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Synthesis and screening of bicyclic carbohydrate-based compounds: A novel type of antivirals

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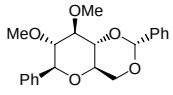
Abstract—A small library of bicyclic carbohydrate derivatives was synthesized and screened. A strong and selective activity against cytomegalovirus was found. Structure–activity relationship for this new type of antivirals is discussed.

Carbohydrates play a significant role in many biological processes. For example, they provide signals for protein targeting and serve as cell-receptors for adhesion of bacteria, viruses, and parasites.[1] As a result, this class of compounds has significant diagnostic and therapeutic potential and promises to be a major source of drug discovery leads.[2]

High-throughput screening (HTS) of a large number of diverse carbohydrate derivatives revealed that product **1**, a bicyclic *C*-glycoside (Fig. 1), shows very good and selective inhibitory activity against human cytomegalovirus (CMV).[3] This member of the herpes virus family is widespread: 50–90% of the population worldwide is infected. Initial infection is followed by persistent, long-term latency, but does not represent a threat for persons with a functional immune system. In immunocompromised persons however, such as newborns, patients involved in organ transplant therapy and AIDS-patients, a CMV-infection can cause severe clinical manifestations which can be life-threatening.[4]

Typical therapies for pathological CMV infections include treatment with ganciclovir (**2**)[5] and cidofovir (**3**)[6] (Fig. 2), two nucleoside analogues that inhibit the viral DNA polymerase. Cidofovir is more potent, but also



1

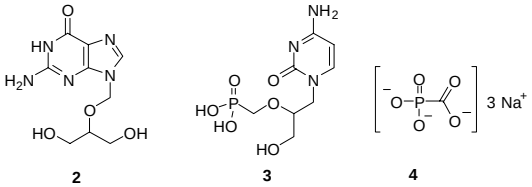
Activity:

CMV- Davis: IC_{50}: **2.4** μg/ml
CMV- AD-169: IC_{50}: **2.0** μg/ml

Figure 1. Parent CMV inhibitor and inhibitory activity.

shows higher toxicity. Foscarnet (**4**),[7] a simple phosphonate-containing compound, inhibits viral growth by blocking the enzymatic phosphate binding pocket. Compounds **2**, **3**, and **4** are administered intravenously. Fomvirsen[8] is a 21-nucleotide antisense product, used for treatment of CMV retinitis. It is more potent than ganciclovir, but has to be administered by intraocular injection. All therapies only help to control the infection but fail to clear the virus. Toxicity and administration present a problem, and prolonged use may result in the emergence of resistance.[9] More efficaceous and patient-friendly therapeutic agents are therefore desirable.

Identification of product **1** as a strong CMV inhibitor was remarkable, as no known antiviral product possess-



2 3 4

Figure 2. Current drugs for treatment of CMV infection.

Keywords: Carbohydrates; Antiviral; Anti-CMV; Cytomegalovirus.
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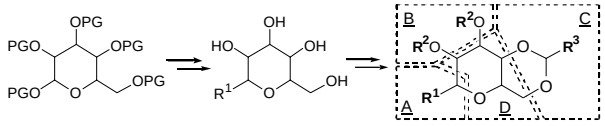


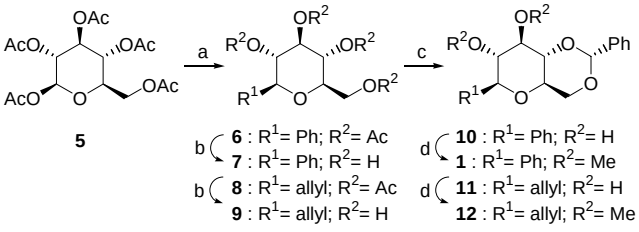
Scheme 1. Synthetic strategy and modifications.

es a similar structure.[10] Synthesis of a large number of analogues was initiated to further explore the scope of this new type of antiviral compound. Structural modifications were made in four different regions (Scheme 1): the anomeric substituent R^1 (A), the *O*-alkyl residues R^2 (B), the acetal substituent R^3 (C), and the configuration of the sugar (D). The synthetic strategy (Scheme 1) includes introduction of the *C*-glycosyl group by the Grignard reaction, followed by acetal formation and alkylation.

