



Cite this: *Org. Biomol. Chem.*, 2017, **15**, 4517

Received 23rd April 2017,
Accepted 7th May 2017

DOI: 10.1039/c7ob00991g

rsc.li/obc

Strained alkynes derived from 2,2'-dihydroxy-1,1'-biaryls; synthesis and copper-free cycloaddition with azides†

Alessandro Del Grosso, Lavrentis-Dimitrios Galanopoulos, Cookson K. C. Chiu, ,
Guy J. Clarkson, , Peter B. O' Connor and Martin Wills *

A series of strained alkynes were prepared from 2,2'-dihydroxy-biaryls. Several were characterised by X-ray crystallography, revealing strained C(sp)–C(sp)–C(sp³) bond angles in the range of 163–167°. Their cycloadditions with azides proceed without a catalyst. Functionalised versions of these reagents have potential applications to materials synthesis and bioconjugations.

Azides react with terminal alkynes to form a triazole in what is commonly referred to as a 'click' reaction.^{1–6} Reactions of this type proceed under mild conditions, in high conversion and their biorthogonal nature allows them to be used to attach functional molecules (*e.g.* dyes, fluorescent groups, drugs *etc.*) to biomolecules, and to create antibody/drug conjugates (ADCs) for therapeutic use.^{7–9} The undesirable requirement for the use of a copper catalyst can be eliminated through the use of reactive strained alkynes in 'Strain-Activated Alkyne Azide Cycloaddition'.¹⁰ Examples of recently-reported strained alkynes are illustrated in Fig. 1.^{11–21}

The majority of reported strained alkynes contain 8-membered rings, however there are some examples of 9-membered ring reagents²⁰ such as the 2-amidobenzene sulphonamide cyclononane²¹ and even a 19-membered crown ether,²² both illustrated in Fig. 1. Thiacycloalkyne and dibenzoselenacycloheptynes have also been reported.²³

Strained alkynes have also been used to conjugate functional polymers, for example to combine^{24a} contrasting chains and to form crosslinks in the formation of hydrogels.^{24b} They have also been used to create modified pharmaceutical agents.²⁵ Of the reported strained reagents, DIBO, DIBAC and BCN, due to their high reactivity, have been particularly widely adopted for bioconjugation reactions.¹¹ In this paper, we describe the synthesis and applications of a class of strained alkyne which can be prepared through a short and facile synthetic sequence from readily available and inexpensive starting materials.

Following a single precedent in the literature,^{26a} we investigated the reaction of biaryl diols with 1,4-ditosyl-but-2-yne, which resulted in the formation of cyclic alkynes 1–4 (Fig. 2). A literature search revealed just two other reports of this heterocyclic structure, both binaphthyl-derived,^{26b,c} whilst their reactions with azides have not been reported. The 2,2'-biphenols and their strained-alkyne derivatives are racemic, however compound 2 was prepared as a single enantiomer derived from (*R*)-BINOL. Compounds 2–4^{27,28} were characterised by X-ray crystallography (Fig. 3) which served to confirm the strained nature of the triple bonds in each product. The sp angles at the triple bonds (C(sp)–C(sp)–C(sp³)) in 3 and 4 were *ca.* 166–167°, however, due to the presence of three crystallo-

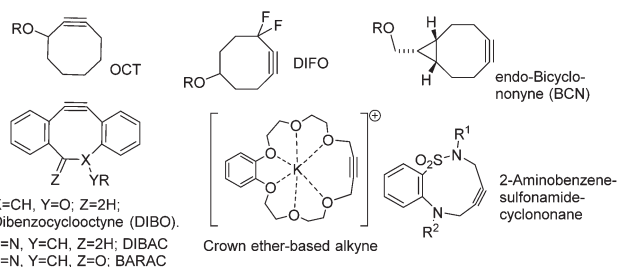


Fig. 1 Examples of strained alkynes which react with azides without the requirement for a copper catalyst. R represents a point of attachment to either a biomolecule or other functional group.

Department of Chemistry, The University of Warwick, Coventry, CV4 7AL, UK.
E-mail: m.wills@warwick.ac.uk

†Electronic supplementary information (ESI) available: Full experimental details, NMR spectra, X-ray crystallographic structures and kinetic data (pdf). CCDC 1515150–1515156. For ESI and crystallographic data in CIF or other electronic format see DOI: 10.1039/c7ob00991g

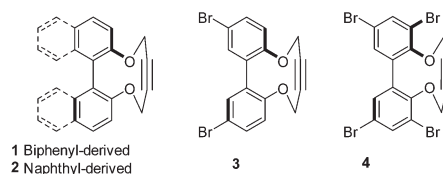


Fig. 2 Strained alkynes 1–4 derived from biaryl and binaphthyl diols.



