Organic & Biomolecular Chemistry

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/c5ob00984g

Self-immolative base-mediated conjugate release from triazolylmethylcarbamates†

Christopher A. Blencowe,[a] David W. Thornthwaite,[b] Wayne Hayes*[a] and Andrew T. Russell*[a]

Received 14th May 2015,
Accepted 7th July 2015

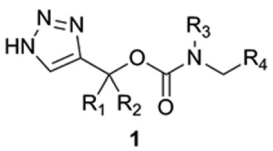
DOI: 10.1039/c5ob00984g

www.rsc.org/obc

A range of carbamate functionalized 1,4-disubstituted triazoles featuring a base sensitive trigger residue, plus a model aromatic amine reporter group, were prepared *via* copper(I) catalysed azide–alkyne cycloaddition and evaluated for their self-immolative characteristics. This study revealed a clear structure–reactivity relationship, *via* Hammett analysis, between the structure of the 1,4-disubstituted triazole and the rate of self-immolative release of the amine reporter group, thus demonstrating that under basic conditions this type of triazole derivative has the potential to be employed in a range of chemical release systems.

It is well-established that the activity of a compound (*e.g.* drug or fragrance, referred to here as a 'reporter' group) can be attenuated by attachment to a protecting moiety (referred to here as a 'trigger' group).[1–4] The spatial proximity of trigger and reporter groups can be significant, especially for scissile bonds requiring enzyme mediated cleavage.[5,6] This steric effect can be negated by inserting a linker unit between trigger and reporter moieties, of which 'self-immolative' linkers have become an important development in recent years, especially in the area of polymeric drug delivery systems.[7–11] In this approach, the linker forms a scissile bond with the trigger moiety and a stable bond to the reporter group – the latter unit is released *via* solvolysis upon removal of the trigger group which effects rapid irreversible disassembly of the three components through a cascade of elimination processes. This phenomenon has been observed for polysubstituted, electron-rich aromatic species that feature an electron-releasing substituent in conjugation with a suitable leaving group at a benzylic position and has been extended recently to heterocyclic systems.[12–15] In particular, 1,4-disubstituted triazoles exhibit self-immolative characteristics under acidic conditions[16] and have been exploited for the controlled release of alcohols,[17] amines[18] and anilines.[19] Recently, an acid-sensitive triazole-based self-immolative linker has been applied by Ackermann *et al.* to the directed, iron-catalysed, arylation of arenes and alkenes, conferring both high regio- and diastereoselectivity, and facilitating traceless cleavage under mild conditions.[20] In contrast, self-immolative triazole linkers designed to degrade under alkaline conditions have not been reported to date. In the light of the development of the efficient copper(I) catalyzed [3 + 2] cycloaddition between azides and alkynes, 1,2,3 triazoles have been studied extensively for a wide range of applications in recent years.[21,22] This paper reports the synthesis of a range of novel degradable triazoles *via* a copper-catalysed azide–alkyne cycloaddition approach and details the study of their self-immolative ability under basic conditions. This linker technology was designed with a broad application spectrum in mind, focussing initially on the controlled release of amines. In this study, we demonstrate that the generic 1*H*-triazole **1** (Fig. 1) is capable of displaying self-immolative characteristics upon formation of the corresponding triazolyl anion. Furthermore, we show that the rate of self-immolative elimination was tunable by the degree and nature of substitution at the triazole α-methine position (**1**, R_1 and R_2).

