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High throughput 'catch-and-release' synthesis within spatially discrete gel arrays

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ABSTRACT

A tetrafluorophenol acrylamide monomer unit was synthesised, co-polymerised and grafted onto a glass slide to form individual gel spots. As a proof of principle study, a small library of amides was rapidly synthesised within these gel spots using 'catch-and-release' chemistry, including the biologically interesting quorum sensing acyl-homoserine lactones. The tetrafluorophenol acrylamide gel provides an efficient platform to synthesise and screen small molecules for biological activity.

Combinatorial chemistry has developed over the past 20 years into a tool for the high throughput synthesis of small molecules, driven in part by the demands of the pharmaceutical industry to generate rapidly large arrays of compounds for biological screening.[1] A number of solid phase chemistry techniques have been developed, for example, tetrafluorophenol (TFP) catch-and-release beads for the synthesis of amide libraries.[2] The TFP beads are made from polystyrene, coupled to tetrafluoro-hydroxybenzoic acid. In the catch-and-release process the TFP beads are firstly treated with carboxylic acids to form the activated esters using either standard coupling reagents or a direct reaction with pre-formed acid chlorides ('catch'). Secondly, these activated esters are reacted with nucleophiles such as amines to generate the free amides in solution ('release').

Several on-chip cell-screening assays have been developed including an array of transfected cells expressing defined locations of DNA.[3] In a similar approach, slides coated with a biodegradable polymer have been used to screen small molecule activity in mammalian cells.[4] Additionally, a library of acrylate polymers has been attached to a chip and screened for biomaterial suitability in terms of cell attachment, growth and differentiation.[5] The development of a methodology that would enable the incorporation of high throughput compound synthesis followed by high throughput biological screening on the same chip would be a significant advancement. This sequential synthesis-screening technique has the potential to be a more cost effective and rapid process for the identification of early hits to feed into drug discovery programs. A powerful synergy can be realised as the synthesis and screening processes become more streamlined, faster and cost-effective. In this context we have developed a polyethylene glycol (PEG) TFP acrylamide 3D gel, which was grafted onto a microscope slide to form spatially discrete spots. This array of gel spots acts as a series of wall-less test tubes for the TFP synthesis of the amide library. Each amide is held non-covalently within the gel spots but can diffuse out easily for analytical detection or testing in cell-based assays.

Acrylamide gels are typically formed by a vinyl-radical polymerisation between an individual monomer and cross-linking units. They provide a robust and easily derivatisable platform for a wide variety of applications from the electrophoresis of proteins and DNA, to the solid-phase synthesis of small molecules.[6,7]

Our research began with the synthesis of the TFP monomer unit needed to prepare the TFP acrylamide gel (Scheme 1). Boc-anhydride was reacted with excess ethanediamine (**1**) to generate mono Boc-protected amine **2**. Subsequently, amine **2** was coupled with acryloyl chloride under Schotten–Baumann conditions to form acrylamide **3** in an excellent yield. The Boc-group was removed using trifluoroacetic acid (TFA) and the isolated amine salt was coupled directly with tetrafluoro-4-hydroxy-benzoic acid (**4**) to produce the TFP acrylamide monomer unit (**5**) in a 43% overall yield for the four-step sequence.

The TFP monomer unit (**5**) was initially co-polymerised to form the free TFP gel. The polymerisation mixture consisted of PEG_{1900}-Da cross-linker (**6**), TFP acrylamide monomer unit (**5**) and 2-hydroxy-2-methylpropiophenone (**7**) as the UV initiator (Scheme 2). The PEG_{1900}Da cross-linker (**6**) is made up of repeating ethylene glycol units (= 1900 Da molecular weight) and has been used in a variety of applications including 3D hydrogel small-molecule microarrays and solid-supported peptide synthesis.[6,8] The free gel was formed upon irradiation of the polymerisation mixture with UV-light. The gel was then characterised in terms of its swelling and loading capacity. Despite the presence of the hydrophilic PEG groups, the gel did not swell considerably in water (1 mL/g). The hydrophobic nature of the TFP group could account for the lack of swelling in water. This hypothesis was confirmed as a control gel containing

