Structure–Activity Relationships of the Antimalarial Agent Artemisinin. 2. Effect of Heteroatom Substitution at O-11: Synthesis and Bioassay of *N*-Alkyl-11-aza-9-desmethylartemisinins

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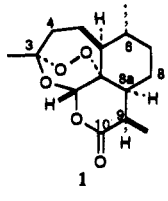
A novel class of artemisinin analogs, *N*-alkyl-11-aza-9-desmethylartemisinins **17–29**, were synthesized via ozonolysis and acid-catalyzed cyclization of precursor amides **5–16**. These amides were prepared through condensation of an activated ester of the known intermediate acid **2** with the corresponding primary amine. The analogs were tested *in vitro* against W-2 and D-6 strains of *Plasmodium falciparum* and found in some cases to be more active than artemisinin. A comparison of the *in vitro* testing methods of Milhous and Makler was conducted and gave similar relative antimalarial activities for these artemisinin analogs. Log *P* values were determined for most of the compounds, but no apparent correlation between log *P* and *in vitro* activity was found.

Malaria parasites, *Plasmodium falciparum* in particular, are increasing in resistance to traditional chemotherapeutics.[1] The toll on economic and health welfare within tropical climes is on an alarming rise for a malady formerly considered controlled and treatable,[2,3] thus sparking a resurgence in the search for alternative approaches and new, structurally novel antimalarials.[3,4] Accordingly, the unique sesquiterpene antimalarial (+)-artemisinin (**1**) is the focus of international medicinal chemistry efforts by numerous groups[5,6] including ourselves.[7,8] Increasing structural variation among artemisinin and its congeners ultimately aims to improve pharmacokinetic attributes.[9] Our ongoing structure–activity relationship (SAR) program[7,8] has now yielded lactam congeners with intriguing activity, as described herein.



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Early efforts at improved artemisinin analogs preserved the entire artemisinin skeleton. These dihydroartemisinin compounds are now strongly represented by the clinical candidates[9–13] artesunate, artemether, arteether, and artelinic acid.[14] But even these heavily studied congeners need improved potency, lower toxicity, oral activity,[9] and, in some cases, stability.[15,16] Departures from the tetracyclic framework have had mixed success, and despite much effort, the accumulated results are not yet complete enough to define a minimal pharmacophore, especially to capitalize upon for drug development. Notable among the structurally simpler analogs are the efforts by Posner et al., who have achieved good activity with trioxanes that were designed to structurally stabilize hypothetical biradical intermediates arising from scission of the peroxide bond.[17] The biological relevance of biradicals and the mode of action remains the subject of active speculation.[18] Recent experiments continue to reveal that artemisinin and analogs specifically alkylate certain parasitic components such as hemin[19] and other unidentified proteins.[20]

Meanwhile, SAR studies in our laboratory simultaneously truncated and elaborated artemisinin.[8] Simplification from tetra- to tricyclic congeners was examined. For 4,5-secoartemisinin[21] and its relatives,[22,23] conformational flexibility and unimpressive activity were experimentally observed, suggesting that the mobility of the peroxide bond was detrimental. In analogs that had a more fixed peroxide geometry, such as 8a,9-secoartemisinin and its derivatives,[24–26] activities varied widely and highlighted the impact of subtle changes in the D-ring.[27] The removal of one[8] or two[28] of the methyl groups at either the C-9 or both the C-6 and C-9 positions, as well as epimerization at C-9[7,29] on the full artemisinin skeleton, did not cause any substantial loss of activity.

We felt that the increasing abundance of diverse analogs could aid us in the development of new analogs, and thus approaches to define overall SAR were explored. A comparative molecular field analysis (CoMFA) was indeed practical—but due to the analogs included, was somewhat restricted to additional C-9-substituted analogs.[7] Therefore, for new analog designs and a more encompassing SAR, novel analogs of artemisinin bearing substitution at positions other than C-9 are desirable. Because we had ready access, via our total synthetic manifold,[30] to a late synthetic intermediate carboxylic acid, it was easy to envision a variety of amides that could undergo our cyclization protocol and furnish tetracycles with the heteroatomic substitution of nitrogen for oxygen at the 11 position. The additional

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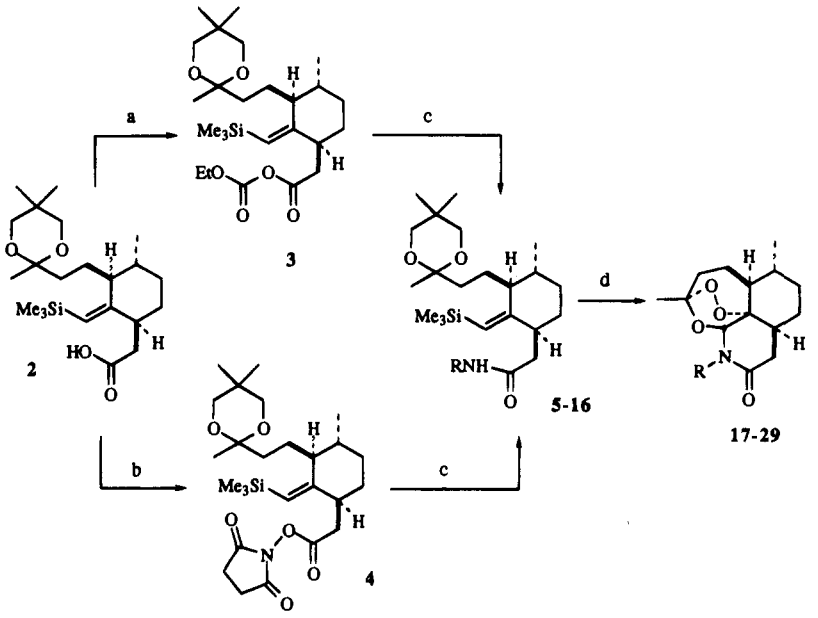
Scheme 1[a]



5, R = CH_3; **6**, R = $CH_2CH_2CH_3$; **7**, R = C_4H_9(i); **8**, R= C_5H_{11}(n); **9**, R= C_5H_{11}(i); **10**, R= $(CH_3)_2NCH_2CH_2$; **11**, R= $(CH_3)_3CO_2CCH_2$; **12**, R= $HO_2C(CH_2)_5$; **13**, R= $C_6H_5CH_2$; **14**, R= *p*-$ClC_6H_4CH_2$; **15**, R= $C_6H_5(CH_2)_2$; **16**, R= $C_6H_5(CH_2)_3$.

[a] (a) Et_3N, $ClCO_2Et$, CH_2Cl_2, 0 °C; (b) *N*-hydroxysuccinimide, DCC, CH_2Cl_2, room temperature; (c) RNH_2, CH_2Cl_2; (d) O_3, −78 °C then SiO_2 followed by 15% H_2SO_4.

valence of nitrogen would also provide a site for alkyl/aryl substituents and their contributions to SAR. Previously, we had started to explore this avenue, gathering bioassay and quantitative structure−activity relationship (QSAR) data on a number of 11-aza analogs;[8] recently another approach has also been reported.[31] We now fully describe the synthesis and evaluation of these derivatives.

Chemistry

Straightforward methodology was employed to prepare the tetracycles **17**−**29**, as shown in Scheme 1. From the total synthetic intermediate **2**, *in situ* formation of the corresponding mixed anhydride with ethyl chloroformate and triethylamine was followed by condensation with the appropriate primary amines to give ketal amides **6**−**11** and **13**−**16**. Exposure to ozone and subsequent acid-catalyzed cyclization gave the desired products in 20−65% overall yields.[30]

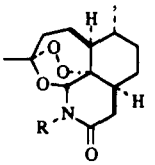
Some targets required further attention. For example, the *tert*-butyl ester derivative **11** underwent ozonolysis/cyclization to the tetracyclic ester **25**, which was subsequently hydrolyzed with trifluoroacetic acid to give acid **23**. Amide **5** was synthesized by addition of aqueous methylamine to **4**, the *N*-hydroxysuccinimide-activated ester of ketal acid **2**. Conversion to the peroxide **17** with ozone/acid ensued in good yield.