To facilitate this study, triazoles featuring both the base labile pivaloyloxymethyl moiety and the alkaline stable benzyl protecting group were prepared and evaluated – the latter to demonstrate the importance of triazolyl anion formation as a



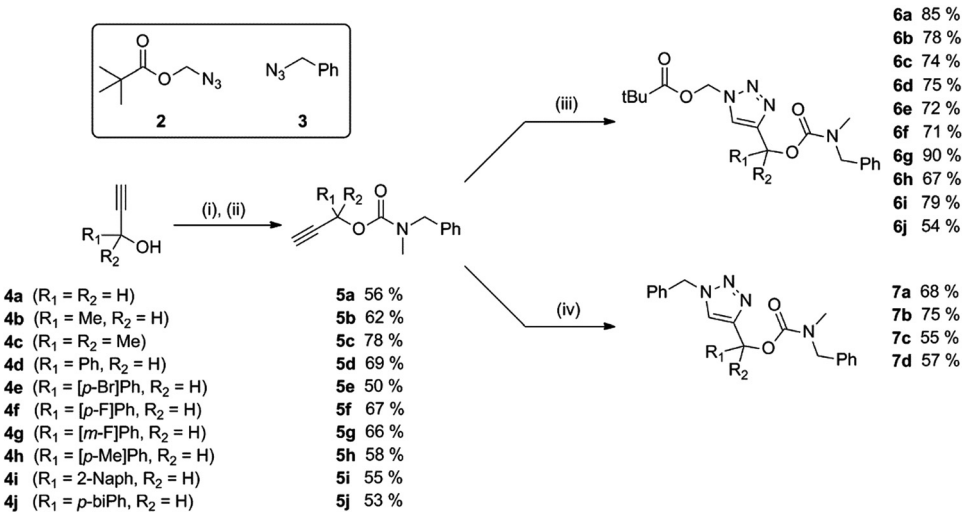
1

Fig. 1 Self-immolative 1*H*-triazole.

[a]*Department of Chemistry, University of Reading, Reading, Berkshire RG6 6AD, UK. E-mail: a.t.russell@reading.ac.uk; Fax: +44 (0)118 378 6331; Tel: +44 (0)118 378 6234*

[b]*Unilever Research and Development, Quarry Road East, Bebington, Wirral CH63 3JW, UK. E-mail: david.thornthwaite@unilever.com; Fax: +44 (0)151 641 1852; Tel: +44 (0)151 641 3257*

†Electronic supplementary information (ESI) available: Experimental and characterization data of triazoles **6a–6j** and **7a–7d** and additional mechanistic details. See DOI: 10.1039/c5ob00984g



Scheme 1 Synthesis of POM protected triazoles **6a–6j** and benzyl protected triazoles **7a–7d**. *Reagents and conditions*: (i) *p*-nitrophenylchloroformate, pyridine, CH_2Cl_2, rt, 16–24 h, (ii) NHMeBn, pyridine, CH_2Cl_2, rt, 12–16 h, (iii) **2**, CuI (5–10 mol%), pyridine, rt, 2–12 h, (iv) **3**, CuI (5–10 mol%), pyridine, rt, 2–12 h.

prerequisite for self-immolative elimination. Pivaloyloxymethyl (POM) azide[23] **2** and benzyl azide **3** were prepared from their corresponding halides. *N*-Methylbenzylamine was chosen as the model reporter group as the *N*-methyl substituent could be used as a marker in the degradation studies and removed any ambiguity regarding the site of deprotonation. To elucidate structure–reactivity relationships in the degradation of the triazole conjugates, a structurally diverse range of alkyl- and aryl-substituted propargyl *N*-methylbenzylcarbamate precursors were targeted. Propargyl alcohols **4d–4j** were synthesized by Grignard reaction of ethynyl magnesium bromide and the corresponding aldehydes *via* a known method (Scheme 1).[24]

The corresponding triazoles were prepared in acceptable yields (54–90%) following the method devised by Bertrand and Gesson.[16] In each case, the formation of the triazole adduct was followed by ^{1}H NMR spectroscopy which revealed the characteristic downfield shift of the acetylene proton upon aromatization. In addition, AB system resonances correlating to the benzyl- and POM- methylene protons, respectively, were also observed as a result of the asymmetric centre in triazole **6** or **7** (other than **6**/**7c**).

