



Visible-light unmasking of heterocyclic quinone methide radicals from alkoxyamines†

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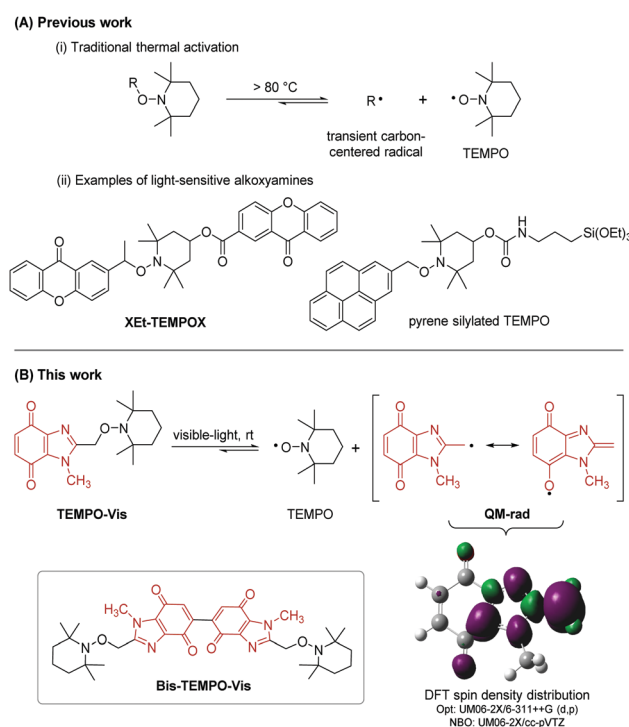
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In nature, the unmasking of heterocyclic quinones to form stabilized quinone methide radicals is achieved using reductases (bioreduction). Herein, an alternative controllable room-temperature, visible-light activated protocol using alkoxyamines and bis-alkoxyamines is provided. Selective synthetic modification of the bis-alkoxyamine, allowed chromophore deactivation to give one labile alkoxyamine moiety.

Nitroxides are bench-stable free radicals and anti-oxidants with a broad range of applications. The most widely used is commercial (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO) with applications in synthesis and catalysis,^{1a} spin labelling,^{1b} magnetic resonance imaging (MRI),^{1c} fluorescence,^{1d} electrochemistry,^{1e} high-tech polymers,^{1f} and radical scavenging.^{1g} Nitroxides mask reactive carbon-centered radicals in the form of alkoxyamines. Homolysis of alkoxyamines is traditionally achieved *via* thermal activation, which for TEMPO usually requires temperatures above 80 °C (Scheme 1A).² The high temperature reversible trapping of initiator-derived alkyl and polymer radicals by nitroxide, is a process made popular by controlled/living nitroxide-mediated polymerization (NMP).³ There are examples of NMP using UV-light driven photodissociation of alkoxyamines.⁴ From a biomedical applications perspective, alkoxyamine activation at or near physiological temperature is essential. Low or ambient temperature activation using UV/visible-light has been reported using alkoxyamine derivatives of 4-hydroxy-TEMPO (4-OH-TEMPO),^{4c,5} including XEt-TEMPOX.^{5c} Guillauneuf and co-workers coupled benzylic derivatives of naphthalene, benzophenone, coumarin, anthraquinone and pyrene with 4-OH-TEMPO to give a series of UV/visible-light sensitive



Scheme 1 (A) Literature alkoxyamines (B) DFT-predicted controllable unmasking of heterocyclic quinone methide radicals using visible-light.

alkoxyamines (including the silylated TEMPO derivative).^{5b} For reported light-sensitive alkoxyamines,^{4c,5} the formation of thermodynamically stabilized benzylic radicals promotes the loss of TEMPO. Comparably, the generation of a quinone methide drives enzymatic bioreduction of mitomycin C (MMC) to allow aziridiny ring-opening and elimination of the carbamate functionality to give reactive sites for cross-linking with DNA.⁶ Benzimidazolequinone anti-tumor alternatives to MMC have been designed as prodrugs to form quinone methide upon bioreduction,⁷ or with adjustment of pH.⁸ Herein, we introduce heterocyclic benzimidazolequinone-based alkoxyamines; the simplest is TEMPO-Vis, where visible-light activated homolysis is

