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The development of new, clean and biocompatible reactions for green pharmaceutical and biomolecule conjugate synthesis is one of the most challenging endeavors in organic synthesis. Accordingly, there has been much effort directed towards the development of new organic transformations that are highly chemoselective, and work in aqueous media and under mild reaction conditions. Among them, the carbon–nitrogen bond formation reaction has attracted the most attention since the amino group features widely in many pharmaceuticals and bioconjugates such as drugs,^{1,2} glycoconjugates, antibody conjugates, *etc.* (Fig. 1). Unfortunately, most of the reported methods for C–N bond forming reactions require the use of expensive metal catalysts, work under harsh reaction conditions, suffer from poor atom economy and are not environmentally friendly. As such, there is an urgent need to develop a metal-free and highly chemoselective C–N bond formation reaction that can work under biocompatible reaction conditions. If successful, this newly developed method will provide efficient and green access to many pharmaceuticals. In addition, bioconjugation with proteins can be potentially achieved where the tertiary structure of proteins can be preserved during the process of the reaction.

Over the years, our group and others have contributed significantly to the green chemistry field through the development of catalyst-free water-based reactions. These include C–C bond forming reactions *via* the Mukaiyama–aldol strategy,³ and

Catalyst-free C–N bond formation under biocompatible reaction conditions†

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A C–N bond formation reaction under biocompatible conditions for the amination of allenic ketone compounds to access a diversity of β -keto enamines is developed. This reaction is atom economical, green, and highly regioselective and works well with many structurally important amines such as amino sugar and amino acid esters or peptides. A wide array of β -keto enamines was obtained in modest to excellent yields with wide functional group tolerance using this protocol. A gram-scale synthesis of an antimicrobial agent was also realized using this strategy under green reaction conditions.

C–P and C–S bond reactions^{4,5b} with allylic alcohols, using activated dichloro-acetophenones⁶ and 2*H*-azirines⁷ as linkers for disulphide bioconjugation. Inspired by our previous work on C–S bond formation reactions⁵ with allenic amide as an efficient linker for cysteine bioconjugation, we envisage that amines may react with highly reactive allenic carbonyl compounds under biocompatible conditions to yield the corresponding enaminones which are also versatile building blocks for pharmaceutical synthesis (Fig. 2).

The amino functional group features in many bioconjugation reactions with biomolecules such as proteins. As such, there has been a considerable amount of attention paid to amino chemical conjugation strategies such as lysine conjugation. The most common lysine conjugation strategies are conjugation with the *N*-hydroxysuccinimide (NHS) ester,

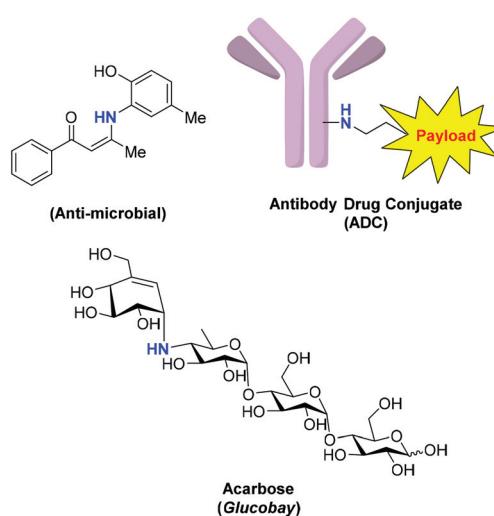


Fig. 1 Structurally crucial nitrogen compounds.

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Previous works: C-S Bond Formation in Aqueous Media

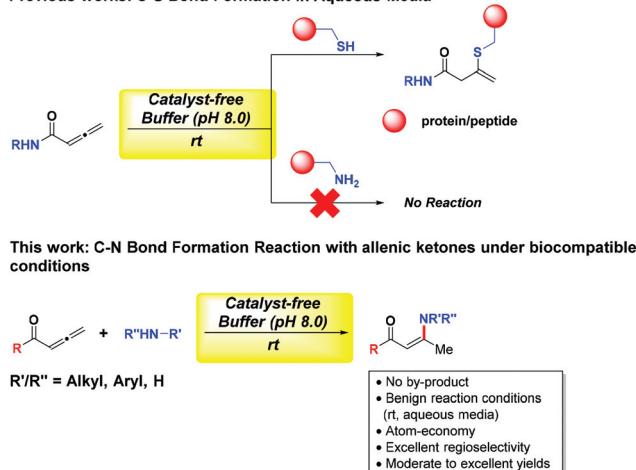


Fig. 2 C–N bond formation reaction via alkenic ketones and amines under biocompatible conditions.

