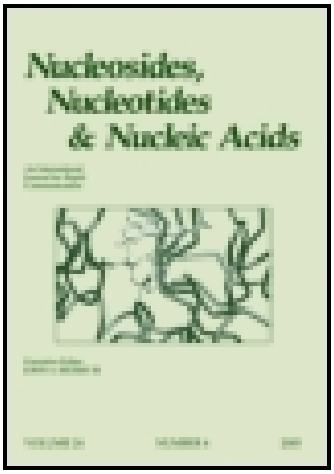

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Efficient Synthesis of Benzamide Riboside, a Potential Anticancer Agent

Laurent F. Bonnac [a], Guang-Yao Gao [a], Liqiang Chen [a], Steven E. Patterson [a], Hiremagalur N. Jayaram [b] & Krzysztof W. Pankiewicz [a]

[a] Center for Drug Design, University of Minnesota, Minneapolis, Minnesota, USA

[b] Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana, USA

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EFFICIENT SYNTHESIS OF BENZAMIDE RIBOSIDE, A POTENTIAL ANTICANCER AGENT

Laurent F. Bonnac, Guang-Yao Gao, Liqiang Chen, and Steven E. Patterson
□ *Center for Drug Design, University of Minnesota, Minneapolis, Minnesota, USA*

Hiremagalur N. Jayaram □ *Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, Indiana, USA*

Krzysztof W. Pankiewicz □ *Center for Drug Design, University of Minnesota, Minneapolis, Minnesota, USA*

□ *An efficient five step synthesis of benzamide riboside (BR) amenable for a large scale synthesis has been developed. It allows for extensive pre-clinical studies of BR as a potential anticancer agent.*

Keywords Benzamide riboside; anticancer agent

Benzamide riboside (BR) belongs to the C-nucleoside family of nucleosides, which has a ribofuranosyl moiety linked to the heterocyclic or aromatic base through a stable carbon-carbon bond rather than a carbon-nitrogen bond as in natural nucleosides[1−4] (Figure 1). BR exhibits potent antitumor activity in a variety of cultured human tumor cells.[1,2]

BR is a prodrug that is phosphorylated by adenosine kinase to the corresponding 5′-mononucleotide, which is converted to the active metabolite, benzamide adenine dinucleotide (BAD), an analog of nicotinamide adenine dinucleotide (NAD), by the action of nicotinamide mononucleotide adenylyltransferase (NMNAT) (Scheme 1).[1]

The primary target of BR is IMP-dehydrogenase (IMPDH), the rate-limiting enzyme in the purine nucleotide pathway of guanine nucleotide synthesis. Inhibition of IMPDH causes depletion of GTP and dGTP pools and results in inhibition of cancer cell proliferation.[1,5] An ideal anticancer drug is one which produces minimal toxic side effects and exhibits its anticancer properties through selective triggering of apoptosis in tumor cells. BR has been shown to induce apoptosis selectively in human ovarian

Address correspondence to Krzysztof W. Pankiewicz, Center for Drug Design, University of Minnesota, 516 Delaware Street SE, Minneapolis, MN 55455. E-mail: panki001@umn.edu

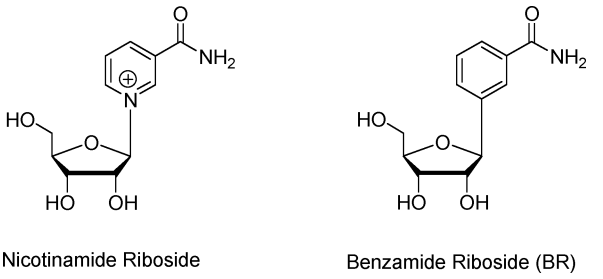
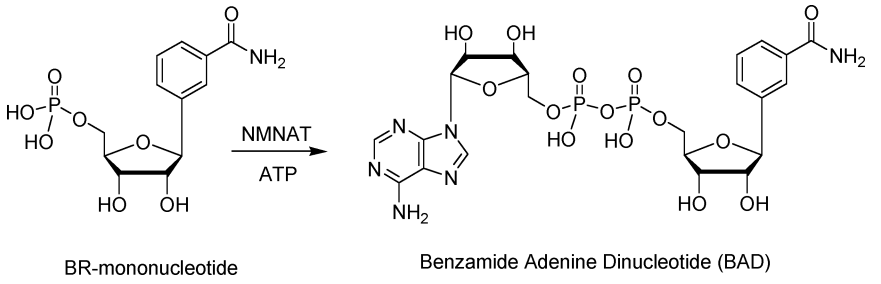


FIGURE 1 Structure of nicotinamide riboside and benzamide riboside.

