


 Cite this: *RSC Adv.*, 2020, 10, 29373

Stereoselective synthesis of Gly-Gly-type (*E*)-methylalkene and (*Z*)-chloroalkene dipeptide isosteres and their application to 14-mer RGG peptidomimetics†

 Hikari Okita,^{‡a} Yuna Kato,^{‡b} Tatsuki Masuzawa,^c Kosuke Arai,^b Sayuri Takeo,^a Kohei Sato,^{id ab} Nobuyuki Mase,^{id abd} Takanori Oyoshi^c and Tetsuo Narumi^{*abe}

 Received 6th July 2020
Accepted 30th July 2020

DOI: 10.1039/d0ra06554d

rsc.li/rsc-advances

Stereoselective and efficient synthesis of Gly-Gly-type (*E*)-methylalkene and (*Z*)-chloroalkene dipeptide isosteres is realized by organocuprate-mediated single electron transfer reduction. The synthetic isosteres can be used in Fmoc-based solid phase peptide synthesis, resulting in the preparation of the 14-mer RGG peptidomimetics containing an (*E*)-methylalkene or a (*Z*)-chloroalkene unit.

Glycylglycine (Gly-Gly) is the smallest dipeptide and has been synthesized by many approaches in the last 100 years.¹ Due to its versatile nature and ready availability, glycylglycine has been used as a chemical probe² and buffer³ in biochemical studies and also as a reagent which can enhance the solubility of overexpressed proteins.⁴ In addition to the utility of Gly-Gly itself, oligoglycine (oligo-Gly) motifs are notable for being more flexible and less-functionalized than any combination of other amino acids. These features are useful in bioconjugation strategies which link multiple biomolecules without interfering with the function of each biomolecule, allowing synthesis of bioconjugated artificial molecules including dimeric, multi-domain, and fusion proteins.⁵ The flexibility of oligo-Gly enables the formation of unusual secondary structures of peptides and proteins.⁶ Thus, the oligo-Gly motif can be found in the biologically important peptides and proteins such as Met/Leu-enkephalin (YGGFM and YGGFL), the C-terminus of ubiquitin (⁷⁰VLRLRGG⁷⁶), ctenidin,⁷ shepherdin I,⁸ and DNA/RNA-binding proteins with repeated RGG sequences related to the various physiological processes *via*

protein–protein and protein–nucleic acids interactions.⁹ These proteins relate with gene expression, DNA damage signal and apoptosis, however, the detail effects of Gly-Gly with steric and electronic factors to these functions are unknown. Given the importance of oligo-Gly in various fields, non-hydrolyzable peptidomimetics of oligo-Gly could be attractive building blocks for the synthesis of novel bioconjugated molecules and complex peptidomimetics with improved chemical stability and functionality. For example, even the Gly-Gly dipeptide mimic with the tetra-substituted alkene unit replacing the Gly-Gly peptide bond has been shown to promote the β -hairpin formation, and is thus the smallest peptidomimetic that is known to control a peptide structure.¹⁰ There are two reports of the synthesis of Gly-Gly-type fluoroalkene dipeptide isosteres.¹¹ However, the poor synthetic access to such molecules has hindered their application to the peptidomimetics. Our long-standing interest in the drug discovery with amide-to-alkene isosteric switching prompted this investigation into the stereoselective synthesis of Gly-Gly-type (*E*)-methylalkene and (*Z*)-chloroalkene dipeptide isosteres.

In this report, we describe the beginning of our oligo-Gly-based peptidomimetic study with the stereoselective synthesis of Gly-Gly-type (*E*)-methylalkene and (*Z*)-chloroalkene dipeptide isosteres and their use in Fmoc-based solid phase peptide synthesis (SPPS). In the first application of isosteres of this type to access complex peptidomimetics, we synthesize 14-mer RGG peptidomimetics containing (*E*)-methylalkene or (*Z*)-chloroalkene unit as surrogates for a Gly-Gly peptide bond. These two isosteres were selected because the potentials of both isosteres have not been fully uncovered, though there are several promising examples.^{12,13} Since the carbonyl oxygen equivalents of those isosteres are similar in their size¹⁴ but differ in their electronic properties,¹⁵ comparative studies of these isosteres would have the advantage of exploring

^aDepartment of Applied Chemistry and Biochemical Engineering, Faculty of Engineering, Shizuoka University, Shizuoka, 432-8561, Japan. E-mail: narumi.tetsuo@shizuoka.ac.jp

^bDepartment of Engineering, Graduate School of Integrated Science and Technology, Shizuoka University, Shizuoka, 432-8561, Japan

^cDepartment of Chemistry, Graduate School of Integrated Science and Technology, Shizuoka University, Shizuoka, 422-8529, Japan

^dGreen Energy Research Division, Research Institute of Green Science and Technology, Shizuoka University, Shizuoka, 432-8561, Japan

^eGreen Chemistry Research Division, Research Institute of Green Science and Technology, Shizuoka University, Shizuoka, 432-8561, Japan

† Electronic supplementary information (ESI) available. See DOI: 10.1039/d0ra06554d

‡ These authors contributed equally to this work.

