Journal of Medicinal Chemistry

pubs.acs.org/jmc

Article

Evaluation of Manassantin A Tetrahydrofuran Core Region Analogues and Cooperative Therapeutic Effects with EGFR Inhibition

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Cite This: https://dx.doi.org/10.1021/acs.jmedchem.0c00151

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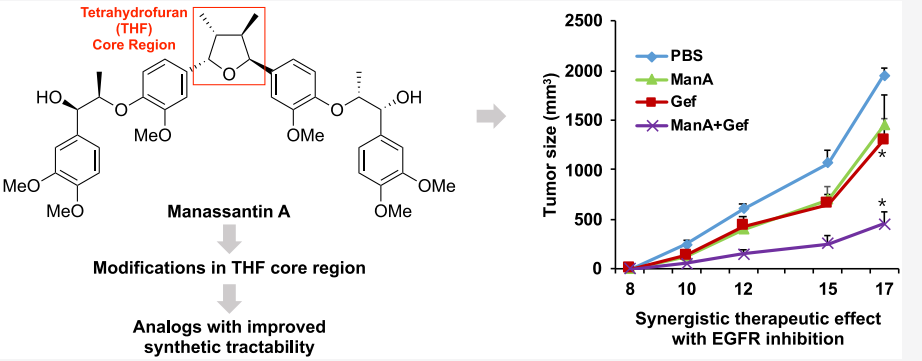

ABSTRACT: Tumors adapt to hypoxia by regulating angiogenesis, metastatic potential, and metabolism. These adaptations mediated by hypoxia-inducible factor 1 (HIF-1) make tumors more aggressive and resistant to chemotherapy and radiation. Therefore, HIF-1 is a validated therapeutic target for cancer. In order to develop new HIF-1 inhibitors for cancer chemotherapy by harnessing the potential of the natural product manassantin A, we synthesized and evaluated manassantin A analogues with modifications in the tetrahydrofuran core region of manassantin A. Our structure−activity relationship study indicated that the α,α'-*trans*-configuration of the central ring of manassantin A is critical to HIF-1 inhibition. We also demonstrated that a combination of manassantin A with an epidermal growth factor receptor inhibitor shows cooperative antitumor activity (~80% inhibition for combination vs ~30% inhibition for monotherapy). Our findings will provide important frameworks for the future therapeutic development of manassantin A-derived chemotherapeutic agents.

INTRODUCTION

According to estimates from the International Agency for Research on Cancer (IARC), there were 17.0 million new cancer cases and 9.5 million cancer deaths worldwide in 2018.[1] Globally, about 1 in 6 deaths is due to cancer. By 2040, the global burden is expected to grow to 27.5 million new cancer cases and 16.3 million cancer deaths simply due to the growth and aging of the population. The exact global economic impact of cancer is unknown but is thought to be in the hundreds of billions of dollars per year. In the United States alone, the estimated direct medical cost for cancer in 2015 was $80 billion.[2] The estimated annual health care expenditure for cancer in Europe in 2014 was €83 billion (about $110 billion).[3] Therefore, there is an urgent need to develop effective treatment strategies for a wide range of cancers.

The normal oxygen level in most mammalian tissues is 2−9%. However, hypoxia is defined by <2% of tissue oxygen levels, and it occurs in various pathological conditions, including cancer progression. Mammalian tissues utilize hypoxia-inducible factor 1 (HIF-1) to cope with the stress of hypoxia.[4] HIF-1 is an α/β heterodimeric transcription factor[5] and activates several hundred genes involved in angiogenesis (*VEGF*), glucose transport (*GLUT1*), glycolytic pathways (*LDH-A*), and other processes.[6]

HIF-1 helps tumors adapt to hypoxia, subsequently making tumors more aggressive and resistant to chemotherapy and radiation,[7−11] thereby resulting in poor clinical outcomes.[12−14] Histological and clinical evidence suggests that HIF-1 is a promising drug target for cancer.[15] Thus, it is crucial to advance our understanding of the HIF-1 regulation in tumor development and progression, which will lead to significant

Received: February 2, 2020

therapeutic benefits. However, our understanding of HIF-1 regulation is still limited due to the complexity of HIF-1 regulatory mechanisms. Therefore, a need exists for chemical probes with novel mechanisms of action to delineate the regulatory mechanisms of HIF-1 and hypoxia signaling.

The dineolignan manassantin A (**1**, Figure 1) is a natural product isolated from an aquatic plant, *Saururuscernuus* L.

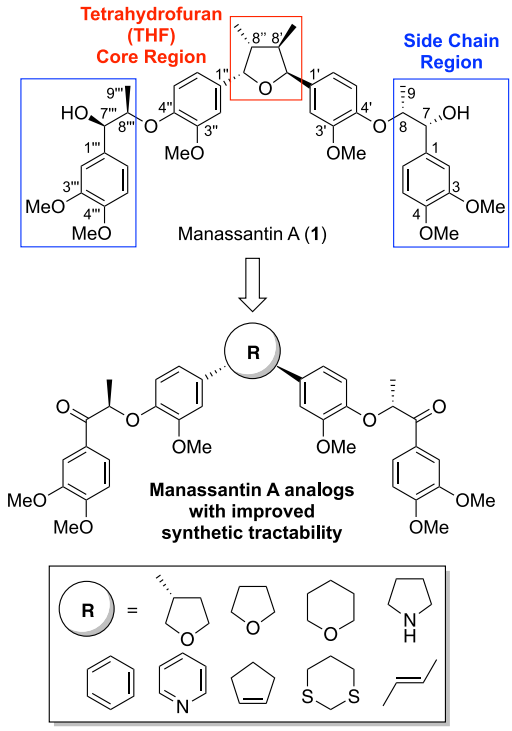

Figure 1. Structure and SAR plan of manassantin A.

(Saururaceae).[16] Manassantin A (**1**) is a potent inhibitor of HIF-1 activity (IC_{50} ~ 10 nM) *in vitro*.[17,18] Intrigued by the great potential of manassantin A as a HIF-1 chemical probe for studying the hypoxia signaling pathway, we devised a convergent synthetic route to manassantin A that is amenable to the development of manassantin A-derived chemical probes.[19] Upon the completion of manassantin A synthesis, we evaluated the potential of manassantin A as a HIF-1 inhibitor. Manassantin A potently reduces the HIF-1α protein expression under hypoxia and significantly decreases the expression of hypoxia-induced HIF-1 downstream target genes, such as vascular endothelial growth factor (*VEGF*) and cyclin-dependent kinase 6 (*Cdk6*).[20] Our preliminary structure–activity relationship (SAR) study of manassantin A analogues with modifications in the side chains identified potent manassantin A analogues with reduced structural complexity and increased ligand efficiency.[20,21]

In order to define the pharmacophore of manassantin A and develop analogues with increased potency and selectivity for future preclinical and clinical studies, it is required to define the role of the tetrahydrofuran (THF) core of manassantin A in HIF-1 inhibition. Herein, we describe our efforts toward the synthesis and SAR study of manassantin A analogues with modifications in the THF core region to guide the design of chemically more tractable manassantin A analogues for anticancer therapeutic development. We also report the cooperative antitumor activity of manassantin A with a clinically used epidermal growth factor receptor (EGFR) inhibitor.

RESULTS AND DISCUSSION

Chemistry. Manassantin A (**1**) consists of two regions (Figure 1): tetrahydrofuran (THF) core and side chain regions. Following our previous study on the role of the manassantin A side chain regions in the HIF-1 inhibition by **1**,[20] we envisioned the preparation of analogues with modifications in the THF core region in an effort to complete a comprehensive SAR of **1**. As the asymmetric synthesis of the THF core of **1** requires a lengthy synthetic sequence,[19] we also aimed to identify manassantin A analogues with reduced structural complexity and improved chemical tractability. We anticipated that our effort would help understand the effect of ring type and size, conformation, heteroatom, and hydrogen bonding on the HIF-1 inhibition by **1**. Such information will allow structural modifications to increase specificity, decrease off-target effects, and improve drug performance.

First, to evaluate the effect of the two methyl groups on HIF-1 inhibition, two tetrahydrofuran analogues (**4** and **11**) were designed. Since the C7 and C7‴ ketone analogue of **1** was as potent as **1**,[20] we prepared the corresponding ketone analogues for SAR studies. Our previous synthesis of **1**[19] provided the basis for the synthesis of the C8″ monomethyl THF analogue **4** (Scheme 1A). Starting from the known monomethyl THF **2**,[19] Bn deprotection followed by BEMP-mediated S_N2-coupling of the resulting bis-phenol with the tosylate **3** provided the desired C8″ monomethyl THF analogue **4** in 58% for two steps.

To further simplify the structure of manassantin A (**1**), we prepared the C8′ and C8″ desmethyl THF analogue **11** (Scheme 1B). The synthesis began with the cross-metathesis–isomerization[22] of the known allyl alcohol **5**[23] with methyl acrylate in the presence of Grubbs II and PMHS to afford the γ-keto ester **6**. The Corey–Bakshi–Shibata (CBS) reduction[24] of **6** afforded the (*S*)-γ-hydroxy alcohol **7** (80% ee).[25] The PPTS-catalyzed lactonization of **7** gave the lactone **8** in 86%, which was converted to the corresponding hemiketal by the addition of an aryl lithium reagent generated *in situ* from the aryl bromide **9**.[26] The reductive deoxygenation[19] of the resulting hemiketal successfully provided the 2,5-*trans*-THF **10** as a single diastereomer (49%). The stereochemistry of **10** was determined to be *trans* by NMR analysis.[19] TBS deprotection and coupling of the resulting bis-phenol with **3** completed the synthesis of the C8′ and C8″ desmethyl THF analogue **11**.

To gain an insight into the role of the ring size in HIF-1 inhibition, we prepared the tetrahydropyran (THP) analogue **15** of **1** (Scheme 1C). Treatment of the Weinreb amide **12**[27] with **9** and *n*-BuLi followed by $LiAlH_4$ reduction of the resulting 1,5-diketone afforded the 1,5-diol **13** in 56% yield. Next, for the synthesis of the THP core, the Au(III)-catalyzed cyclization reaction[28] of **13** provided the 2,6-*cis*-tetrahydropyran **14** as a single diastereomer.[29,30] Final TBS deprotection of **14** and coupling with **3** provided the desired THP analogue **15**.