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driven by the formation of a quinone methide-type radical (**QM-rad**), stabilized by resonance delocalization (Scheme 1B). Bis-alkoxyamines are described, and visible-light is used for the first time to release up to two equivalents of TEMPO per molecule, using **Bis-TEMPO-Vis**. Bis- or bifunctional alkoxyamine activation has only been previously achieved by thermal means (at 70–140 °C),^{3,9} which for non-TEMPO bis-alkoxyamines leads to nitroxide decomposition.^{9c}

The premise for this room temperature homolysis was obtained through DFT investigation of the level of delocalization of the unpaired electron in **QM-rad** (Fig. S1, ESI†). DFT supported the traditional resonance structures with a shortening of the 2C–CH₂ bond relative to **TEMPO-Vis**, indicating partial double bond character (Table S1, ESI†), and significant distribution of spin density into the benzimidazolequinone ring system, including onto the quinone 7O-atom. The bond dissociation energy (BDE), and the lowest triplet energy level (E_T) of **TEMPO-Vis** were estimated using DFT. The model-alkoxyamine (**TEMPO-Vis**) was found to have a suitably low BDE (85.1 kJ mol⁻¹) compared to benzylic thermally labile (92.7–108.7 kJ mol⁻¹),¹⁰ and UV-light activated (80–122 kJ mol⁻¹)^{4a} alkoxyamines, with enough driving force in the E_T to support the observed bond dissociation: $\Delta G_d = \text{BDE} - E_T = -122.3 \text{ kJ mol}^{-1}$ (see later discussion).

The preparation of **TEMPO-Vis** and **Bis-TEMPO-Vis** from 4,7-dimethoxybenzimidazole alkoxyamine precursor **1**, using one-step oxidation was investigated (Table 1). Previous work used NBS in combination with H₂SO₄ to convert 4,7-dimethoxybenzimidazole into benzimidazolequinone.¹¹ In the present work, **TEMPO-Vis** was isolated in 52% yield, with some undesirable bromination of **1** observed. PIFA [(CF₃CO₂)₂IPh] improved the yield of **TEMPO-Vis** to 78%. Oxidative dimerization of dimethoxybenzenes to dibenzoquinones is reported through the use of CAN [Ce(NH₄)₂(NO₃)₆],¹² and one report exists of a benzimidazolequinone dimer in low yield.¹³ Treatment of **1** with CAN (2 equiv.) afforded bis-alkoxyamine **2** in 86% yield, as the product of oxidative coupling of dimethoxybenzimidazole **TEMPO-Vis** with dimethoxybenzimidazole **1**. Increased amounts of CAN (3.2 equiv.) gave the fully oxidized **Bis-TEMPO-Vis** in 82% yield. The selective dimerization at C5–C5' was confirmed by X-ray crystallography of **Bis-TEMPO-Vis**, with alternative couplings at C6–C6', C5–C6' and C6–C5' not observed (Table 1).

To investigate the necessity in **Bis-TEMPO-Vis** of the benzimidazolequinone chromophore for the release of both attached TEMPO residues, a selective epoxidation strategy was devised for single chromophore deactivation (Scheme 2). Given its asymmetric aromatic/non-aromatic nature, **2** was deemed a good substrate for selective quinone functionalization. Subjecting **2** to the Langlois reaction (using NaSO₂CF₃),¹⁴ gave the electrophilic trifluoromethylated quinone bis-alkoxyamine **3** in 54% yield. The oxidative demethylation of **3** using NBS and H₂SO₄ (using Table 1 conditions), followed by mild epoxidation at the CF₃-containing quinone moiety, *via* air oxidation under basic conditions, furnished epoxide-quinone **4** in 78% yield.

Visible-light activated alkoxyamine homolysis was carried out under an O₂ atmosphere to trap carbon-centered radicals,^{4a,5b,15} while monitoring alkoxyamine decay and TEMPO release by HPLC (Table 2).^{9b,16} Under blue LED (420–520 nm), 87% conversion of **TEMPO-Vis** to TEMPO was observed after 2.25 min. The decay of **TEMPO-Vis** alkoxyamine was first-order (Fig. 1A), from which the dissociation rate constant (k_d) was determined, and corresponded to a half-life ($t_{1/2}$) of 6 s. By monitoring [TEMPO] growth over time (Fig. 1B), eqn (1) may be fitted to the plot, to provide an alternative method to determine k_d , which also gave a $t_{1/2}$ of 6 s.

$$[\text{TEMPO}] = [\text{TEMPO}]_{\text{max}}(1 - e^{-k_d t}) \quad (1)$$

Alkoxyamines were indefinitely stable in the absence of light, and the on/off switchable nature of homolysis was demonstrated by alternating periods of light and dark for **TEMPO-Vis** using blue LED (Fig. S2, ESI†).

