

Cite this: *Chem. Sci.*, 2022, 13, 6309

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

Received 14th March 2022

Accepted 21st April 2022

DOI: 10.1039/d2sc01466a

rsc.li/chemical-science

An economical approach for peptide synthesis via regioselective C–N bond cleavage of lactams†

Wataru Muramatsu * and Hisashi Yamamoto *

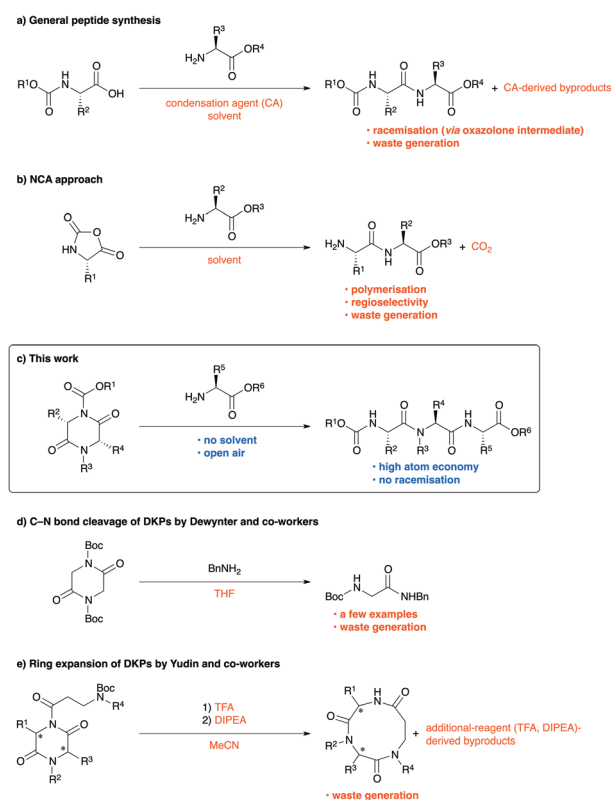
An economical, solvent-free, and metal-free method for peptide synthesis via C–N bond cleavage using lactams has been developed. The method not only eliminates the need for condensation agents and their auxiliaries, which are essential for conventional peptide synthesis, but also exhibits high atom economy. The reaction is versatile because it can tolerate side chains bearing a range of functional groups, affording up to >99% yields of the corresponding peptides without racemisation or polymerisation. Moreover, the developed strategy enables peptide segment coupling, providing access to a hexapeptide that occurs as a repeat sequence in spider silk proteins.

Introduction

Peptide chemistry has evolved remarkably in the past century, and peptides are being extensively exploited in pharmaceuticals, materials, and cosmetics.¹ Because peptide synthesis largely focuses on developing general condensation agents,² realising high reactivity, and ensuring low risk of racemisation,³ issues such as health hazards,⁴ waste generation,^{4,5} and atom economy⁶ have remained neglected and must be urgently addressed. In fact, “Amide formation avoiding poor atom economy reagents,” listed as one of the 12 key green chemistry research areas in 2006, was retained as “General methods for catalytic/sustainable (direct) amide or peptide formation” and was regarded as one of the 10 key green chemistry research areas in 2018.⁷

Peptides are usually synthesised by repeating a condensation-agent-mediated amidation reaction, followed by deprotection. Because of the importance of condensation agents, more valuable condensation-agent-mediated peptide synthesis methods are still being actively developed.² However, condensation-agent-mediated peptide syntheses have low atom economy and occasionally face undesired racemisation (Scheme 1a).³ More importantly, the condensation agents may trigger allergic reactions in the user.^{4,8} Although a number of catalytic approaches have been reported in the past few decades to address these concerns,⁹ significant improvement is still necessary. Using amino acid *N*-carboxy anhydride (NCA) is among the most efficient approaches for peptide synthesis;¹⁰ however, suppression of polymerisation is quite difficult. Consequently, this method is generally used for the synthesis of

polypeptides.¹¹ Furthermore, the copious carbon dioxide generated at the industrial level is a serious concern, particularly in a modern society that is striving toward carbon neutrality. In addition, the preparation of NCAs requires considerable skill because of the use of phosgene, an extremely poisonous chemical (Scheme 1b).¹²



Scheme 1 Strategies for peptide chain elongation.

Peptide Research Center, Chubu University, 1200 Matsumoto-cho, Kasugai, Aichi 487-8501, Japan. E-mail: muramatsu@isc.chubu.ac.jp; hyamamoto@isc.chubu.ac.jp

† Electronic supplementary information (ESI) available: Experimental procedures, characterisation data, and copies of ¹H and ¹³C NMR spectra for all compounds. See <https://doi.org/10.1039/d2sc01466a>

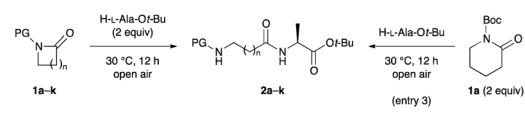


In this study, we have developed a new strategy for peptide synthesis *via* regioselective C–N bond cleavage reaction using lactams, including diketopiperazines (DKPs), as building blocks (Scheme 1c). We believe that most concerns in peptide synthesis can be addressed by our strategy. First, our strategy requires no metals, reaction solvents, or condensation agents that present health hazards. Second, conventional peptide syntheses using condensation agents always generate wastes derived from condensation agents, whereas our developed reaction does not (high atom economy and low *E* factor).¹³ Third, although various condensation agents and catalysts have been developed to minimise racemisation, the risk of racemisation *via* the formation of oxazolone intermediates, which is the major cause of racemisation,^{3d} still remains. By contrast, the risk of racemisation *via* the generation of oxazolone intermediates is completely eliminated in our new approach. To the best of our knowledge, similar studies have been reported so far only on amidation using BnNH₂ by the Dewynter group (Scheme 1d)¹⁴ and ring expansion by the Yudin group (Scheme 1e).¹⁵ Although the two groups have conducted pioneering studies on metal-free C–N bond cleavage of lactams, reactions such as intermolecular peptide bond formation have not yet been examined, and there is significant scope for improvement in terms of substrate limitation and versatility.

