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Synthesis and biological evaluation of new pyrazole- and tetrazole-related *C*-nucleosides with modified sugar moieties

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Abstract—3(5)-Carboxamido-4-(β-D-ribofuranosyl)pyrazoles bearing 2′-benzamido (**15**) and 3′-mesyloxy (**29**) isosteric groups, as well as the tetrazole *C*-nucleosides with 2-benzamido-2-deoxy-β-D-ribofuranose (**19**) and 3-azido-3-deoxy-β-D-xylofuranose (**36**) as sugar segments, have been synthesized starting from D-glucose, by utilizing the 2,5-anhydro-D-glucose ethylene acetal derivatives **1** and **20** as divergent intermediates. The *C*-nucleosides **15** and **36** were shown to be moderate inhibitors of the in vitro growth of both N2a and BHK 21 tumour cell lines, whereas **29** showed a selective, although not potent cytotoxic activity against N2a cells. Compound **29** also showed a moderate in vitro antiviral activity towards the rabies virus.

1. Introduction

A number of nucleoside analogues have been found to show a broad spectrum of biological activity and have stimulated considerable interest as potential antitumour and/or antiviral agents.[1] Significant antitumour and antiviral activity of some pyrazole-related nucleosides[2] prompted us to prepare the corresponding *C*-nucleoside analogues. A number of 4-(β-D-ribofuranosyl)pyrazoles,[3] along with several 4- and 5-(D-lyxofuranosyl)pyrazoles[4] and a pyrazole homo-*C*-nucleoside,[5] have been already synthesized. Conversely, the synthesis of *C*-nucleosides containing the pyrazole-5-carboxamide moiety and a modified sugar segment has not been described in the literature so far. Some *C*-(glycopyranosyl)tetrazoles[6,7] were also synthesized as potential inhibitors of early steps in the shikimate pathway,[7] whereas certain 5-(glycofuranosyl)-tetrazoles[8,9] were designed as possible bioisosteres of the well known antiviral agent ribavirin.[9] However, the data related to their antiviral and/or cytotoxic activities were not reported so far. In this paper we describe the synthesis of the pyrazole-related *C*-nucleosides **15** and **29**,[10] as well as the tetrazole-related *C*-nucleosides **19** and **36**, along with their preliminary biological evaluations, including their in vitro cytotoxic and antiviral activities.

Keywords: *C*-nucleosides; pyrazoles; tetrazoles; cytotoxic activity; antiviral activity; 2,5-Anhydro sugars; X-ray crystallography.
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2. Results and discussion

2.1. Chemistry

The crucial problem in the synthesis of all targets **15**, **19**, **29** and **36** was the establishment of the requisite β-configuration at the anomeric position. In a previous paper we have described[11] the conversion of D-glucose to the 2,5-anhydro-D-glucose derivative **1** (Scheme 1), a compound already containing the desired β-D-glycosidic bond, as well as the functionalities suitable for further introduction of diversity into the nucleoside carbohydrate segment.

The 2,5-anhydro-D-glucose derivative **1** reacted with benzyl bromide in ether, in the presence of silver(I)-oxide as a catalyst, to afford the expected 4-*O*-benzyl derivative **2** in 87% yield. Reaction of **2** with potassium benzoate in *N*,*N*-dimethylformamide gave the corresponding 6-*O*-benzoyl derivative **3** in 83% yield. Compound **3** readily reacted with sodium azide in dimethyl sulfoxide to afford the desired 3-azido-3-deoxy derivative **5** as a major reaction product (77%) accompanied with a small amount of the corresponding 2,3-unsaturated derivative **4** (10%). Catalytic reduction of **5** over 10% Pd/C according to the procedure developed by Secrist and Logue,[12] gave the amine hydrochloride **6** as a product of sequential azide reduction/benzyl ether hydrogenolysis process. Treatment of crude **6** with benzoyl chloride in pyridine gave the fully benzoylated product **7** (71% from **5**). O-Debenzoylation of **7** with sodium methoxide in methanol afforded the diol **8** in 86%

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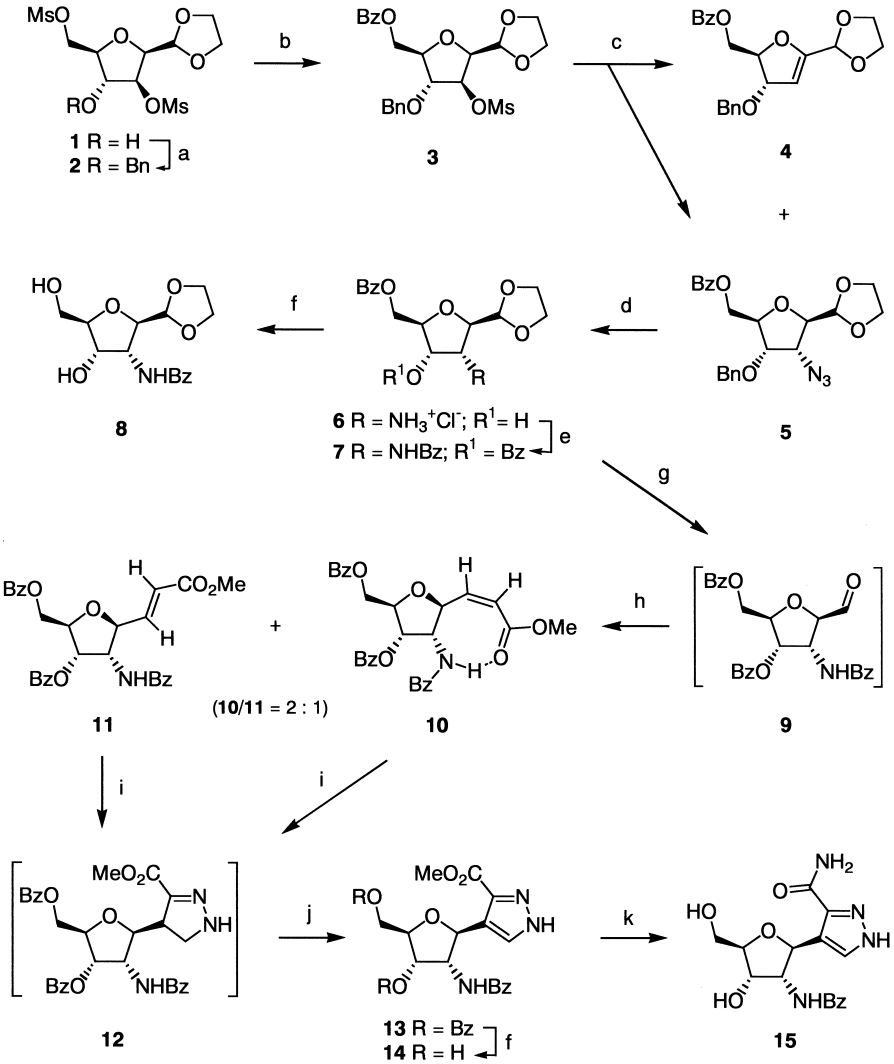
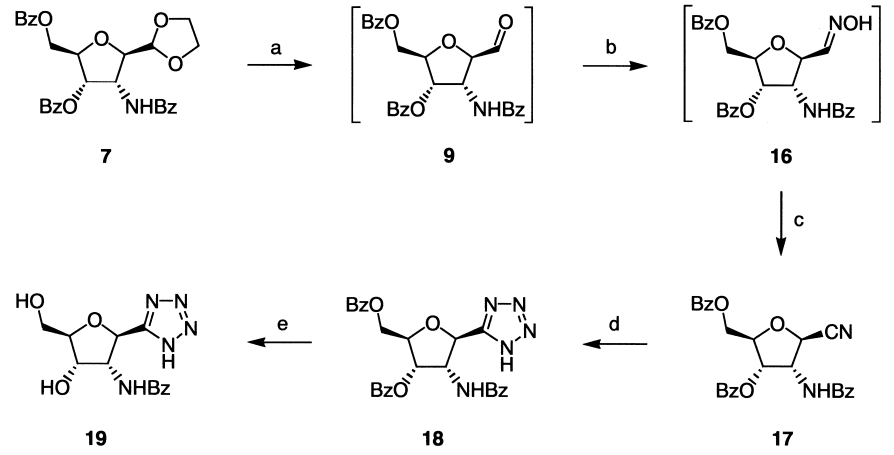
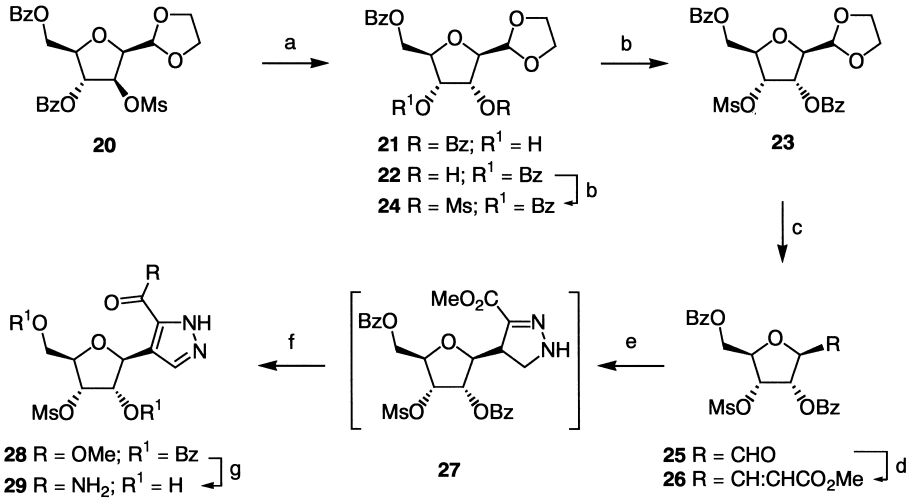


Scheme 1. (a) BnBr, Ag_2O, Et_2O, reflux, 22 h, 87%; (b) KOBz, DMF, 100°C, 48 h, 83%; (c) NaN_3, DMSO, 120°C, 47 h, 10% of **4**, 77% of **5**; (d) H_2, Pd/C, $CHCl_3$ (cat.), EtOH, rt, 120 h; (e) BzCl, Py, rt, 96 h, 71% from **5**; (f) NaOMe, MeOH, rt, 2 h for **7**, 86% of **8**, 1.5 h for **13**, 45% of **14** from **10**; (g) 4:1 CF_3CO_2H–6 M HCl, +4°C, 96 h; (h) $Ph_3P{:}CHCO_2Me$, CH_2Cl_2, rt, 19 h, 85% from **7**; (i) CH_2N_2, Et_2O, 0°C, 2 h for **10**, 45 h for **11**; (j) Cl_2/CCl_4, CH_2Cl_2, rt, 2.5 h, 97% from **10**, 98% from **11**; (k) NH_3, MeOH, rt, 7 days, 86%.



