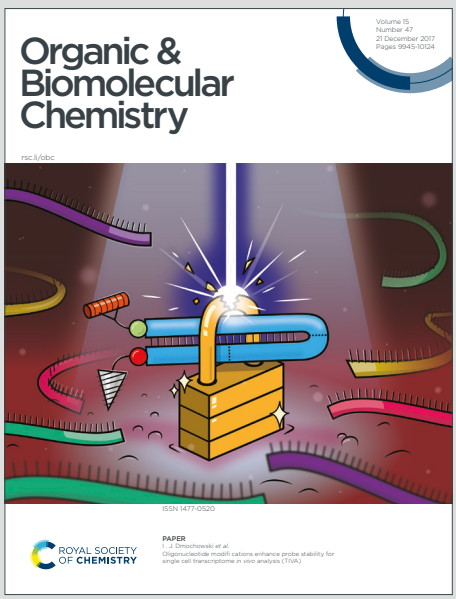
Organic & Biomolecular Chemistry

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: Z. Yao, X. Wu, X. Zhang, Q. Xiong, S. Jiang and Z. Yu, *Org. Biomol. Chem.*, 2019, DOI: 10.1039/C9OB00898E.



ROYAL SOCIETY OF CHEMISTRY

rsc.li/obc

View Article Online
DOI: 10.1039/C9OB00898E

Journal Name

COMMUNICATION

Synthesis and Evaluation of Photo-activatable β-Diarylsydnone-L-alanines for Fluorogenic Photo-click Cyclization of Peptides

Zhuojun Yao, Xueting Wu, Xiaocui Zhang, Qin Xiong, Shichao Jiang and Zhipeng Yu*

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Herein, we designed and synthesized a series of photo-activatable β-diarylsydnone-L-alanines (DASAs), which have excellent photo-reactivity with high fluorescence turn-on toward alkenes in bio-compatible environment. Environment sensing property of the resulting fluorescent pyrazoline-alanine facilitates its probing capability. By introducing the DASA residue on side chain of linear peptides, the macrocyclic peptides resulting from the *in-situ* photo-cyclization toward alkene residue exhibited its fluorogenic translocation through live cell membranes.

Photo-triggered chemoselective bioconjugation reactions are landmark tools for tracing molecules in living cells,[1] constructing biomaterials[2] and perturbating drug potency,[3] in possession of spatiotemporal controllability. The photo-induced ligation reactions currently have been investigated including: photo-click 1,3-dipolar cycloaddition between alkene and tetrazole[4] or azirine,[5] photo-induced *o*-naphthoquinone methides-ene hetero-Diels-Alder coupling,[6] photo-triggered thiol-olefin[7] or alkyne[8] addition, photo-irradiated strain-promoted azide–alkyne cycloaddition,[9] photo-induced [4+2] cycloaddition of phenanthrenequinone-alkene,[10] photo-oxidative inverse-electron-demand Diels-Alder ligation,[11] and strain-loadable alkene-azide photo-click chemistry.[12] These paired photo-reactive reagents with unique functionality were introduced into proteins or peptides by either chemical modification of natural amino acid residues[13] or biological[14] incorporation of synthetic unnatural amino acids (UAAs)[15] to exert their practicability. Due to the high abundance of competitive reactivity from natural amino acids as well as undesired perturbation resulted in non-specific modifications on proteins, genetic incorporation of UAAs provided a more versatile and precise way for site-selective modification of proteins or peptides in living system, primarily through the supressing of the amber stop codon.[16] Particularly, UAAs for photo-click ligation such as *p*-(2-tetrazole)-phenylalanine (*p*-Tpa)[17] and methyl-2-pyrrole-5-carboxy-tetrazolelysine (mPyTK)[18] as dipole; N^{ε}-(1-methylcycloprop-2-enecarboxyamido)lysine (CpK)[19] and spiro[2.3]hex-1-ene modified lysine (SphK)[20] containing unsaturated C-C bonds, have been incorporated into proteins for subsequent live cell dynamic study via bioorthogonal ligation.[21] In spite of the exciting developments in recent years, the discovery of photoclickable UAAs are highly demanded, which would provide a powerful chemical foundation for research on proteins *in vivo*.

