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Efficient Syntheses of Thiadiazole Peptides

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

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

katritzky@chem.ufl.edu

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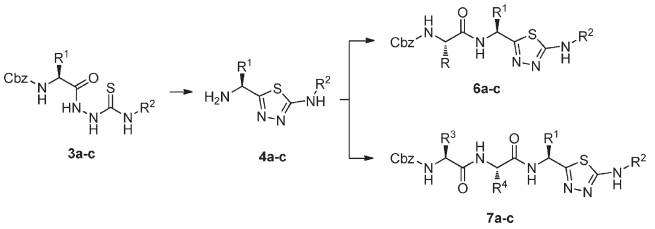

Novel *N*-(Cbz-aminoacyl)thiosemicarbazides **3a**−**c** were cyclized by treatment with sulfuric acid to give 1,3,4-thiadiazoles **4a**−**c**. Compounds **4a**−**c** reacted with *N*-(Cbz-aminoacyl)- and -dipeptidoylbenzotriazoles to afford chirally pure 1,3,4-thiadiazol-2-yl-substituted amino acids **6a**−**c** and dipeptides **7a**−**c**.

Attempts to discover peptide analogues with increased chemical stability and oral availability have replaced peptide fragments such as −NHCO−CHR− by a wide variety of alternative structural moieties. In particular, heterocyclic moieties attached to peptides and α-amino acids have attracted considerable interest.[1−4]

Among important five-membered heterocyclic synthetic building blocks in medicinal, agricultural, and materials chemistry, 1,3,4-thiadiazoles exhibit diverse biological properties, including antibacterial,[5] antimicrobial,[6] antiarrhythmic,[7] anticancer,[8,9] anti-inflammatory,[10] antidepressant[11] and anti-HIV[12] activities.

In previous efforts to develop new chiral nonproteinogenic α-amino acids and peptides that could be incorporated into heterocycles, we synthesized chiral 1,2,4-oxadiazoles[13] utilizing *N*-protected (α-aminoacyl)benzotriazoles.[14] We now report the synthesis of chiral 1,3,4-thiadiazolo-substituted amino acids and peptides as potential building blocks. Similar compounds with 2-amino-1,3,4-thiadiazoles as coupling units to peptides are known to be potent APN inhibitors[15] and metalloprotease inhibitors.[16] Moreover, reports exist of the synthesis of thiadiazoles from amino acids for biological testing,[12,17] which disclosed low toxicity and, in the case of phenylalanine derivatives, anti-inflammatory activity.[18] Isothiocyanates are often used to form thiosemicarbazide intermediates for conversion to 1,3,4-thiadiazoles.[5,12,17,18]

Here, we convert amino acid derivatives **1a**−**c** (**1c**+**1c**′) under microwave irradiation first into thiosemicarbazides **3a**−**c** (**3c**+**3c**′); we next cyclized **3** to afford enantiomerically pure 1,3,4-thiadiazoles **4a**−**c**, which in turn provide the first synthesis of chirally pure peptidoylaminothiadiazoles.

Compounds **1** and **5** (Tables 3 and 4) were prepared following established procedures.[14,19] Thiosemicarbazides **2** were synthesized from 1-(alkylthiocarbamoyl)benzotriazoles and hydrazine[20] (Table 5).

N-(Cbz-α-aminoacyl)benzotriazoles **1a**−**c** and (**1c**+**1c**′) reacted with thiosemicarbazides **2a**,**b** under microwave irradiation to yield precursors **3a**−**c** and (**3c**+**3c**′) (68−79%). The enantiopurity of compound **3c** was confirmed by HPLC-UV; as expected, HPLC analysis of **3c** showed a single peak with nearly the retention time (7.31 min) as that of one of the two peaks (7.10 and 7.38 min) obtained from the racemic mixture (**3c**+**3c**′). Compounds **3a**−**c** and the racemic mixture (**3c**+**3c**′) each underwent concurrent cyclization and deprotection of the Cbz group on treatment with concentrated sulfuric acid[12,17] to produce 1,3,4-thiadiazoles **4a**−**c** and (**4c**+**4c**′) in yields of 53−73% (Scheme 1, Table 1).

