JOC The Journal of Organic Chemistry

Article

pubs.acs.org/joc

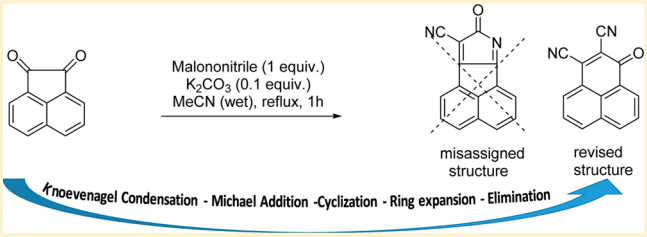
1-Oxo-1*H*-phenalene-2,3-dicarbonitrile Heteroaromatic Scaffold: Revised Structure and Mechanistic Studies

Romaric Lenk, Arnaud Tessier, Pierre Lefranc, Virginie Silvestre, Aurélien Planchat, Virginie Blot, Didier Dubreuil, and Jacques Lebreton*

Université de Nantes, CNRS, CEISAM, UMR CNRS 6230, Faculté des Sciences et des Techniques, 2 rue de la Houssinière, BP 92208, 44322 Nantes Cedex 3, France

Supporting Information

ABSTRACT: Synthesis of the originally proposed 8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrol-9-carbonitrile led to a structural revision, and the product has now been identified as unknown compound 1-oxo-1*H*-phenalene-2,3-dicarbonitrile. The structural assignment was corroborated by detailed NMR studies and unambiguously confirmed by X-ray diffraction. A mechanism is proposed to explain the formation of this original heterocyclic scaffold. In addition, some new chemical transformations involving this compound are presented.



INTRODUCTION

The preparation of substituted and fused polycyclic aromatic compounds and their corresponding heteroaromatic derivatives is an important field of organic chemistry with various applications from medicinal chemistry[2] to materials sciences.[3]

As a continuation of our investigations into the preparation of a new substituted heterocyclic scaffold, we were interested in the acenaphthopyrrole derivatives such as compound **1** (Figure 1).[1] Qian's research group first described the synthesis of the

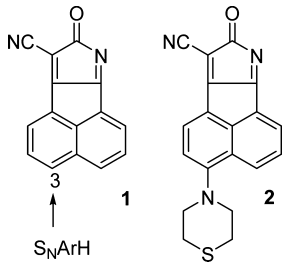


Figure 1. 8-Oxo-8*H*-acenaphtho[1,2-*b*]pyrrol-9-carbonitrile **1** and its thiomorpholine acenaphtho[1,2-*b*]pyrrole derivative **2**.

presumed 8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrol-9-carbonitrile **1** and reported the strong fluorescence properties of such derivatives.[4] Since then, these structures have also appeared in other major publications, which describe their great optical properties for fluorescent chemosensor devices.[5] Regarding their biological activity, these structures were also recently reported for their promising effect as potent inhibitors of Bcl-2 (B-cell lymphoma 2) and Mcl-1 (Myeloid cell leukemia 1) proteins as well as for selective FGFR-1 (fibroblast growth factor receptor 1) inhibition.[6] In this context, compound **2** (Figure 1) was identified as a very promising antitumor drug, acting as the first authentic BH3-interacting domain death agonist mimetic with a dual inhibitor effect on both Bcl-2 and Mcl-1.[7] This family of compounds was readily available from 8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrol-9-carbonitrile **1** by simple treatment with nucleophiles such as primary and secondary amines (e.g., thiomorpholine) and thiols. The reaction proceeds with nucleophilic aromatic substitution of hydrogen (S_NArH)[8] at the C-3 position.

RESULTS AND DISCUSSION

In the context of our current medicinal chemistry programs, this structure, featuring a cyano group[9] as a versatile precursor of other functional groups,[10] was identified as an excellent platform to provide more complex molecules that also offers the possibility of introducing substituents at the C-3 position. Because our approach required access to a substantial amount of compound **1**, initial efforts were directed toward development of robust and efficient synthesis of this known starting material.

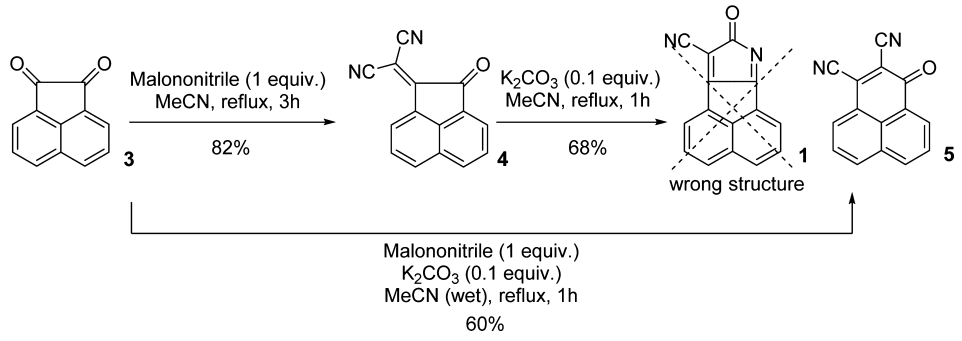
Starting material **1** was prepared following a two-step sequence described in the literature.[4,5d] Treatment of acenaphthylene-1,2-dione **3** with malononitrile in refluxing acetonitrile provided the Knoevenagel adduct **4** in 82% yield (Scheme 1). In the second step, compound **4** was treated with 10 mol % K_2CO_3 at refluxing acetonitrile, instead of 10 equiv as mentioned by Spange et al.,[5d] to provide carbonitrile **1** in 68% yield. At this point, we spent some time finding more suitable conditions to develop an efficient two-step, one-pot sequence.

A key aspect of this two-step, one-pot sequence was the compatibility of bases with the Knoevenagel condensation.[11] Nevertheless, this should not be a problem since alkali metal hydroxides (e.g., NaOH and KOH)[12] or organic bases[13] such as

Received: August 21, 2014
Published: September 26, 2014

   dx.doi.org/10.1021/jo5016932 | *J. Org. Chem.* 2014, 79, 9754–9761

Scheme 1. Synthesis of 1-Oxo-1*H*-phenalene-2,3-dicarbonitrile by a Two- or One-Step Sequence



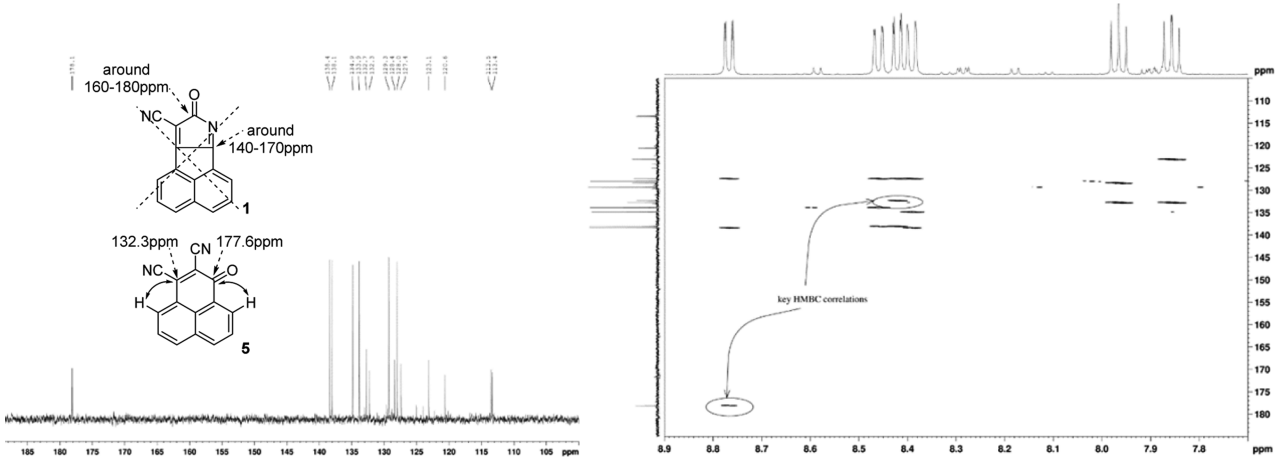


Figure 2. ^{13}C NMR and HMBC correlations for compound **5**.

pyridine or piperidine are the traditional catalysts used in this reaction. Unfortunately, we investigated various bases (DBU, Al_2O_3, Cs_2CO_3, NaOH) in acetonitrile at different temperatures without success. To our surprise, reaction of acenaphthylene-1,2-dione **3** with malononitrile with catalytic amounts of K_2CO_3 at reflux in wet acetonitrile, instead of dry acetonitrile under a nitrogen atmosphere, provided the desired carbonitrile derivative **1** as a sole product in 60% yield. Spectral properties of this resulting material **1** were identical in all respects to those reported in the literature.[4,5d] However, we were intrigued by the absence of NMR data to assign the chemical structure reported in the literature for compound **1** as well as any mechanistic arguments to explain its formation. Consequently, we decided to reinvestigate the structure of compound **1** using NMR spectral studies.

