Novel synthetic route to the C-nucleoside, 2-deoxy benzamide riboside

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ABSTRACT

2-Deoxy-C-nucleosides are a subcategory of C-nucleosides that has not been explored extensively, largely because the synthesis is less facile. Flexible synthetic procedures giving access to 2-deoxy-C-nucleosides are therefore of interest. To exemplify the versatility and highlight the limitations of a synthetic route recently developed to that effect, the first synthesis of 2-deoxy benzamide riboside is reported. Biological properties of this novel C-nucleoside are also discussed.

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C-Nucleosides are an important class of hydrolytically and enzymatically stable compounds and have been found to be important probes in the pursuit of novel antiviral and antiproliferative drugs.1 There are several known ribose-based C-nucleosides that are potent antiproliferatives. Famous examples are formycin, tiazofurin and benzamide riboside (Fig. 1).2 Carboxamide derivatives of C-nucleosides, such as, benzamide riboside and tiazofurin, are based on nicotinamide riboside (NR), one of three NAD precursor vitamins.3 As prodrugs, these compounds must gain entry to target cells, be phosphorylated by NR kinase 1 or NR kinase 23a and then be adenylylated by NMN adenylyltransferase (NMNAT)4 to a derivative of NAD.5 Benzamide riboside (BR) has been shown, as its benzamide adenine dinucleotide (BAD) metabolite, to exhibit significant cytotoxicity against a variety of human tumour cell lines.6 The mechanism of cytotoxicity of BAD and TAD depends on inhibition of inosine monophosphate dehydrogenase (IMPDH),5 thus resulting in the depletion of the intracellular GTP pool.7

Additionally, the discovery that select nucleosides with L-configuration have biological activity has lead to a breakthrough in antiviral chemotherapy. The antiviral mechanism has shown that such L-nucleosides were phosphorylated by cellular kinases, and selectively interacted with viral polymerases, while remaining relatively innocuous to normal cellular processes.8 These results highlight the need for new synthetic strategies generating both enantiomers of nucleoside analogues equally well, while retaining the possibility for synthetic desymmetrisation, if either or both analogues show interesting antiproliferative properties.8

The syntheses of C-nucleosides are, in general, quite difficult, low yielding and do not allow access to large series of derivatives for SAR studies, in particular with regards to the aromatic moiety.9 Strategies have remained linear, resulting in a limited number of options with regards to the possible structural and functional combinations that can potentially enhance pharmacodynamic/pharmacokinetic properties of the novel compounds.

Our goal is to synthesise analogues of BR to probe each step in the process of NR to NAD biotransformation: stability of the glycosidic linkage to hydrolysis/phosphorolysis,9b import, phosphorylation by NR kinases,3a,d adenylylation,4 and inhibition of subsequent target enzymes. Definition of NR analogue specificity may allow probes to be developed for cancer or for dissection of the metabolic effects of NR.3h Analogues with variation on the aromatic moiety, such as tiazofurin and its selenium equivalent, selenofurin have yielded precursors to potent IMPDH inhibitors. Similarly, the 2’ position of tiazofurin was shown to be tolerant to manipulation, with various compounds retaining cytotoxicity, for example, the 2’-amido-tiazofurin analogue.10 Similarly the epoxide functionality across the 2–3’ positions displayed the most pronounced increase in cytotoxic activity against certain lymphoma cell lines, being almost 30 times more potent than the parent compound tiazofurin.11 While BR and tiazofurin share similar biological profiles and potencies, similar analogues have not been reported for BR. We therefore proposed that BR-analogues incorporating different carbohydrate substitution could also offer novel biological opportunities. As a
result, 2- and 4-deoxypyranoside analogues of BR have been generated.\textsuperscript{12} We also proposed to test whether biological properties might be retained in the 2-deoxyribose series of C-nucleosides.

To access this type of novel 2-deoxy C-nucleoside furanosides, we have devised a synthetic methodology involving key exo-alkene intermediates.\textsuperscript{13} Herein, we report the synthesis of the meta-vinyl substituted phenyl exo-alkene intermediate which provided the necessary chemical flexibility to access the meta-carboxamide substituent essential to BR analogues. The meta-vinyl group was chosen as a direct result of the electron withdrawing nature of the preferred amide or nitrile aromatic functionality, which had proven incompatible to the TMSNTf\textsubscript{2} mediated cyclization conditions necessary to access the exo-alkene furanoside intermediates.\textsuperscript{13}

