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A Practical Synthesis of the Enantiomers of Hydroxychloroquine

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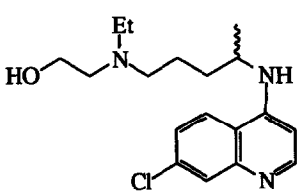
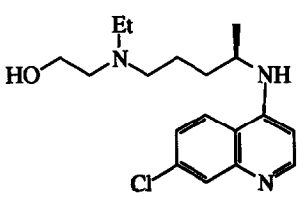
Abstract: An efficient synthesis of the enantiomeric forms of hydroxychloroquine is described. The method is suitable for the production of multigram quantities of the dihydrogenphosphate salts **(R)-1b** and **(S)-1b**.

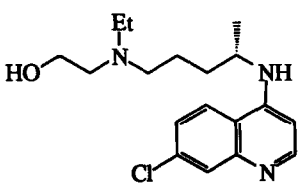
It has been known for some years that, in addition to its antimalarial activity,[1] hydroxychloroquine (Plaquenil, ***rac*-1**; primarily used as the sulfate, **1c**) is of value in the palliation of rheumatoid arthritis.[2,3] As the use of ***rac*-1** in this indication has increased in recent years, so has interest in the biology of its enantiomers. Recent reports by several groups[3-6] of potentially useful differences in biological activity between **(R)-1** and **(S)-1** has prompted us to develop a method for their preparation in multigram quantities, so as to enable more complete evaluation.



