Potential Photoaffinity Labels for Tubulin. Synthesis and Evaluation of Diazocyclohexadienone and Azide Analogs of Colchicine, Combretastatin, and 3,4,5-Trimethoxybiphenyl

John D. Olszewski, Mary Marshalla, Michal Sabat, and Richard J. Sundberg*

Department of Chemistry, University of Virginia, Charlottesville, Virginia 22901

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Analogs of tubulin assembly inhibitors such as colchicine, combretastatin, and 3,4,5-trimethoxybiphenyl which incorporate a 6-diazo-2,4-cyclohexadienone (*o*-quinone diazide) ring have been synthesized and characterized. The compounds synthesized include 6-diazo-4-(2′,3′,4′-trimethoxyphenyl)cyclohexa-2,4-dien-1-one and its 3-methyl and 3-ethyl analogs (**1a–c**), 6-diazo-3-(2,3,4-trimethoxyphenyl)-2,4-cyclohexadien-1-one and its 4-methyl and 4-ethyl derivatives (**2a–c**), 4-[*Z*-2-(3′,4′,5′-trimethoxyphenyl)ethenyl]-6-diazocyclohexa-2,4-dienone (**3**), 3-[*Z*-2-(3′,4′,5′-trimethoxyphenyl)ethenyl]-6-diazocyclohexa-2,4-dien-1-one (**4**), the corresponding dihydro derivatives (**5**, **6**) and two isomeric diazocyclohexadienones derived from *N*-acetylcolchinol (**7**, **8**). Compounds in which the cyclohexadienone oxygen is approximately isostructural with carbonyl or hydroxy functions of the parent compounds exhibit good activity in the tubulin assembly inhibition assay. 2′-Alkyl-4′-azido-3,4,5-trimethoxy-1,1′-biphenyls also show good activity as tubulin assembly inhibitors.

Colchicine has a unique place in the study of antimitotic substances which act by affecting the tubulin assembly–disassembly process.[1] Indeed, the colchicine-binding property of tubulin was the basis of its initial isolation.[2] Subsequently, many other naturally-occurring and synthetic compounds which bind at the "colchicine site" and inhibit tubulin assembly have been identified. One subset of these has in common the trimethoxybenzene ring found in colchicine and includes the natural products podophyllotoxin,[3] steganacin,[4] and combretastatin A4.[5] Several types of related synthetic compounds have similar activity.[6] Various synthetic analogs of colchicine also are tubulin assembly inhibitors.[7] The trimethoxybenzene ring is considered to be involved in the binding of these compounds at the "colchicine site".[8]

Although the amino acid sequence of tubulin is known for numerous organisms, it has so far resisted crystallographic structure determination so that only indirect methods for characterization of the colchicine-binding site have been available.[9] Several affinity and photoaffinity labeling studies have so far failed to provide definitive structural information. Although most of the results suggest that the binding site is on the β-subunit, it may overlap with a part of the α-subunit.[10] One potential ambiguity which is associated with most of the tubulin photoaffinity labels studied to date is that they incorporate a photoactive group which extends beyond the structure of the inhibitor itself and, therefore, may label a region adjacent to, rather than within, the binding site. In order to address this particular aspect of the photoaffinity labeling problem, we decided to synthesize diazocyclohexadienone analogs of colchicine, combretastatin, and related trimethoxybiphenyls. We reasoned that the diazocyclohexadienone ring might be an adequate structural replacement for the methoxytropone and hydroxyphenyl components of colchicine and combretastatin A4. If so, the diazocyclohexadienone ring could be photoactivated within the inhibitor binding site. Although only limited studies have so far been directed at diazocyclohexadienones as photoaffinity labels,[11] the colchicine/combretastatin series seemed to be an excellent case with which to evaluate their potential. The compounds chosen for synthesis are shown in Scheme 1.

Synthesis

The isomeric series **1a–c** and **2a–c** are structurally the simplest of the compounds selected for study. It was decided to approach these compounds by Pd-catalyzed aryl cross-coupling.[12] Two complementary routes were examined. The coupling could be carried out on a protected phenol, with subsequent introduction of the nitrogen substituent, or by use of a nitro aromatic with subsequent introduction of the oxygen substituent. In

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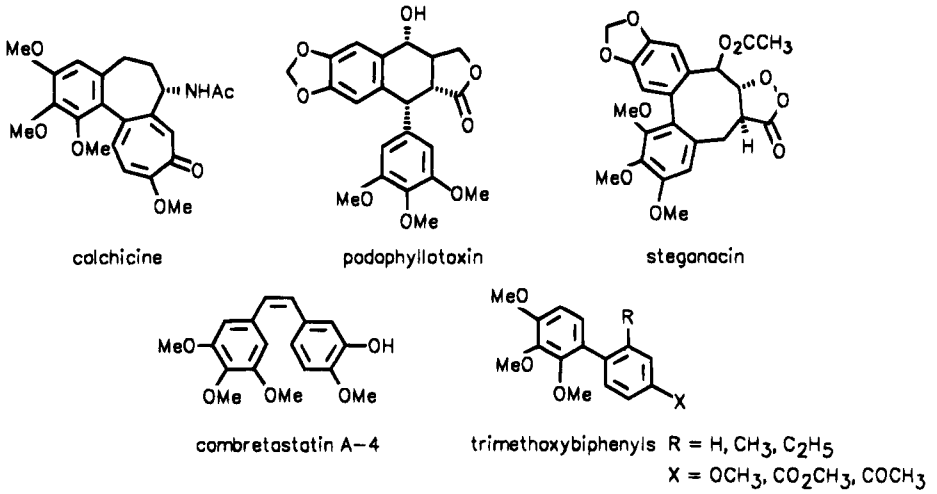
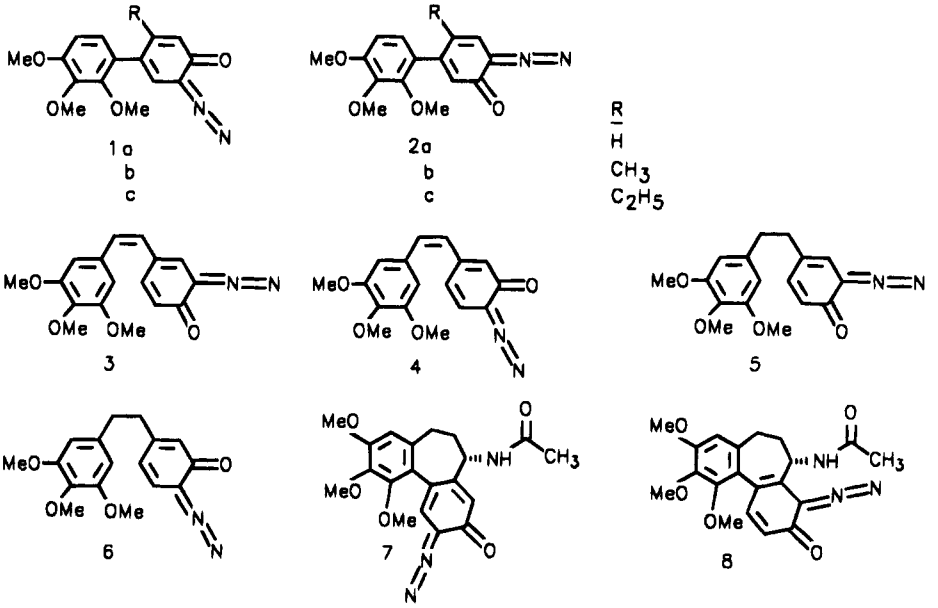
Chart 1



Scheme 1



the event, series **1a–c** turned out to be accessible by the former route while **2a–c** were best obtained by the latter method.

4-Bromophenol, 4-iodo-3-methylphenol, and 3-ethyl-4-iodophenol were converted to the corresponding methanesulfonates and subjected to palladium-catalyzed cross-coupling with tri-*n*-butyl-2,3,4-trimethoxyphenylstannane. Copper(I) iodide was included as a cocatalyst.[13] Yields for the coupling step were generally 20–30%. These compounds were converted to the corresponding phenols by alkaline hydrolysis and then subjected to azo coupling. The azo coupling for **13a** was straightforward using 4-nitrobenzenediazonium ion to afford **14a**. However, for **13b** and **13c**, yields tended to be lower and two isomeric azo coupling products were formed. More highly substituted diazonium ions such as those from 2-isopropyl or 2,6-diisopropylaniline were explored briefly in an effort to achieve better regioselectivity but without success. Instead, the isomeric mixtures from **13b** and 4-nitrobenzenediazonium ion and from **13c** and 2-methyl-4-nitrobenzenediazonium ion, which could be obtained in satisfactory yield, were reduced to the corresponding aminophenols by sodium dithionite. The 5-aminophenols **15b** and **15c** were then separated by chromatography from the isomeric 3-aminophenols. Diazotization with isoamyl nitrite in the presence of TFA gave the diazocyclohexadienones **1a–c** in good yield.[14]

For the isomeric series of diazocyclohexadienones **2a–c**, the nitrobiphenyls **17a–c** were selected as appropriate intermediates. Among other considerations, it was expected that the nitro-substituted compounds would be favorable for palladium-catalyzed cross-coupling. 4-Bromonitrobenzene (**16a**) and 2-bromo-5-nitrotoluene (**16b**) are commercially available. 2-Ethylphenol was converted to the triflate **16c** by nitration followed by reaction with triflic anhydride. Optimum conditions found for coupling of **16a** with tri-*n*-butyl-2,3,4-trimethoxyphenylstannane involved use of 5% $Pd(PPh_3)_4$ and 8% CuI in NMP at 70 °C, which gave **17a** in 65% yield. Similar coupling conditions resulted in a 50% yield for **17b**. Coupling the triflate **16c** was more difficult to accomplish in adequate

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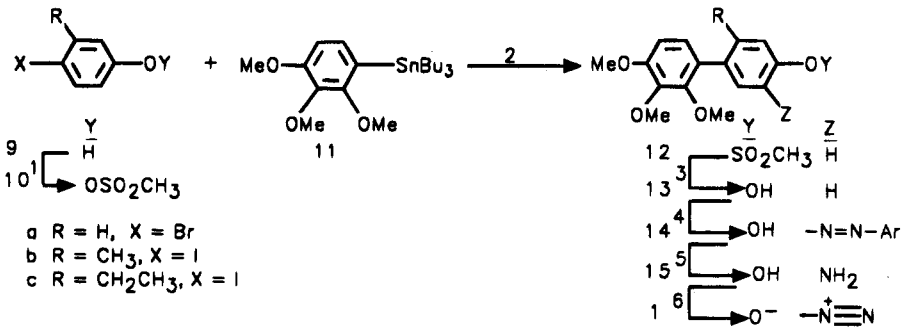
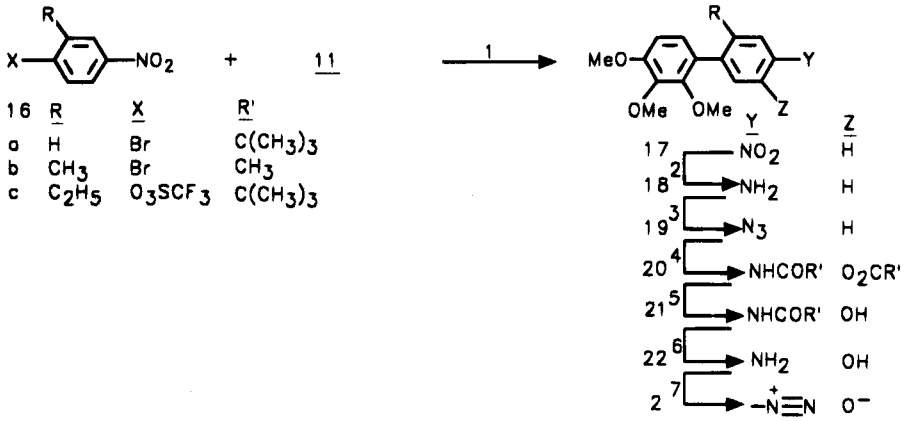
Scheme 2[a]



[a] 1: CH_3SO_2Cl, pyridine; 2: 5% $Pd(PPh_3)_4$, 8% CuI; 3: 40% NaOH; 4: ^+N_2Ar; 5: $Na_2S_2O_4$; 6: $C_5H_{11}ONO$, TFA.

