PAPER

CrossMark
click for updates

A quantum chemical study of HOCl-induced transformations of carbamazepine†

Tana Tandarić,[a] Valerije Vrček*[b] and Davor Šakić*[b]

The antiepileptic drug carbamazepine (**CBZ**) is one of the most persistent pharmaceuticals in the environment. Its chemical fate is influenced by the type of wastewater treatment. This study sets out to determine the degradation mechanism and products in the reaction between **CBZ** and hypochlorous acid (HOCl), which is the main chlorinating species in water. In the search for the most feasible pathways of HOCl-induced transformations of **CBZ**, a quantum chemical approach was employed. Chlorination and epoxidation of **CBZ** are two initial, competitive processes that result in two key intermediates: *N*-chloramide and 10,11-epoxide. The calculated free energy barriers ($\Delta G^{\ddagger}_{293}$) for these reactions are 105.7 and 95.7 kJ mol^{-1} resp., which is in agreement with the experimental energy barrier of 98.2 kJ mol^{-1}. All transformation products detected in chlorination experiments were located by computational models, and the reaction mechanism underlying their formation was described in detail. Different computational methods (density functional and *ab initio* theory) were applied, and the double hybrid B2-PLYPD functional was found to be superior in terms of efficiency and accuracy. Of special interest are oxoiminostilbene and formylacridine, which are the final products in the degradation cascade. Their exceptional thermodynamic stability, as predicted by quantum chemical methods, suggests that these structures should be considered as recalcitrants in chlorinated waters. Fruitful interplay between computational models and experimental data proves that the quantum chemical approach can be used as a predictive tool in environmental degradation studies.

Introduction

Carbamazepine (**CBZ**) is a pharmaceutical compound that is commonly used as an anticonvulsant and mood stabilizing drug.[1] **CBZ** is one of the most frequently detected pharmaceuticals in the aquatic environment.[2,3] It is present in surface waters and treated wastewaters at concentrations of up to 6 μg L^{-1}.[4–7] Although there are only preliminary data indicating that **CBZ** has a negative impact on the environment,[8–10] its recalcitrance is worrisome especially as its usage has seen an upward trend in recent years.[11–13]

CBZ is persistent in the environment and is negligibly degraded *via* biotic mechanisms.[14–18] Beyond the biological treatments, a variety of other processes have been probed to eliminate **CBZ** from the environment. Physicochemical processes such as coagulation–flocculation and flotation did not give better results,[19,20] but advanced oxidation processes and different photolytic processes resulted in higher percentages of **CBZ** degradation.[21–25] However, the main drawback is the formation of a wide range of undesirable and toxic by-products.[26,27]

As chlorination remains to be a primary method for wastewater effluent disinfection, it is imperative that further studies be performed to examine the potential reactions between **CBZ** and free chlorine. The performance of chlorination treatment is somewhat controversial, with earlier studies describing the chlorination as an inefficient procedure for **CBZ** elimination,[28,29] whereas recent results indicate that the reaction of **CBZ** and chlorinating agents is a feasible process.[30–32]

In this work we focus our investigation on the transformation reactions of **CBZ** induced exclusively with hypochlorous acid (HOCl), which is the main chlorinating agent in a neutral or slightly acidic aqueous environment (pH = 6–7). Quantum chemical models (density functional and *ab initio* theory) have been extensively used to identify major intermediates and reaction products. To assess the performance of selected computational models, all relevant structures located by the theory were compared with the products detected in chlorination experiments. Two recent experimental studies suggested the formation of 8 different products in the reaction between **CBZ** and HOCl (Chart 1).[30,32] However, the final structures and reaction mechanisms proposed in these two reports do not match,

[a] Ruđer Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia
[b] Faculty of Pharmacy and Biochemistry, University of Zagreb, Ante Kovačića 1, 10000 Zagreb, Croatia. E-mail: dsakic@pharma.hr, vvrcek@pharma.hr
†Electronic supplementary information (ESI) available: Coordinates for all the calculated structures and corresponding energies. See DOI: 10.1039/c6ob02166b
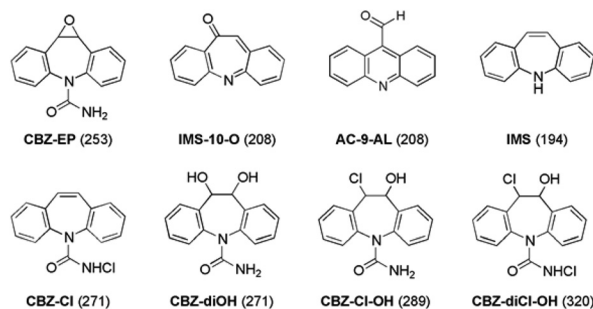
10866 | *Org. Biomol. Chem.*, 2016, **14**, 10866–10874

This journal is © The Royal Society of Chemistry 2016

Chart 1 Proposed structures of products, formed in the reaction between **CBZ** and HOCl, assigned to the respective *m/z* signals (in parentheses) from experimental mass spectra (ref. 30 and 32).

although experiments are carried out under similar reaction conditions. Here we show that the use of quantum-chemical models is suitable to explain and interpret the discrepancy between the two sets of experimental data. The use of quantum chemical methods in solving the issues of ecological concern appears only rarely in the literature.[33–36] We propose that an approach which combines computational techniques with available experimental data has to be employed more often when addressing environmental problems, *e.g.* to identify the major pathways of degradation, and to assist in experimental assignments of transformation products.

Computational details

Quantum chemical calculations were performed using the Gaussian09 suite of programs.[37] All structures were fully optimized with the B3LYP functional.[38,39] The standard split valence and polarized 6-31G(d) basis set was used for geometry optimizations and frequency calculations. All energies are reported at 293.15 K in order to compare the calculated and experimental results. Thermal corrections to Gibbs free energies have been calculated at the same level using the rigid rotor/harmonic oscillator model. Improved energetics have been calculated using the double-hybrid methods B2K-PLYP[40] and B2-PLYPD[41] in combination with the 6-311+G(3df,2p) basis set. The former DFT method shows the best overall performance for calculating barrier heights for water-catalyzed proton-transfer reactions,[42] whereas the latter has "high accuracy and extended applicability".[43] Both DFT models accurately reproduce the results obtained by high-level reference procedures (*e.g.* G3B3),[31,44] with B2-PLYPD values being discussed throughout this text. In addition, single point energies have been calculated using second-order Møller–Plesset perturbation theory (MP2)[45] with 6-311+G(3df,2p) and G3MP2Large basis sets. Energies calculated at the B2-PLYPD level agree well with the values obtained at the MP2 level of theory.