^{1}H NMR spectroscopy was also used to monitor the kinetics of the self-immolative processes and provided an insight to the degradation pathways. A non-aqueous polar protic system (sodium methoxide-d_3 in methanol-d_4) was chosen in conjunction with TMS as the calibrant for the degradation studies. De Groot *et al.* confirmed the self-immolative elimination of 2,6-bis(hydroxymethyl)phenol based linkers in an analogous manner.[25] The excess amount of NaOMe-d_3 used was based upon the observations of Sharpless *et al.*, who determined that 2.2 equivalents of base were required to effect efficient pivaloyloxymethyl deprotection.[23] In the case of triazoles **6a–6j** a common degradation profile was observed spectroscopically (see Fig. 2).

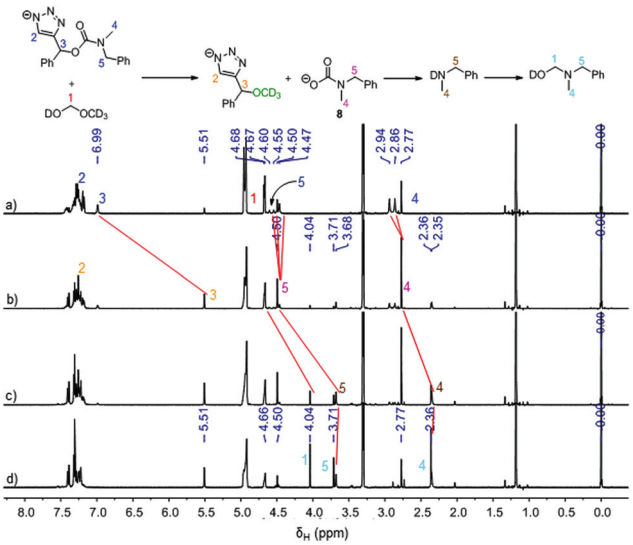


Fig. 2 ^{1}H NMR spectra showing self-immolative release from triazolyl anion intermediate from **6d**; (a) *t* = 5 min. (b) *t* = 15 min. (c) *t* = 46 min. (d) *t* = 102 min.

^{1}H NMR spectra obtained were interpreted by reference to chemical shift data of standards (such as (1*H*-triazol-4-yl)phenylmethyl methyl ether-d_3, (1*H*-triazol-4-yl)phenylmethyl alcohol, methyl *N*-methylbenzyl-carbamate and *N*-hydroxymethyl-*N*-methylbenzylcarbamate) in order to determine the presence of the triazole linker fragment and amine reporter. The carbamate anion **8** was also identified by an HMBC spectrum. For triazoles **6a–6j** the deprotection of the POM group was found to be rapid and complete within the timescale required to make the first measurement (*ca.* 4 minutes). Using triazole **6d** as a representative example (Fig. 2), formation of

the triazolyl anion *in situ* was characterized by an upfield shift of the triazole proton from rotameric resonances at δ_H 8.02 and 7.85 ppm, respectively, into the aromatic region (*ca.* δ_H 7.30 ppm) and the singlet α-methine proton resonance at δ_H 6.99 ppm (see ESI, Fig. S27†). Rotameric resonances for the *N*-methylene (AB pattern) and *N*-methyl protons were observed at δ_H 4.65–4.47, and 2.94–2.86 ppm, respectively, their positions not affected by deprotection. Degradation of the triazolyl anion fragment was observed by the decrease in intensity and disappearance of the singlet and rotameric resonances corresponding to the α-methine and *N*-methyl protons over the course of approximately two hours.

