*Synthetic Communications*®, 35: 1881–1888, 2005

ISSN 0039-7911 print/1532-2432 online
DOI: 10.1081/SCC-200064913

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# Template Synthesis of Peptidomimetics Composed of Aspartic Acid Moiety by Ugi Four-Component Condensation Reaction

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**Abstract:** Caspase-3 inhibitors were supposed to be of benefit to both acute and chronic neurodegenerative conditions. In this work, a CPP32-peptidomimetic-inhibitor library was designed. The necessary aspartate moiety was reserved at the C terminal. A facile and versatile method based on Ugi-4CR was developed to build up the library. The aspartic acid–derived isocyanide **3**, the most important component of the four, was effectively synthesized from protected aspartic acid. This Ugi procedure was tested by synthesizing a small library.

**Keywords:** Aspartic acid, isocyanide, peptidomimetics, Ugi four-component reaction (Ugi-4CR)

## INTRODUCTION

The concept of apoptosis was introduced in 1972 by Keer et al.[1] as a physiologic type of cell death with an important function, complementary to mitosis, in tissue homeostasis. Abnormal apoptosis is related to a lot of diseases such as tumors, AIDS, self-immuno diseases, and degenerate diseases.[2] Apoptotic cell death plays an important role in neuronal cell death. Both in vitro and in vivo evidence implicates ICE (Interlukin-1b Converting Enzyme) as an important factor in neuronal apoptosis, especially under pathological conditions.[3] Activation of the cysteine protease caspase-3 (CPP32) appears to

Received in Japan January 17, 2005
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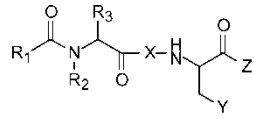


X = linear and cyclic spacer
Y = COOH, COOR, CONHR
$SO_3H$, $SO_2NHR$, $PO(OH)NH_2$, $PO(OH)OC_2H_5$
Z = $N(OCH_3)CH_3$, H, COOR, $CH_2F$, $CH_2OCOC_6H_3Cl_2$

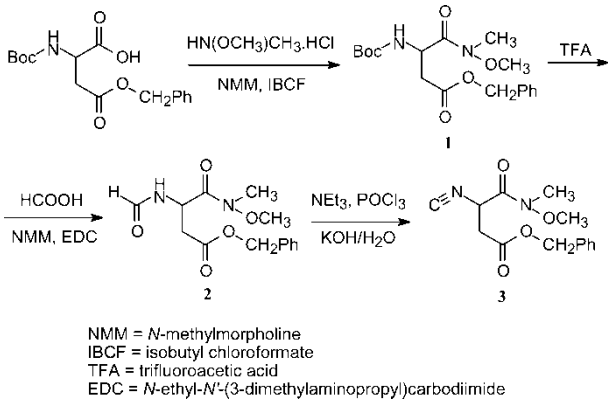
***Figure 1.*** CPP32 inhibitor library template.

be a key event in the execution of apoptosis in the central nervous system. CPP32 inhibitors may become part of a practical therapeutic approach for both acute and chronic neurodegenerative conditions.[4] Based on one of the most effective CPP32 peptide aldehyde inhibitors, Ac-DEVD-CHO,[5] and the three-dimensional structure of human CPP32 in complex with the irreversible tetrapeptide inhibitor Ac-DVAD fluoromethyl ketone,[6] a CPP32 peptidomimetic inhibitor library was designed in our project. The necessary aspartate moiety was reserved at the C terminal. The structure of one of the library templates is shown in Figure 1. The purpose of this work is to build up an applicable synthetic method for this library.

## RESULTS AND DISCUSSION

The multicomponent condensation reactions, especially Ugi four-component condensation reaction (Ugi-4CR), have been used as a powerful tool in combinatorial synthesis.[7] In this article, a facile and versatile method was introduced to build up one template of the library. This method was based on Ugi-4CR. The required components of aldehydes, acids, and amines were commercially available. Hence, the important works were the preparation of the isocyanide component and development of the Ugi-4CR procedure.