Scheme 2. (a) 4:1 CF_3CO_2H–6 M HCl, +4°C, 120 h; (b) $NH_2OH{\cdot}HCl$, NaOAc, EtOH, rt, 2 h; (c) MsCl, Py, −15°C→rt, 2.5 h, 63% from **7**; (d) NaN_3, NH_4Cl, DMF, 100°C, 4 h, 55%; (e) NaOMe, MeOH, rt, 3.5 h, 72%.

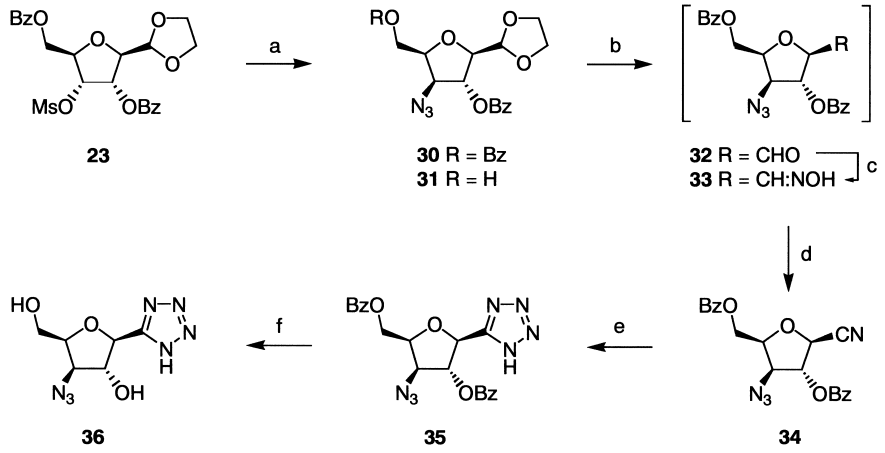


Scheme 3. (a) DMF, H_2O (5%), $CaCO_3$, 155–160°C, 20 h, 86%; (b) MsCl, Py, +4°C, 48 h, for **23**: 41% from **20**; (c) 4:1 CF_3CO_2H–6 M HCl, +4°C, 24 h; (d) Ph_3P:$CHCO_2Me$, CH_2Cl_2, rt, 24 h, 48% from **23**; (e) CH_2N_2, Et_2O, 0°C, 5 h; (f) Cl_2/CCl_4, CH_2Cl_2, rt, 3 h, 91% from **26**; (g) NH_3, MeOH, rt, 7 days, 86%.

yield. Hydrolytic removal of the dioxolane protective group in **7** was achieved with 4:1 mixture of trifluoroacetic acid and 6 M hydrochloric acid at +4°C, whereupon the corresponding aldehyde **9** was obtained. Due to its instability the crude **9** was immediately treated with (carbomethoxymethylene)-triphenylphosphorane in dichloromethane to afford a 2:1 mixture of the corresponding *Z* and *E* unsaturated esters **10** and **11** in 85% combined yield. The unsaturated esters **10** and **11** were readily separated by column chromatography and converted to the *C*-nucleoside **15** according to the methodology developed by Moffatt et al.[13] Both **10** and **11** readily underwent 1,3-dipolar cycloaddition with an excess of diazomethane in ether at 0°C to yield the 2-pyrazoline **12** as the only reaction product. The transformation of **10** to **12** was complete in 2 h, while the conversion of **11** to **12** required 45 h for completion. The enhanced reactivity of the *Z*-isomer **10** is presumably due to the activation of its α,β-unsaturated system by the intramolecular hydrogen bond between H (amide) and O (ester carbonyl). Indeed, the ^1H NMR spectrum of **10** showed a significant downfield shift of the amide proton signal (δ_{NH} 7.67) in contrast to the corresponding signal in the spectrum of **11**, which appeared at a highfield position (δ_{NH} 6.78). This result is consistent with the presence of an intramolecular hydrogen bond in **10**. Without purification or further characterization the intermediate **12** was treated with a saturated solution of chlorine in carbon tetrachloride to give the pyrazole derivative **13**. Quite unexpectedly, pure **13** was isolated as highly hygroscopic syrup. However, the corresponding di-O-deprotected product **14**, prepared by treatment of **13** with sodium methoxide in methanol, was a highly crystalline stable compound, which was fully characterized by corresponding spectral and analytical data. Finally, treatment of **13** with methanolic ammonia afforded the *O*-deprotected *C*-nucleoside **15**, ready for biological testing. Under these reaction conditions both intermediates **10** and **11** were converted to the target **15** in 97 and 85% overall yields, respectively.

2,5-Anhydro derivative **7** has been conveniently used as a divergent intermediate for preparation of the corresponding tetrazole *C*-nucleoside **19** (Scheme 2).

Hydrolytic removal of the dioxolane protective group from **7**, followed by subsequent treatment of the liberated aldehyde **9** with hydroxylamine hydrochloride, in the presence



Scheme 4. (a) NaN_3, DMSO, 120°C, 144 h, 62% of **30**, 9% of **31**; (b) 4:1 CF_3CO_2H–6 M HCl, +4°C, 96 h; (c) NH_2OH·HCl, NaOAc, EtOH, rt, 2 h; (d) MsCl, Py, −15°C→rt, 2.5 h, 22% from **30**; (e) NaN_3, NH_4Cl, DMF, 100°C, 2.5 h, 58%; (f) NaOMe, MeOH, rt, 2.5 h, 91%.

of sodium acetate in ethanol, gave an inseparable mixture of the corresponding *E*- and *Z*-oxime **16**. As the mixture **16** appeared as a single spot in TLC, it was not purified further, but was immediately treated with mesyl chloride in pyridine to afford the corresponding nitrile **17** in an overall yield of 63% with respect to **7**. The intermediate **17** was then reacted with an excess of sodium azide in the presence of ammonium chloride, under the Buchanan reaction conditions,[7] to give the corresponding tetrazole derivative **18** in good yield. Moreover, an action of sodium methoxide onto **18** furnished the *O*-deprotected *C*-nucleoside **19** in 72% yield.

Synthesis of the pyrazole *C*-nucleoside **29** bearing the 3-*O*-mesyl functionality as an isostere is outlined in Scheme 3.

Solvolysis of **20**[11] in wet *N*,*N*-dimethylformamide (5% of water), in the presence of calcium carbonate as a proton acceptor, gave a mixture of 3,6- and 4,6-di-*O*-benzoyl derivatives **21** and **22** in 86% combined yield. The regioisomers **21** and **22** could not be separated by column chromatography, presumably due to their rapid interconversion caused by silica gel. However, direct crystallization of the crude reaction mixture from benzene–hexane afforded pure **21**, which was subsequently mesylated to give the key intermediate **23**. The addition of mesyl chloride in pyridine to the mother liquor, which remained after crystallization of **21** afforded an additional quantity of **23**. The total yield of **23** was 41% with respect to **20**.

The intermediate **23** was converted to the target molecule **29** by using the same five-step sequence already applied for the conversion of **7** into the *C*-nucleoside **15** (Scheme 1). In this way the 4-*O*-mesyl derivative **23** has been transformed into the target **29** in 38% overall yield.

Finally, the intermediate **23** was converted into the tetrazole *C*-nucleoside **36** as shown in Scheme 4. The sequence started with nucleophilic displacement of the C-4 mesyloxy group with azide anion, whereupon the 4-azido-4-deoxy derivative **30** was obtained as a major reaction product (62%) accompanied with small amount of the 6-*O*-debenzoylated derivative **31** (9%). The intermediate **30** was then converted to the tetrazole **36** by using the same five-step sequence already applied for the conversion of **7** into the *C*-nucleoside **19** (Scheme 2).

2.2. X-Ray analysis

An X-ray diffraction analysis of compound **15** (Fig. 1) unambiguously confirmed its structure providing a proof that all intermediates generated by the multistep sequence **7→15** retained the required β-configuration at the anomeric position. The values of torsion angles C3′–C2′–C1′–C4=162.9(5°) and C1′–O–C4′–C5′=147.6(5°) are consistent with the β-D-configuration of **15**. The torsion angle of O–C1′–C4–C5 is −145.8(6)° that indicates that pyrazole-5-carboxamide moiety in **15** has the *anti* orientation. The five membered furanose ring adopts an envelope conformation [φ=41.2(9)°], with C1′ below the best plane [0.261(4) Å] of the ring. The distance between the H-atom from N7 (amide) and O6 (carbonyl) of 2.176 Å is considerably less then the sum of their van der Waals radii (2.72 Å)[14]

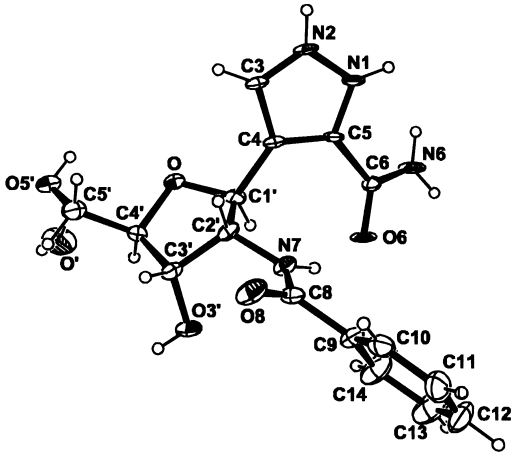


Figure 1. ORTEP drawing of 3(5)-carboxamido-4-(2-benzamido-2-deoxy-β-D-ribofuranosyl)pyrazole (**15**·H_2O) with non-H labelling scheme. The displacement ellipsoids were drawn at 30% probability.

and consistent with a strong intramolecular hydrogen bond between them.

Two tautomeric forms with hydrogen atoms at N1 and N2 are randomly present in a three dimensional lattice. The final refinement gave the bond lengths of N1–C5=1.338(7) Å and N2–C3=1.330(8) Å, as well as the population of N1–H and N2–H tautomers in an approximate ratio of 3:7, respectively. Both tautomers and a water molecule are built into the three-dimensional crystal lattice by intermolecular hydrogen bonds whereby the water molecule is included as a proton donor of two hydrogen bonds (O′–HA···O3′=2.802 Å, HA···O3′=1.703 Å, ∠O′–HA···O3′=166.8°; O′-HB···O5′=2.782 Å, HB···O5′=2.030 Å, ∠O′–HB···O5′=165.4°), as well as a proton acceptor of one hydrogen bond (N1–H1···O′=3.164 Å, H1···O′=2.340 Å, ∠N1–H1···O′=160.8°). There are no significant deviations from the expected bond lengths and angles.

