SYNTHESIS OF A NOVEL SERIES OF β-NAD$^+$-ANALOGUES.

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In order to get an insight in the factors influencing enzymatic oxido-reduction reactions involving β-NAD$^+$, several β-NAD$^+$ analogues were tested upon their enzymatic activity(1). In the past many of these analogues were obtained through an enzymatic exchange reaction involving a DPN-ase. A few were obtained via pure synthetic efforts but then were modified at the adenine moiety of the molecule. Since in the theory of the enzymatic reduction of ketones by β-NADH, as suggested by Prelog and others the carbamoyl function on the pyridinium part of the molecule seemed to play an important role, a novel series of β-NAD$^+$-analogues, modified at the pyridinium ring, was synthesised. To obtain such analogues the following strategy was followed :

1. Synthesis of modified pyridine derivatives :

   The synthesis of these pyridine derivatives, resulting in a series of original 3,5-disubstituted pyridines is described elsewhere(2).

2. Coupling of these pyridine compounds with a protected sugar halide :

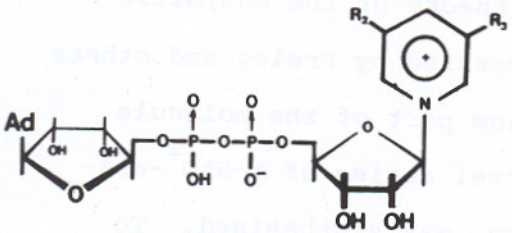
   This coupling involves two main problems : it should be highly stereospecific and the yields should be high.

Both purposes were attained by using 3,5-di-O-benzoyl-β-D-ribofuranosyl chloride.

3. Deblocking of the sugar and phosphorylation of the 5'OH group.

4. Coupling of these nucleotides with AMP:

The coupling of the nucleotides obtained by the reactions described above, with AMP, offers the following difficulties if direct coupling of the nucleotides is done under the influence of DCC, both symmetric and asymmetric dinucleotides are obtained. The synthesis of symmetric dinucleotides can be suppressed by using either the morpholidate or the 5'-phosphoric di-n-butyl-thiophosphinic anhydride of AMP. By using both procedures the following β-NAD^+-analogues were obtained:



I. R_2=CH_3CO; R_3=Me yield: 20%
II. R_2=CH_3CO; R_3=Et yield: 33%
III. R_2=CH_3CO; R_3=prop yield: 22%
IV. R_2=CH_3CO; R_3=i-prop yield: 26%

Ad=adenine

5. Purification of the reaction mixture:

The solvent is evaporated, the residu triturated with dry acetone resulting in the precipitation of a white solid which is filtered of. Further purification is done by HPLC: LiCrosorb 10 RP 8; column length 25cm, I.D.: 22.7 mm, eluent: 97% 0.05M NH_4OAC/3% methanol; flow rate: 24 ml:min; detection U.V.: 254 nm.

6. Structure identification was done by 360 MHz ^{1}H-NMR

References:

1. B.J.Jones, C.J.Sih, D.Perlman, "Applications of Biochem.Sys. in Org.Chem" partI, John Wiley &Sons New-york (1976).
2. E.L.Esmans, F.C.Alderweireldt, Bull.Chem.Soc.Belges, 82, 435 (1973)