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Kinetic investigation of the effect of the amino acid side chains in the selective acidolysis of *N*-acyl-*N*,α,α-trialkyl glycine amides

SIDE CHAIN EFFECTS IN *N*-ACYL-*N*,α,α-TRIALKYL GLYCINE AMIDE ACIDOLYSIS

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Accurate kinetic measurements of the rate constants for the acidolysis of five *N*-acetyl-*N*-(4-methoxybenzyl)-α,α-trialkyl glycine cyclohexyl amides in TFA were performed at 25.00 ºC and the reactions monitored by HPLC. The results were in all cases consistent with a first order behaviour with respect to the substrate. No direct correlation was obtained with this data between rate constant values and structure, but a good correlation coefficient was obtained when a multiple regression analysis was applied by taking advantage of a Taft equation using appropriate polar and steric substituent parameters. In a plot of the values observed for log *k* against those calculated by this equation all five points fell very close to the line of perfect correlation. The calculated sensitivity coefficients to polar and steric contributions were used to discuss the experimental results and showed that the acidolyses are comparatively less affected by steric effects than what one would expect.

Introduction

Steric crowding and/or hindrance to rotation usually recognised in α,α-dialkyl glycines make these compounds good candidates for incorporation into the peptide chain of peptidomimetics for eventual pharmacological use; however, the same features often make such incorporation become problematic, mainly when the amino acid side chains are larger than methyl [1-3]. Difficulties involving the use of these amino acids, most of which cannot be obtained from commercial sources, usually start with their synthesis or that of their derivatives suitable for incorporation into a peptide chain. By successive improvements of our method to use the Ugi-Passerini four-component condensation for the preparation of these compounds [4,5] and by taking advantage of the lability to acid of the amide bond at the C-terminus of α,α-dialkyl glycine derivatives, we were able to investigate a promising strategy to incorporate residues of these amino acids into peptide chains [5]. This consists in cleaving with neat trifluoroacetic acid (TFA) the 4-methoxybenzyl group together with the C-terminal amide bond of an *N*-acyl-*N*-(4-methoxybenzyl)-α,α-dialkyl glycine derivative. The product is the corresponding *N*-acylamino acid or *N*-acylpeptide acid required for elongation towards the C-terminus. Later, we also found that this double cleavage can be performed selectively in two steps [6]; by using sufficiently diluted TFA only the amide bond cleaves to give the corresponding *N*-acyl-*N*,α,α-trialkyl glycine ready for the same purpose. Undoubtedly, the worth of this strategy will be highly dependent on the ability to cleave the amide bond; thus, we carried out a thorough investigation of the behaviour of two sets of differently substituted derivatives of α,α-dimethyl and α,α-dibenzyl glycine, respectively, during acidolysis in order to evaluate the effect of the various substituents [7]. In the course of our work we were able to detect formation of the intermediate oxazolonium that had been predicted by Creighton *et al*. [8] and also by Keating *et al.* [9] and isolate a fairly stable oxazolone resulting from decomposition of this intermediate. We now present the results of accurate kinetic measurements at 25.00 ºC we have undertaken with the aid of HPLC monitoring of the selective cleavage of the C-terminal amide bond in a set of five α,α-dialkyl glycine derivatives (**1a**-**1e**) differing from each other only on the size of their α-alkyl groups (Scheme 1). The *N*-acetyl group is sufficiently stable under acidolyses to act as a model for peptidyl, the *N*-alkyl group chosen (4-methoxybenzyl – Pmb) can be cleaved under forcing acidolytic conditions if required [5-7] and the choice of cyclohexyl for the C-terminus allowed the use of cyclohexyl isonitrile, which is fairly stable and commercially available.

Results and Discussion

Substrates **1a**-**1e** were synthesised by a Ugi-Passerini reaction using acetic acid, cyclohexyl isonitrile, 4-methoxybenzyl amine and the appropriate ketone to generate the required amino acid side chains according to the methodologies described elsewhere [5]; the yields are presented in Table 1. When these substrates were treated with semi-concentrated HCl the only products isolated were the acids **3a**-**3d** resulting from selective cleavage of the C-terminal amide bond (Scheme 1). Good yields (78-86%, Table 2) were obtained with all compounds but the dibenzyl substrate, which gave only a trace of **3e**; in this case 48% hydrobromic had to be used but no more than a fair yield (45%) could be obtained, which was related to the low solubility of this substrate in water. The acidolyses were also conducted in organic medium with 2% TFA in acetonitrile to give quantitative or almost quantitative yields (Table 2) with all substrates. When the reactions were monitored by HPLC clean chromatograms were obtained showing only the reagent and a single product, which proved to be acid **3**. The assignments were performed by chromatographing aliquots of the reaction mixture to which an equal amount of a solution of the corresponding compound **3** had been added; a similar procedure was used with regard to the reagent. Differently from what had been previously observed when a higher concentration of TFA was used in similar reactions [7], no oxazolonium peaks could now be detected possibly because with 2% TFA the acidolyses are sufficiently slow to allow complete hydrolysis of the intermediate cyclic species. Thus, it is understood that the mechanism of the reactions under investigation is that proposed by Creighton *et al.* [8] and supported by our own results [6,7], i.e. that the acydolytic cleavage yields an oxazolonium species that undergoes subsequent hydrolysis with trace water in the solvent (Scheme 1).