2.3. Biological evaluation

The biological activities of compounds **8**, **15**, **29** and **36** were evaluated as follows: a preliminary in vitro evaluation for cytotoxic activity against mouse neuroblastoma (N2a) and baby hamster kidney (BHK 21) continuous cell lines, as well as for antiviral activity against rabies virus.

As shown in Table 1, the *C*-glycoside **8** was found to be inactive against both N2a and BHK 21 cells. Among the pyrazole-related *C*-nucleosides, compound **15** showed a

Table 1. Cytotoxic activity of synthesized compounds **8**, **15**, **29** and **36**

Compounds	IC_{50}[a] (μM)	
	N2a	BHK 21
8	3500	>5000
15	220	170
29	440	1300
36	430	630

[a] IC_{50} values represent the compound concentration required to inhibit 50% of the cells growth.

Table 2. Antiviral activity of synthesized compounds **8**, **15**, **29** and **36**

Final conc. (μM).	GM (%)	0.5% Tween 80 (%)	**8** (%)	**15** (%)	**29** (%)	**36** (%)
5000	70	70[a]	70	NCP[b]	<1[a]	70[a]
2500	70	70[a]	70	70[a]	1[a]	70[a]
1250	70	70[a]	70	70[a]	10[a]	70[a]
625	70	70[a]	70	70	50	70
312	70	70	70	70	70	70

[a] Significant cytotoxic effect.
[b] NCP-no cells present.

moderate cytotoxic effect against both N2a and BHK 21 cells, whereas compound **29** showed a selective, although not potent cytotoxic activity against the N2a cells. Moreover, the tetrazole *C*-nucleoside **36** showed a weak cytotoxic activity towards both N2a and BHK 21 cell lines.

The results related to antiviral activities of **8**, **15**, **29** and **36**, are shown in Table 2.

Obviously, only the pyrazole related *C*-nucleoside **29** showed a slight antiviral activity towards the rabies virus, while other compounds were devoid of any antiviral activity. Each compound in higher concentrations exhibited a notable cytotoxicity as evidenced by cell rounding of a significant proportion of cells (>5%). Due to the cytotoxic effect of **29**, its virus-inhibitory activity is of negligible practical value.

3. Conclusions

In summary, we have synthesized novel pyrazole- and tetrazole-related *C*-nucleosides bearing the 2′-benzamido (**15** and **19**), 3′-mesyloxy (**29**) and 3′-azido group (**36**) as isosteres. The *C*-nucleosides **15** and **36** were shown to be moderate inhibitors of the in vitro growth of both N2a and BHK 21 tumour cell lines, whereas **29** showed a moderate cytotoxic activity only against N2a cells. Compound **29** also showed a moderate in vitro antiviral activity towards the rabies virus. Molecules of type **15** or **19** might be of wider medicinal interest because they are partly related to certain 2′-benzamido-2′-deoxy adenosines, the potential agents for treatment of sleeping sickness.[15] In addition the protected 5-(glycofuranosyl)tetrazoles **18** and **35** might be used as convenient synthons for preparation of a range *C*-nucleosides bearing the 4,5-substituted pyrazoles or 1,3,4-oxadiazoles as heterocyclic aglycons.[16]

4. Experimental

4.1. General methods

Melting points were determined on a Büchi 510 apparatus and were not corrected. Optical rotations were measured on a Perkin Elmer 141 MC polarimeter. IR spectra were recorded with a Specord 75IR spectrophotometer. NMR spectra were recorded on a Bruker AC 250 E instrument and chemical shifts are expressed in ppm downfield from tetramethylsilane. Low resolution mass spectra were recorded on Finnigan-MAT 8230 (CI) and VG AutoSpec (FAB) mass spectrometers. High resolution mass spectra were taken on a Micromass LCT KA111 spectrometer. TLC was performed on DC Alufolien Kieselgel 60 F_{254} (E. Merck). Column chromatography was carried out using Kieselgel 60 (under 0.063 mm; E. Merck). All organic extracts were dried with anhydrous Na_2SO_4. Organic solutions were concentrated in a rotary evaporator under diminished pressure at a bath temperature below 35°C.

4.1.1. 2,5-Anhydro-4-*O*-benzyl-3,6-di-*O*-methanesulfonyl-D-glucose ethylene acetal (2). Compound **1**[11] (9.31 g, 25.72 mmol) was dissolved in dry ether (620 mL), and then benzyl bromide (32.12 mL, 268.95 mmol) and Ag_2O (59.30 g, 255.78 mmol) were added. The reaction mixture was stirred at reflux temperature for 22 h, then filtered and the precipitate was washed successively with toluene (100 mL) and Me_2CO (250 mL). The organic solutions were evaporated and the oily residue was submitted to column chromatography (toluene; 4:1→7:3 toluene–EtOAc) to furnish pure **2** (10.13 g, 87%) as a colorless syrup: $[\alpha]_D$=+95 (*c*, 1.23 in $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 2.98 and 3.09 (2×s, 3H each, 2×$MeSO_2$), 3.89 (m, 1H, $J_{4,5}$=4.5 Hz, H-5), 3.9–4.1 (m, 4H, 2×CH_2–dioxolane), 4.20–4.27 (m, 4H, H-2, H-4 and 2×H-6), 4.69 (2×d, 2H, J_{gem}=11.8 Hz, PhCH_2), 5.09 (d, 1H, $J_{2,3}$=3.2 Hz, H-3), 5.12 (d, 1H, $J_{1,2}$=6.8 Hz, H-1), 7.3–7.4 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$): δ 37.51 and 38.35 (2×$MeSO_2$), 65.27 and 65.36 (2×CH_2–dioxolane), 67.75 (C-6), 72.31 (PhCH_2), 80.77 (C-2), 82.57 (C-3), 82.81 and 83.54 (C-4 and C-5), 101.35 (C-1), 127.94, 128.2, 128.56 and 136.73 (Ph); FAB-MS: *m*/*z* 453 (M^++1).

4.1.2. 2,5-Anhydro-6-*O*-benzoyl-4-*O*-benzyl-3-*O*-methanesulfonyl-D-glucose ethylene acetal (3). To a solution of **2** (10.13 g, 22.41 mmol) in DMF (143 mL) was added potassium benzoate (15.11 g, 94.39 mmol). The mixture was stirred for 48 h at 100°C, then poured into cold water (300 mL) and the emulsion was extracted with CH_2Cl_2 (4×200 mL). The combined extracts were washed successively with water (200 mL) and satd aq. $NaHCO_3$ (200 mL), dried and the solvents were evaporated. The oily residue solidified after triturating with EtOH. Recrystallization from EtOH gave pure **3** (8.90 g, 83%) as colorless crystals: mp 88–89°C; $[\alpha]_D$=+70.9 (*c*, 1.54 in $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 3.08 (s, 3H, $MeSO_2$), 3.82–4.04 (m, 4H, 2×CH_2–dioxolane), 4.02 (m, 1H, $J_{2,3}$=4 Hz, H-2), 4.30 (m, 1H, $J_{5,6}$=4.5 Hz, H-5), 4.31 (bs, 1H, $J_{3,4}$<1 Hz, H-4), 4.43 (m, 2H, 2×H-6), 4.70 (2×d, 2H, J_{gem}=12 Hz, PhCH_2), 5.15 (d, 1H, H-3), 5.17 (d, 1H, $J_{1,2}$=7.3 Hz, H-1), 7.25–8.05 (m, 10H, 2×Ph); ^{13}C NMR ($CDCl_3$): δ 38.17 ($MeSO_2$), 63.69 (C-6), 65.21 and 65.79 (2×CH_2–dioxolane), 72.16 (PhCH_2), 80.51 (C-2), 83.05 and 84.41 (C-4 and C-5), 83.18 (C-3), 101.4 (C-1), 127.71, 127.82, 128.20, 128.56,

129.84, 133.01 and 136.82 (2×Ph), 166.01 (Ph*C*O). Anal. Calcd for $C_{23}H_{26}O_9S$: C 57.74, H 5.48, S 6.68. Found: C 57.33, H 5.72, S 6.04.

4.1.3. 2,5-Anhydro-3-azido-6-*O*-benzoyl-4-*O*-benzyl-3-deoxy-D-allose ethylene acetal (5). A mixture of **3** (1.35 g, 2.82 mmol) and sodium azide (1.8464 g, 28.41 mmol) in DMSO (48 mL) was stirred at 115–120°C for 47 h. The mixture was poured into cold water and the resulting emulsion was extracted with 1:1 benzene–hexane (4×80 mL). The combined extracts were washed with water (2×100 mL), dried and evaporated to yellow oil. Column chromatography (toluene; 97:3 toluene–EtOAc) of the residue gave pure **4** (0.1095 g, 10%) as a colorless syrup: $[\alpha]_D$=+477.6 (*c*, 1.45 in $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 3.90–4.06 (m, 4H, 2×CH_2–dioxolane), 4.38 (m, 2H, $J_{5,6}$=5.3 Hz, 2×H-6), 4.54 (s, 2H, PhCH_2), 4.74 (dd, 1H, $J_{3,4}$=2.3 Hz, $J_{4,5}$=3.2 Hz, H-4), 4.88 (m, 1H, H-5), 5.35 (dd, 1H, $J_{1,3}$=0.8 Hz, H-3), 5.55 (s, 1H, H-1), 7.25–8.06 (m, 10H, 2×Ph); ^{13}C NMR ($CDCl_3$) δ 64.30 (C-6), 65.10 and 65.20 (2×CH_2–dioxolane), 69.82 (Ph*C*H_2), 82.61 (C-4), 84.69 (C-5), 97.39 (C-1), 98.69 (C-3), 127.73, 128.35, 128.41, 129.68, 133.16 and 137.77 (2×Ph), 159.40 (C-2), 166.18 (Ph*C*O). Further elution gave **5** (0.92 g, 77%) as a colorless oil: $[\alpha]_D$=+78 (*c*, 1.11 in $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 3.82–4.00 (m, 5H, $J_{2,3}$=4.3 Hz, $J_{3,4}$=5.5 Hz, H-3 and 2×CH_2–dioxolane), 4.14 (dd, 1H, $J_{1,2}$=2.8 Hz, H-2), 4.18 (dd, 1H, $J_{4,5}$=6.1 Hz, H-4), 4.30 (m, 1H, $J_{5,6a}$=4.5 Hz, $J_{5,6b}$=3.4 Hz, H-5), 4.34 (dd, 1H, $J_{6a,6b}$=11.5 Hz, H-6a), 4.50 (m, 1H, H-6b), 4.68 (2×d, 2H, J_{gem}=11.7 Hz, PhCH_2), 4.96 (d, 1H, H-1), 7.25–8.04 (m, 10H, 2×Ph); ^{13}C NMR ($CDCl_3$) δ 60.48 (C-3), 63.50 (C-6), 65.10 and 65.26 (2×CH_2–dioxolane), 72.79 (Ph*C*H_2), 78.93 (C-4), 79.11 (C-5), 81.85 (C-2), 102.29 (C-1), 127.79, 127.91, 128.08, 128.27, 129.45, 129.57, 132.84 and 135.78 (2×Ph), 165.89 (Ph*C*O); FAB-MS: *m*/*z* 448 (M^++Na), 426 (M^++H).

