

Cite this: *Chem. Sci.*, 2020, 11, 7609

All publication charges for this article have been paid for by the Royal Society of Chemistry

Received 4th March 2020
Accepted 9th March 2020

DOI: 10.1039/d0sc01330g

rsc.li/chemical-science

Synthesis of secondary and tertiary amides without coupling agents from amines and potassium acyltrifluoroborates (KATs)[†]

Anne Schuhmacher,^a Tomoya Shiro,^{‡a} Sarah J. Ryan^b and Jeffrey W. Bode^{ID}*^a

Although highly effective for most amide syntheses, the activation of carboxylic acids requires the use of problematic coupling reagents and is often poorly suited for challenging cases such as *N*-methyl amino acids. As an alternative to both secondary and tertiary amides, we report their convenient synthesis by the rapid oxidation of trifluoroborate iminiums (TIMs). TIMs are easily prepared by acid-promoted condensation of potassium acyltrifluoroborates (KATs) and amines and are cleanly and rapidly oxidized to amides with hydrogen peroxide. The overall transformation can be conducted either as a one-pot procedure or *via* isolation of the TIM. The unique nature of the neutral, zwitterionic TIMs makes possible the preparation of tertiary amides *via* an iminium species that would not be accessible from other carbonyl derivatives and can be conducted in the presence of unprotected functional groups including acids, alcohols and thioethers. In preliminary studies, this approach was applied to the late-stage modifications of long peptides and the iterative synthesis of short, *N*-methylated peptides without the need for coupling agents.

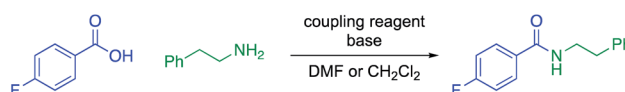
Introduction

Amide bonds are the key connections between amino acids in peptides and are present in many marketed drug molecules.¹ The synthesis of amides is typically achieved by the reaction of an activated carboxylic acid with an amine,² a process that employs stoichiometric amounts of coupling reagents and environmentally concerning solvents, *e.g.* *N,N*-dimethylformamide or dichloromethane (Fig. 1a).³ While recycling of solvents on large scale can reduce the environmental impact of peptide synthesis, the use and handling of coupling agents results in acute safety hazards due to their explosive and allergenic nature.⁴ Current approaches are therefore suitable for making most amide-based structures, but sustainable approaches to peptides and other amides requires new reactions that do not rely on these problematic reagents.

As part of a program aimed at new amide-forming methods, our group has identified potassium acyltrifluoroborates (KATs) as functional groups that form amide bonds with azides,⁵ hydroxylamines⁶ or *in situ* generated *N*-chloroamines.⁷ These methods, however, are

mechanistically limited to the synthesis of secondary amides; the preparation of tertiary amides is not possible. In this report we document the oxidative amidation of KATs to

a) Traditional amide coupling with carboxylic acids and amines



b) This work: Tertiary and secondary amides by oxidation of TIMs

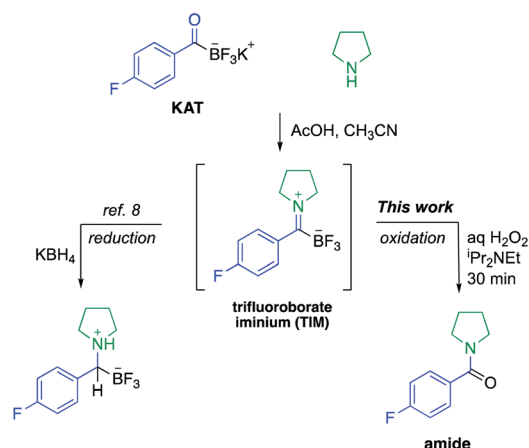


Fig. 1 Amide-forming reaction with potassium acyltrifluoroborates (KATs) and amines *via* trifluoroborate iminiums (TIMs). (a) Traditional amide coupling methods using coupling reagents. (b) Formation of TIMs and oxidative conversion to amides.

^aLaboratory of Organic Chemistry, Eidgenössische Technische Hochschule (ETH) Zurich, Vladimir-Prelog-Weg 3, 8093 Zurich, Switzerland. E-mail: bode@org.chem.ethz.ch

^bSmall Molecule Design and Development, Eli Lilly and Company Indianapolis, IN 46285, USA

[†] Electronic supplementary information (ESI) available. See DOI: 10.1039/d0sc01330g

[‡] Current address: Sumitomo Dainippon Pharma Co., Ltd., 3-1-98 Kasugade-naka, Konohana-ku, Osaka 554-0022, Japan.

