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# Chemoselective fragment condensation between peptide and peptidomimetic oligomers†

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**We report the first example of chemoselective fragment condensation, through native amide bond formation, between peptoid and peptide oligomers. Peptoid oligomers bearing C-terminal salicylaldehyde esters were synthesized and ligated to peptides containing N-terminal serine or threonine residues. We investigate the ligation efficiency of peptoid oligomers varying in length, sequence, and C-terminal steric bulk. These protocols enhance accessibility of structurally complex peptoid–peptide hybrids and will facilitate the design new semi-synthetic proteins with unique attributes.**

A general route for the synthesis of structurally complex sequence-specific heteropolymers capable of performing sophisticated functions, such as enzyme-like catalysis, remains a fundamental challenge. While significant attention has been devoted to total chemical synthesis of native proteins, recent advances have also established synthetic routes to generate hybrid biomacromolecules, such as proteins bearing abiotic oligomer constituents.[1] These semi-synthetic biomolecules have the potential to exhibit new modes of molecular recognition and enhanced thermodynamic stability. Previously, single-atom or small abiotic substitutions have been incorporated into native protein frameworks.[2] We now seek to establish new efficient routes to hybrids between diverse abiotic oligomers and polypeptide sequences.

Conventional linear solid-phase peptide synthesis (SPPS) protocols can routinely generate large polypeptides of sizable chain lengths (20–50 monomers). Limitations in the size and structural diversity of the synthetic polypeptide products can restrict their ability to exhibit particular functions, such as enzymatic catalysis. There are limited chemoselective synthetic routes for ligating abiotic and peptide oligomers through native amide linkages.[2] Discovering new methods for conducting chemoselective fragment condensation to incorporate abiotic constituents into protein constructs will facilitate the development of hybrid biomacromolecules that can establish unprecedented structural motifs, enhanced stability, and novel functions.

*N*-Substituted glycine oligomers, or 'peptoids,' are an important class of foldamer compounds composed of tertiary amide linkages (Fig. 1).[3] The ability to incorporate extensive chemical diversity into the peptoid side chains facilitates design strategies that allow for a wide range of applications, such as enantioselective catalysis, molecular recognition, and intracellular delivery.[4–6] Importantly, numerous studies have identified peptoid side chains that can control backbone conformational ordering, establishing the potential to fold peptoids into distinct secondary structures that are not populated by polypeptides.[7] More recently, two well-ordered peptoid secondary structure modules were assembled *via* triazole linkages to generate rudimentary peptoid tertiary structures.[8] Although this strategy permits the design of peptoids that begin to resemble small folded proteins, we seek the capability to craft functional peptoid-peptide hybrids joined through native amide bonds.

Native chemical ligation (NCL) has proven to be a valuable technique for the construction of synthetic proteins of extraordinary complexity.[9] NCL typically involves the reaction between a C-terminal peptide thioester and a peptide bearing an N-terminal cysteine residue. Following a selective transthioesterification reaction, a spontaneous S→N acyl transfer affords the native amide bond at the ligation site. Extensive developments in cysteine-based NCL have allowed a multitude

