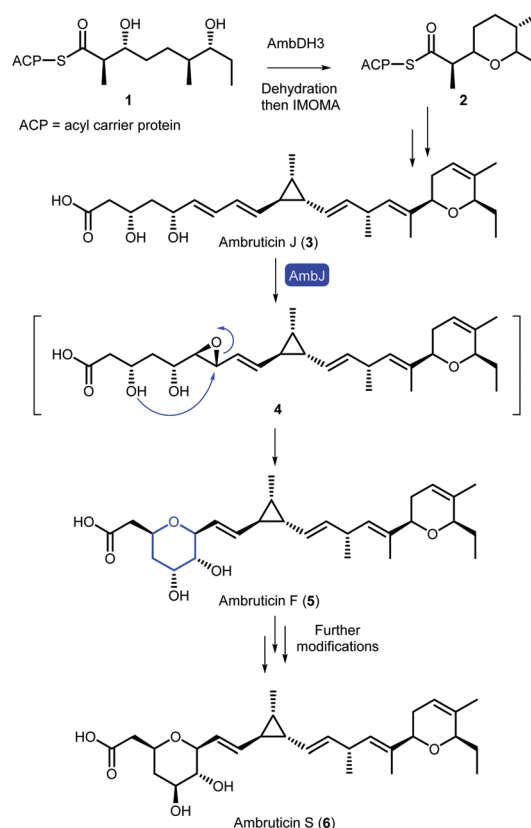




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In 2006, Reeves proposed a biosynthetic pathway to the ambruticins, based on results from analysis of the biosynthetic gene cluster alongside gene knockout experiments.¹⁴ More recently, Hahn and co-workers have revealed that the DHP is formed *via* dehydration followed by an intramolecular oxa-Michael addition (IMOMA) catalysed by AmbDH3 of linear polyketide **1** to give THP **2** (Scheme 1).¹⁵ Oxidation to the DHP is proposed to occur later in the pathway. The value of AmbDH3 as a biocatalyst for use in synthesis has been reported recently.¹⁶

Oxygen heterocycles, including dihydropyran (DHPs) and tetrahydropyran (THPs), are common structural features of biologically active natural products and FDA approved small molecule drugs.¹ The ambruticins (*e.g.* ambruticins F and S) are an interesting family of myxobacterial polyketide-derived natural products, which contain both these heterocycles (Scheme 1).^{2,3} Ambruticin S exhibits potent antifungal activity against a variety of fungal pathogens including *Coccidioides immitis*, *Histoplasma capsulatum* and *Blastomyces dermatitidis*.^{4–6} The antimycotic activity originates from interaction with the high-osmolarity glycerol (HOG) protein kinase signalling pathway, and no toxicity was observed in mice dosed with ambruticin S.^{7,8} Due to this promising antifungal activity, and fascinating structure, ambruticin S has been a target for several total syntheses.^{9–13}



Scheme 1 Proposed biosynthesis of ambruticin S.

†Electronic supplementary information (ESI) available: Synthetic procedures and NMR spectra. See DOI: 10.1039/d1ob00883h

However, a detailed understanding of the formation of the THP in ambruticin biosynthesis remains elusive. The gene *ambJ* encodes for a flavin-dependent monooxygenase and cultures of the $\Delta ambJ$ mutant of *Sorangium cellulosum* yielded *syn*-diol ambruticin J (3).¹⁴ Hence it was proposed that THP formation occurs through epoxidation of ambruticin J to 4, catalysed by AmbJ, followed by selective 6-*endo* epoxide ring opening.¹⁷

Creation of THPs *via* regioselective cyclisation of epoxy-alcohols has been investigated in the biosynthesis of several natural products. For example, we have shown that in mupirocin biosynthesis the Rieske oxidase MupW catalyses an initial oxidation of acyclic precursor 7 to epoxide 8, and the epoxide hydrolase MupZ is required for regioselective THP formation. Without MupZ, tetrahydrofuran 9 is exclusively formed *via* a 5-*exo* cyclisation (Scheme 2).¹⁸ Similar cyclisations have been reported for lasalocid biosynthesis in which Lsd18 catalyses alkene epoxidation and the epoxide hydrolase (Lsd19) is required for THP formation.¹⁹

