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Niphatenones, Glycerol Ethers from the Sponge *Niphates digitalis* Block Androgen Receptor Transcriptional Activity in Prostate Cancer Cells: Structure Elucidation, Synthesis, and Biological Activity

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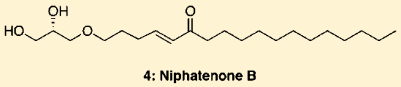
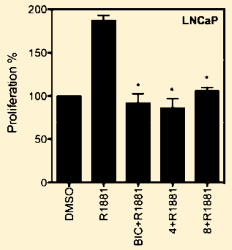
Supporting Information

ABSTRACT: Extracts of the marine sponge *Niphates digitalis* collected in Dominica showed strong activity in a cell-based assay designed to detect antagonists of the androgen receptor (AR) that could act as lead compounds for the development of a new class of drugs to treat castration recurrent prostate cancer (CRPC). Assay-guided fractionation showed that niphatenones A (**3**) and B (**4**), two new glycerol ether lipids, were the active components of the extracts. The structures of **3** and **4** were elucidated by analysis of NMR and MS data and confimed via total synthesis. Biological evaluation of synthetic analogues of the niphatenones has shown that the enantiomers **7** and **8** are more potent than the natural products in the screening assay and defined preliminary SAR for the new AR antagonist pharmacophore, including the finding that the Michael acceptor enone functionality is not required for activity. Niphatenone B (**4**) and its enantiomer **8** blocked androgen-induced proliferation of LNCaP prostate cancer cells but had no effect on the proliferation of PC3 prostate cancer cells that do not express functional AR, consistent with activity as AR antagonists. Use of the propargyl ether **44** and Click chemistry showed that niphatenone B binds covalently to the activation function-1 (AF1) region of the AR *N*-terminus domain (NTD).



4: Niphatenone B



INTRODUCTION

One man in six will face the sobering prospect of being diagnosed with prostate cancer at some point during his life.[1] There are approximately 240,000 new diagnoses and 33,000 deaths resulting from prostate cancer each year in the USA alone. First line treatment for localized low grade prostate cancer involves radical prostectomy and/or radiation therapy.[2] Roughly 30% of these patients will eventually have recurrence of their cancer accompanied by a sharp rise in tumor burden and serum concentrations of prostate-specific antigen (PSA). Treatment of this second stage of the disease involves androgen ablation by chemical or surgical castration and administration of antiandrogens, resulting in effective tumor regression and a drop in PSA concentrations. Eventually, after a period of up to a few years, the cancer will reemerge in all of these patients as indicated by a second sharp rise in PSA and tumor burden. This last stage of the disease is referred to as castration recurrent prostate cancer (CRPC). The only treatments available to patients with CRPC are administration of cytotoxic agents (taxanes) or sipuleucel-T (a vaccine), which only prolong life for 2 to 6 months. Men with CRPC usually succumb to their disease within 2 to 3 years.

Growth of prostate cancer cells is driven by the transcriptional activity of the androgen receptor (AR) at all stages of the disease.[2,3] The AR comprises a C-terminal ligand binding domain (LBD), a DNA binding domain (DBD), a hinge region, and an *N*-terminus domain (NTD), containing the activation function-1 (AF1) region. AR LBD can bind ligands in the absence of the NTD, but no AR transcriptional activity is possible without a functioning NTD AF1 region. During the normal growth of prostate gland tissue and in the early stages of prostate cancer, transcriptional activity of the AR is initiated by binding of the endogenous ligand dihydrotestosterone to the LBD. Androgen ablation by castration and treatment with potent LBD antagonists (antiandrogens) such as bicalutamide prevents AR transactivation by endogenous androgens, thereby providing a highly beneficial although transient therapeutic effect in the early stages of the disease. All current therapies that

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target the AR LBD, either by reducing ligand or directly with antiandrogens, eventually fail presumably through mechanisms involving gain-of-function mutations in the LBD, intratumoral de novo androgen synthesis, expression of constitutively active AR splice variants that lack LBD, or activation of signaling pathways that bypass the LBD and target the NTD such as interleukin-6 (IL6) and protein kinase A (PKA).[4] Recognition of the essential role that the AF1 region plays in the transactivation of the AR identified the AR NTD as a novel drug target for treating CRPC. Decoy proteins provided the first animal model proof-of-principle (POP) demonstration that blocking activation of the AR NTD was a viable method to control the growth of CRPC.[5]

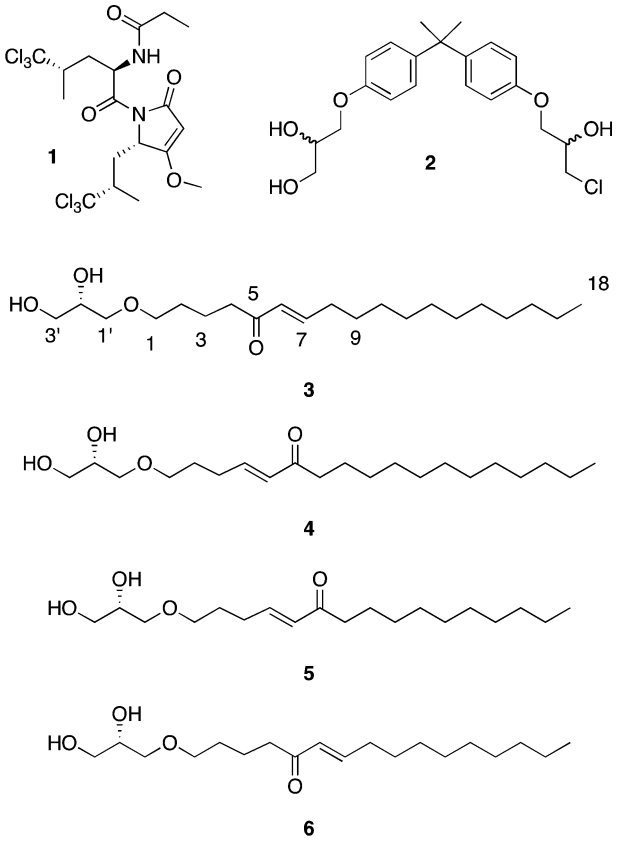
As part of a program designed to find small molecule antagonists of the AR NTD as lead compounds for the development of drugs to treat CRPC, we have screened a library of marine invertebrate extracts with an assay that uses LNCaP prostate cancer cells containing an engineered PSA gene with a luciferase reporter.[3,6] Stimulation of the cells with forskolin to stimulate PKA or IL6 activates the AR NTD leading to AR-dependent production of PSA-luciferase that generates light upon addition of luciferin. Antagonists of the AR inhibit light production, a positive hit in the assay. Use of this assay enabled the discovery of the sintokamides (i.e., **1**)[6] and EPI-001 (**2**),[3] the first known small molecule antagonists of the AR NTD. Extensive characterization of the bioactivity of EPI-001 has provided the first POP demonstration that small molecule antagonists of the AR NTD are highly active in vivo in LNCaP mouse models of CRPC.[3]

Crude MeOH extracts of the marine sponge *Niphates digitalis* collected in Dominica showed strong activity in the screening assay. Bioactivity-guided fractionation of the extracts led to the isolation of the glycerol ethers niphatenones A (**3**) and B (**4**) as the active components. The niphatenones represent a new structural class of AR antagonists that bind covalently to the AF1 region of the NTD. Details of the isolation, structure elucidation, synthesis of the natural products **3** and **4** and various analogues, and the biological activities of the natural products and synthetic analogues are presented below.

■ ISOLATION AND STRUCTURE ELUCIDATION

Specimens of *Niphates digitalis* (Lamarck, 1814) were collected by hand using SCUBA on shallow reefs near Pennville in the Commonwealth of Dominica and frozen on site for transport to UBC. Freshly thawed sponge (160 g) was extracted repeatedly with MeOH, and the combined extracts were evaporated in vacuo to give a residue that was partitioned between H_2O and EtOAc. The bioactive EtOAc soluble material was fractionated by assay-guided sequential application of reversed-phase flash chromatography, Sephadex LH20 chromatography, and C_{18} reversed-phase HPLC to give small amounts of pure niphatenones A (**3**) (0.1 mg) and B (**4**) (0.1 mg).

Niphatenone A (**3**) was isolated as a clear oil that gave a $[M + Na]^+$ ion in the HRESIMS at m/z 379.2808 consistent with a molecular formula of $C_{21}H_{40}O_4$ (calcd for $C_{21}H_{40}O_4Na$, 379.2824), requiring 2 sites of unsaturation. The limited quantity of niphatenone A (**3**) that was available precluded a measurement of its specific rotation or a 1D ^{13}C NMR spectrum. A pair of olefinic methine resonances at δ 6.01 (H-6) and 6.69 (H-7) in the ^1H NMR spectrum of **3** were correlated to each other in the COSY spectrum and to a carbon resonance at δ 198.5 in the HMBC spectrum, indicating the presence of an $\alpha\beta$-unsaturated ketone substructure in **3**. The enone



accounted for the two sites of unsaturation required by the molecular formula.

An isolated linear ^1H spin system in **3** comprising four contiguous methylenes (H_2-1 to H_2-4) was identified from the COSY data. A multiplet at δ 3.12 (H_2-1) was correlated to a pentet at δ 1.40 (H_2-2) that was in turn correlated to a pentet at δ 1.64 (H_2-3), which showed a correlation to a triplet at δ 2.23 (H_2-4). The triplet at δ 2.23 (H_2-4) and the pentet at δ 1.64 (H_2-3) both showed strong HMBC correlations to the ketone carbonyl resonance at δ 198.5 (C-5), demonstrating that C-4 of the linear methylene chain was attached to the ketone carbon.

