EDGE ARTICLE

CrossMark
← click for updates

Large ring-forming alkylations provide facile access to composite macrocycles†

Tristan E. Rose, Kenneth V. Lawson and Patrick. G. Harran*

Macrocyclic compounds have potential to enable drug discovery for protein targets with extended, solvent-exposed binding sites. Crystallographic structures of peptides bound at such sites show strong surface complementarity and frequent aromatic side-chain contacts. In an effort to capture these features in stabilized small molecules, we describe a method to convert linear peptides into constrained macrocycles based upon their aromatic content. Designed templates initiate the venerable Friedel–Crafts alkylation to form large rings efficiently at room temperature – routinely within minutes – and unimpeded by polar functional groups. No protecting groups, metals, or air-free techniques are required. Regiochemistry can be tuned electronically to explore diverse macrocycle connectivities. Templates with additional reaction capabilities can further manipulate macrocycle structure. The chemistry lays a foundation to extend studies of how the size, shape and constitution of peptidyl macrocycles correlate with their pharmacological properties.

Macrocyclic small molecules have potential to expand the repertoire of lead compounds for drug discovery, and have special utility for investigating challenging biological targets.[1] Large ring structures can scaffold extended pharmacophores and typically have improved pharmacological properties relative to linear counterparts.[2] Macrocyclic natural products have a longstanding history in medicine, and an increasing number of *de novo* designed macrocycles are being advocated for development,[3] including thirteen that have recently entered into clinical trials.[4] This trend should accelerate as pharmaceutical research delves further into control of biological processes mediated at protein surfaces.[5]

Peptide-derived macrocycles are of particular interest because their composition can logically reflect a consensus binding sequence.[6] Nonetheless, transforming short peptides into probe molecules and therapeutics is a perennial challenge. High-resolution structures, mutagenesis, and computational studies of peptide–protein interaction surfaces have identified features that drive binding, and which may be targeted by small molecules. Despite relatively large contact areas and variable secondary structure elements involved, binding energy is often localized to so-called hot-spots.[7] Aromatic amino acid side chains are prevalent and contribute significantly to binding and recognition at these sites.[8] Peptide–protein interfaces are also characterized by tight complementarity of surface shape and charge.[9] In an effort to capture these features in stabilized small molecules, we have sought methods to convert linear peptides into constrained macrocycles based upon their aromatic content.

Recent developments in peptide cyclization methods have improved generality and efficiency over conventional lactamizations. Intramolecular azide–alkyne cycloadditions,[10] olefin metatheses[11,10b] and cysteine-based ligations[12] have been widely studied in this regard, and used successfully to prepare combinatorial libraries and to stabilize α-helices[13] and β-strands.[14] These techniques are valuable, yet often require substrate tailoring and reaction optimization in order to prepare variants of the macrocyclic core. DNA-templated macrocyclizations[15] and genetically encoded biosynthetic methods,[16] have also been reported. An alternative approach involves late-stage synthetic incorporation of templates that induce cyclization by bridging native functional groups.[17] This allows macrocycle shape, size and character to be influenced by template structure. It also opens the possibility for multi-step sequences wherein the template modifies the oligomer more substantially and in increments.[18]

Initially, we designed templates to form macrocycles by reacting with polar functional groups in the peptide.[19] Palladium-catalyzed activation of a cinnamyl carbonate in the template gave internal linkages to phenols, imidazoles, amines, anilines and carboxylic acids.[20] In the case of tyrosine *O*-allylations, the ether products would ring contract when treated with acid.[18] This gave stable *C–C* linked rings through electrophilic aromatic substitution. The macrocyclic ether derived from model peptide Trp-Trp-Tyr yielded mainly the product of alkylation *ortho* to the phenol after acid treatment.[21]

Department of Chemistry and Biochemistry, University of California Los Angeles, 607 Charles E. Young Drive East, Los Angeles, USA. E-mail: harran@chem.ucla.edu

