



Cite this: *Org. Biomol. Chem.*, 2019, **17**, 2103

Received 28th August 2018,
Accepted 19th September 2018

DOI: 10.1039/c8ob02102c

rsc.li/obc

Stereoselective C-terminal peptide elongation from Chan–Lam–Evans reaction generated isopropenyl esters†

Luuk Steemers and Jan H. van Maarseveen *

C-Terminal dipeptide isopropenyl esters were synthesised by a Cu(II)-mediated Chan–Lam–Evans enol esterification of peptide carboxylic acids and isoprenyl boroxine. These shelf stable peptide esters could be coupled stereoselectively with a variety of amino acid and dipeptide nucleophiles in high yield and purity in the presence of pyrazole/DBU as the catalyst.

In current pharmaceutical research peptides play an increasingly important role.¹ As a result, in recent years several peptides and analogs thereof were registered as drugs.² By using both solid and solution phase techniques peptides are synthesized in the C → N direction exclusively. In other words, the N-terminus is elongated by consecutive coupling of single N-protected amino acids. Commonly employed carboxyl group activating reagents, for example uronium, phosphonium and carbodiimide based, form a highly reactive intermediate, that is usually transformed *in situ* into less reactive HOBt or HOAt esters to avoid racemisation.³ The mesomerically stabilised and, as compared to an amide group, less nucleophilic N-carbamate protective group suppresses racemisation of the stereogenic center by hampering enolisation or oxazolone formation. Especially for peptide fragment couplings or peptide cyclisation C-terminal peptide activation is required. However, coupling of two peptide fragments or cyclisation with traditional coupling reagents inevitably leads to epimerisation of the α-carbon of the C-terminally carboxyl-activated residue. As a result, in current ligation or cyclisation strategies, usually a C-terminal glycine is chosen as the site of carboxyl activation.⁴ In stark contrast, both ribosomally and non-ribosomally synthesised peptides are elongated in the N → C direction, *via* aminolysis of the growing peptide chain of the activated C-terminus. Nature uses very mild carboxyl group activation such as the connecting ester bond between the amino acid

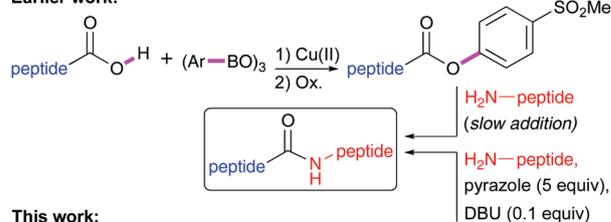
and tRNA, or as a thioester while linked at the peptidyl carrier protein in the case of non-ribosomal peptide synthesis. Aminolysis of the rather unreactive secondary ester at the tRNA occurs due to the proximity of the incoming amine nucleophile to this ester, induced by the ribosome, underscoring the power of pre-organisation for certain chemical transformations.

Synthetic strategies employing elongation in the N → C direction are scarce. Katritzky has shown that peptide benzotriazole esters may be prepared in the cold using SOCl₂/HOBt and subsequently coupled to other peptide fragments.⁵ More recently, the Bode group has shown that unprotected peptides bearing a C-terminal keto acid ligate with peptides modified with a hydroxylamine at the N-terminus.⁶ Another viable method used for fragment couplings is the synthesis of peptides that deliver a stable C-terminal ester serving as a substrate for chemoenzymatic coupling.⁷ In the search for catalytic carboxyl group activation methods to substitute the traditional stoichiometric coupling reagents we have reported earlier the use of mildly activated 4-methylsulfonylphenyl esters, obtained *via* a Cu(II)-catalysed Chan–Lam–Evans (CLE) reaction⁸ and subsequent oxidation, as suitable substrates for epimerisation-free coupling (Scheme 1).⁹

Van 't Hoff Institute for Molecular Sciences, University of Amsterdam, Science Park 904, 1098 XH Amsterdam, The Netherlands. E-mail: j.h.vanmaarseveen@uva.nl

† Electronic supplementary information (ESI) available: Detailed experimental procedures and copies of the ¹H- and ¹³C-NMR spectra and chiral HPLC traces. See DOI: 10.1039/c8ob02102c

Earlier work:



Scheme 1 CLE-mediated peptide esterification and follow-up elongation by aminolysis.