isocyanates, isothiocyanates, 4-azidobenzoyl fluoride (ABF) *etc.*⁸ While these strategies offer great selectivity and are carried out under biocompatible conditions, isolation of the desired product can prove to be cumbersome. Therefore, there is an urgent need to achieve a strategy that works under both green and biocompatible conditions while ensuring that the entire process is easy to operate, and is able to isolate the pure products without the need for liquid–liquid extraction or column chromatography.

1-Phenylbuta-2,3-dien-1-one (**1a**) and benzyl amine (**2a**) were chosen as model substrates for the aza-Michael reaction (Table 1). A variety of solvents were utilized to screen the reaction (refer to the ESI†), affording the desired product **3a** in

modest to excellent yields. Compound **3a** was obtained in 35% yield when H_2O was used as the solvent (Table 1, entry 6). The yields can be improved modestly (51–55%) when phosphate buffered saline (PBS) at pH 7.0 and 8.0 were employed as solvents for the reaction (Table 1, entries 7 and 8). Further investigations on the optimization of the reaction in PBS buffer were carried out extensively and the optimal conditions were determined (Table 1, entry 12). Remarkably, this reaction shows an atom economy of 100% and an *E*-factor of 58 based on green chemistry metrics. The results show that this strategy could potentially be employed for bioconjugation reactions with proteins or peptides. As with all other reported “in water” or “on water” reactions,⁹ the continuous and intense stirring proved to be vital for the organic reagents to interact efficiently during the process of the reaction.

A competitive study between different alkenic carbonyl compounds (*i.e.* alkenic ketones/esters/amides) was first performed (Scheme 1). The reactions were performed in phosphate buffer (pH 8.0). The yields were determined by NMR analyses using CH_2I_2 as the internal standard. The results showed that the alkenic ketone is the most reactive species under the reaction conditions; the substrate was fully converted in the reaction and the corresponding enaminone was obtained in 81% yield. In contrast, the alkenic ester and amide variants failed to react under these conditions; this observation was expected due to the lower reactivity of these alkenic carbonyl variants as compared to alkenic ketones.

The substrate scope of the amines was subsequently explored in the optimized phosphate buffer (pH 8.0) (Scheme 2). A variety of aryl and aliphatic amines were investigated. Aryl amines bearing electron-donating groups (**3b–3i**) reacted smoothly to afford the corresponding enaminones in good to excellent yields. Even electron-withdrawing substituents present on the aryl rings (**3c** and **3j**) worked well under these conditions. In addition, aliphatic amines (**3k–3m**) also reacted with ease to give the enaminones in modest yields. Interestingly, a series of chiral amines (**3o–3v**) such as protected glucosamine (**3p**) and amino acid/peptide esters (**3r–3v**) also reacted well in this transformation (Scheme 3).

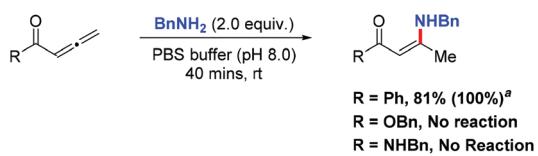
Subsequently, the substrate scope of the alkenic ketones was explored (Scheme 4). The aryl rings with electron-donating groups (**3w–3z**) reacted smoothly under the optimized conditions to give the desired enaminones in modest yields. Even with an electron-withdrawing substituent on the aryl ring, enaminone **3aa** was obtained in 51% yield. Enaminone **3ab**