***rac*-1**



(R)-1



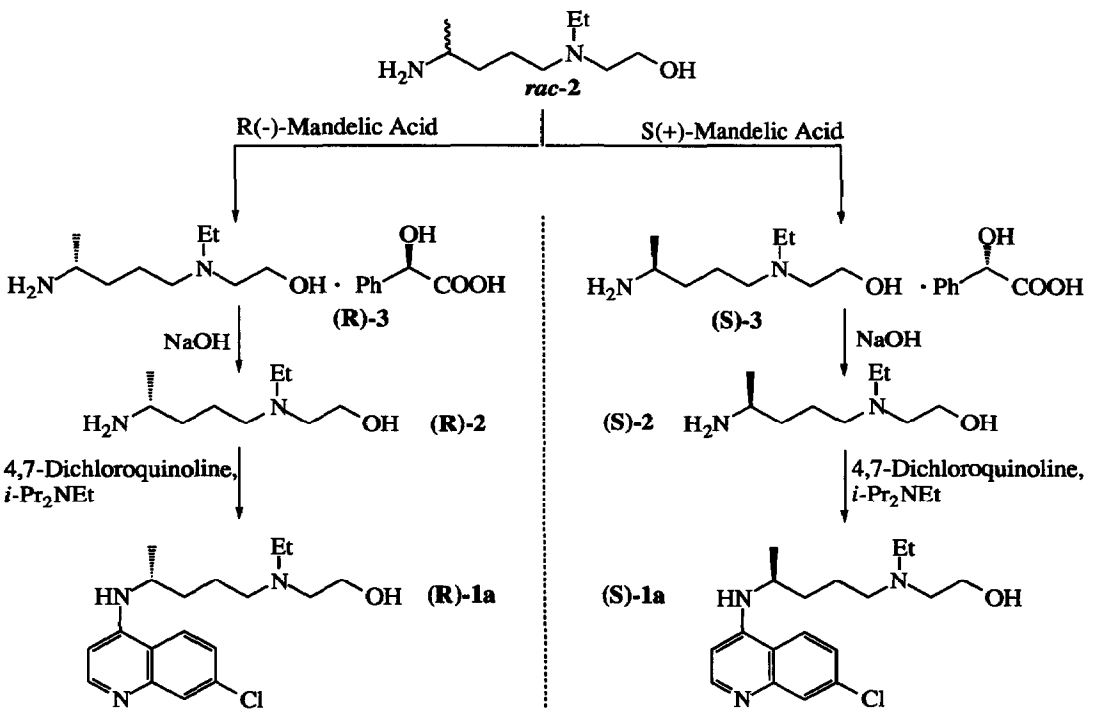
(S)-1

a. free base; b. bis(dihydrogenphosphate); c. sulfate

None of the available routes to **(R)-1** and **(S)-1** is suitable for the preparation of multigram quantities of both. The methods reported by the groups of Tett[4] and Wainer[5] rely on HPLC using a homochiral stationary phase. Ansari and Craig[7] have developed a route to both enantiomers, based on their successful resolution of chloroquine,[8] and have established the absolute configurations of the enantiomers of **1** as S(+) and R(-) from circular dichroism data.

RESULTS

Synthesis of the enantiomers **(R)**- and **(S)-1a** is summarised in Scheme 1. The racemic diamine ***rac* -2** is resolved by crystallisation of its salt with S(+)-mandelic acid. Subsequent coupling with 4,7-dichloroquinoline gives S(+)-hydroxychloroquine (**(S)-1a**). Similarly, using the opposite enantiomer of mandelic acid gives **(R)-2** and R(-)-hydroxychloroquine (**(R)-1a**).

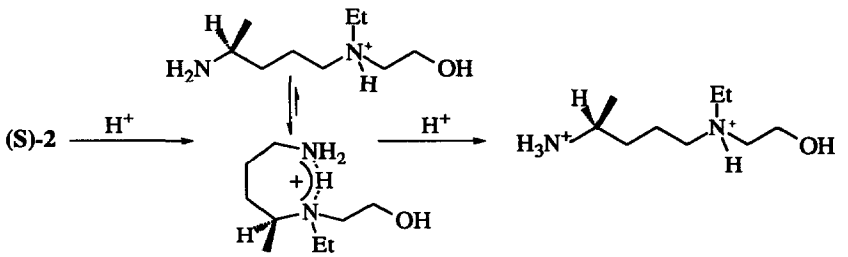


Scheme 1. Resolution of **2** and formation of **(R)**- and **(S)-1a**.

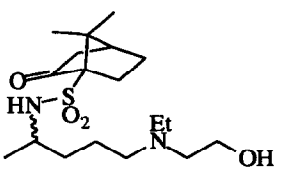
An effective process for resolution of **2** involved crystallisation of its diastereomeric mandelate salts from *iso*-propanol. Using 0.5 molar equivalents of S(+)-mandelic acid and seeding the mixture with pure diastereomer at 45°C, 67% of **(S)-3** was recovered after a single crystallisation with a diastereomeric excess (d.e.) of 92%. When the mixture was not seeded, the recovery dropped to 63% and the d.e. to 90%. After a further two crystallisations, the recovery of **(S)-3** was 55.5%, with a d.e. above 99%. Attempts were made to

improve the recovery and/or initial d.e. of the diamine salt by varying the ratio of diamine to mandelic acid but these led to no improvement in either the yield or enantiomeric purity of the resolved diamine. Ratios and yields are calculated without taking into account the presence of a variable quantity (up to 10%) of water in **2**.

After hydrolysis of **3** to the corresponding diamine **2**, it was necessary to develop a reliable, accurate, and convenient procedure for assaying the enantiomeric purities of **(R)-** and **(S)-2**. Fully resolved **2** appears to have an $[\alpha]_D$ in the region of 6. In addition to its small specific rotation, the hygroscopic nature of **2** results in the presence of varying amounts of water in samples, and therefore polarimetry is an inappropriate technique for determination of its enantiomeric purity. Reliable methods were, however, developed using ^{1}H-NMR of diastereomeric derivatives of **2**. Figure 1 shows the result of addition of (R)-α-methoxy-α-trifluoromethylphenylacetic acid (MTPA) to chloroform solutions of **2**: addition of one molar equivalent of MTPA causes resonances due to H^a in the two diastereomers to broaden and move to higher frequency, while excess MTPA forms diastereomeric salts in which the resonances due to H^a and H^b change position. In the latter case, there is a large separation between the resonances due to H^b of the two diastereomers. This technique allows detection of as little as 1% of the minor enantiomer. It is suggested that, as illustrated in Scheme 2, monoprotonation gives a species which exists in a pseudocyclic form, whereas the diprotonated species exists in an acyclic form. Similarly, in the ^{1}H NMR spectrum of the diastereomeric camphorsulfonamides **4**, the resonances due to the terminal methyl group of the diamine moiety were fully resolved, even at low field strength.



