Fluoroartemisinin: Trifluoromethyl Analogues of Artemether and Artesunate

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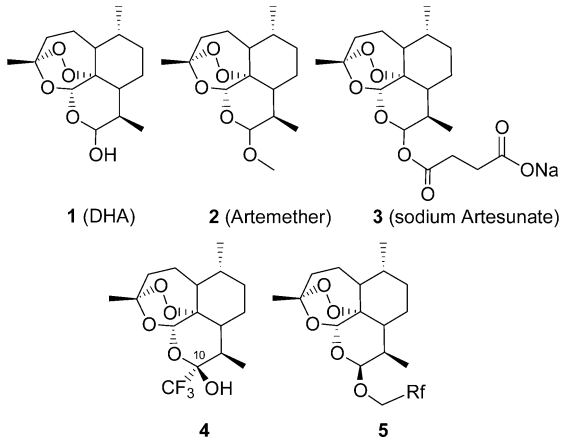
The synthesis of a series of C-10 trifluoromethyl ethers of artemisinin has been achieved from key bromide **8**, itself carried out in two steps from artemisinin. The substitution of **8** with methanol, ethanol, or succinic acid allowed the access of C-10 CF_3 analogues of β-artemether, β-arteether, or artesunate, respectively, in good yields (up to 89%). The presence of the CF_3 group at C-10 of artemisinin clearly increased the chemical stability under simulated stomach acid conditions. For example, the CF_3 analogue of arteether was found to be around 45 times more stable than arteether itself. The influence of the CF_3 moiety on biological activity was also highlighted. CF_3 analogues of artemether and arteether exhibited a high in vivo antimalarial activity on mice infected with *Plasmodium berghei* NK173, with a complete clearance of the parasitemia during the entire observation period (25 days).

Introduction

Endoperoxides are a promising class of antimalarial drugs. Artemisinin (ART), the lead natural compound, is a sesquiterpenic lactone.[1] Because of its poor pharmacological profile, scientists rapidly developed derivatives from dihydroartemisinin (DHA) **1** (Chart 1) with better properties, in particular better solubility in oil or water.[2] The semisynthetic ethers or esters, such as artemether **2**,[3] arteether,[4] and artesunate **3**,[5] are being widely used for therapy in Asia and in Africa.[6] Artelinate is still in clinical development.[7] These endoperoxides have several advantages over existing antimalarial drugs. First, they clear the peripheral blood from parasites very rapidly, and second, there is no cross-resistance with other antimalarial drugs. However, they also have some disadvantages, such as short plasmatic half-lives, and the effective concentrations in plasma are sustained for only relatively brief periods. Artemisinin derivatives undergo in vivo cytochrome P450 oxidation (artemether, arteether) and/or are hydrolyzed under mild acidic conditions (in particular artesunate). Both processes provide active dihydroartemisin, which has itself a short plasmatic half-life.[8] As a result, a short-course treatment is generally associated with a high rate of recrudescent parasitemia.[9] An urgent objective is thus to design new artemisinin derivatives that enable prolonged efficiency and to target a reasonable plasmatic half-life that should be long enough to avoid the unacceptable recrudescence of parasitemia, to allow shorter treatments or smaller doses while preventing the emergence of resistance.

Fluorinated substituents (Rf) are known to impart greater protection to molecules from metabolic (oxidative and hydrolytic) degradation,[10] due to a strong C−F bond. We have shown that the introduction of a fluoroalkyl group onto a crucial position for metabolism of artemisinin derivatives, for instance, on carbon C-10 (compound **4**) or on the alkoxy chain of ethers of DHA (compound **5**), resulted in excellent in vitro and in vivo activities by both intraperitoneal and oral routes, surpassing those of DHA and its ethers **2** and **3**.[11,12] Comparative measurement of plasmatic half-lives of CF_3 hemiketal **4** ($T_{1/2}$ = 85.9 min) and DHA ($T_{1/2}$ = 26.3 min) after intravenous administration to rats seems to indicate that the CF_3 group at C-10 effectively protects ART derivatives against oxidation or hydrolytic metabolism.[13]

Chart 1



Considering the facile cleavage of the acetal function of DHA ethers in acidic solution, we decided to use the fluorine approach to design hydrolytically stable analogues of artemether and arteether. The easy hydrolysis of DHA acetals and esters is the consequence of the easy formation and the great stability of oxonium ion **6a** (Chart 2). The destabilization of this oxonium ion by an electron-withdrawing substituent should strongly disfavor any pathway where it could be involved. In connection with this process, we have already shown that on more simple molecules the formation of oxonium ion **7a** is strongly destabilized by the presence of a trifluoromethyl group compared to **6a**.[14] In an artemisi-

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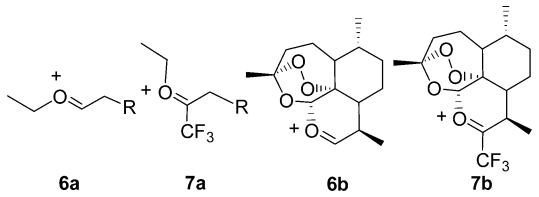
Chart 2



nin series, oxonium ion **7b** would be much more difficult to generate by hydrolysis from the CF_3 analogue of artemether **8** than the ion **6b** from artemether itself.