It was found that the CLE-type aryl esterification of peptides proceeded with complete stereoretention. However, to obtain high de's in the aminolysis reaction the amine had to be added slowly to avoid too basic conditions. Recently, we disclosed the efficient enol esterification of carboxylic acids *via* the CLE reaction and demonstrated the compatibility with all proteogenic amino acids, except methionine.¹⁰ Furthermore, no racemisation of the C-terminus was observed during the CLE-type enol esterification of amino acids. This prompted us to study the possibility of stereoselective elongation of peptides at the C-terminus employing isopropenyl esters as mild activating groups. As compared to 4-methylsulfonylphenyl esters, isopropenyl esters are less reactive and only react slowly in direct aminolysis reactions. Recently, the group of Birman reported anionic azoles as efficient catalysts for the aminolysis of both phenyl and isopropenyl esters.¹¹ Remarkably, in a landmark paper that was far ahead of its time, the group of Beyerman reported back in the 1960s the use of neutral azoles as additives for the stereoselective aminolysis of peptide vinyl esters that were made *via* Pd-catalysis.¹² Although vinyl esters can be efficiently synthesized *via* the CLE reaction from commercially available trivinylboroxine¹³ but also by Pd(II)¹⁴ or Hg(II)¹⁵ catalysed transesterification with vinyl acetate, the acetaldehyde side product released after aminolysis is far more reactive than acetone, the side product resulting from amide bond formation from isopropenyl esters.

For our studies, six model dipeptide acids were synthesized, *i.e.* Boc-Phe-Ala-OH (**1a**), Boc-Phe-Phe-OH (**1c**) and Boc-Phe-Val-OH (**1e**) and, to allow facile monitoring of the stereointegrity, their diastereomers **1b**, **1d** and **1f**, (see Table 1).

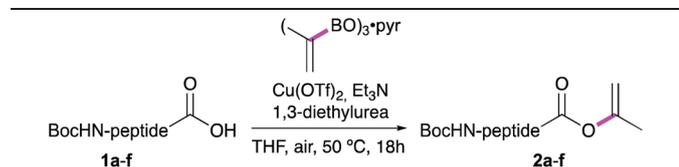
Ala, Phe and Val were selected as the C-terminal residues due to their increasing steric properties. Transformation of the peptide acids **1a–f** into the isopropenyl esters **2a–f** was carried out smoothly by the previously reported CLE conditions, using tri(isopropenyl)boroxine pyridine complex¹⁶ as the coupling partner. All peptide isoprenyl esters were obtained in excellent yields as shelf-stable solid compounds. To determine the stereointegrity, the most epimerisation sensitive peptide esters

2c and **2d**, bearing a C-terminal phenylalanine, were subjected to chiral HPLC analysis showing no detectable loss of stereo-integrity. For the same reason, for our optimisation studies of the aminolysis reaction, Boc-Phe-Phe-OC(CH₃)=CH₂ (**1c**) was chosen as the C-terminally activated peptide to deliver, by using Ala-*Ot*Bu as the nucleophile, Boc-Phe-Phe-Ala-*Ot*Bu (**3k**) as the target tripeptide (Table 2).

The optimisation studies started using CH₂Cl₂ as solvent and by adding a slight excess of 1,2,4-triazole as a catalyst and 10 mol% DBU as the base to generate the active anionic azolate species. Although the aminolysis reaction was clean and did not require further purification besides a simple acidic washing step, only a moderate de of 69% was found (Table 2, entry 1). By using pyrazole as the catalyst, a similar outcome of the reaction was found (entry 2). A significant improvement of the de was achieved by increasing the amount of azole to five equiv. (entry 3). By adding two equiv. of the amine nucleophile (entry 4) the de went further up to 89%. Switching to toluene as a more apolar solvent helped to improve the de even further to 97% (entry 5 *vs.* 1 and entry 6 *vs.* 4). Also in this case pyrazole performed slightly better than 1,2,4-triazole (entry 7 *vs.* 6). Raising the amount of amine or using an even more apolar solvent did not lead to a further increase of the de (entries 8 and 9). Also 1,2,3-triazole and benzotriazole were screened as catalysts, however these reactions either did not reach full conversion, had lower de and/or produced unidentified byproducts (not shown).

