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# Stereoselective synthesis of nicotinamide β-riboside and nucleoside analogs

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**Abstract**—The β-anomers of *N*-ribofuranosylnicotine-3-carboxamide (β-NAR) and its nicotinic acid analog (β-NaR) were obtained by stereoselective synthesis via glycosylation of the presilylated bases under Vorbruggen's protocol. A NAR analog, methylated in position 3 of the ribosylic moiety, is also reported.


## 1. Introduction

Nicotinamide riboside (β-NAR) is an intermediate in one biosynthetic pathway by which nicotinamide is converted into NAD. In fact, nicotinamide or nicotinamide riboside derived from degradation of NAD can be reused by nicotinamide phosphoribosyl transferase or ribosylnicotinamide kinase, respectively, to form the ribotide NMN that is adenylated to NAD by nicotinamide mononucleotide adenylyltransferase (NMNAT).[1] Human NMNAT is an indispensable enzyme in both de novo and salvage/recycling pathways for NAD biosynthesis, catalyzing the conversion of NMN or its deamidated form NaMN into NAD or NaAD, respectively. On the other hand, it is known that many bacterial NMNATs strongly prefer the deamidated NaMN as a substrate.[2] NAD is a co-factor in numerous enzyme-catalyzed redox reactions in all living organisms and plays a fundamental role in cellular metabolic processes. It is crucial thus that proper levels of NAD are regulated and maintained for cellular survival.[2] There is evidence that hNMNAT is weakly expressed in tumor cells, determining thus interest in the enzyme as a target for anticancer drugs. In this respect, some nicotinamide riboside analogs have been described as antitumor agents involving their metabolic conversion to NAD analogs by NMNAT activity.[3]

Kinetic and structural studies of both human and bacterial NMNAT require the β-anomer of nicotinamide monoribotide as a substrate and of nicotinamide riboside as a product of the NAD degradation pathway. The stereospecificity of NAD-mediated reactions is determinant in all living organisms because the pyridine ribotide moiety of NAD reacts only in the β-configuration.

In order to develop the study of ribonucleoside and nucleotide analogs as chemotherapeutic agents, we needed to synthesize pyridinium ribosidic derivatives. From a survey of the literature, we found that nicotinamide and nicotinic acid ribosides could be obtained from their respective mononucleotides by enzymatic reaction.[4] In addition, a number of synthetic approaches to form NAR and its derivatives have been described.[5] However, most of the reported procedures gave the nicotinamide riboside as an α/β anomeric mixture.

Recently, a simple but nonstereoselective chemical synthesis of β-NAR starting from 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose was reported by Tanimori et al.[6] in this journal. At first, we repeated this synthetic strategy, but all attempts to isolate the NAR were unsuccessful. Thus we decided to reinvestigate the above methodology making changes in some parameters to set up a stereoselective synthesis of both nicotinamide and nicotinic acid β-ribosides (β-NAR **1**, and β-NaR **2**, respectively).

The key elements of our synthesis involved the formation of silylated nicotinamide as starting material, and

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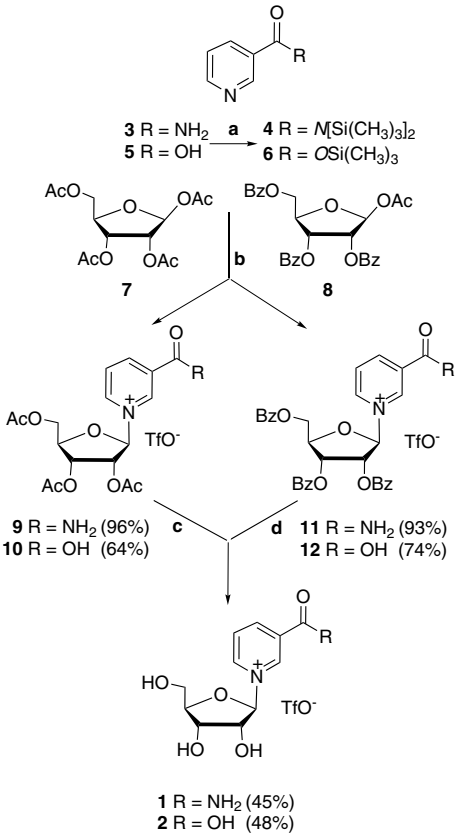
then its coupling with peracylated sugars in the presence of trimethylsilyl trifluoromethanesulfonate. However, we found that when a large excess of trimethylsilyl chloride in hexamethyldisilazane was used to silylate the nicotinamide, a poor yield of the desired nucleoside was obtained and the purification of 3-(carbamoyl)-1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl) pyridinium triflate (**9**) was very difficult. In addition, the amount of TMSOTf proved to be crucial in this reaction.

