Biocompatible Ligations

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Metal-Free, Regioselective Triazole Ligations that Deliver Locked *cis* Peptide Mimetics**

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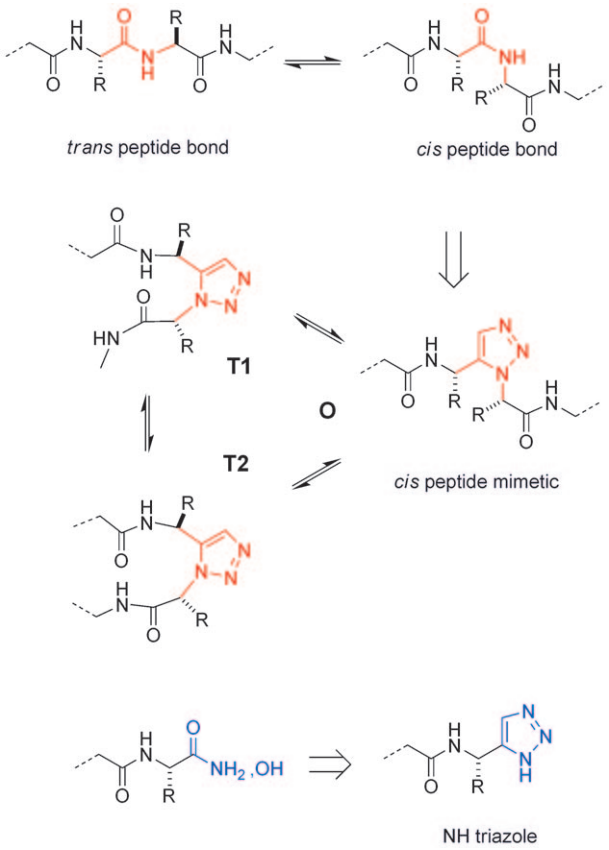
Chemical ligation reactions have become popular during recent years as they enable formation of chemoselective linkages under mild, often aqueous reaction conditions, and with high yields.[1] Chemical ligations are especially useful if they can couple complex functionalized molecules without protecting groups under physiological conditions or in the presence of living cells. Reversible ligations have been used successfully in fragment-based screening and drug discovery.[2] A ligation strategy furnishing conformationally locked, biologically active molecules would be an exciting extension of current methodology. Such a strategy could be especially valuable for the structure-based design and synthesis of inhibitors of protein–protein interactions and peptidyl isomerases.[3]

Our aim was to establish the direct incorporation of 1,5-disubstituted triazole rings into peptides starting from standard amino acid building blocks. A reaction yielding these products would allow straightforward access to locked *cis* peptide mimetics, or "clack peptides" (Scheme 1).[4] The 5-peptidyl-(1*H*-1,2,3-triazol-1-yl) peptides thus obtained should allow the study and exploitation of the effects of *cis* peptide geometry, either for open-chain conformations (**O**) or for peptide turns (**T1** and **T2**) induced by the *cis* peptide bond (Scheme 1).[5] Moreover, the 5-peptidyl-1*H*-1,2,3-triazoles (NH triazoles) that are unsubstituted in the 1-position, which have not been prepared synthetically until now, might serve as proteolytically stable bioisosters of the C termini of peptides and proteins (Scheme 1, bottom).

1,2,3-Triazoles are attractive ligation products as they are thermodynamically and physiologically stable. Accordingly, they are found in several orally administered drugs.[6,7] 1,3-Dipolar cycloadditions that deliver 1,2,3-triazoles from alkynes and azides were first reported by Huisgen.[8] These additions became especially popular after they were found to proceed regioselectively with a base and with copper(I) as



Scheme 1. Replacement of a peptide amide bond with 1,5-disubstituted 1,2,3-triazole, furnishing a 5-peptidyl-1*H*-1,2,3-triazolyl peptide as a "locked *cis* peptide mimetic" or "clack peptide". It is expected that *cis* peptide mimetics would populate either open-chain conformations (**O**) or turn structures (e.g. **T1**, **T2**). 5-Peptidyl-1*H*-1,2,3-triazoles ("NH-triazoles") are expected to constitute an enzymatically stable C terminus with a H-bonding donor and acceptor that is similar to the wild type.