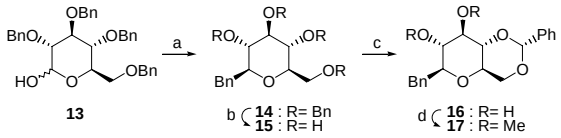
Analogues with varying substituents at the anomeric position (R_1), including the parent compound **1**, were synthesized by Grignard substitution of an anomeric bromide[11] (Scheme 2). β-D-Glucose penta-acetate (**5**) was converted into 1-phenyl- (**7**) or 1-allyl-1-deoxy-β-D-glucose (**9**) by bromination at the anomeric position, followed by nucleophilic substitution with phenylmagnesium bromide or allylmagnesium bromide, respectively, and subsequent acetylation–deacetylation to facilitate purification.

A similar procedure for the synthesis of the 1-benzyl analogue starting from **5** leads to excessive formation of the 1-*ortho*-tolyl derivative in the Grignard substitution.[12] This side reaction could be avoided by starting from 2,3,4,6-tetra-*O*-benzyl-D-glucose (Scheme 3). Anomeric bromination followed by Grignard reaction yielded protected *C*-glycoside **14**, which after deprotection afforded tetrol **15**. Selective transformation of **7**, **9**, and **15** into the corresponding 4,6-*O*-benzylidene acetals and methylation of the remaining hydroxyl groups furnished **1**, **12**, and **17**, respectively.

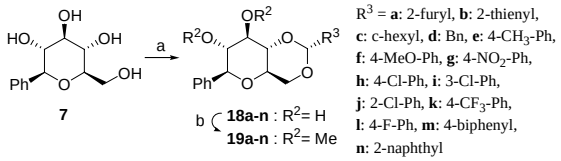
Analogues with different acetal substituents (R^3) were prepared from 1-phenyl-1-deoxy-β-D-glucose **7** by acid-catalyzed condensation with an aldehyde, with removal of water (Scheme 4). A variety of aldehydes were used,



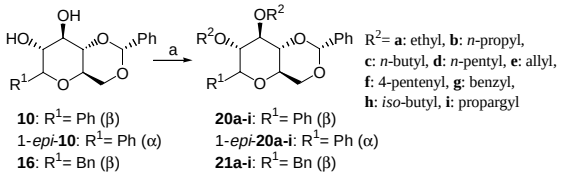
Scheme 2. Reagents and conditions: (a) i—HBr, HOAc, rt, 30 min; ii—R^1MgBr, Et_2O, 0 °C to rt, R^1 = Ph: 72 h, R^1 = allyl: 23 h; iii—Ac_2O, pyridine, rt, R^1 = Ph: 63 h, R^1 = allyl: 24 h; iv—column chromatography, R^1 = Ph: 56%, R^1 = allyl: 69%; (b) K_2CO_3, THF, MeOH, rt, 21 h, R^1 = Ph: 90%, R^1 = allyl: 100%; (c) $PhCH(OMe)_2$, CSA, CH_3CN, rt, 22 h, R^1 = Ph: 97%, R^1 = allyl: 92%; (d) i—NaH, DMF, 0 °C, 30 min; ii—MeI, rt, overnight, R^1 = Ph: 93%, R^1 = allyl: 98%.



Scheme 3. Reagents and conditions: (a) i—oxalylbromide, CH_2Cl_2, rt, 1 h; ii—benzylmagnesium-chloride, Et_2O, rt, 18 h, 50%; (b) 3–4 atm H_2/Pd-C, EtOH, rt, 5 h, 100%; (c) $PhCH(OMe)_2$, CSA, CH_3CN, rt, 24 h, 70%; (d) i—NaH, DME, 0 °C, 30 min; ii—MeI, rt, overnight, 91%.



Scheme 4. Reagents and conditions: (a) R^3CHO, CSA, $CuSO_4$.anh, CH_3CN, reflux, 33–90%; (b) i—NaH, DMF, 0 °C, 30 min; ii—MeI, rt, overnight, 35–96%.

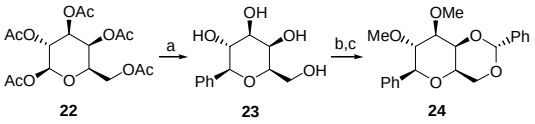


Scheme 5. Reagents and conditions: (a) for **a**–**g**: i—NaH, DMF, 0 °C, 30 min; (ii) R^2X; for **h**: i—NaH, DMF, 0 °C, 30 min; ii—*i*-BuOTs, 50 °C; for **i**: i—NaH, THF, 0 °C to reflux, 15 h; ii—propargyl bromide, TBAI, rt (**a**–**i**: 45–96%).