Scheme 2. Monoprotonated and Diprotonated forms of **(S)-2**.



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Conversion of resolved diamines **(R)-2** and **(S)-2** to the enantiomers of hydroxychloroquine, **(R)-1a** and **(S)-1a** involved heating **2** with 4,7-dichloroquinoline in the presence of diisopropylethylamine. Optimum conditions were different for the two enantiomers: at 135°C, **(S)-2** was consistently more prone to degradation than **(R)-2**, and the conversion was correspondingly less clean. However, when the **(S)-2** was coupled at

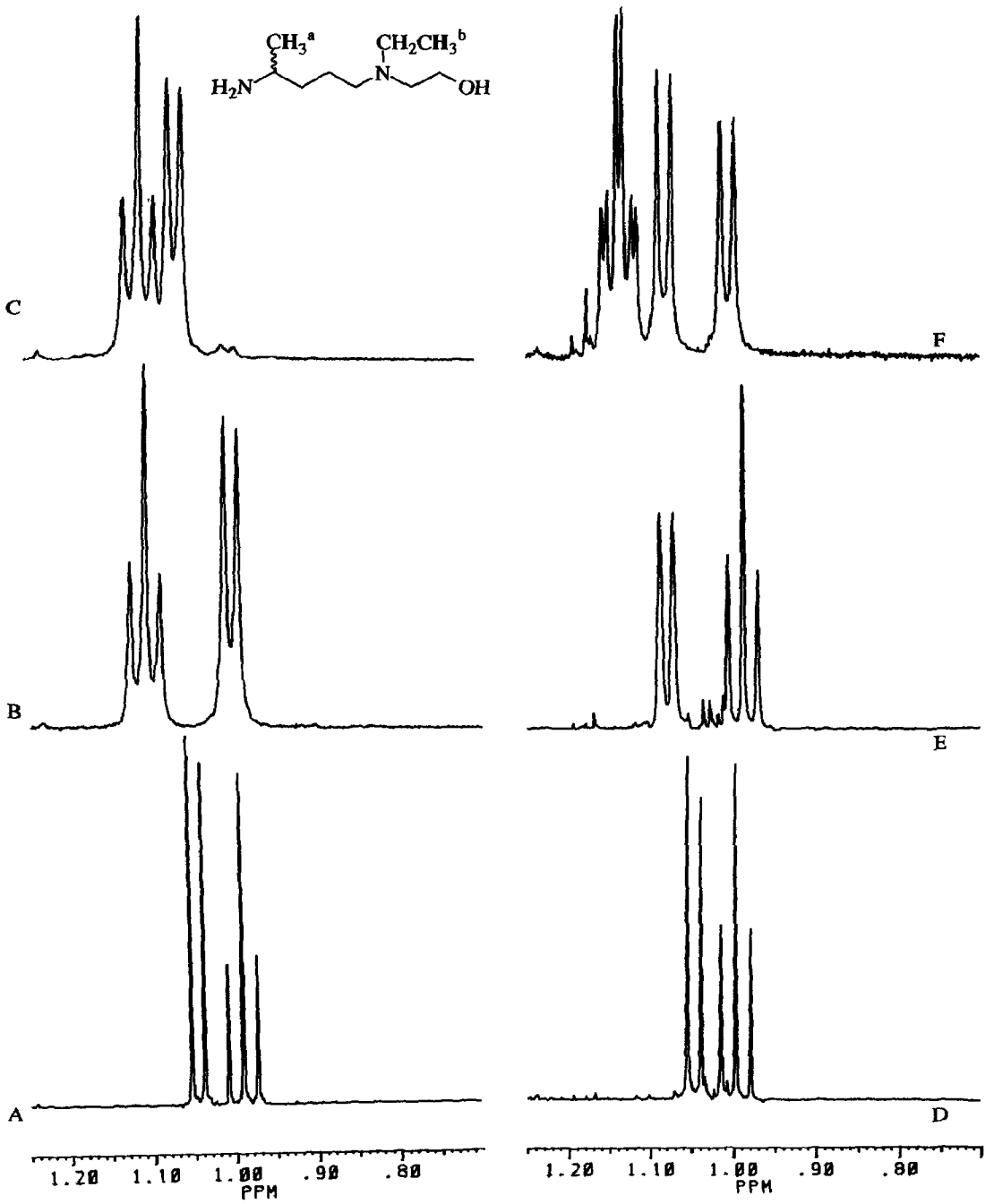

Figure 1. Examples of upfield regions in ^{1}H-NMR spectra of **2** (in $CDCl_3$ at 400 MHz). A. **(R)-2**. B. **(S)-2** (>98% e.e.) with excess MTPA. C. **(R)-2** (96% e.e.; resonance due to H^a of minor diastereomer is indicated) with excess MTPA. D. ***rac*-2**. E. ***rac*-2** with MTPA (1:1). F. ***rac*-2** with excess MTPA.

125°C over a longer period, the reaction was much improved. This may result from incorporation of some impurity into batches of (S)-**2** during resolution.

On a small scale, it is convenient to purify **1a** by column chromatography. Alumina is the preferred adsorbent, although both florisil and silica gel (using a basic system) are adequate. On a larger scale, chromatography is unsatisfactory, and so a purification method was developed using acid-base extraction. Below pH 5, excess dichloroquinoline was removed from the aqueous phase, and the remaining impurities were removed by further extraction between pH 7 and pH 8, followed by charcoal treatment of the aqueous phase to remove a trace of highly-coloured material. Above pH 8 (the most convenient pH being around 12), pure hydroxychloroquine was extracted. Both (R)- and (S)-**1** are oils, and require protection from light during storage, developing a yellow colour otherwise.

