Nucleoside Conjugates. 7. Synthesis and Antitumor Activity of 1-β-D-Arabinofuranosylcytosine Conjugates of Ether Lipids[1]

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Three new 1-β-D-arabinofuranosylcytosine conjugates of ether lipids (alkyl glycerols) linked by a pyrophosphate diester bond have been prepared and evaluated against mouse leukemia L1210 and P388. These include *ara*-CDP-*rac*-1-*O*-hexadecyl-2-*O*-palmitoylglycerol (**9a**) (*ara*-CDP = 1-β-D-arabinofuranosylcytosine 5′-diphosphate), *ara*-CDP-*rac*-1-*O*-octadecyl-2-*O*-palmitoylglycerol (**9b**), and *ara*-CDP-*rac*-1-*O*-octadecyl-2-*O*-methylglycerol (**9c**). Among them, conjugate **9a** produced significant increase in life span (200–293%) in mice bearing ip and ic implanted L1210 lymphoic leukemia at a total dose of 400–500 mg (406–508 μmol/kg). Significant schedule dependence was not observed when the conjugate was given ip once daily on day 1, days 1, 5, and 9, and days 1–5. The new conjugates are water soluble by sonication.

Ether lipids (alkyl glycerols) occur widely in nature. A most interesting observation is that many tumors contain elevated concentrations of ether lipids as compared to normal tissues, since 1-*O*-alkyl cleavage enzymes are present in normal tissues, but characteristically absent from neoplastic cells.[2] However, incubation with certain metabolically stable ether lipid derivatives leads to progressive destruction of tumor cells.[3] 1-*O*-Alkyllysophospholipids, which are phospholipid derivatives of alkyl glycerols, and their synthetic analogues, e.g., *rac*-1-*O*-octadecyl-2-*O*-methylglycerophosphocholine (**1**) (Figure 1), have shown prophylactic and therapeutic effects on the growth and the metastasis of different animal tumors[4] and have shown immunomodulating activity.[5] They are also a new class of biological response modifier.[5] These results, coupled with the improved antitumor activities exhibited by 1-β-D-arabinofuranosylcytosine (*ara*-C)[6] conjugates of phosphatidic acids, e.g., *ara*-CDP-L-dipalmitin (**2**)[7-10] and *ara*-CDP-DL-dipalmitin (**3**)[11,12] (Figure 1), provided an excellent rationale for synthesis of *ara*-C conjugates of ether lipids.

As an extension of our previous work, this present paper describes the detailed synthetic procedures for new 1-*O*-alkyl analogues (**9a–c**) of *ara*-CDP-L-dipalmitin (Figure 2). Their antitumor activities against L1210 and P388 mouse leukemia in vivo are compared to those for *ara*-C, *ara*-CDP-dipalmitins, 5′-*O*-palmitoyl-*ara*-C,[13] and N^4-palmitoyl-*ara*-C.[14]

Chemistry. The protected 1-*O*-alkyl glycerols **4a** and **4b** (Scheme I) were prepared as reported earlier.[15-17] These compounds were then acylated with palmitoyl chloride and pyridine or methylated with CH_3I and $NaNH_2$, and the resulting compounds (**5a–c**) were purified by crystallization from a large amount of boiling 95% EtOH. The trityl group was removed by treatment of **5a** and **5b** in CH_2Cl_2 with BF_3–MeOH at 0 °C,[18] and 1-*O*-alkyl-2-*O*-palmitoylglycerols (**6a** and **6b**) were obtained in good yield (77–80%) (Table I). The acyl migration was minimal and produced only a trace of isomeric 1-*O*-alkyl-3-*O*-palmitoylglycerols. The benzyl group of **5a–c** was removed by catalytic hydrogenolysis with 10% Pd/C.

Structural assignments of **6a** and **6b** were confirmed by ^1H NMR spectrometry. The *sn*-2-methine proton of **6a** and **6b** gave a first-order quintet at 5.04–5.20 ppm, whereas it was a second-order multiplet at 3.83–3.85 ppm in 1-*O*-alkyl glycerols. These values are in good agreement with those for isomeric lysophosphatidylcholines reported previously.[19] Compounds **6a** and **6b** were phosphorylated with $POCl_3$ and Et_3N at 0–5 °C as reported earlier[20] with some modifications of solvent and purification. Com-

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(6) Abbreviations used are as follows: *ara*-C, 1-β-D-arabinofuranoylcytosine: *ara*-CDP, 1-β-D-arabinofuranosylcytosine 5′-diphosphate; *ara*-CDP-L-(or DL)-dipalmitin, *ara*-CDP-L-(or DL)-1,2-dipalmitin; *ara*-CMP morpholidate, 1-β-D-arabinofuranosylcytosine 5′-monophosphoromorpholidate; ip, intraperitoneally; ic, intracerebrally; ILS, increase in life span.
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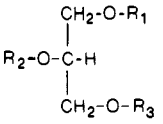
Table I. Ether Lipids



compd	R_1	R_2	R_3	method	mp, °C	yield, %	formula	anal.
6a	$C_{16}H_{33}$	$C_{15}H_{31}CO$	H	A	66–67	77	$C_{35}H_{70}O_4$	C, H
				B		91		
6b	$C_{18}H_{37}$	$C_{15}H_{31}CO$	H	A	70–71	80	$C_{37}H_{74}O_4$	C, H
				B		95		
6c	$C_{18}H_{37}$	CH_3	H		42–44.5[a]	44	$C_{22}H_{46}O_3$	
7a	$C_{16}H_{33}$	$C_{15}H_{31}CO$	$P(O)(ONa)_2$	C	60–62	50	$C_{35}H_{69}O_7P{\cdot}2Na$	C,[b] H, P[c]
7b	$C_{18}H_{37}$	$C_{15}H_{31}CO$	$P(O)(OH)_2$	C	59–60	45	$C_{37}H_{75}O_7P$	C, H, P
7c	$C_{18}H_{37}$	CH_3	$P(O)(ONa)_2$	D	57–59	55	$C_{22}H_{45}O_6P{\cdot}2Na$	C,[d] H, P

[a] Reference 15 gives mp 47 °C for the L isomer. [b] C: calcd, 61.92; found, 62.58. [c] P: calcd, 4.56; found, 5.19. [d] C: calcd, 54.75; found, 52.68.