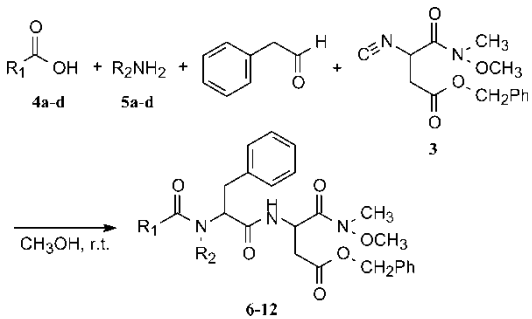
The aspartic acid–derived isocyanide **3** was designed to be synthesized by the method described in Scheme 1. The first step converted the starting material Boc-Asp(OBzl)-OH to its Weinreb amide **1**. According to Ref. 8, this reaction should be carried out under nitrogen protection. However, we found that the same result was produced without nitrogen. Then, the Boc protection of Weinreb amide **1** needed to be deprotected before the $\alpha$-amino group was formulated. Several deprotection procedures have been tried, and it was found that the Boc protecting group in compound **1** was removed thoroughly with a solution of 50% TFA (trifluoroacetic acid) and $CH_2Cl_2$. The produced TFA salt of deprotected Weinreb amide **1** was then neutralized with NMM (*N*-methylmorpholine). The following formulation reaction was performed with anhydrous formic acid in the presence of EDC (*N*-ethyl-*N*′-[3-dimethylaminopropyl]carbodiimide). The formulation failed if DCC (dicyclohexylcarbodiimide) was used instead of EDC. The formamide **2** was




***Scheme 1.*** Synthetic route of isocyanide **3**.

dehydrated with $POCl_3$ to yield the isocyanide **3**, which is a key component for the succedent Ugi-4CR. In this dehydration reaction $NEt_3$ and KOH were used to capture the released HCl because the resulting isocyano group was not stable in acid conditions. The isocynaide has an active methine, which might be susceptible to base-mediated racemization, theoretically. In our experiment only one product was separated, which showed no rotation. The structure of isocyanide **3** was characterized by $^1$H NMR, IR, and MS.

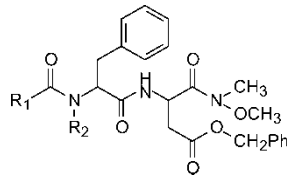
After obtaining the isocyanide **3**, an Ugi-4CR model reaction was carried out to produce compound **6** as a template synthesis (Scheme 2). $\beta$-Phenyl propionic acid (**4a**), 3-methylbutyl amine (**5a**), phenylacetaldehyde, and isocyanide **3** were used first. The ratio of the four components was 1:1:1:1 for acid/amine/aldehyde/isocyanide. The reaction was done fluently in MeOH solution at room temperature, which was the ordinary condition of Ugi-4CR. According to the mechanism of Ugi reaction, to produce the condensation product **6**, the amino group of 3-methylbutyl amine first reacted with

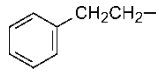
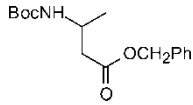
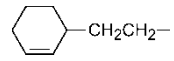
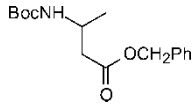
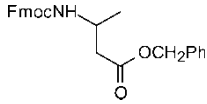
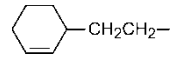
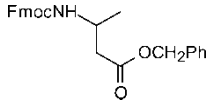
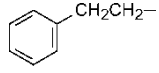
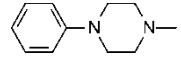



