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A mild and concise synthesis of aryloxy phosphoramidate prodrug of alcohols via transesterification reaction†

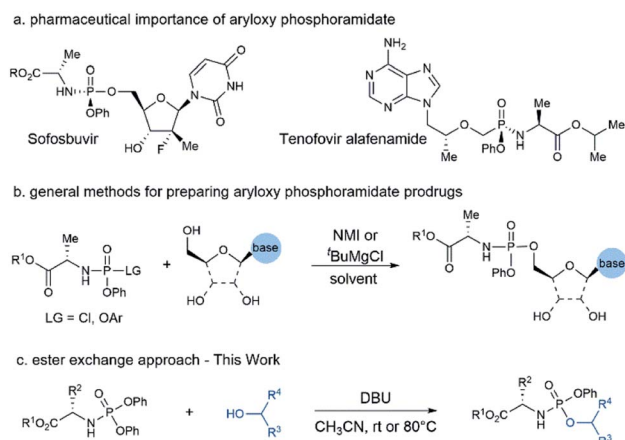
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A synthesis of aryloxy phosphoramidate prodrug of alcohols enabled by a transesterification strategy is described here. This reaction operates under mild conditions and thus has excellent functional group tolerance. This method provides an efficient and practical solution to the rapid construction of the aryloxy phosphoramidate prodrugs library for potential SAR studies.

The phosphate and phosphonate prodrug strategy is widely recognized as an effective approach to improve the physico-chemical and pharmacological properties of therapeutic nucleosides and sugars.¹ Over the last few decades, the ProTide prodrug strategy pioneered by Prof. Chris McGuigan has emerged as a powerful platform for developing nucleotide therapeutics.² This prodrug approach has been extensively studied in the antiviral and anticancer fields, enabling the discovery and development of three FDA-approved antiviral drugs and several clinical candidates (Fig. 1a).³ Whereas early efforts focused mainly on nucleoside-based medicines, many recent discoveries suggested that this technology can be applied to non-nucleoside substrates as well.⁴ The nature of different components of the phosphoramidate core is essential for the prodrug's potency, especially for the case of non-nucleoside drug candidates in which other amino acid motifs are more effective than the commonly used L-alanine.⁵ As a result, SAR studies of amino acid ester and aryl moieties would be necessary to identify the optimal combination of these masking groups when applying ProTide technology to a new therapeutic chemical entity. Consequently, an efficient method capable of rapidly assembling aryloxy phosphoramidate prodrug library from parent drug would be very attractive to the discovery of new ProTide prodrugs.

Current methods for the preparation of aryloxy phosphoramidate structures include: (a) phosphorylating the nucleoside with phosphorochloridate reagent,⁶ (b) ester exchange between nucleoside and a diarylphosphite followed by oxidative amination,⁷ (c) coupling of the amino acid ester with a nucleoside aryl phosphate or its derivatives.⁸ Among these methods, coupling nucleosides with phosphorochloridate reagents in the presence

of either *N*-methylimidazole (NMI) or *tert*-butyl magnesium chloride (*t*BuMgCl) is the most popular strategy for ProTide prodrug construction (Fig. 1b). Although this method has enabled the synthesis of numerous nucleoside prodrugs, regioselectivity and diastereoselectivity issues associated with this approach remained challenging.⁹ As a result, numerous efforts have been devoted to develop methods with high regioselectivity¹⁰ and diastereoselectivity.¹¹ While these advances offer a variety of choices on batch synthesis of designated compounds, other issues remain to be addressed. First, the current method mainly focused on accessing phosphoramidate prodrugs containing L-alanine and nucleoside. The compatibility of these methods towards other amino acid motif and non-nucleosides substrates are less investigated. Second, compatible substrate nucleoside bases are limited due to the high reactivity of phosphorylating reagents such as phosphorochloridate or diarylphosphite, which have to involve protection of the nucleobase moiety sometimes.



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Fig. 1 Pharmaceutical importance and the synthetic methods of aryloxy phosphoramidate prodrugs.



Given the growing interest in expanding ProTide technology to other therapeutic areas and the unmet need for rapid access to the prodrug library for hit screenings or SAR studies, we wonder if we can develop a practical method capable of preparing aryloxy phosphoramidates prodrugs from both nucleoside and non-nucleoside substrates. We reasoned that *N*-diphenyl phosphoryl amino acid ester could serve as a mild phosphorylating reagent and afford aryloxy phosphoramidate prodrugs *via* a simple transesterification process. Herein, we report our discovery on a DBU promoted synthesis of aryloxy phosphoramidates prodrugs from stable and readily available *N*-diphenylphosphoryl amino acid esters and alcoholic substrates (Fig. 1c).