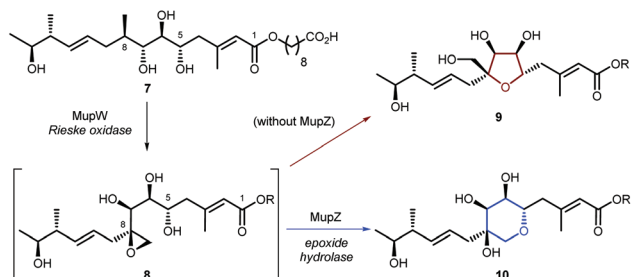
In accord with these biosynthetic studies, it has been shown that in the absence of structural features which stabilise a 6-*endo* transition state or destabilise the 5-*exo* transition state, cyclisation of 4,5-epoxy alcohols predominantly form the favoured smaller tetrahydrofuran ring.^{20–23} No epoxide hydrolase has been identified in the ambruticin biosynthetic gene cluster. Hence, it raises the intriguing question as to whether AmbJ catalyses both formation and cyclisation of epoxide 4 or if THP formation occurs spontaneously with the adjacent alkene in 6,7-epoxy-ambruticin J (4) stabilising the 6-*endo* transition state leading to ambruticin F (5).^{24,25}

With our ongoing interest in the synthesis and biosynthesis of THP containing natural products, we aim to elucidate the mechanism of THP formation in ambruticin biosynthesis.^{26,27} Herein, we describe our approach for the total synthesis of ambruticin J required as a substrate for AmbJ as well as cyclisation studies on model unsaturated alcohols. Whilst this manuscript was in preparation, Taylor and co-workers reported the first total synthesis of ambruticin J which uses a similar strategy to that which we have developed.²⁸

Results and discussion

Total synthesis of ambruticin J

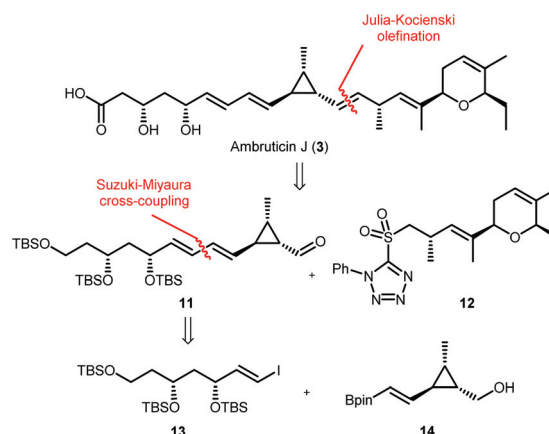
Our approach for the total synthesis of ambruticin J needed to be flexible to enable the preparation of analogues for later sub-



Scheme 2 THP ring formation in mupirocin biosynthesis.

strate specificity studies with AmbJ. Due to the reported instability of ambruticin J to conjugate elimination and cyclopropane ring opening, we opted to introduce the carboxylic acid in the final synthetic step.¹⁴ Our strategy took inspiration from a number of natural product syntheses which led to a modular approach, whereby three fragments could be united at a late stage of the synthesis (Scheme 3). The framework of ambruticin J was to be constructed through a Julia–Kocienski olefination between aldehyde 11 and the known sulfone 12¹¹ (prepared using a phosphonamide anion olefination previously developed by Hanessian in the total synthesis of ambruticin S).¹³ Aldehyde 11 was to be prepared *via* a Suzuki–Miyaura cross-coupling of vinyl iodide 13 and vinyl boronic ester 14 with the latter being constructed using cationic cyclopropane methodology developed by Taylor.²⁹

