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Systhesis and characterization of analogues of the antimalaria alkaloid febrifugine

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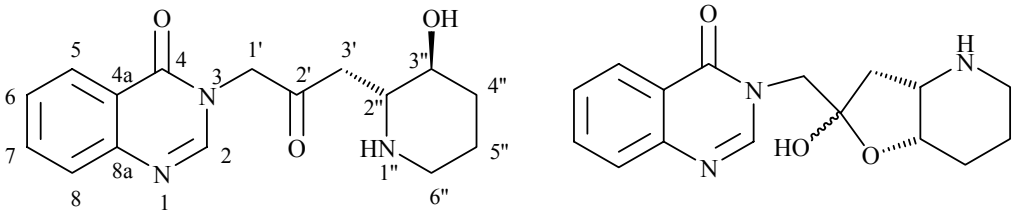
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CHAPTER 1

INTRODUCTION TO FEBRIFUGINE

1.1. History of febrifugine

The alkaloids febrifugine **1** and isofebrifugine **2** (Figure 1) were originally isolated in 1947 from the roots of the Chinese shrub Ch'ang Shan (*Dichroa febrifuga*, Figure 2) in 0.005-0.03 % yield as the active ingredients in antimalarial preparations from this plant [1]. As seen from Figure 1, these alkaloids contain the 4(3*H*)-quinazolinone aromatic moiety. It was determined in 1971-72 [2] that, independent of the place of growth, the content of **1** and **2** was 0.02-0.05% in the roots and 0.5-0.7% in the leaves of *D. febrifuga*.



Febrifugine (+)-**1** Isofebrifugine (+)-**2**

Figure 1: The accepted structures of febrifugine **1** (showing the atomic numbering scheme used in this text) and isofebrifugine **2**.

Soon after the first isolation, **1** and **2** were also found to be present in the related common hydrangea (*H. umbellata*, Figure 2) [3], and eventually in other hydrangea species (eg. *H. macrophylla* [4] and *H. chinensis* [5] also pictured in Figure 2). All these plant species belong to the family Saxifragaceae which generally comprises a diverse group of hardy shrubs.



Dichroa febrifuga



Hydrangea umbellata



Hydrangea macrophylla "cardinal"



Hydrangea macrophylla



Hydrangea chinensis

Figure 2: Plant species from which **1** and **2** have been isolated.

The current interest in these alkaloids stems from their potent antimalarial activity and novel mechanism of action as will be outlined later. Malaria is the most important tropical disease worldwide, endemic in 91 countries and affecting 40% of the world's population. There are an estimated 300 to 500 million clinical cases of malaria annually and malaria causes 0.5 to 2.5 million human deaths every year. More than 90% of the world's malaria occurs in sub-Saharan Africa. Perhaps most disturbing when considering positive global economic growth is that malaria is currently undergoing resurgence and claiming more deaths now than it did 30 years ago [6].

There exists now a desperate need for new antimalarial drugs which function by novel mechanisms. Owing to their long-term usage, most of the available clinical drugs have caused the development of parasitic resistance. In fact, some strains of *P. falciparum* have developed resistance to all currently used drugs with the exception of the artemisinin derivatives [6]. Currently the most promising treatment strategy is the use of drug combinations. However, this approach can only in the short term delay the usually rapid onset of parasitic resistance.

Extracts of **1** and **2** have been used traditionally for over 4000 years in China [7], and during the Vietnam war [8] as a prophylaxis and treatment for malaria. Owing to its proposed novel mechanism of action (see later), no parasitic resistance against **1** has been reported. This fact together with its other reported therapeutic properties (see later) makes **1** and its derivatives currently extremely desirable synthetic targets in medicinal chemistry research. The important drawback is febrifugine's strong emetic effects, which has up to now prevented its use as a clinical drug. This has led to a flurry of synthetic strategies towards obtaining potentially superior analogues with increased chemotherapeutic index (see later in Section 1.4.).

This project aims to add a simple and versatile synthetic approach to the growing list of synthesis entries for febrifugine derivatives found in the literature today. It will become clear that our approach is both economically viable and environmentally friendly, which makes this a worthwhile research effort.

1.2. Structural elucidation of febrifugine and isofebrifugine

Starting in the late 1940s, structural studies on **1** and **2** proved to be a daunting task. Table 1 summarizes the history of proposed structures for natural febrifugine (+)-**1**.

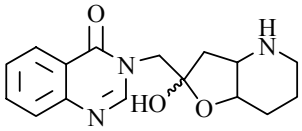
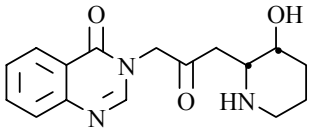
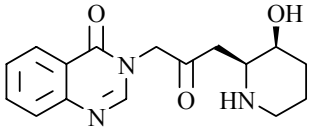
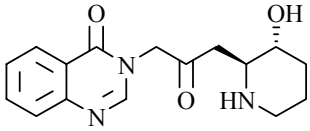
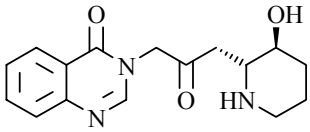
Structure	Year	Proposer
	1950	Koepfli *et al.* [9]
 *cis*-2",3"	1953	Baker *et al.* [10]
	1962	Hill and Edwards [11]
	1973	Barringer *et al.* [12]
	1999	Kobayashi *et al.* [13]

Table 1: Proposed structures of (+)-**1**.

Koepfli *et al.* [14], through standard degradation and titration procedures, were the first to perform structural elucidation studies on the isomers **1** and **2**. Although highly stable under conditions of acidic hydrolysis (70% H_2SO_4, 100 ºC, 1 h), both alkaloids were very susceptible to alkaline hydrolysis and liberated 2-aminobenzoic acid and formic acid. Coupled with the finding that quinazolin-4(3*H*)-one was formed during alkaline permanganate oxidation, it was concluded that **1** and **2** contain the

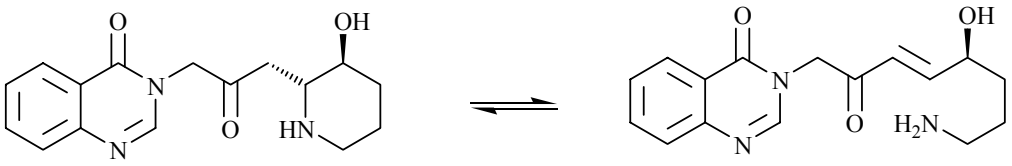
quinazolone moiety, a rare feature at the time. Furthermore, it was suggested by Koepfli's group that, upon oxidation using periodate, **1** and **2** produce a ring-opened aldehyde [9], the structure of which points to the presence of a piperidine ring, 3-substituted by oxygen. On the basis of this, combined with an erroneous negative ketone test for **1**, Koepfli proposed that **1** and **2** both possess the planar structure of **2** (Figure 1), and that they differ only in configuration about the hemiketal carbon atom.

In 1952, Hutchings *et al*. [15] established the presence both of a ketone group (by preparing the oxime using hydroxylamine) in the side-chain of **1**, and of a secondary amine in **1** in a position beta to the ketone. The presence of a hydroxyl group was confirmed by the preparation of a stable *O*-acetylated derivative of **1**. However, they were uncertain about whether **1** contained a piperidine or a pyrrolidine ring and also about the position of the alcohol group on the ring.

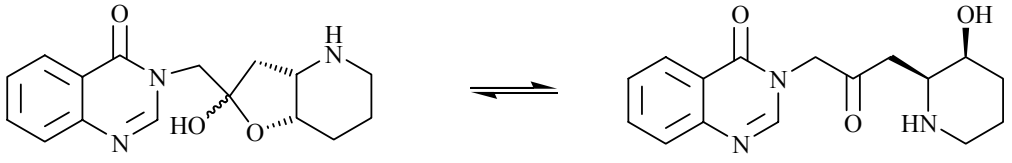
Baker's group [16] unequivocally determined the gross structure of **1** by completing the first total synthesis of (±)-**1**, the antimalarial activity of which was one-half that of natural (+)-**1**. All the other hydroxyl regioisomers prepared were inactive against malaria (see later in Section 1.4.2.).

Substantial synthetic research approaches towards **1** only recently gathered momentum owing to much ambiguity up until 1999 surrounding the absolute structures of **1** and **2**. The ready interconversion of these two naturally occurring isomers by a reversible Michael reaction (Scheme 1) [17] initially made structure determination a puzzling task. It is now known that complete isomerization between (+)-**1** and (+)-**2** occurs in water within 15 min at 80 ºC to give a 2:1 mixture of (+)-**1** and (+)-**2**. When measured at rt, 30 % of (+)-**1** changes to (+)-**2**, and 40 % of (+)-**2** changes to (+)-**1** within 2 days in aqueous solution. A supporting detailed NMR study [18] revealed that **1** tends to adopt the 2′-keto form regardless of solvent polarity, but that **2** adopts its hemi-ketal form exclusively in non-polar solvents ($CDCl_3$) yet isomerizes to give a mixture of **1** and **2** in polar solvents (DMSO-d_6 and CD_3COOD, CD_3COONa, D_2O buffer).

The early workers in the field were of course, unaware of these stereochemical ambiguities, which gradually became apparent. The sequence in which the stereochemical subtleties were elucidated is interesting in itself.



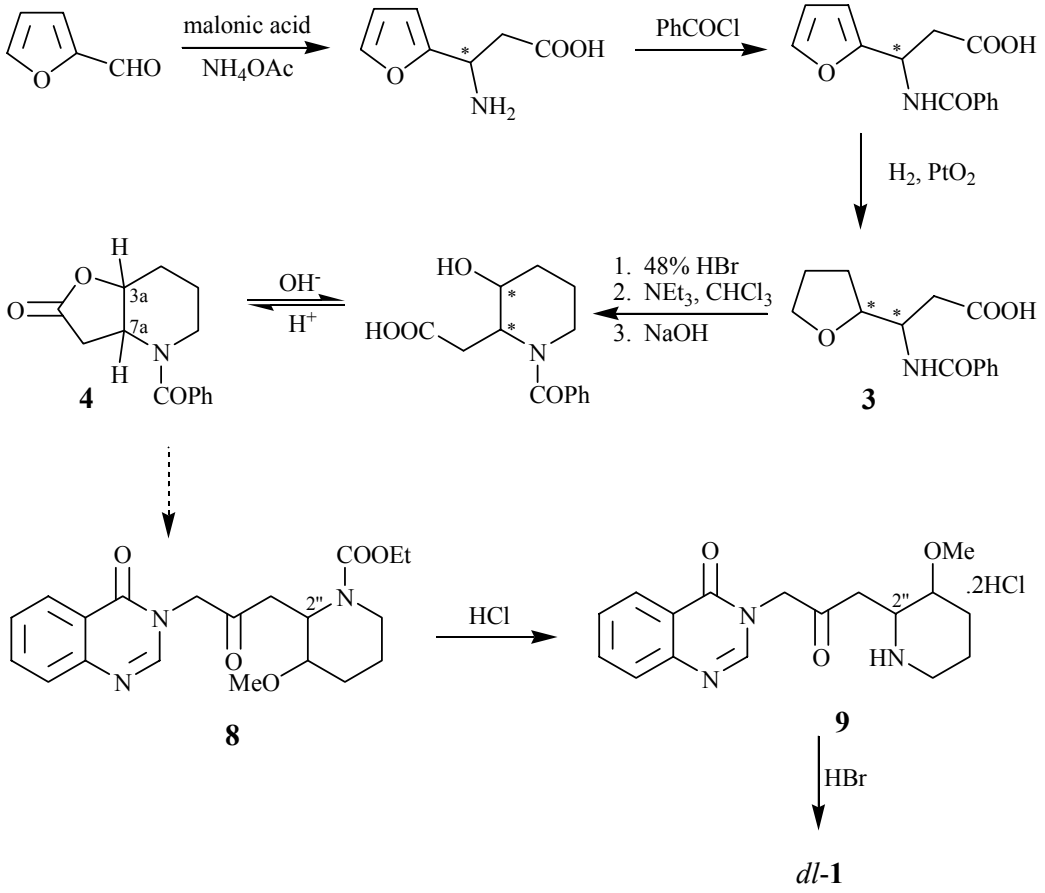
Febrifugine (+)-**1**



Isofebrifugine (+)-**2**

Scheme 1: Mechanism of the interconversion between febrifugine and isofebrifugine.

Although Baker's group correctly proposed the planar structure of **1** and also isolated **2** during their second synthesis [10], they were still uncertain about the structure of **2** and were unable to determine the absolute configurations about the asymmetric carbons in (+)-**1**. Baker furthermore erroneously deduced the relative stereochemistry of natural **1** to be 2″,3″-*cis* (numbering scheme, Figure 1) in the piperidine moiety of **1** based on the structural determination of a key intermediate **4** [which they assigned as *cis*-**4** owing to the Pt hydrogenation reaction employed to produce **3** obtained in their synthesis of (±)-**1** (Scheme 2) [10]].

