Indian Journal of Chemistry
Vol. 62, May 2023, pp. 513-517
DOI: 10.56042/ijc.v62i5.1431

Serendipitous late-stage modification of dipeptide by using AIBN and thioacetic acid

Sambasivarao Kotha*, Naveen Kumar Gupta & Saima Ansari
Department of Chemistry, Indian Institute of Technology Bombay, Powai, Mumbai 400 076, India
*E-mail: srk@chem.iitb.ac.in

Received 9 January 2023; accepted (revised) 13 March 2023

Post-assembly peptide modifications have become an integral part of medicinal chemistry nowadays. This exercise is useful in finding novel bioconjugates, therapeutic peptides and protein labelling. Therefore, in our attempts towards the modification of peptides, we have tried to insert thiophene moiety in dipropargyl dipeptide in the presence of AIBN and thioacetic acid. Surprisingly, instead of thiophene group introduction, we observe the serendipitous functionalization of amide group *i.e.* –$NHCOCH_3$ is converted into –NHCHO group.

Keywords: Peptide modification, Azodiisobutyronitrile (AIBN), Thiophene, Serendipity, Propargyl group

Peptides and proteins are essential components of different biological processes and they are natural messengers in biological systems. Peptides play a crucial role in the living systems. Interestingly, various hormones, neurotransmitters, neuromodulators, function and transfer of message in the form of protein from one place to another place in the biological systems[1]. Peptides, proteins and their analogues are very useful in medical field as therapeutic agents[2]. Nowadays, medicinal chemists are designing peptide based drugs with minimum side effects, increased selectivity and improved oral bioavailability, metabolic stability, function specificity and decreased enzymatic degradation. These aspects of the biological systems are realized without the compromise of pharmacological activity of the parent pharmacophore[3]. After modification of peptide, one can improve the efficiency as well as selectivity so that in the near future, activity related to peptide-based drugs is likely to increase[4]. These methods are applicable for modification of peptide and accelerate the peptide-based drug discovery process.

Several times, unique amino acids (AAAs) have been added to peptides to change their shape and increase their stability. These modified peptides are proven to be more physiologically active than their unmodified counterparts[5-9]. The importance of 'building block approach' is well established in modifying peptides by using commercially available glycine equivalent, ethyl isocyanoacetate[10,11]. In this regard, our group is interested in preparing different unusual AAA derivatives and carry out modification of peptides by post-assembly chemical methods. Inspired by the work of Zade *et al.*[12] (Fig. 1a), we attempted to modify diyne dipeptide by inserting a thiophene unit *via* a radical pathway utilizing azobisisobutyronitrile (AIBN) and thioacetic acid (Fig. 1b). Surprisingly, the expected modified thiophene containing peptide was not observed and a H-atom was introduced in the place of -CH_3 group in –NHAc moiety.

Results and Discussion

Our group has vigorously studied the preparation of unusual AAAs and peptide modification *via* a [2+2+2] cycloaddition strategy[13,14], sultine formation[15], Diels–Alder chemistry[16], triazole formation[17], Suzuki–Miyaura cross-coupling[18], Negishi cross-coupling[19], olefin metathesis[20] by using different transition metal-based catalysts[21-23]. Along similar lines, we initiated another strategy by preparing di-alkyne derivative starting with a glycine equivalent, ethyl isocyanoacetate **1**. Treating ethyl isocyanoacetate **1** with propargyl bromide **2** in the presence of DBU as a base and under refluxing acetonitrile produced dipropargyl derivative **3**. Later, the dipropargyl ethyl isocyanoacetate **3** was hydrolyzed in the presence of conc. HCl (33-35%) in ethanol to afford the dipropargyl amino ester **4**. This aminoester derivative **4** was further protected by employing acetic