To begin, vinyl iodide 13 was prepared *via* an aldol reaction of aldehyde 16 with acylated thiazolidinethione auxiliary 15 followed by a decarboxylative Claisen condensation with ethyl potassium malonate 18 to afford β -ketoester 19 (Scheme 4).³⁰ Selective reduction of 19 gave *syn*-diol 20 in 71% yield over the three steps.^{31,32} Attempted reduction of ester 20 with DIBAL-H resulted in unwanted lactol formation. Hence, a sequence of silyl protection, reduction and further protection was used giving protected triol 22 in 73% over three steps. Conversion of vinyl silane 22 to vinyl iodide 13 proved problematic, with classical conditions such as NIS in MeCN providing the required vinyl iodide in <50% yield and as a 2:1 mixture of *E/Z* isomers.³³ Adopting Zakarian's conditions with hexafluoroisopropanol as the solvent increased the yield to 55% but with a similar *E/Z* ratio.³⁴ However, excellent (*E*)-selectivity was achieved using a three step sequence developed by Sato.³⁵ Initial epoxidation of vinyl silane 22 with *m*CPBA afforded a diastereomeric mixture of epoxides 23 in 73% yield. Regioselective epoxide ring opening with LiSnBu₃ and subsequent Peterson olefination provided the *trans*-vinyl stannane which was readily converted to (*E*)-vinyl iodide 13 in 34% overall yield from simple starting materials on a multigram scale.³⁶



Scheme 3 Retrosynthetic analysis of ambruticin J.



Scheme 4 Synthesis of vinyl iodide **13**.Scheme 5 Synthesis of vinyl boronic ester **14**.

Our attention then turned to the synthesis of the vinyl boronic ester coupling partner **14** (Scheme 5) and our approach to this fragment proved to be similar to that described in the recently released paper by Taylor.²⁸ Homoallylic alcohol **27** was prepared from boronic ester **24** in two steps utilising Hall's protocol,³⁷ beginning with a Matteson homologation of boronic ester **24**, followed by *in situ* Grignard addition to give allyl boronic ester **25**. Lewis acid catalysed allylation with aldehyde **26** (prepared in two steps from ethylene glycol) provided the required homoallylic alcohol **27** in 75% yield over the two steps. Formation of vinyl cyclopropane **28** proceeded in 96% yield by treatment of alcohol **27** with triflic anhydride.²⁹ The rate of addition of triflic anhydride proved key to the success of this reaction. If addition was too slow, competitive silylation of the starting material by TMSOTf, generated in the reaction, was observed. Conversely, faster rates of addition led to an exotherm and reduced the stereocontrol. Dihydroxylation of alkene **28** followed by cleavage with NaIO₄ gave aldehyde **29** required for the Seyferth–Gilbert homologation with Ohira–Bestmann reagent followed by silyl deprotection to alcohol **32**.^{38,39} Finally, hydroboration with Schwartz's reagent provided vinyl boronic ester **14** in 97% yield.⁴⁰ Overall, this eight step synthetic route gave boronic ester **14** in 51% yield on a multi-gram scale.

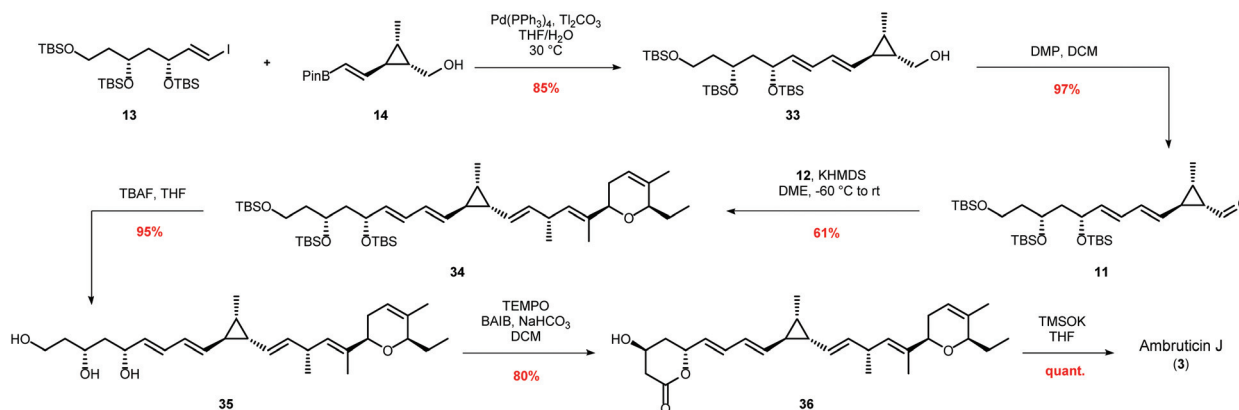
Sulfone **12** (Scheme 3) was prepared as previously described by Hanessian and Markó.^{41,42} However some of the steps were optimised and the modified procedures are reported in the ESI.† With all three fragments **12**, **13** and **14** in hand the key carbon–carbon bond forming reactions were investigated (Scheme 6).

