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# Total synthesis of quinolactacin-H from marine-derived *Penicillium* sp. ENP701 and biological activities†

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The quinolactacins are a family of pyrroloquinoline-type natural products from *Penicillium* sp. From the organic extract of *Penicillium* sp. ENP701 fermentation broth, a microorganism from the east China sea, one new quinolactacin was isolated and named quinolactacin-H. The structure of quinolactacin-H was determined by spectroscopic analysis and the absolute configurations by X-ray crystallographic analysis. Enantioselective total synthesis of (*R*)-(+)-quinolactacin-H and (*S*)-(–)-quinolactacin-H was achieved. When assayed through crystal violet (CV) microtiter plate biofilm, both (*R*) and (*S*)-quinolactacin-H showed a strong inhibition and dispersion of *Pseudomonas aeruginosa* PAO1 biofilms. Thus, quinolactacins could be proposed and developed as natural anti-biofilm agents in order to solve the problem of microbial resistance in future.

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Quinolones have a wide range of biological activities like anti-cancer, antibacterial,<sup>1</sup> antimalarial,<sup>2</sup> antifungal<sup>3</sup> and anti-HIV activities (Fig. 1a).<sup>4</sup> Nalidixic acid is the first generation of 4-quinolone antibiotics, which was discovered as a byproduct during the synthesis of chloroquine.<sup>5</sup> Therein, 4-quinolone antibiotics have become the second dominant drugs used in anti-infective chemotherapy (Fig. 1b).<sup>6</sup> Natural sources of quinolones have adequate quinolone motifs that act as structural subunits of a large number of complexes in natural products,<sup>1b</sup> but the only problem is the high cost of obtaining quinolones from nature. Rahme and co-workers reported 3,4-dihydroxy-2-heptylquinoline (PQS) as a signal molecule for *Pseudomonas aeruginosa* to coordinate their population behaviour (Fig. 1c).<sup>7</sup>

Quinolactacins are the first reported pyrroloquinoline-type natural compounds and are considered to be novel quinolone antibiotics. The first example of quinolactacins was reported by Norihiro Kakinuma and co-workers in 2000, which was isolated from the cultured broth of an unidentified *Penicillium* sp. EPF-6. Quinolactacin A has inhibitory activity against tumor necrosis factor (TNF) production induced by murine peritoneal macrophages and macrophage-like J774.1 cell stimulated with lipopolysaccharide. At that moment, the stereochemistry of

quinolactacin-A, -B and -C were unknown.<sup>8</sup> In 2001, Kin and co-workers discovered (*S*)-quinolactacin-A and (*R*)-quinolactacin-A as diastereomers from solid-state fermentation of *Penicillium citrinum* 90648.<sup>9</sup> (*S*)-Quinolactacin-D and (*R*)-quinolactacin-D were isolated from a solid phase fermentation of an Australian *P. citrinum* strain in 2006.<sup>10</sup> Abe and co-workers discovered a new compound named quinolactacide from *P. citrinum* F 1539

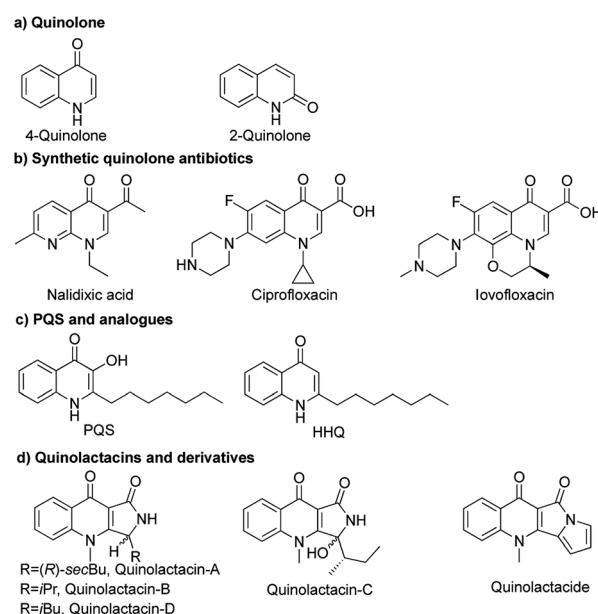


Fig. 1 Structures of quinolones and derivative.