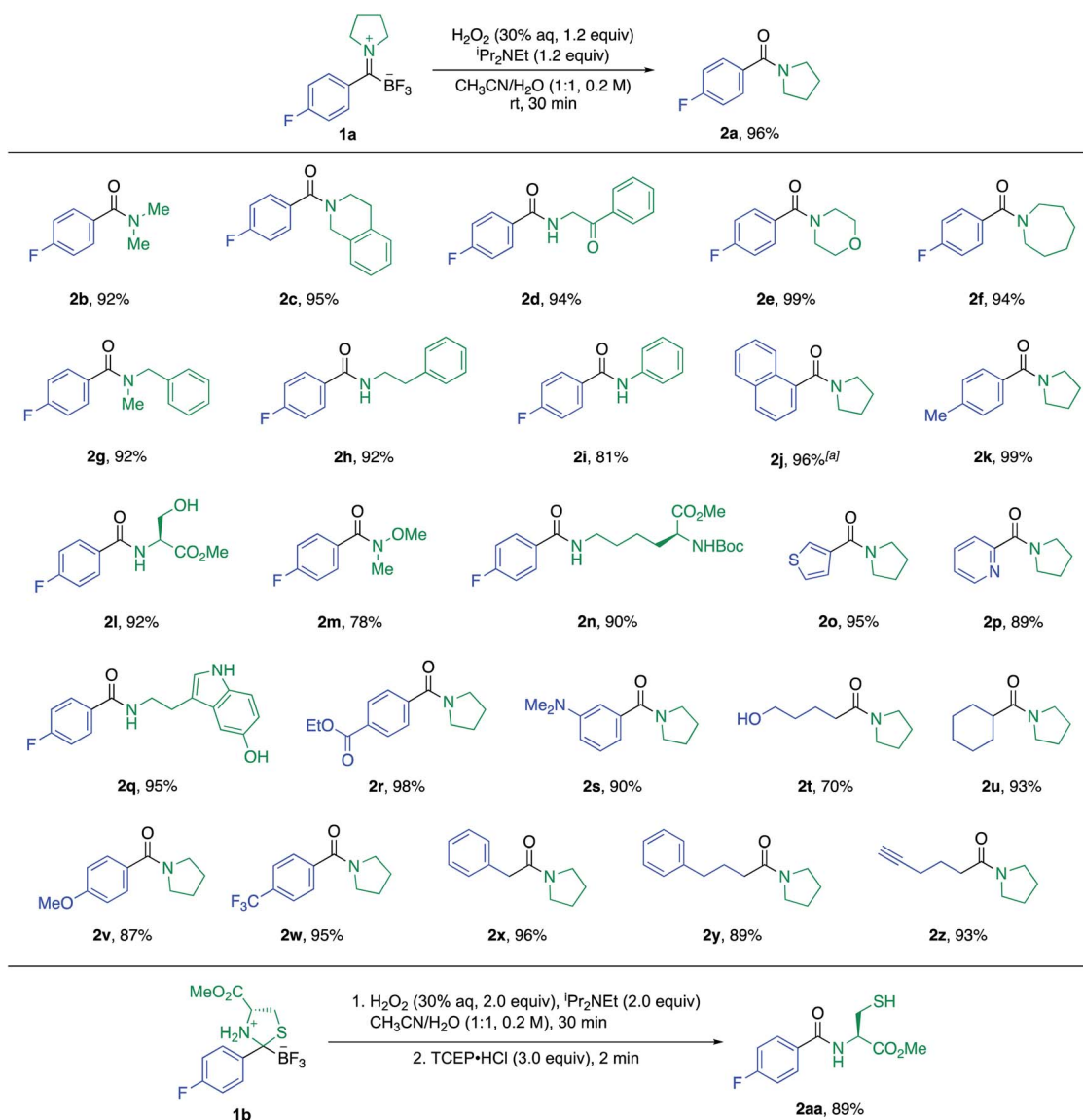


form tertiary and secondary amides from amines *via* trifluoroborate iminiums (TIMs) (Fig. 1b). The TIMs are formed under simple conditions by mixing KATs and amine salts and their oxidation occurs rapidly and chemoselectively.⁸ Although KATs are far less common starting materials than carboxylic acids, they are increasingly available from commercial sources or a plethora of recently reported methods for conversion of common starting materials to KATs. In the past few years, their syntheses from aryl halides,⁹ Grignard reagents,¹⁰ aldehydes,¹¹ vinyl boronic acids,¹² alkynes,¹³ and acid chlorides¹⁴ have been reported. The important class of amino acid-derived KATs is expanding and has been shown to be configurationally stable.^{9b,11b} Anticipating that these amino acid derivatives will become widely available in the near future, we sought to identify protocols for peptide synthesis under conditions where no activating agents are required.

