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THE DOMINO BLOCKS: A SIMPLE SOLUTION FOR PARALLEL SOLID-PHASE ORGANIC SYNTHESIS

Viktor Krchňák[a,b*] and Víteček Paděra[b]

[a]*SIDDCO, 9040 South Rita Rd., Tucson, AZ 85747, U.S.A.*
[b]*Torviq, 3251 West Lambert Ln., Tucson, AZ 85742, U.S.A.*

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Abstract: The domino block is a reaction block for manual and semi-automatic parallel solid-phase organic synthesis that simplifies liquid exchange and integrates common synthetic steps. The domino block consists of enclosed reaction vessels, polypropylene syringes, attached to a manifold that clamps the syringes and connects them to a common port. Liquid is removed from the closed reaction vessels by vacuum connected to the common port. The vacuum formed inside each reaction vessel is subsequently used to draw fresh solvent or reagent into each reaction vessel.

The most striking feature of combinatorial chemistry is the capability to produce large numbers of compounds in a relatively short period of time.[1] For practical reasons, there is a balance between quantity of material and numbers of compounds synthesized. The current technology can support two scenarios: weekly production of (i) hundreds of compounds in larger quantities (10 to 100 mg) or (ii) thousands of compounds in smaller quantities (1 to 5 mg). These two approaches require different logistics and synthetic "hardware".

The reaction vessel of choice is either Richard Houghten's ingenious T-bag[2] or a syringe[3] for the manual synthesis of compounds in larger quantities. The advantage of T-bags is the easy handling of large numbers of reaction vessels; the disadvantage is a larger volume of solvent/solution and difficulty of automation. Syringes require only as small volume as necessary; however, managing a large number of syringes is cumbersome.

The integration of reaction vessels into a reaction block has become popular. In the late eighties Krchňák and Vágner designed the MultiBlock, the first reaction block for parallel solid-phase synthesis.[4] The explosion of combinatorial chemistry motivated the construction of variety of reaction blocks.[5] To further simplify and accelerate the parallel manual synthesis on solid phase, we have designed and constructed a domino block (the name reflects resemblance to a domino piece), a very simple semi-automatic device that integrates reaction vessels for common steps of parallel synthesis and simplifies liquid exchange in reaction vessels.

The concept of liquid exchange using the domino block is based on a simple principle (Figure 1). Initially, a single outlet reaction vessel (e.g., a plastic syringe) loaded with a resin and solvent is connected to an evacuated container for collecting waste solvent. External vacuum draws the liquid from the reaction vessel into a waste container creating vacuum inside the vessel. The evacuated reaction vessel is then connected to a solvent reservoir. The vacuum draws the liquid from the reservoir into the reaction vessel. To perform this liquid exchange with a number of reaction vessels

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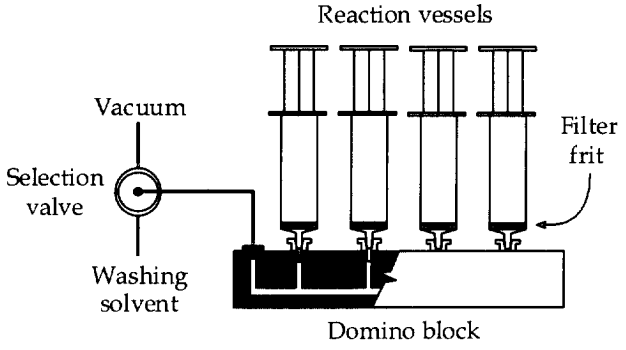
at the same time, the syringes were coupled to a liquid distribution manifold that connected them to one common port.[6]

Figure 1. Principle of the domino block.



To switch the connection of reaction vessels from the evacuated waste container to the liquid reservoir we used a 4-port distribution valve (Hamilton, Reno, NV) equipped with female Luer lock fittings (Figure 2). The central port was connected to the domino block. Two opposite ports A and B were connected to the evacuated waste container and solvent reservoir, respectively. The third port C was used to introduce a small volume of air/inert gas (ca 20% of reaction vessel volume) into the reaction vessel in order to insure reliable agitation and removal of liquid from the syringe. An HPLC syringe filter was attached to this port in order to limit gas flow. When performing oxygen/moisture sensitive reactions a source of inert gas (a balloon) was connected to port C.[7]

The fourth port D of the distribution valve was used for introduction of common reagents. The reagents solution was placed into a syringe attached to port D. Reaction vessels were evacuated via port A and the solution drawn into the reaction vessels by connection to port D. If different building blocks are required to be introduced into individual reaction vessels, each syringe was disconnected from the block and the particular solution was drawn into the syringe manually by moving the plunger.