The oxygen atom in the THF core of **1** is expected to play an important role in manassantin's binding to its molecular target(s) by functioning as a hydrogen bond acceptor, mediating polar interactions, or adopting a specific ring conformation. To explore the role of the oxygen atom in HIF-1 inhibition, we designed the two pyrrolidine analogues

https://dx.doi.org/10.1021/acs.jmedchem.0c00151
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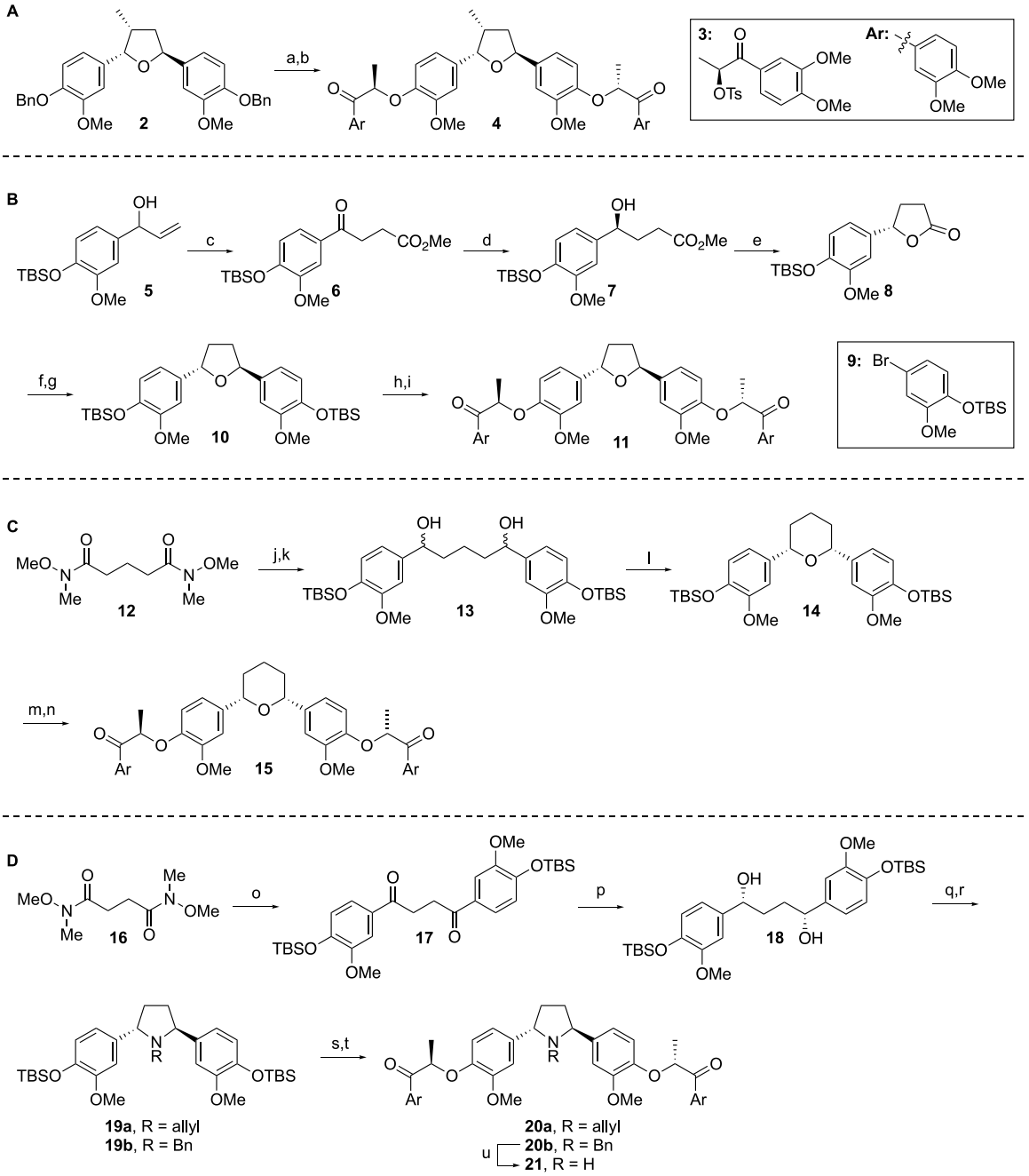
Scheme 1. Synthesis of the Analogues 4, 11, 15, 20a, and 21[a]



[a]Reagents and conditions: (a) Pd/C, H_2, EtOAc/EtOH, 25 °C, 24 h, 71%; (b) **3**, BEMP, CH_2Cl_2, 0−25 °C, 1 h, 83%; (c) methyl acrylate, Grubbs' II, PMHS, toluene, 110 °C, 30 min, 130 °C, 12 h, 45%; (d) (*R*)-2-Me-CBS-oxazaborolidine, $BH_3{\cdot}SMe_2$, THF, 0 °C, 30 min, 58%, 80% ee; (e) PPTS, toluene, 70 °C, 12 h, 86%; (f) **9**, *t*-BuLi, THF, −78 °C, 1 h, 20%; (g) $BF_3{\cdot}OEt_2$, $NaBH_3CN$, CH_2Cl_2/CH_3CN, −78 °C, 1 h, 49%; (h) TBAF, THF, 0−25 °C, 1 h, 88%; (i) **3**, BEMP, CH_2Cl_2, 0 to 25 °C, 1 h, 73%; (j) **9**, *n*-BuLi, THF, −78 °C, 4 h, 64%; (k) $LiAlH_4$, THF, 0 to 25 °C, 10 min, 56%; (l) $AuCl_3$, CH_3CN, 25 °C, 10 min, 57%; (m) TBAF, THF, 0−25 °C, 10 min, 47%; (n) **3**, BEMP, CH_2Cl_2, 0−25 °C, 1.5 h, 76%; (o) **9**, *t*-BuLi, THF, −78 °C, 6 h, 40%; (p) (*S*)-*α*,*α*-diphenyl-2-pyrrolidinemethanol, $B(OMe)_3$, $BH_3{\cdot}SMe_2$, THF, 25 °C, 1 h, 80%, 97% ee; (q) MsCl, Et_3N, CH_2Cl_2, −20 °C, 3 h; (r) RNH_2, 25 °C, 12 h, (**19a**) 73%, (**19b**) 64%; (s) TBAF, THF, 0 to 25 °C, 10 min (R = allyl, 55%; R = Bn, 82%); (t) **3**, BEMP, CH_2Cl_2, 0 to 25 °C, 1 h, (**20a**) 72%, (**20b**) 26%; (u) Pd/C, H_2, HOAc, 25 °C, 24 h, 50%.

(**20a** and **21**). As shown in Scheme 1D, the addition of an aryl lithium reagent, generated *in situ* from **9** and *t*-BuLi, to the known Weinreb amide **16**[31] gave the 1,4-diketone **17** in 40%. For the enantioselective reduction of **17**, (*S*)-*α*,*α*-diphenyl-2-pyrrolidinemethanol was used in combination with $BH_3{\cdot}SMe_2$ as a reducing agent to afford the 1,4-*syn*-diol **18** in 97% ee (see the Supporting Information for details).[32] Mesylate formation of **18** followed by cyclization in the presence of allylamine or benzylamine afforded the *N*-allyl (**19a**)- and *N*-benzyl (**19b**)-protected pyrrolidines. TBS deprotection of **19a** and **19b** followed by BEMP-mediated coupling of the resulting bisphenols and **3** successfully provided **20a** and **20b**, respectively. Treatment of **20b** with Pd/C for debenzylation gave the pyrrolidine analogue **21**.

https://dx.doi.org/10.1021/acs.jmedchem.0c00151
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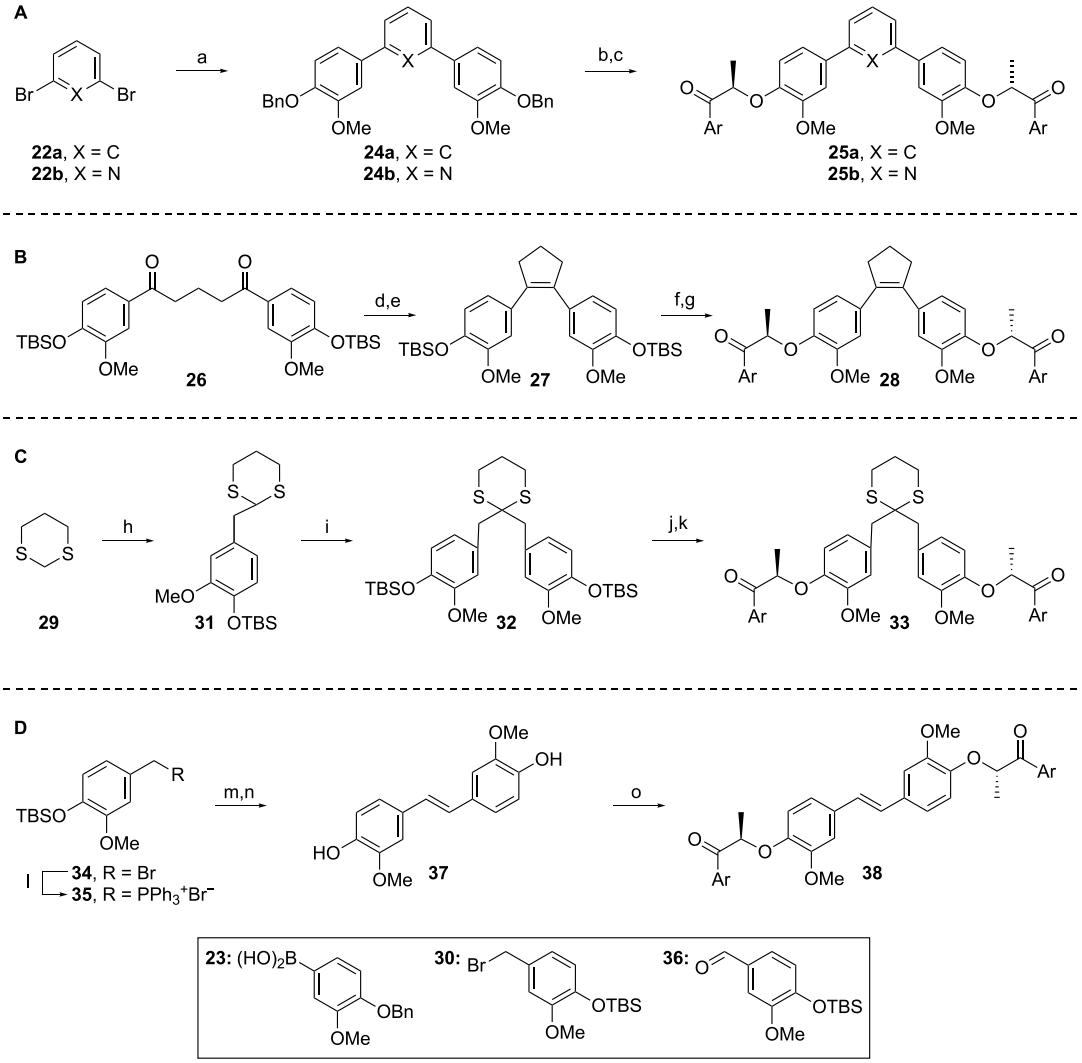
Scheme 2. Synthesis of the Analogues 25a, 25b, 28, 33, and 38[a]



[a]Reagents and conditions: (a) **23**, $Pd(dppf)Cl_2$, K_2CO_3, DMF, 60 °C, 2−4 h, (**24a**) 99%, (**24b**) 8%; (b) Pd/C, H_2, EtOH, 25 °C, 24 h, X = C: 34%, X = N: 89%; (c) **3**, BEMP, CH_2Cl_2, 0−25 °C, 1 h, (**25a**) 27%, (**25b**) 43%; (d) $TsNHNH_2$, MeOH, 60 °C, 15 h, quant.; (e) *t*-BuXphosAuCl, LiO*t*-Bu, NaBArF, 1,2-dichloroethane, reflux, 16 h, 13%; (f) TBAF, THF, 0−25 °C, 1 h, 88%; (g) **3**, BEMP, CH_2Cl_2, 0−25 °C, 1 h, 73%; (h) **30**, *n*-BuLi, THF, −78 °C, 1 h, 39%; (i) **30**, *t*-BuLi, THF/HMPA, −78 °C, 1 h, 63%; (j) TBAF, THF, 0−25 °C, 40 min, 79%; (k) **3**, BEMP, CH_2Cl_2, 0−25 °C, 1 h, 86%; (l) PPh_3, toluene, reflux, 2 h, 60%; (m) **36**, LHMDS, THF, −78 to 0 °C, 5.5 h, 8.4%; (n) TBAF, THF, 0−25 °C, 1 h, 55%; (o) **3**, BEMP, CH_2Cl_2, 0−25 °C, 1 h, 45%.

We envisioned that a chemically more tractable ring system that can recapitulate the overall linear-shaped conformation of the 2,3-*cis*-3,4-*trans*-4,5-*cis*-configuration of the THF core of manassantin A (**1**) may retain the potency of **1** toward the hypoxia signaling pathway. To test this hypothesis, we prepared several 5- and 6-membered ring analogues, **25a**, **25b**, **28**, and **33** (Scheme 2).

As shown in Scheme 2A, preparation of the benzene (**25a**) and pyridine (**25b**) analogues started with the Suzuki coupling reaction of boronic acid **23** with 1,3-dibromobenzene (**22a**) and 1,3-dibromopyridine (**22b**), respectively. Debenzylation of **24a**,**b** and BEMP-mediated coupling of the resulting bis-phenols with **3** provided the desired benzene (**25a**) and pyridine (**25b**) core analogues.