Results and discussion

Amines and KATs condense to form TIMs – chromatographically stable, zwitterionic species that can be reduced with hydride sources or alkylated with organometallic reagents to give α -aminoboronates (Fig. 1).⁸ Given their ease of formation, we hypothesized that TIMs could also serve as intermediates to secondary and tertiary amides *via* oxidation. Using TIM **1a** as a model substrate, we screened oxidation conditions and found successful formation of the desired tertiary amide with several oxidants and basic additives under a variety of conditions (detailed screening data can be found in the ESI†). As TIMs can hydrolyze to give the corresponding KATs under basic aqueous conditions, we were pleased to find that the oxidation is fast and occurs without competing hydrolysis of the TIM.

We selected aqueous H₂O₂ and *N,N*-diisopropylethylamine in 1 : 1 CH₃CN/H₂O for 30 min as the standard conditions for



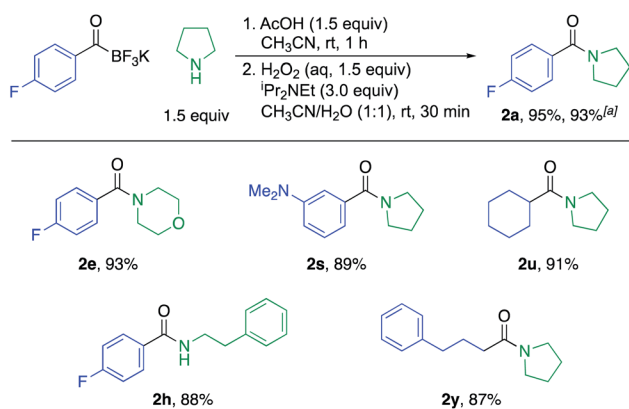
Scheme 1 Substrate scope for the amide formation from trifluoroborate iminiums (TIMs). The yields are isolated yields. ^a Reaction time of 2 h.



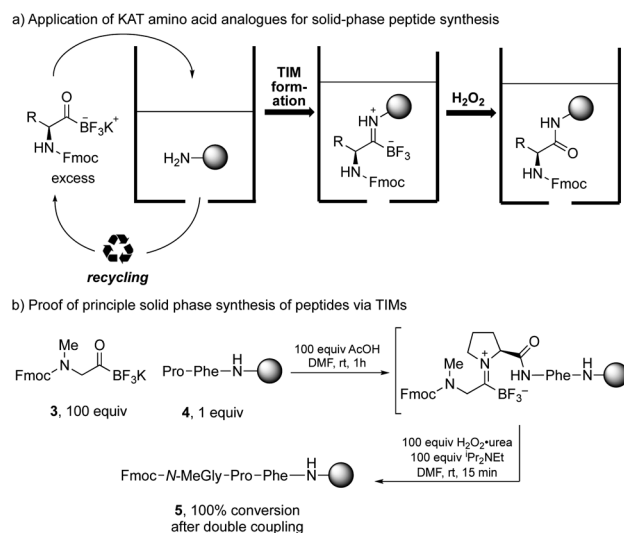
further experiments. This method allows for the fast preparation of secondary and tertiary amides, including those derived from aliphatic or aromatic KATs with different primary and secondary amines. All underwent smooth oxidation to give the amide in good yields (Scheme 1). Unprotected functional groups including alcohols, indoles, and alkynes are not affected by the oxidation step. The reaction between a KAT and cysteine yielded the *S,N*-acetal **1b** instead of a TIM. We found that the oxidation of **1b** is possible under slightly modified conditions using 2 equiv. of hydrogen peroxide to achieve full conversion and adding TCEP before work-up to reduce the disulfide formed during the reaction.

