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Nature's hydrides: rapid reduction of halocarbons by folate model compounds

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Halocarbons R–X are reduced to hydrocarbons R–H by folate model compounds under biomimetic conditions. The reactions correspond to a halide–hydride exchange with the methylenetetrahydrofolate (MTHF) models acting as hydride donors. The MTHF models are also functional equivalents of dehalohydrogenases but, unlike these enzymes, do not require a metal cofactor. The reactions suggest that halocarbons have the potential to act as endocrinological disruptors of biochemical pathways involving MTHF. As a case in point, we observe the rapid reaction of the MTHF models with the inhalation anaesthetic halothane. The ready synthetic accessibility of the MTHF models as well as their dehalogenation activity in the presence of air and moisture allow for the remediation of toxic, halogenated hydrocarbons.

Introduction

The structural similarity between methylenetetrahydrofolate (MTHF) **2H** [1–5] and Thauer's hydrogenase **3H** [6] has led us to investigate imidazolidines **1H** as model compounds for the reactivity of these fascinating biomolecules (Fig. 1).

A type of reactivity shared by **2H** and **3H** is the transfer of negatively charged "hydridic" hydrogen leading to the very stable cationic species $[2]^+$ and $[3]^+$.

In addition to its function as a hydride donor, MTHF is involved in several other biochemical pathways including folate-dependent DNA methylation, purine, pyrimidine, and amino acid syntheses, as well as the methylation of homocysteine to methionine. MTHF is also the precursor to 5-methyltetrahydrofolate, an important intermediate in its own right.[1–5]

The recent discovery that the hydride donor system **2H**/$[2]^+$ acts as a photobiological cofactor in the light-activated repair enzyme DNA photolyase[5] demonstrates that MTHF offers significant surprises even after more than sixty years of extensive research. For comprehensive reviews on Thauer's hydrogenase **3H** see ref. 6a–d.

Results

Reduction of halogenated hydrocarbons

During our initial studies,[7] we were surprised to find that imidazolidines **1H** (R: methyl, *tert*-butyl)[8] are unstable in chloroform and resemble many organometallic compounds and hydrido complexes in this respect. A closer examination revealed that the reason for this instability was the reduction of chloroform to dichloromethane by **1H** acting as a hydride donor. By-product of the reduction is the salt $[1]^+Cl^-$ (Scheme 1). We now report that this type of reduction is remarkably general and allows for the dehalogenation of a wide variety of halogenated hydrocarbons including those of toxicological, environmental, and pharmacological significance.

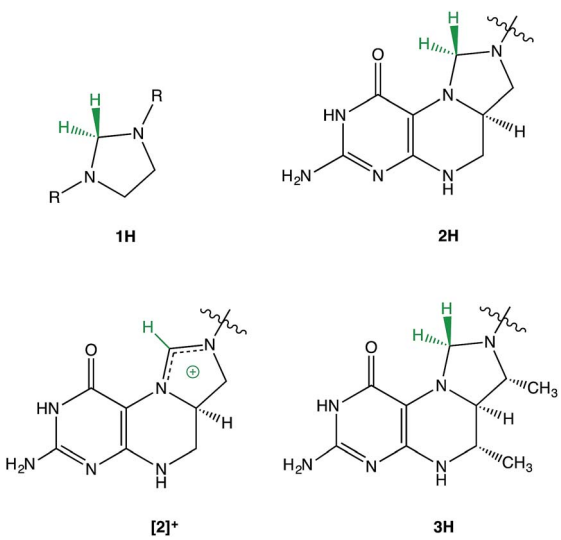

Fig. 1 Structural relationship between imidazolidines **1H**, methylenetetrahydrofolate (MTHF) **2H**, and Thauer's hydrogenase **3H**. Different structural formulae resulting from different N-protonation positions can be found in the literature for **2H**. The structure presented in this figure is the one most commonly used.[1]

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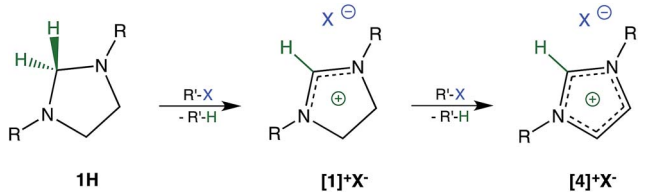
To further explore the scope of the reductive dehalogenation, the reactivity of **1H** towards a series of chloromethanes and bromomethanes was investigated. With the exceptions of dichloromethane, chloromethane, and bromomethane, all chlorine- and bromine-substituted methanes are reduced by **1H**. The reductions occur at room temperature, but the time required for a complete conversion ranges from weeks for chloroform to only minutes for bromoform and carbon tetrabromide. Reactions of **1H** with allyl bromide and ethyl 2-bromoacetate resulted in the formation of complex product mixtures. Details about the reaction as well as the compatibility of functional groups are the subject of on-going studies and will be reported in a separate publication.

