DIASTEREOFACIAL ADDITIONS TO A β-SUBSTITUTED GLYCAL, ANHYDRODIHYDROARTEMISININ‡

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Abstract - Triphenylphosphine hydrobromide catalyzed electrophilic addition of ethanol to the carbon-carbon double bond of anhydrodihydroartemisinin occurs predominately from the β-face of the molecule.

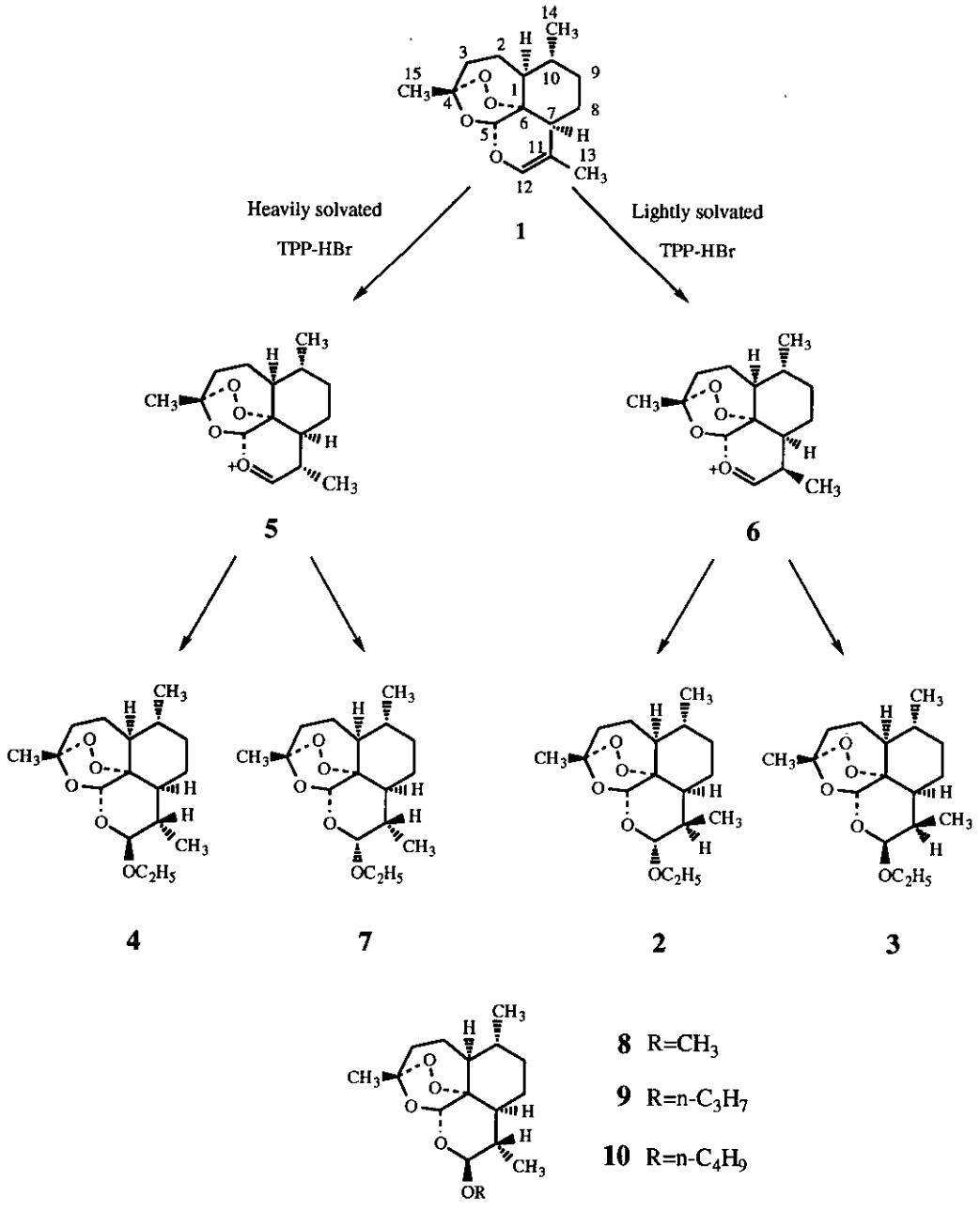
INTRODUCTION While efforts to develop vaccines against malaria progress slowly,[1] an urgent need for new effective antimalarial agents has prompted a host of structure-activity relationships (SAR) of artemisinin derivatives. Artemisinin, the active principle of the Chinese medicinal herb Artemisia annua, was shown to be effective in treating patients with drug resistant strains of Plasmodium falciparum.[2] SAR studies of artemisinin derivatives possessing an unnatural stereochemistry at one or more centers have been carried out by Avery et al.[3] using intermediates prepared by total synthesis. Few transformations of artemisinin into derivatives containing unnatural configurations at C-10, or C-11 have been reported. A report by Franck et al.[4] that triphenylphosphine hydrobromide (TPP-HBr), catalyzes the addition of alcohols to glycals, suggested that it might be possible to prepare 11-epiartemisinin derivatives from anhydrodihydroartemisinin (**1**), readily accessible from dihydroartemisinin by literature methods.[5] Franck et al.[4] found that the proton adding to the double bond of several glycals became an equatorial atom. However, none of the glycals they examined possessed a β–alkyl group so it was uncertain whether the specificity observed by Franck et al. would lead to formation of 11-epidihydroartemisinin derivatives. In addition to providing a route to the desired compounds, the results from TPP-HBr catalyzed additions to **1** could be used to evaluate the ability of Polarized π-Frontier Molecular Orbital Theory (PFMOT) to predict which face of a glycal is more reactive to electrophiles. [6] Finally, the in vitro antimalarial activities of new artemisinin derivatives prepared in these studies have been determined.