Biological Evaluation

The analogs **17**−**24** and **26**−**29** were tested *in vitro* in parasitized whole blood (human) against drug-resistant strains of *P. falciparum* at the Walter Reed Army Institute of Research by a modification of the procedure of Desjardins that monitors uptake of tritiated hypoxanthine[32,33] (Table 1). Two *P. falciparum* malaria parasite clones, designated as Indochina (W-2) and Sierra Leone (D-6), were used in susceptibility testing. The W-2 clone is chloroquine-resistant and mefloquine-sensitive, while the D-6 clone is chloroquine-sensitive and mefloquine-resistant. The relative potencies for the analogs (Table 1) were derived as follows: [(IC_{50} of artemisinin divided by IC_{50} of analog) × (MW of analog divided by MW of of artemisinin)] × 100% (i.e., the relative activity of artemisinin = 100%).

The analogs **19**, **20**, and **26**−**29** were also tested *in vitro* against the D-6 and W-2 strains of *P. falciparum*

Table 1. Relative Potencies of 11-Azaartemisinin Analogs[a]



compd	R	relative potency (%) Indochina (W-2)	Sierra Leone (D-6)	formula	anal.
1		100	100		
17	CH_3	500	213	$C_{15}H_{23}NO_4$	C,H
18	$CH_3CH_2CH_2$	111	75	$C_{17}H_{27}NO_4$	C,H,N
19	$C_4H_9(i)$	105	145	$C_{18}H_{29}NO_4$	C,H
20	$C_5H_{11}(n)$	63	110	$C_{19}H_{31}NO_4$	C,H
21	$C_5H_{11}(i)$	92	149	$C_{19}H_{31}NO_4$[b]	C,H
22	$(CH_3)_2NCH_2CH_2$		37	$C_{18}H_{30}N_2O_4$	C,H,N
23	HO_2CCH_2		6	$C_{16}H_{23}NO_6$	C,H,N
24	$HO_2C(CH_2)_5$	2	12	$C_{20}H_{31}NO_6$	C,H
25	$(CH_3)_3CO_2CCH_2$[c]			$C_{19}H_{31}NO_5$	C,H,N
26	$C_6H_5CH_2$	217	189	$C_{21}H_{27}NO_4$	C,H,N
27	*p*-$ClC_6H_4CH_2$	69	111	$C_{21}H_{26}NO_4Cl$	C,H
28	$C_6H_5(CH_2)_2$	143	197	$C_{22}H_{29}NO_4$	C,H
29	$C_6H_5(CH_2)_3$	105	205	$C_{23}H_{31}NO_4$	C,H

[a] Relative potency = [(IC_{50} of artemisinin/IC_{50} of analog) × (MW of analog/MW of artemisinin)] × 100%. [b] As hydrate, $^1/_4H_2O$. [c] Not tested.

Table 2. Comparison of Relative Potencies of 11-Azaartemisinin Analogs between Walter Reed (WR) and the University of Mississippi (UM)[a]

		relative potency (%)			
		W-2		D-6	
compd	R	WR	UM	WR	UM
19	$C_4H_9(i)$	105	227	145	155
20	$C_5H_{11}(n)$ 63	133	110	139	
26	$C_6H_5CH_2$	217	252	189	146
27	p-$ClC_6H_4CH_2$	69	311	111	128
28	$C_6H_5(CH_2)_2$	143	204	197	187
29	$C_6H_5(CH_2)_3$	105	281	205	147

[a] Relative potency = [(IC_{50} of artemisinin/IC_{50} of analog) × (MW of analog/MW of artemisinin)] × 100%.

Table 3. log P Values

	log P		activity	
compd	found	calcd[a]	W-2	D-6
23	1.75	1.688	0	6
dihydroartemisinin	2.68	1.778	358	575
17	2.76	1.554	500	213
artemisinin	2.90	2.250	100	100
10-deoxoartemisinin	3.16	2.585	597	693
18	3.41	2.612	111	75
artemether	3.53	2.239	192	234
19	3.66	3.011	105	145
28	3.94	3.501	143	197
arteether	4.03	2.768	218	258
21	4.16	3.540	92	149
20	4.24	3.670	63	110
27	4.32	3.956	69	111
29	4.39	4.030	105	205

[a] (a) Calculated using CLOGP, ref 38.

at the University of Mississippi using the parasite lactate dehydrogenase (pLDH) assay developed by Makler (Table 2).[34,35] This assay is based on the ability of pLDH enzyme of *P. falciparum* to reduce APAD to APADH. This reaction is carried out at a slow rate by human red blood cell LDH. The formation of APADH was monitored colorimetrically by the addition of nitroblue tetrazolium which was reduced to a blue formazan product. The relative potencies for the analogs (Table 2) were derived as described for Table 1.

Determination of log P Values. In an attempt to gain a better overall understanding of the SAR involved, we determined the log P values for the majority of these compounds using known literature procedures and standard values (Table 3).[36-38] The values were also calculated using CLOGP.[39]

Results and Discussion

The presence of a nitrogen instead of the 11-oxygen allowed us to investigate the effects of substituents in the southwest quadrant (as drawn) of the pharmacophore of **1**, which was heretofore unexplored. The data from Table 1 indicate that for 11-substituents (designated R), the optimum allowable chain length may be relatively short (1–3 carbons, see **17**, **18**) and a phenyl terminus seems best (see **26**, **28**, **29**). Coincidentally, these results are fairly similar to SAR at C-9,[7] perhaps revealing a maximum size for the pharmacophore in a putative active site or receptor. Since the activity improves about 6-fold upon removal of the C-9 methyl of artemisinin,[7] we were optimistic that incorporating this alteration would further enhance the potency of 11-azaartemisinins, but this was unsuccessful. Overall this novel class of 11-azaartemisinin analogs was favorably comparable to artemisinin against resistant strains of *P. falciparum*, and in particular the *N*-methyl analog **17** was more than 5 times as potent as parent **1** in the W-2 strain.

To our knowledge, these two distinct bioassay methods are directly compared for the first time on artemisinin analogs. Several issues warrant comparison of the two methods. First, any tendencies in relative potency expressed by both assays raise the confidence level in the accuracy of the relative potencies. The avoidance of using radioactive labeling ([^{3}H]hypoxanthine) is of obvious advantage, while the utility of the pLDH assay lies in its speed and lower cost. In our case, the comparison serves to add a degree of assurance that the 11-aza compounds are approximately as active as artemisinin. However, in ranking the compounds according to activity (from lowest to highest), each method ranked the compounds in a significantly different order. While some measure of uncertainty undoubtedly arises from the different nature of the two assays and how they are conducted (such as differences in percent parasitemia and percent hematocrit), the small range of activities (<1 order of magnitude) may also be partly responsible. Therefore, testing of other analogs with considerably different activities is underway to further evaluate the extent of correlation between the two procedures.

A number of interesting scenarios emerge in analyzing the log P data. In general, the calculated values fluctuated in terms of accuracy but demonstrated essentially the same trend (as observed values) in lipophilicity. Interestingly, every calculated value was low, although the notice "possibly underpredicted" appeared in the calculation details. A partial explanation for this resides in the fact that the 3-dimensionality of the compounds is not taken into account, and therefore polar fragment interactions (especially through conformational restrictions) can be underestimated. Not surprisingly, the data did not provide any immediately evident correlation between log P and *in vitro* activity; admittedly, any relationship found may not hold true *in vivo*, since it would not take into account distribution, metabolism, or transport mechanisms thought to play a role *in vivo*. The possibility exists that there is no correlation between log P and activity, whether *in vitro or in vivo*—a log P value within a certain range may only fulfill one requirement of several that are necessary for activity. Nevertheless, a statistically significant trend could provide new information regarding SAR; currently, values for a large variety of artemisinin derivatives spanning a broad range of activity values are being determined, and statistical (multiple regression) analysis of the resulting data will be reported in due course.

These analogs uniquely contribute to our evolving CoMFA model[7,8] and aid our efforts to provide a cohesive SAR model for artemisinin and its analogs in order to design improved, effective antimalarials.

Experimental Section

All solvents were purchased as HPLC grade and where appropriate were distilled from CaH_2 prior to use. Solvent and reagent transfers were accomplished via dried syringe, and all reactions were routinely conducted under an inert atmosphere, unless otherwise indicated. Flash chromatography was accomplished using silica gel (Whatman 60, 230–400

mesh). Preparative thin-layer chromatography utilized 1-, 1.5-, or 2-mm thick Analtech Uniplates with F-256, and 250-μm silica gel thin-layer chromatography plates were also purchased from Analtech. Unless otherwise noted, all NMR analyses were conducted in $CDCl_3$, on a Varian VXR-300 spectrometer, and referenced to chloroform at δ 7.27. IR spectra were recorded on a Digilab FTS-40 or Perkin-Elmer 1610 FTIR spectrometer. MS were obtained on a VG 7070E-HF or Reibermag R-10-10-C spectrometer. Elemental analyses are within ±0.4% as determined by Desert Analytics, Tucson, AZ. Determination of log *P* values was as follows: Partition coefficient values for each compound were estimated from corresponding HPLC retention indices[36,37] as was done for similar compounds by Ramu and Baker.[38] A Waters HPLC LC1 module equipped with a 150-cm ODS column (5 μm) was used for the analysis with a mobile phase of 50% methanol in water and a flow rate of 1 mL/min. A wavelength of 330 nm was used to assay the ketone standards, and a wavelength of 216 nm was used for the artemisinin derivatives. Samples were analyzed in triplicate.

