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Synthesis of the Fungal Metabolite YWA1 and Related Constructs as Tools to Study MelLec-Mediated Immune Response to *Aspergillus* Infections†

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ABSTRACT: We describe the chemical synthesis of the fungal naphthopyrones YWA1 and fonsecin B, as well as their functionalization with an amine-spacer arm and the conjugation of the resulting molecules to three different functional tags (i.e., biotin, Oregon green, 1-[3-(succinimidyloxycarbonyl)benzyl]-4-[5-(4-methoxyphenyl)-2-oxazolyl]pyridinium bromide (PyMPO)). The naphthopyrone-biotin and -PyMPO constructs maintained the ability to bind the C-type lectin receptor MelLec, whose interaction with immunologically active fungal metabolites (i.e., 1,8-dihydroxynaphthalene-(DHN)-melanin and YWA1) is a key step in host recognition and induction of protective immune responses against *Aspergillus fumigatus*. The fluorescent Fonsecin B-PyMPO construct **21** was used to selectively visualize MelLec-expressing cells, thus validating the potential of this strategy for studying the role and functions of MelLec in immunity.

INTRODUCTION

Naphthopyrones are important aromatic polyketides displaying a characteristic polyhydroxynaphthalene system fused to a pyran-4-one ring. These fungal metabolites have attracted a great deal of interest due to their broad range of biological activities, encompassing immunomodulatory,[1] antiproliferative,[2] cytotoxic,[3] and antibacterial[4] properties (Figure 1a). The hemiketal naphthopyrone YWA1, whose structure was unambiguously determined by spectroscopic methods two decades ago,[5] was isolated from the human opportunistic pathogen *Aspergillus fumigatus* and other fungal species expressing specific types of nonreducing polyketide synthases (NR-PKS). These multidomain enzymes assemble acyl and malonyl units in a polyketide chain and successively catalyze regiospecific cyclization/aromatization reactions to convert the polyketide chain into the YWA1 aromatic scaffold.[6,7] YWA1 is the first intermediate in the biosynthetic pathway leading to 1,8-dihydroxynaphthalene-(DHN)-melanin (Figure 1b), a highly insoluble conidial pigment formed by polymerization of 1,8-DHN monomers that confers fungal cell resistance against the host immune response, oxidative stress, and ultraviolet light.[8] Although melanin represents an important protective factor contributing to fungal virulence and pathogenesis, we recently demonstrated that recognition of DHN-melanin components by the host immune system has a crucial role in the control of *A. fumigatus* infections in both mice and humans.[9] Fungal DHN-melanin and its metabolic precursors (YWA1, THN, and DHN) are sensed by the host immune system through the C-type lectin receptor (MelLec).[9] Our findings indicate that the interaction of MelLec with immunologically active components of DHN-melanin is a key step in host recognition and induction of protective immune responses against *A. fumigatus*. To investigate the interaction of the melanin-sensing receptor MelLec with YWA1 and evaluate how structural modifications of the naphthopyrone scaffold could affect the ligand–receptor binding properties, we have now developed the first synthesis of YWA1. To date, YWA1—whose structure offers very limited options for chemical modification—could only be obtained in

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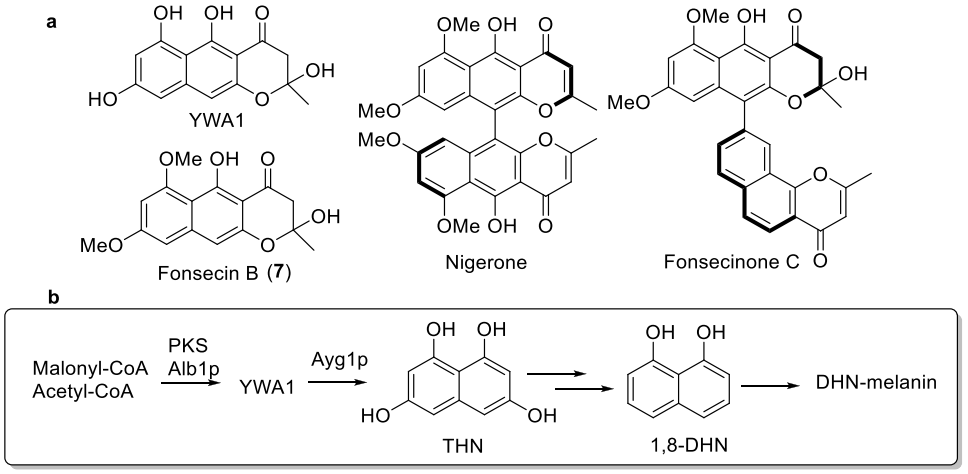
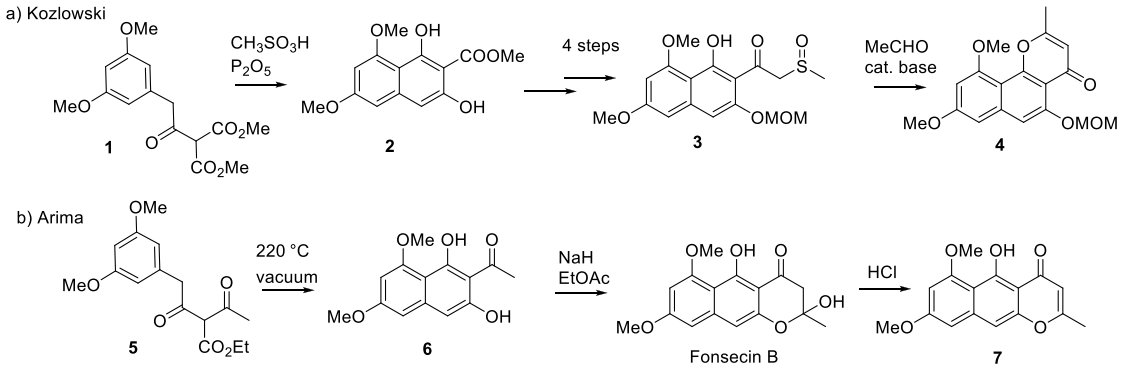
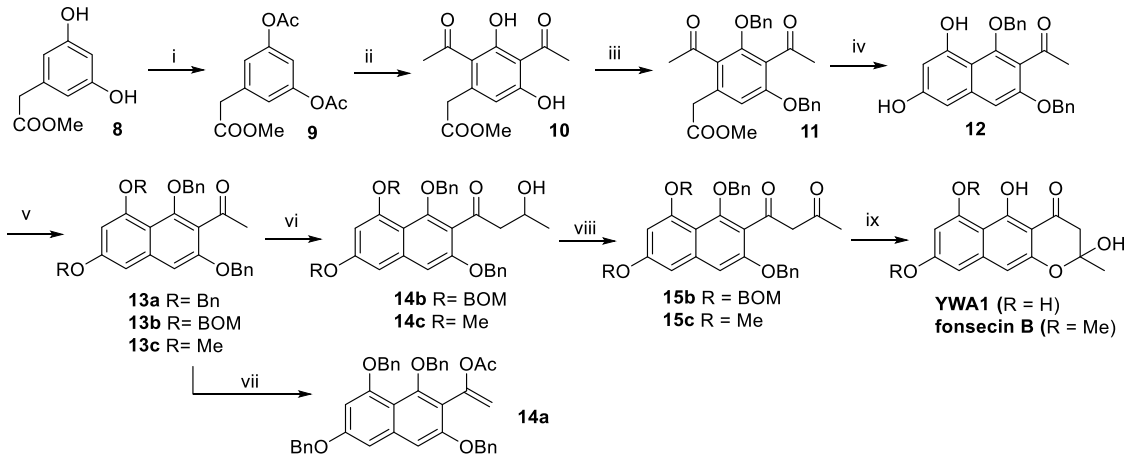


Figure 1. (a) Structures of YWA1 and other biologically active fungal metabolites incorporating a naphthopyrone system: fonsecin B (stabilizing ligand of c-myc G-quadruplex DNA),[2] nigerone (cytotoxic agent),[3] and fonsecinone C (antibiotic).[4] (b) Biosynthetic pathway to DHN-melanin.

Scheme 1. Examples of Syntheses of Polyketide Naphthopyrones



Scheme 2. Syntheses of YWA1 and Fonsecin B[a]



[a]Reagents and conditions: (i) Ac_2O, pyridine, r.t., overnight (70%); (ii) $AlCl_3$, 150 °C, 1.5 h, and then MeOH, H_2SO_4, r.t., 4 h (60%); (iii) BnBr, K_2CO_3, acetone, 60 °C, overnight (70%); (iv) lithium bis(trimethylsilyl)amide (LiHMDS), tetrahydrofuran (THF), 0 °C, 2 h (90%); (v) (a) BnOH, PPh_3, diisopropyl azodicarboxylate (DIAD), Et_2O, r.t., 4 h for compound **13a** (17%); (b) BOMCl, *N*,*N*-diisopropylethylamine (DIPEA), CH_2Cl_2, r.t., overnight for compound **13b** (50%); (c) Me_2SO_4, K_2CO_3, acetone, 60 °C, overnight for compound **13c** (70%); (vi) LiHMDS, THF, −40 °C, acetaldehyde, 15 min (90%); (vii) LiHMDS, THF, 0 °C, Ac_2O, 15 min (50%); (viii) Dess−Martin periodinane (DMP), $CHCl_3$, 40 °C; (ix) H_2, $Pd(OH)_2$ 20 wt % on C, AcOH, MeOH, r.t., 3−12 h (90%).

tiny amounts by extraction from *Aspergillus* conidia. Despite the biological importance of polyketide naphthopyrones, very few methods have been described for their preparation,[10−13] and none of these turned out to be suitable for our aims, which were (i) to have ready access to sufficient quantities of YWA1 for biological studies and (ii) to generate YWA1-based functional chemical probes for studying the activity of MelLec in cells, with the view of enabling the study of MelLec-

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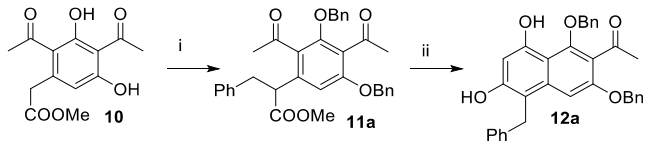
mediated immune responses in vivo and the biosynthesis of DHN-melanin in fungi.

Herein, we report (1) a concise synthesis of YWA1 and some of its structural analogues, which are recognized by MelLec protein;[9] (2) the functionalization of the YWA1 scaffold with an amine-spacer arm and its conjugation with Oregon green (**19**), biotin (**20**), and 1-[3-(succinimidyloxycarbonyl)benzyl]-4-[5-(4-methoxyphenyl)-2-oxazolyl]-pyridinium bromide (PyMPO) (**21**), without significantly altering its ability to bind MelLec; and (3) the capacity of the fluorescent conjugate **21** to specifically visualize MelLec in cell cultures.

