Mechanisms and prevention of trifluoroacetylation in solid-phase peptide synthesis

(termination/resin-bound functionalities/intersite reactions/phenylacetamidomethyl-resin)

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Contributed by Bruce Merrifield, February 14, 1979

ABSTRACT A novel mechanism for trifluoroacetylation in solid-phase peptide synthesis, independent of the coupling step, has been elucidated. It involves the presence of trifluoroacetoxyethyl methoxymethyl groups on the resin support, which react with resin-bound amines by an intersite nucleophilic reaction. The trifluoroacetoxyethyl groups are generated from preexisting hydroxymethyl sites during treatment with trifluoroacetic acid in dichloromethane or by acidolysis of the benzyl ester bond between the peptide and the resin. The transfer of trifluoroacetyl from hydroxyl amine occurs during the subsequent neutralization with tertiary amine. The mechanism was first elucidated by model studies with aminomethyl-resins. Then the expected transfer of trifluoroacetyl groups from trifluoroacetoxyethyl-resin to the α-amino group of N’-benzoylcarbonyllysyl benzyl ester in solution was demonstrated; k2, 6 x 10^{-4} M^{-1} sec^{-1}. Lysine-resins were used to examine the extent of trifluoroacetylation under the conditions of solid-phase peptide synthesis. After a series of acid/base cycles simulating synthetic conditions but without coupling, the poorly nucleophilic α-amino group was approximately 1-2% trifluoroacetylated per cycle when attached to resins already containing hydroxymethyl groups. Standard benzyl ester resins without preexisting hydroxymethyl groups gave comparable levels of trifluoroacetylation after the first few synthetic cycles because of gradual acid cleavage of the ester and accumulation of trifluoroacetoxyethyl sites. Peptide chain termination resulting from trifluoroacetylation by this mechanism could be prevented (<0.02% per cycle) by the use of aminomethyl-4(oxymethyl)-phenylacetamidomethyl-resin support, which can be synthesized free from extraneous functionalities and which is stable to trifluoroacetic acid under the conditions of solid-phase peptide synthesis.

The majority of reported solid-phase peptide syntheses (1-3) have involved the stepwise addition of tert-butoxycarbonyl (Boc) amino acids to a peptide chain that is anchored to the polymeric support through the COOH-terminal amino acid. During the assembly of the protected peptide chain, the Boc group is removed at each step under conditions that leave other protecting groups unaffected, most commonly by the use of 50% (vol/vol) trifluoroacetic acid in dichloromethane for 15-30 min. Cleavage of the α-amino group of the growing peptide chain, to give N’-trifluoroacetyl peptides, has been reported on several occasions (4-7). This potentially general side reaction has serious consequences because the N’-trifluoroacetyl peptides formed are stable to the conditions of solid-phase peptide synthesis, thus lowering the overall yield of the desired product and complicating its purification from the accompanying terminated peptides. Assumed explanations for trifluoroacetylation include physical carryover of trifluoroacetic acid vapors into the coupling step (6), incomplete neutralization (4), the presence of trifluoroacetic acid salts of quaternary sites on the resin (8), and physical entrapment of trifluoroacetic acid in components of the apparatus or within the polystyrene beads used as the support for peptide synthesis (9).

These proposals all assume that trifluoroacetic acid is activated by dicyclohexylcarbodiimide in the coupling step and subsequently reacts with the α-amino group. This has been reported to occur in solution, where it was found that use of coupling conditions that minimized activation of trifluoroacetic acid substantially decreased trifluoroacetylation (10). A similar approach has been used in solid-phase peptide synthesis with use of preformed Boc-amino acid symmetric anhydrides for the coupling reaction (6). This has decreased, but not entirely eliminated, trifluoroacetylation (7). Up to now it has been thought that this is a general side reaction which usually occurs at a low level, although occasionally to a more serious extent, and that no method exists for preventing it.

Preliminary results in this laboratory showed that trifluoroacetylation also occurred independently of the coupling step. An N’-benzoylcarbonyllysine-resin (Lys(Z)-resin) that had been subjected to repeated alternate treatments with trifluoroacetic acid and tertiary amine showed a low (<1%) but significant level of N’-trifluoroacetylsine after HF cleavage from the resin. Similar experiments using N’-Z-Lys-resin gave about 10% N’-trifluoroacetylation. These totally unexpected observations were inconsistent with all explanations previously proposed and suggested that there must exist an additional, radically different, explanation for trifluoroacetylation.