We obtained a stereoselectivity in the synthesis of NAR by condensation of nicotinamide with both 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (**7**) and 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (**8**) using carefully controlled conditions. In this procedure we used 2 equiv of TMSCl to silylate the dry nicotinamide under reflux. The intermediate **4** was directly coupled with the protected sugars (1 eqiv) under Vorbruggen's[7] conditions in the presence of a catalytic amount of TMSOTf in 1,2-dichloroethane, to give **9**[8] and **11**[9] in high yield (Scheme 1). Only β-anomers of the protected *N*-nucleosides were obtained. β-NAR[10] (**1**) was then obtained by basic hydrolytic deblocking of compounds **9** or **11** at −5 °C. The low temperature was required to minimize cleavage of the glycosidic linkage. In the case of compound **11**, for removal of benzoyl groups, two days were required under methanolic ammonia. The nucleoside **1** was then purified by chromatography on activated charcoal and isolated as a white solid.




**Scheme 1.** Reagents and conditions: (a) TMSCl, HMDS, 120 °C, 5 h; (b) TMSOTf, $ClCH_2CH_2Cl$ anhydrous, 45 °C, 2 h; (c) $NH_3/CH_3OH$, −5 °C, 6 h; (d) $NH_3/CH_3OH$, −5 °C, 48 h.

In order to validate the above stereoselective procedure, we investigated the ribosylation of 3-nicotinic acid using both the sugars **7** and **8**. In this case, for the *O*-silylation of **5**, 1 equiv of TMSCl was used. Both the glycosylations provided the tri-*O*-acylated ribosides **10**[11] and **12**[12] uniquely as β-anomers. Nucleoside **2**[13] was obtained as a white solid after deprotection of **10** or **12** as reported for **1**.

The stereoselectivity of the synthesis may be explained in the following way (Fig. 1). In the first step, the TMSOTf catalyst converts peracylated sugars **7** and **8** into the corresponding 1,2-acyloxonium salts as a unique electrophilic sugar moiety. Under these reversible and thus thermodynamically controlled conditions, the nucleophilic silylated bases **4** and **6** can attack only the furanose cations from the top (the β-side) to afford the β-nucleosides **9**–**12**. Only the silylated bases will react with the electrophilic sugar cations to form the final products; since an excess of TMSCl blocks the $N^1$ of nicotinic bases, a slow and poor reactivity was observed when more than 2 equiv of TMSCl were used.

