Ru(II)-Catalyzed C(sp^2)–H Activation Annulation: Synthesis of Fluorescent Benzoisoquinolonyl Acetate/Peptides from *N*-Arylamides and Ethyne at Room Temperature

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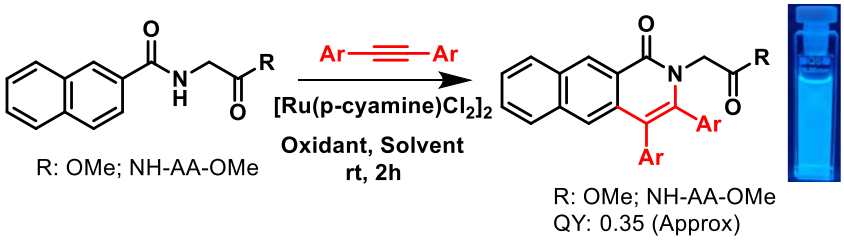


ABSTRACT: Benzoisoquinolones are aryl ring extended isoquinolinone derivatives, constituents of alkaloid natural products. This report describes the synthesis of benzoisoquinolones amino acids/peptides derivatives from respective *N*-aryl amino esters /peptides through Ru-catalyzed C(sp^2)–H annulation at room temperature. Herein, the amino acid ester/amide residue acts as a directing group for annulation at the aryl ring, and then cyclization occurs at the amide NH. Importantly, these benzoisoquinolinones exhibit fluorescence in protic polar solvents. Hence, this methodology could be helpful to transform standard amino acids/peptides into respective fluobenzoisoquinolinones conjugated fluorescent peptide derivatives at room temperature, which could be applicable for leveling amino acid/peptides effectively.

Isoquinolone and its derivatives are *N*-heterocylic aromatic molecules that are constituents of various alkaloid natural products, which have a wide range of pharmacological and physiological activities.[1-4] Their chemical synthesis is well documented by following the traditional synthetic methodology including annulation high atom-utilization and step-economy.[5-8] Recently, the modern synthetic method C-H activation/functionalization has emerged as a powerful technique for the synthesis of substituted heterocyclic molecules from the core skeleton.[9] The transition metal-catalyzed regioselective C-H activations of core aromatic/aliphatic molecules are extensively reported by installing directing groups such as auxiliary, intrinsic, transition, non-covalent interactions.[10] Various transition metal catalyst have been used for the synthesis of isoquinolone derivatives from aryl amide and ethyne molecules via C-H activation annulation reaction.[11] Recently, Ru(II)-catalyzed C−H activation via [4 + 2] annulation is performed with *N*-chlorobenzamides and 1,3- diynes at high temperature that yield the isoquinolone derivatives.[12] The specificity and efficacy of small drug molecules have significantly improved by conjugation with biomacromolecules peptides/protein.[13-16] Fluorophore conjugated therapeutic peptides have been synthesized to examine their cell permeability, cellular localization, and interaction with other biomolecules in real time inside cells with fluorescence microscopes. To treat cancer and deliver medications to specific locations peptide conjugation to bioactive substances including lipids, carbohydrates, and pharmaceuticals is widespread practise.[17-18] Real-time tracking in physiological settings requires peptide ligation to fluorescent labels, which is in great demand.[19-20] Selective peptide modification is a desirable strategy in drug discovery to enhance bioavailability, metabolic stability, and membrane permeability.[21-24] Post synthesis modifications of peptides are being accomplished by C-H activation that provide an opportunity for the synthesis of fluorescent/drug molecule conjugated peptides. Structurally, isoquinolone molecule has conjugated double bond skeleton comprising electron donor (phenyl) and acceptor (amide ring) groups that exhibit fluorescence properties due to push-pull effects. Peptide backbone diversification employing the amide bonds of peptide backbone acting as the bidentate directing group through Ru(II)-catalyzed C−H activation/annulation (Figure-1a).[25] Lys-based peptides are modified chemo- and site selectively using Rh(III)-catalyzed C-H activation/annulation (Figure-1b).[26] Since these peptide modification requires harsh reaction condition or installation of directing group, here in we report eco-friendly and versatile Ru-catalysed C-H activation/annulation in *N*-

napthoyl amino ester/peptides in room temperature in a minimal duration of 2h. The modification of a peptide backbone by a Ru-catalyst at room temperature is being reported for the first time in this work.

