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Acetylene – azide click macrocyclization of peptides

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This review is focused on the synthesis of peptide macrocycles based on the intramolecular click reaction — [2 + 3]-cycloaddition of alkynes and azides giving a 1,2,3-triazole moiety. The importance of this class of macrocyclic peptides and the general approach to the synthesis of these compounds are discussed. The review summarizes published data on these reactions proceeding in the presence of catalysts based on copper(I) and ruthenium(II) salts and thermal cyclization reactions that allow the synthesis of 1,4- and 1,5-disubstituted 1,2,3-triazoles. Special attention is given to the Ugi/click strategy for the synthesis of macrocyclic peptides. This approach is shown to be efficient for the preparation of various macrocycles.
The bibliography includes 111 references.

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1. Introduction

Many peptides and depsipeptides (peptides that contain both ester and amide linkages) exhibit high physiological activity and are of great interest for the development of new drugs. Physiologically active peptides are regulators of vital functions of the body and its certain functional systems, such as immune, digestive, nervous and cardiovascular.[1] The main characteristic feature of these agents is that they consist of non-toxic amino acids, which are, in turn, the basis for all life. Despite high physiological activity of peptides and their great pharmacological potential, only 2% of the currently used pharmaceuticals are based on peptides, and the annual global sales of all the approved peptide drugs are only about 20 billion US dollars.[2] This is because most of peptide-based drugs have low membrane-penetrating ability and metabolic stability and, as a consequence, poor oral activity, because of their peptidase-mediated hydrolysis in the body. One solution to this problem is to synthesize cyclic peptides. Cyclic peptides are resistant to hydrolysis by peptidases because this process occurs at amino acid sequence termini. Besides, cyclic peptides are conformationally less flexible than their linear analogues.[3,4] That is why cyclic peptides are widely used in pharmaceutics, particularly as antibiotics. Among them are colistin, cyclosporine, daptomycin, gramicidin S, octreotide, valinomycin, *etc.* (Fig. 1).

The classical approach to peptide synthesis is based on sequential assembly from amino acids. The synthesis of cyclic peptides starts with the preparation of a linear sequence followed by ring closure *via* amide bond formation in the final step (Scheme 1). However, a number of problems arise when implementing this strategy.

Main challenges are the epimerization resulting in a change in absolute configuration of the terminal amino acid (Scheme 2), oligomerization and polymerization of the starting peptide. Apart from the major monomeric product, the cyclization often affords di- and trimerization products and polymers. To suppress side processes, the reactions are performed at very high dilutions (substrate concentrations are 10^{-4} – 10^{-6} mol litre^{-1}) or on a solid support (pseudodilution effect).[5] It should be emphasized that the preorganization of a linear molecule directly influences the successful macrocyclization.

Consequently, the formation of macrocyclic peptides *via* amide bond formation is a difficult task. The development of new approaches to the synthesis of biologically active compounds resulted in the elaboration of efficient macrocyclization methods. The intramolecular formation of 1,2,3-triazoles *via* azide – alkyne cycloaddition, often referred to

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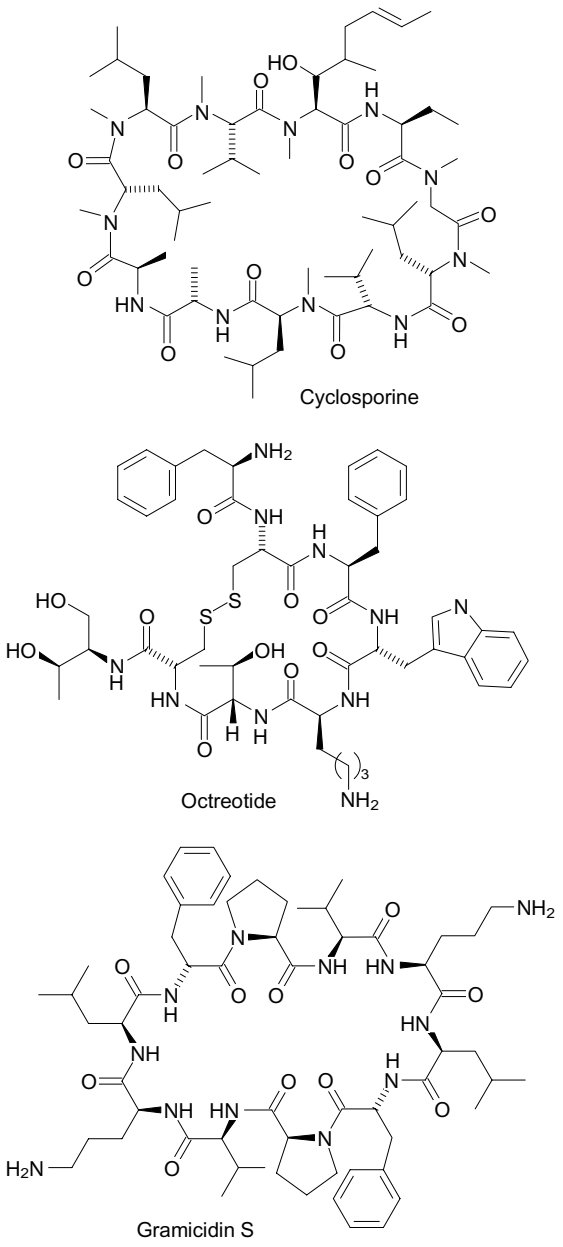
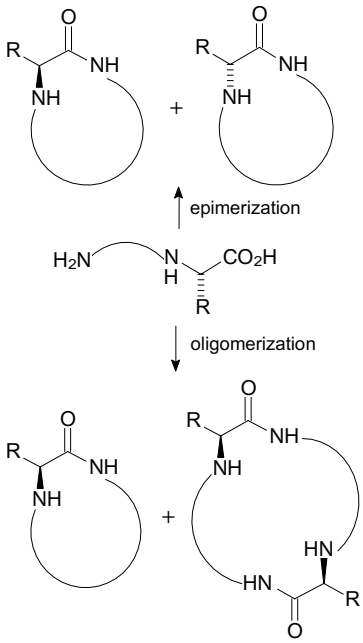


Figure 1. Structures of peptide antibiotics.

as the click reaction, is utilized as a synthetic tool for ring closure.[6–8] Since 1,2,3-triazole is a bioisostere of the amide group and it is more stable under physiological conditions, the click reaction is widely used as an efficient method for the synthesis of macrocyclic peptidomimetics.

In view of the fact that cyclic peptides are difficult to synthesize by macrolactamization, attention is given to

Scheme 2



elaboration of alternative strategies for the synthesis of macrocyclic structures. The development of efficient methods of ring closure, which is the last and most problematic step, has attracted extensive research interest. This review is concerned with the click macrocyclization of peptides and their derivatives *via* azide–alkyne cycloaddition.

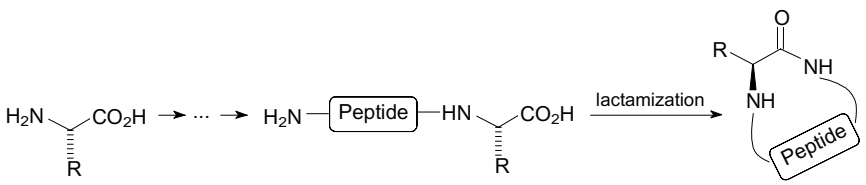
2. Strategies for peptide macrocyclization

The rings are traditionally classified into small (three- and four-membered), normal (five-, six- and seven-membered), medium (8–11-membered) and bigger (macrocycles, 12-membered or larger). Therefore, peptides containing four or more amino acid residues are generally assigned to macrocyclic peptides.

Depending on the structure and functional groups, a peptide can be cyclized in four different ways:[9] head-to-tail (C-terminus to N-terminus) (type I), head-to-side chain (type II), side chain-to-tail (type III) and side chain-to-side chain (type IV) (Fig. 2 *a*). The approaches to ring closure (types II–IV) involving one or several side groups are most often based on reactions giving rise to disulfide bridges,[10] lactones[11] or lactams[12] (see Fig. 2 *b–d*) and are most facile. For example, this strategy is suitable for the condensation of two cysteine residues, resulting in the S–S bond formation, or for the reaction between the side chains of glutamic acid and lysine.

The most commonly used ring-closing strategy is based on the type I route, namely, the reaction of the head with the tail part of the molecule, resulting in the formation of a

Scheme 1



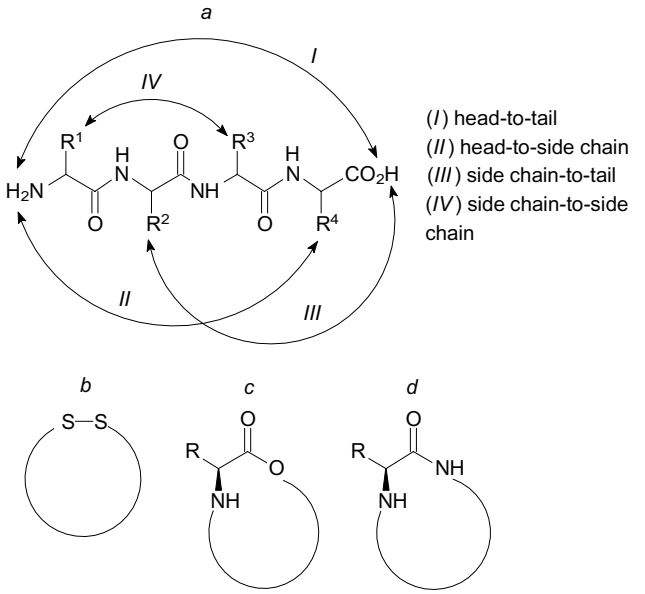


Figure 2. Schematic representation of the possible ways for macrocyclization: four types of cyclization of a linear peptide sequence (*a*), formation of a disulfide bridge (*b*) and aza analogues of the lactone (*c*) and lactam moieties (*d*).

new cyclic peptide bond. However, this method of cyclization is inefficient and accompanied by epimerization of the C-terminal amino acid and formation of dimers and oligomers.

In the general case, the success of peptide chain cyclization depends on numerous factors. The preorganization of a linear molecule and its structure play a key role in the efficiency of the synthesis. The outcome of macrocyclization depends on the conformation and configuration of the linear precursor, which ensure close spatial proximity of two reaction sites and facilitate the reaction. This effect is particularly pronounced for macrocycles comprising seven or less amino acid residues. Hence, the ring size also plays an important role. Cyclic tri-, tetra- and pentapeptides are most difficult to synthesize, while the efficiency of cyclization is generally enhanced with increasing number of amino acids in the chain.

The energy of the peptide ring is determined by all types of interactions (*e.g.*, van der Waals contacts, dipole – dipole interactions, hydrogen bonds) and depends on the energy of internal rotation of amino acid residues about single bonds.[13] Calculations of all factors in order to model the ideal molecular conformation is a challenging problem. Therefore, there are no clear rules for the synthesis of a particular cyclic peptide. Only some limitations and recommendations can help to perform the desired macrocyclization. Some of these issues are considered below.