RESULTS AND DISCUSSION

Syntheses of YWA1 and Fonsecin B. Existing synthetic procedures for the preparation of tricyclic oxygen-heterocycles, similar to YWA1, generally start with the formation of a naphthalene unit followed by condensation of a pyran-4-one ring.[10−12] In their work on the total synthesis of nigerone, Kozlowski et al. used a 2-naphthoate precursor (**2**) to generate the naphthopyrone heterocycle **4** (Scheme 1a).[10] According to the authors, the formation of the pyrone ring was the most challenging part of the synthesis, where the obvious disconnection involving addition of acetone to the methyl ester of **2** failed to supply the requisite pyrone ring and an alternative approach—based on the addition of a dimesyl anion and condensation of acetaldehyde—was instead required. Our attempts to adapt this strategy to prepare YWA1 from the naphthol intermediate **2** were unsuccessful, mainly because the pyrone ring could not be obtained in its hemiketal form, characteristic of YWA1. Additionally, this route seemed to lack the synthetic flexibility we required to develop YWA1 analogues suitable for bioconjugation to chemical handles and functional probes. In earlier studies based on the preparation of naphthopyrones, Arima and coworkers used a carboxy-diketone precursor (**5**) to access the 2-acetyl naphthol **6**, which was then readily converted into Fonsecin B by Claisen condensation with EtOAc (Scheme 1b).[11] Although this latter strategy seemed particularly well-suited for the synthesis of our target molecule, numerous attempts to convert **5** into the desired **6** turned out to be unsuccessful in our hands. In a more recent publication,[6] a concise approach to the 2-acetyl naphthol unit **12** was described (Scheme 2), comprising (i) the Fries rearrangement of phenyl diacetate **9**, (ii) benzylation of the key intermediate **10**, and (iii) Dieckmann's cyclization of the nonsymmetrical penta-substituted phenyl ester **11**. Following this procedure and optimizing the reaction conditions for the Fries rearrangement, we successfully obtained **12** in satisfactory yields. When the Fries rearrangement was conducted using $AlCl_3$ in refluxing dichloroethane (DCE) for 72 h, as previously reported, we could only obtain small quantities of the desired product **10** (15% yield). A considerable amount of the unreacted starting material was recovered along with a complex mixture of byproducts resulting from the hydrolysis of the *O*-acetyl/methyl ester groups. Significant improvements were made by treating neat **9** with $AlCl_3$ at 150 °C for 1 h and then performing a Fisher esterification to repristinate the methyl ester partially hydrolyzed during the acylation step. O-Benzylation of **10** followed by intramolecular Dieckmann condensation of **11** afforded the desired naphthol **12**. During the O-benzylation step, the acidic character of the methylene protons of **10** led to the formation of the overalkylated side-product **11a** (Scheme 3). The nature of the solvent was found to have a remarkable effect on the chemoselectivity of the process, with O-benzylation being favored in acetone, while C-alkylation waspredominant in dimethylformamide (DMF). Interestingly, the overalkylated byproduct **11a** could also undergo intramolecular Dieckmann cyclization, giving the 5-benzyl substituted naphthalene analogue **12a** (Scheme 3). The newly formed hydroxyl groups of **12** were then fully protected before testing the formation of the hemiketal pyrone ring. While O-methylation of **12** to **13c** was achieved smoothly, O-benzylation proved to be particularly laborious. Treatment of **12** with BnBr under different reaction conditions did not afford the desired fully protected intermediate **13a**, while Mitsunobu alkylation gave low yields (17%). In both cases, C-alkylation of the naphthol ring in position 5 was the main side-reaction observed.

Scheme 3. Synthesis of 12a[a]



[a]Reagents and conditions: (i) BnBr, K_2CO_3, DMF, r.t., 4 h (95%); (ii) LiHMDS, THF, −40 °C, acetaldehyde, 2 h (90%).

Better results were obtained using the benzyloxymethyl (BOM) group as an alternative to benzyl protection (**13b**). With the protected 2-acetyl naphthols **13b**, **13c** in our hands, we then tried to access the final product YWA1 and its methylated analogue fonsecin B by Claisen condensation with EtOAc and subsequent hydrogenolysis of the protecting groups. Surprisingly, numerous attempts to install a diketone moiety via Claisen condensation were unsuccessful. A variety of solvents, bases, and temperatures were screened to condense **13b**, **13c** to EtOAc, but only the starting material was recovered, while an enol acetate was formed (**14a**, see the Supporting Information) when Ac_2O and LiHMDS were used. Applying a different strategy based on (i) aldol condensation of **13b**, **13c** with acetaldehyde, (ii) oxidation of β-hydroxyketones **14b**, **14c**, and (iii) final hydrogenolysis of the resulting 1,3-diketones **15b**, **15c**, we finally accomplished the synthesis of YWA1 (10.7% overall yield) and its *O*-dimethyl analogue fonsecin B (15.0% overall yield), both in eight steps. Various procedures were tested for oxidizing the β-hydroxyketone group in **14b**, **14c** (Swern, Parikh−Doering, pyridinium chlorochromate (PCC), 2-iodoxybenzoic acid (IBX)), but only DMP led to the conversion of the secondary alcohol into the desired diketone moiety.

Design and Synthesis of Bioconjugated Derivatives. This synthetic strategy was used to functionalize YWA1 with an amine-spacer arm and perform a chemoselective ligation of the naphthopyrone scaffold to different tags (Scheme 4). Aldol condensation of the fully protected intermediate **13b**, **13c** with 8-azido octanal **22** and subsequent oxidation of the β-hydroxyketones **16b**, **16c** with DMP (or IBX for **16c**) afforded the desired diketones **17b**, **17c**. Hydrogenolysis of the protecting groups of **17c**, concomitantly with azide reduction, quantitatively produced intermediate **18b**, while cleavage of the BOM protecting groups from **17b** was impaired by the presence of the newly formed amino group.

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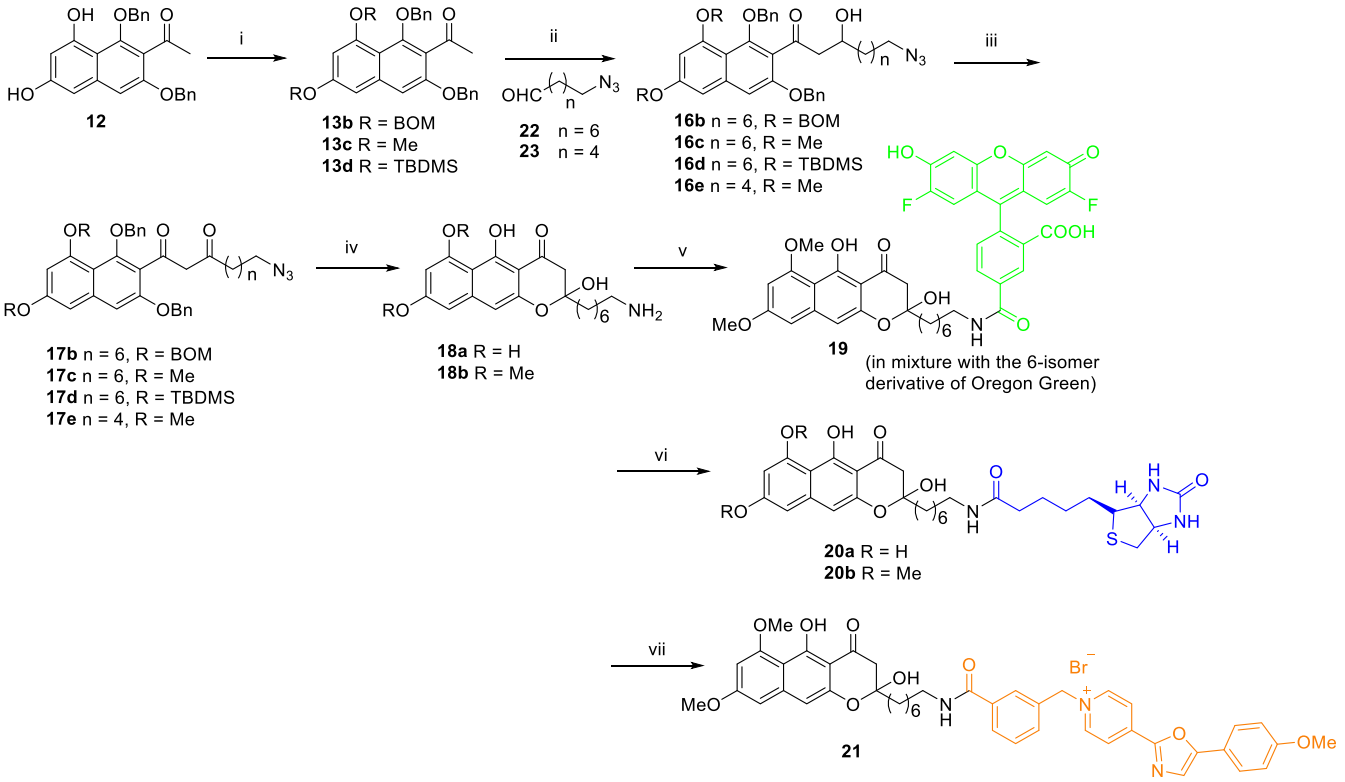
Scheme 4. Synthesis of Bioconjugated Derivatives[a]



[a]Reagents and conditions: (i) (a) BOMCl, DIPEA, CH_2Cl_2, r.t., overnight for compound **13b** (50%); (b) Me_2SO_4, K_2CO_3, acetone, 60 °C, overnight, for compound **13c** (70%); (c) *tert*-butyldimethylsilyl chloride (TBDMSCl), DIPEA, DMF, r.t., overnight for compound **13d** (99%); (ii) lithium diisopropylamide (LDA), THF, −78 °C, 45 min, and then 22, −78 °C, 15 min (99%); (iii) DMP, $CHCl_3$, 40 °C or IBX, EtOAc, 80 °C overnight (for **16c**); (iv) (a) tetra-*n*-butylammonium fluoride (TBAF), THF, 0 °C, 3 h, and then H_2, $Pd(OH)_2$ on carbon 20%, AcOH, MeOH, r.t., 3 h for compound **18a**; (b) H_2, $Pd(OH)_2$ on carbon 20%, AcOH, MeOH, r.t., 3 h for compound **18b**; (v) Oregon Green-*N*-hydroxysuccinimide (NHS), DIPEA, DMF, r.t., 0.5 h (50%); (vi) biotin-NHS, DIPEA, DMF, r.t., 0.5 h (50%); (vii) PyMPO-NHS, DIPEA, DMF, r.t., 0.5 h (60%).

Attempts to force the removal of the BOM groups by adding more catalyst or acetic acid led to the formation of a complex mixture of byproducts. To overcome this problem, we decided to protect the hydroxyl groups of **12** as *tert*-butyldimethylsilyl (TBDMS) ethers. The protected intermediate **13d** was successfully condensed with 8-azido octanal to afford **16d**, which was then oxidized to give the diketone **17d**. Treatment of **17d** with TBAF followed by hydrogenolysis of the Bn groups afforded **18a**. The final intermediates (**18a**, **18b**) equipped with an amine-spacer arm were successfully conjugated to the NHS active esters of Oregon green (commercial mixture of 5- and 6-isomers), biotin, and PyMPO affording the constructs **19** (9.2% overall yield), **20a** (13.0% overall yield), **20b** (9.2% overall yield), and **21** (11.0% overall yield), each in nine steps. It should be noted that fonsecin B and derivatives are more stable than YWA1 counterparts, and therefore more reliable tools for performing biological studies, as OH-group methylation prevents oxidation to the corresponding quinones.

It is also worth noting that the spacer's arm length is important for the synthetic accessibility of these constructs: hydrogenolysis of an azido diketone analogue **17e** synthesized using ω-azido hexanal **23**, instead of ω-azido octanal **22**, produced a complex mixture of unidentified products.