4.1.4. 2,5-Anhydro-3-benzamido-4,6-di-*O*-benzoyl-3-deoxy-D-allose ethylene acetal (7). A solution of **5** (5.4 g, 12.7 mmol) in a mixture of dry EtOH (185 mL) and $CHCl_3$ (9.2 mL) was hydrogenated over 10% Pd/C (12 g) for 120 h at room temperature. The mixture was filtered and the catalyst washed with EtOH. The filtrate and washings were combined and evaporated to give crude **6** as a colorless solid. The crude product **6** was dissolved in anhydrous pyridine (165 mL) and benzyl chloride (13.6 mL, 116.84 mmol) was added to the solution. The mixture was left at room temperature for 96 h, then poured onto ice and acidified with 6 M HCl (to pH 3–4) whereupon a white precipitate was formed. The precipitate was collected by filtration, washed with water and dried. Recrystallization from MeOH gave pure **7** (4.67 g, 71%) as white crystals: mp 177–177.5°C; $[\alpha]_D$=−110.1 (*c*, 1.02 in $CHCl_3$); ν_{max} (KBr): 3350, 1730, 1640, 1600 cm^{-1}; ^{1}H NMR (DMSO-d_6): δ 3.80–3.98 (m, 4H, 2×CH_2–dioxolane), 4.34 (dd, 1H, $J_{1,2}$=2.9 Hz, $J_{2,3}$=8.1 Hz, H-2), 4.43 (dd, 1H, $J_{5,6a}$=3.8 Hz, $J_{6a,6b}$=11.0 Hz, H-6a), 4.49 (m, 1H, $J_{4,5}$=3.3 Hz, $J_{5,6b}$=3.6 Hz, H-5), 4.56 (dd, 1H, H-6b), 4.87 (m, 1H, $J_{3,4}$=6.2 Hz, $J_{3,NH}$=8.5 Hz, H-3), 5.00 (d, 1H, H-1), 5.56 (dd, 1H, H-4), 7.72–8.09 (m, 15H, 3×Ph), 8.76 (d, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 51.68 (C-3), 64.47 (C-6), 65.11 and 65.28 (2×CH_2–dioxolane), 73.9 (C-4), 80.24 (C-2), 80.46 (C-5), 102.62 (C-1), 127.57, 127.71, 127.91, 128.35, 128.47, 128.82, 129.02, 129.31, 129.51, 129.56, 129.61, 129.72, 131.12, 131.68, 133.08, 133.77, 133.88 and 134.23 (3×Ph), 165.28, 165.86 and 167.2 (2×Ph*C*OO and Ph*C*ONH). Anal. Calcd for $C_{29}H_{27}NO_8$: C 67.30, H 5.26, N 2.71. Found: C 67.54, H 5.33, N 2.60.

4.1.5. 2,5-Anhydro-3-benzamido-3-deoxy-D-allose ethylene acetal (8). To a solution of **7** (0.1029 g, 0.2 mmol) in anhydrous MeOH (10 mL) was added a solution of 5% NaOMe in MeOH (0.05 mL). The mixture was stirred for 2 h at room temperature and then neutralized by stirring with Amberlite IR-120[H^+] resin at room temperature for 30 min. The mixture was filtered and the resin was washed with MeOH. The combined organic solutions were evaporated to an oil, which solidified upon triturating with 3:2 toluene–EtOAc (5 mL). Recrystallization from CH_2Cl_2–hexane afforded pure **8** (0.0526 g, 86%) as white crystals: mp 157–157.5°C; $[\alpha]_D$=−64.9 (*c*, 1.28 in MeOH); ^{1}H NMR (acetone-d_6):δ 3.55 (dd, 1H, $J_{5,6a}$=4.9 Hz, $J_{6a,6b}$=11.9 Hz, H-6a), 3.62 (dd, 1H, $J_{5,6b}$=4.8 Hz, H-6b), 3.80–4.00 (m, 6H, 2×CH_2–dioxolane, OH and H-5), 4.06 (dd, 1H, $J_{1,2}$=2.9 Hz, $J_{2,3}$=8.3 Hz, H-2), 4.26 (dd, 1H, $J_{3,4}$=5.9 Hz, $J_{4,5}$=3 Hz, H-4), 4.61 (m, 1H, $J_{3,NH}$=6.1 Hz, H-3), 5.04 (d, 1H, H-1), 7.52–8.00 (m, 6H, Ph and NH); ^{13}C NMR (acetone-d_6): δ 53.87 (C-3), 63.55 (C-6), 65.93 and 65.98 (2×CH_2–dioxolane), 72.64 (C-4), 81.91 (C-2), 87.38 (C-5), 104.27 (C-1), 128.09, 129.02, 131.96 and 135.59 (Ph), 167.51 (Ph*C*ONH). Anal. Calcd for $C_{15}H_{19}NO_6$×0.5MeOH: C 57.17, H 6.51, N 4.30. Found: C 57.04, H 6.57, N 4.08.

4.1.6. Methyl *Z*-4,7-anhydro-5-benzamido-6,8-di-*O*-benzoyl-2,3,5-trideoxy-D-allo-oct-2-enoate (10) and methyl *E*-4,7-anhydro-5-benzamido-6,8-di-*O*-benzoyl-2,3,5-trideoxy-D-allo-oct-2-enoate (11). A solution of **7** (3 g, 5.8 mmol) in a mixture of CF_3CO_2H (18 mL) and 6 M HCl (4.5 mL) was kept at +4°C for 96 h. The mixture was concentrated to 5–6 mL and poured into satd aq. $NaHCO_3$ (15 mL). The aqueous solution was rendered alkaline with solid $NaHCO_3$ to pH 8–9 and extracted with CH_2Cl_2 (4×80 mL). The combined extracts were washed successively with satd aq. $NaHCO_3$ (50 mL) and water, dried and evaporated to an unstable oil. The crude aldehyde **9** was immediately dissolved in anhydrous CH_2Cl_2 (55 mL) and treated with (carbomethoxymethylene)triphenylphosphorane (4.87 g, 14.6 mmol) in dry CH_2Cl_2 (16 mL). After 19 h at room temperature the solvent was evaporated and the residue was chromatographed on a column of silica gel (20:1→10:1→5:1 toluene–EtOAc). Pure *Z*-isomer **10** (1.4 g, 56%) was isolated as a white crystalline solid. Recrystallization from CH_2Cl_2–hexane gave an analytical sample **10**: mp 114–115°C; $[\alpha]_D$=−54 (*c*, 0.85 in $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 3.76 (s, 3H, OCH_3), 4.52 (td, 1H, $J_{6,7}$=1.5 Hz, $J_{7,8a}$=4.1 Hz, $J_{7,8b}$=3.6 Hz, H-7), 4.61 (m, 1H, $J_{4,5}$=10.1 Hz, $J_{5,NH}$=6.7 Hz, $J_{5,6}$=5.5 Hz, H-5), 4.63 (dd, 1H, $J_{8a,8b}$=11.9 Hz, H-8a), 4.75 (dd, 1H, H-8b), 5.77 (ddd, 1H, $J_{2,4}$=1.3 Hz, $J_{3,4}$=7.8 Hz, H-4), 5.84 (dd, 1H, H-6), 6.02 (dd, 1H, $J_{2,3}$=11.7 Hz, H-2), 6.35 (dd, 1H, H-3), 7.67 (d, 1H, NH), 7.33–8.21 (m, 15H, 3×Ph); ^{13}C NMR ($CDCl_3$) δ 52.07 (OCH_3), 57.51 (C-5), 64.54 (C-8), 74.54 (C-4), 75.67 (C-6), 83.18 (C-7), 122.40 (C-2), 127.03, 128.45, 128.56, 129.48, 129.63, 129.81, 131.63, 133.16, 133.36 and 133.40 (ArC), 146.92 (C-3), 165.57, 166.28,

167.18 and 167.66 (4×C=O). Anal. Calcd for $C_{30}H_{27}NO_8$: C 68.04, H 5.14, N 2.65. Found: C 67.55, H 5.03, N 3.21. Further elution with 5:1 toluene–EtOAc gave pure *E*-isomer **11** (0.74 g, 29%) which crystallized from CH_2Cl_2–hexane to afford colorless needless mp 165°C; $[\alpha]_D$=−256.6 (*c*, 0.96 in $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 3.67 (s, 3H, OCH_3), 4.52–4.70 (m, 3H, $J_{6,7}$=2.2 Hz, $J_{7,8a}$=3.7 Hz, $J_{7,8b}$=4.8 Hz, $J_{8a,8b}$=12.4 Hz, 2×H-8 and H-7), 4.71 (m, 1H, $J_{2,4}$= 1.5 Hz, $J_{3,4}$=5.0 Hz, $J_{4,5}$=9.2 Hz, H-4), 4.92 (m, 1H, $J_{5,NH}$=8.9 Hz, $J_{5,6}$= 5.9 Hz, H-5), 5.66 (dd, 1H, H-6), 6.21 (dd, 1H, $J_{2,3}$=15.7 Hz, H-2), 6.78 (bs, 1H, NH), 7.04 (dd, 1H, H-3), 7.29–8.15 (m, 15H, 3×Ph); ^{13}C NMR ($CDCl_3$) δ 51.57 (OCH_3), 55.39 (C-5), 64.20 (C-8), 74.82 (C-6), 80.05 (C-4), 81.99 (C-7), 122.28 (C-2), 126.88, 128.44, 128.53, 128.58, 128.89, 129.08, 129.34, 129.48, 129.67 131.84, 133.16, 133.50 and 133.70 (ArC), 143.50 (C-3), 165.22, 166.11, 166.21 and 167.39 (4×C=O). Anal. Calcd for $C_{30}H_{27}NO_8$: C 68.04, H 5.14, N 2.65. Found: C 67.98, H 5.07, N 2.59.