Moreover, the presence of a trifluoromethyl group could also slow the rate of the oxidative dealkylation of the alkoxyl at C-10, since it has been demonstrated that the protection of oxidative processes, provided by a fluoroalkyl group, could extend to β and even γ CH or CH_2 groups.[10]

In this paper, we report our findings on the acid stability and the high in vitro and in vivo antimalarial activities of the C-10 trifluoromethyl analogues of artemether and other ethers and esters.

Chemistry

Trifluoromethyl-substituted artemether and other parent compounds were prepared from the bromo derivative **8**, available itself in 80% yield from trifluorohemiketal **4** through a reaction with thionyl bromide followed by an intramolecular substitution.[15] Bromide **8** was first substituted with methanol through a silver salt assisted solvolysis. Under these conditions, compound **9** was obtained in a 55% yield as a 52/48 mixture of both diastereoisomers **9**α (where OMe is α) and **9**β, accompanied by a large amount (43%) of an elimination product, glycal **10**. The yield was improved to 79% when 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) was used as a bromide activator in place of silver salt (as a cosolvent with CH_2Cl_2).[16] A better chemical selectivity was also obtained with only 5% of glycal **10** observed. Moreover, with HFIP, **9**β was formed as the major diastereoisomer (>95% de). These conditions were then applied to introduce various other alcohols, one acid, one amine, and even hydrogen peroxide (Chart 3). Yields in substitution were moderate to good. In all cases except for the amine **17**, only a small amount of glycal was obtained and the substitution reaction was completely stereoselective with only the β isomer formed.

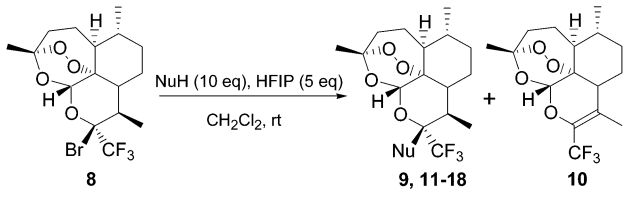
Although the role of HFIP as an activating agent has not been well elucidated, its use as a cosolvent clearly improved the rate and the course of the reaction.

Subsequent basic hydrolysis of methyl ester **12** provided the trifluoromethyl analogue **19** of artelinic acid in a 53% overall yield from **8**. The allyl ether **13** was also submitted to various conversions to obtain water-soluble compounds. Dihydroxylation with a catalytic amount of OsO_4 afforded the corresponding diol **20** in 60% yield (Scheme 1). Compound **13** was also readily converted into carboxylic acid **21** using RuO_4, generated in situ from $RuCl_3$ and $NaIO_4$, as an oxidant system, or oxidized by ozonolysis into the corresponding aldehyde **22** (80% yield).

Chemical Stability

DHA, artemether, arteether, sodium artesunate, and artelinic acid are known to be sensitive to moisture and gastric pH conditions and often lead to inactive metabolites.[8c,12b] Their half-lives did not exceed 20 h at pH 2.[17] The stability of their fluorinated analogues **4**, **9**, **11**, **16**, and **19** was evaluated to check if the trifluoromethyl substituent is actually able to improve the stability of artemisinin compounds, as postulated. Since fluorinated compounds can be detected by ^{19}F NMR, the analysis described in the literature[17] was simplified. The fluorinated derivative **4** (C-10 trifluoromethyl analogue of DHA) was first placed under the described simulated stomach acid conditions (1 mg/mL in 0.01 N HCl (pH 2) at 37 °C) in an NMR tube in which trifluoromethanesulfonic acid was added as an internal standard. Its stability was evaluated by following the disappearance of its ^{19}F NMR signal. The half-life of compound **4** was found to be around 40 times higher (∼700 h) than the DHA itself (17 h) (Figure 1). However, because of the lower solubility of other fluorinated compounds in water, conditions were slightly modified with the addition of acetonitrile. The pH was then adjusted in order to obtain the same degradation rate for **4** (760 h) as in the previous conditions: 1:1 v/v mixture of 0.1 N HCl solution (pH 1) and acetonitrile at 37 °C. All other fluorinated compounds (2 mg/mL) were thus completely soluble and analyzable by ^{19}F NMR. Compounds **9**, **11**, and **19** also exhibited high acid stability with half-lives

Chart 3



Nu	Compounds	Yields[a] (%)	Olefin **10**[b] (%)
MeO-	**9**	79	5
EtO-	**11**	76	6
4-(MeO_2C)-C_6H_4-CH_2O-	**12**[c]	(76)	6
CH_2=CH-CH_2O-	**13**	73	5
$HOCH_2$-CH_2O-	**14**	89	2
CF_3-CH_2O-	**15**	46	12
HO_2C-$(CH_2)_2$-CO_2-	**16**	68	5
4-MeO-C_6H_4-NH-	**17**	25	62
HOO- (from H_2O_2/urea)	**18**	83	0

[a] Isolated yield. [b] crude NMR ratio. [c] Non isolated, directly hydrolysed into CF_3-artelinic acid **19** (53% from **8**).