Biology. We have previously shown that MelLec is able to recognize 1,8-DHN-melanin and other structural isomers in the DHN-melanin biosynthetic pathway due to the presence of the naphthalene-diol unit.[9] To test our compounds, we used the Fc-MelLec probe (the extracellular domain of MelLec, fused to the Fc-portion of human Ig),[9] which was pretreated with or without YWA1, 1,8-DHN, fonsecin B, 2′,6′-dihydroxyacetophenone (2,6-DHAP), **21**, and **20b**, and then incubated with *A. fumigatus* ΔrodA conidia (Figure 2). Fc-MelLec bound to the conidia was detected with allophycocyanin (APC)-conjugated donkey antihuman antibody. Binding of Fc-MelLec to these compounds was determined by flow cytometry, where lack of a signal on the *A. fumigatus* conidia indicates inhibition of binding through competitive inhibition. As we had described previously,[9] YWA1 inhibited binding of Fc-MelLec to ΔrodA conidia, whereas the structurally unrelated 2,6-DHAP had no effect on binding. Notably, fonsecin B and conjugates, **20b** and **21**, also inhibited the recognition of A. fumigatus ΔrodA conidia by Fc-MelLec. This shows that addition of the amine-spacer arm and subsequent conjugation do not impair the ability to bind the receptor. Immunofluorescence experiments showed that, compared to the untreated cells, RAW 264.7 cells expressing hMelLec can bind to the fluorescent conjugate **21** (Figure 3), and the binding is concentration-dependent (data not shown). The binding of **21** was shown to be specific by treating RAW 264.7 cells expressing hMelLec in the presence of competing unlabeled YWA1 (Figure 3), which resulted in a strong decrease of fluorescence at the highest concentrations, such as 25 and 50 μM (for further competition experiments and data, see Figure S1, Supporting Information).

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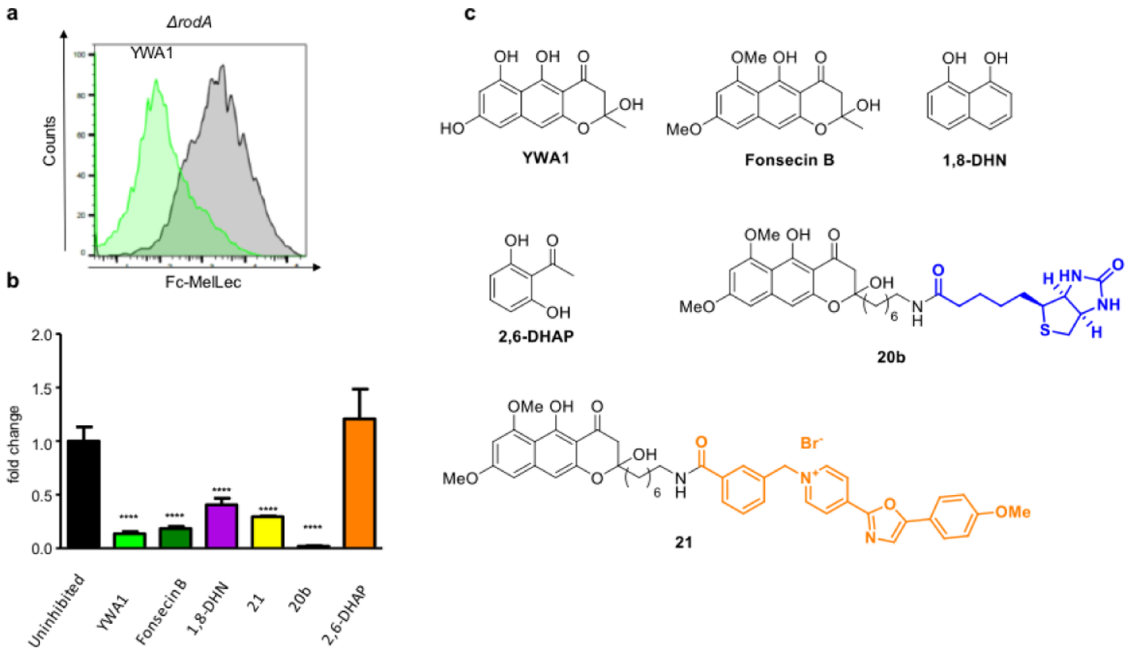

Figure 2. Fc-MelLec binds to 1,8-DHN-melanin precursors and other structurally related bioconjugates. (a) Flow cytometry histogram showing that YWA1 (1,8-DHN-melanin precursor) inhibits recognition of *A. fumigatus* Δ*rodA* conidia by Fc-MelLec (green) compared to the uninhibited Fc-MelLec control (gray). (b) Inhibition of Fc-MelLec binding to Δ*rodA* conidia of *A. fumigatus* by 1,8-DHN-melanin precursors (YWA1, 1,8-DHN), Fonsecin B, and the bioconjugates **21** and **20b** compared to the uninhibited Fc-MelLec control. 2,6-DHAP is included as a negative control as it lacks the naphthol unit. The experiment was repeated independently twice, with similar results. Two technical replicates were used for each sample in each experiment. Data shown here is pooled from the two independent experiments. Fold change was calculated by dividing the mean value of pretreated Fc-MelLec with each compound by the mean value of the uninhibited Fc-MelLec. Values show mean ± standard error of the mean (SEM); ****$p < 0.05$ (one-way analysis of variance (ANOVA) and Bonferroni post hoc test comparing inhibition of Fc-MelLec binding to Δ*rodA* conidia by the different compounds versus uninhibited Fc-MelLec). (c) Structures of the compounds tested.

CONCLUSIONS

We described an efficient synthesis of YWA1 and other structurally related fungal naphthopyrones. The flexibility of the synthetic approach allowed us to functionalize the YWA1 scaffold with an amine-spacer arm and conjugate the resulting molecules to three different functional tags (i.e., biotin, Oregon green, PyMPO) without impairing the ability to bind to MelLec. The ability of MelLec to recognize YWA1 and its structural analogues was determined by flow cytometry experiments, using an Fc-MelLec probe.[9] The capacity to bind to MelLec was determined as the ability of naphthopyrone ligands to inhibit the interaction of Fc-MelLec with melanin components localized on the surface of *A. fumigatus* conidia. The fluorescent probe **21** was then used to selectively visualize MelLec-expressing cells using fluorescence microscopy, thus validating the potential of this strategy for studying *Aspergillus* infections. These compounds therefore offer exciting new tools to study the cellular and immunological functions of MelLec in vitro and in vivo.

EXPERIMENTAL SECTION

General Information. Dry solvents were obtained from commercial sources and used without further purification. The reactions performed under a nitrogen atmosphere were carried out in dry solvents. Reactions requiring heating were performed using an oil bath. Reactions were monitored by thin-layer chromatography (TLC), unless otherwise noted. TLCs were performed on Merck silica gel glass plates (60 F_{254}). Visualization was accomplished by UV light (254 nm) or staining with ceric ammonium molybdate or $KMnO_4$ solution. Flash chromatography was performed manually using silica gel (60 Å, particle size 40−63 μm) purchased from Merck or automatically using Interchim PuriFlash instrument and normal-phase silica columns. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE III 400 NMR spectrometer and calibrated using residual undeuterated solvent as an internal reference. ^1H δ = 7.26 ($CDCl_3$), ^{13}C δ = 77.16 ($CDCl_3$), ^1H δ = 3.31 (CD_3OD), ^{13}C δ = 49.15 (CD_3OD), ^1H δ = 2.05 ($(CD_3)_2CO$), ^{13}C δ = 29.84 ($(CD_3)_2CO$), ^1H δ = 2.50 ($(CD_3)_2SO$), ^{13}C δ = 39.52 ($(CD_3)_2SO$). ^{19}F NMR spectra were recorded on a Bruker AVANCE III 400 NMR spectrometer and were referenced to $CFCl_3$. ^{13}C NMR spectra were recorded with complete proton decoupling. Chemical shifts (δ) are reported in parts per million (ppm), coupling constants (J) are given in hertz (Hz), and multiplicity is described using the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad; or combinations thereof. Mass analyses were performed using an Agilent 1200 high-performance liquid chromatography (HPLC) system coupled to an Agilent G6120 single quadrupole detector equipped with an electrospray ionization (ESI) source in direct infusion modality. ESI-MS spectra were recorded in the positive mode. Reverse-phase (RP)-HPLC-MS analyses were performed with an Agilent 1200 HPLC system equipped with a diode array detector (DAD) and an ESI-MS detector. High-resolution mass spectra (HRMS) were recorded using a Thermofisher Q-Exactive orbitrap ion trap mass spectrometer. HPLC conditions for analytical analyses: Phenomenex Luna C18 column, 5 μm, 100 Å, 250 × 4.6 mm^2 (L × ID) injected volume 10 μL, flow rate 1 mL/min, unless otherwise specified. HPLC preparative purification was performed using an Agilent 1260 HPLC system equipped with a DAD detector. HPLC conditions for preparative purification were as follows: Phenomenex Luna C18 column, 5 μm, 100 Å, 250 × 21.2 mm^2 (L × ID), flow rate 20 mL/min.

*5-(2-Methoxy-2-oxoethyl)-1,3-phenylene Diacetate (**9**).* Commercially available methyl 3,5-dihydroxyphenylacetate (12.0 g, 65.9 mmol, 1.0 equiv) was dissolved in Ac_2O (31.2 mL, 0.33 mol, 5.0 equiv); then, pyridine (26.5 mL, 0.33 mol, 5.0 equiv) was added dropwise at 0 °C. The reaction was stirred at room temperature (r.t.)

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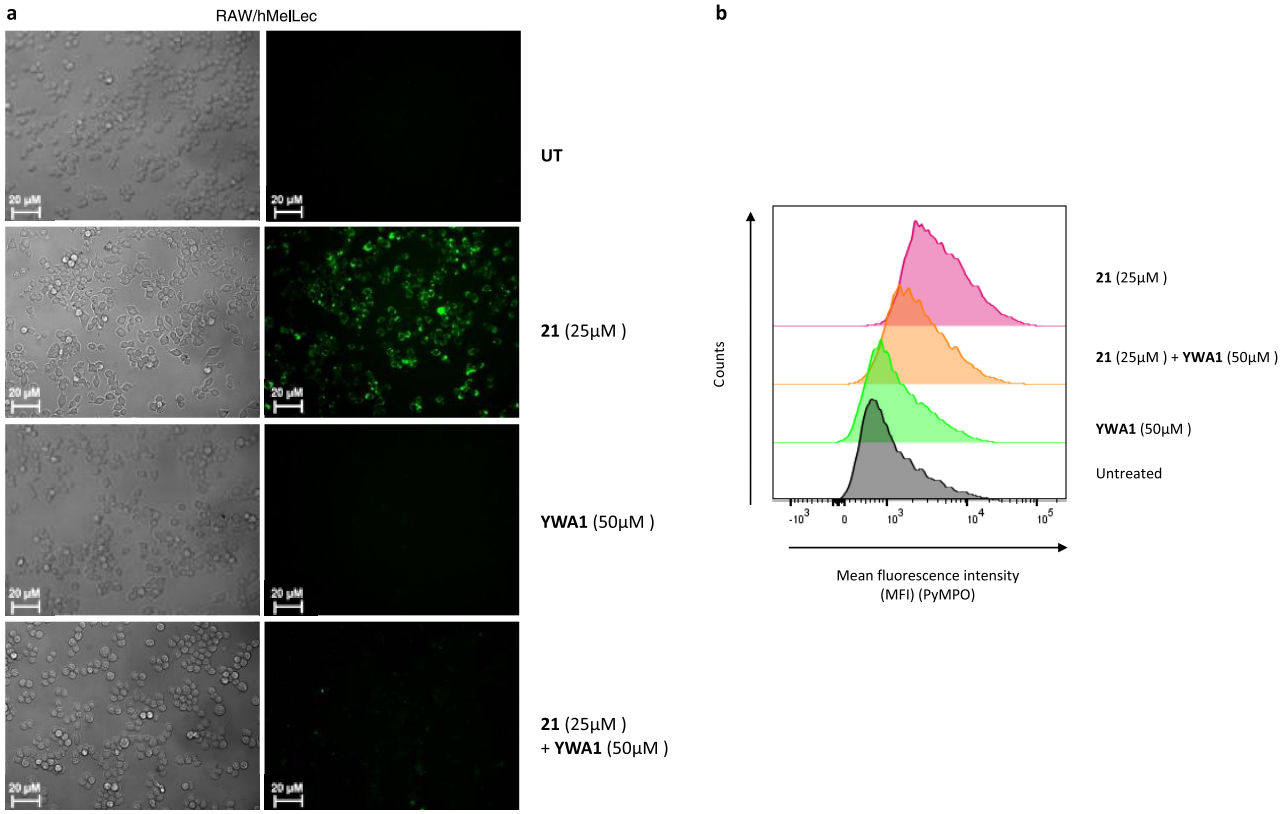

