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Stereospecific synthesis of two novel cytotoxic pyrazole *C*-nucleosides from D-glucose

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Abstract

A multistep stereospecific synthesis of two novel pyrazole *C*-nucleosides **12** and **21** has been achieved starting from D-glucose, by utilizing the 2,5-anhydro-D-glucose ethylene acetal derivative **1** as a divergent intermediate. The *C*-nucleoside **12** was shown to be a moderate inhibitor of the in vitro growth of N2a and BHK 21 tumor cell lines, whereas **21** showed a moderate cytotoxic activity only against N2a cells. © 2000 Published by Elsevier Science Ltd.

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Introducing diversity into the carbohydrate and/or base subunit of nucleosides represents a promising strategy for the design of novel chemotherapeutic agents.[1] Significant antitumor and antiviral activity displayed by certain pyrazole-related nucleosides[2] prompted us to prepare the related *C*-nucleoside analogues. The synthesis of several 4-(β-D-ribofuranosyl)pyrazoles,[3] including a pyrazole homo-*C*-nucleoside, has already been reported.[4] 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. Herein we report a divergent synthesis of two novel pyrazole-related *C*-nucleosides **12** and **21** from D-glucose, along with preliminary results related to their in vitro cytotoxic activity against mouse neuroblastoma (N2a) and baby hamster kidney (BHK 21) tumor cell lines.

The crucial problem in the synthesis of both targets **12** and **21** was the establishment of the requisite β-configuration at the anomeric position. In a previous paper we have described[5] the conversion of D-glucose to the 2,5-anhydro-D-glucose ethylene acetal derivative **1** (Scheme 1), a compound already containing the desired β-*C*-glycosidic bond, as well as the functionalities suitable

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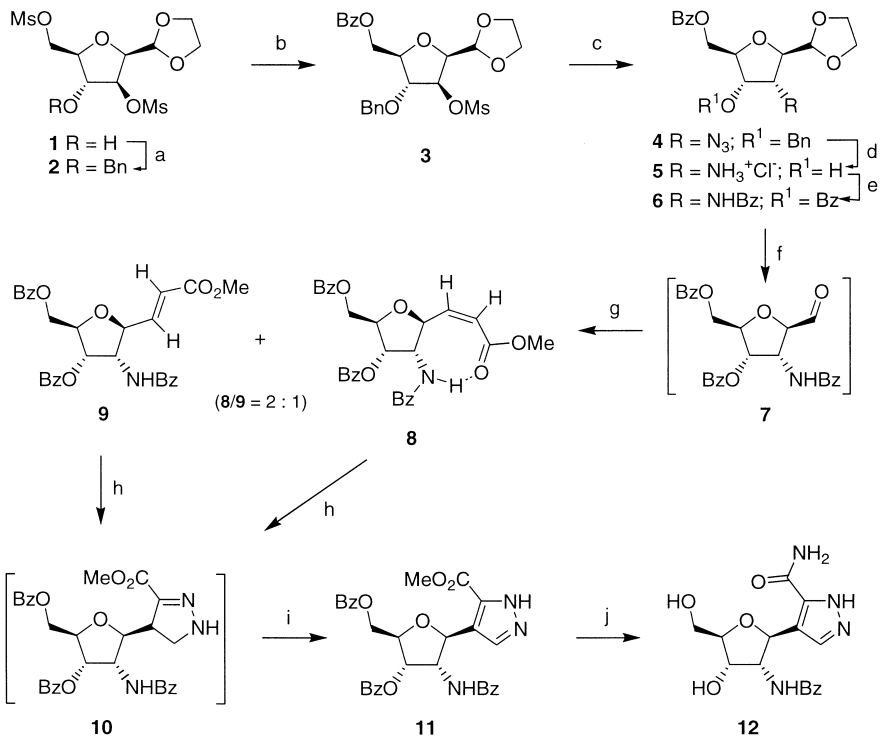


Scheme 1. (a) BnBr, Ag_2O, Et_2O, reflux, 22 h, 87%; (b) KOBz, DMF, 100°C, 48 h, 83%; (c) NaN_3, DMSO, 120°C, 47 h, 77%; (d) H_2, Pd/C, $CHCl_3$ (cat.), EtOH, rt, 120 h; (e) BzCl, Py, rt, 96 h, 71% from **4**; (f) 4:1 CF_3CO_2H:6 M HCl,

