Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 2807–2810

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

A Two-Step, One-Pot Synthesis of Diverse *N*-Pyruvoyl Amino Acid Derivatives Using the Ugi Reaction

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Received 31 July 2000; accepted 4 October 2000

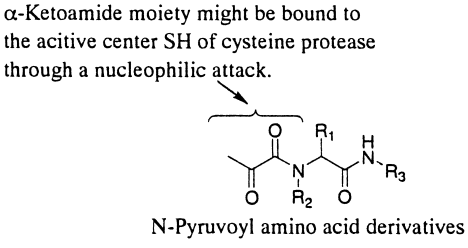
Abstract—A 100-member combinatorial library of α-ketoamides, which was designed to search potent cysteine protease inhibitors, was generated by a parallel solution-phase synthesis. A two-step one-pot synthesis, in which the Ugi reaction followed by pyridinium dichromate (PDC) oxidation was employed in the same reaction vessel, easily afforded the α-ketoamides in a short time.

Introduction

In recent years, combinatorial chemistry has emerged as a powerful tool for rapid identification and optimization of new lead compounds in drug discovery.[1] In this field, the multi-component reaction (MCR) has been used very efficiently to generate chemical diversity in a few reaction steps.[2] The Ugi reaction, which is the most representative example among the MCRs, is a condensation reaction of four components, i.e., carboxylic acid, amine, aldehyde and isocyanide, at a time.[3] Several groups succeeded in creating Ugi-libraries of benzodiazepines,[4] β-lactams,[5] pyrroles,[6] imdazoles[7] and piperazinediones,[8] etc.

Cysteine proteases play important roles in various biological processes.[9] Many diseases, such as central nervous system (CNS) disease,[10] muscular dystrophy,[11] osteoporosis,[12] and cataracts,[13] are associated with elevated proteolytic activity of these enzymes. Therefore, much attention has been paid to the rational design and synthesis of selective inhibitors for the cysteine proteases. Peptidyl aldehyde,[14] α-ketoamide[15] and diaminoketone[16] are reversible inhibitors, which form hemithioacetal or ketal with the active SH of cysteine residue of the enzymes.

Our interests involved using the Ugi reaction to synthesize diverse compounds with an active α-ketoamide moiety in search for cysteine protease inhibitors. In this communication, we describe a convenient method for the generation of an α-ketoamide library in solution-phase synthesis.



N-Pyruvoyl amino acid derivatives

Results and Discussion

We designed *N*-pyruvoyl amino acid derivatives as a synthetic target and planned the synthetic route of two steps as shown in Scheme 1. The first step was the Ugi reaction using a DL-lactic acid as an acid component in methanol to give Ugi compounds (**Ugi{x,y,z}**). The second step was PDC oxidation[17] in dichloromethane to form α-ketoamides (**Keto{x,y,z}**). For a model experiment, five α-ketoamides were prepared as shown in Table 1. The yields of all the compounds except **Ugi{3,3,2}** in the two steps were moderate, ranging from 53 to 75%. The low yield of **Ugi{3,3,2}** may be caused by steric hindrance of aldehyde **3**. This result is compatible with a published report that the Ugi reaction using sterically hindered ketone instead of aldehyde needed high pressure and a long time for completion of the reaction.[18]

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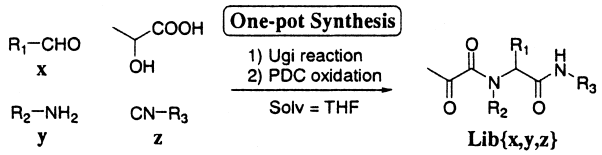
0960-894X/00/$ - see front matter
PII: S0960-894X(00)00577-1