Table II. *ara*-C Conjugates of Ether Lipids

compd	mp, °C	yield, %	formula[a]	UV_{max}, nm ($\epsilon \times 10^{-3}$) neutral[b]	acid[c]	base[d]
9a	202–205 dec	30	$C_{44}H_{81}N_3O_{14}P_2{\cdot}2Na$	271 (5.96)	282 (7.95)	270 (5.52)
9b	200–202	29	$C_{46}H_{87}N_3O_{14}P_2$[e]	272 (5.99)	282 (9.71)	271 (6.14)
9c	217–221	43	$C_{31}H_{59}N_3O_{13}P_2{\cdot}0.5H_2O$[f]	277 (5.90)	282 (8.45)	271 (5.76)

[a] Analyses for C, H, N, and P for all compounds listed unless otherwise noted. [b] $CHCl_3$–MeOH–H_2O (2:3:1). [c] $CHCl_3$–MeOH–0.6 N HCl (2:3:1). [d] $CHCl_3$–MeOH–0.6 N NaOH (2:3:1). [e] N: calcd, 4.34; found, 3.55. [f] N: calcd, 5.58; found 2.49. P: calcd, 8.23; found 6.67.

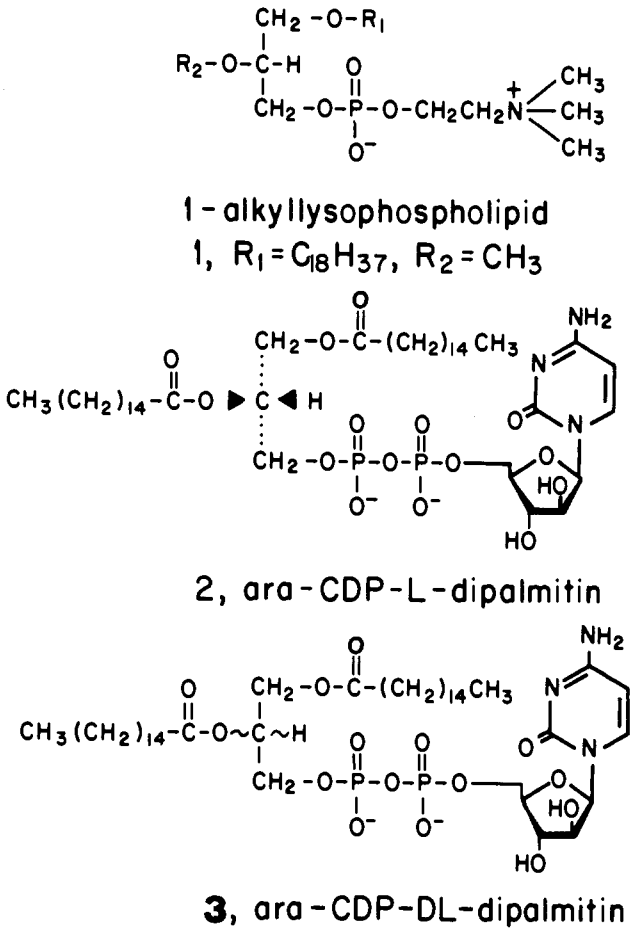


Figure 1. Structures of 1-alkyllysophospholipid and *ara*-CDP-dipalmitins.

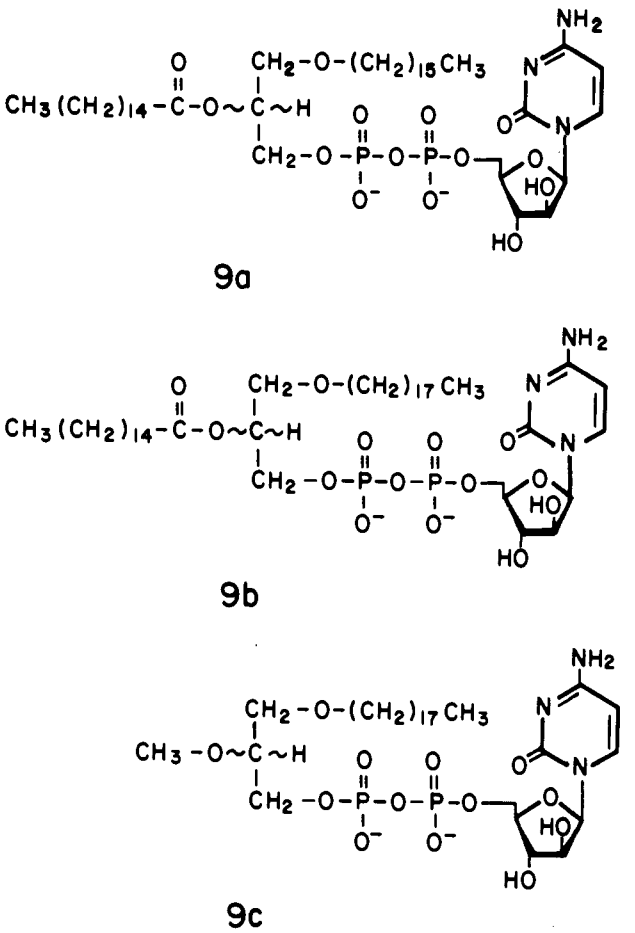


Figure 2. Structures of *ara*-C conjugates of ether lipids.

pounds **7a** and **7b** were purified by successive crystallization from hexanes and Et_2O, and the final yield was 45–50% (Table I). Compound **7c** was obtained by phosphorylating **6c** with phenyl dichlorophosphate followed by removal of the phenyl group by catalytic hydrogenation. Condensation of **7a–c** with *ara*-CMP morpholidate (**8**) in pyridine gave conjugates **9a–c** in overall yields of 30–40% (Table II). Structures were verified by elemental analysis, ^1H NMR, and UV spectrometry.

Biological Results. In Vivo Studies. Table III summarizes the effects of *ara*-C, conjugates **9a–c** and **2**, mix-

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Scheme I. Synthetic Scheme of *ara*-C Conjugates of Ether Lipids

