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Synthetic Silvestrol Analogues as Potent and Selective Protein Synthesis Inhibitors

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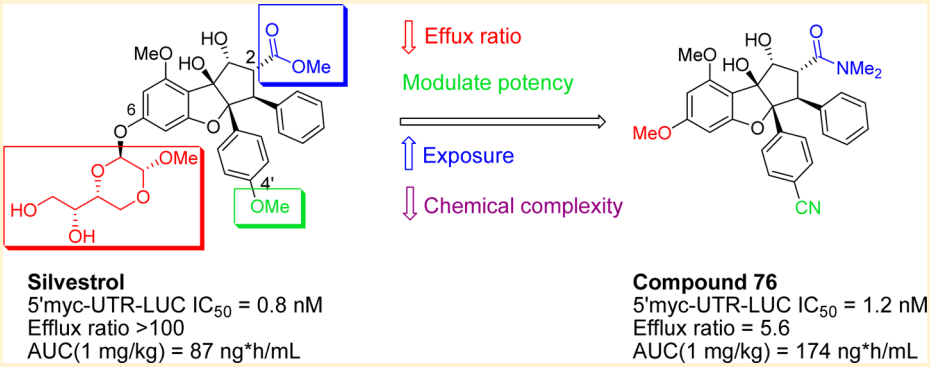
Supporting Information



ABSTRACT: Misregulation of protein translation plays a critical role in human cancer pathogenesis at many levels. Silvestrol, a cyclopenta[*b*]benzofuran natural product, blocks translation at the initiation step by interfering with assembly of the eIF4F translation complex. Silvestrol has a complex chemical structure whose functional group requirements have not been systematically investigated. Moreover, silvestrol has limited development potential due to poor druglike properties. Herein, we sought to develop a practical synthesis of key intermediates of silvestrol and explore structure−activity relationships around the C6 position. The ability of silvestrol and analogues to selectively inhibit the translation of proteins with high requirement on the translation−initiation machinery (i.e., complex 5′-untranslated region UTR) relative to simple 5′UTR was determined by a cellular reporter assay. Simplified analogues of silvestrol such as compounds **74** and **76** were shown to have similar cytotoxic potency and better ADME characteristics relative to those of silvestrol.

INTRODUCTION

Misregulation of protein translation plays a critical role in human cancer pathogenesis at many levels.[1,2] Aberrant activation of oncogenic pathways such as PI3K/Akt/mTOR and Ras/Raf/MEK enhance the availability and/or the activity of translation initiation factors, increasing the efficiency of protein synthesis.[3] Among all initiation factors, eIF4E has been the most studied for its role in cancer in the initiation and progression of the disease.[4,5] Overexpression of eIF4E is commonly observed in human cancer.[6] In experiments with tumor cells, overexpression of eIF4E results in rapid cell proliferation, suppression of apoptosis, and malignant transformation.[7] These phenotypic changes are caused by enhancement of translation of mRNAs that code for proteins involved in cell survival and growth (c-myc, cyclin D1), angiogenesis (VEGF, FGF2), and invasion and metastasis (MMP-9, heparanase). The expression and phosphorylation state of eIF4E's natural repressor 4E-BP play a major role in integrating oncogenic pathways and translation initiation.[8,9] While the role of eIF4G and eIF4A in cancer has not been studied extensively, the tumor suppressor programmed cell death protein 4 (PDCD4) binds eIF4A and blocks protein translation initiation.[10] Loss or reduced expression of PDCD4 has been implicated in the growth of human cancers.[11]

In addition to the tight control exerted on the translation machinery components, the efficiency of protein synthesis can also be regulated at the mRNA level. The degree of complexity of 5′-untranslated region (5′-UTR) of the mRNA correlates with the requirement for helicase activity of eIF4A.[12] Long and structured 5′UTRs are typically stable secondary structures that impede the ribosomal scanning process. It is estimated that around 10% of all mRNAs contain atypically long and complex 5′-UTR. Several of these encode oncoproteins, growth factors, transcription factors, signal transduction proteins, and a variety of receptors.[13]

The involvement of translation initiation in the etiology of malignant diseases, as well as the differential translation

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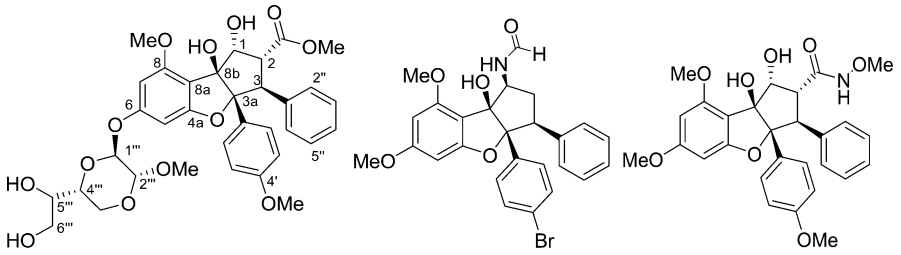


Figure 1. Structures of silvestrol (**1**) and synthetic flavagine analogues **2** and **3**.

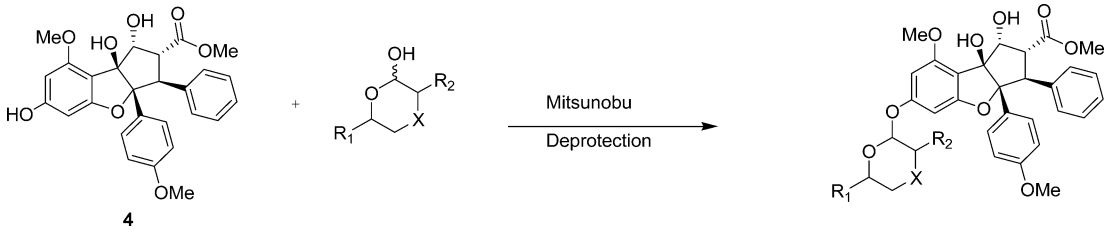
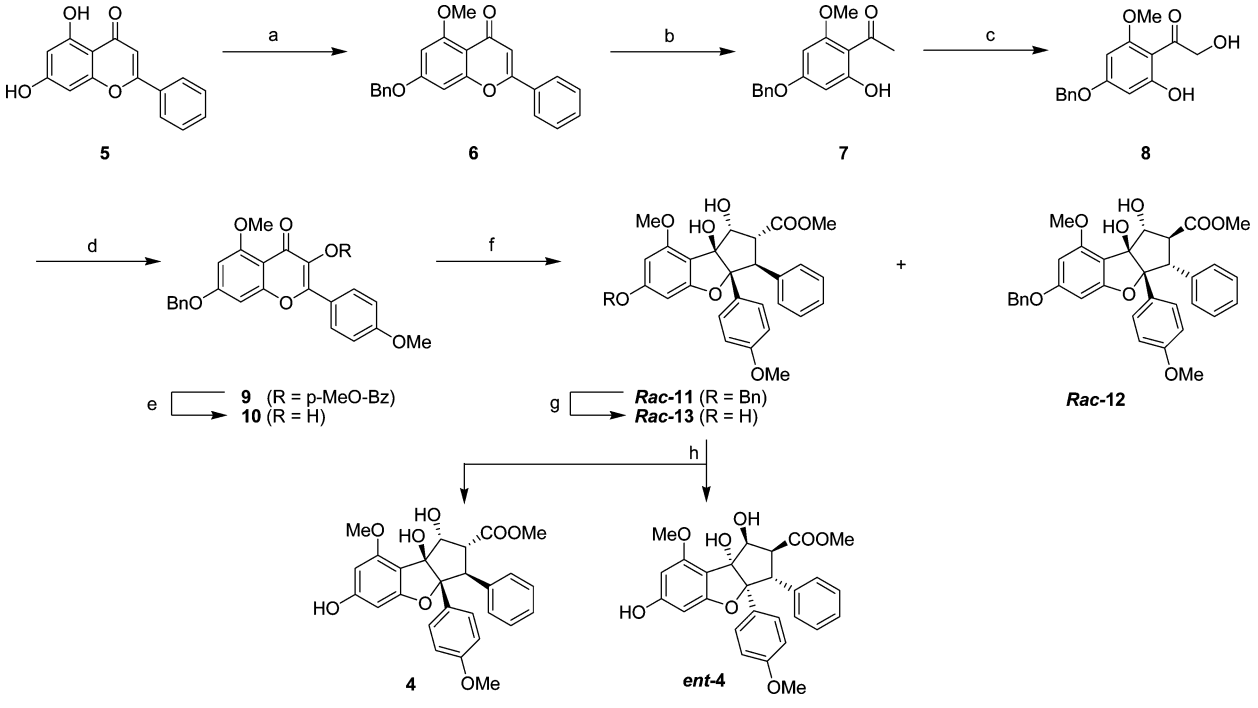


Figure 2. General synthetic approach to silvestrol analogues.

Scheme 1[a]



[a]Reagents and conditions: (a) (i) BnBr, K_2CO_3, acetone; (ii) Me_2SO_4; (b) KOH/pyridine, diethylene glycol; (c) (i) TBSOTf, Et_3N, CH_2Cl_2; (ii) *m*-CPBA, $NaHCO_3$, CH_2Cl_2; (iii) *p*-TSA, THF/H_2O; (d) (i) (*p*-OMe)BzCl, Et_3N, DMAP, CH_2Cl_2; (ii) LiHMDS, THF; (iii) H_2SO_4, AcOH; (e) NaOH, EtOH/water; (f) (i) methyl cinnamate, CH_2Cl_2/CH_3CN/MeOH, UV lamp; (ii) NaOMe/MeOH; (iii) $Me_4NBH(OAc)_3$, AcOH, CH_3CN; (g) H_2, Pd/C; (h) chiral supercritical fluid chromatography.

efficiency based on the mRNA secondary structure, provides a rationale to target protein synthesis for the treatment of proliferating diseases such as cancer.[14] Recently, small-molecule inhibitors of translation initiation with various modes of action have been identified.[15] Among them, plant-derived silvestrol (**1**, Figure 1) and structurally simplified flavaglines were shown to affect the composition of the eIF4F complex by acting on eIF4A, the ATP-dependent RNA-helicase subunit of the eIF4F complex.[16,17] Moreover, silvestrol has shown in vivo antitumor activity in a variety of murine models of solid tumors[17−19] and hematological cancers.[16,20,21]

Silvestrol, isolated from *Aglaia lepthanta*[19] or *Aglaia foveolata*[18] species, belongs to the family of cyclopenta[*b*]-benzofuran natural products such as rocaglates and rocaglamides. Naturally occurring cyclopenta[*b*]benzofurans from a variety of species (also known as flavaglines) display a wide range of biological effects.[22−24] Slightly over 60 cyclopenta[*b*]-benzofuran natural products have been isolated from different

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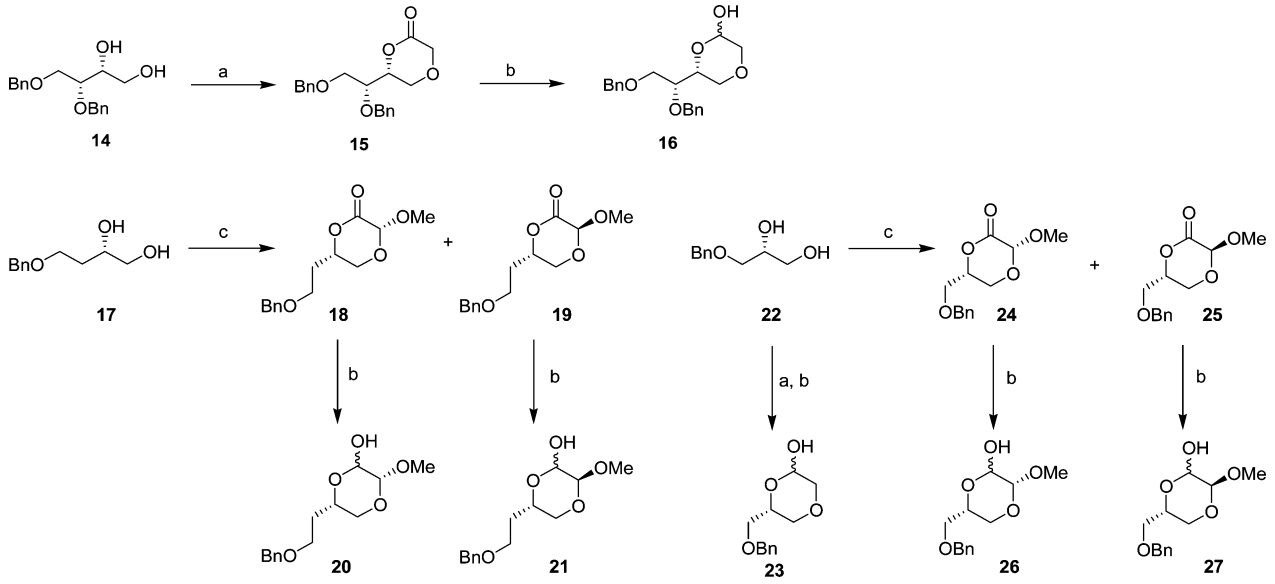
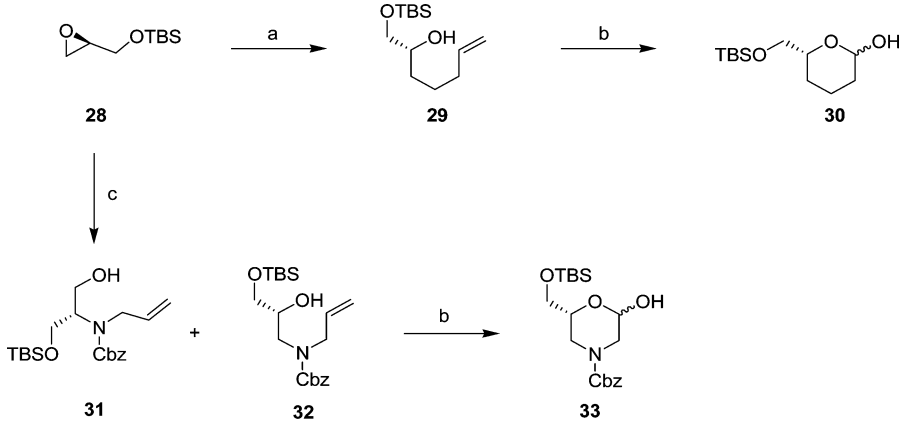
Scheme 2[a]



[a]Reagents and conditions: (a) Bu_2SnO, Bu_4NI, $BrCH_2COOEt$; (b) DIBALH, toluene; (c) Bu_2SnO, Bu_4NI, $BrCH(OMe)COOEt$.

Scheme 3[a]



[a]Reagents and conditions: (a) $BrMgCH_2CH_2CH{=}CH_2$, Li_2CuCl_4; (b) (i) O_3, $NaHCO_3$; (ii) PPh_3; (c) (i) $NH_2CH_2CH{=}CH_2$; (ii) Cbz-Cl.

Aglaia species, but silvestrol and a small number of related epimeric structures have a unique feature with the presence of a dioxanyloxy moiety at the C6 position.[23] While most cyclopenta[*b*]benzofuran rocaglate displays cytostatic effects on cells,[25,26] silvestrol and C4‴-episilvestrol are cytotoxic in their mode of action, suggesting the importance of the dioxanyloxy group for this effect.[19] Several studies with either synthetic analogues[27−30] or naturally occurring epimers of silvestrol[18,31] suggested a crucial role of stereochemistry at the C1‴ and C2‴ on the biological activity. While silvestrol's mode of action has been studied,[17,32,33] the nature of the interaction of the dioxanyloxy moiety with eIF4A, as well as its contribution to the cytotoxic action of this compound, is not well understood. This unusual chemical moiety contributes considerably to the synthetic complexity of the molecule[34,35] and negatively impacts druglike properties of silvestrol. Of note, Désaubry et al.[36] and Porco et al.[37] have recently unveiled flavagline compounds **2** and **3** (Figure 1) with similar potency relative to that of silvestrol, supporting the need for further understanding the structure−activity relationship of silvestrol.

We set out to generate selective oncoprotein translation inhibitors based on the silvestrol scaffold. Consequently, systematic exploration of structure−activity relationships around the dioxanyl moiety and the cyclopenta[*b*]benzofuran core was warranted to identify simpler and better analogues of silvestrol. Herein we report explorative studies that led to the discovery of rocaglate analogues with improved pharmaceutical properties and similar potencies relative to those of silvestrol.

■ CHEMISTRY

Similar to total syntheses of silvestrol described previously by Porco's group[27] and Rizzacasa's group,[28,29] a convergent approach was elaborated that features a late-stage glycosylation by a Mitsunobu reaction to assemble the flavagline and the dioxanyl moiety. This approach enabled access to a diverse set of silvestrol analogues by varying the dioxanyl moiety (Figure 2).

In order to support this late-stage diversification strategy, a robust, multigram synthesis of 6-phenolic flavagline **4** was implemented (Scheme 1). As pioneered by Porco's group,[27,38] hydroxyflavones such as **10** form oxidopyryliums upon UV light

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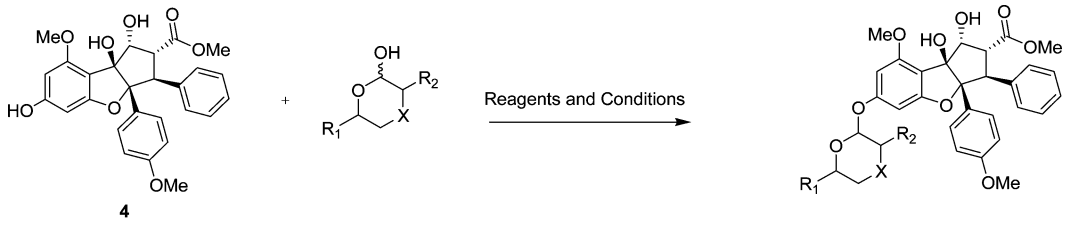
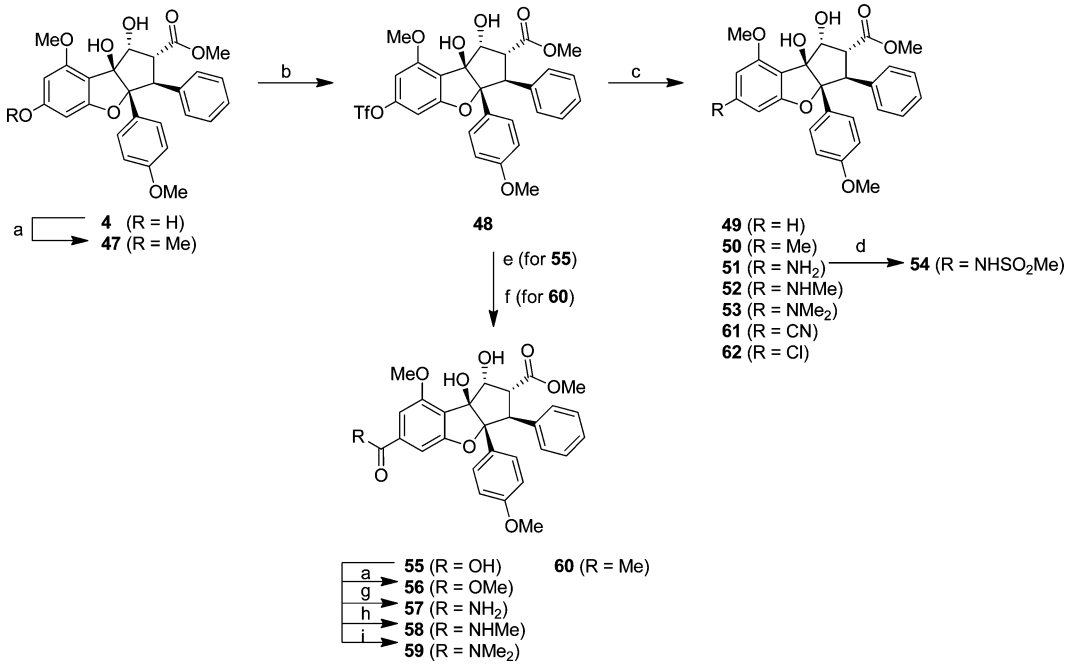
Scheme 4. Coupling of Flavagline 4 with Lactols[a]



Lactols	Reagents and Conditions	Final products
16	a, b	**34**
20	a, b	**35** and **36**
21	a, b	**37** and **38**
26	a, b	**39** and **40**
27	a, b	**41** and **42**
23	a, b	**43**
30	a	**44**
33	a, d, c	**45**
33	a, d, e, c	**46**

[a]Reagents and conditions: (a) DMEAD, PPh_3, toluene; (b) H_2, $Pd(OH)_2/C$; (c) TBAF, THF; (d) H_2, Pd/C; (e) formaldehyde, $NaBH(OAc)_3$, THF.