General Procedure for Formation of Ketal Amides 6–11 and 13–16. To a stirred solution of ketal acid **2** (250 mg, 1.0 mmol) in CH_2Cl_2 (15 mL) was added Et_3N (2.2 equiv). After cooling to 0 °C, ethyl chloroformate (1.1 equiv) was added dropwise and the mixture stirred for 20 min. Then desired amine (1.5 equiv) was added dropwise, and the mixture was allowed to warm to ambient temperature and stir for 6–8 h. A solution of 10% HCl/saturated aqueous NH_4Cl (5 mL, 1:15, v/v) was added and the mixture stirred for 5 min. The CH_2Cl_2 layer was separated, dried with $MgSO_4$, and concentrated *in vacuo* to yield the crude desired amide, which was purified by flash column chromatography with ethyl acetate/hexanes as eluent.

General Procedure for Formation of *N*-Alkyl-11-aza-9-desmethylartemisinin Analogs. To a stirred solution of ketal amide (~0.4 mmol) in 40 mL of CH_2Cl_2 at −78 °C was bubbled O_3/O_2 (4 psi, 0.04 L/min, 80 V) until a light blue color persisted. The mixture was purged with O_2 (20 min) and then with N_2 (20 min). SiO_2 (~4.0 g) and 15% H_2SO_4 (200 μL) were added, and the mixture was allowed to warm to ambient temperature. After 2–3 days, the solids were filtered off and washed with CH_2Cl_2 (3 × 20 mL) and ethyl acetate (1 × 20 mL). The resultant filtrate was washed with saturated aqueous $NaHCO_3$ (1 × 25 mL) and saturated aqueous NaCl (1 × 25 mL), dried over $MgSO_4$, and concentrated *in vacuo* to provide the crude product. Purification via flash column chromatography or preparative TLC with ether or ethyl acetate/hexanes as eluent provided the pure product.

(1″*S*,3″*S*,5″*R*)-2-[[3″-[*N*-(1-Carboxy-5-pentyl)-2‴-acetamido]-6-methyl-(2″*Z*)-[(trimethylsilyl)methylene]cyclohexyl]ethyl]-2,5,5-trimethyl-1,3-dioxane (12). To a stirred solution of ketal acid **2** (0.83 g, 2.09 mmol) in dry CH_2Cl_2 (30 mL) at 0 °C under Ar was added Et_3N (0.7 mL, 2.2 equiv). After 10 min, ethyl chloroformate (240 μL) was added. After stirring for 1 h at 0 °C, 6-aminohexanoic acid (400 mg), Et_3N (2 mL), and DMF (3 mL) were sequentially added. After 6 h, DMAP (10 mg) was added. After stirring overnight, a solution of 10% HCl/saturated aqueous NH_4Cl (5 mL, 1:15, v/v) was added. After 5 min, the CH_2Cl_2 layer was separated, dried with $MgSO_4$, and concentrated *in vacuo* to yield the crude desired amide (40%), which was purified by flash column chromatography with 1% HOAc/50% ethyl acetate/hexanes as eluent.

(1″*S*,3″*S*,5″*R*)-2-[[3″-(*N*-Succinimido-2‴-acetoxy)-6-methyl-(2″*Z*)-[(trimethylsilyl)methylene]cyclohexyl]ethyl]-2,5,5-trimethyl-1,3-dioxane (4). To a solution of ketal acid **2** (0.216 g, 0.54 mmol) in 5 mL of CH_2Cl_2 was added DCC (0.125 g, 0.60 mmol) and *N*-hydroxysuccinimide (0.07 g, 0.60 mmol). The reaction mixture was allowed to stir overnight. It was filtered, washed with CH_2Cl_2, and concentrated *in vacuo* to give the crude product which was purified by flash column chromatography. Elution with 30:70 EtOAc/hexanes afforded 0.212 g (81%) of pure product as an oil.

(1″*S*,3″*S*,5″*R*)-2-[[3″-(*N*-Methyl-2‴-acetamido)-6-methyl-(2″*Z*)-[(trimethylsilyl)methylene]cyclohexyl]ethyl]-2,5,5-trimethyl-1,3-dioxane (5). To a solution of ester **4** (212 mg, 0.44 mmol) in MeOH (5 mL) was added 40% aqueous $MeNH_2$ (3 mL). The reaction mixture was allowed to stir at ambient temperature for 2 h, concentrated *in vacuo*, and extracted with EtOAc (2 × 30 mL). The organic extracts were washed with water, dried over Na_2SO_4, and concentrated *in vacuo* to give the crude product which was purified by flash column chromatography eluting with 60:40 EtOAc/hexanes to provide 0.158 g (87%) of the pure product as an oil.

(−)-Octahydro-3,6-dimethyl-3,12-epoxy-11-(carboxymethyl)-12*H*-pyridino[4,3-*j*]-1,2-benzodioxepin-10(3*H*)-one (23). To a solution of *tert*-butyl ester **25** (135 mg, 0.354 mmol) in 10 mL of CH_2Cl_2 was added 0.50 mL of trifluoroacetic acid. After 3 h at ambient temperature, the resultant solution was washed with H_2O (4 × 20 mL) and brine (25 mL), dried over Na_2SO_4, and concentrated under reduced pressure to a white solid, which recrystallized from EtOAc/hexanes to provide 47 mg (41%) of white cubic prisms in successive crops.

(1″*S*,3″*S*,5″*R*)-2-[[3″-(*N*-Succinimido-2‴-acetoxy)-6-methyl-(2″*Z*)-[(trimethylsilyl)methylene]cyclohexyl]ethyl]-2,5,5-trimethyl-1,3-dioxane (4): ^{1}H NMR (400 MHz) δ 5.46 (s, 1H), 3.52–3.59 (m, 2H), 3.36–3.48 (m, 2H), 2.66–2.95 (br m, 7H), 2.14 (m, 1H), 1.48–1.98 (br m, 9H), 1.36 (s, 3H), 1.02 (s, 3H), 0.94 (d, 3H, J = 7.0 Hz), 0.11 (s, 9H).

(1″*S*,3″*S*,5″*R*)-2-[[3″-(*N*-Methyl-2‴-acetamido)-6-methyl-(2″*Z*)-[(trimethylsilyl)methylene]cyclohexyl]ethyl]-2,5,5-trimethyl-1,3-dioxane (5): mp 78–80 °C; ^{1}H NMR (300 MHz) δ 6.10 (br s, 1H), 5.43 (s, 1H), 3.60–3.71 (m, 2H), 3.37–3.45 (m, 2H), 2.90 (m, 1H), 2.74 (d, 3H, J = 4.8 Hz), 2.38–2.48 (m, 2H), 2.22–2.31 (m, 2H), 2.12–2.20 (m, 1H), 1.61–1.98 (m, 9H), 1.40 (s, 3H), 1.11 (s, 3H), 0.96 (d, 3H, J = 7.0 Hz), 0.86 (s, 3H), 0.09 (s, 9H).

(1″*S*,3″*S*,5″*R*)-2-[[3″-(*N*-Propyl-2‴-acetamido)-6-methyl-(2″*Z*)-[(trimethylsilyl)methylene]cyclohexyl]ethyl]-2,5,5-trimethyl-1,3-dioxane (6): prepared from *n*-propylamine; pure product as a yellow oil (81%); $[\alpha]^{22}_D$ = +39.9° (c = 5.17, CH_2Cl_2); ^{1}H NMR (400 MHz) δ 6.06 (br s, 1H), 5.42 (s, 1H), 3.62 (dd, 2H, J = 5.0, 11.4 Hz), 3.41 (dt, 2H, J = 1.9, 11.4 Hz), 3.09–3.25 (m, 2H), 2.83–2.91 (m, 1H), 2.44 (dd, 2H, J = 9.2, 13.6 Hz), 2.26 (dd, 1H, J = 6.4, 13.6 Hz), 2.11–2.17 (m, 1H), 1.60–1.75 (m, 8H), 1.40–1.51 (m, 3H), 1.39 (s, 3H), 1.09 (s, 3H), 0.88–0.98 (m, 6H), 0.86 (s, 3H), 0.08 (s, 9H); IR 3300, 2980, 2965, 2880, 1655, 1255, 860, 750 cm^{-1}; EIMS m/z (rel intensity) 437 (10), 422 (21), 281 (78), 129 (83), 73 (100).

