Amino Acids
DOI 10.1007/s00726-013-1663-1

1 ORIGINAL ARTICLE

2 Design and synthesis of anti-cancer cyclopeptides containing 3 triazole skeleton

4 **Fatemeh Tahoori · Saeed Balalaie · Reza Sheikhnejad ·**
5 **Mahnaz Sadjadi · Parvin Boloori**

REVISED PROOF

6 Received: 4 November 2013 / Accepted: 29 December 2013
7 © Springer-Verlag Wien 2014

8 **Abstract** We describe the design and synthesis of some 9 hypothetical heptapeptides specifically to overcome the 10 neoplastic activity of ras oncogene and their anti-cancer 11 activities were studied. To improve the anti-cancer activity 12 of the synthesized peptides, their structure modifications 13 were done based on a sequential Ugi/Huisgen 1,3-Dipolar 14 cyclization reaction. The cyclopeptides which contained 15 triazole skeleton showed significant anti-cancer activity 16 against cancer cells with mutated ras oncogene such as 17 A549, PC3 and C26 cells. This study clearly shows the 18 importance of triazole skeleton in biological activity of the 19 peptides. It might be possible to overcome the difficulties 20 involved in making complex peptides by employing this 21 22 elegant chemistry.

23 **Keywords** Ugi ligation · Ligation of peptides · Anti- 24 cancer activity · Cyclopeptides · Click reaction · Huisgen 25 1,3-Dipolar reaction

26 Introduction

27 Several monoclonal antibodies such as Rituximab (anti- 28 CD20 antibody) and Herceptin (anti-HER-2 antibody) have 29 been approved for the treatment of some cancers. The 30 efficacy of this cancer immunotherapy is, however, limited 31 by its large size and its nonspecific binding to the reticu- 32 loendothelial system that causes many undesirable side 33 effects (Aina et al. 2007). Furthermore, the drug research 34 and development has become very expensive and the 35 number of approved drugs has been declining in recent 36 years. Therefore, the demands for alternative approaches 37 are very high. This has contributed to the revival of pep- 38 tides as potential therapeutic drugs. A large number of 39 peptide-based drugs are now being marketed because new 40 synthetic strategies have been developed in recent years 41 (Vlieghe et al. 2010).

42 One classical strategy used in drug design is based on 43 the structure of receptor-binding pocket, called "rational 44 structure-based design" (Shoichet et al. 1993; Von Itzstein 45 et al. 1993). Most peptide drugs are designed this way. 46 Here we have used a novel strategy based on DNA–protein 47 binding criteria to design anti-cancer drugs. We focused 48 our interest on finding specific DNA–protein binding sites 49 along the promoter elements of ras oncogene. The precise 50 interactions between amino acid motifs of our designed 51 peptides and ras-specific regulatory sites within the CpG 52 islands might interfere with ras activity at transcriptional 53 level. The most active peptide is then selected based on its 54 in vitro anti-cancer activity to optimize its pharmaceutical 55 value by means of different chemical approaches. One such 56 approach would be the reduction of conformational space 57 by cyclization.

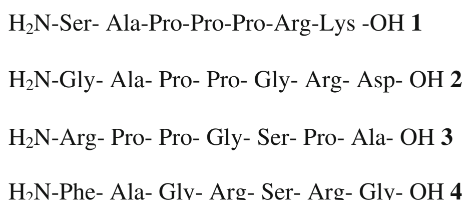
58 Several hypothetical heptapeptides were designed based 59 on DNA–protein binding criteria known for regulation of 60 gene expression at transcriptional level. These peptides 61 designed to perhaps suppress ras oncogenic activities in 62 human cancer cells. The designed peptides **1–4** were tested 63 for their anti-cancer activities against A549, human lung

A1 **Electronic supplementary material** The online version of this A2 article (doi:10.1007/s00726-013-1663-1) contains supplementary A3 material, which is available to authorized users.

A4 F. Tahoori · S. Balalaie (✉)
A5 Peptide Chemistry Research Center, K. N. Toosi University
A6 of Technology, P. O. Box 15875-4416, Tehran, Iran
A7 e-mail: balalaie@yahoo.com

A8 R. Sheikhnejad · M. Sadjadi · P. Boloori
A9 Tofigh Daru Res. & Eng. Co., 61st. km 18 Karaj Highway,
A10 37515-375 Tehran, Iran

Journal : Large 726 | Dispatch : 9-1-2014 | Pages : 14
Article No. : 1663 | ☐ LE | ☐ TYPESET
MS Code : AMAC-D-13-00527 | ☑ CP | ☑ DISK



Scheme 1 Compositions of the designed heptapeptides **1–4**

cancer cells in vitro. The most active compound **1** is proline-rich peptide (Ball et al. 2005) selected to further improve its pharmaceutical potential with some specific chemical modifications to create a cyclic peptide (Scheme 1).

Cyclic peptides are a unique class of compounds that have made great contributions to the treatment of certain diseases such as cancer. Penicillin, vancomycin, cyclosporin, and echinocandins are well-known cyclic peptides. Cilengitide is also a cyclic pentapeptide currently in clinical trial for brain cancer, glioblastomas, and some other cancers (Katsara et al. 2006; Mas-Moruno et al. 2010; Boger 2001; Nicolaou et al. 1999; Rao et al. 1995; Chatterjee et al. 2005). Cyclic peptides, compared to linear peptides, have been considered to have greater potential as therapeutic agents. This may be due to their increased chemical stability, receptor selectivity, as well as improved pharmaco-dynamic properties. Considering these facts, we decided to use one of the known cyclization methods to prepare a unique cyclic heptapeptide. A reaction that would seem ideal for conjugation of peptides and oligonucleotides, due to the compatibility with many other functional groups, is the copper(I) catalyzed 1,3-Dipolar cycloaddition between an azide and an alkyne, commonly referred to as click chemistry (Rostovtsev et al. 2002; Kolb et al. 2001; Moses and Moorhouse 2007; Wu and Fokin 2007; Kolb and Sharpless 2003; Meldal and Tornoe 2008; Tornoe et al. 2002). Moreover, triazole-modified peptidomimetics have been shown as assembling protein-like oligomers and nonpeptidic protein-mimetic foldamers (Angelo and Arora 2005, 2007). Therefore, triazole-modified peptidomimetics have gained considerable attention for designing biological effectors or foldamers (Horne et al. 2004; Kuijpers et al. 2004; Cantel et al. 2008). The synthesis of 1,2,3-triazoles has grown in importance in medicinal (Chabre and Roy 2008; Colombo and Peretto 2008; Hanselmann et al. 2010; Moumne et al. 2010), material (Li et al. 2005; Rozkiewicz et al. 2006; Wyszogrodzka and Haag 2008; Gadzikwa et al. 2009; Golas and Matyjaszewski 2010; Bronisz 2005; Yue et al. 2007; Fazio et al. 2008; Fletcher et al. 2008; Hua and Flood 2010; Rawal et al. 2010), and biological researches (Hahn and Muir 2005; Heal et al. 2008; Ahsanullah et al. 2009; Schneider 2010; Chemama et al. 2009; Nahrwold et al. 2010; Michaels et al. 2010; Mamidyala and Finn 2010). Furthermore, a number of these compounds show a broad spectrum of biological activities, displaying, for example, antibacterial (Genin et al. 2000), herbicidal, fungicidal (Wamhoff 1984), antiallergic (Buckle et al. 1986), or anti-HIV (Alvarez et al. 1994) properties. Recently, 1,2,3-triazoles have also been used as catalysts and ligands in transition metal-based catalyst systems (Chan et al. 2004; Liu et al. 2005; Detz et al. 2006; Colasson et al. 2007; Beyer et al. 2009; Hein et al. 2009; Duan et al. 2009; Mager and Zeiler 2010). Since peptide **1** showed the best anti-cancer activity, we decided to modify its structure using functionalized amino acids which could form triazole scaffold.

Multicomponent reactions (MCR) have become important tools in the preparation of structurally diverse chemical libraries of drug-like polyfunctional compounds. However, to ensure sufficient molecular diversity and complexity of new chemical entities, there is a continuous need for novel reactions with high efficiency and selectivity in novel reaction media (Dömling 2005, 2006; Slobbe et al. 2012; Ruijter et al. 2011; Dömling et al. 2012; Tietze et al. 2006; Tietze and Hauner 2000). We intend to use the Ugi-4CR to construct products with further functional groups which are prone to additional ring closure reactions. This strategy allows us to prepare in a very economic and ecologic way complex systems (Bararjanian et al. 2010, 2011; Balalaie et al. 2011, 2012). Recently, we showed that the Ugi-4CR is an efficient approach for the synthesis of some novel GnRH analogs with better anti-cancer activity (Arabanian et al. 2009; Saleh-Abady et al. 2010). The 2D-NMR spectroscopic data showed that this reaction affects the structure of molecule on folding (Tahoori et al. 2010).

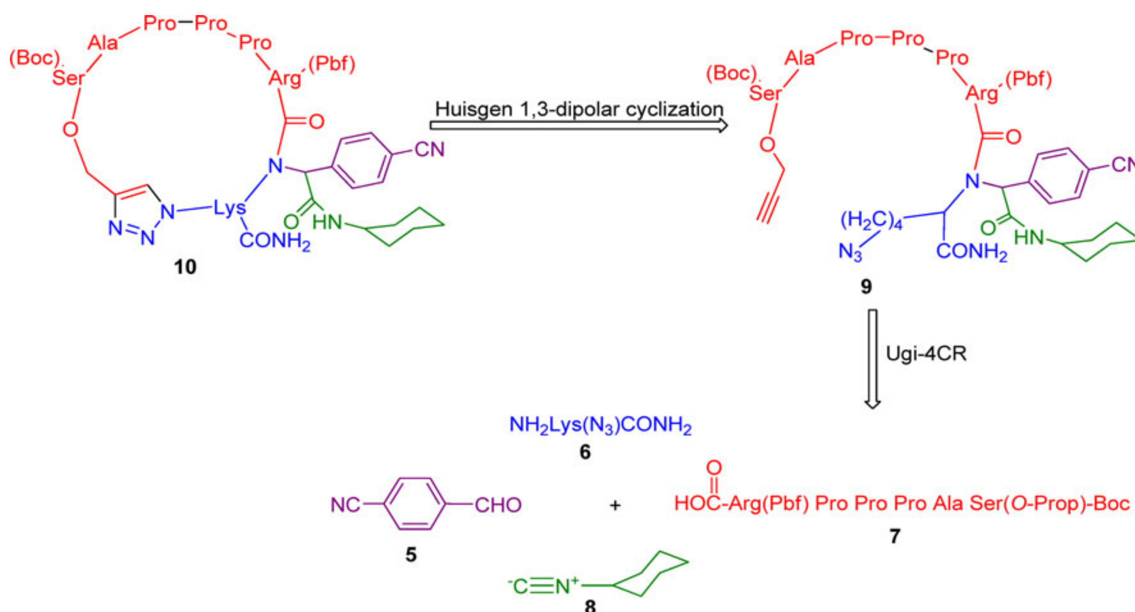
We report the design and synthesis of novel cyclopeptides through the Ugi ligation/click reaction to construct cyclopeptides which have a triazole moiety and also lipophilic moieties (Scheme 2).