catalyst, yielding 1,4-disubstituted 1,2,3-triazoles.[9] In constrast, 1,5-disubstituted 1,2,3-triazoles can be formed regioselectively from alkynes and azides using a Ru^{II} catalyst.[10] Unfortunately, the published methods for regioselective triazole ligations, both for the 1,4- and 1,5-isomers, rely on the use of heavy metal salts, thus excluding applications in the presence of living cells. Therefore, the development of a metal-free, and thus biocompatible, regioselective triazole ligation method was considered as highly attractive target.[11]

We envisioned that 5-peptidyl-(1*H*-1,2,3-triazol-1-yl) peptides should be accessible by solid-phase synthesis starting from the polymer-supported phosphoranylidene acetate **1** (Scheme 2). Racemization-free C-acylations of polymer-bound phosphoranylidene acetates with Fmoc-protected

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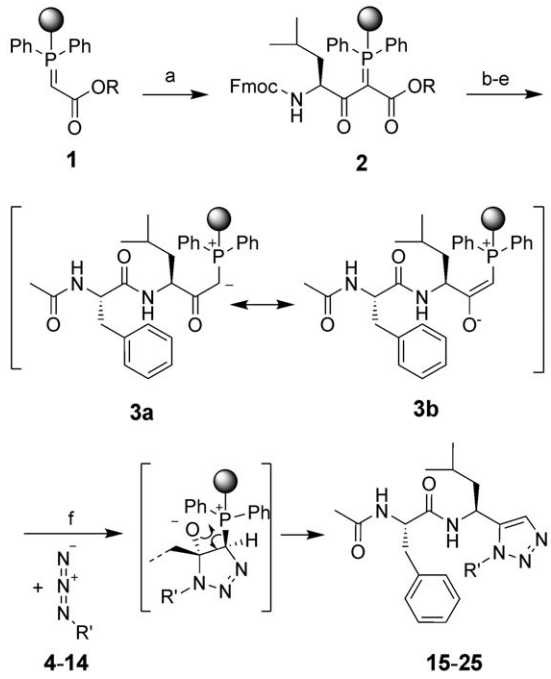


Scheme 2. Reaction conditions: a) Fmoc-Leu-OH and MSNT, lutidine in CH_2Cl_2; or: BTFFH, DIPEA, DMF (R = TMSE or *tert*-butyl), 14 h; b) 20% piperidine/DMF; c) Fmoc-Phe-OH, DIC, HOBT, DMF, 2 h; d) 20% Ac_2O/DMF; e) TAS-F/DMF or TFA/CH_2Cl_2 (R = TMSE or *tert*-butyl); f) **4**–**14**, CH_2Cl_2, or THF. MSNT = 1-(mesitylene-2-sulfonyl)-3-nitro-1*H*-1,2,4-triazole, BTFFH = bis(tetramethylen)fluoroformamidinium hexafluorophosphate, DIPEA = *N*,*N*-diisopropylethylamine, TMSE = trimethylsilylethyl, HOBt = 1-hydroxybenzotriazole, TAS-F = tris(dimethylamino)sulfonium difluorotrimethylsilicate, TFA = trifluoroacetic acid.

amino acids have been recently shown to yield Fmoc amino acyl phosphoranylidene acetates **2**.[12,13] Following Fmoc cleavage of **2**, the peptide could be elongated via the free amino group by standard peptide chemistry. Deprotection of the trimethylsilylethyl (TMSE) or *tert*-butyl ester group furnished the decarboxylated peptidyl phosphorane **3** as the only product.

Herein we report reactions of peptidyl phosphorane **3** with azides **4**–**14**. At room temperature, polymer **3** reacted smoothly with 4-toluenesulfonyl azide **4**, furnishing the 5-peptidyl-(1*H*-1,2,3-triazol-1-yl) tosylate **15** in high yield. The product was obtained in excellent purity solely by washing the resin, followed by evaporation of the solvent. The second product, triphenylphosphane oxide, remained attached to the polymer. Neither epimerized byproducts nor traces of the 1,4-substituted triazole were detected. Product formation can be rationalized by a 1,3-dipolar cycloaddition reaction by considering the phosphorous ylide **3a** as phosphonium enolate in accordance with the resonance structure **3b**. The cyclic intermediate can be formed either by a concerted or a stepwise mechanism. To the best of our knowledge, this reaction of peptidyl phosphoranes has not been reported to date, and is also the first account of a dipolar cycloaddition of phosphoranes on a polymeric support. Earlier work has reported 1,3-dipolar cycloadditions with electron-rich olefins,[14] and the transition states of such reactions have been calculated, thus explaining the observed 1,5-selectivity by interaction of the olefin HOMO with the azide LUMO.[14c]