Results and discussion

We began our investigation by reacting lactams **1** with H-L-Ala-Ot-Bu.¹⁶ Various solvents and ring sizes were tested for this reaction to determine the optimal conditions (Table 1). When **1a** was reacted with H-L-Ala-Ot-Bu (2 equiv.) in the absence of solvents under air at 30 °C for 12 h, desired peptide **2a** was regioselectively obtained in 99% yield, with >99 : 1 er and ideal atom economy (entry 1). On a gram-scale (6 mmol), the C–N bond cleavage reaction proceeded with essentially identical yield and purity as for that conducted on a 0.50 mmol-scale (97% yield; entry 2). When **1a** (2 equiv.) was reacted with H-L-Ala-Ot-Bu under the same reaction conditions, the yield of **2a** decreased slightly to 92% (entry 3). When the reaction was carried out in less polar solvents, the C–N bond cleavage reaction afforded **2a** in 47–74% yields, with no byproducts (entries 4–7). The use of ethers bearing sterically bulky substituents such as CPME, 2-Me-THF, or TBME, instead of toluene, led to a similar outcome (entries 8–10). The yields were lower than that in toluene when less hindered ethers such as 1,4-dioxane and DME or highly polar solvents such as DMF and DMSO were used (entries 11–13).¹⁷ The tolerance of several other protecting groups was evaluated.¹⁸ Contrary to expectation, the reaction did not proceed in some cases, while the yields were low or negligible relative to that with Boc as the protecting group (entries 14–19). This is because lactams **1b–e** are unreactive, and the reaction of lactams **1b–e** largely proceeds to cleave the corresponding protecting groups *via* C–N bond cleavage at the exocyclic amide moieties. Regardless of the ring size, lactams **1h–k** gave satisfactory yields of the corresponding dipeptides involving Gly homolog residues **2h–k**, without loss of stereochemical integrity and without side reactions (entries 20–23).