Figure 3. Specificity control of the binding of the fluorescent conjugate **21** to hMelLec-expressing cells with or without competition by YWA1. (a) Representative light microscopy images and immunofluorescence micrographs of hMelLec-expressing RAW 264.7 cells untreated or treated with 25 μM concentration of **21** (green) for 3 h at 37 °C 5% CO_2 with or without YWA1 (50 μM) or with YWA1 (50 μM) alone. (b) Histograms showing the binding of MelLec-expressing RAW 264.7 cells to **21** compared to untreated cells, or treated with YWA1 alone, or treated with both **21** and YWA1, as determined by flow cytometry.

overnight, quenched with 10 mL of an ice-cold aqueous solution of HCl (2 M), and extracted with Et_2O (3 × 100 mL). The combined organic phases were washed with 50 mL of brine and 20 mL of an aqueous solution of HCl (1 M), dried over Na_2SO_4, and concentrated under vacuum. The crude product was purified by flash chromatography (EtOAc/*n*-hexane gradient from 10% EtOAc to 40% EtOAc) to afford compound **9** as a pale-yellow oil (12.0 g, 70% yield). R_f = 0.6, *n*-hexane/EtOAc, 1:1. Analytical and spectroscopic data were consistent with those reported in the literature.[14]

*Methyl 2-(2,4-Diacetyl-3,5-dihydroxyphenyl)acetate (**10**).* A mixture of $AlCl_3$ (5.20 g, 39.4 mmol, 3.5 equiv) and **9** (3.0 g, 11.2 mmol, 1.0 equiv) was stirred at 150 °C for 1.5 h. After cooling, the reaction was poured into a solution of ice and aqueous HCl (2 M) and the whole suspension was stirred for 10 min. The mixture was extracted with EtOAc (3 x 100 mL), and the combined organic phases were dried over Na_2SO_4 and concentrated under vacuum. To repristinate the partially hydrolyzed ester, the crude mixture was submitted to Fisher esterification using H_2SO_4 (0.6 mL, 11.2 mmol, 1.0 equiv) in MeOH (20 mL) and stirring at room temperature for 4 h. The product was then extracted with EtOAc (3 × 100 mL), washed with brine (50 mL), and dried over Na_2SO_4. The solvent was removed under vacuum, and the crude product was purified by automated flash chromatography (100% DCM) to give product **10** as a white solid (1.9 g, 60% yield). R_f = 0.5, *n*-hexane/EtOAc, 1:1. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 15.33 (s, 1H), 13.94 (br, 1H), 6.35 (s, 1H), 3.92 (s, 2H), 3.76 (s, 3H), 2.77 (s, 3H), 2.64 (s, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ: 205.6, 203.6, 170.4, 169.5, 168.6, 143.3, 116.4, 114.1, 109.6, 52.6, 42.1, 33.6, 31.9. MS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{13}H_{15}O_6$ 267.08; found 267.1. Analytical and spectroscopic data were consistent with those reported in the literature.[14]

*Methyl 2-(2,4-Diacetyl-3,5-bis(benzyloxy)phenyl)ace-tate (**11**).* To a suspension of **10** (1.10 g, 4.20 mmol, 1.0 equiv) and K_2CO_3 (1.20 g, 8.40 mmol, 2.0 equiv) at room temperature in acetone (25 mL), BnBr (1.4 g, 8.4 mmol, 2.0 equiv) was added. The suspension was heated to 60 °C and stirred overnight, quenched with 10 mL of an ice-cold aqueous solution of HCl (1 M), and extracted with Et_2O (3 × 100 mL). The combined organic phases were washed with brine (50 mL), dried over Na_2SO_4, and concentrated under vacuum. The crude product was then purified by automated flash chromatography (EtOAc/*n*-hexane gradient from 10% EtOAc to 40% EtOAc) to afford compound **11** as a white solid (1.3 g, 70% yield). R_f = 0.3, *n*-hexane/EtOAc, 7:3. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.46−7.34 (m, 10H), 6.70 (s, 1H), 5.14 (s, 2H), 4.85 (s, 2H), 3.74 (s, 2H), 3.71 (s, 3H), 2.57 (s, 3H), 2.54 (s, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ: 204.0, 201.3, 171.2, 156.5, 154.3, 136.0, 135.7, 135.1, 129.6, 128.7, 128.6, 128.5, 128.3, 127.3, 125.8, 111.2, 79.5, 70.7, 52.2, 38.6, 32.5, 32.2. MS (ESI) *m*/*z*: $[M + H]^+$ calcd for $C_{27}H_{27}O_6$ 447.17; found 447.2. Analytical and spectroscopic data were consistent with those reported in the literature.[14]

*Methyl 2-(2,4-Diacetyl-3,5-bis(benzyloxy)phenyl)-3-phenylpro-panoate (**11a**).* To a suspension of **10** (1.10 g, 4.20 mmol, 1.0 equiv) and K_2CO_3 (1.70 g, 12.6 mmol, 3.0 equiv) in DMF (25 mL), BnBr (2.5 g, 14.7 mmol, 3.5 equiv) was added. This suspension was stirred at room temperature for 4 h, quenched with 10 mL of an ice-cold aqueous solution of HCl (1 M), and extracted with Et_2O (3 × 30 mL). The organic phase was washed with brine (10 mL), dried over Na_2SO_4, and concentrated under vacuum. The mixture was then purified by automated flash chromatography (EtOAc/*n*-hexane gradient from 10% EtOAc to 40% EtOAc) to afford compound **11a** as a white solid (2.0 g, 95% yield). R_f = 0.4, *n*-hexane/EtOAc, 7:3. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.34−7.03 (m, 13H), 6.98−6.93 (m, 2H), 6.85 (s, 1H), 5.06 (s, 2H), 4.72 (d, J = 10.4, 1H), 4.68 (d, J = 10.4, 1H), 3.92 (dd, J = 8.2, 6.8 Hz, 1H), 3.48 (s, 3H), 3.19 (dd, J = 13.5, 8.2 Hz, 1H), 2.87 (dd, J = 13.5, 6.8 Hz, 1H), 2.38 (s, 3H), 2.16

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(s, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ: 204.4, 201.2, 173.3, 156.3, 153.4, 138.7, 138.4, 136.0, 135.9, 129.1, 128.8, 128.61, 128.56, 128.52, 128.50, 128.3, 127.4, 126.7, 125.7, 107.8, 79.3, 70.7, 52.3, 48.8, 40.1, 32.6, 32.5. MS (ESI) m/z: $[M + H]^+$ calcd for $C_{34}H_{33}O_6$ 537.22; found 537.2.

General Procedure for the Dieckman Condensation (Procedure A). To a solution of **11** or **11a** (1.0 equiv, 0.28 M in THF), LiHMDS (1.0 M in THF, 3.0 equiv) was added dropwise at 0 °C. The resulting orange suspension was stirred at 0 °C under a nitrogen atmosphere for 2 h, quenched with an aqueous solution of HCl (1 M), extracted with Et_2O (50 mL x 3), washed with brine (20 mL), dried over Na_2SO_4, and concentrated under vacuum to afford the title compound **12** or **12a**.

1-(1,3-Bis(benzyloxy)-6,8-dihydroxynaphthalen-2-yl)ethan-1-one (12). From compound **11** (1.23 g, 2.76 mmol) according to general procedure A, product **12** was obtained as a white solid (1.0 g, 90%). R_f = 0.2, *n*-hexane/EtOAc, 7:3. ^1H NMR (400 MHz, $(CD_3)_2CO$) δ: 9.19 (s, 1H), 8.71 (s, 1H), 7.55−7.32 (m, 10 H), 7.12 (s, 1H), 6.72 (d, J = 2.3 Hz, 1H), 6.41 (d, J = 2.3 Hz, 1H), 5.27 (s, 2H), 5.15 (s, 2H), 2.58 (s, 3H). $^{13}C\{^1H\}$ (101 MHz, $(CD_3)_2CO$) δ: 200.8, 158.0, 155.9, 153.8, 152.1, 138.8, 136.8, 135.8, 128.9, 128.8, 128.7, 128.5, 128.0, 127.6, 122.2, 107.7, 103.2, 101.1, 100.8, 80.0, 70.2, 32.2. MS (ESI) m/z: $[M + H]^+$ calcd for $C_{26}H_{23}O_5$ 415.15; found 415.2. Analytical and spectroscopic data were consistent with those reported in the literature.[14]

1-5(Benzyl-1,3-bis(benzyloxy)-6,8-dihydroxynaphthalen-2-yl)-ethan-1-one (12a). From compound **11a** (1.00 g, 1.86 mmol) according to general procedure A, product **12a** was obtained as a yellow solid (850 mg, 90% yield). R_f = 0.3, *n*-hexane/EtOAc, 7:3. ^1H NMR (400 MHz, $(CD_3)_2CO$) δ: 9.12 (s, 1H), 8.71 (s, 1H), 7.41−6.94 (m, 16H), 6.47 (s, 1H), 5.01 (s, 2H), 4.98 (s, 2H), 4.16 (s, 2H), 2.41 (s, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $(CD_3)_2CO$) δ: 200.8, 155.3, 154.4, 153.6, 152.5, 141.7, 137.1, 136.7, 135.6, 129.0, 128.9, 128.7, 128.6, 128.3, 128.2, 128.0, 127.6, 125.5, 121.9, 110.3, 108.2, 101.2, 100.6, 80.2, 70.2, 32.3, 30.3. HRMS (ESI), m/z: $[M + Na]^+$ calcd for $C_{33}H_{28}O_5Na$ 527.1829; found 527.1828.

1-(1,3,6,8-Tetrakis(benzyloxy)naphthalen-2-yl)ethan-1-one (13a). To a solution of **12** (230 mg, 0.56 mmol, 1.0 equiv), PPh_3 (308 mg, 1.2 mmol, 2.1 equiv), and BnOH (122 μL, 1.17 mmol, 2.1 equiv) in Et_2O (10 mL) at 0 °C, DIAD (232 μL, 1.17 mmol, 2.1 equiv) was added dropwise. The reaction was stirred for 4 h at room temperature and then purified by automated flash chromatography (EtOAc/*n*-hexane gradient from 0% EtOAc to 30% EtOAc) to afford compound **13a** as a white solid (50.0 mg, 17% yield). R_f = 0.3, *n*-hexane/EtOAc, 8:2. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.51−7.14 (m, 20H), 6.95 (s, 1H), 6.79 (d, J = 2.2 Hz, 1H), 6.62 (d, J = 2.2 Hz, 1H), 5.21 (s, 2H), 5.19 (s, 2H), 5.15 (s, 2H), 5.02 (s, 2H), 2.51 (s, 3H). $^{13}C\{1H\}$ NMR (101 MHz, $CDCl_3$) δ: 203.0, 158.2, 156.9, 153.7, 152.9, 138.8, 137.4, 136.6, 136.5, 136.3, 128.7, 128.63, 128.59, 128.2, 128.0, 127.8, 127.7, 127.5, 127.1, 125.5, 111.8, 103.8, 100.2, 99.6, 78.7, 71.3, 70.2, 70.1, 32.8. HRMS (ESI), m/z: $[M + Na]^+$ calcd for $C_{40}H_{34}O_5Na$ 617.2298; found 617.2298.

