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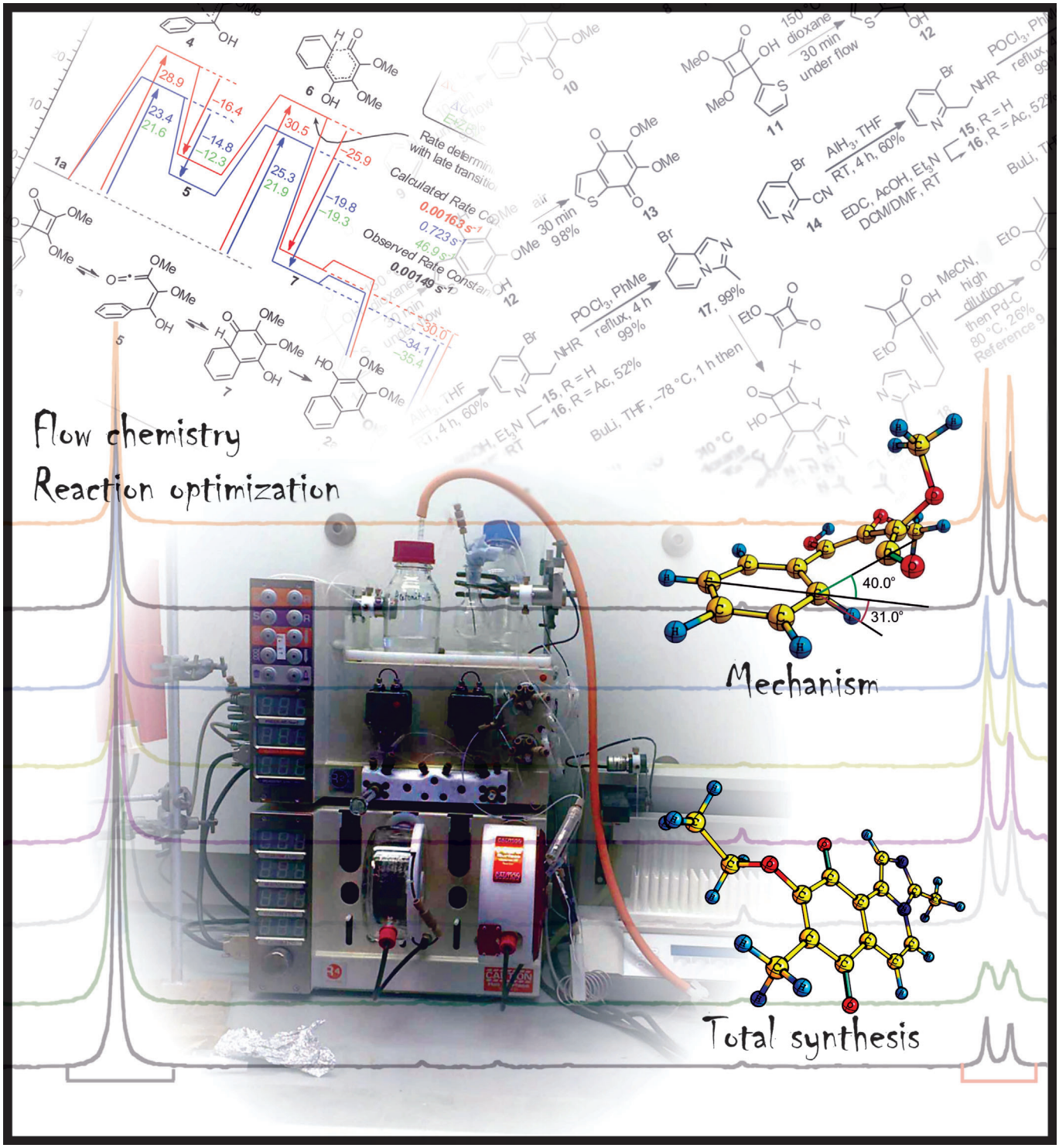
New Insights into Cyclobutenone Rearrangements: A Total Synthesis of the Natural ROS-Generating Anti-Cancer Agent Cribrostatin 6**

Mubina Mohamed,[a] Théo P. Gonçalves,[a] Richard J. Whitby,[a] Helen F. Sneddon,[b] and David C. Harrowven*[a]



Abstract: Aryl- and heteroarylcyclobutenone rearrangements proceed in excellent yield under continuous-flow conditions. The former shows a Hammett correlation with σ_I providing strong evidence that electrocyclisation is the rate-determining step and has a late transition state. The reaction has been modelled by using DFT and CCSD(T) methods, with the latter giving excellent correlation with the experimental rate constant. A short and efficient total synthesis of cribrostatin 6, an anti-neoplastic and anti-microbial agent, provides a topical demonstration of the value of this method.

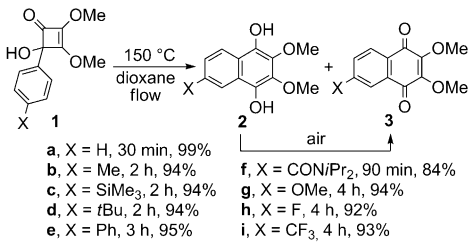
Keywords: density functional calculations · flow chemistry · Hammett correlation · reaction mechanisms · thermochemistry

