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TRIALKYLSILANES AS SCAVENGERS FOR THE TRIFLUOROACETIC ACID DEBLOCKING OF PROTECTING GROUPS IN PEPTIDE SYNTHESIS

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Abstract: Triethylsilane was used as a scavenging agent in peptide synthesis for the removal of protecting groups with TFA. The efficiency of scavenging ability was compared with anisole and ethanedithiol in a kinetic experiment. It was also found that triethylsilane/TFA reduces the indole ring of tryptophan.

Trialkylsilanes have been known for some time to be mild reducing agents when used in combination with trifluoroacetic acid as a solvent system.[1] However, we have found little mention of their use as scavengers[2] with no mention of efficacy or limitations. We wish to report the use of triethylsilane and triisopropylsilane as carbocation scavengers in the acidic deblocking of protecting groups in peptide synthesis. We have also compared the efficiency of their ability to scavenge the t-butyl cation with the scavengers ethanedithiol and anisole.

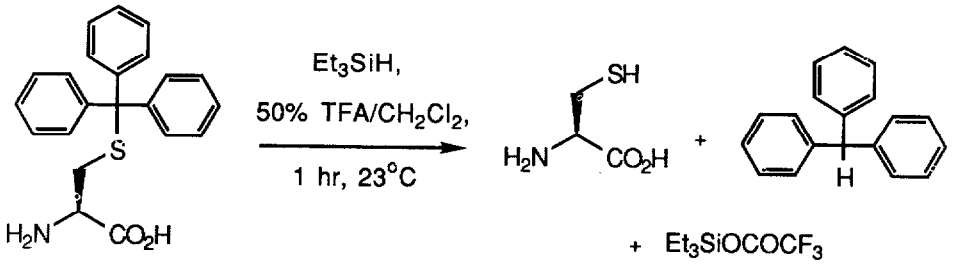
We first became interested in using trialkylsilanes as scavengers when attempting to synthesize peptides containing a high percentage of cysteines (up to 30% by amino acid composition) for use as heavy metal chelators. When the trityl group was used for sulfhydryl protection, most of the common scavengers we tried gave routinely poor results. Reverse-phase HPLC indicated that the crude peptides produced were contaminated with high levels of partially deprotected peptide. Even when the extremely efficient scavenger ethanedithiol was used, pronounced amounts of these impurities were in evidence. Discouraged by these results, an investigation into the use of silanes as scavengers was initiated.

Trialkylsilanes under the appropriate acidic conditions can be considered hydride donors in an ionic hydrogenation (Scheme 1). The side products formed contain a hydrogen substituted where the functional group was attached to the protecting group. The reaction is irreversible and confers the advantage that once the protecting group is deblocked and scavenged, it can not react again with the peptide. This is especially important if there are on the peptide a number of functional groups in proximity to each other which can also react with carbocations. If this is the case, the residence time of a protecting group on the peptide would be expected to increase dramatically as intramolecular interactions play a more prominent role. In this instance the use of an irreversible scavenger can be extremely important as will be seen later in the deprotection of high-cysteine content peptides. Although not looked for in the course of this investigation, it is presumed that triethysilyl trifluoroacetate is also formed and later hydrolysed to triethylsilanol in an aqueous workup.

Further advantages in the use of trialkylsilanes as scavengers are that they are volatile compounds (Et_3SiH boiling point = 108°) and the side products formed are either volatile enough

or non-polar enough to be easily removed from the desired peptide obtained from the deprotection.

Scheme 1



Kemp, et. al.[3], studied carbocation scavengers in the deblocking of N-α-biphenyloxycarbonyl (Bpoc) amino acids with 0.5% TFA/methylene chloride. It was found that even with the most efficient scavenger studied, benzyl mercaptan, a reservoir of 2-(p-biphenylyl)-propene (5% with 10 equivalents thiol) existed in solution. This indicates incomplete trapping of the intermediate carbocation. When we studied the deblocking of N-α-Bpoc glycine under similar conditions (0.002M amino acid, 0.010M triethylsilane, 0.5% TFA/dichloromethane) in an NMR experiment, we saw no evidence of 2-(biphenylyl)-propene and instead saw only 2-(p-biphenylyl)-propane produced as a result of the carbocation scavenging.

