Synthesis of Glycosides of Nicotinamide and Nicotinamide Mononucleotide[1]

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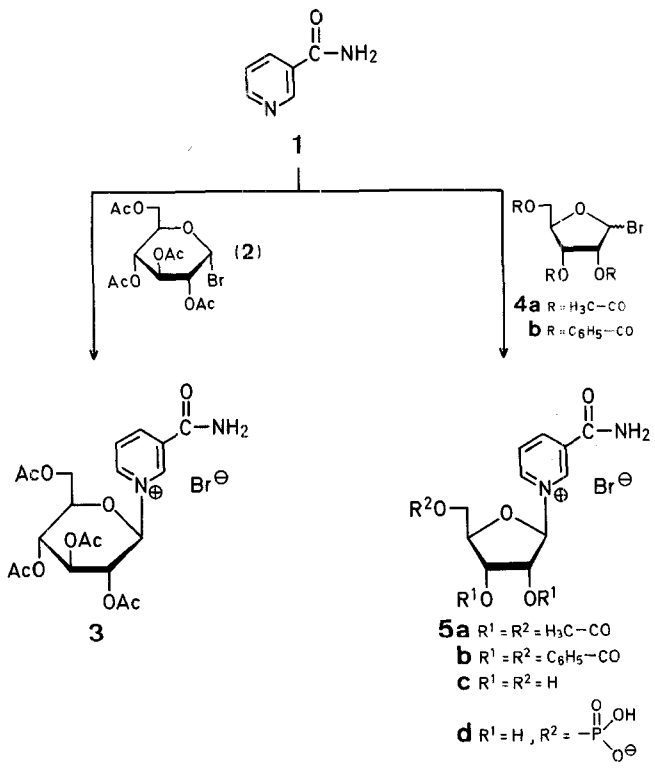
$NAD^{\oplus}$ (nicotinamide adenine dinucleotide) analogues modified at different moieties of the molecule are important tools in the study of mechanism of the functioning of $NAD^{\oplus}$-dependent enzymes[2,3]. Both chemical[4] and enzymic[3] methods of synthesis of the $NAD^{\oplus}$ analogues involve the hardly accessible nicotinamide mononucleotide (NMN) as a component. The preparation of NMN via cleavage of the $NAD^{\oplus}$ under the action of organic pyrophosphatase from potato has been described[5]. Chemical methods of the NMN synthesis are rather laborious and result in low, not reproducible product yields[4]. It appeared reasonable, therefore, to reinvestigate the condensation of nicotinamide with peracylated halo sugars and the phosphorylation of nicotinamide riboside (NAR) to develop an improved NMN synthesis.

There are two essentially different routes to the synthesis of nicotinamide glycosides: (a) via condensation of 1-amino sugars with N^1-(2,4-dinitrophenyl)-3-aminocarbonylpyridinium halogenides[4,6,7] and (b) via condensation of peracylated halo sugars with nicotinamide (**1**)[8-11]. Our attention was drawn to the latter route because of the availability of peracyl sugars and nicotinamide. The condensation of α-acetobromoglucose (**2**) with nicotinamide (**1**) has been studied as a model system. It was found that in sulpholane at room temperature the reaction finishes in 7 h to afford N^1-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-3-aminocarbonylpyridinium bromide (**3**) in practically quantitative yield. However, the isolation of nucleoside **3** in the pure state was hindered by the high boiling point of sulpholane and the good solubility of both the solvent and **3** in water and many organic solvents; thus, the nucleoside **3** was isolated in only 40–42% yield.



The condensation of 1-bromo-2,3,5-tri-*O*-acetyl-D-ribofuranose (**4a**) with nicotinamide (**1**) in sulpholane was accompanied by an intense darkening of the reaction mixture which hindered the isolation of the desired nucleoside **5a** in a pure state, although it was formed (detected by T.L.C.). A substitution of sulpholane by the liquid sulphur dioxide resulted in the formation of **5a** in practically quantitative yield. The application of nitromethane as solvent allowed us to isolate N^1-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-3-aminocarbonylpyridinium bromide (**5a**) in 50% yield contaminated with the parent nicotinamide. The nucleoside **5a** thus obtained contained up to 5% of the α-anomer as determined by ^{1}H-N.M.R. spectroscopy.

