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Solution and Solid-Supported Synthesis of 3,4,5-Trisubstituted 1,2,4-Triazole-Based Peptidomimetics

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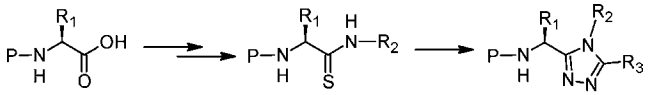
ABSTRACT



3,4,5-Trisubstituted 1,2,4-triazoles were synthesized in solution from various thioamides and hydrazides in smooth experimental conditions leading to peptidomimetic scaffolds. This strategy was found to be compatible with the usual peptide synthesis protecting groups. This methodology was then applied on solid support by anchoring α-amino acids through their amino function to an activated carbonate resin.

Molecules containing a 1,2,4-triazole moiety have elicited considerable interest among medicinal chemists because they display a wide range of antifungal[1] and antibacterial[2] activities. This moiety was also found in potent agonist or antagonist receptor ligands.[3−6] 1,2,4-Triazole derivatives have been used as mimics[5−8] or isosteres[9,10] of the amide bond in attempts to increase bioavailability of the parent bioactive molecules. They have also been incorporated into peptides to surrogate cis amide bonds.[11] Different approaches have been reported for the preparation of such heterocycles,[12−14] but the more explored strategy involved cyclization of an acylamidrazone intermediate at high temperature.[15−17]

As far as we know, this last strategy was the only one involving α-amino acids to synthesize 3,5-disubstituted 1,2,4-

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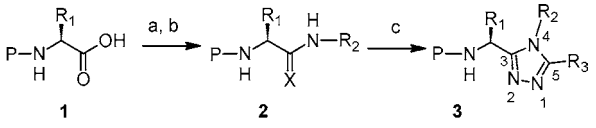
Scheme 1. Preparation of 1,2,4-Triazoles[a]



P = Boc, Fmoc or Z
R_1 = Indol-3-yl-methyl
R_2 = CH_2-CH(Ph)$_2$, $(CH_2)_5$-CH_3, $CH(CH_3)COOtBu$, indol-3-yl-ethyl, CH_3, 4-OMe-phenyl-1-methyl
R_3 = H, phenyl, benzyl, 4-N-Boc-piperidinyl, Indol-3-yl-methyl, Indol-3-yl-ethyl

[a] Reagents and conditions: (a) Coupling reaction with H_2N-R_2, X = O. (b) Lawesson's reagent, X = S. (c) $H_2N-HN-COR_3$, $Hg(OAc)_2$, rt.

triazole (AA-Gly) dipeptide mimetics in moderate yields (13−52%).[18] We chose an alternative synthetic pathway recently described by Hitostuyanagi et al.[19] to prepare 1,2,4-triazole derivatives. It consists of condensation of a thionotripeptide at room temperature with an excess of formic hydrazide in the presence of a thiophile metal salt such as mercury(II) acetate. Commercial availability of a large selection of primary amines and acids, including α-amino acids, and the mild reaction conditions used for the heterocycle formation make this reaction very attractive. Despite the apparent synthetic possibilities allowed by this strategy, to the best of our knowledge, the synthetic scope and the functional group tolerance of this reaction have not been fully exploited. Hitosuyanagi et al. started from Boc-thionotripeptides and performed cyclization only with formic hydrazide. We decided to explore this route for the synthesis of 3,4- and 3,5-disubstituted and 3,4,5-trisubstituted 1,2,4-triazoles starting from enantiomerically pure Fmoc-, Z-, or Boc-amino acids and various hydrazides.

