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Solid phase synthesis of ω-aspartic thioacid containing peptides

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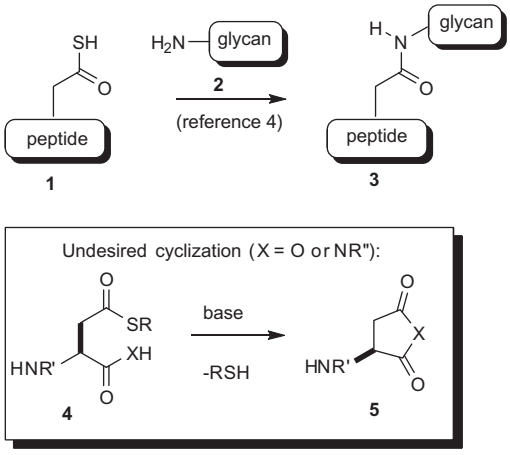
ABSTRACT

The Fmoc-based solid phase synthesis of unprotected ω-aspartic thioacid containing peptides is demonstrated. The method involves the novel use of a silyl ester as a carboxylate surrogate for mild peptide bond formation in the presence of a reactive ω-aspartyl thioester. The resulting peptide thioacids may be used in N-glycoligation and other thioacid-mediated conjugation reactions.

The thioacid functional group provides a functional handle for the chemoselective formation of peptide and related amide bonds.[1] Convergent segment coupling applications of such methods benefit from the fact that C-terminal thioacids are available via solid phase peptide synthesis (SPPS)[2] and biosynthetically using recombinant techniques.[3] The unique properties of this functional group may also be exploited for the site-specific modification of peptides, most notably for the introduction of an intact glycan at a particular aspartic acid residue to form N-glycopeptides (Scheme 1, **1** + **2** → **3**).[4,5] Currently, the only practical convergent N-glycopeptide synthesis is the Lansbury aspartylation[6] even though it has significant limitations associated with chemoselectivity and aspartimide (Asi) formation.[7] While the thioacid-mediated glycoligation methods are encouraging in terms of their convergency and the Cu(II)-promoted variant can be performed on fully unprotected peptides, they still suffer from the need to convert an orthogonally-protected peptide ω-aspartate ester to its corresponding thioacid. This transformation is accomplished via the intermediate ω-aspartic acid-containing peptide post-SPPS in solution, not only requiring additional steps but also placing restrictions on the SPPS linker. Furthermore, any intermediate active ester will be susceptible to aspartimide formation. An attractive alternative approach to ω-aspartate thioester-containing peptides involves the incorporation of a suitably masked ω-aspartate thioester building block into an Fmoc-based SPPS sequence, which would produce the desired peptide thioacid directly upon acidic global deprotection. However, any attempt to implement such a strategy must deal with the propensity of thioesters to undergo



Scheme 1. Thioaspartic acid mediated N-glycosylation.

nucleophilic acyl substitution. In the case of a ω-aspartate thioester-containing peptide, this reactivity would likely manifest itself in the form of aspartimide side-product (Scheme 1, **4** → **5**, X = NR″). We now describe a novel Fmoc-SPPS approach to ω-aspartic thioacid containing peptides. The resulting methodology provides the basis for a more streamlined synthetic entry to N-glycopeptides via glycoligation.

The first task involved identifying an appropriately functionalized aspartic acid-derived thioester building block corresponding to **4** (X = O) that could be used with an Fmoc-based SPPS strategy. In order to mitigate unwanted nucleophilic attack at the thioester carbonyl, it was decided that the substituent R should be sterically