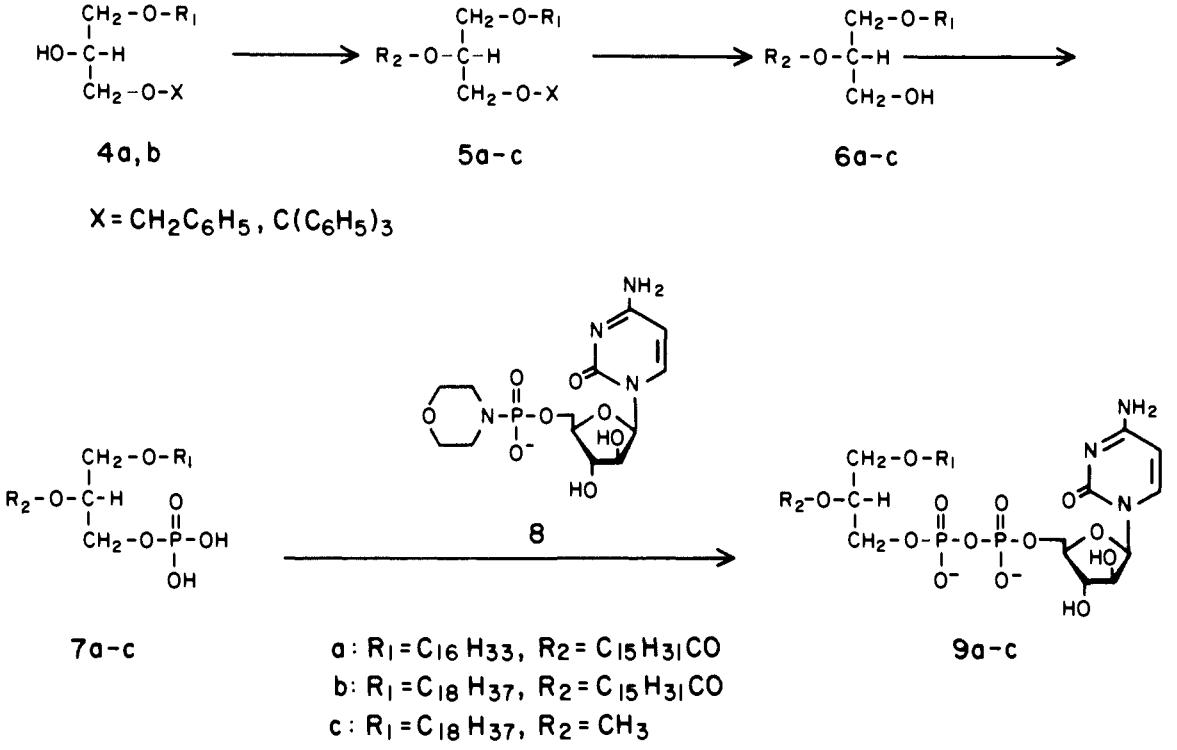

Table III. Antitumor Activity against ip Implanted L1210 Leukemia in Mice[a]

compd	treatment schedule, qd	active dose range,[b] mg (μmol)/kg per day	optimal dose,[c] mg (μmol)/kg per day	wt change (g/mouse) on day 8	survival days			45-day survivor
					range	median (T/C)[d] %	ILS[e]	
ara-C	1		400 (1644)	+2.37	9–11	9.5/8.0	19	0
	1–5	5 (21)–350 (1439)	200 (822)	−2.90	15–17	17.0/7.0	143	0
9a	1	300 (305)–400 (406)	400 (406)	+1.47	20–33	27.5/7.0	293	0
	1–5	40 (41)–80 (81)	80 (81)	+0.33	21–30	25.5/7.0	264	0
ara-CMP + **7a**[f]	1		131 & 258 (406)	−0.80	8–9	8.0/8.0	0	0
	1–5		26 & 51 (81)	+1.10	11–12	11.0/8.0	38	0
9b	1	300 (296)–500 (494)	400 (395)	−1.00	15–29	25.0/7.0	257	0
	1–5	60 (59)–100 (99)	100 (99)	−0.95	18–32	23.0/7.0	229	0
ara-CMP + **7b**[f]	1		128 & 262 (395)	+3.20	7–8	8.0/8.0	0	0
	1–5		32 & 66 (99)	+3.50	10–12	11.0/8.0	38	0
9c	1		300 (381)	+2.58	9–10	9.5/8.0	12	0
	1–5	10 (13)–120 (152)	80 (102)	−1.53	17–18	18.0/7.0	157	0
ara-CDP-L-dipalmitin (**2**)	1	50 (50)–400 (401)	300 (301)	−0.62	14–>45	20.5/6.5	215	1
	1–5	10 (10)–80 (80)	40 (40)	−1.90	24–>45	30.0/8.0	275	1
5′-*O*-palmitoyl-*ara*-C	1	100 (208)–250 (519)	200 (415)	+2.00	12–>45	17.5/7.0	150	1
N^4-palmitoyl-*ara*-C	1	100 (208)–250 (519)	200 (415)	−0.50	14–23	19.5/7.0	179	0
ET-18-OCH_3 (**1**)	1–5		5 (9.5)		8–9	9.0/8.0	13	0

[a] Each group of 6 DBA/2J mice (wt 23–28 g) received ip inoculation of 1×10^6 cells on day 0. Treatments (ip) were initiated 24 h after tumor inoculation. Animals were observed daily until death or 45 days. [b] Tested doses producing an increase in life span ≥25% over the controls. [c] Dose producing greatest increase in life span. [d] Calculated from the survivors according to the NCI protocols (ref 21). Median survival days for the control studies (102 mice) were 6.5–8.0 days. [e] Percent increase in life span: (T/C − 1) × 100. [f] Mixture of *ara*-CMP and *rac*-1-*O*-hexadecyl-2-*O*-palmitoylglycerol 3-phosphate (**7a**) or *rac*-1-*O*-octadecyl-2-*O*-palmitoylglycerol 3-phosphate (**7b**).

tures of *ara*-CMP and **7a** or **7b**, lipophilic prodrugs, and ET-18-OCH_3 (**1**) against ip implanted L1210 lymphoid leukemia in DBA/2J mice according to the procedures outlined in the NCI protocols[21] with some modifications.[22] The untreated mice died on days 6–8 after tumor implantation. When the optimum dose of *ara*-C ((200 mg or 822 μmol/kg per day) was given ip daily for 5 successive days, the increase in life span (ILS) was 143%. In contrast, after the administration of **9a** and **9b** at their optimal doses given by either qd×1 or qd×5, the ILS values were 229–293%, while mixtures of *ara*-CMP and **7a** or **7b** at the same molar doses as the corresponding conjugates produced ILS values of 0–38%. These new conjugates **9a** and **9b** showed strong activity at a single dose of 400 mg [(406 and 395 μmol)/kg, 293 and 257% ILS, respectively]. In comparison, 5′-*O*-palmitoyl-*ara*-C and N^4-palmitoyl-*ara*-C at 200 mg (415 μmol)/kg produced ILS values of 150 and 179%, respectively. *ara*-CDP-L-dipalmitin (**2**) at its optimal doses given qd×1 or qd×5 provided ILS values of 215–275%. However, **9c** was found to be effective (157% ILS) only when the optimum daily dose (80 mg or 102 μmol/kg per day) was given for 5 days. A synthetic analogue of alkyl lysophopholipid, ET-18-OCH_3 (**1**), was found to be ineffective. Table IV shows effects of the new con-

