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Ruthenium-catalyzed intermolecular alkene–alkyne couplings in biologically relevant media†

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Cationic cyclopentadienyl Ru(II) catalysts can efficiently promote mild intermolecular alkyne–alkene couplings in aqueous media, even in the presence of different biomolecular components, and in complex media like DMEM. The method can also be used for the derivatization of amino acids and peptides, therefore proposing a new way to label biomolecules with external tags. This C–C bond-forming reaction, based on simple alkene and alkyne reactants, can now be added to the toolbox of bioorthogonal reactions promoted by transition metal catalysts.

Introduction

The development of bioorthogonal reactions has brought a paradigm shift in the potential of synthetic chemistry to impact the fields of chemical biology and biomedicine.[1] Within the "toolbox" of bioorthogonal reactions, those involving transition metal catalysis are particularly appealing, as they avoid the need of strained reactants and benefit from the versatility and tuning possibilities of the transition metal reagents.[2] Progress in this area has been sluggish, mostly because of the established notion that transition metal catalysts are not compatible with aqueous and biological milieu. However, recent years have witnessed a substantial growth of the field, especially in the development of uncaging reactions entailing bond-breaking processes, such as the removal of *N*-alloc groups.[3]

Related bioorthogonal reactions involving bond-forming processes are much less common and, so far, mainly restricted to the construction of carbon–heteroatom bonds, especially using Click-like cycloadditions.[4] Accordingly, the development of transition metal catalyzed reactions that forge carbon–carbon bonds in biorelevant media has clearly lagged behind. Most reported examples consist of Suzuki and related C–C cross-couplings promoted by palladium catalysts.[5] A handful of other reactions that form C–C bonds in biological settings, including gold-promoted cyclizations or ruthenium-catalyzed metathesis, have also been sporadically described.[6,7] In this context, we have recently reported a ruthenium catalyzed carbon–carbon bond-forming reaction using alkynes as reaction partners. Specifically, we demonstrated the viability of carrying out formal (2 + 2 + 2) annulation between diynes and alkynes, under biologically relevant environments, using CpRu-based catalysts (Fig. 1a).[6e] The excellent bioorthogonality of the reaction stems from the absence of alkyne functional groups in native biomolecules, and from the intrinsic metal chelating effect of tethered 1,*n*-diynes, which are well posed to generate the required ruthenacyclic intermediates **I** (Fig. 1a).

We next questioned whether simpler, monounsaturated alkyne or alkene precursors could also be used for fully intermolecular C–C ligation reactions in aqueous and biological buffers. Towards this aim, we paid attention to the ruthenium-promoted cross-coupling between alkenes and alkynes, an Alder-ene type of process that proceeds *via* ruthenacyclopentane intermediates of type **II** (Fig. 1b).[8] Although the reaction has been widely used in synthetic chemistry, in organic solvents, it has also proven compatible with protic solvents and with small

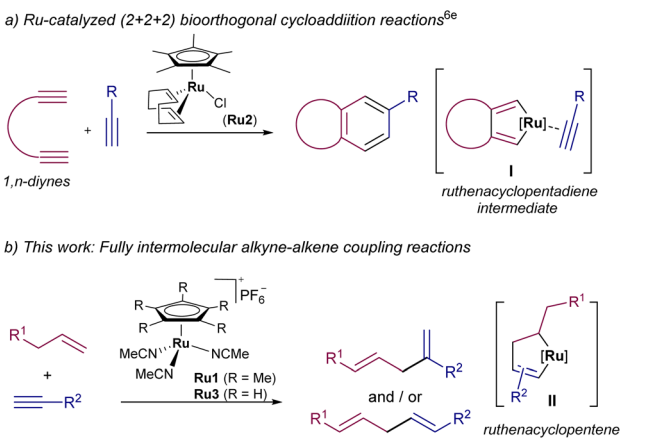

Fig. 1 Ru-catalyzed biocompatible C–C bond forming processes involving ruthenacyclic intermediates.