Kinetic investigation of substrates 1a-1e. Acidolyses carried out prior to the kinetic measurements with various concentrations of TFA in acetonitrile showed that a concentration of 2% would allow using the same experimental conditions with all compounds and simultaneously secure both an acceptable amount of points for each kinetic plot and reactions reaching the end within a reasonable period of time, i.e. 30 minutes for the fastest reaction (substrate **1b**) and 12 hours for the slowest one (substrate **1e**). All substrates were reacted under controlled temperature (25.00 ± 0.01 ºC) and samples were collected at regular intervals for HPLC monitoring with chromatogram recording until all the reagent had been consumed; peak areas were those printed by the chromatographic machine as the result of automatic integration of each chromatographic peak. An example of the plots of peak areas, A, *vs.* time is presented in Figure 1 for a single experiment with the α,α-dimethyl glycine derivative (**1a**). Previously, peak areas were measured for each substrate **1a**-**1e** 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. The analysis of these data revealed

an excellent linear relationship between concentrations and reagent peak areas, which allowed calculation of reaction rate constants directly from peak areas. All reactions exhibited pseudo-first order behaviour with respect to the amino acid derivative, which is shown by the also excellent linear variation of ln A as a function of time as obtained from the relationship $\ln A = \ln A_0 - kt$, where A is the peak area at time t, A_0 is the peak area for $t = 0$ and k is the rate constant. As an example, ln A *vs*. t plots for one experiment concerning each substrate are presented in Figure 2. The observed rate constants, k, were calculated by the linear least squares methodology 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; dk represent their mean deviations. For comparative purposes, in addition to k values half-live periods, $t_{1/2}$, are also listed. The dependence of reaction rates on the concentration of TFA was also investigated; thus, in a few experiments carried out with 1% or 5% TFA, the reaction rates were found to be directly proportional to the concentration of acid.

As no direct correlation between rate constant values and structure was obtained, a regression analysis was applied by taking advantage of the following multiparametric Taft equation [10,11]

$$\log k = \log k_0 + \rho^* \sigma^* + \delta E_s$$

where σ^* and E_s are Taft's polar and steric substituent parameters, respectively (Table 4). By fitting the experimental values for compounds **1a**-**1e** in this equation, the following fairly good correlation was found

$$\log k = -2.834 - 3.799\sigma^* + 0.7675E_s$$

with a correlation coefficient of 0.984, a standard deviation of 0.15 and a confidence level of 95% as calculated by the statistical Fisher test. A plot of the values of σ^* *vs.* E_s used [9,10] shows a correlation coefficient (r) as low as 0.188, thus meaning that there is no appreciable collinearity in these explanatory variables; this indicates that the explanatory variables σ^* and E_s are independent and, thus, allows using them in the same equation to quantify two different effects (polar and steric, respectively). In order to demonstrate the validity of the above numeric multiparametric Taft equation, the values observed for log k were plotted against those calculated by this equation, which is shown in Figure 3; all five points fall very closely to the bisectrix of perfect correlation. According to the above correlation coefficient, standard deviation and confidence level, we may

conclude that the fit obtained describes well the acidolysis of compounds **1a**-**1e**, which is in agreement with an acid catalysed process.

Conclusions. It was found that the C-terminal amide bond of the Ugi-Passerini adducts investigated can be cleaved selectively not only with TFA in acetonitrile as previously reported [5-7] but also with aqueous hydrogen halides, although in this case lower yields were obtained possibly due to insufficient solubility of the compounds in water. The kinetic measurements showed that the rate constants depend very much on the structure of the amino acid side chains. Thus, substrates having linear side chains at the α-carbon atom (**1a**-**1c**) show larger rate constants than those having branched chains (**1d** and **1e**); the dibenzyl substrate is that presenting the lowest value. The use of a multiparametric Taft equation allowed to quantify both polar and steric substituent effects. The coefficients in the above equation show that the reactions are more sensitive to polar than to steric effects. Although the reaction rate of acid catalysed processes usually depends almost only on steric effects [12], our results show that this is not the case in the acidolysis of the C-terminal amide bond of α,α-dialkyl glycine derivatives where steric hindrance seems to play a fairly unimportant role as compared with polar contributions (*cf*. position of Me in the plot of Figure 3). Having in mind to use the reaction products for coupling at their C-terminus, this behaviour ensures that independently from the size and degree of branching of the amino acid side chains, one may expect selective cleavage to occur satisfactorily.

Experimental Section

All ketones but dibenzyl ketone were freshly distilled. Methanol and toluene were dried by standard procedures. All other solvents and reagents were used as obtained from commercial sources. Tri-distilled and de-ionised water was used in HPLC experiments. TLC analyses were carried out on 0.25 mm thick precoated silica plates (Merck Fertigplatten Kieselgel $60F_{254}$) and spots were visualised under UV light or by exposure to vaporised iodine. Preparative chromatography was carried out on Merck Kieselgel 60 (230-400 mesh). All melting points were measured on a Gallenkamp melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded at 25 °C in ~5% $CDCl_3$ solution on a Varian 300 Unity Plus spectrometer; all shifts are given in δ ppm using δ_H $Me_4Si = 0$ and *J*-values are given in Hz, and assignments were made by comparison of chemical shifts, peak multiplicity and *J*-values. ^{13}C NMR spectra were recorded with the same instrument at 75.4 MHz and using the solvent peak as internal reference; assignments were carried out by the

DEPT 135 technique. Elemental analyses were carried out on a Leco CHNS 932 instrument. 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 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 ºC with the aid a Precision® thermometer ranging from 20.00 to 30.00 ºC.