Scheme 3[a]



[a] 1: 5% $Pd(PPh_3)_4$, 8% CuI, LiCl for **17c**; 2: $SnCl_2$; 3: TFA, $NaNO_2$, NaN_3; 4: $[(CH_3)CCO]_2O$ for **20a**, **20c**, $(CH_3CO)_2O$ for **20b**; 5: $MeOH/H_2O$, $NaHCO_3$; 6: 5% HCl; 7: $C_5H_{11}ONO$, TFA.

yield. Variations in the phosphine ligands[15] and addition of lithium chloride[16] have been found to be beneficial in related couplings. Exploration of these issues eventually resulted in 60–75% yields of **17c** being achieved by use of 5% $Pd(II)Cl_2(CH_3CN)_2$ and 3 equiv of LiCl along with 8% CuI in DMF or NMP.

The nitrobiphenyls **17a–c** were reduced with $SnCl_2$ to the amines **18a–c** and then converted to the azides **19a–c**. The plan was to then effect Bamberger rearrangement in acetic anhydride.[17] Because of byproduct formation, pivalic anhydride was found to be preferable for **19a**. Heating **19a** in a pivalic anhydride gave **20a** in 50–55% yield. It was converted to the aminophenol **22a** by a two-stage hydrolysis. Diazotization led to **2a**.

For **19b** and **19c** the formation of regioisomers in the Bamberger rearrangement becomes an issue. Acetic anhydride turned out to give the best results for **19b**. A mixture of acetoxy amides was obtained and deacetylated to **21b** and **21b′**. These were separable by chromatography. The desired intermediate **21b** was then deacetylated to **22b** and diazotized, affording **2b**. For **19c**, pivalic anhydride gave the best results, with **20c** being the major product of Bamberger rearrangement. Deacylation and diazotization to **2c** proceeded satisfactorily.

For the combretastatin analogs **3** and **4**, the synthetic strategy which was adopted followed one developed by Cushman for synthesis of combretastatin analogs.[18] This involves Wittig condensation and the main experimental challenge was to achieve acceptable *Z:E* ratios for the stilbene forming reaction. 4-Hydroxybenzaldehyde was converted to **27** following methodology developed by Laughton.[19] It was then converted to the phosphonium salt **28**. The best results for condensation with 3,4,5-trimethoxybenzaldehyde involved use of NaHMDS as the base in THF at −78 °C. A 54% yield of stilbenes with a 7:3 *Z:E* ratio was obtained. The desired isomers **30** could be separated by chromatography and deprotected with TBAF. After removal of the TBDMS group, the phenol **31** was coupled with 4-nitrophenyldiazonium ion and the azo compound reduced to give **33**. Diazotization with isoamyl nitrite afforded **3**. The dihydrostilbene **34** was obtained by catalytic hydrogenation of **32**. On diazotization it gave **5**.

Attempts to use the same synthesis for **4** were thwarted by poor yields at the azo coupling stage. As a result, the phosphonium salt already incorporating a nitro group was prepared. However, poor *Z:E* ratios were observed on reaction with 3,4,5-trimethoxybenzaldehyde. The two components of the Wittig reaction were then switched. The aldehyde **37** was prepared and condensed with the 3,4,5-trimethoxybenzylphosphonium salt **41**. This gave a 1:1 mixture of the *Z* and *E* isomers of **42** in excellent yield. The two isomers *Z*-**42** and *E*-**42** could be separated either by chromatography or by crystallization. Reduc-

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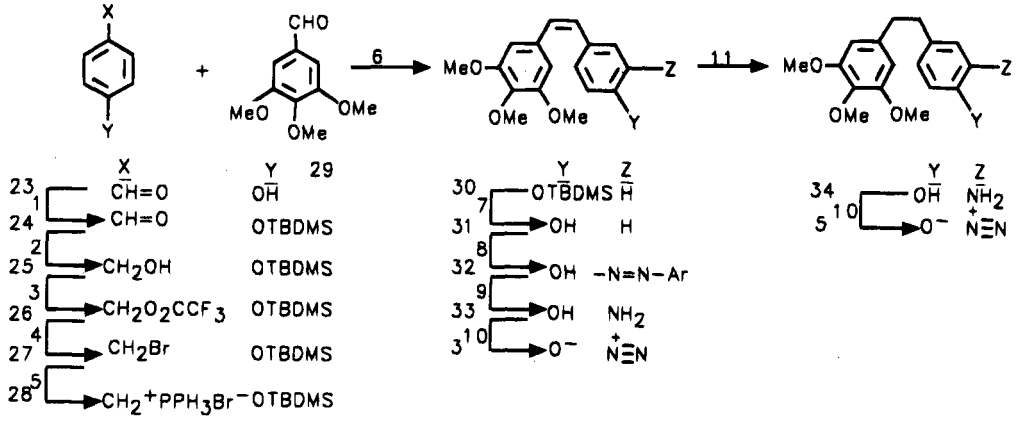
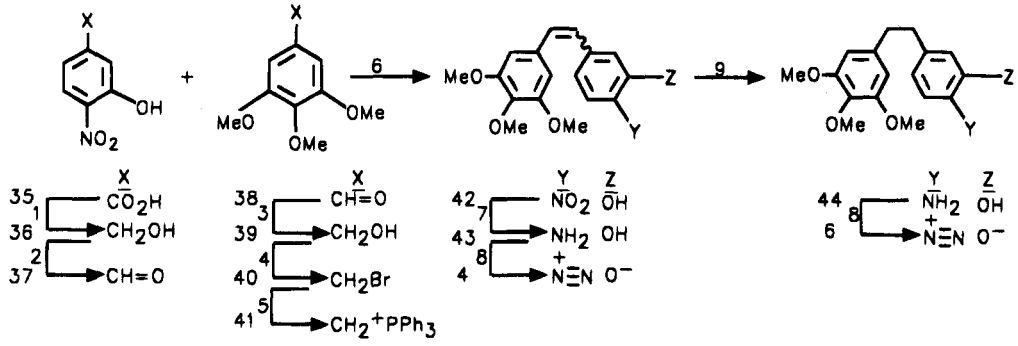
Scheme 4[a]



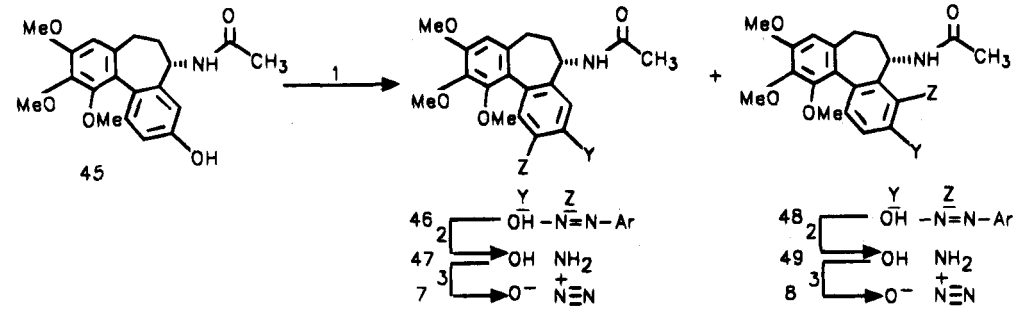
[a] 1: TBDMS-Cl, imidazole; 2: $LiAlH_4$; 3: $(CF_3CO)_2O$; 4: LiBr; 5: PPh_3; 6: NaHMDS; 7: $Bu_4N^+F^-$; separate isomers; 8: ArN_2^+; 9: $Na_2S_2O_4$; 10: $C_5H_{11}ONO$, TFA; 11: H_2, Pd/C.

Scheme 5[a]



[a] 1: BH_3-SMe_2; 2: MnO_2; 3: LAH; 4: CBr_4, PPh_3; 5: PPh_3; 6: NaHMDS, separate isomers; 7: $Na_2S_2O_4$; 8: $C_5H_{11}ONO$, TFA; 9: H_2, Pd/C.

Scheme 6[a]



[a] 1: ArN_2^+; 2: $Na_2S_2O_4$; 3: $C_5H_{11}ONO$, TFA.

tion with sodium dithionite, followed by diazotization gave **4**. The dihydro analog **6** was obtained by hydrogenating *E*-**42** to give **44** which was then subjected to the standard diazotization conditions.

For synthesis of the colchicine analogs, colchicine was hydrolyzed and subjected to ring contraction to *N*-acetylcolchinol (**45**) following known methods.[20] Yields from azo coupling were better for 2-methyl-4-nitrobenzenediazonium ion than with 4-nitrobenzenediazonium ion. A mixture of the regioisomers **46** and **48** was obtained and separated by chromatography. The azophenols were then reduced to **47** and **49** and finally diazotized affording **7** and **8**.

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All of the diazocyclohexadienone analogs showed diazo bands near 2100 cm^{-1}. The ^1H-NMR spectra were consistent with expectation and the chemical ionization mass spectra usually showed a weak molecular ion and a strong ion corresponding to loss of nitrogen and/or CO. Specific spectroscopic features are included in the Experimental Section.

Tubulin Binding and Assembly Inhibition

Two standard assays were used to determine the tubulin binding and assembly inhibition activity of the diazocyclohexadienones. Because of their potential as photoaffinity analogs, the azide intermediates **19a–c** were also examined as assembly inhibitors. The first assay used was that for tubulin assembly inhibition.[21] Tubulin solutions (~3 mg/mL protein) containing the test

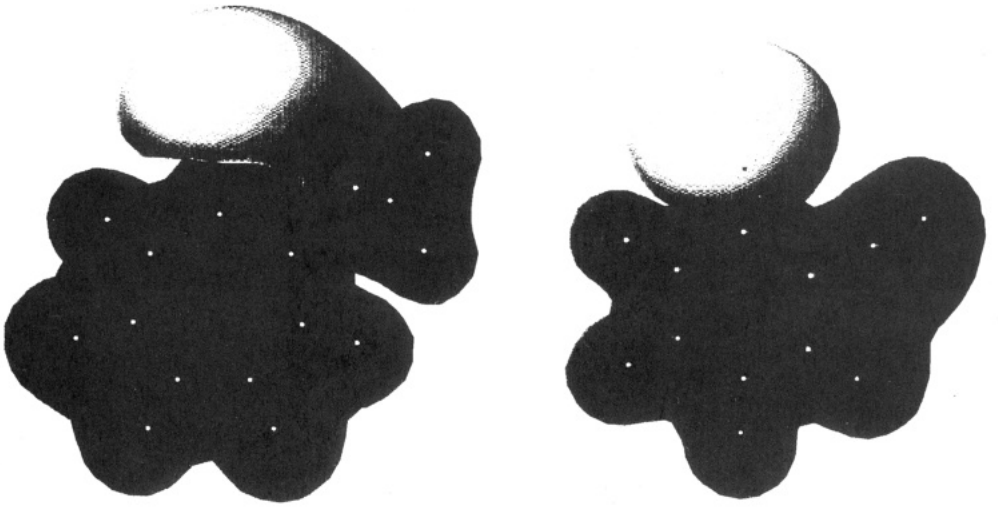


Figure 1. Electrostatic maps for 2-methoxytropolone (left) and 2-diazocyclohexa-3,5-dienone (right) from MOPAC computation.

Table 1. Tubulin Assembly and Colchicine Binding Inhibition

	IC_{50} (μM)	inhibition of ^{3}H-colchicine,[b] %
1a	22	–
1b	11	+5
1c	3	+22
2a	26	–
2b	26	–
2c	74	–
3	25	–
4	5	+17
5	20	–
6	38	–
7	2	+6
8	47	–
19a	3	–7
19b	4	+27
19c	0.8	+10
colchicine	0.9 ± 0.3[a]	

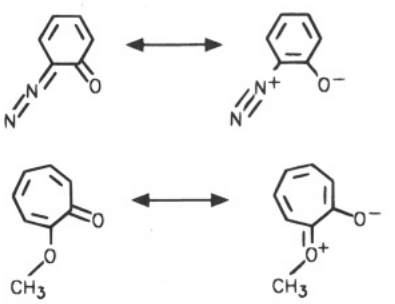
[a] Average of seven determinations. [b] Relative to an equimolar concentration of colchicine.

compound at appropriate concentrations were incubated at ambient temperature for 20 min. Assembly was then initiated by adding GTP to a final concentration of 1 mM and warming to 37 °C. Absorbance was followed at 351 nm for 20 min. The extent of polymerization was compared to a control sample containing no inhibitor. A total of six concentrations of each inhibitor was run and each experiment was done in duplicate. An IC_{50} value was determined from a third-order regression of the data using Sigma Plot 4.0. The data are reported in Table 1. For the most active assembly inhibitors a competitive binding assay was conducted by incubating equimolar amounts of the test compound and ^{3}H-colchicine with tubulin for 2 h at 37 °C. The solution was then filtered to collect tubulin–inhibitor complex and the filter disc was counted.[22] These results are reported in Table 1 as % inhibition of ^{3}H-colchicine binding relative to colchicine.