4.1.7. 3(5)-Carbomethoxy-4-(2-benzamido-2-deoxy-β-D-ribofuranosyl)pyrazole (14). To a stirred and cooled (0°C) solution of **10** (0.2512 g, 0.48 mmol) in anhydrous ether (4.5 mL) was added a solution of diazomethane (generated from 1.729 g, 11.75 mmol of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine) in Et_2O (37.5 mL). After 2 h at 0°C the volatiles were evaporated whereupon the pyrazoline **12** remained as a white foam. The crude **12** was dissolved in dry CH_2Cl_2 (15 mL) and oxidized with a freshly prepared saturated solution of Cl_2 in CCl_4 (55 mL). The mixture was stirred at room temperature for 2.5 h and then evaporated. Column chromatography (7:3 toluene–EtOAc) of the residue gave pure pyrazole **13** (0.2072 g, ~100%) as a colorless hygroscopic syrup: ^{1}H NMR ($CDCl_3$): δ 3.88 (s, 3H, OMe), 4.50–4.63 (m, 2H, $J_{1',2'}$=10.4 Hz, $J_{2',3'}$=5.6 Hz, $J_{3',4'}$=1.5 Hz, $J_{4',5a'}$=4.1 Hz, $J_{4',5b'}$=3.2 Hz, H-2′ and H-4′), 4.73 (dd, 1H, $J_{5a',5b'}$=11.9 Hz, H-5a′), 5.79 (d, 1H, H-1′), 5.92 (dd, 1H, H-3′), 7.34–8.18 (m, 17H, 3×Ph, H-3 and N_3′–H), 12.96 (bs, 1H, N_1–H); ^{13}C NMR ($CDCl_3$): δ 52.36 (OMe), 60.43 (C-2′), 64.50 (C-5′), 73.78 (C-1′), 74.77 (C-3′), 82.76 (C-4′), 123.43 and 138.43 (C-4 and C-5), 127.01, 128.15, 128.40, 128.50, 128.54, 128.96, 129.41, 129.50, 129.59, 129.65, 131.68, 133.19, 133.26 and 133.40 (3×Ph), 163.92, 165.66, 166.39 and 167.32 (2×Ph*C*OO, Ph*C*ONH and *C*OOMe). To a solution of **13** in dry MeOH (10 mL) was added a solution of 5% NaOMe in MeOH (0.3 mL). The mixture was stirred at room temperature for 1.5 h, then neutralized with Amberlite IR-120 [H^+] resin, and filtered. The resin was washed with MeOH and the combined filtrate and washings were evaporated to dryness. Trituration of the residue with benzene (2×10 mL) gave crude **14** as a white solid. Recrystallization from MeOH afforded pure **14** (0.0766 g, 45% from **10**) as colorless crystals: mp 238°C; $[\alpha]_D$=−76.4 (*c*, 1.02 in MeOH); ^{1}H NMR (methanol-d_4): δ 3.74 (dd, 1H, $J_{4',5a'}$= 4.6 Hz, $J_{5a',5b'}$=11.9 Hz, H-5a′), 3.80 (s, 3H, OMe), 3.81 (dd, 1H, $J_{4',5b'}$=3 Hz, H-5b′), 4.05 (m, 1H, $J_{3',4'}$=2.6 Hz, H-4′), 4.35 (dd, 1H, $J_{2',3'}$=5.8 Hz, H-3′), 4.44 (dd, 1H, $J_{1',2'}$=9.4 Hz, H-2′), 5.49 (d, 1H, H-1′), 7.40–7.84 (m, 5H, Ph), 7.95 (s, 1H, H-3); ^{13}C NMR (methanol-d_4) δ 52.44 (OMe), 61.26 (C-2′), 63.61 (C-5′), 72.74 (C-3′), 75.04 (C-1′), 88.27 (C-4′), 124.83 (C-3), 128.31, 129.54, 132.86 and 135.22 (Ph), 169.86 and 169.97 (Ph*C*ONH and *C*OOMe). Anal. Calcd for $C_{17}H_{19}N_3O_6$: C 56.51, H 5.30, N 11.63. Found: C 56.40, H 5.35, N 11.54.

4.1.8. 3(5)-Carboxamido-4-(2-benzamido-2-deoxy-β-D-ribofuranosyl)pyrazole (15). Procedure A: A solution of compound **13** (0.2072 g, 0.47 mmol) in saturated methanolic solution of NH_3 (60 mL) was stored at room temperature for 6 days. The volatiles were evaporated and the remaining residue was purified by column chromatography (4:1 toluene–EtOAc, 4:1 EtOAc–MeOH) whereupon pure **15** (0.1594 g, 97%) was obtained as a white solid. Recrystallization from EtOH–MeOH gave an analytical sample **15** as colorless crystals.

Procedure B: Compound **11** (0.5798 g, 1.1 mmol) was dissolved in a mixture of anhydrous ether (7 mL), freshly distilled dioxane (17 mL) and dry CH_2Cl_2 (1 mL). To the cooled (0°C) and stirred mixture was added a solution of diazomethane (generated from 3.325 g, 22.6 mmol, of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine) in dry ether (75 mL). The mixture was stirred on an ice bath for 45 h. After workup as described above (preparation of **14**) pure **13** (0.6111 g; 98%) was obtained which was immediately treated with saturated methanolic solution of NH_3 (50 mL) for 7 days at room temperature. The usual workup (procedure A) gave pure **15** (0.3206 g, 85% from **11**) as colorless needless: mp 133°C; $[\alpha]_D$=−116.6 (*c*, 1.08MeOH). ^{1}H NMR (DMSO-d_6): δ 3.54 (d, 2H, $J_{4',5'}$=4.6 Hz, 2×H-5′), 3.82–3.95 (m, 2H, $J_{1',2'}$=10.4 Hz, $J_{2',3'}$=5.2 Hz, $J_{3',4'}$= 1.5 Hz, H-4′ and H-2′), 3.40–4.60 (bs, 2H, 2×OH), 4.25 (dd, 1H, H-3′), 5.39 (d, 1H, H-1′), 7.38–7.84 (m, 5H, Ph), 7.60 and 7.90 (2×bs, 1H each, $CONH_2$), 7.85 (s, 1H, H-3), 8.54 (d, 1H, $J_{2',NH}$=6.1 Hz, NH); ^{13}C NMR (DMSO-d_6): δ 61.17 (C-2′), 62.06 (C-5′), 70.77 (C-3′), 72.34 (C-1′), 86.85 (C-4′), 121.30 (C-3), 127.11, 128.41, 131.46 and 133.83 (ArC), 130.20 and 142.82 (C-4 and C-5), 164.97 (*C*ONH_2), 166.24 (Ph*C*ONH).; HRMS (EI): Calcd for $C_{16}H_{18}N_4O_5$: 346.1277. Found: *m*/*z* 346.1307 (M^+). Anal. Calcd for $C_{16}H_{18}N_4O_5$: C 55.49, H 5.24, N 16.18; Found: C 55.78, H 5.53, N 16.23.

4.1.9. 2,5-Anhydro-3-benzamido-4,6-di-*O*-benzoyl-3-deoxy-D-allononitrile (17). A solution of **7** (1.2575 g, 2.43 mmol) in a mixture of TFA (9 mL) and 6 M HCl (2.7 mL) was left at +4°C for 120 h. The workup as described above (preparation of **10** and **11**) gave crude aldehyde **9** which was immediately dissolved in a mixture of EtOH (2 mL) and CH_2Cl_2 (6 mL) and treated with NH_2OH×HCl (0.3724 g, 5.36 mmol) and NaOAc (0.6487 g, 7.91 mmol) while stirring at room temperature for 2 h. The mixture was evaporated and the residue extracted with CH_2Cl_2 (4×40 mL). The extract was dried and evaporated to afford crude oxime **16** (1.12 g) as a mixture of the corresponding *E*- and *Z*-isomers. To a cooled (−15°C) and stirred solution of crude **16** (1.12 g), in anhydrous pyridine (4.4 mL) was added dropwise during 30 min a cold solution of MsCl (0.96 mL, 12.46 mmol) in dry pyridine (2.65 mL). The mixture was warmed to room temperature, stirred for the next 2 h, then poured into a 1:1 mixture of ice and conc. HCl (pH~2). The emulsion was extracted with CH_2Cl_2 (4×80 mL), the combined extracts were washed with water (50 mL), satd aq. $NaHCO_3$ (50 mL) and again with water (50 mL). The extract was

dried and evaporated, and the residue was purified on a column of silica gel (4:1 cyclohexane–Me_2CO). Pure **17** (0.7171 g, 63%) was obtained as a white solid. Recrystallization from MeOH–EtOAc gave an analytical sample **17**: mp 204°C; $[\alpha]_D=-82.4$ (*c*, 1.1 in Me_2CO). ^{1}H NMR (acetone-d_6): δ 4.67 (dd, 1H, $J_{5,6a}=3.8$ Hz, $J_{6a,6b}=12.2$ Hz, H-6a), 4.75 (dd, 1H, $J_{5,6b}=3.4$ Hz, H-6b), 4.81 (m, 1H, $J_{4,5}=2.3$ Hz, H-5), 5.23 (d, 1H, $J_{2,3}=9.5$ Hz, H-2), 5.58 (m, 1H, $J_{3,NH}=8.5$ Hz, $J_{3,4}=5.8$ Hz, H-3), 5.89 (dd, 1H, H-4), 7.33–8.27 (m, 15H, 3×Ph), 8.42 (d, 1H, NH); ^{13}C NMR (acetone-d_6): δ 56.71 (C-3), 64.92 (C-6), 68.65 (C-2), 74.25 (C-4), 84.05 (C-5), 118.12 (CN), 128.19, 129.17, 129.39, 129.50, 130.14, 130.45, 130.56, 132.57, 134.19, 134.45 and 134.54 (3×Ph), 165.90, 166.51 and 168.08 (2×Ph*C*OO and Ph*C*ONH). Anal. Calcd for $C_{27}H_{22}N_2O_6$: C 68.93, H 4.71, N 5.95. Found: C 68.52, H 5.11, N 5.55.

