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Reference Data

NMR spectral data for ester prodrugs of ganciclovir

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ABSTRACT: A series of 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine mono- and diesters, **2a–i**, **3a–d** and **4a–d**, were synthesized as potential prodrugs of ganciclovir and both ^{1}H NMR and ^{13}C NMR spectra were assigned to these esters based on spectral comparison with compounds of similar structure. Copyright © 2000 John Wiley & Sons, Ltd.

KEYWORDS: NMR; ^{1}H NMR; ^{13}C NMR; ganciclovir; prodrug; esterification

INTRODUCTION

9-[(1,3-Dihydroxy-2-propoxy)methyl]guanine (ganciclovir, **1**, Scheme 1) is a potent and selectively active agent against cytomegalovirus.[1] Since the administration of ganciclovir intravenously and orally is not optimal, various prodrugs have been synthesized and evaluated with the aim of improving its delivery and half-life.[2–6] The use of esters to improve the bioavailablity of acyclovir, another antiherpes agent, has many precedents.[7–10] Therefore, we synthesized a series of esters of ganciclovir to investigate their effectiveness in improving pharmacokinetic properties and enhancing their intraocular retention after intravitreal administration. During the synthesis, the product structures were confirmed by their NMR chemical shifts, mass spectrometry (MS) and high-performance liquid chromatography (HPLC). NMR spectral data for several series of novel acyclovir prodrugs are reported. The NMR assignments of the carbon signals are based on spectral comparison with compounds of similar structure.[2,11–16]

EXPERIMENTAL

Compounds

All melting points were measured in open capillaries and are uncorrected. Ganciclovir solution in dimethylformamide (DMF) was obtained by heating ganciclovir in DMF on a steam-bath and cooling to 23 °C. Column chromatography was performed with CH_2Cl_2–CH_3OH (9 : 1) as the eluent.

General procedure for synthesis of compounds 2a–d. DMAP (40 mg, 0.33 mmol, 0.2 equiv.) and acid anhydride (6.3 mmol, 4 equiv.) were added to a solution of ganciclovir (400 mg, 1.57 mmol) in DMF (40 ml). After stirring at 23 °C for 2–4 h, water (2 ml) was added and the solvent was evaporated *in vacuo*. The resulting oil was chromatographed on a column of silica gel to yield compounds **2a–d**.

General procedure for synthesis of compounds 2e–g. To a solution of ganciclovir (500 mg, 1.96 mmol) in DMF (50 ml) were added acid (4.90 mmol, 2.5 equiv.), DMAP (50 mg, 0.41 mmol, 0.2 equiv.) and *N,N*-dicyclohexylcarbodiimide (DCC) (1.2 g, 5.88 mmol, 3 equiv.). The resulting solution was stirred at 23 °C for 24 h and the same amounts of acid, DMAP and DCC were recharged. The reaction mixture was stirred at 23 °C for 24 h and quenched by addition of water (2 ml). The solvent was removed *in vacuo* and the resulting oil was chromatographed on a column of silica gel to afford **2e–g**.

General procedure for synthesis of compounds 2h and i. To a solution of ganciclovir (500 mg, 1.96 mmol) in DMF (50 ml) were added acid (4.90 mmol, 2.5 equiv.), DMAP (50 mg, 0.41 mmol, 0.2 equiv.) and DCC (1.2 g, 5.88 mmol, 3 equiv.). The resulting solution was stirred at 23 °C for 24 h and quenched by addition of water (2 ml). The solvent was removed *in vacuo* and the resulting oil was chromatographed on a column of silica gel to afford **2h** and **i**.

General procedure for synthesis of compounds 3a–d and 4a–d. To a solution of ganciclovir (500 mg, 1.96 mmol) in DMF (50 ml) were added trimethyl orthoacetate (0.45 ml, 3.5 mmol, 1.7 equiv.) and trifluoroacetic acid (TFA) (0.16 ml, 2.08 mmol, 1.06 equiv.). The resulting solution was stirred at 23 °C for 2 h and quenched by addition of water (4 ml). After stirring at 23 °C overnight, the solvent was removed *in vacuo* and the resulting oil was chromatographed on a column of silica gel to afford **3a–d** and **4a–d**.