The nature of the employed solvent (ethanol, hexanes), an excess of **1H**, or an excess of the halogenated methane do not significantly influence the reaction rates. Remarkably, the dehalogenation reactions are not inhibited by air or moisture.

An excess of the halogenated hydrocarbon and prolonged reaction times lead to further oxidation of $[\mathbf{1}]^{+}\mathbf{X}^{-}$ to $[\mathbf{4}]^{+}\mathbf{X}^{-}$ (Scheme 1).



Scheme 1 Reduction of carbon–halogen bonds R′–X by imidazolidine **1H** (R: *tert*-butyl).

The unsaturated imidazoline analogues of **1H** were previously found to be incompatible with biomimetic conditions due to their high sensitivity towards moisture.[7c] To investigate if the lack of reduction observed for some of the halogenated substrates is due to thermodynamic reasons or is the result of kinetic factors the reaction energies ΔG° for the halide–hydride exchange were determined by quantum chemical calculations (Table 1).[9] A computational solvation model for water was added to simulate biological conditions (see Methods).[10]

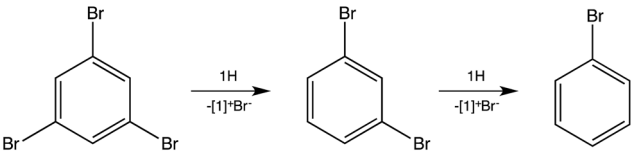
The calculated energies show that all reactions are thermodynamically favourable. The lack of reactivity observed for bromomethane, chloromethane, and dichloromethane is accordingly only the result of low reaction rates.

Table 1 Computational free energies ΔG° (CBS-QB3 level,[11] H_2O-SMD solvent model,[10] kcal mol^{-1}) for the reductions of selected halomethanes (A → B) by **1H** (R: *tert*-butyl)

A	B	X:Cl	X:Br
CX_4	CHX_3	−48.3	−37.9
CHX_3	CH_2X_2	−44.3	−39.1
CH_2X_2	CH_3X	−41.0	−36.5
CH_3X	CH_4	−39.8	−29.3
PhX	PhH	−35.9	−31.3

The observed reaction rates show no correspondence to the calculated reaction energies ΔG° but closely match the order of decreasing carbon–halogen bond energies (CBS-QB3 level, kcal mol^{-1}): CH_3Br (77.1), $CHCl_3$ (77.0), CH_2Br_2 (71.7), CCl_4 (71.9), $CHBr_3$ (65.3), CBr_4 (62.9).

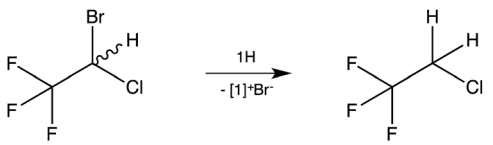
To test for the ability of **1H** to reduce polybrominated commercial flame retardants,[12] 1,3,5-tribromobenzene was investigated (Scheme 2). Dehalogenation to 1,3-dibromobenzene occurred within minutes, even at room temperature, although the total reduction to benzene requires prolonged heating at 150 °C.



Scheme 2 Reaction of imidazolidines **1H** with 1,3,5-tribromobenzene.

As carbon–halogen bonds are found in many medicinal drugs, the dehalogenation reactions observed for the MTHF model **1H** may well occur *in vivo* with MTHF itself, with obvious implications for drug metabolism as well as toxicology.

As a test case, the reaction of the inhalation anaesthetic halothane, $CF_3CH(Br)Cl$, with **1H** was examined (Scheme 3). Dehalogenation was observed (NMR) at room temperature within minutes with 2-chloro-1,1,1-trifluoroethane, CF_3CH_2Cl,[13] being formed as the sole reduction product.



Scheme 3 Reaction of imidazolidines **1H** with 2-bromo-2-chloro-1,1,1-trifluoroethane (halothane).