Initial attempts to couple vinyl iodide **13** and vinyl boronic ester **14** via a Pd(PPh₃)₄ catalysed Suzuki–Miyaura cross-coupling with various potassium and sodium bases were low yielding. However, using thallium carbonate as a base gave the required *E,E*-diene in 85% yield.⁴³ Oxidation of alcohol **33** with DMP to aldehyde **11** followed by a Julia–Kocienski olefination using equimolar amounts of sulfone **12** and aldehyde **11** with KHMDS in DME gave *E*-alkene **34** in only 44% isolated yield.⁴⁴ However, using a slight excess (1.3 eq.) of sulfone **12** improved the yield to 61%. With the complete skeleton of ambruticin **J** now assembled, **34** was deprotected with TBAF to liberate triol **35** and selective oxidation of the primary alcohol was achieved using TEMPO–BAIB to give lactone **36**.⁴⁵ Careful monitoring of this oxidation was required to prevent formation of unwanted products. Finally, lactone **36** was hydrolysed under mild conditions to give ambruticin **J** (**3**). Potassium trimethylsilanolate was selected for this final transformation as Reeves *et al.* had reported that ambruticin **J** is unstable even to weak acid and base.^{14,46} The spectroscopic data for ambruticin **J** were in accord with that reported by Taylor.²⁸

Model cyclisation studies

Factors which control cyclisation of 4,5-epoxy alcohols to selectively give either tetrahydropyrans or tetrahydrofurans are of widespread interest, stimulated in part by a fascination in the biosynthetic origin of polyether natural products such as the monensins and brevotoxins.⁴⁷ In the absence of structural features which either stabilise a 6-*endo* transition state or destabi-





Scheme 6 Completing the synthesis of ambruticin J via key thallium-accelerated Suzuki–Miyaura cross-coupling and Julia–Kocienski olefination.

lise a 5-*exo* transition state, cyclisations of 4,5-epoxy alcohols tend to favour the smaller THF rather than the THP.^{20–23} As no epoxide hydrolase has been identified in the ambruticin biosynthetic gene cluster, cyclisation of putative *trans* epoxide 4 to ambruticin F may be catalysed by either AmbJ or a further as yet unknown enzyme; alternatively an enzyme may not be required (Scheme 1). Indeed, it is possible that THP ring formation may be favoured over the more typical smaller heterocycle (THF) due to stabilisation of the 6-*endo* transition state by the 8,9-double bond, in accord with early studies by Nicolaou.^{24,25} Such cyclisations have been used to good effect in synthesis as exemplified in the recent total synthesis of the THP containing natural product meayamycin B.⁴⁸ Thus, model studies were undertaken to gain an insight into the preferred mode of cyclisation of simple 4,5-epoxy alcohols.

