On resin multicomponent 1,3-dipolar cycloaddition of ciclopentanone-proline enamines and sulfonylazides as an efficient tool for the synthesis of amidino depsipeptide mimics

Raffaella Bucci [aγ]*, Federico Dapiaggi[bγ], Helena Macut[aγ], Stefano Pieraccini[b], Maurizio Sironi[b], Maria Luisa Gelmi[a], Emanuela Erba[a], Sara Pellegrino*[a]

[a] DISFARM-Dipartimento di Scienze Farmaceutiche, Sezione Chimica Generale e Organica ''A. Marchesini'', Università degli Studi di Milano, Via Venezian 21, 20133, Milano, Italy

[b] Dipartimento di Chimica, Università degli Studi di Milano, Via Golgi 19, 20133, Milano, Italy.

e-mail: sara.pellegrino@unimi.it
raffaella.bucci@unimi.it

Author Contributions
γ These authors equally contributed to this work

Abstract

Depsipeptides are biologically active peptide derivatives that possess a high therapeutic interest. The development of depsipeptide mimics characterized by a chemical diversity could lead to compounds with enhanced features and activity. In this work, an on resin multicomponent procedure for the synthesis of amidino depsipeptide mimics is described. This approach exploits a metal-free 1,3-dipolar cycloaddition of cyclopentanone - proline enamines and sulfonylazides. In this reaction, the obtained primary cycloadduct undergoes a ring opening and molecular rearrangement giving access to a linear sulfonyl amidine functionalized with both a peptide chain and a diazoalkane. The so obtained diazo function "one pot" reacts with the carboxylic group of *N*-Fmoc protected amino acids leading to amidino depsipeptide mimics possessing a C4 aliphatic chain. An important advantage of this procedure is the possibility to easily obtain amidino functionalized derivatives that are proteolitically stable peptide bond bioisosters. Moreover, the conformational freedom given by the alkyl chain could promote the obtainment of cyclic depsipeptide with a stabilized secondary structure as demonstrated with both in silico calculations and experimental conformational studies. Finally, labelled depsipeptide mimics can be also synthesized using a fluorescent sulfonylazide in the multicomponent reaction.

Keywords 1,3-dipolar cycloaddition, depsipeptide mimics, multicomponent reaction, solid phase synthesis

Introduction

Depsipeptides are an important class of naturally occurring bioactive compounds, strictly related to peptides, containing at least one ester bond in place of an amide (Stamm et al. 2016). Their core structure consists thus of both amide and ester bonds

(Hamada et al. 2019). The presence of the ester function gives rise to a different conformational behavior compared to peptides, leading to disparate biological activities, from immunosuppressant to antibiotics, from antifungines to anticancers (Sarabia et al. 2004). Depsipeptides are also used as models for macromolecular dendrons (Buschhaus et al. 2003) and as biochemical tools (Taliani et al. 1996). Many depsipeptides have already evaluated in clinical trials (e.g. *Kahalalides F* or *romidepsin*) and, interestingly, cyclic derivatives showed high biological activities (Hamann et al. 1996; Isaka et al. 2007; Lemmens-Gruber et al. 2009), good resistance to hydrolyzing enzymes, and enhanced oral bioavailability (Sivanathan and Scherkenbeck 2014). On the other hand, linear depsipeptides, such as dolastatin 15, possess cytostatic activity (Bai et al. 1992). The insertion of not natural core in depsipeptide skeleton could be a valuable strategy to obtain peptide mimics characterized by a high chemical diversity, and enhanced features (Sefler et al. 1997; de la Torre et al. 2016). It is indeed known that the insertion of not natural scaffold in peptides could increase proteolytic stability (Bucci et al. 2017; Contini et al. 2017), induce specific secondary structure conformation (Pellegrino et al. 2017; Bucci et al. 2018) while introducing active functional groups (Bucci et al. 2019; Oliva et al. 2019).

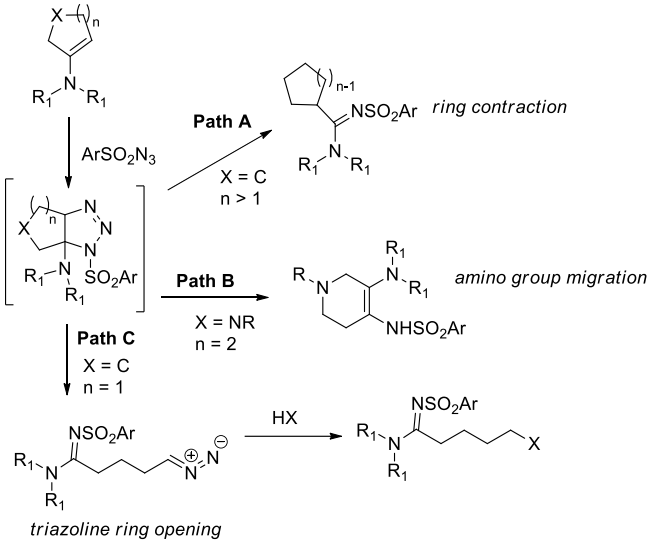
The main source of depsipeptides are fermentations of various bacteria, actinomycetes and fungi or semi-synthesis (Oku et al. 2008; Tsukimoto et al. 2011). In fact, depsipeptides represent a challenging synthetic attempt due to their structural complexities owing to the macrocyclic domain and the complex side chains (Bera and Dhananjoy 2019).