With 1-bromo-2,3,5-tri-*O*-benzoyl-D-ribofuranose (**4b**) as the carbohydrate component in the condensation with nicotinamide (**1**) in liquid sulphur dioxide the nucleoside **5b** was obtained in practically quantitative yield. The product **5b** did not contain the α-anomer. In nitromethane, the reaction also proceeded stereospecifically and **5b** was isolated in 90% yield.

The removal of benzoyl protecting groups was most effectively achieved by the action of ammonia in anhydrous methanol. The isolation of pure NAR (**5c**) was simplified and its yield amounted to 50% if benzamide was removed by the three-fold precipitation of NAR from a methanolic solution with ether and acetone.

The phosphorylation of NAR with phosphoryl chloride in anhydrous trimethyl phosphate[12] and subsequent chromatography on Dowex 1 × 2 ($AcO^{\ominus}$ form) with water elution gave NMN (**5d**) in 30–60% yield. The present method was checked repeatedly on a scale up to 3 g of parent NAR (**5c**) and provides a facile, high-yield synthesis of NMN (**5d**) starting from nicotinamide.

Structural assignments for the compounds obtained were confirmed by spectral data and by a comparison with those published in Refs.[5,6,7,13].

Nicotinamide (purum, Khimreactive, Erevan, U.S.S.R.) was used without further purification, but was dried at 50 °C for 8 h prior to use. Sulpholane (Merck) was distilled three times in vacuo from sodium hydroxide. Nitromethane and acetonitrile were purified by distillation from phosphorus pentoxide. Trimethyl phosphate and phosphoryl chloride were distilled in vacuo before use.

N^1-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-3-aminocarbonylpyridinium Bromide (3):

Method A: A mixture of nicotinamide (**1**; 0.3 g, 2.4 mmol) and acetobromoglucose (**2**; 1.02 g, 2.5 mmol) in sulpholane (20 ml) is stirred for 7 h at room temperature with exclusion of moisture. The reaction mixture is treated with water (20 ml); the unreacted sugar and sulpholane are extracted from the water layer with chloroform (4 × 20 ml); the water layer is evaporated and the residue is crystallised from ethanol; yield: 40–42%.

Method B: A mixture of nicotinamide (**1**; 6.6 g, 54 mmol) and acetobromoglucose (**2**; 22.5 g, 54.7 mmol) is boiled for 4 h in acetonitrile (100 ml)[11]. The reaction mixture is cooled, the precipitated residue is filtered off, and recrystallised from ethanol to give the bromide **3**; yield: 18.4 g (70%); m.p. 200 °C (dec.) (Lit.[9], m.p. 193–195 °C); $[\alpha]_D^{20}$: −24.9° (*c* 1.85, water) (Lit.[9], $[\alpha]_D$: −20.5° (*c* 2.09, water).

$C_{20}H_{25}BrN_2O_{10}$	calc.	C 45.04	H 4.72	N 5.25	Br 14.98
(533.3)	found	44.81	4.79	5.30	14.98

U.V. (H_2O): λ_{max} = 266 nm.

^{1}H-N.M.R. (D_2O): δ = 5.90 (t, H-4'); 5.98 (t, H-2', $J_{H-2',H-3'}$ = 8.4 Hz); 6.62 (t, H-3', $J_{H-3',H-4'}$ = 8.4 Hz); 6.82 (d, H-1', $J_{H-1',H-2'}$ = 8.4 Hz); 8.78 (dd, H-5); 9.56 (dt, H-4, $J_{H-4,H-5}$ = 8.0 Hz); 9.78 (dt, H-6, $J_{H-6,H-4}$ = 1.5 Hz, $J_{H-6,H-5}$ = 6.6 Hz); 10.06 ppm (t, H-2, $J_{H-2,H-4}$ = $J_{H-2,H-6}$ = 1.5 Hz).