First 3,5-dihydroxy ester 40 required as a short chain analogue of ambruticin J was prepared in 4 steps by an analogous route to that used for the synthesis of vinyl iodide 13 (Scheme 7A). Asymmetric aldol condensation of unsaturated aldehyde 37 with acylated thiazolidinethione 15 followed by a decarboxylative Claisen condensation cleaved the auxiliary to give β -hydroxy-ketone 39. Stereoselective Narasaka–Prasad reduction of 39 delivered the required dihydroxy-ester in 54% overall yield.^{31,32} Next a selective epoxidation of diene 40 was required. Using the free alcohol to direct Sharpless epoxidation, allylic alcohol 40 was assumed to give epoxide 41, although it could not be detected even in the NMR spectrum of the crude product as cyclisation had occurred giving THP 42.^{49,50} It is well established that such epoxy alcohols are unstable and readily cyclise, for example as described by Oishi in studies towards the synthesis of yessotoxin using Sharpless epoxidation conditions.⁵¹ The structure of 42 was confirmed by acetylation to 43 and subsequent NMR analysis (see ESI†). No further products, including THF 44, were detected. This result is in accord with the proposal that THP formation is favoured by the presence of the adjacent π -system stabilising the 6-*endo* transition state in conjunction with destabilisation of the 5-*exo* transition state by inductive effects of the C-5 alcohol (Fig. S2†).²⁵



Scheme 7 (A) Synthesis and epoxidation of diene 40 (B) Epoxidation of ester 45 and (C) Epoxidation of ester 49.



To gain further support for this proposal two further model unsaturated alcohols were prepared. The first, dihydroxy ester **45** lacking the second double bond, was synthesised by an analogous route to that used for **40** but starting from crotonal (Scheme 7B). Sharpless epoxidation of **45** gave a mixture of THP and THF products **47** and **48** in 44% and 19% isolated yield respectively, with no further products detected (Scheme 7B). Finally, unsaturated alcohol **49** was prepared and treated with *m*CPBA to give a mixture of epoxides **50** which were not purified but on addition of CSA gave THFs **51** and **52** arising from the expected 5-*exo* cyclisation (Scheme 7C). These results taken together are in accord with the proposal that both the C-5 alcohol and 8,9-alkene of ambruticin J may play a role in directing intramolecular epoxide ring opening to form ambruticin F. Hence, if a non-enzymatic cyclisation of epoxide **4** occurs then, based on these simple model studies, formation of the THP would be reasonable.

Conclusions

AmbJ is a flavin-dependent monooxygenase proposed to catalyse oxidation of ambruticin J to epoxide **4** (Scheme 1).¹⁴ However, as no epoxide hydrolase has been identified in the ambruticin biosynthetic gene cluster, it is not clear if AmbJ also catalyses a 6-*endo* epoxide ring opening to give ambruticin F or if THP formation occurs spontaneously. The results of model studies described herein on the chemical epoxidation and cyclisation of unsaturated alcohols (**40**, **45** and **49**) are in accord with the proposal that both the C-5 alcohol and 8,9-alkene of ambruticin J may play a role in directing 6-*endo* intramolecular ring opening to form ambruticin F. However, studies with AmbJ are essential to fully understand the biosynthesis of ambruticin F. To facilitate these biochemical investigations, we have completed a convergent total synthesis of ambruticin J. The approach involved construction of the carbon framework *via* the synthesis of three fragments **12**, **13** and **14** which were united *via* a thallium-accelerated Suzuki–Miyaura cross-coupling and Julia–Kocienski olefination. A global deprotection of **34**, selective oxidation to lactone **36** and finally hydrolysis under mild conditions gave ambruticin J. This modular route may be readily adapted to generate ambruticin J analogues to investigate the substrate specificity of AmbJ and these studies are currently underway in our laboratories.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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Notes and references