Here we present an efficient solid phase synthesis of different depsipeptide mimics containing an amidino moiety, a proteolitically stable peptide bond bioisoster (Inokuchi et al. 2011; Vastl et al. 2016). The key step of this synthesis is a metal free multicomponent 1,3-dipolar cycloaddition.

Multicomponent reactions (MCRs) are defined as the combination of at least three reactants in the same pot to generate a product containing most of the atoms of the starting materials (Ugi 1962; Dömling 2006; Zhu and Bienaymè 2006; Zhu et al. 2015). In the organic synthesis, they are abundantly used as they allow the production of small molecules with good atom/step economy, efficiency and structural diversity. The development of MCRs for the construction of biologically interesting molecules thus exhibits opportunities and challenges due to the simple experimental procedures and one-pot ecofriendly synthetic method (Bienaymé et al. 2000; Tempest et al. 2003; Pando et al. 2011; Morejón et al. 2016; Zhang et al. 2019). Cycloadditions have been exploited as MCRs, especially in domino or cascade processes (Padwa and Bur 2007). Among them, copper catalyzed 1,3-dipolar cycloadditions of azides have been widely used in the preparation of biologically active heterocycles (Mancebo-Aracil et al. 2013), in the functionalization of polymers (Kakuchi and Theato 2013), and in the development of smart materials (Lee et al. 2009).

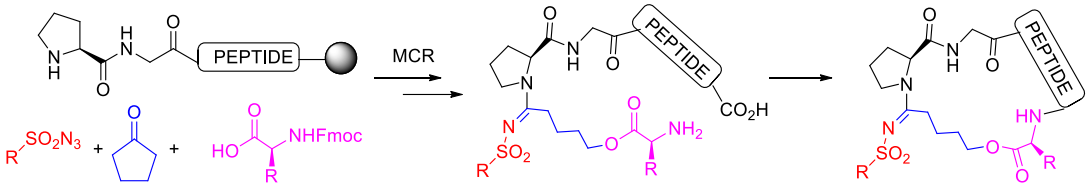
In our group, we have a long time experience in the study of metal free MCR 1,3-dipolar cyloadditions between aryl-azides and enamines of various aldehyde and ketones (Fusco et al. 1963; Almirante et al. 1986; Battistini et al. 1993; Cassani et al. 2004; Contini et al. 2008, 2012; Contini and Erba 2012; Pellegrino et al. 2014). As depicted in **Scheme 1**, performing the cycloaddition with cyclic enamines and sulfonylazides, the triazoline cycloadduct is not stable and spontaneously undergoes a rearrangement, leading to different types of products, depending on the starting carbonyl derivative (Contini et al. 2008). When carbocyclic ketones are employed, a ring contraction with nitrogen loss occurs leading to exocyclic amidines (Fusco et al. 1963) (**Scheme 1, Path A**). On the other hand, mono-sufonyl di-en-amines are obtained from *N*-substituted pirrolydinone, through amino group migration and nitrogen loss (Contini and Erba 2012; Pellegrino et al. 2014) (**Scheme 1, Path B**). Finally, linear amidines functionalized with a diazoalkane derive from cyclopentanone. In this case, the triazoline cycloadduct rearranges through ring opening (Contini et al. 2012) (**Scheme 1, Path C**). The obtained diazo-functionality directly reacts

with acidic compounds, such as inorganic acids, carboxylic acids and electron-poor phenols, affording differently 5-substituted pentyl amidines.



Scheme 1 MCRs on enamines from cyclic ketones and sulfonylazides

Here, we envisaged that the above-described MCR could be applied in the synthesis of amidino depsipeptides mimics. In particular, we planned to perform the MCR directly on the resin, taking advantage from the efficiency of the solid phase peptide synthesis. Thus, peptides bearing *L*-Proline at the *N-terminus* were on resin reacted with cyclopentanone, sulfonylazide and an amino acid (Scheme 2). Using this approach, it could be possible to obtain depsipeptide mimics possessing a C4 aliphatic chain. This flexible alkyl chain could promote the head to tail cyclization giving access to molecules of biological interest as cyclic depsipeptides. Interestingly, labelled depsipeptide mimics could be produced using a fluorescent sulfonylazide in the MCR.