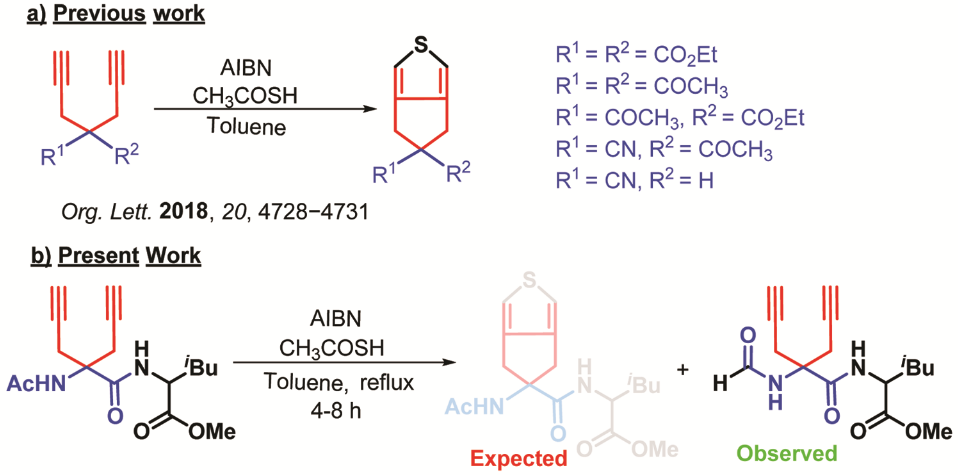
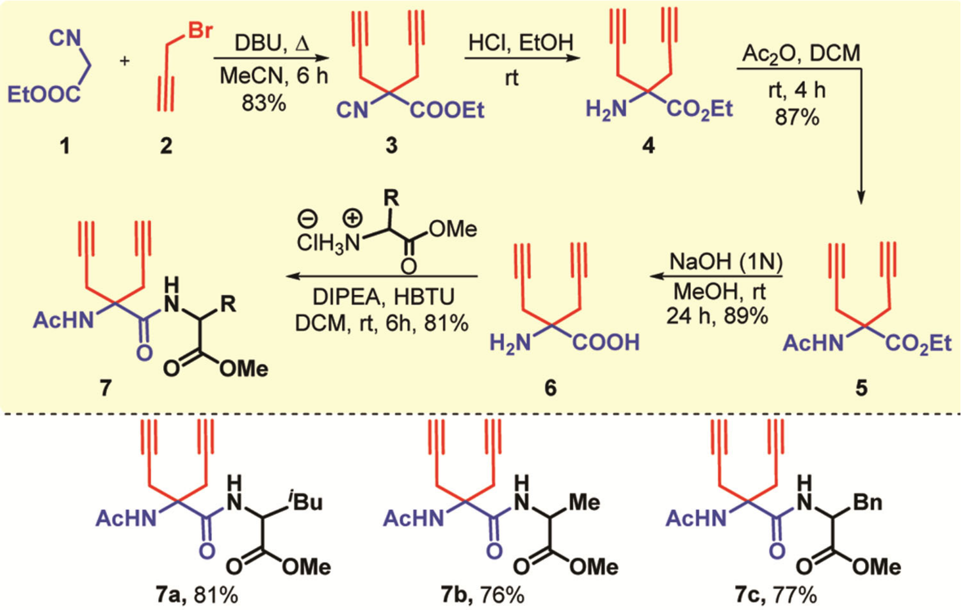


Fig. 1 — Reactivity of diyne in the presence of thioacetic acid and AIBN



Scheme 1 — Synthesis of diyne dipeptide **7** from ethyl isocyanoacetate **1**

anhydride (Ac_2O) at RT in dichloromethane to deliver the *N*-acetyl diyne **5**.

Afterwards, the ester group present in *N*-acetyl diyne amino derivative **5** was hydrolysed using sodium hydroxide (1N) in methanol to afford the free –COOH containing diyne amino acid **6** as a key building block. After obtaining free –COOH containing amino acid **6**, we attempted peptide coupling sequence in the presence of L-Leucine methyl ester hydrochloride, with the aid of *N*,*N*-diisopropylethylamine (DIPEA), and 3-[bis(dimethylamino)methyliumyl]-3*H*-benzotriazol-1-oxide hexafluorophosphate (HBTU) in dichloromethane at RT to deliver various dipeptides **7** in good yields (Scheme 1)[24].