In the ^{1}H NMR, the characteristic peaks of the NH protons at δ 9.40 and 8.24 ppm of compound **3c** disappeared when thiadiazole **4c** was formed. The removal of the Cbz protecting group was also evident from the aromatic region

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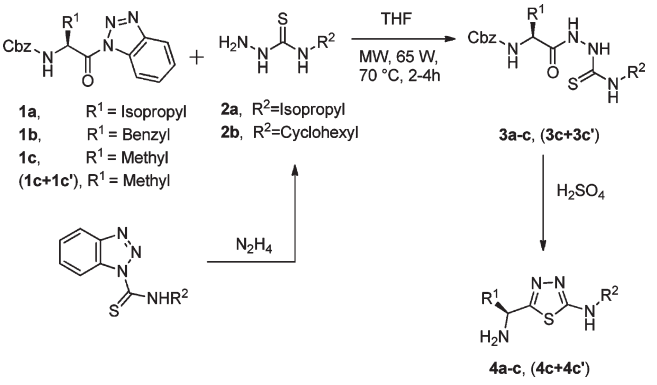
TABLE 1. Preparation of Substituted Amino Acid Thiosemicarbazides 3 and 1,3,4-Thiadiazoles 4

		compounds **3**			compounds **4**			
reactants **1**	R^2	entry	yield (%)	mp (°C)	entry	yield (%)	mp (°C)	$[\alpha]^{25}_D$
Z-L-Val-Bt	isopropyl	**3a**	68	140−142	**4a**	68	60−62	+13.0
Z-L-Phe-Bt	cyclohexyl	**3b**	74	164−165	**4b**	53	158−159	+5.1
Z-L-Ala-Bt	cyclohexyl	**3c**	79	193−195	**4c**	73	138−139	−12.9
Z-DL-Ala-Bt	cyclohexyl	(**3c**+**3c**′)	72	189−190	(**4c**+**4c**′)	67	133−135	0.0

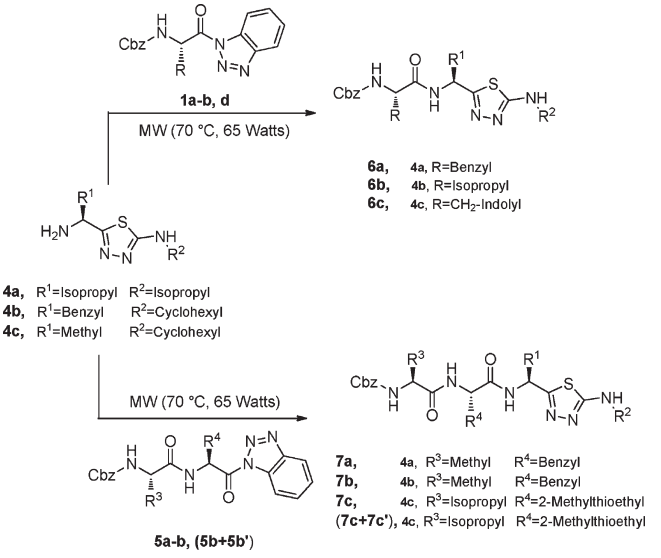
TABLE 2. Preparation of 1,3,4-Thiadiazolo-Substituted Amino Acids 6 and Dipeptides 7

reactants **4**	reactants **1** and **5**	products	conditions	yield (%)	mp (°C)
4a	Z-L-Phe-Bt, **1b**	**6a**	TEA, DMF, 2.5 h	62	74−75
4b	Z-L-Val-Bt, **1a**	**6b**	TEA, DMF, 3 h	60	205−206
4c	Z-L-Trp-Bt, **1d**	**6c**	THF, 2.5 h	60	112−114
4a	Z-L-Ala-L-Phe-Bt, **5a**	**7a**	THF, 2 h	57	208−209
4b	Z-L-Ala-L-Phe-Bt, **5a**	**7b**	THF, 3 h	67	229−230
4c	Z-L-Val-L-Met-Bt, **5b**	**7c**	THF, 0.5 h	65	237−238
(**4c**+**4c**′)	Z-L-Val-DL-Met-Bt, (**5b**+**5b**′)	(**7c**+**7c**′)	THF, 1.5 h	61	233−234