Scheme 2 MCR approach toward depsipeptide mimics

Materials and methods

2-Chloro-trityl resin, Rink-amide resin, Fmoc-protected (L)-amino acids, HBTU, HOBT, DIEA, piperidine, solvents, piperidine and other reagents were purchased from Zentek (Italy) or Sigma Aldrich (Italy). Peptide **7** was synthesized using a CEM Liberty peptide synthesizer. All the compounds were purified using RP-HPLC with a Jasco BS-997-01 instrument and a DENALI C-18 column from GRACE VYDAC (10 µm, 250 x 22 mm). HRMS spectra were recorded on ESI Q-Tof Mass Spectrometer SYNAPT G2-Si & MassLynx software (Waters). NMR spectra were acquired on a Bruker Advance 300 Spectrometer.

Peptide synthesis on 2-Chloro-trityl chloride resin

Loading of the first amino acid. Fmoc-Gly (0.65 eq) was dissolved in a DIPEA solution (2M in N-methyl pyrrolidinone, 5 eq). The solution was added to 125 mg of 2-chloro-trityl chloride resin (1.6 mmol/gram loading) and kept under stirring for 1 h. Then 1 mL of MeOH was added and the reaction was stirred for 1 h. The liquid phase was removed and the resin washed with 5 x DMF and 5 x CH_2Cl_2. A final loading of 0.6 mmol/gram was obtained (UV analysis).

Coupling reactions. A fivefold molar excess of Fmoc-protected amino acids (0.2 M in N-methyl pyrrolidinone), and HOBT/HBTU/DIEA (5 : 5 : 10 eq) as activators were used. Coupling reactions were performed for 2 h at room temperature under stirring. The liquid phase was removed and the resin washed with DMF (5 x 5 mL) and CH_2Cl_2 (5 x 5 mL).

Fmoc deprotection. Deprotection was performed twice using 20% piperidine in DMF (30 and 15 min each). The liquid phase was removed and the resin washed with DMF (5 x 5 mL).

On resin MCR. Cyclopentanone (2 eq) was added to the preloaded resin swelled in DMF. The reactor was kept under stirring for 15 min. The selected azide (2 eq) were added and the mixture was stirred for 30 min. Fmoc-AA (1 eq) was added and the reactor was kept under stirring overnight. The liquid phase was removed and the resin washed with DMF (5 x 5 mL) and CH_2Cl_2 (5 x 5 mL).

Cleavage from the resin. The cleavage mixture for compounds **1** and **4** (2 mL, CH_2Cl_2/TFE/AcOH, 7/2/1) was added to the dry resin. The reactor was kept under stirring for 1 h. The liquid phase was then collected in a round bottom flask and the solvent was removed. For compound **2** milder conditions were used: (4 mL, CH_2Cl_2/HFIP 3:1) was added to the dry resin and left stirring for 1 hour. The liquid phase was collected in a round bottom flask and the same fresh aliquot of cleavage cocktail was then added to the resin for an additional time of 30 min. The liquid phase was recollected and the combined one was concentrated under reduced pressure.

Side chain deprotection. Side chain protecting groups were removed by adding TFA (2.5 mL), TIS (0.2 mL), TAN (0.2 mL) and H_2O (0.1 mL) to the full protected peptide (0.1 mmol). The deprotected peptide was precipitated and washed using ice-cold anhydrous ethyl ether.

Peptide purification. The peptides were purified by RP-HPLC using a gradient elution of 5–70% solvent B (solvent A: water/acetonitrile/trifluoroacetic acid 95:5:0.1; solvent B: water/acetonitrile/trifluoroacetic acid 5:95 :0.1) over 20 min. at a flow rate of 20 mL/min. The purified peptides were freeze-dried and stored at 0 °C. They were then analyzed by analytical HPLC and HRMS spectrometry (see Supporting Information).

Cyclization reaction. A solution of full protected peptide **1** or **3** in DCM/DMF (10:1, 0.4M) was pre-activated with HOBt and HBTU (3 eq each). This mixture was then added dropwise to a solution of DIPEA (5 eq) in DCM (0.01 M final concentration of the peptide). The reaction was kept under stirring at room temperature overnight. The organic solution was washed with H_2O (2 x 20 mL), dried on anhydrous Na_2SO_4 and the solvent was removed under vacuum. Side chain deprotection and purification were performed as above described.

Synthesis of peptide 7 on Rinkamide resin. Peptide **7** (PGSWEEYLDW) was prepared by automated microwave-assisted solid phase synthesis on Rinkamide resin (0.57 mmol/g substitution) as solid support. A fivefold molar excess of Fmoc-protected amino acids (0.2 M in N-methyl pyrrolidinone), and HOBT/HBTU/DIEA (5 : 5 : 10 eq) as activators were used. Coupling reactions were performed for 5 min at 40 W with a maximum temperature of 75 °C. Deprotection was performed twice using 20% piperidine in dimethylformamide (5 and 10 min each). Cleavage from the resin was performed using 10 mL

of Reagent K (trifluoroacetic acid/phenol/water/thioanisole/1,2-ethanedithiol; 82.5:5:5:5:2.5) for 180 min. Following cleavage, the peptide was precipitated and washed using ice-cold anhydrous ethyl ether. Compound **7** was then purified by RP-HPLC as above described.