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† Electronic supplementary information (ESI) available. CCDC crystallographic data of quinolactacin-H have been deposited at the Cambridge Crystallographic Data Centre (CCDC) under the reference number CCDC 1517245. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/d0ra05244b



which showed 88% mortality against green peach aphids at 250 ppm (Fig. 1d).<sup>11</sup>

The particularity of the marine ecological environment has created vast biodiversity and unique chemical diversity, hence the formation of the secondary metabolites of miscellaneous, unique structure and its variations, and the activity of the high efficiency, caused the extensive attention of pharmacologist, chemists and biologists. Ocean source of raw materials has become an important source of new drug discovery. There has been a sharp upward swing in the number of new natural products reported from marine microorganisms. The number of reported new marine natural products has steadily grown from 332 in 1984 to 1340 in 2015.<sup>12</sup> 57% of brand new marine natural products reported in 2017 were from marine microorganisms.<sup>13</sup>

During screening for new bioactive compounds from marine microorganism, we successfully discovered a new quinolactacin named quinolactacin-H from the cultured of *Penicillium* sp. ENP701, which was originated from the water sample from the East China Sea. So far, all the quinolactacins were isolated from *Penicillium* sp. However, it is interesting that the new quinolactacin-H comes from a marine source of *Penicillium* for the first time. Different from other quinolactacins, the structure of the new compound consists of a molecular (*R*)-quinolactacin-H and a molecular (*S*)-quinolactacin-H coordinating to a central metal of magnesium ion. This special structure may be formed under the condition of microbial source. The absolute configuration of quinolactacin-H was determined by X-ray crystallographic analysis (Fig. 2). The structure kindled our interest in magnesium complex as research target for its total synthesis.

In 2003, Zhang and co-workers reported an enantioselective total synthesis of (*S*)-quinolactacins-A, (*R*)-quinolactacins-A and (*R*)-quinolactacins-B.<sup>14</sup> The most important synthetic procedure is asymmetric Pictet–Spengler reaction.<sup>15</sup> The  $\beta$ -carboline structures achieved by Pictet–Spengler reaction and quinolone skeleton could be assembled by Winterfeldt oxidation.<sup>16</sup> The diastereoselective syntheses of chiral  $\beta$ -carboline by employing

*N,N*-phthaloyl-protected *tert*-leucine chlorides as chiral auxiliaries.<sup>17</sup> On this basis, we designed asymmetric total synthesis routes for (*R*)-quinolactacin-H (Scheme 1).

Based on the same synthetic route, we synthesized the marine natural products (*R*)-quinolactacin-H by applying the asymmetric Pictet–Spengler reaction to get chiral  $\beta$ -carboline intermediates. *N*-protected  $\beta$ -carboline **12b** was obtained from tryptamine-derived imine **10** by Waldmann's asymmetric Pictet–Spengler reaction using *N,N*-phthaloyl-protected *t*-leucine chlorides as chiral auxiliary (Scheme 2).<sup>15</sup> Diastereomers of carboline **12a** and **12b** were fully separated by silica gel column chromatography and crystallization in 80% yield in 2 : 1 ratio. The chiral auxiliary on carboline **12b** was removed by LiAlH<sub>4</sub> in dry THF, obtaining tetrahydro- $\beta$ -carboline in 36% yield. We also investigated other methodology to get (*S*)-quinolactacin-H in high yield and high enantioselectivity. We screened a series of organocatalysts, which were successfully applied in asymmetric Pictet–Spengler reaction<sup>18</sup> previously. Hence, we were attracted by asymmetric Pictet–Spengler reaction catalysed by chiral thiourea catalyst. Jacobsen *et al.* developed a highly enantioselective catalytic Pictet–Spengler reaction to synthesize a range of *N*-acetyl  $\beta$ -carbolines in high enantioselectivity.<sup>19</sup> Asymmetric acetyl-Pictet–Spengler cyclization of imine **10** was demonstrated by using chiral thiourea catalyst **A** in the presence of AcCl and 2,6-lutidine in diisopropyl ether (DIPE) at  $-30\text{ }^{\circ}\text{C}$  to afford the *N*-acetyl tetrahydro- $\beta$ -carboline **10** in 63% yield and 99.5% enantiomeric excess (ee) (Scheme 2). Tetrahydro- $\beta$ -carboline could be obtained by reductive deacetylation of *N*-acetyl tetrahydro- $\beta$ -carboline (*S*)-**4** with LiH<sub>2</sub>NBH<sub>3</sub> in THF in 46% yield. We then total synthesized (*S*)-quinolactacin-H *via* the same strategy.

