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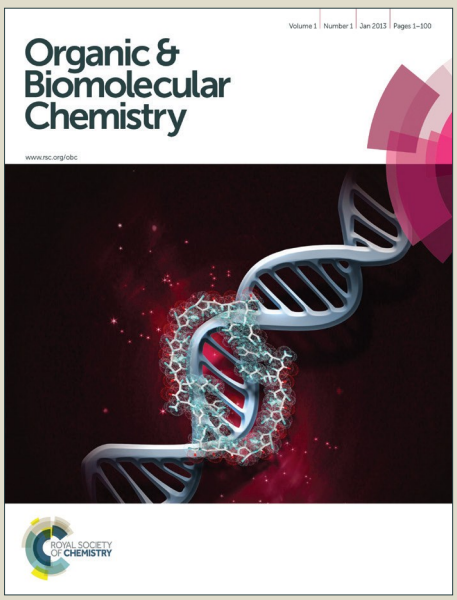

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Highly Efficient Synthesis of Flavonol 5-*O*-glycosides with Glycosyl ortho-Akynylbenzoates as Donors

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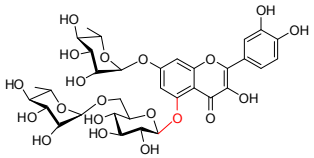
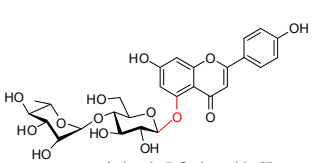
With glycosyl ortho-alkynylbenzoates as donors, the highly efficient glycosylation of flavonoid 5-OH which are notorious for their low reactivity due to their involvement in the formation of strong intramolecular H-bonds were achieved under the catalysis of Au(I) complex. Thus, a series of flavonoid 5-*O*-glycosides, including a kaempferol 5-*O*-disaccharide, were synthesized with goog to excellent yields.

Flavonoid *O*-glycosides are widely spread in the plant kingdom,[1] and they play a variety of important roles in the growth and development of plants, e.g., as interspecies signal-transferring molecules.[2] Especially, they have also been demonstrated to possess a wide spectrum of activities beneficial to humans, such as antimicrobial, anticancer, and radical-scavenging activities.[1,2] Thus far, more than 1500 flavonoid *O*-glycosides have been characterized, and the number is still increasing rapidly. As a special type of flavonoid *O*-glycosides, flavonol 5-*O*-glycosides are seldom found in nature, and so far only around 10 of them including brachyside (I) and apigenin 5-*O*-glycoside (II) have been isolated and characterized (Figure 1).[3] In spite of the scarcity of flavonol 5-*O*-glycosides, some known members of them have been proven to possess promising pharmaceutical activities. For example, apigenin 5-*O*-glycoside (II), isolated from *Cephalotaxus sinensis*, was demonstrated to have significant antihyperglycemic effect, although limited by accessibility the function mechanism was not deciphered.[3a] Besides, as reference compounds, flavonol 5-*O*-glycosides are highly demanded in metabolic research of flavonol compounds in humans. Structurally, flavonol 5-*O*-glycosides are distinguished from their cousins by the existence of flavonol 5-*O*-glycosidic linkages, which pose considerable challenge for synthetic chemists equipped with modern synthetic arsenals. The difficulty associated with the construction of 5-OHs glycosidic linkages resides on the extraordinary low reactivity of these phenolic OHs since they are involved in the formation of intramolecular H-bonds with C-4 carbonyl group. The strength of this kind of intramolecular H-bonds is so strong that makes simply protecting group manipulations become difficult, not alone sophisticated glycosylation reactions. Of course, the electron-withdrawing property of 4-carbonyl groups can further diminish the nucleophilicity of 5-OHs. In addition, because of the high tendency to form stable intramolecular H-bond system, flavonol 5-*O*-glycosidic linkages are so acid sensitive that even mild acid can induce rapid glycosidic linkage cleavage, which further increase the difficulty in fashion such special bonds.[4]

