Chemical Ligation

DOI: 10.1002/ange.200903050

Triblock Peptide and Peptide Thioester Synthesis With Reactivity-Differentiated Sulfonamides and Peptidyl Thioacids**

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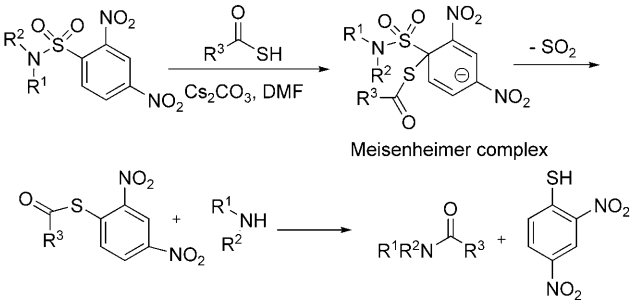
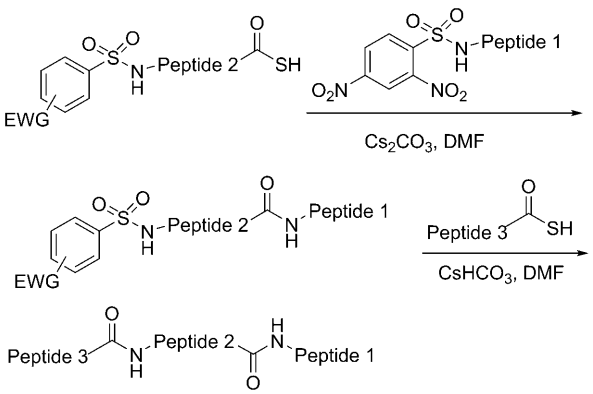
Dedicated to Professor A. Paul Schaap

With the construction of peptides by solid-phase peptide synthesis limited, for practical reasons, to chains of around fifty residues,[1] the development of methods for the assembly of peptide blocks into longer sequences is of importance. Kent's concept[2] of native chemical ligation was a major advance in this area, and has been considerably extended and optimized since its introduction in 1994.[1,3] A significant number of these improvements have addressed the development of methods for peptidyl thioester synthesis and the limitations posed by the mechanistic requirement of using an N-terminal 2-mercaptoethylamine, typically cysteine.[4] The most important modification, however, was the introduction, by Kent and co-workers,[5] of the thiazolidine group as a means of protection for N-terminal cysteine moieties. This approach is compatible with the native chemical ligation, and permits the block assembly of three or more peptides into a single entity. Our group has been engaged in the development of an alternative method of block synthesis for peptides in which a C-terminal peptidyl thioacid reacts with an electron-deficient N-terminal sulfonamide to yield a native amide bond.[5] The mechanism of this reaction, which is not limited to the use of any particular amino acid, involves nucleophilic aromatic substitution by the thiocarboxylate on the electron-deficient sulfonamide to give a highly reactive thioester and, after loss of sulfur dioxide, an amine leading ultimately to the amide product (Scheme 1). A variant on the method employs a free amine and an electron-deficient aryl halide, such as the Sanger or Mukaiyama reagents, as the condensing agent in place of the sulfonamide.[7,8]



Scheme 1. Amide-forming reaction.



Scheme 2. Triblock peptide synthesis.

To convert this method into one capable of enabling the controlled coupling of three blocks into a single segment with minimal protecting-group manipulation we required a set of two sulfonamides with differential reactivity toward thiocarboxylates (Scheme 2).

A series of *N*-sulfonylphenylalanine derivatives was therefore prepared (see Supporting Information) and screened for reactivity toward thioacetic acid under a standard set of conditions related to those used in our peptide synthesis (Table 1).

Under the conditions employed, a single electron-withdrawing group was found to be insufficient to induce reaction, as was the presence of two trifluoromethyl groups in the 3- and 5-positions. However, 2,4-disubstituted systems in which a single nitro group was complemented by a second, but less potent electron-withdrawing group functioned well (Table 1). Nevertheless, all three such systems investigated (denoted as ENS, CNS, and FNS) proved significantly less reactive than the 2,4-dinitrobenzenesulfonamide (DNS) employed originally, and therefore met our reactivity criteria.

