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# Efficient and Facile Synthesis of Acrylamide Libraries for Protein-Guided Tethering

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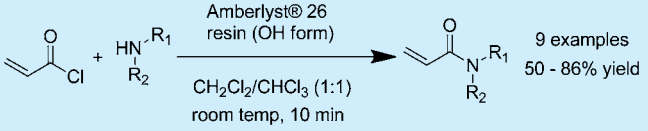
Supporting Information

**ABSTRACT:** A kinetic template-guided tethering (KTGT) strategy has been developed for the site-directed discovery of fragments that bind to defined protein surfaces, where acrylamide-modified fragments can be irreversibly captured in a protein-templated conjugate addition reaction. Herein, an efficient and facile method is reported for the preparation of acrylamide libraries from a diverse range of amine fragments using a solid-supported quaternary amine base.




Over the past decade, tethering strategies have been successfully developed for the site-directed discovery of fragments that bind to defined protein surfaces.[1−4] These include kinetic template-guided tethering (KTGT),[1] where upon fragment binding a Cys-residue adjacent to the protein binding site irreversibly captures an acrylamide-modified fragment in a protein templated, conjugate addition reaction. KTGT is able to identify fragments with low affinity for the protein of interest, which has the potential to provide accurate and relevant hit matter, enabling the development of fragments into inhibitors or antagonists of challenging targets, such as protein−protein interactions (PPIs).

Acrylamides have also been successfully used as electrophilic capture groups in the development of covalent inhibitors,[5] as exemplified by the EGFR/ERBB2 inhibitor neratinib, which is currently in phase III clinical trials for the treatment of metastatic breast cancer, Figure 1.[5c]

In order to add value to KTGT as a fragment screening technique, an efficient and high-yielding procedure for the preparation of structurally relevant acrylamide libraries from their corresponding amines was sought. Solution-phase methods for acrylamide synthesis are well documented but are often low yielding and unsuitable for high-throughput library synthesis. There have been previous attempts to overcome the current limitations, in particular through the use of solid-phase syntheses,[6,7] but these often require a large number of steps for a simple chemical transformation. Moreover, the commercial availability of chemically diverse, acrylamide-functionalized fragments is relatively low compared to other structural classes such as amines or carboxylic acids. Herein, we describe the design of an acrylamide library for use in KTGT, and the optimization of an efficient and facile method for the preparation of acrylamide libraries from a diverse range of amine fragments, suitable for fragment based drug discovery(FBDD), using a solid-supported quaternary amine base.

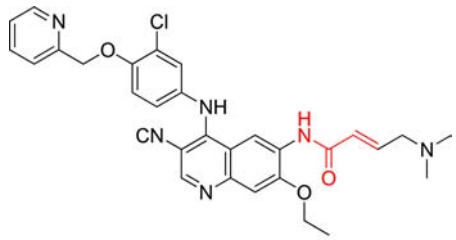



**Figure 1.** Structure of neratinib, a covalent inhibitor of EGFR/ERBB2, currently in phase III clinical trials.

A library of 192 fragment amines was designed for modification to the corresponding acrylamide for use in a KTGT screening campaign. The amines consisted of primary, secondary, aromatic and aliphatic fragments, covering a broad range of chemical space and structural diversity, with many compounds falling within the "Rule of 3" guidelines suggested for FBDD (see the Supporting Information).[8,9] All fragments with known "toxicophores" were removed. By selecting a diverse amine library, a robust synthetic methodology compatible with a wide range of fragments could be investigated for KTGT libraries as well as for the development of covalent inhibitors.

To prepare the acrylamides for screening, the 192-member amine library was initially subjected to standard acylation conditions using acryloyl chloride and triethylamine base using an automated liquid-handling system.[10] Unfortunately, the success rate (determined as desired product isolated from the reaction) was only 33%, and the average isolated product yield

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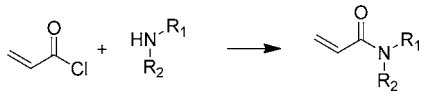
was a disappointing 24%, Table 1. The low success rate and product yield were attributed to the susceptibility of

**Table 1. Comparison of Standard Acylation *versus* Our Conditions for Acrylamide Synthesis**



| method | no. of amines | total successful[c] | success rate (%) | average yield[d] (%) |
|---|---|---|---|---|
| standard acylation[a] | 192 | 64 | 33 | 24 |
| Amberlyst resin[b] | 30 | 25 | 83 | 54 |

[a]Reagents and conditions: acryloyl chloride (0.3 mmol), amine (0.3 mmol), $NEt_3$ (0.9 mmol), $CH_2Cl_2$ (1 mL). [b]Reagents and conditions: acryloyl chloride (0.3 mmol), amine (0.3 mmol), Amberlyst 26 (OH form) (45 mg), $CH_2Cl_2/CHCl_3$ (1 mL). [c]Success determined as desired product isolated. [d]Isolated, purified yield.

acrylamides to polymerize, as well as the need for extensive purification to remove side products formed during the reaction, due to the reaction of acryloyl chloride with triethylamine.