Results and discussion

Reduction of conformational flexibility is important to increase the affinity of a peptide for its natural receptor. The first convenient approach to achieve this goal is head-to-tail cyclization. To achieve this goal, chemical modification of starting materials is important for many applications in biology and biotechnology. In order to synthesize cyclopeptides, different strategies were studied. Applying the well-known click chemistry is a known approach to synthesize cyclopeptides with triazole moiety. Functionalizing the scaffold with an alkyne moiety, and also an azide group, to form triazole skeleton is an approach to the synthesis of cyclopeptides.

REVISED PROOF

Journal : Large 726 | Dispatch : 9-1-2014 | Pages : 14
Article No. : 1663 | LE | TYPESET
MS Code : AMAC-D-13-00527 | CP | DISK



Scheme 2 Retrosynthetic approach for the synthesis of cyclopeptide **10** through sequential Ugi ligation/Huisgen 1,3-Dipolar reaction

The click reactions have the potential to be further fortified when combined with multicomponent reactions. The idea of using MCRs followed by a Huisgen [3⇓2] copper-catalyzed reaction was investigated and a number of pharmaceutically relevant heterocyclic compounds were synthesized via classical multicomponent reactions combined with click chemistry in separate steps such as sequential Ugi/intermolecular alkyne–azide cycloaddition (IAAC) (Ramachary and Barbas 2004; Akritopoulou-Zanze et al. 2004).

The sequence of Ugi isocyanide multicomponent reaction, followed by post-condensation transformations, constitutes an extremely powerful synthetic tool for the preparation of structurally diverse complex molecules, especially heterocyclic compounds (Orru and Ruijter 2010; Aravind et al. 2011; De Graaff et al. 2012). Ultimately, this one-pot sequential combination of multi-catalysis and multicomponent approach should reduce the cost and waste associated with pharmaceutical synthesis.

The wide variation in starting materials available for IMCRs (isocyanide multicomponent reactions) opens up versatile opportunities for the synthesis of compound libraries. The significant potential of isocyanides for the development of multicomponent reactions is a result of their ability to take part in diverse bond formation processes, their functional group tolerance, and the high levels of chemo-, regio-, and stereoselectivity often observed. According to our design, the copper(I)-catalyzed 1,3-Dipolar cycloaddition between an azide and an alkyne was used. The first moiety to be inserted in the Huisgen reaction is an alkyne group. The alkyne moiety was added to the side chain of serine and the other moiety which was prepared for click chemistry is the amino acid which contains the azide group.

Since peptide **1** showed better anti-cancer activity compared to other heptapeptides, compound **1** was selected as a druggable molecule to be chemically modified. At first, heptapeptide **1** was divided into a hexapeptide **7** which contained an alkyne moiety and an amidated C-terminal Lysine **6** which contains an azide moiety.

The procedure for the synthesis of Fmoc-Lys(N_3)-$CONH_2$ **6** is shown in Scheme 3 in which the amine group in the side chain was converted to azide moiety. To access this molecule, Fmoc-Lys(Boc)-OH was selected as starting material and the synthesis was done in four steps: (a) Amidation of terminal carboxylic acid was done using ammonium chloride in the presence of TBTU as coupling reagent and NMM as base to form compound **12**. (b) The Boc-protecting group was removed with trifluoroacetic acid **13**. (c) The conversion of side-chain amine group to azide could be done using imidazoliumsulfonylazide **14** to obtain compound **15**. (d) Fmoc deprotection using 25 % piperidine afforded H_2N-Lys(N_3)-$CONH_2$ **6**.

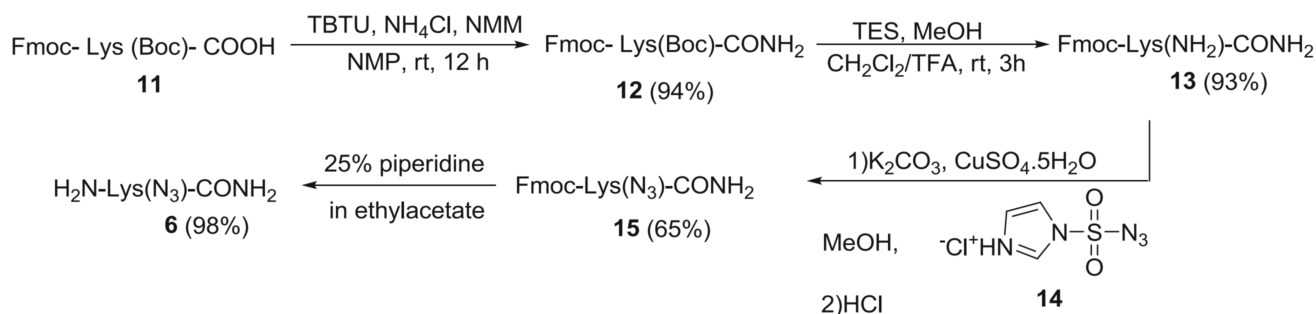
The imidazoliumsulfonylazide **14** was synthesized according to the reported method (Johnasson and Pedersen 2012).

As shown in Scheme 4, Boc-Ser(*O*-Prop)-OH was prepared through the reaction of Boc-Ser-OH and sodium hydride followed by propargyl bromide reaction which led to the desired Boc(*O*-Prop)-OH with 72 % yield.

The linear peptide analogue Boc-Ser(*O*-Prop)-Ala-Pro-Pro–Pro-Arg(pbf)-OH was synthesized by standard solid-phase peptide synthesis (SPPS) strategy on resin. It should be noted that the initial experiments with Fmoc-Arg(Pbf)-

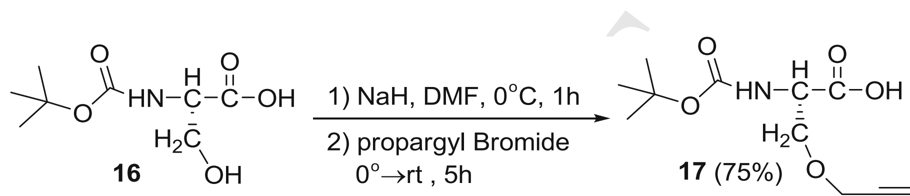
REVISED PROOF

Journal : Large 726 | Dispatch : 9-1-2014 | Pages : 14
Article No. : 1663 | □ LE | □ TYPESET
MS Code : AMAC-D-13-00527 | ✔ CP | ✔ DISK

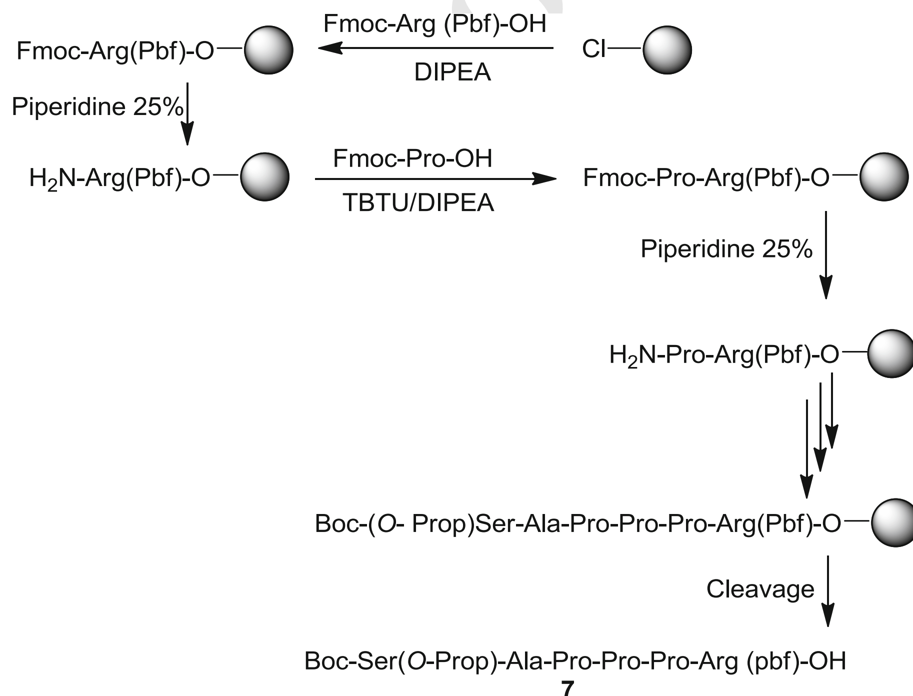


Scheme 3 Synthesis of H_2N-Lys(N_3)-$CONH_2$ **6**

Scheme 4 Synthesis of Boc-Ser(*O*-Prop)-OH **17**



Scheme 5 Solid phase hexapeptide synthesis of **7**

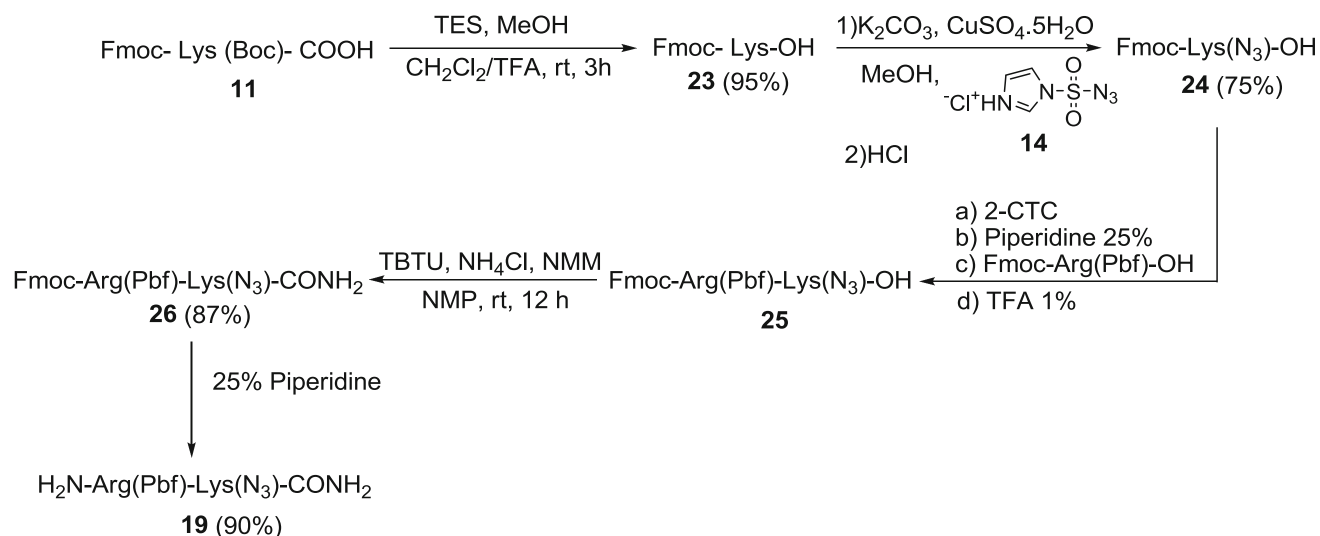


OH coupled to the 2-chlorotrityl chloride resin (2-CTC resin) and the peptides elongated more efficiently with high purity. Coupling reactions were performed using protected amino acids, activated with TBTU in the presence of diisopropyl ethyl amine (DIPEA). Then, three *S*-proline and one L-alanine was used in this sequence of the desired peptide and finally Boc-Ser(*O*-Prop)-OH was added to the peptide sequence to afford the protected hexapeptide **7**. Cleavage of the peptide from the surface of resin was done using TFA (1 %) (Scheme 5).

To twist and bring the two ends closer together for the click reaction, based on our previous experience, we decided to use Ugi ligation. Four-component reaction of hexapeptide with carboxylic acid containing the propargyl group, amidated functionalized lysine containing azide moiety in the side chain as an amine group, 4-cyano-benzaldehyde and cyclohexyl isocyanide in methanol led to Ugi-ligated product **9**.

