Chapitre 4

Synthèse de macrocycles peptidiques sur support solide par ancrage du squelette peptidique par réaction de Ugi

Avant-propos

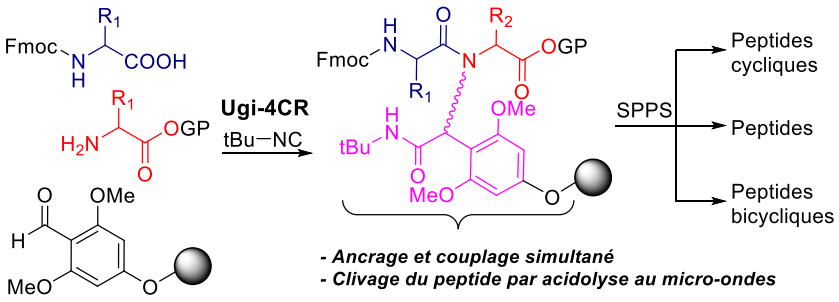
Le chapitre 4 inclut un manuscrit qui sera soumis sous forme de communication au journal *Tetrahedron Letters*.

Mon directeur de recherche, Éric Biron, a supervisé et fait le design des travaux en donnant des idées novatrices à expérimenter en laboratoire. Il a également participé très activement à la rédaction du manuscrit.

Pour ma part, j'ai effectué toutes les manipulations que l'on retrouve dans cet article, en plus d'avoir rassemblé les résultats dans la partie expérimentale et de rédiger le plan et lebrouillon de l'article.

Résumé

La synthèse de macrocycles peptidiques sur support solide par cyclisation tête-à-queue via la formation d'un lien amide entre le N- et le C-terminal nécessite habituellement l'utilisation d'ancrages par une chaîne latérale ou le squelette peptidique. Dans cet article, nous décrivons l'utilisation de la réaction multicomposante de Ugi pour lier deux acides aminés sur un support solide par ce même squelette en une seule étape simple et rapide. En effectuant la réaction, la formation d'un ancrage clivable par acidolyse s'effectue simultanément. En plus de se faire plus rapidement et efficacement, tout en utilisant moins d'excès de réactifs que l'amination réductive, cette nouvelle méthode d'ancrage de peptides par leur lien amide ajoute également un degré d'orthogonalité à la synthèse, puisque l'utilisation de micro-ondes est nécessaire au clivage du support solide. Cette approche simple et efficace a été optimisée et appliquée à la synthèse de peptides linéaires, cycliques et bicycliques, donnant généralement les produits en rendements modérés et avec de bonnes puretés, ce qui est bien compte tenu de la complexité des produits formés.



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Solid-phase synthesis of macrocyclic peptides by backbone anchoring using a traceless Ugi multicomponent approach

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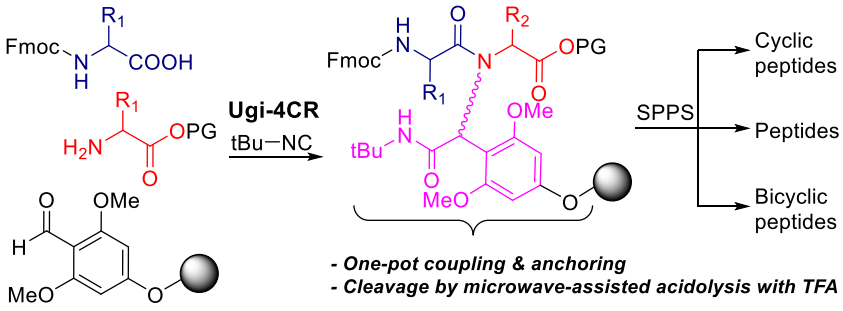
Abstract

A new methodology to anchor peptides by their backbone to a solid support in a single step using an isocyanide-based multicomponent reaction is described. The approach uses a microwave-assisted Ugi four-component reaction to simultaneously condense and bind an *N*-protected amino acid and an amino ester to a supported aldehyde. Afterward, the generated backbone anchored dipeptide can be used in solid-phase peptide synthesis to prepare head-to-tail cyclic peptides. We also show that the backbone anchored peptide can be efficiently released from the resin by microwave-assisted acidolysis with trifluoroacetic acid. This straightforward one-pot Ugi reaction anchoring approach was also applied to condense fragments and prepare a variety of linear and macrocyclic peptides.