This reaction mode suggests a negative effect of azide electron density on the reaction rate. Thus we investigated the scope of this reaction by using azides with different electron densities (Scheme 2, Table 1). Azides substituted with electron-withdrawing substitutents, such as sulfonyl and acyl, reacted rapidly in dichloromethane and yielded the 1,5-disubstitued triazolyl sulfonamides, and carboxamides, respectively. 4-Nitrobenzoyl-azide (**6**) led to the 1-acyl-1,2,3-triazole **16**. The aromatic azide 4-azidobenzoic acid (**7**) required prolonged reaction times to yield the 1-aryl-5-peptidyl triazole **17** with high purity and yield. Employing trifluoromethanesulfonyl azide (TfN_3; **5**) as dipole directly furnished 5-peptidyl-1*H*-1,2,3-triazole **18** after aqueous work-up. Aliphatic azides, such as benzylazide (**8**), methyl-2-azido-acetate (**9**), and 2-azido-acetamide (**10**), did not react in dichloromethane, even after several days. More polar solvents, such as tetrahydrofuran or DMF, however, enabled efficient reactions at slightly elevated temperatures, cleanly furnishing triazoles **19**–**21**. The observed reactivity trends of azides and the effect of solvent polarity are in accordance with a polar transition state and with a LUMO-controlled 1,3-dipolar cycloaddition reaction.

The polymer-supported peptidyl phosphorane **3** also has the potential for direct access to 5-peptidyl-(1,2,3-triazol-1-yl) peptides. To verify this hypothesis, 2-azido acids were synthesized from unprotected amino acids by a diazo transfer reaction employing trifluoromethanesulfonyl azide **5**.[15] The N-terminal azidopeptide amides **11**–**13** were prepared on Rink resin using the respective 2-azido acids in the final coupling step, whereas N-terminal azidopeptide acid **14** was prepared on 2-chlorotrityl resin. Azidopeptides **11**–**14** were reacted in excess with the peptidylphosphorane under the conditions defined for electron-rich azides, and the desired peptidyl triazolyl peptides **22**–**25** were isolated in good yields by reverse-phase HPLC.

All the new products were purified by column chromatography if necessary, and were characterized by HR-MS and by full assignment of their ^1H and ^{13}C NMR spectra. The potential of peptidyl triazolyl peptides to form stable conformations in solution was investigated with product **22** in DMSO using NMR spectroscopy. A complete assignment of all the ^1H and ^{13}C resonances was obtained from a set of two-dimensional spectra. Based on this assignment, distances were extracted from a 2D-ROESY experiment. More than 30 distances could be obtained in this way, and they were used as distance restraints in a molecular dynamics simulation. Sets of structures were calculated by a simulated annealing procedure. An overlay of ten calculated structures indicated consistently a turn-like bend of the triazole-flanking amino acid residues (Figure 1), which was characterized by the inward orientation of the −1 amide carbonyl group and the +1 amide NH group (numbering in N- to C-terminal direction). Therefore, the preferred conformation of peptidyl triazol-1-yl peptide **22** in DMSO solution most closely resembled the **T2** structure in Scheme 1. (Precise rotational angles and their corresponding standard deviations are

Table 1: Selected triazole ligation products.