For the synthesis of the cyclopentene core analogue **28**, the 1,5-diketone **26** was treated with *p*-toluenesulfonyl hydrazide to provide the bis-tosylhydrazone intermediate (Scheme 2B). The subsequent base-promoted/gold-catalyzed intramolecular detosylative cyclization[33] provided the cyclopentene **27**. TBS deprotection, followed by BEMP-mediated coupling, completed the synthesis of **28** in 64% in two steps.

To access the dithiane core analogue **33**, we used the 1,3-dithiane umpolung chemistry. Monoalkylation of 1,3-dithiane (**29**) with the benzyl bromide **30**[34] afforded **31**, which was coupled to **30** to give the bis-alkylated dithiane **32** (Scheme 2C). Final TBS deprotection and the BEMP-mediated coupling reaction accomplished the synthesis of the dithiane core analogue **33** in a 68% yield.

Next, we replaced the THF core of **1** with a double bond to determine if the THF core is a side chain tether or serves a larger role in mediating molecular target interactions. The known benzyl bromide **34**[34] was converted to the corresponding phosphonium salt **35** by treating with PPh_3 (Scheme 2D). The Wittig reaction of **35** with aldehyde **36**,[34] followed by TBS-deprotection, produced the *trans*-alkene **37** as determined

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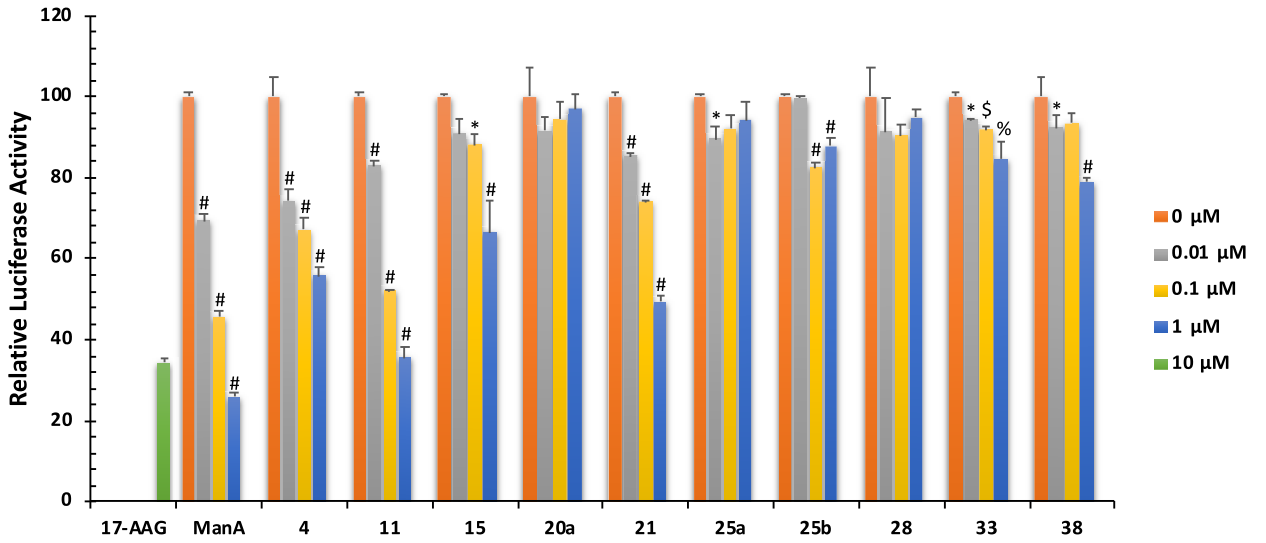

Figure 2. Activity of manassantin A (ManA) and analogues for HIF-1 inhibition. HEK-293T cells were transfected with pGL3-5HRE-VEGF-luciferase and pRL-SV40 plasmids, and treated with 0, 0.01, 0.1, and 1 μM of ManA (manassantin A) and analogues or 10 μM of 17-AAG (17-(allylamino)-17-demethoxygeldanamycin), as a positive control under hypoxia for 24 h. Relative luciferase activity was determined after normalized to the Renilla luciferase activity. Three independent experiments were performed. # $P < 0.0001$ versus vehicle control group, % $P < 0.001$ versus vehicle control group, \$ $P < 0.01$ versus vehicle control group, and * $P < 0.1$ versus vehicle control group.

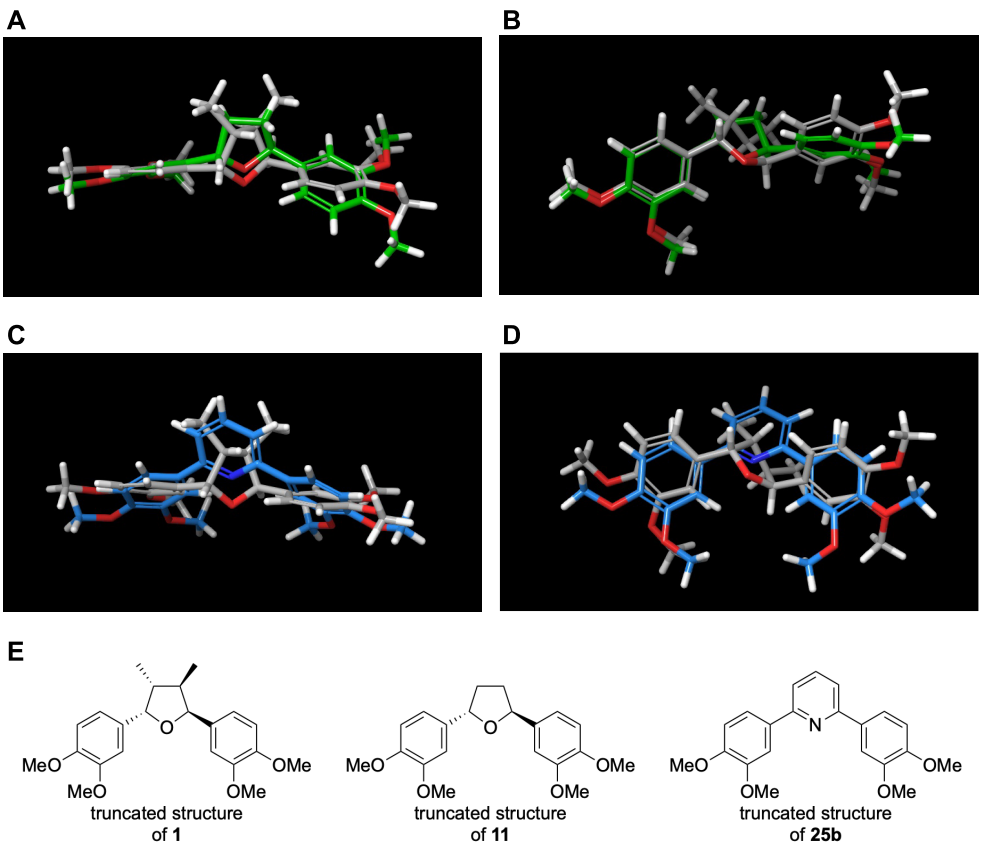

Figure 3. Overlays of optimized conformations of manassantin A (**1**) and analogues (**11** and **25b**). To avoid any unnecessary complications and/or exaggeration by the flexible side chains, truncated structures of **1**, **11**, and **25b** were used instead of the full structures. Initial geometries were determined by conformational search based on MMFF and OPLS3. (A and B) Superimposition of an active manassantin A analog (gray: **1**; green: **11**). (C and D) Superimposition of an inactive manassantin A analog (gray: **1**; sky blue: **25b**). (E) Truncated structures **1**, **11**, and **25b**.

by NMR analysis.[35] The BEMP-mediated coupling of **37** and **3** afforded the *trans*-alkene analogue **38**.

Biological Characterization. After the completion of analogue synthesis, we performed a dual luciferase-reporter assay to assess the effect of manassantin A analogues on HIF-1 transactivation activity. We used HEK-293T cells transiently transfected with both pGL3-5 × HRE-Luc and pRL-SV40 encoding Renilla-luciferase (pRen-Luc) vectors to investigate

https://dx.doi.org/10.1021/acs.jmedchem.0c00151
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the effect of manassantin analogues on HIF-1 transactivation activity. HEK-293T cells were treated with hypoxia (1% O_2) and serially diluted compounds for 24 h. The luciferase signals were normalized to the activity of Renilla luciferase and quantified as relative light units (RLU).

Several manassantin analogues such as **4**, **11**, and **21** reduced the luciferase signal in a dose-dependent manner (Figure 2). The luciferase assay provided several valuable insights into the SAR of **1**. First, the C8″ monomethyl (**4**) and C8′,8″ desmethyl analogues (**11**) potently inhibited HIF-1 transactivation activity. In particular, the potency of **11** was comparable to that of **1**, suggesting the methyl groups of the THF core of manassantin A are not crucial to HIF-1 inhibition. These analogues lacking the methyl groups, in particular **11**, significantly shortens the overall synthesis. The 2,6-*cis*-THP core analogue **15** was less potent than manassantin A. The lack of activity of **15** may be attributed to the change in ring size or conformation by the replacement of 2,5-*trans*-THF with 2,6-*cis*-THP (**11** vs **15**). The pyrrolidine analogue **21** was slightly less potent than **11**. However, the *N*-allyl substituted pyrrolidine analogue **20a** was completely inactive. This is likely due to the loss of hydrogen bonding capability or the bulkiness of the *N*-allyl group. This data indicated that the THF core of manassantin A can be replaced by an α,α'-*trans*-disubstituted ring without significant loss of activity, but the ring is required to have hydrogen bonding capability.

The chemically more tractable analogues such as the benzene and pyridine analogues (**25a** and **25b**) were completely inactive due to their flat geometry, confirming our previous finding that the relative orientation of the side chains is crucial to the activity of **1**. The cyclopentene analogue **28**, 1,3-dithiane analogue **33**, and *trans*-alkene analogue **38** were all inactive.

To characterize the effect of ring conformation of manassantin A (**1**) on HIF-1 inhibition, we optimized the conformations of truncated structures[36] of manassantin A (**1**) and analogues (**11** and **25b**) using Maestro 11.2 (Schrödinger, NY). As shown in Figure 3, manassantin A (**1**) adopted a nearly linear conformation due to the 2,5-*trans*-configuration of the THF ring. An active analogue **11** also adopted a linear conformation like **1**. However, an inactive analogue, **25b**, adopted a shape remarkably different from that of **1**. The conformational analysis of **1**, **11**, and **25b** clearly showed that the side chains are required to position in the correct orientation for high potency, which suggests that the α,α'-*trans*-configuration of **1** is critical to HIF-1 inhibition. Thus, designing a ligand that mimics the overall conformation of **1** may improve the potency toward the hypoxia signaling pathway.

Cooperative Therapeutic Effect of Manassantin A with EGFR Inhibition. Although precision-based targeting of specific growth factors/receptors and/or pathways has been effective in certain tumor types, most of the cancers, if not all, treated with targeted drugs eventually develop resistance. In addition, intratumoral heterogeneity and clonal evolution, which are common in solid tumors, are also likely to contribute to resistance to some targeted therapies. Therefore, the emergence of resistant cancer cells in several targeted therapies indicates that simultaneous administration of multiple agents may be essential for the successful treatment of human cancers.[37−39] In this regard, HIF-1 inhibitors can be extremely useful in maximizing the therapeutic benefits of standard treatments. For example, targeting blood vessels in tumors results in starvation of the tumor from oxygen and nutrients.[40] As tumor vasculatures have leaky and chaotic structures, hypoxic regions are generated again as tumors grow.[41] In turn, HIF-1α is overexpressed by recurrent hypoxia and increases the expression of HIF target genes involved in angiogenesis and chemoresistance. Therefore, we hypothesized that the use of HIF-1α inhibitors in combination with anticancer agents is likely to improve the treatment outcome.

