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COMMUNICATION

Studies of the H–D exchange mechanism of malonganenone B†

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Malonganenone B (1) exhibits an unusual H–D exchange of a formyl proton when in deuteric-NMR solvents. Synthetic and kinetic investigations were made to probe the mechanism of this exchange, which appears to occur *via* an uncommon and transient amine–amide NHC intermediate.

The isolation of malonganenone B (**1**) was first reported from the gorgonian *Leptogorgia gilchristi* collected at a reef near Ponto Malongane, Mozambique,[1] and again shortly after from *Euplexaura nuttingi* (both Order Alcyonacea) collected from Uvinage, Tanzania.[2] The original structural elucidation of **1** was problematic as mass spectral analysis, following NMR acquisition in CD_3OD, resulted in two quasi-molecular ion peaks differing by one mass unit, implying substitution of a proton for a deuteron. Further, ^{13}C NMR data suggested the presence of two isotopomeric formamide carbonyl carbon resonances that, in addition to reduced proton integration for this functionality, indicated formyl H–D exchange had occurred (Fig. 1).

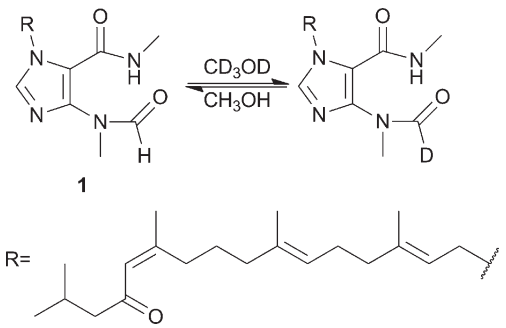

Fig. 1 Formyl H–D exchange in malonganenone B (**1**).

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†Electronic supplementary information (ESI) available: Three proposed mechanisms for H–D exchange in dialkylated formamide acetals. Experimental procedures for the preparation and characterisation of **3**, **4**, **6**, **8**, **9**, **11** and **13–15**. Kinetic analyses and data for **4** and **11**. ^1H and ^{13}C NMR spectra for **3**, **4**, **9**, **11**, **14** and **15**, ^1H NMR for **13** and both detected and simulated MS spectra for **15**. Details of molecular modelling of pK_a values. See DOI: 10.1039/c2ob06926a

H–D exchange of carbonyl-bound protons is very unusual, especially in the absence of any catalyst, but a related exchange had been observed previously in *N*,*N*-dialkylformamide acetals (**2**).[3] The effects upon the H–D exchange of changing alkyl substituents, solvent and addition of exogenous acid or base were examined.[3] Carbene, self-protonation and ylide mechanisms were proposed (see Scheme 1 in ESI†) with the ylide example deemed the best fit to the data. However, not all of the experimental observations could be reconciled with this mechanism, especially the putative base inhibition that should in fact enhance the reaction kinetics.

Since then, progress in the field of *N*-heterocyclic carbenes (NHC) has added credence to the alternative carbene mechanism previously proposed (Fig. 2).[3] With this knowledge in mind, we sought to determine if either the proposed ylide or carbene mechanisms were responsible for the H–D exchange observed in **1**. We report here our evidence supporting an NHC-based mechanism for the exchange, which involves a rare mixed amine–amide NHC intermediate.

Our hypothesis for exchange was the fully reversible attack of the *N*-methyl amide of **1** upon the formamide carbonyl followed by nitrogen-assisted elimination of water that would generate an NHC precursor in a similar manner to the proposed carbene mechanism for **2** (Scheme 1).

To test this theory, models of **1** were synthesised for kinetic studies. Analogue **3** had a methyl group in place of the tetraprenyl tail while **4** contained a phenyl ring instead of the imidazole (Scheme 2). Hydrolysis of caffeine (**5**) yielded intermediate **6** that was then formylated using a mixed anhydride to yield **3** in 29% overall yield.[4] Difficulties in the purification of **3**, however, prompted the synthesis of a related analogue for further studies. *N*-Methylisatoic anhydride (**7**) was attacked by methylamine to give intermediate **8**,[5] which was similarly formylated with the mixed anhydride to give **4** in an 84% overall yield.