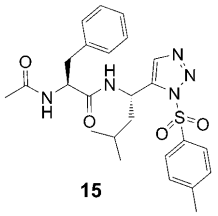
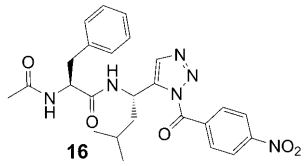
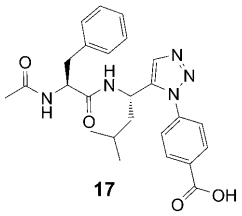
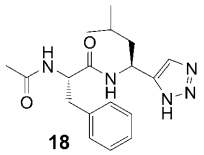
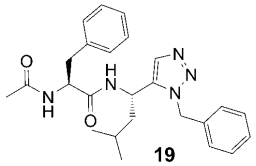
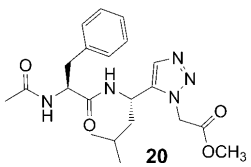
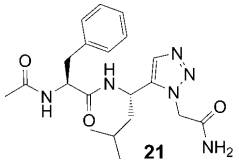
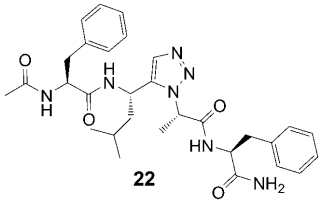
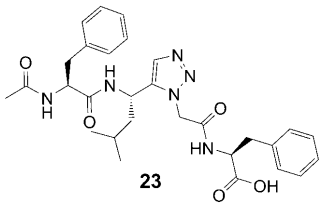
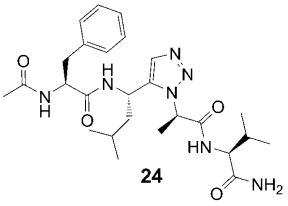
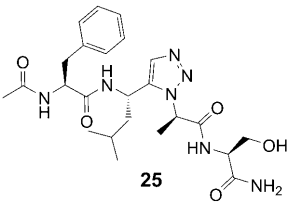
Entry	Product	Yield [%]	Purity[c] [%]
1	 **15**	88[a]	96
2	 **16**	95[a]	74[d]
3	 **17**	80[a]	92
4	 **18**	88[a]	92[d]
5	 **19**	62[b]	88
6	 **20**	70[b]	91
7	 **21**	72[b]	95
8	 **22**	68[b]	82
9	 **23**	72[b]	96

Table 1: (Continued)

Entry	Product	Yield [%]	Purity[c] [%]
10	 **24**	74[b]	97
11	 **25**	73[b]	98

[a] Reaction in CH_2Cl_2 at RT. [b] Reaction in THF at 60 °C. [c] HPLC purities were determined at 220 nm with UV/Vis spectroscopy. Compounds **15** and **17**–**21** were more than 80% pure in crude products after trituration with acetonitrile. [d] Purity of the crude product; compound **16** degraded partially to **18** during purification or storage.

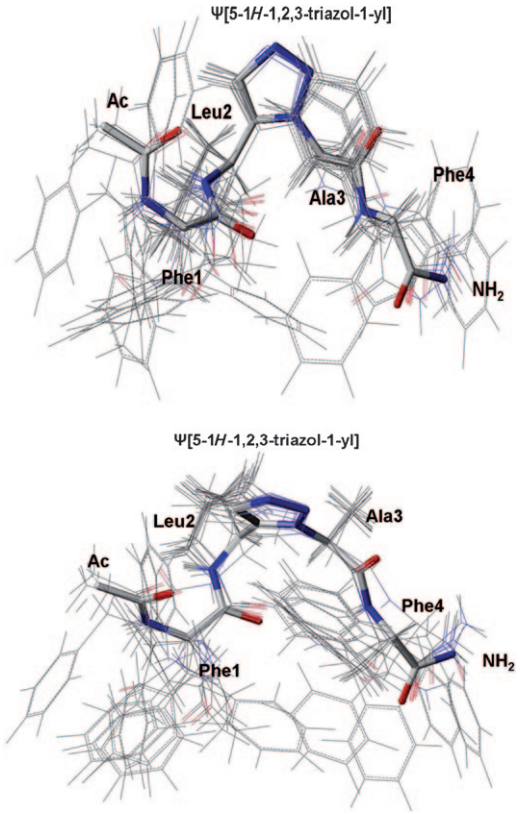


Figure 1. The solution structure of 5-peptidyl-triazol-1-yl peptide **22** as determined in DMSO by ROESY NMR spectroscopy. Measured average distances were used as distance constraints in a molecular dynamics simulation. An overlay of 10 structures obtained by simulated annealing is shown. All the structures display a bent-like turn structure. Amino acids flanking the triazole motif are relatively rigid, with the carbonyl of the −1 amino acid and the NH of the +1 amino acid pointing inwards in all ten structures.

summarized in the Supporting Information). More detailed studies concerning the effects of elongated peptide tails, of amino acid stereochemistry, and cyclization of 1,5-triazolyl peptides are under way and will deliver a more comprehensive picture of the potential of this compound class in conformation control.

In summary, we have presented the first metal-free regioselective triazole ligation. The reaction has a broad scope and, most relevantly, allows full integration into peptide synthesis, thus avoiding the use of tediously prepared amino acid alkynes. The new triazole ligation can be employed to yield products with carefully controlled conformations. Details of structural investigations into the ligation products will be reported in due course.

Experimental Section

Synthetic procedures and analytical data (HRMS; ^{1}H, ^{13}C NMR) of all new compounds are given in the Supporting Information.

