Fmoc Chemistry Compatible Methods for Thio-Ligation Assembly of Proteins.

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Discussion

Two methods are compared that enable the assembly of proteins from peptide thioester fragments prepared by Fmoc chemistry-mediated solid phase synthesis. The first, which utilizes *iso*-thiouronium salts [1], allows formation of thiophenyl esters directly from partially protected peptides, either in solution or on resin. The chloride salt, named CTTU, reacts far more rapidly than does the variant bearing a non-nucleophilic counterion, TTTU. This observation suggests active participation of the chloride ion during thiophenyl ester formation (Figure 1), and that use of CTTU may give rise to significant levels of racemization. Solution and on resin derivatization have been shown to be satisfactory and equivalent methods in the synthesis of SDF-1β 47-64 (Figure 2).

Recent publications [2,3] have demonstrated straightforward application of the Backes/Ellman safety-catch method to ligation assembly of several complex peptides. Our initial studies with this procedure gave very low to no yield of target peptides. Rather than continue with the SDF sequence our evaluations switched to a variant of a difficult test sequence, tBoc-Gly-Tyr(tBu)-Leu-Phe-Glu(OtBu)-Val-Asn(Trt), which still maintained a C-terminal Asn(Trt).

Results

Application of the simultaneous comparative approach yielded the following results:

- Activation by methylation is effective, iodoacetonitrile is not;
- Thiolytic cleavage only occurs in the presence of sodium thiophenoxide;
- Thiolytic cleavage with mercaptopropionic acid ethyl ester is safest, a significant side-reaction occurs with benzylmercaptan and thiophenol due to thioester migration during base treatment (the biproducts from all side reactions are identified in Figure 3);
- The Kenner safety catch linker is as effective as the sulfamoylbutyrl linker of Backes/Ellman;
- PS and NovaGel supports are equally effective.

Conclusions

- CTTU is an effective, readily prepared and non-noxious reagent which can form thiophenyl esters on resin or in solution. Its use may result in significant levels of racemization.
- Safety catch approaches are subject to steric effects at the C-terminus, and may require catalysis with sodium thiophenoxide. Thiolysis is best performed with mercaptopropionic acid ethyl ester, use of which avoids a thioester migration side-reaction.

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[1] Hudson and Thompson, US Patent allowed 2001.

[2] Ingenito, R., Bianchi, E., Fattori, D., Pessi, A. *J. Am. Chem. Soc.* **121** 11369 (1999).

[3] Shin, Y., Winans, K.A., Backes, B.J., Kent, S.B.H., Ellman, J.A., Bertozzii, C.R. *J. Am. Chem. Soc.* **121** 11684 (1999).

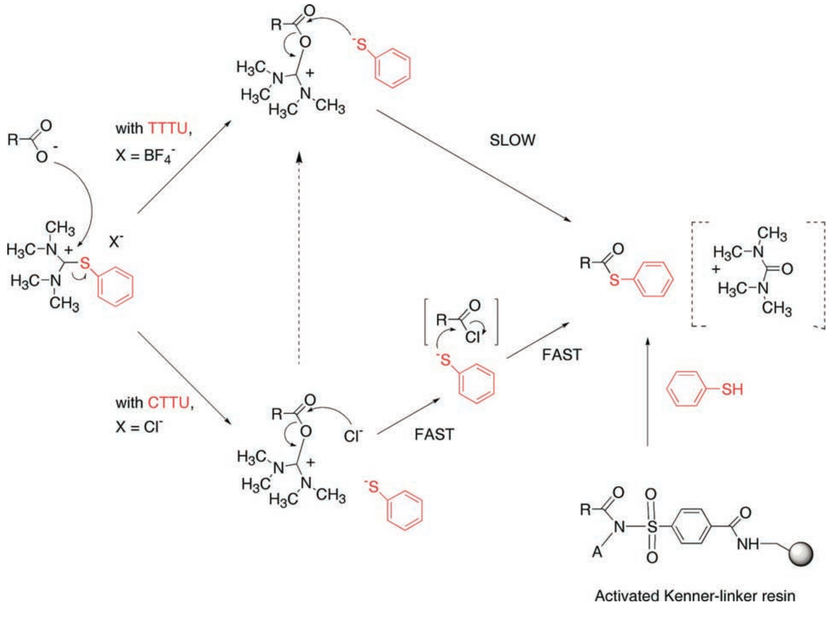

Figure 1. Schematic representation of the formation of thiophenyl esters via the use of isothiouronium salts (suggested mechanism), and using an activated Kenner sulfonamide resin; **R** represents a protected peptide chain, and **A** either a methyl or cyanomethyl group.