Analytical vibrational analyses at the B3LYP level were performed to characterize each stationary point as a minimum (NImag = 0) or first-order saddle point (NImag = 1). Intrinsic

reaction coordinate (IRC) calculations were performed to identify the minima connected through the transition state.[46]

Gibbs energies of solvation were determined using the CPCM continuum solvation model at the B3LYP/6-31G(d) level, with the UFF atomic radii and electrostatic scaling factor (alpha value) set to 1.1 for all atoms (default values in Gaussian09).[47] The solvent relative permittivity of $\varepsilon = 78.4$ (water) was used. To correctly describe chemical systems in water the inclusion of bulk and specific solvent effects is mandatory. We have found that the addition of explicit water molecules substantially lowers the calculated free energy barriers for all the processes investigated. The number of explicit water molecules is varied in order to identify the most stable structure.[31] In this work three explicit water molecules were included in all calculations throughout the text. For clarity, explicit water molecules are not included in Schemes 1–3.

The most stable forms of water-complexed species are located by placing water molecules in a variety of locations to sample the different arrays of interaction networks available between the reactants and water. Initial configurations were created using a locally modified version of the stochastic search method.[48,49] According to Pliego,[50] the reactant and the explicit water molecules form a rigid cluster that constitutes a distinct chemical species. Therefore, the relative energy of reactants complexed with an optimal number of water molecules is set to zero. All other structures, intermediates and products, reported throughout the text also include extra water molecules.

In order to account for the entropic effect of the presence of solvent molecules around a solute, the cell model presented by Ardura *et al.* was used.[51] This model is proposed in order to explicitly evaluate the effect of the loss of translation degrees of freedom in solution on the Gibbs activation energy in bimolecular (or higher order of molecularity) reaction.[31]

Results and discussion

According to LC-MS analysis, four structures were detected and proposed as products of the reaction between **CBZ** and HOCl (Chart 1): carbamazepine 10,11-epoxide (**CBZ-EP**, *m/z* = 253), acridine-9-carboxaldehyde (**AC-9-AL**, *m/z* = 208), 10-oxo-iminostilbene (**IMS-10-O**, *m/z* = 208), and iminostilbene (**IMS**, *m/z* = 194).[32] The two latter signals were also observed by Soufan *et al.* together with three other peaks (Chart 1) corresponding to carbamazepine 10,11-chlorohydrin (**CBZ-Cl-OH**, *m/z* = 289), carbamazepine 10,11-diol (**CBZ-diOH**, *m/z* = 271), and carbamazepine *N*-chloramide (**CBZ-Cl**, *m/z* = 271).[30] In addition, one signal having 320 *m/z* was termed "unknown".

In this work, our goal is to verify if the calculated potential energy landscape for the reaction between **CBZ** and HOCl involves the same set of structures as predicted by the two chlorination experiments. The computational search for energetically viable transformations of **CBZ** in chlorinated water (HOCl-induced reactions) revealed the two reaction pathways as the most feasible: chlorination and epoxidation of **CBZ**. In

This journal is © The Royal Society of Chemistry 2016

Org. Biomol. Chem., 2016, **14**, 10866–10874 | **10867**

the first reaction pathway the *N*-chloramide **CBZ-Cl** is formed, whereas the second parallel reaction results in the 10,11-epoxide **CBZ-EP**. Once formed, both **CBZ-Cl** and **CBZ-EP** can undergo rearrangements that result in a series of additional transformation products.

In the following sections, we will discuss the mechanism of the initial reactions (chlorination and epoxidation of **CBZ**) responsible for carbamazepine loss, and then we will explain the formation of additional transformation products observed in the chlorination/oxidation experiments.

N-Chlorination of CBZ

Contrary to older reports,[28,29] it has been found that **CBZ** can be chlorinated with HOCl.[32] This is in agreement with a recent study by Soufan *et al.*,[30] and also with our high-level computational results.[31] In our detailed description of the reaction mechanism, the *N*-chloramide product **CBZ-Cl** is formed in a two-step manner (Scheme 1).[31] In the first step, carbamazepine undergoes tautomerization in which the iminol intermediate **CBZ-I** is formed. The less stable (Table 1) but more reactive
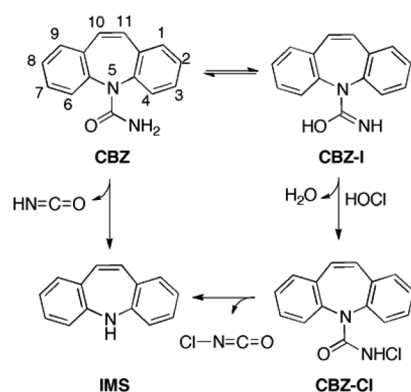

Scheme 1 Tautomerisation of carbamazepine (**CBZ → CBZ-I**), chlorination of its iminol form (**CBZ-I → CBZ-Cl**), and formation of **IMS** from **CBZ-Cl** (chloroisocyanate elimination), and from **CBZ** (deamidation process).

iminol reacts with HOCl in the second step **CBZ-I → CBZ-Cl**. The latter reaction is the rate-determining step and the calculated Gibbs free energy of activation (see below) is within the barrier limits set by experimental measurements.