***N*-Acetyl-*N*-(4-methoxybenzyl)-α,α-dimethyl glycine cyclohexyl amide, 1a**. Acetic acid (4.02 g, 67 mmol) and 4-methoxybenzyl amine (9.13 g, 67 mmol) were dissolved in acetone (50 ml). After stirring at room temperature for 15 min, cyclohexyl isocyanide (7.27 g, 67 mmol) was added and the reaction mixture stirred for three days at room temperature, in the dark and under nitrogen. The precipitate formed during the reaction was filtered off and the filtrate evaporated to dryness. The residue obtained was triturated with petroleum ether (40-60) °C and the second crop thus obtained was combined with the previous one and recrystallised from ethyl acetate/diethyl ether to yield **1a** (18.5 g, 80%) as a white solid, mp 135-136 °C (Found: C, 69.1; H, 8.5; N, 8.1. $C_{20}H_{30}N_2O_3$ requires C, 69.3; H, 8.7; N, 8.1%). ^{1}H NMR ($CDCl_3$) δ ppm: 1.17 (m, 3H , cHex); 1.38 (m, 2H, cHex): 1.41 (s, 6H , 2 × CH_3); 1.63 (m, 3H, cHex); 1.93 (m, 2H, cHex); 2.09 (s, 3H, CH_3); 3.77 (m, 1H, cHex); 3.80 (s, 3H, OCH_3); 4.58 (s, 2H, CH_2-Ar); 5.52 (d, 1H, NH); 6.91 (d, 2H, *J* 8.7, Ph-$H_{2,6}$); 7.38 (d, 2H, *J* 8.7, Ph-$H_{3,5}$) ^{13}C NMR (DMSO-d_6, DEPT) δ ppm: 23.04 (2 × CH_3); 24.37 (CH_3); 24.84 (2 × CH_2); 25.56 (CH_2); 32.99 (2 × CH_2); 47.65 (CH_2); 48.19 (CH); 55.23 (OCH_3); 62.15 (C^{α}); 114.19 (Ph-$C_{2,6}$); 127.19 (PH-$C_{3,5}$); 130.41 (Ph-C_1); 158.69 (Ph-C_4); 171.67 (NC=O); 174.10 (NHC=O).

General Method 1: Synthesis of Ugi-Passerini adducts 1b-1e

A freshly distilled Schiff base (prepared by azeotropic reflux of the corresponding ketone and 4-methoxybenzyl amine [5]) (0.1 mol) and acetic acid (0.1 mol) were added to a flask containing dry MeOH (50 ml). After stirring at room temperature for 15 min, cyclohexyl isocyanide (0.1 mol) was added. The reaction mixture was stirred for 3-7 days at room temperature, in the dark and under nitrogen. If a precipitate had formed either during the reaction or by concentration at the end, it was filtered off, the filtrate evaporated under reduced pressure and the residue triturated with petroleum ether (40-60) °C. The second crop thus obtained was combined with the previous one and recrystallised.

***N*-Acetyl-*N*-(4-methoxybenzyl)-α,α-diethyl glycine cyclohexyl amide, 1b**. The reaction was carried out on a 0.1-molar scale and the crude product recrystallised from ethyl acetate/diethyl ether

to yield **1b** (32.11 g, 86%) as a white solid, mp 113-115 °C (Found: C, 70.3; H, 9.0; N, 7.6. $C_{22}H_{34}N_2O_3$ requires C, 70.5; H, 9.1; N, 7.5%). ^{1}H NMR ($CDCl_3$) δ ppm: 0.84 (6 H, t, *J* 7.4, 2 × C*H*$_3$CH$_2$), 1.06-1.24 (3 H, m, cHex), 1.28-1.45 (2 H, m, cHex), 1.55-1.76 (5 H, m, cHex + CH$_3$C*H*$_2$), 1.90-2.00 (2 H, m, cHex), 2.03 (3 H, s, CH$_3$CO), 2.04-2.18 (2 H, m, CH$_3$C*H*$_2$), 3.80 (3 H, s, CH$_3$O), 3.80-3.92 (1 H, m, cHex), 4.57 (2 H, s, NCH$_2$), 5.55 (1 H, d, *J* 8.7, NH), 6.93 (2 H, d, *J* 8.4, NCH$_2$Ph-*H*$_{3,5}$), 7.57 (2 H, d, *J* 8.4, NCH$_2$Ph-*H*$_{2,6}$). ^{13}C NMR ($CDCl_3$, DEPT) δ ppm: δ_C (75.4 MHz; $CDCl_3$) 8.31 (*C*H$_3$CH$_2$), 23.14 (*C*H$_3$CO), 23.78 (CH$_3$*C*H$_2$), 24.81 (cHex-*C*$_{3,5}$), 25.53 (cHex-*C*$_4$), 33.11 (cHex-*C*$_{2,6}$), 48.09 (cHex-*C*$_1$), 48.82 (NCH$_2$), 55.14 (CH$_3$O), 68.36 (C^{α}), 114.09 (NCH$_2$Ph-*C*$_{3,5}$), 127.12 (NCH$_2$Ph-*C*$_{2,6}$), 131.15 (NCH$_2$Ph-*C*$_1$), 158.45 (NCH$_2$Ph-*C*$_4$), 171.90 (CH$_3$*C*O), 172.72 (CONH).