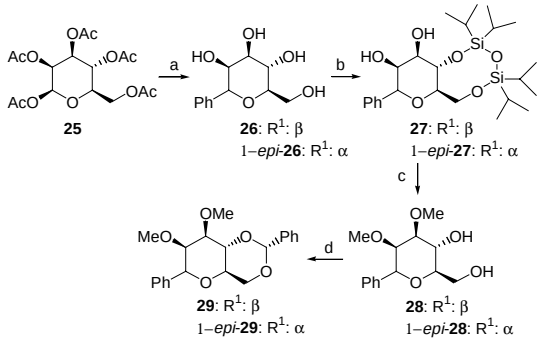
focussing mostly on aromatic aldehydes. The resulting diols were subsequently O-methylated using standard alkylation procedures.

A series of 2,3-*O*-dialkyl analogues was synthesized from **10** by alkylation with the appropriate alkyl bromide (Scheme 5). In this way, 2,3-di-*O*-ethyl, -propyl, -butyl, -pentyl, -allyl, -4-pentenyl, and -benzyl ethers were obtained. The *iso*-butyl group was introduced by treatment with *iso*-butyl tosylate at elevated temperatures.[13] The introduction of the propargyl group required in situ conversion of propargyl bromide to the more reactive iodide by addition of a catalytic amount of tetra-*n*-butylammonium iodide. The same alkylation procedures were used to prepare a series of compounds derived from **16** and 1-*epi*-**10**, obtained as above via acetalyzation of 1-*epi*-**7** (a byproduct of the synthesis of **7**).

D-Galactose (C-4 epimer) and D-mannose (C-2 epimer) stereoisomers of lead compound **1** were also prepared. D-Galactose derivative **24** (Scheme 6) was obtained from β-D-galactose penta-acetate **22**, following the same synthetic strategy as described above for the preparation of **1**. For the synthesis of analogues with the D-mannose configuration (Scheme 7), the axial orientation of the C-2 substituent influenced the stereoselectivity of the Grignard substitution: both α and β anomers were obtained in a 1:1 ratio. Moreover, subsequent formation



Scheme 6. Reagents and conditions: (a) i—HBr, HOAc, rt, 30 min; ii—PhMgBr, Et_2O, 0 °C to rt, 34 h; iii—Ac_2O, pyridine, rt, 6 h; iv—column chromatography, 43%; v—K_2CO_3, THF, MeOH, rt, overnight, 96%; (b) $PhCH(OMe)_2$, CSA, CH_3CN, rt, 1.5 h, 95%; (c) i—NaH, DMF, 0 °C, 30 min; ii—MeI, rt, overnight, 94%.



Scheme 7. Reagents and conditions: (a) i—HBr, HOAc, rt, 30 min; (ii) PhMgBr, Et_2O, 0 °C to rt, over weekend; iii—Ac_2O, pyridine, rt; iv—column chromatography, 73% (α:β 1:1); v—K_2CO_3, THF, MeOH, rt, 16 h, β: 93%, α: 94%; (b) dichloro-tetra-*iso*-propyl-disiloxane, pyridine, −15 °C to rt, 24 h, β: 74%, α: 84%; (c) i—Ag_2O, MeI, reflux, 22–72 h, β: 98%, α: 93%; ii—TBAF, THF, rt, 15 h, β: 92%, α: 86%; (d) $PhCH(OMe)_2$, CSA, CH_3CN, rt, 20 h, β: 91%, α: 91%.

of the benzylidene acetal function did not proceed selectively: due to the *syn* orientation of the hydroxyl groups at C-2 and C-3, competitive 2,3-*O*-acetal formation occurred.

The required selectivity was obtained by treatment of tetrol **26** with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane[14] to selectively protect the C-4 and C-6 hydroxyl functions. Methylation of the remaining hydroxyl groups under mild conditions using silver(I)oxide,[14] followed by deprotection and acetal-formation, yielded mannose-based analogue **29**. The same procedure was used for the synthesis of the α-epimer 1-*epi*-**29**.

All analogues of **1**, as well as their synthetic intermediates, were evaluated for their activity against the CMV-strains Davis and AD-169.[15] The most interesting results are shown in Table 1. All active compounds possess the D-glucose configuration: changing stereochemistry at C-2 (**24**) or C-4 (**29**) annihilates activity.

Interestingly, the intermediate diols **18g**, **18h**, **18k**, and **18n** also show activity. The compounds with R^3 = 4-biphenyl (**19m**) or R^3 = 2-naphthyl (**19n**) are the most potent analogues bearing an anomeric phenyl group, displaying good inhibitory activity for doses below 1 μg/ml.

