Journal of Peptide Science
J. Peptide Sci. **11**: 472–480 (2005)
Published online 10 January 2005 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/psc.640

JOURNAL OF PEPTIDE SCIENCE

Kinetic and mechanistic investigation of the selective acidolysis of the *C*-terminal amide bond of *N*-acyl-*N*,α,α-trialkyl glycine amides

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Received 3 June 2004; Revised 28 July 2004; Accepted 28 October 2004

Abstract: Accurate rate constants were calculated from HPLC kinetic measurements of the selective acidolysis of the *C*-terminal amide bond of eight *N*-acyl-*N*-(4-methoxybenzyl)-α,α-trialkyl glycine amides in TFA at 25.00 °C. The results were in all cases consistent with a first order behaviour with respect to the substrate and, apparently, also to the acid, and a clear relationship between reactivity and structure could be observed. The data collected also allowed experimental evidence to be obtained for the first time in support of the previously postulated formation of an intermediate oxazolonium salt. In the case of the more crowded species this intermediate compound undergoes slow hydrolytic ring opening, which takes place in competition with cleavage of the *N*-alkyl group to give another oxazolonium derivative that hydrolysed still more slowly. The stability of the intermediate cyclic compounds may result either from conjugation of the phenyl group with the oxazolonium ring in the case of *N*-benzoyl derivatives, or from conformational assistance imparted by the bulky amino acid side chains of the α,α-dialkyl glycine species, or both. The loss of the *N*-alkyl group also seems to be assisted by the bulkiness of the amino acid side chains, which thus tends to decrease the selectivity of cleavage. Copyright © 2005 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: *N*-acyl-*N*-(4-methoxybenzyl)-α,α-trialkyl glycine amides; selective acidolysis; rate constants; reaction mechanism; oxazolonium salts; Ugi-Passerini adducts

INTRODUCTION

N-Alkylamino acid residues are useful for replacing proline with advantages in peptidomimetics. In fact, like proline, they are helix-breaking amino acids as they do not allow hydrogen bonding at the amino acid nitrogen atom. However, in contrast to proline, they tolerate rotation at their N–C$^\alpha$ bond, thus imparting increased flexibility to the peptide chain, which can be modulated by the length and bulk of the alkyl group at the amino acid nitrogen atom. Frequently, the synthetic modifications of natural peptides and peptidomimetics needed for biological or medical applications require the generation or imposition of restrictions to backbone flexibility [1,2]. The steric crowding and/or hindrance to rotation typical of α,α-dialkyl and *N*,α,α-trialkyl glycines make these compounds good candidates for incorporation into peptide chains with the aim of granting them such conformational features. Sarcosine and other *N*-methyl amino acids have been used for this purpose and can be prepared by *N*-methylation of the parent amino acid with methyl iodide [3]. However, the preparation of amino acids with alkyl groups larger than methyl at their nitrogen atom requires elaborate synthetic methods [4] involving electrochemical techniques [5] that are not available in most organic chemistry laboratories; in addition, these methods have never been tested on α,α-dialkyl glycines, where steric hindrance might prevent formation of the required products. In contrast, this difficulty could be overcome by taking advantage of the Ugi-Passerini four-component condensation [6,7], mainly in the case of *N*,α,α-trialkyl glycines [8], since any primary amine is suitable for generating an *N*-alkyl group in a Ugi-Passerini adduct. To our knowledge this reaction has never been used for such a purpose in the field of amino acid or peptide chemistry, possibly due to inherent difficulties that only recently have been overcome [9]. In fact, by successive improvements of our method that uses the Ugi-Passerini reaction [6,7] and by taking advantage of the lability to acid of the amide bond at the *C*-terminus of the Ugi-Passerini adducts, it was possible to develop a promising strategy suitable for preparing *N*-acyl-*N*-(4-methoxybenzyl)-α,α-dialkyl glycine derivatives as reagents for incorporation of residues of these amino acids into peptide chains [10]. This consists of synthesizing an N-protected *N*-(4-methoxybenzyl) amide of the required α,α-dialkyl glycine followed by treatment with neat trifluoroacetic acid (TFA) to remove the 4-methoxybenzyl group and simultaneously cleaving selectively the *C*-terminal amide bond to yield an N-protected amino acid or

