Pergamon

Bioorganic & Medicinal Chemistry 8 (2000) 1407–1413

BIOORGANIC & MEDICINAL CHEMISTRY

Synthesis of Acyclic 6,7-Dihaloquinolone Nucleoside Analogues as Potential Antibacterial and Antiviral Agents

Najim A. Al-Masoudi,[a,*] Yaseen A. Al-Soud,[b] Michael Eherman[c] and Erik De Clercq[d]

[a]*Faculty of Chemistry, University of Konstanz, PO Box 5560, D-78434 Konstanz, Germany*
[b]*Department of Chemistry, University of Al al-Bayt, Al-Mafraq, Jordan*
[c]*Faculty of Biology, University of Konstanz, PO Box 5560, D-78434 Konstanz, Germany*
[d]*Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium*

Received 12 August 1999; accepted 15 February 2000

Abstract—Reaction of the quinolone carboxylic acids **1** and **2** with (2-acetoxyethoxy)methyl chloride **3** in the presence of *n*-Bu_4NI afforded the *N*-alkylated products **4** and **6**, which could be deblocked to the free nucleoside analogues **5** and **7**, respectively. The alkylated quinolone carboxylic acids **9** and **10** were obtained by condensation of **1** and **2** with 1,4-dichlorobut-2-ene **8** in the presence of NaH. Hydrolysis of **9** gave the alcohol **11**. Similar treatment of **1** with **8** in the presence of K_2CO_3 at relatively high temperature furnished **12**. Prolonged heating of the ester **13** with **8** in NaH/DMF afforded the conjugated-diene **15**. Treatment of **1** and **2** with dimethyl acetylenedicarboxylate **16** furnished the pyrano[4,3-*b*]quinolones **17** and **18**, respectively. Antibacterial and antiviral evaluations of the new products are reported.

Introduction

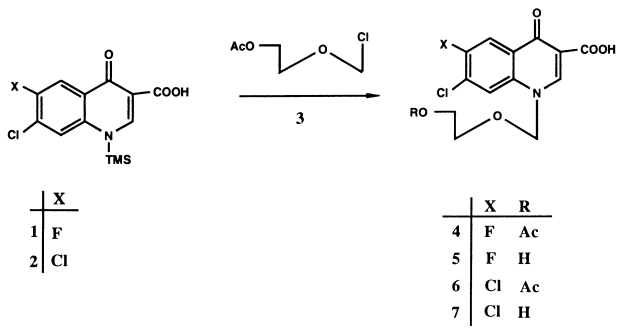
The biological properties of several quinolones as antibacterial,[1] like nalidixic acid,[2] or antiviral agents[3] and dehydrogenase inhibitors[4] have stimulated considerable interest in the synthesis of various modified clinically applied antibacterial quinolones such as norfloxacine,[5] ciprofloxacine,[6] amifloxacine,[7] and sparfloxacine.[8] The fact that various types of natural *N*-nucleosides exhibited antiviral and anticancer activities[9] prompted us to join other laboratories[10,11] in their efforts to synthesize quinolone *N*-nucleosides carrying ribose, 2-deoxyribose and azido-ribose moieties.[12] Among these nucleosides are acyclonucleosides, which are of interest because of their known antiviral activities.[13,14] Examples of such nucleosides are 9-[(2-hydroxyethoxy)methyl]guanine (acyclovir, Zovirax®), a powerful antiherpetic agent of clinical significance,[15] 9-[(1,3-dihydroxy-2-propoxy)-methyl]guanine (Ganciclovir, Cymevene®),[16,17] a potent inhibitor of the cytomegalovirus replication, and PMEA (Adefovir), which demonstrates activity against herpes, retro and hepadna viruses.[18] Other antiviral compounds[19] are derived from nucleosides by replacing the sugar portion by an olefinic bond (CH=CH),[19–24] and/or are carrying an acetylenic alcohol residue.[20,25] It has been reported[23] that 9-[(*E*)-4-chlorobut-2-enyl]adenine inhibits the growth of P388 mouse lymphoid leukemia cells in culture ($ED_{50} = 5 \pm 0.1$ μg/mL). Zemlicka and his coworkers[26,27] reported numerous examples of unsaturated nucleosides as antitumor and antiviral agents. As a part of our program for searching for biologically active quinolone nucleosides, we report here syntheses of *N*-oxy-methyl and *N*-allyl substituted quinolones along with the result of their antibacterial and antiviral evaluation.

Chemistry

The synthesis of the acyclic nucleosides **4** and **6** (Scheme 1) was achieved using a silylation procedure.[28] Thus, the quinolones were refluxed in hexamethyldisilazane and the resulting silylated bases **1** and **2** were treated with (2-acetoxyethoxy)methyl bromide **3**[29] in the presence of *n*-Bu_4NI as catalyst in dry CH_3CN as solvent at 23 °C to afford **4** (77%) and **6** (74%), respectively. Compound **4** was prepared previously,[30] in 13% yield, by condensation of the silylated quinolone **1** with acetoxyethylacetoxymethyl ether[31] in the presence of $SnCl_4$. Deacetylation to the free acyclic nucleosides **5** and **7** (90 and 92% yields, respectively) was achieved with NH_3/MeOH at 23 °C. The free nucleoside **5** was prepared,[30] in 92% yields, by deblocking of the ethyl carboxylic acid analogue in refluxing 1 N NaOH and MeOH. The

*Corresponding author. Tel.: +49-7531-34435; fax: +49-7531-34435; e-mail: masoudi@chclu.chemie.uni-konstanz.de

0968-0896/00/$ - see front matter
PII: S0968-0896(00)00067-5

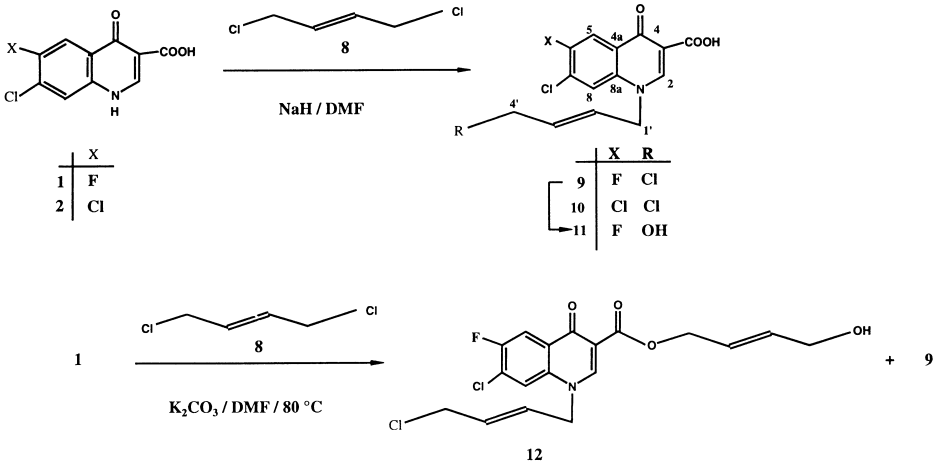


Scheme 1.