It should be noted that *trans*-peptide bonds predominate in native peptides and proteins (Fig. 3 *a*). However, proline and sarcosine (see Fig. 3 *b*) are incapable of stabilizing such bonds. Therefore, the incorporation of these residues into the peptide chain induces the formation of a *cis*-amide bond, resulting in turn-structure motifs, thereby facilitating macrocyclization.[14] Previous studies demonstrated[15] that the synthesis of cyclic peptides comprising only L- or D-amino acids is a difficult task because of the extended conformation of the molecule, placing its termini far away

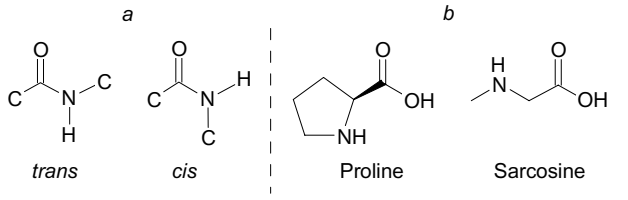


Figure 3. Peptide bond configuration (*a*) and structural formulae of proline and sarcosine (*b*).

from each other. For example, the synthesis of the cyclic tetrapeptide cyclo-[Pro-Val-Pro-Tyr] has not met with success, and only its epimerized form cyclo-[Pro-Val-Pro-D-Tyr] was prepared.[16] In this form, the last amino acid undergoes a conformational change, thereby making this cyclization possible. Besides, the product yield of the reaction largely depends on the choice of the site of cyclization. Bulky substituents, such as the isopropyl and isobutyl groups in valine and isoleucine, as well as N-methyl-substituted amino acids, at the termini of a peptide reduce the efficiency of the synthesis. Meanwhile, the cyclization between D- and L-amino acids proceeds faster than that between amino acids having the same configuration.[17]

Therefore, as mentioned above, the peptide macrocyclization is a difficult-to-tackle problem. The development of alternative approaches to ring closure can help in addressing this issue. These approaches include the introduction of additional moieties into a linear molecule[18] or cyclization *via* click chemistry,[19] metathesis[20] and multicomponent reactions.[21]

3. Click chemistry as a macrocyclization tool

Many biologically active cyclic molecules isolated from marine organisms contain various five-membered heterocycles (oxazoles, thiazoles, *etc.*). The presence of these moieties not only influences biological activity but also imposes conformational restrictions on the structures of the corresponding macrocycles.[22]

The triazole ring is geometrically similar to the peptide bond and, depending on the fusion mode, triazole is a bioisostere of the *cis*- or *trans*-amide bond (Fig. 4 *a*).[23]

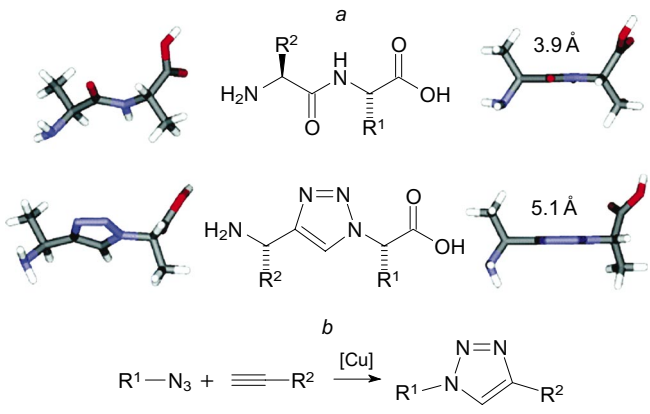


Figure 4. Triazole ring as a bioisostere of the *cis*- or *trans*-amide bond (*a*) and scheme of 1,3-dipolar cycloaddition of substituted azide to terminal alkyne (*b*).

This heterocycle can be efficiently synthesized by 1,3-dipolar cycloaddition of substituted azides to terminal alkynes (see Fig. 4 *b*).[24] The triazole ring is known to have high physiological stability and low toxicity. The introduction of this moiety into biologically active molecules is of interest for the development of new drugs.

The 1,3-dipolar cycloaddition is one of the most important methods for the synthesis of five-membered heterocyclic compounds, including functionally substituted derivatives. Huisgen [25] systematically analyzed experimental data on this reaction and elaborated a general synthetic algorithm for applying the reaction between azides and alkynes to prepare 1,2,3-triazoles. In 2002, Sharpless and co-workers [26] and the Meldal group [19] independently discovered the copper(I)-catalyzed version of azide–alkyne Huisgen cycloaddition classified as a click reaction. This gave further strong impetus to the development of this process. The copper-catalyzed reaction yields exclusively 1,4-disubstituted 1,2,3-triazoles,[27] as opposed to its non-catalytic version,[28] which is not regioselective and generally requires rather drastic experimental conditions. Therefore, this reaction can serve as an efficient alternative to the classical peptide macrocyclization based on amide bond formation.

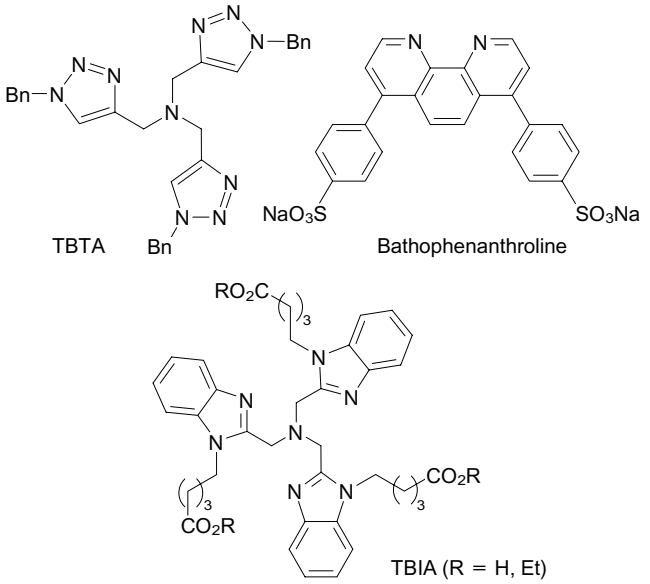


Figure 5. Structures of ligands for Cu catalysts for the click reaction.

3.1. Conditions of the click reaction

It should be noted that in this review, the term 'click reaction' is used only to indicate the copper(I)- or ruthenium(II)-catalyzed azide–alkyne cycloaddition. These reactions are commonly abbreviated as CuAAC (Cu-catalyzed azide–alkyne cycloaddition) and RuAAC (Ru-catalyzed azide–alkyne cycloaddition), respectively. Much of the research has been focused on Cu-catalyzed reactions considered below.

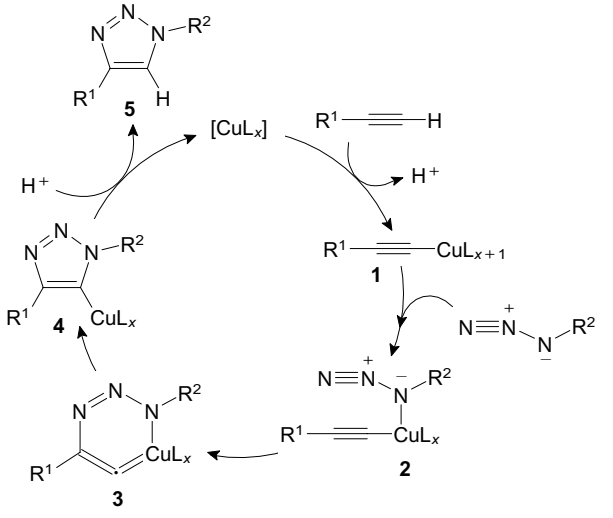
Click reactions are performed in different solvents (including water) in a wide pH range (from 5 to 12) at 0–160 °C. Polar solvents (both protic and aprotic) are most popular for this process.[29]

Different methods are available for the preparation of efficient catalysts for 1,3-dipolar cycloaddition.[29,30] The reduction of Cu^{II} salts, *e.g.*, $CuSO_4$, *in situ* giving copper(I) salts is most commonly employed for this purpose. Sodium ascorbate, hydrazine and tris(2-carboxyethyl)phosphine (TCEP) are utilized as reducing agents. Another method is based on the introduction of copper(I) salts, such as CuBr, CuI, CuOAc, CuOTf PhH [OTf is trifluoromethanesulfonate (triflate)], *etc.*, into the reaction. In this case, the reaction should be performed in an inert atmosphere in the presence of a base or an appropriate ligand. Yet another method implies the use of Cu^0 (copper turnings, powder or nanoparticles). Different copper complexes, such as $[Cu(MeCN)_4]PF_6$, $[Cu(MeCN)_4]OTf$, $(EtO)_3P-CuI$, and copper salts, *e.g.*, $Cu(OAc)_2$, can serve as the catalysts in the absence of a reducing agent.

Bases (*N*,*N*-diisopropylethylamine, triethylamine, 2,6-lutidine, *etc.*) are added to increase the basicity of the medium. Various ligands, for example, tris(1-benzyl-1*H*-1,2,3-triazol-4-ylmethyl)amine (TBTA), bathophenanthroline, tris(benzimidazol-2-ylmethyl)amine (TBIA) (Fig. 5), are utilized to increase the reaction rate and protect copper(I) from oxidation.

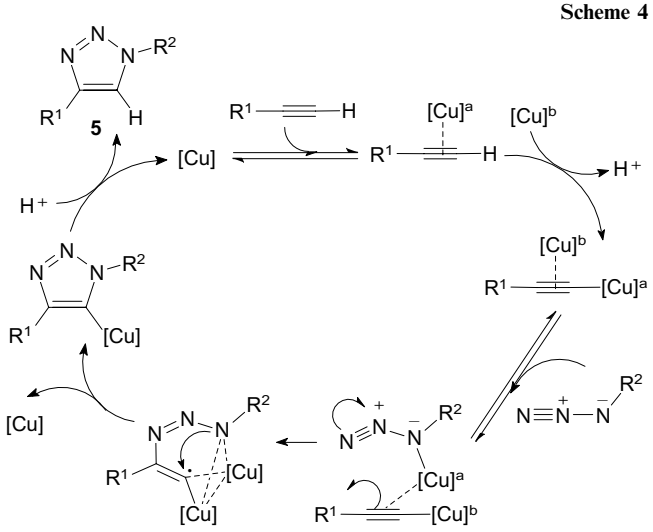
In 2005, Himo *et al.*[31] proposed the first putative reaction mechanism (Scheme 3) based on experimental data and results of molecular modelling. In terms of this classical description, it is assumed that a single copper atom is involved in the catalytic cycle. The initial event in these transformations includes the formation of an alkyne π-complex with a copper compound and elimination of the terminal hydrogen atom giving copper acetylide **1**. In the next step, azide replaces one of the ligands (L) and binds to the copper atom *via* the nitrogen atom proximal to the carbon atom, resulting in the formation of intermediate **2**. Then the distal nitrogen atom of the azide attacks the carbon atom of the acetylide to form strained metallacycle **3**, which is transformed into triazole **4**. The protonation of the latter produces triazole **5**.

