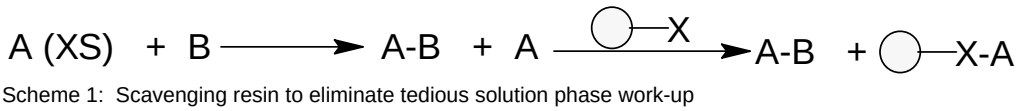
Supported Nucleophiles for Rapid Removal of Bsmoc-Amino Protecting Group Utilizing Microwave Heating

Shahnaz Ghassemi
Biotage, Discovery Chemistry Group1725 Discovery Drive Charlottesville, VA 22911

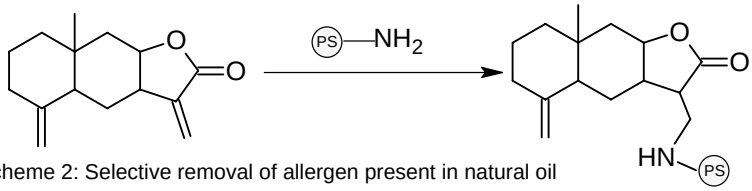
Introduction

The need to increase the rate of drug discovery is the driving force for innovative new techniques for rapid synthesis and isolation. Although solid-phase organic synthesis is one of the most innovative techniques used to increase the rate of drug discovery, the extra steps required for anchoring the target molecule to the resin, its cleavage from the resin, the need for a large excess of reagents to overcome the slow reaction kinetic and difficulty in monitoring the reaction progresses are few of the shortcomings of this technique. In recent years the inverse Merrifield approach, where the target molecule is synthesized in solution and solid-supported molecules are used as reagents, catalysts or scavengers, has became an alternative to SPOS. Since this technique offers many of the advantages of solid supported organic synthesis in terms of ease of reaction workup and product purification in addition to the advantages associated with traditional solution-phase chemistry (e.g. the ease of monitoring the progress of the reaction by simply applying LC-MS or TLC techniques).



Scheme 1: Scavenging resin to eliminate tedious solution phase work-up

This concept was utilized in 1980 by Frechet[1] who utilized solid-bonded primary amines for the selective removal of allergens present in natural oils (scheme 2). Carpino later reported the use of solid bonded nucleophile (supported cyclic secondary amines) in the simultaneous de-blocking and scavenging of the 9-fluorenylmethyloxycarbonyl (Fmoc-) amino-protecting group and its by-product dibenzofulvene (DBF)[2]. These techniques simplify work-up and purification, thus facilitating rapid solution phase synthesis.



Scheme 2: Selective removal of allergen present in natural oil

The main disadvantage of using solid bonded reagents and scavengers is their relative slow reaction rate. Recently It has been reported, that controlled microwave irradiation can effectively accelerate the rate of reactions involving solid bonded reagents and scavengers[3].

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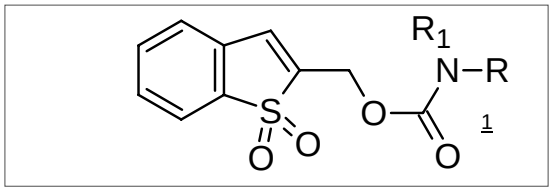
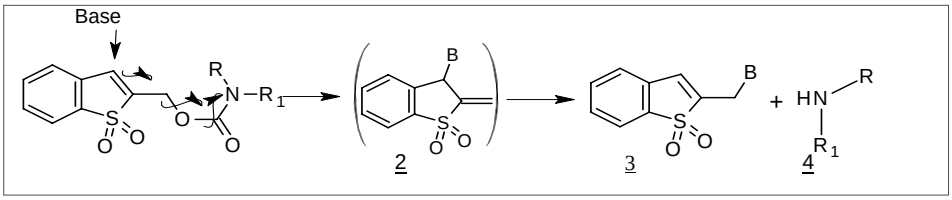
Solid bonded nucleophiles in conjunction with microwave heating for simultaneous de-blocking and scavenging of Bsmoc- group

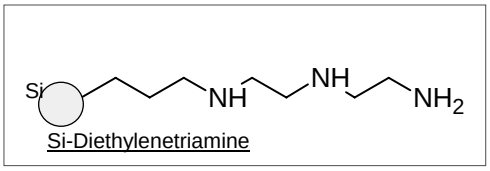
1,1-dioxobenzo[b]thiophene-2-ylmethyloxycarbonyl (Bsmoc-) (1) is a base sensitive amino protecting group used commonly in rapid solution phase peptide synthesis[4].