Introduction

Thermal rearrangements of aryl- and heteroarylcyclobutenones have become established as useful methods for the de novo synthesis of many polyaromatic and heteroaromatic ring systems, especially those with dense substitution patterns.[1–3] Though widely used, little is known about the factors that influence the course of these reactions, or indeed the optimal conditions for effecting them. Herein we present a detailed study of the rearrangement and show how it is possible to achieve near-quantitative conversions under continuous flow. In turn, this has allowed us to establish a Hammett relationship for the reaction which,[4] in conjunction with an in silico study, provides new insights into the mechanistic course. Extensions to heteroarylcyclobutenone rearrangements include a short and efficient total synthesis of the marine natural product cribrostatin 6,[5–9] which displays useful anti-microbial and anti-cancer activity through a reactive-oxygen species (ROS)-generating mechanism.[5,7]

Results and Discussion

Reaction optimization under continuous flow and establishment of a Hammett relationship: Our investigation began with a survey of the arylcyclobutenone rearrangement, **1**→**2**+**3** (Scheme 1).[1] In batch, reactions of this type are usually conducted in xylenes at reflux (ca. 135 °C) and typically give yields of 70–85 % after 2–10 h.[1–3] By contrast, under continuous flow on a Vapourtec R4/R2+ instrument with stainless-steel tubing of 1 mm diameter,[10] it was possible to employ dioxane as the solvent at 150 °C to induce rearrangement of cyclobutenone **1a** to benzohydroquinone **2a** in 99 % isolated yield in less than 1 h.



Scheme 1. Arylcyclobutenone rearrangements under continuouse flow (with isolated yields quoted for the formation of **3** from **1**).

By reasoning that the marked improvement in efficiency was due to a tight control of temperature across the narrow tubing, we were pleased to observe similar results for the rearrangement of a range of related substrates **1b–i** (Scheme 1). In each case, reactions took longer to proceed to completion than the parent compound **1a**, with electron-withdrawing substituents (F, CF_3, amide) and some electron donors (OMe) slowing the reaction down significantly. To delineate the nature of substituent effects, the progress of each reaction was determined at various residence times by using ^{1}H NMR analysis to assess the extent of conversion.[11] Though complicated by incomplete aerial oxidation of the product hydroquinones **2** to the respective benzoquinones **3**, the method proved reliable in establishing that each rearrangement exhibited first-order kinetics (Figure 1).

With these data a Hammett relationship for the reaction was sought. No correlation was evident with either the σ_m or σ_p parameter sets, as would be expected if the electrocyclic opening of the cyclobutenone were involved in the rate-determining step (Figure 2, **1a**→**5**). A reasonable correlation was given with the σ_I (inductive) parameter set (R^2=0.8584, Figure 3, grey line),[4b] with the parent compound **1a** (X= H) and those with large substituents (*t*Bu, Me_3Si, CF_3) showing greatest deviation from the line of best fit. This suggested a significant steric component to the reaction.[4,12] Indeed, by introducing a small steric correction factor (σ_I−6 % E_s) the correlation was improved to R^2=0.989 (Figure 3, bold line).[4,12]

Computational studies on the reaction mechanism: The Hammett relationship observed suggests that the electrocyc-

[a] M. Mohamed, T. P. Gonçalves, Prof. R. J. Whitby, Prof. D. C. Harrowven
Chemistry, University of Southampton
Highfield, Southampton, Hampshire, SO17 1BJ (UK)
Fax: (+44) 23-8059-6805
E-mail: dch2@soton.ac.uk

[b] Dr. H. F. Sneddon
c/o GlaxoSmithKline Medicines Research Centre
Gunnels Wood Road, Stevenage, SG1 2NY (UK)

[**] ROS = reactive-oxygen species

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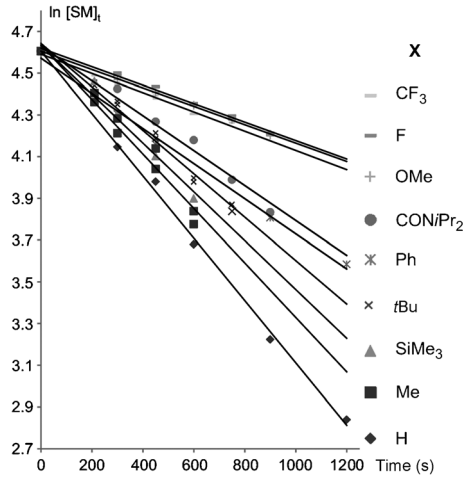


Figure 1. Determination of rate constants for **1**→**2**+**3**. X=CF_3: $k=4.4\times10^{-4}\,s^{-1}$, $R^2=0.96$; X=F: $k=4.6\times10^{-4}\,s^{-1}$, $R^2=0.98$; X=OMe: $k=6.6\times10^{-4}\,s^{-1}$, $R^2=0.99$; X=CON*i*Pr_2: $k=8.0\times10^{-4}\,s^{-1}$, $R^2=0.98$; X=Ph: $k=8.2\times10^{-4}\,s^{-1}$, $R^2=1.00$; X=*t*Bu: $k=10.2\times10^{-4}\,s^{-1}$, $R^2=0.99$; X=$SiMe_3$: $k=12.1\times10^{-4}\,s^{-1}$, $R^2=0.98$; X=Me: $k=13.1\times10^{-4}\,s^{-1}$, $R^2=0.97$; X=H: $k=14.9\times10^{-4}\,s^{-1}$, $R^2=1.00$. $[SM]_t$=concentration of starting material at a given time.