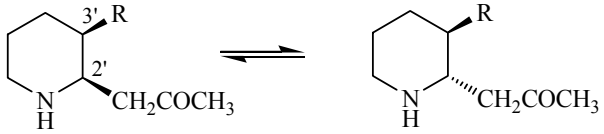


Scheme 2: Representation of the second synthesis [10] of *dl*-febrifugine by Baker's group.

In 1955, the Baker group prepared the natural isomer (+)-**1** by optical resolution of an early intermediate in the synthesis [19]. Baker again for the same reason assigned the *cis*-configuration to a critical intermediate **5** (R= OCH_3, Scheme 3) in support of their previous structural assignment of natural **1**. Based on Baker's assumption, Hill and Edwards [11] suggested the 2″*S*, 3″*S* configuration for **1** in 1962. The natural isomer was consequently called *cis*-febrifugine.

In 1973 Barringer *et al.* [12] queried Baker's structural assignment of **1** by studying the ready conversion upon heating between *cis*-3′-substituted, 2′-piperidyl 2-propanones **5** and their *trans* isomers **6** (Scheme 3). The 2′,3′-substituted piperidines **5** were prepared by Barringer by the hydrogenation of the corresponding pyridines over Rhodium on alumina using aqueous hydrobromic acid. Barringer assumed that he prepared the *cis*-isomers **5** based on literature precedence only, e.g. the studies

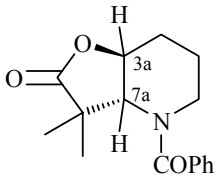
conducted by Adkins *et al*. [20] and Robinson [21], without providing any ^{1}H NMR spectroscopic evidence. Note that epimerization occurs at position 2′ which becomes 2″ in **1**. They consequently reasoned that isomerization from *cis*-**5** (R = OCH_3) to *trans*-**6** (R= OCH_3) had occurred during Baker's third synthesis [19]. The same reasoning could be applied to a very similar intermediate in Baker's first synthesis [16]. In a second paper, Barringer *et al.* [22] then reported their synthesis of what they proposed was theoretically *cis*-**4** (Scheme 2) and conducted a detailed ^{1}H NMR study of this compound. They primarily used the coupling constant between H_{3a} and H_{7a} ($J_{3a\text{-}7a}$ = 8.5 Hz) to deduce that the relative configuration was instead *trans*-**4**, not *cis*-**4**, as predicted theoretically and as proposed by Baker [10]. They deduced from their previous study [12] that epimerization occurs at C_{7a} (which becomes position 2″ in **1**). Because Hill and Edwards [11] were convinced that the configuration around C2″ of (+)-**1** was (*S*), Barringer *et al.* proposed that the absolute stereochemistry in active (+)-**1** must be 2″*S*, 3″*R*.



5 **6**

R= OCH_3 (Baker's intermediate), CH_3

Scheme 3: Interconversion of the piperidines (including Baker's [10] intermediate) prepared by Barringer *et al.* [12]



trans-**7**

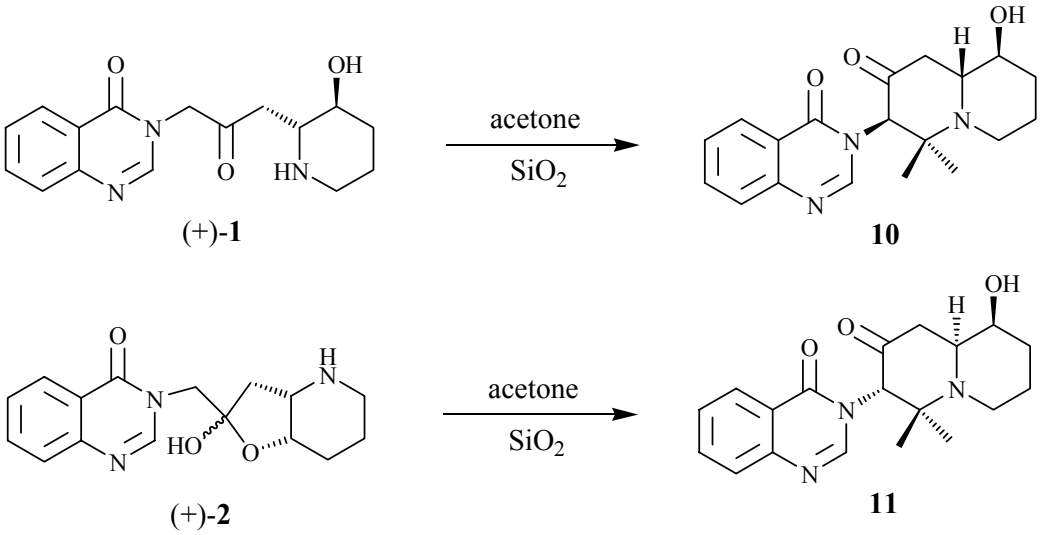
Recently, Kobayashi's group synthesized *trans*-**7** [23] shown here. The observed coupling constant $J_{3a\text{-}7a}$ was found to be 10.4 Hz, even higher than that previously reported for **4**. Thus, Barringer was probably wrong in assuming the *trans* configuration for **4** based only on $J_{3a\text{-}7a}$ = 8.5 Hz.

Takeuchi *et al*. then conducted a detailed synthetic study in 2003 [24] which solved the puzzle surrounding the earlier attempts at explaining relative stereochemistry in (+)-**1**. They proved that epimerization in the Baker syntheses most probably occurred later on during the penultimate *N*-deprotection step, i.e. forming **9** from **8** (Scheme 2) using HCl. This acid-catalyzed epimerization occurred at C2″ (Scheme 2).

In 1999, Kobayashi *et al.* [13,25] published the first unambiguous total syntheses of all four antipodes of **1** and **2** (see details later). They concluded that the correct absolute configurations of natural and active (+)-**1** and (+)-**2** were respectively (2′*R*, 3′*S*) and (2′*S*, 3′*S*) as depicted in Figure 1.

1.3. Other alkaloids related to febrifugine

The acetone adducts of **1** and **2** respectively, namely **10** and **11** (depicted in Scheme 4), were synthesized in 1999 by Takaya *et al.* [26] These Mannich reactions were carried out by eluting **1** and **2**, in two distinct experiments on silica gel, using a mixture of acetone, hexane and MeOH. Both synthetic quinolizidines **10** and **11** showed similar activity to **1** and **2** when tested *in vitro* against *P. falciparum* (see later). However, **10** and **11** tested considerably weaker than **1** and **2** *in vivo* against the *P. berghei* strain used. Furthermore, **11** was 25 times less effective *in vivo* than **10**. This difference in activity was explained by differences in the rate of metabolism of **10** and **11** *in vivo*. Interestingly, the discovery of **10** and **11** was made before the isolation of the natural product(s) described below.



(+)-**1** **10**

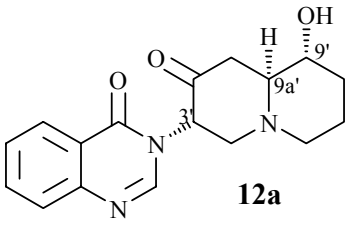
(+)-**2** **11**

Scheme 4: The synthesis of the acetone adducts, **10** and **11**.

In 2000, Deng *et al.* [27] isolated a new quinazolinone alkaloid **12a** as a crystalline solid, along with **1** and **2**, from *Dichroa febrifuga*. The structure of **12a**, which they named (+)-neodichroine, was proposed as shown in Figure 3. It can be seen that **12a** bears a

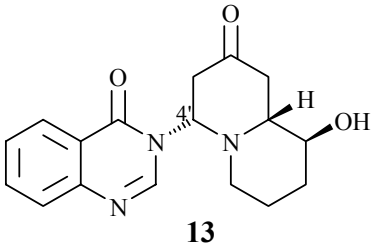
remarkable resemblance to **10** and **11** (Scheme 4). Deng used ^{1}H NMR spectroscopy coupling constants and NOESY correlations to assign the relative stereochemistry in **12a** as *trans*-diaxial 9a′H, 9′H (J = 10.1 Hz). Furthermore, the large coupling constant (J = 11.3 Hz) for 3′H indicated that the quinazoline moiety was equatorial in **12a**. They conclusively confirmed the connectivity in **12a** by synthesizing the same compound through the Mannich reaction between **1** and formaldehyde.

A year later, in 2001, Patnam *et al.* [28] published the structure **13** (Figure 3) for hydrachine A, another new alkaloid which was isolated as a semi-solid from the roots of *Hydrangea chinensis*. Patnam's group also observed large coupling constants for 9a′H, 9′H and 4′H which indicated their axial orientations in **13**.



12a

$[\alpha]_D$ + 198.8 (*c* 0.17, MeOH)



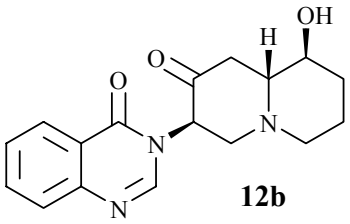
13

$[\alpha]_D$ + 25.3 (*c* 0.2, $CHCl_3$)

Figure 3: The structures and optical rotations of **12a** and **13**, showing the numbering scheme for the asymmetric carbons.

Michael [29] published a review in 2002 in which he commented on the similarities between **12** and **13**. He suggested that the latter structure was wrong, and that the two alkaloids might be identical since both could be related to febrifugine **1**, which is present in both the plant species studied. However, the NMR spectra of **13** were recorded in $CDCl_3$, which makes comparison to Deng's spectra (recorded in deuterated pyridine) tricky. Michael also noticed that Deng *et al.* [27] incorrectly assumed the absolute configurations of C9a′ and C9′ in **12a** to be 2″*S*, 3″*R* respectively, based on the erroneous assignment of natural (+)-**1** by Barringer *et al.* [12]; they were seemingly unaware of Kobayashi's correction of the absolute structure of (+)-**1** [13] in 1999.

A year later, Chang *et al*.[30] admitted that the structure of **13** in their previous paper[28] was probably incorrect with regards to the quinazolinyl linkage to C4′. In this subsequent paper, Chang reported the following optical rotation for **13**: $[\alpha]_D$ − 25.3 (*c* 0.20, MeOH). This was opposite in orientation to the original measurement (+ 25.3, Figure 3) in $CDCl_3$ published by Patnam from the same group. It is implied by Chang *et al*. that the same compound was isolated by both groups, but that its optical rotation is negative (i.e. the original Deng and Patnam measurements were faulty!). Their revised structure of (−)-neodichroine **12b** is shown here. In conclusion, it cannot be argued with total certainty what the absolute stereochemistry of **12a** or **12b** is, but the planar structure of neodichroine is now probably solved. The definitive deduction should come from the fact that **12b** can be easily correlated with febrifugine **1**, in which case the correct structure should be as shown here.



12b

The Patnam group carried out only antitumour tests on **12b** and found that, unlike **1** (see later), **12b** was inactive against cancer. Further studies on **12b** would be useful in order to confirm its structure (preferably by single crystal X-ray diffraction of a prepared derivative), and also to test its antimalarial activity seeing that **12b** is similar to the promising acetone adducts **9** and **10** discussed earlier.

1.4. Antimalarial properties of febrifugine and its derivatives

1.4.1. Overview

Human malaria is caused by four distinct *Plasmodium* (*P*.) species[6], a protozoan transmitted by the female *Anopheles* mosquito. The incidence of infection by the two rarer species, *P. ovale* and *P. malariae*, is low. *P. vivax*, which is widely spread throughout Asia, Africa, the Middle East, Oceania, South America and even found in Eastern Europe, is known to cause recurring and debilitating infections but rarely kills its human host. The most aggressive species is *P. falciparum* which is especially

common in sub-Saharan Africa. *P. falciparum* can progress to severe and complicated malaria and almost all deaths result from this species.

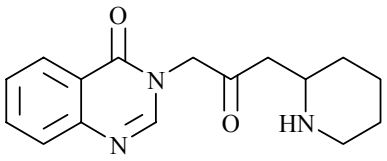
In Table 2, the antimalarial activities and toxic selectivities of febrifugine derivatives and selected clinically used drugs, are compared [31]. It is at once apparent that **1** exhibits extreme potency against *P. falciparum*. Its activity is approximately 10-fold that of the effective antimalarial artemisinin, and 25-fold that of the well-known drug chloroquine. However, the toxic selectivity of **1** is considerably lower than that of both the clinical drugs mentioned.

Racemic febrifugine (±)-**1** was reported to be about one-half as effective as (+)-**1** [13,16] and it was concluded that the antipode of febrifugine therefore possesses practically no antimalarial activity.

Compound	***P. falciparum*** **EC_{50} (μM)**	**Toxic Selectivity**
Febrifugine (**1**)	0.0007	253
Isofebrifugine (**2**)	0.0034	56
(±)-Deoxyfebrifugine (**14**)	0.1	21
Quinine	0.11	909
Chloroquine	0.018	1778
Pyrimethamine	0.001	120
Artemisinin	0.0079	1266

Table 2: Antimalarial activity and toxic selectivity of febrifugine and its derivatives compared to clinically used drugs.