A further series of experiments with more elaborate thioesters revealed the reactivity of both the CNS and ENS sulfonamides to be adequate for coupling with primary thioacids but not with electron-deficient or more hindered

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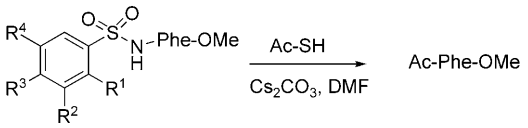
[**] We thank the NIH (GM62160) for partial support of this work and Albert A. Bowers for preliminary experiments with heteroaryl sulfonamide systems.

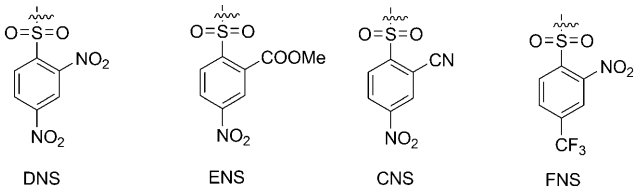
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200903050.

Table 1: Reactivity of thioacetic acid towards *N*-sulfonylphenylalanine derivatives.[a]

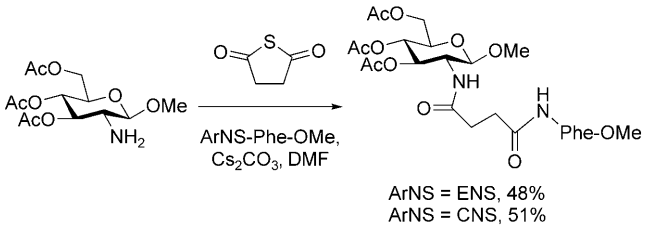


Entry	R^1	R^2	R^3	R^4	*t* [h]	Yield [%]
1	H	H	Ac	H	12	–
2	H	H	CN	H	12	–
3	CF_3	H	H	H	12	–
4	NO_2	H	H	H	12	–
5	H	CF_3	H	CF_3	12	–
6	COOMe	H	NO_2	H	6	85
7	CN	H	NO_2	H	6	87
8	NO_2	H	CF_3	H	4	88
9	NO_2	H	NO_2	H	0.07[b]	94

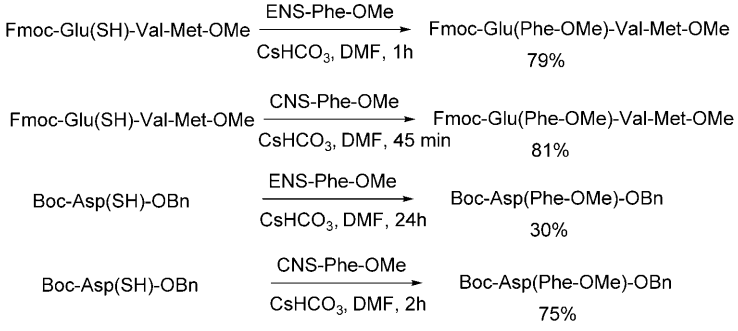
[a] All reactions employed 0.15 M sulfonamide in DMF with thioacetic acid (1.5 equiv), and Cs_2CO_3 (1.5 equiv) as base (See Supporting Information). [b] 4 min.



ones. Thus, while both the CNS and ENS class of sulfonamides were amenable to reaction with primary thioacids (Schemes 3 and 4) they either failed to react or reacted only very slowly with peptide-based thioacids such as Alloc-Gly-Gly-SH.



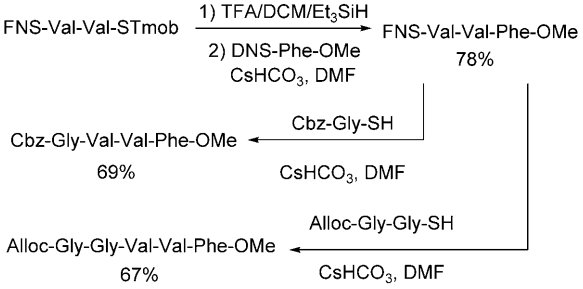
Scheme 3. Three-component coupling.



Scheme 4. Reaction with glutamic and aspartic acid side chain thioacids.