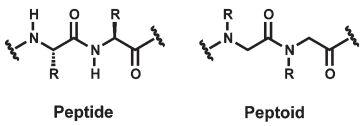


**Fig. 1** Comparison of peptide and peptoid structures.

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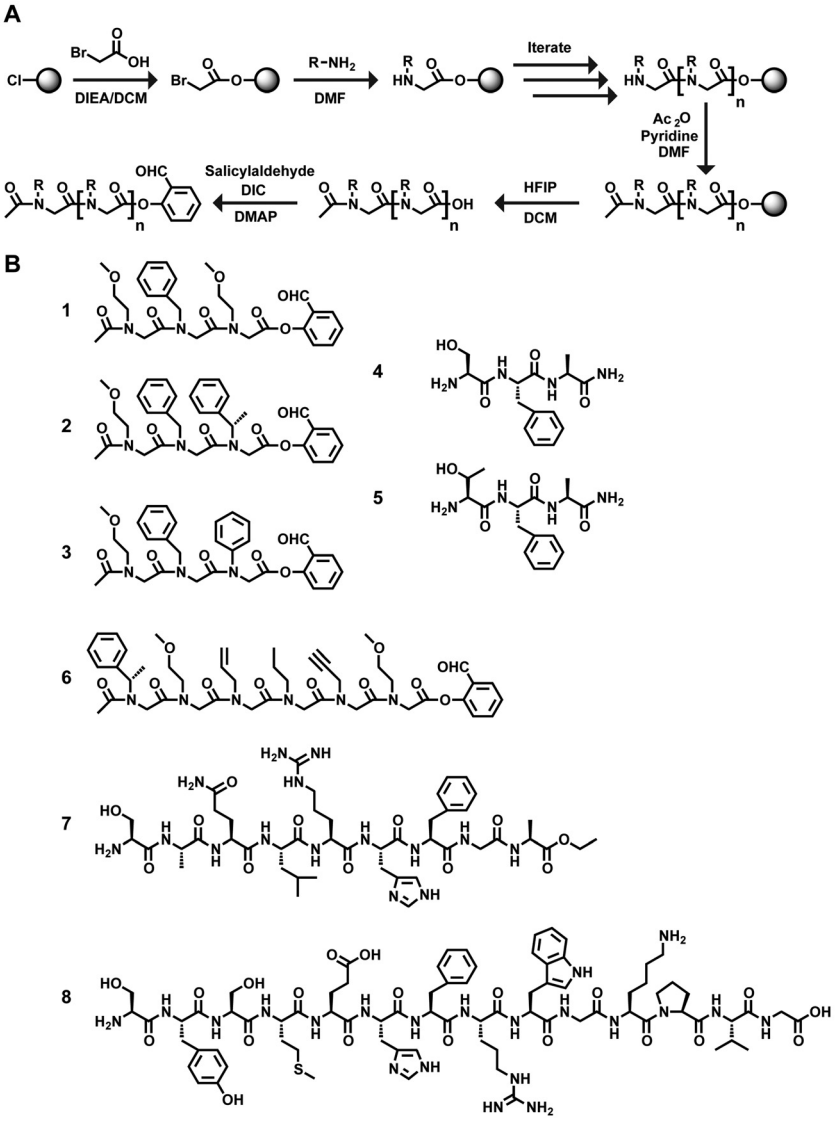



**Fig. 2** Synthesis of C-terminal peptoid salicylaldehyde esters and the library of fragments utilized in ligation studies. Iterated steps include bromoacetylation using DIC as a coupling agent, followed by displacement with a primary amine. (A) Peptoids bearing C-terminal salicylaldehyde esters are synthesized from 2-chlorotrityl resin. (B) Library of peptoid and peptide fragments utilized in chemoselective fragment condensation reactions.

of variations in this methodology, including the use of C-terminal phenyl-esters and hydrazide thioester surrogates.[10,11] However, the rare presence of cysteine (1.4% content in proteins) and challenges associated with synthesizing thioesters have limited the utility of cysteine-based NCL.[12] For this reason, new protocols have been introduced that include post-ligation desulfurization to establish native amide linkages at additional amino acid residues (*i.e.*, Arg, Gln, Val, Leu, Lys, and Pro).[13]