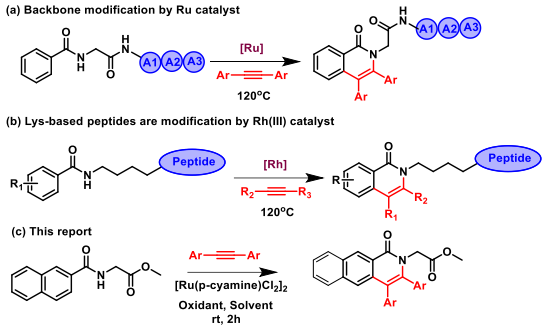


Figure 1. Annulation reactions of various isoquolone

Recently, Erik V. Van der Eycken have reported the Ru catalyzed C(sp^2)–H activation/annulation of amino acid derivative at high temperature (100°C)[27-28]. It would be economical to achieve such reactions with amino acids/peptides at room temperature. Herein we planned to investigate the Ru(II) catalyzed C(sp^2)–H activation/annulation of amino acid derivatives at high temperature (100°C). In Scheme 1, 2-Napthoic acid (**1a**) was conjugated with the amine group of amino acid ester derivatives (Gly-OMe/Val-OMe/Pne-OMe) and alkyl amine (octyl/^{tert}Butyl) under peptide coupling conditions that produced respective 2-Napthylamides (**1b-1f**). First, we explored the annulation reactions at Napthoic acid (**1a**) with diphenyl acetylene (**2**) in presence of catalyst [Ru(cymene)Cl_2]$_2$ and oxidant $Cu(OAc)_2$ in HFIP at rt for 2 h that gave lactone (**3a**) owing to the C(sp^2)–H activation/annulation reactions. We examined similar Ru(II) catalyzed annulation reactions with diphenyl acetylene (**2**) at 2-Napthylamide glycine ester (**1b**) under different solvents and oxidants at room temperature as described in Table 1 (Entry 1-8) and Table S1. The optimized condition of Ru(II)-catalyzed annulation reaction with 2-Napthylamide (**1b**) and ethyne (**2**) is [Ru(*p*-cymene)Cl_2]$_2$ (5.0 mol%) and oxidant $Cu(OAc)_2$ in solvent HFIP at room temperature that produced benzoisoquinolone derivative (**3b**) in good yield (Table 1, Entry 11). The product (**3b**) was characterized by NMR and ESI-HRMS. Pleasantly, we obtained the crystal structure by X-ray diffractometer data analyses with CCCD no. 2310339. Other solvents, such as TFE, DCE, ^{t}AmOH gave lower yields whereas solvents like DMF, DMSO, ^{t}BuOH and Toluene gave trace amount of product (Table 1). We performed the similar Ru(II) catalyzed annulation reactions with other 2-Napthylamides (**1c-1f**) and ethyne (**2**) but could not encouraging results as 2-Napthylamides of Valine ester (**1c**) and octyl amine (**1f**) gave in trace amount while no annulated product from Phenylalanine ester and *tert*-butylamine, possibly due to steric factors of bulky group.

Next, we evaluated the scope of the methodology by performing the similar C-H annulation with other substrates 1-Napthamides (**4a-4c**) and diphenyl 1,2-diphenyl acetylene (**2**) under optimized conditions of annulation reactions (Scheme 2a). We noticed the formation of annulated products lactone (**5a)** and benzoisoqunolinone (**5b**) in good yield at room temperature while trace amount of **5c** from respective 1-Napthamides.