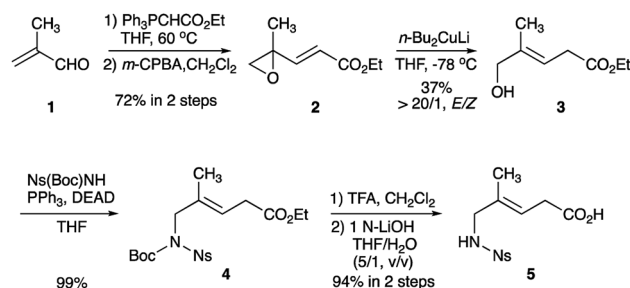


the role of peptide bonds in terms of their steric and electronic natures. This work also uncovered the unique ability of the Gly-Gly-type (*Z*)-chloroalkene isostere to induce β -turn structures in the almost unfolded peptides (Fig. 1).

The main challenges in this study include the stereoselective formation of the (*E*)-methylalkene and (*Z*)-chloroalkene moieties as the surrogates of the Gly-Gly *trans*-peptide bond, together with the control of the olefine isomerization under the condition of the isostere synthesis and Fmoc-based SPPS. There are several synthetic approaches toward tri-substituted alkene-type isosteres, including the Overman rearrangement,¹³ the S_N2' -type opening of alkenylaziridines,^{12a} Cu-mediated CF_3 -coupling of vinyl iodide,¹⁶ and cross-couplings of vinyl stannane.¹⁷ Organocuprate-mediated reactions of α,β -unsaturated carbonyl compounds with γ -leaving group(s) are also powerful methods to produce tri-substituted alkene-type isosteres, and are particularly suitable for the stereoselective synthesis of (*Z*)-fluoroalkene¹⁸ and (*Z*)-chloroalkene isosteres.¹⁹

The preparation of Gly-Gly-type alkene dipeptide isosteres was facilitated by the organocuprate-mediated SET reduction that was used in the stereoselective formation of the (*E*)-methylalkene and (*Z*)-chloroalkene moieties. Scheme 1 shows the synthesis of (*E*)-methylalkene isosteres (5). A Wittig reaction of methacrolein (1) with ethyl (triphenylphosphoranylidene) acetate followed by epoxidation with *m*-CPBA afforded the alkenyl oxirane (2) which, treated with Gillman reagent (*n*-Bu₂-CuLi), produced the allylic alcohol (3) with high *E*-selectivity. A Mitsunobu reaction of 3 with Ns(Boc)NH and deprotection provided the Gly-Gly-type (*E*)-methylalkene dipeptide isostere 5 that can be applied to the Fmoc-based SPPS. All reactions leading to the synthesis of 5 were performed on a gram scale.

Synthesis of chloroalkene isostere (14) is shown in Scheme 2. Based on previous results for the successful, efficient synthesis of Val-Xaa-type chloroalkene isosteres,^{19c} we assumed that a similar protocol would allow for the synthesis of 14. However, our attempts revealed that the Gly substrate used has different reactivity and selectivity compared to the other substrates,



Scheme 1 Synthesis of Gly-Gly-type (*E*)-methylalkene dipeptide isosteres (5).

