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Solid- and solution-phase synthesis of bioactive dihydropyrimidines*

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Abstract: With the emergence of high-throughput screening in the pharmaceutical industry over a decade ago, synthetic chemists were faced with the challenge of preparing large collections of molecules to satisfy the demand for new screening compounds. The unique exploratory power of multicomponent reactions such as the Biginelli three-component reaction was soon recognized to be extremely valuable to produce compound libraries in a time- and cost-effective manner. The present review summarizes synthetic advances from our laboratories for the construction of Biginelli libraries via solution- and solid-phase strategies that are amenable to a high-throughput or combinatorial format.

INTRODUCTION

Multicomponent reactions (MCRs) are of increasing importance in organic and medicinal chemistry. In times where a premium is put on speed, diversity, and efficiency in the drug discovery process, MCR strategies offer significant advantages over conventional linear-type syntheses. In such reactions, three or more reactants come together in a single reaction vessel to form new products that contain portions of all the components. In an ideal case, the individual building blocks are commercially available or are easily synthesized, and cover a broad range of structural variations. MCRs are providing products with the diversity needed for the discovery of new lead compounds or lead optimization employing combinatorial chemistry techniques. MCRs leading to interesting heterocyclic scaffolds are particularly useful for the creation of diverse chemical libraries of "drug-like" molecules for biological screening, since the combination of three or more small-molecular-weight building blocks in a single operation leads to high combinatorial efficacy. Over the last decade, industrial and academic researchers have made such powerful MCR strategies into one of the most efficient and cost-effective tools for combinatorial and parallel synthesis [1].

One prominent MCR that produces an interesting class of nitrogen heterocycles is the venerable Biginelli dihydropyrimidine synthesis. In 1893, P. Biginelli reported on the acid-catalyzed cyclocondensation reaction of ethyl acetoacetate (**1**), benzaldehyde (**2**), and urea (**3**) [2]. The reaction was carried out by simply heating a mixture of the three components dissolved in ethanol with a catalytic amount of HCl at reflux temperature. The product of this novel one-pot, three-component synthesis that precipitated on cooling of the reaction mixture was identified as 3,4-dihydropyrimidin-2(1*H*)-one **4** (Scheme 1), and this reaction is nowadays referred to as "Biginelli reaction", "Biginelli condensation", or as "Biginelli dihydropyrimidine synthesis" [3–5].

While the early examples of this cyclocondensation process typically involved a β-ketoester, aromatic aldehyde, and urea, the scope of this heterocycle synthesis has now been extended considerably by variation of all three building blocks, allowing access to a large number of multifunctionalized

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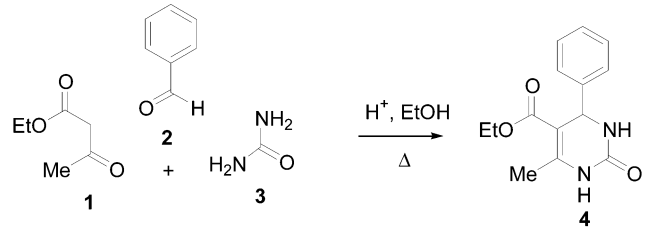
pyrimidine derivatives [6]. For this particular heterocyclic scaffold, the acronym DHPM has been adopted in the literature and is also used throughout this review. Due to the importance of multicomponent reactions in combinatorial chemistry, there has been a renewed interest in the Biginelli reaction, and the number of publications and patents describing the synthesis of novel DHPM analogs is constantly growing. In the present review article, we focus on synthetic methods that are suitable for the generation of DHPM libraries in a high-throughput or combinatorial format.



Scheme 1 The original Biginelli dihydropyrimidine synthesis.