Mass spectrometry

The compounds were dissolved in CH_3OH and injected at 20 μl min^{-1} into the electrospray source using a Harvard Apparatus Model 22 syringe pump. Electrospray ionization (ESI) mass spectra were obtained on a Finnigan TSQ-700 triple-quadrupole mass spectrometer equipped with an electrospray source. The mass spectra were acquired at 4 s per scan and 2–4 scans were averaged. A capillary temperature of 200 °C was used. The spray voltage was 4.5 kV. Dry nitrogen was used as the sheath gas and auxiliary gas for the analysis.

NMR spectroscopy

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 250-modified Tecmag DSPect Fourier transform NMR spectrometer at 250 and 63 MHz, respectively, for 0.25–0.5 M DMSO-d_6 solutions at 23 °C. Chemical shifts are expressed in ppm relative to tetramethylsilane (TMS) as internal standard. For the ^{13}C NMR spectra, the spectral width was 20 000 Hz and the number of data points was 16 000, generating a 2.5 Hz per point digital resolution. The flip angle was 4 μs (90°) and the acquisition time was 0.409 s with a pulse delay of 3 s; 500–1000 scans were accumulated for each spectrum.

RESULTS AND DISCUSSION

Syntheses

The simple short-chain alkyl (*O*-acyl) diesters of {9-[(1,3-dihydroxy-2-propoxy)methyl]guanine} (ganciclovir, **1**, Scheme 1) were prepared in a single-step reaction, using a modified procedure of Martin *et al*.[2] A solution of ganciclovir in DMF was treated with acid anhydride in the presence of a catalytic amount of 4-dimethylaminopyridine (DMAP) at room temperature for 2–4 h. Triacyl derivatives of acyclovir were observed to be the major product using this literature protocol.[2] For longer chain diesters of ganciclovir, the corresponding acid was activated with DCC according to literature protocol.[2] However, the monoester was found to be the major product along with the diester product. Reactivation of the acid, DMAP and DCC is necessary to convert the monoester to the diester.

The preparation of the mono-*O*-acyl derivative of ganciclovir was reported in a three-step procedure by Martin *et al*.[2] However, we found that the first step, selective protection of one of the two hydroxyl functions in ganciclovir by trityl chloride, gave only a 20% instead of a 38% yield.[2] Using 1 equiv. of acid anhydride produced a significant amount ganciclovir and diester byproduct. Finally, a one-step procedure[17] by reaction of ganciclovir with an excess of trimethyl orthoacetate followed

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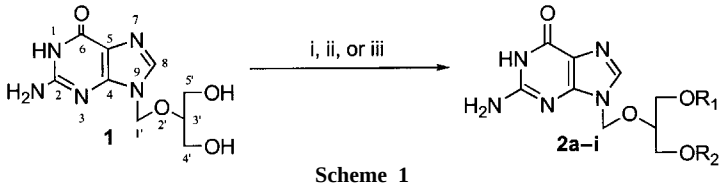
Reference Data



Scheme 1

Compound	R_1	R_2	Yield (%)	M P (°C)	MS $[M+1]^+$
2a	CH_3CO	CH_3CO	90%	243–245	340(92)
2b	C_2H_5CO	C_2H_5CO	88%	190–192	368(100)
2c	*n*-C_3H_7CO	*n*-C_3H_7CO	80%	198–200	396(100)
2d	*i*-C_3H_7CO	*i*-C_3H_7CO	80%	208–210	396(100)
2e	*n*-$C_8H_{17}CO$	*n*-$C_8H_{17}CO$	53%	155–157	536(100)
2f	*n*-$C_{10}H_{21}CO$	*n*-$C_{10}H_{21}CO$	78%	160–162	592(100)
2g	*n*-$C_{12}H_{25}CO$	*n*-$C_{12}H_{25}CO$	74%	160–162	648(50)
2h	*n*-$C_8H_{17}CO$	H	23%	190–192	396(32)
2i	*n*-$C_{12}H_{25}CO$	H	22%	218–220	452(100)