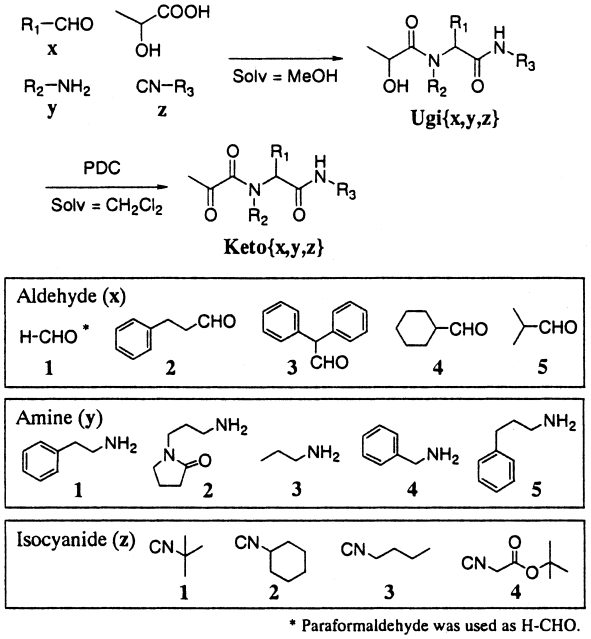
^{1}H NMR spectra of the five α-ketoamides indicate slow interconversion between *cis–trans* isomers in both DMSO-d_6 and $CDCl_3$ at room temperature. This phenomenon complicated the structural characterization of the compounds. A variable-temperature (VT) NMR experiment for these compounds in DMSO-d_6 supported this isomerism. The isomeric NMR peaks of pyruvoyl proton in **Keto{2,2,1}** became closer with raising the temperature, and almost merged at 90 °C. Similar results were observed in VT experiments for **Keto{1,1,1}**, **Keto{3,3,2}** and **Keto{4,4,3}**, although the isomeric NMR peaks didn't merge until 90 °C.

The model experiment showed that it was possible to synthesize 100 α-ketoamides from all the combinations of three components, i.e., five aldehydes, five amines and four isocyanides. As a next experiment, we made an effort to develop a simpler and more rapid method for the generation of combinatorial library. A one-pot synthesis, that is a treatment of Ugi reaction followed by PDC oxidation in the same reaction vessel, was employed to synthesize 100 α-ketoamides **Lib{x,y,z}** as shown in Scheme 2. THF was selected as the reaction solvent of one-pot synthesis, because it is impossible to use methanol in PDC oxidation, although methanol is a general reaction solvent in the Ugi reaction. The reaction and work up were performed with manual synthesizer QUEST-210® (Argonaut Technologies), which enabled us to prepare 10 samples at a time. Removal of solvent was carried out with a centrifuged evaporator. Purification such as column chromatograph and crystallization was avoided. The generation of a 100-member library of α-ketoamides was accomplished by employing the one-pot synthesis and the rapid work up for 12 days.

The purities of five α-ketoamides, **Lib{1,1,1}**, **Lib{2,2,1}**, **Lib{3,3,2}**, **Lib{4,4,3}** and **Lib{5,5,4}** were examined by HPLC analysis. To determine the precise purities, we quantified them using corresponding external standards **Keto{x,y,z}** which were obtained in the model experiment. As shown in Table 2, the purities of the five α-ketoamides, ranging from 68 to 90% (average 80%), were sufficient for initial biological testing. To confirm these structures, the five α-ketoamides were analyzed by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDITOF-MS) using α-cyano-4-hydroxycinnamic acid as the matrix. The desired molecular ions for these compounds were detected as the sodium adducts (Table 2). Furthermore, 17 α-ketoamides were randomly selected among the



Scheme 2.



Scheme 1.

Table 1. Isolated yields of **Ugi{x,y,z}** and **Keto{x,y,z}**

	Ugi{x,y,z} (%)	**Keto{x,y,z}** (%)
{1,1,1}	60	58
{2,2,1}	67	53
{3,3,2}	28	53
{4,4,3}	75	60
{5,5,4}	62	61

Table 2. The purity and MALDITOF-MS analysis data of five α-ketoamides **Lib{x,y,z}**

		$[M+Na]^+$	
Lib{x,y,z}	Purity (%)	Calcd	Found
{1,1,1}	68	327.168	327.176
{2,2,1}	85	452.253	452.244
{3,3,2}	76	457.247	457.252
{4,4,3}	90	395.231	395.232
{5,5,4}	81	441.237	441.232

Table 3. MALDITOF-MS analysis data of randomly selected α-ketoamides **Lib{x,y,z}**

	$[M+Na]^+$	
Lib{x,y,z}	Calcd	Found
{1,1,3}	327.168	327.175
{1,2,1}	348.190	348.188
{1,5,2}	367.200	367.200
{2,2,2}	478.268	478.255
{2,4,4}	475.221	475.275
{3,2,1}	514.268	514.216
{3,2,3}	514.268	514.276
{3,4,2}	505.247	505.253
{3,5,1}	507.262	507.257
{4,3,1}	347.231	347.232
{4,3,2}	373.247	373.246
{4,4,1}	395.231	395.231
{4,5,4}	481.268	481.263
{5,2,4}	448.242	448.235
{5,3,1}	307.200	307.208
{5,4,1}	355.200	355.195
{5,4,2}	381.215	381.213

library, and analyzed by MALDITOF-MS in the same way. As a result, all the compounds were characterized as shown in Table 3. The MALDITOF-MS analysis provided unambiguous evidence that the generation of the α-ketoamide library was a success.