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Table IV. Antitumor Activity against ip Implanted P388 Leukemia in Mice[a]

compd	treatment schedule, qd	active dose range,[b] mg (μmol)/kg per day	optimal dose,[c] mg (μmol)/kg per day	wt change[d] (g/mouse) on day 8	survival days			45-day survivor
					range	median (T/C)[e] %	ILS[f]	
ara-C	1	100 (411)–600 (2467)	500 (2056)	+0.72	14–15	15.0/11.0	36	0
	1–5	20 (82)–200 (822)	200 (822)	−3.62	22–24	23.0/11.0	109	0
9a	1	300 (305)–500 (508)	300 (305)	−1.51		>45.0/11.0	>309	6
	1, 5, 9	67 (68)–133 (136)	133 (136)	−0.25	23–>45	>45.0/11.0	>309	4
	1–5	40 (41)–60 (61)	60 (61)	−3.30		>45.0/11.0	>309	6
9b	1	200 (198)–500 (494)	500 (494)	−0.07	23–>45	>45.0/11.0	>309	4
	1, 5, 9		67 (66)	+0.63	21–31	25.5/11.0	132	0
	1–5	40 (40)–80 (79)	60 (59)	−0.12	21–24	23.0/11.0	109	0
9c	1		300 (381)	+0.38	3–18	12.5/11.0	14	0
	1–5	60 (76)–80 (102)	60 (76)	+0.04	18–21	21.0/12.0	75	0
ara-CDP-L-dipalmitin (**2**)	1	200 (200)–500 (501)	500 (501)	−0.07	35–>45	>45.0/11.0	>309	4
	1, 5, 9	100 (100)–167 (167)	133 (134)	−1.43	31–>45	>45.0/11.0	>309	4
	1–5	40 (40)–80 (80)	60 (60)	−1.92	35–>45	>45.0/11.0	>309	5
ara-CDP-DL-dipalmitin (**3**)	1	200 (200)–400 (401)	300 (301)	−1.70	39–>45	>45.0/11.0	>309	4
	1, 5, 9	100 (100)–167 (167)	133 (134)	+0.37		>45.0/11.5	>291	6
	1–5	40 (40)–80 (80)	60 (60)	−3.27		>45.0/11.5	>291	6
5′-*O*-palmitoyl-*ara*-C	1	50 (104)–100 (208)	100 (208)	+0.30	28–>45	>45.0/12.0	>275	4
N^4-palmitoyl-*ara*-C	1	200 (415)–300 (624)	200 (415)	+0.75	24–>45	>45.0/12.0	>275	5

[a] Each group of 6 DBA/2J mice (wt 23–33 g) received ip inoculation of 1×10^6 cells on day 0. Treatments (ip) were initiated 24 h after tumor inoculation. Animals were observed daily until death or 45 days. [b] Tested doses producing an increase in life span ≥25% over the controls. [c] Dose producing greatest increase in life span. [d] Weight changes for the control studies of 42 DBA/2J mice averaged +2.15 ± 1.07 (SD) g/mouse. [e] Calculated from the survivors according to the NCI protocols (ref 21). Median survival days of 42 mice for the control studies were 11–12 days. [f] Percent increase in life span: (T/C − 1) × 100.

Table V. Antitumor Activity against ic Implanted L1210 Lymphoid Leukemia in Mice[a]

compd	treatment schedule, qd	active dose range,[b] mg (μmol)/kg per day	optimal dose,[c] mg (μmol)/kg per day	wt change[d] (g/mouse) on day 8	survival days		
					range	median (T/C)[e] %	ILS[f]
ara-C	1–5	50 (206)–300 (1233)	300 (1233)	−6.10	15–18	16.0/7.0	129
	1–9	100 (411)–300 (1233)	100 (411)	−5.80	11–18	14.0/7.0	100
9a	1	200 (203)–500 (508)	500 (508)	−2.07	18–23	23.0/7.0	229
	1, 5, 9	100 (102)–167 (170)	167 (170)	−1.15	14–22	21.0/7.0	200
	1–5	40 (41)–100 (102)	100 (102)	−2.03	21–24	22.0/7.0	214
ara-CDP-L-dipalmitin (**2**)	1	200 (200)–400 (401)	300 (301)	−0.88	19–23	21.0/7.0	200
	1, 5, 9	67 (67)–133 (133)	133 (133)	−1.11	15–22	20.0/8.0	150
	1–5	40 (40)–80 (80)	80 (80)	−5.24	11–24	22.0/8.0	175
5′-*O*-palmitoyl-*ara*-C	1		100 (208)	−2.03	14–18	17.5/7.0	150
N^4-palmitoyl-*ara*-C	1		300 (623)	−2.04	14–18	14.5/7.0	107

[a] Each group of 6 DBA/2J mice (wt 26–32 g) received ic inoculation of 1×10^5 cells on day 0. Treatments (ip) were initiated 24 h after tumor inoculation. Animals were observed daily until death. [b] Tested doses producing an increase in life span ≥25% over the controls. [c] Dose producing greatest increase in life span. [d] Weight change for the surviving mice (5 out of 42 mice) for the control studies averaged −5.80 ± 0.18 (SD) g/mouse. [e] Calculated from the survivors according to the NCI protocols (ref 21). Median survival days for the controls were 7–8 days. [f] Percent increase in life span: (T/C − 1) × 100.

jugates, the previous ones, and lipophilic prodrugs against ip implanted P388 leukemia in DBA/2J mice. The untreated mice died on days 10–12 after tumor implantation. The maximum ILS found for *ara*-C with 5-day treatment of 200 mg (822 μmol)/kg per day was 109%. The new conjugate **9a** resembled the previously studied *ara*-C derivatives **2** and **3** in being curative on either the qd×1 or qd×5 schedule. These conjugates produced ILS values from greater than 291% to greater than 309% with 4–6 45-day survivors. Conjugate **9b** showed significant activity (>309% ILS) on the qd×1 schedule; conjugate **9c** displayed marginal activity (75% ILS) on the qd×5 schedule. The lipophilic prodrugs also exhibited strong antitumor activity.

Thus, against the treatment system used in ip implanted L1210 and P388 mouse leukemia in DBA/2J mice, **9a** was found to be much more effective than *ara*-C and to be comparable to the previously studied conjugates, *ara*-CDP-L-dipalmitin (**2**) and *ara*-CDP-DL-dipalmitin (**3**). Furthermore, it appeared that toxicity as reflected in weight loss was relatively low.

Table V shows the effects of **9a** and appropriate controls against ic implanted L1210 lymphoid leukemia in DBA/2J mice. The untreated control mice lost about 6 g, or 20%, of their initial weight and died near days 7–8. This suggests that weight loss is not a reliable parameter of drug toxicity when the tumor is intracranial. All the animals developed severe exophthalmos reflecting expanding intracranial mass of the tumor. The most effective dosage schedule of *ara*-C was 300 mg (1233 μmol)/kg per day for 5 days, and the resulting ILS was 129%. After treatments with **9a** at the optimal total dose of 500 mg (508 μmol)/kg given on day 1, days 1, 5, and 9, and days 1–5, the ILS values found were 229, 200, and 214%, respectively. Thus, on a qd×5 schedule this conjugate was 2-fold more effective than *ara*-C with only $^1/_{12}$ of the total molar dose. Furthermore, the efficacy did not appear to be strongly dependent on treatment schedule. On the whole, relatively comparable results were obtained by treatment with *ara*-CDP-L-dipalmitin (**2**). However, the lipophilic prodrugs displayed somewhat less activity than the conjugates at the single doses.