The high reactivity and selectivity observed suggests that **1H** may become a new substitute for the tin hydrides[18] and hydrosilanes[19] previously used for selective debrominations.

Discussion

While *in vivo* studies will inevitably be required to further study the biochemical implications of these findings, the reactivity of **1H** does suggest that MTHF-dependent biochemical pathways are likely to be disrupted by halogenated hydrocarbons. It is of interest to note in this context that MTHF is the precursor to 5-methyltetrahydrofolate – a compound under current investigation for the treatment of depression and schizophrenia.[4]

The reported reductive dehalogenation reactions are highly unusual for a simple, neutral, and stable organic molecule like

1H, but have precedence in the fascinating reactivity of dehalohydrogenases.[15]

Dehalohydrogenases have been isolated from a variety of microorganisms[14–16] and have attracted great interest for their ability to reduce a number of highly toxic, halogenated hydrocarbons like the notorious 2,3,5,6-tetrachlorodibenzodioxin.[16] A notable difference between our system **1H** and dehalohydrogenases reported to date is that the latter require metal cofactors like iron-sulphur proteins or cobalamines.[17] The fact that dehalohydrogenase-type activity is possible with metal-free folate models **1H** suggests that metal-free dehalohydrogenases can exist as well.

Methods

Quantum chemical calculations

All calculations were carried out with Gaussian 09, Revision D.01.[9] The energies were obtained from full optimizations with the CBS-QB3 method[11] and the SMD solvent model[10] for water. Minima were verified by the absence of virtual frequencies.

Starting materials

The imidazolines **1H** (R: *tert*-butyl, methyl, *para*-methyphenyl) were prepared from the respective 1,2-diaminoethanes as described elsewhere.[7] Halothane and 1,3,5-tribromobenzene were purchased from Sigma-Aldrich Inc.

Reduction of halocarbons

To the best of our knowledge the only previously reported reduction of a halogenated hydrocarbon by a MTHF analogue is that of CCl_4.[8] Reductions were carried out with a slight excess of the imidazolidines **1H** (1.1 equivalent per reducible halogen). In nonpolar solvents like hexanes or diethyl ether, the onset of the reduction is indicated by the appearance of turbidity with subsequent precipitation of the salts $[\mathbf{1}]^+\mathbf{X}^-$. Control experiments in polar solvents or under inert gas (99.995% argon) with degassed materials did not show noticeably different reaction rates or products. For the less reactive aryl halides and chloromethanes, heating was required to drive the reaction to completion. The progress of the reductions was monitored by NMR and GC-MS. After completion of the reaction, the salts were isolated by filtration under inert gas, washed with diethyl ether, and dried *in vacuo*. Yield 85–95%. Traces of $[\mathbf{4}]^+\mathbf{X}^-$ could be removed from $[\mathbf{1}]^+\mathbf{X}^-$ by recrystallization from 95% ethanol.

Reduction of halothane

To retain the highly volatile product 2-chloro-1,1,1-trifluoroethane (bp +6 °C)[13a] the reduction of halothane was monitored in a flame-sealed NMR tube.

Spectroscopic data

2-Bromo-2-chloro-1,1,1-trifluoroethane (halothane). ^{1}H-NMR (600 MHz, C_6D_6): δ 4.58 [q, $^3J(^1H,^{19}F) = 5.4$ Hz]. ^{13}C-NMR (600 MHz, C_6D_6): δ 50.2 [q, $^2J(^{13}C,^{19}F) = 40.9$ Hz, CHClBr], 121.6 [q, $^1J(^{13}C,^{19}F) = 278.0$ Hz, CF_3]. ^{19}F-NMR (600 MHz, C_6D_6 *vs.* $CFCl_3$): δ −76.1 [d, $^3J(^1H,^{19}F) = 5.3$ Hz].

2-Chloro-1,1,1-trifluoroethane. ^{1}H-NMR (600 MHz, C_6D_6): δ 2.78 [q, $^3J(^1H,^{19}F) = 8.5$ Hz]. ^{13}C-NMR (600 MHz, C_6D_6): δ 40.1 [q, $^2J(^{13}C,^{19}F) = 37.7$ Hz, CH_2], 123.1 [q, $^1J(^{13}C,^{19}F) = 275.7$ Hz, CF_3]. ^{19}F-NMR (600 MHz, C_6D_6 *vs.* $CFCl_3$): δ −71.6 [t, $^3J(^1H,^{19}F) = 8.5$ Hz].

