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Regioselective synthesis of various prodrugs of ganciclovir

Hongwu Gao and Ashim K. Mitra *

Division of Pharmaceutical Sciences, School of Pharmacy, University of Missouri-Kansas City, 5005 Rockhill Road, Kansas City, MO 64100-2499, USA

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Abstract

High-yield regioselective syntheses of 9-[(1,3-dihydroxy-2-propoxy)methyl]guanine mono-, di- and tri-substitution derivatives as potential prodrugs were accomplished via one or multisteps. Two amino acid esters of ganciclovir were synthesized as water-soluble prodrugs, which form protonated cations in pH 7.4 phosphate buffer.

9-[(1,3-Dihydroxy-2-propoxy)methyl]guanine (ganciclovir **1**, Scheme 1) is a potent and selectively active agent against cytomegalovirus.[1] Since the oral administration of ganciclovir may not provide optimal therapeutic levels, various prodrugs were synthesized and evaluated with the aim of improving its delivery and apparent half-life.[2–6] Ganciclovir has two identical primary hydroxyl groups and one amino group which may result in five possible combinations of ester and amide prodrug derivatives (mono-*O*-, mono-N^2-, di-*O*-, di-N^2,*O*-, tri-N^2,*O*,*O*-acyl derivatives). In this paper, we wish to report various strategies for regioselective syntheses of these ganciclovir derivatives as potential prodrugs to investigate their effectiveness in improving pharmacokinetic properties and enhancing their intraocular retention after direct intravitreal administration. Biochemical conversion and in vivo pharmacokinetic studies of acyclovir prodrugs in ocular tissues have revealed that the isobutyrate prodrug was most effective because of its optimum hydrolysis profile producing sustained therapeutic levels of acyclovir after intravitreal administration.[7] Thus, ganciclovir isobutyrate derivatives were selected for further studies and development. The product structures were confirmed by their NMR chemical shifts, MS and HPLC retention profiles.

The simple di-*O*-isobutyrate ester of ganciclovir **2a** was prepared in a single-step reaction as reported previously from this laboratory (Scheme 1).[8] A solution of ganciclovir in dimethylformamide (DMF) was treated with isobutyric anhydride in the presence of a catalytic amount of 4-dimethylaminopyridine (DMAP) at room temperature for 4 hours. Chromatographic purification generated compound **2a** in 80% yield.

The synthesis of mono-*O*-propionate ester of ganciclovir **2b** could be achieved by reaction of ganciclovir with an excess of trimethyl orthopropionate followed by acidic hydrolysis of the cyclic orthoester intermediate (Scheme 1).[8]

* Corresponding author.

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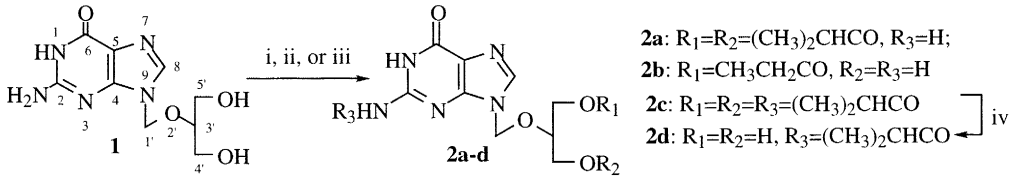
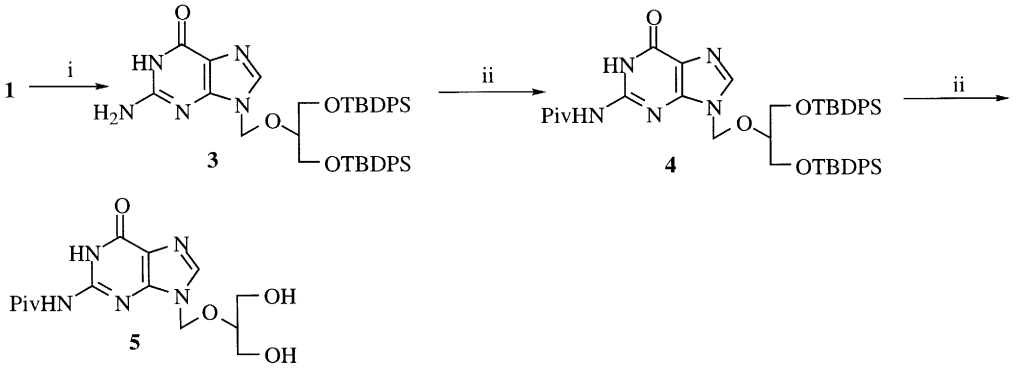