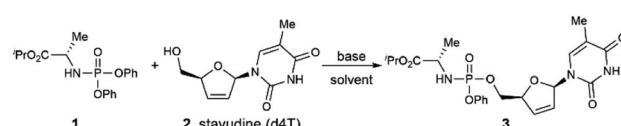
We began our study by examining the reaction of phosphoramidate **1** and stavudine (d4T, anti-HIV drug) in the presence of diisopropylethylamine (DIPEA) as the base in acetonitrile (CH₃CN) at 25 °C (Table 1, entry 1). Unfortunately, no product was detected, and all starting materials were recovered. After screening several other bases (entries 2–4), we found that inorganic bases such as K₂CO₃ and NaOH promoted the reaction but in low yield. In contrast, strong organic base 1,8-diazabicyclo 5.4.0 undec-7-ene (DBU) afforded desired product in a much higher yield. This result suggests that efficient deprotonation of the hydroxyl group is essential to drive this reaction. Screening of other similar bases such as 1,5-diazabicyclo [4.3.0]non-5-ene (DBN), 1,1,3,3-tetramethyl-guanidine (TMG), and 1,5,7-triazabicyclo [4.4.0]dec-5-ene (TBD) did not improve the reaction efficiency.¹² Increasing the equivalents of phosphorylating reagent **1** to 2.0 equivalent or 3.0 equivalent afforded the target product in 91% and 92% yield, respectively (entries 5–6). Stoichiometry optimization on base revealed that

2.0 equivalent of DBU was optimal, with 1.0 or 3.0 equivalent of DBU gave impaired yield (entries 7–8). In addition, solvent is proved to be vital for a productive reaction, as replacing CH₃CN with DCM, DMF, and THF only afford product **3** in 27% to 71% yield (entries 9–11). To test the scalability of this strategy, we conducted a 1.5 mmol scale synthesis of product **3** under the standard conditions. This reaction afforded the desired product in 85% isolated yield, demonstrating the scalability and potential synthetic application of this protocol.¹³

While previous studies suggested that L-alanine was optimal for antiviral and anticancer ProTide prodrugs, some new SAR data obtained in the studies of non-nucleoside drug candidates indicated that other amino acids, in some cases, might be more effective than L-alanine.⁵ Therefore, we decided to explore the scope of *N*-diphenylphosphoryl amino acid esters (Table 2). Under standard conditions, a variety of L-alanine esters were tolerated, affording desired aryloxy phosphoramidates prodrugs in good yields (products **4–6**). Moreover, substrates containing nonpolar amino acid residue such as valine, isoleucine, phenylalanine proceeded smoothly to afford the target products with good efficiency (products **7–9**). In addition, *N*-diphenylphosphoryl tryptophan or *O*-protected serine were well tolerated in the standard conditions, affording desired product in high yield (products **10–11**).

Satisfied with the scope of *N*-diphenylphosphoryl amino acid esters, we then investigated the generality of this protocol

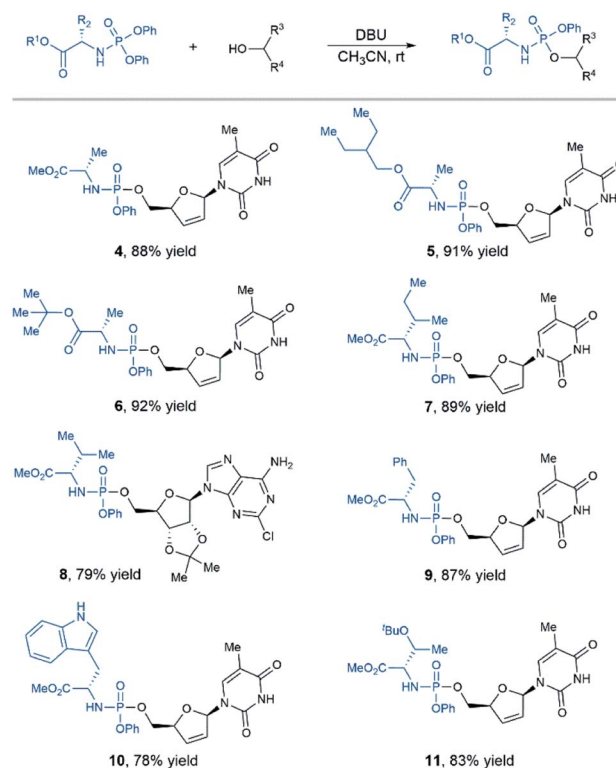
Table 1 Optimization of the reaction conditions^a