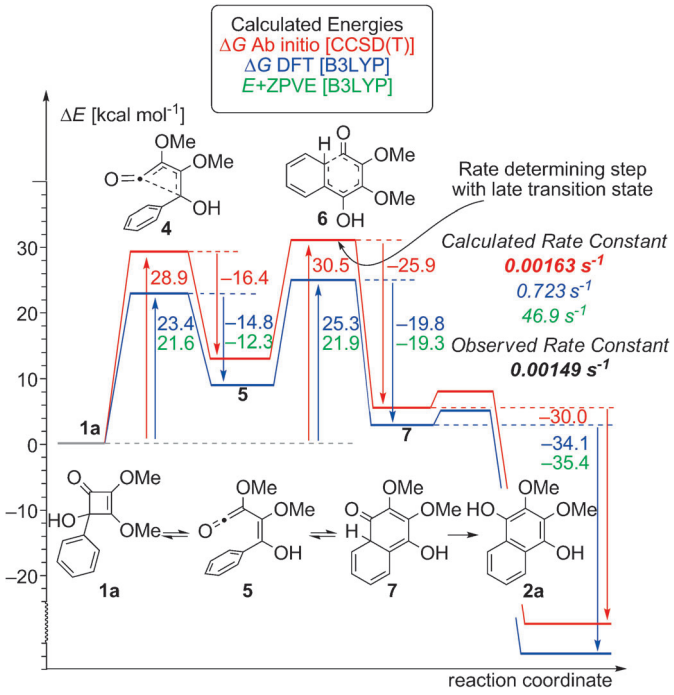


Figure 2. Summary of the energies calculated for the rearrangement **1a**→**2a** in the gas phase at 150°C computed by Δ(*E*+ZPE) UB3LYP/6-311G(d,p) (in green), Δ*G*(UB3LYP/6-311G(d,p)) (in blue) and Δ*G*(RCCSD(T)/6-31G(d)//UB3LYP/6-311G(d,p)) (in red) as well as associated rate constants.[15–18]

lisation of ketene **5** to bicyclic ketone **7** is rate determining and has a late transition state (i.e., **6** is more akin to intermediate **7** than precursor ketene **5**, Figure 2). The rate of reaction is thus dictated by the ease with which the sigma

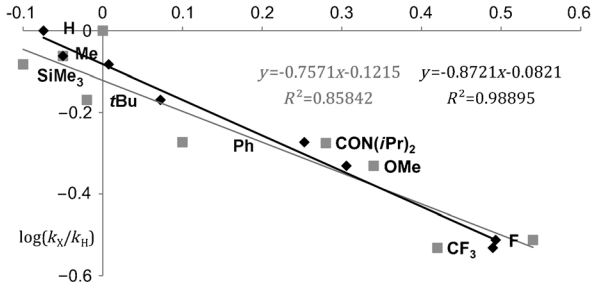


Figure 3. Hammett plot for the arylcyclobutenone rearrangement **1**→**2**+**3** with the σ_I parameter set (■) and with a steric correction (σ_I–6% E_s, ◆).[13]

bond, which is developing between the arene and the carbonyl in **6**, is established; this explains the observed influence of inductive rather than resonance effects. To test this hypothesis, the course of the reaction was modelled by DFT calculations at the UB3LYP/6-311G(d,p) level with the Gaussian 09 program.[14,15] The estimated *E*+ZPVE values (Figure 2, in green) were interesting in that they showed little difference between the activation barrier for electrocyclic ring opening (21.6 kcal mol^{-1}) and ring closing (21.9 kcal mol^{-1}). However, when these were corrected to reflect free energy, the calculated values for Δ*G* at 150°C (23.4 and 25.3 kcal mol^{-1}, respectively) supported our postulate that electrocyclisation of vinylketene **5**→**7** is rate determining. Importantly, they also showed the electrocyclic opening of **1a**→**5** to be reversible with the equilibrium favouring the cyclobutenone rather than the ketene.

A limitation of the DFT method was exposed when we sought to relate the predicted Δ*G* values to the reaction rates observed experimentally for the rearrangement **1a**→**2a**. The calculated values implied a reaction rate substantially faster than the observed one, underestimating the energy requirements by nearly 5 kcal mol^{-1}. Consequently, we refined our analysis further by employing high ab initio single-point energy calculations [RCCSD(T)/6-31G(d)] with the GAMESS(US) package.[17,18] The results attained predicted a rate constant for the rearrangement of **1a**→**2a** of 0.0016 s^{-1} after correction for the free energy at 150°C; this is in excellent agreement with the observed value (0.00149 s^{-1}). In addition, these calculations reaffirmed that the electrocyclic closure **5**→**7** is rate limiting (30.5 kcal mol^{-1} compared with 28.9 kcal mol^{-1} for **1a**→**5**).

The calculated geometry for transition-state **6** (Figure 4) is also instructive as it shows an angle of incidence of 40.0° between the developing σ bond and the plane of the arene. The angle is reduced to 22.1° as the reaction progresses to intermediate **7**. Thus, interaction between this nascent σ bond and the residual π system is limited as it develops to become part of the σ framework—an observation that is consistent with a late transition state under the influence of inductive rather than resonance effects.