After purification, enantiomers **1a** were converted to the bis(dihydrogenphosphate) salts **1b** by treatment with phosphoric acid (two molar equivalents). Where triethylamine remained from the preceding chromatographic step, this was removed by trituration with acetone to leave a deliquescent hydrate. Dehydration by heating in ethanolic suspension gave (R)- and (S)- **1b** as friable white solids. Not surprisingly, the melting points of (R)- and (S)-**1b** are identical; both anhydrous samples and monohydrates melt at 192°C, substantially higher than the melting point of 168-170°C reported for the racemate.[9] The enantiomers of **1** give substantial and reproducible rotations, and as little as 0.5% of the minor enantiomer can be detected by HPLC using a chiral AGP stationary phase.[5] Thus, both polarimetry and HPLC are suitable for determination of the optical purity of **1**.

EXPERIMENTAL

Analyses were carried out by CHN Analysis Ltd., South Wigston, Leicester. High-resolution mass spectra were recorded by Dr. T. Dransfield (University of York) using a VG Autospec magnetic sector instrument; low-resolution mass spectra were recorded by Dr. J. Firth using a Finnigan MAT TSQ700 triple quadrupole instrument. NMR spectra were recorded using Bruker WH-400 and Jeol GSX-270 instruments. Optical Rotations were measured using an Optical Activity AA-1000 polarimeter, and enantiomeric ratios were measured by HPLC using a chiral AGP column (Chromtech; 10x0.46 cm) eluting with 2:98 acetonitrile - 50 mM pH 7.0 phosphate buffer containing 3 mM *N,N*-dimethyloctylamine. Retention times were 12 min and 16 min for (R)-**1** and (S)-**1** respectively.[5]

