Organic & Biomolecular Chemistry

COMMUNICATION

View Article Online
View Journal

Cite this: DOI: 10.1039/c4ob02242d

Received 22nd October 2014,
Accepted 10th November 2014

DOI: 10.1039/c4ob02242d

www.rsc.org/obc

Developing piperine towards TRPV1 and $GABA_A$ receptor ligands – synthesis of piperine analogs *via* Heck-coupling of conjugated dienes†

Laurin Wimmer,[a] David Schönbauer,[a] Peter Pakfeifer,[b] Angela Schöffmann,[b] Sophia Khom,[b] Steffen Hering[b] and Marko D. Mihovilovic*[a]

Piperine, the pungent alkaloid of black pepper, and several of its derivatives are modulators of γ-amino butyric acid type A ($GABA_A$) receptors. Concomitantly, this natural product has also been reported to activate transient receptor potential vanilloid type 1 (TRPV1) receptors. We have developed a Heck cross-coupling reaction of conjugated dienamides enabling the rapid assembly of piperine derivatives containing a modified aromatic core. Upon assessment of a focussed compound library, key aromatic substituents were identified selectively affecting either the $GABA_A$ or the TRPV1 receptor.

Piperine, the pungent alkaloid of *piper nigrum*, was recently identified as a positive allosteric modulator of γ-amino butyric acid type A ($GABA_A$) receptors.[1] Pharmaceutical compounds modulating this receptor and thus enhancing neuronal GABAergic inhibition, like benzodiazepines, are widely used as anxiolytics, sleep-inducing agents as well as for the treatment of convulsive disorders and other disease states.[2]

The pungency of piperine is caused by its ability to activate transient receptor potential vanilloid type 1 (TRPV1) receptors.[3] These receptors are non-selective cation channels which serve as sensors for pain-inducing stimuli like capsaicin, acidic conditions and heat and are also involved in temperature regulation of the human body.[4] Due to the receptors' involvement in pain processing, TRPV1 agonists and antagonists are currently under investigation as agents for the treatment of neuropathic pain and other diseases.[5] With regard to a further role of piperine derivatives in the formation of a prospective pharmacological lead compound, selectivity for either of these receptors would be highly desirable. However, the simultaneous interaction of piperine and (potentially) its derivatives with $GABA_A$ and TRPV1 receptors could lead to unwanted side effects.

In a most recent study[6] we have modified the amide functionality as well as the linker region of the natural product to investigate the effect of such structural modifications on its pharmacological activity. Analyzing the modulation of GABA-induced chloride currents through the $GABA_A$ activity of these derivatives has revealed a strong preference for di-*n*-butyl and di-*n*-propyl amide. The scaffold has proven to be highly sensitive to modifications of the linker region – all attempted modifications led to a significant loss of efficiency.

With the goal of synthesizing a library of aryl-modified piperine derivatives in mind, we required a robust synthetic method which would allow us to synthesize the desired aryldienamides in a minimum number of steps and a high level of modularity with respect to the aryl residues.

Although at present a plethora of methods for the assembly of 1-carbonyl-4-aryl substituted dienes exist, there is a demand for the development of modern and efficient methods,[7] including Wittig reactions,[8] metathesis,[9] transition-metal catalyzed ene–ene[10] and ene–yne[11] coupling reactions and C–H activation reactions.[12] These methods typically assemble the 1,3-diene from 2 + 2 or 3 + 1 carbon synthons with the requirement for pre-functionalization of both coupling partners. In this context, coupling reaction of suitably substituted dienoic acid derivatives with an aryl coupling partner is attractive. Such a reaction was recently reported by Maulide and coworkers:[13] they prepared 5-halodienoic derivatives from cyclobutene lactones and coupled these compounds in a Suzuki–Miyaura cross-coupling reaction with arylboronic acids.

In this project, we chose to employ a Heck cross-coupling reaction, which is appealing for several reasons: good atom economy, the diene coupling partner can be easily prepared in a single step from commercial material, substituted arylbromides are abundantly available and the reaction can be expected to be *E*-selective.[14]

From the arsenal of metal assisted C–C bond formation strategies, the Heck-cross coupling reaction, *i.e.* the palladium-

[a]*Institute of Applied Synthetic Chemistry, Vienna University of Technology, Getreidemarkt 9, 1060 Vienna, Austria. E-mail: marko.mihovilovic@tuwien.ac.at; Fax: +43 1 58801 154 99; Tel: +43 1 58801 163615*