In this paper, we report our investigations of trifluoroacetylation that have led to the elucidation of this novel mechanism. We discuss the levels of occurrence of trifluoroacetylation under various conditions and show that it can readily be prevented in solid-phase peptide synthesis.

EXPERIMENTAL

Trifluoroacetic acid was obtained from Halocarbon and used without further purification. Dichloromethane (ACS reagent grade) was obtained from Eastman and distilled from sodium carbonate before use. Other chemicals were reagent grade. The resins used were copoly(styrene-1% divinylbenzene) beads, 200-400 mesh. Chloromethyl-resins were from Pierce, Lab Systems, and Bio-Rad; unsubstituted resin was Bio-Beads S-X1 from Bio-Rad. All substitutions are given as mmol/g of unsubstituted polystyrene resin. Infrared (IR) spectra of resins were taken on KBr pellets.

Aminomethyl-resin was synthesized from unsubstituted resin by direct amidolkylation followed by hydrazinolysis (11) or from chloromethyl-resin by a Gabriel synthesis (12). Hydromethyl-resin was made from chloromethyl-resin by reaction with acetate followed by transesterification (13). Hy-

Abbreviations: iPr₂EtN, N,N-diisopropylethylamine; IR, infrared; Lys(Z), N’-(benzoylcarbonyl)lysine; OCH₂Pam, 4(oxymethyl)-phenylacetamidomethyl; Boc, tert-butoxycarbonyl; DCC, dicyclohexylcarbodiimide; DMF, N,N-dimethylformamide.
droxymethyl-resin was converted to the trifluoroacetoxy-
methyl-resin by reaction with the p-nitrophenyl ester of tri-
fluoroacetic acid in N,N-dimethylformamide in the presence of
N,N-diisopropylethylamine (iPr2EtN). This procedure
was used to determine hydroxymethyl sites on the resin: The
intensity of the 1785 cm$^{-1}$ band relative to the 1601 cm$^{-1}$
band of polystyrene divided by an empirical factor of 2.2 gave the
substitution in mmol/g. The detection limit was <0.02 mmol/g.
Hydroxymethyl-resin was converted to acetoxyethyl-resin
by reaction with acetic anhydride in pyridine and to benzoyl-
oxymethyl-resin by reaction with benzoyl chloride and pyri-
dine in CH$_2$Cl$_2$ (14). Levels of free amine on the resin were
determined by titration with picric acid (15) or imidazolium
picrate (16).

Boc-Lys(Z)-OCH$_2$-resin was prepared from aminomethyl-resin
by the cesium salt procedure (8). Boc-Lys(Z)-4-(oxyethyl)-
phenylacetamidomethyl-resin [Boc-Lys(Z)-4-OCH$_2$-Pam-resin]
(12) was prepared from aminomethyl-resin and preformed
Boc-Lys(Z)-OCH$_2$-phenylacetic acid by dicyclohexylcarbo-
diimide coupling (17). A simulated synthetic cycle without
coupling consisted of: CH$_2$Cl$_2$, three times, 1 min each; 50% (vol/vol)
trifluoroacetic acid/CH$_2$Cl$_2$, 1 min; 50% trifluoro-
acetic acid/CH$_2$Cl$_2$, 30 min; CH$_2$Cl$_2$, six times, 1 min each;
5% (vol/vol) iPr$_2$EtN/CH$_2$Cl$_2$, 5 min; CH$_2$Cl$_2$, three times, 1
min each; 5% iPr$_2$EtN/CH$_2$Cl$_2$, 5 min; and CH$_2$Cl$_2$, three
times, 1 min each. All volumes were 20 ml/g of starting resin.
To exchange the salt to the hydrochloride, the trifluoroacetic
acid step was followed by: CH$_2$Cl$_2$, six times, 1 min each; 0.13 M
tetraethylammonium chloride in CH$_2$Cl$_2$, 2 min; CH$_2$Cl$_2$,
three times, 1 min each; 0.13 M tetraethylammonium chloride
in CH$_2$Cl$_2$, 2 min; CH$_2$Cl$_2$, three times, 1 min each; then
the first iPr$_2$EtN, as above. To couple, the simulated synthetic cycle
was followed by: 4 equivalents of Boc-amino acid in CH$_2$Cl$_2$, 30
min (do not filter); 4 equivalents of DCC in CH$_2$Cl$_2$, 0.5-2 hr;
CH$_2$Cl$_2$, three times, 1 min each.