Scheme 3



In 2013, Worrell *et al.*[32] published experimental evidence for the mechanism involving a six-membered metallacycle and a dinuclear copper acetylide complex (Scheme 4). The authors used isotopically enriched ^{63}Cu

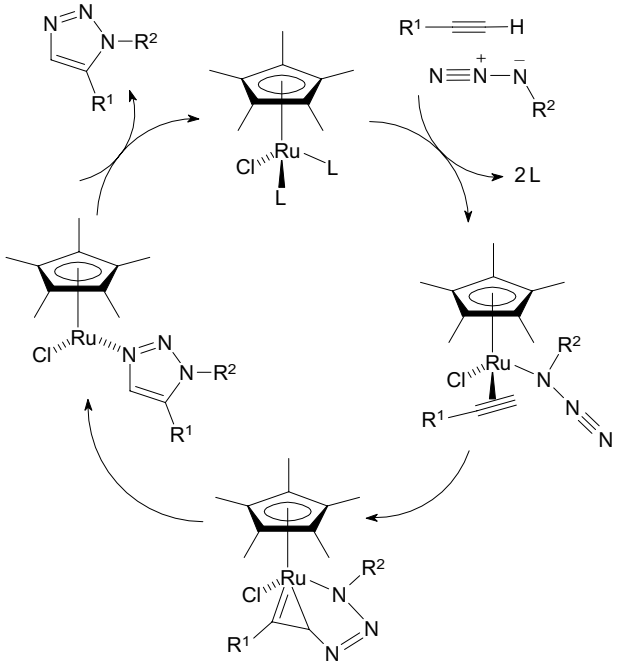
Scheme 4



and ^{65}Cu reagents, which allowed them to ultimately confirm the involvement of two copper atoms (denoted by the superscripts 'a' and 'b') in the catalytic cycle.

The reaction of alkynes with azides in the presence of divalent ruthenium complexes affords 1,5-disubstituted triazoles.[33] As opposed to the copper-catalyzed reaction, the latter transformations can be applied to synthesize fully substituted triazoles. Initially, 1,5-disubstituted triazoles were synthesized using other metals (Na, Mg, Sn, Ge) in stoichiometric amounts. The employment of ruthenium made it possible to substantially decrease the amount of metal in the reaction and increase the product yield. The reactions are generally performed in polar and nonpolar aprotic solvents (toluene, DMF, THF, acetonitrile, *etc.*); the complexes $Cp^*RuCl(PPh_3)_2$, [Cp*Ru(cod)Cl] and $[Cp^*RuCl]_4$, where Cp* = η-C_5Me_5 and cod is cycloocta-1,5-diene, are the most commonly used catalysts

Scheme 5



(Scheme 5).[33] The putative reaction mechanism involves the following four steps: 1) the coordination of alkyne and azide to a ruthenium complex, 2) the formation of bonds between the alkyne and azide, 3) the formation of a ruthenium complex with triazole, 4) elimination of triazole.[34]

3.2. Cu ^{I}-Catalyzed macrocyclization giving 1,4-disubstituted triazole

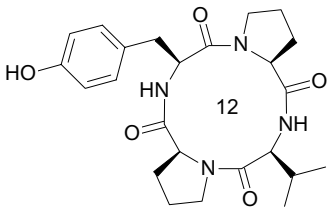
There are quite a number of examples of the application of the copper-catalyzed 1,3-dipolar cycloaddition (click reaction) as an efficient macrocyclization method. Such products are synthesized using ddifferent ring closure techniques: head-to-tail,[35,36] head-to-side chain[37,38] and side chain-to-side chain.[39–43] In most cases, the reactions produce monomeric structures — intramolecular transformation products. As a rule, this is characteristic of molecules containing more than 12 atoms in the ring. Otherwise, the intermolecular click reaction takes place due to the ring strain of the molecule, giving rise to dimers. The outcome of the click reaction depends on conformational preferences,[44] the nature of the catalyst and the solvent,[23] the substrate concentration,[45] the number of stereocentres in the molecule,[46] the employment of the solid-phase supported synthesis[47] and specific features of the cyclization mechanism.[48,49]

3.2.1. Intramolecular click macrocyclization

Click macrocyclization favours the formation of cyclic monomers. It is especially important that intramolecular cyclization allows the stabilization of a particular conformation of a peptide or peptidomimetic (*e.g.*, α-helices[50–52] or β-structures[53–58]). Intramolecular click macrocyclization is applicable to the synthesis of bicyclic peptidomimetics[59,60] and various cyclic peptide-like structures.

The successful synthesis of triazole-containing analogues of the cyclic tetrapeptide cyclo-[L-Pro-L-Val-L-Pro-L-Tyr] (**6**) was described in the literature.[61] This peptide cannot be synthesized by the classical method with retention of the all-L configuration (hereinafter, the figure at the centre of the macrocycle indicates the number of atoms).

Structure 6

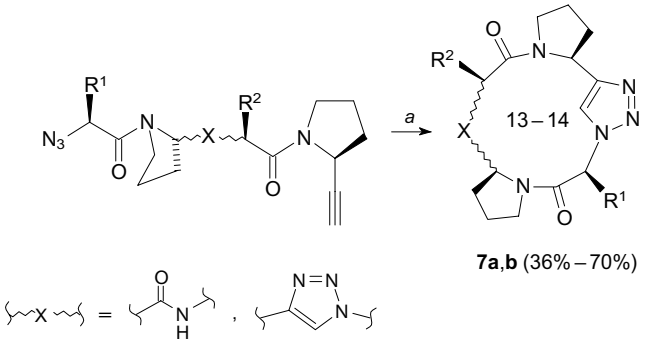


6

Van Maarseveen and co-workers[23,62] reported a versatile method for the synthesis of target compounds **7a**,**b** containing a different number of triazole moieties at different positions (Scheme 6; hereinafter, the substrate concentration is given in parentheses after the solvent). The optimization of the reaction conditions was performed to achieve the selective synthesis of the only monomeric product. Thus, very high dilution (the substrate concentration is 1 mmol litre^{-1}; hereinafter, mM) is essential for the reactions in toluene in the presence of 0.2 equiv. of CuBr and 3 equiv. of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) under reflux. The ring closure *via* the click reaction proved

to be much more efficient than classical macrolactamization of related linear molecules containing incorporated heterocycles. In the latter case, the reactions produced only di- and oligomerization products. The evaluation of tyrosinase inhibitory activity of triazole analogues showed[62] that benzyl deprotection of compounds **7a**,**b** [X = C(O)NH] led to the enhancement of activity [the half-maximal inhibitory concentration (IC_{50}) was 0.5 and 0.6 mmol litre^{-1}, respectively] compared to natural peptide **6** (IC_{50} = 1.5 mmol litre^{-1}).

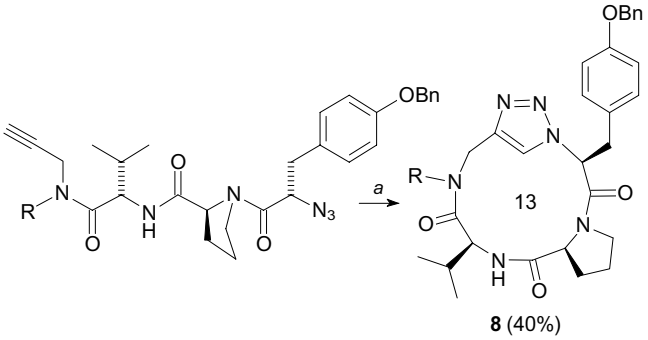
Scheme 6



(*a*) CuBr (0.2 equiv.), DBU, PhMe (1 mM), 110 °C, 16 h;
R^1 = 4-$BnOC_6H_4CH_2$, R^2 = Pr^i (**a**); R^1 = Pr^i, R^2 = 4-$BnOC_6H_4CH_2$ (**b**)

Compound **8**, the tetrazole derivative of cyclo-[Pro-Val-Pro-Tyr], was synthesized both by the solid-phase method and in solution.[63] The cyclization of the peptide attached to the solid support was unsuccessful, while the ring closure in solution gave the product in 40% yield. To achieve this, the solid-supported synthesized linear peptides were cleaved prior to cyclization (Scheme 7).

Scheme 7



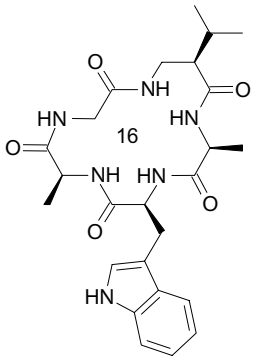
(*a*) CuBr (0.2 equiv.), DBU, PhMe (1 mM), 110 °C;
R = 4-$(BnO_2C(CH_2)_3O)$-2-$MeOC_6H_3CH_2$

This method was also used to prepare a bioisostere of segetalin B (**9**) — cyclic pentapeptide, which exhibits biological activity similar to that of estrogen.[63]

In this case, the cyclization was performed in the presence of the bis(oxazoline) ligand pybox; 17-membered macrocycle **10** was obtained in moderate yield (Scheme 8).

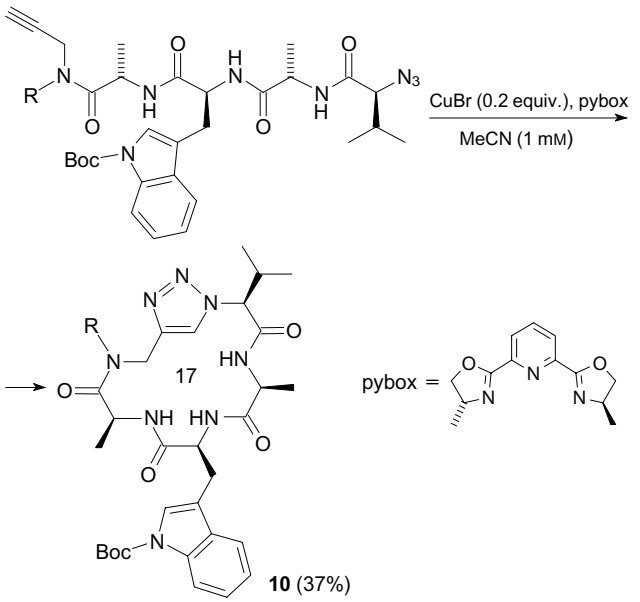
The solid-phase click reaction was applied to accomplish macrocyclization of tetra-, penta-, hexa- and heptapeptides containing leucine residues.[64] The reaction proceeded in the

Structure 9



9 (segetalin B)

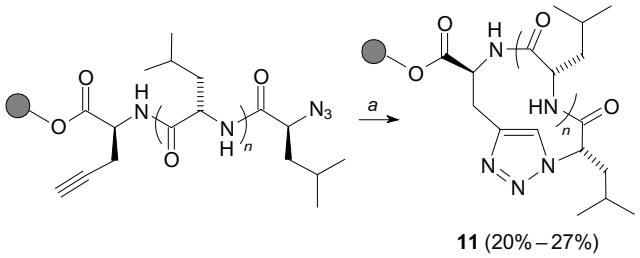
Scheme 8



R = 4-$(BnO_2C(CH_2)_3O)$-2-$MeOC_6H_3CH_2$; Boc = $Bu^tOC(O)$

presence of sodium ascorbate, Pr^i_2NEt and 2,6-lutidine in DMF at room temperature. An increase in the amount of the catalyst CuBr to 1 equiv. made it possible to decrease the reaction time to 6 h and reduce the contribution of trimerization side reactions. The cyclization furnished mainly monomers **11** with 14-, 17-, 20- and 23-membered

Scheme 9

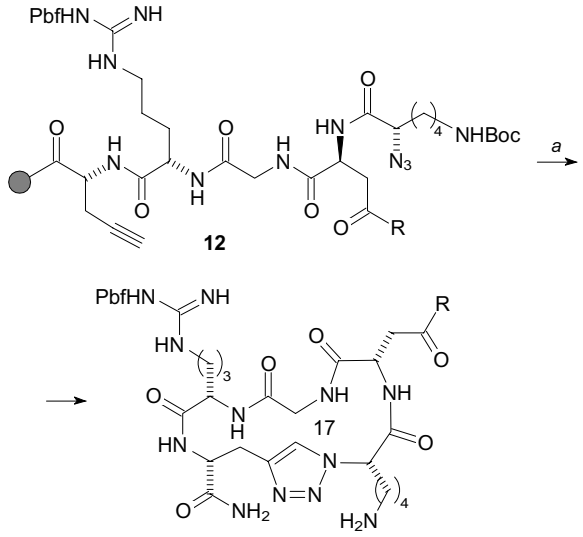


(*a*) CuBr (1 equiv.), sodium ascorbate, Pr^i_2NEt, 2,6-lutidine, DMF, 25 °C, 6 h; *n* = 2 – 5; ● is a polymer

rings ($n = 2-5$, respectively), cyclooligomeric by-products being formed in small amounts (Scheme 9).