particularly in the SET reduction step. Consequently, Gly-specific reaction conditions are necessary. The nucleophilic addition of the lithium enolate of methyl dichloroacetate to the *N*-sulfinylaldimine (7), prepared from (\pm)-*tert*-butylsulfonamide (6) and paraformaldehyde and *m*-CPBA oxidation of 6 gave the *N*-*tert*-butylsulfonyl (Bus)-protected α,α -dichloro- β -amino ester (8). Precise control of the amount of DIBAL-H at low temperatures enables the partial reduction of 8, and this is followed by a Horner–Wadsworth–Emmons reaction to produce the corresponding (*E*)-enoate (9). Initial efforts to apply our established conditions for SET reduction with Me₂CuLi identified the poor *Z*-selectivity of the reaction and also its propensity to form the α -methylated side products 11 and 12 (Table 1, entry 1). Changing the methyl groups on the copper to *n*-butyl groups decreased the formation of (*E*)-chloroalkene compounds and allowed the easy separation of 10-*Z* from side products (entry 2). Addition of Lewis bases such as HMPA, NMP, or DMSO proved to be ineffective for the reductive dechlorination step (entries 3–5). Bulky alkyl groups on copper can improve the product selectivity and favor the desired (*Z*)-chloroalkene product (10-*Z*) (entries 6–8). Although the organocuprates derived from *tert*-BuLi and *tert*-BuMgCl gave slightly better yields and selectivity (entries 7 and 8), we favoured the organocuprate derived from *sec*-BuLi due to

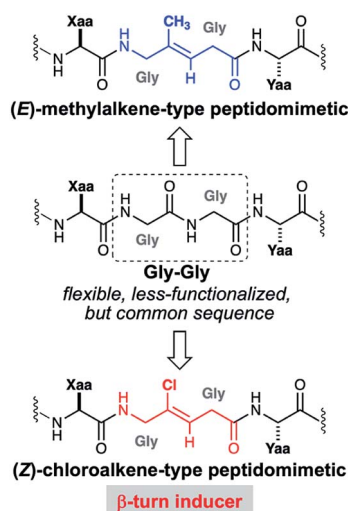
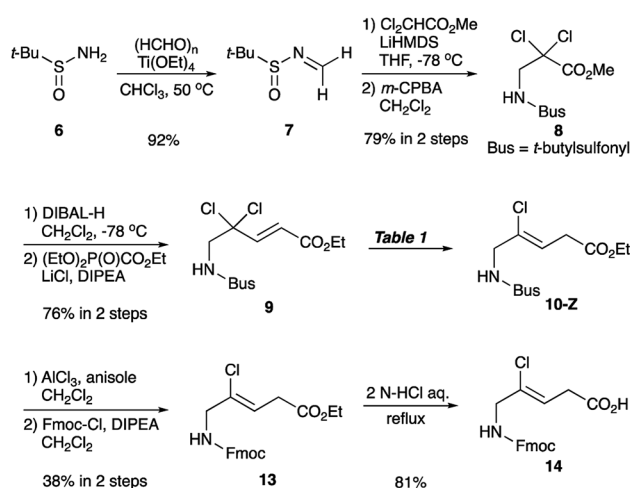
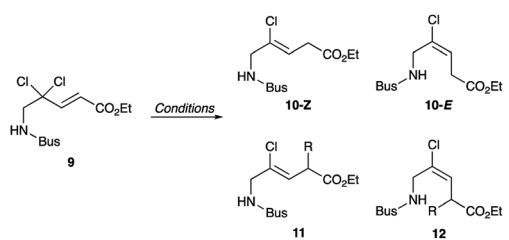


Fig. 1 Gly-Gly peptide and its alkene-type peptidomimetics.



Scheme 2 Synthesis of Gly-Gly-type (*Z*)-chloroalkene dipeptide isosteres (14).

Table 1 Reactivity of (*E*)-enoate (**9**) with organocuprates^a


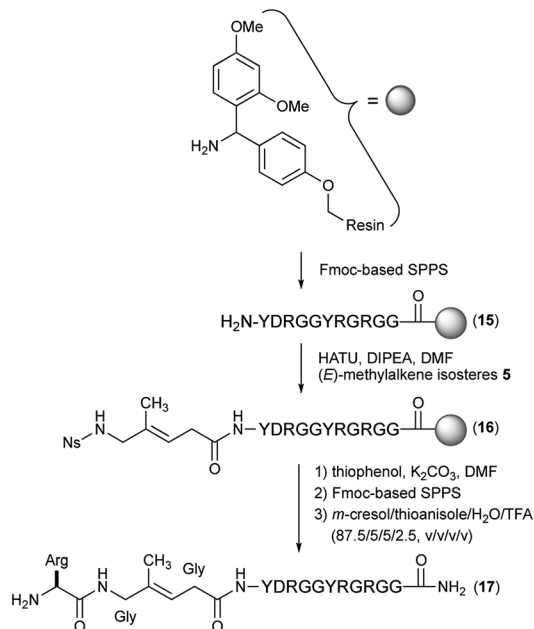
Entry	Conditions	Yield ^b (%)			
		10-Z	10-E	11	12
1	Me ₂ CuLi	48	19	15 ^c	1 ^c
2	<i>n</i> -Bu ₂ CuLi	55	0	25 ^d	20 ^d
3	<i>n</i> -Bu ₂ CuLi, HMPA	0	0	46 ^d	13 ^d
4	<i>n</i> -Bu ₂ CuLi, NMP	44	0	29 ^d	6 ^d
5	<i>n</i> -Bu ₂ CuLi, DMSO	46	7	19 ^d	2 ^d
6	<i>sec</i> -Bu ₂ CuLi	67	6	27 ^e	0
7	<i>tert</i> -Bu ₂ CuLi	74	4	2 ^f	0
8	<i>tert</i> -Bu ₂ CuMgCl	63	0	0	0