† Electronic supplementary information (ESI) available: Fig. S1–S4, list of macrocycles prepared, experimental procedures, and spectroscopic data. CCDC 1023942. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c4sc03848g
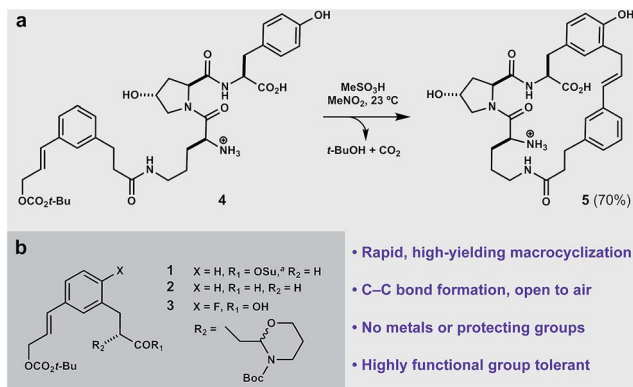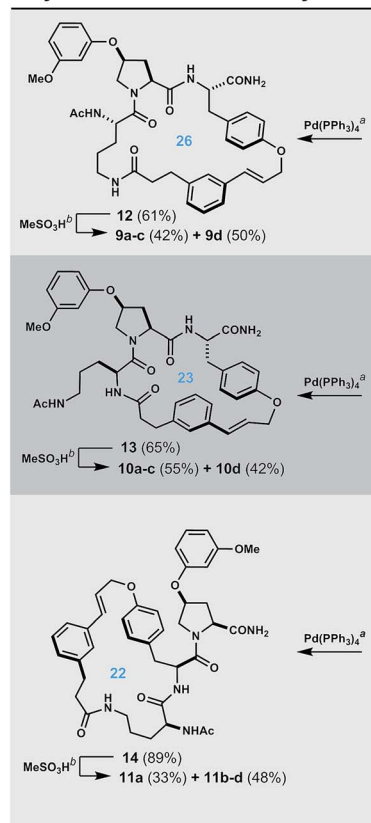
Fig. 1 Unprotected peptides acylated with a cinnamyl carbonate-containing template readily macrocyclize in acidic media. (a) Large ring-forming Friedel–Crafts alkylation is insensitive to polar protic functional groups. (b) Template structures used in this work. ^{a}75 mM MeSO$_3$H in MeNO$_2$, 5 mM in **4**, for 2 h. ^{b}Su = *N*-succinimidyl.

residues. Indole and phenol alkylations, historically a nuisance in peptide synthesis, had created new large ring systems. Ring closures had occurred at non-polar aryl side chains such as those found at hot-spots.

Despite being one of the oldest and most studied organic reactions, Friedel–Crafts alkylation has seldom been used in complex settings and rarely to form large rings.[22] However, the reaction shown in Fig. 1a made clear its potential to do so. Acylation of Orn-Hyp-Tyr with template **2** provided isolable intermediate **4**. Treatment of this compound with methanesulfonic acid in nitromethane gave composite macrocycle **5** in 70% yield. Ionization of the mixed carbonate caused selective internal substitution *ortho* to the tyrosyl phenol. The free primary amine, the carboxylic acid, and the secondary alcohol were unaffected. No metals, ligands, protecting groups or air-free techniques were required, and ring closure occurred rapidly at room temperature. The simplicity and utility of the reaction prompted us to; (1) survey reaction efficiency, electronic tunability, and kinetics for a variety of ring constitutions and arene nucleophiles, (2) compare reaction trajectories of direct macrocyclizations to the isomerization of cognate cinnamyl tyrosyl ethers, and (3) evaluate the cinnamyl carbocation