[b]*Department of Pharmacology and Toxicology, University of Vienna, Althanstr. 14, 1090 Vienna, Austria*

†Electronic supplementary information (ESI) available. See DOI: 10.1039/c4ob02242d

catalyzed cross-coupling of olefins and aromatic or vinylic (pseudo)halides, has become an integral part of modern cross-coupling methods.[15] The palladium-catalyzed arylation[14] and vinylation[16] of conjugated dienes was first reported by Heck and coworkers in the late 1970s and early 1980s. In the case of arylation of pentadienoic acid it has been shown that the reaction occurs at the terminal olefinic position providing *E*,*E*-dienes as products.[14]

Given the importance of dienes as synthetic intermediates and final products, there are only a few precedents of direct coupling of dienes with suitable coupling partners in the literature. Arylation has been reported in the presence of silver or thallium salts,[17] in ionic liquids[18] or under C–H activation conditions with benzoxazole as a coupling partner[19] as well as in the total synthesis of galanthamine.[20] Vinylation has been reported under oxidative coupling conditions[10] or in rhodium(I) catalysis[21] using boron compounds as coupling partners and in a tandem hydrozirconation-coupling process.[22] Trapping of the intermediate Pd-π-allyl species by nucleophiles has been utilized for carbo- and heteroannulation reactions.[23]

The conditions for the arylation of dienes initially published by Heck were not suitable for our purpose: reactions are conducted without a solvent, which, on a small reaction scale, leads to impractically small volumes. In our hands, the diene substrate was also prone to polymerization under these conditions. In the present study we report the optimization of reaction conditions and the synthesis of a focused library of aryl-modified piperine derivatives. Demonstrating the potential of this facile access to a compound library for biological assessment, the modulation of currents through $GABA_A$ and TRPV1 receptors, expressed in *Xenopus laevis* oocytes by these compounds was analyzed by means of the 2-microelectrode clamp technique.

Results and discussion

Based on our previous findings and with the aim of further improving activity of the hit structure towards $GABA_A$ modulation,[6] we focused on the preparation of piperine derivatives bearing the non-natural dibutylamide function.

Pentadienoic acid[24] was readily converted into its acid chloride *in situ* by treatment with oxalylchloride/DMF, followed by the addition of dibutylamine. Attempts to isolate the acid chloride led to decomposition in our hands. Alternatively, pentadienoic acid was smoothly converted into the required amide in the presence of EDCI·HCl. When kept at −20 °C the amide displayed a storage stability of several months without significant degradation.

As a starting point for the optimization of the metal assisted C–C bond formation reaction, the coupling of 4-bromotoluene was conducted employing the standard Heck-reaction conditions ($Pd(OAc)_2$, $(o\text{-tolyl})_3P$, NEt_3, MeCN, 70 °C).[25]

The reaction proceeded slowly, giving 52% yield after 72 hours (Table 1, entry 1). Throughout the screening process, reactions at temperatures at or below the boiling point of the