Article

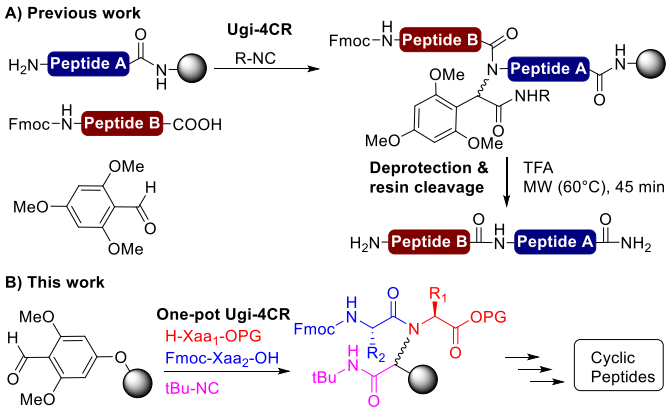
Peptide macrocycles are a very attractive class of bioactive compounds that have gained significant interest in drug discovery.[1-4] Compared to their linear counterparts, cyclic peptides are more resistant to proteases.[5, 6] Moreover, their conformational rigidity makes them tighter-binding to a given macromolecule and allow functional and conformational fine-tuning.[2,7-9] Therefore, peptide macrocycles are very useful scaffolds in structure-activity relationship studies and valuable lead compounds in drug development.[1,4,10-12] For these reasons, a wide variety of synthetic methodologies have been developed over the years to perform peptide macrocyclization.[2,12-17]

Compared to normal peptide bond formation, ring-closing reactions usually proceed much more slowly and side reactions, such as oligomerization and cyclodimerization of linear peptide precursors, may be observed and even predominate.[18-20] To minimize these intermolecular processes, the cyclization reaction must be performed under high dilution conditions (10^{-4} to 10^{-3} M) or using “pseudo-high” dilution methods with syringe pumps.[21] Another strategy is to perform the cyclization step while the peptide is still bound to the solid support. With limited mobility, resin-bound peptides are less prone to encounter one another creating a pseudo-dilution phenomenon that favours intramolecular cyclization over undesired intermolecular side reactions.[22-24]

On-resin macrocyclization generally requires at least three dimensions of orthogonal protecting groups to allow selective deprotection of the reactive ends (N- or C-terminus or side chain) and ring-closing on solid support.[25-30] While side chain-to-tail, head-to-side chain and side chain-to-side chain cyclization can be performed with an anchored C-terminal and commonly used linkers, head-to-tail cyclization implies the anchoring of the linear peptide precursor via: (i) a side chain functional group, (ii) a backbone amide or (iii) C-terminal bonding on latent inducible linkers (safety-catch) for cyclative cleavage. Unlike side chain anchoring which requires the presence of a trifunctional amino acid such as Asp, Glu, Lys, Ser or Tyr in the sequence, backbone anchoring can be performed with any amino acid.[31-33] This approach known as the backbone amide linker strategy (BAL) involves the coupling of an α-amino ester (H-Xaa-OPG) to an aromatic aldehyde linker by reductive amination followed by acylation of the generated benzylic amine with the next amino acid under standard coupling conditions.[32,33] Backbone anchoring by reductive amination usually involves large excess of reagents, long reaction time and difficult

acylation on a bulky secondary amine. In an effort to reduce reagents equivalents, reaction time and the number of steps, we decided to use a multicomponent reaction to simultaneously link the C-protected amino acid, N-protected amino acid and aldehyde linker. Herein we report a simple and affordable one-pot approach for the backbone anchoring of protected peptides and solid-phase synthesis of cyclic and bicyclic peptides.