We observed transient species following self-immolative elimination of the triazolyl anion, for example, solvolysis at the α-methine position led to the release of the *N*-methylbenzylcarbamate anion **8**, which had characteristic resolution of the rotameric methylene resonances to a sharp singlet resonance at δ_H 4.50 ppm. This intermediate anion then collapsed to afford the reporter unit, *N*-methylbenzylamine and carbon dioxide. The former reacted further with formaldehyde generated as a by-product of POM deprotection, to afford *N*-hydroxymethyl-*N*-methylbenzylamine (Scheme 2). For comparison, benzyl protected triazoles **7a–7d** were evaluated under the same conditions. Cleavage of the carbamate linker or release of *N*-methylbenzylamine was not observed, even after several months. In this case treatment of triazoles **7a–7d** with NaOMe-d_3 in methanol-d_4 resulted in slow deuteration of the triazole ring at the C-5 position (see ESI, Fig. S30†).

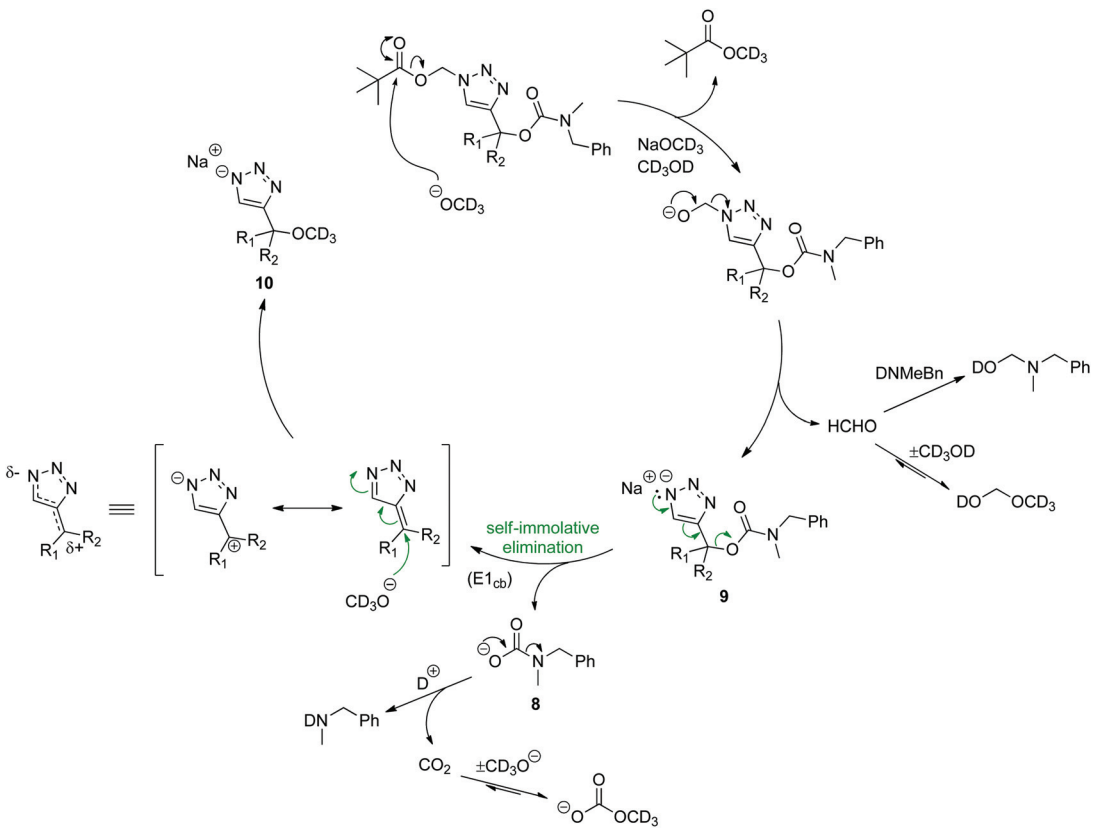
Table 1 Rate constants and half-lives for triazoles **6a–6j**