Our study began with the preparation of various 3,4-disubstituted 1,2,4-triazoles as shown in Scheme 1 using formic hydrazide for the cyclization step. Because tryptophane residue or indole moiety is involved in various small peptidomimetic ligands of receptors,[20,21] we incorporated this residue in the first position (R_1 = indol-3-yl-methyl). After coupling of this *N*-protected amino acid **1** with an amine, the amide was thionated by using Lawesson's reagent.[22] The obtained thioamide **2** was then submitted to the conditions reported by Hitosuyanagi et al. which were slightly modified (5 equiv of formic hydrazide and 1.1 equiv of mercury(II) acetate in acetonitrile). The completion of this step was followed by reversed-phase HPLC, showing that cyclization into triazoles **3** was achieved within 3 h. After purification,

Table 1. Formation of 1,2,4-Triazoles from Thioamides (R_1 = Indol-3-yl-methyl)

compd no.	P	R_2	R_3	yield[a] (%)
3a	Boc	2,2-diphenylethyl	benzyl	64
3b	Z	*n*-hexyl	phenyl	47
3c	Z	*n*-hexyl	4-*N*-Boc-piperidinyl	31
3d	Fmoc	$CH(CH_3)COOtBu$[b]	H	76[c]
3e	Fmoc	$CH(CH_3)COOtBu$[b]	benzyl	80
3f	Boc	indol-3-yl-ethyl	H	51[c]
3g	Boc	methyl	indol-3-yl-methyl	50[c]
3h	Boc	methyl	indol-3-yl-ethyl	51[c]
3i	Boc	4-methoxybenzyl	indol-3-yl-methyl	49
3j	Boc	2,4-dimethoxybenzyl	indol-3-yl-methyl	55

[a] Yields were calculated after purification. [b] From L-alanine *tert*-butyl ester. [c] Reactions were performed in CH_3CN as solvent.

the desired compounds were obtained in at least 50% yield (see Table 1, entries d and f).

From these results, we decided to investigate the synthesis of 3,4,5-trisubstituted 1,2,4-triazoles. Starting from various thioamides **2**, the cyclization step was performed at room temperature in the presence of various selected hydrazides (R_3 in Table 1) in slight excess (1.1 equiv) in THF. Acylamidrazone intermediates were readily obtained but cyclization to trisubstituted triazoles occurred more slowly than with formic hydrazide. After completion of the reaction and purification, the desired compounds **3** were obtained in reasonable yields (see Table 1). All compounds were chemically characterized by mass spectrometry and ^1H and/or ^{13}C NMR.[23] In these experiments, we clearly demonstrated the following: (i) it was possible to introduce substitution in position 5 of the triazole by using substituted hydrazides in place of formic hydrazide; (ii) the nature of the introduced group could be alkyl, aryl, or cycloalkyl; (iii) trisubstituted 1,2,4-triazoles could be obtained by this approach in smooth conditions but required longer reaction time;[24] and (iv) the optical purity of final products showed an ee superior to 98%, indicating that configuration of the starting α-amino acid was not affected during the process. To check the optical purity, both enantiomers of **3g** and **3h** were synthesized and their optical purities were checked by chiral HPLC (Chiralcel OD at 30 °C, 1 mL/min, detection at 280 nm, isocratic run with 80/20/0.1 (v/v/v) hexane/2-propanol/Et_2NH as eluent). **3g**: (S enantiomer) t_R = 19.89 min, (R enantiomer) t_R = 14.12

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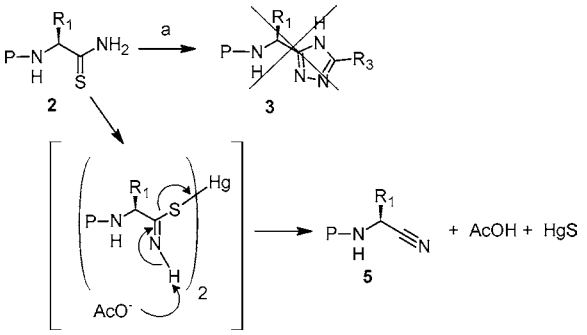
(23) Typical chemical shifts of triazole could be observed. For example, with R_3 = H, the unique proton shift ranged from 8.2 to 8.5 ppm and its corresponding carbon shift (C5) was close to 142 ppm. In the other cases, quaternary carbon shifts of triazole were observed between 150 and 156 ppm.
(24) **General Procedure for the Conversion of Thioamide to Trisubstituted 1,2,4-Triazole.** A stirred solution of 1.0 eq. of thioamide and 1.1 equiv of hydrazide in THF or ACN (0.2 M) was treated at room temperature with 1.1 equiv of $Hg(OAc)_2$. The reaction was monitored by RP-HPLC and after completion (usually within 3 days), it was filtered through a pad of Celite. The filtrate was concentrated under vacuo and the residue was dissolved in EtOAc. This solution was sequentially washed with a 1 M $KHSO_4$ aqueous solution and brine, dried over Na_2SO_4, and concentrated under reduced pressure. The resulting compound was purified by flash chromatography on silica gel to yield the expected 1,2,4-triazole derivative.