It is important to note that (±)-deoxyfebrifugine (**14**), the simple 3′-unsubstituted model compound of **1**, exhibits similar activity to quinine against *P. falciparum*. Although the toxic selectivity of **14** leaves much to be desired, it was one of the very few derivatives (among a large number prepared by Baker's group in the 1950s) which displayed some activity and continues to represent an important lead compound for the development of potentially superior febrifugine derivatives.



14

1.4.2. Pre-1999 structure-activity studies on the antimalarial activity of febrifugine

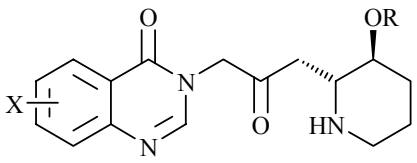
The isomerization between (+)-**1** and (+)-**2** also hampered progress regarding structure-activity relationship studies of febrifugine. In earlier work, numerous derivatives of febrifugine, many of which were optically inactive, were prepared in order to establish which structural features of **1** were essential for its powerful antimalarial activity.

Variations of the piperidine moiety proved to be fruitless, e.g. replacing the 3″-hydroxypiperidine ring by unsubstituted or 3″-hydroxy- or 3″-methoxypyridine residues resulted in complete loss of activity [32]. Baker's group synthesized various derivatives, eg. 3″-methyl- and 3″-hydroxymethylpyrrolidine analogues [33], but none of the resulting compounds was particularly active. It is important to note that the only piperidine derivatives of **1** prepared up until 2001 (see later) included regioisomers of the 3″-hydroxy group on the piperidine ring (Figure 1) and substitution at this position by other groups, all of which displayed poor activities. After many studies, it was apparent that the 3″-oxygen was necessary for good antimalarial activity (in the substituted piperidine derivatives).

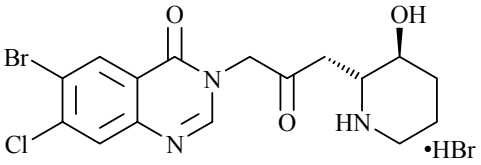
In contrast, variations of the benzenoid portion of the quinazolinone moiety often led to promising results. In 1951, Baker *et al.* [34] used their synthesis of (±)-**1** to obtain a variety of derivatives bearing substituents (mostly alkyl, halogen, methoxy groups

etc.) on the benzene ring of **1**. It was found that, for the monosubstituted derivatives, only those substituted at C_5 (Figure 1, numbering scheme) were superior both in activity and chemotherapeutic index to **1**. Monosubstitution at C_6 increased activity but lowered or did not affect the chemotherapeutic index. Disubstitution at C_6, C_8, or C_7, C_8 lowered activity greatly, while at C_5, C_7, or C_6, C_7 or C_7, C_8 chemotherapeutic index decreased and activity in some cases decreased. Disubstitution at C_5, C_6, however, increased activity by 30% and, in the case of the 5,6-dimethyl derivative, also doubled the chemotherapeutic index. It is important to note that, of the abundant derivatives synthesized, none were hydroxyl substituted.

Using Baker's asymmetric synthesis [19], a series of methylenedioxy derivatives **15** (Figure 4) was synthesized by Chien and Cheng [35], all of which were found to exhibit similar activity but significantly lower cytotoxicity *in vivo* than **1**. Another derivative, halofuginone **16** [(±)-6-bromo-7-chloro-**1** ·HBr, Figure 4], was found to be a useful coccidiostat (see later in Section 1.4.6.).



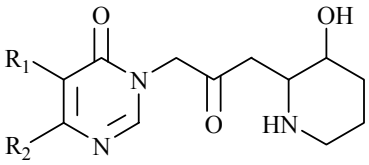
methylenedioxy analogues (**15**)
X= 5,6-, 6,7- or 7,8-OCH_2O
R= H or OCH_3



Halofuginone (**16**),
(*dl*-6-bromo-7-chloro-**1** •HBr)

Figure 4: The structures of some promising benzenoid derivatives (**15** and **16**) of febrifugine.

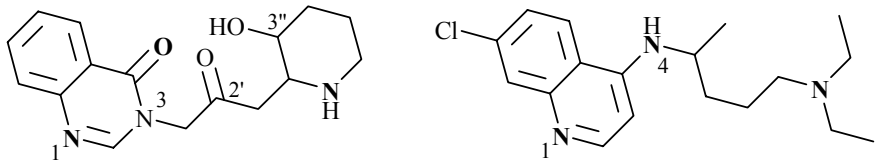
Seeing that 4-quinazolinone may be regarded as 4-pyrimidinone substituted at positions 5 and 6, a series of 5,6-alkyl and aryl substituted pyrimidinone derivatives (**17**) of **1** (Figure 5), was prepared by Baker *et al*. [36], all of which exhibited reduced or no activity against malaria.



pyrimidinone analogues (**17**)
R_1, R_2= various alkyl and aryl groups

Figure 5: Pyrimidinone analogues of **1**.

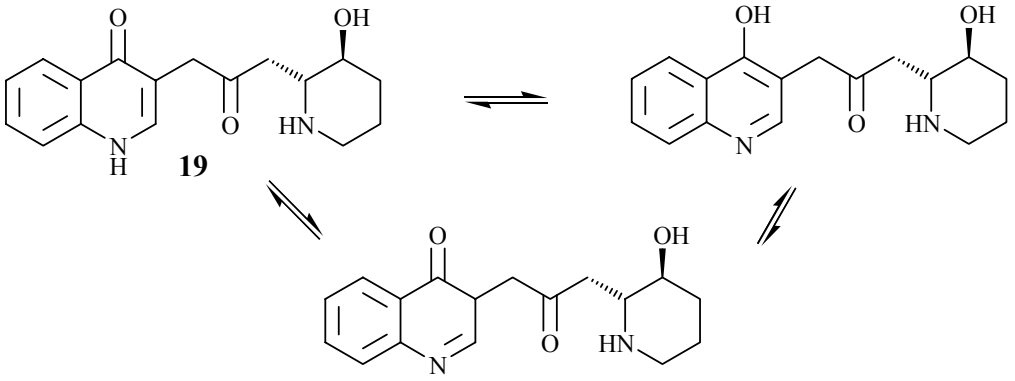
In an interesting study by Cheng in 1976 [7], the structures of **1** and chloroquine (**18**) were compared using Dreiding molecular models. It was found that when the quinoline ring of **18** and the quinazolinone ring of **1** were superimposed such that both nitrogens at the 1-position overlap, the quinoline N-4 is in the vicinity of the quinazolinone *O*. Furthermore, both the side chains can be turned along their axes in such a way that the tertiary nitrogen of **18** and the piperidinyl nitrogen of **1**, in addition to the carbon side chains of both, overlap (Figure 6). Based on these conformational and heteroatom similarities, Cheng concluded that **1** and **18** might share common sites for *in vivo* binding to enzymes involved in their antimalarial function.



1 **18**

Figure 6: Structural similarity between febrifugine (**1**) and chloroquine (**18**). Overlapping heteroatoms are shown in bold print.

Cheng further noticed that many alkaloids displaying emetic properties share the N-3, O-2′, O-3″ relationship contained in **1** (Figure 6). In fact, the natural product emetine also contains a similar triangular feature between two oxygen atoms and one nitrogen atom [37]. It can be seen that N-3 of the quinazolinone moiety of **1** does not overlap with a heteroatom in **18**. He thus theoretically proposed that the structure **19** shown below in Scheme 5 might be an important analogue of **1** which does not display the emetic properties that have hampered the clinical use of **1**.



Scheme 5: The resonance structures of **19**, the compound Cheng suggested might not cause emeticism. Note the absence of N-3 in the quinazolinone moiety.

1.4.3. Post-1999 structure-activity studies on the antimalarial activity of febrifugine

Once the absolute structure of (+)-**1** was unequivocally determined in 1999, more significant structure-activity studies soon followed. The *in vitro* activities against *P. falciparum* of natural (+)-**1**, (+)-**2** and their antipodes, published by Kobayashi *et al*. [13], are tabulated below.

It can be seen from Table 3 that natural (+)-**1** and (+)-**2** are significantly more active and selective than their synthetic antipodes against the *Plasmodium* parasite. From these results, it was concluded that the stereochemistry at positions 2″ and 3″ of **1** and **2** (Figure 1) is essential for the antimalarial activity of these compounds.

Compound	***P. falciparum*** **EC_{50} (M)**	**Selectivity**
2″*R*, 3″*S*-febrifugine (+)-**1**	7.6×10^{-11}	2763
2″*S*, 3″*R*-febrifugine (–)-**1**	2.0×10^{-7}	105
2″*S*, 3″*S*-isofebrifugine (+)-**2**	2.9×10^{-10}	2517
2″*R*, 3″*R*-isofebrifugine (–)-**2**	1.6×10^{-7}	119

Table 3: *In vitro* antimalarial activities and selectivities of natural (+)-**1**, (+)-**2** and their synthetic antipodes (–)-**1** and (–)-**2**.

In 2001, Takeuchi *et al*. [38] prepared the first *N* regioisomers of the piperidine ring of (+)-**1** (**20**, **22** and **24** in Figure 7) and (+)-**2** (**21** and **23** in Figure 7). Both **20** and **21** were inactive against *P. falciparum in vitro*, whereas **22**, **23** and **24** displayed very weak activity compared to (+)-**1** and (+)-**2**. It was concluded that both the presence and the position of N1″ in the piperidine ring of **1** (Figure 1) were essential for its antimalarial potency.

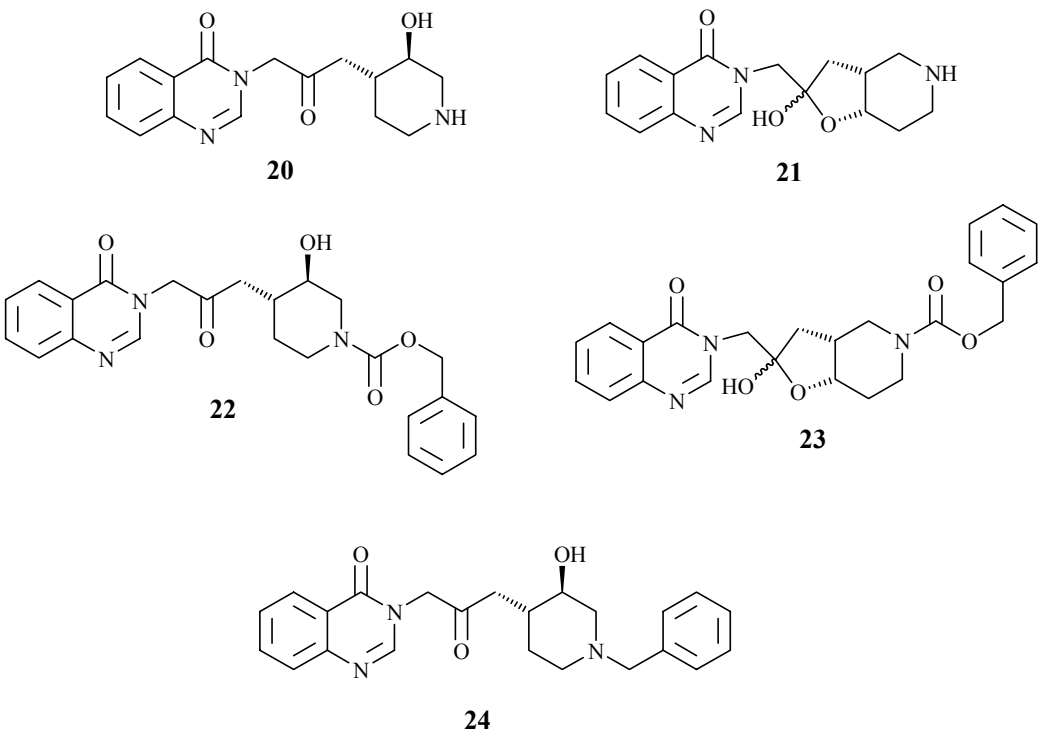
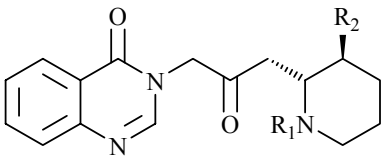
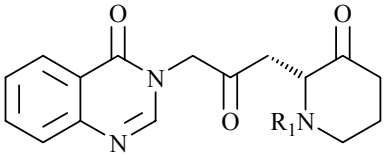


Figure 7: Piperidine *N*-regioisomers of **1** prepared by Takeuchi's group.

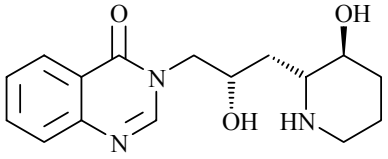
In 2002, Kikuchi *et al.* [39] published an extensive paper on the *in vitro* antimalarial test results of a few simple derivatives they prepared from natural **1** and **2** (i.e. compounds **25** to **31**, Figure 8a), in addition to pyrimidone or isoquinolone derivatives they prepared by using Hatakeyama's method (see later in Section 1.5.1., i.e. compounds **32** to **35**, Figure 8b), as well as derivatives prepared from synthetic **10** and **11** (see Scheme 4, i.e. compounds **36** to **50** in Schemes 6a and 6b).