Triazole	α-Substituent	$t_{1/2}$ (minutes)	k_0 (min^{-1})
6a	CH_2	86 413[a] ± 2493	$8.02 \times 10^{-6} \pm 2.4 \times 10^{-7}$
6b	Me	534.9 ± 19.9	0.0016 ± 0.0006
6g	[*m*-F]Ph	87.2 ± 1.0	0.0084 ± 0.0002
6e	[*p*-Br]Ph	27.1 ± 0.6	0.0253 ± 0.0004
6d	Ph	15.3 ± 1.3	0.0439 ± 0.002
6f	[*p*-F]Ph	13.2 ± 0.8	0.0582 ± 0.002
6i	2-Naph	9.5 ± 0.2	0.0727 ± 0.003
6j	*p*-biPh	8.1 ± 0.7	0.0856 ± 0.008
6h	[*p*-Me]Ph	5.4 ± 0.3	0.1426 ± 0.021
6c	*Gem*-diMe	<2	ND[b]

[a] Calculated based on rate constant. Errors expressed as first standard deviation of values measured in triplicate. [b] Not determined.

The nature of the substituent at the α-methine position of the triazole had a profound effect upon the fragmentation rate of the α-methine-carbamate C–O bond (see Table 1).[18]

In order for degradation of the protected triazoles to occur at rates amenable to applications in controlled delivery systems, secondary or tertiary triazole α-carbon linkages were required. The use of variously substituted phenyl groups offered potential to fine-tune such release and so were exam-



Scheme 2 Proposed mechanism for the self-immolative elimination of *N*-methylbenzylamine from triazoles **6a–6j**.

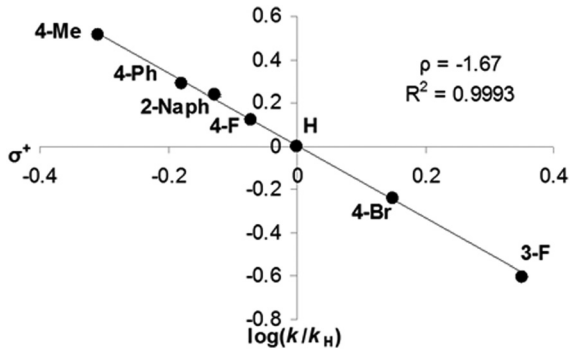


Fig. 3 Hammett plot of the logarithm of relative rate *vs.* σ+ values.

ined as part of this study. The inclusion of a phenyl substituent provided an immediately measurable degradation rate, whilst the presence of electron releasing or electron-withdrawing substituents resulted in further incremental changes.[18,19]

Under these conditions the *gem*-dimethyl derivative **6c** underwent such rapid self-immolative elimination that accurate kinetic data was not obtained by the ^{1}H NMR spectroscopic assay. In the case of aryl substituted triazoles **6d–6j**, their degradation rates were correlated with their Hammett substituent constants σ+ (Fig. 3), σ and σ-values.[26–28] Using these values and the rate constants obtained, Hammett analysis afforded a graph whose slope gave the reaction constant ρ, which is a measure of the susceptibility of the reaction to electronic effects.

Excellent correlation was obtained when the modified Hammett equation (Brown σ+ values) was used, to account for the through conjugation of the aromatic substituents to the reaction centre. The negative value obtained for ρ (−1.67) indicated that the self-immolative elimination is enhanced by electron-releasing substituents, whilst electron-withdrawing substituents have the opposite effect.[29] The negative sign being consistent with a transition state where the degree of negative charge is decreasing. The linearity of the Hammett plot implied that a common rate determining step is observed for all triazoles across the series. The relative rate obtained for triazole **6f** with respect to triazole **6d** also provided important information regarding the electronic character of the transition state. The overall effect of the fluorine (χ = 3.98) substituent can change significantly depending on the electronics of the transition state, and provide useful insight regarding the reaction mechanism. The rate enhancement observed for triazole **6f** suggested that there is a decrease in negative charge in the transition state of the rate limiting step, which correlated with the interpretation of the Hammett plot. In the case of triazole **6i**, in lieu of a known literature value for σ+, the Hammett plot plus the experimentally determined rate constant was used to define this parameter (σ+ = 1.20).

