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Synthesis of tripeptides containing a very crowded α,α-disubstituted glycine with pyridine rings by solid-phase Ugi reaction

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Abstract—Tripeptides containing a novel α,α-disubstituted glycine with two pyridine rings, α,α-di(2-pyridyl)glycine (2Dpy), were synthesized by the solid-phase Ugi reaction using di(2-pyridyl)methanimine attached directly to a Rink amide resin. Thereby, yields of the tripeptides, Z-AA_1-2Dpy-AA_3-OMe (AA_1 and AA_3 = Gly or Aib), were markedly improved, compared with yields by the solution method.

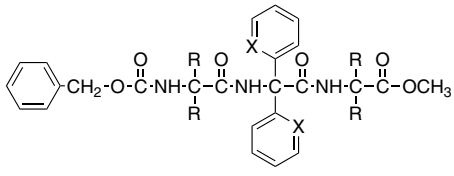
α,α-Disubstituted glycines (DSGs) have been described as a useful tool for restricting the conformational mobility of a peptide backbone.[1] As an application of the ability of DSGs to control conformation, we recently reported the quaternary ammonium-binding ability,[2] and metal ion-binding ability[3] of DSG-containing tetrapeptides.

On the other hand, novel DSGs, which have additional functions are of growing interest: 4-amino-1,2-dithiolane-4-carboxylic acid,[4] 2′,1′:1,2;1″,2″:3,4-dinaphthocyclohepta-1,3-diene-6-amino-6-carboxylic acid,[5] 2,2′-dihydroxy-1,1′-binaphthyl-based crown-carrier DSGs,[6] 1-amino-cyclohenicosanecarboxylic acid,[7] etc.,[8–10] have been reported. However, the synthesis of peptides containing DSGs still presents challenging problems because of the difficulty arising from steric hindrance in the conventional synthesis of the peptides.[11]

Previously, we synthesized a variety of fully protected tripeptides containing a very crowded DSG, α,α-diphenylglycine (Dph), by the modified Ugi reaction,[12–16] and were able to clarify that the Ugi reaction[17] is very useful and potent for the synthesis of sterically hindered peptides containing DSGs. In connection with Dph, we recently synthesized peptides containing α,α-di(2-pyridyl)glycine (2Dpy) as a novel DSG which is expected to have additional functionality.[18] ^{1}H NMR analysis of 2Dpy-containing tripeptides (Z-AA_1-2Dpy-AA_3-OMe (**1**): AA_1, AA_3 = Gly, Aib; see Scheme 1) indicates that **1** adopts unique conformations stabilized by the novel hydrogen bonding between a pyridine nitrogen and an amide hydrogen.[18] We have also found that **1b** is able to self assemble in the presence of a Cu(II) ion, by coordination of the metal ion to pyridine nitrogens.[19]

Though these tripeptides were synthesized by the modified Ugi reaction, their yields were very low. The reaction mixture colorized very darkly and many by-products were produced. Therefore, purification of the desired Ugi product was very troublesome. This may be due to the steric crowding in the reaction intermediate and the reactivity of the pyridine ring itself.[18]

Generally, it is said that the solid-phase synthesis is a powerful tool for organic synthesis and combinatorial chemistry.[20–22] Recently, multicomponent condensation



1a R=H, X=N: Z-Gly-2Dpy-Gly-OMe
1b R=CH_3, X=N: Z-Aib-2Dpy-Aib-OMe
2a R=H, X=CH: Z-Gly-Dph-Gly-OMe
2b R=CH_3, X-CH: Z-Aib-Dph-Aib-OMe

Scheme 1. Structures of 2Dpy/Dph-containing tripeptides.

Keywords: α,α-Disubstituted glycine; α,α-Di(2-pyridyl)glycine (2Dpy); α,α-Diphenylglycine (Dph); Solid-phase synthesis; Ugi reaction.
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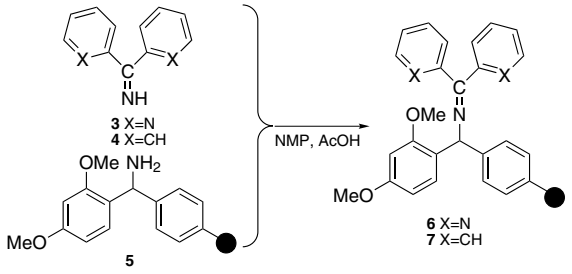
reactions such as the Ugi reaction have also been developed in the area of the solid-phase synthesis.[23–25] Many by-products, which occur from several side reactions, can be separated from the resin by washing with excess solvents. Now, we report a solid-phase synthesis of the very crowded DSGs (2Dpy and Dph)-containing tripeptides by the Ugi reaction.

