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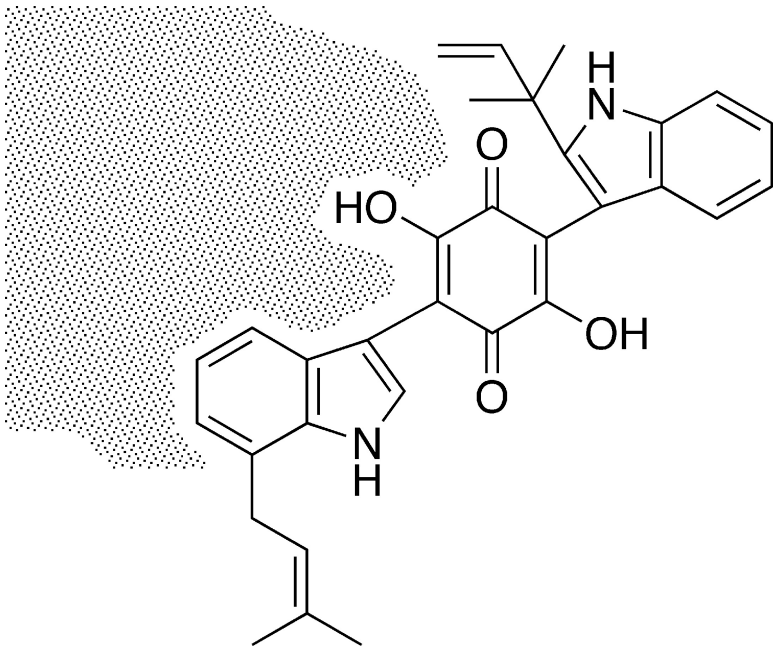

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Methyl Scanning: Total Synthesis of Demethylasterriquinone B1 and Derivatives for Identification of Sites of Interaction with and Isolation of Its Receptor(s)

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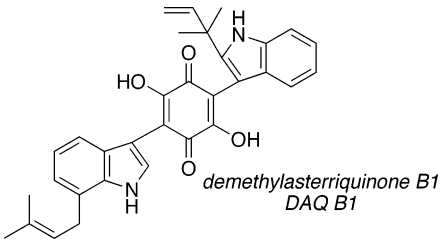
Abstract: The principle of methyl scanning is proposed for determination of the sites of interaction between biologically active small molecules and their macromolecular target(s). It involves the systematic preparation of a family of methylated derivatives of a compound and their biological testing. As a functional assay, the method can identify the regions of a molecule that are important (and unimportant) for biological activity against even unknown targets, and thus provides an excellent complement to structural biology. Methyl scanning was applied to demethylasterriquinone B1, a small-molecule mimetic of insulin. A new, optimal total synthesis of this natural product was developed that enables the family of methyl scan derivatives to be concisely prepared for evaluation in a cellular assay. The results of this experiment were used to design a biotin−demethylasterriquinone conjugate for use as an affinity reagent. This compound was prepared in tens of milligram quantities in a four-step synthesis.

Introduction

Natural products have consistently provided novel, interesting chemical structures with provocative biological activities. Because it is practical to culture bacterial and tumor cells and examine crude natural product extracts for activity in inhibiting their growth, this paradigm for biologically active natural product discovery applies particularly well to anti-bacterial and anti-cancer agents. With greater effort, it can be applied to the discovery of other types of desired biological activities. Such was the case with the remarkable discovery of an orally active, small-molecule insulin mimic, demethylasterriquinone B1 (DAQ B1).[1] The biological screen was based on Chinese hamster ovary



demethylasterriquinone B1
DAQ B1

cells expressing human insulin receptor (hIR), which has three domains: extracellular insulin binding, transmembrane, and tyrosine kinase domains. Upon insulin binding, the receptor is autophosphorylated on tyrosine residues, activating it for binding of phosphotyrosine-binding proteins and for tyrosine phosphorylation of substrate proteins. Natural product extracts were examined for insulin receptor activation in cells by immunoprecipitation of the receptor followed by measurement of its kinase activity. In a heroic effort, over 50 000 extracts were tested in this screen to discover DAQ B1. Three experiments implied that DAQ B1 activates the hIR by binding directly to its cytoplasmic tyrosine kinase domain: (1) DAQ B1 does not compete with insulin for receptor binding; (2) DAQ B1 alters the pattern of proteolysis at K1030 near the ATP binding site in the tyrosine kinase domain; and (3) DAQ B1 can activate a chimeric receptor consisting of the hIR tyrosine kinase domain fused to the transmembrane and ligand-binding domains of another receptor. It should be emphasized, however, that these experiments do not establish that the hIR is the exclusive or even most important target of action of DAQ B1. The wide variety of biological activities[2] that earlier have been ascribed to members of the asterriquinone family of natural products

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(1) Zhang, B.; Salituro, G.; Szalkowski, D.; Li, Z.; Zhang, Y.; Royo, I.; Vilella, D.; Diez, M. T.; Pelaez, F.; Ruby, C.; Kendall, R. L.; Mao, X.; Griffin, P.; Calaycay, J.; Zierath, J. R.; Heck, J. V.; Smith, R. G.; Moller, D. E. *Science* **1999**, *284*, 974−977.
(2) Shimizu, S.; Yamamoto, Y.; Koshimura, S. *Chem. Pharm. Bull*. **1982**, *30*, 1896−1899. Fredenhagen, A.; Petersen, F.; Tintelnot-Blomley, M.; Rosel, J.; Mett, H.; Hug, P. *J. Antibiot.* **1997**, *50*, 395−401. Kaji, A.; Saito, R.; Nomura, M.; Miyamoto, K.-i.; Kiriyama, N. *Biol. Pharm. Bull*. **1998**, *21*, 945−949. Alvi, K. A.; Pu, H.; Luche, M.; Rice, A.; App, H.; McMahon, G.; Dare, H.; Margolis, B. *J. Antibiot.* **1999**, *52*, 215−223. Wilkie, N.; Wingrove, P. B.; Bilsland, J. G.; Young, L.; Harper, S. J.; Hefti, F.; Ellis, S.; Pollack, S. J. *J. Neurochem.* **2001**, *78*, 1135−1145.

suggest that many intracellular targets are possible. We ourselves examined[3] a hypothesis that a primary mode of action of DAQ B1 was as an inhibitor of PTP1B, the phosphatase responsible for deactivation of the hIR,[4] but were unable to gain evidence in its favor. It is still quite reasonable that DAQ B1 acts on other cellular proteins not yet identified.

To further explore and develop the biological properties of DAQ B1, it is important to determine all of the cellular proteins against which it may act. Affinity chromatography and photoaffinity labeling are traditional methods for identifying targets of drug action. One novel method is phage display cloning, which notably has been useful in identifying heretofore undiscovered binding partners for even well-known drugs (with thought-to-be well-known modes of action, such as doxorubicin).[5] All of these methods require a chemical link between the drug and another molecule or a surface. It is crucial that this link not interfere with the binding of the drug to *any* of its targets. This principle is well known for the development of affinity ligands for single proteins, and structure-based ligand design can be applied to develop such ligands.[6] However, structure-based design of affinity ligand attachment is useful only if the target of a drug is known, there is only a single target, and the structure of the drug−target complex has been determined. [No structural studies or attempts to crystallize DAQ B1 with the hIR tyrosine kinase domain have been reported.] Structure-based design is useless if affinity techniques are to be used for the *identification* of the targets of a drug. This situation creates a paradox in that knowledge of target−ligand interactions is necessary to design a ligand that can be used to identify the targets. This paradox can be resolved only by identifying sites on the molecule through which affinity ligands can be attached without interfering with the overall actions of the drug, that is to say, against all possible targets. The actions of affinity ligand-modified drugs must be investigated in the milieu in which the drug acts so that the modified drugs have an opportunity to interact with all targets in the cell. Thus, in vitro assays against single targets cannot provide the information sought. Assessment of ligand-modified drug action must be based on function in a cell-based readout. For example, in the case of DAQ B1 the desired function is activation of the insulin receptor. This process might occur through direct action of the compound on the receptor or by inhibition of the phosphatase that deactivates the receptor (vide supra). Examination of candidate affinity ligands for interactions with the proteins themselves might be misleading.

These issues have certainly been faced by earlier workers investigating targets of drug action.[7] Generally, their solutions have involved a serial approach based upon preparation of a limited number of ligand-modified drug derivatives. These compounds were examined for cell-based activity until a derivative was found with activity comparable to that of the parent drug. This strategy is valid and has been effective. However, we considered that a more systematic approach to identify "null" sites on a molecule, where chemical modification does not significantly affect activity, would offer several attractions. This strategy draws directly on earlier concepts for the systematic investigation of protein ligand−receptor interactions, and specifically to the identification of "hot spots" for the interaction of human growth hormone with its receptor.[8] Alanine-scanning mutagenesis uses genetic engineering to replace each amino acid residue, in turn, by alanine. Those positions at which the change to alanine has no effect on activity must be unrelated to the action of the ligand. Those positions at which the change to alanine affects activity are the hot spots. Subsequent structural studies have supported the idea that hot spots are involved in ligand−receptor contacts, but "null spots" are not.[9] This method has been applied to mapping the interaction of insulin with insulin receptor.[10]

For analysis of small molecule/natural product−protein interactions, such as those of DAQ B1 with its target(s), the alanine-scanning mutagenesis method can be translated into a "methyl scanning" method. That is, methyl scanning replaces each hydrogen in a molecule in turn with a methyl group, based upon the thinking that if there is a close contact between target protein(s) and a particular site, a methyl group at that site will sterically exclude binding. Sites at which addition of a methyl group decreases activity are the hot spots. Methyl substitution may be subtle enough that drastically different physicochemical properties or toxicity of analogues, which could certainly affect cell-based activity, is unlikely. Kazmaier et al. reported studies on derivatives of the anti-bacterial agent furanomycin that are similar to methyl scanning, though they did not use that term.[11] *N*-Methyl scanning is a common methodology for determination of structure−activity relationships in synthetic bioactive peptides.[12] The term has also been used to describe incorporation of a methyl group at each position in a linker between two peptide domains of a thrombin inhibitor.[13] A related concept of fluorine scanning has been used to systematically modify the pK_a of the amidinium group in thrombin inhibitors and investigate "fluorophilicity" in the thrombin active site.[14]

Whether substitution of a hydrogen with a methyl group is sufficient to disrupt small molecule−macromolecule interactions

(3) Rudolph, J.; Pirrung, M. C., unpublished.
(4) Tonks, N. K. *FEBS Lett.* **2003**, *546*, 140−148. Espanel, X.; Walchli, S.; Gobert, R. P.; El Alama, M.; Curchod, M. L.; Gullu-Isler, N.; van Huijsduijnen, R. H. *Endocrine* **2001**, *15*, 19−28. Liu, G.; Trevillyan, J. M. *Curr. Opin. Investig. Drugs*. **2002**, *3*, 1608−1616. Ramachandran, C.; Kennedy, B. P. *Curr. Top. Med. Chem.* **2003**, *3*, 749−757.
(5) Sche, P. P.; McKenzie, K. M.; White, J. D.; Austin, D. J. *Chem. Biol.* **1999**, *6*, 707−716. Savinov, S. N.; Austin, D. J. *Comb. Chem. High Throughput Screen.* **2001**, *4*, 593−597. Tanaka, H.; Ohshima, N.; Hidaka, H. *Mol. Pharmacol.* **1999**, *55*, 356−363. Jin, Y.; Yu, J.; Yu, Y. G. *Chem. Biol.* **2002**, *9*, 157−162. McKenzie, K. M.; Videlock, E. J.; Splittgerber, U.; Austin, D. J. *Angew. Chem., Int. Ed.* **2004**, *43*, 4052−4055.
(6) Baumann, H.; Ohrman, S.; Shinohara, Y.; Ersoy, O.; Choudhury, D.; Axen, A.; Tedebark, U.; Carredano, E. *Protein Sci.* **2003**, *12*, 784−793.
(7) Sin, N.; Meng, L.; Auth, H.; Crews, C. M. *Bioorg. Med. Chem.* **1998**, *6*, 1209−1217. Radeke, H. S.; Snapper, M. L. *Bioorg. Med. Chem.* **1998**, *6*, 1227−1232.
(8) Bass, S. H.; Mulkerrin, M. G.; Wells, J. A. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 4498−4502. Cunningham, B. C.; Wells, J. A. *Science* **1989**, *244*, 1081−1085.
(9) Clackson, T.; Ultsch, M. H.; Wells, J. A.; de Vos, A. M. *J. Mol. Biol.* **1998**, *277*, 1111−1128.
(10) Pillutla, R. C.; Hsiao, K. C.; Beasley, J. R.; Brandt, J.; Ostergaard, S.; Hansen, P. H.; Spetzler, J. C.; Danielsen, G. M.; Andersen, A. S.; Brissette, R. E.; Lennick, M.; Fletcher, P. W.; Blume, A. J.; Schaffer, L.; Goldstein, N. I. *J. Biol. Chem.* **2002**, *277*, 22590−22594.
(11) Kazmaier, U.; Pahler, S.; Endermann, R.; Habich, D.; Kroll, H. P.; Riedl, B. *Bioorg. Med. Chem.* **2002**, *10*, 3905−3913.
(12) Rajeswaran, W. G.; Hocart, S. J.; Murphy, W. A.; Taylor, J. E.; Coy, D. H. *J. Med. Chem.* **2001**, *44*, 1305−1311. Miller, S. C.; Scanlan, T. S. *J. Am. Chem. Soc.* **1997**, *119*, 2301−2302. Cerusoa, M. A.; McComseya, D. F.; Leoa, G. C.; Andrade-Gordona, P.; Addoa, M. F.; Scarborough, R. M.; Oksenberg, D.; Maryanoff, B. E. *Bioorg. Med. Chem.* **1999**, *7*, 2353−2371.
(13) Slon-Usakiewicz, J. J.; Purisima, E.; Tsuda, Y.; Sulea, T.; Pedyczak, A.; Fethiere, J.; Cygler, M.; Konishi, Y. *Biochemistry* **1997**, *36*, 13494−13502.
(14) Olsen, J. A.; Banner, D. W.; Seiler, P.; Obst-Sander, U.; D'Arcy, A.; Stihle, M.; Muller, K.; Diederich, F. *Angew. Chem., Int. Ed.* **2003**, *42*, 2507−2511. Olsen, J.; Seiler, P.; Wagner, B.; Fischer, H.; Tschopp, T.; Obst-Sander, U.; Banner, D. W.; Kansy, M.; Muller, K.; Diederich F. *Org. Biomol. Chem.* **2004**, *2*, 1339−1352.

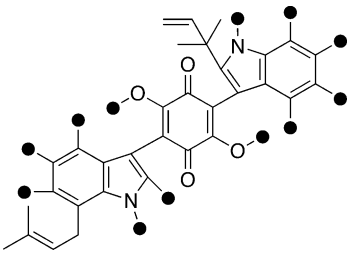


Figure 1. Positions of substitution on demethylasterriquinone B1 for methyl scanning.

can certainly be questioned. Related concepts can be applied to larger substituents ("propyl scan") if desired. Likewise, knowledge that a methyl group can be tolerated at a particular site in the molecule may not necessarily mean that larger alkyl groups, such as linkers to affinity reagents, can also be tolerated. Methyl scanning has intellectual progenitors in the "bump-and-hole" method of engineering specific protein−ligand interactions.[15] That technique works best when structural information is available for the protein−ligand interaction, though homology modeling can also serve well.[16] Structurally modified compounds that are inactive in a methyl scanning experiment could also be useful in the design or selection of proteins with compensatory mutations. Identification of a hot spot also does not prove that that site is in direct contact with target protein(s). It is possible that substitution at a site remote from small molecule−macromolecule contacts might change the molecular conformation to a less active form.

To apply the methyl scanning concept to a small natural product molecule, it should be accessible by an efficient synthesis, enabling the production of methylated variants from methylated synthetic precursors that should be readily available. Ideally, the synthesis would be modular so that the preparation of a methylated variant would merely involve substitution of a methylated precursor into a synthetic sequence that is otherwise identical to that used to prepare the parent compound. Repeated execution of linear synthetic sequences to obtain the methyl scan targets would be tedious. In the case of DAQ B1, the design of the methyl scan study involved 12 derivatives substituted at all aromatic carbons as well as the nitrogens and oxygens (Figure 1). Several syntheses of DAQ B1 have been developed,[17] including two in our own laboratory.[18] Some of these syntheses are modular, but none were really adequate for the intended methyl scanning study. Our first synthesis is brief and modular but regiochemically uncontrolled. Our second synthesis requires one of the indole building blocks to be converted to an organotin reagent in a multistep process. Tatsuta's synthesis is brief, modular, and regiochemically controlled, but does not permit *N*-substitution. Thus, a new total synthesis was sought that would add two different indole building blocks (without prior derivatization) to a quinone core.

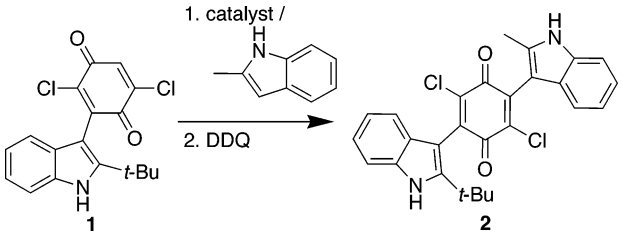
(15) Clemons, P. A.; Gladstone, B. G.; Seth, A.; Chao, E. D.; Foley, M. A.; Schreiber, S. L. *Chem. Biol*. **2002**, *9*, 49−61. Bishop, A.; Buzko, O.; Heyeck-Dumas, S.; Jung, I.; Kraybill, B.; Liu, Y.; Shah, K.; Ulrich, S.; Witocki, L.; Yang, F.; Zhang, C.; Shokat, K. M. *Annu. Rev. Biophys. Biomol. Struct.* **2000**, *29*, 577−606.

(16) Bishop, A. C.; Ubersax, J. A.; Petsch, D. T.; Matheos, D. P.; Gray, N. S.; Blethrow, J.; Shimizu, E.; Tsien, J. Z.; Schultz, P. G.; Rose, M. D.; Wood, J. L.; Morgan, D. O.; Shokat, K. M. *Nature* **2000**, *407*, 395−401.