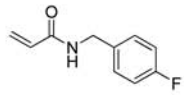
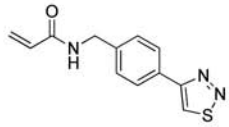
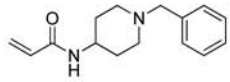
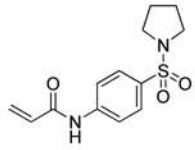
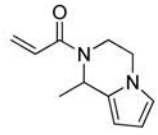
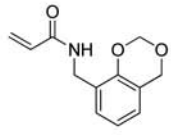
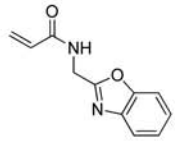
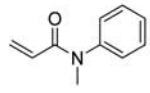
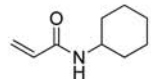
We therefore chose to investigate alternative reaction conditions that would enable acrylamide isolation without the need for an aqueous workup or purification and in sufficient purity for use in KTGT. Methods investigated included solid-phase syntheses,[6,7] boron-mediated amidations,[11,12] solid-supported amide coupling agents,[13] as well as Lewis acid mediated transformations.[14] Unfortunately, these methods did not overcome the limitations of low conversion, side product formation, and polymerization. Attempts to mask the acrylamide functionality, in order to prevent any residual amine from reacting with the product acrylamide prior to purification, did not deliver an efficient and high-throughput method.

In order to prevent the unwanted side reactions under standard acylation conditions between acryloyl chloride and the amine base (in this case $NEt_3$), inorganic and solid-supported bases were investigated. Inorganic bases proved to be inefficient due to their poor solubility in organic solvents and the hydrolysis of acryloyl chloride under aqueous conditions. Gratifyingly, ion-exchange resin Amberlyst A26 (OH-form) provided an efficient method of scavenging the HCl generated during the reaction, without reacting with acryloyl chloride to generate unwanted side products. An optimized method was therefore developed.[15] It was envisaged that this method would enable the rapid generation of acrylamide libraries from amine starting points suitable for KTGT, particularly as the method was suitable for relatively small-scale reactions (0.1−0.3 mmol amine).

In order to provide a robust and thorough test for the Amberlyst A26 method, a sample of 30 fragment amines were selected from the initial 192 member library. **This selection was of amines that failed to react under standard acylation conditions.**[10] They were subjected to the improved Amberlyst A26 resin method.[15] The 30-member test set once again contained a range of primary, secondary, aromatic, and aliphatic amines for diversity (see the Supporting Information). Of the 30 fragment amines, 25 reacted successfully, required no further purification and gave a satisfactory average yield of 54%. All examples were isolated in >95% purity as determined by HPLC. Therefore, the improved Amberlyst A26 resin methodology provided access to acrylamide fragments that would otherwise be unobtainable using the standard conditions, thus enabling the inclusion of greater chemical diversity in our acrylamide library.

To exemplify the utility of the method, selected examples from our library are outlined in Table 2. Entries 1−3 highlight

**Table 2. Selected Examples Prepared Using the Amberlyst A26 Method[15]**

| entry | structure | product | yield | lit yield[a] |
|---|---|---|---|---|
| 1 |  | 1 | 86% | 74% |
| 2 |  | 2 | 59% | 39% |
| 3 |  | 3 | 66% | 31% |
| 4 |  | 4 | 54% | - |
| 5 |  | 5 | 73% | - |
| 6 |  | 6 | 50% | - |
| 7 |  | 7 | 61% | - |
| 8 |  | 8 | 72% | - |
| 9 |  | 9 | 80%[b] | - |

[a]Isolated yields reported in ref 1. [b]Average isolated yield when prepared on a 0.3, 1.2, 5.0, and 10.0 mmol scale.

the improvement in yield when using our Amberlyst A26 resin method compared with the standard acylation conditions. The selection confirms that a range of functional groups are tolerated and that primary and secondary amines react equally well under these conditions. Often the presence of basic amine centers can be problematic due to quaternization; however, *N*-(1-benzylpiperidin-4-yl)acrylamide was prepared in 66% yield



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without the need for purification compared with the reported literature yield of 31% (entry 3). In order to test whether this methodology was applicable to the scale-up of acrylamide fragments, cyclohexylamine was subjected to the Amberlyst A26 resin method up to 10 mmol in comparable yields, Table 2 (entry 9).

Synthetic methods have been investigated for the rapid conversion of amine libraries to acrylamides that are suitable for use in KTGT screening campaigns. The reactive nature of the acrylamide functional group poses challenges to the purification and isolation of the desired fragment acrylamides. This was overcome through the use of ion-exchange resin Amberlyst A26 (OH-form), which drives the reaction to completion without the formation of unwanted side products. The short reaction times and experimental simplicity of this method ideally lend it for use in high-throughput combinatorial chemistry as well as for the efficient synthesis of specific acrylamide-bearing compounds that could be used as potential covalent inhibitors against relevant therapeutic targets.

## ■ ASSOCIATED CONTENT

### Supporting Information

Experimental procedures, general methods, and spectroscopic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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(10) Standard acylation conditions: 192 amines (300 $\mu$mol) were placed in 24 well trays, and the addition of $Et_3N$ (1 mL, 0.3 M in $CH_2Cl_2$) was automated using parallel equipment. The vials were shaken for 15 min. The vials were charged by hand with acryloyl chloride (1 mL, 0.3 M in $CH_2Cl_2$). The vials were shaken for 16 h. The samples were concentrated under reduced pressure, redissolved in DMSO (1000 $\mu$L) and MeOH (200 $\mu$L), and then purified by preparative HPLC. The fractions containing product were collected and concentrated to dryness to give desired product.

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(15) Amberlyst A26 resin method conditions: 30 amines (300 $\mu$mol) were placed in 24 well trays and dissolved in 900 $\mu$L of $CHCl_3$. To each vial was added acryloyl chloride (100 $\mu$L, 3 mM in $CH_2Cl_2$), and the vials were shaken for 5 min, whereupon a solid precipitate was formed. Subsequently, Amberlyst A26 (OH-form) (80 mg) was added to each vial, which was shaken for a further 5 min. Amberlyst A26 resin was removed in parallel using vacuum filtration and subsequently washed with $CH_2Cl_2$ (5 mL), and the filtrate was concentrated under reduced pressure to provide the desired product in >95% purity.




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