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The effect of peptide length on the cleavage kinetics of 2-chlorotrityl resin-bound ethers

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Abstract: Different characteristics of cleavage kinetics of resin-bound amino alcohols and their peptide derivatives were observed in acid containing protic and aprotic solvent mixtures. The hydrolysis reactions are hindered by steric crowding around the cleaving C–O bond and accelerated by the special solvation effect of CF_3CH_2OH on the peptide chain as well as the increase of the strength and concentration of the acid. In trifluoroacetic acid containing mixtures, trifluoroacetylation of the peptide alcohols was detected. The appearance of *O*-trifluoroacetyl serine and threonine derivatives is detected in cleavage mixtures containing trifluoroacetic acid in anhydrous solvent. Copyright © 2006 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: trityl resin; cleavage; kinetics; peptide alcohol; trifluoroacetylation

INTRODUCTION

Two-phase (liquid–liquid or solid–liquid) reactions are of increasing interest in modern synthetic chemistry. In solid phase chemistry, widely utilized in combinatorial chemistry, molecules synthesized are attached to an insoluble support, either gel or surface. A large number of molecules are tested in high throughput test systems, either testing on the bead or after cleavage from the support in solution. The yield of the cleavage and the side reactions can also affect the apparent screening efficiency of the molecules under investigation. An optimization of the reagents and cleavage conditions (e.g. time, reagent concentration) is necessary to obtain reliable results in the screening method. When an active compound is found, its structure can be determined by suitable analytical techniques.

In this paper we describe our findings on the factors, e.g. temperature, the cleavage reagent, concentration and the nature of bond between the molecule and the resin, as well as the structure of the attached molecule, which affect the kinetics and the efficiency of the cleavage.

The kinetics of the cleavage is dependent on the chemical structure of the resin and of the molecule attached, therefore the cleavage conditions need to be optimized on a case by case basis. The cleavage of carbamate, urea, and sulfonamide molecules has been studied on benzyl, benzhydryl, and indole containing linkers in a solvent containing high concentrations of trifluoroacetic acid by Yan and his coworkers [1]. The optimization of cleavage conditions allowed the selective cleavage of Boc protecting group from the *N*-terminal of the peptide anchored to an acid labile Wang resin [2].

In this study, we present our results on the cleavage kinetics observed on 2-chlorotrityl resin [3]. This resin is a versatile support that can be utilized in the synthesis of carboxylic acids, alcohols [4] or thiols [5]. It is widely used in solid phase peptide synthesis with Fmoc-technique [6].

We found that the product composition and yields were dependent on the cleavage mixtures used [7,8].

To characterize the effect of variables and to establish the optimal conditions, the kinetics of the peptide alcohol or peptide acid resin-bond cleavage was studied quantitatively as a function of peptide length, the terminal amino acid, and the cleavage mixture applied.

The rates of cleavage were determined in four solvent mixtures as follows:

(a) Dichloromethane : methanol : acetic acid = 8 : 1 : 1 (v/v/v). This mixture is routinely used in our laboratory for the cleavage of smaller peptide acid fragments (mixture a).
(b) Dichloromethane:2,2,2-trifluoroethanol : acetic acid = 4 : 1 : 1 (v/v/v). Described by Barlos and his coworkers [6], this mixture is used for the cleavage of large, otherwise insoluble fragments. It has been a very powerful, efficient, and widely applied cleavage mixture (mixture b).
(c) Dichloromethane containing 0.1% trifluoroacetic acid. This solution is widely used for the cleavage of protected peptide acids from SASRIN [9,10] and other acid sensitive resins because it slowly attacks Boc groups (mixture c).
(d) Dichloromethane containing 0.2% trifluoroacetic acid. A more acidic variant of mixture c. Boc groups with longer contact time may be damaged (mixture d).

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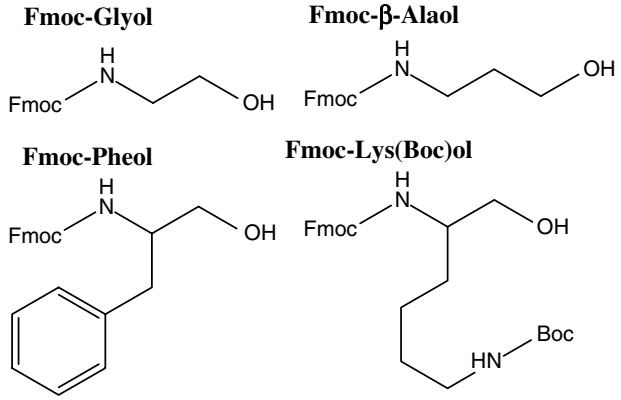

Figure 1 The structures of the amino alcohols studied.

The rate of the ether bond cleavage which links the attached molecule to the resin of four Fmoc-amino acid derived alcohol [Fmoc-Glyol, Fmoc-Pheol, Fmoc-Lys(Boc)ol, Fmoc-β-Alaol (Figure 1)], and their *N*-terminal Fmoc-protected peptide derivatives were investigated in detail.

