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Performing the Synthesis of a Complex Molecule on Sequentially Linked Columns: Toward the Development of a "Synthesis Machine"

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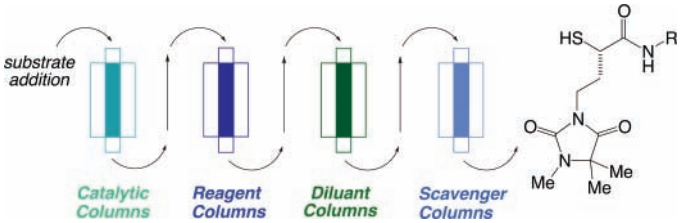
ABSTRACT



We describe the diastereoselective synthesis of a pharmaceutically active drug candidate via a column-based system. This methodology is complementary to classical solid-phase synthesis; individual columns are packed with resin-bound reagents and then linked in sequence and/or in parallel. In contrast to the traditional solid-phase approach, substrates are introduced in the mobile phase where they build up chemical complexity by percolating through the linked columns, ultimately eluting as the desired product.

It is no exaggeration to say that automated solid-phase synthesis has brought about a revolution in organic chemistry over the past 40 years. In its basic form, a substrate is attached to a solid support, while reagents and catalysts are introduced in solution.[1] A series of manipulations can be performed such that, upon detachment from the support, the final product can often be obtained in good chemical yield.[2,3] Imagine instead a system in which the fundamental roles are reversed: catalysts and reagents are attached to solid supports[4] in individual columns (which serve as reaction vessels) that are lined up in sequence and/or in parallel. The substrate is introduced in the mobile phase, where it builds up chemical complexity by flowing through the linked columns. Such a system, once optimized, could allow for the rapid and efficient synthesis of potentially complex molecules without the need for laborious, and often yield-limiting, intermediate isolation steps.

Previously, we reported the efficient synthesis of optically pure *cis*-β-lactams using a simple sequentially linked reaction column apparatus.[5] Currently, we are engaged in the development of truly complex systems such as the one pictured in Figure 2, wherein columns loaded with reagents and catalysts affixed to solid supports are used to modify

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(2) (a) *Solid-Phase Organic Synthesis*; Burgess, K., Ed; John Wiley & Sons: New York, 2000. (b) Seneci, P. *Solid-Phase Synthesis and Combinatorial Technologies*; John Wiley & Sons: New York, 2000.

(3) For a review of organic synthesis using polymer-supported reagents, catalysts, and scavengers in laboratory flow systems, see: Hodge, P. *Curr. Opin. Chem. Biol.* **2003**, *7*, 362−373.

(4) (a) De Miguel, Y. R. *J. Chem. Soc., Perkin Trans. 1* **2000**, 4213−4221. (b) Ley, S. V.; Baxendale, I. R.; Bream, R. N.; Jackson, P. S.; Leach, A. G.; Longbottom, D. A.; Nesi, M.; Scott, J. S.; Storer, R. I.; Taylor, S. J. *J. Chem. Soc., Perkin Trans. 1* **2000**, 3815−4195. (c) Gladysz, J. A. *Chem. Rev.* **2002**, *102*, 3215−3892.

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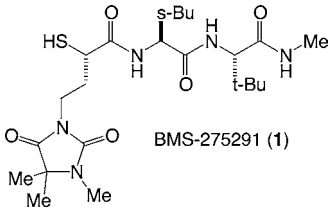
and combine multiple substrates in a systematic fashion. The reaction sequence is orchestrated via a regulated flow system, eventually yielding the desired product and allowing for easy regeneration and reuse of the resin-bound reagents; in essence, a "synthesis machine." We are actively pursuing this as the ultimate goal of our sequentially linked column assembly methodology.