structures of the newly synthesized quinolone nucleosides were confirmed by comparison of the ^{1}H and ^{13}C NMR spectra with those of the structurally proven quinolone ribonucleosides.[11,12] In the ^{1}H NMR spectra ($CDCl_3$) the singlets in the region of δ_H 5.70–5.91 were attributed to the geminal protons at C-1′, while the two triplets at δ_H 3.8–3.53 and δ_H 4.24–3.45 represent the geminal protons at C-3′ and C-4′, respectively. In the ^{13}C NMR spectra the resonances for C-1′, C-3′, and C-4′ appeared between δ_C 83.4 and 59.8.

Alkylation of the bases **1** and **2** with (*E*)-1,4-dichlorobut-2-ene (**8**) led to the open-chain analogues (Scheme 2). Thus, condensation of compounds **1** and **2** with an excess of **8** in the presence of NaH afforded after chromatographic purification the products **9** and **10** in 59 and 37% yields, respectively. The allyl chloride **9** was transformed into the allyl alcohol **11** by treatment with fresh CuCl in the presence of 1.5% hydrochloric acid. The site of alkylation was assigned from the UV data by comparison with suitable alkyl models previously reported[32] and some known N-1 alkylated quinolones[10,12] and was confirmed by an NOE experiment as well as by mass spectra. In the ^{1}H NMR (HMQC) spectra (DMSO-d_6) of **9**–**11**, the two multiplets appearing in the region of δ_H 5.86–6.06 (δ_C 127.9–131.0) could be assigned to H-2′ and H-3′ (C-2′ and C-3′) by spin–spin decoupling experiments. These ^{1}H NMR data are in agreement with those of 9-[(*E*)-4-chloro-2-butene-1-yl]adenine and its 4′-hydroxy analogue.[23,24] Prolonged heating of **1** (80 °C for 10 h) with excess of K_2CO_3 and olefine **8** resulted in esterification of the carboxylic group at C-3 as well as in alkylation at N-1, furnishing the chloro-alcohol **12** (33%). Obviously, this reaction proceeded via formation of the dichloro intermediate followed by hydrolysis of the allylic chlorine atom. The site of alkylation of **12** at N-1 was visible in the ROSY spectrum, where the CH_2-1′ protons showed cross signals to both H-2 and H-8, but not to H-5 of the quinolone ring. The proton spin system of **12** was further identified from DFQ-COSY[33] spectrum, where the singlets of the olefinic protons H-2′, H-3′, H-2″, H-3″ were found as doublets at δ_H 5.79, 5.85, 6.01, and 5.79, respectively, and correlated to the singlets at δ_C 122.3, 129.7, 134.8 and 128.5 for C-2′, C-3′, C-2″ and C-3″, respectively. The CH_2-4′ protons resonated at relatively higher field (δ_H 4.26) in comparison to the CH_2-4″ (δ_H 4.20) and these data are in agreement with those of 9-[(*E*)-4-chlorobut-2-enyl]adenine and its 4′-hydroxy analogue.[23] From the gradient selected HMBC spectrum of **12**, C-1′ at δ_C 32.9 shows a heteronuclear long-correlation to the CH_2-4′ methylene group at δ_H 4.26. Furthermore, C-1′ appeared at higher field in comparison to C-1″, which resonated at lower field (δ_C 43.2) due to the deshielding nature around it. These arguments might explain the retention of chlorine atom at C-4′, whereas the other chlorine at C-4″ is hydrolyzed to the hydroxyl group. The assignment of protons and carbons of the quinolone ring was deduced from the previously reported data.[33,34] This evidence proved that **12** carried two allylic groups with the presumed structure shown in Scheme 2.

When the ester **13** was treated with the dichloride **8** in the presence of NaH the *N*-butadienyl derivative **15** (35%) was obtained as the result of a dehydrochlorination of the intermediate **14**. The structural assignment follows from the mass spectrum and a 2-D NMR spectrum showing overlapping signals for the CH_2-1′ and H-2′ at δ_H 6.55 and 6.56 correlated with δ_C 125.0 and 132.4 (C-1′ and C-2′), respectively. Irradiation at δ_H 6.55 produced a 17% NOE effect on the signal at δ_H 7.53 assigned to the H-8, indicating that the conjugated diene side-chain is attached to N-1. It proved to be



Scheme 2.

difficult to assign the configuration of the *N*-vinyl group on the basis of the NMR spectra (Scheme 3).

Treatment at 80 °C of the quinolones **1** and **2** with excess of dimethyl acetylenedicarboxylate **16** resulted in the formation of the heterocycles **17** (23%) and **18** (29%), respectively. The structural assignments of these products were based on the COSY-HMQC NMR and mass spectra. In the ^{1}H NMR spectra ($CDCl_3$) compounds **17** and **18** each showed signals for five methyl ester groups and no signal assignable to H-2. The ^{13}C NMR spectra supported the proposed structures, since the carbons of the quinolone ring were deduced by comparison with those of compounds **4**–**7** and the structurally proven quinolone ribonucleosides[11,12] (see Experimental). A rationale explaining the products **17** and **18** is shown in Scheme 4.

Biological Assays

Antibacterial activity

The in vitro antibacterial activity of compounds **5**–**7**, **9**, and **10** was tested by using the wild-type *Escherichia coli* K12 wild-type strain D10, a Gram-negative bacterium, and wild-type *Bacillius subtilis*, a Gram-positive bacterium: 10^4 cells/mL were incubated into LB medium containing the indicated amount of a given compound. After growth overnight at 37 °C, the optical density of the culture was determined. The minimal inhibitory concentration (MIC) was determined by assaying the effect of each compound at concentrations of 0.1, 0.5, 1, 10, 50, 100, 200, and 500 μg/mL. The results of these experiments are summarized in Table 1.