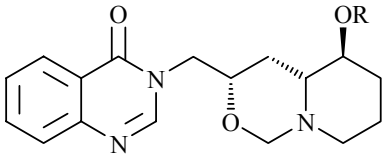
25 R_1= Ac, R_2= OAc
26 R_1= CO_2Et, R_2= OH



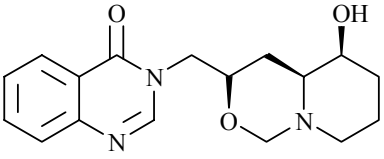
27



28

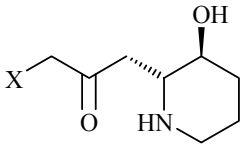


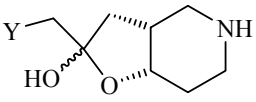
29 R=H
30 R= MOM

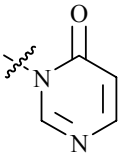


31

Figure 8a: Derivatives prepared from natural **1** and **2**.





32 and **33**, X and Y= 

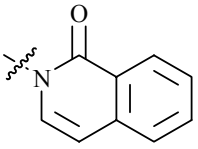
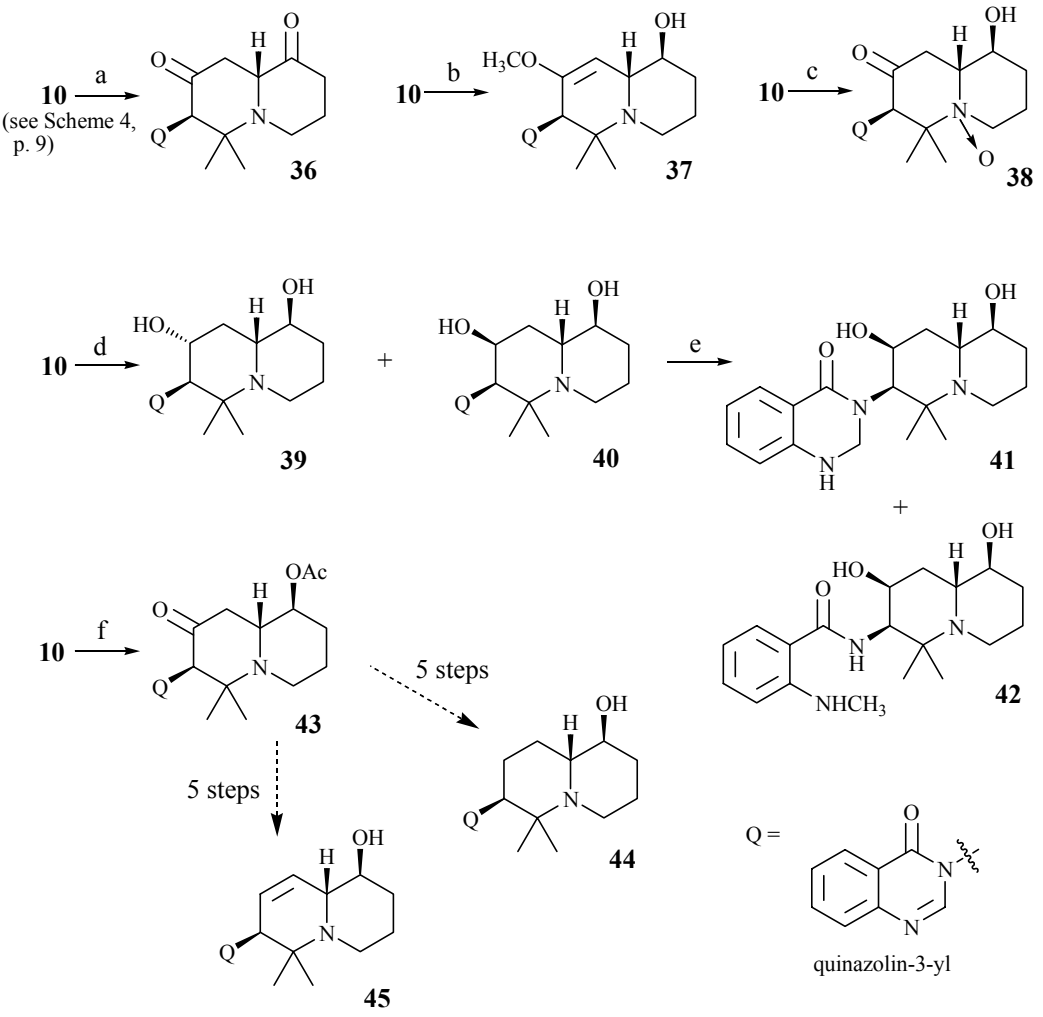
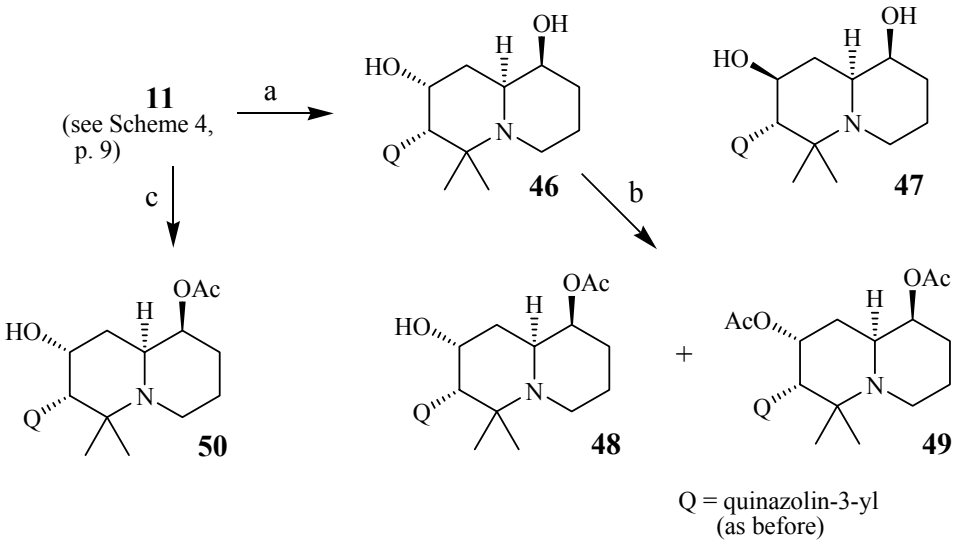
34 and **35**, X and Y= 

Figure 8b: Pyrimidone (**32** and **33**) and isoquinolone (**34** and **35**) derivatives of **1** and **2**.



Reagents and conditions: (a) Dess-Martin periodinane, CH_2Cl_2 (90%); (b) K_2CO_3, MeOH, 0 ºC (79%); (c) *m*-CPBA, K_2CO_3, CH_2Cl_2, –78 ºC; (d) $NaBH_4$, MeOH, 0 ºC (**40**, 66%; **39**, 14%); (e) LAH, THF, reflux (**41**, 25%; **42**, 20%); (f) Ac_2O, pyridine, 0 ºC (93%).

Scheme 6a: Derivatives of **10** and their preparation.



Reagents and conditions: (a) $NaBH_4$, MeOH, 0 ºC (**46**, 61%; **47**, 14%); (b) Ac_2O, pyridine (**48**, 41%, **49**, 28%); (c) Ac_2O, pyridine (62%)

Scheme 6b: Derivatives of **11** and their preparation.

Interestingly, all the compounds tested (**25** to **50**) displayed some antimalarial activity *in vitro*. However, none of them (with the exception of **36**) was more active than the lead compounds, i.e. **1**, **2**, **10** and **11**, against the *P. falciparum* FCR-3 strain tested. In addition, the toxic selectivities varied greatly (see Table 4).

For simplicity, because so many compounds were tested in this paper, the biological properties of only those candidates which are essential in this discussion are compared to the lead compounds and two clinical drugs in Table 4 below. The most promising new derivatives are indicated by asterisks in this table.

Compound	**Figure no.**	**Antimalarial activity EC_{50} (M)**	**Toxic selectivity**
Chloroquine	-	1.8×10^{-8}	1778
Artemisinin	-	1.0×10^{-8}	1000
1	1	7.0×10^{-10}	253
2	1	3.4×10^{-9}	53
10	Scheme 4	1.6×10^{-9}	238
11	Scheme 4	2.8×10^{-9}	857
25	8a	9.1×10^{-7}	32
26	8a	2.0×10^{-8}	500
27 *	8a	2.0×10^{-8}	750
28	8a	2.0×10^{-8}	750
29 *	8a	3.7×10^{-9}	1027
30	8a	8.6×10^{-9}	291
32	8b	6.0×10^{-7}	32
33	8b	4.0×10^{-8}	175
34	8b	5.0×10^{-7}	32
36 *	Scheme 6a	1.9×10^{-9}	3105
43	Scheme 6a	3.6×10^{-9}	361
50	Scheme 6b	1.9×10^{-8}	368

Table 4: Antimalarial activities of selected compounds prepared by Kikuchi *et al.* compared to the activities of **1**, **2**, chloroquine and artemisinin (* most promising new derivatives).

From Table 4 it can be suggested that protection of the oxygen at position 3″ of the piperidine ring in **1** usually leads to a decrease in activity and selectivity (**26** and **30**). Importantly, **27** (containing the 3″-oxo substituent instead of the hydroxy group) and **28** (containing the 2′-hydroxy group instead of the oxo group) not only showed promising activities, but they both proved to be more selective cytotoxically than **1**. This result disproved the general consensus that the (3″*S*) absolute constitution (hydroxyl group) and stereochemistry were essential for the antimalarial activity of (+)-**1**. In fact, in a recent review on new antimalarial drugs [40], 3″-ketofebrifugine (**27**) is cited as a good candidate for clinical trials. Both the benzenoid moiety and the N-1 atom of the 4-quinazolinone ring are important in determining the potency of **1** as **32**, **33** and **34** showed both weaker activity and selectivity than **1**.

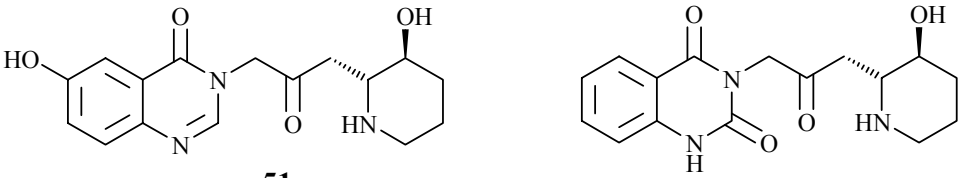
The results obtained from the derivatives of **10** and **11** followed less general trends. Compound **50** (acetylated **11**) was both less active and less selective in action than **11** which further supports the trend that protection of the 3″-hydroxy group in **1**, **10** or **11**, usually suppresses biological activity. However, **43** (acetylated **10**) and **10** tested equally promisingly which contradicted this trend to a minor extent.

Two compounds were particularly impressive in their antimalarial function. Compound **29**, the product formed by cyclization *via* methylene incorporation between N1″ and the oxygen at position C2′ of **28**, exhibited similar antimalarial activity but much higher selectivity than **1**. Perhaps most promising and most surprising were the excellent activity and selectivity of **36**, the 3″-oxo derivative formed by Dess-Martin oxidation of the 3″-hydroxyl group in **10**.

In summary, it was established conclusively by the Kikuchi study [39] that the three heteroatoms (O2′, N1″ and O3″) in the "side-chain" moiety of **1** and its most potent derivatives confer activity to these compounds. Furthermore, the 4(3*H*)-quinazolinone moiety is necessary in its full constitution if highly active antimalarial derivatives of **1** are to be sought. The stereochemistry at position 3″ is not as crucial as was originally thought. In fact, two of the best derivatives prepared (**36** and **27**) contained a ketone group at the corresponding 3″ position. It became clear, though, that positions 2′ and 3″ do need to be oxygen substituted. Notably, several compounds with improved toxic

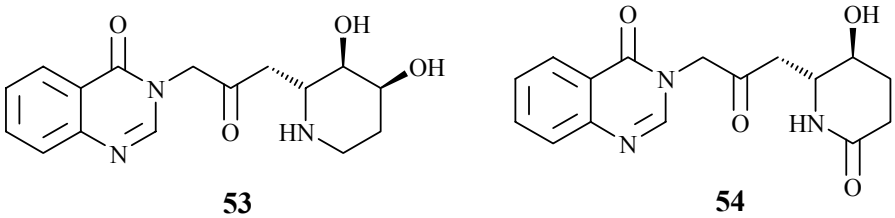
selectivity over **1** were synthesized (see Table 4), which indicates the importance of continued research into the synthesis of febrifugine derivatives.

In 2003, Hirai *et al.* [41] reported the first results on the metabolism of febrifugine analogues when incubated with mouse liver S9 cells. Febrifugine (**1**) was converted into **51** and **52** (Figure 9a), while the acetone adduct derivative **10** produced **53** and **54** (Figure 9b). Synthetic analogues (**55** and **56**, Figure 9c) of **51** and **53**, respectively, were also prepared and tested against malaria. The *in vitro* antimalarial properties of these metabolites are given in Table 5.