A second isolated spin system identified in the COSY spectrum of **3** started with a methylene resonance at δ 3.19 (H_2-1′; HSQC correlation to δ 72.6) that showed a correlation to a methine at δ 3.63 (H-2′; HSQC correlation to δ 70.7), which was in turn weakly correlated to a methylene resonance at δ 3.42 (H-3′; HSQC correlation to δ 64.2). The resonance at δ 3.42 (H-3′) also showed an HSQC correlation to the carbon resonance at δ 64.2 (C-3′) and a strong COSY correlation to the resonance at δ 3.51 (H-3′), assigned to its geminal partner. This isolated spin system was assigned to a glycerol moiety. HMBC correlations observed between the glycerol methylene resonance at δ 3.19 (H_2-1′) and the C-1 carbon resonance at δ 71.2, and between the H_2-1 methylene resonance at δ 3.12 and the C-1′ glycerol resonance at δ 72.6 showed that the glycerol moiety was connected to C-1 via an ether linkage.

The remaining fragment of **3** had to be saturated, acyclic, and account for $C_{11}H_{23}$. A COSY correlation was observed between the resonance at δ 6.69 (H-7), assigned to the β proton of the $\alpha\beta$-unsaturated ketone, and a methylene resonance at δ 1.87 (H_2-8). The H_2-8 (δ 1.87) resonance showed a COSY correlation into an unresolved envelope of resonances at $\approx \delta$ 1.1 to 1.3 in the COSY spectrum. A lone triplet methyl resonance at δ 0.92 (H_3-18) was also correlated in the COSY

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Table 1. NMR Data (600 MHz, C_6D_6) for Natural Niphatenones A (3) and B (4)

	3		4	
position	δ_C	δ_H(J in Hz)	δ_C	δ_H(J in Hz)
1	71.2	3.12, m	70.5	3.01, m
2	29.4	1.40, p (7.1)	28.3	1.33, nd[a]
3	20.9	1.64, p (7.1)	29.2	1.88, m
4	39.8	2.23, t (7.1)	145.1	6.66, dt (15.8, 7.0)
5	198.5		130.7	5.99, d (15.8)
6	130.6	6.01, d (15.8)	198.6	
7	146.3	6.69, dt (15.8, 7.0)	40.6	2.28, t (7.4)
8	32.5	1.87, m	24.4	1.68, m
9	28.5	1.23, nd	29.6	1.27, nd
10	29.5−30.1	1.1−1.3	29.5−30.0	1.18−1.35
11	29.5−30.1	1.1−1.3	29.5−30.0	1.18−1.35
12	29.5−30.1	1.1−1.3	29.5−30.0	1.18−1.35
13	29.5−30.1	1.1−1.3	29.5−30.0	1.18−1.35
14	29.5−30.1	1.1−1.3	29.5−30.0	1.18−1.35
15	29.5−30.1	1.1−1.3	29.5−30.0	1.18−1.35
16	32.3	1.30, nd	32.2	1.31, nd
17	23.1	1.30, nd	23.1	1.31, nd
18	14.4	0.92, t (6.8)	14.4	0.91, t (6.7)
1′	72.6	3.19, m	72.6	3.15, m
2′	70.7	3.63, m	70.6	3.61, m
3′	64.2	3.42, dd (10.7, 5.2) 3.51, m	64.1	3.39, m 3.48, m

[a]Multiplicity was not determined due to the overlapping signals/chemical shifts determined from 2D data.

spectrum of **3** into the unresolved envelope of resonances at ≈ δ 1.1 to 1.3. This data was consistent with the $C_{11}H_{23}$ fragment of niphatenone A being a linear saturated hydrocarbon chain attached to C-7 of the $\alpha\beta$-unsaturated ketone as shown in **3**. The observed H-6/H-7 scalar coupling constant of 15.8 Hz indicated that the $\Delta^{6,7}$-alkene had the *E* configuration.

Niphatenone B (**4**) was isolated as a clear oil that gave a $[M + Na]^+$ ion in the HRESIMS at m/z 379.2811 consistent with a molecular formula of $C_{21}H_{40}O_4$ (calcd for $C_{21}H_{40}O_4Na$, 379.2824), identical to the molecular formula of niphatenone A (**3**). The ^{1}H NMR spectrum recorded for **4** showed a very strong resemblance to the corresponding spectrum obtained for **3** (Table 1), suggesting that **3** and **4** were closely related isomers. A pair of scalar coupled (J = 15.8 Hz) olefinic methine resonances at δ 5.99 (H-5) and 6.66 (H-4) were correlated in the HMBC spectrum to a carbonyl resonance at δ 198.6 (C-6), revealing that niphatenone B also contained an $\alpha\beta$-unsaturated ketone substructure with the *E* configuration.

The COSY, HMBC, and HSQC data confirmed the presence of a glycerol moiety in niphatenone B (**4**) [δ 3.15 (2H), 72.6 (C-1′); 3.61 (1H), 70.6 (C-2′); 3.39 (1H), 3.48 (1H), 64.1 (C-3′)]. COSY correlations were observed between the H-4 alkene methine resonance at δ 6.66 and a methylene quartet at δ 1.88 (H_2-3). The methylene quartet was further correlated in the COSY spectrum to a poorly resolved methylene resonance at δ 1.33 (H_2-2) that was in turn correlated to a deshielded methylene resonance at δ 3.01 (H_2-1). Both of the methylene proton resonances at δ 1.88 (H_2-3) and 1.33 (H_2-2) showed HMBC correlations to the olefinic methine carbon resonance at δ 145.1 (C-4) and the methylene carbon resonance at δ 70.5 (C-1), in agreement with the presence of three contiguous methylenes attached to the β carbon of the enone substructure. HMBC correlations observed between the H_2-1 resonance at δ 3.01 and the C-1′ resonance at δ 72.6, and between the H_2-1′ resonance at δ 3.15 and the C-1 resonance at δ 70.5 showed that the distal terminus of the C-1 to C-3 methylene chain was linked to the glycerol fragment via a C-1 to C-1′ ether bond as shown in **4**.

The remaining fragment of niphatenone B had to be saturated, acyclic, and account for $C_{12}H_{25}$. A triplet methylene resonance at δ 2.28 (H_2-7) and a methylene multiplet at δ 1.68 (H_2-8), which were coupled to each other in the COSY spectrum, both showed HMBC correlations to the C-6 ketone carbonyl resonance (δ 198.6). The H_2-8 methylene resonance (δ 1.68) and a methyl triplet at δ 0.91 (H_3-18) both showed COSY correlations into an unresolved envelope between δ 1.18 and 1.35. The above data was consistent with a linear 12 carbon alkyl group attached to the enone carbonyl to give the structure **4** for niphatenone B.

Niphatenones A (**3**) and B (**4**) are new glycerol ether sponge natural products. They are homologues of the ceratodictyols A (**5**) and B (**6**) isolated by Matsunaga and co-workers from the red alga/sponge assemblage *Ceratodictyon spongiosum*/*Haliclona cymaeformis*.[7] The ceratodictyols were reported to have modest in vitro cytotoxicity against a human cervical cancer cell line (IC_{50} ≈ 67 μM). Niphatenones A (**3**) and B (**4**) showed strong activity in a cell-based screening assay designed to find anatagonists of AR NTD.[3,5] They represent a new pharmacophore with this promising biological activity, and they are, therefore, potential lead compounds for the development of a new class of drugs to treat CRPC.

SYNTHESIS OF NIPHATENONES A (3) AND B (4)

The extremely small amounts of niphatenones A (**3**) and B (**4**) available from the *N. digitalis* extract made it impossible to determine the absolute configurations of the natural products or carry out further evaluation of their biological activities. In order to provide reference materials for the absolute configuration determination and biological evaluation, we have completed a total synthesis of *S*-(**3**) and (*R*)-niphatenone A (**7**) and *S*-(**4**) and (*R*)-niphatenone B (**8**).

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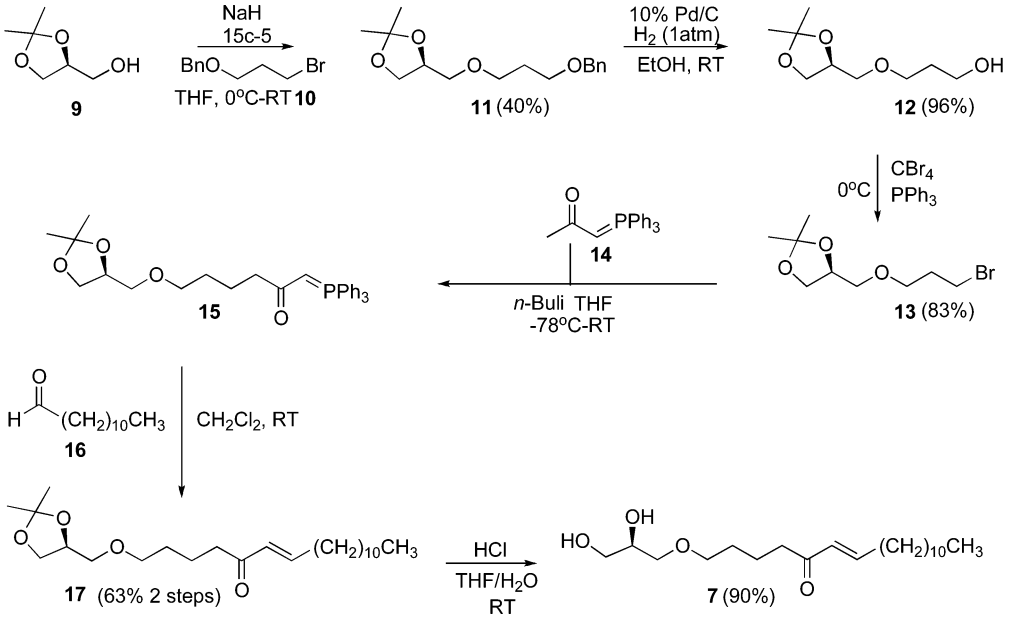
Scheme 1



The synthesis of (*R*)-niphatenone A (**7**) started with commercially available (*S*)-4-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (**9**) as shown in Scheme 1. Deprotonation of **9** with sodium hydride in the presence of crown ether followed by treatment with the primary bromide **10** gave the protected glycerol ether **11** in acceptable yield.[8] Hydrogenolysis gave the primary alcohol **12** that was converted to the primary bromide **13** by treatment with carbon tetrabromide and triphenylphosphine. Deprotonation of the phosphonium salt **14** with *n*-butyl lithium followed by alkylation with the bromide **13** gave the Wittig reagent **15**.[9] Reaction of **15** with dodecanal **16** gave the enone **17**. Deprotection of **17** with HCl in H_2O/THF gave (*R*)-niphatenone A (**7**). Repeating the synthesis using (*R*)-4-hydroxymethyl-2,2-dimethyl-1,3-dioxolane as the starting material gave (*S*)-niphatenone A (**3**).