(1″*S*,3″*S*,5″*R*)-2-[[3″-[*N*-(2‴-Methylpropyl)-2‴-acetamido]-6-methyl-(2″*Z*)-[(trimethylsilyl)methylene]cyclohexyl]ethyl]-2,5,5-trimethyl-1,3-dioxane (7): prepared from isobutylamine; pure product as a yellow oil (84%); ^{1}H NMR (300 MHz) δ 6.14 (br s, 1H), 5.19 (s, 1H), 3.36 (dd, 2H, J = 3.4, 11 Hz), 3.16 (d, 2H, J = 13 Hz), 2.60–2.91 (m, 3H), 2.02–2.27 (m, 2H), 1.92 (br s, 1H), 1.20–1.71 (m, 8H), 1.16 (s, 4H), 0.84 (s, 3H), 0.59–0.76 (m, 13H), −0.15 (s, 9H); ^{13}C NMR (75 MHz) δ 171.9, 159.0, 128.6, 98.5, 70.0, 69.9, 47.5, 46.5, 43.9, 42.9, 38.0, 32.9, 29.6, 29.1, 28.2, 26.0, 22.9, 22.5, 22.0, 20.6, 19.9, 19.5, 19.5, −0.5; IR (neat) 3305, 3081, 2955, 2872, 1650, 1470, 1212, 1086, 916, 850, 733 cm^{-1}; DCIMS-NH_3 m/z 452 (M + H$^+$), 392.

(1″*S*,3″*S*,5″*R*)-2-[[3″-[*N*-Pentyl-2‴-acetamido)-6-methyl-(2″*Z*)-[(trimethylsilyl)methylene]cyclohexyl]ethyl]-2,5,5-trimethyl-1,3-dioxane (8): prepared from *n*-amylamine; pure product as a yellow oil (91%); ^{1}H NMR (300 MHz) δ 5.97 (br s, 1H), 5.34 (s, 1H), 3.53 (dd, 2H, J = 3.0, 11.4 Hz), 3.35 (d, 2H, J = 12 Hz), 3.10 (m, 2H), 2.80 (br s, 1H), 1.95–2.42 (m, 3H), 0.73–1.91 (m, 30H), 0.015 (s, 9H); ^{13}C NMR (75 MHz) δ 172.2, 158.7, 129.4, 98.8, 47.6, 44.3, 43.3, 39.4, 38.4, 33.5, 29.9, 29.4, 29.4, 29.1, 26.5, 23.2, 22.8, 22.3, 22.3, 19.9, 19.8, 13.9, 0.8; IR (neat) 3292, 3087, 2958, 2868, 1643, 1510 cm^{-1}; LRFAB MS m/z 466 (M + H$^+$), 380, 310, 157, 129.

(1″*S*,3″*S*,5″*R*)-2-[[3″-[*N*-(3‴-Methylbutyl)-2‴-acetamido]-6-methyl-(2″*Z*)-[(trimethylsilyl)methylene]cyclohexyl]ethyl]-2,5,5-trimethyl-1,3-dioxane (9): prepared from isoamylamine; pure product as a yellow oil (78%); ^{1}H NMR (300 MHz) δ 6.05 (br s, 1H), 5.24 (s, 1H), 3.43 (d, 2H, J = 11.5 Hz), 3.22 (d, 2H, J = 10.9 Hz), 3.02 (q, 2H, J = 7 Hz), 2.71 (br s, 1H), 1.93–2.31 (m, 3H), 1.31–1.80 (m, 8H), 1.22 (s, 4H), 0.91 (s, 3H), 0.64–0.82 (m, 15H), 0.09 (s, 9H); ^{13}C NMR (75 MHz) δ 171.9, 156.6, 129.1, 98.5, 70.1, 70.0, 47.5, 44.1, 43.0, 38.4,

38.2, 37.4, 33.3, 29.6, 29.1, 26.3, 25.6, 23.0, 22.7, 22.3, 22.3, 22.1, 19.6, 0.9; IR (neat) 3302, 3087, 2954, 2869, 1650, 1247, 1122, 853, 787 cm^{-1}; FABMS *m/z* 466 (M + H^+), 380, 310, 157, 129.

(1″*S*,3″*S*,5″*R*)-2-[[3″-[*N*-[(*N*,*N*′-Dimethylamino)ethyl]-acetamido]-6-methyl-(2″*Z*)-[(trimethylsilyl)methylene]-cyclohexyl]ethyl]-2,5,5-trimethyl-1,3-dioxane (10): prepared from *N*,*N*-dimethylethylenediamine; product as a colorless oil (71%); ^1H NMR (400 MHz) δ 6.19 (br m, 1H), 5.41 (s, 1H), 3.58 (d, 2H, J = 10.8 Hz), 3.43 (d, 2H, J = 10.8 Hz), 3.31 (ddd, 2H, J = 5.9, 5.9, 13.0 Hz), 2.36 (m, 3H), 2.23 (s, 6H), 2.00–2.17 (m, 1H), 1.45–1.95 (m, 7H), 1.06 (s, 3H), 0.89–1.05 (m, 6H), 0.88 (s, 3H), 0.09 (s, 9H); IR 3300, 2960, 2860, 2820, 2770, 1645, 1605, 1550, 1465, 1380, 1253, 1220, 1200, 1120, 1100, 1045, 855 cm^{-1}; EIMS *m/z* (rel intensity) 466 (1), 396 (6), 129 (10), 71 (70), 58 (100).

(1″*S*,3″*S*,5″*R*)-2-[[3″-[*N*-(*tert*-Butylacetoxy)acetamido]-6-methyl-(2″*Z*)-[(trimethylsilyl)methylene]cyclohexyl]-ethyl]-2,5,5-trimethyl-1,3-dioxane (11): obtained from glycine *tert*-butyl ester; $[\alpha]^{22}_D$ = +40.3° (c = 16.2, CH_2Cl_2); ^1H NMR (400 MHz) δ 6.33 (br m, 1H), 5.42 (s, 1H), 4.01 (dd, 1H, J = 4.7, 18.3 Hz), 3.79 (dd, 1H, J = 4.7, 18.3 Hz), 3.60 (d, 2H, J = 11.5 Hz), 3.44 (ddd, 2H, J = 1.6, 6.3, 11.5 Hz), 2.82–2.90 (br m, 1H), 2.46 (dd, 1H, J = 8.1, 13.9 Hz), 2.39 (dd, 1H, J = 8.1, 13.9 Hz), 2.15 (br t, 1H, J = 6.9 Hz), 1.56–1.94 (m, 8H), 1.47 (s, 9H), 1.37 (s, 3H), 1.12–1.20 (br m, 1H), 1.07 (s, 3H), 0.93 (d, J = 7.0 Hz), 0.87 (s, 3H), 0.08 (s, 9H); IR 3310, 2950, 2870, 1745, 1655, 1605, 1525, 1455, 1370, 1250, 1165, 1120, 1090, 1043, 917, 855, 735 cm^{-1}; EIMS *m/z* (rel intensity) 509 (7), 494 (10), 438 (11), 366 (10), 353 (42), 297 (42), 129 (100).

(1″*S*,3″*S*,5″*R*)-2-[[3″-[*N*-(1-Carboxy-5-pentyl)-2‴-acetamido]-6-methyl-(2″*Z*)-[(trimethylsilyl)methylene]cyclohexyl]ethyl]-2,5,5-trimethyl-1,3-dioxane (12): ^1H NMR (400 MHz) δ 5.41 (s, 1H), 3.59 (dd, 2H, J = 3.6, 11.3 Hz), 3.47 (m, 2H), 3.25 (quintet, 2H, J = 7.0 Hz), 2.86 (m, 2H), 2.36 (m, 4H), 2.14 (m, 1H), 1.45–1.95 (m, 11H), 1.41 (s, 3H), 1.10–1.40 (m, 5H), 1.05 (s, 3H), 0.93 (d, 3H, J = 7.0 Hz), 0.91 (s, 3H), 0.82 (s, 9H); IR (neat) 3318, 2954, 2867, 1713, 1644, 1552, 1246, 1093, 851, 747, 688 cm^{-1}; DCIMS-NH_3 *m/z* 510 (M + H^+), 424, 406, 354, 334, 301, 174, 157, 129.