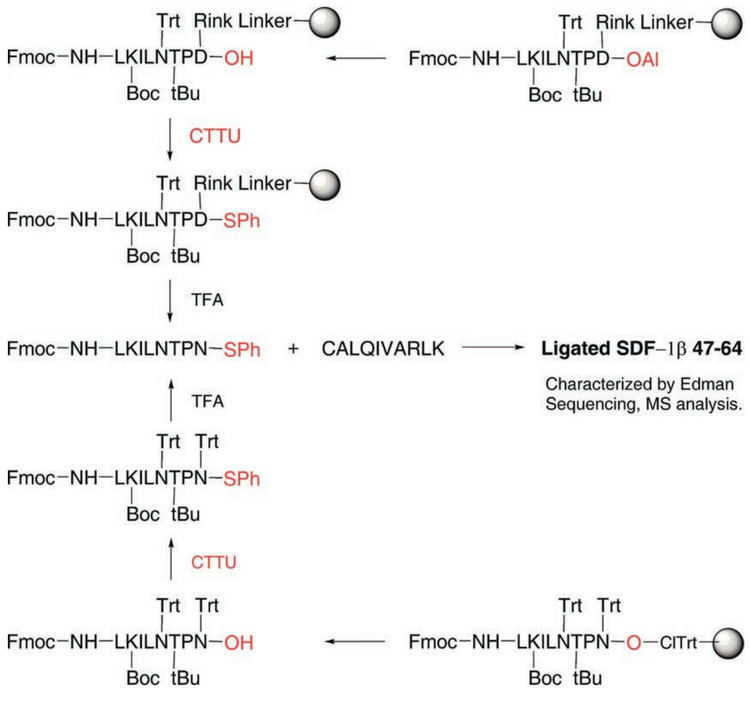

Figure 2. Schematic representation of the synthesis of SDF-1β 47-64 via on resin (top path) and in solution (bottom path) formation of thio ester fragments.

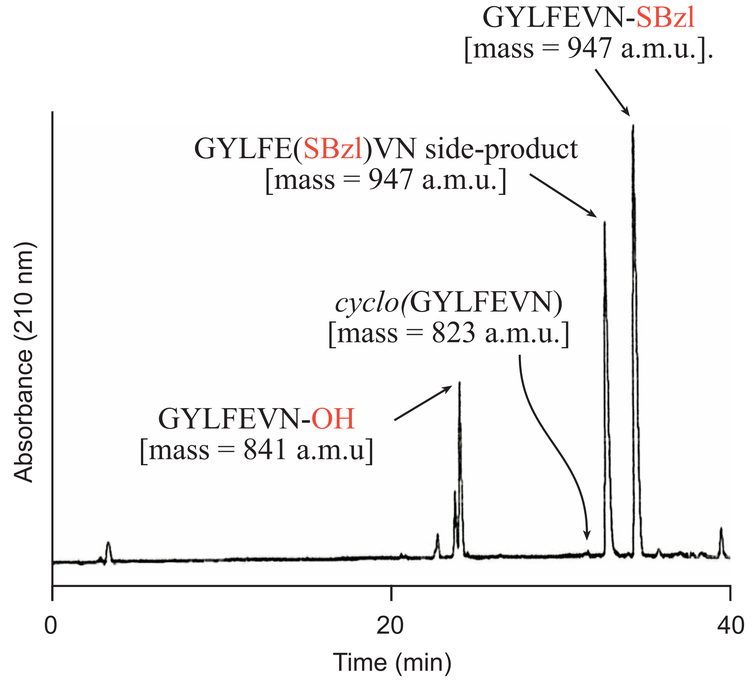

Figure 3. Analytical HPLC profile of GYLFEVN thiobenzyl ester prepared on 4-sulfamoylbutyryl-aminomethyl polystyrene identifying 4 components isolated by prep. HPLC.