Further exemplifications of the method and a total synthesis of cribrostatin 6: Our attention next turned to the rear-

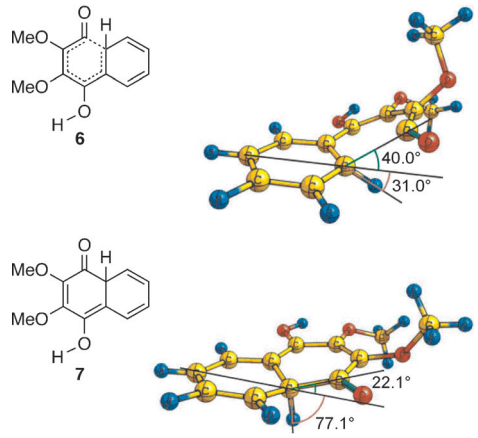
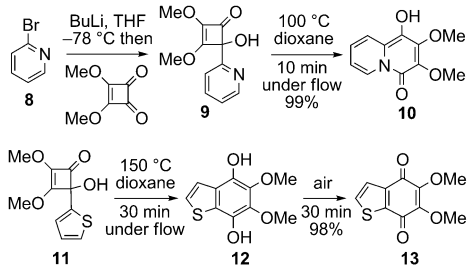


Figure 4. Calculated structures for the transition state **6** and intermediate **7**.[16]

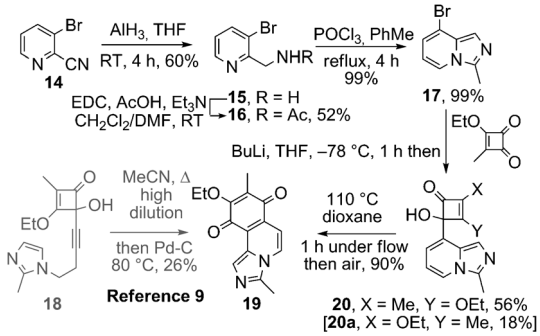
rangement of heteroarylcyclobutenones. With a myriad of options available, we limited our study to representative electron-rich (thiophene) and electron-poor (pyridine) systems, and an exemplification through total synthesis. The thermolysis of (2-pyridyl)cyclobutenones provides rapid access to quinolizidones,[2a] for example, **9**→**10**, which are used widely in medicinal chemistry as isosteres for naphthalenes. In spite of this, the reaction has found little favour, perhaps due to modest yields (29–60%) and a need to protect the alcohol moiety formed on addition of a 2-lithiopyridine to a cyclobutendione. Under continuous-flow conditions, the thermal rearrangement of pyridylcyclobutenone **9** in dioxane at 100°C gave quinolizidone **10** in quantitative yield after a residence time of just 10 min without the need for alcohol protection (Scheme 2). Although the related synthesis of quino[*b*]thiophene **13** from thiophene **11** required a higher temperature (150°C) and an aerial oxidation, it too proceeded efficiently, to give a 98% yield over the two steps (Scheme 2).[19]



Scheme 2. Representative heteroarylcyclobutenone rearrangements under continuous-flow conditions.[17,18]

To demonstrate the value of the method, we chose to tackle the synthesis of the marine natural product cribrostatin 6 (**19**), a popular target since it was identified by Pettit et al. in 2003, that exhibits useful anti-neoplastic and antimicrobial activity.[5,6] In 2010, cribrostatin 6 was reported to induce death in cancer cells by inducing oxidative stress and the build-up of ROS.[20] As an approach to cancer chemotherapy, ROS-inducing therapies are still in their infancy. Esclomol, for example, was recently advanced to phase III clinical trials in combination with taxol, though these were halted because of increased mortality.[20] To date, four total syntheses of **19** have been reported.[6–9,20] The shortest, by Knueppel and Martin, was reported in 2009 and featured an alkynylcyclobutenone rearrangement as a key step, namely, **18**→**19**.[9] Although this step gave a low yield (26%), it allowed the total synthesis to be completed in just five linear steps.

Our plan was to achieve a synthesis of cribrostatin 6 in a similar step count, but with greater efficiency. To that end, nitrile **14** was reduced to the corresponding amine **15** with alane (AlH_3) (Scheme 3).[21] Acylation to **16** was followed by cyclisation with $POCl_3$ to give imidazopyridine **17** in a near-quantitative yield. Halogen–lithium exchange then facilitated the union of **17** and 2-ethoxy-3-methylcyclobutendione, to give a separable 3:1 mixture of adduct **20** and a regioisomer **20a** derived from addition to the vinylogous ester carbonyl. Finally, thermolysis of **20** in dioxane for 1 h at 110°C under continuous flow, followed by exposure to air for a further 45 min, gave **19** in 90% yield after purification by column chromatography.



Scheme 3. A short total synthesis of cribrostatin 6 (**19**). EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.

Conclusion

In conclusion, we have shown that aryl- and heteroarylcyclobutenone rearrangements can be conveniently performed under continuous flow in dioxane at 150°C and proceed with excellent yields. The approach has allowed us to determine a Hammett relationship for the reaction. This, in conjunction with DFT and ab initio modelling,[11] provides strong evidence that the electrocyclisation of ketene **5** to bicyclic ketone **7** is rate determining in arylcyclobutenone rearrangements and has a late transition state. From a computational perspective, the excellent performance of RCCSD(T)/6-31G(d)//UB3LYP/6-311G(d,p) in predicting reaction kinetics is notable. The short and efficient total syn-

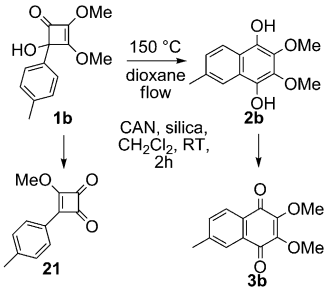
thesis of cribrostatin 6 (**19**), a useful anti-neoplastic and anti-microbial agent, provides a topical demonstration of the value of the method for the rapid construction of condensed quinones.

Experimental Section

General procedure for continuous-flow reactions: Aliquots of **1a–i** (2 mL) in dioxane were taken from bulk solutions (0.25 g in 25 mL) and heated at 150 °C under continuous flow in stainless-steel tubing (internal diameter 1 mm, capacity 10 mL) for the stated residence time by using a Vapourtec R4/R2+ device. The resulting solutions were concentrated in vacuo then analyzed by ^{1}H NMR to determine the composition (**1**, **2** and **3**) by comparison of the respective integrals as indicated in the Supporting Information.