To ensure the results of our structural analysis, 1D ^1H and ^{13}C NMR were performed as well as 2D experiments, both homonuclear (COSY) and heteronuclear (HSQC and HMBC) correlation spectroscopy (Figure 2).[14] Regarding the proton NMR in a nonviscous solvent, the presumed 8-oxo-8*H*-acenaphto[1,2-*b*]pyrrol-9-carbonitrile **1** displays a very simple ^1H NMR spectrum, namely, six doublets of doublets with chemical shifts around 7.0 and 9.5 ppm in which each doublet of a doublet counts for one ^1H. Indeed, four of the six doublets of doublets are composed of one $^3J_{H-H}$ and one $^4J_{H-H}$, and two of the six doublets of doublets are composed of two $^3J_{H-H}$. However, several other indications revealed that the previous assignments cannot be in agreement with the proposed structure. In fact, the 15 expected peaks of the ^{13}C NMR spectrum for the presumed 8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrol-9-carbonitrile **1** could be described as follows: six aromatic CH groups (δ around 110−140 ppm), one imine function C═N (δ around 140−170 ppm), one carbonyl function CO (δ around 160−180 ppm), one nitrile function C≡N (δ around 110−130 ppm), and six quaternary carbons, with four of them being aromatic (δ around 140−170 ppm) and the (CN)−C═C group (δ around 110−140 and 140−170 ppm). However, among the 15 signals observed in our 1D ^{13}C NMR spectra, only one presents a chemical shift above 140 ppm, and two signals have their chemical shift around 110−115 ppm (Figure 2). According to these data, it could be suggested that the structure of the compound would better match with two nitrile functions and one carbonyl group rather than an imine group.

In addition, our further investigation by an HMBC (heteronuclear multiple-bond correlation) experiment revealed that the correlation peaks cannot be explained by 8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrol-9-carbonitrile compound **1** (Figure 2). Surprisingly, the HMBC experiment revealed correlations between one of the aromatic ^1H (δ = 8.77 ppm, dd, J = 1.2 Hz, J = 7.5 Hz) and the carbonyl group (δ = 178.8 ppm) and one of the aromatic ^1H (δ = 8.42 ppm, dd, J = 1.0 Hz, J = 7.4 Hz) and a quaternary carbon (δ = 132.3 ppm). The only structure that can exhibit such HMBC is the 1-oxo-1*H*-phenalene-2,3-dicarbonitrile **5**. Finally, the structure of 1-oxo-1*H*-phenalene-2,3-dicarbonitrile **5** was then unambiguously confirmed by X-ray diffraction (Figure 3).

It is worth noting that the X-ray structure of the presumed compound **2** was also unambiguously reassigned according to the revised structure 1-oxo-6-thiomorpholino-1*H*-phenalene-2,3-dicarbonitrile.[15]

dx.doi.org/10.1021/jo5016932 | *J. Org. Chem.* 2014, 79, 9754−9761

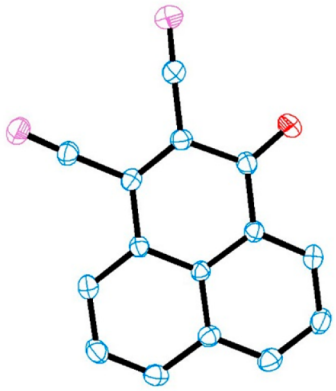
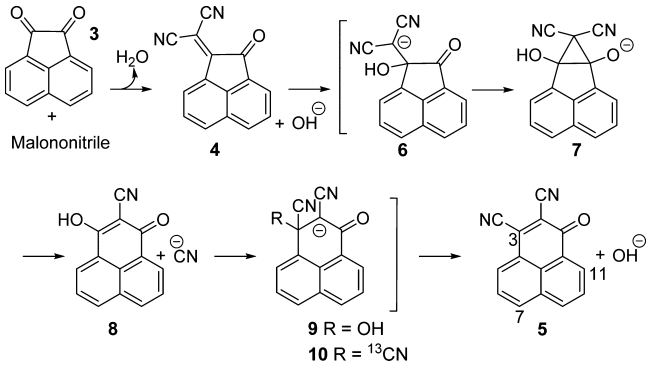


Figure 3. ORTEP drawing of the X-ray crystallographic structure of **5**.

To explain the formation of 1-oxo-1*H*-phenalene-2,3-dicarbonitrile **5**, we postulated the mechanistic pathway disclosed in Scheme 2. As previously mentioned, this two-step, one-pot sequence was successfully run with wet acetonitrile to provide compound **5**. This crucial point supported the hypothesis that the Knoevenagel condensation followed by a Michael addition of water (principally coming from the solvent versus water formed during the Knoevenagel condensation) or hydroxide on adduct **4** gave the corresponding stabilized anion derivative **6**. Then, we proposed a reaction pathway involving a cyclization to provide transient intermediate **7**. At this stage, the resulting *β*-hydroxyenone **8** was formed from **7** by ring extension and elimination of cyanide. Ultimately, the final step involved a Michael addition of cyanide (released from the previous step) on intermediate **8** to furnish adduct **9**, which by a retro-Michael elimination of hydroxide, afforded compound **5**.

Scheme 2. Proposed Mechanistic Pathway

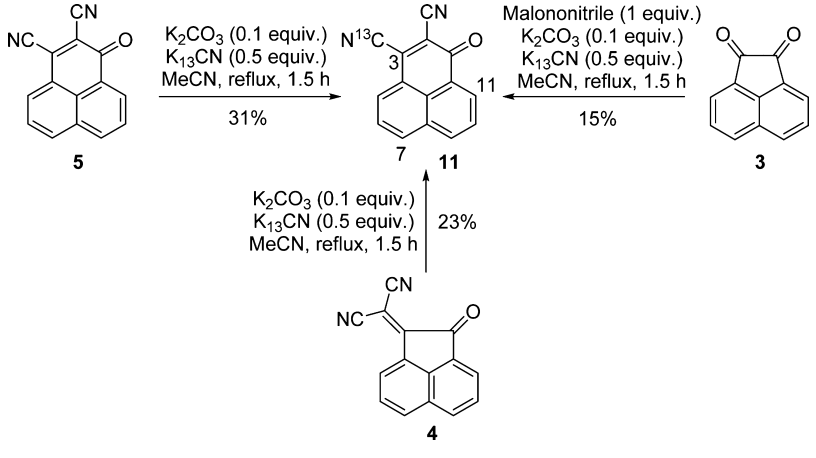


To investigate the proposed mechanistic pathway, we sought to investigate several experiments with $K^{13}CN$, as depicted in Scheme 3.

First, treating **5** with 0.5 equiv $K^{13}CN$ afforded corresponding derivative **11** enriched only at the C-3 position, confirming the proposed mechanistic pathway. Indeed, the 1D and ^{13}C NMR spectra clearly indicate that the ^{13}C signal at δ = 113.4 ppm, corresponding to one nitrile function **C≡N**, was enriched because of its high intensity (see Supporting Information). As described in Scheme 2, this result may come from a cyanide exchange via a Michael addition−retro-Michael reaction sequence involving the 2,3,3-trinitrile analogue adduct of **9** (compound **10** R = CN, depicted in Scheme 2). On the other hand, a mixture of acenaphthylene-1,2-dione **3** and malononitrile was treated with 10 mol % of K_2CO_3 at refluxing acetonitrile with 0.5 equiv of $K^{13}CN$ to furnish the corresponding ^{13}C-enriched derivative **11** only at the C-3 position. Similarly, this enriched derivative **11** was once again obtained by using these reaction conditions starting from adduct **4**. If these three last experiments were in accordance with the proposed mechanistic pathway, then it is noteworthy that the addition of $K^{13}CN$ in the two-step, one-pot sequence improved neither the yield nor the reaction time as would be expected when increasing the concentration of the nucleophilic species. In fact, the yield of these experiments remained modest due to the formation of byproducts resulting from the addition of cyanide following a S_NArH process mainly at the C-7 and C-11 positions.[16] It should be pointed out that the slow release of cyanide as the reaction progressed (transformation of **7** into **8** in Scheme 2) prevented the formation of byproducts from subsequent S_NArH reactions at the C-7 and C-11 positions.

