DOI: 10.1002/ejoc.201201731

# Syntheses of Hydrazino Peptides and Conjugates

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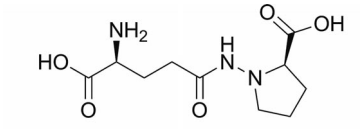
**Keywords:** Synthetic methods / Acylation / Microwave chemistry / Peptides / Conjugates

(α-Benzyloxycarbonyl-aminoacyl)benzotriazolides (Cbz = benzyloxycarbonyl) underwent a coupling reaction with α-hydrazino acids under microwave irradiation to form hybrid hydrazino dipeptides (42–71 %). Chiral acylations of β-*N*-Cbz-α-hydrazino acylbenzotriazolides were successfully carried out with N-, S-, O-, and C-nucleophiles in yields of 49–88 %.

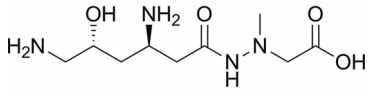
## Introduction

The replacement of one or more α-amino acids by β-amino acid units is a well-known technique in the search for pharmacologically active peptides.[1] Further replacement of the C-α and/or C-β atom of the β-amino acid constituent by a hetero atom is another attractive extension of the β-peptide concept. The replacement of the C-β atom by a nitrogen can be accomplished by incorporating one or more α-hydrazino acid units ($H_2N^{\beta}$-$N^{\alpha}$H-CH(R)-COOH)[2] into a peptide, which produces hydrazino peptides, that is, peptide analogues in which one or more of the peptidic bond(s) [-HN-CO-CH(R)-] is replaced by one or more hydrazidic bond(s) [HN-NH-CH(R)-].[3] The vitamin B6 antagonist linatine (**1**) and the antibiotic negamycin (**2**) are naturally occurring peptides that contain such a α-hydrazino acid moiety (see Figure 1).[4,5]

Unlike the corresponding amino acids [$NH_2$CH(R)-COOH], α-hydrazino acids [$H_2$N-NH-CH(R)-COOH] can inhibit the enzymes that metabolize amino acids.[6] Peptide analogs that contain an N-terminal achiral hydrazino acetic acid residue display anticancer properties[7] and are intermediates in the preparation of lipopeptides.[8] Hydrazino peptides are also building blocks in the syntheses of antiviral peptidomimetics,[9] substrates for hydrazone chemical ligation,[10] and solvent gelators.[11] Peptides that contain an *N*-alkyl-, *N*-aryl-, or *N*-acyl-hydrazino group undergo reaction with peptide aldehydes to give high molecular weight conjugates.[12]




**1**




**2**

Figure 1. Linatine (**1**) and negamycin (**2**).

Structurally, a hydrazone fragment in a peptide chain induces a "hydrazino turn",[13,14] which folds the peptide backbone locally by way of well-defined intramolecular bifurcated hydrogen bonding. Quantum and molecular mechanics calculations[2] reveal that oligomers of α-hydrazino acids adopt a wide variety of secondary structures that are characteristic of foldamers.[15]

Published preparations of hydrazino acids **8** include (i) the Hofmann rearrangement of hydantoic acids **3**,[6,17] (ii) the hydrogenation of diacyl hydrazino acid **4**,[18,19] (iii) the amination of chiral α-amino acids with *N*-alkoxycarbonyl-3-phenyloxaziridines **5**,[20] (iv) the asymmetric hydrogenation of *N*-acylhydrazones derived from α-keto acids **6**,[21] and (v) the synthesis from α-bromo acids **7** (see Scheme 1).[22–24] Cheguillaume et al. reported the synthesis of protected hydrazine peptides from protected hydrazine and esters of bromoacetate.[25] Killian et al. incorporated hydrazinophenylalanine into modified peptide and protein analogues through ribosomes.[26] Despite their interesting biological properties and diverse methods for their preparation, hydrazino peptides have not been widely studied, probably because of the difficulties associated with their synthesis, which include tedious reaction procedures,[18] low yields of the employed aminating agents,[20] and incomplete chiral characterization of the products.[23]

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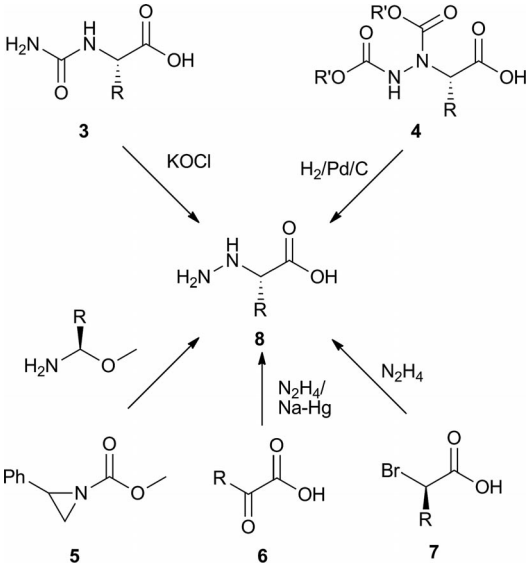
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201201731.



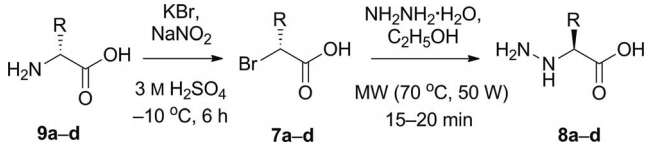

Scheme 1. Synthesis of unprotected α-hydrazino acids.

We recently disclosed the efficient benzotriazole-mediated syntheses of α-aminoxy acids and peptides.[27,28] Following an analogous pathway, we herein report an alternate route to synthesize chirally pure α-hydrazino acids.[22] This was accomplished by using microwave irradiation in the displacement reaction of α-bromo acids with hydrazine hydrate followed by the solution-phase conversion into chirally pure α-hydrazino hybrid dipeptides and α-hydrazino acid conjugates through *N*-, *O*-, *S*-, and *C*-acylation reactions.

## Results and Discussion

### Preparation of α-Hydrazino Acids 8a–8d

α-Hydrazino acids **8a**–**8d** were prepared through the nucleophilic substitution of the corresponding α-bromo acids **7a**–**7d**[29] with hydrazine hydrate at 70 °C under microwave irradiation for 15–20 min. According to the literature procedure, the displacement reaction took place at 20 °C in 24 h, and the crucial step was the purification and recrystallization of the desired α-hydrazino acid. By changing the procedure and carrying out to the reaction under microwave conditions, the reaction took less time, and the products were isolated in fairly good yields (see Scheme 2 and Tables 1 and 2). The chiral purity of **8a**–**8d** was confirmed by the products formed from coupling **8a**–**8d** with benzyloxycarbonyl-protected (Cbz = benzyloxycarbonyl) α-(aminoacyl)benzotriazoles **10a**–**10f** as described below.