Our strategy was based on a traceless-Ugi multicomponent reaction we have recently described to efficiently couple peptide fragments on solid support (Scheme **1a**).[34] In this previous study, an on-resin Ugi four-component reaction (Ugi-4CR) was used to attach a carboxyl free peptide to a supported peptide bearing a free N-terminal amine via the formation of an *N*-protected amide bond at the ligation site. Afterward, the generated backbone amide protecting group was efficiently removed by microwave-assisted acidolysis with trifluoroacetic acid (TFA) to afford a fully deprotected peptide. Among tested aldehyde derivatives, the 2,4,6-trimethoxybenzaldehyde gave the best Ugi-4CR and deprotection yields. This high degree of similarity with the tris(alkoxy)benzaldehyde BAL linker led us to design the present study where the aldehyde component is supported and used to anchor a peptide by its backbone with a Ugi-4CR and allow the release of the final compound by acidolysis.

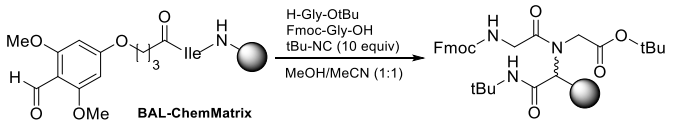


Scheme 1. Solid-phase traceless-Ugi-4CR ligation and backbone anchoring.

To evaluate the on-resin Ugi-4CR with a supported aldehyde, the BAL linker 4-(4-formyl-3,5-dimethoxyphenoxy)butanoic acid was coupled to H-Ile-ChemMatrix® (CM) resin.[33] CM resin was selected to perform the anchoring reaction because of its compatibility with polar solvents such as MeOH usually used in Ugi-4CR.[35] The efficiency of the reaction was

determined by comparing the post-Ugi and initial (Fmoc-Ile-CM) loadings obtained by dosage of the Fmoc-group using the method reported by Gude *et al.* (Table **1**).[36] Initial Ugi-4CR anchoring were performed on BAL-CM resin with *tert*-butyl isocyanide (10 equiv), Fmoc-Gly-OH (5 equiv) and H-Gly-OtBu hydrochloride salt (2.5 equiv) in MeOH/CH_3CN (1:1) with microwave (MW) heating at 60°C in a sealed vial for 60 min. Unfortunately, no resin-bound product was observed and the hydrochloride salt was suspected to prevent imine formation during the Ugi-4CR. The addition of DIEA to the mixture did not allow the formation of dipeptide on the resin. However, the use of the free amine H-Gly-OtBu in the Ugi-4CR anchoring yielded a very good loading (Table **1**, entry 1). The results showed that the reaction is nearly completed in 30 min with MW heating at 60°C (entry 2) and that the use of more H-Gly-OtBu did not increase the yield (entry 3). On the other hand, fewer equivalents of Fmoc-Gly-OH or both amine and acid components yielded significantly lower loadings (entries 4 and 5). The use of MW heating at 100°C did not increase the loading (entry 6), which might be caused by degradation of the desired product. The Ugi-4CR anchoring was also evaluated at room temperature (entries 7-9) and the results showed that the reaction was nearly completed after 48 h with 97% yield while completion was observed after 72 h. This first evaluation clearly showed the efficiency of MW irradiation to accelerate the on-resin Ugi-4CR where completion is reached after 30 min instead of 48 to 72 h at room temperature. The results observed with the best Ugi-4CR conditions were similar to the loading obtained by reductive amination (entry 10). However, the Ugi-4CR anchoring was performed in a single step and required less time and reagents equivalents. Based on these results, the next experiments were conducted with reaction conditions used for entry 2, i.e. H-Xaa-OPG (2.5 equiv), Fmoc-Xaa-OH (5 equiv), *tert*-butyl isocyanide (10 equiv) in MeOH/CH_3CN (1:1) and MW heating at 60°C for 30 min.