SOLUTION-PHASE LIBRARY SYNTHESIS

Recently, several groups have reported on protocols for the solution-phase generation of Biginelli libraries [7]. For example, we have reported the automated generation of a library of 48 DHPM analogs by employing robotic dispensing of individual building block solutions into microwave reaction vials that were then irradiated in a microwave cavity under sealed vessel conditions [8]. This work employed a commercially available single-mode microwave reactor with a robotics interface including a liquid handler and a gripper. The liquid handler allows dispensing of reagents into the Teflon-sealed reaction vials, while the gripper moves each sealed vial in an out of the microwave cavity after irradiation. Here, a diverse set of 25 aldehydes, 8 urea/thioureas, and 17 CH-acidic carbonyl compounds was used in the preparation of the DHPM library. Out of the full set of 3400 possible DHPM derivatives, a representative subset of 48 analogs was prepared using automated mixing of building blocks. For most building block combinations, 10 min of microwave heating at 120 °C using AcOH/EtOH (3:1) and 10 mol% $Yb(OTF)_3$ as solvent/catalyst system proved successful, leading to an average isolated yield of 52 % of DHPMs with >90 % purity. Given the unattended automation capabilities of the microwave synthesizer, a library of this size was synthesized in 12 h [8]. More recently, our group has been involved with high-speed scaffold decorations of this heterocyclic core, exploring the diversity on at least six points around the scaffold [9–11]. This opens up the preparations of a very large number of analogs given the commercial availability of the building blocks that are used in the functionalization process (Fig. 1). The use of resin-bound catalysts, reagents, and scavengers in conjunction with microwave heating is a standard technology used in this context.

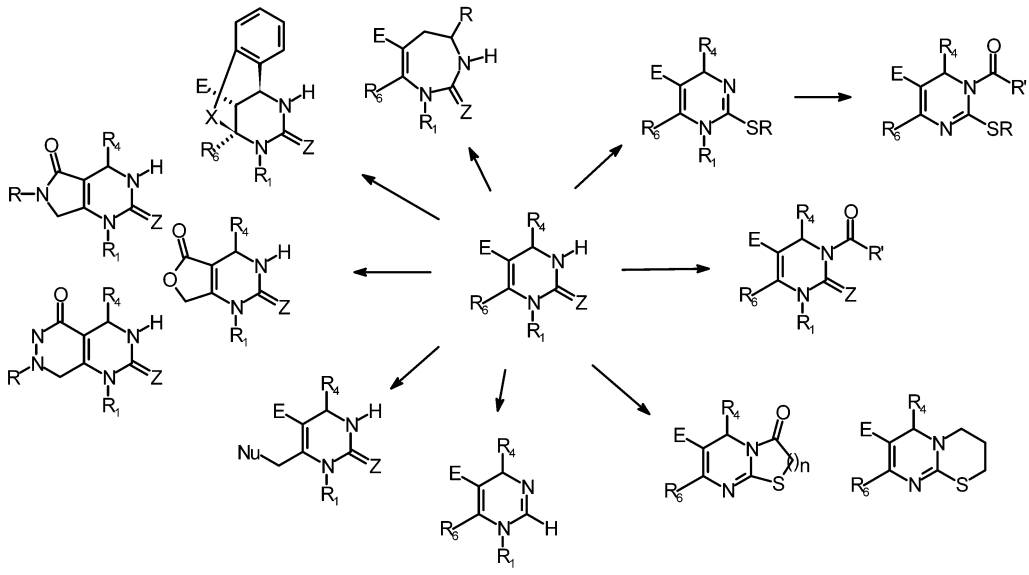
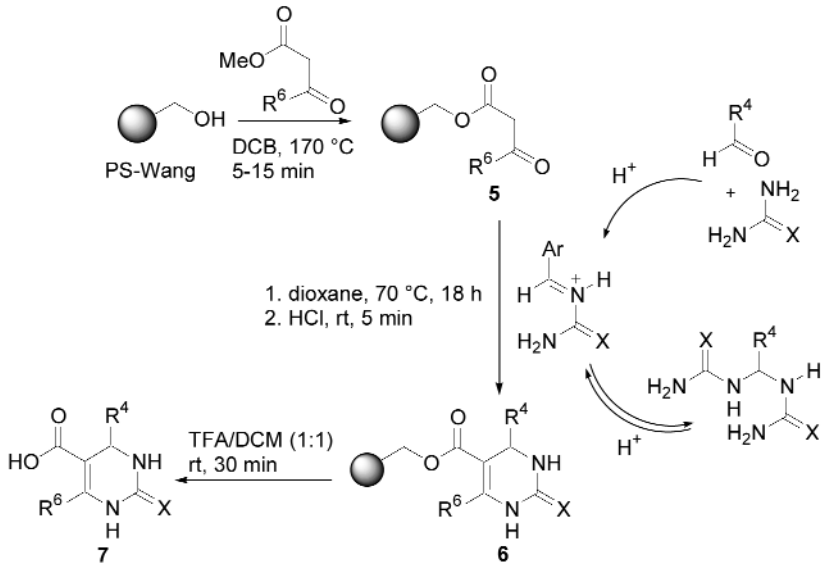