Entry	1 (eq.)	2 (eq.)	Base (eq.)	Solvent	Yield ^b (%)
1	1.5	1.0	DIPEA (2.0)	CH ₃ CN	—
2	1.5	1.0	K ₂ CO ₃ (2.0)	CH ₃ CN	—
3	1.5	1.0	NaOH (2.0)	CH ₃ CN	11
4	1.5	1.0	DBU (2.0)	CH ₃ CN	78
5	2.0	1.0	DBU (2.0)	CH ₃ CN	91(88)
6	3.0	1.0	DBU (2.0)	CH ₃ CN	92
7	2.0	1.0	DBU (1.0)	CH ₃ CN	65
8	2.0	1.0	DBU (3.0)	CH ₃ CN	83
9	2.0	1.0	DBU (2.0)	DCM	71
10	2.0	1.0	DBU (2.0)	DMF	27
11	2.0	1.0	DBU (2.0)	THF	36

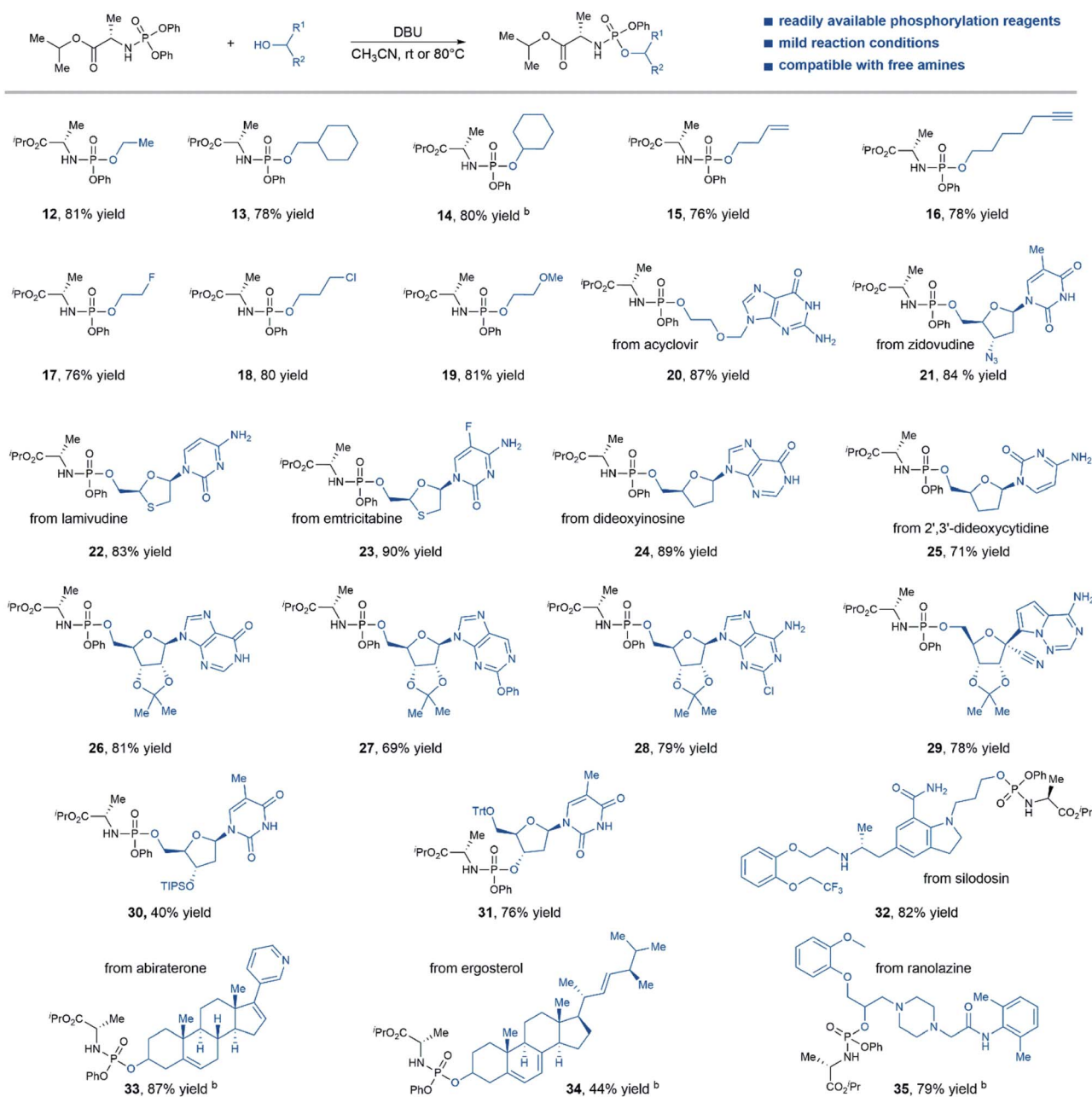
^a Reaction conditions: phosphorylating reagent **1** (0.3–0.6 mmol), stavudine **2** (0.2 mmol), base (1.0–3.0 equiv.) and solvent (1 mL) at room temperature, *t* = 36 h. ^b NMR yield using triethyl phosphate as the internal standard. The yield shown in parentheses is isolated yield, and the product is a 1 : 1 mixture of *R_p* and *S_p* diastereoisomers.