***Scheme 2.*** Ugi-4CR for template synthesis.

the carbonyl group of phenylacetaldehyde to form a iminium intermediate state, which was attached nucleophile by isocyanide carbon to produce a new chiral center. Because the attachment of the isocyanide was equal from the two sides of the $sp^2$ plane in the transition state, the product **6** might be formed equally in a pair of diastereomers. However, in our experiment, **6** was yielded as one compound and all the condensed products **7–12** did so. No structural data were obtained to show the stereostructures. The structure of the Ugi reaction product **6** was confirmed by $^1$H NMR and MS. This successful synthesis of template compound laid a foundation for building up our library. This procedure was tested more by synthesizing a small library (compounds **6**–**12**, Table 1). Because all these pure products were obtained

***Table 1.*** Structures of template compounds




| Compd. | $R_1$ | $R_2$ | Formula (Mw.) | Yield (%) |
|---|---|---|---|---|
| **6** |  | $(CH_3)_2CHCH_2CH_2-$ | $C_{36}H_{45}N_3O_6$ 615.77 | 20 |
| **7** |  |  | $C_{46}H_{58}N_4O_{10}$ 826.97 | 8.6 |
| **8** |  | $(CH_3)_2CHCH_2CH_2-$ | $C_{43}H_{56}N_4O_{10}$ 788.93 | 12 |
| **9** |  |  | $C_{56}H_{60}N_4O_{10}$ 949.10 | 30 |
| **10** |  | $(CH_3)_2CHCH_2CH_2-$ | $C_{53}H_{58}N_4O_{10}$ 911.05 | 16 |
| **11** |  |  | $C_{41}H_{47}N_5O_6$ 705.84 | 12 |
| **12** |  | $CH_3CH_2CH_2CH_2-$ | $C_{40}H_{41}N_5O_6$ 574.67 | 10 |

by prepared TLC developed three times, the yields of these compounds (10–30%) were not good. The structures of compounds **7–12** was characterized by $^1$H NMR and MS.

## CONCLUSION

In conclusion, an Ugi reaction–based method was developed to build up one template of the designed CPP32 peptidomimetic inhibitor library. The key component of the reaction, aspartate-derived isocyanide, has to be synthesized before use. The applicability of this method was limited by preparation of diverse aspartate derived isocyanide.

## EXPERIMENTAL

Unless otherwise mentioned, reagents were obtained commercially and used without further purification. The petroleum ether used had a boiling point range of 60–90°C. Melting points were taken with an X-4 apparatus and are uncorrected. IR (KBr), $^1$H NMR, MS, and element data were taken with Perkin-Elmer983, JNM-AL300 FT NMR System, VG-ZAB-HS20–250, and Flash EA 1112 instruments respectively.

### *N*-(*tert*-Butoxycarbonyl)-L-aspartic Acid 4-Benzyl Ester *N*′-Methoxy-*N*′-methylamide (1)

Boc-Asp(OBzl)-OH (3.23 g, 0.01 mol) was dissolved in $CH_2Cl_2$ (15 mL). The solution was cooled to $-13$°C. Under a nitrogen atmosphere, *N*-methylmorpholine (2.2 mL, 0.02 mol) was added, followed by dropwise addition of isobutyl chloroformate (1.3 mL, 0.01 mol). The reaction mixture was stirred vigorously for 15 min. *N*,*O*-Dimethylhydroxylamine hydrochloride (0.97 g, 0.01 mol) was added, then the ice bath was removed, and the reaction mixture was stirred for another 1 h. After filtration the solution was purified by silica-gel column chromatography (EtOAc/petroleum ether = 3/2) to afford **1** as a colorless liquid in 94% yield (3.44 g). $^1$H NMR ($CDCl_3$) $\delta$ 7.344 (m, 5H, –Ph), 5.440 (s, 1H, –NH–), 5.113 (s, 2H, $-OCH_2Ph$), 5.031 (m, 1H, –CH–), 3.747 (s, 3H, $-OCH_3$), 3.177 (s, 3H, $-NCH_3$), 2.817 (m, 2H, $-CH_2$), 1.429 (s, 9H, Boc). MS (ESI): 366.8 ($M^+$).

