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Utilization of some non coded amino acids as isosters of peptide building blocks

Review Article

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Summary. In our study on non coded amino acids and their utilization in peptide chemistry we synthesized methylene-thio (CH_2—S) and methylene-oxy (CH_2—O) group containing amino acids and pseudodipeptides which could be used as building blocks for the construction of peptide hormone analogues. The (CH_2—S) isoster of peptide bond exhibits increased flexibility, lipophility and resistance to proteolytic enzymes. This group exhibits similar properties as the isosteric disulfide bond in the side chain of cystine residue. The (CH_2—O) isoster is moreover similar in its geometry to extended conformation of peptide bond. As a consequence, the changed profile of biological activities could be expected for peptide hormone analogues containing such isosteric moiety. The (CH_2—S) isosters of the peptide bond were prepared by alkylation of thiolates of 2-mercaptocarboxylic acids, the disulfide bond by alkylation of cysteine or homocysteine. The (CH_2—O) isosters were prepared by $(AcO)_4Rh_2$ catalyzed addition of carbenes of alkyl diazocarboxylates to N-protected aminoalcohols. Pseudodipeptides H—Leu—ψ(CH_2—S)—Gly—NH_2 and H—Leu—ψ(CH_2—O)—Gly—NH_2 were introduced into the C-terminal part of the oxytocin molecule using solution methods of peptide chemistry. Both inserted isosteric bonds were resistant against proteolytic degradation, the first one was found to decrease an enzymic cleavage of the distant Tyr^2-Ile^3 bond in the corresponding analogue, too. The (CH_2—S) isosters of the disulfide bond containing an orthogonal protection of their α-amino (Fmoc) and α-(OAll, OH) or ω-(OBu^+, OH) carboxylic groups were applied in the solid phase synthesis of the aminoterminal 1-deamino-15-pentadecapeptide of endothelin-I which represents a strong vasoactive agent. The solid phase synthesis was carried out by the step-wise protocol on the Rink or Merrifield type resin using orthogonally protected carba cystine building blocks.

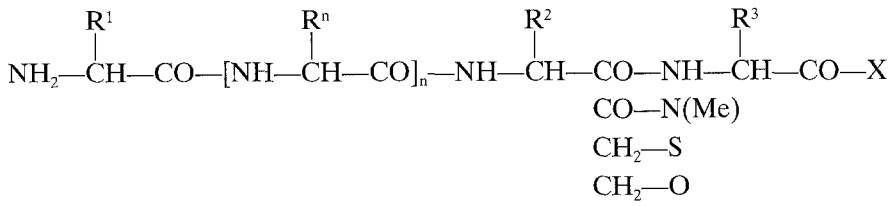
Keywords: Non coded amino acids – Peptide bond isosters – Oxytocin – Vasopressin – Cholecystokinin – Endothelin – GHRP – Peptides synthesis – Biological activity

Introduction

Non coded amino acids have been found as an important tool for the synthesis and investigation of biological and physicochemical properties of peptide hormone analogues which can be potentially used as peptide drugs in human and veterinary medicine. We have been using them also in our laboratory for many years when dealing with a synthesis of biologically active peptide analogues, like oxytocin, vasopressin (Hlaváček, 1987; Hlaváček and Frič, 1989; Frič et al., 1990), cholecystokinin (Čeřovský et al., 1990; Hlaváček et al., 1991a,b,c, 1993a,b; Maletínská et al., 1992), or growth-hormone releasing hexapeptide (Hlaváček et al., 1993c, 1994) to mention some of them.

During our synthetic work, non coded amino acids (or more generally non coded structures) were inserted into a peptide chain to replace original amino acid residues (Nomenclature and Symbolism for Amino Acids and Peptides, 1984) in the corresponding peptides (Fig. 1). The aim of such replacement was to influence a conformation and consequently an interaction of the peptide with its biological counterpart, like receptors or proteolytic enzymes and to modify the peptide bioactivity and/or bioavailability for enzyme degradation. In comparison with a relatively large molecule of the corresponding peptide such structure alteration is very small and as a rule is located to limited part of the peptide molecule. However, its effect can dramatically change a biological activities profile of such peptide.

