Developing Piperine towards TRPV1 and GABAA Receptor Ligands - Synthesis of Piperine Analogs via Heck-Coupling of Conjugated Dienes

Journal:	*Organic & Biomolecular Chemistry*
Manuscript ID:	OB-COM-10-2014-002242.R1
Article Type:	Communication
Date Submitted by the Author:	07-Nov-2014

Complete List of Authors:	Wimmer, Laurin; Vienna University of Technology, Institute of Applied Synthetic Chemistry Schönbauer, David; Vienna University of Technology, Institute of Applied Synthetic Chemistry Pakfeifer, Peter; University of Vienna, Department of Pharmacology and Toxicology Schöffmann, Angela; University of Vienna, Department of Pharmacology and Toxicology Khom, Sophia; University of Vienna, Department of Pharmacology and Toxicology Hering, Steffen; University of Vienna, Department of Pharmacology and Toxicology Mihovilovic, Marko; Vienna Universit of Technology, Institute for Applied Synthetic Chemistry

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

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]

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX
DOI: 10.1039/b000000x

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 dien-amides 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 elaboration of piperine derivatives towards 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.

Within 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 pharmacological activity. Analyzing the modulation of GABA—induced chloride currents trough $GABA_A$ activity by these derivatives has revealed a strong preference for the 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 with 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 exists, 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 on 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.

Within 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-catalyzed cross-coupling of olefines 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 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]

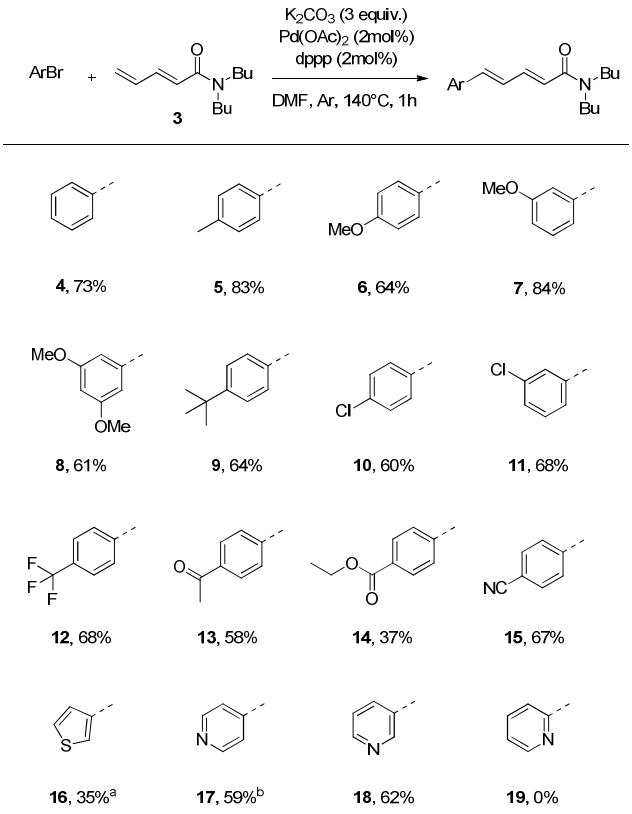
Given the importance of dienes as synthetic intermediates and final products, there are only few precedents of direct coupling of dienes with suitable coupling partners in the literature. Arylation has been reported in the presence of silver or thallium salts[17], in ionic liquids[18] or under C-H activation conditions with benzoxazole as coupling partner[19] as well as in the total synthesis of galanthamine[20]. Vinylation has been reported under oxidative coupling conditions[10] or rhodium(I) catalysis[21] using boron