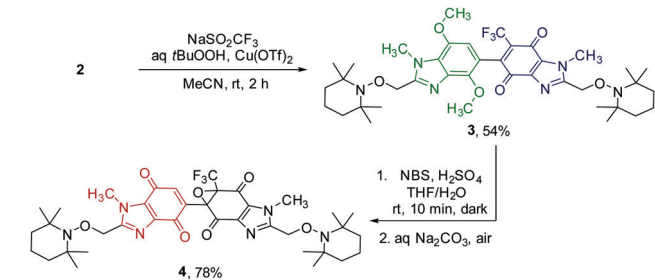
For the bis-alkoxyamine, **Bis-TEMPO-Vis**, there are two possible dissociation rate constants, k_{d1} and k_{d2} corresponding to TEMPO release from the starting compound, and from the monoalkoxyamine O₂-trapped intermediate(s), with hydroperoxide and aldehyde intermediates detected by HPLC-MS (Fig. S3, ESI†). Just over twice as much TEMPO was released from **Bis-TEMPO-Vis** compared to **TEMPO-Vis**, with 175% released after the same period of 2.25 min. The k_{d1} was directly measured from the first-order decay plot of **Bis-TEMPO-Vis**, and corresponded to a $t_{1/2}$ of 15 s in blue LED. The slower photolysis of **Bis-TEMPO-Vis** was supported by the DFT-derived ΔG_d being 11.7 kJ mol⁻¹ less favorable compared to **TEMPO-Vis** (Table 2). The rate of overall TEMPO release attained by fitting eqn (1) to the [TEMPO] vs. time plot (Fig. 1B),

Table 1 Oxidation of dimethoxybenzimidazoles to visible-light sensitive benzimidazolequinone-alkoxyamines^{a,b}

Oxidant	Equiv.	TEMPO-Vis (%)	2 (%)	Bis-TEMPO-Vis (%)
NBS ^c	1.1	52 ^d	—	—
PIFA ^e	1.5	78	—	—
CAN ^f	2.0	—	86	—
CAN ^f	3.2	—	—	82

^a Isolated yields. ^b Performed in the absence of light. ^c H₂SO₄ (1.7 equiv.), THF/H₂O, rt, 10 min. ^d Brominated **1** and recovered **1** detected by HPLC-MS. ^e rt, 3 h. ^f 0 °C, 20 min.





Scheme 2 Chromophore deactivation.

combines release from the starting bis-alkoxyamine (k_{d1}), as well as from the O_2 -trapped intermediate alkoxyamine(s) (k_{d2}). The rate constant derived in this way was in good agreement with the rate of **Bis-TEMPO-Vis** decay (Fig. 1A and Table 2). This infers that for **Bis-TEMPO-Vis**, $k_{d1} \approx k_{d2}$, and the homolysis of the alkoxyamine in the O_2 -trapped intermediate(s) occurs at an almost identical rate to that of the starting bis-alkoxyamine.

By removing one of the chromophores of **Bis-TEMPO-Vis** in epoxide-quinone **4**, the release of <1 equiv. TEMPO occurred over the same time period (Table 2). The homolysis of one alkoxyamine of **4** was detected by HPLC-MS, with singly-homolyzed O_2 -trapped adducts observed (Fig. S3, ESI[†]), and no products of double alkoxyamine homolysis detected. Single bond homolysis occurred despite the BDE of the alkoxyamine of the epoxide part mirroring the BDE of **Bis-TEMPO-Vis**. TD-DFT calculations (see below) supported the localization of the frontier molecular orbitals on only the fully-conjugated quinone moiety of **4** (Fig. 2). The k_d of the labile alkoxyamine of **4** is less than half that of **Bis-TEMPO-Vis** in blue LED, and given that the ΔG_d values of the quinone-alkoxyamine in **4** and **Bis-TEMPO-Vis** are similar (at about -110 kJ mol⁻¹), the observed reduction in rate may be attributed to the lower absorption of the partially deactivated **4** in the visible region (Fig. S4, ESI[†]).