The replacement of the isolated amino acid residue can be performed either by means of non coded amino acid which maintain the peptide bond and alter mostly the side chain of the original amino acid residue or by insertion of a partial structure isosteric with the peptide bond. In the first



R^2 or R^3 = Tle, Neo, Cle, Cpa, Phe(2-Me), Phe(2,4-diMe), Phe(2,4,6-triMe), Phe(2,3,4,5,6-pentaMe)

Peptide bond = MeLeu, MeIle, MeVal, MeAla, MeArg, MeOrn

Peptide bond isosters: CH_2—S, CH_2—O

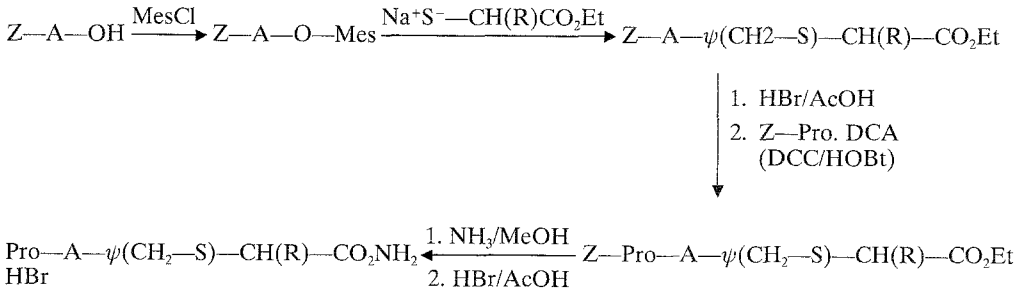
Fig. 1. Side-chain and backbone non coded structures in peptides

case, for example, Leu derivatives like tert. leucine, neopentylglycine and cycloleucine, or Phe derivatives methylated at its aromatic nucleus like Phe (2-Me), Phe(2,6-diMe), Phe(2,4,6-triMe), Phe(2,3,4,5,6-pentaMe) were used. On the other hand we have also introduced into the molecule of some peptide hormone analogues the N-methyl surrogates of the peptide bond using some N-methylamino acids (Hlaváček et al., 1977). Such methylation demonstrates one type of the peptide backbone modification which can, in general, lead to stable biotransportable peptide drugs. Some advantages resulting from the modification of the peptide bond (Spatola, 1983) can consist in the enhancement of metabolic stability, increase of the selectivity towards subtypes of receptors, change of agonistic versus antagonistic activities as well as alterations in pharmacokinetic properties of such peptides, e.g. the increase of the oral bioavailability, prolonged duration of action and improved penetration into the CNS.

In contrast to the N-methylation, other surrogates of the peptide bond are mostly created inside of the dipeptide unit which can be used as a building block in the construction of peptide hormone analogues. Among the numerous surrogates of the natural peptide bond the methylene-thio and methyleneoxy modifications were of our interest and this review will briefly describe some of our work on this topic.

Peptide building blocks with the CH_2—S isoster of the peptide bond

The methylene-thio isoster exhibits increased flexibility, lipophility and resistance to proteolytic enzymes. We carried out the synthesis of the methylene-thio isosters (Hlaváček et al., 1984) according to scheme in Fig. 2. The N-protected amino acid methyl ester (Abbreviations, 1995) of the corresponding amino acid was reduced to the derivative of aminoalcohol and this product was characterized as mesylate. In addition to good crystalline properties a further advantage of such derivatization was the high reactivity of the mesylate towards the thiolates of 2-mercaptocarboxylic acid methyl esters. This reaction yielded the protected pseudodipeptide presenting a peptide building block with a methylene-thio isoster. This partial structure can be conveniently introduced into larger peptides.