(i) $(R)_2O$ (4 equiv.), DMAP (0.2 equiv.), DMF, rt, 2–4 h, **2a–d**.
(ii) ROH (2.5 equiv.), DMAP (0.2 equiv.), DCC (3 equiv.), DMF, rt, 24 h, then, recharge ROH (2.5 equiv.), DMAP (0.2 equiv.), DCC (3 equiv.), rt, 24 h, **2e–g**.
(iii) ROH (2.5 equiv.), DMAP (0.2 equiv.), DCC (3 equiv.), DMF, rt, 24 h, **2h–i**.

Table 1. ^{1}H NMR data for ganciclovir and its di-*O*-acyl derivatives

Proton	**1**[13]	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**[a]	**2g**
$OCH(OCH_2)$ 3′, 4′ and 5′	3.35 (m, 4H) 4.63 (p, 1H)	3.95–4.10 (m, 5H)	3.99–4.15 (m, 5H)	4.03–4.10 (m, 5H)	3.99–4.17 (m, 5H)	4.03–4.09 (m, 5H)	4.14–4.26 (m, 5H)	4.04–4.09 (m, 5H)
NCH_2O, 1′	5.44 (s)	5.42 (s)	5.44 (s)	5.42 (s)	5.45 (s)	5.43 (s)	5.56 (s)	5.44 (s)
2-NH_2	6.50 (bs)	6.52 (bs)	6.55 (bs)	6.54 (bs)	6.56 (bs)	6.54 (bs)	6.77 (bs)	6.46 (bs)
H-8	7.81 (s)	7.83 (s)	7.85 (s)	7.84 (s)	7.87 (s)	7.83 (s)	7.81 (s)	7.77 (s)
H-1	10.64 (bs)	10.65 (bs)	10.75 (bs)	10.71 (bs)	10.78 (bs)	10.75 (bs)	12.42 (bs)	10.73 (bs)
*R*COO		1.90 (s, 6H)	0.97 (t, 6H) 2.20 (q, 4H)	0.85 (d, 6H) 1.47 (bs, 4H) 2.15 (bs, 4H)	1.03 (d, 12H) 2.42 (m, 2H)	0.85 (bs, 6H) 1.23–1.44 (m, 24H) 2.17 (t, 4H)	0.85–1.57 (m, 38H) 2.28 (bs, 4H)	0.86 (bs, 6H) 1.24–1.47 (m, 40H) 2.17 (t, 4H)

[a] Solvent: $CDCl_3$.

Table 2. ^{1}H NMR data for ganciclovir mono-*O*-acyl derivatives

Proton	**3a**	**3b**	**3c**	**3d**	**2h**	**2i**
$OCH(OCH_2)$ 3′ and 4′	3.82–4,10 (m, 3H)	3.94–4.09 (m, 3H)	3.84–4.09 (m, 3H)	3.85–4.12 (m, 3H)	3.83–4.08 (m, 5H)	3.81–4.07 (m, 3H)
CH_2OH 5′	3.42 (bs)	3.45 (bs)	3.45 (bs)	3.45 (bs)	3.44 (d)	3.41 (bs)
NCH_2O, 1′	5.44 (s)	5.46 (s)	5.46 (s)	5.46 (s)	5.45 (s)	5.43 (s)
2-NH_2	6.76 (bs)	6.76 (bs)	6.76 (bs)	6.74 (bs)	6.59 (bs)	6.64 (bs)
H-8	7.84 (s)	7.87 (s)	7.86 (s)	7.86 (s)	7.84 (s)	7.82 (s)
H-1	9.70 (bs)	10.18 (bs)	11.02 (bs)	10.35 (bs)	10.80 (bs)	10.72 (bs)
*R*COO	1.86 (s, 3H)	0.94 (t, 3H) 2.15 (q, 2H)	0.83 (bs, 3H) 1.43 (bs, 2H) 2.09 (bs, 2H)	0.85 (t, 3H) 1.22–1.39 (m, 4H) 2.09 (t, 2H)	0.85 (bs, 3H) 1.22–1.40 (m, 12H) 2.10 (t, 2H)	0.86 (bs, 3H) 1.23 (bs, 20H) 2.11 (t, 2H)

by acidic hydrolysis of the cyclic orthoester intermediate yielded satisfactory results (74%). The other three mono-*O*-acyl derivatives were prepared by the same strategy.