Discussion

The diazocyclohexadienones **1a–c** and **2a–c** are analogous to a series of synthetic trimethoxybiphenyls.[6] The most active of these compounds is 4-acetyl-2′,3′,4′-trimethoxy-1,1′-biphenyl which was found to inhibit tubulin assembly with an IC_{50} value of 0.6 μM. The tubulin assembly inhibition data in Table 1 indicate the **2** series has relatively low activity. On the other hand, **1b** and **1c** are quite active as assembly inhibitors. This implies that the **1** series has a better fit for the colchicine-binding site than the **2** series.

The diazocyclohexadienone ring system is a resonance hybrid of an *o*-diazoniophenolate structure. As such, it bears some resemblance to the charged resonance contributor to the tropone ring system of colchicine. Figure 1 compares the electrostatic maps of the diazocyclohexadienone and methoxytropone rings.[23] This feature of the structure, along with the considerable variation of functionality which can be tolerated in ring C of colchicine,[7,24] suggest that the diazocyclohexadienone structure would be well accommodated by the colchicine binding site.



If one overlaps the trimethoxyphenyl ring of **1** and **2** with colchicine, a better match of the cyclohexadienone oxygen with the colchicine carbonyl group is obtained for structure **1** than for structure **2**. This was explored using the fit feature of the CAChe molecular modeling program.[25] The aromatic rings were input in conformations similar to those found in the crystal structures of colchicine[26] and a related biphenyl.[6] The colchicine structure minimized to a structure very similar to that found

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(23) Andreu, J. M.; Gorbunoff, M. J.; Medrano, F. J.; Rossi, M.; Timasheff, S. N. *Biochemistry* **1991**, *30*, 3777–3786.
(24) Electrostatic potentials were calculated after the geometries were optimized by using the MOPAC program of the CAChe Worksystem 3.5 with the PM3 parameters. The electrostatic potential maps were computed by the multipole expansion method.
(25) CAChe Worksystem Version 3.5, CAChe Scientific Inc., Beaverton, OR.
(26) Lessinger, L.; Margulis, T. N. *Acta Crystallogr.* **1978**, *B34*, 578–584.

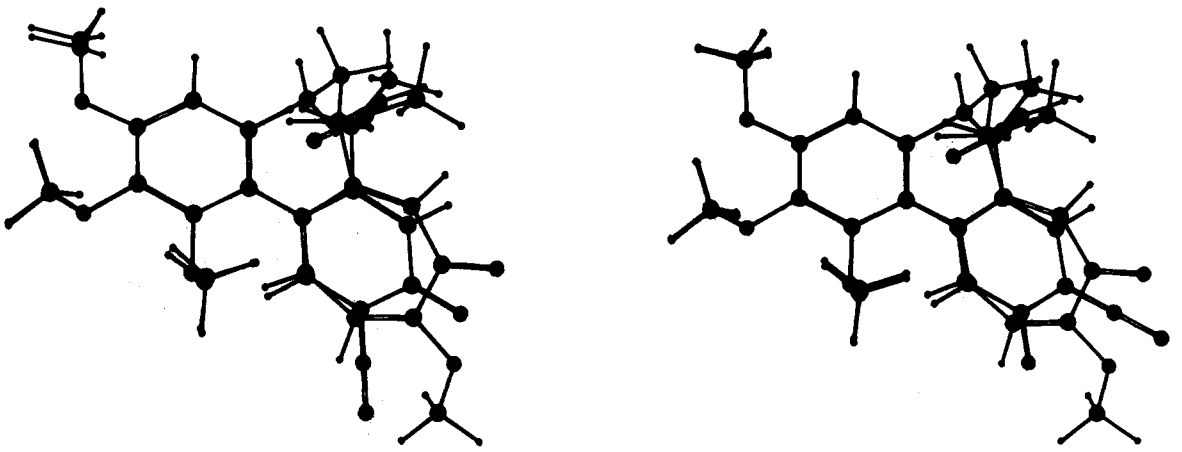

Figure 2. Comparison of overlap of compound **1c** (left) and **2c** (right) with colchicine.

crystallographically with a ring A–ring C dihedral angle of 55°. The biphenyl minimized with larger dihedral angles and were fit to the colchicine template by adjusting the dihedral angle to 53°. Figure 2 shows the comparison of fit of colchicine with **1c** and **2c**. The diazocyclohexadienone oxygen is closer to the tropolone oxygen in **1c** than in **2c**. Since **1c** is a more active assembly inhibitor than **2c**, this suggests that this oxygen makes a significant contribution to tubulin binding. Compound **2c** may be thought of as analogous to isocolchicine which is a much weaker tubulin assembly inhibitor.[27]

Of the isomeric colchicine analogs **7** is much more active than **8**. The carbonyl groups are similarly disposed in these two isomers and the lower activity of **8** suggests that the diazo substituent at C8 interferes with binding at the colchicine site. The minimized conformation of the tricyclic ring system in **8** is not appreciably different from that in colchicine.

The biphenyl azides **19a–c** also show inhibitory activity both in the assembly and colchicine binding assays. The azide **19b** and quinonediazide **7** have been synthesized in radioactive form and further studies on these compounds are in progress.

Experimental Section

Melting points were determined in capillary tubes and are uncorrected. High resolution mass measurements were performed by Mr. Kim Harich, VPISU, under methane chemical ionization conditions using a polydimethylsiloxane mixture as an internal standard for mass measurement. THF was distilled from sodium/benzophenone. CH_2Cl_2, *N*-methylpyrrolidinone (under vacuum), Et_3N, and CH_3CN were all distilled from CaH_2 under nitrogen. Cuprous iodide was recrystallized from saturated aqueous sodium iodide. Tris(dibenzylideneacetone)palladium(0) chloroform complex and bis(acetonitrile)-palladium(II) chloride were gifts from Dr. William Pitts of SKB. Product isolation was normally done by extraction with Et_2O or EtOAc followed by purification by flash chromatography if necessary. Silica gel used for flash column chromatography was Fisher 200–425 mesh, grade 633, type 60A or Baker 230–400 mesh, type 60. The following general synthetic procedures were used and will be referred to by method.

Method A. Procedure for Iodination of Phenols. Using the procedure of Edgar and Falling,[28] the phenol, sodium iodide (1 equiv), and crushed NaOH (1 equiv) were dissolved in MeOH (25 mL/g of phenol) and cooled to 0 °C. Sodium hypochlorite (1 equiv, 5.25% bleach) was added dropwise over 30–60 min with stirring at 0 °C. After the addition was complete, the reaction mixture was allowed to warm to room temperature and stirred overnight (17 h). After acidification with 10% HCl, the product was isolated by precipitation or extraction with Et_2O.

Method B. Procedure for Protection of Phenols as Mesylates. Using the method of Bordwell and Boutan,[29] the phenol was dissolved in dry pyridine and cooled to 0 °C. Methanesulfonyl chloride (2 equiv) was added dropwise with stirring. After stirring at 0 °C for 10–15 min the flask was stoppered and stored at 5 °C overnight. The contents were then poured into 10% HCl and the product filtered or extracted.

Method C. Procedure for Palladium Coupling Reactions. Using Liebeskind and Fengl's modification of the Stille coupling reaction,[13] the mesyl-protected phenol or *p*-nitrophenyl halide was dissolved in dry NMP with tetrakis(triphenylphosphine)palladium(0) (5 mol %), and copper iodide (8 mol %) was added with stirring. Tri-*n*-butyl-3,4,5-trimethoxyphenylstannane (1.1 equiv) was added and the reaction mixture warmed to 70 °C with stirring. After 48 h, the mixture was cooled to room temperature, diluted with EtOAc, and filtered through Celite. The filtrate was washed three times with 10% NH_4OH, once with 10% KF, once with water, twice with brine, dried over $MgSO_4$, filtered, and concentrated. The resulting oil was purified via flash chromatography.

Method D. Procedure for Deprotection of Mesylated Biphenyl Compounds. Using the method of Bordwell and Boutan,[29] the (mesyloxy)biphenyl was suspended in 40% NaOH (w/v) and heated at 70 °C with constant stirring for 24–48 h. After cooling to 0 °C, the reaction mixture was acidified with concd H_2SO_4 and the product extracted with EtOAc.

Method E. Procedure for Azo Coupling Reactions.[30] The phenolate was dissolved in 15% NaOH (w/v) and cooled to 0 °C. In a separate flask, 2-methyl-4-nitroaniline or 4-nitroaniline (2 equiv) was dissolved in 50% HCl (v/v) and cooled to 0 °C, and sodium nitrite (2.5 equiv) was added with vigorous stirring. The diazotized aniline solution was added dropwise to the phenol solution at 0 °C with constant stirring while maintaining a basic pH by monitoring the color (basic = purple; acidic = orange red) and periodic addition of 15% NaOH (w/v). After the addition was complete, stirring was continued at room temperature for 24 h. The solution was then acidified with HCl and the product extracted with CH_2Cl_2 and purified via flash column chromatography.

Method F. Procedure for Reduction of Azo Com-

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pounds. Using a procedure similar to that of Vogel,[31] the azo compound was dissolved in 2:1 acetone/water and heated to 60 °C. Sodium dithionite (20 equiv) was added with stirring. Stirring was continued for 30 min, the reaction mixture cooled to room temperature and diluted with water, and the product extracted with EtOAc.

Method G. Procedure for Preparation of Diazocyclohexadienone Compounds. Using a procedure similar to that of Sundberg and co-workers,[14] the aminophenol was dissolved in 95% EtOH, and the flask was wrapped with aluminum foil and cooled to 0 °C. Isoamyl nitrite (3.7 equiv) and TFA (to 10% total volume) were added with stirring. After 15 min at 0 °C, stirring was continued at room temperature for 1.5 h. The mixture was then concentrated and purified via flash column chromatography over silica.

Method H. Procedure for Reduction of Nitrobiphenyls. Using the method of Bellamy and Ou,[32] the nitrobiphenyl was dissolved in 2:1 95% EtOH/EtOAc. Tin(II) chloride (5 equiv) was added with stirring and the mixture heated at 70 °C for 24 h with stirring. The reaction mixture was then cooled to room temperature, poured into ice–water, and brought to pH = 8 with saturated $NaHCO_3$. The product was then extracted with EtOAc.

Method I. Procedure for Conversion of Aminobiphenyls to Azidobiphenyls. Applying the method of Pinney and Katzenellenbogen,[33] the aminobiphenyl was dissolved in neat TFA and cooled to 0 °C. Sodium nitrite (1 equiv) was added at 0 °C and the mixture stirred for 10 min. Sodium azide (10 equiv) was dissolved in a minimal amount of water and added dropwise to the reaction mixture at 0 °C, with stirring and protection from light. The reaction mixture was stirred for 15 min at 0 °C and then for 10 min at room temperature. After dilution with two times the volume of water, the product was extracted with ether.

Method J. Procedure for Bamberger Rearrangement of Azidobiphenyls. Using the method of Smalley and Suschitzky,[34] the azidobiphenyl was refluxed in acetic anhydride or heated at 135 °C in pivalic anhydride for 15 h. The excess anhydride was removed under reduced pressure and the product mixture purified via flash column chromatography over silica.

Method K. Procedure for Deacetylation of the Hydroxyl Group. Using the conditions of Büchi and Weinreb,[35] the 2-acetoxy-4-(2,3,4-trimethoxyphenyl)acetanilide was dissolved in 2:1:1 MeOH/saturated aqueous $NaHCO_3$/water, stirred at room temperature for 6 h and then acidified with 10% HCl. The product was extracted with EtOAc.

Method L. Procedure for Deacetylation of the Acetamido Group. Using the method of Dilbeck and co-workers,[36] the 2-hydroxy-4-(2,3,4-trimethoxyphenyl)acetanilide was refluxed in 5% HCl for 10 h, cooled to room temperature, and neutralized with 10% $NaHCO_3$. The product was obtained by filtration or extracted with EtOAc.

Method M. Procedure for Hydrogenation of Stilbenes. Using the procedure of Cushman and co-workers,[18] the stilbene (50–100 mg) was dissolved in 20 mL of dry EtOAc and palladium on carbon (20 mg) added. The flask was charged to 40 psi of hydrogen and shaken gently for 4.5 h at room temperature. The reaction mixture was filtered through Celite, washing with EtOAc, EtOH, and CH_2Cl_2 and the filtrate concentrated.