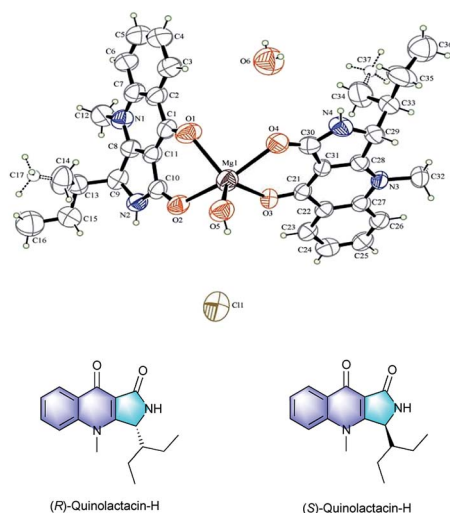
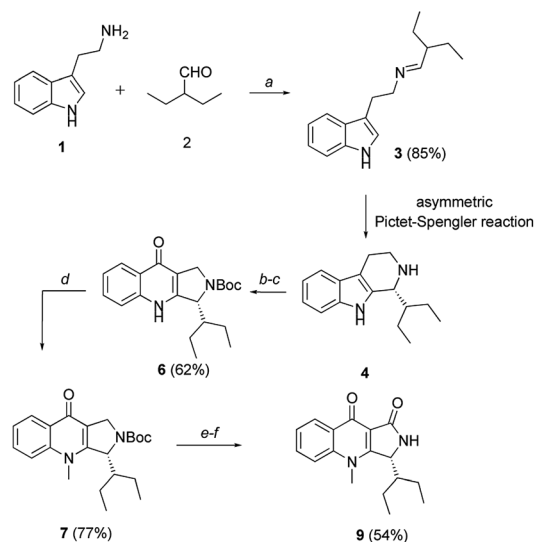
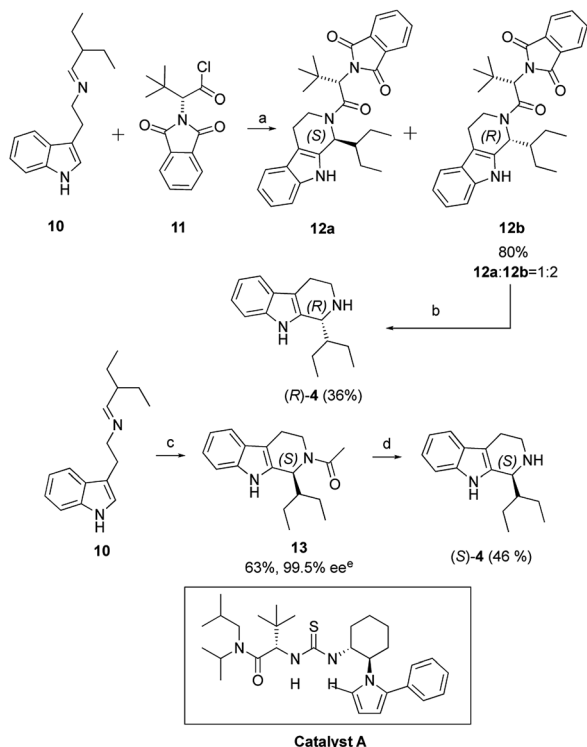


Fig. 2 Structures of the (*S*)-quinolactacin-H and (*R*)-quinolactacin-H.