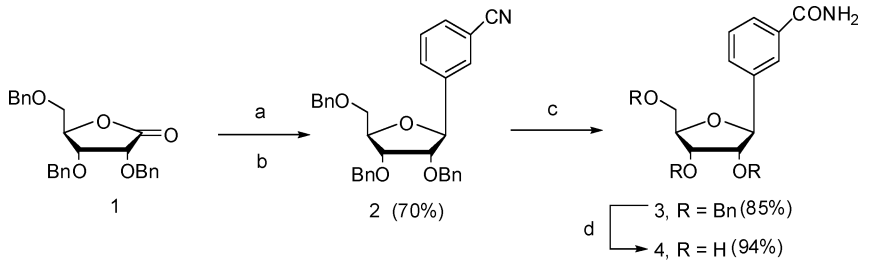
carcinoma-N-1 cells, lung cancer (H520) cells, and in leukemic cells (Hl-60) in culture.[5–7]

The first synthesis of BR reported by Krohn and coworkers[8,9] is a ten-step procedure not amenable for large scale preparation. We report herein a new efficient synthesis of BR in five steps from a commercially available D-(+)-ribonic-γ-lactone, protected as the 2,3,5-tri-O-benzyl lactone **1**[10] (Scheme 2). The key reaction is a stereo-specific coupling of the Grignard reagent[11] prepared from 3-iodobenzonitrile with **1** followed by reduction to give the β anomer **2** exclusively. The nitrile group is stable during coupling reaction and is easy to hydrolyze. Indeed, treatment of **2** with Me_3SiOK afforded the carboxyamido derivative **3**, which was deprotected with BBr_3 to give the desired BR in 56% overall yield.

In Krohn's synthesis the protected ribonolactone was obtained from ribose by selective protection of the anomeric hydroxyl group followed by the further benzylation of the sugar OH groups. Then removal of the anomeric protective group and oxidation gave the protected ribonolactone (**1**). For coupling Krohn et al. used an oxazoline protected compound obtained in three steps from the benzoic acid. The choice of this protection offered stability during organometalic coupling reaction, but protection and deprotection adds seven steps to the process.



SCHEME 1 Enzymatic synthesis of benzamide adenine dinucleotide.



Reagents: a) iPrMgCl, 3-iodobenzonitrile, THF; b) BF_3OEt_2, Et_3SiH, CH_2Cl_2; c) Me_3SiOK, THF; d) BBr_3, CH_2Cl_2

SCHEME 2 Synthesis of benzamide riboside.

EXPERIMENTAL

General Methods

All commercial reagents (Sigma-Aldrich, Acros) were used as provided unless otherwise indicated. An anhydrous solvent dispensing system (J. C. Meyer) using two packed columns of neutral alumina was used for drying THF, Et_2O, and CH_2Cl_2, while two packed columns of molecular sieves were used to dry DMF. Solvents were dispensed under argon. Nuclear magnetic resonance spectra were recorded on a Varian 600 MHz with Me_4Si or DDS as the internal standard for ^1H. Chemical shifts are reported in ppm, and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), bs (broad singlet), and dd (double doublet). Values given for coupling constants are first order. High resolution mass spectra were recorded on an Agilent TOF II TOF/MS instrument equipped with either ESI or APCI interface.