4.1.10. 5-(2-Benzamido-3,5-di-*O*-benzoyl-2-deoxy-β-D-ribofuranosyl)tetrazole (18). A mixture of **17** (0.5574 g, 1.18 mmol), NaN_3 (0.1534 g, 2.36 mmol) and NH_4Cl (0.1203 g, 2.25 mmol) in DMF (4.3 mL) was stirred at 100°C for 2.5 h. An additional amount of NaN_3 (0.0394 g, 0.61 mmol) and NH_4Cl (0.0298 g, 0.56 mmol) was added to the reaction mixture, the stirring was continued at 100°C for the next 1.5 h, and the solvent was evaporated. The residue was extracted with CH_2Cl_2, and the extract was filtered and evaporated. Column chromatography (4:1 EtOAc–MeOH) of the residue gave pure **18** (0.3339 g, 55%) as a pale yellow solid. Recrystallization from MeCN–MeOH gave an analytical sample **18** as colorless crystals: mp 262–263°C; $[\alpha]_D=-196.2$ (*c*, 0.98 in pyridine). ^{1}H NMR (acetone-d_6): δ 4.73 (d, 2H, $J_{4',5'}=4$ Hz, 2×H-5′), 4.86 (m, 1H, $J_{3',4'}=2.2$ Hz, H-4′), 5.46 (ddd, 1H, $J_{1',2'}=8.8$ Hz, $J_{2',NH}=8.4$ Hz, $J_{2',3'}=5.7$ Hz, H-2′), 5.84 (d, 1H, H-1′), 5.94 (dd, 1H, H-3′), 7.32–8.24 (m, 16H, 3×Ph and NH-tetrazole), 8.49 (d, 1H, Ph*C*ONH); 13 C NMR (acetone-d_6): δ 57.36 (C-2′), 65.54 (C-5′), 73.99 (C-1′), 75.18 (C-3′), 83.72 (C-4′), 127.85, 128.23, 128.31, 128.98, 129.06, 129.38, 129.44, 130.46, 130.66, 130.72, 132.31, 134.07, 134.36 and 134.95 (3×Ph), 166.14, 166.73 and 168.08 (2×Ph*C*OO and Ph*C*ONH). HR-MS: Calcd for $C_{27}H_{23}N_5O_6$: 513.1648. Found: *m*/*z* 513.1661.

4.1.11. 5-(2-Benzamido-2-deoxy-β-D-ribofuranosyl)-tetrazole (19). To a solution of **18** (0.2392 g, 0.47 mmol) in dry MeOH (20 mL) was added 5% solution of MeONa in MeOH (0.2 mL) and the mixture was stirred at room temperature for 2.5 h. An additional amount of the base (0.2 mL) was added to the mixture and stirring at room temperature was continued for the next 1 h. The workup as described above (preparation of **8**) gave crude **19** as a yellow oil. Silica gel column chromatography (EtOAc, 4:1, 7:3 EtOAc–MeOH) gave pure **19** (0.1029 g, 72%) as a white solid. Recrystallization from MeOH–EtOAc gave an analytical sample **19** as colorless crystals: mp 188°C; $[\alpha]_D=-118.8$ (*c*, 0.99 in MeOH). ^{1}H NMR (methanol-d_4): δ 3.75 (dd, 1H, $J_{4',5a'}=4.3$ Hz, $J_{5a',5b'}=12.3$ Hz, H-5a′), 3.87 (dd, 1H, $J_{4',5b'}=3.3$ Hz, H-5b′), 4.14 (m, 1H, $J_{3',4'}=2.8$ Hz, H-4′), 4.42 (dd, 1H, $J_{2',3'}=5.9$ Hz, H-3′), 4.75 (dd, 1H, $J_{1',2'}=9.2$ Hz, H-2′), 5.33 (d, 1H, H-1′), 7.32–7.82 (m, 5H, Ph); ^{13}C NMR (methanol-d_4): δ 59.76 (C-2′), 63.44 (C-5′), 72.30 (C-3′), 75.78 (C-1′), 88.79 (C-4′), 128.48, 129.39, 132.70 and 135.32 (Ph), 161.58 (C-5), 170.22 (Ph*C*ONH). FAB-MS: *m*/*z* 350 (M^++2Na−H), 328 (M^++Na). HR-MS: Calcd for $C_{13}H_{15}N_5NaO_4$: 328.1022. Found: *m*/*z* 328.1031. Anal. Calcd for $C_{13}H_{15}N_5O_4\times2.5H_2O$: C 44.57, H 5.71, N 20.00. Found: C 44.23, H 5.12, N 20.41.

4.1.12. 2,5-Anhydro-3,6-di-*O*-benzoyl-D-allose ethylene acetal (21). To a solution of **20**[11] (3.55 g, 7.21 mmol) in 95% aq. DMF (73 mL) was added $CaCO_3$ (1.44 g, 14.0 mmol). The mixture was stirred for 20 h at 155–160°C and then filtered. The filtrate was diluted with water (300 mL) and extracted with 1:1 mixture of benzene–hexane (3×200 mL). The combined extracts were washed with water (2×50 mL) dried and filtered. The organic solution was left at room temperature for 10 h whereupon pure **21** was obtained as a white solid. Recrystallization from CH_2Cl_2–hexane gave an analytical sample **21** (0.72 g, 24%) as colorless crystals: mp 135°C; $[\alpha]_D=-2.8$ (*c*, 0.6 in $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 2.45 (d, 1H, $J_{OH,4}=5.8$ Hz, OH-4), 3.79–4.10 (m, 4H, 2×CH_2–dioxolane), 4.28 (ddd, 1H, $J_{4,5}=10.9$ Hz, $J_{5,6a}=5.1$ Hz, $J_{5,6b}=2.9$ Hz, H-5), 4.37 (t, 1H, $J_{1,2}=J_{2,3}=2.3$ Hz, H-2), 4.45–4.56 (m, 2H, $J_{3,4}=5.4$ Hz, $J_{6a,6b}=12.2$ Hz, H-4 and H-6a), 4.69 (dd, 1H, H-6b), 5.09 (d, 1H, H-1), 5.51 (dd, 1H, H-3), 7.39–8.16 (m, 10H, 2×Ph); ^{13}C NMR ($CDCl_3$): δ 64.14 (C-6), 65.50 and 65.62 (2×CH_2–dioxolane), 71.65 (C-4), 74.12 (C-3), 80.46 (C-5), 82.69 (C-2), 102.35 (C-1), 128.32, 128.50, 129.29, 129.79, 133.08 and 133.51 (2×Ph), 166.22 and 166.53 (2×Ph*C*OO). Anal. Calcd for $C_{22}H_{22}O_8$: C 63.76, H 5.35. Found: C 63.96, H 5.50. Mother liquor was evaporated to afford an inseparable mixture of **21** and **22** (1.86 g, 62%) as colorless syrup, which was used for further work without additional purification.

4.1.13. 2,5-Anhydro-3,6-di-*O*-benzoyl-4-*O*-methanesulfonyl-D-allose ethylene acetal (23). Procedure A: To a stirred and cooled (0°C) solution of **21** (2.0122 g, 4.86 mmol) in dry pyridine (50 mL) was added MsCl (1.25 mL, 16.08 mmol). The mixture was left at +4°C for 48 h, then poured onto ice, acidified with conc. HCl (to pH 2) and the resulting solution was extracted with CH_2Cl_2 (4×100 mL). The combined extracts were washed with water (100 mL), satd aq. $NaHCO_3$ (100 mL) and again with water (100 mL), dried and evaporated to give crude **23** as a pale yellow crystalline mass. Recrystallization from EtOH gave pure **23** (2.27 g, 95%) as colorless crystals: mp 107°C; $[\alpha]_D=+15.15$ (*c*, 1.2 in $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 2.98 (s, 3H, $MeSO_2$), 3.78–4.05 (m, 4H, 2×CH_2–dioxolane), 4.40 (dd, 1H, $J_{1,2}=2.0$ Hz, $J_{2,3}=4.0$ Hz, H-2), 4.48 (dd, 1H, $J_{5,6a}=4.1$ Hz, $J_{6a,6b}=12.1$ Hz, H-6a), 4.57 (m, 1H, H-5), 4.75 (dd, 1H, $J_{5,6b}=3$ Hz, H-6b), 5.10 (d, 1H, H-1), 5.37 (dd, 1H, $J_{3,4}=5.5$ Hz, $J_{4,5}=6.5$ Hz, H-4), 5.65 (dd, 1H, H-3), 7.40–8.20 (m, 10H, 2×Ph); ^{13}C NMR ($CDCl_3$): δ 38.11 ($MeSO_2$), 62.71 (C-6), 65.50 and 65.62 (2×CH_2–dioxolane), 71.35 (C-3), 76.62 (C-4), 79.36 (C-5), 81.97 (C-2), 101.85 (C-1), 128.35, 128.57, 129.71, 129.75, 133.12 and 133.67 (2×Ph), 165.40 and 166.00 (2×Ph*C*OO). Anal. Calcd for $C_{23}H_{24}O_{10}S$: C 56.10, H 4.87, S 6.50. Found: C 56.26, H 4.75, S 6.46.

Procedure B: To a stirred and cooled (0°C) solution of crude mixture **21** and **22** (mother liquor, 1.42 g, 3.43 mmol) in dry pyridine (50 mL) was added MsCl (0.81 mL, 10.41 mmol).

The solution was stored at +4°C for 48 h. After workup as described above (procedure A) the crude mixture was obtained which was purified on a column of silica gel (5:1 toluene–EtOAc). Physical and spectral data of thus obtained sample **23** (0.56 g, 33%) were in full agreement with the corresponding values reported above (procedure A). Further elution gave pure **24** (0.21 g, 12.5%) as a colorless oil: $[\alpha]_D$=+52.9 (*c*, 1.12 in $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 2.97 (s, 3H, $MeSO_2$), 3.92–4.05 (m, 4H, 2×CH_2–dioxolane), 4.46 (t, 1H, $J_{1,2}$=2.3 Hz, $J_{2,3}$=2.8 Hz, H-2), 4.49 (dd, 1H, $J_{5,6a}$=4.5 Hz, $J_{6a,6b}$=11.8 Hz, H-6a), 4.58 (m, 1H, $J_{4,5}$=7.5 Hz, $J_{5,6b}$=3.5 Hz, H-5), 4.70 (dd, 1H, H-6b), 5.09 (d, 1H, H-1), 5.38 (dd, 1H, $J_{3,4}$=5.5 Hz, H-3), 5.53 (dd, 1H, H-4), 7.32–8.11 (m, 10H, 2×Ph); ^{13}C NMR ($CDCl_3$): δ 38.34 ($MeSO_2$), 63.49 (C-6), 65.53 and 65.64 (2×CH_2–dioxolane), 71.93 (C-4), 77.54 (C-3), 78.27 (C-5), 83.07 (C-2), 101.59 (C-1), 128.28, 128.54, 129.70, 129.83, 133.08 and 133.66 (2×Ph), 165.53 and 166.07 (2×Ph*C*OO). LRMS (EI): *m*/*z* 492 (M^+).