1,3,5-Tribromobenzene. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.61 [s]. ^{13}C-NMR (400 MHz, $CDCl_3$): δ 123.4 [C-2,4,6], 133.0 [C-1,3,5]. GC-MS: t_r = 10.28 min *m*/*z* (rel. int%): 314(100)[M^+], 235(30), 156(15), 74(32).

1,3-Dibromobenzene. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 7.67 [m], 7.43 [m], 7.11 [m]. ^{13}C-NMR (400 MHz, $CDCl_3$): δ 123.1 [C-1,3], 130.3 [C-4,6], 131.2 [C-5], 134.2 [C-2]. GC-MS: t_r = 8.57 min *m*/*z* (rel. int%): 236(100)[M^+], 155(41), 75(32).

1,3-Di-*tert*-butylimidazolidinium chloride. Colourless crystals, mp 202–203 °C (from chloroform). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.55 [18H, s, $C(CH_3)_3$], 4.05 [4H, s, CH_2CH_2], 8.85 [1H, s, N_2CH^+]. ^{13}C-NMR (400 MHz, $CDCl_3$): δ 28.2 [$C(\underline{C}H_3)_3$], 45.3 [CH_2CH_2], 57.2 [$C(\underline{C}H_3)_3$], 154.1 [N_2CH^+].

1,3-Di-*tert*-butylimidazolidinium bromide. Colourless crystals, mp 230–231 °C (dec.) (from chloroform). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.56 [18H, s, $C(CH_3)_3$], 4.10 [4H, s, CH_2CH_2], 8.46 [1H, s, N_2CH^+]. ^{13}C-NMR (400 MHz, $CDCl_3$): δ 28.3 [$C(\underline{C}H_3)_3$], 45.5 [CH_2CH_2], 57.2 [$\underline{C}(CH_3)_3$], 153.1 [N_2CH^+].

1,3-Di-*tert*-butylimidazolium chloride. Colourless crystals, mp 209–210 °C (from chloroform). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.80 [18H, s, $C(CH_3)_3$], 7.54 [4H, s, HC=CH], 10.54 [1H, s, N_2CH^+]. ^{13}C-NMR (400 MHz, $CDCl_3$): δ 30.3 [s, $C(\underline{C}H_3)_3$], 60.8 [$\underline{C}(CH_3)_3$], 119.7 [HC=CH], 134.5 [N_2CH^+].

1,3-Di-*tert*-butylimidazolium bromide. Colourless crystals, mp 219–220 °C (from chloroform). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.85 [18H, s, $C(CH_3)_3$], 7.39 [4H, s, HC=CH], 10.37 [1H, s, N_2CH^+]. ^{13}C-NMR (400 MHz, $CDCl_3$): δ 30.4 [$C(\underline{C}H_3)_3$], 61.2 [$\underline{C}(CH_3)_3$], 118.9 [HC=CH], 135.2 [N_2CH^+].

1,3-Dimethylimidazolidinium bromide. Colourless crystals, mp 189–191 °C (from chloroform). ^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.35 [18H, s, NCH_3], 4.01 [4H, s, NCH_2], 9.54 [1H, s, N_2CH^+]. ^{1}H-NMR (400 MHz, DMSO-D_6): δ 3.08 [18H, s, NCH_3], 3.85 [4H, s, NCH_2], 8.41 [1H, s, N_2CH^+]. ^{13}C-NMR (400 MHz, $CDCl_3$): δ 35.2 [NCH_3], 50.9 [CH_2CH_2], 159.4 [N_2CH^+]. ^{13}C-NMR (400 MHz, DMSO-D_6): δ 34.1 [NCH_3], 50.2 [CH_2CH_2], 158.3 [N_2CH^+].

1,3-Di-*para*-methylphenylimidazolidine. Colourless crystals, mp 157–158 °C. ^{1}H-NMR (400 MHz, $CDCl_3$): δ 2.28 [6H, s, C–CH_3], 3.59 [4H, s, CH_2CH_2], 4.60 [2H, s, N_2CH_2], 6.58 [4H, d, ^3J = 8.5 Hz, *meta*-CH], 7.10 [4H, d, ^3J = 8.5 Hz, *ortho*-CH]. ^{13}C-NMR (400 MHz, $CDCl_3$): δ 20.4, [C–$\underline{C}H_3$], 46.8 [CH_2CH_2], 66.4 [N_2CH_2], 112.5, 126.7, 129.8, 144.4. GC-MS: t_r = 17.9 min *m*/*z* (rel. int%): 252(72)[M^+], 251(100), 133(39), 118(16), 105(81), 91(30), 65(10).