(17) Liu, K.; Wood, H. B.; Jones, A. B. *Tetrahedron Lett*. **1999**, *40*, 5119−5122. Tatsuta, K.; Mukai, H.; Mitsumoto, K. *J. Antibiot*. **2001**, *54*, 105−108.

(18) Pirrung, M. C.; Park, K.; Zhao, Y.; Webster, N. J. G. *ChemBioChem* **2002**, *3*, 379−385. Pirrung, M. C.; Li, Z.; Park, K.; Zhu, J. *J. Org. Chem*. **2002**, *67*, 7919−7926.

Table 1. Acid-Promoted Addition of 2-Methylindole to **1**



acid	1 equiv	2 equiv	4 equiv
$HClO_4$	complex	complex	complex
HBF_4	complex	complex	complex
H_2SO_4	90% yield of **2**	90% yield of **2**	90% yield of **2**
$C_6H_5SO_3H$	90% yield of **2**	90% yield of **2**	90% yield of **2**
CF_3COOH	slow	slow	slow
HCOOH	slow	slow	slow
CH_3COOH	slow	43% yield of **2**	73% yield of **2**

Results

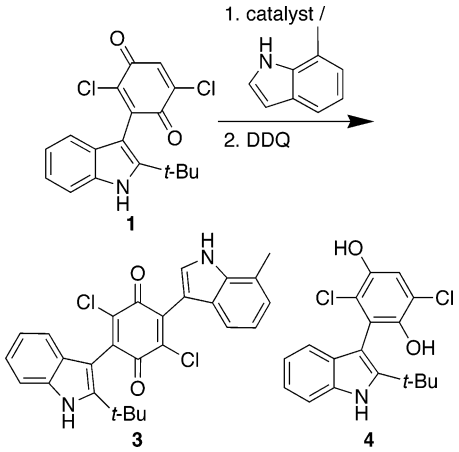
Our earlier work has described efficient methods to unite diverse indoles with our core quinone synthon, 2,5-dichlorobenzoquinone, through protic acid catalysis.[19] We required only a method to add a second indole to the resulting monoindolylquinone to complete a concise DAQ B1 synthesis (after hydrolysis of the bisindolyldichlorobenzoquinone). Strategically, it was desirable to add the 2-isoprenylindole first, since our earlier work showed that the monoindolylquinone derived from 7-prenylindole is unstable in concentrated form.[20] Thus, the second indole would be added to a monoindolylquinone bearing a bulky 2-substitutent. Such molecules are expected for steric reasons to prefer a conformation in which the indole and quinone rings are perpendicular, a conformation that should minimize the conjugation between the two rings. A readily accessible model compound **1** was selected for use in reaction surveys for introduction of a second indole. On the basis of its good reactivity in our earlier work with protic acid catalysts, 2-methylindole was selected as a model second indole. As summarized in Table 1, a combinatorial grid of acids with pK_a's ranging from −10 to 4.7 as one dimension and 1, 2, and 4 equiv of acids as the other dimension was used for pilot studies with initial thin-layer chromatography (TLC) monitoring. The oxidant in these reactions was dichlorodicyanoquinone, and the solvent was tetrahydrofuran (THF). With this asterriquinone family of compounds, TLC is quite useful because of the prominent colors of reactants (yellow) and products (blue-green to purple), and the hydroquinone byproduct (reddish; vide infra). For reactions that gave promising TLCs, the product **2** was isolated and purified.

On the basis of the results in Table 1, the reaction promoted by sulfuric acid (1 equiv) in THF was examined with several other indoles. With 5-chloro-, 5-methyl-, and 5-methoxy-2-methylindole, the isolated yields were 95%, 78%, and 78%, respectively, but with indole and 7-methylindole, very low conversion was observed. These results are somewhat surprising, as no such dependence on indole structure was seen in our earlier work on indole−quinone conjugate additions.[18] It was expected that the reactivity of **1** should be quite similar to that of

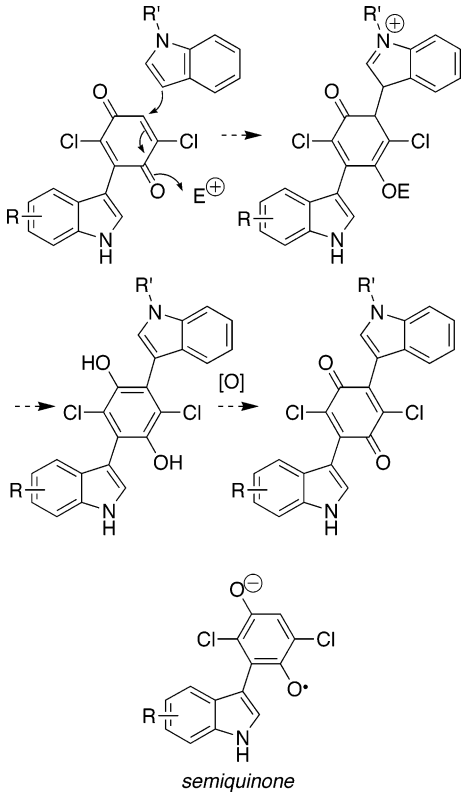
(19) Pirrung, M. C.; Deng, L.; Li, Z.; Park, K. *J. Org. Chem*. **2002**, *67*, 8374−8388.

(20) Pirrung, M. C.; Park, K.; Li, Z. *Org. Lett*. **2001**, *3*, 365−367.

Scheme 1



Scheme 2



dichlorobenzoquinone, since the π-system of the indole is orthogonal to the π-system of the quinone and would not be expected to exert much electronic influence on its reactivity. Nevertheless, it was apparent that a model reactant closer to the intended 7-substituted indole should be used, so 7-methylindole was used in promoter screening (Scheme 1). Interestingly, many of these pilot reactions (with $HClO_4$, H_2SO_4, and $C_6H_5SO_3H$) gave the reduction product **4**. We have earlier discussed an alternative to the polar mechanism (Scheme 2) for the conjugate addition of an indole to a quinone, electron transfer to produce a radical ion pair. If a second electron is transferred to the semiquinone in this alternative mechanism, presumably from another molecule of indole, **4** would be produced after protonation. Such reduction products are occasionally noted as minor components in other of our indole−quinone conjugate addition reactions. Among all of the protic acid promoters examined for this reaction, only formic and acetic acids seemed promising, but preparative experiments with these promoters provided **3** in only low yield.

Table 2. Lewis Acid-Promoted Addition of 7-Methylindole to **1**[a]

acid	reaction conditions	yield (%)
$Dy(OTf)_3$ (1 equiv)	CH_3OH, N_2, reflux, 5h	28
$BF_3{\cdot}OEt_2$ (1 equiv)	THF, N_2, rt, 17 h	0 (**4** produced)
$Cu(OTf)_2$ (1 equiv)	CH_3CN, N_2, rt, 1 h	0 (**4** produced)
$Sc(OTf)_3$ (1 equiv)	CH_3CN, N_2, rt, 1 h	0 (**4** produced)
$Zn(OTf)_2$ (1 equiv)	CH_3CN, N_2, reflux, 9 h	44
$Zn(OTf)_2$ (1 equiv)	THF, N_2, reflux, 26 h	80
$Zn(OTf)_2$ (1 equiv)	nitromethane, N_2, 70 °C, 3 h	10
$Zn(OTf)_2$ (1 equiv)	dioxane, N_2, reflux, 48 h	40
$Zn(OTf)_2$ (1 equiv)	diglyme, N_2, 100 °C, 27 h	43
$Zn(OTf)_2$ (1 equiv)	1,2-dichloroethane, N_2, reflux, 45 h	slow
$Zn(OTf)_2{\cdot}H_2O$ (1 equiv)	THF, N_2, reflux, 24 h	64
$Zn(OTf)_2{\cdot}H_2O$ (2 equiv)	THF, N_2, reflux, 12 h	82

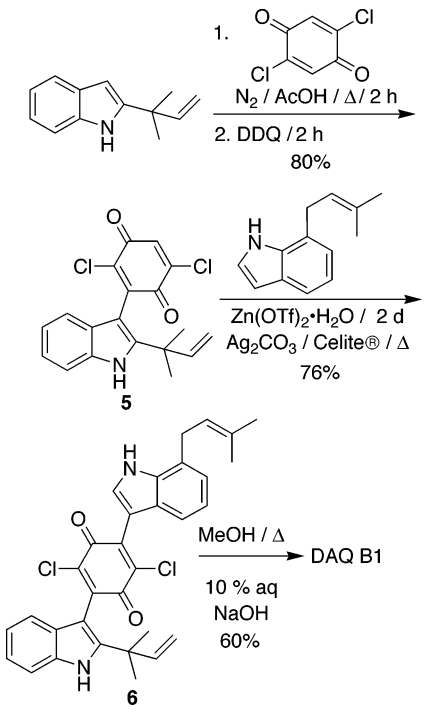
[a] On a 0.20 mmol scale in 1 mL of solvent.

We then examined Lewis acid promoters for the reaction of **1** with 7-methylindole; a selection is shown in Table 2. Reaction mixtures were first stirred at room temperature and monitored by TLC. If a reaction was slow, it was carried out at higher temperature. For promising reactions, the product was isolated and the yield was determined. Initial results revealed zinc triflate as a promising catalyst, and further optimization showed THF was the superior solvent. Surprisingly, zinc triflate monohydrate, which is much more convenient to use than the highly hygroscopic anhydrous zinc triflate, was found to be an even better promoter. In all of these reactions, DDQ was added following the consumption of the starting material to oxidize the conjugate addition product to the quinone. If any **4** was present in the reaction mixture, it was converted back to **1**, which required chromatography to be removed. In our earlier work on the conjugate addition of indoles to dichlorobenzoquinone,[18,19] it was important that oxidant be excluded during the initial addition step, since maintaining the product as the hydroquinone form prevents conjugate addition of a second molecule of indole (in fact, the process we are attempting to achieve here). For this reaction, a second addition is not possible, so it is not necessary to exclude oxidant during the addition step. In fact, the presence of oxidant during the addition step has a benefit: if reduction of **1** to **4** by the indole occurs, **4** can be oxidized back to **1**. This helps to ensure minimal recovery of **1** after the reaction. Silver carbonate on Celite[21] (Fetizon's reagent) was found to be a convenient oxidant in our preparation of monoindolylquinones,[18] as it can be removed by simple filtration, and it worked similarly well here.

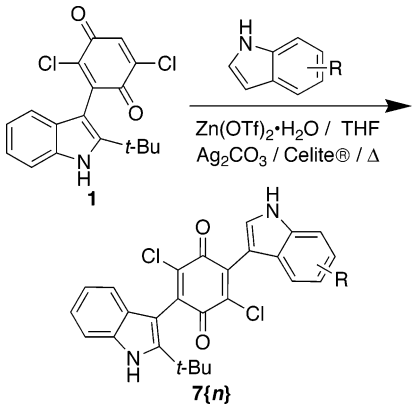
With this method in hand, the fully regiocontrolled total synthesis of DAQ B1 could be completed in only three steps from the indoles in the overall yield of 36% (Scheme 3). This synthesis was applied to the preparation of 10 of the 12 methyl scan targets (vide infra). It was also apparent that this synthesis would be amenable to solution-phase, parallel synthesis of DAQ B1 combinatorial libraries. Toward this end, 20 diverse indoles were condensed with **1** under the zinc triflate/Fetizon's reagent conditions in parallel reactions on a Quest 210 synthesizer, and the products **7**{***n***} were purified using parallel flash chromatography (Scheme 4). The yields ranged from 24 to 86%, the

(21) Balogh, V.; Fetizon, M.; Golfier, M. *J. Org. Chem.* **1971**, *36*, 1339−1341.

Scheme 3



Scheme 4



compounds were produced in 24−91 mg amounts, and the purities (determined by HPLC) were excellent (Table 3), with the only problematic indoles being those with 4-substitution. Our experience has shown that these indoles have reduced reactivity (for obvious steric reasons) in all of the methods we have examined for uniting indoles with quinones.

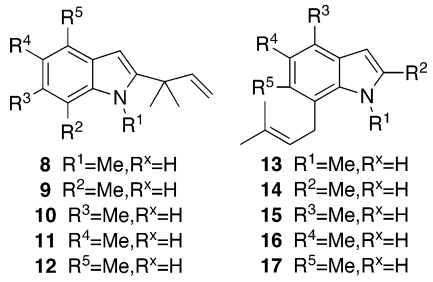
Five derivatives each of the two indoles required for the synthesis of the methyl scan derivatives of DAQ B1 (**8**−**17**, where R^x means all of the non-methylated positions; Chart 1) were prepared by modifications of previously described procedures.[22,23] The *N*-methyl derivative **8** of 2-isoprenylindole was prepared by a known method (*tert*-BuOK/18-crown-6/MeI) for indole *N*-methylation.[24] The Fisher indole synthesis of 2-isoprenylindole was modified by the use of three tolylhydrazines. *o*-Tolylhydrazine gives 7-methyl-2-isoprenylindole **9**, but in low efficiency as expected on the basis of precedent. *p*-Tolylhydrazine gives 5-methyl-2-isoprenylindole **11**, and *m*-tolylhydrazine gives a mixture of 4-methyl- and 6-methyl-2-isoprenylindole **12** and **10**, as precedented.[25] These two compounds could be separated by careful chromatography.

Table 3. Zinc Triflate-Promoted Addition of Indoles to **1**[a]

7{*n*}	indole	yield (%)	purity (%)
1	parent	60	99
2	*N*-methyl-	86	99
3	2-methyl-	74	99
4	2-cyclopropyl-	58	99
5	2-isopropyl-	53	99
6	2-*tert*-butyl-	79	99
7	2-phenyl-	84	93
8	4-methoxy-	24	99
9	4-benzyloxy-	34	99
10	5-fluoro-	53	99
11	5-methoxy-	58	98
12	5-benzyloxy-	60	99
13	5-methyl-	67	99
14	6-fluoro-	45	98
15	6-methyl-	25	99
16	7-methyl-	43	98
17	7-*tert*-butyl-	80	98
18	2,5-methyl-	84	95
19	5-methoxy-2-methyl-	68	98
20	5-chloro-2-methyl-	76	97

[a] These products are all known compounds from our earlier work.[18]

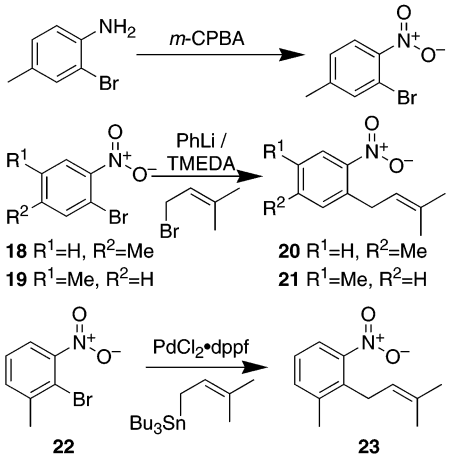
Chart 1



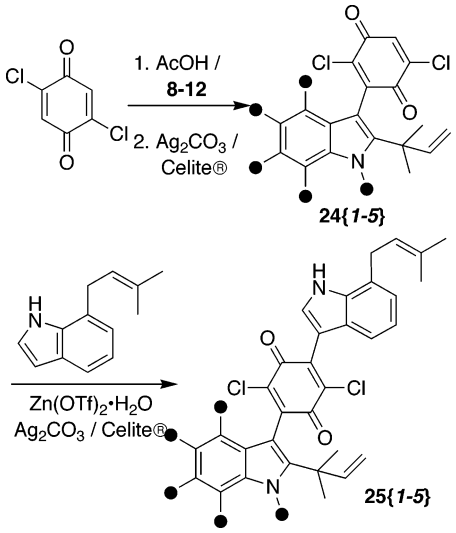
Our synthesis of 7-prenylindole converts 2-bromonitrobenzene to the lithium reagent, which is alkylated with prenyl bromide.[22] Bartoli indole synthesis using vinyl Grignard under modified conditions that we developed gives the target. Application of the foregoing methylation procedure yields *N*-methyl-7-prenylindole **13**, and substitution of isopropenyl Grignard for vinyl Grignard gives 2-methyl-7-prenylindole **14**. For preparation of the other methylated indoles, the corresponding methylated bromonitrobenzenes are required (Scheme 5). Two of them, 1-bromo-4-methyl-2-nitrobenzene and 2-bromo-1-methyl-3-nitrobenzene, are commercially available. The third (**18**) was prepared by oxidation of the corresponding aniline. Several oxidants were tested (dimethyldioxirane,[26] peroxyacetic acid,[27] and *meta*-chloroperbenzoic acid[27]). Dimethyldioxirane (prepared in situ from oxone and acetone) yielded the nitro compound in 67% yield. Better results were obtained with peroxyacetic acid (90%) and *m*-CPBA (84%). Although peroxyacetic acid gives a higher yield, *m*-CPBA is easier to handle and the reaction is much faster. This reaction was also easily scaled up (to 5 g). The metalation/alkylation reaction was straightforward for nitrobenzenes **18** and **19**, resulting in quantitative yields, though tetramethylethylenediamine (TME-

(22) Sanz-Cerveral, J. F.; Glinka, T.; Williams, R. M. *Tetrahedron* **1993**, *49*, 8471−8482.
(23) Pirrung, M. C.; Wedel, M.; Zhao, Y. *Synlett* **2002**, 143−145.
(24) Guida, W. C.; Mathre, D. J. *J. Org. Chem.* **1980**, *45*, 3172−3176.
(25) Bistochi, G.; De Meo, G.; Ricci, A.; Croisy, A.; Jacquignon, P. *Heterocycles* **1978**, *9*, 247−255. Baccolini, G.; Marotta, E. *Tetrahedron.* **1985**, *41*, 4615−4620.
(26) Lemek, T.; Makosza, M.; Golinski, J. *Tetrahedron* **2001**, *57*, 4753−4757.
(27) Smith, M. B.; March, J. *Advanced organic chemistry: reactions, mechanisms, and structure*; Wiley: New York, 2001.