(1″*S*,3″*S*,5″*R*)-2-[[3″-(*N*-Benzyl-2‴-acetamido)-6-methyl-(2″*Z*)-[(trimethylsilyl)methylene]cyclohexyl]ethyl]-2,5,5-trimethyl-1,3-dioxane (13): prepared from benzylamine; pure product as a yellow oil (95%); $[\alpha]^{22}_D$ = +73.9° (c = 2.90, CH_2Cl_2); ^1H NMR (400 MHz) δ 7.22–7.36 (m, 5H), 6.52 (m, 1H), 5.47 (s, 1H), 4.42 (ddd, 1H, J = 5.3, 14.6, 14.6 Hz), 4.37 (ddd, 1H, J = 5.3, 14.6, 14.6 Hz), 3.54 (d, 1H, J = 11.4 Hz), 3.40 (d, 1H, J = 11.4 Hz), 3.34 (dd, 1H, J = 1.7, 11.4 Hz), 3.23 (dd, 1H, J = 1.8, 11.2 Hz), 2.90–2.98 (m, 1H), 2.50–2.64 (m, 1H), 2.31 (dd, 1H, J = 6.0, 13.7 Hz), 2.12–2.18 (m, 1H), 1.56–2.01 (m, 7H), 1.44 (d, H, J = 13.3 Hz), 1.09–1.23 (m, 4H), 1.05 (s, 3H), 0.94 (d, 3H, J = 6.9 Hz), 0.78 (s, 3H), 0.09 (s, 9H); IR (CH_2Cl_2) 3450, 3330, 2960, 2875, 1660, 1510, 1460, 1380, 1218, 1117, 1088, 933, 860 cm^{-1}; EIMS *m/z* (rel intensity) 485 (13), 470 (20), 329 (88), 91 (100).

(1″*S*,3″*S*,5″*R*)-2-[[3″-[*N*-[(4-Chlorophenyl)methyl]-2‴-acetamido]-6-methyl-(2″*Z*)-[(trimethylsilyl)methylene]-cyclohexyl]ethyl]-2,5,5-trimethyl-1,3-dioxane (14): prepared from *p*-chlorobenzylamine; pure product as a yellow oil (82%); ^1H NMR (300 MHz) δ 7.11 (d, 2H, J = 8.4 Hz), 7.04 (d, 2H, J = 8.4 Hz), 6.69 (br s, 1H), 5.29 (s, 1H), 4.19 (m, 2H), 3.34 (dd, 2H, J = 11.1, 13.0 Hz), 3.15 (dd, 2H, J = 11.4, 12.1 Hz), 2.77 (br s, 1H), 2.11–2.42 (m, 2H), 1.94–2.05 (m, 1H), 1.17–1.90 (m, 5H), 1.05 (s, 4H), 0.90 (s, 3H), 0.80 (d, 3H, J = 6.8 Hz), 0.65 (s, 6H), −0.04 (s, 9H); ^{13}C NMR (75 MHz) δ 172.1, 156.4, 137.1, 132.7, 129.4, 129.0, 128.4, 98.6, 70.0, 69.9, 47.4, 44.2, 42.8, 42.4, 38.2, 33.5, 29.6, 29.0, 26.6, 23.0, 22.6, 22.0, 19.5, 19.3, 0.7; IR (neat) 3298, 3069, 2959, 2872, 1651, 1247, 1096, 1019, 915, 848, 732 cm^{-1}; FABMS *m/z* 520 (M + H^+), 434, 364, 235.

(1″*S*,3″*S*,5″*R*)-2-[[3″-[*N*-(2‴-Phenylethyl)-2‴-acetamido]-6-methyl-(2″*Z*)-[(trimethylsilyl)methylene]cyclohexyl]-ethyl]-2,5,5-trimethyl-1,3-dioxane (15): prepared from phenethylamine; pure product as a yellow oil (78%); ^1H NMR (300 MHz) δ 7.01–7.19 (m, 5H), 6.25 (br s, 1H), 5.29 (s, 1H), 3.06–3.47 (m, 6H), 2.55–2.80 (m, 3H), 2.11–2.30 (m, 2H), 2.02 (br s, 1H), 1.48–1.78 (m, 7H), 1.30 (m, 1H), 1.25 (s, 3H), 1.04 (m, 1H), 0.91 (s, 3H), 0.82 (d, 3H, J = 6.8 Hz), 0.69 (s, 3H), −0.04 (s, 9H); ^{13}C NMR (75 MHz) δ 172.0, 158.7, 138.9, 128.9, 128.4, 128.2, 126.0, 98.5, 70.0, 69.9, 47.4, 44.0, 42.9, 40.4, 38.1, 35.7, 33.2, 29.6, 29.0, 25.9, 22.9, 22.5, 22.0, 19.5, −0.6; IR (neat) 3307, 3063, 3030, 2956, 2869, 1648, 1379, 1251, 1094, 917, 850, 737 cm^{-1}; DCIMS-NH_3 *m/z* 500 (M + H^+), 452, 386, 324.

(1″*S*,3″*S*,5″*R*)-2-[[3″-[*N*-(3‴-Phenylpropyl)-2‴-acetamido]-6-methyl-(2″*Z*)-[(trimethylsilyl)methylene]cyclohexyl]ethyl]-2,5,5-trimethyl-1,3-dioxane (16): prepared from 3-phenylpropylamine; pure product as a yellow oil (80%); ^1H NMR (300 MHz) δ 7.01–7.23 (m, 5H), 6.09 (br s, 1H), 5.32 (s, 1H), 3.50 (d, 2H, J = 12 Hz), 3.32 (d, 2H, J = 12 Hz), 3.12 (m, 2H), 2.78 (m, 1H), 2.53, (t, 2H, J = 7 Hz), 2.11–2.38 (m, 2H), 2.05 (m, 1H), 1.55–1.83 (m, 11H), 1.27 (s, 3H), 0.98 (s, 3H), 0.84 (d, 3H, J = 6.8 Hz), 0.74 (s, 3H), −0.02 (s, 9H); ^{13}C NMR (75 MHz) δ 172.1, 158.8, 141.4, 129.2, 128.2, 128.2, 125.8, 98.7, 70.2, 70.1, 47.6, 44.2, 43.1, 38.8, 38.2, 33.3, 33.2, 31.4, 29.8, 29.3, 26.3, 23.1, 22.7, 22.2, 19.8, 19.7, −0.7; IR (neat) 3302, 3085, 3070, 3034, 2957, 2866, 1651, 1606, 1461, 1376, 1255, 1096, 909, 858, 741 cm^{-1}; DCIMS-NH_3 *m/z* 514 (M + H^+), 452, 392, 324.

(−)-Octahydro-3,6-dimethyl-3,12-epoxy-11-methyl-12*H*-pyridino[4,3-*j*]-1,2-benzodioxepin-10(3*H*)-one (17): $[\alpha]^{22}_D$ = −12.2° (c = 1.17, $CHCl_3$); ^1H NMR (400 MHz) δ 5.19 (s, 1H), 3.11 (m, 2H), 2.97 (s, 3H), 2.41 (m, 2H), 1.37-2.06 (br m, 7H), 1.36 (s, 3H), 1.02–1.34 (br m, 2H), 0.99 (d, 3H, J = 6.1 Hz); ^{13}C NMR (75 MHz) δ 168.7, 104.7, 79.9, 79.5, 51.3, 39.1, 37.9, 36.5, 34.0, 33.5, 29.0, 28.9, 25.3, 25.0, 19.8; IR ($CHCl_3$) 3005, 2940, 2880, 1640, 1455, 1410, 1385, 1330, 1295, 1260, 1160, 1150, 1095, 1035, 950, 895, 870 cm^{-1}; DCIMS-NH_3 *m/z* 299 (M + NH_4^+), 282 (M + H^+), 264, 240, 222.