*2,3-Dimethoxynaphthalene-1,4-dione (**3a**)*:[1a,f] Compound **3a** could be formed in a near-quantitative yield by using the general procedure with a residence time of 30 min and stirring the resulting solution in air for 1 h. M.p.: 116–118 °C (Et_2O/petroleum ether; previously reported: 115–117 °C (Et_2O));[1a,f] ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.71 (m, 2H), 8.07 (m, 2H), 4.13 ppm (s, 6H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 182.0 (2×C), 147.5 (2×C), 133.8 (2×CH), 130.8 (2×C), 126.3 (2×CH), 61.5 ppm (2×CH_3); IR ($CHCl_3$): $\tilde{\nu}$ = 3385, 2954, 1772, 1601, 1468, 1337, 1047, 994, 858 cm^{-1}; MS (ES$^+$): *m/z* (%): 241 $[M+Na]^+$ (46), 219 $[M+H]^+$ (16). See Figure 5 and Table 1.

*2,3-Dimethoxy-6-methylnaphthalene-1,4-dione (**3b**)*:[1a] Compound **3b** could be formed in 94% yield by using the general procedure with a residence time of 2 h and stirring the resulting solution in air for 3 h. M.p.: 89–91 °C (MeOH; previously reported: 90–92 °C (aq MeOH));[1a] ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.96 (d, J = 7.8 Hz, 1H), 7.87 (br s, 1H), 7.53–7.47 (ddq, J = 7.8, 1.5, 0.8 Hz, 1H), 4.12 (s, 3H), 4.10 (s, 3H), 2.48 ppm (br s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 182.3 (C), 181.8 (C), 147.6 (C), 144.9 (C), 134.4 (C), 130.8 (CH), 128.6 (C), 126.7 (CH), 126.4 (CH), 119.7 (C), 61.4 (2×CH_3), 21.8 ppm (CH_3); IR ($CHCl_3$): $\tilde{\nu}$ = 2950, 1659, 1611, 1600, 1306, 1269, 1040 cm^{-1}; MS (ES$^+$): *m/z* (%): 482 $[2M+NH_4]^+$ (60), 233 $[M+H]^+$ (100). See Scheme 4, Figure 6 and Table 2.



Scheme 4. Synthesis of **3b**. CAN = ceric ammonium nitrate.

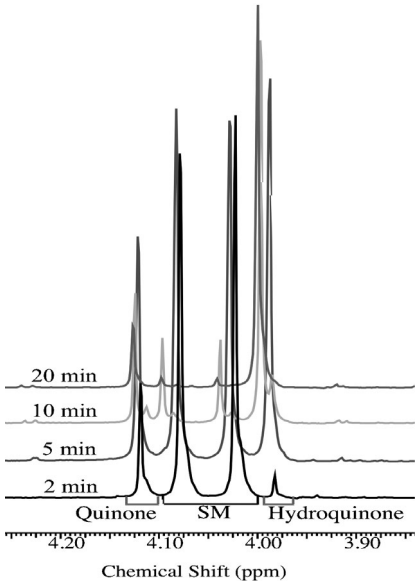


Figure 5. NMR spreads used to extract data presented in Table 1.

Table 1. Concentration of **1a** after thermolysis in dioxane at 150 °C for the stated residence time, as determined by NMR integration.

Time [s]	**1a** [%]	ln[SM]
0	100	4.61
300	63.2	4.15
450	53.5	3.98
600	39.6	3.68
900	25.1	3.22
1200	17.1	2.84

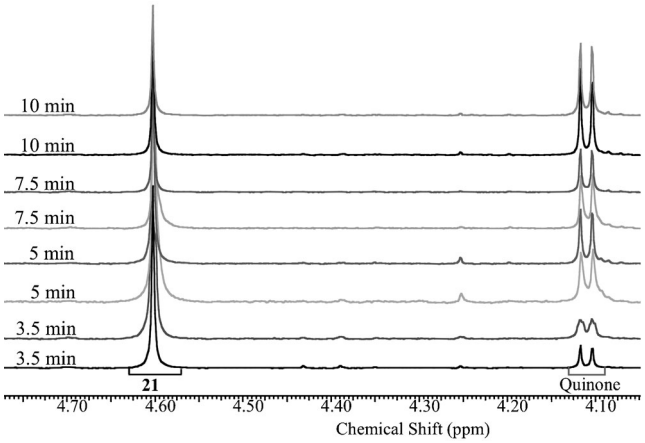


Figure 6. NMR spectra used to extract data presented in Table 2.

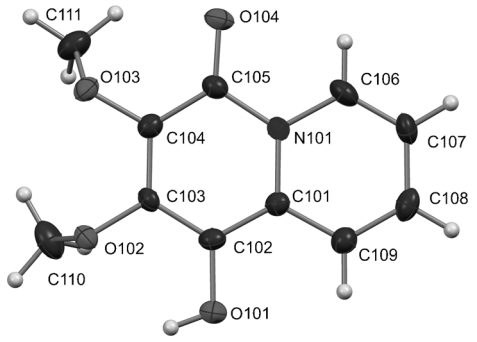
Table 2. Concentration of **1b** after thermolysis in dioxane at 150 °C for the stated residence time, as determined by NMR integration; the reactions were run in duplicate.