Scheme 1. Reagents and conditions: (i) $((CH_3)_2CHCO)_2O$, DMAP, DMF, rt, 4 h, **2a**: 80%; (ii) $CH_3CH_2C(OCH_3)_3$, TFA, DMF, rt, 2 h, then, H_2O, overnight, **2b**: 87%; (iii) $((CH_3)_2CHCO)_2O$, DMAP, pyridine, reflux, 1 h, **2c**: 99%; (iv) 8 M NaOMe, MeOH, rt, 16 min, **2d**: 79%

Triisobutyl derivatization of ganciclovir **2c** was successfully carried out with isobutyric anhydride in refluxing pyridine using DMAP as a catalyst (Scheme 1). During the synthesis of acyclovir prodrugs, the use of strong base (8 M $NaOCH_3/CH_3OH$) resulted in a selective *O*-deacylation in the presence of N^2-acyl as reported previously.[9] The same protocol when applied to compound **2c** produced mono-N^2-isobutyrate amide of ganciclovir **2d** in 79% yield.

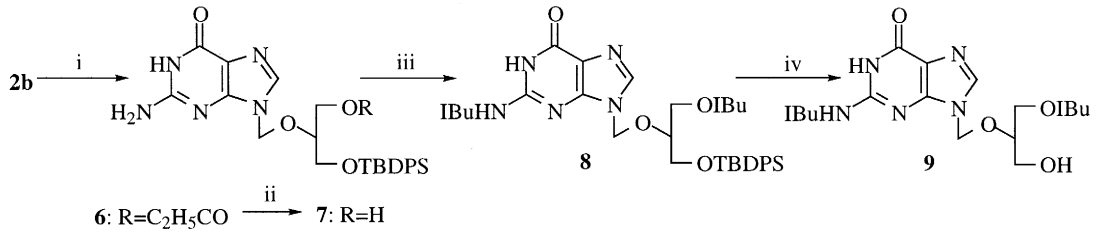
To avoid potential difficulty in selective hydrolysis of *O*-acyl groups, preparation of mono-N^2-pivalate amide of ganciclovir **5** was accomplished in a three-step procedure (Scheme 2). This preparation required the protection of both primary hydroxyl functions, which was reported by direct silylation of ganciclovir in DMF with *tert*-butyldimethyl silyl chloride (TBDMSCl) in the presence of imidazole.[2] However, our investigation revealed that TBDMS groups were unstable at reflux in pivalic anhydride. The more acidic stable silyl protecting group, *tert*-butyldiphenylsilyl, was satisfactory. Treatment of the bis-*O*-*tert*-butyldiphenylsilyl derivative **3** with trimethyl acetic anhydride in pyridine, followed by removal of the silyl protecting group with tetrabutylammonium fluoride (TBAF) in DMF, gave the desired mono-N^2-pivaloyl derivative **5** in 52% yield.



Scheme 2. Reagents and conditions: (i) TBDPSCl, imidazole, DMF, rt, 24 h, **3**: 91%; (ii) trimethylacetic anhydride, DMAP, pyridine, reflux, 1 h, **4**; (iii) TBAF, THF, DMF, 1 h, **5**: 52% for two steps