Fig. 1 Scaffold decoration of dihydropyrimidines.

SOLID-PHASE STRATEGIES

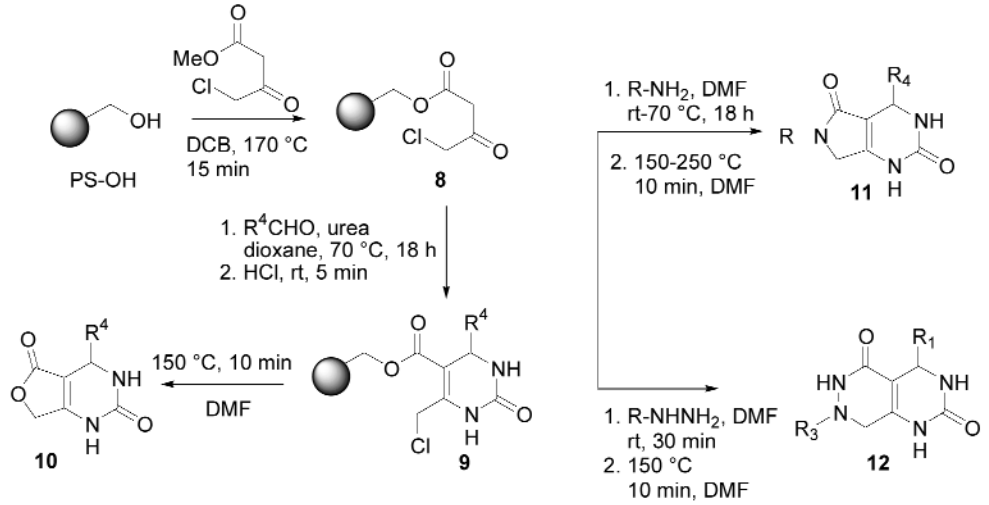
Solid-phase organic synthesis remains one of the cornerstones of combinatorial chemistry, since this technique allows the chemist to take full advantage of the powerful principles (i.e., split-and-mix synthesis) offered by combinatorial technologies. For a multicomponent reaction such as the Biginelli condensation, various solid-phase strategies can be envisaged, and, in fact, a number of different approaches have been disclosed in recent years, utilizing different resin-bound building blocks and linker combinations [12]. Given the regioselectivity encountered in using *N*-substituted urea building blocks in the Biginelli condensation [3], a solid-phase modification where the urea component is linked to the solid support via the amide nitrogen is an obvious choice. Wipf and Cunningham first described this strategy in 1995 [13].