Table 1 Optimization of the aza-Michael reaction^a

| Entry | Solvent | Time (h) | Conversion ^b (%) | Yield ^b (%) |
|-----------------|----------------------------|-------------|-----------------------------|----------------------------|
| 1 | PhCF ₃ | 12 | 100 | 82 |
| 2 | Et ₂ O | 12 | 100 | 70 |
| 3 | EtOAc | 12 | 100 | 80 |
| 4 | CH ₃ CN | 12 | 100 | 75 |
| 5 | EtOH | 12 | 100 | 67 |
| 6 | H ₂ O | 12 | 100 | 35 |
| 7 | PBS buffer (pH 7.0) | 12 | 100 | 51 |
| 8 | PBS buffer (pH 8.0) | 12 | 100 | 55 |
| 9 ^c | PBS buffer (pH 8.0) | 12 | 100 | 58 |
| 10 ^d | PBS buffer (pH 8.0) | 12 | 100 | 59 |
| 11 ^d | PBS buffer (pH 8.0) | 1 | 100 | 79 |
| 12 ^d | PBS buffer (pH 8.0) | 0.67 | 100 | 81 (70)^e |

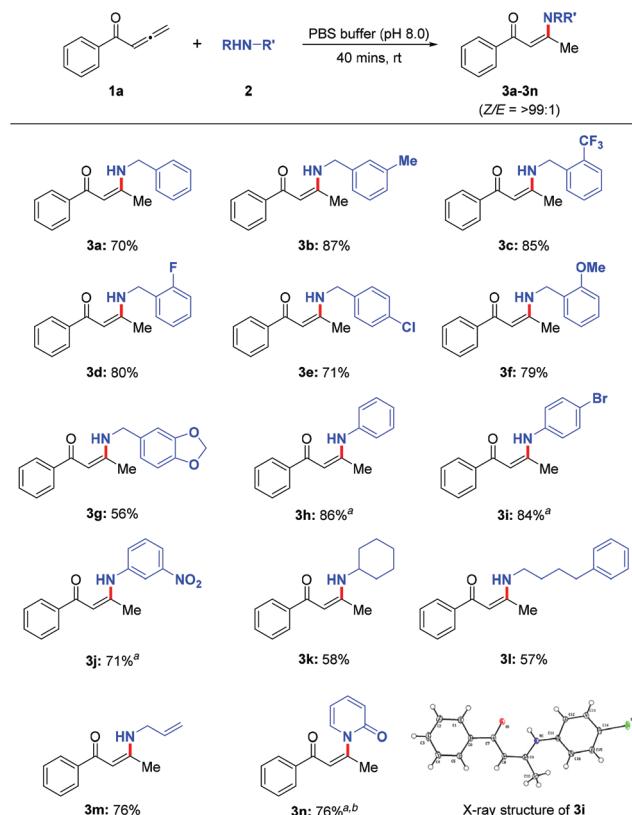
^a Experimental conditions: **1a** (0.20 mmol) and **2a** (0.30 mmol) in the specified solvent (2 mL) at room temperature. ^b Conversions and yields were determined by ¹H NMR using CH_2I_2 as the internal standard.

^c 1.0 equiv. of **2a** was used. ^d 2.0 equiv. of **2a** was used. ^e Isolated yield.



Scheme 1 Competitive study between various alkenes. Experimental conditions: **1** (0.20 mmol) and **2a** (0.40 mmol) in PBS buffer (pH 8.0) (2 mL) at room temperature for 40 min. ^a Conversions and yields were determined by ¹H NMR using CH_2I_2 as the internal standard.



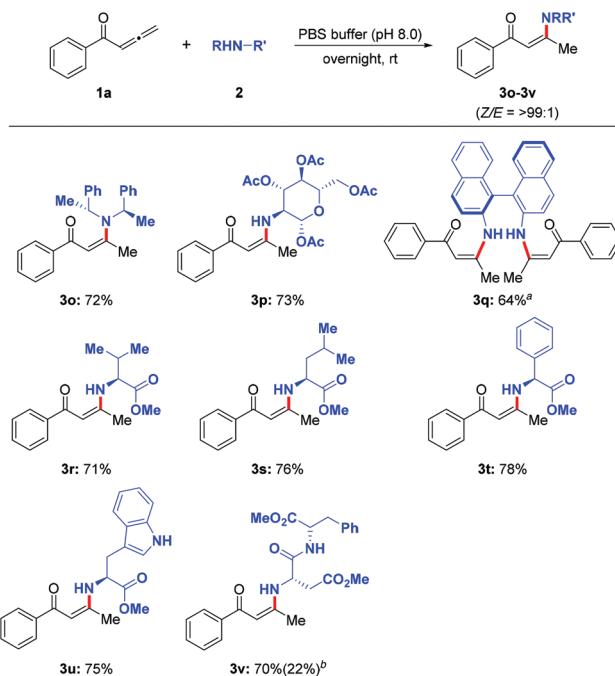


Scheme 2 Substrate scope of the amines. Experimental conditions: **1a** (0.20 mmol) and **2** (0.40 mmol) in PBS buffer (pH 8.0) (2 mL) at room temperature for 40 min. Isolated yields. ^a Reaction stirred overnight. ^b Reaction carried out in EtOAc.