It has been estimated recently that the rate constant for an elementary reaction between **CBZ** and HOCl at 20 (±2) °C is <1.0 × 10^{-4} M^{-1} s^{-1}, which corresponds to the Gibbs free energy of activation slightly higher than 94 kJ mol^{-1}.[30] In an earlier study,[32] the experimental Gibbs free energy of activation ($\Delta G^{\ddagger}_{293}$) for the reaction between **CBZ** and HOCl is 98.2 kJ mol^{-1}, which is in agreement with the estimation of Soufan *et al.* ($\Delta G^{\ddagger}_{293} > 94$ kJ mol^{-1}).[30]

A similar free energy barrier has been obtained by quantum-chemical methods (Table 1). The calculated Gibbs free energy of activation for chlorination of **CBZ** ranges from 99.6 kJ mol^{-1} (MP2 level) to 115.6 kJ mol^{-1} (B2K-PLYP level), which proves that selected theoretical models can successfully reproduce the experimental results. Gibbs free energies of activation, determined from the difference in energy between the transition state structure **CBZ-TS-Cl** and reactants (Table 1), govern the reaction rate according to the transition state theory.[52] The structure **CBZ-TS-Cl** in which three water molecules assist the chlorination of the iminol form **CBZ-I** is presented in Fig. 1. This structure is essential to explain the experimental observation that the *N*-chloramide **CBZ-Cl** is one of the major transformation intermediates of carbamazepine.[30] It is known that chloramides are reactive species,[53,54] and can undergo fast rearrangements. Their detection, therefore, depends on the reaction conditions employed. In the experiment reported by Soufan *et al.* the *N*-chloramide **CBZ-Cl** corresponds to 271 *m/z* in the mass spectrum.[30]

10,11-Epoxidation of CBZ

Among 30 metabolites of **CBZ** isolated in biological systems, carbamazepine 10,11-epoxide (**CBZ-EP**) is the most important from a clinical point of view. In addition, this metabolite is a transformation product of **CBZ** frequently detected in the aqueous and soil environment,[55] as well in experiments

Table 1 Relative Gibbs energy (ΔG_{293}, kJ mol^{-1}) of reactants,[a] intermediates, products, and transition state structures involved in *N*-chlorination and epoxidation of carbamazepine, calculated at different levels of theory in water as the model solvent[b]

Entry	B3LYP[c]	B2K-PLYP[d]	B2-PLYPD[e]	MP2[f]	MP2[g]
CBZ + HOCl(H$_2$O)	0.0	0.0	0.0	0.0	0.0
CBZ-I + HOCl(H$_2$O)	62.6	60.2	58.9	56.9	56.2
CBZ-Cl + (H$_2$O)$_2$	−33.0	−44.0	−48.5	−67.8	−64.0
CBZ-EP + HCl(H$_2$O)	−93.7	−94.4	−93.3	−114.0	−108.0
IMS HNCO + HOCl(H$_2$O)	31.9	33.7	26.4	41.5	39.8
IMS + ClNCO + (H$_2$O)$_2$	4.7	−3.7	−6.6	−2.2	−2.3
CBZ-TS-I + HOCl(H$_2$O)	62.2	70.3	64.8	64.4	62.2
CBZ-TS-Cl	107.4	115.6	105.7	99.6	100.0
CBZ-TS-EP	88.9	125.9	95.7	95.5	96.3
CBZ-TS-IMS + HOCl(H$_2$O)	147.8	165.8	127.8	122.2	122.0
CBZ-Cl-TS-IMS + (H$_2$O)$_2$	79.2	32.2	−1.6	−1.1	0.8

[a] Gibbs free energy of reactants (**CBZ** complexed with two water molecules and HOCl complexed with one water molecule) set to zero; intermediates and products are complexed with two water molecules. [b] CPCM(UFF, α = 1.1)//B3LYP/6-31G(d) level (ε = 78.4). [c] B3LYP/6-31G(d). [d] B2K-PLYP/6-311+G(3df,2p)//B3LYP/6-31G(d). [e] B2PLYP-D/6-311+G(3df,2p). [f] MP2/6-311+G(3df,2p)//B3LYP/6-31G(d). [g] MP2/G3MP2Large//B3LYP/6-31G(d).
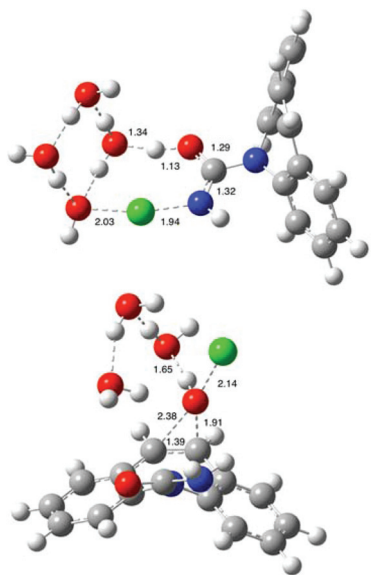
10868 | *Org. Biomol. Chem.*, 2016, **14**, 10866–10874

This journal is © The Royal Society of Chemistry 2016

Fig. 1 B3LYP/6-31G(d) optimized structures of transition states for *N*-chlorination (**CBZ-TS-Cl**) and epoxidation (**CBZ-TS-EP**) of carbamazepine by HOCl. Bond distances are in angstroms.

performed under different reaction conditions (aerobic degradation in soil or a bioreactor, UV-radiation, UV/H$_2$O$_2$ treatment, oxidation with Cl$_2$O or Cl$_2$...).[24,30,56] Therefore, various reactants can mediate the epoxidation of **CBZ**, including HOCl, which is predominately used in water treatment procedures.

We set out to investigate computationally the epoxidation of **CBZ**, which is a process competitive to the *N*-chlorination of **CBZ** (see above) in chlorinated water. The mechanism of carbamazepine epoxidation by HOCl follows a similar reaction mechanism reported earlier for the parent system, *i.e.* ethylene.[57] Oxygen is transferred onto the C10–C11 double bond of **CBZ** (atom numbering in Scheme 1) *via* a transition state structure **CBZ-TS-EP** (Fig. 1), in which the three atoms (C10, C11, and O) form vertices of a scalene triangle (C10–C11: 1.39 Å, C10–O: 2.38 Å and C11–O: 1.91 Å).

This process is assisted by three explicit water molecules which facilitate proton transfer between O and Cl atoms in HOCl. Oxygen is transferred onto the C10–C11 double bond of **CBZ** (atom numbering in Scheme 1) *via* a transition state structure **CBZ-TS-EP** (Fig. 1), in which the three atoms (C10, C11, and O) form vertices of a scalene triangle (C10–C11: 1.39 Å, C10–O: 2.38 Å and C11–O: 1.91 Å). The calculated free energy barrier for **CBZ** epoxidation is 95.7 kJ mol^{-1} (Table 1), which is very similar to the corresponding barrier for ethylene epoxidation ($\Delta G^{\ddagger}$ = 94.6 kJ mol^{-1}) (see the ESI†). To compare the epoxidation power of HOCl, four typical epoxidizing agents have been selected: peroxynitrous acid, performic acid, oxaziridine, and peroxide.[58] In the reaction between **CBZ** and each of the oxidants the product **CBZ-EP** has been obtained.