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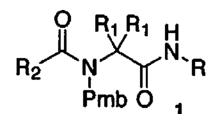
peptide acid ready for elongation towards its *C*-terminus. Later, it was found that cleavage of the *C*-terminal amide bond is faster than the loss of the 4-methoxybenzyl (Pmb) group at the amino acid nitrogen atom if diluted TFA in acetonitrile is used; this allowed cleavages to be carried out with further selectivity and various *N*-acyl-*N*-(4-methoxybenzyl)-α,α-dialkyl glycines to be isolated in fair to good yields. However, our results with some of the bulkier compounds showed that reaction yields, path and selectivity are greatly influenced by the nature of the substituents and are concentration dependent with respect to acid, thus suggesting kinetically controlled processes. Although a reaction scheme and rationale were proposed to explain this behaviour, they were based exclusively on qualitative considerations and did not allow a clear understanding of why the yields and reaction products could vary as much as was found. The Pmb group can be cleaved with TFA from the nitrogen atom of a peptide bond at any stage of a synthesis, as was used previously to prevent the so called 'sixth residue effect' during solid phase peptide synthesis with common amino acids [11]. Thus, *N*-(4-methoxybenzyl)-α,α-dialkyl glycines may become particularly important for peptidomimetics, their *N*-alkyl group playing the role either of a permanent or of a temporary feature. Bearing this in mind, a further investigation was undertaken of the behaviour of these compounds and this paper now presents (i) the results of accurate kinetic measurements of the amide bond selective cleavage of the same model compounds (**1a**–**1h**, Scheme 1, Figure 1) with the purpose of quantitatively evaluating the effect of substituents on reactivity and (ii) HPLC quantitative data supporting and further clarifying the cleavage mechanism and the reaction paths previously proposed. On the one hand, the use of *N*-benzoyl compounds permits comparison of our kinetic measurements with those performed by Creighton *et al.* [12] on various substituted *N*-benzoyl-*N*,α,α-trimethyl glycine amides; on the other hand, as the *N*-phenylacetyl group is a suitable model for a peptidyl moiety, our results may be useful in the future planning of syntheses of peptides with α,α-dialkyl glycine residues by taking advantage of the selective cleavage of the *C*-terminal amide bond of Ugi-Passerini adducts.



1a: R^1 = Me, R^2 = $PhCH_2$, R^3 = 4-CH_3O-C_6H_4-CH_2 (Pmb)
1b: R^1 = Me, R^2 = Ph, R^3 = Pmb
1c: R^1 = Me, R^2 = $PhCH_2$, R^3 = C_6H_{11}
1d: R^1 = Me, R^2 = Ph, R^3 = C_6H_{11}
1e: R^1 = $PhCH_2$, R^2 = $PhCH_2$, R^3 = Pmb
1f: R^1 = $PhCH_2$, R^2 = Ph, R^3 = Pmb
1g: R^1 = $PhCH_2$, R^2 = $PhCH_2$, R^3 = C_6H_{11}
1h: R^1 = $PhCH_2$, R^2 = Ph, R^3 = C_6H_{11}

Scheme 1

RESULTS AND DISCUSSION

Substrates **1a**–**1h** were prepared according to the procedures described in our previous paper [10] and acidolysed under controlled temperature (25.00 ± 0.01 °C) with a solution of TFA in acetonitrile; samples were collected at regular intervals for HPLC using an aqueous solution of acetonitrile as the eluent in connection with a reverse phase column monitored at 260 nm wavelength. Preliminary treatment of each compound with different concentrations of TFA in acetonitrile showed that a 5% solution would yield an acceptable number of points for each of the required kinetic measurements.

Assignment of chromatographic peaks

As shown in Table 1, for six of the eight compounds under investigation, several chromatographic peaks were obtained in addition to that corresponding to the starting material. Peak assignments were carried out by injecting a mixture of an aliquot of the reaction mixture with an acetonitrile solution of each of the possible reaction products **3** or **6** (Scheme 2), i.e. **3a** and **6a** for **1a** and **1c, 3b** and **6b** for **1b** and **1d, 3e** and **6e** for **1e** and **1g**, and **3f** and **6f** for **1f** and **1h**. Most of these standards were obtained by preparative scale acidolyses with 5% TFA under a procedure similar to that described elsewhere [10] but without adding base at the end of the reactions, as described in the Experimental section, in order to maintain an excess of TFA during the work-up procedures. In the case of substrates **1b, 1d, 1e** and **1g** this allowed in a single preparation not only product **3** resulting from scission of the amide bond (Scheme 2, Path A) to be obtained but also product **6** from further cleavage of the Pmb group at the amino acid nitrogen atom. In the case of substrates **1f** and **1h** almost only oxazolone **5f** could be obtained by this procedure, a solution of this compound in acetonitrile containing 5% TFA being used to assign the chromatographic peak corresponding to **4f**. Consequently, compounds **3f** and **6f** were prepared by the procedure described elsewhere [10], the same applying to **6a**, which could not be obtained by the above modified procedure due to the high stability of **3a** under the corresponding experimental conditions. Commercial 4-methoxybenzyl ammonium (**8**) was used to assign a low retention time peak found in the chromatograms obtained with compounds **1a, 1b, 1e** and **1f**. By not bearing an aromatic moiety, the corresponding fragment from substrates **1c, 1d, 1g** and **1h**, i.e. cyclohexyl ammonium, could not be detected at the frequency used in the HPLC monitor.