Table 1 Optimisation of the C–N bond cleavage reaction^a

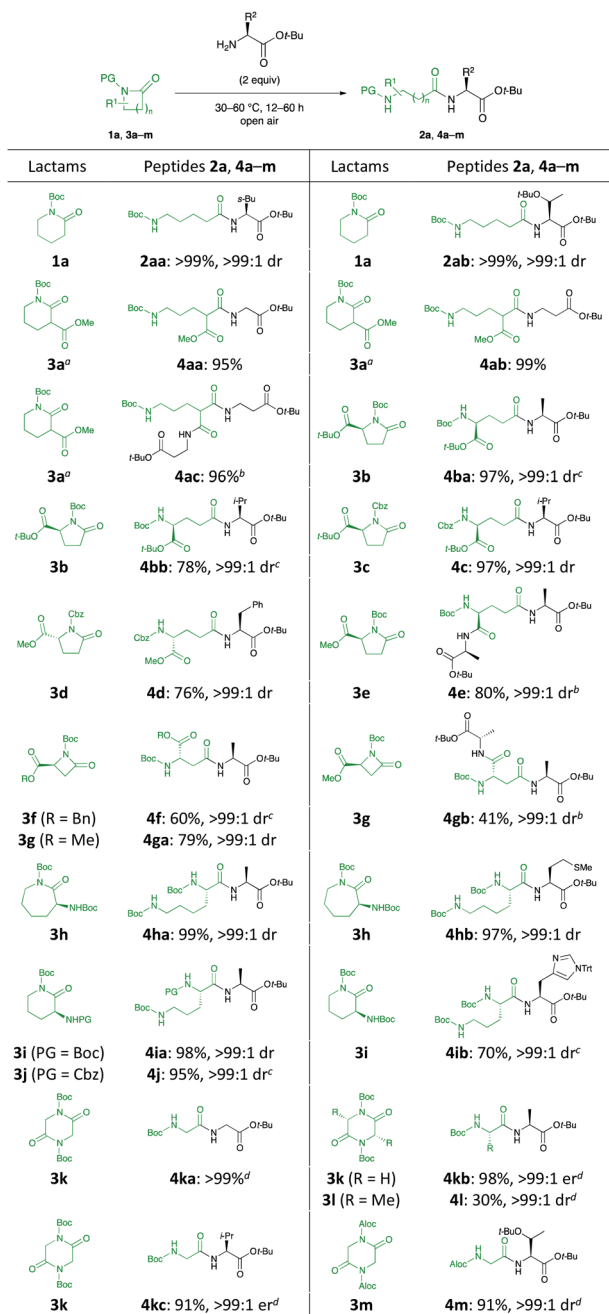


Entry	Lactam	PG	<i>n</i>	Solvent	Yield of 2a–k (%)	er of 2a–k
1	1a	Boc	3	—	99	>99 : 1
2 ^b	1a	Boc	3	—	97	>99 : 1
3	1a	Boc	3	—	92	>99 : 1
4	1a	Boc	3	Toluene	74	>99 : 1
5	1a	Boc	3	Benzene	58	>99 : 1
6	1a	Boc	3	CHCl ₃	47	>99 : 1
7	1a	Boc	3	DCM	52	>99 : 1
8	1a	Boc	3	CPME	88	>99 : 1
9	1a	Boc	3	2-Me-THF	69	>99 : 1
10	1a	Boc	3	TBME	76	>99 : 1
11	1a	Boc	3	DME	58	>99 : 1
12	1a	Boc	3	DMF	52	>99 : 1
13	1a	Boc	3	DMSO	29	>99 : 1
14	1b	H	3	—	0	—
15	1c	Me	3	—	0	—
16	1d	Bn	3	—	0	—
17	1e	Ac	3	—	0	—
18	1f	Bz	3	—	5	—
19	1g	Cbz	3	—	43	>99 : 1
20 ^c	1h	Boc	1	—	91	>99 : 1
21 ^d	1i	Boc	2	—	73	>99 : 1
22	1j	Boc	4	—	90	>99 : 1
23 ^d	1k	Boc	10	—	65	>99 : 1

^a Percentage represents the isolated yield. Ers were determined by HPLC. ^b Yield obtained *via* the gram-scale synthesis of **2a**. ^c 50 °C. ^d H-L-Ala-Ot-Bu (3.0 equiv.) at 50 °C for 24 h.

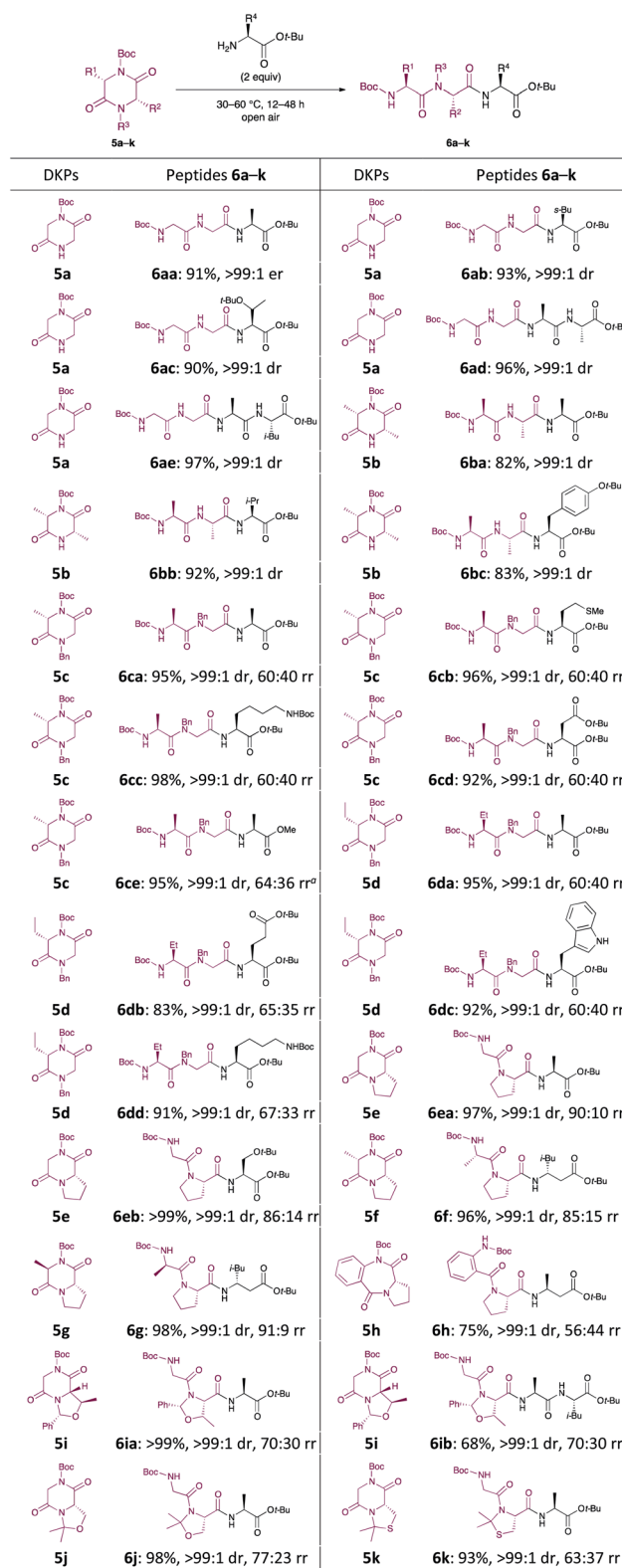
With the optimal reaction conditions in hand, lactams bearing an array of functional groups were successfully coupled with a wide variety of amino acid esters that served as the nucleophilic components for peptide formation (Scheme 2). Amino acid esters possessing a branched or relatively bulky side chain (*e.g.*, Leu, Thr, and His) were compatible with this reaction, providing the desired dipeptides (**2aa**, **2ab**, and **4ib**, respectively) in excellent yields. When lactams comprising esters and protected amino groups **3a–d** and **3f–j** were employed with a variety of amino acid esters, the corresponding dipeptides **4aa**, **4ab**, **4ba**, **4bb**, **4c**, **4d**, **4f**, **4ga**, **4ha**, **4ia**, and **4j** were successfully furnished in 60–99% yields. Fortunately, an independent parallel C–N bond cleavage reaction and our original tantalum-catalysed amidation¹⁹ could furnish the desired tripeptides **4ac**, **4e**, and **4gb** in 96%, 80%, and 41% yields, respectively. The unsatisfactory yield of **4gb** was attributed to the incomplete conversion. The activity of the metal catalyst used in peptide bond-forming reactions is often poisoned by the sulfur atoms in amino acids such as Met, Cys, and their derivatives because of their strong binding affinities.²⁰ Such substrate limitations were not observed in our system because it did not require any additional reagents and metals. Moreover, H-L-Met-Ot-Bu was compatible under the reaction conditions, affording **4hb** in 97% yield without the occurrence of either epimerisation or side reactions.