To test the hypothesis, we evaluated a combination of gefitinib (an EGFR inhibitor) and manassantin A in a Lewis lung carcinoma (LLC) allograft experiment. The tumor microenvironment including abnormal surrounding blood vessels is an important factor determining the effect of anticancer drugs. It is highly challenging to precisely replicate the tumor microenvironment in an *in vitro* model. Therefore, we directly tested the combination of manassantin A and gefitinib in an *in vivo* lung cancer model and evaluated the efficacy of combination therapy. LLC cells were inoculated in the mouse flank subcutaneously (s.c.) and treated with manassantin A and/or gefitinib intraperitoneally (i.p.) every 3 days for 15 days. Manassantin A or gefitinib alone inhibited tumor growth by ∼30% (Figure 4A). However, manassantin A, together with gefitinib, suppressed tumor growth in a cooperative manner (∼80% inhibition). Decreased tumor weight in manassantin A-treated and/or gefitinib-treated mice showed similar phenomena (Figure 4B), whereas the body weight did not significantly change (Figure S3). Although several manassantin A-related compounds are known to be effective in tumor xenograft models,[42−44] there has been no report of the *in vivo* antitumor activity of manassantin A. To the best of our knowledge, this is also the first report of the cooperative therapeutic effect of a small molecule HIF-1 inhibitor with EGFR inhibition. Our encouraging *in vivo* testing result warrants further exploration of the therapeutic potential of manassantin A in combination with other treatment methods such as radiation and cancer immunotherapy.

CONCLUSION

To explore the therapeutic potential of manassantin A (**1**) for cancer treatment, we prepared and evaluated THF core analogues of manassantin A. Our SAR study of manassantin A analogues provided valuable insights into the role of the THF core of manassantin A in HIF-1 inhibition. Among the analogues tested, **4**, **11**, and **21** with reduced structural complexity and enhanced chemical tractability inhibited HIF-1 transactivation activity under hypoxia. Moreover, manassantin A cooperatively inhibited tumor growth in combination with an EGFR inhibitor, demonstrating its therapeutic potential in combination therapies. We expect that our findings will allow further structural modifications of manassantin A in order to enhance the potency and specificity of manassantin A analogues for novel anticancer drug development.

EXPERIMENTAL SECTION

General Chemistry Procedures. All reactions were conducted in oven-dried glassware under nitrogen. Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. All solvents were American Chemical Society (ACS) grade or better and used without further purification, except tetrahydrofuran (THF), which was freshly distilled from sodium/benzophenone each time before use. Analytical thin-layer chromatography (TLC) was performed with glass-backed silica gel (60 Å) plates

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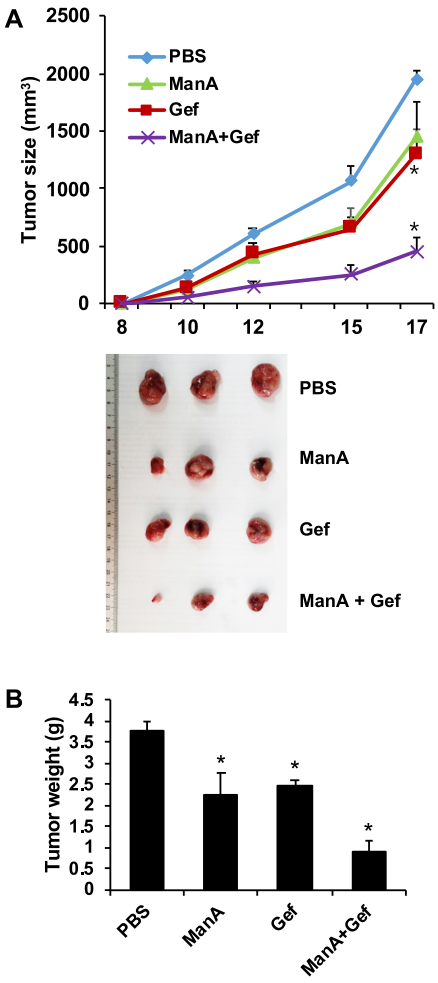

Figure 4. Manassantin A (ManA) cooperatively inhibits tumor growth in combination with an EGFR inhibitor, gefitinib (Gef). Lewis lung carcinoma cells (2×10^5) were inoculated into both flanks of C57Bl/6 mice (Day 0). From two days after inoculation, mice ($n = 5$ per group) were treated intraperitoneally with manassantin A (5 mg/kg body weight) and/or gefitinib (5 mg/kg body weight) three times a week for 17 days and then sacrificed. Tumor size (A) and tumor weight (B) were measured three times a week and on the final day, respectively. Tumor masses were photographed and shown in a box right the graph. * $P < 0.05$ versus vehicle control group.

with a fluorescent indication (Whatman). Visualization was accomplished by UV irradiation at 254 nm and/or by staining with *p*-anisaldehyde solution. Flash column chromatography was performed by using silica gel (particle size 230–400 mesh, 60 Å). All ^{1}H and ^{13}C NMR spectra were recorded with a Varian 400 (400 MHz) and a Bruker 500 (500 MHz) spectrometer. All NMR δ values are given in parts per million (ppm) and are referenced to the residual solvent signals (δ = 7.26 ppm of $CDCl_3$; δ = 2.05 ppm of CD_3COCD_3) for ^{1}H NMR spectra, or the solvent signals for ^{13}C spectra. Coupling constants (J) are given in hertz (Hz), and multiplicities are indicated using the conventional abbreviation (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet or overlap of nonequivalent resonances, br = broad). Electrospray ionization (ESI) mass spectrometry (MS) was recorded with an Agilent 1100 series (LC/MSD trap) spectrometer in order to obtain the molecular masses of compounds. Optical rotation values were measured with a Rudolph Research Analytical (A21102 API/1W) polarimeter. The purity of final compounds used in bioassays was determined by analytical HPLC and was found to be 90.7–99.6%. The analysis was performed on an HPLC (Phenomenex Kinetix C18 column, 3 × 30 mm, mobile phase A = $H_2O/CH_3OH/HCO_2H$ (100:2:0.3), and mobile phase B = $H_2O/CH_3CN/HCO_2H$ (2:100:0.3). Flow injections are in 50:50 mobile phase A/mobile phase B at 0.5 mL/min).

Synthesis of Manassantin A Analogues. *General Procedure for Bn Deprotection (GP1).* To a stirred solution of the aryl benzyl ether (1 equiv) in an appropriate solvent was added 10% palladium on activated carbon. After stirring for 24 h under a H_2 atmosphere at 25 °C, the reaction mixture was filtered through a pad of Celite, and the filtrate was concentrated *in vacuo*. The residue was purified as indicated.

General Procedure for Coupling Reaction (GP2). To a cooled (−78 °C) solution of a substrate (1 equiv) in an appropriate solvent was added dropwise *n*-BuLi (2.5 M in hexanes, 1–4 equiv) or *t*-BuLi (1.7 M in pentane, 1–4 equiv). After stirring for 1 h at −78 °C, the reaction mixture was treated with an electrophile (1–6 equiv) in anhydrous THF. After stirring for 1–6 h at −78 °C, the reaction was quenched by the addition of saturated aqueous NH_4Cl, and the resulting mixture was diluted with EtOAc. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified as indicated.

General Procedure for TBS Deprotection (GP3). To a cooled (0 °C) solution of the TBS silyl ether (1 equiv) in anhydrous THF (0.03 M) was added TBAF (1 M in THF, 3 equiv). After stirring for 5 min at 0 °C, the reaction mixture was warmed to 25 °C. After stirring for 10 min to 1 h, the reaction was quenched by an addition of saturated aqueous NH_4Cl, and the resulting mixture was extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified as indicated.

General Procedure for the Suzuki Coupling Reaction GP4. To a solution of the 1,3-dibromo benzene or pyridine (1 equiv) in anhydrous DMF (0.39 M) was added the aryl boronic acid (2 equiv), K_2CO_3 (2 equiv), and $Pd(dppf)Cl_2$ (0.5 mol %) at 25 °C. The resulting mixture was heated to 60 °C and stirred for 2–4 h. After cooling to 0 °C, the reaction was quenched by the addition of H_2O, and the resulting mixture was diluted with CH_2Cl_2. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified as indicated.

General Procedure for the BEMP-Mediated Coupling Reaction (GP5). To a cooled (0 °C) solution of the bis-phenol (1 equiv) in anhydrous CH_2Cl_2 (0.05 M) was added dropwise BEMP (2.3 equiv). The reaction mixture was stirred at the same temperature for 10 min before the tosylate **3** (2.5–4 equiv) was added. After stirring for 1 h at 25 °C, the reaction was quenched by the addition of saturated aqueous NH_4Cl and extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified as indicated.

*(2R,2′R)-2,2′-((((2S,3R,5S)-3-Methyltetrahydrofuran-2,5-diyl)bis-(2-methoxy-4,1-phenylene))bis(oxy))bis(1-(3,4-dimethoxyphenyl)-propan-1-one) (Analogue **4**).* The bis-phenol was synthesized from the aryl benzyl ether **2** (13 mg, 0.02 mmol) and 10% palladium on activated carbon in EtOAc/EtOH (3:1, 3 mL) according to GP1. Purification by column chromatography (silica gel, hexanes/EtOAc, 4:1) afforded the bis-phenol (6 mg, 71%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.95 (s, 1H), 6.92–6.84 (m, 4H), 6.78 (d, J = 8.1 Hz, 1H), 5.54 (s, 2H), 5.35–5.26 (m, 2H), 3.90 (s, 6H), 2.64–2.52 (m, 1H), 2.21–2.13 (m, 2H), 0.70 (d, J = 7.0 Hz, 3H); HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{19}H_{22}O_5$ 331.1540, found 331.1543. The analogue **4** was synthesized from the bis-phenol (10 mg, 0.03 mmol) and the tosylate **3** (44.1 mg, 0.12 mmol) according to GP5. Purification by column chromatography (silica gel, hexanes/EtOAc, 1:1) afforded the analogue **4** (18 mg, 83%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86–7.76 (m, 2H), 7.66 (s, 2H), 6.92 (s, 1H), 6.89–6.83 (m, 3H), 6.81–6.73 (m, 3H), 6.70 (d, J = 8.8 Hz, 1H), 5.46 (q, J = 4.0 Hz, 2H), 5.23 (dd, J = 7.2, 5.7 Hz, 2H), 3.95 (s, 6H), 3.93 (s, 6H), 3.83 (s, 6H), 2.55–2.48 (m, 1H), 2.15–2.07 (m, 2H), 1.70 (d, J = 7.0 Hz, 6H), 0.64 (d, J = 7.1 Hz, 3H); HRMS (ESI) m/z $[M + Na]^+$ calcd for $C_{41}H_{46}O_{11}$ 737.2932, found 737.2941; purity 95.07%, t_R 7.693 min.

https://dx.doi.org/10.1021/acs.jmedchem.0c00151
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*Methyl 4-(4-((tert-Butyldimethylsilyl)oxy)-3-methoxyphenyl)-4-oxobutanoate (**6**).* To a solution of the allyl alcohol **5** (1.3 g, 4.4 mmol) in anhydrous toluene (100 mL) were added methyl acrylate (3.9 mL, 44 mmol) and Grubbs' second-generation catalyst (186 mg, 0.22 mmol). After stirring for 30 min at 110 °C, the remaining methyl acrylate was removed *in vacuo*. PMHS (1.3 mL, 0.88 mmol) was added and stirred for 12 h at 130 °C on reflux conditions. After that, all volatiles were removed *in vacuo*, and the residue was purified by column chromatography (silica gel, hexanes/EtOAc, 8:1) to afford **6** (700 mg, 45%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.56−7.46 (m, 2H), 6.87 (d, J = 8.0 Hz, 1H), 3.84 (s, 3H), 3.69 (s, 3H), 3.27 (t, J = 6.6 Hz, 2H), 2.73 (t, J = 6.6 Hz, 2H), 0.98 (s, 9H), 0.16 (s, 6H); ^{13}C NMR (125 MHz, CD_3COCD_3) δ 196.14, 172.81, 150.98, 149.65, 131.13, 122.15, 120.27, 111.11, 54.97, 50.83, 32.71, 27.66, 25.15, 18.22, −5.28; HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{18}H_{28}O_5Si$ 353.1785, found 353.1785.