A—OH = leucinol, valinol, isoleucinol, alaninol

R = H, Me

Fig. 2. Synthesis of the methylene-thio isoster bond

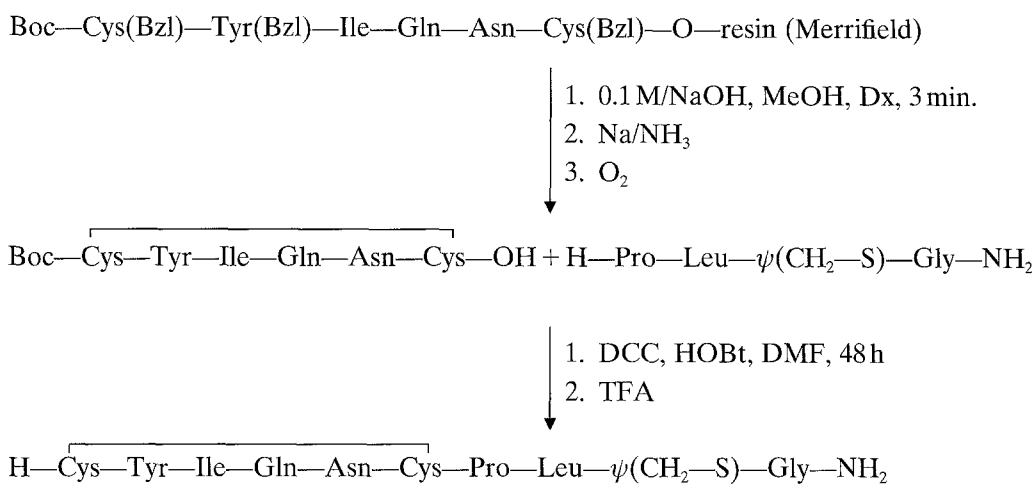


Fig. 3. Fragment synthesis of [Leu^8-ψ(CH_2-S)-Gly^9-NH_2] oxytocin

To prove the usefulness of such a procedure we modified the C-terminal dipeptide part of the peptide hormone oxytocin using the building block H—Leu—ψ(CH_2—S)—Gly—NH_2. Figure 3 shows the coupling of the corresponding C-terminal pseudotripeptide with N-tert.butyloxycarbonyl tocinoic acid, this sequence was built up on the Merrifield resin. The acid itself was prepared in several steps. After deprotection and purification by preparative HPLC the bioassay revealed that this analogue exhibits decreased uterotonic and galactogogic (10 I.U. and 16 I.U. respectively) but no pressoric activity, when assayed in a rat. The analogue also exhibited a decreased enzymic cleavage of the distant Tyr^2-Ile^3 bond. The thiomethylene bond could be very easily oxidized by sodium periodate to give a mixture of diastereoisomeric sulfoxides which, after HPLC separation, exhibited only insignificant oxytocin activity.

A difference in the biological potency of this analogue and oxytocin is probably caused by the different geometry of the amide and methylene-thio bond containing the larger sulfur atom in comparison with the amide nitrogen. For instance, the distances between adjacent C^α-atoms are 3.8 A for amide and 4.2 A for methylene-thio linkage, respectively. However, the geometry is very close in the case of the methylene-oxy surrogate (3.72 A). Therefore, more similar bioactivities to those of oxytocin could be expected from the corresponding analogue.

Peptide building blocks with the CH_2—O isoster of the peptide bond

In the synthesis of the methylene-oxy derivatives of the peptide bond shown on the scheme in the Fig. 4 a quite different synthetic approach has been used (Hlaváček and Král, 1992). We utilized a known procedure based on insertion of carbenes into the OH group of alcohols mediated by rhodium acetate as a catalyst (Paulissen et al., 1973). We have chosen the methyl or ethyl diazocarboxylates prepared by the acid catalyzed reaction of the Gly, Ala,