NMR spectroscopy

The ^{1}H NMR spectral data and assignments for di-*O*-acyl derivatives (**2a–g**), mono-*O*-acyl derivatives (**3a–d**, **2h** and **i**) and di-*N*,*O*-acyl derivatives (**4a–d**) are listed in Tables 1, 2 and 3, respectively. The ^{13}C NMR data for **2a–g**, **3a–d**, **2h** and **i** and **4a–d** are listed in Tables 4, 5 and 6, respectively. Table 7 summarizes the substituent effects of various carbonyl groups on the parent compound **1** (ganciclovir), calculated from the chemical shifts in Tables 1–6. Their structures are illustrated in Schemes 1 and 2. All NMR assignments are based on comparison with compounds of similar structure.

Reference Data

Table 3. ^{1}H NMR data for ganciclovir diacyl derivatives

Proton	**4a**	**4b**	**4c**	**4d**
OCH(OCH_2) 3′ and 4′	3.82–4,17 (m, 3H)	3.84–4.08 (m, 3H)	3.85–4.07 (m, 3H)	3.85–4.04 (m, 3H)
CH_2OH 5′ NCH_2O, 1′	3.17 (bs) 5.54 (s)	3.17 (bs) 5.54 (s)	3.18 (bs) 5.55 (s)	3.19 (bs) 5.58 (s)
2-NH	12.11 (bs)	12.11 (bs)	12.15 (bs)	12.24 (bs)
H-8	8.14 (s)	8.14 (s)	8.15 (s)	8.17 (s)
H-1	11.86 (bs)	11.81 (bs)	11.85 (bs)	11.94 (bs)
*R*COO	1.81 (s, 3H)	0.90 (t, 3H) 2.08 (q, 2H)	0.79 (t, 3H) 1.38 (m, 2H) 2.02 (t, 2H)	0.82–0.92 (m, 7H) 2.04 (t, 2H)
*R*CNH	2.19 (s, 3H)	1.08 (t, 3H) 2.48 (q, 2H)	0.92 (t, 3H) 1.62 (m, 2H) 2.46 (t, 2H)	1.34–1.59 (m, 7H) 2.52 (t, 2H)

As stated in Table 1, conversion of OH to OR_1 and OR_2 (aliphatic esters) leads to deshielded ^{1}H NMR chemical shifts of 0.65–0.72 ppm for the protons of the carbon (CH_2OCO) to which these groups are attached and the β-situated (OCH) proton (compare 1 with **2a–g**). In comparsion with ganciclovir (**1**), the remaining protons showed no chemical shift change.

As summarized in Table 2, derivatization one of two OHs to OR leads to lower deshielded ^{1}H NMR chemical shifts on the 9-side-chain. The protons of the carbon (CH_2OCO) to which these groups are attached and the β-situated (OCH) proton are deshielded by 0.51–0.55 ppm. The protons of the carbon attached to a hydroxyl group revealed no appreciable chemical shift change.

As depicted in Table 3, additional derivatization of H_2N— to RHN— leads to the same chemical shift changes on the 9-side-chain. In addition, both H-1 and H-8 are deshielded, by 1.14–1.27 and 0.32–0.35 ppm, respectively. The 2-amino group disappeared from the spectra with the loss of its 6.52 ppm signal, and the amide proton appeared between 12.11 and 12.24 ppm.