Scheme 1

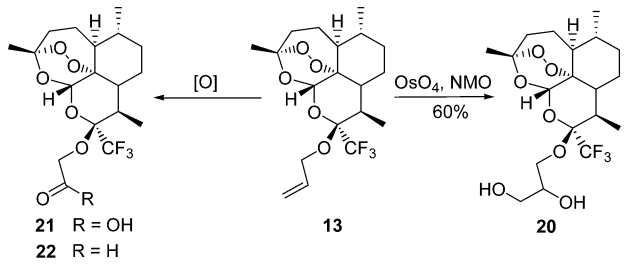


Table 1. In Vitro Antimalarial Activity against *Plasmodium falciparum* FCB1 Strain

	compound												
	2 (ref)	**4**	**9**	**11**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
IC_{50} (nM)	3.5	9.4	0.8	3.3	0.9	8.3	4.1	12.3	13.2	10.6	0.9	30.4	21.4
SD (±)	1.2	2.7	0.3	1.1	0.2	2.4	1.2	2.8	0.9	3.0	0.3	6.0	3.0

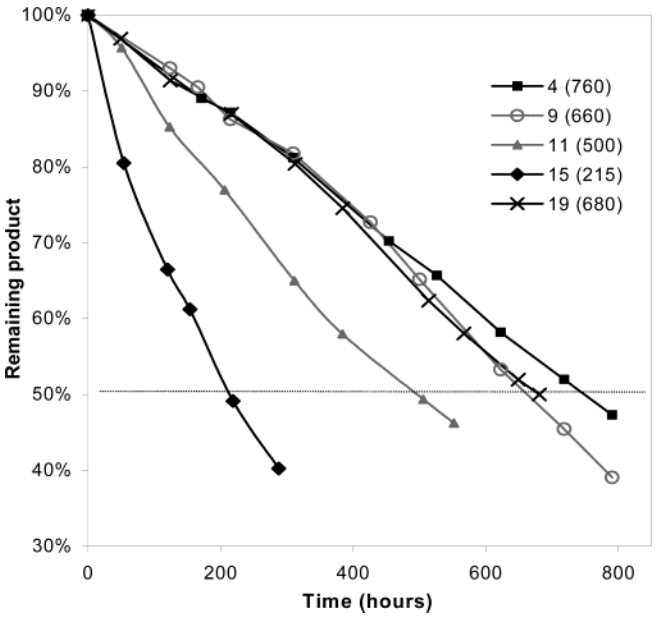


Figure 1. Stability of C-10 trifluoromethyl compounds under acidic conditions.

of 660, 500, and 680 h, respectively. Compound **15** was a little less stable, with a half-life of 215 h. Conversely compounds **16** and **17** were found to be much less stable under these acid conditions (pH 1) than C-10 trifluoromethyl ethers, with half-lives of 30 h. However, trifluoromethyl-substituted artesunic acid **16** was found to be more stable at pH 2 (80 h) or at pH 1 (30 h) than artesunate at pH 7−8 (∼10 h), which decomposed mainly into DHA.[18] It must be noted that similarly the ^{19}F NMR signal of **16** was in part replaced by the signal corresponding to **4**. For **17**, the short half-life suggests that the first step of hydrolysis occurs at a different site where the CF_3 substituent has no influence.

Biological Evaluation

The in vitro antimalarial activities of compounds **4**, **9**, **11**, and **14**−**22** were determined using the FCB1 strain of *Plasmodium falciparum*. This strain is resistant to chloroquine (IC_{50} = 115 ± 25 nM). IC_{50} values are shown in Table 1.

All derivatives exhibited good in vitro activity compared to artemether. All these compounds were subjected to in vivo assays in mice according to Peters' protocol (*Plasmodium berghei* NK 173 infected mice, intraperitoneal administration for 4 days at 35.5 μmol·kg^{-1}).[19] Results are reported in Table 2. By use of this procedure, artemether ensured a nearly complete clearance of parasitemia at the end of the treatment (day 4), but the parasitemia then gradually increased. Most of the fluorinated derivatives were more efficient than artemether in decreasing parasitemia. More importantly, with the C-10 trifluoromethyl analogues of artemether and arteether (**9** and **11**) and with **15**, there

Table 2. In Vivo Antimalarial Activity against *P. berghei* NK 173 at 35.5 μmol/kg

	% parasitemia (% infected erythrocytes)					
drug	day 4	day 7	day 9	day 11	day 13	day 25[a]
2 (ref)	2.2	5.5	39.2		48.1	
4	4.0	10.7		22.1		
9	0	0		0		0
11	0	0		0		3.8[b]
14	8.8	14.8		27.3		
15	0	0		0		0
16	0.5	3.9	36.8			
17	0	0	0.1		8.1	0
18	5.5	28.3	41.7		36.8	
19	25.9	26.8	34.9		25.6	
20	2.1[c]	12.1	28.8	46.3		
21	47.5					
22	0.2	7.8	26.8		46.8	29.6[d]

[a] Values only for experiments where all mice survived. [b] 0% of parasitemia for 80% of mice. [c] Measured on day 3. [d] Measured on day 23.

Table 3. In Vivo Antimalarial Activity of **9** and **2**

	ED_{50}		ED_{90}	
drug	mg/kg	μmol/kg	mg/kg	μmol/kg
2	2.5	8.4	8.5	28.5
9	1.25	3.4	6.4	17.5

is a complete clearance of parasitemia in mice treated, not only at the end of the treatment but from day 4 to day 25.