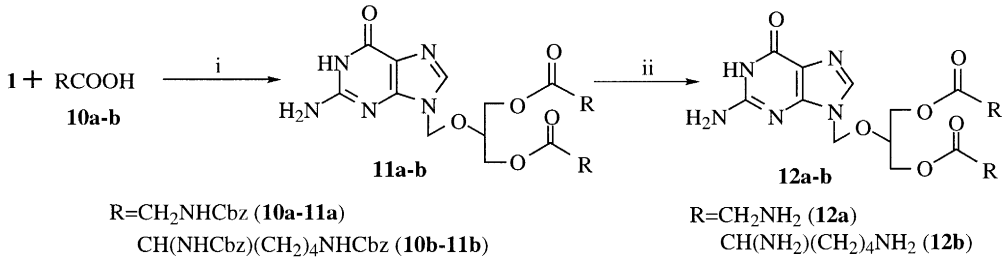
Synthesis of di-N^2,*O*-diisobutyl ganciclovir derivative **9** required selective protection of one of two primary hydroxyl functions in ganciclovir (Scheme 3). This was achieved by treating mono-*O*-propionate ester **2b** in DMF with TBDPSCl in the presence of imidazole, followed by removal of the propionate ester with strong base (8 M $NaOCH_3/CH_3OH$). The resulting mono-*O*-*tert*-butyldimethylsilyl derivative **7** was then treated with isobutyric anhydride in refluxing pyridine in the presence of DMAP to give fully protected product **8**. Removal of the TBDPS silyl group with TBAF provided the desired di-N^2,*O*-diisobutyl ganciclovir derivative **9** in 62% yield.

Two di-*O*-amino acid esters (**12a** and **12b**) were prepared in two steps according to the same method as for preparation of acyclovir amino acid derivatives (Scheme 4).[10,11] A solution of ganciclovir in DMF was treated with the *N*-carbobenzyloxy-protected amino acid (**10a** and **10b**) using the coupling agent DCC and DMAP as a catalyst. The resulting *N*-protected ester derivatives (**11a** and **11b**) were purified



Scheme 3. Reagents and conditions: (i) TBDPSCl, imidazole, DMF, rt, 24 h, **6**: 93%; (ii) 8 M NaOMe, MeOH, rt, 16 min, **7**: 85%; (iii) $((CH_3)_2CHCO)_2O$, DMAP, pyridine, reflux, 1 h, **8**; (iv) TBAF, THF, DMF, 1 h, **9**: 62% for two steps

by column chromatography on silica gel and subsequently subjected to catalytic hydrogenation in the presence of HCl in order to avoid hydrolysis of the labile ester function. The target esters, **12a** and **12b**, as the hydrochloride salts were isolated from a water:ethanol mixture (80:20) in 97% and 96% yields, respectively (Scheme 4).



Scheme 4. Reagents and conditions: (i) acid **10a**–**10b**, DCC, DMAP, DMF, rt, 24 h, then, recharge acid, DCC, DMAP, rt, 24 h, **11a**: 81%, **11b**: 68%; (ii) H_2, 10% Pd/C, ethanol/water, rt, 4 h, **12a**: 97%, **12b**: 96%

The ^{1}H NMR spectral data and assignments for compounds **2a**–**2d**, **3**–**5** and **6**–**9**, **11a**–**12b** are listed in Tables 1 and 2, respectively. The ^{13}C NMR spectral data and assignments for compounds **2a**–**2d**, **3**–**5** and **6**–**9**, **11a**–**12b** are listed in Tables 3 and 4, respectively. The physiological data and MS data of compounds **2a**–**2d**, **3**–**9** and **11a**–**12b** are listed in Ref. 12.

Table 1
^{1}H NMR data for ganciclovir and its derivatives (**2a**–**2d**, **3**–**5**)

Proton	**1**	**2a**	**2b**	**2c**	**2d**	**3**	**4**	**5**
OCH, 3' OCH_2 4'	3.30-3.54 (m, 5H)	3.99-4.17 (m, 5H)	3.94-4.09 (m, 3H)	3.99-4.17 (m, 5H)	3.35-3.62 (m, 5H)	3.70-3.88 (m, 5H)	3.69-3.80 (m, 5H)	3.28-3.56 (m, 5H)
OCH_2 5'			3.45(bs)					
NCH_2O, 1'	5.45(s)	5.45(s)	5.46(s)	5.56(s)	5.60(s)	5.52(s)	5.58(s)	5.61(s)
2-NH_2(NH)	6.52(bs)	6.56(bs)	6.76(bs)	12.12(bs)	12.03(bs)	6.36(bs)	12.24(bs)	12.28(bs)
H-8	7.82(s)	7.87(s)	7.87(s)	8.17(s)	8.16(s)	7.52(s)	8.95(s)	8.14(s)
H-1	10.67(bs)	10.78(bs)	10.18(bs)	11.77(bs)	12.03(bs)	12.09(bs)	11.83(bs)	11.27(bs)
*R*COO TBDPS	4.64 (t, 2H, OH)	1.03 (d, 12H) 2.42 (m, 2H)	0.94 (t, 3H) 2.15 (q, 2H)	0.99 (d, 12H) 2.38 (m, 2H)	4.77(bs, 2H,OH)	0.99-1.00 (d, 18H) 7.35-7.59 (m, 20H)	0.99 (m, 18H) 7.35-7.61 (m, 20H)	------
*R*CONH	------	------	------	1.12 (d, 6H) 2.79 (m, 1H)	1.13 (bs, 6H) 2.83 (t, 1H)	------	1.26 (bs, 9H)	1.26 (bs, 9H)