N^1-(2,3,5-Tri-*O*-acetyl-β-D-ribofuranosyl)-3-aminocarbonylpyridinium Bromide (5a):

Through a solution of β-D-ribofuranose tetraacetate (8.5 g, 26.7 mmol) in dry dichloromethane (150 ml) a current of hydrogen bromide gas is

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passed for 2–3 h up to saturation at −20 °C. The solution is allowed to warm to room temperature. The solvent is evaporated and the remaining syrup is co-evaporated with dry toluene (5 × 50 ml). The residue is dissolved in liquid sulphur dioxide (50 ml). Nicotinamide (**1**; 3.1 g, 25.4 mmol) is added. The reaction mixture is allowed to stand overnight. The remaining light-yellow oil is dissolved in dry chloroform (50 ml) and the product precipitated with dry ether (500 ml) to give **5a** as a hygroscopic powder; yield: 11 g (96%); $[\alpha]_D^{20}$: −44.7° (*c* 1.93, water).

$C_{17}H_{21}BrN_2O_8$	calc.	C 44.26	H 4.58	N 6.07	Br 17.32
(461.3)	found	44.08	4.62	6.12	17.20

U.V. (H_2O): λ_{max} = 266 nm.

^{1}H-N.M.R. (D_2O): δ = 3.95 (m, H-4′); 5.53 (t, H-3′, $J_{H\text{-}3',H\text{-}4'}$ = 5.0 Hz); 5.57 (dd, H-2′, $J_{H\text{-}2',H\text{-}3'}$ = 5.0 Hz); 6.73 (d, H-1′, $J_{H\text{-}1',H\text{-}2'}$ = 4.2 Hz); 8.31 (dd, H-5); 9.09 (dt, H-4, $J_{H\text{-}4,H\text{-}5}$ = 8.0 Hz); 9.28 (dt, H-6, $J_{H\text{-}6,H\text{-}4}$ = 1.5 Hz, $J_{H\text{-}6,H\text{-}5}$ = 6.2 Hz); 9.50 ppm (t, H-2, $J_{H\text{-}2,H\text{-}4} = J_{H\text{-}2,H\text{-}6}$ = 1.5 Hz).

N^1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-3-aminocarbonylpyridinium Bromide (5b):

A current of hydrogen bromide gas is passed for 2–3 h through a solution of 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-ribofuranose (1.5 g, 3.41 mmol) in dry dichloromethane (50 ml) up to saturation at −20 °C. The solution is allowed to warm to room temperature. The solvent is evaporated, the remaining syrup is co-evaporated with dry toluene (5 × 20 ml). The residue is dissolved in liquid sulphur dioxide (20 ml). Nicotinamide (**1**; 0.4 g, 3.28 mmol) is added and the reaction mixture is allowed to stand overnight. The remaining oil is dissolved in dry chloroform (10 ml) and dry ether (200 ml) is added. The precipitated residue is filtered off to give **5b** as an amorphous powder; yield: 1.9 g (90%); $[\alpha]_D^{20}$: −36.8° (*c* 1.52, methanol {Lit.[8], $[\alpha]_D$: −42.0° (*c* 1.15, methanol)}.

$C_{32}H_{27}BrN_2O_8$	calc.	C 59.36	H 4.20	N 4.33	Br 12.34
(647.5)	found	59.04	4.12	4.51	12.55

U.V. (CH_3OH): λ_{max} = 270 nm.

^{1}H-N.M.R. (CD_3OD): δ = 5.15 (m, H-4′); 6.1 (m, H-2′, H-3′); 7.14 (d, H-1′, $J_{H\text{-}1',H\text{-}2'}$ = 4.0 Hz); 8.40 (dd, H-5); 9.10 (dt, H-4); 9.60 (dt, H-6); 9.84 ppm (t, H-2).

N^1-(β-D-Ribofuranosyl)-3-aminocarbonylpyridinium Bromide (5c):

N^1-(2,3,5-Tri-*O*-benzoyl-β-D-ribofuranosyl)-3-aminocarbonylpyridinium bromide (**5b**; 1 g, 3 mmol) dissolved in methanol (50 ml, previously saturated at room temperature with ammonia gas dried over potassium hydroxide). The reaction mixture is allowed to stand for 72 h at −18 °C. Methanol is evaporated in vacuo. The residue is dissolved in absolute methanol (10 ml) and dry acetone (100 ml) + dry ether (100 ml) are added. Precipitation is repeated three times to give **5c** as an amorphous, hygroscopic powder; yield: 0.3 g (55%); $[\alpha]_D^{20}$: −33.0° (*c* 1.42, water).