In preliminary experiments, we found that the cleavage of Fmoc-amino acids followed a first-order release and can be described by simple exponential saturation kinetics. However, for the protected amino acid, or peptide alcohols, the cleavage kinetics cannot be interpreted by the model described for the Fmoc-amino acids. A new compound resulted from the trifluoroacetylation of the alcohols appeared in the reaction mixture and proved to be responsible for the observed kinetics.

RESULTS AND DISCUSSION

Characterization of the Cleavage Kinetics of Fmoc-amino Alcohols and Peptide Alcohols

As a reference, the cleavage rates of four Fmoc-amino acids anchored to the same 2-chlorotrityl resin were determined in the cleavage mixtures (a) and (b).

The cleavage rate of Fmoc-amino acids in cleavage mixture (a) and (b) was slow enough to be determined by the HPLC methodology applied, whereas in mixtures (c) and (d), the rate was too fast to obtain reliable and accurate data.

The cleavage of Fmoc-amino acids followed a first-order release and can be described by simple exponential saturation kinetics. The rate of cleavage was determined by fitting Eqn (1) to the observed time-Fmoc-amino acid concentration pairs.

$$C = C_0 + C_1(1 - e^{-k_1 t}) \quad (1)$$

where C is the observed Fmoc-amino acid concentration in solution, C_0 and C_1 are fitting parameters, k_1 is the rate of Fmoc-amino acid-resin cleavage and t is the time in minutes from the beginning of the cleavage. As shown in Figure 2, Eqn (1) describes simple saturation kinetics, where the compound released from the solid phase reaches its maximum concentration at infinite time. C_0 can be correlated to the initial amount of Fmoc-amino acid present in the reaction mixture, whereas C_1 can be visualized as the maximal amount of Fmoc-amino acid that can be cleaved from the resin. Ideally, the sum of C_0 and C_1 should be proportional to the nominal capacity of the resin.

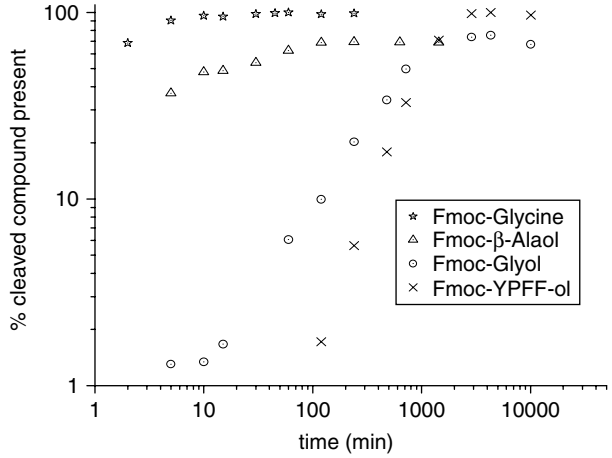

Figure 2 The release of 2-chlorotrityl-bound compounds as a function of time in solvent mixture (b).

On the basis of these results, we attempted to extend the investigation to the Fmoc-amino alcohol or peptide alcohol resin cleavage. As expected from the well-known differences between the acid lability of trityl esters and ethers, the rate of Fmoc-amino alcohol or peptide alcohol resin cleavage was considerably slower than that of the peptide acids.

In mixture (a), very slow and complex reaction kinetics were observed. In mixture (b) (Figure 2), the cleavage kinetics of the peptide alcohols were similar

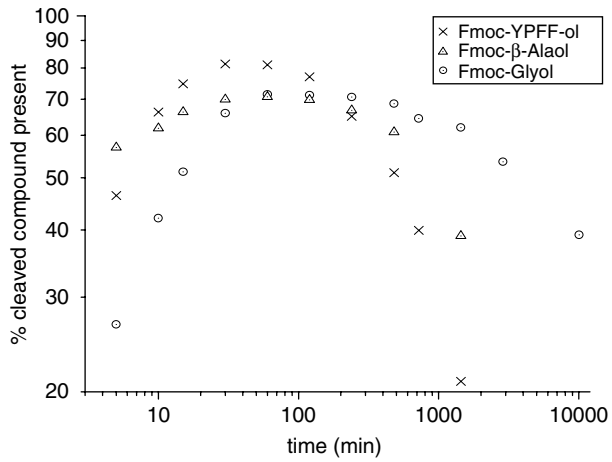

Figure 3 The concentration of the cleaved amino alcohol or peptide alcohol as a function of time in cleavage mixture (c).

to that of the acids, but the rate of cleavage was much slower.

However, the cleavage kinetics (Figure 3) cannot be interpreted by the model described for the Fmoc-amino acids [Eqn (1)]. It is obvious that the release of the alcohol from the resin cannot be described by Eqn (1), which describes a simple saturation kinetics reaching a maximum value at infinite time. This behavior in mixtures (c) and (d) was also very characteristic. It is apparent from Figure 3 that the time when the concentration of cleaved product is maximal in the cleavage mixture is different for each compound. From a preparative point of view, this means that the optimal cleavage time to obtain the maximum amount of compound is different for each compound investigated.

In attempts to interpret the observed time-concentration correlations (Figure 3), it has been hypothesized that two independent processes are involved, the first is the normal acid catalyzed cleavage of the Fmoc-alcohol from the resin, the second process is some side reaction consuming the Fmoc-alcohol present.