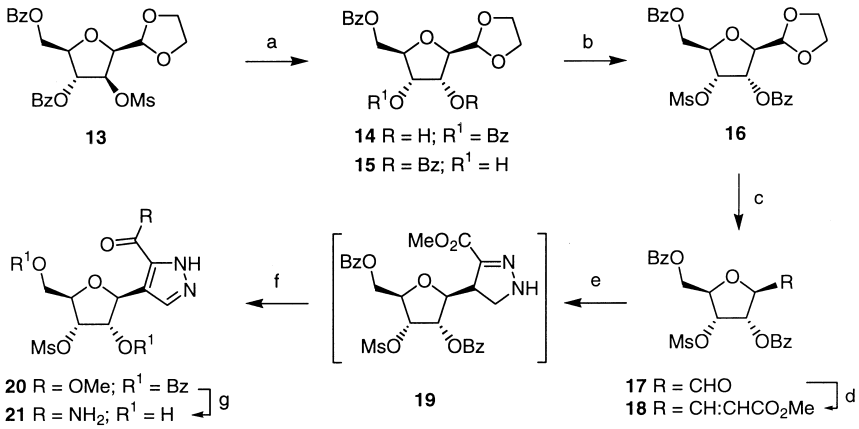
for further introduction of diversity into the nucleoside carbohydrate segment. The 2,5-anhydro-D-glucose derivative **1** reacted with benzyl bromide in ether, in the presence of silver(I)-oxide as a catalyst, to afford the expected 4-*O*-benzyl derivative **2** in 87% yield. Reaction of **2** with potassium benzoate in *N,N*-dimethylformamide gave the corresponding 6-*O*-benzoyl derivative **3** (83%), which was further treated with sodium azide in dimethyl sulfoxide to afford the 3-azido-3-deoxy derivative **4** in 77% yield.

Catalytic reduction of **4** over 10% Pd/C, in ethanol containing a catalytic amount of chloroform, gave the amine hydrochloride **5** as a product of a sequential azide reduction/benzyl ether hydrogenolysis process.[6] Treatment of crude **5** with benzoyl chloride in pyridine yielded the key intermediate **6** as the major reaction product (71% from **4**). Hydrolytic removal of the dioxolane protective group in **6** was achieved with a 4:1 mixture of trifluoroacetic acid and 6 M hydrochloric acid at +4°C, whereupon the corresponding aldehyde **7** was obtained. Due to its instability it was not characterized further, but was immediately treated with (carbomethoxymethylene)-triphenylphosphorane in dichloromethane to afford a 2:1 mixture of the corresponding *Z* and *E* unsaturated esters **8** and **9** in 85% combined yield.

The unsaturated esters **8** and **9** were readily separated by column chromatography and converted to the *C*-nucleoside **12** according to the methodology developed by Moffatt et al.[7] Both **8** and **9** readily underwent 1,3-dipolar cycloaddition with an excess of diazomethane in ether at 0°C to yield the 2-pyrazoline **10** as the only reaction product. The transformation of **8** to **10** was completed within 2 h, while the conversion of **9** to **10** required 15 h for completion. The enhanced

reactivity of the *Z*-isomer **8** 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 ^{1}H NMR spectrum of **8** showed a significant downfield shift of the amide proton signal (δ_{NH} 7.67) in contrast to the corresponding signal in the spectrum of **9**, which appeared at a highfield position (δ_{NH} 6.78). This result is consistent with the presence of an intramolecular hydrogen bond in **8**. Without purification or further characterization the intermediate **10** was treated with a saturated solution of chlorine in carbon tetrachloride to give the pyrazole derivative **11**. Finally, treatment of **11** with methanolic ammonia afforded the *O*-deprotected *C*-nucleoside **12**,[8] ready for biological testing. Under these reaction conditions the intermediates **8** and **9** were converted into the target **12** in 97 and 85% overall yields, respectively.