Diarylsydnone (DASyd), a class of mesoionic 5-membered heterocycles, reacting with various dipolarophiles underwent [3+2] cycloaddition affording pyrazoles (Pyes)[22] or pyrazolines (Pyis) by means of nitrile imine intermediation upon photo-irradiation.[23] We recently reported a photo-induced DASyd-alkene cycloaddition reaction which provided fluorogenic performance for protein labelling without tagging any exogenous fluorophore.[24] Although sydnone, as a remarkable dipole, has attracted a lot of interests in the bioorthogonal research field recently,[25] DASyd cored amino acids are rarely

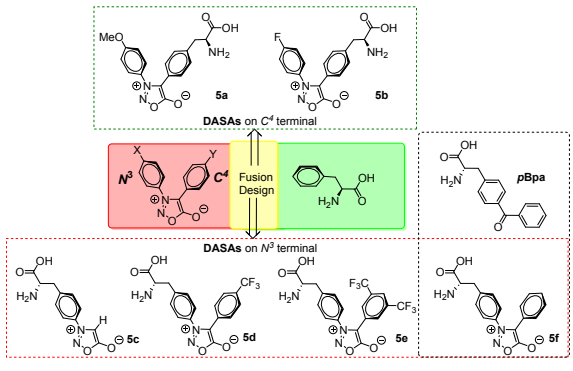
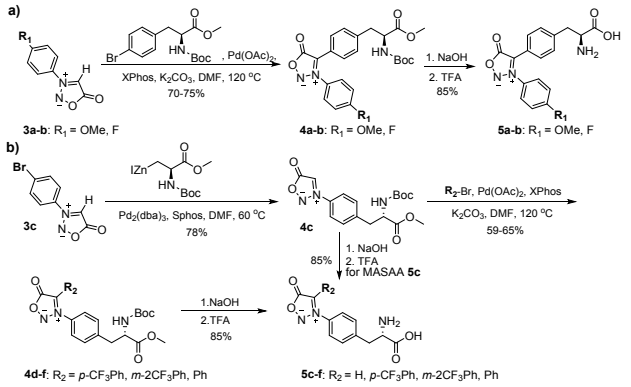


Figure 1. Design and structures of sydnone amino acids, **5a–f**.

[a] *Key Laboratory of Green Chemistry and Technology of Ministry of Education, College of Chemistry, Sichuan University, 29 Wangjiang Road, Chengdu 610064, P. R. China, E-mail: zhipengy@scu.edu.cn*

† Electronic Supplementary Information (ESI) available: Containing details on experimental procedures, spectra property, and characterization of all new compounds. See DOI: 10.1039/x0xx00000x

View Article Online DOI: 10.1039/C9OB00899F



Scheme 1. Synthetic routes for the sydnone amino acids, **5a–f**.

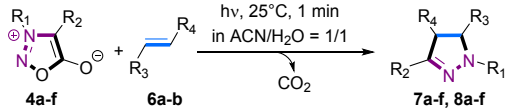
explored. Inspired by its attractive features, herein we report the synthesis of a set of DASAs and the subsequent evaluation of their photo-reactivity, photophysical properties of the resulting Pyis, in search of its application as photo-linking tool on peptide framework.