We also established a protocol for the one-pot preparation of amides from KATs and amines by TIM formation and subsequent oxidation (Scheme 2). This one-pot procedure offers both operational simplicity and slightly higher overall yields by obviating the isolation of the TIM intermediate. Since KATs are commercially available or easily prepared by several routes from commercial starting materials, this one step protocol offers a practical alternative to the formation of many simple amides. For substrate **2a**, we demonstrated the applicability of the reaction on a larger scale (3.0 mmol) without a significant decrease in yield.

The success of the one-pot amide formation from KATs and amines encouraged us to make preliminary studies towards a solid-phase peptide synthesis (SPPS) method based on this chemistry. In standard SPPS, the carboxylic acid is used in excess and must be activated with a coupling reagent. This activation step prevents it from being recovered after the coupling reaction. This both contributes to the large volume of waste produced during standard SPPS¹⁵ and limits the number of equivalents that can be used to drive the reaction. The amide-forming reaction between KATs and amines does not require any activating agent and the KAT can be recovered and reused if used in excess. We hypothesized that SPPS could be performed using KAT amino acids as monomers by TIM formation and subsequent oxidation, allowing the excess KAT to be recycled (Scheme 3a).



Scheme 2 Substrate scope for the one-pot amide formation from potassium acyltrifluoroborates (KATs) and scale-up experiment. The yields are isolated yields. ^a Yield for the reaction at 3.0 mmol scale instead of 0.2 mmol scale.



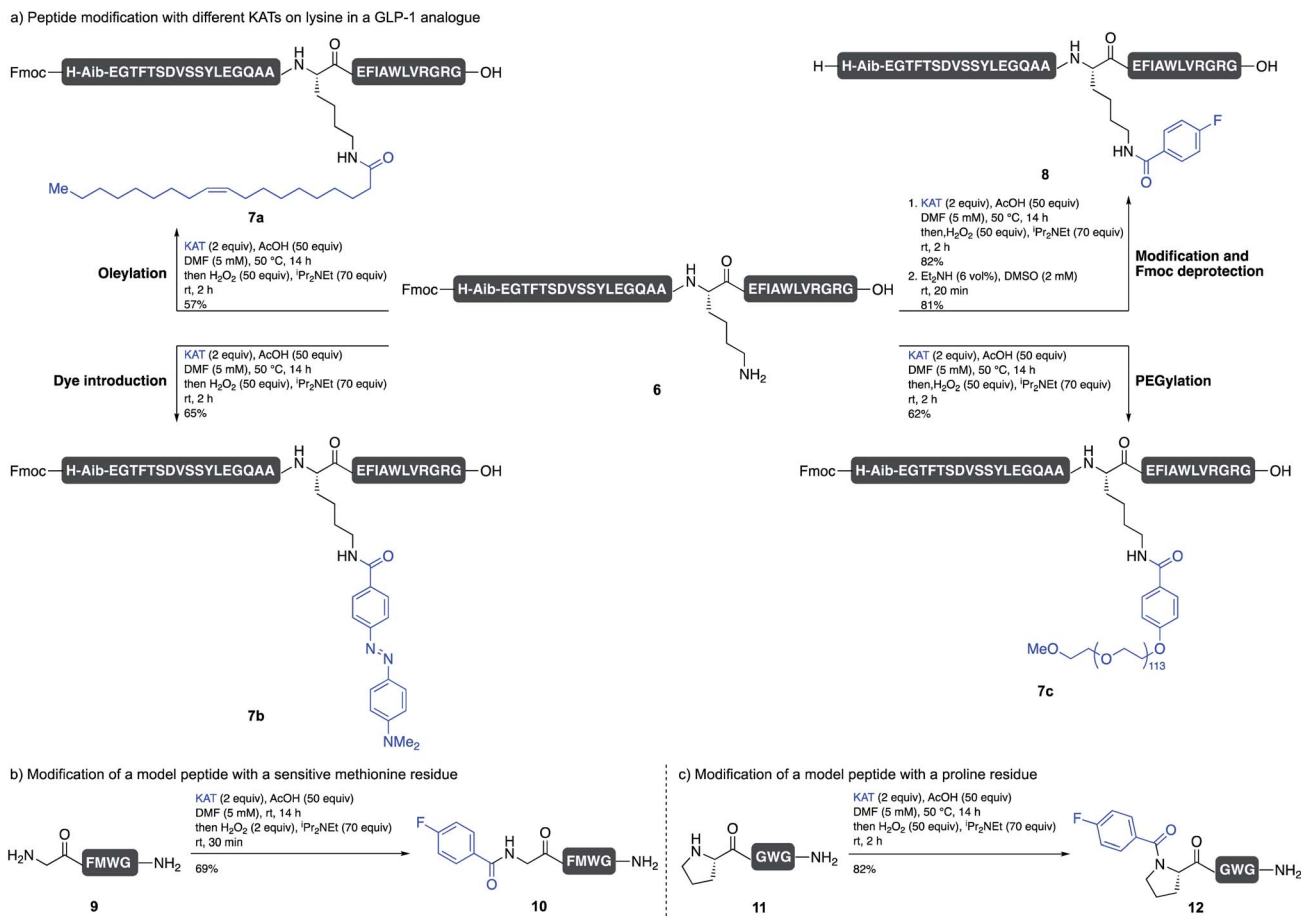
Scheme 3 Preliminary studies toward solid-phase peptide synthesis (SPPS) using KAT amino acids as monomers. (a) Schematic representation of the application of KATs in SPPS. (b) Model synthesis of a peptide on solid support via TIMs. Conversion was determined by Fmoc loading test and HPLC analysis after cleavage from the resin.