Cell Culture Studies. Table VI summarizes the effect of *ara*-C, ET-18-OCH_3 (**1**), conjugate **9c**, and a mixture of *ara*-C and ET-18-OCH_3 on the viability of three human leukemias and three human solid tumors in culture by trypan blue dye exclusion.[23] The concentration of each

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Table VI. Influence of the Compounds on the Viability of Cells from Various Types of Human Leukemias and Solid Tumors in Vitro

cell line	concn, μM, of 50% loss of viability at 48 h (IC_{50}) for given compd			
	Et-18-OCH_3	*ara*-C	**9c**	*ara*-C + ET-18-OCH_3
AMML[a]	3.3	2.3	2.3	1.35
CML/BC[b]	1.5	0.21	1.2	0.25
HL-60 leukemia	2.5	0.13	13.0	0.26
glioblastoma	20.0	>100	47.0	27.0
small cell lung cancer	24.0	>100	>100	38.0
non-small cell lung cancer	21.0	34.0	38.0	34.0

[a] Acute myelomonocytic leukemia. [b] Chronic myeloid leukemia, blast crisis.

compound that resulted in 50% inhibition (IC_{50}) at 48 h was estimated from response curves of three separate experiments.[24] ET-18-OCH_3, *ara*-C, and the mixture was moderately toxic (IC_{50} = 0.25–3.3 μM) to leukemic cells and blast crisis of CML/BC. However, conjugate **9c** did not show a stronger effect when compared with its parent compounds. The results in cells of the human solid tumors showed that ET-18-OCH_3 and the mixture were quite effective, while the efficacy of *ara*-C and the conjugate was variable. The conjugate was more effective than *ara*-C against glioblastoma cells (IC_{50} = 47 vs. >100 μM).

Discussion

Results obtained in this study and the previous work with *ara*-CDP-L-dipalmitin[7-10] and its racemic mixture[11,12] demonstrate that the majority of the *ara*-C conjugates of phospholipids are highly effective against both ip and ic implanted L1210 lymphoid leukemia in mice, exceeding the activity of *ara*-C. The superior antitumor activity of the new conjugates over *ara*-C, generally independent of the treatment schedules, might be due partially to their sustained release effect. Several other explanations for the improved responses achieved by the new and previous conjugates can include (1) resistance to hydrolysis by cytidine deaminase,[9] (2) rapid interaction with serum lipoproteins, which would have a possible role in the transport of the conjugate,[25] (3) rapid uptake by cells, (4) effects on lipid biosynthesis, and (5) hydrolysis of the phosphate bond or transformation to phosphatidyl compounds to release intracellular *ara*-CMP and the higher phosphorylated nucleotides.[11,12] Even though some of the above have been demonstrated, most of them remain to be investigated. In addition to the improved responses, the new 1-*O*-alkyl analogues **9a** and **9b** might have an additional advantage for their utilization in cancer chemotherapy because of their possible release of the biologically active lysophospholipids. 1-*O*-Alkyl-2-acyl phosphatidic acids (**7a** and **7b**), released by hydrolysis of the pyrophosphate bond of conjugates **9a** and **9b**, are converted to 1-*O*-alkyl-2-acylglycerols (**6a** and **6b**) by phosphohydrolase, and the latter serve as a substrate for cholinephosphotransferase (EC 2.7.8.2) to produce 1-*O*-alkyl-2-acylphosphatidylcholines.[26] These phospholipids are converted to the 1-*O*-alkyllysophospholipids, 1-*O*-alkyllysophosphatidylcholines, via a phospholipase A_2 catalyzed reaction.[27] Analogues of these compounds have demonstrated activity against the growth and metastasis of animal tumors[4] and have shown immunomodulating activity.[5] Thus, the novel *ara*-C conjugates **9a** and **9b** contain lipophilic 1-*O*-alkylphospholipid carriers that may themselves produce tumor inhibition once the pyrophosphate bond is cleaved.

Unlike the other lipophilic prodrugs of *ara*-C, the conjugates are water soluble by sonication. The preliminary results demonstrate that a new 1-*O*-alkyl analogue may have potential in cancer chemotherapy in general and especially in some forms of brain cancer.

Experimental Section

Synthesis. Melting points were taken on a Mel-Temp capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^1H NMR) spectra were recorded on a Varian Associates EM-390 spectrometer. The chemical shift values are expressed in δ values (ppm) relative to tetramethylsilane as an internal standard. The presence of water as indicated by elemental analysis was verified by ^1H NMR. UV absorption spectra were recorded on a Varian-Cary Model 219 spectrophotometer. AG1-X8 (Bio-Rad), (diethylamino)ethylcellulose (DE-52, Whatman), Dowex 50W-X8 (Bio Rad), and Amberlite CG-50 (Mallinckrodt) were used for column chromatography. Evaporation was performed in vacuo at 30 °C. TLC was performed on glass plates coated with a 0.25-mm layer of silica gel PF-254 (Brinkman) and on polygram sil G/UV 254 (Brinkman) using the following solvent systems: (A) $CHCl_3$–MeOH (95:5), (B) $CHCl_3$–MeOH–H_2O–HOAc (25:15:4:2), (C) *i*-PrOH–H_2O–concentrated NH_4OH (7:2:1), and (D) $CHCl_3$–MeOH–H_2O (10:10:1). UV-absorbing compounds were detected by visualization under a UV lamp (254 nm), and phosphorus-containing compounds were detected with a modified Dittmer-Lester spray.[28] The organic compounds were also detected by charring after spraying with the above reagent. Elemental analyses were performed by Galbraith Laboratories, Knoxville, TN. When analyses are reported only by the element symbols, results are within ±0.4% of the theoretical values unless noted otherwise.

***rac*-1-*O*-Hexadecylglycerol (DL-Chimyl Alcohol) and *rac*-1-*O*-Octadecylglycerol (DL-Batyl Alcohol).** These compounds were prepared in an analogous manner for 3-*O*-hexadecyl-*sn*-glycerol[15,16] and 3-*O*-octadecyl-*sn*-glycerol.[16]

***rac*-1-*O*-Hexadecyl-3-*O*-tritylglycerol (4a, X = $C(C_6H_5)_3$).** This compound was prepared by a procedure reported previously.[16] A mixture of 31.6 g (0.1 mol) of *rac*-1-*O*-hexadecylglycerol and 39.0 g (0.14 mol) of trityl chloride in 200 mL of anhydrous pyridine was stirred at room temperature for 24 h. The solvent was evaporated, and the residue was dissolved in 500 mL of Et_2O. The ether layer was extracted with ice-cold H_2O (200 mL) and dried over Na_2SO_4. The solvent was evaporated, and the oily residue was dissolved in 300 mL of Me_2CO. After cooling at −10 °C overnight, the white solids were filtered and washed with cold Me_2CO. The crude product, essentially homogenous by TLC, weighed 56 g (yield, 100%) and was used for the next step without further purification: ^1H NMR ($CDCl_3$) δ 0.90 (3, t, CH_3), 1.27–1.73 (28, m, $(CH_2)_{14}$), 3.07–3.70 (6, m, 1-CH_2, 2-CH_2, CH_2OCH_2), 3.97 (1, quintet, *J* = 5 Hz, 2-C*H*), 7.30 (15, m, $(C_6H_5)_3$).

rac-1-*O*-Octadecyl-3-*O*-tritylglycerol (**4b**, X = $C(C_6H_5)_3$) was prepared in an analogous manner.