Scheme 2 Scope and limitation of lactams. Percentage represent the isolated yield. Ers were determined by HPLC. Drs determined by ¹H NMR spectroscopy. ^aRacemic lactam was used as the starting material. ^bAmino acid *tert*-butyl esters (4 equiv.) and Ta(OMe)₅ (10 mol%). ^cAmino acid *tert*-butyl esters (3 equiv.). ^dAmino acid *tert*-butyl esters (4 equiv.).

Next, the reaction was extended to the C–N bond cleavage reaction of 2,5-diketopiperazines (DKPs) for dipeptide synthesis. The C–N bond cleavage of cyclo-(PG-Gly-PG-Gly-) **3k** and **3m** with some amino acid esters smoothly generated the corresponding dipeptides **4ka**, **4kb**, **4kc**, and **4m** in excellent yields. Cyclo-(Boc-*L*-Ala-Boc-*L*-Ala-) **3l** was less reactive than **3k** and **3m**.



Scheme 3 Scope and limitation of DKPs. Percentage represent the isolated yield. Er was determined by HPLC. Drs and rrs (ratio of rotation isomers)²⁴ were determined by ¹H NMR spectroscopy. ^aH-*L*-Ala-OMe·HCl (3 equiv.) and Et₃N (3 equiv.) were used.



Subsequently, we focused our attention on the applicability of DKPs bearing an array of functional groups for the formation of peptide bonds with several amino acid esters and peptides as nucleophilic components (Scheme 3).²¹ In contrast to **3l**, whose reactivity was relatively low, **5a** and **5b** reacted smoothly with a variety of amino acid esters and dipeptides to afford the corresponding tri- and tetrapeptides **6aa–ae**, **6ba–bc** in high yields, without loss of stereochemical integrity. Similar to **5a**, DKPs bearing a Bn group at the 4-position (**5c** and **5d**) reacted to give the desired products **6ca–ce** and **6da–dd** in high yields. This is probably because the approach of amines to the carbonyl groups at the endocyclic amide moieties of **3l** is prevented by steric hindrance of the *t*-Bu groups, which is caused by the twisting of the exocyclic amide moieties (Fig. 1).²²

In general, hydrochloride salts of amino acid esters can be stored for a longer time period than the corresponding free amines because of the stability and ease of handling of the former. Since free amines such as H-Gly-OMe and H-L-Ala-OMe are volatile and prone to spontaneous polymerisation, it is quite difficult to handle them directly for peptide synthesis.^{16a} Thus, when H-L-Ala-OMe, formed by the *in situ* neutralisation of its HCl salt using Et₃N, was reacted with **5c**, the desired product **6ce** was obtained in 95% yield, without epimerisation. A broad variety of six-five bicyclic DKPs, serving as electrophilic counterparts, smoothly participated in this C–N bond cleavage reaction, and DKPs **5e–g** reacted with α - and β -amino acid esters to form new peptides in excellent yields.²³ The stereochemistry of the DKPs did not affect this C–N bond cleavage reaction, and both **5f** and **5g** gave **6f** and **6g**, respectively, in excellent yields without any apparent epimerisation. Unfortunately, a moderate yield (75%) of **6h** was obtained when seven-five bicyclic compound **5h** was used. This is due to the occurrence of an unexpected C–N bond cleavage reaction (cleavage of the Boc group) that produced undesired Boc-L- β -HoAla-*Ot*-Bu and cyclo(-Abz-L-Pro-). DKPs **5i–k**, possessing ether or sulfide side chains, were also tolerated under the reaction conditions and were smoothly transformed into **6ia**, **6ib**, **6j**, and **6k** in high yields. Thus, the developed method can be used to synthesise peptides in high yields while circumventing the commonly encountered problems in peptide synthesis.

Finally, we demonstrated convergent synthesis of a hexapeptide (**11**: Gly-L-Ser-Gly-L-Ala-Gly-L-Ala) *via* this C–N bond cleavage as a key reaction because peptides that mimic parts of biologically-derived proteins or that incorporate parts of

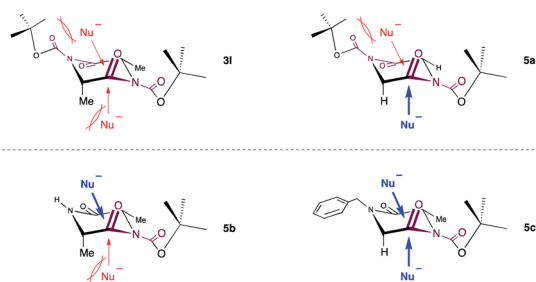
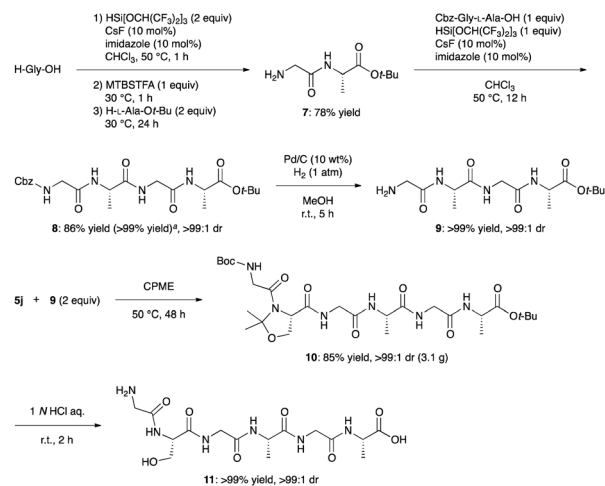