***N*-Acetyl-*N*-(4-methoxybenzyl)-α,α-dipropyl glycine cyclohexyl amide, 1c**. The reaction was carried out on a 0.025-molar scale and the crude product recrystallised from ethyl acetate/petroleum ether (40-60) °C to yield **1c** (9.23 g, 92%) as a white solid, mp 128-130 °C (Found: C, 71.6; H, 9.5; N, 7.2. $C_{24}H_{38}N_2O_3$ requires C, 71.6; H, 9.5; N, 7.0%). ^{1}H NMR ($CDCl_3$) δ ppm: 0.89 (6 H, t, *J* 7.2, 2 × C*H*$_3$CH$_2$CH$_2$), 1.08-1.44 (10 H, m, 2 × CH$_3$C*H*$_2$CH$_2$ + cHex), 1.55-1.76 (6 H, m, CH$_3$CH$_2$C*H*$_2$ + cHex), 1.93-2.12 (2 H, m, CH$_3$CH$_2$C*H*$_2$), 2.00 (3 H, s, CH$_3$CO), 3.80 (3 H, s, CH$_3$O), 3.80-3.90 (1 H, m, cHex), 4.54 (2 H, s, NCH$_2$), 5.54 (1 H, d, *J* 7.8, NH), 6.92 (2 H, d, *J* 8.4, NCH$_2$Ph-*H*$_{3,5}$), 7.59 (2 H, d, *J* 8.4, NCH$_2$Ph-*H*$_{2,6}$). ^{13}C NMR ($CDCl_3$, DEPT) δ ppm: 14.46 (*C*H$_3$CH$_2$CH$_2$), 17.35 (CH$_3$*C*H$_2$CH$_2$), 23.22 (*C*H$_3$CO), 24.86 (cHex-*C*$_{3,5}$), 25.54 (cHex-*C*$_4$), 33.14 (cHex-*C*$_{2,6}$), 34.22 (CH$_3$CH$_2$*C*H$_2$), 48.11 (cHex-*C*$_1$), 48.68 (NCH$_2$), 55.14 (CH$_3$O), 67.63 (C^{α}), 114.08 (NCH$_2$Ph-*C*$_{3,5}$), 127.17 (NCH$_2$Ph-*C*$_{2,6}$), 131.22 (NCH$_2$Ph-*C*$_1$), 158.42 (NCH$_2$Ph-*C*$_4$), 171.92 (CH$_3$*C*O), 172.95 (CONH).

***N*-Acetyl-*N*-(4-methoxybenzyl)-α,α-diisobutyl glycine cyclohexyl amide, 1d**. The reaction was carried out on a 0.025-molar scale and the crude product recrystallised from ethyl acetate/petroleum ether (40-60) °C to yield **1d** (9.13 g, 85%) as a white solid, mp 90-92 °C (Found: C, 72.3; H, 9.7; N, 6.7. $C_{26}H_{42}N_2O_3$ requires C, 72.5; H, 9.8; N, 6.5%). ^{1}H NMR ($CDCl_3$) δ ppm: 0.91 (12 H, t, *J* 6.8, 2 × (C*H*$_3$)$_2$CH), 1.05-1.45 (7 H, m, 2 × (CH$_3$)$_2$C*H* + cHex), 1.54-1.76 (7 H, m, CHC*H*$_2$ + cHex), 1.95-2.15 (2 H, m, CHC*H*$_2$), 2.05 (3 H, s, CH$_3$CO), 3.75-3.90 (1 H, m, cHex), 3.81 (3 H, s, CH$_3$O), 4.54 (2 H, s, NCH$_2$), 5.54 (1 H, d, *J* 7.8, NH), 6.94 (2 H, d, *J* 8.7, NCH$_2$Ph-*H*$_{3,5}$), 7.63 (2 H, d, *J* 8.5, NCH$_2$Ph-*H*$_{2,6}$). ^{13}C NMR ($CDCl_3$, DEPT) δ ppm: 23.48 (*C*H$_3$CO), 23.52 (CH$_3$*C*H), 24.53 (*C*H$_3$CH), 24.90 (cHex-*C*$_{3,5}$), 25.27 (*C*H$_3$CH), 25.58 (cHex-*C*$_4$), 33.11 (cHex-*C*$_{2,6}$), 40.37 (*C*H$_2$CH), 48.58 (cHex-*C*$_1$), 48.65 (NCH$_2$), 55.14 (CH$_3$O), 67.31 (C^{α}), 114.13 (NCH$_2$Ph-*C*$_{3,5}$), 127.27 (NCH$_2$Ph-*C*$_{2,6}$), 131.15 (NCH$_2$Ph-*C*$_1$), 158.48 (NCH$_2$Ph-*C*$_4$), 171.79 (CH$_3$*C*O), 172.78 (CONH).