Several criteria guided the choice of our target molecule. We wanted it to be a structure with a degree of complexity that could be assembled from readily available starting materials in four or more steps on the columns. We also wanted one of the steps to be a new use of an asymmetric reaction that we had developed in our own labs. In other steps, we wished to make new uses of solid-phase reagents. Finally, the target had to have notable medicinal activity, be a currently investigated drug candidate, or be a natural product of importance. Along those lines, the metalloproteinase inhibitor BMS-275291 (**1**), which is now in stage III clinical trials as a treatment for cancer, seemed an attractive candidate.[6]



BMS-275291 (**1**)

To assemble **1** on a sequentially linked system, we envisaged an apparatus containing columns linked both in parallel and in series, which reflects the convergent nature of the proposed synthesis (Figure 1). While the concept is intriguing, there are several limitations to this approach: most notable is the necessity for a "base solvent" that is introduced at the top of the assembly in which all subsequent reactions must be conducted (although quantities of different solvents can be introduced along the way, producing admixtures). For our purposes, we were lucky to discover that THF was a nearly ideal solvent/cosolvent for all of our transformations.

In one branch of the assembly, acid halide precursor **8** undergoes asymmetric chlorination[7] to yield the active ester **9** in 88% ee[8] in 3 h. This is the first instance in which our cinchona alkaloid-catalyzed asymmetric halogenation reaction has been performed on a solid-phase system. Quinine-loaded Wang resin-based beads **2** were packed into a jacketed addition funnel, saturated with THF, and then cooled to 0 °C. Acid chloride **8** and chlorinating agent **7** are then added in sequence to the top of the system and allowed to drip through the column. The cinchona alkaloid serves as both a

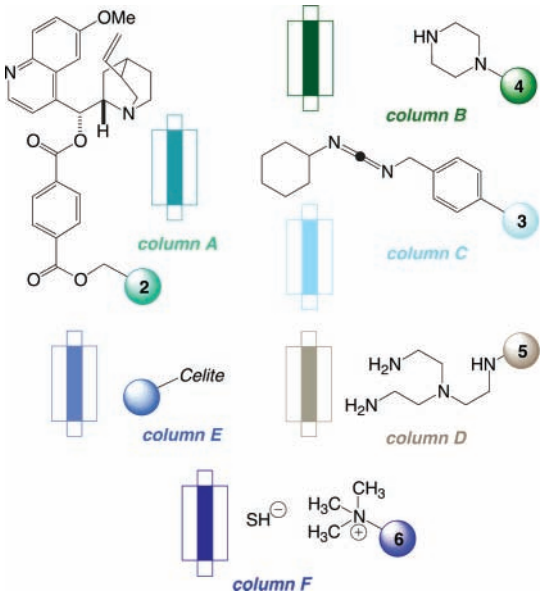


Figure 1. Components of the solid-phase "synthesis machine".

stoichiometric base for dehydrohalogenation and as the catalyst for the chlorination (the beads can be regenerated by a simple flush cycle with Hünig's base, followed by THF). The reaction mixture then flows into column B (Figure 1), containing piperazino resin **4**, to remove any remaining acid chloride.[9] Next, the reaction mixture encounters column E, where it is mixed with the peptide that is synthesized on columns C and D (the second branch of the system). Two simple amino acid derivatives are coupled on a column containing the versatile carbodiimide-based resin **3** to yield peptide **10** using THF as the flow solvent. Removal of the Fmoc group from product **10** is accomplished on column D, which contains tris-(2-aminoethyl)amine resin **5**.[10] Both of these reactions are accomplished in relatively short periods of time, making them ideal for a column based flow system. This balance between the residence time of the substrate on the column and the flow rate of the mobile phase is key to the success of such a system. Rapid elution does not allow for complete conversion, while excessive residence times often promote undesired side reactions.[11] It is also important to note that in all cases, the results appear to be better when a column with a smaller inner diameter is employed (e.g., 13 mm was found to be optimal for many steps); this allows for maximum interaction with the reactant beads as the substrate percolates through the solid phase.

