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The Use of Power Ultrasound Coupled with Magnetic Separation for the Solid Phase Synthesis of Compound Libraries

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Abstract—Enhanced reaction rates are observed when power ultrasound is utilized as a substitute for mixing during solid phase organic chemical reactions on a paramagnetic support. Power ultrasound is also used to facilitate the washing of the paramagnetic support as it is magnetically separated from the reaction mixture. Selective examples from a library targeting the κ-opioid receptor are presented.

Solid phase organic chemistry continues to play a powerful role in the synthesis of combinatorial libraries either through a mix-and-split or a multiple parallel synthesis strategy. Over the past several years, very little has changed in the way one goes about running a solid phase organic chemical reaction. For example, most solid phase reactions are filtered at the end of the reaction to separate the insoluble support from the soluble components of the reaction mixture. Recently, we introduced a novel high-loading paramagnetic support for practical solid phase organic chemistry.[1] Instead of filtration, a magnetic field is used to separate the paramagnetic support from the soluble as well as insoluble (i.e. precipitants) components of the reaction mixture. Another common practice in running a solid phase reaction is the application of some sort of agitation such as shaking, gas bubbling, stirring, or vortexing.[2,3] None of these individual methods have been conclusively shown to be better at directly affecting the solid phase reaction rate as compared to any of the other mixing methods. One form of agitation that has been found to enhance the solid phase reaction rate is high energy ultrasound or power ultrasound.[4] Application of power ultrasound in solid phase peptide synthesis has been found in some specific cases to accelerate the observed peptide coupling and cleavage reaction rates.[5–7] We have been interested in applying power ultrasound to the production of compound libraries targeting the κ-opioid receptor. We were specifically interested in producing compounds that could mimic the properties of the selective κ-agonist **1** (ICI-199,441).[8] For this reason we chose scaffold **2** as our initial entry into this area (Fig. 1).

Incorporation of power ultrasound into a multiple parallel solid phase synthesis format is relatively easy when combined with the use of a paramagnetic support since only an external magnetic field is required to separate the support from the soluble components of the reaction mixture. Those non-magnetic components of the reaction mixture are then removed by aspiration.[9] Other benefits of using power ultrasound in solid phase organic synthesis include solvent degassing while the reaction is running as well as giving one an easy method to control the reaction temperature via the sonicating water bath.

Starting with the hydroxymethyl derivatized, paramagnetic support **3** (0.51 mmol hydroxymethyl/g of support), an excess of fluorenylmethoxycarbonyl (Fmoc) protected L-proline, benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium-hexafluorophosphate (PyBop) coupling reagent and diisopropylethylamine (DIEA) in dimethylformamide (DMF) was added under the presence of ultrasound to give the resulting resin bound Fmoc protected proline (0.32 mmol Fmoc/g of support).[10] Ultrasound was applied to the coupling reaction via an immersible 600 watt, 25 kHz ultrasonic transducer at 30% power for 24 h.[11] Deprotection of the Fmoc protecting group was accomplished using 50% piperidine in DMF under similar ultrasonic conditions to give the resin bound L-proline **4** (Scheme 1).

Alkylation of the resin bound L-proline with 2-nitrobenzyl bromide **5** and DIEA in the presence of power ultrasound gave the resulting *N*-benzyl adduct **6**. It was

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possible to monitor by UV spectrophotometry the disappearance of the 2-nitrobenzyl bromide **5** in solution under varying agitation conditions.[12] Figure 2A shows the course of the reaction as a function of time in the presence of ultrasound, stirring and neither ultrasound nor stirring. The results show that at constant temperature ($t = 25\,^{\circ}C$) an enhancement in the reaction rate was observed when ultrasound was applied ($t_{1/2} = 12.4$ min) to the solid phase reaction as compared to stirring ($t_{1/2} = 20.1$ min).[13] We believe the observed rate enhancement is a result of the ultrasound's ability to decrease the time for solute molecules located within the inner and outer surfaces of the support to reach equilibrium. This idea is further supported by Figure 2B which shows the release of non-covalently bound 2-nitrobenzyl bromide from a support that had been previously saturated with the compound.[14] Figure 2B shows that beads exposed to ultrasound release 2-nitrobenzyl bromide faster ($t_{1/2} = 30$ s) than when stirred ($t_{1/2} = 45$ s).