Table 1. Selection of the optimal reaction conditions for backbone anchoring by Ugi-4CR



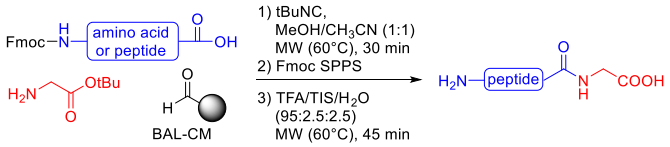
Entry	Time (h)	Temperature (°C)	Amine (equiv.)	Acid (equiv.)	Loading (mmol/g)[a]	Yield (%)[b]
1	1	MW (60°C)	2.5	5	0.298	>99
2	0.5	MW (60°C)	2.5	5	0.293	98
3	0.5	MW (60°C)	5	5	0.293	98
4	0.5	MW (60°C)	5	2.5	0.213	72
5	0.5	MW (60°C)	1	1.2	0.161	54
6	0.5	MW (100°C)	2.5	5	0.107	36
7	24	rt	2.5	5	0.191	64
8	48	rt	2.5	5	0.286	97
9	72	rt	2.5	5	0.296	>99
10[c]	3 + 3	rt	10	5	0.295	>99

[a]The experimental loading was determined by dosage of the Fmoc group.[36] [b]Yields were calculated according to the experimental loading for Fmoc-Ile-CM (0.297 mmol/g). [c]Performed by reductive amination.[32] *Reagent and conditions*: i) $NaBH_3CN$ (10 equiv), HCl·H-Gly-OtBu (10 equiv), DMF, rt, 3 h; ii) Fmoc-Gly-OH (5 equiv), HATU (5 equiv), NMM (10 equiv), DMF, 3 h, rt.

To assess the compatibility of the methodology with standard Fmoc solid–phase peptide synthesis (SPPS), various peptides were prepared by iterative amino acids coupling or by fragment ligation (Table **2**). First, C-terminal dipeptide anchoring was performed as described above and the peptides assembled by standard Fmoc solid-phase peptide synthesis. Afterward, the peptides were simultaneously deprotected and released from the resin in presence of TFA with MW heating at 60°C for 45 min to be analyzed and purified by HPLC. The results showed that peptides **1**-**3** were obtained with excellent crude purities and in good yields. Compared to the peptide obtained after reductive amination with 81%

crude purity and in 53% yield, peptide **1** prepared by Ugi-4CR anchoring and MW-assisted cleavage showed a crude purity of 92% and was isolated in 72% yield.

Table 2. Crude purities and isolated yields for peptides prepared by Ugi-4CR backbone anchoring



Oligomer	Sequence[a]	Purity[b] (%)	Yield[c] (%)
1[d]	GYKLGG	81	53
1	GYKLGG	92	72
2	GYKLAG	92	36
3	GYKLFG	93	51
4[e]	GFGYLGG	95	60
5[e]	GFGYLFG	82	58
6[e]	GFGYLGGFGYLG-NHPr	72	5

[a]Ligation site is underlined. [b]Crude purities were determined by UV absorbance at 220nm. [c] Isolated yields after preparative HPLC purification. Based on the experimental loading for Fmoc-Ile-CM resin (0.297 mmol/g). [d] Anchoring by reductive amination. [e] Fragment coupling with Fmoc-GFGY(tBu)LG-OH, Fmoc-GFGY(tBu)LF-OH or H-GFGY(tBu)LG-NHPr.

Peptide **3** was prepared to evaluate the presence of epimerization at the ligation site. A Phe residue was used at the ligation site because it is particularly prone to C-terminal epimerization.[37] However, only one diastereoisomer was observed, suggesting that the stereochemistry of the C-terminal residue is conserved. This first series of results was very interesting and suggest that the described approach offers several advantages over the reductive amination procedure such as one-pot reaction, shorter reaction time and fewer reagent equivalents.

