a cascade of matched synthesisers in which each machine performs a single step and provides feedstocks for the next instrument. The utility of this approach is illustrated by the efficient construction of a large array by a 4 step solution synthesis.

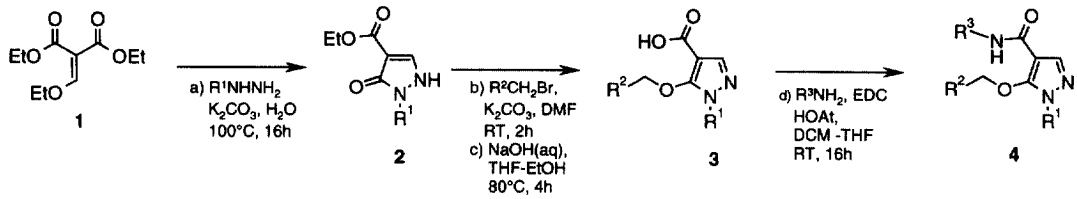
The mix-split paradigm[1] was introduced to enable the construction of huge product diversity with high synthetic efficiency and was the impetus for the combinatorial chemistry revolution that has occurred in drug discovery research groups. In recent years the inherent limitations of this approach, which requires a deconvolution strategy to obtain the complete information content of the synthesised compounds, combined with the rapid advances in automation technology[2] have led to parallel synthesis becoming the predominant strategy for most combinatorial chemistry groups.[3] However, the throughput, as well as the range of the chemistry that can be carried out, will be determined by the capability of the particular synthesiser and the major influence on the output of automated parallel synthesis is synthetic efficiency. For single step processes, parallelisation requires no duplication and is thus maximally efficient, but few syntheses comprise a single step, particularly as the goal is to introduce diversity into the products. Multi-component chemistry[4] which allows the introduction of multiple diversity elements in a single process is an exception, and for this reason has become an active area of research. However, to develop an approach that does not require purpose-designed chemistry, a more general strategy for automated parallel synthesis is necessary. We now describe a "split-split" strategy that employs the co-ordinated use of multiple synthesisers to obtain high efficiency, high throughput solution phase synthesis.

Previously in our group, MacLachlan[5] has reported the synthesis of solution phase combinatorial libraries based on a pyrazole template using a 4 step synthesis (figure 1). Using a split-mix approach 1000 compounds were synthesised in pools of 100 compounds. The 4 step synthesis required 40 reactions and represents the highest synthetic efficiency possible for this library. We now wanted to construct a similar library as an array by automated parallel synthesis. If the above library were constructed by complete

0040-4039/99/$ - see front matter
PII: S0040-4039(98)02620-3

parallelisation, it would be necessary to carry out 4000 reactions which represents the lowest synthetic efficiency, due to the high degree of duplication in the early steps of the synthesis.

Figure 1. Pyrazole library synthesis



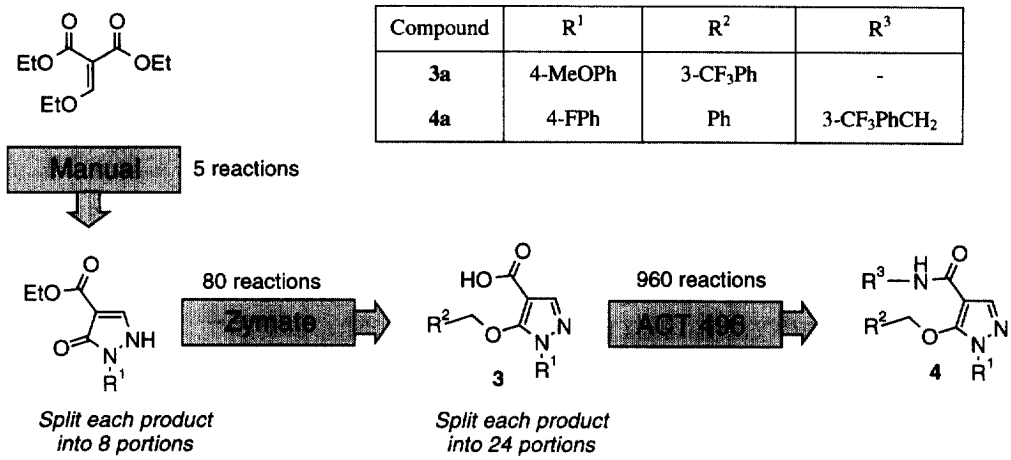
Alternatively, if instead of parallel synthesis each diversity step was carried out once and the product from these reactions then split for subsequent diversity steps, all duplication in the synthesis would be eliminated. However, although fewer reactions would be carried out in the early stages in the synthetic sequence, these reactions would have to be carried out on a larger scale which can present difficulties for automation since synthesisers have defined reaction numbers and reaction scales which generally cannot be varied by orders of magnitude. Thus in order to automate this "split-split" strategy it is necessary to link different synthesisers with capabilities appropriate to each step in the synthesis in terms of reaction scale and reaction numbers. The practical requirements for each step to construct a 960 member 5 R^1 x 8 R^2 x 24 R^3 library to give 40μmol of each final compound by the "split-split" approach are shown below.