Cleavage from the resin was performed in anhydrous
HF/10% (vol/vol) anisole for 1 hr at room temperature (only
for these model studies). N$^\alpha$-Trifluoroacetyllysine was unaffected
by these conditions. Amino acid analysis on a Beckman
121 analyzer gave N$^\alpha$-trifluoroacetyllysine at the position of
leucine, with 22% of the color yield. Leu-Ala-Gly-Val-OCH$_2$-
resin and Leu-Ala-Gly-Val-OCH$_2$-Pam-resin were prepared by
standard-solid phase peptide synthesis as described (17).

RESULTS AND DISCUSSION

Mechanism of trifluoroacetylation

Initial Observations. Aminomethyl-resin was prepared from
commercial chloromethyl-resin (Bio-Rad) and subjected to
simulated synthetic cycles consisting of acid and base treat-
ments, but without the coupling step. The resin was examined
by IR spectroscopy at the end of each cycle and showed high
levels of the trifluoroacetamide. Subsequently, samples of the
resin from individual steps in the simulated synthetic cycle were
examined. A species with a strong carbonyl absorption at 1785
cm$^{-1}$ was present after trifluoroacetic acid treatment, in ad-
tion to the trifluoroacetate salt at 1680 cm$^{-1}$. On neutral-
ization, the 1785 cm$^{-1}$ species decreased in proportion to the
amount of trifluoroacetamide (1725 cm$^{-1}$) formed. From this,
it was hypothesized that the 1785 cm$^{-1}$ species was a resin-
bound activated form of trifluoroacetic acid and was responsible
for the trifluoroacetamide formation. Other evidence suggested
that a likely candidate for the 1785 cm$^{-1}$ species was the tri-
fluoroacetyl-OCH$_2$-resin ester. The proposed mechanism of
trifluoroacetylation is shown in Fig. 1.

![Diagram of mechanism of trifluoroacetylation](image)

FIG. 1. Mechanism of trifluoroacetylation of resin-bound amines
in the neutralization step of solid-phase peptide synthesis. The
transfer of the trifluoroacetyl moiety occurs via direct interniste
nucleophilic reaction within a resin bead. The same mechanism
was shown to occur with resin-bound a-amino groups of peptides.
The vertical wavy line represents the polystyrene backbone. The sub-
ituents are not necessarily on adjacent styrene units but are located
randomly throughout the polystyrene network.

Generation of Trifluoroacetoxyethyl Sites. Hydroxymethyl-
resin was treated at room temperature with 50% (vol/vol)
trifluoroacetic acid in dichloromethane. Resin samples were
withdrawn at intervals and rinsed with ethanol in dichloro-
methane, and the amount of trifluoroacetyl ester formed (Fig
2A) was measured by IR spectroscopy. The reaction showed
first-order kinetics with a rate constant of 8 x 10$^{-4}$ sec$^{-1}$ (t$_{1/2}$
15 min). When aminomethyl sites were also present on the resin,
the esterification was slightly slower (rate constant, 6 x 10$^{-4}$
sec$^{-1}$; t$_{1/2}$ 20 min).

Leu-Ala-Gly-Val-OCH$_2$-resin (0.3 mmol/g) was shaken at
room temperature with 50% (vol/vol) trifluoroacetic acid in
dichloromethane. After 7 hr, a sample of the resin was rinsed
and examined by IR spectroscopy. This showed that 61% of
the tetrapeptide was cleaved and replaced by an equivalent
number of trifluoroacetoxyethyl sites (Fig 2B). The observed
extent of cleavage was consistent with the reported rate (12) of
acidolysis in 50% trifluoroacetic acid/dichloromethane.
An identical experiment using Leu-Ala-Gly-Val-OCH$_2$-Pam-resin
(0.54 mmol/g) containing the more acid-resistant 4-(carboxa-
midomethyl)benzyl ester linkage showed no detectable
cleavage (<2%) after 65 hr and no trifluoroacetoxyethyl
formation. The calculated extent of cleavage, based on the
literature cleavage rate (12), was only 1.35%.