Molecular Modelling. Compounds **5**, **6**, **7** were build using GaussView 627. Molecular dynamics simulations were carried out using the GROMACS-5.0.7 program suite28, using explicit solvent and periodic boundary conditions. The systems have been described with the AMBER99SB-ildn29. The systems were solvated with TIP3P30 water and neutralized adding a proper number of Na+ or Cl- ions. The LINCS31 algorithm was employed to constrain all bonds involving hydrogen to their equilibrium length, allowing a time step of 2 fs. The systems were submitted to 10000 steps of geometry optimization with the steepest descent method. Then they were equilibrated for 200 ps in NVT conditions (T = 300 K) and subsequently for 200 ps in NPT conditions, in order to equilibrate system density. Then the production phase of molecular dynamics were performed in NPT conditions (1 bar, 300 K). Temperature and pressure were kept constant to their reference values using the velocity rescale algorithm32 and the Berendsen barostat33 respectively. A 14 Å cutoff was applied for non-bonded interactions and the Particles Mesh algorithm34 was employed to calculate long range electrostatic interactions. The cluster analysis was carried out using the GROMOS35 clustering method with a 0.1 nm threshold.

2-((S)-1-((E)-5-(((S)-2-amino-3-methylbutanoyl)oxy)-1-(tosylimino)pentyl)pyrrolidine-2-carboxamido)acetic acid (1): White crystalline solid (121 mg, 77%); ^{1}H NMR (300 MHz, CD_3OD) δ 7.71 (d, J = 8.2 Hz, 2H), 7.32 (d, J = 8.2 Hz, 2H), 4.52-4.45 (m, 1H), 4.41-4.25 (m, 1H), 4.04-3.35 (m, 6H), 3.11-2.77 (m, 2H), 2.42 (s, 3H), 2.40-2.17 (m, 2H), 2.09 (s, 3H), 2.03-1.94 (m, 3H), 1.93-1.68 (m, 3H), 1.09 (d, J = 6.8 Hz, 6H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 172.7, 171.0, 168.6, 166.9, 142.3, 140.9, 129.0, 125.7, 65.1, 62.0, 60.8, 58.0, 40.4, 31.2, 29.5, 29.4, 27.6, 23.9, 22.2, 19.7, 17.0, 16.6 ppm; HRMS (ESI-TOF): calcd for $C_{24}H_{36}N_4O_7S$ 524.63, found: 525.2384 $[M+H]^+$.

(6R,9R,12R)-1-((S)-1-((E)-5-(((S)-2-amino-3-phenylpropanoyl)oxy)-1-(tosylimino)pentyl)pyrrolidin-2-yl)-12-benzyl-6-isobutyl-9-isopropyl-1,4,7,10-tetraoxo-2,5,8,11-tetraazatridecan-13-oic acid (2): White crystalline solid (335 mg, 60%); ^{1}H NMR (300 MHz, CD_3OD) δ 7.99–7.62 (m, 4 H), 7.43–7.05 (m, 10 H), 4.61-4.42 (m, 2 H), 4.30-4.12 (m, 2H), 4.04-3.76 (m, 4H), 3.91-3.20 (m, 7H), 2.42-2.25 (m, 3H), 2.05-1.80 (m, 5H), 1.80-1.45 (m, 5H), 0.91-1.02 (m, 12H) ppm ; ^{13}C NMR (75 MHz, CD_3OD) δ 175.0, 173.6, 171.2, 170.4, 170.1, 168.7, 158.5, 150.1, 139.8, 137.2, 136.0, 134.9, 130.0, 129.0, 127.3, 125.0, 121.3, 120 .0, 63.5, 61.4, 56.7, 55.4, 48.6, 44.1, 38.9, 36.8, 31.0, 30.0, 27.4, 26.3, 25.1, 25.0, 22.54, 22. 51, 22.50, 21.3, 18.54, 18.52, 1 8.4, 16.6, 16.0 ppm; HRMS (ESI-TOF): calcd for $C_{48}H_{65}N_7O_{10}S$ 931.15, found: 932.4570 $[M+H]^+$; 954.4387 $[M+Na]^+$.

(2R,5R,8R,11R,14R,17R)-2-((1H-indol-3-yl)methyl)-17-((R)-2-((R)-2-(2-((S)-1-((E)-5-(((S)-2-amino-3-methylbutanoyl)oxy)-1-(tosylimino)pentyl)pyrrolidine-2-carboxamido)acetamido)-3-hydroxypropanamido)-3-(1H-indol-3-yl)propanamido)-14-(2-carboxyethyl)-5-(carboxymethyl)-11-(4-hydroxybenzyl)-8-isobutyl-4,7,10,13,16-pentaoxo-3,6,9,12,15-pentaazaicosane-1,20-dioic acid (3): White crystalline solid (351 mg, 65%); HRMS (ESI-TOF): calcd for $C_{78}H_{100}N_{14}O_{23}S$ 1633.77, found: 1631.6772 $[M+H]^+$.