Step	Reaction	Reaction scale	Number of reactions
a	Pyrazolinone formation	20 mmol	5
b	Alkylation	2 mmol	40
c	Ester hydrolysis	2 mmol	40
d	Amidation	40 μmol	960

In addition to these parameters, the chemistry requirements must also be taken into account in selecting the appropriate automated synthesiser. For this synthesis a Zymate solution phase chemistry system[6] and an ACT 496 MOS synthesiser were available. The Zymate synthesiser has the capability to synthesise 40 compounds at a time on a 1-5g scale, and seemed appropriate for carrying out steps b) and c) to synthesise intermediates **3**. The ACT is a 96 position synthesiser capable of making 10-100mg of compound, and would be suitable for step d) to make the final compounds **4**. The synthesis of intermediates **2** could have been automated using multiple runs on the Zymate, but because >10g was needed of each intermediate it was decided to prepare the compounds manually since the synthesis can be carried out on a multigram scale without chromatography. Thus by the use of the "split-split" strategy, the synthesis to construct the 960 compound could be carried out in 1045 reactions (figure 2). The 5 starting pyrazolinones were synthesised manually according to the published procedure[7] and all products were >95% pure by NMR and HPLC. These pyrazolinones were then divided into 8 portions and the *O*-alkylation and hydrolysis steps were carried out on the Zymate.[8] An advantage of the "split-split" process is that convenient purification steps can be introduced to early stages in the synthesis. Thus all 40 pyrazole acids obtained from the Zymate

were manually recrystallised. A purification step is necessary since small amounts of *N*-alkylation occur in step b) and these by-products must be removed from the synthesis. Thus all intermediates **3** were obtained as >90% pure crystalline solids and used in the acylation step which was carried out using the ACT 496[9] with purification by automated solid phase extraction using a Hamilton Microlab 2200. This final step was carried out by 10 x 96 compound runs, in which 8 pyrazole acids and 12 amines were used in each run.

Figure 2. Split-Split Pyrazole library synthesis



Compound	R^1	R^2	R^3
3a	4-MeOPh	3-CF_3Ph	-
4a	4-FPh	Ph	3-CF_3PhCH_2

The products from each run were analysed by LC-MS using a MicroMass Open-Lynx Diversity system. In total 823 (86%) out of 960 compounds synthesised were of sufficient purity to be submitted for screening. It is noteworthy that the high submission rate is a direct consequence of the "split-split" strategy since it is highly unlikely that the multi-step protocol could have been carried out by parallel synthesis using any single instrument. Apart from removing duplication, the approach meant that a quality control process was implemented for each step of the synthesis prior to commencement of the next. Furthermore, a "pre-final step" purification was conveniently carried out by manual recrystallisation to give pure intermediates which could allow the synthesis of compounds in the final step in acceptable purity without purification. If purification were limited to the final step of the synthesis, the *N*-alkylation by-products would have had to have been removed by a technique such as preparative HPLC to process 960 samples for which instruments are only recently becoming available.

Clearly common-sense indicates that full parallelisation is not the optimum approach for the automated synthesis of large libraries *via* multi-step synthesis. However, most groups when considering an automated parallel synthesis strategy will be constrained by the capability of the automated instruments they have at their disposal. In many cases laboratories have several of the same instrument, and are thus forced into full-parallelisation for multi-step synthesis. The success of the "split-split" approach relies instead on having a set of different, complementary synthesisers whose capabilities match the requirements of each step, both in scale of reaction and number of reactions. The concept of a compound factory[10] is gaining ground for high throughput synthesis and the "split-split" multi-synthesiser approach is a factor that should be considered when establishing such a unit.

In conclusion, we have developed a "split-split" approach for the multi-step solution phase synthesis of a pyrazole library by combination of manual and robotic synthesis using Zymate and ACT 496 synthesisers. This process not only enabled efficient high throughput synthesis to be carried out, but allowed for the introduction of purification and quality control steps to be introduced into the synthesis resulting in a high submission rate of the synthesised products. Work is now in progress to apply this strategy to solid phase synthesis and these results will be reported in due course.

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8. Zymate synthesis: Each pyrazolinone **2** was dissolved in DMF as a 0.8M solution and 5ml was robotically added to 8 reaction tubes containing 1.1eq potassium carbonate and stirred. The 8 benzyl bromides were prepared as 0.88M solutions in DMF and added robotically to each of the pyrazolinone solutions. The mixtures were stirred overnight at room temperature and then quenched with water and extracted with ethyl acetate (3x 10ml). The organic layers were evaporated using a Savant vacuum centrifuge to give oily products and a mixture of 2M NaOH /THF/EtOH (2:2:1) was added to each. The mixtures were heated to 80°C for 2h and allowed to cool then extracted with ether/hexane. The aqueous phases were manually acidified to pH1 with concentrated hydrochloric acid and extracted with dichloromethane. The organic solutions were evaporated and the residues recrystallised manually from ethanol or ethyl acetate /hexane. **3a** ^{1}H (d^6 DMSO) δ 3.79 (3H, s, OCH_3), 5.48 (2H, s, CH_2), 7.00 (2H, d, Ar H), 7.36 (2H, d, Ar H), 7.52-7.65 (4H, m, Ar H), 7.90 (1H, s, pyrazolinone H), 12.60 (1H, brs, CO_2H).
9. ACT 496 synthesis: a 0.1M solution of each pyrazole acid **3** in THF was prepared and added to 12 reaction vessels. A mixed solution of 0.1M EDC (1.9eq.) and 0.018M HOAt (0.03eq.) in DCM was added to each vessel, followed by 0.15M solution of the 12 amines in DCM (1.9eq) and the block shaken overnight. The products were drained into vials and then purified by automated solid phase extraction on SCX cartrigdes using a Hamilton Microlab 2200. Evaporation of the resulting solutions using a Savant vacuum centrifuge afforded the products. **4a** ^{1}H ($CDCl_3$) δ 4.61 (2H, s, CH_2N), 4.95 (2H, s, CH_2) 6.50 (1H, br, NH), 6.79-7.57 (13H, m, Ar H), 7.94 (1H, s, pyrazole H). MH+ 488. HPLC purity (215nm) 97%.
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