Employing the powerful glycosyl trichloroacetimidate donor,[5] Schmidt et al. made the first try to overcome this daunting problem.[6] Under the catalysis of TMSOTf, a low yield of the desired flavonol 5-*O*-glycoside was obtained. The inefficiency can probably be ascribed to the high acid-sensitivity of the incipient formed flavonol 5-*O*-glycosidic linkage to TMSOTf used as glycosylation catalyst. To avoid the detrimental effect of Lewis acid, alternative PTC protocol with glycosyl bromide as donors was introduced by the groups of Dangles[7a] and Rolando,[7b] respectively. The intramolecular H-bond prohibits the release of the 5-OH protons, therefore the glycosylation yields recorded are far from satisfactory. We also made some efforts in this field, and capitalizing on Yu glycosylation[8] the rhamnosyl residues were incorporated onto the 5 and 4'-OH**s** of kaempferol at the same time.[7c] Nevertheless, the method is seriously suffered from narrow substrate scope, only rhamnosyl *ortho*-alkynylbenzoate donor could offer practically useful yield. Therefore, a highly efficient approach to forge flavonol-5-*O*-glycosidic linkages is highly demanded.



Brachyside (I)



Apigenin 5-*O*-glycoside (II)

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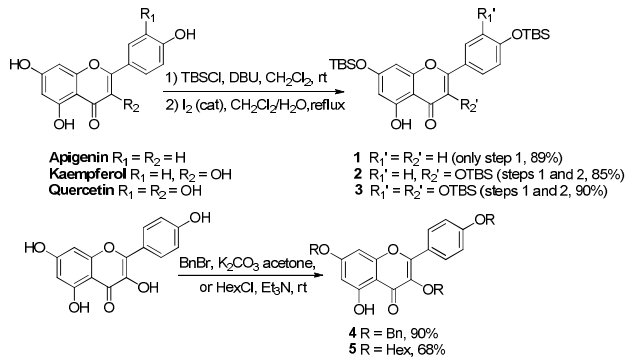
Electronic Supplementary Information (ESI) available: Copies of ^{1}H NMR and ^{13}C spectra of all new compounds and 2D spectra for **2**, **4**, **5**, **10**, and **28** are provided. See DOI: 10.1039/x0xx00000x

Figure 1 The chemical structures of representative flavonoid 5-O-glycosides.

In line with our continuous interest in complex bioactive flavonoid glycosides synthesis, we decided to make a systematic investigation in construction of the challenging flavonol 5-*O*-glycosidic linkages. Although have encountered some unwanted problems in our preliminary studies with glycosyl ortho-alkynylbenzoate as donor,[7c] we still believe that the advent of this conceptually new glycosylation protocol can bring promise to solve this long-lasting problems, since the glycosylation protocol is powerful while the promotion conditions are extremely mild. Herein, we would like to report on an efficient approach to fashion flavonol 5-*O*-glycosidic linkages with glycosyl *ortho*-alkynylbenzoates as donors. Based on the established protocol, the first synthesis of the proposed apigenin 5-*O*-disaccharide was also accomplished.

To facilitate the screening of the optimal condtitions, a series of flavonol 5-OH acceptors were synthesized (Scheme 1). The discrimination of 5-OHs became feasible with the help of intramolecular H-bonds. Thus, treated with TBSCl and DBU, apigenin, kaempferol, and quercetin were thoroughly silylated. After general work-up, the fully silylated kaempferol and quercetin were then subjected to regioselective desilylation of 5-OTBS, which was facilitated by propensity of the oxygen atoms of 5-OTBS to coordinate a proton under the aid of C-4 carbonyl group to form stable intramolecular H-bond systems, to generate **2**[9a] and **3** in excellent yields (85% and 90%, 2 steps). For the synthesis of apigenin analogues **1**,[10] the 5-*O*-desilylation step was not required because it was so labile that the general work-up could remove it to afford **1** in a good 89% yield. Replacing the strong DBU base with mild ones such as K_2CO_3 and Et_3N could lead to the removal of all phenolic protons except for 5-OH protons restricted in intramolecular H-bonds, thus at the presence of BnCl or Hexanoyl chloride **4**[9c] and **5** were generated conveniently. To confirm the structures of all acceptors, the ^{1}H NMR data of known compounds including **1**, **2**, and **4** were compared with those reported in literature, and no evident discrepancy was detected. NOE spectra of **3** and **5** were made, the proton signals of the free phenolic OHs residing at 12.68 and 12.70 ppm only had cross-correlations with one of the two signals assigned to H-6 and H-8, which demonstrated that the free phenolic OH was 5-OH. If the free phenolic OH resided on C-7, then its correlations with both H-6 and H-8 should be detected.[11]