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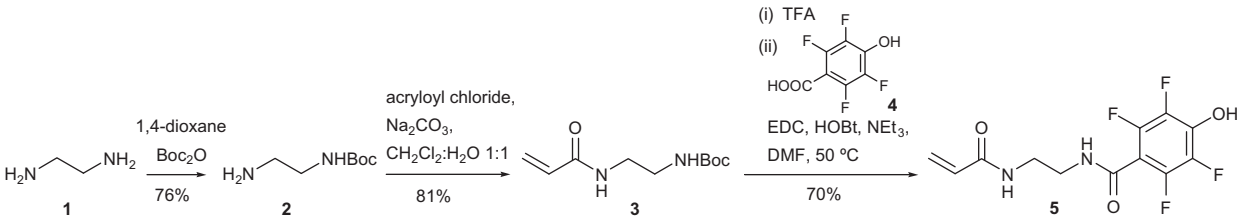
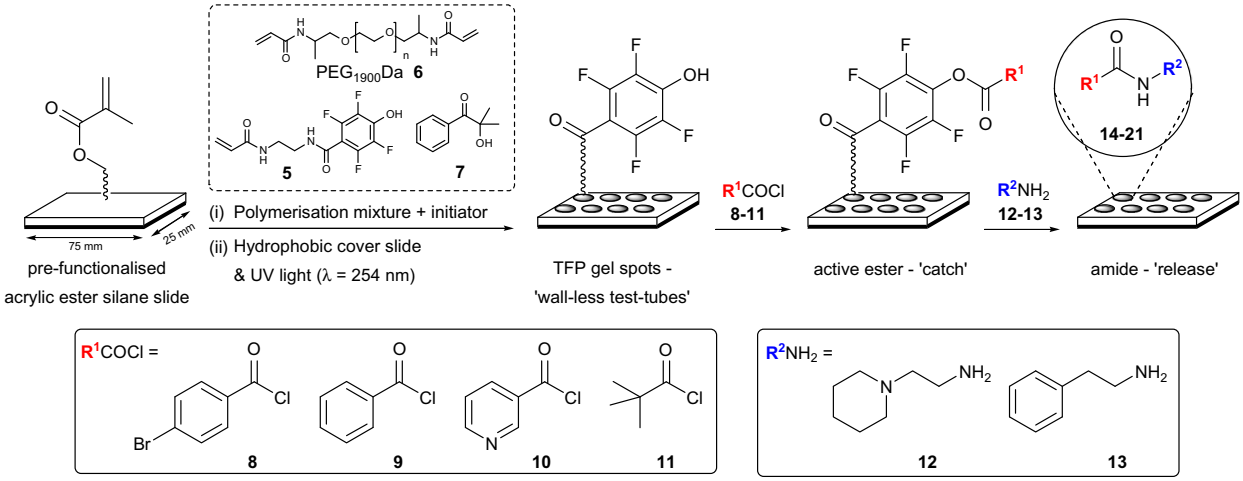


Scheme 1. Synthesis of the TFP (**T**etra**F**luoro**P**henol) acrylate monomer unit (**5**). TFA = trifluoroacetic acid, Boc = *tert*-butoxycarbonyl, EDC = 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, HOBt = 1-hydroxybenzotriazole.



Scheme 2. Generation of TFP 3D gel spots for 'catch' and 'release' synthesis. A pre-functionalised glass slide was generated by treatment with methylacryloxypropyltrimethoxysilane in acidic ethanol/water mixture. The pre-polymerisation mixture which consisted of the PEG cross-linker (**6**), TFP monomer (**5**) and 1% UV-initiator (**7**) in DMF was applied in 4 μL volume using a Gilson pipette to create an array of up to 16 spots/slide. A hydrophobic slide was used to cover the spots before polymerisation using UV light. The cover slide was removed and the spots were treated with one of four acid chlorides (R^1COCl, **8**–**11**), followed by one of two amines (R^2NH_2, **12**–**13**). LCMS spectra of washings were used to show evidence for synthesis of the amides (**14**–**21**).

two standard acrylamide monomer units but no TFP acrylamide monomer unit (**5**) swelled considerably in water (7.9 mL/g). The TFP acrylamide gel was found to swell effectively in either DMSO or DMF solutions (DMF = 4.6 mL/g). The loading capacity of the TFP acrylamide gel was calculated to be 0.7 mmol/g; this compared favourably with reported loadings of 1.0 mmol/g for the commercial TFP beads.[9]

After polymerisation the TFP acrylamide gel was incorporated onto glass slides as gel spots. In order to co-polymerise the gel onto the glass platform, the slides were first derivatised with 3-methylacryloxypropyltrimethoxysilane as shown in Scheme 2. The functionalised glass slides were then spotted with the polymerisation mixture and covered with a hydrophobic cover slide to prevent excessive exposure to the air. It is essential to prevent excess air getting into the reaction as oxygen inhibits the free radical polymerisation. The glass slides were finally irradiated with UV light to generate the gel spot array.