DISCUSSION Since both α– and β-arteethers (**2** and **3**) were known compounds, the TPP-HBr catalyzed

‡ Dedicated to Dr. Arnold Brossi on the occasion of his 70th birthday.

addition of 1-2 equivalents of ethanol to anhydrodihydroartemisinin (**1**) was examined and found to yield a 3:5 mixture of **3** and a new product (**4**). To avoid a difficult separation, the effect of different reaction conditions on the ratio of **4:3** was examined and showed a dependence of the ratio upon the number of equivalents of ethanol

Scheme 1



employed. As the number of equivalents of ethanol relative to the compound was increased from 1-2 to 6-20, the apparent rate of reaction decreased. However, the selectivity of proton addition increased, i.e. the ratio of **4:3** increased to 10:1. In addition to simplifying the purification of **4**, the above results enabled us to employ this catalyzed addition to prepare radiochemically labelled **3** by employing 1-2 equivalents of EtO^3H.[5]

Table 1

Equivalents of ethanol:**1**	Ratio of **4:3**	Total yield of products
1	0.4	39%
2	0.7	42%
3	1.4	36%
6	10	32%
20	10	72%

One explanation for the dependence of the addition stereochemistry on the quantity of alcohol employed is that in the presence of additional equivalents of alcohol the latter solvate the triphenylphosphonium ion and the peroxide moiety. As the solvation shell about the ion increases, it becomes bulkier and thus more sensitive to small differences in energetic and steric barriers of approaching the α– and β– faces of the glycal. Hydrogen bonding between the alcohol and peroxide group increases the barrier toward approach from the α–face. Thus, solvation of the triphenylphosphonium ion and the substrate facilatate proton addition to the β–face of the glycal as shown in Scheme 1. The anomeric effect then determines the stereochemistry of the products derived from **5** and **6**. When 20 equivalents of ethanol were employed, **4** was isolated in 75% yield and purified by flash chromatography. Its molecular weight was identical to that of **3**, but its carbon nmr spectrum (Table 2) differed from those of **2** and **3**. The chemical shift of H-13 (methyl) in **4** was 0.20 ppm downfield from the same methyl group in **3** (Table 3). A similar downfield shift (0.25 ppm) was reported by Acton and Klayman [7] for H-13 in 11-epiartemisinin as compared to that for H-13 in artemisinin. In the normal series, **2** and **3**, the stereochemistry