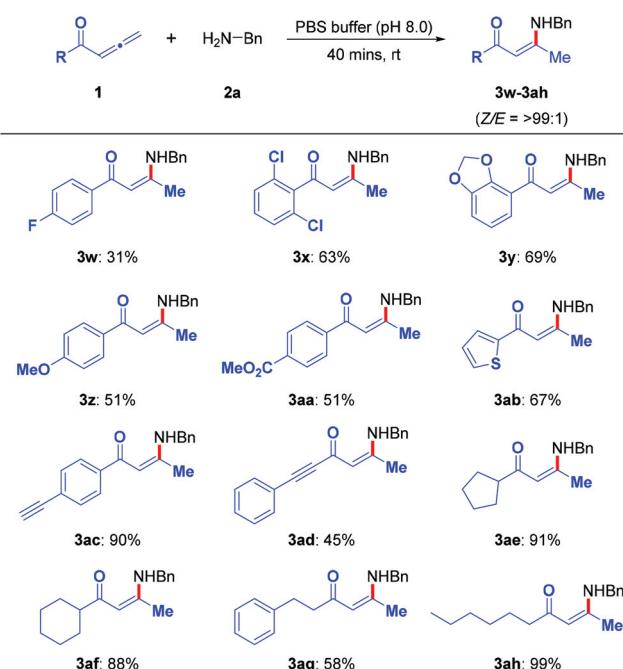
was obtained in 67% yield when a heterocyclic allenic ketone was employed in the reaction. The presence of an alkyne in the substrates (**3ac** and **3ad**) did not appear to hinder the outcome of the reaction; the desired enaminones were obtained in 90% and 45% yields, respectively. Aliphatic allenic ketones (**3ae**–**3ah**) also reacted smoothly to afford the corresponding enaminones in modest to excellent yields.

The synthetic utility of the enaminones was subsequently explored (Fig. 3). Enaminone **3a** was thiolated with Lawesson's reagent to afford enaminothione **4** in 59% yield.¹⁰ Treating enaminone **3a** with the phenyl vinyl ketone gave the corresponding cyclohexa-1,3-diene **5** in 56% yield.¹¹ A gram-scale synthesis of the anti-microbial drug **6** was realized under benign conditions, yielding the desired product in 90% yield by removing the solvent under reduced pressure. Enaminone **3ac** can participate in a Cu(i)-catalyzed [3 + 2] cycloaddition¹² with tosyl azide to give compound **7** in 88% yield. Enaminone **3ac** can also undergo a click reaction^{5c} with the anti-viral drug, Zidovudine,¹³ giving compound **8** in 27% yield.

The proposed mechanism for the formation of the enaminone is illustrated in Fig. 4. The initial aza-Michael attack by the nucleophilic benzylamine regioselectively at the β -position of the allenic ketone **1a** afforded an enol intermediate. This enol tautomer was proposed to be stabilized through a



Scheme 3 Substrate scope of chiral amines. Experimental conditions: **1a** (0.20 mmol) and **2** (0.40 mmol) in PBS buffer (pH 8.0) (2 mL) at room temperature overnight. Isolated yields. ^a Conditions: **1a** (0.44 mmol) and **2** (0.2 mmol) in EtOAc (2 mL). ^b Reaction carried out in EtOAc. The side product formed in 22% yield.



Scheme 4 Substrate scope of the allenic ketones. Experimental conditions: **1** (0.20 mmol) and **2a** (0.40 mmol) in PBS buffer (pH 8.0) (2 mL) at room temperature for 40 min. Isolated yields.