2-((S)-1-(5-(((S)-2-amino-3-methylbutanoyl)oxy)-1-(((5-(dimethylamino)naphthalen-1-1yl)sulfonyl)imino)pentyl)pyrrolidine-2-carboxamido)acetic acid (4): White crystalline solid (313 mg, 80%); ^{1}H NMR (300 MHz, CD_3OD) δ 8.6 (d, J = 8.9 Hz, 1 H), 8.3 (d, J = 8.9 Hz, 1 H), 8.2 (dd, J = 7.6 Hz, 1.2 Hz, 1H), 7.6-7.5 (m, 2H), 7.3 (d, J = 7.6 Hz, 1H), 4.25-4.45 (m, 4H), 3.03-3.88 (m, 2H), 3.83-3.64 (m, 4H), 3.12-2.99 (m, overl., 4H), 2.91 (s, overl. 6H), 2.4-1.8 (m, 5H), 1.09 (d, overl, J = 6.8 Hz, 6H) ppm; ^{13}C NMR (75 MHz, CD_3OD) δ 172.2, 170.9, 168.6, 167.3, 151.1, 139.1, 129.7, 129.6, 128.9, 127.1, 126.0, 122.9, 121.0, 115.0, 65.1, 61.9, 58.1, 48.3, 44.6, 44.5, 39.9, 31.5, 29.5, 28.0, 23.8, 22.3, 17.0, 16.9 ppm; ^{13}C NMR (50 MHz, Acetone d6): δ = 18.3, 19.0, 20.8, 22.5, 24.8, 28.5, 32.4, 32.7, 43.1, 49.9, 59.7, 61.5, 63.9, 126.4, 129.3, 141.8, 143.2, 167.3, 169.7, 170.5, 172.6 ppm. HRMS (ESI-TOF): calcd for $C_{29}H_{41}N_5O_7S$ 603.73, found: 604.2811 $[M+H]^+$; 626.2625 $[M+Na^+]^+$.

(E)-N-((6S,17aS)-6-isopropyl-1,4,7-trioxotetradecahydropyrrolo[2,1-i][1,4,7,10]oxatriazacyclopentadecin-13(1H)-ylidene)-4-methylbenzenesulfonamide (5): White crystalline solid (35 mg, 80%); NMR (300 MHz, Acetone d-6) δ 8.10-8.03 (m, 1H), 7.79 (d, J = 8.0 Hz, 2H), 7.79-7.68 (m, 1H), 7.32 (d, J = 8.0 Hz, 2H), 4.91 (d, J = 7.2 Hz, 1H), 4.51-4.44 (m, 2H), 4.08-4.11 (m, 1H), 3.79-3.75 (m, 1H), 3.60-3.53 (m, 2H), 3.39 (dd, J = 14.8 Hz, 3.5 Hz, 1H), 3.20-2.80 (m, 1H) 2.50-2.48 (m, 1H), 2.40 (s, 3H), 2.36-2.39 (m, 1H) 2.15-1.96 (m, 4H), 1.80-1.61 (m, 4H), 0.99-0.97 (m, 6H); MS (ESI-TOF): calcd for $C_{24}H_{34}N_4O_6S$ 506.22, found: 507.2 $[M+H]^+$; 529.4 $[M+Na^+]^+$.

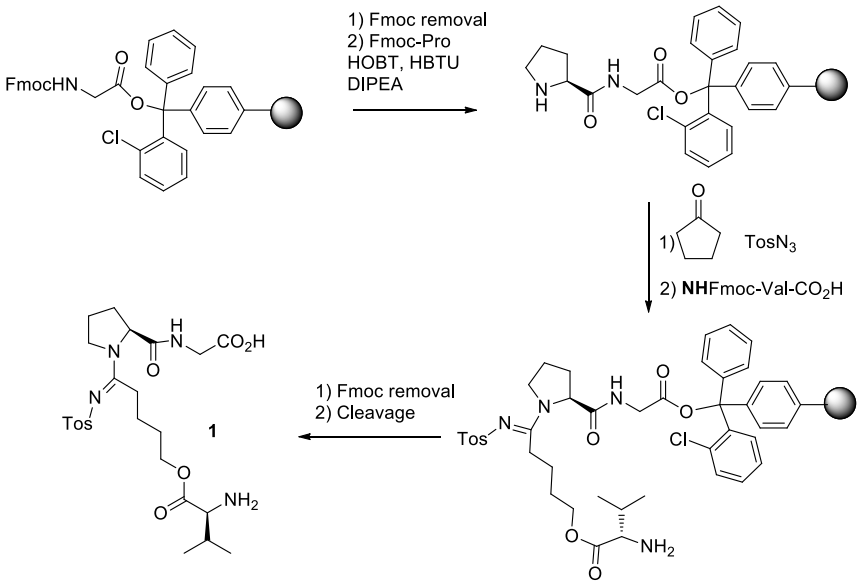
3,3'-((6R,9R,12R,15R,18R,21R,24R,27R,30S,41aS,E)-9,27-bis((1H-indol-3-yl)methyl)-24-(carboxymethyl)-18-(4-hydroxybenzyl)-6-(hydroxymethyl)-21-isobutyl-30-isopropyl-1,4,7,10,13,16,19,22,25,28,31-undecaoxo-37-(tosylimino)tetracontahydropyrrolo[2,1][1,4,7,10,13,16,19,22,25,28,31,34]oxaundecaazacyclononatriacontine-12,15-diyl)dipropanoic acid (6): White crystalline solid (12 mg, 35%); HRMS (ESI-TOF): calcd for $C_{78}H_{98}N_{14}O_{22}S$ 1614.67, found: 1613.6659 $[M-H]^+$; 806.3278 $[M+2]^+/2$.