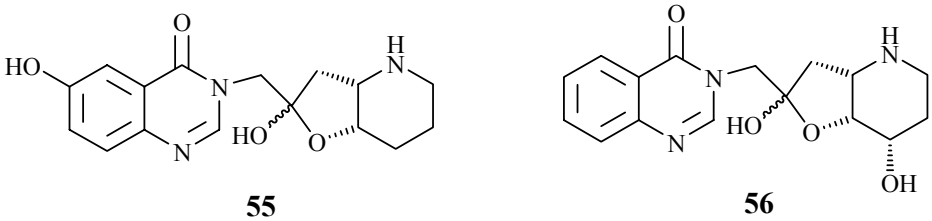
51 **52**

Figure 9a: Major metabolic products from **1** incubated with mouse liver S9.



53 **54**

Figure 9b: Metabolic products isolated, together with **51** and **52** (Figure 9a), from **10** incubated with mouse liver S9.



55 **56**

Figure 9c: Synthetic analogues of **51** and **53**.

Compound	*P. falciparum* EC_{50}, M	Toxic selectivity
1	7.0×10^{-10}	253
2	3.4×10^{-9}	53
51	2.2×10^{-9}	123
52	6.6×10^{-6}	>13
53	2.2×10^{-8}	1636
54	$>5.2 \times 10^{-5}$	-
55	2.7×10^{-10}	10 741
56	1.5×10^{-7}	167

Table 5: *In vitro* antimalarial activities and selectivities of the compounds from Figures 9a-9c.

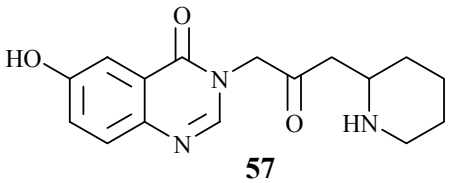
It is at once obvious from Table 5 that the basicity of N1″ is essential for good antimalarial function as the amide analogue **54** was practically inactive. The low activity of **52**, containing an amido group at C2, emphasized once again that the quinazolinone residue should be unchanged if good activity is to be observed.

Furthermore both 6-hydroxy substituted quinazolinone analogues **51** and **55** were active, although the selectivity of **51** was low. This was one of the very few cases reported thus far in which the isofebrifugine analogue (**55**) was superior to the febrifugine analogue (**51**).

Substitution at C4″ by a hydroxyl group proved to retain good activity and increased selectivity over **1** as seen for **53**. It was not studied, however, whether the 3″,4″-*cis* dihydroxyl substitution was necessary over the *trans* orientation to retain activity. In this case, the isofebrifugine analogue **56** again proved to be inferior.

Most remarkable was compound **55**. This was the first derivative of **1** reported in the literature to test both more active and much more selective against malaria than **1**. The slight discouragement here was that when *in vivo* studies in mice infected with *P. berghei* were conducted, the results of which are not shown here, the ED_{50} for **55** was found to be higher (6.0 mg/kg) than for **1** (0.3 mg/kg). However, the other 6-hydroxyl substituted analogue **51** performed very pleasingly (0.6 mg/day).

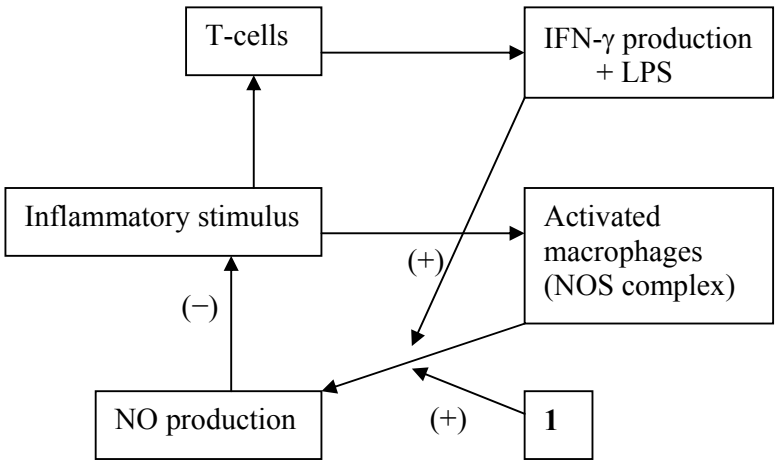
It can be concluded that both **51** and **55** are outstanding new lead compounds for the development of potent antimalarials. On the basis of this study, it is suggested that a simple 6-hydroxy analogue such as **57** shown here be prepared and tested against malaria. A simple method for the synthesis of (±)-deoxyfebrifugine **14** (p. 13) might be easily applied to achieve this end.



57

1.4.5. Mechanism of the antimalarial activity of febrifugine

In 1998, Murata *et al.* [42] reported that **1** functions by causing an increase in the production of nitric oxide (NO) gas during the immunological response. This process is represented in Scheme 7.



Scheme 7: Mechanism of **1**.

NO is a free radical gas which has been implicated in a variety of biological functions, e.g. neurotransmission, vascular homeostasis, and antimicrobial and antitumour activities. The release of NO in the body is controlled by the NOS (nitric oxide synthase) enzyme complex. Macrophages contain a transcriptionally inducible isoform of NOS which may be activated to produce NO over a long period of time in order to exert cytotoxic activity against e.g. viruses, protozoa, tumour cells etc.

In the case of malaria infection, the inflammatory stimulus in Scheme 7 is provided by the sporozoites injected into the host by the *Anopheles* mosquito. This triggers the T-cells to produce interferon-γ (IFN-γ), an immunological mediator which, in conjunction with lipopolysaccharide (LPS), stimulates the activated macrophages to produce NO. This effect is greatly enhanced by the presence of **1** as discussed below.

In the first study [42], mice were immunized with a bacillus bacterial strain (BCG) 3 days after administering **1** orally. It was found that the alkaloidal fraction (containing **1** and **2**) of a MeOH extract from *D. febrifuga* potentiated NO production by 103% at a dosage of 1mg/kg/day.

After purifying the MeOH extract mentioned above, the three components (**1**, **2** and 4(3*H*)-quinazolinone) obtained were individually tested for their NO potentiating ability. It was found that NO production was increased in the following order: **2** (22%) < 4(3*H*)-quinazolinone (29%) < **1** (91%) at dosages of 1 mg/kg/day. None of the other antimalarial drugs (quinine, chloroquine and artemisinin) tested in the same manner increased the production of NO.

In the second study by Murata *et al*. [43], **1** was administered two days before and/or several days after mice were infected with *P. berghei* parasitized erythrocytes. Again it was found that **1** greatly enhanced the production of NO by monitoring the plasma NO_3^- (a degradation product of NO) levels. Mortality and the level of parasitemia were significantly reduced by **1** during treatment at a dosage of 1 mg/kg/day. In fact, all the mice were rescued during treatment. However, **1** caused toxic death at a dosage of 10 mg/kg. Furthermore, four days after the last administration of **1** parasitemia and

mortality rate increased and by day 17 all the mice died. A more recent study [44] reported similar observations.

Murata *et al*. also found that NOS inhibitors (*l*-NMA and aminoguanidine) prevented the NO potentiating and rescuing effects of **1**. Importantly in another study [45] it was found that the simultaneous use of quinine and desferrioxamine B (an iron chelator that also increases NO production in macrophages) improved host defense against *Plasmodium* infection over the use of quinine alone.

The above results indicate that **1** acts by causing an increase in NO production during the immunological response. Furthermore, **1** has the potential for malaria prophylaxis as it strengthens host defense against malaria. The therapeutic effect of other antimalarial drugs might be enhanced by **1**. Importantly, owing to its novel mode of action, no resistant strains of the malaria parasite against **1** have been found.

1.4.6. Other biological activities of febrifugine

Owing to its novel mechanism of action, it is not surprising that **1** has exhibited other useful biological activities. In 1960, **1** was shown to possess good antitumour properties [46]. *In vitro* tests showed that **1** destroyed up to 90% of Ehrlich ascites cells within 3 h at 37 ºC in a 0.25% solution. The growth of other cancer cells was also inhibited by **1** to extents dependent on the concentration used. Recently it was claimed that **1** might present a cure for cardiac arrhythmia [8].

The powerful coccidiostatic properties of a derivative of **1**, halofuginone **16** (Figure 4, p. 14, *dl*-6-bromo-6-chloro-**1** hydrobromide) are well known [47,48]. The commercial antiparasitic feed additive Stenorol (Roussel Uclaf. Co. Ltd.) [49] contains **16** as the active ingredient used to treat coccidiosis in chickens. Natural products **1** and **2** have also been tested for their coccidiostatic properties. Isofebrifugine (**2**) initially tested *ca*. 10-fold less active against coccidiosis in chickens [50] than febrifugine (**1**), but was more recently shown to be completely inactive, even at 25 times the effective dose of **1** [4]. Recently, **16** was shown to inhibit collagen production and is currently under clinical trials for the treatment of scleroderma in humans [51].

1.4.7. Conclusions

It can be speculated from the above discussion that, to date, the following features in (+)-**1** are necessary for, or may serve to enhance, the antimalarial activity of (+)-**1**:

1. The piperidine ring;
2. The central 2-propanoyl unit;
3. The benzenoid (unsubstituted or substituted) part and N-1 of the quinazolinone moiety;
4. All the heteroatoms, except for O-4 and N-3. Both N-1 and N-1″ need to be basic (amino or imino groups), but O-2′ and O-3″ may both be either in the keto- or the hydroxy- forms.

To summarize these findings, the crucial parts of (+)-**1** are indicated in Figure 10.

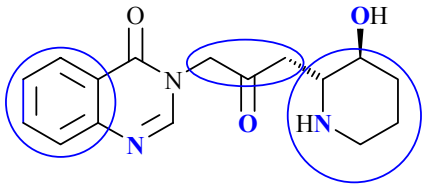
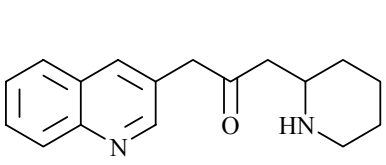


Figure 10: Parts (indicated in blue) of (+)-**1** thought to be responsible for potentiating its antimalarial activity.

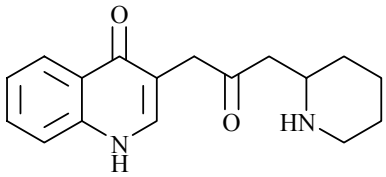
Although it is thought that the bicyclic structure of the quinazolinone moiety is also good for the antimalarial activity of **1**, it can be seen from Figure 10 that the need now arises for the synthesis of derivatives in which two of the heteroatoms in this moiety, N-3 and O-4, are either absent or substituted by other atoms, in agreement with the proposal by Cheng [7] (see section 1.4.2.).

It might be worthwhile in future to synthesize, in addition to the proposed compound **57** (mentioned before on p. 26), some simple quinoline and quinolinone analogues (e.g. **58** and **59** in Figure 11**)** of (±)-deoxyfebrifugine **14** using an easy synthesis of **14**, before attempting to synthesize similar analogues of **1** by complex procedures. Even if the 3″-hydroxy group and absolute stereochemistry are absent, the biological

activities of analogues such as **58** and **59** will probably shed more light on the structure-function relationships in febrifugine (**1**).



58



59

Figure 11: Proposed analogues of **14**.

1.5. Published syntheses of febrifugine and isofebrifugine

1.5.1. Overview

The first syntheses of **1** and **2** by Baker's group in the 1950s [10,16,19] were briefly discussed in a previous section. Surprisingly after the Baker syntheses, very little new work on febrifugine was published until a total synthesis of (±)-**1** was reported by Burgess *et al*. [52] in 1996. As this was a racemic synthesis (5 steps, 1.0% overall yield) of 2″, 3″-*trans*-**1**, they were still unaware of the incorrect assignments (2″*S*, 3″*R*) by Barringer [12].

Since the absolute structure of (+)-**1** was unambiguously determined in 1999, many syntheses of **1** and **2** have been reported. For simplicity, we choose to discuss, in the upcoming sections, only a few of these in order to illustrate generally how synthetic chemists have approached the preparation of these alkaloids. However, for the sake of completeness the latest syntheses of **1** and **2** are tabulated in Table 6, including where applicable, the number of steps involved and the respective overall yields from a commercially available or simple starting material.

From Table 6 it can be seen that, in general, all the reported syntheses require many steps and are low-yielding. When looking at the seemingly simple structures of **1** and **2** (both containing few asymmetric carbons) this is quite surprising. However, considering the relatively large number of contrasting functional groups and reactive

centres in the structures of **1** and **2**, one might envisage problems with side-reactions and the general stability of intermediates. Furthermore, the reported syntheses mostly require toxic reactants (e.g. heavy metal catalysts) that are environmentally hazardous, and the required chemicals are also often very expensive, specialized species.

