Supporting Information

Efficient Syntheses of Thiadiazole Peptides

*Alan R. Katritzky, Claudia El-Nachef, Kiran Bajaj, Jonathan Kubik and Danniebell N. Haase

Center for Heterocyclic Compounds, University of Florida, Department of Chemistry,
Gainesville, Florida 32611-7200, USA

katritzky@chem.ufl.edu

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* Ph. (352) 392-0554, fax (352)-392-9199
General Methods

Melting points were determined on a hot-stage apparatus and are uncorrected. $^1$H NMR (300 MHz) spectra were recorded in CDCl$_3$, (CD$_3$)$_2$CO or DMSO-$d_6$ with TMS as the internal standard and CDCl$_3$, (CD$_3$)$_2$CO or DMSO-$d_6$ as the internal standard for $^{13}$C NMR (75 MHz). Column chromatography was conducted on flash silica gel (200-425 mesh). Visualization of TLC plates was done via UV and phosphomolybdic acid staining. Anhydrous THF was obtained by distillation immediately prior to use, from sodium/benzophenone ketyl. HPLC was done using Chiralcel OD-H and Chirobiotic-T column. In the case of compound 3, Chiralcel OD-H column and a mixture of ethanol:hexane (8:2) were used. As per compound 4, Chiralcel OD-H column and a mixture of 2-propanol:hexane (1:9) were used. Finally, for compound 7, Chirobiotic-T column with a mixture of methanol:water (9:1) were used.

**Benzyl((S)-1-(((S)-1-(1H-benzo[d][1,2,3]triazol-1-yl)-4-(methylthio)-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate 5b.** Recrystallized from diethyl ether yielding the product as white microcrystals (70%, 145-147 °C); $^1$H NMR (CDCl$_3$) $\delta$ 8.25 (d, $J$ = 8.1 Hz, 1H), 8.14 (d, $J$ = 8.8 Hz, 1H), 7.68 (t, $J$ = 7.7 Hz, 1H), 7.54 (t, $J$ = 7.8 Hz, 1H), 7.42-7.28 (m, 5H), 7.20 (d, $J$ = 7.2 Hz, 1H), 6.14-6.02 (m, 1H), 5.47 (d, $J$ = 9.0 Hz, 1H), 5.20 - 5.08 (m, 2H), 4.21 - 4.10 (m, 1H), 2.71 - 2.60 (m, 2H), 2.54 - 2.40 (m, 1H), 2.40 - 2.12 (m, 2H), 2.06 (s, 3H), 1.00 (d, $J$ = 6.9 Hz, 3H), 0.96 (d, $J$ = 6.9 Hz, 3H); $^{13}$C NMR (CDCl$_3$) $\delta$ 171.7, 170.8, 156.7, 146.2, 136.3, 131.3, 131.0, 128.7, 128.4, 128.2, 126.8, 120.6, 114.6, 67.4, 60.6, 53.0, 31.9, 31.2, 30.3, 19.4, 18.1, 15.6. Anal. Calcd for C$_{24}$H$_{29}$N$_5$O$_4$S: C, 59.61; H, 6.04; N, 14.48; found: C, 59.85; H, 6.18; N, 14.52.

**Benzyl((2S)-1-(((1H-benzo[d][1,2,3]triazol-1-yl)-4-(methylthio)-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (5b+5b').** Recrystallized from diethyl ether yielding the product as white microcrystals (79 %, 143-145 °C); $^1$H NMR (CDCl$_3$) $\delta$ 8.31-8.20 (m, 1H), 8.20-8.07 (m, 1H), 7.67 (t, $J$ = 7.4 Hz, 1H), 7.53 (t, $J$ = 7.4 Hz, 1H), 7.43-7.28 (m, 6H), 6.17-6.01 (m, 1H), 5.59 (d, $J$ = 8.1 Hz, 1H), 5.20-5.09 (m, 2H), 4.32-4.14 (m, 1H), 2.75-2.59 (m, 2H), 2.54-2.38 (m, 1H), 2.30-2.10 (m, 2H), 2.04 (s, 3H), 0.98 (dd, $J$ = 12.5, 6.8 Hz, 6H); $^{13}$C NMR (CDCl$_3$) $\delta$ 171.9, 170.8, 156.7, 146.1, 136.3,
131.2, 130.9, 128.7, 128.1, 126.7, 120.5, 114.5, 67.3, 60.5, 52.9, 52.8, 31.8, 31.3, 30.3, 19.5, 19.4, 18.1, 17.9, 15.5. Anal. Calcd for C$_{24}$H$_{29}$N$_5$O$_4$S: C, 59.61; H, 6.04; N, 14.48; found: C, 59.55; H, 6.05; N, 14.43.