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amounts of water,[8c] which encouraged us to explore the viability of this C–C bond forming reaction in biorelevant aqueous media.[9] In addition to the intrinsic challenges of bioorthogonality and aqueous compatibility, the reaction may also present chemo- and regioselectivity issues. However, in case of success, it would be a valuable new ligation tool in chemical biology, particularly considering the simplicity of the coupling partners, and the ease with which alkenes and alkynes can be directly incorporated into different types of biomolecules.[10]

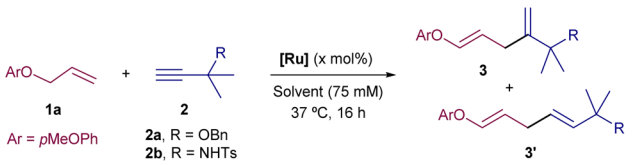
Herein, we demonstrate the viability of the approach, by demonstrating that the intermolecular coupling between alkenes (**1**) and alkynes (**2**) to deliver 1,4-dienes (**3** and or **3′**) can be carried out in aqueous buffers or in complex media like DMEM, using the Ru(II) complex **Ru1**. Interestingly, the regioselectivity of the process [*i.e.* formation of branched (**3**) *vs.* linear (**3′**) isomers] can be controlled by appropriate selection of the catalyst and/or the type of reactants. Finally, we also show that the reaction can be used to selectively label peptides in water at low micromolar concentrations, which provides good prospects for its further use as bioconjugation tool.

Results and discussion

Our first experiments were carried out with the allyl ether **1a** and the propargyl benzyl ether **2a** as model substrates, using the cationic complex **Ru1** as catalyst (10 mol%).[8] In consonance with the reported precedents,[8] we observed that the reaction could be performed in organic solvents, such as THF, acetone and CH_2Cl_2 (75 mM) although the yields of **3aa** were modest, from 30 to 40% (Table 1, entries 1–3). The reaction can also be performed in water, with similar yields (entry 4), but more importantly, the incorporation of THF as co-solvent (water : THF = 8 : 2) allowed to increase the yield up to a good 70% (entry 5). In all these cases, the regioselectivity was high, favouring the formation of the branched product **3aa**, which was exclusively obtained as *E*-isomer.

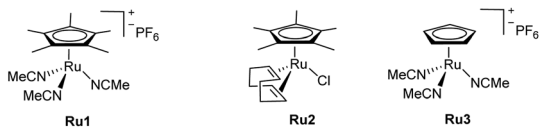
Under otherwise identical reaction conditions, the use of the neutral complex Cp*Ru(cod)Cl (**Ru2**) led to complete recovery of the starting materials (entry 6). With the cationic trisacetonitrile complex **Ru1**, we observed that the amounts of co-solvent and catalyst could be decreased down to 10% vol (THF) and 5 mol% (**Ru1**), without causing a severe impact on the yield and/or selectivity (entry 7). On the other hand, the use of a more polar alkyne precursor, such as the sulphonyl amide derivative **2b**, led to a more efficient reaction, probably due to its higher solubility in aqueous mixtures. Thus, the coupling of **1a** with **2b** in a H_2O : THF mixture (8 : 2), promoted by 5 mol% of **Ru1**, led to the corresponding diene product **3ab** in an excellent 99% yield (entry 8). Lowering the amount of THF (entry 9), using other cosolvents such as EtOH, *t*BuOH or DMSO (entries 10–12), and even performing the reaction in pure water (entry 13) was also possible, leading in all cases to the desired product, **3ab**, in moderate to excellent yields. On the other hand, although slightly higher yields are obtained under an atmosphere of N_2, or using degassed solvents, the reaction is perfectly efficient under air and open-bottle solvents (entries 14 and 15). Interestingly, the regioselectivity of the coupling could be inverted by using the related catalyst **Ru3**, which features a less bulky cyclopentadienyl (Cp) ligand, instead of the pentamethyl derivate (Cp*, entries 16 and 17). This divergence can be tentatively rationalized considering the putative ruthenacycle intermediates that respectively evolve to the branched (**3**) and linear (**3′**) isomers (**Int1** and **Int-1′**, Fig. 2). In particular, the steric clash in **Int-1′**, between the bulkier Cp* ligand and the alkyne propargylic substituents, would hamper the evolution towards the more stable linear isomer, **3′** (Fig. 2).[11]

