Articles

Phenyl β-Methoxyacrylates: A New Antimalarial Pharmacophore

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Phenyl β-methoxyacrylates, linked to an aromatic ring via an olefinic bridge, have been identified as novel, potentially inexpensive, antimalarial agents. The compounds are believed to exert their activity by inhibition of mitochondrial electron transport at the cytochrome bc_1 complex. A series of compounds have been synthesized to define structure−activity relationships affecting antimalarial activity. It was found that the β-methoxyacrylate was required *ortho* to the linker and the optimal bridge was (*E*,*E*)-butadiene. Compounds in which the second aromatic ring was *ortho*-substituted or *ortho*,*para*-disubstituted gave optimal potency. Several compounds were identified with potency that is superior to that of chloroquine both in culture and in a murine malaria model.

Introduction

The rapidly increasing resistance of *Plasmodium falciparum* malaria parasites to commonly used inexpensive drugs, such as chloroquine and sulfadoxine-pyrimethamine, and the recent appearance of resistance to more expensive chemotherapeutic agents and prophylactic drugs, such as mefloquine and halofantrine, indicate the urgent need for new classes of antimalarial compounds.[1−4]

Atovaquone (**1**) is a hydroxynaphthoquinone analogue that was recently developed as an antimalarial drug in combination with proguanil.[5,6] This compound is an analogue of ubiquinone which is a critical component in mitochondrial electron transfer. It has been demonstrated that atovaquone inhibits the transfer of electrons at the cytochrome *c* reductase complex (cytochrome bc_1) of malaria parasites.[7] This results in the collapse of the mitochondrial membrane potential in the parasite.[8] A further consequence is the inhibition of parasite dihydroorotate dehydrogenase, which transfers electrons to cytochrome bc_1 and requires a functional mitochondrial electron-transport chain.[9,10] This enzyme is a key component in pyrimidine biosynthesis, and due to its inhibition parasites are no longer able to synthesize and replicate their DNA.

Although the atovaquone plus proguanil combination is effective against malaria, atovaquone's high cost is likely to limit its widespread use in disease-endemic countries, especially those of sub-Saharan Africa. Less-expensive inhibitors of the malaria cytochrome *c* reductase complex might therefore find application as antimalarial therapies.

Another class of compounds known to inhibit cytochrome *c* reductase complex are the β-methoxyacrylates, which have found use as agrochemical agents.[11−14] They have not been previously reported to inhibit human pathogens, but their ability to inhibit the mitochondrial respiration process of certain fungal plant pathogens and insects is widely reported. We rationalized that β-methoxyacrylates could constitute a new class of antimalarial agents and proceeded to investigate this possibility.

A series of β-methoxyacrylates was screened for antimalarial activity, both in culture against *P. falciparum*-infected erythrocytes, and in a rodent *P. berghei* model. Full details of our methodology have been reported previously.[15] Compound **2** was found to have reasonable antimalarial activity in culture, with a 50% inhibitory concentration (IC_{50}) against the chloroquine-resistant *P. falciparum* strain K1 of 29 ng/mL and an IC_{50} against the chloroquine-sensitive *P. falciparum* strain NF54 of 5.5 ng/mL. No in vivo activity was observed in the rodent *P. berghei* model. Consequently, we initiated a synthesis program to explore structure−activity relationships (SAR) of the new lead compound with the objective of improving the in vitro activity and obtaining oral in vivo activity.

In this paper we describe the effects of modifying the linker, the substituent pattern on the aromatic rings A + B, and the isosteric replacement of β-methoxyacrylate. We report here the synthesis and antimalarial activities of a series of β-methoxyacrylates to define the optimal structural features of this novel antimalarial pharmacophore.

Chemistry

Compounds **2** and **7** were prepared from **3a**[16] and **4a**,[17] respectively, as outlined in Scheme 1. Thus, nucleophilic displacement of bromine ion **3a** with *o*-trifluorothiophenol (**5**) gave the phenylsulfanyl acrylate **2**, and a Wittig−Horner reaction between phosphonate **4a** and *o*-(trifluoromethyl)benzaldehyde (**6**) gave the ethylene-bridged acrylate **7**.

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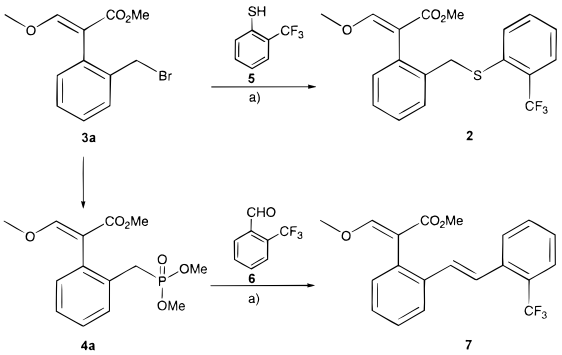
Scheme 1[a]



[a] (a) NaH, THF.

Similarly compounds **12**, **15a**−**o**, and **18** were made as depicted in Schemes 2 and 3. The intermediate aromatic propenals **14a**−**o** were prepared by Wittig oxopropenylation of the appropriate benzaldehyde derivatives using (1,3-dioxan-2-ylmethyl)tributylphosphonium bromide.[18] Catalytic hydrogenation converted butadiene **15a** to butane **16**.

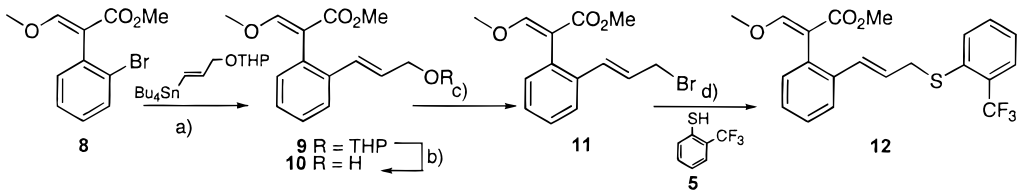
The methoxyiminoacetate derivatives were made as shown in Schemes 4 and 5. *o*-(Trifluoromethyl)benzaldehyde (**6**) was transferred to *gem*-dibromoolefin **19** which, in turn, was treated with diethyl phosphite in the presence of triethylamine[19] to give monobromoalkene **20** (*trans:cis* = 8:1).

Vinylstannane **24** was prepared from **23**[20] as an inseparable mixture of *E*- and *Z*-isomers (1:1) using the radical hydrostannylation condition (Bu_3SnH/AIBN). When hydrostannylation was performed in the presence of Pd(0)/Bu_3SnH, only regioisomer **25** was obtained. Coupling vinylstannane **24** with vinyl bromide **20** gave (*E*,*E*)-butadiene **26**. Butenyne derivative **27** was made by coupling vinyl bromide **20** with alkyne **23** (Scheme 4) using $Pd_2[diba]_3$ and CuI in the presence of triethylamine.

Although oxime **23** has been reported,[20] no final proof for the assigned *Z*-stereochemistry of the oxime moiety was given. To obtain both isomers for comparison by ^{1}H NMR, we attempted to isomerize oxime **23** with various acids but were unsuccessful. Compound **23** either was recovered unchanged or underwent decomposition. However, in the reaction of methoxylamine with acetylene **29**, a 6:1 mixture of two isomers was obtained which could be separated by column chromatography. The major product had an ^{1}H NMR spectrum identical to that of **23**. The major difference in ^{1}H NMR spectra of both isomers is the chemical shift values of the acetylenic proton: the minor product resonates at 3.27 ppm, whereas the major product resonates at 3.17 ppm. Irradiation of OMe protons in the ^{1}H NMR spectra in both isomers, unfortunately, did not show any NOE effects. However, it is fair to assume that the trimethylsilyl group on the acetylene in **21** leads to exclusive formation of the *Z*-isomer (assigned as **23**) due to large steric repulsion, whereas the smaller proton of acetylene **29** allows also some formation of the *E*-isomer (assigned as **30**).