Scheme 2. Decomposition of Primary Thioamide to Nitrile in the Presence of $Hg(OAc)_2$[a]

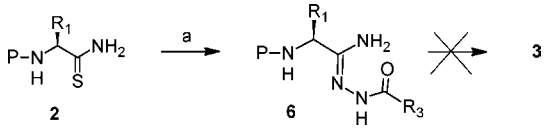


[a] Reagents and conditions: (a) $H_2N-HN-COR_3$, $Hg(OAc)_2$, rt.

min; ee **3g(R)** ≥ 98%. **3h**: (R enantiomer) t_R = 11.30 min, (S enantiomer) t_R = 16.03 min; ee **3h(R)** ≥ 98%.

When R_2 = H, formation of the expected compounds was not observed and examination of LC/MS spectra revealed the presence of the corresponding nitriles **5** (Scheme 2). This "desulfuration" of nonsubstituted thioamides was already described on simple aliphatic and aromatic primary thioamides,[25] showing that the use of mercury(II) acetate is not suitable for the preparation of 3,5-disubstituted 1,2,4-triazoles. When mercury(II) acetate was replaced by its chloride analogue, the intermediate acylamidrazone could be observed by LC/MS but did not cyclize into the expected triazole at room temperature (Scheme 3) even after addition

Scheme 3. Formation of Acylamidrazone in the Presence of $Hg(Cl)_2$[a]



[a] Reagents and conditions: (a) $H_2N-HN-COR_3$, $HgCl_2$, rt.

of sodium acetate. When the corresponding isolated acylamidrazone **6** was heated under refluxing toluene, no cyclization occurred. To obtain 3,5-disubstituted 1,2,4-triazoles, we decided to introduce on the primary amide function a protecting group that could be cleaved after the formation of the desired triazole moiety. In our first attempt, we used 4-methoxybenzylamine for this purpose (entry **3i**, Table 1). The expected compound was obtained in 49% yield after purification. Various experimental conditions (TFA, CAN, H_2 Pd/C) were tested to remove the 4-methoxybenzyl protecting group but unfortunately, no deprotection occurred. We next introduced a more acid-labile protecting group, 2,4-dimethoxybenzylamine. The presence of a second methoxy group on the benzylamine ring did not interfere with the

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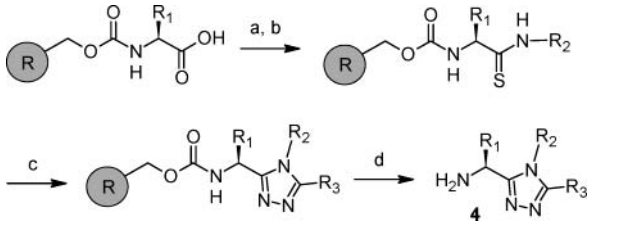
Scheme 4. Solid-Phase Synthesis of Triazoles[a]



[a] Reagents and conditions: (a) Coupling with an amine. (b) Lawesson's reagent, THF, under reflux. (c) $H_2N-HN-COR_3$, $Hg(OAc)_2$, DMF, rt. (d) TFA/DCM/H_2O/TIS.

formation of the triazole moiety and expected compound **3j** was obtained in similar yield. This group could be removed by TFA to yield 3,5-disubstituted 1,2,4-triazoles.