Parallel to the decrease of the cleaved alcohol, the appearance of a new component was detected by HPLC. For Fmoc-β-Ala-ol, the unknown compound was isolated and identified by mass spectrometry as the trifluoroacetylated derivative of the amino alcohol released from the resin. Its structure was also verified by synthesis and coinjection of trifluoroacetyl ester of Fmoc-β-Ala-ol.

The interpretation of the results is shown in Figure 4. The acid catalyzed cleavage of the trityl ether is the first step in the reaction sequence, which is followed by trifluoroacetylation of the cleaved alcohol in the cleavage mixture.

This reaction sequence can be modeled satisfactorily by a

$$\mathbf{A} \xrightarrow{k_1} \mathbf{B} \xrightarrow{k_2} \mathbf{C}$$

A = resin-bound alcohol
B = released alcohol in solution
C = trifluoroacetylated alcohol in solution

reaction scheme, where ***A*** is the resin-bound Fmoc-alcohol, ***B*** is the Fmoc-amino or peptide alcohol in solution, and ***C*** is the trifluoroacetylated Fmoc-alcohol. For the description of the cleaved alcohol concentration (which in the scheme is the concentration of component B) in the cleavage solution, the following equation was used.

$$C = C_0 + C_1 \cdot \frac{k_1}{k_2 - k_1} \cdot (e^{-k_1 \cdot t} - e^{-k_2 \cdot t}), \qquad (2)$$

where C is the observed alcohol concentration in solution, C_0 and C_1 are fitting parameters, k_1 is the rate of resin-Fmoc-alcohol cleavage, k_2 is the rate of the Fmoc-alcohol trifluoroacetylation present in the cleavage mixture.

(If there is no trifluoroacetylation in the reaction mixture, then, $k_2 = 0$; in this case Eqn (2) describes the same process as Eqn (1) with a little different parameterization, i.e. Eqn (1) is a special form of Eqn (2).)

The Effect of Steric Crowding and the Size of the Molecule

In another series of experiments, we studied and compared the resin cleavage rates in different cleavage mixtures as a function of the side chain (in case of amino acid derivatives) or peptide length (in case of peptide derivatives). As the side chain modification of the amino acid derivatives has only minor effect on the cleavage rates, either in the case of Fmoc-amino acids (Figures 5, 6; Tables 1, 2) or alcohols (Figures 6, 7; Table 3), we thought that the little differences in the cleavage rates might be attributed to the steric hindrance in the vicinity of the reaction center. Shifting the crowded group further from the bond to be cleaved, we expected an increased rate of cleavage. This can be demonstrated, e.g. in mixture (b) (Figure 7; Table 3), where the rate of cleavage for Fmoc-β-Alaol is more than one order of magnitude higher than for Fmoc-Glyol. This difference seems to be diminished in more acidic solvent [mixture (c) (Figure 7; Table 4) and mixture (d) (Figure 7; Table 5)].

The structures of the amino acids, amino alcohols and the peptide derivatives of amino alcohols attached

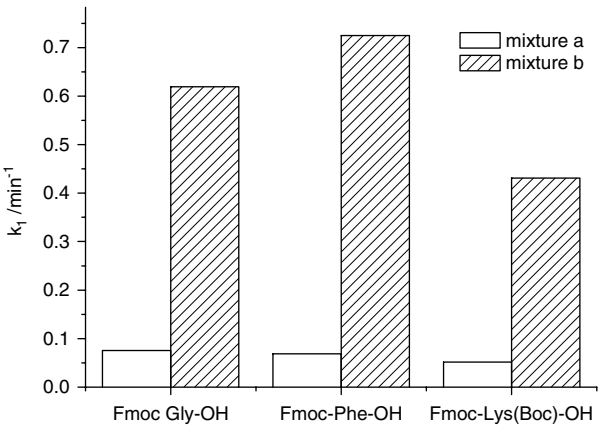

Figure 5 Cleavage rates of resin-bound fmoc-amino acids.

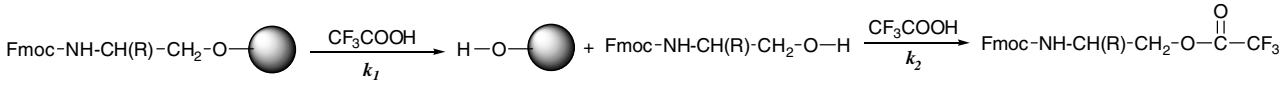

Figure 4 Trifluoroacetylation of fmoc-amino alcohols after cleavage from the resin in the presence of trifluoroacetic acid.