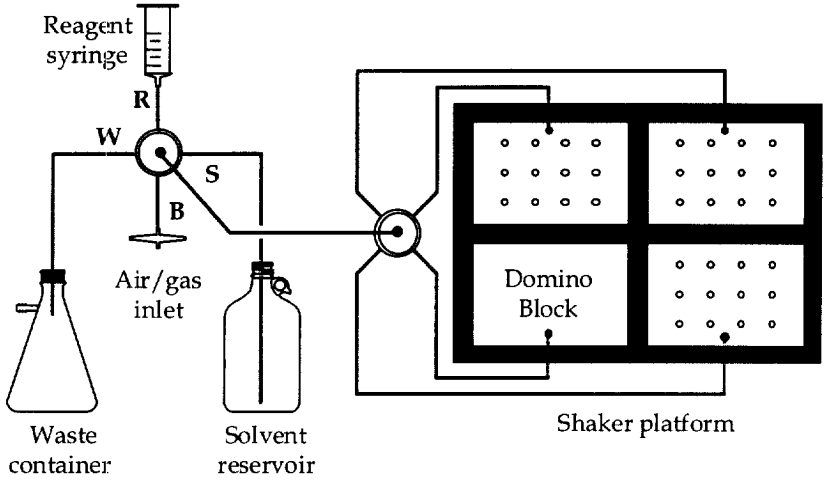
During the addition and removal of liquid the domino block was shaken to equilibrate the resin with the incoming solvent. We have used a Titer-plate shaker (Lab-Line Instruments) with a platform that can house four domino blocks. We connected all four domino blocks to an additional 4-port distribution valve that enabled us to wash one domino block after another (Figure 2). While the solvent was exchanged in reaction vessels of one domino block, the resin in other three blocks was shaken with the washing solvent. It is necessary that the resin beads be exposed to the fresh solvent for at least one minute to allow diffusion of soluble compounds out of the beads. Since the entire washing cycle requires only switching the connection between vacuum and solvent reservoir, the washing process can be easily automated.

An additional benefit of the domino block is its complete enclosure. The contents of the reaction vessels are never exposed to the atmosphere, and therefore any chemistry requiring an inert atmosphere can be performed on the block. Dry solvent for conditioning of resins, or oxygen/moisture sensitive reagents may be introduced directly from a septum-sealed container via a syringe needle. Alternatively, an enclosed syringe with the sensitive solution is coupled to the reagent port and the solution is introduced into reaction vessels.

The volume of reaction vessels is adjustable by moving the syringe plunger. Therefore different amounts of resin can be placed into individual syringes on the same block. Different sizes of syringes can be attached to the same block as well; they will always be filled since the volume of

the evacuated reaction vessel determines the volume of liquid introduced. The vacuum does not move the plunger and there is no need to fix the position of the plunger. However, the syringe must be wetted by solvent before use in order to restrict plunger movement.

Figure 2. Scheme of a synthesizer consisting of four domino blocks.



Combinatorial solid-phase synthesis in domino blocks is analogous to the synthesis in T-bags[2] or MicroKans.[8] T-bags that receive identical reagent are placed in the same reaction vessels. Analogously, domino blocks integrate syringes that undergo the same chemical transformation. After finishing one chemical reaction the T-bags are redistributed into different reaction vessels for the next chemical step. Similarly, syringes containing different resin-bound intermediates are redistributed into one domino block to receive the same reagents.

To illustrate the usefulness of the domino blocks for parallel synthesis, we describe here the construction of a small three step combinatorial library of 1,728 N-(alkoxyacyl)amino acids.[9] The TentaGel resin was esterified with 12 amino acids. In the second combinatorial step the resin-bound amino groups were acylated with 12 aromatic hydroxy acids. Twelve alcohols were used in the last step for polymer-supported Mitsunobu etherification. The first two combinatorial steps yielding 144 resin-bound intermediates were performed in domino blocks, the last step was performed in 96-well plates.[10] We have developed the synthesis in deep-well plates to accelerate the high throughput parallel synthesis on solid phase.[11] Typically resin beads are separated from solvent using filtration through porous material. We used an alternative procedure; resin beads were allowed to settle and the solvent above the resin bed was aspirated using a suction manifold. This method allows the synthesis of thousands of compounds in mg quantities per day.[10,12]

In summary, we have designed and constructed the domino block, a reaction block for solid-phase organic synthesis that integrates reaction vessels for common operations and simplifies the liquid exchange. Vacuum removes liquid from an enclosed reaction vessel and then the evacuated reaction vessel draws a liquid from a reservoir into the vessel. The solid-phase synthesis in domino blocks allows semi-automatic preparation of compounds in batches of hundreds with a minimum investment and minimum risk of fatigue and error.