Anomeric purity of β-NAR and β-NaR was revealed to be 100% by reverse phase HPLC and the structure of these nucleosides was confirmed by mass spectrometric analysis. Unambiguous assignment of the configuration of ribonucleosides was best accomplished by $^1$H NMR data and proton nuclear Overhauser enhancement (NOE) effects.[9–13] It is worth noting that the $^1$H NMR spectrum of compound **1** was different from that reported by Tanimori et al.,[6] but similar to that described by Jarman and Ross[14] for the corresponding chloride. Moreover, the coupling constant $J_{1'-2'}$ and the proton chemical shift for NAR (**1**) corresponded to values reported by Oppenheimer and Japlan.[15] The proton spectrum of both the protected compounds **9**–**12**[8,9,11,12] and nucleosides **1**[10] and **2**[13] contained a unique doublet, which was attributed to the anomeric proton (H1′) of the ribose moiety. The absence of a second doublet in this region provided strong evidence for the essential anomeric purity of the nucleosides. The β-configuration was confirmed through the coupling constants ($J_{1'-2'}$) value and by proton NOE data. Because of the overlapping of the proton signals of H2′, and H4′ in the deprotected ribosides **1** and **2**, the NOE spectrum was performed in the corresponding protected nucleosides. In the one-dimensional NOE spectrum, irradiation of the anomeric protons of **9**–**12** showed an enhancement of H2′ and H4′ in the ribose moiety, while the intensity enhancement of the H3′ signal was zero, supporting a spatial arrangement where H1′ and H4′ protons are on the same side as ribofuranose, as would be the case in β-configuration. Moreover an enhancement of H2 and H6 signals in the pyridine ring was also observed. The correct structure of β-NAR was also confirmed by the $^{13}$C NMR spectrum on the basis of reported values for NMN and NAD,[16,17] mass spectrometric analysis, and through a C*H* COSY diagram ($D_2O$, 300 MHz).

A sugar *C*-methylated in position 3 of the ribofuranosyl moiety was also used to obtain a new class of nicotin-

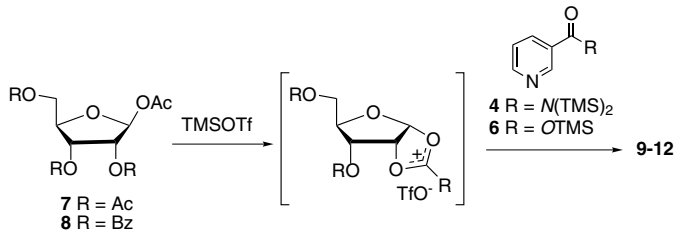
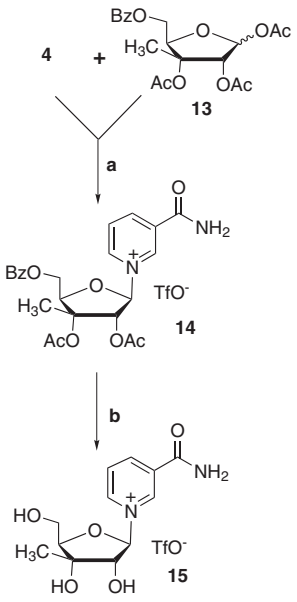



**Figure 1.** Glycosylation mechanism of pyridinic nucleosides.




**Scheme 2.** Reagents and conditions: (a) TMSOTf, $ClCH_2CH_2Cl$ anhydrous, 45 °C, 2 h; (b) $NH_3/CH_3OH$, −5 °C, 48 h, (yield 46%).

amide riboside derivatives using the above-described methodology. Thus, the synthesis of *N*-pyridinium 3-(carbamoyl)-1-(3′-*C*-methyl)-β-D-ribofuranose (NA3′-*C*-MeR) is reported (Scheme 2). Coupling of 1,2,3-tri-*O*-acetyl-5-*O*-benzoyl-β-D-(3-*C*-methyl)ribofuranose[18] (**13**) with silylated nicotinamide (**4**) followed by direct basic deblocking of nucleoside **14**, gave nucleoside **15** as a white solid, after purification as reported above. The structure of **15**[19] was determined by $^1$H NMR and mass spectrometric analysis and β-configuration of this nucleoside was confirmed by proton NOE.

In summary, a stereoselective reaction for β-NAR and its analogs via TMSOTf-mediated glycosylation of the silylated nicotinic bases using controlled conditions was shown.

In order to obtain novel NAD analogs as substrate/inhibitors of key enzymes involved in nucleotide biosynthesis, other NAR derivatives are currently under investigation.

