## Efficient use of the Dmab protecting group: applications for the solid-phase synthesis of *N*-linked glycopeptides<sup>†</sup>

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An efficient protocol for the chemoselective removal of Dmab esters on the solid phase is reported; this method has been successfully utilised for the convergent solid phase synthesis of *N*-linked glycopeptides.

The  $4-{N-[1-(4,4-dimethy]-2,6-dioxocyclohexylidene)-3-methylbutyl]-amino} benzyl (Dmab) ester has recently been introduced as an orthogonal protecting group for the modification of peptides on resin.<sup>1</sup> Aspartic and glutamic acids bearing this group on their side chains or$ *C*-termini are commercially available in their Fmoc protected form ready for incorporation into solid phase peptide synthesis (SPPS). The Dmab group contains a 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)-3-methylbutyl (ivDde) group which can be conveniently removed by treatment with 2% hydrazine. Following ivDde removal, the residual aminobenzyl moiety is reported to spontaneously eliminate to afford the corresponding free carboxylate (Scheme 1).<sup>2</sup>



Scheme 1 Mechanism of Dmab deprotection with hydrazine.

The Dmab group possesses several notable advantages over the allyl ester, which is the traditional choice of orthogonal protecting group in the solid phase synthesis of modified peptides, cyclic peptides and glycopeptides. These include facile (hydrazine) deprotection conditions, which negate the need for expensive palladium catalysts. This is especially important in cases where biological evaluation of the ensuing peptide derivatives may be hampered by catalyst contamination. In addition, the Dmab deprotection reaction generates a UV active indazole moiety, thereby providing a convenient means by which to follow the reaction progress spectrophotometrically (Scheme 1).<sup>3</sup>

To date, several peptides protected with a *C*-terminal Dmab ester have been synthesised on the solid phase and converted to cyclic peptides in an efficient manner.<sup>4-7</sup> However, it is somewhat surprising that similar approaches to the synthesis of modified peptides involving Dmab-derivatised aspartate and glutamate side chains have proved problematic.<sup>2,3,8,9</sup> This can be attributed to either the formation of undesired side products<sup>2,8-10</sup> or, perhaps, incomplete removal of the protecting group under standard deprotection conditions.<sup>3</sup> In the context of synthetic endeavours within this laboratory directed towards the construction of *N*linked glycopeptides on the solid phase, we required a protecting group orthogonal to the allyl ester. We were hopeful that the development of improved conditions for the incorporation and removal of the Dmab ester would facilitate this goal.

To this end, synthetic efforts commenced with the preparation of a model resin bound peptide bearing a Dmab protected aspartic acid residue 1 (Scheme 2). A dimethoxybenzyl (Dmb)-protected glycine residue was incorporated adjacent to the aspartic acid residue to prevent aspartimide formation.8 So as to gauge the efficiency of the Dmab deprotection and a subsequent aspartylation reaction, we prepared an analogous resin bound model peptide 2 bearing an allyl protected aspartic acid residue, which is known to be efficiently removed and derivatised on the solid phase.<sup>11</sup> Both resin bound peptides were synthesised on Wang resin following the Fmoc-strategy, to initially afford resin bound tripeptide 3 (see ESI<sup>†</sup>). Fmoc-Gly(Dmb)-OH was coupled as the penultimate amino acid followed by Fmoc-Asp(ODmab)-OH or Fmoc-Asp(OAllyl)-OH for the synthesis of 1 and 2 respectively. Yields of amino acid coupling steps were ascertained to be quantitative in all cases, as determined by measuring the piperidine-fulvene adduct at  $\lambda = 301$  nm. Finally, a capping step was employed to acetylate the N-terminus of the resin bound peptides to afford 1 and 2.

Deallylation of **2** was smoothly achieved on the solid phase by treatment with  $Pd(PPh_3)_4$  and phenylsilane.<sup>12</sup> Subsequent aspartylation of amino-sugar **5**<sup>13</sup> using PyBOP and NMM, followed by acidolytic side chain deprotection and cleavage from the resin, gave the desired glycopeptide **6** which was analysed by LC-MS and purified by preparative HPLC. As expected, the desired

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Scheme 2 Solid phase synthesis of N-linked glycopeptides.

*N*-linked glycopeptide was produced in high yield, without the formation of aspartimides or truncated products. In contrast, Dmab deprotection using the reported conditions (2% hydrazine),<sup>1</sup> followed by aspartylation of **5** with PyBOP and NMM, returned only 5% of glycopeptide **6**.

Since the solid phase aspartylation of allyl protected peptide **2** proceeded in high yield, it was postulated that the Dmab ester was not cleaved in an efficient manner under the reported conditions. As the ivDde moiety was found to be completely removed upon five treatments with 2% hydrazine solution, it was evident that spontaneous elimination of the aminobenzyl ester was very slow (Scheme 1).

