Inhibitors of Sir2: Evaluation of Splitomicin Analogues

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Splitomicin (**1**) and 41 analogues were prepared and evaluated in cell-based Sir2 inhibition and toxicity assays and an in vitro Sir2 inhibition assay. Lactone ring or naphthalene (positions 7–9) substituents decrease activity, but other naphthalene substitutions (positions 5 and 6) are well-tolerated. The hydrolytically unstable aromatic lactone is important for activity. Lactone hydrolysis rates were used as a measure of reactivity; hydrolysis rates correlate with inhibitory activity. The most potent Sir2 inhibitors were structurally similar to and had hydrolysis rates similar to **1**.

Introduction

Epigenetic regulation of gene transcription is often accomplished via posttranslational modification of histones.[1] Histone lysine methylation, acetylation and deactylation, and serine phosphorylation are essential to this regulation, which has been described as a "histone code" that complements the genetic code.[2] Prominent among the enzymes responsible for this regulation are the histone deacetylases, which promote gene silencing via cleavage of acetyl-lysine (Ac-K) on histone tails. Two classes of histone deacetylases have been identified.[3] One class, known as the HDACs, incorporates a divalent Zn atom in the active site that activates a water molecule, which hydrolyzes the amide bond. The other class of histone deacetylases is known as Sirtuins after their founding member Sir2 in *S. cerevisiae*. The Sirtuins consume NAD^+ in the deacetylation reaction to afford free lysine, nicotinamide, and O-acetyl ADP ribose. Both classes of histone deacetylases have also been shown to deacetylate and modulate the activity of nonhistone proteins. For example, p53, a tumor suppressor protein involved in the modulation of a cell's response to DNA damage, is inactivated via deacetylation on lysine 382 by a human Sir2.[4] Furthermore, the function of the transcriptional repressor BCL6, implicated in the pathogenesis of B-cell lymphomas, critically depends on its deacetylation by HDAC and Sir2-like enzymes.[5] Because of their role in a variety of cellular pathways relating to cancer, inhibitors of histone deacetylases have been an active area of research. Indeed, inhibitors of these enzymes have been shown to induce growth arrest, differentiation, and/or apoptotic cell death in transformed cells.[6]

We used *S. cerevisiae* as a model organism to identify and characterize inhibitors of NAD-dependent deacetylases. Sir2 is required for epigenetic silencing at three sites in the yeast genomes: genes adjacent to telomeres, the ribosomal DNA (r-DNA) locus, and the silent mating loci. Using a cell-based screen for inhibitors of telomeric silencing from a library of 6000 compounds,[7] we recently identified splitomicin (**1**) as a micromolar inhibitor of Sir2. Treatment of wild-type cells with splitomicin created a faithful phenocopy of a Sir2 mutant. Approximately 130 analogues of splitomicin were evaluated including analogues of sirtinol.[8] Here, we report the convenient syntheses for splitomicin and 41 analogues and the analysis of their activity. This analysis revealed that the hydrolytically unstable lactone ring of splitomicin is critically important for its activity.

Chemistry

Splitomicin and numerous derivatives were synthesized by Friedel–Crafts alkylation of the appropriate naphthol with an excess of acrylic acid and Amberlyst 15 ion exchange resin as the acid catalyst (Scheme 1). The product was purified by first filtering off the catalyst and then removing the solvent, followed by column chromatography. This synthesis with its good atom economy afforded numerous methoxy- and hydroxy-substituted versions of splitomicin with typical yields of 50–85%. However, the limited solubility of 1,6-dihydroxynaphthalene in refluxing toluene resulted in only a 20% yield of **32**. Dipyranones (e.g., **28**, **36**, and **39**) were also isolated from reactions with dihydroxynaphthalenes, although in disappointingly low yields (4–11%). This procedure was also successful with 2-naphthalenethiol affording the thiopyranone (**10**) in 40% yield. The regioselectivity of alkylation followed that expected for naphthalenes, i.e., preferred alkylation at the 1-, 4-, 5-, and 8-positions. However, substitution at the less-favored carbons also occurred with two 1-naphthols to afford **6** and **40**, albeit in generally lower yields (10–37%). In cases where competing alkylation could occur (e.g., 1,3- or 1,6-dihydroxynaphthalene), only products with the "preferred" alkylation patterns were observed. Friedel–Crafts alkylation using propiolic acid in place of acrylic acid was also successful. For example, alkylation of 2-naphthol afforded the unsaturated pyranone **2** (58%) and alkylation of **24** afforded the partially saturated dipyranone **29** (35%).

Nitrogen and oxygen nucleophiles readily reacted with the aromatic lactone ring of **1**, which facilitated the synthesis of analogues **12**–**16**. Deprotonation (DBU)

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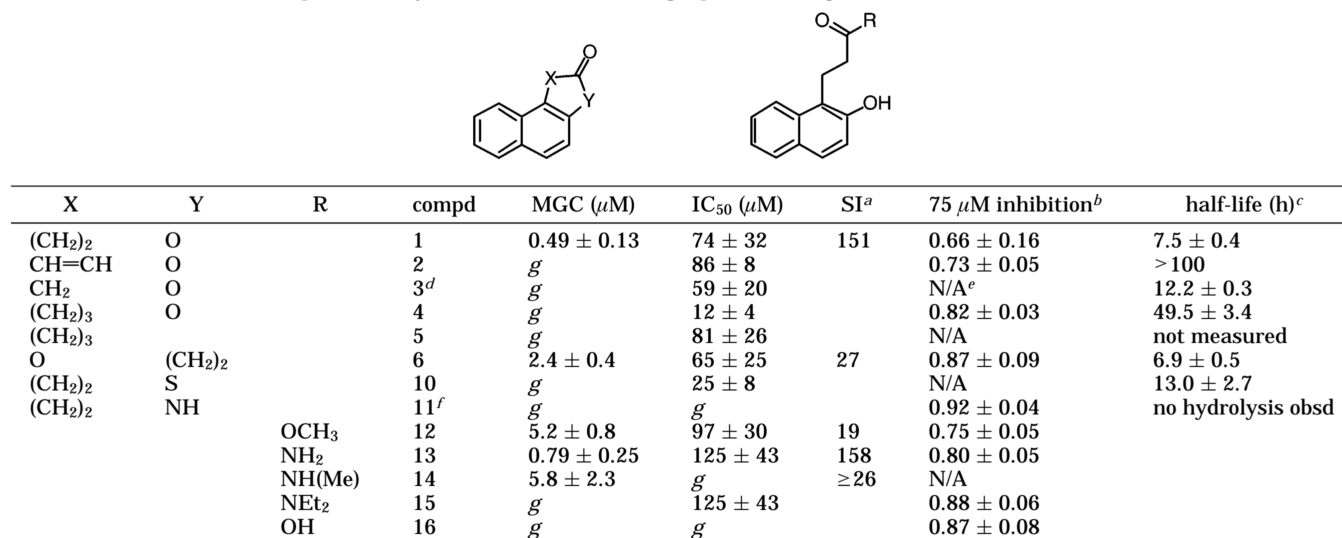
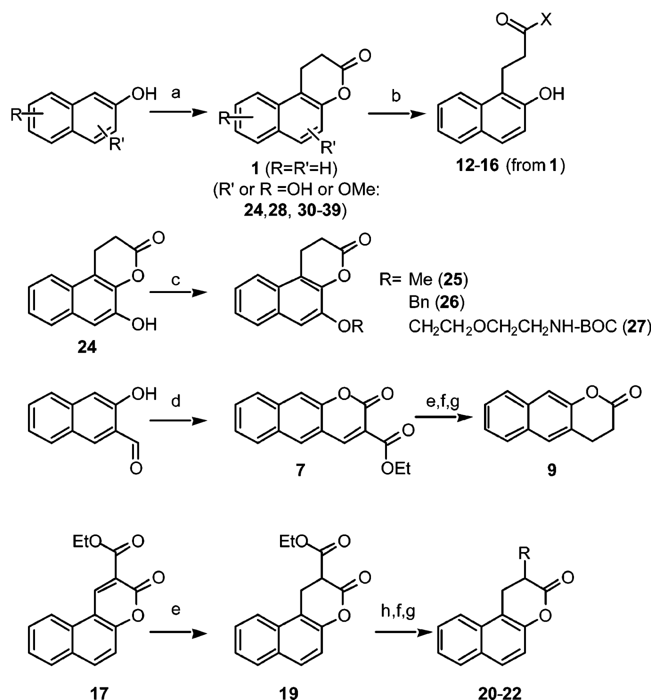
Table 1. Structures and Properties of Pyranone Variant and Ring-Opened Analogues



X	Y	R	compd	MGC (μM)	IC$_{50}$ (μM)	SI[a]	75 μM inhibition[b]	half-life (h)[c]
$(CH_2)_2$	O		1	0.49 ± 0.13	74 ± 32	151	0.66 ± 0.16	7.5 ± 0.4
CH=CH	O		2	*g*	86 ± 8		0.73 ± 0.05	>100
CH_2	O		3[d]	*g*	59 ± 20		N/A[e]	12.2 ± 0.3
$(CH_2)_3$	O		4	*g*	12 ± 4		0.82 ± 0.03	49.5 ± 3.4
$(CH_2)_3$			5	*g*	81 ± 26		N/A	not measured
O	$(CH_2)_2$		6	2.4 ± 0.4	65 ± 25	27	0.87 ± 0.09	6.9 ± 0.5
$(CH_2)_2$	S		10	*g*	25 ± 8		N/A	13.0 ± 2.7
$(CH_2)_2$	NH		11[f]	*g*	*g*		0.92 ± 0.04	no hydrolysis obsd
		OCH_3	12	5.2 ± 0.8	97 ± 30	19	0.75 ± 0.05	
		NH_2	13	0.79 ± 0.25	125 ± 43	158	0.80 ± 0.05	
		NH(Me)	14	5.8 ± 2.3	*g*	≥26	N/A	
		NEt_2	15	*g*	125 ± 43		0.88 ± 0.06	
		OH	16	*g*	*g*		0.87 ± 0.08	

[a] SI = IC$_{50}$/MGC. [b] Activity of in vitro assay at 75 μM, normalized to DMSO control. [c] Determined from the pseudo-first-order hydrolysis rate constant, measured in yeast growth media, pH 4.3. [d] Described in ref 10. [e] No activity (≥95% of DMSO controls). [f] Described in ref 11. [g] ≥150 μM.