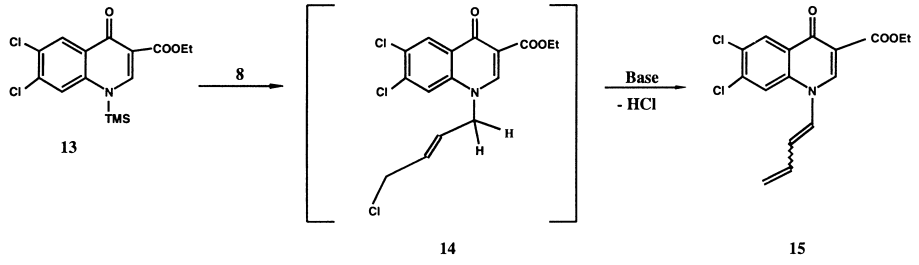
It is noteworthy that compound **9** has the same antibacterial potency against *B. subtilis* as nalidixic acid (1.0 μg/mL).

Antiviral activity

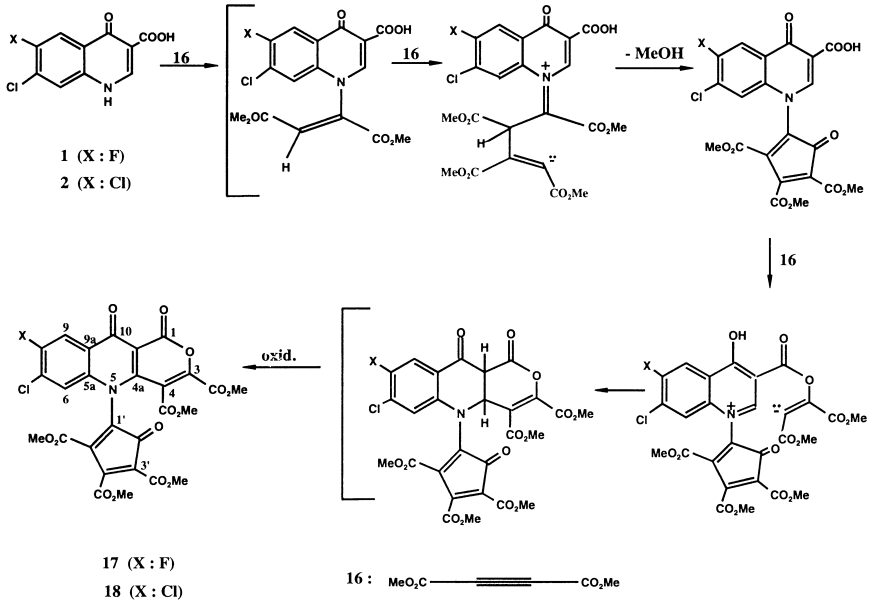
Compounds **5**–**7** and **9** were evaluated for their anti-HIV activity in vitro by using the III_B strain for HIV-1 and the ROD strain for HIV-2, and monitored by the inhibition of the virus-induced cytopathic effect in MT-4 cells. The results are shown in Table 2.

Among these nucleoside analogues, none was found to inhibit HIV-1 or -2 replication, in vitro, at EC_{50} lower than the CC_{50}. Thus, no selective anti-HIV activity could be witnessed.

Compounds **5**–**7** and **9** were also evaluated against various other viruses: herpes simplex viruses [HSV-1 (KOS



Scheme 3.



Scheme 4.

Table 1. In vitro antibacterial screening[a]

Compd	*E. coli*	*B. subtilis*
5	500	100
6	500	50
7	10	50
9	50	1
10	> 500	> 500
Nalidixic acid[2]	1	1

[a]Numbers indicate the minimal inhibitory concentrations in μg/mL of the cell cultures.

Table 2. Anti-HIV-1[a] and HIV-2[b] activity (EC_{50}, μg/mL) and cytotoxicity (CC_{50}, μg/mL) in MT-4 cells

Compound	Strain	$(EC_{50})^c$	$(CC_{50})^d$	SI[e]
5	(IIIB)	> 35	= 35.3	< 1
	(ROD)	> 27	= 27.3	< 1
6	(IIIB)	> 46	= 45.5	< 1
	(ROD)	> 34	= 33.6	< 1
7	(IIIB)	> 47	= 47	< 1
	(ROD)	> 39	= 38.7	< 1
9	(IIIB)	> 2	= 2.2	< 1
	(ROD)	> 1	= 1.3	< 1
Zidovudine[36]	(IIIB)	0.005	= 110	22,000

[a]Anti-HIV-1 activity measured with strain IIIB.
[b]Anti-HIV-2 activity measured with strain ROD.
[c]Effective concentration of compound required to achieve 50% protection of MT-4 cells against the cytopathic effect of HIV.
[d]Cytotoxic concentration of compound required to reduce the viability of mock-infected MT-4 cells by 50%.
[e]Selectivity index: ratio of CC_{50}/EC_{50}.

strain), HSV-2 (G strain)]; human cytomegalovirus (HCMV); vaccinia virus, in E_6SM cell cultures; vesicular stomatitis virus; Coxsackie virus, B4; respiratory syncytical virus, in HeLa cell cultures; parainfluenza-3 virus; Reovirus-1 and Sindbis virus, in Vero cell cultures. As compared to the known antiviral agents Ganciclovir[16,17] and Lidofovir,[35] the results shown in Tables 3 and 4 indicated little or no activity for any of the compounds at nontoxic concentrations. Compound **9** was clearly the most cytotoxic of the series: its 50% inhibitory effect on MT-cell viability and HEL cell growth was 1.3–2.2 and 6.0 μg, respectively (Tables 3 and 4).

Experimental

The melting points are uncorrected. The UV spectra were recorded on a Perkin–Elmer spectrophotometer Lambda 5. The ^1H NMR and ^{13}C NMR spectra were recorded on Bruker AC-250, WM-250 and DRX 600 spectrometers with tetramethylsilane as an internal standard; δ scale in ppm; coupling constants in Hz. The signal assignments for protons were verified by selective proton decoupling or by COSY spectra. Heteronuclear assignments were verified by ^1H–^{13}C COSY or HMQC experiments. TLC was performed on silica gel 60 F254 sheet layers (Merck) with eluents: (a) $CHCl_3$:MeOH (9:1); (b) $CHCl_3$:MeOH (4:1). EI and FAB mass spectra were recorded on an MAT 312 mass spectrometer using 3-nitrophenol (NBOH) or glycerol as matrices. Some molecular ions were detected by doping the sample with Na^+ ion.