Aminomethyl-Resin Model. Chloromethyl-resin containing
hydroxymethyl sites was converted to aminomethyl-resin (0.7
mmol/g) containing hydroxymethyl sites (0.53 mmol/g). In
order to investigate the mechanism of the trifluoroacetylation
of resin-bound amines, this resin was subjected to various con-
ditions and samples were examined by IR spectroscopy and
picric acid titration (15, 16) (Table 1). After a simulated syn-
thetic cycle without coupling, about 30% of the aminomethyl
groups had been trifluoroacetylated. No change was observed when the time of iPr2EtN treatment was varied. The total time of neutralization and washing was sufficient for all possible trifluoroacetylation to occur. When the trifluoroacetic acid step was followed by treatment with tetraethylammonium chloride in dichloromethane, which was shown to convert the initially formed trifluoroacetate salt to the hydrochloride, the same level of trifluoroacetamide formation was observed after the iPr2EtN step. This rules out the participation of the trifluoroacetate salt of the amine. A synthetic cycle omitting the neutralization steps gave no trifluoroacetamide. When 5% benzylamine in dichloromethane was used in the simulated synthetic cycle, the extent of trifluoroacetamidomethyl-resin formation was substantially decreased. This is consistent with the lability of the trifluoroacetyl ester on the resin toward primary amines in solution (ks, 0.15 M⁻¹ sec⁻¹ for benzylamine).

Aminomethyl-resin containing no hydroxymethyl sites and hydroxymethyl-resin containing no aminomethyl sites were mixed together and subjected to a single simulated synthetic cycle without coupling. Although IR spectroscopy showed the formation of the 1785 cm⁻¹ species after trifluoroacetic acid treatment, no trifluoroacetamide was formed during the neutralizations (Table 1). This rules out the existence of a long-lived soluble intermediate trifluoroacetylated species.

Occurrence and prevention of trifluoroacetylation

Screening for Hydroxymethyl Sites. Examination of unsubstituted and chloromethylated resins from this laboratory and the commercial sources listed above revealed only two lots that contained hydroxymethyl groups: chloromethylated Bio-Beads S-X1 (Lot 13668, 0.75 mmol/g by total chloride analysis) contained 0.36 mmol of hydroxymethyl per g; Bio-Beads S-X1 (lot 14591, 0.84 mmol/g by total chloride analysis) contained 0.33 mmol of hydroxymethyl per g.

Various Boc-aminocyl-OCH₂-resins, prepared from chloromethyl-resins by the cesium salt method (8), were found to contain no hydroxymethyl sites. Aminomethyl-resins prepared by the early procedure (12) from chloromethyl-resins in some cases showed additional hydroxymethyl sites that had been generated during the Gabriel synthesis. In contrast, screening of a large number of aminomethyl-resins with up to 1.8 mmol/g substitution that had been prepared from unsubstituted Bio-Beads S-X1 by direct amidoalkylation (11) showed no detectable hydroxymethyl groups in any instance.

**Blocked Hydroxymethyl Sites.** Samples of hydroxymethyl-resin were blocked as acetoxymethyl-resin and benzoxymethyl-resin and were treated with 50% (vol/vol) trifluoroacetic acid in dichloromethane at room temperature. The rate of cleavage was determined by IR spectroscopy of resin samples withdrawn at intervals. Pseudo-first-order rate constants were 50 × 10⁻⁵ sec⁻¹ for acetoxymethyl-resin and 40 × 10⁻⁷ sec⁻¹ for benzoxymethyl-resin. These compare with 58 × 10⁻⁷ sec⁻¹ (t₁/₂, 33 hr) for Leu-Ala-Gly-Val-OCH₂-resin and 0.58 × 10⁻⁷ sec⁻¹ (t₁/₂, 3300 hr) for Leu-Ala-Gly-Val-OCH₂-Pam-resin under the same conditions (12). Within the limits of experimental uncertainty, all the cleaved sites formed trifluoroacetoxymethyl groups on the resin.