^a All reactions were carried out at $-78\text{ }^{\circ}\text{C}$ for 30 min on a 0.25 mmol scale with 4 equiv. of organocuprates in the presence of metal salts.

^b Yield is determined by ¹H NMR analysis of the crude mixture utilizing mesitylene as an internal standard. ^c R = Me. ^d R = *n*-Bu. ^e R = *sec*-Bu. ^f R = *tert*-Bu.

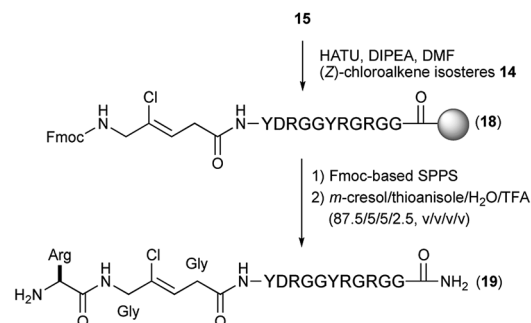
its safety and reproducibility; treatment of the enoate (**9**) with *sec*-Bu₂CuLi gave the (*Z*)-chloroalkene product (**10-Z**) in 67% yield (entry 6). Manipulations of the amine protecting group followed by acidic hydrolysis produced the Gly-Gly-type (*Z*)-chloroalkene dipeptide isostere (**14**). During the conversion of both esters **4** and **13** to the isosteres **5** and **14**, suitable for Fmoc-based SPPS, we observed no olefin isomerization products.

With these isosteres in hand, we explored their use in Fmoc-based SPPS for the preparation of peptidomimetics of the 14-mer RGG peptide derived from translocation in lipo-sarcoma/fused in sarcoma (TLS/FUS) related to the RNA processing (Schemes 3 and 4).²⁰ Starting from the Rink Amide ChemMatrix resin, standard Fmoc-based SPPS with DIC/Oxyma for peptide couplings and 20% (v/v) piperidine/DMF for Fmoc removals were performed for the construction of the peptide resin (**15**). The synthesized isosteres were incorporated into the peptide-chain by HATU/DIPEA in DMF affording the peptide resins **16** and **18**. For the synthesis of (*E*)-methylalkene-type peptidomimetic, deprotection of Ns group with thiophenol/K₂CO₃ in DMF and chain elongation followed by global deprotection with TFA/*m*-cresol/thioanisole/H₂O (87.5/5/5/2.5, v/v/v/v) provided the desired (*E*)-methylalkene-type peptidomimetic (**17**). Synthesis of the (*Z*)-chloroalkene-type peptidomimetic (**19**) was achieved using standard conditions. NMR analysis of the purified peptidomimetics revealed that although (*Z*)-chloroalkene-type peptidomimetic (**19**) can be purified solely, a trace amount of olefin isomerized compounds of **17**, possibly generated under the coupling, is observed as a side product and was difficult to remove from the desired product.²¹ Since we used a single

Scheme 3 Synthesis of Gly-Gly-type (*E*)-methylalkene-type peptidomimetic (**17**).

coupling protocol with HATU for this study, optimization for the coupling without olefin isomerization is likely to be possible.

It has been demonstrated that D-Ala-L-Ala-type (*E*)-methylalkene isostere sequence shows a higher preference for a type-II' β -turn than the corresponding (*E*)-alkene isostere.^{12a} To determine whether amide-to-alkene isosteric switching in Gly-Gly peptide bonds affects the ability of a peptide to form a β -turn structure, CD spectra were obtained for peptidomimetics (**17** and **19**) in 50 mM Tris-HCl (pH 7.5) with 100 mM KCl (Fig. 2). The native peptide (**20**) was included as the control. The CD spectra analysis of turn structures has been discussed in the literature, albeit with lower accuracies.²² Although (*E*)-methylalkene-type peptidomimetic (**17**) appears to be random coil, the spectra of **19** exhibited a minimal absorbance peak at 202 nm, which is a typical characteristic of a β -turn conformation.²⁰ On the other hand, the peptide **20** appears to form a β -turn conformation slightly. These results indicated that

Scheme 4 Synthesis of (*Z*)-chloroalkene-type peptidomimetic (**19**).