- 1 R. D. Taylor, M. MacCoss and A. D. G. Lawson, *J. Med. Chem.*, 2014, **57**, 5845.
- 2 S. M. Ringel, R. C. Greenough, S. Roemer, D. Connor, A. L. Gutt, B. Blair, G. Kanter and M. von Strandtmann, *J. Antibiot.*, 1977, **30**, 371.
- 3 V. Michelet and J.-P. Genet, *Curr. Org. Chem.*, 2005, **9**, 405.
- 4 G. Höfle, H. Steinmetz, K. Gerth and H. Reichenbach, *Liebigs Ann. Chem.*, 1991, **1991**, 941.
- 5 F. Hahn and F. M. Guth, *Nat. Prod. Rep.*, 2020, **37**, 1300.
- 6 S. Shadomy, D. M. Dixon, A. Espinel-Ingroff, G. E. Wagner, H. P. Yu and H. J. Shadomy, *Antimicrob. Agents Chemother.*, 1978, **14**, 99.
- 7 H. B. Levine, S. M. Ringel and J. M. Cobb, *Chest*, 1978, **73**, 202.
- 8 P. Knauth and H. Reichenbach, *J. Antibiot.*, 2000, **53**, 1182.
- 9 A. S. Kende, Y. Fujii and J. S. Mendoza, *J. Am. Chem. Soc.*, 1990, **112**, 9645.
- 10 T. A. Kirkland, J. Colucci, L. S. Geraci, M. A. Marx, M. Schneider, D. E. Kaelin and S. F. Martin, *J. Am. Chem. Soc.*, 2001, **123**, 12432.
- 11 P. Liu and E. N. Jacobsen, *J. Am. Chem. Soc.*, 2001, **123**, 10772.
- 12 E. Lee, S. J. Choi, H. Kim, H. O. Han, Y. K. Kim, S. J. Min, S. H. Son, S. M. Lim and W. S. Jang, *Angew. Chem., Int. Ed.*, 2002, **41**, 176.
- 13 S. Hanessian, T. Focken, X. Mi, R. Oza, B. Chen, D. Ritson and R. Beaudegnies, *J. Org. Chem.*, 2010, **75**, 5601.
- 14 B. Julien, Z.-Q. Tian, R. Reid and C. D. Reeves, *Chem. Biol.*, 2006, **13**, 1277.
- 15 G. Berkhan and F. Hahn, *Angew. Chem., Int. Ed.*, 2014, **53**, 14240.
- 16 T. Hollmann, G. Berkhan, L. Wagner, K. H. Sung, S. Kolb, H. Geise and F. Hahn, *ACS Catal.*, 2020, **10**, 4973.
- 17 In this paper, 6-*endo* refers to the cyclisation of an epoxy-alcohol in which a 6-membered ring (THP) is formed, and the parent epoxide C–C bond is located inside the newly formed oxygen heterocycle. In contrast, 5-*exo* refers to cyclisation of an epoxy-alcohol where the epoxide C–C bond is outside the newly formed THF ring.
- 18 L. Wang, A. Parnell, C. Williams, N. A. Bakar, M. R. Challand, M. W. van der Kamp, T. J. Simpson, P. R. Race, M. P. Crump and C. L. Willis, *Nat. Catal.*, 2018, **1**, 968.
- 19 Y. Shichijo, A. Migita, H. Oguri, M. Watanabe, T. Tokiwano, K. Watanabe and H. Oikawa, *J. Am. Chem. Soc.*, 2008, **130**, 12230.
- 20 J. E. Baldwin, *J. Chem. Soc., Chem. Commun.*, 1976, 734.
- 21 K. Gilmore, R. K. Mohamed and I. V. Alabugin, *Wiley Interdiscip. Rev.: Comput. Mol. Sci.*, 2016, **6**, 487.
- 22 J. Na, K. N. Houk, C. G. Shevlin, K. D. Janda and R. A. Lerner, *J. Am. Chem. Soc.*, 1993, **115**, 8453.
- 23 I. Vilotijevic and T. F. Jamison, *Science*, 2007, **317**, 1189.
- 24 K. C. Nicolaou, M. E. Duggan, C.-K. Hwang and P. K. Somers, *J. Chem. Soc., Chem. Commun.*, 1985, **111**, 1359.