In preliminary studies, we achieved coupling between Fmoc-protected *N*-methylglycine KAT and solid-supported proline with full conversion. The conversion was determined after coupling of the *N*-methylglycine KAT analogue by Fmoc loading test of the resin and HPLC analysis after cleavage from the resin (Scheme 3b).

The acidic reaction conditions for TIM formation provides a unique environment for amide formation on unprotected substrates, such as peptides. The selective modification of peptides is important for the development of new peptide drugs, as most modern peptide therapeutics are modified with lipids or PEGs to increase their half-life and stability.¹⁶ Our method allows for the direct derivatization of an amine residue (lysine or N terminus), even when all other side chain functional groups are unprotected. We chose to investigate the modification of a lysine residue of an anti-diabetic GLP-1 analogue. Several GLP-1 analogues, *e.g.* Liraglutide or Semaglutide, are approved drugs for the treatment of diabetes.¹⁷ In both cases, the peptide is modified with a lipid side chain for binding to serum albumin.¹⁸ We were able to achieve the introduction of different KATs on Lys12 of peptide **6** with good conversions and yields, including KATs bearing a PEG chain (**7c**), an oleyl side chain (**7a**) and a diazo dye (**7b**) (Scheme 4a). Although it was necessary to preserve the N-terminal Fmoc group, this could be easily removed with Et₂NH in DMSO to give target peptide **8**.

Amino acid side chains sensitive to oxidizing conditions in peptide **6**, *i.e.* tryptophan, tyrosine, or histidine, tolerated the conditions and we did not observe any oxidation side products. We also showed that peptide **9**, which contains methionine, tolerates slightly modified oxidation conditions. Using only 2 equiv. of hydrogen peroxide and quenching the reaction with Na₂SO₃ after 30 min gave the desired product

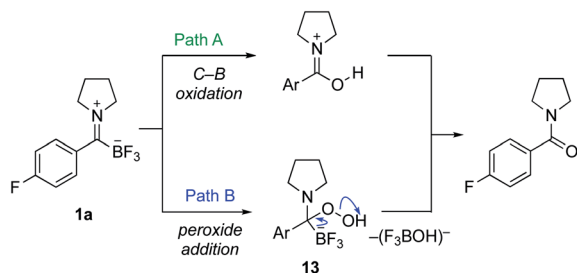




Scheme 4 Application of the trifluoroborate iminium (TIM) formation and subsequent oxidation for the modification of different peptides. (a) Modification of a GLP-1 analogue with different KATs. (b) Modification of a peptide containing methionine. (c) Modification of a proline residue in a peptide.

10 in good yield. The oxidized methionine residue was only observed as a minor side product (Scheme 4b). Since this method allows for the facile formation of tertiary amides, we furthermore showed that the modification of an N-terminal proline residue is possible and peptide **12** was obtained in high yield (Scheme 4c).

