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Solid-phase based synthesis of biologically promising triazolyl aminoacyl (peptidyl) penicillins

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ABSTRACT

An efficient and versatile methodology for the preparation of valuable triazolyl aminoacyl (peptidyl) penicillins is described. Solid-phase Cu(I)-catalyzed Hüisgen 1,3-dipolar cycloaddition was used as the key step showing general applicability and excellent regioselectivity either with CuI or $[Cu(CH_3CN)_4]PF_6$ as Cu(I) source.

The main challenge in medicinal chemistry is to build libraries of compounds during the process of drug discovery, so the development of methodologies for a rapid and efficient construction of molecular diversity has been a priority during the last decade.[1] One of those methodologies is the parallel solid-phase synthesis that has been an important tool in order to improve the efficiency of drug discovery. After its initial use in the preparation of peptides, solid-phase organic synthesis is now extensively employed for the preparation of a range of biologically interesting molecules including heterocycles and natural product scaffolds.[2]

Hybrid constructs from the entities of known biological activity could be another important source for molecular diversity. This is a very promising approach in the development of leads for medicinal chemistry applications, which benefits from the intrinsic activity of all or part of the components of the hybrid.[3]

As part of our interest in the application of solid-phase techniques to biologically interesting molecules,[4] we envisaged that 1,3-dipolar cycloaddition could be useful for the conjugation of peptides to a penicillin derivative,[5] resulting in the triazole compounds bearing several points of diversity as shown in Figure 1.

The synthesis of 1,2,3-triazoles is clearly the most useful 'click reaction' discovered so far,[6,7] providing a very attractive possibility for (bio)conjugation reactions since, in general, it can be performed at mild conditions as well as biological media. Also, the 1,2,3-triazole moiety has several good properties: high chemical stability (hydrolytic, oxidant, and reducing conditions), aromatic character, good hydrogen-bond-accepting ability and this moiety is relatively resistant to metabolic degradation. This structure is found in a lot of biological active compounds: antimicrobial,[8] antiallergic,[9] anti-inflammatory, anticancer,[10] and anti-HIV agents,[11] as well as a new series of selective human β_3-adrenergic receptor agonists.[12] Additionally, 1,2,3-triazoles are found in herbicides, fungicides,[13] and dyes. Click reactions have been very successful in the synthesis of (bio)polymeric materials for biomedical and pharmaceutical applications.[14] Among other applications, peptides are used to facilitate transport across the cell membranes and to produce protein–protein interactions.[15] Besides, peptidotriazoles were reported as novel inhibitors of the growth of *Leishmania mexicana*.[16] On the other hand, β-lactam is arguably one of the most important heterocyclic skeletons in organic chemistry.[17] Since the discovery of penicillin, more than seven decades ago, a number of monocyclic and bicyclic β-lactams have found broad applicability in antibacterial therapy.[18,19] β-lactams can be referred as 'privileged structure' since many non-antibacterial activities have been found in their derivatives: inhibition of cholesterol absorption,[20] prostate specific antigen,[21] thrombin,[22] human cytomegalovirus protein,[23] cysteine protease,[24] and human fatty acid amide hydrolase,[25] as well as anticancer activity[26] and neuroprotective action.[27]

To achieve our objectives, several conditions of catalyzed 1,3-dipolar cycloaddition between terminal alkynes on solid support and penicillin azide were investigated.[28] Thermal cycloaddition conditions have a very high activation energy, therefore more energetic reaction conditions are required, leading to a mixture of both 1,4- and 1,5-regioisomers of 1,2,3-triazole. Softer conditions and better regioselectivity could be achieved using catalysis, such as the Cu(I)-catalyzed azide–alkyne cycloaddition (CuAAC).[16,29] The major drawback of this reaction in the