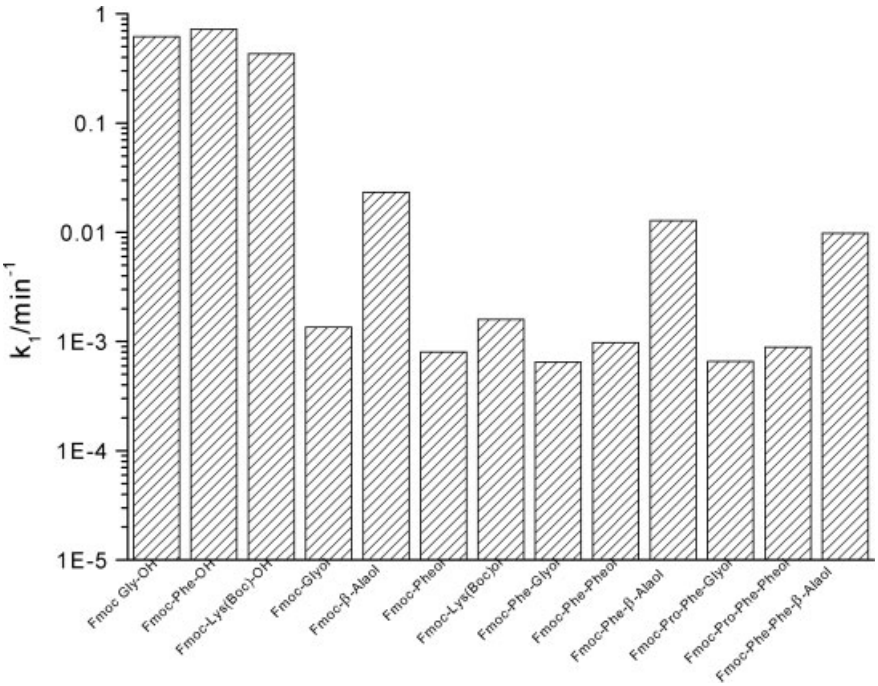

Figure 6 Cleavage rate constants (k_1) of resin-bound esters and ethers in mixture b.

to the resin are analogous to those of the trityl esters and ethers, respectively. Trityl esters hydrolyze very easily in acidic media, by the $A_{Al}1$ mechanism [11,12], with the cleavage of $(Ph_3)C-O$ bond and the formation of a resonance stabilized *tert.*-alkyl cation and a carboxylic acid. The *tert.*-alkyl cation reacts very rapidly with the anion nucleophiles present in the media or form ethers if the reaction is carried out in alcohols. The protonation of the ester is not necessary for the reaction, the substrates dissociate to ions if H-bonds are formed in protic solvents. For example, triphenylmethyl benzoate was found to hydrolyze in an uncatalyzed ionization reaction in ethanol with the formation of benzoic acid and ethyl triphenylmethyl ether. Reactions were faster in the presence of acids [13].

Fmoc-amino acids coupled to the resin hydrolyzed even in mixture (a), and the rate of the reaction increased with one order of magnitude if methanol was changed for the more acidic 2,2,2-trifluoroethanol. The ratio of the protic components of the solvent was higher in mixture (b). The different side chains of the amino acids had no marked influence on the hydrolysis (Figure 5; Tables 1,2).

The cleavage of ethers in acidic media is considerably slower than that of esters (compare the k_1 values in Table 2 and Table 3 or in Figure 7) and usually much stronger acids are needed for the reaction [14,15]. Only *tert.*-alkyl ethers hydrolyze easily by aqueous acids in an S_N1 reaction by the protonation or H-bond formation of the oxygen atom, with the cleavage of the bond of the oxygen and the *tert.*-carbon atom. The products of this rate determining step of the reactions are a *tert.*-carbocation and a primary or secondary alcohol, depending on the structure of the substrate [14,15].

Table 1 Cleavage rates of resin-bound Fmoc-amino acids in mixture (a)

Amino acid	k_1 (min^{-1})		
	Average	RSD (number of determinations)	Average R^2
Fmoc-Gly-OH	0.0753	0.00153 (3)	0.979
Fmoc-Phe-OH	0.068	0.00649(3)	0.959
Fmoc-Lys(Boc)OH	0.052	0.00264 (3)	0.965

Table 2 Cleavage rates of resin-bound amino acids in mixture (b)

Amino acid	k_1 (min^{-1})		
	Average	RSD (number of determinations)	Average R^2
Fmoc-Gly-OH	0.619	0.198 (3)	0.960
Fmoc-Phe-OH	0.725	0.107 (3)	0.955
Fmoc-Lys(Boc)OH	0.431	0.028 (3)	0.955

In mixture (b), Fmoc-amino alcohols attached to the resin react faster than their peptide derivatives (Figure 7; Table 3). The limited accessibility of the oxygen atom of the ether seems to have an important role in the reaction, as derivatives of β-Ala react with