Interestingly, when the acyclic carbonate precursor to the same cyclic ether was treated with acid, we obtained macrocycles directly. These resulted from competing alkylations at all three
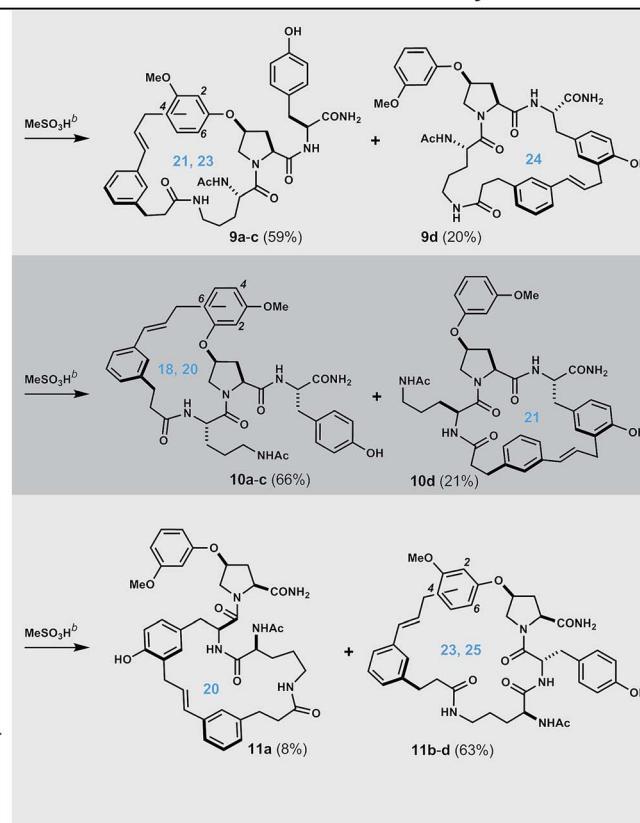

Fig. 2 Cyclized product distributions track arene electronics and are responsive to conformational pre-organization. Linear peptidic precursors **6–8** undergo facile large ring-forming Friedel–Crafts alkylations, or tyrosine *O*-alkylation when treated with protic acid or Pd^0, respectively. Acidolysis of conformationally pre-organized macrocyclic cinnamyl tyrosyl ethers **12–14** favors intra-side-chain O → C_{ortho} isomerization in comparison to direct ring-forming reactions. ^{a}10 mol% Pd(PPh$_3$)$_4$, DMF, 5 mM in substrate, 1–2 h, 23 °C. ^{b}75 mM MeSO$_3$H in MeNO$_2$, 5 mM in substrate, 2 h, 23 °C.

as one module in a multi-functional template able to transform linear peptides into polycyclic products.

To further probe Friedel–Crafts alkylations within **4**, related macrocyclizations were examined in three isomeric settings. In each of these, the secondary hydroxyl group was replaced with a *meta*-methoxyphenoxy group in order to assess relative rates of competing internal alkylations. Substrates **6** and **7** (Fig. 2) varied in their connectivity to template **1**, whereas compound **8** reversed the order of the aryloxyproline and tyrosine residues relative to **6**. Acidolyses of **6–8** yielded twelve distinct macrocyclic isomers comprising 18, 20, 21, 22, 23, 24 and 25-membered rings (**9–11a-d**, as shown in Fig. 2). No *meta*-alkylation relative to electron donating groups, formation of branched α-phenylallyl products, or dimerization was observed. Whereas Friedel–Crafts reactions can be reversible, no equilibration was detected when isolated macrocycles **9–11a-d** were independently re-subjected to the reaction conditions. Linear substrates **6**, **7**, and **8** were converted to macrocyclic cinnamyl tyrosyl ethers **12**, **13** and **14**, respectively, by treatment with catalytic amounts of Pd(PPh$_3$)$_4$. Acidolyses of these cyclic ethers afforded the same twelve *C–C*-linked products, also in good yield, but in different ratios than from linear substrates **6–8**. These data further implied products were formed kinetically, and showed that conformational pre-organization as tyrosyl ethers can alter the trajectory of the intramolecular alkylation reaction.