Scheme 5



Scheme 6



DA) had to be used as an additive. This reaction sequence fails utterly with the remaining nitrobenzene **22**, evidently due to steric hindrance, even though metal−halogen exchange occurs (as shown by recovery after acidic workup of the dehalogenated nitrobenzene, identical to an authentic sample). An inversion of the charge polarity in this reaction provided the solution. A Stille coupling of 2-bromo-1-methyl-3-nitrobenzene with commercially available prenyltributyltin under palladium catalysis yields the target nitrobenzene in 74% yield. This reaction proved to be very sensitive to temperature variations: below 90 °C the reaction was very slow, and above 120 °C almost no product was isolated. These three nitrobenzenes were converted to the indoles **15**, **16**, and **17** by treatment with vinyl Grignard.

The 10 indoles emerging from the foregoing preparations were used in two synthetic routes. The methylated 2-isoprenylindoles **8**−**12** were condensed with dichlorobenzoquinone (Scheme 6). Coupling reactions were very successful under the standard acetic acid conditions[17] with *N*-, 5-, and 7-methyl-2-isoprenylindoles, but gave low yields for 4- and 6-methyl-2-isoprenylindoles. Further investigation showed that a solvent mixture of THF and acetic acid gave better results for those compounds. The reaction of 4-methyl-2-isoprenylindole was much slower than the others due to steric hindrance, which might also be responsible for the modest yield. The resulting compounds **24**{***1***−***5***} were coupled with 7-prenylindole using the

Scheme 7

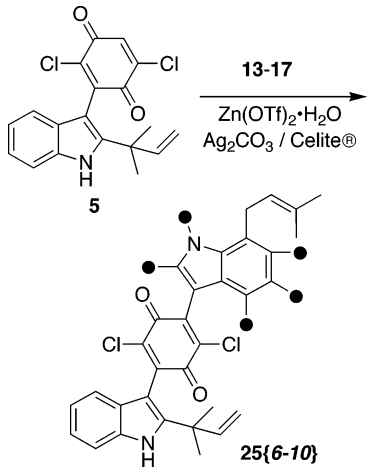
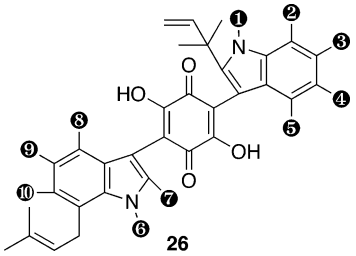


Table 4. Preparation of Methyl Scan Library of DAQ B1 Derivatives

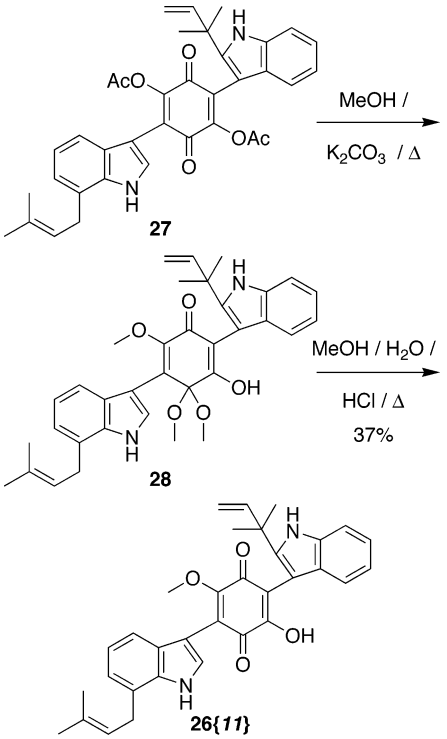
indole	*n*	yield (%) of **25**{*n*}	yield (%) of **26**{*n*}
8	***1***	68	47
9	***2***	39	45
10	***3***	71	55
11	***4***	75	68
12	***5***		9 (two steps)
13	***6***	86	70
14	***7***	59	82
15	***8***		15 (two steps)
16	***9***		12 (two steps)
17	***10***		14 (two steps)

same procedure used in the DAQ B1 total synthesis. Intermediate **5** from that work could be used as a common intermediate in a divergent synthesis with the methylated 7-prenylindoles (Scheme 7). Some of these products **25**{***6***−***10***} are not stable in concentrated form for significant periods even at room temperature. Because this lowered yields appreciably, these compounds were hydrolyzed directly after purification by column chromatography and removal of solvent. Summary results are in Table 4.

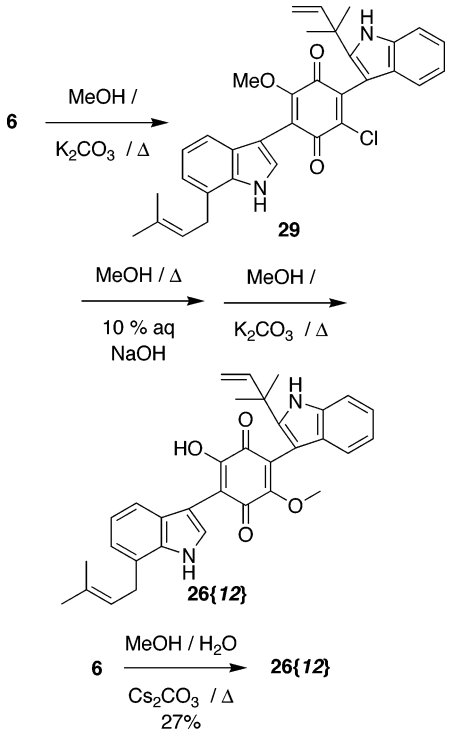
The hydrolysis of many 3,6-bisindolyl-2,5-dichloro-[1,4]-benzoquinones has been successfully conducted using a procedure developed in our laboratory[19] for hydrolysis of monoindolyl-2,5-dichloro-[1,4]benzoquinones: addition of sodium hydroxide to their dilute methanol solutions under reflux. This method applied to the majority of compounds **25** also. However, under the standard reaction conditions, compounds **25**{***8***}, **25**{***9***}, and **25**{***10***} give only a small amount of **26**{***8***−***10***}, with comparable amounts of methoxyl compounds revealed by NMR. The hydrolysis of **25**{***8***}, **25**{***9***}, and **25**{***10***} in refluxing methanol was examined with slow addition of an aqueous sodium hydroxide solution via a syringe pump over 30 min, and adequate results were obtained.



Scheme 8



Scheme 9



The two *O*-methyl derivatives are not simple to prepare from DAQ B1 since the two sites of reactivity are very similar, but several methods were developed to access **26**{***11***} and **26**{***12***}. Attempts to selectively methylate DAQ B1 (with MeI) or selectively hydrolyze the *O*,*O*-dimethyl compound prepared from DAQ B1 with diazomethane gave complex mixtures. It has been reported that treatment of 2,5-diacetoxy-3,6-bis(*N*-isoprenylindolyl)-[1,4]benzoquinone (a DAQ A1 derivative) with alcohols in the presence of potassium carbonate results in formation of the monomethyl ether through the intermediacy of the quinone acetal.[28] Under these reaction conditions, the diacetate of DAQ B1 **27** was converted to acetal **28** (Scheme 8). Presumably, this product is formed by nucleophilic addition−elimination of the less hindered acetate and methanolysis of the more hindered acetate. Formation of the acetal under basic/nucleophilic conditions is difficult to describe mechanistically, but the observed reaction does occur preferentially at the less hindered carbonyl group in **27** that should be more reactive to nucleophilic attack. Subsequent hydrolysis of **28** by mineral acid gives the target compound **26**{***11***}.

During investigations of the hydrolysis of dichloroquinone **6**, a reaction sequence was discovered that provides the monomethyl derivatives of DAQ B1. When treated with 1 equiv of potassium carbonate in methanol, **6** gives a mixture of chloromethoxyquinone **29**, its regioisomer, and a dimethoxyquinone, which can be separated by column chromatography (Scheme 9). The structural assignment for compound **29** is based on a nuclear Overhauser effect (NOE) between the methoxyl group and both the 2-H and 4-H of the 7-prenylindole. Hydrolysis of this material leads, somewhat surprisingly, to loss of the methoxyl group. Further treatment with methanol and potassium carbonate replaces the more hindered chloride, giving the desired **26**{***12***}. This compound was reported by earlier investigators[29] as the product of selective hydrolysis of asterriquinone B1, but spectral data were not available for comparison, and their structural assignment was based on an assumption of the greater reactivity of the less hindered methyl ether. Its structure was confirmed by an NOE study. When the methoxyl group is irradiated, a positive NOE is observed on the 4-H and vinyl proton of the 2-isoprenylindole, proving the methoxyl group is on the isoprenylindole side. Further investigations of the hydrolysis of **6** enabled us to discover reaction conditions (aqueous methanol/cesium carbonate) that generate **26**{***12***} selectively.

Table 5. Activation of the Insulin Receptor in hIRcB Cells Treated for 10 min with Methylated Compounds **26**{***1***−***12***} at 30 μM[a]

compound	mean	SEM	compound	mean	SEM
DAQ B1	58	13			
1	40	15	***7***	86	30
2	81	44	***8***	25	9
3	66	30	***9***	22	14
4	63	25	***10***	22	10
5	45	23	***11***	9	5
6	42	21	***12***	11	7

Biological Data

Each compound **26**{***1***−***12***} was examined for activation of hIR tyrosine kinase autophosphorylation in a rat fibroblast cell line overexpressing the receptor (hIRcB).[16a] Complete dose−response studies were performed, and the percent activation by each compound at 30 μM is presented in Table 5. Statistical analysis gave significant differences from DAQ B1 for the ***8***−***10***, ***11***, and ***12*** compounds. The hot spots for protein−small molecule interaction identified in this experiment are the 7-substituted indole (except the 1- and 2-positions) and the OH's (Figure 2).

(28) Kaji, A.; Saito, R.; Hata, Y.; Kiriyama, N. *Chem. Pharm. Bull*. **1999**, *47*, 77−82. Kaji, A.; Saito, R.; Shinbo, Y.; Kiriyama, N. *Chem. Pharm. Bull*. **1996**, *44*, 2340−2341.

(29) Wood, H. B.; Black, R.; Salituro, G.; Szalkowski, D.; Li, Z.; Zhang, Y.; Moller, D. E.; Zhang, B.; Jones, A. B. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 1189−1192.

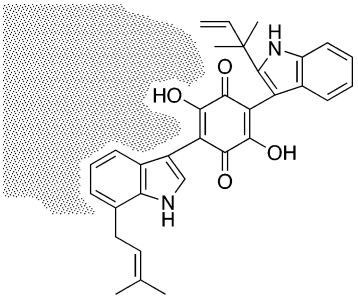
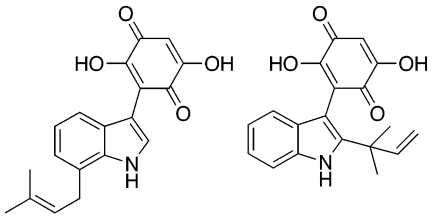


Figure 2. Contact surfaces of demethylasterriquinone B1 and its target receptors ("hot spots") identified by methyl scanning.

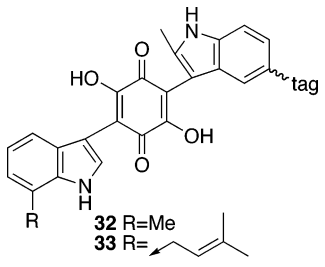
While the potencies listed in Table 5 are modest, the reported EC_{50} of DAQ B1 for insulin receptor (hIR) activation in cell-based assays is in the low micromolar range.[1,17a,30] Further, it is known that the asterriquinones penetrate cells poorly. When P388 leukemia cells are exposed to an extracellular concentration of 30 μM of the related compound demethylasterriquinone A1 for 1 h, the intracellular concentration is only 60 pM.[31] In a similar experiment, its more hydrophobic dimethyl derivative asterriquinone A1 shows an increased intracellular concentration of 200 pM, but even the monomethyl derivative showed an intracellular concentration of only 70 pM. The asterriquinones are obviously not very cell-permeant. One hypothesis was that cell penetration is impeded by hydroxyl group ionization at assay pH, since the first pK_a of demethylasterriquinone A1 is 6.93. To address this issue, assays were performed at pH 6.5, hoping to minimize the fraction of charged compound. However, the results were the same as those obtained at pH 7.4.[32] Therefore, we believe that the results from this cell-based study are valid even though the concentrations used are high relative to those used in most studies of drug action. There is no evidence that the methylated derivatives of DAQ B1 have reduced solubility in buffer below 100 μM based on observations of precipitation or turbidity. All compounds were dissolved in dimethylsulfoxide (DMSO) and diluted into buffer. The *C* log *P* values for the set of methylated DAQ derivatives (Table S1, Supporting Information) are all very similar, so different solubilities cannot explain the different activities of these derivatives. Likewise, there is no evidence that methylation reduces their ability to penetrate cells, or that the methylated DAQ B1 derivatives are differentially toxic or have other negative effects on cellular viability. The treatment with compounds is also so short (10 min) that toxic effects are unlikely to be involved.

The function of growth factor receptors generally involves dimerization and autophosphorylation of their tyrosine kinase domains.[33] Small molecules that have earlier been shown to mimic the action of other protein therapeutics[34] are symmetric, two-domain structures, perhaps suggesting that these properties dovetail with receptor dimerization and kinase signaling. We have therefore considered the idea that the action of DAQ B1, a two-domain molecule that at least approaches symmetry, is based on its ability to dimerize the hIR kinase domain, perhaps bridging two protein molecules through its indole units. A well-known test for protein dimerization by two-domain molecules is the ability of one-domain "half-molecules" to inhibit the action of the full molecule.[35] In the course of developing synthetic methods toward monoindolylquinones,[18] molecules **30** and **31**, which represent the two halves of DAQ B1, were prepared. The availability of these compounds permitted the dimerization hypothesis to be readily tested. Compound **31** at 30 μM has no effect on the activation by DAQ B1 of hIR in hIRcB cells, whereas **30** enhances receptor activation at this concentration. Control experiments in the absence of DAQ B1 show that **30** itself is capable of activating hIR in hIRcB cells, though it is somewhat less potent, with an EC_{50} of ~100 μM. This study provides information on the DAQ B1 pharmacophore that is discussed later.



30 **31**

Information from both of these studies was used in the design of affinity reagent **33**. The main pharmacophore (**30**) is present in **33**, with modifications being made to the 2-substituted indole ring of DAQ B1 for synthetic ease. Initial studies showed that omitting a 2-alkyl group was very costly to the yield in the hydrolysis of the dichloroquinone (J. May, unpublished), in concert with data showing low yields in bisindolyldichloroquinone hydrolysis when neither indole bears a 2-substituent.[36] A definitive explanation for this observation is not available, but any indole 2-alkyl substituent strongly favors a nonplanar conformation of the 3-indolylquinone.[18] A reasonable hypothesis is that a nonplanar conformation of at least one of the indole−quinone bonds is necessary for high yields in the hydrolysis, but mechanistic understanding of why this should be so is unavailable. While any of the aromatic carbons in the 2-substituted indole should serve for the position of tag attachment, the choice of the 5-position was based on the commercial availability of 2-methyl-5-aminoindole. The tag chosen was biotin linked to the indole via an aminocaproic acid unit. As a model compound for the synthesis and a negative control for affinity binding studies, compound **32** with a 7-methyl group replacing the 7-prenyl group was also targeted.



32 R=Me
33 R=

(30) Qureshi, S. A.; Ding, V.; Li, Z.; Szalkowski, D.; Biazzo-Ashnault, D. E.; Xie, D.; Saperstein, R.; Brady, E.; Huskey, S.; Shen, X.; Liu, K.; Xu, L.; Salituro, G. M.; Heck, J. V.; Moller, D. E.; Jones, A. B.; Zhang, B. B. *J. Biol. Chem.* **2000** 275, 36590−36595. Liu, K.; Xu, L.; Szalkowski, D.; Li, Z.; Ding, V.; Kwei, G.; Huskey, S.; Moller, D. E.; Heck, J. V.; Zhang, B. B.; Jones, A. B. *J. Med. Chem.* **2000**, *43*, 3487−3494.

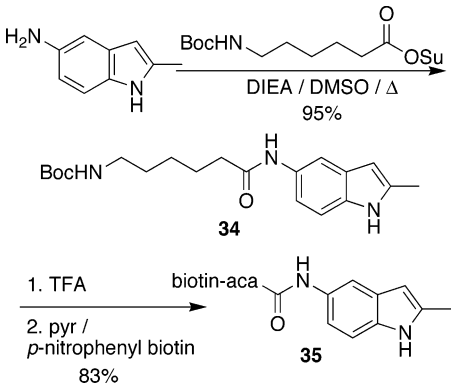
(31) Kaji, A.; Saito, R.; Nomura, M.; Miyamoto, K.-i.; Kiriyama, N. *Biol. Pharm. Bull.* **1998**, *21*, 945−949.

(32) In vitro studies that we have performed suggest the potency of DAQ B1 for direct action on the hIR is in the nanomolar range.

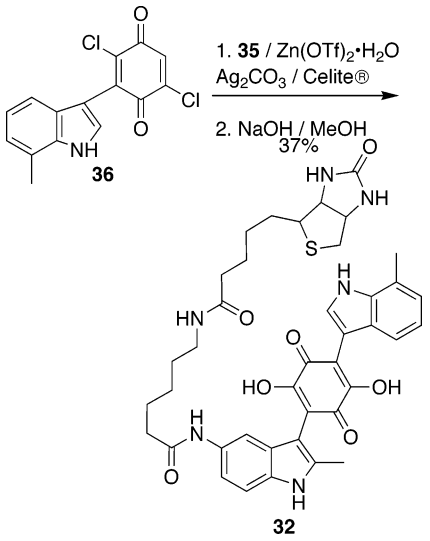
(33) McInnes, C.; Sykes, B. D. *Biopolymers* **1997**, *43*, 339−366.

(34) Laborde, E.; Manchem, V. P. *Curr. Med. Chem.* **2002**, *9*, 2231−2242. Tian, S. S.; Lamb, P.; King, A. G.; Miller, S. G.; Kessler, L.; Luengo, J. I.; Averill, L.; Johnson, R. K.; Gleason, J. G.; Pelus, L. M.; Dillon, S. B.; Rosen, J. *Science* **1998**, *281*, 257−259.