Scheme 1 Synthesis of flavonol 5-OH acceptors.

Following known procedures, representative glcosyl *ortho*-alkynylbenzoates donors including perbenzoylated glucosyl *ortho*-cyclopropylethynylbenzoate **6**,[9] 6-OH selectively protected glucosyl *ortho*-cyclopropylethynylbenzoate **7**,[9] galactosyl *ortho*-cyclopropylethynylbenzoate **8**,[9] and its rhamnosyl counterpart **9**[9] were obtained without any event (Figure 2). In all donors **6**-**9**, the 2-OHs were protected with benzyol groups which could control the stereoselectivity via anchimeric participation in the following glycosylation steps so as to afford 1,2-*trans*-glycosides solely.

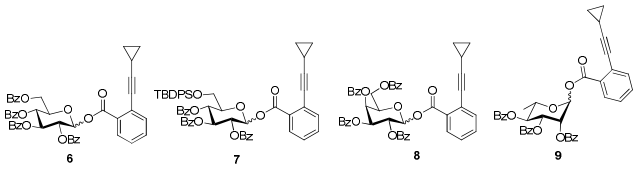
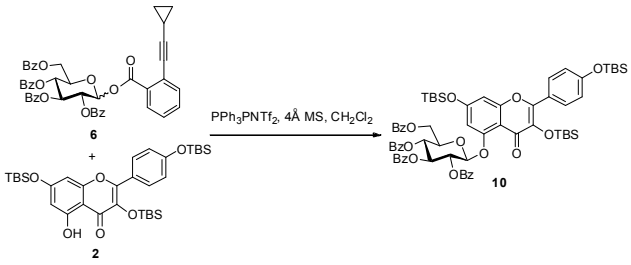


Figure 2 Chemical strucutures of glycosyl ortho-alkynylbenzoates donors.

With both glycosyl donors and acceptors in hand, now the stage was set for the pivotal glycosydic linkage construction (Table 1). Glycosylation between donor **6** and acceptor **2** was selected as model reaction to screen the optimal conditions. The selection of TBS protected acceptor **2** was based on the conjecture that the high electron-donating property of TBS protecting groups may increase the nucleophilicity of 5-OH, therefore reasonable glycosylation yield may be expected. Thus, treated with 0.2 equivalents of PPh_3AuNTf_2, acceptor **2** was subjected to condense with donor **6** (1.5 equiv.) at room temperature (10 ^oC). Disappointedly, no desired glycosylation product was detected, and the only detectable side-reaction was partial hydrolysis of donor **6** (entry 1). It is notable that the reaction mixture turned to darkness after being stirred at the presence of PPh_3AuNTf_2 for 1 h. This phenomenon was likely resulted from the appearance of silver metal via reduction of Ag(I) contained in PPh_3AuNTf_2 by reductive **2**.[12] The contamination of Au(I) complex by Ag(I) salt is inevitable since only a filtration through a pad of celite work-up is adopted to get the PPh_3AuNTf_2 catalyst in standard preparation procedure.[13] In order to prevent the adverse effect of excess Ag(I) in the desired glycosylation step, a slight modification of Au(I) catalyst preparation was made, that is, an additional filtration through a pad of silica gel was introduced. Thus obtained PPh_3AuNTf_2 , as a greyish-white solid, was then tried for glycosylation between **2** and **6**. To our delight, 35% yield of desired glycoside **10** was isolated (entry 2). In addition, no abnormal darkness was noticed any more in the reaction mixture. Upon raising the reaction temperature to 30 ^oC, we were rewarded by a dramatic enhancement in chemical yield (90%,entry 3). The optimal reaction conditions were thus fixed as follow: under the effect of 0.2 equivalents of PPh_3AuNTf_2 generated by the slightly modified procedure, donor (1.5 equiv.) condensed with acceptor in CH_2Cl_2 in the presence of activated 4Å MS at 30 ^oC to afford the desired glycoside. The chemical structure of **10** was ascertained by 1D NMR spectra