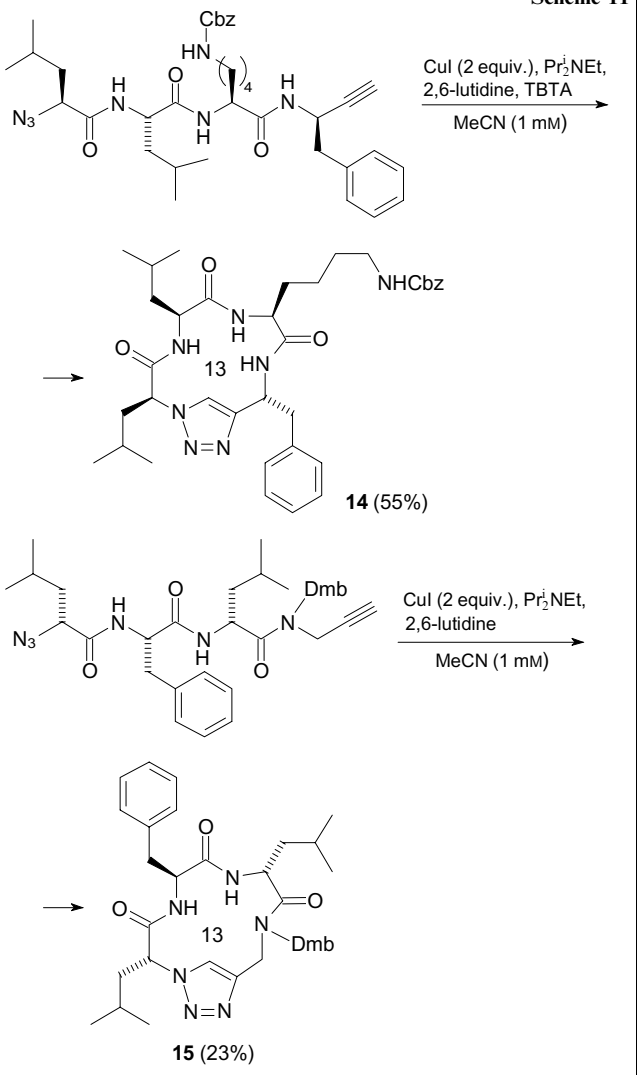
Another example of the solid-phase synthesis of macrocyclic peptides is the click transformation of linear RGD and NGR peptide derivatives **12** (Scheme 10).[65] Short amino acid sequences, such as Arg-Gly-Asp (RGD) and Asn-Gly-Arg (NGR), are attractive for targeted delivery of anticancer drugs because they can selectively bind to receptors on the cancer cell surface. However, in the linear form, these peptides are prone to degradation, limiting their use.[66] Macrocyclization of compounds **12** *via* azide – alkyne cycloaddition makes them metabolically stable and leads to a several-fold increase in the affinity for receptors.[67] In the cyclization step, the yields of products **13** were up to 30%. Peptides **13** modified by triazole heterocycles retain receptor activity.

Scheme 10



(*a*) 1) CuBr, ascorbic acid, Pr^i_2NEt, 2,6-lutidine, DMF, MeCN; 2) CF_3CO_2H, *m*-cresol; R = NH_2, OH;
Pbf is 2,2,4,6,7-pentamethyl-2,3-dihydrobenzofuran-5-sulfonyl

Scheme 11



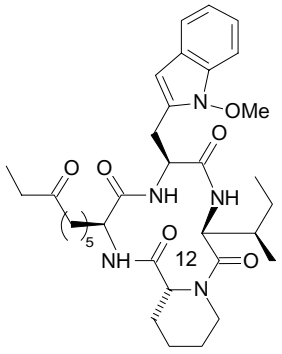
Dmb is 2,4-dimethoxybenzyl, Cbz is benzyloxycarbonyl

As mentioned above, 1,4-disubstituted triazoles are *trans*-amide bond isosteres. Cyclic tetrapeptides with high affinity for the somatostatin receptor (somatostatin is a hormone that inhibits the secretion of several other hormones in the body) were reported.[68] These cyclic peptides, having similar conformations, can bind to the receptor as efficiently as the natural somatostatin. The incorporation of one or two triazole moieties into cyclic tetrapeptides (see compounds **14** and **15** in Scheme 11) was found to have no significant effect on the conformation of macromolecules. Therefore, they can be used as three-dimensional scaffolds for drug design. It should be noted that pseudopeptides have a single conformation even at elevated temperatures.

The cyclic tetrapeptide apicidin (**16**) is a non-specific histone deacetylase inhibitor. This compound holds promise for the treatment of different cancers.[69]

Several research groups attempted to synthesize analogues of **16** by click chemistry. Singh *et al.*[70] prepared three cyclic pseudotetrapeptides **17a** – **c** *via* azide – alkyne cycloaddition (Scheme 12). The reaction was performed in the $CuSO_4$ (0.3 equiv.) – sodium ascorbate – Pr^i_2NEt system in a

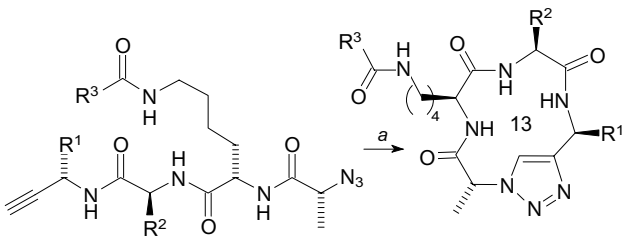
Structure 16



16 (apicidin)

$MeOH-H_2O$ solvent mixture (1 : 1); the concentration of the starting linear peptidomimetic was 5 mmol litre^{-1}. The incorporation of the triazole heterocycle into the structure of the molecule was shown to improve its inhibitory activity. However, the yields of peptides **17a** – **c** were lower than 10% in the macrocyclization step.

Scheme 12

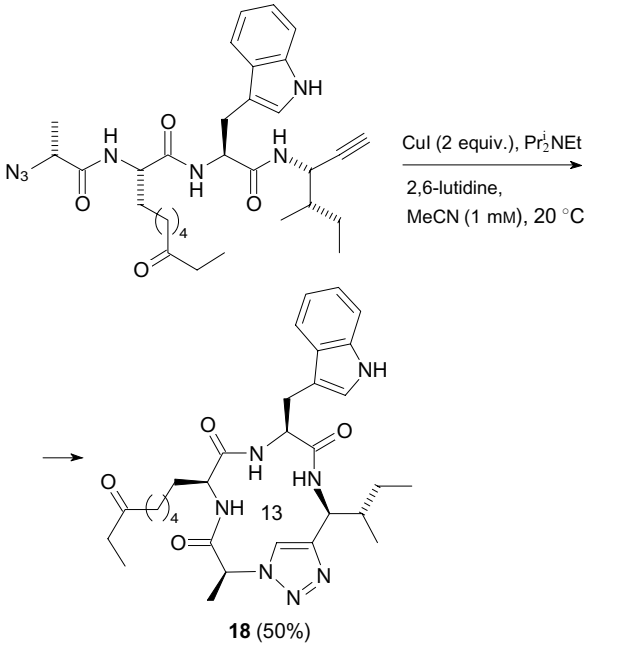


17a–c (3%–9%)

(*a*) $CuSO_4$ (0.3 equiv.), Pr^i_2NEt, sodium ascorbate, MeOH–H_2O (1 : 1) (5 mM); R^1 = Bn, R^2 = Et: R^3 = Me (**a**), CF_3 (**b**); R^1 = Bu^i, R^2 is 2-methyl-1*H*-indol-3-yl, R^3 = Me (**c**)

Horne *et al.*[71] developed a more efficient procedure for the synthesis of apicidin derivative **18**. An analogue containing 1,4-disubstituted triazole was synthesized. The macrocyclization proceeds *via* the Cu^I-catalyzed click reaction in the presence of the above-described CuI (2 equiv.)–Pr^i_2NEt–2,6-lutidine–MeCN (1 mM) system (Scheme 13). (The authors also synthesized a cyclic derivative bearing the 1,5-substituted triazole moiety, but without using click chemistry in the final step.)

Scheme 13

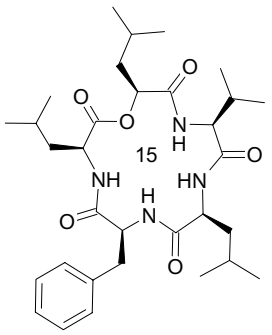


18 (50%)

The synthesis and evaluation of biological activity of structural analogues of sansalvamide A (**19**) — the cyclic pentapeptide isolated from the marine fungus *Fusarium*[72] have attracted considerable attention. Sansalvamide A exhibits selective cytotoxicity towards a number of cancer cell lines and could be a potential agent for anticancer therapy.

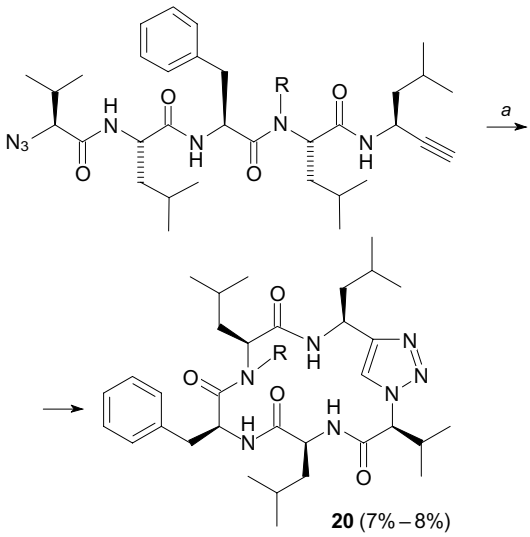
For instance, peptides containing five-membered heterocycles, such as triazoles, thiazoles, oxazoles and pseudoprolines, were synthesized from on sansalvamide A.[73] In this study, the triazole moiety was constructed by click chemistry. The cycloaddition was performed in the presence of the $CuSO_4$–ascorbic acid–$NaHCO_3$ system in a

Structure 19



19 (sansalvamide A)

Scheme 14



20 (7%–8%)

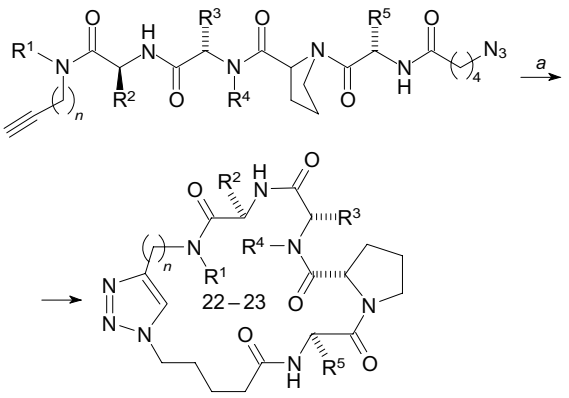
(*a*) $CuSO_4 \cdot H_2O$ (0.3 equiv.), ascorbic acid, $NaHCO_3$, MeOH–H_2O (1 : 1) (7 mM); R = H, Me

MeOH–H_2O solvent mixture (1 : 1). Unfortunately, the authors failed to achieve high yields of products **20** in this step (Scheme 14).

