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A convenient synthesis of 1,3,4-thiadiazole and 1,3,4-oxadiazole based peptidomimetics employing diacylhydrazines derived from amino acids

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ABSTRACT

Synthesis of novel orthogonally protected 1,3,4-thiadiazole and 1,3,4-oxadiazole tethered dipeptide mimetics is described. Both the heterocycles are prepared via a set of diacylhydrazines derived from amino acids. 1,3,4-Thiadiazoles are synthesized by dehydrosulfurization using Lawesson's reagent while 1,3,4-oxadiazoles are obtained by EDC mediated cyclodehydration.

The changes in conformation, polarity and metabolic stability generated by structural modifications of peptide backbone have prompted the synthesis of several new classes of peptide mimics, which contain unnatural linkages as replacements for the native peptide bonds. These molecules have shown enhanced potency, specificity and oral bioavailability than the natural peptides and are considered to be potential candidates in drug discovery.[1] One such important class of molecules is peptides substituted with heterocycles, also referred to as peptide heterocycles. The heterocyclic unit in these molecules can introduce conformational constraints to the structure that enhances the activity and alters the structure–activity-relationships.[2] Thus several heterocycles such as 1,2,3- and 1,2,4-triazole,[3] oxazole,[4] thiazole, and tetrazole,[5] have been inserted en route to the design of new peptidomimetics. 2-Alkyl amino-1,3,4-oxadiazole based peptidomimetics have been described earlier by our group.[6] In view of the above and as a part of our ongoing work on the synthesis of amino acids and peptide derivatives containing heterocyclic units,[7] we herein report the facile synthesis of novel *N,N′*-orthogonally protected 1,3,4-thiadiazolo- and 1,3,4-oxadiazolo-substituted peptides. Substances bearing these heterocycles have attracted significant interest in medicinal and bio-organic chemistry. For example, thiadiazole derivatives are known to be potent aminopeptidase inhibitors[8] and metalloprotease inhibitors.[9] Similarly, biologically relevant entities containing the oxadiazole motif include HIV integrase inhibitors and the angiogenesis inhibitors.[10,11]

Although numerous reports are available for the construction of 1,2,4-oxadiazole peptidomimetics,[7a,12] the reports on the synthesis of 1,3,4-oxadiazole and 1,3,4-thiadiazoles containing peptides are rare. Recently Katritzky et al.,[13] described a related class of compounds which are amino acid derivatives containing 2-alkyl amino-1,3,4-thiadiazole units. The synthesis consisted of coupling N-protected amino acid hydrazides with alkyl isothiocyanates followed by dehydration of the resulting thiosemicarbazides. Several others have reported the synthesis of molecules containing a 1,3,4-thiadiazole unit starting from alkyl and aryl diacylhydrazines.[14]

The standard method for the synthesis of 1,3,4-thiadiazoles involves cyclization of *N′*-(acyl)thiohydrazide[15a] or thiosemicarbazides[15b] employing usually $POCl_3$, PCl_5, MsOH, or H_2SO_4 as dehydrating agents. The other important route is via the exchange of the oxygen atom in 1,3,4-oxadiazole to sulfur using thiourea and tetraphosphorus decasulfide.[16] Following the former route, Katritzky et al.,[13] have used H_2SO_4 for the cyclization of N^{α}-Cbz protected amino acid derived thiosemicarbazides and have obtained the thiadiazole products with concomitant removal of the Cbz-group. We envisaged that the use of strong acidic conditions for cyclization, apart from being harsh, is not suitable for amino acid substrates when they bear acid sensitive protecting group(s). In this regard, we sought a mild and effective route for the insertion of 1,3,4-thiadiazole into peptides.

Luthman[17] and co-workers reported the synthesis of Phe-Gly dipeptidomimetics containing 1,3,4-oxadiazole and 1,2,4-oxadiazole and Gly mimetics containing 1,3,4-oxadiazoles[18] through

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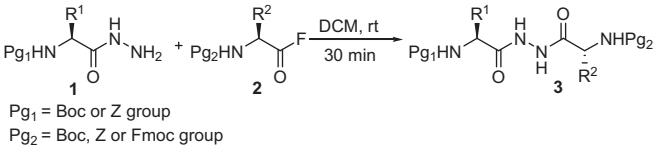
cyclodehydration of diacylhydrazines derived from Boc-Phe-$NHNH_2$ and methyl malonyl chloride. Huryn and co-workers,[19] synthesized S-linked 2,5-disubstituted oxadiazoles through chemoselective alkylation of oxadiazolethiones obtained from Boc-Trp-$NHNH_2$. Synthesis of amino acid derived oxadiazoles via the dehydrothiolative cyclization of thiosemicarbazide has also been reported.[20] The present report deals with the synthesis of 1,3,4-thiadiazole and oxadiazole based peptidomimetics through a facile route which involves *N,N′*-di-α-aminoacyl hydrazines as common precursors.