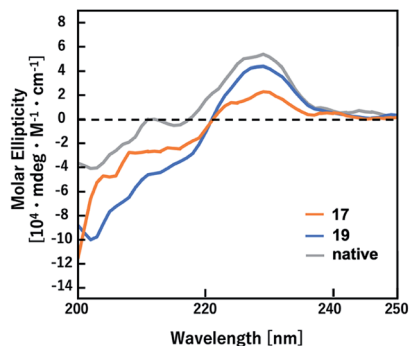


Fig. 2 CD spectra of peptidomimetics (17 and 19) and the corresponding native peptide (20).

isosteric switching of Gly-Gly peptide bond with a (*Z*)-chloroalkene unit can induce a β -turn conformation in the secondary structure of peptides and also that the β -turn inducing ability of (*Z*)-chloroalkene isosteres is superior to that of (*E*)-methylalkene isosteres. To the best of our knowledge, this is the first example of such drastic structural control effects of (*Z*)-chloroalkene isosteres on peptides. We speculated that the electronic effects of the chlorine substituent are responsible for the superior β -turn inducing ability. Efforts to determine their biological activity, including DNA/RNA-binding affinity, are currently in progress.

Conclusions

In conclusion, we have achieved the synthesis of amide-to-alkene 14-mer peptidomimetics containing (*E*)-methylalkene or (*Z*)-chloroalkene dipeptide isosteres employing a combination of a key organocuprate-mediated SET reduction and Fmoc-based SPPS. Organocuprate-mediated reduction enables the rapid construction of two-types of isostere skeletons in good yields with high *trans*-selectivity. The use of organocuprates with bulky alkyl groups was found to improve the chemical yields and the product selectivity of the reductive dechlorination for the (*Z*)-chloroalkene isostere. The esters obtained in this ways can be converted to the Ns/Fmoc-protected acids without isomerization of the olefin and can be coupled with the N-terminus of peptides on a resin, affording the 14-mer RGG peptidomimetics containing an (*E*)-methylalkene or a (*Z*)-chloroalkene unit. Also, we have identified the β -turn inducing ability of the Gly-Gly-type (*Z*)-chloroalkene isostere. Such oligo-Gly motifs are common in peptides and proteins and show unique characteristics. Thus, the synthesized Gly-Gly-type isosteres can offer new opportunities for development of enzymatically stable and structurally defined bioconjugated molecules and peptidomimetics. Continuing studies on the applications of both isosteres to the synthesis of mimics of peptides and proteins are ongoing.

Conflicts of interest

There are no conflicts to declare.