We designed two types of sydnone amino acids (SAAs) in which phenylalanine moiety, as one of the aryl components, was linked at C^4- (**5a**-**b**) or N^3-terminal (**5d**-**f**) of the sydnone core, respectively (Figure 1). The N^3-π-EDG was essential for excellent photo-reactivity, including N^3-*p*-MeOPh with polarized electron density, while trifluoromethyl modified C^4-aryl ring exhibited outstanding fluorescence turn-on according to our recent study.[24] Notably, **5b** and **5f** which are akin to *p*-benzoyl-L-phenylalanine (*p*Bpa),[26] a genetic encodable photo-cross-linker, will be the target substrates for directed evolution of tRNA synthetase to incorporate DASAs into proteins in future research. Therefore, two synthetic routes were explored to construct six SAAs (Scheme 1). First, monoarylsydnones (MASyds) **3a**-**c** were obtained through a straightforward transformation from *N*-aryl glycine via *N*-nitrosylation and intramolecular cyclization promoted by trifluoroacetic anhydride (TFAA).[27] Then the protocol to obtain DASAs **4a**-**b** were proved to be easily achieved by Heck-type cross-coupling[28] between C^4-H MASyd and a protected 4-bromophenylalanine (Scheme 1a). For N^3-sydnone modified phenylalanine, an alternative approach was developed. Iodo-alanine derived organo-zinc complex was treated with 4-bromophenyl-sydnone **3c** to form monoarylsydnone amino acid (MASAA) **4c** via Negishi coupling,[29] followed by Heck-coupling reactions[28] with the various aryl halides to generate DASAs **4d**-**f** successively (Scheme 1b). Finally, **5a**-**f** were obtained stepwise by deprotection with sodium hydroxide and TFA in high yields.

To study the photo-reactivity of SAAs, **4a**-**f** were chosen to examine the UV-Vis spectroscopic properties, because of their protected α-amino acid framework mimicking the peptide side-chain environment. All SAAs except MASAA **4c** exhibited maximum absorption wavelength around 332 nm (Figure S1). Then, we explored their photo-reactivity toward methyl methacrylate (MMA) or *trans*-cyclooct-4-en-1-ol (TCO) in ACN:H_2O = 1:1 under irradiation with a 311 nm UV lamp or 371 nm or 405 nm LED array by HPLC-MS analysis (Table 1, Figures S2-3). While all six SAAs were consumed under 311 nm within 1 min photo-illumination, only DASAs **4a**-**b** and **4d**-**f** provided ≥99% conversions to generate the desired Pyi cycloadducts. In addition, even though 371 or 405 nm photo-illumination leaded to lower conversions compared to 311 nm, **4a** still afforded ≥90% and ≥12% conversions, respectively, making it a more suitable choice for chemo-selective bioconjugation propose. No cycloaddition was observed for 12 h in dark (Figure S4). Subsequently, all Pyi adducts were micro-prepared, isolated and validated via NMR spectra analyses (Table 1, columns 9-10).

Since MMA is readily accessible (Table 1), we explored the photophysical properties of the resulting Pyis **7a-b** and **7d-f** (Table 2, Figures 2a and S6). Based on the fluorescence emission spectra, **7a** harboured a red-shifted emission maximum in comparison with other Pyis, accompanied by the largest Stokes shift (Table 2. entry 1 *vs.* others). More importantly, when excited, **7a-b** and **7d-f** showed higher fluorescence quantum yields (Table 2. Φ_F 0.71-0.90, Figure 2a) in EtOAc *vs.* those in ACN:PB = 1:1, and varying degrees of fluorescence turn-on during the photo-click process, ranging from 85-fold (**7f**) to >460-fold (**7e**). Encouraged by these results, we next examined the photo-ligation quantum yield (Φ_R) to evaluate the efficiency

Table 1. Photo-induced 1,3-Dipolar Cycloaddition of **4a**-**4f** with MMA or TCO



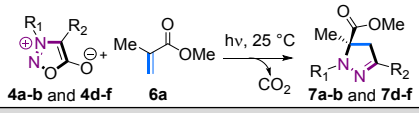
4a: R_1 = *p*-MeOPh; R_2 = Boc-L-Phe-Ome
4b: R_1 = *p*-FPh; R_2 = Boc-L-Phe-Ome
4c: R_1 = Boc-L-Phe-OMe; R_2 = H
4d: R_1 = Boc-L-Phe-OMe; R_2 = *p*-CF_3Ph
4e: R_1 = Boc-L-Phe-OMe; R_2 = *m*-$2CF_3$Ph
4f: R_1 = Boc-L-Phe-OMe; R_2 = Ph