This overall reactivity pattern was sufficient to enable a first series of triply convergent reactions in which an amino acid or peptide, protected at the N-terminus by an ENS or CNS group and carrying a 2,4,6-trimethoxybenzyl[9] (Tmob) thioester at the C-terminus, was first treated with triethylsilane and trifluoroacetic acid to release the C-terminal thioacid before exposure to a DNS-protected peptide and a mild base (Table 2). This first coupling resulted in the formation of a new peptide bearing the ENS or CNS group at the N-terminus ready for a final coupling with a thioacid, albeit necessarily a primary one (Table 2). This critical series of experiments established the feasibility of generation of a thioacid in the presence of a moderately electron-deficient sulfonamide and the ability of that thioacid to undergo subsequent and selective condensation with the more reactive DNS class of sulfonamides. The reaction sequence was applied successfully to the synthesis of simple di- and tripeptides (Table 2) and to the synthesis of model octapeptides (Table 2, entries 5 and 6). Finally, the sequence was shown to be amenable to the use of aqueous buffered media, rather than DMF as solvent, with little loss of yield as is clear from a comparison of entries 4 and 7 of Table 2.[10]

Further investigation revealed the FNS group to be somewhat more reactive than the CNS and ENS groups toward the less reactive α-amino-derived thiocarboxylates as illustrated by the examples in Scheme 5. A critical point in the use of the FNS group in this manner, however, was the switch from cesium carbonate to cesium hydrogencarbonate at the level of the first coupling reaction.[11]

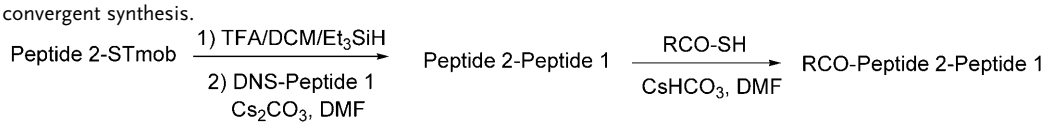


Scheme 5. Tricomponent couplings employing the FNS group. TFA = trifluoroacetic acid, DCM = dichloromethane.

The block synthesis strategy that we present here is complementary to the methods developed by Kent based on native chemical ligation. However, for maximum flexibility in approaching future targets, the ability to incorporate both approaches into a single scheme is desirable. For this, the compatibility of thioesters with our thiocarboxylate–sulfonamide coupling approach is required. The triblock synthesis set out in Scheme 6, in which a fluorenylmethyl (Fm)[6a] thioester is carried through two coupling steps, nicely illustrates that such is the case.

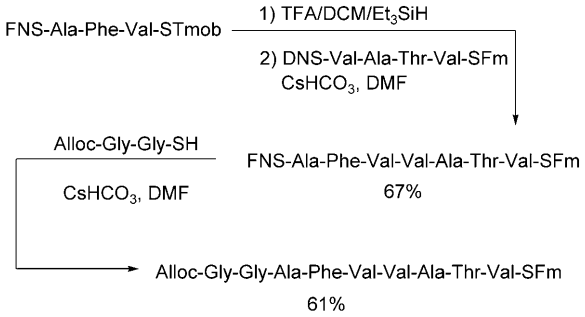
In addition to the "right to left" strategy for block peptide synthesis set out above, with its necessary reliance on the use of a series of sulfonamides of decreasing reactivity, we have briefly investigated an

Table 2: Triply convergent synthesis.