Table 3. Activity against human cytomegalovirus (HMCV) in human embryonic lung (HEL) cells

Compd	Antiviral activity IC_{50} (μg/mL)[a]		Cytotoxicity (μg/mL)	
	AD-169 strain	Davis strain	Cell morphology (MCC)[b]	Cell growth $(CC_{50})^c$
5	> 20	> 20	50	> 50
6	32	32	⩾ 50	> 50
7	> 50	> 50	> 50	> 50
9	> 2	2	5	6
Ganciclovir[16,17]	1.4	1.3	> 50	> 50
Lidofovir[35]	0.2	0.3	> 50	> 50

[a]Inhibitory concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units.
[b]Minimuim cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.
[c]Cytotoxic concentration required to reduce cell growth by 50%. Lidofovir: (*S*)-9-(3-hydroxy-2-phosphonylmethoxypropyl)cytocine [(*S*)-HPMPC].

1-[(2-Acetoxyethoxy)methyl]-6,7-dialkyl-1,4-dihydro-4-oxoquinoline-3-carboxylic acids

General procedure. A suspension of the quinolone base (1.24 mmol) in hexamethyldisilazane (20 mL) and a few crystals of $(NH_4)_2SO_4$ were heated under reflux for 10 h. After cooling, the solution was evaporated to dryness and the residue was dissolved in dry CH_3CN (20 mL). Chloride **3** (0.17 g, 0.86 mmol) in dry CH_3CN (10 mL) was added dropwise followed by addition of Bu_4NI (0.17 g). After stirring at 23 °C for 5 h, the solution was evaporated to dryness and the residue was partitioned between $CHCl_3$ (3×20 mL) and H_2O (20 mL). The combined organic extracts were dried ($MgSO_4$) and evaporated to dryness. Crystallization from EtOH/hexane afforded the pure nucleosides.

1-[(2-Acetoxyethoxy)methyl]-6-chloro-7-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (4). From **1** (0.30 g). Yield: 0.34 g, 77%; mp 183–185 °C (dec.); (lit.[30] mp 184–185 °C; yield: 13%); R_f (a) = 0.27. UV λ_{max} (log ε) (MeOH) 329 (3.90), 316 (3.92), [305] [3.81], 288 (4.34), 258 (4.35), [225] [4.20], 215 (4.23). ^1H NMR (600 MHz, $CDCl_3$) δ 8.88 (H-2), 8.24 (d, $J_{H\text{-}5,F}$ = 8.5 Hz, H-5), 8.00 (d, $J_{H\text{-}8,F}$ = 5.9 Hz, H-8), 5.71 (CH_2-1′), 4.25 (t, $J_{H\text{-}4',5'}$ = 4.5 Hz, CH_2-4′), 3.80 (t, $J_{H\text{-}3',4'}$ = 4.5 Hz, CH_2-3′), 2.01 (OAc); ^{13}C NMR ($CDCl_3$) δ 177.7 (CO_2H), 166.1 (C-4), 156.5 (d, $J_{C\text{-}6,F}$ = 247.7 Hz, C-6), 148.5 (C-2), 136.8 (C-8a), 128.3 (d, $J_{C\text{-}4a,F}$ = 5.7 Hz, C-4a), 126.3 (d, $J_{C\text{-}7,F}$ = 20 Hz, C-7), 120.2 (C-8), 119.5 (C-3), 113.2 (d, $J_{C\text{-}5,F}$ = 22.5 Hz, C-5), 81.6 (C-1′), 67.5 (C-3′), 62.4 (C-4′), 20.6 (CH_3). Anal. calcd for $C_{15}H_{13}FClNO_6$ (357.7): C, 50.36; H, 3.66; N, 3.92: Found: C, 50.24; H, 3.59; N, 3.81; *m*/*z* (EI) 357 $(M)^+$.

1-[(2-Acetoxyethoxy)methyl]-6,7-dichloro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (6). From **2** (0.32 g). Yield: 0.33 g, 74%; mp 186–187 °C (dec.); R_f (a) = 0.39.

Table 4. Activity test against different viruses other than HIV and HCMV

Compd	CC_{50} (μg/mL)[a]	EC_{50} (μg/mL)[b]								
		HSV-1 (KOS)	HSV-2 (G)	Vaccinia virus	Vesicular Stomatitis virus	Coxsackie virus B4	Respiratory Syncytical virus	Parainfluenza-3	Reovirus-1	Sindbis virus
5	⩾400	>80	>80	>80	>80	>80	>80	>80	>80	>80
6	400	>80	>80	>80	>80	>80	>80	>80	>80	>80
7	>400	>400	>400	>400	>80	>80	>80	>400	>400	>400
8	400	>80	>80	>80	>80	>80	>80	>80	>80	>80
Brivudin[37]	400	0.0768	>80	3.2	>80	>400	>400	>400	>400	>400
(*S*)-DHPA[38]	>400				240	>400	>400	48	48	>400
Ribavirin[39]	>400	240	240	48	96	48	0.64	16	48	48

[a]Required to cause a microscopically detectable alteration of normal cell morphology.
[b]Required to reduce virus-induced cytopathogenicity by 50%. Brivudin: (*E*)-5-(2-bromovinyl)-2′-deoxyuridine (BVDU). (*S*)-DHPA: (*S*)-9-(2,3-dihydroxypropyl)adenine.

UV_{max} (log ε) (MeOH) 333 ((3.90), 320 (3.94), [309] [3.88], 261 (4.43), 253 (4.42), [227] [4.38], 220 (4.42). ^{1}H NMR ($CDCl_3$) δ 8.87 (H-2), 8.59 (H-5), 8.02 (H-8), 5.70 (CH_2-1′), 4.24 (t, $J_{H\text{-}4',5'}=4.6$ Hz, CH_2-4′), 3.80 (t, $J_{H\text{-}3',4'}=4.6$ Hz, CH_2-3′), 2.02 (OAc). Anal. calcd for $C_{15}H_{13}Cl_2NO_6$ (374.2): C, 48.15; H, 3.50; N, 3.74. Found: C, 47.89; H, 3.42; H, 3.42; N, 3.82; *m/z* (FAB > 0) 374/376 $(MH)^+$.

6,7-Dialkyl-1,4-dihydro-1-[(2-hydroxyethoxy)methyl]-4-oxoquinoline-3-carboxylic acids. A solution of the acetate **4, 6** (2.21 mmol) in 16% NH_3/MeOH (10 mL) was stirred at 23 °C for 5 h. The solution was evaporated to dryness and the residue was stirred with ether (3×20 mL), and filtered. Crystallization from EtOH afforded the free nucleoside.