and NOE 2D spectrum (for the anomeric proton: 5.71 ppm, J = 7.2 Hz; correlation of the anomeric proton with H-6 was observed in NOE spectrum).[11]

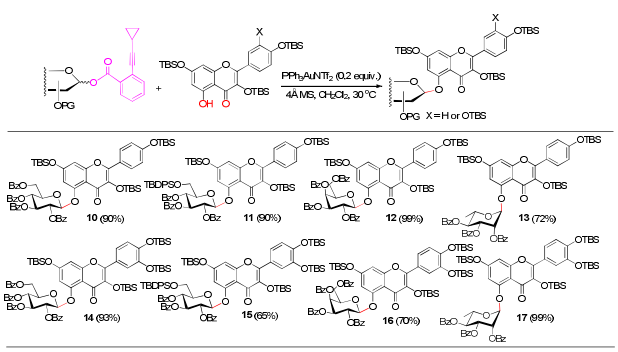


Entries	Equivalents of donor	Catalyst (equiv)	Temperature (°C)	Yield[a]
1	1.5	PPh_3AuNTf_2 (0.2)[b]	10	NP
2	1.5	PPh_3AuNTf_2 (0.2)[c]	10	35%
3	1.5	PPh_3AuNTf_2 (0.2)[c]	30	90%

[a] Isolated yields. [b] Ag(I) contaminated catalyst. [c] Pure Au(I) catalyst.

Table 1 The optimizations of flavonol 5-OH glycosylation.

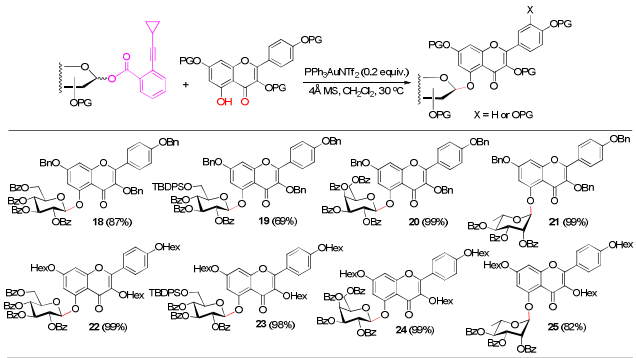
After the optimal reaction conditions were established, the substrate generality and limitation were subsequently checked (Scheme 2). Besides **6**, the more reactive glucosyl donor **7** could offer excellent yield of product glycoside as well, when coupled with **2** under the optimized glycosylation conditions. Donor **8**, the galactosyl counterpart of **6**, was proved to be an even better substrate for the protocol, and almost quantitative yield of **12** was recorded. As a representative of L-series sugar donor, L-rhamnosyl donor **9** condensed with **2** fluently, affording **13** in a good 72% yield. Not only enjoy quite broad substrate scope in terms of glycosyl donors, the glycosylation conditions could also be applicable to quercetin acceptor protected with TBS groups. When glycosylated with donors **6**-**9** respectively under standard conditions, acceptor **3** afforded quercetin 5-*O*-glycosides **14**-**17** in yields ranging from 65% to 99%. Surprisingly, condensation of **1**, the simplest one in the TBS protected acceptor series, and **6** under the identical conditions was found to proceed sluggishly, only trace amount of apigenin 5-*O*-glucoside product was detected after stirring 20 h at 30 °C.



Scheme 2 Construction of TBS protected flavonoid 5-OH glycosidic linkages with glycosyl ortho-alkynylbenzoates as donors.