SCHEME 1



SCHEME 2



of the ^{1}H NMR spectrum, where the multiplet at δ 7.40−7.33 ppm for the precursor **3c** disappeared in the product thiadiazole **4c**. The enantiomeric purity of compound **4c** was confirmed by HPLC studies; thus using a Chiralcel-OD column, **4c** showed a single peak (11.47 min), whereas racemic mixture (**4c**+**4c**′) showed two peaks (11.87 and 12.54 min), one of which had nearly the same retention time as the single peak from **4c**. To justify the small difference in retention times, we ran the HPLC of the 1:1 ratio mixture of **4c** and (**4c**+**4c**′) and observed two peaks at 11.43 and 12.26 min in a ratio of 3:1, respectively, which confirmed the previous analysis. The chiral integrity of **4a** and **4b** is proven by the chiral purity of **7a** and **7b**, which is discussed later in this paper. The measured optical rotations (Table 1) support this conclusion.

1,3,4-Thiadiazoles **4a**−**c** were each reacted with acylbenzotriazoles **1** and **5** under microwave irradiation to afford 1,3,4-thiadiazolo-substituted amino acids **6a**−**c** and dipeptides **7a**−**c**, respectively (Scheme 2, Table 2).

The ^{13}C NMR spectrum of the diastereomeric mixture (**7c**+**7c**′) derived from the coupling of the diastereomeric mixture of dipeptides (**5b**+**5b**′) with enantiomerically pure thiadiazole **4c** showed two peaks at δ 14.7 and 14.5 ppm for the methionine methyl carbons, while a single peak at δ 14.6 ppm was observed for the pure chiral compound **7c**.

HPLC-UV analysis of compound **7c** and the diastereomeric mixture (**7c**+**7c**′) further supported the above conclusions; thus **7c** showed a single peak with a retention time (12.17 min) nearly the same that one of the two peaks (retention times of 11.63 and 12.16 min) disclosed by the diastereomeric mixture (**7c**+**7c**′). We further analyzed a 1:1 mixture of **7c** and (**7c**+**7c**′) and as expected observed an intensity ratio of 1:3 for the two peaks at 11.72 and 12.30 min; this confirms the above HPLC conclusions.

The HPLC evidence of **7c** confirms the ^{13}C evidence for the chiral integrity of **7c**; by analogy, we conclude that the ^{13}C NMR spectra for **6a**−**c** and for **7a**−**b** are evidence for their chiral purity.

In conclusion, novel *N*-(Cbz-aminoacyl)thiosemicarbazides **3a**−**c** and racemate (**3c**+**3c**′) underwent concurrent cyclization and deprotection with retention of chirality to give 2,5-disubstituted 1,3,4-thiadiazoles **4a**−**c** and (**4c**+**4c**′) each possessing a free amino group. The free amino group in the substituted thiadiazoles **4a**−**c** coupled with diverse acylbenzotriazoles under microwave conditions to give chirally pure thiadiazolyl amino acids **6a**−**c** and dipeptides **7a**−**c** and (**7c**+**7c**′) (57−67%). Considering the high selectivity and the fact that there is no other known method to make such compounds, this method represents a promising route to the preparation of various thiadiazole-substituted peptides.