Result and discussion

2-Cl-trytil chloride was chosen to study the feasibility of selected MCR on the resin. The obtainment of a free carboxylic acid at *C-terminus* was essential for the final cyclization of the depsipeptides. In addition, the weakly acidic cleavage conditions could prevent the hydrolysis of the ester bond.

The optimization of the 'on resin MCR' did not result trivial (**Scheme 3**). Firstly, we directly used the *N*-Fmoc-Proline preloaded resin (1.2 mmol/gram loading). After N-Fmoc deprotection using piperidine (20% in DMF), cyclopentanone (2 eq.) and tosylazide (2 eq.) were added to the resin swelled in DMF and the mixture was kept under shaking for 30 minutes. After that, *N*-Fmoc-Valine (1 eq. in DMF) was added but the MCR did not occur. We hypothesized that the resin steric hindrance prevented the formation of the desired compounds, thus we performed the 1,3-dipolar cycloaddition on the *N*-Fmoc-(Pro-Gly) preloaded resin (1.2 mmol/gram loading), but also in this case we did not achieve the expected results. Precisely, after the cleavage, a dimeric diazoalkane was isolated in trace amounts. Therefore, we envisage that the tosylazide reacted with the proline-cyclopentanone enamine, but the high resin loading, probably, allowed the dimerization reaction, preventing the reaction of the diazo group with *N*-Fmoc-Valine (See Scheme S1 in SI for the details).

Finally, we succeed in performing the MCR, decreasing the loading of *N*-Fmoc-Gly preloaded resin (0.65 mmol/gram loading) and obtaining compound **1** with 77% yield. N-Fmoc Ala, Leu and Ser preloaded resins were then tested, but the MCR failed in all cases, as a further confirmation that steric hindrance could have a detrimental effect on the reaction outcome.



Scheme 3 Synthetic pathway of the synthesized linear depsipeptides

The performance of our MCR was tested on peptides of different length. In particular, the PGLVF pentapeptide led to compound **2** in 60% yield (**Figure 1**). Compound **3** was obtained in good yield from a 10-mer sequence designed containing the SWEEYLDW sequence from the PCSK9 peptide ligand, interesting for successive conformational studies (Zhang et al. 2014). We tested then the on resin MCR using a fluorescent dansylazide to obtain a labelled depsipeptide. Compound **4** was obtained, proving that the MCR is not sensitive to the azide type. The possibility to use a fluorescent dye is of particular interest for biological applications, because fluorescent compounds are usually used as cell membrane permeability assays (Goncalves 2009).