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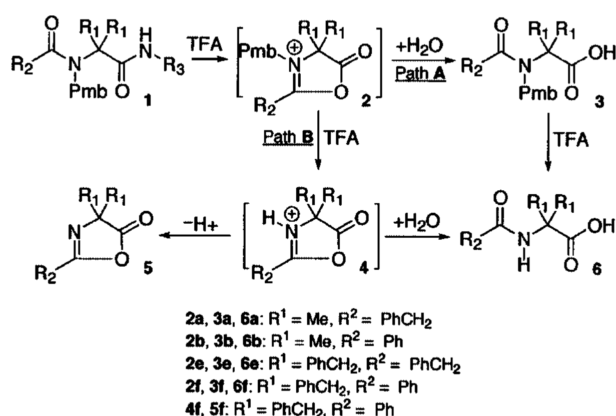
Table 1 Retention Times and Assignment of the Various Peaks Observed in the Acidolysis of Substrates **1a**–**1h**

Reagent	Retention times (min) and assignment							Eluent	
	8[a]	**6**	**2**	**3**	**7**	**4**	**1**	Composition[b]	Flow (ml s^{-1})
1a	1.2	—	—	1.8	—	—	2.3	AcN/water, 3 : 1	26.7
1b	1.7	2.1	2.5[c]	4.7	—	7.5[c]	9.5	AcN/water, 1 : 1	26.7
1c	—	—	—	3.0	—	—	7.5	AcN/water, 2 : 1	26.7
1d	—	2.1	2.5[c]	4.7	—	7.5[c]	11.4	AcN/water, 1 : 1	26.7
1e	1.7	2.2	2.6[c]	4.1	—	4.8[c]	7.5	AcN/water, 2 : 1	33.3
1f	1.7	2.2	3.3[c]	4.7	4.7	7.7	9.7	AcN/water, 3 : 1	26.7
1g	—	2.2	2.6[c]	4.1	—	4.8[c]	17.0	AcN/water, 3 : 1	26.7
1h	—	2.2	3.3[c]	4.7	4.7	7.7	12.1	AcN/water, 2 : 1	30.0

[a] 4-Methoxybenzyl ammonium.
[b] AcN, acetonitrile.
[c] Tentative assignment.



Scheme 2

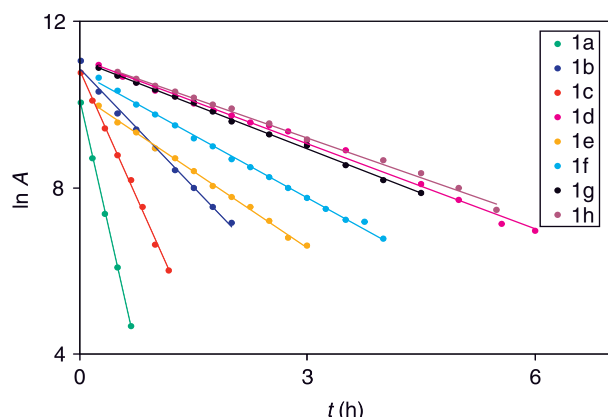


Figure 1 Linear variation of ln *A*, where *A* is a peak area, with time (*t*) for one experiment with each substrate **1a**–**1h**.

In order to complete the assignment of the chromatographic peaks, the reaction mixtures concerning acidolyses of substrates **1b** and **1d**–**1h** were submitted to quenching experiments with a large excess of water when most or all of the starting material had been consumed and the results are presented in Table 2. In all cases fading of two chromatographic peaks was observed, thus indicating that all the reactions yielded two compounds that were unstable in water when TFA was present. In the case of compounds **1b** and **1d** the quenching time was similar or shorter than the retention time; this must be due to differences in the TFA concentration, which in the chromatographic column must decrease rapidly owing to separation of the reaction components and thus allow observation of the corresponding peaks. With substrates **1f** and **1h** one of these compounds was oxazolonium **4f** as described above; the other was tentatively assigned to oxazolonium **2f**, from which **4f** must have formed by acid catalysed loss of the *N*-Pmb group. In fact, as anticipated by Creighton *et al.* [12] and supported by our own results [10], selective cleavage of the *C*-terminal amide bond would proceed in all cases *via* an oxazolonium-type intermediate; this would undergo hydrolytic cleavage with trace water in the solvent to give the corresponding open chain derivative (**3** in Scheme 2). It is noteworthy that, in order to make sure that the reactions had stopped at the thus greatly diminished concentration of acid, in their experiments these authors submitted all their samples to quenching before injection in the chromatographic column; this might have prevented them from detecting the formation of the postulated compounds. By combining the data of Table 1 with that of Table 2 it was possible to reach a tentative assignment of the fading peaks in the chromatograms of the remaining substrates, as shown.