Proline plays an essential role in the structure, conformation and biological functions of peptides. The introduction of the proline moiety affects the rigidity and secondary structure of the peptide molecule.[14] Therefore, investigations of the possibility of cyclization of proline-containing peptides are of great importance. Chouhan and James[74] developed a versatile procedure for the synthesis of proline-containing triazole analogues of cyclic peptides **21** in good yields (Scheme 15). Click reactions were performed in dichloromethane (the concentration of the starting linear compounds was 10 mM) at elevated temperature using the $Cu(MeCN)_4BF_4$ (5 mol.%)–TBTA (5 mol.%) catalytic system.

Depsipeptides — peptides that contain, apart from amide groups, one or several ester groups — comprise a unique class of peptidomimetics. The common characteristic feature of most of natural depsipeptides is the presence of moieties other than α-amino acid residues. To demonstrate the potential of click chemistry, Agrawal *et al.*[75] synthesized a series of 14-membered cyclic depsipeptides

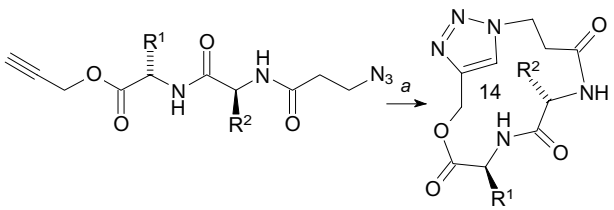
Scheme 15



21 (39% – 79%)

(*a*) $Cu(MeCN)_4BF_4$ (5 mol.%), TBTA (5 mol.%), CH_2Cl_2 (10 mM), 55 °C, 12 h;
R^1 , R^4 = H, Me; R^2 = H, Pr^i, Bu^i, $AllCH_2$; R^3 = Bu^i, Bn;
R^5 = Bu^i, 4-$ClC_6H_4CH_2$, 3-$F_3CC_6H_4CH_2$, $AllCH_2$; *n* = 1, 2

Scheme 16



22 (58% – 64%)

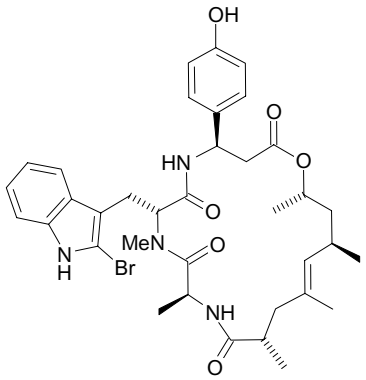
(*a*) CuBr (0.2 equiv.), DBU, PhMe (1.2 mM), 110 °C, 14 h;
R^1 = Pr^i, Bu^i, Bu^s; R^2 = H, Me, Pr^i, Bu^i, Bu^s, Bn, 4-$HOC_6H_4CH_2$, $BzOCH_2$

containing a triazole ring. The azide – alkyne cycloaddition was carried out under reflux in toluene in the presence of 0.2 equiv. of CuBr and DBU. In the macrocyclization step, products **22** were obtained in moderate yields (Scheme 16).

A click reaction was also used in the synthesis of a triazole analogue of the cyclodepsipeptide jasplakinolide (**23**) exhibiting antitumour activity.

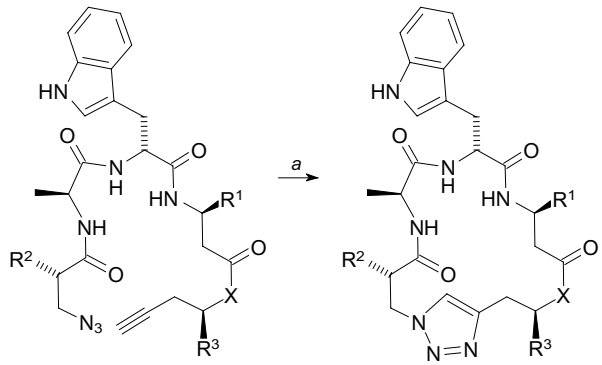
The reaction was performed in a THF – acetonitrile solvent mixture using copper(I) iodide, 2,6-lutidine and

Structure 23



23 [(+)-jasplakinolide]

Scheme 17



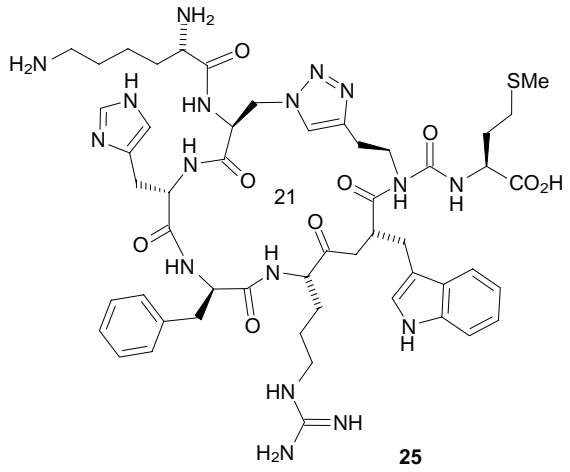
24 (57% – 92%)

(*a*) 1) CuI (0.1 – 0.5 equiv.), Pr^i_2NEt; 2) Bu^n_4NF, 2,6-lutidine, MeCN – THF (1 mM); R^1 = H, 4-$Bu^tMe_2SiOC_6H_4$; R^2, R^3 = H, Me; X = O, NH

Hünig's base as the catalyst (Scheme 17).[76] The macrocyclization was not accompanied by the formation of dimeric structures as by-products and gave target products **24** in yields quite high for such transformations.

Apart from the intramolecular head-to-tail cyclization reactions considered above, reactions involving side-chain moieties of peptides bearing azide and alkyne groups were described in the literature. This type of reactions was performed for the first time in 2004 by Meldal and co-workers.[77] The authors performed the solid-phase synthesis of cyclic peptidomimetic **25**, which contains a peptide moiety that is present in α-melanotropin (a hormone that increases skin pigmentation and protects skin against ultraviolet-induced damage).

Structure 25

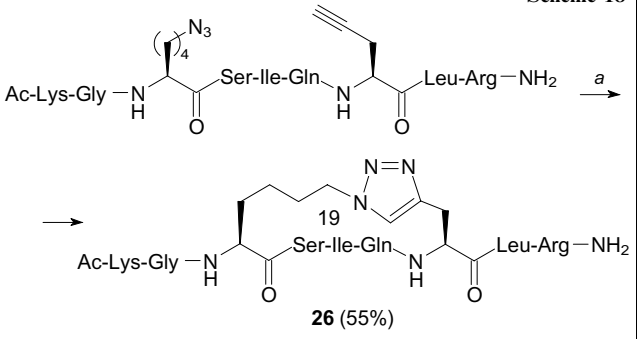


25

Intramolecular side chain-to-side chain cyclization was employed in the synthesis of macrocyclic peptidomimetic **26**, the cyclic part of which has an α-helix-like structure (Scheme 18).[50]

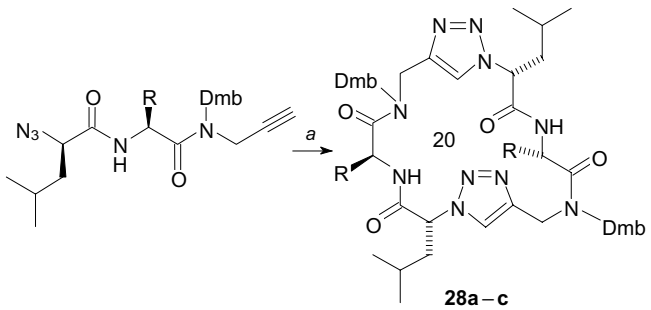
It should be emphasized that in this case, an attempt to use classical click chemistry conditions, such as the CuI – Pr^i_2NEt – 2,6-lutidine – MeCN and CuBr – DBU – PhMe systems, was not successful and did not give the target product. These reactions were performed in the

Scheme 18



(*a*) $CuSO_4 \cdot 5H_2O$ (4 equiv.), ascorbic acid, $Bu^tOH - H_2O$ (1 : 2) (0.9 mM)

Scheme 20

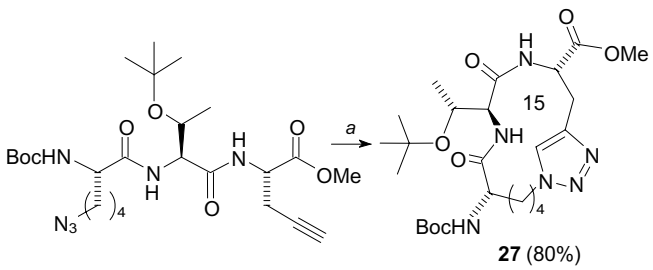


(*a*) CuI (2 equiv.), Pr^i_2NEt, 2,6-lutidine, MeCN (1 mM), argon, 20 °C; R = Bn (**a**, 77% yield), $(CH_2)_2Boc$ (**b**, 85%), $(CH_2)_2NHBoc$ (**c**, 67%)

$Bu^tOH - H_2O$ binary system (1 : 2) in the presence of ascorbic acid and $CuSO_4$ as the catalyst. More recently, Scrima *et al.*[78] employed this method to synthesize a series of 1,4-disubstituted 1,2,3-triazole-containing cyclic peptidomimetics and studied the influence of the linker length and the location of the triazole moiety on the conformational characteristics of the molecules in solution.

The development of anticancer drugs based on cyclic peptides has attracted ever-growing interest. Chen *et al.*[79] endeavoured to prepare cyclic 15-membered pseudopeptide **27** as the basis for the subsequent synthesis of an anticancer drug. This peptidomimetic, which was cyclized by the side chain-to-side chain click reaction, shows inhibitory activity against some molecular targets responsible for tumour growth (Scheme 19).