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References and notes

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3. Krchňák, V.; Vágner, J. *Peptide Res.* **1990**, *3*, 182.
4. The MultiBlock is commercially available from CSPS, San Diego, CA (http://www.5z.com).
5. For recent overview of automation/integration of SPOS see e.g. by Cargill, J. F.; Lebl, M. *Curr.Opin.Chem.Biol.* **1997**, *1*, 67.
6. Description of the domino block: The domino block (Torviq, Tucson AZ) is Teflon made liquid distribution manifold (footprint of a microplate) that has two functions: (i) to clamp reaction vessels and (ii) to connect all reaction vessels to one common port. This common port was used to introduce and to remove a liquid. Plastic polypropylene syringes equipped with a porous disk at the bottom were used as reaction vessels. The syringes were attached via a male Luer fitting to the liquid distribution manifold arrayed with a female Luer lock fittings. We constructed blocks for 12 (4 rows of 3 syringes) and 24 (6 rows of 4 syringes) reaction vessels. The first block accommodated syringe size up to 20 mL; the 24-block was useful for 2.5 and 5-mL syringes.
7. Description of operation: the common port of the distribution valve was connected to the port A to connect the domino block with reaction vessels to an evacuated waste container (Figure 2). After the syringes were emptied, which typically took less than 10 sec, the valve was turned to the port C for a fraction of a second. A small amount of air/inert gas was introduced into syringes via this port equipped with a syringe filter. Then the valve was turned to the port B to connect the domino block with the solvent container. The vacuum created inside reaction vessels drawn the liquid into the vessels. We also used an alternative way of introducing an air gap into syringes. After the syringes were evacuated, the valve was directly switched to the port B. The common port of the valve was then connected to the port C, which introduced the air, before the syringes were completely filled with the solvent. This washing operation was repeated as many times as washes were required. One washing liquid was used in the above-described configuration. For a comfortable selection of washing solvents a distribution valve can be inserted between the 4-port valve and solvent reservoirs.
8. Radiofrequency tagged T-bag-like reaction vessels were described by Moran, E. J.; Sarshar, S.; Cargill, J. F.; Shahbaz, M. M.; Lio, A.; Mjalli, A. M. M.; Armstrong, R. W. *J. Am. Chem. Soc.* **1995**, *117*, 10787. Nicolaou, K. C.; Xiao, X. Y.; Parandoosh, Z.; Senyei, A.; Nova, M. P. *Angew. Chem. Int. Ed.* **1995**, *34*, 2289.
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10. Briefly, twelve domino blocks, each composed of twelve 10-mL syringes, were charged with 240 mg of hydroxy TentaGel and the resin was esterified with 12 Fmoc protected amino acids[9] (Ala, Phe, His(Trt), Ile, Lys(Boc), Leu, Met, Asn, Pro, Ser(tBu), Val, and Trp). After washing the resin with DMF, the Fmoc group was removed by 5-min treatment with 50% piperidine in DMF. Deprotected resin beads were washed with DMF. For the next combinatorial step the syringes were rearranged the way that each domino block received syringes with twelve different amino acid resins. Aromatic hydroxy acids were coupled to resin bound amino groups,[9] each domino block receiving one hydroxy acid (salicylic, 3-hydroxybenzoic, 4-hydroxybenzoic, 4-hydroxy-3-nitrobenzoic, 3-hydroxy-4-methoxybenzoic, 4-aminosalicylic, 4-hydroxyphenylacetic, 4-hydroxy-3-chloro-phenylacetic, 3-(4-hydroxyphenyl)propionic, 4-hydroxycinnamic, 2-(4-hydroxyphenoxy)propionic, and 6-hydroxynicotinic acid). In this manner, 144 compounds were synthesized in two days. Then the resin bound intermediate from each syringe was split into 12 wells of one row of a 96-well plate (8 syringes/plate) and the resin-bound phenolic hydroxyl groups were alkylated under Mitsunobu conditions to yield alkylaryl ethers.[11] Twelve different alcohols were distributed into the wells, one per each column (methanol, ethanol, 2-propanol, allyl alcohol, 1,3-propanediol, 1-(2-hydroxyethyl)pyrrolidine, 1-piperidineethanol, 1-(2-hydroxyethyl)-2-pyrrolidinone, benzyl alcohol, 4-chlorbenzylalcohol, 4-nitrobenzylalcohol, and phenethylalcohol). After an overnight reaction and washing with DMF and MeOH the products were cleaved by 0.5 M NaOH and extracted into 50 % MeOH in water.
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