Scheme 2. Preparation of α-hydrazino acids **8a**–**8d** (MW = microwave).

Table 1. Preparation of α-bromo acids **7a**–**7d**.

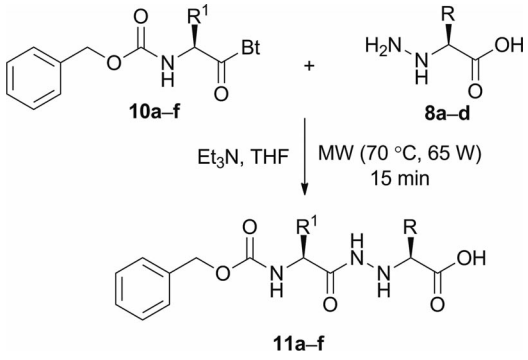
| Product | R | M.p. [°C] | Yield [%] | $[\alpha]_D^{22}$ ($c$ = 1, MeOH) |
|---|---|---|---|---|
| **7a** | $CH_3$ | oil | 68 | +41 |
| **7b** | $CH_2CH(CH_3)_2$ | oil | 67 | +17 |
| **7c** | $CH_2Ph$ | oil | 70 | +20 |
| **7d** | $CH(CH_3)_2$ | oil | 60 | +20 |

Table 2. Preparation of α-hydrazino acids **8a**–**8d**.

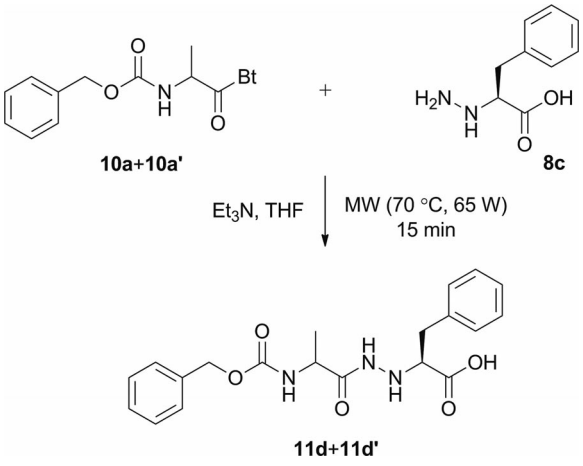
| Product | M.p. [°C] | Yield [%] | $[\alpha]_D^{22}$ (6 N HCl) | Ref.[17] $[\alpha]_D$ |
|---|---|---|---|---|
| **8a** | 214–215[16] | 48 | –32 ($c$ = 1) | –26.5 ($c$ = 1.2) |
| **8b** | 214–217 | 43 | –12 ($c$ = 1) | –13.2 ($c$ = 1) |
| **8c** | 203–206[24] | 45 | –22 ($c$ = 1) | –15.8 ($c$ = 0.5) |
| **8d** | 232–235[21] | 40 | –3 ($c$ = 1) | –17.1 ($c$ = 0.8) |

### Preparation of Hybrid α-Hydrazino Dipeptides 11a–11f

The coupling reaction between α-hydrazino acids **8a**–**8d** and Cbz-protected α-aminoacylbenzotriazoles **10a**–**10f**[30] under microwave irradiation (70 °C and 65 W) for 15 min afforded new hydrazino hybrid dipeptides **11a**–**11f** (42–71%). Comparatively low yields were obtained in case of compounds **11b** and **11c** as a result of purifying these compounds by column chromatography, but no side products were obtained after this step except for some unreacted starting material (see Schemes 3 and 4 and Table 3).




Scheme 3. Preparation of hybrid α-hydrazino dipeptides **11a**–**11f** (Bt = benzotriazolyl, THF = tetrahydrofuran).




Scheme 4. Preparation of hybrid α-hydrazino dipeptide (**11d** + **11d′**).

Table 3. Preparation of dipeptides **11a**–**11f** that contain one hydrazino acid unit.

| Product | R | $R^1$ | M.p. [°C] | Yield [%] |
|---|---|---|---|---|
| **11a** | $CH_3$ | $CH(CH_3)_2$ | 140–141 | 61 |
| **11b** | $CH(CH_3)_2$ | $CH_2Ph$ | 126–128 | 42 |
| **11c** | $CH_2CH(CH_3)_2$ | $(CH_2)_4NH(Cbz)$ | 93–95 | 45 |
| **11d** | $CH_2Ph$ | $CH_3$ | 113–114 | 68 |
| **11d + 11d′** | $CH_2Ph$ | $CH_3$ | 145–150 | 71 |
| **11e** | $CH_2Ph$ | $CH_2S(Bz)$ | 72–73 | 60 |
| **11f** | $CH_2CH(CH_3)_2$ | $CH_2$(3-indolyl) | 81–83 | 50 |

The coupling of α-hydrazino valine **8d** with the benzotriazole derivative of Cbz-protected phenylalanine **10c** to prepare compound **11b** did give a product that displayed duplicate peaks in the NMR spectra. This suggests incomplete inversion of the chirality, and, thus, **8d** was accompanied by enantiomer **8d′** in a ratio of 2:1 (as inferred by integrations in the $^1$H NMR spectrum of **11**).