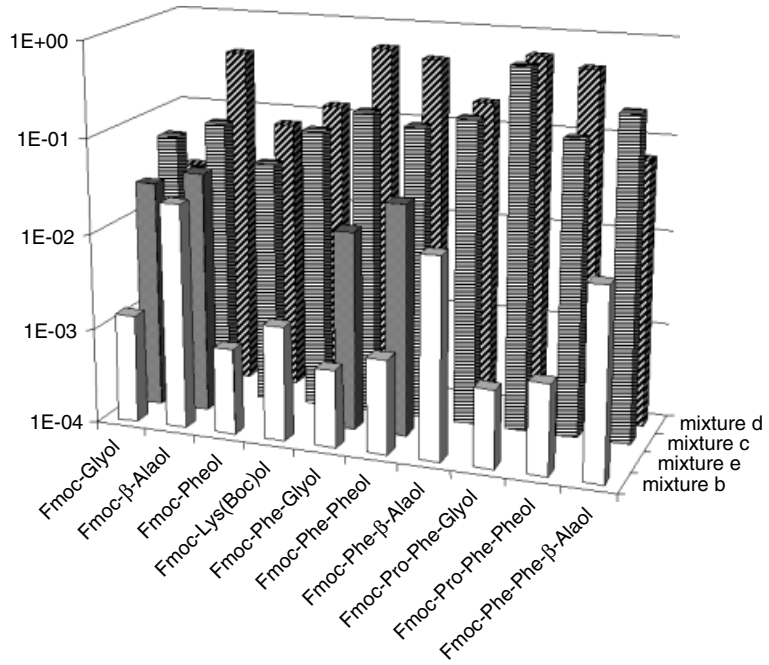

Figure 7 Cleavage rate constants (k_1) of resin-bound ethers.

Table 3 Cleavage rates of resin-bound ethers in mixture (b)

Amino alcohol	k_1 (min^{-1})		
	Average	RSD (number of determinations)	Average R^2
Fmoc-Glyol	1.35×10^{-3}	2.12×10^{-4} (3)	0.997
Fmoc-β-Alaol	2.33×10^{-2}	8.18×10^{-3} (4)	0.970
Fmoc-Lysol	1.59×10^{-3}	3.11×10^{-4} (3)	0.996
Fmoc-Pheol	8.00×10^{-4}	5.57×10^{-5} (3)	0.994
Fmoc-Glyol	1.35×10^{-3}	2.12×10^{-4} (3)	0.997
Fmoc-Phe-Glyol	6.50×10^{-4}	1.00×10^{-5} (3)	0.990
Fmoc-Phe-Pheol	9.77×10^{-4}	7.23×10^{-5} (3)	0.987
Fmoc-Pro-Phe-Glyol	6.57×10^{-4}	4.04×10^{-5} (3)	0.991
Fmoc-Pro-Phe-Pheol	8.90×10^{-4}	3.61×10^{-5} (3)	0.988
Fmoc-Tyr-Pro-Phe-Pheol	7.93×10^{-4}	3.215×10^{-5} (3)	0.986
Fmoc-β-Alaol	2.33×10^{-2}	8.18×10^{-3} (4)	0.970
Fmoc-Phe-β-Alaol	1.28×10^{-2}	1.09×10^{-3} (3)	0.994
Fmoc-Pro-Phe-β-Alaol	9.77×10^{-3}	3.03×10^{-4} (3)	0.983

one order of magnitude faster than the derivatives of α-amino acids (Figure 7; Table 3). In compounds prepared with β-Ala, there are two CH_2 groups between the oxygen atoms. Peptide derivatives of amino alcohols have lower reactivity than Fmoc-amino alcohols both in the α and β series (Figure 7; Table 3).

The Effect of Solvent Composition and Acidity

Relative reactivities remain the same even in those cases when CF_3COOH is added to the cleavage mixture [mixture (e)] (Table 6). The lower reactivity of the peptide derivatives can be explained by the strong solvation effect of CF_3CH_2OH on peptides [16,17]. Similarly, to the observation of the trifluoroacetylation rate of the amino alcohol derivatives (see below), In the case of β-alaninol-containing peptides, not only is the rate of cleavage much higher but also the rate of trifluoroacetylation.

In CF_3COOH containing CH_2Cl_2 (Figure 7; Tables 4 and 5), the rate of the reaction increases with the concentration of the acid. In the absence of special solvation effects, the reactivity is determined only by the steric hindrance. Amino alcohols having the bulky Fmoc group closer to the splitting C–O bond cleaved more slowly from the resin than β-alaninol and amino alcohol peptide derivatives of α-amino acids.

The Rate of Trifluoroacetylation

The Fmoc-alcohols obtained after the cleavage are acylated with CF_3COOH in CH_2Cl_2. The rate of this reaction (k_2 in Tables 4, 5 and 6; Figure 8) is increased with the concentration of trifluoroacetic acid. The reaction is also hindered by the proximity of the bulky Fmoc group. Fmoc-Glyol, Fmoc-Pheol, and Fmoc-Lys(Boc)ol react more slowly than peptide derivatives of amino alcohols prepared from α-amino acids, as well as β-Alaol and its amino acid derivatives, which have practically the same rate. In accordance with previous observations for the resin-peptide alcohol cleavage rates, both the rate of resin-alcohol bond cleavage and the rate of trifluoroacetylation decrease

Table 4 Cleavage (k_1) and trifluoroacetylation rates (k_2) of Fmoc-amino alcohols and peptides of resin-bound ethers in mixture (c). The values are the mean of three independent determinations followed by parameter fitting