**Aminomethyl-Resin Model.** Unsubstituted resin was converted to aminomethyl-resin by direct amidoalkylation followed by hydrazinolysis (11). This resin was subjected repeatedly to a simulated synthetic cycle without coupling, for a total of 20 cycles to amplify any low-level reaction. The result is shown in Table 1. No trifluoroacetamide formation (1725 cm⁻¹) was observed (<0.15% per cycle), consistent with the complete absence of the 1785 cm⁻¹ species after treatment with trifluoroacetic acid.

(*) Protected lysine-Resin Model. A number of different lysine-resins were prepared and subjected to simulated synthetic cycles without coupling, repeated 5–10 times to amplify any low-level reaction. After HF cleavage, which does not affect the trifluoroacetamide group, amino acid analysis by standard ion exchange methods was used to separate and quantitate lysine and Nα-trifluoroacetyl-lysine (Table 2). As little as 0.01% Nα-trifluoroacetylation per cycle could be detected. A standard benzyl ester-resin with no initial hydroxymethyl sites gave a significant level of trifluoroacetylation (0.6% per cycle, averaged over five cycles), which increased to about 1.5% per cycle after prior treatment of the loaded resin for 5 hr with trifluoroacetic acid in dichloromethane. Both the standard benzyl ester-resin and the Pam-resin (12) gave levels of trifluoroacetylation of about 1.5% per cycle when prepared from resins.

### Table 1. Trifluoroacetylation of aminomethyl-resin in simulated synthetic cycles without coupling

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Amount of trifluoroacetamide formed</th>
<th>By IR spectrum</th>
<th>By picrole</th>
<th>%</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simulated cycle</td>
<td>0.21</td>
<td>30</td>
<td>0.19</td>
<td>28</td>
<td></td>
</tr>
<tr>
<td>Simulated cycle, exchange to HCl salt</td>
<td>0.19</td>
<td>28</td>
<td>0.22</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Simulated cycle, no neutralization</td>
<td>0</td>
<td>&lt;2</td>
<td>0</td>
<td>&lt;2</td>
<td></td>
</tr>
<tr>
<td>Simulated cycle, mixed resins</td>
<td>0</td>
<td>&lt;5</td>
<td>—</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Simulated cycle, repeated 20 times</td>
<td>0</td>
<td>&lt;0.15</td>
<td>—</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* In mmol/g of polystyrene.

† Aminomethyl-resin prepared from commercial chloromethyl-resin containing hydroxymethyl sites.

§ Equal amounts of aminomethyl-resin without hydroxymethyl sites and hydroxymethyl-resin without aminomethyl sites.

‡ Aminomethyl-resin (0.54 mmol/g) prepared from unsubstituted polystyrene by direct amidoalkylation.
that already contained hydroxymethyl groups, and exchange of the trifluoroacetate salt to the hydrochloride salt did not affect the level of trifluoroacetylation. However, lysine-OCH2-Pam-resins, prepared by using aminomethyl-resin from direct amidolylation of unsubstituted resin containing no hydroxymethyl sites, gave an amount of trifluoroacetylation (0.02% per cycle) that was the same as background. Pretreatment with trifluoroacetic acid in dichloromethane for the equivalent of 65 deprotection cycles (20 min each) caused only a slight increase, to 0.1% per cycle. This very low background is thought to be due to the traces of trifluoroacetic acid anhydride detected by gas chromatography in the trifluoroacetic acid (18).