We considered several possible mechanisms for the formation of amides from TIMs with basic hydrogen peroxide. We showed with ^{18}O -labelled hydrogen peroxide that the



Scheme 5 Possible mechanisms for the oxidative amide formation from trifluoroborate iminiums (TIMs).

oxygen in the amide is incorporated from the hydrogen peroxide exclusively and no exchange with water is observed. With this in mind, we focused our preliminary studies on the two most likely pathways (Scheme 5). Hydrogen peroxide as well as other oxidants are well known to oxidize organotrifluoroborates and organoboronates to hydroxyl groups, making Path A a viable route. While there are many reports concerning the oxidation of organoboronates,¹⁹ only a few examples of the direct oxidation of organotrifluoroborates are known. Hu reported a copper-catalyzed oxidation of phenyltrifluoroborate,²⁰ Fensterbank a TEMPO-promoted procedure,²¹ and Ochiai employed a hypervalent iodonium complex.²² Of greatest relevance are Molander's oxidation conditions using Oxone²³ and Kandasamy's report using hydrogen peroxide in lactic acid for the oxidation of phenyltrifluoroborate.²⁴ These conditions, however, are relatively harsh and not consistent with the rapid, clean oxidations we observed.

As an alternative, we considered nucleophilic attack of peroxide anion to the TIM (Path B). We have already established that TIMs are good electrophiles, which would lead to tetrahedral intermediate **13**.⁷ Loss of BF_3OH^- , in a manner



similar to the postulated mechanism of KAT ligations with hydroxylamines, would lead directly to the amide.⁶ In order to test this, we attempted the oxidation with NaClO₂, which is used in the Pinnick oxidation *via* a similar mechanism.²⁵ Upon exposure of TIMs to standard Pinnick conditions, the amide product was cleanly formed. We also conducted a competition experiment between potassium phenyltrifluoroborate and TIM **1a** under our standard oxidation conditions. TIM **1a** was oxidized to give the amide while potassium phenyltrifluoroborate was recovered unchanged (see ESI† for details). Takemoto and co-workers recently published oxidative amide formation from α -ketoacids and amines. They propose a similar mechanism *via* imine formation and subsequent oxidation *via* a tetrahedral intermediate.²⁶ Together, these observations support Path B as the likely route for amide formation.

Conclusions

In summary, we have reported the oxidative synthesis of secondary and tertiary amides from KATs or TIMs under aqueous conditions. It offers a unique reactivity and chemoselectivity profile, features that should make it a viable alternative to classical methods for amide bond formation and a promising approach to chemoselective modification of peptides and proteins. The unique ability to use two stable precursors – KATs and amines – without any additional reagents to form the TIM intermediate provides a promising approach to a new form of iterative peptide synthesis that avoids problematic coupling reagents.

Experimental section

General procedure for the oxidation of TIMs to amides

TIM (1.0 equiv.) was dissolved in CH₃CN/H₂O (1 : 1, 0.2 M), H₂O₂ (30% aq., 1.2 equiv.) and *N,N*-diisopropylethylamine (1.2 equiv.) were added and the reaction was allowed to stir for 30 min. The reaction was diluted with H₂O and EtOAc and the aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. If necessary, the crude product was purified with flash column chromatography.

General procedure for the one-pot TIM formation and oxidation

KAT (1.0 equiv.) was dissolved in CH₃CN (0.5 M), AcOH (1.5 equiv.) and the amine (1.5 equiv.) were added and the reaction was stirred for 1 hour. H₂O (0.5 M), H₂O₂ (aq, 30%, 1.5 equiv.), and *N,N*-diisopropylethylamine (3.0 equiv.) were added and the reaction was allowed to stir for an additional 30 min. The reaction was diluted with H₂O and EtOAc and the aqueous layer was extracted three times with EtOAc. The combined organic layers were washed with brine, dried over MgSO₄, filtered and concentrated under reduced pressure. If necessary, the crude product was purified with flash column chromatography.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Financial support was provided by Eli Lilly through the Lilly Research Award Program (LRAP). A. Schuhmacher acknowledges the Scholarship Fund of the Swiss Chemical Industry (SSCI) for a fellowship. Support for T. Shiro was provided by Sumitomo Dainippon Pharma. Co., Ltd. We thank the Molecular and Biomolecular Analytical Services (MoBIAS) and the LOC NMR service for analyses. Alessandro Fracassi (ETH Zurich) is acknowledged for providing oleyl KAT. Dino Wu (ETH Zurich) is acknowledged for developing the synthesis of potassium (*N*-(*tert*-butoxycarbonyl)-*N*-methylglycyl)trifluoroborate.