A striking result is that compounds bearing a polar functionality, except amine **17**, did not ensure a prolonged antimalarial activity, even when they were able to clear parasitemia at the end of the treatment, exhibiting thus a profile similar to that of artemether. For example, compound **14** was not able to suppress parasitemia or to maintain it at a low level while compound **11**, which differs from **14** only by an alcohol function, completely cured mice from day 4 to day 25. While compound **16** protected mice against *P. Berghei* until day 7 (3.9% of parasitemia), a rapid increase of parasitemia suggests a rapid metabolism and/or elimination of the drug. The difference between **21** (acid functionality) and **22** (aldehyde functionality) is also striking and could be due to the inadequate intraperitoneal route of the administration of the acid. The addition of another peroxide site onto artemisinin (**18**) did not bring any beneficial effect compared to **4**.

The improvement of antimalarial activity when a trifluoromethyl group is incorporated at C-10 on the artemisinin skeleton is also indicated with the ED_{50} and ED_{90} values, evaluated for artemether and its 10-trifluoromethyl analogue (on *P. Berghei* NK 173, ip administration) (Table 3). The CF_3-artemether was found to be approximately 2 times more active than artemether. It is worth noting that ED_{50} and ED_{90} values, which are measured at day 4 (at the end of treatment), cannot give any information on the recrudescence of parasitemia, which is the main drawback of artemether.

Better antimalarial activity can be attributed to several phenomena such as the demonstrated hydrolytic stability and a decreased rate of oxidation by cytochrome P450 enzymes, due to the influence of CF_3 on a metabolism course or a different distribution. Furthermore, considering the calculated values of log *P*, C10-trifluoromethyl artemether **9** (ClogP = 4.19)[20] is very likely more lipophilic than artemether itself (ClogP = 2.91).[20] This change in the lipophilic character can induce a greater accumulation of fat cells and thus modify the distribution of the drug.

Conclusion

In conclusion, new C-10 trifluoromethyl substituted artemisinin derivatives were easily obtained in a three-step process from artemisinin, thus giving the opportunity to evaluate more quantitatively the incidence of the introduction of a trifluoromethyl group at C-10 on the stability and antimalarial activity. C-10 trifluoromethyl hydroartemisinin **4** was found to be 40 times more stable than DHA under acid conditions (pH 2), and the trifluoromethyl analogue of arteether was found to be 45 times more stable than arteether itself. This study demonstrated the increased hydrolytic stability brought to the ketal function by the trifluoromethyl substituent. Higher stability of these compounds under acidic conditions should increase their half-lives in stomach and in the acidic parasite food vacuole, where artemisinin derivatives function as alkylating agents, reacting with heme and parasite proteins.[9] The beneficial effect of the trifluoromethyl group was also observed in the biological evaluations. All new prepared compounds exhibited good in vitro activities against a chloroquine-resistant strain. In vivo, three of them, in particular the 10-CF_3 analogues of artemether and arteether, completely cleared parasitemia in mice infested with *Plasmodium berghei*, from the end of treatment to day 25, which, to our knowledge, has never been observed with artemether itself at the concentration tested of 35.5 μmol/kg, whatever the strain used. This improvement in biological activity could be attributed to several factors: better hydrolytic stability, higher oxidative metabolism stability, and higher lipophilic character. These compounds deserve further evaluation and additional biological data in order to exploit their remarkable activity and to have a better appreciation of the influence of the CF_3 group.

Experimental Section

In Vitro Assays. Chloroquine-resistant *P. falciparum* strain FCB1 (Colombia) was maintained in a continuous culture of human erythrocytes as described by Trager and Jensen.[21] In vitro antiplasmodial activity of our compounds was determined using a modification of the semiautomated microdilution technique of Desjardins et al.[22] Stock solutions of tested compounds were prepared in methanol or DMSO. Drug solutions were serially diluted with the culture medium and added to parasite cultures synchronized at the ring stage (1% parasitemia and 1% final hematocrit) in 96-well plates. Parasite growth was assessed by adding 0.5 μCi of [^{3}H]-hypoxanthine (10−30 Ci/mmol, Amersham Biosciences Europe Gmbh) to each well. Plates were incubated for 48 h at 37 °C in the appropriate atmosphere. Immediately after incubation, the plates were frozen and thawed to lyse erythrocytes. The contents of each well were collected on filter microplates, washed using a cell harvester, and dried. Scintillation cocktail was added to each filter, and radioactivity incorporated by the parasites was measured using a scintillation counter. The growth inhibition for each drug concentration was determined by comparison of the radioactivity incorporated in the treated culture with that in the control culture (without drug). The drug concentration causing 50% inhibition (IC_{50}) was determined by nonlinear regression analysis of log(dose)−response curves. Values are the average of three experiments. DMSO and methanol introduced into the cultures never exceeded 0.1% and did not affect parasite growth.