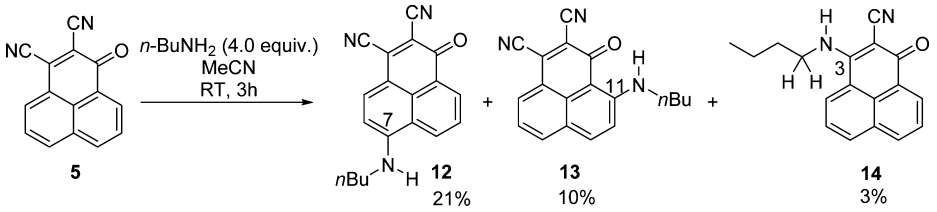
In parallel, we reinvestigated the S_NArH reaction of primary amines on the highly electron-deficient heteroaromatic compound **5** as previously reported (Scheme 4).[17] With *n*-butylamine, the desired adduct **12** was isolated in 21% yield after purification. C-11 adduct **13** was also isolated in 10% yield after careful silica gel chromatography purification.[18] More interestingly, a small amount, typically about a 3% yield of a second byproduct, was isolated, and mass spectrometry and

Scheme 3. Mechanistic Elucidations by Key Experiments in the Presence of ^{13}C-Labeled Cyanide Potassium



dx.doi.org/10.1021/jo5016932 | J. Org. Chem. 2014, 79, 9754−9761

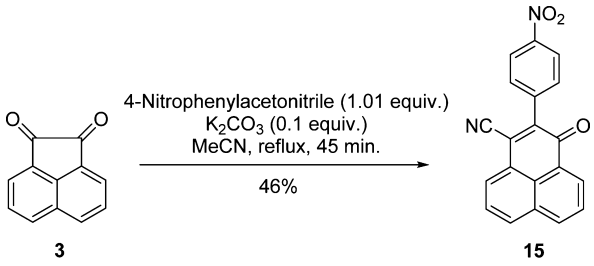
Scheme 4. S_NArH Reaction of 1-Oxo-1*H*-phenalene-2,3-dicarbonitrile 5 with *n*-Butylamine



NMR spectroscopy demonstrated that compound **14** was, in fact, formed by Michael addition of the primary amine at C-3 of the α,β-unsaturated ketone moiety of **5** followed by retro-Michael addition of cyanide. Regarding the NMR structure assignment, the 3-bond coupling between the methylene hydrogen (-(CH_2)-NH-) and C-3, which unambiguously defined the attachment point of the *n*-BuNH- chain, was particularly diagnostic (see Supporting Information). Despite the rather low yields,[19] this last experiment clearly indicates the reactivity of this 1-oxo-1*H*-phenalene-2,3-dicarbonitrile scaffold toward primary nucleophiles. As previously described for the synthesis of thiomorpholine-substituted 1-oxo-1*H*-phenalene-2,3-dicarbonitrile **2**, both regioisomers **12** and **13** substituted at the C-7 and C-11 positions, respectively, are the main products isolated.[20] Analogous with our mechanistic experiments, the formation of compound **14**, resulting from a nucleophilic attack by a primary amine at the C-3 position, revealed the tendency of this position to react with nucleophiles.

Therefore, the question then arises as to whether any methylene active compounds having a cyano group could be used in this two-step, one-pot sequence; if so, then this will be additional proof in favor of the proposed mechanism in which the cyano group acts as a leaving group and a nucleophile. To answer this question and to complete our mechanism investigations as well as to extend the scope of this reaction, acenaphthylene-1,2-dione **3** was treated with active methylene compounds having a cyano group, such as 4-nitrophenylacetonitrile or methyl cyanoacetate. Under optimized conditions with 4-nitrophenylacetonitrile, as outlined in Scheme 5, nitro derivative **15** was formed as a single regioisomer following a two-step, one-pot sequence in 46% yield after purification.

Scheme 5. Knoevenagel Condensation of Acenaphthylene-1,2-dione 3 with 4-Nitrophenylacetonitrile



In contrast with this previous result, all attempts to prepare cyanoester derivative **18** by direct treatment of acenaphthylene-1,2-dione **3** with methyl cyanoacetate failed, and the desired product was isolated in low yields (Scheme 6). The targeted compound **18** was then prepared in two steps: first, the known Knoevenagel condensation reaction of dione **3** with methyl cyanoacetate[21] provided the geometric isomers (*E*) and (*Z*) of 1-cyano-1-carbethoxymethylene acenaphthen-2-one **16** and **17**, respectively. Attempts to carry out the second step under standards conditions (K_2CO_3 in refluxing acetonitrile) were unsuccessful, and only low yields of **18** were obtained, along with unidentified byproducts. Finally, a mixture of **16** and **17** was treated with 10 mol % of K_2CO_3 in acetonitrile under microwave irradiation at 100 °C with a short reaction time to afford cyanoester compound **18** in 23% yield.[22]

Finally, the two last examples, with compounds **15** and **18** obtained from Knoevenagel condensation using methylene active compounds bearing a cyano group (4-nitrophenylacetonitrile and methyl cyanoacetate), showed similar reactivity to the malononitrile. On the basis of the above-mentioned mechanistic insights using malononitrile, it is plausible to propose the same mechanism (Scheme 2) for these two other examples in which the cyanide anion may likewise proceed from its first elimination as a leaving group followed by its attack as a nucleophile on β-hydroxyenone, similar to intermediate **8**.

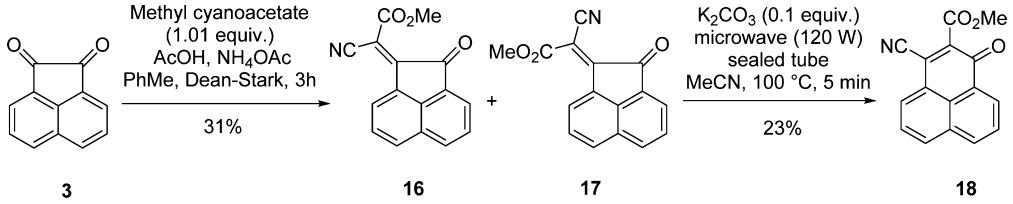
To end this work, we evaluated the reactivity of the cyano groups of **5** toward acidic hydrolysis into its corresponding dicarboxylic derivative (Scheme 7). Such transformations were also reported in the literature by treatment of the phthalonitrile derivative with sodium in ethanol followed by subsequent hydrolysis of the 1*H*-isoindol-1-imine-3-ethoxy intermediate with diluted nitric acid.[23] Treatment of compound **5** in concentrated sulfuric acid at 40 °C was carried out and gave the corresponding imide derivative **19** in high yield. The structure of compound **19** was also unambiguously confirmed by X-ray diffraction.[24] It is worth noting that the structure of the compound **19** was wrongly assigned in the literature.[17] In fact, compound **21** was depicted instead of compound **19** because their acidic treatment was described from the incorrect structure **1** as starting material. The subsequent N-alkylation of the imide moiety of compound **19** was further achieved in the presence of methyltosylate and cesium carbonate, affording the corresponding *N*-methylnaphthoisoindoletrione **20**. Such an original structure of **20** was also confirmed by X-ray diffraction.[24]

■ CONCLUSIONS

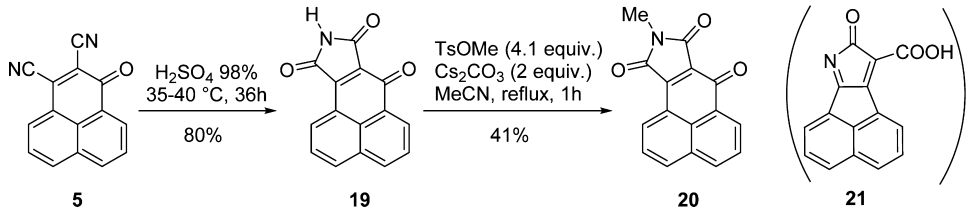
Although the past half century has witnessed a remarkable improvement in our ability to isolate and characterize complex organic molecules, mistakes are still a relatively common occurrence. Whereas our recent investigations were focused on the synthesis of 8-oxo-8*H*-acenaphtho[1,2-*b*]pyrrol-9-carbonitrile compound **1**, we discovered that the initial structure was misassigned and that the structural backbone was 1-oxo-1*H*-phenalene-2,3-dicarbonitrile **5**. We also developed an efficient two-step, one-pot sequence from acenaphthylene-1,2-dione **3** and malononitrile. The mechanism of the formation of 1-oxo-1*H*-phenalene-2,3-dicarbonitrile **5** has been studied by the use of $K^{13}CN$ and different active methylene compounds having a cyano group as well as by careful analysis of the byproducts

dx.doi.org/10.1021/jo5016932 | J. Org. Chem. 2014, 79, 9754−9761

Scheme 6. Knoevenagel Condensation of Acenaphthylene-1,2-dione 3 with Methyl Cyanoacetate



Scheme 7. Acidic Hydrolysis of 1-Oxo-1*H*-phenalene-2,3-dicarbonitrile 5



formed during the reaction. On the basis of these studies, a mechanistic pathway for the formation of this original scaffold is proposed in which the cyanide plays a crucial role as a leaving group in the ring expansion step and as a nucleophile at the last stage. We are actively investigating the chemistry of this new family of (poly)heteroaromatic compounds, and we will report our findings in due course.

