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Synthesis of dicarba-cyclooctapeptide Somatostatin analogs by conventional and MW-assisted RCM: A study about the impact of the configuration at C^{α} of selected amino acids

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ABSTRACT

This work describes the synthesis of thirteen cyclooctapeptides dicarba-analogues of Somatostatin, containing L- or D-allylglycine (Agl) residues at the termini of the peptide chain, through on resin Ring Closing Metathesis (RCM) of the linear octapeptides. We investigated the influence of the stereochemistry of some strategic amino acids on the propensity to give the cyclic compounds in mild conditions (refluxing DCM). Systematic individual replacement of $Phe^{6,7,11}$ residues with the corresponding enantiomers, strongly favoured the ring closure by conventional heating. The yield of the cyclic products was strictly correlated to the position of this amino acid on the peptide chain. In particular substitution of Phe^{6} by Tyr in peptides which did not give the cyclic compounds, allowed the ring formation. The effect of the phenolic –OH function of Tyr side chain on the proximity of the terminal Agl residue was studied by NMR techniques. All the linear precursors gave cyclic somatostatin dicarba-analogues, in good to high yields and in short reaction times, by microwave-assisted RCM, performed with the 2nd generation Grubbs catalyst. The unsaturated dicarba-tether resulted in a mixture of *E* and *Z* stereoisomers in a variable ratio, depending on the sequence and the cyclization method. The *E* isomer was largely the most abundant in all but one the described product.

1. Introduction

Cyclic peptide chemistry and biology is an emerging multidisciplinary approach to medicinal chemistry and molecular biology. Native cyclic peptides are present in animals, plants and bacteria and were found in several classes of natural compounds like hormones, antibiotics, and toxins. In medicinal chemistry, synthetic cyclopeptides showed many advantages over their linear counterparts: increased metabolic stability, better bioavailability and receptor selectivity, higher resistance to enzymatic degradation. Furthermore, due to their restricted conformational freedom, the cyclopeptides are valuable tools in the study of receptor-ligand interactions. Cyclisation methods cover head-to-tail linkage, side-chain and backbone cyclisation with all possible connections [1]. A

Abbreviations: Agl, Allylglycine; COSY, Correlation Spectroscopy; D-2-Nal, (2-naphthyl)-D-alanine; DIC, *N,N′*-Diisopropylcarbodiimide; DMF, N,N-Dimethylformamide; HPLC-ESI–MS, High Pressure Liquid Chromatography – Electrospray Ionization – Mass Spectrometry; Msa, 3-mesityl alanine; MW, microwave; MW-SPPS, Microwave-assisted Solid-Phase Peptide Synthesis; ODN-8, des-$AA^{1,2,4,5,12,13}$[D-$2Nal^{8}$]-somatostatin-14; ODT-8, des-$AA^{1,2,4,5,12,13}$[D-Trp^{8}]-somatostatin-14; Oxyma, Ethyl(2-cyano-2-(hydroxyimino)acetate); RCM, Ring Closing Methatesis; RP-HPLC, Reverse Phase – High Pressure Liquid Chromatography; SPPS, Solid Phase Peptide Synthesis; SRIF, Somatotropin Release-Inhibiting Factor; TFA, trifluoroacetic acid; TIS, Triisopropyl silane.

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frequent structural motif found in side-chain-to-side-chain bridged natural peptides is the cystine (S-S) moiety which forms inter- and intra-molecular tethers of fundamental importance for the structure of biomolecules and hence for their activity [1–4]. Among the natural hormones, somatostatin-14 (SRIF-14), an S-S tethered cyclotetradecapeptide, was discovered to exert anti-secretory and anti-proliferative activity through the binding to five receptor subtypes, $sstr_{1\text{-}5}$ [5]. In many cases, especially in oligopeptides, intramolecular disulphide bridges act as a constraint maintaining active secondary structures like helices or turns. However, the S-S bridge is highly sensitive to exo- and endogenous attack of oxidant or reducing agents (i. e. disulfide reductase enzymes). Therefore, the native cystine tether has been substituted, in time, by other linkages like thioether and ester groups, peptide bond, *N*-backbone linkages, or triazolic rings. In the last decade, the substitution of the disulfide group by isosteric, non-reducible, dicarba-bonds has grown of importance after the discovery of the Ru-catalysts mediated Ring-Closing Metathesis (RCM) of alkylidene substrates, proposed by Grubbs and other researchers [6–9]. Nowadays, the researchers can handle efficient Ru-catalysts representing useful and flexible tools for building a variety of dicarba-bridges in bioactive peptides, intended to stabilize the molecules and/or to rigidify their active conformation [10–14]. Recently, we applied the RCM on hexapeptides containing L-allylglycine residues and, by using 1st or 2nd generation Grubbs catalysts with conventional heating, we successfully installed an intramolecular C=C bridge in the cyclic peptides. Subsequent coupling of the terminal NH_2 with D-Phe afforded a series of robust octreotide (Sandostatin) mimics, containing $C^{\alpha}_{(i)} \rightarrow C^{\alpha}_{(i+5)}$ [m = 4, (*E*)/(*Z*) tethers] especially suitable for ^{99m}Tc or ^{188}Re labeling methods which require reducing media [15]. Some of them, mainly *Z* stereoisomers, showed high affinity and selectivity for sst2/sst5 receptor subtypes [16–18]. Studies performed by Reubi *et al.* [19] on the structure-activity relationship of Somatostatin-14 (SRIF-14) mimic ligands, paid attention to the role of the ring size, and discovered that some disulfide bridged cyclo-octapeptides, rich in aromatic and hetero-aromatics side-chains, showed antagonist activity towards SRIF receptors, making these molecules very interesting for the clinical use as radio-labeled drugs [20]. Two well performing examples are depicted in Fig. 1.