N-Deprotected peptide **11** in THF then flows to meet α-chloroester **9** in "junction" column E, which is loaded with a simple diluant (Celite) that acts as a benign reaction medium. A longer residence time at this step is necessary to

(6) (a) Heath, E. I.; Grochow, L. B. *Drugs* **2000**, *59*, 1043−1055. (b) Marshall, J. L.; Baidas, S.; Bhargava, P.; Rizvi, N. *IDrugs* **2000**, *3*, 518−524.

(7) (a) Wack, H.; Taggi, A. E.; Hafez, A. M.; Drury, W. J., III; Lectka, T. *J. Am. Chem. Soc.* **2001**, *123*, 1531−1532. (b) France, S.; Wack, H.; Taggi, A. E.; Hafez, A. M.; Wagerle, T. R.; Shah, M. H.; Dusich, C. L.; Lectka, T. *J. Am. Chem. Soc.* **2004**, *126*, 4245−4255.

(8) Enantiomeric excess was determined by HPLC. An aliquot of the reaction mixture (which ordinarily would flow to the next column) was taken and analyzed.

(9) Since secondary amines do not react with our α-halo esters, the acid chloride scavenging step can be performed very rapidly (<1 h).

(10) Tris-(2-ethylamino)amine acts as an effective deblocking agent and scavenger for dibenzofulvene while suppressing formation of precipitates or emulsions: Carpino, L. A.; Sadat-Aalaee, D.; Beyermann, M. *J. Org. Chem.* **1990**, *55*, 1673−1675.

(11) Weber, A. P. *Chem. Eng.* **1969**, *76*, 79−80.

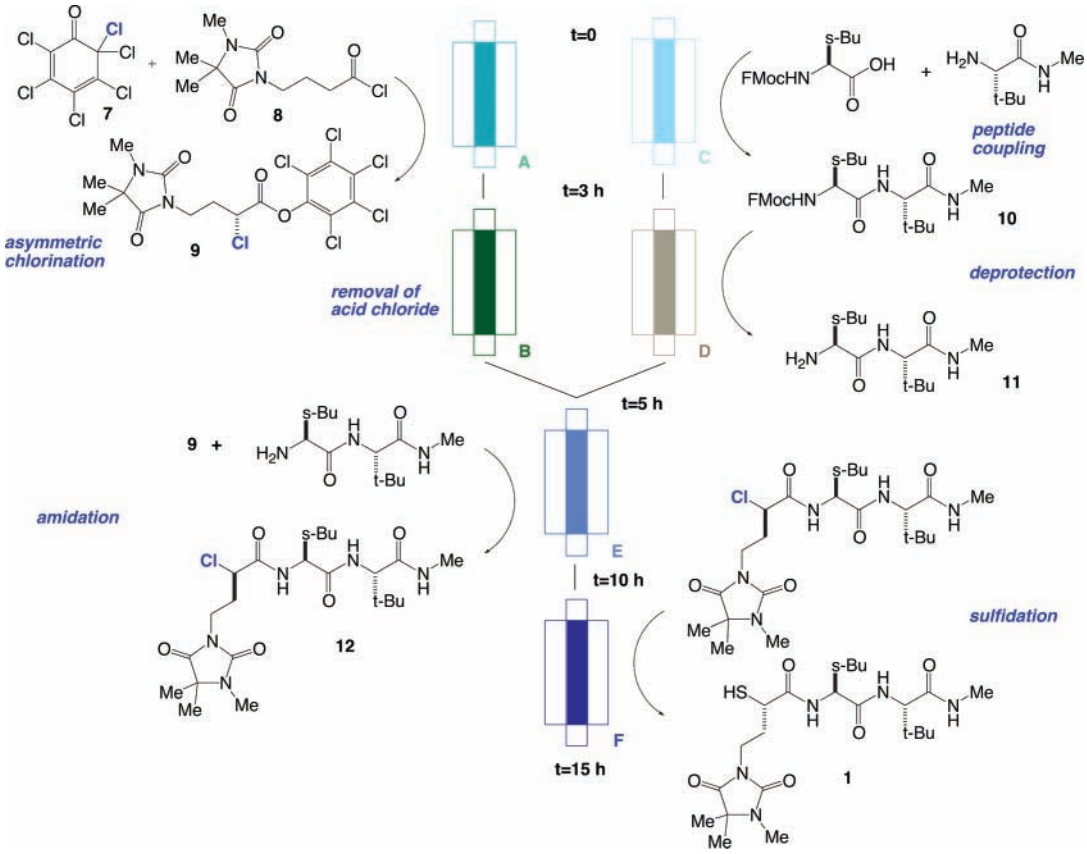


Figure 2. Apparatus for the solid-phase synthesis of BMS-275291 (**1**).