In order to evaluate the progress of the reactions following acidolysis, the reaction mixtures were monitored by HPLC for a period of time at least twice as long as that required to consume all the starting material. Figures 2–4 show examples of plots of peak areas against time for the acidolysis of substrates **1b, 1e** and **1f** and these are presented in two different scales (A

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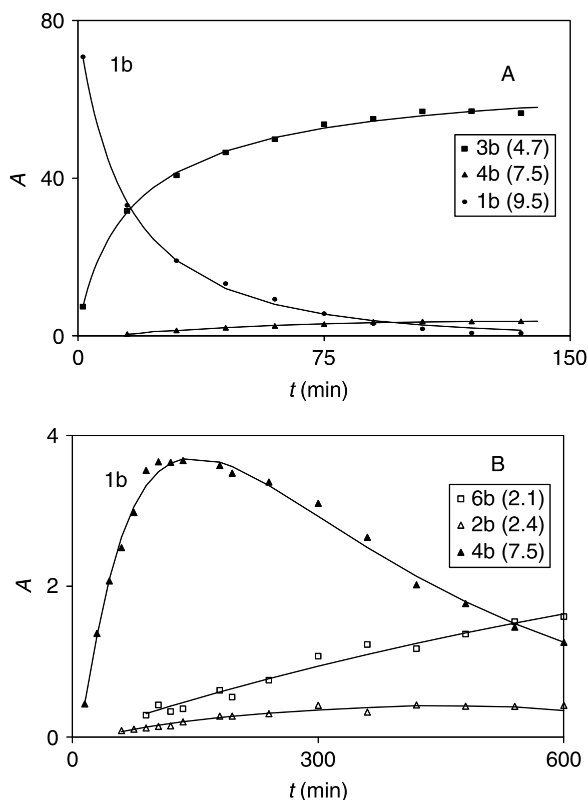

Figure 2 Plots of peak area (A) against reaction time (t) for an acidolysis experiment with substrate **1b** (A and B). The figures in brackets are retention times (min).

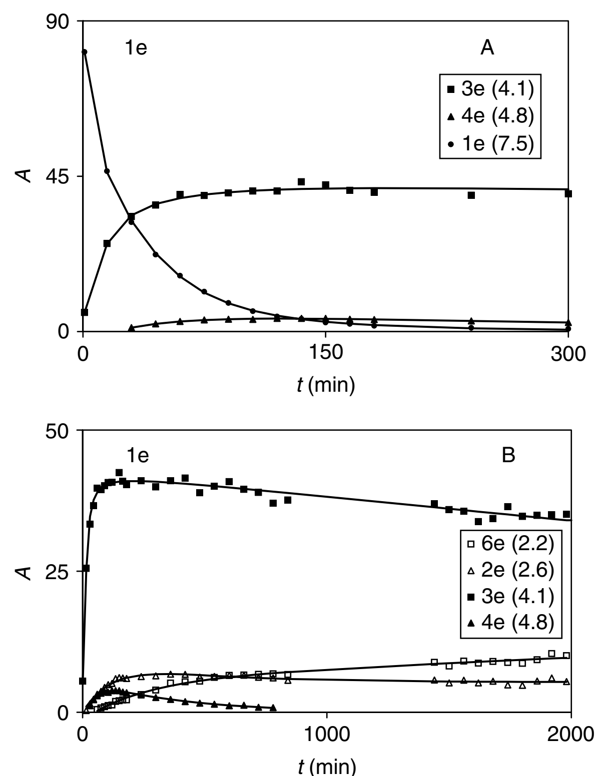

Figure 3 Plots of peak area (A) against reaction time (t) for an acidolysis experiment with substrate **1e** (A and B). The figures in brackets are retention times (min).

and B) in order to show the relevant features; compounds **1d, 1g** and **1h** behaved similarly to the above three substrates, respectively. The shape of the curve labelled with **4f** in Figure 4 is typical of a final product and differs from that labelled with **2f**, which is typical of an intermediate compound and consistent with a precursor of **4f**. The labels **2b, 2e, 4b** and **4e** in Figures 2 and 3 were assigned by comparison with the data in Tables 1 and 2, and also with similar assignments in Figure 4 and the corresponding curves are also typical of intermediate species in agreement with their expected instability.

Observation of plot B in Figure 4 in detail reveals that the first part of the curve related to compound **3f** has a maximum appearing very early followed by a very pronounced slope. However, instead of decreasing to zero when the reagent had been consumed, the peak areas become approximately constant at a value corresponding to the yield estimated in the end of the reaction for the above compound (~10%). Therefore, this must be a combination of two overlapping curves, one for **3f** whose concentration should increase along the process and another for an unknown intermediate whose concentration would increase to a maximum and then decrease to zero; with the support presented below, this intermediate was assigned tentatively the structure of **7**, as shown in Scheme 3, a precursor of **2f** according to the mechanism postulated by Creighton *et al.* [12]. In quenching experiments carried out during the first hour of the reaction, this effect could not be observed owing to the large, fast and steady increase of the corresponding peak area caused by hydrolytic formation of **3f**.