Scheme 19



(*a*) $CuSO_4$, sodium ascorbate, $Bu^tOH - H_2O$

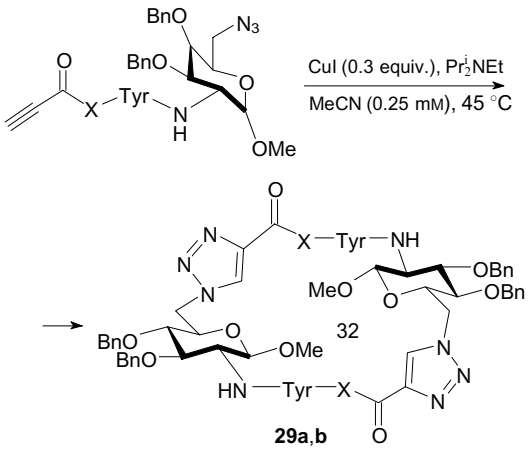
3.2.2. Click macrocyclization accompanied by oligomerization

The azide – alkyne cycloaddition often affords, apart from monomers, cyclic di- and trimeric by-products. Punna *et al.*[48] suggested that dimeric products are formed due to a specific coordination of two alkynes prior to cycloaddition and(or) the binding of the catalyst to the peptide chain. These hypotheses were later rejected, and the hydrogen bonding between peptide chains was identified as the key factor that affects cyclodimerization of grafted peptides.[80]

Van Maarseveen *et al.*[81] examined the possibility of preparing C_2-symmetric cyclic pseudopeptides **28a – c** by dimerization of the appropriate linear precursor (Scheme 20). Horne *et al.*[82] demonstrated that these molecules can self-assemble to form peptide nanotubes. The reactions were performed using the CuI (2 equiv.) – Pr^i_2NEt – 2,6-lutidine – MeCN (1 mM) catalytic system. This approach proved to be much more efficient than macrolactamization of a linear precursor bearing both triazole moieties due to the selective formation of target dimers in rather high yields.

An efficient method for the synthesis of macrocyclic glycopeptides **29a**,**b** (Scheme 21) was developed based on an intermolecular click reaction. These hybrid dimers containing amino acids and carbohydrates can be employed as artificial receptors for small biomolecules. The CuI (0.3 equiv.) – Pr^i_2NEt – MeCN system proved to be the conditions of choice for click macrocyclization. The reactions performed at elevated temperature for several days gave cyclic products **29a**,**b** in moderate yields.[83]

Scheme 21



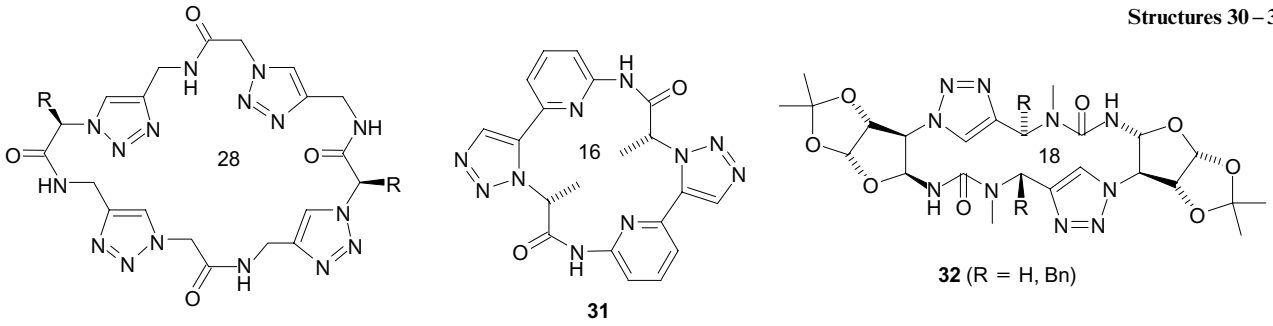
X = Tyr (**a**, 64% yield), Arg(Mtr) (**b**, 33%);
Mtr is 4-methoxy-2,3,6-trimethylbenzenesulfonyl

The intramolecular click macrocyclization was also applied to prepare other peptidomimetics bearing several triazole moieties, *e.g.*, compounds **30 – 32**.[84 – 86]

3.3. Ru^{II}-Catalyzed macrocyclization giving 1,5-disubstituted triazole derivatives

1,5-Disubstituted triazoles can be synthesized regioselectively using ruthenium(II)-based catalysts. (In this Section, examples of CuAAC are given for comparison with RuAAC.) Like 1,4-disubstituted triazoles, which are geometric analogues of the *trans*-amide bond, 1,5-disubstituted

Structures 30 – 32



30 (R = H, CH_2NH_2)

31

32 (R = H, Bn)

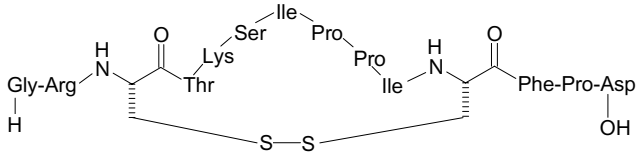
triazoles can serve as isosteres of the *cis*-amide bond. The incorporation of 1,5-disubstituted triazoles into cyclic peptides and peptidomimetics affects the molecular conformation by inducing the peptide chain to adopt a turn.[87] In a number of studies, cyclic pseudopeptides were synthesized *via* both copper- and ruthenium-catalyzed azide – alkyne cycloaddition.

A great diversity of compounds of the same structural class is a necessary condition for the development of new drugs. The lack of a general method for the synthesis of macrocyclic peptidomimetics makes the synthesis of large libraries of such derivatives impossible and, consequently, hinders the discovery of drugs on their basis.

Isidro-Llobet *et al.*[88,89] proposed a strategy for the synthesis of a series of cyclic peptides and their derivatives by assembling different readily available building blocks in varying combinations. More than 200 compounds were synthesized by combinatorial chemistry varying the chain length, ways of assembly and methods of macrocyclization. The construction of macrocycles from linear molecules was achieved by click chemistry through the formation of 1,4- and 1,5-disubstituted triazoles. The former derivatives were synthesized by refluxing the reactants in THF (1.2 mM) in the presence of the CuI (2 equiv.) – Pr^i_2NEt catalytic system. A procedure was developed for the efficient synthesis of 1,5-disubstituted triazole macrocyclic structures using 10% $[Cp^*RuCl]_4$ and toluene (1.2 mM) as the solvent. Compounds **33** and **34**, which were synthesized by the cited authors, are shown as an example in Scheme 22. In some cases, the product yields are nearly quantitative, which is quite unusual for macrocyclization reactions.

Both 1,4- and 1,5-disubstituted triazoles can serve as good substitutes for disulfide bridges in cyclic peptides. This modification can improve their properties because the S – S bond is unstable and susceptible to oxidation and reduction. The possibility of this replacement was shown for the protein SFTI-1 (**35**) — the sunflower trypsin inhibitor.[90]

Structure 35



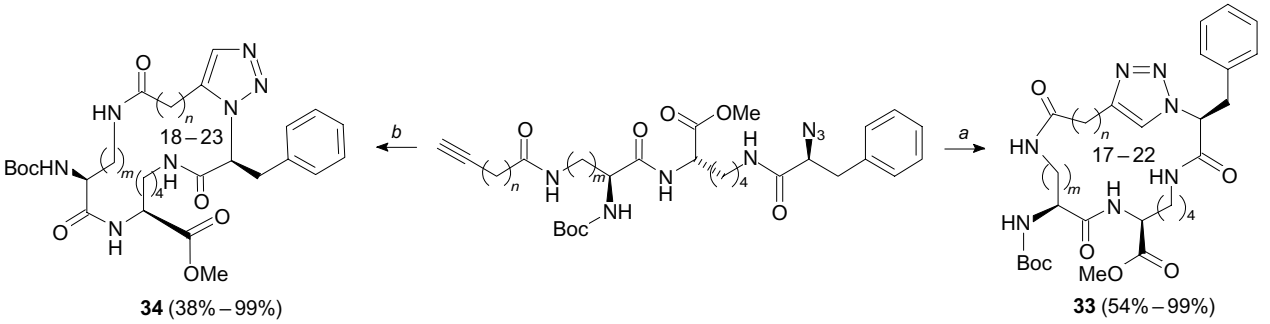
35 (SFTI-1)

Scheme 23 shows the structures of products **36** and **37** prepared by click chemistry. The macrocyclization giving 1,4-triazole was performed in the presence of the $CuSO_4$ – Pr^i_2NEt – sodium ascorbate – H_2O system. The [Cp*Ru(cod)Cl] – THF mixture was the system of choice for the click reaction yielding the 1,5-disubstituted moiety. Subsequently, triazole analogues **36** and **37** were examined for binding to trypsin and matriptase.[91] The inhibition constants of these triazole macrocycles proved to be higher compared to the natural peptide SFTI-1, which attests to their high inhibitory activity.

C_3-Symmetric macrocyclic pseudopeptides **38a**,**b** containing pyridine and triazole rings were synthesized.[92,93] Due to the converging arrangement of nitrogen atoms, these molecules exhibit anion receptor properties. Click macrocyclization was also performed using Ru or Cu catalysts (Scheme 24). However, the yield of 24-membered macrocycle **38b** (75%) was much higher than that of 27-membered macrocycle **38a** (10%).

Cyclic peptidomimetics are widespread in nature. There are both simple and very complex molecules, which are

Scheme 22

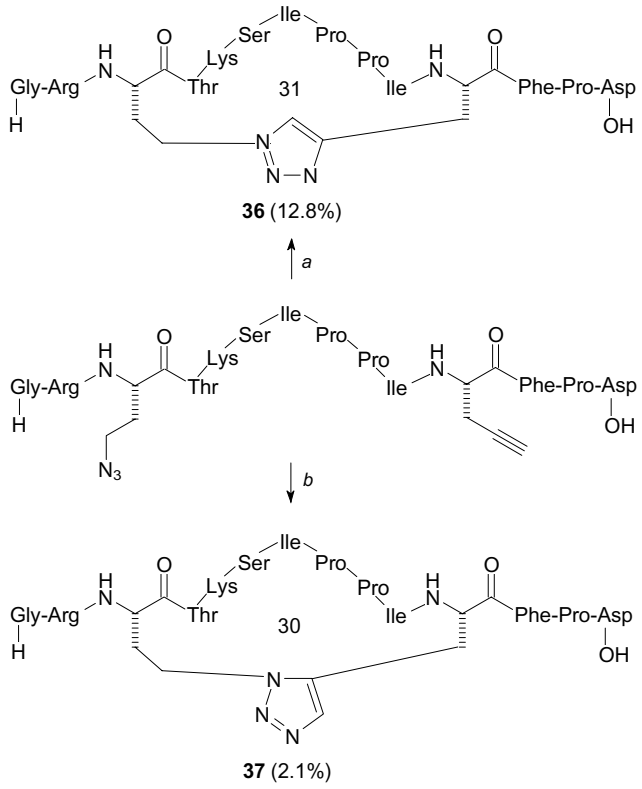


34 (38% – 99%)

33 (54% – 99%)

(*a*) CuI (2 equiv.), Pr^i_2NEt, THF (1.2 mM), Δ, 18 h; (*b*) $[Cp^*RuCl]_4$ (0.1 equiv.), PhMe (1.2 mM), Δ, 18 h; n = 1 – 4, m = 1 – 3