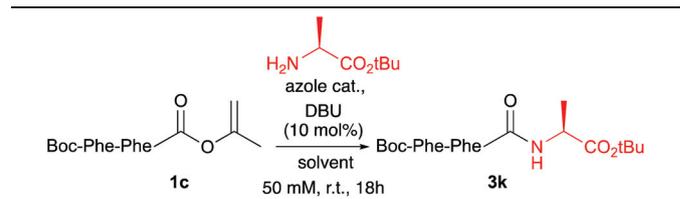
The proposed reaction mechanism for the pyrazole-catalysed aminolysis reaction starts by deprotonation of pyrazole **I** by DBU to form the highly nucleophilic azole anion **II** (Scheme 2). Nucleophilic attack of the anionic nitrogen at the isopropenyl ester provides acylpyrazole **III**, thereby irreversibly releasing acetone and regenerating DBU. The incoming amine nucleophile can coordinate to the pyrazole 2-nitrogen atom *via* H-bonding forming complex **IV** showing a favourable 5-mem-

Table 1 Transformation of the dipeptide carboxylic acids into their isoprenyl esters using the CLE-reaction



| Starting dipeptide | Starting peptide acid | Starting peptide product | Yield (%) | de (%) |
|--------------------|-----------------------|--------------------------|-----------|--------|
| 1a | Boc-Phe-Ala-OH | 2a | 92 | nd |
| 1b | Boc-Phe-D-Ala-OH | 2b | 91 | nd |
| 1c | Boc-Phe-Phe-OH | 2c | 90 | >99 |
| 1d | Boc-Phe-D-Phe-OH | 2d | 97 | >99 |
| 1e | Boc-Phe-Val-OH | 2e | 80 | nd |
| 1f | Boc-Phe-D-Val-OH | 2f | 91 | nd |

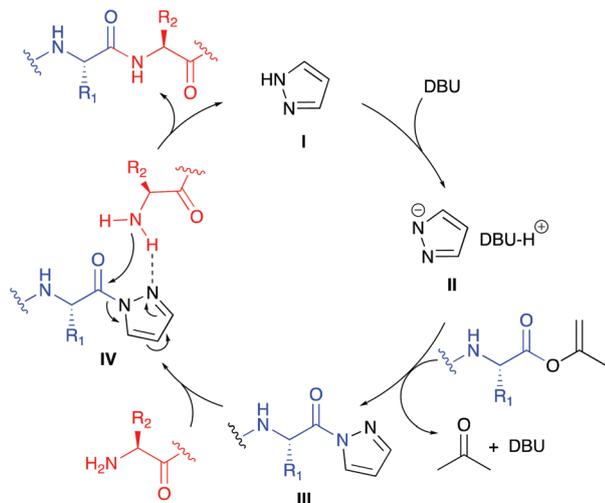
Table 2 Optimisation of the aminolysis reaction



| Entry | Solvent | Azole (equiv.) | Equiv. H-Ala- <i>Ot</i> Bu | de ^a (%) |
|-------|---------------------------------|----------------------|----------------------------|---------------------|
| 1 | CH ₂ Cl ₂ | 1,2,4-Triazole (1.2) | 1.2 | 69 |
| 2 | CH ₂ Cl ₂ | Pyrazole (1.2) | 1.2 | 72 |
| 3 | CH ₂ Cl ₂ | 1,2,4-Triazole (5) | 1.2 | 79 |
| 4 | CH ₂ Cl ₂ | 1,2,4-Triazole (5) | 2 | 89 |
| 5 | PhCH ₃ | 1,2,4-Triazole (1.2) | 1.2 | 84 |
| 6 | PhCH ₃ | 1,2,4-Triazole (5) | 2 | 95 |
| 7 | PhCH ₃ | Pyrazole (5) | 2 | 97 |
| 8 | PhCH ₃ | Pyrazole (5) | 4 | 96 |
| 9 | PhH | Pyrazole (5) | 2 | 95 |

^a Determined by chiral HPLC.





Scheme 2 Proposed catalytic cycle for the pyrazole catalysed aminolysis of isoprenyl esters.

bered transition state for the aminolysis step to give the amide product and releasing pyrazole **I**.

