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Microwave Assisted Combinatorial Chemistry
Synthesis of Substituted Pyridines

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Abstract: A new highly efficient MICROCOS technology (Microwave-assisted Combinatorial Synthesis) for generating combinatorial libraries is described. The technology is applied to the high throughput, automated, one-step, parallel synthesis of diverse substituted pyridines using the Hantzsch synthesis. The advantages of microwave-assisted chemistry for combinatorial synthesis include a broad range of available chemistries, simple reaction setup and product recovery readily amenable to automation, extremely short reaction times, and high product yields.
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Microwave-assisted organic synthesis (MAOS) is a new and quickly developing area in synthetic organic chemistry.[1] This synthetic technique is based on the empirical observation that some organic reactions proceed much faster and with higher yields under microwave irradiation[2] compared to conventional heating. In many cases reactions that normally require many hours at reflux temperature under classical conditions can be completed within several minutes or even seconds in a microwave oven, even at comparable reaction temperatures. While different hypotheses have been proposed to account for the effect of microwaves on organic reactions,[3] the reason for such dramatic acceleration effects remains largely unknown. Regardless of the exact origin of the microwave effect, it is extremely efficient and applicable to a very broad range of practical syntheses.

One of the most useful implementations of MAOS is the so-called "solvent-free" or "dry media" synthesis.[4] In this case, a solid support capable of absorbing microwave radiation (such as clay or alumina) is first impregnated with a solution of reactants in a volatile solvent. The solvent is removed by evaporation, and the solid support with adsorbed reagents is irradiated by microwaves. In some cases a small amount of N,N'-dimethylformamide is added to the reaction as an energy transfer medium.[5] The reaction takes place in the solid phase, and the products are then extracted from the support using an appropriate solvent. The great advantage of the solvent-free technique is that it can be conducted in open vessels, making it simple and safe to perform, and does not require specialized equipment (e.g. sealed digestion bombs) to accommodate high pressures which develop in heated liquid reaction systems.

Microwave-assisted organic reactions allow rapid product generation in high yield under uniform conditions. Therefore, they are ideally suited for combinatorial chemistry, which has emerged during the past decade as a powerful tool for producing large chemical libraries for biological screening and drug discovery.[6] *In the present work we demonstrate the first practical application of microwave technology in combinatorial chemistry.* The new synthetic technology, Microwave-assisted Combinatorial Synthesis (MICROCOS), employs solvent-free MAOS performed in a 96-well plate format for high-throughput, automated production of combinatorial libraries.

In its most productive version, MICROCOS utilizes multicomponent reactions[7] for synthesis of large and diverse compound libraries. In this work, MICROCOS is demonstrated by the synthesis of a library of substituted pyridines using a three-component Hantzsch synthesis. The pyridine scaffold is an essential structural element of various drugs, including antihistamines, antiseptics, antirheumatics, and numerous other agents.[8]

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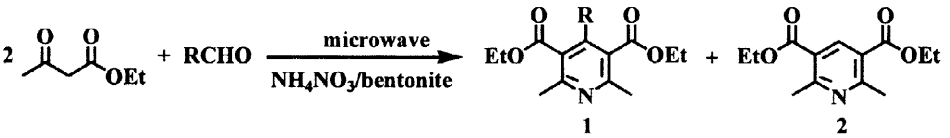
The Hantzsch synthesis is a condensation reaction between 2 moles of β-keto ester, 1 mole of aldehyde and 1 mole of ammonia.[9] The product of this reaction is a 1,4-dihydropyridine, which can be oxidized to the corresponding pyridine derivative.[10] Recently, a synthetic method was reported to obtain pyridines on a multigram scale in a one pot microwave reaction using bentonite clay as a support and ammonium nitrate as the source of ammonia and oxidant (nitric acid) as shown in Scheme 1.[11] However, good yields of C4-substituted pyridine products (compound **1**) could only be obtained when alkyl aldehydes were used. For example, only 5% yield of the 4-phenyl pyridine derivative was obtained when benzaldehyde was used, with a 75% yield of the C4-unsubstituted pyridine (compound **2**).