Table 2 Scope of *N*-diphenylphosphoryl amino acid esters^a



^a Standard reaction conditions, 36 h. The yield shown here is isolated yield, and the product is a 1 : 1 mixture of *R_p* and *S_p* diastereoisomers.



Table 3 Scope of nucleosides and alcohols^{ab}

^a Standard reaction conditions, 36 h. The yield shown here is isolated yield, and the product is a 1 : 1 mixture of *R*_p and *S*_p diastereoisomers. ^b For the synthesis of products **14**, **33**, **34** and **35**, reactions were run at 80 °C instead of room temperature.

regarding the coupling of various alcohol substrates with phosphorylation reagent **1** (Table 3). A range of alcohols with different steric environments were smoothly converted to the desired products in consistently good yields (products **12**–**14**). In the case of the secondary alcohol, an elevated temperature (80 °C) was required for efficient transformation (product **14**). Substrates bearing functional groups such as alkene (product **15**), alkyne (product **16**), halides (product **17**–**18**), and ether (product **19**) were well tolerated in this protocol and delivered the desired product with good efficiency (76–81% yield).

Nucleoside substrates that contain only 5'-hydroxyl group, such as acyclovir (product **20**), zidovudine (product **21**), lamivudine (product **22**), emtricitabine (product **23**), dideoxyinosine (product **24**), and 2',3'-dideoxycytidine (product **25**) were all viable substrates that afforded desired aryloxy triester phosphoramidate prodrugs in good yields. Notably, the nucleophilic nitrogens on those nucleosides were well tolerated under our reaction condition, no *N*-phosphorylation by-products were observed. Moreover, nucleoside substrates with protected 2',3'-hydroxyl groups were also effective for product formation



(products 26–29, 69–81% yield). Low conversion was observed when substrates bearing 2',3'-hydroxyl groups were directly subjected to the reaction conditions, likely due to poor solubility of this type of substrates in CH₃CN. Although treating nucleoside bearing unprotected 3',5'-hydroxyl groups with the standard reaction conditions gave a mixture of regioisomers, mono-phosphorylation of 5' or 3'-hydroxyl group can be achieved by protecting the other hydroxyl group (products 30 and 31). In addition, non-nucleoside medicines such as silodosin, abiraterone, ergosterol, and ranolazine were also viable substrates for this reaction, afforded desired prodrug products in good yields (products 32–35), except for ergosterol. The amine and amide functional groups in these complex drugs were well tolerated in this protocol without side reactions, thus demonstrating the potential application of this method to the synthesis of aryloxy phosphoramidates prodrug of non-nucleoside drug candidates.

In conclusion, we have developed a mild and concise protocol for synthesizing aryloxy phosphoramidate prodrugs of alcohols and nucleosides. This method utilizes diphenyl phosphoramidates as the phosphoryl donor to realize the phosphorylation of a range of primary or secondary alcohols. This method operates under mild conditions and has good functional group tolerance, thus enabling the synthesis of a range of prodrug analogues of nucleosides containing nucleophilic functional groups. We believe this work will provide a valuable tool for the rapid construction of the aryloxy phosphoramidate prodrug library for hit screenings or SAR studies, which is essential for discovery of potent ProTide drug candidates.