Zade and co-workers have reported an intramolecular radical cascade reaction of diynes to generate the thiophene moiety by using two diyne groups in a single step[11]. In this regard, they used thioacetic acid and azobisisobutyronitrile (AIBN) as a radical initiator in toluene under reflux conditions to assemble thiophene derivatives. In this context, we applied Zade's methodology with our diyne dipeptide **7** to convert to thiophene moiety using a similar reaction condition and we observed a new spot in thin layer chromatography (TLC) plate. By using silica gel column chromatography purification and recording the NMR data, we were unable to identify the signature peaks of thiophene unit in ^{1}H and ^{13}C NMR spectra. Moreover, we found that -COCH$_3$ peak is missing and -CHO peak in ^{1}H NMR as well as in ^{13}C NMR spectroscopy. Upfield shifting of carbonyl peak was observed indicating the formation of diyne dipeptide **9** (Fig. 2, Scheme 2). This result was unexpected, therefore, the structure of the dipeptide **9** was unambiguously established by single crystal X-ray diffraction studies (Fig. 3).

This observation was unexpected and we ventured towards finding a plausible reaction mechanism for the conversion of the –COCH$_3$

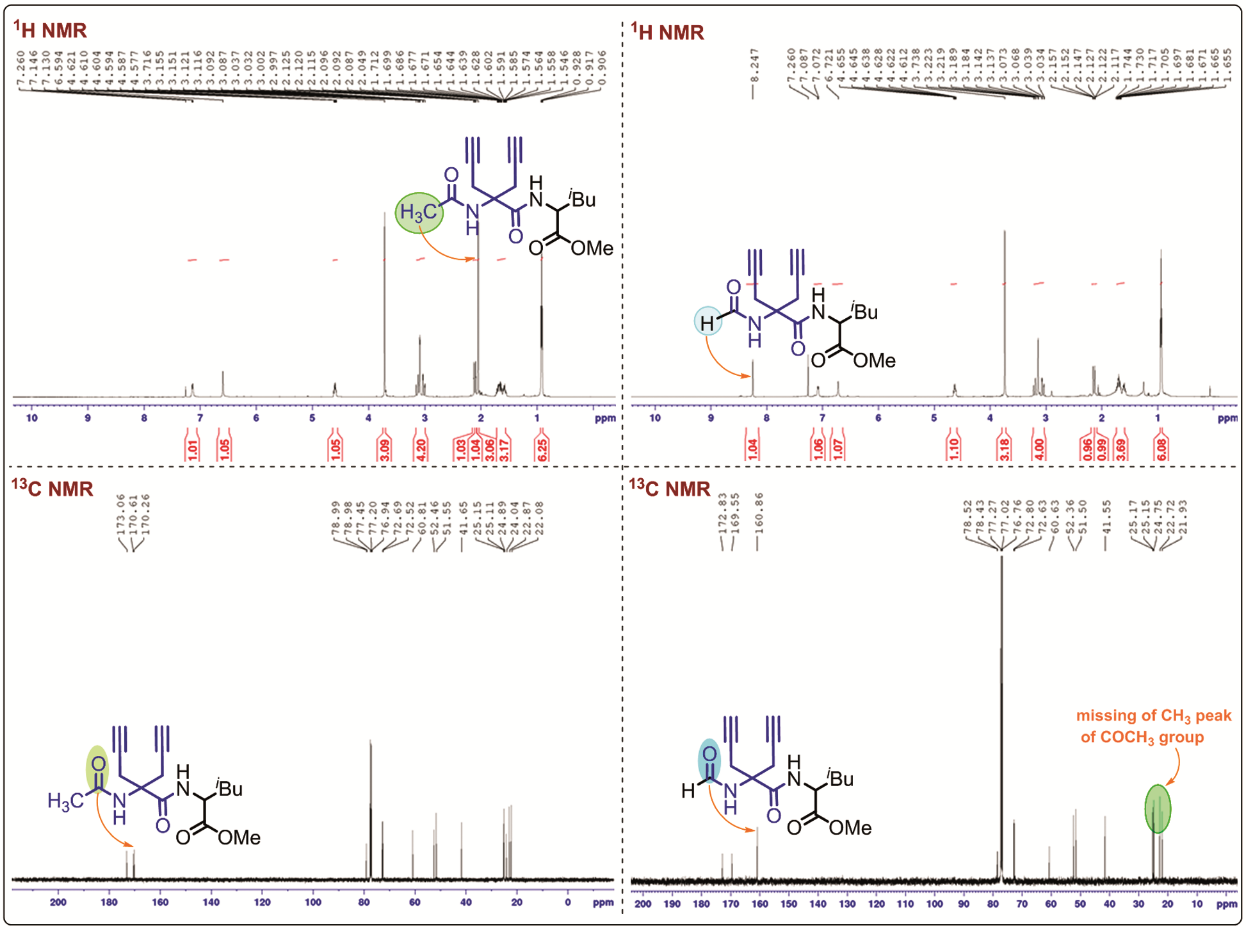
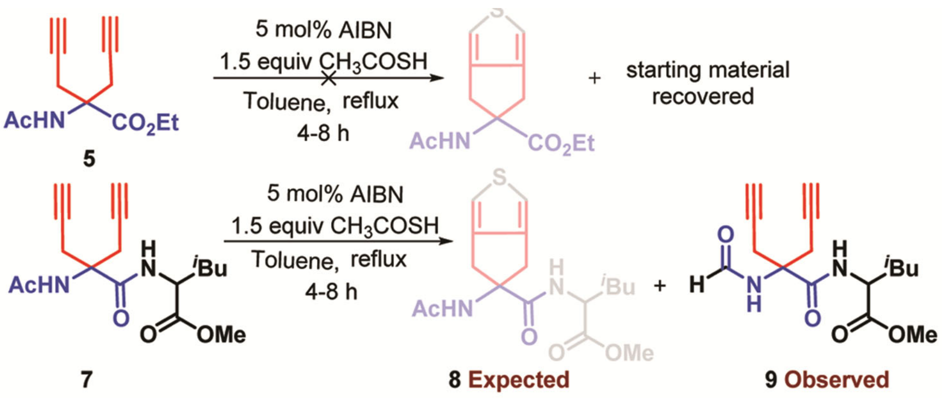