Scheme 1[a]



[a] Reagents and conditions: (a) Acrylic acid, Amberlyst 15, toluene, reflux. (b) Nu. (c) DBU, RX. (d) Diethyl malonate, pyridine, piperidine (catalytic), reflux. (e) NaBH$_4$, pyridine. (f) 1 N LiOH, THF. (g) TsOH, benzene, reflux or room temperature, no solvent. (h) LDA, RX.

of the hydroxyl group of **24** and subsequent alkylation with alkyl halides or alkyl methanesulfonates were a useful route to several analogues (**25**−**27**) obtained in fair to excellent (37−95%) yield.

A lengthier route to analogues of **1** involved Knoevenagel condensation between a malonate ester and a hydroxynaphthaldehyde, subsequent NaBH$_4$ reduction of the unsaturated β-diester in pyridine, ester hydrolysis, and decarboxylation/ring closure steps (Scheme 1). This was the only route to compound **9**, which was obtained from 2-hydroxy-3-naphthaldehyde[9] in 48% overall yield. Analogues with substituents at the 2-position of the pyran ring (**20**−**22**) were produced by alkylation of the anion of the β-diester of **19**, followed by ester hydrolysis and decarboxylation/ring closure steps in poor to good (27−71%) overall yields. Relactonization was achieved via acid catalysis under dehydrating conditions (e.g., catalytic TsOH or HCl in refluxing benzene). Spontaneous relactonization was observed when the ring-opened versions were stored neat for several days (e.g., **16** and the hydroxy acid precursors to **21** and **22**).

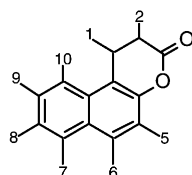
The naphtho-oxepinone, **4**, was produced by Bayer−Villager oxidation of the tetrahydrophenanthrenone (**5**) in 49% yield. Borohydride reduction of **2** in MeOH afforded the fully reduced naphthol (**18**, 94%). Structures of all the compounds are given in Tables 1 and 2, Figure 1 and Scheme 1.

Biology: Results and Discussion

Sir2 inhibitory activity of splitomicin analogues was first evaluated in vivo using a functional assay for telomeric silencing in yeast. The test strain contains the URA3 gene in close proximity to the left telomere of chromosome VII, where the expression of URA3 is repressed through Sir2-dependent silencing[12] (Figure 2). Because Ura3 is required for uracil biosynthesis, this strain cannot grow in media lacking uracil. Compound-mediated inhibition of Sir2 eliminates silencing and activates the URA3 gene, which allows the cells to grow in media lacking uracil.

In this cell-based screen, the measured output (yeast growth) includes effects of Sir2 inhibition, compound-mediated toxicity, and the compounds' individual physicochemical parameters, such as solubility and lipophilicity, which can affect drug transport. Thus, a compound's Sir2 inhibitory activity is expressed as minimum growth-stimulating concentration (MGC, 10% of maximal growth in media lacking uracil, seen with compound **41**) (Tables 1 and 3), the concentration at which toxicity is least pronounced. A compound's toxicity (expressed as IC$_{50}$) was evaluated separately by

Table 2. Structures of the Substituted Naphthopyranones



compd	position 1	2	5	6	7	8	9	10
1	H	H	H	H	H	H	H	H
19	H	CO_2Et	H	H	H	H	H	H
20	H	Me	H	H	H	H	H	H
21	H	$CH_2CH{=}CH_2$	H	H	H	H	H	H
22	H	$CH_2CH(Me)_2$	H	H	H	H	H	H
23[a]	Ph	H	H	H	H	H	H	H
24	H	H	OH	H	H	H	H	H
25	H	H	OMe	H	H	H	H	H
26	H	H	OBn	H	H	H	H	H
27	H	H	$O(CH_2)_2O(CH_2)_2NHBOC$	H	H	H	H	H
28	H	H	$OC(O)(CH_2)_2$-§[b]	†	H	H	H	H
29	H	H	OC(O)CH=CH-§	†	H	H	H	H
30	H	H	H	OH	H	H	H	H
31	H	H	H	OMe	H	H	H	H
32	H	H	H	H	OH	H	H	H
33	H	H	H	H	OMe	H	H	H
34	H	H	H	H	H	OH	H	H
35	H	H	H	H	H	OMe	H	H
36	H	H	H	H	$(CH_2)_2C(O)O$-§	†	H	H
37	H	H	H	H	H	H	OH	H
38	H	H	H	H	H	H	OMe	H
39	H	H	H	H	H	H	$OC(O)(CH_2)_2$-§	†

[a] Obtained from the NCI. [b] §, denotes attachment at next (†) position.

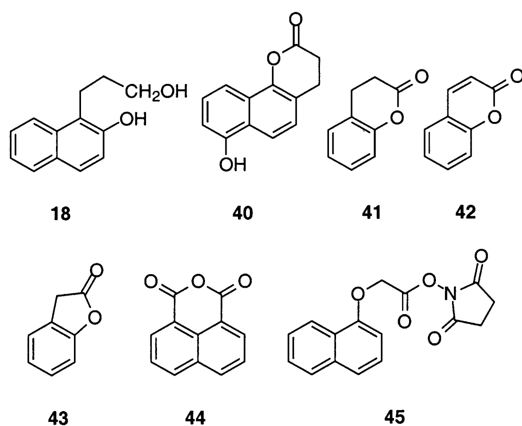


Figure 1. Miscellaneous analogues of **1**.

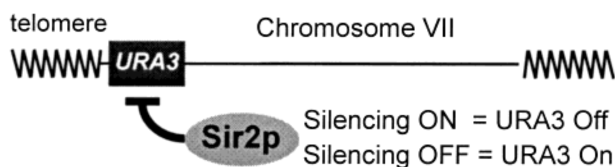


Figure 2. Sir2 regulates telomeric silencing.

assessing the effect on growth of the same strain in complete medium. Representative dose–response curves can be found in Figures 3 and 4. The ratio of the MGC to IC_{50} value afforded a selectivity index (SI) for each of the compounds. Examination of splitomicin and its analogues' activities in vivo have helped to elucidate the structure–activity relationships, described below.

Variations of and Substitutions on the Lactone Ring. The saturated six-membered lactone ring of splitomicin is important to its activity. The unsaturated lactone (**2**) and tetrahydrophenanthrenone (**5**) do not stimulate growth. However, the five-membered lactone (**3**)[10] and oxepinone (**4**) induce slight growth at concentrations >10 and >20 μM, respectively, but neither induce a sufficient growth density to determine the MGC (see Figures 3 and 4). Oxepinone **4** is quite toxic (IC_{50} = 12 μM), which may mask its inhibition of Sir2. Changing the lactone to a thiolactone (**10**) or lactam (**11**)[11] also eliminates growth stimulation. The orientation of the lactone ring is also important. The [1,2]-lactone (**6**) and [2,3]-lactone (**9**) are ca. 5- and 31-fold less active than **1** ([2,1]-lactone), respectively. Similarly, the 6-hydroxy [1,2]-lactone (**40**) is also less active than its [2,1]-lactone homologue (**32**, discussed below). Hydroxypropyl naphthol (**18**), which is incapable of lactonization, shows no growth stimulation. That the lactone ring orientation is important and that substituents in the 7–9 positions greatly reduce the growth stimulation (see below) suggest that a splitomicin interacts with its target in a particular orientation, perhaps in a binding pocket.

Several ring-opened analogues (**12**, **14**–**16**) have markedly decreased activity (MGCs 5 to >150 μM). Hydroxyamide **13** is potent, but subsequent analysis of this compound indicated that **13** in aqueous solution is converted to **1** over time (see Supporting Information) and that a significant concentration of **1** accumulates during the growth stimulation experiment. Substitution on the lactone ring is poorly tolerated as demonstrated by the marked increase in MGC (5.4 to >150 μM) from methyl (**20**), allyl (**21**), isobutyl (**22**), carboxyethyl (**19**), and phenyl (**23**) substitution on the lactone ring.

One interpretation of these data is that an analogue's growth stimulating activity is dependent upon the

Table 3. Biological Activity of the Substituted Naphthopyranones and Miscellaneous Analogues

no.	MGC ± SD (μM)	IC_{50} ± SD (μM)	SI[a]	75 μM inhibitor ± SD[b]
1	0.49 ± 0.13	74 ± 32	151	0.66 ± 0.16
19	*f*	*f*		0.88 ± 0.09
20	5.4 ± 0.7	52 ± 17	10	0.94 ± 0.13
21	*f*	27 ± 14		0.86 ± 0.04
22	*f*	60 ± 37		N/A[c]
23[d]	*f*	43 ± 37		0.90 ± 0.15
24	0.69 ± 0.19	111 ± 48	161	N/A
25	1.5 ± 0.5	128 ± 44	85	N/A
26	0.97 ± 0.63	27 ± 28	29	0.17 ± 0.06
27	6.4 ± 0.4	86 ± 8	13	N/A
28	4.9 ± 1.8	89 ± 34	18	0.80 ± 0.03
29	5.1 ± 0.2	*f*	≥29	0.83 ± 0.07
30	11 ± 1	131 ± 47	12	N/A
31	1.8 ± 0.3	44 ± 26	24	N/A
32	16 ± 6	*f*	≥107	N/A
33	5.5 ± 2.0	39 ± 19	7	N/A
34	38 ± 10	*f*	≥39	0.85 ± 0.08
35	13.8 ± 4.4	73 ± 30	5	0.84 ± 0.03
36	*f*	70 ± 26		0.87 ± 0.09
37	48 ± 5	134 ± 14	3	N/A
38	*f*	41 ± 15		0.84 ± 0.08
39	*f*	*f*		N/A
		miscellaneous analogues[e]		
9	15 ± 5	*f*	10	0.85 ± 0.03
18	*f*	*f*		0.88 ± 0.04
40	21 ± 1	*f*	≥7	N/A
41	0.85 ± 0.05	122 ± 11	144	0.15 ± 0.03
42	*f*	*f*		0.74 ± 0.05
43	24 ± 4	*f*	≥6	0.89 ± 0.11
44	*f*	8.8 ± 1.7		0.94 ± 0.02
45	*f*	*f*		N/A

[a] SI = IC_{50}/MGC. [b] Activity of in vitro assay at 75 μM, normalized to DMSO control. [c] No activity (≥95% of DMSO controls). [d] Obtained from the NCI. [e] For structures, see Scheme 1 (compound **9**) and Figure 1 (compounds **18** and **40**–**45**). [f] ≥150 μM.