Table 1 Optimization of coupling conditions

Entry	Solvent	Base	Ligand	*T*/°C	Time	GC-yield
1	MeCN	NEt_3	$(o\text{-Tolyl})_3P$	70	72 h	52%
2	MeCN	NEt_3	$(o\text{-Tolyl})_3P$	140, mw	3 h	31%
3	MeCN	NEt_3	$(o\text{-Tolyl})_3P$	160, mw	3 h	31%
3	MeCN	NEt_3	$(o\text{-Tolyl})_3P$	160, mw	1 h	13%
4	MeCN	NaOAc	$(o\text{-Tolyl})_3P$	160, mw	1 h	4%
5	MeCN	$NaHCO_3$	$(o\text{-Tolyl})_3P$	160, mw	1 h	3%
6	MeCN	K_2CO_3	$(o\text{-Tolyl})_3P$	160, mw	1 h	16%
7	PhMe	NEt_3	$(o\text{-Tolyl})_3P$	160, mw	1 h	3%
8	PhMe	NaOAc	$(o\text{-Tolyl})_3P$	160, mw	1 h	1%
9	PhMe	$NaHCO_3$	$(o\text{-Tolyl})_3P$	160, mw	1 h	1%
10	PhMe	K_2CO_3	$(o\text{-Tolyl})_3P$	160, mw	1 h	4%
11	THF	NEt_3	$(o\text{-Tolyl})_3P$	160, mw	1 h	2%
12	THF	NaOAc	$(o\text{-Tolyl})_3P$	160, mw	1 h	3%
13	THF	$NaHCO_3$	$(o\text{-Tolyl})_3P$	160, mw	1 h	2%
14	THF	K_2CO_3	$(o\text{-Tolyl})_3P$	160, mw	1 h	48%
15	DMF	NEt_3	$(o\text{-Tolyl})_3P$	160	1 h	16%
16	DMF	NaOAc	$(o\text{-Tolyl})_3P$	160	13 h	49%
17	DMF	$NaHCO_3$	$(o\text{-Tolyl})_3P$	160	1 h	53%
18	DMF	K_2CO_3	$(o\text{-Tolyl})_3P$	160	1 h	79%
19	DMF	K_2CO_3 + NEt_4Cl	$(o\text{-Tolyl})_3P$	160	1 h	76%
20	DMF	K_2CO_3 + NEt_4Br	$(o\text{-Tolyl})_3P$	160	1 h	75%
21	DMF	K_2CO_3	$(o\text{-Tolyl})_3P$	140	1 h	77%
22	DMF	K_2CO_3	$(2\text{-Furyl})_3P$	140	1 h	3%
23	DMF	K_2CO_3	$(p\text{-ClPh})_3P$	140	1 h	8%
24	DMF	K_2CO_3	$(1\text{-Naphthyl})_3P$	140	1 h	21%
25	DMF	K_2CO_3	$Pd(PPh_3)_4$	140	1 h	42%
26	DMF	K_2CO_3	Dppf	140	1 h	76%
27	DMF	K_2CO_3	Cy_3P	140	1 h	79%
28	DMF	K_2CO_3	Dppp	140	1 h	87%
29	DMF	K_2CO_3	JohnPhos	140	1 h	89%

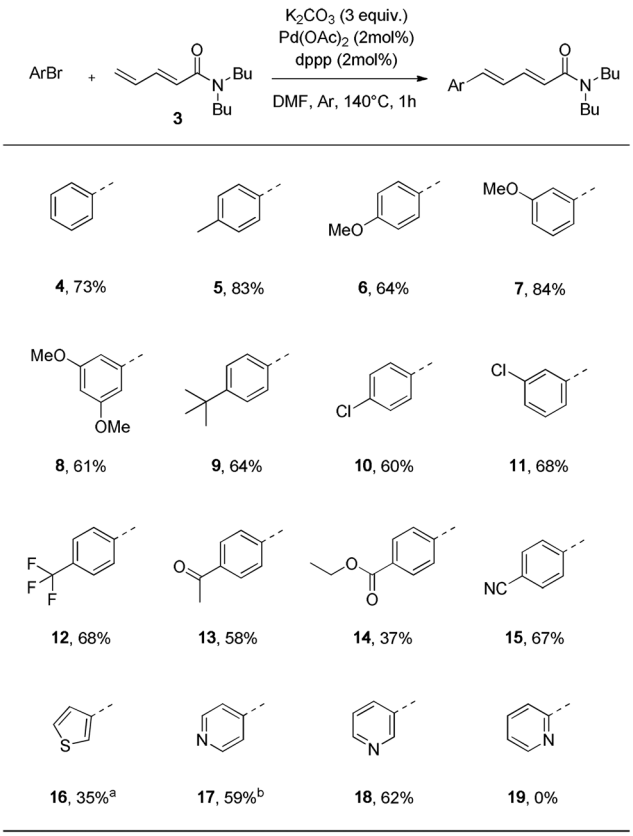
reaction solvent were carried out in screw-cap vials heated in a metal block. Reactions which required higher temperatures were carried out in a microwave reactor, which facilitates automated and safe handling of pressurized vessels (an experiment comparing these heat-sources showed that they can be used interchangeably for this transformation). Increasing the temperature to 140 °C or 160 °C both gave 31% of GC-yield after only three hours (entries 2 and 3). An extension of the reaction time was not attempted in these cases, since the mass balance indicated significant decomposition of the starting materials. First, a set of four bases (NEt_3, $NaHCO_3$, K_2CO_3 and NaOAc) and four solvents (MeCN, toluene, THF and DMF) were evaluated (entries 3–18). While toluene and THF did not facilitate coupling in combination with most bases, the best results were obtained with DMF as the solvent (entries 15–18). Out of the set of bases tested, K_2CO_3 proved most effective in all solvents (entries 6, 10, 14, 18), particularly in DMF (79% GC yield, entry 18). Quaternary ammonium salts as additives, which can be beneficial in Heck-couplings,[26] did not improve the reaction (entries 19 and 20).