Some observations in Table 1 can be explained by this proposed mechanism. At first, in polar solvents DBU is more likely to deprotonate the α -H within **III** to give an enolate resulting in epimerisation. Secondly, the proposed H-bonds in **IV** are stronger in apolar solvents, thereby increasing the rate of the aminolysis reaction thus suppressing epimerisation. The positive influence of using an excess of pyrazole on the de may be explained by a buffering effect lowering the basicity of the reaction mixture. With the optimised aminolysis conditions in hand we set out to investigate the scope of the reaction. Because we have shown earlier the compatibility of the CLE-reaction with appropriately protected canonical amino acids, we aimed at a limited but representative set of target tripeptides.¹⁰ As the C- and N-terminal residues Gly, Ala, Phe and Val, representing a gradual increase in steric hindrance, were selected of which the outcome may be extrapolated to the other canonical amino acids.¹⁷ At the C-terminus also the D-configured analogs were included to allow precise HPLC-analysis to determine the stereointegrity of the aminolysis reaction. The results of this screening by reacting the six dipeptide isopropenyl esters **2a–f** with Gly-*Ot*Bu, Ala-*Ot*Bu, Phe-*Ot*Bu and Val-*Ot*Bu as the model amino acid nucleophiles are summarised in Table 3. It is worth mentioning that for all reactions a simple acidic extraction was sufficient to remove all non-volatiles such as excess amine nucleophile, pyrazole, and DBU. The tripeptides **3a–x** thus obtained were virtually pure and needed no further purification, as determined by ¹H- and ¹³C-NMR spectroscopy. The screening showed that all products were obtained in good to excellent yields. The diastereomeric excess for all peptides **3a–u** is >96% and in most cases exceeds 98%. For the aminolysis of dipeptides **2e** and **2f** with the sterically challenging valine at the C-terminus the reaction gave either incomplete or no conversion at all at room tem-

Table 3 Synthesis of tripeptides

| $\text{2a-f} \xrightarrow[\text{toluene (50 mM), rt, 18h}]{\text{pyrazole (5 equiv), DBU (10 mol\%)}}$ | | $\text{BocHN-peptide} \xrightarrow[\text{3a-x}]{\text{AA-CO}_2\text{tBu}}$ | | |
|--|--|--|---------------------|-----|
| Starting dipeptide | Tripeptide product | Yield (%) | de ^a (%) | |
| 2a | Boc-Phe-Ala-Gly- <i>Ot</i> Bu | 3a | 96 | 97 |
| 2b | Boc-Phe-D-Ala-Gly- <i>Ot</i> Bu | 3b | 98 | 98 |
| 2a | Boc-Phe-Ala-Ala- <i>Ot</i> Bu | 3c | 99 | 99 |
| 2b | Boc-Phe-D-Ala-Ala- <i>Ot</i> Bu | 3d | 93 | 99 |
| 2a | Boc-Phe-Ala-Phe- <i>Ot</i> Bu | 3e | 95 | 99 |
| 2b | Boc-Phe-D-Ala-Phe- <i>Ot</i> Bu | 3f | 95 | 99 |
| 2a | Boc-Phe-Ala-Val- <i>Ot</i> Bu | 3g | 92 | 99 |
| 2b | Boc-Phe-D-Ala-Val- <i>Ot</i> Bu | 3h | 98 | 98 |
| 2c | Boc-Phe-Phe-Gly- <i>Ot</i> Bu | 3i | 95 | 97 |
| 2d | Boc-Phe-D-Phe-Gly- <i>Ot</i> Bu | 3j | 93 | >99 |
| 2c | Boc-Phe-Phe-Ala- <i>Ot</i> Bu | 3k | 90 | 97 |
| 2d | Boc-Phe-D-Phe-Ala- <i>Ot</i> Bu | 3l | 88 | 98 |
| 2c | Boc-Phe-Phe-Phe- <i>Ot</i> Bu | 3m | 88 | 98 |
| 2d | Boc-Phe-D-Phe-Phe- <i>Ot</i> Bu | 3n | 94 | 97 |
| 2c | Boc-Phe-Phe-Val- <i>Ot</i> Bu | 3o | 90 | 99 |
| 2d | Boc-Phe-D-Phe-Val- <i>Ot</i> Bu | 3p | 94 | 97 |
| 2e | Boc-Phe-Val-Gly- <i>Ot</i> Bu ^b | 3q | 92 | 99 |
| 2f | Boc-Phe-D-Val-Gly- <i>Ot</i> Bu ^b | 3r | 90 | 99 |
| 2e | Boc-Phe-Val-Ala- <i>Ot</i> Bu ^b | 3s | 96 | 97 |
| 2f | Boc-Phe-D-Val-Ala- <i>Ot</i> Bu ^b | 3t | 82 | 97 |
| 2e | Boc-Phe-Val-Phe- <i>Ot</i> Bu ^c | 3u | 90 | 98 |
| 2f | Boc-Phe-D-Val-Phe- <i>Ot</i> Bu ^c | 3v | 79 | 94 |
| 2e | Boc-Phe-Val-Val- <i>Ot</i> Bu ^c | 3w | 77 | 90 |
| 2f | Boc-Phe-D-Val-Val- <i>Ot</i> Bu ^c | 3x | 85 | 95 |