### *N*-Formyl-L-aspartic Acid 4-Benzyl Ester *N*′-Methoxy-*N*′-methylamide (2)

Compound **1** (3.66 g, 0.01 mol) was stirred with 50% TFA/$CH_2Cl_2$ (20 mL) for 0.5 h to remove the Boc protection, and then the solution was evaporated in vacuum. The residue was dissolved in a solution of *N*-methylmorpholine

(2.2 mL, 0.02 mol) in $CH_2Cl_2$ (50 mL). *N*-Ethyl-*N*′-(3-dimethylaminopropyl)-carbodiimide (3.82 g, 0.02 mol) was added to a solution of formic acid (2.1 g, 0.05 mol) in $CH_2Cl_2$ (50 mL) and the mixture was stirred for 15 min. The two solutions were combined and stirred overnight at room temperature. The reaction mixture was washed with 5% HCl, saturated $NaHCO_3$, and NaCl in sequence. The organic layer was dried over $Na_2SO_4$, and then evaporated to give **2** as a yellow oil in 96% yield (2.85 g). $^1$H NMR ($CDCl_3$) $\delta$ 8.168 (s, 1H, –CHO), 7.354 (m, 5H, –Ph), 6.646 (bs, 1H, –CONH–), 5.373 (m, 1H, N–CH), 5.117 (s, 2H, $-OCH_2Ph$), 3.756 (s, 3H, $-OCH_3$), 3.190 (s, 3H, $-NCH_3$), 2.850 (m, 2H, $-CH_2$).

## 2-Isocyano-succinic acid 4-Benzyl Ester *N*′-Methoxy-*N*′-methylamide (3)

Compound **2** (1 g, 3.4 mmol) was dissolved in $CH_2Cl_2$ (15 mL), and the solution was cooled in an ice bath. Triethyl amine (0.7 mL, 5.6 mmol) was dropped into the solution; then $POCl_3$ (0.4 mL, 4 mmol) was added. After the mixture had been stirred for 1 h, a solution of KOH (500 mg) in water (5 mL) was added and stirred for another 1 h. The layers were separated and the organic layer was washed with water and dried over $Na_2SO_4$. After evaporation, the residue was purified by silica-gel column chromatography (EtOAc/P.ether = 3/2) to obtain **3** as a yellow oil in 83% yield (780 mg). $^1$H NMR ($CDCl_3$) $\delta$ 7.360 (m, 5H, –Ph), 5.211 (q, 2H, $-OCH_2Ph$), 5.056 (t, 1H, CN–CH–), 3.814 (s, 3H, $-OCH_3$), 3.261 (s, 3H, $-NCH_3$), 2.978 (m, 2H, $-CH_2$). IR (KBr) $cm^{-1}$: 2923.89, 2141.26 (CN–), 1735.24, 1677.61. MS (ESI): 276.2 ($M^+$).

## General Procedure for Template Synthesis (6–12)

$\beta$-Phenyl propionic acid (**4a**, 150 mg, 1 mmol), 3-methylbutyl amine (**5a**, 40 mg, 1 mmol), and phenylacetaldehyde (120 mg, 1 mmol) were dissolved in methanol (5 mL). Then, isocyanide **3** (276 mg, 1 mmol) was added to this solution, and the mixture was stirred for 48 h at room temperature. The reaction mixture was separated by prepared TLC ($CH_2Cl_2$/ethanol = 3/1 as eluent) three times to produce **6** as a soft solid in 20% yield (123 mg), mp 30°C. $^1$H NMR ($CDCl_3$) $\delta$ 8.589 (s, 1H, CO–NH–), 7.828–7.518 (m, 15H, 3Ph–), 5.299 (m, 2H, 2N–CHCO–), 5.161 (s, 2H, $-OCH_2Ph$), 4.306 (t, 2H, $PhCH_2-$), 4.217 (t, 2H, $PhCH_2-$), 4.131 (t, 2H, $NCH_2-$), 4.078 (s, 3H, $-OCH_3$), 3.736 (s, 3H, $-NCH_3$), 2.302 (m, 4H, $2COCH_2-$), 1.302 (m, 1H, –CH–), 1.253 (m, 2H, $CH_2-$), 1.053 (d, 3H, $-CH_3$), 0.996 (d, 3H, $-CH_3$). MS (ESI): 615.6 ($M^+$).