An improvement in the side-product profile as judged by GC was achieved by lowering the reaction temperature to 140 °C, while the reaction yield was unaffected (entries 18 and 21).

Finally, a set of eight mono- and bidentate phosphine ligands were tested in combination with palladium(II) acetate. The use of ($Pd(dba)_2$) as a palladium source was also investigated, but it gave generally lower conversions (see ESI†). With respect to the ligands the best results were obtained with JohnPhos and dppp (87–89% GC yield, Table 1, entries 28 and 29). Compared to JohnPhos, dppp has a lower price and was therefore selected for the final reaction protocol.

After establishing an optimized set of reaction parameters for the required reaction, the robustness of the protocol was investigated (Scheme 1). Coupling proceeded smoothly for a variety of aryl bromides bearing electron donating (**4**, **6–9**) or electron withdrawing substituents (**12–15**). In the reactions of bromochlorobenzenes the chloro-substituent was inert under the reaction conditions (**10** and **11**). In the case of 3-bromothiophene the product was obtained in a low yield of 35%. 3- and 4-bromopyridines were well accepted giving products **17** and **18** in 59% and 62% yield, respectively. However, 2-substituted heterocycles (aimed at compound **19**) failed to undergo coupling. The same was observed in the cases of 2-bromothiophene and 2-bromothiazole. This indicates that complexation by the neighboring heteroatom could be responsible for the detrimental effect on the reaction in these cases. Concerning regio- and stereoselectivity of the reaction, all final products were isolated as the 2*E*,4*E*-dienamides. However, GC-MS analysis of the crude reaction mixture typically showed several minor peaks with the same *m*/*z* ratio as the products, which are likely to be stereo- and regioisomers. These side products occurred only in trace amounts and we were therefore unable to isolate sufficient quantities for their characterization.

The effect of aryl-modifications on the enhancement of GABA-induced chloride currents (I_{GABA}) through $\alpha_1\beta_2\gamma_{2S}$ recep-



Scheme 1 Compound library. (a) 2 equiv. of 3-bromothiophene, 100 °C, 16 h; (b) 44 h, 2 mol% of catalyst added after 1 h, 1 equiv. of 4-bromopyridine added after 16 h.

tors was studied at 100 μM. Compared to the natural product piperine, compounds **4** (783 ± 72%, $p < 0.001$), **6** (883 ± 70%, $p < 0.001$), **15** (570 ± 113, $p < 0.05$), **16** (970 ± 244%, $p < 0.001$) and **18** (782 ± 62%, $p < 0.001$) displayed a significantly more pronounced I_{GABA} enhancement, while I_{GABA} modulation by the other prepared compounds did not significantly differ from that of piperine (226 ± 26% at 100 μM; data taken from ref. 1, see Fig. 1A).

Likewise, the effect on the modulation of capsaicin-induced currents through the TRPV1 receptors was studied at a concentration of 100 μM. As illustrated in Fig. 1B, compound **8** (80 ± 22%, $p < 0.001$) significantly enhanced the currents through TRPV1 channels, while compounds **4** (−90 ± 2%, $p < 0.0015$), **5** (−59 ± 6%; $p < 0.05$), **7** (−63 ± 16%; $p < 0.01$), **9** (−73 ± 10%; $p < 0.001$), **10** (65 ± 7%; $p < 0.01$) and **11** (87 ± 2%, $p < 0.001$) effectively inhibited them. Products **6**, **12**, **13**, **14**, **15**, **16**, **17** and **18** did not display any significant modulation of the TRPV1 receptors (representative traces for the modulation of GABA- and capsaicin-induced currents, respectively, by selected compounds, see Fig. 1C).

Collectively, these data indicate that slight modifications in the natural product piperine can lead to a high selectivity for either the $GABA_A$ or the TRPV1 channels.

Most strikingly, compound **8** significantly enhanced $I_{capsaicin}$ (80 ± 22%, $p < 0.001$), while it was nearly inactive on