The synthesis of (*R*)-niphatenone B (**8**) also started with (*S*)-4-hydroxymethyl-2,2-dimethyl-1,3-dioxolane (**9**) as shown in Scheme 2. Deprotonation of **9** with sodium hydride followed by alkylation with the primary bromide **18** gave ether **19**. Removal of the benzyl protecting group in **19** via hydrogenolysis gave the primary alcohol **20** that was oxidized with DMP to give the aldehyde **21**.[10] The starting material for the preparation of the Horner–Wadsworth–Emmons (HWE) Wittig reagent **22** was epoxide **23**. Treatment of **23** with cerous chloride and sodium bromide gave bromohydrin **24**,[11] which was oxidized with DMP to give bromo ketone **25**. Reaction of **25** with triethylphosphite gave the HWE Wittig reagent precursor **22**.[12] Deprotonation of **22** with barium hydroxide followed by the addition of aldehyde **21** gave enone **26**.[13] Removal of the acetonide protecting group in **26** with HCl in H_2O/THF gave authentic (*R*)-niphatenone B (**8**).[14] Repeating the synthesis with (*R*)-4-hydroxymethyl-2,2-dimethyl-1,3-dioxolane as the starting material gave authentic (*S*)-niphatenone B (**4**).

Comparison of the NMR and MS data obtained for the synthetic enantiomers of niphatenones A (**3** and **7**) and B (**4** and **8**) with the data collected for the natural products **3** and **4** showed that they were identical (Supporting Information), confirming the constitutions assigned to the natural products via NMR analysis. Chiral HPLC was able to resolve the synthetic enantiomers of both niphatenones A and B. Using the Chiral HPLC method, we showed that naturally occurring niphatenones A (**3**) and B (**4**) both had the *S* configuration.

The synthetic niphatenone enantiomers **3**, **4**, **7**, and **8** were tested in an assay that measures AR transcriptional activity (Figure 1). Compounds **4**, **7**, and **8** showed strong activity in the assay. It is interesting to note that the configuration of the glycerol C-2′ chiral center is an important determinant of potency in the niphatenones, with the unnatural *R* enantiomers **7** and **8** being more active than the natural *S* enantiomers **3** and **4**, respectively.

SYNTHESIS OF ANALOGUES OF NIPHATENONES A AND B

A number of analogues of niphatenones A and B were prepared in order to explore the SAR for this new AR antagonist pharmacophore. The niphatenones contain enone functionalities that are potential Michael acceptors. In order to probe if covalent bonding of the niphatenones to the AR played a role in their biological activity, (*R*)-dihydroniphatenone B (**28**) was prepared from acetonide **26** via catalytic hydrogenation to give the saturated ketone **27**,[15] followed by acid catalyzed hydrolysis of the acetonide as shown in Scheme 3.[14]

To examine the effect of changing the length of the linear saturated alkyl substituent on the ketone functionality in niphatenone B (**4**), the analogue (*R*)-**32** with a C-18 ketone substituent and the analogue (*R*)-**35** with a one carbon ketone substituent were prepared using the same approach as that followed in the synthesis of (*R*)-niphatenone B (**8**) as shown in Scheme 4.

The niphatenones are very nonpolar compounds that are poorly soluble in water. In an attempt to make a more water-soluble version, analogue (*S*)-**41** containing a PEG tail attached to the glycerol moiety was prepared as shown in Scheme 5. (*S*)-Glycidol (**36**) was deprotonated by treatment with NaH and crown ether in THF, and the resulting alkoxide was alkylated with the primary bromide **18** to give the ether (*R*)-**37**. Hydrogenolysis removed the benzyl protecting group in **37** to

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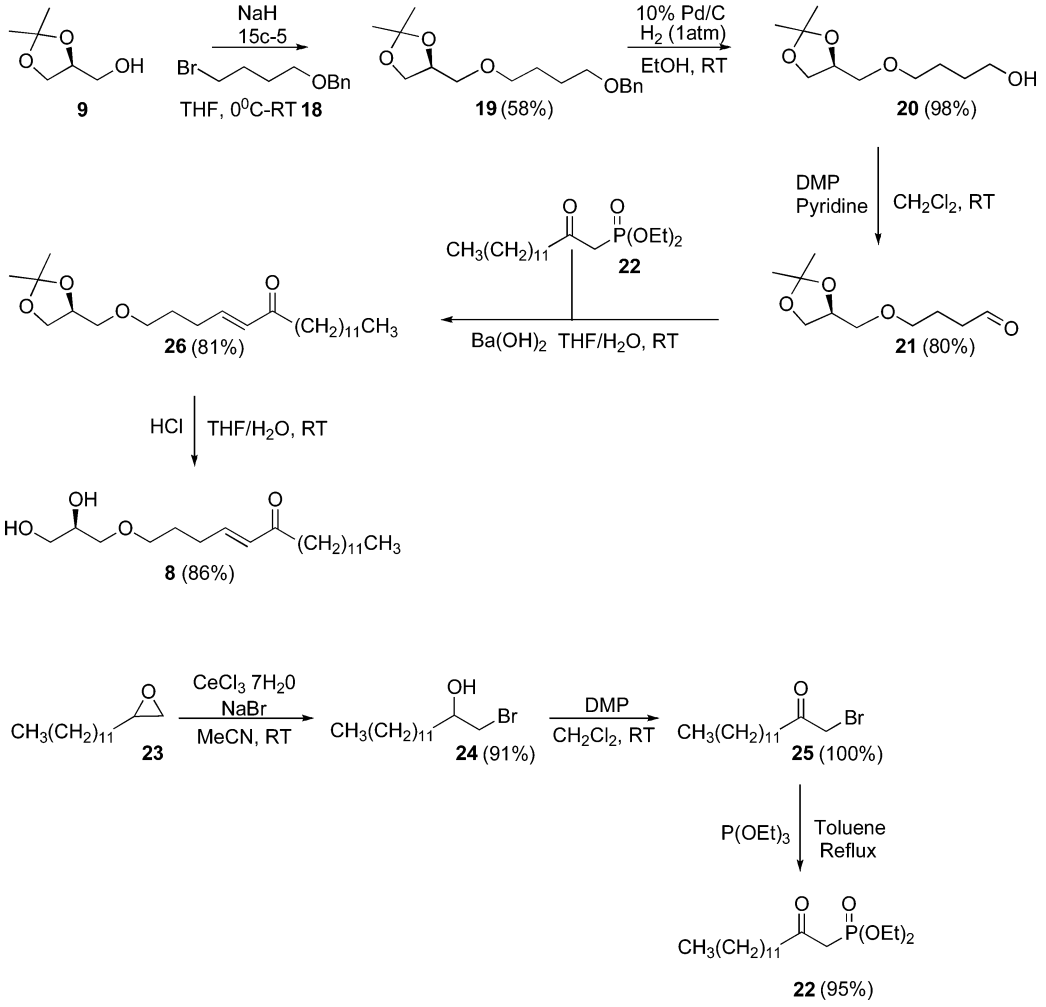
Scheme 2



give the primary alcohol (*R*)-**38**. Oxidation of the primary alcohol **38** with DMP to give to the corresponding aldehyde followed by a HWE Wittig reaction with deprotonated **22** gave the epoxide (*R*)-**39**. Opening of the epoxide in **39** with bismuth triflate in the PEG diol **40** as a solvent gave the desired PEG ether (*S*)-**41**. The glycerol fragment of the niphatenones is also found in EPI-001 (**2**), a highly effective antagonist of the AR NTD.[3] The chlorohydrin functionality in **2** is required for its AR-NTD blocking properties. Therefore, we decided to make the EPI-001/niphatenone B hybrid analogue (*S*)-**42** from the epoxide (*R*)-**39** by opening with cerium(III) chloride heptahydrate as shown in Scheme 5. In order to explore the possibility that the niphatenones bind covalently to the AR via the Michael acceptor enone substructures, we prepared analogue (*S*)-**44** containing an alkyne functionality by opening epoxide (*R*)-**39** with bismuth triflate in propargyl alcohol as a solvent (Scheme 5). It was anticipated that Click chemistry could be used to add biotin or fluorescent tags to this probe after exposure to target proteins in order to provide direct evidence for binding of the niphatenones to the AR NTD.

Finally, we made the all carbon chain analogue (*S*)-**45** as shown in Scheme 6. Primary alcohol (*S*)-**46** was converted to the primary bromide (*S*)-**47** by treatment with triphenylphosphine in carbon tetrabromide. Conversion of bromide **18** into the corresponding Grignard reagent followed by Cu catalyzed coupling with **47** gave the benzyl protected dioxolane **48**. Removal of the benzyl protecting group in **48** via hydrogenolysis gave the primary alcohol **49** that was oxidized with DMP to the aldehyde **50**. A HWE Wittig reaction between **50** and deprotonated **22** gave enone **51**. Removal of acetonide in **51** with HCl/H_2O gave the all carbon chain niphatenone B analogue (*S*)-**45**.

BIOLOGICAL ACTIVITY OF THE NIPHATENONES AND SYNTHETIC ANALOGUES

Figure 1 shows the biological activities of the natural niphatenones [A (**3**) and B (**4**)] and a series of synthetic analogues in an assay that measures AR transcriptional activity. This assay consisted of transfecting LNCaP human prostate cancer cells that express functional AR with luciferase reporter that is regulated by AR in response to androgen. All the compounds were tested at a concentration of 7 μM with the exception of **32**, which was limited to 3.5 μM due to poor solubility. Niphatenone B (**4**) shows ≈50% inhibition at the test concentration, while niphatenone A (**3**) shows weaker activity that is not statistically different from that of the negative control.