Amino alcohol	k_1 [min^{-1}][a]	k_2 (RSD) [min^{-1}]	R^2
Fmoc-Glyol	6.0×10^{-2}	7.33×10^{-5} (5.80×10^{-6})	0.975
Fmoc-β-Alaol	8.9×10^{-2}	1.52×10^{-3} (2.55×10^{-4})	0.877
Fmoc-Lys(Boc)ol	9.0×10^{-2}	2.80×10^{-4} (4.58×10^{-5})	0.960
Fmoc-Pheol	3.67×10^{-2}	5.67×10^{-5} (1.16×10^{-5})	0.959
Fmoc-Glyol	6.0×10^{-2}	7.33×10^{-5} (5.80×10^{-6})	0.975
Fmoc-Phe-Glyol	1.57×10^{-1}	9.87×10^{-4} (1.07×10^{-4})	0.990
Fmoc-Phe-Pheol	1.21×10^{-1}	1.91×10^{-3} (2.23×10^{-4})	0.993
Fmoc-Pro-Phe-Glyol	6.0×10^{-1}	1.13×10^{-3} (2.08×10^{-5})	0.877
Fmoc-Pro-Phe-Pheol	1.23×10^{-1}	1.60×10^{-3} (2.51×10^{-4})	0.982
Fmoc-Tyr-Pro-Phe-Pheol	1.23×10^{-1}	1.27×10^{-3} (1.47×10^{-4})	0.994
Fmoc-β-Alaol	8.9×10^{-2}	1.52×10^{-3} (2.55×10^{-4})	0.877
Fmoc-Phe-β-Alaol	1.60×10^{-1}	1.44×10^{-3} (7.62×10^{-4})	0.950
Fmoc-Pro-Phe-β-Alaol	2.43×10^{-1}	1.71×10^{-3} (1.48×10^{-4})	0.976

[a] This parameter was calculated numerically from the k_2 values and the observed time of maximal cleaved alcohol concentration using the equation $t_{max} = \frac{1}{k_2 - k_1} \cdot \ln \frac{k_2}{k_1}$.

by increasing the length of the peptide to be cleaved in solvent mixtures containing higher amounts of protic solvent (Table 6).

CONCLUSIONS

Different characteristics of the cleavage kinetics were observed in protic and aprotic cleavage mixtures by increasing the length of the peptide attached to the resin-bound ethers because of the specific solvation effects. The rate of cleavage is decreased by increasing peptide length if 2,2,2-trifluoroethanol is present in the cleavage mixture because of a specific solvation effect. In cleavage mixtures containing dichloromethane and trifluoroacetic acid, the rate of the resin-bound alcohol cleavage is determined only by the steric hindrance. Careful selection of the cleavage conditions is necessary to obtain optimal yield.

Table 5 Cleavage (k_1) and trifluoroacetylation rates (k_2) of Fmoc-amino alcohols and peptides of resin-bound ethers in mixture (d). The values are the mean of three independent determinations followed by parameter fitting. The relative standard deviations of the values are in parentheses

Amino alcohol	k_1 [min^{-1}][a]	k_2 (RSD) [min^{-1}]	R^2
Fmoc-Glyol	2.20×10^{-2}	7.33×10^{-5} (5.80×10^{-6})	0.980
Fmoc-β-Alaol	4.04×10^{-1}	1.47×10^{-3} (5.19×10^{-4})	0.987
Fmoc-Lys(Boc)ol	1.27×10^{-1}	2.80×10^{-4} (2.08×10^{-5})	0.932
Fmoc-Pheol	7.33×10^{-2}	3.60×10^{-5} (7.81×10^{-5})	0.959
Fmoc-Glyol	2.20×10^{-2}	7.33×10^{-5} (5.80×10^{-6})	0.980
Fmoc-Phe-Glyol	5.61×10^{-1}	2.15×10^{-3} (8.54×10^{-4})	0.997
Fmoc-Phe-Pheol	4.67×10^{-1}	3.07×10^{-3} (1.56×10^{-4})	0.995
Fmoc-Pro-Phe-Glyol	6.0×10^{-1}	1.13×10^{-3} (2.08×10^{-5})	0.977
Fmoc-Pro-Phe-Pheol	4.83×10^{-1}	3.17×10^{-3} (1.40×10^{-4})	0.998
Fmoc-Tyr-Pro-Phe-Pheol	4.57×10^{-1}	2.19×10^{-3} (3.46×10^{-5})	0.995
Fmoc-β-Alaol	4.04×10^{-1}	1.47×10^{-3} (5.19×10^{-4})	0.987
Fmoc-Phe-β-Alaol	1.83×10^{-1}	2.68×10^{-3} (3.72×10^{-4})	0.984
Fmoc-Pro-Phe-β-Alaol	6.33×10^{-2}	2.02×10^{-3} (4.21×10^{-4})	0.929

[a] This parameter was calculated numerically from the k_2 values and the observed time of maximal cleaved alcohol concentration using the equation $t_{max} = \frac{1}{k_2 - k_1} \cdot \ln \frac{k_2}{k_1}$.

In the presence of trifluoroacetic acid, the trifluoroacetylation side reaction cannot be significantly suppressed even in the presence of higher concentration of primary alcohol. Longer cleavage time not only increases the amount of compound cleaved from the resin but also increases the amount of trifluoroacetylated alcohol. These findings may have implications for the cleavage conditions of unprotected serine and threonine -containing peptides. Without trifluoroacetic acid, however, the rate of the cleavage is much lower, therefore the replacement of trifluoroacetic acid could slow down the resin-peptide cleavage rate. As trifluoroacetic acid is widely used in peptide and combinatorial chemistry as an acidic reagent in different cleavage mixtures, special attention should be paid to this side reaction.