Analogues with an anomeric benzyl group also show potent activity, giving the best results for the compounds with allyl- (**21e**) and propargyl ether substituents (**21i**). The latter compound was the most active one found. The only active compound with α-configuration at the anomeric center is 1-*epi*-**20h**. Absolute requirements for activity seem to be the presence of an aromatic functionality at the anomeric position (R^1), and a correct orientation of the substituents imposed by the sugar scaffold. It is not unlikely that the alkyl substituents at C-2 and C-3 also influence the orientation of the anomeric substituent and in this way affect the inhibitory activity.

Table 1. Activity against human cytomegalovirus (CMV), strains Davis and AD-169, in human embryonic lung (HEL) cells[15]

Compound	R^1	R^2	R^3	Sugar	CMV Davis IC_{50}[a] (μg/ml)	CMV AD-169 IC_{50}[a] (μg/ml)	Cell morphology (MCC) (μg/ml)	Cell growth (CC_{50}) (μg/ml)
1	Ph (β)	Me	Ph	D-Glc	2.4	2.0	n.d.	n.d.
12	All (β)	Me	Ph	D-Glc	>50	>50	n.d.	n.d.
17	Bn (β)	Me	Ph	D-Glc	5.0	20.0	20	>50
18g	Ph (β)	H	4-NO_2-Ph	D-Glc	9.3	10.0	50	>50
18h	Ph (β)	H	4-Cl-Ph	D-Glc	10.0	14.0	⩾100	>50
18k	Ph (β)	H	4-CF_3-Ph	D-Glc	1.6	1.6	400	35
18n	Ph (β)	H	2-Naphthyl	D-Glc	1.8	1.3	⩾400	21
19c	Ph (β)	Me	*c*-Hexyl	D-Glc	3.5	3.2	20	28
19h	Ph (β)	Me	4-Cl-Ph	D-Glc	4.0	5.0	⩾100	>50
19i	Ph (β)	Me	3-Cl-Ph	D-Glc	3.2	5.0	⩾40	n.d.
19k	Ph (β)	Me	4-CF_3-Ph	D-Glc	3.1	1.5	⩾16	>50
19m	Ph (β)	Me	4-Biphenyl	D-Glc	0.6	0.8	⩾16	>50
19n	Ph (β)	Me	2-Naphthyl	D-Glc	0.8	0.47	⩾16	>50
20d	Ph (β)	*n*-Pentyl	Ph	D-Glc	2.4	>3.2	16	182
20i	Ph (β)	Propargyl	Ph	D-Glc	3.0	5.0	40	n.d.
21a	Bn (β)	Ethyl	Ph	D-Glc	3.0	2.6	>120	>50
1-*epi*-**20h**	Ph (α)	*i*-Butyl	Ph	D-Glc	3.2	2.6	40	n.d.
21e	Bn (β)	Allyl	Ph	D-Glc	1.0	1.2	20	>50
21i[b]	Bn (β)	Propargyl	Ph	D-Glc	0.5	0.5	40	n.d.
24	Ph (β)	Me	Ph	D-Gal	>50	>50	>400	n.d.
29	Ph (β)	Me	Ph	D-Man	>50	>50	400	n.d.

n.d. = not determined

[a] Reference compound is cidofovir (**3**)[6]: IC_{50} (Davis) = 0.6 μg/ml; IC_{50} (AD-169) = 0.7 μg/ml.

[b] See Ref. 16 for experimental data.

Cytotoxicity levels are good, especially for compound **19n**, which combines a very good activity with a low toxicity. Some products have a smaller therapeutic window, with the non-alkylated compounds showing higher toxicity. Further improvement of the antiviral activity of the lead structures is currently investigated. Preclinical tests are pursued for the most active compounds.

In conclusion, a strong and selective activity of bicyclic carbohydrate derivative **1** against CMV was discovered. A small library of analogues of lead compound **1** was synthesized and screened. Several more potent CMV growth inhibitors were identified. Specific conclusions toward SAR are difficult to make. The mechanism of activity is yet unknown and is the subject of further investigation.