Scheme 1. Ru-catalyzed annulation reaction of 2-Napthamides

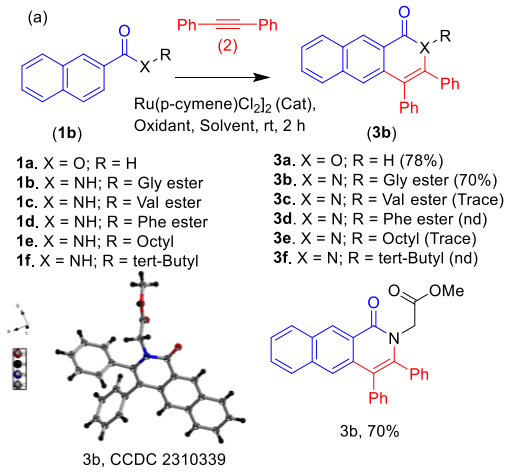


Table 1. Optimized reaction conditions

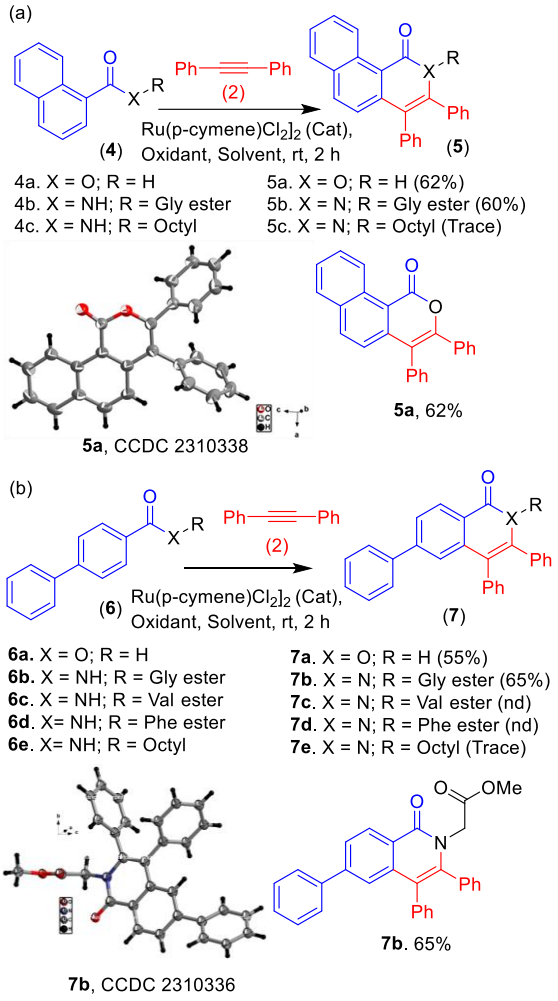
Entry	Solvent	Oxidant	T (°C)	Yield (%)
1	TFE	AgOAc	rt	60
2	t-Am-OH	AgOAc	rt	50
3	TFE	Ag_2CO_3	rt	53
4	TFE	CsOAc	rt	45
5	HFIP	$Cu(OAc)_2$	rt	70
6	DCE	AgOAc	rt	40
7	HFIP	$Cu(OAc)_2$	rt	43
8	HFIP	$Cu(OAc)_2$	rt	54

1a (50 mg, 0.2 mmol, 1 eq.), **2** (54.9 mg, 0.3 mmol, 1.5 eq.), [Ru(*p*-cymene)Cl_2]$_2$ (6.3 mg, 0.05 mmol, 5 mol%).

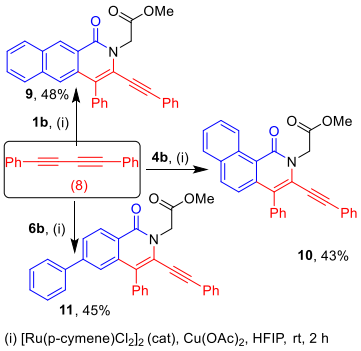
The isolated products were characterized by NMR and HRMS**.** We also obtained the single crystal of aryl lactone derivative (**5a**) and studied by X-ray diffractometer that confirmed structure of lactone **5a** with CCDC 2310338. In repertoire of substrate scope expansion, we 1,4-biphenyl carboxylic acid (**6a**) with amino group of amino acid ester (Gly-OMe, Val-OMe, Phe-OMe)/octylamine under peptide coupling conditions that produced respective arylamides (**6b-6e**). These derivatives (**6a-6e**) were treated with diphenyl acetylene in under optimized conditions of Ru(II)-catalyzed C-H annulation. We obtained the annulated aryl lactone (**7a**) and phenyl isoquinolone ester (**7b**) in good yield. Interestingly, the structure of compound **7b** was characterized by single crystal X-ray analyses with CCDC 2310336. However, arylamide **6c/6d** did not give desired products owing to the steric hindrance at α-carbon amino ester and aliphatic amines. Aryl amide (**6e**) of octyl amine gives in trace amount of phenyl isoquinolone (**7e**).

Next, we examined the C-H annulation with different alkyne 1,4-diphenylbuta-1,3-diyne (**8**) and glycinate derivatives **1b/4b/6b** that formed the corresponding benzoisoquinolones (**9-11)** at room temperature through Ru(II) catalyzed C-H annulation (Scheme 3)**.**