The change in kinetic product ratio implies that cyclic tyrosyl ethers conformationally bias the incipient cinnamyl carbocation towards the transition state leading to tyrosine alkylation. Results indicate a path-dependence consistent with kinetic quenching, rather than Curtin–Hammett kinetics.[23] Presumably, a low barrier to *C–C* bond formation leads to alkylation at a comparable or faster rate than conformational relaxation. Competing solvolysis or ion pair return would be expected to permit relaxation of the quasi-macrocyclic conformation biased towards tyrosine alkylation. These side reactions appeared minimal under these conditions (*vide infra*). Notably, the ring expansion from tyrosyl ether **14** to products **11b-d** was also possible, whereas **9a-c** and **10a-c** demonstrated ring contractions. These data suggest that ring closure rate, while sensitive to substrate conformation, is governed primarily by arene reactivity, and not by ring size. This was born out in a series of desmethoxy analogs of **6** incorporating an aliphatic spacer between *cis*-4-phenoxy-L-proline and tyrosine (Fig. S1†). Consistent with kinetic studies reported for ring-forming Friedel–Crafts acylations,[24] we observed little change in product distribution as the spacer was lengthened from four to twelve atoms, yielding 28- and 36-membered rings **S4b** and **S6b**, respectively. In conjunction with the reactivity observed for isomeric substrates **6–8**, these data anticipate cyclization of extended peptides of diverse sequence will be possible, and that a turn-inducing motif is not required.

Nine additional derivatives of substrate **6** were prepared to survey electronic requirements and reaction regioselectivity. In the absence of a competing arene, ring closure occurred selectively at tyrosine (entry 1, Table 1). The desmethoxy analog of **6** (entry 2) showed high selectivity for *para*-alkylation of the phenoxy group on proline (site III, Fig. 3) and comparable reactivity
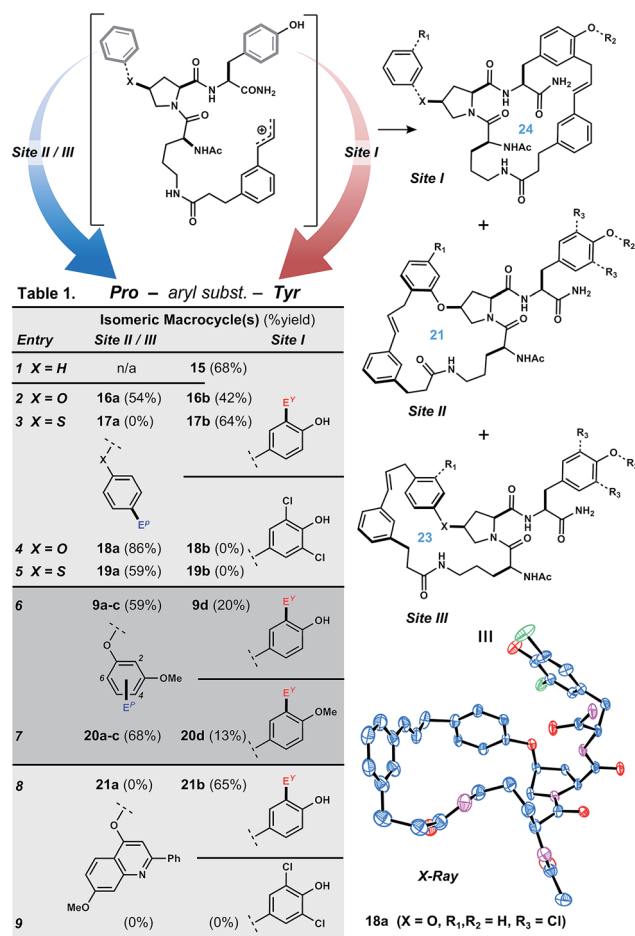

Table 1. Pro – aryl subst. – Tyr

Entry	Isomeric Macrocycle(s) (%yield) Site II / III	Site I
1 X = H	n/a	**15** (68%)
2 X = O	**16a** (54%)	**16b** (42%)
3 X = S	**17a** (0%)	**17b** (64%)
4 X = O	**18a** (86%)	**18b** (0%)
5 X = S	**19a** (59%)	**19b** (0%)
6	**9a-c** (59%)	**9d** (20%)
7	**20a-c** (68%)	**20d** (13%)
8	**21a** (0%)	**21b** (65%)
9	(0%)	(0%)