Principal Authors	**Year of publication**	**Compound(s) synthesized**	**No. of steps**	**Overall yield (%)**
Kobayashi [13,25]	1999	(+)-**1**, (–)-**1**, (+)-**2**, (–)-**2**	13	2.4 [for (+)-**1**]
Takeuchi [53]	1999	(±)-**2**, (±)-**1**	8, 9	7.2, 5.2
Takeuchi [54,55]	2000	(+)-**2**, (+)-**1**	9, 10	5.2, 3.8
Ogasawara [56]	2000	(+)-**1**	25	11
Kobayashi [57]	2001	(+)-**2**	n. d.	n. d.
Kobayashi [58]	2001	(+)-**2**	n. d.	n. d.
Kobayashi [59]	2001	(+)-**2**	11	8.4
Hatakeyama [60]	2001	(+)-**1**, (+)-**2**	13	9.0, 4.2
Huang [61]	2003	(+)-**2**, (+)-**1**	n. d.	n. d.
Honda [62]	2004	(+)-**1**	n. d.	n. d.
Caprio [63]	2005	(+)-**1**	n. d.	n. d.

Table 6: List of post-1999 syntheses reported of **1** and **2**. (n. d. = not determined, since non-commercially available precursors were used).

1.5.2. Synthesis of febrifugine by Kobayashi and co-workers

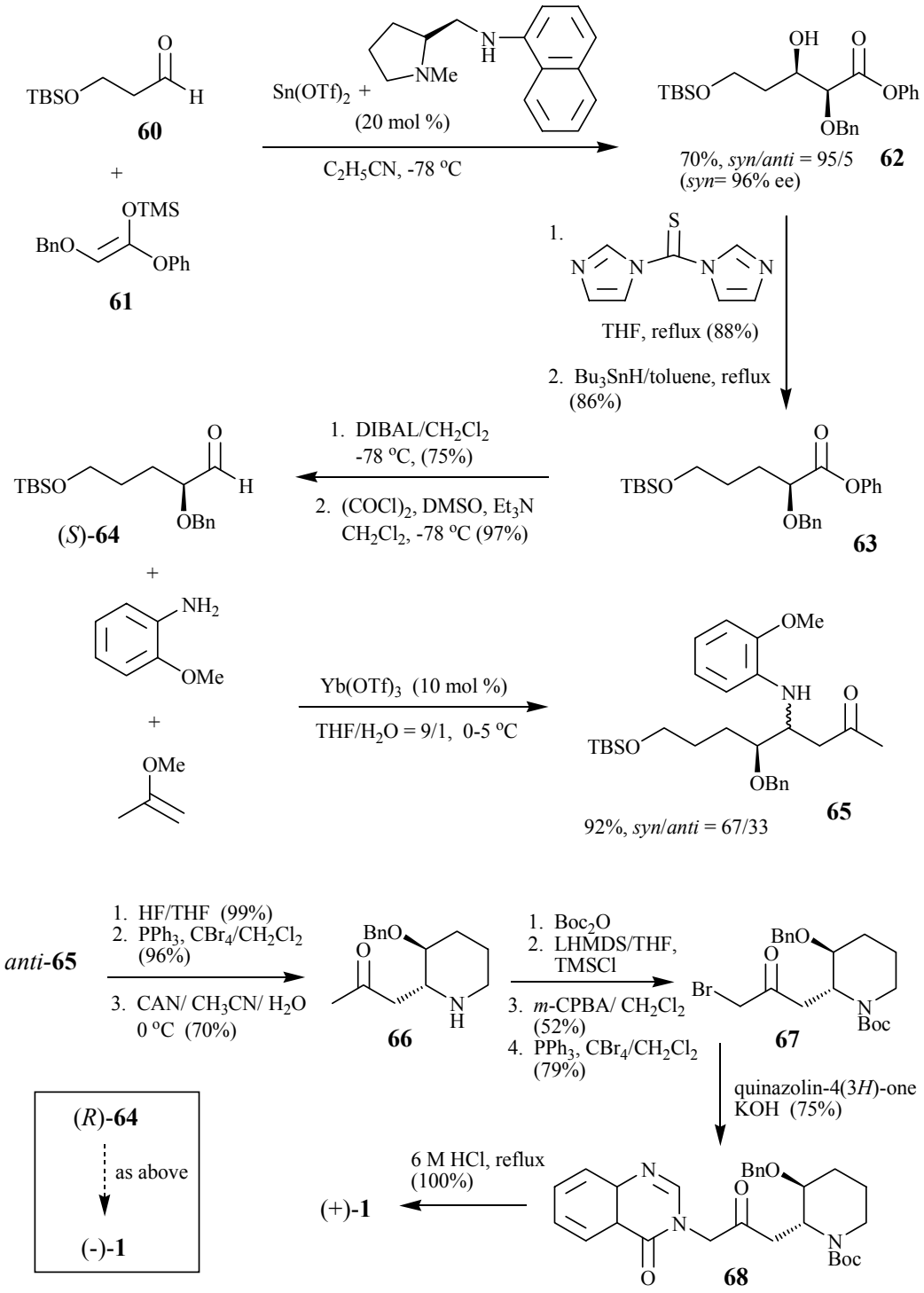
Represented in Schemes 8 and 9 is the interesting synthesis of all the antipodes of **1** and **2** reported by Kobayashi *et al.* [13,25] in 1999. The syntheses of natural (+)-**1** and (+)-**2** are described below.

A well-known stereoselective Sn(II)-catalyzed Mukaiyama aldol reaction was used to obtain *syn*-**62** in 96% ee from the reaction between simple achiral aldehyde **60** and *Z* silyl enol ether **61** (Scheme 8). Two more steps removed the β-hydroxyl group to yield **63** in 86% yield. Reduction of ester **63** using DIBAL, followed by Swern oxidation of the intermediate primary alcohol yielded key aldehyde (*S*)-**64**.

The unique three-component reaction in aqueous medium between (*S*)-**63**, 2-methoxyaniline and 2-methoxypropene in the presence of a lanthanide catalyst (ytterbium triflate) proceeded in excellent yield to afford the Mannich-type adduct **65** as a mixture of diastereomers. The major product (*syn*-**65**) was used to prepare (+)-**2** (see Scheme 9) and *anti*-**65** was used to prepare (+)-**1**.

After removal of the TBS group in *anti*-**65** using HF, followed by bromination, the spontaneously cyclized *N*-(2-methoxyphenyl)-protected piperidine intermediate was deprotected using CAN to afford **66**. After *N*-Boc protection of **66**, the intermediate silyl enol ether was formed by lithiation followed by *O*-protection using TMSCl, and subsequently oxidized followed by bromination to give **67** in 41% yield over 4 steps. Simple alkylation of quinazolin-4(3*H*)-one with **67** using KOH yielded **68**, which was doubly deprotected in refluxing 6 M HCl to afford (+)-**1**.

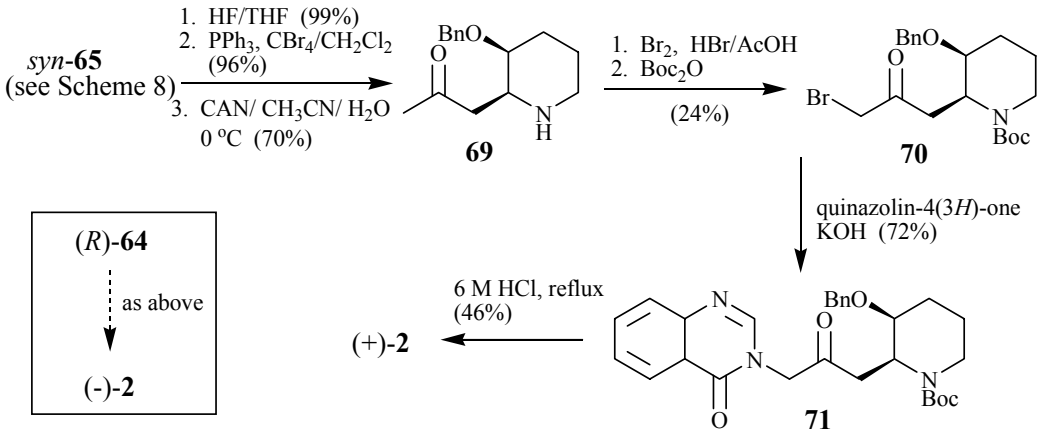
Similarly, (–)-**1** could be prepared by changing the configuration of the chiral diamine in the Sn(II)-catalyzed reaction to form (2*R*, 3*S*)-*syn*-**62**, and subsequently preparing (*R*)-**64** by completing the synthetic sequence.



Scheme 8: Synthesis of (+)-**1** and (–)-**1** by Kobayashi and co-workers.

To confirm the absolute configuration of key aldehyde (*R*)-**64** in the synthesis of (–)-**1**, the authors prepared **64** from D-glutamic acid and obtained the same optical rotation. It was therefore unequivocally proven that active (+)-**1** possesses the (2″*R*, 3″*S*) configuration.

The synthesis of (+)-**2** and (–)-**2** is illustrated in Scheme 9. In this case, similar reactions were used as described above for **1**, except that *syn*-**65** was used and that the bromination of **69** (the stereoisomer of **66**) using Br_2 in HBr/AcOH afforded a substantially decreased yield of bromide **70** compared to its isomer **67** (Scheme 8). Owing to the known isomerization of **2** to **1** in protic solvents, the yield of deprotection of **71** to (+)-**2** was low (46%). Similarly (–)-**2** was prepared from (*R*)-**64** (Scheme 9).



Scheme 9: Synthesis of (+)-**2** and (–)-**2** by Kobayashi and co-workers.

1.5.3. Synthesis of febrifugine by Takeuchi and co-workers

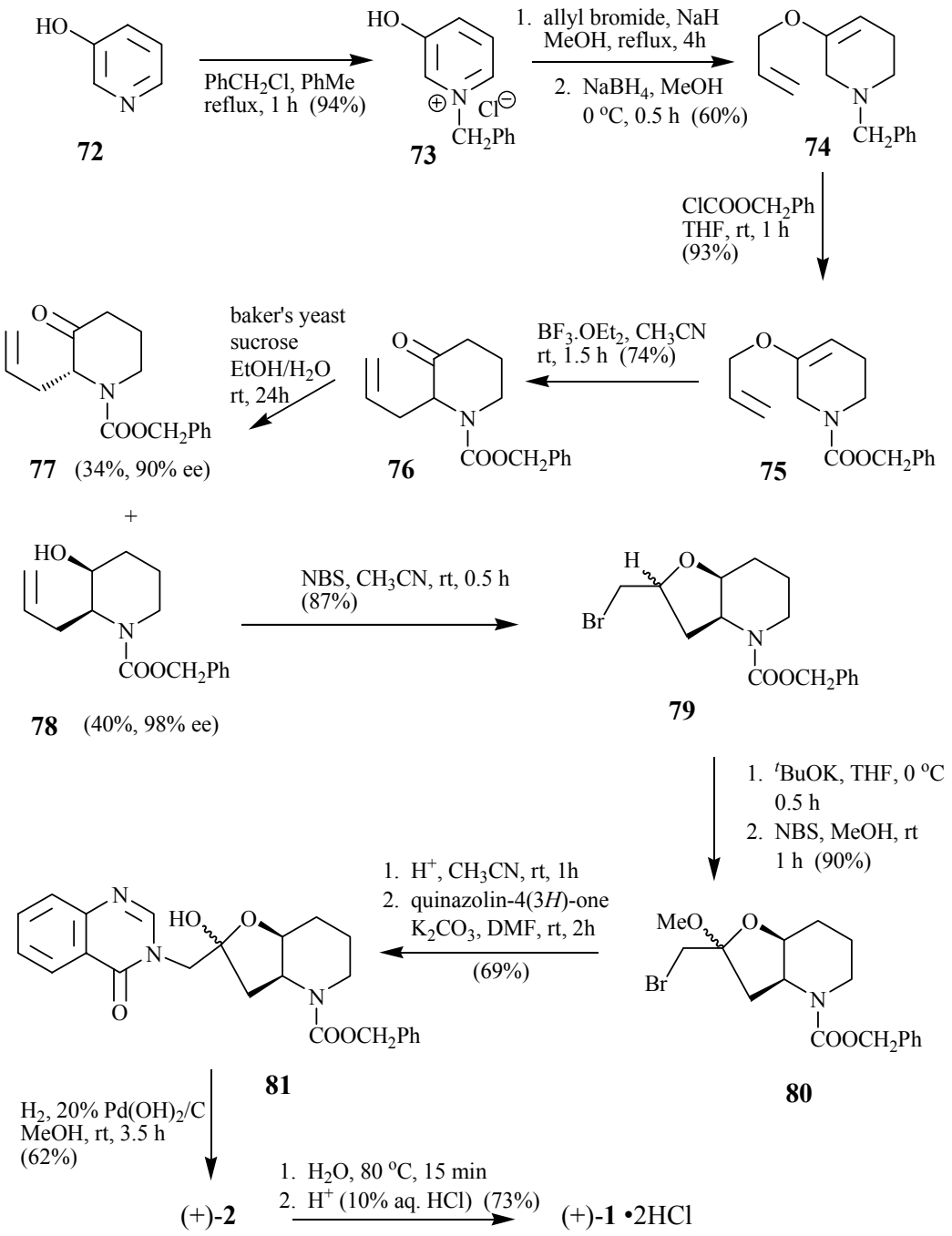
Soon after Kobayashi *et al*., Takeuchi's group published their asymmetric synthesis of (+)-**2** and (+)-**1** (Scheme 10) using yeast reduction as a chiral resolution step [54,55].