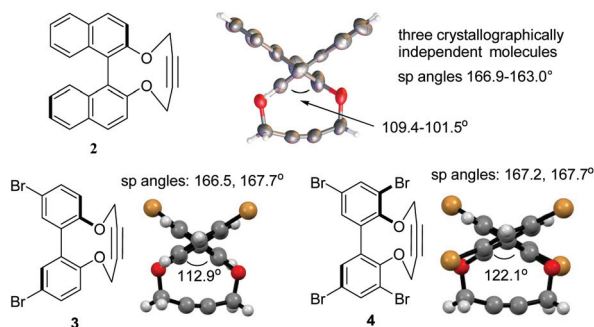
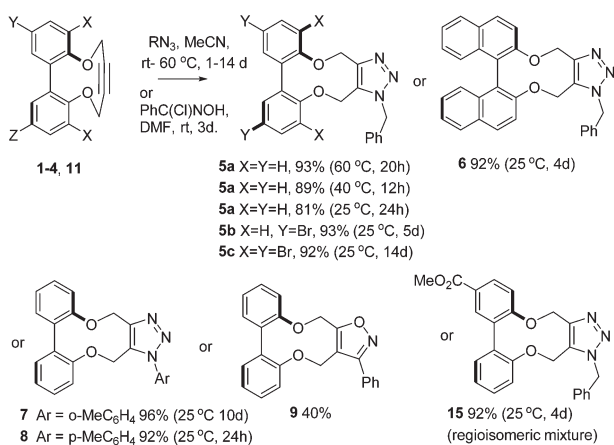


Fig. 3 X-ray crystallographic structures of strained alkynes 2–4.

graphically independent molecules in the unit cell of 2, the sp bond angles in this molecule ranged from 166.9–163.0° with an average of *ca.* 165°. The high aryl/aryl torsion angles (Fig. 3) reflect the wide spacing required to accommodate the linear butyne component in the ring. This angle increased to 112.5° in the dibromo 3 and 122.1° in the tetrabromo compound 4.

The strain in the alkyne is reflected in the reactivity of 1–4 towards benzyl azide and arylazides (Scheme 1), which resulted in formation of cycloaddition products 5–8 in high yields, at room temperature, and without the need for a metal catalyst. The X-ray crystallographic structure of cycloadduct 5a (Fig. 4) revealed a low torsion angle between the aromatic rings of just 43°, reflecting the greater flexibility of the triazolylidimethylene bridge.



Scheme 1 Cycloadditions of strained alkynes with benzyl azide, aryl azides and a nitrile oxide.

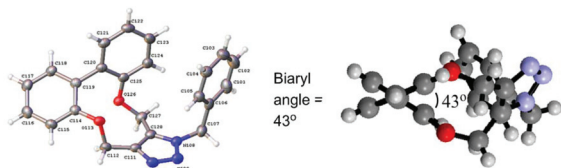


Fig. 4 X-ray crystallographic structure of cycloadduct 5a.

Conversions to cycloadducts were typically *ca.* 90% or higher at 40 °C within 24 h in several cases, however the reactions could be run for several days without substrate decomposition, to generate products in high yield even at the lower temperature. The cycloaddition of a nitrile oxide (generated *in situ* from the chloride precursor)²⁹ to give adduct 9, was also completed (Scheme 1).

The second-order rate constants for a number of the reactions were determined (Table 1, ESI†). The reactions of the unsubstituted derivative 1 with both benzyl azide and *p*-tolylazide, were followed over time at three temperatures by ¹H NMR to allow calculations of thermodynamic data. For the unsubstituted substrate 1, 81% conversion was observed after 24 h at 25 °C, and 89% after 12 h at 40 °C (Table 1). The rate constants are higher than for a potassium complex of a cyclic 1,4-diether containing a but-2-yne (Fig. 1; $1.3 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$), which is a rare example of a strained alkyne based on 1,4-but-2-yne diether structure.²² The reactivity of the dibromide 3 is similar to that of 1, however the tetrabromo 4 was of lower reactivity, possibly due to steric hindrance caused by the large bromine atoms. Arylazide cycloaddition was also achieved without the need for a catalyst, and *p*-tolylazide was more reactive than *o*-tolylazide; again this may be due to higher steric hindrance in the *ortho*-substituted azide.