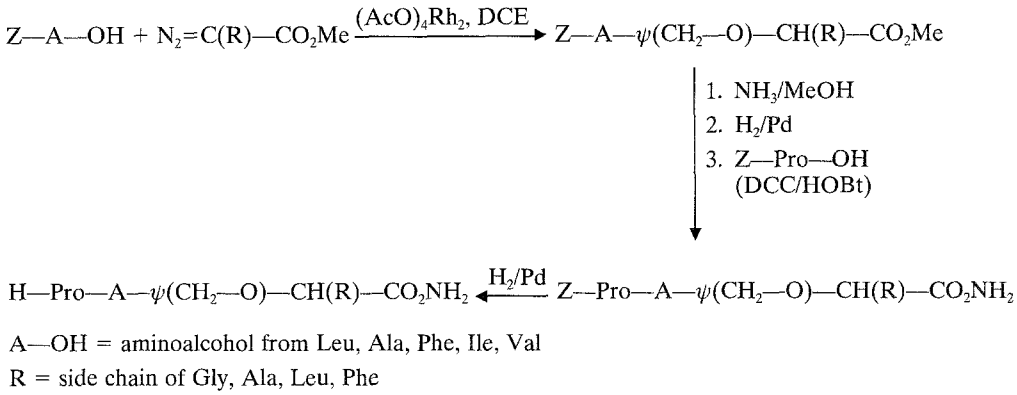


Fig. 4. Synthesis of the methylene-oxy isoster

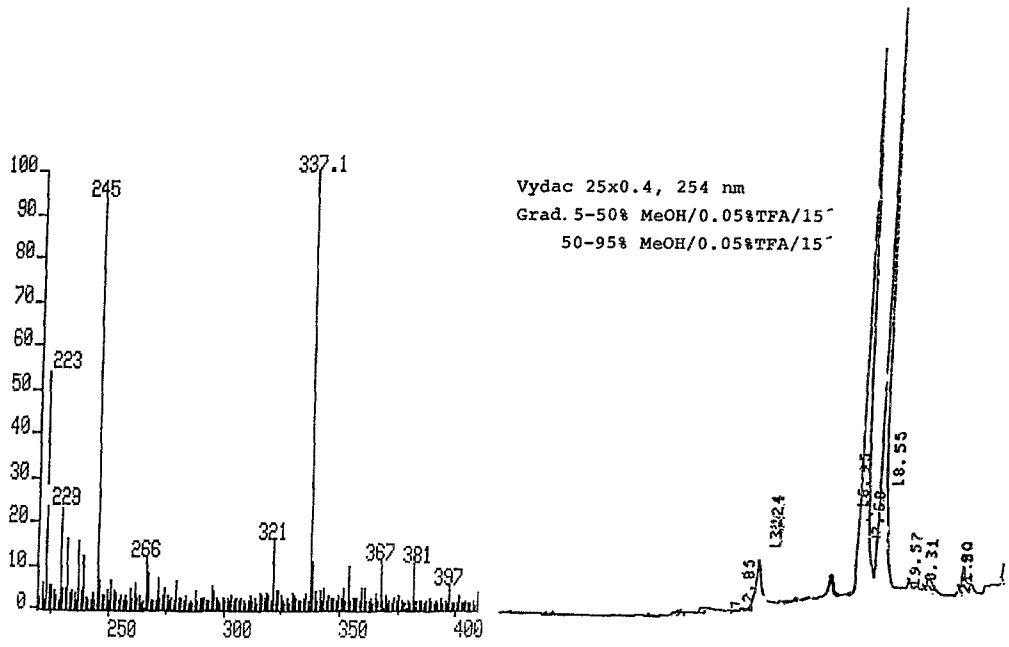


Fig. 5. HPLC and mass spectrum of the Z—Leu—ψ(CH_2—O)—Ala—O—Me

Leu and Phe esters with sodium nitrite (Curtius, 1898; La Forge et al., 1952). The carbenes obtained from the diazocarboxylates were reacted with N-benzyloxycarbonyl derivatives of the corresponding aminoalcohols which were derived in this first study from Leu, Ala, Phe, Ile, and Val. The fully protected pseudodipeptides were ready for use in the peptide synthesis as peptide building blocks after deprotection, either on the amino or carboxy terminus, and insertion of the block unit into the peptide chain.