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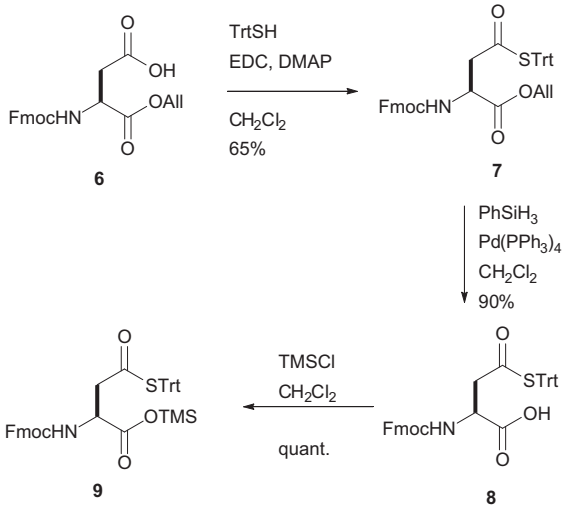
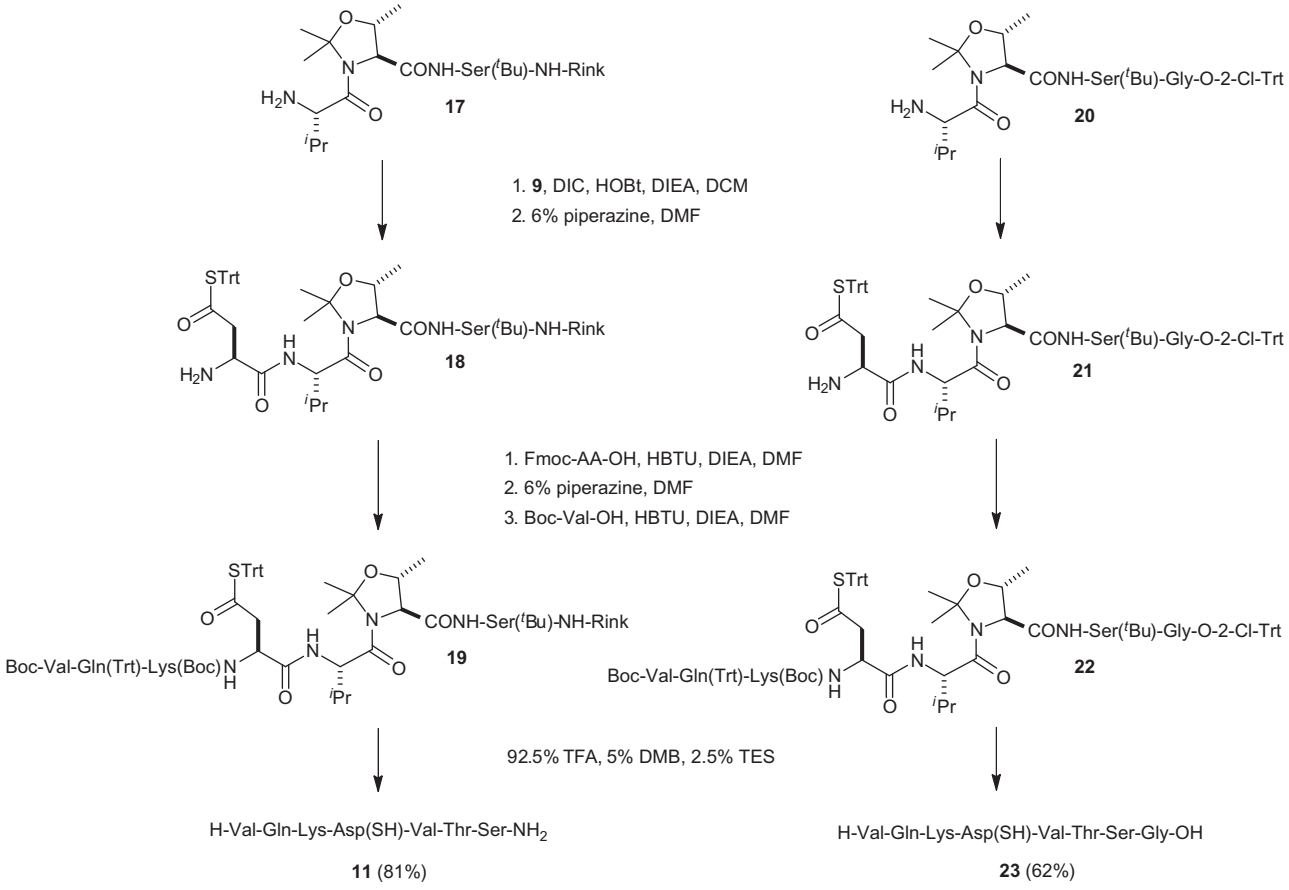


Scheme 2. Synthesis of thioaspartic acid building block **9**.

bulky yet readily removable using a standard acidic global deprotection cocktail. This line of reasoning eventually led us to consider the use of a trityl thioester for this purpose. Our choice of this protecting group for the thioacid was guided by the knowledge that tertiary thioesters are known to resist Pd-mediated Fukuyama reduction[8] as well as nucleophilic acyl substitution during standard Fmoc-deprotection.[9] Thus, Fmoc-Asp(STrt)-OH (**8**) was synthesized from the known aspartic acid derivative **6**[10] via Steglich thioesterification with TrtSH[11] followed by Pd-mediated deallylation to give **8** (Scheme 2).