1,3-Di-*para*-methylphenylimidazolium bromide. Colourless crystals, mp 292–293 °C (from methanol). ^{1}H-NMR (400 MHz, DMSO-D_6): δ 2.35 [6H, s, C–CH_3], 4.56 [4H, s, CH_2CH_2], 7.36 [4H, d, ^3J = 8.4 Hz, *meta*-CH], 7.53 [4H, d, ^3J = 8.4 Hz, *ortho*-CH], 9.89 [1H, s, N_2CH^+]. ^{13}C-NMR (400 MHz, DMSO-D_6): δ 20.3, [C–$\underline{C}H_3$], 48.2 [CH_2CH_2], 118.1, 129.9, 133.6, 136.4, 150.9 [N_2CH^+].

Conclusions

Our observation that dehalohydrogenase activity is possible with a simple, metal-free MTHF model **1H** suggests that metal-free dehalohydrogenases may likewise exist.

The ready synthetic accessibility of the model compounds **1H** as well as their dehalogenation activity in the presence of air and moisture suggest their use for the remediation of toxic, halogenated hydrocarbons. The reactions suggest that halocarbons are likely to act as endocrinological disruptors of folate dependent pathways.

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Notes and references

1 R. G. Kallen and W. P. Jencks, *J. Biol. Chem.*, 1966, **241**, 5851–5863.

2 (*a*) L. Jaenicke, *Angew. Chem.*, 1961, **73**, 449–480; (*b*) L. Jaenicke, *Annu. Rev. Biochem.*, 1964, **33**, 287–312; (*c*) J. T. Fox and P. J. Stover, *Folic Acid and Folates*, ed. G. Litwack, Elsevier Academic Press Inc., San Diego, 1st edn, 2008, vol. 79, ch. 1, pp. 1–44; (*d*) E. P. Quinlivan, A. D. Hanson and J. F. Gregory, *Anal. Biochem.*, 2006, **348**, 163–184.

3 (*a*) E. R. Werner, N. Blau and B. Thoöny, *Biochem. J.*, 2011, **438**, 397–414; (*b*) M. Födinger, W. H. Hörl and G. Sunder-Plassmann, *J. Nephrol.*, 2000, **13**, 20–33; (*c*) K. Robien and C. M. Ulrich, *Am. J. Epidemiol.*, 2003, **157**, 571–582.

4 (*a*) A. L. Miller, *Altern. Med. Rev.*, 2008, **13**, 216–226; (*b*) M. Fava and D. Mischoulon, *J. Clin. Psychiatry*, 2009, **70**(5), 12–17; (*c*) S. M. Stahl, *CNS Spectr.*, 2007, **12**, 739–744.

5 (*a*) A. Sancar, *Chem. Rev.*, 2003, **103**, 2203–2237; (*b*) R. F. Pauszek III, G. Kodali and R. J. Stanley, *J. Phys. Chem. A*, 2014, **118**, 8320–8328.

6 (*a*) S. Shima and R. K. Thauer, *Chem. Rec.*, 2007, **7**, 37–46; (*b*) B. H. Geierstanger, T. Prasch, C. Griesinger, G. Hartmann, G. Buurman and R. K. Thauer, *Angew. Chem., Int. Ed. Engl.*, 1998, **37**, 3300–3303; (*c*) S. Shima, E. J. Lyon, M. Sordel-Klippert, M. Kauß, J. Kahnt, R. K. Thauer, K. Steinbach, X. Xie, L. Verdier and C. Griesinger, *Angew. Chem., Int. Ed. Engl.*, 2004, **43**, 2547–2551; (*d*) E. J. Lyon, S. Shima, G. Buurman, S. Chowdhuri, A. Batschauer, K. Steinbach and R. K. Thauer, *Eur. J. Biochem.*, 2004, **271**, 195–204.

7 (*a*) M. K. Denk, S. Gupta, J. Brownie, S. Tajammul and A. J. Lough, *Chem.–Eur. J.*, 2001, **7**, 4477–4486; (*b*) M. K. Denk, A. Hezarkhani and F. Zheng, *Eur. J. Inorg. Chem.*, 2007, 3527–3534; (*c*) M. K. Denk, J. M. Rodezno, S. Gupta and A. J. Lough, *J. Organomet. Chem.*, 2001, **617–618**, 242–253.