(S)-Benzy1(1-(2-(isopropylcarbamothioyl)hydrazinyl)-3-methyl-1-oxobutan-2-yl)carbamate 3a. Recrystallized from diethyl ether yielding the product as white microcrystals (68%, 140-142 °C); $^1$H NMR (CD$_3$COCD$_3$) δ 9.41 (br s, 1H), 8.48 (br s, 1H), 7.40-7.31 (m, 6H), 6.90 (br s, 1H), 5.16 (d, A part of AB system, $J = 12.3$ Hz, 1H), 5.08 (d, B part of AB system, $J = 12.3$ Hz, 1H), 4.56-4.40 (m, 1H), 3.82-3.74 (m, 1H), 1.20 (d, $J = 6.6$ Hz, 3H), 1.13 (d, $J = 6.6$ Hz, 3H), 1.05 (d, $J = 6.9$ Hz, 3H), 1.02 (d, $J = 6.9$ Hz, 3H); $^{13}$C NMR (CD$_3$COCD$_3$) δ 182.7, 171.7, 158.2, 137.8, 129.4, 128.9, 128.8, 67.3, 61.5, 47.1, 22.4, 19.5, 19.3. Anal. Calcd for C$_{17}$H$_{26}$N$_4$O$_3$S: C, 55.71; H, 7.15; N, 15.29; found: C, 56.06; H, 7.43; N, 15.13.

(S)-Benzy1(1-(2-(cyclohexylcarbamothioyl)hydrazinyl)-1-oxo-3-phenylpropan-2-yl)carbamate 3b. Purified by washing with diethyl ether to yield the product as white microcrystals (74 %, 164-165 °C); $^1$H NMR (CD$_3$COCD$_3$) δ 9.42 (br s, 1H), 8.33 (br s, 1H), 7.40-7.00 (m, 11H), 6.93 (d, $J = 5.4$ Hz, 1H), 5.20-4.90 (m, 2H), 4.30-4.20 (m, 1H), 4.20-4.05 (m, 1H), 3.17 (dd, $J = 13.8$, 6.2 Hz, 1H), 3.01 (dd, $J = 13.6$, 8.9 Hz, 1H), 2.00-1.80 (m, 2H), 1.78-1.50 (m, 3H), 1.40-1.00 (m, 5H); $^{13}$C NMR (CD$_3$COCD$_3$) δ 183.2, 172.1, 158.2, 138.6, 130.7, 129.8, 129.3, 129.1, 128.1, 128.7, 54.7, 38.1, 33.6, 31.2, 26.9, 26.4. Anal. Calcd for C$_{24}$H$_{30}$N$_4$O$_3$S: C, 57.41; H, 6.65; N, 12.32; found: C, 63.37; H, 6.99; N, 12.31.