In order to circumvent difficulties associated with cysteine-based NCL, alternative strategies to achieve chemoselective fragment condensation, through native amide bond formation, have been introduced. These include α-ketoacid-hydroxylamine (KAHA) ligation and traceless Staudinger ligation.[14,15] Of particular interest is a chemoselective serine/threonine-based ligation that utilizes a C-terminal peptide salicylaldehyde ester. Reversible imine formation with an N-terminal serine or threonine residue produces a stable *N*,*O*-benzylidene acetal intermediate followed by irreversible amide bond formation through a 1,5 O→N acyl shift (ESI Fig. 1†). Upon deprotection, the intermediate is readily converted into a native serine/threonine linkage (12.7% content in proteins) at the ligation site.[16] Importantly, β-branched amino acids (*e.g.*, Val, Pro, and Ile) at the C-terminus do not hinder rapid coupling kinetics, a common drawback observed with cysteine-based NCL (which can require coupling times exceeding 48 h). Importantly, the chemoselective serine/threonine-based ligation reaction is orthogonal to cysteine-based NCL and has been utilized for total protein chemical synthesis.[16]

Although initial synthetic strategies for peptoid ligation have been reported, such as disulfide bond formation and Cu-catalyzed azide–alkyne [3 + 2] cycloaddition (CuAAC) 'click'

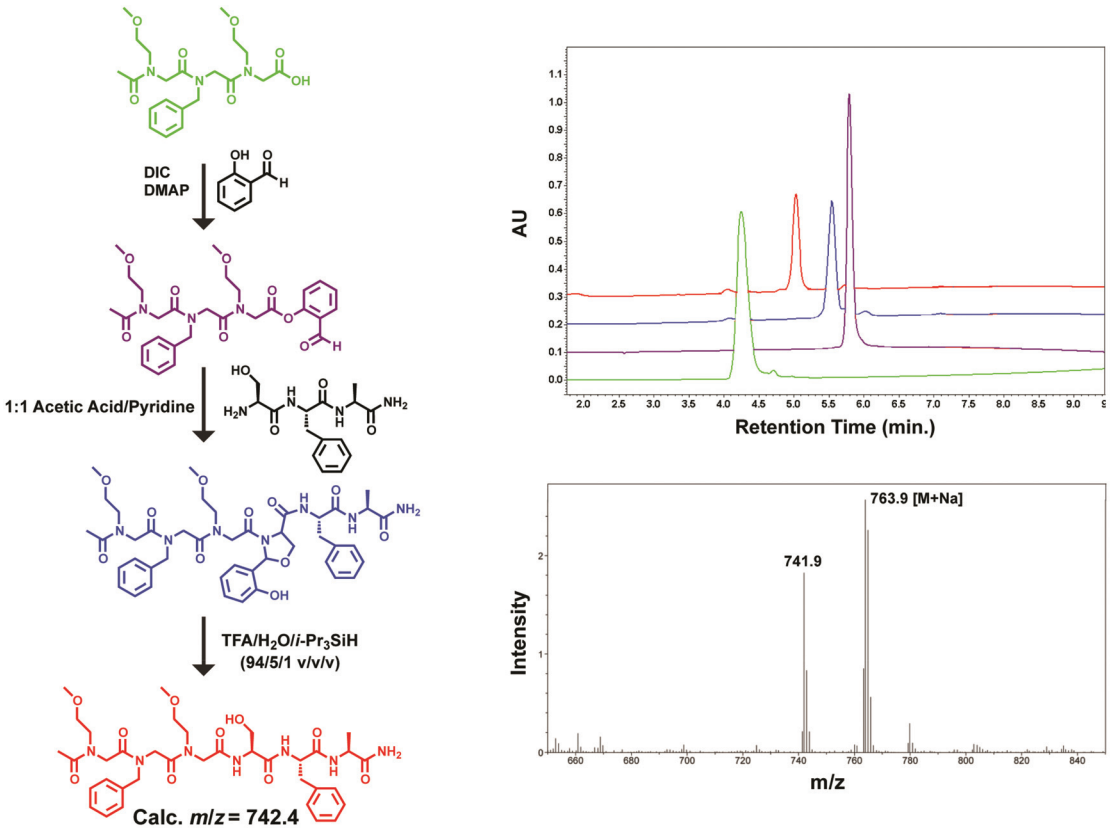



**Fig. 3** Fragment condensation between C-terminal peptoid salicylaldehyde ester **1** and peptide **4**. Upper traces of peptoid **1** (purple), intermediate (blue), and product containing native serine (red) are offset in *y*-direction for clarity. Reaction monitored by HPLC (214 nm) and LCMS. AU indicates absorbance units.