Scheme 2. Ru-catalyzed annulation reaction of 1-Napthamides

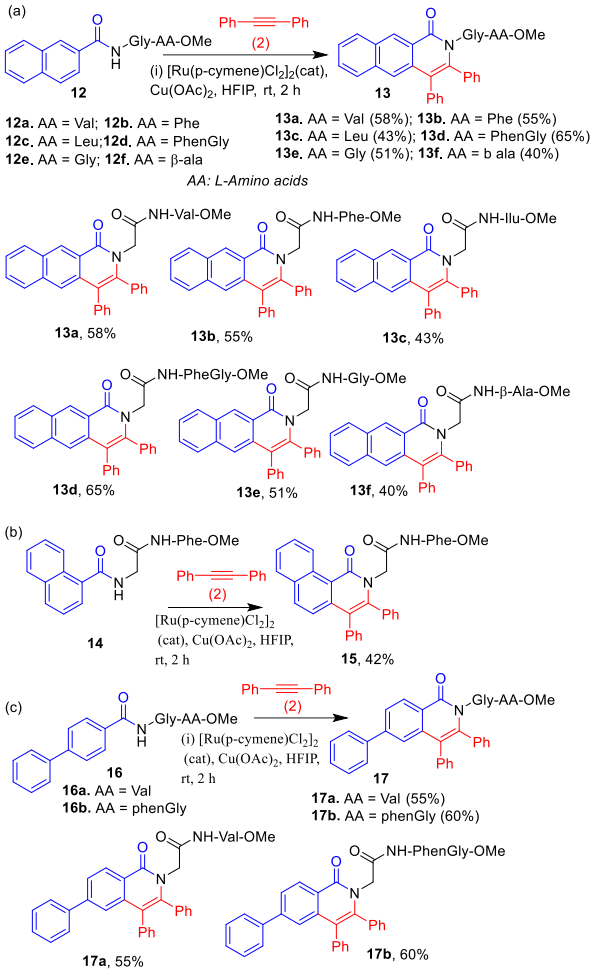


Scheme 3. Ru-catalyzed annulation reaction with other ethyne



These results encouraged us to explore annulation reactions at peptides. Thus we synthesized 2-Napthamide-dipeptides (**12a-12f**) and then treated with diphenyl ethyne (**2**) under Ru(II) catalyzed optimized conditions (Scheme 3a). 2-Napthamide-dipeptides (**12a-12f**) derived from glycine and other amino acid derivatives (Phe-OMe, Leu-OMe, Phenglycine-OMe, Gly-OMe, *β*-Ala-OMe). These aryl dipetides (**12a-12f)** gave respective benzoisoquinolones (**13-13f)** in good yields (40−65%). Their characterization data are provided in SI. In Scheme 3b, we also synthesized 1-Napthamide-dipeptides (**14)** and demonstrated the formation of benzoisoquinolone peptide derivative (**15**) under optimized condition Ru(II) catalyzed annulation reactions. In Scheme 3c, 1,4-biphenylamide-dipeptides (**16a-16b**), prepared from respective dipeptides Gly-Phe-OMe and Gly-Phengly-OMe, were subjected for Ru(II) catalyzed annulation under optimized conditions with diphenyl ethyne (**2**) that gave respective phenyl isoquinolone peptide derivatives (17**a-17b**) in good yield.

Scheme 3. Ru-catalyzed annulation reaction in peptides



Based on the above results and previous reports, we proposed a plausible mechanism (Figure 2). First, $[RuCl_2(p\text{-cymene})]_2$ undergoes ligand exchange with $Cu(OAc)_2$ to give the active catalytic species, which coordinates to the nitrogen atom of the N-benzoyl glycine ester moiety via NH deprotonation. This is followed by C-H activation through elimination of AcOH, forming the five membered ruthenacycle. Further coordination of ethyne (**2**), followed by insertion and reductive elimination afforded the final product isoquinolone derivative (**3**). The active catalyst species is then regenerated by $Cu(OAc)_2$ and air for the next catalytic cycle. The extended phenyl ring at benzamide enhances the coordination of Ru(II)-peptide complex that felicitate the C(sp^2)-H activation room temperature possibly due to lowering energy barrier.

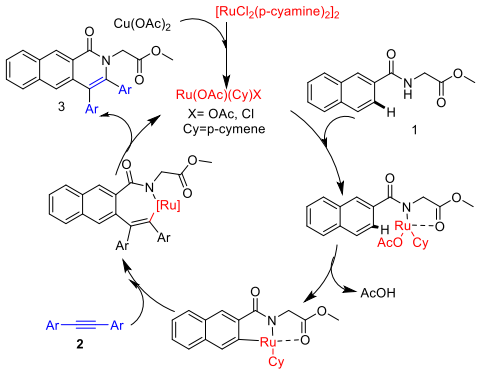


Figure 2. Catalytic cycle of Ru-catalyzed annulation reactions at isoquinolone of amino acid derivatives.