However, the chiral integrity of compounds **11a** and **11c**–**11f** was supported by the NMR spectroscopic data. Notably, there was no duplication of peaks in the $^1$H and $^{13}$C NMR spectra of **11a** and **11c**–**11f**. For further proof, we

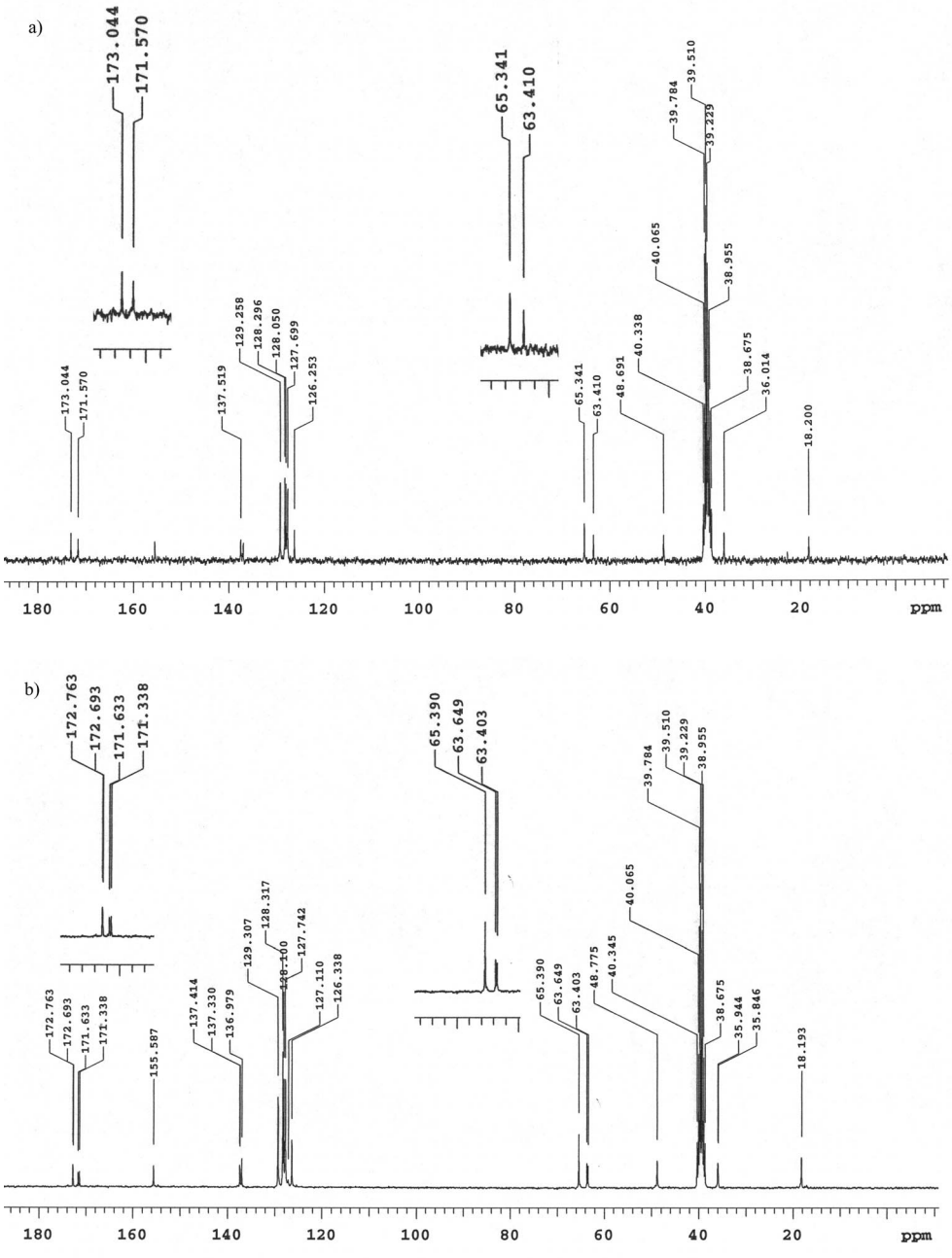


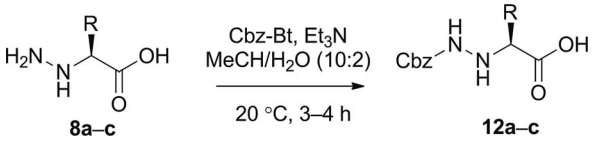

Figure 2. $^{13}$C NMR spectra of compound (a) **11d**; (b) **11d + 11d′**.

coupled compound **8c** with the racemate (**10a** + **10a′**) to give the expected mixture of diastereomers (**11d** + **11d′**, see Scheme 4). The $^{13}C$ NMR spectrum displayed the duplication of all of the carbonyl peaks, which were originally singlets in the spectrum of chirally pure isomer **11d** (see Figures 2, a and b). The chirality of all the compounds was confirmed by the optical rotation and chiral HPLC analysis.

### Preparation of β-*N*-Cbz-hydrazino Acids

β-*N*-Cbz-hydrazino acids **12a**–**12c** were prepared in good yields (68–92%) by treating the unprotected α-hydrazino acids with Cbz-Bt[31] at 20 °C in the presence of triethylamine (see Scheme 5 and Table 4).



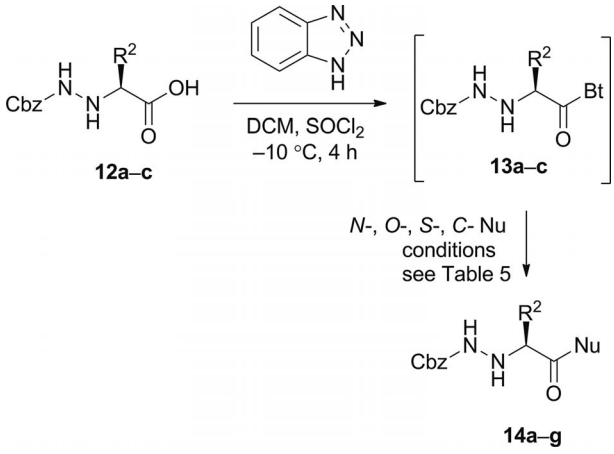

Scheme 5. Preparation of β-*N*-Cbz-hydrazino acids **12a**–**12c**.

Table 4. Preparation of *N*-Pg-hydrazino acids **12a**–**12c** (Pg = protecting group).

| Product | R | M.p. [°C] | Yield [%] |
|---|---|---|---|
| **12a** | $CH_3$ | 110–112 | 68 |
| **12b** | $CH_2CH(CH_3)_2$ | 119–120 | 74 |
| **12c** | $CH_2Ph$ | 158–160 | 92 |

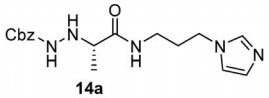
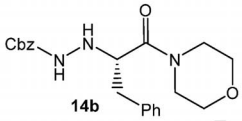
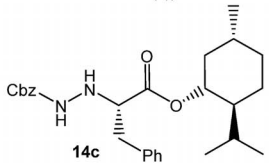
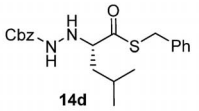
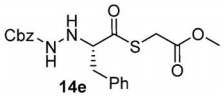
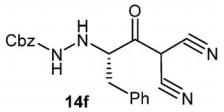
### Chiral Acylation of Compounds 13a–13c

Protected hydrazino acids **12a**–**12c** were then converted into their benzotriazolides **13a**–**13c**, which were highly hygroscopic and used without further purification for the reaction with nucleophiles. Under the appropriate conditions, benzotriazolides **13a**–**13c** of the β-*N*-Cbz-hydrazino acids underwent successful acylations with N-, O-, S-, and C-nucleophiles to give products with retention of chirality. During the acylation, we did not observe any evidence of oligomerization, which indicates that the free NH of hydrazine group was not affected under these reaction conditions (see Scheme 6 and Table 5).