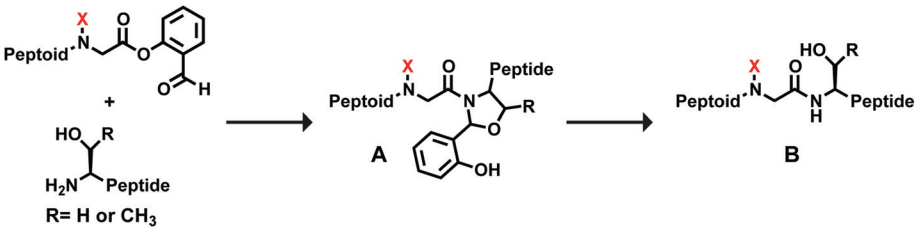
reactions, none have provided native amide bond formation.[17,18] Protease-mediated ligation of peptoid oligomers has yielded sequence-defined macromolecular polymers, but of heterogeneous chain lengths.[19] Recently, cysteine-based NCL was attempted to develop peptoid–peptide hybrids of bovine pancreatic ribonuclease A.[20] This study included attempts to synthesize peptoid C-terminal thioesters, which were unsuccessful using either MBHA or hydrazinobenzoyl resin due to incompatibilities with standard peptoid synthesis protocols (*e.g.*, prolonged exposure to primary amines or bromoacetic acid). Instead, the authors successfully utilized the thiazolidine ligation strategy (which does not form native amide bonds) to generate semi-synthetic mimics of bovine pancreatic ribonuclease A and assess their catalytic activity.

Due to the challenges associated with NCL between peptoid and peptide fragments (*vide supra*), we decided to investigate the applicability of serine/threonine-based NCL to achieve chemoselective condensation using small peptoid and peptide fragments. Employing modified solid-phase peptoid synthesis protocols, we synthesized linear peptoid oligomers containing C-terminal free acids from 2-chlorotrityl resin (Fig. 2A).[19] Following C-terminal phenolysis conversion in solution and purification, a small library of peptoid salicylaldehyde esters were evaluated for ligation efficiency utilizing serine/threonine-based NCL with a small set of peptide fragments (Fig. 2B). Beginning with a simple trimer model system, we coupled peptoid trimer **1** to tripeptide **4**, forming the corresponding *N*,*O*-benzylidene acetal intermediate. This reaction was monitored by HPLC and LCMS, which established that coupling was complete after 1 hour (Fig. 3 and ESI Table 2†). Upon removal of the acetal group under acidic conditions, the intermediate was rapidly and quantitatively converted to the native amide product.

Because C-terminal β-branched amino acids commonly hinder peptide coupling in cysteine-based NCL reactions, we evaluated ligation efficiency of peptoid salicylaldehyde esters that include steric bulk at the C-terminus (Table 1, entries 1–4). Ligation reactions were conducted between N-terminal serine/threonine peptide fragments and peptoid oligomers incorporating *N*-alkyl or *N*-aryl side chains at their C-termini (Table 1). The presence of the bulky C-terminal peptoid side-chains does not abrogate rapid coupling kinetics, as these reactions were conducted with ~99% conversion after 1 h. These *N*-alkyl or *N*-aryl peptoid side-chains can promote polyproline I- or polyproline II-type helix formation, respectively, establishing the ability to generate conformationally ordered architectures, including secondary structure motifs that may be of marginal stability for polypeptides.[21,22]