Scheme 5[a]



[a]Reagents and conditions: (a) $TMSCHN_2$, MeOH, toluene; (b) Tf_2O, *i*-Pr_2EtN, CH_2Cl_2. (c) For **49**: HCOOH, Et_3N, $Pd(OAc)_2$, PPh_3. For **50**: $Pd(OAc)_2$, Cs_2CO_3, $MeB(OH)_2$. For **51**: $Pd(OAc)_2$, $Ph_2C{=}NH$, then NH_2OH. For **52**: $Pd(OAc)_2$, P(*t*-Bu)$_3$, Cs_2CO_3, $MeNH_2$. For **53**: $Pd(OAc)_2$, P(*t*-Bu)$_3$, Cs_2CO_3, $HNMe_2$. For **61**: $Pd_2(dba)_3$, dppf, $Zn(CN)_2$. For **62**: $PdCl_2(dppf)_2$ bis(pinacolato)diboron, then $CuCl_2$; (d) BtMs, Et_3N, DMAP; (e) $Pd(OAc)_2$, dppf, CO; (f) $Pd(OAc)_2$, PPh_3, $Bu_3Sn((C{=}CH_2)OEt)$; (g) EDCI, HOBt, NH_4OAc; (h) EDCI, HOBt, $MeNH_2$; (i) EDCI, HOBt, $HNMe_2$.

activation and are very productive partners in [3 + 2] cycloaddition with dipolarophiles like cinnamates to give the flavagline cores. To access large quantities of hydroxyflavone **10**, we devised a synthetic route that could (1) offer flexibility in the substitution pattern at the C6 and C4′ of the flavagline, (2) generate the hydroxyflavone under mild conditions, and (3) give opportunities for crystalline intermediates. Accordingly, selective benzylation of chrysin **5** followed by methylation gave protected chrysin **6**. Base-mediated fragmentation of **6** yielded the acetophenone **7**, which was converted to the hydroxyketone

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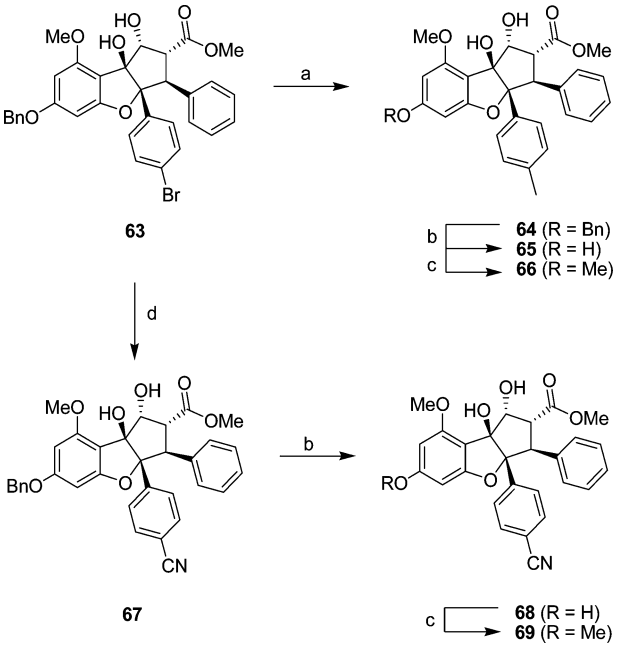
8 through a Rubottom oxidation.[39] Condensation of 8 with *p*-methoxybenzoyl chloride and Baker–Venkataraman rearrangement[40,41] gave intermediate 9, which generated the hydroxyflavone 10 upon basic treatment. Hydroxyflavone 10 was submitted to a known sequence of photocycloaddition, ketol rearrangement, and directed stereoselective reduction to give a separable diastereoisomeric mixture (~3/1) of flavagline *rac*-11 and *rac*-12. Notably, the throughput and robustness of the photoaddition reaction were increased by flow chemistry (see Experimental Section) in such a way that we could produce tens of grams of *rac*-11 from hydroxyflavone 10 in a few hours. With large quantities of this racemic mixture in hand, chiral supercritical fluid chromatography (SFC) performed on the phenol *rac*-13 gave multigram amounts of optically pure flavagline 4.

The syntheses of dioxanyl building blocks were performed by a tandem stannylene acetal alkylation–lactonization sequence[27] involving various chiral diols with α-bromoesters (Schemes 2 and 3). Glycosylation reactions with flavagline 4 were performed using a modified diazodicarboxylate[42] to directly give final compounds or access them through deprotection of penultimate intermediates (Scheme 4).

In addition to silvestrol analogues, flavagline 4 was used as a synthetic precursor to produce C6-modified rocaglate analogues (Scheme 5). The triflate 48 served as an intermediate in various Pd-mediated cross-coupling reactions (Suzuki, amination, and carbonylation) that provided access to C-or N-substituted analogues at the C6-position (49–62). Likewise, a small set of C4′-analogues (Scheme 6) were synthesized from 4′-bromo precursor 63 prepared according to a method similar to that for 4 (Supporting Information). Suzuki cross-coupling followed by a deprotection–protection sequence yielded the 4′-methyl analogue 66. Palladium-mediated cyanation provided intermediate 67 which was converted to the 6-methyl ether analogue 69.

Scheme 6[a]



[a]Reagents and conditions: (a) $Pd(OAc)_2$, Cs_2CO_3, $MeB(OH)_2$; (b) H_2, Pd/C; (c) $TMSCHN_2$, MeOH, toluene; (d) $Pd_2(dba)_3$, dppf, $Zn(CN)_2$.

RESULTS AND DISCUSSION

Identification of Simplified Silvestrol Analogues. Silvestrol inhibits protein translation initiation by targeting the ATP-dependent RNA helicase eIF4A in the eIF4F complex.[17] This helicase unwinds the secondary structure of the mRNA and facilitates scanning of the 43S preinitiation complex through the 5′ untranslated region (5′UTR) for the AUG start codon. As a consequence, the translation of mRNAs flanked with long and highly structured 5′UTR should be more sensitive to silvestrol relative to mRNAs with short and unstructured 5′UTRs. To evaluate the potency of our new silvestrol analogues as inhibitors of protein translation initiation, two reporter assays were developed in human MDA-MB-231 breast cancer cells (Figure 3). These assays take advantage of the differential translation of luciferase reporters flanked by the highly structured 5′UTR of c-myc (myc-LUC) versus the short 5′UTR of tubulin (tub-LUC). The inhibitory effect on luciferase reporter protein translation was measured after a 24 h treatment with compounds, whereas proliferation of MDA-MB-231 cells was measured with the MTS assay after 72 h. Generally, synthetic analogues of silvestrol and rocaglates were shown to be selective inhibitors of complex mRNA protein translation with tub-LUC/myc-LUC EC_{50} ratios ranging from 5 to 50. In contrast and as expected, a ratio of 1 was consistently observed with the translational elongation inhibitor cycloheximide (Figure 3) or the polypeptide chain initiation inhibitor verrucarin A (data not shown). Data for silvestrol analogues and rocaglate analogues are summarized in Tables 1 and 2, respectively. A good correlation was observed between translation inhibition and growth inhibition, together suggesting that the observed effect on cells is due to inhibition of protein translation initiation. Interestingly, flavagline 4 retains potency despite the absence of the dioxanyl moiety, albeit approximately 10-fold weaker than silvestrol.

A convergent synthetic approach allowed us to systematically probe the importance of functional groups and stereochemistry of the dioxanyl moiety on the potency of silvestrol analogues. Naturally occurring 2‴-episilvestrol was reported to be significantly less cytotoxic than silvestrol (>3000-fold) on the HT-29 human colon cancer cell line.[31] Likewise, synthetic 2‴,5‴-diepisilvestrol was also shown to be considerably less active than the corresponding 5‴-episilvestrol.[30] Consequently, it was anticipated that altering this portion of the molecule would have a dramatic effect on activity. However, deletion of the 2‴-MeO group does not have merely the same influence as the inversion of configuration, since 34 was only ~3-fold less potent than 1. Presumably, changing the 2‴-MeO group from an axial to equatorial configuration may either influence the overall conformational equilibrium of the dioxanyl fragment (Figure S1, Supporting Information)[43] or bring significant bulk near the flavagline portion, thereby disrupting binding to eIF4A. Deletion of the 5‴-hydroxy group (35) or 5‴-hydroxymethyl group (39) or deletion of the 2‴-MeO group (43) has little influence on activity. The latter observation correlates well with the reported data on 5‴-episilvestrol, which is essentially equipotent to silvestrol. However, inversion configuration at the 1‴ and/or 2‴ position on these latter compounds resulted in significant loss of activity (36–38 and 40–42), which is also consistent with the previous report on

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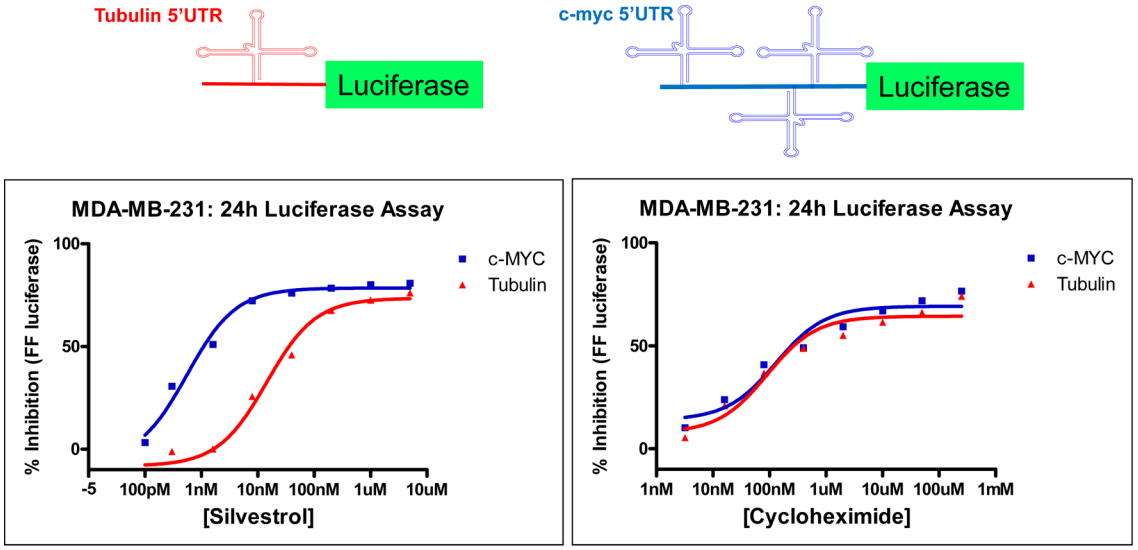
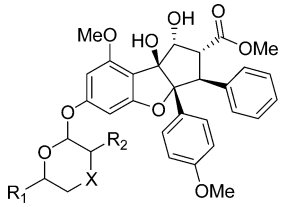


Figure 3. Differential translation reporter assay in intact cells. MBA-MB-231 cell lines were stably transfected by luciferase reporters flanked by either the highly structured 5′UTR of c-myc (myc-LUC) or the short 5′UTR of tubulin (tub-LUC). Silvestrol potently inhibits the translation of luciferase in myc-LUC cells relative to tub-LUC cells. In contrast, cycloheximide does not differentiate between the two assay mRNA constructs.

Table 1. Translation Initiation Inhibitory and Antiproliferative Effects of Silvestrol Analogues



compd	R_1	R_2	X	1‴	myc-LUC EC_{50}, nM (*N*)	tub-LUC EC_{50}, nM (*N*)	tub-LUC/myc-LUC ratio	growth inh EC_{50}, nM (*N*)
1	$HOCH_2$(*R*-CHOH)(*R*-CH)-	(*R*)-OMe	O	*S*	0.8 ± 0.5 (19)	7 ± 7 (19)	~9	3 ± 2 (19)
4					10 ± 7 (11)	200 ± 100 (11)	~20	20 ± 10 (11)
34	$HOCH_2$(*R*-CHOH)(*R*-CH)-	H	O	*S*	3 ± 2 (3)	14 ± 5 (3)	~5	9 ± 5 (4)
35	$HOCH_2CH_2$(*S*-CH)-	(*R*)-OMe	O	*S*	1 ± 0.5 (3)	5 ± 5 (3)	~5	1.5 ± 0.5 (2)
36	$HOCH_2CH_2$(*S*-CH)-	(*R*)-OMe	O	*R*	>200 (2)	>3500 (2)	~18	>500 (2)
37	$HOCH_2CH_2$(*S*-CH)-	(*S*)-OMe	O	*S*	35 (1)	275 (1)	~8	70 (1)
38	$HOCH_2CH_2$(*S*-CH)-	(*S*)-OMe	O	*R*	120 ± 30 (2)	>2000 (2)	nd	300 ± 200 (2)
39	$HOCH_2$(*S*-CH)-	(*R*)-OMe	O	*S*	3 ± 2 (2)	60 ± 10 (2)	~20	15 ± 1 (2)
40	$HOCH_2$(*S*-CH)-	(*R*)-OMe	O	*R*	35 ± 25 (2)	240 ± 120 (2)	~7	60 ± 40 (2)
41	$HOCH_2$(*S*-CH)-	(*S*)-OMe	O	*S*	170 ± 150 (2)	>2500 (2)	nd	>100 (2)
42	$HOCH_2$(*S*-CH)-	(*S*)-OMe	O	*R*	>2000	>5000	nd	>5000
43	$HOCH_2$ (*S*-CH)-	H	O	*S*	3 ± 2 (4)	40 ± 20 (3)	~13	9 ± 5 (4)
44	$HOCH_2$ (*S*-CH)-	H	CH_2	*S*	50 ± 30 (4)	2000 ± 1000 (4)	~40	200 ± 100 (4)
45	$HOCH_2$(*S*-CH)-	H	NH	*S*	80 ± 40 (3)	3500 ± 1000 (3)	~43	350 ± 100 (4)
46	$HOCH_2$(*S*-CH)-	H	NMe	*S*	>5000	>5000	nd	>5000

2‴-episilvestrol. With compound **43** being a considerably simpler and potent starting point, we next probed the importance of the dioxanyl ring system. Replacement of dioxanyl by tetrahydropyranyl (**44**) or morpholine (**45**) reduced the potency by 15-fold and 25-fold, respectively, while the activity was abolished by N-methylation of the morpholine (**46**).

Our initial SAR study suggested that the structure of silvestrol can be simplified dramatically while maintaining very respectable potencies as protein translation inhibitors and antiproliferative agents. Indeed, an approximately 3-fold potency difference exists between flavagline **4** and dioxanyl-containing **5**. These data, together with the synthetic complexity and the pharmacological liabilities associated to the dioxanyl moiety, prompted us to expand our SAR at the C6-position with simpler functional groups. Data are summarized in Table 2. Generally, synthetic rocaglates were shown to be less potent than silvestrol with a few notable exceptions bearing electron-withdrawing group at the 6-position (**56**, COOMe; **60**, C(O)Me; **61**, CN; **62**, Cl). These encouraging data prompted us to explore the 4′-position, rationalizing that replacement of the methoxy with a different functional group could enhance potency and metabolic stability. In this regard, disclosure of compound **2** by Désaubry and co-workers[36,44] reinforced our hypothesis. Once again, the addition of the electron-withdrawing nitrile group (**69**) resulted

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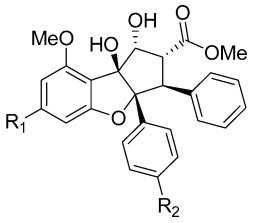
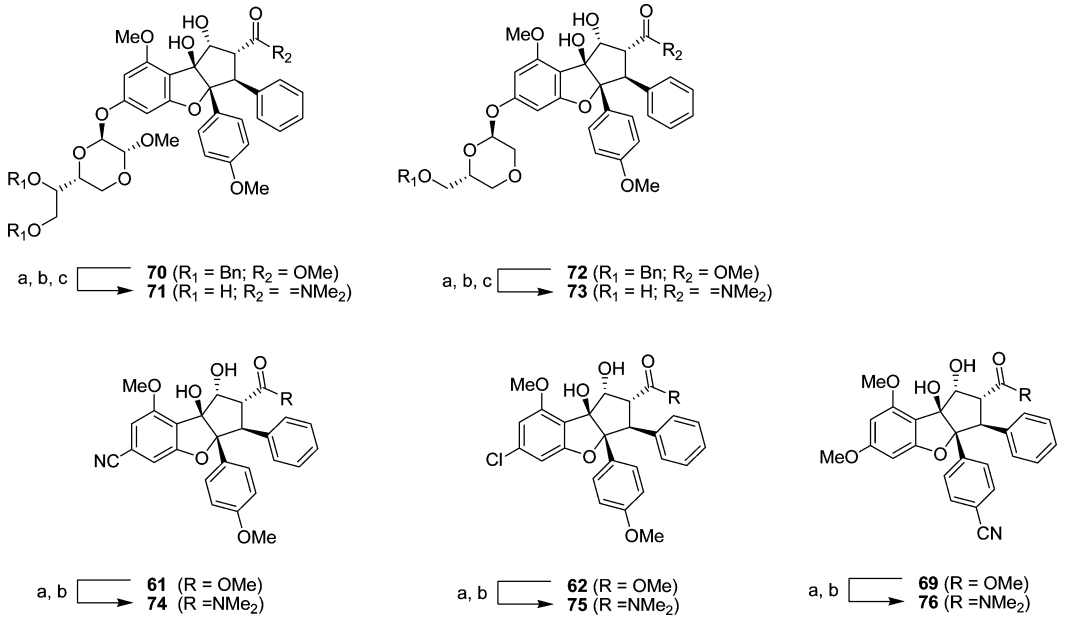
Table 2. Translation Initiation Inhibitory and Antiproliferative Effects of Rocaglate Analogues



compd	R_1	R_2	myc-LUC EC_{50}, nM (*N*)	tub-LUC EC_{50}, nM (*N*)	tub-LUC/myc-LUC ratio	growth inh EC_{50}, nM (*N*)
1	$HOCH_2$(*R*-CHOH)(*R*-CH)dioxanyloxy-	OMe	0.8 ± 0.5 (19)	7 ± 7 (19)	~9	3 ± 2 (19)
4	OH	OMe	10 ± 7 (11)	200 ± 100 (11)	~20	20 ± 10 (11)
47	OMe	OMe	10 ± 3 (6)	90 ± 40 (6)	~9	16 ± 9 (6)
49	H	OMe	>2000 (3)	>5000 (3)	nd	90 (1)
50	Me	OMe	>5000	>5000	nd	>5000
51	NH_2	OMe	40 ± 20 (5)	>2000	nd	50 ± 30 (5)
52	NHMe	OMe	11 ± 9 (4)	700 ± 300 (4)	~63	120 ± 60 (4)
53	NMe_2	OMe	>2000 (3)	>5000 (3)	nd	>4000 (1)
54	$NHSO_2Me$	OMe	>5000	>5000	nd	>5000
55	COOH	OMe	>1000	>5000	nd	>5000
56	COOMe	OMe	0.2 ± 0.1 (3)	4 ± 2 (3)	~20	0.3 ± 0.2 (3)
57	$CONH_2$	OMe	29 ± 2 (2)	450 ± 100 (2)	~16	80 ± 10 (2)
58	CONHMe	OMe	35 ± 5 (2)	>2000	nd	100 ± 50 (2)
59	$CONMe_2$	OMe	>5000	>5000	nd	>5000
60	C(O)Me	OMe	1 ± 0.5 (2)	30 ± 20 (2)	~30	1 ± 0.5 (2)
61	CN	OMe	0.9 ± 0.7 (7)	15 ± 10 (7)	~17	1.8 ± 0.9 (7)
62	Cl	OMe	4 ± 2 (4)	70 ± 50 (4)	~18	5 ± 2 (3)
66	OMe	Me	5 ± 2 (2)	>100	>20	32 ± 5 (2)
69	OMe	CN	2 ± 1 (2)	17 ± 10 (2)	~9	3 ± 1 (2)

Scheme 7. Synthesis of Dimethylamide Analogues[a]



[a]Reagents and conditions: (a) LiOH; (b) EDCI, HOBt, $HNMe_2$; (c) H_2, $Pd(OH)_2$, THF.

in a notable increase in potency, whereas the 4′-methyl analogue **66** showed similar potency as the 4′-OMe (**47**). Among the rocaglate analogues, 6-cyano **61**, 6-chloro **62**, and 4′-cyano **69** analogues were chosen for further evaluation alongside the simplified dioxanyl-containing silvestrol analogue **43**, since they had potencies very similar to that of silvestrol.