Table 1 Optimization of coupling conditions

Entry	Solvent	Base	Ligand	T/°C	Time	GC-yield
1	MeCN	NEt_3	(*o*-tolyl)$_3$P	70	72h	52%
2	MeCN	NEt_3	(*o*-tolyl)$_3$P	140, mw	3h	31%
3	MeCN	NEt_3	(*o*-tolyl)$_3$P	160, mw	3h	31%
3	MeCN	NEt_3	(*o*-tolyl)$_3$P	160, mw	1h	13%
4	MeCN	NaOAc	(*o*-tolyl)$_3$P	160, mw	1h	4%
5	MeCN	$NaHCO_3$	(*o*-tolyl)$_3$P	160, mw	1h	3%
6	MeCN	K_2CO_3	(*o*-tolyl)$_3$P	160, mw	1h	16%
7	PhMe	NEt_3	(*o*-tolyl)$_3$P	160, mw	1h	3%
8	PhMe	NaOAc	(*o*-tolyl)$_3$P	160, mw	1h	1%
9	PhMe	$NaHCO_3$	(*o*-tolyl)$_3$P	160, mw	1h	1%
10	PhMe	K_2CO_3	(*o*-tolyl)$_3$P	160, mw	1h	4%
11	THF	NEt_3	(*o*-tolyl)$_3$P	160, mw	1h	2%
12	THF	NaOAc	(*o*-tolyl)$_3$P	160, mw	1h	3%
13	THF	$NaHCO_3$	(*o*-tolyl)$_3$P	160, mw	1h	2%
14	THF	K_2CO_3	(*o*-tolyl)$_3$P	160, mw	1h	48%
15	DMF	NEt_3	(*o*-tolyl)$_3$P	160	1h	16%
16	DMF	NaOAc	(*o*-tolyl)$_3$P	160	13h	49%
17	DMF	$NaHCO_3$	(*o*-tolyl)$_3$P	160	1h	53%
18	DMF	K_2CO_3	(*o*-tolyl)$_3$P	160	1h	79%
19	DMF	K_2CO_3+ NEt_4Cl	(*o*-tolyl)$_3$P	160	1h	76%
20	DMF	K_2CO_3+ NEt_4Br	(*o*-tolyl)$_3$P	160	1h	75%
21	DMF	K_2CO_3	(*o*-tolyl)$_3$P	140	1h	77%
22	DMF	K_2CO_3	(2-furyl)$_3$P	140	1h	3%
23	DMF	K_2CO_3	(*p*-ClPh)$_3$P	140	1h	8%
24	DMF	K_2CO_3	(1-naphtyl)$_3$P	140	1h	21%
25	DMF	K_2CO_3	$Pd(PPh_3)_4$	140	1h	42%
26	DMF	K_2CO_3	dppf	140	1h	76%
27	DMF	K_2CO_3	Cy_3P	140	1h	79%
28	**DMF**	**K_2CO_3**	**dppp**	**140**	**1h**	**87%**
29	DMF	K_2CO_3	JohnPhos	140	1h	89%

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 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, respectively, expressed in *Xenopus laevis* oocytes by these compounds was analyzed by means of the 2-microelectrode clamp technique.



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

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 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, coupling of 4-bromotoluene was conducted employing standard Heck-reaction conditions ($Pd(OAc)_2$, (o-tolyl)$_3$P, NEt_3, MeCN, 70°C).[25]