S(+)-5-[N-Ethyl-N-(2-hydroxyethyl)amino]-2-pentanamine ((S)-2). A solution of ***rac*-2** (200g, 1.15 mol) in 2-propanol (350 ml) was added to a solution of (+)-mandelic acid (87.4g, 0.575 mol) in 2-propanol (500 ml). Additional 2-propanol was added to bring the total volume to 900ml and the solution was stirred overnight at room temperature. Filtration gave white crystals (235g) which were recrystallised twice more from 2-propanol (1800 ml and 1600 ml respectively) to afford (S)-**3** (145.4g) as white crystals. The solid was suspended in 35% aqueous sodium hydroxide (350 ml) and extracted with *tert*-butyl methyl ether (5 x 600 ml). The extracts were combined, dried ($MgSO_4$) and concentrated to give (S)-**2** (55.5g, 55%) as a colourless

oil. ^{1}H NMR ($CDCl_3$) δ 0.98 (3H, t, J = 7.1 Hz), 1.025 (3H, d, J = 6.3 Hz), 1.25-1.35 (2H, m), 1.35-1.55 (2H, m), *ca.* 1.9 (3H, br s), 2.42 (2H, t, J = 7.3 Hz), 2.51 (2H, q, J = 7.1 Hz), 2.54 (2H, t, J = 5.5 Hz), 2.85 (1H, tq, J = 6.3 and 5.2 Hz), 3.50 (2H, t, J = 5.5 Hz); MS (CI, Ammonia) 175 ([MH]$^+$). HRMS Calc. for $C_9H_{22}N_2O$: 175.181039; Found: 175.180493.

R(-)-5-[N-Ethyl-N-(2-hydroxyethyl)amino]-2-pentanamine ((R)-2). The mother liquor from the first crystallisation above was concentrated. The residue was suspended in 35% aqueous sodium hydroxide (250 ml) and extracted with *tert*-butyl methyl ether (5 x 550 ml). The combined extracts were dried ($MgSO_4$)and concentrated to give a yellow oil (70.6 g). This was redissolved in 2-propanol (200 ml) and added to a solution of (-)-mandelic acid (64.00 g, 0.421 mol) in 2-propanol (300 ml). Additional 2-propanol was added to bring the total volume to 600ml and the solution was stirred overnight at room temperature. Filtration gave white crystals (111g) which were recrystallised twice more from 2-propanol (1100 ml and 800 ml respectively) to afford **(R)-3** (77.2 g) as white crystals. ^{1}H NMR (DMSO-d_6) δ 0.92 (3H, t), 1.09 (3H, d), 1.35-1.55(4H, m), 2.3-2.55 (6H, m), 3.03 (1H, tq), 3.43 (2H, t), 4.48 (1H, s), 7.1-7.25 (3H, m), 7.39 (2H, dd). **(R)-3** was suspended in 35% aqueous sodium hydroxide (200 ml) and extracted with *tert*-butyl methyl ether (5 x 400ml). The extracts were combined, dried ($MgSO_4$) and concentrated to give **(R)-2** (29.3g, 29%) as a colourless oil. ^{1}H NMR ($CDCl_3$) δ 0.97 (3H, t, J = 7.1 Hz), 1.025 (3H, d, J = 6.3 Hz), 1.25-1.35 (2H, m), 1.35-1.5 (2H, m), *ca.* 2.1 (3H, br s), 2.41 (2H, t, J = 7.3 Hz), 2.51 (2H, q, J = 7.1 Hz), 2.53 (2H, t, J = 5.5 Hz), 2.85 (1H, tq, J = 6.3 and 5.2 Hz), 3.49 (2H, t, J = 5.5 Hz); HRMS (CI-Ammonia) Calc. for $C_9H_{22}N_2O$: 175.181039; Found: 175.180390.