^a Determined by chiral HPLC. ^b Reaction run at 50 °C. ^c Reaction run at 80 °C.

perature. For Gly-*Ot*Bu or Ala-*Ot*Bu as nucleophiles, heating to 50 °C was sufficient to complete the reaction, giving tripeptides **3q–t** in excellent yields and diastereomeric excess. However, in the case of using Phe-*Ot*Bu or Val-*Ot*Bu as nucleophiles heating to 80 °C was required to ensure completion of the reaction. Although the yields of products **3v–x** were still very good, we had to accept that the de's showed a small but significant drop. Presumably this is caused by the longer living epimerisation-prone acylpyrazole intermediate **III** (see Scheme 2).

To mimic more closely a true peptide ligation reaction, the dipeptide isopropenyl esters **2a–f** were subjected to pyrazole-catalysed aminolysis by reaction with dipeptide H-Phe-Phe-*Ot*Bu as the nucleophile.¹⁸ By application of the optimised conditions, Boc-Phe-Ala-Phe-Phe-*Ot*Bu (**4a**) and Boc-Phe-D-Ala-Phe-Phe-*Ot*Bu (**4b**) were isolated in yields of 68% and 79%, respectively. This drop of the yield as compared to using amino acid esters as the nucleophile was caused by the fact that the excess of apolar H-Phe-Phe-*Ot*Bu could not be removed by simply acidic washing. Moreover, chromatographic purification was unsuccessful due to the similar *R_F* values of H-Phe-Phe-*Ot*Bu and the tetrapeptide products. The problem was overcome by reacting the crude mixture with Boc₂O to transform the excess of unreacted nucleophile into Boc-Phe-Phe-*Ot*Bu, which could be separated *via* column chromato-



Table 4 Synthesis of tetrapeptides

| Starting dipeptide | Tetrapeptide product | Yield (%) | de ^a (%) |
|--------------------|---|-----------|---------------------|
| 2a | Boc-Phe-Ala-Phe-Phe-OtBu 4a | 68 | 99 |
| 2b | Boc-Phe-D-Ala-Phe-Phe-OtBu 4b | 79 | 99 |
| 2c | Boc-Phe-Phe-Phe-Phe-OtBu 4c | 69 | 93 |
| 2d | Boc-Phe-D-Phe-Phe-Phe-OtBu 4d | 69 | 95 |
| 2e | Boc-Phe-Val-Phe-Phe-OtBu ^a 4e | 47 | 95 |
| 2f | Boc-Phe-D-Val-Phe-Phe-OtBu ^a 4f | 52 | 97 |

^a Reaction run at 70 °C.

graphy. Fortunately, the de's of tetrapeptide **4a** and its diastereomer **4b** were both 99%, which is comparable to the tripeptide series. Similarly, tetrapeptides Boc-Phe-Phe-Phe-Phe-OtBu **4c** and Boc-Phe-D-Phe-Phe-Phe-OtBu **4d** were both isolated in a yield of 69% and gave de's of 93% and 95%, respectively. For obtaining tetrapeptide **4e** and its diastereomer **4f**, resulting from a coupling at C-terminal valine, the reaction mixture had to be warmed up to 70 °C to reach full conversion. Although a moderate yield had to be accepted, we were pleased that only little loss of de had occurred (Table 4).

Conclusions

Robust, high yielding and scalable methodology was developed to transform the C-terminal carboxylic acid of dipeptides with complete stereoretention into isopropenyl esters *via* a CLE reaction. Further elongation into tri- and tetrapeptides was carried out *via* a mild pyrazole catalysed aminolysis reaction liberating acetone as an inert side product. For the tripeptide series, high yields of crude product were obtained, which required no further purification. Excellent de's (>96%) were observed for almost all tested tripeptides. Only hindered couplings, such as for making the connecting peptide bond within Val-Phe and Val-Val showed a small drop in de. For the tetrapeptide series similar results were observed, however in this case the Phe-Phe coupling also showed a small drop in de. Future work will focus on applications in peptide cyclizations and the ligation of larger, more diverse peptide fragments.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors would like to thank The Netherlands Organization for Scientific Research (NWO-CW, ECHO grant

number 711.014.010 to J. v. M.). E. Zuidinga and J. M. Ernsting (University of Amsterdam) are acknowledged for mass spectrometry and NMR assistance.