The rate constants for 1, within the temperature range examined, are similar to that reported for benzyl azide with ‘OCT’ ($2.4 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$), but lower than those of other cyclooctyne reagents illustrated in Fig. 1 (DIFO; 7.6×10^{-2} , DIBO; 0.17, DIBAC; 0.31, BCN; 0.11–0.29, BARAC; $0.96 \text{ M}^{-1} \text{ s}^{-1}$). The relative reactivities are also reflected in the distorted sp bond angles of 161/151° (DIFO2) and 153° (BARAC).³⁰ However the reactivity of 1 and its derivatives is sufficient for it to be a practical reagent for use in synthetic chemistry applications under mild, Cu-free conditions.

Whilst following the cycloaddition of 1 with benzylazide at 60 °C, we observed that cycloadduct 5a exhibited significant peak broadening in the ¹H-NMR spectrum whilst the starting

Table 1 Summary of second-order rate constants for cycloaddition reactions

| Product | Temp./°C | Solvent | [alkyne] ^a /M | Rate constant |
|-----------------|----------|-------------------|--------------------------|---|
| 5a ^b | 25 | MeCN | 0.25 | $1.85 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$ |
| 5a ^c | 40 | MeCN | 0.25 | $7.56 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$ |
| 5a ^d | 60 | MeCN | 0.25 | $3.12 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$ |
| 5a | 25 | CDCl ₃ | 0.10 | $1.28 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$ |
| 5b | 25 | CDCl ₃ | 0.10 | $1.41 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$ |
| 5c | 25 | CDCl ₃ | 0.10 | $3.64 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$ |
| 15 | 25 | MeCN | 0.25 | $2.50 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$ |
| 7 ^e | 25 | CDCl ₃ | 0.10 | $3.07 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$ |
| 8 ^f | 25 | CDCl ₃ | 0.10 | $1.24 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$ |
| 8 | 40 | CDCl ₃ | 0.10 | $5.00 \times 10^{-4} \text{ M}^{-1} \text{ s}^{-1}$ |
| 8 ^g | 60 | CDCl ₃ | 0.10 | $1.69 \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$ |

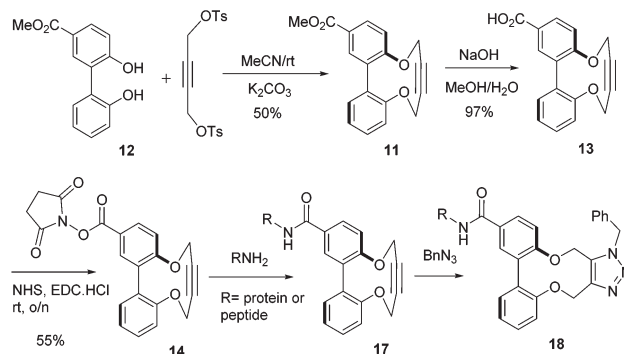
^a Alkyne : azide = 1 : 1. ^b 81% conv. in 24 h. ^c 89% conv. in 12 h. ^d Benzyl azide adduct 1; $\Delta E^\ddagger = 15.9 \text{ kcal mol}^{-1}$, $\Delta H^\ddagger = 15.2 \text{ kcal mol}^{-1}$, $\Delta S^\ddagger = -24.3 \text{ cal mol}^{-1} \text{ K}$. ^e 96% yield in 10 days. ^f 92% yield in 24 h. ^g *p*-Tolylazide adduct 8; $\Delta E^\ddagger = 14.6 \text{ kcal mol}^{-1}$, $\Delta H^\ddagger = 14.0 \text{ kcal mol}^{-1}$, $\Delta S^\ddagger = -29.2 \text{ cal mol}^{-1} \text{ K}$.