4-Iodo-3-methylphenol (9b). Using method A, *m*-cresol (10 g, 0.093 mol) was iodinated and allowed to crystallize from the crude filtrate after concentrating. The product was isolated in 53% yield as white crystals: mp 96–98 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.61 (d, 2H, J = 8.4 Hz), 6.76 (s, 1H, J = 3 Hz), 6.42 (dd, 1H, J = 8.4, 3 Hz), 4.66 (s, 1H), 2.37 (s, 3H); CIMS (methane) m/e 235 (MH^+, 100), 234 (43).

3-Ethyl-4-iodophenol (9c). Using method A, 3-ethylphenol (5 g, 0.041 mol) was iodinated and crystallization achieved by diluting the concentrated filtrate with petroleum ether, cooling to −78 °C and slowly adding hexane while scratching with a glass rod. The yield ranged from 50 to 80%: mp 53–54 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.62 (d, 1H, J = 8.4 Hz), 6.75 (d, 1H, J = 3 Hz), 6.43 (dd, 1H, J = 8.4, 3 Hz), 4.65 (s, OH), 2.66 (q, 2H), 1.19 (t, 3H), CIMS (methane) m/e 248 (MH^+ − 1, 100), 122 (33).

Tri-*n*-butyl-(2,3,4-trimethoxyphenyl)stannane (11). 3,4,5-Trimethoxybenzene (5 g, 0.03 mol) was dissolved in dry THF and cooled to −78 °C. 1.6 M *n*-Butyllithium (0.0328 mol, 20.5 mL) in hexanes was added with stirring and the solution was maintained at −78 °C for 30 min. The reaction mixture was allowed to warm to room temperature and stirring was continued for 2 h. The mixture was then cooled to −78 °C and tri-*n*-butyltin chloride (9.7 g, 0.0298 mol, 8.08 mL) added slowly with stirring. After the addition was complete, stirring was continued for 20 min at −78 °C and then for 2 h at room temperature. The mixture was poured into saturated NH_4Cl and extracted three times with ether. The product was purified by flash column chromatography over silica using 100% hexane followed by 10% EtOAc in hexane to give the product as a tan oil in 55% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.98 (d, 1H, J = 8.4 Hz), 6.68 (d, 1H, J = 8.4 Hz), 3.87 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H), 1.80–0.80 (m, 27H); CIMS (methane) m/e 401 (MH^+ − 57, 65), 399 (53), 397 (25), 291 (100), 289 (82), 287 (45).

4′-(Methanesulfonyloxy)-2,3,4-trimethoxy-1,1′-biphenyl (12a). 4-Bromophenol (2 g, 0.012 mol) was protected via method B. Fine white needles were collected via Büchner filtration in 89% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.54 (d, 2H, J = 9 Hz), 7.18 (d, 2H, J = 9 Hz), 3.15 (s, 3H). The methanesulfonate **10a** (500 mg, 1.99 mmol) was subjected to the conditions of method C. The product was obtained as a white solid in 17% yield (NOTE: smaller scale reactions usually result in higher yields): mp 173–175 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.54 (d, 2H, J = 8.7 Hz), 7.31 (d, 2H, J = 8.7 Hz), 7.01 (d, 1H, J = 8.7 Hz), 6.74 (d, 1H, J = 8.7 Hz), 3.93 (s, 3H), 3.90 (s, 3H), 3.69 (s, 3H), 3.18 (s, 3H).

4′-(Methanesulfonyloxy)-2′-methyl-2,3,4-trimethoxy-1,1′-biphenyl (12b). The phenol **9b** (910 mg, 3.87 mmol), was protected via method B and the product was isolated in 90% yield as white crystals: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.82 (d, 1H, J = 8.7 Hz), 7.18 (d, 1H, J = 3 Hz), 6.84 (dd, 1H, J = 8.7, 3 Hz), 3.14 (s, 3H), 2.45 (s, 3H). The methanesulfonate **10b** (500 mg, 1.60 mmol) was subjected to the conditions of method C. The product was obtained as a white solid in 33% yield: mp 139-143 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.22 (d, 1H, J = 8.7 Hz), 7.17 (d, 1H, J = 2.1 Hz), 7.14 (dd, 1H, J = 8.7, 2.1 Hz), 6.81 (d, 1H, J = 8.4 Hz), 6.72 (d, 1H, J = 8.4 Hz), 3.92 (s, 3H), 3.91 (s, 3H), 3.59 (s, 3H), 3.18 (s, 3H), 2.18 (s, 3H); CIMS (methane) m/e 353 (MH^+, 100).

4′-(Methanesulfonyloxy)-2′-ethyl-2,3,4-trimethoxy-1,1′-biphenyl (12c). The phenol **9c** (3.13 g, 0.013 mol) was protected via method B. The product **10c** did not crystallize but was extracted from the 10% HCl with EtOAc and obtained as a light yellow oil in 94% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.81 (d, 1H, J = 8.7 Hz), 7.14 (d, 1H, J = 2.7 Hz), 6.84 (dd, 1H, J = 8.7, 2.7 Hz), 3.13 (s, 3H), 2.71 (q, 2H), 1.20 (t, 3H). The methanesulfonate **10c** (500 mg, 1.53 mmol) was subjected to the conditions of method C. The product was obtained as a tan oil in 21–31% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.21 (d, 1H, J = 8.7 Hz), 7.20 (d, 1H, J = 2.7 Hz), 7.12 (dd, 1H, J = 8.7, 2.7 Hz), 6.81 (d, 1H, J = 8.7 Hz), 6.71 (d, 1H, J = 8.7 Hz), 3.91 (s, 3H), 3.90 (s, 3H), 3.60 (s, 3H), 3.18 (s, 3H), 2.50 (m, broad, 2H), 1.08 (t, 3H).

4′-Hydroxy-2,3,4-trimethoxy-1,1′-biphenyl (13a). The methanesulfonate **12a** (100 mg, 0.30 mmol) was subjected to the conditions of method D. A white solid was obtained in 97% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.38 (d, 2H, J = 8.4 Hz), 7.00 (d, 1H, J = 8.4 Hz), 6.87 (d, 2H, J = 8.4 Hz), 6.72 (d,

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1H, J = 8.4 Hz), 4.82 (s, broad, OH), 3.92 (s, 3H), 3.89 (s, 3H), 3.66 (s, 3H).

4′-Hydroxy-2′-methyl-2,3,4-trimethoxy-4′1,1′-biphenyl (13b). The methanesulfonate **12b** (93 mg, 0.26 mmol) was subjected to the conditions of method D. The product was obtained as a white solid in 88% yield: mp 139−143 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.05 (d, 1H, J = 8.1 Hz), 6.82 (d, 1H, J = 8.4 Hz), 6.73 (d, 1H, J = 2.7 Hz), 6.70 (d, 1H, J = 8.4 Hz), 6.68 (dd, 1H, J = 8.1, 2.7 Hz), 3.92 (s, 3H), 3.90 (s, 3H), 3.56 (s, 3H), 2.12 (s, 3H); CIMS (methane) m/e 275 (MH^+, 100).

2′-Ethyl-4′-hydroxy-2,3,4-trimethoxy-1,1′-biphenyl (13c). The methanesulfonate **12c** (187 mg, 0.51 mmol) was subjected to the conditions of method D. The product was obtained as a tan solid in 94% yield: mp 117−120 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.03 (d, 1H, J = 8.7 Hz), 6.82 (d, 1H, J = 8.4 Hz), 6.78 (d, 1H, J = 2.7 Hz), 6.69 (d, 1H, J = 8.4 Hz), 6.68 (dd, 1H, J = 8.7, 2.7 Hz), 4.71 (s, broad, OH), 3.91 (s, 3H), 3.90 (s, 3H), 3.57 (s, 3H), 2.45 (m, broad, 2H), 1.06 (t, 3H).

3′-Amino-4′-hydroxy-2,3,4-trimethoxy-1,1′-biphenyl (15a). The hydroxybiphenyl **13a** (56.5 mg, 0.217 mmol) was subjected to the conditions of method E. The product was purified via flash column chromatography over silica using CH_2Cl_2, and **14a** was obtained in a 55% yield as a red-brown solid: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.40 (d, 2H, J = 9 Hz), 8.15 (d, 1H, J = 2.1 Hz), 8.03 (d, 2H, J = 9 Hz), 7.63 (dd, 1H, J = 8.7, 2.1 Hz), 7.10 (d, 2H, J = 8.4 Hz), 6.78 (d, 1H, J = 8.7 Hz), 5.30 (s, OH), 3.96 (s, 3H), 3.92 (s, 3H), 3.74 (s, 3H). The azobiphenyl **14a** (88.5 mg, 0.216 mmol) was subjected to the conditions of method F. A red solid was obtained in quantitative yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.98 (d, 1H, J = 8.7 Hz), 6.94 (d, 1H, J = 2.1 Hz), 6.79 (dd, 1H, J = 2.1, 8.6 Hz), 6.70 (d, 1H, J = 8.7 Hz), 6.57 (s, 1H), 3.92 (s, 3H), 3.88 (s, 3H), 3.66 (s, 3H).

5′-Amino-4′-hydroxy-2′-methyl-2,3,4-trimethoxy-1,1′-biphenyl (15b). The hydroxybiphenyl **13b** (80 mg, 0.292 mmol) was subjected to the conditions of method E. The product was purified via flash column chromatography over silica using CH_2Cl_2. The 3′ and 5′ azo coupling products were obtained in a combined 47% yield as a red-brown solid in approximately a 1:1 ratio, but proved to be inseparable: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.37 (d, 2H, J = 9 Hz), 8.38 (d, 2H, J = 9 Hz), 7.98 (d, 2H, J = 9 Hz), 7.97 (d, 2H, J = 9 Hz), 7.80 (s, 1H), 7.29 (d, 1H, J = 8.4 Hz), 6.95 (s, 1H), 6.91 (d, 1H, J = 8.7 Hz), 6.89 (hidden, d, 1H), 6.75 (d, 1H, J = 8.7 Hz), 3.94 (s, 6H), 3.92 (s, 6H), 3.65 (s, 6H), 2.56 (s, 3H), 2.23 (s, 3H). The mixture containing **14b** (93 mg, 0.220 mmol) was subjected to the conditions of method F. The resulting isomers were separated via flash column chromatography over silica using 1:1 EtOAc/hexane. The desired isomer **15b** was obtained in 45% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.81 (d, 1H, J = 8.7 Hz), 6.68 (d, 1H, J = 8.7 Hz), 6.65 (s, broad, 2H), 3.91 (s, 3H), 3.89 (s, 3H), 3.58 (s, 3H), 2.03 (s, 3H).

5′-Amino-2′-ethyl-4′-hydroxy-2,3,4-trimethoxy-1,1′-biphenyl (15c). The hydroxybiphenyl **13c** (145 mg, 0.49 mmol) was subjected to the conditions of method E, using 2-methyl-4-nitroaniline. Purification was achieved via flash column chromatography over silica using 100% CH_2Cl_2. A mixture of regioisomers was isolated in 82% yield as a red oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 13.87 (s, 1H), 12.90 (s, 1H), 8.26 (d, 1H, J = 3 Hz), 8.24 (d, 1H, J = 3 Hz), 8.18 (dd, 1H, J = 9, 3 Hz), 8.15 (dd, 1H, J = 9, 3 Hz), 7.90 (d, 1H, J = 9 Hz), 7.88 (d, 1H, J = 9 Hz), 7.80 (s, 1H), 7.26 (d, 1H, obscured), 7.00 (s, 1H), 6.92 (d, 1H, J = 8.7 Hz), 6.91 (d, 1H, J = 8.7 Hz), 6.74 (d, 1H, J = 8.7 Hz), 3.95 (s, 6H), 3.94 (s, 6H), 3.67 (s, 3H), 3.66 (s, 3H), 2.75 (s, 3H), 2.72 (s, 3H), 2.55 (m, broad, 2H), 1.05−1.20 (overlapping triplets, 3H each). The azobiphenyl mixture (185 mg, 0.42 mmol) was subjected to the conditions of method F. The resulting regioisomers were separated via flash column chromatography over silica using 2:1 hexane/EtOAc followed by 1:1 hexane/EtOAc. The desired product **15c** was isolated as a yellow oil in 48% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.83 (s, 1H) 6.77 (d, 1H, J = 8.1 Hz), 6.75 (s, 1H), 6.67 (d, 1H, J = 8.1 Hz), 3.89 (s, 6H), 3.57 (s, 3H), 2.36 (m, broad, 2H), 1.02 (t, 3H); CIMS (methane) m/e 304 (MH^+, 87), 303 (65), 115 (55), 101 (100).