Following the same procedure as with **6**, from Boc-Asp(Obzl)-OH (**4b**, 160 mg, 0.5 mmol), 2-(1-cyclohexenyl)ethylamine (**5b**, 65 mg, 0.5 mmol),

phenylacetaldehyde (60 mg, 0.5 mmol), and **3** (140 mg, 0.5 mmol), **7** was obtained as a colorless oil in 8.6% yield (35 mg). $^1$H NMR ($CDCl_3$) $\delta$ 8.451 (s, 1H, CO–NH–), 7.768–7.547 (m, 15H, 3Ph–), 5.631 (m, 1H, –CH═), 5.541 (s, 1H, BocNH–), 5.487 (m, 1H, –CH═), 5.302 (m, 3H, 3N–CH–CO), 5.147 (s, 4H, $2OCH_2Ph$), 4.128 (t, 2H, $NCH_2$–), 4.104 (s, 3H, $-OCH_3$), 4.011 (t, 2H, $PhCH_2$–), 3.678 (s, 3H, $-NCH_3$), 2.324 (m, 4H, $2COCH_2$–), 1.950 (m, 1H, –CH–), 1.435 (s, 9H, Boc), 1.323 (m, 8H, $4CH_2$–). MS (ESI): 827.0 ($M^+$).

Following the same procedure as with **6**, from Boc-Asp(Obzl)-OH (**4b**, 160 mg, 0.5 mmol), 3-methylbutyl amine (**5a**, 20 mg, 0.5 mmol), phenylacetaldehyde (60 mg, 0.5 mmol), and **3** (140 mg, 0.5 mmol), **8** was obtained as a colorless oil in 12% yield (47 mg). $^1$H NMR ($CDCl_3$) $\delta$ 8.497 (s, 1H, CO–NH–), 7.813–7.585 (m, 15H, 3Ph–), 5.481 (s, 1H, BocNH–), 5.311 (m, 3H, 3N–CH–CO), 5.094 (s, 4H, $2OCH_2Ph$), 4.204 (t, 2H, $NCH_2$–), 4.082 (s, 3H, $-OCH_3$), 4.043 (t, 2H, $PhCH_2$–), 3.757 (s, 3H, $-NCH_3$), 2.895 (m, 4H, $2COCH_2$–), 1.435 (s, 9H, Boc), 1.388 (m, 1H, –CH–), 1.235 (m, 2H, $-CH_2$–), 1.108 (d, 3H, $-CH_3$), 1.002 (d, 3H, $-CH_3$). MS (ESI): 789.0 ($M^+$).

Following the same procedure as with **6**, from Fmoc-Asp(Obzl)-OH (**4c**, 205 mg, 0.5 mmol), 2-(1-cyclohexenyl)ethylamine (**5b**, 65 mg, 0.5 mmol), phenylacetaldehyde (60 mg, 0.5 mmol), and **3** (140 mg, 0.5 mmol), **9** was obtained as a colorless oil in 30% yield (142 mg). $^1$H NMR ($CDCl_3$) $\delta$ 8.468 (s, 1H, CO–NH–), 7.852–7.523 (m, 23H, Ar–), 5.834 (s, 1H, FmocNH–), 5.627 (m, 1H, –CH═), 5.573 (m, 1H, –CH═), 5.338 (m, 3H, 3N–CH–CO), 5.205 (s, 4H, $2OCH_2Ph$), 4.709 (d, 2H, $OCH_2$–), 4.461 (m, 1H, ArCHAr), 4.102 (t, 2H, $NCH_2$–), 4.092 (s, 3H, $-OCH_3$), 3.996 (t, 2H, $PhCH_2$–), 3.711 (s, 3H, $-NCH_3$), 2.469 (m, 4H, $2COCH_2$–), 1.976 (m, 1H, –CH–), 1.550 (m, 8H, $4CH_2$–). MS (ESI): 949.7 ($M^+$).