2. Material and methods

2.1. General procedures

Fmoc-protected with amino acids were purchased from Novabiochem (Laufelfingen, Switzerland) and from Iris Biotech (Marktredwitz, Germany), Rink Amide AM resin was purchased from Iris Biotech. Second generation Grubbs catalyst was obtained from Aldrich (St. Louis, MO, USA). Fmoc-Agl-OH was purchased from Polypeptide Laboratories (Strasbourg, France). Peptide grade DMF was from Scharlau (Barcelona, Spain). Oxyma Pure was purchased from CEM (Matthews, NC, USA) All the other solvents and reagents used for SPPS were of analytical quality and used without further purification. The syntheses of the linear peptides were performed on a CEM Liberty Blue™ automated peptide synthesizer, equipped with a CEM single-mode MW reactor (Matthews, NC, USA). The desired cyclic peptides were obtained by using a CEM Discovery® microwave reactor (Matthews, NC, USA). Analytical RP-HPLCs were performed on a Thermo Finnigan (Waltham, MA, USA) Surveyor instrument equipped with an UV detector and a Thermo Finnigan LTQ Advantage mass spectrometer.

2.1.1. Micro- w ave assisted synthesis of the linear peptides I-XIII

The linear peptides **I-XIII** were synthesized on a CEM Liberty Blue™ automated peptide synthesizer, by MW-assisted strategies (Scheme 1) and using the conventional Fmoc/*t*Bu protection scheme.

The reactions were performed in a Teflon vessel and mixed by nitrogen bubbling. Reaction temperature was measured by an internal fiber-optic sensor. The general MW-SPPS protocol was structured as follow: before the coupling of the first amino acid the resin was swelled in DMF for 2 min. Afterwards, two deprotection steps were performed at 75 °C using 155 W for 15 s for the first and 30 W for 30 s for the second one. The coupling steps were performed at 90 °C, using 170 W for 15 s and 30W for 110 s for all amino acids. After each coupling cycle, the resin was washed tree times with DMF.

The syntheses were performed on Rink-amide AM resin (0.5 mmol/g, 200 mg). During the general coupling cycle, the *N*-terminal Fmoc-protecting group was removed with a solution of

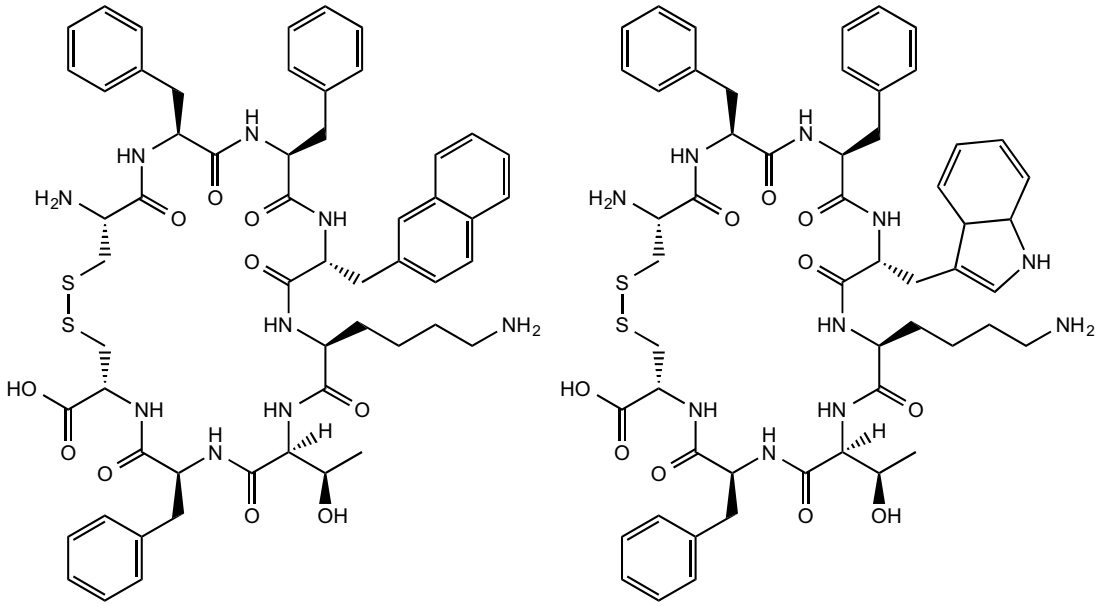

Fig. 1. ODN-8 and ODT-8 structures.

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