Table 2
^{1}H NMR data for ganciclovir derivatives (**6–9**, **11a–12b**)

Proton	**6**	**7**	**8**	**9**	**11a**	**11b**	**12a**	**12b**
OCH, 3' OCH_2 4'	3.97-4.27 (m, 3H)	3.50-3.82 (m, 5H)	4.19(d) 3.89(t)	3.94-4.10 (m, 3H)	4.08 (m, 5H)	4.05-4.23 (m, 7H)	4.23-4.34 (m, 5H)	3.95-4.45 (m, 7H)
OCH_2 5'	3.69(m)		3.66(d)	3.49(bs)				
NCH_2O, 1'	5.47(s)	5.48(s)	5.48(s)	5.63(s)	5.44(s)	5.85(s)	5.70(s)	5.74(s)
2-NH_2(NH)	6.29(bs)	6.54(bs)	12.19(bs)	12.16(bs)	6.56(bs)	6.34(bs)	6.47(bs)	7.57(bs)
H-8	7.72(s)	7.88(s)	8.63(s)	8.28(s)	7.85(s)	7.90 (bs, 3H)	7.53(bs)	7.57(bs)
H-1	10.04(bs)	10.74(bs)	12.19(bs)	11.97(bs)	10.74(bs)	12.47(bs)	11.93(bs)	12.00(bs)
*R*COO *R*CONH TBDPS	0.9-1.04 (m, 12H) 2.17 (q, 2H) 7.11-7.65 (m, 10H)	0.92 (bs, 9H) 7.43-7.56 (m, 10H)	1.02-1.26 (m, 21H) 2.41-2.73 (m, 2H) 7.30-7.62 (m, 10H)	0.93-1.14 (m, 12H) 2.30 (m, 1H) 2.91 (m, 1H)	3.72 (bs, 4H)	1.37-1.65 (m, 12H) 3.08 (bs, 4H) 4.05-4.23 (m, 7H)	3.78 (bs, 4H)	1.45-1.80 (m, 12H) 2.78 (bs, 4H) 3.95-4.45 (m, 7H)
*CH_2*Ar	------	------	------	------	5.05 (bs, 4H)	5.02 (bs, 4H)	------	------
CH_2*Ar*	------	------	------	------	7.35 (bs, 10H)	7.24 (bs, 20H)	------	------
*NH*Cbz NH_3^+	------	------	------	------	7.73(bs)	7.90 (bs,3H)	8.70 (bs, 6H)	8.30 (bs, 6H) 8.85 (bs, 6H)

Table 3
^{13}C NMR data for ganciclovir and its derivatives (**2a–2d**, **3–5**)