In the various Ugi reactions by the solid-phase method, Rink amide resin (**5**) has often been used as an amine component.[26,27] As expected, attachment of sterically hindered ketone onto a resin is very difficult compared with that of aldehyde. In order to overcome the difficulty, we attempted to attach the ketone on the resin using transiminum reaction.[28–30] Reaction of Rink amide resin with imines (**3**, **4**) in AcOH/*N*-methylpyrrolidone (NMP) for a day gave the imine-bound Rink amide resins (**6**, **7**), as shown in Scheme 2. Yield of loading of the imine on the resin was estimated by the measurement of UV absorbance after hydrolysis of the resin (**6**, **7**) with 1 N HCl/THF (3/1, v/v), as shown in Table 1. The results indicated that both **6** and **7** which were derived from the imine (**3** and **4**) and Rink amide resin might be used as the imine component.

The resin-bound imine component (**6**, **7**) was subjected to the modified Ugi reaction conditions along with Fmoc amino acid and the isocyanide as shown in Scheme 3.[31] These results are summarized in Table 2. Yields of the 2Dpy-containing tripeptides (**1a**, **1b**) were markedly improved by using the solid-phase method. Many by-products were also produced, but the purification of **1** could be easily performed. Although the yields of the Dph-containing tripeptides (**2a**, **2b**) were not improved by using the solid-phase method, this is not unexpected. In the solution method, **2a** and **2b** were obtained in a higher yield in DCM than in a polar solvent, such as MeOH.[12,15] The slightly lower yields of **2** in the

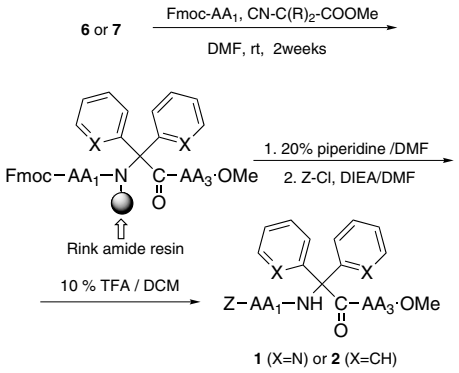


Scheme 2. Transiminum approach.

Table 1. Loading of imine onto Rink amide resin

Run	Imine	X	Loading of imine (mmol/g)	Yield (%)
1	**6**[a]	N	0.56	87.5
2	**6**	N	0.57	89.0
3	**6**	N	0.53	82.8
4	**7**[b]	CH	0.54	84.3
5	**7**	CH	0.51	79.6
6	**7**	CH	0.52	81.2

[a] Determined by UV absorbance at 268 nm.
[b] Determined by UV absorbance at 252 nm.



Scheme 3. Synthetic route of tripeptides.

Table 2. Synthesis of 2Dpy- and Dph-containing tripeptides

	AA_1	R (AA_3)	Method	Yield (%)
1a	Gly	H (Gly)	Solution[a]	7.2[b]
1a	Gly	H (Gly)	Solid-phase	28.2
1b	Aib	Me (Aib)	Solution[a]	12.8[b]
1b	Aib	Me (Aib)	Solid-phase	21.8
2a	Gly	H (Gly)	Solution[a]	31.0[c]
2a	Gly	H (Gly)	Solid-phase	29.6
2b	Aib	Me (Aib)	Solution[a]	35.0[d]
2b	Aib	Me (Aib)	Solid-phase	21.1

[a] In DCM.
[b] See Ref.18.
[c] See Ref.14.
[d] See Ref.15.

solid-phase synthesis seem to be due to a solvent effect related to the high solvent polarity of DMF.[12] Thus, this procedure is particularly effective in preparing 2Dpy-containing tripeptides where many by-products were formed.