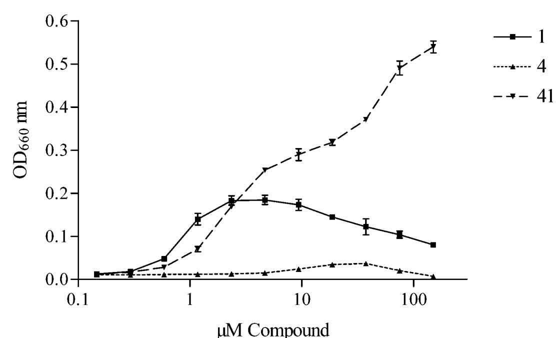


Figure 3. Representative yeast proliferation assay dose–response curves.

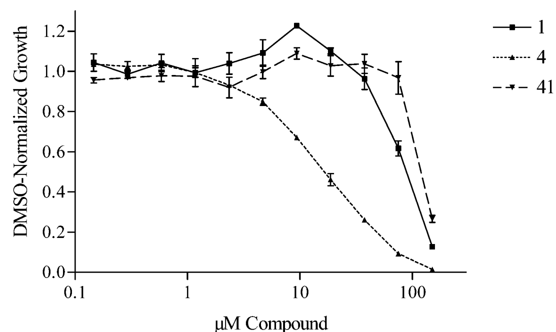


Figure 4. Representative toxicity dose–response curves.

reactivity (electrophilicity) of the aromatic lactone in forming a covalent adduct with a nucleophile (e.g., RNH_2, RSH, and ROH). The nucleophile may be an amino acid residue of Sir2 itself or another component critical to Sir2's function. Efforts to identify covalent splitomicin adducts have so far proven unsuccessful. However, the fact that **13** can be converted to **1** suggests that adduct formation may be a reversible reaction and adducts are only present transiently.

In the absence of a biologically relevant nucleophile, we have used the hydrolysis rate of selected analogues in growth media as a relative measure of lactone reactivity (Table 1). Indeed, compounds **2**–**4**, **10**, and **11** have slower hydrolysis rates than **1** and all are less active than **1**. Compound **6** ([1,2]-lactone) is hydrolyzed only slightly faster than **1**, and its higher MGC may be due to the orientation of the lactone ring. Compound **9** ([2,3]-lactone), in contrast, has a hydrolysis rate (24.1 ± 4.5 h) ca. three times that of **1**, and its poor activity may be due to its slower hydrolysis rate or the lactone ring's orientation. However, it is not possible to evaluate the relative importance of these two factors from these data. Interestingly, compound **41** also has a much longer half-life (20.5 ± 1.6 h) than **1** but has a similar MGC to that of **1**. One explanation for this discrepancy is that a phenyl ring in place of the naphthalene ring alters the solubility and lipophilicity sufficiently to afford a greater concentration of **41** at the active site in the cell. As such, the significant structural changes from **41** and **1** may preclude their direct comparison in an SAR study.

Substitutions on the Naphthalene Ring. The activity of analogues with naphthalene ring substituents depends on the position of substitution as is illustrated by the series of hydroxy- (**24**, **30**, **32**, **34**, and **37**) and methoxy-substituted (**25**, **31**, **33**, **35**, and **38**) analogues. The MGC values decrease systematically in the series of compounds with substituents at the 5–9-positions (from 0.7 to 48 μM for hydroxy substitution and from 1.5 to >150 μM for methoxy substitution). A similar trend is observed for the bis-lactone series (**28**, **36**, and **39**). The hydroxy- and methoxy-naphthols used to prepare these analogues show no growth stimulating activity. Interestingly, the 5-position tolerates even large groups such as O-benzyl (**26**), an N-BOC-aminoethoxyethyl chain (**27**), and the bis-lactones (**28** and **29**) without large increases in MGC values.

The hydrolysis half-lives of methoxy-substituted analogues (**33**, **35**, and **38**) and the 9-hydroxy analogue (**37**) were determined to be 9.5 ± 0.1, 9.5 ± 0.3, 7.8 ± 0.5, and 8.3 ± 0.3 h, respectively. These data suggest that the increased MGC values of these analogues are due to the substituents' interference at the site of action and not to a change in the hydrolysis rates, as these half-lives are very similar to that of splitomicin.

Miscellaneous Analogues. Dihydrocoumarin (**41**) induces the most growth in our yeast proliferation assays (Figure 3) and its toxicity is low (IC_{50} = 122 μM, Figure 4), which likely contributed to its potency. As such, **41** was used as the reference for determination of the MGC values (10% of maximal growth). Like **2**, coumarin (**42**) also shows no growth induction; however, coumaranone (**43**) has a modest MGC (24 μM).

Two other analogues (**44** and **45**), which, like splitomicin, have the potential to act as acylating reagents, show no growth stimulation. However, **44** has the

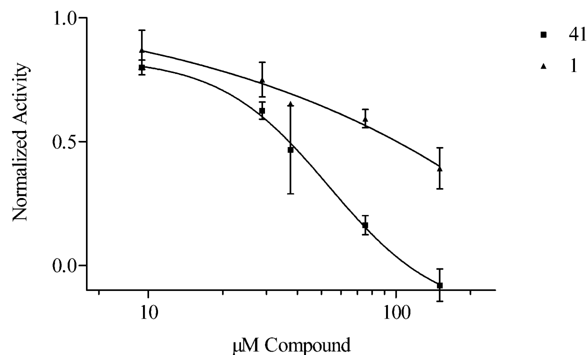


Figure 5. In vitro HDAC assay for **1** and **41**.

greatest toxicity of all of the compounds evaluated in this screen and this may have masked any growth induction.

Toxicity and Selectivity Index (SI). Most analogues with MGC values below 50 μM exhibited low toxicity (IC_{50} > 60 μM), but no clear correlation of toxicity to the MGCs was observed. This is consistent with the observation that Sir2 activity is not required for viability. Indeed, yeast cells lacking all five NAD^+-dependent deacetylases are viable.[13] The toxicity:MGC ratio yields a SI, a ratio that was useful in comparing different compounds. Splitomicin (**1**), **13** (which is converted to **1** in situ), **24**, and **41** had the highest SI values.

In Vitro HDAC Inhibition. The in vitro inhibition of Sir2 was evaluated using yeast whole cell lysates from a strain overexpressing Sir2 as described previously.[7] Inhibition of NAD^+-dependent deacetylase activity was first examined at 75 μM (Tables 1 and 3), and dose−response curves were determined for **1** and **41** (IC_{50} = 100 and 31 μM, respectively; Figure 5). There was no clear correlation between the activity in the yeast assay and the in vitro HDAC assay, which is consistent with our previous observations comparing in vivo and in vitro inhibition of Sir2.[7] Nevertheless, many of the compounds that were highly active in vivo (e.g., **1**, **26**, and **41**) inhibited Sir2 in vitro, affording 15−66% of the activity relative to dimethyl sulfoxide (DMSO) control at 75 μM. The concentrations sufficient for the in vivo effect are lower than those required for the in vitro inhibition. This might be due to higher stability of the compounds in the acidic growth medium in the in vivo assay. In synthetic complete medium, pH 4.3−4.5, the half-life of **1** is 7.5 h; the pH of the in vitro reaction is ~7.5 and **1** has a half-life of 1.5 ± 0.1 h (measured in phosphate-buffered saline, data not shown). Alternatively, the discrepancy between in vivo and in vitro growth stimulating concentrations could be a reflection of the differences between the histone peptide substrate in vitro and the native nucleosomes in the context of chromatin. A similar rationale has been proposed to explain different in vitro vs in vivo properties of the Sir2-R275 mutant.[14] This Sir2 allele contains a point mutation in the putative peptide binding site, where splitomicin is proposed to act,[7,15] and encodes for a fully active histone deacetylase in vitro. Although the mutant Sir2 protein is well-expressed and is appropriately recruited to chromatin, it is incapable of establishing a silenced state at the target loci.

Conclusions

Forty-one analogues of splitomicin (**1**) were prepared and evaluated for their relative Sir2 growth stimulating activity in vivo and in vitro, and their toxicity profiles were determined in vivo. The structure−activity relationships show that substituents are not well-tolerated on the lactone nor at the 7−9-positions on the naphthalene ring, and the most potent analogues are structurally similar to **1**. Furthermore, the naphthalene ring is not required for the activity as it can be replaced with a benzene ring (e.g., **41**). Although the aromatic lactone is hydrolytically unstable, it is central to splitomicin's activity as an inhibitor of Sir2. Analogues with modified lactone rings (e.g., contracted, oxidized, or substituted with a heteroatom) are poor inhibitors and are less reactive (i.e., have slower hydrolysis rates than **1**), suggesting that the reactivity of the lactone ring was critical to the inhibition of Sir2. Our studies will serve as a starting point toward the development of specific inhibitors of Sir2-like deacetylases.

