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AUTOMATED SOLID-PHASE ORGANIC SYNTHESIS IN MICRO-PLATE WELLS. SYNTHESIS OF N-(ALKOXY-ACYL)AMINO ALCOHOLS[1]

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Abstract: The technique of concurrent solid-phase organic synthesis in 96-deep-well micro-titer plates is outlined and documented with the synthesis of N-(alkoxy-acyl)amino alcohols. Typically resin beads are separated from solvent using filtration approach that complicates automation. Instead we describe a method employing settling of resin, followed by aspiration of supernatant.

Multiple solid-phase peptide synthesis has been reported and is being widely used, including full automation of the entire process (e.g., reference[3] and references cited therein). Currently, in the drug discovery process, parallel and automated synthesis of non peptide, drug-like molecules is of great interest. To make the parallel synthesis of compounds on solid phase more simple, efficient, and economical, we have developed a protocol for concurrent synthesis of 96 compounds in commercially available polypropylene 96-well micro-plates.

The customary way to separate the resin beads from liquid is filtration through a porous material, usually a glass or plastic frit. Automated solid-phase synthesis in micro-titer plates has been described by Schnorrenberg and Gerhardt.[4] The tip of their washing device was protected by a narrow stainless steel net. Solid-phase synthesis in modified micro-titer plates where each well is equipped with a frit has also been reported.[5]

We have developed a method that enables solid-phase organic synthesis in micro-titer wells not equipped with any kind of porous material at the bottom to facilitate the separation of solid resin beads from a solvent. Each well of a standard polypropylene deep-well micro-titer plate (Deep Plate Titer Plate Polypropylene, Beckman) was charged with derivatized resin beads, typically 10 mg per well. The solvent was aspirated by suction from the surface of the liquid after the resin beads had settled (typically 1 min). Vacuum was applied to 1 mm bore stainless steel needles that were slowly immersed into the wells. Liquid was removed from the surface without disturbing the settled resin and no resin beads were sucked by the needles. Typically, 1 mm of solvent above the resin beads was left in the reaction vessel, corresponding to cca 30 μL of solvent.