Conclusion

We established a convenient method for the generation of a combinatorial α-ketoamide library, in which the Ugi reaction and PDC oxidation were successively carried out in the same reaction vessel. The Ugi reaction was found to be very effective for generating a library of chemically diverse compounds. Although the confusing NMR spectrum of α-ketoamides due to the slow amide rotation made the characterization of structure difficult, MALDITOF-MS analysis enabled the exact characterization of the structure of α-ketoamides. The biological activities of this library against various cysteine proteases will be evaluated in our laboratory, and generation of similar libraries is now in progress.

Experimental

^{1}H NMR spectra show that the five Ugi compounds and the five α-ketoamides exist as a mixture of *cis–trans* isomers at room temperature. The major and minor peaks of the same proton were separately listed, in case each peak was identified. The assignments of **Ugi{2,2,1}**, **Ugi{3,3,2}**, **Ugi{4,4,3}** and **Ugi{5,5,4}** were not completed, because the NMR peaks of these compounds were extremely broadened due to the presence of diastereomers and *cis–trans* isomers.

General procedure for the preparation of Ugi compounds, Ugi{1,1,1}

Paraformaldehyde (80%) (0.37 g, 10 mmol), 2-phenylethylamine (1.4 g, 12 mmol), DL-lactic acid (0.90 g, 10 mmol) and *t*-butylisocyanide (0.83 g, 10 mmol) were added successively to MeOH (30 mL). The mixture was stirred at room temperature for 48 h, and concentrated in vacuo. The residue was dissolved in EtOAc, and the solution was washed with 1 M HCl, saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$, and then concentrated in vacuo. The residual oil was crystallized from hexane to give **Ugi{1,1,1}** (1.8 g, 60%) as a white solid. Mp 66–68 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ (major/minor) 1.12/1.10 (d/d, 3H, J = 6.6/6.3), 1.25/1.24 (s/s, 9H), 2.72/2.82 (dd/t, 2H, J = 8.7, 6.9/7.7), 3.38–3.43/3.45–3.61 (m/m, 2H), 3.85/3.79 (d/d, 1H, J = 16.8/16.2), 4.00/3.92 (d/d, 1H, J = 16.8/16.1), 4.27 (m, 1H), 4.91/4.92 (d/d, 1H, J = 6.9/7.5), 7.17–7.31 (m, 5H), 7.70/7.38 (s/s, 1H), (major:minor = 1.4:1). MALDITOF-MS $[M+Na]^+$ calcd 329.184, found 329.185.

General procedure for the preparation of α-ketoamides, Keto{1,1,1}

To a solution of **Ugi{1,1,1}** (1.7 g, 5.5 mmol) in CH_2Cl_2 (30 mL) were added PDC (3.1 g, 8.3 mmol) and Celite (2.0 g). After stirring the mixture at room temperature for 24 h, diethyl ether was added, inorganic compound was filtered off, and the filtrate was concentrated in vacuo. The residue was dissolved in EtOAc, and the solution was washed with 1 M HCl, saturated $NaHCO_3$ and saturated NaCl, dried over $MgSO_4$, and concentrated in vacuo. The residual oil was purified by preparative TLC (silica gel, hexane:EtOAc = 1:2), and crystallized from hexane to give **Keto{1,1,1}** (0.98 g, 58%) as a white solid. Mp 69–70 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ (major/minor) 1.22/1.25 (s/s, 9H), 2.28/2.01 (s/s, 3H), 2.73/2.81 (dd/t, 2H, J = 8.7, 6.9/7.4), 3.43–3.50 (m, 2H), 3.94 (s, 2H), 7.15–7.33 (m, 5H), 7.77/7.63 (s/s, 1H), (major:minor = 2.3:1). MALDITOF-MS $[M+Na]^+$ calcd 327.169, found 327.168. Anal. calcd for $C_{17}H_{24}N_2O_3$: C, 67.08; H, 7.94; N, 9.20, found: C, 67.05; H, 8.03; N, 9.24. Purity (HPLC A/A) 99.8%.