It comes out that HOCl is less effective than peroxynitrous acid, but comparable to performic acid, and more effective

than oxaziridine and peroxide (for details, see Table S4 in the ESI†). Therefore, the computational results on the epoxidation reactivity of HOCl support experimental observation that epoxidation of **CBZ** ($\Delta G^{\ddagger}_{293}$ = 95.7 kJ mol^{-1}) occurs competitively with the *N*-chlorination process ($\Delta G^{\ddagger}_{293}$ = 105.7 kJ mol^{-1}) in chlorinated water. The latter process is somewhat slower due to a higher free energy barrier, and is thermodynamically less feasible. Chlorination products (**CBZ-Cl** and H$_2$O) have been calculated to be 48.5 kJ mol^{-1} more stable than reactants (**CBZ** and HOCl), whereas epoxidation products (**CBZ-EP** and HCl) are 93.3 kJ mol^{-1} more stable than the starting reactants (Table 1).

In conclusion, the two parallel reactions, epoxidation and *N*-chlorination, contribute the most to the HOCl-induced degradation of carbamazepine. The calculated free energy barriers (95.7 and 105.7 kJ mol^{-1}, resp.) for these reaction pathways are in agreement with the experimental value of 98.2 kJ mol^{-1}. The similar barrier heights (for example, $\Delta\Delta G^{\ddagger}_{293}$ at MP2 levels is only 4 kJ mol^{-1}) suggest that the rates for the formation of the respective products (**CBZ-EP** and **CBZ-Cl**) are comparable. Their structures have been proposed and the respective peaks at *m/z* 253 and 271, resp., have been detected in the MS spectra.

Reaction cascade from *N*-chloramide CBZ-Cl

Formation of iminostilbene (IMS). Transformation of **CBZ** to **IMS** has been observed in several studies, both analytical and biochemical, but no details on the reaction mechanism have been reported. The minor metabolic pathway undergone by carbamazepine in biological systems is its oxidation by cytochrome P450 enzymes, which generates iminostilbene.[59] This metabolite (dibenz[*b,f*]azepine) is easily detected by liquid chromatography-mass spectrometry methods as a fragment ion at *m/z* 194 (protonated molecular ion).[60] In some cases, iminostilbene has been recorded as a byproduct resulting from the thermal degradation of carbamazepine in the GC liner.[61] However, this artifactual formation of iminostilbene can be prevented if high temperature is avoided during sample analysis.

In this study we set out to rationalize the two different mechanisms underlying the formation of iminostilbene from (i) carbamazepine or (ii) *N*-chlorinated carbamazepine. In the first reaction, iminostilbene could have resulted from the cleavage of the HNCO fragment (deamidation). In the second reaction, the elimination of the Cl–NCO fragment from **CBZ** is a HOCl-mediated process. The first reaction could take place under metabolic conditions or as a by-product of the ion source at high temperatures, whereas the second process could undergo in the chlorinated aqueous environment. Several possible pathways for the elimination of the isocyanate fragment from **CBZ** and **CBZ-Cl** have been considered (see the ESI†), but only the most plausible reaction channels are presented in each case (Table 1 and Scheme 1).

The calculated free energy barrier for elimination of the isocyanate moiety from **CBZ** is 205.9 kJ mol^{-1}. If two explicit
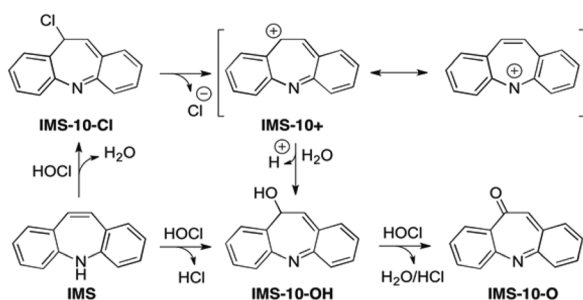
water molecules are included in the calculation, the free energy barrier is lowered to 127.8 kJ mol^{-1} (Table 1). It is still a high barrier to overcome, suggesting that either strong (bio) catalysis is involved or elevated temperature is needed for reaction **CBZ → IMS** to occur (*e.g.* in the GC liner). The transition state structure **CBZ-TS-IMS** for this process has one imaginary frequency (979i cm^{-1}) which corresponds to the proton transfer from the NH$_2$ to N5 atom simultaneously with N5–C bond cleavage (Fig. S1 in the ESI†). The analogous mechanism has been established for water-assisted decomposition of urea,[62,63] which is the parent compound of carbamazepine.

In the case of *N*-chlorinated carbamazepine **CBZ-Cl**, the calculated free energy barrier for elimination of *N*-chloro-isocyanate is only 46.9 kJ mol^{-1} (energy difference between the corresponding transition states **CBZ-Cl-TS-IMS** and **CBZ-Cl** in Table 1), which is 81 kJ mol^{-1} lower than the barrier for the corresponding process (isocyanate elimination) in **CBZ**. This suggests that the formation of iminostilbene from carbamazepine is kinetically strongly favored if carbamazepine exists in its *N*-chlorinated form **CBZ-Cl**. The evidence for the intermediacy of **CBZ-Cl** in the degradation of carbamazepine has been provided earlier.[30]

To summarize this part, the occurrence of **IMS** is not limited only to metabolic degradation or high-temperature induced side reactions, but is also related to HOCl-mediated degradation of **CBZ** in the aqueous environment. During water treatment the chlorination of **CBZ** gives rise to *N*-chloramide **CBZ-Cl**, which may easily undergo ClNCO cleavage forming **IMS**.

Formation of oxoiminostilbene (IMS-10-O). Once formed, **IMS** can undergo different transformation reactions promoted by HOCl in the aqueous environment. Several pathways have been considered: C10–C11 double-bond and phenyl ring epoxidation, *N*- and *C*-chlorination, and C10–C11 double-bond and phenyl-ring hydroxylation (see details in Scheme S2 in the ESI†). The two most feasible processes are additions of the chlorine or hydroxyl group on the C10–C11 double bond in **IMS** (Scheme 2). The calculated barriers for chlorination and hydroxylation processes are 41.8 and 84.7 kJ mol^{-1}, respectively, (Table 2) which suggests that the addition products, 10-chloroiminostilbene (**IMS-10-Cl**) and 10-hydroxyiminostilbene (**IMS-10-OH**), can easily be formed.