S(+)-Hydroxychloroquine ((S)-1a) A mixture of **(S)-2** (55.47 g, 0.32 mol), 4,7-dichloroquinoline (63.03 g, 0.32 mol) and diisopropylethylamine (63.9 ml, 0.37 mol) was heated at 125°C under reflux in a nitrogen atmosphere for four days. After cooling, the mixture was transferred into a separating funnel using 1M aqueous sodium hydroxide (500ml) and dichloromethane (500 ml). The organic phase was separated and the aqueous phase was re-extracted with dichloromethane (2 x 500 ml). The organic phases were combined, dried ($MgSO_4$) and concentrated to give a yellow oil (116 g) which was chromatographed on silica gel in 95:3:2 dichloromethane:triethylamine:methanol to give **(S)-1a** (73 g, 78%) as a pale yellow oil. Alternatively, the crude product was chromatographed on alumina in 2:2:1 acetone:hexane:methanol to give **(S)-1a** as a colourless oil. ^{1}H NMR ($CDCl_3$) δ 0.99 (3H, t, J = 7 Hz, CH_2CH_3), 1.285 (3H, d, J = 6 Hz, $CHCH_3$), 1.45-1.85 (4H, m), 2.35-2.75 (6H, m), 3.4-3.95 (3H, m), 5.18 (1H, br d, J = 8 Hz, NH), 6.37 (1H, d, J = 6 Hz, 3-H), 7.28 (1H, dd, J_o = 9 Hz, J_m = 2 Hz, 6-H), 7.74 (1H, d, J = 9 Hz, 5-H), 7.91 (1H, d, J = 2 Hz, 8-H), 8.465 (1H, d, J = 6 Hz, 2-H); ; ^{13}C NMR ($CDCl_3$) 11.5 (CH_2CH_3), 20.2 (CH-CH_3), 23.9 ($CH_2CH_2CH_2N$), 34.2 ($CH_2CH_2CH_2N$), 47.5 (CH_2CH_3), 48.3 (CH), 53.1 ($CH_2CH_2CH_2N$), 54.9 (CH_2CH_2OH), 58.5 (CH_2CH_2OH), 99.0 (C3), 117.2 (C4a), 121.3 (C6), 125.0 (C8), 128.4 (C5), 134.7 (C7), 149.1 (C4), 151.65 (C2); MS (EI, 70EV) 337, 335 (15, 44%, $M^{+\cdot}$), 306, 304 (20, 62%, [M-·CH_2OH]$^+$), 247 (81%), 102 (100%); HRMS Calc. for $C_{18}H_{26}ClN_3O$: 335.176440; Found: 335.175518.

R(-)-Hydroxychloroquine ((R)-1a). A mixture of **(R)-2** (29.34 g, 0.168 mol), 4,7-dichloroquinoline (33.34 g, 0.168 mol) and diisopropylethylamine (33.8 ml, 0.194 mol) was heated at 135°C under reflux in a nitrogen atmosphere for three days. Work-up and purification as described for **(S)-1a** gave **(R)-1a** (39.8 g, 84%) as a pale yellow oil. *Alternative Purification:* crude **(R)-1a** (18.7 g) was dissolved in hydrochloric acid (1M, 50