Keto{2,2,1}. Mp 69–71 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ (major/minor) 1.25 (s, 9H), 1.66–1.74 (m, 2H), 1.80–1.95 (m, 3H), 2.05–2.20 (m, 3H), 2.32/2.73 (s/s, 3H), 2.46–2.52 (m, 2H), 3.06–3.35 (m, 6H), 4.05/4.61 (t/t, 1H, J = 7.4/7.5), 7.16–7.31 (m, 5H), 7.56/7.65 (s/s, 1H), (major:minor = 1.2:1). MALDITOF-MS $[M+Na]^+$ calcd 452.253, found 452.247. Anal. calcd for $C_{24}H_{35}N_3O_4$: C, 67.11; H, 8.21; N, 9.78, found: C, 67.36; H, 8.27; N, 9.71. Purity (HPLC A/A) 98.9%.

Keto{3,3,2}. Mp 60–65 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ (major/minor) 0.68/0.72 (t/t, 3H, J = 7.5/7.5), 0.80–1.57 (m, 12H), 1.79/2.13 (s/s, 3H), 3.40–3.50/2.82–2.92 (m/m, 2H), 3.22–3.34 (m, 1H), 4.73/4.74 (d/d, 1H, J = 12.0/11.7), 5.72/4.84 (d/d, 1H, J = 12.3/11.7), 7.12–7.44 (m, 10H), 8.13/7.58 (d/d, 1H, J = 7.8/8.1), (major:minor = 2.4:1). MALDITOF-MS $[M+Na]^+$ calcd 457.247, found 457.240. Purity (HPLC A/A) 98.6%.

Keto{4,4,3}. Mp 78–79 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ (major/minor) 0.74–1.65 (m, 14H), 0.85/0.83 (t/t, 3H, J = 6.6/6.6), 1.58/2.38 (s/s, 3H), 1.89 (m, 1H), 2.97–3.03 (m, 2H), 4.67/3.67 (d/d, 1H, J = 10.8/10.5), 4.44/4.68 (d/d, 1H, J = 15.9/15.3), 5.00/4.76 (d/d, 1H, J = 16.2/15.0), 7.05–7.32 (m, 5H), 8.46/8.06 (t/t, 1H, J = 5.4/5.6), (major:minor = 1.3:1). MALDITOF-MS $[M+Na]^+$ calcd 395.231, found 395.243. Anal. calcd for $C_{22}H_{32}N_2O_3$: C, 70.94; H, 8.65; N, 7.52, found: C, 70.97; H, 8.72; N, 7.57. Purity (HPLC A/A): 99.8%.

Keto{5,5,4}. ^{1}H NMR (300 MHz, $CDCl_3$) δ (major/minor) 0.80/0.89 (d/d, 3H, J = 6.6/6.3), 0.91/1.00 (d/d, 3H, J = 6.3/6.3), 1.44/1.43 (s/s, 9H), 1.65–1.93 (m, 2H), 2.40 (m, 1H), 2.51/2.38 (s/s, 3H), 2.49–2.63 (m, 2H), 3.10–3.60 (m, 2H), 3.63/4.05 (d/d, 1H, J = 10.8/11.1), 3.81–4.02 (m, 2H), 7.06–7.30 (m, 6H), (major:minor = 1.4:1). MALDITOF-MS $[M+Na]^+$ calcd 443.254, found 443.239. Anal. calcd for $C_{23}H_{34}N_2O_5$: C, 66.01; H, 8.18; N, 6.69, found: C, 66.19; H, 8.30; N, 6.69. Purity (HPLC A/A) 95.6%.

Procedure for the one-pot synthesis

Equal amounts of 1 M solution of four components in THF (0.8 mL) were combined in a reaction vessel, and

the mixture was stirred at room temperature for 24 h, then PDC (0.36 g) was directly added to the mixture. After stirring for 3 h at room temperature, diethyl ether was added, inorganic compound was filtered off, and the filtrate was diluted with EtOAc. The solution was washed with 1 M HCl, saturated $NaHCO_3$ and saturated NaCl, passed through a silica gel column cartridge (YMC Dispo SPE®), and concentrated in vacuo to give α-ketoamide **Lib{x,y,z}** (Yd: 23–77%).

Acknowledgements

We gratefully thank Mr. M. Hanyu for MS analysis. We also thank Dr. Y.-S. Cui and Mr. Y. Sakai for helpful discussions.

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