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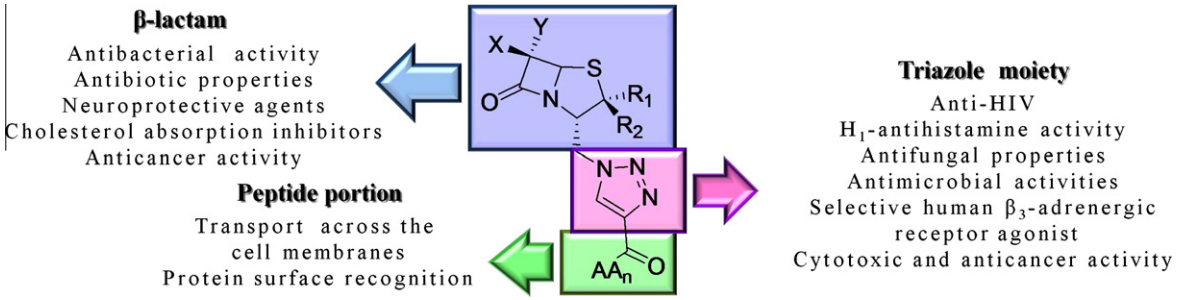
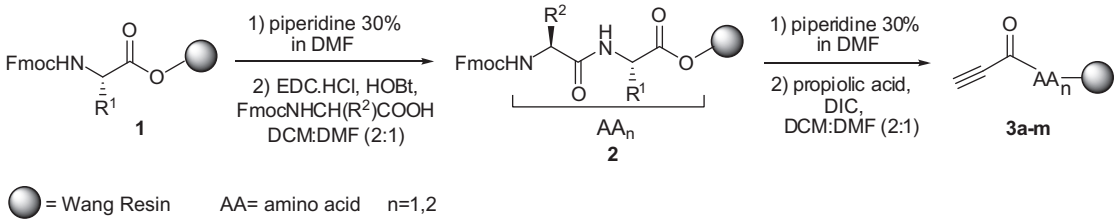


Fig. 1. Intrinsic biological properties of the building blocks.



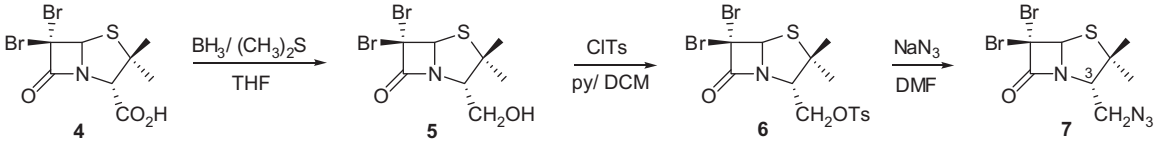
Scheme 1. Synthesis of alkyne-supported component.

homogeneous phase is the homocoupling products from two terminal alkynes.[30] Having the alkyne anchored to solid support, site isolation makes the homocoupling a considerably less favorable process.

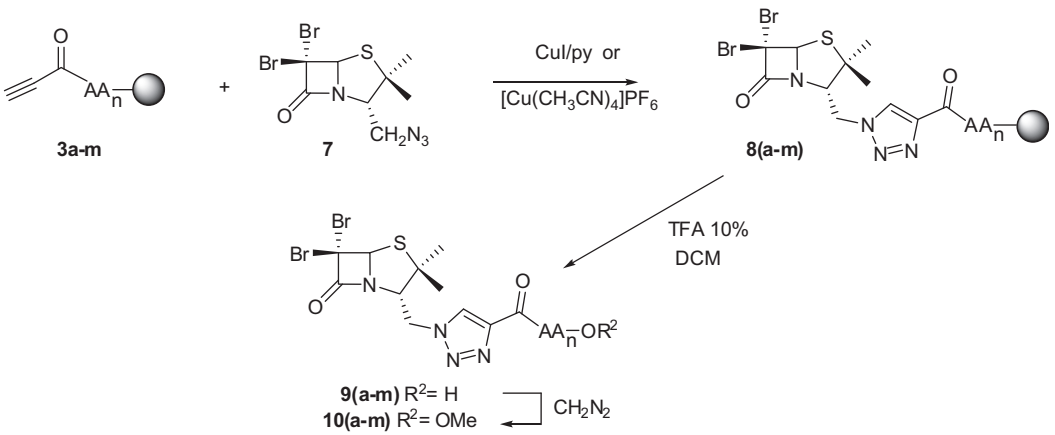
Our strategy started with the Fmoc protected-amino acid preloaded to Wang resin (**1**) which was treated with 30% piperidine in DMF to free the amino group (Scheme 1). In case that a second amino acid was coupled, standard solid-phase peptide synthesis conditions (EDC, HOBt) were used. Then, the resulting immobilized amino acid (peptide) was acylated with propiolic acid and *N*,*N*′-diisopropylcarbodiimide (DIC) as activating agent, leading to the terminal alkyne **3**.