Fig. 1 C–N bond cleavage at a twisted amide moiety.



Scheme 4 Convergent synthesis of a unique fragment of spider silk proteins. ^aCbz-Gly-L-Ala-OH, H-Gly-L-Ala-*Ot*-Bu (1.5 equiv.), HSi [OCH(CF₃)₂]₃ (1.3 equiv.), CHCl₃, 50 °C, 24 h.

biologically-derived proteins have attracted increasing attention in recent years as new bio-based materials for potential applications due to their remarkable properties such as a balance of toughness, strength, and extensibility.²⁵ This hexapeptide is a unique repeat sequence observed in spider, bagworm, and *Bombyx mori* silk proteins.^{25f,g,26} One-pot amidation of H-Gly-OH with H-L-Ala-*Ot*-Bu through transient masking with silylating agents produced **7** in 78% yield.²⁷ In the presence of HSi [OCH(CF₃)₂]₃,²⁸ CsF, and imidazole,^{27,29} peptide segment coupling of **7** with Cbz-Gly-L-Ala-OH, which can be obtained by classical methods in two steps from commercially available Cbz-Gly-OH and H-L-Ala-OMe,³⁰ followed by hydrogenation furnished **9** in excellent yields. The subsequent C–N bond cleavage/new amide bond formation/deprotection sequence afforded target peptide **11** with >99 : 1 dr (Scheme 4).

Conclusions

In summary, we have demonstrated that C–N bond cleavage reaction using lactams as building blocks is an economical design principle for peptide synthesis. This method tolerates amino acid side chains bearing various functional groups and can be effectively employed to avoid racemisation during peptide bond formation. Moreover, this is competent for peptide segment coupling to provide a unique fragment of spider silk proteins. Important features of our strategy are as follows: (1) no use of metals, condensation agents, auxiliaries, or reaction solvents. (2) The reactions do not produce byproducts (high atom economy) and potentially, they may be run on an industrial scale without waste generation (low *E* factor). (3) No racemisation. (4) Mild reaction conditions and simple operation. (5) The present synthetic approach is not only the first example of a metal-free C–N bond cleavage reaction for intermolecular peptide bond formation but also the most economical convergent peptide synthesis. (6) DKPs can be easily prepared according to known methods^{16b,31} and are recognised



to have unique bioactivities.³² In addition, they are also attracting attention as new functional ingredients in foods and beverages.³³ (7) Superfluous amino acid esters can be readily removed by aqueous extraction or using an oil rotary vacuum pump/oil diffusion pump. (8) DKPs can be stored for long periods and are easy to handle because they are relatively stable. Considering the operational simplicity and high atom economy of this approach for new peptide synthesis with high optical purity and the potential applicability of these peptides as pharmaceutically relevant scaffolds, we expect this method to be widely adopted in academia and industry.

Data availability

Experimental procedures, characterisation data, and copies of ¹H and ¹³C NMR spectra for all compounds have been included in the ESI.†

Author contributions

W. M. and H. Y. conceived and designed the project. W. M. performed the experiments and collected the data. W. M. prepared the manuscript with input from all contributing authors.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Support was provided by a grant-in-aid from the New Energy and Industrial Technology Development Organisation (NEDO, JPNP14004) and Daiko Foundation (No. 9226), Japan.