Entry	No.	311 nm[a]		371 nm[a]		405 nm[a]		Isolated yield[b]	
		6a	6b	6a	6b	6a	6b	6a	6b
1	**4a**	>99[c]	>99[c]	92.6[c]	97.7[c]	12.9	12.4	75	80.4
2	**4b**	>99[c]	>99	81.5[c]	90.7	8.1	11.9	71.3	56.6
3	**4c**	U.P.[d]	U.P.[d]	N.R.[e]	N.R.[e]	-	-	-	-
4	**4d**	>99[c]	>99[c]	56.8	70.1	4.6	6.4	72	80
5	**4e**	>99[c]	>99	52.0	66.3	0.4	0.5	70	67.9
6	**4f**	>99[c]	>99[c]	70.9[c]	81.6[c]	3.7	7.3	71	79.8

[a]HPLC-MS conversion (%). 50 μM SAAs and 500 μM alkenes in ACN:H_2O = 1:1 were photo-irradiated with corresponding light sources for 1 min. See Figures S2-3 in ESI for details. [b]Micropreparations were conducted. 30 mg of SAAs and 20 equiv. MMA (**6a**) or 5 equiv. TCO (**6b**) in 50 mL ethyl acetate (EtOAc) with 311 nm UV light for 2 h. [c]Trace amount of by-products formed. [d]U.P. = All converted into undesired products. [e]N.R. = No photo-reaction.

DOI: 10.1039/C9OB00898E

Table 2. Photophysical properties of the MMA-Pyis[a]



Entry	No.	DASAs R1	DASAs R2	$\lambda_{ex}/\lambda_{em}$ (nm)	Φ_R[b]	Φ_F[c] EtOAc/ACN:PB (1:1)	F. E.[d]
1	**7a**	*p*-MeOPh	Boc-L-Phe-OMe	362/497	0.26	0.90/0.09	>90
2	**7b**	*p*-FPh	Boc-L-Phe-OMe	356/459	0.32	0.71/0.27	>270
3	**7d**	Boc-L-Phe-OMe	*p*-CF_3Ph	376/484	0.15	0.87/0.44	220
4	**7e**	Boc-L-Phe-OMe	*m*-2CF_3Ph	380/497	0.17	0.72/0.46	>460
5	**7f**	Boc-L-Phe-OMe	Ph	360/461	0.29	0.88/0.34	85

[a]25 μM **7a-b** and **7d-f** in ACN:PB = 1:1. [b]Reaction quantum yield in ACN:PB = 1:1. [c]Fluorescence quantum yield. [d]Fluorescence enhancement by comparing the fluorescence quantum yields of Pyis *vs*. DASAs (Figure S7) in ACN:PB = 1:1.

of the photochemistry. In general, the photo transformation quantum yields of DASAs were determined to be 0.26-0.32 with EDG on N^3-terminal better than EWG on C^4-terminal (Table 2. entries 1, 2, 5 *vs.* 3, 4). Since Pyis were solvatochromic fluorophores,[30] we investigated the solvent dependent fluorescence variation (Figure 2b, Figure S5). To our satisfaction, all Pyi fluorophores were sensitive to the micro-environment change that lied in the dielectric constant of the solvent (Figure S5). Significantly, **7a** displayed a sharp increasing of fluorescence intensity in non-protonic solvent, like: dichloromethane (DCM), EtOAc and acetonitrile (ACN); the ratio of fluorescence elevation effect from protonic methanol (MeOH) to EtOAc was 18-fold (Figure 2b). Therefore, it was beneficial to probe the micro-environment of the cycloadduct when on peptides or proteins via comparing the relative fluorescence brightness.