The synthetic enantiomers (*R*)-niphatenone A (**7**) and (*R*)-niphatenone B (**8**) are both more active than the corresponding natural *S* isomers **3** and **4**, respectively, and (*R*)-niphatenone B (**8**) is the most active compound tested in this series. (*R*)-Dihydroniphatenone B (**28**) is less active than (*R*)-niphatenone

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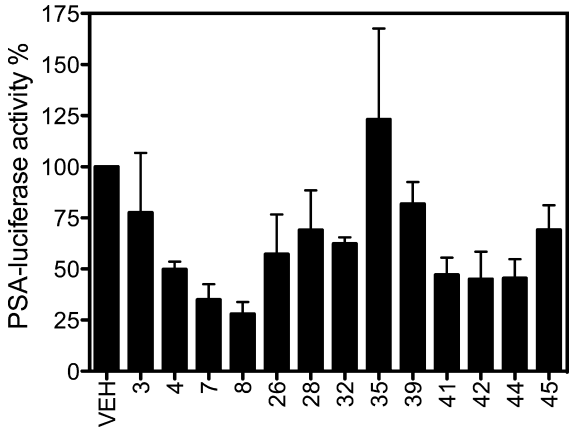
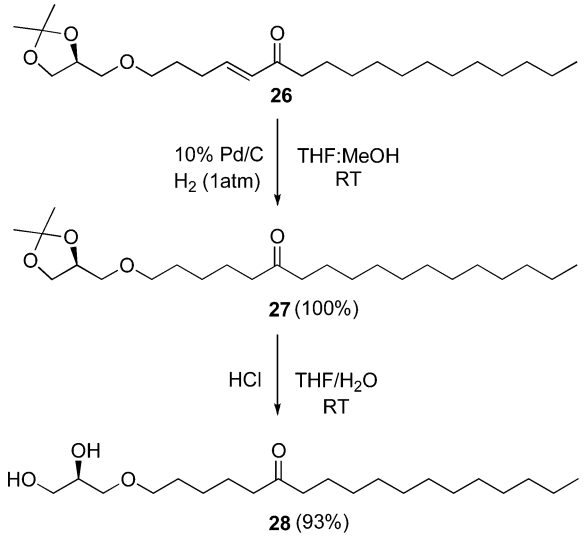


Figure 1. Key functional groups or structures within the niphatenone analogues correlate with reduced endogenous AR activity at the AR-driven promoter–luciferase reporter construct in LNCaP cells. LNCaP cells were transiently transfected with PSA (p6.1)-luciferase (0.5 μg/well in 12-well plates) and empty vector (1 μg/well) for 24 h prior to a 1 h pretreatment with niphatenones at 7 μM or for **32** at 3.5 μM. Cells were then treated with R1881 (1 μM) with DMSO vehicle (VEH) or R1881 (1 μM) with niphatenones for 48 h before harvesting and measurement of luciferase activities and protein concentrations. Luciferase activity was normalized to the predicted maximal activity induction (i.e., 100% in the absence of test compounds with VEH at 0 μM niphatenone). Error bars represent the mean ± SD of three separate experiments, with each treatment condition performed in triplicate.

Scheme 3



B (**8**) but only slightly less active than (*S*)-niphatenone B (**4**), demonstrating that although the potentially reactive enone plays a role in the potency of **8**, it is not an absolute requirement for activity. This suggests that covalent binding to the AR is not required for the biological activity of the niphatenones in the assay. The analogue (*R*)-**32** with a longer alkyl substituent on the ketone is about half as active as (*R*)-niphatenone B (**8**) at half the dose, while the analogue (*R*)-**35** with a one carbon alkyl substituent on the ketone has lost essentially all activity at the test concentration. Removing the glycerol ether oxygen atom from the linear skeleton of (*R*)-niphatenone B (**8**) to give the all carbon chain analogue (*R*)-**45** produced a compound with roughly half the activity of **8**. Therefore, the glycerol ether linkage seems important to the activity. Similarly, the acetonide (*R*)-**26** and the epoxide (*R*)-**39** are less active than **8**, suggesting that the alcohol functionalities on the glycerol moiety are important for activity. Finally, the PEG ether (*R*)-**41**, chlorohydrin (*R*)-**42**, and alkyne ether (*R*)-**44** analogues are all less active than (*R*)-niphatenone B (**8**) and comparable in activity to the natural product (*S*)-niphatenone B (**4**). The attenuation in activity observed for analogues **41**, **42**, and **44** can perhaps be attributed to the loss of the glycerol primary alcohol.

The inhibitory effects of niphatenones on the AR-driven luciferase reporter were not due to nonspecific toxicity as shown by comparing the proliferation of cells that are dependent on AR for growth and proliferation to cells that do not express functional AR, such as PC3 shown in Figure 2. (*S*)-Niphatenone B (**4**) and (*R*)-niphatenone B (**8**) blocked androgen-induced proliferation of LNCaP cells at comparable concentrations to the clinical drug bicalutamide. These same concentrations of either (*S*) or (*R*) niphatenone B had no effect on the proliferation of PC3 cells that do not express functional AR.

EVIDENCE FOR COVALENT BINDING OF THE NIPHATENONE B ALKYNYL ETHER ANALOGUE 44 TO THE AF1 REGION OF THE AR NTD

The objective of the screening program that identified the niphatenones was to discover new antagonists of the AR NTD. The differential effects of both (*R*)-**8** and (*S*)-**4** niphatenone B on the proliferation of LNCaP prostate cancer cells versus the proliferation of PC3 prostate cancer cells described above, and their activity in the screening assay, were consistent with niphatenone B being an androgen receptor antagonist. In order to determine if niphatenone B would bind to the AR NTD, we exposed recombinant NTD AF1 protein to the niphatenone B analogue **44** that contains a propargyl ether. Analogue **44** was active in the screening assay (Figure 1) indicating that it shared the AR antagonistic properties of niphatenone B. After 50 min of exposure at 0 °C, Click chemistry (Supporting Information Scheme 1) was used to add a fluorescein tag, and the protein was analyzed on a SDS–PAGE gel. Figure 3 shows that the band corresponding to the AF1 protein is labeled with the fluorescent tag, demonstrating that probe **44** had bound covalently to the NTD AF1 protein.

CONCLUSIONS

The niphatenones represent a new natural product pharmacophore that could serve as a lead for the development of drugs to treat CRPC. Synthesis has shown that the unnatural (*R*) analogues are more potent than the natural products. Examination of a series of synthetic analogues of (*R*)-niphatenone B (**8**) revealed that the enone functionality is not required for activity and that the glycerol ether oxygen, glycerol alcohols, and a reasonable length saturated alkyl chain substituent on the ketone are important. These findings show that there is clear SAR for this pharmacophore, and they suggest that it should be possible to design more potent analogues of the natural products that do not contain the undesirable enone Michael acceptor. The ability of niphatenone B (**4**) and its enantiomer **8** to block androgen-induced proliferation of LNCaP prostate cancer cells but not the proliferation of PC3 prostate cancer cells that do not express functional AR supports target-specific antiproliferative effects.

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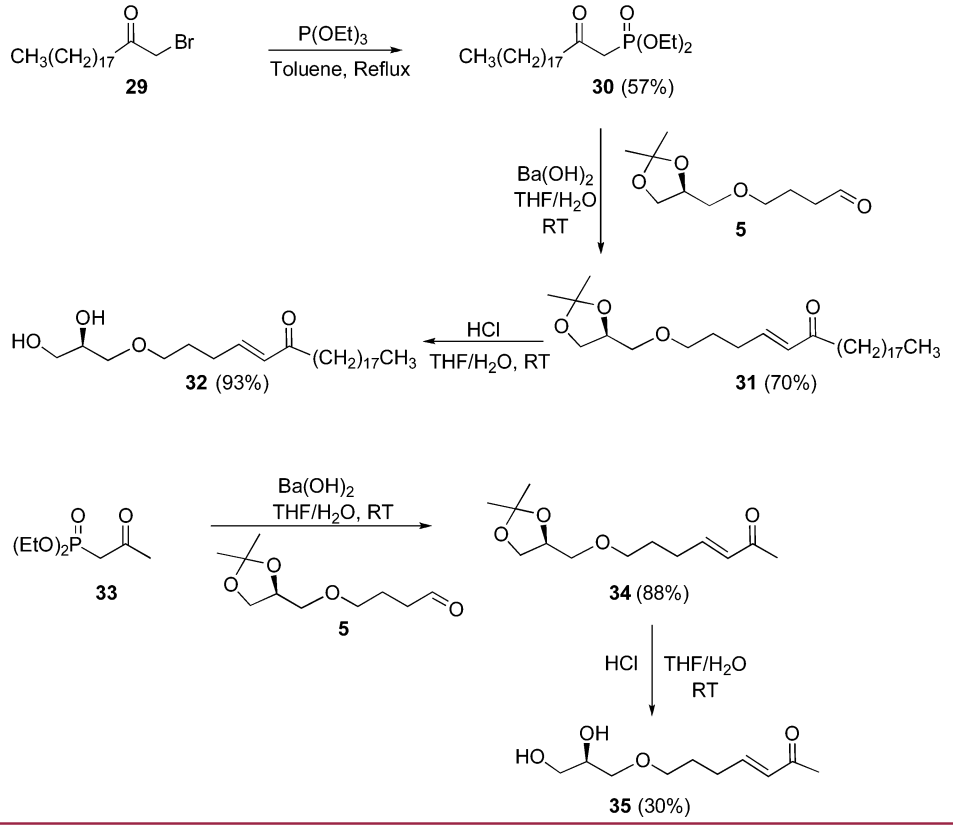
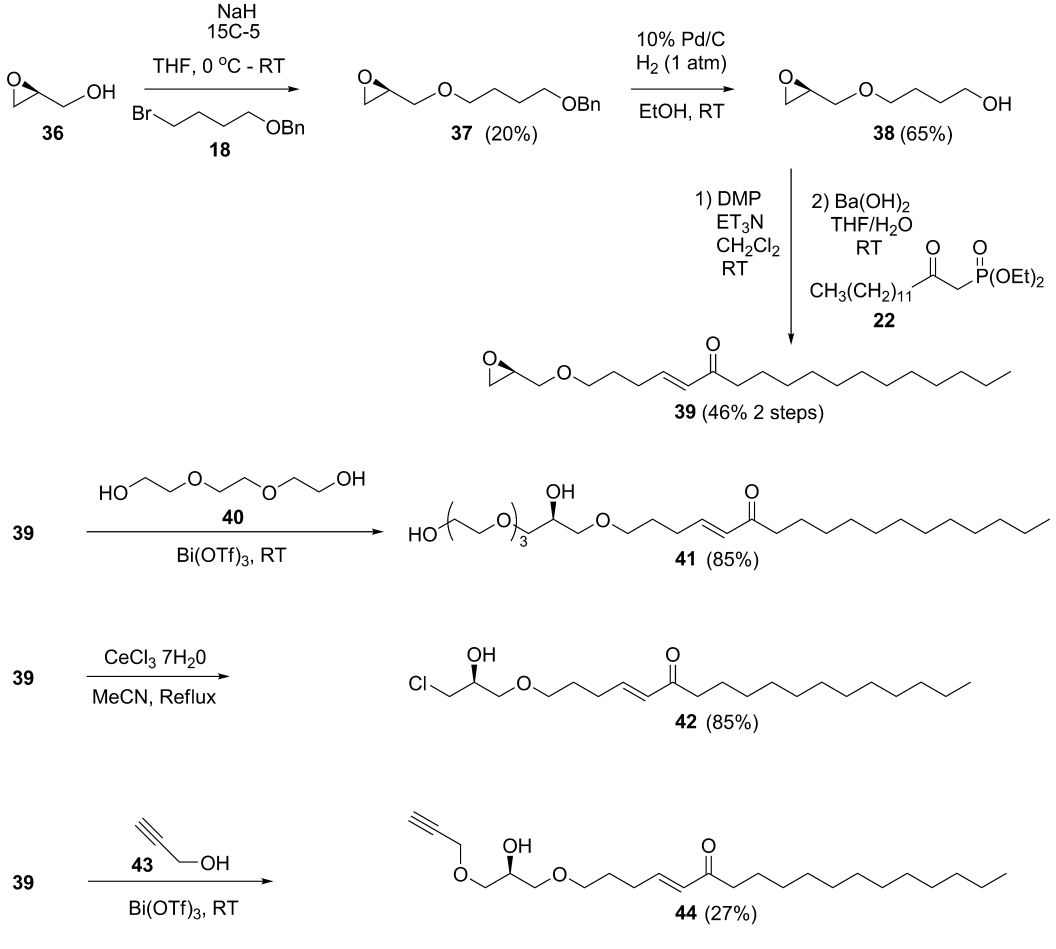
Scheme 4