Table 1Conditions (a-k) used for the elimination of the aminobenzylester. The efficiency of elimination was determined from the yield of thesolid phase aspartylation of 5



It has been reported that sluggish elimination of the aminobenzyl moiety can be remedied by treatment with an aqueous solution of N,N-diisopropylethylamine (DIPEA).<sup>3</sup> In this case, treatment of the resin bound peptide with hydrazine, 20% DIPEA in 9:1 v/v DMF/H<sub>2</sub>O for 12 hours, followed by aspartylation of **5**, provided the desired glycopeptide **6** in an improved yield of 50% (entry 1, Table 1). Extending the base treatment to 48 h (entry 2, Table 1) prior to aspartylation afforded **6** in 90% yield. Although a vast improvement, incomplete removal of the aminobenzyl moiety would complicate separation of the glycopeptide from the peptide containing the free side chain carboxylate, especially significant in the case of longer peptide sequences. Additionally, we were concerned that prolonged exposure to aqueous basic conditions may lead to epimerisation and aspartimide formation with delicate amino acid sequences.

In an effort to optimise reaction conditions, we examined a range of organic bases in the absence of water. These included DIPEA (entries 3 and 4, Table 1), pyridine (entry 5, Table 1), piperidine (entry 6, Table 1) and triethylamine (entry 7, Table 1). Unfortunately, these conditions resulted in low yields of glycopeptide 6 (15-30%), suggesting water was required to facilitate the deprotection. The next step was to employ an inorganic aqueous base (entries 8-11, Table 1). Gratifyingly, treatment of the resin with 5 mM sodium hydroxide in methanol and water led to rapid elimination of the aminobenzyl ester. A time course experiment revealed that a 3 hour treatment with 5 mM NaOH resulted in complete deprotection (entry 11, Table 1). Additionally, all protecting groups commonly employed in Fmoc-strategy SPPS, as well as the ester linkage to Wang resin, are stable to this low concentration of hydroxide (see ESI<sup>+</sup>). The exception is the Fmoc protecting group which is partially cleaved under the conditions,

however, this has no bearing on the utility of the reaction since peptides are fully assembled prior to Dmab deprotection (see ESI<sup>†</sup>). In addition, no side products relating to epimerisation or aspartimide formation were formed under these optimised reaction conditions, even after prolonged treatments (see ESI<sup>†</sup>).

We have observed that the nature of the amino acid C-terminal to the site of derivatisation has a dramatic effect on the efficiency of solid phase reactions of aspartic acid and glutamic acid side chains. As such, our next goal was to investigate the potential scope of this improved solid phase aspartylation protocol, via the incorporation of alternative amino acids C-terminal to the Dmab-protected aspartic acid residue. Resin bound tripeptide 3 served as the starting point for the synthesis of three resin bound peptides (Scheme 3). Peptides bearing penultimate glycine, proline and valine residues were synthesised by SPPS. Glycine was incorporated in its Dmb-protected form (again to prevent aspartimide formation). The inability of proline to form aspartimides, and the sterically hindered nature of the valine side chain, meant that the backbone amides were not protected with Dmb groups in these cases. Treatment of the resin bound pentapeptides with hydrazine, followed by 5 mM NaOH, gave the corresponding free acids 4, 7 and 8, which were subsequently reacted with 5. Side chain deprotection and cleavage from the resin using an acidic cocktail gave the desired glycopeptides 6, 9 and 10 in high yields (quant., 95% and 96%, respectively) as determined by LC-MS analysis. Isolation of these glycopeptides by preparative HPLC provided 6, 9 and 10 in moderate to high yields.



Scheme 3 Solid phase synthesis of N-linked glycopeptides 6, 9 and 10.

Finally, to demonstrate the applicability of our solid-phase aspartylation method for the preparation of longer glycopeptides bearing an internal glycan, we embarked on the synthesis of **11** (Scheme 4). Synthesis of fully protected peptide **12** was achieved from **3** using standard Fmoc-SPPS followed by Dmab-deprotection of the internal aspartic acid residue (see ESI<sup>†</sup>). Solid phase aspartylation of **5** and acidolytic deprotection and cleavage from the resin gave the desired glycopeptide **11** in 61% isolated yield based on the Fmoc loading of **3**. This example clearly demon-



Scheme 4 Solid phase synthesis of N-linked glycopeptide 11.

strates the orthogonal nature of the deprotection/aspartylation conditions to a large range of protecting groups commonly employed in solid-phase chemistry including Boc, *t*Bu ethers, *t*Bu esters and trityl (Trt) groups and illustrates the utility of the solid phase aspartylation method for the synthesis of more complex glycopeptides.

In summary, improved conditions for the removal of Dmab esters from the side chain of aspartic acid residues on the solid phase have been developed and implemented in a novel solidphase strategy for the simple, rapid and efficient construction of *N*-linked glycopeptides in a convergent manner. Applications of this procedure are ongoing in this laboratory for the synthesis of *N*-linked glycopeptide fragments which can be used for the ligatory assembly of therapeutic glycopeptides and glycoproteins. In addition, it is anticipated that the Dmab deprotection method will find application in a host of synthetic endeavours, including the synthesis of cyclic peptides.

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