Notes and references

- (a) G. Klebe, *Drug Design*, Springer, Berlin, Heidelberg, 2013; (b) A. Henninot, J. C. Collins and J. M. Nuss, *J. Med. Chem.*, 2018, **61**, 1382–1414; (c) A. Harrington and Y. Tal-Gan, *Future Med. Chem.*, 2019, **11**, 2759–2763; (d) S. Kumari, A. V. Carmona, A. K. Tiwari and P. C. Trippier, *J. Med. Chem.*, 2020, **63**, 12290–12358.
- (a) F. Albericio, R. Chinchilla, D. J. Dodsworth and C. Nájera, *Org. Prep. Proced. Int.*, 2001, **33**, 203–303; (b) E. Valeur and M. Bradley, *Chem. Soc. Rev.*, 2009, **38**, 606–631; (c) A. El-Faham and F. Albericio, *Chem. Rev.*, 2011, **111**, 6557–6602; (d) J. R. Dunetz, J. Magano and G. A. Weisenburger, *Org. Process Res. Dev.*, 2016, **20**, 140–177; (e) F. Albericio and A. El-Faham, *Org. Process Res. Dev.*, 2018, **22**, 760–772; (f) J. B. Sperry, C. J. Minter, J. Y. Tao, R. Johnson, R. Duzguner, M. Hawksworth, S. Oke, P. F. Richardson, R. Barnhart, D. R. Bill, R. A. Giusto and J. D. Weaver III, *Org. Process Res. Dev.*, 2018, **2**, 1262–1275.
- (a) M. B. A. Bodanszky, *Chem. Commun.*, 1967, 591–593; (b) I. Antonovics and G. T. Young, *J. Chem. Soc. C*, 1967, 595–601; (c) N. Izumiya and M. Muraoka, *J. Am. Chem. Soc.*, 1969, **91**, 2391–2392; (d) D. S. Kemp and J. Rebeck Jr, *J. Am. Chem. Soc.*, 1970, **92**, 5792–5793.
- (a) L. Wigman, T. Remarchuk, S. R. Gomez, A. Kumar, M. W. Dong, C. D. Medley and N. Chetwyn, *Am. Pharm. Rev.*, 2014, **17**; (b) O. A. Musaimi, B. G. de la Torre and F. Albericio, *Green Chem.*, 2020, **22**, 996–1018; (c) K. Wegner, D. Barnes, K. Manzor, A. Jardine and D. Moran, *Green Chem. Lett. Rev.*, 2021, **14**, 153–164.
- (a) C. Jimenez-Gonzalez, C. S. Ponder, Q. B. Broxterman and J. B. Manley, *Org. Process Res. Dev.*, 2011, **15**, 912–917; (b) J. H. Rasmussen, *Bioorg. Med. Chem.*, 2018, **26**, 2914–2918; (c) A. Isidro-Llobet, M. N. Kenworthy, S. Mukherjee, M. E. Kopach, K. Wegner, F. Gallou, A. G. Smith and F. Roschangar, *J. Org. Chem.*, 2019, **84**, 4615–4628.
- B. M. Trost, *Science*, 1991, **254**, 1471–1477.
- (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. Pearman, A. Wells, A. Zaks and T. Y. Zhang, *Green Chem.*, 2007, **9**, 411–420; (b) B. M. Monks and A. Whiting, *Sustainable Catalysis: Challenges and Practices for the Pharmaceutical and Fine Chemical Industries*, John Wiley & Sons, Inc., Hoboken, NJ, 2013, pp. 89–110.
- K. J. McKnelly, W. Sokol and J. S. Nowick, *J. Org. Chem.*, 2020, **85**, 1764–1768.
- (a) V. R. Pattabiraman and J. W. Bode, *Nature*, 2011, **480**, 471–479; (b) T. Krause, S. Baader, B. Erb and L. J. Gooßen, *Nat. Commun.*, 2016, **7**, 11732; (c) M. T. Sabatini, L. T. Boulton, H. F. Sheddon and T. D. Sheppard, *Nat. Catal.*, 2019, **2**, 10–17; (d) Handoko, S. Satishkumar, N. R. Ranigrahi and P. S. Arora, *J. Am. Chem. Soc.*, 2019, **141**, 15977–15985; (e) M. Todorovic and D. M. Perrin, *J. Pept. Sci.*, 2020, **112**, e24210; (f) W. Muramatsu, T. Hattori and H. Yamamoto, *Bull. Chem. Soc. Jpn.*, 2020, **93**, 759–767; (g) W. Muramatsu, T. Hattori and H. Yamamoto, *Chem. Commun.*, 2021, **57**, 6346–6359.
- V. Declerck, P. Nun, J. Martinez and F. Lamaty, *Angew. Chem., Int. Ed.*, 2009, **48**, 9318–9321.
- (a) H. R. Kricheldorf, *Angew. Chem., Int. Ed.*, 2006, **45**, 5752–5784; (b) N. Hadjichristidis, H. Iatrou, M. Pitsikalis and G. Sakellariou, *Chem. Rev.*, 2009, **109**, 5528–5578; (c) T. J. Deming, *Adv. Polym. Sci.*, 2013, **262**, 1–37; (d) T. J. Deming, *Chem. Rev.*, 2016, **116**, 786–808; (e) Y. Zhang, R. Liu, H. Jin, W. Song, R. Augustine and I. Kim, *Commun. Chem.*, 2018, **1**, 40; (f) A. R. Mazo, S. Allison-Logan, F. Karimi, N. J.-A. Chan, W. Qiu, W. Duan, N. M. O'Brien-Simpson and G. G. Qiao, *Chem. Soc. Rev.*, 2020, **49**, 4737–4834; (g) Y. Wu, K. Chen, X. Wu, L. Liu, W. Zhang, Y. Ding, S. Liu, M. Zhou, N. Shao, Z. Ji, J. Chen, M. Zhu and R. Liu, *Angew. Chem., Int. Ed.*, 2021, **60**, 26063–26071.
- (a) F. Fuchs, *Ber. Dtsch. Chem. Ges. B*, 1922, **55**, 2943; (b) A. C. Farthing, *J. Chem. Soc.*, 1950, 3213–3217; (c) R. Katakai and Y. Iizuka, *J. Org. Chem.*, 1985, **50**, 715–716; (d) R. Wilder and S. Mobashery, *J. Org. Chem.*, 1992, **57**, 2755–2756; (e) Y. Otake, H. Nakamura and S. Fuse, *Angew. Chem., Int. Ed.*, 2018, **57**, 11389–11393.