The required diacylhydrazines were synthesized as follows. Initially, the *N*-Boc/Z-protected amino acids were converted to their corresponding hydrazides **1** following the reported protocol.[21] N-Acylation of the latter with a second amino acid to furnish the diacylhydrazine was achieved by the treatment with *N*-Boc/Z protected α-amino acid fluorides. Unlike amino acid chlorides, *N*-urethane protected amino acid fluorides are excellent coupling reagents for both solution and solid phase synthesis. Their most impressive application is in the synthesis of extremely hindered dipeptides.[22] The N-protected amino acid fluorides **2** used were prepared through the reaction of protected amino acids with Deoxo-fluor[23] in the presence of *N*-methylmorpholine (NMM). In a typical example, Fmoc-Ala-F was treated with Boc-Phe-$NHNH_2$ in dry CH_2Cl_2 at room temperature for 30 min. The resulting peptidyl diacylhydrazine **3b** was isolated and purified (Scheme 1).[24] The list of N-protected diacylhydrazines prepared is summarized in Table 1.

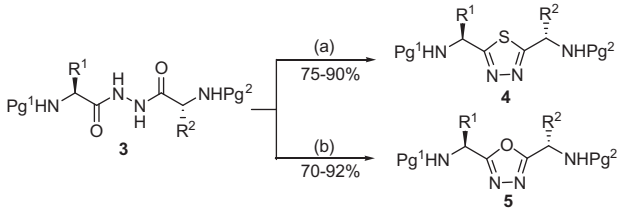
Lawesson and co-workers, have described the thionation of 1,2-diacylhydrazines with 2,4-bis(4-methoxypheny1)-1,2,3,4-dithiadiphosphetane (Lawesson's reagent, LR), followed by spontaneous ring closure through dehydrosulfurization leading to the formation of thiadiazole ring.[25] Their study dealt mainly with the preparation of dialkyl thiadiazoles. We sought to extend this route for the synthesis of thiadiazole tethered *N,N′*-orthogonally protected dipeptidomimetics **4** through the direct cyclization of diacylhydrazines (without the requirement of a separate thionation step).

In a typical experiment, the diacylhydrazine **3b** was refluxed with LR in THF for 3 h which yielded the 1,3,4-thiadiazolo-dipeptide 4b. The same protocol was used to prepare several differentially protected 1,3,4-thiadiazolo-peptides **4a–j** containing *N,N′*-orthogonal amino protecting groups (Scheme 2, Table 2, Fig 1).[26]

The synthesis of oxadiazolo-peptides **5a–j** was then undertaken. 1,3,4-Oxadiazoles have been synthesized by several approaches, involving dehydrocyclization of diacylhydrazines and



Scheme 1. Synthesis of diacylhydrazines **3**.



Scheme 2. Synthesis of 1,3,4-thiadiazoles **4** and 1,3,4-oxadiazoles **5**. Reagents and conditions: (a) LR, THF, reflux, 3 h, (b) EDC/TEA, DCM, reflux, 3 h.

Table 2
List of 1,3,4-thiadiazoles **4**/oxadiazoles **5** prepared

Entry	Mp (°C)	Yield (%)	Mass	
			Calcd	Obsd
4a	128	82	651.2253	651.2257[b]
5a	83	73	613.2662	613.2668[a]
4b	Gum	94	593.2	593.3[d]
5b	131	81	555.2607	555.2610[a]
4c	123	90	593.2198	593.2196[b]
5c	148	86	577.2427	577.2426[b]
4d	Gum	76	593.2	593.2[d]
5d	113	80	555.2641	555.2646[a]
4e	148	95	467.1787	467.1792[a]
5e	Gum	91	451.2	451.2[c]
4f	197	83	647.3	647.8[c]
5f	56	81	631.3	631.2[c]
4g	152	96	633.2	633.3[c]
5g	141	88	639.2583	639.2588[b]
4h	78	83	586.2675	586.2679[b]
5h	Gum	71	570.3	570.8[d]
4i	145	79	435.2	435.0[c]
5i	126	88	441.2	441.3[d]
4j	Gum	90	340.1	340.8 [c]
5j	93	94	346.3357	346.3362[b]

[a] HRMS [M+H⁺].
[b] HRMS [M+Na⁺].
[c] ESI-MS [M+H⁺].
[d] ESI-MS [M+Na⁺].