***rac*-1-*O*-Hexadecyl-3-*O*-benzylglycerol (4a, X = $CH_2C_6H_5$).** This compound was prepared by a literature procedure,[15] *rac*-3-*O*-Benzylglycerol was alkylated with 1 M equiv of 1-bromohexadecane in the presence of $NaNH_2$ in refluxing toluene. After removing the starting materials at 100–134 °C/0.5 mmHg, the crude product was purified on a dry-packed silica gel column with hexanes, $CHCl_3$, and solvent A as eluents: yield, 36.4%; ^1H NMR ($CDCl_3$) δ 0.85 (3, t, CH_3), 1.23–1.62 (28, m, $(CH_2)_{14}$), 3.34–3.65 (7, m, 1-CH_2, 3-CH_2, CH_2OCH_2, O*H*), 3.92 (1, quintet, *J* = 5 Hz, 2-C*H*), 4.51 (2, s, $CH_2C_6H_5$), 7.29 (5, s, C_6H_5).

rac-1-*O*-Octadecyl-3-*O*-benzylglycerol (**4b**, X = $CH_2C_6H_5$) was prepared in an analogous manner.

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***rac*-1-*O*-Hexadecyl-2-*O*-palmitoylglycerol (6a). Method A.** To a mixture of 50.3 g (0.09 mol) of **4a** (X = $C(C_6H_5)_3$), 8.3 mL (0.10 mol) of pyridine, and 500 mL of toluene was added dropwise 27.5 g (0.10 mol) of palmitoyl chloride, and the mixture was stirred at room temperature for 1 day. The mixture was then mixed with 300 mL of Et_2O and 300 mL of H_2O. The organic layer was separated and washed with 0.5 N H_2SO_4, saturated $NaHCO_3$, and H_2O, dried over Na_2SO_4, and evaporated to dryness. The oily residue was crystallized from a large volume (4 L) of boiling 95% EtOH. Compound **5a** (X = $C(C_6H_5)_3$) weighed 61.0 g (yield, 85%). This compound was dissolved in 500 mL of CH_2Cl_2 and treated with an equimolar amount of BF_3 (10.16 g of BF_3–2MeOH) at 0 °C for 30 min. The mixture was then extracted 3 times with ice-cold H_2O (total 100 mL) and evaporated immediately to dryness. The residue was crystallized from 95% EtOH–hexanes (5:1): yield, 38.4 g (77% based on **4a**, X = $C(C_6H_5)_3$); mp 66–67 °C; ^1H NMR ($CDCl_3$) δ 0.87 (6, t, J = 6 Hz, 2 CH_3), 1.27–1.77 (54, m, $(CH_2)_{14}$, $(CH_2)_{13}$), 2.33 (2, t, J = 6 Hz, CH_2CH_2CO), 3.43 (2, t, J = 6 Hz, OCH_2CH_2), 3.60 (2, d, J = 5 Hz, 1-CH_2), 3.78 (2, d, J = 5 Hz, 3-CH_2), 5.00 (1, quintet, J = 5 Hz, 2-CH).

Method B. To a mixture of **4a** (X = $CH_2C_6H_5$, 22.2 g, 0.55 mol), pyridine (8.4 g, 0.11 mol), and benzene (100 mL) was added dropwise palmitoyl chloride (27.5 g, 0.1 mol) at room temperature. The mixture was heated at 70 °C overnight and cooled to room temperature. Compound **5a** (X = $CH_2C_6H_5$) isolated by the above procedure weighed 35 g (yield, 99%). This compound (20 g) was hydrogenated with 10% Pd/C (1 g) in HOAc and *n*-hexane (125 mL each) at 50 psi for 3 days. The mixture was warmed, and the catalyst was removed by filtration. The product was crystallized from the filtrate at 0–5 °C: yield, 15.8 g (91% based on **4a**, X = $CH_2C_6H_5$).

rac-1-*O*-Octadecyl-2-*O*-palmitoylglycerol (**6b**) was prepared in an analogous manner.

***rac*-1-*O*-Octadecyl-2-*O*-methylglycerol (6c).** This compound was synthesized in a manner analogous to 1-*O*-octadecyl-2-*O*-methyl-*sn*-glycerol.[15] *rac*-3-*O*-Benzylglycerol was alkylated with 1-bromooctadecane in the presence of $NaNH_2$, and the resulting *rac*-1-*O*-octadecyl-3-*O*-benzylglycerol (**4b**, X = $CH_2C_6H_5$) was methylated with methyl iodide and $NaNH_2$. Debenzylation of **5c** (X = $CH_2C_6H_5$) by catalytic hydrogenolysis with 10% Pd/C gave product **6c** in 44.3% yield based on *rac*-3-*O*-benzylglycerol: mp 42–44.5 °C; ^1H NMR ($CDCl_3$) δ 0.87 (3, t, CH_3), 1.27–1.87 (32, m, $(CH_2)_{16}$), 3.49 (6, m, 1-CH_2, 3-CH_2, CH_2O-CH_2), 3.66 (3, s, OCH_3), 3.84 (1, quintet, J = 5 Hz, 2-CH).

***rac*-1-*O*-Hexadecyl-2-*O*-palmitoylglycerol 3-Phosphate (7a). Method C.** To an ice-cold mixture of 4.5 g (0.03 mol) of $POCl_3$, 3.0 g (0.03 mol) of Et_3N, and 30 mL of *n*-hexane was added 11.1 g (0.02 mol) of **6a** in 200 mL of toluene for a period of 1 h, and then the mixture was stirred at room temperature for 18 h. Water (10 mL) was added to the mixture, and the suspension was stirred at room temperature for 1 h. The mixture was then partitioned between Et_2O (600 mL) and H_2O (200 mL). The organic layer was dried over Na_2SO_4 and evaporated to dryness. The residue was crystallized from *n*-hexane: yield, 6.4 g (50.4%). The anatlytical sample was prepared by recrystallization from Et_2O at 0–5 °C followed by conversion to the disodium salt using an Amberlite CG-50 (Na^+) column: mp 60–62 °C (disodium salt); ^1H NMR ($CDCl_3$) δ 0.87 (6, t, J = 6 Hz, 2 CH_3), 1.27–1.67 (54, m, $(CH_2)_{14}$, $(CH_2)_{13}$), 2.33 (2, t, J = 6 Hz, CH_2CH_2CO) 3.33–4.07 (6, m, 1-CH_2, 3-cH_2, CH_2OCH_2), 5.10 (1, m, 2-CH).

rac-1-*O*-Octadecyl-2-*O*-palmitoylglycerol 3-phosphate (**7b**) was prepared in an analogous manner.