- 13 (a) R. A. Sheldon, *Chem. Ind.*, 1992, 903–906; (b) R. A. Sheldon, *Green Chem.*, 2007, **9**, 1273–1283; (c) R. A. Sheldon, *Chem. Commun.*, 2008, **44**, 3352–3365; (d) R. A. Sheldon, *Green Chem.*, 2017, **19**, 18–43.
- 14 D. Farran, D. Echalié, J. Martínez and G. Dewynter, *J. Pept. Sci.*, 2009, **15**, 474–478.
- 15 R. Mendoza-Sanchez, V. B. Corless, Q. N. N. Nguyen, M. Bergeron-Brelek, J. Frost, S. Adachi, D. J. Tantillo and A. K. Yudin, *Chem.–Eur. J.*, 2017, **23**, 13319–13322.
- 16 We chose amino acid *tert*-butyl esters as nucleophilic components because side reactions such as the self-intermolecular condensation or intramolecular cyclisation of peptides can be avoided by the virtue of the steric hindrance from the *tert*-butyl moiety. See: (a) G. W. Anderson and F. M. Callahan, *J. Am. Chem. Soc.*, 1960, **82**, 3359–3363; (b) N. Funasaki, S. Hada and S. Neya, *Anal. Chem.*, 1993, **65**, 1861–1867.
- 17 More data for the solvent screening is shown in ESI.†
- 18 We attempted to prepare *N*-Fmoc-2-piperidone according to the reference. Unfortunately, the desired compound could not be obtained. See, A. Barco, S. Benetti, C. D. Risi, P. Marchetti, G. P. Pollini and V. Zanirato, *J. Comb. Chem.*, 2000, **2**, 337–340.
- 19 W. Muramatsu, T. Hattori and H. Yamamoto, *J. Am. Chem. Soc.*, 2019, **141**, 12288–12295.
- 20 (a) P. Forzatti and L. Lietti, *Catal. Today*, 1999, **52**, 165–181; (b) C. H. Bartholomew, *Appl. Catal., A*, 2001, **212**, 17–60.
- 21 Asn and Gln derivatives can be applied to this method in CPME as nucleophilic components. Unfortunately, the corresponding peptides were obtained in unsatisfactory yields because of their insolubility.
- 22 (a) C. L. L. Chai, D. C. R. Hockless and A. R. King, *Aust. J. Chem.*, 1996, **49**, 1229–1233; (b) A. P. Mendham, J. Spencer, B. Z. Chowdhry, T. J. Dines, M. Mujahid, R. A. Palmer, G. J. Tizzard and S. J. Coles, *J. Chem. Crystallogr.*, 2011, **41**, 1323–1327; (c) L. Lelo, V. Pace, W. Holzer, M. M. Rahman, G. Meng, R. Szostak and M. Szostak, *Chem.–Eur. J.*, 2020, **26**, 16246–16250; (d) G. Meng, J. Zhang and M. Szostak, *Chem. Rev.*, 2021, **121**, 12746–12783.
- 23 β -Amino acids, β -peptides, and α,β -hybrid peptides possess unique properties in biological and structural aspects. See, (a) E. A. Porter, X. Wang, H.-S. Lee, B. Weisblum and S. H. Gellman, *Nature*, 2000, **404**, 565; (b) O. M. Stephens, S. Kim, B. D. Welch, M. E. Hodsdon, M. S. Kay and A. Schepartz, *J. Am. Chem. Soc.*, 2005, **127**, 13126–13127; (c) T. Beke, C. Somlai and A. Perczel, *J. Comput. Chem.*, 2006, **27**, 20–38; (d) A. J. Karlsson, W. C. Pomerantz, K. J. Neilsen, S. H. Gellman and S. P. Palecek, *ACS Chem. Biol.*, 2009, **4**, 567–579; (e) F. Kudo, A. Miyanaaga and T. Eguchi, *Nat. Prod. Rep.*, 2014, **31**, 1056–1073; (f) C. Cabrele, T. A. Martinek, O. Reiser and L. Berlicki, *J. Med. Chem.*, 2014, **57**, 9718–9739.
- 24 Rotational isomers were observed in CDCl₃. On the other hand, they were rarely observed in CD₃OD. This is probably due to the effects of intramolecular hydrogen bonding.
- 25 (a) S. Osaki, *Nature*, 1996, **384**, 419; (b) E. S. Place, N. D. Evans and M. M. Stevens, *Nat. Mater.*, 2009, **8**, 457–470; (c) M. Heim, D. Keerl and T. Scheibel, *Angew. Chem., Int. Ed.*, 2009, **48**, 3584–3596; (d) A. Thathe, M. Ghodke and P. Nikalje, *Int. J. Pharm. Pharm. Sci.*, 2010, **2**, 19–23; (e) F. Teulé, Y.-G. Miao, B.-H. Sohn, Y.-S. Kim, J. J. Hull, M. J. Fraser Jr, R. V. Lewis and D. L. Jarvis, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, 923–928; (f) G. A. Knoll, S. M. Romanelli, A. M. Brown, R. M. Sortino and I. A. Banerjee, *J. Nanosci. Nanotechnol.*, 2016, **16**, 2464–2473; (g) T. Yoshioka, T. Tsubota, K. Tashiro, A. Jouraku and T. Kameda, *Nat. Commun.*, 2019, **10**, 1469; (h) A. P. Kiseleva, P. V. Krivoschapkin and E. F. Krivoschapkina, *Front. Chem.*, 2020, **8**, 554; (i) R. Balu, N. K. Dutta, A. K. Dutta and N. R. Choudhury, *Nat. Commun.*, 2021, **12**, 149.
- 26 (a) N. D. Lazo and D. T. Downing, *Macromolecules*, 1999, **32**, 4700–4705; (b) C. Z. Zhou, F. Confalonieri, N. Medina, Y. Zivanovic, C. Esnault, T. Yang, M. Jacquet, J. Janin, M. Duguet, R. Perasso and Z.-G. Li, *Nucleic Acids Res.*, 2000, **28**, 2413–2419; (c) Z. Megeed, J. Cappello and H. Ghandehari, *Adv. Drug Delivery Rev.*, 2002, **54**, 1075–1091; (d) J. G. Hardy and T. R. Scheibel, *Biochem. Soc. Trans.*, 2009, **37**, 677–681; (e) T. Asakura, H. Nishi, A. Nagano, A. Yoshida, Y. Nakazawa, M. Kamiya and M. Demura, *Biomacromolecules*, 2011, **12**, 3910–3916.
- 27 W. Muramatsu and H. Yamamoto, *J. Am. Chem. Soc.*, 2021, **143**, 6792–6797.
- 28 W. Muramatsu, C. Manthena, E. Nakashima and H. Yamamoto, *ACS Catal.*, 2020, **10**, 9594–9603.
- 29 L. Horner and J. Mathias, *J. Organomet. Chem.*, 1985, **282**, 155–174.
- 30 P. Singh, S. Kaur, J. Kaur, G. Singh and R. Bhatti, *J. Med. Chem.*, 2016, **59**, 3920–3934.
- 31 (a) H. Thajudeen, K. Park, S.-S. Moon and I. S. Hong, *Tetrahedron Lett.*, 2010, **51**, 1303–1305; (b) Nonappa, K. Ahonen, M. Lahtinen and E. Kolehmainen, *Green Chem.*, 2011, **13**, 1203–1209; (c) J.-C. M. Monbaliu, F. K. Hansen, L. K. Beagle, M. J. Panzner, P. J. Steel, E. Todadze, C. V. Stevens and A. R. Katritzky, *Chem.–Eur. J.*, 2012, **18**, 2632–2638; (d) H. Abe, C. Sakashita, M. Kawada, A. Nomoto, T. Watanabe and M. Shibasaki, *Chem. Pharm. Bull.*, 2015, **63**, 463–468; (e) A. Mollica, R. Costante, S. Mirzaie, S. Carradori, G. Macedonio, A. Stefanucci and E. Novellino, *J. Heterocycl. Chem.*, 2016, **53**, 2106–2110; (f) H. Yu, Y. Zong and T. Xu, *Chem. Sci.*, 2020, **11**, 656–660; (g) C. G. Pappas, N. K. Wijerathne, J. K. Sahoo, A. Jain, D. Kroiss, I. R. Sasselli, A. S. Pina, A. Lampel and R. V. Uljijn, *ChemSystemsChem*, 2020, **2**, e2000013.
- 32 (a) A. K. Szardenings, V. Antonenko, D. A. Campbell, N. DeFrancisco, S. Ida, L. Shi, N. Sharkov, D. Tien, Y. Wang and M. Navre, *J. Med. Chem.*, 1999, **42**, 1348–1357; (b) F. Fdhila, V. Vázquez, J. L. Sánchez and R. Riguera, *J. Nat. Prod.*, 2003, **66**, 1299–1301; (c) A. I. Faden, S. M. Knobloch, I. Cernak, L. Fan, R. Vink, G. L. Araldi, S. T. Fricke, B. L. Roth and A. P. Kozikowski, *J. Cereb. Blood Flow Metab.*, 2003, **23**, 342–354; (d) D. R. Houston,