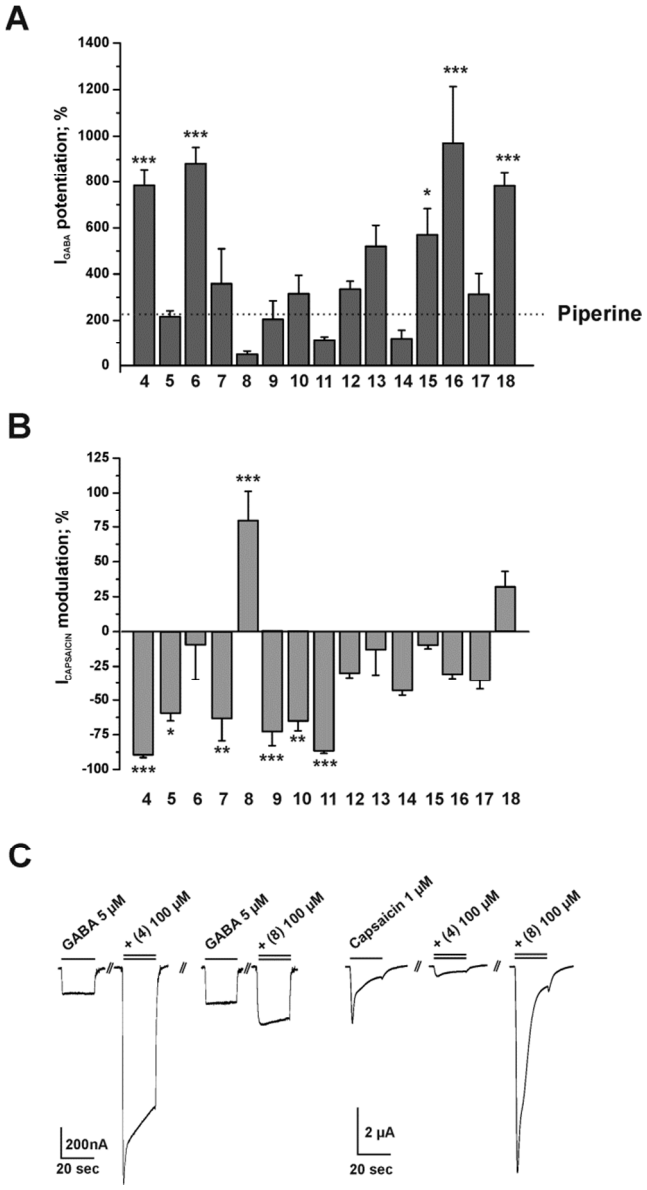


Figure 1 (A) Modulation of GABA-induced currents through $\alpha_1\beta_2\gamma_{2S}$ $GABA_A$ receptors by 100 µM of the indicated compound. Dashed line represents the I_{GABA} enhancement by the natural product piperine 100µM[1a]. Each data point represents a mean±SEM; Asterisks indicate statistical significance calculated by one-way ANOVA followed by a Dunnet 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 Dunnet 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 100µM of the indicated derivative are illustrated.

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 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). Extension of the reaction time was not attempted in these cases, since the mass balance indicated significant decomposition of 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, best results were obtained with DMF as 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 of the side-product profile as judged by GC was achieved by lowering the reaction temperature to 140°C, while reaction yield was unaffected (entry 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 palladium sources was also investigated, but gave generally lower conversions (see Supporting Information). 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 2). Coupling proceeded smoothly for a variety of aryl bromides bearing electron donating (**4**, **6-9**) or electon withdrawing substituents (**12-15**). Within reactions of bromochlorobenzenes the chloro-substituent was inert under the reaction conditions (**10** and **11**). In case of 3-bromothiophene the product was obtained in 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 case 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 product, 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 $\alpha_1\beta_2\gamma_{2S}$ receptors was studied at 100μM. 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 100μM; data taken from[1], see Fig.1A).

Likeweise, the effect on the modulation of capsaicin-induced currents through TRPV1 receptors was studied at a concentration of 100μM. 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) efficaciously inhibited them. Products **6, 12, 13, 14, 15, 16, 17** and **18** did not display any significant modulation of 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 of the natural product piperine can lead to high selectivity for either $GABA_A$ or TRPV1 channels.

Most strikingly, compound **8** significantly enhanced $I_{caspsaicin}$ (80±22%, p<0.001), while it was nearly inactive on $GABA_A$ 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 TRPV1 receptors. The most efficacious inhibition of $I_{capsaicin}$ was observed for compound **4** (-90±2%)**,** however, this derivative also efficaciously modulated $GABA_A$ receptors (783±72%) and was thus not selective for either receptor type. Finally, compound **6** was identified as a novel piperine-derived efficacious $GABA_A$ receptor modulator (883±70%), that does not affect 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, usage 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 high efficacy and selectivity for either $GABA_A$ or TRPV1. These results are very promising and a full pharmacological characterization of test compounds is currently under way in our laboratories to be published in due course.