ml) and washed with ethyl acetate (2 x 50 ml) to remove 2,7-dichloroquinoline. After neutralisation to pH 7.5 with 1M aqueous sodium hydroxide, the aqueous phase was washed again with ethyl acetate (2 x 50ml), then stirred overnight with activated charcoal. After filtration through celite, the mixture was basified to pH 12 and extracted with ethyl acetate (4 x 50 ml). The extracts were combined, dried ($MgSO_4$) and concentrated to give **(R)-1a** as a pale yellow oil (17.2 g). ^1H NMR ($CDCl_3$) δ 0.99 (3H, t), 1.285 (3H, d), 1.45-1.85 (4H, m), 2.35-2.75 (6H, m), 3.4-3.95 (3H, m), 5.18 (1H, br d), 6.37 (1H, d), 7.28 (1H, dd), 7.74 (1H, d), 7.91 (1H, d), 8.465 (1H, d); ^{13}C NMR ($CDCl_3$) δ 11.5, 20.2, 23.9, 34.2, 47.5, 48.3, 53.1, 54.9, 58.5, 99.0, 117.2, 121.3, 125.0, 128.4, 134.7, 149.1, 151.65; MS (thermospray) 338, 336 ($[MH]^+$); major fragment ions at 247 (100%, $[M\text{-}EtNHCH_2CH_2OH]^{+\cdot}$), 158.

S(+)-Hydroxychloroquine bis(dihydrogenphosphate) ((S)-1b). Phosphoric acid (19.7 ml, 0.29 mol) was added to **(S)-1a** (43.4 g, 0.144 mol) with ice cooling to moderate the reaction. The resulting gum was ground under acetone and the resulting deliquescent solid was filtered quickly, suspended immediately in fresh acetone (200 ml), then stirred overnight. Rapid filtration gave a white powder which was transferred immediately into a flask containing ethanol (200 ml). The resulting suspension was refluxed for four days, then filtered, and the solid washed with ethanol. After drying under vacuum to constant weight, the yield of **(S)-1b** was 52.6 g (69%). m.p. 191.5-193°C; $[\alpha]_D^{20}$ +86.5 (c 0.95, H_2O). Calc. for $C_{18}H_{32}ClN_3O_9P_2$: C, 40.65; H, 6.06; N, 7.90; P, 11.65; Cl, 6.67; Found: C, 40.50; H, 6.03; N, 7.88; P, 11.62; Cl, 6.83; IR (KBr disk) ν_{max} 2500-2200 (OH, NH), 1610, 1100, 970 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.33 (3H, t, J = 7 Hz, CH_2CH_3), 1.48 (3H, d, J = 6 Hz, $CHCH_3$), 1.75-2.15 (4H, m), 3.15-3.55 (6H, m), 3.94 (2H, t, J = 5 Hz, CH_2OH), 4.0-4.4 (1H, m, -NHCH(CH_3)-), 6.86 (1H, d, J = 7 Hz, 3-H), 7.52 (1H, dd, J_o = 9 Hz, J_m = 2 Hz, 6-H), 7.67 (1H, d, J = 2 Hz, 8-H), 8.12 (1H, d, J = 9 Hz, 5-H), 8.31 (1H, d, J = 7 Hz, 2-H); ^1H NMR (D_2O at 75°C, 80MHz) d 1.81 (3H, t, CH_2CH_3), 1..95 (3H, d), 2.2-2.6 (m), 3.6-4.0 (m), 4.43 (2H, t), 4.8 (m), 7.39 (1H, d, 3-H), 8.18 (1H, dd, 6-H), 8.35 (1H, br s, 8-H), 8.76 (1H, d, 5-H), 8.86 (1H, d, 2-H); ^{13}C NMR (DMSO-d_6) 10.8 (CH_2CH_3), 21.6 (CH-CH_3), 22.9 ($CH_2CH_2CH_2N$), 34.9 ($CH_2CH_2CH_2N$), 51.3 (CH_2CH_3), 52.35 (CH), 55.0 ($CH_2CH_2CH_2N$), 56.7 (CH_2CH_2OH), 58.2 (CH_2CH_2OH), 101.4(C3), 117.9 (C4a), 121.8 (C6), 126.9 (C8), 130.1 (C5), 140.8 (C7), 142.0 (C8a), 145.1 (C2), 158.1 (C4); MS (CI, ammonia) 338, 336 ($[MH]^+$).