Acknowledgments

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15. For determination of antiviral activity against CMV, human embryonic lung fibroblast cells grown in 96-well microtiter plates were infected with 20 PFU virus/well. After 2 h of incubation at 37 °C, the infected cells were replenished with 0.1 ml of medium containing serial dilutions of the test compound. On day 7 the plaques were counted microscopically after staining the cells with Giemsa's solution. The minimum antiviral concentration was expressed as IC_{50}, the dose required to inhibit virus-induced plaque formation by 50%. For cytotoxicity measurements, confluent monolayers of HEL cells, as well as growing HEL cells in 96-well microtiter plates, were treated with different concentrations of the experimental drugs. Growing cells were incubated for 3 days. At that time, the cells were trypsinized, and the cell number was determined using a Coulter counter. The 50% cytotoxic concentration (CC_{50}) was defined as the compound concentration required to reduce the cell growth by 50%. During the antiviral assay, the minimum cytotoxic concentration (MCC) was evaluated at day 7 as the lowest compound concentration giving microscopically visible cytotoxicity on the confluent cells. Neyts, J.; Andrei, G.; Snoeck, R.; Meerbach, A.; De Clercq, E. In *Cytomegalovirus Protocols*; Sinclair, J., Ed.; Humana Press: Totowa, NJ, 2000; pp 129–152.
16. *Synthesis of compound **21i***: To a solution of compound **15** (1.0 g, 3.93 mmol) in DMF (38.6 ml) were added benzaldehyde dimethylacetal (708 μl, 1.2 eq) and camphorsulfonic acid (274 mg, 0.3 eq). After 2 h, ethyl acetate (150 ml) was added and the mixture was washed with 1 N NaOH solution (2× 150 ml), saturated $NaHCO_3$ solution (2× 100 ml), and brine (2× 100 ml), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography (eluent CH_2Cl_2: MeOH 99:1), affording acetal **16** (940 mg; 70% yield). To a solution of **16** (80 mg, 0.234 mmol) in THF (410 μl) was added sodium hydride (23 mg, 60% dispersion in mineral oil, 2.2 eq) at 0 °C, and the mixture was subsequently heated to reflux temperature. After 1.5 h, the mixture was cooled to room temperature and tetra-*n*-butylammonium iodide (1.8 mg, 0.02 eq) and propargyl bromide (57 μl, 2.2 eq) were added. After stirring overnight at room temperature, extra sodium hydride (1.1 eq), tetra-*n*-butylammonium iodide (0.02 eq), and propargyl bromide (1 eq) were added. After stirring for another 2.5 h, the reaction mixture was poured into H_2O (50 ml) and extracted with ether (3× 40 ml). The combined organic layers were washed with brine (50 ml), dried over $MgSO_4$, filtered, and concentrated. The residue was purified by column chromatography (eluent pentane:Et_2O 85:15), to yield compound **21i** (83 mg; 86% yield). *Spectral data of compound **21i***: ^1H NMR (500 MHz, $CDCl_3$): δ (ppm) 7.47–7.45 (2H, m), 7.39–7.35 (3H, m), 7.31–7.21 (5H, m), 5.51 (1H, s), 4.62 (1H, dd, $J = 15.7$, 2.4 Hz), 4.52 (1H, dd, $J = 15.7$, 2.4 Hz), 4.50 (1H, dd, $J = 15.7$, 2.4 Hz), 4.43 (1H, dd, $J = 15.7$, 2.4 Hz), 4.25 (1H, dd, $J = 10.5$, 5.0 Hz), 3.83 (1H, dd, $J = 9.1$, 8.6 Hz), 3.67 (1H, dd [app. t], $J = 10.3$, 10.3 Hz), 3.58 (1H, dd [app. t], $J = 9.4$, 9.4 Hz), 3.55 (1H, ddd [app. dt], $J = 9.0$, 9.0, 2.2 Hz), 3.36–3.27 (3H, m), 2.73 (1H, dd, $J = 14.5$, 8.7 Hz), 2.50 (1H, t, $J = 2.4$ Hz), 2.48 (1H, t, $J = 2.4$ Hz); APT-NMR (125 MHz, $CDCl_3$) : δ (ppm) 138.4 (C), 137.2 (C), 129.7 (CH), 129.0 (CH), 128.3 (CH), 128.1 (CH), 126.3 (CH), 126.0 (CH), 101.1 (CH), 82.6 (CH), 82.2 (CH), 80.1 (CH), 79.5 (CH), 74.7 (C), 74.5 (C), 69.8 (CH), 68.8 (CH_2), 60.2 (CH_2), 59.7 (CH_2), 38.1 (CH_2); mp = 81–83 °C; $[\alpha]_D^{20}$ −45.0 (*c* 1.02, $CHCl_3$).