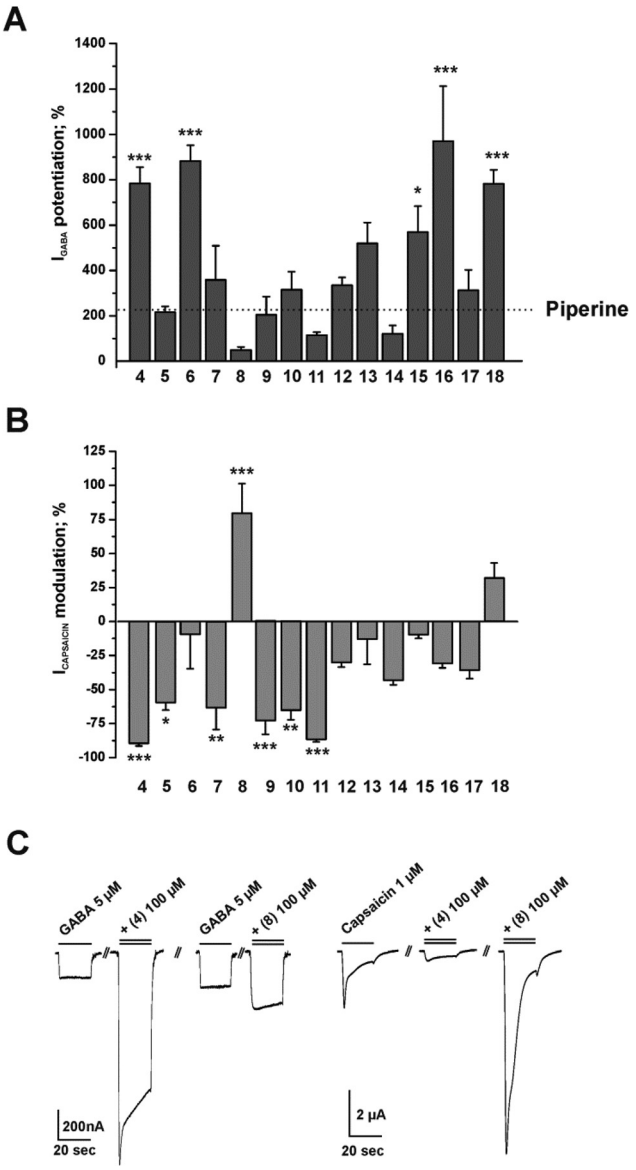


Fig. 1 (A) Modulation of GABA-induced currents through $\alpha_1\beta_2\gamma_{2s}$ $GABA_A$ receptors by 100 μM of the indicated compound. Dashed line represents the I_{GABA} enhancement by the natural product piperine 100 μM.[1a] Each data point represents a mean ± SEM; asterisks indicate statistical significance calculated by one-way ANOVA followed by a Dunnett mean comparison test. (B) Modulation of capsaicin-induced currents through TRPV1 receptors by 100 μM of the indicated compound. Each data point represents a mean ± SEM; asterisks indicate statistical significance calculated by one-way ANOVA followed by a Dunnett mean comparison test. (C) Representative currents for the modulation of GABA-induced currents (left panel) and capsaicin-induced currents (right panel), respectively, by co-application of 100 μM of the indicated derivative are illustrated.

the $GABA_A$ receptors. Likewise, products **11** and **14** displayed only weak I_{GABA} enhancement, however – in contrast to compound **8** – they significantly reduced capsaicin-induced currents through the TRPV1 receptors. The most effective inhibition of $I_{capsaicin}$ was observed for compound **4** (−90 ± 2%), however, this derivative also effectively modulated the $GABA_A$ receptors (783 ± 72%) and was thus not selective for either receptor type. Finally, compound **6** was identified as a novel piperine-derived effective $GABA_A$ receptor modulator (883 ± 70%), that did not affect the TRPV1 receptors (−10 ± 3%).

Conclusions

We have developed a facile protocol for the arylation of dienamides which facilitates rapid and stereoselective access to 2*E*,4*E*-products through operational simplicity and short reaction times. Compared to other protocols, the use of arylbromides instead of boronic acids,[13] alkynes,[11] alkenes[10] or aldehydes[8] comprises a significant advantage in terms of price and commercial availability. Applying this protocol we have synthesized a library of 15 compounds. Biological testing has revealed compounds with a high efficacy and selectivity for either the $GABA_A$ or the TRPV1 receptors. These results are very promising and a full pharmacological characterization of the test compounds is currently underway in our laboratories to be published in due course.