Fig. 2 — Comparison of ^{1}H NMR and ^{13}C NMR spectra of compound **7** and **9**



Scheme 2 — Attempts to synthesize thiophene containing dipeptide **8** and preparation of compound **9**

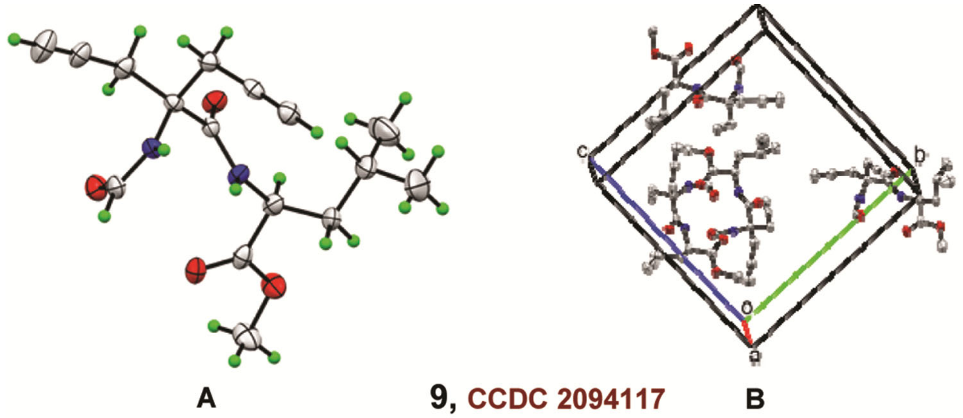
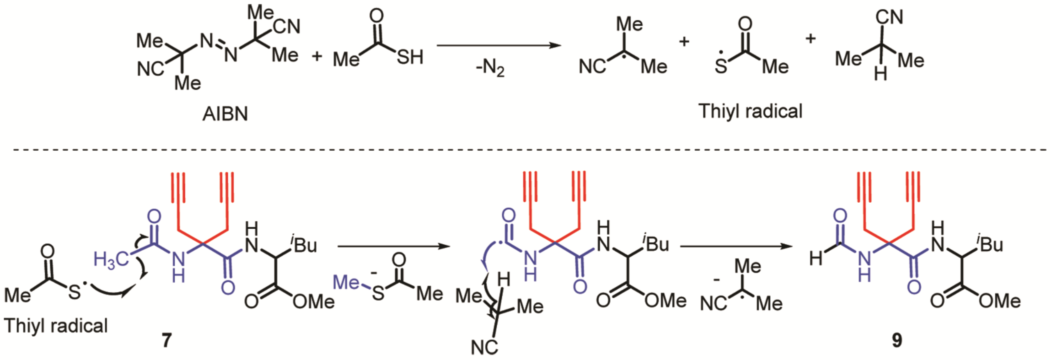