The formation of trifluoroacetyl esters in the presence of trifluoroacetic acid can give reasonable explanation for the appearance of trifluoroacetylated deletion products during Boc solid phase peptide synthesis

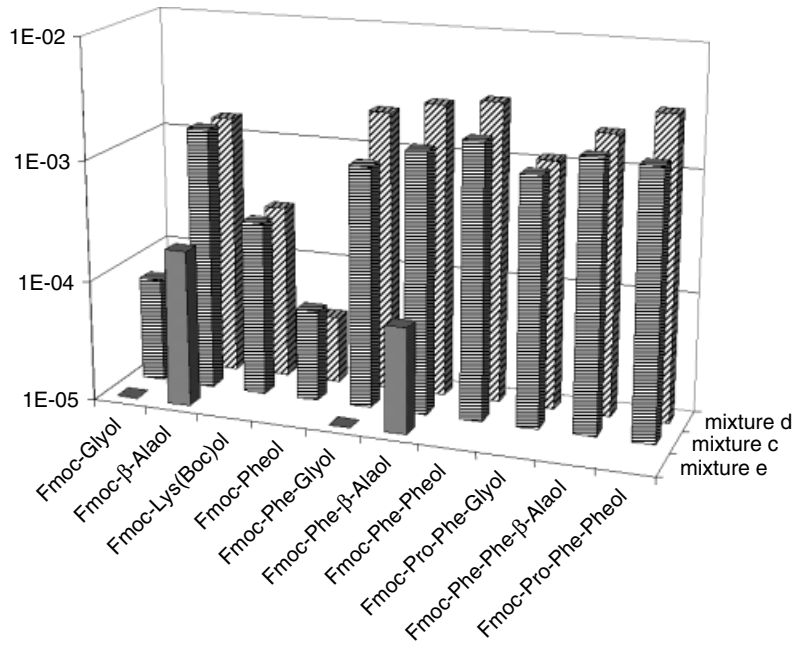

Figure 8 Trifluoroacetylation rates (k_2) of fmoc-amino alcohols and peptides of resin-bound ethers.

Table 6 Cleavage (k_1) and trifluoroacetylation rates (k_2) of Fmoc-amino alcohols and peptides of resin-bound ethers in dichloromethane : 2,2,2-trifluoroethanol : acetic acid = 4 : 1 : 1 containing 0.1% trifluoroacetic acid. The values are the mean of two independent determinations followed by parameter fitting. The relative standard deviations of the values are in parentheses

Amino alcohol	k_1 (RSD) [min^{-1}]	k_2 (RSD) [min^{-1}]	R^2
Fmoc-Glyol	2.50×10^{-2} (7.07×10^{-5})	Not observed	0.995
Fmoc-β-Alaol	3.54×10^{-2} (2.36×10^{-3})	1.95×10^{-4} (7.78×10^{-5})	0.994
Fmoc-Phe-Glyol	1.23×10^{-2} (1.87×10^{-3})	Not observed	0.998
Fmoc-Phe-β-Alaol	2.69×10^{-2} (1.91×10^{-4})	7.50×10^{-5} (7.07×10^{-6})	0.996

on Merrifield resin. This resin always contains variable amounts of benzyl alcohol, which is formed during the synthesis of the resin or during the attachment of the first amino acid. The polymer-bound benzyl alcohol is trifluoroacetylated during the cleavage of the Boc group (20–50% trifluoroacetic acid in dichloromethane). This resin-bound trifluoroacetyl benzyl ester may acylate the free *N*-terminus of the growing peptide chain by transamination. This can be one reasonable explanation for the appearance of trifluoroacetylated deleted peptides in the crude peptide after HF cleavage, especially on resins of low quality.

EXPERIMENTAL

Abbreviations

All of the abbreviations that were used, were defined in the literature [18].

Materials

N,*N*-dimethyl formamide (DMF; distilled from ninhydrin), methanol (MeOH), diethyl ether (Et_2O; distilled from $LiAlH_4$), dichloromethane (DCM; distilled from phosphorus pentoxide), 2-propanol (iPrOH), 2-chlorotrityl chloride resin (nominal capacity: 1.3 mmol/g resin), Fmoc-Ala-OH, Fmoc-β-Ala-OH, Fmoc-Lys(Boc)-OH, Fmoc-Phe-OH, Fmoc-OSu, trifluoroacetic acid (TFA) and Diisopropyl ether (DIPE; distilled from $LiAlH_4$) were purchased from Reanal (Budapest, Hungary). Diisopropylethylamine (DIEA; distilled from ninhydrin) was purchased from Fluka (Buchs, Switzerland). Tetrahydrofuran (THF; distilled from Na and K in the presence of benzophenone), ethanolamine and HPTLC pre-coated silica gel 60 F_{254} glass plates (Cat. No: 105 629) were obtained from Merck (Hohenbrunn, Germany). HPLC quality *acetonitrile* (MeCN) was purchased from Lab-Scan (Dublin, Ireland).