EXPERIMENTAL SECTION

General Information. Solvents were purified and dried by standard methods prior to use; alternatively, the MB SPS-800-dry solvent system was used to dry THF and DCM. When needed, dry acetonitrile was obtained by refluxing solvant on calcium hydride for an hour and distilling under argon, which was stored over activated molecular sieves (3 Å) for a week. For the preparation of 1-oxo-1*H*-phenalene-2,3-dicarbonitrile compounds, HPLC grade acetonitrile was used without any drying procedure. ^{1}H and ^{13}C NMR spectra were recorded with 300 MHz (fitted with a 5 mm i.d. BBO probe), 400 MHz (fitted with a 5 mm i.d. BBFO + probe, probe temperature set at 303 K), 500 MHz (fitted with a 5 mm i.d. ^{13}C/^{1}H dual cryoprobe, probe temperature set at 303 K), and 700 MHz (fitted with a 5 mm i.d. QXI cryoprobe, probe temperature set at 298 K) NMR spectrometers. The spectra are referenced to the solvent in which they were run (7.26 ppm for ^{1}H $CDCl_3$ and 77.16 ppm for ^{13}C $CDCl_3$, 5.32 ppm for ^{1}H CD_2Cl_2 and 53.84 ppm for ^{13}C CD_2Cl_2, 2.50 ppm for ^{1}H DMSO-d_6 and 39.52 ppm for ^{13}C DMSO-d_6). Chemical shifts (δ) are given in ppm, and coupling constants (J) are given in Hz. All assignments were confirmed with the aid of two-dimensional ^{1}H, ^{1}H (COSY), or ^{1}H,^{13}C (HSQC, HMBC) experiments using standard pulse programs. All reactions were monitored by TLC on commercially available precoated plates (Kieselgel 60 F254), and the products were visualized by UV (254 nm), by autofluorescence, or with $KMnO_4$ solution [$KMnO_4$ (3 g), K_2CO_3 (20 g), NaOH (5% aq.; 5 mL), H_2O (300 mL)]. Kieselgel 60, 230−400 mesh (Merck), was used for column chromatography. Melting points were determined on a RCH (C. Reichert) microscope equipped with a Kofler heating system. Optical rotations were measured at 20 ± 1 °C in the indicated solvents, and concentrations are expressed in grams/100 mL. FTIR spectra were obtained in the 500−4000 cm^{-1} range. Elemental analyses were performed using an analyzer with detection by a catharometer (thermal conductivity detector). Mass spectra were measured using CI with NH_3 on a quad instrument. HRMS spectra were measured with an LCT spectrometer (flow: MeOH/H_2O, 50:50, 0.2 mL/min). Alternatively, for electrospray ionization HRMS spectra, the measurements were performed using a mass spectrometer equipped with a linear trap coupled to an Orbitrap in positive mode. For MALDI HRMS spectra, the measurements were performed on a MALDI-TOF/TOF spectrometer in positive-ion mode. For UV/vis experiments, the ground-state absorption spectra were recorded at room temperature. The fluorescence excitation and emission spectra were obtained using a spectrofluorimeter where all spectra were recorded in quartz cuvettes with a 1 × 1 cm cross section at room temperature.

2-(2-Oxo-2*H*-acenaphthylene-1-ylidene)-malononitrile (4). Compound **4** was synthesized as described earlier by Zhang et al.[5b] Acenaphthene-1,2-dione **3** (3.64 g, 1 equiv, 20 mmol) and malononitrile (1.32 g, 1 equiv, 20 mmol) were dissolved in acetonitrile (60 mL). The solution was refluxed for 3 h under an inert atmosphere and strong magnetic stirring. Note that the yellow mixture slowly took a deep green color upon heating. After cooling at 0 °C, filtration of the reaction mixture yielded a green dark powder. Purification of the crude over silica gel column chromatography afforded a bright orange powder (3.79 g, yield = 82%). The polarity of the starting eluent (petroleum ether/dichloromethane = 30:70) was slowly increased up to 100% dichloromethane. The resulting compound is slightly soluble in acetonitrile, dichloromethane, or chloroform. Single crystals (orange cubes) were obtained by slow evaporation from a saturated solution of the expected compound **4** in dichloromethane. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 8.58 (d, J = 7.3 Hz, 1H), 8.25 (dd, J = 2.0, 8.2 Hz, 2H), 8.17 (d, J = 6.9 Hz, 1H), 7.88 (dd, J = 7.0, 8.1 Hz, 1H), 7.87 (dd, J = 7.4, 8.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 185.9, 155.4, 143.1, 132.7, 132.4, 130.7, 129.5, 129.4, 129.2, 128.5, 124.7, 123.9, 112.7, 110.9, 81.9. $C_{15}H_6N_2O$ LRMS: CI (NH_3) $[M]^+$ = 230.03 g/mol, $[M + NH_4]^+$ = 248.02 g/mol, R_f = 0.50 (PE/CH_2Cl_2, 3:7)

1-Oxo-1*H*-phenalene-2,3-dicarbonitrile (5). Compound **5** was synthesized as described earlier by Hofmann et al.[5d] To a suspension of 2-(2-Oxo-2*H*-acenaphthylene-1-ylidene)-malononitrile **4** (3.79 g, 1 equiv, 16.47 mmol) and potassium carbonate (226 mg, 0.1 equiv, 1.60 mmol) in acetonitrile (60 mL) was refluxed for 1 h. The mixture was cooled at 0 °C and was filtered off. The precipitate was washed several times with cold acetonitrile until a brown powder could be observed. The resulting pure compound **5** as bright orange flakes (2.60 g, yield = 68%) was obtained by chromatography on silica gel (eluent 100% CH_2Cl_2). For elucidation of the structure, see the Supporting Information. ^{1}H NMR (500.13 MHz, CD_2Cl_2): δ 8.77 (dd, J = 1.2, 7.5 Hz, 1H), 8.46 (dd, J = 1.0, 8.1 Hz, 1H), 8.42 (dd, J = 1.0, 7.4 Hz, 1H), 8.40 (dd, J = 0.5, 8.3 Hz, 1H), 7.96 (dd, J = 7.7, 7.7 Hz, 1H), 7.86 (dd, J = 7.4, 8.2 Hz, 1H). ^{13}C NMR (125.76 MHz, CD_2Cl_2): δ 178.1, 138.4, 138.1, 134.9, 133.9, 132.7, 132.3, 129.3, 128.4, 128.0, 127.4, 123.1, 120.6, 113.5, 113.4. Additional data in DMSO-d_6: ^{1}H NMR (500.13 MHz, DMSO-d_6): δ 8.71 (dd, J = 0.8, 8.1 Hz, 1H), 8.67 (dd, J = 1.0, 7.4 Hz, 1H), 8.63 (broad d, J = 8.0 Hz, 1H), 8.42 (dd, J = 0.8, 7.4 Hz, 1H), 8.06 (dd, J = 7.7, 7.7 Hz, 1H), 7.98 (dd, J = 7.5, 8.1 Hz, 1H). ^{13}C NMR (125.76 MHz, DMSO-d_6): δ 177.6, 138.3, 137.7, 134.4, 132.7, 131.8, 131.4, 129.0, 128.0, 127.4, 126.1, 122.3, 119.8, 113.9, 113.5. LRMS: CI (NH_3) $[M + H]^+$ = 231.0 g/mol, $[M + NH_4]^+$

dx.doi.org/10.1021/jo5016932 | J. Org. Chem. 2014, 79, 9754−9761

= 248 g/mol, $[M]^-$ = 230.0 g/mol. HRMS (MALDI; solvent, CH_2Cl_2; matrix, DCTB): $C_{15}H_7N_2O$ $[M + H]^+_{measured}$ = 231.0543 g/mol, $[M + H]^+_{calculated}$ = 231.0558 g/mol. mp 294−300 °C. Anal. Calcd: C, 78.26; H, 2.63; N, 12.17. Found: C, 78.23; H, 2.83; N, 12.15. Solubility in common solvents: soluble in DMSO and boiling acetonitrile, slightly soluble in cold acetonitrile, dichloromethane, and chloroform, sparingly soluble in toluene, and insoluble in alkanes and cyclohexane.