***N*-Acetyl-*N*-(4-methoxybenzyl)-α,α-dibenzyl glycine cyclohexyl amide, 1e**. The reaction was carried out on a 0.011-molar scale, using 1.1 equivalent of the correponding Schiff base, and the crude product recrystallised from ethyl acetate/diethyl ether to yield **1e** (3.63 g, 73%) as a white solid, mp 193-194 °C (Found: C, 77.0; H, 7.7; N, 5.7. $C_{32}H_{38}N_2O_3$ requires C, 77.0; H, 7.7; N, 5.6%). ^{1}H NMR ($CDCl_3$) δ ppm: 0.76-0.96 (2 H, m, cHex), 1.00-1.15 (1 H, m, cHex), 1.20-1.35 (2 H, m, cHex), 1.50-1.65 (5 H, m, cHex), 2.01 (3 H, s, CH_3CO), 2.96 (2 H, d, *J* 12.0, $C^{\alpha}CH_2$), 3.39 (2 H, d, *J* 11.5, $C^{\alpha}CH_2$), 3.52-3.68 (1 H, m, cHex), 3.61 (2 H, s, NCH_2), 3.77 (3 H, s, CH_3O), 5.02 (1 H, d, *J* 7.8, NH), 6.88 (2 H, d, *J* 9.0, NCH_2Ph-$H_{3,5}$), 7.28-7.40 (10 H, m, 2 × Ph), 7.57 (2 H, d, *J* 8.4, NCH_2Ph-$H_{2,6}$). ^{13}C NMR ($CDCl_3$, DEPT) δ ppm: 22.25 (CH_3CO), 24.76 (cHex-$C_{3,5}$), 25.51 (cHex-C_4), 32.52 (cHex-$C_{2,6}$), 36.03 ($C^{\alpha}CH_2$), 48.02 (cHex-C_1), 48.16 (NCH_2), 55.11 (CH_3O), 69.04 (C^{α}), 113.96 (NCH_2Ph-$C_{3,5}$), 126.95 (NCH_2Ph-$C_{2,6}$), 127.01 (Ph-C_4), 128.25 (Ph-$C_{3,5}$), 130.76 (NCH_2Ph-C_1), 130.84 (Ph-$C_{2,6}$), 135.60 (Ph-C_1), 158.32 (NCH_2Ph-C_4), 170.89 (CH_3CO), 172.57 (CONH).

General Method 2: Preparation of compounds 3a-3e by selective acidolysis of the C-terminal amide bond of 1a-1e with aqueous hydrochloric acid

Compounds **1a**-**1e** (0.01 mol) were suspended in 6M HCl (100 ml) and left stirring at room temperature for 24h, the reaction being followed by TLC. The solid thus formed was filtered off, purified by column chromatography and recrystallised.

General Method 3: Preparation of compounds 3a-3e by selective acidolysis of the C-terminal amide bond of 1a-1e with 2% TFA in acetonitrile

Compounds **1a**-**1e** (1.5 mmol) were dissolved in 75.0 ml of dry acetonitrile followed by addition of the amount of TFA necessary to give a 2% solution of acid. This was kept at room temperature until no more starting material could be observed by HPLC. The solvent was evaporated at 30 ºC 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 compound.

***N*-Acetyl-*N*-(4-methoxybenzyl)-*α,α*-dimethyl glycine, 3a**.

A reaction by General Method 3 on a 1.5-mmolar scale was purified by column chromatography with ethyl acetate/dichloromethane 7:2 as the eluent. Recrystallisation from methanol yielded **3a** (321 mg, 96%). mp 198-199 °C. (Found: C, 63.4; H, 7.1; N, 5.4. $C_{14}H_{19}NO_4$ requires C, 63.4; H, 7.2; N, 5.3%). ^{1}H NMR (DMSO-d_6) δ ppm: 1.25 (s, 6H, 2 × CH_3); 1.94 (s, 3H, CH_3CO); 3.73 (s, 3H, OCH_3); 4.57 (s, 2H, CH_2); 6.93 (d, 2H, *J* 8.0, Ph-$H_{2,6}$); 7.33 (d, 2H, *J* 8.0, Ph-$H_{3,5}$); 11.96 (br,

1H, OH). ^{13}C NMR (DMSO-d$_6$, DEPT) δ ppm: 22.27 (CH_3); 23.31 (CH_3); 46.62 (CH_2); 54.96 (OCH_3); 60.22 (C^{α}); 113.83 (Ph-$C_{2,6}$), 126.86 (Ph-$C_{3,5}$); 130.96 (Ph-C_1); 157.98 (Ph-C_4); 170.27 (NC=O); 175.14 (OC=O). The same product (567 mg, 86%) was obtained in a reaction by General Method 2 on a 2.5-mmolar scale.