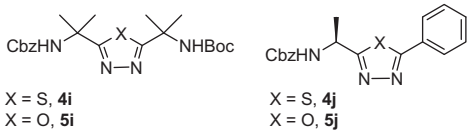


Figure 1.

oxidation of acylhydrazones. In the former route regents such as $Et_2O{\cdot}BF_3$,[27] hexamethyldisilazane,[28] Tf_2O,[29] polyphosphoric acid,[30] $SOCl_2$. and $POCl_3$[31] have been frequently used as dehydrating agents for cyclization. However, the use of these reagents is not always compatible with amino acid/peptides due to their ability to

Table 1
List of diacyl hydrazines **3** prepared

Compd	Pg^1	Amino acid (R^1)	Pg^2	Amino acid (R^2)	Mp (°C)	Yield (%)
3a	Boc	Gly	Fmoc	Glu(Bzl)	Gum	78
3b	Boc	Phe	Fmoc	Ala	178	90
3c	Boc	Phe	Fmoc	D-Ala	178	90
3d	Boc	Ile	Z	Cys(Bzl)	151	81
3e	Boc	Ala	Z	Met	Gum	84
3f	Z	Phe	Fmoc	Leu	205	89
3g	Z	Val	Fmoc	Phe	167	96
3h	Z	Lys(Boc)	Boc	Ala	147	76

cause undesired reactions like cleavage of protecting groups. The mild reagents generally used are carbodiimides,[32] TsCl/pyridine[33], trimethyl silylchloride[34], and Burgess reagent.[35] Therefore, we focused on employing ethyl-3-(3-dimethylaminoprophyl)carbodiimide (EDC) as cyclodehydrating agent. EDC was particularly attractive because of easy handling, simplicity associated to the workup of the product, efficiency in terms of product yield and the familiarity with its use in peptide and peptidomimetic(s) synthesis. In a typical procedure, the reaction of the *N*-Boc, *N*′-Fmoc protected diacylhydrazine **3b** in dry CH_2Cl_2 was refluxed in the presence of 1 equiv of EDC and 1.5 equiv of triethylamine (TEA) for 3 h which resulted in the formation of 1,3,4-oxadiazole derivative **5b** in almost quantitative yield. Extending the protocol further, a series of Boc, Z and Fmoc-protected 1,3,4-oxadiazole containing dipeptidomimetics **5a–j** were prepared starting from the corresponding diacylhydrazides (Scheme 2, Table 2, Fig. 1).[36]

Using the above protocols, the 1,3,4-thiadiazole/oxadiazole moieties were also inserted between an orthogonally protected sterically hindered dipeptide Z-Aib-CONHNHCO-Aib-Boc (Fig. 1, **4i** and **5i**). In another example Z-Ala-$CONHNH_2$ was coupled to C_6H_5COF and subsequently cyclized into 1,3,4-thiadiazole/oxadiazole derivative (Fig. 1, **4j** and **5j**).

Finally, the possibility of racemization during the synthesis of title thiadiazoles and oxadiazoles was studied by determination of the chiral purity of the samples of **4b**, **5b**, **4c,** and **5c** prepared via the present protocol by means of ^{1}H NMR and HPLC analyses.[37,38] The studies revealed that the tested samples were optically homogenous, and further the described protocol is free from racemization.

In conclusion, the current protocol is a simple and straightforward route for the insertion of 1,3,4-thiadiazolo- and 1,3,4-oxadiazolo-units into peptides. The synthesis makes use of amino acid substituted diacylhydrazines as common intermediate which on treatment with Lawesson's reagent and EDC furnishes the thiadiazolyl and oxadiazolyl compounds, respectively. The procedures give good yields and good purities with no detectable enantiomerization.

