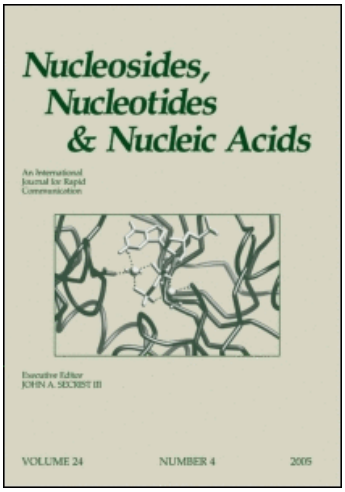

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A Novel Preparation of Nicotinamide Mononucleotide

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NOTE

A NOVEL PREPARATION OF NICOTINAMIDE MONONUCLEOTIDE

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Abstract. Nicotinamide mononucleotide is conveniently prepared from nicotinamide adenine dinucleotide by specific hydrolysis of the pyrophosphate bond using the Zr^{4+} ion as catalyst.

Nicotinamide mononucleotide (NMN) is usually prepared by the enzymatic cleavage of nicotinamide adenine dinucleotide (NAD) with NAD pyrophosphatase[1-4]. Material prepared in this way is expensive. Recently, the specific hydrolysis of pyrophosphate bonds using heavy-metal ions such as Zr^{4+} and Th^{4+} as catalysts has been reported[5]. Here we describe a simple preparation of NMN from NAD using this chemistry.

NAD from yeast (98% pure) was purchased from Sigma, zirconium tetrachloride (99.5+%) from Aldrich, and NMN and Dowex 50W x 8 from Sigma. We prepared 50 ml of a solution 0.01 M in NAD and 0.05 M in $ZrCl_4$. The solution was maintained at 50 °C for 30 mins. Hydrolysis was monitored by TLC on silica gel 60 F_{254} from Merck Darmstadt. (Solvent: 95% ethanol : 1 M NH_4Ac at pH 5, in a ratio of 7:3, [EDTA] = 10^{-5} M. NAD: R_f=0.64; AMP: R_f=0.75; NMN: R_f=0.42). After 30 min, only AMP and NMN could be detected. The reaction mixture was quenched by adding 23.4 ml of a 0.5 M solution of Na_3PO_4 (pH ~12.6). The pH was adjusted to 7.0 with 2 N HCl and the precipitate was separated by high-speed centrifugation (8 x

1000 RPM, 10 min). The precipitate was washed twice with 100 ml of water, and the combined supernatant concentrated to 50 ml *in vacuo* at 30 °C. This solution was applied to a Dowex 50W x 8 column (100-200 mesh, H^+ form, 28 x 1.7 cm). The column was eluted with water. NMN appeared in a volume of 300 ml, and was obtained as a powder by evaporating the eluate to a small volume, and then removing the remaining water by freeze-drying. The yield was 115 mg (~70%). A very similar yield was obtained in a larger scale preparation from 5 gms of NAD.

The final product was shown to be identical to authentic material by thin-layer chromatography, UV spectroscopy (ε=4200 at 266 nm) and by proton and phosphorus NMR spectroscopy.

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