For comparison, the calculated barriers for epoxidations at the C10–C11 and C2–C3 positions (Scheme S2 in the ESI†) are more than 50 kJ mol^{-1} higher in energy, and therefore less favorable than the two electrophilic reactions at the C-10 position (**IMS → IMS-10-Cl** and/or **IMS-10-OH**).

The transient species **IMS-10-OH** was detected earlier by Li *et al.*[55] They recorded a signal of *m/z* 210 [M + H] and assigned it to **IMS-10-OH**. On the contrary, the existence of the chlorinated intermediate **IMS-10-Cl** has never been confirmed. It is kinetically very unstable, considering that the ionization of the C10–Cl bond occurs rapidly through the aromatic carbocation intermediate **IMS-10+** (presented with two resonance structures in Scheme 2), which in the aqueous environment undergoes fast addition of water to give **IMS-10-OH**.[64] The 10-hydroxyiminostilbene is by 97.6 kJ mol^{-1} (Table 2) more stable than the starting **IMS**, but represents a short-lived intermediate that can easily be oxidized by HOCl to 10-oxoiminostilbene (**IMS-10-O**). It is known that HOCl is an effective oxidant for conversion of a secondary alcohol to the corresponding ketone.[65] The oxidation step **IMS-10-OH → IMS-10-O** is both kinetically and thermodynamically driven: the calculated free energy barrier is 53.7 kJ mol^{-1} (the energy difference between **IMS-10-OH** and the transition state **IMS-10-OH-TS-O**, Table 2), and the final product **IMS-10-O** is 353.3 kJ mol^{-1} more stable than **IMS**. This large change in energy is due to the resonance energy, since **IMS-10-O** is an aromatic structure, unlike its parent compound **IMS**. Similar oxidation has been reported in the course of the transformation of 2-hydroxyiminostilbene to 2-oxoiminostilbene. However, this process is relevant only for hepatic metabolism of **IMS** mediated by cytochrome P450.[66,67]

The experimental (MS signal at *m/z* = 208) and our computational results suggest that the formation of 10-oxoiminostilbene (**IMS-10-O**) is an important process for the chemical fate of iminostilbene in chlorinated water.

Reaction cascade from epoxide CBZ-EP

Formation of carbamazepine 10,11-chlorohydrin (CBZ-Cl-OH) and carbamazepine 10,11-diol (CBZ-diOH). Similarly to chloramide **CBZ-Cl** obtained in the reaction of **CBZ** and HOCl (see above), the reactivity of epoxide **CBZ-EP** is also relatively high. Several rearrangement pathways for **CBZ-EP** have been considered computationally (see detailed Scheme S3 in the ESI†), but only the most feasible one has been described herewith.

The opening of the three-membered ring in epoxide **CBZ-EP** (Scheme 3) has the lowest free energy barrier ($\Delta G_{293}^{\ddagger}$ = 128.7 kJ mol^{-1}, at the B3LYP level, Table 3). This process is mediated by HOCl (or H$_2$O), and results in the formation of the carbocation intermediate **CBZ-EP+**.
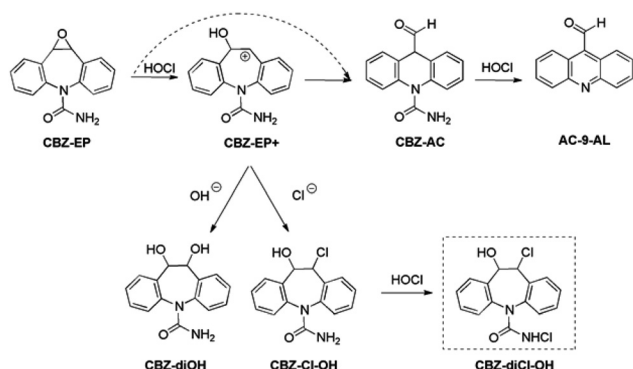
The formed carbocation can easily react with the hydroxide or chloride ion producing 10,11-diol **CBZ-diOH** or 10,11-chlorohydrin **CBZ-Cl-OH**, respectively. Both structures were detected in mass spectrometry as respective signals at *m/z* = 271 and *m/z* = 289. The latter intermediate can undergo

Scheme 2 HOCl-induced transformation of **IMS** in the aqueous environment.

Table 2 Relative Gibbs energy (ΔG_{293}, kJ mol^{-1}) of reactants,[a] intermediates, products, and transition state structures involved in 10-oxoiminostilbene formation, calculated at different levels of theory in water as the model solvent[b]

Entry	B3LYP[c]	B2K-PLYP[d]	B2-PLYPD[e]	MP2[f]	MP2[g]
IMS + HOCl(H$_2$O)	0.0	0.0	0.0	0.0	0.0
IMS-10-Cl + (H$_2$O)$_2$	−82.1	−143.3	−134.8	−161.2	−160.0
IMS-10-OH + HCl(H$_2$O)	−57.2	−48.2	−97.6	−116.5	−105.4
IMS-TS-10-Cl	69.5	72.2	41.8	48.8	50.2
IMS-TS-10-OH	80.9	90.1	84.7	97.9	92.8
IMS-10-OH-TS-O[h]	−5.3	−22.9	−43.9	−40.1	−49.3
IMS-10-O + HCl(H$_2$O)[h]	−336.4	−371.7	−353.3	−360.5	−354.8

[a] Gibbs free energy of reactants (**IMS** complexed with two water molecules and HOCl complexed with one water molecule) set to zero; intermediates and products are complexed with two molecules. [b] CPCM(UFF, α = 1.1)//B3LYP/6-31G(d) model (ε = 78.4). [c] B3LYP/6-31G(d). [d] B2K-PLYP/6-311+G(3df,2p)//B3LYP/6-31G(d). [e] B2PLYP-D/6-311+G(3df,2p). [f] MP2/6-311+G(3df,2p)//B3LYP/6-31G(d). [g] MP2/G3MP2Large//B3LYP/6-31G(d). [h] Relative to **IMS** + 2(HOCl(H$_2$O)).

Scheme 3 Rearrangement of **CBZ-EP** to chlorohydrin, dihydroxy, and acridine derivatives mediated by HOCl. Dashed arrow describes the process in which no carbocationic intermediate **CBZ-EP+** exists.

additional *N*-chlorination resulting in the proposed product **CBZ-diCl-OH** (structure in the dashed rectangle in Scheme 3) which can be assigned to the unknown compound detected in the MS spectrum (peak at *m/z* = 320).