In Vivo Assays. The antimalarial activity was studied in mice (female ICR (CD-1), 18−20 g; Harlan, Gannat, France) infected with *P. berghei* (NK 173 strain) ($15-10^6$ red cells) according to the protocol of Peters.[19] Each group contained four to five mice. Treatments with drugs were performed over 4 days, beginning with the day of infection, by intraperitoneal route. The drugs were given once a day at 0.0355 mmol·kg^{-1} as a suspension in an aqueous solution of carboxymethyl cellulose (1%). The untreated group received the same amount of DMSO in 1% carboxymethyl cellulose. Efficient doses to inhibit 50% and 90% of parasite growth (ED_{50} and ED_{90}) were determined according to Peters by counting parasitemia at day 4 for drug concentrations ranging from 0.5 to 10 mg/kg.[19] The percentage of inhibition was calculated by comparison to the parasitemia determined in control infected mice.

Stability Tests of Artemisinin Analogues in Simulated Stomach Acid. Sample Preparation and Measurement of Stability. 1. Water-Soluble Compounds. A 50 μL portion of an artemisinin prodrug (0.5 mg) stock solution (in acetonitrile) was added to 500 μL of a freshly prepared 0.01 N HCl aqueous solution (pH 2). An amount of 20 μL of a freshly prepared internal standard solution (10 mg of trifluoromethanesulfonic acid per milliliter of D_2O) was also added to the mixture.

2. Insoluble Compounds. A 250 μL portion of an artemisinin prodrug (1 mg) stock solution (in acetonitrile) was added to 250 μL of a freshly prepared 0.1 N HCl aqueous solution (pH 1). An amount of 40 μL of a freshly prepared internal standard solution (10 mg of trifluoromethanesulfonic acid per milliliter of D_2O) was also added to the mixture.

The NMR tube was sealed to prevent evaporation and maintained at 37 °C in a water bath. ^{19}F NMR (128 scans) measurements were made at regular time intervals up to 30 days. The disappearance of the artemisinin derivative was evaluated by comparing integration with that of the internal standard.

Chemistry. NMR spectra were recorded using Bruker AC 200 and ARX 400 (^{1}H, 200 or 400 MHz; ^{19}F, 188 MHz; ^{13}C, 50 or 100 MHz) spectrometers, in $CDCl_3$ solutions. Chemical shifts are reported in ppm relative to Me_4Si and $CFCl_3$ (for ^{19}F NMR) as internal standards. In the ^{13}C NMR data, reported signal multiplicities are related to C−F coupling. For the determination of fine coupling constants, an acquisition of 16K data points, a Lorentz−Gauss transformation of the free induction decay (FID), and a zero filling to 64K were performed in order to obtain a minimum of resolution of 0.2 Hz/point (^{1}H) or 0.5 Hz/point (^{13}C). Complete assignments in NMR resulted from *J* module, COSY, HMQC, and HMBC experiments performed on a multinuclear probe head equipped with a *Z*-gradient coil. Optical rotations were measured at 589 nm on a Polartronic E-Schmidt-Haensch apparatus. TLC was performed on Merck 60 F_{254} silica plates (vanilin−MeOH−H_2SO_4). Column chromatography was carried out on Merck SiO_2 (70−230 mesh). The IUPAC nomenclature of **6** is (1*S*,4*R*,5*R*,8*R*,9*R*,10*R*,12*R*,13*R*)-1,5,9-trimethyl-10-(trifluoromethyl)-11,14,15,16-tetraoxatetracyclo[10.3.1.0.^{4,13}.0^{8,13}]-hexadecane-10-ol. However, for commodity reasons we have opted for the usual nomenclature 10β-hydroxy-10α-trifluoromethyldeoxoartemisinin for **6** and similar nomenclature for all other compounds described below. Compounds **9**, **11**−**16**, **18**, and **19** were prepared as previously described.[16]

Preparation of 10β-(4-Methoxyphenylamino)-10α-trifluoromethyldeoxoartemisinin (17). Bromide **8** (1.128 g, 2.72 mmol) was dissolved in CH_2Cl_2 (15 mL). The solution was stirred under an Ar stream at room temperature, and *p*-

anisidine (3.34 g, 27.2 mmol) and HFIP (1.4 mL, 13.6 mmol) were successively added. After being stirred for 1 night at room temperature, the mixture was diluted with ethyl acetate, washed with a saturated aqueous solution of $NaHCO_3$, and dried over $MgSO_4$. Evaporation of the solvent afforded the crude product, which was purified on an SiO_2 column (9:1 petroleum ether/AcOEt). Compound **16** was obtained (315 mg, 25%) as a brown foam. $[\alpha]_D$ +93.6 (*c* 0.34, MeOH); ^{1}H NMR δ 0.95 (3H, d, $J = 5.5$ Hz), 1.11 (3H, d, $J = 7.5$ Hz), 1.47 (3H, s), 0.83−2.17 (10H, m), 2.39 (1H, m), 3.05 (1H, m), 3.63 (1H, s broad), 3.75 (3H, s), 5.56 (1H, s), 6.81 (2H, m), 7.04 (2H, m); ^{13}C NMR δ 12.6, 20.0, 23.5, 24.5, 25.7, 30.1, 34.3, 36.2, 37.2, 46.3, 52.2, 55.2, 80.1, 88.3 (q, $J_{C-F} = 28$ Hz), 90.0, 104.3, 114.2, 119.2, 123.8 (q, $J_{C-F} = 294$ Hz), 136.4, 154.0; ^{19}F NMR δ −75.5 (s, 3F, CF_3). Anal. ($C_{23}H_{30}F_3NO_5$) C, H, N.