Fig. 3 — (A) The single-crystal XRD structure of the compound **9**. Displacement ellipsoids are drawn at the 50% probability level. (B) Crystal packing of the compound **9** viewed along *a*-axis without H-atom for clarity.



Scheme 3 — Plausible mechanism of the preparation of diyne dipeptide **9**

into –CHO group. It might be initiated by the thiyl radical through a radical cascade at the C–C bond of the $-COCH_3$ in dipeptide **7** and generated carbonyl radical $-\dot{C}O$. After carbonyl radical formation, it takes hydrogen radical ($\dot{H}$) from isobutyronitrile and delivers the desired compound **9** (Scheme 3).

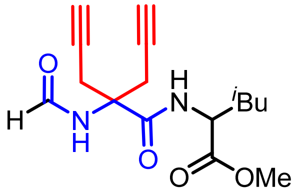
Experimental Section

All commercially available reagents were used as received unless otherwise mentioned. Commercial sources such as Aldrich, Avra and Spectrochem were used for buying chemicals such as ethyl isocyanoacetate, DBU, HBTU, DIPEA, and propargyl bromide. Air or moisture sensitive reactions were carried out under inert atmosphere using a nitrogen balloon and in anhydrous and/or degassed solvents. The reaction progress was monitored by thin-layer chromatography using appropriate combination of ethyl acetate (EtOAc) and petroleum ether. By utilizing an appropriate mixture of EtOAc and petroleum ether, column chromatography was performed by using Acme's silica gel (100–200 mesh) for the purification and isolation of compounds from the reaction mixture. High-resolution mass spectrometry (HRMS) measurements of unknown compounds were done by using Bruker or Micromass Q-ToF spectrometers. The characterization of newly synthesized compounds *via* NMR spectroscopy were accomplished by using Bruker 500 MHz and Bruker 400 MHz (AVANCE IITM) spectrometers. NMR data is expressed in chemical shift (δ, ppm), 0-10 ppm for ^{1}H NMR and 0-250 ppm for ^{13}C NMR and coupling constants *J* in Hertz (Hz). Bruker diffractometer equipped with graphite monochromated MoKα radiation was used for single-crystal X-ray diffraction data collection and CrysAlis and OLEX were used for data reduction and refinement. The melting point (mp's) of solid compounds were recorded from a Buchi 560 melting point instrument and are uncorrected.

Synthesis of peptide 9

A 50 mL round-bottom flask fitted with a reflux condenser was taken and under nitrogen atmosphere, the diyne **7** (100 mg, 1.0 equiv.) and 15 mL dry toluene was transferred to the flask using septum and the mixture was allowed to stir at RT. Thioacetic acid (1.5 equiv. with respect to diyne **7**) and AIBN (5 mol % with respect to diyne **7**) were added to the reaction mixture and the reaction mixture was refluxed. After completion of the reaction (TLC monitoring), the reaction was cooled and the solvent was evaporated under high vacuum. The residue of reaction mixture was washed with water, K_2CO_3 solution and extracted with ethyl acetate. Organic layer was concentrated under high vacuum pressure using rotary evaporator. Further, the crude product was purified by silica gel column chromatography by using ethyl acetate and petroleum ether to afford the compound **9**.

Methyl (2-formamido-2-(prop-2-yn-1-yl)pent-4-ynoyl)-L-leucinate, 9



9

Yield 84 mg (79%). Appearance: colorless solid. m.p.144-146°C. $R_f = 0.6$ (50% EtOAc-petroleum ether); ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.25 (s, 1H), 7.08 (d, $J = 7.5$ Hz, 1H), 6.72 (s, 1H), 4.66-4.61 (m, 1H), 3.74 (s, 3H), 3.22-3.03 (m, 4H), 2.15 (t, $J = 2.5$ Hz, 1H), 2.12 (t, $J = 2.5$ Hz, 1H), 1.74-1.58 (m, 3H), 0.95-0.92 (m, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.8, 169.6, 160.9, 78.5, 78.4, 72.8, 72.6, 60.6, 52.4, 51.5, 41.6, 25.2, 25.2, 24.8, 22.7, 21.9; HRMS (ESI, Q-ToF): m/z Calcd for $C_{16}H_{22}N_2NaO_4$: $[M+Na]^+$ 329.1472. Found: 329.1471.