Fig. 3 Nucleophile survey within the scaffold Ac-Orn(**1**)-Pro(X-Ar)-Tyr. Macrocyclization can be tuned for selective or divergent outcome using substituent effects or blocking groups. Alkylation at sites I, II and III accesses 24, 21 and 23-membered rings, respectively (ring size indicated in blue; E^P = aryloxyprolyl linkage, E^Y = tyrosyl linkage).

between this ring and tyrosine (site I). Dichlorination of the tyrosyl phenol also led to selective formation of the site III core yielding **18a**. X-ray crystallographic analysis of this molecule confirmed the structure assigned from NMR correlation spectra (Fig. 3). Compound **23**, the macrocyclic core of **16a** and **18a** (site III), was selectively prepared on gram scale in 84% yield from truncated dipeptidyl substrate **22** (Fig. 4). 4-(Phenylthio)proline did not compete with tyrosine for alkylation (entry 3, Table 1), but underwent *para*-alkylation in good yield when tyrosine alkylation was blocked by dichlorination (entry 5). Lack of a suitably reactive arene led to intractables over a several hour period (entry 9). Conversely, substituting tyrosine in substrate **6** with electron rich (4-hydroxy-2-methoxyphenyl)alanine enhanced the rate of reaction at this side chain, as expected. Unanticipated, however, was the production of two branched diastereomeric α-phenylallyl products in this case. These potentially derive from rotamer preferences in the side chain, transient *O*-alkylation and Claisen rearrangement,[25] or a combination thereof (Fig. S2†). Large ring-forming alkylations to give products **15–21** show that cyclization typically gives
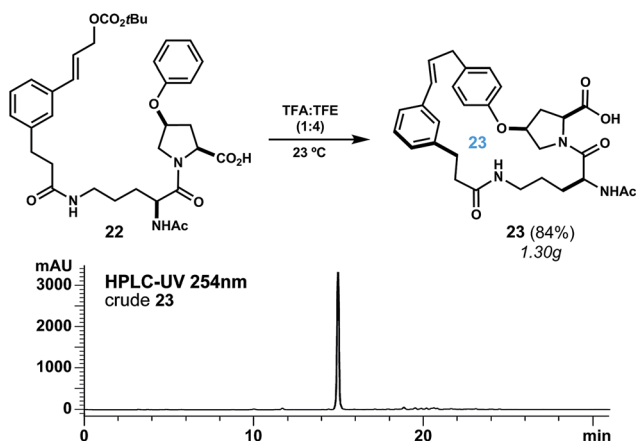
Fig. 4 Selective, gram scale synthesis of the 23-membered ring **23**, the macrocyclic core of compounds **16a** and **18a**. HPLC-UV analysis (C18, 4.6 × 250 mm 5μ, 30 → 60% ACN + 0.1% TFA, 1 mL min^{-1}) following concentration of the crude reaction mixture. TFE = 2,2,2-trifluoroethanol.

unbranched cinnamylated products, and that regioselectivity can be tuned with substituent effects about the arene nucleophile.[26]