Scheme 10



Scheme 11

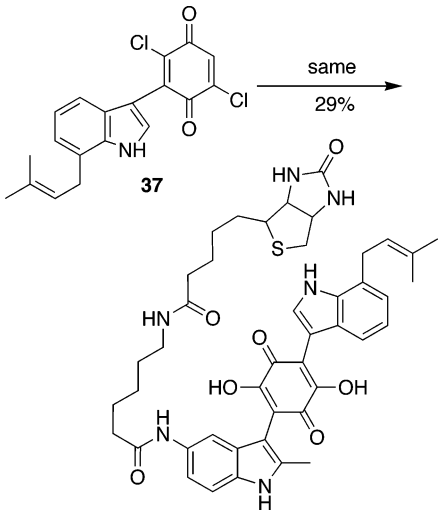


Syntheses of **32** and **33** begin with acylation of 2-methyl-5-aminoindole with the commercially available active ester of aminocaproic acid to give **34**. The low nucleophilicity of the aniline nitrogen requires vigorous reaction conditions. Removal of the Boc group, neutralization, and acylation give the biotin-conjugated indole **35** (Scheme 10). Its zinc triflate-promoted condensation with the known[18] dichloroindolylbenzoquinone **36** proceeds in 82% yield, and hydrolysis to **32** under standard conditions proceeds in 45% yield (Scheme 11). Likewise, condensation of the known[18] dichloroindolylbenzoquinone **37** with **35** proceeds in 81% yield, and hydrolysis to **33** under standard conditions proceeds in 36% yield (Scheme 12). These compounds were characterized by full spectroscopic analysis, including the ESI MS spectrum of **33** shown in Figure 3.

Discussion

The concept of methyl scanning described here provides a general approach to achieving an essential step in the identification of targets of novel natural products with cell-based activity, particularly anti-bacterial and anti-tumor activity. While valuable in identifying sites available for structural modification, methyl scanning cannot provide information concerning the basis of the modified potency of methylated derivatives. For example, methylation at the 6-position of 7-prenylindole, which reduces

Scheme 12



potency, might act by influencing the conformation of the adjacent prenyl group rather than by exerting a direct effect on a protein contact. The electronic properties of the quinone might be modified by methylation in ***11*** and ***12***. However, we have investigated the redox properties of compounds in this family and do not believe that they relate to biological activity.[37]

This work differs from conventional structure−activity correlations, such as have earlier been described for DAQ B1,[29] regarding both the study's purpose and the commitment to a systematic approach of studying alkyl substitution at every available position. Good agreement is noted between the hot spots identified through methyl scanning and a pharmacophore model that emerges from study of the half-molecules **30** and **31**. That is, the main locus of activity in DAQ B1 is the 7-substituted indole ring and the quinone; the 2-isoprenylindole unit is much less important. Interestingly, methylation at the 2-position of the 7-substituted indole, in compound **26**{***7***}, enhances potency slightly. Methyl scanning had been envisioned as a modification that would decrease activity or leave it unchanged, but it is certainly conceivable that activity could increase by happenstance. Based on our earlier studies concerning the conformation and barrier to rotation of 3-indolylbenzoquinones,[18] methylation at the 2-position of the indole is sure to increase the rotational barrier and strongly favor a perpendicular conformation in the ground state. The enhanced potency of this compound may relate to an increase in the population of the conformer in which DAQ B1 is bound to its target(s). Potency enhancement by conformational restriction is certainly a well-appreciated strategy in drug development, though it more often involves rings than acyclic conformations. The pharmacophore model that emerges from this work is shown in Figure 2.

Using extensions of the synthetic methods developed here and earlier, a biotin conjugate of DAQ B1 has been prepared. This material can be used as an indicator to identify DAQ B1-binding proteins in blotting procedures, as a ligand in affinity chromatography, and as bait in phage display cloning.[5]

This work has provided a general strategy to the synthesis of DAQ B1 and related molecules based on a first, protic acid-promoted condensation of indoles with dichlorobenzoquinone

(35) Spencer, D. M.; Graef, I.; Austin, D. J.; Schreiber, S. L.; Crabtree, G. R. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 9805−9809.
(36) Li, Z. Ph.D. Thesis, Duke University, 2003.
(37) Sohn, J.; Kiburz, B.; Li, Z.; Deng, L.; Safi, A.; Pirrung, M. C.; Rudolph, J. *J. Med. Chem.* **2003**, *46*, 2580−2588.

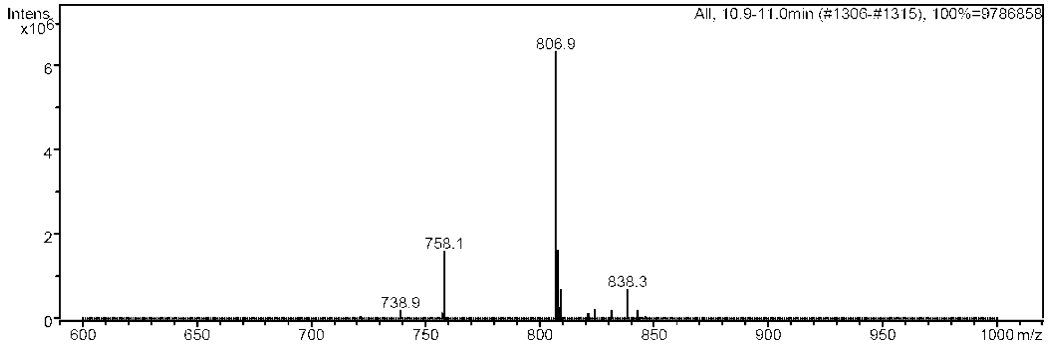


Figure 3. ESI MS spectrum of **33**.

and a second, Lewis acid-promoted condensation of indoles with the (3-indolyl)benzoquinone produced in the first step. The ineffectiveness of Brønsted acids in promoting the second reaction is actually advantageous in that it allows selective, sequential introduction of two different indoles. An earlier asterriquinone synthesis[38] could not control the rate of addition of the first and second indoles to a quinone core under basic conditions, with the result being that only symmetrical compounds could be prepared. While the three-step total synthesis of DAQ B1 we developed was applied in this work to methyl scanning, it should be straightforward to use these methods, as described in the pilot study to prepare **7**, in the solution-phase preparation of combinatorial libraries of asterriquinones.[36] As exemplified by the poor reactivity of 4-substituted indoles in the acid-promoted additions to quinones observed here, a general challenge to the use of combinatorial methods for the preparation of diverse structures is that modifications to the building blocks that introduce diversity also often modify their chemical reactivity. The variation in structure that is essential to combinatorial chemistry can frustrate the preparation of structurally diverse targets.

The choice of a methyl group to probe the interaction surface between a drug and its receptor is a very practical one, in that many methylated versions of the building blocks used in target-directed synthesis are likely to be commercially available. For those that are not commercial, the introduction of a methyl group, through reactive nucleophilic and electrophilic synthons, should be among the most efficient organic reactions. Methyl scanning provides information on the contacts between drugs and their protein targets, but does not provide compounds to serve as affinity ligands. A more advanced concept that should be pursued is allyl or propargyl scanning. While perhaps fewer allyl or propargyl building blocks will be commercially available, both nucleophilic and electrophilic allyl and propargyl synthons[39] are well known. Molecules that retain activity in allyl or propargyl scanning experiments can themselves be taken forward through reactions such as cross-metathesis[40] or copper-catalyzed azide−alkyne [3 + 2] cycloaddition[41] to convert them to affinity reagents.

Ultimate validation of reagent **33** might be proposed to come from examination of its biological activity in activating the insulin receptor; however, such a proposal overlooks important differences between affinity techniques and receptor activation. While phage display, blotting, and affinity chromatography are intrinsically in vitro techniques, performed on cell-free extracts, insulin receptor activation is measured in intact cells. The ability of **33** to function in vitro as designed is not informed by its activity in a cell-based assay, though of course this issue was examined. Neither **32** nor **33** affected insulin receptor activation in two cell lines. These data are readily explained if the target(s) of DAQ B1 are intracellular (a significant body of evidence already exists that DAQ B1 affects the cytoplasmic tyrosine kinase domain of the insulin receptor) and addition of the affinity tag makes the compound unable to penetrate the cell membrane.

In summary, the molecular recognition of the natural product demethylasterriquinone B1 by its cellular receptor(s) has been studied using a systematic approach involving synthesis of a complete set of methylated derivatives and evaluation of their activity in cells. In comparison to structural methods that might be used to design affinity reagents, methyl scanning offers the advantage that it is a functional assay that reports on interactions between the small molecule and *all* proteins in the cell rather than just a single identified protein. Methyl scanning should be applicable to the identification of the hot spots on any biologically active compound that can be synthesized. Knowledge of the hot spots also presents opportunities to make compensating changes in one protein ("bump-and-hole" method) to engineer exquisite specificity into the actions of the small molecule.

Experimental Section

General Procedure for the Coupling of Indoles with 2,5-Dichloro-[1,4]benzoquinone, with Acetic Acid as the Catalyst and Silver Carbonate as the Oxidizing Reagent. A mixture of 2,5-dichloro-[1,4]-benzoquinone (1.00 mmol), indole (0.50 mmol), and acetic acid (3 mL) was stirred at 70 °C under nitrogen until indole was completely consumed as indicated by TLC. After the reaction mixture was cooled to room temperature, silver carbonate (50 wt % on Celite, 1 mmol) was added and the mixture was stirred overnight. It was transferred into a larger flask by washing with ethyl acetate and evaporated to dryness in vacuo with a small amount silica gel. The pure product was obtained after purification by flash chromatography (silica gel, ethyl acetate:hexanes 1:10) and removal of solvent in vacuo.

3-(2-*tert*-Butyl-1*H*-indol-3-yl)-2,5-dichloro[1,4]benzoquinone (1).[17] Blue solid, yield 90%. ^{1}H NMR (acetone-d_6): δ 10.45 (1H, s), 7.48 (1H, s), 7.36−7.34 (1H, m), 7.25−7.22 (1H, m), 7.11−7.05 (1H, m), 6.99−6.93 (1H, m), 1.38 (9H, s).

2,5-Dichloro-3-[2-(1,1-dimethylallyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (5). Blue solid, yield 80%. Mp: 187−188 °C. ^{1}H NMR (acetone-d_6): δ 10.45 (1H, s), 7.42 (1H, s), 7.39−7.36 (1H, m), 7.25−7.22 (1H, m), 7.12−7.07 (1H, m), 7.00−6.95 (1H, m), 6.06 (1H, dd,

(38) Harris, G. D.; Nguyen, A.; App, H.; Hirth, P.; McMahon, G.; Tang, C. *Org. Lett.* **1999**, *1*, 431−434.
(39) Teobald, B. J. *Tetrahedron* **2002**, *58*, 4133−4170.
(40) Roy, R.; Dominique, R.; Das, S. K. *J. Org. Chem.* **1999**, 64, 5408−5412.
(41) Speers, A. E.; Adam, G. C.; Cravatt, B. F. *J. Am. Chem. Soc.* **2003**, *125*, 4686−4687.

J = 17.7, 10.5 Hz), 5.08−5.02 (2H, m), 1.484 (3H, s), 1.480 (3H, s). ^{13}C NMR (acetone-d_6): δ 178.0, 177.8, 145.7, 144.2, 143.4, 142.6, 141.7, 136.0, 133.7, 127.1, 121.9, 119.8, 118.8, 112.5, 111.3, 103.0, 39.4, 27.9, 26.1. IR (KBr): 3418, 3057, 2979, 1678, 1663, 1575, 1458, 1431, 1250, 1029 cm^{-1}. Anal. Calcd for $C_{19}H_{15}Cl_2NO_2$: C, 63.35; H, 4.20; N, 3.89. Found: C, 63.39; H, 4.43; N, 3.90.

2-(2-*tert*-Butyl-1*H*-indol-3-yl)-3,6-dichloro-5-(2-methyl-1*H*-indol-3-yl)-[1,4]benzoquinone (2). A mixture of 2-methylindole (63 mg, 0.48 mmol), compound **1** (140 mg, 0.40 mmol), and sulfuric acid (90 μL, 1.6 mmol) was stirred in THF (2 mL) at room temperature under nitrogen for 4 h. To the reaction mixture was added DDQ (120 mg, 0.53 mmol). After being stirred for 1 h, the reaction mixture was diluted with ethyl acetate (20 mL), washed with saturated aqueous sodium bicarbonate solution (20 mL × 2) and brine (20 mL × 2), dried with sodium sulfate, and purified with flash chromatography (silica gel, ethyl acetate:hexanes 1:5). The title compound was obtained as a dark blue solid, 174 mg (91%). Mp: 283−284 °C. ^{1}H NMR (acetone-d_6): δ 10.63 (1H, s), 10.43 (1H, s), 7.41−6.95 (8H, m), 2.44 and 2.40 (3H, s), 1.45 and 1.43 (9H, s). ^{13}C NMR (acetone-d_6): δ 178.6, 177.3, 145.4, 143.1, 142.6, 140.2, 140.1, 137.7, 137.4, 136.1, 135.8, 127.6, 121.6, 121.4, 120.2, 119.9, 119.5, 118.7, 118.6, 111.1, 111.0, 102.2, 33.5, 30.0, 13.1. IR (KBr): 3417, 2971, 1678, 1619, 1585, 1459, 1426, 1309, 1260, 1037 cm^{-1}. Anal. Calcd for $C_{27}H_{22}Cl_2N_2O_2$: C, 67.93; H, 4.65; N, 5.87. Found: C, 67.77; H, 4.83; N, 5.73.

2-(2-*tert*-Butyl-1*H*-indol-3-yl)-3,6-dichloro-5-(7-methyl-1*H*-indol-3-yl)-[1,4]benzoquinone (3). A mixture of 7-methylindole (26 mg, 0.20 mmol), compound **1** (70 mg, 0.20 mmol), and zinc triflate monohydrate (152 mg, 0.40 mmol) was stirred in THF at reflux under nitrogen for 12 h. After the mixture cooled to room temperature, DDQ (50 mg, 0.22 mmol) was added in a single portion. After being stirred for 1 h, the reaction mixture was diluted with ethyl acetate (20 mL), washed with saturated aqueous sodium bicarbonate solution (20 mL) and brine (20 mL × 2), dried with sodium sulfate, and purified with flash chromatography (silica gel, ethyl acetate:hexanes 1:5). The title compound was obtained as a blue solid, 78 mg (82%). Mp: 268−269 °C. ^{1}H NMR (acetone-d_6): δ 11.00 (1H, s), 10.42 (1H, s), 7.77−6.95 (8H, m), 2.58 (3H, s), 1.43 (9H, s). ^{13}C NMR (acetone-d_6): δ 178.3, 177.8, 145.3, 142.7, 142.5, 139.4, 136.9, 135.9, 135.7, 130.2, 127.6, 125.8, 122.8, 121.6, 120.5, 119.6, 119.5, 118.6, 107.4, 102.4, 33.7, 30.1, 16.4. IR (KBr): 3418, 2967, 1661, 1615, 1571, 1461, 1426, 1341, 1260, 1116 cm^{-1}. Anal. Calcd for $C_{27}H_{22}Cl_2N_2O_2$: C, 67.93; H, 4.65; N, 5.87. Found: C, 67.52; H, 4.83; N, 5.77.

2,5-Dichloro-3-[2-(1,1-dimethylallyl)-1*H*-indol-3-yl]-6-[7-(3-methyl-but-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (6). A mixture of zinc triflate monohydrate (533 mg, 1.4 mmol), silver carbonate (on Celite, 50wt %, 386 mg, 0.70 mmol), compound **5** (126 mg, 0.35 mmol), and 7-prenylindole (130 mg, 0.70 mmol) was stirred in THF at reflux under nitrogen for 2 d. After cooling to room temperature, the reaction mixture was filtered, washed with ethyl acetate (20 mL), and purified with flash chromatography (silica gel, ethyl acetate:hexanes 1:5). The title compound was obtained as a blue solid, 145 mg, 76%. Mp: 141−142 °C. ^{1}H NMR (acetone-d_6): δ 10.86 (1H, s), 10.42 (1H, s), 7.74−6.98 (8H, m), 6.14 (1H, dd, J = 13.2, 7.8, Hz), 5.51−5.47 (1H, m), 5.15−5.09 (2H, m), 3.68 (2H, d, J = 5.4 Hz), 1.79−1.77 (6H, m), 1.53−1.52 (6H, s). ^{13}C NMR (acetone-d_6): δ 178.4, 178.0, 145.7, 143.2, 142.4, 141.7, 139.1, 137.3, 136.0, 135.3, 133.1, 130.1, 127.3, 126.1, 125.4, 122.1, 121.8, 121.7, 120.6, 119.7, 119.6, 118.9, 112.4, 111.3, 107.3, 103.5, 39.4, 29.6, 27.9, 26.3, 25.2, 17.3. IR (KBr): 3408, 2967, 1669, 1570, 1457, 1431, 1245, 1215, 1183, 1129 cm^{-1}. Anal. Calcd for $C_{32}H_{28}Cl_2N_2O_2$: C, 70.72; H, 5.19; N, 5.15. Found: C, 70.46; H, 45.29; N, 4.97.

Representative Procedure for Lewis Acid-Promoted Addition: 2-(2-*tert*-Butyl-1*H*-indol-3-yl)-3,6-dichloro-5-(7-methyl-1*H*-indol-3-yl)-[1,4]benzoquinone (7{*16*}). A mixture of 7-methylindole (26 mg, 0.20 mmol), compound **1** (70 mg, 0.20 mmol), and zinc triflate monohydrate (152 mg, 0.40 mmol) was stirred in THF at reflux under nitrogen for 12 h. After the mixture cooled to room temperature, DDQ (50 mg, 0.22 mmol) was added in a single portion. After being stirred for 1 h, the mixture was diluted with ethyl acetate (20 mL), washed with saturated aqueous sodium bicarbonate solution (20 mL) and brine (20 mL × 2), dried with sodium sulfate, and purified with flash chromatography (silica gel, ethyl acetate:hexanes 1:5). This compound is the same as compound **3**.