Notes and references

- (a) A. Henninot, J. C. Collins and J. M. Nuss, *J. Med. Chem.*, 2018, **61**, 1382–1414; (b) J. L. Lau and M. K. Dunn, *Bioorg. Med. Chem.*, 2018, **26**, 2700–2707.
- S. S. Usmani, G. Bedi, J. S. Samuel, S. Singh, S. Kalra, P. Kumar, A. A. Ahuja, M. Sharma, A. Gautam and G. P. S. Raghav, *PLoS One*, 2017, **12**, e0181748.
- (a) C. Griehl, A. Kolbe and S. Merkel, *J. Chem. Soc., Perkin Trans. 2*, 1996, 2525–2529; (b) S.-Y. Han and Y.-A. Kim, *Tetrahedron*, 2004, **60**, 2447–2467; (c) A. El-Faham and F. Albericio, *Chem. Rev.*, 2011, **111**, 6557–6602.
- (a) Q. Wan, J. Chen, Y. Yuan and S. J. Danishefsky, *J. Am. Chem. Soc.*, 2008, **130**, 15814–15816; (b) Q. Wan and S. J. Danishefsky, *Angew. Chem., Int. Ed.*, 2007, **46**, 9248–9252.
- A. Abdelmajeid, S. R. Tala, M. S. Amine and A. R. Katritzky, *Synthesis*, 2011, 2995–3005.
- (a) J. W. Bode, R. M. Fox and K. D. Baucom, *Angew. Chem., Int. Ed.*, 2006, **45**, 1248–1252; (b) J. W. Bode, *Acc. Chem. Res.*, 2017, **50**, 2104–2115.
- (a) H. Schröder, G. A. Strohmeier, M. Leybold, T. Nuijens, P. J. L. M. Quaedflieg and R. Breinbauer, *Adv. Synth. Catal.*, 2013, **355**, 1799–1807. For an overview of chemoenzymatic peptide ligations see: (b) M. Schmidt, A. Toplak, P. J. L. M. Quaedflieg, J. H. van Maarseveen and T. Nuijens, *Drug Discovery Today*, 2017, **26**, 11–16.
- For a recent overview on the CLE-reaction see: (a) P. Y. S. Lam, *Synthetic methods in drug discovery*, 2016, ch. 7, vol. 1, p. 242. See also: (b) J. Ohata, M. B. Minus, M. E. Abernathy and Z. T. Ball, *J. Am. Chem. Soc.*, 2016, **138**, 7472–7475.
- S. Popovic, H. Bieräugel, R. J. Detz, A. M. Kluwer, J. A. A. Koole, D. E. Streefkerk, H. Hiemstra and J. H. van Maarseveen, *Chem. – Eur. J.*, 2013, **19**, 16934–16937.
- L. Steemers, L. Wijsman and J. H. van Maarseveen, *Adv. Synth. Catal.*, DOI: 10.1002/adsc.201800914.
- X. Yang and V. B. Birman, *Org. Lett.*, 2009, **11**, 1499–1502.
- H. C. Beyerman, W. M. van den Brink, F. Weygand, A. Prox, W. König, L. Schmidhammer and E. Nintz, *Recl. Trav. Chim. Pays-Bas*, 1965, **84**, 213–231.
- F. Kerins and D. F. O'Shea, *J. Org. Chem.*, 2002, **67**, 4968–4971.
- S. Martinez-Montero, S. Fernandez, Y. S. Sanghvi, V. Gotor and M. Ferrero, *Org. Biomol. Chem.*, 2011, **9**, 5960–5966.
- X.-Q. Cai, N. Wang and X.-F. Lin, *J. Mol. Catal. B: Enzym.*, 2006, **40**, 51–57.
- S. M. Tan, A. C. Willis, M. N. Paddon-Row and M. S. Sherburn, *Angew. Chem., Int. Ed.*, 2016, **55**, 3081–3085.
- S. M. King and S. L. Buchwald, *Org. Lett.*, 2016, **18**, 4128–4131.
- U. Filp, A. Pekosak, A. J. Poot and A. D. Windhorst, *Eur. J. Org. Chem.*, 2017, 5592–5596.