Macrocyclizations mediated by **1** are rendered selective when arene symmetry or substrate geometry dictates a single outcome. Given current interest in macrocyclic hepatitis C virus NS3/4a protease inhibitors, we sought to employ our methodology to selectively prepare a structural analog of the clinical candidate vaniprevir.[27] Linear precursor **24** (Fig. 5a) containing a 4-methoxyisoindoline was readily prepared, and cyclized to **25** under optimized conditions using TFA : TFE (1 : 1) as the reaction medium. As was observed with isomerization of cyclic tyrosyl ethers, ring closure proceeded faster than solvolysis and ion pair return. Time course HPLC analysis indicated greater than 75% conversion to product within 5 seconds, and concomitant formation of trace products corresponding to the cinnamyl alcohol, its 2,2,2-trifluoroethyl ether, and its trifluoroacetate. These reacted further, but at slower rates, yielding the 21-membered ring **25** as the sole product within 1 hour (Fig. S3†). Hydrogenation followed by condensation with **S31** completed the synthesis of **26**. This analog differs from vaniprevir by one ring atom, the constitution of the bridging hydrocarbon, and the methoxy substituent. Though less potent than vaniprevir itself, compound **26** was active against wild type NS3 in cellular HCV replicon assays of genotypes 1a and 1b with EC$_{50}$ = 1.2 μM and 1.9 μM, respectively. To address whether the regioselectivity observed in reactions of **24** was inherent to this heterocycle, we prepared an analogous 4-methoxyisoindoline carbamate within the *cis*-hydroxyproline framework of **6** (Fig. S4†). In this case, products were obtained from alkylation at both the 7- and 5-positions of the isoindoline to give 24- and 25-membered rings, respectively. These results highlight the broader difficulty in predicting the influence of strain or steric hindrance on the rate of large ring-forming reactions. Nonetheless, these factors can be exploited with good effect.
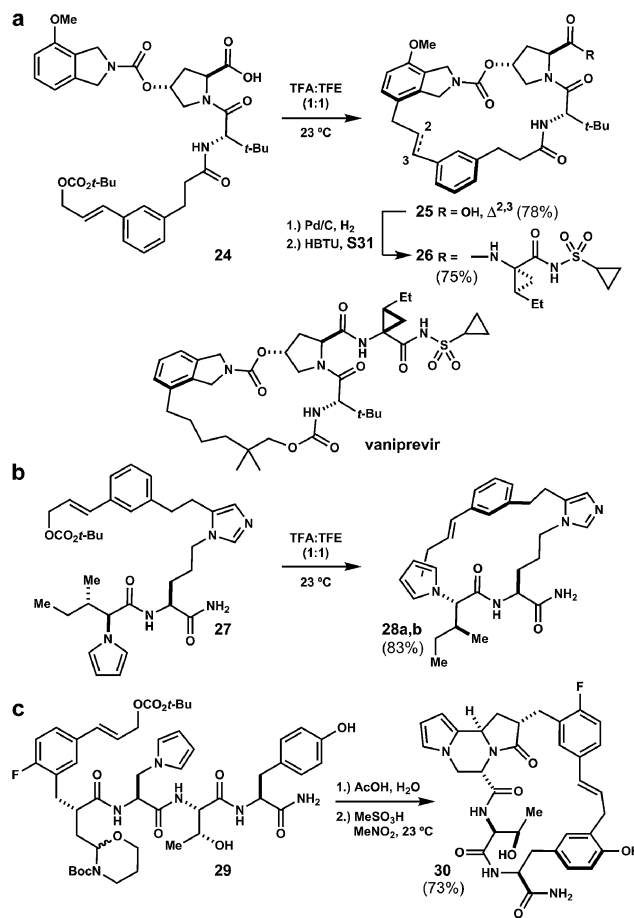

Fig. 5 Template design permits incremental variation of product structure. (a) Template **1** allows facile synthesis of vaniprevir analog **26**, (b) three-component coupling of **2** and macrocyclization accesses imidazole-linked macrocycles (*e.g.* **28a,b**). (c) Bi-functional template **3** initiates P1 cyclization and large ring-forming alkylation (*e.g.* **30**). HBTU = O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate.