Azeotropic drying for a shorter period gave **S(+)-Hydroxychloroquine bis(dihydrogenphosphate) monohydrate ((R)-1b).** m.p. 193-194°C; $[\alpha]_D^{20}$ -80.7 (c 0.95, H_2O). Calc. for $C_{18}H_{34}ClN_3O_{10}P_2$: C, 39.31; H, 6.23; N, 7.64; Found: C, 38.89; H, 6.41; N, 7.49; IR (KBr disk) ν_{max} 3500-2400 (OH, NH), 1610, 1100, 950 cm^{-1}; ^1H NMR (D_2O, 75°C) δ 1.81 (3H, t), 1.95 (3H, d), 2.2-2.6 (4H, m), 3.6-4.0 (6H, m), 4.43 (2H, t), 4.8 (1H, m), 7.39 (1H, d), 8.18 (1H, dd), 8.35 (1H, d), 8.76 (1H, d), 8.86 (1H, d); MS (thermospray) 338, 336 ($[MH]^+$).

Similarly prepared was **R(-)-Hydroxychloroquine bis(dihydrogenphosphate) ((R)-1b).** m.p. 186.5-187°C; $[\alpha]_D^{20}$ -85.8 (c 0.95, H_2O). Calc. for $C_{18}H_{32}ClN_3O_9P_2$: C, 40.65; H, 6.06; N, 7.90; P, 11.65; Cl, 6.67; Found: C, 40.60; H, 6.00; N, 7.82; P, 11.50; Cl, 6.76; IR (KBr disk) ν_{max} 3500-2400 (OH, NH), 1610, 1100, 950 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.21 (3H, t), 1.445 (3H, d), 1.7-2.1 (4H, m), 3.1-3.5 (6H, m), 3.94 (2H, t), 4.0-4.4 (1H, m), 6.85 (1H, d), 7.52 (1H, dd), 7.68 (1H, d), 8.13 (1H, d), 8.28 (1H, d); ^{13}C NMR (DMSO-d_6) 10.8,

21.6, 22.9, 34.9, 51.3, 52.35, 55.0, 56.7, 58.2, 101.4, 117.8, 121.7, 126.8, 130.0, 140.8, 141.9, 145.1, 158.0; MS (thermospray) 338, 336 ($[MH]^+$).

R(-)-Hydroxychloroquine bis(dihydrogenphosphate) monohydrate ((R)-1b). Phosphoric acid (1.08 ml, 18.6 mmol) was added to a solution of **(R)-1a** (3.423 g, 10 mmol) in ethanol (100 ml), and the suspension stirred vigorously overnight. Rapid filtration gave a solid which was stirred for a further 24 h in acetone (100 ml), then filtered again. The resulting solid was suspended in ethanol (100 ml) and the mixture refluxed for two days, then filtered, and the solid washed with ethanol and dried under vacuum. The yield of **(R)-1b.H_2O** was 4.0 g (73%). m.p. 192°C; $[\alpha]_D^{20}$ -80.4 (c 0.95, H_2O). Calc. for $C_{18}H_{34}ClN_3O_{10}P_2$: C, 39.31; H, 6.23; N, 7.64; Found: C, 39.19; H, 5.96; N, 7.61; IR (KBr disk) ν_{max} 3500-2400 (OH, NH), 1610, 1100, 950 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.10 (3H, t), 1.24 (3H, d), 1.5-1.8 (4H, m), 2.8-3.0 (6H, m), 3.62 (2H, t), 3.7-3.85 (1H, m), 6.85 (1H, d, NH), 7.36 (1H, d), 7.45 (1H, dd), 7.79 (1H, d), 8.38 (1H, d), 8.45 (1H, d); ^{13}C NMR (D_2O) δ 10.8, 21.6, 22.85, 34.7, 51.3, 52.3, 55.0, 56.7, 58.2, 101.4, 118.0, 121.85, 126.9, 130.1, 142.1, 145.05, 158.2; MS (thermospray) 338, 336 ($[MH]^+$).

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