Synthesis of 1-Oxo-1*H*-phenalene-2,3-dicarbonitrile (5) in a One-Pot Sequence. To a suspension of malononitrile (72 mg, 1 equiv, 1.09 mmol) in acetonitrile (4 mL) were added successively acenaphtylene-1,2-dione **3** (200 mg, 1 equiv, 1.09 mmol) and K_2CO_3 (14 mg, 0.1 equiv, 0.10 mmol). After 1 h of reflux, the solvent was removed under reduced pressure, and the crude was purified on silica gel (CH_2Cl_2 100%). The expected product **5** was isolated as bright orange flakes (151 mg, yield = 60%). See above for characterization data.

Mechanistic Elucidation by Isotopic Assignment of 1-Oxo-1*H*-phenalene-2,3-dicarbonitrile (11). 1-Oxo-1*H*-phenalene-2,3-dicarbonitrile **11** from compound **5** was synthesized as follows: To a suspension of 1-oxo-1*H*-phenalene-2,3-dicarbonitrile **5** (150 mg, 1.0 equiv, 0.65 mmol) in acetonitrile (5 mL) were successively added K_2CO_3 (9 mg, 0.1 equiv, 0.065 mmol) and $K^{13}CN$ (21 mg, 0.5 equiv, 0.325 mmol). After 1.5 h of reflux, the solvent was removed under reduced pressure, and the crude was purified on silica gel (eluent: CH_2Cl_2 100%). The expected product **11** was isolated as bright orange flakes (45 mg, yield = 31%).

1-Oxo-1*H*-phenalene-2,3-dicarbonitrile **11** from compound **3** was synthesized as follows: To a suspension of malononitrile (54.4 mg, 1 equiv, 0.824 mmol) in acetonitrile (5 mL) were added successively acenaphtylene-1,2-dione **3** (150 mg, 1.0 equiv, 0.824 mmol), K_2CO_3 (10 mg, 0.1 equiv, 0.082 mmol), and $K^{13}CN$ (27 mg, 0.5 equiv, 0.412 mmol). After refluxing the suspension over 1.5 h, the solvent was removed under reduced pressure, and the crude was purified on silica gel (eluent: CH_2Cl_2 100%). The expected product **11** was isolated as bright orange flakes (28 mg, yield = 15%).

1-Oxo-1*H*-phenalene-2,3-dicarbonitrile **11** from compound **4** was synthesized as follows: To a suspension of 2-(2-oxo-2*H*-acenaphthylene-1-ylidene)-malononitrile **4** (128 mg, 1.0 equiv, 0.56 mmol) in acetonitrile (4 mL) were added K_2CO_3 (7.7 mg, 0,1 equiv, 0.056 mmol), and $K^{13}CN$ (18,5 mg, 0.5 equiv, 0.28 mmol). After refluxing the suspension for 1.5 h, the solvent was removed under reduced pressure, and the crude was purified on silica gel (eluent: CH_2Cl_2 100%). The expected product **11** was isolated as a bright orange flakes (30 mg, yield = 23%).

All NMR spectra of the enriched derivative **11** were recorded and displayed the following data:[25] ^{1}H NMR (500.13 MHz, CD_2Cl_2): δ 8.77 (dd, J = 1.0, 7.5 Hz, 1H), 8.46 (dd, J = 0.9, 8.0 Hz, 1H), 8.42 (dd, J = 0.8 Hz, 7.4 Hz, 1H), 8.39 (d, J = 8.2 Hz, 1H), 7.96 (dd, J = 7.7, 7.7 Hz, 1H), 7.86 (dd, J = 7.6, 8.0 Hz, 1H). ^{13}C NMR (125.76 MHz, CD_2Cl_2): 178.1, 138.4, 138.1, 134.9, 133.8, 132.7, 132.3, 129.3, 128.4, 128.0, 127.4, 123.1, 120.6, 113.5, 113.4.

Synthesis of the Newly Assigned 1-Oxo-6-thiomorpholino-1*H*-phenalene-2,3-dicarbonitrile (2). Compound **2** was synthesized according to the procedure described by Liu et al.[5a] A mixture of thiomorpholine (1.31 mL, 1.43 g, 4 equiv, 13.9 mmol) and 1-oxo-1*H*-phenalene-2,3-dicarbonitrile **5** (800 mg, 1.0 equiv, 3.47 mmol) in acetonitrile (140 mL) was stirred for 2 to 3 h at room temperature. After mixing, the reaction mixture turned to a green color and then to a purple color. After reaction completion, acetonitrile was removed under reduced pressure, and the crude was purified by column chromatography on silica gel (eluent: from CH_2Cl_2 100% to CH_2Cl_2 99.9%/acetone 0.1%). Compound **2** was obtained as a dark-purple powder (596 mg, yield = 52%) and was recrystallized in chloroform. R_f = 0.60 (CH_2Cl_2/acetone: 98/2). ^{1}H NMR (DMSO-d_6, 400 MHz, 30 °C, high dilution): δ 8.66 (m, 2H), 8.18 (d, J = 8.9 Hz, 1H), 7.96 (dd, J = 7.4 Hz, 1H), 7.42 (d, J = 8.4 Hz, 1H), 3.94 (m, 4H), 2.97 (m, 4H) or ^{1}H NMR (CD_2Cl_2, 400 MHz, 30 °C): δ 8.81 (dd, J = 1.2, 7.5 Hz, 1H), 8.53 (dd, J = 1.0, 8.4 Hz, 1H), 8.24 (d, J = 8.4 Hz, 1H), 7.89 (d, J = 8.0 Hz, 1H), 7.21 (d, J = 8.5 Hz, 1H), 3.81 (m, 4H), 2.98 (m, 4H). ^{13}C NMR (CD_2Cl_2, 100 MHz, 30 °C): δ 178.6, 161.4, 136.8, 134.3, 133.8, 130.5, 130.1, 129.7, 128.0, 127.0, 117.1, 116.4, 114.7, 114.6, 114.1, 56.6, 28.3. LRMS MALDI (solvent, CH_2Cl_2; matrix, DCTB) $[M + Na]^+$ = 455.1 g/mol. HRMS MALDI (CH_2Cl_2, Matrix DCTB, Standard: PEG200): $C_{19}H_{13}N_3OSNa$ $[M + Na]^+_{measured}$ = 354.0665 g/mol, $[M + Na]^+_{calculated}$ = 354.0671 g/mol. Single crystal preparation: Crystals (dark-violet needles) were prepared by diffusion of liquid cyclohexane through a saturated solution of **2** in dichloromethane. UV−visible (solvent: dichloromethane): λ_{max1} = 575 nm, λ_{max2} = 540 nm, λ_{max3} = 306 nm. Fluorescence: λ^{em}_{max} = 606 nm. Φ_f = 0.03 (standard: Rhodamine B in 2 mL of MeOH + 1 drop of TFA, Φ_f = 0.40). Note that sonication or trituration of the crude in dichloromethane followed by filtration may remove most of the unreacted compound **5** (slightly soluble in dichloromethane). Dry loading on silica gel must be avoided because lower yields were obtained. A solution of compound **2** in $CDCl_3$ showed significant decomposition (TLC analysis 10 min after solubilization; analysis of the solution after room temperature overnight storage showed that compound **2** was the minor species in the NMR tube). Compound **2** is soluble in dichloromethane, hot chloroform, acetonitrile, and DMSO, slightly soluble in cold chloroform, acetone, and toluene, sparingly soluble in diethyl ether and a dichloromethane 50%/diethyl ether 50% mixture, and insoluble in alkanes and cyclohexane.