With diazocompounds derived from Ala, Leu and Phe, pairs of the diasteroisomeric pseudodipeptides were obtained, which could be separated by HPLC. Figure 5–7 show how the side-chains of the amino acids participating in the methylene oxy surrogates could influence the ratio of both diastereoisomers. While the combination Leu-Ala yielded nearly equal mix-

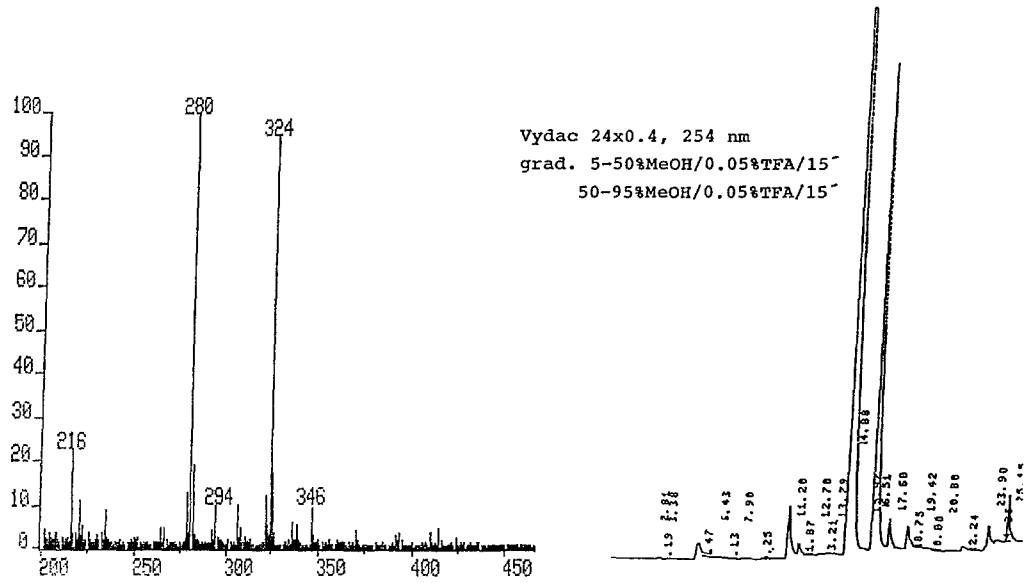


Fig. 6. HPLC and mass spectrum of the Z—Val—ψ(CH_2—O)—Ala—O—Me

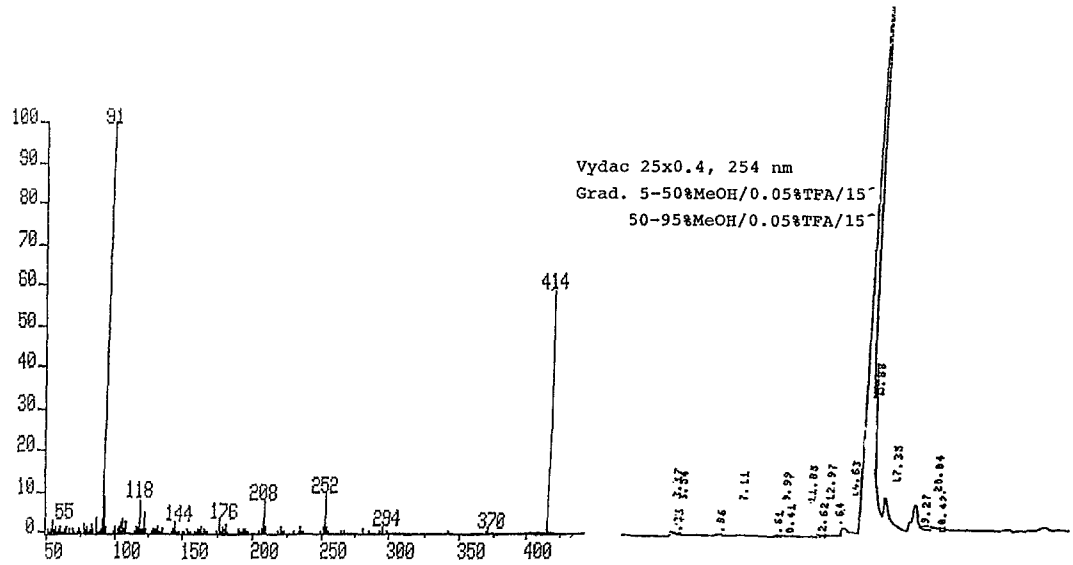


Fig. 7. HPLC and mass spectrum of the Z—Leu—ψ(CH_2—O)—Phe—O—Me

ture diastereoisomers characterized by a molecular peak in the mass spectrum, m/z 337.1 (M^++1), combination Val-Ala (m/z 324, M^++1) shows one of the diastereoisomers considerably prevailing. Finally, the combination Leu-Phe (m/z 414, M^++1) is presented where the one diastereoisomer is nearly extincted. At present, the configuration of both optical isomers in the corresponding compounds is investigated in detail by NMR.