Acknowledgments

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24. Typical experimental procedure for the synthesis of diacylhydrazines **3**.
To a solution of hydrazide **1** (10 mmol), in DCM (10 mL) was added a solution of acid fluoride **2** (10 mmol) in DCM (5 mL) and the mixture was stirred at rt for 30 min or till the completion of the reaction (TLC analysis). The solvent was evaporated under reduced pressure and the residue was extracted with EtOAc. The organic layer was washed with citric acid (10%, 10 mL × 2), Na_2CO_3 (10%, 10 mL × 2), water (10 mL) and brine (10 mL) and finally dried over anhydrous Na_2SO_4.
The solvent was removed under reduced pressure and the resulting crude compound was purified by column chromatography. Spectroscopic data for **3b**: ^{1}H NMR ($CDCl_3$, 300 MHz,) δ 1.41 (s, 9H), 1.46 (d, J = 5.8 Hz, 3H), 3.00–3.04 (m, 1H), 3.12–3.16 (m, 1H), 4.20 (t, J = 6.2 Hz, 1H), 4.39–4.43 (m, 3H), 4.82 (t, J = 6.2 Hz, 1H), 6.30 (br, 1H), 7.03–7.64 (m, 13H), 8.76 (br, 1H); ^{13}C NMR ($CDCl_3$,75 MHz) δ 16.2, 28.7, 33.2, 42.8, 48.3, 53.4, 64.6, 79.8, 126.1, 126.7, 127.6, 128.0, 128.1, 128.5, 128.9, 137.6, 140.3, 143.1, 155.3, 155.8, 169.2, 170.1; HRMS calcd for $C_{32}H_{36}N_4O_6$ m/z 595.2533 [M+Na$^+$], found 595.2537 [M+Na$^+$].
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26. Typical experimental procedure for the synthesis of 1,3,4-thiadiazoles **4**.
To a solution of diacylhydrazine **3** (10 mmol) in THF, Lawesson's reagent (2.85 g, 15 mmol) was added and the solution was refluxed for 3 h. After completion of the reaction (monitored by TLC), the reaction mixture was filtered and the solvent was removed in vacuo. The resulting crude compound was purified by column chromatography (EtOAc/*n*-hexane, 2:8) to afford analytically pure product. Spectroscopic data for **4i**: ^{1}H NMR ($CDCl_3$, 300 MHz,) δ 1.40 (s, 9H), 1.55 (s, 12H), 5.08 (s, 2H), 6.18 (br, 1H), 6.78 (br, 1H), 7.08–7.19 (m, 5H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 28.1, 28.5, 48.6, 55.9, 64.6, 80.2, 127.2, 127.4, 127.9, 135.5, 155.6, 155.8, 167.9, 169.5; ESI-MS calcd for $C_{21}H_{30}N_4O_4S$ m/z 435.2 [M+H$^+$], found 435.0 [M+H$^+$].
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36. Typical experimental procedure for the synthesis of 1,3,4-oxadiazoles **5**.
To a solution of diacylhydrazide **3** (10 mmol) in DCM, EDC (1.91 g, 10 mmol) and TEA (2.09 mL, 15 mmol) were added and the reaction mixture was refluxed for 3 h (monitored by TLC). The solvent was removed in vacuo and the crude was dissolved in EtOAc (10 mL × 2) and it was washed successively with citric acid (10%, 10 mL × 2), Na_2CO_3 (5%, 10 mL), water (2 × 10 mL) and brine (10 mL) and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum and purified by column chromatography (EtOAc/*n*-hexane, 2:8) to afford pure oxadiazole.
Spectroscopic data for **5e**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.39 (s, 9H), 1.46 (d,

J = 6.0 Hz, 3H), 2.01 (s, 3H), 2.28 (m, 2H), 2.39 (m, 2H), 4.61 (m, 1H), 4.98 (m, 1H), 5.10 (s, 2H), 6.33 (br, 1H), 7.05–7.17 (m, 5H), 8.35 (br, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 15.2, 17.2, 28.7, 29.0, 33.7, 48.3, 51.0, 64.1, 79.9, 126.8, 127.3, 128.2, 139.8, 154.9, 155.8, 166.8, 169.7; ESI-MS calcd for $C_{21}H_{30}N_4O_5S$ m/z 451.2 [M+H$^+$], found 451.2 [M+H$^+$].

37. ^{1}H NMR analysis of samples **3b** and **3c** revealed that methyl group appeared as doublet at δ 1.29 and 1.33 ppm for **3b** and at δ 1.30 and 1.34 ppm for **3c**, respectively. Similarly, the ^{1}H NMR of samples of thiadiazoles Boc-Phe-ψ-[C_2N_2S]-Ala-Fmoc **4b**, **4c** and oxadiazoles Boc-Phe-ψ-[C_2N_2O]-Ala-Fmoc **5b**, **5c** were studied. The alanyl methyl groups were resonated as distinct doublets at δ 1.30 and 1.34 for **4b** and at δ 1.28 and 1.32 for **4c**, at δ 1.31 and 1.35 for **5b** and at δ 1.28 and 1.32 for **5c** supporting their enantiopurity.
38. The enantiopurity of each of the above compounds was further supported by the HPLC analysis by using column: Agilent Eclipse XDB-C18 (flow rate 0.5 mL/min; eluting with 0.1% TFA water–acetonitrile; 30–100% in 30 min). Diacylhydrazines **3b** and **3c** showed single peak with retention time at 12.48 and 12.06 min, respectively. Similarly in the case of 1,3,4-thiadiazole derivatives, single retention time at 9.55 min for **4b** and at 9.43 min for **4c** were observed, whereas the corresponding enantiopure 1,3,4-oxadiazoles **5b** and **5c** showed single retention times at 11.73, 11.67 min, respectively.