TABLE 3. Synthesis of N-Protected (α-Aminoacyl)benzotriazoles 1a−d

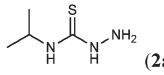
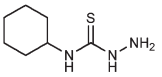
reactants	products	yield (%)	mp (°C)	lit mp (°C)
Z-L-Val-OH	Z-L-Val-Bt (**1a**)	87	107−108	73−74
Z-L-Phe-OH	Z-L-Phe-Bt (**1b**)	85	149−151	151−152
Z-L-Ala-OH	Z-L-Ala-Bt (**1c**)	85	115−117	114−115
Z-DL-Ala-OH	Z-DL-Ala-Bt (**1c**+**1c′**)	83	116−118	112−113
Z-L-Trp-OH	Z-L-Trp-Bt (**1d**)	88	99−100	100−101

TABLE 4. Preparation of Dipeptidoylbenzotriazoles 5a,b and (5b+5b′)

reactants	products	yield (%)	mp (°C)
Z-L-Ala-L-Phe-OH	Z-L-Ala-L-Phe-Bt (**5a**)	68	148−149[a]
Z-L-Val-L-Met-OH	Z-L-Val-L-Met-Bt (**5b**)	70	145−147
Z-L-Val-DL-Met-OH	Z-L-Val-DL-Met-Bt (**5b**+**5b′**)	79	143−145

[a]Literature mp of **5a** is 148−149 °C.[19]

TABLE 5. Preparation of Thiosemicarbazides 2a,b

product	yeild %	mp (°C)	lit mp (°C)
 (**2a**)	50	76-78	82-83.[21]
 (**2b**)	84	142-144	142.[20]

Experimental Section

N-Protected (α-aminoacyl)benzotriazoles **1a−d** and *N*-protected (dipeptidoyl)benzotriazoles **5a**,**b** were synthesized following our established procedure[14,19] (Tables 3 and 4).

Thiosemicarbazides **2a**,**b** were synthesized following our established procedure[20] (Table 5).

General Procedure for the Preparation of 1,3,4-Thiadiazole Intermediates 3a−c and (3c+3c′). *N*-Protected (α-aminoacyl)-benzotriazoles **1a−c** and (**1c**+**1c′**) (2.5 mmol) in tetrahydrofuran (5 mL) were each irradiated by microwave at 70 °C, 65 W, together with thiosemicarbazide **2a** or **2b**. Upon completion of the reaction (monitored by TLC) (2−4 h), the solvent was evaporated and the residue was washed with methylene chloride or diethyl ether then filtered to afford **3a−c** and (**3c**+**3c′**).

(*S*)-Benzyl (1-(2-(Cyclohexylcarbamothioyl)hydrazinyl)-1-oxopropan-2-yl)carbamate (3c): Purified by washing with methylene chloride to yield the product as white microcrystals (79%, 193−195 °C); ^{1}H NMR (300 MHz, CD_3COCD_3) δ 9.41 (br s, 1H), 8.25 (br s, 1H), 7.45−7.22 (m, 5H), 7.00 (br s, 1H), 5.22−5.05 (m, 2H), 4.26−4.12 (m, 1H), 4.12−3.99 (m, 1H), 2.85 (br s, 1H), 2.00−1.87 (m, 2H), 1.80−1.65 (m, 2H), 1.65−1.54 (m, 1H), 1.37 (overlapped d, J = 6.6 Hz, 3H), 1.34−1.04 (m, 5H); ^{13}C NMR (75 MHz, CD_3COCD_3) δ 183.1, 173.0, 158.2, 138.3, 129.8, 129.3, 129.0, 67.6, 54.7, 51.6, 33.5, 26.8, 26.4, 26.3, 17.7. Anal. Calcd for $C_{18}H_{26}N_4O_3S$: C, 57.12; H, 6.92; N, 14.80. Found: C, 57.11; H, 7.06; N, 14.92.