That no reduction of the thioester to aldehyde was observed in this reaction was taken as initial support for our steric argument. However, at this stage we reached an impasse. All attempts to couple the carboxylic acid **8** with even a simple amine to produce an amide in good yield met with failure. The attempted reaction using a carbodiimide coupling reagent was illustrative, producing a mixture that indicated competitive formation of a reactive anhydride (**5**, R′ = Fmoc, X = O) which triggered other undesired reactions. We conjectured that this entropically-favored cyclization must proceed through a carboxylate anion and that a weaker nucleophile might shut down (or at least mitigate) this unwanted pathway. Since the Pd-catalyzed de-allylation reaction had presumably passed through a silyl ester intermediate without a problem, we decided to explore the use of an analogous silyl ester as a neutral carboxylate surrogate in a peptide coupling context.[12] In the event, trimethylsilyl ester **9**, produced quantitatively by the action of TMSCl on **8**, underwent a very clean reaction with benzylamine (DIC + HOBt in DCM) to give Fmoc-Asp(STrt)-NHBn (**10**) in 79% yield. We were now poised to apply this reaction to the SPPS of ω-aspartic thioacid-containing peptides.

We chose the heptapeptide thioacid H-Val-Gln-Lys-Asp(SH)-Val-Thr-Ser-NH_2 (**11**) as our first target since it contains the progenitor to a typical N-glycopeptide consensus sequence (Asn(glycan)-Xaa-Thr/Ser). Starting with a Rink amide resin (Scheme 3), the resin-bound tetrapeptide Fmoc-Asp(STrt)-Val-Thr(^{t}Bu)-Ser(^{t}Bu)-NH-Rink (**12**) was assembled without problem using standard Fmoc removal and HATU coupling conditions for the first three amino acids followed by DIC-HOBt-DIEA coupling of the TMS ester **9**. LC/MS analysis after micro-cleavage from the resin confirmed formation of the expected tetrapeptide thioacid. No aspartimide was detected. However, the situation changed



Scheme 3. Synthesis of ω-thioaspartic acid containing peptides.

from this point on in the SPPS. Exposure of **12** to 20% piperidine produced significant amounts of aspartimide and piperidine-derived amide in addition to the expected N-terminal amine as determined after microcleavage. Initial attempts to modulate the basicity and/or nucleophilicity of the deprotection reagent did not seem to help. In the end, a brief treatment with a 2% piperidine solution cleanly produced H-Asp(STrt)-Val-Thr(^{t}Bu)-Ser(^{t}Bu)-NH-Rink (**13**). We again encountered the aspartimide problem during coupling of the next amino acid, obtaining mixtures of Fmoc-Lys(Boc)-Asp(STrt)-Val-Thr(^{t}Bu)-Ser(^{t}Bu)-NH-Rink (**14**) and Fmoc-Lys(Boc)-Asi-Val-Thr(^{t}Bu)-Ser(^{t}Bu)-NH-Rink (**15**).

At this point, it was decided to retool the SPPS and replace the Thr(^{t}Bu) residue with a Mutter pseudoproline (Ψpro). This protecting group had been shown to greatly reduce aspartimide formation during Lansbury aspartylation, which proceeds through an active ester intermediate.[13] The Ψpro modification was expected to likewise protect the ω-thioester from aspartimide formation and would be unmasked during the global deprotection. In the event (Scheme 3), the dipeptide Fmoc-Val-Thr(Ψpro)-OH (**16**)[14] was incorporated into the SPPS to give resin-bound peptide **17** after standard Fmoc removal. Addition of the building block **9** as described above proceeded uneventfully to give **18** after a brief treatment with 6% piperazine/DMF. These deprotection conditions worked well with the Ψpro-containing peptides and were used to remove each Fmoc group commencing with the Asp(STrt) residue. Completion of the SPPS produced resin-bound heptapeptide **19** which was subjected to a modified Reagent I cleavage cocktail[15] to give the ω-aspartic thioacid containing peptide H-Val-Gln-Lys-Asp(SH)-Val-Thr-Ser-NH_2 (**11**) in 81% isolated yield after HPLC purification. No aspartimide was detected. To evaluate the method's versatility, the SPPS was also performed on a 2-chlorotrityl resin to give the octapeptide thioacid H-Val-Gln-Lys-Asp(SH)-Val-Thr-Ser-Gly-OH (**23**) in 62% yield. However, the method as it stands does have its limits. This was ascertained using consensus sequences that are highly susceptible to aspartimide formation. Thus, the SPPS was repeated using Fmoc-Gly-Thr(Ψpro)-OH (**24**) and Fmoc-Ala-Thr(Ψpro)-OH (**25**) instead of **16**. In the former case, the aspartimide H-Val-Gln-Lys-Asi-Gly-Thr-Ser-NH_2 (**26**) was produced exclusively in 78% yield. In the latter case, a 3:1 mixture of aspartimide H-Val-Gln-Lys-Asi-Ala-Thr-Ser-NH_2 (**27**) and thioacid H-Val-Gln-Lys-Asp(SH)-Ala-Thr-Ser-NH_2 (**28**) was isolated in 70% yield.