Scheme 6. Chiral acylation of compounds **13a**–**13c**.

Table 5. *N*-, *O*-, *S*-, and *C*-acylations utilizing compounds **13a**–**13c**.

| Entry | Reactant **13** | Product **14** | Conditions | M.p. (°C) | Yield (%) |
|---|---|---|---|---|---|
| 1 | Cbz-NH-L-Ala-Bt **13a** |  **14a** | THF, $Et_3N$, 20 °C, 4 h | oil | 71 |
| 2 | Cbz-NH-L-Phe-Bt **13b** |  **14b** | THF, $Et_3N$, 20 °C, 4 h | oil | 69 |
| 3 | Cbz-NH-L-Phe-Bt **13c** |  **14c** | THF, DMAP, MW (60 °C, 50 W), 1 h | 112–114 | 49 |
| 4 | Cbz-NH-L-Leu-Bt **13b** |  **14d** | THF, $Et_3N$, 20 °C, 4 h | oil | 61 |
| 5 | Cbz-NH-L-Phe-Bt **13c** |  **14e** | THF, $Et_3N$, 20 °C, 4 h | oil | 63 |
| 6 | Cbz-NH-L-Phe-Bt **13c** |  **14f** | THF, DIPEA, MW (60 °C, 50 W), 30 min. | 233–235 | 88 |

## Conclusions

In conclusion, an efficient general method for the preparation of hybrid α-hydrazino peptides was developed by treating *N*-(Pg-α-aminoacyl)benzotriazolides with α-hydrazine acids. Similarly, α-hydrazino acid conjugates were prepared by treating β-*N*-Cbz-α-hydrazino aminoacylbenzotriazolides with nucleophiles without any evidence of oligomerization. All of the hydrazino derivatives were obtained under mild reaction conditions in good yields and with no detectable racemization.

## Experimental Section

**General Remarks:** Melting points were recorded with a capillary point apparatus equipped with a digital thermometer. The NMR spectroscopic data were recorded in $D_2O$, $CF_3CO_2D$, $CDCl_3$ or [$D_6$]DMSO with Mercury, Gemini NMR spectrometers that operated at 300 MHz for $^1H$ NMR (with TMS as an internal standard) and 75 MHz for $^{13}C$ NMR. Elemental analyses were performed with a Carlo–Erba EA1108 instrument. All microwave-assisted reactions were carried out with a single-mode cavity Discover Microwave Synthesizer (CEM Corporation, NC). The reaction mixtures were transferred into a 10 mL glass pressure microwave tube equipped with a magnetic stir bar. The tube was sealed with a silicon septum, and the reaction mixture was subjected to microwave irradiation (Discover mode; run time: 60 s; PowerMax-cooling mode).

**General Procedure for the Synthesis of α-Bromo Acids 7a–7c**: The compounds were synthesized by following our established procedure.[29]

**General Procedure for the Synthesis of α-Hydrazino Acids 8a–8d:** Hydrazine hydrate (4.7 equiv.) was dissolved in ethanol (3 mL), and the solution of α-bromo acid **7a–7c** in ethanol (1 equiv.) was added dropwise with water cooling. The reaction mixture was irradiated under MW at 70 °C (internal probe) and 50 W for 15–20 min. The resulting solid was washed with diethyl ether, dried under vacuum, and recrystallized to give the corresponding α-hydrazino acids.

**(*S*)-2-Hydrazinylpropanoic Acid (8a):** Recrystallized (ethanol/water) to give white microcrystals (0.33 g, 48%), m.p. 214–215 °C; ref.[16] m.p. 186–188 °C. $^1H$ NMR ($D_2O$): $\delta$ = 3.80–3.73 (m, 1 H), 1.48 (d, $J$ = 7.2 Hz, 3 H) ppm. $^{13}C$ NMR ($D_2O$/[$D_6$]DMSO): $\delta$ = 175.8, 61.7, 15.4 ppm.

**(*S*)-2-Hydrazinyl-4-methylpentanoic Acid (8b):** Recrystallized (ethanol/water) to give white microcrystals (0.32 g, 43%); m.p. 214–217 °C. $^1H$ NMR ($D_2O$): $\delta$ = 3.64 (t, $J$ = 6.8 Hz, 1 H), 1.78–1.59 (m, 3 H), 0.93 (d, $J$ = 6.3 Hz, 6 H) ppm. $^{13}C$ NMR ($D_2O$/[$D_6$]DMSO): $\delta$ = 176.4, 65.1, 25.9, 23.4, 23.3 ppm. $C_6H_{14}N_2O_2$ (146.19): calcd. C 49.30, H 9.65, N 19.16; found C 49.29, H 10.08, N 19.01.

**(*S*)-2-Hydrazinyl-3-phenylpropanoic Acid (8c):** Recrystallized (hot water) to give white microcrystals (0.35 g, 45%), m.p. 203–206 °C; ref.[24] m.p. 191–194 °C. $^1H$ NMR ($D_2O$): $\delta$ = 7.42–7.18 (m, 5 H), 3.88 (t, $J$ = 6.3 Hz, 1), 3.19 (dd, $J$ = 14.4, 5.7 Hz, 1 H), 3.11 (dd, $J$ = 14.6, 7.1 Hz, 1 H) ppm. $^{13}C$ NMR ($CF_3CO_2D$): $\delta$ = 179.1, 135.7, 132.1, 131.5, 131.2, 64.3, 38.3 ppm. $C_9H_{12}N_2O_2$ (180.21): calcd. C 59.99, H 6.71, N 15.54; found C 60.33, H 6.95, N 15.73.