Carbon	**1**	**2a**	**2b**	**2c**	**2d**	**3**	**4**	**5**
2	153.71	153.87	153.99	148.74	148.98	153.95	148.32	148.63
4	151.19	151.35	151.38	148.20	148.32	151.97	147.85	148.40
5	116.29	116.53	116.33	120.29	119.94	116.76	121.15	120.02
6	156.74	156.86	157.05	154.76	155.23	159.19	155.19	154.92
8	137.68	137.92	137.88	140.40	140.09	137.45	139.47	139.97
NCH_2O, 1'	71.38	70.88	71.31	71.42	72.12	72.12	72.70	72.08
CH_2O, 5'	60.75	62.49	60.32	62.38	60.94	63.85	63.81	60.83
CH_2O, 4'			63.23					
CHO, 3'	79.88	73.75	76.86	73.83	80.43	79.50	79.50	80.47
TBDPS	------	------	------	------	------	19.10 26.75 127.71 129.73 133.10 135.51 137.45	19.06 26.75 127.75 129.84 132.91 135.39 137.33	------
*R*COO	------	18.55 32.99	8.77 26.47	18.52 32.96	------			------
*R*CONH	------	------	------	18.79 34.66	18.94 34.78	------	27.09 40.29	26.20 39.94
R*COO*	------	175.76	173.43	175.68	------	------	------	------
R*CONH*	------	------	------	180.19	180.19	------	180.26	181.23

Table 4
^{13}C NMR data for ganciclovir derivatives (**6**–**9**, **11a**–**12b**)

Carbon	**6**	**7**	**8**	**9**	**11a**	**11b**	**12a**	**12b**
2	154.26	153.75	148.94	148.90	153.87	154.30	153.75	153.48
4	151.89	151.19	148.24	148.20	151.35	151.73	149.83	149.72
5	116.84	116.49	121.03	120.21	116.37	116.37	109.23	108.53
6	159.19	156.78	155.34	154.99	156.43	156.43	155.58	155.62
8	137.61	137.49	139.16	140.36	137.72	138.58	137.60	137.84
NCH_2O, 1'	71.81	71.46	72.35	71.77	71.27		72.78	72.86
CH_2O, 5'	63.23	60.40	62.73	60.32	63.23	63.54	63.81	63.97
CH_2O, 4'		63.47	63.43	63.50				
CHO, 3'	76.59	79.77	76.59	77.05		73.67	73.87	74.10
TBDPS	19.14 26.75 127.82 129.92 132.91 135.55	18.59 26.40 127.78 129.72 134.93 135.08	19.10 26.71 127.78 129.96 132.75 135.43	------	------	------	------	------
*R*COO	8.93 27.25	------	18.83 33.81	18.87 33.03	41.84	22.48 29.22 36.37 40.29	48.44	21.12 26.05 29.04 38.08 51.51
*R*CONH	------	------	18.94 36.26	18.94 34.70	------	------	------	------
R*COO*	174.29	------	177.04	175.80	169.86	172.31	167.14	168.93
R*CONH*	------	------	181.62	180.30	156.74	156.78	------	------
*CH_2*Ar	------	------	------	------	65.56	66.30 66.73	------	------
CH_2*Ar*	------	------	------	------	127.71 128.29 136.79	127.59 127.86 128.37 136.25 136.67	------	------

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12. **2a**: 80%. M.p. 208–210°C. MS (ESI): 396 $[M+1]^+$ (100). **2b**: 87%. M.p. 138–140°C. MS (ESI): 312 $[M+1]^+$ (78), 126 (100). **2c**: 99%. M.p. 153–155°C. MS (ESI): 466 $[M+1]^+$ (100). **2d**: 79%. Foamy solid. MS (ESI): 326 $[M+1]^+$ (100). **3**: 91%. M.p. 188–190°C. MS (ESI): 732 $[M+1]^+$ (100). **4**: Foamy solid. MS (ESI): 816 $[M+1]^+$ (100). **5**: 52%. M.p. 203–205°C. MS (ESI): 340 $[M+1]^+$ (100). **6**: 93%. M.p. 158–160°C. MS (ESI): 550 $[M+1]^+$ (100). **7**: 85%. M.p. 223–225°C. MS (ESI): 494 $[M+1]^+$ (100). **8**: Foamy solid. MS (ESI): 634 $[M+1]^+$ (100). **9**: 62%. Foamy solid. MS (ESI): 396 $[M+1]^+$ (100). **11a**: 81%. Foamy solid. MS (ESI): 638 $[M+1]^+$ (50), 306 (100). **11b**: 68%. Foamy solid. MS (ESI): 1048 $[M+1]^+$ (40), 306 (100). **12a**: 97%. Foamy solid. MS (ESI): 370 $[M+1]^+$ (100). **12b**: 96%. Foamy solid. MS (ESI): 512 $[M+1]^+$ (100).