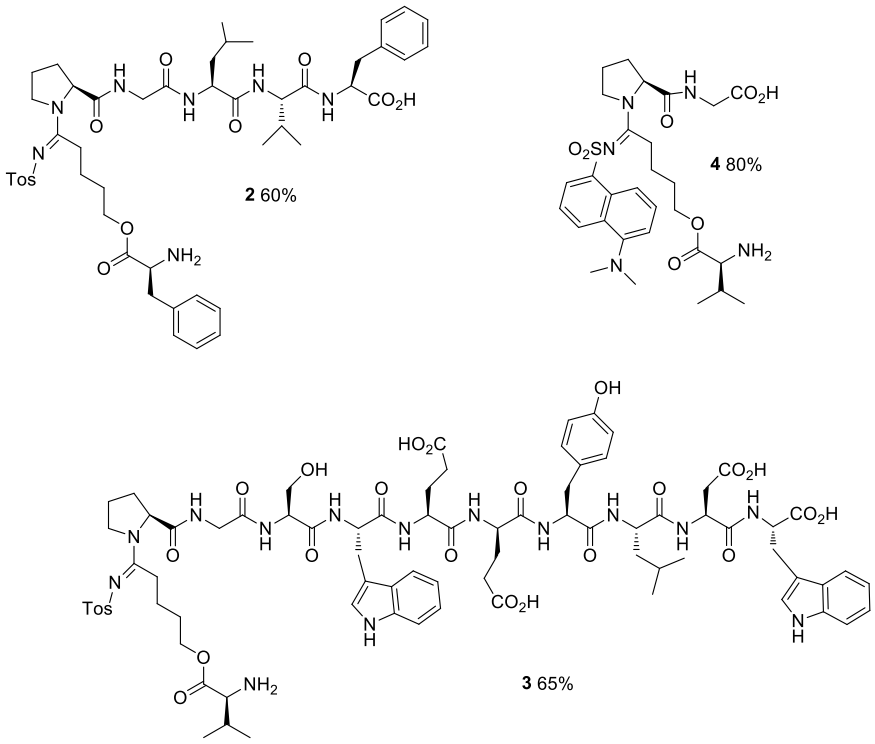


Figure 1 Synthesized depsipeptides with their isolated yields

Finally, we studied the possibility to obtain cyclic depsipeptide mimics taking advantage of the flexible alkyl chain. The cyclization was performed for fully protected compounds **1** and **3** to evaluate the influence of the peptide length on the reaction outcome. First, a solution of the peptide **1** or **3** was dissolved in DCM/DMF (10:1, 0.4 M) and activated using HOBt (3 eq.) and HBTU (3 eq.). After 30 min. this mixture was added to a solution of DIPEA (5 eq.) in DCM (0.01 M as final concentration of the peptide). The reaction was kept stirring overnight and the desired products (**5** and **6** in **Figure 2**) were obtained after purification by semi-preparative HPLC.

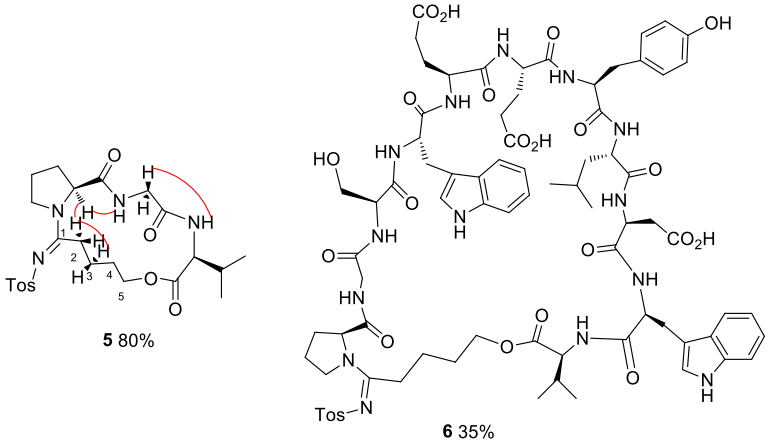


Figure 2 Structure of the obtained cyclic depsipeptides **5** and **6**

In solution conformational analysis. It is known that the cyclization could often influence the secondary structure of the peptide (De Araujo et al. 2014). For this reason, the conformation of compounds **5** and **6** was investigated.

Compound **5** was fully characterized by NMR (Acetone-d6, 300 MHz, for NMR spectra see SI). Spatial proximities (NOESY experiment, tmix 600ms) between αH-Pro and both NH-Gly and H-2 alkyl chain protons were found, proving the presence of a turn conformation (**Figure 2 and Table TS2 in Supporting Information**). Since the solubility of compound **6** was very poor, the conformational study by NMR spectroscopy was not possible. For this reason, circular dichroism experiments were performed both in PBS buffer (pH = 7.4) and in 50% trifluoroethanol (TFE). In **Figure 3** the CD spectra were reported in comparison with the linear wild type PCSK9 peptide sequence **7** (PGSWEEYLDW) synthesized using microwave assisted solid phase peptide synthesis. (Pellegrino et al. 2012)

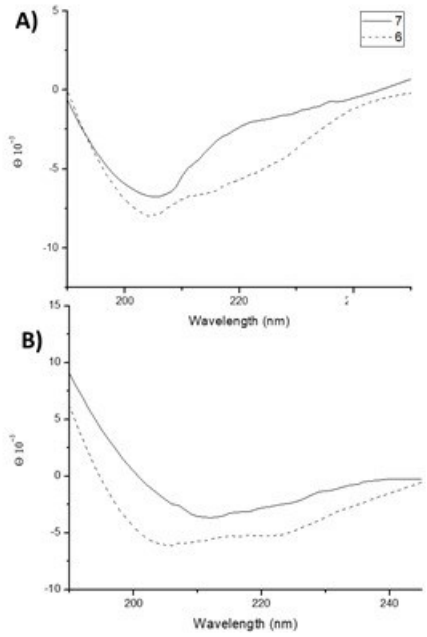


Figure 3 A) CD spectra of compounds **6** and **7** (50 μM) in PBS and B) in 50% TFE

In PBS (**Figure 3A**), compounds **6** and **7** did not adopt a single preferred secondary structure, as evicted by the absence of ellipticity at around 190 nm. Accordingly, secondary structure analysis of the CD spectrum with the CONTIN algorithm (Sreerama and Woody 2000) indicated the presence of 51% and 96% unordered fraction for compound **6** and **7**, respectively. In 50% TFE (Figure 3B), the random conformation decrease to 26% and 50%, while the helix content increased (60% for compound **6**, 30% for compound **7**). Surprisingly, a 20% of beta-conformation was observed in both cases. Taken together these data, we can conclude that the cyclization stabilizes the secondary structure, increasing the ordered fraction both in buffer and in 50% TFE.