$C_{11}H_{15}BrN_2O_5$	calc.	C 39.42	H 4.51	N 8.36	Br 23.84
(335.2)	found	39.32	4.60	8.20	23.77

U.V. (H_2O): λ_{max} = 266 nm.

^{1}H-N.M.R. (D_2O): δ = 6.23 (d, H-1′, $J_{H\text{-}1',H\text{-}2'}$ = 4.2 Hz); 8.24 (dd, H-5); 9.02 (dt, H-4, $J_{H\text{-}4,H\text{-}5}$ = 8.0 Hz); 9.29 (dt, H-6, $J_{H\text{-}6,H\text{-}4}$ = 1.5 Hz, $J_{H\text{-}6,H\text{-}5}$ = 6.8 Hz); 9.33 ppm (t, H-2, $J_{H\text{-}2,H\text{-}4} = J_{H\text{-}2,H\text{-}6}$ = 1.5 Hz).

Nicotinamide Mononucleotide (5d):

To N^1-(β-D-ribofuranosyl)-3-aminocarbonylpyridinium bromide (**5c**; 100 mg, 0.3 mmol) is added a phosphorylating mixture cooled to 0 °C [0.42 ml; consisting of phosphoryl chloride (0.06 ml) and trimethyl phosphate (0.36 ml)]. The mixture is stirred for 4 h at 0 °C, a few drops of water are added, then the mixture is neutralised with Dowex resin 1 × 8 ($HCO_3^{\ominus}$ form, 20–50 mesh) to pH 5. The resin is washed with water (200 ml). The aqueous solution is evaporated to 10 ml and placed on a column of Dowex resin 1 × 2 ($CH_3COO^{\ominus}$ form, 200–400 mesh; 8 × 1 cm) and eluted with water. The fractions chromatographically identical to natural samples of **5d** are combined, the water is evaporated at 30 °C, and **5d** is precipitated with acetone as an amorphous powder; yield: 30–60 mg (32–64%); $[\alpha]_D^{20}$: −31.4° (*c* 1.94, water).

$C_{11}H_{15}N_2O_8P$	calc.	C 39.53	H 4.52	N 8.38
(334.2)	found	39.84	4.23	8.50

U.V. (H_2O): λ_{max} = 266 nm.

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[1] Modified Nucleosides; 19. Part 18: A. A. Akhrem, N. B. Khripach, I. A. Mikhailopulo, *Chem. Heterocycl. Comp. (Russ.)*, 1427 (1979); *C. A.* **92**, 111262 (1980).

[2] For a comprehensive review see: C. Woenckhaus, in: *Topics in Current Chemistry* **1979**, 208.

[3] R. J. Suhadolnik et al., *J. Biol. Chem.* **252**, 4125 (1977), and references cited therein.

[4] For a general survey, see:
R. Jeck, C. Woenckhaus, in: *Methods in Enzymology*, S. P. Colowick, N. O. Kaplan, Eds., Vol. 66, Academic Press Inc., New York, 1979, p. 62–70.

[5] R. Jeck, P. Heik, C. Woenckhaus, *FEBS Lett.* **42**, 161 (1974).

[6] E. L. Esmans, J. A. Lepoivre, F. C. Alderweireldt, *J. Carbohyd. Nucleosides Nucleotides* **3**, 93 (1976).

[7] E. J. Freyne, J. A. Lepoivre, F. C. Anderweireldt, *J. Carbohyd. Nucleosides Nucleotides* **3**, 113 (1976).

[8] L. J. Haynes, N. A. Hughes, G. W. Kenner, A. R. Todd, *J. Chem. Soc.* **1957**, 3727.

[9] L. M. Melnikowa, V. M. Beresovskii, *J. Gen. Chem. U.S.S.R. (Engl. Trans.)* **37**, 1507 (1967).

[10] M. Jarman, W. C. J. Ross, *J. Chem. Soc.* [C] **1969**, 199.

[11] R. U. Lemieux, J. W. Lown, *Can. J. Chem.* **41**, 889 (1963).

[12] M. Yoshikawa, T. Kato, T. Takehishi, *Bull. Chem. Soc. Jpn.* **42**, 3505 (1969).

[13] N. J. Oppenheimer, N. O. Kaplan, *Biochemistry* **15**, 3981 (1976).