Kinetic measurements with substrates 1a–1h

Peak areas (A) were measured for each substrate **1a–1h** at five to seven different concentrations in neat acetonitrile within the interval 0.005–0.035 mol dm^{-3} and at least three results were obtained for each concentration. As expected for diluted solutions, analysis of these data revealed a linear relationship between the concentrations and peak areas, which allowed calculation of the reaction rate constants directly from the peak areas. All reactions revealed a pseudo-first order behaviour with respect to the substrate, as shown by the excellent linear variation of ln A with time and exemplified in Figure 1 for one experiment with each substrate. The measured rate constants, k, were calculated by the least squares method for a straight line (the standard deviation of the fits never exceeded 1% of the corresponding k value). The results obtained are presented in Table 3 and are the mean values of at least three independent kinetic experiments. As a wide range of reaction rates was

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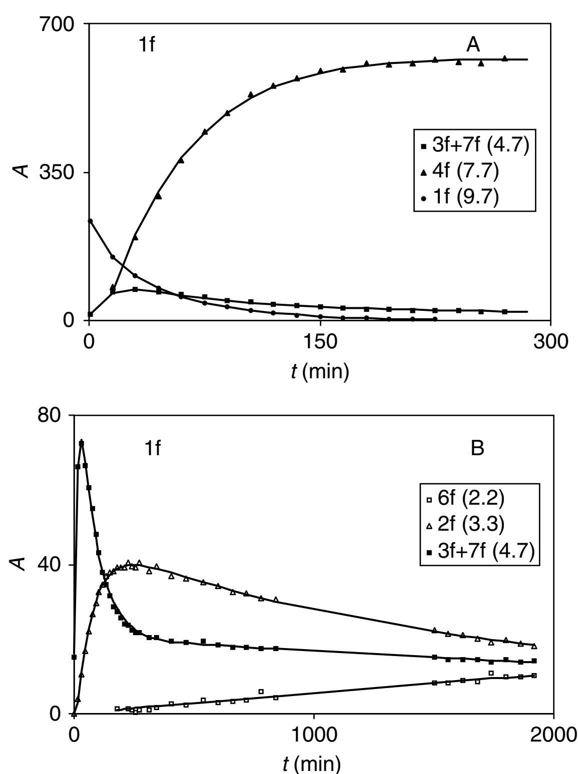

Figure 4 Plots of peak area (A) against reaction time (t) for an acidolysis experiment with substrate **1f** (A and B). The figures in brackets are retention times (min).

found for the various substrates, in order to obtain an acceptable number of points for the individual plots within an equally acceptable time, in the case of faster reactions the eluent was enriched in acetonitrile and its flow rate adjusted accordingly. Since samples had to be collected from the reaction vessel for injection in the chromatographic column, a larger error should be expected in the case of the faster reactions as a consequence of the sampling procedure and this is partially reflected in the values of the mean deviations (dk). For comparative purposes, in addition to k values half-life periods ($t_{1/2}$) are also listed. The dependence of reaction rate constants on the concentration of TFA was equally investigated; in a few experiments carried out with 2% or 10% TFA, the value of the reaction rate constant was found to be directly proportional to the concentration of acid.

Comparison of the k values presented in Table 3 reveals that there are two classes of compounds, namely those having a phenylacetyl group at the N-terminus, whose rate constants decrease in the order **1a** > **1c** > **1e** > **1g**, and those with a benzoyl group, their rate constants decreasing in the order **1b** > **1f** > **1d** ≅ **1h**. Acidolysis of the compounds in the former series is in all cases faster than that of their analogues in the latter series, but the differences among the α,α-dibenzyl glycine derivatives are less pronounced than those found among their α,α-dimethyl glycine analogues. The difference of behaviour between the above two classes of compounds must be related to a decreased nucleophilicity of the carbonyl group in the benzoyl derivatives, possibly due to steric hindrance within the conformation required for internal attack [10,12]. Replacement of the 4-methoxybenzyl group bonded to the reaction centre by cyclohexyl (in compounds **1a, 1b, 1e** and **1f**) decreases the reaction rates to approximately one half; this must be due to the larger bulkiness of the cyclohexyl group compared with Pmb at the C-terminus. Finally, α,α-dimethyl glycines **1a**–**1d** undergo faster acidolysis than the corresponding dibenzyl analogues **1e**–**1h**. On the whole, increased bulkiness of the amino acid side chains makes the reactions much slower as one would expect, but the low reactivity of the benzoyl derivatives seems to decrease drastically the sensitivity of these compounds to the nature and bulkiness of the other substituents.