material **1** did not. A systematic study of their variable temperature $^1\text{H-NMR}$ spectra, in $d^6\text{-DMSO}$ was completed and this indicated that **1** did not undergo helical inversion even up to $100\text{ }^\circ\text{C}$, whereas **5a** exhibited coalescence of the OCH_2 peaks at *ca.* $56\text{ }^\circ\text{C}$. The ΔG^\ddagger required for atropisomer interconversion was calculated to be $16.3\text{ kcal mol}^{-1}$ (ESI^\dagger). The related aliphatic compound **10** was independently synthesised³¹ and its OCH_2 peak signals were found to broaden and coalesce at *ca.* $74\text{ }^\circ\text{C}$ (Fig. 5; biaryl angle 55.6° , ΔG^\ddagger required for atropisomer interconversion *ca.* $16.7\text{ kcal mol}^{-1}$). For **5a** in CD_3CN solvent, coalescence was at a similar temperature of *ca.* $60\text{ }^\circ\text{C}$. This confirms a much higher level of rigidity in **1** (and hence **2–4**) compared to their more flexible cycloadducts and to compound **10** (Fig. 5, ESI^\dagger). Hence the well-defined structures of the cyclic strained alkynes offers the potential for them to be used as the basis of asymmetric ligands, and possibly as building blocks for extended materials.

There is potential for the attachment of derivatives of **1** to proteins and functional groups through the introduction of a linking functional group. In order to demonstrate the potential for this, the ester-functionalised alkyne **11** was prepared in one step from the diphenol **12**³² in an unoptimised 50% yield (some of the $[2+2]$ and $[3+3]$ products were also isolated; see ESI^\dagger for structures). Hydrolysis of the ester to acid **13** was followed by formation of the activated NHS ester **14** (Scheme 2). Fig. 6 illustrates the X-ray crystallographic structures of both **11** and **13**, which exhibit very similar biaryl torsion angles and strained sp bond angles to each other and to **1–4**. The reaction of **11** with benzyl azide proceeded (Scheme 1) without a catalyst, giving product **15** in 92% yield at room temperature after a reaction period of 4 days. The second order rate constant for the cycloaddition was similar to that for **1** (Table 1), indicating that the introduction of the ester had not significantly diminished the reactivity of the alkyne.

Reduction of **14** with DIBAL resulted in formation of alcohol **16**, which also has the potential to be converted to a conjugation reagent. Although lower in reactivity than the most active strained alkynes (Fig. 1), we sought to obtain preliminary evidence of its potential for bioconjugation. To



Scheme 2 Synthesis of activated NHS ester **14** and subsequent reaction with peptides and a protein.

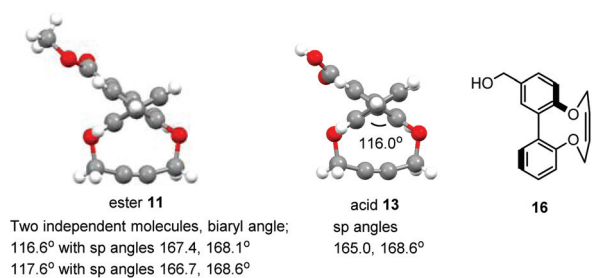


Fig. 6 X-ray crystallographic structure of ester **11** and acid **13**, and structure of alcohol **16**.

obtain a proof of principle in this respect, **14** was reacted with the small peptides substance P, vasopressin and bombesin, and the protein myoglobin, the reactions being followed using FTICR mass spectrometry (Scheme 2). The reactions of peptides with the activated ester **14** in water/methanol solution resulted in partial functionalization to give adducts **17** (see ESI^\dagger). MS/MS studies confirmed that the lysine residue in each peptide had been functionalised. The subsequent reaction with an excess of benzyl azide in each case occurred with $>95\%$ conversion to the 'clicked' product **18**.

Solutions of myoglobin and **14** in methanol/water were prepared, and after standing for one day at rt were analysed by FTICR. Since myoglobin contains multiple lysine residues, the protein was only partly functionalised (*ca.* 10%) in order to avoid formation of a range of functionalised proteins, which would be more difficult to analyse. An excess of benzyl azide in the same solvent was then added and after 7 days the solutions were analysed by FTICR (Fig. 7 features an expansion of one charged form at each stage). Significantly, the subsequent cycloaddition reaction of the fraction of functionalised protein had undergone $>95\%$ conversion to the 'click' cycloadduct.