From the identity of intermediate and product fragments, a detailed mechanism could be postulated (Scheme 2, also see ESI, Fig. S32†). From rapid methanolysis of the POM group, the formation of methyl pivalate-d_3 and formaldehyde were confirmed, the latter of which existed predominantly in the methoxymethanol-d_3 hemiacetal form. As the reaction progressed, *N*-methylbenzylamine was liberated, however, as a result of the 'closed system', reaction of this amine with formaldehyde led to the formation of *N*-hydroxymethyl-*N*-methylbenzylamine. The very low reactivity of **7a–d** implies that self-immolative elimination was facilitated by the formation of triazole anion **9** (1,2,3-triazole pK_a 9.4).[30] The proposed intermediate formed from **9** was not detected, presumably being quenched rapidly, with restoration of aromaticity, by methanol/methoxide addition at the electron deficient α-methine position to afford triazolyl anion intermediate **10**. Using triazole **6d** as a representative example, the identity of the product fragment was confirmed by NMR doping experiments (see ESI, Fig. S28 and S29†). The relative stability of the *N*-methylbenzylcarbamate anion **8** under basic conditions meant that it could be observed and characterized by NMR spectroscopy (ESI, Fig. S31†). Subsequent decarboxylation led to the expulsion of carbon dioxide, which was quenched with methoxide to afford methylcarbonate anion, and liberation of the reporter group *N*-methylbenzylamine. The presence of formaldehyde-methoxymethanol-d_3 formed *via* POM deprotection, resulted in further reaction of *N*-methylbenzylamine to give *N*-hydroxymethyl-*N*-methylbenzylamine.

The absence of the hydroxy analogue of **10** strongly suggested that direct attack of methoxide at the carbamate carbonyl carbon, by a '$B_{AC}2$' type mechanism, could be discounted. Hence, from the Hammett analysis and degradation profiles observed spectroscopically, it was concluded that a single mechanism is operative.

Conclusions

We have demonstrated that triazolylmethylcarbamates are susceptible to base mediated self-immolative elimination. The stability of triazoles **7a–7d** confirmed that formation of the triazolyl anion is a prerequisite step in this degradation process. A structure–reactivity relationship study highlighted that the reaction proceeds with reduction of negative charge in the transition state of the rate limiting step and degrades *via* an '$E1_{cb}$' type 1,4-elimination. By altering the nature of the triazole α-methine substituent, predictable changes in degradation rate were realized and quantified. Work investigating the scope of this triazole linker for the controlled release of alcohols under acidic/basic conditions, and the synthesis and evaluation of self-immolative triazole–polymer conjugates, will be the subject of further publications.

Acknowledgements

We gratefully acknowledge EPSRC, Unilever and the University of Reading for funding (PhD studentship for CAB). The authors also acknowledge the University of Reading for access

to analytical instrumentation within the Chemical Analysis Facility.