In order to utilize the DASA-alkene photo-click ligation for the fluorogenic tracing of peptides in live cell via imaging, we selected methacrylamide modified lysine and DASA **5a** to replace i and i+4 sites of a Balaram's 3_{10}-helix[31] peptide, respectively (Figure 3a). Both Grubbs[32] and Qin[33] have studied the stapling of this α-helical peptide model via chemically modified lysine residues. Different from previous studies, the incorporation of DASA **5a** into the peptide sequence with more deeply embedded DASyd moiety would arouse more rigidity in resulting macrocyclic peptide after photo-click reaction, which might provide strengthened control on the conformation of the peptide. With the peptide **P1** synthesized, we attempted to

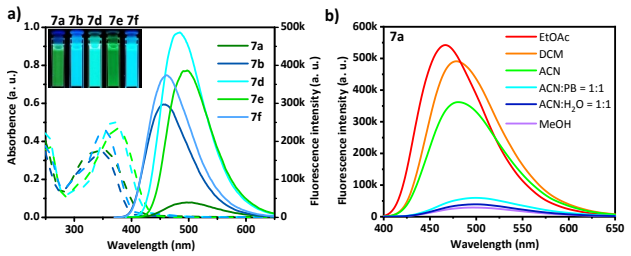


Figure 2. Characterization of the absorbance and fluorescence properties of the MMA-Pyis. (a) Absorption (dashed lines) and fluorescence emission spectra (solid lines) of 30 μM Pyis **7a-f** in ACN:PB = 1:1. λ_{ex} = 360 nm for **7a, 7b**, **7f** and 378 nm for **7d**, **7e**. Inset: Images of the fluorescence of MMA-Pyis in ACN:PB = 1:1 with irradiation at 365 nm. (b) Fluorescence spectra for 30 μM **7a** in various solvents. λ_{ex} = 360 nm.

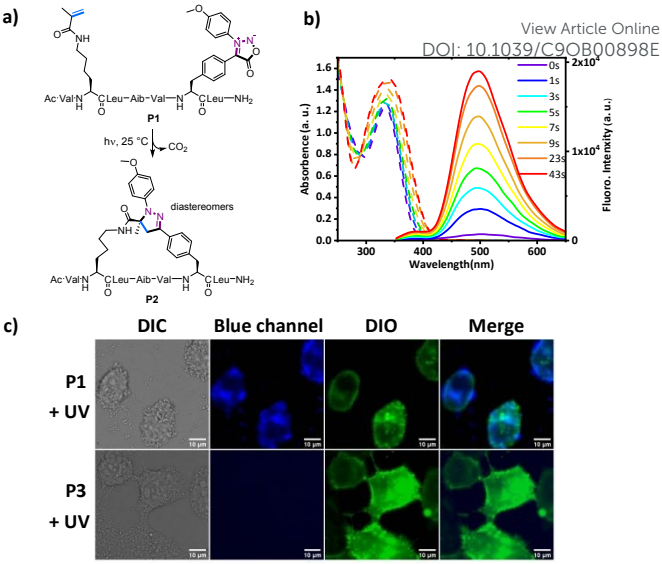


Figure 3. (a) Cyclization of peptide **P1** bearing 1,3-dipolar cycloaddition in positions i and i+4. (b) Time-dependent changes in absorption (dashed lines) and fluorescence emission spectra (solid lines) of 150 μM linear peptide **P1** in ACN:PB = 1:1 (pH = 7.4) under irradiation of UV light. (c) Fluorescence images of live A549 cells for *in-situ* cyclization of 150 μM **P1** in the medium after 371 nm irradiation. **P3** as the control group.