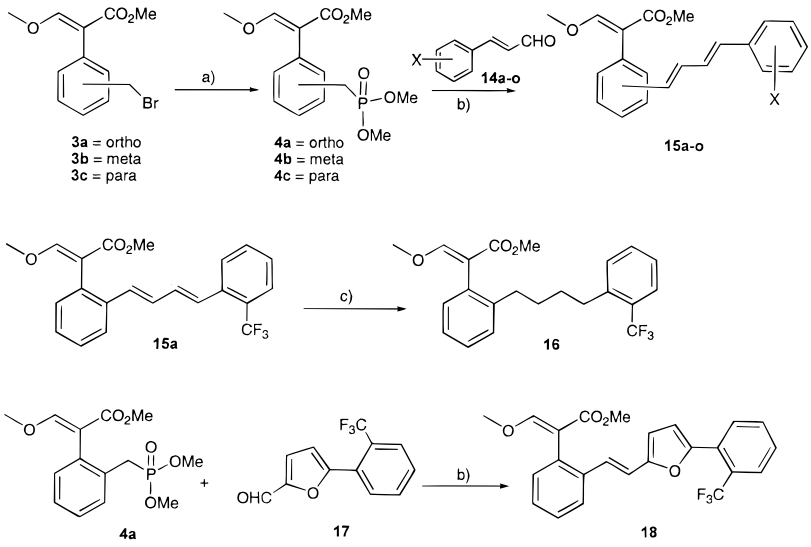
Different olefin bridges were also prepared, as outlined in Scheme 5. Oximes **23** and **30** were coupled with bromoalkyne **28**, prepared by the dehydrobromination of dibromoolefin **19** using Ratovelomanana's condition,[21] in the presence of Pd[diba]$_3$/CuI to afford butadiynes **31** and **32**, respectively. Treatment of butadiyne **31** with

Scheme 2[a]



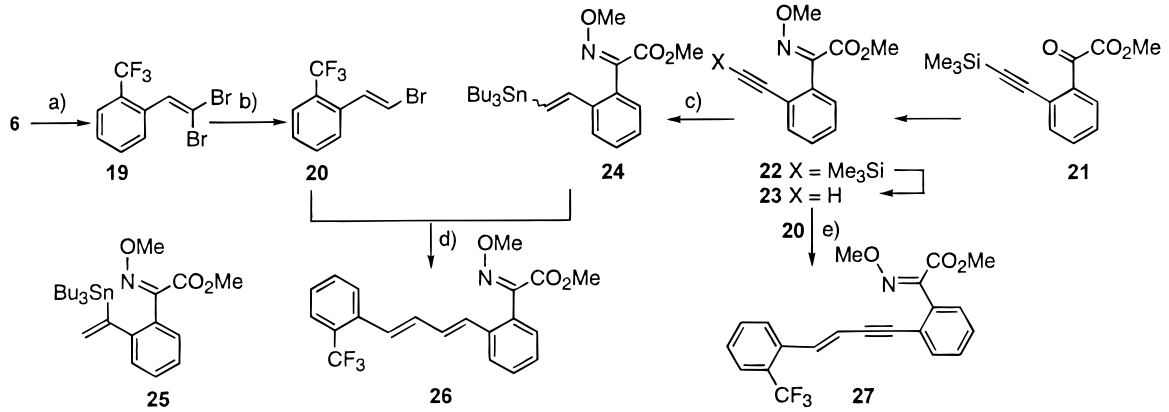
[a] (a) $Pd[PPh_3]_4$, toluene; (b) toluene-4-sulfonic acid, EtOH; (c) CBr_4, PPh_3, Et_2O; (d) NaH, THF.

Scheme 3[a]



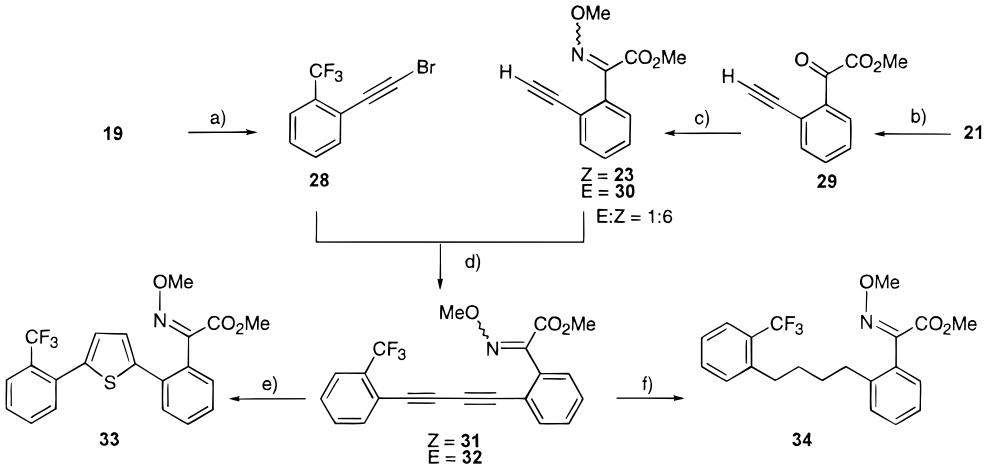
[a] (a) $P(OMe)_3$, toluene; (b) NaH, THF; (c) Pd/C, H_2, CH_2Cl_2.

Scheme 4[a]



[a] (a) CBr_4, PPh_3; (b) $HPO(EtO)_2$, Et_3N; (c) Bu_3SnH, AIBN; (d) $Pd[PPh_3]_4$, toluene; (e) Et_3N, $Pd_2[diba]_3$, CuI, toluene.

Scheme 5[a]



[a] (a) DBU, DMSO; (b) Bu_4NF, THF; (c) $MeONH_2.HCl$, NaOAc, MeOH, H_2O; (d) Et_3N, CuI, $Pd_2[diba]_3$; (e) $Na_2S{\cdot}9H_2O$, MeOEtOH, H_2SO_4, MeOH; (f) 10% Pd/C, H_2, CH_2Cl_2.

$Na_2S{\cdot}9H_2O$ in 2-methoxyethanol followed by H_2SO_4/MeOH gave thiophene **33**. Reduction of both triple bonds in **30** with H_2/Pd yielded **34**.

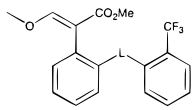
Biological Results and Discussion

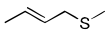
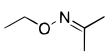
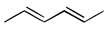
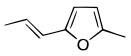
All new compounds were evaluated for antimalarial activity in vitro (i.e. in culture against parasite-infected erythrocytes) against chloroquine-sensitive (NF54) and -resistant (K1) strains of *P. falciparum*. They were also tested for in vivo efficacy in a rodent *P. bergehei* model (both po and sc) using a single-dose application of 50 or 100 mg/kg, 24 h after infection. The dose of those compounds showing in vivo activity was titrated down to a dose of 10 mg/kg in order to investigate differences. The methodology used for these studies has previously been described in detail.[15] If in vivo efficacy in the rodent model, as measured by growth inhibition (GI) of parasites, was >99% compared to control untreated mice 72 h after infection, further studies were undertaken to determine the effective doses for parasite reduction in the mice of 50% or 90%, respectively ($ED_{50/90}$ values).