Experimental Section

General Methods. Mass spectrometry (electrospray ionization) was performed on a Bruker Esquire ion trap mass spectrometer or an HP Series 1100 MSD. Gas chromatography−mass spectrometry (GC-MS) analyses (EI) were performed on an HP 5890 GC with a HP 5970 detector and 30 m DB5 column (0.25 mm i.d., 0.25 μm film) (method A), a HP 5989 detector and 60 m DB1 column (0.32 mm i.d., 0.25 μm film) (method B), or a 15 m SPB-35 column (0.25 mm i.d., 0.25 μm film) (method C). Each GC-MS analysis was performed at 15 L/min (He) with a starting temperature of 70 °C and a gradient of 15 °C/min from 3 to 18 min. ^1H NMR spectra were obtained on a Tecmag 300 MHz spectrometer, and ^1H chemical shifts are reported in ppm (δ). Flash column chromatography was performed using silica gel (Merck, grade 9385, 230−400 mesh). Analytical thin-layer chromatography (TLC) was performed using silica gel Analtech GF plates (0.25 mm), and products were visualized using UV light. Unless otherwise noted, reagents were purchased from Sigma-Aldrich Company or Lancaster Synthesis. Solvents were ACS reagent grade or better, and anhydrous solvents (Aldrich) were used as received unless otherwise indicated. 1,2,3,4-Tetrahydro-1-phenanthrenone (**5**), 1,2-dihydro coumarin (**41**), coumarin (**42**), 2-coumaranone (**43**), 1,8-dinaphthoic anhydride (**44**), and N-hydroxysuccinimidyl (1-oxynaphthyl)acetate (**45**) were purchased from Sigma-Aldrich Company and were used without further purification. Compound **23** (1-phenyl-1,2-dihydro-3*H*-naphtho[2,1-*b*]pyran-3-one, NSC no. 17364) was obtained from the National Cancer Institute. In general, the dihydronaphthopyranones were susceptible to hydrolysis and were kept as solids until preparing the DMSO stock solutions (30 mM) used in the yeast proliferation assay, described below. The DMSO solutions were kept tightly capped at 3−4 °C before use, and as such, hydrolysis was less than a few percent over a period of 2−3 weeks.

Yeast Proliferation Assay. The yeast proliferation assay was performed as described previously.[7] Briefly, DMSO stock solutions were used to prepare stock solutions of each compound in 5% DMSO in water (1.5 mM to 0.73 μM). Aliquots of these solutions were transferred to 96 well plates containing yeast cultures (strain UCC2210 *Mat*α *ppr1 adh4::URA3-TEL* (VII-L), ca. 13 500 cells per well) in synthetic complete media lacking uracil to achieve 150 μM to 73 nM concentrations (150 μL total). The maximum concentration of DMSO in the yeast cultures was 2%. Background absorbance measurements (OD_{660nm}) were taken at the beginning of the assay to account for any compound precipitation at high concentrations. The cultures were incubated at 30 °C until the OD_{660nm} for the culture of **41** reached ca. 0.5, which was typically reached by 40−48 h. The initial absorbance measurements were sub-

Table 4. HPLC Elution Methods

A			B			C		
time (min)	% H_2O^a	% CH_3CN^a	time (min)	% (50 mM NH_4OAc, 5 mM $Et_3N{\cdot}HOAc$)^b	% MeOH	time (min)	% H_2O^a	% CH_3CN^a
0	80	20	0	55	45	0	65	35
3	80	20	23	55	45	3	65	35
13	0	100	24	5	95	6	0	100
20	0	100	29	5	95	10	0	100
			30	55	45			
			35	55	45			
flow rate = 0.3 mL/min			flow rate = 0.25 mL/min			flow rate = 0.36 mL/min		

[a] With 0.1% TFA. [b] pH 3.5.

tracted from the final readings, and these values were plotted vs the corresponding concentrations. MGC values were determined by assessing the concentration on the dose−response curve at which the OD_{660nm} was 10% of the growth observed with **41** (typically 0.5−0.55 AU at 150 μM). Each compound was evaluated in triplicate; uncertainties in Tables 1 and 3 and Figure 3 represent ±1 SD.

Yeast Toxicity Assay. The yeast cultures were incubated with each compound as described above but in synthetic complete media containing uracil. Initial OD_{660nm} measurements were taken just after preparing the plates, and the plates were incubated at 30 °C. The final OD_{660nm} measurements were taken when the DMSO control wells had reached 0.35−0.4 AU. The final OD_{660nm} values were subtracted from the initial values, and the data were normalized to the DMSO controls and plotted on a dose−response curve. The IC_{50} values were determined using the software program Prism. Each compound was evaluated in triplicate; uncertainties in Tables 1 and 3 and Figure 4 represent ±1 SD.

In Vitro HDAC Assay. Histone deacetylase activity in the presence or absence of splitomicin analogues was assessed by measuring the NAD^+-dependent release of free [^{3}H]acetate from [^{3}H]-acetylated histone H4 peptide in the presence of whole cell lysate prepared from an *hst2*Δ strain overexpressing Sir2 as described previously.[7] The *hst2*Δ strain contains a 2 μm plasmid with galactose inducible *SIR2*,[16] and the extract contained robust NAD-dependent deacetylase activity derived exclusively from Sir2. Histone H4 peptide was acetylated chemically using the HDAC assay kit (Upstate Biotechnology, NY). Whole cell yeast lysate (10 μg) was incubated with [^{3}H]-acetylated histone H4 peptide (40 000 cpm) with or without 500 μM NAD^+ in a 20 μL reaction buffered with 150 mM NaCl, 50 mM Tris-HCl, pH 8.0, and 1 mM DTT. Reactions were incubated at 30 °C for 23 h and stopped by the addition of 5 μL of 1 N HCl and 0.15 N acetic acid. Released [^{3}H]acetate was extracted with 400 μL of ethyl acetate, and the activity in 200 μL of this extract was determined using a scintillation counter. All analogues were initially evaluated at 75 μM in deacetylation reactions with 500 μM NAD^+. Compounds **1** and **41** were also evaluated over a concentration range of 150 to 9.4 μM. Each deacetylation assay was performed in triplicate; uncertainties in Tables 1 and 3 and Figure 5 represent ±1 SD.

High-Performance Liquid Chromatography (HPLC). HPLC analyses were performed on HP1050 and HP1100 HPLC systems using a Supelcosil LC-18 column (25 cm × 2.1 mm, 0.3 μm; Supelco). To determine compound purity, aliquots (1 μL) of 0.4 mM solutions (2:1 DMSO:MeOH) were analyzed (at 230 nm) with gradient (method A) and isocratic then step gradient (method B) elution protocols as described in Table 4. Method C was used to monitor (280 nm) the hydrolysis rates of selected compounds: DMSO stock solutions (30 mM) of selected compounds were used to prepare 100 μM solutions in complete media lacking uracil (pH 4.2−4.3, unadjusted). The solutions were analyzed by HPLC several times over a 2−3 day period, and the peak area of unhydrolyzed product was determined. A plot of ln(unhydrolyzed peak area) vs time afforded a slope of the pseudo-first-order hydrolysis rate constant. Each hydrolysis experiment was performed in triplicate; uncertainties in Table 1 represent ±1 SD.

General Method: Friedel−Crafts Alkylation. The naphthol (1 mmol), acrylic acid (2 mmol), and Amberlyst 15 ion exchange resin (75−100 mg) were refluxed in toluene (2−3 mL) overnight. The catalyst was filtered off and washed with hot toluene (5 mL), and the solvent was removed under reduced pressure. Flash chromatography of the residue afforded the purified product.

1,2-Dihydro-3*H*-naphtho[2,1-*b*]pyran-3-one (1). Friedel−Crafts alkylation of 2-naphthol afforded **1** (85%) as a white solid. R_f = 0.6 (CH_2Cl_2). ^{1}H NMR ($CDCl_3$): [2.89 (d, J = 7.0), 2.93 (d, J = 6.5 Hz), 2H], 3.35 (t, J_1 = 7.5, J_2 = 7.3 Hz, 2H), 7.23 (d, J = 8.8 Hz, 1H), 7.47 (overlapping dd, J_1 = 6.7 Hz, J_2 = 1.3 Hz, 1H), 7.58 (overlapping dd, J_1 = 6.9 Hz, J_2 = 1.5 Hz, 1H), 7.76 (d, J = 8.8 Hz, 1H), 7.84−7.90 (m, 2H). The HPLC, GC-MS, and electrospray MS data for **1** and all other compounds can be found in Table 5.

3*H*-Naphtho[2,1-*b*]pyran-3-one (2). Compound **2** was prepared by Friedel−Crafts alkylation of 2-naphthol using propiolic acid in place of acrylic acid, and the mixture was refluxed for 4 h. Some 2-naphthol (21%) was recovered, and the yield of **2** was 73% based on 79% conversion. R_f = 0.45 (hexanes:EtOAc 3:2). ^{1}H NMR ($CDCl_3$): 6.54 (d, J = 9.9 Hz, 1H), 7.41 (d, J = 8.8 Hz, 1H), 7.55 (t, J = 8.8 Hz, 1H), 7.67 (t, J = 8.3 Hz, 1H), 7.88 (d, J = 7.8 Hz, 1H), 7.94 (d, J = 8.8 Hz, 1H), 8.17 (d, J = 8.3 Hz, 1H), 8.43 (d, J = 9.9 Hz, 1H).

2,3-Dihydro-1*H*-naphtho[2,1-*b*]oxepin-4-one (4). *m*-CPBA (≥77% *m*-CPBA, remainder chlorobenzoic acid and H_2O, 0.63 g, 2.5 equiv) was added in 3 aliquots over 12 h to a solution of 1,2,3,4-tetrahydro-1-phenanthrenone (**5**, 0.196 g, 1 mmol) in CH_2Cl_2 (5 mL) while the solution was refluxed. Then, the solvent was removed and the residue was flash-chromatographed to afford **4** (0.104 g, 49%). R_f = 0.7 (CH_2Cl_2). ^{1}H NMR ($CDCl_3$): 2.15−2.30 (m, 2H), 2.39−2.46 (m, 2H), 3.20 (t, J = 7.0 Hz, 2H), 7.19 (d, J = 8.8 Hz, 1H), 7.36−7.53 (m, 2H), 7.70 (d, J = 8.8 Hz, 1H), 7.79 (dd, J_1 = 8.2 Hz, J_2 = 1.3 Hz, 1H), 7.94 (d, J = 8.4 Hz, 1H).