**2-Hydrazinyl-3-methylbutanoic Acid (8d):** Recrystallized (hot water) to give white microcrystals (0.29 g, 40%), m.p. 232–235 °C; ref.[22] m.p. 250 °C. $^1H$ NMR ($D_2O$): $\delta$ = 3.47 (dd, $J$ = 4.5, 0.9 Hz, 1 H), 2.25–2.12 (m, 1 H), 1.00 (d, $J$ = 6.9 Hz, 6 H) ppm. $^{13}C$ NMR ($D_2O$/$CD_3OD$): $\delta$ = 147.7, 72.1, 29.5, 19.0 ppm. $C_5H_{12}N_2O_2$ (132.16): calcd. C 45.44, H 9.15, N 21.20; found C 45.82, H 9.44, N 21.11.

**General Procedure for the Synthesis of Cbz-Protected α-(Aminoacyl)benzotriazoles (10a–10f)**: The compounds were synthesized by following our established procedure.[28]

**(*S*)-Benzyl [1-(1*H*-Benzo[*d*][1,2,3]triazol-1-yl)-3-(benzylthio)-1-oxopropan-2-yl]carbamate [Cbz-L-Cys(Bz)-Bt, 10f]:** White microcrystals (0.54 g, 84%); m.p. 92–93 °C. $^1H$ NMR ($CDCl_3$): $\delta$ = 8.24 (d, $J$ = 8.1 Hz, 1 H), 8.13 (d, $J$ = 8.1 Hz, 1 H), 7.67 (t, $J$ = 7.7 Hz, 1 H), 7.53 (t, $J$ = 7.5 Hz, 1 H), 7.40–7.31 (br. s, 5 H), 7.16–7.10 (m, 5 H), 5.98–5.89 (m, 2 H), 5.13 (br. s, 2 H), 3.71 (s, 2 H), 3.17 (dd, $J$ = 14.1, 5.1 Hz, 1 H), 3.03 (dd, $J$ = 14.3, 6.8 Hz, 1 H) ppm. $^{13}C$ NMR ($CDCl_3$): $\delta$ = 170.1, 155.9, 146.2, 137.1, 136.1, 131.1, 129.0, 128.7, 128.4, 128.3, 127.4, 126.8, 126.1, 120.6, 115.1, 114.5, 67.6, 53.9, 36.4, 33.8 ppm. $C_{24}H_{22}N_4O_3S$ (446.52): calcd. C 64.56, H 4.97, N 12.55; found C 64.41, H 5.03, N 12.71.

**General Procedure for the Synthesis of α-Hydrazino Hybrid Dipeptides 11a–11f:** A dried thick-walled Pyrex tube containing a small stir bar was charged with Cbz-protected aminoacyl benzotriazole (1.0 equiv.), α-hydrazino acid (1.0 equiv.), and a catalytic amount of triethylamine dissolved in THF (3 mL). The reaction mixture was exposed to microwave irradiation (65 W) at 70 °C (internal probe) for 15 min. The reaction mixture was cooled until the temperature dropped below 30 °C (approximately 10 min). The solvent was removed under reduced pressure, and the residue was washed with diethyl ether and then recrystallized with dichloromethane (DCM). In a few cases, the residue was subjected to a silica gel column (DCM/methanol) to remove the benzotriazole and the hybrid dipeptide.

**(*S*)-2-(2-((*S*)-2-(((Benzyloxy)carbonyl)amino)-3-methylbutanoyl)-hydrazinyl)propanoic Acid (Cbz-L-Val-NH-L-Ala-OH, 11a):** White microcrystals (0.12g, 61%); m.p. 140–141 °C. $^1H$ NMR ([$D_6$]-DMSO): $\delta$ = 9.42 (s, 1 H), 7.40–7.25 (m, 6 H), 5.02 (br. s, 2 H), 3.83–3.75 (m, 1 H), 3.55–3.45 (m, 1 H), 1.90–1.82 (m, 1 H), 1.16 (d, $J$ = 6.9 Hz, 3 H), 0.85–0.79 (m, 6 H) ppm. $^{13}C$ NMR ([$D_6$]-DMSO): $\delta$ = 174.4, 170.3, 156.0, 137.1, 128.3, 127.7, 127.6, 65.4, 58.8, 57.1, 30.2, 19.1, 18.4, 16.1 ppm. $C_{16}H_{23}N_3O_5$ (337.37): calcd. C 56.96, H 6.87, N 12.45; found C 56.64, H 7.05, N 12.08.

**(*S*)-2-(2-((*S*)-2-(((Benzyloxy)carbonyl)amino)-3-phenylpropanoyl)-hydrazinyl)-3-methylbutanoic Acid (Cbz-L-Phe-NH-D/L-Val-OH, 11b):** White powder (0.09 g, 42%); 126–128 °C. $^1H$ NMR ([$D_6$]-DMSO): $\delta$ = 9.53 (d, $J$ = 6.3 Hz, 1 H), 7.53 (t, $J$ = 9.2 Hz, 1 H), 7.37–7.15 (m, 10 H), 4.97–4.87 (m, 2 H), 4.25–4.12 (m, 1 H), 3.17 (d, $J$ = 5.1 Hz, 1 H), 3.05 (d, $J$ = 4.8 Hz, 1 H), 2.93–2.81 (m, 1 H), 2.80–2.67 (m, 1 H), 1.95–1.80 (m, 1 H), 0.96–0.87 (m, 6 H) ppm. $^{13}C$ NMR ([$D_6$]DMSO): $\delta$ = 173.6, 170.2, 155.7, 137.8, 137.0, 129.2, 128.3, 128.1, 127.7, 127.5, 126.3, 67.9, 65.2, 54.8, 37.6, 29.3, 18.7 ppm. $C_{22}H_{27}N_3O_5$ (413.47): calcd. C 63.91, H 6.58, N 10.16; found C 63.67, H 6.80, N 10.55.

**(*S*)-2-(2-((*S*)-2,6-Bis(((benzyloxy)carbonyl)amino)hexanoyl)-hydrazinyl)-4-methylpentanoic Acid [Cbz-L-Lys(Z)-NH-L-Leu-OH, 11c]:** White microcrystals (0.09 g, 45%); m.p. 93–95 °C. $^1H$ NMR ([$D_6$]DMSO): $\delta$ = 9.36 (s, 1 H), 7.39–7.26 (m, 12 H), 7.22 (t, $J$ = 5.7 Hz, 1 H), 5.05–4.91 (m, 4 H), 3.96–3.83 (m, 1 H), 3.46–3.37 (m, 1 H), 3.00–2.90 (m, 2 H), 1.78–1.66 (m, 1 H), 1.55–1.44 (m, 2 H), 1.43–1.15 (m, 6 H), 0.87 (t, $J$ = 6.8 Hz, 6 H) ppm. $^{13}C$ NMR ([$D_6$]-DMSO): $\delta$ = 174.7, 171.0, 156.1, 155.9, 137.3, 137.0, 128.4, 127.8, 65.4, 65.2, 60.7, 53.3, 31.7, 29.1, 24.4, 22.8, 22.7, 22.4 ppm. $C_{28}H_{38}N_4O_7$ (542.63): calcd. C 61.98, H 7.06, N 10.32; found C 62.07, H 7.31, N 10.46.