First we investigated the influence of the linker on antimalarial activity, keeping the 2-trifluoromethyl substituent on ring B constant (Table 1). All compounds in this series showed an enhancement of in vitro activity over the lead compound **2**, against both sensitive (NF54) and resistant (K1) strains of *P. falciparum*. Consistent improvement of in vitro activity was observed upon increasing the length of the linker from two to four atoms. In some cases this also resulted in a demonstrable in vivo activity. The (*E*,*E*)-butadiene-linker (compound **15a**) conferred the best activity. Conjugation through the linker appears beneficial for activity as indicated by the lower potency of the saturated derivative **16** in comparison to **15a**, in both in vitro and in vivo models. Substitution of one of the double bonds in **15a** by a heterocycle, such as furan in **18**, gave derivatives that had good in vitro activity but which were toxic when applied to mice. Compound **15a** exhibited 140-fold increase in potency against *P. falciparum* in vitro compared to the lead compound **2**. In correlation with its excellent in vitro activity, **15a** also gave the best results for in vivo efficacy.

In the next step, the substituents as well as the substitution pattern on ring B were optimized, keeping the (*E*,*E*)-butadiene linker constant (Table 2). Replacement of the trifluoromethyl group in **15a** with a chloro substituent did not improve potency but retained some in vivo activity in contrast to strongly electron-withdrawing substituents such as the cyano group (**15d**). Compounds having corresponding substituents in the *meta*- or *para*-position (**15e**−**h**) exhibited diminished potency and had no in vivo activity. A series of disubstituted analogues (**15i**−**m**) were also prepared and

Table 1. Antimalarial Activities of β-Methoxyacrylates with Different Linkers Against Chloroquine-Sensitive (NF54) and Chloroquine-Resistant (K1) *P. falciparum* (in vitro) and *P. berghei* (in vivo)[a]



No.	L	in vitro		in vivo	
		IC50[ng/ml]		GI%	
		NF54	K1	po	sc
2		5.50	29.0	0	0
7		3.01	7.80	0	0
12		1.75	2.51	46	91
13		0.17	0.65	0	0
15a		0.06	0.15	100	100
16		0.54	1.53	82	99
18		0.39	1.8	tox	tox

[a] The dose used in the in vivo experiments was 100 mg/kg. GI, growth inhibition; tox, toxic; sc, subcutaneous; po, per os.

tested. In general, the *o*-*β*-methoxyacrylate derivatives with *o*-*p*-disubstitution pattern on ring B were extremely potent, in vitro and in vivo. In particular, **15i**−**k** warranted further investigation.

The substitution pattern on ring A was also evaluated, shifting the *β*-methoxyacrylate pharmacophore into *meta*- and *para*-positions (Table 3). The results clearly demonstrated the superiority of *o*-*β*-methoxyacrylates.

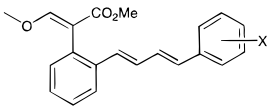
In addition, we investigated structural modifications in the pharmacophore. Isosteric replacement of carbon by nitrogen led to methoxyimino analogues such as **26** (Table 4). Many of these compounds gave reasonable activity in vitro. However, none displayed significant in vivo activity, and so they were not pursued further.

The $ED_{50/90}$ values were determined for the most active compounds, and these are presented, together with their in vitro IC_{50} values, in Table 5 and are compared to both chloroquine and atovaquone. An outstanding feature of this series is their potent in vitro activity, surpassing that of chloroquine by a factor of 100. These are among the lowest IC_{50} values we have ever recorded. The in vivo potency also exceeds that of chloroquine in the rodent *P. berghei* model, but to a lesser extent. A compound which incorporated both the butadiene linker and the 2,4-bis-trifluoromethyl substituents (**15i**) was found to be the most potent compound of the series with an $ED_{50/90}$ value of 0.42/1.44 mg/kg (po) and 0.28/0.96 mg/kg (sc). The $ED_{50/90}$ value of compound **15i** is superior to that of chloroquine by a factor of 5 but is a factor of 10 lower than that for atovaquone.

Conclusion

This work has identified a new class of compounds with antimalarial activity. A series of compounds have been designed, synthesized, and subjected to pharmacological evaluation. Some compounds have been identified with very potent oral antimalarial activities. SAR confirmed the role of the *β*-methoxyacrylate as pharmocophore. Several criteria have been established for the further design of *β*-methoxyacrylate analogues: i.e., (a) *o*-*β*-methoxyacrylate on the aromatic ring A, (b) 2,4-substitution pattern on the aromatic ring B, and (c) (*E*,*E*)-butadiene linker.

Table 2. Antimalarial Activities of β-Methoxyacrylates **15**, Containing a Butadiene Linker, Against Chloroquine-Sensitive (NF54) and Chloroquine-Resistant (K1) *P. falciparum* (in vitro) and *P. berghei* (in vivo)[a]



No.	X	in vitro		in vivo		
		IC50 [ng/ml]			GI%	
		NF54	K1	mg/kg	po	sc
15a	2-CF_3	0.06	0.15	10	99	100
15b	H	0.80	3.7	50	0	0
15c	2-Cl	0.09	0.39	10	41	77
15d	2-CN	0.52	1.60	100	0	0
15e	3-F	1.5	8.4	50	0	0
15f	3-CF_3	7.8	16.7	50	0	0
15g	3-Br	6.1	31.4	50	0	0
15h	4-Cl	0.52	2.0	50	0	0
15i	2,4-di-CF_3	0.06	0.13	10	100	100
15j	2,4-di-Cl	0.03	0.10	10	100	100
15k	2,4-di-Me	0.03	0.05	10	100	100
15l	2-Cl,4-F	0.06	0.19	10	92	100
15m	3-MeO,2-NO_2	0.11	0.58	100	98	tox

[a] For the in vivo experiments the compounds were initially tested at either 50 or 100 mg/kg. If growth inhibitory activity was greater than 99%, they were further tested at 10 mg/kg and this value was recorded.

Table 3. Antimalarial Activities of β-Methoxyacrylates **15l**,**t**,**u** Against Chloroquine-Sensitive (NF54) and Chloroquine-Resistant (K1) *P. falciparum* (in vitro) and *P. berghei* (in vivo)

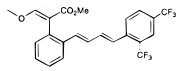
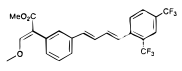
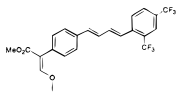
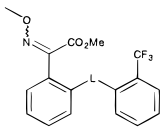
No.	Structure	in vitro		in vivo		
		IC50 [ng/ml]			GI%	
		NF54	K1	mg/kg	po	sc
15i		0.06	0.13	100	100	99.97
15n		175.9	396.4	100	0	0
15o		>5000	>5000	100	0	0

Table 4. Antimalarial Activities of α-Methoxyiminoacetates with Different Linkers Against Chloroquine-Sensitive (NF54) and Chloroquine-Resistant (K1) *P. falciparum* (in vitro) and *P. berghei* (in vivo)



No.	Config.	L	in vitro IC50 [ng/ml]		in vivo GI%		
			NF54	K1	mg/kg	po	sc
26	Z		0.4	1.4	100	46	44
27	Z		3.2	5.7	10	0	0
31	Z		10.8	25.8	100	0	0
32	E		1387	2276	100	0	0
33	Z		23.7	60.8	100	0	0
34	Z		6.4	15.0	100	0	0

Table 5. $ED_{50/90}$ on *P. berghei* (in vivo) and Antimalarial Activities Against Chloroquine-Sensitive (NF54) and Chloroquine-Resistant (K1) *P. falciparum* (in vitro) of the Most Active Compounds Compared to Chloroquine and Atovaquone

No.	$ED_{50/90}$, mg/kg po	$ED_{50/90}$, mg/kg sc	in vitro IC50 [ng/ml]	
			NF54	K1
15a	2.16/6.66	0.6/1.4	0.06	0.15
15c	n.t.	1.55/25.64	0.09	0.39
15i	0.42/1.44	0.28/0.96	0.06	0.13
15j	1.07/4.03	0.69/1.8	0.03	0.10
15k	1.11/2.65	0.33/0.72	0.03	0.05
Chloroquine	2.4/5.0	1.5/3.3	6.4	160.8
Atovaquone	0.05/0.1	0.04/0.9	0.11	0.41

Literature evidence suggests that β-methoxyacrylates act on the cytochrome bc_1 complex similarly to atovaquone.[11−14] Preliminary data also confirm that they act by this mechanism against malaria parasites (Arnulf Dorn, unpublished data).