On the other hand, the synthesis of azide component starts with the selected penicillanic acid **4** that was reduced with borane–dimethyl sulfide complex to afford the corresponding alcohol (**5**) (Scheme 2).[31] Tosylation of **5** followed by azide displacement gave the desired 3-(azidomethyl)penam derivative **7**.

Although the solid-phase Cu(I)-catalyzed Hüisgen 1,3-dipolar cyclization usually proceeds in high yields, it has been reported to be highly dependent on the substrate and reaction conditions.[32] Using the immobilized *N*-propiolyl glycine (**3a**, AA = Gly, $n = 1$, Scheme 3) as a model alkyne, the most suitable conditions to carry out the cyclization with azide **7** were explored. The immobilized penicillin–triazole conjugate obtained was cleavage from the solid



Scheme 2. Synthesis of azide component.



Scheme 3. 1,3-Dipolar cycloaddition between azides and immobilized alkynes.

support by the treatment with TFA (10% in DCM), to afford the crude product, which was methylated using diazomethane and purified. The complete consumption of alkynyl group was verified by the disappearance of the absorption band at 2110 cm^{-1} (C≡C) in the FT-IR spectrum. The most relevant results of this study are shown in Table 1. At the beginning, 0.1 equiv of Cu(I) was used in the presence of *N*,*N*-diisopropylethylamine (DIPEA) and pyridine, which was added to dissolve and stabilize the cuprous iodide. After 2 h at room temperature and releasing for the resin according to the above procedure, the desired product was obtained in 15% overall isolated yield for the five steps (based on the manufacturer's loading of the Wang resin) (entry 1). When the amount of Cu(I) was increased to 0.5 equiv, the yield improved significantly (43%) (entry 2). Interestingly, a further increase in the amount of Cu(I) did not improve the yield; on the contrary, it decreased to 9% isolated yield (entry 3). The excess of Cu(I) probably caused a diminution in the performance due to the deposition of copper species on the resin.[28] The addition of ascorbic acid to avoid Cu(I) oxidation did not improve the yield of the sequence (entry 4). Similarly, attempts to increase yields using microwave irradiation were unsuccessful, and decomposition was mostly observed (entry 5). When commercially available $[Cu(CH_3CN)_4]PF_6$ was used as an organic soluble Cu(I),[29d,33] instead of cuprous iodide, the outcomes were comparable to the best achieved before (44%) (entry 6).

After establishing the optimal conditions (Table 1, entries 2 and 6) we decided to build a small library of compounds with potential pharmacological activity (Table 2).[34] A series of immobilized *N*-propiolyl aminoacids and dipeptides (**3a–o**) were tested for the reaction with the penicillin azide, using cuprous iodide[35] or $[Cu(CH_3CN)_4]PF_6$.[36] Mostly, yields remained good for the whole synthetic sequence, and regioselectivity was excellent in all cases, leading exclusively to the 1,4-regioisomeric product. Products were analyzed by ^1H, ^{13}C, HSQC NMR, and HRMS. The resultant 1,4-regioisomer was confirmed through ROESY spectroscopy. The ROESY spectrum shows cross-peaks between the triazole proton and the *N*-substituted methylene group, providing evidence for the formation of the 1,4-triazole (Fig. 2). Such regioselectivity agrees with that found in the literature, and can be explained by the high steric hindrance at the solid supported alkyne leading to the obtention of the less hindered triazole.