Reagent **1** provides a small glimpse of possibilities for templates. Even minor modifications of the substance permit variations in product structures. For example, its aldehyde congener **2** (Fig. 1) was ligated to a Leu-Orn dipeptide derivative using a van Leusen three-component condensation to afford imidazole **27**.[28] This substance smoothly underwent macrocyclization *via* pyrrole alkylations in TFA : TFE to give **28a,b** (Fig. 5b) in high yield. This was a remarkable result given the lability and reactivity of monosubstituted pyrroles. We next investigated a third variant of **1**, namely **3**, harboring a tetrahydrooxazine as a latent aldehyde positioned to initiate N-acyliminium ion chemistry.[29]

Condensation of **3** with a pyrrolic tripeptide gave intermediate **29** (Fig. 5c). When this material was treated with aqueous acetic acid, the oxazine was hydrolyzed, promoting a diastereoselective Pictet–Spengler cyclization with the adjacent pyrrole *in situ*. Exposure of the crude product to methanesulfonic acid initiated Friedel–Crafts alkylation of the phenol to afford complex macrocycle **30** in good yield. Template **3** forms products based on the position of the phenol and the identity of the

N-terminal amino acid residue. Related multi-step processes can be designed that remodel a peptide consensus binding motif through template variations rather than modifications to the oligomer.

Large ring-forming alkylations have special potential as a tool to generate peptidomimetic leads for medicinal chemistry programs. As template designs become more sophisticated, we expect it will be possible to derive unique macrocycles from bioactive peptides with unmatched ease. Analyses of those that retain function of the unmodified peptide promise to help correlate structure with target affinity, selectivity, and importantly with favorable pharmacological profiles. The latter has considerable implications, but is not well understood at present.

Acknowledgements

Notes and references

1 E. M. Driggers, S. P. Hale, J. Lee and N. K. Terret, *Nat. Rev. Drug Discovery*, 2008, **7**, 608.

2 (a) J. E. Bock, J. Gavenonis and J. A. Kritzer, *ACS Chem. Biol.*, 2013, **8**, 488; (b) E. Marsault and M. L. Peterson, *J. Med. Chem.*, 2011, **54**, 1961.

3 (a) T. R. White, C. M. Renzelman, A. C. Rand, T. Rezai, C. M. McEwen, V. M. Gelev, R. A. Turner, R. G. Linington, S. S. F. Leung, A. S. Kalgutkar, J. N. Bauman, Y. Zhang, S. Liras, D. A. Price, A. M. Mathiowetz, M. P. Jacobson and R. S. Lokey, *Nat. Chem. Biol.*, 2011, **7**, 810; (b) A. C. Rand, S. S. Leung, H. Eng, C. J. Rotter, R. Sharma, A. S. Kalgutkar, Y. Zhang, M. V. Varma, K. A. Farley, B. Khunte, C. Limberakis, D. A. Price, S. Liras, A. M. Mathiowetz, M. P. Jacobson and R. S. Lokey, *MedChemComm*, 2012, **3**, 1282; (c) J. P. Maianti, A. McFedries, Z. H. Foda, R. E. Kleiner, X. Q. Du, M. A. Leissring, W. J. Tang, M. J. Charron, M. A. Seeliger, A. Saghatelian and D. R. Liu, *Nature*, 2014, **511**, 94.

4 (a) S. Vendeville and M. D. Cummings, in *Annual Reports in Medicinal Chemistry*, ed. M. C. Desai, Elsevier, San Diego, 2013, vol. 48, pp. 371–386; (b) F. Giodanetto and J. Kihlberg, *J. Med. Chem.*, 2014, **57**, 278.

5 (a) A. Mullard, *Nat. Rev. Drug Discovery*, 2012, **11**, 173; (b) J. A. Wells and C. L. McClendon, *Nature*, 1997, **450**, 1001.

6 M. C. Souroujon and D. Mochly-Rosen, *Nat. Biotechnol.*, 1998, **16**, 919.

7 (a) T. Clackson and J. A. Wells, *Science*, 1995, **267**, 383; (b) B. N. Bullock, A. L. Jochim and S. A. Arora, *J. Am. Chem. Soc.*, 2011, **133**, 14220; (c) A. M. Watkins and P. S. Arorra, *ACS Chem. Biol.*, 2014, **9**, 1747.