6-(*n*-Butylamino)-1-oxo-1*H*-phenalene-2,3-dicarbonitrile (12), 9-(Butylamino)-1-oxo-1*H*-phenalene-2,3-dicarbonitrile (13), and 3-(*n*-Butylamino)-1-oxo-1*H*-phenalene-2,3-dicarbonitrile (14). To a suspension of 1-oxo-1*H*-phenalene-2,3-dicarbonitrile **5** (500 mg, 1 equiv, 2.17 mmol) in acetonitrile (90 mL) was added *n*-butylamine (1.07 mL, 792 mg, 4 equiv, 10.8 mmol), and the reaction mixture was stirred for 3 h at room temperature. A few minutes after adding *n*-butylamine, the reaction mixture became deep blue. The solvent was removed under reduced pressure, and the resulting black solid was purified by silica gel column chromatography (from CH_2Cl_2 100% to CH_2Cl_2 95%/MeOH 5%). 6-(Butylamino)-1-oxo-1*H*-phenalene-2,3-dicarbonitrile **12** was obtained as a dark purple powder (138 mg, yield = 21%). ^{1}H NMR (CD_2Cl_2 93%/CD_3OD 7%, 500 MHz, 30 °C): δ 8.69 (dd, J = 1.1 Hz, J = 7.6 Hz, 1H), 8.67 (dd, J = 1.0 Hz, J = 8.1 Hz, 1H), 8.13 (d, J = 9.0 Hz, 1H), 7.82 (d, J = 7.9 Hz, J = 7.9 Hz, 1H), 6.84 (d, J = 9.1 Hz, 1H), 3.57 (t, J = 7.4 Hz, 2H), 1.79 (quintuplet, J = 7.4 Hz, 2H), 1.49 (sextuplet, J = 7.4 Hz, 2H), 1.00 (t, J = 7.4 Hz, 4H). ^{13}C NMR (CD_2Cl_2 93%/CD_3OD 7%, 125 MHz, 30 °C): δ 178.7, 156.9, 140.0, 133.9, 131.99, 131.97, 129.5, 128.0, 127.7, 123.1, 116.2, 114.8, 113.3, 108.0, 106.7, 44.7, 31.1, 20.8, 14.0. HRMS: ESI, $C_{19}H_{16}N_3O$ $[M + H]^+_{measured}$ = 302.1290 g/mol, $[M + H]^+_{calculated}$ = 302.1293 g/mol. UV−visible spectroscopy (solvent: dichloromethane): λ_{max1} = 573 nm, ε (λ_{max1}) = 47 717 L mol^{-1} cm^{-1}, λ_{max2} = 534 nm, ε (λ_{max2}) = 19 491 L mol^{-1} cm^{-1}, λ_{max3} = 303 nm, ε (λ_{max3}) = 12 159 L mol^{-1} cm^{-1}. Fluorescence spectroscopy (solvent: dichloromethane): λ^{em}_{max1} = 586 nm, Φ_f = 0.60 (standard: Rhodamine B in 2 mL of MeOH + 1 drop of TFA, Φ_f = 0.40). 9-(*n*-Butylamino)-1-oxo-1*H*-phenalene-2,3-dicarbonitrile **13** was also present in the reaction mixture but could not be separated from the starting material. A pure analytic fraction could be separated for its characterization (65 mg, 10%). ^{1}H NMR (CD_2Cl_2, 400 MHz, 30 °C): δ 12.68 (broad singlet, 1H), 8.35 (dd, J = 1.0, 7.9 Hz, 1H), 8.14 (dd, J = 1.0, 7.5 Hz, 1H), 8.10 (d, J = 9.4 Hz, 1H), 7.64 (dd, J = 7.7 Hz, 1H), 7.32 (d, J = 9.5 Hz, 1H), 3.68 (m, 2H), 1.86 (m, 2H), 1.03 (t, J = 7.3 Hz, 3H). ^{13}C NMR (CD_2Cl_2, 100 MHz, 30 °C): δ 176.3, 158.5, 141.0, 136.6, 132.3, 129.4, 126.2, 125.4, 124.2, 121.2, 118.3, 116.2, 115.1, 114.5, 110.0, 43.9, 31.7, 20.8, 14.0. HRMS: ESI, $C_{19}H_{16}N_3O$ $[M + H]^+_{measured}$ = 302.1288 g/mol, $[M + H]^+_{calculated}$ = 302.1293 g/mol. 3-(*n*-Butylamino)-1-oxo-1*H*-phenalene-2,3-dicarbonitrile **14** (18 mg, 3%) was also isolated. ^{1}H NMR ($CDCl_3$, 400 MHz, 30 °C): δ 8.55 (dd, J = 1.3 Hz, J = 7.2 Hz, 1H), 8.18 (m, 2H), 7.99 (d, 7.5 Hz, 1H), 7.76 (dd, J = 7.3 Hz, J = 8.0 Hz, 1H), 7.70 (dd, J = 7.8 Hz, J = 7.8 Hz, 1H), 4.06 (m, 2H), 1.85 (m, 2H), 1.56 (m, 2H), 1.02 (t, J = 7.4 Hz, 3H).^{13}C NMR ($CDCl_3$, 100 MHz, 30 °C): δ 176.3, 158.5, 141.1, 136.6, 132.3, 129.4, 126.2, 125.4, 124.2, 121.2, 118.3, 116.2, 115.1, 114.5, 110.0, 43.9, 31.7, 20.8, 14.0. (ESI-QTOF) $C_{18}H_{16}N_2O$ $[M + H]^+_{measured}$ = 277.1331 g/mol, $[M + H]^+_{calculated}$ = 277.1335).

dx.doi.org/10.1021/jo5016932 | J. Org. Chem. 2014, 79, 9754−9761

2-(4-Nitrophenyl)-1-oxo-1*H*-phenalene-3-carbonitrile (15). To a solution of 4-nitrophenylacetonitrile (870 mg, 1.01 equiv, 5.3 mmol) in acetonitrile (20 mL) were added successively acenaphtylene-1,2-dione **3** (1 g, 1 equiv, 5.4 mmol) and K_2CO_3 (76 mg, 0.1 equiv, 0.54 mmol). The resulting suspension was heated at reflux over 45 min under strong stirring. After few minutes of stirring, the reaction mixture progressively turned to a purple color and then became greenish. After heating, the reaction mixture was concentrated under vacuum. The resulting black solid was purified on silica gel by column chromatography (CH_2Cl_2/PE 80:20 and then pure CH_2Cl_2). The expected compound **15** was obtained as a yellow solid (803 mg, 46%). R_f = 0.71 (eluent DCM: 100%). ^{1}H NMR (700.28 MHz, CD_2Cl_2): δ 8.71 (dd, J = 1.0, 7.4 Hz, 1H), 8.42 (dd, J = 0.8 Hz, 8.0 Hz, 1H), 8.36–8.41 (m, 3H), 8.27 (d, J = 8.2 Hz, 1H), 7.93 (dd, J = 7.7, 7.7 Hz, 1H), 7.81 (dd, J = 7.4, 8.2 Hz, 1H), 7.79 (d, J = 8.8 Hz, 2H). ^{13}C NMR (176.08 MHz, CD_2Cl_2): δ 181.7, 148.9, 144.8, 140.3, 137.1, 134.6, 132.6, 132.55, 132.2, 131.8, 129.2, 128.6, 127.7, 127.1, 124.3, 123.8, 123.3, 115.2. IR 2224, 1709, 1642, 1616.5, 1601, 1580, 1573, 1322, 1274, 1262, 1223, 1195, 1140, 880, 859, 808, 786, 701. HRMS: ESI, $C_{20}H_{11}N_2O_3$ $[M + H]^+_{measured}$ = 327.0766 g/mol, $[M + H]^+_{calculated}$ = 327.0764 g/mol.

Methyl 3-Cyano-1-oxo-1*H*-phenalene-2-carboxylate (18). The mixture of methyl (2*Z*)-cyano(2-oxoacenaphtylen-1(2*H*)-ylidene)acetate (**16**) and methyl (2*E*)-cyano(2-oxoacenaphtylen-1(2*H*)-ylidene)acetate (**17**) was obtained according to the procedure described by Kollia et al.[21] To a suspension of these compounds (**16** and **17**) (150 mg, 1.0 equiv., 0.57 mmol) in a microwave sealed tube were added acetonitrile (4 mL) and K_2CO_3 (7.8 mg, 0.1 equiv, 0.05 mmol). The tube was set up in a microwave reactor (Multisynth Milestone), and the following reaction conditions were applied: temperature rampe from 20 to 100 °C over 3 min (power limit of 120 W). The temperature was further kept at 100 °C over 5 min, and cooling was applied from 100 °C until 40 °C. The solvent was removed under reduced pressure, and the crude was purified by silica gel column chromatography (petroleum ether 30%/CH_2Cl_2 70%). The expected compound **18** was obtained as a pulverulent yellow solid (35 mg, yield = 23%). ^{1}H NMR ($CDCl_3$, 400 MHz, 30 °C): δ 8.67 (dd, J = 1.2, 7.4 Hz, 1H), 8.32 (m, 2H), 8.22 (dd, J = 0.7, 8.3 Hz, 1H), 7.87 (dd, J = 7.6, 7.9 Hz, 1H), 7.76 (dd, J = 7.3, 8.3 Hz, 1H), 4.06 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz, 30 °C): δ 179.6, 164.0, 139.1, 137.0, 135.3, 132.7, 132.5, 132.3, 128.8, 128.5, 127.4, 126.9, 123.3, 123.1, 113.6, 53.6. HRMS: MALDI (solvent, CH_2Cl_2; matrix, DHB) $C_{16}H_9NO_3Na$ $[M + Na]^+_{measured}$ = 286.0463 g/mol, $[M + Na]^+_{calculated}$ = 286.0475 g/mol. Single crystal preparation: Crystals (orange octahedron) could be prepared by diffusion of liquid cyclohexane through a saturated solution of the product in dichloromethane. ORTEP view was obtained (see the Supporting Information), but these data were not submitted to the Cambridge Crystallographic Data Centre due to incorrect refinement of the structure in terms of convergence, although the other parameters were validated.