7-Chloro-6-fluoro-1,4-dihydro-1-[(2-hydroxyethoxy)methyl]-4-oxoquinoline-3-carboxylic acid (5). From **4** (0.79 g). Yield: 0.63 g, 90%; mp 276–277 °C (dec.); (lit.[30] mp 272–275 °C; yield: 92%); R_f (b) = 0.42. UV λ_{max} (log ε) (MeOH) 333 (3.90), 320 (3.98), [307] [3.94], [293] [3.78], 263 (4.44), 254 (4.41), [229] [4.38], 221 (4.42). ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.76 (CO_2H), 9.08 (H-2), 8.27 (d, $J_{H\text{-}8,F}=6.1$ Hz, H-8), 8.21 (d, $J_{H\text{-}5,F}=4.1$ Hz, H-5), 5.91 (CH_2-1′), 4.50 (bs, OH), 3.54 (t, $J_{H\text{-}3',4'}=4.5$ Hz, CH_2-3′), 3.45 (*t*, CH_2-4′); ^{13}C NMR (DMSO-d_6) δ 176.6 (CO_2H), 165.6 (C-4), 156.7 (d, $J_{C\text{-}6,F}=247.5$ Hz, C-6), 149.6 (C-2), 136.9 (C-8a), 127.7 (d, $J_{C\text{-}4a,F}=5.6$, C-4a), 126.4 (d, $J_{C\text{-}7,F}=20.0$ Hz, C-7), 121.4 (C-8), 112.0 (d, $J_{C\text{-}5,F}=22.1$ Hz, C-5), 111.7 (C-3), 83.3 (C-1′), 70.4 (C-3′), 59.8 (C-4′). Anal. calcd for $C_{13}H_{11}FClNO_5$ (315.6): C, 49.46; H, 3.51; N, 4.43. Found: C, 49.31; H, 3.43; N, 4.31; *m/z* (FAB > 0) 338/340 $(MNa)^+$.

6,7-Dichloro-1,4-dihydro-1-[(2-hydroxyethoxy)methyl]-4-oxoquinoline-3-carboxylic acid (7). From **6** (0.82 g). Yield: 0.67 g, 92%; mp 208–210 °C (dec.); R_f (b) = 0.51. UV λ_{max} (log ε) (MeOH) 330 (3.90), 317 (4.01), [4.01], [305] [3.93], [303] [3.92], 259 (4.46), 251 (4.42), [243] [4.32], [227] [4.31], 215 (4.31). ^{1}H NMR (600 MHz, HMQC, DMSO-d_6) δ 12.80 (CO_2H), 9.15 (H-2), 8.40 (H-5), 8.38 (H-8), 5.91 (CH_2-1′), 4.70 (OH), 3.53 (m, CH_2-3′), 3.43 (m, CH_2-4′); ^{13}C NMR (DMSO-d_6) δ 176.9 (CO_2H), 165.3 (C-4), 150.4 (C-2), 138.3 (C-7), 136.9 (C-8a), 129.7 (C-6), 126.8 (C-5), 125.4 (C-4a), 121.0 (C-8), 108.3 (C-3), 83.4 (C-1′), 70.5 (C-3′), 59.8 (C-4′). Anal. calcd for $C_{13}H_{11}Cl_2NO_5$ (332.1): C, 47.01; H, 3.34; N, 4.22. Found: C, 46.71; H, 3.21; N, 4.65; *m/z* (FAB > 0) 354/356 $(MNa)^+$.

7-Chloro-1-[(*E*)-4-chloro-2-butene-1-yl]-1,4-dihydro-6-fluoro-4-oxoquinoline-3-carboxylic acid (9). A suspension of **1** (100 mg, 0.41 mmol) in DMF (5 mL) and NaH (12 mg, 0.50 mmol) was stirred at −40 °C for 1 h. (*E*)-1,4-Dichlorobut-2-ene (**8**) (300 mg, 2.40 mmol) was added and the suspension was stirred at 23 °C for 12 h. Filtration, evaporation of the filtrate and chromatographic purification of the residue [silica gel (30 g); MeOH (0–15%) in $CHCl_3$ as gradient eluent] furnished **9** as a powder (80 mg, 59%); mp 195–198 °C (dec.); R_f (a) = 0.44. UV λ_{max} (log ε) (MeOH) 334 (3.97), 321 (3.98), [306] [3.94], 261 (4.25), 252 (4.21), 215 (4.38). ^{1}H NMR (600 MHz, HMQC, DMSO-d_6) δ 9.08 (H-2), 8.30 (d, $J_{H\text{-}8,F}=5.9$ Hz, H-8), 8.21 (d, $J_{H\text{-}5,F}=9.2$ Hz, H-5), 6.06 (dt, $J_{2',3'}=15.3$ Hz, H-2′), 6.00 (dt, $J_{3',4'}=7.3$ Hz, H-3), 5.28 (d, 1H, $J_{1',2'}=5.6$ Hz, CH_2-1′), 4.12 (d, CH_2-4′); ^{13}C NMR (DMSO-d_6) δ 176.7 (CO_2H), 165.4 (C-4), 154.9 (d, $J_{C\text{-}6,F}=250.3$ Hz, C-6), 150.2 (C-2), 136.5 (C-8a), 130.8 (C-3′), 128.6 (C-2′), 127.3 (d, $J_{C\text{-}7,F}=20.2$ Hz, C-7), 126.0 (d, $J_{C\text{-}4a,F}=5.8$ Hz, C-4a), 121.4 (C-8), 112.0 (d, $J_{C\text{-}5,F}=22.5$ Hz, C-5), 108.1 (C-3), 54.3 (C-4′), 32.5 (C-1′). Anal. calcd for $C_{14}H_{10}FCl_2NO_3$ (330.1): C, 50.93; H, 3.05; N, 4.24. Found: C, 50.82; H, 3.00; N, 4.19; *m/z* (FAB > 0) 330/332 $(MH)^+$.

6,7-Dichloro-1-[(*E*)-4-chloro-2-butene-1-yl)]-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (10). From **2** (0.30 g, 1.16 mol) in the manner described for **9**. Yield 149 mg (37%) as a colorless powder; mp 220–223 °C (dec.); R_f (a) = 0.48. UV λ_{max} (log ε) (MeOH) [340] [4.03], 329 (4.03), [313] [3.98], 266 (4.44), [258] [4.37], [213] [3.98]. ^{1}H NMR (600 MHz, HMQC, DMSO-d_6) δ 8.17 (H-2), 8.00 (H-5), 7.28 (H-8), 5.97 (d, $J_{2',3'}=16.6$ Hz, H-2′), 5.86 (d, $J_{3',4'}=3.0$ Hz, H-3′), 5.18 (d, $J_{1',2'}=5.5$ Hz, CH_2-1′), 4.14 (CH_2-4′); ^{13}C NMR (DMSO-d_6) δ 173.5 (CO_2H), 165.2 (C-4), 147.2 (C-2), 136.8 (C-8a), 130.5 (C-3′), 128.3 (C-5), 128.1 (C-7), 127.9 (C-2′), 127.6 (C-6), 123.0 (C-4a), 118.2 (C-8), 112.6 (C-3), 59.8 (C-4′), 31.8 (C-1′). Anal. calcd for $C_{14}H_{10}Cl_3NO_3$ (346.6): C, 48.52; H,

2.91; N, 4.04. Found: C, 48.31; H, 2.82; N, 3.89; *m/z* (FAB > 0) 347/349 $(MH)^+$.