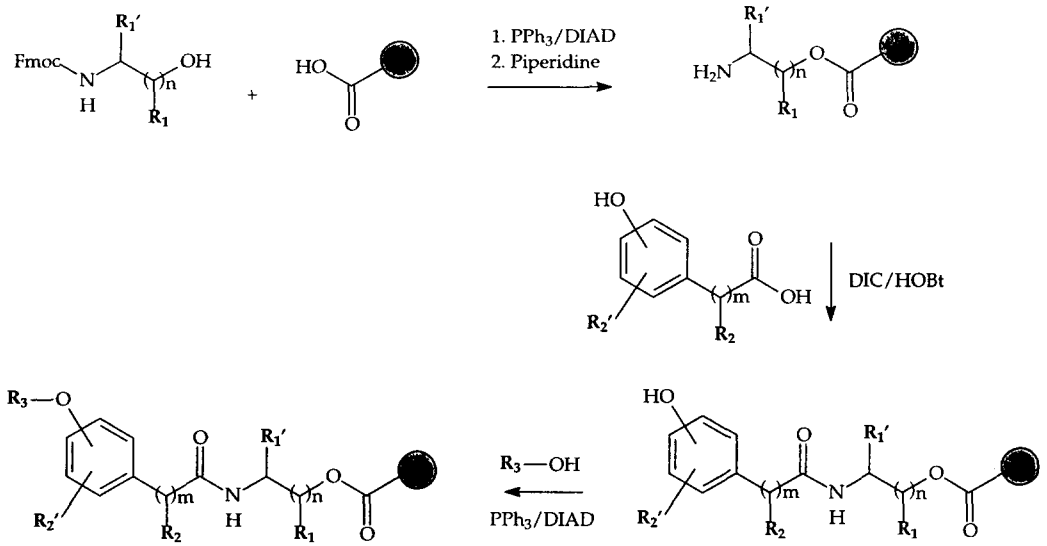

Figure 1. Synthetic scheme for N-(alkoxy-acyl)amino alcohols

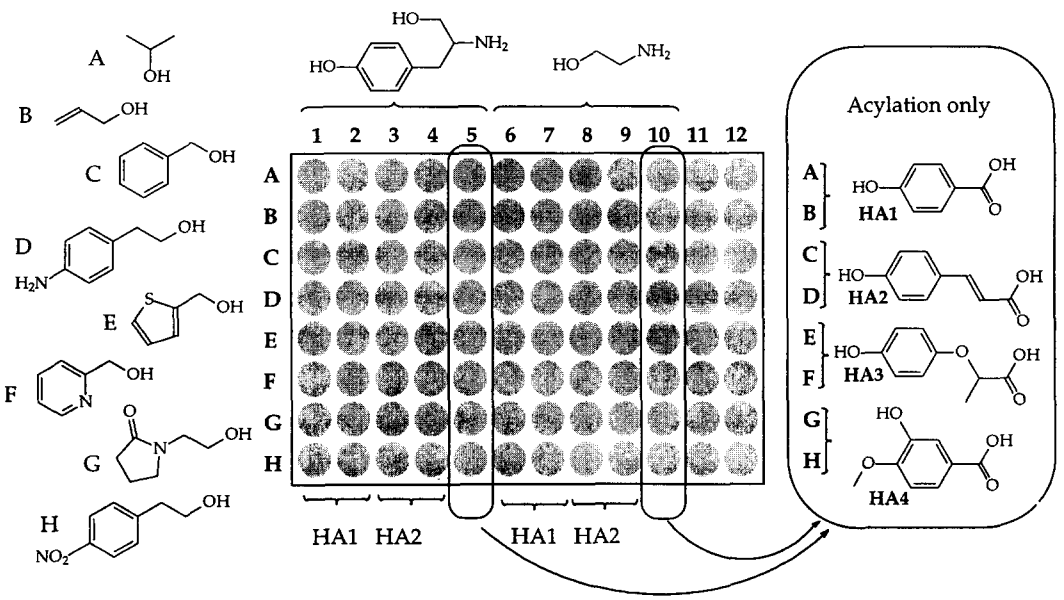

Figure 2. Distribution of building blocks on a model plate.

As proof of concept, a combinatorial array of 80 N-(alkoxy-acyl)amino alcohols[6] was synthesized. The reaction scheme is shown in Figure 1. The esterification of the carboxylate resin was done manually.[7] Well mixed resin was uniformly distributed into wells as a slurry in DMF. In the automated, micro-scale synthesis we performed two reactions: (i) acylation of amino alcohols by aromatic hydroxy carboxylic acids, and (ii) ether formation by the Mitsunobu reaction. The synthesis was performed in a polypropylene 96 deep-well micro-titer plate, containing 1 mL volume per well. Distribution of resin and reagents was done in automated fashion using a multi-channel pipetter (Zymark Zypettor Pipetting Station) that handles reagents and resin distribution in micro-scale, and a multi-channel washing station (Zymark Reagent Addition Station) that washes the resin between reaction steps. The modification of the Zymark Reagent Station included the use of solvent resistant parts (Teflon and stainless steel) and longer needles (4 cm).

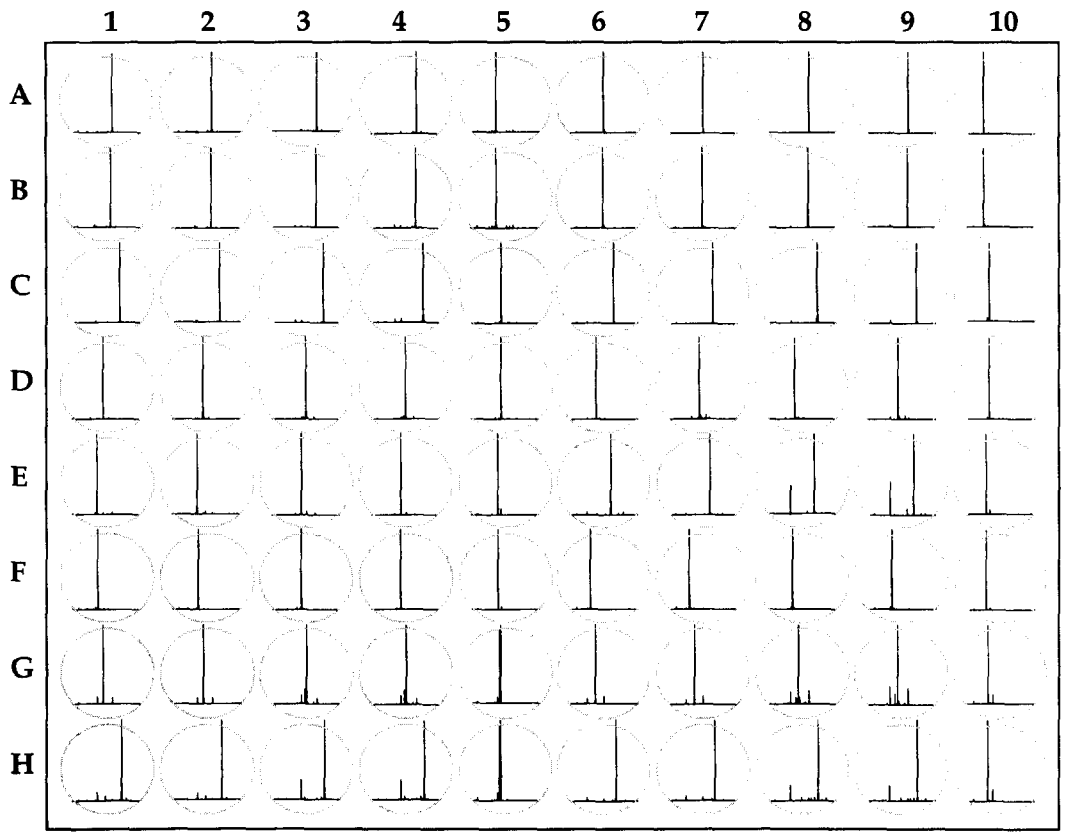

Figure 3. HPLC traces of crude compounds. For structure of compounds in "wells" see Figure 2.