Table 1 Preliminary screening of the Ru-catalyzed alkyne–alkene coupling under aqueous conditions[a]



Entry	2	Solvent	[Ru] (*x* mol%)	Regio (**3** : **3′**)	**3**, % yield
1	**2a**	THF	**Ru1**, 10	>9 : 1	**3aa**, 32
2	**2a**	Acetone	**Ru1**, 10	>9 : 1	**3aa**, 30
3	**2a**	CH_2Cl_2	**Ru1**, 10	>9 : 1	**3aa**, 40
4	**2a**	H_2O	**Ru1**, 10	>9 : 1	**3aa**, 36
5	**2a**	H_2O/THF (8 : 2)	**Ru1**, 10	>9 : 1	**3aa**, 70[b]
6	**2a**	H_2O/THF (8 : 2)	**Ru2**, 10	>9 : 1	**3aa**, 0
7	**2a**	H_2O/THF (9 : 1)	**Ru1**, 5	>9 : 1	**3aa**, 56
8	**2b**	H_2O/THF (8 : 2)	**Ru1**, 5	>9 : 1	**3ab**, 99
9	**2b**	H_2O/THF (9 : 1)	**Ru1**, 5	>9 : 1	**3ab**, 68
10	**2b**	H_2O/EtOH (8 : 2)	**Ru1**, 5	5 : 1	**3ab**, 88
11	**2b**	H_2O/^{t}BuOH (8 : 2)	**Ru1**, 5	>9 : 1	**3ab**, 77
12	**2b**	H_2O/DMSO (8 : 2)	**Ru1**, 5	>9 : 1	**3ab**, 45
13	**2b**	H_2O	**Ru1**, 10	>9 : 1	**3ab**, 53
14[c]	**2b**	H_2O/THF (8 : 2)	**Ru1**, 10	>9 : 1	**3ab**, 97
15[d]	**2b**	H_2O/THF (8 : 2)	**Ru1**, 10	>9 : 1	**3ab**, 78
16	**2a**	H_2O/THF (8 : 2)	**Ru3**, 10	1 : 6	**3aa′**, 40[e]
17	**2b**	H_2O/THF (8 : 2)	**Ru3**, 10	1 : 7	**3ab′**, 99[f]

[a] Conditions: alkene **1a** (0.075 mmol), alkyne **2** (0.075 mmol), the degassed solvent (1.0 mL) and the [**Ru**] catalyst (*x* mol%) were stirred under N_2 at 37 °C for 16 h, under otherwise noted. Yields and branched to linear (**3** : **3′**) ratios determined by ^{1}H-NMR using dimethylsulfone as internal standard. [b] 61% isolated yield. [c] Carried out with non-degassed solvents. [d] Carried out under air and non-degassed solvents. [e] 33% isolated yield. [f] 78% isolated yield.