1-(1,3-Bis(benzyloxy)-6,8-bis((benzyloxy)methoxy)naphthalen-2-yl)ethan-1-one (13b). To a solution of **12** (930 mg, 2.24 mmol, 1.0 equiv) in CH_2Cl_2 (16 mL), DIPEA (3.8 mL, 22.4 mmol, 10.0 equiv) and BOMCl (1.2 mL, 8.96 mmol, 4.0 equiv) were added at 0 °C. The reaction was stirred at room temperature overnight under a nitrogen atmosphere. The mixture was quenched with 10 mL of a saturated aqueous solution of NH_4Cl, extracted with Et_2O (3 × 50 mL), washed with brine (20 mL), dried over Na_2SO_4, and concentrated under vacuum. The crude product was purified by automated flash chromatography (EtOAc/*n*-hexane gradient from 0% EtOAc to 30% EtOAc) to afford compound **13b** as a yellow oil (730 mg, 50% yield). R_f = 0.6, *n*-hexane/EtOAc, 8:2. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.43−7.11 (m, 20H), 6.97 (d, J = 2.2 Hz, 1H), 6.88−6.83 (m, 2H), 5.29 (s, 2H), 5.21 (s, 2H), 5.10 (s, 2H), 4.98 (s, 2H), 4.68 (s, 2H), 4.54 (s, 2H), 2.46 (s, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ: 202.7, 156.5, 155.2, 153.6, 152.2, 138.6, 137.8, 137.2, 137.1, 136.4, 128.6, 128.5, 128.4, 128.2, 128.0, 127.9, 127.8, 127.7, 127.1, 125.9, 112.1, 104.0, 103.9, 102.9, 93.6, 92.2, 78.5, 70.3, 70.2, 70.2, 32.8. HRMS (ESI), m/z: $[M + Na]^+$ calcd for $C_{42}H_{38}O_7Na$ 677.2510; found 677.2510.

1-(1,3-Bis(benzyloxy)-6,8-dimethoxynaphthalen-2-yl)ethan-1-one (13c). Me_2SO_4 (0.8 mL, 8.90 mmol, 10.0 equiv) and K_2CO_3 (616 mg, 4.46 mmol, 5.0 equiv) were added to a solution of **12** (370 mg, 0.89 mmol, 1.0 equiv) in acetone (15 mL), and the reaction was stirred overnight at 60 °C. The mixture was quenched with 10 mL of an aqueous solution of NaOH (1 M) and extracted with Et_2O (3 × 50 mL). The solvent was removed under vacuum, and the residue was stirred with 10 mL of an aqueous solution of NH_4OH (1 M) for 2 h. The aqueous phase was extracted with Et_2O (3 × 50 mL), and the combined organic phases were washed with H_2O (2 × 30 mL) and brine (1 × 30 mL), dried over Na_2SO_4, and concentrated under vacuum. The crude product was purified by automated flash chromatography (EtOAc/*n*-hexane gradient from 10% EtOAc to 20% EtOAc) to afford product **13c** as a yellow oil (275 mg, 70% yield). R_f = 0.6, *n*-hexane/EtOAc, 7:3. ^1H NMR (400 MHz, $CDCl_3$) δ 7.54−7.32 (m, 10H), 6.96 (s, 1H), 6.68 (d, J = 2.2 Hz, 1H), 6.46 (d, J = 2.2 Hz, 1H), 5.21 (s, 2H), 5.02 (s, 2H), 3.92 (s, 3H), 3.88 (s, 3H), 2.58 (s, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ: 202.9, 159.2, 157.6, 153.7, 152.5, 138.8, 137.6, 136.46, 128.6, 128.4, 128.2, 128.0, 127.9, 127.1, 125.2, 111.2, 103.7, 98.6, 97.4, 78.9, 70.2, 55.8, 55.3, 32.8. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{28}H_{26}O_5Na$ 465.1672; found 465.1672.

1-(1,3-Bis(benzyloxy)-6,8-bis((tert-butyldimethyl-silyl)oxy)-naphtalen-2-yl)ethan-1-one (13d). TBDMSCl (85.1 mg, 0.56 mmol, 2.5 equiv) and DIPEA (0.1 mL, 0.68 mmol, 3.0 equiv) were added to a solution of **12** (100 mg, 0.23 mmol, 1.0 equiv) in DMF (1 mL), and the reaction was stirred at room temperature overnight under a nitrogen atmosphere. The mixture was extracted with Et_2O (3 × 10 mL), washed with 10 mL of brine, dried over Na_2SO_4, and concentrated under vacuum. The crude product was purified by automated flash chromatography (EtOAc/*n*-hexane gradient from 0% EtOAc to 20% EtOAc) to afford compound **13d** as a yellow oil (140 mg, 99% yield). R_f = 0.6, *n*-hexane/EtOAc, 9:1. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.45−7.37 (m, 10 H), 6.90 (s, 1H), 6.81 (d, J = 2.3 Hz, 1H), 6.48 (d, J = 2.3 Hz, 1H), 5.19 (s, 2H), 5.08 (s, 2H), 2.50 (s, 3H), 1.07 (s, 9H), 0.94 (s, 9H), 0.31 (s, 6H), 0.11 (s, 6H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ: 202.4, 154.9, 153.4, 153.3, 153.0, 138.6, 137.7, 136.5, 128.6, 128.1, 128.0, 127.5, 127.5, 127.1, 125.0, 113.8, 110.8, 109.0, 103.1, 78.0, 70.1, 32.9, 26.21, 25.7, 18.9, 18.3, −4.0, −4.2. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{38}H_{50}O_5Si_2Na$ 665.3089; found 665.3088.

1-(1,3,6,8-Tetrakis(benzyloxy)naphtalen-2-yl)vinyl Acetate (14a). LiHMDS (33.0 μL, 0.17 mmol, 2.0 equiv) was added to a solution of **13a** (50.0 mg, 0.08 mmol, 1.0 equiv) in THF (2 mL) at 0 °C, and the reaction was stirred for 15 min. Then, acetic anhydride (9.5 mg, 0.09 mmol, 1.1 equiv) was added dropwise and the reaction was monitored by liquid chromatography−mass spectrometry (LC-MS), confirming the complete conversion of the starting material into the desired product after 2 h. The reaction was quenched with H_2O (5 mL), and the mixture was extracted with EtOAc (3 × 20 mL), washed with 10 mL of brine, dried over Na_2SO_4, and concentrated under vacuum. The crude product was then purified by flash chromatography (*n*-hexane/EtOAc 8:2) to afford **14a** as a white solid (25.0 mg, 50% yield). R_f = 0.4, *n*-hexane/EtOAc, 7:3. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.51−7.26 (m, 14H), 7.23−7.13 (m, 6H), 6.90 (s, 1H), 6.71 (d, J = 2.2 Hz, 1H), 6.54 (d, J = 2.2 Hz, 1H), 5.45 (d, J = 1.3 Hz, 1H), 5.31 (d, J = 1.3 Hz, 1H), 5.20 (s, 2H), 5.12 (s, 2H), 5.11 (s, 2H) 5.05 (s, 2H), 1.94 (s, 3H). $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ: 168.5, 158.1, 157.0, 155.5, 155.3, 146.3, 138.5, 138.2, 136.9, 136.7, 136.4, 128.7, 128.5, 128.4, 128.1, 128.0, 127.8, 127.71, 127.67, 127.4, 127.3, 127.1, 118.1, 112.1, 108.3, 103.6, 100.0, 99.6, 76.4, 71.2, 70.2, 70.0, 21.3. MS (ESI) m/z: $[M + H]^+$ calcd for $C_{42}H_{37}O_6$ 637.25; found 637.2.

General Procedure for Aldol Condensation with Acetaldehyde (Procedure B1). To a solution of **13b** or **13c** (1.0 equiv, 0.11 M in THF), LiHMDS (1 M in THF, 3.0 equiv) was added dropwise at −40 °C under a nitrogen atmosphere. The solution was stirred for 10 min, and then acetaldehyde (5 M in THF, 4.0 equiv) was added. The

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reaction was stirred for 15 min keeping the temperature at −40 °C and then quenched with a saturated aqueous solution of NH_4Cl. The mixture was extracted with Et_2O, washed with brine, dried over Na_2SO_4, and concentrated under vacuum. The crude product was purified by flash chromatography eluting with the indicated solvent.

*1-(1,3-Bis(benzyloxy)-6,8-bis((benzyloxy)methxy)naphtalen-2-yl)-3-hydroxybutan-1-one (**14b**).* Compound **13b** (750 mg, 1.14 mmol) was reacted according to the general procedure B1. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 8:2) to afford compound **14b** as a yellow oil (700 mg, 90% yield). R_f = 0.3, *n*-hexane/EtOAc, 8:2. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.49−7.25 (m, 18H), 7.24−7.18 (m, 2H), 7.05 (d, J = 2.2 Hz, 1H), 6.96 (s, 1H), 6.95 (d, J = 2.2 Hz, 1H), 5.38 (s, 2H), 5.29 (s, 2H), 5.17 (s, 2H), 5.08 (d, J = 10.5 Hz, 1H), 5.04 (d, J = 10.5 Hz, 1H), 4.76 (s, 2H), 4.62 (s, 2H), 4.30−4.21 (m, 1H), 3.13 (br, 1H), 3.02 (dd, J = 17.1, 2.7 Hz, 1H), 2.86 (dd, J = 17.1, 9.0 Hz, 1H), 1.12 (d, J = 6.4 Hz, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ 206.0, 156.7, 155.2, 153.4, 152.3, 138.8, 137.4, 137.14, 137.07, 136.09, 128.7, 128.52, 128.48, 128.40, 128.18, 128.16, 128.0, 127.9, 127.8, 127.7, 127.2, 125.1, 112.0, 104.1, 103.9, 103.0, 93.6, 92.2, 78.78, 70.4, 70.36, 70.27, 64.4, 53.7, 22.3. MS (ESI) m/z: $[M + H]^+$ calcd for $C_{44}H_{43}O_8$ 699.29; found 699.3.

*1-(1,3-Bis(benzyloxy)-6,8-dimethoxynaphtalen-2-yl)-3-hydroxy-buta-1-one (**14c**).* Compound **13c** (275 mg, 0.62 mmol) was reacted according to the general procedure B1. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 8:2) to afford compound **14c** as a yellow oil (272 mg, 90% yield). R_f = 0.5, *n*-hexane/EtOAc, 8:2. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.42−7.21 (m, 10H), 6.86 (s, 1H), 6.57 (d, J = 2.2 Hz, 1H), 6.35 (d, J = 2.2 Hz, 1H), 5.09 (s, 2H), 4.93 (d, J = 10.5 Hz, 1H), 4.88 (d, J = 10.5 Hz, 1H) 4.19 (m, 1H), 3.81 (s, 3H), 3.77 (s, 3H), 2.96 (dd, J = 17.2, 2.7 Hz, 1H), 2.78 (dd, J = 17.2, 9.0 Hz, 1H), 1.04 (d, J = 6.3 Hz, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ: 206.2, 159.4, 157.63, 153.60, 152.7, 139.0, 137.3, 136.2, 128.7, 128.5, 128.2, 128.1, 128.0, 127.1, 124.4, 111.1, 103.9, 98.6, 97.6, 79.1, 70.3, 64.3, 55.8, 55.4, 53.6, 22.3. MS (ESI) m/z: $[M + H]^+$ calcd for $C_{30}H_{31}O_6$ 487.20; found 487.2.