Fmoc-Glycinol (Fmoc-Glyol) was synthesized according to the procedure described by D. Jonssonn, A. Unden [19] and by T. S. Rao *et al.* [20], to obtain a compound melting at 146–149 °C (lit : 144–145 °C); R_f(DCM:MeOH = 9 : 1) = 0.79; R_f(Pyridine:AcOH:water:EtOAc = 20 : 6 : 11 : 240) = 0.78; R_f(EtOAc:hexane = 3 : 1) = 0.38.

Fmoc-Phenylalanilol (Fmoc-Pheol) was obtained following the procedure of M. Souček, J. Urban, and D. Saman [21], melting point: 145.5–146 °C (lit: 146 °C); R_f(DIPE) = 0.18; R_f(EtOAc:hexane = 1 : 1) = 0.32.

Fmoc-Lys(Boc)ol has been described by K. Burgess *et al.* [22] and A. Boeijen *et al.* [23].

The compound had a melting point: of 130–132 °C (lit : 130–131 °C). R_f(EtOAc : hexane = 2 : 1) = 0.63, R_f(DCM: hexane = 4 : 1) = 0.79

Fmoc-β-Alaol was synthesized following the procedure described by Giordano [24], and A. Boeijen *et al.* [23]. The compound melts at 128–129 °C (lit : 128–130 °C); R_f(EtOAc:hexane = 2 : 1) = 0.57 R_f(DCM:hexane = 4 : 1) = 0.72

Anchoring the Amino Alcohols to 2-Chlorotrityl Resin

The amino alcohol (4 equivalents calculated for the nominal capacity of the resin) was dissolved in a minimal amount of freshly distilled, dry DMF or THF and added to the unswollen resin. The mixture was shaken until the resin was swollen completely. The first part of pyridine (0.5 equivalents to amino alcohol used) was added, then after 5 min the remaining pyridine (2 equivalents of amino alcohol) was added to the mixture under stirring. Samples were taken after 1, 3, 6, 12, 24, 48 and 72 h. The samples were washed with DMF (5x), DCM(2x), iPrOH(2x), MeOH(2x), DCM(2x), iPrOH(2x), MeOH(2x), and diethyl ether (2x), then dried *in vacuo*. The capacity of the resin was determined photometrically from the amount of Fmoc chromophore released upon treatment of the resin with piperidine/DMF [25]. The capacity of the Fmoc-amino alcohol loaded resins were the fallows Fmoc-Glyol 0.41, Fmoc-β-Alaol 0.33, Fmoc-Lys(Boc)-ol 0.32, Fmoc-Pheol 0.38 mmol/g resin.

Synthesis of Resin-bound Fmoc-peptidyl-amino Alcohol Derivatives

After anchoring the Fmoc-amino alcohols to the resin, the synthesis was carried out by Fmoc/^{t}Bu technique using our standard Fmoc protocol [26]. In the chain elongation steps, 2 equivalents of Fmoc-amino acid, 1.9 equivalents of HBTU, and 5 equivalents of DIEA dissolved in DMF were used for 30 min. The Fmoc protection was removed by 2% DBU and 2% piperidine in DMF for 3 + 17 min. After building up of the peptide chain the resins were washed with DMF, DCM, iPrOH, MeOH, and ether, and finally dried *in vacuo*.

Cleavage of Fmoc-protected Amino Alcohols and Peptide Alcohols from the Resin

To 20 mg resin calculated amount of internal standard, Fmoc-Pro-OH was added. The molar amount of the internal standard was equal to the theoretically cleavable amount of the amino alcohol and peptide alcohol, which was determined from the loaded resin capacity. 3 cm^3 of the cleavage mixture [(a) DCM : MeOH : AcOH = 8 : 1 : 1; or (b) DCM : TFE : AcOH = 4 : 1 : 1; or (c) 0.1% TFA in DCM; or (d) 0.2% TFA in DCM] was added and 100 µl samples were withdrawn for analysis after 2, 5, 10, 15, 30, 60, 120, 240, 480, 720, and 1440 min. 100 µl acetonitrile was added to each sample, and the supernatant was injected to HPLC.

HPLC Analysis

Hplc system. Shimadzu 6 HPLC gradient system with Vydac 218 TP 250 mm × 4.6 mm (C_{18}, 5 µm, 300 Å) column. *Eluent*: A binary gradient was used from 70% A (water containing 0.045% TFA) and 30% B (acetonitrile containing 0.036% TFA) to 45% A and 55% B in 13 min and from 45% A and 55% B to 5% A and 95% B in 2 min at a flow rate of 1 cm^3/min. The chromatograms were recorded at 220 nm. An internal standard method using Fmoc-proline was applied.

Curve Fitting

The relative amount of the cleaved Fmoc or Fmoc-peptide amino alcohols in the mixture was plotted as the function of time. The best fit of parameters in Eqn (1) or Eqn (2) to the experimental data was estimated using the nonlinear curve-fitting algorithm of Microcal's Origin software (6.0 SP2) for each series of determination. The means of the fitted parameters obtained are listed in Tables 1–6 along with the standard deviation of the fitted parameters and the mean of the R^2 values of the curve-fitting algorithm.

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