In conclusion, we have demonstrated that strained alkynes based on diether derivatives of 2,2'-diphenol react with azides without the need for a metal catalyst. The strained alkyne can be attached to a biomolecule or other group, hence demonstrating the potential for the reagents to be used broadly in synthetic reactions where a Cu catalyst must be avoided, and

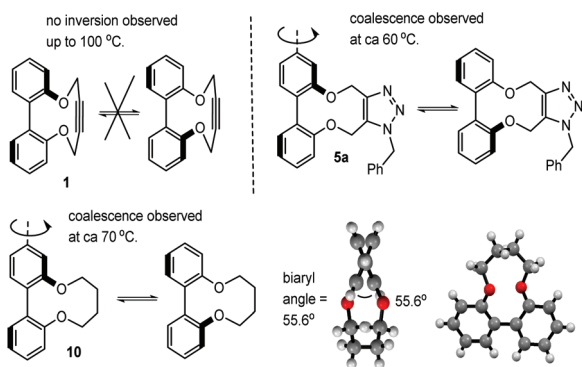


Fig. 5 Restricted rotation of strained alkyne **1**, adduct **5a** and aliphatic compound **10**. Also shown is the X-ray crystallographic structure of **10**.



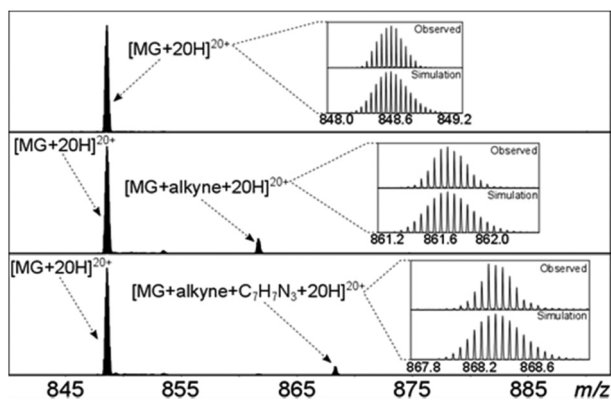


Fig. 7 The FTICR-MS spectra of one charge state of myoglobin. Top; without **14**, middle; following addition of **14**, bottom; following addition of PhCH_2N_3 .

with potential for use in bioconjugation applications. Importantly, the strained alkynes described herein benefit from extreme ease of synthesis, from readily available and inexpensive starting materials.

Acknowledgements

The EPSRC are thanked for funding ADG (project grant ref no. EP/M006670/1) and CKCC (DTG PhD Studentship). Bruker (UK) Ltd are also thanked for support of CKCC through a CASE collaboration. Warwick University are thanked for supporting LG through the Analytical Sciences MSc programme. The X-ray diffraction instrument was obtained through the Science City Project with support from the AWM and part funded by the ERDF.