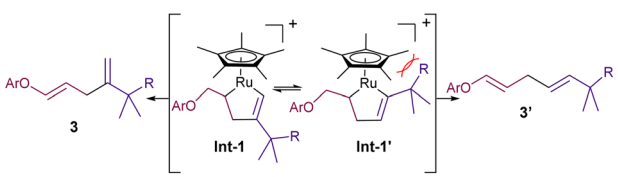
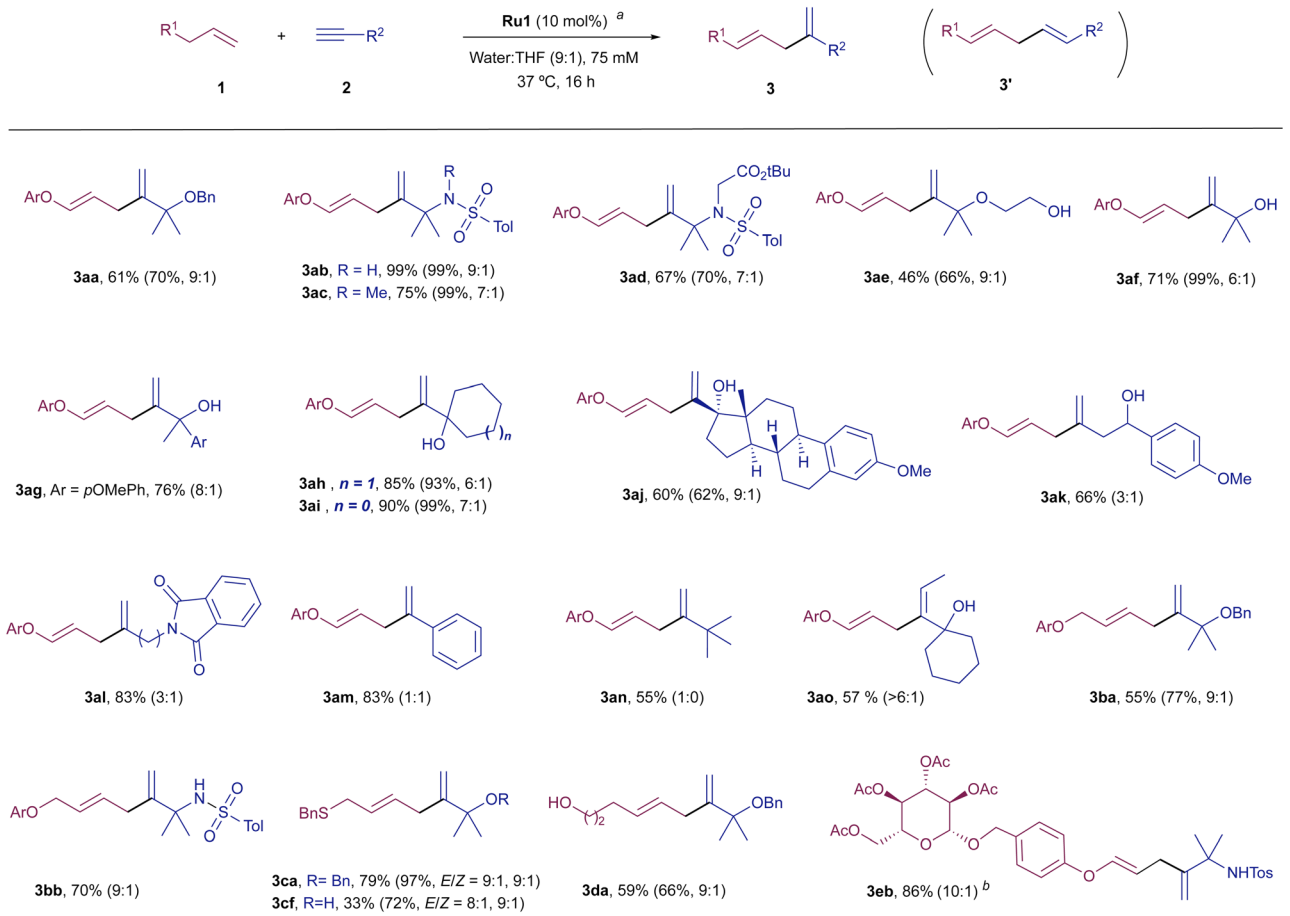