3-(2,3,5-Tri-*O*-benzyl-1-β-D-ribofuranosyl)benzonitrile (2). A solution of 3-iodobenzonitrile (3 g, 13.1 mmol) in dry THF (195 mL, 15 mL/mmol) under nitrogen was cooled to −78°C, a solution of *i*PrMgCl (6.55 mL, 2 M in THF) was added, and the mixture was stirred for 1 hour at −78°C. The mixture was transferred (via canule) into a solution of 2,3,5-tri-*O*-benzyl-D-ribono-1,4-lactone (**1**)[10] (5.47 g, 13.1 mmol) in dry THF (35 mL, 2.5 mL/mmol) under nitrogen cooled at −78°C and stirred at −78°C for 1 hour. It allowed to reach room temperature and then stirred for 4 hours to give a pink-red solution. A saturated solution of $NaHCO_3$ was added and the mixture was extracted with ether. The organic layer was separated, dried over Na_2SO_4, filtered, and concentrated. The residue was diluted with dry CH_2Cl_2 (26 mL, 2 mL/mmol) under nitrogen, cooled to −78°C and BF_3Et_2O (3.3 mL, 26.2 mmol) was slowly added (5 minutes) to the solution followed by Et_3SiH (4.2 mL, 26.2 mmol). After 1 hour at −78°C the solution was allowed to reach room temperature and then stirred overnight.

After addition of sat.$NaHCO_3$ the mixture was extracted with CH_2Cl_2. The organic layer is separated, dried over Na_2SO_4, filtered, concentrated, and chromatographed on a silica gel column with CH_2Cl_2 to give compound **2** (4.64 g, 70%) as an oil. ^1H NMR (δ, $CDCl_3$, 600 MHz), 7.65 (s, 1H), 7.59 (d, J = 7.2 Hz, 1H), 7.51 (d, J= 7.8 Hz, 1H), 7.37–7.12 (m, 16H), 4.97 (d, J= 8.2 Hz, 1H), 4.61–4.51 (m, 16H), 4.36 (m, 1H), 4.00 (dd, J= 4.8, 3.0 Hz, 1H), 3.73 (dd, J= 7.8, 4.8 Hz, 1H), 3.65 (dd, J= 10.2, 4.2 Hz, 1H), 3.58 (dd, J= 10.2, 3.6 Hz, 1H). HRMS calcd for $C_{33}H_{30}NO_4$ $[M\text{-}H]^-$504.2180 found 504.2166.

3-(2,3,5-Tri-*O*-benzyl-1-β-D-ribofuranosyl)benzamide (3). To a solution of compound **2** (0.397 g, 0.78 mmol) in dry THF (2 mL, 2. 5 mL/mmol, dry toluene can also be used) placed in a pressure tube Me_3SiOK (200 mg, 1.56 mmol) was added in one portion and the mixture was refluxed for 24 hours. After reaching room temperature EtOH (20 mL) was added and the mixture was concentrated. The residue was purified on a silica gel column with CH_2Cl_2/MeOH(5%) to give compound **3** (350 mg, 85%) as a white solid. ^1H NMR (δ, $CDCl_3$, 600 MHz), 7.78 (s, 1 H), 7.75 (d, J= 7.8 Hz, 1H), 7.50 (d, J= 7.8 Hz, 1H), 7.33–7.17 (m, 16H), 5.95 (m, 2H), 5.05 (d, J= 6.0 Hz, 1H), 4.62–4.52 (m, 6H), 4.36 (m, 1H), 4.04 (t, J= 4.8 Hz, 1H), 3.82 (dd, J= 6.0, 5.4 Hz, 1H), 3.73 (dd, J= 10.8, 4.2 Hz, 1H), 3.64 (dd, J= 10.2, 4.2 Hz, 1H). HRMS calcd for $C_{33}H_{34}NO_5$ $[M\text{-}H]^+$ 524.2431 found 524.2483.

3-(1-β-D-ribofuranosyl)benzamide (4, BR). Compound **3** (2.15 g, 4.11 mmol) was diluted with dry CH_2Cl_2 (85 mL, 20 mL/mmol) under nitrogen at −78°C and a 1 N solution of BBr_3 in CH_2Cl_2 (16.5 mL) was added slowly (15 minutes). The reaction was stirred for 1 hour at −78°C and then overnight at room temperature. The reaction was quenched with a mixture of Et_2O and MeOH (4/1, 200 mL), stirred for 20 minutes. and concentrated. The residue was chromatographed on a silica gel column with CH_2Cl_2-MeOH (25%), the fractions containing **4** were treated with charcoal and filtered through celite pad to give **4** (980 mg, 94%) as a white solid rather than an oil reported previously.[8] Spectral and biological properties of **4** were identical to those reported earlier.

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