Scheme 23



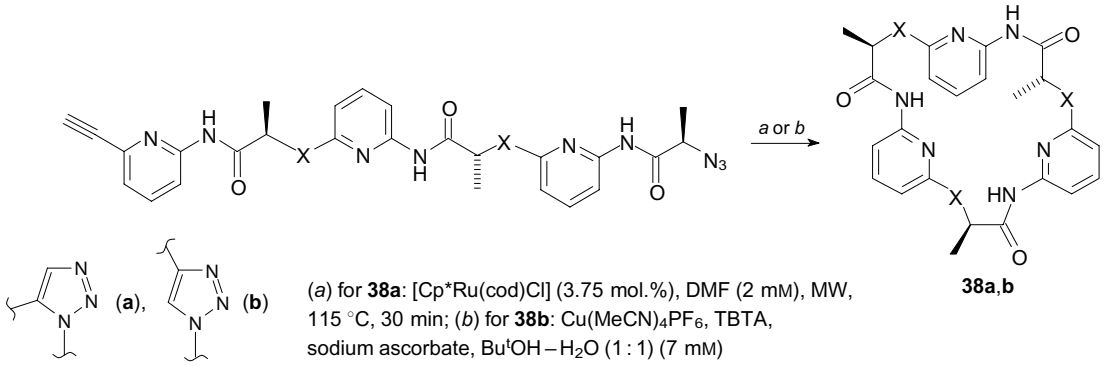
(*a*) $CuSO_4$ (1 equiv.), Pr^i_2NEt, sodium ascorbate, H_2O (1 mg ml^{-1}), 20 °C, 12 h; (*b*) [Cp*Ru(cod)Cl] (0.2 equiv.), THF, MW, 60 °C, 5 h; MW is microwave irradiation

difficult to synthesize. Among these compounds is the tricyclic glycopeptide antibiotic vancomycin isolated from the strain *Amycolatopsis orientalis*.[94] A procedure was proposed for the synthesis of vancomycin derivatives (*e.g.*, compound **39**) bearing 1,5-disubstituted triazoles instead of aryl groups in the bicyclic moiety (Scheme 25).[95] Macrocyclization was performed in the THF – MeOH (5 mM) mixture at 55 °C in the presence of 0.3 equiv. of the catalyst $[Cp^*RuCl]_4$. In this step, the yield of product **39** was up to 40%. Previously, Zhang *et al.*[96] studied this modification in the presence of Cu catalysts; however, this cyclization produced mainly cyclic dimers.

4. Synthesis of macrocyclic peptidomimetics *via* a combination of the Ugi reaction and click chemistry

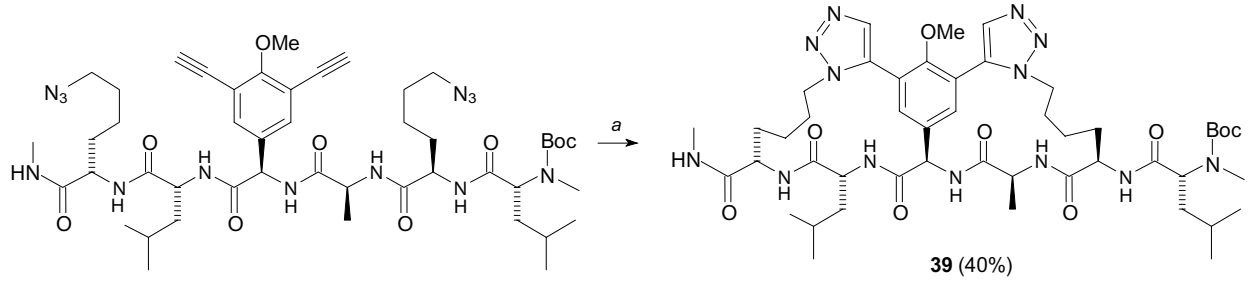
The Ugi reaction provides an efficient approach to the construction of a linear peptide.[97–99] This is a multicomponent reaction, which involves the reaction of amine, aldehyde (or ketone), isonitrile and carboxylic acid giving amino acid amide (peptide) (Fig. 6 *a*). Linear peptides bearing both azide and ethynyl groups can be used for subsequent macrocyclization (see Fig. 6 *b*). Each participant of the Ugi reaction can contain a peptide moiety (the central figure in the structures in Fig. 6 *c*), the azide group or the terminal triple bond. Precursors for click cyclization can be synthesized in twelve different ways by varying positions of the azide and ethynyl groups in the starting compounds (carboxylic acid, amine, carbonyl compound, isocyanide). Figure 6 *c* shows possible combinations of substrates for the Ugi reaction, which produces peptide molecules bearing two reactive groups necessary for subsequent click cyclization.

Scheme 24



(*a*) for **38a**: [Cp*Ru(cod)Cl] (3.75 mol.%), DMF (2 mM), MW, 115 °C, 30 min; (*b*) for **38b**: $Cu(MeCN)_4PF_6$, TBTA, sodium ascorbate, $Bu^tOH – H_2O$ (1 : 1) (7 mM)

Scheme 25



(*a*) $[Cp^*RuCl]_4$ (0.3 equiv.),THF – MeOH (19 : 1) (5 mM), 50 °C, 24 h

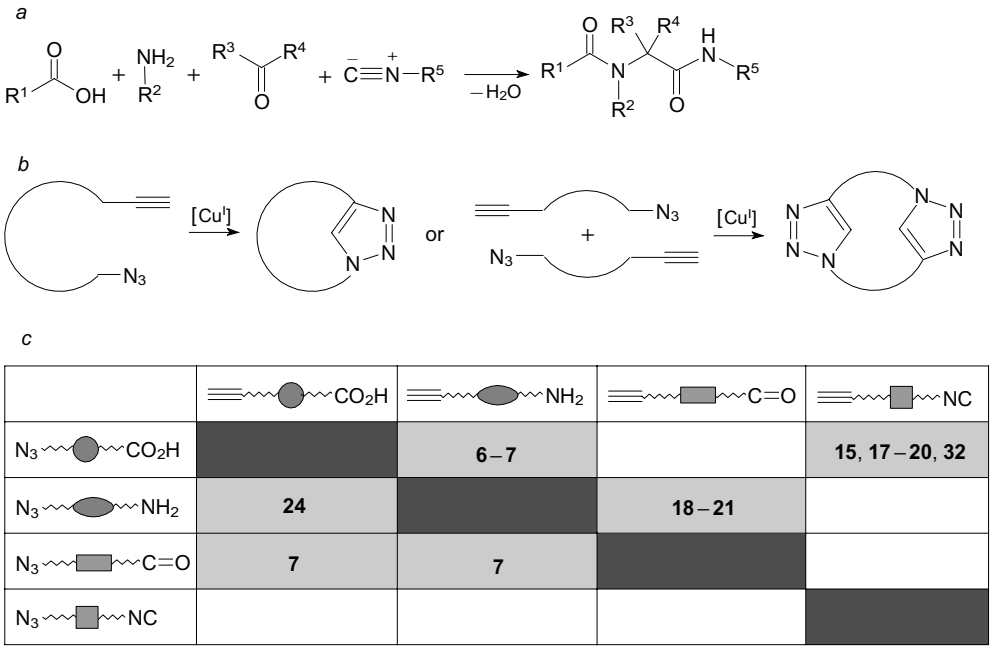


Figure 6. General schemes of the Ugi reaction (a) and intramolecular click cyclization (*b*) and possible combinations of reactants for the synthesis of substrates for the click reaction (*c*).
Black cells indicate impossible combinations of reagents; white cells mean a lack of literature data; the figures in the grey cells indicate the sizes of the rings formed.

Some versions of the synthesis by the Ugi reaction of linear peptidomimetics containing alkyne and azide moieties are described in the literature (see Fig. 6 *c*). The sizes of the resulting rings vary from 6 – 7 to 24 atoms in the intramolecular reaction and to 32 atoms in the intermolecular reaction. Therefore, the Ugi – click strategy can also be applied to synthesize macrocycles.

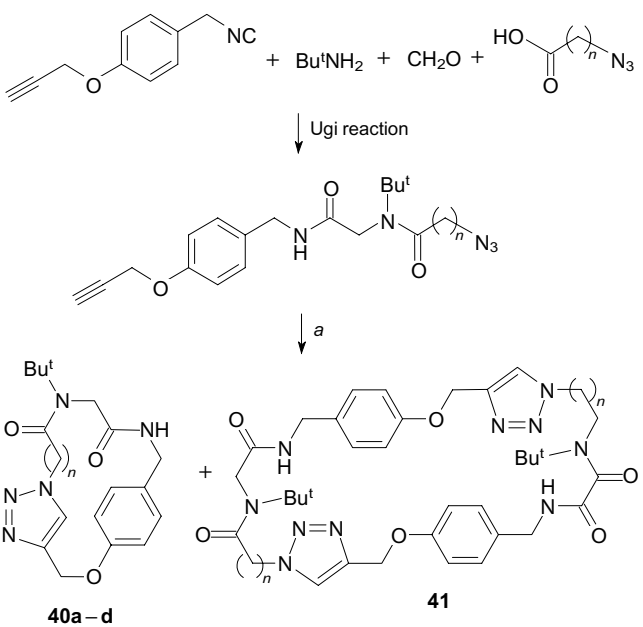
An efficient approach to macrocyclization of linear peptide derivatives was developed based on CuAAC.[100] Linear molecules synthesized by the Ugi reaction contained an alkyne group in the isocyanide moiety and an azide group in the acidic portion of the molecule. The final macrocycles were synthesized by a continuous multistep strategy in a flow microreactor made of a copper coil at elevated temperature and pressure without the isolation of intermediates (Scheme 26). Depending on the structure of the linear chain, the reaction affords mono- (**40a – d**) or dimeric macrocycles (**41**).

Linear peptides were synthesized based on propargylamine and azide-containing acids.† Evidently, the insertion of reactive groups by this route does not allow the formation of macrocycles because the azide and alkyne groups are at a moderate distance from each other; however, this approach offers promise for the construction of six- and seven-membered triazole-containing heterocycles. This procedure was also employed to prepare compounds **42a**,**b** with the dihydrotriazolopyrazine or triazolodiazepine scaffold (Scheme 27).

Dihydrotriazolopyrazinones and benzotriazolodiazepines can also be synthesized *via* the construction of linear

† Although these compounds are not macrocycles, they are considered in the review because such examples are scarce.