These compounds were then subjected to kinetic analyses using NMR spectroscopy. Upon dissolution in D_2O or CD_3OD, the measured integral of the formyl proton resonance of each

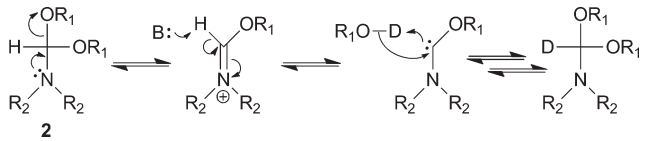
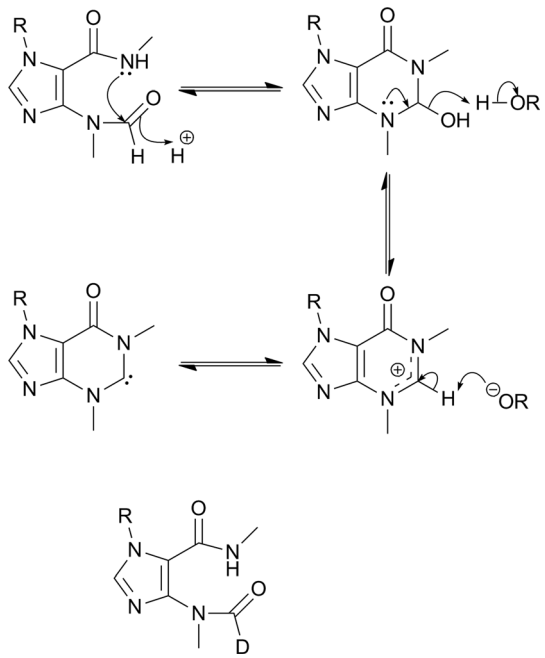
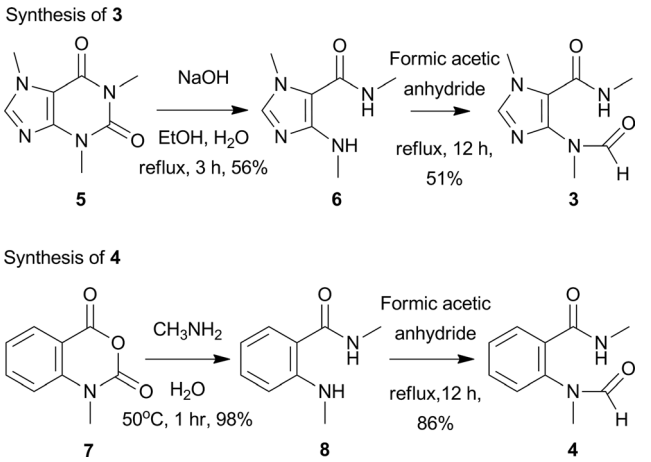


Fig. 2 Proposed carbene mechanism of H–D exchange in formamide acetals.



Scheme 1 Proposed mechanism of H–D exchange for **1**.

species decayed with time, exchanging for a deuteron, as shown by the loss of the formyl proton resonance of **4** (Fig. 3)[6] and by MS. These results confirmed the H–D exchange of



Scheme 2 Synthesis of *N*-methyl imidazole (**3**) and benzene (**4**) analogues.

malonganenone B is independent of the tetraprenyl tail of **1**. Addition of exogenous acid or base had opposing effects on the exchange; triethylamine was catalytic whereas acetic acid-d_4 was inhibitory (Fig. 4). This contradicts the previously reported observations and is consistent with both the proposed ylide and carbene mechanisms.[3]

Evidence of cyclisation was sought to confirm our hypothesis that the proposed mechanisms for H–D exchange of *N*,*N*-