4.1.14. 3(5)-Carbomethoxy-4-(2,5-di-*O*-benzoyl-3-*O*-methanesulfonyl-β-D-ribofuranosyl)pyrazole (28). A solution of **23** (2.8261 g, 5.74 mmol) in a mixture of TFA (28 mL) and 6 M HCl (7 mL) was kept at +4°C for 24 h. The workup as described earlier (preparation of **10** and **11**) gave crude aldehyde **25** which was immediately dissolved in dry CH_2Cl_2 (50 mL) and allowed to react with a solution of (carbomethoxymethylene)triphenylphosphorane (4.7916 g, 14.37 mmol) in dry CH_2Cl_2 (15 mL) for 24 h at room temperature. The mixture was evaporated and purified by column chromatography (10:1 toluene–EtOAc) whereupon the oily unsaturated ester **26** (1.1346 g, 48%) was obtained as a 4:1 mixture of *E*- and *Z*-isomers: $[\alpha]_D$=−61.2 (*c*, 1.11 in $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 2.95 (s, $MeSO_2$, *E*-isomer), 3.00 (s, $MeSO_2$, *Z*-isomer), 3.69 (s, OMe, *Z*-isomer), 3.70 (s, OMe, *E*-isomer), 4.55 (dd, $J_{7,8a}$=3.2 Hz, $J_{8a,8b}$=12 Hz, H-8a, *E*-isomer), 4.67 (m, $J_{6,7}$=4.5 Hz, $J_{7,8b}$=3.1 Hz, H-7, *E*-isomer), 4.74 (dd, H-8b, *E*-isomer), 4.85 (m, $J_{2,4}$=1.5 Hz, $J_{3,4}$=4.8 Hz, $J_{4,5}$=6.5 Hz, H-4, *E*-isomer), 5.21 (t, $J_{5,6}$=5.6 Hz, H-5, *E*-isomer), 5.37 (t, H-6, *E*-isomer), 5.97 (dd, $J_{2,3}$=11.6 Hz, $J_{2,4}$=1.2 Hz, H-2, *Z*-isomer), 6.17 (dd, $J_{3,4}$=7.9 Hz, H-3, *Z*-isomer), 6.24 (dd, $J_{2,3}$=15.7 Hz, H-2, *E*-isomer), 7.00 (dd, H-3, *E*-isomer), 7.40–8.18 (m, 10H, 2×Ph); ^{13}C NMR ($CDCl_3$): δ 38.08 ($MeSO_2$, *E*-isomer), 38.22 ($MeSO_2$, *Z*-isomer), 51.55 (OMe, *Z*-isomer), 51.69 (OMe, *E*-isomer), 62.80 (C-8, *E*-isomer), 63.04 (C-8, *Z*-isomer), 74.59 (C-5, *E*-isomer), 76.84 (C-6, *E*-isomer), 79.14 (C-4, *E*-isomer), 80.85 (C-7, *E*-isomer), 122.97 (C-2, *E*-isomer), 123.48 (C-2, *Z*-isomer), 125.21, 128.14, 128.39, 128.50, 128.68, 128.94, 129.27, 129.62, 129.66, 129.79, 129.90, 133.30, 133.69 and 133.95 (2×Ph), 142.36 (C-3, *E*-isomer), 144.11 (C-3, *Z*-isomer), 165.51, 165.94 and 165.98 (2×Ph*C*OO and *C*OOMe). To a stirred and cooled (0°C) solution of **26** (0.419 g, 0.83 mmol) in dry Et_2O (4 mL) was added a solution of diazomethane (generated from 1.596 g, 10.85 mmol, of *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine) in Et_2O (36 mL). The mixture was stirred at 0°C for 5 h and then evaporated. The remaining crude 2-pyrazoline **27** was dissolved in a mixture of dry CCl_4 (10 mL) and CH_2Cl_2 (4 mL) and treated with a freshly prepared saturated solution of Cl_2 in CCl_4 (15 mL) for 3 h at room temperature. The mixture was evaporated and the remaining crude **28** was purified on a column of silica gel (4:1, 7:3, 3:2 toluene–EtOAc). Pure product **28** (0.41 g, 48% from **23**) was isolated as colorless syrup: $[\alpha]_D$=+9.4 (*c*, 0.91 in $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 2.99 (s, 3H, $MeSO_2$), 3.73 (s, 3H, OMe), 4.59–4.72 (m, 2H, $J_{4',5a'}$=3.8 Hz, $J_{5a',5b'}$=14.5 Hz, H-4′ and H-5a′), 4.85 (m, 1H, H-5b′), 5.43 (t, 1H, $J_{2'3'}$=5.4 Hz, $J_{3',4'}$=5.6 Hz, H-3′), 5.61 (t, 1H, $J_{1',2'}$=5.2 Hz, H-2′), 5.75 (d, 1H, H-1′), 7.35–8.17 (m, 10H, 2×Ph), 7.85 (s, 1H, H-3), 13.42 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$): δ 38.23 ($MeSO_2$), 52.09 (OMe), 62.85 (C-5′), 75.77 (C-1′), 76.22 (C-2′), 76.38 (C-3′), 79.53 (C-4′), 121.57 (C-4), 128.53, 128.65, 128.75, 128.99, 129.40, 129.67, 129.86, 133.33, 133.82 (2×Ph), 130.24 (C-3), 138.55 (C-5), 161.82 (*C*OOMe) 165.49 and 166.15 (2×Ph*C*OO).

4.1.15. 3(5)-Carboxamido-4-(3-*O*-methanesulfonyl-β-D-ribofuranosyl)pyrazole (29). A solution of **28** (0.5304 g, 0.97 mmol) in saturated methanolic solution of NH_3 (50 mL) was left at room temperature for 7 days, and then evaporated. The remaining crude mixture was triturated with benzene. The benzene solution was decanted, and the remaining crude oil was purified by preparative TLC (7:3 $CHCl_3$–MeOH). Pure **29** (0.313 g, 86%) was thus obtained as colorless oil: $[\alpha]_D$=+26.4 (*c*, 1.03 in MeOH). Crystallization from MeOH–^{i}Pr_2O gave an analytical sample **29** as colorless crystals: mp 110–111°C. ^{1}H NMR (DMSO-d_6): δ 3.21 (s, 3H, $MeSO_2$), 3.51 (dd, 1H, $J_{5a',5b'}$=12.1 Hz, $J_{4',5a'}$=4.1 Hz, H-5a′), 3.56 (dd, 1H, $J_{4',5b'}$=4.0 Hz, H-5b′), 4.05 (m, 1H, $J_{3',4'}$=3.2 Hz, H-4′), 4.13 (dd, 1H, $J_{1',2'}$=7.7 Hz, $J_{2',3'}$=5.2 Hz, H-2′), 4.88 (dd, 1H, H-3′), 5.11 (d, 1H, H-1′), 3.42 and 5.84 (2×bs, 1H each, 2×OH), 7.38 and 7.64 (2×bs, 1H each, $CONH_2$), 7.86 (s, 1H, H-3), 13.28 (bs, 1H, NH); ^{13}C NMR (DMSO-d_6): δ 38.21 ($MeSO_2$), 60.92 (C-5′), 74.96 (C-2′), 75.44 (C-1′), 81.21 (C-3′), 82.51 (C-4′), 120.44 (C-3), 130.15 and 143.05 (C-4 and C-5), 164.47 ($CONH_2$). FAB-MS: *m*/*z* 344 (M^++Na), 322 (M^++H). Anal. Calcd for $C_{10}H_{15}N_3O_7S$×0.5H_2O: C 36.36, H 4.88, N 12.72. Found: C 36.20, H 5.00, N 12.38.

4.1.16. 2,5-Anhydro-4-azido-3,6-di-*O*-benzoyl-4-deoxy-D-gulose ethylene acetal (30). To a solution of **23** (3.02 g, 6.14 mmol) in DMSO (60 mL) was added NaN_3 (4.24 g, 65.23 mmol). The resulting suspension was stirred at 118–120°C for 144 h and then poured into cold water (100 mL) and extracted with 1:1 benzene–hexane (4×50 mL). The extract was washed with water (2×20 mL), dried and evaporated. The remaining crude mixture was purified by column chromatography (99:1, 11:4 toluene–EtOAc) to afford pure **30** (1.68 g, 62%) as a colorless oil: $[\alpha]_D$=−6.4 (*c*, 1.59 in $CHCl_3$). ν_{max} (film): 2100 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 3.86–4.15 (m, 4H, 2×CH_2–dioxolane), 4.18 (dd, 1H, $J_{1,2}$=4.7 Hz, $J_{2,3}$=3 Hz, H-2), 4.30 (dd, 1H, $J_{3,4}$=1.1 Hz, $J_{4,5}$=4.1 Hz, H-4), 4.46 (m, 1H, $J_{5,6a}$=5.7 Hz, $J_{5,6b}$=5.9 Hz, H-5), 4.55–4.69 (m, 2H, 2×H-6), 5.17 (d, 1H, H-1), 5.58 (dd, 1H, H-3), 7.40–8.13 (m, 10H, 2×Ph); ^{13}C NMR ($CDCl_3$): δ 62.64 (C-6), 65.35 and 65.68 (2×CH_2–dioxolane), 66.70 (C-4), 78.14 (C-3), 78.30 (C-5), 83.76 (C-2), 102.25 (C-1), 127.96, 128.14, 128.53, 128.57, 129.26, 129.31, 132.76 and 133.28 (2×Ph). Further eluting gave pure **31** (0.18 g, 9%) as a colorless syrup: ^{1}H NMR ($CDCl_3$): δ 2.72 (bs, 1H, exchangeable with D_2O, OH), 3.75 (t, 1H, $J_{1,2}$=5.1 Hz, $J_{2,3}$=5.3 Hz, H-2), 3.81–4.08 (m, 4H, 2×CH_2–dioxolane),

4.18 (dd, 1H, $J_{3,4}$=4.2 Hz, $J_{4,5}$=5.3 Hz, H-4), 4.29–4.60 (m, 4H, H-3, H-5 and 2×H-6), 5.00 (d, 1H, H-1), 7.30–8.13 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$): δ 63.21 (C-6), 65.21 and 65.35 (2×CH_2–dioxolane), 68.10 (C-4), 76.42 (C-3), 77.50 (C-5), 84.63 (C-2), 102.97 (C-1), 128.29, 128.45, 129.53, 129.59 and 133.09 (Ph), 166.25 (Ph*C*OO).