Synthesis of the pyrazole *C*-nucleoside **21** bearing the 3′-mesyloxy group as an isostere is outlined in Scheme 2. Solvolysis of **13**[5] in wet *N,N*-dimethylformamide (5% of water), in the presence of calcium carbonate as a proton acceptor, gave a mixture of 4,6- and 3,6-di-*O*-benzoyl derivatives **14** and **15** in 86% combined yield. The regioisomers **14** and **15** could not be separated by column chromatography, presumably due to their rapid inter-conversion affected by silica gel. However, direct crystallization of crude reaction mixture from benzene–hexane afforded pure **15**, which was subsequently mesylated to give the key intermediate **16**. The addition of mesyl chloride in pyridine to the mother liquor, which remained after crystallization of **15**, afforded an additional quantity of **16**. The total yield of **16** was 41% with respect to **13**. The intermediate **16** was converted to the target molecule **21**[9] by using the same five-step sequence already applied for the conversion of **6** into the *C*-nucleoside **12**. In this way the 4-*O*-mesyl derivative **16** has been transformed into the target **21** in 38% overall yield.



Scheme 2. (a) DMF, H_2O (5%), $CaCO_3$, 155–160°C, 20 h, 86%; (b) MsCl, Py, +4°C, 48 h, 41% from **13**; (c) 4:1 CF_3CO_2H-6M HCl, +4°C, 24 h; (d) Ph_3P:$CHCO_2Me$, CH_2Cl_2, rt, 24 h, 48% from **16**; (e) CH_2N_2, Et_2O, 0°C, 5 h; (f) Cl_2/CCl_4, CH_2Cl_2, rt, 3 h, 91% from **18**; (g) NH_3, MeOH, rt, 7 days, 86%.

The *C*-nucleosides **12** and **21** were preliminarily tested in vitro for cytotoxic activity against mouse neuroblastoma (N2a) and baby hamster kidney (BHK 21) tumor cell lines. As shown in Table 1, the *C*-nucleoside **12** showed a moderate cytotoxic effect against both N2a and BHK 21 cells. Compound **21** displayed no significant cytotoxicity towards BHK 21, but showed some cytostatic activity against N2a cells. Studies on further biological evaluation of these compounds will be reported in due course.

Table 1
Cytotoxic activity of synthesized *C*-nucleosides **12** and **21**

Cell lines	IC_{50}[a] (μM)	
	12	**21**
N2a	220	440
BHK 21	170	1300

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

In summary, a stereospecific synthesis of cytotoxic pyrazole-related *C*-nucleosides bearing the 2-benzamido (**12**) or the 3-mesyloxy group (**21**) as isosteres has been achieved starting from D-glucose, via the 2,5-anhydro-D-glucose derivative **1** as a divergent intermediate. Molecules of type **12** might be of a wider medicinal interest because they are partly related to certain 2′-benzamido-2′-deoxy adenosines, the potential agents for treatment of sleeping sickness.[10]

Acknowledgements

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6. Standard conditions for this transformation are: 1 atm, 10 mmol of **4**, 146 mL of absolute EtOH, 7.2 mL of $CHCl_3$, and 9.5 g of 10% Pd/C. Decreasing the quantity of catalyst (214 mg/1 mmol) resulted in complete reduction of the azide function, but only to partial removal of the benzyl protective group.
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8. Compound **12**: m.p. 133°C (from MeOH−EtOAc); $[\alpha]_D$ –116.6 (*c*, 1.08 in MeOH); ^{1}H NMR (250 MHz, 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, $J_{2',3'}$ = 5.2, $J_{3',4'}$ = 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, NHBz), 13.26 (bs, 1H, NH); ^{13}C NMR (62.5 MHz, 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 ($CONH_2$), 166.24 (PhCO).
9. Compound **21**: m.p. 110–111°C (from MeOH–^{i}Pr$_2$O); $[\alpha]_D$ +26.4 (*c*, 1.03 in MeOH); ^{1}H NMR (250 MHz, DMSO-d_6): δ 3.21 (s, 3H, CH_3SO_2), 3.51 (dd, 1H, $J_{5a',5b'}$ = 12.1, $J_{4',5a'}$ = 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, $J_{2',3'}$ = 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 (62.5 MHz, DMSO-d_6): δ 38.21 (CH_3SO_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 ($CONH_2$).
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