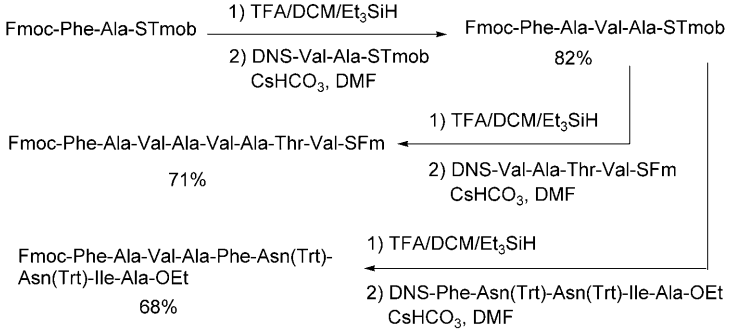
Entry	Peptide 2-STmob	DNS-Peptide 1	Peptide 2-Peptide 1 (yield [%])	RCO-SH	RCO-Peptide 2-Peptide 1 (yield [%])
1	ENS-Trp-STmob	DNS-Phe-OMe	ENS-Trp-Phe-OMe	Ac-SH	Ac-Trp-Phe-OMe (60)
2	CNS-Phe-STmob	DNS-Phe-OMe	CNS-Phe-Phe-OMe (84)	Boc-Glu(SH)-O*t*Bu	Boc-Glu(Phe-Phe-OMe)-O*t*Bu (81)
3	CNS-Phe-STmob	DNS-Phe-OMe	CNS-Phe-Phe-OMe (84)	Boc-Asp(SH)-OBn	Boc-Asp(Phe-Phe-OMe)-OBn (78)
4	ENS-Ala-Phe-STmob	DNS-Phe-OMe	ENS-Ala-Phe-Phe-OMe (81)	Boc-Glu(SH)-O*t*Bu	Boc-Glu(Ala-Phe-Phe-OMe)-O*t*Bu (83)
5	ENS-Ala-Phe-STmob	DNS-Val-Met-Val-Pro-Ala-OEt	ENS-Ala-Phe-Val-Met-Val-Pro-Ala-OEt (81)	Boc-Glu(SH)-O*t*Bu	Boc-Glu(Ala-Phe-Val-Met-Val-Pro-Ala-OEt)-O*t*Bu (71)
6	ENS-Ala-Val-STmob	DNS-Val-Met-Val-Pro-Ala-OEt	ENS-Ala-Val-Val-Met-Val-Pro-Ala-OEt (78)	Boc-Glu(SH)-O*t*Bu	Boc-Glu(Ala-Val-Val-Met-Val-Pro-Ala-OEt)-O*t*Bu (65)
7[a]	ENS-Ala-Phe-STmob	DNS-Phe-OMe	ENS-Ala-Phe-Phe-OMe (74)	Boc-Glu(SH)-O*t*Bu	Boc-Glu(Ala-Phe-Phe-OMe)-O*t*Bu (80)

[a] Both coupling steps in this example were conducted in an aqueous buffer: 4:1 v/v NMP: 6 M Gn·HCl, 1 M HEPES, pH ≈ 8, NMP = *N*-Methylpyrrolidone; Gn = guanidine.



Scheme 6. Compatibility of the thioesters with the thioacid–sulfonamide block synthesis.

alternative "left to right" strategy. This approach enables the more reactive DNS sulfonamide to be employed in all coupling steps but requires the compatibility of the Tmob thioester function with the sulfonamide coupling reaction. The triblock syntheses set out in Scheme 7 show such a sequence and thereby establish the compatibility of the Tmob thioester. Again with a view to potential interconnection with a native chemical ligation strategy, one of the examples is terminated by the incorporation of a further thioester.



Scheme 7. "Left to right" strategy showing compatibility with thioesters.

Overall, we present a combination of versatile new methods for the block synthesis of peptides based on the reaction of thioacids with electron-deficient sulfonamides. The assembly of the various blocks may be conducted in a "right to left" or "left to right" manner and may be arranged in such a way as to provide a peptide thioester ready for incorporation in a native chemical ligation sequence.

Received: June 6, 2009
Revised: August 11, 2009
Published online: September 8, 2009

Keywords: chemical ligation · nucleophilic aromatic substitution · peptide synthesis · thioacids · thioesters

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[10] Within the limits of our NMR and UPLC detection methods the couplings reported in this manuscript proceed without racemization. In preliminary studies on the thioacid–sulfonamide method, and on the related thioacid–amine–Sangers/Mukaiyama approach, this was rigorously established through the synthesis of authentic samples of epimeric products.[6a,7]
[11] With cesium carbonate the N-FNS protected di- and higher peptide thioacids were observed by mass spectrometry to undergo cyclization, with retention of the FNS group, rather than condensation when exposed to a DNS peptide and cesium carbonate. This was attributed to the acidity of the FNS sulfonamide NH group and so was circumvented by the use of the milder base. The use of pyridine as base afforded a similar result to cesium carbonate.