Notes and references

1 A. Albert, *Nature*, 1958, **182**, 421–422.
2 N. J. Harper, *J. Med. Pharm. Chem.*, 1959, **1**, 467–500.
3 A. K. Sinhababu and D. R. Thakker, *Adv. Drug Delivery Rev.*, 1996, **19**, 241–273.
4 V. J. Stella and K. J. Himmelstein, *J. Med. Chem.*, 1980, **23**, 1275–1282.
5 P. K. Chakravarty, P. L. Carl, M. J. Weber and J. A. Katzenellenbogen, *J. Med. Chem.*, 1983, **26**, 638–644.
6 F. M. H. De Groot, A. C. W. de Bart, J. H. Verheijen and H. W. Scheeren, *J. Med. Chem.*, 1999, **42**, 5277–5283.
7 P. L. Carl, P. K. Chakravarty and J. A. Katzenellenbogen, *J. Med. Chem.*, 1981, **24**, 479–480.
8 P. D. Senter, W. E. Pearce and R. S. Greenfield, *J. Org. Chem.*, 1990, **55**, 2975–2978.
9 G. Le Corre, E. Guibe-Jampel and M. Wakselman, *Tetrahedron*, 1978, **34**, 3105–3112.
10 L. D. Taylor, J. M. Grasshoff and M. Pluhar, *J. Org. Chem.*, 1978, **43**, 1197–1200.
11 (*a*) S. Gnaim and D. Shabat, *Acc. Chem. Res.*, 2014, **47**, 2970–2984; (*b*) C. A. Blencowe, A. T. Russell, F. Greco, W. Hayes and D. Thornthwaite, *Polym. Chem.*, 2011, **2**, 773–790.
12 M. P. Hay, R. F. Anderson, D. M. Ferry, W. R. Wilson and W. A. Denny, *J. Med. Chem.*, 2003, **46**, 5533–5545.
13 M. P. Hay and W. A. Denny, *Tetrahedron Lett.*, 1997, **38**, 8425–8428.
14 W. K. Anderson, D. Bhattacharjee and D. M. Houston, *J. Med. Chem.*, 1989, **32**, 119–127.
15 M. A. Naylor, S. A. Everett, K. B. Patel, M. R. L. Stratford and P. Wardman, *Bioorg. Med. Chem. Lett.*, 1999, 1267–1272.
16 P. Bertrand and J. P. Gesson, *J. Org. Chem.*, 2007, **72**, 3596–3599.
17 M. Mondon, R. Delatouche, C. Bachmann, G. Frapper, C. Len and P. Bertrand, *Eur. J. Org. Chem.*, 2011, 2111–2119.
18 R. Delatouche, M. Mondon, A. Gil, G. Frapper, C. Bachmann and P. Bertrand, *Tetrahedron*, 2011, **67**, 401–407.
19 R. Delatouche, C. Bachmann, G. Frapper and P. Bertrand, *Synthesis*, 2012, 1090–1094.
20 Q. Gu, H. H. Al Mamari, K. Graczyk, E. Diers and L. Ackermann, *Angew. Chem., Int. Ed.*, 2014, **53**, 3868–3871.
21 V. V. Rostovtsev, L. G. Green, V. V. Fokin and K. B. Sharpless, *Angew. Chem., Int. Ed.*, 2002, **41**, 2596–2599.
22 C. W. Tornoe, C. Christensen and M. Meldal, *J. Org. Chem.*, 2002, **67**, 3057–3064.
23 J. C. Loren, A. Krasinski, V. V. Fokin and K. B. Sharpless, *Synlett*, 2005, 2847–2850.
24 (*a*) S. Chassing, M. Kueny-Stotz, G. Isorez and R. Brouillard, *Eur. J. Org. Chem.*, 2007, 2438–2448; (*b*) J. A. Baccile, M. A. Morrell, R. M. Falotico, B. T. Milliken, D. L. Drew and F. M. Rossi, *Tetrahedron Lett.*, 2012, **53**, 1933–1935.
25 F. M. H. De Groot, C. Albrecht, R. Koekkoek, P. H. Beusker and H. W. Scheeren, *Angew. Chem., Int. Ed.*, 2003, **42**, 4490–4494.
26 H. C. Brown and Y. Okamoto, *J. Am. Chem. Soc.*, 1958, **80**, 4979–4987.
27 H. H. Jaffe, *Chem. Rev.*, 1953, **53**, 191–254.
28 M. B. Smith and J. March, in *March's Advanced Organic Chemistry*, J. Wiley & Sons, NJ, 2007, pp. 401–412, and references therein.
29 P. Sykes, in *A Guidebook to Mechanism in Organic Chemistry*, Longman Group Ltd, NY, 1965, pp. 345–381.
30 J. A. Joule and K. Mills, in *Heterocyclic Chemistry*, Blackwell Publishing, Oxford, 4th edn, 2000, p. 505.