Scheme 1 Total synthesis of (*R*)-quinolactacin-H. Reagents and conditions: (a) **1** (50 mmol), **2** (1.2 equiv.), DCM (200 mL), MgSO<sub>4</sub> (10 g), r.t.; (b) **4** (4.1 mmol), Boc<sub>2</sub>O (1.2 equiv.), TEA (1.5 equiv.), DMF (15 mL), r.t.; (c) KO<sub>2</sub> (4.0 equiv.), 18-Crown-6 (1.0 equiv.), DMF (15 mL), r.t., 62% over two steps; (d) **6** (1.4 mmol), MeI (2.0 equiv.), K<sub>2</sub>CO<sub>3</sub> (2.0 equiv.), DMF (8 mL), r.t.; (e) **7** (0.5 mmol), CuBr (0.2 mmol, 40 mol%), *t*-BuOOH (2.0 equiv, 5.5 M in decane), PhMe (5 mL), 50  $^{\circ}\text{C}$  to r.t.; (f) TFA (4.0 equiv.), DCM (4 mL), r.t., 54% over two steps.

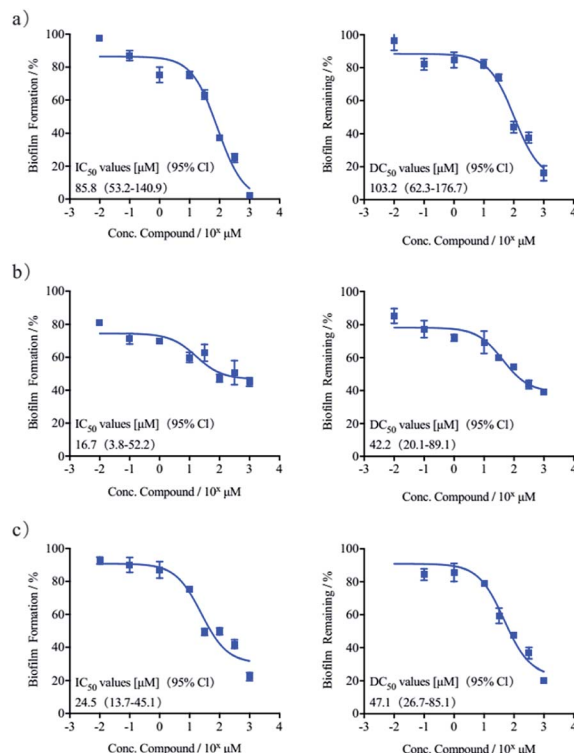




**Scheme 2** Asymmetric Pictet–Spengler reaction. Reagents and conditions: (a) **10** (24.8 mmol), **11** (1.2 equiv.),  $\text{Ti}(\text{O}-n\text{-Pr})_4$  (1.2 equiv.), dry DCM (200 mL), r.t., (b) **12b** (2.06 mmol),  $\text{H}_4\text{AlLi}$  (LAH) (2.0 equiv.), Dry THF (20 mL), reflux, (c) **10** (2.0 mmol), **Catalyst A** (0.1 mmol, 5 mol%),  $\text{AcCl}$  (1.05 equiv.), 2,6-lutidine (1.05 equiv.), DIPE (40 mL),  $-80^\circ\text{C}$  to  $-30^\circ\text{C}$ , (d)  $\text{LiH}_2\text{NBH}_3$ , Dry THF. (e) The ee was determined by HPLC analysis on the Chiralcel OD-H column.

With the enantiopure (*S*)-quinolactacin-H and (*R*)-quinolactacin-H in hand, we turned towards determination of their *in vitro* antimicrobial activity. Unfortunately, both enantiomers of quinolactacin-H showed weak antimicrobial activity. However, in the meantime, we accidentally found that biofilm growth of microorganisms was inhibited. Biofilms are extremely difficult to be cleared because they are encased in a protective, impermeable extracellular matrix that makes biofilm-related bacteria resistant to both the antibiotic and host's immune response.<sup>20</sup> In fact, biofilm clearance typically requires about 1000-fold more conventional antibiotics than planktonic bacteria.<sup>21</sup> *Pseudomonas aeruginosa* is a Gram-negative opportunistic pathogen that poses a threat to immunocompromised patients, and is a cause of death in patients with cystic fibrosis, AIDS and cancer.<sup>22</sup> It is also one of the most studied models of biofilm formation.