Preparation of 10*β*-(2,3-dihydroxypropoxy)-10α-trifluoromethyldeoxoartemisinin (20). Compound **13** (396 mg, 1.0 mmol) was dissolved in a mixture of *tert*-butanol (20 mL) and water (2 mL). 4-Methylmorpholine *N*-oxide (150 mg, 1.1 mmol) was then added to the solution, followed by osmium tetroxide (13 mg, 0.05 mmol). After being stirred for 3 h at room temperature, the mixture was diluted with AcOEt, successively washed with an aqueous solution of $NaHSO_3$ and $NaHCO_3$, and dried over $MgSO_4$ and the solvents were removed under vacuum. Purification of the crude on silica gel (petroleum ether/AcOEt 8:2) afforded a white foam of **20** (257 mg, 60%) as a mixture (40:60) of two diastereoisomers. ^{1}H NMR δ 0.95 (3H, d, $J = 6.0$ Hz), 0.99 (3H, d, $J = 7.5$ Hz), 1.41 (3H, s), 2.37 (1H, ddd, $J = 14.5$ Hz, $J = 14.0$ Hz, $J = 4.5$ Hz), 0.76−2.59 (12H, m), 2.86 (1H, m), 3.41−4.05 (5H, m), 5.48 (H_{mino}, s), 5.55 (H_{majo}, s); ^{13}C NMR δ 11.7, 19.7, 22.6, 22.8, 24.3, 25.2, 29.3, 34.4, 35.8, 36.85, 36.90, 45.6, 45.7, 51.8, 63.3, 63.5, 63.6, 64.2, 70.6, 71.0, 79.7, 79.8, 88.7, 88.8, 98.22 (q, $J_{C-F} =$ 29 Hz), 98.25 (q, $J_{C-F} = 29$ Hz), 104.0, 122.2 (q, $J_{C-F} = 293$ Hz); ^{19}F NMR δ −76.1 (40%), −76.2 (60%). Anal. ($C_{19}H_{29}F_3O_7$) C, H.

Preparation of 10*β*-carboxymethoxy-10α-trifluoromethyldeoxoartemisinin (21). Compound **13** (595 mg, 1.5 mmol) was dissolved in a mixture of CCl_4 (3 mL), CH_3CN (3 mL), and water (5 mL). Sodium periodate (1.6 g, 7.6 mmol) was then added to the solution, followed by ruthenium(III) chloride (8 mg, 0.03 mmol). After being stirred for 1 night at room temperature, the mixture was filtered over Celite, diluted with AcOEt, successively washed with an aqueous solution of $NaHSO_3$ and $NaHCO_3$, and dried over $MgSO_4$ and the solvents were removed under vacuum. Purification of the crude on silica gel (petroleum ether/AcOEt 7:3) afforded **21** (353 mg, 60%) as a white foam. $[\alpha]_D$ +71.2 (*c* 0.80, MeOH); ^{1}H NMR δ 0.90 (1H, m, H-7ax), 0.95 (3H, d, $J_{15-6} = 6.0$ Hz, CH_3-15), 1.05 (3H, d, $J_{16-9} = 7.0$ Hz, CH_3-16), 1.05 (1H, m, H-5a), 1.35 (1H, m, H-6), 1.40 (3H, s, CH_3-14), 1.45 (1H, m, H-5), 1.55 (1H, m, H-8a), 1.65 (1H, dq, $J_{7eq-7ax} = 13.0$ Hz, $J_{7eq-6} = J_{7eq-8ax} = J_{7eq-8eq} =$ 2.5 Hz, H-7eq), 1.75 (1H, m, H-8), 1.9 (1H, m, H-5), 2.00 (1H, dt, $J_{4eq-4ax} = 14.5$ Hz, $J_{4eq-5ax} = J_{4eq-5eq} = 3.0$ Hz, H-4eq), 2.15 (1H, m, H-8), 2.40 (1H, ddd, $J_{4ax-4eq} = 14.5$ Hz, $J_{4ax-5a} = 14.0$ Hz, $J_{4ax-5eq} = 4.0$ Hz, H-4ax), 2.90 (1H, dq, $J_{9-16} = J_{9-8a} = 6.0$ Hz, H-9), 4.20 (1H, d, $J_{17-17} = 16.5$ Hz, H-17), 4.50 (1H, s, OH), 4.55 (1H, d, $J_{17-17} = 16.5$ Hz, H-17), 5.50 (1H, s, H-12); ^{13}C NMR δ 12.1 (C-16), 20.2 (C-15), 22.8 (C-8), 24.7 (C-5), 25.7 (C-14), 29.5 (C-9), 34.7 (C-7), 36.2 (C-4), 37.3 (C-6), 45.9 (C-8a), 52.1 (C-5a), 60.7 (C-17), 80.2 (C-12a), 89.6 (C-12), 98.9 (q, $J_{C-F} = 29$ Hz, C-10), 104.7 (C-3), 122.4 (q, $J_{C-F} = 292$ Hz, CF_3), 175.5 (C-18); ^{19}F NMR δ −76.1 (s, 3 F, CF_3); IR ν_{max} 3200, 1728 cm^{-1}. Anal. ($C_{18}H_{25}F_3O_7$) C, H.