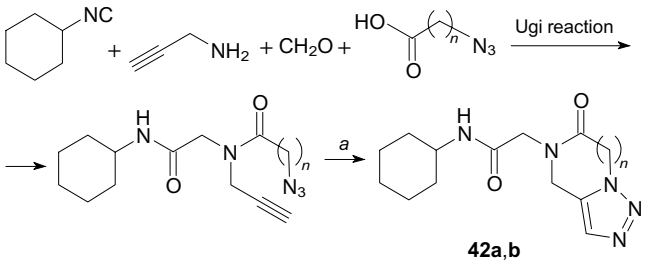
Scheme 26



(*a*) Cu coil, MeOH – MeCN (1 : 1) (2 mM, continuous flow), 140 °C, 10 bar, 25 min

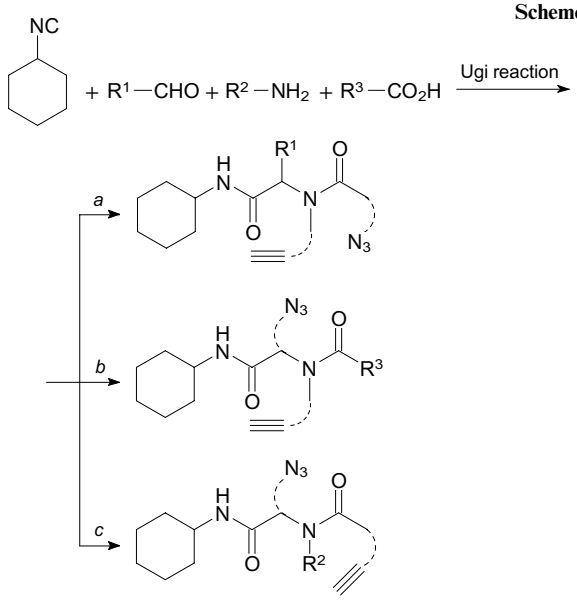
n	Product	Ring size	Yield (%)
1	**41**	32	74
2	**40a**	17	81
3	**40b**	18	81
4	**40c**	19	80
5	**40d**	20	83

Scheme 27



(*a*) Cu coil, MeOH–MeCN (1 : 1) (2 mM, continuous flow), 140 °C, 10 bar, 25 min; n = 1 (**a**, 86% yield), 2 (**b**, 91%)

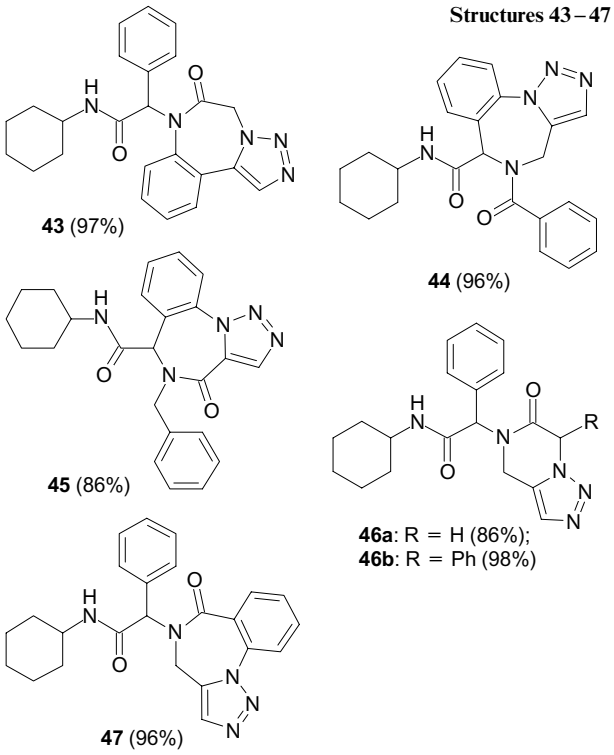
Scheme 28



molecules by other methods (Scheme 28).[101, 102] The structural diversity of the products was achieved by varying the position of the azide group in carboxylic acid (path *a*) and in aldehyde (paths *b*, *c*), as well as the position of the terminal triple bond in amine (paths *a*, *b*) or in carboxylic acid (path *c*).

Due to the ease of formation of six- or seven-membered rings, azide–alkyne cycloaddition proceeds even in the absence of catalysts to give products in high yields. For instance, the heating of the appropriate intermediates (see

Structures 43–47



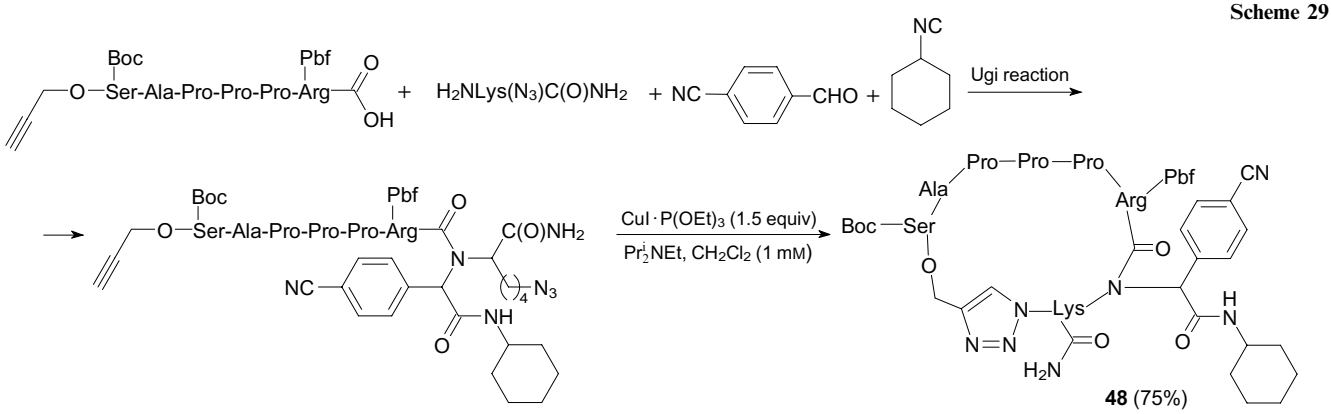
Scheme 28) under reflux in benzene (4–18 h) resulted in the formation of heterocycles **43**–**47**.

The Ugi–click strategy was employed to synthesize cyclic hexapeptide **48** with anticancer activity (Scheme 29).[103] This procedure was applied as an alternative to the classical macrolactamization because the latter did not give the target product. Besides, the presence of the triazole moiety in the cyclic structure enhances biological activity of the molecule. The click reaction was performed using the CuI–$P(OEt)_3$ (1.5 equiv.)–Pr^i_2NEt–CH_2Cl_2 system.

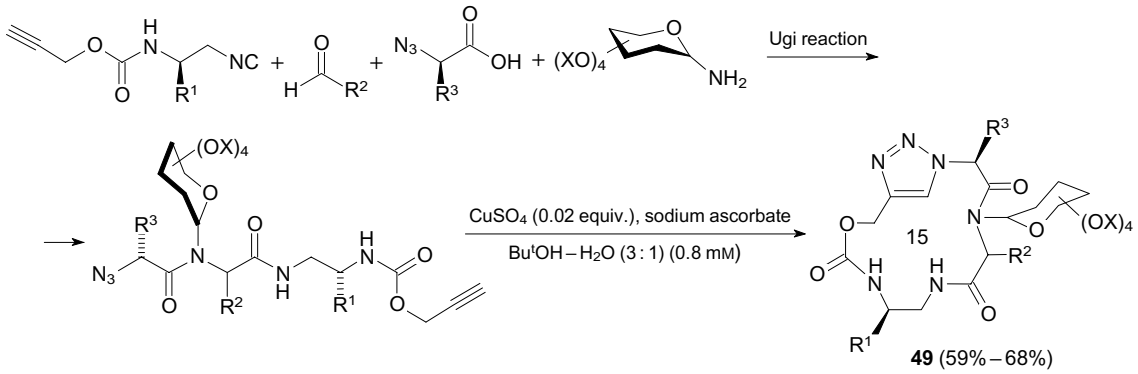
The Ugi reaction allows the preparation of complex peptide-like molecules starting from simple compounds. Necessary functional groups (for example, saccharides) can easily be introduced into the substrates in order to be finally incorporated into linear or cyclic peptidomimetics.

Glycopeptides are widespread in nature and have attracted considerable research interest.[104] Due to the presence of saccharide moieties, cyclic glycopeptides have high membrane-penetrating ability and metabolic stabil-

Scheme 29



Scheme 30



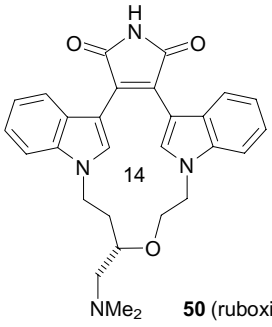
X = Ac, Bn; R^1 = H, Me, Pr^i, Bu^i, Bu^s, Bn; R^2 = H, Pr^i, Bu^i, Bn, Ph; R^3 = H, Me, Pr^i, Bu^i, Bn

ity.[105] A series of cyclic glycopeptides **49** were synthesized by successive Ugi–click transformations (Scheme 30).[106]

Azide and alkyne groups were introduced into a linear precursor using acidic and isonitrile components, respectively. In this case, both these functional groups necessary for the click reaction are most remote from each other, resulting in the formation of macrocycles with the largest number of atoms in the ring.

The Ugi–click strategy was employed in the synthesis of tryptamine-containing macrocycles.[107] The indole moiety is an important structural unit of many natural and synthetic molecules, including macrocycles. For instance, ruboxistaurin (**50**) is used for the treatment of diabetic retinopathy and macular oedema.[108]

Structure 50

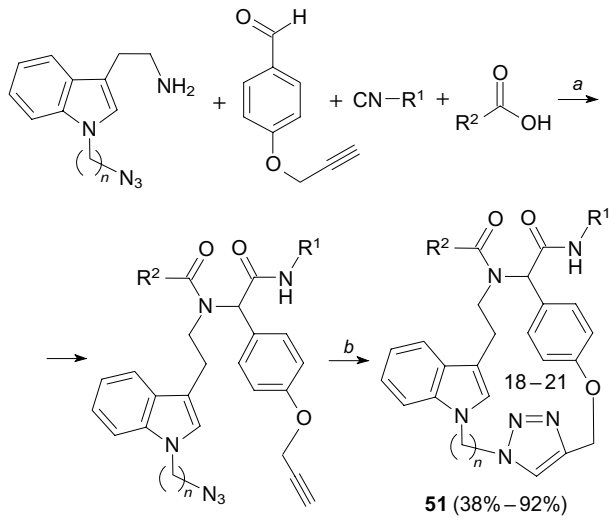


50 (ruboxistaurin)

Chavez-Acevedo and Miranda [107] synthesized a series of products by the Ugi reaction using *p*-propargyloxybenzaldehyde, azide derivatives of tryptamine, different isonitriles and carboxylic acids. The subsequent macrocyclization *via* click chemistry produced compounds **51** (16 examples) in moderate to high yields (Scheme 31).

As can be seen from the data on the application of the Ugi–click strategy in the synthesis of macrocyclic compounds, a combination based on azidoisonitriles and ethynyl-containing carboxylic acids holds the most promise.[109–111] This combination enables the arrangement of the triple bond and the azide group far apart from each other, thereby making it possible to prepare medium-size rings and macrocycles, which are of most applied and theoretical interest.

Scheme 31



(*a*) MeOH (1 mM), 20 °C, 48 h; (*b*) CuBr (0.4 equiv.), DBU, PhMe (5 mM), MW, 110 °C;
R^1 = Bu^t, cyclo-C_6H_{11}, 2,6-$Me_2C_6H_3$; R^2 = Me, Ph; *n* = 2–5

* * *

Data summarized in this review show that the click reaction is of great value as an alternative method for macrocyclization of peptides and peptidomimetics. Over the past years, considerable progress has been made in the application of azide–alkyne cycloaddition in the synthesis of macrocyclic peptides, and dozens of papers on this issue have been published. This method has two important advantages. First, this method allows the more efficient synthesis of peptide macrocycles bearing a triazole ring, avoiding the problem of racemization. Second, many macrocycles exhibit biological activity comparable to or even higher than that of natural analogues. The synthesis of macrocyclic compounds by a combination of the Ugi reaction and CuAAC is a rather new field of research, with less than a dozen publications to date. The application of this strategy opens up a route to the fast and efficient synthesis of macrocyclic peptide derivatives, including biologically active products. This approach holds considerable promise in the synthesis of macrocyclic peptides containing a triazole ring.

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