**(*S*)-2-(2-((*S*)-2-(((Benzyloxy)carbonyl)amino)propanoyl)hydrazinyl)–3-phenylpropanoic Acid (Cbz-L-Ala-NH-L-Phe-OH, 11d):** White microcrystals (0.16 g, 68%); m.p. 113–114 °C. $^1$H NMR ([D$_6$]-DMSO): $\delta$ = 9.45 (br. s, 1 H), 7.45 (d, $J$ = 7.2 Hz, 1 H), 7.40–7.32 (m, 5 H), 7.30–7.16 (m, 6 H), 5.06–4.96 (m, 2 H), 4.10–3.98 (m, 1 H), 3.68 (t, $J$ = 6.3 Hz, 1 H), 2.87 (d, $J$ = 6.0 Hz, 2 H), 1.17 (d, $J$ = 8.4 Hz, 3 H) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 173.0, 171.6, 155.6, 137.5, 137.0, 129.3, 128.3, 128.1, 127.7, 126.3, 65.3, 63.4, 48.7, 36.0, 18.2 ppm. $C_{20}H_{23}N_3O_5$ (385.42): calcd. C 62.33, H 6.01, N 10.90; found C 62.09, H 6.14, N 10.84.

**(2*S*)-2-(2-(2-(((Benzyloxy)carbonyl)amino)propanoyl)hydrazinyl)-3-phenylpropanoic Acid (Cbz-L-Ala-NH-L-Phe-OH, 11d + 11d′):** White powder (0.17 g, 71%); m.p. 145–150 °C. $^1$H NMR ([D$_6$]-DMSO): $\delta$ = 9.64 (br. s, 1 H), 7.48 (d, $J$ = 7.2 Hz, 1 H), 7.40–7.16 (m, 11 H), 5.01 (br. s, 2 H), 4.10–3.98 (m, 1 H), 3.74 (t, $J$ = 6.6 Hz, 0.5 H), 3.69 (t, $J$ = 6.3 Hz, 0.5 H), 2.90 (d, $J$ = 6.6 Hz, 2 H), 1.18 (d, $J$ = 6.9 Hz, 3 H) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 171.8, 172.7, 171.6, 171.3, 155.6, 137.4, 137.3, 137.0, 129.3, 128.3, 128.1, 127.7, 127.1, 126.3, 65.4, 63.6, 63.4, 48.8, 35.9, 36.0, 18.2 ppm. $C_{20}H_{23}N_3O_5$ (385.42): calcd. C 62.33, H 6.01, N 10.90; found C 62.09, H 5.93, N 10.74.

**(*S*)-2-(2-((*S*)-2-(((Benzyloxy)carbonyl)amino)-3-(benzylthio)-propanoyl)hydrazinyl)-3-phenylpropanoic Acid [Cbz-L-Cys(Bz)-NH-L-Phe-OH, 11e]:** White solid (0.14 g, 60%); m.p. 72–73 °C. $^1$H NMR ([D$_6$]DMSO): $\delta$ = 9.89 (s, 1 H), 7.63 (d, $J$ = 8.1 Hz, 1 H), 7.37–7.19 (m, 16 H), 5.06–5.04 (m, 2 H), 4.37–4.21 (m, 1 H), 3.76 (s, 2 H), 3.73–3.71 (m, 1 H), 2.94–2.90 (m, 2 H), 2.72–2.63 (m, 1 H), 2.59–2.50 (m, 1 H) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 172.7, 169.2, 155.8, 138.3, 137.3, 136.9, 129.3, 128.9, 128.3, 128.1, 127.8, 127.7, 126.8, 126.4, 65.5, 63.5, 52.8, 35.9, 35.0, 33.0 ppm. HRMS [ESI(+)-TOF]: calcd. for $C_{27}H_{29}N_3O_5NaS$ [M + Na]$^+$ 530.1720; found 530.1745.

**(*S*)-2-(2-((*S*)-2-(((Benzyloxy)carbonyl)amino)-3-(1*H*-indol-3-yl)-propanoyl)hydrazinyl)-4-methylpentanoic acid (Cbz-L-Trp-NH-L-Leu-OH, 11f):** White microcrystals (0.11 g, 50%); m.p. 81–83 °C. $^1$H NMR ([D$_6$]DMSO): $\delta$ = 10.79 (s, 1 H), 9.54 (s, 1 H), 7.62 (d, $J$ = 7.8 Hz, 1 H), 7.38 (d, $J$ = 8.4 Hz, 1 H), 7.33–7.21 (m, 7 H), 7.13–7.12 (m, 1 H), 7.04 (t, $J$ = 7.1 Hz, 1 H), 6.95 (t, $J$ = 7.4 Hz, 1 H), 4.96–4.86 (m, 2 H), 4.27–4.22 (m, 1 H), 3.46–3.33 (m, 1 H), 3.00 (dd, $J$ = 14.6, 4.2 Hz, 1 H), 2.88 (dd, $J$ = 14.4, 9.6 Hz, 1 H), 1.80–1.65 (m, 1 H), 1.40 (t, $J$ = 6.9 Hz, 2 H), 0.87 (d, $J$ = 6.6 Hz, 3 H), 0.85 (d, $J$ = 6.9 Hz, 3 H) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 174.6, 170.8, 155.7, 137.0, 136.0, 128.3, 127.7, 127.5, 127.2, 123.8, 120.8, 118.5, 118.2, 111.3, 110.0, 65.2, 60.7, 54.1, 28.0, 24.4, 22.6, 22.4 ppm. HRMS [ESI(–)-TOF]: calcd.for $C_{25}H_{30}N_4O_5$ [M – H]$^+$ 465.2143; found 465.2164.

**General Procedure for the Synthesis of Cbz-Hydrazino Acids 12a–12c:** To a solution of the hydrazino acid (1.0 equiv.) in water (2 mL) were added $Et_3N$ (2.0 equiv.) and a solution of Cbz-Bt (1.0 equiv.) in acetonitrile (10 mL). The reaction mixture was stirred at room temperature for 3–4 h (monitored by TLC). The mixture was then acidified with HCl (4 N solution, 2 mL). Acetonitrile was removed under reduced pressure, and the resulting crude product was subjected to column chromatography (hexanes/ethyl acetate) to give the desired product.