Author contributions

F. W. and F. N. directed the project and wrote the manuscript. F. W. and F. N. conceived the idea and designed the experiments. H. Y. and J. Y. performed the experiments. All the authors participated in the preparation of the manuscript.

Conflicts of interest

There are no conflicts to declare.

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Notes and references

- For selected reviews, see: (a) A. J. Wiemer and D. F. Wiemer, *Top. Curr. Chem.*, 2015, **360**, 115–160; (b) K. M. Heidel and C. S. Dowd, *Future Med. Chem.*, 2019, **11**, 1625–1643.
- For selected reviews (a) D. Cahard, C. McGuigan and J. Balzarini, *Mini-Rev. Med. Chem.*, 2004, **4**, 371–381; (b) A. S. Alanazi, E. James and Y. Mehellou, *ACS Med. Chem. Lett.*, 2019, **10**, 2–5; (c) M. Serpi and F. Pertusati, *Expert Opin. Drug Discovery*, 2021, **16**, 1149–1161.

- (a) P. J. Thornton, H. Kadri, A. Miccoli and Y. Mehellou, *J. Med. Chem.*, 2016, **59**, 10400–10410; (b) Y. Mehellou, H. S. Rattan and J. Balzarini, *J. Med. Chem.*, 2018, **61**, 2211–2226.
- (a) C. McGuigan, M. Serpi, R. Bibbo, H. Roberts, C. Hughes, B. Caterson, A. T. Gibert and C. R. A. Verson, *J. Med. Chem.*, 2008, **51**, 5807–5812; (b) M. Serpi, R. Bibbo, S. Rat, H. Roberts, C. Hughes, B. Caterson, M. J. Alcaraz, A. T. Gibert, C. R. Verson and C. McGuigan, *J. Med. Chem.*, 2012, **55**, 4629–4639; (c) N. Hamon, M. Quintiliani, J. Balzarini and C. McGuigan, *Bioorg. Med. Chem. Lett.*, 2013, **23**, 2555–2559; (d) N. Hamon, M. Slusarczyk, M. Serpi, J. Balzarini and C. McGuigan, *Bioorg. Med. Chem.*, 2015, **23**, 829–838; (e) E. James, F. Pertusati, A. Brancale and C. McGuigan, *Bioorg. Med. Chem. Lett.*, 2017, **27**, 1371–1378; (f) L. Osgerby, Y.-C. Lai, P. J. Thornton, J. Amalfitano, C. S. Le Duff, I. Jabeen, H. Kadri, A. Miccoli, J. H. R. Tucker, M. M. K. Muqit and Y. Mehellou, *J. Med. Chem.*, 2017, **60**, 3518–3524; (g) M. S. Davey, R. Malde, R. C. Mykura, A. T. Baker, T. E. Taher, C. S. Le Duff, B. E. Willcox and Y. Mehellou, *J. Med. Chem.*, 2018, **61**, 2111–2117; (h) N. A. Lentini, B. J. Foust, C.-H. C. Hsiao, A. J. Wiemer and D. F. Wiemer, *J. Med. Chem.*, 2018, **61**, 8658–8669.
- (a) L.-J. Gao, S. De Jonghe, D. Daelemans and P. Herdewijn, *Bioorg. Med. Chem. Lett.*, 2016, **26**, 2142–2146; (b) C. Morozzi, J. Sedláková, M. Serpi, M. Avigliano, R. Carbajo, L. Sandoval, Y. Valles-Ayoub, P. Crutcher, S. Thomas and F. Pertusati, *J. Med. Chem.*, 2019, **62**, 8178–8193.
- For selected examples of NMI-mediated coupling of nucleoside with phosphorochloridate reagents, see: (a) C. McGuigan, R. N. Pathirana, N. Mahmood, K. G. Devine and A. J. Hay, *Antiviral Res.*, 1992, **17**, 311–321; (b) P. Franchetti, L. Cappellacci, M. Grifantini, L. Messini, G. A. Sheikha, A. G. Loi, E. Tramontano, A. De Mantis, M. G. Spiga and P. La Colla, *J. Med. Chem.*, 1994, **37**, 3534–3541; (c) C. McGuigan, P. W. Sutton, D. Cahard, K. Turner, G. O'Leary, Y. Wang, M. Gumbleton, E. De Clercq and J. Balzarini, *Antiviral Chem. Chemother.*, 1998, **9**, 473–479.
- For selected examples of *t*-BuMgCl-mediated synthesis of aryloxyphosphoramidate prodrugs, see: (d) C. McGuigan, O. M. Wedgwood, E. De Clercq and J. Balzarini, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 2359–2362; (e) Y. Mehellou, C. McGuigan, A. Brancale and J. Balzarini, *Bioorg. Med. Chem. Lett.*, 2007, **17**, 3666–3669.
- For selected examples, see: (a) X. Li, H. Fu, Y. Jiang, Y. Zhao and J. Liu, *Synlett*, 2004, **14**, 2600–2602; (b) M. Ora, J. Ojanpera and H. Lonnberg, *Chem.-Eur. J.*, 2007, **13**, 8591–8599.
- For selected examples, see: (a) H. Chapman, M. Kernan, E. Prisbe, J. Rohloff, M. Sparacino, T. Terhorst and R. Yu, *Nucleosides, Nucleotides Nucleic Acids*, 2001, **20**, 621–628; (b) R. L. Mackman, A. S. Ray, H. C. Hui, L. Zhang, G. Birkus, C. G. Boojamra, M. C. Desai, J. L. Douglas, Y. Gao, D. Grant, G. Laflamme, K.-Y. Lin, D. Y. Markevitch,