Notes and references

- (a) *The Amide Linkage: Selected Structural Aspects in Chemistry, Biochemistry and Material Science*, ed. A. Greenburg, C. M. Breneman and J. S. Liebman, Wiley, New York, 2000; (b) J. L. Lau and M. K. Dunn, *Bioorg. Med. Chem.*, 2018, **26**, 2700–2707.
- C. A. G. N. Montalbetti and V. Falque, *Tetrahedron*, 2005, **61**, 10827–10852.
- (a) D. J. C. Constable, P. J. Dunn, J. D. Hayler, G. R. Humphrey, J. L. Leazer Jr, R. J. Linderman, K. Lorenz, J. Manley, B. A. Pearlman, A. Wells, A. Zaks and T. Y. Zhang, *Green Chem.*, 2007, **9**, 411–420; (b) D. S. MacMillan, J. Murray, H. F. Sneddon, C. Jamieson and A. J. B. Watson, *Green Chem.*, 2013, **15**, 596–600; (c) M. T. Sabatini, L. T. Boulton, H. F. Sneddon and T. D. Sheppard, *Nat. Catal.*, 2019, **2**, 10–17.
- K. J. McKnelly, W. Sokol and J. S. Nowick, *J. Org. Chem.*, 2020, **85**, 1764–1768.
- G. A. Molander, J. Raushel and N. M. Ellis, *J. Org. Chem.*, 2010, **75**, 4304–4306.
- (a) A. M. Dumas, G. A. Molander and J. W. Bode, *Angew. Chem., Int. Ed.*, 2012, **51**, 5683–5686; (b) H. Noda, G. Erős and J. W. Bode, *J. Am. Chem. Soc.*, 2014, **136**, 5611–5614.
- A. Osuna Gálvez, C. P. Schaack, H. Noda and J. W. Bode, *J. Am. Chem. Soc.*, 2017, **139**, 1826–1829.
- T. Shiro, A. Schuhmacher, M. K. Jackl and J. W. Bode, *Chem. Sci.*, 2018, **9**, 5191–5196.
- (a) G. Erős, Y. Kushida and J. W. Bode, *Angew. Chem., Int. Ed.*, 2014, **53**, 7604–7607; (b) D. Wu, N. A. Fohn and J. W. Bode, *Angew. Chem., Int. Ed.*, 2019, **58**, 11058–11062.
- S. M. Liu, D. Wu and J. W. Bode, *Org. Lett.*, 2018, **20**, 2378–2381.
- (a) A. M. Dumas and J. W. Bode, *Org. Lett.*, 2012, **14**, 2138–2141; (b) J. Taguchi, T. Takeuchi, R. Takahashi, F. Masero and H. Ito, *Angew. Chem., Int. Ed.*, 2019, **58**, 7299–7303.
- (a) Z. He, P. Trinchera, S. Adachi, J. D. S. Denis and A. K. Yudin, *Angew. Chem., Int. Ed.*, 2012, **51**, 11092–11096; (b) S. Adachi, S. K. Liew, C. F. Lee, A. Lough, Z. He, J. D. S. Denis, G. Poda and A. K. Yudin, *Org. Lett.*, 2015, **17**, 5594–5597; (c) D. Tan, Y. Cai, Y. Zeng, W. Lv, L. Yang, Q. Li