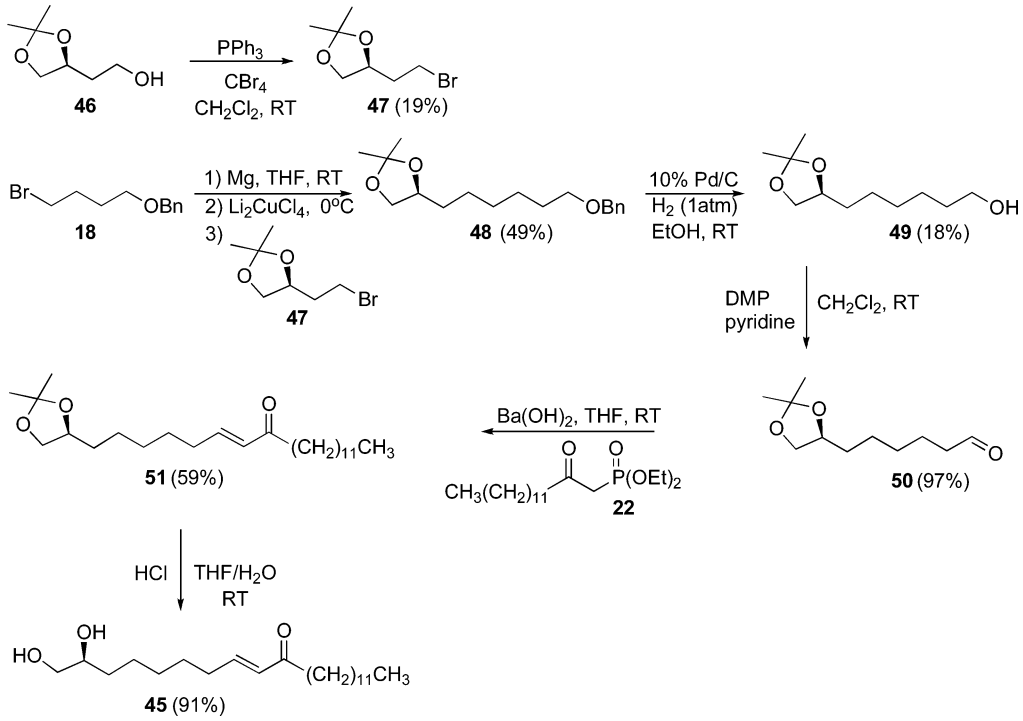


Scheme 5



dx.doi.org/10.1021/jm2014056 | J. Med. Chem. 2012, 55, 503−514

Scheme 6



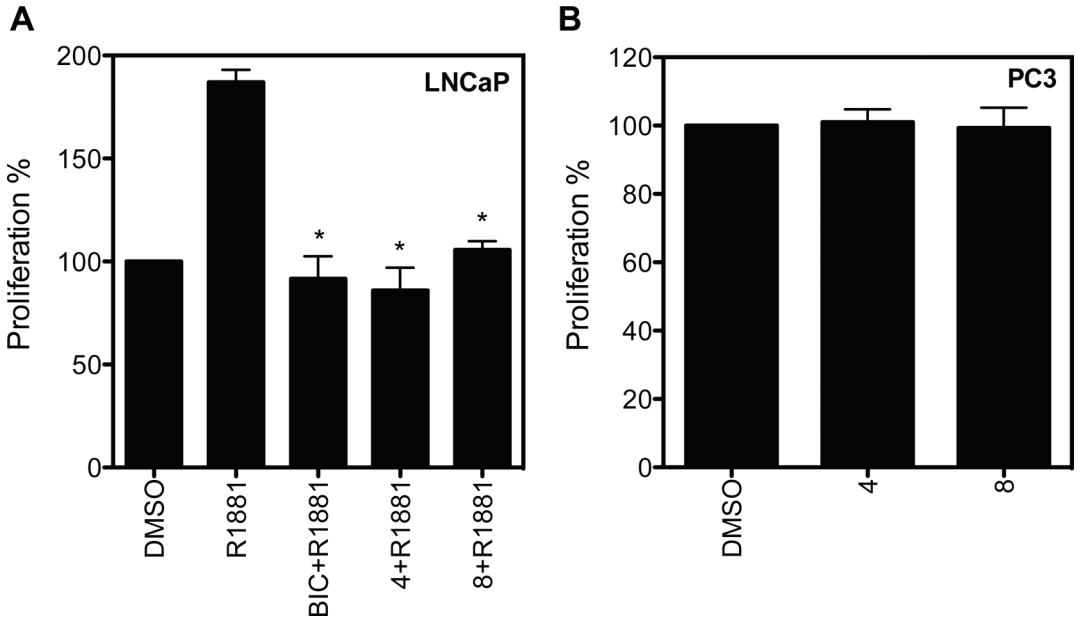


Figure 2. Niphatenones inhibit androgen-dependent proliferation at concentrations that do not reduce proliferation of cells that do not express function AR. (A) (*S*)-Niphatenone B (**4**) and (*R*)-niphatenone B (**8**) inhibit androgen-dependent proliferation. LNCaP cells were pretreated with antiandrogen bicalutamide (BIC, 10 μM), (*S*)-niphatenone B (**4**) (14 μM), and (*R*)-niphatenone B (**8**) (14 μM) for 1 h prior to treatment with 0.1 nM R1881 in LNCaP cells. BrdU incorporation was measured 72 h after treatment. (B) (*S*)-Niphatenone B (**4**) and (*R*)-niphatenone B (**8**) (14 μM) did not affect the proliferation of PC3 cells. BrdU incorporation was measured 24 h after treatment. Error bars represent the mean ± SD of three separate experiments, with each treatment condition performed in 6 technical replicates. Student's *t* test comparing each treatment to R1881. * = $p < 0.05$.

Use of the niphatenone analogue **44** demonstrated that niphatenone B binds covalently to the AF1 region of the AR NTD. Therefore, niphatenone B has the desired profile of an AR NTD antagonist drug candidate. The availability of a synthetic route to the niphatenones has provided material for further in vitro and in vivo biological evaluation of these promising lead compounds and analogues in disease models of CRPC. Results from these evaluations will be reported elsewhere.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured using a Jasco P-1010 Polarimeter with sodium light

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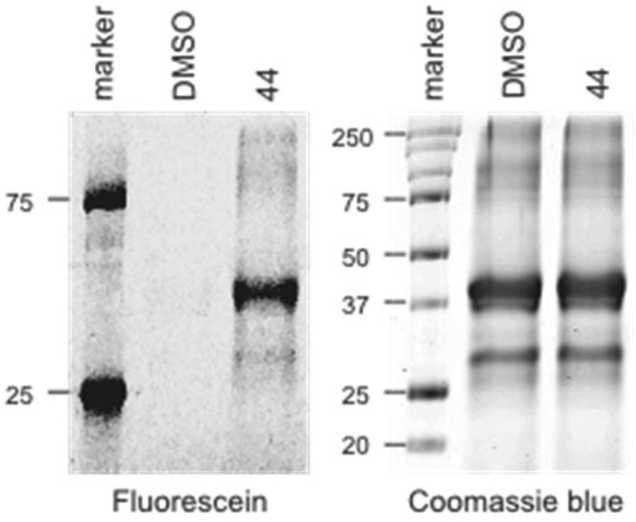

Figure 3. Binding between niphatenone analogue **44** and AR AF1 protein was detected by fluorescein labeling. Fluorescein was visualized by an image analyzer, and the image was modified by Adobe Photoshop to adjust the band intensity. Equal amounts of proteins were loaded as shown on the Coomassie blue stained gel. A representative gel is shown (n = 2).