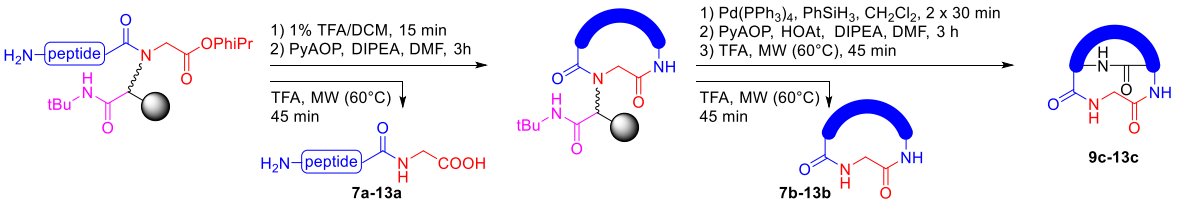
To perform peptide fragment anchoring, N-terminal fragments were beforehand assembled on 2-chlorotrityl chloride resin and released with a solution of 1,1,1,3,3,3-

hexafluoroisopropanol (HFIP) in DCM to yield protected hexapeptides with free C-terminal carboxylic acids. Backbone anchoring of the N-terminal fragment with H-Gly-OtBu was performed as described above. After protecting group removal and cleavage from the resin, peptides **4** and **5** were obtained in 95% and 82% crude purities and isolated in 60% and 58% yields, respectively. These results showed that the Ugi-4CR anchoring can also be efficiently achieved with peptide fragments. Finally, to evaluate if peptide fragment ligation can be performed by Ugi-4CR with the BAL-CM resin, a C-terminal fragment was prepared on HMBA resin and release with *N*-propylamine to yield a protected hexapeptide with a free N-terminal amine and a C-terminal *N*-propylamide. Coupling of the C- and N-terminal fragments by Ugi-4CR afforded 12-mer peptide **6** with 72% crude purity but in a very poor 5% isolated yield. This suggests that conditions are not the same for peptides fragments and for amino acids. Therefore, a further optimization for fragments coupling would be necessary.

Finally, to evaluate if the increased steric hindrance of the anchoring site affects cyclization efficiency, backbone anchored peptides were prepared by Ugi-4CR and standard SPPS to be submitted to macrocyclization (Table **3**). As on-resin cyclization requires a third degree of orthogonal protecting groups, the acid sensitive 2-phenylpropan-2-yl (PhiPr) group was used as C-terminal protection. The amino ester H-Gly-OPhiPr was prepared as described by Virta *et al*.[38] and used in the Ugi-4CR anchoring. In this case, the PhiPr ester is very useful because it can prevent the formation of diketopiperazines during Fmoc removal on the anchored dipeptide as observed with methyl, benzyl and allyl esters.[33,39] Moreover, the PhiPr ester can be cleaved with very mild acidic conditions such as 1% TFA in DCM to avoid peptide release from the resin. After their synthesis, a small portion of the supported linear precursors were cleaved by MW-assisted acidolysis to be analyzed and purified by HPLC. The results showed that peptides **7a**-**13a** were obtained in crude purities ranging from 73% to 95% and isolated in 40% to 77% yields. Afterward, the supported linear precursors were submitted to selective N-terminal Fmoc-group removal with 20% piperidine in DMF and C-terminal PhiPr ester cleavage with a solution of 1% TFA in DCM to allow on-resin head-to-tail macrocyclization with PyAOP and DIPEA in DMF for 3 h. After their release from the resin, cyclic peptides **7b**-**13b** were obtained in crude purities ranging from 57% to 79% and isolated in 21% to 42% yields after their purification by HPLC. For each case, a 50% decrease in isolated yields was observed with the linear