Reduction of the resin bound nitro compound **6** was accomplished with $SnCl_2{\cdot}2H_2O$ in DMF and in the presence of ultrasound to give after magnetic separation and aspiration the resulting amine **7** (Scheme 2).[15] Using the resin bound amine, it is now possible to incorporate a diverse set of side chains through the process of amide formation. For example, treating the resin bound amine **4** with an acyl chloride such as benzoyl chloride gives the resulting resin bound benzamide **8a** (Scheme 2). Conversely, one can couple free acids such as 3-nitrophenylacetic acid using a coupling reagent such as diisopropylethylamine (DIC) and dimethylaminopyridine (DMAP) in the presence of ultrasound to give the

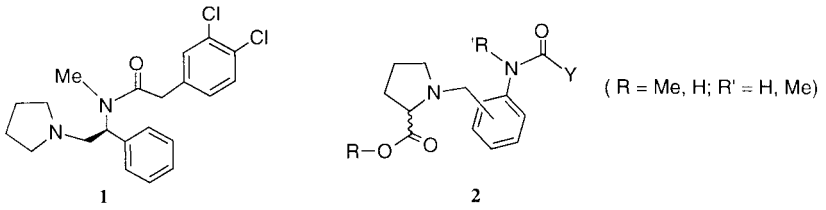
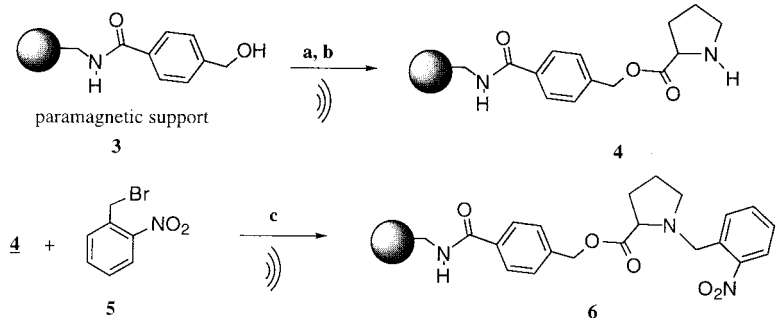


Figure 1. Known opioid receptor agonist **1** and combinatorial library scaffold **2**.



Scheme 1. Reagents: (a) Fmoc-L-Proline, PyBop, DIEA, 30% power; (b) 50% piperidine-DMF, 30% power; (c) DIEA, DMF, 100% power.