General Procedure for the Preparation of 1,3,4-Thiadiazoles 4a−c and (4c+4c′). Concentrated sulfuric acid (2 mL) was added dropwise to (2.0 mmol) of thiosemicarbazides **3a−c** and (**3c**+**3c′**). The resulting mixture was stirred at room temperature for 8−12 h. The solution was quenched with ice and then extracted with methylene chloride (2 × 10 mL). The aqueous portion was collected and neutralized with sodium hydroxide and then extracted with methylene chloride (3 × 10 mL). The organic layers were combined, washed with water (1 × 10 mL) and brine (1 × 10 mL), and then dried over anhydrous magnesium sulfate. The solvent was removed in vacuo to yield 1,3,4-thiadiazoles **4a−c** and (**4c**+**4c′**).

(*S*)-5-(1-Aminoethyl)-*N*-cyclohexyl-1,3,4-thiadiazol-2-amine (4c): White microcrystals (73%, 138−139 °C); ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.82 (br s, 1H), 4.40−4.30 (m, 1H), 3.29 (br s, 1H), 2.22−1.96 (m, 2H), 1.78−1.55 (m, 5H), 1.49 (dd, J = 6.6, 2.4 Hz, 3H), 1.44−1.10 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.7, 166.3, 56.5, 48.1, 33.2, 25.6, 24.9, 24.6. Anal. Calcd for $C_{10}H_{18}N_4S$: C, 53.06; H, 8.02; N, 24.75. Found: C, 52.79; H, 8.18; N, 24.51.

General Procedure for the Preparation of 1,3,4-Thiadiazole Derivatives 6a−c, 7a−c, and (7c+7c′). 1,3,4-Thiadiazoles **4** (0.3 mmol) and acylbenzotriazoles **1** or **5** (0.3 mmol) were dissolved in anhydrous THF (4 mL). The reaction mixture was exposed to microwave irradiation at 70 °C, 65 W until completion of the reaction (monitored by TLC). After the solvent was evaporated, ethyl acetate was added (4 mL) to give a white precipitate. This solid was filtered and dried to afford 1,3,4-thiadiazolo-substituted amino acids **6a−c** and dipeptides **7a−c** and (**7c**+**7c′**). In the case of **6a** and **6b**, anhydrous DMF and triethylamine proved to be the best conditions for a shorter reaction time and a short flash column with a gradient elution of hexanes/ethyl acetate was used to purify the product.

Benzyl ((*S*)-1-(((*S*)-1-(((*S*)-1-(5-(Cyclohexylamino)-1,3,4-thiadiazol-2-yl)ethyl)amino)-4-(methylthio)-1-oxobutan-2-yl)amino)-3-methyl-1-oxobutan-2-yl)carbamate (7c): White microcrystals (60%, 237−238 °C); ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.60 (d, J = 7.8 Hz, 1H), 8.01 (d, J = 7.8 Hz, 1H), 7.59 (d, J = 6.9 Hz, 1H), 7.50−7.22 (m, 6H), 5.17−4.94 (m, 3H), 4.48−4.26 (m, 1H), 3.96−3.80 (m, 1H), 3.52−3.38 (m, 1H), 2.49−2.30 (m, 2H), 2.02 (s, 3H), 2.00−1.86 (m, 4H), 1.86−1.74 (m, 1H), 1.74−1.61 (m, 2H), 1.61−1.50 (m, 1H), 1.44 (d, J = 6.9 Hz, 3H), 1.39−1.10 (m, 5H), 0.88−0.82 (m. 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.9, 170.1, 167.7, 159.9, 156.0, 136.9, 128.2, 127.5, 65.3, 60.1, 53.5, 51.5, 44.3, 32.0, 30.0, 29.3, 25.2, 24.2, 19.5, 19.1, 18.0, 14.6. Anal. Calcd for $C_{28}H_{42}N_6O_4S_2$: C, 56.92; H, 7.17; N, 14.22. Found: C, 56.56; H, 7.45; N, 14.15.

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Supporting Information Available: Compound characterization data for **3a**, **3b**, (**3c**+**3c′**), **4a**, **4b**, (**4c**+**4c′**), **5b**, (**5b**+**5b′**), **6a**, **6b**, **7a**, **7b**, and (**7c**+**7c′**). This material is available free of charge via the Internet at http://pubs.acs.org.