Table 3

Selected ^1H nmr data on the isomeric arteethers

Compound	H-5	H-12	H-13	H-14	H-15
3	5.41	4.80	0.90	0.95	1.44
4	5.39	4.93	1.10	0.88	1.35
2	5.34	4.43	0.88	0.95	1.44
7	5.26	4.78	1.28	0.94	1.45

at C-12 could readily be assigned from data on $J_{11,12}$, since H-11 is axial. However, since H-11 is equatorial in the 11-epi isomers, the e-a and e-e couplings become too similar to allow secure assignments. The stereochemistry at C-12 in **4** was therefore initially assigned from mechanistic arguments. The anomeric effect favors axial addition of ethanol to the β-face of the oxonium intermediate (**5**) to yield **4**. Brossi et al. [8] obtained a 3:1 mixture of β- to α-arteethers from the reaction of ethanol with **6** (under acidic conditions).

The possibility that one or both α- ethers might be present in the crude reaction mixture prompted us to search for 11-epi-α-arteether which had not previously been prepared. An analysis by thin layer chromatography of the crude reaction mixture revealed the presence of small quantities of two additional dihydroartemisinin derivatives. The compounds were isolated by flash chromatography and their ^{13}C nmr spectra are reported in Table 2. A comparison of the proton and carbon spectra of the more abundant minor product with that of **2** showed that they were identical. The second minor product displayed mass and ^{13}C nmr spectra showing it was isomeric with the other arteethers (Table 2). Its stereochemistry was therefore initally assigned as **7**. The assignment was consistent with chemical shift of C-13 and that of H-13 in their nmr spectra. These assignments were verified by a comparison of 2D NOE spectra of **4** and **7**. Molecular models show that H-5 and H-12 are both axial and on the same face of the molecule only for the 12α- isomer (**7**). The compound assigned this structure exhibited small NOE interactions between H-5 and H-12, as well as between H-11 and H-8. No such interactions were observed for these protons in the NOE spectrum of **4**. Thus, the stereochemical assignments for **4** and **7** based on mechanistic grounds are supported by spectroscopic data, i.e. the 2D NOE spectral measurements of the two compounds. The major products formed in TPP-HBr catalyzed additions of methanol, n-propanol, and n-butanol to **1** were isolated and their stereochemistries were assigned as 11-epi β-ethers from an analysis of their ^{1}H and ^{13}C nmr spectra. The data are summarized in Table 2. Their in vitro antimalarial activities were determined and the results are summarized in Table 4. Luo and Shen [2b] reported that the suppressive doses (SD_{50})of several α- and β-ethers of dihydroartemisinin varied by less than a factor of two. A similar insensitivity of the antimalarial activities of the 11-epi compounds was shown by our compounds.

CONCLUSION The TPP-HBr catalyzed addition of alcohols to anhydrodihydroartemisinin has been used to prepare a series of 11-epidihydroartemisinin β-ethers. Their in vitro antimalarial activities are very similar but less than those of the corresponding isomers with a β-C-11 methyl. In all cases the proton adding to the β-carbon of the glycal becomes equatorial. Thus, the presence of the β-methyl group on the glycal does not alter the stereochemistry for the proton addition described by Franck et al. [4]

Table 4

Summary of in vitro antimalarial data on D-6

Compound	IC_{50} compound/ control (artemisinin)
2	0.4
7	2.0
8	4.0
3	5.0
9	4.6
10	4.9

EXPERIMENTAL

Anhydrodihydroartemisinin (**1**): Dihydroartemisinin was prepared by reduction of artemisinin with sodium borohydride in 87% yield. Into a 250 ml 2-necked round bottom flask were placed dihydroartemisinin (1.5 g, 5.28 mmol), 200 ml of dried CH_2Cl_2, and 1.5 g (10 mmol) of phosphorus pentoxide. The solution was stirred for 30 min at room temperature at which time the starting material had been consummed. The solution was concentrated and the crude product was purified by flash chromatography (hexane:ether, 15:1) to yield **1** (1.1 g) in 80% yield. Its ^1H nmr spectrum was identical to that reported by Brossi et al. [8]