Similarly to the methylene-thio building block this isosteric unit was introduced into a model oxytocin analogue according to scheme in the Fig. 8. The construction of the C-terminal tripeptide derivative was similar to that chosen

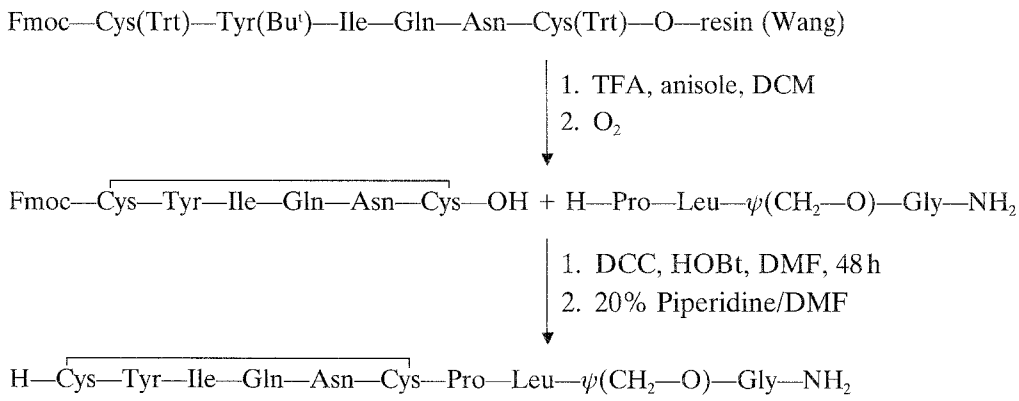


Fig. 8. Fragment synthesis of [Leu^8—ψ(CH_2—O)—Gly^9—NH2] oxytocin

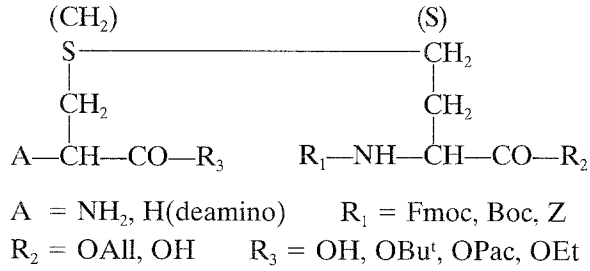


Fig. 9. Protected carba isosters of cystine and deamino cystine

for the methylene-thio analogue. However, we have used N-Fmoc protection for tocinoic acid in the coupling process with this isosteric tripeptide amide due to the suspected lability of the methylene-oxy bond to TFA. On the other hand the Fmoc group is cleaved by the secondary amine for example by piperidine. Preliminary bioassay of this analogue has confirmed our supposition about a similar biological activity profile to oxytocin.