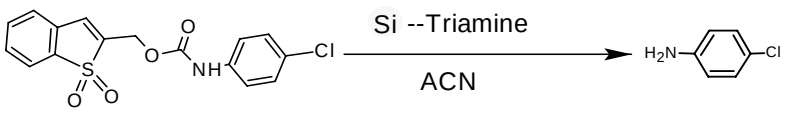
This protecting group is cleaved by a Michael-like addition of the base to the Bsmoc thiophene ring resulting in the cleavage of the Bsmoc-urethane bond and releasing the free amine (4) along with a reactive intermediate (2) which rearranges to the stable by-product (3).



Typically, the Bsmoc-urethane bond is cleaved upon stirring the Bsmoc-amine with tris(2-aminoethyl)amine (20 equiv) at room temperature for 15 minutes followed by reaction workup, which involves several extractions with saturated NaCl. While the use of a solid bonded base, such as piperazine-functionalized silica gel for Bsmoc- removal, has helped eliminate the aqueous extractions during workup, it has, however, increased the reaction time from minutes to hours. We have investigate the effect of microwave heating for the rapid removal of Bsmoc-PCA with Si-Diethylenetriamine.



Bsmoc-PCA was used as a test probe, to investigate the effect of solvent, time and temperature on the removal of the Bsmoc- amino protecting group using Si-Diethylene triamine (scheme 3). A mixture of Bsmoc-PCA (35 mg, 0.1mm) in 1mL acetonitrile and 1g of Si-Diethylenetriamine was stirred at room temperature, The RP-HPLC of this mixture after one hour showed 50% unreacted starting material.. When the same reaction mixture was heated using microwaves at 90 ºC for 4 minutes less than 1% of unreacted starting material remained .figure 1).



Scheme 3: De-blocking of the Bsmoc- protecting group using Si- Diethylenetriamine

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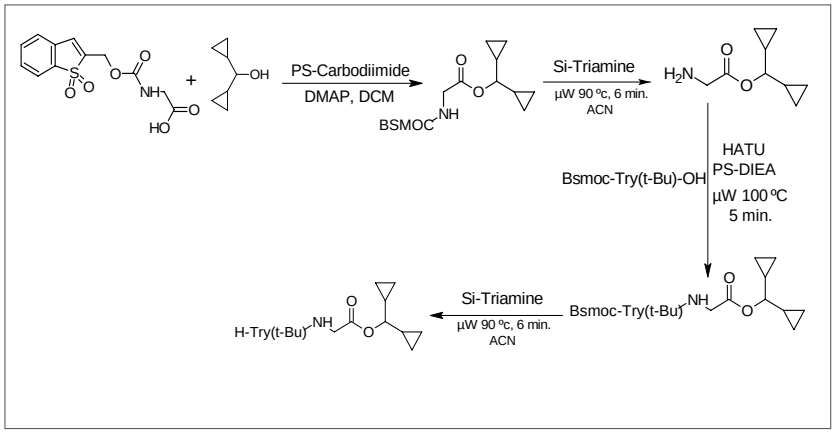
Different solvents such as DMSO, DMF and DCM were evaluated for this reaction. Although the reaction rate was faster in DMSO and DMF (90 ºC in 3 minutes), CH_3CN and DCM were preferred, due to their lower boiling points (ease of evaporation in multi-step synthesis) and ease of the monitoring reaction progress by TLC (figure 2). In order to investigate the scope of this technique, a range of Bsmoc- dipeptides with varying protecting groups such as, DMCP-amide[5], Bzl, Ethyl, DCPM[5] and t-Butyl ester were prepared ,through microwave assisted multi-step synthesis, involving coupling and deblocking (scheme 4). The Bsmoc-group was cleaved from these compounds in high yields and purity using Si-Diethylenetriamine in conjunction with microwave heating (Table 1).