Preparation of 10*β*-(2-Oxoethoxy)-10α-trifluoromethyldeoxoartemisinin (22). A solution of **13** (298 mg, 0.76 mmol) in dichloromethane (40 mL) was cooled at −78 °C. Ozone gas was passed through the solution at that temperature until it became light-blue. The color was diminished as oxygen gas was bubbled through the solution. The reaction was quenched with methyl sulfide (85 μL, 1.14 mmol), and the resultant reaction solution was evaporated under reduced pressure to give a colorless oil. Purification of the crude on silica gel (petroleum ether/AcOEt 9:1) afforded **22** (238 mg, 80%) as a white foam. $[\alpha]_D$ +94.0 (*c* 0.32, MeOH); ^{1}H NMR δ 0.94 (3H, d, $J = 5.5$ Hz), 1.04 (3H, d, $J = 7.0$ Hz), 1.39 (3H, s), 2.35 (1H, m), 2.88 (1H, m), 4.35 (1H, dm, $J = 18.5$ Hz), 4.54 (1H, d, $J = 18.5$ Hz), 5.28 (1H, s), 9.6 (1H, s); ^{13}C NMR δ 12.0, 20.1, 23.1, 24.5, 25.6, 29.4, 34.5, 36.0, 37.3, 45.6, 51.8, 68.4, 79.9, 89.4, 98.8 (q, $J_{C-F} = 29$ Hz), 104.6, 122.2 (q, $J_{C-F} = 292$ Hz), 197.8; ^{19}F NMR δ −76.3 (s, 3 F, CF_3); IR ν_{max} 1740 cm^{-1}. Anal. ($C_{18}H_{25}F_3O_6$) C, H.

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References

(1) Klayman, D. L. Qinghaosu (Artemisinin): An Antimalarial Drug from China. *Science* **1985**, *228*, 1049−1055.

(2) Wu, Y. L.; Li, Y. The Chemistry, Pharmacology, and Clinical Applications of Qinghaosu (Artemisinin) and Its Derivatives. *Med. Chem. Res.* **1995**, *5*, 599−586.

(3) (a) Li, Y.; Yu, P. L.; Chen, Y. X.; Li, L. Q.; Gai, Y. Z.; Wang, D. S.; Zheng, Y. P. Studies on Analogs of Artemisinin I Synthesis of Ethers, Carboxylate Esters and Carbonates of Dihydroartemisinin. *Acta Pharm. Sin.* **1981**, *16*, 429−439. (b) Wang, T.; Xu, R. Clinical studies of treatment of falciparum malaria with arthemether, a derivative of qinghaosu. *J. Tradit. Chin. Med.* **1985**, *5*, 240−242.

(4) Brossi, A.; Venugopalan, B.; Gerpe, L.; Yeh, H. J. C.; Flippen-Anderson, J. L.; Buchs, P.; Luo, X. D.; Milhous, W.; Peters, W. Arteether, a New Antimalarial Drug: Synthesis and Antimalarial Properties. *J. Med. Chem.* **1988**, *31*, 645−650. Arteether is registered as Artemotil: *TDR News*, **2000**, June (No. 62).

(5) China Cooperative Research Group on Qinghaosu and Its Derivatives as Antimalarials. The chemistry and synthesis of qinghaosu derivatives. *J. Tradit. Chin. Med.* **1982**, *2*, 9−16.

(6) Hien, T. T.; White, N. J. Qinghaosu. *Lancet* **1993**, *341*, 603−608.

(7) Lin, A. J. L.; Klayman, D. L.; Milhous, W. K. Antimalarial Activity of New Water-Soluble Dihydroartemisinin Derivatives. *J. Med. Chem.* **1987**, *30*, 2147−2150.

(8) (a) Leskovac, V.; Theoharides, A. D. Hepatic metabolism of artemisinin drugs: I. Drug metabolism in rat liver microsomes. *Comp. Biochem. Physiol.* **1991**, *C99*, 383−390. (b) Leskovac, V.; Theoharides, A. D. Hepatic metabolism of artemisinin drugs: II. Metabolism of arteether in rat liver cytosol. *Comp. Biochem. Physiol.* **1991**, *C99*, 391−396. (c) Baker, J. K.; McChesney, J. D.; Chi, H. T. Decomposition of Arteether in Simulated Stomach Acid Yielding Compounds Retaining Antimalarial Activity. *Pharm. Res.* **1993**, *10*, 662−666. (d) Grace, J. M.; Aguilar, A. J.; Trotman, K. M.; Brewer, T. G. Metabolism of *β*-arteether to dihydroqinghaosu by human liver microsomes and recombinant cytrochrome P450. *Drug Metab. Dispos.* **1998**, *26*, 313−317. (e) Navaratnam, V.; Mansor, S. M.; Sit, N. W.; Grace, J.; Li, Q. G.; Olliaro, P. Pharmacokinetics of artemisinin-type compounds. *Drug Dispos.* **2000**, *39*, 255−570.