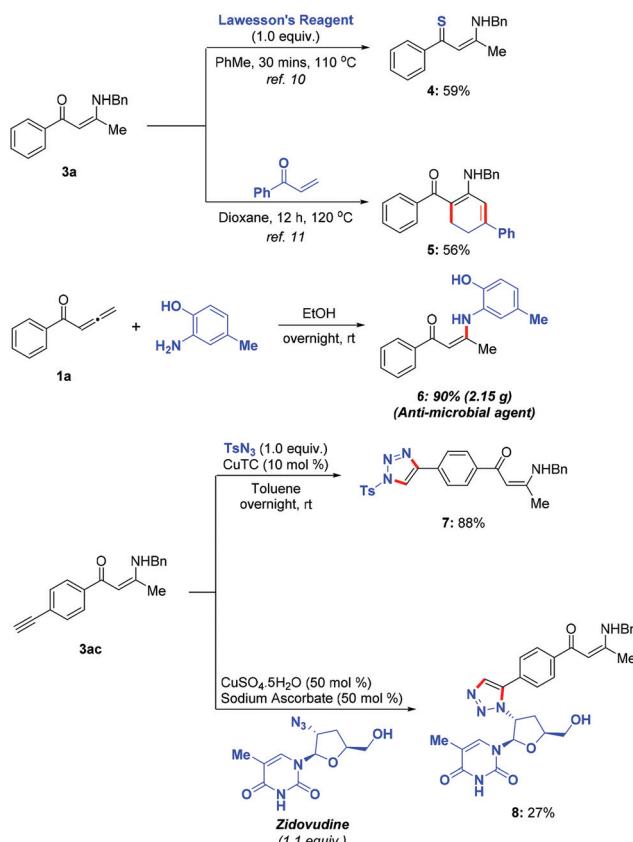


Fig. 3 Synthetic utility and functionalization of enaminones.

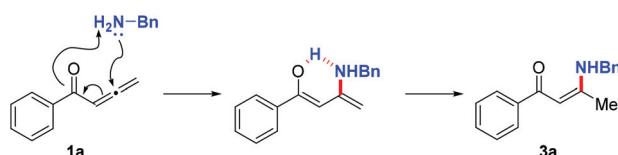


Fig. 4 Proposed mechanism for the enaminone formation.

six-membered transition state *via* intramolecular hydrogen bonding, which readily tautomerized to yield (*Z*)-enaminone **3a** exclusively as the desired product.

In summary, we have developed a practical and efficient strategy for the amination of allenic ketones in a green and biocompatible manner. The reaction is carried out in a phosphate buffer system (pH 7–8) under catalyst-free conditions and at room temperature. The special features of this method are: (1) no protection of sensitive functional groups is required; (2) applicable for glycoconjugation; (3) dual-linker functionality; (4) gram scale synthesis of an anti-microbial agent with no usage of metals and organic solvents and no column chromatography is required for its purification. With this novel strategy, we have once again taken a step towards increasing our toolbox of green synthetic and bioconjugation methods.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

- (a) W. Masocha, S. B. Kombian and I. O. Edafiogho, Evaluation of the antinociceptive activities of enaminone compounds on the formalin and hot plate tests in mice, *Sci. Rep.*, 2016, **6**, 21582; (b) J. Apraku and C. O. Okoro, Design, synthesis and anticonvulsant evaluation of fluorinated benzyl amino enaminones, *Bioorg. Med. Chem.*, 2019, **27**, 161–166.
- (a) M. Bhat, M. Al-Omar, A. Naglah and A. Khan, Enaminone-derived pyrazoles with antimicrobial activity, *J. Chem.*, 2019, 2467970; (b) I. J. Amaye, R. D. Haywood, E. M. Mandzo, J. J. Wirick and P. L. Jackson-Ayotunde, Enaminones as building blocks in drug development: Recent advances in their chemistry, synthesis, and biological properties, *Tetrahedron*, 2021, **83**, 131984.
- (a) T.-P. Loh, L.-C. Feng and L.-L. Wei, Water-Accelerated Aldol Reaction of Ketene Silyl Acetals with Carbonyl Compounds, *Tetrahedron*, 2000, **56**, 7309–7312; (b) J. Alam, T. H. Keller and T.-P. Loh, Functionalization of Peptides and Proteins by Mukaiyama Aldol Reaction, *J. Am. Chem. Soc.*, 2010, **132**, 9546–9548; (c) J. Xiao, Y.-L. Liu and T.-P. Loh, The Organocatalytic Enantioselective Michael Addition of Aldehydes to Vinyl Sulfones in Water, *Synlett*, 2010, 2029–2032.
- P. Xie, W. Fu, Y. Wu, X. Cai, Z. Sun, S. Li, C. Gao, X. Yang and T.-P. Loh, Allylic Phosphorus Ylides Directly Generated from Alcohols with Water as the Only Byproduct, *Org. Lett.*, 2019, **21**, 4168–4172.
- (a) A. Abbas, B. Xing and T.-P. Loh, Allenamides as Orthogonal Handles for Selective Modification of Cysteine in Peptides and Proteins, *Angew. Chem., Int. Ed.*, 2014, **53**, 7491–7494; (b) P. Xie, J. Wang, Y. Liu, J. Fan, X. Wo, W. Fu, Z. Sun and T.-P. Loh, Water-promoted C–S bond formation reactions, *Nat. Commun.*, 2018, **9**, 1321; (c) J. Goh, M. Maraswami and T.-P. Loh, Synthesis of Vinylic Sulfones in Aqueous Media, *Org. Lett.*, 2021, **23**, 1060–1065.
- L.-H. Wu, S. Zhou, Q.-F. Luo, J.-S. Tian and T.-P. Loh, Dichloroacetophenone Derivatives: A Class of