(589 nm) in EtOAc. The ^{1}H and ^{13}C NMR spectra were recorded on either a Bruker AV-400 spectrometer or a Bruker AV-600 spectrometer with a 5 mm CPTCI cryoprobe. ^{1}H chemical shifts are referenced to the residual C_6D_6 or $CDCl_3$ signals (δ 7.15 and 7.24 ppm, repectively), and ^{13}C chemical shifts are referenced to the C_6D_6 or $CDCl_3$ solvent peak (δ 128.0 and 77.0 ppm, respectively). Low and high resolution ESI-QIT-MS were recorded on a Bruker-Hewlett-Packard 1100 Esquire−LC system mass spectrometer. Merck Type 5554 silica gel plates and Whatman MKC18F plates were used for analytical thin layer chromatography. Flash chromatography was carried out on Silicycle SiliaFlash 60A. Reversed-phase HPLC purifications were performed on a Waters 600E System Controller liquid chromatograph attached to a Waters 996 Photodiode Array Detector. All solvents used for HPLC were of Fisher HPLC grade. All final compounds tested for biological activity had a purity of >95% by HPLC analysis.

Sponge Material. Specimens of *Niphates digitalis* (Lamarck, 1814) were collected by hand using SCUBA at a depth of 10−15 m on gently sloping reefs east of Pennville, Commonwealth of Dominica on September 3, 2000 (N 15° 37.7′, W 61° 25.5′). A voucher sample has been deposited at the Zoological Museum of Amsterdam (ZMA POR. 19968).

Extraction of *Niphates digitalis* and Isolation of Niphatenones A (3) and B (4). Freshly collected sponge was frozen on site and transported to UBC frozen in a portable chest freezer. The sponge material (160 g) was cut into small pieces and immersed in and subsequently extracted repeatedly with MeOH (3 × 100 mL) at RT. The combined methanolic extracts were concentrated in vacuo, and the resultant extract was then partitioned between EtOAc (3 × 30 mL) and H_2O (100 mL). The combined EtOAc extract was evaporated to dryness, and the resulting oil was fractionated using reversed-phase Si gel flash chromatography, employing a step gradient from 1:1 MeOH/H_2O to MeOH with a final CH_2Cl_2 wash. A 30.2 mg fraction, eluting with 9:1 MeOH/H_2O, elicited activity and was chromatographed on Sephadex LH-20 with MeOH as eluent. Purification of the resulting active fraction via C_{18} reversed-phase HPLC using a CSC-Inertsil 150A/ODS2, 5 μm 25 × 0.94 cm column, with a linear gradient of 65:35 MeCN/H_2O to MeCN over 60 min, gave pure samples of the two minor compounds niphatenone A (**3**) (∼0.1 mg) and niphatenone B (**4**) (∼0.1 mg) (with retention times of 37.5 and 36.5 min, respectively).

*Niphatenone A (**3**).* Isolated as a clear oil; $[\alpha]^{25}_D$ not possible to measure accurately; ^{1}H and ^{13}C NMR, see Table 1; (+)-HRESIMS $[M + Na]^+$ m/z 379.2808 (calcd for $C_{21}H_{40}O_4Na$, 379.2824).

*Niphatenone B (**4**).* Isolated as a clear oil; $[\alpha]^{25}_D$ not possible to measure accurately; ^{1}H and ^{13}C NMR, see Table 1; (+)-HRESIMS $[M + Na]^+$ m/z 379.2811 (calcd for $C_{21}H_{40}O_4Na$, 379.2824).

Synthesis of *R* and *S* Niphatenones A and B. *Preparation of **11**.* Sodium hydride (436 mg, 10.9 mmol, 60% suspension in oil) was washed twice with hexanes (20.0 mL total), to which was added 45 mL of THF, and the suspension was cooled to 0 °C. To this suspension was added alcohol **9** (720 mg, 5.45 mmol) neat, along with 15C-5 (240 mg, 1.09 mmol). The mixture was then allowed to stir at RT for 1 h, after which bromide **10** (2.5 g, 10.9 mmol) was added dropwise. After 4.5 h, the reaction mixture was cooled to 0 °C and quenched with the addition of H_2O, and the aqueous phase was extracted 3 times with CH_2Cl_2. The organic extracts were dried with $MgSO_4$ and concentrated. The crude mixture was purified with flash chromatography (hexanes/EtOAc 9:1) to give **11** as a clear oil (623 mg, 2.22 mmol, 41%). $[\alpha]^{24}_D$ = +13.6 (c 0.12); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 (m, 4H), 7.28 (m, 1H), 4.50 (s, 2H), 4.25 (quin, J = 5.8 Hz, 1H), 4.04 (dd, J = 6.5, 1.7 Hz, 1H), 3.71 (dd, J = 6.1, 2.1 Hz, 1H), 3.56 (m, 5H), 3.42 (dd, J = 5.5, 4.4 Hz, 1H), 1.90 (quin, J = 6.1 Hz, 2H), 1.42 (s, 3H), 1.37 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.7, 128.5, 127.8, 127.7, 109.5, 74.9, 73.1, 72.1, 68.8, 67.3, 66.9, 30.2, 26.9, 25.6; HRESIMS $[M + H]^+$ calcd for $C_{16}H_{25}O_4$ 281.1753; found, 281.1747.

*Preparation of **12**.* To compound **11** (575 mg, 2.05 mmol) dissolved in 2.6 mL of EtOH was added 10% Pd/C (133 mg), in a round-bottom flask and the system flushed with H_2. The reaction mixture was then exposed to 1 atm of H_2 (balloon) overnight. Upon completion, the heterogeneous mixture was filtered and the filtrate washed three times with EtOH (60 mL total). The organic extracts were concentrated, and the crude mixture was purified with flash chromatography (hexanes/EtOAc 1:1), to give **12** as a clear oil (375 mg, 1.97 mmol, 96%). $[\alpha]^{24}_D$ = +16.3 (c 0.1); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.16 (quin, J = 5.8 Hz, 1H), 3.94 (dd, J = 8.2, 6.5 Hz, 1H), 3.62 (m, 3H), 3.54 (t, J = 6.0 Hz, 2H), 3.39 (m, 2H), 3.02 (s, 1H), 1.72 (quin, J = 6.0 Hz, 2H), 1.31 (s, 3H), 1.25 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 109.5, 74.7, 71.9, 69.9, 66.6, 60.6, 32.2, 26.7, 25.4; HRESIMS $[M + Na]^+$ calcd for $C_9H_{18}O_4Na$ 213.1103; found, 213.1105.

*Preparation of **13**.* To alcohol **12** (150 mg, 0.788 mmol) dissolved in 2 mL of CH_2Cl_2 was added PPh_3 (248 mg, 0.946 mmol), and the solution was cooled to 0 °C. CBr_4 was added to this solution, and it was stirred for 1 h after which the mixture was concentrated under a stream of nitrogen. The crude mixture was purified with flash chromatography (hexanes/EtOAc 12:1), to give **13** as a clear oil (166 mg, 0.657 mmol, 83%).^{1}H NMR (400 MHz, $CDCl_3$) δ 4.19 (quin, J = 6.0 Hz, 1H), 3.98 (dd, J = 6.5, 1.7 Hz, 1H), 3.65 (dd, J = 6.5, 1.7 Hz, 1H), 3.54 (td, J = 5.9, 2.6 Hz, 2H), 3.43 (m, 4H), 2.04 (td, J = 2.9, 6.2 Hz, 2H), 1.35 (s, 3H), 1.29 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 109.5, 74.7, 72.2, 69.0, 66.7, 32.8, 30.6, 26.9, 25.5; HRESIMS $[M + H]^+$ calcd for $C_9H_{18}O_3{}^{79}Br$ 253.0439; found, 253.0439.

*Preparation of **17**.* Phosphorane **14** (248 mg, 0.78 mmol) was dissolved in 5 mL of THF, and the solution was cooled to −78 °C. To this solution was added 1.6 M n-Buli (0.53 mL, 0.86 mmol), and it was stirred for 20 min. To this solution, bromide **13** (166 mg, 0.66 mmol) dissolved in 0.5 mL CH_2Cl_2 was added dropwise, and the mixture was allowed to warm from 0 °C to RT overnight. The reaction mixture was then diluted with H_2O and extracted 3 times with CH_2Cl_2. The organic extracts were dried with $MgSO_4$ and concentrated. The crude phosphorane was then used immediately in the follow up Wittig reaction by dissolving it in 1 mL of CH_2Cl_2 and adding aldehyde **16** (121 mg, 0.657 mmol) before stirring at RT overnight. The reaction mixture was concentrated under a stream of N_2. The crude mixture was purified with flash chromatography (hexanes/EtOAc 7:1), to give **17** as a clear oil (165 mg, 0.415 mmol, 63% over 2 steps). $[\alpha]^{24}_D$ = +11.4 (c 0.09); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.19 (dt, J = 16.0, 7.2 Hz, 1H), 6.01 (d, J = 16.0 Hz, 1H), 4.19 (quin, J = 6.1 Hz, 1H), 3.99 (dd, J = 8.2, 6.5 Hz, 1H), 3.66 (dd, J = 6.5, 1.7 Hz, 1H), 3.42 (m, 4H), 2.50 (t, J = 7.2 Hz, 2H), 2.15 (q, J = 6.8 Hz, 2H), 1.57 (m, 4H), 1.40

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(m, 1H), 1.35 (s, 3H), 1.30 (s, 3H), 1.21 (m, 17H), 0.82 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 200.4, 147.5, 130.3, 109.4, 74.8, 71.9, 71.5, 66.9, 39.7, 32.5, 32.0, 29.7 (2C), 29.6, 29.5, 29.4, 29.3, 29.2, 28.2, 26.9, 25.5, 22.8, 20.9, 14.2; HRESIMS $[M + H]^+$ calcd for $C_{24}H_{45}O_4$ 397.3318; found, 397.3310.