In conclusion, a successful solid phase synthetic approach to ω-aspartic thioacid containing peptides has been developed. The method involves the novel use of a silyl ester as a carboxylate surrogate for mild peptide bond formation in the presence of a reactive ω-aspartyl thioester moiety. The resulting peptide thioacids may be used in N-glycoligation as well as other thioacid-mediated conjugation reactions.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2015.05.064.

References and notes

1. (a) Cronyn, M. W.; Jiu, J. *J. Am. Chem. Soc.* **1952**, *74*, 4726; (b) Sheehan, J. C.; Johnson, D. A. *J. Am. Chem. Soc.* **1952**, *74*, 4726; (c) Blake, J. *Int. J. Peptide Protein Res.* **1981**, *17*, 273; (d) Blake, J.; Li, C. H. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4055; (e) Tam, J. P.; Lu, Y. A.; Liu, C. F.; Shao, J. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 12485; (f) Liu, C.-F.; Rao, C.; Tam, J. P. *Tetrahedron Lett.* **1996**, *37*, 933; (g) Shangguan, N.; Katukojvala, S.; Greenberg, R.; Williams, L. J. *J. Am. Chem. Soc.* **2003**, *125*, 7754; (h) Zhu, X.; Pachamuthu, K.; Schmidt, R. R. *Org. Lett.* **2004**, *6*, 1083; (i) Crich, D.; Sana, K.; Guo, S. *Org. Lett.* **2007**, *9*, 4423; (j) Crich, D.; Sharma, I. *Angew. Chem., Int. Ed.* **2009**, *48*, 7591; (k) Yuan, Y.; Zhu, J.; Li, X.; Wu, X.; Danishefsky, S. J. *Tetrahedron Lett.* **2009**, *50*, 2329; (l) Rao, Y.; Li, X.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2009**, *131*, 12924; (m) Sasaki, K.; Crich, D. *Org. Lett.* **2010**, *12*, 3254; (n) Pan, J.; Devarie-Baez, N. O.; Xian, M. *Org. Lett.* **2011**, *13*, 1092; (o) Karmakar, P.; Talan, R. S.; Sucheck, S. J. *Org. Lett.* **2011**, *13*, 5298; (p) Wu, W.; Shang, Z.; Liebeskind, L. S. *J. Am. Chem. Soc.* **2011**, *133*, 14256; (q) Dyer, F. B.; Park, C.-M.; Joseph, R.; Garner, P. *J. Am. Chem. Soc.* **2011**, *133*, 20033; (r) Mali, S. M.; Jadhav, S. V.; Gopi, H. N. *Chem. Commun.* **2012**, 7085; (s) Chen, W.; Shao, J.; Hu, M.; Yu, W.; Giulianotti, M. A.; Houghten, R. A.; Yu, Y. *Chem. Sci.* **2013**, *4*, 970; (t) Mali, S. M.; Gopi, H. N. *J. Org. Chem.* **2014**, *79*, 2377.
2. Boc-SPPS: (a) Schwabacher, A. W.; Maynard, T. L. *Tetrahedron Lett.* **1993**, *34*, 1269; (b) Canne, L. E.; Walker, S. M.; Kent, S. B. H. *Tetrahedron Lett.* **1995**, *36*, 1217; (c) Gaertner, H.; Villain, M.; Botti, P.; Canne, L. *Tetrahedron Lett.* **2004**, *45*, 2239. Fmoc-SPPS; (d) Tan, X. H.; Zhang, X.; Yang, R.; Liu, C. F. *ChemBioChem* **2008**, *9*, 1052; (e) Zhang, X.; Lu, X.-W.; Liu, C.-F. *Tetrahedron Lett.* **2008**, *49*, 6122; (f) Crich, D.; Sana, K. *J. Org. Chem.* **2009**, *74*, 7383; (g) Shigenaga, A.; Sumikawa, Y.; Tsuda, S.; Sato, K.; Otaka, A. *Tetrahedron* **2010**, *66*, 3290; (h) Raz, R.; Rademann, J. *Org. Lett.* **2012**, *14*, 5038; (i) Pira, S. L.; Boll, E.; Melnyk, O. *Org. Lett.* **2013**, *15*, 5346.