It has been reported previously that trialkylsilanes in acidic media reduce indoles[4]. We therefore checked the sensitivity of tryptophan reduction to triethylsilane and triisopropylsilane in 90% TFA/methylene chloride. Trytophan was rapidly reduced with triethlsilane (5 equiv.) such that after 2 hours at 23°, NMR studies indicated 85% reduction. However, this reduction was markedly slowed by using triisopropylsilane as the scavenger. Using the same conditions as those above,there was only 4% reduction in evidence. It should be mentioned that the use of protected tryptophan derivatives that might retard the decomposition of indole were not looked into. When histidine and cystine were subjected to the conditions above, there were no products of reduction seen.

Kinetic experiments were performed to compare triethylsilane and triisopropylsilane with the two commonly used scavengers anisole and ethanedithiol. The same protocol as set forth by Lundt, et. al.[5] was used in determining rate constants. A 0.2M solution of t-butyl trifluoroacetate in deuteromethylene chloride was prepared and to 0.50 ml of this was added 0.1 mmol of trialkylsilane followed by the addition of 0.50 ml of trifluoroacetic acid. The reaction was followed by NMR at 20° and the gradual decrease of the singlet at 1.50 ppm corresponding to the t-butyl ester of t-butyl trifluoroacetate and the increase of the doublet centered at 0.87 ppm corresponding to 2-methylpropane were monitored by integration. The second-order rate constant was calculated from the following formula and the results tabulated below.

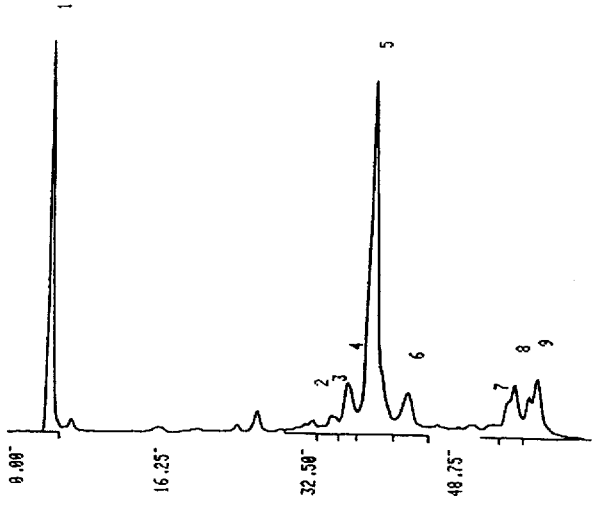
	k_2 (M^{-1} min^{-1})
ethanedithiol	4.9×10^{-2}
triethylsilane	6.1×10^{-3}
anisole	4.2×10^{-3}
triisopropylsilane	2.3×10^{-3}

$$k_2 = \frac{\left(\frac{1}{a-x} - \frac{1}{a}\right)}{t}$$

a = initial conc. (mol/l)
(a-x) = conc. at time t (min)
k_2 = second order rate constant

Finally, the peptide H-CysGlyGluCysGlyGluGlyGlyCysGlyGluCysGly-OH (GEC-4) was synthesized on a R[a]MPS peptide synthesizer by standard stepwise solid-phase synthesis methodology starting from glycine supported on Wang resin and utilizing the Fmoc protecting group for N-terminal amine protection. The glumatic acid side chains were protected as t-butyl esters and the cysteine side chains were protected as S-trityls. The cleavage/deprotection was performed in neat trifluoroacetic acid for 1 hour with 2 equivalents of triethylsilane per protecting group used as the scavenger. At the end of this time the TFA and other volatiles were removed under a stream of nitrogen and the residue taken up in water and washed with ether. Recoveries were high and, as seen from the reverse-phase HPLC shown below, the crude product (product is peak 5) was relatively pure. This example is typical of other high cysteine-content peptides we have synthesized by this method. The peaks with a retention time of 55 to 60 minutes are side products resulting from partial detritylation. These peaks were much more pronounced when other scavengers (even thiol containing scavengers) were used.

flow rate = 1.0 ml/min
A = 0.1% TFA (aq), B = 0.1% TFA/90% CH_3CN (aq)
0-5 min 0% B; 5-45 min 0-15% B; 45-60 min 15% B; 60-70 min 50% B



Crude GEC-4 after deprotection and cleavage from resin

In conclusion, for the deprotection of high cysteine-content peptides, triethylsilane proved to be a better scavenger than ethanedithiol even though the rate constant of the latter is approximately an order of magnitude higher. This is probably due to the irreversibility of the scavenging reaction when triethylsilane is used. Care should be taken when using this system in the presence of tryptophan containing peptides. The use of the more hindered triisopropylsilane or careful titration of carbocations with triethylsilane is warranted.

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