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BENZOYL CYANIDE - A NEW BENZOYLATING AGENT IN NUCLEOSIDE AND NUCLEOTIDE CHEMISTRY

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In the chemistry of nucleosides and nucelotides, the benzoyl group is often used to protect hydroxylic or amino functions of the compounds mentioned. Mostly, benzoyl chloride in pyridine, or benzoic anhydride in various solvents have been used for this purpose. The benzoylation procedures with the use of both the reagents mentioned require often, especially with polyfunctional molecules, a prolonged treatment or an elevated temperature which might not be suitable for the use with sensitive molecules.

The reaction mixture contains pyridine hydrochloride or benzoic acid and is often contamined by a variety of coloured substances. This circumstance brings about the necessity of a rather laborious purification of the material to be obtained. We wish to report the application of another benzoylating agent in nucleic acid chemistry, which obviates the difficulties mentioned above.

Benzoyl cyanide was observed[1] to react smoothly with alcoholic, phenolic and mercapto functions with or without base catalysis, depending on the character of the respective function. In aprotic solvents, benzoyl cyanide does not react even at elevated temperatures with nucleoside derivatives (including derivatives with an amino function on the heterocyclic base moiety). On the contrary, in the presence of a catalytic amount of a base (triethylamine, tri-n-butylamin), a smooth reaction takes place, affording a quantitative conversion of the starting material to the benzoylated derivative within a few minutes at room temperature. The small excess of the agent, if any, is then decomposed with methanol. In most cases, an analytically pure product

crystallizes directly from the reaction mixture or can be purified by silica column chromatography.

The reaction is accompanied by evolution of hydrogen cyanide. There are no by-products present in the reaction mixture, except for a trace of the base and methyl benzoate which can be easily removed. On a prolonged treatment, yellow and red dyes are formed; it is advisable to work-up the reaction mixture immediately after the reaction has been finished.

In a typical experiment, thymidine (1.2 g, 5 mmol) and benzoyl cyanide (1.44 g, 11 mmol) were stirred in acetonitrile (10 ml). Upon addition of tri-n-butylamine (50 µl), the nucleoside quickly dissolved and, after 5 min. stirring at room temperature, 3′,5′-di-O-benzoylthymidine crystallized. After additional 10 min., methanol (0.5 ml) was added, the crystalline material collected with suction, washed with ethanol and recrystallized from the same solvent. Yield, 1.73 g (77%). M.p. 194-5°C (the literature[2] gives 193.5°C) $[\alpha]_D^{25}$ -36.6 (c 0.5, dimethylformamide). $C_{24}H_{22}N_2O_7$ (450.4) calculated 64.00% C, 4.92% H, 6.22% N; found 63.89% C, 4.52% H, 6.52% N.

2′-Deoxyuridine afforded its 3′,5′-di-O-benzoate in a 86% yield, under the same conditions. M.p. 225-6°C (ethanol), $[\alpha]_D^{25}$ -17.0 (c 0.5, dimethylformamide) (literature[3] gives 220°C). $C_{23}H_{20}N_2O_7$ (436.4) calculated 63.29% C, 4.62% H, 6.42% N; found 63.62% C, 5.08% H, 6.37% N.

5′-O-Tritylthymidine[4] was transformed to 3′-O-benzoyl-5′-O-tritylthymidine under the above conditions with 10% molar excess of the reagent. Yield, 76%. M.p. 166-169°C (acetonitrile). $C_{36}H_{32}N_2O_6$ (588.6) calculated 73.45% C, 5.48% H, 4.76% N; found 73.80% C, 5.43% H, 4.82% N. After removal of the trityl group from this derivative on refluxing in 80% aqueous acetic acid for 30 min., evaporating to dryness and crystallizing the residue from ethanol, 3′-O-benzoylthymidine was obtained in 89% yield. M.p. 114-6°C, $[\alpha]_D^{25}$ -2.2° (c 0.5, dimethylformamide). $C_{17}H_{18}N_2O_6$ (346.3) calculated 58.95% C, 5.42% H, 8.09% N; found 59.21% C, 5.14% H, 8.35% N.