7-Chloro-1-[(*E*)-4-hydroxy-2-butene-1-yl]-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylic acid (11). To a solution of **9** (200 mg, 0.61 mmol) in DMF (2 mL) and 1.5% HCl (20 mL) was added 5 mL of a fresh aqueous CuCl solution (3.5 mg of CuCl/mL). The mixture was kept at 40 °C for 5 h, neutralized to pH 1 with 1 M NaOH and evaporated to dryness. The residue was purified by chromatography on SiO_2 (10 g) with MeOH (0–60%) in $CHCl_3$ as gradient eluent to give **11** (51 mg, 27%) as a colorless amorphous powder; mp 139–146 °C (dec.); R_f (a) = 0.38. UV λ_{max} (log ε) (MeOH): 333 (3.92), 320 (3.97), [308] [3.94], 260 (4.20), 250 (4.25), 214 (4.36). ^{1}H NMR (DMSO-d_6) δ 9.09 (H-2), 8.21 (d, $J_{H\text{-}8,F}$ = 6.0 Hz, H-8), 8.20 (d, $J_{H\text{-}5,F}$ = 9.0 Hz,-H-5), 6.00 (dt, $J_{2',3'}$ = 15.0 Hz, H-2), 4.01 (ddd, $J_{3',4'}$ = 5.2 Hz, H-4′), 5.20 (d, $J_{1',2'}$ = 4.4 Hz, CH_2-1′), 4.62 (d, J = 5.6 Hz, $C_{4'}$-OH), 3.90 (m, CH_2-4′); ^{13}C NMR (DMSO-d_6) δ 176.3 (CO_2H), 164.1 (C-4), 153.8 (d, $J_{C\text{-}6,F}$ = 249.2 Hz, C-6), 150.0 (C-2), 135.2 (C-8a), 131.0 (C-3′), 128.4 (C-2′), 126.8 (d, $J_{C\text{-}7,F}$ = 20 Hz, C-7), 125.7 (d, $J_{C\text{-}4a,F}$ = 5.7 Hz, C-4a), 121.1 (C-8), 112.0 (d, $J_{C\text{-}5,F}$ = 21.5 Hz, C-5), 107.7 (C-3), 36.1 (C-4′), 31.7 (C-1′). Anal. calcd for $C_{14}H_{11}FCl_2NO_4$ (311.6): C, 53.95; H, 3.56; N, 4.49. Found: C, 53.70; H, 3.48; N, 4.38; *m/z* (FAB > 0) 312/314 $(MH)^+$.

[(*E*)-4-Hydroxy-2-butene-1-yl]-7-chloro-1-[(*E*)-4-chlorobutene-1-yl]-6-fluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate (12). A mixture of **1** (0.40 g, 1.6 mmol), K_2CO_3 (0.55 g, 4.0 mmol) and the olefine **8** (1.0 g, 8.0 mmol) in abs. DMF (5 mL) was stirred under N_2 at 80 °C for 10 h. After cooling, the mixture was acidified with 1 M HCl to pH 1 and then diluted with H_2O (50 mL). Extraction with ethyl acetate (6×20 mL) and work up of the combined organic extracts afforded 0.38 g of an oil, which was purified by column chromatography on SiO_2 (50 g) with MeOH (0–10%) in $CHCl_3$ as gradient eluent to give **12** (0.12 g, 33%) as a colorless powder; mp 110–14 °C; R_f (a) = 0.62. UV λ_{max} (log ε) (MeOH): 335 (3.94), 323 (4.00), [306] [3.95], 262 (4.32), 253 (4.28), [244] [4.12], 215 (4.40). ^{1}H NMR (600 MHz, HMQC, DMSO-d_6) δ 8.73 (H-2), 8.06 (d, $J_{H\text{-}8,F}$ = 5.7, H-8), 8.03 (d, $J_{H\text{-}5,F}$ = 9.0 Hz, H-5), 6.01 (m, H-3″), 5.85 (dt, $J_{3'\text{-}4'}$ = 5.0 Hz, H-3′), 5.79 (m, $J_{2',3'}$ = 15.2 Hz, H-2′), 5.09 (d, $J_{1'',2''}$ = 5.2 Hz, CH_2-1″), 4.74 (CH_2-1′), 4.26 (m, CH_2-4′), 4.20 (m, CH_2-4″); ^{13}C NMR (DMSO-d_6) δ 171.4 (CO_2H), 164.0 (C-4), 154.0 (d, $J_{C\text{-}6,F}$ = 250.0 Hz, C-6), 149.9 (C-2), 136.1 (C-8a), 134.8 (C-2″), 129.7 (C-3′), 128.5 (C-3″), 127.1 (d, $J_{C\text{-}7,F}$ = 21.0 Hz, C-7), 125.4 (d, $J_{C\text{-}4a,F}$ = 5.5 Hz, H-4a), 122.3 (C-2′), 120.2 (C-8), 112.3 (C-5), 97.0 (C-3), 53.3 (C-4′), 43.8 (C-4″), 43.2 (C-1″), 32.9 (C-1′). Anal. calcd for $C_{18}H_{16}FCl_2NO_4$ (400.2): C, 54.02; H, 4.03; N, 3.50. Found: C, 53.82; H, 3.95; N, 3.38; *m/z* (FAB > 0) 400/402 $(MH)^+$, 422/424 $(MNa)^+$.