The strikingly different performance of **1** in comparison to **2** and **3** in glycosylation reaction probably originates from the lack of the 3-OH. The presence of 3-OH in acceptors **2** and **3** may probably weaken the strength of the intramolecular H-bond, in turn facilitating the glycosylation of the 5-OH.[9c,14] This hypothesis was confirmed by comparison of the chemical shifts of the 5-OHs, indicatives of intramolecular H-bond strength.[15] The 5-OH of **1** appeared at 12.72 ppm, while the corresponding protons of **2** and **3** had chemical shifts of 12.67 ppm and 12.68 ppm, respectively. Evident up-field shifts of 5-OH protons were observed for acceptors **2** and **3**, indicating the strength decrease of the corresponding intramolecular H-bonds. Actually, a hint of intramolecular H-bond strength difference between **1** and **2**, **3** was also provided during the synthesis of them. The stronger H-bond in **1** made the regioselective removal of 5-OTBS operation unnecessary, while in **2** and **3** cases, the desilylation manipulation is required.

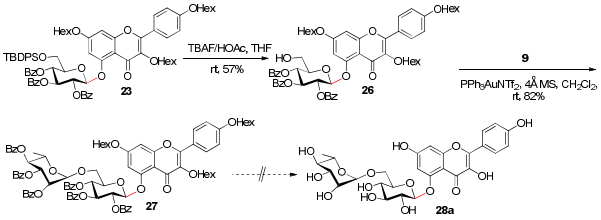
To further extend the substrate scope of the method in terms of glycosyl accepters, compounds **4** and **5** carrying benzyl and hexanoyl protecting groups were subsequently studied. Although Bn is also regarded as electron-donating protecting group, the electron-donating propensity of it is much less than that of TBS. Hexanoyl group is a typical electron-withdrawing protecting group. Thus, by means of checking the possibility of compounds **4** and **5** as acceptors in the established protocol, the electronic effect exerted by protecting groups on acceptor activities could be deduced. Catalysed by Au(I) complex, acceptor **4** reacted with donors **6**-**7** efficiently, providing glycosides **18**-**21** in good to excellent yields (Scheme 3). In terms of electron-deficient compound **5**, it was also proved to be a vital acceptor for the new glycosylation protocol, and above 80% yields of **22**-**25** were obtained. Therefore, at this juncture, a conclusion that the electronic property of the protecting groups has little effect on the reactivity of the acceptors could be draw from these results.



Scheme 3 Glycosylation of flavonol 5-OH protected with benzyl and Hexanoyl groups.

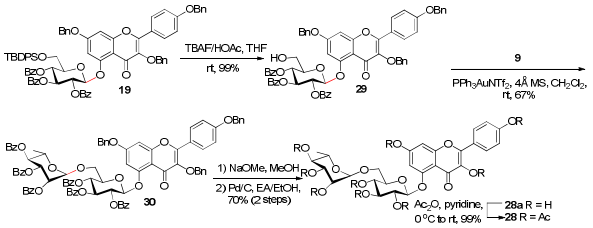
To test the synthetic potential of the glycosylation protocol in natural product synthesis, a synthetic study toward kaempferol 5-*O*-disaccharide **28a**, isolated from *Nerium oleander*,[16] was undertaken. As depicted in scheme 4, the synthesis commenced with **23**. Selective removal of the TBDPS group on sugar residue was achieved under buffered TBAF condition to yield **26** in a moderate 57% yield.[17] The main by-product of this conversion was the 7,6''-OHs free glycoside, derived from simultaneous removal of basic labile 7-*O*-hexanol and 6'-*O*-TBDPS groups. Given the acidic labile nature of the glucosydic linkage in **26**, donor **9** was applied for the

installation of rhamnosyl residue. Under standard conditions, disaccharide **27** was isolated in a good 82% yield. What remained to complete the synthesis of **28a** was the global deprotection of all acyl groups in **27**, a process proved to be quite problematic. Saponification with generally applied NaOMe/MeOH system only led to a complex mixture due to the instability of the electron-rich free flavonol nuclei in basic conditions.[18] Changing the base from NaOMe to mild K_2CO_3[19] and $Mg(OMe)_2$[20] didn't bring about considerable improvement, therefore, this route was finally abandoned.