Optimization of Simplified Silvestrol Analogues. In addition to its structural complexity, druglike properties of silvestrol are suboptimal. Silvestrol has poor metabolic stability, resulting in high plasma clearance in mice.[45] Interestingly, the hydrolysis product silvestric acid was found in equal amount as the parent in plasma, 1 h after intravenous administration. Moreover, silvestrol is a Pgp-substrate and expression of these

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Table 3. In Vitro Potency, Metabolic Stability, Permeability, and PK Properties of Optimized Silvestrol Analogues

				plasma stability		microsome stability		Caco-2			
compd	myc-LUC EC_{50}, nM (*N*)	tub-LUC EC_{50}, nM (*N*)	growth inh EC_{50}, nM (*N*)	M (min)[a]	H (min)[a]	M (min)[a]	H (min)[a]	A–B permeability[d]	efflux ratio	$t_{1/2}$ (h)	AUC_{last} (ng·h/mL)
1	0.8 ± 0.5 (19)	7 ± 7 (19)	3 ± 2 (19)	160	>240	80	97	0.05	>100	0.7	87[b]
71	3 ± 1 (6)	19 ± 7 (6)	3 ± 1 (6)	>240	>240	>180	160	ND[e]	>100	1.3	255[b]
43	3 ± 2 (4)	40 ± 20 (3)	9 ± 5 (4)	48	>240	>180	>180	0.26	62		
73	3 ± 1 (2)	23 ± 3 (2)	5	>240	>240	>180	>180	ND[e]	>100		
61	0.9 ± 0.7 (7)	15 ± 10 (7)	1.8 ± 0.9 (7)	92	>240	75	32	2.8	2.3		
74	0.8 ± 0.2 (2)	35 ± 15 (2)	2					3.5	7.3	2	184
62	4 ± 2 (4)	70 ± 50 (4)	5 ± 2 (3)					1.8	1.4		
75	0.6 ± 0.4 (2)	15 ± 10 (2)	0.4					15.5	2.0	0.7	108
69	2 ± 1 (2)	17 ± 10 (2)	3 ± 1 (2)					5.2	1.7		
76	1.2 ± 0.2 (2)	40 ± 10 (2)	7					2.9	5.6	1.2	174[c]

[a]M: mouse. H: human. [b]Dose is 1 mg/kg, iv. [c]Dose is 1 mg/kg, ip. [d]Permeability × 10^{-6} cm/s [e]ND: not detected, very low permeability.

transporters confers resistance to this agent.[46] Consequently, we aimed at improving druglike properties and drug delivery to the target by reducing potential metabolic sites and reducing its interaction with P-glycoprotein.

The synthesis of dimethylamide derivatives of the most promising analogues (**1**, **43**, **61**, **62**, and **69**) was performed to modulate hydrolysis in vivo (Scheme 7). Generally, this transformation resulted in a marginal decrease in potency and an improvement in metabolic stability (Table 3, **1** vs **71**; **43** vs **73**). While rocaglate analogues have similar PK characteristics relative to silvestrol analogues, removal of the dioxanyl moiety dramatically reduces their interaction with Pgp. The cellular efflux ratio as measured by the bidirectional transport from basolateral to apical (B/A) divided by apical to basolateral (A/B) in Caco-2 cell monolayers was consistently higher with silvestrol analogues relative to the rocaglate analogues (Table 3). An efflux ratio greater than 2 indicates the compound is a Pgp substrate. In addition, the A to B permeability was very low with silvestrol analogues (**1** and **43**) and low to moderate with rocaglate analogues (e.g., **75**). Finally, concentration–time PK profiles in mice were similar for all these compounds after intravenous administration, with newer compounds having slightly better plasma exposure than silvestrol.

Pharmacology and Tolerability. Having potent silvestrol analogues with acceptable pharmacokinetic profile, we next evaluated whether these compounds would demonstrate antiproliferative activity in vivo. After screening of a large panel of cell lines, the human pancreatic cancer cell line L3.6pl and human myeloma cell line RPMI-8226 were found to be very sensitive to silvestrol (Figure S2, Supporting Information). Notably, loss of viability was observed by 7-AAD exclusion in MDA-MB-231, L3.6pl, and RPMI-8226 cells tested with silvestrol and **76**. Similar to MDA-MB-231,[17] treatment of these cell lines with silvestrol in culture rapidly reduces the protein levels of c-myc, while the levels of the housekeeping protein β-actin remain unchanged (Figure 4). This is consistent with our reporter assay and could provide a pharmacodynamic measurement of target modulation *in vivo*. For these reasons, L3.6pl and RPMI-8226 subcutaneous xenograft models were selected for the evaluation of silvestrol and various analogues in vivo .

In order to inform dose selection for the efficacy studies, tolerability to silvestrol treatment was assessed in L3.6pl-bearing BALB/c nude mice. In this strain, daily intraperitoneal (ip) dosing (q.d.) of 1 mg/kg silvestrol (formulated in 5.2% PEG-400, 5.2% Tween 80, 2% DMSO) was tolerated over 5

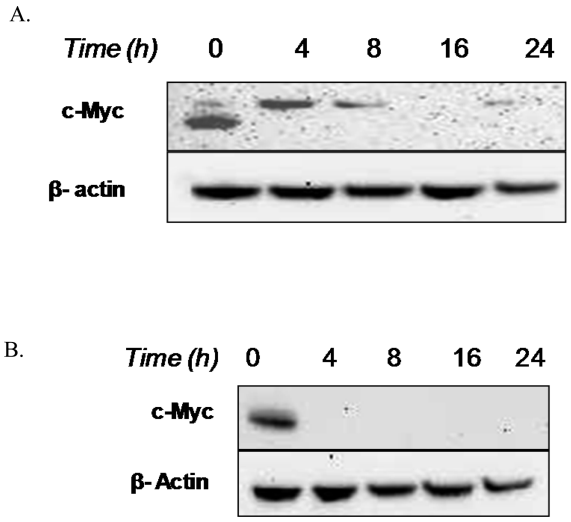

Figure 4. Inhibition of c-myc protein levels in pancreatic L3.6pl cells (A) and myeloma RMPI-8226 cells (B) in culture after treatment with silvestrol (100 nM).

days. Likewise, a larger bolus of 2.5 mg/kg (ip) could be administered on a twice-a-week (b.i.w.) regimen for the same period of time. Conversely, tolerability to silvestrol treatment in NOD/SCID mice (used for RPMI-8226 xenografts) was lower and the maximum tolerated dose was determined at 0.2 mg/kg q.d. × 5 days (ip) in this strain.

Although silvestrol is very potent (cytotoxicity $LC_{50} \approx 4$ nM; CellTiter-Glo) in L3.6pl cells in culture, it failed to demonstrate any tumor growth delay in L3.6 tumor-bearing mice when administered at the maximum tolerated dose. No difference was observed between the vehicle and the treatment arms regardless of dosing regimens (1 mg/kg q.d. × 5 days × 2 weeks or 2.5 mg/kg b.i.w. × 2 weeks). In a PK–PD experiment performed in L3.6 tumor-bearing mice treated with silvestrol (1 mg/kg, ip), total drug concentrations were measured at 100 and 15 ng of silvestrol per gram of tumor tissues after 6 and 24 h postdose, but no modulation of c-myc level was observed in the tumor at these time-points. Furthermore, despite the fact that silvestrol and analogues **74**–**76** were extremely cytotoxic ($LC_{50} \approx 0.06$ nM; CellTiter-Glo) to RMPI-8226 cells in culture, no effect was observed on the growth of RMPI-8226 xenografts in NOD/SCID mice after daily intraperitoneal administration of silvestrol or rocaglate compound **76** when dosed at their maximum tolerated doses (0.2 mg/kg) for 3 weeks (q.d. × 5

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days × 3 weeks). Dose-limiting toxicities led to insufficient target coverage, as little to no modulation of the c-myc protein levels were observed in the RPMI-8226 tumors (not shown). In an attempt to understand whether this narrow therapeutic window was compound- or target-related, C1‴-episilvestrol was used as a probe. This analogue, isolated as a side product of silvestrol synthesis, was inactive in the translation reporter assays and had similar PK profile as silvestrol. Unlike silvestrol, C1‴-episilvestrol was well tolerated in tumor-bearing mice (5 mg/kg ip, q.d. × 5) with no change in body weight. This result suggests that the toxicity of silvestrol is related to the inhibition of translation in vivo, albeit different off-target or metabolite profiles for these compounds cannot be totally ruled out. Nevertheless, our studies show that the therapeutic window of silvestrol and analogues that inhibit translation is too narrow to warrant further optimization without a better understanding of silvestrol pharmacology.

CONCLUSION

We postulated that translation initiation factors could be good targets for cancer therapy, since the protein translation of many oncogenes is dependent on these factors in cells. In vitro studies with compounds such as the natural product silvestrol indicate that it may be feasible to inhibit such factors. However, the druglike properties of silvestrol are suboptimal, limiting the ability to evaluate its therapeutic potential. In addition, sources of silvestrol are not readily available, which further complicates comprehensive preclinical studies. In this study, efficient and versatile synthetic routes were devised to access simplified, potent, and selective protein translation inhibitors based on the silvestrol scaffold. As an example, compound **76** displays very similar in vitro potency and PK characteristics compared to silvestrol. However, simplified analogues exemplified by compound **76** also show a similar toxicity profile despite the absence of the dioxanyl moiety of the molecule. Arguments for on-target based toxicity can be offered to explain the narrow therapeutic window that limits the single agent activity of silvestrol analogues. For all analogues tested in vitro, tub-LUC/myc-LUC IC_{50} ratios range from 5 to 50. It is noteworthy that c-myc and tubulin 5′UTR structures represent extreme cases with melting energy (ΔG) of approximately −164 and −5.5 kcal/mol, respectively.[47] Consequently, the translation of other proteins essential for cell viability could be affected at concentrations and amplitudes similar to those of highly structured 5′UTR-containing oncoproteins. Moreover, the lack of toxicity observed with the inactive C1‴-episilvestrol provides additional evidence that the toxicity could be related to the inhibition of translation in vivo. We considered whether target modulation by silvestrol within the tumor was insufficient to provide a therapeutic benefit. While silvestrol produces a toxic effect in normal cells, we speculated that low permeability and/or Pgp mediated efflux[46] in tumor cells may result in intracellular drug concentrations too low to achieve target modulation and reduction of tumor growth. However, compound **76** did not perform better than silvestrol despite improved permeability and efflux ratio relative to silvestrol. Therefore, it seems unlikely that reduced permeability was a significant contributor to the lack of efficacy in the two models described.

It was originally thought that approaches to target eIF4A would result in broad-acting therapeutics due to the convergence of many oncogenic pathways that result in enhanced mRNA translation. The broad and potent activity of silvestrol in many cancer cell lines in culture seemed to indicate that this would indeed be the case. The lack of correlation between silvestrol sensitivity in vitro and in vivo is confounding. For instance, silvestrol was shown to significantly reduce the growth of prostate cancer PC-3 and breast cancer MDA-MB-231 cell lines grown as xenografts.[17,48] However, in culture, silvestrol had modest activity in MDA-MB-231 relative to L3.6pl and RPMI-8226 cells, which were chosen for in vivo evaluation in this study. It was expected that doses of the drugs required to affect growth of L3.6pl and RPMI-8226 tumors would be well tolerated. However, the growth of L3.6pl and RPMI-8226 tumors in vivo was unaffected by silvestrol treatment at the MTD in contrast to the MDA-MB-231 xenografts reported in the literature and reproduced in our laboratories.[48] It has been reported that silvestrol is ineffective as a single agent in PTEN$^{\pm}$Eμ-myc and Eμ-myc/eIF4E lymphoma models but acts as sensitizer to doxorubicin.[16] It is plausible that the degree of addiction to altered mRNA translation rates dictates the sensitivity of these tumor cells to silvestrol, and dose-limiting toxicities are often observed before these mechanisms can be affected to produce a therapeutic benefit.

Our results bring the drug potential of eIF4A inhibitors into question because of the narrow therapeutic window observed with silvestrol and compound **76**. In recent literature, a protein toxin from *Burkholderia pseudomallei* was found to inhibit the helicase activity of eIF4A and was lethal to BALB/c mice.[49] By contrast, some results with other alternative eIF4A inhibitors appear more encouraging. Hippuristanol, a polyoxygenated steroid with a similar mode of action as that of silvestrol, is highly effective and well-tolerated at 7.5 mg/kg q.d. × 28 days in a model of T-cell leukemia.[50]

Although more work is required to understand the optimal settings for the use of these translation inhibitors as single agents, or in combination with other agents, our results underscore the possibility of using these simplified silvestrol analogues to modulate the level of oncoproteins otherwise difficult to target with conventional therapies. Therefore, these compounds represent a unique class of agents with anticancer therapeutic potential worthy of further investigation.

EXPERIMENTAL SECTION

Chemical Synthesis. *General Methods.* Silvestrol was prepared from **4** using procedure described by Porco et al.[27] All physical data (Supporting Information) match an authentic sample (Prof. Mark A. Rizzacasa). Commercial reagents and solvents were used as received without further purification or drying. All experiments involving water-sensitive compounds were carried out under argon and scrupulously dry conditions, using commercially available anhydrous solvents. Thin-layer chromatography was performed on glass-backed precoated Merck silica gel (60 F254) plates, and compounds were visualized using UV light, ceric ammonium molybdate, or 2% aqueous potassium permanganate solution. Silica gel column chromatography was carried out using Merck silica gel 60. Flash chromatography was run using silica gel (200−400 mesh) from Sorbent Technologies. The purity of tested compounds was determined by analytical liquid chromatography performed by methods A, B, or C. Method A consisted of the following: Agilent 1100 HPLC system equipped with an XBridge C18 column (2.5 μm, 4.6 mm × 50 mm). The mobile phases were (A) 10 mM ammonium formate buffer, pH 3.8, and (B) 0.05% formic acid in CH_3CN. After injection the gradient holds were at A/B (25%/75%) for 2 min followed by a gradient to A/B (5%/95%) over 16 min, a 2 min organic flush at 5%/95% (A/B), and a 2 min re-equilibration at a flow rate of 1.0 mL/min, column temperature at 40 °C, and detection wavelength at 215 nm. Method B consisted of the following: Waters

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Alliance 2795XE LC with a Waters 2996 photodiode array detector equipped with a Waters Symmetry C18 column (3.5 μm, 2.1 mm × 50 mm). The mobile phases were (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. After injection the gradient holds were at A/B (75%/15%) for 0.5 min followed by a gradient to A/B (0%/100%) over 5 min, a 1 min flush at 0%/100% (A/B), and a 2 min re-equilibration at A/B (75%/15%) at a flow rate of 1.5 mL/min and column temperature at 40 °C. Method C consisted of the following: Waters Alliance 2795XE LC with a Waters 2996 photodiode array detector equipped with an Waters Symmetry C18 column (3.5 μm, 2.1 mm × 30 mm). The mobile phases were (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile. After injection the gradient change was from A/B (95%/5%) to A/B (0%/100%) over 3 min, with a 0.5 min flush at 0%/100% (A/B) and a 0.5 min re-equilibration at A/B (95%/5%) at a flow rate of 1.5 mL/min and column temperature at 40 °C. ^{1}H NMR spectra were recorded on a Bruker 400 spectrometer (400 MHz). Chemical shifts are reported in ppm with the solvent resonance as the internal standard ($CHCl_3$, δ 7.26). Data are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, dd = doublet of doublet, ddd = doublet of doublet of doublet, t = triplet, q = quartet, br = broad, m = multiplet), and coupling constants (Hz). ^{13}C NMR spectra were recorded on a Bruker 400 spectrometer (100 MHz) with complete proton decoupling. Chemical shifts are reported in ppm with the solvent as the internal reference ($CDCl_3$, δ 77.16). Electrospray positive ionization mass spectrometry (ESI-MS) was performed on samples using a reversed phase HPLC−MS system. The system was equipped with a diode array UV detector and a single quadrupole mass spectrometer.

Preparation of Compounds in Scheme 1. *7-(Benzyloxy)-5-methoxy-2-phenyl-4H-chromen-4-one (**6**).* A suspension of chrysin **5** (255 g, 1.0 mol, 1 equiv) in acetone (3 L) was treated with potassium carbonate (415 g, 3.0 mol, 3 equiv) and benzyl bromide (129 mL, 1.0 mol, 1 equiv). The mixture was heated to 60 °C and stirred for 3 h. The mixture was cooled to 40 °C, treated with dimethyl sulfate (287 mL, 3.0 mol, 3 equiv), and stirred for 3 days. The mixture was cooled to room temperature and the solid removed by filtration on a Buchner funnel. The cake was partitioned in dichloromethane/water and combined with the filtrate that was previously concentrated to dryness. The pH of the aqueous phase was adjusted to 2 with a 2 N aqueous hydrochloric acid solution, and the aqueous phase was extracted with dichloromethane. The solvent was removed under reduced pressure and coevaporated with ethyl acetate. The residue was suspended in ethyl acetate and heated to reflux for 1 h. The mixture was cooled to room temperature, filtered on a Buchner funnel and the solid washed with ethyl acetate. The solid was air-dried to constant weight to give the desired protected chrysin **6** (303 g, 0.85 mol, 84%). NMR δ_H (400 MHz, $CDCl_3$) 7.9−7.8 (m, 2H), 7.5−7.35 (m, 8H), 6.69 (s, 1H), 6.66 (d, J = 2.4 Hz, 1H), 6.47 (d, J = 2.4 Hz, 1H), 5.16 (s, 2H), 3.96 (s, 3H); NMR δ_C (100 MHz, $CDCl_3$) 177.59, 163.57, 161.36, 160.96, 159.92, 135.63, 131.51, 131.21, 129.01, 128.83, 128.53, 127.67, 126.12, 108.94, 108.43, 97.01, 93.85, 70.65, 56.49; ESI-MS m/z = 359.3 $[M + H]^+$.

*1-(4-(Benzyloxy)-2-hydroxy-6-methoxyphenyl)ethanone (**7**).* Protected chrysin **6** (150 g, 419 mmol, 1 equiv) was added to a mixture of 50% aqueous sodium hydroxide solution (1172 g, 14.6 mol, 35 equiv) and pyridine (677 mL, 8.3 mol, 20 equiv). The mixture was vigorously stirred and treated with diethylene glycol (800 mL, 8.3 mol, 20 equiv). The mixture was heated to 100 °C and stirred for 2 h. The mixture was cooled to <20 °C, and the pH was adjusted to 1 with a 12 N aqueous hydrochloric acid solution. The aqueous portion was extracted with ethyl acetate. The combined organic phase was washed with saturated aqueous sodium bicarbonate, dried with sodium sulfate, and the solvent was removed under reduced pressure. The residue was suspended in methanol and heated to reflux for 1 h. The solution was cooled to room temperature, filtered on a Buchner funnel, and the crystals were washed with cold methanol. The solid was air-dried to constant weight to give the desired acetophenone **7** (79 g, 290 mol, 70%). NMR δ_H (400 MHz, $CDCl_3$) 13.99 (s, 1H), 7.41−7.32 (m, 5H), 6.13 (d, J = 2.4 Hz, 1H), 5.99 (d, J = 2.4 Hz, 1H), 5.05 (s, 2H), 3.83 (s, 3H), 2.60 (s, 3H); NMR δ_C (100 MHz, $CDCl_3$) 203.19, 167.57, 165.21, 162.99, 135.97, 128.75, 128.37, 127.68, 106.15, 94.49, 91.27, 70.23, 55.58, 32.97; ESI-MS m/z = 273.3 $[M + H]^+$.