All structures were confirmed based on spectroscopic data and also high resolution mass spectrometry. Using Ugi-4CR, a new stereocenter was created in the product and the products formed as two diastereomers; the ratio of diastereomers was 80:20. The diastereomers were separated using column chromatography and compound **10** was

Journal : Large 726 | Dispatch : 9-1-2014 | Pages : 14
Article No. : 1663 | LE | TYPESET
MS Code : AMAC-D-13-00527 | CP | DISK



Scheme 6 Synthesis of functionalized dipeptide H_2N-Arg(Pbf)-Lys(N_3)-$CONH_2$ **19**

246 separated as a major and pure stereoisomer. The anti-cancer activity of major and minor diastereomers was investigated and only the major diastereomer showed good biological activity and will be discussed. The minor diastereomer did not show good biological activity. 250

251 1,3-Dipolar cycloaddition reaction between an azide and alkyne takes place in the presence of a Cu (I) catalyst under mild conditions, resulting in the formation of a triazole link connecting the two molecules. In peptide chemistry, the increasing popularity of the click reaction is largely a result of the unique properties of both azides and the resulting triazoles. Interestingly, the triazole moiety formed by click reaction has a unique similarity to an amide bond. The relative planarity, strong dipole moments, and hydrogen bonding ability of triazole linkage make it as attractive as an amide bond with added advantage that it is less prone to hydrolytic cleavage. Triazole unit may impart rigidity, lipophilicity, enhanced absorption and protease stability, and act as an amide bond. Thus, the incorporation of the triazole unit in the structure of peptide is an added advantage. 265

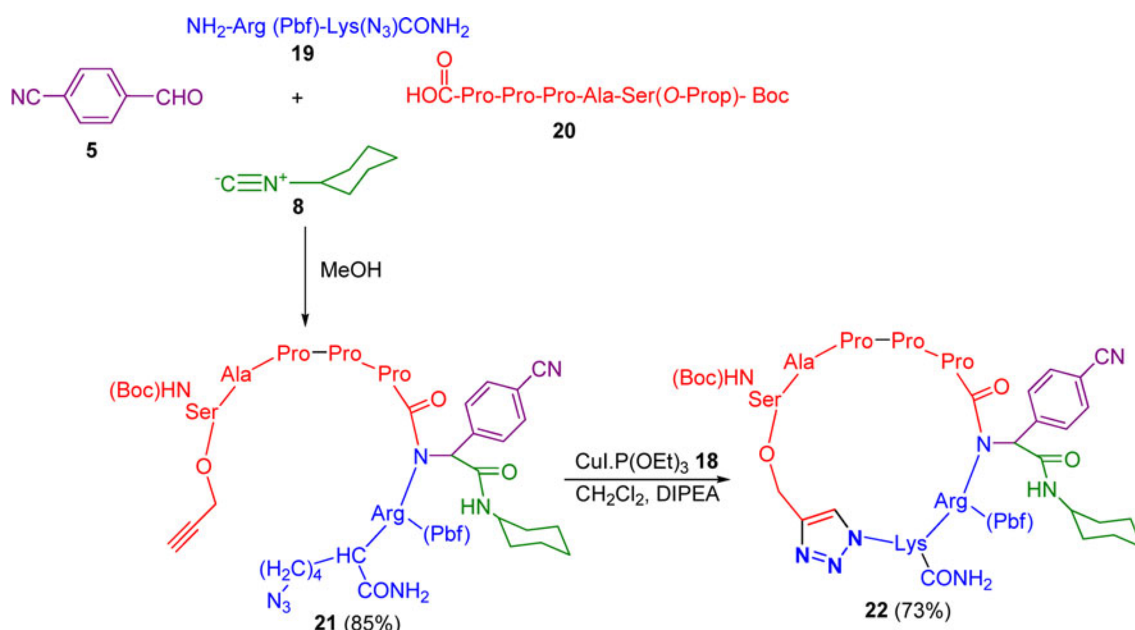
266 There are many reports for the cycloaddition of azides and acetylenes which was done in the presence of CuI or $Cu(OAc)_2$/sodium ascorbate. The reaction was checked according to the reported methods based on CuI and $Cu(OAc)_2$, but the yields were low (Johnasson and Pedersen 2012). Finally, CuI.P(OEt)$_3$ was used as a reagent for the click reaction and cyclopeptide **10** was obtained in 75 % yield. 272

273 Based on the result of the experiment mentioned above and based on the amino acid sequence in the structure of peptide **1**, another Ugi-4CR was designed. The two pentapeptide **20** and dipeptide **19** segments were selected as the carboxylic acid **20** and amine moiety **19**, respectively. The reaction sequences for the synthesis of dipeptide H_2N-Arg(Pbf)-Lys(N_3)-$CONH_2$ **19** are shown in Scheme 6. The amine group in the side chain of lysine was converted to azide moiety using **14** and Fmoc-Lys(N_3)-OH **23** was loaded on the surface of 2-chlorotrityl chloride resin in the presence of DIPEA. Then Fmoc deprotection was done using piperidine 25 % in DMF and Fmoc-Arg(Pbf)-OH was added to the sequence using TBTU as coupling reagent in the presence of DIPEA. The cleavage of the protected dipeptide was done using 1 % TFA. The amidation of C-terminal was done using ammonium chloride and TBTU in the presence of NMM as base. Finally, Fmoc deprotection was carried out using 25 % piperidine and the desired dipeptide **19** was formed. 291

292 The pentapeptide **20** was synthesized based on the standard SPPS method. The Ugi-4CR of pentapeptide **20** as carboxylic acid, dipeptide **19** as amine, 4-cyano-benzaldehyde **5**, cyclohexyl isocyanide **8** led to the formation of compound **21** and finally using CuI.P(OEt)$_3$ **18** (Langille and Jamison 2006), cyclization was carried out and cyclopeptide **22** was obtained (Scheme 7). 298

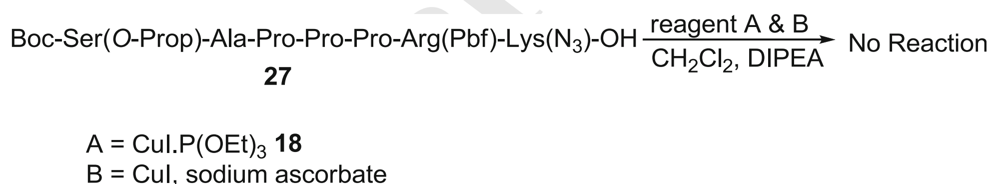
299 To access a cyclopeptide, at first the heptapeptide **27** was synthesized based on SPPS strategy, the sequence of amino acids in this compound is the same as heptapeptide **1**. There are only two changes: instead of Lys and Ser in the sequence, Fmoc-Lys(N_3)-OH and Boc-Ser(*O*-Prop)-OH were used in C- and N-terminal, respectively. The 1,3-Dipolar cycloaddition of peptide **27** was done according to the standard method (CuI) as well as CuI. P(OEt)$_3$ **18**, but in both cases the cyclization was not successful (Scheme 8). It seems that the distance between alkyne and azide moieties is very far. It shows that the Ugi-4CR could affect the folding of molecule and cause efficient cyclization. 311

Journal : Large 726 | Dispatch : 9-1-2014 | Pages : 14
Article No. : 1663 | LE | TYPESET
MS Code : AMAC-D-13-00527 | CP | DISK



Scheme 7 Sequential Ugi ligation/Huisgen 1,3-Dipolar reaction to construct cyclopeptide **22**

Scheme 8 Try for cyclization of peptide **27**



Peptide **27** had no biological activity. The click reaction did not occur; perhaps due to the amino acid sequence of this peptide or because the two ends failed to connect. Compound **10** was highly active against lung cancer cells, which induced apoptosis at a much lower dose than the original heptapeptides. Yet, the same compound had no anti-cancer activity before the click reaction (compound **9**). The chemical modifications before click reaction actually affected the original heptapeptides **1** negatively. All products were less soluble in water; therefore, they were dissolved in a small volume of dimethyl sulfoxide (DMSO) and then diluted with water for in vitro assays. Interestingly, the products **21** and **22** had no anti-cancer activities; this result shows that the selection of suitable segments for Ugi-4CR has an important role in the biological activity of products.

The biological activities of products **1** (the unmodified heptapeptide), **9**, **10**, **21**, **22**, and **27** were determined by their effects on A549, human lung cancer cell line. The results (Fig. 1) clearly indicate that cyclopeptide **10**, the final product in Scheme 9, shows significant anti-cancer activity. The cyclization of our original heptapeptides by this method has improved its biological activity up to 20-fold. The dose–response assay (Fig. 2) shows that 0.5 µg of compound **10** had the same or greater anti-cancer activity than 10 µg of compound **1** (Fig. 1). The biological activity of compound **10** was further examined by its effect on different cell lines: A549 used was a well-characterized human lung carcinoma cell line, known to have mutated ras oncogene; C26, an aggressive colorectal cancer cell line that contains constitutively activated ras oncogene; PC3 is a cell line characteristic of prostatic small cell carcinoma that also has mutated ras oncogene; and noncancerous CHO, Chinese hamster ovary cell line that does not have mutated ras oncogene. Cyclopeptide **10** showed great specificity against these cell lines. The results (Fig. 3) show that **10** had significant anti-cancer activity against all the three different cancer cell lines that have mutated ras oncogene. Meanwhile, it had little or no effect on noncancerous CHO cells and normal human leukocytes.

In conclusion, we have designed a hypothetical heptapeptides specifically to overcome the neoplastic activity of ras oncogene. This peptide showed the potential to be druggable by inducing apoptosis in some specific cancer cells known to have mutated ras oncogene. To improve

Journal : Large 726 Dispatch : 9-1-2014 Pages : 14
Article No. : 1663 LE TYPESET
MS Code : AMAC-D-13-00527 CP DISK

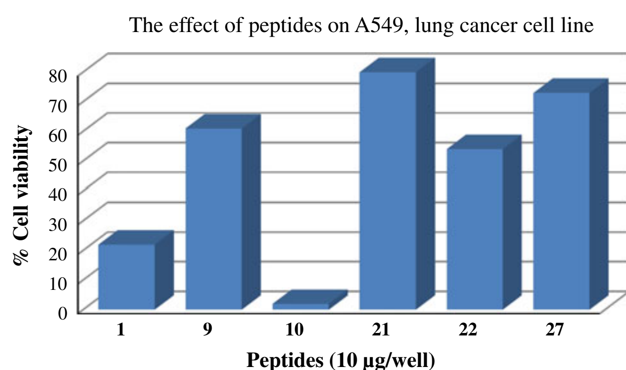


Fig. 1 The anti-cancer activities of synthesized peptides against human lung carcinoma cells, A549

the anti-cancer activity of this peptide, we chemically modified it by cyclization reaction. The reaction could be categorized as a sequential Ugi/Huisgen 1,3-Dipolar cyclization reaction. We have now constructed a cyclopeptide that contains a triazole motif. The existence of a triple bond opens an avenue to a diversity of subsequent compounds accessible by different reactions. The anti-cancer activity of all products was examined in vitro and only cyclopeptide **10** with triazole skeleton showed significant anti-cancer activity against cancer cells with mutated ras oncogene such as A549, PC3 and C26 cells. Cyclopeptide **10** had little or no activity on Chinese hamster cells, CHO that does not express ras oncogene. This study clearly shows the importance of triazole skeleton in biological activity of the peptides. It might be possible to overcome the difficulties involved in making complex peptides by employing this elegant chemistry.