3,4-Dihydro-2*H*-naphtho[1,2-*b*]pyran-3-one (6). Friedel−Crafts alkylation (5 h reflux) of 1-naphthol afforded **6** (37%) and a trace of unreacted 1-naphthol. **6**: R_f = 0.65 (EtOAc: hexanes 1:2). ^{1}H NMR ($CDCl_3$): 2.88−2.90 (m, 2H), 3.13−3.17 (m, 2H), 7.27 (d, J = 8.3 Hz, 1H), 7.47−7.58 (m, 2H), 7.60 (d, J = 8.3 Hz, 1H), 7.81−7.84 (m, 1H), 8.23−8.26 (m, 1H).

Compound **6** was also be prepared in 9% overall yield by the Knoevenagel condensation, borohydride reduction, and decarboxylation procedure, described below for **9**, using 1-hydroxy-2-naphthaldehyde.[17]

1,2-Dihydro-3*H*-naphtho[2,3-*b*]pyran-3-one (9). Knoevenagel condensation: 3-Hydroxy-2-naphthalenecarboxaldehyde[9] (0.11 g, 0.58 mmol), diethylmalonate (0.18 mL, 1.2 mmol), and piperidine (1 drop) were dissolved in pyridine (3 mL), and the mixture was refluxed overnight. The solvent was removed, the residue was washed with H_2O (20 mL), and the product was extracted into EtOAc (2 × 25 mL). The organic extract was dried (Na_2SO_4), and the solvent was removed. The residue was purified by flash chromatography (hexanes:EtOAc, 2:1 to 3:2) to afford ethyl 3*H*-naphtho[2,3-*b*]pyran-3-one-2-carboxylate (**7**, 0.145 g, 93%). R_f = 0.35 (2:1 hexanes:EtOAc). ^{1}H NMR ($CDCl_3$): 1.44 (t, J = 7.1 Hz, 3H), 4.44 (q, J = 7.1 Hz, 2H), 7.49−7.57 (m, 1H), 7.60−7.68 (m, 1H), 7.73 (s, 1H), 7.87−7.97 (m, 2H), 8.17 (s, 1H), 8.65 (s, 1H).

Table 5. HPLC, GC-MS, and Electrospray MS Data

	R_t HPLC A (% purity)	R_t HPLC B (% purity)	GC-MS[a] R_t (method, purity), m/z (intensity, assignment)	ESI-MS (−/+) m/z (intensity, assignment)
1	14.70 (99)	16.55 (96)	12.15 min (B, >99%), 198 (100, M$^+$), 170 (60), 128 (70).	
2	10.60 (>99)	7.97 (>99)	12.4 min (A, >99%), 196 (90, M$^+$), 168 (100), 139 (60)	
3[b]	11.89 (>99)	8.33 (>99)		
4	16.08 (96)	9.57 (>99)	13.1 min (A, >99%), 212 (40, M$^+$), 184 (40), 157 (100), 129 (40)	
6	13.93 (>99)	18.08 (98)	11.9 min (B, 99%), 190 (100, M$^+$), 170 (50), 156 (90), 128 (50)	
7	13.27 (>99)	31.37 (>99)	15.4 min (B, >99%), 268 (100, M$^+$), 223 (70), 196 (90), 139 (70)	
8	16.22 (>99)	20.87 (>99)		(+) 563 (20, 2M + Na), 293 (80, M + Na), 242 (100, M + H−CH$_2$=CH$_2$)
9	12.03 (>99)	19.24 (>99)	12.2 min (B, >99%), 198 (100, M$^+$), 170 (50), 128 (50)	
10	15.88 (>99)	21.37 (>99)	13.1 min (B, >99%), 214 (50, M$^+$), 186 (100)	
11[c]	6.57 (>99)	16.73 (>99)	14.3 min (A, >99%), 197 (100, M$^+$), 168 (85)	
12	10.33 (>99)	24.62 (95)		(+) 269 (20, M + K), 253 (50, M + Na), 231 (10, M + H), 199 (100, M + H−MeOH)
13	5.79 (>99)	7.11 (>99)		(−) 214 (100, M − H)
14	13.29 (98)	6.78 (>99)		(−) 228 (100, M − H)
15	12.60 (>99)	18.00 (98)		(+) 272 (100, M + H), 294 (5, M + Na)
16	6.09 (>99)	13.33 (>99)		(−) 215 (100, M − H)
17	6.18 (>99)	23.76 (>99)		(+) 307 (15, M + K), 291 (100, M + Na), 269 (5, M + H)
18	5.78 (98.7)	7.36 (95)		(−) 201 (100, M − H)
19	17.38 (>99)	17.42 (98)		(+) 309 (60, M + K), 293 (100, M + Na), 271 (30, M + H)
20	15.97 (>99)	35.04 (>99)	12.9 min (A, 98%), 212 (80, M$^+$), 184 (30), 169 (100), 128 (60)	
21	17.38 (97)	37.15 (>99)	12.8 min (B, 97%), 238 (80, M$^+$), 196 (100), 169 (50), 198 (50)	
22	18.06 (95)	36.94 (>99)	14.1 min (A, 90%), 254 (100, M$^+$), 198 (85), 183 (80), 157 (100), 128 (100)	
23[d]	17.96 (99)	37.11 (>99)		
24	5.93 (96.5)	7.48 (98)		(−) 213 (100, M − H)
25	13.84 (>99)	14.74 (>99)	17.3 min (A, >99%), 228 (100, M$^+$), 200 (30), 186 (45).	
26[e]	16.17 (91)	36.18 (94)	17.9 min (A, >99%), 304 (50, M$^+$), 91 (100)	
27	15.32 (95)	35.8 (95)		(+) 424 (100, M + Na), 440 (10, M + K)
28[e]	9.80 (94)	13.86 (92)	18.2 min (C, 79%), 268 (100, M$^+$), 198 (50)	
29	8.28 (>99)	8.87 (99)		(+) 555 (100, 2M + Na), 289 (40, M + Na)
30	6.84 (>99)	8.31 (>99)		(−), 213 (100, M − H)
31	16.11 (97)	31.61 (>99)	14.3 min (A, 99%), 228 (100, M$^+$)	
32	5.83 (>99)	6.38 (97)	13.9 min (A, 99%), 214 (100, M$^+$), 186 (40), 172 (75)	
33	15.20 (>99)	18.50 (98)	14.3 min (A, 91%), 228 (100, M$^+$), 200 (40), 186 (95)	
34	5.42 (>99)	6.48 (>99)		(−) 213 (100, M − H)
35	14.69 (96.7)	16.52 (99)	13.9 min (C, 99%), 228 (100, M$^+$), 186 (90)	
36	9.19 (97.7)	13.32 (95)	17.7 min (C, >99%), 268 (100, M$^+$), 226 (50), 198 (50)	
37	5.42 (98)	7.66 (97)		(−) 213 (100, M − H)
38	15.46 (95)	18.57 (>99)	13.8 min (C, 90%), 228 (100, M$^+$), 200 (70)	
39	7.93 (97.4)	13.25 (>99)	17.6 min (C, 99%), 268 (M$^+$, 100), 240 (50), 197 (70)	
40	5.95 (>99)	9.29 (>99)		(−) 213 (100, M − H)

[a] If GC-MS showed a purity <95%, the sample was repurified by chromatography before HPLC analysis and biological evaluation. [b] Described in ref 10. [c] Described in ref 11. [d] Obtained from the NCI. [e] Purity was 90−95% even after subsequent purification. The biological assays were performed with compound as is.

$NaBH_4$ Reduction. Compound **7** (50 mg, 0.19 mmol) was dissolved in dry pyridine (1 mL) at 0 °C, and $NaBH_4$ was added (5 mg). The solution was stirred for 1 h at 0 °C and then for 30 min at room temperature. The mixture was poured into 1 N HCl (10 mL), and the precipitated product was filtered off and subsequently washed with H_2O (50 mL). The precipitate was dissolved in a minimum of EtOAc:hexanes (1:1) and passed through a plug of silica. Solvent removal afforded 43 mg (86%) of ethyl 1,2-dihydro-3*H*-naphtho[2,3-*b*]pyran-3-one-2-carboxylate (**8**), which was used without further purification. Compound **8**: ^{1}H NMR ($CDCl_3$): 1.19 (t, $J = 7.2$ Hz, 3H), 3.30−3.65 (m, 2H), 3.86 (d of d, $J_1 = 7.7$ Hz, $J_2 = 5.8$ Hz, 1H), 4.18 (q, $J = 7.2$ Hz, 2H), [7.45−7.49 (m) overlapping with 7.51 (s), 3H], 7.70 (s, 1H), 7.77−7.82 (m, 2H).

Decarboxylation. Compound **8** (20 mg, 75 μmol) was dissolved in THF (1 mL), and 1 N LiOH (1 mL) was added. The mixture was stirred overnight, then the solution was acidified to pH 4 with 1 N HCl, brine (5 mL) was added, and

the product was extracted into EtOAc. The solvent was removed, and the residue was then dissolved in benzene (10 mL) acidified with TsOH (1−2 mg). The mixture was refluxed overnight, and then, the solvent was removed. The product was purified by chromatography (CH_2Cl_2) to afford **9** (9 mg, 45 μmol, 60%). Compound **9**: $R_f = 0.55$ (CH_2Cl_2). ^1H NMR ($CDCl_3$): 2.85−2.90 (m, 2H), 3.21 (t br, $J_1 = 7.5$ Hz, $J_2 = 5.7$ Hz, 2H), [7.44−7.51 (m) overlapping with 7.49 (s), 3H)], 7.69 (s, 1H), 7.78−7.81 (m, 2H).