(−)-Octahydro-3,6-dimethyl-3,12-epoxy-11-propyl-12*H*-pyridino[4,3-*j*]-1,2-benzodioxepin-10(3*H*)-one (18): crystallized from EtOAc/hexanes (24%); mp 125.0–125.5 °C; $[\alpha]^{22}_D$ = −15.7° (c = 0.890, $CHCl_3$); ^1H NMR (400 MHz) δ 5.28 (s, 1H), 3.58 (ddd, 1H, J = 6.1, 10.0, 13.2 Hz), 3.41 (ddd, 1H, J = 5.3, 10.0, 13.2 Hz), 3.14 (dd, 1H, J = 6.0, 17.5 Hz), 2.37–2.47 (m, 1H), 2.12 (dd, 1H, J = 1.3, 17.4 Hz), 1.95–2.07 (m, 2H), 1.40–1.79 (m, 7H), 1.39 (s, 3H), 1.20–1.37 (m, 2H), 1.04–1.16 (m, 1H), 1.01 (d, 3H, J = 6.2 Hz), 0.93 (t, 3H, J = 7.4 Hz); IR (CH_2Cl_2) 2940, 1640, 1065, 1045 cm^{-1}; CIMS (NH_3) *m/z* (rel intensity) 327 (M + NH_4^+, 12), 310 (M + H^+, 100).

(−)-Octahydro-3,6-dimethyl-3,12-epoxy-11-(2′-methylpropyl)-12*H*-pyridino[4,3-*j*]-1,2-benzodioxepin-10(3*H*)-one (19): crystallized from EtOAc/hexanes; yield 61%; mp 127.5–129 °C; $[\alpha]^{25}_D$ = −9.5° (*c* = 0.515, $CHCl_3$); ^1H NMR (300 MHz) δ 5.22 (s, 1H), 3.61 (dd, 1H, J = 8.8, 13.4 Hz), 3.21 (dd, 1H, J = 6.6, 13.4 Hz), 3.09 (dd, 1H, J = 6.1, 17.5 Hz), 2.35 (dt, 1H, J = 4.0, 14.0 Hz), 1.57–2.13 (m, 8H), 1.33–1.56 (m, 2H), 1.32 (s, 3H), 0.96–1.31 (m, 2H), 0.95 (d, 3H, J = 6.8 Hz), 0.89 (d, 3H, J = 6.8 Hz), 0.83 (d, 3H, J = 6.8 Hz); ^{13}C NMR (75 MHz) δ 168.9, 104.7, 80.0, 78.2, 51.5, 47.9, 39.1, 38.0, 36.8, 34.0, 33.8, 29.1, 26.0, 25.3, 25.0, 20.5, 20.3, 19.8; IR (neat) 2958, 2929, 2872, 1661, 1252, 1024, 957, 890 cm^{-1}; DCIMS-NH_3 *m/z* 324 (M + H^+).

(−)-Octahydro-3,6-dimethyl-3,12-epoxy-11-pentyl-12*H*-pyridino[4,3-*j*]-1,2-benzodioxepin-10(3*H*)-one (20): yield 51%; $[\alpha]^{25}_D$ = −24.9° (c = 0.438, $CHCl_3$); ^1H NMR (300 MHz) δ 5.15 (s, 1H), 3.20–3.58 (m, 2H), 3.04 (dd, 1H, J = 6.0, 18.0 Hz), 2.34 (dt, 1H, J = 4.0, 14.0 Hz), 1.33–2.08 (m, 18H), 1.32 (s, 3H), 0.95–1.31 (m, 3H), 0.92 (d, 3H, J = 6.8 Hz), 0.73–0.91 (m, 4H); ^{13}C NMR (75 MHz) δ 168.2, 104.6, 79.7, 78.1, 51.4, 41.8, 39.3, 37.9, 36.6, 33.9, 33.7, 29.3, 28.8, 26.8, 25.4, 25.0, 22.4, 19.8, 14.0; IR (neat) 2924, 2866, 1654, 1464, 1252, 1137, 1023, 946, 836, 728 cm^{-1}; FABMS *m/z* 338 (M + H^+), 322, 305, 234, 165, 137.

(−)-Octahydro-3,6-dimethyl-3,12-epoxy-11-(3′-methylbutyl)-12*H*-pyridino[4,3-*j*]-1,2-benzodioxepin-10(3*H*)-one (21): yield 65%; ^1H NMR (300 MHz) δ 5.15 (s, 1H), 3.23–3.58 (m, 2H), 3.02 (dd, 1H, J = 6.1, 17.5 Hz), 2.30 (dt, 1H, J = 4.0, 14.0 Hz), 1.31–2.05 (m, 9H), 1.30 (s, 3H), 0.92-1.29 (m, 5H), 0.91 (d, 3H, J = 6.8 Hz), 0.83 (d, 6H, J = 6.8 Hz); ^{13}C NMR (75 MHz) δ 168.0, 104.5, 79.7, 77.9, 51.4, 40.2, 39.2, 37.8, 36.6, 35.8, 33.8, 33.6, 28.7, 26.4, 25.4, 24.9, 22.5,

19.7; IR (neat) 2957, 2929, 2876, 1657, 1467, 1188, 989, 810, 732, 658 cm^{-1}; FABMS m/z 338 (M + H^+), 322, 305, 294, 278, 261.

(−)-Octahydro-3,6-dimethyl-3,12-epoxy-11-[2′-(dimethylamino)ethyl]-12*H*-pyridino[4,3-*j*]-1,2-benzodioxepin-10(3*H*)-one (22): $[\alpha]^{22}_D = -16.4°$ (c = 5.54, CH_2Cl_2); ^1H NMR (400 MHz) δ 5.45 (s, 1H), 3.77 (ddd, 1H, J = 5.1, 7.7, 13.5 Hz), 3.56 (quintet, 1H, J = 6.8 Hz), 3.13 (dd, 1H, J = 6.0, 17.4 Hz), 2.61−2.70 (m, 1H), 2.35−2.51 (m, 2H), 2.28 (s, 6H), 2.11 (dd, 1H, J = 1.4, 17.4 Hz) 1.94−2.06 (m, 2H), 1.60−1.79 (m, 3H), 1.30−1.54 (m, 7H), 1.03−1.18 (m, 1H), 1.00 (d, 3H, J = 6.2 Hz); ^{13}C NMR (100 MHz) δ 167.2, 119.2, 103.3, 91.9, 78.5, 77.5, 55.6, 50.1, 44.2, 38.1, 37.6, 36.6, 35.3, 32.7, 32.4, 27.2, 23.7, 18.5; IR 2980, 2930, 1708, 1465, 1260, 1160, 1039 cm^{-1}; CIMS (NH_3) m/z (rel intensity) 339 (M + H^+,100), 323 (15), 307 (18), 281 (20), 240 (17), 117 (29).

(−)-Octahydro-3,6-dimethyl-3,12-epoxy-11-(carboxymethyl)-12*H*-pyridino[4,3-*j*]-1,2-benzodioxepin-10(3*H*)-one (23): mp 169−172 °C dec; $[\alpha]^{22}_D = -26°$ (c = 0.730, $CHCl_3$); ^1H NMR (400 MHz) δ 5.39 (s, 1H), 4.52 (d, 1H, J = 17.4 Hz), 4.21 (d, 1H, J = 17.4 Hz), 3.19 (dd, 1H, J = 6.2, 17.5 Hz), 2.43 (ddd, 1H, J = 3.6, 14.3, 14.6 Hz), 2.21 (dd, 1H, J = 1.2, 17.6 Hz), 1.95−2.08 (m, 2H), 1.83 (br dt, 1H, J = 4.5,13.7 Hz), 1.72 (ddd, 1H, J = 3.1, 6.9, 13.6 Hz), 1.68 (ddd, 1H, J = 3.1, 6.9, 13.6 Hz), 1.39--1.61 (m, 3H), 1.38 (s, 3H), 1.14 (dd, 1H, J = 3.7, 12.8 Hz), 1.07 (dd, 1H, J = 3.7, 12.8 Hz), 1.05 (d, 3H, J = 6.2 Hz); IR ($CHCl_3$) 3500−3150, 2945, 1725, 1648, 1265, 1165, 1138, 1038 cm^{-1}; CIMS (NH_3) m/z (rel intensity) 343 (M + NH_4^+, 5), 326 (M + H^+, 100), 240 (50).

(−)-Octahydro-3,6-dimethyl-3,12-epoxy-11-(1-carboxy-5-pentyl)-12*H*-pyridino[4,3-*j*]-1,2-benzodioxepin-10(3*H*)-one (24): mp 172−174 °C; $[\alpha]^{22}_D = -22.4°$ (c = 0.367, CH_2Cl_2); ^1H NMR (400 MHz) δ 5.27 (s, 1H), 3.56 (ddd, 1H, J = 5.9, 10.0, 13.2 Hz), 3.48 (ddd, 1H, J = 5.3, 9.7, 13.6 Hz), 3.14 (dd, 1H, J = 6.1, 17.4 Hz), 2.44 (dd, 1H, J = 3.9, 13.3 Hz), 2.37 (t, 2H, J = 7.5 Hz), 2.12 (dd, 1H, J = 1.1, 17.4 Hz), 1.95−2.07 (m, 2H), 1.39−1.80 (m, 13H), 1.38 (s, 3H), 1.02−1.37 (m, 1H), 1.00 (d, 3H, J = 6.2 Hz); IR (Nujol) 2919, 1722, 1606, 1460, 1376, 1196, 1012, 826, 764 cm^{-1}.