Preparation of ***7****.* To acetonide **26** (45 mg, 0.11 mmol), dissolved in 1.65 mL of THF/H_2O (5:1), was added 12.4 M HCl (0.045 mL, 0.56 mmol), and the solution was stirred at RT for 30 min. The reaction mixture was then quenched with the addition of saturated $NaHCO_3$, and the aqueous phase was extracted 3 times with CH_2Cl_2. The organic extracts were dried with $MgSO_4$ and concentrated. The crude mixture was purified with flash chromatography (4:1 EtOAc/hexanes) to give **27** as a white solid (35 mg, 0.099 mmol, 90%). $[\alpha]^{24}_D$ = +2.8 (c 0.07); ^{1}H NMR (600 MHz, C_6D_6) δ 6.70 (dt, J = 15.6, 7.2 Hz, 1H), 6.03 (d, J = 15.6 Hz, 1H), 3.91 (bs, 1H), 3.81 (bs, 1H), 3.73 (m, 1H), 3.66 (m, 1H), 3.41 (m, 2H), 3.26 (m, 2H), 3.28 (bs, 1H), 2.29 (t, J = 7.2 Hz, 2H), 1.90 (q, J = 6.6 Hz, 2H), 1.67 (quin, J = 7.2 Hz, 2H), 1.48 (quin, J = 6.6 Hz, 2H), 1.28 (m, 18H), 0.91 (t, J = 4.8 Hz, 3H); ^{13}C NMR (150 MHz, C_6D_6) δ 199.2, 146.6, 130.6, 72.6, 71.3(2C), 64.4, 39.8, 32.5, 32.3, 30.0, 30.0, 29.9, 29.8, 29.8, 29.6, 29.4, 28.4, 23.1, 21.0, 14.3; HRESIMS $[M + Na]^+$ calcd for $C_{21}H_{40}O_4Na$ 379.2824; found, 379.2814.

Preparation of ***3****.* Procedures identical to those used to prepare **7** were used but only using (*R*)-4-hydroxymethyl-2,2-dimethyl-1,3-dioxolane as the starting material. Synthetic **3**: $[\alpha]^{24}_D$ = −2.5 (c 0.09); HRESIMS $[M + Na]^+$ calcd for $C_{21}H_{40}O_4Na$ 379.2824; found, 379.2813.

Preparation of ***19****.* NaH (330 mg, 8.2 mmol, 60% suspension in oil) was washed twice with hexanes (20 mL total) and added to 4 mL of THF to give a suspension that was cooled to 0 °C. To this suspension was added alcohol **9** (271 mg, 2.1 mmol) dissolved in 1 mL of THF along with 15C-5 (90 mg, 0.41 mmol). The mixture was then allowed to stir at RT for 30 min after which bromide **18** (1.0 g, 4.1 mmol) was added dropwise. After 4.5 h, the reaction mixture was cooled to 0 °C and quenched with the addition of H_2O, and the aqueous phase was extracted 3 times with CH_2Cl_2. The organic extracts were dried with $MgSO_4$ and concentrated. The crude mixture was purified with flash chromatography (hexanes/EtOAc 9:1), to give **19** as a clear oil (350 mg, 1.18 mmol, 58%). $[\alpha]^{24}_D$ = +9.9 (c 0.35); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (m, 2H), 7.31 (m, 2H), 7.25 (m, 1H), 4.47 (s, 2H), 4.23 (quin, J = 6.0 Hz, 1H), 4.01 (dd, J = 8.2, 6.5 Hz, 1H), 3.70 (dd, J = 8.2, 6.5 Hz 1H), 3.45 (m, 5H), 3.39 (m, 1H), 1.66 (m, 4H), 1.41 (s, 3H), 1.34 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 138.7, 128.5, 127.7, 127.6, 109.4, 74.9, 72.9, 71.9, 71.6, 70.2, 67.0, 26.9 (2C), 26.5 (2C), 25.5; HRESIMS $[M + Na]^+$ calcd for $C_{17}H_{26}O_4Na$ 317.1729; found, 317.1733.

Preparation of ***20****.* To compound **19** (760 mg, 2.6 mmol) dissolved in 4 mL of EtOH in a round-bottom flask was added 10% Pd/C (200 mg), and the system was flushed with H_2. The reaction mixture was then exposed to 1 atm of H_2 (balloon) overnight. Upon completion, the heterogeneous mixture was filtered and the filtrate washed three times with EtOH (60 mL total). The organic extracts were concentrated, and the crude mixture was purified with flash chromatography (hexanes/EtOAc 2:1), to give **20** as a clear oil (515 mg, 2.52 mmol, 98%). $[\alpha]^{24}_D$ = +12 (c 0.09); ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.20 (quin, J = 5.8 Hz, 1H), 3.99 (dd, J = 8.2, 6.5 Hz, 1H), 3.66 (dd, J = 8.4, 6.3 Hz, 1H), 3.57 (t, J = 5.8 Hz, 2H), 3.46 (m, 3H), 3.39 (m, 1H), 2.59 (bs, 1H), 1.60 (m, 4H), 1.36 (s, 3H), 1.31 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 109.5, 74.7, 71.9, 71.7, 66.9, 62.5, 29.9, 26.8, 26.4, 25.5; HRESIMS $[M + Na]^+$ calcd for $C_{10}H_{20}O_4Na$ 227.1259; found, 227.1265.

Preparation of ***21****.* To Dess–Martin periodinane (622 mg, 1.46 mmol) was added 9 mL of CH_2Cl_2, followed by pyridine (387 mg, 4.9 mmol). To this mixture was added alcohol **20** (190 mg, 0.97 mmol) dissolved on 0.5 mL of CH_2Cl_2, and the solution was stirred at RT for 30 min. The crude mixture was then concentrated and purified with flash chromatography (hexanes/EtOAc 2:1), to give **21** as a clear oil (150 mg, 0.74 mmol, 80%). $[\alpha]^{24}_D$ = +13 (c 0.03); ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.75 (s, 1H), 4.21 (quin, J = 6.0 Hz, 1H), 4.02 (dd, J = 8.4, 6.7 Hz, 1H), 3.66 (dd, J = 8.2, 6.5 Hz, 1H), 3.48 (m, 3H), 3.39 (m, 1H), 2.49 (td, J = 7.2, 1.2 Hz, 2H), 1.89 (quin, J = 6.8 Hz, 2H), 1.39 (s, 3H), 1.33 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.3, 109.5, 74.8, 71.9, 70.6, 66.8, 40.9, 26.8, 25.5, 22.5; HRESIMS $[M + Na]^+$ calcd for $C_{10}H_{18}O_4Na$ 225.1103; found, 225.1098.

Preparation of ***26****.* To phosphonate **22** (353 mg, 1.0 mmol) dissolved in 3.0 mL of THF was added $Ba(OH)_2$ (254 mg, 0.80 mmol), and the suspension was stirred at RT for 30 min. To the suspension was added aldehyde **21** (205 mg, 1.0 mmol), dissolved in 5.2 mL of THF/H_2O (40:1), and it was stirred at RT for 1 h. The reaction mixture was then diluted with H_2O, and the aqueous phase was extracted 3 times with CH_2Cl_2. The organic extracts were dried with $MgSO_4$ and concentrated. The crude mixture was purified with flash chromatography (hexanes/EtOAc 6:1) to give **26** as a clear oil (326 mg, 0.82 mmol, 81%). $[\alpha]^{24}_D$ = +10 (c 0.01); ^{1}H NMR (400 MHz, C_6D_6) δ 6.65 (dt, J = 15.6, 7.2 Hz, 1H), 6.02 (d, J = 15.6 Hz, 1H), 4.12 (quin, J = 5.2 Hz, 1H), 3.83 (dd, J = 6.4, 1.6 Hz, 1H), 3.67 (dd, J = 6.4, 2.0 Hz, 1H), 3.32 (dd, J = 4.8, 4.8 Hz, 1H), 3.19 (dd, J = 6.0, 3.6 Hz, 1H), 3.11 (t, J = 6.8 Hz, 2H), 2.27 (t, J = 7.3 Hz, 2H), 1.96 (q, J = 7.6 Hz, 2H), 1.66 (m, 2H), 1.42 (s, 3H), 1.38 (m, 2H), 1.30 (s, 3H), 1.28 (m, 18H), 0.90 (t, J = 6.8 Hz, 3H); ^{13}C NMR (100 MHz, C_6D_6) δ 198.5, 145.2, 130.8, 109.3, 75.1, 72.1, 70.5, 67.1, 40.4, 32.3, 30.0, 30.0, 29.9, 29.9, 29.7, 29.7, 29.7, 29.1, 28.5, 27.1, 25.6, 24.4, 23.0, 14.3; HRESIMS $[M + Na]^+$ calcd for $C_{24}H_{44}O_4Na$ 419.3124; found, 419.3130.

Preparation of ***8****.* To acetonide **26** (218 mg, 0.55 mmol) dissolved in 8 mL of THF/H_2O (5:1) was added 12.4 M HCl (0.22 mL, 2.7 mmol) and the solution was stirred at RT for 30 min. The reaction mixture was then quenched with the addition of saturated $NaHCO_3$, and the aqueous phase was extracted 3 times with CH_2Cl_2. The organic extracts were dried with $MgSO_4$ and concentrated. The crude mixture was purified with flash chromatography (EtOAc) to give **8** as a white solid (168 mg, 0.47 mmol, 86%). $[\alpha]^{24}_D$ = +2.9 (c 0.17); ^{1}H NMR (600 MHz, C_6D_6) δ 6.69 (dt, J = 14.4, 7.2 Hz, 1H), 6.02 (d, J = 15.6 Hz, 1H), 3.87 (m, 1H), 3.70 (m, 1H), 3.63 (m, 2H), 3.33 (m, 3H), 3.16 (m, 2H), 2.31 (t, J = 6.6 Hz, 2H), 1.99 (q, J = 6.6 Hz, 2H), 1.65 (m, 2H), 1.45 (quin, J = 7.2 Hz, 2H), 1.28 (s, 18H), 0.91 (t, J = 7.2 Hz, 3H); ^{13}C NMR (150 MHz, C_6D_6) δ 199.3, 145.8, 130.7, 72.5, 71.2, 70.5, 64.3, 40.4, 32.3, 30.1, 30.1, 30.0, 30.0, 29.9, 29.8, 29.7, 29.2, 28.3, 24.4, 23.0, 14.3; HRESIMS $[M + Na]^+$ calcd for $C_{21}H_{40}O_4Na$ 379.2835; found, 379.2830.