As shown in Table 4, conversion of OH to OR (aliphatic esters) has the effect of deshielding the carbons (CH_2OR) directly attached by the substituent (4′,5′-positions) by 1.55–1.82 ppm, but the adjacent carbon (3′-position) is shielded by 6.09–6.28 ppm. The rest of the carbons do not exhibit any appreciable chemical shift changes.

As summarized in Table 5, derivatization of one of two OHs to OR leads to the different chemical shift changes on the 9-side-chain. The carbons (CH_2OCO) to which these groups are attached is deshielded by 2.33–2.56 ppm. The adjacent carbon (3′-position) is shielded by 2.98–3.06 ppm. The carbons bonded with hydroxyl group are shielded by 0.39–0.47 ppm. The ^{13}C NMR shielding effect on the 3′-carbon can be attributed to steric repulsion of electron density in the C—H bond towards the carbon. Crowding of the 3′-carbon is more prominent in the di-*O*-acyl derivatives in Table 4 which exhibit the largest shielding effect (−6.1 versus −3.0 ppm)

As depicted in Table 6, a similar change of OH to OR and H_2N—

Table 5. ^{13}C NMR data for ganciclovir mono-*O*-acyl derivatives

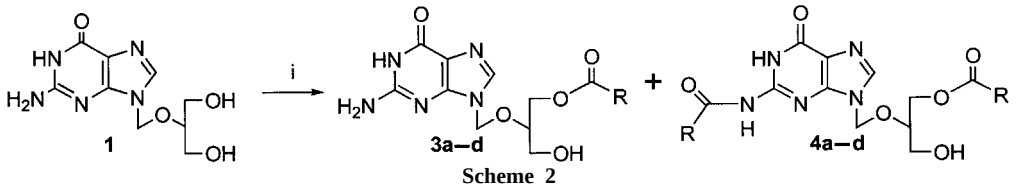
Carbon	**3a**	**3b**	**3c**	**3d**	**2h**	**2i**
2	154.02	153.99	153.99	153.99	153.87	153.79
4	151.38	151.38	151.38	151.42	151.35	151.27
5	116.29	116.33	116.33	116.37	116.41	116.41
6	156.97	157.05	157.01	157.05	156.90	156.78
8	137.72	137.88	137.72	137.80	137.68	137.57
NCH_2O, 1′	71.35	71.31	71.27	71.31	71.27	71.23
CH_2OH, 5′	60.28	60.32	60.32	60.36	60.32	60.28
CH_2O, 4′	63.31	63.23	63.12	63.15	63.12	63.08
CHO, 3′	76.82	76.86	76.86	76.90	76.86	76.78
*R*COO	20.30	8.77 26.47	13.28 17.74 35.01	13.55 21.58 26.43 32.92	13.90 22.09 24.30 28.41 28.57 28.65 31.25 33.15	13.90 22.09 24.30 28.41 28.72 28.92 29.04 31.29 33.11
R*COO*	170.13	173.43	172.54	172.73	172.69	172.62

Table 4. ^{13}C NMR data for ganciclovir and its di-*O*-acyl derivatives

Carbon	**1**[13]	**2a**	**2b**	**2c**	**2d**	**2e**	**2f**[a]	**2g**
2	153.78	153.71	153.83	153.79	153.87	153.87	154.41	153.91
4	151.27	151.27	151.31	151.27	151.35	151.35	152.05	151.38
5	116.40	116.37	116.45	116.49	116.53	116.49	116.49	116.60
6	156.87	156.66	156.78	156.74	156.86	156.82	159.23	156.97
8	137.62	137.84	137.84	137.84	137.92	137.80	137.64	137.53
NCH_2O, 1′	71.48	71.07	71.03	71.00	70.88	71.07	71.54	71.00
CH_2O, 4′ 5′	60.90	62.57	62.53	62.42	62.49	62.42	62.73	62.30
CHO, 3′	80.00	73.67	73.79	73.71	73.75	73.75	74.30	73.60
*R*COO		20.22	8.73 26.43	13.28 17.70 34.94	18.55 32.99	13.90 22.09 24.25 28.41 28.57 28.69 31.25 33.15	14.13 22.67 24.84 29.15 29.35 29.50 29.58 31.91 34.04	13.94 22.24 24.38 28.57 28.88 29.04 29.15 31.44 33.30
R*COO*		169.94	173.24	172.38	175.76	172.50	173.47	172.46

[a] Solvent: $CDCl_3$.