2-(1,1-Dimethylallyl)-1-methyl-1*H*-indole (8). To a solution of 18-crown-6 (26 mg, 0.10 mmol) in anhydrous ether (2 mL) were added potassium *tert*-butoxide (130 mg, 1.2 mmol) and 2-isoprenylindole (185 mg, 1.0 mmol). The suspension was stirred at room temperature for 15 min, and a solution of iodomethane (69 μL, 156 mg, 1.1 mmol) in anhydrous ether (1 mL) was added. After being stirred for 2 h, the reaction was quenched with water (10 mL), extracted with ether (10 mL × 3), washed with brine (10 mL), dried with sodium sulfate, and purified with flash chromatography (silica gel, ethyl acetate:hexanes 1:9). The title compound was obtained as a light-yellow oil, 164 mg (82%). ^{1}H NMR (acetone-d_6): δ 7.48 (1H, d, J = 7.5 Hz), 7.25 (1H, d, J = 8.1 Hz), 7.09 (1H, t, J = 7.2 Hz), 6.99 (1H, t, J = 7.2 Hz), 6.33 (1H, s), 6.05 (1H, dd, J = 17.4, 10.5 Hz), 5.07 (1H, d, J = 10.5 Hz), 4.98 (1H, d, J = 17.4 Hz), 3.67 (3H, s), 1.48 (6H, s). ^{13}C NMR (acetone-d_6): δ 146.5, 146.3, 139.0, 127.6, 121.0, 120.1, 119.2, 112.2, 109.1, 98.9, 38.7, 31.8, 28.2.

3,3-Dimethylpent-4-en-2-one. The synthesis was conducted as in the literature.[20] The ketone was obtained in 62% yield and exhibited spectral properties consistent with the assigned structure. ^{1}H NMR ($CDCl_3$): δ 1.22 (6H, s), 2.11 (3H, s), 5.11−5.17 (2H, m), 5.87−5.96 (1H, m).

2-(1,1-Dimethylallyl)-5-methyl-1*H*-indole (11). In a 100 mL round-bottom flask fitted with a Dean−Stark distillation head, 0.878 g of 3,3-dimethylpent-4-en-2-one, 5 mL of toluene, 1.078 g of sodium acetate, and 1.242 g of *p*-tolylhydrazine hydrochloride were combined. The contents were refluxed at 140 °C until water was no longer formed (45 min). The solid was removed by filtration, and the toluene was removed in vacuo. The hydrazone was subjected to vacuum overnight. The crude hydrazone was dissolved in 5 mL of absolute diglyme, and 2.18 g of anhydrous $ZnCl_2$ was added quickly with stirring. The reaction was heated at 180−185 °C at reflux for 9 h under N_2. After reflux, 5 mL of toluene was added while the contents continued to stir and cool. The $ZnCl_2$ was removed through filtration and washed with hot toluene. The toluene and diglyme were removed in vacuo, and the product **11** was recovered through column chromatography (silica gel, toluene). The indole was obtained in the second fraction, producing 0.534 g (34%). It eluted as a yellow oil that darkened on exposure to air. ^{1}H NMR ($CDCl_3$): δ 1.47 (6H, s), 2.42 (3H, s), 5.07−5.14 (2H, m), 6.04 (1H, dd, J = 10.2, 17.4 Hz), 6.23 (1H, d, J = 2.1 Hz), 6.94 (1H, d, J = 8.1 Hz), 7.19 (1H, d, J = 8.4 Hz), 7.33 (1H, s), 7.78 (1H, s, br). ^{13}C NMR ($CDCl_3$): δ 146.3, 146.0, 134.3, 128.9, 123.0, 120.0, 112.3, 110.3, 97.7, 77.7, 77.3, 76.9, 38.6, 27.8, 21.8. IR 3416, 3083, 2967, 1640, 1586, 1469 cm^{-1}. LRMS: 199 (M^+). HRMS: calcd for $C_{14}H_{17}N$ 199.1361, found 199.1356 (M^+).

2-(1,1-Dimethylallyl)-7-methyl-1*H*-indole (9). The procedure was repeated as above with *o*-tolylhydrazine hydrochloride to produce **9**. This method yielded 0.162 g (10%) of the indole.^{1}H NMR ($CDCl_3$): δ 1.50 (6H, s), 2.47 (3H, s), 5.11−5.17 (2H, m), 6.06 (1H, dd, J = 10.8, 17.4 Hz), 6.33 (1H, d, J = 2.1 Hz). ^{13}C NMR ($CDCl_3$): δ 146.3, 145.5, 135.6, 128.2, 122.1, 120.0, 119.8, 118.0, 112.5, 98.8, 77.7, 77.3, 76.9, 38.6, 27.9, 17.1. IR: 3466, 2967, 1653, 1544, 1458 cm^{-1}. LRMS: 199 (M^+). HRMS: calcd for $C_{14}H_{17}N$ 199.1361, found 199.1354 (M^+).

2-(1,1-Dimethylallyl)-6-methyl-1*H*-indole (10) and 2-(1,1-Dimethylallyl)-4-methyl-1*H*-indole (12). The procedure was repeated with *m*-tolylhydrazine hydrochloride to yield a mixture of **10** and **12** after elution from the column of silica gel and toluene. The mixture of indoles was produced in 33% yield (0.517 g). A second column (silica gel, CH_2Cl_2−C_6H_{14} 1:4) mostly separated the indoles, with the 2-(1,1-

dimethylallyl)-6-methyl-1*H*-indole eluting first. The 2-(1,1-dimethylallyl)-6-methyl-1*H*-indole was isolated in a 0.260 g portion (17%), and the 2-(1,1-dimethylallyl)-4-methyl-1*H*-indole was isolated in a 0.200 g portion (13%). 2-(1,1-Dimethylallyl)-4-methyl-1*H*-indole: ^{1}H NMR ($CDCl_3$) δ 1.49 (6H, s), 2.53 (3H, s), 5.10−5.15 (2H, m), 6.05 (1H, dd, J = 10.2, 17.4 Hz), 6.31 (1H, d, J = 2.1 Hz), 6.87 (1H, d, J = 7.2 Hz), 7.03 (1H, t, J = 6.9 Hz), 7.14 (1H, d, J = 8.1 Hz), 7.89 (1H, s, br); ^{13}C NMR ($CDCl_3$) δ 146.3, 145.2, 135.7, 129.8, 128.4, 121.6, 120.0, 112.4, 108.3, 96.6, 77.7, 77.3, 77.9, 38.6, 27.8, 19.2; IR 3416, 3082, 2967, 1638, 1537, 1453 cm^{-1}; LRMS 199 (M^+); HRMS calcd for $C_{14}H_{17}N$ 199.1361, found 199.1359 (M^+). 2-(1,1-Dimethylallyl)-6-methyl-1*H*-indole: ^{1}H NMR ($CDCl_3$) δ TMS 1.46 (6H, s), 2.43 (3H, s), 5.07−5.13 (2H, m), 6.03 (1H, dd, J = 10.2, 17.4 Hz), 6.25 (1H, d, J = 2.4 Hz), 6.90 (1H, d, J = 8.1 Hz), 7.09 (1H, s), 7.42 (1H, d, J = 7.8 Hz), 7.74 (1H, s, br); ^{13}C NMR ($CDCl_3$) δ 146.4, 145.2, 136.5, 131.2, 126.4, 121.4, 119.9, 112.3, 110.7, 97.9, 77.7, 77.3, 76.9, 38.5, 27.8, 22.1; IR 3422, 3082, 2968, 1622, 1541, 1461 cm^{-1}; LRMS 199 (M^+); HRMS calcd for $C_{14}H_{17}N$ 199.1361, found 199.1356 (M^+).

1-Methyl-7-(3-methylbut-2-enyl)-1*H*-indole (13). This compound was synthesized analogously to compound **8**, giving 187 mg (99%) of a light-yellow oil. ^{1}H NMR (acetone-d_6): δ 7.38−7.35 (1H, m), 7.00 (1H, d, J = 3.0 Hz), 6.90−6.87 (1H, m), 6.35 (1H, d, J = 3.0 Hz), 5.32−5.26 (1H, m), 3.95 (3H, s), 3.75 (2H, d, J = 6.6 Hz), 1.73−1.71 (6H, m). ^{13}C NMR (acetone-d_6): δ 139.9, 135.2, 131.5, 130.9, 125.4, 125.0, 123.1, 119.5, 119.2, 100.9, 36.3, 31.6, 25.4, 17.8. IR (thin film): 3052, 2935, 1636, 1530, 1469, 1379, 1356, 1332, 1313, 1237 cm^{-1}. Anal. Calcd for $C_{14}H_{17}N$: C, 84.37; H, 8.60; N, 7.03. Found: C, 84.40; H, 8.74; N, 7.12.

2-Bromo-4-methylnitrobenzene (18). 2-Bromo-4-methylaniline (5.00 g, 26.9 mmol) and *m*-CPBA (33 g of 70 wt %, 134 mmol) were dissolved in toluene (100 mL) and heated under reflux for 6 h. After the mixture cooled to room temperature, the solid formed was filtered and washed with ether (100 mL). The filtrate was washed with sodium hydroxide (10%). After evaporation of the solvent, the residue was purified by flash chromatography (SiO_2, hexanes:ethyl acetate 9:1), yielding the known (CAS registry no. 40385-54-4) nitro compound as a light brown solid (4.85 g, 84%). ^{1}H NMR ($CDCl_3$): δ 2.39 (3H, s), 7.21 (1H, m), 7.49 (1H, m), 7.75 (1H, d, J = 8.4 Hz). ^{13}C NMR ($CDCl_3$): δ 21.45, 114.66, 125.89, 129.03, 135.63, 145.02, 147.53.

General Procedure for the Formation/Alkylation of Lithionitrobenzenes. Phenyllithium (2.3 mmol) was dissolved in THF (25 mL) under argon and the solution cooled to −78 °C (ethanol/N_2). 2-Bromo-5-methylnitrobenzene (2.3 mmol) dissolved in THF (5 mL) was added dropwise via syringe. After 5 min prenyl bromide (1.15 mmol) was added all at once, followed by TMEDA (2.3 mmol). The solution was kept at −78 °C for 3 h. After the reaction was quenched with saturated NH_4Cl (20 mL), the aqueous phase was extracted with ether (2 × 20 mL). The combined ethereal fractions were filtered through cotton wool, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (SiO_2, hexanes:ethyl acetate 9:1), yielding the alkylated nitrobenzene as a brown oil (236 mg, 100%).

2-(3-Methylbut-2-ene)nitrobenzene.[22] Yield: 100%. ^{1}H NMR ($CDCl_3$): δ 1.71, 1.74 (2 s, each 3H), 3.61 (2H, d, J = 7.2 Hz), 5.24 (1H, m), 7.31 (2H, m), 7.47 (1H, m), 7.84 (1H, d, J = 9 Hz). ^{13}C NMR ($CDCl_3$): δ 18.27, 26.09, 31.63, 120.86, 124.64, 127.01, 131.57, 132.99, 134.72, 136.64, 149.52. MS (GC/EI): 191 [M^+]. HRMS: calcd for $C_{11}H_{13}NO_2$ 191.094629, found 191.0947.

2-(3-Methylbut-2-ene)-4-methylnitrobenzene (20). Yield: 100%. ^{1}H NMR ($CDCl_3$): δ 1.68 (s, 3H), 1.71, (s, 3H), 2.36 (s, 3H), 3.58 (2H, d, J = 7.2 Hz), 5.20 (1H, m), 7.08 (2H, m), 7.78 (1H, d, J = 8.1 Hz). ^{13}C NMR ($CDCl_3$): δ 18.14, 21.65, 25.95, 31.72, 121.08, 125.04, 127.61, 129.62, 132.15, 134.46, 136.98, 144.16. MS (GC/EI): 205 [M^+]. HRMS: calcd for $C_{12}H_{15}NO_2$ 205.110279, found 205.1098.

2-(3-Methylbut-2-ene)-5-methylnitrobenzene (21). Yield: 100%. ^{1}H NMR ($CDCl_3$): δ 1.69 (s, 3H), 1.70, (s, 3H), 2.37 (s, 3H), 3.56 (2H, d, J = 7.2 Hz), 5.22 (1H, m), 7.23 (1H, d, J = 7.8 Hz), 7.30 (1H, dd, J = 8.1, 1.8 Hz), 7.67 (1H, s). ^{13}C NMR ($CDCl_3$): δ 18.26, 21.02, 26.08, 31.28, 121.13, 124.94, 131.39, 133.65, 133.79, 134.43, 137.20, 149.37. MS (GC/EI): 205 [M^+]. HRMS: calcd for $C_{12}H_{15}NO_2$ 205.110279, found 205.1109.

2-(3-Methylbut-2-ene)-3-methylnitrobenzene (23). The commercial bromide **22** (200 mg, 0.93 mmol) and $PdCl_2$(dppf) (155 mg, 0.19 mmol, 20 mol %) were dissolved in DMF (16 mL) under argon. Prenyltributyltin (0.41 mL, 1.2 mmol) was added and the solution heated for 23 h at 100 °C. The solution was filtered through Celite. Addition of 50 mL of water to the organic phase was followed by extraction with ether (3 × 15 mL) and washing with saturated KF solution and water. After filtration through cotton wool and evaporation of the solvent, the residue was purified by flash chromatography (SiO_2, hexanes:ethyl acetate 9:1), yielding the product as a brown oil (127 mg, 67%). ^{1}H NMR ($CDCl_3$): δ 1.70 (3H, d, J = 1.5 Hz), 1.73, (3H, d, J = 1.2 Hz), 2.36 (3H, s), 3.44 (2H, d, J = 7.8 Hz), 5.07 (1H, m), 7.21 (1H, t, J = 7.8 Hz), 7.35 (1H, d, J = 7.5 Hz), 7.54 (1H, dd, J = 8.1, 0.9 Hz). ^{13}C NMR ($CDCl_3$): δ 18.38, 20.26, 26.01, 28.39, 120.68, 121.91, 126.54, 133.53, 133.85, 134.21, 139.60. MS (GC/EI): 205 [M^+]. HRMS: calcd for $C_{12}H_{15}NO_2$ 205.110279, found 205.1098.

General Procedure for Bartoli Reactions. Vinylmagnesium bromide (12 mmol, 12 mL of a 1 M solution in THF) and dimethoxyethane (DME, 6 mL) were cooled under argon to −40 °C (ethanol/dry ice). The nitrobenzene (2 mmol) dissolved in THF (5 mL) was added dropwise via a syringe. The solution was kept at −40 °C until TLC and GC showed no remaining starting material (2−4 h). After the reaction was quenched with saturated NH_4Cl (20 mL), the aqueous phase was extracted with ether (3 × 20 mL). The combined ethereal fractions were filtered through cotton wool, and the solvent was evaporated under reduced pressure. The residue was purified by flash chromatography (SiO_2, hexanes:ethyl acetate 9:1), yielding the indole.

2-Methyl-7-(3-methylbut-2-ene)-1*H*-indole (14). Yield: 24%. ^{1}H NMR ($CDCl_3$): δ 1.85 (3H, d, J = 1.2 Hz), 1.88 (3H, m), 2.49 (3H, s), 3.58 (2H, d, J = 7.2 Hz), 5.48 (1H, m), 6.28 (1H, m), 6.98 (1H, d, J = 7.2 Hz), 7.07 (1H, t, J = 7.5 Hz), 7.44 (1H, d, J = 7.8 Hz), 7.85 (1H, br s). ^{13}C NMR ($CDCl_3$): δ 14.23, 18.40, 26.14, 31.01, 101.08, 117.90, 120.05, 120.80, 122.57, 123.34, 129.23, 133.34, 134.80, 135.40. MS (GC/EI): 199 [M^+]. HRMS: calcd for $C_{14}H_{17}N$ 199.136099, found 199.1357.

4-Methyl-7-(3-methylbut-2-ene)-1*H*-indole (15). Yield: 49%. ^{1}H NMR ($CDCl_3$): δ 1.83 (3H, s), 1.87 (3H, s), 2.59 (3H, s), 3.58 (2H, d, J = 7.2 Hz), 5.45 (1H, m), 6.62 (1H, m), 6.90 (1H, d, J = 7.2 Hz), 6.96 (1H, d, J = 7.2 Hz), 7.21 (1H, t, J = 3 Hz), 8.15 (1H, br s). ^{13}C NMR ($CDCl_3$): δ 18.37, 19.01, 26.14, 31.01, 101.70, 120.23, 121.68, 121.80, 122.77, 123.49, 127.89, 128.30, 133.27, 134.88. MS (GC/EI): 199 [M^+]. HRMS: calcd for $C_{14}H_{17}N$ 199.136099, found 199.1368.

5-Methyl-7-(3-methylbut-2-ene)-1*H*-indole (16). Yield: 43%. ^{1}H NMR ($CDCl_3$): δ 1.78 (3H, s), 1.81 (3H, s), 2.42 (3H, s), 3.53 (2H, d, J = 7.2 Hz), 5.40 (1H, m), 6.46 (1H, m), 6.82 (1H, s), 7.12 (1H, m), 7.29 (1H, s), 8.02 (1H, br s). ^{13}C NMR ($CDCl_3$): δ 18.18, 21.64, 25.95, 31.04, 102.62, 118.48, 122.58, 123.48, 123.76, 124.11, 128.32, 129.39, 133.35, 133.62. MS (GC/EI): 199 [M^+]. HRMS: calcd for $C_{14}H_{17}N$ 199.136099, found 199.1363.

6-Methyl-7-(3-methylbut-2-ene)-1*H*-indole (17). Yield: 60%. ^{1}H NMR ($CDCl_3$): δ 1.73 (3H, s), 1.86 (3H, s), 2.41 (3H, s), 3.56 (2H, d, J = 6.6 Hz), 5.24 (1H, m), 6.49 (1H, m), 6.94 (1H, d, J = 8.1 Hz), 7.13 (1H, t, J = 3 Hz), 7.39 (1H, d, J = 8.1 Hz), 8.07 (1H, br s). ^{13}C NMR ($CDCl_3$): δ 18.46, 19.56, 26.04, 27.77, 103.02, 118.38, 121.95, 122.65, 123.17, 123.66, 126.40, 129.02, 132.68, 136.07. MS (GC/EI): 199 [M^+]. HRMS: calcd for $C_{14}H_{17}N$ 199.136099, found 199.1365.