Our group has reported a conceptually different solid-phase approach in 2001 (Scheme 2) [14]. In contrast to the previous strategy employed by Wipf and Cunningham, here the acetoacetate building block was linked to the solid support. Acetoacetates could either be attached by treatment of Wang resin with diketene (R^6 = Me), or by transesterification of Wang resin with β-ketoesters (R^6 = alkyl, aryl). This latter procedure can be conveniently carried out by parallel high-speed microwave-assisted chemistry at 170 °C in 1,2-dichlorobenzene (DCB) and obviously offers a higher degree of diversity (R^6) in terms of the number of different polymer- bound β-ketoester building blocks that can be generated [15]. The resin-bound β-ketoesters **5** were then reacted with urea/thiourea and an aldehyde building block in dioxane/HCl. Within minutes after the addition of the reagents, a colorless bisureide precipitate formed, resulting from trapping of the initially formed *N*-acyliminium ion intermediate [5] by excess urea present in the reaction medium. Upon longer reaction times and under acidic catalysis, the iminium ion intermediates are regenerated from the bisureides and subsequently are intercepted (irreversibly) by the resin-bound β-ketoesters, resulting in the formation of polymer-bound DHPMs **6** (Scheme 2). Treatment with concentrated HCl at room temperature dissolved any remaining bisureide by-product and allowed clean filtration of the resin. No release of DHPM product was observed under these conditions. Subsequent cleavage with TFA/DCM (1:1) led to the desired DHPM-5-carboxylic acids **7** in 52–81 % yield and high purity. Biginelli condensations involving thiourea needed considerable longer reaction times in accordance with solution-phase reactions and required the presence of a suitable scavenger such as thiophenol in the cleavage step [14].



Scheme 2 Solid-phase synthesis of DHPM-5-carboxylic acids.

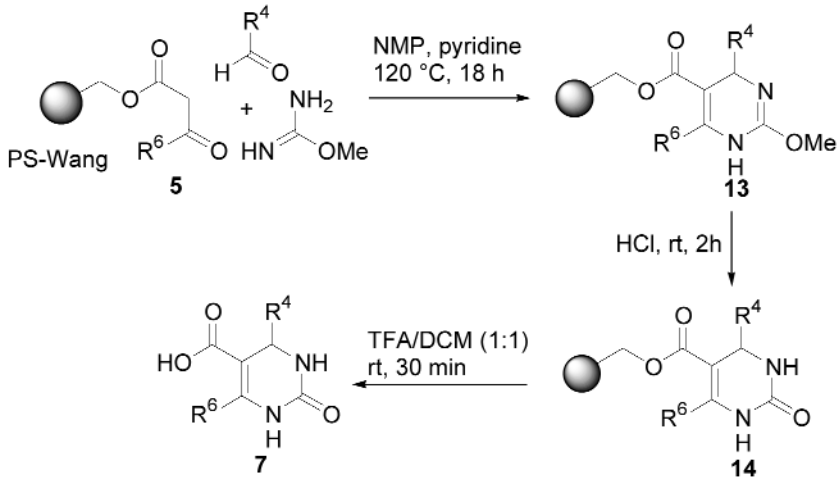
In the context of increasing the complexity generating-power of the classical Biginelli approach, we have recently disclosed an extension of the solid-phase strategy highlighted in Scheme 2 toward the synthesis of bicyclic DHPM scaffolds, employing commercially available methyl 4-chloroacetoacetate as a β-ketoester building block (Scheme 3) [16]. The 4-chloroacetoacetate precursor immobilized on hydroxymethyl polystyrene (PS-OH) **8** was subjected to a Biginelli-type three-component condensation employing urea and 12 diverse aromatic aldehydes utilizing the same reaction conditions as described above. The resulting 6-chloromethyl-functionalized resin-bound DHPMs **9** served as common chemical templates for the generation of three different heterobicyclic scaffolds using three different traceless cyclative cleavage strategies [16]. The corresponding furo[3,4-d]pyrimidines **10** were obtained by microwave heating in a rapid, thermally triggered cyclative release. Complete cleavage of material from the resin was achieved within 10 min at 150 °C utilizing DMF as a solvent. These heterocycles were obtained in 10–77 % overall yield over the three solid-phase reaction steps in high purity. Treatment of



Scheme 3 Solid-phase synthesis of bicyclic DHPM derivatives.