*General Procedure for the Synthesis of 8-Azido Octanal (**22**) or 8-Azido Hexanal (**23**).* Commercially available 8-bromo-1-octanol (100 mg, 0.48 mmol, 1.0 equiv) or 6-bromo-1-hexanol (87.0 mg, 0.48 mmol, 1.0 equiv) was dissolved in DMF (4 mL), followed by the addition of NaN_3 (31.2 mg, 0.96 mmol, 2.0 equiv). The reaction was stirred at room temperature for 12 h. The mixture was extracted with Et_2O (3 × 50 mL), washed with brine (10 mL), dried over Na_2SO_4, and evaporated. The crude product was directly submitted to the next step without further purification. DMP (140 mg, 0.72 mmol, 1.5 equiv) was added to the solution of the azide in $CHCl_3$ (7 mL). The reaction was stirred at room temperature for 30 min, and the crude product was purified by flash chromatography (*n*-hexane/CH_2Cl_2 4:6) to afford product **22** or **23** as a pale oil (99% yield for both **22** and **23**). **22**: R_f = 0.5, *n*-hexane/CH_2Cl_2, 3:7. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 9.79 (t, J = 1.8 Hz, 1H), 3.28 (t, J = 6.9 Hz, 2H), 2.45 (td, J = 7.3, 1.8 Hz, 2H), 1.71−1.58 (m, 4H), 1.44−1.34 (m, 6H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ: 202.60, 51.36, 43.77, 28.94, 28.85, 28.72, 26.47, 21.88. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_8H_{15}N_3ONa$ 192.1107; found 192.1107. For **23**, analytical and spectroscopic data were consistent with those reported in the literature.[15]

*General Procedure for Aldol Condensation with **22** or **23** (Procedure B2).* LDA (1 M in THF) was freshly prepared according to the following procedure: 280 μL of 1,2-diisopropylamine was added to 920 μL of THF in dry conditions under an inert atmosphere and cooled to −78 °C, and then 800 μL of *n*-BuLi (2.5 M in *n*-hexane) was added and the mixture was stirred at 0 °C for 40 min. LDA (1 M in THF, 1.5 equiv) was added dropwise to a solution of **13b**, **13c**, or **13d** (1.0 equiv, 0.04 M in THF) at −78 °C, and the reaction was stirred for 45 min under nitrogen keeping the temperature at −78 °C. Then, **22** or **23** (2.0 equiv), freshly prepared and stored under an inert atmosphere until needed, was added dropwise to the solution and the reaction was stirred for 15 min at −78 °C. The reaction was quenched with a saturated aqueous solution of NH_4Cl, extracted with Et_2O (3 × 30 mL), washed with brine (5 mL), dried over Na_2SO_4, and evaporated. The crude product was purified by flash chromatography or directly submitted to the following step without further purification.

*10-Azido-1-(1,3-bis(benzyloxy)-6,8-bis((benzyloxy)methoxy)-naphthalen-2-yl)-3-hydroxydecan-1-one (**16b**).* Compound **13b** (30.0 mg, 0.04 mmol) was reacted according to general procedure B2. The crude product was purified by automated flash chromatography (EtOAc/*n*-hexane gradient from 0% EtOAc to 20% EtOAc) to afford compound **16b** as a yellow oil (38.0 mg, 99%). R_f = 0.4, *n*-hexane/EtOAc, 8:2. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.42−7.09 (m, 20H), 6.98 (d, J = 2.2 Hz, 1H), 6.88 (s, 1H), 6.87 (d, J = 2.2 Hz, 1H), 5.30 (s, 2H), 5.21 (s, 2H), 5.09 (s, 2H), 5.02 (d, J = 10.5 Hz, 1H), 4.96 (d, J = 10.5 Hz, 1H), 4.68 (s, 2H), 4.54 (s, 2H), 3.98 (br, 1H), 3.15 (t, J = 7.0 Hz, 2H), 3.02 (d, J = 3.1 Hz, 1H), 2.97 (dd, J = 17.1, 2.5 Hz, 1H), 2.77 (dd, J = 17.1, 9.1 Hz, 1H), 1.53−1.14 (m, 12H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ: 206.2, 156.7, 155.2, 153.4, 152.3, 138.8, 137.5, 137.1, 137.07, 136.1, 128.7, 128.52, 128.46, 128.40, 128.2, 128.1, 128.0, 127.88, 127.85, 127.81, 127.6, 127.2, 125.1, 112.1, 104.1, 103.9, 103.0, 93.6, 92.2, 78.7, 70.4, 70.3, 70.3, 68.1, 52.2, 51.5, 36.3, 29.4, 29.1, 28.8, 26.6, 25.3. MS (ESI) m/z: $[M + H]^+$ calcd for $C_{50}H_{54}N_3O_8$ 824.38; found 824.4.

*10-Azido-1-(1,3-bis(benzyloxy)-6,8-dimethoxynaphthalen-2-yl)-3-hydroxydecan-1-one (**16c**).* Compound **13c** (450 mg, 1.0 mmol) was reacted according to general procedure B2. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 8:2) to afford compound **16c** as a yellow oil (588 mg, 99%). R_f = 0.4, *n*-hexane/EtOAc, 7:3. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.49−7.31 (m, 10H), 6.95 (s, 1H), 6.66 (d, J = 2.2 Hz, 1H), 6.44 (d, J = 2.2 Hz, 1H), 5.17 (s, 2H), 5.03 (d, J = 10.5 Hz, 1H), 4.97 (d, J = 10.5 Hz, 1H) 4.09 (br, 1H), 3.89 (s, 3H), 3.85 (s, 3H), 3.24 (t, J = 7.0 Hz, 2H), 3.08 (dd, J = 17.1, 2.5 Hz, 1H), 2.86 (dd, J = 17.1, 9.1 Hz, 1H), 1.62−1.11 (m, 12H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ: 206.4, 159.4, 157.6, 153.6, 152.7, 139.0, 137.4, 136.2, 128.7, 128.5, 128.2, 128.1, 128.0, 127.1, 124.5, 111.1, 103.9, 98.6, 97.6, 79.1, 70.3, 68.0, 55.8, 55.4, 52.2, 51.5, 36.3, 29.4, 29.1, 28.8, 26.6, 25.3. MS (ESI) m/z: $[M + H]^+$ calcd for $C_{36}H_{42}N_3O_6$ 612.30; found 612.3.

*10-Azido-1-(1,3-bis(benzyloxy)-8-((tert-butyldimethylsilyl)oxy)-6-((isopropyldimethylsilyl)oxy)naphthalen-2-yl)-3-hydroxydecan-1-one (**16d**).* Compound **13d** (130 mg, 0.2 mmol) was reacted according to general procedure B2. The crude product was purified by flash chromatography (*n*-hexane/EtOAc 9:1) to afford compound **16d** as a yellow oil (162 mg, 99%). R_f = 0.3, *n*-hexane/EtOAc, 9:1. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.35−7.17 (m, 10H), 6.78 (s, 1H), 6.68 (d, J = 2.2 Hz, 1H), 6.35 (d, J = 2.2 Hz, 1H), 5.05 (s, 2H), 4.98 (d, J = 10.5 Hz, 1H), 4.93 (d, J = 10.5 Hz, 1H), 3.91 (br, 1H), 3.15 (t, J = 7.0 Hz, 2H), 2.98 (br, 1H), 2.90 (dd, J = 17.1, 2.4 Hz, 1H), 2.64 (dd, J = 17.1, 9.1 Hz, 1H), 1.54−1.44 (m, 2H), 1.30−1.13 (m, 10H), 0.94 (s, 9H), 0.82 (s, 9H), 0.18 (s, 6H), −0.00 (d, J = 2.4 Hz, 6H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ: 205.8, 155.1, 153.5, 153.25, 153.16, 138.7, 137.3, 136.3, 128.6, 128.2, 128.1, 127.6, 127.2, 124.1, 113.7, 111.0, 109.1, 103.2, 78.3, 70.2, 67.9, 52.2, 51.5, 36.3, 29.4, 29.1, 28.8, 26.7, 26.20, 26.16, 25.7, 25.4, 18.9, 18.3, −3.89, −3.93, −4.19. MS (ESI) m/z: $[M + H]^+$ calcd for $C_{46}H_{66}N_3O_6Si_2$ 812.44; found 812.4.

*8-Azido-1-(1,3-bis(benzyloxy)-6,8-dihydroxynaphthalen-2-yl)-3-hydroxyoctan-1-one (**16e**).* Compound **13c** (450 mg, 1.0 mmol) was reacted following procedure B2. The crude product was directly submitted to the next step without further purification. R_f = 0.4, *n*-hexane/EtOAc, 7:3. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.47−7.32 (m, 10H), 6.94 (s, 1H), 6.66 (d, J = 2.2 Hz, 1H), 6.44 (d, J = 2.2 Hz, 1H), 5.17 (s, 2H), 5.02 (d, J = 10.5 Hz, 1H), 4.96 (d, J = 10.5 Hz, 1H), 4.05 (br, 1H), 3.90 (s, 3H), 3.86 (s, 3H), 3.20 (t, J = 7.0 Hz, 2H), 3.14 (br, 1H), 3.05 (dd, J = 17.1, 2.5 Hz, 1H), 2.84 (dd, J = 17.1, 9.1 Hz, 1H), 1.57−1.11 (m, 8H). MS (ESI) m/z: $[M + H]^+$ calcd for $C_{34}H_{38}N_3O_6$ 584.27; found 584.3.

General Procedure for the Dess−Martin Oxidation (Procedure C). To a solution of β-hydroxyketone **14b**, **14c** or **16b−e** (1.0 equiv, 0.04 M in $CHCl_3$), DMP (1.5 equiv) was added in small portions. The suspension was stirred at 40 °C or at room temperature until complete consumption of the starting material (reaction monitored by

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TLC). The crude product was filtered on a silica pad to remove the excess of DMP and directly submitted to the next step without further purification.

*(Z)-1-(1,3-Bis(benzyloxy)-6,8-bis((benzyloxy)methoxy)-naphthalen-2-yl)-3-hydroxybut-2-en-1-one (**15b**).* Compound **14b** (700 mg, 1.0 mmol) was reacted according to general procedure C. The crude product was filtered on a silica pad to give **15b**, which was directly submitted to the next step without further purification. R_f = 0.4, *n*-hexane/EtOAc, 8:2. Ketone **15b** is in equilibrium with its enolic form. According to the NMR, the enol is predominant. The signals of the ketone are labeled with an asterisk when clearly distinguishable from the enol. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 15.49 (s, 1H), 7.41−7.10 (m, 26H), 6.95 (d, J = 2.2 Hz, 1.3H), 6.87−6.83 (m, 2.6H), 5.73 (s, 1H), 5.28 (s, 2.6H), 5.20 (s, 2.6H), 5.10 (s, 2H), 5.06* (s, 0.6H), 4.98 (s, 2.6H), 4.66 (s, 2.6H), 4.55 (s, 2H), 4.51* (s, 0.6H), 3.81* (s, 0.6H), 1.96 (s, 3H) 1.95* (s, 0.9H). MS (ESI) m/z: $[M + H]^+$ calcd for $C_{44}H_{41}O_8$ 697.27; found 697.3.