6-Diazo-4-(2,3,4-trimethoxyphenyl)-2,4-cyclohexadien-1-one (1a). The 4′-amino-3′-hydroxybiphenyl **15a** (59.4 mg, 0.216 mmol) was subjected to the conditions of method G. The product was isolated as an orange-brown solid in 52% yield: IR 2115, 1642 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.56 (dd, 1H, J = 9.6, 2.4 Hz), 7.29 (d, 1H, J = 2.4 Hz), 6.96 (d, 1H, J = 8.7 Hz), 6.76 (d, 1H, J = 9.6 Hz), 6.71 (d, 1H, J = 8.7 Hz), 3.92 (s, 3H), 3.89 (s, 3H), 3.74 (s, 3H); CIMS (methane) m/e 287 (MH^+, 52), 261 (27), 259 (45), 100 (100); HRMS found 287.1042, calcd for MH^+ $C_{15}H_{15}N_2O_4$ 287.1032.

6-Diazo-3-methyl-4-(2,3,4-trimethoxyphenyl)-2,4-cyclohexadien-1-one (1b). The 5′-amino-4′-hydroxybiphenyl **15b** (19.1 mg, 0.066 mmol) was subjected to the conditions of method G. The product was purified by flash column chromatography over silica using 2:1 EtOAc/hexane followed by 100% EtOAc and isolated in 61% yield as a red solid: IR 2109, 1639 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.98 (s, broad, 1H), 6.78 (d, 1H, J = 8.4 Hz), 6.68 (d, 1H, J = 8.4 Hz), 6.67 (s, broad, 1H), 3.89 (s, 6H), 3.70 (s, 3H), 2.04 (s, 3H); CIMS (methane) m/e 272 (MH^+ − 28, 13), 227 (100), 113 (35); HRMS found 301.1201, calcd for MH^+ $C_{16}H_{17}N_2O_4$ 301.1188.

6-Diazo-3-ethyl-4-(2,3,4-trimethoxyphenyl)-2,4-cyclohexadien-1-one (1c). The 5′-amino-4′-hydroxybiphenyl **15c** (35.1 mg, 0.116 mmol) was subjected to the conditions of method G. The product was purified by flash column chromatography over silica using 2:1 EtOAc/hexane followed by 100% EtOAc and isolated in quantitative yield as a red solid: FTIR 2110, 1626 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.02 (s, 1H), 6.86 (s, broad, 1H), 6.79 (d, 1H, J = 8.7 Hz), 6.68 (d, 1H, J = 8.7 Hz), 3.90 (s, 6H), 3.72 (s, 3H), 2.37 (m, broad, 2H), 1.04 (t, 3H); CIMS (methane) m/e 315 (MH^+, 100), 305 (34), 289 (95), 287 (37), 272 (28), 115 (45), 65 (63).

2-Ethyl-4-nitro-1-[(trifluoromethanesulfonyloxy)]-benzene (16c). Using the nitration method of Crivello,[37] 2-ethylphenol (1.0 g, 82 mmol) was dissolved in dry acetonitrile, and ammonium nitrate (656 mg, 8.2 mmol) was added with stirring. After cooling to 0 °C, trifluoroacetic anhydride (1.89 g, 9.0 mmol) was added with stirring. The reaction mixture was allowed to warm to room temperature slowly as the ice bath melted, stirred for 6 h, and quenched with water. After extracting with CH_2Cl_2, the extract was washed with brine, dried, and concentrated. The residue was purified via flash column chromatography over silica using 2:1 CH_2Cl_2/hexane followed by 4:1 CH_2Cl_2/hexane. The product was isolated as a light orange solid in 25−30% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.08 (d, 1H, J = 3 Hz), 8.02 (dd, 1H, J = 9, 3 Hz), 6.82 (d, 1H, J = 9 Hz), 5.42 (s, 1H), 2.70 (q, 2H), 1.29 (t, 3H); CIMS (methane) m/e 168 (MH^+, 100). Using a procedure similar to that of Echavarren and Stille,[16] 2-ethyl-4-nitrophenol (1.0 g, 6.0 mmol) was dissolved in dry CH_2Cl_2 and triethylamine (667 mg, 6.6 mmol) was added with stirring at room temperature. After stirring for 5 min the reaction mixture was cooled to −78 °C and triflic anhydride (1.86 g, 6.6 mol) was added with stirring. Stirring was continued while allowing the reaction to warm to room temperature for 3 h. The reaction mixture was then washed three times with 10% HCl and once with brine, dried, and concentrated to an orange oil. The product was isolated in 92−97% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.25 (d, 1H, J = 2.7 Hz), 8.16 (dd, 1H, J = 8.7, 2.7 Hz), 7.44 (d, 1H, J = 8.7 Hz), 2.85 (q, 2H), 1.34 (t, 3H); CIMS (methane) m/e 300 (MH^+, 100).

4′-Nitro-2,3,4-trimethoxy-1,1′-biphenyl (17a). 4-Nitrobromobenzene (1.24 g, 6.1 mmol) was subjected to the conditions of method C. The product was purified via flash column chromatography over silica using 5% EtOAc/hexane followed by 10% EtOAc/hexane and was obtained as a bright yellow solid in 45% yield: mp 108−111 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.25 (d, 2H, J = 8.7 Hz), 7.68 (d, 2H, J = 8.7 Hz), 7.06 (d, 1H, J = 8.4 Hz), 6.78 (d, 1H, J = 8.4 Hz), 3.94 (s, 3H), 3.92 (s, 3H), 3.70 (s, 3H); CIMS (methane) m/e 290 (MH^+, 100); Anal. Calcd for $C_{15}H_{15}NO_5$: C, 62.27; H, 5.23; N, 4.86. Found: C, 62.14; H, 5.22; N, 4.78.

2′-Methyl-4′-nitro-2,3,4-trimethoxy-1,1′-biphenyl (17b). 2-Bromo-5-nitrotoluene (700 mg, 3.24 mmol) was subjected to

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the conditions of method C. The product was purified via flash column chromatography over silica using 5% EtOAc/hexane followed by 10% EtOAc/hexane and crystallized from EtOAc/hexane after being kept in the freezer for several days. It was isolated as pale yellow needles in 51% yield: mp 87–89 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.13 (d, 1H, J = 2.7 Hz), 8.06 (dd, 1H, J = 8.4, 2.7 Hz), 7.34 (d, 1H, 8.4 Hz), 6.81 (d, 1H, J = 8.7 Hz), 6.74 (d, 1H, J = 8.7 Hz), 3.93 (s, 3H), 3.92 (s, 3H), 3.60 (s, 3H), 2.27 (s, 3H); Anal. Calcd for $C_{16}H_{17}NO_5$: C, 63.35; H, 5.65; N, 4.64. Found: C, 63.29; H, 5.68, N, 4.63.

2′-Ethyl-4′-nitro-2,3,4-trimethoxy-1,1′-biphenyl (17c). The triflate **16c** (500 mg, 1.672 mmol) was subjected to the conditions of method C, also adding dry lithium chloride (213 mg, 5.02 mmol). The product was collected as a yellow solid in 77% yield. It could be recrystallized from EtOAc/hexane in the freezer: mp 115–117 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.17 (d, 1H, J = 3 Hz), 8.06 (dd, 1H, J = 3, 8.4 Hz), 7.32 (d, 1H, J = 8.4 Hz), 6.81 (d, 1H, J = 9 Hz), 6.73 (d, 1H, J = 9 Hz), 3.92 (s, 6H), 3.61 (s, 3H), 2.60 (m, 2H), 1.14 (t, 3H); CIMS (methane) m/e 318 (MH^+, 100). Anal. Calcd for $C_{17}H_{19}NO_5$: C, 64.33; H, 6.03; N, 4.43. Found: C, 64.39; H, 6.06; N, 4.46.

4′-Azido-2,3,4-trimethoxy-1,1′-biphenyl (19a). The 4′-nitrobiphenyl **17a** (300 mg, 1.04 mmol) was subjected to the conditions of method H. Upon concentration, the product **18a** was isolated as a tan solid in 96% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.31 (d, 2H, J = 8.4 Hz), 6.99 (d, 1H, J = 9 Hz), 6.73 (d, 2H, J = 8.4 Hz), 6.70 (d, 1H, J = 9 Hz), 3.92 (s, 3H), 3.88 (s, 3H), 3.66 (s, 3H); CIMS (methane) m/e 260 (MH^+, 100), 89 (55). The 4′-aminobiphenyl **18a** (160 mg, 0.62 mmol) was subjected to the conditions of method I. The product was isolated as an orange-brown semisolid in quantitative yield: IR 2120, 2100 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.50 (d, 2H, J = 8.7 Hz), 7.06 (d, 2H, J = 8.7 Hz), 7.01 (d, 1H, J = 8.7 Hz), 6.74 (d, 1H, J = 8.7 Hz), 3.93 (s, 3H), 3.90 (s, 3H), 3.67 (s, 3H); CIMS (methane) m/e 286 (MH^+, 67), 258 (100).

4′-Azido-2′-methyl-2,3,4-trimethoxy-1,1′-biphenyl (19b). The 4′-nitrobiphenyl **17b** (500 mg, 1.65 mmol) was subjected to the conditions of method H. Upon concentration, the product **18b** was isolated as a tan solid in 90% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.97 (d, 1H, J = 8.1 Hz), 6.83 (d, 1H, J = 8.7 Hz), 6.69 (d, 1H, J = 8.7 Hz), 6.61 (d, 1H, J = 2.1 Hz), 6.56 (dd, 1H, J = 8.1, 2.1 Hz), 3.93 (s, 3H), 3.90 (s, 3H), 3.62 (s, broad, NH_2), 3.56 (s, 3H), 2.09 (s, 3H); CIMS (methane) m/e 274 (MH^+, 100). The 4′-aminobiphenyl **18b** (263 mg, 0.96 mmol) was subjected to the conditions of method I. The product was isolated as an orange-brown semisolid in 95% yield: IR 2112 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.17 (d, 1H, J = 8.4 Hz), 6.92 (d, 1H, J = 2.1 Hz), 6.88 (dd, 1H, J = 8.4, 2.1 Hz), 6.81 (d, 1H, J = 8.4 Hz), 6.71 (d, 1H, J = 8.4 Hz), 3.92 (s, 3H), 3.90 (s, 3H), 3.57 (s, 3H), 2.16 (s, 3H); CIMS (methane) m/e 300 (MH^+, 25), 272 (100).

4′-Azido-2′-ethyl-2,3,4-trimethoxy-1,1′-biphenyl (19c). The 4′-nitrobiphenyl **17c** (460 mg, 1.45 mmol) was subjected to the conditions of method H which gave **18c** in 91% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.98 (d, 1H, J = 8.1 Hz), 6.82 (d, 1H, J = 8.4 Hz), 6.70 (d, 1H, J = 2.4 Hz), 6.68 (d, 1H, J = 8.4 Hz), 6.62 (dd, 1H, J = 2.4, 8.1 Hz), 3.91 (s, 3H), 3.89 (s, 3H), 3.57 (s, 3H), 2.43 (m, 2H), 1.05 (t, 3H); CIMS (methane) m/e 288 (MH^+, 100), 287 (90). The 4′-aminobiphenyl **18c** (330 mg, 1.15 mmol) was subjected to the conditions of method I. The product was purified via flash column chromatography over silica using 6% EtOAc/hexane followed by 12% EtOAc/hexane and obtained as a yellow solid in 72% yield: FTIR 2110 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.14 (d, 1H, J = 8.1 Hz), 6.95 (d, 1H, J = 2.1 Hz), 6.89 (dd, 1H, J = 2.1, 8.1 Hz), 6.81 (d, 1H, J = 8.4 Hz), 6.70 (d, 1H, J = 8.4 Hz), 3.91 (s, 3H), 3.90 (s, 3H), 3.58 (s, 3H), 2.48 (m, 2H), 1.07 (t, 3H); CIMS (methane) m/e 314 (MH^+, 35), 313 (40), 286 (100).