***rac*-1-*O*-Octadecyl-2-*O*-methylglycerol 3-Phosphate (7c). Method D.** To an ice-cold solution of **6c** in 70 mL of toluene and 1 mL of pyridine was added dropwise a solution of 4.22 g (0.02 mol) of phenyl dichlorophosphate in 40 mL of toluene, and then the mixture was stirred at room temperature for 2 days. Water (1 mL) was added to mixture, and the suspension was stirred at room temperature for 1 h. The mixture was then partitioned between Et_2O (30 mL) and H_2O (30 mL). The organic layer was washed with H_2O (3 × 50 mL), dried over Na_2SO_4, and evaporated to dryness. The residue was recrystallized from 95% EtOH (yield, 3 g). This protected phosphate was then dissolved in 50 mL of toluene and 50 mL of HOAc and hydrogenated in the presence of PtO_2 and PdO (400 mg each). After the catalysts were removed, the solvent was evaporated to dryness and the residue was recrystallized from 95% EtOH: yield, 2.4 g (55%): mp 57–59 °C (disodium salt).

***ara*-CDP-*rac*-1-*O*-hexadecyl-2-*O*-palmitoylglycerol (9a).** An anhydrous mixture of **7a** (4.19 g, 6.6 mmol) and 3.43 g (5 mmol) of *ara*-CMP morpholidate 4-morpholine *N,N'*-dicyclohexylcarboxamidinium salt[7] (8) in 300 mL of pyridine was stirred at room temperature for 5 days. The mixture was then evaporated to dryness, and the residual pyridine was removed by coevaporation with toluene (2 × 20 mL). The residue was stirred in 10% HOAc in $CHCl_3$–MeOH–H_2O (2:3:1) (150 mL) at room temperature for 1 h, and then $CHCl_3$ (250 mL) was added. The organic layer was evaporated to dryness, and the coevaporation of residual pyridine with toluene (3 × 10 mL) was repeated. The residue was then dissolved in 100 mL of $CHCl_3$–MeOH–H_2O (2:3:1) (filtered if necessary), and the solution was applied to a DE-52 (AcO^-) column (2.5 × 50 cm, jacketed, 5 °C) prepacked in H_2O and equilibrated in $CHCl_3$–MeOH–H_2O (2:3:1). The column was first eluted with the same solvent (800 mL) and then with a linear gradient of 0–0.15 M ammonium acetate in the same solvent (1500 mL each). Fractions between 900 and 1500 mL were pooled and evaporated to a small volume (30 mL). The solid was filtered and washed with H_2O and Me_2CO. This (NH_4 salt of **9a**) was dissolved in a minimum volume of $CHCl_3$–MeOH–H_2O (2:3:1), and the solution was passed through a CG-50 (Na^+) column (2.5 × 15 cm). The column was eluted further with the same solvent, and the eluate containing the product was evaporated to dryness and the residue was recrystallized from $CHCl_3$–Me_2CO. The product was dried in vacuo over P_2O_5: yield, 1.47 g (30%); mp 202–205 °C dec; ^1H NMR ($CDCl_3$–CD_3OD–D_2O, 2:3:1) δ 0.87 (6, t, J = 6 Hz, 2CH_3) 1.07–1.78 (54, m, $(CH_2)_{14}$, $(CH_2)_{13}$), 2.35 (2, t, J = 6 Hz, $CH_2CH_2CO_2$), 3.27–4.35 (11, m, *H*-2′, *H*-3′, *H*-4′, *H*-5′, 1-CH_2, 3-CH_2, CH_2OCH_2), 5.15 (1, m, 2-CH) 5.92 (1, d, J = 7 Hz, cytosine *H*-5), 6.14 (1, d, J = 5 Hz, H-1′), 7.88 (1, d, J = 7 Hz, cytosine *H*-6).

ara-CDP-*rac*-1-*O*-octadecyl-2-*O*-palmitoylglycerol (**9b**) and *ara*-CDP-*rac*-1-*O*-octadecyl-2-*O*-methylglycerol (**9c**) were prepared in an analogous manner.

Biological Studies. Tumor Cells and Animals. L1210 lymphoid leukemia cells were purchased from Arthur D. Little, Inc. (Cambridge, MA), and P388 lymphocytic leukemia was obtained from NCI. The cells were routinely transplanted into DBA/2J mice, which were supplied by Roswell Park Memorial Institute.

Antitumor Activity in Vivo. DBA/2J mice in groups of six (av wt = 25 g) were inoculated ip with 1 × 10^6 L1210 lymphoid leukemia or P388 lymphocytic leukemia cells[21] (or ic with 1 × 10^5 L1210 cells),[29,30] and a sonicated solution[9] of the conjugates or suspension of the reference prodrugs of Tables III–V in 0.9% NaCl containing 0.5% Tween-80 was given ip as reported earlier.[10] Each drug was tested over a wide range of doses. The active dose ranges reported in Tables III–V are those giving ILS values ≥25%. Optimum doses are those producing the greatest increase in life span in the designated treatment schedule.

In Vitro Studies. The trypan blue dye exclusion test[23] was performed to assess cytotoxic effects of the drugs. The tumor cells of human origin were grown and passaged according to the method reported previously.[31]

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Registry No. **4a** (X = CPh_3), 82002-20-8; **4a** (X = CH_2Ph), 103475-80-5; **4b** (X = CPh_3), 86334-56-7; **4b** (X = CH_2Ph),

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103475-82-7; **5a** (X = CPh_3), 103383-63-7; **5a** (X = CH_2Ph), 103476-86-4; **5c** (X = CH_2Ph), 90940-90-2; **6a**, 103475-81-6; **6b**, 103475-83-8; **6c**, 88929-32-2; **7a**, 103383-64-8; **7b**, 103383-65-9; **7c**, 103475-84-9; 8, 69467-87-4; **9a** (NH_3 salt), 103421-96-1; **9a**, 103383-66-0; **9b**, 103383-67-1; **9c**, 103383-68-2; (±)-1-*O*-hexadecylglycerol, 6145-69-3; (±)-3-*O*-benzylglycerol, 13071-59-5; 1-bromohexadecane, 112-82-3; palmitoyl chloride, 112-67-4; 1-bromooctadecane, 112-89-0; methyl iodide, 74-88-4.