The general procedure for microwave assisted Bsmoc-urethane bond cleavage involves heating the Bsmoc-amine in CH_3CN in the presence of Si-Diethyelentriamine(10 equiv) at 90 ºC for 5 minutes.



Scheme 4: The dipeptide H-Try(t-Bu)-Gly-ODMCP was prepared in rapid multi-step solution phase technique utilizing microwave irradiation with PS-Carbodiimide[6] and PS-DIEA[6] and HATU[7] for coupling steps and Si-Triamine for de-blocking steps.

Cleaving the Bsmoc- moiety from the dipeptide using the technique described above, did not affect acid labile protecting groups (t-Bu; DCPM) (figure 3) .

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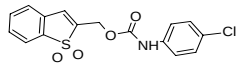
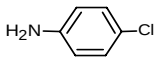
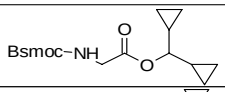
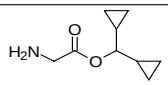
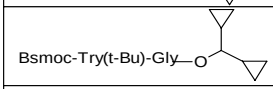
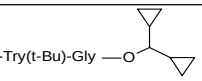
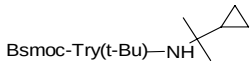
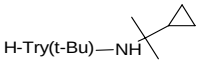
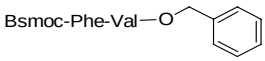
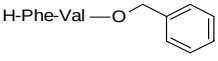
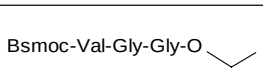
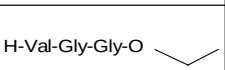
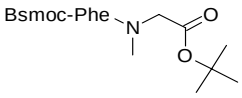
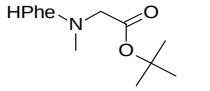
BsmocAmine	Amine	Isolated Yield	MS M+1
		**95 %**	**128.1**
		**90 %**	**170.1**
		**91 %**	**389.3**
		**89%**	**319.2**
		**94%**	**355.1**
		**87%**	**260.1**
		**87%**	**293.1**

Table 1. Reactions were performed in the Biotage EMRYS™ Liberator microwave system in 2-5 mL reaction vials at 90 °C for 6 min.. [a] Yield and purity determined by LC/MASS with UV(220 and 254 nm). [b] LC/MASS was carried out on a XTerra® MS C18 3x50 mm, 3.5 µm, Mobile phase: A: 0.5 mM CH_3COONH_4 in 5% MeCN, B: 0.5mM CH_3COONH_4 in MeCN, Gradient: 10 to 100 B in 5 min. Micromass® ZQ (Waters).

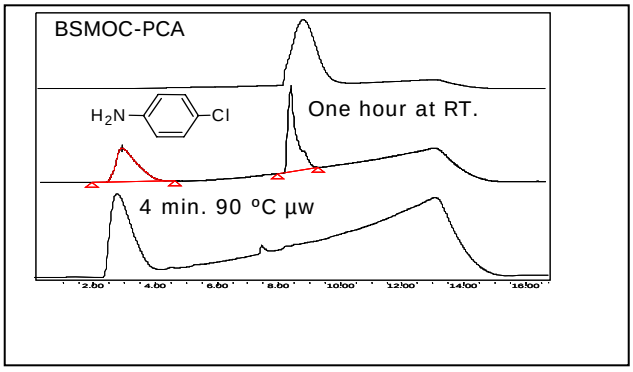


Figure 1: Deblocking of Bsmoc-PCA at room temperature and Microwave heating at 90 ºC