Time [s]	**1b** [%]	ln[SM]
0	100	4.61
	100	4.61
210	81.9	4.41
	78.4	4.36
300	72.4	4.28
	67.5	4.21
450	62.7	4.14
	56.9	4.04
600	46.5	3.84
	43.7	3.78

As an alternative to the general method, the crude reaction mixture could be concentrated in vacuo, dissolved in CH_2Cl_2 then exposed to 23% CAN on silica. The procedure facilitates the quantitative oxidation of the benzohydroquinone **2b** to benzoquinone **3b** with remaining starting-material **1b** converted to 3-methoxy-4-(4-methylphenyl)cyclobuten-1,2-dione (**21**). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.94$ (d, $J=8.1$ Hz, 2H), 7.32 (d, $J=8.1$, Hz, 2H), 4.60 (3H, s), 2.44 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=193.8$ (C), 184.7 (C), 174.9 (C), 143.9 (C), 129.9 (2×CH), 127.9 (2×CH), 125.0 (C), 116.3 (C), 61.6 (CH_3), 22.0 ppm (CH_3); IR ($CHCl_3$): $\tilde{\nu}=1784$, 1595, 1369 cm^{-1}; MS (ES$^+$): *m/z* (%): 266 $[M+MeCN+Na]^+$ (100), 203 $[M+H]^+$ (2).[22]

*4-Hydroxy-2,3-dimethoxy-4-(pyridin-2-yl)cyclobut-2-enone (**9**)*: *n*BuLi (3.52 mL, 2.40 M solution in hexane, 8.45 mmol) was added to a solution of 2-bromopyridine (0.82 mL, 8.45 mmol) in THF (30 mL) at −78 °C over 5 min. After 15 min the resulting solution was added, through a cannula, to a solution of dimethyl squarate (1.00 g, 7.04 mmol) in THF (10 mL) at −78 °C, giving a red solution. After 30 min, saturated NH_4Cl (20 mL) was added. The reaction mixture was allowed to warm to RT then extracted with CH_2Cl_2 (50 mL×3). The combined organic layers were washed with brine (50 mL×2), dried ($MgSO_4$), filtered, concentrated in vacuo and purified by flash column chromatography (5%→50% EtOAc/petroleum ether with 2% NEt_3) to afford the title-compound **9** as a pale brown oil (0.97 g, 4.37 mmol, 62%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.62$ (d+fine splitting, $J=4.5$ Hz, 1H), 7.77 (td, $J=7.5$, 1.5 Hz, 1H), 7.48 (d, $J=7.5$ Hz, 1H), 7.30 (ddd, $J=7.5$, 4.5, 1.0 Hz, 1H), 6.07 (s, 1H), 4.09 (s, 3H), 3.98 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=182.6$ (C), 164.4 (C), 154.6 (CH), 148.3 (C), 137.6 (C), 137.4 (CH), 123.2 (CH), 120.0 (CH), 80.0 (C), 59.9 (CH_3), 58.8 ppm (CH_3); IR ($CHCl_3$): $\tilde{\nu}=3463$, 2951, 1776, 1632, 1468, 1337, 1061 cm^{-1}; MS (ES$^+$): *m/z* (%): 222 $[M+H]^+$ (100); HRMS (ES$^+$): *m/z*: calcd for $C_{11}H_{12}NO_4$: 222.0775 $[M+H]^+$; found: 222.0766.

*1-Hydroxy-2,3-dimethoxy-4H-quinolizin-4-one (**10**)*: A solution of **9** (5.0 mg, 0.023 mmol) in dioxane (2 mL) was heated at 100 °C in stainless-steel tubing for a residence time of 10 min with a Vapourtec R4/R2+ device. The resulting solution was stirred in air for 1 h and then concentrated in vacuo to give **10** as a pale brown solid (4.9 mg, 0.022 mmol, 99%). M.p.: 110–112 °C (EtOAc/petroleum ether); ^{1}H NMR (400 MHz, [D$_6$]DMSO): $\delta=8.88$ (s, 1H), 8.70 (d, $J=7.6$ Hz, 1H), 7.73 (d, $J=9.0$ Hz, 1H), 7.16 (dd, $J=8.0$, 6.6 Hz, 1H), 6.86–6.97 (m, 1H), 4.03 (s, 3H), 3.87 ppm (s, 3H); ^{13}C NMR (100 MHz, [D$_6$]DMSO): $\delta=152.1$ (C), 150.2 (C), 131.7 (C), 127.2 (C), 126.7 (C), 125.4 (CH), 125.2 (CH), 120.0 (CH), 114.0 (CH), 60.9 (CH_3), 59.3 ppm (CH_3); IR ($CHCl_3$): $\tilde{\nu}=2955$, 2925, 1733, 1634, 1595, 1457, 1288, 1122 cm^{-1}; MS (ES$^+$): *m/z* (%): 222 $[MH]^+$ (100); HRMS (ES$^+$): *m/z*: calcd for $C_{11}H_{12}NO_4$: 222.0775 $[M+H]^+$; found: 222.0766.