***N*-Acetyl-*N*-(4-methoxybenzyl)-α,α-diethyl glycine, 3b**. The crude product of a reaction carried out by General Method 2 on a 0.01-molar scale was purified by column chromatography with ethyl acetate/dichloromethane 7:2 as the eluent. Recrystallisation from diethyl ether yielded **3b** (2.49 g, 85%) as a white solid, mp 177-179 °C. (Found: C, 65.7; H, 7.8; N, 4.8. $C_{16}H_{23}NO_4$ requires C, 65.5; H, 7.9; N, 4.8%). ^{1}H NMR ($CDCl_3$) δ ppm: 0.86 (6 H, t, *J* 7.2, 2 × CH_3CH_2), 1.78 (2 H, sextet, *J* 7.2, CH_3CH_2), 2.06 (3 H, s, CH_3CO), 2.17 (2 H, sextet, *J* 7.2, CH_3CH_2), 3.82 (3 H, s, CH_3O), 4.60 (2 H, s, NCH_2), 6.93 (2 H, d, *J* 8.4, NCH_2Ph-$H_{3,5}$), 7.43 (2 H, d, *J* 8.4, NCH_2Ph-$H_{2,6}$). ^{13}C NMR ($CDCl_3$, DEPT) δ ppm: 8.24 (CH_3CH_2), 22.59 (CH_3CO), 23.59 (CH_3CH_2), 49.06 (NCH_2), 55.22 (CH_3O), 67.70 (C^{α}), 114.17 (NCH_2Ph-$C_{3,5}$), 126.85 (NCH_2Ph-$C_{2,6}$), 130.43 (NCH_2Ph-C_1), 158.54 (NCH_2Ph-C_4), 172.36 (CH_3CO), 177.20 (COOH). The same product (438 mg, 100%) was obtained in a reaction by General Method 3 on a 1.5-mmolar scale.

***N*-Acetyl-*N*-(4-methoxybenzyl)-α,α-dipropyl glycine, 3c.** The crude product of a reaction carried out by General Method 2 on a 0.0125-molar scale was purified by column chromatography with ethyl acetate/ dichloromethane 7:2 as the eluent. Recrystallisation from diethyl ether yielded **3c** (3.18 g, 79%) as a white solid, mp 167-168 °C (Found: C, 67.4; H, 8.3; N, 4.5. $C_{18}H_{27}NO_4$ requires C, 67.3; H, 8.5; N, 4.4%). ^{1}H NMR ($CDCl_3$) δ ppm: 0.89 (6 H, t, *J* 6.9, 2 × $CH_3CH_2CH_2$), 1.20-1.35 (4 H, m, 2 × $CH_3CH_2CH_2$), 1.65-1.75 (2 H, m, $CH_3CH_2CH_2$), 1.98-2.10 (2 H, m, $CH_3CH_2CH_2$), 2.03 (3 H, s, CH_3CO), 3.83 (3 H, s, CH_3O), 4.57 (2 H, s, NCH_2), 6.93 (2 H, d, *J* 8.7, NCH_2Ph-$H_{3,5}$), 7.43 (2 H, d, *J* 8.7, NCH_2Ph-$H_{2,6}$). ^{13}C NMR ($CDCl_3$, DEPT) δ ppm: 14.43 ($CH_3CH_2CH_2$), 17.21 ($CH_3CH_2CH_2$), 22.65 (CH_3CO), 34.18 ($CH_3CH_2CH_2$), 48.93 (NCH_2), 55.21 (CH_3O), 66.94 (C^{α}), 114.18 (NCH_2Ph-$C_{3,5}$), 126.86 (NCH_2Ph-$C_{2,6}$), 130.47 (NCH_2Ph-C_1), 158.51 (NCH_2Ph-C_4), 172.40 (CH_3CO), 177.43 (COOH). The same product (465 mg, 97%) was obtained in a reaction by General Method 3 on a 1.5-mmolar scale.

***N*-Acetyl-*N*-(4-methoxybenzyl)-α,α-diisobutyl glycine, 3d**. The crude product of a reaction carried out by General Method 2 on a 0.005-molar scale was purified by column chromatography with chloroform/methanol 9:1 as the eluent. Recrystallisation from diethyl ether yielded **3d** (1.37 g, 78%) as a white solid, mp 141-142 °C (Found: C, 68.5; H, 8.7; N, 4.2. $C_{20}H_{31}NO_4$ requires C, 68.7; H, 8.9; N, 4.0%). ^{1}H NMR ($CDCl_3$) δ ppm: 0.93 (12 H, t, *J* 6.0, 2 × $(CH_3)_2CH$), 1.65-1.78 (4 H, m, 2 × $(CH_3)_2CH$ + $CHCH_2$), 2.01 (3 H, s, CH_3CO), 2.13 (2 H, q, *J* 9.0, $CHCH_2$), 3.83 (3 H, s, CH_3O), 4.59 (2 H, s, NCH_2), 6.93 (2 H, d, *J* 8.7, NCH_2Ph-$H_{3,5}$), 7.46 (2 H, d, *J* 8.4, NCH_2Ph-$H_{2,6}$). ^{13}C

NMR ($CDCl_3$ + DMSO-d_6, DEPT) δ ppm: 23.57 (CH_3CH), 24.15 (CH_3CH), 41.86 (CH_2), 46.16 (NCH_2), 54.69 (OCH_3), 68.62 (C^{α}), 113.48 (NCH_2Ar-$C_{3,5}$), 124.68 (NCH_2Ar-C_1), 130.88 (NCH_2Ar-$C_{2,6}$), 159.37 (NCH_2Ar-C_4), 173.44 (CH_3CO). The same product (514 mg, 98%) was obtained in a reaction by General Method 3 on a 1.5-mmolar scale.