11-Epi-β-arteether, **4**: A solution of **1** (106 mg, 0.40 mmol), triphenylphosphine hydrobromide (7.0 mg, 0.02 mmol) and absolute ethanol (0.37 ml, 8.0 mmol) in CH_2Cl_2 (8.0 ml) in a 25 ml round bottom flask was stirred at RT for 8 hr. It was washed with 10 mL of a saturated aqueous $NaHCO_3$ solution, dried and concentrated. The crude reaction mixture was purified by flash chromatography (hexane:ethyl acetate, 94:6) to yield a 9:1 mixture (77 mg) of **4** and **3**. A pure sample of **4** was obtained by hplc, on a reverse phase column, using methanol:H_2O (85:15), mp 60-62°C; $[\alpha]_D$ +140° (c 0.50, $CHCl_3$), 130° (c 0.50, EtOH); ^1H nmr ($CDCl_3$) 5.39 (1H, s, H-5), 4.93 (1H, d, H-11 $J_{11,12}$ = 5.3Hz)), 3.85, 3.51 (2H, m, H-16), 2.24 (1H, ddd, H-11 $J_{11,13}$=7.3, $J_{11,12}$=5.3, $J_{7,11}$=1.5 Hz,), 1.35 (3H, s, H-15), 1,11 (3H, d, H-13, J=7.3 Hz), 0.88 (3H, d, H-14 J=5.9 Hz). CI-ms (NH_3) 330, 5%; 267, 100%. Anal. Calcd for $C_{17}H_{28}O_5$, C 65.36, H 9.03. Found C 65.33, H 9.05.

Arteether (**3**). A sample of **3** was isolated using the above reaction except the following ratio of reactants; **1** (318 mg, 1.2 mmol), TPP-HBr (21 mg, 0.06 mmol), absolute alcohol (0.11 ml, 1.8 mmol) in CH_2Cl_2 (36 ml). The product was purified by hplc on a reverse phase column with methanol:water (85:15). The compound's ^1H and ^{13}C nmr spectra were in agreement with published data .[8]

11-Epi-α-arteether (**7**): Thin layer analysis on silica gel using hexane:ethyl acetate (94:6) showed the presence of a dihydro-artemisinin derivative with an R_f of 0.28 compared to 0.22 for an authentic sample of α-arteether (**2**). A sample of material was isolated and purified by preparative thin layer chromatography to yield a solid, mp 136-138°C. Its specific rotation, $[\alpha]_D$ was +30° (c 0.45, $CHCl_3$) and +25° (c 0.45, EtOH). An authentic sample of **2** was prepared as described by Brossi et al.[8]

General Procedure for Preparation of 12β-Ethers of 11-Epidihydroartemisinin: To a solution of **1** (106 mg, 0.40 mmol) in dry CH_2Cl_2 (8.0 ml) was added TPP-HBr (7.0 mg, 0.02 mmol) and dry alcohol (methanol, n-propanol, and n-butanol, 8.0 mmol). The solution was stirred for 6-20 h at room temperature. The solution was washed with 1% aqueous $NaHCO_3$, dried over Na_2SO_4 and concentrated. The product was initially purified by flash chromatography over silica gel using hexane:ethyl acetate (94:6). The final purification of the 12β-11-epiethers was accomplished by hplc on a reverse phase column using methanol:water (85:15).