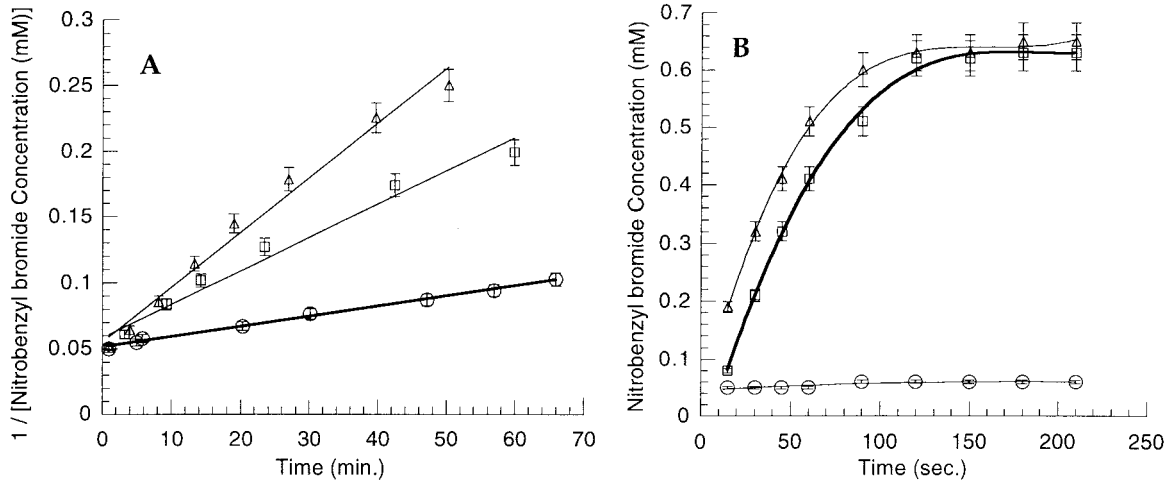
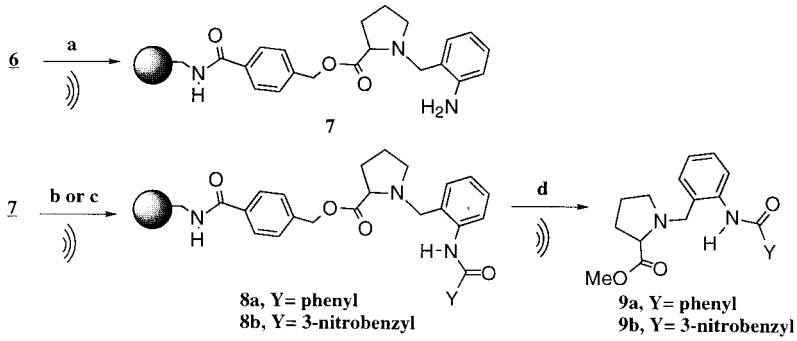


Figure 2. (A) Plot of 1/[2-nitrobenzyl bromide concentration] as a function of time and in the presence of **4** and DIEA while exposed to ultrasound, stirring and no agitation (unstirred). (B) Plot of the release of 2-nitrobenzyl bromide in DMF as a function of time in the presence of ultrasound, stirring and no agitation. △ = 25 KHz ultrasound, □ stirring, ○ = no agitation (unstirred).



Scheme 2. Reagents: (a) $SnCl_2 \cdot 2H_2O$, DMF, 60% power; (b) benzoyl chloride, DIEA, 15% power; (c) 3-nitrophenylacetic acid, DIC, DMAP, 15% power; (d) NaOMe, MeOH-THF, 40% power.

corresponding nitrophenylacetamide **8b** (Scheme 2). The resin bound amides were then ultrasonically cleaved in the presence of sodium methoxide to give the resulting methyl esters which were then subsequently purified using preparative thin layer chromatography to give **9a** and **9b** in overall yields of 44 and 40%, respectively (starting from the resin bound proline **3**).[16]

In conclusion, we have found that power ultrasound is a good substitute for mechanical mixing and, in the case of solid phase alkylation reactions, can enhance both the observed reaction rate and the efficiency of resin washing. Additionally, we have found that applying power ultrasound to a solid phase organic chemical reaction is far easier when using magnetic separation as compared to standard filtration, since separation of the support requires only a series of magnets and solvent removal can be accomplished by simple aspiration. The advantages to combining power ultrasound with magnetic separation in solid phase synthesis will become more apparent with the introduction of both large numbers of simultaneous solid phase reactions and automation to the system, a project that is curently ongoing in our laboratory.