***N*-Acetyl-*N*-(4-methoxybenzyl)-α,α-dibenzyl glycine, 3e**. The crude product of a reaction carried out by General Method 2 on a 0.005-molar scale, but using 48% aqueous HBr instead of 6M HCl, was purified by column chromatography with dichloromethane/methanol 32:1 as the eluent. Recrystallisation from methanol/diethyl ether yielded **3e** (0.94 g, 45%) as a white solid, mp 215-217 °C (Found: C, 74.5; H, 6.5; N, 3.4. $C_{26}H_{27}NO_4$ requires C, 74.8; H, 6.5; N, 3.4%). ^{1}H NMR ($CDCl_3$) δ ppm: 2.06 (3 H, s, CH_3CO), 2.99 (2 H, d, J 13.6, $C^{\alpha}CH_2$), 3.46 (2 H, d, J 13.8, $C^{\alpha}CH_2$), 3.68 (2 H, s, NCH_2), 3.78 (3 H, s, CH_3O), 6.86 (2 H, d, J 9.0, NCH_2Ph-$H_{3,5}$), 7.32 (2 H, d, J 8.7, NCH_2Ph-$H_{2,6}$), 7.30-7.40 (10 H, m, 2 × Ph). ^{13}C NMR ($CDCl_3$, DEPT) δ ppm: 22.70 (CH_3CO), 36.24 ($C^{\alpha}CH_2$), 48.54 (NCH_2), 55.19 (CH_3O), 68.66 (C^{α}), 114.17 (NCH_2Ph-$C_{3,5}$), 126.68 (NCH_2Ph-$C_{2,6}$), 127.12 (Ph-C_4), 128.47 (Ph-$C_{3,5}$), 130.08 (NCH_2Ph-C_1), 130.80 (Ph-$C_{2,6}$), 135.64 (Ph-C_1), 158.47 (NCH_2Ph-C_4), 173.05 (CH_3CO), 176.05 (COOH). The same product (610 mg, 98%) was obtained in a reaction by General Method 3 on a 1.5-mmolar scale.

General Method 4: Kinetic measurements

Each Ugi-Passerini adduct (**1a**-**1e**) was dissolved in dry acetonitrile, which was followed by addition of the amount of TFA necessary to give a 0.02 M solution of substrate containing the acid at a concentration of 2%. These solutions were kept under controlled temperature (25.00±0.01 ºC) 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 the fluctuation of temperature. The detection was made at the wavelength of 260 nm and eluents of acetonitrile/water mixtures with compositions of 1:1, 2:1 and 3:1 (v/v) were used.

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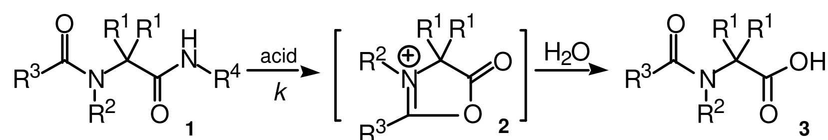

1a, 2a, **3a**: R^1 = Me, R^2 = 4-MeC$_6$H$_4$CH$_2$ (Pmb), R^3 = Me, R^4 = C_6H_{11}
1b, 2b, **3b**: R^1 = Et, R^2 = Pmb, R^3 = Me, R^4 = C_6H_{11}
1c, 2c, **3c**: R^1 = Pr, R^2 = Pmb, R^3 = Me, R^4 = C_6H_{11}
1d, 2d, **3d**: R^1 = *i*Bu, R^2 = Pmb, R^3 = Me, R^4 = C_6H_{11}
1e, 2e, **3e**: R^1 = Bn, R^2 = Pmb, R^3 = Me, R^4 = C_6H_{11}

Scheme 1

Table 1 Synthesis of Ugi-Passerini adducts **1a-1e**

Product	R^1	Yield (%)
1a	CH_3	80
1b	CH_2CH_3	86
1c	$CH_2CH_2CH_3$	92
1d	$CH_2CH(CH_3)_2$	85
1e	CH_2Ph	73

Table 2 Selective cleavage of Ugi-Passerini adducts **1a-1e** with aqueous acid and in 2% TFA in acetonitrile

Reagent	Product	R^1	Acid[a]	Yield (%)	Acid[b]	Yield (%)
1a	**3a**	CH_3	6M HCl	86	2% TFA	96
1b	**3b**	CH_2CH_3	6M HCl	85	2% TFA	100
1c	**3c**	$CH_2CH_2CH_3$	6M HCl	79	2% TFA	97
1d	**3d**	$CH_2CH(CH_3)_2$	6M HCl	78	2% TFA	98
1e	**3e**	CH_2Ph	48% HBr	45	2% TFA	98

[a] Aqueous solution; [b] in acetonitrile.