*1-(4-(Benzyloxy)-2-hydroxy-6-methoxyphenyl)-2-hydroxyethanone (**8**).* A solution of acetophenone **7** (53.5 g, 197 mmol, 1 equiv) in dichloromethane (500 mL) was cooled to 0 °C, treated with triethylamine (69 mL, 492 mmol, 2.5 equiv) and *tert*-butyldimethylsilyl trifluoromethanesulfonate (104 mL, 452 mmol, 2.3 equiv). The mixture was stirred at 0 °C for 1 h. The solution was charged with saturated aqueous sodium bicarbonate solution and warmed to room temperature. The organic phase was separated, dried with sodium sulfate and the solvent removed under reduced pressure. The residue was coevaporated with dichloromethane and used directly for the next step. The residue, dissolved in dichloromethane (200 mL), was added to a cooled (0 °C) mixture of anhydrous *m*-chloroperbenzoic acid (76 g, 310 mmol, 1.6 equiv) and sodium bicarbonate (40.7 g, 484 mmol, 2.5 equiv) in dichloromethane (800 mL). The mixture was warmed to room temperature and vigorously stirred for 4 h. The mixture was diluted with dichloromethane and washed successively with a saturated aqueous sodium bicarbonate solution and water. The solvent was removed under reduced pressure and the residue used directly for the next step. A suspension of crude product in tetrahydrofuran (1 L) and water (0.1 L) was treated with *p*-toluenesulfonic acid (3.7 g, 19 mmol, 0.1 equiv). The solution was heated to reflux and stirred for 6 h. The mixture was cooled to room temperature and partitioned between ethyl acetate and a saturated aqueous sodium bicarbonate solution. The organic phase was dried with sodium sulfate and the solvent removed under reduced pressure. The residue was suspended in ethanol and heated at reflux for 1 h. The solution was cooled to room temperature. The precipitated solid was filtered on a Buchner funnel and the solid washed with cold ethanol. The solid was air-dried to constant weight to give the desired hydroxyacetophenone **8** (36 g, 126 mmol, 65% over three steps). NMR δ_H (400 MHz, $CDCl_3$) 13.19 (s, 1H), 7.4−7.3 (m, 5H), 6.18 (d, J = 2.4 Hz, 1H), 6.00 (d, J = 2.4 Hz, 1H), 5.07 (s, 2H), 4.70 (s, 2H), 3.84 (s, 3H); NMR δ_C (100 MHz, $CDCl_3$) 167.19, 166.15, 163.21, 135.73, 128.78, 128.46, 127.68, 103.49, 94.73, 91.46, 70.41, 68.65, 55.71; ESI-MS m/z = 289.16 $[M + H]^+$.

*7-(Benzyloxy)-5-methoxy-2-(4-methoxyphenyl)-4-oxo-4H-chromen-3-yl 4-methoxybenzoate (**9**).* A solution of hydroxyacetophenone **8** (36.2 g, 126 mmol, 1 equiv) in dichloromethane (350 mL) was treated with dimethylaminopyridine (0.767 g, 6.3 mmol, 0.05 equiv) and triethylamine (52.5 mL, 377 mmol, 3 equiv). The mixture was cooled to 0 °C, treated with 4-methoxybenzoyl chloride (43.3 g, 254 mmol, 2 equiv), warmed to room temperature, and stirred for 3 h. The solution was quenched with 1 N aqueous hydrochloric acid, and the phases were separated. The aqueous phase was back-extracted with dichloromethane, and the combined organic phases were dried with sodium sulfate and concentrated under reduced pressure. The desired bisbenzoate residue was used directly for the next step. A solution of the crude bisbenzoate in tetrahydrofuran (700 mL) was cooled to −70 °C and was treated with a 1 M solution of lithium hexamethyldisilazane in tetrahydrofuran (377 mL, 377 mmol, 3 equiv). The mixture was stirred below −60 °C for 1 h and then warmed to −20 °C for 1 h. The solution was quenched with a saturated aqueous ammonium chloride solution and warmed to room temperature. The aqueous phase was extracted with ethyl acetate, and the combined organic phases were dried with sodium sulfate and concentrated under reduced pressure. The desired phenol residue was used directly for the next step. A suspension of crude phenol in acetic acid (1.5 L) was treated with sulfuric acid (33.5 mL, 628 mmol, 5 equiv) and stirred at room temperature for 20 h. The reaction mixture was poured over water/ice and stirred for 15 min. The mixture was filtered on a Buchner funnel and the cake washed with water. The wet cake was suspended in ethanol and heated to reflux for 1 h. The mixture was cooled to room temperature, filtered on a Buchner funnel, and washed with cold ethanol. The solid was air-dried to constant weight to give the desired protected 3-hydroxyflavone **9** (62.3 g, 116 mmol, 92% over three steps). NMR δ_H (400 MHz, $CDCl_3$) 8.17 (d, J = 9.2 Hz, 2H), 7.86 (d, J = 9.2 Hz, 2H), 7.41−7.32 (m, 5H), 6.96 (d, J = 9.2 Hz, 2H), 6.94 (d, J = 9.2 Hz, 2H), 6.63 (d, J = 2.4 Hz, 1H), 6.45 (d, J = 2.4 Hz,

1H), 5.16 (s, 2H), 3.91 (s, 3H), 3.89 (s, 3H), 3.83 (s, 3H); NMR δ_C (100 MHz, $CDCl_3$) 170.72, 163.96, 163.79, 163.31, 161.53, 161.18, 159.06, 153.25, 135.79, 133.88, 132.72, 129.67, 128.76, 128.41, 127.68, 122.28, 121.45, 114.07, 113.83, 108.95, 96.53, 93.59, 70.51, 56.27, 55.52, 55.37; ESI-MS m/z = 539.42 $[M + H]^+$.

*7-(Benzyloxy)-3-hydroxy-5-methoxy-2-(4-methoxyphenyl)-4H-chromen-4-one (**10**).* A suspension of benzoate **9** (31 g, 57.6 mmol, 1 equiv) in ethanol (715 mL) was treated with a 5% aqueous sodium hydroxide solution (86 g, 108 mmol, 1.875 equiv). The suspension was heated to 80 °C and stirred for 1 h. The reaction mixture was cooled to room temperature and treated with a 1 N aqueous hydrochloric acid solution (108 mL, 108 mmol, 1.875 equiv). The resulting suspension was filtered on a Buchner funnel and washed with ethanol. The solid was air-dried to constant weight to give the desired 3-hydroxyflavone **10** (22 g, 54.4 mmol, 95%). NMR δ_H (400 MHz, $CDCl_3$) 8.16 (d, J = 9.2 Hz, 2H), 7.5−7.35 (m, 5H), 7.02 (d, J = 9.2 Hz, 2H), 6.64 (d, J = 2.4 Hz, 1H), 6.44 (d, J = 2.4 Hz, 1H), 5.15 (s, 2H), 3.97 (s, 3H), 3.88 (s, 3H); NMR δ_C (100 MHz, $CDCl_3$) 171.92, 163.41, 160.70, 160.59, 158.79, 142.32, 137.52, 135.75, 128.95, 128.88, 128.58, 127.78, 123.61, 114.06, 106.40, 96.21, 93.38, 70.64, 56.47, 55.45; ESI-MS m/z = 405.31 $[M + H]^+$.

*(1,2,3,3a,8b)-Methyl 6-(Benzyloxy)-1,8b-dihydroxy-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]-cyclopenta[d]furan-2-carboxylate (rac-**11**).* A solution of 3-hydroxyflavone **10** (15.9 g, 39.3 mmol, 1 equiv) and methyl cinnamate (73.3 g, 452 mmol, 11.5 equiv) in dichloromethane (800 mL), acetonitrile (320 mL), and methanol (320 mL) was placed in a jacketed flask (temperature controlled) equipped with a recirculation pump. The reaction mixture was pumped (∼50−100 mL/min) through a coil wrapped around a jacketed glass cylinder containing a UV lamp. The jacket temperature was adjusted so that the internal reaction temperature was 0−5 °C while the UV light was turned on. The reaction mixture was irradiated for 10 h and then warmed to room temperature. The solvent was removed under reduced pressure and the residue purified using a plug of silica gel (EtOAc/hexanes) to remove excess cinnamate (low polarity). The remaining material was concentrated under reduced pressure and used directly for the next step. The crude residue was suspended in methanol (300 mL) and treated with a 25% methanolic sodium methoxide solution (22 g, 102 mmol, 2.6 equiv). The mixture was heated to 60 °C and stirred for 1 h. The mixture was cooled to room temperature, and approximately half of the methanol was removed under reduced pressure. The resulting mixture was filtered on the a Buchner funnel. The solids were washed with cold methanol and air-dried to constant weight. The cake was then partitioned between ethyl acetate and a saturated aqueous ammonium chloride solution. The organic phase was separated, dried with sodium sulfate, and concentrated under reduced pressure to give the desired keto ester in a 3/1 ratio along with the undesired regioisomer (8.46 g, 15.76 mmol, 40%). Additional material can be obtained by processing the mother liquor as follows: residue from concentrated mother liquor (∼9.5 g) was partitioned between ethyl acetate and a saturated aqueous ammonium chloride solution. The organic phase was separated, dried with sodium sulfate, and concentrated under reduced pressure. The residue was purified using silica gel chromatography (EtOAc/hexanes). The product containing fractions were pooled, concentrated, and crystallized from ethanol to give the additional desired keto ester as a single regioisomer (1.5 g, 2.65 mmol, 7%). A regioisomeric mixture (3/1) of keto ester (8.93 g, 15.76 mmol, 1 equiv) was charged to a solution of tetramethylammonium triacetoxyborohydride (24.9 g, 95 mmol, 6 equiv) in acetonitrile (300 mL) and acetic acid (9 mL, 158 mmol, 10 equiv). The resulting mixture was stirred for 48 h and then partitioned between a saturated aqueous sodium bicarbonate solution and dichloromethane. The organic layer was separated, dried over sodium sulfate, and concentrated. The residue was purified using silica gel chromatography (EtOAc/hexanes) to give the desired alcohol *rac*-**11** (6.4 g, 11.3 mmol, 72%) and the undesired regioisomer *rac*-**12** (2.1 g, 3.63 mmol, 23%). Data for *rac*-**11**: NMR δ_H (400 MHz, $CDCl_3$) 7.50−7.035 (m, 5H), 7.12−7.05 (m, 5H), 6.90−6.86 (m, 2H), 6.65 (d, J = 9 Hz, 2H), 6.37 (d, J = 2 Hz, 1H), 6.21 (d, J = 2 Hz, 1H), 5.08 (s, 2H), 5.03 (d, J = 7 Hz, 2H), 4.32 (d, J = 15 Hz, 1H), 3.90 (dd, J = 15, 7 Hz, 2H), 3.83 (s, 3H), 3.70 (br s, 1H) 3.66 (s, 3H), 3.65 (s, 3H); NMR δ_C (100 MHz, $CDCl_3$) 170.63, 163.25, 160.88, 158.76, 157.13, 137.08, 136.60, 129.05, 128.79, 128.27, 127.93, 127.80, 127.67, 126.64, 126.57, 112.79, 108.10, 101.94, 93.77, 93.44, 90.58, 79.66, 70.57, 55.84, 55.14, 55.08, 52.05, 50.57; ESI-MS m/z = 569.22 $[M + H]^+$.

*(1R,2R,3S,3aR,8bS)-Methyl 1,6,8b-Trihydroxy-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]-cyclopenta[d]furan-2-carboxylate (**4**).* A solution of benzyl ether *rac*-**11** (6.40 g, 11.26 mmol, 1 equiv) and 5% palladium on carbon (1.25 g, 20 wt %) in tetrahydrofuran (120 mL) was placed under hydrogen atmosphere and stirred for 18 h at room temperature. The reaction mixture was filtered on Celite and the filtrate concentrated to dryness to give the desired phenol *rac*-**13** (5.10 g, 10.66 mmol, 95%). This material was purified by chiral supercritical fluid chromatography (column, 3.0 cm × 25.0 cm ChiralPak AD-H (Chiral Technologies); isocratic CO_2/EtOH (35%) at 80 mL/min; 100 bar) to obtain enantiomerically enriched (>95% ee) phenol **4**. NMR δ_H (400 MHz, $CDCl_3$) 7.09−7.02 (m, 5H), 6.90−6.87 (m, 2H), 6.30 (d, J = 9 Hz, 2H), 6.17 (d, J = 1 Hz, 1H), 5.97 (s, 1H), 5.03 (d, J = 7 Hz, 1H), 4.30 (d, J = 14 Hz, 1H), 3.90 (dd, J = 14, 6 Hz, 1H), 3.76 (s, 3H), 3.65 (s, 3H), 3.64 (s, 3H), 3.20 (s, 1H); NMR δ_C (100 MHz, $CDCl_3$) 171.28, 160.88, 160.66, 158.76, 157.14, 136.89, 129.07, 128.06, 127.91, 126.78, 126.62, 112.87, 107.20, 101.79, 93.78, 93.18, 92.33, 79.30, 55.84, 55.22, 52.31, 50.59, 27.07; ESI-MS m/z = 479.19 $[M + H]^+$.

Preparation of Compounds in Scheme 4. *General Procedure for Coupling Lactols to Phenolic Intermediate of Rocaglate.* A solution of phenol (1 equiv), lactol (2−3 equiv), and triphenylphosphine (2−3 equiv) in dry toluene was stirred at 0 °C for 10 min. Molecular sieves, 3 Å, were added, and then a solution of di-2-methoxyethyl azodicarboxylate (DMEAD) (2−3 equiv) in dry toluene is added. The mixture is stirred until acceptable conversion is observed. The reaction mixture is then concentrated, and the residue is purified by silica gel column chromatography.

*(1R,2R,3S,3aR,8bS)-Methyl 6-((2S,6R)-6-((R)-1,2-Dihydroxyethyl)-1,4-dioxan-2-yloxy)-1,8b-dihydroxy-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]cyclopenta[d]-furan-2-carboxylate (**34**).* A mixture of phenol **4** (35.6 mg, 0.074 mmol, 1.0 equiv), 3 Å molecular sieves (101 mg), the lactol **16** (67 mg, 0.19 mmol, 2.6 equiv), and triphenylphosphine (50 mg, 0.19 mmol, 2.6 equiv) in 1.8 mL of toluene was stirred for 30 min at room temperature, cooled to 0−5 °C, and treated dropwise with a solution of di-2-methoxyethyl azodicarboxylate (DMEAD) (50 mg, 0.21 mmol, 2.8 equiv) in 0.16 mL of toluene over a period of 6 min. The mixture was stirred for 19 h. The molecular sieves were removed by filtration though a pad of Celite. The Celite pad was washed with EtOAc (2 × 10 mL). The combined filtrates were washed with water (2 × 10 mL) and dried (Na_2SO_4). The drying agent was filtered off, and the solvents were removed in vacuo. The residue was purified by chromatography on silica gel (4 g). The product containing fractions were concentrated in vacuo. The residue was further purified on silica gel by preparative TLC using 10% EtOAc in DCM to give 18 mg (0.022 mol, 30%) of bis-benzyl ether as a white foam (ESI-MS m/z = 805.3 $[M + H]^+$). A mixture of bis-benzyl ether (18.0 mg, 0.022 mmol, 1.0 equiv) and 5% palladium hydroxide on carbon (14.0 mg, 0.005 mmol, 0.26 equiv) in 3 mL of THF was evacuated and filled with hydrogen four times and stirred under hydrogen for 40 min. The hydrogen supply was disconnected, flushed with nitrogen, and the catalyst was filtered off. The filtrate was concentrated in vacuo and the residue was purified by preparative TLC on silica gel (40% acetone in DCM) and lyophilized to give 7.8 mg (0.012 mmol, 65%) of **34**. NMR δ_H (400 MHz, $CDCl_3$) 7.12−7.05 (m, 5H), 6.87−6.84 (m, 2H), 6.70−6.67 (m, 2H), 6.46 (d, J = 1.6 Hz, 1H), 6.31 (d, J = 2.0 Hz, 1H), 5.46 (brs, 1H), 5.04 (dd, J = 6.8, 1.6 Hz, 1H), 5.04 (dd, J = 6.8, 1.6 Hz, 1H), 4.29 (d, J = 14.4 Hz, 1H), 4.25−4.21 (m, 1H), 4.00 (d, J = 14.4 Hz, 1H), 3.92 (m, 1H), 3.89 (brs, 1H), 3.88 (s, 3H), 3.85−3.79 (m, 2H), 3.74 (brs, 1H), 3.72 (s, 3H), 3.67−3.60 (m, 3H), 3.65 (s, 3H); ESI-MS m/z = 625.2 $[M + H]^+$. Purity (method A): 98% AUC.

(1R,2R,3S,3aR,8bS)-Methyl 1,8b-Dihydroxy-6-((2S,3R,6S)-6-(2-hydroxyethyl)-3-methoxy-1,4-dioxan-2-yloxy)-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]-

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*cyclopenta[d]furan-2-carboxylate (**35**) and (1R,2R,3S,3aR,8bS)-Methyl 1,8b-Dihydroxy-6-((2R,3R,6S)-6-(2-hydroxyethyl)-3-methoxy-1,4-dioxan-2-yloxy)-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]cyclopenta[d]furan-2-carboxylate (**36**).* A solution of phenol **4** (15 mg, 0.031 mmol), lactol **20** (21 mg, 0.07 mmol), and triphenylphosphine (21 mg, 0.078 mmol) in toluene (3 mL) was stirred at 0 °C for 10 min. Molecular sieves, 3 Å (25 mg), were added, and then a solution of di-2-methoxyethyl azodicarboxylate (21 mg, 0.088 mmol) in toluene (0.5 mL) was added over a period of 5 min. The mixture was stirred for an additional 2 h. The reaction mixture was then concentrated, and the residue was purified by silica gel column chromatography (4 g) and eluted using 30−50% EtOAc−hexanes to provide the desired compound benzyl ether (8 mg) and its corresponding 1‴ epimer (7 mg). Both epimers were independently subjected to hydrogenolysis and HPLC purification to give compounds **35** (4 mg) and **36** (4 mg). Compound **35**: NMR δ_H (400 MHz, $CDCl_3$) 7.10 (d, J = 8.8 Hz, 2H), 7.07−7.05 (m, 3H), 6.89−6.87 (m, 2H), 6.68 (d, J = 8.8 Hz, 2H), 6.47 (d, J = 1.6 Hz, 1H), 6.32 (d, J = 1.6 Hz, 1H), 5.30 (brs, 1H), 5.03 (d, J = 6.4 Hz, 1H), 4.61 (s, 1H), 4.40−4.35 (m, 1H), 4.32 (d, J = 14 Hz, 1H), 3.92 (dd, J = 6.4, 14 Hz, 1H), 3.87 (s, 3H), 3.75−3.70 (m, 2H), 3.71 (s, 3H), 3.65 (s, 3H), 3.56−3.54 (m, 1H), 3.50 (s, 3H), 1.90 (brs, 1H), 1.70−1.60 (m, 2H); ESI-MS m/z = 639.4 $[M + H]^+$. Purity (method B): >95% AUC. Compound **36**: NMR δ_H (400 MHz, $CDCl_3$) 7.10 (d, J = 8.8 Hz, 2H), 7.07−7.05 (m, 3H), 6.88−6.85 (m, 2H), 6.68 (d, J = 8.8 Hz, 2H), 6.40 (d, J = 1.6 Hz, 1H), 6.30 (d, J = 1.6 Hz, 1H), 5.27 (d, J = 1.6 Hz, 1H), 5.03 (dd, J = 1.6, 6.8 Hz, 1H), 4.30 (d, J = 14.4 Hz, 1H), 4.250−4.18 (m, 1H), 3.90 (dd, J = 6.4, 14.4 Hz, 1H), 3.86 (s, 3H), 3.85−3.75 (m, 3H), 3.71 (s, 3H), 3.65 (s, 3H), 3.57 (s, 3H), 3.49 (dd, J = 2.8, 11.2 Hz, 1H), 1.90 (brs, 1H), 1.89−1.81 (m, 1H), 1.78−1.69 (m, 2H); ESI-MS m/z = 639.4 $[M + H]^+$. Purity (method B): >95% AUC.