The procedure began with the synthesis of acetal 3 from the reaction between easily accessible intermediates allylsilane 1 and chloroacetal 2 in 59% yield.\textsuperscript{13} Subsequently, using optimized conditions, acetal 3 was intramolecularly cyclised to provide a 1:3:1 (cis:trans) mixture of furanoside 4 using 2 equiv of TMSNTf\textsubscript{2} in 54% combined yield.\textsuperscript{14} With this intermediate mixture in hand, the double oxidation of the unsaturated functionalities was performed using optimized conditions for ozonolysis,\textsuperscript{15} to achieve the cis and trans isomers of keto-aldehyde product 5 in 87% combined yields. This diastereomeric mixture of 5 was easily separated on silica with the desired cis-isomer isolated in 50% yield. Unfortunately, attempts failed to convert the trans-isomer to the cis-isomer by epimerisation. Density Functional Theory (DFT) calculations showed that the trans-isomer was 16 kcal/mol lower in energy relative to the cis-isomer,\textsuperscript{15} and this was therefore the preferred configuration for the compound, with too high an energy difference to easily achieve efficient epimerisation from trans- to cis-configuration without decomposition.

Difficulties in accessing the essential amide functionality directly from aldehyde 5 led us to proceed through the carboxylic acid derivative 6. An oxidising system of sodium chlorite, hydrogen peroxide and sodium dihydrogen phosphate was successfully implemented to obtain 6 in high yields.\textsuperscript{16} At this point some conflicting issues arose: the amide formation was inevitably going to involve an amine, which had the potential to epimerise the stereocentre adjacent to the ketone, as observed in previous studies.\textsuperscript{13}

The most straightforward route from a carboxylic acid to an amide is via an acid chloride and subsequent reaction with the required amine. The latter was successfully carried out using aqueous ammonia. Gratifyingly, this reaction did not cause noticeable epimerisation of the vulnerable stereocentre and quantitatively provided compound 7.\textsuperscript{17}

Deprotection of the TBDPS moiety could not be carried out using standard protocols, either by using a fluoride source or other basic conditions without causing the epimerisation.\textsuperscript{16} Indeed, attempts to use TBAF gave a mixture of products in low yields, with complete scrambling of the stereocentre. However, when using p-toluene sulfonic acid, the silyl protecting group was cleaved at 60 °C in DMF, albeit in 56% yield. The final reduction using sodium triacetate-oxyborohydride proceeded smoothly, giving the final compound, 2-deoxy-benzamide riboside cis-8, in 95% yields, with the relative stereochemistry confirmed by NOE experiments, (Scheme 1).\textsuperscript{13,19}

To the best of our knowledge, this compound has not yet been synthesised, and thus never been tested for targeted biological and enzymatic activity. It was anticipated that the removal of the 2'-hydroxyl group on the ribose would affect the ability of the compound to hydrogen bond with polar residues of the binding pockets in NRK,\textsuperscript{20} NMNAT\textsuperscript{20} and IMPDH.\textsuperscript{21} Therefore to retain activity, it is vital that the resulting NAD analogue structure not only fit into the IMPDH pocket to achieve inhibition, but that its metabolic precursors be recognised and metabolised effectively by the enzymes that convert it into an NAD analogue.\textsuperscript{7,22}

The target molecule cis-8 was therefore tested for activity against a number of cancerous cell lines. The cell lines were: RKO—Human colon carcinoma, HT29 - Human colorectal adenocarcinoma, HCT116—Human colorectal carcinoma, H460 - Human large lung cell carcinoma, MCF7—Human breast adenocarcinoma (pleural effusion metastatic site), T47D Human breast ducetal carcinoma, PC3—Human prostate adenocarcinoma (bone metastatic site) and DU145—Human prostate carcinoma (brain metastatic site). Some of these cell lines have been shown to be sensitive to tiazofurin and benzamide riboside at concentrations that range from 50 \mu M (benzamide riboside in MCF7) to 500 \mu M (cell line PC-3).\textsuperscript{23} Despite incubation at concentrations up to 1 mM, no cell line displayed a significant level of inhibition upon incubation with cis-8 compared to the blank sample of pure DMSO.