Experimental section

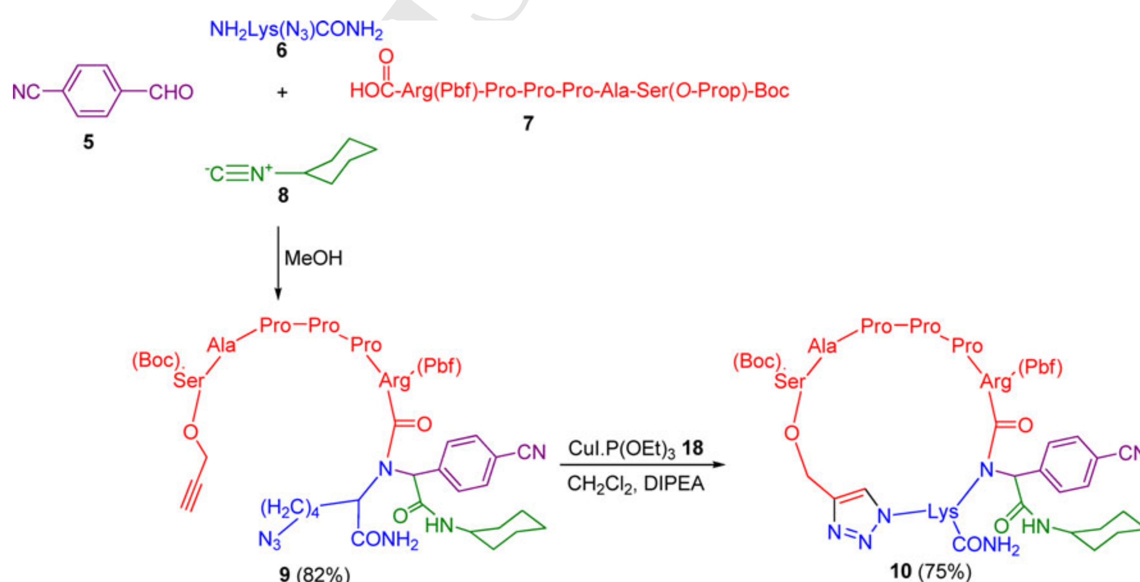
General

Commercially available chemicals were used as received unless otherwise stated. Flash column chromatography was carried out using silica Gel 60 (particle size 0.04–0.06 mm/230–400 mesh). The abbreviations are given in separate place. The mass spectra were recorded by EI-mass (70 eV), mass (ESI-triple quadrupole), mass (ESI-ion trap), HRMS (ESI-FT-ICR), HRMS (MALDI-FT-ICR). The purification of peptides was done using preparative HPLC (column C18, 7 μm). NMR spectra were recorded at 500, 300 MHz in $CDCl_3$, DMSO-d_6 and D_2O.

Synthetic procedures

*General procedure for the synthesis of heptapeptides-COOH (**1–4**)*

Synthesis was carried out using 2-chlorotrityl chloride resin (1.0 mmol/g) following the standard Fmoc strategy. Fmoc-Lys(Boc)-OH (4.687 g, 10 mmol) was attached to the 2-CTC resin (5.000 g) with DIPEA (6.85 mL, 40 mmol) in anhydrous DCM:DMF (50 mL, 1:1) at room temperature for 2 h. After filtration, the remaining trityl chloride groups were capped by a solution of DCM/MeOH/DIPEA (17:2:1, 120 mL) for 30 min. Then, it was filtered and washed thoroughly with DCM (1 × 20 mL), DMF (4 × 20 mL) and MeOH (5 × 20 mL). The loading capacity was determined by weight after drying the resin under vacuum and was 1.0. The resin-bound Fmoc-amino acid was



Scheme 9 Sequential Ugi ligation/Huisgen 1,3-Dipolar reaction to construct cyclopeptide **10**

Journal : Large 726 | Dispatch : 9-1-2014 | Pages : 14
Article No. : 1663 | LE | TYPESET
MS Code : AMAC-D-13-00527 | CP | DISK

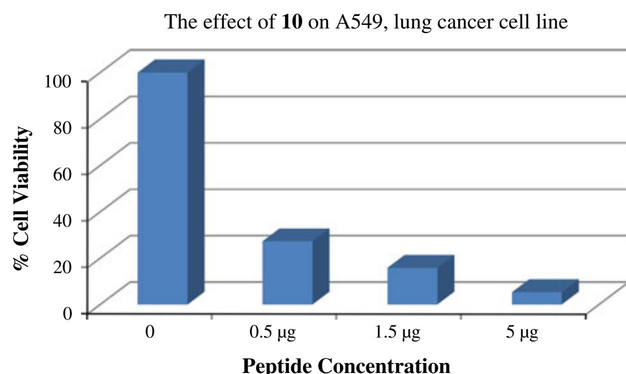


Fig. 2 The dose-dependent anti-cancer activity of cyclopeptide **10** against human lung carcinoma cells, A549

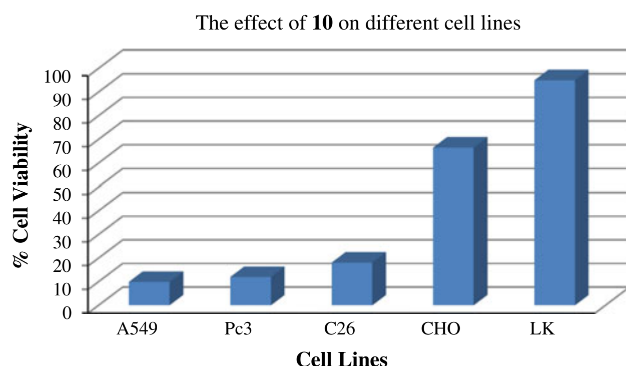


Fig. 3 The specificity of cyclopeptide **10** against different cell lines, A549, PC3, C26, CHO, and LK

washed with DMF (3 × 20 mL) and treated with 25 % piperidine in DMF (65 mL) for 30 min and the resin was washed with DMF (3 × 20 mL). Then a solution of Fmoc-Arg(Pbf)-OH (4.866 g, 7.5 mmol), TBTU (2.407 g, 7.5 mmol), and DIPEA (3.0 mL, 17.5 mmol) in 30 mL DMF was added to the resin-bound free amine and shaken for 1 h at room temperature. After completion of coupling, resin was washed with DMF (4 × 20 mL). The coupling was repeated as in the same way as for other amino acids of their sequences. In all cases for the presence or absence of free primary amino groups, Kaiser Test was used. Fmoc determination was done using UV spectroscopy method. After completion of couplings, resin was washed with DMF (4 × 20 mL). The produced heptapeptide was cleaved from resin by treatment of TFA (1 %) in DCM (275 mL) and neutralization with pyridine (4 %) in MeOH (85 mL). The solvent was removed under reduced pressure and precipitated in water. The precipitate was filtered and dried. Final deprotection was done using TFA (95 %) and reagent K (TFA/TES/Water 95:2.5:2.5). The excess TFA/DCM was removed under reduced pressure. The desired peptide was precipitated in diisopropyl ether. The purification was done using preparative HPLC (Column C18). The same procedure was used for the synthesis of peptide **2–4**.

This procedure was used for the synthesis of hexapeptide **7**, only Fmoc-Ser(*O*-prop)-OH was used instead of Fmoc-Ser(^{t}Bu)-OH.

HRMS (ESI) heptapeptides

1 *m/z* $[M+H]^+$ Calcd for $C_{33}H_{58}N_{11}O_9$ 752.44189, Found 752.44183. $[M+Na]^+$ Calcd for $C_{33}H_{57}N_{11}NaO_9$ 774.42409, Found 774.42400.

2 *m/z* $[M+H]^+$ Calcd for $C_{27}H_{45}N_{10}O_{10}$ 669.33284, Found 669.33266.

3 *m/z* $[M+H]^+$ Calcd for $C_{29}H_{49}N_{10}O_9$ 681.36893, Found 681.36879.

4 *m/z* $[M+H]^+$ Calcd for $C_{31}H_{52}N_{13}O_9$ 750.40298, Found 750.40269.

HRMS (ESI-FT-ICR)

HRMS (ESI-FT-ICR) 7 *m/z*: $[M+H]^+$ Calcd for $C_{48}H_{72}N_9O_{13}S$ 1,014.49667, Found 1,014.49665, $[M+Na]^+$ Calcd for $C_{48}H_{71}N_9NaO_{13}S$ 1,036.47904, Found 1,036.47899, $[M+K]^+$ Calcd for $C_{48}H_{71}KN_9O_{13}S$ 1,052.45305, Found 1,052.45299.

Fmoc-Lys(Boc)-CONH$_2$ **12**

A solution of Fmoc-Lys(Boc)-OH **11** (4.396 g, 9.4 mmol) and ammonium chloride (1.069 g, 20 mmol) in *N*-methyl-2-pyrrolidinone (3 mL) was magnetically stirred and then TBTU (4.815 g, 15 mmol) in *N*-methyl morpholine (5.5 mL, 50 mmol) was added to the mixture. The mixture was stirred for 12 h at room temperature.

A yellow solution was formed. The reaction progress was monitored using thin layer chromatography (1:2:10, H_2O:MeOH:ethyl acetate). The reaction was completed after 12 h. Then, by slow addition of 70 mL H_2O, a yellow discretion was formed. The deposition was filtered and dried. Mass of discretion was 4.830 g (9.4 mmol) with 94 % yield (Arabanian et al. 2010).

m.p. 158–161 °C; ^{1}HNMR (300 MHz, DMSO-d_6) $\delta = 1.26$–1.28 (m, 2H, CH$_2$), 1.35 (s, 9H, ^{t}Bu), 1.56–1.59 (m, 2H, CH$_2$), 1.89 (quin, 2H, CH$_2$), 2.85–2.90 (m, 2H, C*H*$_2$NH), 3.28 (t, 1H, $J = 7.0$ Hz, CH fluorene), 4.20–4.29 (m, 3H, CHα and CH$_2$O), 6.76 (brs, 1H, N*H*–CO–CH$_2$fluorenyl), 6.96 (brs, 1H, N*H*-Boc), 7.31 (t, 1H, $J = 7.3$ Hz, H–Ar), 7.32–7.37 (m, 2H, H–Ar), 7.40 (t, 1H, $J = 7.0$ Hz, H–Ar), 7.71 (d, 1H, $J = 6.3$ Hz, H–Ar), 7.82 (d, 1H, $J = 7.5$ Hz, H–Ar), 7.87 (d, 2H, $J = 7.3$ Hz, H–Ar) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) $\delta = 17.2$, 22.9 (CH$_2$), 28.3 (C(*C*H$_3$)$_3$), 29.2 (CH$_2$), 31.5 (*C*H$_2$NH),46.7 (CH fluorene), 54.4 (*C*H$^\alpha$–CONH$_2$), 65.6 (*C*H$_2$O–), 77.3

REVISED PROOF

Journal : Large 726 | Dispatch : 9-1-2014 | Pages : 14
Article No. : 1663 | LE | TYPESET
MS Code : AMAC-D-13-00527 | CP | DISK

(–O*C*(CH_3)$_3$), 120.1, 125.3, 127.0, 127.6, 140.7, 143.8, 143.9 (C Ar), 155.5 (fluorenyl-CH_2-*C*ONH-), 155.9 (–O*C*O^{t}Bu), 173.9 (*C*ONH$_2$) ppm; IRν_{max}(neat) 3,313, 3,055 (NH), 1,687 (C=O), 1,511 cm^{-1}; MS (70 eV): *m/z* (%):467 (10) [M$^+$], 394 (74) [M$^+$-C_4H_9O], 366 (67) [M$^+$-$C_5H_9NO_2$].