Bioconjugation Reagents for Disulfide Bridging, *Org. Lett.*, 2020, **22**, 8193–8197.

7 Y. Chen, W. Yang, J. Wu, W. Sun, T.-P. Loh and Y. Jiang, 2*H*-Azirines as Potential Bifunctional Chemical Linkers of Cysteine Residues in Bioconjugate Technology, *Org. Lett.*, 2020, **22**, 2038–2043.

8 M. S. Kang, T. W. S. Kong, J. Y. X. Khoo and T.-P. Loh, Recent developments in chemical conjugation strategies targeting native amino acids in proteins and their applications in antibody–drug conjugates, *Chem. Sci.*, 2021, **12**, 13613–13647.

9 (a) A. Chanda and V. V. Fokin, Organic Synthesis “On Water”, *Chem. Rev.*, 2009, **109**, 725–748; (b) R. N. Butler and A. G. Coyne, Water: Nature’s Reaction Enforcer—Comparative Effects for Organic Synthesis “In-Water” and “On-Water”, *Chem. Rev.*, 2010, **110**, 6302–6337; (c) S. Narayan, J. Muldoon, M. G. Finn, V. V. Fokin, H. C. Kolb and K. B. Sharpless, “On Water”: Unique Reactivity of Organic Compounds in Aqueous Suspension, *Angew. Chem., Int. Ed.*, 2005, **44**, 3275–3279; (d) Y. Yang, L. Tang, S. Zhang, X. Guo, Z. Zha and Z. Wang, Catalyst-free sulfonylation of activated alkenes for highly efficient synthesis of mono-substituted ethyl sulfones in water, *Green Chem.*, 2014, **16**, 4106–4109; (e) G. Lu, C. Cai, F. Chen, R. Ye and B. Zhou, Facile Sulfa-Michael Reactions with Sodium Arylsulfinate in Water: The Promotion of Water on the Reaction, *ACS Sustainable Chem. Eng.*, 2016, **4**, 1804–1809.

10 Z. Liu, P. Wu, Y. He, T. Yang and Z. Yu, [4 + 1] Cycloaddition of Enaminothiones and Aldehyde *N*-Tosylhydrazones Toward 3-Aminothiophenes, *Adv. Synth. Catal.*, 2018, **360**, 4381–4392.

11 T. Feng, M. Tian, X. Zhang and X. Fan, Tunable Synthesis of Functionalized Cyclohexa-1,3-dienes and 2-Aminobenzophenones/Benzoate from the Cascade Reactions of Allenic Ketones/Allenoate with Amines and Enones, *J. Org. Chem.*, 2018, **83**, 5313–5322.

12 T. Miura, T. Nakamuro, K. Hiraga and M. Murakami, The stereoselective synthesis of α -amino aldols starting from terminal alkynes, *Chem. Commun.*, 2014, **50**, 10474–10477.

13 V. Gogineni, R. F. Schinazi and M. T. Hamann, Role of Marine Natural Products in the Genesis of Antiviral Agents, *Chem. Rev.*, 2015, **115**, 9655–9706.