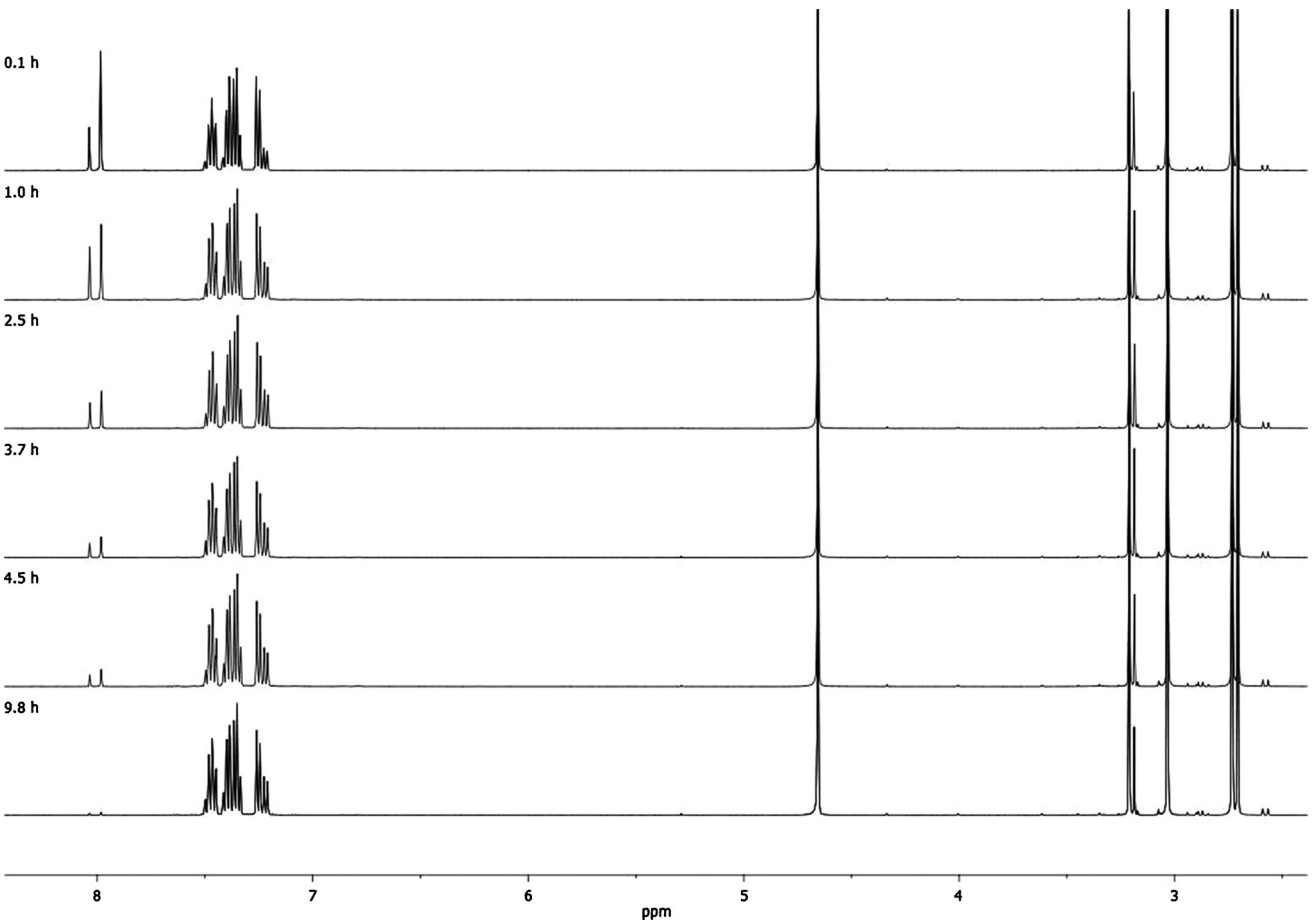


Fig. 3 ^{1}H NMR (D_2O, 600 MHz) spectrum demonstrating H–D exchange of the formyl proton resonance of **4**.[6]