precursor as the major impurity. Finally, to demonstrate the compatibility of the anchoring moiety with allyl ester and alloc group hydrogenolysis, an additional cyclization was performed between Lys and Glu side chains after their deprotection. With their increased structural rigidity, bicyclic peptides can be very useful scaffolds in combinatorial libraries and drug discovery.[40-42] Bicyclic peptides were prepared from supported cyclic peptides **9b**-**13b** after Lys(Alloc) and Glu(Oall) side chain deprotection with $Pd(PPh_3)_4$ and phenylsilane in DCM followed by side chain-to-side chain cyclization with PyAOP. Compound release from the resin by MW-assisted cleavage with TFA afforded bicyclic peptides **9c**-**13c** in 55-97% crude purities and moderate 11-31% yields. For some bicyclic peptides, the observed decrease in isolated yields could be explained by the increased rigidity of the cyclic precursor making the reaction between the reactive ends more difficult. Overall, the peptides, whether linear, cyclic or bicyclic, prepared by Ugi-4CR backbone anchoring were obtained in very good crude purities and good yields. These results showed that the Ugi-4CR backbone anchoring and the MW-assisted acidolysis are compatible with protecting groups used in standard Fmoc SPPS and can be used to efficiently prepare peptide macrocycles.

Table 3. Crude purities and purified yields for linear (**7a-13a**), cyclic (**7b-13b**) and bicyclic (**9c-13c**) oligomers



#	Linear peptides (**7a**-**13a**)			Cyclic peptides (**7b**-**13b**)			Bicyclic peptides (**9c**-**13c**)		
	Sequence[a]	Purity (%)[b]	Yield (%)[c]	Sequence	Purity (%)[b]	Yield (%)[c]	Sequence	Purity (%)[b]	Yield (%)[c]
7	GYKLGG	92	66	*c*[GYKLGG]	66	36			
8	GFGYLGG	95	48	*c*[GFGYLGG]	71	29			
9	GLK*PYKE*GG	76	53	*c*[GLK*PYKE*GG]	74	31	*c*[GL*c*[KPYKE]GG]	74	31
10	GLAK*YPAKGE*GG	73	51	*c*[GLAK*YPAKGE*GG]	57	25	*c*[GLA*c*[KYPAKGE]GG]	82	22
11	GLK*PYKGE*G	85	77	*c*[GLK*PYKGE*G]	79	42	*c*[GL*c*[KPYKGE]G]	75	24
12	GAK*YLKE*GG	79	41	*c*[GAK*YLKE*GG]	70	21	*c*[GA*c*[KYLKE]GG]	55	11
13	K*GAPYKAE*GG	79	40	*c*[K*GAPYKAE*GG]	66	32	*c*[*c*[KGAPYKAE]GG]	97	12

[a]Ligation site is underlined; K* = Lys(Alloc); E* = E(OAll); *c* = cyclo. [b]Crude purities were determined by HPLC. [c]Isolated yield after purification by preparative HPLC. Based on the experimental loading of 0.297 mmol/g for Fmoc-Ile-CM.

In summary, a convenient one-pot Ugi-4CR was developed to anchor peptides by their backbone to a solid support. The study showed that the use of MW irradiations significantly accelerated the Ugi-4CR anchoring and allowed an efficient peptide release from the resin in presence of TFA. Compared to the reductive amination procedure, the described Ugi-4CR anchoring can be performed in a single step of 30 min with less reagents equivalents. The results obtained in this work demonstrate that the described approach is compatible with standard Fmoc solid-phase peptide synthesis and can be used to prepare linear and macrocyclic peptides. Parameters such as the impact of MW temperature, the efficiency of C- and N-terminal amino acid residues at the anchoring site; and the effect of reagents equivalents are currently under investigation to expand the applicability of the approach and allow one-pot peptide ligation-anchoring and macrocyclization-anchoring. Simple and affordable, the described procedure is likely to become a useful method to perform backbone anchoring and prepare peptide macrocycles by the backbone anchoring strategy.

Acknowledgments

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