Next, we investigated the solvent effect in the preparation of **1**. The results are summarized in Table 3. Yields of both **1a** and **1b** were improved by addition of DCM into NMP (entries 7 and 15), but synthesis of **1** could

Table 3. Survey of conditions for the solid-phase Ugi reaction

Entry		AA_1	R (AA_3)	Solvent	Yield (%)
1	**1a**	Gly	H (Gly)	DMF	28.2
2	**1a**	Gly	H (Gly)	NMP	27.5
3	**1a**	Gly	H (Gly)	TFE	9.5
4	**1a**	Gly	H (Gly)	TFE–DCM (4/1 v/v)	15.3
5	**1a**	Gly	H (Gly)	TFE–DCM (2/1 v/v)	29.6
6	**1a**	Gly	H (Gly)	TFE–DCM (1/1 v/v)	31.4
7	**1a**	Gly	H (Gly)	NMP–DCM(1/1)	32.4
8	**1a**	Gly	H (Gly)	DMF–DCM(1/1)	31.9
9	**1b**	Aib	Me (Aib)	DMF	21.8
10	**1b**	Aib	Me (Aib)	NMP	19.6
11	**1b**	Aib	Me (Aib)	TFE	7.6
12	**1b**	Aib	Me (Aib)	TFE–DCM (4/1 v/v)	12.3
13	**1b**	Aib	Me (Aib)	TFE–DCM (2/1 v/v)	22.9
14	**1b**	Aib	Me (Aib)	TFE–DCM (1/1 v/v)	23.4
15	**1b**	Aib	Me (Aib)	NMP–DCM(1/1)	24.0
16	**1b**	Aib	Me (Aib)	DMF–DCM(1/1)	23.6

not be performed using only DCM as a solvent, because Fmoc-AA_1 hardly dissolved in DCM. In the case of the reaction in 2,2,2-trifluoroethanol (TFE), yields of both **1a** and **1b** were low (entries 3 and 11). This may be due to the poor swelling level of the resin. Yields of both **1a** and **1b** in the case where DCM was added into TFE (entries 6 and 14) were almost the same as in both cases of the addition of DCM into NMP (entries 7 and 15) and DCM into DMF (entries 8 and 16). These results suggested that the control of solvent polarity seems to be an important factor when the synthesis of 2Dpy-containing tripeptides is performed by the solid-phase method.

Although it is said that addition of TFE into an apolar solvent can generally accelerate the reaction rate of the Ugi reaction,[32,33] our results indicated that the yield was reduced by the addition of TFE into DCM (entries 6–3, and 14–11) in the synthesis of the super-hindered peptides containing DSGs by the solid-phase modified Ugi reaction.

In conclusion, yields of 2Dpy-containing tripeptides were markedly improved by using the solid-phase modified Ugi reaction. This method can open up a new way to prepare sterically hindered peptides containing DSGs with pyridine rings.

Acknowledgments

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31. General procedure for the solid-phase synthesis: To a screw-cap fitted reaction vessel was charged the Fmoc-Rink amide resin (500 mg, 0.64 mmol/g, 0.32 mmol), and the resin was treated with 20% piperidine/DMF (6 ml, 2 × 15 min). Then, the solvent was drained and the resin was washed with NMP (5 ml × 5), DCM (5 ml × 5) and dried in vacuo for 30 min. To the reaction vessel was added di(2-pyridyl)methanimine (**3**) (3.2 mmol, 484 mg) or diphenylmethanimine (**4**) (3.2 mmol, 483 mg), AcOH (7.8 mmol, 448 μl) and NMP (6 ml). After shaking for 24 h at room temperature, the solvent was drained and the resin was washed with NMP/DIEA (5 ml × 5, 10:1), NMP (5 ml × 5), MeOH (5 ml × 5) and DCM (5 ml × 5), successively. To a