*(1R,2R,3S,3aR,8bS)-Methyl 1,8b-Dihydroxy-6-((2S,3S,6S)-6-(2-hydroxyethyl)-3-methoxy-1,4-dioxan-2-yloxy)-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]-cyclopenta[d]furan-2-carboxylate (**37**) and (1R,2R,3S,3aR,8bS)-Methyl 1,8b-Dihydroxy-6-((2R,3S,6S)-6-(2-hydroxyethyl)-3-methoxy-1,4-dioxan-2-yloxy)-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]cyclopenta[d]furan-2-carboxylate (**38**).* A solution of phenol **4** (11 mg, 0.023 mmol), lactol **21** (15 mg, 0.057 mmol), and triphenylphosphine (15 mg, 0.057 mmol) in toluene (3 mL) was stirred at 0 °C for 10 min. Molecular sieves, 3 Å (25 mg), were added, and then a solution of di-2-methoxyethyl azodicarboxylate (15 mg, 0.064 mmol) in toluene (0.5 mL) was added over a period of 5 min. The mixture was stirred for an additional 2 h. The reaction mixture was then concentrated and the residue was purified by silica gel column chromatography (4 g) and eluted using 30−50% EtOAc−hexanes to provide the desired compound benzyl ether (7 mg) and its corresponding 1‴ epimer (6 mg). Both epimers were independently subjected to hydrogenolysis and HPLC purification to give compound **37** (4 mg) and compound **38** (5 mg). Compound **37**: NMR δ_H (400 MHz, $CDCl_3$) 7.10 (d, J = 9.2 Hz, 2H), 7.07−7.05 (m, 3H), 6.89−6.87 (m, 2H), 6.67 (d, J = 9.2 Hz, 2H), 6.42 (d, J = 1.6 Hz, 1H), 6.26 (d, J = 1.6 Hz, 1H), 5.04 (d, J = 5.2 Hz, 1H), 5.01 (d, J = 1.6 Hz, 1H), 4.46 (d, J = 4.8 Hz, 1H), 4.30 (d, J = 14.4 Hz, 1H), 4.11−4.06 (m, 1H), 3.98 (dd, J = 2.8, 11.6 Hz, 1H), 3.91 (dd, J = 6.4, 14.4 Hz, 1H), 3.88 (s, 3H), 3.81−3.75 (m, 2H), 3.71 (s, 3H), 3.65 (s, 3H), 3.64−3.62 (m, 1H), 3.58 (s, 3H), 1.89 (brs, 1H), 1.88−1.78 (m, 2H), 1.67 (brs, 1H); ESI-MS m/z = 639.4 $[M + H]^+$. Purity (method B): >95% AUC. Compound **38**: NMR δ_H (400 MHz, $CDCl_3$) 7.09 (d, J = 8.8 Hz, 2H), 7.06−7.04 (m, 3H), 6.87−6.85 (m, 2H), 6.67 (d, J = 8.8 Hz, 2H), 6.50 (d, J = 1.6 Hz, 1H), 6.40 (d, J = 1.6 Hz, 1H), 5.32 (brs, 1H), 5.30 (s, 1H),5.03 (d, J = 6.8 Hz, 1H), 4.29 (d, J = 14 Hz, 1H), 4.27−4.25 (m, 1H), 4.01 (dd, J = 2.4, 11.6 Hz, 1H), 3.89 (dd, J = 6.8, 14.4 Hz, 1H), 3.86 (s, 3H), 3.71 (s, 3H), 3.65 (s, 3H), 3.61 (s, 3H), 1.98 (brs, 1H), 1.68−1.64 (m, 2H); ESI-MS m/z = 639.4 $[M + H]^+$. Purity (method B): >95% AUC.

*(1R,2R,3S,3aR,8bS)-Methyl 1,8b-Dihydroxy-6-((2S,3R,6S)-6-(hydroxymethyl)-3-methoxy-1,4-dioxan-2-yloxy)-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]-cyclopenta[d]furan-2-carboxylate (**39**) and (1R,2R,3S,3aR,8bS)-Methyl 1,8b-Dihydroxy-6-((2R,3R,6S)-6-(hydroxymethyl)-3-methoxy-1,4-dioxan-2-yloxy)-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]cyclopenta[d]furan-2-carboxylate (**40**).* A solution of phenol **4** (15 mg, 0.031 mmol), lactol **26** (20 mg, 0.078 mmol), and triphenylphosphine (21 mg, 0.078 mmol) in toluene (3 mL) was stirred at 0 °C for 10 min. Molecular sieves, 3 Å (25 mg), were added, and then a solution of di-2-methoxyethyl azodicarboxylate (21 mg, 0.088 mmol) in toluene (0.5 mL) was added over a period of 5 min. The mixture was stirred for an additional 2 h. The reaction mixture was then concentrated, and the residue was purified by silica gel column chromatography (4 g) and eluted using 30−50% EtOAc−hexanes to provide the desired compound benzyl ether (14 mg) and its corresponding 1‴ epimer (2 mg). Both epimers were independently subjected to hydrogenolysis and HPLC purification provide the desired compound **39** (4 mg) and compound **40** (1 mg). Compound **39**: NMR δ_H (400 MHz, $CDCl_3$) 7.11 (d, J = 8.8 Hz, 2H), 7.06−7.04 (m, 3H), 6.86−6.80 (m, 2H), 6.69 (d, J = 8.8 Hz, 2H), 6.49 (d, J = 1.6 Hz, 1H), 6.28 (d, J = 1.6 Hz, 1H), 5.24 (s, 1H), 5.04 (d, J = 6.4 Hz, 1H), 4.59 (s, 1H), 4.31 (d, J = 14.4 Hz, 1H), 4.26−4.22 (m, 1H), 4.10 (dd, J = 11.2, 11.2 Hz, 1H), 3.91 (dd, J = 6.4, 14 Hz, 1H), 3.87 (s, 3H), 3.71 (s, 3H), 3.68 (brs, 1H), 3.60 (s, 3H), 3.63−3.561 (m, 2H), 3.50 (s, 3H), 1.90 (brs, 1H), 1.8−1.7 (m, 1H); ESI-MS m/z = 625.4 $[M + H]^+$. Purity (method B): 92% AUC. Compound **40**: NMR δ_H (400 MHz, $CDCl_3$) 7.11 (d, J = 8.8 Hz, 2H), 7.07−7.06 (m, 3H), 6.86−6.80 (m, 2H), 6.69 (d, J = 8.8 Hz, 2H), 6.43 (d, J = 1.6 Hz, 1H), 6.31 (d, J = 1.6 Hz, 1H), 5.32 (s, 1H), 5.03 (d, J = 6.8 Hz, 1H), 4.66 (s, 1H), 4.29 (d, J = 14.4 Hz, 1H), 4.13−4.10 (m, 1H), 3.97−3.94 (m, 1H), 3.86 (s, 3H), 3.78−3.75 (m, 1H), 3.71 (s, 3H), 3.69 (brs, 1H), 3.65 (s, 3H), 3.58 (s, 3H), 3.55−3.51 (m, 1H), 2−1.9 (m, 1H), 1.81 (brs, 1H); ESI-MS m/z = 625.4 $[M + H]^+$. Purity (method B): 97% AUC.

*(1R,2R,3S,3aR,8bS)-Methyl 1,8b-Dihydroxy-6-((2S,3S,6S)-6-(hydroxymethyl)-3-methoxy-1,4-dioxan-2-yloxy)-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]-cyclopenta[d]furan-2-carboxylate (**41**) and (1R,2R,3S,3aR,8bS)-Methyl 1,8b-Dihydroxy-6-((2R,3S,6S)-6-(hydroxymethyl)-3-methoxy-1,4-dioxan-2-yloxy)-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]cyclopenta[d]furan-2-carboxylate (**42**).* A solution of phenol **4** (15 mg, 0.031 mmol), lactol **27** (20 mg, 0.078 mmol), and triphenylphosphine (21 mg, 0.078 mmol) in toluene (3 mL) was stirred at 0 °C for 10 min. Molecular sieves, 3 Å (25 mg), were added, and then a solution of di-2-methoxyethyl azodicarboxylate (21 mg, 0.088 mmol) in toluene (0.5 mL) was added over a period of 5 min. The mixture was stirred for an additional 2 h. The reaction mixture was then concentrated and the residue was purified by silica gel column chromatography (4 g) and eluted using 30−50% EtOAc−hexanes to provide the desired benzyl ether (12 mg) and its corresponding 1‴ epimer (2 mg). Both epimers were independently subjected to hydrogenolysis and HPLC purification to provide the desired compound **41** (5 mg) and compound **42** (1 mg). Compound **41**: NMR δ_H (400 MHz, $CDCl_3$) 7.11 (d, J = 8.8 Hz, 2H), 7.06−7.05 (m, 3H), 6.88−6.85 (m, 2H), 6.67 (d, J = 8.8 Hz, 2H), 6.47 (d, J = 1.6 Hz, 1H), 6.26 (d, J = 1.6 Hz, 1H), 5.15 (d, J = 3.2 Hz, 1H), 5.02 (dd, J = 1.6, 6.8 Hz, 1H), 4.52 (d, J = 3.2 Hz, 1H), 4.29 (d, J = 14 Hz, 1H), 4.10 (dd, J = 3.6, 12 Hz, 1H), 3.95−3.91 (m, 3H), 3.88 (s, 3H), 3.83−3.76 (m, 2H), 3.71 (s, 3H), 3.69 (d, J = 5.6 Hz, 1H), 3.65 (s, 3H), 3.56 (s, 3H), 1.84 (d, J = 14 Hz, 1H); ESI-MS m/z = 625.4 $[M + H]^+$. Purity (method C): 86% AUC. Compound **42**: NMR δ_H (400 MHz, $CDCl_3$) 7.11 (d, J = 8.8 Hz, 2H), 7.06−7.05 (m, 3H), 6.88−6.85 (m, 2H), 6.69 (d, J = 8.8 Hz, 2H), 6.53 (d, J = 1.6 Hz, 1H), 6.39 (d, J = 1.6 Hz, 1H), 5.35 (s, 1H), 5.04 (d, J = 5.6 Hz, 1H), 4.63 (s, 1H), 4.29 (d, J = 14 Hz, 1H), 4.23−4.19 (m, 1H), 4.08 (dd, J = 2.8, 12 Hz, 1H), 3.95−3.91 (m, 2H), 3.88 (s, 3H), 3.81−3.77 (m, 1H), 3.72 (s, 3H), 3.71−3.66 (m, 1H), 3.65 (s, 3H), 3.62 (s, 3H), 1.81 (s, 1H) 1.71−1.69 (m, 1H); ESI-MS m/z = 625.4 $[M + H]^+$. Purity (method C): 79% AUC.

*(1R,2R,3S,3aR,8bS)-Methyl 1,8b-Dihydroxy-6-((2S,6S)-6-(hydroxymethyl)-1,4-dioxan-2-yloxy)-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]cyclopenta[d]furan-2-carboxylate (**43**).* A solution of phenol **4** (40 mg, 0.084 mmol), lactol **23** (47 mg, 0.21 mmol), and triphenylphosphine (55 mg, 0.21 mmol) in

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toluene (3 mL) was stirred at 0 °C for 10 min. Molecular sieves, 3 Å (25 mg), were added, and then a solution of di-2-methoxyethyl azodicarboxylate (55 mg, 0.23 mmol) in toluene (0.5 mL) was added over a period of 5 min. The mixture was stirred for an additional 2 h. The reaction mixture was then concentrated and the residue was purified by silica gel column chromatography (4 g) and eluted using 30−50% EtOAc-CH_2Cl_2 to provide the desired benzyl ether (46 mg). The benzyl ether was hydrogenated as described for compound **46** to generate the desired compound **43** (5 mg) after purification by HPLC. NMR δ_H (400 MHz, $CDCl_3$) 7.11 (d, J = 8.8 Hz, 2H), 7.06−7.05 (m, 3H), 6.88−6.85 (m, 2H), 6.68 (d, J = 8.8 Hz, 2H), 6.52 (d, J = 2 Hz, 1H), 6.32 (d, J = 2 Hz, 1H), 5.40 (s, 1H), 4.31 (d, J = 6.8 Hz, 1H), 4.28−4.21 (m, 1H), 3.97 (dd, J = 12, 12 Hz, 2H), 3.94−3.91 (m, 1H), 3.88 (s, 3H), 3.88−3.86 (m, 1H), 3.78 (dd, J = 2, 12 Hz, 1H), 3.76 (d, J = 12 Hz, 1H), 3.71 (s, 3H), 3.69 (brs, 1H), 3.65 (s, 3H), 1.86 (brs, 1H) 1.71−1.65 (m, 1H); ESI-MS m/z = 595.2 $[M + H]^+$. Purity (method C): 92% AUC.

*(1R,2R,3S,3aR,8bS)-Methyl 1,8b-Dihydroxy-6-((2S,6R)-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yloxy)-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]-cyclopenta[d]furan-2-carboxylate (**44**).* A solution of phenol **4** (25 mg, 0.052 mmol), lactol **30** (32 mg, 0.13 mmol), and triphenylphosphine (34 mg, 0.13 mmol) in anhydrous toluene (3 mL) was stirred at 0 °C for 10 min. Molecular sieves, 3 Å (50 mg), were added, and then a solution of di-2-methoxyethyl azodicarboxylate (34 mg, 0.15 mmol) in toluene (0.5 mL) was added over a period of 5 min. The mixture was stirred for an additional 10 h. The reaction mixture was then concentrated, and the residue was purified by silica gel column chromatography and eluted using 10% EtOAc−hexanes to provide the desired TBS ether adduct (29 mg). To a solution of TBS ether (10 mg, 0.014 mmol) in THF (1 mL) was added a solution of TBAF (56 uL, 1 M) in THF at 0 °C. The mixture was stirred at room temperature for 1 h before the solvent was removed under reduced pressure. The residue was dissolved into DCM, and purification by flash chromatography with EtOAc as eluent gave 7 mg of the desired product **44**. NMR δ_H (400 MHz, $CDCl_3$) 7.12 (d, J = 8.8 Hz, 2H), 7.06−7.04 (m, 3H), 6.87−6.85 (m, 2H), 6.67 (d, J = 8.8 Hz, 2H), 6.50 (d, J = 2 Hz, 1H), 6.26 (d, J = 2 Hz, 1H), 5.53 (brs, 1H), 5.04 (dd, J = 1.6, 6.8 Hz, 1H), 4.30 (d, J = 14 Hz, 1H), 3.90 (d, J = 8.4 Hz, 1H), 3.93−3.91 (m, 1H), 3.88 (s, 3H), 3.71 (s, 3H), 3.65 (s, 3H), 3.54−3.49 (m, 1H), 2.1−2.0 (m, 1H), 1.91 (brs, 1H), 1.92−1.89 (m, 1H), 1.82−1.71 (m, 3H), 1.65−1.56 (m, 2H); ESI-MS m/z = 593.3 $[M + H]^+$. Purity (method B): 90% AUC

*(1R,2R,3S,3aR,8bS)-Methyl 1,8b-Dihydroxy-6-((2S,6R)-6-(hydroxymethyl)morpholin-2-yloxy)-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]cyclopenta[d]-furan-2-carboxylate (**45**).* A suspension of phenol **4** (100 mg, 0.21 mmol), lactol **33** (200 mg, 0.52 mmol), and triphenylphosphine (137 mg, 0.52 mmol) with molecular sieves, 3 Å (35 mg), in toluene (6 mL) was stirred at 0 °C for 10 min. A solution of di-2-methoxyethyl azodicarboxylate (137 mg, 0.59 mmol) in toluene (0.8 mL) was added over a period of 5 min. The mixture was stirred for an additional 6 h at 0 °C. The mixture was filtered and diluted with EtOAc and washed with water and brine and dried over Na_2SO_4, filtered, and concentrated. Purification on silica gel (30−40% EtOAc in hexane) gave the desired Cbz-protected adduct (140 mg). To a solution of Cbz-protected material (15 mg, 0.018 mmol) in MeOH (3 mL) was added (10%) Pd on carbon (10 mg). After three purge−fill cycles, the vessel was placed under 1 atm of hydrogen. After completion of the reaction, the vessel was purged and catalyst was filtered off from Celite. The Celite was washed with THF (3 × 5 mL), and the combined filtrate was concentrated and purified on HPLC to give 10 mg of the desired morpholine. To a solution of TBS ether (5 mg, 0.007 mmol) in THF (2 mL) was added a solution of TBAF (22 μL, 1 M) in THF at 0 °C. The mixture was stirred at room temperature for 1 h before the solvent was removed under reduced pressure. The residue was purified by HPLC to give 3 mg of the desired product **45**. NMR δ_H (400 MHz, $CDCl_3$) 7.11 (d, J = 8.8 Hz, 2H), 7.06−7.05 (m, 3H), 6.87−6.85 (m, 2H), 6.67 (d, J = 8.8 Hz, 2H), 6.51 (d, J = 1.6 Hz, 1H), 6.30 (d, J = 1.6 Hz, 1H), 5.39 (brs, 1H), 5.03 (d, J = 6.4 Hz, 1H), 4.30 (d, J = 14 Hz, 1H), 4.05−3.97 (m, 1H), 3.92 (d, J = 7.2 Hz, 1H), 3.88 (s, 3H), 3.88−3.87 (m, 1H), 3.71 (s, 3H), 3.68 (d, J = 1.6 Hz, 1H), 3.65 (s, 3H), 3.65−3.64 (m, 1H), 3.57 (dd, J = 4.8, 12 Hz, 1H), 3.06 (d, J = 6 Hz, 1H), 2.93−2.91 (m, 2H); ESI-MS m/z = 594.3 $[M^+ + H]$. Purity (method C): 87% AUC.

*(1R,2R,3S,3aR,8bS)-Methyl 1,8b-Dihydroxy-6-((2R,6R)-6-(hydroxymethyl)-4-methylmorpholin-2-yloxy)-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]cyclopenta[d]-furan-2-carboxylate (**46**).* To a solution of the morpholine made during the synthesis of compound **57** (5 mg, 0.007 mmol) in THF (1.5 mL)/MeOH (0.5 mL) were added formaldehyde (37% in water, 21 μL, 0.28 mmol), acetic acid (16 μL, 0.28 mmol), and sodium triacetoxyborohydride (15 mg, 0.07 mmol). The mixture was stirred at room temperature for 3 h. The mixture was purified on HPLC to give 4.5 mg of the desired methylmorpholine derivative. To a solution of TBS ether (4.5 mg, 0.006 mmol) in THF (2 mL) was added a solution of TBAF (34 μL, 1 M) in THF at 0 °C. The mixture was stirred at room temperature for 1 h and worked up with dilution of DCM, washed with sodium bicarbonate, dried over Na_2SO_4, filtered, and concentrated. Flash chromatography on silica gel (DCM, 1−2% MeOH in DCM, 5% MeOH, 5% Et_3N in DCM) gave product **46** (3 mg). NMR δ_H (400 MHz, $CDCl_3$) 7.11 (d, J = 8.8 Hz, 2H), 7.06−7.04 (m, 3H), 6.87−6.85 (m, 2H), 6.68 (d, J = 8.8 Hz, 2H), 6.54 (d, J = 1.6 Hz, 1H), 6.36 (d, J = 1.6 Hz, 1H), 5.51 (brs, 1H), 5.04 (dd, J = 1.2, 6.4 Hz, 1H), 4.30 (d, J = 14 Hz, 1H), 4.24−4.16 (m, 1H), 3.90 (dd, J = 6.8, 14 Hz, 1H), 3.85 (s, 3H), 3.75−3.72 (m, 2H), 3.71 (s, 3H), 3.64 (s, 3H), 3.65−3.62 (m, 1H), 3.03 (d, J = 12 Hz, 1H), 2.37 (s, 3H), 2.34 (dd, J = 2.4, 12 Hz, 1H), 2.22 (dd, J = 11.2, 11.2 Hz, 1H), 1.95−1.91 (m, 1H); ESI-MS m/z = 608.3 $[M + H]^+$. Purity (method C): 92% AUC

Preparation of Compounds in Scheme 5. *(1R,2R,3S,3aR,8bS)-Methyl 1,8b-Dihydroxy-6,8-dimethoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]cyclopenta[d]furan-2-carboxylate (**47**).* A solution of phenol **4** (15 mg, 0.031 mmol, 1 equiv) in toluene (1 mL) and methanol (1 mL) was treated with trimethylsilyldiazomethane (0.470 mL, 0.500 mmol, 30 equiv) and stirred for 3 h at 25 °C. Solvent was removed under vacuum. The residue was purified using silica gel chromatography (40% EtOAc/hexanes) to give the desired rocaglate **47** (14 mg, 90%). All physical data matched the natural product from a commercially available source.