**(*S*)-2-(2-((Benzyloxy)carbonyl)hydrazinyl)propanoic Acid (Cbz-NH-L-Ala-OH, 12a):** White powder (0.31 g, 68%); m.p. 110–112 °C. $^1$H NMR ([D$_6$]DMSO): $\delta$ = 8.63 (br. s, 1 H), 7.91 (br. s, 1 H), 7.40–7.28 (m, 5 H), 5.04 (s, 2 H), 3.60–3.51 (m, 1 H), 1.13 (d, $J$ = 6.0 Hz, 3 H) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 174.5, 156.9, 136.9, 128.3, 127.8, 127.7, 65.5, 16.0 ppm. HRMS [ESI(+)-TOF]: calcd. for $C_{11}H_{14}N_2O_4Na$ [M + Na]$^+$ 261.0846; found 261.0840.

**(*S*)-2-(2-((Benzyloxy)carbonyl)hydrazinyl)-4-methylpentanoic Acid (Cbz-NH-L-Leu-OH, 12b):** White powder (0.28 g, 74%); m.p. 119–120 °C. $^1$H NMR ([D$_6$]DMSO): $\delta$ = 8.56 (br. s, 1 H), 7.40–7.27 (m, 5 H), 5.07 (d, $J$ = 12.6 Hz, 1 H), 5.01 (d, $J$ = 12.6 Hz, 1 H), 3.46 (t, $J$ = 6.8 Hz, 1 H), 1.81–1.66 (m, 1 H), 1.38 (t, $J$ = 6.9 Hz, 2 H), 0.88–0.84 (m, 6 H) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 174.8, 156.8, 136.9, 128.3, 127.8, 127.6, 65.4, 61.0, 39.4, 24.3, 22.6, 22.3 ppm. $C_{14}H_{20}N_2O_4$ (280.32): calcd. C 59.98, H 7.19, N 9.99; found C 59.91, H 7.37, N 9.99.

**(*S*)-2-(2-((Benzyloxy)carbonyl)hydrazinyl)-3-phenylpropanoic Acid (Cbz-NH-L-Phe-OH, 12c):** White powder (0.32 g, 92%); m.p. 158–160 °C. $^1$H NMR ([D$_6$]DMSO): $\delta$ = 8.70 (br. s, 1 H), 7.40–7.13 (m, 11 H), 5.04 (s, 2 H), 3.74 (t, $J$ = 6.3 Hz, 1 H), 2.86 (d, $J$ = 6.3 Hz, 2 H) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 173.2, 156.9, 137.6, 136.9, 129.3, 128.3, 128.0, 127.8, 127.7, 126.2, 65.5, 52.1, 36.0 ppm. $C_{17}H_{18}N_2O_4$ (314.34): calcd. C 64.96, H 5.77, N 8.91; found C 65.02, H 5.70, N 8.95.

**General Procedure for *N*-Acylation: Synthesis of Compounds 14a and 14b:** The N-nucleophile (1 equiv.) and triethylamine (1.5 equiv.) were dissolved in THF (5 mL). The benzotriazole intermediate (**13a** or **13b**, 1 equiv.) was added to the solution, and the mixture was stirred at room temperature for 4 h. The mixture was acidified with HCl (6 N solution), and the resulting solution was concentrated and then diluted with ethyl acetate. The organic layer was washed with HCl (6 N solution), dried with anhydrous $MgSO_4$, filtered, and evaporated to give the desired compound.

**(*S*)-Benzyl 2-(1-((3-(1*H*-Imidazol-1-yl)propyl)amino)-1-oxopropan-2-yl)hydrazinecarboxylate (14a):** Oil (0.14 g, 71%). $^1$H NMR ($CD_3OD$): $\delta$ = 7.53 (s, 1 H), 7.24–7.16 (m, 5 H), 6.99 (s, 1 H), 6.84 (s, 1 H), 4.96 (br. s, 2 H), 3.82 (t, $J$ = 6.9 Hz, 2 H), 3.35 (q, $J$ = 6.9 Hz, 1 H), 3.00 (t, $J$ = 6.5 Hz, 2 H), 1.77 (t, $J$ = 6.8 Hz, 2 H), 1.17 (d, $J$ = 6.9 Hz, 3 H) ppm. $^{13}$C NMR ($CD_3OD$): $\delta$ = 176.8, 159.5, 138.6, 138.1, 129.6, 129.2, 129.1, 129.0, 120.7, 67.9, 61.6, 45.5, 37.1, 31.9, 31.1, 17.3 ppm. HRMS [ESI(+)-TOF]: calcd. for $C_{17}H_{23}N_5O_3$ [M + H]$^+$ 346.1874; found 346.1890.

**(*S*)-Benzyl 2-(1-Morpholino-1-oxo-3-phenylpropan-2-yl)hydrazine-carboxylate (14b):** Oil (0.13 g, 69%). $^1$H NMR ([D$_6$]DMSO): $\delta$ = 10.15 (br. s, 1 H), 8.70 (br. s, 1 H), 7.36–7.10 (m, 10 H), 5.14–5.05 (m, 2 H), 4.05–4.01 (m, 1 H), 3.54–3.28 (m, 8 H), 2.98–2.79 (m, 2 H) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 169.6, 156.7, 137.7, 136.9, 129.3, 128.5, 128.4, 127.9, 127.8, 126.3, 66.6, 65.9, 65.8, 41.9, 36.9, 30.5 ppm. HRMS [ESI(+)-TOF]: calcd. for $C_{21}H_{25}N_3O_4Na$ [M + Na]$^+$ 406.1737; found 406.1731.

**General Procedure for *O*-Acylation: Synthesis of Compound 14c:** A dried thick-walled Pyrex tube containing a small stir bar was charged with the benzotriazole intermediate **13c** (1 equiv.). The O-nucleophile (1.5 equiv.) and 4-(dimethylamino)pyridine (DMAP, 0.1 equiv.) were dissolved in THF (3 mL). The reaction mixture was exposed to microwave irradiation (50 W) at 60 °C (internal probe) for 1 h. The mixture was cooled through a custom-made system until the temperature fell below 30 °C (approximately 10 min). The reaction mixture was quenched with water, and the resulting solution was extracted with EtOAc. The extracts were washed with a 10% $Na_2CO_3$ aqueous solution and then dried with $MgSO_4$. The solvent was removed under reduced pressure, and the residue was subjected to a silica gel column (EtOAc/hexane) to give compound **14c**.