In work preceding the discovery of the NRK-dependent NAD biosynthetic pathway\textsuperscript{3} and the evidence that tiazofurin was an excellent substrate for NRK,\textsuperscript{24} tiazofurin and benzamide riboside were proposed to enter cells via nucleoside transporters. While a nicotinamide riboside specific transporter has been identified in yeast, the human homolog still remains undiscovered. To test the hypothesis that cis-8 fails as a cytotoxic agent because of a deficit in the biosynthesis of NAD rather than or in addition to limited transport, we incubated the compound at 0.5 mM with 1 mM ATP and sufficient NR kinase 2 for complete conversion of 0.5 mM NR to NMN in 30 min. Reactions monitored for 2 h by HPLC and LC–MS indicated that the compound is totally resistant to conversion to the corresponding monophosphate. Earlier a 2-deoxy derivative of tiazofurin\textsuperscript{24} was synthesised and shown to be lacking in cytotoxic activity. Our data strongly suggest that 2-deoxy substitutions are not tolerated by NR kinases,\textsuperscript{34} thereby severely compromising their pharmacological activities.

The present work provides the direction which the chemistry of C-nicotinamide riboside modified on the sugar moiety must take and that is attempting to by-pass the NRK step in accessing in situ NAD analogues, putative inhibitors of IMPDH and other
NAD dependent enzymes. Pre-forming the monophosphate of cis-8 and synthesising the NAD analogue incorporating cis-8 will give an indication as to whether the dinucleotide compound has the ability to inhibit IMPDH, and would substantiate the proposal made by Robins.

This work is ongoing in our laboratory, and will be combined with isolated enzyme kinetic experiments so as to obtain a clear understanding of the activation pathway requirements.

The present results demonstrate the first successful synthesis of 2-deoxy benzamide riboside, cis-8. The vinyl group on the key intermediate 4 was successfully converted to the amide group in the meta-position of the aromatic moiety, proving that the cyclisation reaction developed in our previous work can provide intermediates for a wide range of biologically relevant C-nucleosides. This interesting advancement in the methodology of useful C-nucleosides will increase the existing pool of synthetic procedures available for the pursuit of novel C-nucleosides.

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References and notes


14. Acetal 3 (0.50 g, 0.9 mmol, 1 equiv) was dissolved in dry DCM (1.5 mL/mmole) and the reaction mixture cooled to −78 °C. TMSN2 (2 equiv) was added and the reaction mixture stirred for 5 min. It was then filtered through a pad of silica gel using 25% EtOAc in hexane or pent ether as eluent. The filtrate was concentrated in vacuo and the residue purified by column chromatography (0−5% EtOAc in hexane on silica gel) and yielded 4 (0.22 g, 54%) as a colourless oil.

The diastereomers were not separated. Diastereomeric ratio was measured at a ratio 1.3:1 (cis/trans) based on ‘H NMR. (S-R)-4 (cis): δ 40 (400 MHz; CDCl3): 1.06 (9H, s, C-CH3); 2.57−2.64 (1H, m, CH(CH2)3); 2.89 (1H, dd, J = 5.4, 15.1 Hz, CH(=CH)2); 3.83 (1H, dd, J = 4.5, 10.7 Hz, TBDPSO-CH2); 3.90 (1H, dd, J = 4.2, 10.7 Hz, TBDPSO-CH2); 4.56−4.58 (1H, m, CH(=CH2)); 4.92 (1H, dd, J = 5.4, 10.7 Hz, OCH2-CH2=); 4.99−5.01 (1H, m, CH2); 5.09−5.11 (1H, m, CH2); 6.08 (1H, dd, J = 11.0, 17.6 Hz, CH=C=); 7.22−7.47 (4H, m, H-CH=CH=C=); 7.71−7.75 (4H, m, Ar-H); 1.42 (100 MHz; CDCl3): 19.3 (Si-C(=CH2)); 26.8 (S-C(=CH2)); 42.6 (CH(=CH2)); 66.8 (TBDPSO-CH2); 80.4 (O-CH=Ar); 82.0 (O-CH=C-); 105.8 (E=CH2); 113.9 (CH=C=); 124.1 (Ar=CH); 125.4 (Ar=CH).