Benzyl(1-(2-(cyclohexylcarbamothioyl)hydrazinyl)-1-oxopropan-2-yl)carbamate (3c+3c'). Purified by washing with methylene chloride to yield the product as white microcrystals (72%, 189-190 °C); $^1$H NMR (CD$_3$COCD$_3$) δ 9.42 (br s, 1H), 8.19 (br s, 1H), 7.54-7.22 (m, 6H), 7.05-6.90 (m, 1H), 5.22-5.05 (m, 2H), 4.27-4.11 (m, 1H), 4.11-4.00 (m, 1H), 2.00-1.85 (m, 2H), 1.70-1.64 (m, 2H), 1.64-1.53 (m, 1H), 1.37 (overlapped d, $J = 7.2$ Hz, 3H), 1.35-1.04 (m, 5H); $^{13}$C NMR (CD$_3$COCD$_3$) δ 183.2, 173.1, 158.3, 129.8, 129.3, 129.1, 128.7, 54.7, 51.6, 33.6, 26.9, 26.5, 17.8. Anal. Calcd for C$_{18}$H$_{26}$N$_4$O$_3$S: C, 57.12; H, 6.92; N, 14.80; found: C, 57.17; H, 7.24; N, 15.01.
(S)-5-(1-Amino-2-methylpropyl)-N-isopropyl-1,3,4-thiadiazol-2-amine 4a. White microcrystals (68%, 60-62 °C); $^1$H NMR (CDCl$_3$) $\delta$ 6.22 (br s, 1H), 3.95 (d, $J = 6.3$ Hz, 1H), 3.72-3.55 (m, 1H), 2.08-1.62 (m, 3H), 1.25 (d, $J = 6.6$ Hz, 6H), 0.94 (overlapped d, $J = 6.9$ Hz, 3H), 0.91 (overlapped d, $J = 6.9$ Hz, 3H); $^{13}$C NMR (CDCl$_3$) $\delta$ 169.8, 164.3, 58.0, 49.4, 34.8, 22.9, 19.4, 18.0. HRMS Calcd for C$_9$H$_{19}$N$_4$S [M+H]$^+$ 215.1325; found 215.1315

(S)-5-(1-Amino-2-phenylethyl)-N-cyclohexyl-1,3,4-thiadiazol-2-amine 4b. White microcrystals (53%, 158-159 °C); $^1$H NMR (CDCl$_3$) $\delta$ 7.43-7.20 (m, 5H), 5.46 (br s, 1H), 4.49 (dd, $J = 9.3, 4.5$ Hz, 1H), 3.31 (dd, $J = 13.5, 4.2$ Hz, 2H), 2.87 (dd, $J = 13.5, 9.3$ Hz, 1H), 2.20-2.00 (m, 2H), 1.88-1.53 (m, 5H), 1.50-1.10 (m, 5H); $^{13}$C NMR (CDCl$_3$) $\delta$ 169.7, 165.0, 137.7, 129.6, 128.9, 127.1, 56.5, 53.7, 44.7, 33.2, 25.7, 24.9. Anal. Calcd for C$_{16}$H$_{22}$N$_4$S: C, 63.54; H, 7.33; N, 18.52; found: C, 63.17; H, 7.65; N, 18.88.

5-(1-Aminoethyl)-N-cyclohexyl-1,3,4-thiadiazol-2-amine (4c+4c'). White microcrystals (67%, 133-135 °C); $^1$H NMR (CDCl$_3$) $\delta$ 5.59 (br s, 1H), 4.33-4.25 (m, 1H), 3.31-3.17 (m, 1H), 2.08-1.95 (m, 2H), 1.85-1.65 (m, 4H), 1.50-1.60 (m, 1H), 1.43 (dd, $J = 6.6, 0.6$ Hz, 3H), 1.38-1.08 (m, 5H); $^{13}$C NMR (CDCl$_3$) $\delta$ 169.7, 166.3, 56.5, 48.1, 33.1, 25.6, 24.9, 24.6. HRMS Calcd for C$_{10}$H$_{19}$N$_4$S [M+H]$^+$ 227.1325; found 227.1318.