*Methyl (S)-4-(4-((tert-Butyldimethylsilyl)oxy)-3-methoxyphenyl)-4-hydroxybutanoate (**7**).* A solution of (*R*)-Me-CBS-oxazaborolidine (1 M in toluene, 0.09 mL, 0.09 mmol) was added to $BH_3{\cdot}SMe_2$ (132 μL, 1.39 mmol), and the reaction mixture was stirred under a nitrogen atmosphere at 25 °C before cooling to −20 °C. After stirring 10 min, the solution of γ-keto ester **6** (330 mg, 0.93 mmol) in anhydrous THF (15 mL) was added dropwise at −20 °C. After stirring for 30 min, the reaction was quenched by the addition of saturated aqueous NH_4Cl, and the resulting mixture was diluted with EtOAc. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with H_2O, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 100:1) to afford **7** (190 mg, 58%) as a colorless oil: ^{1}H NMR (400 MHz, CD_3COCD_3) δ 7.00 (d, J = 5.1 Hz, 1H), 6.81 (s, 1H), 6.80 (d, J = 5.2 Hz, 1H), 4.69−4.59 (m, 1H), 4.27−4.20 (m, 1H), 3.83 (s, 3H), 3.61 (s, 3H), 2.46−2.32 (m, 2H), 2.01−1.88 (m, 2H), 1.00 (s, 9H), 0.15 (s, 6H); ^{13}C NMR (125 MHz, CD_3COCD_3) δ 173.38, 150.77, 143.77, 139.40, 120.16, 117.99, 109.85, 72.20, 54.89, 50.71, 34.64, 30.12, 25.30, 18.19, −5.23; HRMS (ESI) m/z $[M + Na]^+$ calcd for $C_{18}H_{30}O_5Si$ 377.1755, found 377.1763 .

*(S)-5-(4-((tert-Butyldimethylsilyl)oxy)-3-methoxyphenyl)-dihydrofuran-2(3H)-one (**8**).* The alcohol **7** (150 mg, 0.42 mmol) in anhydrous toluene (10 mL) was treated with pyridinium *p*-toluene sulfonate (211 mg, 0.84 mmol). After stirring for 12 h at 70 °C, the reaction was quenched by the addition of H_2O, and the resulting mixture was diluted with EtOAc. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were and washed with water, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 6:1) to afford the lactone **8** (115 mg, 86%) as a colorless oil: ^{1}H NMR (400 MHz, CD_3COCD_3) δ 7.06 (br s, 1H), 6.87 (br s, 2H), 5.52−5.42 (m, 1H), 3.83 (s, 3H), 2.72−2.50 (m, 3H), 2.29−2.21 (m, 1H), 1.01 (s, 9H), 0.16 (s, 6H); ^{13}C NMR (125 MHz, CD_3COCD_3) δ 176.23, 151.17, 144.99, 133.76, 120.54, 118.44, 110.06, 81.25, 55.01, 30.77, 25.25, 18.18, −5.26; HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{17}H_{26}O_4Si$ 323.1673, found 323.1678.

*(2S,5S)-2,5-Bis(4-((tert-butyldimethylsilyl)oxy)-3-methoxyphenyl)tetrahydrofuran (**10**).* Compound **8** (60 mg, 0.18 mmol) in anhydrous THF (1 mL) was reacted with the aryl bromide **9** (79 mg, 0.25 mmol) in anhydrous THF (1 mL) and *t*-BuLi (1.7 M in pentane, 0.21 mL, 0.37 mmol) according to GP2. Purification by column chromatography (silica gel, hexanes/EtOAc, 5:1) afforded the cyclic hemiketal (20 mg, 20%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, J = 1.8 Hz, 1H), 7.44 (dd, J = 1.9, 8.2 Hz, 1H), 6.93−6.75 (m, 4H), 4.74 (t, J = 6.5 Hz, 1H), 3.84 (s, 3H), 3.78 (s, 3H), 3.03 (td, J = 6.9, 1.6 Hz, 2H), 2.50 (s, 1H), 2.17 (q, J = 6.8 Hz, 2H), 0.99 (s, 18H), 0.16 (s, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 150.74, 150.50, 150.37, 144.10, 143.64, 140.47, 140.33, 137.05, 120.53, 120.07, 119.95, 118.42, 118.34, 111.17, 110.49, 110.35, 88.75, 81.00, 55.56, 55.42, 55.29, 39.02, 34.96, 25.74, 18.45, −4.61; HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{30}H_{48}O_6Si_2$ 561.3062, found 561.3047. To a cooled (−78 °C) solution of hemiketal (27 mg, 0.04 mmol) in anhydrous CH_2Cl_2 (2 mL) was added $NaBH_3CN$ (6 mg, 0.09 mmol) in anhydrous CH_3CN (2 mL). After stirring for 10 min at −78 °C, $BF_3{\cdot}OEt_2$ (18 μL, 0.14 mmol) was added, and the resulting mixture was stirred for 1 h at −78 °C. The reaction was quenched by the addition of saturated aqueous $NaHCO_3$, and the resulting mixture was diluted with EtOAc. The layers were separated, and the aqueous layer was extracted with EtOAc. The organic layers were washed with water, dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 8:1) to afford **10** (13 mg, 49%) as a colorless oil: $[\alpha]_D^{22.9}$ −27.27 (c 0.18, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.92 (br s, 2H), 6.82 (br s, 4H), 5.16 (t, J = 6.7 Hz, 2H), 3.82 (s, 6H), 2.44−2.40 (m, 2H), 2.02−1.95 (m, 2H), 0.98 (s, 18H), 0.14 (s, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 150.88, 144.17, 137.02, 120.64, 118.04, 109.76, 81.33, 55.53, 35.73, 25.75, 18.46, −4.62; HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{30}H_{48}O_5Si_2$ 545.3113, found 545.3114.

*(2R,2′R)-2,2′-((((2S,5S)-Tetrahydrofuran-2,5-diyl)bis(2-methoxy-4,1-phenylene))bis(oxy))bis(1-(3,4-dimethoxyphenyl)propan-1-one) (Analogue **11**).* The bis-phenol was synthesized from **10** (10 mg, 0.01 mmol) according to GP3. Purification by column chromatography (silica gel, CH_2Cl_2/MeOH, 50:1) afforded the bis-phenol (5 mg, 88%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.96 (s, 2H), 6.92−6.85 (m, 4H), 5.57 (br s, 2H), 5.17 (t, J = 6.7 Hz, 2H), 3.91 (s, 6H), 2.50−2.38 (m, 2H), 2.06−1.91 (m, 2H). The analogue **11** was synthesized from the bis-phenol (5 mg, 0.01 mmol) and the tosylate **3** (23 mg, 0.06 mmol) according to GP5. Purification by column chromatography (silica gel, CH_2Cl_2/MeOH, 50:1) afforded **11** (8.1 mg, 73%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.82 (d, J = 8.7 Hz, 2H), 7.67 (s, 2H), 6.92 (s, 2H), 6.87 (d, J = 8.6 Hz, 2H), 6.76 (s, 4H), 5.44−5.36 (m, 2H), 5.14−5.06 (m, 2H), 3.93 (s, 6H), 3.91 (s, 6H), 3.85 (s, 6H), 2.41−2.32 (m, 2H), 1.92 (s, 2H), 1.71 (d, J = 7.0 Hz, 6H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 153.59, 149.87, 148.99, 146.06, 137.56, 127.37, 123.64, 117.90, 115.67, 111.26, 109.81, 81.03, 55.95, 35.49, 19.27; HRMS (ESI) m/z $[M + Na]^+$ calcd for $C_{40}H_{44}O_{11}$ 723.2913, found 723.2712; purity 93.54%, t_R 7.503 min.

*1,5-Bis(4-((tert-butyldimethylsilyl)oxy)-3-methoxyphenyl)-pentane-1,5-diol (**13**).* The 1,5-diketone was synthesized from the aryl bromide **9** (7.5 g, 24 mmol) in anhydrous THF (36 mL), *n*-BuLi (2.5 M in hexanes, 6.4 mL, 16 mmol), and the Weinreb amide **12** (873 mg, 4 mmol) in anhydrous THF (36 mL) according to GP2. Purified by column chromatography (silica gel, hexanes/EtOAc, 8:1) afforded the 1,5-diketone (1.47 g, 64%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58−7.48 (m, 4H), 6.87 (d, J = 8.0 Hz, 2H), 3.87 (s, 6H), 3.06 (t, J = 7.0 Hz, 4H), 2.22−2.12 (m, 2H), 0.99 (s, 18H), 0.18 (s, 12H); ^{13}C NMR (125 MHz, CD_3COCD_3) δ 197.74, 150.93, 149.48, 131.48, 122.14, 120.21, 111.18, 54.95, 37.06, 25.12, 19.23, 18.19, −5.31; HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{31}H_{48}O_6Si_2$ 573.3062, found 573.3069. The 1,5-diketone (200 mg, 0.34 mmol) in anhydrous THF (20 mL) was added dropwise to a stirred solution of $LiAlH_4$ (2 M in THF, 0.6 mL, 1.22 mmol) at 25 °C. The cloudy solution was stirred for 10 min and then cooled to 0 °C. The reaction was quenched by the addition of 15% NaOH solution, and the resulting mixture was diluted with EtOAc. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 50:1) to afford the 1,5-diol **13** (110 mg, 56%) as a needle-shaped white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83 (s, 2H), 6.80−6.69 (m, 4H), 4.56 (t, J = 8.0 Hz, 2H), 3.79 (s, 6H), 1.85−1.58 (m, 4H), 1.44−1.33 (m, 2H), 0.98 (s, 18H), 0.13 (s, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 151.36, 144.80, 138.69, 121.00, 118.60, 110.08, 74.86, 55.88, 39.16, 26.13, 18.84, −4.22; HRMS (ESI) m/z $[M + Na]^+$ calcd for $C_{31}H_{52}O_6Si_2$ 599.3195, found 599.3193.

*(2R,6S)-2,6-Bis(4-((tert-butyldimethylsilyl)oxy)-3-methoxyphenyl)tetrahydro-2H-pyran (**14**).* To a solution of **13** (100 mg, 0.17 mmol) in anhydrous CH_3CN (30 mL) was added $AuCl_3$ (26 mg, 0.008 mmol). After stirring for 10 min at 25 °C, the reaction

https://dx.doi.org/10.1021/acs.jmedchem.0c00151
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mixture was filtered through a pad of Celite and concentrated. The residue was purified by column chromatography (silica gel, hexanes/EtOAc, 100:1) to afford the 2,6-*cis*-tetrahydropyran **14** (55 mg, 57%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.95 (d, J = 1.9 Hz, 2H), 6.85 (dd, J = 2.0, 8.2 Hz, 2H), 6.79 (d, J = 8.1 Hz, 2H), 4.49 (d, J = 11.0 Hz, 2H), 3.80 (s, 6H), 1.88 (d, J = 13.5 Hz, 2H), 1.67−1.54 (m, 4H), 0.98 (s, 18H), 0.13 (s, 12H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 150.69, 144.12, 137.13, 120.55, 118.25, 110.20, 80.26, 55.49, 33.83, 25.77, 18.46, −4.60; HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{31}H_{50}O_5Si_2$ 559.3270, found 559.3282.