Scheme 1

We have expanded the scope of this microwave-assisted reaction to make it amenable to combinatorial synthesis. **First**, effective library generation benefits from an increased number of sites for structural variation, which enables a broader variety of reagent structures to participate in building the library. In this respect, we found that in contrast to the results previously described [11], undesired products analogous to **2** were not observed under the reaction conditions described herein. Every aryl aldehyde tested (Fig. 1) gave solely pyridine products substituted at C4, as confirmed by mass spectral analysis.

Second, in addition to ethyl acetoacetate a number of other different 1,3-dicarbonyl compounds were identified as efficient components of the microwave-assisted Hantzsch synthesis. These include not only traditional β-keto esters, but also cyclic 1,3-diketones (Fig. 1). The acceptance of the wide variety of aldehydes and 1,3-dicarbonyl compounds as building blocks for the multicomponent Hantzsch synthesis greatly increases the structural diversity of libraries that can be produced using this approach. The diversity is further expanded due to the fact that when two different 1,3-dicarbonyl compounds are used together in a single Hantzsch synthesis, three distinct pyridine derivatives can potentially be formed as illustrated in Scheme 2.[12] The actual ratio of these products, which can be easily separated by HPLC, presumably depends upon the reactivity and steric bulk of the starting 1,3-dicarbonyl compounds. This point is illustrated in Fig. 2, which shows the total ion chromatograms (TIC) from the HPLC/MS analyses on products obtained according to Schemes 2a and 2b from building blocks **3**, **5**, **15** and **3**, **10**, **12**, respectively (Fig. 1).[13]

Third, efficient library generation requires a compact reactor array amenable to reaction work-up and automation. Therefore, 96-well filter-bottom plates were adapted as reactors for the library synthesis. The use of a filter-bottom plate takes advantage of the solvent-free method to allow rapid recovery of products by washing from the solid support with an appropriate solvent into a 96-well receiving plate by applying vacuum or centrifugal force.

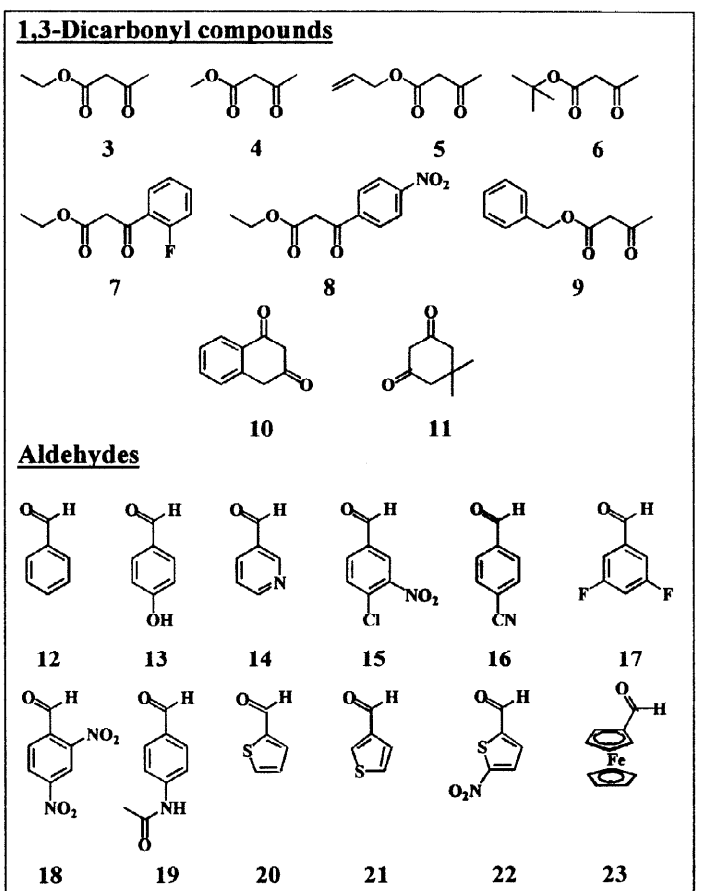
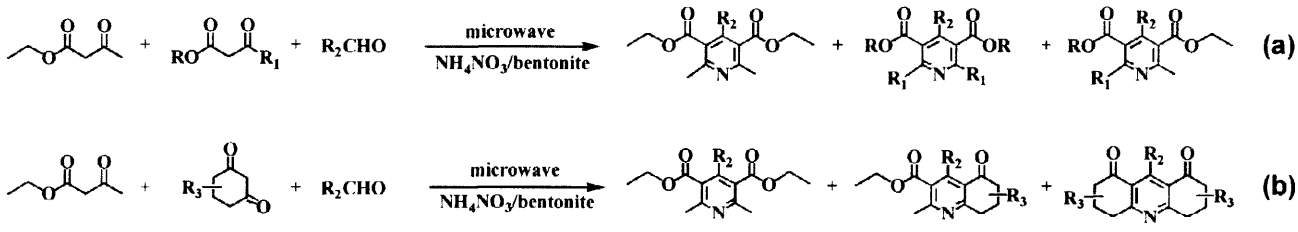