- and H. Wang, *Angew. Chem., Int. Ed.*, 2019, **58**, 13784–13788; (d) S. Lin, L. Wang, N. Aminoleslami, Y. Lao, C. Yagel and A. Sharma, *Chem. Sci.*, 2019, **10**, 4684–4691.
- 13 (a) M. L. Lepage, S. Lai, N. Peressin, R. Hadjerci, B. O. Patrick and D. M. Perrin, *Angew. Chem., Int. Ed.*, 2017, **56**, 15257–15261; (b) J. Taguchi, T. Ikeda, R. Takahashi, I. Sasaki, Y. Ogasawara, T. Dairi, N. Kato, Y. Yamamoto, J. W. Bode and H. Ito, *Angew. Chem., Int. Ed.*, 2017, **56**, 13847–13851.
- 14 (a) Y. Segawa, M. Yamashita and K. Nozaki, *Science*, 2006, **314**, 113–115; (b) J. Campos and S. Aldridge, *Angew. Chem., Int. Ed.*, 2015, **54**, 14159–14163.
- 15 (a) R. B. Merrifield, *J. Am. Chem. Soc.*, 1963, **85**, 2149–2154; (b) M. Amblard, J. Fehrentz, J. Martinez and G. Subra, *Mol. Biotechnol.*, 2006, **33**, 239–254; (c) K. G. Varnava and V. Sarojini, *Chem.–Asian J.*, 2019, **14**, 1088–1097.
- 16 (a) Q. Hu, F. Berti and R. Adamo, *Chem. Soc. Rev.*, 2016, **45**, 1691–1719; (b) N. Yin, M. A. Brimble, P. W. R. Harris and J. Wen, *Med. Chem.*, 2014, **4**, 763–769; (c) O. Boutureira and G. J. L. Bernardes, *Chem. Rev.*, 2015, **115**, 2174–2195.
- 17 (a) A. Andersen, A. Lund, F. K. Knop and T. Vilsbøll, *Nat. Rev. Endocrinol.*, 2018, **14**, 390–403; (b) D. Sharma, S. Verma, S. Vaidya, K. Kalia and V. Tiwari, *Biomed. Pharmacother.*, 2018, **108**, 952–962; (c) S. Dhillon, *Drugs*, 2018, **78**, 275–284.
- 18 (a) D. Irby, C. Du and F. Li, *Mol. Pharmaceutics*, 2017, **14**, 1325–1338; (b) M. T. Larsen, M. Kuhlmann, M. L. Hvam and K. A. Howard, *Mol. Cell. Ther.*, 2016, **4**, 3.
- 19 (a) J. Simon, S. Salzbrunn, G. K. S. Prakash, N. A. Petasis and G. A. Olah, *J. Org. Chem.*, 2001, **66**, 633–634; (b) G. K. S. Prakash, S. Chacko, C. Panja, T. E. Thomas, L. Gurung, G. Rasul, T. Mathew and G. A. Olah, *Adv. Synth. Catal.*, 2009, **351**, 1567–1574; (c) K. S. Webb and D. Levy, *Tetrahedron Lett.*, 1995, **36**, 5117–5118; (d) R. E. Maleczka, F. Shi, D. Holmes and M. R. Smith III, *J. Am. Chem. Soc.*, 2003, **125**, 7792–7793; (e) E. Kianmehr, M. Yahyaee and K. Tabatabai, *Tetrahedron Lett.*, 2007, **48**, 2713–2715; (f) E. Shin, H. Kim, S. Joo, U. S. Shin and S. Kim, *Catal. Lett.*, 2019, **149**, 1560–1564; (g) G. Cheng, X. Zeng and X. Cui, *Synthesis*, 2014, **46**, 295–300.
- 20 J. Xu, X. Wang, C. Shao, D. Su, G. Cheng and Y. Hu, *Org. Lett.*, 2010, **12**, 1964–1967.
- 21 G. Sorin, R. Martinez Mallorquin, Y. Contie, A. Baralle, M. Malacria, J. Goddard and L. Fensterbank, *Angew. Chem., Int. Ed.*, 2010, **49**, 8721–8723.
- 22 M. Ochiai, K. Miyamoto, Y. Yokota, T. Suefuji and M. Shiro, *Angew. Chem., Int. Ed.*, 2005, **44**, 75–78.
- 23 (a) G. A. Molander and L. N. Cavalcanti, *J. Org. Chem.*, 2011, **76**, 623–630; (b) G. A. Molander, S. Z. Siddiqui and N. Fleury-Brégeot, *Org. Synth.*, 2013, **90**, 153–163.
- 24 (a) S. Gupta, P. Chaudhary, L. Seva, S. Sabiah and J. Kandasamy, *RSC Adv.*, 2015, **5**, 89133–89138; (b) S. Gupta, P. Chaudry, V. Srivastava and J. Kandasamy, *Tetrahedron Lett.*, 2016, **57**, 2506–2510.
- 25 (a) B. O. Lindgren and T. Nilsson, *Acta Chem. Scand.*, 1973, **27**, 888–890; (b) G. A. Kraus and B. Roth, *J. Org. Chem.*, 1980, **45**, 4825–4830; (c) B. S. Bal, W. E. Childers Jr and H. W. Pinnick, *Tetrahedron*, 1981, **37**, 2091–2096; (d) V. P. Kudesia, *Bull. Soc. Chim. Belg.*, 1972, **81**, 623–628.
- 26 T. Nanjo, N. Kato, X. Zhang and Y. Takemoto, *Chem.–Eur. J.*, 2019, **25**, 15504–15507.