4.1.17. 2,5-Anhydro-4-azido-3,6-di-*O*-benzoyl-4-deoxy-D-gulononitrile (34). A solution of **30** (1.68 g, 3.83 mmol) in a mixture of TFA (8.5 mL) and 6 M HCl (2.1 mL) was left at +4°C for 96 h. The workup as described earlier (preparation of **10** and **11**) gave crude aldehyde **32**,which was immediately dissolved in a mixture of EtOH (13 mL) and CH_2Cl_2 (2 mL) and allowed to react with NH_2OH×HCl (0.6 g, 8.63 mmol) and NaOAc (0.96 g, 11.58 mmol) by stirring at room temperature for 2 h. After work up as described above (preparation of **17**), crude oxime **33** was obtained as a mixture of *E*- and *Z*-isomers. To a cooled (−15°C) and stirred solution of crude **33** (1.1 g) in anhydrous pyridine (5 mL) was added dropwise precooled (0°C) solution of MsCl (1.29 mL,16.55 mmol) in dry pyridine (3.5 mL). The mixture was warmed to room temperature and stirred for the next 2 h. The usual workup (preparation of **17**) gave crude **34** as brown oil. Chromatographic purification on a column of silica gel (4:1 cyclohexane–Me_2CO) gave pure **34** (0.324 g, 22% from **30**) as colorless syrup: $[\alpha]_D$=−32.7 (*c*, 2.61 in $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 4.51 (dd, 1H, $J_{4,5}$=3.5 Hz, $J_{3,4}$=1.4 Hz, H-4), 4.56–4.76 (m, 3H, $J_{6a,6b}$=11.7 Hz, $J_{5,6a}$=6 Hz, $J_{5,6b}$=5.2 Hz, H-5 and 2×H-6), 4.84 (d, 1H, $J_{2,3}$=1.6 Hz, H-2), 5.74 (t, 1H, H-3), 7.40–8.12 (m, 10H, 2×Ph); ^{13}C NMR ($CDCl_3$): δ 62.29 (C-6), 65.70 (C-4), 70.22 (C-5), 79.86 (C-2), 80.50 (C-3), 115.40 (CN), 127.72, 128.44, 129.18, 129.68, 129.84, 133.39 and 134.30 (2×Ph), 164.88 and 165.99 (2×PhCOO).

4.1.18. 5-(3-Azido-2,5-di-*O*-benzoyl-3-deoxy-β-D-xylofuranosyl) tetrazole (35). A suspension of compound **34** (0.12 g, 0.3 mmol), NaN_3 (0.04, 0.55) and NH_4Cl (0.03, 0.53 mmol) in DMF (1 mL) was stirred at 100°C for 2.5 h. The workup as described earlier (preparation of **18**) gave crude **35**, which was purified by preparative TLC (80:23 $CHCl_3$–MeOH) to afford pure **35** (0.08 g, 58%) as a colorless syrup: $[\alpha]_D$=−29.4 (*c*, 1.85 in $CHCl_3$). ^{1}H NMR (acetone-d_6): δ 4.63 (d, 2H, $J_{4',5'}$=5.5 Hz, 2×H-5′), 4.81 (m, 1H, $J_{3',4'}$=4.3 Hz, H-4′), 4.87 (dd, 1H, $J_{2',3'}$=1.5 Hz, H-3′), 5.62 (d, 1H, $J_{1',2'}$=3.3 Hz, H-1′), 5.96 (dd, 1H, H-2′), 7.43–8.18 (m, 10H, 2×Ph); ^{13}C NMR (acetone-d_6): δ 63.72 (C-5′), 67.47 (C-3′), 77.43 (C-1′), 80.02 (C-4′), 82.53 (C-2′), 129.38, 129.46, 130.02, 130.31, 130.55, 130.65, 134.10 and 134.53 (2×Ph), 157.29 (C-5), 165.96 and 166.52 (2×Ph*C*OO). LR-MS (CI): *m/z* 493 (M^++C_4H_{10}), 436 (M^++H).

4.1.19. 5-(3-Azido-3-deoxy-β-D-xylofuranosyl)tetrazole (36). To a solution of **35** (0.13 g, 0.29 mmol) in dry MeOH (6 mL) was added 5% solution of MeONa in MeOH (0.35 mL) and the mixture was stirred at room temperature for 2.5 h. The workup as described earlier (preparation of **19**) yielded crude **36**, which was separated from methyl benzoate after triturating with benzene. Pure product **36** (0.06 g, 91%) was thus obtained as a colorless syrup: $[\alpha]_D$=−122.6 (*c*, 0.99 in MeOH). ^{1}H NMR (methanol-d_4): δ 3.83 (d, 2H, $J_{4',5'}$=5.8 Hz, 2×H-5′), 4.17 (dd, 1H, $J_{3',4'}$=4.6 Hz, $J_{2',3'}$=2.6 Hz, H-3′), 4.40 (m, 1H, H-4′), 4.58 (t, 1H, $J_{1',2'}$=3 Hz, H-2′), 5.14 (d, 1H, H-1′); ^{13}C NMR (methanol-d_4): δ 61.37 (C-5′), 69.39 (C-3′), 79.57 (C-1′), 81.19 (C-2′), 82.72 (C-4′), 157.69 (C-5). LR-MS (CI): *m/z* 257 (M^++C_4H_{10}−N_2), 228 (M^++H).

4.2. X-Ray analysis[17]

Colorless, transparent single crystal of **15** was mountered on a Enraf–Nonius CAD-4 diffractometer equipped with graphite-monochromated Mo Kα radiation. Cell parameters (Table 3) were determined by least-squares refinement of diffractometer angles for 24 reflections collected in the range 7.0≤θ≤1.0°. Three standard reflections (−1 1 −6), (−1 1 6) and (−1 1 −9) were monitored every 60 min with intensity variations R_{int}=0.00057, $R(\sigma)$=0.0931. Reflections were recorded with ω–2θ scan with Miller indices h_{min}=−1, h_{max}=8, k_{min}=0, k_{max}=15, l_{min}=−9, l_{max}=22 and merged with R=0.0057 to 1806 unique reflections. Data were corrected for Lorentz and polarization effects. The structure was solved by a direct method using the SHELXS86 program.[18] The *E*-map computed from the phase set with best combined figure of merit revealed the positions of all non-hydrogen atoms along with an oxygen atom (O′) from the independent water molecule that was also included into the crystal lattice. Full-matrix least squares refinement of the fractional coordinates of the non-hydrogen atoms with anisotropic atomic displacement parameters was performed with SHELXL97.[19] Positions of hydrogen atoms were generated from the assumed geometry, checked in difference Fourier map and refined isotropically with common displacement parameters U_1=0.053(7) Å^2 and U_2=0.093(12) Å^2. For solvated water hydrogens isotropic displacement parameters were fixed to

Table 3. Crystallographic data and structure refinement of **15**

Crystallographic parameter	
Empirical formula	$C_{16}H_{18}N_4O_5$×H_2O
Formula weight	364.36
Temperature (K)	293(2)
Wavelength (Å)	0.71070
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
Unit cell dimensions	a=7.122(2) Å, α=90° b=13.049(2) Å, β=90° c=19.229(2) Å, γ=90°
Volume (Å^3)	1787.0(6)
Z	4
Density (calculated) (mg m^{-3})	1.354
Absorption coefficient (mm^{-1})	0.105
F(000)	768
Crystal size (mm^3)	0.35×0.25×0.20
Θ range for data collection (°)	2.1–24.95
Index ranges	−1≤h≤8, 0≤k≤15, −9≤l≤22
Reflections collected	1913
Independent reflections	1806 [R(int)=0.0057]
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	1806/0/245
Goodness-of-fit on F2	0.947
Final R indices [I>2σ(I)]	R1=0.0661, wR2=0.1520
R indices (all data)	R1=0.1128, wR2=0.1692
Absolute structure parameter	3(4)
Extinction coefficient	0.012(4)
Largest difference peak and hole (e Å^{-3})	0.321 and −0.290

$1.3U_{eq}$ of the parent atom O′. The uncertainty of the hydrogen position within the pyrazole ring indicated the presence of two tautomeric forms.

The refinement of the occupational factors of hydrogens H1 and H2 bonded to N1 and N2 atoms lead to the final value of 0.28(8) and 0.72(8), respectively. Final *R*-factor was R=0.0661, for 245 parameters using the F^2 values of 1806 ($I>2\sigma(I)$) reflections. The highest and the lowest peaks in the final difference map were 0.321 and 0.290 e $Å^{-3}$. Scattering factors were taken from SHELXL97.

4.3. Cytotoxic activity

Cytotoxic activity was determined for mouse neuroblastoma (N2a) and baby hamster kidney (BHK 21) cells suspended at a density of 10^{-4} cells/0.1 mL. Stock solutions of the tested compounds (c=0.1 mol/L) in growth medium (GM) were prepared as follows: Compound **15** was dissolved in Eagle Minimal Essential Medium (EMEM) supplemented with 10% calf serum. Due to their poor solubilities compounds **8**, **29** and **36** were dissolved in GM containing 0.5% of Tween 80. Eight serial twofold diluted solutions were prepared on a microplate for cell culture (0.1 mL volume per well), starting with 0.01 M concentration for each tested substance. In parallel, GM with and without Tween 80 were included as controls (GM with 0.5% Tween 80 was serially diluted as above). N2a and BHK 21 cell suspensions (0.1 mL) were added separately to each tested solution and controls. The final concentrations of tested compounds were: 5000, 2500, 1250, 625, 312, 156, 78 and 39 μM/L. The microplate was then incubated for 24 h at 37°C in the atmosphere with 5% of CO_2. At the end of the incubation period, the microplate was examined under an inverted optical microscope (magnification 40×) and the percentage of rounded cells recorded for each well. The concentration that resulted in 50% inhibition of growth (IC50) was determined by linear regression technique (Table 1).

4.4. Antiviral activity

Compounds **8**, **15**, **29** and **36** were evaluated for possible inhibition of rabies virus growth in mouse neuroblastoma (N2a) cell culture. The eight serial twofold dilutions of tested compounds were prepared as described above (0.05 mL per well) starting with 0.02 M solution for each substance. In parallel, GM with and without Tween 80 were included as controls. Rabies virus strain CVS was added to each well (0.05 mL) in concentrations which form specific nucleocapsid inclusions in 70% of cells after 24 h incubation in GM control wells. N2a cell suspension (10^4 cells/0.1 mL) was added to each well (0.1 mL), and the microplate was incubated for 24 h at 37°C in the atmosphere with 5% CO_2. The cells were fixed with 80% cold acetone and strained with specific rabies antinucleocapsid FITC-IgG conjugate. The microplate was then examined for presence of specific fluorescent inclusions on a fluorescent microscope (magnification 100×). The percentage of cells containing fluorescent inclusions was recorded for each well, and the results are presented in Table 2.

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no. CCDC 167534. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK (fax: +44-(0)1223-336033 or e-mail: deposit @ccdc.cam.ac.uk).

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