Conclusions

Serendipitous conversion of $-NHCOCH_3$ group to $-NHCHO$ in dipeptide has been observed by using AIBN and thioacetic acid. The final structure of the peptide **9** has been confirmed by NMR spectroscopy and also by single-crystal X-ray diffraction data. Studies on this observation are under investigation in our laboratory. Our results might be applied by chemists working in the area of peptidomimetics and medicinal chemistry.

Supplementary Information

All experimental procedures and spectral data (^{1}H and ^{13}C NMR, and HRMS) of all new compounds are available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

Acknowledgment

NKG and SA are grateful to IIT Bombay for Institute Post-Doctoral Fellowships (IPDF).

References

1 Buxbaum E, *Fundamentals of Protein Structure and Function*, 2nd Edn (Springer Cham, Switzerland) (2015).
2 Dietzen D J, ‘Amino Acids, Peptides, and Proteins’, in *Principles and Applications of Molecular Diagnostics*, edited by Horvath A R and Wittwer C T (Elsevier Inc., Amsterdam, Netherlands) 2018.
3 Otvos Jr L & Wade J D, *Front Chem*, 2 (2014) 62.
4 Gante J, *Angew Chem Int Ed*, 33 (1994) 1699.
5 Craik D J & Adams D J, *Chem Biol*, 2 (2007) 457.
6 Gentilucci L, De Marco R & Cerisoli L, *Cur Pharm Des*, 16 (2010) 3185.
7 Marek P, Woys A M, Sutton K, Zanni M T & Raleigh D P, *Org Lett*, 12 (2010) 4848.
8 Larsen P K & Bundgaard H, *A Textbook of Drug Design and Development* (Harwood Academic Press, Chur, Switzerland) (1992).
9 Balaram P, *Curr Opin Struct Biol*, 845 (1992).
10 Kotha S & Halder S, *Synlett*, 337 (2010).
11 Kotha S & Brahmachary E, *Bioorg Med Chem Lett*, 7 (1997) 2719.
12 Agrawal A R, Kumar N R, Debnath S, Das S, Kumar C & Zade S S, *Org Lett*, 20 (2018) 4728.
13 Kotha S, Mohanraja K & Durani S, *Chem Commun*, 1909 (2000).
14 Kotha S & Brahmachary E, *Bioorg Med Chem*, 10 (2002) 2291.
15 Kotha S & Ghosh A K, *Tetrahedron Lett*, 45 (2004) 2931.
16 Kotha S, Ganesh T & Ghosh A, *Bioorg Med Chem Lett*, 10 (2000) 1755.
17 Kotha S, Goyal D, Bitra A, Thota N, Kruger G & Anand R, *RSC Adv*, 3 (2013) 24447.
18 Kotha S & Lahiri K, *Bioorg Med Chem Lett*, 11 (2001) 2887.
19 Kotha S & Todeti S, *Beilstein J Org Chem*, 15 (2019) 371.
20 Kotha S, Goyal D, Thota N & Sreenivas V, *Eur J Org Chem*, 2012 (2012) 1843.
21 Kotha S, Meshram M & Panguluri N R, *Synthesis*, 51 (2019) 1913.
22 Kotha S, Goyal D & Chavan A S, *J Org Chem*, 78 (2013) 12288.
23 Kotha S, Gupta N K, Sreevani G & Panguluri N R, *Chem Asian J*, 16 (2021) 3649.
24 For preparation and characterization of compounds **1**-**7**: (a) Kotha S & Brahmachary E, *Tetrahedron Lett*, 38 (1997) 3561; (b) Kotha S, Goyal D, Banerjee S & Datta A, *Analyst*, 137 (2012) 2871 and Reference 23.