The rate of homolysis decreased using green (470–600 nm) compared to blue LED by 71-, 19- and 40-fold for **TEMPO-Vis**, **Bis-TEMPO-Vis**, and **4** respectively (Fig. S5, ESI[†]), due to less absorption. The decrease in absorbance is reflected in reduced quantum yields (Φ_h) with the greater intensity green LED used

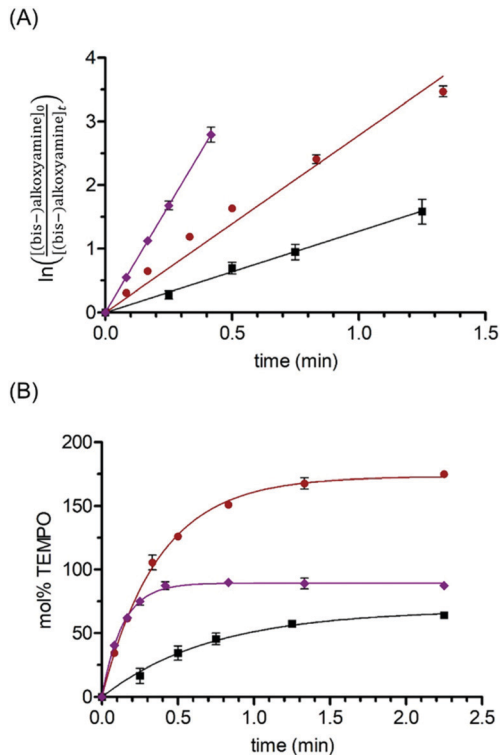


Fig. 1 Kinetics (at rt) of alkoxyamine and bis-alkoxyamine homolysis in blue LED according to (A) (bis-)alkoxyamine decay and (B) TEMPO release. Eqn (1) was fitted to the plots in (B) using GraphPad Prism software. Key: **TEMPO-Vis** (—▲—), **Bis-TEMPO-Vis** (—●—) and **4** (—■—). Conditions according to Table 2.

(see Table S2, ESI[†]). Moreover, **Bis-TEMPO-Vis** underwent photolysis at a faster rate than **TEMPO-Vis** in green LED, in accordance with λ_{max} of the former being red-shifted by 12 nm (Fig. S4, ESI[†]). The result in green LED, is a reversal in the magnitude of k_d that was observed for blue LED for the two compounds. The same conclusion of $k_{d1} \approx k_{d2}$ for **Bis-TEMPO-Vis** was reached for green LED activation.

Bis-alkoxyamine **2** was found to be largely stable under visible-light, having a k_d three orders of magnitude smaller than its fully-oxidized derivative, **Bis-TEMPO-Vis**, in blue and green LED (Table 2). Although **2** possesses a similar first BDE to

Table 2 Kinetics for room-temperature alkoxyamine homolysis under visible-light,^a and DFT-calculated homolysis parameters

Alkoxyamine	LED color	k_d^b via alkoxyamine decay (min ⁻¹)	k_d^c via TEMPO release (min ⁻¹)	mol% ^d TEMPO released (time, min)	BDE ^e (kJ mol ⁻¹)	E_T^e (kJ mol ⁻¹)
TEMPO-Vis	Blue	6.71 ± 0.21	7.29 ± 0.26	87 (2.25)	85.1	207.4
Bis-TEMPO-Vis	Blue	2.78 ± 0.07	2.66 ± 0.09	175 (2.25)	104.9	215.5
4	Blue	1.27 ± 0.16	1.41 ± 0.24	64 (2.25)	99.7, ^f 104.6 ^g	209.7
2	Blue	0.00313 ± 0.00055	0.00417 ± 0.00024	78 (480)	104.1, ^f 111.9 ^g	176.8
TEMPO-Vis	Green	0.0948 ± 0.0032	0.0993 ± 0.0063	80 (25)	—	—
Bis-TEMPO-Vis	Green	0.148 ± 0.002	0.146 ± 0.004	162 (15)	—	—
4	Green	0.0321 ± 0.0007	0.0346 ± 0.0061	43 (65)	—	—
2	Green	$<2 \times 10^{-4}$	—	<5 (480)	—	—