In a second set of experiments, we undertook the polymer-supported synthesis of trisubstituted 1,2,4-triazoles. Solid-phase synthesis of triazoles has already been described. Katritzky et al.[26] reported the synthesis of a 1,2,4-triazole linker on Wang resin leading to a mixture of trisubstituted regioisomers.

Furthermore, as the resin linkage was performed through a phenolic oxygen, no diversity could be achieved at this position of the heterocycle, yielding 3- or 5-*p*-hydroxyphenyl-substituted triazoles. Makara et al.[27] modified Katritzky's approach to obtain amino-triazoles. Larsen et al.[28] proposed the synthesis of disubstituted 1,2,4-triazoles through the use of a traceless linker. With the aim to rapidly generate small peptidomimetic molecules including amino acid residues and with three diversity centers, we needed to anchor amino acids on the resin through their *N*-terminus. An inverse anchoring of three α-amino esters was achieved on a Wang resin through its *p*-nitrophenyl carbonate derivative[29] to yield urethane-linked α-amino -esters. After saponification of the ester function,[30] three different amines were coupled to the corresponding carboxylic function. Conversion of the amide functions into thioamides was performed with Lawesson's reagent as already described,[31] and direct conversion of thioamides to disubstituted and trisubstituted triazoles was achieved on support, using three different hydrazides in 10-fold excess in the presence of 3 equiv of $Hg(OAc)_2$ at room temperature (see Scheme 4). After 3 days and classical washings, resins were treated with TFA/DCM/H_2O/TIS (70/25/2.5/2.5, v/v) to quantitatively release compounds **4** which were examined by LC/MS. Results are gathered in Table 2 and showed that the expected compounds were obtained in excellent purities, except for compound **4o**. This result is not surprising as an aryl group is branched in position 5 of

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Table 2. Supported Synthesis of Triazoles

compd no.	R_1	R_2	R_3	purity[a] (%)
4a	benzyl[b]	benzyl	H	87
4b	benzyl[b]	benzyl	benzyl	95
4c	benzyl[b]	indol-3-yl-ethyl	H	69
4d	benzyl[b]	indol-3-yl-ethyl	benzyl	81
4e	benzyl[b]	$CH_2-CH(Ph)_2$	H	93
4f	benzyl[b]	$CH_2-CH(Ph)_2$	benzyl	87
4g	indol-3-yl-methyl[c]	benzyl	H	87
4h	indol-3-yl-methyl[c]	benzyl	benzyl	83
4i	indol-3-yl-methyl[c]	$CH_2-CH(Ph)_2$	H	89
4j	indol-3-yl-methyl[c]	$CH_2-CH(Ph)_2$	benzyl	88
4k	isobutyl[d]	benzyl	H	81
4l	isobutyl[d]	benzyl	benzyl	87
4m	isobutyl[d]	indol-3-yl-ethyl	H	98
4n	isobutyl[d]	indol-3-yl-ethyl	benzyl	73
4o	isobutyl[d]	indol-3-yl-ethyl	phenyl	45

[a] Purity was checked by LC/MS at 214 nm. [b] From L-phenylalanine. [c] From L-tryptophane. [d] From L-leucine.

the ring, increasing the steric hindrance, and thus maybe required longer reaction time for cyclization.

In summary, we have successfully developed an easy and new method for synthesizing 3,4,5-trisubstituted 1,2,4-triazoles that can be applied to a large variety of acids and amines including α-amino acids. This approach was found to be compatible with the commonly used amine protecting groups. As the delicate tryptophane residue was not sensitive to these experimental conditions, it can be assumed that most of the α-amino acid residues (with the exception of asparagine and glutamine, due to the conversion to thioamides) can be introduced in such a schematic route. Furthermore, optically pure compounds were obtained. Its application on solid support was successful. Consequently, this strategy may provide a useful tool for the synthesis of new scaffolds or peptidomimetics containing trisubstituted triazoles and may allow preparation of triazole libraries.

Supporting Information Available: HPLC monitoring and flash chromatographic conditions, NMR data, and general physicochemical data of obtained compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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