Since the isoquinolone-Lys peptide derivatives reportedly exhibits fluorescence characters.[29] We also noticed that benzoisoquinolone derivatives exhibit fluorescence. Thus, we explored the elementary photophysical properties (Absorbance and Emission) of representative benzoisoquinolone-peptide molecules (**3b/5b/10/13a-13c/15**) in methanol (MeOH) due to better solubility. Their UV-Vis and fluorescence spectra are provided in Figure 3 and SI. We also extracted their photophysical parameters, which are tabulated in Table 1 and Figure S2. The quantum yield of benzo[g]isoquinolones acetate (**3b),** ~30%, is significantly low as compared to its peptides (**13a-13c**), ~35-40%, (Table 1, Entry 1, 4-6). However, the quantum yield of other benzoisoquinolone acetate (**5b**), ~19 is high as compared to its peptide **15,** ~14% but lower than **3b**. Quantum yield of phenyl ethyne benzoisoquinolone acetate (**10**), ~9%, is remarkably lower than other derivatives (**3b**/**5b/13a-c/15)**. The Stokes shift of benzo[g]isoquinolones ester (**3b**)/peptide (**13a-13c**) is ~120 nm higher than other benzoisoquinolones (**5b/10/15**), ~50-70 nm. To examine the enhancement of quantum yield after peptide formation, we calculated the HOMO-LUMO diagram of and its energy difference by using Gaussian software through DFT (B3LYP) calculation using basis set 6-311+G(2D,P).[30] The optimized structure of rationally designed amide derivative (**3b'**) shows the formation of hydrogen bonding, amide N-H with benzoisoquinolone carbonyl (N-H----O=C) as shown (see SI, Figure S77). We also calculated is HOMO-LUMO diagram and energy difference by 3.7eV. Its HOMO diagram shows the major contribution from Benzoisoquinolone residue and minor from amide/side chain phenyl residue. Its LUMO diagram, in contrast, only benzoisoquinolone residue. Its hydrogen bonding possibly felicitating better the push-pull effect as compare to ester derivatives (**3b**) that enhances fluorescence quantum yield. For practical utility as fluorescence biomolecule probe, we investigated cell cytotoxicity and cell internalization with one compound **13b**. Our MTA studies show that this derivative has negligible cell toxicity at low concentration with both normal (HEK-293T) and cancerous (HeLa) cells (Figure S78-80). The cell internalization study by confocal microscope revealed that the derivative (**13b**) entered HeLa cells nucleolus (see SI, Figure S81). Hence benzo[g]isoquinolones could utilize as fluorescence probes.

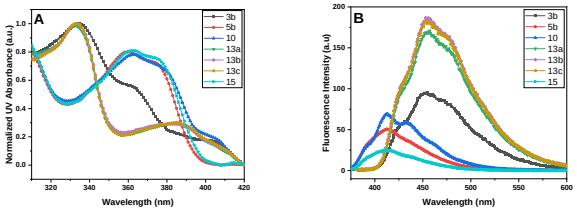


Figure 3. Normalized UV-Vis spectra (A); and Fluorescence Spectra (B) in protic polar solvent MeOH.

Table 2. Photophysical Parameters of Annulated Peptides

Entry	Compound[a] ($\lambda_{ex,\ em}$) nm	Σmax ($M^{-1}cm^{-1}$)	QY[a]	Stokes Shift
1	**3b** ($\lambda_{334,\ 453}$)	9670	0.30	119
2	**5b** ($\lambda_{360,\ 413}$)	8610	0.19	53
3	**10** ($\lambda_{363,\ 433}$)	8440	0.09	70
4	**13a** ($\lambda_{333,\ 454}$)	3730	0.42	121
5	**13b** ($\lambda_{335,\ 454}$)	3120	0.35	119
6	**13c** ($\lambda_{334,\ 455}$)	3940	0.37	121
7	**15** ($\lambda_{362,\ 413}$)	4610	0.14	51

[a]With reference benzoquinine/H_2SO_4 solution. Excitation (ex) and Emission (em); QY: Quantum Yield

In conclusion, we developed a flexible and effective method for chemo selective annulation of *N*-Naphthoyl amino acid/peptides with 1,2-diphenylethyne that offers fair to outstanding regioselectivity in a time-efficient manner in ambient temperature. We suggest that the NH-amido and carbonyl ester/peptide groups both play a significant role in the catalytic reaction based on the experimental findings and spectroscopic investigations. This technique was additionally expanded to include the synthesis of isoquinoline with 1,4-diphenylbuta-1,3-diyne. Importantly, these derivatives exhibit fluorescence with quantum yield of ~42%. and are non-toxic to Hek-293T cell line. They also have nucleus binding property which was evident from cell internalization studies.

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Author Contributions

MKG and NKS have involve in synthesis and characterization. AP has studied fluorescence and cell internalization .

ACKNOWLEDGMENT

We thank NISER-DAE for the planned budget (RIN4002) for the basic research. We thank Dr. Supriya Kumari for helping us in obtaining confocal imaging of Hela cells.

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