Fmoc-Lys-CONH$_2$ **13**

Fmoc-Lys(Boc)-CONH$_2$ **12** (2.935 g, 8.0 mmol) was dissolved in 50 % (v/v) TFA in CH_2Cl_2 (100 mL). Then triethylsilane (1.4 mL, 9.0 mmol) was added to the mixture as scavenger. The reaction mixture was stirred for 3 h at room temperature. Then, the solvent was removed under the reduced pressure, the pH of the mixture was adjusted via addition of NaOH (1 N). The precipitate was filtered and washed with water. 2.490 g of product was achieved. The yield at this step was 93 % (Diaz-Mochon et al. 2005).

^{1}HNMR (300 MHz, DMSO-d_6) $\delta = 1.32$–1.34 (m, 2H, CH_2), 1.43–1.50 (m, 2H, CH_2), 1.89 (quin, 2H, CH_2), 2.67–2.87 (m, 2H, C*H*$_2$–NH$_2$), 3.29 (t, 1H, $J = 7.0$ Hz, CH fluorene), 3.50–3.81 (brs, 2H, NH_2), 4.20–4.27 (m, 3H, –CH_2O, CHα) 6.26 (s, 1H, CONH), 6.95 (m, 2H, CON*H*, fluorenyl CH_2CON*H*), 7.31 (t, 1H, $J = 7.3$ Hz, H–Ar), 7.32–7.37 (m, 2H, H–Ar), 7.40 (t, 1H, $J = 7.0$ Hz, H–Ar), 7.71 (d, 1H, $J = 6.2$ Hz, H–Ar), 7.82 (d, 1H, $J = 7.5$ Hz, H–Ar), 7.87 (d, 2H, $J = 7.4$ Hz, H–Ar) ppm; ^{13}C NMR(75 MHz, DMSO-d_6) $\delta = 17.2$, 22.1, 28.9 (CH_2), 30.1 (CH_2–NH_2), 33.8 (CH fluorene), 53.9 (CH$^\alpha$–CONH$_2$), 109.7, 111.2, 115.2, 119.1, 119.9, 121.3, 123.3, 127.3, 128.9, 137.4, 139.4, 142.5 (C Ar), 173.9 (fluorenyl-CH_2-*C*ONH-), 176.7 (CONH$_2$) ppm; IRν_{max} (neat) 3,055, 2,984 (NH), 2,854 (CH), 1,681 (CO) cm^{-1}; MS (70 eV): *m/z* (%): 366 (6) [M$^+$], 351 (16) [M$^+$-NH_2], 337 (12) [M$^+$-(H_2C=NH_2)].

Imidazole-1-sulfonyl azide hydrochloride **14**

A round bottle containing suspension of sodium azide (1.001 g, 15.4 mmol) and acetonitrile (20 mL) was placed in ice bath. When the reaction mixture was cooled, sulfuryl chloride (1.25 mL, 15.4 mmol) was dropped slowly. Then, ice bath was removed. And reaction mixture was stirred for 20 h at room temperature. The reaction mixture was cooled to 0 °C (ice bath) again and imidazole (2.000 g, 29.3 mmol) was added over 10 min. The reaction mixture was stirred for 4 h at room temperature. Then it was diluted with EtOAc (20 mL) and washed with H_2O (2 × 20 mL). The organic phase was washed by saturated $NaHCO_3$ solution (2 × 20 mL). The organic phase was separated and dried with anhydrous $MgSO_4$ and cooled to 0 °C (ice bath). Acetyl chloride (2.098 mL, 29.4 mmol) was added dropwise to ice cold EtOH (5 mL) over 10–15 min. After stirring for 10 min at 0 °C, the solution was added to the EtOAc solution over 20 min. After stirring for 10 min, the resulting suspension was filtered and the precipitate washed with EtOAc (4 × 10 mL) and dried under suction for 30 min affording the title compound as a white solid (2.250 g, 70 %) with spectral characteristics in accordance with literature data (Goddard-Borger and Stick 2007).

^{1}H NMR(300 MHz, D_2O) $\delta = 7.46$ (dd, 1H, $J = 2.1$ Hz, $J = 1.2$ Hz, CH^4), 7.88 (t, 1H, $J = 2.1$ Hz, CH^5), 9.15 (t, 1H, $J = 1.2$ Hz, CH^2) ppm; ^{13}C NMR (75 MHz, D_2O) $\delta = 119.8$ (C^4), 121.8 (C^5), 137.0 (C^2) ppm; IRν_{max} (KBr) 3,111 (NH), 2,167 (N_3), 1,428 (SO_2) cm^{-1}.

Fmoc-Lys(N_3)-CONH$_2$ **15**

A suspension of Fmoc-Lys-CONH$_2$ **13** (2.910 g, 7.9 mmol) with potassium carbonate (2.722 g, 19.7 mmol) and copper sulfate pentahydrate (0.017 g, 0.07 mmol) in methanol (50 mL) was prepared in a proper round bottle. The imidazole-1-sulfonyl azide hydrochloride (2.000 g, 9.5 mmol) was added to the suspension slowly. The above mixture was stirred for 20 h at room temperature. After completion of reaction, solvent was removed under vacuum, then 120 mL H_2O was added to reaction mixture and pH was diminished to 2 by consumption of concentrated HCl. The achieved acidic solution was extracted by ethylacetate (3 × 20 mL) and finally the whole organic phase was washed by brine. Organic phase was dried by anhydrous $MgSO_4$ and the solvent was distilled. Yellow oil (2.000 g, 65 %) was formed.

^{1}H NMR(300 MHz, DMSO-d_6) $\delta = 1.29$–1.37 (m, 2H, CH_2), 1.42–1.59 (m, 2H, CH_2), 1.59–1.71 (m, 2H, CH_2), 3.32 (t, 2H, $J = 6.9$ Hz, CH_2N_3), 3.68–3.73 (m, 1H, CH fluorene), 3.85–3.92 (m, 1H, C*H*$^\alpha$–CONH$_2$), 4.20–4.25 (m, 2H, -fluorenyl-C*H*$_2$-O), 6.99 (brs, 1H, NH-COOCH$_2$fluorenyl), 7.28–7.35 (m, 3H, H–Ar), 7.36–7.50 (m, 3H, H–Ar and CONH$_2$), 7.59 (brs, 1H, CONH$_2$), 7.72 (d, 1H, $J = 7.2$ Hz, H–Ar), 7.78 (d, 2H, $J = 7.4$ Hz, H–Ar) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) $\delta = 22.5$, 28.3, 31.7 (CH_2), 47.1 (CH fluorene), 51.1 (CH_2N_3), 56.8 (*C*H$^\alpha$–CONH$_2$), 67.2 (CH_2–O–), 120.1, 125.1, 127.1, 127.8, 141.3, 143.6, 143.8 (C–Ar), 156.3 (*C*OOCH$_2$fluorenyl), 177.0 (CONH$_2$) ppm; IRν_{max} (neat) 3,380, 3,351, 2,943 (NH), 2,105 (N_3), 1,775, 1,681 (C=O) cm^{-1}; MS(70 eV): *m/z* (%):393 (70) [M$^+$].

H_2N-Lys(N_3)-CONH$_2$ **6**

Fmoc-Lys(N_3)-CONH$_2$ **15** (2.001 g, 5.1 mmol) was dissolved in ethylacetate (8 mL) and then piperidine (2.0 mL, 20 mmol) was added. The reaction mixture was stirred at room temperature. After 3 h, the reaction mixture was extracted by water (3 × 10 mL). The aqueous phases were

REVISED PROOF

Journal : Large 726 | Dispatch : 9-1-2014 | Pages : 14
Article No. : 1663 | LE | TYPESET
MS Code : AMAC-D-13-00527 | CP | DISK

washed by fresh ethylacetate (1 × 10 mL) again. Aqueous phase was dried. Yellow viscous oil (0.850 g, 98 %) was obtained.

^{1}H NMR (300 MHz, DMSO-d_6) $\delta = 1.32$–1.45 (m, 2H, CH_2), 1.47–1.60 (m, 4H, 2CH_2), 2.92 (m, 3H, CH-NH_2 and CH^{α}CONH$_2$), 3.29 (t, 2H, $J = 6.7$ Hz, CH_2N_3), 6.80 (brs, 2H, CONH$_2$) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) $\delta = 22.5$, 23.9, 28.3 (CH_2), 43.9 (CH_2N_3), 53.6 (CH^{α}), 178.2 (C=O) ppm; IRν_{max} (neat) 3,485, 3,469, 3,420 (NH), 2,098 (N_3), 1,697 (C=O) cm^{-1}; MS (70 eV): *m/z* (%): 170 (5) [M^+-H], 127 (14) [M^+-CONH$_2$], 85 (90) [M^+-CH_2N_4O], 56 (94) [$C_2H_4NO^+$].

Boc-Ser(*O*-Prop)-OH17

N-*tert*-butoxycarbonyl-L-serine **16** (2.000 g, 10 mmol) was dissolved in DMF (5 mL) and the solution was placed in ice bath. Sodium hydride [0.880 g, 22 mmol, 60 % (w/w) dispersion in mineral oil] was added slowly and the reaction mixture stirred for 1 h at 0 °C. Then, propargyl bromide (1.3 mL, 11 mmol) was added dropwise to the mixture; yellow solution was formed. The solution was placed in ice bath for 1 h. Then, ice bath was removed and the solution was stirred at room temperature for 4 h. The progress of reaction was followed by thin layer chromatography (30:20:1, petroleum ether:ethylacetate:acetic acid). Water (15 mL) was added to the mixture which was then washed with diethylether (3 × 10 mL). The aqueous phase was acidified to pH 3 by adding 10 % HCl. The solution was extracted from acidic solution with ethylacetate (3 × 25 mL). The organic phase was dried using magnesium sulfate and the solvent was removed under vacuum. Orange viscous oil was achieved and purified by column chromatography (30:20:1, petroleum ether:ethylacetate:acetic acid). The solvent was evaporated affording a pale yellow, viscous oil (1.820 g, 75 %), with spectral characteristics in accordance with literature data (Brink et al. 2006; Jacobsen et al. 2011).

^{1}H NMR (500 MHz, CDCl$_3$) $\delta = 1.44$ (s, 9H, ^{t}Bu), 2.45 (t, 1H, $J = 2.6$ Hz, CCH, rotamer 1), 2.49 (t, 1H, $J = 2.4$ Hz, CCH, rotamer 2) 3.78 (dd,1H, $J = 9.4$ Hz, $J = 3.6$ Hz, CH_2 serine), 3.98 (dd,1H, $J = 9.5$ Hz, $J = 3.3$ Hz, CH_2 serine), 4.14 (d, 2H, $J = 2.3$ Hz, O–CH_2CCH, rotamer 1), 4.49–4.51(m, 1H, CH^{α}), 4.75 (d, 2H, $J = 2.4$ Hz, O–CH_2CCH, rotamer 2), 5.38 (d, 1H, $J = 8.6$ Hz, NH) ppm; IRν_{max} (neat) 3,440 (CO*OH*), 3,294 (NH), 1,715, 1,692 (C=O) cm^{-1}.