Formation of 9-formylacridine (AC-9-AL). In addition, the carbocation intermediate **CBZ-EP+** can undergo facile ring contraction *via* **CBZ-EP+-TS-AC**, yielding the acridine derivative **CBZ-AC**. The ring contraction process **CBZ-EP → CBZ-AC** can also occur directly (dashed arrow in Scheme 3), without the intermediacy of the carbocation, but the corresponding barrier is 108.5 kJ mol^{-1} (Table 3) higher in energy (process *via* the transition state **CBZ-EP-TS-AC**).

Interestingly, no ring contraction process in carbamazepine metabolites has been observed earlier in the chlorination experiment.[30] This is probably due to the different reaction conditions employed (*e.g.* use of carbonate instead of

Table 3 Relative Gibbs energy (ΔG_{293}, kJ mol^{-1}) of reactants,[a] intermediates, products, and transition state structures involved in oxidation of carbamazepine-10,11-epoxide, calculated at different levels of theory in water as the model solvent[b]

	B3LYP[c]	B2K-PLYP[d]	B2-PLYPD[e]	MP2[f]	MP2[g]
CBZ-EP + HOCl(H$_2$O)	0.0	0.0	0.0	0.0	0.0
CBZ-EP$^+$ + OCl$^-$(H$_2$O)$_2$	44.7	59.4	58.9	62.0	62.2
CBZ-AC + HOCl(H$_2$O)	−81.2	−91.8	−90.5	−89.5	−89.2
CBZ-EP-TS-EP$^+$	128.7	137.6	134.9	129.9	131.4
CBZ-EP$^+$-TS-AC	65.8	71.3	49.7	55.0	51.2
CBZ-EP-TS-AC	136.8	150.4	158.2	172.6	171.2
CBZ-diOH	−26.7	−35.2	−32.3	−41.2	−39.3
CBZ-Cl-OH	−27.6	−48.7	−46.1	−54.8	−52.6
CBZ-Cl-OH-I	35.1	20.7	22.8	12.3	13.8
CBZ-diCl-OH	−38.8	−70.3	−62.9	−91.8	−85.0
CBZ-EP-TS-diOH	171.4	195.3	192.7	181.3	182.0
CBZ-EP$^+$-TS-diOH	142.7	127.3	156.3	149.3	153.1
CBZ-EP-TS-Cl-OH	64.5	82.3	71.4	106.1	102.4
CBZ-EP$^+$-TS-Cl-OH	51.4	62.3	53.6	80.2	79.9
CBZ-Cl-OH-TS-Cl-OH-I	50.9	47.1	45.4	36.0	36.0
CBZ-Cl-OH-I-TS-diCl-OH	90.8	66.7	68.1	52.8	56.7
AC-9-AL + HCl(H$_2$O)	−307.1	−352.9	-339.8	−344.0	−339.4
CBZ-AC-TS-AC-9-AL	32.7	15.1	38.5	43.1	42.3

[a] Gibbs free energy of reactants (**CBZ-EP** complexed with two water molecules and HOCl complexed with one water molecule) set to zero; intermediates and products are complexed with two water molecules. [b] CPCM(UFF, α = 1.1)//B3LYP/6-31G(d) model (ε = 78.4). [c] B3LYP/6-31G(d). [d] B2K-PLYP/6-311+G(3df,2p)//B3LYP/6-31G(d). [e] B2PLYP-D/6-311+G(3df,2p). [f] MP2/6-311+G(3df,2p)//B3LYP/6-31G(d). [g] MP2/G3MP2Large//B3LYP/6-31G(d).

This journal is © The Royal Society of Chemistry 2016

Org. Biomol. Chem., 2016, **14**, 10866–10874 | **10871**

phosphate buffer, change in acidic medium, or variation in the level of chloride ions in the free chlorine stock solution).

On the other hand, **CBZ-AC** has been proposed as a tentative intermediate in photodegradation,[23,68] and advanced oxidation processes.[69] We have found that **CBZ-AC** can undergo oxidation, mediated by HOCl, to give 9-formylacridine (**AC-9-AL**). This process is thermodynamically driven and can be explained by the tendency of **CBZ-AC** to yield an aromatic structure. The final product **AC-9-AL** has been calculated as the most stable intermediate on the corresponding potential energy surface. It is 249.3 and 339.8 kJ mol^{-1} more stable than **CBZ-AC** and starting reactants (**CBZ** and HOCl), respectively. The exceptional stability of **AC-9-AL** suggests that this structure should be considered as a recalcitrant metabolite formed in chlorinated water.

As **IMS-10-O** and **AC-9-AL** are both aromatic, and have the same mass, it is hard to differentiate between those two products. According to our calculations, **AC-9-AL** has been calculated almost 100 kJ mol^{-1} more stable than the former. It is clear that the acridine moiety in **AC-9-AL** provides much better stabilization than the tricyclic system with the azepine ring in **IMS-10-O**. The MS peak corresponding to the mass of either **IMS-10-O** or **AC-9-AL** has been detected earlier in the chlorination experiment,[30] but neither 10-oxoiminostilbene nor acridine-9-carboxaldehyde has been proposed as a possible carbamazepine degradation intermediate. On the contrary, in a number of other analytical or biochemical studies, acridine-9-carboxaldehyde has been discussed as an important product of carbamazepine transformations in both aqueous[58,63] and physiological environments.[62,70]

Conclusions

Quantum chemical investigation of HOCl-mediated transformation of **CBZ** was accomplished. Five different theoretical levels (DFT and MP2 methods) were tested, and B2-PLYPD was selected as superior in terms of better precision and lower computational cost. The experimental Gibbs free energy of activation for the reaction between HOCl and **CBZ** ($\Delta G^{\ddagger}_{293} = 98.2$ kJ mol^{-1}) was successfully reproduced by the B2-PLYPD functional. The two parallel reactions contribute to the overall barrier: C10,C11-epoxidation ($\Delta G^{\ddagger}_{293} = 95.7$ kJ mol^{-1}) and *N*-chlorination ($\Delta G^{\ddagger}_{293} = 105.7$ kJ mol^{-1}) of carbamazepine.