Chiral DNA Gyrase Inhibitors. 1. Synthesis and Antimicrobial Activity of the Enantiomers of 6-Fluoro-7-(1-piperazinyl)-1-(2′-*trans*-phenyl-1′-cyclopropyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic Acid

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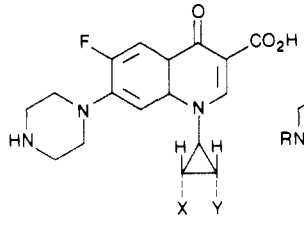
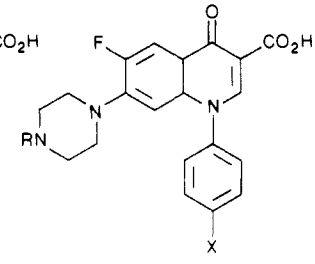
New quinolone antimicrobial agents (racemic, (1′*S*,2′*R*)- and (1′*R*,2′*S*)-6-fluoro-7-(1-piperazinyl)-1-(2′-*trans*-phenyl-1′-cyclopropyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acids) were synthesized, and their in vitro antimicrobial potencies and spectra were determined. As compared to their conceptual parents, these agents retained a considerable amount of the antimicrobial potency and spectra of ciprofloxacin and of 6-fluoro-1-phenyl-7-(1-piperazinyl)-1,4-dihydro-4-oxoquinoline-3-carboxylic acid against Gram-positives. Gram-negatives were considerably less sensitive. The (−)-(1′*S*,2′*R*) analogue was the more potent of the enantiomers, but the degree of chiral discrimination by most bacteria was only 4-fold. The 4-fold chiral discrimination was observed also using purified DNA gyrase obtained from *Micrococcus luteus*, whereas the two enantiomers were essentially equiactive against the enzyme derived from *Escherichia coli*. These results confirm that there is a substantial degree of bulk tolerance available at N-1 of quinolone antimicrobial agents and suggest that electronic factors controlled by substitution at that site are of considerable importance. On the other hand, chiral recognition brought about by attachment of optically active groups to the N-1 position in these derivatives is relatively small.

Recently it has been shown that ciprofloxacin (**1**),[1] difloxacin (**2**),[2,3] and A-56620 (**3**)[3,4] possess attractive in vivo antimicrobial spectra and potencies. These compounds thus take their place among the substantial number of quinol-4-ones with clinical promise as antibacterial agents that have been prepared recently.[5] The attractive antimicrobial features of **1–3** led us to speculate whether incorporation of both of these features in a single molecule would be consistent with antimicrobial activity. Conventional wisdom in this field has held until recently that the optimal aliphatic group to attach to N-1 should be ethyl, vinyl, or a bioisostere of ethyl ($NHCH_3$, OCH_3, etc.).[3]



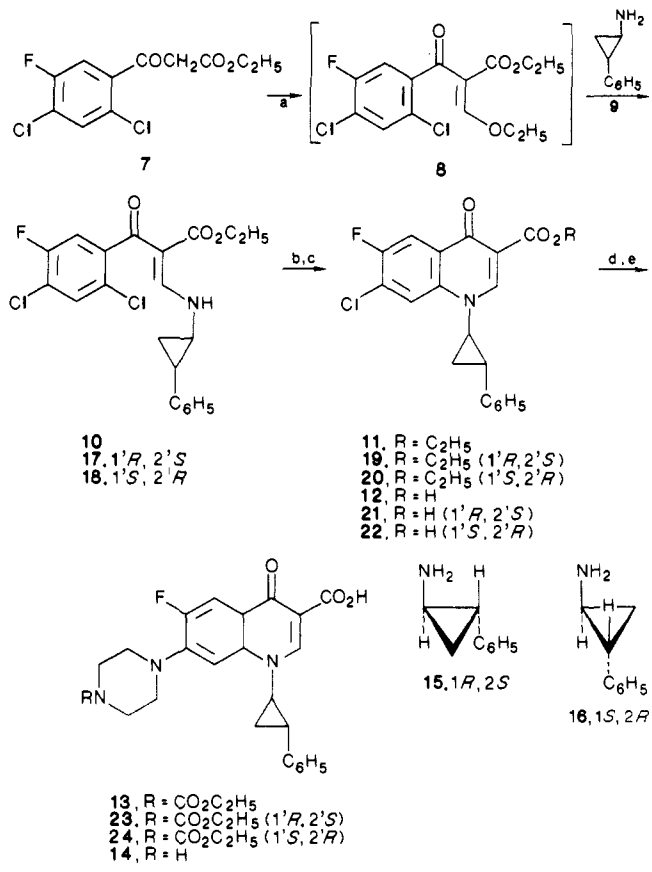
1, X = Y = H
5, X = H; Y = C_6H_5
6, X = C_6H_5; Y = H



2, R = CH_3, X = F
3, R = H; X = F
4, R = X = H

Recently several analogues have appeared whose potency casts doubt on the universality of this concept. Ciprofloxacin (*N*-cyclopropyl),[1] ofloxacin (tricyclic),[6] flumequine (tricyclic),[7] and A-56619 (*N*-aryl)[3] serve as examples. The excellent potency of **2**, **3**, and their congeners has been speculated to be due in part to electronic effects. The aryl group is clearly large compared to the aliphatic N-1 groups mentioned above. Attaching a benzene ring to the cyclopropyl ring of ciprofloxacin would not only preserve a considerable degree of electronic influence, through conjugative effects, but would provide an additional test of the bulk tolerance at N-1 for antimicrobial activity and would also allow the convenient preparation of antipodal

Scheme I



7
8
9
10
17, 1′*R*, 2′*S*
18, 1′*S*, 2′*R*
11, R = C_2H_5
19, R = C_2H_5 (1′*R*,2′*S*)
20, R = C_2H_5 (1′*S*,2′*R*)
12, R = H
21, R = H (1′*R*, 2′*S*)
22, R = H (1′*S*, 2′*R*)
13, R = $CO_2C_2H_5$
23, R = $CO_2C_2H_5$ (1′*R*, 2′*S*)
24, R = $CO_2C_2H_5$ (1′*S*, 2′*R*)
14, R = H
15, 1*R*, 2*S*
16, 1*S*, 2*R*

[a] a = $HC(OC_2H_5)_3$ + Ac_2O + Δ; b = NaH + Δ; c = KOH/H_2O + Δ; d = $HN[(CH_2)_2]_2NCO_2C_2H_5$; e = KOH + Δ

analogues. Chiral preferences of drug candidates is a subject of considerable contemporary interest[8-11] but one

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(1) Wise, R.; Andrews, J. M.; Edwards, L. J. *Antimicrob. Agents Chemother.* **1983**, *23*, 559.

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