Copper(I) iodide triethyl phosphate 18

Copper iodide (1.000 g, 5.3 mmol) prepared by Nishizawa method was added to a round bottle containing triethylphosphite (0.9 mL, 5.3 mmol) and toluene (5.5 mL) in 10 min. The round bottle was sealed to protect from light; after 1 h stirring at room temperature, the reaction mixture was passed through Celite and was concentrated in vacuum. Further purification was done using recrystallization in toluene and hexane; the white crystal (1.100 g, 57 %) was formed (Langille and Jamison 2006).

^{1}H NMR (300 MHz, CDCl$_3$) $\delta = 4.11$ (quin, 2H, CH_2), 1.29 (t, 3H, $J = 7.0$ Hz, CH_3) ppm. [Compare to P (OEt)$_3$: ^{1}H NMR (500 MHz, CDCl$_3$) 3.88 (6H, q, $J = 7.0$), 1.28 (9H, t, $J = 7.0$)].

General procedure for the synthesis of peptide 9 via Ugi-4CR

A solution of Fmoc-Lys(N_3)-CONH$_2$ **6** (0.260 g, 1.5 mmol) and 4-cyano-benzaldehyde (0.200 g, 1.5 mmol) in MeOH (5 mL) was added for the formation of imine. After 1 h, Boc-Ser(*O*-Prop)-Ala-Pro-Pro–Pro-Arg(Pbf)-COOH **7** (1.520 g, 1.5 mmol) was added, this reaction followed with addition of cyclohexyl isocyanide (0.2 mL, 1.5 mmol). The mixture was stirred for 48 h. After completion of the reaction, solvent was evaporated in vacuum. The crude oil was purified by flash column chromatography. The product **9** was obtained as a white solid (1.730 g, 82 %).

HRMS (ESI-FT-ICR) *m/z*: $[M+Na]^+$ Calcd for $C_{69}H_{98}N_{16}NaO_{14}S$, 1,430.72662, Found 1,430.72659.

General procedure for the synthesis of cyclopeptide 10 through Huisgen 1,3-Dipolar cycloaddition reaction

Freshly prepared **9** (1.500 g, 1.07 mmol) was dissolved in CH_2Cl_2 (1,000 mL). *N*,*N*-Diisopropylethylamine (0.55 mL, 3.41 mmol) and copper(I) iodide triethylphosphite **18** (0.370 g, 1.61 mmol) were added to the reaction mixture. The reaction mixture was stirred and protected from light for 5 days at room temperature. The solvent was evaporated. The residue was redissolved in CH_2Cl_2 and purified by flash column chromatography (14:1:1 CH_2Cl_2:acetone:MeOH) affording the title compound as a yellow solid (1.130 g, 75 %). HRMS (ESI-FT-ICR) *m/z*: $[M+Na]^+$ Calcd for $C_{69}H_{98}N_{16}NaO_{14}S$ 1,430.57978; Found 1,430.57972.

Fmoc-Lys-OH 23

A 50 % (v/v) solution of TFA in CH_2Cl_2 (16 mL) was added to Fmoc-Lys(Boc)-OH **11** (4.680 g, 10 mmol) and then triethylsilane (1.6 mL, 10 mmol) as a scavenger was added to reaction mixture. The mixture was stirred for 2 h at room temperature. The solvent and bulk of excess TFA were removed under vacuum. The solution of sodium

REVISED PROOF

Journal : Large 726 Dispatch : 9-1-2014 Pages : 14
Article No. : 1663 LE TYPESET
MS Code : AMAC-D-13-00527 CP DISK

hydroxide (1 M) was added to the residue; the product was precipitated at pH 6. The precipitate was filtered and washed with water and dried affording a white powder (3.500 g, 95 %).

mp: 247–249 °C; ^{1}H NMR (300 MHz, $CDCl_3$) $\delta = 1.41$–1.83 (m, 4H, $2CH_2$), 1.94 (quin, 2H, CH_2), 3.01–3.10 (m, 2H, CH_2NH_2), 3.36 (t, 1H, $J = 7.1$ Hz, CH fluorene),4.18 (t, 1H, $J = 6.5$ Hz, *CH*COOH), 4.35–4.40 (m, 2H, –OCH_2), 4.76 (brs, 2H, NH_2), 5.94 (d, 1H, $J = 7.6$ Hz, N*H*COOCH_2fluorenyl), 7.28 (t, 2H, $J = 7.3$ Hz, H–Ar), 7.37 (t, 2H, $J = 7.3$ Hz, H–Ar), 7.73 (d, 2H, $J = 7.4$ Hz, H–Ar), 7.57 (d, 2H, $J = 6.9$ Hz, H–Ar) ppm; IRν_{max} (KBr): 3,330 (NH, COOH), 3,052 (CH aromatic), 2,940 (CH aliphatic), 1,689 (C=O), 1,596 (C=C) cm^{-1}.

Fmoc-Lys(N_3)-OH **24**

A suspension of Fmoc-Lys-OH **23** (2.947 g, 8 mmol) with potassium carbonate (2.730 g, 19.7 mmol) and copper sulfate pentahydrate (0.017 g, 0.07 mmol) in methanol (50 mL) was prepared in a proper round bottle. The imidazole-1-sulfonyl azide hydrochloride (2.000 g, 9.5 mmol) was added to the suspension slowly. The mixture was stirred for 20 h at room temperature. After completion of reaction, the solvent was removed under vacuum. Then 120 mL H_2O was added to reaction mixture and the pH was diminished to 2 by consumption of concentrated HCl. The achieved acidic solution was extracted by ethylacetate (3 × 20 mL) and finally the whole organic phase was washed by brine. The organic phase was dried by magnesium sulfate and the solvent was distilled. Yellow oil (2.360 g, 75 %) was formed (Sabido 2009).

mp: 224–228 °C; ^{1}H NMR (300 MHz, DMSO-d_6) $\delta = 1.27$–1.73 (m, 6H, $3CH_2$), 3.26 (t, 2H, $J = 6.5$ Hz, CH_2N_3), 4.22 (t, 1H,$J = 7.0$ Hz, CH fluorene), 4.43 (d, 2H, $J = 6.8$ Hz, –OCH_2), 4.50–4.54 (m, 1H, CH^{α}COOH), 5.58 (d, 1H, $J = 8.2$ Hz, fluorenylCH_2OOCN*H*), 7.32 (t, 2H, $J = 7.3$ Hz, H–Ar), 7.41 (t, 2H, $J = 7.3$ Hz, H–Ar), 7.55 (d, 1H, $J = 7.3$ Hz, H–Ar), 7.60 (d, 1H, $J = 7.3$ Hz, H–Ar), 7.76 (d, 2H, $J = 7.3$ Hz, H–Ar) ppm; ^{13}C NMR (75 MHz, DMSO-d_6) $\delta = 22.9$, 27.8, 30.3 (CH_2), 50.5 (CH fluorene), 54.9 (CH_2N_3), 55.5 (*C*H$^{\alpha}$COOH), 61.1(OCH_2), 120.0, 120.1 121.2, 123.9, 125.3, 127.1, 127.6, 129.5, 135.3, 140.7, 143.8, 143.9 (C–Ar), 156.2 (CONH), 171.8 (COOH) ppm; IRν_{max} (KBr): 3,455 (NH, COOH), 3,152 (CH aromatic), 2,089 (N_3), 1,743 (C=O carboxylic acid), 1,670 (*CO*NH) cm^{-1}.

General procedure for preparation of dipeptide **25**

The synthesis of dipeptide was carried out using 2-chlorotrityl chloride resin (1.0 mmol/g) following the standard Fmoc strategy. Fmoc-Lys(N_3)-OH (0.790 g, 2 mmol) was attached to the 2-CTC resin (1.000 g) with DIPEA (1.37 mL, 8 mmol) in anhydrous DCM:DMF (10 mL, 1:1) at room temperature for 2 h. After filtration, the resin was capped by a solution of DCM/MeOH/DIPEA (17:2:1, 24 mL) for 30 min. Then, it was filtered and washed thoroughly with DCM (1 × 7 mL), DMF (4 × 7 mL). The resin-bound Fmoc-amino acid was treated with 25 % piperidine in DMF (15 mL) for 30 min and the resin was washed with DMF (3 × 7 mL). Then a solution of Fmoc-Arg(Pbf)-OH (0.971 g, 1.5 mmol), TBTU (0.480 g, 1.5 mmol), and DIPEA (0.6 mL, 3.5 mmol) in 7 mL DMF was added to the resin-bound free amine and shaken for 1 h at room temperature. After completion of coupling, resin was washed with DMF (4 × 7 mL) and DCM (3 × 7 mL). The produced dipeptide was cleaved from resin by treatment of TFA (1 %) in DCM (55 mL) and neutralization with pyridine (4 %) in MeOH (17 mL). The solvent was removed under reduced pressure and precipitated in water and dried affording a powder (1.202 g).

Mass (ESI-triple quadrupole) *m/z*: $[M+H]^+$ Found for $C_{40}H_{51}N_8O_8S$ 803.10000.

Amidation of C-terminal of dipeptide **26**

The dipeptide **25** (1.200 g, 1.5 mmol) and *N*-methyl morpholine (0.8 mL, 7.5 mmol) were added to a solution of TBTU (0.740 g, 2.3 mmol) and NH_4Cl (0.160 g, 3.0 mmol) in NMP (3 mL). The mixture was stirred overnight. The dipeptide was precipitated in water and the C-terminal amidated dipeptide **26** was dried affording a yellow powder (1.040 g, 87 %).

General procedure for Fmoc deprotection of amidated dipeptide **19**

Purified amidated dipeptide **26** (0.96 g, 1.2 mmol) was added to ethylacetate (2 mL), and then piperidine (0.5 mL, 5 mmol) was added. The reaction mixture was stirred at room temperature. After 3 h, reaction mixture was extracted by water (3 × 10 mL). The aqueous phases were washed by fresh ethylacetate (1 × 10 mL) again. Aqueous phase was dried using natrium sulfate. Yellow viscous oil (0.62 g, 90 %) was obtained.

Mass (ESI-triple quadrupole) *m/z*: $[M+H]^+$ Found for $C_{25}H_{42}N_9O_5S$ 580.10000.

General procedure for the synthesis of pentapeptides-COOH **20**

The synthesis of pentapeptide **25** was done using the standard Fmoc SPPS strategy. At first, Fmoc-Pro-OH was loaded on the surface of resin. The two times Fmoc-Pro-OH, Fmoc-Ala-OH, and Boc-Ser(*O*-Prop)-OH were loaded

REVISED PROOF

Journal : Large 726 | Dispatch : 9-1-2014 | Pages : 14
Article No. : 1663 | LE | TYPESET
MS Code : AMAC-D-13-00527 | CP | DISK

on the surface of resin. The peptide was removed from the surface of 2-CTC resin using 1 % TFA based on the known procedure.

HRMS (ESI-FT-ICR) *m/z* = $C_{29}H_{44}N_6O_9$ $[M+H]^+$ Found 606.31390, Calc. 606.31399, $C_{29}H_{43}N_6NaO_9$ $[M+Na]^+$ Found 628.29535, Calc. 628.29536, $C_{29}H_{44}KN_6O_9$ $[M+K]^+$ Found 644.26965, Calc. 644.26971.