Computational analysis. A 300 ns molecular dynamics (MD) simulation on compound **5** was carried out in order to investigate its structure and dynamic behavior. By comparing the average distances observed during the dynamics with the ones found by NOESY NMR (**Table T23 in SI**), a good agreement between experimental data and the structures sampled in the MD simulation were obtained. The central structure of the principal cluster from the analysis on the MD trajectory is shown in **Figure 4**. A hydrogen bond between the SO_2 of the tosyl group and the NH-Val stabilizes a beta turn-like conformation of

the cyclic peptide. The same H-bond was observed experimentally with NMR at variable temperature ($\Delta\delta/\Delta T_{NHVal}$ = 0.85 ppb/K, **Figure S3 in Supporting Information**).This interaction is also observed during 57% of the dynamics. We performed a 200ns molecular dynamics simulation restraining the interatomic distances from Noesy to their experimental values. Cluster analysis of this trajectory led to a central structure of the principal cluster similar to the previous one obtained without Noesy restraints (**Figure S4 in SI**). This last result suggests that plain MD very well reproduces experimental data and no significant improvement is associated with Noesy restraints implementation.

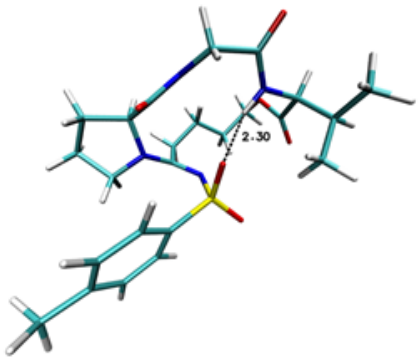


Figure 4 Central structure of 5 arising from the MD simulation. The HB between SO_2 and Val amide nitrogen is highlighted.

The conformational behavior of compound **6** was explored performing a 200 ns molecular dynamic simulation. Our aim was to test if the cyclization could stabilize the secondary structure with respect to linear peptide **7**. The RMSD analysis of compound **6** (**Figure S5 in Supporting Information**) indicated that the system underwent a structural rearrangement in the first 10 ns of the dynamics. Later on, the RMSD reached a stationary value.

The central structure of the most populated cluster (65% of the structures of the dynamics) is represented in **Figure 5**, showing the presence of a helical motif comprising residues EEYLDWV. Hydrogen bonds between Glu4-Leu7 and between Leu7-Val10 were observed along the whole dynamics. On the other hand, the hydrogen bond between residues Glu5-Asp8 is observed in almost 50% of the trajectory snapshots. This is consistent with a helical structure closely related to a 3_{10} helix, characterized by the typical *i*, *i*+3 hydrogen bond interactions. In the case of linear peptide **7**, we performed a 200 ns control MD simulation. From the analysis of the secondary structure (**Figure S6 in Supporting Information**), it was evicted that this peptide does not adopt a preferred secondary structure.

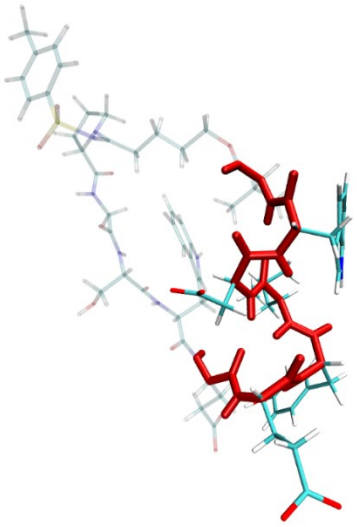


Figure 5 The structure arising from the cluster analysis carried out on the cyclic peptide **6**. The backbone of residues Glu3-Val10 is highlighted in red.

Conclusion

In conclusion, we set up an efficient on resin MCR protocol for the synthesis of amidino depsipeptide mimics. We demonstrated that this protocol does not lose its efficiency changing the peptide length or the type of azide. This methodology gives access to differently functionalized depsipeptides. Furthermore, the C4-alkyl chain, deriving from cyclopentanone ring, assesses the necessary flexibility allowing the head to tail cyclization of the depsipeptide derivatives. The obtained cyclic compounds possess stabilized secondary structures, as demonstrated with both in silico calculations and experimental conformational studies on compounds **5** and **6**.

Disclosure of potential conflicts of interest

The authors declare that they have no conflict of interest.

Research involving Human Participants and/or Animals

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

Informed consent was obtained from all individual participants included in the study.

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