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Note

Improved synthesis of N^4-anisoyldeoxycytidine using Bio-Rex 5 columns

P. CASHION*, H. NOTMAN**, G. SATHE, T. CADGER and T. TRANQUILLA
Biology Department, University of New Brunswick, Fredericton, N.B. E3B 5A3 (Canada)
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In the phosphodiester approach to the chemical synthesis of the 3'-5' internucleotide bond of DNA, the primary amino groups of the bases require protection lest they become involved in complicated side reactions[1]; the commonly used protecting groups are the benzoyl group for deoxyadenylic acid, the anisoyl group for deoxycytidylic acid and the acetyl, isobutyryl or 2-methyl butyryl[2] groups for deoxyguanylic acid. Likewise in the phosphotriester approach, wherein deoxynucleosides are the starting material, these same blocking groups are used[3,4]. In particular the syntheses[5] of dan^{4}C and dbz^{6}A*** involve reaction of the nucleoside with an excess of the respective acid chloride in dry pyridine to form the tri- and tetraderivitized adducts d(an)$_3$C and d(bz)$_4$A respectively, followed by precipitation into water or chloroform–water extraction, selective saponification, neutralization of excess base with pyridinium Dowex 50 resin and after concentrating the Dowex filtrate, extraction between diethyl ether and water (see Fig. 1, part A).

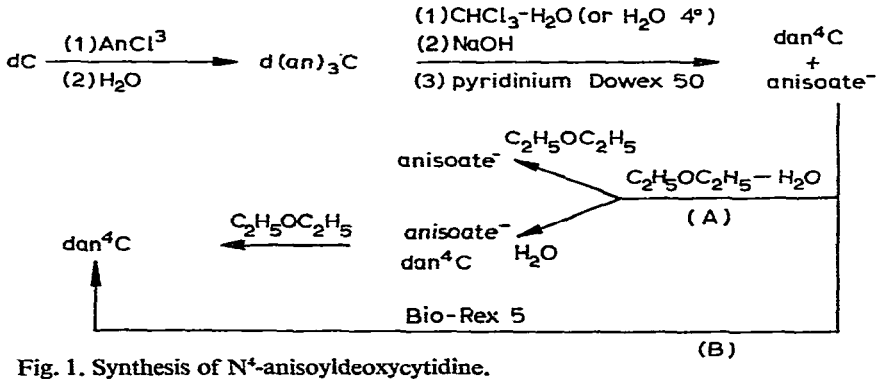

Fig. 1. Synthesis of N^4-anisoyldeoxycytidine.

* To whom correspondence should be addressed.
** Present address: MacDonald College, McGill Univ., Montreal, Quebec.
*** Abbreviations: dan^{4}C = N^4-anisoyldeoxycytidine; dbz^{6}A = N^6-benzoyldeoxyadenosine; AnCl = anisoyl chloride; d(an)$_3$C = O^3,O^5,N^4-trianisoyldeoxycytidine; d(bz)$_4$A = O^3,O^5,N^1,N^6-tetrabenzoyldeoxyadenosine.

In the latter extraction most of the anisic or benzoic acid partitions into the ether phase as the pyridinium salt, with the products —either dan^{4}C or dbz^{6}A-being in the water phase as a heavy white precipitate; the latter suspension is then washed a number of times with ether to remove the sodium salts of anisic or benzoic acid which coprecipitate with the N-protected nucleosides. Isolated yield are in the 60–75% range. We have found that in the workup of dan^{4}C it is very difficult to remove all traces of anisic acid from the final product (as judged by thin-layer chromatography, chloroform–ethanol (9:1)) even with multiple ether washings. Since dan^{4}C is merely the starting point in a long series of tritylation and phosphate condensation reactions involved in DNA synthesis, traces of contaminant at this early stage could cause problems with respect to the yield or purity of the subsequent reactions of dan^{4}C. Moreover the multiple ether washing steps result in a decreased isolated yield of dan^{4}C. Alternate modes of purifying dan^{4}C such as silica gel chromatography are excessively time-consuming. Accordingly we have introduced a simple ion exchange step into the workup procedure for dan^{4}C, whereby the anisoate ion is removed by rapid passage through an anion-exchange resin, thereby eliminating the multiple ether washing steps (see Fig. 1, part B). In detail: following the neutralization of the saponification reaction with an excess of pyridinium Dowex 50 resin the filtrate and washings containing the anisoate anion and product dan^{4}C are passed directly through a Bio-Rex 5 resin (Bio-Rad Labs., Richmond, Calif., U.S.A.; contains tertiary and quaternary amines on a polyalkylenamine lattice; 50–100 mesh, 2.8 mequiv./ml resin bed; HCO_3^- form); by having an excess (2–10-fold) of resin exchange groups relative to the anisoate ion in solution, a rapid flow-rate is possible (2–5 ml/min). Alternatively one can use a Dowex 1 type resin to effect the same separation, however in this case the moderately aromatic product dan^{4}C remains bound to the column along with anisic acid; apparently the vinyl–benzene lattice of Dowex-1 interacts hydrophobically with the aromatic regions of dan^{4}C under the conditions of application (organic solvent–water (*ca.* 1:3)); if subsequently the column is washed with organic solvent (either ethanol or pyridine)–water (1:1), the dan^{4}C elutes immediately. The HCO_3^- form of the Bio-Rex 5 resin is used*. The resin may be regenerated by washing with 1 *M* aqueous ammonium hydrogen carbonate–ethanol (3:1) until no more anisic acid emerges in the eluate (thin-layer chromatography, chloroform–ethanol (9:1), R_F 0.3) and then washed with excess water–ethanol (3:1). No apparent loss of resin capacity has occurred over a 1.5-year period. Recently in the preparation of N^2-isobutyryl-deoxyguanosine using isobutyryl chloride as the acylating reagent a moderate amount of fluorescent side product was seen after saponification with sodium hydroxide and Dowex 50 treatment; this unidentified side product co-partitioned with

* Initially the OH^- form of the resin was used; occassionally one would observe traces of N-deprotection of the product to generate dC and anisoate ion. This observation in turn suggests that unless one uses a considerable excess of pyridinium Dowex 50 the anisoate ions produced during the saponification may exist as the Na^+ rather than the pyridinium salt. Hence the Na anisoate as it encounters the Bio-Rex 5 active grouping $-\overset{|}{\underset{|}{N}}^{\pm}\ OH^-$ will form NaOH which in turn causes the deacylation of dan^{4}C to dC and the sodium salt of anisic acid. If, on the other hand, the original anisoate ion existed entirely as the pyridinium salt, only H_2O would be formed during the anion exchange process and no deprotection would have been observed. In any case by using HCO_3^- as the Bio-Rex 5 counter ion no deprotection has been observed.

the product between either chloroform–water or diethyl ether–water; passage of an aqueous solution of the mixture through a Bio-Rex 5 column, however, removed all of the fluorescent side product. In summary this modification has improved the isolated yield of dan^{4}C to *ca.* 90% (20% increase over literature value[5], reduced the working time *ca.* 15% (2 h), eliminated traces of anisic acid from dan^{4}C and is less costly insofar as no ether is required.

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