The rate of reaction of trifluoroacetoxymethyl-resin with N\textsuperscript{2}-lysine benzyl ester hydrochloride in solution in 10% iPr\textsubscript{2}EtN/dichloromethane was determined, giving a second-order rate constant of 6 \times 10\textsuperscript{-4} M\textsuperscript{-1} sec\textsuperscript{-1}. For the levels of hydroxymethyl groups present in the commercial (Bio-Rad) resin, this gives an apparent first-order rate constant of 4 \times 10\textsuperscript{-5} sec\textsuperscript{-1} (t\textsubscript{1/2}, 5 hr) for the formation of N\textsuperscript{2}-trifluoroacetylyllysine on the resin. The neutralization steps of the standard synthetic cycle involve 10-min exposure to iPr\textsubscript{2}EtN in dichloromethane and would lead to 2.5% N\textsuperscript{2}-trifluoroacetylyllysine on the basis of this rate constant. This is in agreement with the approximately 2% trifluoroacetylation per cycle actually observed in several experiments using this resin (see Table 2). These data indicate that the mechanism of trifluoroacetylation of resin-bound lysine is the same as that deduced for the aminomethyl-resin model, as shown in Fig. 1.

**CONCLUSIONS**

**Mechanism.** We have shown that trifluoroacetylation in solid-phase peptide synthesis can occur by a mechanism unlike any previously proposed. This novel mechanism is independent of the coupling step and involves the reaction of trifluoroacetoxymethyl groups on the resin support with the amino groups of resin-bound peptides, by an intersite aminolysis within a resin bead. The nucleophilic transfer reaction was shown to occur during the neutralization step of the synthetic cycle. This mechanism is shown in Fig. 1. Authentic trifluoroacetoxymethyl-resin has been shown to have properties consistent with those required for the proposed mechanism, and in particular it reacts with primary amines in solution in dichloromethane at the necessary rates. The \(\alpha\)-amino groups of peptides were found to react, but at <1% of the rate of primary alkyl amines.

The trifluoroacetylation was independent of the nature of the amine salt present and did not involve a long-lived soluble intermediate.

Trifluoroacetoxymethyl sites on a resin support can arise from existing hydroxymethyl groups or can be generated by acidi
dysis of the peptidyl-resin ester bond (Fig. 2). Both of these processes occur during the acid deprotection step and are increased by longer exposure to the trifluoroacetic acid. Examination of commercial chloromethyl-resins showed that Bio-Rad resin (nominal loading, 0.7 mmol/g) contained 0.35 mmol of hydroxymethyl groups per g. This particular resin is diluted in solid-phase peptide syntheses. The hydroxymethyl groups react with 50% trifluoroacetic acid/dichloromethane to form the ester with a half-time of about 20 min. The standard peptidyl-resin benzyl ester bond is labile to trifluoroacetic acid in dichloromethane and generates about 1% trifluoroacetoxymethyl sites per 50-min deprotection step.

Occurrence. The chloromethyl-resin already containing these high levels of hydroxymethyl sites gives rise to 1–2% N\textsuperscript{2}-trifluoroacetylation in each standard synthetic cycle. Acidi
dysis of the normal peptidyl-resin benzyl ester bond by trifluoroacetic acid produces trifluoroacetoxymethyl sites, which accumulate during a stepwise synthesis and lead to comparable levels of trifluoroacetylation per cycle within 10 cycles (Table 2).

The extent of trifluoroacetylation in either of the above is rate-limited by the poor nucleophilicity of the peptide \(\alpha\)-amino group. Repeated or prolonged neutralization (7) will lead to more trifluoroacetylation. The IR studies reported here show that neutralization is complete within 30 sec (the shortest time examined), as expected for a proton transfer reaction. Prolonged neutralization is therefore unnecessary. Similarly, repeated picric acid titrations of such a peptidyl-resin will lead to a steady decrease in titer. When proline is the NH\textsubscript{2}-terminal residue, much more extensive trifluoroacetylation can be expected because of the greater nucleophilicity of the \(\alpha\)-imino group which has a \(pK_a\) about 1 unit higher than an \(\alpha\)-amino group. This is why trifluoroacetylation has been most often reported to occur at proline residues (4, 5).