In summary, we have developed an efficient and versatile methodology for the solid-phase synthesis of valuable triazolyl aminoacyl (peptidyl) penicillins, which may be assessed against different pharmacological targets. The key step is the copper(I)-catalyzed 1,3-dipolar cyclization of aminoacyl (peptidyl) alkynes to penicillin azides, which displays remarkably broad scope and excellent regioselectivity. In most of the cases, triazoles were obtained in good overall yield for the five reaction steps. Further extension of this research to generate a larger library of triazolyl

Table 1
Optimization of Cu(I)-catalyzed 1,3-dipolar cyclization on solid support using **3a** and **7** as models[a]

Entry	Catalyst	Solvent	Condition	DIPEA (equiv)	Time (h)	Yield[b] (%)
1	0.1 equiv CuI/py	THF	rt	10	2	15
2	0.5 equiv CuI/py	THF	rt	10	2	43
3	5 equiv CuI/py	THF	rt	10	21	9
4	0.5 equiv CuI, ascorbic acid/lutidine	CH_3CN/DMSO	rt	—	2	14
5	5 equiv CuI/py	THF	Microwave[c]	10	0.07	10[d]
6	0.3 equiv $[Cu(CH_3CN)_4]PF_6$	DCM	rt	—	2	44

[a] Two equivalents of azide **7** were used.
[b] Overall isolated yield of **10a** after releasing into solution with 10% TFA/DCM followed by esterification with diazomethane, and purification by flash column chromatography (based on the initial loading level of Wang resin, five reaction steps). Reactions were performed in 0.1 mmol scale and monitored by FT-IR, checking the disappearance of the alkyne signal.
[c] The reaction was performed at 85 °C and 60 W.
[d] Extensive decomposition was observed.

Table 2
Solid-phase based synthesis of triazolyl aminoacyl (peptidyl) penicillins[a]

Entry	Compound	AA_n	Method	Time (h)	R^2	Yield[b] (%)
1	**10a**	Gly	0.5 equiv CuI/py	2	Me	43
2	**10a**	Gly	0.3 equiv $[Cu(CH_3CN)_4]PF_6$	2	Me	44
3	**10b**	PheGly	0.3 equiv $[Cu(CH_3CN)_4]PF_6$	2	Me	37
4	**10c**	Val	0.5 equiv CuI/py	1.5	Me	50
5	**10d**	Ala	0.5 equiv CuI/py	18	Me	35
6	**10e**	Trp	0.5 equiv CuI/py	2	Me	25
7	**10f**	Tyr	0.5 equiv CuI/py	18	Me	36
8	**10g**	Phe	0.5 equiv CuI/py	18	Me	36
9	**10h**	AlaLeu	0.5 equiv CuI/py	18	Me	13
10	**10i**	AlaGly	0.3 equiv $[Cu(CH_3CN)_4]PF_6$	2	Me	42
11	**10j**	Met	0.3 equiv $[Cu(CH_3CN)_4]PF_6$	21	Me	17
12	**10k**	Thr	0.3 equiv $[Cu(CH_3CN)_4]PF_6$	21	Me	30
13	**10l**	TyrLeu	0.3 equiv $[Cu(CH_3CN)_4]PF_6$	18	Me	30
14	**10m**	PheLeu	0.3 equiv $[Cu(CH_3CN)_4]PF_6$	18	Me	49
15	**9g**	Phe	0.3 equiv $[Cu(CH_3CN)_4]PF_6$	8	H	20[c]
16	**9c**	Val	0.3 equiv $[Cu(CH_3CN)_4]PF_6$	18	H	38[c]

[a] Two equivalents of azide **7** were used.
[b] Overall isolated yield after releasing into solution with 10% TFA/DCM followed by esterification with diazomethane, and purification by column chromatography (based on the initial loading level of Wang resin, five reaction steps). Reactions were performed in 0.1 mmol scale and monitored by the disappearance of the alkyne signal in FT-IR.
[c] No esterification was performed in these cases.