crosslink the side-chain via the photo-induced 1,3-dipolar cycloaddition under 311 nm UV light or 371 nm LED array in a variety of solvents including organic or buffer solution. To our surprise, the side chains of the peptide showed desired cyclization only in the presence of PB containing solvents with a time-dependent fluorescence generation under continuous illumination (Figures 3b, S9), while other solvents, including ACN:H_2O = 1:1, only leaded to side-reactions (solvolysis or nucleophilic additions, Figure S8). By inquiring the circular dichroism (CD) spectra of peptide **P1** in different solvents, we found that the degree of α-helicity was reduced in ACN:PB = 1:1, therefore the phosphate probably behaved as a unique conformational relaxer of the peptide **P1**, causing the proximity of alkene residue toward DASA **5a** in the disorder conformational dynamics (Figure S10a). After purification by reverse-phase HPLC, a pair of macrocyclic peptidic diastereomers **P2** were characterized by CD spectrum analysis in trifluoroethanol (TFE) or ACN:PB = 1:1. Peptide **P3** (Ac-Val-Lys-Leu-Aib-Val-**5a**-Leu-NH_2) was selected as a control in which the lysine residue was unmodified to avoid conformational perturbation during spectral scanning (190-250 nm). We observed that the macrocyclic peptide **P2** lost the α-helical conformation, comparing to the peptide **P3** (Figure S10b), presumably due to the tight control of the macrocyclic space (Figure 3a).

To study the membrane permeability of peptide **P2** via *in-situ* cyclization from **P1** in live cells, A549 cells culturing with 150 μM **P1** *vs.* **P3** were photo-irradiated under 371 nm LED array for 1 min and then incubated for 3 h, followed by DIO staining to visualize the cell membrane. Contrast to the control group treating with **P3** (without alkene residue) which scarcely presented any fluorescence signal in blue channel (lower panel, Figure 3c), accumulated fluorescence was observed

intermittently in the cytoplasm for **P1** cyclization via fluorescence microscopy imaging (upper panel, Figures 3c and S11). This pattern of distribution indicated that the cyclized **P1** might penetrate the cell membrane through the pinocytotic pathway.[34] The cell viability after photo-cyclization of peptide **P1** *in-situ* was determined to be 91 ± 1.7% (Figure S12). Furthermore, we treated the A549 cells with preformed cyclic peptide **P2** *vs.* the linear peptide **P4** (Ac-Val-Lys-Leu-Aib-Val-Pyis-Leu-NH_2) which possesses the fluorescent Pyis residue as the control. Intriguingly, preformed **P2** displayed the distribution of blue fluorescence signal akin to the *in-situ* cyclized **P1**, while the linear **P4** still showed negligible signal (Figure S13), indicating the *in-situ* photo-cyclization of **P1** is a valid mean for tuning the properties of the peptide.

In summary, we reported the design and synthesis of DASAs with minimal spatial occupation. By investigating the properties of corresponding Pyis products formed with MMA under photo-irradiation, prominent fluorescence turn-on was observed. Among them, **5a** harbouring the high photo-reactivity was introduced into an α-helical peptide in combination with MMA-modified lysine to generate fluorescent cyclopeptide via photoclick ligation. The fluorogenic photo-macrocyclization demonstrated the utilization of DASAs as UAAs to explore the biological activity of macromolecules in live cells.

Financial support was provided by the National Natural Science Foundation of China (21502130), the "1000-Youth Talents Program", and the Fundamental Research Funds for the Central Universities.

Conflicts of interest

The authors declare no competing financial interests.