Preparation of ***4****.* Procedures identical to those used to prepare **8** were employed but only using (*R*)-4-hydroxymethyl-2,2-dimethyl-1,3-dioxolane as the starting material. Synthetic **4**: $[\alpha]^{24}_D$ = −2.7 (c 0.05); HRESIMS $[M + Na]^+$ calcd for $C_{21}H_{40}O_4Na$ 379.2824; found, 379.2816.

Chiral HPLC Analysis of the Sponge-Derived Niphatenones A (3) and B (4). The samples of niphatenones A (**3**) and B (**4**) isolated from the sponge *Niphates digitalis* were analyzed by chiral HPLC using a Daicel Chemical Industries Ltd., Chiralcel OD-H 5 μm, 4.6 × 250 mm column. For niphatenone A (**3**) 3:97 isopropanol/hexanes and for niphatenone B (**4**) 1:19 isopropanol/hexanes were used as eluent (flow rate 1 mL/min), with UV detection at 224 nm. The retention times of the peaks in the chromatographic trace were compared to the retention times and photodiode array UV spectra of the synthetically derived *R* and *S* versions of niphatenones A (**3**) and B (**4**) and by coinjection. Retention times (min) are given in parentheses: (*R*)-niphatenone A (**7**) (31.62); (*S*)-niphatenone A (**3**) (29.75); (*R*)-niphatenone B (**8**) (19.77); (*S*)-niphatenone B (**4**) (18.30). The results of the analysis indicated that the sponge derived niphatenones A (**3**) and B (**4**) both had the *S* configuration.

Preparation of ***24****.* To epoxide **23** (2.55 g, 11.9 mmol) dissolved in 150 mL of MeCN was added NaBr (1.45 g, 14.1 mmol) followed by cerium(III) chloride heptahydrate (5.26 g, 14.1 mmol), and the solution was stirred at RT for 24 h. The reaction mixture was then concentrated and extracted three times with EtOAc (150 mL total). The EtOAc fractions we combined, concentrated in vacuo, and purified with flash chromatography (hexanes/EtOAc gradient 15:1, 10:1, and 7:1) to give bromohydrin **24** as a white solid (3.19 g, 10.9 mmol, 92%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.78 (bs, 1H), 3.53 (dd, J = 6.8, 3.2 Hz, 1H), 3.38 (dd, J = 6.8, 3.2 Hz, 1H), 2.26 (m, 1H), 1.53

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(m, 2H), 1.26 (m, 20H), 0.88 (t, J = 7.2 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 71.2, 40.8, 35.3, 32.1, 29.83/29.80/29.72/29.67/29.52 (all five signals account for a total of 7C), 25.7, 22.8, 14.3. Elemental analysis: theoretical, 57.33% C and 9.97% H; found, 57.51% C and 10.04% H.

Preparation of ***25****.* To bromohydrin **24** (158 mg, 0.54 mmol) dissolved in 6 mL of CH_2Cl_2 at RT was added Dess–Martin periodinane (455 mg, 1.07 mmol), and the mixture was stirred for 1 h. The reaction mixture was then quenched with the addition of saturated $NaHCO_3$, and the aqueous phase was extracted 3 times with CH_2Cl_2. The organic extracts were dried with $MgSO_4$ and concentrated in vacuo. The crude mixture was purified with flash chromatography (hexanes/EtOAc 30:1), to give **25** as a white solid (126 mg, 0.43 mmol, 100%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.89 (s, 2H), 2.64 (t, J = 7.4 Hz, 2H), 1.60 (m, 2H), 1.26 (m, 18H), 0.88 (t, J = 6.7 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 202.2, 39.9, 34.4, 32.0, 31.7, 29.8, 29.7, 29.6(2C), 29.5, 29.2, 24.0, 22.9, 14.2; HRESIMS $[M + Na]^+$ calcd for $C_{14}H_{27}ONa^{79}Br$ 313.1143; found, 313.1136.

Preparation of ***22****.* To bromide **25** (80 mg, 0.27 mmol) dissolved in 1 mL of toluene was added triethylphosphite (228 mg, 1.4 mmol). The reaction mixture was refluxed for 24 h, after which it was cooled to RT and concentrated under a stream of N_2. The crude mixture was purified with flash chromatography (hexanes/EtOAc 1:1), to give **22** as a clear oil (89 mg, 0.25 mmol, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.09 (t, J = 7.2 Hz, 4H), 3.02 (m, 2H), 2.56 (t, J = 7.2 Hz, 2H), 1.53 (m, 2H), 1.28 (t, J = 7.0 Hz, 6H), 1.19 (m, 18H), 0.82 (t, J = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 202.3, 62.6, 44.2, 43.1, 41.8, 32.0, 29.7, 29.6(2C), 29.5, 29.48, 29.43, 29.1, 23.5, 22.8, 16.4, 16.3, 14.2; HRESIMS $[M + Na]^+$ calcd for $C_{18}H_{38}O_4P$ 349.2508; found, 349.2517.

AR Transcriptional Activity. AR transcriptional activity was measured using the PSA (6.1 kb)–luciferase reporter gene construct transiently transfected into LNCaP human prostate cancer cells which express functional AR. This reporter contains several well-characterized androgen response elements to which the AR specifically binds to increase luciferase activity in response to androgen such as R1881. LNCaP (2.5×10^4 cell/well) cells were seeded on 24-well plates overnight before transfection with PSA (6.1 kb)-luc, (0.5 μg/well) in serum-free, phenol red-free media using lipofectin (Invitrogen) according to published methods.[4b] For SAR analysis, LNCaP cells in 12-well plates were pretreated 1 h with (*S*)-niphatenone B (**4**), (*R*)-niphatenone B (**8**), or their SAR analogues (all added at 7 μM, with the exception of **32**, which was tested at 3.5 μM due to solubility limitations) prior to the addition of 1 nM R1881. After 48 h of exposure, cells were harvested, luciferase activity measured, and normalized to protein concentration determined by the Bradford assay.

Proliferation Assays. Experiments using LNCaP human prostate cancer cells were done in phenol red-free RPMI 1640 medium with 0.5% (v/v) fetal bovine serum, while for PC3 human prostate cancer cells, they were done in phenol red DMEM medium with 5% (v/v) fetal bovine serum. Both media were supplemented with 100 units/mL penicillin and 100 mg/mL streptomycin. Cells were seeded in 96-well plates for 24 h before pretreatment for 1 h with bicalutamide (10 μM), (*S*)-niphatenone B (**4**), and (*R*)-niphatenone B (**8**) (~14 μM) before treatment with 0.1 nM R1881 for LNCaP cells. LNCaP cells were incubated for 72 h with R1881, while the duration of the experiment was 24 h for PC3 cells. BrdU was added to the cells for an additional 2 h. Cells were fixed prior to incubation for 1.5 h with the anti-BrdU-POD antibody (Roche). BrdU incorporation was measured at 570 nm via VersaMax ELISA Microplate Reader (Molecular Devices).

Expression and Purification of Recombinant Protein. AR AF1 recombinant protein was expressed and purified as described previously.[3,16] The Ni^{2+}-agarose affinity chromatography purified recombinant AR AF1 protein was further purified by size exclusion chromatography.

In Vitro Binding. The binding reaction was carried out by incubating 10 μM AR AF1 protein with 20 μM compound **44** containing an alkyne group solubilized in DMSO, or just DMSO, on ice for 50 min. The binding reaction was diluted in half (i.e., 5 μM AR AF1 protein and 10 μM compound **44**) for fluorescein labeling on compound. Labeling was done by a Click chemistry reaction with 10 μM fluorescein azide, 0.1 mM ascorbic acid, and 0.1 mM copper(II)–TBTA complex (Lumiprobe) and incubated at RT for 30–40 min. Samples were separated on 12.5% SDS–PAGE. Fluorescein was detected by an image analyzer (Fujifilm FLA-7000, GE Healthcare). The same gel was stained with Coomassie blue.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures for the synthesis of the niphatenone analogues and NMR spectra for natural **3** and **4** and all synthetic compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

AR, androgen receptor; CRPC, castration recurrent prostate cancer; PC3, androgen independent human prostate cancer cell line; LNCaP, androgen-sensitive human prostate cancer cell line; SAR, structure–activity relationship; PSA, prostate-specific antigen; LBD, ligand binding domain; DBD, DNA binding domain; NTD, *N*-terminus domain; AF-1, activation function-1; POP, proof of principle; MeOH, methanol; EtOH, ethanol; EtOAc, ethyl acetate; CH_2Cl_2, dichloromethane; MeCN, acetonitrile; SCUBA, self-contained underwater breathing apparatus; IL6, interleukin-6; HPLC, high peformance liquid chromatography; HRESIMS, high resolution electrospray mass spectrometry; NMR, nuclear magnetic resonance; COSY, correlation spectroscopy; HSQC, heteronuclear single-quantum correlation; HMBC, heteronuclear multiple bond correlation; THF, tetrahydrofuran; HWE, Horner–Wadsworth–Emmons; DMP, Dess–Martin periodinane; PEG, polyethylene glycol; RT, room temperature; h, hour; min, minutes; PKA, protein kinase A; ELISA, enzyme-linked immunosorbent assay; BrdU, bromodeoxyuridine; DMEM, Dulbecco's modified Eagle's medium; RPMI, Roswell Park Memorial Institute; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; TBTA, tris[(1-benzyl-1*H*-1,2,3-triazol-4-yl)methyl]amine; 15c5, 1,4,7,10,13-pentaoxacyclopentadecane

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■ NOTE ADDED AFTER ASAP PUBLICATION

After this paper was published online December 28, 2011, a spelling error was corrected in the name of author Nicole J. de Voogd. The revised version was published January 4, 2012.