Scheme 1. Complete synthetic route to the novel C-nucleoside 2-deoxy benzamide riboside, cis-8. (i) TMSCH2Li, THF −78 °C; (ii) TBDPSI, Et3N, DMAP, DCM, rt; (iii) Et3N, DCM, 0 °C, rt; (iv) TMSNTf2, DCM, −78 °C; (v) O3; (vi) PPh3, DCM; (vii) NaN3, H2O, NaH2PO4 MeCN, H2O; (viii) SOCl2, PhMe, (ix) NH4OH, (x) pTSA, DMF, 60 °C, (xi) NaBH(OAc)3, AcOH, MeCN, 0 °C.
125.7 (Ar-CH), 127.6 (Ar-CH), 127.7 (Ar-CH), 128.5 (Ar-CH), 129.6 (Ar-CH), 129.7 (Ar-CH), 129.8 (Ar-CH), 130.7 (Ar-CH), 131.5 (Ar-CH), 132.1 (Ar-CH), 135.8 (Ar-CH), 136.6 (Ar-CH), 136.9 (Ar-CH), 137.3 (Ar-CH), 137.7 (Ar-CH), 140.2 (Ar-CH), 149.2 (C=CH2), 165.0 (trans): ωH (400 MHz; CDCl3); 1.07 (9H, s, C-(CH3)2), 2.57-2.64 (1H, m, CH=CH2), 3.05 (1H, m, CH=CH2), 3.78 (1H, dd, J= 5.0, 10.9 Hz, TBDPSO-CH2), 3.82 (1H, dd, J=4.0, 10.9 Hz, TBDPSO-CH2), 4.15 (1H, br s, O-CH=C), 4.98 (1H, dd, J=2.1, 4.1 Hz, C=CH2), 5.08-5.11 (1H, m, CH=C), 5.13 (1H, t, J= 7.2 Hz, O-CH-Ar), 5.25 (1H, d, J= 11.0 Hz, CH=CH2), 5.75 (1H, d, J= 17.6 Hz, CH=CH2), 7.63 (1H, dd, J=11.0, 17.6 Hz, CH=CH2), 7.72-7.75 (4H, m, Ar-H). ωC (125 MHz; CDCl3): 19.3 (Si-C(CH3)), 26.9 (Si-CH3), 41.6 (CH=Ar=CH2), 66.0 (TBDPSO-CH2), 79.6 (O-CH=Ar), 81.8 (1H-CH=CH2), 113.9 (CH=CH2), 123.8 (Ar-CH), 125.3 (Ar-CH), 125.7 (Ar-CH), 127.6 (Ar-CH), 127.7 (Ar-CH), 128.6 (Ar-CH), 129.6 (Ar-CH), 129.6 (Ar-CH), 133.4 (Ar-C), 133.6 (Ar-C), 135.7 (Ar-CH), 135.8 (Ar-CH), 136.9 (CH=CH2), 137.7 (Ar-C), 142.9 (Ar-C). 148.6 (C=O), νmax/cm-1: 3059 (ω), 3280 (ω), 1245 (ω), 1113 (ω), 907 (m), 702 (s). m/z (ES): 397.1629 (M+H+), calcd for C28H30O5Si: 397.1624 (1.3 ppm). 

15. Calculation carried out using the Gaussian 03, Revision D.01. 

16. Keto-aldehyde compound 6 (0.36 g, 0.75 mmol, 1 equiv) was dissolved in MeCN (15 mL) and the resulting solution cooled to 0°C. A solution of NaH2PO4 (43 mg, 0.4 mmol, 1 equiv) in water (1 mL) was added, followed by H2O2 (0.14 mL, 20% in water, 1.1 mmol, 1.8 equiv) and a solution of NaNO2 (0.12 mg, 1.5 mmol, 2 equiv) in water (1 mL). The reaction mixture was stirred at rt for 2 h, before quenching with Na2S2O3 (0.20 g, 1.5 mmol, 2 equiv) and extraction with EtOAc (3 x 20 mL). The combined organic layers were dried with MgSO4, filtered and concentrated in vacuo to yield compound 6 (0.32 g, 90%) as a colourless oil. 

17. Acid 6 (0.36 g, 0.75 mmol, 1 equiv) was dissolved in dry toluene (30 mL) and added SOCl2 (0.1 mL, 1.4 mmol, 1.5 equiv). This was heated to reflux for 2 h before removing volatiles in vacuo and adding cold NH4OH (15 mL, 35% NH4OH w/w) to the residue. The reaction mixture was then stirred for 1 h at rt. The product was extracted with EtOAc (3 x 15 mL), and the combined organic layers were dried with MgSO4, filtered and concentrated in vacuo to yield title compound 7 (0.34 g, 95%) as a colourless oil. 


19. Amide 7 (0.24 g, 0.51 mmol, 1 equiv) was dissolved in DMF (25 mL) and p-toluenesulfonyl chloride (0.13 g, 0.8 mmol, 1.5 equiv) was added. The reaction mixture was stirred at 60°C for 4 days. The reaction was then quenched with saturated aqueous NaHCO3 (25 mL) and the aqueous layer extracted with EtOAc (3 x 50 mL). The combined organic layers were dried with MgSO4, filtered and concentrated in vacuo. Purification by column chromatography (0-15% MeOH in DCMA) yielded 8 (55%) as a white solid. 