(−)-Octahydro-3,6-dimethyl-3,12-epoxy-11-(2′-*tert*-butylacetoxy)-12*H*-pyridino[4,3-*j*]-1,2-benzodioxepin-10(3*H*)-one (25): crystallized from EtOAc/hexanes (35%); mp 116−117 °C; $[\alpha]^{22}_D = -13.3°$ (c = 9.55, CH_2Cl_2); ^1H NMR (400 MHz) δ 5.43 (s, 1H), 4.52 (d, 1H, J = 17.4 Hz), 3.94 (d, 1H, J = 17.4 Hz), 3.16 (dd, 1H, J = 5.9, 17.4 Hz), 2.38−2.48 (m, 1H), 2.18 (dd, 1H, J = 1.3, 17.4 Hz), 1.94−2.07 (m, 2H), 1.63−1.84 (m, 4H), 1.43−1.56 (m, 15H), 1.30−1.42 (m, 4H), 1.02−1.16 (m, 1H), 1.00 (d, 3H, J = 6.3 Hz); IR (CH_2Cl_2) 2930, 1735, 1650, 1367, 1227, 1160, 1135, 1035 cm^{-1}; CIMS (NH_4^+) m/z (rel intensity) 399 (M + NH_4, 7), 382 (M + H^+, 30), 326 (100).

(−)-Octahydro-3,6-dimethyl-3,12-epoxy-11-benzyl-12*H*-pyridino[4,3-*j*]-1,2-benzodioxepin-10(3*H*)-one (26): crystallized from EtOAc/hexanes (25%); mp 177−179 °C; $[\alpha]^{22}_D$ = −3.0° (c = 0.500, CH_2Cl_2); ^1H NMR (400 MHz) δ 7.19−7.39 (m, 5H), 5.14 (s, 1H), 5.06 (d, 1H, J = 14.6 Hz), 4.60 (d, 1H, J = 14.6 Hz), 2.33−2.42 (m, 1H), 2.22 (dd, 1H, J = 1.4, 17.6 Hz), 1.89−2.01 (m, 2H), 1.75−1.82 (m, 1H), 1.57−1.69 (m, 3H), 1.17−1.47 (m, 4H), 1.15 (s, 3H), 0.97−1.12 (m, 1H), 0.94 (d, 3H, J = 6.2 Hz); IR (CH_2Cl_2) 2940, 1643, 1135, 1080, 1027, 917 cm^{-1}; CIMS (NH_3) m/z (rel intensity) 375 (M + NH_4^+, 5), 358 (M + H^+, 100).

(−)-Octahydro-3,6-dimethyl-3,12-epoxy-11-[(4-chlorophenyl)methyl]-12*H*-pyridino[4,3-*j*]-1,2-benzodioxepin-10(3*H*)-one (27): crystallized from EtOAc; yield 42%; mp 163.5−164.5 °C; $[\alpha]^{25}_D = +1.9°$ (c = 0.431, $CHCl_3$); ^1H NMR (300 MHz, acetone-d_6) δ 7.31 (s, 4H), 5.35 (s, 1H), 4.69 (s, 2H), 3.02 (dd, 1H, J = 6.0, 16.0 Hz), 2.80 (d, 2H, J = 9 Hz), 2.29 (dt, 1H, J = 4.0, 14.0 Hz), 1.58−2.01 (m, 4H), 1.02−1.49 (m, 5H), 1.01 (s, 3H), 0.93 (d, 3H, J = 6.0 Hz); ^{13}C NMR (75 MHz) δ 168.6, 138.6, 132.8, 130.6, 128.9, 105.4, 80.8, 79.4, 52.3, 45.4, 40.1, 38.4, 37.4, 34.6, 34.3, 25.7, 25.1, 20.0; IR (neat) 3059, 2948, 2921, 2864, 1653, 1495, 1133, 1022, 993, 801, 766, 658 cm^{-1}; DCIMS-NH_3 m/z 392 (M + H^+), 331.

(−)-Octahydro-3,6-dimethyl-3,12-epoxy-11-(2′-phenylethyl)-12*H*-pyridino[4,3-*j*]-1,2-benzodioxepin-10(3*H*)-one (28): crystallized from hexanes (83%); mp 101.0−102.0 °C; $[\alpha]^{25}_D = +8.9°$ (c = 0.392, $CHCl_3$); ^1H NMR (300 MHz) δ 7.16 (m, 5H), 4.90 (s, 1H), 3.78 (m, 1H), 3.62 (m, 1H), 2.96 (m, 2H), 2.93 (m, 1H), 2.25 (dt, 1H, J = 4.0, 14.0 Hz), 1.76−2.00 (m, 3H), 1.26−1.62 (m, 4H), 1.27 (s, 3H), 0.84 (m, 4H), 0.83 (d, 3H, J = 5.4 Hz); ^{13}C NMR (75 MHz) δ 168.2, 139.2, 128.7, 128.2, 126.0, 79.5, 78.2, 78.0, 51.0, 42.4, 39.9, 37.4, 36.3, 33.6, 33.4, 28.2, 25.3, 25.2, 24.7, 19.5; IR (neat) 2931, 2876, 1651, 1460, 1249, 1023, 948, 808, 746 cm^{-1}; DCIMS-NH_3 m/z 372 (M + H^+).

(−)-Octahydro-3,6-dimethyl-3,12-epoxy-11-(3′-phenylpropyl)-12*H*-pyridino[4,3-*j*]-1,2-benzodioxepin-10(3*H*)-one (29): crystallized from petroleum ether (78%); mp 99.0−99.5 °C; $[\alpha]^{25}_D = -22.0°$ (c = 0.323, $CHCl_3$); ^1H NMR (300 MHz) δ 7.14 (m, 5H), 5.18 (s, 1H), 3.50 (m, 2H), 3.05 (dd, 1H, J = 6.1, 17.3 Hz), 2.58 (t, 2H, J = 7.0 Hz), 2.32 (dt, 1H, J = 4.0, 14.0 Hz), 1.30−2.07 (m, 11H), 1.29 (s, 3H), 0.92−1.28 (m, 2H), 0.91 (d, 3H, J = 6.9 Hz); ^{13}C NMR (75 MHz) δ 168.3, 141.7, 128.1, 125.7, 104.6, 79.7, 78.1, 51.3, 41.7, 39.2, 37.8, 36.5, 33.8, 33.6, 33.5, 28.9, 28.7, 25.4, 24.9, 19.7; IR (neat) 2926, 2870, 1658, 1648, 1450, 1252, 1133, 752, 698 cm^{-1}; DCIMS-NH_3 m/z 386 (M + H^+), 324.