1,2-Dihydro-3*H*-naphtho[2,1-*b*]thiopyran-3-one (10). Friedel−Crafts alkylation of 2-naphthalene thiol, as described for **1** (48 h of reflux), afforded **10** in 40% yield. $R_f = 0.9$ (hexanes:EtOAc 3:2). ^1H NMR ($CDCl_3$): 3.09−3.14 (m, 2H), 3.27−3.32 (m, 2H), 7.28 (d, $J = 8.6$ Hz, 1H), 7.45 (t, $J = 8.0$ Hz, 1H), 7.6 (t of d, $J_1 = 8.3$ Hz, $J_2 = 1.3$ Hz, 1H), 7.74 (d, $J = 7.8$ Hz, 1H), 7.78 (d, $J = 8.8$ Hz, 1H), 9.16 (d, $J = 8.8$ Hz, 1H).

Methyl 3-(2-Hydroxynaphth-1-yl)propionate (12). Splitomicin (**1**, 100 mg, 0.5 mmol) and TsOH (5 mg) were disssolved in MeOH (5 mL), and the mixture was stirred overnight. Then, the solvent was removed and the residue was purified by flash chromatography (CH_2Cl_2) to yield 0.103 g (0.45 mmol, 89%) of **12** as a white powder. $R_f = 0.55$. ^1H NMR ($CDCl_3$): 2.9 (dd, $J_1 = 5.7$ Hz, $J_2 = 6.2$ Hz, 2H), 3.3 (dd, $J_1 = 5.7$ Hz, $J_2 = 6.0$ Hz, 2H), 3.69 (s, 3H), 7.21 (d, $J = 8.8$ Hz, 1H), 7.34 (t, $J = 6.4$ Hz, 1H), 7.50 (t, $J = 6.4$ Hz, 1H), 7.67 (d, $J = 8.8$ Hz, 1H), 7.9 (overlapping d's, $J = 8.2$ Hz, 2H), 8.13 (s, 1H).

3-(2-Hydroxynaphth-1-yl)propionamide (13). Splitomicin (100 mg, 0.5 mmol) and NH_4OAc (150 mg, ca. 2 mmol) were dissolved in DMF (1 mL), and the mixture was stirred overnight. The mixture was poured into water (25 mL), brine (15 mL) was added, and the product was extracted into EtOAc (2 × 25 mL). The solvent was dried (Na_2SO_4) and then removed under reduced pressure. The residue was chromatographed using EtOAc to afford 12 mg of recovered **1** and **13** (89 mg, 0.41 mmol, 94% based on 88% conversion). $R_f = 0.55$ (EtOAc). ^1H NMR (acetone-d_6): 2.79−2.83 (m, overlapping with residual H_2O, 2H), 3.27 (dd, $J_1 = 6.0$ Hz, $J_2 = 6.2$ Hz, 2H), 6.6 (s br, 1H), 7.11 (d, $J = 8.8$ Hz, 1H), 7.30 (t of d, $J_1 = 8.0$ Hz, $J_2 = 1.0$ Hz, 1H), 7.45 (t of d, $J_1 = 8.3$ Hz, $J_2 = 1.0$ Hz), 7.65 (d, $J = 8.6$ Hz, 1H), 7.78 (d, $J = 8.3$ Hz, 1H), 7.88 (d, $J = 8.6$ Hz, 1H), 9.70 (s, 1H).

***N*-Methyl-3-(2-hydroxynaphth-1-yl)propionamide (14).** Splitomicin (50 mg, 0.25 mmol) was dissolved in CH_3CN (2 mL), and two aliquots of methylamine (2.0 M solution in MeOH, 15 μL each) were added at 0 and 3 h. The solution was stirred overnight, during which time a precipitate formed. The solvent was removed, and the residue was chromatographed (CH_2Cl_2:MeOH 25:1) to afford unreacted **1** (38 mg) and **14** (13 mg, 0.057 mmol, 95% based on 24% conversion). $R_f = 0.35$. ^1H NMR ($CDCl_3$): 2.18 (t, $J = 7.5$ Hz, 2H), 2.93 (t, $J = 7.5$ Hz, 2H), 4.34 (s, 3H), 6.71 (d, $J = 8.8$ Hz, 1H), 6.87 (overlapping d of d, $J_1 = 7.0$ Hz, $J_2 = 1.0$ Hz, 1H), 7.05 (overlapping d of d, $J_1 = 7.0$ Hz, $J_2 = 1.6$ Hz, 1H), 7.20 (d, $J = 8.8$ Hz, 1H), 7.33 (d, $J = 7.3$ Hz, 1H), 7.50 (d, $J = 8.6$ Hz, 1H), 9.90 (s, 1H).

***N*,*N*-Diethyl-3-(2-hydroxynaphth-1-yl)propionamide (15).** Splitomicin (**1**, 15 mg, 76 μmol) and diethylamine (0.2 mL, 0.19 mmol) were combined and stirred for 1 h. The solvent was removed, and the residue was chromatographed (CH_2Cl_2: MeOH 100:1) to afford **15** (7.5 mg, 28 μmol) in 36% yield. $R_f = 0.4$. ^1H NMR (acetone-d_6): 1.01−1.09 (overlapping t's, $J = 7.0$ Hz, 6H), 2.93−2.97 (m, 2H), 3.30−3.40 (m, 6H), 7.09 (d, $J = 8.8$ Hz, 1H), 7.28 (t of d, $J_1 = 6.8$, $J_2 = 1.5$ Hz), 7.65 (d, $J = 8.6$, 1H), 7.77 (d, $J = 8.3$ Hz, 1H), 7.93 (d, $J = 8.6$ Hz, 1H), 10.30 (s, 1H).

3-(2-Hydroxynaphth-1-yl)propionic Acid (16). Splitomicin (**1**, 0.138 g, 0.7 mmol) was dissolved in THF (5 mL), and 1 N LiOH (2.5 mL) was added. The mixture was stirred overnight, then the pH was adjusted to ∼3 (1 N HCl), and the product was extracted into EtOAc. The solvent was dried (Na_2SO_4) and removed under reduced pressure to afford **16** (0.145 g, 96%) as an oil. ^1H NMR (DMSO-d_6): 2.39−3.45 (overlapping d's, $J_1 = 8.6$ Hz, $J_2 = 7.7$ Hz, 2H), 3.15−3.23 (overlapping d's, $J_1 = 8.6$, $J_2 = 7.9$ Hz, 2H), 7.15 (d, $J = 8.8$ Hz, 1H), 7.26 (t of d, $J_1 = 7.0$ Hz, $J_2 = 0.9$ Hz, 1H), 7.44 (t of d, $J_1 = 7.0$ Hz, $J_2 = 1.4$ Hz, 1H), 7.63 (d, $J = 8.8$ Hz, 1H), 7.75 (d, $J = 7.2$ Hz, 1H), 7.87 (d, $J = 8.6$ Hz), 9.6 (s, br, 1H), 12.1 (s, br, 1H).

As a neat oil, **16** slowly relactonizes to **1**. Thus, preparation of **16** for biological evaluation was more conveniently carried out by treating a DMSO solution of **1** with 5% NaOH. After several minutes, HPLC indicated only **16** (data not shown).

1-(3-Hydroxypropyl)-2-hydroxynaphthalene (18). $NaBH_4$ (40 mg) was added to a solution of **2** (25 mg, 0.13 mmol) in MeOH (4 mL). The mixture was stirred for 5 h and then slowly added to 1 N HCl (10 mL). The product was extracted into EtOAc, and the solvent was removed after drying (Na_2SO_4). The residue was purified by flash chromatography (EtOAc) to afford **18** (12 mg, 46%). $R_f = 0.35$. ^1H NMR (CD_3CN): 1.87 (m, 2H), 3.13 (d, $J = 7.3$ Hz, 2H), 3.53 (d, $J = 6.2$ Hz, 2H), 7.14 (d, $J = 8.8$ Hz, 1H), 7.34 (t of d, $J_1 = 8.0$, $J_2 = 1.0$, 1H), 7.49 (t of d, $J_1 = 8.6$, $J_2 = 1.6$, 1H), 7.67 (d, $J = 8.8$ Hz, 1H), 7.82 (d, $J = 8.0$ Hz, 1H), 7.98 (d, $J = 8.6$ Hz, 1H).

Ethyl 1,2-Dihydro-3*H*-naphtho[2,1-*b*]pyran-3-one-2-carboxylate (19). Diethylmalonate and 2-hydroxy-1-naphthaldehyde were subjected to conditions of the Knoevanagel condensation described for **7** above. Purification by flash chromatography and subsequent recrystallization from EtOH afforded ethyl 3*H*-naphtho[2,1-*b*]pyran-3-one-2-carboxylate (**17**) in 92% yield. Compound **17** was reduced using $NaBH_4$ in pyridine, as described for **8** above, to afford **19** in 85% yield. Compound **17.** $R_f = 0.6$ (1:1 EtOAc:hexanes). MS (ESI$^+$): m/z 307(10, M + K), 291 (M + Na), 269 (5, M + H). ^1H NMR ($CDCl_3$): 1.33 (t, $J = 7.1$ Hz, 3H), 4.40 (q, $J = 7.1$ Hz, 2H), 7.44 (d, $J = 9.1$ Hz, 1H), 7.58−7.63 (m, 1H), 7.71−7.77 (m, 1H), 7.92 (d, $J = 7.3$ Hz, 1H), 8.08 (d, $J = 9.1$ Hz, 1H), 8.30 (d, $J = 8.3$ Hz, 1H), 9.29 (s, 1H). Compound **19**. $R_f = 0.75$ (1:1 EtOAc:hexanes). ^1H NMR ($CDCl_3$): 1.20 (t, $J = 7.1$ Hz, 3H), 3.5 (dd, $J_1 = 6.8$ Hz, $J_2 = 16.0$ Hz, 1H), 3.62−3.91 (m, 2H), 4.2 (overlapping q's, each with $J = 7.1$ Hz, 2H), 7.23 (d, $J = 8.2$ Hz, 1H), 7.48 (ABC spin system, $J_{AB} = 8.1$ Hz, $J_{AC} = 7.0$ Hz, $J_{BC} = 1.0$ Hz, 1H), 7.58 (ABC spin system, $J_{AB} = 8.3$ Hz, $J_{AC} = 7.0$ Hz, $J_{BC} = 1.3$ Hz, 1H), 7.78 (d, $J = 8.8$ Hz, 1H), 7.84 (d, $J = 7.5$ Hz, 1H), 7.90 (d, $J = 7.5$ Hz, 1H).