General procedure for the synthesis of peptide 21 via Ugi-4CR

A solution of H_2N-Arg(Pbf)-Lys(N_3)-NH_2 **19** (0.500 g, 0.86 mmol) and 4-cyano-benzaldehyde (0.120 g, 0.86 mmol) in MeOH (3 mL) was added for the formation of imine. After 1 h, Boc-Ser(*O*-Prop)-Ala-Pro-Pro–Pro-COOH **20** (0.520 g, 0.86 mmol) was added, this reaction was followed with addition of cyclohexyl isocyanide (0.11 mL, 0.86 mmol). The mixture was stirred for 48 h. Further purification was done using flash column chromatography. The desired product was achieved as yellow oil (1.030 g, 85 %).

Mass (ESI-ion trap) *m/z*: $[M+Na]^+$ Found for $C_{69}H_{98}N_{16}NaO_{14}S$ 1430.33.

General procedure for the synthesis of cyclopeptide 22 via Huisgen 1,3-Dipolar cycloaddition reaction

Freshly prepared **21** (1.100 g, 0.8 mmol) was dissolved in CH_2Cl_2 (900 mL). Then, *N,N*-diisopropylethylamine (0.4 mL, 2.4 mmol) and copper(I) iodide triethylphosphite (0.272 g, 1.2 mmol) were added to reaction mixture. The reaction mixture was stirred and protected from light for 5 days at room temperature. The solvent was evaporated. The residue was redissolved in CH_2Cl_2 and purified by flash column chromatography (14:1:1 CH_2Cl_2:acetone:MeOH) affording the title compound as a yellow solid (0.820 g, 73 %).

Mass (ESI-ion trap) *m/z*: $[M+H]^+$Found for $C_{69}H_{98}N_{16}O_{14}S$ 1408.13.

HRMS (ESI-FT-ICR) *m/z*: $[M-(C_6H_{10}N_3)]^+$ Calcd for $C_{63}H_{88}N_{13}O_{14}S$ 1,282.55259, Found 1,282.55248; $[M-(C_{18}H_{29}N_3O_2S)]^+$ Calcd for $C_{51}H_{69}N_{12}O_{10}$ 1009.49428, Found 1,009.49420; $[M-(C_{31}H_{50}N_8O_4S)]^+$ Calcd for $C_{38}H_{48}N_8O_{10}$ 776.24264, Found 776.24257.

General procedure for the synthesis of heptapeptides-COOH (27)

The synthesis of heptapeptide **27** was carried out using the general Fmoc SPPS strategy using 2-CTC. At first, Fmoc-Lys (N_3)-OH **24** was attached on the surface of resin and then Fmoc-Arg(Pbf)-OH, three times Fmoc-Pro-OH, Fmoc-Ala-OH and finally Fmoc-Ser(*O*-Prop)-OH were added on the surface. The peptide was removed from the surface of resin using TFA (1 %) based on the known procedure.

HRMS (MALDI-FT-ICR) *m/z*: $[M+H]^+$ Calcd for $C_{54}H_{82}N_{13}O_{14}S$ 1168.58514, Found 1,168.58489; $[M+Na]^+$ Calcd for $C_{54}H_{81}N_{13}NaO_{14}S$ 1,190.57059, Found 1,190.57048; $[M+K]^+$ Calcd for $C_{54}H_{81}KN_{13}O_{14}S$ 1,206.52828, Found 1,206.52901.

General in vitro experiments

Cancer cells were all seeded at 5,000 cells/well in a 96-well plate and the culture was maintained in RPMI 1640 supplemented with 10 % fetal bovine serum, 1 % L-glutamine, 100 units/mL penicillin, and 100 µg/mL streptomycin overnight. The media was replaced with fresh media containing up to 50 µg of peptides and incubated for 48 h in a humidified atmosphere of 95 % air and 5 % CO_2 at 37 °C until the control cultures were confluent. The media was then removed and the plate was washed two times with phosphate-buffered saline (PBS). Serum-free media (100 µL) containing 0.5 mg/mL MTT dye was added into each well and incubated at 37 °C for 2 h. The media with dye was removed, washed with PBS and the reactive dye was solved by addition of 100 µL dimethylsulfoxide (DMSO). The absorbance was read using an automatic multiwell spectrophotometer. The experiment was always performed in triplicates.

Acknowledgments S. B. gratefully acknowledges Iran National Science Foundation (INSF). We are also thanking Iranian ministry of health and medicinal education and also presidential office deputy of science and technology for financial support.

Conflict of interest Meanwhile, I certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

References

Ahsanullah MP, Schmieder P, Kuhne R, Rademann J (2009) Metal-free, regioselective triazole ligations deliver locked *cis*-peptide mimetics. Angew Chem Int Ed 48:5042–5045

Aina OH, Liu R, Sutcliffe JL, Marik J, Pan CX, Lam KS (2007) From combinatorial chemistry to cancer-targeting peptides. Mol Pharm 4:631–651

Akritopoulou-Zanze I, Gracias V, Djuric SW (2004) A versatile synthesis of fused triazolo derivatives by sequential Ugi/alkyne-azide cycloaddition reactions. Tetrahedron Lett 45:8439–8441

Alvarez R, Velazquez S, San-Felix A, Aquaro S, DeClercq E, Perno CF, Karlsson A, Balzarini J, Camarasa MJ (1994) 1,2,3-triazole-[2,5-*bis*-*O*-(tert-butyldimethylsilyl)-.beta.-D-ribofuranosyl]-3′-spiro-5″-(4″-amino-1″,2″-oxathiole 2″,2″-dioxide) (TSAO) analogs: synthesis and anti-HIV-1 activity. J Med Chem 37:4185–4194

Angelo NG, Arora PS (2005) Nonpeptidic foldamers from amino Acids: synthesis and characterization of 1,3-substituted triazole oligomers. J Am Chem Soc 127:17134–17135

REVISED PROOF

Journal : Large 726 Dispatch : 9-1-2014 Pages : 14
Article No. : 1663 □ LE □ TYPESET
MS Code : AMAC-D-13-00527 ✔ CP ✔ DISK

Angelo NG, Arora PS (2007) Solution and solid phase synthesis of triazole oligomers that display protein-like functionality. J Org Chem 72:7963–7967

Arabanian A, Mohammadnejad M, Balalaie S, Gross JH (2009) Synthesis of novel GnRH analogues using Ugi-4CR. Bioorg Chem Lett 19:887–890

Arabanian A, Mohammadnejad M, Balalaie S (2010) A novel and efficient approach for the amidation of C-terminal peptides. J Iran Chem Soc 7:840–845

Aravind A, Kumar PS, Sankar MG, Baskaran S (2011) Diversity-oriented synthesis of useful chiral building blocks from D-mannitol. Eur J Org Chem 6980–6988

Balalaie S, Bararjanian M, Rominger F, Hosseinzadeh S, Bijanzadeh HR, Wolf E (2011) Designing a sequential Ugi/Ullmann type reaction for the synthesis of indolo[1,2-a]quinoxalinones catalyzed by CuI/L-proline. Tetrahedron 67:7294–7300

Balalaie S, Motaghedi H, Tahmassebi D, Bararjanian M, Bijanzadeh HR (2012) Afacile and efficient synthesis of 2,2,2-trifluoroethyl 2-[(E)-*N*-phenylcinnamamido]-2-phenylacetates in trifluoroethanol via sequential Ugi four-component reaction/esterification. Tetrahedron Lett 52:6177–6181

Ball LJ, Kühne R, Schneider-Mergener J, Oschkinat H (2005) Recognition of proline-rich motifs by protein–protein interaction domains. Angew Chem Int Ed 45:2852–2862

Bararjanian M, Balalaie S, Rominger F, Movassagh B, Bijanzadeh HR (2010) Six-component reactions for the stereoselective synthesis of 3-arylidene-2-oxindoles via sequential one-pot Ugi/Heck carbocyclization/Sonogashira/nucleophilic addition. J Org Chem 75:2806–2812

Bararjanian M, Hosseinzadeh S, Balalaie S, Bijanzadeh HR, Wolf E (2011) Palladium catalyzed stereoselective synthesis of 3-(aminoarylmethylene)-2-oxindoles through Ugi/Heck/Buchwald reaction sequences. Tetrahedron Lett 52:3329–3332

Beyer B, Ulbricht C, Escudero D, Friebe C, Winter A, González L, Schubert US (2009) Phenyl-1*H*-[1,2,3]-triazoles as new cyclometalating ligands for iridium(III) complexes. Organometallics 28:5478–5488

Boger DL (2001) Vancomycin, teicoplanin, and ramoplanin: synthetic and mechanistic studies. Med Res Rev 21:356–381

Brink HTT, Rijkers DTS, Liskamp RMJ (2006) Synthesis of alkyne-bridged cyclic tripeptides toward constrained mimics of vancomycin. J Org Chem 71:1817–1824

Bronisz R (2005) 1,4-Di(1,2,3-triazol-1-yl)butane as building block for the preparation of the iron(II) spin-crossover 2D coordination polymer. Inorg Chem 44:4463–4465

Buckle DR, Rockell CJM, Smith H, Spicer BA (1986) Studies on 1,2,3-triazoles. 13. (Piperazinylalkoxy)-[1]benzopyrano[2,3-d]-1,2,3-triazol-9(1H)-ones with combined H1-antihistamine and mast cell stabilizing properties. J Med Chem 29:2262–2267

Cantel S, Isaad ALC, Scrima M, Levy JJ, DiMarchi RD, Rovero P, Halperin JA, D'Ursi AM, Papini AM, Chorev M (2008) Synthesis and conformational analysis of a cyclic peptide obtained via i to $i + 4$ intramolecular side-chain to side-chain azide-alkyne 1,3-Dipolar cycloaddition. J Org Chem 75:5663–5674

Chabre YM, Roy R (2008) Recent trends in glycodendrimer syntheses and applications. Curr Top Med Chem 8:1237–1285

Chan TR, Hilgraf R, Sharpless KB, Fokin VV (2004) Polytriazoles as copper(I)-stabilizing ligands in catalysis. Org Lett 6:2853–2855

Chatterjee C, Paul M, Xie L, van der Donk WA (2005) Biosynthesis and mode of action of antibiotics. Chem Rev 105:633–684

Chemama M, Fonvielle M, Arthur M, Valéry JM, Etheve-Quelquejeu M (2009) Synthesis of stable aminoacyl-tRNA analogues containing triazole as a bioisoster of esters. Chem Eur J 15:1929–1938

Colasson B, Save M, Milko P, Roithova J, Schroder D, Reinaud O (2007) A ditopic calix[6]arene ligand with *N*-methylimidazole and 1,2,3-triazole substituents: synthesis and coordination with zn(ii) cations. Org Lett 9:4987–4990

Colombo M, Peretto I (2008) Chemistry strategies in early drug discovery: an overview of recent trends. Drug Discov Today 13: 677–684

De Graaff C, Ruijter E, Orru RVA (2012) Recent developments in asymmetric multicomponent reactions. Chem Soc Rev 41: 3969–4009

Detz RJ, Heras SA, de Gelder R, van Leeuwen P, Hiemstra H, Reek JNH, van Maarseveen JH (2006) "Clickphine": a novel and highly versatile P, N ligand class via click chemistry. Org Lett 8:3227–3230

Diaz-Mochon JJ, Bialy L, Watson J, Sanchez-Martin RM, Bradley M (2005) Synthesis and cellular uptake of cell delivering PNA–peptide conjugates. Chem Commun 26:3316–3318

Dömling A (2005) In: Zhu J, Bienayme H (eds) Multicomponent reactions. Wiley, Weinheim, pp 76–94

Dömling A (2006) Recent developments in isocyanide based multicomponent reactions in applied chemistry. Chem Rev 106:17–89