8 E. Rabe and H. W. Wanzlick, *Liebigs Ann. Chem.*, 1973, 40–44.

9 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery Jr, J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, *GAUSSIAN 09 (Revision D.01)*, Gaussian Inc., Wallingford, CT, 2013.

10 A. Marenich, C. J. Cramer and D. G. Truhlar, *J. Phys. Chem. B*, 2009, **113**, 6378–6396.

11 J. A. Montgomery, M. J. Frisch, J. W. Ochterski and G. A. Petersson, *J. Chem. Phys.*, 2000, **112**, 6532–6542.

12 (*a*) C. A. de Wit, *Chemosphere*, 2002, **46**, 583–624; (*b*) The Stockholm convention (2001) and its annexes list polychlorinated and brominated hydrocarbons that have been phased out due to their persistence in the environment, their bioaccumulation, their toxicity and capability to act as endocrinological disruptors (http://chm.pops.int/). Excluding the list of newly proposed compounds, the list now (2015) comprises 23 chlorinated and brominated hydrocarbons.

13 (*a*) J. Chapman, R. Hill, J. Muir, C. W. Suckling and D. J. Viney, *J. Pharm. Pharmacol.*, 1967, **19**, 231–239; (*b*) F. J. Weigert and J. K. Karel, *J. Fluorine Chem.*, 1987, **37**, 125–149.

14 J. Dolfing, A. J. van den Wijngaard and D. B. Janssen, *Biodegradation*, 1993, **4**, 261–282.

15 (*a*) P. Adriaens and D. Grbić-Galić, *Chemosphere*, 1994, **29**, 2253–2259; (*b*) A. Neumann, G. Wohlfarth and G. Diekert, *J. Biol. Chem.*, 1996, **271**, 16515–16519; (*c*) X. Maymó-Gatell, Y. T. Chien, J. M. Gossett and S. H. Zinder, *Science*, 1997, **276**, 1568–1571; (*d*) W. Schumacher, C. Holliger, A. J. Zehnder and W. R. Hagen, *FEBS Lett.*, 1997, **409**, 421–425; (*e*) G. Wohlfarth and G. Diekert, *Curr. Opin. Biotechnol.*, 1997, **8**, 290–295; (*f*) C. Holliger, G. Wohlfarth and G. Diekert, *FEMS Microbiol. Rev.*, 1998, **22**, 383–398; (*g*) A. Mägli, M. Messmer and T. Leisinger, *Appl. Environ. Microbiol.*, 1998, **64**, 646–650; (*h*) L. Adrian, U. Szewzyk, J. Wecke and H. Görisch, *Nature*, 2000, **408**, 580–583.

16 (*a*) M. Bunge, L. Adrian, A. Kraus, M. Opel, W. G. Lorenz, J. R. Andreesen, H. Görisch and U. Lechner, *Nature*, 2003, **421**, 357–360; (*b*) H. Smidt and W. M. de Vos, *Annu. Rev. Microbiol.*, 2004, **58**, 43–73.

17 (*a*) M. Bommer, C. Kunze, J. Fesseler, T. Schubert, G. Diekert and H. Dobbek, *Science*, 2014, **346**, 455–458; (*b*) K. A. Payne, C. P. Quezada, K. Fisher, M. S. Dunstan, F. A. Collins, H. Sjuts, C. Levy, S. Hay, S. E. Rigby and D. Leys, *Nature*, 2015, **517**, 513–516.

18 (*a*) R. J. Strunk, P. M. DiGiacomo, K. Aso and H. G. Kuivila, *J. Am. Chem. Soc.*, 1970, **92**, 2849–2856; (*b*) A. Sanders and W. P. Giering, *J. Org. Chem.*, 1973, **38**, 3055; (*c*) T. Ando, F. Namigata, H. Yamanaka and W. Funasaka, *J. Am. Chem. Soc.*, 1967, **89**, 5719–5721.

19 (*a*) C. Chatgilialoglu, D. Griller and M. Lesage, *J. Org. Chem.*, 1988, **53**, 3641–3642; (*b*) M. Ballestri, C. Chatgilialoglu, K. B. Clark, D. Griller, B. Geise and B. Kopping, *J. Org. Chem.*, 1991, **56**, 678–683; (*c*) C. Chatgilialoglu, *Chem.–Eur. J.*, 2008, **14**, 2310–2320.