Scheme 4 Synthetic attempt toward the proposed naturally occurring kaempferol 5-*O*-glycoside **28a**.

To avoid exposure of free flavonol subunit to basic conditions, a two-step deprotection procedure was adopted (Scheme 5). Compound **19** was subjected to TBAF mediated desilylation to furnish **29** which was ready for sugar chain elongation (99%). The condensation between **29** and **9** proceeded fluently under standard glycosylation conditions, yielding kaempferol disaccharide **30** (67%). Saponification was followed by hydrogenation to generate **28a** successfully (70%, 2 steps). Unfortunately, evident discrepancy was observed between ^{1}H NMR of **28a** and that reported in literature.[16] To eliminate the difficulties possibly brought about by H-bonds in spectrum discrimination[21] and to facilitate the final structure determination, **28a** was thus acetylated to afford **28**. All 1D and 2D spectra supported that the obtained compound had the correct structure of **28**, in turn the structure of **28a** was also verified. In HMBC spectrum, correlation between C-5 and H''-1 was observed, which demonstrated that the sugar chained was attached to 5-OH. C''-5, discriminated from DEPT spectrum, correlated with H'''-1 in HMBC spectrum, which proved that the rahmnosyl residue was attached to the 6-OH of the glucosyl residue. NOE spectrum could offer further evidence to support the attaching position of the sugar chain, and correlations between H-6 and H''-1 and H'''-1 were detected.[11] In fact, in the isolation literature, a quite similar compound to **28** was also made except for the free 3-OH. Evident spectroscopic discrepancies of **28** and its 3-OH analogue were also noticed. In **28**, for all protons attached to carbons substituted with acetylated hydroxyl groups appeared in reasonable area (for protons of H''-2,3,4 and H'''-2,3,4 in **28** appeared at above 5.0 ppm; while in the original document, protons of H''-2,3,4 and H'''-4 were assigned to resonate at below 5.0 ppm). The H''-5 and H'''-5 of **28** are easily discriminated at 3.93 and 3.85 ppm in multiple form (these two protons were assigned to 5.1 and 5.24 ppm, respectively in the isolation paper). Additional support could be obtained from quercetin 5-*O*-2'',3'',4'',6''-tetra-*O*-acetyl-glucoside synthesized by Rolando et al.,[6b] whose H''-2, 3 and 4 resided between 5.11-5.49 ppm, while H''-5 appeared at 3.76 ppm. The diagnostic ^{13}C chemical shifts for the two anomeric carbons C''-1 and C'''-1 in **28** were 106.2 and 100.5 ppm, respectively, in construct to the reported 104.5 and 102.4 ppm for these two carbons.



Scheme 5 Synthesis of kaempferol 5-*O*-gycoside **28**.

In summary, capitalizing on Yu glycosylation, an efficient protocol to construct the challenging flavonol 5-OH glycosidic linkages was established. The protocol enjoys broad substrate scope in terms of both donors and acceptors. Employing this protocol the synthesis of kaempferol 5-*O*-disaccharide was achieved for the first time. To the best of our knowledge, this is the first time that flavonol 5-*O*-disaccharide was obtained by synthetic method.

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Notes and references

1 (a) J. B. Harborne, and C. A. Williams, *Nat. Prod. Rep*. 1995, **12**, 639. (b) J. B. Harborne, and C. A. Williams, *Nat. Prod. Rep*. 1998, **15**, 631. (c) J. B. Harborne, and C. A. Williams, *Nat. Prod. Rep*. 2001, **18**, 310. (d) N. C. Veitch, and R. J. Grayer, *Nat. Prod. Rep*. 2008, **25**, 555.

2 J. B. Harborne, and H. Baxter, T*he Handbook of Natural Flavonoids*; John Wiley & Sons: Chichester, UK, 1999, Vol. 1.

3 (a) S. Perveen, A. M. Al-Taweel, N. Al-Musayeib, G. A. Fawzy, A. Khan, R. Mehmood, and Malik, A. *Nat. Prod. Res*. 2015, **29**, 615. (b) R.-J. Dai, Y.-H. Yu, L. Li, C.-M. Wu, W.-W. Luan, W.-W. Meng, X.-S. Zhang, and Y.-L. Deng, *Biol. Pharm. Bull*. 2007, **30**, 1123.