Reference Data



Scheme 2

Compound	R	Yield(%)	M P (°C)	MS $[M+1]^+$
3a	CH_3	74%	133–135	298(100)
3b	C_2H_5	87%	138–140	312(78)
3c	n-C_3H_7	83%	135–137	326(100)
3d	n-C_4H_9	90%	180–182	340(100)
4a	CH_3	17%	glass	340(100)
4b	C_2H_5	6%	glass	368(16)
4c	n-C_3H_7	15%	glass	396(100)
4d	n-C_4H_9	5%	glass	424(28)

(i) $RC(OCH_3)_3$ (1.7 equiv.), TFA (1 equiv.), DMF, rt, 2 h, then H_2O, overnight, **3a–d**, **4a–d**.

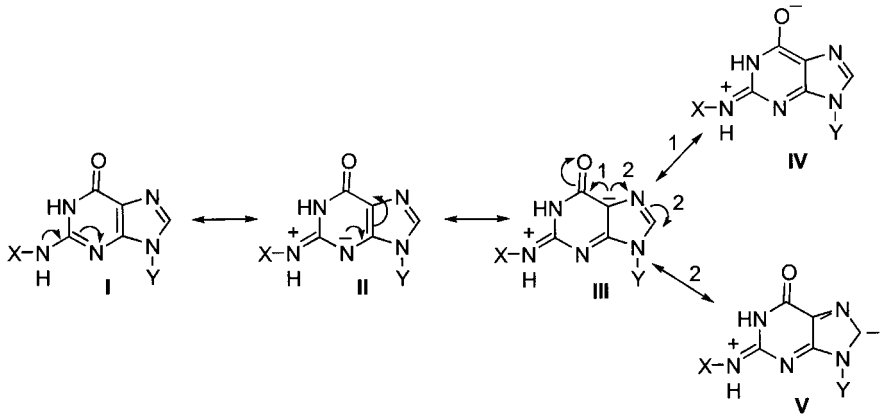


Figure 1

Table 6. ^{13}C NMR data for ganciclovir diacyl derivatives

Carbon	4a	4b	4c	4d
2	148.78	148.82	148.82	148.94
4	148.01	148.01	148.01	148.09
5	120.02	119.40	119.44	119.44
6	154.88	154.88	154.92	154.99
8	140.32	140.17		140.55
NCH_2O, 1′	71.89		71.85	72.08
CH_2OH, 5′	60.28	60.28	60.28	60.40
CH_2O, 4′	63.23	63.15	63.12	63.23
CHO, 3′	77.09	77.13	77.09	77.21
*R*COO	20.22	8.66 26.36	13.24 17.70 37.69	13.51 21.58 26.40 32.88
*R*CONH	23.72	8.66 29.23	13.31 17.89 37.69	13.63 21.58 26.55 35.63
R*COO*	170.02	173.28	172.42	172.62
R*CONH*	173.51	177.00	176.30	176.54

to RHN— leads to similar chemical shift changes on the 9-side-chain compared with Table 5. C-2, C-4 and C-6 are shielded by 4.77–4.93, 3.10–3.18 and 1.75–1.86 ppm, respectively. On the other hand, C-5 and C-8 are deshielded by 3.11–3.73 ppm and 2.49–2.87 ppm, respectively. These deshielding effects can be explained by the resonance structures as shown in Fig. 1. The 2-amide substitutions decrease the participation of contributors **II–V**, resulting in a reduction in shielding at C-5 and C-8. The ester and amide moieties generated two sets of data. The chemical shift substituent effects generally decrease as the carbon positions are progressively removed from the carbonyl groups.

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