The synthetic protocol consisted of eight steps: (1) distribution of the resin esterified by amino alcohols,[8] (2) acylation by aromatic hydroxy acids,[9] (3) washing, (4) Mitsunobu etherification,[10] (5) washing, (6) side chain deprotection,[11] (7) washing, and (8) release of compounds by basic hydrolysis.[12] The distribution of building blocks for the model plate synthesis is schematically

shown in Figure 2. Each compound was synthesized twice, to evaluate the reproducibility of the process. The purity of crude compounds was evaluated in an analytical gradient HPLC, the traces for individual compounds are shown in Figure 3. The major peak corresponded to the expected product (correct molecular weight, NMR of selected compounds).

These results suggest that automation of solid-phase organic synthesis in micro-titer plates can be reliable and reproducible, and can provide compounds of high purity. This technique allows one to synthesize rapidly mg quantities of organic compounds. Furthermore it is amenable to automation accelerating the quest for pharmaceutically interesting compounds.

References and notes

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7. Amino TentaGel resin derivatized with glutaric anhydride (3.5 mL of a slurry, bead size 90 μm, substitution 0.25 mmol NH_2/g) was esterified manually in syringes by Fmoc-tyrosinol(*t*Bu) and Fmoc-glycinol according to the procedure described elsewhere.[6] Fmoc groups were removed by piperidine and quantified. Substitution of 0.18 mmol/g was found.
8. The esterified resin was distributed into 96 deep-well polypropylene micro-titer plate by the pipetting station. The resin (400 mg) was suspended in DMF (total volume 8 mL) and 200 μL of stirred suspension was pipetted and delivered, i.e., each well contained 50 μL of settled resin slurry (ca. 2 μmol). Fmoc-tyrosinol and Fmoc-glycinol derivatized resins were each distributed into 40 wells A1-H5, A6-H10 respectively. No compounds were synthesized in columns 11 and 12. Building block distribution is schematically shown in Figure 2.
9. Polymer-supported amino groups were acylated by 4 different aromatic hydroxy acids: p-hydroxybenzoic, p-hydroxycinnamic, 2-(4-hydroxyphenoxy)propionic, and 3-hydroxy-4-methoxybenzoic acids. 0.1 mL of 0.5 M solution of acid and HOBt was added to each well, followed by addition of 200 μL of 0.25 M DIC in DMF (total volume of liquid per well was 350 μL, final concentration of acid was ca 0.15 mM, the excess was 50 μmol/2 μmol, i.e., 25-fold). The plate was shaken on a IKA-Schutter MTS 4 Vibrax shaker for 3 h at rt, washed 6 x with DMF and stored overnight in DMF containing 1% of HOBt.
10. The resin in all plates was washed 5 x with DMF and 5 x with THF. Eight different alcohols were used for the Mitsunobu etherification: i-propanol, allyl alcohol, benzyl alcohol, 4-amino-phenethyl alcohol, 2-thiophenemethanol, 2-(hydroxymethyl) pyridine, 1-(2-hydroxyethyl)-2-pyrrolidinone, and 4-nitrophenyl ethyl alcohol (building block distribution Figure 2). 250 μL of 1 M solution of alcohol in 0.5 M PPh_3 in THF was added to each well (final concentration of alcohol was 0.65 mM, the excess was 125-fold), immediately followed by the addition of 62 μL of 2 M solution of DIAD in THF (added in two portions 2 x 31 μL) at rt. The plate was left overnight and then washed 6x with THF. No etherification was done in columns 5 and 10.
11. The *t*Bu side-chain protecting groups were cleaved (rows 1-5 only) by TFA. The resin was washed 5 x with methyl-t-butyl ether (500 μL per well), then 200 μL of a 70% TFA in DCM containing 5% of p-cresol was added and left for 1 h at rt. The resin was washed 5 x with methyl-*t*-butyl ether, 5 x with DMF, 5 x with MeOH, 5 x with methyl-t-butyl ether and then dried in dessicator for 2 h.
12. The compounds were released from the resin by NaOH. 200 μL of 0.5% NaOH in 50% MeCN was added to each well. After 3 h the solution was neutralized with 200 μL of 20% aqueous AcOH.

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