Mechanistic Interpretation of the Results

By comparison with chromatograms of standard solutions of genuine samples the yields of the various products obtained in the acidolysis of all substrates were estimated immediately after all the starting

Table 2 Quenching Experiments Carried out by Treatment of Substrates **1b** and **1d**–**1h** with a Large Excess of Water

Substrate	R^1	R^3	Peak no.	Retention time[a] (min)	Tentative assignment	Quenching time
1b, 1d	Me	Ph	1	2.4	**2b**	<1 min[b]
			2	7.5	**4b**	≈ 7.5 min[b]
1e, 1g	Bn	$PhCH_2$	1	2.6	**2e**	20 min
			2	4.8	**4e**	3 h
1f,1h	Bn	Ph	1	3.3	**2f**	8 h
			2	7.7	**4f**[c]	10 h

[a] As shown in Figures 2–4 (eluent compositions and flow rates as indicated in Table 1).
[b] See explanation in the text.
[c] This peak compared well with that obtained with a solution of oxazolone **5f** in 2% TFA in acetonitrile.

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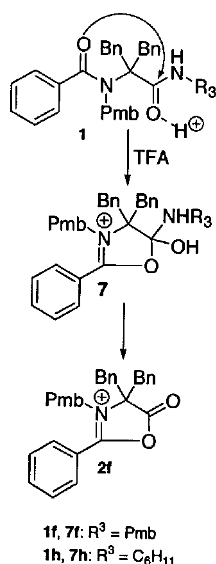
Table 3 Rate Constants (k) and Half-life Period ($t_{1/2}$) for the Reactions of Cleavage of the *C*-terminal Amide Bond in α,α-Dialkyl Glycine Derivatives with 5% TFA in Acetonitrile at 25.00 °C (eluent compositions and flow rates as indicated in Table 1)

Substrate	R^1	R^3	R^4	$(k \pm dk) \times 10^4 (s^{-1})$	$t_{1/2}$ (h)	No. of experiments
1a	Me	$PhCH_2$	Pmb	23.14 ± 0.99	0.08	6 (1 deleted)
1b	Me	Ph	Pmb	5.07 ± 0.35	0.38	5
1c	Me	$PhCH_2$	C_6H_{11}	11.56 ± 0.44	0.17	4
1d	Me	Ph	C_6H_{11}	1.97 ± 0.10	0.98	5
1e	Bn	$PhCH_2$	Pmb	3.38 ± 0.08	0.57	4
1f	Bn	Ph	Pmb	2.91 ± 0.06	0.66	6 (2 deleted)
1g	Bn	$PhCH_2$	C_6H_{11}	1.96 ± 0.04	0.98	5
1h	Bn	Ph	C_6H_{11}	1.95 ± 0.01	0.99	3



Scheme 3

material had been consumed and also a few hours later (Table 4). This showed that formation of **6** results from the acid-promoted loss of the *N*-Pmb group from compound **3** and seems to be assisted by crowding within the amino acid side chains (**1e** and **1g** as compared with **1a** and **1c**) and, to a lesser extent, also by the phenyl group in the benzoyl derivatives (**1b** and **1d** as compared with **1a** and **1c**). In the case of the *N*-benzoyl-α,α-dibenzyl compounds (**1f** and **1h**), where both effects can occur, the major product is oxazolone **5f** as obtained by treatment of the reaction mixture with base [10]. In this case the loss of *N*-Pmb occurs directly from oxazolonium **2** (Scheme 2) and the product is stable on standing, which may be related to steric crowding. No such species could be obtained with the other substrates, possibly because they are not sufficiently crowded and thus are too unstable to be isolated under our conditions.

As reported by Creighton *et al.* [12] and in agreement with some evidence previously reported [10], crystallographic data showed that in two *N*,α,α-trimethyl glycine derivatives the oxygen atom of the *N*-acyl group (O1) is very close to the carbon atom (C6) of the amino acid carbonyl group and this would account for a facile internal nucleophilic attack yielding an oxazolonium derivative. Figure 5 shows an ORTEP diagram for compound **1h**, where the distance between O1 and C6 was found to be still shorter (2.57 Å) than those reported by the above authors (2.61 and 2.69 Å). This is consistent with the increased tendency of amino acid derivatives having bulky side chains to cyclize, as is the case of α,α-dibenzyl glycine compounds.

Conclusions

Our kinetic measurements for substrates **1a**–**1h** showed that while the rate of acidolysis of cyclohexyl amides is one half of that for the corresponding 4-methoxybenzyl compounds, replacement of methyl by benzyl at the amino acid side chains decreases the rate of acidolysis to approximately one sixth. The HPLC data obtained during and after acidolysis allowed the formation of two intermediate compounds to be followed during acidolysis of each of six of the eight substrates investigated. The low stability of these compounds towards hydrolysis led us to assign them oxazolonium structures; this was the first time that these intermediate compounds have been detected and supports the reaction paths depicted in Scheme 2. They are consistent with and would also support and complement the mechanism proposed by Creighton *et al.* [12] for the cleavage of the *C*-terminal amide bond in the case of substituted *N*-benzoyl-*N*,α,α-trimethyl glycine amides. An amide bond cleavage with a similar ancillary participation of a neighbouring amide through a cyclic intermediate as proposed by the authors was recently reported [13]; the 'attacking amide' is conformationally constrained as its fully substituted nitrogen atom belongs to a heterocyclic ring. As previously discussed [10], full substitution of this nitrogen atom seems to be required for a facile cleavage; however, in our case the conformational constraint provided by steric crowding seems to act as a hindering factor that tends to stabilize the