Dömling A, Wang W, Wang K (2012) Chemistry and biology of multicomponent reactions. Chem Rev 112:3083–3155

Duan H, Sengupta S, Petersen JL, Akhmedov NG, Shi X (2009) Triazole-Au(I) complexes: a new class of catalysts with improved thermal stability and reactivity for intermolecular alkyne hydroamination. J Am Chem Soc 131:12100–12102

Fazio MA, Lee OP, Schuster DI (2008) First triazole-linked porphyrin-fullerene dyads. Org Lett 10:4979–4982

Fletcher JT, Bumgarner BJ, Engels ND, Skoglund DA (2008) Multidentate 1,2,3-triazole-containing chelators from tandem deprotection/click reactions of (trimethylsilyl)alkynes and comparison of their ruthenium(II)complexes. Organometallics 27:5430–5433

Gadzikwa T, Farha OK, Malliakas CD, Kanatzidis MG, Hupp JT, Nguyen SBT (2009) Selective bifunctional modification of a noncatenated metal-organic framework material via "click" chemistry. J Am Chem Soc 131:13613–13615

Genin MJ, Allwine DA, Anderson DJ, Barbachyn MR, Emmert DE, Garmon SA, Graber DR, Grega KC, Hester JB, Hutchinson DK, Morris J, Reischer RD, Ford CW, Zurenko GE, Hamel JC, Schaadt RD, Stapert D, Yagi BH (2000) Substituent effects on the antibacterial activity of nitrogen-carbon-linked (azolylphenyl) oxazolidinones with expanded activity against the fastidious gram-negative organisms haemophilus influenzae and moraxella catarrhalis. J Med Chem 43:953–970

Goddard-Borger ED, Stick RV (2007) An efficient, inexpensive and shelf-stable diazotransfer reagent: imidazole-1-sulfonyl azide hydrochloride. Org Lett 9:3797–3800

Golas PL, Matyjaszewski K (2010) Marrying click chemistry with polymerization: expanding the scope of polymeric materials. Chem Soc Rev 39:1338–1354

Hahn ME, Muir TW (2005) Manipulating proteins with chemistry: a cross-section of chemical biology. Trends Biochem Sci 30: 26–34

Hanselmann R, Job GE, Johnson G, Lou RL, Martynow JG, Reeve MM (2010) Synthesis of an antibacterial compound containing a 1,4-substituted 1*H*-1,2,3-triazole: a scalable alternative to the "click" reaction. Org Process Res Dev 14:152–158

Heal WP, Wickramasinghe SR, Leatherbarrow RJ, Tate EW (2008) *N*-Myristoyl transferase-mediated protein labelling in vivo. Org Biomol Chem 6:2308–2315

Hein JE, Tripp JC, Krasnova LB, Sharpless KB, Fokin VV (2009) Copper(I)-catalyzed cycloaddition of organic azides and 1-iodoalkynes. Angew Chem Int Ed 48:8018–8021

Horne WS, Yadav MK, Stout CD, Ghadiri MR (2004) Heterocyclic peptide backbone modifications in an alpha-helical coiled coil. J Am Chem Soc 126:15366–15367

REVISED PROOF

994 Hua YR, Flood AH (2010) Click chemistry generates privileged CH hydrogen-bonding triazoles: the latest addition to anion supramolecular chemistry. Chem Soc Rev 39:1262–1271
997 Jacobsen Ø, Maekawa H, Ge NH, Görbitz H, Rongved P, Ottersen OP, Amiry-Moghaddam M, Klaveness J (2011) Stapling of a 310-Helix with click chemistry. J Org Chem 76:1228–1238
1000 Johnasson H, Pedersen DS (2012) Azide- and alkyne-derivatised α-amino acids. Eur J Org Chem 2012:4267–4281
1002 Katsara M, Tselios T, Deraos S, Deraos G, Matsoukas MT, Lazoura E, Matsoukas J, Apostolopoulos V (2006) Round and round we go: cyclic peptides in disease. Curr Med Chem 13:2221–2232
1005 Kolb HC, Sharpless KB (2003) The growing impact of click chemistry on drug discovery. Drug Discov Today 8:1128–1137
1007 Kolb HC, Finn MG, Sharpless KB (2001) Click chemistry: diverse chemical function from a few good reactions. Angew Chem Int Ed 40:2004–2021
1010 Kuijpers BHM, Groothuys S, Keereweer AR, Quaedflieg PJLM, Blaauw RH, Van Delft FL, Rutjes FPJT (2004) Expedient synthesis of triazole-linked glycosyl amino acids and peptides. Org Lett 6:3123–3126
1014 Langille NF, Jamison TF (2006) Trans-hydroalumination/alkylation: one-pot synthesis of trisubstituted allylic alcohols. Org Lett 8:3761–3764
1017 Li HM, Cheng FO, Duft AM, Adronov A (2005) Functionalization of single-walled carbon nanotubes with well-defined polystyrene by "click" coupling. J Am Chem Soc 127:14518–14524
1020 Liu D, Gao WZ, Dai Q, Zhang XM (2005) Triazole-based monophosphines for Suzuki–Miyaura coupling and amination reactions of aryl chlorides. Org Lett 7:4907–4910
1023 Mager I, Zeiler K (2010) Efficient, enantioselective iminium catalysis with an immobilized, recyclable diarylprolinol silyl ether catalyst. Org Lett 12:1480–1483
1026 Mamidyala SK, Finn MG (2010) In situ click chemistry: probing the binding landscapes of biological molecules. Chem Soc Rev 39:1252–1261
1029 Mas-Moruno C, Rechenmacher F, Kessler H (2010) Cilengitide: the first anti-angiogenic small molecule drug candidate design, synthesis and clinical evaluation. Med Chem 10:753–768
1032 Meldal M, Tornoe CW (2008) Cu-catalyzed azide-alkyne cycloaddition. Chem Rev 108:2952–3015
1034 Michaels HA, Murphy CS, Clark RJ, Davidson MW, Zhu L (2010) 2-Anthryltriazolyl-containing multidentate ligands: zinc-coordination mediated photophysical processes and potential in live-cell imaging applications. Inorg Chem 49:4278–4287
1038 Moses JE, Moorhouse AD (2007) The growing applications of click chemistry. Chem Soc Rev 36:1249–1262
1040 Moumne R, Larue V, Seijo B, Lecourt T, Micouin L, Tisne C (2010) Fragment-based design of ligands: influence of the tether on binding properties. Org Biomol Chem 8:1154–1159
1043 Nahrwold M, Bogner T, Eissler S, Verma S, Sewald N (2010) Clicktophycin-52: a bioactive cryptophycin-52 triazole analogue. Org Lett 12:1064–1067
1046 Nicolaou KC, Boddy CNC, Bräse S, Winssinger N (1999) Chemistry, biology, and medicine of the glycopeptide antibiotics. Angew Chem Int Ed 38:2096–2152
1049 Orru RVA, Ruijter E (2010) Synthesis of heterocycles via multicomponent reactions I. Springer, Heidelberg
1051 Ramachary DB, Barbas CF (2004) Towards organo-click chemistry: development of organocatalytic multicomponent reactions through combinations of Aldol. Chem Eur J 10:5323–5331
1054 Rao AVR, Gurjar MK, Reddy KL, Rao AS (1995) Studies directed toward the synthesis of vancomycin and related cyclic peptides. Chem Rev 95:2135–2167
1056
1057 Rawal GK, Zhang P, Ling C (2010) Controlled synthesis of linear α-cyclodextrin oligomers using copper-catalyzed Huisgen 1,3-Dipolar cycloaddition. Org Lett 12:3096–3099
1060 Rostovtsev VV, Green LG, Fokin VV, Sharpless KB (2002) A stepwise Huisgen cycloaddition process: copper(I)-catalyzed regioselective ligation of azides and terminal alkynes. Angew Chem Int Ed 41:2596–2599
1064 Rozkiewicz DI, Jańczewski D, Verboom W, Ravoo BJ, Reinhoudt DN (2006) Click chemistry by microcontact printing. Angew Chem Int Ed 45:5292–5296
1067 Ruijter E, Scheffelaar R, Orru RVA (2011) Multicomponent reaction design in the quest for molecular complexity and diversity. Angew Chem Int Ed 50:6234–6246
1070 Sabido E (2009) Using peptidyl aldehydes in activity-based proteomics. Bioorg Med Chem Lett 19:3752–3755
1072 Saleh-Abady MM, Naderi-Manesh H, Alizadeh A, Shamsipour F, Balalaie S, Arabanian A (2010) Anticancer activity of a new gonadotropin releasing hormone analogue. Biopolymers (Pept Sci) 94:292–298
1076 Schneider G (2010) Virtual screening—and endless staircase. Nat Rev Drug Discov 9:273–276
1078 Shoichet BK, Stroud RM, Santi DV, Kuntz ID, Perry KM (1993) Structure-based discovery of inhibitors of thymidylate synthase. Science 259:1445–1450
1081 Slobbe P, Ruijler E, Orru RVA (2012) Recent applications of multicomponent reactions in medicinal chemistry. Med Chem Commun 3:1189–1218
1084 Tahoori F, Erfani Moghaddam M, Arabanian A, Balalaie S (2010) Molecular modeling of novel GnRH analogues using NMR spectroscopy and relation with their anti-cancer activities. In: Proceedings of the 31st European Peptide Symposium, Copenhagen, Denmark, pp 576–577
1089 Tietze LF, Hauner F (2000) In: Shibasaki M, Stoddart JF (eds) Stimulating concepts in chemistry. Wiley, Weinheim
1091 Tietze LF, Brasche G, Gericke KM (2006) Domino reactions in organic synthesis. Wiley, Weinheim
1093 Tornoe CW, Christensen C, Meldal M (2002) Peptidotriazoles on solid phase: [1,2,3]-triazoles by regiospecific copper(I)-catalyzed 1,3-Dipolar cycloadditions of terminal alkynes to azides. J Org Chem 67:3057–3064
1097 Vlieghe P, Lisowski V, Martinez J, Khrestchatisky M (2010) Synthetic therapeutic peptides: science and market. Drug Discov Today 15:40–56
1100 Von Itzstein M, Wu WY, Kok GB, Pegg MS, Dyason JC, Jin B, Van Phan T, Smythe ML, White HF, Oliver SW, Colman PM, Vargheses JN, Ryan DM, Woods JM, Bethell RC, Hotham VJ, Cameron JM, Penn CR (1993) Rational design of potent sialidase-based inhibitors of influenza virus replication. Nature 363:418–423
1106 Wamhoff H (1984) In: Katritzky AR, Rees CW (eds) Comprehensive heterocyclic chemistry, vol. 5. Pergamon, Oxford
1108 Wu P, Fokin VV (2007) Catalytic azide-alkyne cycloaddition: reactivity and applications. Aldrichimica Acta 40:7–17
1110 Wyszogrodzka M, Haag R (2008) A convergent approach to biocompatible polyglycerol "click" dendrons for the synthesis of modular core–shell architectures and their transport behavior. Chem Eur J 14:9202–9214
1114 Yue YF, Wang BW, Gao EQ, Fang CJ, He C, Yan CH (2007) A novel three-dimensional heterometallic compound: templated assembly of the unprecedented planar "Na ⊂ [Cu_4]" metalloporphyrin-like subunits. Chem Commun 2034–2036
1118

REVISED PROOF

Journal : Large 726 | Dispatch : 9-1-2014 | Pages : 14
Article No. : 1663 | LE | TYPESET
MS Code : AMAC-D-13-00527 | ✔ CP | ✔ DISK