Ethyl 6,7-dichloro-1-[(*E*)-buta-1,3-dienyl]-1,4-dihydro-4-oxoquinoline-3-carboxylate (15). To a stirred solution of **13** (0.50 g, 1.84 mmol) in abs. DMF (10 mL) was added dry NaH (0.13 g, 5.41 mmol). When the evolution of H_2 had ceased, the mixture was stirred at 23 °C for 1 h. After addition of **8** (0.92 g, 7.35 mmol), the mixture was stirred under N_2 at 60 °C for 2 h. Work up in the manner described for **12** furnished **15** (231 mg, 35%) as a colorless powder; mp 90–95 °C (dec.); R_f (a) = 0.76. UV λ_{max} (log ε) (MeOH): [338] [3.96], 328 (3.97), [310] [3.93], 265 (4.34), [257] [4.31], 222 (4.50). ^{1}H NMR (600 MHz, HMQC, DMSO-d_6) δ 8.52 (H-5), 8.36 (H-2), 7.53 (H-8), 6.56 (m, H-1′, H-2′), 6.26 (dt, $J_{3',4'}$ = 1.0 Hz, H-3), 5.65 (dd, $J_{4',4''}$ = 14.0 Hz, H-4′), 5.48 (dd, H-4″), 4.39 (q, J = 7.2 Hz, CH_2CH_3), 1.39 (t, CH_2CH_3); ^{13}C NMR (DMSO-d_6) δ 173.4 (CO_2H), 165.2 (C-4), 148.9 (C-2′), 138.2 (C-7), 132.4 (C-2′), 137.5 (C-8a), 128.9 (C-5), 125.7 (C-4′), 125.2 (C-4a), 125.0 (C-1′), 118.7 (C-8), 112.2 (C-3), 61.2 (CH_2CH_3), 14.3 (CH_2CH_3). Anal. calcd for $C_{16}H_{13}Cl_2NO_3$ (338.2): C, 56.82; H, 3.87; N, 4.14. Found: C, 56.61; H, 3.79; N, 4.03; *m/z* (FAB) 337/339 $(M)^+$.

Dimethyl 7-chloro-8-fluoro-5,10-dihydro-1,10-dioxo-5-[1-oxo-3,4,5-tris(methoxycarbonyl)-cyclopentadien-2-yl]-1*H*-pyrano[4,3-*b*]quinoline-3,4-dicarboxylate (17). To a suspension of **1** (0.50 g, 2.07 mmol) and K_2CO_3 (0.71 g, 5.17 mmol) in DMF (10 mL) the acetylene **16** (1.47 g, 10.35 mmol) was added. The mixture was stirred at 80 °C for 20 min. Evaporation of solvent and partitioning of the residue between $CHCl_3$ (3×20 mL) and H_2O (30 mL) afforded after work up an orange oil, which was purified by chromatography on silica gel (50 g) with $CHCl_3$ as eluent. Compound **17** was isolated as an orange amorphous powder (0.30 g, 23%); mp 104–110 °C; R_f (a) = 0.84. UV λ_{max} (log ε) (MeOH): 373 (4.07), [299] [4.08], 258 (4.49), 220 (4.55). ^{1}H NMR ($CDCl_3$) δ 8.08 (d, $J_{H\text{-}6,F}$ = 8.2 Hz, H-6), 7.63 (d, $J_{H\text{-}9,F}$ = 5.5 Hz, H-9), 3.88, 3.84, 3.81, 3.77, 3.75 (5s, CO_2Me); ^{13}C NMR (600 MHz, $CDCl_3$) δ 166.2, 165.5, 165.0, 164.6, 163.9, 163.5, 163.0 (C=O), 157.0 (d, $J_{C\text{-}8,F}$ = 249.0 Hz, C-8), 140.6 (C-4a), 137.2 (C-5a), 134.7 (d, $J_{C\text{-}9a,F}$ = 5.5 Hz, C-9a), 134.5 (C-4), 131.8 (C-1′), 128.5 (d, $J_{C\text{-}7,F}$ = 20.0 Hz, C-7), 124.5 (C-4′), 121.6 (C-6), 121.4 (C-3′), 119.3 (C-5′), 116.2 (C-10a), 113.4 (d, $J_{C\text{-}9,F}$ = 22.0 Hz, C-9), 113.2 (C-3), 54.0, 53.4, 53.3, 52.5, 52.1 (CO_2Me). Anal. calcd for $C_{26}H_{17}FClNO_{14}$ (621.8): C, 50.22; H, 2.76; N; 2.25. Found: C, 50.00; H, 2.68; N, 2.09; *m/z* (FAB > 0) 622/624 $(MH)^+$, 644/646 $(MNa)^+$.

Dimethyl 7,8-dichloro-5,10-dihydro-1,10-dioxo-5-[1-oxo-3,4,5-tris(methoxycarbonyl)-cyclopentadien-2-yl]-1*H*-pyrano[4,3-*b*]quinoline-3,4-dicarboxylate (18). Compound **18** was prepared from **2** (0.18 g, 0.69 mmol) and **16** (0.45 g, 3.33 mmol) in the manner described for **17**. Yield: 0.13 g (29%); mp 93–102 °C; R_f (a) = 0.88. UV λ_{max} (log ε) (MeOH): [372] [4.05], [299] [4.07], 262 (4.32), 253 (4.41), 218 (4.51). ^{1}H NMR ($CDCl_3$) δ 8.59 (H-6), 8.49 (H-9), 3.84, 3.77, 3.72, 3.68, 3.65 (CO_2Me); ^{13}C NMR (600 MHz, $CDCl_3$) δ 165.2, 165.0, 164.7, 164.0, 163.6, 161.1 (C-4), 140.3 (C-4a), 137.0 (C-5a), 134.4 (C-9a), 134.1 (C-4), 132.0 (C-1′), 128.1 (C-7), 127.5 (C-8), 125.0 (C-4′), 121.7 (C-6), 121.0 (C-3′), 118.8 (C-5′), 116.0 (C-10a), 112.8 (C-9), 112.2 (C-3), 53.8, 53.2, 53.0, 52.5, 52.0 (CO_2Me). Anal. calcd for $C_{26}H_{17}Cl_2NO_{14}$ (638.3): C, 48.92; H, 2.68; N, 2.19. Found: C, 48.64; H, 2.57; N, 2.08; *m/z* (FAB > 0) 660/662 $(MNa)^+$.

Acknowledgements

We thank Dr. A. Geyer, Faculty of Chemistry, University of Konstanz (Germany), for the 2-D NMR measurements and Mrs. M. J. Quelle and Mr. K. Hägele for the mass spectroscopic analyses.