The recent success of atovaquone in combination with proguanil as a malaria therapy[6] further demonstrates that the β-methoxyacrylates, either alone or in combination, could become valuable antimalarial drugs. The compounds have the notable advantage over atovaquone of a less-expensive synthetic route. Thus, whereas atovaquone−proguanil is limited to high-cost therapy, and potentially prophylaxis, β-methoxyacrylates have the potential for use in poorer populations, such as in sub-Saharan Africa.

On the basis of the studies outlined here, compounds **15i**−**k** were selected for further investigations, which will be reported elsewhere.

Experimental Section

General Methods. TLC was performed on silica gel plates (Merck, silica gel 60 F254). Column chromatography was carried out with Merck silica gel, 40−63 μm, 230−400 mesh. Melting points were determined on a Buchi 510 apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker FT AC-250 instrument (250 MHz); chemical shifts are reported as δ values (ppm) downfield from internal Me_4Si in the solvent shown. IR spectra were recorded on a Nicolet FT IR20 SX spectrophotometer. EI mass spectra were recorded on a Perking Elmer Siex API III instrument.

Methyl (*E*)-3-Methoxy-2-[(2-trifluoromethylphenylsulfanylmethyl)phenyl]acrylate (2). Sodium hydride (85% suspension in mineral oil, 74 mg, 2.62 mmol) was added to a solution of 2-(trifluoromethyl)thiophenol (190 μL, 1.05 mmol) in THF (5 mL). The mixture was stirred at room temperature for 10 min and treated with **3a** (250 mg, 0.87 mmol) for 1 h. After treatment with acetic acid, the reaction mixture was diluted with ethyl acetate and brine. The organic phase was separated and dried over Na_2SO_4. Evaporation of the solvent afforded **2** (330 mg, 98%) as white crystals: mp 92−93 °C; IR (KBr) 2924, 1708, 1634 cm^{-1}; MS (EI) 350 (M − MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.70 (s, 3H, MeO), 3.83 (s, 3H, MeO), 4.04 (2H, s), 7.11 (1H, d), 7.25 (6H, m), 7.60 (1H, s), 7.62 (1H, d) ppm.

Methyl (*E*)-2-[2-[(*E*)-2-(2-Trifluoromethylphenyl)vinyl]-phenyl]-3-methoxyacrylate (7). Sodium hydride (21 mg, 0.88 mmol) was added to a cooled (0 °C) solution of **4a** (233 mg, 0.74 mmol) in THF (2 mL). The cooling bath was removed and the mixture stirred at room temperature for 20 min. *o*-(Trifluoromethyl)benzaldehyde (**6**) (97 μL, 0.81 mmol) was added and the mixture heated to 60 °C for 4 h. The reaction mixture was allowed to cool to room temperature, treated with acetic acid (1 mL), diluted with ethyl acetate, washed with brine, and dried over Na_2SO_4. Evaporation of the solvent and purification of the residue by column chromatography (7:1 hexane/ethyl acetate eluant) afforded **7** (100 mg, 37%) as white crystals: mp 99−100 °C; IR (KBr) 1705, 1629 cm^{-1}; MS (EI) 62 (M); ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.67 (s, 3H, MeO), 3.79 (s, 3H, MeO), 7.00 (1H, d, J = 16 Hz), 7.25 (5H, m), 7.50 (1H, t), 7.62 (1H, s), 7.64 (3H, m) ppm.

Methyl (*E*)-3-Methoxy-2-{(*E*)-2-[3(*R*)- and 3-(*S*)-(tetrahydropyran-2-yloxy)propenyl]phenyl}acrylate (9). Tetrakis(triphenylphosphine)palladium (58 mg, 0.05 mmol) was added to a solution of (*E*)-tributyl-[3(*RS*)-(tetrahydropyran-2-yloxy)propenyl]stannane (2.2 g, 5.1 mmol) and **8** (1.38 g, 5.1 mmol) in toluene (20 mL). The mixture was stirred at 110 °C for 2 days, cooled to room temperature, extracted with toluene, washed with brine, and dried over Na_2SO_4. Evaporation of the solvent and purification of the residue by column chromatography (9:1 hexane/ethyl acetate eluant) afforded **9** (884 mg, 52%) as white crystals: mp 66−68 °C; IR (KBr) 1711, 1633 cm^{-1}; MS (EI) 216 (M − MeOH − dihydropyran); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.8 (6H, m), 3.47 (1H, m), 3.67 (3H, s), 3.80 (3H, s), 3.90 (1H, m), 4.17 (1H, dd, J = 12, 6.8 Hz), 4.55 (1H, dd, J = 12, 5.0 Hz), 4.70 (1H, t), 6.22 (1H, ddd, J = 16.0, 6.8, 5.0 Hz), 6.6, (1H, d, J = 16.0 Hz), 7.1 (1H, d), 7.26 (1H, m), 7.57 (1H, d) ppm.

Methyl (*E*)-2-[2-[(*E*)-3-Hydroxypropenyl]phenyl]-3-methoxyacrylate (10). Toluene-4-sulfonic acid monohydrate (1.11 g, 0.4 mmol) was added to a solution of **9** (4.86 g, 14 mmol) in ethanol (70 mL). The mixture was stirred for 6 h and neutralized with saturated K_2CO_3 solution. The solvent was evaporated and the residue treated with ethyl acetate. The organic phase was separated, washed with brine, and dried over Na_2SO_4. Evaporation of the solvent afforded **10** (3.4 g, 93%) as white crystals: mp 78−80 °C; IR (KBr) 1683, 1619 cm^{-1}; MS (EI) 216 (M − MeOH); ^{1}H NMR (250 MHz, $CDCl_3$) δ 1.52 (OH, t, J = 6.0 Hz), 3.67 (3H, s), 3.8 (3H, s), 4.26 (2H, m), 6.3 (1H, td, J = 15.0, 6.0 Hz), 6.55 (1H, d, J = 15.0 Hz), 7.1 (1H, d, J = 8.0 Hz), 7.25 (2H, m), 7.58 (1H, d), 7.59 (1H, s) ppm.

Methyl (*E*)-2-[2-[(*E*)-3-Bromopropenyl]phenyl]-3-methoxyacrylate (11). A solution of **10** (3.84 g, 14 mmol) in diethyl ether (60 mL) was added to a solution of carbon tetrabromide (6.26 g, 18.2 mmol) and triphenylphosphine (4.96 g, 18.2 mmol) in diethyl ether (30 mL). The resulting white suspension was stirred for 20 h and filtered through a pad of Celite. Evapora-

tion of the solvent and purification of the residue by column chromatography (9:2 hexane/ethyl acetate eluant) afforded **11** (3 g, 63%) as white crystals: mp 88 °C; IR (KBr) 1708, 1632 cm^{-1}; MS (EI) 231 (M − Br); ^1H NMR (250 MHz, $CDCl_3$) δ 3.69 (3H, s), 3.83 (3H, s), 4.12 (2H, d, J = 7.5 Hz), 6.32 (1H, td, J = 15.0 Hz), 6.62 (1H, d, J = 15.0 Hz), 7.1 (1H, d, J = 8.0 Hz), 7.25 (2H, m), 7.58 (1H, d), 7.6 (1H, s) ppm.