Notes and references

1 (a) P. An, Z. Yu and Q. Lin, *Chem. Commun.*, 2013, **49**, 9920; (b) F. Li, H. Zhang, Y. Sun, Y. Pan, J. Zhou and J. Wang, *Angew. Chem. Int. Ed.*, 2013, **52**, 9700.
2 M. He, J. Li, S. Tan, R. Wang and Y. Zhang, *J. Am. Chem. Soc.*, 2013, **135**, 18718.
3 M. M. Madden, A. Muppidi, Z. Li, X. Li, J. Chen and Q. Lin, *Bioorg. Med. Chem. Lett.*, 2011, **21**, 1472.
4 Y. Wang, C. I. Rivera Vera and Q. Lin, *Org. Lett.*, 2007, **9**, 4155.
5 (a) A. Padwa, *Acc. Chem. Res.*, 1976, **9**, 371; (b) R. K. V. Lim and Q. Lin, *Chem. Commun.*, 2010, **46**, 7993.
6 (a) S. Arumugam and V. V. Popik, *J. Am. Chem. Soc.*, 2011, **133**, 15730; (b) S. Arumugam, S. V. Orski, J. Locklin and V. V. Popik, *J. Am. Chem. Soc.*, 2012, **134**, 179.
7 K. L. Killops, L. M. Campos and C. J. Hawker, *J. Am. Chem. Soc.*, 2008, **130**, 5062.
8 R. M. Hensarling, V. A. Doughty, J. W. Chan and D. L. Patton, *J. Am. Chem. Soc.*, 2009, **131**, 14673.
9 (a) A. A. Poloukhtine, N. E. Mbua, M. A. Wolfert, G. J. Boons and V. V. Popik, *J. Am. Chem. Soc.*, 2009, **131**, 15769; (b) S. Arumugam and V. V. Popik, *J. Org. Chem.*, 2014, **79**, 2702.
10 J. Li, H. Kong, L. Huang, B. Cheng, K. Qin, M. Zheng, Z. Yan and Y. Zhang, *J. Am. Chem. Soc.*, 2018, **140**, 14542.
11 H. Zhang, W. S. Trout, S. Liu, G. A. Andrade, D. A. Hudson, S. L. Scinto, K. T. Dicker, Y. Li, N. Lazouski, J. Rosenthal, C. Thorpe, X. Jia and J. M. Fox, *J. Am. Chem. Soc.*, 2016, **138**, 5978.
12 K. Singh, C. J. Fennell, E. A. Coutsias, R. Latifi, S. Hartson and J. D. Weaver, *Chem*, 2018, **4**, 124.
13 W. Song, Y. Wang, J. Qu, M. Madden and Q. Lin, *Angew. Chem. Int. Ed.*, 2008, **47**, 2832.
14 J. T. Ngo, B. M. Babin, J. A. Champion, E. M. Schuman and D. A. Tirrell, *ACS Chem. Biol.*, 2012, **7**, 1326.
15 W. Song, Y. Wang, J. Qu and Q. Lin, *J. Am. Chem. Soc.*, 2008, **130**, 9654.
16 (a) L. Wang and P. G. Schultz, *Angew. Chem. Int. Ed.*, 2005, **44**, 34; (b) J. W. Chin, *Annu. Rev. Biochem*, 2014, **83**, 379; (c) H. S. Park, M. J. Hohn, T. Umehara, L. T. Guo, E. M. Osborne, J. Benner, C. J. Noren, J. Rinehart and D. Söll, *Science*, 2011, **333**, 1151.
17 J. Wang, W. Zhang, W. Song, Y. Wang, Z. Yu, J. Li, M. Wu, L. Wang, J. Zang and Q. Lin, *J. Am. Chem. Soc.*, 2010, **132**, 14812.
18 Y. Tian, M. P. Jacinto, Y. Zeng, Z. Yu, J. Qu, W. R. Liu and Q. Lin, *J. Am. Chem. Soc.*, 2017, **139**, 6078.
19 Z. Yu, Y. Pan, Z. Wang, J. Wang and Q. Lin, *Angew. Chem. Int. Ed.*, 2012, **51**, 10600.
20 Z. Yu and Q. Lin, *J. Am. Chem. Soc.*, 2014, **136**, 4153.