Commercially available 3-hydroxypyridine **72** was transformed into pyridinium chloride **73**, followed by *O*-allylation and regioselective reduction of **73** to afford in 60% yield the 3-allyl-*N*-benzyl derivative **74**. As the *N*-benzyl group was found to be unsuitable for the remainder of the synthesis, it was replaced by the benzyloxycarbonyl group using benzyl chloroformate in THF. The product **75** was subjected to an unusual Claisen rearrangement reaction in the presence of boron trifluoride-diethyl ether complex at rt to afford key piperidin-3-one intermediate **76** in 74% yield.

After **76** was stirred for 25 h in EtOH/H_2O in the presence of Baker's yeast and sucrose, it was found that (2*S*)-**76** was selectively reduced to afford (3*S*)-hydroxy derivative **78** in 40% yield in 98% ee. From the reaction extracts was also recovered (2*R*)-**77** in 34% yield and 90% ee.

Bromination of **78** using NBS followed by intramolecular etherification yielded **79** which was first transformed by dehydrobromination and re-etherification into 2-methoxy derivative **80**. Acid-catalyzed demethylation of **80** followed by the substitution reaction with quinazolin-4(3*H*)-one in the presence of K_2CO_3 afforded *N*-benzyloxycarbonyl isofebrifugine **81** in 62% yield over 4 steps from **79**. A reduced yield (40%) of **81** was obtained when **79** was reacted directly with quinazolin-4(3*H*)-one in one step.

Finally, deprotection of **81** using Pearlman's catalyst afforded (+)-**2** which was converted into (+)-**1** dihydrochloride in 73% yield by stirring in H_2O at 80 ºC for 15 min, followed by exposure to HCl and recrystallization from EtOH:H_2O (9:1).



Scheme 10: Synthesis of (+)-**2** and (+)-**1** by Takeuchi and co-workers.

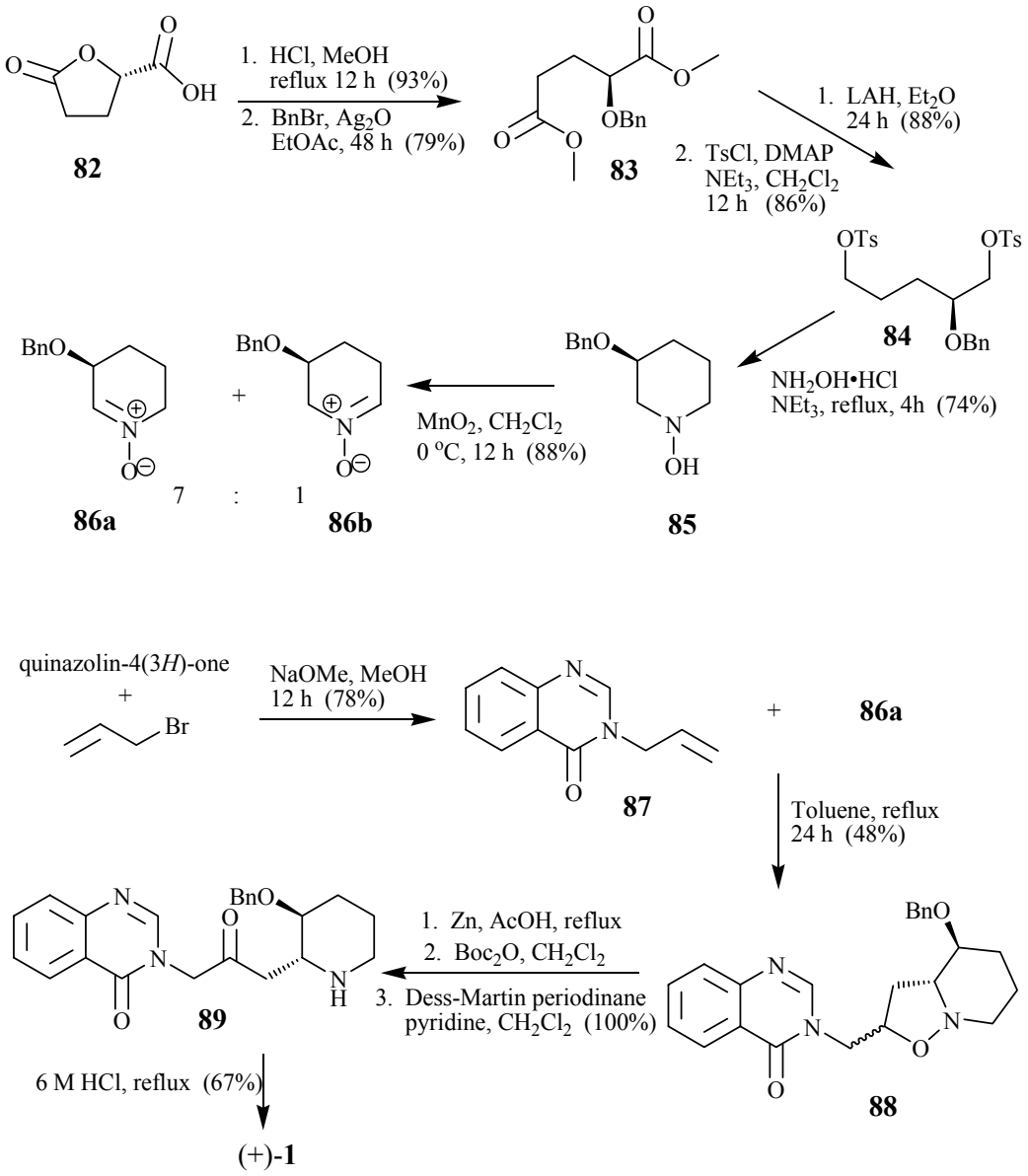
1.5.4. Synthesis of febrifugine by Ashoorzadeh and Caprio

Outlined in Scheme 11 is the most recently published asymmetric synthesis of (+)-**1** [63].

Firstly, enantiomerically pure lactone acid **82** was prepared by an existing procedure from naturally occurring L-glutamic acid. Benzyloxydiester **83** was obtained in 93% yield by acid-catalyzed ring-opening of **82**, followed by *O*-benzylation which was found to proceed optimally in the presence of 1.5 eq. Ag_2O to afford **83**. Diester reduction using LAH, followed by di-*O*-tosyl protection by a standard procedure yielded **84**, which was cyclized using $NH_2OH{\cdot}HCl$ in NEt_3. The obtained *N*-hydroxypiperidine **85** was converted into a mixture of separable regioisomeric nitrones **86a** and **86b** by oxidation using MnO_2.

Stable dipolar nitrone **86a** was subjected to 1,3-dipolar cycloaddition with dipolarophile 3-allylquinazolin-4(3*H*)-one **87** to afford cycloadduct **88** in 48% yield. The major product was found to possess the desired regiochemistry in addition to the required *trans*-stereochemistry, which was verified by NOE correlations.

The N-O bond of isoxazolidine **88** was reductively cleaved using Zn and AcOH to yield the crude hydroxylamine intermediate, which was first *N*-Boc protected followed by a Dess-Martin oxidation procedure to afford the ketone **89** in quantitative yield over 3 steps. Finally, double deprotection using the Kobayashi conditions (6 M HCl, reflux) afforded (+)-**1**, the characterization data of which agreed with the natural product, in 67% yield.



Scheme 11: Synthesis of (+)-**1** by Ashoorzadeh and Caprio.

1.5.5. Comments on the existing syntheses of febrifugine

The Kobayashi synthesis is a classic example of asymmetric synthesis based on the implementation of a chiral metal-based catalyst. Although this synthesis was extremely useful and easily adaptable for the synthesis of all the stereoisomers of **1** and **2**, it is a low-yielding and expensive synthesis. The starting materials shown in

Scheme 8 are not commercially available, thus the effective overall yield is even lower than 2.4%. It is never desirable to replace one protecting group by another (conversion of **65** to **67**, Scheme 8) as this adds two additional steps to a synthesis. Many toxic (e.g. the Sn-complex, the lanthanide salt) and expensive reagents were used in this synthesis. Most importantly, it is not a general synthesis, which may easily be adapted to the synthesis of diverse febrifugine derivatives, as metal catalysts are often very substrate-specific.

The Takeuchi synthesis depends on the enzymatic resolution of a key intermediate which significantly reduces the overall yield. As in the Kobayashi synthesis, a protecting group needs to be replaced by a more suitable group (transformation **74** to **75**, Scheme 10) and three additional steps were employed to optimize the yield of **81**. However, this is certainly a simpler, more economical and environmentally more friendly synthesis than the Kobayashi synthesis. In fact, the Takeuchi synthesis has been used for the preparation of potent febrifugine derivatives [41] which proves its generality. However, most enzymes are quite substrate-specific and this route might become more limiting when attempting to synthesize derivatives of **1** which differ considerably in the piperidine moiety.

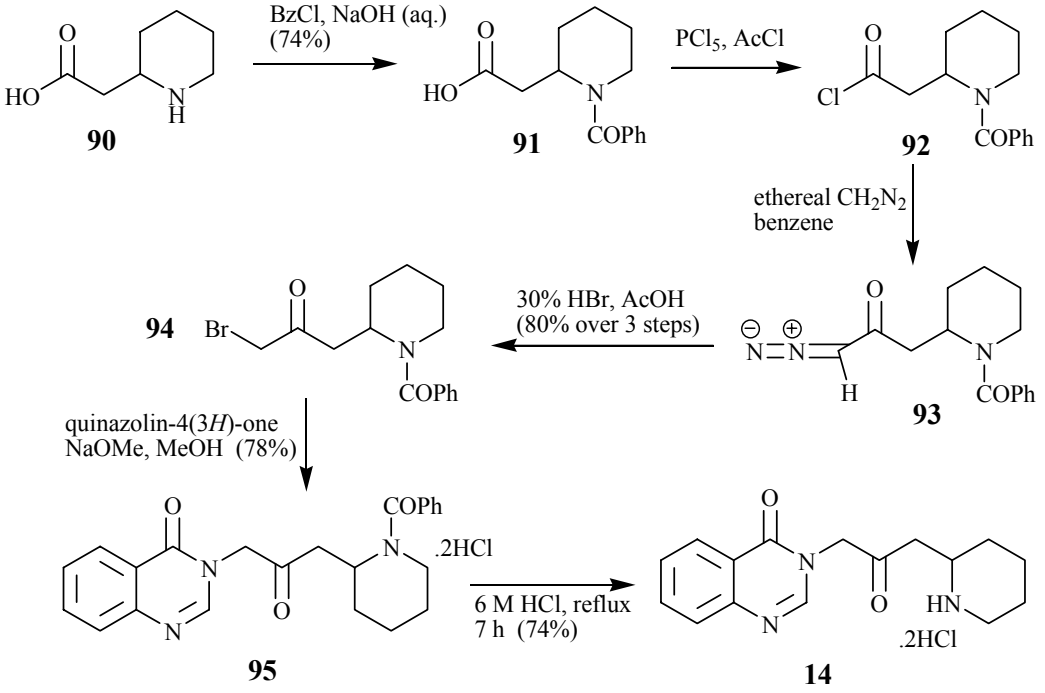
Ashoorzadeh and Caprio used the most desirable approach to asymmetric synthesis, i.e. starting from enantiomerically pure and cheaply available natural material (L-glutamic acid). However, this synthesis has a moderate overall yield and many steps (11.6%, 11 steps) considering that **82** (Scheme 11) first needs to be prepared from L-glutamic acid.

In conclusion it can be said that new, more general and more efficient syntheses of **1** and its derivatives are still currently required regardless of the large number of recent published syntheses of **1**. This is further supported by the findings that many analogues of **1**, the structures of which are substantially different to that of **1**, are now promising lead compounds for the development of good antimalarials (see Section 1.4).

1.6. Published syntheses of (±)-deoxyfebrifugine

1.6.1. Baker's first synthesis of (±)-deoxyfebrifugine

The 3″-unsubstituted model compound of **1**, (±)-deoxyfebrifugine (**14**), is an important analogue of **1** and an appealing synthetic target for reasons mentioned before. The first synthesis of **14** by Baker's group in 1952 [64] is represented in Scheme 12.



Scheme 12: Baker's first synthesis of **14**.

The starting material, racemic 2-(piperidin-2-yl)acetic acid **90** was *N*-benzoylated to afford **91** in 74% yield which was subsequently converted into the acid chloride **92** using PCl_5 in AcCl. Crude **92** was converted into the desired bromomethyl ketone **94** by reaction first with diazomethane to obtain crude **93**, followed by direct bromination using HBr/AcOH. The yield of **94** over 3 steps was 80%. After alkylative coupling of **94** to quinazolin-4(3*H*)-one to afford **95**, acidic hydrolysis of the *N*-benzoyl protecting group yielded the dihydrochloride salt of **14**.