Following the same procedure as with **6**, from Fmoc-Asp(Obzl)-OH (**4c**, 205 mg, 0.5 mmol), 3-methylbutyl amine (**5a**, 20 mg, 0.5 mmol), phenylacetaldehyde (60 mg, 0.5 mmol), and **3** (140 mg, 0.5 mmol), **10** was obtained as a colorless oil in 16% yield (73 mg). $^1$H NMR ($CDCl_3$) $\delta$ 8.531 (s, 1H, CO–NH–), 7.864–7.539 (m, 23H, Ar–), 5.893 (s, 1H, FmocNH–), 5.347 (m, 3H, 3N–CH–CO), 5.340 (s, 4H, $2OCH_2Ph$), 4.716 (d, 2H, $OCH_2$–), 4.458 (m, 1H, ArCHAr), 4.097 (t, 2H, $NCH_2$–), 4.052 (s, 3H, $-OCH_3$), 3.988 (t, 2H, $PhCH_2$–), 3.696 (s, 3H, $-NCH_3$), 2.880 (m, 4H, $2COCH_2$–), 1.405 (m, 1H, –CH–), 1.261 (m, 2H, $-CH_2$–), 1.079 (d, 3H, $-CH_3$), 0.992 (d, 3H, $-CH_3$). MS (ESI): 911.0 ($M^+$).

Following the same procedure as with **6**, from $\beta$-phenyl propionic acid (**4a**, 75 mg, 0.5 mmol), 4-amino-1-phenylpiperazine (**5c**, 89 mg, 0.5 mmol), phenylacetaldehyde (60 mg, 0.5 mmol), and **3** (140 mg, 0.5 mmol), **11** was obtained as a colorless oil in 12% yield (42 mg). $^1$H NMR ($CDCl_3$) $\delta$ 8.564 (s, 1H, CO–NH–), 7.749–6.607 (m, 20H, 4Ph–), 5.286 (m, 2H, 2N –CHCO–), 5.193 (s, 2H, $-OCH_2Ph$), 4.313 (t, 2H, $PhCH_2$–), 4.248 (t, 2H, $PhCH_2$–), 4.101 (s, 3H, $-OCH_3$), 3.825 (s, 3H, $-NCH_3$), 3.470 (t, 4H,

2NCH$_2$–), 2.778 (t, 4H, 2NCH$_2$–), 2.630 (m, 4H, 2COCH$_2$–). MS (ESI): 705.7 ($M^+$).

Following the same procedure as with **6**, from nicotinic acid (**4e**, 62 mg, 0.5 mmol), 1-butylamine (**5d**, 37 mg, 0.5 mmol), phenylacetaldehyde (60 mg, 0.5 mmol), and **3** (140 mg, 0.5 mmol), **12** was obtained as a colorless oil in 10% yield (29 mg). $^1$H NMR ($CDCl_3$) $\delta$ 9.171 (s, 1H, Py), 8.843 (d, 1H, Py), 8.572 (s, 1H, CO–NH–), 8.324 (d, 1H, Py), 7.638–7.359 (m, 11H, 2Ph–, Py), 5.289 (m, 2H, 2N–CHCO–), 5.091 (s, 2H, –OCH$_2$Ph), 3.972 (t, 2H, PhCH$_2$–), 4.135 (t, 2H, NCH$_2$–), 3.997 (s, 3H, –OCH$_3$), 3.723 (s, 3H, –NCH$_3$), 2.882 (m, 2H, COCH$_2$–), 1.356 (m, 4H, –2CH$_2$–), 0.969 (t, 3H, –CH$_3$). MS (ESI): 574.5 ($M^+$).

## ACKNOWLEDGMENT

This work was supported by the National Natural Science Foundation of China (20272004).

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