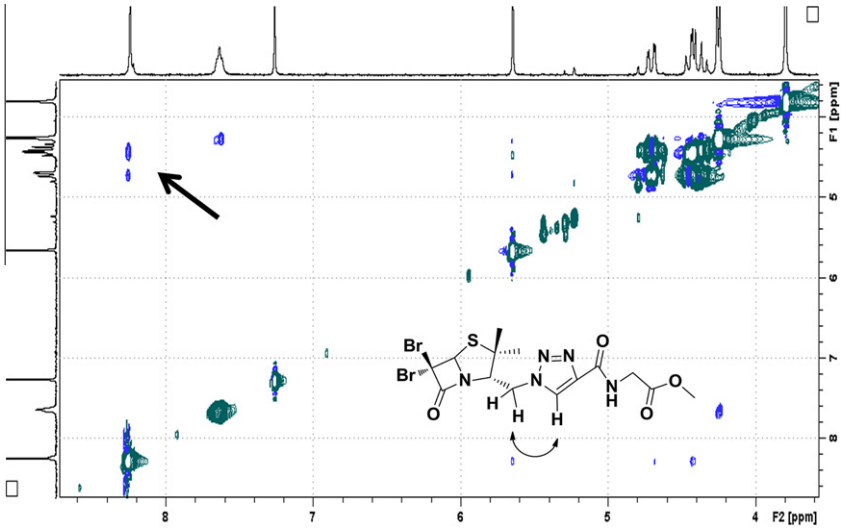


Fig. 2. ROESY spectrum of **10a**. Arrows show the cross-peaks that prove the regiospecific 1,4-triazole formation.

peptidyl penicillins, and the biological evaluation of these compounds against different targets, is currently in progress in our laboratory.