*(1R,2R,3S,3aR,8bS)-Methyl 1,8b-Dihydroxy-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-6-(trifluoromethylsulfonyloxy)-2,3,3a,8b-tetrahydro-1H-benzo[b]cyclopenta[d]furan-2-carboxylate (**48**).* To a solution of phenol **4** (506 mg, 1.06 mmol, 1.0 equiv) and diisopropylethylamine (0.3 mL, 1.7 mmol, 1.7 equiv) in 11 mL of DCM at −10 °C was added trifluoromethanesulfonic anhydride (0.21 mL, 1.22 mmol, 1.15 equiv) dropwise over a period of 4 min. The mixture was aged for 45 min and partitioned between 25 mL of saturated sodium bicarbonate. The lower organic layer was collected. The aqueous layer was extracted with DCM (20 mL). The combined organic layers were washed successively with 10% citric acid (20 mL), water (20 mL), brine (20 mL) and dried (Na_2SO_4). The drying agent was filtered off. The filtrate was concentrated in vacuo, and the residue was chromatographed on silica gel (4g) using 0% → 10% ethyl acetate in DCM to give the desired triflate **48** (515 mg, 1.06 mmol, 76%) as a white powder. NMR δ_H (400 MHz, $CDCl_3$) 7.08−7.10 (m, 5H), 6.90−6.92 (m, 2H), 6.66−6.70 (m, 3H), 6.46 (d, J = 1.0 Hz, 1H), 5.03 (d, J = 6.0 Hz, 1H), 4.38 (d, J = 14.0 Hz, 1H), 3.96 (dd, J = 14.0, 6.0 Hz, 1H), 3.94 (s, 3H), 3.71 (s, 3H), 3.68 (s, 3H), 3.48 (brs, 1H), 2.02 (brs, 1H), 1.59 (brs, 1H); NMR δ_C (100 MHz, $CDCl_3$) 170.56, 160.22, 158.97, 157.25, 152.15, 136.28, 128.78, 127.84, 127.77, 126.78, 125.52, 120.30, 117.12, 115.41, 112.90, 102.71, 98.67, 98.14, 93.38, 79.43, 56.38, 55.49, 55.10, 52.16, 50.42; ESI-MS m/z 633.1 $[M + Na]^+$. Purity (method A): 95% AUC.

*(1R,2R,3S,3aR,8bS)-Methyl 1,8b-Dihydroxy-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]-cyclopenta[d]furan-2-carboxylate (**49**).* To a solution of the triflate **48** (20 mg, 0.033 mmol) in dimethylformamide (2 mL) were added triphenylphosphine (9 mg, 0.033 mmol), palladium acetate (3.6 mg, 0.016 mmol), triethylamine (10 mg, 0.1 mmol), and formic acid (3

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mg, 0.066 mmol). The mixture was slowly warmed to 60 °C and stirred for an additional 2 h at that temperature. The reaction was quenched by adding brine (5 mL), and the aqueous portion was extracted using EtOAc (20 mL). Combined organic layers were dried over Na_2SO_4, filtered, and concentrated to provide a residue that was further purified by a preparatory TLC plate using 50% EtOAc−hexanes as an eluent. The central band was removed and further purified using preparative HPLC. The desired compound **49** was obtained as a white solid after lyophilization (2.1 mg, 14%). NMR δ_H (400 MHz, $CDCl_3$) 7.34 (t, J = 8.0 Hz), 7.15 (dd, J = 8.0, 2.0 Hz, 2H), 7.07−7.10 (m, 3H), 6.89−6.92 (m, 2H), 6.74 (d, J = 8.0 Hz, 1H), 6.70 (dd, J = 8.0, 2.0 Hz, 2H), 6.57 (d, J = 8.0 Hz, 1H), 5.09 (d, J = 6.0 Hz, 1H), 4.35 (d, J = 14.0 Hz, 1H), 3.96 (dd, J = 14.0, 6.0 Hz, 1H), 3.94 (s, 3H), 3.77 (brs, 1H), 3.72 (s, 3H), 3.67 (s, 3H), 1.98 (brs, 1H), 1.59 (brs, 1H); NMR δ_C (100 MHz, $CDCl_3$) 170.51, 15.87, 158.72, 156.56, 136.84, 132.44, 128.96, 127.69, 126.53, 126.30, 115.26, 112.70, 105.15, 103.59, 101.10, 93.75, 79.77, 55.78, 55.06, 51.97, 50.41; ESI-MS m/z 463.1 $[M + H]^+$. Purity (method A): 99% AUC.

*(1R,2R,3S,3aR,8bS)-Methyl 1,8b-Dihydroxy-8-methoxy-3a-(4-methoxyphenyl)-6-methyl-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo-[b]cyclopenta[d]furan-2-carboxylate (**50**).* To a mixture of triflate **48** (20 mg, 0.033 mmol), palladium acetate (1.8 mg, 8.19 μmol), cesium carbonate (32 mg, 0.098 mmol), X-phos (7.8 mg, 0.016 mmol), and methylboronic acid (5.9 mg, 0.098 mmol) was added toluene (2 mL), and the mixture was heated at 100 °C overnight . The reaction mixture was filtered through a 0.2 μm filter, concentrated, and then redissolved in CH_2Cl_2 (2 mL). Purification by preparative TLC using 20% EtOAc−hexanes provided the desired product **50**, which was isolated as a white solid after lyophilization (1.8 mg, 12%). NMR δ_H (400 MHz, $CDCl_3$) 7.14 (d, J = 8.8 Hz, 2H), 7.07−7.08 (m, 3H), 6.89−6.92 (m, 2H), 6.70 (d, J = 8.8 Hz, 1H), 6.58 (s, 1H), 6.38 (s, 1H), 5.07 (d, J = 6.0 Hz, 1H), 4.33 (d, J = 14.4 Hz, 1H), 3.95 (dd, J = 14.0, 6.0 Hz, 1H), 3.94 (s, 3H), 3.78 (brs, 1H), 3.72 (s, 3H), 3.66 (s, 3H), 2.40 (s, 3H), 1.87 (brs, 1H), 1.59 (brs, 1H); NMR δ_C (100 MHz, $CDCl_3$) 170.56, 159.99, 158.73, 156.18, 143.42, 136.95, 128.99, 127.83, 127.70, 126.53, 126.44, 112.72, 112.53, 105.66, 104.72, 101.30, 93.67, 79.70, 55.70, 5.10, 55.00, 51.98, 50.44, 22.22; ESI-MS m/z 477.2 $[M + H]^+$. Purity (method A): 93% AUC.

*(1R,2R,3S,3aR,8bS)-Methyl 6-Amino-1,8b-dihydroxy-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]-cyclopenta[d]furan-2-carboxylate (**51**).* A mixture of triflate **48** (263 mg, 0.431 mmol, 1 equiv), 2,2′-bis(diphenylphosphino)-1,1′-binaphthyl (BINAP) (148 mg, 0.237 mmol, 0.55 equiv), diphenylimine (625 mg, 3.45 mmol, 8 equiv), palladium acetate (24 mg, 0.108 mmol, 0.25 equiv), and cesium carbonate (332 mg, 1.728 mmol, 4 equiv) was suspended in toluene (25 mL) and heated to 100 °C for 20 h. The resulting mixture was cooled and partitioned between ethyl acetate and a saturated aqueous sodium chloride solution. The organic layer was separated, dried over sodium sulfate, and concentrated. The residue was dissolved in methanol (25 mL) and treated with sodium acetate (1.0g, 12.0 mmol, 28 equiv) and hydroxylamine hydrochloride (800 mg, 12.0 mmol, 28 equiv). The mixture was stirred for 3 h at room temperature. The mixture was partitioned between ethyl acetate and water. The organic layer was separated, dried over sodium sulfate, and concentrated. The residue was purified using silica gel chromatography (EtOAc/hexanes) to give the desired aniline **51** (153 mg, 0.320 mmol, 74%). NMR δ_H (400 MHz, $CDCl_3$) 7.12 (d, J = 8.0, 2H), 7.04−7.07 (m, 3H), 6.85−6.87 (m, 2H), 6.68 (d, J = 8.0 Hz, 1H), 5.99 (s, 1H), 5.83 (s, 1H), 5.02 (d, J = 6.0 Hz, 1H), 4.30 (d, J = 14.0 Hz, 1H), 3.91 (dd, J = 14.0, 6.0 Hz, 1H), 3.83 (s, 3H), 3.71 (brs, 1H), 3.64 (s, 3H), 3.67 (s, 3H), 2.01 (brs, 1H), 1.61 (brs, 2H); NMR δ_C (100 MHz, $CDCl_3$) 170.59, 161.11, 158.65, 157.25, 150.91, 137.10, 129.02, 127.84, 127.66, 126.77, 126.47, 112.65, 105.66, 101.56, 93.64, 91.83, 91.20, 79.53, 55.58, 55.10, 54.84, 51.93, 50.50; ESI-MS m/z 478.1 $[M + H]^+$. Purity (method C): >95% AUC

*(1R,2R,3S,3aR,8bS)-Methyl 1,8b-Dihydroxy-8-methoxy-3a-(4-methoxyphenyl)-6-(methylamino)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]cyclopenta[d]furan-2-carboxylate (**52**).* A solution of the triflate **48** (11 mg, 0.018 mmol) in toluene (2 mL) was treated with methylamine (0.090 mL, 0.180 mmol), palladium acetate (1 mg, 4.50 μmol), *tert*-butylphosphine (4 mg, 9.01 μmol), and cesium carbonate (18 mg, 0.054 mmol). The mixture was heated at 100 °C for 12 h in a sealed tube. The reaction mixture was cooled to room temperature, and CH_2Cl_2 (4 mL) was added. The mixture was filtered using a 0.45 μm HPLC filter, and the filtrate was concentrated. The residue was redissolved in acetonitrile (2 mL) and purified by preparative HPLC to provide the desired **52** (4 mg, 45%). NMR δ_H (400 MHz, $CDCl_3$) 7.12 (d, J = 8.0, 2H), 7.07−7.10 (m, 3H), 6.89−6.92 (m, 2H), 6.74 (d, J = 8.0 Hz, 1H), 5.99 (s, 1H), 5.80 (s, 1H), 5.02 (d, J = 6.0 Hz, 1H), 4.34 (d, J = 14.0 Hz, 1H), 3.96 (dd, J = 14.0, 6.0 Hz, 1H), 3.86 (s, 3H), 3.76 (brs, 1H), 3.74 (s, 3H), 3.67 (s, 3H), 2.79 (s, 3H), 1.80 (brs, 1H), 1.59 (brs, 2H); NMR δ_C (100 MHz, $CDCl_3$) 170.66, 161.21, 158.62, 157.02, 154.85, 137.22, 129.02, 127.86, 127.66, 126.84, 126.43, 112.66, 103.48, 101.44, 93.83, 89.21, 88.50, 79.53, 55.4, 55.09, 54.73, 51.92, 50.53, 40.79; ESI-MS m/z 492.1 $[M + H]^+$. Purity (method A): 95% AUC.

*(1R,2R,3S,3aR,8bS)-Methyl 6-(Dimethylamino)-1,8b-dihydroxy-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]cyclopenta[d]furan-2-carboxylate (**53**).* A solution of the triflate **48** (15 mg, 0.025 mmol) in toluene (2 mL) was treated with dimethylamine (0.123 mL, 0.246 mmol, 2 M in THF), palladium acetate (1.4 mg, 6.14 μmol), *tert*-butylphosphine (5 mg, 0.012 mmol), and cesium carbonate (24 mg, 0.074 mmol). The mixture was heated at 100 °C for 12 h in a sealed tube. The reaction mixture was filtered using a 0.45 μm HPLC filter and then purified by preparative TLC plate using 50% EtOAc−hexanes. The desired product **53** was obtained as a white solid after lyophilization (4 mg, 31%). NMR δ_H (400 MHz, $CDCl_3$) 7.15 (d, J = 8.0, 2H), 7.05−7.09 (m, 3H), 6.88−6.90 (m, 2H), 6.67 (d, J = 8.0 Hz, 1H), 6.07 (s, 1H), 5.88 (s, 1H), 5.05 (d, J = 6.0 Hz, 1H), 4.33 (d, J = 14.0 Hz, 1H), 3.92 (dd, J = 14.0, 6.0 Hz, 1H), 3.89 (s, 3H), 3.78 (brs, 1H), 3.71 (s, 3H), 3.65 (s, 3H), 3.01 (s, 6H), 2.05 (brs, 1H), 1.85 (brs, 1H); NMR δ_C (100 MHz, $CDCl_3$) 170.61, 161.20, 158.61, 157.02, 154.83, 137.25, 129.04, 127.87, 127.65, 126.88, 126.42, 112.64, 101.43, 103.82, 93.82, 89.25, 88.54, 79.54, 76.71, 55.4, 55.09, 54.73, 51.92, 50.53, 40.79; ESI-MS m/z 506.2 $[M + H]^+$. Purity (method A): 93% AUC.

*(1R,2R,3S,3aR,8bS)-Methyl 1,8b-Dihydroxy-8-methoxy-3a-(4-methoxyphenyl)-6-(methylsulfonamido)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]cyclopenta[d]furan-2-carboxylate (**54**).* To a solution of aniline **51** (10 mg, 0.021 mmol) in DMF (2 mL) were added DMAP (2.6 mg, 0.021 mmol), 1-(methylsulfonyl)-1*H*-benzo[*d*]-[1,2,3]triazole (9 mg, 0.042 mmol), and Et_3N (11 mg, 0.105 mmol). The reaction mixture was stirred for 4 h at room temperature. Brine (10 mL) was added, and the aqueous portion was extracted with EtOAc (20 mL). The organic layer was separated, dried, and concentrated. The residue was purified by preparative HPLC to provide the sulfonamide **54** as a white solid after lyophilization (8 mg, 68%). NMR δ_H (400 MHz, $CDCl_3$) 7.16 (d, J = 8.0, 2H), 7.01−7.07 (m, 3H), 6.93−6.95 (m, 2H), 6.67 (d, J = 8.0 Hz, 1H), 6.63 (s, 1H), 6.52 (s, 1H), 5.11 (d, J = 6.0 Hz, 1H), 4.37 (d, J = 14.0 Hz, 1H), 3.98 (dd, J = 14.0, 6.0 Hz, 1H), 3.93 (s, 3H), 3.74 (brs, 1H), 3.69 (s, 3H), 3.05 (s, 3H), 1.91(brs, 1H), 1.58 (brs, 2H); NMR δ_C (100 MHz, $CDCl_3$) 170.78, 160.33, 159.07, 157.21, 141.10, 136.37, 128.95, 127.88, 126.90, 125.88, 112.99, 112.40, 102.10, 97.13, 93.71, 91.23, 79.85, 56.18, 55.64, 55.20, 52.22, 50.40, 39.34; ESI-MS m/z 556.2 $[M + H]^+$. Purity (method A): 94% AUC.

*(1R,2R,3S,3aR,8bS)-1,8b-Dihydroxy-8-methoxy-2-(methoxycarbonyl)-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]cyclopenta[d]furan-6-carboxylic Acid (**55**).* Carbon monoxide was bubbled through a mixture of the triflate **48** (102 mg, 0.17 mmol, 1.0 equiv), DPPF (18.6 mg, 0.034 mmol, 0.2 equiv), potassium carbonate (116 mg, 0.838 mmol, 5.0 equiv), and palladium acetate (3.8 mg, 0.017 mmol, 0.1 equiv) in 2.0 mL of DMF for 15 min. The resulting reddish orange solution was heated at 100 °C for 2 h, cooled, diluted with brine (5 mL), acidified with 1 N HCl to pH 1−2, and partitioned between EtOAc (10 mL). The aqueous layer was extracted with EtOAc (2 × 10 mL), and the combined organic layers were washed with water (10 mL) and brine (10 mL). The mixture was dried (Na_2SO_4). The drying agent was filtered off. The solvents were evaporated in vacuo and the residue was purified by preparative TLC

using 5% MeOH in DCM as the developing solvent to give the desired acid which was then taken up in 2 mL of 90% *tert*-butanol, frozen, and lyophilized to give 63 mg of **55** (0.125 mmol, 74%) as an off white solid. NMR δ_H (400 MHz, $CDCl_3$) 7.47 (brs, 1H), 7.29 (brs, 1H), 7.13−7.07 (m, 5H), 6.92 (d, J = 7.6 Hz, 2H), 6.68 (d, J = 8.8 Hz, 2H), 5.06 (d, J = 6.4 Hz, 1H), 4.37 (d, J = 14 Hz, 1H), 3.98−3.93 (m, 4H), 3.71 (s, 3H), 3.67 (s, 3H); NMR δ_C (100 MHz, $CDCl_3$) 170.66, 169.91, 159.81, 158.88, 156.46, 136.49, 133.51, 128.87, 127.81, 126.69, 125.87, 120.58, 112.85, 107.13, 105.55, 101.84, 93.46, 79.76, 56.14, 55.46, 55.10, 52.13, 50.38; ESI-MS m/z 507.1 $[M + H]^+$. Purity (method A): >99% AUC.

*(1R,2R,3S,3aR,8bS)-Dimethyl 1,8b-Dihydroxy-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]-cyclopenta[d]furan-2,6-dicarboxylate (**56**).* To a solution of the acid **55** (12 mg, 0.024 mmol, 1.0 equiv) in 1 mL of methanol at 22 °C was added a 1.0 M solution of trimethylsilyldiazomethane (0.25 mL, 0.237 mmol, 10 equiv), and the mixture was stirred for 1 h. The mixture was concentrated in vacuo and the residue was purified by preparative TLC (10% EtOAc in DCM) to give 8.0 mg (0.015 mmol, 63%) of **56**. NMR δ_H (400 MHz, $CDCl_3$) 7.41 (d, J = 1 Hz, 1H), 7.25 (d, J = 1 Hz, 1H), 7.12−7.06 (m, 5 H), 6.91−6.89 (m, 2H), 6.69−6.66 (m, 2H), 5.05 (d, J = 6.4 Hz, 1H), 4.34 (d, J = 14.0 Hz, 1H), 3.98 (s, 3H), 3.96−3.92 (m,1H), 3.95 (s, 3H) 3.71 (s, 3H), 3.66 (s, 3H), 2.00 (s, 1H). ESI-MS m/z 521.1 $[M + H]^+$. Purity (method A): 93.6% AUC.

*(1R,2R,3S,3aR,8bS)-Methyl 6-Carbamoyl-1,8b-dihydroxy-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]cyclopenta[d]furan-2-carboxylate (**57**).* To a mixture of the acid **55** (10 mg, 0.02 mmol, 1.0 equiv), HOBt (16 mg, 0.13 mmol, 6.5 equiv), ammonium acetate (12.7 mg, 0.16 mmol, 8 equiv), and EDCI (21 mg, 0.1 mmol, 5.0 equiv) in DCM at 22 °C was added diisopropylethylamine (35 μL, 0.2 mmol, 10.0 equiv) before diluting with DCM (2 mL) and 10% citric acid (2 mL). The organic layer was collected. The aqueous layer was extracted with DCM (5 mL). The combined organic layers were washed with saturated $NaHCO_3$ (2 mL), water (2 mL) and dried (Na_2SO_4). The drying agent was filtered off. The filtrate was concentrated in vacuo and the crude was purified by preparative TLC (5% MeOH in DCM) to give 5.9 mg (0.012 mmol, 59%) of **57**. NMR δ_H (400 MHz, $CDCl_3$) 7.11−7.03 (m, 7H), 6.91−6.88 (m, 2H), 6.69−6.66 (m, 2 H), 6.10 (brs, 1H), 5.64 (brs, 1H), 5.04 (d, J = 6.4 Hz, 1H), 4.34 (d, J = 14.4 Hz, 1H), 3.96 (s, 3H), 3.94 (dd, J = 14.4, 6.4 Hz, 1H), 3.71 (s, 3H), 3.66 (s, 3H), 3.48 (s, 2H). ESI-MS m/z = 528.2 $[M + Na]^+$. Purity (method A): >99% AUC.