4′-Amino-3′-hydroxy-2,3,4-trimethoxy-1,1′-biphenyl (22a). The 4′-azidobiphenyl **19a** (125 mg, 0.439 mmol) was subjected to the conditions of method J using pivalic anhydride. The product mixture was separated by chromatography and gave **20a** in 40% yield. The compound was recrystallized from EtOAc/hexane, mp 118–119 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.27 (d, 1H, J = 8.7 Hz), 7.53 (s, broad, NH), 7.37 (dd, 1H, J = 8.7, 3 Hz), 7.27 (d, 1H, J = 3 Hz), 7.03 (d, 1H, J = 8.7 Hz), 6.72 (d, 1H, J = 8.7 Hz), 3.92 (s, 3H), 3.89 (s, 3H), 3.65 (s, 3H), 1.41 (s, 9H), 1.30 (s, 9H); CIMS (methane) m/e 444 (MH^+, 100), 443 (61), 360 (55). Anal. Calcd for $C_{25}H_{33}NO_6$: C, 67.70; H, 7.50; N, 3.16. Found: C, 67.64; H, 7.51; N, 3.13. The intermediate **20a** (114 mg, 0.257 mmol) was subjected to the conditions of method K. Pure **21a** was isolated in 95% yield as a yellow oil after concentration of the extracts: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.82 (s, broad, 1H), 7.60 (s, broad, 1H), 7.17 (d, 1H, J = 2.1 Hz), 7.06 (dd, 1H, J = 8.7, 2.1 Hz), 7.02 (d, 1H, J = 8.7 Hz), 7.01 (d, 1H, J = 8.7 Hz), 6.73 (d, 1H, J = 8.7 Hz), 3.93 (s, 3H), 3.89 (s, 3H), 3.66 (s, 3H), 1.37 (s, 9H). The intermediate **21a** (59 mg, 0.164 mmol) was subjected to the conditions of method L. Upon standing after neutralization, the product precipitated and was isolated in 64–66% yield as a tan solid (Extraction with EtOAc yielded an additional 20%.): ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.98 (d, 1H, J = 8.7 Hz), 6.96 (d, hidden, 1H), 6.92 (dd, 1H, J = 8.4, 1.8 Hz), 6.79 (d, 1H, J = 8.4 Hz), 6.70 (d, 1H, J = 8.7 Hz), 3.92 (s, 3H), 3.88 (s, 3H), 3.65 (s, 3H).

4′-Amino-5′-hydroxy-2′-methyl-2,3,4-trimethoxy-1,1′-biphenyl (22b). The 4′-azidobiphenyl **19b** (290 mg, 0.970 mmol) was subjected to the conditions of method J using acetic anhydride. The product was isolated in 42–52% yield as a mixture of isomers **20b** and **20b′**. The mixture was subjected to the conditions of method K. The regioisomers **21b** and **21b′** were separated by flash column chromatography over silica using ethyl acetate/hexane **21b**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.52 (bs, 1H), 7.46 (bs, broad, 1H), 6.88 (s, 1H), 6.84 (s, 1H), 6.81 (d, 1H, J = 8.7 Hz), 6.70 (d, 1H, J = 8.7 Hz), 3.91 (s, 3H), 3.90 (s, 3H), 3.58 (s, 3H), 2.28 (s, 3H), 2.05 (s, 3H); mp 215 °C; Calcd for $C_{18}H_{21}NO_5$: C, 65.24; H, 6.39; N, 4.23. Found: C, 65.36; H, 6.40; N, 4.25. **21b′**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.57 (bs, 1H), 7.42 (bs, 1H) 6.86 (d, 1H), 6.82 (d, 1H), 6.74 (d, 1H), 6.71 (d, 1H); mp 174 °C. Anal. Calcd for $C_{18}H_{21}NO_5$: C, 65.24; H, 6.39, N, 4.23. Found: C, 65.23; H, 6.41; N, 4.20. Intermediate **21b** (60 mg, 0.181 mmol) was subjected to the conditions of method L. The solution was extracted three times with EtOAc, and the organic layers were combined, dried over $MgSO_4$, and concentrated to give **22b** a reddish-brown solid in 95% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.04 (s, 1H), 6.92 (s, 1H), 6.81 (d, 1H, J = 8.7 Hz), 6.70 (d, 1H, J = 8.7 Hz), 3.94 (s, 3H), 3.93 (s, 3H), 3.61 (s, 3H), 2.15 (s, 3H).

4′-Amino-2′-ethyl-5′-hydroxy-2,3,4-trimethoxy-1,1′-biphenyl (22c). The 4′-azidobiphenyl **19c** (263 mg, 0.84 mmol) was subjected to the conditions of method J using pivalic anhydride. After cooling to room temperature, the remaining anhydride was removed by vacuum distillation and the residue purified via flash column chromatography over silica using 20% EtOAc/hexane. The product **20c** was isolated as a yellow oil in 23% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.20 (s, 1H), 7.51 (s, broad, NH), 6.89 (s, 1H), 6.83 (d, 1H, J = 8.4 Hz), 6.67 (d, 1H, J = 8.4 Hz), 3.91 (s, 3H), 3.89 (s, 3H), 3.58 (s, 3H), 2.49 (m, 2H), 1.38 (s, 9H), 1.31 (s, 9H), 1.08 (t, 3H); CIMS (methane) m/e 472 (MH^+, 100), 370 (29). The intermediate **20c** (37 mg, 0.079 mmol) was subjected to the conditions of method K. Concentration gave the product **21c** in 73% yield: mp 159 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 8.65 (s, broad, OH) 7.62 (s, broad, NH), 6.88 (s, 1H), 6.86 (s, 1H), 6.86 (d, 1H, J = 8.7 Hz), 6.69 (d, 1H, J = 8.7 Hz), 3.91 (s, 3H), 3.90 (s, 3H), 3.58 (s, 3H), 2.39 (m, 2H), 1.39 (s, 9H), 1.02 (t, 3H). Anal. Calcd for $C_{22}H_{29}NO_5$: C, 68.19; H, 7.54; N, 3.62. Found: C, 67.68; H, 7.56; N, 3.52. The intermediate **21c** (40 mg, 0.103 mmol) was subjected to the conditions of method L. The product was extracted with EtOAc. The organic layers were combined, washed with brine, and concentrated and the residue purified via flash column chromatography over silica with 30–50% EtOAc/hexane. The product **22c** was isolated in 50% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.27 (s, 1H, hidden), 6.80 (d, 1H, J = 9 Hz), 6.66 (d, 1H, J = 9 Hz), 6.62 (s, 1H), 3.90 (s, 3H), 3.89 (s, 3H), 3.58 (s, 3H), 2.37 (m, broad, 2H), 1.01 (t, 3H); CIMS (methane) m/e 304 (MH^+, 100), 303 (31).

6-Diazo-3-(2,3,4-trimethoxyphenyl)-2,4-cyclohexadien-1-one (2a). The aminophenol **22a** (107 mg, 0.39 mmol) was subjected to the conditions of method G. The product was purified via flash column chromatography over silica, with minimal light exposure, using 2:1 EtOAc/hexane followed by

100% EtOAc. It was isolated in 82% yield as a red solid: IR 2110, 1631 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.16 (d, 1H, J = 9 Hz), 7.02 (d, 1H, J = 8.7 Hz), 6.73 (d, 1H, J = 8.7 Hz), 6.80 (d, 1H, J = 1.2 Hz), 6.56 (dd, 1H, J = 8.7, 1.2 Hz), 3.91 (s, 3H), 3.90 (s, 3H), 3.76 (s, 3H); CIMS (methane) m/e 287 (MH^+, 74), 261 (49), 259 (27), 115 (100); HRMS found 287.1035, calcd for MH^+ $C_{16}H_{17}N_2O_4$ 287.1032.

6-Diazo-4-methyl-3-(2,3,4-trimethoxyphenyl)-2,4-cyclohexadien-1-one (2b). The aminophenol **22b** (34 mg, 0.12 mmol) was subjected to the conditions of method G. The product was purified via flash column chromatography over silica, with minimal light exposure, using 2:1 EtOAc/hexane followed by 100% EtOAc. The product was collected as an orange-red semisolid in 30% yield: FTIR 2108, 1624 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 6.96 (s, broad, 1H), 6.76 (d, 1H, J = 8.4 Hz), 6.70 (d, 1H, J = 8.4 Hz), 6.64 (s, broad, 1H), 3.90 (s, 3H), 3.89 (s, 3H), 3.70 (s, 3H), 1.94 (s, 3H); CIMS (methane) m/e 301 (MH^+, 100), 275 (41), 273 (27); HRMS found 301.1196, calcd for MH^+ $C_{16}H_{17}N_2O_4$ 301.1188.

6-Diazo-4-ethyl-3-(2,3,4-trimethoxyphenyl)-2,4-cyclohexadien-1-one (2c). The aminophenol **22c** (32 mg, 0.106 mmol) was subjected to the conditions of method G. Purification was achieved by flash column chromatography over silica, with minimal light exposure, using 30% EtOAc/hexane followed by 50% EtOAc/hexane followed by 100% EtOAc. The product was isolated as a yellow solid in 84% yield: FTIR 2205, 1679 cm^{-1}; ^1H NMR (MeOH-d_4, 300 MHz) δ 7.32 (s, 1H), 6.82 (d, 1H, J = 8.7 Hz), 6.77 (d, 1H, J = 8.7 Hz), 6.47 (s, 1H), 3.86 (s, 3H), 3.82 (s, 3H), 3.64 (s, 3H), 2.27 (m, broad, 2H), 0.95 (t, 3H); CIMS (methane) m/e 315 (MH^+, 100), 289 (95), 287 (40), 115 (45), 65 (55).

4-[(*tert*-Butyldimethylsilyl)oxy]benzaldehyde (24).[19] 4-Hydroxybenzaldehyde (15 g, 123 mmol) and imidazole (16.7 g, 25 mol) were dissolved in dry THF. TBDMS-Cl (20.4 g, 135 mmol) was added and the mixture stirred for 24 h at room temperature. The reaction mixture was diluted with hexane and washed once with water, twice with 5% NaOH, and once with brine. The organic layer was dried over $MgSO_4$, filtered, concentrated, and purified by flash column chromatography over Florisil using 100% hexane followed by 5% EtOAc/hexane. The product was isolated as a cloudy, white oil in 87% yield: ^1H NMR ($CDCl_3$, 300 MHz) δ 9.87 (s, 1H, CHO), 7.78 (d, 2H, J = 8.4 Hz), 6.94 (d, 2H, J = 8.4 Hz), 0.99 (s, 9H), 0.24 (s, 6H).

4-[(*tert*-Butyldimethylsilyl)oxy]benzyl Alcohol (25). The benzaldehyde **24** (404 mg, 1.71 mmol) was dissolved in dry THF and cooled to 0 °C and lithium aluminum hydride (65.1 mg, 1.71 mmol) was added slowly with stirring. After 2–3 h at 0 °C, the remaining lithium aluminum hydride was destroyed[38] and the grey precipitate filtered and washed with hexane. The filtrate was dried over $MgSO_4$ and concentrated to give the product as a light yellow oil in 85% yield: ^1H NMR ($CDCl_3$, 300 MHz) δ 7.23 (d, 2H, J = 8.7 Hz), 6.82 (d, 2H, J = 8.7 Hz), 4.60 (d, 2H, J = 6 Hz), 0.99 (s, 9H), 0.19 (s, 6H).

4-[(*tert*-Butyldimethylsilyl)oxy]benzyl Trifluoroacetate (26).[19] The benzyl alcohol **25** (12.9 g, 54 mmol) was dissolved in dry THF, trifluoroacetic anhydride (13.6 g, 65 mmol) was added with stirring, and the mixture was refluxed for 20–30 min. After cooling to room temperature, the mixture was diluted with ether and washed three times with saturated $NaHCO_3$. The ether layer was dried, filtered, and concentrated. The product was obtained as a clear yellow oil in 98% yield: ^1H NMR ($CDCl_3$, 300 MHz) δ 7.27 (d, 2H, J = 8.7 Hz), 6.85 (d, 2H, J = 8.7 Hz), 5.29 (s, 2H), 0.99 (s, 9H), 0.21 (s, 6H).

4-[(*tert*-Butyldimethylsilyl)oxy]benzyl Bromide (27).[19] The benzyl trifluoroacetate **26** (17.9 g, 53 mmol) was dissolved in dry THF, and dry lithium bromide (5.06 g, 58 mmol) was added with stirring. The mixture was refluxed overnight (18 h), cooled, diluted with acetonitrile, and extracted three times with hexane. The hexane layers were combined, dried, filtered, and concentrated leaving a white oil in 88% yield: ^1H NMR ($CDCl_3$, 300 MHz) δ 7.25 (d, 2H, J = 8.7 Hz), 6.79 (d, 2H, J = 8.7 Hz), 4.48 (s, 2H), 0.98 (s, 9H), 0.20 (s, 6H).

[4-[(*tert*-Butyldimethylsilyl)oxy]benzyl]triphenylphosphonium Bromide (28). The benzyl bromide **27** (17.9 g, 59 mmol) was dissolved in dry THF, triphenylphosphine (20.1 g, 77 mmol) was added with stirring, and the mixture was refluxed overnight (22 h). A white solid precipitated after cooling to room temperature and was collected by filtration and washed with hexane and ether. The product was obtained as a white powder in 81% yield: ^1H NMR (DMSO-d_6, 300 MHz) δ 7.90–7.55 (m, 15H), 6.81 (d, 2H, J = 8.7 Hz), 6.67 (d, 2H, J = 8.7 Hz), 5.01 (d, 2H, J = 15 Hz), 0.86 (s, 9H), 0.12 (s, 6H).