These processes give rise to epoxide **CBZ-EP** and chloramide **CBZ-Cl**, respectively, which undergo a series of fast rearrangements resulting in an additional set of intermediates. The two chlorination experiments[30,32] revealed 8 degradation products, with some structures tentatively assigned or unknown. Full assignment of signals in mass spectra was performed by assistance of computational models. In addition to epoxide **CBZ-EP** and chloramide **CBZ-Cl**, the suggested structures include iminostilbene (**IMS**), carbamazepine 10,11-diol (**CBZ-diOH**), carbamazepine 10,11-chlorohydrin (**CBZ-Cl-OH**), and its *N*-chlorinated derivative (**CBZ-diCl-OH**), oxoiminostil-bene (**IMS-10-OH**), and formylacridine (**AC-9-AL**). The two latter are of special importance due to their exceptional thermodynamic stability (326.9 kJ mol^{-1} and 433.1 kJ mol^{-1}, respectively, more stable than **CBZ**). Unlike other intermediates, **IMS-10-O** and **AC-9-AL** have planar aromatic structures which can be associated with their (bio)recalcitrant properties.

In this work the fruitful interplay between computational models and available experimental data was implemented in assessing the environmental fate of carbamazepine. The measured kinetic parameters for reactions ($\Delta G^{\ddagger}$) and signals observed in mass spectra were used as a guide for calculations of reaction mechanisms underlying the HOCl-induced transformations of carbamazepine.

Acknowledgements

Notes and references

1 E. J. Fertig and R. H. Mattson, in *Epilepsy: A Comprehensive Textbook*, ed. J. Engel and T. A. Pedley, Lippincott Williams & Wilkins, Philadelphia, Pa, 2007, p. 1543.

2 R. Loos, G. Locoro, S. Comero, S. Contini, D. Schwesig, F. Werres, P. Balsaa, O. Gans, S. Weiss, L. Blaha, M. Bolchi and B. M. Gawlik, *Water Res.*, 2010, **44**, 4115.

3 H. D. Zhou, C. Y. Wu, X. Huang, M. J. Gao, X. H. Wen, H. Tsuno and H. Tanaka, *Water Environ. Res.*, 2010, **82**, 2239.

4 S. Kern, K. Fenner, H. P. Singer, R. P. Schwarzenbach and J. Hollender, *Environ. Sci. Technol.*, 2009, **43**, 7039.

5 X. S. Miao, J. J. Yang and C. D. Metcalfe, *Environ. Sci. Technol.*, 2005, **39**, 7469.

6 S. D. Richardson and T. A. Ternes, *Anal. Chem.*, 2011, **83**, 4614.

7 A. Bahlmann, W. Brack, R. J. Schneider and M. Krauss, *Water Res.*, 2014, **57**, 104.

8 B. Ferrari, N. Paxéus, R. Lo Giudice, A. Pollio and J. Garric, *Ecotoxicol. Environ. Saf.*, 2003, **55**, 359.

9 A. Almeida, V. Calisto, V. I. Esteves, R. J. Schneider, A. M. V. M. Soares, E. Figueira and R. Freitas, *Aquat. Toxicol.*, 2014, **156**, 74.

10 R. Andreozzi, R. Marotta, G. Pinto and A. Pollio, *Water Res.*, 2002, **36**, 2869.

11 R. Mojtabai and M. Olfson, *Arch. Gen. Psychiatry*, 2010, **67**, 26.

12 J. M. Nicholas, L. Ridsdale, M. P. Richardson, M. Ashworth and M. C. Gulliford, *Seizure*, 2012, **21**, 466.

13 C. J. Landmark, H. Fossmark, P. G. Larsson, E. Rytter and S. I. Johannessen, *Epilepsy Res.*, 2011, **95**, 51.

10872 | *Org. Biomol. Chem.*, 2016, **14**, 10866–10874

This journal is © The Royal Society of Chemistry 2016

14 M. Bernhard, J. Muller and T. P. Knepper, *Water Res.*, 2006, **40**, 3419.

15 Y. Zhang and S. U. Geiben, *J. Hazard. Mater.*, 2010, **176**, 1089.

16 M. Clara, B. Strenn and N. Kreuzinger, *Water Res.*, 2004, **38**, 947.

17 M. Carballa, F. Omil, T. Ternes and J. M. Lema, *Water Res.*, 2007, **41**, 2139.

18 M. D. Celiz, S. Perez, D. Barcelo and D. S. Aga, *J. Chromatogr. Sci.*, 2009, **47**, 19.

19 S. Suarez, M. Carballa, F. Omil and J. M. Lema, *Rev. Environ. Sci. Biotechnol.*, 2008, **7**, 125.

20 M. Carballa, F. Omil, J. M. Lema, M. Llompart, C. Garcıa-Jares, I. Rodrıguez, M. Gomez and T. Ternes, *Water Res.*, 2004, **38**, 2918.

21 S. Esplugas, D. M. Bila, L. G. T. Krause and M. Dezotti, *J. Hazard. Mater.*, 2007, **149**, 631.

22 T. A. Ternes, M. Meisenheimer, D. McDowell, F. Sacher, H. J. R. Brauch, B. Haist-Gulde, G. Preuss, U. Wilme and N. Zulei-Seibert, *Environ. Sci. Technol.*, 2002, **36**, 3855.

23 E. De Laurentiis, S. Chiron, S. Kouras-Hadef, C. Richard, M. Minella, V. Maurino, C. Minero and D. Vione, *Environ. Sci. Technol.*, 2012, **46**, 8164.

24 D. Vogna, R. Marotta, R. Andreozzi, A. Napolitano and M. D'Ischia, *Chemosphere*, 2004, **54**, 497.

25 D. C. McDowell, M. M. Huber, M. Wagner, U. von Gunten and T. A. Ternes, *Environ. Sci. Technol.*, 2005, **39**, 8014.

26 M. Petrović and D. Barcelo, *Trends Anal. Chem.*, 2007, **26**, 486.

27 S. Chiron, C. Minero and D. Vione, *Environ. Sci. Technol.*, 2006, **40**, 5977.

28 T. Okuda, Y. Kobayashi, R. Nagao, N. Yamashita, H. Tanaka, S. Tanaka, S. Fujii, C. Konishi and I. Houwa, *Water Sci. Technol.*, 2008, **57**, 65.

29 J. Gibs, P. E. Stackelberg, E. T. Furlong, M. Meyer, S. D. Zaugg and R. L. Lippincott, *Sci. Total Environ.*, 2007, **373**, 240.