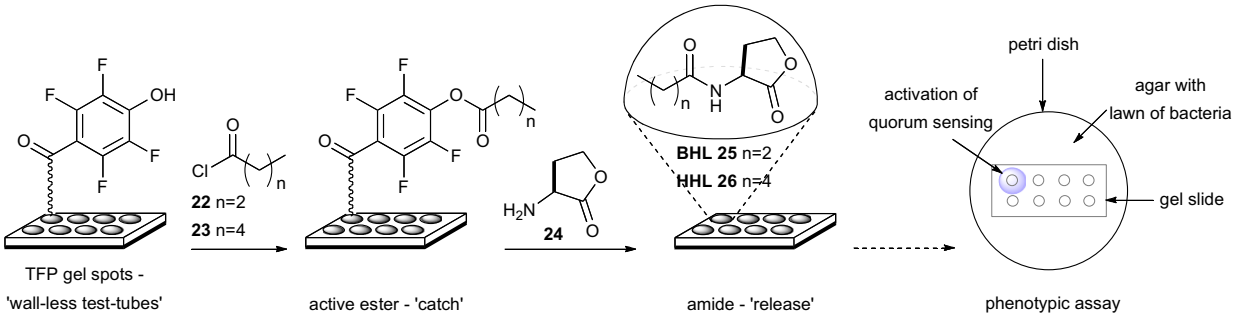
Following the generation of the gel spot array, the amides were synthesised within each gel spot using the catch-and-release TFP chemistry. The same amides were also synthesised in solution for analytical comparison to those produced using the TFP acrylamide gel. Each individual spot was treated with one of four different acid chlorides (**8–11**) to generate the intermediate activated esters. It should be noted that carboxylic acids were also successfully loaded onto the TFP acrylamide gel using diisopropylcarbodiimide-mediated coupling; however, acid chlorides were favoured as the electrophile as coupling reagents were not required.

Every gel spot containing a different active ester was reacted with one of the two primary amines (**12** or **13**) to generate eight distinct amides (**14–21**), one in each gel spot. The successful synthesis of the amide library within the gel was confirmed by comparison to those amides made in solution; the recorded mass and retention times of the amides were identical independent of the method used to form them. The approximate yield was calculated to be in the range 40–60%.

Quorum sensing is the chemical language used by bacteria to communicate and thus co-ordinate behaviour.[10] The gram negative bacteria *Serratia* and *Chromabacterium violaceum* use the acyl-homoserine lactone (AHL)-signalling molecules butanoyl-L-homoserine-lactone (BHL) and hexanoyl-L-homoserine lactone (HHL), respectively, to communicate. The inhibition of AHL signalling and consequently quorum sensing could provide the next generation of anti-bacterial agents against multi drug resistant bacteria.[11] To expand upon our proof of principle study we next examined the synthesis of biologically active quorum-sensing small molecules in the TFP acrylamide gel. In a similar strategy, a recent report by Praneenararat et al. describes the synthesis of quorum-sensing molecules on planar cellulose supports.[12]

Pleasingly, the natural quorum-sensing signalling molecules BHL and HHL were successfully synthesised within the TFP acrylamide gel spots and this was confirmed by LCMS analysis (Scheme 3, compounds **25** and **26**, respectively). This approach offers the potential to synthesise and screen[13] sequentially the gel spots containing the AHL analogues for phenotypic activity on the same chip. Quorum sensing is an ideal pathway for cell-based screening because its activation leads to the expression of certain detectable phenotypes.[14]

In summary, we have developed a new and effective method for the synthesis of small molecules using TFP acrylamide 3D gels. A library of amides were generated within TFP-based gel spots using



Scheme 3. Synthesis of BHL (butanoyl-L-homoserine lactone) and HHL (hexanoyl-L-homoserine lactone) in TFP acrylamide gel spots. Gel spots were loaded with butyryl chloride (**22**) or hexanyl chloride (**23**) before reaction with aminobutyrolactone (**24**) to produce BHL (**25**) and HHL (**26**), which were validated by LCMS. BHL and HHL could be detected using quorum sensing phenotypic assays using *Serratia* sp. ATCC39006 (BHL) or *Chromabacterium violaceum* (HHL). For example, *C. violaceum* produces a purple violaceum halo under HHL mediated quorum sensing.

catch-and-release chemistry. Bioactive quorum-sensing molecules were successfully synthesised in preparation for phenotypic assay screening.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.09.024.

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