Acknowledgement

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

Experimental

Experimental procedures for compounds synthesis and biological testing, as well as compound characterization data can be found in the supplementary material.

Notes and references

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

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

† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

1(a) J. Zaugg, I. Baburin, B. Strommer, H.-J. Kim, S. Hering and M. Hamburger, *Journal of Natural Products*, 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, *Current Opinion in Pharmacology*, 2006, **6**, 24; (b) J. Riss, J. Cloyd, J. Gates and S. Collins, *Acta Neurologica Scandinavica*, 2008, **118**, 69; (c) U. Rudolph and H. Mohler, *Annual Review of Pharmacology and Toxicology*, 2014, **54**, 483.

3 F. N. McNamara, A. Randall and M. J. Gunthorpe, *British Journal of Pharmacology*, 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, *Journal of Neuroscience*, 2007, **27**, 3366.

5 M. J. Gunthorpe and A. Szallasi, *Current Pharmaceutical Design*, 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, *Journal of Medicinal Chemistry*, 2014, **57**, 5602.

7 M. De Paolis, I. Chataigner and J. Maddaluno, *Topics in Current Chemistry*, 2012, **327**, 87.

8 C. J. O'Brien, F. Lavigne, E. E. Coyle, A. J. Holohan and B. J. Doonan, *Chemistry - A European Journal*, 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, *Journal of the American Chemical Society*, 2013, **135**, 8460.

11 T. Schabel and B. Plietker, *Chemistry - A European Journal*, 2013, **19**, 6938.

12 Z.-K. Wen, Y.-H. Xu and T.-P. Loh, *Chemical Science*, 2013, **4**, 4520.

13 C. Souris, F. Frebault, A. Patel, D. Audisio, K. N. Houk and N. Maulide, *Organic Letters*, 2013, **15**, 3242.

14 B. A. Patel, J. E. Dickerson and R. F. Heck, *Journal of Organic Chemistry*, 1978, **43**, 5018.

15(a) K. C. Nicolaou, P. G. Bulger and D. Sarlah, *Angewandte Chemie, International Edition*, 2005, **44**, 4442; (b) I. P. Beletskaya and A. V. Cheprakov, *Chemical Reviews (Washington, D. C.)*, 2000, **100**, 3009.

16 W. Fischetti, K. T. Mak, F. G. Stakem, J. Kim, A. L. Rheingold and R. F. Heck, *Journal of Organic Chemistry*, 1983, **48**, 948.

17 T. Jeffery, *Tetrahedron Letters*, 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, *Chemical Communications (Cambridge, United Kingdom)*, 2014, **50**, 3671.

20 J. Choi, H. Kim, S. Park and J. Tae, *Synlett*, 2013, **24**, 379.
21 G. De la Herran, C. Murcia and A. G. Csakye, *Organic Letters*, 2005, **7**, 5629.
22 G. Wang, S. Mohan and E.-i. Negishi, *Proceedings of the National Academy of Sciences of the United States of America*, 2011, **108**, 11344.
23(a) R. C. Larock and C. A. Fried, *Journal of the American Chemical Society*, 1990, **112**, 5882; (b) R. C. Larock, N. Berrios-Pena and K. Narayanan, *Journal of Organic Chemistry*, 1990, **55**, 3447.
24 K. Sparrow, D. Barker and M. A. Brimble, *Tetrahedron*, 2011, **67**, 7989.
25 R. F. Heck, *Organic Reactions (New York)*, 1982, **27**, 345.
26 T. Jeffery, *Tetrahedron*, 1996, **52**, 10113.