Methyl (*E*)-3-Methoxy-2-[2-[(*E*)-3-(2-trifluoromethylphenylsulfanyl)propenyl]phenyl]acrylate (12). Sodium hydride (85% suspension in mineral oil, 24 mg, 0.85 mmol) was added to a solution of **5** (81 mg, 0.45 mmol) in THF (1 mL). The mixture was stirred for 10 min and treated with **11** (127 mg, 0.38 mmol). After stirring 10 min, acetic acid was added and diluted with ethyl acetate. The organic phase was washed with brine and dried over Na_2SO_4. Evaporation of the solvent afforded **12** (147 mg, 0.36 mmol, 95%) as a colorless oil: IR (neat) 2948, 1708, 1633, 1593 cm^{-1}; MS (EI) 408 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 3.65 (3H, s), 3.73 (2H, d), 3.77 (3H, s), 6.2 (1H, dt), 6.4 (1H, d), 7.11 (1H, d), 7.25 (4H, m), 7.48 (2H, m), 7.53 (1H, s), 7.62 (1H, d) ppm.

Methyl (*E*)-2-[3-(Dimethoxyphosphorylmethyl)phenyl]-3-methoxyacrylate (4b). 4a[17] was prepared according to the method described for **4b.** At room temperature, a catalytic amount of 2,2′-azobis(isobutyronitrile) was added to a solution of methyl 2-(3-methylphenyl)-3-methoxyacrylate (4 g, 19.4 mmol) and *N*-bromosuccinimide (3.9 g, 21.3 mmol) in carbon tetrachloride (100 mL). The mixture was refluxed for 4 h, cooled to 0 °C, and filtered. The filtrate was evaporated and the residue dissolved in toluene (2.5 mL). Trimethyl phosphite (2.5 mL, 21.3 mmol) was added and the solution heated 20 h under reflux. The solvent was evaporated and the product was purified by column chromatography (ethyl acetate) to afford **4b** (850 mg, 14%) as white crystals:^1H NMR (250 MHz, $CDCl_3$) δ 3.17 (2H, d, J = 21.0 Hz), 3.64 (3H, s), 3.68 (3H, s), 3.73 (3H, s), 3.85 (3H, s), 7.26 (4H, m), 7.55 (1H, s) ppm.

Methyl (*E*)-2-[4-(dimethoxyphosphorylmethyl)phenyl]-3-methoxyacrylate (4c) was obtained analogously to **4b**: ^1H NMR (250 MHz, $CDCl_3$) δ 3.15 (2H, d, J = 21.0 Hz), 3.66 (3H, s), 3.7 (3H, s), 3.73 (3H, s), 3.84 (3H, s), 7.28 (4H, m), 7.54 (1H, s) ppm.

(*E*)-3-(2-Chloro-4-fluorophenyl)propenal (14l). Sodium methoxide (266 mg, 4.92 mmol) was added to a solution of (1,3-dioxan-2-ylmethyl)tributylphosphonium bromide (5.7 mL, 1 M in DMF, 5.7 mmol) and 2-chloro-4-fluorobenzaldehyde (600 mg, 3.78 mmol) in 10 mL of THF. The mixture was heated 6 h at 90 °C and poured into water. The phases were separated and the aqueous phase was extracted with diethyl ether. The combined organic phases were washed with brine and dried over Na_2SO_4. The solvent was evaporated and the residue was dissolved in THF (10 mL) and treated with 2 N HCl (10 mL). After stirring for 45 min at room temperature, THF was evaporated under reduced pressure, and the remaining aqueous solution was extracted with diethyl ether. The organic phase was washed with brine and dried over Na_2SO_4. Evaporation of the solvent and purification of the residue by column chromatography (2:1 hexane ethyl acetate eluant) afforded **14l** (385 g, 55%) as colorless crystals: mp 83 °C; IR (KBr) 1689, 1598 cm^{-1}; MS (EI) 180 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 6.65 (1H, dd, J = 7.5, 15.0 Hz), 7.07 (1H, td), 7.21 (1H, dd, J = 3.0, 8.0 Hz), 7.66 (1H, dd, J = 5.0, 7.5 Hz), 7.9 (1H, d, J = 15 Hz), 9.75 (1H, d, J = 7.5 Hz) ppm.

General Procedure for the Preparation of Compounds of Type 15: Methyl (*E*)-2-[2-(*E*,*E*)-[4-(2,4-Bis-trifluoromethylphenyl)buta-1,3-dienyl]phenyl]-3-methoxyacrylate (15i). Sodium hydride (85% suspension in mineral oil, 90 mg, 3.18 mmol) was added to a cooled (0 °C) solution of **4a** (500 mg, 1.6 mmol) in THF (10 mL). The mixture was stirred for 20 min at room temperature and treated with (*E*)-3-(2,4-bis-trifluoromethylphenyl)propenal[22] (426 mg, 1.6 mmol) in THF (2 mL). The mixture was stirred for a further 30 min at room temperature and heated under reflux for 2 h. The solvent was evaporated and the residue taken up in ethyl acetate. The solution was washed with brine and water and dried over Na_2SO_4. Evaporation of the solvent and purification of the residue by column chromatography (8:2 hexane/ethyl acetate elvant) afforded **15i** (245 mg, 33%) as white crystals: mp 176−177 °C; IR (KBr) 1703, 1630 cm^{-1}; MS (EI) 456 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 3.69 (3H, s), 3.82 (3H, s), 6.76 (1H, d, J = 15.0 Hz), 6.97 (3H, m), 7.15 (1H, dd, J = 2.0, 8.0 Hz), 7.3 (2H, m), 7.63 (1H, s), 7.7 (1H, d, J = 8.0 Hz), 7.75 (1H, d, J = 8.0 Hz), 7.83 (1H, d, J = 8.0 Hz), 7.87 (1H, s) ppm.

The following compounds were obtained similarly.

Methyl (*E*)-3-methoxy-2-{2-[(*E*,*E*)-4-(2-trifluoromethylphenyl)buta-1,3-dienyl]phenyl}acrylate (15a): MS (EI) 388 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 3.69 (3H, s), 3.81 (3H, s), 6.67 (1H, d, J = 15.0 Hz), 6.95 (3H, m), 7.15 (1H, dd, J = 2.0, 8.0 Hz), 7.28 (3H, m), 7.5 (1H, t, J = 8.0 Hz), 7.62 (1H, s), 7.63 (1H, d, J = 8.0 Hz), 7.73 (2H, m) ppm.

Methyl (*E*)-3-methoxy-2-[2-(*E*,*E*)-(4-phenylbuta-1,3-dienyl)phenyl]acrylate (15b): MS (EI) 320 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 3.69 (3H, s), 3.81 (3H, s), 6.66 (2H, m), 6.93 (2H, m), 7.2 (6H, m), 7.43 (2H, d), 7.62 (1H, s), 7.68 (1H, d, J = 8.0 Hz) ppm.

Methyl (*E*)-2-{2-[(*E*,*E*)-4-(2-chlorophenyl)buta-1,3-dienyl]phenyl}-3-methoxyacrylate (15c): MS (EI) 354 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 3.69 (3H, s), 3.81 (3H, s), 6.69 (1H, d, J = 15.0 Hz), 6.9−7.4 (9H, m), 7.58 (1H, d, J = 8.0 Hz), 7.62 (1H, s), 7.63 (1H, d, J = 8.0 Hz) ppm.

Methyl (*E*)-2-[2-[(*E*,*E*)-4-(2-cyanophenyl)buta-1,3-dienyl]-phenyl]-3-methoxyacrylate (15d): mp 161−162 °C; IR (KBr) 1706, 1628 cm^{-1}; MS (EI) 345 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 3.69 (3H, s), 3.82 (3H, s), 6.74 (1H, d, J = 15.0 Hz), 7.0 (3H, m), 7.18 (1H, d, J = 8.0 Hz), 7.3 (3H, m), 7.55 (1H, t, J = 8.0 Hz), 7.6 (1H, d, J = 8.0 Hz), 7.63 (1H, s), 7.7 (2H, d) ppm.