*(Z)-1-(1,3-Bis(benzyloxy)-6,8-dimethoxynaphthalen-2-yl)-3-hydroxybut-2-en-1-one (**15c**).* Compound **14c** (395 mg, 0.81 mmol) was reacted according to general procedure C. The crude product was filtered on a silica pad to afford **15c**, which was directly submitted to the next step without further purification. R_f = 0.4, *n*-hexane/EtOAc, 7:3. Ketone **15c** is in equilibrium with its enolic form. According to the NMR, the enol is predominant. The signals of the ketone are labeled with an asterisk when clearly distinguishable from the enol. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 15.50 (s, 1H), 7.42−7.20 (m, 14H), 6.85 (s, 1.4H), 6.56 (d, J = 2.2 Hz, 1.4H), 6.34 (d, J = 2.2 Hz, 1.4H), 5.77 (s, 1H), 5.13 (s, 2H), 5.09* (s, 0.8 H), 4.93* (s, 0.8 H), 4.90 (s, 2H), 3.82 (s, 4.2H), 3.79 (s, 4.2H), 1.98 (s, 4.2H). MS (ESI) m/z: $[M + H]^+$ calcd for $C_{30}H_{29}O_6$ 485.19; found 485.2.

*(Z)-10-Azido-1-(1,3-bis(benzyloxy)-6,8-dimethoxynaphthalen-2-yl)-3-hydroxydec-2-en-1-one (**17c**).* Compound **16c** (269 mg, 0.44 mmol) was reacted according to general procedure C. The crude product was filtered on a silica pad to afford **17c**, which was directly submitted to the next step without further purification. R_f = 0.5, *n*-hexane/EtOAc, 7:3. Ketone **17c** is in equilibrium with its enolic form. According to the NMR, the enol is predominant. The signals of the ketone are labeled with an asterisk when clearly distinguishable from the enol. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 15.61 (s, 1H), 7.48−7.30 (m, 14H), 6.93 (s, 1.4H), 6.64 (d, J =2.2 Hz, 1.4H), 6.42 (d, J = 2.2 Hz, 1.4), 5.83 (s, 1H), 5.20 (s, 2H), 5.17* (s, 0.8H), 4.99* (s, 0.8H), 4.98 (s, 2H), 3.90 (s, 4.2H), 3.86 (s, 4.2H), 3.23 (t, J = 7.0, 2.8H), 2.35* (t, J = 7.4 Hz, 0.8H), 2.27 (t, J = 8.0 Hz, 2H), 1.55−1.23 (m, 14H). MS (ESI) m/z: $[M + H]^+$ calcd for $C_{36}H_{40}N_3O_6$ 610.28; found 610.3.

Alternative Procedure. Stabilized 2-iodoxybenzoic acid (275 mg, 0.98 mmol, 3 equiv) was added to compound **16c** (200 mg, 0.33 mmol) in EtOAc (10 mL) at room temperature. The reaction mixture was heated to 80 °C and stirred overnight. After cooling to room temperature, the reaction was filtered on a silica pad and washed with EtOAc. The filtrate was washed with saturated $NaHCO_3$ (3 × 10 mL), dried ($MgSO_4$), filtered, and concentrated under vacuum to give **17c** as a yellow oil.

*(Z)-10-Azido-1-(1,3-bis(benzyloxy)-8-((tert-butyldimethylsilyl)-oxy)-6-((isopropyldimethylsilyl)oxy)naphthalen-2-yl)-3-hydroxy-dec-2-en-1-one (**17d**).* Compound **16d** (140 mg, 0.17 mmol) was reacted according to general procedure C (room temperature). The crude product was filtered on a silica pad to afford **17d**, which was directly submitted to the next step without further purification. R_f = 0.4, *n*-hexane/EtOAc, 9:1. Ketone **17d** is in equilibrium with its enolic form. According to the NMR, the enol is predominant. The signals of the ketone are labeled with an asterisk when clearly distinguishable from the enol. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 15.52 (br, 1H), 7.46−7.27 (m, 14H), 6.88 (s, 1.4H), 6.77 (d, J = 2.2 Hz, 1.4H), 6.45 (d, J = 2.2 Hz, 1.4H), 5.73 (s, 1H), 5.19 (s, 2H), 5.15* (s, 0.8H), 5.07 (s, 2.8H), 3.84* (s, 0.8H), 3.25 (t, J = 6.9 Hz, 2.8H), 2.36* (t, J = 8 Hz, 0.8H), 2.26 (t, J = 8 Hz, 2H), 1.62−1.54 (m, 5.6H), 1.42−1.25 (m, 8.4H), 1.05 (s, 12.6H), 0.92 (s, 12.6H), 0.29 (s, 8.4H), 0.08 (s, 8.4H). MS (ESI) m/z: $[M + H]^+$ calcd for $C_{46}H_{64}N_3O_6Si_2$ 810.43; found 810.4.

*(Z)-8-Azido-1-(1,3-bis(benzyloxy)-6,8-dihydroxynaphthalen-2-yl)-3-hydroxyoct-2-en-1-one (**17e**).* Compound **16e** (255 mg, 0.44 mmol) was reacted according to general procedure C. The crude product was filtered on a silica pad to afford **17e**, which was directly submitted to the next step without further purification. R_f = 0.5, *n*-hexane/EtOAc, 7:3. Ketone **17e** is in equilibrium with its enolic form. According to the NMR, the enol is predominant. The signals of the ketone are labeled with an asterisk when clearly distinguishable from the enol. ^{1}H NMR (400 MHz, $CDCl_3$) δ 15.61 (br, 1H), 7.55−7.29 (m, 14H), 6.94 (s, 1.4H), 6.65 (d, J = 2.2 Hz, 1.4H), 6.43 (d, J = 2.2 Hz, 1.4H), 5.85 (s, 1H), 5.20 (s, 2H), 5.16* (s, 0.8H), 5.00 (s, 2.8H), 3.92* (s, 0.8H), 3.89 (s, 4.2H), 3.86 (s, 4.2 H) 3.21−3.15 (m, 2.8H), 2.36* (t, J = 8 Hz, 0.8H), 2.29 (t, J = 8 Hz, 2H), 1.64−1.38 (m, 8.4H). MS (ESI) m/z: $[M + H]^+$ calcd for $C_{34}H_{36}N_3O_6$ 582.25; found 582.2.

General Procedure for Hydrogenolysis (Procedure D). Glacial AcOH (1.0 equiv) and a catalytic amount of $Pd(OH)_2$ on carbon 20% were added to a suspension of 1,3-diketone **15b**, **15c** or **17b−d** (1.0 equiv, 0.014 M in MeOH) under nitrogen. The reaction was stirred under a hydrogen atmosphere and monitored via LC-MS using a gradient from 30% acetonitrile (ACN) (containing 0.1% trifluoroacetic acid (TFA)) to 100% ACN (containing 0.1% TFA) in water over 10 min (method 1), confirming the complete conversion of the starting material into the desired product. $Pd(OH)_2$ was removed by filtration on a cotton pad, and the solvent was slowly evaporated at low temperature under vacuum. The crude product was purified by preparative RP-HPLC using a gradient from 30% ACN (containing 0.05% TFA) to 100% ACN (containing 0.05% TFA) in water over 10 min (method 2) to obtain the final products YWA1 and fonsecin B. Intermediates **18a** and **18b** instead, due to the reactivity of the amine group, proved to be unstable in solution and therefore were used immediately in the next conjugation step without further purification.

YWA1. Compound **15b** (100 mg, 0.14 mmol) was reacted according to general procedure D. The reaction was monitored by LC-MS (method 1), confirming the complete conversion of the starting material to the desired product in 12 h. The product was submitted to preparative RP-HPLC (method 2) and isolated as a yellow solid (36.0 mg, 90% yield), r.t. = 7.8 min. ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ: 15.37 (s, 1H), 9.45 (s, 1H), 9.20 (s, 1H), 6.54 (d, J = 2.2 Hz, 1H), 6.47 (s, 1H), 6.30 (d, J = 2.2 Hz, 1H), 6.05 (s, 1H), 3.19 (d, J = 17.1 Hz, 1H), 2.88 (d, J = 17.1 Hz, 1H), 1.74 (s, 3H). ^{13}C{^{1}H} NMR (101 MHz, $(CD_3)_2CO$) δ: 198.8, 165.0, 162.8, 160.8, 154.1, 143.6, 105.0, 102.9, 102.8, 102.4, 101.3, 100.8, 47.8, 28.4. MS (ESI) m/z: $[M + H]^+$ calcd. for $C_{14}H_{13}O_6$ 277.06; found 277.1. Analytical and spectroscopic data were consistent with those reported in the literature.[5]

Fonsecin B. Compound **15c** (100 mg, 0.21 mmol) was reacted according to general procedure D. The reaction was monitored by LC-MS (method 1), confirming the complete conversion of the starting material into the desired product in 3 h. The product was submitted to preparative RP-HPLC (method 2) and isolated as a yellow solid (56.0 mg, 90% yield), r.t. = 8.5 min. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 14.21 (s, 1H), 6.45 (s, 1H), 6.39 (d, J = 2.2 Hz, 1H), 6.24 (d, J = 2.2 Hz, 1H), 3.89 (s, 3H), 3.83 (s, 3H), 2.95 (d, J = 17.0 Hz, 1H), 2.85 (d, J = 17.0 Hz, 1H), 1.68 (s, 3H). ^{13}C{^{1}H} NMR (101 MHz, $CDCl_3$) δ: 196.0, 164.9, 162.5, 161.4, 153.1, 143.3, 107.4, 103.1, 102.6, 100.0, 98.6, 96.7, 56.1, 55.4, 47.0, 28.7. HRMS (ESI), m/z: $[M + H]^+$ calcd for $C_{16}H_{16}O_6Na$ 327.0839; found 327.0840.

General Procedure for the Coupling Reaction (Procedure E). Intermediate **18a** or **18b** (1.0 equiv) was dissolved in DMF, and then the desired fluorescent tag (1.5 equiv) was added in one portion. After the addition of DIPEA (2.0 equiv), the solution was stirred for 0.5 h under a nitrogen atmosphere. The reaction was monitored by LC-MS using a gradient from 30% ACN (containing 0.1% TFA) to 100% ACN (containing 0.1% TFA) in water over 10 min (method 1), confirming the total conversion of the starting material into the desired product. The compound was then purified by preparative RP-HPLC with a gradient from 30% ACN (containing 0.05% TFA) to 100% ACN (containing 0.05% TFA) in water over 10 min (method 2).