Experimental

The experimental procedures for compound synthesis and biological testing, as well as the compound characterization data can be found in the ESI.†

Acknowledgements

L. W. and A. S. were supported by the Austrian Science Fund (FWF doctoral program "Molecular drug targets" W1232).

Notes and references

1 (*a*) J. Zaugg, I. Baburin, B. Strommer, H.-J. Kim, S. Hering and M. Hamburger, *J. Nat. Prod.*, 2010, **73**, 185; (*b*) M. E. Pedersen, B. Metzler, G. I. Stafford, J. van Staden, A. K. Jager and H. B. Rasmussen, *Molecules*, 2009, **14**, 3833.
2 (*a*) P. J. Whiting, *Curr. Opin. Pharmacol.*, 2006, **6**, 24; (*b*) J. Riss, J. Cloyd, J. Gates and S. Collins, *Acta Neurol. Scand.*, 2008, **118**, 69; (*c*) U. Rudolph and H. Mohler, *Annu. Rev. Pharmacol. Toxicol.*, 2014, **54**, 483.
3 F. N. McNamara, A. Randall and M. J. Gunthorpe, *Br. J. Pharmacol.*, 2005, **144**, 781.
4 N. R. Gavva, A. W. Bannon, S. Surapaneni, D. N. Hovland Jr., S. G. Lehto, A. Gore, T. Juan, H. Deng, B. Han, L. Klionsky, R. Kuang, A. Le, R. Tamir, J. Wang, B. Youngblood, D. Zhu, M. H. Norman, E. Magal, J. J. S. Treanor and J.-C. Louis, *J. Neurosci.*, 2007, **27**, 3366.
5 M. J. Gunthorpe and A. Szallasi, *Curr. Pharm. Des.*, 2008, **14**, 32.
6 A. Schoeffmann, L. Wimmer, D. Goldmann, S. Khom, J. Hintersteiner, I. Baburin, T. Schwarz, M. Hintersteininger,

P. Pakfeifer, M. Oufir, M. Hamburger, T. Erker, G. F. Ecker, M. D. Mihovilovic and S. Hering, *J. Med. Chem.*, 2014, **57**, 5602.
7 M. De Paolis, I. Chataigner and J. Maddaluno, *Top. Curr. Chem.*, 2012, **327**, 87.
8 C. J. O'Brien, F. Lavigne, E. E. Coyle, A. J. Holohan and B. J. Doonan, *Chem. – Eur. J.*, 2013, **19**, 5854.
9 B. H. Lipshutz, S. Ghorai and Z. V. Boskovic, *Tetrahedron*, 2008, **64**, 6949.
10 J. H. Delcamp, P. E. Gormisky and M. C. White, *J. Am. Chem. Soc.*, 2013, **135**, 8460.
11 T. Schabel and B. Plietker, *Chem. – Eur. J.*, 2013, **19**, 6938.
12 Z.-K. Wen, Y.-H. Xu and T.-P. Loh, *Chem. Sci.*, 2013, **4**, 4520.
13 C. Souris, F. Frebault, A. Patel, D. Audisio, K. N. Houk and N. Maulide, *Org. Lett.*, 2013, **15**, 3242.
14 B. A. Patel, J. E. Dickerson and R. F. Heck, *J. Org. Chem.*, 1978, **43**, 5018.
15 (*a*) K. C. Nicolaou, P. G. Bulger and D. Sarlah, *Angew. Chem., Int. Ed.*, 2005, **44**, 4442; (*b*) I. P. Beletskaya and A. V. Cheprakov, *Chem. Rev.*, 2000, **100**, 3009.
16 W. Fischetti, K. T. Mak, F. G. Stakem, J. Kim, A. L. Rheingold and R. F. Heck, *J. Org. Chem.*, 1983, **48**, 948.
17 T. Jeffery, *Tetrahedron Lett.*, 1992, **33**, 1989.
18 L. Beccaria, A. Deagostino, C. Prandi, C. Zavattaro and P. Venturello, *Synlett*, 2006, 2989.
19 W.-C. Lee, T.-H. Wang and T.-G. Ong, *Chem. Commun.*, 2014, **50**, 3671.
20 J. Choi, H. Kim, S. Park and J. Tae, *Synlett*, 2013, 379.
21 G. De la Herran, C. Murcia and A. G. Csakye, *Org. Lett.*, 2005, **7**, 5629.
22 G. Wang, S. Mohan and E.-i. Negishi, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 11344.
23 (*a*) R. C. Larock and C. A. Fried, *J. Am. Chem. Soc.*, 1990, **112**, 5882; (*b*) R. C. Larock, N. Berrios-Pena and K. Narayanan, *J. Org. Chem.*, 1990, **55**, 3447.
24 K. Sparrow, D. Barker and M. A. Brimble, *Tetrahedron*, 2011, **67**, 7989.
25 R. F. Heck, *Org. React.*, 1982, **27**, 345.
26 T. Jeffery, *Tetrahedron*, 1996, **52**, 10113.