Synthesis of a 5-peptidyl-1*H*-1,2,3-triazole: **18**: An excess of freshly prepared trifluoromethanesulfonyl azide **5** (3 equiv) in CH_2Cl_2 (5 mL) was added to phosphorane resin **3** (200 mg, 0.254 mmol), and the mixture was stirred for 5 h at room temperature. The polymer support was separated by filtration and washed with CH_2Cl_2. The solvent were removed under vacuum and the crude product was dissolved in acetonitrile. Water (1 mL) was added, and the mixture was lyophilized to deliver **18** as a pale yellow solid (99 mg, 88 %). ^{1}H NMR (300 MHz, CD_3CN): $\delta = 0.83$, 0.88 (2d, $J = 6.1$, 6.7, 6H, 2CH_3, Leu), 1.46–1.63 (m, 3H, *CH*, CH_2, Leu), 1.86 (s, 3H, CH_3, acetyl), 2.86–3.11 (m, 2H, CH_2, Phe), 4.23–4.32 (m, 1H, *CH*, Phe), 4.55–4.62 (m, 1H, *CH*, Leu), 6.93–7.49 ppm (m, 6H, arom.). ^{13}C NMR: (75.5 MHz, $CDCl_3$): $\delta = 22.0$, 23.2, 25.0, 30.7, 38.5, 46.1, 54.8, 117.7, 122.0, 125.9, 129.0, 129.8, 136.4, 171.2, 177.6 ppm. HRMS (ESI): *m*/*z* calcd for $C_{18}H_{25}N_5O_2$ $[M+H]^+$: 344.20865; found: 344.20845.

Synthesis of a 5-peptidyl-(1*H*-1,2,3-triazol-1-yl)-peptide: **22**: Phosphorane resin **3** (100 mg, 0.127 mmol) was pre-swollen in THF (1 mL) in a glass vial, and azidopeptide **11** (1.5 equiv, 0.19 mmol), dissolved in THF (1 mL), was added to the vial. The mixture was heated overnight at 60 °C in sealed glass vial. After cooling to room temperature, the polymer support was filtered off and washed with THF and CH_2Cl_2. Solvents were removed under vacuum, and **22** was isolated by preparative reverse-phase HPLC to remove remains of the azidopeptide reagent. Compound **22** was obtained as a white lyophilisate (48 mg, 68 %). ^{1}H NMR: (300 MHz, [D$_6$]DMSO): $\delta =$ 0.82, 0.85 (2d, $J = 6.1$ Hz, 6H, CH_3, Leu), 1.35–1.52 (m, 2H, Leu), 1.60 (d, 3H, $J = 7.3$ Hz, CH_3, Ala), 1.65–1.67 (m, 1H, $C^{\beta}H$, Leu), 1.73 (s, 3H, CH_3, acetyl), 2.75–3.02 (m, 4H, 2CH_2, Phe), 1.53–1.87 (m, 3H, CH_2, *CH*, Leu), 2.50–3.31 (m, 4H, 2CH_2, Phe), 4.39–4.49 (m, 2H, 2*CH*, Phe), 5.04–5.12 (m, 1H, $C^{\alpha}H$, Leu), 5.43 (q, $J = 7.3$, 1H, *CH*, Ala), 7.02 (d, $J = 7.4$, 2H, NH_2), 7.11–7.24 (m, 10H, arom., Phe), 7.55 (s, 1H, *CH*, triazole), 8.01, 8.04, 8.53 ppm (3d, $J = 7.9$, 8.3, 7.9, 3H, 3*NH*). ^{13}C NMR: (75.5 MHz, [D$_6$]DMSO): $\delta = 17.7$, 21.6, 22.3, 22.6, 24.2, 37.2, 41.4, 43.2, 53.7, 54.3, 56.7, 126.1, 127.9, 129.0, 131.6, 137.5, 139.7, 167.5, 169.1, 171.1, 172.2 ppm. HRMS (ESI): *m*/*z* calcd for $C_{30}H_{39}N_7O_4$ $[M+H]^+$: 562.31418; found: 562.31419.

The structure of compound **22** was calculated using repeated simulated annealing with a heating phase of 2000 fs up to a temperature of 1000 K followed by an exponential cooling for 10000 fs up to 0 K, and using NMR-derived range constraints with a force constant of 200 kcal mol^{-1}. In all structures presented in Figure 1, the range constraints were violated less than 0.2 Å. All calculations were carried out using SYBYL7.3 (Tripos Inc.).

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