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Triblock Peptide and Peptide Thioester Synthesis by the Use of Reactivity-Differentiated Sulfonamides and Peptidyl Thioacids

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As is the norm for a transformational new paradigm, Kent's concept[1] of native chemical ligation enabling the block synthesis of peptides has been considerably extended and optimized since its introduction in 1994.[2] 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 a *N*-terminal 2-mercaptoethylamine, typically cysteine.[3] Perhaps the most important modification, however, was the introduction by Kent and co-workers[4] of the thiazolidine group as a means of protection for *N*-terminal cysteine moieties compatible with the native chemical ligation itself, and permitting the ligation of three or more peptides into a single entity. Our laboratory 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.[6]

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 were therefore prepared (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).

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Dedicated to Professor A. Paul Schaap

Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.

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 nicely (Table 1). Nevertheless, all three such systems investigated proved significantly less reactive than the 2,4-dinitrobenzenesulfonamide 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 ones. Thus, while both the CNS and ENS class of sulphonamides 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-SH.

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[7] (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, entry 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.[8]

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 bicarbonate at the level of the first coupling reaction.[9]

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, harmonious 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)[5a] 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 alternative left to right strategy. This approach enables the more reactive DNS sulphonamide to be employed in all coupling steps but requires the compatibility of the Tmob thioester function with the sulphonamide 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.

Overall, we present a combination of powerful 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.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. a) Dawson PE, Muir TW, Clark-Lewis I, Kent SBH. *Science*. 1994; 266:776–779. [PubMed: 7973629] b) Tam JP, Lu Y-A, Liu CF, Shao J. *Proc Natl Acad Sci, USA*. 1995; 92:12485–12489. [PubMed: 8618926] c) Dawson PE, Kent SBH. *Ann Rev Biochem*. 2000; 69:923–960. [PubMed: 10966479]
2. a) Kent SBH. *Chem Soc Rev*. 2009; 38:338–351. [PubMed: 19169452] b) Hackenberger CPR, Schwarzer D. *Angew Chem Int Ed*. 2008; 47:10030–10078. c) Bode JW. *Curr Op Drug Disc Dev*. 2006; 9:765–775. d) Macmillan D. *Angew Chem Int Ed*. 2006; 45:7668–7672.
3. a) Crich D, Banerjee A. *J Am Chem Soc*. 2007; 129:10064–10065. [PubMed: 17658806] b) Botti, P.; Tchertchian, S. *WO 2006/133962*. 2006. c) Okamoto R, Kajihara Y. *Angew Chem Int Ed*. 2008; 47:5402–5406. d) Quaderer R, Hilvert D. *D Chem Commun*. 2002:2620–2621. e) Hondal RJ, Nilsson BL, Raines RT. *J Am Chem Soc*. 2001; 123:5140–5141. [PubMed: 11457362] f) Haase C, Rohde H, Seitz O. *Angew Chem Int Ed*. 2008; 47:6807–6810. g) Payne RJ, Ficht S, Greenberg WA, Wong CH. *Angew Chem Int Ed*. 2008; 47:4411–4415.
4. a) Bang D, Kent SBH. *Angew Chem Int Ed*. 2004; 43:2534–2538. b) Pentelute BL, Gates ZP, Dashnau JL, Vanderkooi JM, Kent SBH. *J Am Chem Soc*. 2008; 130:9702–9707. [PubMed: 18598026]
5. a) Crich D, Sana K, Guo S. *Org Lett*. 2007; 9:4423–4426. [PubMed: 17900128] b) Crich D, Bowers AA. *Org Lett*. 2007; 9:5323–5325. [PubMed: 17979281] c) Crich D, Sasaki K, Rahaman MdY, Bowers AA. *J Org Chem*. 2009; 74:3886–3893. [PubMed: 19385609]
6. Crich D, Sharma I. *Angew Chem Int Ed*. 2009; 48:2355–2358.
7. Vetter S. *Synth Commun*. 1998; 28:3219–3223.
8. 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 companion thioacid-amine-Sangers/Mukaiyama approach, this was rigorously established through the synthesis of authentic samples of epimeric products.[5a,6]
9. 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 the same result as cesium carbonate.

Scheme 1.
Amide Forming Reaction

Scheme 2.
One Pot Triblock Peptide Synthesis

Scheme 3.
Three Component Coupling

Scheme 4.

Reaction with Glutamic and Aspartic Acid Side Chain Thioacids

Scheme 5.
Tricomponent Couplings Employing the FNS Group

Scheme 6.
Comatibility of the Thioesters with the Thioacid-Sulfonamide Block Synthesis

Scheme 7.

Left to Right Strategy Showing Compatibility with Thioesters

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

^[a] All reactions employed 0.15 M sulfonamide in DMF with thioacetic acid (1.5 equiv), and Cs₂CO₃ (1.5 equiv) as base (See supporting information).

Table 2

Triply convergent synthesis:

Entry	Peptide 2-STmob	DNS-Peptide 1	Peptide 2-Peptide 1 (Yield, %)	Peptide 3-SH	Peptide 3-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)-OtBu	Boc-Glu(Phe-Phe-OMe)-OtBu (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)-OtBu	Boc-Glu(Ala-Phe-Phe-OMe)-OtBu (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)-OtBu	Boc-Glu(Ala-Phe-Val-Met-Val-Pro-Ala-OEt)-OtBu (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)-OtBu	Boc-Glu(Ala-Val-Val-Met-Val-Pro-Ala-OEt)-OtBu (65)
γ [a]	ENS-Ala-Phe-STmob	DNS-Phe-OMe	ENS-Ala-Phe-Phe-OMe (74)	Boc-Glu(SH)-OtBu	Boc-Glu(Ala-Phe-Phe-OMe)-OtBu (80)

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