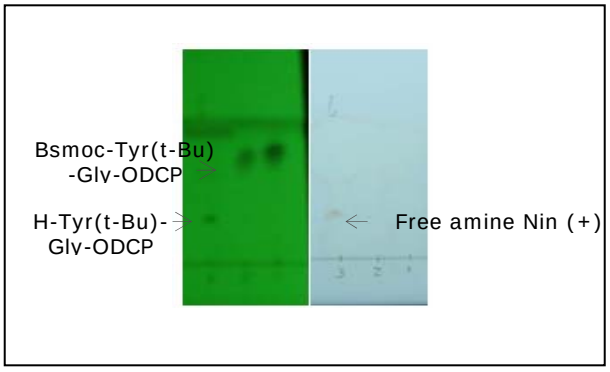


Figure 2: TLC plate monitoring, reaction progress. Solvent EtOAc, Hexane 4:6

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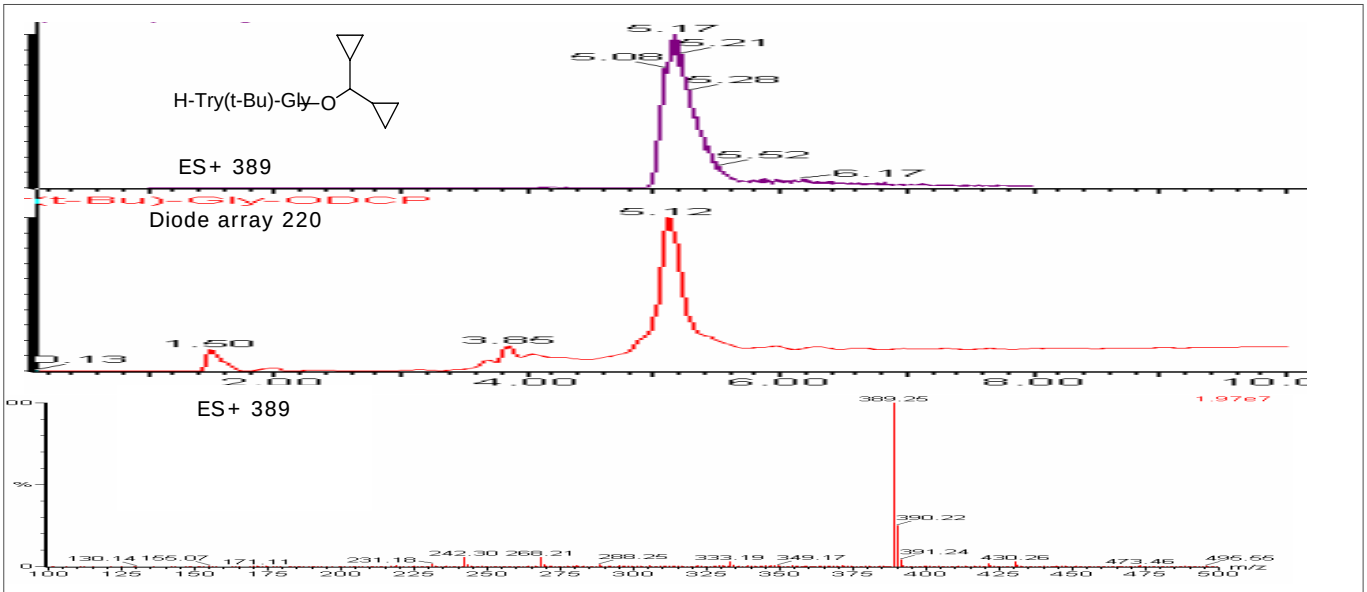

Figure 3: The dipeptide H-Try(t-Bu)-Gly-ODMCP was prepared in high yield and purity through a multi-step rapid solution phase technique utilizing microwave irradiation and Si-Triamine . LC/MASS was carried out on a XTerra® MS C18 3x50 mm, 3.5 µm, Mobile phase: A: 0.5 mM CH_3COONH_4 in 5% MeCN, B: 0.5mM CH_3COONH_4 in MeCN, Gradient: 10 to 100 B in 5 min. Micromass® ZQ (Waters).

Conclusion

Solid supported solution phase synthesis has emerged as an important enabling technique in rapid multi-step synthesis. This technique in conjunction with microwave heating has been employed for simultaneous de-blocking and scavenging of Bsmoc- groups in rapid solution phase synthesis. It is clear that using solid bonded reagents in conjunction with microwave irradiation will gain greater importance and will become significant techniques in speeding up the drug development process, especially since the combination is easy to automate.

Refferences:

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