^a Conditions: alkoxyamine (0.25 mM, DCE) illuminated at rt using blue (1 × 9 W) or green (2 × 9 W) LED bulbs under O_2 balloon with HPLC analysis. Experiments performed in triplicate. ^b Dissociation rate (k_d) derived from slope of Fig. 1A and Fig. S5A (ESI). ^c Derived from fit of eqn (1) to Fig. 1B and Fig. S5B (ESI). ^d HPLC yield based on starting alkoxyamine. ^e M06-2X, or UM06-2X for radicals, 6-311++G (d,p) in the gas phase. ^f BDE at benzimidazolequinone part. ^g BDE at epoxide/dimethoxybenzimidazole part.



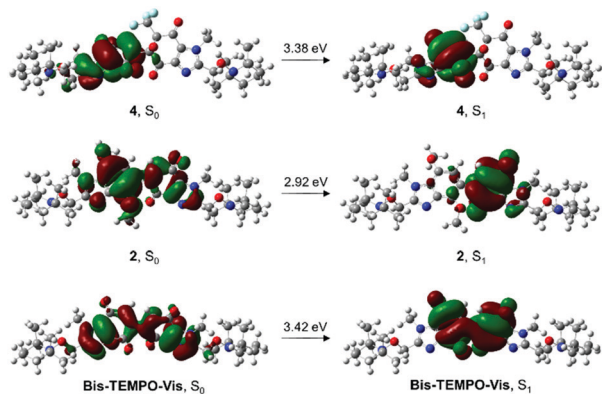


Fig. 2 TD-DFT analysis of ground and excited state orbital delocalization in epoxide-quinone **4**, *p*-dimethoxybenzene-coupled quinone **2**, and **Bis-TEMPO-Vis**. Conditions: PCM/M06-2X/6-311++G (d,p), using DCE as solvent and the natural transition orbital (NTO)¹⁷ method for visualization.

Bis-TEMPO-Vis, its E_T was more than 30 kJ mol⁻¹ lower than the other alkoxyamines. The λ_{\max} of **2** was red-shifted by 96 nm compared to **Bis-TEMPO-Vis**, suggesting the presence of a low-lying charge-transfer (CT) state (Fig. S4, ESI[†]). Cyclic voltammetry on **2** and its constituent alkoxyamines **1** and **TEMPO-Vis**, supported the localization of the HOMO and LUMO to the dimethoxybenzimidazole and the benzimidazolequinone motifs respectively (Fig. S6, ESI[†]). Time-dependent density functional theory (TD-DFT)¹⁸ provided graphical representation of spatially-separated ground and excited state orbitals (Fig. 2). The ground state (S_0) of **2** is primarily localized on the dimethoxybenzimidazole, while the density of the first excited state (S_1) is entirely localized on the quinone, with limited overlap between the two states. In comparison, the CT effect is not observed in the analogous TD-DFT of **Bis-TEMPO-Vis**.

In conclusion, alkoxyamines of heterocyclic quinones are introduced with room temperature visible-light homolysis providing an alternative to nature's bioreductive activation of prodrugs, as a means of unmasking the transient quinone methide. This includes an alkoxyamine that can release up to two equivalents of nitroxide per molecule using visible-light activation, and that does so sequentially with $k_{d1} \approx k_{d2}$. Facile synthetic deactivation of one chromophore limited TEMPO release to <1 equiv. For blue LED, the rates of bond homolysis can largely be rationalized by thermodynamics, while for green LED variations in absorbance become more important. The placement of an electron-rich substituent on the electron-deficient quinone gives a charge-transfer state that stabilizes the quinone under visible-light. The benzimidazole-quinone alkoxyamines offer the possibility of wide-ranging applications from visible-light activated anti-tumour cytotoxins to radical initiators for vinyl monomer photopolymerizations giving polymers end-functionalized with antibiotics.

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Conflicts of interest

There are no conflicts to declare.