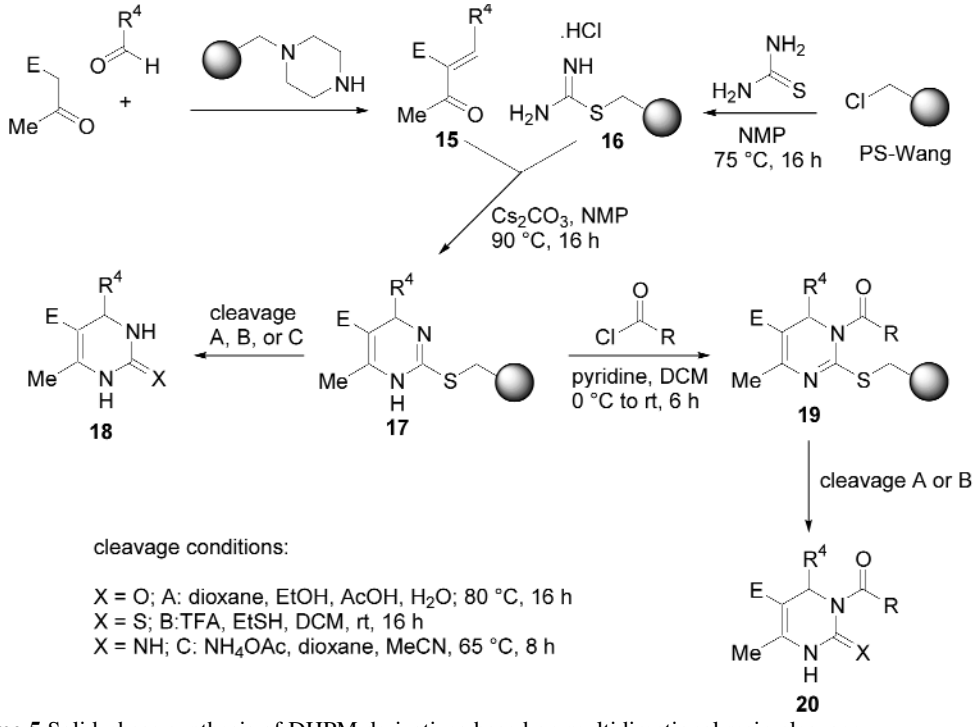
the chloromethyl DHPM intermediates with a variety of primary amines followed by high-temperature microwave heating furnished the anticipated pyrrolo[3,4-d]pyrimidine scaffolds **11** via nucleophilic cyclative cleavage. A small library was prepared in 25–55 % overall yield (4 steps) and generally ca. 85 % purity. For aromatic amines, cleavage temperatures of 250 °C were necessary in order to release the desired products from the polymer support. In a similar way, reaction with monosubstituted hydrazines resulted in the formation of pyrimido[4,5-d]pyridazines **12**, although here yields and purities were considerable lower. The same strategies could also be utilized in solution-phase synthesis [16].

In addition to solid-phase adaptations of the traditional three-component Biginelli condensation, solid-phase variations of the so-called "Atwal modification" [3] of the Biginelli reaction have also been reported. Thus, as an alternative to the acid-catalyzed Biginelli three-component assembly of the DHPM ring system outline in Scheme 2, an "orthogonal" strategy, where an *O*-methylisourea salt is condensed with CH-acidic carbonyl and aldehyde components under *mildly basic* conditions has been introduced (Scheme 4) [14]. Polymer-bound ketoesters were treated with *O*-methylisourea hydrogensulfate and an aromatic aldehyde in *N*-methylpyrrolidone (NMP) at 120 °C using pyridine as a base. After hydrolysis of the initially formed resin-bound *O*-methyl-1,4-dihydropyrimidine intermediates **13** with a large excess of aqueous HCl, standard cleavage from Wang resin furnished the desired DHPM-5-carboxylic acids **7** in acceptable overall yields (30–66 % over four steps) and high purity. The yields in this base-catalyzed version of the Biginelli condensation were however somewhat lower than utilizing the classic acid-catalyzed pathway (see Scheme 2).



Scheme 4 Solid-phase synthesis of DHPM-5-carboxylic acids.