Herein we tested the ability of (*S*)-quinolactacin-H and (*R*)-quinolactacin-H to inhibit biofilm growth in a wild-type *P. aeruginosa* strain (PAO1) using standard static biofilm growth assays. Biofilms were grown in a Luria-Bertani (LB) media in 96-well microtiter plates, and quantified biofilm growth after 24 hours by using crystal violet staining of the surface-associated biomass.<sup>23</sup> Dose-response curves of crystal-violet-stained biofilms in the presence of (*S*)-quinolactacin-H and (*R*)-



**Fig. 3** Dose-response curves of crystal violet biofilm inhibitory and dispersion in *P. aeruginosa*: (a) benzimidazole, (b) (*S*)-quinolactacin-H, (c) (*R*)-quinolactacin-H.

quinolactacin-H are shown in Fig. 3, benzimidazole used as positive control.<sup>24</sup> Follow-up dose-response experiments revealed that (*S*)-quinolactacin-H had half-maximal inhibitory concentration ( $\text{IC}_{50}$ ) values of  $16.7\ \mu\text{M}$  against *P. aeruginosa* PAO1. Moreover, (*R*)-quinolactacin-H also showed strong inhibitory activity against *P. aeruginosa* biofilm growth ( $\text{IC}_{50} = 24.5\ \mu\text{M}$ ).

The new natural products quinolactacins are capable of not only inhibiting bacteria biofilm growth, but also dispersing preformed biofilms. We used the crystal violet staining assay to test the ability of (*S*)-quinolactacin-H and (*R*)-quinolactacin-H to disperse 24 hour-old *P. aeruginosa* biofilms. The results showed that (*S*)-quinolactacin-H and (*R*)-quinolactacin-H strongly disperse *P. aeruginosa* biofilms, with half-maximal dispersion ( $\text{DC}_{50}$ ) values of  $42.2$  and  $47.1\ \mu\text{M}$ , respectively (Fig. 3).

## Conclusions

In conclusion, we discovered the new natural product named quinolactacin-H, isolated from a *Penicillium* which came from the east China sea. The absolute configuration was determined by X-single crystal diffraction. Total synthesis (*S*)-quinolactacin-H and (*R*)-quinolactacin-H by asymmetric Pictet–Spengler reaction as one of the most important steps in the whole route. The preliminary biological results obtained for this series of quinolactacin-H suggests that quinolactacins as potent anti-biofilm agents in *P. aeruginosa*. Both (*S*)-quinolactacin-H and (*R*)-quinolactacin-H show strong activity in inhibiting the



growth of biofilms and dispersing preformed biofilms. This is the first report that quinolactacins strongly inhibit and disperse bacterial biofilm. Ongoing work in our group is directed towards the study of other quinolactacins and derivatives, as well as assessing their biofilm inhibitory activities across an expanded set of bacterial species and investigate the mechanism by which the quinolactacins elicits its biofilm inhibitory activity in *P. aeruginosa*.

## Conflicts of interest

There are no conflicts to declare.