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8. Compound **9**: TLC ($BuOH/H_2O/AcOH$, 7:2:1) $R_f$ = 0.40. $^1$H NMR (300 MHz DMSO-$d_6$): $\delta$ (ppm) 2.08 (s, 9H, $CH_3$), 4.45 (d, $J$ = 3.3 Hz, 2H, H5′), 4.70 (m, 1H, H4′), 5.40 (t, $J$ = 6.0 Hz, 1H, H3′), 5.65 (dd, $J$ = 3.5, 5.7 Hz, 1H, H2′), 6.60 (d, $J$ = 3.3 Hz, 1H, H1′), 8.20 (br s, 1H, NH), 8.40 (dd, $J$ = 6.2, 8.1 Hz, 1H, H5), 8.60 (br s, 1H, NH), 9.0 (d, $J$ = 8.4 Hz, 1H, H4), 9.35 (d, $J$ = 6.2 Hz, 1H, H6), 9.40 (s, 1H, H2). MS (API-ESI) *m/z* 381.2 [M−TfO$^-$]. HPLC [DAD and MS detector HP 1100 MSD, G19/46 A]; conditions: column: $C_{18}$ reverse phased 3.9×300 mm, 4 μm, Nova Pak (Waters), isocratic flow rate 0.7 mL/min, injection volume: 1 μl/min, 30% MeOH in 100 mM ammonium acetate buffer; 254 nm DAD detection: retention time: 3.35 min. The other compounds were followed the same above conditions for HPLC. Anal. calcd for $C_{17}H_{21}N_2O_8 \cdot CF_3SO_3^-$: C, 40.76; H, 3.99; N, 5.28. Found: C, 40.95; H, 4.11; N, 5.14.
9. Compound **11**: TLC ($BuOH,/H_2O/AcOH$, 8:1:1) $R_f$ = 0.56. $^1$H NMR (300 MHz DMSO-$d_6$): $\delta$ (ppm) 4.90 (m, 2H, H5′), 5.20 (m, 1H, H4′), 6.0 (t, $J$ = 6.1 Hz, 1H, H3′), 6.15 (m, 1H, H2′), 7.0 (d, $J$ = 3.4 Hz, 1H, H1′), 7.20–8.0 (m, 15H, arom.), 8.10 (br s, 1H, NH), 8.30 (t, $J$ = 7.3 Hz, 1H, H5), 8.60 (br s, 1H, NH), 9.05 (d, $J$ = 8.3 Hz, 1H, H4), 9.45 (d, $J$ = 5.9 Hz, 1H, H6), 9.65 (s, 1H, H2); MS (API-ESI) *m/z* 567.2 [M−TfO$^-$]. HPLC: retention time: 3.62 min. Anal.

calcd for $C_{32}H_{27}N_2O_8 \cdot CF_3SO_3^-$: C, 55.31; H, 3.80; N, 3.91. Found: C, 55.50; H, 3.68; N, 4.07.