**Table 1** Ligation efficiency of peptoid salicylaldehyde esters displaying steric bulk at the C-terminus with peptides containing N-terminal serine or threonine residues




| Entry | Peptoid | Peptide | **A**[a] (%) | **B**[b] (%) | **B** calc. *m*/*z* | **B** obs. *m*/*z* |
|---|---|---|---|---|---|---|
| 1 | **2** | **4** | 99 | 99 | 787.4 | 787.9 |
| 2 | **2** | **5** | 99 | 99 | 801.4 | 801.7 |
| 3 | **3** | **4** | 99 | 99 | 759.4 | 759.9 |
| 4 | **3** | **5** | 99 | 99 | 773.4 | 773.9 |

[a] Reaction conversion after 1 h. The conversion was monitored by HPLC and was calculated based on the consumption of the salicylaldehyde. [b] Deprotection conversion after 10 min. The conversion was monitored by HPLC and was calculated based on the consumption of the *N*,*O*-benzylidene acetal intermediate. X denotes an (*S*)-*N*-1-phenylethyl glycine (peptoid **2**) or an *N*-aryl moiety (peptoid **3**).

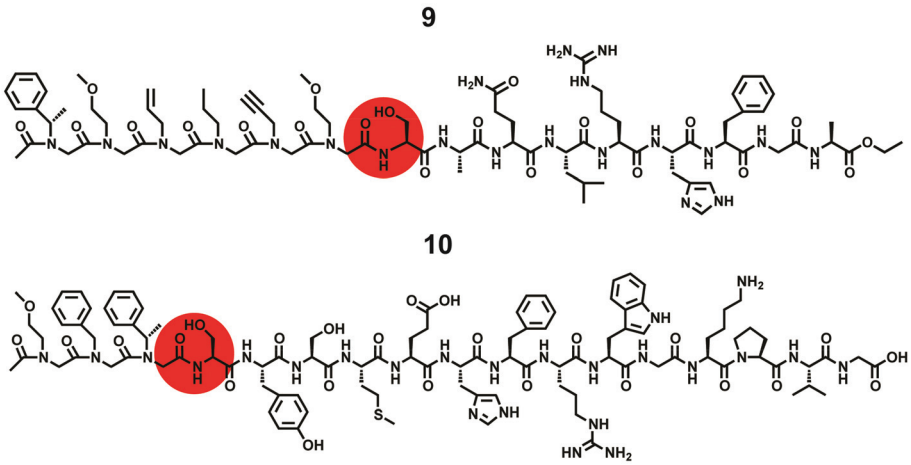



**Fig. 4** Chemical structures of peptoid–peptide hybrids **9** (ligation product between peptoid **6** and peptide **7**) and **10** (ligation product between peptoid **2** and peptide **8**). Red circle indicates native amide linkage formed by the ligation reaction.

In order to demonstrate that peptoid oligomers, including diverse side chain functional groups, can be ligated using unprotected peptide fragments, coupling was conducted with sequence-diverse peptoid **6** and peptide **7**. Electrospray ionization mass spectrometry confirmed the initial formation of the *N*,*O*-benzylidene acetal ligation intermediate (calc. *m*/*z*: 1842.0; obs. *m*/*z*: 1842.0) and the peptoid–peptide hybrid product **9** (Fig. 4, calc. *m*/*z*: 1738.9; obs. *m*/*z*: 1738.8, see ESI Fig. 2†). In addition, peptide **8**, which incorporates numerous reactive side-chains (*i.e.*, Lys, Glu, Tyr, and Ser) was successfully coupled to peptoid **2**, affording the desired ligated product **10** (Fig. 4, calc. *m*/*z*: 2145.0; obs. *m*/*z*: 2144.9). Based on previous studies, the expectation is that cysteine is not compatible with this ligation method.[16] The ability to conduct fragment condensation with peptoids bearing diverse side chains will further assist in the development of structurally complex sequence-specific heteropolymers capable of performing sophisticated protein-like functions.