Peptide building blocks with the CH_2—S isoster of the disulfide bond

Among the building blocks for amino acids the isosters of disulfide bond in cystine, so called carba analogues, are of current interest (Fig. 9). These derivatives have been usually prepared by S-alkylation of cysteine or homocysteine. The corresponding protected derivatives of S-alkoxycarbonyl propyl cysteine or S-alkoxycarbonyl ethyl homocysteine are used as building blocks in peptide synthesis (Jošt, 1987).

Recently, we have used some of the orthogonally protected carba units in the synthesis of carba analogues of the strong vasoactive peptide endothelin (Doherty, 1992), (Fig. 10). The conformation of this peptide family is relatively rigid due to two disulfide bonds spanning the positions 1–15 and 3–11 which can be replaced by carba building block. We have chosen two routes (Hlaváček et al., 1995) for the synthesis of the N-terminal cyclic pentadecapeptide of the endothelin sequence containing this carba building block.

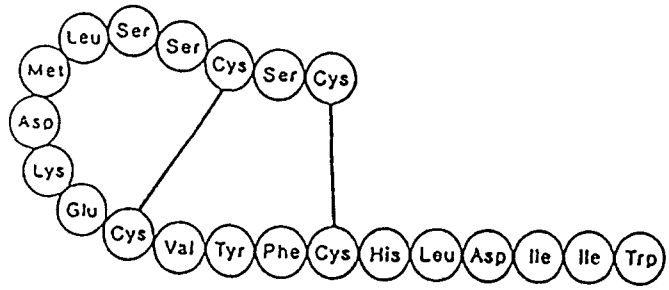


Fig. 10. Endothelin-I (Human, porcine, dog, rat)

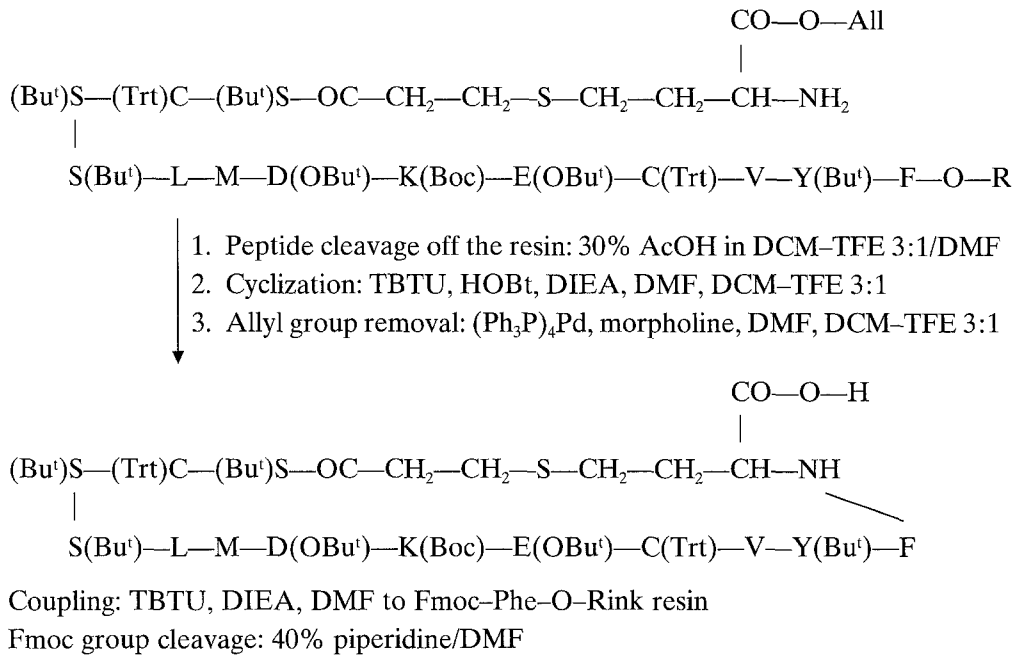


Fig. 11. Synthesis of the protected N-terminal 1-deamino-15-carba-pentadecapeptide of endothelin (Route 1)