Notes and references

- (a) H. A. Beejapur, Q. Zhang, K. Hu, L. Zhu, J. Wang and Z. Ye, *ACS Catal.*, 2019, **9**, 2777; (b) M. M. Haugland, J. E. Lovett and E. A. Anderson, *Chem. Soc. Rev.*, 2018, **47**, 668; (c) J. Wahsner, E. M. Gale, A. Rodríguez-Rodríguez and P. Caravan, *Chem. Rev.*, 2019, **119**, 957; (d) A. Kaur, J. L. Kolanowski and E. J. New, *Angew. Chem., Int. Ed.*, 2016, **55**, 1602; (e) J. E. Nutting, M. Rafiee and S. S. Stahl, *Chem. Rev.*, 2018, **118**, 4834; (f) K.-A. Hansen and J. P. Blinco, *Polym. Chem.*, 2018, **9**, 1479; (g) E. G. Bagryanskaya and S. R. A. Marque, *Chem. Rev.*, 2014, **114**, 5011.
- T. Fukuda, T. Terauchi, A. Goto, K. Ohno, Y. Tsujii, T. Miyamoto, S. Kobatake and B. Yamada, *Macromolecules*, 1996, **29**, 6393.
- J. Nicolas, Y. Guillauneuf, C. Lefay, D. Bertin, D. Gigmes and B. Charleux, *Prog. Polym. Sci.*, 2013, **38**, 63.
- (a) D.-L. Versace, Y. Guillauneuf, D. Bertin, J. P. Fouassier, J. Lalevée and D. Gigmes, *Org. Biomol. Chem.*, 2011, **9**, 2892; (b) Y. Guillauneuf, D. Bertin, D. Gigmes, D.-L. Versace, J. Lalevée and J.-P. Fouassier, *Macromolecules*, 2010, **43**, 2204; (c) S. Hu, J. H. Malpert, X. Yang and D. C. Neckers, *Polymer*, 2000, **41**, 445.
- (a) A. Goto, J. C. Scaiano and L. Maretta, *Photochem. Photobiol. Sci.*, 2007, **6**, 833; (b) M. Baron, J. C. Morris, S. Telitel, J.-L. Clément, J. Lalevée, F. Morlet-Savary, A. Spangenberg, J.-P. Malval, O. Soppera, D. Gigmes and Y. Guillauneuf, *J. Am. Chem. Soc.*, 2018, **140**, 3339; (c) M. Herder and J.-M. Lehn, *J. Am. Chem. Soc.*, 2018, **140**, 7647.
- P. D. Bass, D. A. Gubler, T. C. Judd and R. M. Williams, *Chem. Rev.*, 2013, **113**, 6816.
- (a) W. G. Schulz, R. A. Nieman and E. B. Skibo, *Proc. Natl. Acad. Sci. U. S. A.*, 1995, **92**, 11854; (b) C. Flader, J. Liu and R. F. Borch, *J. Med. Chem.*, 2000, **43**, 3157.
- E. B. Skibo, *J. Org. Chem.*, 1992, **57**, 5874.
- (a) I. Q. Li, B. A. Howell, R. A. Koster and D. B. Priddy, *Macromolecules*, 1996, **29**, 8554; (b) G. Ananchenko and K. Matyjaszewski, *Macromolecules*, 2002, **35**, 8323; (c) J. Ruehl, N. L. Hill, E. D. Walter, G. Millhauser and R. Braslau, *Macromolecules*, 2008, **41**, 1972.
- J. L. Hodgson, L. B. Roskop, M. S. Gordon, C. Y. Lin and M. L. Coote, *J. Phys. Chem. A*, 2010, **114**, 10458.
- L. O'Donovan, M. P. Carty and F. Aldabbagh, *Chem. Commun.*, 2008, 5592.
- B. E. Love, J. Bonner-Stewart and L. A. Forrest, *Synlett*, 2009, 813.
- A. Gellis, H. Kovacic, N. Boufatah and P. Vanelle, *Eur. J. Med. Chem.*, 2008, **43**, 1858.
- B. R. Langlois, in *Modern Synthesis Processes and Reactivity of Fluorinated Compounds*, ed. H. Groult, F. R. Leroux and A. Tressaud, Elsevier, 2017, ch. 5, pp. 125.
- D. W. Grattan, D. J. Carlsson, J. A. Howard and D. M. Wiles, *Can. J. Chem.*, 1979, **57**, 2834.
- G. Moad and E. Rizzardo, *Macromolecules*, 1995, **28**, 8722.
- R. L. Martin, *J. Chem. Phys.*, 2003, **118**, 4775.
- A. D. Laurent and D. Jacquemin, *Int. J. Quantum Chem.*, 2013, **113**, 2019.