30 M. Soufan, M. Deborde, A. Delmont and B. Legube, *Water Res.*, 2013, **47**, 5076.

31 D. Šakić, P. Šonjić, T. Tandarić and V. Vrček, *J. Phys. Chem. A*, 2014, **118**, 2367.

32 K. Kotcharaksa, *In the Thesis: The Mechanisms, Products, and Kinetic of Carbamazepine-Free Chlorine Reactions*, Virginia Polytechnic Institute, 2008. Accessed on September 1, 2016 at: http://theses.lib.vt.edu/theses/available/etd-12232008-141520/.

33 J. Blotevogel, A. N. Mayeno, T. C. Sale and T. Borch, *Environ. Sci. Technol.*, 2011, **45**, 2236.

34 W. J. Barr, T. Yi, D. Aga, O. Acevedo and W. F. Harper Jr., *Environ. Sci. Technol.*, 2012, **46**, 760.

35 H. Zhang, H. Xie, J. Chen and S. Zhang, *Environ. Sci. Technol.*, 2015, **49**, 1552.

36 D. Šakić, F. Achrainer, V. Vrček and H. Zipse, *Org. Biomol. Chem.*, 2013, **11**, 4232.

37 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, *et al.*, *Gaussian 09, Revision D.01*, Gaussian, Inc., Wallingford, CT, 2009.

38 C. Lee, W. Yang and R. G. Parr, *Phys. Rev. B: Condens. Matter*, 1988, **37**, 785.

39 A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648.

40 A. Tarnopolsky, A. Karton, R. Sertchook, D. Vuzman and J. M. L. Martin, *J. Phys. Chem. A*, 2008, **112**, 3.

41 S. Grimme, *J. Chem. Phys.*, 2006, **124**, 034108.

42 A. Karton, R. J. O'Reilly and L. Radom, *J. Phys. Chem. A*, 2012, **116**, 4211.

43 T. Schwabe and S. Grimme, *Phys. Chem. Chem. Phys.*, 2007, **9**, 3397.

44 D. Šakić, H. Zipse and V. Vrček, *Org. Biomol. Chem.*, 2011, **9**, 4336.

45 M. J. Frisch, M. Head-Gordon and J. A. Pople, *Chem. Phys. Lett.*, 1990, **166**, 275.

46 H. P. Hratchian and H. B. Schlegel, *J. Chem. Phys.*, 2004, **120**, 9918.

47 V. Barone and M. Cossi, *J. Phys. Chem. A*, 1998, **102**, 1995; M. Cossi, G. Scalmani, N. Rega and V. Barone, *J. Chem. Phys.*, 2002, **117**, 43.

48 D. Šakić and V. Vrček, *J. Phys. Chem. A*, 2012, **116**, 1298.

49 V. Vrček, O. Kronja and M. Saunders, *J. Chem. Theory Comput.*, 2007, **3**, 1223.

50 J. R. Pliego Jr., *Chem. Phys.*, 2004, **306**, 273.

51 D. Ardura, R. López and T. L. Sordo, *J. Phys. Chem. B*, 2005, **109**, 23618.

52 H. P. Hratchian and H. B. Schlegel, Finding minima, transition states, and following reaction pathways on ab initio potential energy surfaces, in *Theory and Applications of Computational Chemistry: The First Forty years*, ed. C. Dykstra, G. Frenking, K. Kim and G. Scuseria, Elsevier, Amsterdam, 2005, p. 195.

53 M. D. Rees, C. L. Hawkins and M. J. Davies, *J. Am. Chem. Soc.*, 2003, **125**, 13719.

54 C. L. Hawkins and M. J. Davies, *Free Radical Biol. Med.*, 1998, **24**, 1396.

55 J. Li, L. Dodgen, Q. Ye and J. Gan, *Environ. Sci. Technol.*, 2013, **47**, 3678.

56 A. Jelic, C. Cruz-Morató, E. Marco-Urea, M. Sarrà, S. Perez, T. Vicent, M. Petrović and D. Barcelo, *Water Res.*, 2012, **46**, 955.

57 M. Srnec, M. Ončak and R. Zahradnik, *J. Phys. Chem. A*, 2008, **112**, 3631.

58 K. N. Houk, J. Liu, N. C. DeMello and K. R. Condroski, *J. Am. Chem. Soc.*, 1997, **119**, 10147.

59 J. Csetenyi, K. M. Baker, A. Frigerio and P. L. Morselli, *J. Pharm. Pharmacol.*, 1973, **25**, 340.

60 H. Breton, M. Cociglio, F. Bressolle, H. Peyriere, J. P. Blayac and D. Hillaire-Buys, *J. Chromatogr., B: Biomed. Appl.*, 2005, **828**, 80.

61 T. Kosjek, H. R. Andersen, B. Kompare, A. Ledin and E. Heath, *Environ. Sci. Technol.*, 2009, **43**, 6256.

62 A. N. Alexandrova and W. L. Jorgensen, *J. Phys. Chem. B*, 2007, **111**, 720.

This journal is © The Royal Society of Chemistry 2016

Org. Biomol. Chem., 2016, **14**, 10866–10874 | **10873**

63 G. Estiu and K. M. Merz Jr., *J. Phys. Chem. B*, 2007, **111**, 6507.

64 J. P. Richard, T. L. Amyes and M. M. Toteva, *Acc. Chem. Res.*, 2001, **34**, 981.

65 R. V. Stevens, K. T. Chapman and H. N. Weller, *J. Org. Chem.*, 1980, **45**, 2030.

66 R. E. Pearce, J. P. Uetrecht and J. S. Leeder, *Drug Metab. Dispos.*, 2005, **33**, 1819.

67 C. Ju and J. P. Uetrecht, *J. Pharmacol. Exp. Ther.*, 1999, **288**, 51.

68 P. Calza, C. Medana, E. Padovano, V. Giancotti and C. Baiocchi, *Rapid Commun. Mass Spectrom.*, 2012, **26**, 1687.

69 O. S. Keen, S. Baik, K. G. Linden, D. S. Aga and N. G. Love, *Environ. Sci. Technol.*, 2012, **46**, 6222.

70 S. M. Furst and J. P. Uetrecht, *Biochem. Pharmacol.*, 1993, **45**, 1267.

10874 | *Org. Biomol. Chem.*, 2016, **14**, 10866–10874

This journal is © The Royal Society of Chemistry 2016