screw-cap fitted reaction vessel was charged the imine-bound resin (**6** or **7**) (300 mg, 0.56 mmol/g, 0.18 mmol), Fmoc-Gly or Fmoc-Aib (1.8 mmol) and NMP/DCM (5 ml, 1:1). After shaking for 2 h, methyl isocyanoacetate (1.8 mmol) or methyl 2-isocyano-2-methylpropanoate (1.8 mmol) was added to the mixture, which was shaken for 14 days at room temperature. The solvent was drained and the resin was washed with NMP (5 × 5 ml), MeOH (5 × 5 ml) and DCM (5 × 5 ml), successively. The deprotection of the Fmoc group was carried out by treatment with 20% piperidine/DMF (6 ml, 2 × 15 min). After that, the solvent was drained and the resin was washed with NMP (5 ml × 5), DCM (5 ml × 5). To this reaction vessel was added Z-Cl (0.9 mmol), DIEA (1.8 mmol) and DMF (5 ml). After shaking for 24 h, the resin was drained and the resin washed with NMP/DIEA (5 × 5 ml, 10:1), NMP (5 × 5 ml), MeOH (5 × 5 ml) and DCM (5 × 5 ml). The products were cleaved from the resin with 10% TFA/DCM (2 × 5 ml, 30 min), and the resin was finally washed with DCM (5 × 5 ml). The combined solutions were concentrated and dried under reduced pressure. The crude product was purified by preparative thin-layer chromatography using $CHCl_3$–MeOH (10:1) as an eluting solvent to give **1a** (27.7 mg, 28.2%), **1b** (20.8 mg, 21.8%), **2a** (8.3 mg, 15.9%) or **2b** (11.8 mg, 11.3%), respectively. Both ^{1}H NMR spectra and mp of **1** and **2** well coincided with the data previously reported.[14,15,18] **1a**: ^{13}C NMR (125 MHz, $CDCl_3$) δ = 41.9 (Gly_1-αC), 45.0 (Gly_3-αC), 52.1 (–OMe), 66.5(Z-CH_2), 68.9 (2Dpy-αC), 122.5 (2Dpy-C5), 123.9 (2Dpy-C3), 127.7 (ϕ-*o*C), 128.1 (ϕ-*p*C), 128.5 (ϕ-*m*C), 136.4 (ϕ-*ipso*C), 136.6 (2Dpy-C4), 147.6 (2Dpy-C6), 156.6 (Z-C=O), 158.1 (2Dpy-C2), 168.4 (2Dpy C=O), 172.8 (Gly_1C=O), 174.8 (Gly_3C=O). MALDI-TOF: *m/z* Calcd for $C_{25}H_{25}N_5O_6$ M + H^+ 492.1883, M + Na^+ 514.1702; Found: M + H^+ 492.1855, M + Na^+ 514.1463. **1b**: ^{13}C NMR (125 MHz, $CDCl_3$) δ = 24.7 (Aib_3-βC), 25.2 (Aib_1-βC), 52.0 (–OMe), 56.4 (Aib_3-αC), 57.2 (Aib_1-αC), 66.5 (Z-CH_2), 68.9 (2Dpy-αC), 122.5 (2Dpy-C5), 123.9 (2Dpy-C3), 127.7 (ϕ-*o*C), 128.1 (ϕ-*p*C), 128.5 (ϕ-*m*C), 136.4 (ϕ-*ipso*C), 136.6 (2Dpy-C4), 147.6 (2Dpy-C6), 156.7 (Z, C=O), 158.2 (2Dpy-C2), 168.4 (2Dpy C=O), 172.6 (Aib_1C=O), 174.9 (Aib_3C=O). MALDI-TOF: *m/z* Calcd for $C_{29}H_{33}N_5O_6$ M + H^+ 548.2509, M + Na^+ 570.2328; Found: M + H^+ 548.2459, M + Na^+ 570.2315. **2a**; ^{13}C NMR (125 MHz, $CDCl_3$) δ = 41.9 (Gly_1-αC), 45.0 (Gly_3-αC), 52.1 (–OMe), 66.5 (Z-CH_2), 70.4 (Dph-αC), 127.86, 128.02, 128.11, 128.17, 128.21, 128.23, 128.34, 128.41(arom), 136.22 (Z-*ipso*C), 139.1 (Dph-C1), 156.6 (Z, C=O), 167.2 (Dph C=O), 169.5 (Gly_1C=O), 171.8 (Gly_3C=O). MALDI-TOF: *m/z* Calcd for $C_{27}H_{27}N_3O_6$ M + H^+ 490.1798, M + Na^+ 512.1798; Found: M + H^+ 490.1785, M + Na^+ 512.1793. **2b**; ^{13}C NMR (125 MHz, $CDCl_3$) δ = 24.3 (Aib_3-βC), 25.0 (Aib_1-αC), 52.3 (–OMe), 56.8 (Aib_3-αC), 57.2 (Aib_1-αC), 66.6 (Z-CH_2), 69.7 (Dph-αC), 127.77, 128.02, 128.12, 128.20, 128.52 (arom), 136.16 (Z-*ipso*C), 140.10 (Dph-C1), 155.0 (Z, C=O), 170.23 (Dph C=O), 172.2 (Aib_1C=O), 174.49 (Aib_3C=O). MALDI-TOF: *m/z* Calcd for $C_{31}H_{35}N_3O_6$ M + H^+ 546.2604, M + Na^+ 568.2424; Found: M + H^+ 546.259, M + Na^+ 568.2412.

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