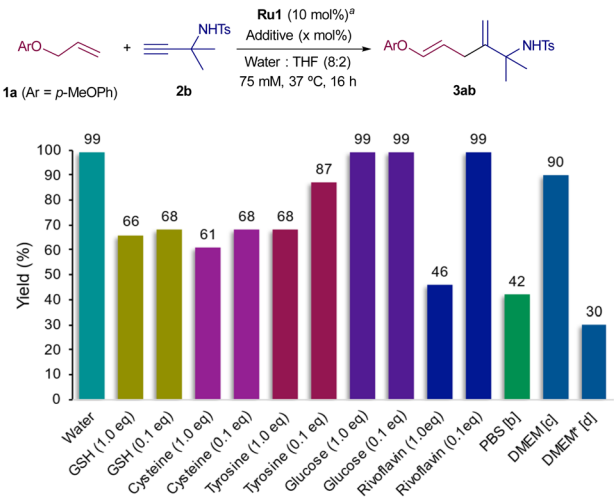
Fig. 2 Proposed ruthenacyclic intermediates leading to **3** and **3′** regioisomers.



Scheme 1 [a]Conditions: alkene (**1**, 0.15 mmol), alkyne (**2**, 0.15 mmol), H_2O : THF (8 : 2, 2.0 mL) and **Ru1** (10 mol%). Yields of pure branched product **3** (**3** : **3′** > 10 : 1) unless otherwise noted; only the branched structure is represented. Ar = *p*MeO(C_6H_4). NMR yield and branched: linear ratios (**3** : **3′**) using dimethyl sulfone as internal standard are given in parenthesis. [b]Carried out using 2.0 equiv. of alkene (0.30 mmol).

With these optimal conditions in hand, we analysed the scope of the Ru-catalyzed coupling with different alkene and alkyne partners (Scheme 1). Regarding the alkyne, several other propargylic systems, including sulphonamides **2b–2d**, other ethers like **2e** and propargylic alcohols (**2f–2j**), efficiently participate in the coupling with the model alkene **1a**, providing their respective diene products **3ab–3aj**, in good to excellent yields and with high branched selectivity. Therefore, both alkyl and aryl substituents are allowed at the propargylic position.

The presence of two of these substituents at the propargylic center is key to achieve a good branched-to-linear ratio. Thus, the Ru-catalyzed coupling of **1a** with alkynes that hold secondary carbons at the propargylic position, such as **2k** or **2l**, provided the corresponding dienes, **3ak** and **3al**, with a lower branched-to-linear ratio (**3 : 3′** = 3 : 1), although in good overall yield (66 and 83%, respectively). The presence of an aromatic moiety, such as in phenylacetylene led to a non-regioselective coupling but proceeded in good overall yield (**3am : 3am′** = 1 : 1, 83% yield). Gratifyingly, we were pleased to observe that the presence of heteroatoms at the propargylic position is not mandatory to achieve good selectivity. Thus, the reaction of **1a** with 3,3-dimethylbut-1-yne, **2n**, provided the diene product **3an**.



Scheme 2 Bioorthogonality of the Ru-catalyzed alkene–alkyne coupling. [a] Conditions: alkene (**1**, 0.075 mmol), alkyne (**2**, 0.075 mmol), water : THF (8 : 2, 1.0 mL) and **Ru1** (10 mol%) unless otherwise noted. NMR yield using dimethylsulphone as internal standard; [b] PBS used as solvent instead of water; [c] DMEM used as solvent (DMEM = Dulbecco's modified eagle medium); [d] DMEM* used as solvent (DMEM* = DMEM + 10 fetal bovine serum + 1% antibiotics).

Indeed, even an internal alkyne can engage quite efficiently in the reaction, provided that it bears an adjacent fully substituted propargylic center, such as in **3ao**.[12]

With respect to the alkene partner, the reaction of model alkynes **2a** and **2b** could also be performed with an homoallyl ether like **1b**, rendering their respective products (**3ba** and **3bb**) in good yields (9 : 1 ratio). The use of a homoallyl thioethers is also possible, so that products **3ca** and **3cf** were obtained in moderate to good yields. Curiously, in these cases, small amounts of their respective *Z*-isomers could also be detected. Structurally simple alkenyl precursors, such as hex-5-en-1-ol, or more complex derivatives, like the pyranoside **1e** are equally efficient partners, so that their corresponding products, **3da** and **3eb** were respectively obtained in good yields (59 and 86% yield) and high regioselectivities.[13]