References and Notes

1. Smith, T. J.; Lewin, C. S. In *The Quinolones*; Andriole, V. T., Ed.; Academic: London, 1988; Chapter 2.
2. Lesher, G. Y.; Froelich, E. J.; Gruett, M. D.; Bailey, J. H.; Brundage, R. P. *J. Med. Chem.* **1962**, *5*, 1063.
3. Nasr, M.; Drach, J. C.; Smith, S. H.; Shipman, C.; Burckhalter, J. H. *J. Med. Chem.* **1988**, *31*, 1347.
4. Baker, B. R.; Bramhall, R. R. *J. Med. Chem.* **1972**, *15*, 230.
5. Koga, H.; Itoh, A.; Murayama, S.; Suzue, S.; Ivikura, T. *J. Med. Chem.* **1980**, *23*, 1358.
6. Wise, R.; Andrews, J. M.; Edwards, L. *J. Antimicrob. Agents Chemother.* **1983**, *23*, 559.
7. Wentland, M. P.; Bailey, D. M.; Cornett, J. B.; Dobson, R. A.; Powles, R. G.; Wagner, R. B. *J. Med. Chem.* **1984**, *27*, 1103.
8. Miyamoto, T.; Matsumoto, J.; Chiba, K.; Egawa, E.; Shibamori, K.; Minamida, A.; Nishimura, Y.; Okada, H.; Kataoka, M.; Fuzita, M.; Hirose, T.; Nakano, J. *J. Med. Chem.* **1990**, *33*, 1645.
9. Fujita, M.; Egawa, H.; Miyamoto, T.; Nakano, J.; Matsumoto, J. I. *Chem. Pharm. Bull* **1996**, *44*, 987.
10. de la Cruz, A.; Elguero, J.; Goya, P.; Martinez, A.; De Clercq, E. *J. Chem. Soc., Perkin. Trans. 1* **1993**, 845 and references therein.
11. da Matta, A. D.; Bernardino, A. M. R.; Romeiro, G. A.; de Oliveira, M. R. P.; de Souza, M. C. B. V.; Ferreira, V. F. *Nucleosides Nucleotides* **1996**, *15*, 889.
12. Al-Masoudi, N. A.; Al-Soud, Y. A.; Ehermann, M.; DeClercq, E. *Nucleosides Nucleotides* **1998**, *17*, 2255.
13. Schaeffer, H. J.; Beauchamp, L.; De Miranda, P.; Elion, G. B.; Bauer, D. J.; Collins, P. *Nature (London)* **1987**, *272*, 583.
14. Martin, J. C.; Dvorak, C. A.; Smee, D. F.; Mattews, T. R.; Verheyden, J. P. H. *J. Med. Chem.* **1983**, *26*, 759.
15. Keller, P. M.; Fyfe, J. A.; Beauchamp, L.; Lubbers, C. M.; Furman, P. A.; Schaeffer, H. J.; Elion, G. B. *Biochem. Pharmacol.* **1981**, *30*, 3071.
16. Reines, E. D.; Gross, P. A. *Antiviral Agents Med. Clin. North Am.* **1988**, *72*, 691.
17. Kelsey, J. E.; Biron, K. K.; Collins, P.; Selway, J.; Lin, J.-C.; Schaeffer, H. J. *J. Med. Chem.* **1988**, *31*, 144.
18. De Clercq, E. *Clin. Microbiol. Rev.* **1997**, *10*, 674.
19. Ashton, W. T.; Canning, L. F.; Wagner, A. F.; Cantone, C.; Walton, E.; Patel, G. F.; Tolman, R. L.; Karkas, J. D.; Field, A.K. 192nd National Meeting of the American Chemical Society, Anaheim, CA, 7–12 September, 1986.
20. Hagberg, C.-E.; Johansson, K. N.-G.; Kovacs, Z. M. I.; Stening, G. B. European Patent 55 239; Bulletin 1982 82/26.
21. Johansson, K. N.-G.; Lindborg, B. G.; Noren, J.-O. European Patent 146 516; *Bulletin* **1985**, 85/26.
22. Haines, D. R.; Tseng, C. K. H.; Marques, V. E. *J. Med. Chem.* **1987**, *30*, 943.
23. Hua, M.; Korkowski, P. M.; Vince, R. *J. Med. Chem.* **1987**, *30*, 198.
24. Phadtare, S.; Zemlicka, J. *J. Med. Chem.* **1987**, *30*, 437.
25. Phadtare, S.; Zemlicka, J. *Nucleic Acids, Symp. Ser. No. 18*, **1987**, 25.
26. Phadtare, S.; Fessel, D.; Zemlicka, J. *Nucleosides Nucleotides* **1989**, *8*, 907.
27. Zemlicka, J. *Nucleosides Nucleotides* **1997**, *16*, 1003.
28. Niedballa, U.; Vorbrüggen, H. *J. Org. Chem.* **1974**, *39*, 3654.
29. Robins, M. J.; Hatfield, P. W. *Can. J. Chem.* **1982**, *60*, 547.
30. de la Cruz, A.; Elguero, J.; Goya, P.; Martinez, A.; Gotor, V.; Moris, F.; De Clercq, E. *J. Chem. Res. (S)* **1992**, 216; and *J. Chem. Res. (M)* **1992**, 1682.
31. (a). Rosowsky, A.; Kim, A.; Wick, M. *J. Med. Chem.* **1982**, *24*, 1177. (b) Robins, M. J.; Hatfield, P. W. *Can. J. Chem.* **1982**, *60*, 547.
32. de la Cruz, A.; Elguero, J.; Goya, P.; Martinez, A.; Pfleiderer, W. *Tetrahedron* **1992**, *48*, 6135 and references therein cited.
33. Al-Masoudi, N. A.; Al-Soud, Y. A.; Geyer, A. *Spectroscopy Lett.* **1998**, *31*, 1031.
34. Katrizky, A. R.; Ellison, J; Frank, J.; Ra'ko'czy, P.; Radics, L.; Ga'cs-Baitz, E. *Org Mag. Res.* **1981**, *18*, 280.
35. (a) De Clercq, E. *Rev. Med. Virol.* **1993**, *3*, 85. (b) Snoeck, R.; Sakuma, T.; De Clercq, E.; Rosenberg, I.; Holy, A. *Antimicrob. Agents Chemother.* **1988**, *32*, 1839.
36. (a) Schinazi, R. F.; Mead, J. R.; Feorino, P. M. *AIDS Res. Hum. Retroviruses* **1992**, *8*, 963. (b) Schinazi, R. F. *Perspect. Drug Discovery Des.* **1993**, *1*, 151–180.
37. De Clercq, E.; Descamps, J.; De Somer, P.; Barr, P. J.; Jones, A. S.; Walker, R. T. *Proc. Natl. Acad. Sci. USA* **1979**, *76*, 2947.
38. De Clercq, E. *Biochem. Pharmacol.* **1991**, *42*, 963.
39. Sidwell, R. W.; Huffman, J. H.; Khare, G. P.; Allen, L. B.; Witkowski, J. T.; Robins, R. K. *Science* **1972**, *177*, 705.