10. Compound **1**: TLC (BuOH/$H_2O$/AcOH, 5:3:2) $R_f = 0.38$. $^1H$ NMR (300 MHz DMSO-$d_6$): $\delta$ (ppm) 3.60–3.80 (double q, $J = 12.2$ Hz, 2H, H5′), 4.10 (m, 1H, H3′), 4.20–4.35 (m, 1H, H4′, H2′), 5.40 (t, $J = 4.4$ Hz, 1H, OH), 5.50 (d, $J = 5.1$ Hz, 1H, OH), 5.90 (d, $J = 5.9$ Hz, 1H, OH), 6.18 (d, $J = 4.4$ Hz, 1H, H1′), 8.20 (br s, 1H, NH), 8.35 (t, $J = 7.1$ Hz, 1H, H5), 8.60 (br s, 1H, NH), 9.0 (d, $J = 8.4$ Hz, 1H, H4), 9.35 (d, $J = 6.2$ Hz, 1H, H6), 9.55 (s, 1H, H2); $^1H$ NMR (300 MHz $D_2O$): $\delta$ (ppm) 3.75 (dd, $J = 3.6$, 12.8 Hz, 1H, H5′), 3.90 (dd, $J = 2.9$, 13.2 Hz, 1H, H5′), 4.20 (t, $J = 4.6$ Hz, 1H, H3′), 4.25–4.40 (m, 2H, H2′, H4′), 6.08 (d, $J = 4.2$ Hz, 1H, H1′), 8.10 (dd, $J = 6.2$, 8.0 Hz, 1H, H5), 8.80 (d, $J = 7.7$ Hz, 1H, H4), 9.10 (d, $J = 5.9$ Hz, 1H, H6), 9.45 (s, 1H, H2); $^{13}C$ NMR (300 MHz $D_2O$): $\delta$ (ppm) 60.35 (C5′), 69.91 (C3′), 77.61 (C2′), 87.80 (C4′), 100.10 (C1′), 128.59 (C5), 134.10 (C3), 140.53 (C2), 142.78 (C6), 145.81 (C4). MS (API-ESI) *m/z* 255.2 [M−TfO$^-$]. HPLC: retention time: 2.86 min. Anal. calcd for $C_{11}H_{15}N_2O_5 \cdot CF_3SO_3^-$: C, 35.64; H, 3.74; N, 6.93. Found: C, 35.88; H, 3.61; N, 6.78.
11. Compound **10**: TLC (BuOH/$H_2O$/AcOH, 7:2:1) $R_f = 0.38$. $^1H$ NMR (300 MHz DMSO-$d_6$): $\delta$ (ppm) 2.10 (s, 9H, $CH_3$), 4.42 (d, $J = 2.9$ Hz, 2H, H5′), 4.70 (m, 1H, H4′), 5.40 (t, $J = 5.9$ Hz, 1H, H3′), 5.60 (dd, $J = 3.3$, 5.9 Hz, 1H, H2′), 6.70 (d, $J = 2.9$ Hz, 1H, H1′), 8.40 (m, 1H, H5), 9.10 (d, $J = 8.5$ Hz, 1H, H4), 9.30 (d, $J = 6.2$ Hz, 1H, H6), 9.50 (s, 1H, H2); MS (API-ESI) *m/z* 382.1 [M−TfO$^-$]. HPLC: retention time: 3.32 min. Anal. calcd for $C_{17}H_{20}NO_9 \cdot CF_3SO_3^-$: C, 40.68; H, 3.79; N, 2.63. Found: C, 40.75; H, 3.91; N, 2.55.
12. Compound **12**: TLC (BuOH/$H_2O$/AcOH, 8:1:1) $R_f = 0.50$. $^1H$ NMR (300 MHz DMSO-$d_6$): $\delta$ (ppm) 4.85 (d, $J = 3.3$ Hz, 2H, H5′), 5.20 (m, 1H, H4′), 6.0 (t, $J = 5.6$ Hz, 1H, H3′), 6.15 (dd, $J = 3.3$, 5.8 Hz, 1H, H2′), 7.05 (d, $J = 3.6$ Hz, 1H, H1′), 7.20–8.15 (m, 15H, arom.), 8.25 (t, $J = 7.6$ Hz, 1H, H5), 9.0 (d, $J = 8.4$ Hz, 1H, H4), 9.40 (d, $J = 6.6$ Hz, 1H, H6), 9.70 (s, 1H, H2); MS (API-ESI) *m/z* 568.1 [M−TfO$^-$]. HPLC: retention time: 3.62 min. Anal. calcd for $C_{32}H_{26}NO_9 \cdot CF_3SO_3^-$: C, 54.47; H, 3.71; N, 1.98. Found: C, 54.59; H, 3.60; N, 2.16.