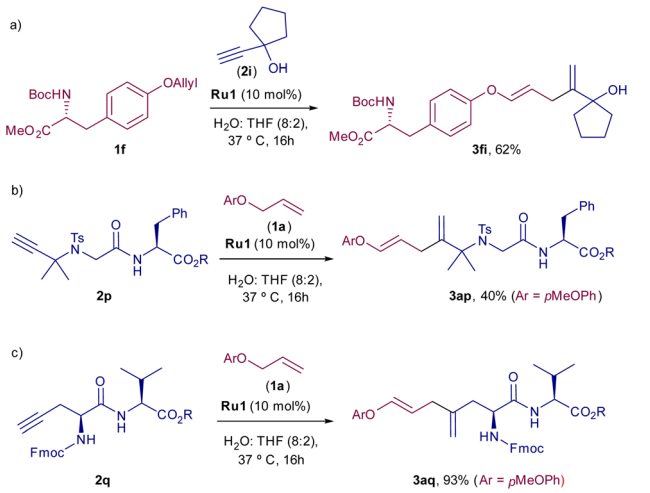
Next, we focused on studying the bioorthogonality of the above cross-couplings, by using aqueous media containing biologically relevant additives (Scheme 2). Gratifyingly, the coupling between **1a** and **2b**, promoted by **Ru1** (10 mol%), proceeds with similar yields in the presence of 0.1 equivalents of different amino acids like tyrosine or cysteine, and of biorelevant thiols such as glutathione. The presence of other additives such as glucose or vitamins like riboflavin neither affected the reaction. Moreover, increasing the amount of any these biomolecules up to 1 equivalent (10 times the amount of catalyst) did not compromise the alkyne–alkene coupling, so that the corresponding product, **3ab**, was obtained in yields varying from 46 to 99%.

Importantly, we found that the reaction is also feasible in PBS (phosphate buffer solution 1×. pH = 7.4), as well as in a cell culture milieu like DMEM, to give in this case **3ab** in an excellent 90% yield. When the reaction was carried out in DMEM*, which includes 10% of fetal bovine serum and a few antibiotics, the product could still be obtained, albeit in a lower 30% yield, which is still satisfactory considering that this serum is a cocktail of hormones, lipids, and different type of proteins.

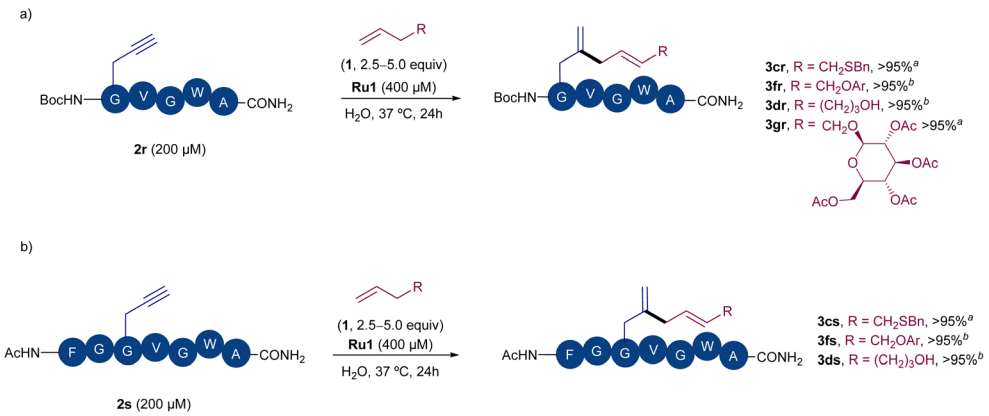
Overall, the observed efficiencies compare favorably with other bioorthogonal reactions catalyzed by ruthenium complexes, such as the hydrosylilation of alkynes or alkene cross metatheses.[14]