## Conclusions

In this report, we describe a facile chemoselective synthetic route to establish native amide bond formation between peptoid and peptide oligomers. Fragment condensation was rapid, including peptoids containing β-branched side-chains at their C-termini, and the intermediates were quantitatively converted to native amide products. In addition, unprotected oligomer fragments varying in length and sequence were efficiently coupled, establishing the ability to generate highly complex peptoid–peptide hybrids. We anticipate this method will be used to synthesize macromolecules that integrate

structural and functional attributes from both polypeptides and peptoids.

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## References

1 U. Arnold, M. P. Hinderaker, B. L. Nilsson, B. L. Huck, S. H. Gellman and R. T. Raines, *J. Am. Chem. Soc.*, 2002, **124**, 8522.

2 (*a*) S. Batjargal, Y. J. Yang, J. M. Goldberg, R. F. Wissner and E. J. Petersson, *J. Am. Chem. Soc.*, 2012, **134**, 9172; (*b*) U. Arnold, M. P. Hinderaker, J. Köditz, R. Ulbrich-Hofmann and R. T. Raines, *J. Am. Chem. Soc.*, 2003, **125**, 1700; (*c*) A. Tam, U. Arnold, M. P. Hinderaker and R. T. Raines, *J. Am. Chem. Soc.*, 2007, **129**, 12670; (*d*) X. Lu, S. K. Olsen, A. D. Capili, J. S. Cisar, C. D. Lima and D. S. Tan, *J. Am. Chem. Soc.*, 2010, **132**, 1748; (*e*) A. A. Fuller, D. Du, F. Liu, J. E. Davoren, G. Bhabha, G. Kroon, D. A. Case, H. J. Dyson, E. T. Powers, P. Wipf, M. Gruebele and J. W. Kelly, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 11067; (*f*) I. E. Valverde, F. Lecaille, G. Lalmanach, V. Aucagne and A. F. Delmas, *Angew. Chem., Int. Ed.*, 2012, **51**, 718; (*g*) R. David, R. Günther, L. Baumann, T. Lühmann, D. Seebach, H.-J. Hofmann and A. G. Beck-Sickinger, *J. Am. Chem. Soc.*, 2008, **130**, 15311; (*h*) B. R. Green, P. Catlin, M. M. Zhang, B. Fiedler, W. Bayudan, A. Morrison, R. S. Norton, B. J. Smith, D. Yoshikami, B. M. Olivera and G. Bulaj, *Chem. Biol.*, 2007, **14**, 399.

3 R. N. Zuckermann, J. M. Kerr, S. B. H. Kent and W. H. Moos, *J. Am. Chem. Soc.*, 1992, **114**, 10646.

4 G. Maayan, M. D. Ward and K. Kirshenbaum, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, **106**, 13679.

5 D. G. Udugamasooriya, S. P. Dineen, R. A. Brekken and T. Kodadek, *J. Am. Chem. Soc.*, 2008, **130**, 5744.

6 J. E. Murphy, T. Uno, J. D. Hamer, F. E. Cohen, V. Dwarki and R. N. Zuckermann, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 1517.

7 (*a*) B. Yoo and K. Kirshenbaum, *Curr. Opin. Chem. Biol.*, 2008, **12**, 714; (*b*) J. R. Stringer, J. A. Crapster, I. A. Guzei and H. E. Blackwell, *J. Am. Chem. Soc.*, 2011, **133**, 15559.

8 S. B. L. Vollrath, S. Bräse and K. Kirshenbaum, *Chem. Sci.*, 2012, **3**, 2726.

9 (*a*) P. Dawson, T. Muir, I. Clark-Lewis and S. Kent, *Science*, 1994, **266**, 776; (*b*) J. M. Monbaliu and A. R. Katritzky, *Chem. Commun.*, 2012, **48**, 11601.

10 (*a*) G.-M. Fang, H.-K. Cui, J.-S. Zheng and L. Liu, *ChemBioChem*, 2010, **11**, 1061; (*b*) Q. Wan, J. Chen, Y. Yuan and S. J. Danishefsky, *J. Am. Chem. Soc.*, 2008, **130**, 15814.