In the first synthesis (Fig. 11), the Fmoc-amino acids and tert.butyl side chain protection was used with exception of Cys residues where S-Trt protection was introduced. The amino acids were assembled step-wise on acid labile Rink resin (Rink, 1987) by means of TBTU, DIEA in DMF. Then the building block derived from homocysteine was coupled via its non protected side-chain carboxyl to the amino group of the Ser in position 2. After cleavage of the Fmoc group by piperidine in DMF, the peptide was split off the resin by 30% AcOH in the mixture DCM-TFE in DMF and cyclized by TBTU, HOBt, DIEA in the same solvents. In the last step, the allyl ester group was removed by $(Ph_3P)_4Pd$ in the presence of morpholine.

The second synthesis (Fig. 12) was performed on Merrifield resin (Barany and Merrifield, 1980). Fmoc amino acids were protected in their side chains with benzyl and cyclohexyl groups. In contrary to the first route, this solid

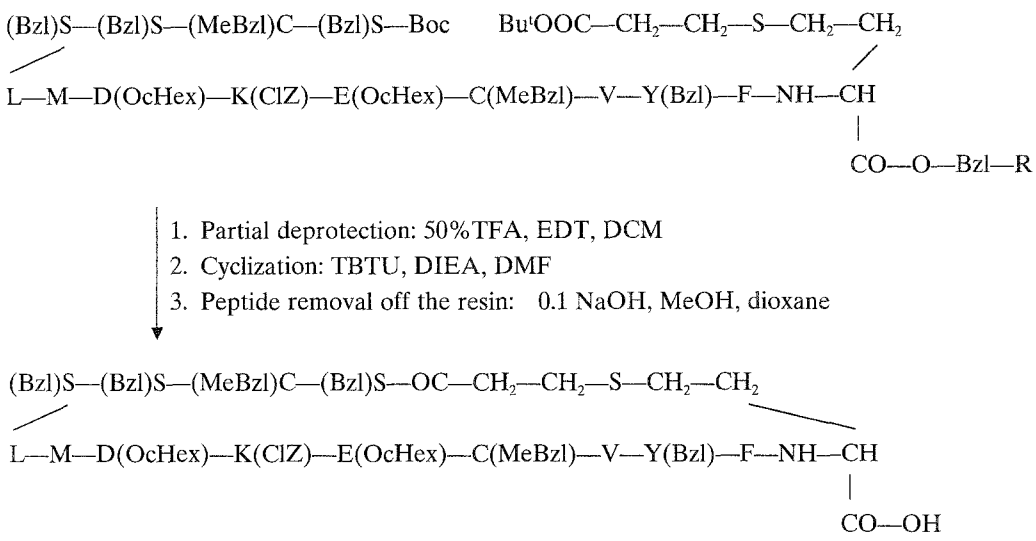


Fig. 12. Synthesis of the protected N-terminal 1-deamino-15-carba-pentadecapeptide of endothelin (Route 2)

phase synthesis starts with the carba building block presented by N$^{\alpha}$-Fmoc-Hcy derivative protected in its side chain as tert.butyl ester. The coupling was carried out with DIC and HOBt in DMF. The closure of the 15-carba bridge was performed on the resin by TBTU, HOBt, DIEA in DMF after a simultaneous cleavage of the tert.butyl protection groups by 50% TFA in DCM. The protected cyclopeptide was split off the resin under alkaline conditions in MeOH, dioxane mixture. The corresponding acid obtained via these two routes described could be further used in a convergent coupling with the C-terminal hexapeptide of endothelin.

Acknowledgements

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