*(2R,2′R)-2,2′-((((2R,6S)-Tetrahydro-2H-pyran-2,6-diyl)bis(2-methoxy-4,1-phenylene))bis(oxy))bis(1-(3,4-dimethoxyphenyl)-propan-1-one) (Analogue **15**).* The bis-phenol was synthesized from **14** (55 mg, 0.09 mmol) according to GP3. Purification by column chromatography (silica gel, hexanes/EtOAc, 2:1) afforded the bis-phenol (19 mg, 47%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.97 (s, 2H), 6.93−6.84 (m, 4H), 5.58 (s, 2H), 4.49 (d, J = 11.0 Hz, 2H), 3.89 (s, 6H), 2.05−1.99 (m, 1H), 1.93−1.84 (m, 3H), 1.70−1.60 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 146.33, 144.78, 135.60, 118.88, 114.01, 108.78, 80.29, 55.90, 33.69, 24.46, 14.21; HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{19}H_{22}O_5$ 331.1540, found 331.1549. The analogue **15** was synthesized from the bis-phenol (19 mg, 0.05 mmol) and the tosylate **3** (83.8 mg, 0.23 mmol) according to GP5. Purification by column chromatography (silica gel, CH_2Cl_2/MeOH, 50:1) afforded **15** (30 mg, 76%) as a beige solid: ^{1}H NMR (400 MHz, CD_3COCD_3) δ 7.84 (d, J = 8.5 Hz, 2H), 7.65 (d, J = 2.2 Hz, 2H), 7.11−7.00 (m, 4H), 6.87−6.81 (m, 2H), 6.80−6.74 (m, 2H), 5.65−5.56 (m, 2H), 4.47 (d, J = 11.4 Hz, 2H), 3.89 (s, 6H), 3.85 (s, 6H), 3.82 (s, 6H), 2.01−1.90 (m, 2H), 1.87−1.80 (m, 2H), 1.59 (d, J = 6.7 Hz, 6H), 1.55−1.41 (m, 2H); ^{13}C NMR (125 MHz, CD_3COCD_3) δ 196.49, 154.00, 149.80, 149.26, 146.10, 137.90, 127.51, 123.34, 117.74, 115.14, 111.23, 110.55, 79.49, 76.75, 55.13, 33.67, 18.21; HRMS (ESI) m/z $[M + Na]^+$ calcd for $C_{41}H_{46}O_{11}$ 737.2932, found 737.2937; purity 99.59%, t_R 7.813 min.

*1,4-Bis(4-((tert-butyldimethylsilyl)oxy)-3-methoxyphenyl)-butane-1,4-dione (**17**).* The 1,4-diketone was synthesized from the aryl bromide **9** (927 mg, 2.93 mmol) in anhydrous THF (3 mL), *t*-BuLi (1.7 M in pentane, 1.15 mL, 1.95 mmol), and the known Weinreb amide **16** (100 mg, 0.48 mmol) in anhydrous THF (4 mL) according to GP2. Purification by column chromatography (silica gel, hexanes/EtOAc, 8:1) afforded the 1,4-diketone **17** (108 mg, 40%) as a yellow solid: ^{1}H NMR (400 MHz, CD_3COCD_3) δ 7.67 (dd, J = 8.2, 2.0 Hz, 2H), 7.61 (d, J = 2.0 Hz, 2H), 7.00 (d, J = 8.2 Hz, 2H), 3.91 (s, 6H), 3.45−3.35 (m, 4H), 1.01 (s, 18H), 0.20 (s, 12H); ^{13}C NMR (125 MHz, CD_3COCD_3) δ 150.64, 143.52, 140.20, 120.02, 118.05, 109.91, 73.26, 54.84, 36.10, 25.26, 18.16, −5.28; HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{30}H_{46}O_6Si_2$ 559.2906, found 559.2914.

*(1R,4R)-1,4-Bis(4-((tert-butyldimethylsilyl)oxy)-3-methoxyphenyl)butane-1,4-diol (**18**).* To a solution of (*S*)-α,α-diphenyl-2-pyrrolidinemethanol (3.8 mg, 0.01 mmol) in anhydrous THF (1 mL) was added $B(OMe)_3$ (1.98 μL, 0.01 mmol). The resulting mixture was stirred under a nitrogen atmosphere at 25 °C for 1 h and transferred to a solution of **17** (50 mg, 0.08 mmol) and $BH_3{\cdot}SMe_2$ (2 M in THF, 0.53 mL, 1.06 mmol) in anhydrous THF (1 mL). After stirring for 1 h, the reaction was quenched by the addition of 2 M HCl solution, and the resulting mixture was diluted with EtOAc. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel, CH_2Cl_2/MeOH, 50:1) to afford the 1,4-diol **18** (40 mg, 80%) as a white solid: ^{1}H NMR (400 MHz, CD_3COCD_3) δ 6.98 (br s, 2H), 6.81−6.75 (m, 4H), 4.59 (br s, 2H), 4.22 (d, J = 4.0 Hz, 2H), 3.80 (s, 6H), 1.90−1.78 (m, 2H), 1.73−1.63 (m, 2H), 1.00 (s, 18H), 0.14 (s, 12H); ^{13}C NMR (125 MHz, CD_3COCD_3) δ 150.64, 143.53, 140.22, 120.02, 118.07, 109.91, 73.28, 54.83, 36.22, 25.25, 18.15, −5.29; HRMS (ESI) m/z $[M + Na]^+$ calcd for $C_{30}H_{50}O_6Si_2$ 585.3219, found 585.3041. The enantiomeric excess of **18** was determined by chiral HPLC separation (see Figure S2 for details).

*(2S,5S)-1-Allyl-2,5-bis(4-((tert-butyldimethylsilyl)oxy)-3-methoxyphenyl)pyrrolidine (**19a**).* To a cooled (−20 °C) solution of **18** (240 mg, 0.42 mmol) in anhydrous CH_2Cl_2 (10 mL) was added Et_3N (0.29 mL, 2.13 mmol) and MsCl (0.16 mL, 2.13 mmol). After stirring for 3 h at −20 °C, an excessive amount of allylamine (3.1 mL, 4.2 mmol) was added. The resulting mixture was allowed to warm to 25 °C and stirred for 12 h. The reaction was quenched by an addition of H_2O, and the resulting mixture was diluted with CH_2Cl_2. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Purification by column chromatography (silica gel, hexanes/EtOAc, 100:1) afforded **19a** (492 mg, 73%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.01 (s, 2H), 6.88 (d, J = 8.0 Hz, 2H), 6.79 (d, J = 8.0 Hz, 2H), 5.74−5.61 (m, 1H), 4.91−4.74 (m, 2H), 3.82 (s, 6H), 3.75−3.67 (m, 2H), 3.01 (d, J = 6.8 Hz, 2H), 2.20−2.06 (m, 2H), 1.81−1.70 (m, 2H), 0.98 (s, 18H), 0.15 (s, 12H); HRMS (ESI) m/z $[M + H]^+$ calcd for C_{33} $H_{53}NO_4Si_2$ 584.3586, found 584.3594.

*(2R,2′R)-2,2′-((((2S,5S)-1-Allylpyrrolidine-2,5-diyl)bis(2-methoxy-4,1-phenylene))bis(oxy))bis(1-(3,4-dimethoxyphenyl)propan-1-one) (Analogue **20a**).* The bis-phenol was synthesized from **19a** (20 mg, 0.03 mmol) according to GP3. Purification by column chromatography (silica gel, hexanes/EtOAc, 1:1) afforded the bis-phenol (11 mg, 55%) as a colorless oil: ^{1}H NMR (400 MHz, CD_3COCD_3) δ 7.38 (s, 2H), 7.15 (s, 2H), 6.92 (d, J = 7.9 Hz, 2H), 6.79 (dd, J = 8.0, 8.0 Hz, 2H), 5.84−5.67 (m, 1H), 4.94−4.79 (m, 2H), 3.88 (s, 6H), 3.81−3.73 (m, 4H), 3.04 (d, J = 6.8 Hz, 2H), 2.19−2.08 (m, 2H), 1.80−1.68 (m, 2H); ^{13}C NMR (125 MHz, CD_3COCD_3) δ 147.42, 145.45, 136.25, 134.24, 119.87, 116.34, 114.57, 110.49, 66.41, 55.26, 52.37, 34.03; HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{21}H_{25}NO_4$ 356.1856, found 356.1864. The analogue **20a** was synthesized from the bis-phenol (44 mg, 0.12 mmol) and the tosylate **3** (270.63 mg, 0.74 mmol) according to GP5. Purification by column chromatography (silica gel, hexanes/EtOAc, 1:1) afforded the analogue **20a** (40 mg, 72%) as a white solid: ^{1}H NMR (400 MHz, CD_3COCD_3) δ 7.86 (dd, J = 8.5, 2.1 Hz, 2H), 7.66 (s, 2H), 7.17 (d, J = 8.2 Hz, 2H), 7.05 (d, J = 8.4 Hz, 2H), 6.89 (ddd, J = 10.4, 8.3, 1.9 Hz, 2H), 6.78 (dd, J = 8.1, 4.4 Hz, 2H), 5.82−5.54 (m, 3H), 4.82 (dd, J = 10.6, 18.2 Hz, 2H), 3.92−3.81 (m, 18H), 3.76 (t, J = 6.4 Hz, 2H), 2.98 (d, J = 6.9 Hz, 2H), 2.13−2.06 (m, 2H), 1.72−1.65 (m, 2H), 1.60 (d, J = 6.7 Hz, 6H); ^{13}C NMR (125 MHz, CD_3COCD_3) δ 196.53, 154.00, 150.13, 149.27, 146.04, 138.81, 134.15, 127.57, 123.36, 119.31, 116.47, 115.39, 115.24, 111.62, 111.49, 111.26, 110.70, 76.74, 66.33, 55.33, 33.99, 18.24; HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{43}H_{49}NO_{10}$ 740.3429, found 740.3446; purity 94.21%, t_R 6.147 min.

*(2S,5S)-1-Benzyl-2,5-bis(4-((tert-butyldimethylsilyl)oxy)-3-methoxyphenyl)pyrrolidine (**19b**).* To a cooled (−20 °C) solution of **18** (240 mg, 0.42 mmol) in anhydrous CH_2Cl_2 (10 mL) were added Et_3N (0.29 mL, 2.13 mmol) and MsCl (0.16 mL, 2.13 mmol). After stirring for 3 h at −20 °C, an excessive amount of benzylamine (4.58 mL, 42 mmol) was added. The resulting mixture was allowed to warm to 25 °C and stirred for 12 h. The reaction was quenched by the addition of H_2O, and the resulting mixture was diluted with CH_2Cl_2. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Purification by column chromatography (silica gel, hexanes/EtOAc, 100:1) afforded **19b** (170 mg, 64%) as a colorless oil: ^{1}H NMR (400 MHz, CD_3COCD_3) δ 7.26−7.05 (m, 4H), 6.99−6.90 (m, 4H), 6.83−6.76 (m, 3H), 3.83 (s, 3H), 3.73 (s, 3H), 3.71−3.65 (m, 2H), 3.58 (s, 2H), 2.14−2.01 (m, 2H), 2.00−1.87 (m, 2H), 1.00 (s, 18H), 0.16 (s, 12H); HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{37}H_{55}NO_4Si_2$ 634.3742, found 634.3756.

*4,4′-((2S,5S)-1-Benzylpyrrolidine-2,5-diyl)bis(2-methoxyphenol) (**20b**).* The bis-phenol was synthesized from **19b** (100 mg, 0.15 mmol) according to GP3. Purification by column chromatography (silica gel, CH_2Cl_2/MeOH, 50:1) afforded the bis-phenol (50 mg, 82%) as a colorless oil: ^{1}H NMR (400 MHz, CD_3COCD_3) δ 7.38 (s, 1H), 7.23−7.06 (m, 4H), 7.03−6.89 (m, 3H), 6.85−6.74 (m, 3H), 3.88 (s, 6H), 3.80 (s, 2H), 3.71−3.66 (m, 4H), 2.11−1.99 (m, 2H), 1.80−1.68 (m, 2H); HRMS (ESI) m/z $[M + H]^+$ calcd for

https://dx.doi.org/10.1021/acs.jmedchem.0c00151
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$C_{25}H_{27}NO_4$ 406.2013, found 406.2018. Compound **20b** was synthesized from the bis-phenol (33 mg, 0.08 mmol) and the tosylate **3** (116 mg, 0.32 mmol) according to GP5. Purification by column chromatography (silica gel, hexanes/EtOAc, 1:1) afforded **20b** (35 mg, 26%) as a white solid: ^{1}H NMR (400 MHz, CD_3COCD_3) δ 7.88 (d, J = 8.5 Hz, 2H), 7.74−7.64 (m, 2H), 7.18 (dd, J = 9.3, 2.1 Hz, 2H), 7.10−7.03 (m, 5H), 6.97−6.88 (m, 3H), 6.86−6.72 (m, 3H), 5.67−5.56 (m, 2H), 4.07 (q, J = 7.2 Hz, 2H), 3.94−3.82 (m, 18H), 3.53 (s, 2H), 2.13−2.02 (m, 2H), 1.77−1.68 (m, 2H), 1.63−1.58 (m, 6H).