11 G.-M. Fang, Y.-M. Li, F. Shen, Y.-C. Huang, J.-B. Li, Y. Lin, H.-K. Cui and L. Liu, *Angew. Chem., Int. Ed.*, 2011, **50**, 7645.

12 J. B. Blanco-Canosa and P. E. Dawson, *Angew. Chem., Int. Ed.*, 2008, **47**, 6851.

13 (*a*) L. R. Malins, K. M. Cergol and R. J. Payne, *ChemBioChem*, 2013, **14**, 559; (*b*) S. D. Townsend, Z. Tan, S. Dong, S. Shang, J. A. Brailsford and S. J. Danishefsky, *J. Am. Chem. Soc.*, 2012, **134**, 3912; (*c*) P. Siman, S. V. Karthikeyan and A. Brik, *Org. Lett.*, 2012, **14**, 1520; (*d*) Z. Tan, S. Shang and S. J. Danishefsky, *Angew. Chem., Int. Ed.*, 2010, **49**, 9500; (*e*) Z. Harpaz, P. Siman, K. S. A. Kumar and A. Brik, *ChemBioChem*, 2010, **11**, 1232; (*f*) R. L. Yang, K. K. Pasunooti, F. P. Li, X. W. Liu and C. F. J. Liu, *J. Am. Chem. Soc.*, 2009, **131**, 13592; (*g*) K. S. A. Kumar, M. Haj-Yahya, D. Olschewski, H. A. Lashuel and A. Brik, *Angew. Chem., Int. Ed.*, 2009, **48**, 8090; (*h*) J. Chen, Q. Wan, Y. Yuan, J. Zhu and S. J. Danishefsky, *Angew. Chem., Int. Ed.*, 2008, **47**, 8521; (*i*) C. Hasse, H. Rohde and O. Seitz, *Angew. Chem., Int. Ed.*, 2008, **47**, 6807; (*j*) Q. Wan and S. J. Danishefsky, *Angew. Chem., Int. Ed.*, 2007, **46**, 9248.

14 J. W. Bode, R. M. Fox and K. D. Baucom, *Angew. Chem., Int. Ed.*, 2006, **45**, 1248.

15 (*a*) E. Saxon, J. I. Armstrong and C. R. Bertozzi, *Org. Lett.*, 2000, **2**, 2141; (*b*) B. L. Nilsson, R. J. Hondal, M. B. Soellner and R. T. Raines, *J. Am. Chem. Soc.*, 2003, **125**, 5268.

16 (*a*) X. Li, H. Y. Lam, Y. Zhang and C. K. Chan, *Org. Lett.*, 2010, **12**, 1724; (*b*) Y. Zhang, C. Xu, H. Y. Lam, C. L. Lee and X. Li, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, 6657.

17 T. Horn, B.-C. Lee, K. A. Dill and R. N. Zuckermann, *Bioconjugate Chem.*, 2004, **15**, 428.

18 H. K. Murnen, A. R. Khokhlov, P. G. Khalatur, R. A. Segalman and R. N. Zuckermann, *Macromolecules*, 2012, **45**, 5229.

19 B. Yoo and K. Kirshenbaum, *J. Am. Chem. Soc.*, 2005, **127**, 17132.

20 B.-C. Lee and R. N. Zuckermann, *ACS Chem. Biol.*, 2011, **6**, 1367.

21 N. H. Shah, G. L. Butterfoss, K. Nguyen, B. Yoo, R. Bonneau, D. L. Rabenstein and K. Kirshenbaum, *J. Am. Chem. Soc.*, 2008, **130**, 16622.

22 K. Kirshenbaum, A. E. Barron, R. A. Goldsmith, P. Armand, E. K. Bradley, K. T. V. Truong, K. A. Dill, F. E. Cohen and R. N. Zuckermann, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 4303.

 