Fig. 1. Building blocks used in the microwave-assisted combinatorial Hantzsch synthesis.

These developments were employed as a basis for automated synthesis of a library of pyridines to demonstrate the feasibility and advantages of the MICROCOS technology. Twelve aldehydes and eight 1,3-dicarbonyl compounds (Fig. 1, all obtained from Aldrich, Milwaukee, WI) were used as building blocks for library synthesis according to Scheme 2. In each reaction, ethyl acetoacetate **3** was used as one of the components of the Hantzsch synthesis, whereas the second 1,3-dicarbonyl compound and the aldehydes were used in all possible combinations (one unique combination per well). Each well of the glass filled polypropylene 96-well filter plate reactors (10 μm polypropylene filter, 2ml/well, Polyfiltronics, Rockland, MA) contained 100 mg of a bentonite/ammonium nitrate (5:1 w/w) mixture.



Scheme 2

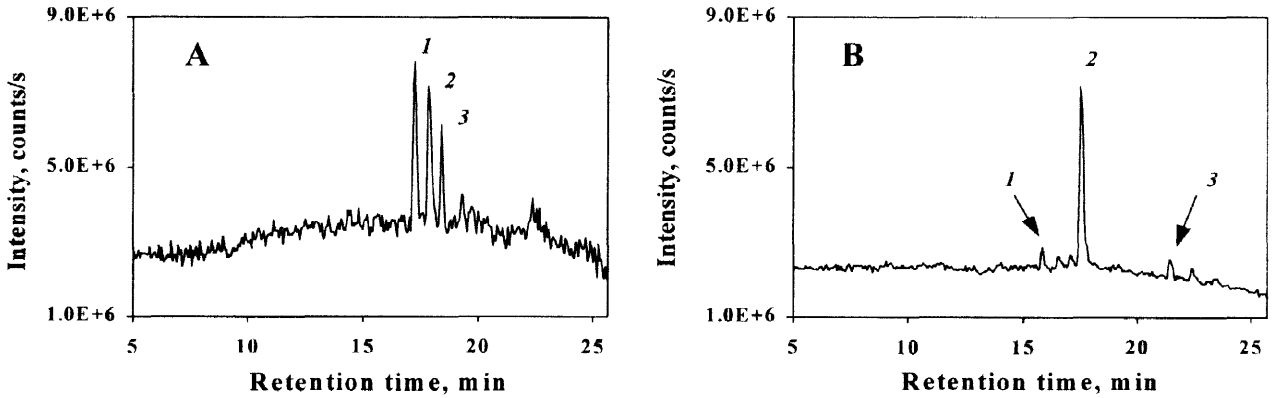


Fig. 2. Total ion chromatograms from HPLC/MS runs on products obtained according to Schemes 2a and 2b. (A) From building blocks **3**, **5**, and **15**: *1*, **3+3+15** (M+H = 407); *2*, **3+5+15** (M+H = 419); *3*, **5+5+15** (M+H = 431); (B) From building blocks **3**, **10**, and **12**: *1*, **3+3+12** (M+H = 328); *2*, **3+10+12** (M+H = 343); *3*, **10+10+12** (M+H = 360).