In an effort to increase the molecular diversity that can be achieved in solid-phase syntheses of DHPM scaffolds utilizing the Atwal concept, an approach was elaborated, where initially an isothiourea building block was attached to resin-bound 4-(benzyloxy)-benzyl chloride ("chloro Wang resin") by treatment of the functionalized polymer with thiourea in NMP at 75 °C (Scheme 5) [17]. The resin-bound isothiouronium salt **16** was then condensed with enones **15**, which themselves can be readily prepared in high-throughput fashion by modified Knoevenagel condensation involving a polymer-supported piperazine catalyst [18]. The key condensation step leading to resin-bound 1,4-dihydropyrimidines **17** was carried out in NMP at 90 °C in the presence of Cs_2CO_3 as a base utilizing excess of the enone. The polymer bound dihydropyrimidine can then be directly cleaved from the resin employing different cleavage strategies (mutidirectional resin cleavage). Thus, three types of DHPMs **18** (X = O, S, and NH) can be obtained applying the appropriate cleaving conditions A (hydrolytic), B (benzylic cleavage), or C (aminolysis). The DHPM derivatives **18** were produced in 55–71 % overall yield (3 steps) and showed > 95 % purity. A key feature of this method is the multidirectional



Scheme 5 Solid-phase synthesis of DHPM derivatives based on multidirectional resin cleavage.

cleavage of the thiouronium-derived Wang linker, allowing the introduction of an additional element of diversity in the cleavage step, which multiplies the number of DHPMs that can be generated via this pathway by three. Furthermore, another diversity element can be attached onto the pyrimidine nucleus by regioselective *N*3-acylation of the polymer-bound DHPM intermediate with suitable electrophiles (e.g., acyl chlorides, RCOCl). Applying different cleavage strategies on this substrate (**19**), the corresponding *N*3-functionalized DHPMs **20** were obtained in moderate yields. This solid-phase approach is therefore particularly attractive for the preparation of pharmacologically active *N*3-acylated analogs and should be useful for the generation of targeted libraries of this heterocyclic scaffold.

Figure 2 summarizes the possible anchoring strategies that have been described for attaching DHPMs to a polymeric or fluorous support. These are the result of either employing the corresponding supported acetoacetate or urea/isothiourea building blocks in a Biginelli or Atwal type condensation (A–C), or are derived from anchoring an existing DHPM core to a polymeric support by suitable derivatization strategies (D). In terms of linking and cleaving methods, acid-labile linkers (i.e., Wang linker) have been used most frequently, but other strategies such as traceless cyclative cleavage, safety-catch release, or cleavage by nucleophiles have also been reported by some authors.

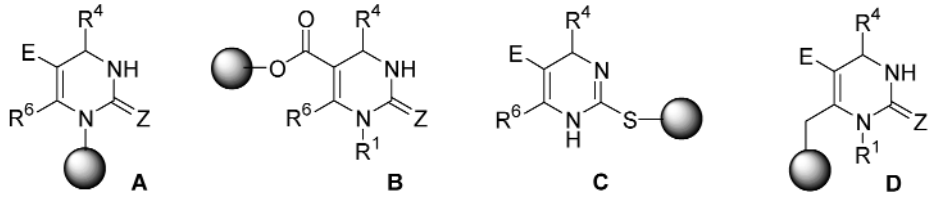


Fig. 2 Possible attachment points of DHPM scaffolds to solid-, soluble polymer-, or fluorous supports.

CONCLUSION

In its 110 years of existence, the Biginelli dihydropyrimidine synthesis has evolved from a little-known name reaction developed in the late 19th century, to one of the most often used multicomponent strategies today, successfully making the transition from academic research laboratories to the high-throughput labs of many pharmaceutical and biotech companies. Because of the pharmacological potency of the DHPM scaffold, novel dihydropyrimidines with important biological properties will undoubtedly be discovered by combining combinatorial synthesis and high-throughput screening (HTS) techniques [19].

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