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34. Attempts to perform this reaction with Ru catalysts to obtain 1,5-regioisomers were unsuccessful. Two ruthenium catalyst complexes [$Cp^*RuCl(PPh_3)_2$ and $Cp^*RuCl(COD)$] were tested under different solvents, reaction times and temperatures, including microwave irradiation. Results were poor in yield and regioselectivity, giving a mixture of the 1,4 and 1,5-regioisomers. For a leading reference on Ru(II)-catalyzed azide-alkyne cycloaddition (RuAAC), see: Boren, B. C.; Narayan, S.; Rasmussen, L. K.; Zhang, L.; Zhao, H.; Lin, Z.; Jia, G.; Fokin, V. V. *J. Am. Chem. Soc.* **2008**, *130*, 8923.
35. Representative experimental procedure for the synthesis of compound **10c** (Table 2, entry 4): The immobilized *N*-propiolyl valine (**3c**) (0.1 mmol, 147 mg, 0.681 mmol/g) was suspended in THF (3 ml) and DIPEA (10 equiv, 1 mmol, 174 μl), CuI (0.5 equiv of a 0.1 M solution in pyridine), and azide 7 (2 equiv, 0.2 mmol, 51.1 mg) were added to the resin and the reaction was stirred for 1.5 h at rt. The resin was then washed with THF, DMF, piperidine 10% in DMF, CH_2Cl_2 and finally drying in vacuo to afford the immobilized compound **8c**. A suspension of the resin **8c** in 10% TFA solution in CH_2Cl_2 (3 ml) was stirred at room temperature for 50 min. The mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 and treated with diazomethane solution in ether at 0 °C. After methylation was completed, solvent was evaporated and the crude purified by silica gel flash column chromatography with hexane: ethyl acetate (4:6), to afford the triazolyl aminoacyl penicillin **10c** (27.65 mg, 50%). ^{1}H RMN ($CDCl_3$, 300 MHz) δ 1.00 (d, 3H, J = 3.4 Hz,Val CH_3), 1.02 (d, 3H, J = 3.4 Hz, Val CH_3), 1.57 (s, 3H, CH_3), 1.59 (s, 3H, CH_3), 2.28 (m, 1H, Val CH), 3.77 (s, 3H, OCH_3), 4.31 (m, 1H, H-8), 4.41 (m, 1H, H-3), 4.70 (m, 1H, H-8), 4.72 (m, 1H, Val CH), 5.68 (s, 1H, H-5), 7.57 (d, 1H, J = 9 Hz, NH) 8.32 (s, 1H, CH triazole). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 17.9 (Val CH_3), 19.1(Val CH_3), 24.5 (CH_3), 31.4 (CH), 33.3 (CH_3), 48.3 (CH_2), 52.3 (OCH_3), 57.1 (CH_3), 58.6(C), 63.5 (C), 67.9 (CH), 78.4 (CH), 126.4 (CH), 143.2 (C), 159.7 (CO), 165.6 (CO), 171.7 (CO). IR: (film) 1789 cm^{-1}(β-lactam), 1745 cm^{-1} (ester), 1666 cm^{-1} (amide). HRMS (ESI): m/z $[M+Na]^+$ calcd for $C_{15}H_{19}Br_2N_5NaO_4S$: 573.9735, found 573.9729.
36. Representative experimental procedure for the synthesis of compound **10a** (Table 2, entry 2): The immobilized *N*-propiolyl glycine (**3a**) (0.1 mmol, 125 mg, 0.795 mmol/g) was suspended in CH_2Cl_2 (3 ml) and tetrakis-(acetonitrile)copper(I)hexafluoro-phosphate (0.3 equiv, 0.03 mmol, 11.2 mg) and azide **7** (2 equiv, 0.2 mmol, 51.1 mg) were added to the resin and the mixture stirred for 2 h at rt. The resin was then washed with THF, DMF, piperidine 10% in DMF, CH_2Cl_2 and finally drying in vacuo to afford the immobilized triazolyl aminoacyl penicillin **8a**. A suspension of **8a** in 10% TFA solution in CH_2Cl_2 (3 ml) was stirred at room temperature for 50 min. The mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was dissolved in CH_2Cl_2 and treated with diazomethane solution in ether at 0 °C. After methylation was completed, solvent was evaporated and the crude product was purified by flash column chromatography using hexane:ethyl acetate (4:2), to give **10a** (22.48 mg, 44%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.57 (s, 3H, CH_3), 1.59 (s, 3H, CH_3), 3.79 (s, 3H, OCH_3), 4.25 (d, 2H, J = 5.64 Hz, Gly CH_2), 4.37 (dd, 1H, J = 12.6, 3 Hz, H-8), 4.42 (m, 1H, H-3), 4.70 (dd, 1H, J = 12.6, 3 Hz, H-8), 5.64 (s, 1H, H-5), 7.63 (t, 1H, J = 5.64 Hz, NH), 8.23 (s, 1H, CH triazole). ^{13}C NMR ($CDCl_3$, 75 MHz) δ 24.5 (CH_3), 33.2 (CH_3), 40.9 (CH_2), 48.3 (CH_2), 52.5 (OCH_3), 58.5(C), 63.6 (C), 67.9 (CH), 78.4 (CH), 126.1 (CH), 143.1 (C), 159.9 (CO), 165.6 (CO), 169.7 (CO). IR: (film) 1789 cm^{-1} (β-lactam), 1747 cm^{-1} (ester), 1672 cm^{-1} (amide). HRMS (ESI): m/z $[M+Na]^+$ calcd for $C_{14}H_{17}Br_2N_5O_4S$: 531.9265, found 531.9260.