Notes and references

- E. M. Sletten and C. R. Bertozzi, *Angew. Chem., Int. Ed.*, 2009, **48**, 6974–6998.
- P. Thirumurugan, D. Matosiuk and K. Jowiak, *Chem. Rev.*, 2013, **113**, 4905–4979.
- J. M. Palomo, *Org. Biomol. Chem.*, 2012, **10**, 9309–9318.
- X. Zhang and Y. Zhang, *Molecules*, 2013, **18**, 7145.
- E. Haldon, M. C. Nicasio and P. J. Perez, *Org. Biomol. Chem.*, 2015, **13**, 9528–9550.
- Y. Gong and L. Pan, *Tetrahedron Lett.*, 2015, **56**, 2123–2332.
- H. Li, R. Aneja and I. Chaiken, *Molecules*, 2013, **18**, 9797–9817.
- D. Y. Jackson, *Org. Process. Res. Dev.*, 2016, **20**, 852–866.
- J. D. Thomas, H. Cui, P. J. North, T. Hofer, C. Rader and T. R. Burke Jr., *Bioconjugate Chem.*, 2012, **23**, 2007–2013.
- G. Wittig and A. Krebs, *Chem. Ber.*, 1961, **94**, 3260–3275.
- J. Dommerholt, F. P. J. T. Rutjes and F. L. van Delft, *Top. Curr. Chem. (Z)*, 2016, **374**, 16.
- J. C. Jewett and C. R. Bertozzi, *Chem. Soc. Rev.*, 2010, **39**, 1272–1279.
- M. F. Debets, S. S. van Berkel, J. Dommerholt, A. J. Dirks, F. P. J. T. Rutjes and F. L. van Delft, *Acc. Chem. Res.*, 2011, **44**, 805–815.
- OCT: N. J. Agard, J. A. Prescher and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2004, **126**, 15046–15047.
- DIFO: J. M. Baskin, J. A. Prescher, S. T. Laughlin, N. J. Agard, P. V. Chang, I. A. Miller, A. Lo, J. A. Codelli and C. R. Bertozzi, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, **104**, 16793–16797.
- DIBO: X. Ning, J. Guo, M. Wolfert and G.-J. Boons, *Angew. Chem., Int. Ed.*, 2008, **47**, 2253–2255.
- BCN: J. Dommerholt, S. Schmidt, R. Temming, L. J. A. Hedriks, F. P. J. T. Rutjes, J. C. M. van Hest, D. J. Lefeber, P. Friedl and F. L. van Delft, *Angew. Chem., Int. Ed.*, 2010, **49**, 9422–9425.
- BCN: G. Stefanetti, Q.-Y. Hu, A. Usera, Z. Robinson, M. Allan, A. Singh, H. Imase, J. Cobb, H. Zhai, D. Quinn, M. Lei, A. Saul, R. Adamo, C. A. MacLennan and F. Micoli, *Angew. Chem., Int. Ed.*, 2015, **54**, 13198–13203.
- (a) DIBAC: M. F. Debets, S. S. van Berkel, S. Schoffelen, F. P. J. T. Rutjes, J. C. M. van Hest and F. L. van Delft, *Chem. Commun.*, 2010, **46**, 97–99; (b) BARAC: J. C. Jewett, E. M. Sletten and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2010, **132**, 3688–3690.
- J. Tummatorn, P. Batsomboon, R. J. Clark, I. V. Alabugin and G. B. Dudley, *J. Org. Chem.*, 2012, **77**, 2093–2097.
- Aminobenzene sulphonamide: K. Kaneda, R. Naruse and S. Yamamoto, *Org. Lett.*, 2017, **19**, 1096–1099.
- Crown ether: B. Gold, P. Batsomboon, G. B. Dudley and I. V. Alabugin, *J. Org. Chem.*, 2014, **79**, 6221–6232.
- (a) Thiacycloalkynes: G. de Almeida, E. M. Sletten, H. Nakamura, K. K. Palaniappan and C. R. Bertozzi, *Angew. Chem., Int. Ed.*, 2012, **51**, 2443–2447; (b) Dibenzoselenacycloheptynes: G. de Almeida, L. C. Townsend and C. R. Bertozzi, *Org. Lett.*, 2013, **15**, 3038–3041.
- (a) J. C. Brendel, G. Gody and S. Perrier, *Polym. Chem.*, 2016, **7**, 5536–5543; (b) X. Su, L. Bu, S. Fu, R. Zhiu and Z. Zhong, *RSC Adv.*, 2016, **6**, 2904–2909.
- (a) L. Qian, C.-J. Zhang, J. Wu and S. Q. Yao, *Chem. – Eur. J.*, 2017, **23**, 360–369; (b) S. Ho, D. L. Sackett and J. L. Leighton, *J. Am. Chem. Soc.*, 2015, **137**, 14047–14050.
- (a) U. Koch-Pomeranz, H.-J. Hansen and H. Schmid, *Helv. Chim. Acta*, 1971, **56**, 2981–3004; (b) G. Proni, G. P. Spada, P. Lustenberger, R. Welti and F. Diederich, *J. Org. Chem.*, 2000, **65**, 5522–5527; (c) P. Lustenberge, E. Martinborough, T. M. Denti and F. Diederich, *J. Chem. Soc., Perkin Trans. 2*, 1998, 747–760.
- S. Facchetti, D. Losi and A. Iuliano, *Tetrahedron: Asymmetry*, 2006, **17**, 2993–3003.
- A. Alexakis, D. Polet, S. Rosset and S. March, *J. Org. Chem.*, 2004, **69**, 5660–5667.
- F. Heaney, *Eur. J. Org. Chem.*, 2012, 3043–3058.



- 30 C. G. Gordon, J. L. Mackey, J. C. Jewett, E. M. Sletten, K. N. Houk and C. R. Bertozzi, *J. Am. Chem. Soc.*, 2012, **134**, 9199–9208.
- 31 (a) D. W. Allen, P. N. Braunton, I. T. Millar and J. C. Tebby, *J. Chem. Soc. C*, 1971, 3454–3468; (b) P. N. Braunton, I. T. Millar and J. C. Tebby, *J. Chem. Soc., Perkin Trans. 2*, 1972, 138–412; (c) J. E. Simpson, G. H. Daub and F. N. Hayes, *J. Org. Chem.*, 1973, **38**, 1771.
- 32 B. Schmidt, M. Riemer and M. Karras, *J. Org. Chem.*, 2013, **78**, 8680–8688.