ensure good conversion since the two substrates are interacting with each other and not the beads. Given the overall complexity of the column assembly, we feared that flow rates would have to be timed like a "ballet dance." Conversely, we found that, in many cases, a slow drip (0.1 mL/min) ensured smooth reaction. In the junction column, of course, we had to time the arrival of compound **9** to coincide with that of compound **11**, but otherwise flow rates posed no special problems. On first attempts, the reaction took ~18 h to reach completion in THF, however, upon introducing MeOH, the amidation could be performed smoothly in less than 5 h, affording *N*-chloroamide **12**, which then proceeds to column F in the THF/MeOH solution.

Finally, Cl^- is displaced by SH^- on column F. Since most hydrogen sulfide salts are insoluble in THF, we found it necessary to find a way to immobilize SH^- on a solid support.[12] We exchanged Cl^- for SH^- on the inexpensive quaternary ammonium-based resin Amberlite-400(Cl) to yield a solid-phase-based reagent **6** in which SH^- could be suitably solubilized in THF, at least enough to provide a smooth reaction.[13] Initial attempts in THF[14] offered poor conversion (~30−40%) after extended times of elution. The cosolvent mixture from the previous step solved the problem by helping to solubilize the SH^- ions. We found that the desired final product **1** eluted from column F in 34% overall yield (~ 55 mg of product, starting from 100 mg of acid chloride **8**) and 83% diastereomeric excess, thus completing the "proof of principle" for our synthesis machine concept.[15] The complete synthesis can be performed in less than 1 day (~15 h) on the solid-phase system as compared to several days in our labs via "traditional" reactions.[16] While the amount of product recovered in our initial system may seem small, the system is fully scalable. The size of the reaction is relative to the size of the glassware we used. For this trial case, we chose columns with only a 20 cm inner diameter. If columns with larger inner diameters are used with greater amounts of the various resins, the scale of the reaction can be increased proportionally.

To ready the apparatus for another catalytic cycle, the columns were separated and regenerated. The quinine-loaded

(12) Choi, J.; Yoon, N. M. *Synthesis* **1995**, *4*, 373−375.

(13) Use of tetraalkylammonium hydrogen sulfides, both on solid phase and in solution, should provide a general method for S_N2-based halide displacement in organic solvents. Further studies on the displacement of optically active α-halo compounds will be reported in due course.

(14) In initial test reactions, this step was performed independently of the others using previously isolated starting materials.

(15) Elution times longer than 5 h for this step resulted in variable amounts of disulfide formation.

(16) An alternative approach could involve the use of these resin-bound reagents in a batch reaction. However, an important limitation of the batch method is degradation of the polymer-bound reagents caused by mechanical stirring. We have observed severe loss of activity from batch to batch due to this phenomenon, as compared to our column method (for example, after >100 runs, our asymmetric catalyst beads display consistent reactivity).

Wang resin was regenerated for subsequent use directly on the column by first washing with Hunig's base in THF and then with THF itself. The piperazino and tris-(2-aminoethyl)-amine resins were washed with ammonia and dried under high vacuum. Celite (being extremely inexpensive) was discarded and replaced. Finally, the hydrogen sulfide beads could easily be regenerated by stirring in MeOH with excess NaSH. Due to the nature of the amino acid coupling reaction, the carbodiimide beads were discarded and replaced.

Further studies targeting the machine synthesis of even more complex molecules (such as natural products) are to be reported in due course.

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Supporting Information Available: General experimental procedures and compound characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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