- R. Mishra, M. McDermott, R. Pakdaman, O. V. Petrakovsky, J. E. Vela and T. Cihlar, *Bioorg. Med. Chem.*, 2010, **18**, 3606–3617.
- 9 For a comprehensive review, see: (a) U. Pradere, E. C. Garnier-Amblard, S. J. Coats, F. Amblard and R. F. Schinazi, *Chem. Rev.*, 2014, **114**, 9154–9218. For the challenges and recent progress of controlling *P*-stereogenic centers, see: (b) X. Ye, L. Peng, X. Bao, C.-H. Tan and H. Wang, *Green Synth.*, 2021, **2**, 6–18.
- 10 For selected examples see: (a) B. Simmons, Z. Liu, A. Klapars, A. Bellomo and S. M. Silverman, *Org. Lett.*, 2017, **19**, 2218–2221; (b) D. A. Glazier, J. M. Schroeder, S. A. Blaszczyk and W. Tang, *Adv. Synth. Catal.*, 2019, **361**, 3729–3732.
- 11 For selected examples see: (a) F. Pertusati and C. McGuigan, *Chem. Commun.*, 2015, **51**, 8070–8073; (b) K. Tran, G. L. Beutner, M. Schmidt, J. Janey, K. Chen, V. Rosso and M. D. Eastgate, *J. Org. Chem.*, 2015, **80**, 4994–5003; (c) D. A. DiRocco, Y. Ji, E. C. Sherer, A. Klapars, M. Reibarkh, J. Dropinski, R. Mathew, P. Maligres, A. M. Hyde, J. Limanto, A. Brunskill, R. T. Ruck, L.-C. Campeau and I. W. Davies, *Science*, 2017, **356**, 426–430; (d) E. Cini, G. Barreca, L. Carcone, F. Manetti, M. Rasparini and M. Taddei, *Eur. J. Org. Chem.*, 2018, 2622–2628; (e) M. Wang, L. Zhang, X. Huo, Z. Zhang, Q. Yuan, P. Li, J. Chen, Y. Zou, Z. Wu and W. Zhang, *Angew. Chem., Int. Ed.*, 2020, **59**, 20814–20819; (f) V. Gannedi, B. Villuri, S. N. Reddy, C.-C. Ku, C.-H. Wong and S.-C. Hung, *J. Org. Chem.*, 2021, **86**, 4977–4985; (g) A. Klapars, J. Y. L. Chung, J. Limanto, R. Calabria, L.-C. Campeau, K. R. Campos, W. Chen, S. M. Dalby, T. A. Davis, D. A. DiRocco, A. M. Hyde, A. M. Kassim, M. U. Larsen, G. Liu, P. E. Maligres, A. Moment, F. Peng, R. T. Ruck, M. Shevlin, B. L. Simmons, Z. J. Song, L. Tan, T. J. Wright and S. L. Zultanski, *Chem. Sci.*, 2021, **12**, 9031–9036.
- 12 See ESI (S3) for details.†
- 13 Details about reaction can be found in the ESI (S34).†