The desired 1,3-dicarbonyl compounds and aldehydes were distributed to each well using a Cyberlab C-200 robotic liquid handler (Brookfield, CT) to give 0.1 mmol of each reagent per well (35 μl of a 2.86 M solution in N,N'-dimethylformamide). The 96-well reactor was then placed in a household microwave oven (Kenmore 1300 W, 2450 MHz) and irradiated for 5 minutes at 70% power level. The plate was allowed to cool to room temperature and the wells were filled with 1 ml ethyl acetate to extract the reaction products. A 96-well solid bottom plate was then fitted below the reaction filter plate and centrifugal force (1000 rpm) was used to wash the product solution off the support into the receiving plate. The solvent was removed under vacuum using a Savant SpeedVac Plus centrifugal evaporator with a microplate rotor (Holbrook, NY) to afford the desired products. Before evaporation, an aliquot was taken from each well and analyzed by HPLC/MS and/or high-throughput MS (flow injection at ca. 1 sample/min).[13] The analysis showed that the reactions were uniformly successful across the 96-well reactor plate, thus indicating that the outcome of parallel reactions in a multiple reactor array is not adversely affected by the presence of microwave-absorbing material in adjacent wells and/or a potentially uneven distribution of the microwave energy inside the oven. No unreacted starting material was detected in any of the reactions,[14] and the HPLC purity of the products was ≥ 70 %. All of the expected 96 nonsymmetrical pyridines were synthesized. In addition, 70 out of 108 symmetrical pyridines distinguishable by MS were formed.[15] Evidently, some bulky 1,3-dicarbonyl components react slower, and therefore the corresponding symmetrical products are not observed. This is the first successful demonstration of parallel synthesis using microwave-assisted chemistries.

In conclusion, the applicability of the MICROCOS technology for generating combinatorial libraries has been demonstrated using the Hantzsch synthesis of pyridine derivatives as an example. The advantages of MAOS, especially of its solvent-free version, provides a dramatic impact on combinatorial synthesis, as summarized in Table 1. Furthermore, in contrast to the traditional solid-phase synthesis,[16] the microwave technology does not require the development of solid phase linking and cleaving chemistries. Similar to solution-phase combinatorial synthesis, the MICROCOS procedure produces compounds in a soluble form immediately available for biological screening. Additional microwave chemistries are currently being developed in our laboratory to extend the MICROCOS technology and allow the synthesis of novel structurally diverse libraries. Results of this work will be reported in due course.

Table 1. Impact of MAOS on Combinatorial Synthesis

Characteristic	Impact
• Applicability to wide range of organic reactions	• Increased diversity of libraries
• Simple reaction setup, uniform conditions	• Simplified and efficient automation
• Very short reaction times	• Dramatically increased synthetic throughput
• Increased yield, no excess reagents, decreased side products	• Simplified and efficient purification

ACKNOWLEDGMENTS

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2. In its simplest (and most widely used) version MAOS is performed in domestic microwave ovens, although more sophisticated microwave reactors are now commercially available.
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12. Note that in the case of nonsymmetrical cyclic 1,3-dicarbonyl compounds such as **10** six distinct pyridine derivatives can potentially be formed, including regioisomers that cannot be distinguished by MS analysis.
13. Analyses were performed on a Perkin Elmer SCIEX API100 electrospray LC/MS system with thermo ion spray ionization. Column: IB-SIL 3 μm C18, 2 x 100 mm. Gradient 20% MeCN in water to 100% MeCN in 20 minutes, flow rate 0.4 ml/min. UV detection at 260 nm.
14. In a separate experiment it was confirmed that the starting reagents were fully stable under reaction conditions, and therefore their absence in the final product indicates that they completely reacted rather than decomposed under microwave irradiation.
15. Theoretically, 144 symmetrical pyridines could be formed in the library synthesis. However, 36 of those (*viz.* regioisomers arising from **10**) are not distinguishable by MS analysis.[12]
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