13. Compound **2**: TLC (BuOH/$H_2O$/AcOH, 5:3:2) $R_f = 0.36$. $^1H$ NMR (300 MHz DMSO-$d_6$): $\delta$ (ppm) 3.60–3.80 (double q, $J = 12.1$ Hz, 2H, H5′), 4.10 (m, 2H, H3′, H4′), 4.22 (d, $J = 3.6$ Hz, 1H, H2′), 5.20 (br s, 1H, OH), 5.25 (br s, 1H, OH), 6.15 (d, $J = 5.1$ Hz, 1H, H1′), 6.25 (br s, 1H, OH), 8.10 (t, $J = 6.2$ Hz, 1H, H5), 8.80 (d, $J = 7.6$ Hz, 1H, H4), 9.20 (d, $J = 6.2$ Hz, 1H, H6), 9.30 (s, 1H, H2); $^1H$ NMR (300 MHz $D_2O$): $\delta$ (ppm) 3.86 (dd, $J = 3.8$, 13.0 Hz, 1H, H5′), 4.02 (dd, $J = 2.9$ Hz, 12.8 Hz, 1H, H5′), 4.30–4.36 (t, $J = 4.6$ Hz, 1H, H3′), 4.41–4.50 (m, 2H, H2′, H4′), 6.20 (d, $J = 4.8$ Hz, 1H, H1′), 8.15 (t, $J = 6.8$ Hz, 1H, H5), 8.90 (d, $J = 8.1$ Hz, 1H, H4), 9.15 (d, $J = 5.9$ Hz, 1H, H6), 9.40 (s, 1H, H2); MS (API-ESI) *m/z* 256.1 [M−TfO$^-$]. HPLC: retention time: 2.81 min. Anal. calcd for $C_{11}H_{14}NO_6 \cdot CF_3SO_3^-$: C, 33.59; H, 3.59; N, 3.56. Found: C, 33.47; H, 3.68; N, 3.41.
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19. Compound **15**: TLC (BuOH/$H_2O$/AcOH, 5:3:2) $R_f = 0.44$. $^1H$ NMR (300 MHz DMSO-$d_6$): $\delta$ (ppm) 1.25 (s, 3H, $CH_3$), 3.60–3.80 (m, 2H, H5′), 4.0 (d, $J = 6.9$ Hz, 1H, H4′), 4.10 (s, 1H, H2′), 4.40–4.60 (br s, 1H, OH), 5.40 (br s, 1H, OH), 5.50 (br s, 1H, OH), 6.18 (d, $J = 6.9$ Hz, 1H, H1′), 8.20 (br s, 1H, NH), 8.35 (t, $J = 7.1$ Hz, 1H, H5), 8.70 (br s, 1H, NH), 9.0 (d, $J = 7.7$ Hz, 1H, H4), 9.40 (d, $J = 6.6$ Hz, 1H, H6), 9.65 (s, 1H, H2); $^1H$ NMR (300 MHz $D_2O$): $\delta$ (ppm) 1.23 (s, 3H, $CH_3$), 3.70 (dd, $J = 4.1$, 12.7 Hz, 1H, H5′), 3.85 (dd, $J = 3.2$, 12.7 Hz, 1H, H5′), 4.18 (d, $J = 6.7$ Hz, 1H, H2′), 4.30 (t, $J = 3.7$ Hz, 1H, H4′), 6.0 (d, $J = 7.0$ Hz, 1H, H1′), 8.15 (q, $J = 6.2$ Hz, 1H, H5), 8.85 (d, $J = 8.2$ Hz, 1H, H4), 9.15 (d, $J = 6.1$ Hz, 1H, H6), 9.44 (s, 1H, H2); MS (API-ESI) *m/z* 269.2 [M−TfO$^-$]. HPLC: retention time: 2.95 min. Anal. calcd for $C_{12}H_{17}N_2O_5 \cdot CF_3SO_3^-$: C, 37.32; H, 4.09; N, 6.70. Found: C, 37.48; H, 3.91; N, 6.82.