Uridine gave on treatment with 4 equivs. of benzoyl cyanide under the above conditions (after purification on a loose layer of silica, elution with 5% ethanol in chloroform, and crystallization from ethanol) 2′,3′,5′-tri-O-

-benzoyluridine as the sole product, (yield, 69%). M.p. 143°C (literature[2] 143°C).

2′-Deoxycytidine was converted to its N^4, $O^{3'}$,$O^{5'}$-tribenzoyl derivative (4 equivs. of benzoyl cyanide, 30 min. at room temperature). Yield, 65% (chromatography on a loose layer of silica in 5% ethanol in chloroform and crystallisation from ethanol). M.p. 185-6°C. $[\alpha]_D^{25}$ +1.8 (c 0.5, dimethylformamide). $C_{30}H_{25}N_3O_7$ (539.5) calculated 66.78% C, 4.67% H, 7.79% N; found 67.15% C, 4.46% H, 7.90% N.

2,2′-Anhydro-(1-β-D-arabinofuranosyl)uracil[5] (5 mmol) and benzoyl cyanide (11 mmol) were dissolved in dimethylformamide (10 ml). Upon addition of tri-n-butylamine (100 µl), 2,2′-anhydro-(3,5-di-O-benzoyl-1-β-D-arabinofuranosyl) uracil crystallized. After 30 min. stirring, the white crystalline material was collected by suction, washed successively with methanol and ether, and dried in vacuo. Yield, 88% of the analytically pure compound. M.p. 312°C. $[\alpha]_D^{25}$ -38.0 (c 0.5, dimethylformamide). $C_{23}H_{18}N_2O_7$ (434.4) calculated 63.59% C, 4.17% H, 6.45% N; found 63.39% C, 4.42% H, 6.23% N.

The utility of benzoyl cyanide in nucleotide benzoylations can be demonstrated by the following experiments: Uridine 2′, 3′-cyclic phosphate ammonium salt[6] (1 mmol), benzoyl cyanide (3 mmol), and tri-n-butylamine (2.5 mmol) were stirred in dimethylformamide (5 ml) for 1 hour at room temperature and the clear solution added dropwise into ether (100 ml). The product was collected with suction, washed with ether and dried in vacuo. Yield, 85% of 5′-O-benzoyluridine 2′,3′-cyclic phosphate tri-n-butylammonium salt, analytically and chromatographically pure. Its cleavage by pancreatic ribonuclease afforded 5′-O-benzoyluridine 3′-phosphate as the sole product.

Uridine 3′-phosphate (freeze-dried ammonium salt, 2 mmol) gave with benzoyl cyanide (5 mmol) and tri-n-butylamine (5 mmol) in dimethylformamide (10 ml) under the same condition of the work-up 2′,5′-di-O-benzoyluridine 3′-phosphate tri-n-butylammonium salt (analytically and chromatographically pure) in 78% yield. The isomeric purity of this material was proved by reaction of its pyridinium salt with 2′,3′-di-O-benzoyluridine under the usual conditions of the N,N′-dicyclohexylcarbodiimide condensation[7], followed by

removal of benzoyl groups. Uridylyl-(3′⟶5′)-uridine thus obtained (yield, 35%) was completely degraded by pancreatic ribonuclease.

The above experiments demonstrate the wide applicability of benzoyl cyanide as benzoylating agent in the nucleoside and nucleotide chemistry. It should be pointed out that the benzoylation of uridine 3′-phosphate is not accompanied by any isomerisation of the phosphate group and can be therefore applied as a suitable procedure for the preparation of 2′-O-benzoylated 3′-ribonucleosides even on a larger scale. Thus, the laborious procedure described for this purpose[8] can be avoided. The extremely mild conditions and short time of the benzoylation make possible other syntheses, which involve the benzoyl derivatives as intermediates[9].

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