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## Notes and references

- (a) G. F. Zhang, S. Zhang, B. Pan, X. Liu and L. S. Feng, *Eur. J. Med. Chem.*, 2018, **144**, 710; (b) S. E. Rossiter, M. H. Fletcher and W. M. Wuest, *Chem. Rev.*, 2017, **117**, 12415; (c) E. Fernández-Álvarez, W. D. Hong, G. L. Nixon, P. M. O'Neill and F. Calderón, *J. Med. Chem.*, 2016, **59**, 5587.
- (a) Z. Xu, S. J. Zhao, Z. S. Lv, F. Gao, Y. Wang, F. Zhang, L. Bai and J. L. Deng, *Eur. J. Med. Chem.*, 2019, **162**, 396; (b) Y. Q. Hu, C. Gao, S. Zhang, L. Xu, Z. Xu, L. S. Feng, X. Wu and F. Zhao, *Eur. J. Med. Chem.*, 2017, **139**, 22; (c) Y. L. Fan, X. W. Cheng, J. B. Wu, M. Liu, F. Z. Zhang, Z. Xu and L. S. Feng, *Eur. J. Med. Chem.*, 2018, **146**, 1.
- F. Gao, P. Wang, H. Yang, Q. Miao, L. Ma and G. Lu, *Eur. J. Med. Chem.*, 2018, **157**, 1223.
- (a) K. C. Sekgota, S. Majumder, M. Isaacs, D. Mnkandhla, H. C. Hoppe, S. D. Khanye, F. H. Kriel, J. Coates and P. T. Kaye, *Bioorg. Chem.*, 2017, **75**, 310; (b) Z. G. Luo, J. J. Tan, Y. Zeng, C. X. Wang and L. M. Hu, *Mini-Rev. Med. Chem.*, 2010, **10**, 1046.
- M. A. Kohanski, D. J. Dwyer and J. J. Collins, *Nat. Rev. Microbiol.*, 2010, **8**, 423.
- H. A. A. Ezelarab, S. H. Abbas, H. A. Hassan and G. E. D. A. Abuo-Rahma, *Arch. Pharm.*, 2018, **351**, 1.
- (a) E. Deziel, F. Lepine, S. Milot and L. G. Rahme, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 1339; (b) S. P. Diggle, S. Matthijs, V. J. Wright, M. P. Fletcher, S. R. Chhabra, I. L. Lamont, X. Kong, R. C. Hider, P. Cornelis, M. Camara and P. Williams, *Chem. Biol.*, 2007, **14**, 87; (c) H. Huse and M. Whiteley, *Chem. Rev.*, 2011, **111**, 152.
- (a) N. Kakinuma, H. Iwai and A. Nakagawa, *J. Antibiot.*, 2000, **53**, 1247; (b) S. Takahashi, N. Kakinuma and A. Nakagawa, *J. Antibiot.*, 2000, **53**, 1252.
- W. G. Kim, N. K. Song and I. D. Yoo, *J. Antibiot.*, 2010, **33**, 831.
- B. Clark, R. J. Capon and E. Lacey, *Org. Biomol. Chem.*, 2006, **4**, 1512.
- (a) M. Abe, T. Imai, N. Ishii, M. Usui, T. Okuda and T. Oki, *Biosci., Biotechnol., Biochem.*, 2005, **69**, 1202; (b) M. Abe, T. Imai, N. Ishii and M. Usui, *Biosci., Biotechnol., Biochem.*, 2006, **70**, 303.
- J. W. Blunt, B. R. Copp, R. A. Keyzers and M. H. G. Munro, *Nat. Prod. Rep.*, 2015, **32**, 116; J. W. Blunt, B. R. Copp, R. A. Keyzers and M. H. G. Munro, *Nat. Prod. Rep.*, 2016, **33**, 382.
- A. R. Carroll, B. R. Copp, R. A. Davis, R. A. Keyzers and M. R. Prinsep, *Nat. Prod. Rep.*, 2019, **36**, 122.
- X. Q. Zhang, W. Q. Jiang and Z. H. Sui, *J. Org. Chem.*, 2003, **68**, 4523.
- H. Waldmann, G. Schmidt, H. Henke and M. Burkard, *Angew. Chem., Int. Ed.*, 1955, **34**, 2402.
- W. Jiang, X. Zhang and Z. Sui, *Org. Lett.*, 2003, **5**, 43.
- G. Schmidt, H. Waldmann, H. Henke and M. Burkard, *Chem. - Eur. J.*, 1996, **2**, 1565.
- (a) L. Qi, H. Hou, F. Ling and W. H. Zhong, *Org. Biomol. Chem.*, 2018, **16**, 566; (b) D. Huang, F. X. Xu, X. F. Lin and Y. G. Wang, *Chem. - Eur. J.*, 2012, **18**, 3148.
- M. S. Taylor and E. N. Jacobsen, *J. Am. Chem. Soc.*, 2004, **126**, 10558.
- H. C. Flemming and J. Wingender, *Nat. Rev. Microbiol.*, 2010, **8**, 623.
- D. Davies, *Nat. Rev. Drug Discovery*, 2003, **2**, 114.
- (a) R. C. Boucher, *Eur. Respir. J.*, 2004, **23**, 146; (b) R. B. Moss, *Clin. Infect. Dis.*, 1995, **21**, 839.
- (a) G. D. Christensen and W. A. Simpson, *J. Clin. Microbiol.*, 1985, **22**, 996; (b) G. A. O'Toole and R. Kolter, *Methods Enzymol.*, 1999, **310**, 91.
- R. Frei, A. S. Breitbach and H. E. Blackwell, *Angew. Chem., Int. Ed.*, 2012, **51**, 5226.