*4-Hydroxy-2,3-dimethoxy-4-(thiophen-2-yl)cyclobut-2-en-1-one (**11**)*:[1a] 2-Bromothiopene (0.376 mL, 3.87 mmol) was added to a solution of *n*BuLi (2.42 mL, 1.6 M solution in hexane, 3.87 mmol) in THF (15 mL) over 5 min at −78 °C. After 15 min, a solution of dimethyl squarate (0.50 g, 3.52 mmol) in THF (10 mL) was added over 5 min, giving an orange solution. After 1 h, saturated NH_4Cl (20 mL) was added. The reaction mixture was allowed to warm to RT then extracted with CH_2Cl_2 (20 mL×3). The combined organic layers were washed with brine (20 mL×2), dried ($MgSO_4$), filtered, concentrated in vacuo and purified by flash column chromatography (5%→40% EtOAc/petroleum ether with 2% NEt_3) to afford the title-compound **11** as a white solid (0.257 g, 1.14 mmol, 32%). M.p.: 66–68 °C (Et_2O/petroleum ether; previously reported: 68–69 °C (Et_2O));[1a] ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.33$ (dd, $J=5.0$, 1.3 Hz, 1H), 7.11 (dd, $J=3.5$, 1.3 Hz, 1H), 7.02 (dd, $J=5.0$, 3.5 Hz, 1H), 4.11 (s, 3H), 4.02 (s, 3H), 3.56 ppm (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=$ 183.1 (C), 166.0 (C), 140.7 (C), 135.2 (C), 127.2 (CH), 126.2 (CH), 125.0 (CH), 85.7 (C), 60.3 (CH_3), 58.8 ppm (CH_3); IR ($CHCl_3$): $\tilde{\nu}=3400$, 3008, 2956, 1781, 1644, 1635, 1470, 1348, 1040 cm^{-1}; MS (ES$^+$): *m/z* (%): 227 $[M+H]^+$ (100).

*5,6-Dimethoxybenzo[b]thiophene-4,7-dione (**13**)*:[1a] A solution of **11** (5.0 mg, 0.022 mmol) in dioxane (2 mL) was heated at 150 °C in stainless-steel tubing for a residence time of 30 min by using a Vapourtec R4/R2+ device. The resulting solution was stirred in air for 1 h then concentrated in vacuo to give the title-compound **13** as an orange solid (4.9 mg, 0.022 mmol, 98%). M.p.: 169–172 °C (Et_2O/petroleum ether; previously reported: 171.5–173 °C (CH_2Cl_2/petroleum ether));[1a] ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.63$ (d, $J=5.0$ Hz, 1H), 7.50 (d, $J=5.0$ Hz, 1H), 4.09 ppm (apparent s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=178.0$ (C), 176.6 (C), 147.2 (C), 146.7 (C), 141.4 (C), 139.5 (C), 133.4 (CH), 125.9 (CH), 61.5 (CH_3), 61.6 ppm (CH_3); IR ($CHCl_3$): $\tilde{\nu}=3033$, 3008, 2929, 1651, 1340, 1292, 858 cm^{-1}; MS (ES$^+$): *m/z* (%): 225 $[M+H]^+$ (100).

*(3-Bromopyridin-2-yl)methanamine (**15**)*:[21] To a cooled (0 °C) solution of 3-bromo-2-cyanopyridine (0.100 g, 0.546 mmol) in toluene (20 mL) was added alane·Me_2NEt complex (0.5 M solution in toluene, 2.2 mL, 1.093 mmol) over 4 min. The resulting mixture was warmed to RT and then, after 16 h, was re-cooled to 0 °C. Methanol (10 mL) and saturated sodium potassium tartare (50 mL) were cautiously added, then the aqueous phase was separated and extracted with $CHCl_3$ (20 mL×3). The combined organic phases were then washed with brine (20 mL×2), dried ($MgSO_4$), filtered and concentrated in vacuo to afford the title-compound **15** as an yellow oil (60.5 mg, mmol, 60%). ^{1}H NMR (300 MHz, $CDCl_3$): $\delta=8.52$ (dd, $J=5.0$, 1.5 Hz, 1H), 7.85 (dd, $J=7.9$, 1.5 Hz, 1H), 7.11 (dd, $J=7.9$, 5.0 Hz, 1H), 4.14 (s, 2H), 2.77 ppm (s, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta=162.4$ (C), 150.2 (CH), 145.2 (CH), 123.1 (C), 115.9 (CH), 45.2 ppm (CH_2); MS (ES$^+$): *m/z* (%): 189 $[M(^{81}Br)+H]^+$ (100), 187 $[M(^{79}Br)+H]^+$ (100).

*N-((3-Bromopyridin-2-yl)methyl)acetamide (**16**)*:[21] Acetic acid (0.850 mL, 14.83 mmol) and triethylamine (4.13 mL, 29.7 mmol) were added sequentially to a solution of *N*-(3-dimethylaminopropyl)-*N*′-ethyl-carbodiimide hydrochloride (2.84 g, 14.83 mmol) in dichloromethane (30 mL) at RT. After 1 h, a solution of **15** (1.422 g, 5.93 mmol) in DMF (15 mL) and CH_2Cl_2 (10 mL) was added, followed by 2 M sodium carbonate (30 mL) after an additional 1 h. The aqueous phase was separated and extracted with dichloromethane (3×20 mL) and then the organic phases were dried ($MgSO_4$), concentrated in vacuo and purified by column chromatography (EtOAc) to give the title-compound **16** as a white solid (0.730 g, 3.187 mmol, 52%). M.p.: 62–64 °C (EtOAc); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=8.50$ (d, $J=4.4$, 1.3 Hz, 1H), 7.89 (d, $J=$ 7.9, 1.3 Hz, 1H), 7.18 (brs, 1H), 7.16 (dd, $J=7.9$, 4.4 Hz, 1H), 4.62 (d, $J=4.3$, 2H), 2.13 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=170.0$ (C), 153.7 (C), 146.9 (CH), 140.4 (CH), 123.5 (C), 120.3 (CH), 44.1 (CH_2), 23.3 ppm (CH_3); IR ($CHCl_3$): $\tilde{\nu}=3314$, 1642, 1560, 812 cm^{-1}; MS (ES$^+$): *m/z* (%): 231 $[M(^{81}Br)+H]^+$ (100), 229 $[M(^{79}Br)+H]^+$ (100).