2,5-Dichloro-3-[2-(1,1-dimethylallyl)-1-methyl-1*H*-indol-3-yl]-[1,4]benzoquinone (24{*1*}). This compound was synthesized analogously to compound **5**. Yield: 71%. Mp: 162−163 °C. ^{1}H NMR (acetone-d_6): δ 7.42 (1H, s), 7.39 (1H, d, J = 8.1 Hz), 7.28−6.98 (3H, m), 6.16 (1H, dd, J = 17.6, 10.5 Hz), 5.14−5.03 (2H, m), 3.86 (3H, s), 1.48 and 1.46 (6H, s). ^{13}C NMR (acetone-d_6): δ 178.0, 177.7,

146.7, 143.9, 143.2, 142.9, 142.2, 138.2, 134.0, 126.2, 122.2, 120.0, 118.7, 112.7, 109.6, 104.2, 40.2, 33.0, 28.3, 27.6. IR (KBr): 3325, 3053, 2969, 1673, 1590, 1472, 1438, 1353, 1069 cm^{-1}. Anal. Calcd for $C_{20}H_{17}Cl_2NO_2$: C, 64.18; H, 4.58; N, 3.74. Found: C, 64.08; H, 4.79; N, 3.53.

2,5-Dichloro-3-[2-(1,1-dimethylallyl)-7-methyl-1*H*-indol-3-yl]-[1,4]benzoquinone (24{*2*}). This compound was synthesized analogously to compound **5**. Yield: 42%. Mp: 181−182 °C. ^{1}H NMR (acetone-d_6): δ 10.06 (1H, s), 7.41 (1H, s), 7.08−6.86 (3H, m), 6.12−6.03 (1H, m), 5.06−5.00 (2H, m), 2.49 (3H, s), 1.50 (6H, s). ^{13}C NMR (acetone-d_6): δ 177.8, 177.7, 145.8, 144.1, 143.2, 142.7, 141.5, 135.3, 133.6, 126.7, 122.7, 120.8, 120.0, 116.5, 112.5, 103.6, 39.6, 28.2, 26.2, 16.7. IR (KBr): 3428, 2971, 1679, 1620, 1584, 1470, 1437, 1271, 1044 cm^{-1}. Anal. Calcd for $C_{20}H_{17}Cl_2NO_2$: C, 64.18; H, 4.58; N, 3.74. Found: C, 64.39; H, 4.73; N, 3.73.

2,5-Dichloro-3-[2-(1,1-dimethylallyl)-6-methyl-1*H*-indol-3-yl]-[1,4]benzoquinone (24{*3*}). This compound was synthesized analogously to compound **5** except that a mixture of THF/acetic acid (1:2) was used as the solvent. Yield: 64%. Mp: 183−184 °C. ^{1}H NMR (acetone-d_6): δ 10.28 (1H, s), 7.40 (1H, s), 7.18 (1H, s), 6.10 (1H, t, J = 8.4 Hz), 6.82 (1H, d, J = 8.1 Hz), 6.04 (1H, dd, J = 17.8, 10.2 Hz), 5.06−5.00 (2H, m), 2.38 (3H, s), 1.46 (6H, s). ^{13}C NMR (acetone-d_6): δ 177.8, 177.7, 145.7, 144.1, 142.6, 141.5, 136.3, 133.6, 131.3, 125.0, 121.4, 118.6, 112.4, 111.2, 102.9, 39.4, 28.1, 26.3, 21.2. IR (KBr): 3426, 3082, 2967, 1679, 1660, 1582, 1470, 1455, 1272, 1028 cm^{-1}. Anal. Calcd for $C_{20}H_{17}Cl_2NO_2$: C, 64.18; H, 4.58; N, 3.74. Found: C, 64.53; H, 4.82; N, 3.77.

2,5-Dichloro-3-[2-(1,1-dimethylallyl)-5-methyl-1*H*-indol-3-yl]-[1,4]benzoquinone (24{*4*}). This compound was synthesized analogously to compound **5**. Yield: 76%. Mp: 216−217 °C. ^{1}H NMR (acetone-d_6): δ 10.31 (1H, s), 7.41 (1H, s), 7.25 (1H, d, J = 8.1 Hz), 7.02 (1H, t, s), 6.94−6.91 (1H, m), 6.05 (1H, dd, J = 17.7, 10.5 Hz), 5.07−5.01 (2H, m), 2.31 (3H, s), 1.46 (6H, s). ^{13}C NMR (acetone-d_6): δ 177.9, 177.8, 145.7, 144.1, 143.2, 142.7, 141.6, 134.2, 133.6, 128.6, 127.4, 123.3, 118.4, 112.4, 110.0, 102.6, 39.4, 28.0, 26.3, 21.1. IR (KBr): 3416, 3055, 2969, 1677, 1620, 1581, 1471, 1438, 1267, 1027 cm^{-1}. Anal. Calcd for $C_{20}H_{17}Cl_2NO_2$: C, 64.18; H, 4.58; N, 3.74. Found: C, 64.58; H, 4.66; N, 3.76.

2,5-Dichloro-3-[2-(1,1-dimethylallyl)-4-methyl-1*H*-indol-3-yl]-[1,4]benzoquinone (24{*5*}). This compound was synthesized analogously to compound **5** except that a mixture of THF/acetic acid (1:2) was used as the solvent. Yield: 45%. Mp: 217−218 °C. ^{1}H NMR (acetone-d_6): δ 10.41 (1H, s), 7.46 (1H, s), 7.23 (1H, d, J = 7.6 Hz), 6.98 (1H, t, J = 7.5 Hz), 6.76 (1H, d, J = 7.6 Hz), 6.00 (1H, dd, J = 17.7, 10.5 Hz), 5.08−5.00 (2H, m), 2.22 (3H, s), 1.46 (6H, s). ^{13}C NMR (acetone-d_6): δ 178.0, 177.5, 145.7, 144.9, 144.3, 142.7, 141.8, 136.0, 133.9, 128.9, 126.3, 121.8, 121.5, 112.2, 109.4, 102.9, 39.4, 28.6, 27.4, 19.0. IR (KBr): 3407, 3056, 2960, 1681, 1622, 1587, 1456, 1438, 1267, 1028 cm^{-1}. Anal. Calcd for $C_{20}H_{17}Cl_2NO_2$: C, 64.18; H, 4.58; N, 3.74. Found: C, 64.29; H, 4.70; N, 3.80.

2,5-Dichloro-3-[2-(1,1-dimethylallyl-1-methyl)-1*H*-indol-3-yl]-6-[7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (25{*1*}). This compound was synthesized analogously to **6**. Yield: 68%. Mp: 112−113 °C. ^{1}H NMR (acetone-d_6): δ 10.92 (1H, s), 7.75−6.90 (8H, m), 6.22 (1H, dd, J = 17.6, 10.5 Hz), 5.52−5.46 (1H, m), 5.18−5.08 (2H, m), 3.87 (3H, s), 3.68 (2H, d, J = 6.9 Hz), 1.78 (3H, s), 1.77 (3H, s), 1.54 (3H, s), 1.51 (3H, s). ^{13}C NMR (acetone-d_6): δ 178.4, 177.8, 146.8, 142.9, 142.8, 142.1, 139.4, 138.2, 136.9, 135.3, 133.1, 130.4, 130.2, 126.3, 126.0, 125.4, 122.1, 121.6, 120.6, 119.9, 119.7, 118.7, 112.6, 109.6, 107.4, 104.7, 40.3, 33.0, 29.7, 28.2, 27.9, 25.4, 17.5. IR (KBr): 3402, 1685, 1670, 1654, 1560, 1508, 1473, 1458, 1210, 737 cm^{-1}. HRMS: calcd for $C_{33}H_{30}Cl_2N_2O_2$ 556.1684, found 556.1684.

2,5-Dichloro-3-[2-(1,1-dimethylallyl-7-methyl)-1*H*-indol-3-yl]-6-[7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (25{*2*}). This compound was synthesized analogously to **6**. Yield: 39%. Mp: 111−112 °C. ^{1}H NMR (acetone-d_6): δ 10.90 (1H, s), 10.07 (1H, s), 7.74 and 7.73 (1H, s), 7.32−6.89 (6H, m), 6.16 (1H, dd, J = 17.6, 10.5 Hz), 5.52−5.47 (1H, m), 5.14−5.08 (2H, m), 3.68 (2H, d, J = 7.2 Hz), 2.51 (3H, s), 1.79 (3H, s), 1.77 (3H, s), 1.55 (3H, s), 1.54 (3H, s). ^{13}C NMR (acetone-d_6): δ 178.3, 177.9, 145.8, 143.1, 142.5, 141.5, 139.0, 137.3, 135.3, 135.2, 130.1, 126.9, 126.1, 125.4, 122.6, 122.1, 121.6, 120.8, 120.6, 120.0, 119.5, 116.6, 112.4, 110.9, 107.3, 104.1, 39.6, 29.7, 28.2, 26.3, 25.4, 17.5, 16.7. IR (KBr): 3409, 2968, 1670, 1655, 1450, 1323, 1255, 1124, 1042, 919 cm^{-1}. HRMS: calcd for $C_{33}H_{30}Cl_2N_2O_2$ 556.1684, found 556.1684.

2,5-Dichloro-3-[2-(1,1-dimethylallyl-6-methyl)-1*H*-indol-3-yl]-6-[7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (25{*3*}). This compound was synthesized analogously to **6**. Yield: 71%. Mp: 108−109 °C. ^{1}H NMR (acetone-d_6): δ 10.88 (1H, s), 10.28 (1H, s), 7.74 (1H, s), 7.32−6.83 (6H, m), 6.13 (1H, dd, J = 17.6, 10.5 Hz), 5.49 (1H, t, J = 6.9 Hz), 5.14−5.08 (2H, m), 3.68 (2H, d, J = 6.9 Hz), 2.39 (3H, s), 1.79 (3H, s), 1.77 (3H, s), 1.51 (6H, s). ^{13}C NMR (acetone-d_6): δ 178.3, 177.9, 145.8, 142.5, 141.4, 139.0, 137.3, 136.3, 135.2, 133.1, 131.2, 130.1, 126.1, 125.4, 125.2, 122.1, 121.6, 121.3, 120.6, 119.6, 118.7, 112.3, 111.2, 107.3, 103.1, 39.5, 29.8, 28.1, 26.4, 25.4, 21.3, 17.5. IR (KBr): 3409, 2969, 1671, 1570, 1431, 1414, 1245, 1184, 1121, 1035 cm^{-1}. HRMS: calcd for $C_{33}H_{30}Cl_2N_2O_2$ 556.1684, found 556.1689.

2,5-Dichloro-3-[2-(1,1-dimethylallyl-5-methyl)-1*H*-indol-3-yl]-6-[7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (25{*4*}). This compound was synthesized analogously to **6**. Yield: 75%. Mp: 143−144 °C. ^{1}H NMR (acetone-d_6): δ 10.86 (1H, s), 10.29 (1H, s), 7.73 (1H, s), 7.33−6.92 (6H, m), 6.13 (1H, dd, J = 17.6, 10.5 Hz), 5.52−5.47 (1H, m), 5.14−5.07 (2H, m), 3.68 (2H, d, J = 7.2 Hz), 2.34 (3H, s), 1.78 (3H, s), 1.77 (3H, s), 1.51 (3H, s), 1.50 (3H, s). ^{13}C NMR (acetone-d_6): δ 178.3, 177.9, 145.7, 143.2, 142.5, 141.6, 139.0, 137.3, 136.0, 135.3, 133.1, 130.1, 128.6, 127.6, 126.1, 125.4, 123.3, 122.1, 121.6, 120.6, 119.6, 118.5, 112.3, 111.0, 107.3, 103.1, 39.5, 29.9, 28.0, 26.5, 25.4, 21.2, 17.5. IR (KBr): 3409, 2970, 1670, 1571, 1432, 1302, 1246, 1195, 1118 cm^{-1}. Anal. Calcd for $C_{33}H_{30}Cl_2N_2O_2$: C, 71.09; H, 5.42; N, 5.02. Found: C, 71.04; H, 5.74; N, 4.84.

2,5-Dichloro-3-[2-(1,1-dimethylallyl)-1*H*-indol-3-yl]-6-[1-methyl-7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (25{*6*}). This compound was synthesized analogously to **6**. Yield: 86%. Mp: 121−122 °C. ^{1}H NMR (acetone-d_6): δ 10.45 (1H, s), 7.54 (1H, s), 7.40−6.96 (7H, m), 6.13 (1H, dd, J = 17.4, 10.5 Hz), 5.37−5.32 (1H, m), 5.15−5.07 (2H, m), 4.16 (3H, s), 3.86 (2H, d, J = 6.3 Hz), 1.78 (3H, s), 1.76 (3H, s), 1.52 (3H, s), 1.51 (3H, s). ^{13}C NMR (acetone-d_6): δ 178.3, 177.9, 145.7, 143.2, 142.4, 141.6, 138.6, 137.1, 135.9, 135.7, 135.2, 132.1, 127.9, 127.3, 126.3, 124.4, 123.9, 121.8, 120.6, 120.2, 119.7, 118.9, 112.4, 111.3, 105.5, 103.5, 39.5, 37.1, 31.3, 28.1, 26.5, 25.3, 17.8. IR (KBr): 3409, 2968, 1670, 1570, 1458, 1449, 1254, 1187, 1108, 1035 cm^{-1}. HRMS: calcd for $C_{33}H_{30}Cl_2N_2O_2$ 556.1684, found 556.1688.

2,5-Dichloro-3-[2-(1,1-dimethylallyl)-1*H*-indol-3-yl]-6-[2-methyl-7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (25{*7*}). This compound was synthesized analogously to **6**. Yield: 59%. Mp: 115−116 °C. ^{1}H NMR (acetone-d_6): δ 10.52 (1H, s), 10.47 (1H, s), 7.41−6.93 (7H, m), 6.21−6.09 (1H, m), 5.52−5.46 (1H, m), 5.18−5.08 (2H, m), 3.62 (2H, d, J = 7.2 Hz), 2.42 and 2.40 (3H, s), 1.76 (6H, s), 1.54 (3H, s), 1.52 (3H, s). ^{13}C NMR (acetone-d_6): δ 178.4, 177.2, 145.8, 143.1, 142.3, 141.9, 140.4, 140.0, 137.2, 136.8, 135.9, 134.9, 132.8, 127.2, 124.4, 124.3, 122.2, 121.8, 120.8, 120.3, 119.1, 118.0, 117.4, 112.4, 111.3, 103.5, 39.5, 29.5, 28.1, 26.4, 25.4, 17.5, 13.2. IR (KBr): 3409, 3330, 2970, 1654, 1635, 1618, 1473, 1341, 987, 743 cm^{-1}. HRMS: calcd for $C_{33}H_{30}Cl_2N_2O_2$ 556.1684, found 556.1680.

Representative Procedure for Hydrolysis of 2-[2-(1,1-Dimethylallyl)-1*H*-indol-3-yl]-3,6-dihydroxy-5-[7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone To Give DAQ B1. To a refluxing solution of **6** (50 mg, 0.092 mmol) in methanol (6 mL) was added an aqueous sodium hydroxide solution (10%, 3 mL) over 3 min. The reaction mixture was stirred under reflux for 30 min, diluted with water

(20 mL), neutralized with sulfuric acid (10% aq, about 3 mL) to pH 2, extracted with ethyl acetate (20 mL × 2), washed with brine (20 mL), dried with sodium sulfate, and purified by flash chromatography (oxalic acid-coated silica gel, ethyl acetate:hexanes 3:7). The title compound was obtained as a red solid (28 mg, 60%). ^{1}H NMR (acetone-d_6): δ 10.43 (1H, s), 10.07 (1H, s), 9.34 (2H, br s), 7.64−6.92 (8H, m), 6.18 (1H, dd, J = 17.4, 10.8 Hz), 5.50−5.46 (1H, m), 5.10 (1H, d, J = 17.4 Hz), 5.01 (1H, d, J = 10.8 Hz), 3.65 (2H, d, J = 6.9 Hz), 1.78 (3H, s), 1.76 (3H, s), 1.52 (6H, s). ^{13}C NMR (acetone-d_6): δ 146.0, 142.6, 135.8, 135.2, 132.7, 129.2, 127.4, 127.0, 124.5, 122.4, 121.2, 120.9, 119.9, 119.5, 119.0, 118.9, 112.5, 111.6, 110.9, 110.8, 105.3, 100.8, 39.4, 27.0, 25.4, 17.5.

2-[2-(1,1-Dimethylallyl)-1-methyl-1*H*-indol-3-yl]-3,6-dihydroxy-5-[7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (26{*1*}). Yield: 47%. Mp: 114−115 °C. ^{1}H NMR (acetone-d_6): δ 10.47 (1H, s), 9.49 (2H, s), 7.64−6.96 (8H, m), 6.24 (1H, dd, J = 17.6, 10.5 Hz), 5.48 (1H, t, J = 6.9 Hz), 5.16−5.10 (2H, m), 3.84 (3H, s), 3.65 (2H, d, J = 6.9 Hz), 1.78 (3H, s), 1.76 (3H, s), 1.56 (6H, s). ^{13}C NMR (acetone-d_6): δ 147.3, 142.6, 138.1, 135.2, 132.7, 128.2, 127.4, 127.3, 127.0, 124.5, 122.4, 121.6, 120.9, 120.0, 119.5, 119.2, 118.9, 113.5, 111.8, 109.0, 105.4, 102.1, 40.3, 32.8, 28.0, 25.4, 17.4. IR (KBr): 3402, 1685, 1670, 1654, 1560, 1508, 1473, 1458, 1210, 737 cm^{-1}. HRMS: calcd for $C_{33}H_{32}N_2O_2$ 520.2362, found 520.2366.