*(2R,2′R)-2,2′-((((2S,5S)-Pyrrolidine-2,5-diyl)bis(2-methoxy-4,1-phenylene))bis(oxy))bis(1-(3,4-dimethoxyphenyl)propan-1-one) (Analogue **21**).* The analogue **21** was synthesized from the aryl benzyl ether **20b** (21 mg, 0.02 mmol) and 10% palladium on activated carbon in HOAc (1 mL) according to GP1. Purification by column chromatography (silica gel, CH_2Cl_2/MeOH, 50:1) afforded the analogue **21** as a beige solid (6.9 mg, 50%): ^{1}H NMR (400 MHz, CD_3COCD_3) δ 7.85 (ddd, J = 8.4, 2.0, 1.2 Hz, 2H), 7.65 (d, J = 2.1 Hz, 2H), 7.27 (s, 2H), 7.10−7.01 (m, 2H), 6.92 (dd, J = 8.2, 2.0 Hz, 2H), 6.76 (d, J = 8.2 Hz, 2H), 5.64−5.57 (m, 2H), 4.32 (br s, 2H), 3.94−3.75 (m, 18H), 2.26−2.19 (m, 2H), 1.84−1.77 (m, 2H), 1.60 (d, J = 6.8 Hz, 6H); HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{40}H_{45}NO_{10}$ 700.3116, found 700.3128; purity 98.39%, t_R 5.877 min.

*4,4′′-Bis(benzyloxy)-3,3′′-dimethoxy-1,1′:3′,1′′-terphenyl (**24a**).* The coupling of **22a** (0.04 mL, 0.39 mmol) and **23** (203 mg, 0.78 mmol) was carried out according to GP4. Purification by column chromatography (silica gel, hexanes/EtOAc, 10:1) afforded **24a** (199 mg, 99%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.70 (s, 1H), 7.54−7.28 (m, 12H), 7.22−7.05 (m, 4H), 7.04−6.89 (m, 3H), 5.21 (d, J = 8.6 Hz, 4H), 3.97 (s, 6H); HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{34}H_{30}O_4$ 503.2217, found 503.2219.

*(2R,2′R)-2,2′-((3,3′′-Dimethoxy-[1,1′:3′,1′′-terphenyl]-4,4′′-diyl)bis(oxy))bis(1-(3,4-dimethoxyphenyl)propan-1-one) (Analogue **25a**).* The bis-phenol was synthesized from the aryl benzyl ether **24a** (28 mg, 0.04 mmol) in EtOH (0.5 mL) and 10% palladium on activated carbon according to GP1. Purification by column chromatography (silica gel, hexanes/EtOAc, 3:1) afforded the bis-phenol (5 mg, 34%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.68 (s, 1H), 7.52−7.41 (m, 3H), 7.18−7.08 (m, 4H), 7.01 (dd, J = 8.2, 2.2 Hz, 2H), 5.65 (s, 2H), 3.96 (s, 6H); HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{20}H_{18}O_4$ 323.1278, found 323.1269. The analogue **25a** was synthesized from the bis-phenol (5 mg, 0.01 mmol) and the tosyl ketone **3** (14.13 mg, 0.03 mmol) according to GP5. Purification by column chromatography (silica gel, hexanes/EtOAc, 20:1) afforded the analogue **25a** (3 mg, 27%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (dd, J = 8.4, 2.0 Hz, 1H), 7.76−7.66 (m, 5H), 7.60 (s, 1H), 7.56−7.50 (m, 3H), 7.43 (d, J = 1.6 Hz, 2H), 7.10 (d, J = 2.1 Hz, 1H), 7.03 (dd, J = 8.3, 2.1 Hz, 1H), 6.93−6.82 (m, 2H), 5.51−5.43 (m, 2H), 4.09 (s, 3H), 4.07 (s, 3H), 3.94 (s, 6H), 3.92 (s, 3H), 3.89 (s, 3H), 1.76 (d, J = 6.8 Hz, 6H); HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{42}H_{42}O_{10}$ 707.2851, found 707.2865; purity 92.45%, t_R 7.921 min.

*2,6-Bis(4-(benzyloxy)-3-methoxyphenyl)pyridine (**24b**).* The coupling of **22b** (50.2 mg, 0.21 mmol) and **23** (219 mg, 0.85 mmol) was carried out according to GP4. Purification by column chromatography (silica gel, hexanes/EtOAc, 10:1) afforded **24b** (9 mg, 8%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79−7.73 (m, 2H), 7.48 (dd, J = 7.8, 1.0 Hz, 2H), 7.43−7.36 (m, 7H), 7.35−7.28 (m, 5H), 7.23 (d, J = 6.9 Hz, 1H), 6.79−6.73 (m, 2H), 5.76 (s, 4H), 4.01 (s, 6H).

*(2R,2′R)-2,2′-((Pyridine-2,6-diylbis(2-methoxy-4,1-phenylene))bis(oxy))bis(1-(3,4-dimethoxyphenyl)propan-1-one) (Analogue **25b**).* The bis-phenol was synthesized from the aryl benzyl ether **24b** (41 mg, 0.08 mmol) in EtOH (0.67 mL) and 10% palladium on activated carbon according to GP1. Purification by column chromatography (silica gel, hexanes/EtOAc, 2:1) afforded the bis-phenol (23 mg, 89%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.79 (s, 2H), 7.74 (t, J = 7.8 Hz, 1H), 7.62−7.54 (m, 4H), 7.02 (d, J = 8.2 Hz, 2H), 5.76 (br s, 2H), 4.02 (s, 6H); HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{19}H_{17}NO_4$ 324.1230, found 324.1248. The analogue **25b** was synthesized from the bis-phenol (22 mg, 0.06 mmol) and the tosyl ketone **3** (99 mg, 0.27 mmol) according to GP5. Purification by column chromatography(silica gel, hexanes/EtOAc, 1:1) afforded the analogue **25b** (21 mg, 43%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.86 (ddd, J = 8.6, 2.0, 1.1 Hz, 2H), 7.78 (dd, J = 2.2, 1.1 Hz, 2H), 7.73−7.67 (m, 3H), 7.57−7.44 (m, 4H), 6.88 (ddd, J = 8.5, 3.6, 1.1 Hz, 4H), 5.49 (q, J = 6.8 Hz, 2H), 3.97 (s, 6H), 3.95 (s, 6H), 3.93 (s, 6H), 1.78 (dd, J = 6.9, 1.2 Hz, 6H); HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{41}H_{41}NO_{10}$ 708.2803, found 708.2819; purity 91.50%, t_R 7.711 min.

*1,5-Bis(4-((tert-butyldimethylsilyl)oxy)-3-methoxyphenyl)-pentane-1,5-dione (**26**).* The 1,5-diketone **26** was synthesized from the aryl bromide **9** (7.5 g, 24 mmol) in anhydrous THF (36 mL), *n*-BuLi (2.5 M in hexanes, 6.4 mL, 16 mmol), and the Weinreb amide **12** (873 mg, 4 mmol) in anhydrous THF (36 mL) according to GP2. Purified by column chromatography (silica gel, hexanes/EtOAc, 8:1) afforded **26** (1.47 g, 64%) as a yellow oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.58−7.48 (m, 4H), 6.87 (d, J = 8.0 Hz, 2H), 3.87 (s, 6H), 3.06 (t, J = 7.0 Hz, 4H), 2.22−2.12 (m, 2H), 0.99 (s, 18H), 0.18 (s, 12H); ^{13}C NMR (125 MHz, CD_3COCD_3) δ 197.74, 150.93, 149.48, 131.48, 122.14, 120.21, 111.18, 54.95, 37.06, 25.12, 19.23, 18.19, −5.31; HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{31}H_{48}O_6Si_2$ 573.3062, found 573.3069.

*1,2-Bis(4-((tert-butyldimethylsilyl)oxy)-3-methoxyphenyl)-cyclopent-1-ene (**27**).* To the 1,5-diketone **26** (802 mg, 1.5 mmol) in MeOH was added $TsNHNH_2$ (701 mg, 3.7 mmol). After stirring for 15 h at 60 °C, the reaction mixture was concentrated under reduced pressure. The resulting solid was used in the next step without further purification. To a solution of the above hydrazide in 1,2-dichloroethane (20 mL) were added LiO*t*-Bu (185 mg, 2.3 mmol), *t*-BuXphosAuCl (12.5 mg, 0.01 mmol), and NaBArF (16.8 mg, 0.01 mmol). The reaction mixture was heated to 80 °C and stirred for 16 h under reflux conditions. The reaction mixture was cooled to 25 °C; the solvent was removed, and the residue was purified by column chromatography (silica gel, hexanes/EtOAc, 50:1) to afford **27** (52 mg, 13%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.76−6.65 (m, 6H), 3.54 (s, 6H), 2.85 (t, J = 7.2 Hz, 4H), 2.05−1.96 (m, 2H), 0.98 (s, 18H), 0.12 (s, 12H).

*(2R,2′R)-2,2′-(((Cyclopent-1-ene-1,2-diylbis(2-methoxy-4,1-phenylene))bis(oxy))bis(1-(3,4-dimethoxyphenyl)propan-1-one) (Analogue **28**).* The bis-phenol was synthesized from **27** (51 mg, 0.09 mmol) according to GP3. Purification by column chromatography (silica gel, hexanes/EtOAc, 2:1) afforded the bis-phenol (25.5 mg, 88%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.82−6.75 (m, 4H), 6.69−6.60 (m, 2H), 3.65 (s, 6H), 2.85 (t, J = 7.2 Hz, 4H), 2.02−1.96 (m, 2H). Analogue **28** was synthesized from the bis-phenol (11 mg, 0.03 mmol) and the tosylate **3** (51 mg, 0.14 mmol) according to GP5. Purification by column chromatography (silica gel, hexanes/EtOAc, 1:1) afforded analogue **28** (18 mg, 73%): ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (d, J = 8.0 Hz, 2H), 7.65 (s, 2H), 6.88 (d, J = 8.8 Hz, 2H), 6.68−6.66 (m, 6H), 5.39 (q, J = 4.0 Hz, 2H), 3.94 (s, 6H), 3.91 (s, 6H), 3.51 (s, 6H), 2.79 (t, J = 7.2 Hz, 4H), 1.98−1.94 (m, 2H), 1.69 (d, J = 6.7 Hz, 6H); HRMS (ESI) m/z [M + Na]$^+$ calcd for $C_{41}H_{44}O_{10}$ 719.2827, found 719.2842; purity 94.57%, t_R 8.094 min.

*(4-((1,3-Dithian-2-yl)methyl)-2-methoxyphenoxy)(tert-butyl)-dimethylsilane (**31**).* Compound **31** was synthesized from 1,3-dithiane (**29**) (58 mg, 0.48 mmol) in anhydrous THF (4 mL), *n*-BuLi (2.5 M in hexanes, 0.19 mL, 0.48 mmol), and the benzyl bromide **30** (161 mg, 0.48 mmol) in anhydrous THF (0.9 mL) according to GP2. Purification by column chromatography (silica gel, hexanes/EtOAc, 20:1) afforded **31** (71 mg, 39%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.77 (d, J = 7.9 Hz, 1H), 6.72 (d, J = 2.0 Hz, 1H), 6.69 (dd, J = 8.0, 2.1 Hz, 1H), 4.21 (t, J = 7.3 Hz, 1H), 3.79 (s, 3H), 2.93 (d, J = 7.3 Hz, 2H), 2.90−2.77 (m, 4H), 2.17−2.06 (m, 1H), 1.92−1.79 (m, 1H), 0.98 (s, 9H), 0.14 (s, 6H); HRMS (ESI) m/z [M + H]$^+$ calcd for $C_{18}H_{30}O_2S_2Si$ 371.1529, found 371.1532.