- 25 K. C. Nicolaou, C. V. C. Prasad, P. K. Somers and C. K. Hwang, *J. Am. Chem. Soc.*, 1989, **111**, 5330.
- 26 E. R. Venegas and C. L. Willis, *Org. Lett.*, 2020, **22**, 2548.
- 27 P. T. Seden, J. P. H. Charmant and C. L. Willis, *Org. Lett.*, 2008, **10**, 1637.
- 28 R. E. Taylor, K. Trentadue, C. Chang and A. Nalin, *Chem. – Eur. J.*, 2021, DOI: 10.1002/chem.202100975.
- 29 R. E. Taylor, F. C. Engelhardt, M. J. Schmitt and H. Yuan, *J. Am. Chem. Soc.*, 2001, **123**, 2964.
- 30 M. B. Hodge and H. F. Olivo, *Tetrahedron*, 2004, **60**, 9397.
- 31 T. E. Smith, M. Djang, A. J. Velander, C. W. Downey, K. A. Carroll and S. van Alphen, *Org. Lett.*, 2004, **6**, 2317.
- 32 K.-M. Chen, G. E. Hardtmann, K. Prasad, O. Repič and M. J. Shapiro, *Tetrahedron Lett.*, 1987, **28**, 155.
- 33 D. P. Stamos, A. G. Taylor and Y. Kishi, *Tetrahedron Lett.*, 1996, **37**, 8647.
- 34 E. A. Ilardi, C. E. Stivala and A. Zakarian, *Org. Lett.*, 2008, **10**, 1727.
- 35 S. Okamoto, T. Shimazaki, Y. Kobayashi and F. Sato, *Tetrahedron Lett.*, 1987, **28**, 2033.
- 36 P. F. Hudrlik, J. P. Arcoleo, R. H. Schwartz, R. N. Misra and R. J. Rona, *Tetrahedron Lett.*, 1977, **18**, 591.
- 37 F. Peng and D. G. Hall, *J. Am. Chem. Soc.*, 2007, **129**, 3070.
- 38 G. Roth, B. Liepold, S. Müller and H. Bestmann, *Synthesis*, 2003, 59.
- 39 J. Pietruszka and A. Witt, *Synthesis*, 2006, 4266.
- 40 Y. D. Wang, G. Kimball, A. S. Prashad and Y. Wang, *Tetrahedron Lett.*, 2005, **46**, 8777.
- 41 S. Hanessian, T. Focken and R. Oza, *Org. Lett.*, 2010, **12**, 3172.
- 42 J. Pospíšil and I. E. Markó, *J. Am. Chem. Soc.*, 2007, **129**, 3516.
- 43 S. A. Frank, H. Chen, R. K. Kunz, M. J. Schnaderbeck and W. R. Roush, *Org. Lett.*, 2000, **2**, 2691.
- 44 P. R. Blakemore, *J. Chem. Soc., Perkin Trans. 1*, 2002, **2**, 2563.
- 45 T. M. Hansen, G. J. Florence, P. Lugo-Mas, J. Chen, J. N. Abrams and C. J. Forsyth, *Tetrahedron Lett.*, 2003, **44**, 57.
- 46 E. D. Laganis and B. L. Chenard, *Tetrahedron Lett.*, 1984, **25**, 5831.
- 47 I. Vilotijevic and T. F. Jamison, *Angew. Chem., Int. Ed.*, 2009, **48**, 5250.
- 48 R. K. Bressin, S. Osman, I. Pohorilets, U. Basu and K. Koide, *J. Org. Chem.*, 2020, **85**, 4637.
- 49 Y. Gao, J. M. Klunder, R. M. Hanson, H. Masamune, S. Y. Ko and K. B. Sharpless, *J. Am. Chem. Soc.*, 1987, **109**, 5765.
- 50 B. Schmidt and O. Kunz, *Beilstein J. Org. Chem.*, 2013, **9**, 2544.
- 51 T. Oishi, M. Suzuki, K. Watanabe and M. Murata, *Tetrahedron Lett.*, 2006, **47**, 3975.