21 (a) R. K. Lim and Q. Lin, *Chem. Commun.*, 2010, **46**, 1589; (b) E. M. Sletten and C. R. Bertozzi, *Angew. Chem. Int. Ed.*, 2009, **48**, 6974; (c) M. D. Best, *Biochemistry*, 2009, **48**, 6571.
22 (a) C. S. Angadiyavar and M. V. George, *J. Org. Chem.*, 1971, **36**, 1589; (b) H. Gotthardt and F. Reiter, *Tetrahedron. Lett.*, 1971, **12**, 2749.
23 (a) H. Gotthardt and F. Reiter, *Chem. Ber.*, 1979, **112**, 1206; (b) K. Butković, D. Vuk, Ž. Marinić, J. Penić and M. Šindler-Kulyk, *Tetrahedron*, 2010, **66**, 9356; (c) X. S. Wang, Y. Lee and W. R. Liu, *Chem. Commun.*, 2014, **50**, 3176.
24 L. Zhang, X. Zhang, Z. Yao, S. Jiang, J. Deng, B. Li and Z. Yu, *J. Am. Chem. Soc.*, 2018, **140**, 7390.
25 (a) S. Kolodych, E. Rasolofonjatovo, M. Chaumontet, M. C. Nevers, C. Crminon and F. Taran, *Angew. Chem. Int. Ed.*, 2013, **52**, 12056; (b) S. Wallace and J. W. Chin, *Chem. Sci.*, 2014, **5**, 1742; (c) L. Plougastel, O. Koniev, S. Specklin, E. Decuypere, C. Créminon, D. A. Buisson, A. Wagner, S. Kolodych and F. Taran, *Chem. Commun.*, 2014, **50**, 9376; (d) C. Favre, L. de Cremoux, J. Badaut and F. Friscourt, *J. Org. Chem.*, 2018, **83**, 2058; (e) C. Favre and F. Friscourt, *Org. Lett.*, 2018, **20**, 4213; (f) L. C. C. Lee, H. M. H. Cheung, H. W. Liu and K. K. W. Lo, *Chem. Eur. J.*, 2018, **24**, 14064; (g) Z. S. Chinoy, C. Bodineau, C. Favre, K. W. Moremen, R. V. Durán and F. Friscourt, *Angew. Chem. Int. Ed.*, 2019, **58**, 4281.
26 (a) N. Hino, Y. Okazaki, T. Kobayashi, A. Hayashi, K. Sakamoto and S. Yokoyama, *Nat. Methods.*, 2005, **2**, 201; (b) V. K. Lacey, G. V. Louie, J. P. Noel and L. Wang, *Chembiochem*, 2013, **14**, 2100.
27 (a) D. L. Browne, J. F. Vivat, A. Plant, E. Gomez-Bengoa and J. P. A. Harrity, *J. Am. Chem. Soc.*, 2009, **131**, 7762; (b) S. Specklin, E. Decuypere, L. Plougastel, S. Aliani and F. Taran, *J. Org. Chem.*, 2014, **79**, 7772.
28 A. W. Brown and J. P. A. Harrity, *J. Org. Chem.*, 2015, **80**, 2467.
29 (a) I. Rilatt, L. Caggiano and R. F. W. Jackson, *Synlett*, 2005, **18**, 2701; (b) A. J. Ross, H. L. Lang and R. F. W. Jackson, *J. Org. Chem.*, 2010, **75**, 245.
30 P. An, Z. Yu and Q. Lin, *Org. Lett.*, 2013, **15**, 5496.
31 I. L. Karle, J. L. Flippen-Anderson, K. Uma and P. Balaram, *Biopolymers*, 1993, **33**, 827.
32 H. E. Blackwell and R. H. Grubbs, *Angew. Chem. Int. Ed.*, 1998, **37**, 3281.
33 M. M. Madden, C. I. Rivera Vera, W. Song and Q. Lin, *Chem. Commun.*, 2009, **37**, 5588.
34 G. H. Bird, F. Bernal, K. Pitter and L. D. Walensky, *Methods Enzymol.*, 2008, **446**, 369.