*((((1,3-Dithiane-2,2-diyl)bis(methylene))bis(2-methoxy-4,1-phenylene))bis(oxy))bis(tert-butyldimethylsilane) (**32**).* Compound **32** was synthesized from the dithiane **31** (21 mg, 0.05 mmol) in THF/HMPA (10:1, 0.7 mL), *t*-BuLi (1.7 M in pentane, 33 μL, 0.05

https://dx.doi.org/10.1021/acs.jmedchem.0c00151
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mmol), and the benzyl bromide **30** (28 mg, 0.08 mmol) in anhydrous THF (0.4 mL) according to GP2. Purification by column chromatography (silica gel, hexanes/EtOAc, 20:1) afforded **32** (22 mg, 63%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.86 (d, J = 1.7 Hz, 2H), 6.81−6.71 (m, 4H), 3.78 (s, 6H), 3.11 (s, 4H), 2.79−2.68 (m, 4H), 1.89−1.77 (m, 2H), 0.99 (s, 18H), 0.15 (s, 12H); HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{32}H_{52}O_4S_2Si_2$ 621.2918, found 621.2904.

*(2R,2′R)-2,2′-(((((1,3-Dithiane-2,2-diyl)bis(methylene))bis(2-methoxy-4,1-phenylene))bis(oxy))bis(1-(3,4-dimethoxyphenyl)-propan-1-one) (Analogue **33**).* The bis-phenol was synthesized from **32** (20 mg, 0.03 mmol) according to GP3. Purification by column chromatography (silica gel, hexanes/EtOAc, 3:1) afforded bis-phenol (10 mg, 79%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.93−6.74 (m, 6H), 5.55 (br s, 2H), 3.87 (s, 6H), 3.13 (s, 4H), 2.89−2.77 (m, 4H), 1.98−1.79 (m, 2H); HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{20}H_{24}O_4S_2$ 393.1189, found 393.1184. Analogue **33** was synthesized from the bis-phenol (10 mg, 0.02 mmol) and the tosyl ketone **3** (35 mg, 0.1 mmol) according to GP5. Purification by column chromatography (silica gel, hexanes/EtOAc, 1:1 to 1:2) afforded analogue **33** (17 mg, 86%) as a colorless oil: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (d, J = 8.4 Hz, 2H), 7.66 (s, 2H), 6.87 (d, J = 8.5 Hz, 2H), 6.85 (s, 1H), 6.77−6.61 (m, 4H), 5.40 (q, J = 4.0 Hz, 2H), 3.93 (s, 6H), 3.91 (s, 6H), 3.79 (s, 6H), 3.04 (s, 4H), 2.82−2.63 (m, 4H), 1.86−1.76 (m, 2H), 1.71 (d, J = 6.7 Hz, 6H); HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{42}H_{48}O_{10}S_2$ 777.2762, found 777.2764; purity 92.42%, t_R 7.780 min.

*Bromo(4-((tert-butyldimethylsilyl)oxy)-3-methoxybenzyl)-triphenyl-l5-phosphane (**35**).* To a solution of the benzyl bromide **34** (675 mg, 2.04 mmol) in anhydrous toluene (8 mL) was added PPh_3 (1.6 g, 6.11 mmol). The reaction mixture was heated to 80 °C and stirred for 2 h under reflux conditions. The reaction mixture was cooled to 25 °C, and the precipitate was filtered and purified by column chromatography (silica gel, CH_2Cl_2/MeOH 20:1) to afford the phosphonium salt **35** (720 mg, 60%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.84−7.50 (m, 15H), 6.84 (t, J = 2.2 Hz, 1H), 6.59 (d, J = 8.1 Hz, 1H), 6.43 (dt, J = 8.2, 2.5 Hz, 1H), 5.27 (d, J = 13.7 Hz, 2H), 3.46 (s, 3H), 1.93 (m, 9H), 0.07 (m, 6H).

*(E)-1,2-Bis(4-((tert-butyldimethylsilyl)oxy)-3-methoxyphenyl)-ethene (**37**).* To a cooled (−78 °C) solution of **35** (211 mg, 0.36 mmol) in anhydrous THF (2 mL, 0.5 M) was added LHMDS (1 M in THF, 0.36 mL, 0.36 mmol) dropwise. The resulting mixture was stirred at the same temperature for 20 min and treated with a solution of aldehyde **36** (142 mg, 0.53 mmol) in anhydrous THF (1 mL). After stirring for 5.5 h at 0 °C, the reaction was quenched by the addition of saturated aqueous NH_4Cl solution and diluted with EtOAc. The layers were separated, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, hexanes/EtOAc 30:1) to afford the corresponding TBS-protected *trans*-alkene (15 mg, 8.4%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.01 (d, J = 1.9 Hz, 2H), 6.95 (dd, J = 2.0, 8.2 Hz, 2H), 6.90 (s, 2H), 6.82 (d, J = 8.1 Hz, 2H), 3.86 (s, 6H), 1.00 (s, 18H), 0.17 (s, 12H); HRMS (ESI) m/z $[M + NH_4]^+$ calcd for $C_{28}H_{44}O_4Si_2$ 518.3116, found 518.3124. The bis-phenol **37** was synthesized from the TBS-protected *trans*-alkene (83 mg, 0.16 mmol) according to GP3. Purification by column chromatography (silica gel, hexanes/EtOAc 2:1) afforded **37** as a white solid (24 mg, 55%): ^{1}H NMR (400 MHz, CD_3COCD_3) δ 7.64 (s, 2H), 7.17 (d, J = 1.7 Hz, 2H), 6.98 (s, 2H), 6.97−6.96 (m, 2H), 6.79 (d, J = 8.1 Hz, 2H), 3.88 (s, 6H); HRMS (ESI) m/z $[M + H]^+$ calcd for $C_{16}H_{16}O_4$ 273.1121, found 273.1120.

*(S)-1-(3,4-Dimethoxyphenyl)-2-(4-((E)-4-(((R)-1-(3,4-dimethoxyphenyl)-1-oxopropan-2-yl)oxy)-3-methoxystyryl)-2-methoxyphenoxy)propan-1-one (Analogue **38**).* Analogue **38** was synthesized from the bis-phenol **37** (25 mg, 0.09 mmol) and the tosylate **3** (134 mg, 0.36 mmol) according to GP5. Purification by column chromatography (silica gel, hexanes/EtOAc, 10:1) afforded the analogue **38** (27 mg, 45%) as a white solid: ^{1}H NMR (400 MHz, CD_3COCD_3) δ 7.91 (dd, J = 8.4, 1.7 Hz, 2H), 7.72 (d, J = 2.0 Hz, 2H), 7.27 (d, J = 1.9 Hz, 1H), 7.12 (dd, J = 8.5, 1.4 Hz, 2H), 7.09 (d, J = 1.5 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 6.86 (dd, J = 8.4, 1.4 Hz, 5H), 5.78−5.68 (m, 2H), 3.97 (s, 6H), 3.94 (s, 6H), 3.93 (s, 6H), 1.69 (d, J = 6.7 Hz, 6H); HRMS (ESI) m/z $[M + Na]^+$ calcd for $C_{38}H_{40}O_{10}$ 679.2519, found 679.2522; purity 90.65%, t_R 7.557 min.

Dual Luciferase-Reporter Assay. HEK-293T cells were seeded on 96-well plates at a density of 5 × 10^3 cells/well and allowed to attach for 24 h. Cells were transfected with a pGL3-5 × HRE-Luc plasmid containing five copies of HREs in human *VEGF* promoter and pRL-SV40 plasmid encoding Renilla luciferase using the Vivamagic Transfection Reagent (Vivagene, Seongnam, Korea), according to the manufacturer's instructions. Cells were treated with hypoxia (1% O_2) and serially diluted compounds for 24 h after transfection. The luciferase-reporter assay was performed using the Dual-Glo Luciferase Assay System (Promega, Madison, WI, USA) according to the manufacturer's instructions. Briefly, an equal volume of the Dual-Glo Reagent to the culture medium was added to each well, and the mixture was incubated for 10 min. The firefly luminescence was measured using a microplate reader (Infinite M200 Pro, TECAN, Männedorf, Switzerland). The Dual-Glo Stop & Glo Reagent was added, and the mixture was incubated for 10 min before the measurement of Renilla luminescence. The firefly luminescence signals were normalized to the activity of Renilla luciferase. Three independent experiments were performed.

Molecular Overlay. The molecular overlay of manassantin A analogues was generated based on a total number of 2 analogues that were tested for their activity against HIF-1 (Figure 2). The molecular structures were sketched and built with Maestro 11.2 (Schrödinger, NY). Low-energy conformations of manassantin A analogues were generated by LigPrep. LigPrep used the force field OPLS3e and charges in all ligand preparation steps. All possible protonation and ionization states were enumerated for each ligand at pH 7.4. Only the lowest energy conformer was kept for each ligand. Ligand conformation sampling was done by using the MacroModel module of Maestro. The extended cutoff of the MacroModel (van der Waals, 8.0; electrostatic, 20.0; and H-bond, 4.0) was used. The superimpose model was generated by using the Structure Alignment module of Schrödinger Glide. The superimposition of conformers was developed with the "atom pair" method in the Structure Alignment module.

Mice. Animal experiments were performed using C57BL/6J mice (Japan SLC, Shizuoka, Japan), which were handled in strict compliance with the guidelines for care and use of laboratory animals issued (KNU 2017-145) by the Institutional Ethical Animal Care Committee of Kyungpook National University (Daegu, Korea).

***In Vivo* Animal Study.** To generate a Lewis lung carcinoma (LLC) allograft tumor model, suspensions of LLC cells (2 × 10^5 cells in 200 μL of serum-free culture media) were implanted subcutaneously into the dorsal flank regions of 5−6 week old male C57BL/6 mice (Day 0). From 2 days after inoculation, mice (n = 5 per group) were treated with manassantin A (5 mg/kg body weight) and/or gefitinib (5 mg/kg body weight) intraperitoneally three times a week for 17 days. Body weights were measured three times a week for 17 days. Tumor volumes (cm^3) were calculated by multiplying the height by length by depth. Eventually, the mice were anesthetized, and their tumor tissues were harvested at the completion of the study period.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c00151.

Determination of enantiomeric excess of **7** and **18**, body weight, and HPLC traces of final compounds (PDF)

SMILES representations and relative luciferase activity (CSV)

https://dx.doi.org/10.1021/acs.jmedchem.0c00151
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Author Contributions

S.-H.K., T.N.S., H.L., S.M.M., J.H.K., and D.-Y.K. synthesized manassantin A and analogues used in *in vitro* and *in vivo* testing. S.-H.K. performed conformational analysis. H.-E.L. performed *in vivo* testing of manassantin A. Y.G. performed the *in vitro* luciferase assay of manassantin A and analogues. Y.M.L. designed *in vitro* and *in vivo* testing and analyzed the data. J.H. conceived the project, designed the overall experimental strategy, and analyzed and discussed the results. J.H., Y.M.L., and S.-H.K. wrote the manuscript with input from all of the authors.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the American Cancer Society (122057-RSG-12-045-01-CDD to J.H.) and the Korean National Research Foundation (NRF-2017R1A2B3002227 to Y.M.L.). T.N.S. was supported by the NSF Graduate Research Fellowship Program.

ABBREVIATIONS USED

BEMP, 2-*tert*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine; CDK6, cyclin-dependent kinase 6; EGFR, epidermal growth factor receptor; GLUT1, glucose transporter 1; HEK, human embryonic kidney; HIF-1, hypoxia-inducible factor; HRE, hypoxia response element; i.p., intraperitoneally; LLC, Lewis lung carcinoma; PMHS, polymethylhydrosiloxane; RLU, relative light unit; SAR, structure−activity relationship; s.c., subcutaneously; THF, tetrahydrofuran; THP, tetrahydropyran; VEGF, vascular endothelial growth factor

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