4 Y. Du, G. Wei, and R. J. Linhardt *Tetrahedron Lett*. 2003, **44**, 6887.

5 R. R. Schmidt, and J. Michel, *Angew. Chem., Int. Ed. Engl*. 1980, **19**, 731.

6 J. A. Mahling, and R. R. Schmidt, *Synthesis* 1993, 325.

7 (a) B. Alluis, and O. Dangles, *Helv. Chim. Acta* 2001, **84**, 1133. (b) M. Kajjout, and C. Rolando, *Tetrahedron* 2011, **67**, 4731. (c) W. Yang, R. Li, W. Han, W.-D, Zhang, and J. Sun, *Chin. J. Org. Chem*. 2012, **32**, 1067.

8 (a) Y. Li, Y. Yang, and B. Yu, *Tetrahedron Lett*. 2008, **49**, 3604. (b) Y. Zhu, and B. Yu, *Angew. Chem. Int. Ed*. 2011, **50**, 8329. (c) Y. Yang, X. Zhang, and B. Yu, *Nat. Prod. Rep*. 2015, **32**, 1331.

9 (a) W. Yang, J. Sun, W. Lu, Y. Li, L. Shan, W. Han, W.-D Zhang, and B. Yu, *J. Org. Chem*. 2010, **75**, 6879. (b) Y. Li, W. Yang, Y. Ma, J. Sun, L. Shan, W.-D. Zhang, and B. Yu, *Synlett* 2011,

915. (c) J. Yu, J. Sun, Y. Niu, R. Li, F. Zhang, and B. Yu, *Chem. Sci*. 2013, **4**, 3899.
10 K.-i. Oyama, and T. Kondo, *Tetrahedron* 2004, **60**, 2025.
11 See supporting information.
12 M. C. Akilonu, S. L. Bonnet, and van der J. H. Westhuizen, *Org. Lett*. 2008, **10**, 3865.
13 (a) N. Mezailles, L. Ricard, and F. Gagosz, *Org. Lett*. 2005, **7**, 4133. (b) Y. Li, X. Yang, Y. Liu, C. Zhu, Y. Yang, and B. Yu, *Chem. Eur, J*. 2010, **16**, 1871.
14 (a) M. B. Cid, F. Alfonso, I. Alonso, and M. Martin-Lomas, *Org. Biomol. Chem*. 2009, **7**, 1471. (b) M. I. Colombo, E. A. Ruveda, and C. A. Stortz, *Org. Biomol. Chem*. 2011, **9**, 3020.
15 B. Bernet, and A. Vassella, *Helv. Chim. Acta* 2000, **83**, 995.
16 B. S. Siddiqui, N. Khatoon, S. Begum, A. D. Farooq, K. Qamar, H. A. Bhatti, and S. K. Ali, *Phytochemitry* 2012, **77**, 238.
17 S. Kim, A. Tsuruyama, A. Ohmori, and K. Chiba, *Chem. Commun*. 2008, 1816.
18 (a) M. Li, X. Han, and B. Yu, *J. Org. Chem*. 2003, **68**, 6842. (b) J. Sun, S. Laval, and B. Yu, *Synthesis* 2014, 46, 1030.
19 (a) N. Al-Maharik, and N. P. Botting, *Tetrahedron Lett*. 2006, **47**, 8703. (b) Al-Maharik, N.; Botting, N. P. *Eur. J. Org. Chem*. 2008, 5622.
20 (a) Y.-C. Xu, A. Bizuneh, and C. Walker, *Tetrahedron Lett*. 1996, **37**, 455. (b) Y.-F. Li, B. Yu, J.-S. Sun, and R.-X. Wang, *Tetrahedon Lett*. 2015, **56**, 3816.
21 C. Nawrat, C. J. Moody, *Org. Lett*. 2012, **14**, 1484.