Methyl (*E*)-2-{2-[(*E*,*E*)-4-(3-fluorophenyl)buta-1,3-dienyl]-phenyl}-3-methoxyacrylate (15e): MS (EI) 338 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 3.69 (3H, s), 3.82 (3H, s), 6.59 (1H, d, J = 15.0 Hz), 6.64 (1H, d, J = 15.0 Hz), 6.9 (3H, m), 7.18 (3H, m), 7.22 (3H, s), 7.62 (1H, s), 7.66 (1H, d, J = 8.0 Hz) ppm.

Methyl (*E*)-3-methoxy-2-{2-[(*E*,*E*)-4-(3-trifluoromethylphenyl)buta-1,3-dienyl]phenyl}acrylate (15f): MS (EI) 388 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 3.69 (3H, s), 3.82 (3H, s), 6.67 (1H, d, J = 15.0 Hz), 6.77 (1H, d, J = 14.0 Hz), 6.93 (2H, m), 7.14 (1H, dd, J = 2.0, 8.0 Hz), 7.28 (2H, m), 7.45 (2H, d), 7.55 (1H, d), 7.63 (1H, s), 7.66 (2H, d) ppm.

Methyl (*E*)-2-{2-[(*E*,*E*)-4-(3-bromophenyl)buta-1,3-dienyl]phenyl}-3-methoxyacrylate (15g): MS (EI) 399 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 3.69 (3H, s), 3.82 (3H, s), 6.55 (1H, d, J = 15.0 Hz), 6.65 (1H, d, J = 15.0 Hz), 6.8 (2H, m), 7.2 (6H, m), 7.57 (1H, s), 7.62 (1H, s), 7.66 (1H, d, J = 8.0 Hz) ppm.

Methyl (*E*)-2-{2-[(*E*,*E*)-4-(4-chlorophenyl)buta-1,3-dienyl]phenyl}-3-methoxyacrylate (15h): MS (EI) 354 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 3.69 (3H, s), 3.82 (3H, s), 6.62 (1H, d, J = 15.0 Hz), 6.66 (1H, d, J = 15.0 Hz), 6.85 (1H, d, J = 15.0 Hz), 6.9 (1H, d, J = 15.0 Hz), 7.16 (1H, d, J = 8.0 Hz), 7.3 (6H, m), 7.63 (1H, s), 7.67 (1H, d, J = 8.0 Hz) ppm.

Methyl (*E*)-2-[2-[(*E*,*E*)-4-(2,4-dichlorophenyl)buta-1,3-dienyl]phenyl]-3-methoxyacrylate (15j): mp 125−126 °C; IR (KBr) 1702, 1627 cm^{-1}; MS (EI) 348 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 2.30 (3H, s), 2.35 (3H, s), 3.69 (3H, s), 3.81 (3H, s), 6.60 (1H, d, J = 15.0 Hz), 6.8 (3H, m), 7.0 (2H, m), 7.13 (1H, d, J = 8.0 Hz), 7.3 (2H, m), 7.46 (1H, d, J = 8.0 Hz), 7.62 (1H, s), 7.7 (1H, d, J = 8.0 Hz) ppm.

Methyl (*E*)-2-[2-[(*E*,*E*)-4-(2,4-dimethylphenyl)buta-1,3-dienyl]phenyl]-3-methoxyacrylate (15k): mp 125−126 °C; IR (KBr) 1702, 1627 cm^{-1}; MS (EI) 348 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 2.30 (3H, s), 2.35 (3H, s), 3.69 (3H, s), 3.81 (3H, s), 6.60 (1H, d, J = 15.0 Hz), 6.8 (3H, m), 7.0 (2H, m), 7.13 (1H, d, J = 8.0 Hz), 7.3 (2H, m), 7.46 (1H, d, J = 8.0 Hz), 7.62 (1H, s), 7.7 (1H, d, J = 8.0 Hz) ppm.

Methyl (*E*)-2-[2-[(*E*,*E*)-4-(2-chloro-4-fluorophenyl)buta-1,3-dienyl]phenyl]-3-methoxyacrylate (15l): mp 163−165 °C; IR (KBr) 1707, 1629 cm^{-1}; MS (EI) 372 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 3.69 (3H, s), 3.82 (3H, s), 6.63 (1H, d, J = 15.0 Hz), 6.9 (4H, m), 7.1 (1H, dd, J = 2.0, 8.0 Hz), 7.18 (1H, dd,

J = 2.0, 8.0 Hz), 7.3 (2H, m), 7.6 (1H, dd, J = 6.0, 8.0 Hz), 7.63 (1H, s), 7.8 (1H, d, J = 8.0 Hz) ppm.

Methyl (*E*)-3-methoxy-2-[2-[(*E*,*E*)-4-(3-methoxy-2-nitrophenyl)buta-1,3-dienyl]phenyl]acrylate (15m): mp 65−66 °C; IR (KBr) 1707, 1632 cm^{-1}; MS (EI) 395 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 3.68 (3H, s), 3.82 (3H, s), 3.89 (3H, s), 6.47(1H, d, J = 15.0 Hz), 6.7 (1H, d, J = 15.0 Hz), 6.81 (1H, d, J = 15.0 Hz), 6.9 (1H, d, J = 15.0 Hz), 6.91 (1H, d), 7.07 (1H, d, J = 8.0 Hz), 7.3 (4H, m), 7.62 (1H, s), 7.64 (1H, d, J = 8.0 Hz) ppm.

Methyl (*E*)-2-{3-(*E*,*E*)-{[4-(2,4-bistrifluoromethylphenyl)buta-1,3-dienyl]phenyl}-3-methoxyacrylate (15n): mp 130 °C; IR (KBr) 1703, 1629 cm^{-1}; MS (EI) 456 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 3.7 (3H, s), 3.83 (3H, s), 6.71 (1H, d, J = 15.0 Hz), 6.75 (1H, d, J = 15.0 Hz), 6.9 (1H, d, J = 15.0 Hz), 6.93 (1H, d, J = 15.0 Hz), 7.09 (1H, d, J = 8.0 Hz), 7.3 (2H, m), 7.64 (1H, s), 7.7 (1H, d, J = 8.0 Hz), 7.74 (1H, s), 7.83 (2H, s) ppm.

Methyl (*E*)-2-{4-(*E*,*E*)-[4-(2,4-bistrifluoromethylphenyl)-buta-1,3-dienyl]phenyl}-3-methoxyacrylate (15o): IR (neat) 1706, 1627 cm^{-1}; MS (EI) 456 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 3.75 (3H, s), 3.88 (3H, s), 6.82 (1H, d, J = 15.0 Hz), 6.95 (3H, m), 7.34 (2H, d), 7.45 (2H, d), 7.57 (1H, s), 7.76 (1H, d, J = 8.0 Hz), 7.83 (1H, d, J = 8.0 Hz), 7.88 (1H, s) ppm.

Methyl (*E*)-3-Methoxy-2-[2-[4-(2-trifluoromethylphenyl)butyl]phenyl]acrylate (16). 15a (100 mg, 0.25 mmol) in CH_2CL_2 was hydrogenated over Pd/C at room temperature and pressure. Filtration of the catalyst and evaporation of the solvent afforded **16** (100 mg, 99%) as a colorless oil: IR (neat) 1710, 1635 cm^{-1}; MS (EI) 392 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 1.62 (4H, m), 2.5 (2H, m), 2.79 (2H, m), 3.65 (3H, s), 3.75 (3H, s), 7.1 (1H, d), 7.25 (5H, m), 7.4 (1H, d), 7.54 (1H, s), 7.6 (1H, d) ppm.