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Table 4 Cleavage of Ugi-Passerini Adducts **1a**–**1h** with 5% TFA

Substrate	R^1	R^3	Product	Time (min)	Yield (%)[a]	Time (min)	Yield (%)[b]
1a	Me	$PhCH_2$	**3a**	50	Quantitative	600	Quantitative
			6a		0		0
1b	Me	Ph	**3b**	150	70	600	69
			6b		0.5		1
1c	Me	$PhCH_2$	**3a**	100	Quantitative	600	Quantitative
			6a		0		0
1d	Me	Ph	**3b**	400	79	600	80
			6b		2		4
1e	Bn	$PhCH_2$	**3e**	300	71	1500	64
			6e		15		31
1f	Bn	Ph	**3f**	300	10	800	9
			4f		87		86
			6e		1		2
1g	Bn	$PhCH_2$	**3e**	400	71	1500	61
			6e		18		29
1h	Bn	Ph	**3f**	400	10	800	8
			4f		87		90
			6e		1		2

[a] By HPLC (eluent compositions and flow rates as indicated in Table 1) when all the reagent had just been consumed.
[b] By HPLC (eluent compositions and flow rates as indicated in Table 1) a few hours after the reagent had been consumed.

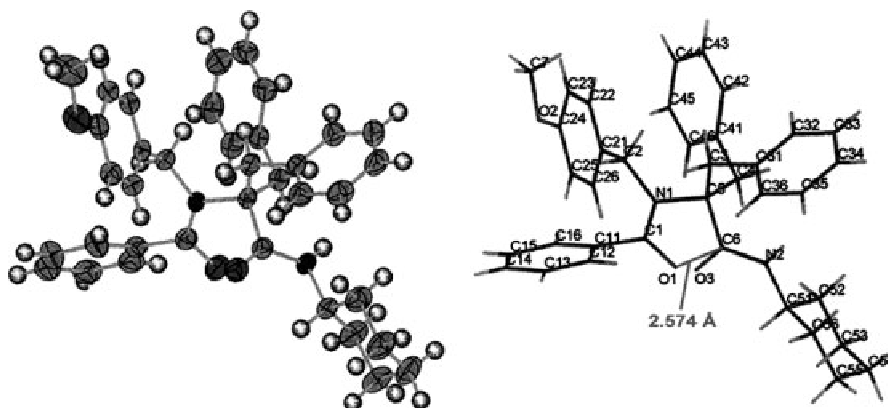

Figure 5 ORTEP diagram and structure for $PhCH_2CO\text{-}N(CH_2C_6H_4OCH_3)C(CH_2Ph)_2CO\text{-}NHC_6H_{11}$ (**1h**).

intermediate cyclic species. In fact, conjugation of the benzoyl phenyl group with the oxazolonium ring of the intermediate cyclic derivatives is not sufficient to explain the fair to high stability of these compounds with regard to hydrolysis. An important role must also be played by the size of the amino acid side chains as observed in all derivatives of α,α-dibenzyl glycine but not in those of α,α-dimethyl glycine. The hindering effect related to the amino acid side chains may take an important part in decreasing the reaction selectivity and in assisting formation of side products. Nevertheless, with the *N*-phenylacetyl substrates appreciable selectivity can still be achieved in cleavage reactions even in the case of the significantly hindered α,α-dibenzyl glycine derivatives, if a judicious choice of the reaction conditions and time is made. Since this group was chosen as a model for a peptidyl moiety, the Ugi-Passerini reaction seems to offer a promising route to peptide syntheses with α,α-dialkyl glycines more crowded than the simple dimethyl analogues.

EXPERIMENTAL

Substrates **1a**–**1h** and reaction products **3f, 6a** and **6f** were prepared according to the procedures published elsewhere [10]. All solvents and reagents were used as obtained from commercial sources. Tri-distilled and de-ionized water was used in HPLC experiments. HPLC measurements were carried out with a Jasco PU-980 intelligent HPLC Pump, a Shimadzu SPD-6AV UV-VIS Spectrophotometric Detector and a Shimadzu C-R6A Chromatopac Printer. A reverse phase

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LiChrospher® 100 RP-18 (5 μm) column was used throughout the work. Temperature stability was maintained throughout the kinetic work with a HAAKE Circulator DL30 set to 25.00 °C with the aid of a Precision® thermometer ranging from 20.00 to 30.00 °C. UV spectra were run with a Hitachi U-2000 spectrophotometer.