1,2-Dihydro-2-methyl-3*H*-naphtho[2,1-*b*]pyran-3-one (20). A solution of **19** (135 mg, 0.5 mmol) in dry THF (2 mL) was cooled to 0 °C, and LDA (0.25 mL of a 2.0 M solution in THF/ethylbenzene) was added. The mixture was stirred for 1 h at 0 °C, and then, CH_3I (0.31 mL, 5 mmol) was added. After it was stirred for another 16 h, the solvent was removed, and the residue was chromatographed (EtOAc:hexanes 1:1) to afford the methylated product ($R_f = 0.85$), The decarboxylation and relactonization were carried out as described for the synthesis of **9**, except the relactonization was accomplished in refluxing (3 h) benzene (25 mL) without adding TsOH. Chromatographic purification (1:1 hexanes:EtOAc) of the residue afforded 90 mg (84% overall yield) of 1,2-dihydro-2-methyl-3*H*-naphtho[2,1-*b*]pyran-3-one-2-carboxylate (**20**) as a white powder. $R_f = 0.55$ (CH_2Cl_2). ^1H NMR ($CDCl_3$): 1.39 (d, 5.5 Hz, 3H), 2.82−2.99 (m, 2H), 3.4 (m, 1H), 7.14 (d, $J = 9.7$ Hz, 1H), 7.34−7.52 (m, 2H), 7.66−7.83 (m, 3H).

1,2-Dihydro-2-allyl-3*H*-naphtho[2,1-*b*]pyran-3-one (21). A solution of **19** (0.108 g, 0.4 mmol) in dry THF (4 mL) was treated with LDA (2.0 M solution in THF/ethylbenzene, 0.2 mL, 0.4 mmol), and the solution was stirred for 1 h at room temperature Allyl bromide (0.69 mL, 8 mmol) was added, and the mixture was stirred for 48 h. The solvent was removed under reduced pressure, and the residue was redissolved in THF (5 mL). The decarboxylation reaction was carried out as described for **9**. As a solid residue, the hydroxy acid product slowly relactonized (TLC), and after 3 days, the residue was purified by chromatography (CH_2Cl_2) to afford **21** (68 mg, 71%). $R_f = 0.65$ (CH_2Cl_2). ^1H NMR ($CDCl_3$): 2.39−2.50 (m, 1H), 2.74−2.90 (m, 2H), [2.96 (d, $J = 10.6$ Hz), 3.01 (d, $J = 10.9$ Hz), 1H], [3.42 (d, $J = 6.2$ Hz), 3.48 (d, $J = 6.0$), 1H], 5.14−5.18 (m, 1H, 5.18−5.21 (m, 1H), 5.84−5.98 (m, 1H), 7.21 (d, $J = 8.8$ Hz, 1H), 7.46 (overlapping d of d, $J_1 = 6.9$ Hz, $J_2 = 1.3$

Hz, 1H), 7.57 (overlapping d of d, J_1 = 6.8 Hz, J_2 = 1.3 Hz, 1H), 7.75 (d, J = 9.1 Hz, 1H), 7.83−7.89 (m, 2H).

1,2-Dihydro-2-(2-methylpropyl)-3*H*-naphtho[2,1-*b*]pyran-3-one (22). LDA (2.0 M in THF/ethylbenzene 0.2 mL) was added to a solution of **19** (0.108 g, 0.2 mmol) in THF (2 mL) at 0 °C, and the solution was stirred for 1 h at room temperature. The solvent was removed, and the residue was redissolved in dry DMF (2 mL). To this solution was added iodo-2-methylpropane (0.9 mL, 8 mmol); the mixture was stirred at room temperature for 24 h and then heated to 60−70 °C for 12 h. The solvent was removed under reduced pressure, and the residue was washed with H_2O (10 mL, acidified to pH 4 with 1 N HCl). Brine (15 mL) was added, and the product was extracted into EtOAc (2 × 25 mL). The EtOAc solution was dried (Na_2SO_4), the solvent was removed, and the residue was flash-chromatographed (CH_2Cl_2). Unreacted **19** (R_f = 0.4, 26 mg, 25%) was recovered, and the new product (R_f = 0.55, 62 mg as an oil), ethyl 1,2-dihydro-2-(2-methylpropyl)-3*H*-naphtho[2,1-*b*]pyran-3-one-2-carboxylate (**22a**), was collected but was not characterized. Compound **22a** (50 mg, 0.15 mmol) was decarboxylated using LiOH as described above for the synthesis of **9**. As a neat residue, the hydroxy acid product slowly relactonized at room temperature. After 3 days, the product was purified by filtering through a plug of silica as a solution in CH_2Cl_2 to afford **22** (22 mg) in 36% overall yield from **19**, based on 75% conversion. Compound **22**: R_f = 0.75 (CH_2Cl_2). ^{1}H NMR ($CDCl_3$): 0.94 (d, J = 6.3 Hz, 3H), 1.00 (d, J = 6.5 Hz, 3H), 1.92−1.98 (m, 2H), 2.85−3.09 (m, 2H), 3.48 (dd, J_1 = 5.8 Hz, J_2 = 15.4 Hz, 1H), 7.23 (d, J = 8.9 Hz), 7.42−7.62 (m, 2H), 7.77 (d, J = 8.9 Hz, 1H), 7.83−7.92 (m, 2H).

1,2-Dihydro-3*H*-5-hydroxynaphtho[2,1-*b*]pyran-3-one (24) and 1,2,7,8-Tetrahydro-3*H*,6*H*-naphtho[2,1-*b*:3,4-*b*′]dipyran-3,6-dione (28). Friedel−Crafts alkylation of 2,3-dihydroxynaphthalene afforded **24** (55%) and **28** (3%) after 3 h of refluxing. Compound **24**: R_f = 0.5 (CH_2Cl_2:acetone 10:1). ^{1}H NMR (DMSO-d_6): [2.88 (d, J = 7.3 Hz), 2.91 (d, J = 6.3 Hz), 2H], 3.32 (t, under residual water peak), 7.18 (s, 1H), [7.35 (d, J = 3.6 Hz), 7.37 (d, J = 2.1 Hz), 2H], 7.69 (m, 1H), 7.87 (m, 1H), 10.05 (s br, 1H). Compound **28**: R_f = 0.9 (CH_2Cl_2: acetone 10:1). ^{1}H NMR ($CDCl_3$): [2.91 (d, J = 7.0 Hz), 2.94 (d, J = 6.2 Hz), 2H], 3.37 (t, J_1 = 7.5 Hz, J_2 = 7.3 Hz, 2H), 7.54 (d, J = 3.4 Hz, 1H), 7.57 (d, J = 3.4 Hz, 1H), 7.87 (d, J = 3.4 Hz, 1H), 7.90 (d, J = 3.4 Hz, 1H).

1,2-Dihydro-3*H*-5-methoxynaphtho[2,1-*b*]pyran-3-one (25). Methyl iodide (25 μL, 0.4 mmol) was added to a solution of **24** (40 mg, 0.19 mmol) and DBU (30 μL, 0.2 mmol) in CH_3CN (2 mL), and the mixture was stirred overnight. Then, the solvent was removed and residue was flash-chromatographed to afford **25** (21 mg, 49%). R_f = 0.85 (20:1 CH_2Cl_2:acetone). ^{1}H NMR ($CDCl_3$): [2.88 (d, J = 7.0 Hz), 2.91 (d, J = 6.2 Hz), 2H], 3.33 (t, J_1 = 7.8, J_2 = 7.0, 2H), 3.98 (s, 3H), 7.11 (s, 1H), 7.42−7.46 (m, 2H), 7.72−7.80 (m, 2H).

1,2-Dihydro-3*H*-5-benzyloxynaphtho[2,1-*b*]pyran-3-one (26). DBU (47 μL, 0.32 mmol) was added to a solution of **24** (50 mg, 0.23 mmol) in CH_3CN (1 mL), and after 15 min of stirring, benzyl bromide (60 μL, 0.5 mmol) was added to the mixture. After it was stirred overnight, the solvent was removed and the mixture was purified by flash chromatography to afford **26** (94% yield). R_f = 0.5 (50:1 CH_2Cl_2:acetone). ^{1}H NMR ($CDCl_3$): [2.90 (d, J = 7.3 Hz), 2.92 (d, J = 6.2 Hz), 2H], 3.35 (t, J_1 = 8.0 Hz, J_2 = 7.0 Hz, 2H), 5.26 (s, 2H), 7.33−7.54 (m, 8H), 7.69−7.72 (m, 1H), 7.78−7.81 (m, 1H).

1,2-Dihydro-3*H*-5-(2-(N-BOC-2-aminoethoxy)ethyloxy)-naphtho[2,1-*b*]pyran-3-one (27). DBU (0.39 mL, 2.6 mmol) and **24** (0.375 g, 1.75 mmol) were combined in dry DMF (4 mL) under Ar, and the mixture was stirred for 20 min at room temperature. Then, 2-(2-*tert*-butoxycarbonylaminoethoxy)-ethanol methanesulfonic ester[18] (0.75 g, 2.6 mmol) was added and the mixture was heated to 80−85 °C for 5 h with stirring. The solvent was removed under reduced pressure, and the mixture was chromatographed on silica/(CH_2Cl_2:acetone 10:1). Unreacted **24** (0.05 g, 13%) eluted, followed by **27** (R_f = 0.5, 0.286 g, 47% based on 87% conversion). ^{1}H NMR (acetone-d_6): 1.39 (s, 9H), [2.92 (d, J = 7.2 Hz), 2.95 (d, J = 6.3 Hz), 2H], 3.27 (q, J = 5.7, 2H), 3.42 (t, J_1 = 8.1 Hz, J_2 = 6.9 Hz, 2H), 3.62 (t, J = 5.7 Hz, 2H), 3.90 (t, J = 4.8 Hz, 2H), 4.30 (t, J = 4.8 Hz, 2H), 5.9 (s, br, 1H), 7.37 (s, 1H), 7.42−7.45 (m, 2H), 7.79−7.83 (m, 1H), 7.89−7.93 (m, 1H).