*8-Bromo-3-methylimidazo[1,5-a]pyridine (**17**)*: Phosphorus oxychloride (1.07 mL, 11.5 mmol) was added to a solution of acetamide **16** (0.730 g, 3.19 mmol) in toluene (10 mL) at RT over 5 min. The reaction mixture was heated at reflux for 4 h then cooled to 0 °C and saturated sodium bicarbonate (30 mL) was added. The aqueous phase was separated and extracted with ethyl acetate (10 mL×3), then the combined organic phases were washed with water (10 mL×2), dried ($MgSO_4$) and concentrated to give the title-compound **17** as a brown oil (0.680 g, 3.222 mmol, 99%, ca. 98% purity). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta=7.68$ (d, $J=7.3$ Hz, 1H), 7.46 (s, 1H), 6.92 (d, $J=6.8$ Hz, 1H), 6.47 (apparent t, $J=7.1$ Hz, 1H), 2.69 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=179.0$ (C), 136.6 (C),

130.0 (C), 120.8 (CH), 119.8 (CH), 119.5 (CH), 112.7 (CH), 12.6 ppm (CH_3); IR ($CHCl_3$): $\tilde{\nu}$ = 3314, 1642, 1560, 812 cm^{-1}; MS (ES^+): *m/z* (%): 213 $[M(^{81}Br)+H]^+$ (100), 211 $[M(^{79}Br)+H]^+$ (100).

*3-Ethoxy-4-hydroxy-2-methyl-4-(3-methylimidazo[1,5-a]pyridin-8-yl)cyclobut-2-enone (**20**)*: *n*BuLi (1.6 M in hexane, 0.79 mL, 1.26 mmol) was added to a solution of imidazo[1,5-*a*]pyridine **17** (242 mg, 1.15 mmol) in THF (5 mL) at −78 °C. After 30 min a solution of 3-ethoxy-4-methyl-3-cyclobutene-1,2-dione (0.161 g, 1.147 mmol)[1,9] in THF (5 mL) was added over 4 min, followed by saturated NH_4Cl (20 mL) after an additional 1 h. After warming to RT the aqueous phase was separated and extracted with dichloromethane (20 mL × 3). The combined organic phases were washed with brine (20 mL × 2), dried ($MgSO_4$), concentrated in vacuo and purified by flash column chromatography (0 % → 5 % methanol/dichloromethane with 1 % NEt_3) gave firstly **20a** (59 mg, 0.204 mmol, 18 %) then the title-compound **20** as a pale orange oil (175 mg, 0.642 mmol, 56 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.64 (d, *J* = 7.0 Hz, 1H), 7.39 (s, 1H), 6.97 (d, *J* = 6.8 Hz, 1H), 6.60 (t, *J* = 6.9 Hz, 1H), 4.45 (dq, *J* = 9.8, 7.1 Hz, 1H), 4.26 (dq, *J* = 9.9, 7.1 Hz, 1H), 2.66 (s, 3H), 1.85 (s, 3H), 1.36 ppm (t, *J* = 7.1 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 189.5 (C), 182.2 (C), 135.5 (C), 127.8 (C), 127.6 (C), 125.2 (C), 120.7 (CH), 118.5 (CH), 116.5 (CH), 112.0 (CH), 91.6 (C), 69.2 (CH_2), 15.0 (CH_3), 12.6 (CH_3), 6.9 ppm (CH_3); IR ($CHCl_3$): $\tilde{\nu}$ = 2928, 2861, 1715, 1731, 1617, 1332, 1135, 1078 cm^{-1}; MS (ES^+): *m/z* (%): 273 $[M+H]^+$ (100); HRMS (ES^+): *m/z*: calcd for $C_{15}H_{17}N_2O_3$: 273.1161 $[M+H]^+$; found: 273.1232.

*9-Ethoxy-3,8-dimethylimidazo[5,1-a]isoquinoline-7,10-dione (**19**, Cribrostatin 6)*: Cyclobutenone **20** (59 mg, 0.217 mmol) in dioxane (2 mL) was heated at 110 °C in stainless-steel tubing for a residence time of 1 h by using a Vapourtec R4/R2+ device. The resulting solution was stirred in air for 30 min then concentrated in vacuo and purified by chromatography (2 % MeOH in CH_2Cl_2) to give **19** as a light-blue solid (52 mg, 0.193 mmol, 90 %). M.p.: 167–169 °C (acetone at −4 °C; previously reported: 165–167 °C (acetone)[8,9], 169–171 °C (acetone)[5], 171–172 °C (acetone)[6,7]); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.52 (s, 1H); 8.10 (d, *J* = 7.6 Hz, 1H), 7.62 (d, *J* = 7.6 Hz, 1H), 4.49 (q, *J* = 6.8 Hz, 2H), 3.09 (br s, 3H), 2.13 (s, 3H), 1.45 ppm (t, *J* = 7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 184.9 (C), 180.7 (C), 156.2 (C), 137. 7 (C), 130.1 (C), 125.9 (C), 125.0 (C), 124.7 (C), 123.9 (CH), 123.5 (CH), 107.6 (C), 69.6 (CH_2), 16.0 (CH_3), 12.6 (CH_3), 9.2 ppm (CH_3); IR ($CHCl_3$): $\tilde{\nu}$ = 2925, 1662, 1626, 1611, 1527, 1172 cm^{-1}; MS (ES^+): *m/z* (%): 271 $[M+H]^+$ (100).

CCDC-808547 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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