Benzyl((S)-1-(((S)-1-(5-(isopropylamino)-1,3,4-thiadiazol-2-yl)-2-methylpropyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate 6a. White microcrystals (63%, 74-75 °C); $^1$H NMR (CDCl$_3$) $\delta$ 7.66 (d, $J = 8.1$ Hz, 1H), 7.32-7.26 (m, 5H), 7.15-7.12 (m, 3H), 7.08-7.06 (m, 2H), 6.00-5.88 (m, 1H), 5.65 (d, $J = 5.1$ Hz, 1H), 5.12-4.96 (m, 3H), 4.74-4.70 (m, 1H), 3.68-3.57 (m, 1H), 3.08-2.98 (m, 2H), 2.28-2.18 (m,1H), 1.32 (overlapped d, $J= 3.0$ Hz, 3H), 1.31 (overlapped d, $J = 3.0$ Hz, 3H), 0.90 (d, $J = 6.3$ Hz, 6H); $^{13}$C NMR (CDCl$_3$) $\delta$ 170.9, 169.6, 157.6, 156.1, 136.5, 129.5, 128.7, 128.2, 128.1, 128.0, 126.9, 67.1, 56.0, 54.3, 49.7, 38.8, 33.2, 22.9, 19.3, 18.5. Anal. Calcd for C$_{26}$H$_{33}$N$_5$O$_3$S: C, 63.01; H, 6.71; N, 14.13; found: C, 62.97; H, 6.50; N, 13.99.
Benzyl((S)-1-(((S)-1-(5-(cyclohexylamino)-1,3,4-thiadiazol-2-yl)-2-phenylethyl)amino)-4-methyl-1-oxopentan-3-yl)carbamate 6b. White microcrystals (60%, 205-206 °C); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 7.80 (d, \(J = 8.4\) Hz, 1H), 7.42-7.27 (m, 5H), 7.23-7.08 (m, 5H), 6.03 (d, \(J = 6.9\) Hz, 1H), 5.60-5.40 (m, 2H), 5.14 (d, A part of AB system, \(J = 12.3\) Hz, 1H), 5.05 (d, B part of AB system, \(J = 12.0\) Hz, 1H), 4.40-4.20 (m, 1H), 3.40-3.20 (m, 2H), 3.15 (br s, 1H), 2.14-1.90 (m, 3H), 1.83-1.68 (m, 2H), 1.68-1.55 (m, 1H), 1.41-1.10 (m, 5H), 0.85 (d, \(J = 6.6\) Hz, 3H), 0.78 (d, \(J = 6.3\) Hz, 3H); \(^13\)C NMR (CDCl\(_3\)) \(\delta\) 171.1, 170.2, 157.6, 156.5, 136.9, 129.7, 128.7, 128.6, 128.3, 128.1, 127.0, 67.2, 60.2, 56.8, 50.2, 40.8, 33.0, 31.5, 25.6, 24.9, 19.4, 17.8. Anal. Calcd for C\(_{29}\)H\(_{37}\)N\(_5\)O\(_3\)S: C, 65.02; H, 6.96; N, 13.07; found: C, 64.88; H, 7.25; N, 13.11.

Benzyl ((S)-1-(((S)-1-(5-(cyclohexylamino)-1,3,4-thiadiazol-2-yl)ethyl)amino)-3-(1H-indol-3-yl)-1-oxopropan-2-yl)carbamate (6c) Beige microcrystals (60%, 112-114 °C); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 8.80 (br s, 1H), 7.55 (d, \(J = 7.1\) Hz, 1H), 7.41-7.20 (m, 7H), 7.19-7.05 (m, 2H), 7.00 (t, \(J = 6.9\) Hz, 1H), 6.89 (s, 1H), 5.85 (d, \(J = 6.0\) Hz, 1H), 5.13-4.99 (m, 3H), 4.68-4.55 (m, 1H), 3.32-3.08 (m, 3H), 2.12-1.90 (m, 2H), 1.64-1.52 (m, 1H), 1.12-1.45 (m, 8H); \(^13\)C NMR (CDCl\(_3\)) \(\delta\) 171.5, 170.0, 159.3, 156.2, 136.5, 128.7, 128.3, 128.2, 127.5, 123.8, 122.2, 119.7, 118.7, 111.6, 109.9, 67.2, 57.0, 55.6, 45.3, 32.8, 28.9, 25.5, 24.8, 19.9. HRMS Calcd for C\(_{29}\)H\(_{35}\)N\(_6\)O\(_3\)S [M+H]+ 547.2486, found 547.2500.