Naphtho[1,8-*ef*]isoindole-7,8,10(9*H*)-trione (19). A solution of 1-oxo-1*H*-phenalene-2,3-dicarbonitrile **5** (250 mg, 1 equiv, 1.08 mmol) in 98% sulfuric acid (1 mL) was heated at 40 °C over 48 h. After reaction completion, the solution was poured on crushed ice. The resulting orange precipitate was filtered, washed several times with water, and then allowed to dry overnight under vacuum (with phosphorus pentoxide as a moisture trap). The resulting dry crude was purified by column chromatography on silica gel. The solid loading was obtained after sonication of the compound in a large excess of acetone, ~25–70 mL, followed by addition of silica and removal under vacuum of the solvent. Unreacted compound **5** was eluted with 100% dichloromethane. The expected compound **19** was further eluted with CH_2Cl_2/MeOH 99:1 and was obtained as an orange–brown fine powder (200 mg, yield = 74%). ^{1}H NMR ($CDCl_3$ 50%/CD_3OD 50%, 500 MHz, 30 °C): δ 8.83 (dd, J = 1.0, 7.3 Hz, 1H), 8.43 (dd, J = 1.2, 7.5 Hz, 1H), 8.11 (dd, J = 1.0, 8.0 Hz, 1H), 8.05 (dd, J = 0.8, 8.2 Hz,1H), 7.61 (dd, J = 7.5, 7.5 Hz, 1H), 7.51 (dd, J = 7.4, 8.2 Hz, 1H). ^{13}C NMR ($CDCl_3$ 50%/CD_3OD 50%, 125 MHz, 30 °C): δ 179.6, 169.7, 168.0, 143.6, 137.3, 136.3, 133.9, 132.5, 131.7, 131.1, 128.9, 127.5, 127.1, 125.5, 121.0. Anal. Calcd for $C_{15}H_7NO_3{\cdot}0.2H_2O$: C, 71.26; H, 2.95; N, 5.54. Found: C, 71.13; H, 3.04; N, 5.70. HRMS MALDI (solvent, acetone; matrix, DCTB): $C_{15}H_7NO_3Na$ $[M + Na]^+_{measured}$ = 272.0324 g/mol, $[M + Na]^+_{calculated}$ = 272.0318 g/mol. Single crystal preparation: Crystals (brown needles) could be prepared by slow evaporation of a saturated solution of **19** in HPLC grade acetone (for X-ray data, see the X-ray section in the Supporting Information).

Synthesis of *N*-Methylnaphtho[1,8-ef]isoindole-7,8,10-trione (20). Cesium carbonate (650 mg, 2 equiv, 2 mmol) was placed under vacuum in a Schlenck tube and heated by heat gun over 15 min. After cooling, naphtho[1,8-*ef*]isoindole-7,8,10(9*H*)-trione **19** (250 mg, 1 equiv, 1 mmol), dry acetonitrile (20 mL), and methyl tosylate (0.62 mL, 762 mg, 4.1 equiv, 4 mmol) were successively added. After 1 h of refluxing, the reaction mixture was cooled, filtered off on cotton wool, and washed with dichloromethane. After solvent removal, the remaining brown solid was purified by silica gel column chromatography (eluent from CH_2Cl_2 100% to CH_2Cl_2 99%/MeOH 1%). Product was isolated as a bright orange powder (107 mg, yield = 41%). ^{1}H NMR ($CDCl_3$, 400 MHz, 30 °C): δ 9.10 (dd, J = 1.1, 7.3 Hz, 1H), 8.73 (dd, J = 1.2, 7.4 Hz, 1H), 8.29 (dd, J = 1.1, 8.1 Hz, 1H), 8.24 (dd, J = 1.0, 8.3 Hz, 1H), 7.85 (dd, J = 7.7, 7.7 Hz, 1H), 7.75 (dd, J = 7.4, 8.2 Hz, 1H), 3.20 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz, 30 °C): δ 178.7, 169.2, 166.8, 143.1, 137.0, 136.2, 134.0, 132.7, 132.2, 131.6, 129.2, 128.0, 127.4, 125.6, 121.4, 24.1. HRMS: MALDI (solvent, CH_3CN; matrix, DHB): $C_{16}H_9NO_3Na$ $[M + Na]^+_{measured}$ = 286.0483 g/mol, $[M + Na]^+_{calculated}$ = 286.0475 g/mol. Crystals (flat orange needles) could be prepared by diffusion of liquid cyclohexane through a saturated solution of the product in dichloromethane or by slow evaporation of a saturated solution in dichloromethane or chloroform (for X-ray data, see X-ray section in the Supporting Information).

■ ASSOCIATED CONTENT

Supporting Information

Elucidation of new structures by ^{1}H, ^{13}C, and ^{2}D NMR spectra and X-ray crystallographic data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: jacques.lebreton@univ-nantes.fr.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The authors are deeply indebted to the Ministère de l'Enseignement Supérieur et de la Recherche, the Centre National de la Recherche Scientifique (CNRS), the Université de Nantes, Biogenouest (Network of Technological Platform) for their support. This work was funded by the program CIMATH2 (Ciblage Moléculaire et Applications Thérapeutiques 2) supported by La Région des Pays de la Loire and IBISA (Infrastructures en Biologie Sante et Agronomie). This project was implemented by CHEM-Symbiose, a technological platform devoted to the synthesis of molecules. The authors would like to thank Dr. Samuel Golten for fruitful discussions and comments. The analytical service of CEISAM is gratefully acknowledged for their support in chemical analysis. The authors also thank the platform "Biopolymères, Intéractions, Biologie Structurale" at INRA Nantes and the LABERCA (Oniris laboratory) in Nantes for supplying the HRMS apparatus.

■ REFERENCES

(1) Part of this present work was published in the following patent: Lebreton, J.; Lenk, R.; Dubreuil, D.; Silvestre, V.; Planchat. A.

Preparation of oxophenalene compounds and their uses in therapy and material fields. Brevet international WO 2013128425 A1 20130906, 2013. This patent was accessible on the 12th of September, 2013. During the completion on this work, in particular the mechanism studies reported in the present article, a correction of all of the structures prepared by the group of Qian appeared in the literature (amendment published online on the 23rd of November, 2013) without citing the work mentioned in our patent, see: Chen, Z.; et al. *J. Med. Chem.* **2014**, *57*, 1621−1624; *Chem. Commun.* **2014**, *50*, 9596−9598;Song, T.; et al. *J. Med. Chem.* **2013**, *56*, 9366−9367 and cited literature therein.

(2) For leading reviews in this field, see: (a) Hu, Y.; Li, C.-Y.; Wang, X.-M.; Yang, Y.-H.; Zhu, H.-L. *Chem. Rev.* **2014**, *114*, 5572−5610. (b) Huang, L.; Yu, R.; Leng, L.; Gong, F.; Zhu, X.; Wan, Y. *Synthesis* **2014**, *46*, 2317−2326. (c) Parikh, P. K.; Marvaniya, H. M.; Sen, D. J. *Int. J. Drug Dev. Res.* **2011**, *3*, 44−50. (d) Verniest, G.; Wang, X.; De Kimpe, N.; Padwa, A. *J. Org. Chem.* **2010**, *75*, 424−433. (e) Bridges, T. M.; Kennedy, J. P.; Hopkins, C. R.; Conn, P. J.; Lindsley, C. W. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5617−5622. (f) Lu, R.-J.; Tucker, J. A.; Pickens, J.; Ma, Y.-A.; Zinevitch, T.; Kirichenko, O.; Konoplev, V.; Kuznetsova, S.; Sviridov, S.; Brahmachary, E.; Khasanov, A.; Mikel, C.; Yang, Y.; Liu, C.; Wang, J.; Freel, S.; Fisher, S.; Sullivan, A.; Zhou, J.; Stanfield-Oakley, S.; Baker, B.; Sailstad, J.; Greenberg, M.; Bolognesi, D.; Bray, B.; Koszalka, B.; Jeffs, P.; Jeffries, C.; Chucholowski, A.; Sexton, C. *J. Med. Chem.* **2009**, *52*, 4481−4487. (g) Eller, G. A.; Haring, A. W.; Datterl, B.; Zwettler, M.; Holzer, W. *Heterocycles* **2007**, *71*, 87−104.