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*Oregon Green Derivative **19**.* Compound **17c** (25.0 mg, 0.04 mmol) was reacted according to general procedure D. The reaction was monitored by LC-MS (method 1), confirming the complete conversion of the starting material into the desired product **18b** in 3 h, r.t. = 5.7 min. MS (ESI, *m*/*z*): $C_{22}H_{30}NO_6$ $[M + H]^+$ calcd 404.20; found 404.2. After removing $Pd(OH)_2$, the solvent was evaporated and the crude product was immediately reacted with Oregon green-NHS (30.0 mg, 0.06 mmol, 1.5 equiv)—previously prepared as reported by Sun et al.[16] (see the Supporting Information)—and DIPEA (15.0 μL, 0.08 mmol, 2.0 equiv) in 1 mL of DMF following general procedure E. The reaction was monitored by LC-MS (method 1), observing the complete conversion of the starting material in 0.5 h. The product was purified by preparative RP-HPLC (method 2) to give **19** as a yellow solid (15.0 mg, 50% yield over two steps), r.t. = 10.1 min. ^{1}H NMR (400 MHz, $(CD_3)_2CO$) *δ*: 14.13 (s, 1H), 8.22 (d, *J* = 8.0, 1H), 8.04 (d, *J* = 8.0 Hz, 1H), 7.96 (t, *J* = 4 Hz, 1H), 7.76 (s, 1H), 6.96 (d, *J* = 7.5 Hz, 2H), 6.64−6.57 (m, 3H), 6.51 (d, *J* = 3.4 Hz, 1H), 6.34 (d, *J* = 2.4 Hz, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.36−3.32 (m, 2H), 3.08 (d, *J* = 16.9 Hz, 1H), 2.75 (d, *J* = 16.9 Hz, 1H), 1.58−1.51 (m, 4H) 1.40−1.23 (m, 8H). HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{43}H_{37}F_2NO_{12}Na$ 820.2176; found 820.2172.

*Biotin Derivative **20a**.* TBAF (1 M in THF, 0.1 mL, 0.13 mmol, 2.5 equiv) was added to a solution of **17d** (45.0 mg, 0.05 mmol, 1.0 equiv) in THF (4 mL) at 0 °C. The reaction was stirred for 3 h under nitrogen at 0 °C, quenched with 5 mL of a saturated aqueous solution of NH_4Cl, extracted with Et_2O (3 × 5 mL), washed with brine (5 mL), and dried over Na_2SO_4, and the solvent was evaporated under vacuum. The crude product was filtered on a silica pad and directly reacted with glacial AcOH (4 μL, 0.05 mmol, 1.0 equiv) and a catalytic amount of $Pd(OH)_2$ on carbon 20% in 3 mL of MeOH under hydrogen according to general procedure D. The reaction was monitored by LC-MS (method 1) confirming the conversion of the starting material into **18a** in 3 h, r.t. = 5.0 min. MS (ESI, *m*/*z*): $C_{20}H_{26}NO_6$ $[M + H]^+$ calcd 376.17; found 376.2. After removing $Pd(OH)_2$, the solvent was evaporated and the crude product was immediately reacted with biotin-NHS (28.0 mg, 0.08 mmol, 1.5 equiv)—previously prepared as reported by Susumu et al.[17]—and DIPEA (10.0 μL, 0.10 mmol, 2.0 equiv) in 1 mL of DMF according to general procedure E. The reaction was monitored by LC-MS (method 1), confirming the complete conversion of the starting material in 0.5 h. The product was purified by preparative RP-HPLC (method 2) to give **20a** as a white solid (15.0 mg, 50% yield over three steps), r.t. = 7.5 min. ^{1}H NMR (400 MHz, CD_3OD) *δ*: 6.33 (s, 1H), 6.30 (d, *J* = 2.1 Hz, 1H), 6.11 (d, *J* = 2.1 Hz, 1H), 4.37 (dd, *J* = 7.8, 4.7 Hz, 1H), 4.18 (dd, *J* = 7.8, 4.7 Hz, 1H), 3.10−3.06 (m, 3H), 2.99−2.88 (m, 1H), 2.81 (ddd, *J* = 12.7, 4.7, 1.3 Hz, 1H), 2.67−2.62 (m, 1H), 2.60 (d, *J* = 12.7 Hz, 1H), 2.10 (t, *J* = 7.3 Hz, 2H), 1.85−1.74 (m, 2H), 1.63−1.29 (m, 16H). HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{30}H_{39}N_3O_8SNa$ 624.2350; found 624.2350.

*Biotin Derivative **20b**.* Compound **17c** (25.0 mg, 0.04 mmol) was reacted according to general procedure D. The reaction was monitored by LC-MS (method 1), confirming the complete conversion of the starting material into the desired product **18b** in 3 h, r.t. = 5.7 min. MS (ESI, *m*/*z*): $C_{22}H_{30}NO_6$ $[M + H]^+$ calcd 404.20; found 404.2. After removing $Pd(OH)_2$, the solvent was evaporated and the crude product was immediately reacted with biotin-NHS (21.0 mg, 0.06 mmol, 1.5 equiv)—previously prepared as reported by Susumu et al.[17]—and DIPEA (8.0 μL, 0.08 mmol, 2.0 equiv) in 1 mL of DMF according to general procedure E. The reaction was monitored by LC-MS (method 1), confirming the complete conversion of the starting material in 0.5 h. The product was purified by preparative RP-HPLC (method 2) to give **20b** as a white solid (13.0 mg, 50% yield over two steps), r.t. = 9.3 min. ^{1}H NMR (400 MHz, $(CD_3)_2CO$) *δ*: 14.02 (s, 1H), 6.92 (br, 1H), 6.52 (d, *J* = 2.2 Hz, 1H), 6.40 (s, 1H), 6.22 (d, *J* = 2.2 Hz, 1H), 5.70 (br, 1H), 5.52 (br, 1H), 4.37 (dd, *J* = 7.4, 4.8 Hz, 1H), 4.20 (dd, *J* = 7.4, 4.8 Hz, 1H), 3.78 (s, 3H), 3.76 (s, 3H), 3.10−3.03 (m, 3H), 2.97 (d, *J* =16.8 Hz, 1H), 2.84−2.76 (m, 2H), 2.65 (d, *J* = 16.8 Hz, 1H), 2.57 (d, *J* = 12.8 Hz, 1H), 2.04 (t, *J* = 7.2 Hz, 2H), 1.88−1.80 (m, 2H), 1.70−1.57 (m, 2H), 1.56−1.18 (m, 14H). HRMS (ESI) *m*/*z*: $[M + Na]^+$ calcd for $C_{32}H_{43}N_3O_8SNa$ 652.2663; found 652.2661.

*PyMPO Derivative **21**.* **17c** (7.0 mg, 0.01 mmol) was reacted according to general procedure D. The reaction was monitored by LC-MS (method 1), confirming the complete conversion of the starting material into **18b** in 3 h, r.t. = 5.7 min. MS (ESI, *m*/*z*): $C_{22}H_{30}NO_6$ $[M + H]^+$ calcd 404.20; found 404.2. After removing $Pd(OH)_2$, the solvent was evaporated and the crude product was immediately reacted with the commercially available PyMPO succinimidyl ester (5.0 mg, 0.01 mmol, 1.5 equiv) and DIPEA (5.0 μL, 0.02 mmol, 2.0 equiv) in 0.5 mL of DMF following procedure E. The reaction was monitored by LC-MS (method 1), confirming the complete conversion of the starting material in 0.5 h. The product was purified by preparative RP-HPLC (method 2) to give 21 as an orange solid (5.0 mg, 60% over two steps), r.t. = 8.0 min. ^{1}H NMR (400 MHz, CD_3OD) *δ*: 9.02 (d, *J* = 6.4 Hz, 2H), 8.44 (d, *J* = 6.4 Hz, 2H), 8.01 (s, 1H), 7.92 (d, *J* = 7.8 Hz, 1H), 7.79 (d, *J* = 8.9 Hz, 2H), 7.76 (s, 1H), 7.70 (d, *J* = 7.8 Hz, 1H), 7.61−7.56 (m, 1H), 7.06 (d, *J* = 8.9 Hz, 2H), 6.45 (d, *J* = 2.2 Hz, 1H), 6.43 (s, 1H), 6.24 (d, *J* = 2.2 Hz, 1H), 5.87 (s, 2H), 3.87 (s, 3H), 3.84 (s, 3H), 3.80 (s, 3H), 3.44 (t, *J* = 6.6 Hz, 2H), 2.95 (d, *J* = 16.9 Hz, 1H), 2.64 (d, *J* = 16.9 Hz, 1H), 1.97−1.81 (m, 2H), 1.73−1.63 (m, 2H), 1.55−1.40 (m, 8H). $^{13}C\{^1H\}$ NMR (101 MHz, CD_3OD) *δ*: 197.7, 167.7, 163.58, 163.56, 162.5, 161.5, 160.9, 156.0, 154.8, 153.8, 145.0, 143.4, 140.9, 135.9, 133.6, 131.8, 129.6, 128.2, 128.1, 126.6, 124.8, 122.8, 118.9, 114.4, 106.4, 103.1, 102.3, 101.6, 98.2, 96.0, 63.4, 54.8, 54.6, 54.5, 45.2, 40.3, 39.3, 28.7, 28.66, 28.1, 25.7, 22.6. HRMS (ESI) *m*/*z*: M^+ calcd for $C_{45}H_{46}N_3O_9^+$ 772.3229; found 772.3228.

Flow Cytometry. *A. fumigatus* Δ*rodA* conidia[18] were incubated in a flow cytometry buffer (1.5% (w/v) bovine serum albumin (BSA) and 5 mM ethylenediaminetetraacetic acid (EDTA) in phosphate-buffered saline (PBS)) on ice for 30 min. Fc-MelLec (5 μg/mL) was pretreated with or without YWA1, Fonsecin B, 2,6-DHAP, 1,8-DHN, **20b**, and **21** (all dissolved in dimethyl sulfoxide (DMSO) and used at 70 μM, resulting in a 1% DMSO solution in PBS) for 30 min at room temperature and then incubated with Δ*rodA* conidia on ice for 40 min. Following incubation, fungal particles were washed in a flow cytometry buffer and Fc-MelLec bound to the conidia was detected with allophycocyanin (APC)-conjugated donkey antihuman IgG antibody (Jackson ImmunoResearch) and then fixed in 1% (v/v) formaldehyde and analyzed by flow cytometry. One-way ANOVA and Bonferroni post hoc tests were performed to compare inhibition of Fc-MelLec binding to Δ*rodA* conidia by the different compounds versus the uninhibited Fc-MelLec control. Fold change was calculated by dividing the mean value of the pretreated Fc-MelLec with each compound by the mean value of the uninhibited Fc-MelLec.

Cell Culture and Growth Conditions. RAW 264.7 cells expressing human MelLec (hMelLec)[9] were maintained at 37 °C and 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal calf serum (FCS), 100 units/mL penicillin, 0.1 mg/mL streptomycin, 2 mM L-glutamine, and 400 μg/mL geneticin (G418) (Life Technologies, Inc.).

Immunofluorescence Microscopy. RAW 264.7 cells expressing human MelLec (hMelLec) (1.25×10^5 cells/well) were seeded in a 48-well plate at 37 °C and 5% CO_2. Next day, the cells were washed with PBS (1×) and then incubated with 10, 20, 50, or 100 μM of **21** at 37 °C and 5% CO_2 for 3 h. The cells were washed with PBS (1×) and then were visualized under an inverted immunofluorescent microscope (Zeiss). Cells without 21 were included as a negative control (untreated).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.joc.0c02324.

Experimental procedure for the synthesis of Oregon Green-NHS (precursor of compound **19**); immunofluorescence microscopy and image analysis experiments

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on binding of **21** to human MelLec-expressing RAW 264.7 cells with competition by YWA1 at different concentrations (1, 5, 25, and 50 μM); copies of ^{1}H and ^{13}C NMR spectra (PDF)

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M.P., I.P., and C.Z. designed and carried out the synthesis of all of the compounds. N.S. repeated the synthesis of all compounds. C.N. and J.A.W. performed the biological studies. G.D.B. and M.Z. designed, supervised, and coordinated the study. M.P., G.D.B., and M.Z. wrote the manuscript. I.P., M.P., N.S., C.Z., C.N., and J.A.W. wrote the Supporting Information.

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Notes

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DEDICATION

†This article is dedicated to the memory of Dr. Leonardo Manzoni.

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