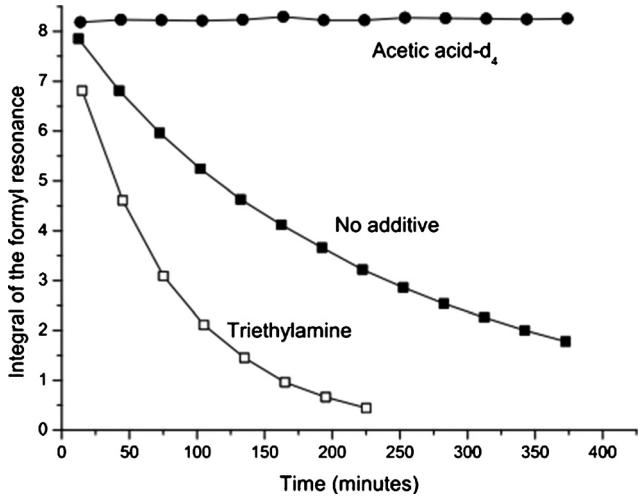


Fig. 4 Effect of acid or base addition on the rate of H–D exchange of **4**.

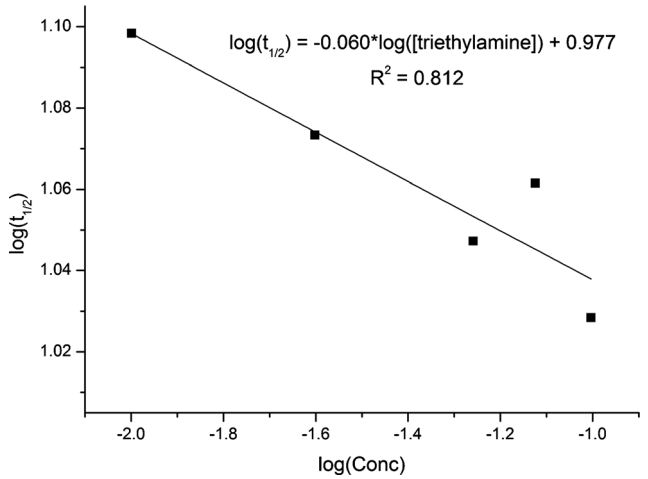
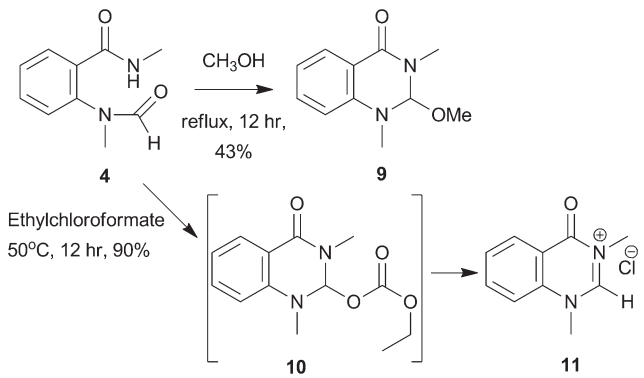


Fig. 5 Determination of the role of base upon the H–D exchange kinetics of cation **11**.



Scheme 3 Formation of aminal ester **9** and quinazolinium cation **11**.

dialkylformamide acetals could be applied here. Reaction of **4** with CH_3OH resulted in the formation of aminal ester **9** (Scheme 3), which was proposed to form *via* amide attack of the formamide followed by exchange of the resultant hydroxide for a methoxide. The lability of the groups around the exchanging centre in **9** is analogous to that of *N*,*N*-dialkylformamide acetals, which supports the application of the proposed NHC-mechanism.