Trifluoroacetylation by the mechanism reported here is se
rious and ubiquitous with existing standard solid-phase meth
ods. Many of the apparent difficulties of solid-phase peptide synthesis undoubtedly can be attributed to unrecognized chemical problems such as this rather than to putative physical problems inherent to the polymer-supported nature of the reactions, as has often been claimed (19–24). The extremely high concentration of hydroxymethyl sites on batches of one of the most commonly used commercial resins, over a period of at least several years, must have caused innumerable difficulties in solid-phase syntheses. Such resins are clearly unacceptable for use in solid-phase peptide syntheses. Similarly, partial esterifi
cation of hydroxymethyl-resin with the COOH-terminal residue, a common route to loaded resins for use in synthesis (25, 26), will lead to substantial termination. The residual hydroxymethyl sites must be blocked before the synthesis is cont

**Table 2.** Trifluoroacetylation of lysine(Z)-resins in simulated synthetic cycles without coupling

<table>
<thead>
<tr>
<th>resin</th>
<th>conditions</th>
<th>extent, % per cycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys(Z)-OCH2-resin†</td>
<td>5 stand. cycles</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5-hr pretreatment,‡</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 stand. cycles</td>
</tr>
<tr>
<td>Lys(Z)-OCH2-resin§</td>
<td>10 stand. cycles</td>
<td>1.3</td>
</tr>
<tr>
<td>Lys(Z)-OCH2-Pam-resin∥</td>
<td>1st stand. cycle</td>
<td>2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10th stand. cycle</td>
</tr>
<tr>
<td>Lys(2ClZ)-OCH2-resin☆</td>
<td>10 stand. cycles</td>
<td>0.02</td>
</tr>
<tr>
<td>Lys(2ClZ)-OCH2-Pam-resin☆</td>
<td>22-hr pretreatment,♯</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 stand. cycles</td>
</tr>
</tbody>
</table>

* % trifluoroacetylation = ([\textsuperscript{18}N\textsuperscript{2}-Tf\textsubscript{2}Lys]/[total Lys]) \times 100.† The original resin was Pierce chloromethyl-resin, 0.7 mmol/g, no hydroxymethyl sites.‡ Pretreatment with 50% (vol/vol) trifluoroacetic acid in dichloromethane at room temperature.§ Original resin was Bio-Rad chloromethyl-resin, 0.7 mmol/g, containing 0.35 mmol hydroxymethyl/g.∥ Aminomethyl-resin, 0.1 mmol/g, from direct amidolylation of unsubstituted resin.☆ Aminomethyl-resin, 0.53 mmol/g, from direct amidolylation of unsubstituted resin.
the extent of trifluoroacetylation but will not eliminate this side reaction even if repeated several times during the synthesis. **Prevention.** On the basis of the studies reported here, we can avoid trifluoroacetylation by the nucleophilic transfer mechanism by using a resin that is initially free of hydroxymethyl sites and that will not generate them during the synthesis. The alternative, blocking these sites, has been discussed above. We have found that neutralization with primary amines prevents trifluoroacetylation by scavenging the trifluoroacetoxyethyl groups generated in each cycle of the synthesis much faster than they can react with the resin-bound peptide. Such primary amine neutralization could be used to minimize trifluoroacetylation in the synthesis of small peptides on standard resins, although this may be undesirable for other chemical reasons not considered here. To avoid trifluoroacetylation by the transfer mechanism, the resin must be derivatized and loaded by unambiguous chemical methods, giving a peptidyl-resin bond that is stable to trifluoroacetic acid in dichloromethane and that does not generate trifluoroacetoxymethyl sites during the synthesis. One such system is the peptidyl-\(\text{OCH}_2\text{Pam-resin, but only when synthesized by the recommended route (17) from preformed Boc-aminooacetyl-\(\text{OCH}_2\text{-phenylacetic acid and aminomethyl-resin prepared from unsubstituted resin by direct amidalkylation (11). The loaded resin prepared in this way does not contain any detectable extraneous functionalities. The peptidyl-resin bond is 100 times more stable to acidolysis in trifluoroacetic acid than is the standard peptidyl-resin benzyl ester bond, and generation of trifluoroacetoxymethyl sites will occur to an extent of less than 1% in 100 synthetic cycles. The successful use of this resin system to eliminate trifluoroacetylation has been demonstrated in the model studies reported here and in actual peptide syntheses (to be reported elsewhere).**

We thank Edward J. Potter for expert technical assistance in this work. This work was supported in part by Grant AM 01260 from the U.S. Public Health Service and by funds from the Hoffmann-La Roche Foundation.