Table 3 Rate constants (k) and half-life period ($t_{1/2}$) for acidolysis of α,α-dialkyl glycine derivatives 1a-1e with 2% TFA in acetonitrile at 25.00 ºC

Compound	$(k \pm dk)\times10^4$ (s^{-1})[a]	$t_{1/2}$ (h)	No. of experiments
1a	11.14 ± 0.22	0.17	3
1b	31.59 ± 0.27	0.06(1)	3
1c	29.74 ± 0.48	0.06(4)	5 (1 deleted)
1d	7.15 ± 0.23	0.27	3
1e	1.25 ± 0.04	1.54	3

[a] $dk = \Sigma|k - k_i|/n$, where k is the calculated mean value of the reaction rate, k_i is the reaction rate obtained in each experiment and n is the number of experiments run for each compound.

Table 4 Experimental log k values and Taft's polar (σ*) and steric (E_s) substituent parameters [9,10] for substrates **1a**-**1e**

Compound	R^1	log k	σ*	E_s
1a	CH_3	–2.952	0	0
1b	CH_2CH_3	–2.500	–0.1	–0.07
1c	$CH_2CH_2CH_3$	–2.527	–0.115	–0.36
1d	$CH_2CH(CH_3)_2$	–3.146	–0.125	–0.93
1e	CH_2Ph	–3.903	–0.215	–0.38

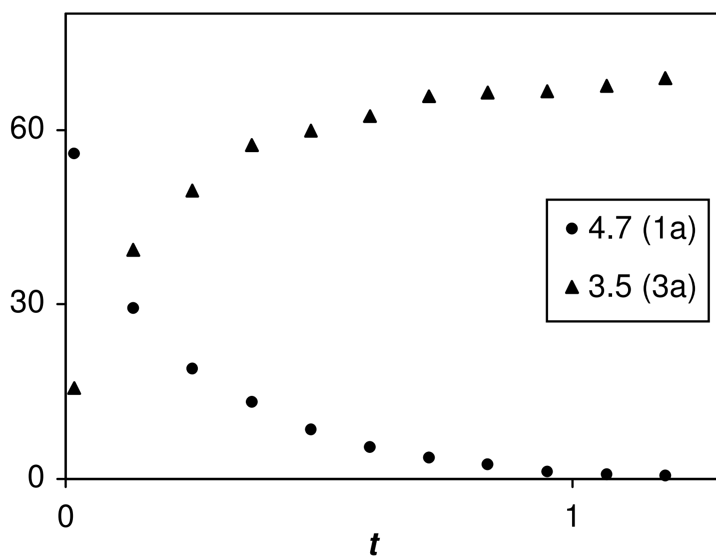

Figure 1 Plot of peak areas, *A*, *vs*. time for an acidolysis experiment with compound **1a**. The figures in the box are the observed HPLC retention times (min).

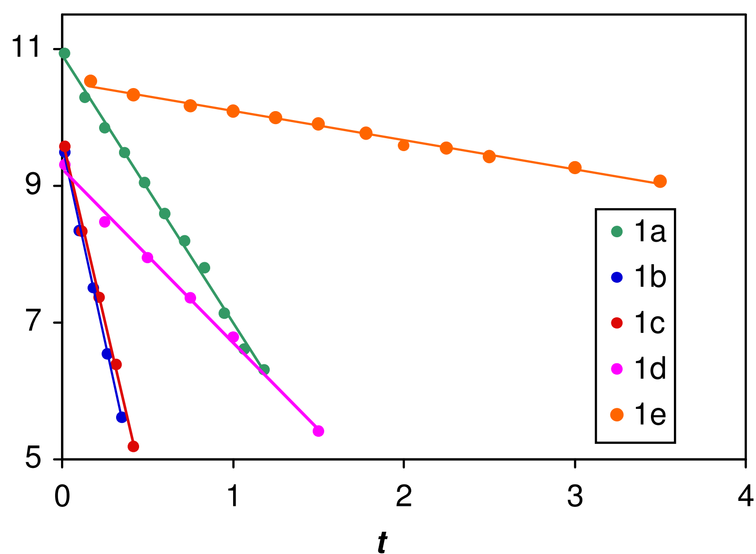

Figure 2 Plots of values of ln *A* *vs*. time for acidolysis of α.α-dialkyl glycine derivatives **1a-1e**.

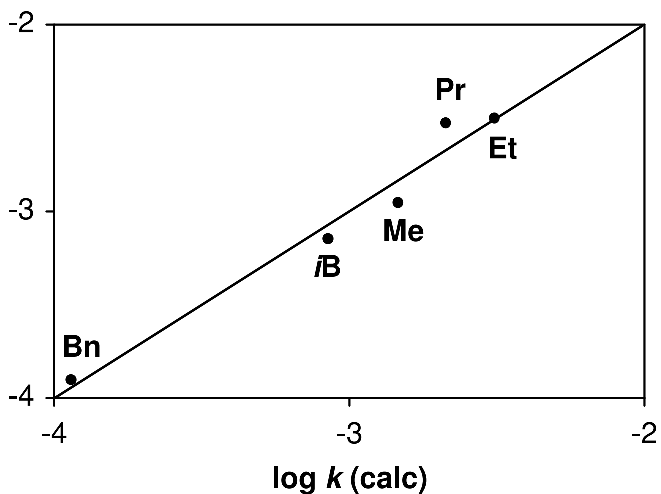

Figure 3 Plot of observed log k values for acidolysis of compounds **1a-1e** against those calculated with $\log k = -2.834 - 3.799\sigma^* + 0.7675E_s$.