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References

(1) Bray, P. G.; Ward, S. A. Malaria chemotherapy: resistance to quinoline containing drugs in *Plasmodium falciparum*. *FEMS Microbiol. Lett.* **1993**, *113*, 1−7.
(2) Oaks, S. C.; Mitchell, V. S.; Pearson, G. W.; Carpenter, C. C. J. *Malaria: obstacles and opportunities*; National Academy Press: Washington, DC, 1991; p 309.
(3) van Es, H. H.; Skamene, E.; Schurr, E. Chemotherapy of malaria: a battle against all odds? *Clin. Invest. Med.* **1993**, *16*, 285−293.
(4) Meshnick, S. R.; Marr, J. J. Intracellular parasites. New developments in chemotherapy. In *Subcellular Biochemistry. Volume 18: Intracellular Parasites*; Avila, J. L., Harris, J. R., Eds.; Plenum Press: New York, 1992; pp 401−441.
(5) White, N. J. Artemisinin: current status. *Trans. R. Soc. Trop. Med. Hyg.* **1994**, *88* (Suppl. 1), 3−4.
(6) Jung, M. Current developments in the chemistry of artemisinin and related compounds. *Curr. Med. Chem.* **1994**, *1*, 35−49.
(7) Avery, M. A.; Gao, F.; Chong, W. K. M.; Mehotra, S.; Milhous, W. K. Structure-activity relationships of the antimalarial agent artemisinin. 1. Synthesis and comparative molecular field analysis of C-9 analogs of artemisinin and 10-deoxoartemisinin. *J. Med. Chem.* **1993**, *36*, 4264−4275.
(8) Avery, M. A.; Gao, F.; Chong, W. K. M.; Mehrotra, S.; Jennings-White, C. The organic and medicinal chemistry of artemsinin and analogs. In *Trends in Organic Chemistry*; Trivandrum: India, 1993; Vol. 4, pp 413−468.
(9) Hien, T. T.; White, N. J. Qinghaosu. *Lancet* **1993**, *341*, 603−608.
(10) Li, G.-Q.; Guo, X.-B.; Fu, L.-C.; Jian, H.-X.; Wang, X.-H. Clinical trials of artemisinin and its derivatives in the treatment of malaria in China. *Trans. R. Soc. Trop. Med. Hyg.* **1994**, *88* (Suppl. 1), 5−6.
(11) Hien, T. T. An overview of the clinical use of artemisinin and its derivatives in the treatment of falciparum malaria in Viet Nam. *Trans. R. Soc. Trop. Med. Hyg.* **1994**, *88* (Suppl. 1), 7−8.
(12) Looareesuwan, S. Overview of clinical studies on artemisinin derivatives in Thailand. *Trans. R. Soc. Trop. Med. Hyg.* **1994**, *88* (Suppl. 1), 9−12.
(13) Salako, L. A.; Walker, O.; Sowunmi, A.; Omokhidion, S. J.; Adio, R.; Oduola, A. M. J. Artemether in moderately severe and cerebral malaria in Nigerian children. *Trans. R. Soc. Trop. Med. Hyg.* **1994**, *88* (Suppl. 1), 13−16.
(14) Lin, A. J.; Klayman, D. L.; Milhous, W. K. Antimalarial activity of a new water soluble dihydroartemisinin derivative. *J. Med. Chem.* **1987**, *30*, 2147−2150.
(15) Brewer, T. G.; Grate, S. J.; Peggins, J. O.; Weina, P. J.; Petras, J. M.; Levine, B. S.; Heiffer, M. H.; Schuster, B. G. Fatal neurotoxicity due to arteether (AE) and artemether (AM). *Am. J. Trop. Med. Hyg.* **1992**, *47* (Suppl.), 93.

(16) Brewer, T. G.; Peggins, J. O.; Grate, S. J.; Petras, J. M.; Levine, B. S.; Weina, P. J.; Swearengen, J.; Heiffer, M. H.; Schuster, B. G. Neurotoxicity in animals due to arteether and artemether. *Trans. R. Soc. Trop. Med. Hyg.* **1994**, *88* (Suppl. 1), 33–36.
(17) Posner, G. H.; Oh, C. H.; Wang, D.; Gerena, L.; Milhous, W. K.; Meshnick, S. R.; Asawamahasadka, W. Mechanism-based design, synthesis, and *in vitro* antimalarial testing of new 4-methylated trioxanes structurally related to artemisinin: the importance of a carbon-centered radical for antimalarial activity. *J. Med. Chem.* **1994**, *37*, 1256–1258.
(18) Meshnick, S. R. The mode of action of antimalarial endoperoxides. *Trans. R. Soc. Trop. Med. Hyg.* **1994**, *88*, 31–32.
(19) Meshnick, S. R.; Thomas, A.; Ranz, A.; Xu, C.-M.; Pan, H.-Z. Artemisinin (qinghaosu): the role of intracellular hemin in its mechanism of antimalarial action. *Mol. Biochem. Parasitol.* **1991**, *49*, 181–190.
(20) Asawamahasadka, W.; Ittarat, I.; Pu, Y. M.; Ziffer, H.; Meshnick, S. R. Reaction of antimalarial endoperoxides with specific parasite proteins. *Antimicrob. Agents Chemother.* **1994**, *38*, 1854–1858.
(21) Avery, M. A.; Chong, W. K. M.; Bupp, J. E. Tricyclic analogs of artemisinin: synthesis and antimalarial activity of (+)-4,5-secoartemisinin and (−)-5-nor-4,5-secoartemisinin. *J. Chem. Soc., Chem. Commun.* **1990**, 1487–1489.
(22) Imakura, Y.; Yokoi, T.; Yamagishi, T.; Koyama, J.; Hu, H.; McPhail, D. R.; McPhail, A. T.; Lee, K. H. Synthesis of desethanoqinghaosu, a novel analogue of the antimalarial qinghaosu. *J. Chem. Soc., Chem. Commun.* **1988**, 372–374.
(23) Imakura, Y.; Hachiya, K.; Ikemoto, T.; Kobayashi, S.; Yamashita, S.; Sakakibara, J.; Smith, F. T.; Lee, K. H. Antimalarial artemisinin analogs: synthesis of 2,3-desethano-12-deoxoartemisinin-related compounds. *Heterocycles* **1990**, *31*, 2125–2129.
(24) Avery, M. A.; Gao, F.; Chong, W. K. M.; Hendrickson, T. F.; Inman, W. D.; Crews, P. Synthesis, conformational analysis, and antimalarial activity of tricyclic analogs of artemisinin. *Tetrahedron* **1994**, *50*, 957–972.
(25) Jefford, C. W.; Velarde, J.; Bernardinelli, G. Synthesis of tricyclic arteannuin-like compounds. *Tetrahedron Lett.* **1989**, *30*, 4485–4488.
(26) Posner, G. H.; Oh, C. H.; Milhous, W. K. Olefin oxidative cleavage and dioxetane formation using triethylsilyl hydrotrioxide: applications to preparation of potent antimalarial 1,2,4-trioxanes. *Tetrahedron Lett.* **1991**, *32*, 4235–4238.
(27) Sharma, R. P.; Zaman, S. S. Some aspects of the chemistry and biological aspects of artemisinin and related antimalarials. *Heterocycles* **1991**, *32*, 1593–1638.
(28) Avery, M. A.; Jennings-White, C.; Chong, W. K. M. Simplified analogues of the antimalarial artemisinin: synthesis of 6,9-desmethylartemisinin. *J. Org. Chem.* **1989**, *54*, 1792–1795.
(29) Acton, N.; Klayman, D. Conversion of artemisinin (qinghaosu) to *iso*-artemisitene and to 9-*epi*-artemisinin. *Planta Med.* **1986**, 266–268.
(30) Avery, M. A.; Chong, W. K. M.; Jennings-White, C. Stereoselective total synthesis of (+)-artemisinin, the antimalarial constituent of *Artemisia annua L. J. Am. Chem. Soc.* **1992**, *114*, 974–979.
(31) Torok, D. S.; Ziffer, H. Synthesis and reactions of 11-azaartemisinin and derivatives. *Tetrahedron Lett.* **1995**, *36*, 829–832.
(32) Milhous, W. K.; Weatherly, N. F.; Bowdre, J. H.; Desjardins, R. E. In vitro activities of and mechanisms of resistance to antifolate antimalarial drugs. *Antimicrob. Agents Chemother.* **1985**, *27*, 525–530.
(33) Desjardins, R. E.; Canfield, C. J.; Haynes, D. E.; Chulay, J. D. Quantitative assessment of antimalarial activity *in vitro* by a a semiautomated microdilution technique. *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.
(34) Makler, M. T.; Ries, J. M.; Williams, J. A.; Bancroft, J. E.; Piper, R. C.; Gibbins, B. L.; Hinrichs, D. J. Parasite lactate dehydrogenase as an assay for *Plasmodium Falciparum* drug sensitivity. *Am. J. Trop. Med. Hyg.* **1993**, *48*, 739–741.
(35) Makler, M. T.; Hinrichs, D. J. Measurement of the lactate dehydrogenase activity of *Plasmodium Falciparum* as an assessment of parasitemia. *Am. J. Trop. Med. Hyg.* **1993**, *48*, 205–210.
(36) Baker, J. K.; Ma, C. Y. Retention index for liquid-liquid chromatography. *J. Chromatogr.* **1979**, 107–115.
(37) Baker, J. K. Estimation of high-performance liquid chromatographic retention indices of glucuronide metabolites. *J. Liq. Chromatogr.* **1981**, 271–278.
(38) Ramu, K.; Baker, J. K. Synthesis, characterization, and antimalarial activity of the glucuronides of the hydroxylated metabolites of arteether. *J. Med. Chem.* **1995**, *38*, 1911–1921.
(39) Daylight Software Release, v. 4.41; DCI Systems: Irvine, CA, 1995.

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