*(1R,2R,3S,3aR,8bS)-Methyl 1,8b-Dihydroxy-8-methoxy-3a-(4-methoxyphenyl)-6-(methylcarbamoyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]cyclopenta[d]furan-2-carboxylate (**58**).* To a mixture of the acid **55** (10 mg, 0.02 mmol, 1.0 equiv), HOBt (16 mg, 0.13 mmol, 6.5 equiv), methylamine hydrochloride (9 mg, 0.133 mmol, 6.6 equiv), and EDCI (20 mg, 0.1 mmol, 5.2 equiv) in 1 mL of DCM at 22 °C was added diisopropylethylamine (35 μL, 0.2 mmol, 10.0 equiv), and the mixture was stirred for 1 h before diluting with DCM (2 mL) and 10% citric acid (2 mL). The organic layer was collected. The aqueous layer was extracted with DCM (5 mL), and the combined organic layers were washed with saturated $NaHCO_3$ (2 mL), water (2 mL) and dried (Na_2SO_4). The drying agent was filtered off and the filtrate was concentrated in vacuo and the crude was purified by preparative TLC (5% MeOH in DCM) to give 7.8 mg (0.015 mmol, 76%) of **58**. NMR δ_H (400 MHz, $CDCl_3$) 7.11 (m, 6H), 6.97 (d, J = 1.2 Hz, 1H), 6.89−6.87 (m, 2H), 6.16 (br m, 1H), 5.04 (d, J = 6.8 Hz, 1H), 4.32 (d, J = 14.0 Hz, 1H), 3.95 (s, 3H), 3.91 (d, J = 6.4 Hz, 1H), 3.71 (s, 3H), 3.65 (s, 3H), 3.48 (s, 3H), 3.02 (d, J = 4.8 Hz, 3H), 2.10 (brs, 1H). NMR δ_C (100 MHz, $CDCl_3$) 170.60, 167.72, 159.78, 158.79, 156.68, 138.84, 136.60, 128.87, 127.77, 127.75, 126.64, 126.01, 118.36, 112.76, 103.44, 103.04, 101.79, 93.43, 79.72, 76.69, 56.07, 55.25, 55.07, 52.06, 50.34, 26.97; ESI-MS m/z = 520.2 $[M + H]^+$. Purity (method A): >99% AUC.

*(1R,2R,3S,3aR,8bS)-Methyl 6-(Dimethylcarbamoyl)-1,8b-dihydroxy-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]cyclopenta[d]furan-2-carboxylate (**59**).* To a mixture of the acid **55** (11 mg, 0.022 mmol, 1.0 equiv), HOBt (16 mg, 0.11 mmol, 5.3 equiv), dimethylamine hydrochloride (12 mg, 0.147 mmol, 6.6 equiv), and EDCI (22 mg, 0.11 mmol, 5.3 equiv) in 1 mL of DCM at 22 °C was added diisopropylethylamine (40 μL, 0.22 mmol, 10.6 equiv). The mixture was stirred for 1 h before diluting with DCM (2 mL) and 10% citric acid (2 mL). The organic layer was collected. The aqueous layer was extracted with DCM (5 mL). The combined organic layers were washed with saturated $NaHCO_3$ (2 mL), water (2 mL) and dried (Na_2SO_4). The drying agent was filtered off and the filtrate was concentrated in vacuo and the crude was purified by preparative HPLC (20% → 85% acetonitrile−water with 0.05% formic acid) to give 5.3 mg (0.099 mmol, 45%) of **59** after lyophilization. NMR δ_H (400 MHz, $CDCl_3$) 7.12−7.06 (m, 5H), 6.89−6.87 (m, 2H), 6.74 (brs, 1H), 6.69−6.66 (m, 2H), 6.60 (brs, 1H), 5.06 (dd, J = 6.4, 1 Hz, 1H), 4.34 (d, J = 14.4 Hz, 1H), 3.94 (dd, J = 14.4, 6.4 Hz, 1H), 3.93 (s, 3H), 3.71 (s, 3H), 3.66 (brs, 4H), 3.13 (s, 3H), 3.05 (s, 3H), 1.92 (s, 1H); ESI-MS m/z = 534.2 $[M + H]^+$. Purity (method A): 97.5% AUC.

*(1R,2R,3S,3aR,8bS)-Methyl 6-Acetyl-1,8b-dihydroxy-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]-cyclopenta[d]furan-2-carboxylate (**60**).* Degassed DMF (2 mL) was added to a 25 mL dry flask. Subsequently, aryl triflate **48** (20 mg, 0.033 mmol), tributyl(1-ethoxyvinyl)stannane (12 mg, 0.033 mmol), palladium acetate (4 mg, 0.016 mmol), and triphenylphosphine (13 mg, 0.049 mmol) were added under an atmosphere of argon. The mixture was stirred for 5 min at room temperature and then heated to 100 °C. The stirring was continued for an additional 3 h. The compound was purified by silica gel chromatography using 50% EtOAc−hexanes to provide the acetophenone **60** (11 mg, 66%). NMR δ_H (400 MHz, $CDCl_3$) 7.29 (s, 1H), 7.17 (s, 1H), 7.13 (d, J = 8.0, 2H), 7.07−7.09 (m, 3H), 6.91−6.93 (m, 2H), 6.66 (d, J = 8.0 Hz, 1H), 5.05 (d, J = 6.0 Hz, 1H), 4.38 (d, J = 14.0 Hz, 1H), 3.97 (s, 3H), 3.94 (dd, J = 14.0, 6.0 Hz, 1H), 3.70 (s, 3H), 3.66 (s, 3H), 2.62 (s, 3H), 2.21 (brs, 1H), 1.62 (brs, 2H); NMR δ_C (100 MHz, $CDCl_3$) 197.22, 170.60, 159.98, 158.87, 156.71, 141.23, 136.55, 128.85, 127.81, 126.69, 125.94, 120.05, 112.84, 105.86, 103.20, 101.92, 93.47, 79.71, 56.10, 55.48, 55.10, 52.10, 50.40, 26.85; ESI-MS m/z = 527.1 $[M + Na]^+$. Purity (method A): 99% AUC

*(1R,2R,3S,3aR,8bS)-Methyl 6-Cyano-1,8b-dihydroxy-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]-cyclopenta[d]furan-2-carboxylate (**61**).* A mixture of the triflate **48** (147 mg, 0.241 mmol, 1.0 equiv), zinc cyanide (62.7 mg, 0.53 mol, 2.2 equiv), DPPF (28.2 mg, 0.05 mmol, 0.21 equiv), and $Pd_2(dba)_3$ (24.8 mg, 0.27 mmol 0.11 equiv) in 6.0 mL of *N*-methylpyrrolidinone (previously degassed) was degassed for 5 min and heated at 98−104 °C for 20 h. The mixture was cooled and diluted with EtOAc (20 mL) and water (20 mL). The organic layer was collected. The aqueous layer was extracted with EtOAc (2 × 15 mL). The combined organic layers were washed with water (20 mL), 20 mL of brine (20 mL) and dried (Na_2SO_4), and the solvent was evaporated in vacuo. The residue was chromatographed on silica gel (12 g) using 0% → 20% EtOAc in DCM to give 107 mg (0.21 mmol, 87%) of **61**. NMR δ_H (400 MHz, $CDCl_3$) 7.11−7.05 (m, 5H), 7.02 (d, J = 1.0 Hz, 1H), 6.93−6.91 (m, 2H), 6.80 (d, J = 1.0 Hz, 1H), 6.90−6.65 (m, 2H), 4.99 (d, J = 6.0 Hz, 1H), 4.37 (d, J = 14.0 Hz, 1H), 3.96 (dd, J = 14.0, 6.0 Hz, 1H), 3.94 (s, 3H), 3.70 (s, 3H), 3.67 (s, 3H), 3.47 (brs, 1H), 2.02 (brs, 1H); NMR δ_C (100 MHz, $CDCl_3$) 170.76, 159.92, 158.99, 157.13, 136.17, 128.70, 127.85, 127.77, 126.78, 125.44, 120.42, 118.42, 115.23, 112.91, 108.89, 107.60, 102.20, 93.39, 79.42, 56.31, 55.70, 55.07, 52.19, 50.38. ESI-MS m/z = 510.0 $[M + Na]^+$. Purity (method A): 97.6% AUC.

*(1R,2R,3S,3aR,8bS)-Methyl 6-Chloro-1,8b-dihydroxy-8-methoxy-3a-(4-methoxyphenyl)-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]-cyclopenta[d]furan-2-carboxylate (**62**).* The aryl triflate **48** (105 mg, 0.17 mmol), bis(pinacolato)diboron (217 mg, 0.86 mmol), $PdCl_2$(dppf) (13 mg, 0.02 mo1%), and Et_3N (120 μL, 0.86 mmol) were added to a flask. Anhydrous dioxane (10 mL) was added to the mixture. The flask was put under vacuum and then refilled with nitrogen. This process was repeated three times before the mixture was heated at reflux for 14 h. The reaction was quenched with water (10 mL), and extraction was with CH_2Cl_2 (3 × 25 mL). After drying (Na_2SO_4), the solvent was removed to give pinacol boronate as a brown solid. To a solution of the boronate ester (90 mg, 0.15 mmol)

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in 3 mL of MeOH was added a water (3 mL) solution of copper(II) chloride (62 mg, 0.46 mmol). The mixture was stirred at reflux for 3 h. The reaction mixture was cooled to room temperature. Then water (10 mL) was added and the residue was extracted with CH_2Cl_2 (3 × 25 mL). After drying (Na_2SO_4) and filtration, the solvent was removed to give a brown solid, which was purified by HPLC to give 35 mg of the desired product **62**. NMR δ_H (400 MHz, $CDCl_3$) 7.10−7.04 (m, 5H), 6.91−6.87 (m, 2H), 6.76 (d, J = 1.6 Hz, 1H), 6.67 (d, J = 9.2 Hz, 2H), 6.54 (d, J = 1.6 Hz, 1H), 5.01 (dd, J = 2, 6 Hz, 1H), 4.32 (d, J = 14.4 Hz, 1H), 3.92 (dd, J = 6.4, 14.4 Hz, 1H), 3.90 (s, 3H), 3.70 (s, 3H), 3.66 (s, 3H), 3.52 (d, J = 1.6 Hz, 1H), 1.87 (s, 1H); NMR δ_C (100 MHz, $CDCl_3$) 170.75, 160.38, 158.97, 156.99, 137.75, 136.75, 129.0, 127.96, 127.94, 126.82, 126.13, 114.13, 112.95, 105.89, 105.11, 102.22, 93.61, 79.64, 56.28, 55.45, 55.24, 55.24, 52.24, 50.62; ESI-MS m/z = 497.2 $[M + H]^+$. Purity (method B): >95% AUC.

Preparation of Compounds in Scheme 6. *(1R,2R,3S,3aR,8bS)-Methyl 1,8b-Dihydroxy-6,8-dimethoxy-3-phenyl-3a-p-tolyl-2,3,3a,8b-tetrahydro-1H-benzo[b]cyclopenta[d]furan-2-carboxylate (**66**).* To a mixture of bromide **63** (10 mg, 0.016 mmol), palladium acetate (0.3 mg, 1.6 μmol), cesium carbonate (21 mg, 0.065 mmol), X-phos (3.6 mg, 0.008 mmol), and methylboronic acid (2 mg, 0.032 mmol) was added toluene (2 mL), and the mixture was heated at 100 °C overnight . The volatiles were removed and purification by normal phase column, eluting with 20−60% EtOAc−hexanes, provided the desired product **64** (6 mg). A solution of compound **64** (25 mg, 0.045 mmol, 1 equiv) and 5% palladium on carbon (5 mg) in tetrahydrofuran (3 mL) was placed under hydrogen atmosphere and stirred for 18 h at room temperature. The reaction mixture was filtered on Celite and the filtrate concentrated to dryness. The residue was purified using silica gel chromatography (40−60% EtOAc/hexanes) to give the desired phenol **65** (15 mg). A solution of phenol **65** (13 mg, 0.028 mmol, 1 equiv) in toluene (1 mL) and methanol was treated with trimethylsilyldiazomethane (0.422 mL, 0.84 mmol, 30 equiv) and stirred for 4 h. The mixture was treated with acetic acid (5 μL) and partitioned between ethyl acetate and a saturated aqueous sodium chloride solution. The organic layer was separated, dried over sodium sulfate, and concentrated. The residue was purified using silica gel chromatography (20−60% EtOAc/hexanes) to give the desired compound **66** (7 mg). NMR δ_H (400 MHz, $CDCl_3$) 7.06−7.04 (m, 5H), 6.96−6.88 (m, 4H), 6.29 (d, J = 2 Hz, 1H), 6.12 (d, J = 2 Hz, 1H), 5.03 (d, J = 7 Hz, 2H), 4.35 (d, J = 15 Hz, 2H), 4.00 (d, J = 15 Hz, 2H), 3.97 (d, J = 7 Hz, 2H), 3.91 (s, 3H), 3.84 (s, 3H), 3.66 (s, 3H); NMR δ_C (100 MHz, $CDCl_3$) 170.79, 164.30, 161.12, 157.20, 137.35, 137.14, 131.46, 128.27, 128.02, 127.87, 127.75, 126.70, 107.82, 102.26, 93.93, 92.83, 89.68, 79.80, 55.95, 55.91, 55.17, 52.18, 50.70, 31.43, 21.18; ESI-MS m/z = 476.99 $[M + H]^+$. Purity (method A): 95.8% AUC.

*(1R,2R,3S,3aR,8bS)-Methyl 3a-(4-Cyanophenyl)-1,8b-dihydroxy-6,8-dimethoxy-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]-cyclopenta[d]furan-2-carboxylate (**69**).* A mixture of bromide **63** (30 mg, 0.049 mmol, 1 equiv), DPPF (5.4 mg, 9.7 μmol, 0.2 equiv), zinc cyanide (11.4 mg, 0.097 mmol, 2 equiv), and $Pd_2(dba)_3$ (4.5 mg, 4.8 μmol, 0.1 equiv) was suspended in toluene (25 mL) and degassed for 15 min. The mixture was heated to 100 °C for 20 h. The resulting mixture was cooled and partitioned between ethyl acetate and a saturated aqueous sodium chloride solution. The organic layer was separated, dried over sodium sulfate, and concentrated. The residue was purified using silica gel chromatography (EtOAc/hexanes) to give the desired cyanide **67** (25 mg, 0.045 mmol, 94%). A solution of compound **67** (20 mg, 0.035 mmol, 1 equiv) and 15% palladium on carbon (20 mg) in tetrahydrofuran (2 mL) was placed under hydrogen atmosphere and stirred for 18 h at room temperature. The reaction mixture was filtered on Celite and the filtrate concentrated to dryness. The residue was purified using silica gel chromatography (EtOAc/hexanes) to give the desired phenol **68** (12 mg, 0.025 mmol, 71%). ESI-MS m/z = 474.00 $[M + H]^+$. A solution of phenol **68** (8 mg, 0.017 mmol, 1 equiv) in toluene (1 mL) and methanol was treated with trimethylsilyldiazomethane (0.250 mL, 0.500 mmol, 30 equiv) and stirred for 4 h. The mixture was treated with acetic acid (5 μL) and partitioned between ethyl acetate and a saturated aqueous sodium chloride solution. The organic layer was separated, dried over sodium sulfate, and concentrated. The residue was purified using silica gel chromatography (EtOAc/hexanes) to give the desired nitrile **69** (5 mg, 10.2 μmol, 60%). NMR δ_H (400 MHz, $CDCl_3$) 7.40 (d, J = 2.4 Hz, 2H), 7.31 (d, J = 2.4 Hz, 2H), 7.04−7.06 (m, 3H), 6.88−6.85 (m 2H), 6.30 (d, J = 2 Hz, 1H), 6.14 (d, J = 2 Hz, 1H), 5.02 (dd, J = 6, 2 Hz, 1H), 4.40 (d, J = 14 Hz, 1H), 3.92 (dd, J = 14, 6 Hz, 1H), 3.88 (s, 3H), 3.85 (s, 3H), 3.67 (s, 3H), 3.51 (d, J = 1 Hz, 1H), 1.96 (s, 1H); NMR δ_C (100 MHz, $CDCl_3$) 170.43, 164.62, 160.76, 157.06, 140.71, 136.24, 130.96, 128.70, 128.20, 127.69, 127.20, 118.89, 111.25, 107.06, 101.87, 94.11, 93.10, 89.68, 79.88, 55.98, 55.51, 52.35, 50.48, 31.43; ESI-MS m/z = 488.01 $[M + H]^+$. Purity (method A): 98.3% AUC.

Preparation of Compounds in Scheme 7. *(1R,2R,3S,3aR,8bS)-6-((2S,3R,6R)-6-((S)-1,2-Dihydroxyethyl)-3-methoxy-1,4-dioxan-2-yloxy)-1,8b-dihydroxy-8-methoxy-3a-(4-methoxyphenyl)-N,N-dimethyl-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]cyclopenta[d]-furan-2-carboxamide (**71**).* A mixture of the *O*,*O*-dibenzylsilvestrol **70**[27] (35 mg, 0.042 mmol, 1.0 equiv) and lithium hydroxide (24 mg, 1.0 mmol, 24 equiv) in 4 mL of 3:1 methanol−water was stirred at 23 °C for 17 h and then heated at 50 C for 4 h. The resulting mixture was cooled and acidfied with 1.0 M HCl to pH 5−6, and extraction was with DCM (3 × 5 mL). The combined DCM layers were dried over sodium sulfate. The solvents were evaporated in vacuo to give the acid (32.3 mg, 0.039 mmol) as a white solid. To a mixture of the acid (20.1 mg, 0.024 mmol, 1.0 equiv), HOBt (9.7 mg, 0.072 mmol, 3.0 equiv), dimethylamine hydrochloride (9.1 mg, 0.111 mmol, 4.65 equiv), and EDCI (12.9 mg, 0.067 mmol, 2.8 equiv) in 1 mL DCM at 22 °C was added diisopropylethylamine (42 μL, 0.288 mmol, 12.0 equiv) for 45 min before diluting with DCM (2 mL) and 10% citric acid (2 mL). The organic layer was collected. The aqueous layer was extracted with DCM (5 mL). The combined organic layers were washed with saturated $NaHCO_3$ (2 mL), water (2 mL) and dried (Na_2SO_4). The drying agent was filtered off and the filtrate was concentrated in vacuo and the crude was purified by silica gel chromatography (4 g, ISCO) using 0% → 5% MeOH in DCM to give the amide (20.2 mg, 0.023 mmol) of as a solid. A mixture of the *O*,*O*-dibenzylamide (19.0 mg, 0.022 mmol, 1.0 equiv) and 5% palladium hydroxide on carbon (21.8 mg, 0.0077 mmol, 0.35 equiv) in 4 mL of THF was evacuated and filled with hydrogen four times and stirred under hydrogen for 1.5 h. The hydrogen supply was disconnected, flushed with nitrogen, and the catalyst was filtered off. The filtrate was concentrated in vacuo and the residue was purified on silica gel (4 g, ISCO), using 0% → 6% MeOH in DCM, and lyophilized to give 12.6 mg (0.019 mmol, 84%) of **71** as a white powder. NMR δ_H (400 MHz, $CDCl_3$) 7.10−7.01 (m, 5H), 6.84−6.82 (m, 2H), 6.70−6.67 (m, 2H), 6.43 (d, J = 2.0 Hz, 1H), 6.26 (d, J = 2.0 Hz, 1H), 5.29 (brs, 1H), 4.96 (d, J = 7.0 Hz, 1H), 4.57 (brs, 1H), 4.49 (d, J = 13.4 Hz, 1H), 4.19−4.09 (m, 3H), 4.02 (dd, J = 13.4, 6.8 Hz, 1H), 3.85 (s, 3H), 3.71 (s, 3H), 3.55−3.45 (m, 3H), 3.48 (s, 3H), 3.29 (s, 3H), 2.92 (s, 3H), 2.13 (brs, 1H). ESI-MS m/z = 668.33 $[M + H]^+$. Purity (method A): 98.4% AUC.