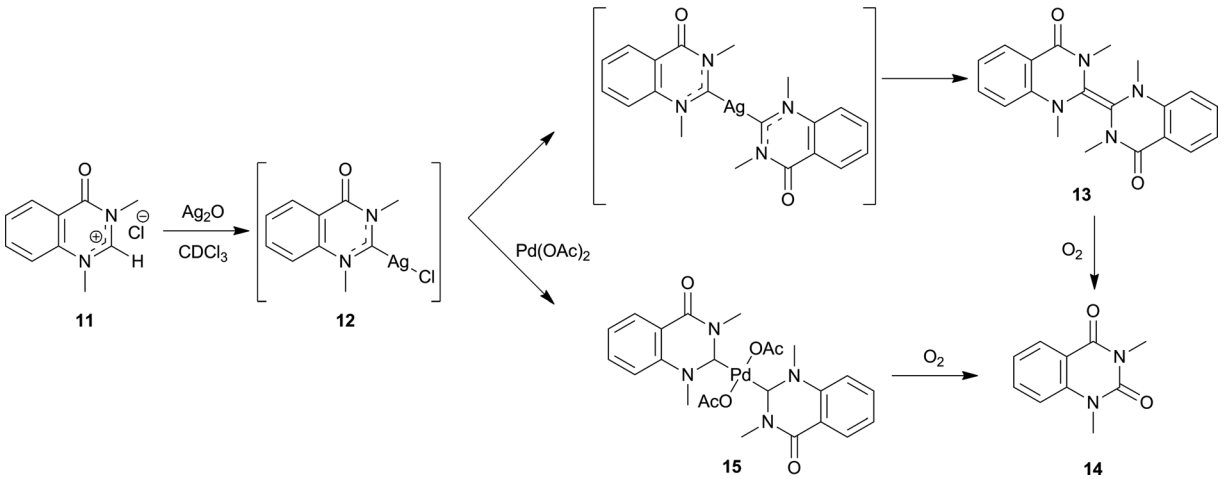
Further attempts to trap **4** as the carbonate **10** with ethylchloroformate had a fortunate outcome, with spontaneous decarboxylation yielding the isolable quinazolinium cation **11** instead (Scheme 3). The apparent stability of this species supports the NHC-mechanism as it represents the substrate for the rate determining step of such a mechanism. Gratifyingly, **11** also underwent H–D exchange in a manner similar to **3** and **4**. Kinetic studies using this compound revealed the exchange is first order with respect to base at low concentrations,‡ concordant with the proposed NHC-mechanism (Fig. 5). The pK_a of the quinazolinium proton in water was estimated to be 19.8 by use of computational methods and calibrated using a linear regression model to match experimentally determined values.[7] The pK_a of the corresponding proton of **3** was determined to be 30.0 using the same model and would explain the reduced rate of exchange of **3** *versus* **4** or **11**. Unfortunately, the order for exchange of **11** could not be established as the range of concentrations required to probe the exchange rate were too dilute for suitable observation by NMR.

More direct evidence of the proposed NHC forming in the exchange was sought. Carbenes have historically been trapped by reaction with an alkene to form stable cyclopropanes; however, attempts to repeat this feat with **3**, **4** or **11** with a variety of alkene traps all failed. Similarly, attempts to form dimerised carbenes by direct base deprotonation of **11** were also unsuccessful.

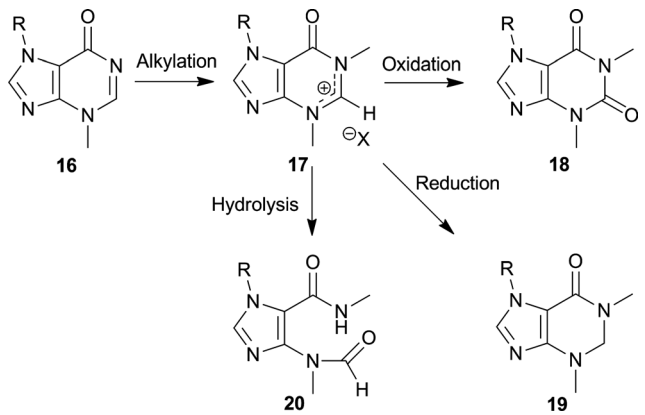
Fortunately, organometallic methods under full Schlenk conditions were more fruitful. Using silver oxide, a silver-NHC complex (**12**) could be generated from **11** (Scheme 4).[8] In the absence of another metal species, fulvalene **13** was observed, which was believed to result from ligand exchange between two molecules of **12** followed by reductive elimination. The structure of **13** was confirmed by MS and NMR (see ESI†), with the observed linking carbon resonance chemical shift of 106.8 ppm being comparable to the literature data of enetetramines.[8] Given the lack of steric protection around the electron-rich alkene, however, this species rapidly degraded to **14** and could not be purified or characterised further.