11-Epi-β-artemether (**8**): The product was obtained in 40% yield as an oil: $[\alpha]_D$ +140° (c 0.50, $CHCl_3$), ^1H nmr ($CDCl_3$) 5.34 (1H, s, H-5), 4.87 (1H, d, H-12, $J_{11,12}$=4.6 Hz) 3.47 (3H, s, H-16), 2.32 (1H, ddd, H-3a, J=13.9, 13.9, 3.9 Hz), 2.03 (1H, ddd, H-3b, J=13.9, 3.9, 3.9 Hz), 1.43 (3H, s, H-15), 1.21 (3H, d, H-13, J=7.3 Hz); CI-ms (NH_3) 316, 10%; 284, 100%; 267, 47%. High resolution mass measured m^+/z for $C_{16}H_{26}O_5$, calcd 298.1780, found 298.1783.

11-Epi-12β-n-propyl ether of dihydroartemisinin (**9**): The product was obtained in 45% yield as an oil: $[\alpha]_D$ +120° (c 5.0, $CHCl_3$), ^1H nmr ($CDCl_3$) 5.45 (1H, s, H-5), 4.97 (1H, d, H-12, $J_{11,12}$=5.1 Hz), 3.83, 3.45 (2H, m, H-16), 2.31 (1H, ddd, H-3a, J=13.9, 13.9, 3.9 Hz), 2.02 (1H, ddd, H-3b, J=13.9, 3.9, 3.9), 1.43 (3H, s, H-15), 1.19 (3H, d, H-13, J=7.3 Hz) 0.95 (3H, d, H-14, J=4.5 Hz), 0.94 (3H, t, H-18, J=7.4 Hz); CI-ms (NH_3) 344, 10%; 298, 5%; 284, 85%; 281, 100%. High resolution mass measured m^+/z for $C_{18}H_{30}O_5$, calcd 326.2092, found 326.2090.

11-Epi-12β-n-butyl ether of dihydroartemisinin (**10**): The product after purification on a reverse phase hplc column yielded an oil in 45% yield, $[\alpha]_D^{\cdot}$ +115° (c 0.60, $CHCl_3$), ^1H nmr ($CDCl_3$) 5.44 (1H, s, H-5), 4.95 (1H, d, H-12 $J_{11,12}$=5.1 Hz), 3.86, 3.48 (2H, m, H-16), 2.32 (1H, ddd, 3-Ha J=13.9, 13.9, 4.0), 2.03 (1H, ddd, H-3b J=13.9, 3.9, 3.9), 1.41 (3H, s, H-15), 1.18 (3H, d, H-13 J=7.1 Hz), 0.94 (3H, d, H-14 J=6.0 Hz), 0.92 (3H, t, H-19 J=7.1 Hz); CI-ms (NH_3) 358, 7%; 295, 98%; 284, 100%; 267, 50%. High resolution mass measured m^+/z for $C_{19}H_{32}O_5$, calcd 340.2248, found 340.2255.

Table 2

Summary of ^{13}C nmr data on isomeric arteethers and 11-epi-ethers

Compounds	Carbon						
	2	**3**	**4**	**7**	**8**	**9**	**10**
1	51.7	52.8	51.9	52.0	52.0	52.0	51.9
2	24.8	24.8	24.8	24.5	24.8	24.8	24.8
3	36.4	36.6	36.6	36.5	36.6	36.6	36.6
4	104.2	104.0	102.9	104.1	103.0	102.9	102.9
5	91.2	87.9	89.2	92.1	88.8	89.0	89.0
6	80.3	81.2	81.7	80.5	81.5	81.6	81.6
7	45.4	44.7	46.7	46.5	46.5	46.6	46.6
8	22.3	24.6	31.7	30.0	31.6	31.6	31.6
9	34.3	34.8	34.5	34.5	34.5	34.5	34.5
10	37.4	37.6	37.4	37.1	37.6	37.8	37.3
11	32.6	31.0	40.0	38.9	39.7	39.8	39.9
12	99.8	101.7	102.3	98.5	104.1	102.7	102.7
13	12.7	13.1	19.5	13.9	19.7*	19.6	19.6
14	20.4	20.4	20.1	20.2	20.2*	20.2	20.2
15	26.1	26.3	26.0	25.9	26.0	26.1	26.0
16	64.4	63.8	64.2	64.8	56.1	70.4	68.5
17	15.2	15.3	15.3	15.0	23.0	31.9	

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