General Method 1: Kinetic Measurements

Each Ugi-Passerini adduct (**1a–1h**) was dissolved in dry acetonitrile followed by the addition of the amount of TFA necessary to give a 0.02 M solution of substrate containing the acid at a concentration of 5% (concentrations of 2% and 10% of acid were also used for investigation of the effect of the TFA concentration on the reaction rate). The above solutions were kept under controlled temperature (25.00 ± 0.01 °C) in a thermostatic bath. Samples were collected for HPLC monitoring at regular intervals of time and injected as quickly as possible to minimize errors due to temperature fluctuations. The detection was made at a wavelength of 260 nm and acetonitrile/water mixtures were used as eluents.

General Method 2: Preparative Acidolysis of Ugi-Passerini Adducts

The required Ugi-Passerini adduct (**1a–1h**) was dissolved in dry acetonitrile followed by the addition of the amount of TFA necessary to give a 0.02 M solution of substrate containing the acid at a concentration of 5%. This was stirred at room temperature until no more starting material could be observed by HPLC. The solvent was evaporated at 30 °C and the crude product purified by column chromatography on Merck 230–400 mesh Kieselgel 60 (dichloromethane/MeOH, 50 : 1). The desired fraction was evaporated to dryness to give the corresponding compounds **6** and/or **3** in the case of **1a–1d**,**1e** and **1g**, or the oxazolone **5f** in the case of **1f** and **1h**.

N-Phenylacetyl-*N*-(4-methoxybenzyl)-α,α-dimethylglycine 3a (from 1a and 1c)

Adduct **1a** (0.92 g, 2.0 mmol) gave acid **3a** (0.57 g, 84%) as a white solid, which compared well with a genuine sample [10]. This compound (0.355 g, 88%) was also obtained from **1c** (0.5 g, 1.19 mmol). When all the starting material had been consumed, HPLC showed in both cases that the reaction had been quantitative with regard to **3a**.

N-Benzoyl-*N*-(4-methoxybenzyl)-α,α-dimethylglycine 3b and *N*-Benzoyl-α,α-dimethyl glycine 6b (from 1b and 1d)

Adduct **1b** (0.446 g, 1.0 mmol) gave acids **3b** (0.18 g, 54%) and **6b** (0.09 g, 44%) as white solids, which compared well with genuine samples [10]; when all the starting material had been consumed, HPLC showed that the crude yield of **3b** was 70%. Compounds **3b** (0.36 g, 46%) and **6b** (0.23 g, 45%) were also obtained from substrate **1d** (1.0 g, 2.45 mmol); when all the starting material had been consumed, HPLC showed that in this case the crude yield of **3b** was 79%.

N-Phenylacetyl-*N*-(4-methoxybenzyl)-α,α-dibenzylglycine 3e and *N*-Phenylacetyl-α,α-dibenzylglycine 6e (from 1e and 1g)

Adduct **1e** (0.60 g, 0.96 mmol) gave acid **6e** (0.235 g, 66%) as a white solid; although when all the starting material had been consumed HPLC had shown that the crude yield of **3e** was as much as 71% (and only 15% of **6e**), merely a small amount of this compound (accounting for less than 10%) could be isolated. Both products compared well with genuine samples [10]. Acid **6e** (0.23 g, 63%) was also obtained from **1g** (0.55 g, 0.96 mmol); again at the end of the reaction HPLC showed that in this case the crude yield of **3e** was 71% (and 18% of **6e**), but only a small amount of this (less than 10%) was obtained.

2-Phenyl-4,4-dibenzyl-1,3-(4*H*)oxazol-5-one 5f (from 1f and 1h)

Adduct **1f** (0.5 g, 0.835 mmol) gave oxazolone **5f** (0.255 g, 90%) as a white solid, which compared well with a genuine sample [10]; when all the starting material had been consumed, HPLC showed that the crude yield of **4f** was 87%. Compound **5f** (0.52 g, 86%) was also obtained from **1h** (1.0 g, 1.79 mmol); again at the end of the reaction HPLC showed that in this case the crude yield of **4f** was also 87%.

Crystal Data for Substrate 1h

$C_{38}H_{44}N_2O_4$, $M = 592.75$, monoclinic, $a = 9.5556(7)$, $b = 26.768(2)$, $c = 13.3038(10)$ Å, $U = 3244.1(4)$ Å^3, $T = 293(2)$ K, space group $P2(1)/c$, $Z = 4$, absorption coefficient = 0.078 mm^{-1}, 18 125 reflections measured, 7279 independent reflections ($R_{int} = 0.0457$). The final $wR(F^2)$ was 0.1838 (all data).

Acknowledgements

We thank the Foundation for Science and Technology (Portugal) for financial support to the Institute of Biotechnology and Fine Chemistry (University of Minho) and CECUL (University of Lisbon), and also for scholarship no. SFRH/BPD/1544/2000 to one of us (W.-Q.).

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