2-[2-(1,1-Dimethylallyl)-7-methyl-1*H*-indol-3-yl]-3,6-dihydroxy-5-[7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (26{*2*}). Yield: 45%. Mp: 135−136 °C. ^{1}H NMR (acetone-d_6): δ 10.46 (1H, s), 9.70 (1H, s), 9.41 (1H, s), 7.64−6.84 (7H, m), 6.20 (1H, dd, J = 17.4, 10.5 Hz), 5.48 (1H, d, J = 7.2 Hz), 5.11−4.97 (2H, m), 3.65 (2H, d, J = 6.9 Hz), 2.48 (3H, s), 1.78 (3H, s), 1.76 (3H, s), 1.54 (6H, s). ^{13}C NMR (acetone-d_6): δ 146.1, 142.5, 135.2, 132.7, 128.8, 127.7, 127.3, 127.0, 124.5, 122.4, 122.1, 120.9, 120.2, 119.8, 119.5, 119.3, 116.7, 112.7, 111.2, 110.8, 105.4, 101.5, 39.5, 29.8, 27.0, 25.4, 17.5, 16.6. IR (KBr): 3378, 3338, 2969, 1693, 1637, 1533, 1438, 1340, 1282, 1261 cm^{-1}. HRMS: calcd for $C_{33}H_{32}N_2O_2$ 520.2362, found 520.2358.

2-[2-(1,1-Dimethylallyl)-6-methyl-1*H*-indol-3-yl]-3,6-dihydroxy-5-[7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (26{*3*}). Yield: 55%. Mp: 141−142 °C. ^{1}H NMR (acetone-d_6): δ 10.46 (1H, s), 9.93 (1H, s), 9.40 (1H, s), 7.63−6.78 (7H, m), 6.16 (1H, dd, J = 17.6, 10.5 Hz), 5.52−5.46 (1H, m), 5.13−4.98 (2H, m), 3.65 (2H, d, J = 7.2 Hz), 2.38 (3H, s), 1.79 (3H, s), 1.76 (3H, s), 1.51 (6H, s). ^{13}C NMR (acetone-d_6): δ 146.1, 141.9, 136.2, 135.2, 132.7, 130.5, 127.3, 127.1, 127.0, 124.5, 122.4, 120.9, 120.7, 119.9, 119.5, 118.7, 112.7, 111.6, 110.8, 105.4, 100.6, 39.4, 29.8, 27.0, 25.4, 21.3, 17.5. IR (KBr): 3402, 3337, 2969, 2915, 1700, 1636, 1458, 1437, 1340, 1282 cm^{-1}. HRMS: calcd for $C_{33}H_{32}N_2O_2$ 520.2362, found 520.2352.

2-[2-(1,1-Dimethylallyl)-5-methyl-1*H*-indol-3-yl]-3,6-dihydroxy-5-[7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (26{*4*}). Yield: 68%. Mp: 109−110 °C. ^{1}H NMR (acetone-d_6): δ 10.43 (1H, s), 9.94 (1H, s), 9.36 (1H, s), 7.63−6.87 (7H, m), 6.16 (1H, dd, J = 17.4, 10.5 Hz), 5.48 (1H, t, J = 6.9 Hz), 5.12−4.98 (2H, m), 3.65 (2H, d, J = 6.9 Hz), 2.33 (3H, s), 1.78 (3H, s), 1.76 (3H, s), 1.51 (6H, s). ^{13}C NMR (acetone-d_6): δ 146.1, 142.7, 135.2, 134.1, 132.7, 129.5, 127.8, 127.4, 127.0, 124.5, 123.0, 122.7, 122.4, 120.9, 119.9, 119.5, 118.6, 110.8, 110.6, 105.4, 103.6, 100.3, 39.4, 29.8, 27.0, 25.4, 21.1, 17.5. IR (KBr): 3413, 3339, 2967, 2915, 1653, 1636, 1437, 1341, 1304, 1244 cm^{-1}. HRMS: calcd for $C_{33}H_{32}N_2O_2$ 520.2362, found 520.2366.

2-[2-(1,1-Dimethylallyl)-4-methyl-1*H*-indol-3-yl]-3,6-dihydroxy-5-[7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (26{*5*}). This compound was synthesized analogously to DAQ B1**,** except that **25**{*5*} was hydrolyzed immediately after preparation, without isolation/characterization, after most of the solvent had been removed in vacuo. Yield: 9% for two steps. Mp: 105−106 °C. ^{1}H NMR (acetone-d_6): δ 10.43 (1H, s), 9.90 (1H, s), 9.38 (2H, s), 7.63−6.78 (7H, m), 6.16 (1H, dd, J = 13.2, 7.8 Hz), 5.48 (1H, t, J = 5.4 Hz), 5.11−4.98 (2H, m), 3.65 (2H, d, J = 5.4 Hz), 2.38 (3H, s), 1.78 (3H, s), 1.76 (3H, s), 1.50 (6H, s). ^{13}C NMR (acetone-d_6): δ 146.2, 141.9, 136.2, 135.2, 132.7, 130.5, 127.7, 127.3, 127.2, 127.0, 124.5, 122.4, 120.9, 120.7, 119.9, 119.5, 118.7, 118.4, 111.1, 110.8, 105.4, 100.6, 39.3, 26.8, 25.2, 21.1, 17.3. IR (KBr): 3410, 3331, 2964, 2923, 1636, 1437, 1341, 1304, 1243, 1127 cm^{-1}. HRMS: calcd for $C_{33}H_{32}N_2O_2$ 520.2362, found 520.2355.

2-[2-(1,1-Dimethylallyl)-1*H*-indol-3-yl]-3,6-dihydroxy-5-[1-methyl-7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (26{*6*}). This compound was synthesized analogously to **26**{**8**}. Yield: 70%. Mp: 109−110 °C. ^{1}H NMR (acetone-d_6): δ 10.10 (1H, s), 9.43 (2H, s), 7.42−6.92 (8H, m), 6.17 (1H, dd, J = 17.2, 10.5 Hz), 5.36 (1H, t, J = 6.3 Hz), 5.13−4.98 (2H, m), 4.12 (1H, s), 3.85 (2H, d, J = 6.3 Hz), 1.78 (3H, s), 1.76 (3H, s), 1.52 (6H, s). ^{13}C NMR (acetone-d_6): δ 146.0, 142.6, 135.8, 135.1, 133.3, 131.7, 129.2, 129.0, 125.4, 124.8, 123.2, 121.2, 120.4, 119.5, 119.0, 118.9, 112.6, 111.3, 110.9, 110.8, 103.6, 100.8, 39.4, 36.6, 31.5, 27.0, 25.3, 17.7. IR (KBr): 3419, 3322, 2968, 2927, 1636, 1541, 1457, 1340, 1245, 990 cm^{-1}. HRMS: calcd for $C_{33}H_{32}N_2O_2$ 520.2362, found 520.2357.

2-[2-(1,1-Dimethylallyl)-1*H*-indol-3-yl]-3,6-dihydroxy-5-[2-methyl-7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (26{*7*}). Yield: 82%. Mp: 214−215 °C. ^{1}H NMR (acetone-d_6): δ 10.12 (1H, s), 10.09 (1H, s), 7.37−6.86 (7H, m), 6.18 (1H, dd, J = 17.8, 9.3 Hz), 5.51−5.46 (1H, m), 5.14−4.99 (2H, m), 3.59 (2H, d, J = 6.9 Hz), 2.83 and 2.37 (1H, s), 1.76 (6H, s), 1.52 (6H, s). ^{13}C NMR (acetone-d_6): δ 169.6, 168.5, 146.1, 142.6, 135.8, 135.2, 134.8, 132.4, 129.2, 128.6, 123.7, 122.6, 121.1, 120.0, 119.3, 119.0, 118.0, 117.6, 112.6, 110.8, 103.1, 101.0, 39.4, 27.0, 25.4, 17.4, 12.9. IR (KBr): 3402, 3330, 2970, 1701, 1654, 1637, 1560, 1459, 1340, 1283 cm^{-1}. HRMS: calcd for $C_{33}H_{32}N_2O_2$ 520.2362, found 520.2350.

2-[2-(1,1-Dimethylallyl)-1*H*-indol-3-yl]-3,6-dihydroxy-5-[4-methyl-7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (26{*8*}). This compound was synthesized analogously to **26**{*7*}**,** except that for the hydrolysis, the sodium hydroxide solution was added over 30 min via a syringe pump. Yield: 15% for two steps. Mp: 75−76 °C. ^{1}H NMR (acetone-d_6): δ 10.26 (1H, s), 10.14 and 10.09 (1H, s), 9.43 (2H, s), 7.35−6.73 (7H, m), 6.17 (1H, dd, J = 13.0, 8.1 Hz), 5.49−5.45 (1H, m), 5.13−4.96 (2H, m), 3.60 (2H, d, J = 5.4 Hz), 2.46 and 2.43 (3H, s), 1.78 (3H, s), 1.76 (3H, s), 1.53 (6H, s). ^{13}C NMR (acetone-d_6): δ 146.0, 145.9, 145.8, 142.6, 135.8, 135.6, 132.5, 129.1, 127.8, 125.4, 122.6, 122.4, 121.5, 121.2, 120.9, 119.5, 119.0, 118.6, 111.4, 111.0, 105.2, 100.8, 39.4, 27.0, 26.7, 25.4, 17.4. IR (KBr): 3402, 2923, 2852, 1654, 1608, 1458, 1274, 934, 914, 805 cm^{-1}. HRMS: calcd for $C_{33}H_{32}N_2O_2$ 520.2362, found 520.2368.

2-[2-(1,1-Dimethylallyl)-1*H*-indol-3-yl]-3,6-dihydroxy-5-[5-methyl-7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (26{*9*}). This compound was synthesized analogously to **26**{**8**}. Yield: 12% for two steps. Mp: 129−130 °C. ^{1}H NMR (acetone-d_6): δ 10.34 (1H, s), 10.09 (1H, s), 9.40 (2H, s), 7.58−6.82 (7H, m), 6.18 (1H, dd, J = 17.4, 10.5 Hz), 5.49−5.44 (1H, m), 5.14−4.99 (2H, m), 3.61 (2H, d, J = 7.2 Hz), 2.38 (3H, s), 1.78 (3H, s), 1.76 (3H, s), 1.53 (6H, s). ^{13}C NMR (acetone-d_6): δ 146.0, 142.6, 135.8, 133.6, 132.5, 129.2, 128.2, 127.4, 127.3, 124.2, 122.6, 122.5, 121.2, 119.6, 119.0, 112.4, 111.8, 111.2, 110.9, 110.8, 104.9, 100.8, 39.4, 29.8, 27.0, 25.4, 21.4, 17.5. IR (KBr): 3413, 2966, 2922, 1653, 1636, 1458, 1341, 1302, 990, 918 cm^{-1}. HRMS: calcd for $C_{33}H_{32}N_2O_2$ 520.2362, found 520.2365.

2-[2-(1,1-Dimethylallyl)-1*H*-indol-3-yl]-3,6-dihydroxy-5-[6-methyl-7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (26-{*10*}). This compound was synthesized analogously to **26**{**8**}. Yield: 14% for two steps. Mp: 179−180 °C. ^{1}H NMR (acetone-d_6): δ 10.32 (1H, s), 10.09 (1H, s), 9.40 (2H, s), 7.59−6.88 (7H, m), 6.12−6.12 (1H, m), 5.19−4.98 (3H, m), 3.64 (2H, d, J = 6.6 Hz), 2.38 (3H, s), 1.84 (3H, s), 1.69 (3H, s), 1.52 (6H, s). ^{13}C NMR (acetone-d_6): δ 146.0, 142.6, 135.9, 135.8, 131.4, 129.2, 128.4, 127.1, 125.5, 122.8, 122.6, 122.3, 121.2, 119.6, 119.0, 118.9, 112.4, 111.8, 110.9, 110.8, 105.3, 100.8, 39.4, 27.1, 27.0, 25.3, 18.6, 17.6. IR (KBr): 3421, 3341, 2968, 1654, 1636, 1617, 1458, 1438, 1340, 1283 cm^{-1}. HRMS: calcd for $C_{33}H_{32}N_2O_2$ 520.2362, found 520.2371.

2-[2-(1,1-Dimethylallyl)-1*H*-indol-3-yl]-3-hydroxy-6-methoxy-5-[7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (26{*11*}). A mixture of DAQ B1 (50 mg, 0.099 mmol), acetic anhydride (0.50 mL, 0.54 g, 5.5 mmol), and pyridine (1 mL, 0.97 g, 12 mmol) was stirred at room temperature for 2 h. The reaction mixture was diluted with 50 mL of ethyl acetate, washed with aqueous sulfuric acid (0.1%, 50 mL × 3) and brine (50 mL), and dried with sodium sulfate. The solvent was removed in vacuo to leave a red solid (**27**), which was dissolved in methanol (5 mL) and stirred with potassium carbonate at room temperature. After 1 h, the potassium carbonate was removed by filtration, and to the reaction mixture were added methanol (20 mL) and mineral acid (aqueous 1 N HCl, 50 mL). After being heated at 50 °C for 4 h, the reaction mixture was extracted with ethyl acetate, washed with water (50 mL) and brine (50 mL), dried with sodium sulfate, and purified with flash chromatography (silica gel, ethyl acetate:hexanes 1:2). The title compound was obtained as a red solid (37%, 19 mg). Mp: 104−105 °C. ^{1}H NMR (acetone-d_6): δ 10.54 (1H, s), 10.08 (1H, s), 9.03 (2H, s), 7.60−6.91 (8H, m), 6.19 (1H, dd, J = 17.6, 10.8 Hz), 5.52−5.46 (1H, m), 5.16−5.01 (2H, m), 3.85 (3H, s), 3.66 (2H, d, J = 7.2 Hz), 1.79 (3H, s), 1.77 (3H, s), 1.53 (6H, s). ^{13}C NMR (acetone-d_6): δ 183.7, 183.5, 156.0, 153.1, 146.2, 142.7, 135.8, 135.2, 132.8, 129.2, 127.9, 127.3, 124.8, 122.3, 121.1, 120.8, 120.0, 119.0, 118.9, 115.0, 110.8, 105.3, 101.4, 60.6, 39.4, 27.4, 26.7, 25.4, 17.5. IR (KBr): 3401, 2968, 1654, 1638, 1458, 1338, 1302, 1268, 1207, 1067 cm^{-1}. HRMS: calcd for $C_{33}H_{32}N_2O_2$ 520.2362, found 520.2365.

2-[2-(1,1-Dimethylallyl)-1*H*-indol-3-yl]-6-hydroxy-3-methoxy-5-[7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (26{*12*}). First Method: 2-Chloro-3-[2-(1,1-dimethylallyl)-1*H*-indol-3-yl]-5-methoxy-6-[7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (29). A mixture of compound **6** (100 mg, 0.18 mmol) and anhydrous potassium carbonate (30 mg, 0.21 mmol) in methanol (8 mL) was heated at reflux for 30 min. The mixture was cooled to room temperature and filtered. The filtrate was collected and purified by silica chromatography (hexane:ethyl acetate 3:1) to give the wrong regioisomer (7 mg), asterriquinone B1 (10 mg), and the desired product **29** (33 mg, 33% yield). Mp: 140−142 °C. IR (KBr): 3458, 2968, 1661, 1588, 1434, 1258, 745. ^{1}H NMR (acetone-d_6): δ 10.68 (1H, br s), 10.40 (1H, br s), 7.66 (1H, d, J = 2.7 Hz), 7.40−7.34 (2H, m), 7.28 (1H, d, J = 7.2 Hz), 7.13−6.96 (4H, m), 6.16 (1H, dd, J = 17.7, 7.8 Hz), 5.49 (1H, m), 5.20−5.10 (2H, m), 3.79 (3H, s), 3.67 (2H, d, J = 7.2 Hz), 1.79 (3H, s), 1.77 (3H, s), 1.54 (3H, s), 1.53 (3H, s).^{13}C NMR (acetone-d_6): δ 181.3, 180.0, 145.7, 145.5, 142.9, 141.5, 140.8, 135.9, 135.1, 132.9, 128.8, 127.5, 127.1, 125.0, 124.9, 122.2, 121.6, 121.2, 120.6, 119.5, 119.1, 118.8, 112.0, 111.2, 107.2, 105.8, 60.2, 39.5, 28.0, 26.5, 25.4, 17.5. HRMS−FAB (*m*/*z*): [M$^+$] calcd for $C_{33}H_{31}ClN_2O_3$, 538.2023; found, 538.2023.

2-Chloro-3-[2-(1,1-dimethylallyl)-1*H*-indol-3-yl]-5-hydroxy-6-[7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone. A mixture of compound **29** (23 mg, 0.043 mmol) in 2-propanol (2 mL) and 1 M $NaHCO_3$ aqueous solution (1 mL) was refluxed for 4 h. The reaction mixture was poured into 1 N HCl solution (5 mL) and extracted with ethyl acetate (5 mL × 3). The organic layers were combined, washed with brine, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the crude product was purified by silica chromatography (hexane:ethyl acetate 3:1) to give the title product (19 mg, 84% yield). Mp: 124−125 °C. IR (KBr): 3430, 1654, 1559, 1508, 1264, 1106, 741 cm^{-1}. ^{1}H NMR (acetone-d_6): δ 10.55 (1H, br s), 10.39 (1H, br s), 9.40−9.40 (1H, br), 7.66 (1H, d, J = 2.7 Hz), 7.43 (1H, d, J = 7.2 Hz), 7.39 (1H, d, J = 7.8 Hz), 7.32 (1H, d, J = 8.1 Hz), 7.14−6.98 (4H, m), 6.14 (1H, dd, J = 17.7, 7.8 Hz), 5.49 (1H, m), 5.15−5.07 (2H, m), 3.66 (2H, d, J = 7.2 Hz), 1.79 (3H, s), 1.77 (3H, s), 1.53 (6H, s). ^{13}C NMR (acetone-d_6): δ 181.7, 179.3, 151.2, 145.6, 143.4, 142.8, 138.8, 135.9, 135.2, 132.7, 128.2, 127.4, 127.0, 124.6, 122.4, 121.7, 121.0, 120.0, 119.7, 119.6, 118.8, 116.0, 112.1, 111.2, 106.0, 39.5, 27.8, 26.5, 25.4, 17.4. HRMS−FAB (*m*/*z*): [M + 2H]$^+$ calcd for $C_{32}H_{31}ClN_2O_3$, 526.2023; found, 526.2017.