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10. The loading capacity was determined according to Green, J.; Bradley, K. *Tetrahedron* **1993**, *49*, 4141.
11. Reactions have also been run using 20 kHz probe-type cup horn transducers with similar results. The percent power level used depended on the reactivity of the overall solid phase reaction. When the reactions are run at higher power levels (>50%) and for longer periods (>10 h) the paramagnetic beads were found to fracture into smaller paramagnetic polymer fragments that were easily separated using a magnetic field. Application of power ultrasound to non-paramagnetic based supports would be problematic since such fragmentation phenomenon would produce fines small enough to clog most frit based filtration systems.
12. Aliquots were taken at timed intervals and the UV absorbance of 2-nitrobenzyl bromide **5** was then measured at a wavelength of 310 nm (extinction coefficient = 1371 L/mols).
13. The reaction was run using equal molar amounts of 5-nitrobenzyl bromide and resin bound **4** in the presence of a large excess of DIEA in DMF. The half-life was calculated using the equations for a bimolecular reaction: $1/[A] - 1/[A_o] = akt$ and $t_{1/2} = 1/(ak[A_o])$ where $[A_o]$ = initial concentration of 5-nitrobenzyl bromide and k = rate constant. By ploting 1/[3-nitrobenzyl bromide concentration] versus time one gets a straight line with a slope equal to ak, and a half-life of the reaction ($t_{1/2}$) equal to the inverse of the initial concentration $[A_o]$ times the slope (ak).
14. Paramagnetic beads were added to a saturated DMF solution of 2-nitrobenzyl bromide. The beads were then poured into water and magnetically separated, repeatedly washed with methanol and then dried. The release of the 2-nitrobenzyl bromide was then monitored spectrophotometrically. The half-life ($t_{1/2}$) of the reaction was calculated following first order reaction kinetics.
15. Experiments in our lab have shown that washing the resin in the presence of ultrasound enhances the removal of residual resin bound tin.
16. **9a** ^{1}H NMR ($CDCl_3$) δ 1.78–2.04 (m, 4H), 2.19–2.42 (m, 1H), 3.01–3.21 (m 5H), 3.70 (dd, 2H, J = 149.0 Hz, J = 15.0 Hz), 6.95–7.49 (m, 6H), 7.93–7.99 (m, 2H), 8.37 (d, 1H, J = 8.3Hz), 11.20 (br. s, 1H); ^{13}C NMR ($CDCl_3$) δ 174.1, 166.0, 138.7, 135.4, 131.4, 129.3, 128.6, 128.2, 127.4, 126.1, 123.3, 120.7, 66.9, 58.51, 53.3, 51.4, 29.5, 22.2; IR (neat film) 2949, 2819, 1730, 1662, 1583, 1527, 1448, 1312, 1194 cm^{-1}; positive ion electrospray ms (M + H) = 339.

9b: ^{1}H NMR ($CDCl_3$) δ 1.78–179 (m, 2H), 1.92–1.96 (m, 1H), 2.11–2.29 (m 1H), 2.72–2.93 (m, 1H), 3.13–3.22 (m, 1H), 3.58 (dd, 2H, $J = 300.0$ Hz, $J = 12.3$ Hz), 3.81 (s, 3H), 3.99 (s, 2H), 0.93–0.13 (m, 1H), 7.04 (d, 1H, $J = 12.3$ Hz), 7.08–7.15 (m, 1H), 7.40–7.47 (m, 1H), 7.84 (d, 1H, $J = 7.6$ Hz), 8.09 (d, 1H, $J = 9.0$ Hz), 8.30 (d, 1H, $J = 9.0$ Hz), 8.36 (s, 1H), 10.60 (br. s, 1H); ^{13}C NMR ($CDCl_3$) δ 170.2, 168.8, 138.2, 138.0, 135.8, 135.7, 129.3, 129.1, 128.4, 124.4, 123.3, 121.7, 120.8, 110.1, 65.5, 57.8, 52.6, 52.3, 43.5, 29.6, 22.9; IR (neat film) 2936, 1730, 1681, 1582, 1519, 1441, 1343, 1194 cm^{-1}; positive ion electrospray ms (M + H) = 398.