(9) Meshnick, S. R.; Taylor, T. E.; Kamchonwongpaisan, S. Artemisinin and the Antimalarial Endoperoxides: From Herbal Remedy to Targeted Chemotherapy. *Microbiol. Rev.* **1996**, *60*, 301−315.

(10) (a) Irurre, J., Jr.; Casas, J.; Ramos, I.; Messeguer, A. Inhibition of Rat Liver Microsomal Lipid Peroxidation Elicited by 2,2-Dimethylchromenes and Chromans Containing Fluorinated Moieties Resistant to Cytochrome P-450 Metabolism. *Bioorg. Med. Chem.* **1993**, *1*, 219−225. (b) Irurre, J., Jr.; Casals, J.; Messeguer, A. Resistance of the 2,2,2-trifluoroethoxy aryl moiety to the cytochrome P-450 metabolism in rat liver microsomes. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 179−182. (c) Edwards, P. N. Uses of Fluorine in Chemotherapy. In *Organofluorine Chemistry: Principles and Commercial Applications*; Banks, R. E., Smart, B. E., Tatlow, J. C., Eds.; Plenum Press: New York, 1994; pp 501−541.

(11) (a) Abouabdellah, A.; Bégué, J. P.; Bonnet-Delpon, D.; Gantier, J. C.; Truong Thi Thanh, N.; Truong Dinh, T. Synthesis and in Vivo Antimalarial Activity of 12α-Trifluoromethyl-hydroartemisinin. *Bioorg. Biomed. Chem. Lett.* **1996**, *6*, 2717−2720. (b)

Nga, T. T.; Ménage, C.; Bégué, J. P.; Bonnet-Delpon, D.; Gantier, J. C.; Pradines, B.; Doury, J. C.; Thac, T. D. Synthesis and antimalarial activities of fluoroalkyl derivatives of dihydroartemisinin. *J. Med. Chem.* **1998**, *41*, 4101−4108. (c) Binh, P. D.; Le Dinh Cong; Nhan, D. H.; Nhu, T. V.; Tien, N. T.; Bégué, J. P.; Bonnet-Delpon, D.; Truong Thi Thanh, Nga. The effect of 10α-Trifluoromethyl Hydroartemisinin on *Plasmodium berghei* Infection and Its Toxicity in Experimental Animals. *Trans. R. Soc. Trop. Med. Hyg.* **2002**, *96*, 677−683.

(12) Previous studies on the introduction of fluorine atoms to other sites have been achieved, but no significant improvement of the in vitro or in vivo activity was claimed. (a) Pu, Y. M.; Torok, D. S.; Ziffer, H.; Pan, X.-Q.; Meshnick, S. R. Synthesis and Antimalarial Activities of Several Fluorinated Artemisinin Derivatives. *J. Med. Chem.* **1995**, *20*, 4120−4124. (b) Luo, X. D.; Shen, C. C. The Chemistry, Pharmacology, and Clinical Applications of Qinghaosu (Artemisinin) and Its Derivatives. *Med. Res. Rev.* **1987**, *7*, 29−52.

(13) Pharmacokinetic studies on **4** and DHA have been performed at Monash University: Charman, W. N.; McIntosh, L. Unpublished results.

(14) Bégué, J. P.; Bonnet-Delpon, D.; Benayoud, F.; Tidwell, T. T.; Cox, R. A.; Allen, A. Comparison of the Formation Energy of Fluorinated Alkoxycarbenium Ions. *Gazz. Chim. Ital.* **1995**, *125*, 399−402.

(15) Chorki, F.; Grellepois, F.; Crousse, B.; Hoang, V. D.; Hung, N. V.; Bonnet-Delpon, D.; Bégué, J. P. First synthesis of 10α-(Trifluoromethyl)deoxoartemisinin. *Org. Lett.* **2002**, *4*, 757−759.

(16) Magueur, G.; Crousse, B.; Ourévitch, M.; Bégué, J. P.; Bonnet-Delpon, D. Preparation of 10-Trifluoromethyl Artemether and Artesunate. Influence of Hexafluoropropan-2-ol on substitution reaction. *J. Org. Chem.* **2003**, *68*, 9763−9766.

(17) Jung, M.; Lee, S. Stability of acetal and non acetal-type analogs of artemisinin in simulated stomach acid. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1003−1006.

(18) (a) Batty, K. T.; Ilett, K. F.; Davis, T.; Davis, M. E. Chemical Stability of Artesunate Injection and Proposal for Its Administration by Intravenous Infusion. *J. Pharm. Pharmacol.* **1996**, *48*, 22−26. (b) In 16, the succinic substituent at C-10 is beta, while it is alpha in artesunate.

(19) Peters, W. Techniques of Drug Evaluation I: Primary Screening. In *Chemotherapy and Drug Resistance in Malaria*; Peters, W., Ed.; Academic Press: London, 1987; Vol. 1, pp 100−123.

(20) The log*P* values were calculated using the http://www.daylight.com/cgi-bin/contrib/pcmodels.cgi program.

(21) Trager, W.; Jensen, J. B. Human malarial parasites in continuous culture. *Science* **1976**, *193*, 673−677.

(22) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, D. J. Quantitive Assessment of Antimalarial Activity in Vitro by a Semiautomated Microdilution Technique. *Antimicrob. Agents Chemother.* **1979**, *16*, 710−718.

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