**Benzyl 2-((*S*)-1-(((1*R*,2*S*,5*R*)-2-Isopropyl-5-methylcyclohexyl)oxy)-1-oxo-3-phenylpropan-2-yl)hydrazinecarboxylate (14c):** White powder (0.11 g, 49%); m.p. 112–114 °C. $^1$H NMR ([D$_6$]DMSO): $\delta$ = 7.33–7.18 (m, 11 H), 6.40 (br. s, 1 H), 5.14–5.00 (m, 2 H), 4.75–

4.60 (m, 1 H), 4.00–3.92 (m, 1 H), 3.10–2.91 (m, 2 H), 1.82–1.60 (m, 4 H), 1.46–1.22 (m, 2 H), 1.08–0.96 (m, 1 H), 0.87–0.77 (m, 8 H), 0.68 (d, $J$ = 7.2 Hz, 3 H) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 172.4, 156.8, 136.4, 136.1, 129.4, 129.3, 128.7, 128.4, 128.2, 127.1, 75.4, 67.3, 64.2, 47.0, 40.8, 37.1, 34.3, 31.5, 26.5, 23.5, 22.2, 20.9, 16.5 ppm. HRMS [APCI(+)-TOF]: calcd. for $C_{27}H_{37}N_2O_4$ [M + H]$^+$ 453.2748; found 453.2744 (APCI = atmospheric pressure chemical ionization).

**General Procedure for *S*-Acylation: Synthesis of Compounds 14d and 14e:** The mercapto nucleophile (1 equiv.) and triethylamine (1.5 equiv.) were dissolved in THF (5 mL). Benzotriazole intermediate (**13b** or **13c**, 1 equiv.) was added to the solution, and the mixture was stirred at room temperature for 4 h and then acidified with HCl (6 N solution). The resulting solution was concentrated, and the residue was diluted with ethyl acetate. The organic layer was washed with HCl (6 N solution), dried with anhydrous $MgSO_4$, filtered, and evaporated to give the desired compound.

**(*S*)-Benzyl 2-(1-(Benzylthio)-4-methyl-1-oxopentan-2-yl)hydrazinecarboxylate (14d):** Oil (0.12 g, 61%). $^1$H NMR ($CDCl_3$): $\delta$ = 7.44–7.20 (m, 10 H), 6.48 (br. s, 1 H), 5.15 (d, $J$ = 12.0 Hz, 2 H), 5.08 (d, $J$ = 12.0 Hz, 1 H), 4.09 (s, 2 H), 3.90–3.78 (m, 1 H), 1.88–1.73 (m, 1 H), 1.49 (t, $J$ = 6.8 Hz, 2 H), 1.10–0.85 (m, 6 H) ppm. $^{13}$C NMR ($CDCl_3$): $\delta$ = 203.0, 157.1, 137.5, 135.9, 129.0, 128.8, 128.6, 128.4, 127.4, 69.2, 67.6, 41.0, 32.9, 25.1, 23.3, 22.2 ppm. HRMS [ESI(+)-TOF]: calcd. for $C_{21}H_{26}N_2O_3SNa$: [M + Na]$^+$ 409.1556; found 409.1572.

**(*S*)-Benzyl 2-(1-((2-Methoxy-2-oxoethyl)thio)-1-oxo-3-phenylpropan-2-yl)hydrazinecarboxylate (14e):** Oil (0.12 g, 63%). $^1$H NMR ([D$_6$]DMSO): $\delta$ = 7.45–7.20 (m, 11 H), 6.68–6.60 (m, 1 H), 5.12–4.96 (m, 2 H), 4.20–4.08 (m, 1 H), 3.71 (s, 3 H), 3.65 (s, 2 H) 3.14–3.06 (m, 1 H), 2.84–2.78 (m, 1 H) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 201.0, 169.3, 156.8, 135.7, 135.2, 129.3, 128.8, 128.6, 128.4, 128.2, 127.4, 70.4, 67.4, 52.8, 37.9, 30.7 ppm. HRMS [ESI(+)-TOF]: calcd. for $C_{20}H_{22}N_2O_5NaS$ [M + Na]$^+$ 425.1142; found 425.1162.

**General Procedure for *C*-Acylation: Synthesis of Compound 14f:** A dried thick-walled Pyrex tube containing a small stir bar was charged with benzotriazole intermediate **13c** (1 equiv.), the N-nucleophile (1 equiv.) dissolved in THF (5 mL), and *N*,*N*-diisopropylethylamine (DIPEA, 1.5 equiv.). The reaction mixture was exposed to microwave irradiation (50 W) at 60 °C (internal probe) for 30 min. The mixture was cooled through an inbuilt system until the temperature fell below 30 °C (approximately 10 min). The reaction mixture was quenched with water, and the resulting solid was filtered and washed with 10% $Na_2CO_3$ and then water to give the desired compound.

**(*S*)-Benzyl 2-(4,4-Dicyano-3-oxo-1-phenylbutan-2-yl)hydrazinecarboxylate (14f):** Light orange solid (0.15 g, 88%); m.p. 233–235 °C. $^1$H NMR ([D$_6$]DMSO): $\delta$ = 8.49 (br. s, 1 H), 7.38 (br. s, 5 H), 7.23–7.14 (m, 5 H), 5.07 (s, 2 H), 4.04 (t, $J$ = 4.7 Hz, 1 H), 3.01–2.86 (m, 2 H) ppm. $^{13}$C NMR ([D$_6$]DMSO): $\delta$ = 188.3, 169.0, 155.0, 136.2, 135.8, 129.7, 128.4, 128.0, 127.8, 126.3, 114.9, 67.3, 66.9, 33.9 ppm. $C_{20}H_{18}N_4O_3$ (362.39): calcd. C 66.29, H 5.01, N 15.46; found C 66.10, H 4.84, N 15.17.

**Supporting Information** (see footnote on the first page of this article): $^1$H and $^{13}$C NMR and CHN/HRMS spectra for all compounds.

## Acknowledgments

The authors thank Dr C. D. Hall for useful suggestions and checking the English. We also thank the University of Florida, The Kenan Foundation, and King Abdulaziz University, Jeddah, Saudi Arabia for their financial support.

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Received: December 21, 2012
Published Online: May 16, 2013

  