(*Z* and *E*)-(*tert*-Butyldimethylsilyl)-4-[2-(3,4,5-trimethoxyphenyl)ethenyl]phenol (*Z*-30, *E*-30). The phosphonium bromide **28** (10 g, 1.8 mmol) was added to dry THF, forming a slurry. After cooling to −78 °C, 1 M sodium bis(trimethylsilyl)amide in THF (1.95 mmol, 1.95 mL) was added. The solution was stirred for 2–3 h at −78 °C, after which 3,4,5-trimethoxybenzaldehyde (347 mg, 1.77 mmol) dissolved in THF was syringed into the reaction flask with stirring. After 3 h at −78 °C the reaction was quenched by addition of saturated NH_4Cl and diluted with hexane. The organic layer was washed twice with saturated NH_4Cl, once with water, dried, filtered, and concentrated to a yellow oil. Separation of the isomers was achieved by flash column chromatography over silica using 1–2% EtOAc in hexane. The product yield was 54% with a ratio of Z to E isomers of approximately 7:3. Both isomers were obtained as cloudy oils: ^1H NMR ($CDCl_3$, 300 MHz) δ *Z*-**30** 7.17 (d, 2H, J = 8.7 Hz), 6.73 (d, 2H, J = 8.7 Hz), 6.51 (s, 2H), 6.50 (ABd, 1H, J = 12 Hz), 6.41 (ABd, 1H, J = 12 Hz), 3.83 (s, 3H), 3.68 (s, 6H), 0.96 (s, 9H), 0.17 (s, 6H); *E*-**30** 7.38 (d, 2H, J = 8.7 Hz), 6.96 (ABd, 1H, J = 15.9 Hz), 6.88 (ABd, 1H, J = 15.9 Hz), 6.83 (d, 2H, J = 8.7 Hz), 6.71 (s, 2H), 3.91 (s, 6H), 3.86 (s, 3H), 0.99 (s, 9H), 0.21 (s, 6H).

(*Z*)-4-[2-(3,4,5-Trimethoxyphenyl)ethenyl]phenol (*Z*-31). Applying the conditions of Corey and Venkateswarlu,[39] the protected stilbene *Z*-**30** (573 mg, 1.43 mmol) was dissolved in dry THF. After cooling to 0 °C, tetrabutylammonium fluoride (750 mg, 2.87 mmol) was added with stirring. After 5 min of stirring at 0 °C, the solution was allowed to warm to room temperature and stirred an additional 30 min. The mixture was diluted with EtOAc and washed three times with water. The organic layer was dried over $MgSO_4$, filtered, and concentrated to a white solid in 74% yield: ^1H NMR ($CDCl_3$, 300 MHz) δ 7.18 (d, 2H, J = 8.7 Hz), 6.72 (d, 2H, J = 8.7 Hz), 6.50 (ABd, 1H, J = 11.7 Hz), 6.49 (s, 2H), 6.41 (ABd, 2H, J = 11.7 Hz), 4.76 (s, 1H, broad), 3.83 (s, 3H), 3.68 (s, 6H).

2-Amino-4-[(*Z*)-2-(3,4,5-trimethoxyphenyl)ethenyl]phenol (33). The phenol *Z*-**31** (100 mg, 0.35 mmol) was subjected to the conditions of method E using 2-methyl-4-nitroaniline. Purification of the coupling product was achieved by flash column chromatography over silica using CH_2Cl_2. The intermediate **32** was isolated as a reddish brown solid in 80% yield: ^1H NMR ($CDCl_3$, 300 MHz) δ 8.38 (d, 2H, J = 9 Hz), 7.98 (d, 2H, J = 9 Hz), 7.94 (d, 1H, J = 2.7 Hz), 7.39 (dd, 1H, J = 8.7, 2.7 Hz), 6.94 (d, 1H, J = 8.7 Hz), 6.58 (s, 2H), 6.55 (s, 2H), 3.86 (s, 3H), 3.72 (s, 6H). The azo compound **32** (122 mg, 0.28 mmol) was subjected to the conditions of method F which resulted in **33** as a light orange-brown solid in quantitative yield: ^1H NMR ($CDCl_3$, 300 MHz) δ 6.72 (s, broad, 1H), 6.62 (s, 2H), 6.54 (s, 2H), 6.44 (ABd, 1H, J = 12 Hz), 6.36 (ABd, 1H, J = 12 Hz), 4.12 (s, broad, NH_2), 3.85 (s, 3H), 3.70 (s, 6H); CIMS (methane) m/e 302 (MH^+, 100).

6-Diazo-4-[(*Z*)-2-(3,4,5-trimethoxyphenyl)ethenyl]-2,4-cyclohexadien-1-one (3). The 3-amino-4-hydroxystilbene **33** (51 mg, 0.17 mmol) was subjected to the conditions of method G. The product was purified by flash column chromatography over silica with minimal exposure to light using 2:1 EtOAc/hexane followed by 100% EtOAc. The product was collected as an orange glassy solid in 87% yield: FTIR 2099, 1730 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 7.25 (dd, hidden, 1H, J = 12.6, 1.8 Hz), 7.04 (d, 1H, J = 1.8 Hz), 6.53 (d, 1H, J = 11.4 Hz), 6.50 (s, 2H), 6.48 (d, 1H, J = 11.4 Hz), 6.28 (d, 1H, J = 12.6

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Hz), 3.86 (s, 3H), 3.76 (s, 6H); CIMS (methane) *m/e* 312 (M, 3.5), 287 (10), 263 (4), 251 (8), 223 (35), 177 (95), 129 (25), 115 (100); HRMS found 313.1202, calcd for MH^+ $C_{17}H_{17}N_2O_4$ 313.1188.

6-Diazo-4-[2-(3,4,5-trimethoxyphenyl)ethyl]-2,4-cyclohexadien-1-one (5). The azo compound **32** (36.2 mg, 0.081 mmol) was subjected to the conditions of method M. The product was purified by flash column chromatography over silica using 2:1 hexane/EtOAc followed by 1:1 hexane/EtOAc and **34** was isolated as a red solid in 45% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.65 (d, lH, J = 7.8 Hz), 6.60 (d, lH, J = 1.5 Hz), 6.49 (dd, 1H, J = 1.5, 7.8 Hz), 6.37 (s, 2H), 3.82 (s, 9H), 2.78 (s, broad, 4H).

The 3-amino-4-hydroxydihydrostilbene **34** (11.1 mg, 0.0366 mmol) was subjected to the conditions of method G. The product was purified by flash column chromatography over silica, with minimal exposure to light, using 2:1 EtOAc/hexane followed by 100% EtOAc followed by 20% MeOH/EtOAc. **5** was isolated as a dark red-brown solid in quantitative yield: FTIR 2110 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.22 (dd, 1H, J = 2.1, 9.6 Hz), 6.85 (s, 1H, broad), 6.74 (d, 1H, J = 9.6 Hz), 6.33 (s, 2H), 3.82 (s, 9H), 2.75 (m, 4H); CIMS (methane) *m/e* 287 (MH^+ − 28, 35), 286 (100), 113 (43); HRMS found 315.1336, calcd for MH^+ $C_{17}H_{19}N_2O_4$ 315.1345

3-Hydroxy-4-nitrobenzyl Alcohol (36). Using the method of Krishnamurthy,[40] 3-hydroxy-4-nitrobenzoic acid (3.0 g, 16 mmol) was dissolved in dry THF, cooled to 0 °C and 10 M borane−dimethyl sulfide complex in THF (4 mL, 0.04 mol) was added. The reaction mixture was refluxed for 3 h and cooled to 0 °C, and an equal volume of MeOH was slowly added with constant stirring. After stirring for several hours, the reaction mixture was made acidic with concd HCl and extracted with ether. The extracts were combined, washed once with brine, dried, filtered, and concentrated to give **36** as an orange solid in 87% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.65 (s, broad, 1H, PhOH), 8.09 (d, 1H, J = 8.7 Hz), 7.18 (s, broad, 1H), 6.97 (dd, 1H, J = 8.7, 1.8 Hz), 4.77 (s, 2H).

3-Hydroxy-4-nitrobenzaldehyde (37). Using the conditions of Highet and Wildman,[41] **36** (594 mg, 3.52 mmol) was dissolved in dry CH_2Cl_2, and manganese dioxide (2.45 g, 28.1 mmol) was added with stirring. The reaction mixture was stirred overnight (20 h) then filtered through Celite, washing with CH_2Cl_2 and EtOH. The filtrate was concentrated to an orange-yellow solid. Yields were typically 95−100%: ^{1}H NMR ($CDCl$ 3, 300 MHz) δ 10.58 (s, 1H, PhOH), 10.07 (s, 1H, CHO), 8.28 (d, 1H, J = 8.7 Hz), 7.66 (d, 1H, J = 1.5 Hz), 7.51 (dd, 1H, J = 8.7, 1.5 Hz).

3,4,5-Trimethoxybenzyl alcohol (39). 3,4,5-Trimethoxybenzaldehyde (1.0 g, 5 mmol) was dissolved in dry THF and cooled to 0 °C. 1 M Lithium aluminum hydride in THF (5.61 mL, 55 mmol) was added slowly with constant stirring. Stirring was continued at 0 °C for 3 h, the remaining lithium aluminum hydride neutralized, and the mixture filtered. The filtrate was dried, filtered, and concentrated to give **39** as a colorless oil. The yield was 82%: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.60 (s, 2H), 4.63 (d, 2H, J = 5.4 Hz), 3.86 (s, 6H), 3.83 (s, 3H).

3,4,5-Trimethoxybenzyl Bromide (40). Using the method of Lan and co-workers,[42] **39** (810 mg, 4.09 mmol), carbon tetrabromide (1.49 g, 4.50 mmol) and triphenylphosphine (2.15 g, 8.18 mmol) were dissolved in dry THF. The reaction mixture was stirred at room temperature for 12 h and filtered. The filtrate was concentrated and purified via flash column chromatography over silica using 5% EtOAc/hexane to give the product as a white solid in 57% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.62 (s, 2H), 4.46 (s, 2H), 3.87 (s, 6H), 3.84 (s, 3H).

(3,4,5-Trimethoxybenzyl)triphenylphosphonium Bromide (41). **40** (517 mg, 1.98 mmol) was dissolved in dry THF, and triphenylphosphine (675 mg, 2.57 mmol) was added with stirring. The reaction mixture was refluxed for 24 h and cooled to room temperature. The resulting white precipitate was collected by filtration and washed with ether and hexane. The white, powdery product was obtained in 95−100% yield: ^{1}H NMR (DMSO-d_6, 300 MHz) δ 7.73 (m, 15H), 6.18 (d, 2H, J = 2.7 Hz), 4.97 (d, 2H, J = 15 Hz), 3.57 (s, 3H), 3.37 (s, 6H).

2-Nitro-5-[(Z and E)-2-(3,4,5-trimethoxyphenyl)ethenyl]phenol (*Z*-42, *E*-42). The benzylphosphonium bromide **41** (778 mg, 1.49 mmol) was suspended in dry THF and cooled to −78 °C. 1.0 M sodium bis(trimethylsilyl)amide in THF (3.27 mL, 3.27 mmol) was added slowly with stirring. After 20 min at −78 °C, the reaction mixture was warmed to room temperature and allowed to stir for 3 h. After cooling to −78 °C, **37** (273 mg, 1.64 mmol), dissolved in a minimal amount of dry THF, was added with stirring. The reaction temperature was maintained at −78 °C for 1 h and then allowed to rise to room temperature over the next 2−2.5 h. The mixture was then poured into saturated NH_4Cl and extracted with EtOAc. The extracts were combined, washed with brine, dried, filtered, and concentrated to an orange, glassy solid. The *E* product was selectively crystallized from CH_2Cl_2/hexane and the byproduct, triphenylphosphine oxide, was removed via crystallization from EtOAc/hexane. The overall yield was 89% with a *E*:*Z* ratio of 55:45. *Z*-**42**: mp 102−104 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.60 (s, 1H, OH), 7.95 (d, 1H, J = 8.7 Hz), 7.09 (d, J = 1.5H_2, 1H), 6.89 (dd, 1H, J = 8.7, 1.5 Hz), 6.73 (ABd, 1H, J = 12 Hz), 6.47 (ABd, 1H, J = 12 Hz), 6.46 (s, 2H), 3.86 (s, 3H), 3.70 (s, 6H). *E*-**42**: mp 185−188 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 10.72 (s, 1H, OH), 8.08 (d, 1H, J = 9 Hz), 7.20 (d, 1H, J = 16.5 Hz), 7.22 (d, 1H, J = 2.7 Hz), 7.13 (dd, 1H, J = 9, 2.7 Hz), 6.95 (d, 1H, J = 16.5 Hz), 6.76 (s, 2H), 3.93 (s, 6H), 3.70 (s, 6H). Anal. Calcd for $C_{17}H_{17}NO_6$: C, 61.62; H, 5.17; N, 4.24. Found: C, 61.55; H, 5.16; N, 4.21.