*(1R,2R,3S,3aR,8bS)-1,8b-Dihydroxy-6-((2S,6S)-6-(hydroxymethyl)-1,4-dioxan-2-yloxy)-8-methoxy-3a-(4-methoxyphenyl)-N,N-dimethyl-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]cyclopenta[d]-furan-2-carboxamide (**73**).* To a solution of ester **72** (46 mg, 0.067 mmol) in MeOH (4 mL) was added 2 mL of 1 M LiOH. This solution was stirred for 16 h at 45 °C, cooled to room, and quenched with 1 N HCl (1 mL). The aqueous solution was extracted with DCM (30 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to afford the crude acid (45 mg) as a white solid. A solution of the crude carboxylic acid (45 mg, 0.0067 mmol), EDCI (31 mg, 0.20 mmol), HOBt (27 mg, 0.020 mmol), and dimethylamine HCl salt (16 mg, 0.20 mmol) in 2 mL of anhydrous CH_2Cl_2 was chilled at 0 °C. After 5 min, Hunig's base (119 μL, 0.67 mmol) was added dropwise. After 10 min at 0 °C, the solution was stirred for an additional 12 h at room temperature. The solution was concentrated and the material was purified by flash chromatography and then by HPLC to give 35 mg of desired amide. To the latter (35 mg, 0.050 mmol) in THF (3 mL) was added (10%) $Pd(OH)_2$ on carbon (5 mg). After three purge−fill cycles, the vessel was placed under 1 atm of hydrogen. After completion of the reaction, the vessel was purged and catalyst was

filtered off from Celite. The Celite was washed with THF (3 × 5 mL) and the combined filtrate was concentrated and purified on silica gel to give 12 mg of the desired product **73**. NMR δ_H (400 MHz, $CDCl_3$) 7.13 (d, J = 8.8 Hz, 2H), 7.02−7.0 (m, 3H), 6.89−6.87 (m, 2H), 6.66 (d, J = 8.8 Hz, 2H), 6.54 (d, J = 2 Hz, 1H), 6.28 (d, J = 2 Hz, 1H), 5.33 (brs, 1H), 4.95 (dd, J = 1.6, 6 Hz, 1H), 4.57 (d, J = 13.6 Hz, 1H), 4.18−4.12 (m, 1H), 4.10 (brs, 1H), 4.08 (dd, J = 13.6, 6 Hz, 1H), 3.94 (d, J = 12 Hz, 1H), 3.89−3.85 (m, 1H), 3.85 (s, 3H), 3.77−3.72 (m, 3H), 3.69 (s, 3H), 3.56−3.53 (m, 2H), 3.32 (s, 3H), 2.94 (s, 3H), 1.89−1.82 (m, 1H); ESI-MS m/z = 608.4 $[M + H]^+$. Purity (method B): 93.4% AUC.

*(1R,2R,3S,3aR,8bS)-6-Cyano-1,8b-dihydroxy-8-methoxy-3a-(4-methoxyphenyl)-N,N-dimethyl-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]cyclopenta[d]furan-2-carboxamide (**74**).* A solution of the ester **61** (95 mg, 0.195 mol, 1.0 equiv) and 0.5 mL of 2.0 M lithium hydroxide (1.0 mmol, 5.1 equiv) in 3 mL of methanol was heated at 50 °C for 2 h. The solution was cooled, acidified with 1.0 M HCl to pH 1−2, and diluted with DCM (10 mL) and water (10 mL). The organic layer was collected. The aqueous layer was extracted with DCM(2 × 10 mL). The combined organic layers were dried (Na_2SO_4). The drying agent was filtered off and the solvent was evaporated in vacuo to give 88.1 mg (0.186 mmol, 95%) of the crude acid. Then 10 mg of the crude was purified by preparative TLC on silica gel (7% MeOH in DCM) to give 6.1 mg of pure acid (ESI-MS m/z 474.1 $[M + H]^+$). To a mixture of the acid (15 mg, 0.032 mmol, 1.0 equiv), HOBt (14.5 mg, 0.10 mmol, 3.1 equiv), dimethylamine hydrochloride (15 mg, 0.18 mmol, 6.0 equiv), and EDCI (22 mg, 0.11 mmol, 3.4 equiv) in 1 mL of DCM at 22 °C was added diisopropylethylamine (34 μL, 0.19 mmol, 6.0 equiv) before diluting with DCM (2 mL) and 10% citric acid (2 mL). The organic layer was collected. The aqueous layer was extracted with DCM (5 mL). The combined organic layers were washed with saturated $NaHCO_3$ (2 mL), water (2 mL) and dried (Na_2SO_4). The drying agent was filtered off. The filtrate was concentrated in vacuo. The crude was purified by preparative TLC on silica gel (5% MeOH in DCM) and the desired product was dissolved in 2 mL of 90% *tert*-butanol, lyophilized to give 9.7 mg (0.019 mmol, 61%) of **74**. NMR δ_H (400 MHz, $CDCl_3$) 7.09−7.03 (m, 5H), 7.00 (d, J = 0.8 Hz, 1H), 6.90 (d, J = 6.8 Hz, 2H), 6.77 (d, J = 0.8 Hz, 1H), 6.67 (d, J = 9.2 Hz, 2H), 4.77 (dd, J = 5.2, 2.3 Hz, 1H), 4.67 (d, J = 13.4 Hz, 1H), 4.20 (dd, J = 13.4, 5.2 Hz, 1H), 3.91 (s, 3H), 3.69 (s, 3H), 3.36 (s, 3H), 2.97 (s, 3H), 2.09 (s, 1H); NMR δ_C (100 MHz, $CDCl_3$); 170.18, 160.28, 158.78, 157.45, 136.78, 128.60, 127.87, 127.70, 126.55, 126.23, 120.20, 118.61, 114.83, 112.85, 108.25, 107.55, 102.00, 93.72, 78.17, 77.31, 56.98, 56.14, 55.02, 46.81; ESI-MS m/z = 501.1 $[M + H]^+$. Purity (method A): 91% AUC.

*(1R,2R,3S,3aR,8bS)-6-Chloro-1,8b-dihydroxy-8-methoxy-3a-(4-methoxyphenyl)-N,N-dimethyl-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo[b]cyclopenta[d]furan-2-carboxamide (**75**).* To a solution of the ester **62** (30 mg, 0.06 mmol) in MeOH (6 mL) was added 1 mL of 1 M LiOH. This solution was stirred for 16 h at 45 °C, cooled to room temperature, and quenched with 1 N HCl (1 mL). The aqueous solution was extracted with DCM (30 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated in vacuo to afford the crude acid (29 mg) as a white solid. A solution of the crude carboxylic acid (29 mg, 0.06 mmol), EDCI (33 mg, 0.22 mmol), HOBt (29 mg, 0.022 mmol), and dimethylamine HCl salt (17 mg, 0.22 mmol) in 2 mL of anhydrous CH_2Cl_2 was chilled at 0 °C. After 5 min, Hunig's base (129 uL, 0.72 mmol) was added dropwise. After 10 min at 0 °C, the solution was stirred for an additional 12 h at room temperature. The solution was concentrated and the material was purified by HPLC to give 24 mg of the desired product **75**. NMR δ_H (400 MHz, $CDCl_3$) 7.09−7.03 (m, 5H), 6.87 (brd, J = 7.2 Hz, 2H), 6.74 (d, J = 1.6 Hz, 1H), 6.67 (d, J = 8.8 Hz, 2H), 6.51 (d, J = 1.6 Hz, 1H), 4.85 (dd, J = 1.6, 5.6 Hz, 1H), 4.60 (d, J = 13.6 Hz, 1H), 3.92 (dd, J = 6, 13.6 Hz, 1H), 4.04 (d, J = 2.4 Hz, 1H), 3.87 (s, 3H), 3.70 (s, 3H), 3.33 (s, 3H), 2.95 (s, 3H), 1.95 (s, 1H). NMR δ_C (100 MHz, $CDCl_3$) 169.88, 160.58, 158.71, 157.15, 137.33, 137.22, 128.78, 127.82, 127.77, 126.67, 126.45, 113.75, 112.83, 105.32, 104.87, 101.92, 93.84, 78.36, 56.51, 56.04, 55.09, 47.26. ESI-MS m/z = 510.1 $[M + H]^+$. Purity (method C): >95%.

*(1R,2R,3S,3aR,8bS)-3a-(4-Cyanophenyl)-1,8b-dihydroxy-6,8-dimethoxy-N,N-dimethyl-3-phenyl-2,3,3a,8b-tetrahydro-1H-benzo-[b]cyclopenta[d]furan-2-carboxamide (**76**).* To a solution of the ester **69** (25 mg, 0.051 mmol) in MeOH (3 mL) was added 0.13 mL of 2 M LiOH. This solution was stirred for 16 h at 45 °C, cooled to room temperature, and quenched with 1 N HCl (1 mL). The aqueous solution was extracted with DCM (30 mL). The organic layer was dried over Na_2SO_4, filtered, and concentrated in vacuo to afford the crude acid as a white solid. A solution of the crude carboxylic acid (23 mg, 0.049 mmol), EDCI (28 mg, 0.15 mmol), HOBt (20 mg, 0.15 mmol), and dimethylamine HCl salt (20 mg, 0.24 mmol) in 2 mL of anhydrous CH_2Cl_2 was chilled at 0 °C. After 5 min, Hunig's base (51 uL, 0.29 mmol) was added dropwise. After 10 min at 0 °C, the solution was stirred for an additional 12 h at room temperature. The solution was concentrated and the material was purified by normal phase column to give 9 mg of the desired product **76**. NMR δ_H (400 MHz, $CDCl_3$) 7.37 (d, J = 8 Hz, 2H), 7.29 (d, J = 8 Hz, 1H), 7.02−7.00 (m, 3H), 6.83−6.81 (m, 2H), 6.27 (s, 1H), 6.10 (d, J = 7 Hz, 1H), 4.87 (d, J = 6 Hz, 1H), 4.62 (d, J = 14 Hz, 1H), 3.90 (dd, J = 14, 6 Hz, 1H), 3.82 (s, 6H), 3.31 (s, 3H), 2.91 (s, 3H); NMR δ_C (100 MHz, $CDCl_3$) 169.44, 164.32, 161.0, 157.5, 141.51, 136.89, 130.86, 128.62, 128.13, 127.61, 126.89, 119.4, 110.90, 107.04, 101.61, 94.50, 92.9, 89.43, 78.88, 56.8, 55.87, 47.7, 37.5, 36.2; ESI-MS m/z = 501.03 $[M + H]^+$. Purity (method A): 98.3% AUC.

Biological Evaluation. *Stable Cell Line Generation.* The c-Myc and tubulin luciferase constructs (see Supporting Information) were subcloned into the pLenti6/R4R2/V5-DEST lentiviral expression vector following the Invitrogen Gateway cloning protocol (A11145). Lentiviral particles were generated in 293FT cells using Invitrogen's ViraPower lentiviral expression kit. MDA-MB-231 (ATCC HTB-26) cells were transduced with lentiviral supernatant according to the manufacturer's protocol. The cells were then selected with 10 μg/mL blasticidin for 5 days to select for positive clones. These stables were then maintained normally in RPMI-1640 medium supplemented with 10% fetal bovine serum.

Differential Translation Assay with c-myc 5′-UTR-Luciferase and Tubulin 5′UTR-Luciferase in MDA-MB-231 Cells. MDA-MB-231 Myc-luciferase and MDA-MB-231 tubulin-luciferase cells stably express luciferase constructs and have been selected with 10 μg/mL blasticidin. On day 1, cells were plated as follows: in a T162 flask, cells were trypsinize with 3 mL of warm trypsin until cells were detached. Then 7 mL of warm medium (RPMI 1640 + 10% FBS + Pen/Strep) was added to neutralize trypsin and cells were counted. Cells were diluted to 100 000 cells/mL and then plated 100 μL/well for a final count of 10 000 cells/well in to CoStar 3903 TC-treated 96-well white-walled polystyrene plate. Cells were incubated at 37 °C (5% CO_2). On day 2, compound dilution plate was prepared by diluting compounds from 10 mM DMSO stocks (stored at room temperaturein the dark), 1:5 in DMSO to 200× final concentration. Intermediate plate was prepared by adding 2 μL of compound stock plate into a fresh 96-well polypropylene plate. Compounds were diluted 1:20 with medium (RPMI 1640 + 10% FBS + Pen/Strep) by adding 40 μL with mixing. Compound from the dilution plate (10 uL) was added to the cells, which were then incubated at 37 °C (5% CO_2). On day 3 (luciferase assay), reconstituted Steady-Glo luciferase assay buffer with substrate (Promega E2520 Steady-Glo luciferase assay kit) (100 μL) was added to the cells, which were incubated at room temperature in the dark for 10 min. Then a white 96-well plate seal (Perkin-Elmer 6005199) was added to the bottom of the plate to prevent leakage of signal. Plates were read on an Envision 0.5s luminescence instrument. Signal is proportional to luciferase expression. On day 5 (72h MTS cell growth assay), the CellTiter 96 AQueous One solution cell proliferation assay (MTS, Promega) was used to measure the metabolic activity of cells by measuring the conversion of the MTS tetrazolium compound to a colored formazan product that is soluble in tissue culture medium. The assay can be used to measure both cell viability and growth. At the end of the incubation period, formazan absorbance is measured at 490 nm according to manufacturer's instructions. The percent inhibition with respect to compound concentration was plotted using Prism graphing

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software on a semilog plot, and EC_{50} values were determined by nonlinear regression analysis with a four-parameter logistic equation.

Cell Viability Assay on L3.6pl and RPMI-8226. L3.6pl cells were maintained in DMEM medium with 10% FBS. RPMI-8226 (ATCC CCL-155) cells were maintained in RPMI-1640 medium with 10% FBS. Cells were treated with compound for 4−24 h at 37 °C and 5% CO_2 in 10 cm tissue culture treated Petri dishes.

The CellTiter-Glo luminescent cell viability assay (Promega) measures the number of viable cells based on the cellular ATP levels. The luminescent signal is proportional to the amount of ATP present which correlates to the number of cells present. The assay is run according to the manufacturer's instructions. Briefly, the cells are plated in 96-well plates and treated with compounds as described above. After the incubation period, the combined lysis and luciferin reagent is added and luciferase is measured on a luminometer. In order to distinguish cell growth inhibition from loss of cell viability, a separate plate was taken for a time zero read that indicates starting cell number to be used to determine level of cell killing at the end of the incubation period.

The 7-aminoactinomycin D (7-AAD) (Millipore) exclusion assay was used to measure cell death. 7-AAD is a cell impermeable nucleic acid dye that is excluded from viable cells but penetrates dead or damaged cells to label DNA. Cells were treated for 72 h with compound followed by incubation with 7-AAD according to the manufacturer's instructions.

Western Blot Analysis. RPMI-8226 (ATCC CCL-155) cells were maintained in RPMI-1640 medium with 10% FBS. L3.6pl cells were maintained in DMEM medium with 10% FBS. Cells were treated with compound for 4−24 h at 37 °C and 5% CO_2 in 10 cm tissue culture treated Petri dishes. After incubation, cells were washed in PBS twice and collected by centrifugation. Cells were lysed in RIPA buffer containing protease inhibitor cocktail (∼100 μL) and incubated for 30 min on ice followed by centrifugation at 20000*g*. Protein concentrations were directly quantified using BCA protein assay kit (Pierce no. 23227) according to the manufacturer's instructions. Samples were diluted in LD loading buffer with reducing agent to a uniform concentration. Samples were denatured by boiling for 5 min. For Western blotting analysis, equal protein quantities were loaded onto a 4−12% Bis-Tris SDS−PAGE gel, and electrophoresis separation was followed by protein transfer onto PVDF membranes (Invitrogen no. LC2005). Membranes were probed with appropriate primary antibodies in BSA saturation buffer (PBS/0.1% Tween, 5% BSA) for anti-c-myc (from rabbit, diluted 1:1000, CST 5605) and antiactin (from rabbit, diluted 1:5000, Santa Cruz, CA). The secondary antibodies corresponding to anti-rabbit were coupled to HRP (from goat, diluted 1:5000, Amersham). The signal was enhanced by chemiluminescence using SuperSignal West Pico kit (Pierce no. 34080) and detected by camera (Biorad).

In Vitro Metabolism Assays and Mouse Pharmacokinetic Studies. *Plasma Stability Assay.* Test compounds and controls (propranolol) were tested at 1000 nM in frozen mouse/human plasma (Bioreclamation LLC, Westbury, NY). Compounds were spiked into mouse/human plasma preincubated to 37 °C in a heated plate shaker (Eppendorf Thermomixer R). Reaction was stopped at designated time points (0, 5, 15, 30, 45, 60, 90, and 120 min) by adding three volumes of acetonitrile containing internal standard. Samples were transferred to a 96-well filter plate (Millipore Multiscreen Solvinert 0.45 μM hydrophobic PTFE) and spun at 1550 rcf for 5 min (Eppendorf centrifuge 5804). The samples were taken and diluted with one volume of water in a 96-well autosampler plate with inert glass inserts (MicroLiter). Samples were analyzed by LC−MS/MS using a 2.0 mm × 25 mm, 5 μm Phenomenex Gemini C18 column (Torrance, CA), and samples were analyzed using an Applied Biosystems 4000QTrap mass spectrometer. Half-lives were determined by the slope of the line for the percent remaining parent compound over time course.

Microsomes Stability Assay. Test compounds and controls (propranolol) were tested at 500 nM in pooled mouse and human liver microsomes (BD Biosciences, Woburn, MA). An incubation solution was made in 100 mM phosphate buffer containing 1 mM EDTA, 3 mM of $MgCl_2$, and 0.5 mg/mL human liver microsomes. Compounds were added to a v-bottom 96-well plate (Costar, Corning, NY). Reaction solution was preincubated at 37 °C for 5 min. Reaction mixture was added to each 96-well plate. To start the reaction, NADPH was added to each plate at a final concentration of 1 mg/mL. Reaction plates were incubated at 37 °C. Reaction was stopped at designated time points (0, 5, 15, 30, 45, and 60 min) by adding two volumes of acetonitrile containing internal standard. Samples were placed in plate centrifuge (Eppendorf) and spun at 1550 rcf for 10 min. The supernatant was taken and diluted with 1 volume of water in a 96-well autosampler plate with inert glass inserts (MicroLiter). Samples were analyzed by LC−MS/MS. A 2.0 mm × 25 mm, 5 μm Phenomenex Gemini C18 column (Torrance, CA) was used, and samples were analyzed using an Applied Biosystems 4000QTrap mass spectrometer. Half-lives were determined by the slope of the line for percent remaining parent compound over time course.

Pharmacokinetic Studies. Dosing. Each compound dissolved in aqueous formulation containing 5.2% PEG-400, 5.2% Tween 80, 2% DMSO was administered intravenously or intraperitoneally to CD-1 mice. At 0.083, 0.25, 0.5, 1, 2, 4, 6, and 24 h postdose, blood was collected and processed to plasma by centrifugation and stored at −80 °C until analysis.

Bioanalytical Analysis. Plasma samples were thawed at room temperature and processed by protein precipitation with two volumes of acetonitrile containing an internal standard. After centrifugation, the supernatants of the precipitated plasma samples were diluted 1:1 with water and analyzed by LC−MS/MS. A standard curve was prepared in plasma (Bioreclamation LLC) from 0.5 to 1000 ng/mL and processed in the same way with internal standard solution as the samples. Sample analysis was performed on an Agilent 1200 series (Foster City, CA) with an API4000 QTrap mass spectrometer from Applied Biosystems (Foster City, CA). Samples were injected on an analytical column (2.0 mm × 25 mm, 5 μm Phenomenex Gemini C18 column (Torrance, CA)), and compounds eluted from the analytical column with a 5 min gradient from 30% to 95% acetonitrile in H_2O, 5 mM ammonium bicarbonate. Mass spectrometric detection of the drug as well as the internal standard was performed by MRM in negative mode. The data were acquired and processed using the software Analyst 1.4 (Applied Biosystems, Foster City, CA). The pharmacokinetics of each compound were analyzed by WinNonlin 5.0.1 (Pharsight, Mountain View, CA) via noncompartmental analysis.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Description of the procedure and physical characterization for synthetic intermediates made in Schemes 2, 3, and 6; PDF copies of physical data (^1H and ^{13}C NMR spectra, mass spectrometry, HPLC) for tested compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

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■ ABBREVIATIONS USED

4E-BP, eIF4E binding protein; Akt, protein kinase B; AUG, adenine−uracil−guanine; BIW, twice a week; c-myc, myelocytomatosis oncogene; eIF4E, eukaryotic initiation factor 4E; eIF4F, eukaryotic initiation factor 4F; LUC, luciferase; mTOR, mammalian target of rapamycin; MEK, mitogen-activated protein kinase kinase; MTS, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium; PDCD4, programmed cell death protein 4; PEG, polyethylene glycol; PTEN, phosphatase and tensin homologue; q.d., daily dosing; *rac*, racemic; Raf, rapidly accelerated fibrosarcoma kinase; Ras, rat sarcoma; UTR, untranslated region

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