8 A. A. Bogan and K. S. Thorn, *J. Mol. Biol.*, 1998, **280**, 1.

9 I. S. Moreira, P. A. Fernandes and M. J. Ramos, *Proteins: Struct., Funct., Bioinf.*, 2007, **68**, 803.

10 (a) A. Isidro-Llobet, T. Murillo, P. Bello, A. Cilibrizzi, J. T. Hodgkinson, W. R. J. D. Galloway, A. Bender, M. Welch and D. R. Spring, *Proc. Natl. Acad. Sci. U. S. A.*, 2011, **108**, 6793; (b) H. S. G. Beckmann, F. Nie, C. E. Hagerman, H. Johansson, Y. S. Tan, D. Wilcke and D. R. Spring, *Nat. Chem.*, 2013, **5**, 861.

11 S. J. Miller, H. E. Blackwell and R. H. Grubbs, *J. Am. Chem. Soc.*, 1996, **118**, 9606.

12 E. Marsault, *et al.*, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 4731.

13 (a) H. E. Blackwell and R. H. Grubbs, *Angew. Chem., Int. Ed.*, 1998, **37**, 3281; (b) C. E. Schafmeister, J. Po and G. L. Verdine, *J. Am. Chem. Soc.*, 2000, **122**, 5891.

14 K. C. H. Chua, M. Pietsch, X. Zhang, S. Hautmann, H. Y. Chan, J. B. Bruning, M. Gütschow and A. D. Abell, *Angew. Chem., Int. Ed.*, 2014, **53**, 7828.

15 Z. J. Gartner, B. N. Tse, R. Grubina, J. B. Doyon, T. M. Snyder and D. R. Liu, *Science*, 2004, **305**, 1601.

16 C. P. Scott, E. Abel-Santos, A. D. Jones and S. J. Benkovic, *J. Biol. Chem.*, 2001, **8**, 801.

17 (a) D. L. Boger and J. B. Myers, *J. Org. Chem.*, 1991, **56**, 5385; (b) S. Jackson, W. DeGrado, A. Dwivedi, A. Parthasarathy, A. Higley, J. Krywko, A. Rockwell, J. Marwalder, G. Wells, R. Wexler, S. Mousa and R. Harlow, *J. Am. Chem. Soc.*, 1994, **116**, 3220; (c) R. Hili, V. Rai and A. K. Yudin, *J. Am. Chem. Soc.*, 2010, **132**, 2889.

18 H. Zhao, L. Negash, Q. Wei, T. G. LaCour, E. Capota, A. A. Pieper and P. G. Harran, *J. Am. Chem. Soc.*, 2008, **130**, 13864.

19 Q. Wei, S. Harran and P. G. Harran, *Tetrahedron*, 2003, **59**, 8947.

20 K. V. Lawson, T. E. Rose and P. G. Harran, *Proc. Natl. Acad. Sci. U. S. A.*, 2013, **110**, E3753.

21 K. V. Lawson, T. E. Rose and P. G. Harran, *Tetrahedron*, 2013, **69**, 7683.

22 T. Kato, K. Nagae and M. Hoshikawa, *Tetrahedron Lett.*, 1999, **40**, 1941.

23 J. I. Seeman, *Chem. Rev.*, 1983, **83**, 83.

24 C. Galli, G. Illuminati and L. Mandolini, *J. Org. Chem.*, 1980, **45**, 311.

25 U. Svanholm and V. D. Parker, *J. Chem. Soc., Perkin Trans. 2*, 1974, **2**, 169.

26 H. C. Brown and Y. Okamoto, *J. Am. Chem. Soc.*, 1958, **80**, 4979.

27 J. A. McCauley, C. J. McIntyre, M. T. Rudd, K. T. Nguyen, J. J. Romano, J. W. Butcher, K. F. Gilbert, K. J. Bush, M. K. Holloway and J. Swestock, *J. Med. Chem.*, 2010, **53**, 2443.

28 A. M. van Leusen, J. Wildeman and O. H. Oldenzie, *J. Org. Chem.*, 1977, **42**, 1153.

29 T. E. Nielsen and M. Meldal, *J. Org. Chem.*, 2004, **69**, 3765.