Methyl (*E*)-3-Methoxy-2-[2-[(*E*)-2-[5-(2-trifluoromethylphenyl)furan-2-yl]vinyl]phenyl]acrylate (18). Sodium hydride (85% suspension in mineral oil, 60 mg, 2.1 mmol) was added to a cooled (0 °C) solution of **4a** (250 mg, 0.79 mmol) in THF (5 mL). The mixture was stirred 30 min at room temperature and cooled to 0 °C before the addition of **17** (191 mg, 0.79 mmol). The mixture was maintained 18 h at 4 °C. The reaction mixture was warmed to room temperature, treated with acetic acid (1 mL), and diluted with ethyl acetate. The organic phase was washed with brine and dried over Na_2SO_4. Evaporation of the solvent and purification of the residue by column chromatography (1:9 hexane/CH_2Cl_2 eluant) afforded **18** (184 mg, 54%) as a yellow oil: IR (neat) 1708, 1632 cm^{-1}; MS (EI) 428 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 3.97 (3H, s), 3.81 (3H, s), 6.42 (1H, d), 6.7 (1H, d), 6.88 (1H, J = 16.0 Hz), 7.17 (1H, d), 7.3 (4H, m), 7.6 (1H, t), 7.64 (1H, s), 7.78 (3H, m) ppm.

1-(2,2-Dibromovinyl)-2-(trifluoromethyl)benzene (19). A solution of carbon tetrabromide (10.8 g, 32 mmol) in CH_2Cl_2 (10 mL) was added dropwise to a stirred, cooled (−10 °C) solution of triphenylphosphine (16.5 g, 64 mmol) in CH_2Cl_2 (40 mL). The resulting orange suspension was stirred 15 min at −10 °C. A solution of 2-(trifluoromethyl)benzaldehyde (2.83 g, 16 mmol) in CH_2Cl_2 (10 mL) was added. The resulting brown solution was stirred for 30 min at −10 °C. Triethylamine (3 mL) and a solution of Na_2CO_3 (2 mL) were added dropwise. The mixture was diluted with diethyl ether (100 mL) and filtered through a short pad of silica gel (eluted with hexane). Evaporation of the solvent afforded **19** (5.29 g, 98%) as a colorless oil: IR (neat) 1316, 1170 cm^{-1}; MS (EI) 330 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 7.46 (1H, m), 7.6 (4H, m) ppm.

1-(2-Bromovinyl)-2-(trifluoromethyl)benzene (20). A solution of **19** (989 mg, 3 mmol) and diethyl phosphite (0.75 mL, 6 mmol) in triethylamine (0.83 mL, 6 mmol) was stirred 4 h at 5 °C. The mixture was diluted with hexane, washed with brine, and dried over Na_2SO_4. Evaporation of the solvent and purification of the residue by column chromatography (hexane) afforded **20** (674 mg, 90%) as a colorless liquid: IR (neat) 1600, 940 cm^{-1}; MS (EI) 251 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 6.78 (1H, d, J = 14.0 Hz), 7.4 (3H, m), 7.63 (2H, m) ppm.

Methyl (*Z*)-Methoxyimino-[2(*E* and *Z*)-(2-tributylstannanylvinyl)phenyl]acetate (1:1) (24). Tributyltin hydride (0.56 mL, 2.1 mmol) was added to a solution of **23** (151 mg, 0.7 mmol) and a catalytic amount of 2,2′-azobis(isobutyronitrile) in toluene (0.5 mL). The mixture was stirred 3.5 h at 80 °C, the solvent was evaporated under reduced pressure, and the residue was purified by column chromatography (9:1 hexane/ethyl acetate eluant) and afforded **24** (155 mg, 44%) as a yellowish liquid: IR (neat) 1731, 1600 cm^{-1}; MS (CI) 525 $(M + NH_4)^+$, 508 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 0.84 (30H, m), 1.3 (12H, m), 1.54 (12H, m), 3.81 (3H, s), 3.84 (3H, s), 4.0 (3H, s), 4.03 (3H, s), 6.2 (1H, d, J = 14.0 Hz), 6.7 (1H, d, J = 19.0 Hz), 6.8 (1H, d, J = 19 Hz), 7.15 (3H, m), 7.33 (5H, m), 7.6 (1H, d) ppm.

Methyl (*Z*)-Methoxyimino-[2-(1-tributylstannanylvinyl)phenyl]acetate (25). Tributyltin hydride (0.16 mL, 0.46 mmol) was added to a solution of tetrakis(triphenylphosphine)-palladium (27 mg, 0.023 mmol) and **23** (100 mg, 0.46 mmol) in THF (2 mL). The mixture was stirred 20 min at room temperature, diluted with ethyl acetate, washed with brine, and dried over Na_2SO_4. Evaporation of the solvent and purification of the residue by column chromatography (9:1 hexane/ethyl acetate eluant) afforded **25** (200 mg, 85%) as a colorless oil: IR (neat) 1730, 1602 cm^{-1}; MS (EI) 508 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 0.87 (15H, m), 1.3 (6H, m), 1.54 (6H, m), 3.80 (3H, s), 4.02 (3H, s), 5.4 (1H, d, J = 2.5 Hz), 5.7 (1H, d, J = 2.5 Hz), 7.0 (1H, d), 7.2 (2H, m), 7.32 (1H, d) ppm.

Methyl (*Z*)-Methoxyimino-{2-[(*E*,*E*)-4-(2-trifluoromethylphenyl)buta-1,3-dienyl]phenyl}acetate (26). Tetrakis-(triphenylphosphine)palladium (21 mg, 0.02 mmol) was added to a solution of **20** (151 mg, 0.6 mmol) and **24** (304 mg, 0.6 mmol) in toluene (5 mL). The mixture was heated 5 h at 110 °C, cooled to room temperature, diluted with toluene, washed with brine, and dried over Na_2SO_4. Evaporation of the solvent and purification of the residue by column chromatography (3:2 hexane/ethyl acetate eluant) afforded **26** (48 mg, 21%) as a brown oil: IR (neat) 1734, 1610 cm^{-1}; MS (EI) 389 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 3.87 (3H, s), 4.06 (3H, s), 6.5 (1H, d, J = 15.0 Hz), 6.9 (1H, d, J = 15.0 Hz), 7.0 (2H, m), 7.16 (1H, d, J = 8.0 Hz), 7.4 (4H, m), 7.71 (1H, d, J = 8.0 Hz), 7.72 (2H, d) ppm.

Methyl (*Z*)-Methoxyimino-{2-[(*E*)-4-(2-trifluoromethylphenyl)but-3-en-1-ynyl]phenyl}acetate (27). Triethylamine (0.22 mL, 1.6 mmol), copper(I) chloride (3 mg, 0.015 mmol), and tris(dibenzylideneacetone)dipalladium chloroform (18 mg, 0.018 mmol) were added to a solution of **23** (129 mg, 0.59 mmol) and **20** (179 mg, 0.71 mmol) in toluene (2 mL). After stirring 4 h at 110 °C, the mixture was cooled to room temperature and treated with NH_4Cl solution (4 mL) for 10 min. The phases were separated and the aqueous phase was extracted with toluene. The combined organic phases were washed with brine and dried over Na_2SO_4. Evaporation of the solvent and purification of the residue by column chromatography (7:3 hexane/ethyl acetate eluant) afforded **27** (28 mg, 12%) as orange crystals: mp 104−107 °C; IR (KBr) 2220, 1744, 1601 cm^{-1}; MS (EI) 387 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 3.88 (3H, s), 4.04 (3H, s), 6.38 (1H, d, J = 15.0 Hz), 7.4 (5H, m), 7.6 (2H, m), 7.69 (2H, d) ppm.