3. Kinsland, C.; Taylor, S. V.; Kelleher, N. L.; McLafferty, F. W.; Begley, T. P. *Protein Sci.* **1839**, *1998*, 7.
4. (a) Wang, P.; Li, X.; Zhu, J.; Chen, J.; Yuan, Y.; Wu, X.; Danishefsky, S. J. *J. Am. Chem. Soc.* **2011**, *133*, 1597; (b) Joseph, R.; Dyer, F. B.; Garner, P. *Org. Lett.* **2013**, *15*, 732.
5. For other approaches to N-glycopeptides, see: Gamblin, D. P.; Scanlan, E. M.; Davis, B. G. *Chem. Rev.* **2009**, *109*, 131.
6. Cohen-Anisfeld, S. T.; Lansbury, P. T., Jr. *J. Am. Chem. Soc.* **1993**, *115*, 10531.
7. Subirós-Funosas, R.; El-Faham, A.; Albericio, F. *Tetrahedron* **2011**, *67*, 8595.
8. Fukuyama, T.; Tokuyama, H. *Aldrichim. Acta* **2004**, *37*, 87.
9. Raz, R.; Rademann, J. *Org. Lett.* **2011**, *13*, 1606.
10. Prepared according to known procedures starting from commercially available Fmoc-Asp(O^{t}Bu)-OH. See: (a) Hamzavi, R.; Dolle, F.; Tavitian, B.; Dahl, O.; Nielsen, P. E. *Bioconjug. Chem.* **2003**, *14*, 941; (b) Ge, J.; Li, L.; Yao, S. Q. *Chem. Commun.* **2011**, 10939.
11. Rohmer, K.; Keiper, O.; Mannuthodikayil, J.; Wittmann, V. In Proceedings of the 31st European Peptide Symposium; Lebl, M.; Meldal, M.; Jensen, K. J.; Hoeg-Jensen, T., Eds; European Peptide Society, 2010; pp 218–19.
12. We first discovered that silyl esters can act as carboxylate surrogates in peptide coupling reactions during the course of our polyoxin synthesis studies. See: Park, J.M., Ph.D. Dissertation, Case Western Reserve University, Cleveland, OH, 1990.
13. (a) Ullmann, V.; Rädisch, M.; Boos, I.; Freund, J.; Pöhner, C.; Schwarzinger, S.; Unverzagt, C. *Angew. Chem., Int. Ed.* **2012**, *51*, 11566; (b) Wang, P.; Aussedat, B.; Vohra, Y.; Danishefsky, S. J. *Angew. Chem., Int. Ed.* **2012**, *51*, 11571.
14. The pseudoproline benzyl esters and their corresponding carboxylic acids were prepared following known procedures. See: (a) Wöhr, T.; Mutter, M. *Tetrahedron Lett.* **1995**, *36*, 3847; (b) Clegg, J. K.; Cochrane, J. R.; Sayyadi, N.; Skropeta, D.; Turner, P.; Jolliffe, K. A. *Austr. J. Org. Chem.* **2009**, *62*, 711.
15. The standard TFA/Et_3SiH/H_2O cocktail gave a significant amount (up to 50%) of alkylated product (M+106) through a known linker decomposition pathway. Modified Reagent I suppresses this side reaction with the addition of DMB (dimethoxybenzene) to the cocktail. See: Stathopoulos, P.; Papas, S.; Tsikaris, V. *J. Pept. Sci.* **2006**, *12*, 227.