Considering the good orthogonality to biological components, we next checked whether the alkene–alkyne cross-coupling could also be used as bioconjugation tool, to chemoselectively modify amino acids containing either an alkene or an alkyne moiety. Gratifyingly, as can be deduced from Scheme 3, the coupling of *N*-Boc *O*-allyl tyrosine **1f** with **2i** proceeded efficiently to provide the diene **3fi** in 62% yield (Scheme 3a). Moreover, the use of amino acids bearing an alkyne moiety is also compatible with the process. Thus, dipeptide **2p**, bearing a pendant alkyne at the N-terminal position, or peptide **2q**, featuring a propargyl glycine, participated efficiently in the coupling with **1a**, with just 10 mol% of the Ru catalyst, leading to their respective products, **3ap** and **3aq**, in moderate to excellent yields (Scheme 3b and c).

Encouraged by these results, we next checked whether the method could also be used to label larger peptides in water.



Scheme 3 (a) Reaction using *O*-allyl tyrosine **1f**. (b) Reaction using dipeptide **2p**. (c) Reaction using dipeptide **2q**.



Scheme 4 Ru-promoted derivatization of peptides in water: (a) reaction with peptide **2r**. (b) Reaction with peptide **2s**, bearing the alkyne in an internal position. Conversions of the precursor peptide (**2**) to the corresponding diene product determined by HPLC-MS (see ESI† for details). Ar = *p*MeOPh. [a] 5 equiv. of alkene **1** were used. [b] 2.5 equiv. of alkene **1** were used.

Peptide **2r**, which bears a N-terminal propargyl glycine was easily prepared by solid phase synthesis. Gratifyingly, after a short optimization of the coupling conditions between this peptide and alkene **1c**, we were able to observe full conversion in water, using a peptide concentration as low as 200 μM, 2.5 fold excess of alkene and a **Ru1** concentration of 400 μM (Scheme 4a). Almost full conversion of the peptide and the exclusive formation of the expected diene peptide product was observed by HPLC-MS. Moreover, other alkenes like **1f**, **1d** or the *O*-allyl pyranoside **1g** could also react to give the corresponding peptide derivatives **3fr–3gr**, as the only coupling products observed by HPLC-MS. The use of a peptide bearing the propargyl glycine residue at an internal position (*i.e.* **2s**) was also tolerated, providing efficiently the products resulting from the coupling with different types of alkene partners (*e.g.* **3cs**, **3fs**, Scheme 4b). Overall, these results argue well for the application of this C–C coupling for bioconjugation and highlight the great potential of Ru catalysts to promote nontrivial C–C bond forming transformations under biorelevant conditions.

Conclusions

In conclusion, we have demonstrated that the ruthenium catalysed Alder-ene coupling between alkenes and alkynes, originally developed in organic solvents, can be efficiently promoted in aqueous and biologically relevant environments, in high yields and with good to excellent regioselectivities. The reaction proved to be tolerant to the presence of a variety of functional groups at the alkyne and alkene partners, and has also been shown to proceed efficiently in the presence of different types of biomolecules as well as in cell cultured complex media. Despite its fully intermolecular nature, the reaction does not generally need excess of any of the two partners and proceeds with low catalyst loadings. Finally, we showed that by adjusting the reaction conditions, it can be applied to the modification of alkyne containing peptides. The structural simplicity of alkene and alkynes, and the ease with which these groups can be incorporated into a wide variety of biomolecules, argue well for the applicability of the method as a new bioconjugation tool in chemical biology.

Data availability

General and synthetic procedures, orthogonality assays, HPLC data and NMR spectra are available in the ESI.†

Author contributions

A. G.-G., F. L. and J. L. M. conceived the study, analyzed the results, and wrote the paper. A. G.-G., D. M.-A., L. G. C. performed the experimental work. All authors discussed the results and edited the paper.

Conflicts of interest

There are no conflicts to declare.

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