- B. Synstad, V. G. H. Eijssink, M. J. R. Stark, I. M. Eggleston and D. M. F. van Aalten, *J. Med. Chem.*, 2004, **47**, 5713–5720; (e) B. Nicholson, G. K. Lloyd, B. R. Miller, M. A. Palladino, Y. Kiso, Y. Hayashi and S. T. C. Neuteboom, *Anti-Cancer Drugs*, 2006, **17**, 25–31; (f) M. Teixidó, E. Zurita, M. Malakoutikhah, T. Tarragó and E. Giralt, *J. Am. Chem. Soc.*, 2007, **129**, 11802–11813; (g) S. Hirano, S. Ichikawa and A. Matsuda, *Bioorg. Med. Chem.*, 2008, **16**, 428–436; (h) E. van der Merwe, D. Huang, D. Peterson, G. Kilian, P. J. Milne, M. V. de Venter and C. Frost, *Peptides*, 2008, **29**, 1305–1311; (i) R. R. King and L. A. Calhoun, *Phytochemistry*, 2009, **70**, 833–841; (j) Z. Song, Y. Hou, Q. Yang, X. Li and S. Wu, *Mar. Drugs*, 2021, **19**, 403.
- 33 (a) T. Mizuma, S. Masubuchi and S. Awazu, *J. Pharm. Pharmacol.*, 1997, **49**, 1067–1071; (b) N. Tsuruoka, Y. Beppu, H. Koda, N. Doe, H. Watanabe and K. Abe, *PLoS One*, 2012, **7**, e50824; (c) M.-K. Kwak, R. Liu, J.-O. Kwon, M.-K. Kim, A. H. Kim and S.-O. Kang, *J. Microbiol.*, 2013, **51**, 836–843; (d) A. D. Borthwick and N. C. D. Costa, *Crit. Rev. Food Sci. Nutr.*, 2017, **57**, 718–742; (e) T. Shimamura, K. Yamamoto, Y. Otsuka, M. Hayashi, T. Kashiwagi and H. Ukeda, *Food Preserv. Sci.*, 2017, **43**, 227–233.