(3) For leading reviews in this field, see: (a) Mohanakrishnan, A. K.; Kumar, N. S.; Amaladass, P. *Tetrahedron Lett.* **2008**, *48*, 4792−4795. (b) Kumar, N. S.; Clement, J. A.; Mohanakrishnan, A. K. *Tetrahedron* **2009**, *65*, 822−830. (c) Raju, B. R.; Firmino, A. D. G.; Costa, A. L. S.; Coutinho, P. J. G.; Gonçalves, M. S. T. *Tetrahedron* **2013**, *69*, 2451−2461.

(4) Xiao, Y.; Liu, F.; Qian, X.; Cui, J. *Chem. Commun.* **2005**, 239−241.

(5) (a) Liu, F.; Xiao, Y.; Qian, X.; Zhang, Z.; Cui, J.; Cui, D.; Zhang, R. *Tetrahedron* **2005**, *61*, 11264−11269. (b) Zhang, M.; Yu, M.; Li, F.; Zhu, M.; Li, M.; Gao, Y.; Li, L.; Liu, Z.; Zhang, J.; Zhang, D.; Yi, T.; Huang, C. *J. Am. Chem. Soc.* **2007**, *129*, 10322−10323. (c) He, C.; Zhu, W.; Xu, Y.; Zhong, Y.; Zhou, J.; Qian, X. *J. Mater. Chem.* **2010**, *20*, 10755−10764. (d) Hofmann, K.; Kahle, I.; Simon, F.; Spange, S. *Beilstein J. Org. Chem.* **2010**, *6*, 79. (e) Chen, Z.; Wang, X.; Zhu, W.; Cao, X.; Tong, L.; Li, H.; Xie, H.; Xu, Y.; Tan, S.; Kuang, D.; Ding, J.; Qian, X. *J. Med. Chem.* **2011**, *54*, 3732−3745.

(6) (a) Zhang, Z.; Wu, G.; Gao, J.; Song, T. *Mol. Pharmaceutics* **2010**, *7*, 1348−1354. (b) Zhang, Z.; Wu, G.; Xie, F.; Song, T.; Chang, X. *J. Med. Chem.* **2011**, *54*, 1101−1105. (c) Zhang, Z.; Yang, H.; Wu, G.; Li, Z.; Song, T.; Li, X. *Eur. J. Med. Chem.* **2011**, *46*, 3909−3916. (d) Song, T.; Li, X.; Chang, X.; Liang, X.; Zhao, Y.; Wu, G.; Xie, S.; Su, P.; Wu, Z.; Feng, Y.; Zhang, Z. *Bioorg. Med. Chem.* **2013**, *21*, 11−20.

(7) Zhang, Z.; Song, T.; Zhang, T.; Gao, J.; Wu, G.; An, L.; Du, G. *Int. J. Cancer* **2011**, *128*, 1724−1735.

(8) For a review in this topic, see: Maükosza, M.; Wojciechowski, K. *Chem. Rev.* **2004**, *104*, 2631−2666.

(9) For interesting reviews on the prevalence of nitrile-containing pharmaceuticals, see: (a) Fleming, F. F.; Yao, L.; Ravikumar, P. C.; Funk, L.; Shook, B. C. *J. Med. Chem.* **2010**, *53*, 7902−7917. (b) Jones, L. H.; Summerhill, N. W.; Swain, N. A.; Mills, J. E. *Med. Chem. Commun.* **2010**, *1*, 309−318 and cited references therein.

(10) For a leading review in this field, see: Collier, S.; Langer, P. *Sci. Synth.* **2004**, *19*, 403−425.

(11) (a) Knoevenagel, E. *Chem. Ber.* **1894**, *27*, 2345−2346. (b) Knoevenagel, E. *Chem. Ber.* **1896**, *29*, 172−174.

(12) (a) Yoon, K. R.; Ko, S.-O.; Lee, S. M.; Lee, H. *Dyes Pigm.* **2007**, *75*, 567−573. (b) Dieskau, A. P.; Holzwarth, M. S.; Plietker, B. *Chem.—Eur. J.* **2012**, *18*, 2423−2429.

(13) (a) Jones, G. *Org. React.* **1967**, *15*, 204−599. For examples of Knoevenagel reaction with pyridine, see: (b) Matsumoto, S.; Samata, D.; Akazome, M.; Ogura, K. *Tetrahedron Lett.* **2009**, *50*, 111−114. (c) Djukiv, B.; Perepichka, D. F. *Chem. Commun.* **2011**, *47*, 12619−12621. (d) Ananthakrishnan, S. J.; Varathan, E.; Ravindran, E.; Somanathan, N.; Subramanian, V.; Mandal, A. B.; Sudha, J. D.; Ramakrishnan, R. *Chem. Commun.* **2013**, *49*, 10742−10744. For examples of Knoevenagel reaction with piperidine, see: (e) Chen, L.; Qian, G.; Cui, Y.; Jin, X.; Wang, Z.; Wang, M. *J. Phys. Chem. B* **2006**, *110*, 19176−19182. (f) Usta, H.; Risko, C.; Wang, Z.; Huang, H.; Deliomeroglu, M. K.; Zhukhovitskiy, A.; Facchetti, A.; Marks, T. J. *J. Am. Chem. Soc.* **2009**, *131*, 5586−5608.

(14) All NMR spectra were recorded on 500 MHz NMR spectrometer fitted with a 5 mm i.d. $^{13}C/^{1}H$ cryoprobe carefully tuned to the recording frequency of 500.13 MHz (for ^{1}H) or 125.76 MHz (for ^{13}C). The temperature of the probe was set at 303 K; the spectra are referenced to the solvent in which they were run (5.32 ppm for ^{1}H CD_2Cl_2 and 53.84 ppm for ^{13}C CD_2Cl_2, 2.50 ppm for ^{1}H DMSO-d_6 and 39.52 ppm for ^{13}C DMSO-d_6). Other ^{1}H NMR, COSY, and HSQC spectra are disclosed in the Supporting Information.

(15) According to the procedure described by Liu et al.,[5a] the synthesis of the reassigned structure **2** was applied and gave the expected compound with 52% yield accompanied with other side products arising from other S_NAr-H on the aromatic moiety. The experimental procedure and analytical data are described in the Experimental Section. For further details of structure elucidation (X-Ray and HMBC experiments), see the Supporting Information.

(16) These side products were not isolated, but their presence is highly probable due to the high propensity of the C-7 and C-11 positions of the aromatic core to react by S_NAr^H with any nucleophile.

(17) Liu, F.; Qian, X.; Cui, J.; Xiao, Y.; Zhang, R.; Li, G. *Bioorg. Med. Chem.* **2006**, *14*, 4639−4644.

(18) These chemical yields are not representative to the global yield of the reaction. It is reasonable to consider a global yield that is much higher than around 40% since two dainty purifications on silica gel were needed to isolate both compounds **12** and **13** as pure materials for fine structural analysis.

(19) The mentioned chemical yields are related to the amount of compounds collected as pure material to complete fine structural analysis.

(20) The same experiment was carried out in presence of *N*-methylmorpholine oxide (1.2 equiv) and gave the same compounds in a different ratio. Both pure regiosisomers **12** and **13** were isolated in similar yields, 15 and 16%, respectively. Once again, compound **14** was also obtained in 6% yield. For more details about oxidative nucleophilic hydrogen substitution (using an oxidant such as *N*-methylmorpholine *N*-oxide), see ref 8.

(21) The same reaction in the presence of ethyl cyanoacetate was previously described in Mhaidat, I.; Hamilakis, S.; Kollia, C.; Tsolomitis, A.; Loizos, Z. *Mater. Lett.* **2007**, *61*, 321−325 and therein the expected compounds were obtained in 80−90% yields.

(22) The same experiment was carried out under classical thermic conditions (7.5 h at 60 °C) and gave rise to compound **18** in 21% yield (isolated as pure compound).

(23) Manley-King, C. I.; Bergh, J. J.; Petzer, J. P. *Bioorg. Med. Chem.* **2011**, *19*, 4829−4840 and cited literature therein.

(24) For confirmation of this structural assignment, see the X-ray section of the Supporting Information.

(25) NMR spectra were recorded on 500 MHz NMR spectrometer fitted with a 5 mm i.d. $^{13}C/^{1}H$ cryoprobe carefully tuned to the recording frequency of 500.13 MHz (for ^{1}H) or 125.76 MHz (for ^{13}C). The temperature of the probe was set at 303 K; the spectra are referenced to the solvent in which they were run (5.32 ppm for ^{1}H CD_2Cl_2 and 53.84 ppm for ^{13}C CD_2Cl_2).