6-Diazo-5-[(*Z*)-3-(3,4,5-trimethoxyphenyl)ethenyl]-2,4-cyclohexadien-1-one (4). The nitro stilbene *Z*-**42** (95.3 mg, 0.288 mmol) was subjected to the conditions of method F. Aminophenol *Z*-**43** was obtained as an orange brown solid in 97% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.72 (d, 1H, J = 8.1 Hz), 6.71 (s, broad, 1H), 6.61 (d, 1H, J = 8.1 Hz), 6.55 (s, 2H), 6.41 (ABd, 1H, J = 12.3 Hz), 6.33 (ABd, 1H, J = 12.3 Hz), 3.83 (s, 3H), 3.70 (s, 6H). The conditions of method G were used starting with Z-**43** (87 mg, 0.29 mmol). The product was purified via flash column chromatography (with minimal exposure to light) over silica using 2:1 EtOAc/hexane followed by 100% EtOAc to give the product as an orange-brown solid in 63% yield: FTIR 2110, 1620 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.97 (d, 1H, J = 9 Hz), 6.68 (d, 1H, J = 12 Hz), 6.65 (d, 1H, J = 2.7 Hz), 6.39 (d, 1H, J = 12 Hz), 6.48 (s, 2H), 6.13 (dd, 1H, J = 9, 1.5 Hz), 3.85 (s, 3H), 3.74 (s, 6H); CIMS (methane) *m/e* 313 (MH^+, 80), 287 (92), 285 (76).[43]

6-Diazo-3-[2-(3,4,5-trimethoxyphenyl)ethyl]-2,4-cyclohexadien-1-one (6). A mixture of the 3-hydroxy-4-nitrostilbenes *E*-**42** and *Z*-**42** (50 mg, 0.15 mmol) was subjected to the conditions of method M. A light orange solid was obtained: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.64 (m, broad, 3H), 6.35 (s, broad, 2H), 3.83 (s, 3H), 3.81 (s, 6H), 2.75 (s, broad, 4H); CIMS (methane) *m/e* 304 (MH^+, 100), 288 (12), 123 (64). The dihydrostilbene **44** (45 mg, 0.15 mmol) was subjected to the conditions of method G. The product was purified by flash column chromatography over silica, with minimal exposure to light, using 2:1 EtOAc/hexane followed by 100% EtOAc followed by 20% MeOH/EtOAc. The product was isolated as a dark red-brown solid in quantitative yield: FTIR 2103, 1690 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.08 (d, 1H, J = 9 Hz), 6.53 (s, broad, 1H), 6.37 (s, 2H), 6.12 (d, broad, 1H, J = 9 Hz), 3.83 (s, 6H), 3.82 (s, 3H), 2.79 (m, 4H); CIMS (methane) *m/e* 315 (MH^+, 5.4), 304 (1.5), 289 (10), 287 (3), 181 (84).[43]

***N*-Acetylcolchinol (45).** *N*-acetylcolchinol was prepared in accordance with the procedure of Cech and Santavy[20b] from colchicine (1.0 g, 2.6 mmol) with minor modifications.[20c] Upon cooling, a precipitate containing a mixture of product and starting material was collected via vacuum filtration. The filtrate was extracted with CH_2Cl_2 and the organic layers were

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combined, washed once with brine, dried, and concentrated. The material recovered by extraction and the collected precipitates were combined and purified by flash column chromatography over silica using 7:3 EtOAc/EtOH. The product was isolated in 29% yield: ^{1}H NMR ($CDCl_3$, 300 MHz) [3:2 ratio of amide rotamers] δ 7.35 (d, 1H, J = 8.4 Hz), 6.79 (s, 1H), 6.78 (d, 1H, J = 8.4 Hz), 6.56 (s, 1H), 5.81 (d, broad, NH), 4.77 (m, broad, 1H), 3.92 (s, 3H), 3.89 (s, 3H), 3.49 (s, 3H), 2.60–2.30 (m, 4H), 2.05 (s, 3H).

***N*-Acetyl-10-[(2-methyl-4-nitrophenyl)azo]colchinol (46) and *N*-Acetyl-8-[(2-methyl-4-nitrophenyl)azo]colchinol (48).** *N*-Acetylcolchinol (**45**) (267 mg, 0.75 mmol) was subjected to the conditions of method E. The resulting isomers were separated by flash column chromatography using 3:1 EtOAc/hexane over silica. The combined yield of both isomers was 48%, both as dark red solids. The 10-isomer **46** predominated by approximately a 1.3:1 ratio. **46**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 13.01 (s, OH), 8.24 (d, 1H, J = 2.1 Hz), 8.16 (dd, 1H, J = 2.1, 9 Hz), 8.15 (s, 1H), 7.93 (d, 1H, J = 9 Hz), 6.99 (s, 1H), 6.60 (s, 1H), 5.79 (d, broad, NH), 4.85 (m, broad, 1H), 3.96 (s, 3H), 3.91 (s, 3H), 3.62 (s, 3H), 2.74 (s, 3H), 2.6–2.4 (m, 4H), 2.07 (s, 3H). **48**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 13.86 (s, OH), 8.23 (s, broad, 1H), 8.19 (dd, 1H, J = 2.1, 8.7 Hz), 8.07 (d, 1H, J = 8.7 Hz), 7.62 (d, 1H, J = 8.7 Hz), 7.06 (d, 1H, J = 8.7 Hz), 6.87 (m, broad, 1H), 6.71 (s, 1H), 5.41 (d, broad, NH), 3.95 (s, 3H), 3.94 (s, 3H), 3.64 (s, 3H), 2.73 (s, 3H), 2.70–2.20 (m, 2H), 1.62 (s, 3H).

***N*-Acetyl-10-aminocolchinol (47).** The azo colchinol **46** (105 mg, 0.20 mmol) was subjected to the conditions of method F. The yield of **47** was quantitative: ^{1}H NMR ($CDCl_3$, 300 MHz) (mixture of rotamers, peaks for major rotamer) δ 6.83 (s, 1H), 6.64 (s, 1H), 6.53 (s, 1H), 6.24 (d, broad) 5.43 (s, broad, NH), 4.64 (m, 1H), 3.89 (s, 3H), 3.87 (s, 3H), 3.50 (s, 3H), 2.60–2.10 (m, 4H), 2.04 (s, 3H).

***N*-Acetyl-8-aminocolchinol (49).** The azo colchinol **48** (81 mg, 0.16 mmol) was subjected to the conditions of method F. The yield of **49** was quantitative: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.78 (ABd, 1H, J = 8.1 Hz), 6.72 (ABd, 1H, J = 8.1 Hz), 6.62 (s, 1H), 5.44 (m, broad, 1H), 5.32 (d, broad, NH), 3.91 (s, 3H), 3.89 (s, 3H), 3.47 (s, 3H), 2.65–2.25 (m, 4H), 2.04 (s, 3H).

***N*-Acetyl-10-diazocolchin-9-one (7) and *N*-Acetyl-8-diazocolchin-9-one (8).** The diazo compounds were prepared from the aminophenols **47** (21 mg, 0.055 mmol) and **49** (40 mg, 0.11 mmol) respectively via method G. Purification was achieved via flash column chromatography over silica using 100% EtOAc followed by 30% EtOH/EtOAc. The 10-substituted product (**7**) was isolated as a red solid in 40% yield. The 8-substituted product **8** was isolated as a red solid in 89% yield. **7**: FTIR 2116, 1624 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.34 (s, 1H), 6.95 (s, 1H), 6.59 (d, broad, NH), 6.54 (s, 1H), 4.68 (m, 1H), 3.91 (s, 1H), 3.89 (s, 3H), 3.64 (s, 3H), 2.60–2.25 (m, 4H), 2.02 (s, 3H); CIMS (methane) m/e 383 (MH^+ − 1, 9), 358 (MH^+ − 26, 25), 329 (30), 119 (52), 115 (52), 60 (100).[43] **8**: FTIR 2110, 1674 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz) δ [3:2 ratio of amide rotamers] 7.67 (d, 1H, J = 9 Hz), 7.12 (d, 1H, J = 9 Hz), 6.88 (d, broad, NH), 6.60 (s, 1H), 5.04–4.88 (m, 1H), 3.93 (s, 3H), 3.90 (s, 3H), 3.64 (s, 3H), 2.76–2.10 (m, 4H), 2.04 (s, 3H); CIMS (methane) m/e 356 (MH^+ − 28, 100), 115 (30), 100 (62).[43]

Tubulin Purification. Bovine brain tubulin was purified via the method developed by Shelanski et al. and Weingarten et al. as modified by Williams and Lee.[44] Briefly, tubulin was subjected to three cycles of polymerization and depolymerization followed by chromatography over phosphocellulose. The purified protein was stored in liquid nitrogen. Purity was assessed by SDS–PAGE on 7.5% gels.[45] Concentration was determined by measuring protein absorbance at 275 nm in 6 molar guanidine hydrochloride and using an extinction coefficient of 1.03.[46]

Polymerization Assays. Stock solutions (10 mM in DMSO) were prepared for all potential photolabels and colchicine. The 10 mM solution was diluted with MeOH (33 μL to 10 mL) in order to obtain a UV–vis spectrum. Dilutions of the 10 mM stock were made such that 4 μL of each dilution could be added to samples to allow different final label concentrations to be tested while maintaining a constant DMSO concentration of 1%. The polymerization buffer was PMED (100 mM PIPES, 2 mM EGTA, 1 mM $MgSO_4$, 2 mM DTT; 0.1 mM GTP is added for PMEDG buffer). High concentration tubulin was used after centrifuging for 2 min to remove aggregates, diluting with fresh PMED buffer to obtain a maximum absorbance of approximately 0.3–0.5 au during a polymerization run (tubulin concentration in each sample was approximately 3–3.5 mg/mL). The total volume for each sample was 400 μL. Each sample containing tubulin, buffer, and inhibitor was incubated at room temperature for 20 min at room temperature. 50 mM GTP was then added to a final concentration of 1 mM, and each sample incubated at 37 °C for 20 min while absorbance was monitored at 351 nm. Four samples were run simultaneously; a control and three different concentrations of inhibitor. A total of six different concentrations were tested and each compound was assayed twice. IC_{50} values (concentration at which polymerization was inhibited by 50%) were determined by interpolation of graphs created by Sigma Plot 4.0 which plotted the third order regression of % polymerization versus log concentration.

Competitive Binding Assays. Stock solutions (in DMSO) were prepared for all compounds, including colchicine and ^{3}H-colchicine, of identical concentration. An amount of 50 μg of tubulin were used for each sample and DMSO concentration was kept at or below 2% by volume. High concentration tubulin was thawed and exchanged into fresh PMEG buffer via a Penefsky column using Sephadex G-50 medium preswollen in buffer. Protein concentration was determined via the guanidine-HCl method.[46] Samples were made in 1.5 mL microcentrifuge tubes using the following order of addition: (1) inhibitor, (2) ^{3}H-colchicine, (3) PMEG buffer and then the samples were mixed for a few seconds, (4) protein followed by mixing. Total volume for each sample was 500 μL. Each sample was incubated for 2 h at 37 °C, and then filtered through Whatman glass microfibre filters (GF/C) after diluting with an equal volume of water, washing with 10–15 mL of water. Each filter disc was then counted for tritium.

Molecular Modeling. Colchicine was input from the X-ray structure (independent molecule b) as a guide and the conformation minimized using the augmented MM2 force field of the CAChe system. The ring A–ring C torsion angle changed little during minimization and the final value was 55.3° as compared to 53.2° in the X-ray structure. Structures **1c** and **2c** were minimized using the block diagonal Newton–Raphson technique and the augmented MM2 force field of the CAChe system. The ring A/C dihedral angle was then adjusted to 55° and the fit feature used to generate Figure 2. Structures **7** and **8** were generated from the colchicine structure and minimized using the procedure described above. The ring A/C dihedral angles in the minimized structures was 58.7° for **7** and 54.4° for **8**.

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Supplementary Material Available: Copies of ^{1}H NMR spectra of **12a**, **12b**, **13a,b**, **15a–c**, and **19a–c** (11 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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