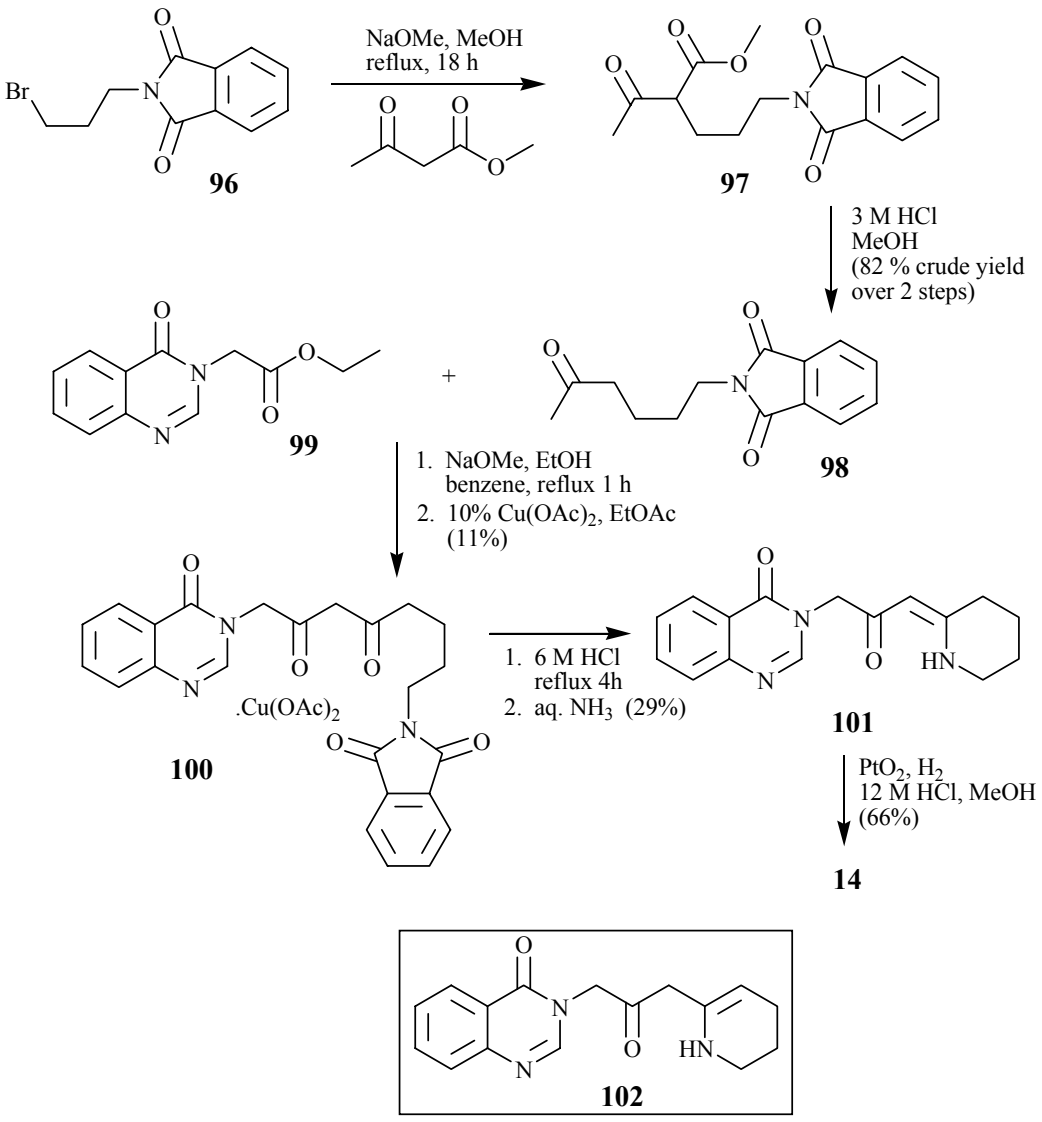
The overall yield of this efficient synthesis was 34% over 6 steps, but the starting material **90** is not a commercially available compound. It can, however, be prepared by Jones oxidation of commercially available piperidinylethanol [65], but this does add an additional step to the synthesis.

1.6.2. Baker's second synthesis of (±)-deoxyfebrifugine

As their first synthesis of **14** was not adaptable to the introduction of a hydroxyl group at different positions on the piperidine ring, Baker's group devised a second more general method to obtain **14** [66]. This procedure is outlined in Scheme 13.

Alkylation of methyl acetoacetate with 3-bromoalkylphthalimide **96**, easily prepared from commercially available 3-bromopropylamine hydrobromide and phthaloyl dichloride, afforded **97** which was subsequently decarbomethoxylated by acid hydrolysis to obtain key intermediate ketone **98**.

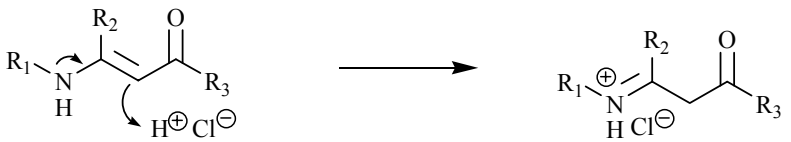
The Claisen condensation reaction between **98** and ethyl 2-(4-oxoquinazolin-3(4*H*)-yl)acetate **99** followed by complexation to $Cu(OAc)_2$ afforded the isolable Cu(II) salt of diketone **100** in poor yield. Acidic hydrolysis of the phthalimide formed the amino-diketone intermediate which underwent spontaneous 1,6-intramolecular cyclization under basic conditions to afford **101** in 29% yield. Hydrogenation over Adams catalyst afforded **14** in 66% yield.



Scheme 13: Baker's second synthesis of **14**.

It is important to note that the authors erroneously and surprisingly assigned enaminone **101** the structure of tetrahydropyridine **102** shown in Scheme 13. The double bond in **101** should be positioned so as to obtain maximum electronic conjugation. Baker did realize that **101** was a highly conjugated compound which exhibited properties different from tetrahydropyridines. According to Baker, the UV spectrum of **101** in NaOH aqueous solution showed "a peak at 312 mμ 8 times the expected intensity for the usual peaks observed at 302 and 315 mμ and which distorted the 267 mμ absorption of 3-alkylquinazolin-4(3*H*)-ones". When measured in

0.1 M aqueous HCl solution, this peak almost disappeared. This occurs because protonation of the enaminone destroys conjugation as illustrated in Scheme 14.



conjugated enaminone unconjugated iminium ion

Scheme 14: Protonation of the enaminone group results in the loss of electronic conjugation.

Furthermore, **101** was found to be resistant to the mild hydrogenation conditions usually employed for tetrahydropyridines which are not conjugated to a carbonyl group. We synthesized **101** (see later in Chapter 3) and used similar hydrogenation conditions to those reported by Baker in order to obtain **14**. There can be no doubt that our compound **101** is identical to the compound which Baker assigned the structure of **102** (Scheme 13).

Baker experimented with protecting groups other than the phthalimido group shown in Scheme 13. It was found that the overall yield was best when using *N*-carbethoxy (20%), compared to *N*-Bz (14%), *N*-CBz (10%) and *N*-phthalimido (4%).

Using this synthetic route, Baker's group managed to prepare the 5″-hydroxy-isomer of **1** [67] and the 2-methyl quinazolinone-substituted analogue of **1** (both found to be completely inactive against malaria as described in a previous section). However, this route was less flexible than originally thought and could not be used to prepare any of the other hydroxyl regioisomers or pyrrolidine analogues [68,69] of **1**.

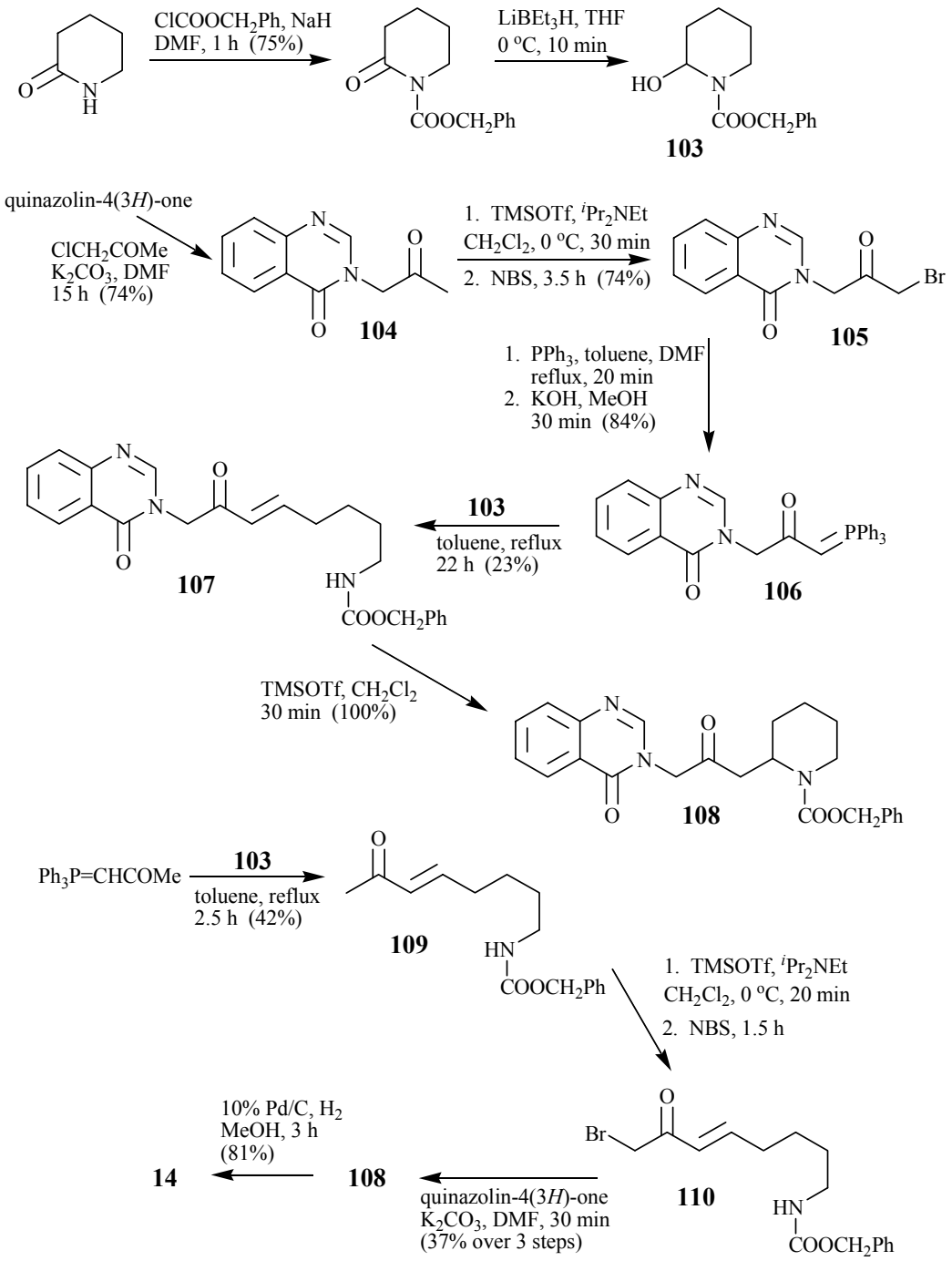
1.6.3. Takeuchi's synthesis of (±)-deoxyfebrifugine

In 1999, Takeuchi *et al*. published a new synthesis of **14** [31], represented in Scheme 15, which is based on the Wittig reaction as key step.

Starting from piperidin-2-one, *N*-Cbz protection followed by amide reduction afforded key *N*-protected cyclic aminal **103**. The Wittig reagent **106** was prepared in 5 steps from quinazolin-4(3*H*)-one. Simple alkylation of quinazolin-4(3*H*)-one using chloroacetone afforded ketone **104** which was converted by two steps (the formation of an intermediate silyl enol ether followed by bromination using NBS) into bromide **105**. Standard conditions (PPh_3 and subsequent deprotonation) were used to convert **105** into **106** in 84% yield. Wittig reaction between **106** and **103** was found to proceed with ring-opening to afford **107** in which the newly formed C=C bond possessed the *E* configuration. Cyclization using TMSOTf afforded **108** in quantitative yield. The overall yield was 7.9% over 9 steps from piperidin-2-one.

A second variation on this method led to an increase in overall yield. The Wittig reaction employing acetylmethylenetriphenylphosphorane and **103** produced ring-opened enone **109**, which was silylated and brominated using the aforementioned method to yield **110**. Subsequent alkyl substitution of quinazolin-4(3*H*)-one afforded again the previously prepared **108**. Finally, *via* hydrogenolysis of the CBz protecting group in **108** over 10% Pd/C, **14** was obtained in 81% yield.

The overall yield of **14** using the first method was 6.4% over 10 steps from piperidin-2-one, while the second method gave an overall yield of 9.4% over 8 steps from acetylmethylenetriphenylphosphorane and piperidin-2-one. This route is less economical and efficient than our route which will be described in the Chapter 3. Furthermore, this route is limiting with regards to the preparation of derivatives of **14** which vary in the piperidine moiety, including febrifugine (**1**).



Scheme 15: Takeuchi's synthesis of **14**.