1,2-Dihydro-3*H*,6*H*-naphtho[2,1-*b*],[3,4-*b*]bispyran-3,6-dione (29). Treatment of **24** with propiolic acid (15 equiv) under the Friedel−Crafts alkylation conditions (24 h reflux) afforded **29** (35%). R_f = 0.35 (1:1 EtOAc:hexanes). ^{1}H NMR (DMSO-d_6): [2.99 (d, J = 7.3 Hz), 3.01 (d, J = 7.0 Hz), 2H], 3.50 (t, J_1 = 7.5 Hz, J_2 = 7.3 Hz), 6.68 (d, J = 9.9 Hz, 1H), 7.68−7.72 (m, 2H), 8.11−8.14 (m, 1H), 8.54−8.57 (m, 1H), 8.96 (d, J = 9.9 Hz, 1H).

1,2-Dihydro-3*H*-6-hydroxynaphtho[2,1-*b*]pyran-3-one (30). Friedel−Crafts alkylation of 1,3-dihydroxynaphthalene afforded **30** (50%) after 3 h of refluxing. R_f = 0.5 (CH_2Cl_2: acetone 10:1). ^{1}H NMR (DMSO-d_6): [2.85 (d, J = 7.0 Hz), 2.88 (d, J = 6.5 Hz), 2H], 3.19 (t, J_1 = 7.8 Hz, J_2 = 7.0.0 Hz, 2H), 6.59 (s, 1H), 7.41 (m, 1H), 7.56 (m, 1H), 7.87 (d, J = 8.3 Hz, 1H), 8.13 (d, J = 8.3 Hz, 1H), 10.48 (s, 1H).

1,2-Dihydro-3*H*-6-methoxynaphtho[2,1-*b*]pyran-3-one (31). Compound **30** was methylated in 30% yield under the conditions used to synthesize **25**. Compound **31**: R_f = 0.85 (20:1 CH_2Cl_2:acetone). ^{1}H NMR ($CDCl_3$): [2.90 (d, J = 7.3 Hz), 2.93 (d, J = 6.2 Hz), 2H)] 3.27 (t, J_1 = 7.0, J_2 = 7.8 Hz, 2H), 3.99 (s, 3H), 6.59 (s, 1H), 7.45 (m, 1H), 7.58 (m, 1H), 7.81 (d, J = 7.3 Hz, 1H), 8.26 (d, J = 7 Hz).

1,2-Dihydro-3*H*-7-hydroxynaphtho[2,1-*b*]pyran-3-one (32). Friedel−Crafts alkylation of 1,6-dihydroxynaphthalene (24 h of reflux) afforded **32** (20%); although all reagents were in solution at the beginning of the reaction, an unidentified precipitate formed during the 24 h reflux. R_f = 0.35 (20:1 CH_2Cl_2:acetone). ^{1}H NMR (acetone-d_6): 2.86−2.91 (m, 2H), 3.11−3.16 (m, 2H), 7.19−7.22 (m, 2H), 7.28 (d, J = 8.6 Hz, 1H), 7.45 (d, J = 8.3 Hz, 1H), 8.01 (d, J = 9.6 Hz, 1H), 8.76 (s, 1H).

1,2-Dihydro-3*H*-7-hydroxymethylnaphtho[2,1-*b*]pyran-3-one (33). Compound **32** was methylated in 40% yield under the conditions used to synthesize **25**. Compound **33**: R_f = 0.85 (20:1 CH_2Cl_2:acetone). ^{1}H NMR ($CDCl_3$): [2.87 (d, J = 7.1 Hz), 2.90 (d, J = 5.4 Hz), 2H], 3.11 (t, J_1 = 7.8 Hz, J_2 = 5.5 Hz, 2H), 3.93 (s, 3H), 7.11−7.24 (m, 3H), 7.48 (d, J = 8.1 Hz, 1H), 8.13 (d, J = 8.5 Hz, 1H).

1,2-Dihydro-3*H*-8-hydroxynaphtho[2,1-*b*]pyran-3-one (34) and 1,2,7,8-Tetrahydro-3*H*,9*H*-naphtho[2,1-*b*:6,5-*b*′]-dipyran-3,9-dione (36). Friedel−Crafts alkylation of 2,6-dihydroxynaphthalene afforded **34** and **36** in 50 and 4% yield, respectively, after chromatographic purification of the products (CH_2Cl_2/acetone 20:1 to 10:1). Compound **34**: R_f = 0.33 (CH_2Cl_2/acetone 20:1). ^{1}H NMR ($CDCl_3$/DMSO-d_6; referenced to DMSO-d_6): [2.85 (d, J = 7.0 Hz), 2.87 (d, J = 7.5 Hz), 2H], [3.28 (t, J_1 = 7.8 Hz, J_2 = 6.8 Hz, 2H) overlapping with water signal], [7.09 to 7.16 (m, 3H); overlapping with residual $CHCl_3$ signal], 7.59 (d, J = 8.8 Hz, 1H), 7.81 (d, J = 9.3 Hz), 9.66 (s, 1H). Compound **36.** R_f = 0.85 (CH_2Cl_2/acetone 20:1). ^{1}H NMR ($CDCl_3$): [2.91 (d, J = 7.0 Hz), 2.93 (d, J = 7.5 Hz), 2H], 3.37 (t, J_1 = 7.0 Hz, J_2 = 7.8 Hz, 2H), 7.31 (d, J = 9.3 Hz, 2H), 7.83 (d, J = 9.2 Hz, 2H).

1,2-Dihydro-3*H*-8-methoxynaphtho[2,1-*b*]pyran-3-one (35). Friedel−Crafts alkylation using 6-methoxy-2-naphthol yielded **35** in 63% yield. R_f = 0.73 (CH_2Cl_2:acetone 10:1). ^{1}H NMR ($CDCl_3$): [2.97 (d, J = 7 Hz), 2.99 (d, J = 6.2), 2H], 3.41 (t, J_1 = 6.7 Hz, J_2 = 7.8 Hz, 2H), 4.00 (s, 3H), 7.23 (d, J = 2.6 Hz, 1H), 7.27 (d, J = 9.1 Hz, 1H), 7.31 (dd, J_1 = 8.8 Hz, J_2 = 2.8 Hz, 1H), 7.73 (d, J = 8.8 Hz, 1H), 7.75 (d, J = 9.1 Hz, 1H).

1,2-Dihydro-3*H*-9-hydroxynaphtho[2,1-*b*]pyran-3-one (37) and 1,2,11,12-Tetrahydro-3*H*,10*H*-naphtho[2,1-*b*:7,8-*b*′]dipyran-3,10-dione (39). Friedel−Crafts alkylation (8 h of reflux) using 2,7-dihydroxynaphthalene afforded **37** (69%) and **39** (11%) after chromatographic purification (CH_2Cl_2/acetone 10:1). Compound **37**: R_f = 0.4 (CH_2Cl_2:acetone 10:1). ^{1}H NMR (DMSO-d_6): 2.87 (d, J = 7.2 Hz, 1H), 2.90 (d, J = 6.2 Hz, 1H), 3.19 (t, J_1 = 7.0 Hz, J_2 = 7.8 Hz, 2H), 6.99 (d, J = 8.8 Hz, 1H), 7.03 (dd, J_1 = 8.8 Hz, J_2 = 2.3 Hz, 1H), 7.14 (d,

J = 2.3 Hz, 1H), 7.71 (d, J = 8.8 Hz, 1H), 7.76 (d, J = 8.8 Hz, 1H), 9.89 (s, 1H). Compound **39.** R_f = 0.75 (CH_2Cl_2:acetone 10:1). ^{1}H NMR ($CDCl_3$): [2.74 (d, J = 6.0 Hz), 2.77 (d, J = 7.2 Hz), 2H], 3.50 (t, J_1 = 7.0 Hz, J_2 = 7.8 Hz, 2H), 7.22 (d, J = 8.8, 2H), 7.74 (d, J = 8.8 Hz, 2H).

1,2-Dihydro-3*H*-9-methoxynaphtho[2,1-*b*]pyran-3-one (38). Friedel–Crafts alkylation with 7-methoxy-2-naphthol yielded **38** (51%); a trace of **39** (8% yield) was also isolated. Compound **38**: R_f = 0.55 (EtOAc:hexanes 1:1). ^{1}H NMR ($CDCl_3$): [3.05 (d, J = 7.0 Hz), 3.08 (d, J = 6.2 Hz), 2H], 3.45 (t, J_1 = 7.0 Hz, J_2 = 7.8 Hz, 2H), 4.08 (s, 3H), 7.23 (d, J = 8.8 Hz, 1H), [overlapping signals 7.27 (dd, J_1 = 9.67 Hz, J_2 = 2.3 Hz, 1H), 7.26 (d,1H)], 7.82 (d, J = 8.8 Hz, 1H), 7.88 (d, J = 9.6 Hz, 1H).

3,4-Dihydro-2*H*-7-hydroxynaphtho[1,2-*b*]pyran-3-one (40). Friedel–Crafts alkylation of 1,5-dihydroxy naphthalene afforded **40** in 10% yield after refluxing for 24 h. R_f = 0.5 (CH_2Cl_2:acetone 10:1). ^{1}H NMR (acetone-d_6): 2.78–2.86 (m, 2H), 3.08–3.15 (m, 2H), 6.86 (d of d, J_1 = 7.7 Hz, J_2 = 0.9 Hz, 1H), 7.22–7.32 (m, 2H), 7.53 (d, J = 8.6 Hz, 1H), 7.90 (d, J = 8.6 Hz, 1H), 9.07 (s, 1H).

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Supporting Information Available: Experimental details describing the conversion of **13** to **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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