Notes and references

- 1 M. S. Dunn, A. W. Butler and T. Deakers, *J. Biol. Chem.*, 1932, **99**, 217.
- 2 For examples: (a) H. Arakawa, S. Saito, M. Kanagawa, H. Kamioka, K. Yano, K. Morimoto and T. Ogihara, *Drug Metab. Pharmacokinet.*, 2014, **29**, 470; (b) D. A. Buckingham, C. E. Davis, D. M. Foster and A. M. Sargeson, *J. Am. Chem. Soc.*, 1970, **92**, 5571; (c) E. Nakashima and A. Tsuji, *J. Pharmacobio-Dyn.*, 1985, **8**, 623.
- 3 M. E. Smith and L. B. Smith, *Biol. Bull.*, 1949, **96**, 233.
- 4 S. Ghosh, S. Rasheedi, S. S. Rahim, S. Banerjee, R. K. Choudhary, P. Chakhaiyar, N. Z. Ehtesham, S. Mukhopadhyay and S. E. Hasnain, *BioTechniques*, 2004, **37**, 418.
- 5 V. P. R. Chichili, V. Kumar and J. Sivaraman, *Protein Sci.*, 2013, **22**, 153.
- 6 K. Mohle, M. Gußmann and H. J. Hofmann, *J. Comput. Chem.*, 1997, **18**, 11141.
- 7 T. Baumann, U. Kämpfer, S. Schürch, J. Schaller, C. Largiadèr, W. Nentwig and L. Kuhn-Nentwig, *Cell. Mol. Life Sci.*, 2010, **67**, 2787.
- 8 C. Remuzgo, T. S. Oewel, S. Daffre, T. R. S. Lopes, F. H. Dyszy, S. Schreier, G. M. Machado-Santelli and M. T. Machini, *Amino Acids*, 2014, **46**, 2573.
- 9 (a) P. Thandapani, T. R. O'Connor, T. L. Bailey and S. Richard, *Mol. Cell*, 2013, **50**, 613; (b) P. A. Chong, R. M. Vernon and J. D. F. Kay, *J. Mol. Biol.*, 2018, **430**, 4650.
- 10 R. R. Gardner, G. B. Liang and S. H. Gellman, *J. Am. Chem. Soc.*, 1995, **117**, 3280.
- 11 (a) S. Sano, Y. Kuroda, K. Saito, Y. Ose and Y. Nagao, *Tetrahedron*, 2006, **62**, 11881; (b) C. Calata, E. Pfund and T. Lequeux, *Tetrahedron*, 2011, **67**, 1398.
- 12 (*E*)-Methylalkene-type: (a) P. Wipf, T. C. Henninger and S. J. Geib, *J. Org. Chem.*, 1998, **63**, 6088; (b) S. Oishi, K. Miyamoto, A. Niida, M. Yamamoto and K. Ajito, *Tetrahedron*, 2006, **62**, 1416.
- 13 (*Z*)-Chloroalkene-type: (a) R. Waelchli, R. Gamse, W. Bauer, H. Meigel, E. Lier and J. H. M. Feyen, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 1151; (b) T. Kobayakawa, Y. Matsuzaki, K. Hozumi, W. Nomura, M. Nomizu and H. Tamamura, *ACS Med. Chem. Lett.*, 2018, **9**, 6.
- 14 (a) R. W. Taft Jr, *J. Am. Chem. Soc.*, 1952, **74**, 2729; (b) R. W. Taft Jr, *J. Am. Chem. Soc.*, 1953, **75**, 4538; (c) R. W. Taft Jr, *Steric Eff. Org. Chem.*, 1956, 556.
- 15 C. Hansch, A. Leo and R. W. Taft, *Chem. Rev.*, 1991, **91**, 165.
- 16 J. Xiao, B. Weisblum and P. Wipf, *J. Am. Chem. Soc.*, 2005, **127**, 5742.
- 17 P. Wipf, J. Xiao and C. R. J. Stephenson, *Chimia*, 2009, **63**, 764.
- 18 (a) A. Otaka, H. Watanabe, E. Mitsuyama, A. Yukimasa, H. Tamamura and N. Fujii, *Tetrahedron Lett.*, 2001, **42**, 285; (b) A. Otaka, H. Watanabe, A. Yukimasa, S. Oishi, H. Tamamura and N. Fujii, *Tetrahedron Lett.*, 2001, **42**, 5443; (c) Y. Nakamura, M. Okada, A. Sato, H. Horikawa, M. Koura, A. Saito and T. Taguchi, *Tetrahedron*, 2005, **61**,

- 5741; (d) T. Narumi, A. Niida, K. Tomita, S. Oishi, A. Otaka, H. Ohno and N. Fujii, *Chem. Commun.*, 2006, 4720.
- 19 (a) T. Narumi, T. Kobayakawa, H. Aikawa, S. Seike and H. Tamamura, *Org. Lett.*, 2012, **14**, 4490; (b) T. Kobayakawa, T. Narumi and H. Tamamura, *Org. Lett.*, 2015, **17**, 2302; (c) T. Kobayakawa and H. Tamamura, *Tetrahedron*, 2016, **72**, 4968; (d) T. Kobayakawa and H. Tamamura, *Tetrahedron*, 2017, **73**, 4464.
- 20 (a) R. Yagi, T. Miyazaki and T. Oyoshi, *Nucleic Acids Res.*, 2018, **46**, 5894; (b) N. Hamad, T. Mashima, Y. Yamaoki, K. Kondo, R. Yoneda, T. Oyoshi, R. Kurokawa, T. Nagata and M. Katahira, *Sci. Rep.*, 2020, **10**, 2629; (c) K. Takahama and T. Oyoshi, *J. Am. Chem. Soc.*, 2013, **135**, 18016.
- 21 See ESI† for the details.
- 22 P. Manavalan and W. C. Johnson Jr, *Anal. Biochem.*, 1987, **167**, 76.