2-[2-(1,1-Dimethylallyl)-1*H*-indol-3-yl]-6-hydroxy-3-methoxy-5-[7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (26{*12*}). A mixture of 2-chloro-3-[2-(1,1-dimethylallyl)-1*H*-indol-3-yl]-5-hydroxy-6-[7-(3-methyl-but-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (19 mg, 0.036 mmol) in methanol (6 mL) and K_2CO_3 (30 mg, 0.21 mmol) was refluxed for 4 h. The reaction mixture was poured into 1 N H_2SO_4 solution and extracted with ethyl acetate (5 mL × 3). The organic layers were combined, washed with brine, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure, and the crude product was purified by silica chromatography (hexane:ethyl acetate 3:1) to give the title compound (17 mg, 90% yield). Mp: 113−115 °C. IR (KBr): 3352, 2978, 1655, 1648, 1637, 1272, 1015 cm^{-1}. ^{1}H NMR (acetone-d_6): δ 10.47 (1H, br s), 10.21 (1H, br s), 9.07 (1H, s), 7.64 (1H, d, J = 2.7 Hz), 7.43 (1H, d, J = 7.2 Hz), 7.35 (2H, m), 7.11−6.98 (4H, m), 6.16 (1H, dd, J = 17.7, 7.8 Hz), 5.49 (1H, m), 5.13−5.01 (2H, m), 3.79 (3H, s), 3.65 (2H, d, J = 7.2 Hz), 1.79 (3H, s), 1.76 (3H, s), 1.53 (6H, s). ^{13}C NMR (acetone-d_6): δ 184.1, 182.5, 157.9, 150.7, 145.8, 142.6, 135.6, 135.1, 132.7, 129.8, 127.7, 127.1, 124.5, 122.4, 121.4, 120.8, 120.0, 119.5, 119.4, 118.7, 111.3, 111.0, 105.9, 60.0, 39.5, 27.4, 26.7, 25.4, 17.4. HRMS−FAB (*m*/*z*): [M$^+$] calcd for $C_{33}H_{32}N_2O_4$, 520.2362; found, 520.2369.

2-[2-(1,1-Dimethylallyl)-1*H*-indol-3-yl]-6-hydroxy-3-methoxy-5-[7-(3-methylbut-2-enyl)-1*H*-indol-3-yl]-[1,4]benzoquinone (26{*12*}). A mixture of compound **6** (16 mg, 0.029 mmol) and 1.0 N aqueous cesium carbonate (1.0 mL) was stirred in 2.0 mL of methanol under reflux. The reaction was monitored by TLC (silica gel, ethyl acetate:hexanes 1:2) until compound **6** was completely consumed (3 h). The reaction mixture was diluted with ethyl acetate (20 mL), washed with saturated aqueous ammonium chloride (20 mL) and brine (20 mL), dried with sodium sulfate, and purified with flash chromatography (silica gel, ethyl acetate:hexanes 1:5). The title compound was obtained as a purple solid (4 mg, 27%). The ^{1}H NMR (acetone-d_6) spectrum was identical to that of an authentic sample prepared in our laboratory using the first method. HRMS: calcd for $C_{33}H_{32}N_2O_2$ 520.2362, found 520.2365.

[5-(2-Methyl-1*H*-indol-5-ylcarbamoyl)pentyl]carbamic Acid *tert*-Butyl Ester (34). To a solution of 5-amino-2-methylindole (1.46 g, 10.0 mmol) and Boc-aminocaproic acid *N*-hydroxysuccinimide ester (3.28 g, 10.0 mmol) in DMSO (50 mL) was added diisopropylethylamine (2.0 mL, 11 mmol) at room temperature. The mixture was then heated at 65 °C and stirred for 2 d. The solution was concentrated, and the residue was purified by silica gel flash chromatography to give the title compound as a clear oil (3.42 g). ^{1}H NMR ($CDCl_3$): δ 1.44 (13H, m), 1.71 (2H, m), 2.29 (2H, t, J = 7.1 Hz), 2.39 (3H, s), 3.07 (2H, t, J = 7.0 Hz), 6.11 (1H, s), 7.12 (2H, m), 7.64 (1H, s), 8.18 (1H, br s). ^{13}C NMR ($CDCl_3$): δ 13.9, 24.9, 26.3, 28.9, 30.1 33.7, 43.9, 67.9, 101.5, 110.1, 111.6, 112.1, 125.7, 130.7, 133.9, 135.6, 157.4, 173.0. IR (KBr): 3387, 2979, 2859, 1672 cm^{-1}. Anal. Calcd for $C_{20}H_{29}N_3O_3$: C, 66.83; H, 8.13. Found: C, 66.77; H, 8.01.

6-(Biotinylamino)hexanoic Acid (2-Methyl-1*H*-indol-5-yl)amide (35). To a solution of **34** (180 mg, 0.500 mmol) in CH_2Cl_2 (5 mL) at 0 °C was added dropwise trifluoroacetic acid (1 mL). The solution was stirred at the same temperature for 1 h, at which time TLC indicated the disappearance of starting material. After being neutralized with 2 N NaOH, the mixture was extracted with EtOAc. The organic phase was washed with brine and dried with anhydrous sodium sulfate. The solvent was removed to give a light yellow oil (130 mg, ∼100%), which was used in the following step without further purification. The resulting 6-aminohexanoic acid (2-methyl-1*H*-indol-5-yl)amide (88 mg, 0.34 mmol) was dissolved in anhydrous pyridine (2 mL). The solution was cooled to 0 °C, and biotin *p*-nitrophenyl ester (72 mg, 0.20 mmol) was added portion wise. The ice bath was removed, and the solution was stirred overnight. The mixture was then loaded onto a silica gel column and purified by flash chromatography to give the title compound as a light yellow oil (80 mg, 83%). ^{1}H NMR ($CDCl_3$): δ 1.4−1.8 (14H, m), 2.17 (3H, m), 2.36 (2H, t, J = 7.2 Hz), 2.38 (3H, s), 2.67 (1H, d,

J = 13.5 Hz), 2.87 (1H, dd, J = 5.5, 13.5 Hz), 3.1−3.2 (3H, m), 3.3 (1H, m), 4.20 (1H, m), 4.43 (1H, m), 6.06 (1H, s), 7.07 (1H, dd, J = 2.0, 9.0 Hz), 7.18 (1H, d, J = 9.0 Hz), 7.58 (1H, d, J = 2.0 Hz), 7.92 (1H, br s). ^{13}C NMR ($CDCl_3$): δ 12.2, 25.8, 25.7, 26.2, 28.1, 28.5, 28.9, 35.6, 36.4, 38.9, 39.8, 55.7, 60.3, 62.0, 102.9, 111.3, 112.9, 126.7, 131.0, 134.9, 136.6, 164.9, 173.0, 174.9. IR (KBr): 3401, 2991, 2878, 1696 cm^{-1}. HRMS (EI): m/z calcd for $C_{25}H_{35}N_5O_3S$ [M$^+$] 485.2461, found 485.2472.

6-(Biotinylamino)hexanoic Acid {3-[2,5-Dichloro-4-(7-methyl-1*H*-indol-3-yl)-3,6-dioxocyclohexa-1,4-dienyl]-2-methyl-1*H*-indol-5-yl}-amide. 2,5-Dichloro-3-(7-methyl-1*H*-indol-3-yl)-[1,4]benzoquinone (77 mg, 0.25 mmol) and **35** (60 mg, 0.12 mmol) were dissolved in a mixture of acetic acid (1 mL) and THF (1 mL). Zinc triflate monohydrate (197 mg, 0.520 mmol) and silver carbonate on Celite (50%, 138 mg, 0.25 mmol) were added. The mixture was allowed to reflux for 2 h and then cooled to room temperature. After filtration, the solvent was removed and the residue was purified by flash chromatography to give the title compound as a purple solid (80 mg, 82%). Mp: 210 °C dec. ^{1}H NMR ($CDCl_3$): δ 1.2−1.8 (15H, m), 2.25 (3H, m), 2.33 (4H, m), 2.5−2.7 (4H, m), 2.8 (1H, m), 3.05 (1H, m), 3.15 (2H, m), 3.3 (1H, m), 4.1 (1H, m), 4.34 (1H, m), 6.94−7.05 (2H, m), 7.19−7.3 (3H, m), 7.46 (1H, m), 7.64 (1H, s), 7.84 (1H, br s). ^{13}C NMR ($CDCl_3$): δ 12.3, 15.5, 25.6, 25.8, 26.2, 28.5, 28.7, 28.9, 35.1, 36.4, 39.0, 39.9, 55.7, 60.3, 62.2, 101.9, 105.0, 110.3, 111.2, 111.9, 112.0, 115.0, 119.2, 119.4, 120.6, 121.9, 126.6, 127.3, 128.6, 130.4, 136.7, 156.8, 157.9, 161.5, 162.9, 164.9, 173.0, 174.5. IR (KBr): 3357, 2938, 2895, 2820, 1672, 1645, 1565, 1275, 1253 cm^{-1}. HRMS (EI): m/z calcd for $C_{40}H_{42}Cl_2N_6O_5S$ [M$^+$] 788.2314, found 788.2311.

6-(Biotinylamino)hexanoic Acid {3-[2,5-Dihydroxy-4-(7-methyl-1*H*-indol-3-yl)-3,6-dioxocyclohexa-1,4-dienyl]-2-methyl-1*H*-indol-5-yl}amide (32). To a refluxing solution of 6-(biotinylamino)hexanoic acid {3-[2,5-dichloro-4-(7-methyl-1*H*-indol-3-yl)-3,6-dioxocyclohexa-1,4-dienyl]-2-methyl-1*H*-indol-5-yl}amide (75 mg, 0.095 mmol) in MeOH (6 mL) was added aqueous NaOH (10%, 3 mL) dropwise. The mixture was refluxed for 30 min and then poured into ice water (20 mL). H_2SO_4 (10% in water) was added to acidify the mixture, which was then extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. The solution was concentrated and purified by flash column chromatography (oxalic acid-coated silica gel) to afford the title compound as a red solid (32 mg, 45%). Mp: 224−225 °C. ^{1}H NMR ($CDCl_3$): δ 1.2−1.8 (15H, m), 2.11 (2H, t, J = 6.9 Hz), 2.32 (5H, m), 2.52 (4H, m), 2.77 (1H, m), 2.86 (1H, m), 3.16 (2H, m), 3.30 (1H, m), 3.99 (1H, m), 4.26 (1H, m), 6.92−6.96 (2H, m), 7.17 (1H, m), 7.23 (1H, m), 7.38 (1H, m), 7.44 (1H, m), 7.54 (1H, s). ^{13}C NMR ($CDCl_3$): δ 12.2, 15.8, 25.4, 25.7, 26.2, 28.1, 28.5, 28.9, 35.6, 36.4, 38.9, 39.8, 55.7, 60.3, 62.0, 102.0, 104.8, 110.2, 111.1, 111.9, 112.0, 115.0, 119.2, 119.3, 120.5, 121.8, 126.4, 127.2, 128.4, 130.4, 133.5, 135.8, 136.7, 164.9, 173.0, 174.9. IR (KBr): 3326, 2983, 2854, 1629, 1540, 1341 cm^{-1}. HRMS (EI): m/z calcd for $C_{40}H_{44}N_6O_7S$ [M$^+$] 752.2992, found 752.2999.

6-(Biotinylamino)hexanoic Acid (3-{2,5-Dichloro-4-[7-prenyl-1*H*-indol-3-yl]-3,6-dioxocyclohexa-1,4-dienyl}-2-methyl-1*H*-indol-5-yl)-amide. 2,5-Dichloro-3-(7-prenyl-1*H*-indol-3-yl)-[1,4]benzoquinone (90 mg, 0.25 mmol) and **35** (60 mg, 0.12 mmol) were dissolved in a mixture of acetic acid (1 mL) and THF (1 mL). Zinc triflate monohydrate (197 mg, 0.520 mmol) and silver carbonate on Celite (50%, 138 mg, 0.25 mmol) were added. The mixture was allowed to reflux for 3 h and cooled to room temperature. After filtration, the solvent was removed and the residue was purified by flash chromatography to give the title compound as a purple solid (82 mg, 81%). Mp: 236 °C dec. ^{1}H NMR ($CDCl_3$): δ 1.3−1.7 (15H, m), 1.77 (6H, m), 2.3−2.4 (5H, m), 2.52 (4H, m), 2.65 (1H, m), 2.84 (1H, m), 3.16 (2H, m), 3.30 (2H, m), 3.62 (1H, m), 4.17 (1H, m), 4.39 (1H, m), 6.9−7.1 (2H, m), 7.2−7.3 (2H, m), 7.38 (1H, m), 7.46 (1H, m), 7.65 (1H, s), 10.95 (1H, br s), 11.22 (1H, br s). ^{13}C NMR ($CDCl_3$): δ 12.5, 15.9, 17.3, 25.1, 25.2, 25.5, 26.6, 27.5, 28.2, 28.7, 35.6, 36.3, 39.0, 39.7, 55.9, 60.4, 62.2, 101.9, 103.2, 104.8, 105.0, 110.2, 111.5, 111.7, 112.0, 115.7, 119.3, 119.4, 120.7, 121.4, 126.5, 127.7, 128.6, 130.8, 136.7, 156.3, 157.9, 161.5, 162.8, 164.7, 173.3, 174.5. IR (KBr): 3406, 3330, 1619, 1355, 1293, 1161, 930 cm^{-1}. HRMS (EI): m/z calcd for $C_{44}H_{48}Cl_2N_6O_5S$ [M$^+$] 842.2784, found 842.2771.

6-(Biotinylamino)hexanoic Acid (3-{2,5-Dihydroxy-4-[7-(3-methyl-but-2-enyl)-1*H*-indol-3-yl]-3,6-dioxocyclohexa-1,4-dienyl}-2-methyl-1*H*-indol-5-yl)amide (33). To a refluxing solution of 6-(biotinylamino)-hexanoic acid (3-{2,5-dichloro-4-[7-prenyl-1*H*-indol-3-yl]-3,6-dioxocyclohexa-1,4-dienyl}-2-methyl-1*H*-indol-5-yl)amide (82 mg, 0.1 mmol) in MeOH (6 mL) was added aqueous NaOH (10%, 3 mL) dropwise. The mixture was refluxed for 30 min and then poured into ice water (20 mL). H_2SO_4 (10% in water) was added to acidify the mixture, which was then extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. The solution was concentrated and purified by flash column chromatography (oxalic acid-coated silica gel) to afford the title compound as a red solid (24 mg, 32%). Mp: 231−232 °C. ^{1}H NMR ($CDCl_3$): δ 1.2−1.6 (15H, m), 1.71 (6H, m), 2.3−2.4 (5H, m), 2.55 (4H, m), 2.66 (1H, m), 2.82 (1H, m), 3.19 (2H, m), 3.35 (2H, m), 3.67 (1H, m), 4.21 (1H, m), 4.45 (1H, m), 6.9−7.1 (2H, m), 7.2−7.3 (2H, m), 7.32 (1H, m), 7.57 (1H, m), 7.68 (1H, s). ^{13}C NMR ($CDCl_3$): δ 12.1, 16.3, 17.8, 25.4, 25.8, 25.9, 26.6, 27.1, 28.1, 28.9, 35.3, 36.6, 39.1, 39.5, 56.3, 61.1, 62.7, 101.3, 103.6, 104.6, 105.0, 109.6, 111.5, 111.8, 112.3, 116.3, 119.3, 119.4, 120.9, 121.5, 126.8, 127.7, 128.2, 129.8, 135.7, 155.9, 158.1, 161.8, 162.9, 164.7, 173.5, 174.2. IR (KBr): 3340, 3150, 2921, 2869, 1615, 1533, 1351 cm^{-1}. HRMS (EI): m/z calcd for $C_{44}H_{50}N_6O_7S$ [M$^+$] 806.3462, found 806.3477.

Immunoblotting. The anti-phosphoIR antibody is from BioSource (Camarillo, CA, catalog no. 44-804). hIRcB cells are grown in DME/F12 medium supplemented with 10% fetal calf serum, 25 mM glucose, 2 mM glutamax, and gentamicin at 37 °C in a 10% CO_2 environment. Cells are serum starved for 72 h in 12-well plates, and then stimulated with increasing concentrations of insulin or asterriquinones in Krebs−Ringer−phosphate−Hepes buffer for 5 or 10 min at 37 °C. The cells are washed with ice-cold phosphate-buffered saline and solubilized in 2× sodium docecyl sulfide (SDS)−sample buffer containing 2 mM sodium orthovanadate and 200 mM sodium fluoride. The proteins are denatured by boiling for 5 min, and then separated by electrophoresis on 7.5% SDS−PAGE and transferred to poly(vinylidene difluoride) (PVDF) membranes. The filter is blocked with 3% bovine serum albumin in Tris-buffered saline (pH 7.4) with 0.1% Tween 20 (T-TBS) for 60 min and incubated with the anti-phosphoIR antibody at a dilution of 1:1000 in blocking buffer for 2 h. The filters are washed with T-TBS for 3 × 10 min and then incubated with an anti-rabbit secondary antibody conjugated to horseradish peroxidase for 60 min in blocking buffer. The filters are washed 5 × 10 min, and enhanced chemiluminescent detection is used to visualize the tyrosine-phosphorylated insulin receptor. The bands are quantified by scanning densitometry and are expressed as percent activation of the insulin receptor by insulin at a concentration of 50 ng/mL.

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Supporting Information Available: Calculated log *P*'s for compounds **26**; ^{1}H NMR spectra for compounds **9**−**12**, **14**−**17**, **20**, **21**, **23**, **25**{***1***−***3***,***6***−***9***}, **26**{***1***−***12***}, **32**, **33**, and **35**. This material is available free of charge via the Internet at http://pubs.acs.org.

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