Silver-NHC complexes have had great use as transmetallation agents to generate other organometallic complexes. When **12** was generated in the presence of palladium acetate, transmetallation occurred to give the transient organometallic complex **15** as a mix of isomers (Scheme 4). This was confirmed by 2D-NMR, where a NHC-Pd carbon resonance of δ_C 199.2 ppm was detected *via* HMBC, consistent with the literature,[9] and MS data that demonstrated the characteristic palladium isotope pattern for a dimerized Pd-system. As before, the lack of protection around the NHC-complex meant this species also rapidly degraded to **14** before further purification or isolation could be achieved. Synthesis of species related to **3** or **11** with increased steric protection has yet to yield more stable organometallic products.

The accumulated evidence suggests that a mixed amine–amide NHC is forming in the H–D exchange of malonganenone B. The observation of cyclic species, the kinetic evidence and the formation of **11**, **13** and **15** all suggest that an NHC is



Scheme 4 Transient formation of fulvalene **13** and organometallic complex **15**.



Scheme 5 Relationships between malonganenones and nuttingins.

generated from these models, which in turn suggests an NHC may be formed as a transient species in solutions of the natural product. To the best of our knowledge, stable mixed NHCs of this nature are rare with only a few recent examples being reported.[10] There are equally few examples of naturally occurring NHC-based reactions or biosyntheses, although thiamine-based carbenes have been implicated in several transketolase reactions.[11] It is therefore possible that further NHC-based mechanisms beyond thiamine may exist in nature that could have profound biosynthetic and bioactive implications, although the reaction kinetics in water would presumably quench such a carbene rapidly and therefore a purely defensive bioactive role for such an NHC is unlikely.

These observations also shed new light on the relationships between the alkaloid moieties of the related malonganenone and nuttingin families (Scheme 5).[1,2] As proposed for the biogenesis of **1** from malonganenone A,[1] alkylation of **16** would yield **17**, the NHC-precursor and common ancestor to the other species. Formation of an NHC from this and subsequent oxidative degradation would yield **18**, while enzymatic reduction could give **19**. Alternatively, hydrolysis of **17** would yield **20**, as found in malonganenone B. It should be noted that the structural motifs of **16** (*e.g.* malonganenone A), **18** (*e.g.* nuttingins A and B) and **19** (*e.g.* nuttingins C–E) have all been reported as marine natural products, with the stable quinazolinium cation nuttingin F (*e.g.* motif **17**) noted as a degradation product. These isolable compounds support our proposed mechanism as plausible intermediates and products, and also imply the key role of cation **17** as a core metabolite linking all of the structural variants of the malonganenone and nuttingin families.

The kinetic data and transient formation of organometallic species using similar analogues of malonganenone B have indicated that an NHC-based carbene mechanism is the likely route for H–D exchange in this molecule. This is at odds with the previously proposed mechanism of exchange in related *N*,*N*-dialkylated formamides. The formation of cation **11** indicates the stability of the direct precursor to the NHC and adds weight to our argument. Further investigations of the chemical implications, both structural and catalytic, and biological activities related to this mechanism are currently underway.

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Notes and references

‡ As analysed by a half-life based method, which is outlined in the supporting information; by B. Cox, in *Modern Liquid Phase Kinetics*, Oxford University Press, Oxford, 1994.

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