1-(Bromoethynyl)-2-(trifluoromethyl)benzene (28). 1,8-Diazabicyclo[5.4.0]undec-7-ene (1.83 mL, 12.3 mmol) was added dropwise to a stirred solution of **19** (2 g, 6.15 mmol) in DMSO (5 mL). The reaction mixture was stirred 45 min at 15 °C, cooled to 0 °C, and treated with 1 N HCl. The mixture was diluted with diethyl ether, the phases were separated, and the organic layer was washed with brine and water and dried over Na_2SO_4. Evaporation of the solvent and purification of the residue by column chromatography (7:1 hexane/ethyl acetate eluant) afforded **28** (1.364 g, 90%) as a colorless oil: IR (neat) 2210, 1319 cm^{-1}; MS (EI) 249 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 7.45 (2H, m), 7.6 (2H, m) ppm.

Methyl (2-Ethynylphenyl)oxoacetate (29). A solution of **21** (400 mg, 1.5 mmol) in THF (2 mL) was added dropwise to a cooled (0 °C) solution of tetrabutylammonium fluoride (146 mg, 0.46 mmol) in THF (5 mL). After 5 min NH_4Cl (5 mL)

solution was added and the mixture stirred for 10 min at room temperature. The mixture was diluted with ethyl acetate, the phases were separated, and the organic layer was washed with NH_4Cl solution and water and dried over Na_2SO_4. Evaporation of the solvent afforded **29** (263 mg, 91%) as an orange oil: IR (neat) 3274, 2130, 1741, 1983 cm^{-1}; MS (EI) 188 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 3.42 (1H, s), 3.93 (3H, s), 7.6 (3H, m), 7.85 (1H, d) ppm.

Methyl (*Z* and *E*)-(2-Ethynylphenyl)-methoxyiminoacetate (23 and 30). Methoxylamine hydrochloride (1.63 g, 18.3 mmol) and anhydrous sodium acetate (1.54 g, 18.3 mmol) were added to a solution of **29** (431 mg, 2.3 mmol) in MeOH (10 mL) and water (1 mL). After stirring 4 h at room temperature the solvent was evaporated under reduced pressure. The residue was dissolved in ethyl acetate; the organic phase was washed with brine and water and dried over Na_2SO_4. Evaporation of the solvent and separation of the mixture by column chromatography (7:3 hexane/ethyl acetate eluant) afforded **23**-(*Z*) (366 mg, 74%) and **30**-(*E*) (50 mg, 10%). Data for **30**: IR (neat) 3281, 2120, 1740 cm^{-1}; MS (EI) 217 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 3.27 (1H, s), 3.85 (3H, s), 4.06 (3H, s), 7.4 (2H, m), 7.5 (1H, d), 7.62 (1H, d**)** ppm.

Methyl (*Z*)-methoxyimino-{2-[4-(2-trifluoromethylphenyl)buta-1,3-diynyl]phenyl}acetate (31): prepared according to the method described for **27**; mp 72−74 °C; IR (KBr) 2210, 1738, 1602 cm^{-1}; MS (EI) 385 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 3.91 (3H, s), 4.1 (3H, s), 7.3 (1H, d), 7.4 (4H, m), 7.65 (3H, m) ppm.

Methyl (*E*)-methoxyimino-{2-[4-(2-trifluoromethylphenyl)buta-1,3-diynyl]phenyl}acetate (32): prepared according to the method described for **27**; IR (neat) 1741, 1603 cm^{-1}; MS (EI) 385 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 3.96 (3H, s), 4.08 (3H, s), 7.4 (21H, m), 7.5 (2H, m), 7.7 (4H, m) ppm.

Methyl (*Z*)-Methoxyimino-{2-[5-(2-trifluoromethylphenyl)thiophene-2-yl]phenyl}acetate (33). Sodium sulfide nonahydrate (195 mg, 0.8 mmol) was added to a solution of **31** (63 mg, 0.16 mmol) in 2-methoxyethanol (1.5 mL). The mixture was heated 1 h at reflux, the solvent was removed under reduced pressure, and MeOH (2 mL) and H_2SO_4 (0.1 mL) were added. The mixture was heated 20 min at reflux and cooled to room temperature and the solvent evaporated. The residue was diluted with ethyl acetate, washed with K_2CO_3 solution and brine, and dried over Na_2SO_4. The solvent was removed under reduced pressure, the residue was dissolved in CH_2Cl_2, and charcoal (0.5 g) was added. The mixture was stirred 1 h at room temperature and filtered through a pad of Celite. Evaporation of the solvent and purification of the residue by column chromatography (7:1 hexane/ethyl acetate eluant) afforded **33** (30 mg, 44%) as a brown oil: IR (neat) 1730, 1603 cm^{-1}; MS (EI) 419 (M); ^1H NMR (250 MHz, $CDCl_3$) δ 3.73 (3H, s), 4.03 (3H, s), 6.93 (1H, d, J = 2.5 Hz), 7.0 (1H, d, J = 2.5 Hz), 7.31−7.59 (7H, m), 7.75 (1H, d, J = 7.5 Hz) ppm.

Methyl (*Z*)-methoxyimino-{2-[4-(2-trifluoromethylphenyl)butyl]phenyl}acetate (34): prepared according to the method described for **16**; IR (neat) 1729, 1607 cm^{-1}; MS (EI) 362 (M − MeO); ^1H NMR (250 MHz, $CDCl_3$) δ 1.55 (4H, m), 2.5 (2H, t), 2.8 (2H, m), 3.83 (3H, s), 3.99 (3H, s), 7.1 (1H, d), 7.3 (5H, m), 7.4 (1H, t), 7.6 (1H, d) ppm.

In Vitro Measurement of *P. falciparum* Parasite Growth Inhibition. *P. falciparum* continuous cultures: Asynchronous stock cultures were maintained in suspension cultures.[23] The culture medium was a variation[24] of that described by Trager and Jensen.[25] It consisted of RPMI 1640 supplemented with 0.5% AlbuMAX (Gibco BRL), 25 mM HEPES, 25 mM $NaHCO_3$ (pH 7.3), 50 μg/mL hypoxanthine, and 50 μg/mL neomycin. Human type A^+ erythrocytes served as host cells. The cultures were kept at 37 °C in an atmosphere of 3% O_2, 4% CO_2, and 93% N_2 ppm.

Parasite growth inhibition in vitro: Drug testing was carried out essentially as described by Desjardins et al.[26] in 96-well microtiter plates. The compounds were dissolved by sonication in DMSO (5 mg/mL), prediluted in the culture medium described above but containing an additional 0.5 μg/mL hypoxanthine, and titrated in duplicates over a 64-fold range. After addition of the parasite cultures with an initial parasitemia of 0.75% in a 2.5% erythrocyte suspension, the test plates were incubated under the conditions described above in humidified modular chambers. Growth of the parasites was measured by the incorporation of radiolabeled [^3H]-hypoxanthine added 16 h prior to the termination of the test. Fifty percent inhibitory concentrations (IC_{50}) were estimated by linear interpolation.[27]

The compounds were tested in vitro against the chloroquine-resistant strain K1 (Thailand) and the sensitive strain NF54 (airport strain of unknown origin).

In vivo antimalarial activity on *P. berghei*: The compounds were tested in vivo as described earlier.[28] Male albino mice (IBM:MORO(SPF); Fullinsdorf), weighing 25 ± 2 g, were infected intravenously with 2×10^7 *P. berghei* (strain ANKA) infected erythrocytes from a donor mouse on day 0 of the experiment. The compounds to be tested were solubilized (or suspended) in 7% Tween-80, 3% ethanol in H_2O, to the desired concentration and injected sc or given po to different groups of mice (0.01 mL/g-mouse, 3 mice/group). The mice were treated at day 1 (24 h after the infection). At day 3 (48 h after the treatment), bloodsmears of all the animals were prepared and stained with Giemsa. The degree of infection (parasitemia expressed in % infected erythrocytes) was determined microscopically. The difference of the mean infection rate of the control group (=100%) to the test group was calculated and expressed as percent reduction. The $ED_{50/90}$ was calculated by nonlinear fitting with the JMP statistical program (Statistical Analysis Institute, Cary, NC).

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