Benzyl ((S)-1-(((S)-1-(5-(isopropylamino)-1,3,4-thiadiazol-2-yl)-2-methylpropyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxopropan-2-yl)carbamate 7a. White microcrystals (57%, 208-210 °C); \(^1\)H NMR (CDCl\(_3\)) \(\delta\) 7.57 (d, \(J = 9.0\) Hz, 1H), 7.40-7.28 (m, 5H), 7.20-7.10 (m, 3H), 7.10-6.98 (m, 3H), 5.71 (d, \(J = 6.3\) Hz, 1H), 5.48 (d, \(J = 7.5\) Hz, 1H), 5.14-4.97 (m, 3H), 4.95-4.84 (m, 1H), 4.32-4.18 (m, 1H), 3.72-3.55 (m, 1H), 3.11-2.98 (m, 2H), 2.36-2.19 (m, 1H), 1.38-1.22 (m, 9H), 0.91 (d, \(J = 7.8\) Hz, 6H); \(^13\)C NMR (CDCl\(_3\)) \(\delta\) 172.3, 170.6, 169.6, 157.8, 156.1, 136.5, 129.5, 128.7, 128.6, 128.4, 128.3, 126.9, 67.2, 54.5, 50.9, 49.7, 38.3, 33.2, 23.0, 19.3, 18.8, 18.5. HRMS Calcd for C\(_{29}\)H\(_{39}\)N\(_6\)O\(_4\)S [M+H]+ 567.2748, found 567.2736.
Benzyl ((S)-1-(((S)-1-((S)-1-(5-(cyclohexylamino)-1,3,4-thiadiazol-2-yl)-2-phenylethyl) amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxopropan-2-yl)carbamate 7b. White microcrystals (67%, 229-230 °C); $^1$H NMR (DMSO-$d_6$) $\delta$ 8.69 (d, $J$ = 7.5 Hz, 1H), 7.88 (d, $J$ = 7.2 Hz, 1H), 7.60 (d, $J$ = 6.3 Hz, 1H), 7.50-7.30 (m, 6H), 7.30-7.20 (m, 5H), 7.20-7.08 (m, 5H), 5.28-5.15 (m, 1H), 5.10-4.93 (m, 2H), 4.55-4.40 (m, 1H), 4.98-3.91 (m, 1H), 3.50-3.38 (m, 1H), 3.30-3.18 (m, 1H), 3.16-3.02 (m, 1H), 2.96-2.83 (m, 1H), 2.80-2.70 (m, 1H), 2.03-1.86 (m, 2H), 1.77-1.64 (m, 2H), 1.62-1.50 (m, 1H), 1.39-1.14 (m, 5H), 1.08 (d, $J$ = 6.3 Hz, 3H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 171.9, 170.2, 168.0, 158.1, 155.6, 137.5, 137.2, 136.9, 129.1, 128.3, 128.1, 127.9, 127.7, 126.3, 126.1, 65.4, 53.6, 50.0, 37.6, 32.1, 25.2, 24.3, 18.1. Anal. Calcd for C$_{36}$H$_{42}$N$_6$O$_4$S: C, 66.03; H, 6.46; N, 12.83; found: C, 66.11; H, 6.74; N, 12.78.

Benzyl ((S)-1-(((S)-1-((S)-1-(5-(cyclohexylamino)-1,3,4-thiadiazol-2-yl)-2-phenylethyl)amino)-1-oxopropan-2-yl)carbamate (7c+7c'). White microcrystals (61%, 233-234 °C); $^1$H NMR (DMSO-$d_6$) $\delta$ 8.58 (d, $J$ = 7.5 Hz, 1H), 7.99 (d, $J$ = 7.5 Hz, 1H), 7.58 (d, $J$ = 7.5 Hz, 1H), 7.40-7.25 (m, 6H), 5.12-4.97 (m, 3H), 4.44-4.30 (m, 1H), 3.94-3.82 (m, 1H), 3.48-3.36 (m, 1H), 2.46-2.36 (m, 2H), 2.01 (s, 3H), 1.97-1.88 (m, 4H), 1.74-1.65 (m, 2H), 1.60-1.51 (m, 1H), 1.44 (d, $J$ = 6.9 Hz, 3H), 1.34-1.14 (m, 6H), 0.85 (t, $J$ = 7.8 Hz, 6H); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 171.6, 171.1, 170.6, 167.4, 160.3, 160.1, 156.4, 156.2, 137.0, 128.3, 127.7, 127.6, 65.4, 60.3, 54.0, 51.6, 44.6, 31.8, 30.1, 29.5, 25.1, 24.1, 19.3, 19.2, 18.7, 18.2, 14.7, 14.5. Anal. Calcd for C$_{28}$H$_{42}$N$_6$O$_4$S$_2$: C, 56.92; H, 7.17; N, 14.22; found: C, 56.84; H, 7.45; N, 14.05.
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(5b+5b')
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(4c+4c')
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