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SYNTHESIS OF N^4-MONO- AND DIALKYL-2'-DEOXYCYTIDINES AND THEIR INSERTION INTO AN OLIGONUCLEOTIDE

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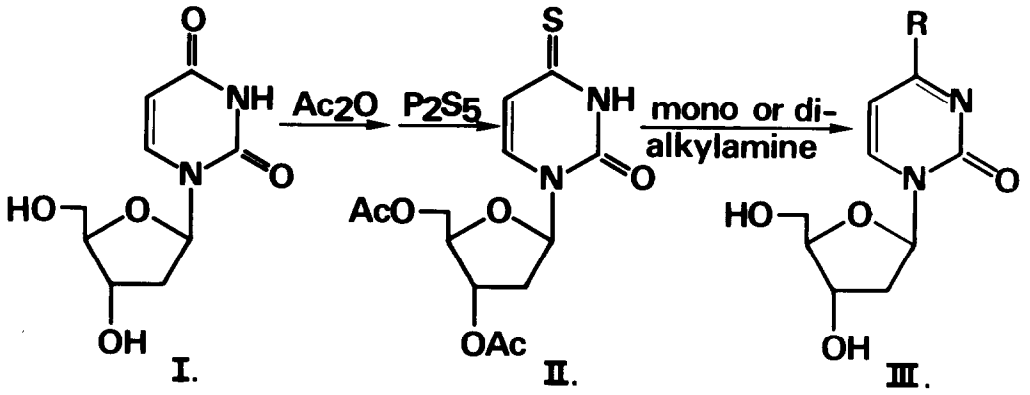
Abstract : Starting with 4-thio-2'-deoxyuridine, the simple synthesis of N^4-mono- and dialkyl-2'-deoxycytidines is described. The N^4-methyl derivative has been incorporated into the oligonucleotide chain d(CGm^4CGCG).

Among so-called unusual nucleosides found in RNAs and DNAs, N^4-alkylated nucleosides are frequently encountered. Their role is not clear but some hypotheses postulate the influence of alkylated nucleoside units on the secondary structure of DNA or RNA which can be specifically recognized by enzymes [1].

Recently, a few methods for the synthesis of alkylated nucleosides have been described [2-5]. The method described by J.J. FOX et al. [7a,b] has been used for synthesis of some N^4-alkyl derivatives of 5'-fluorodeoxycytidine and N^4-methyl, N^4-dimethyl- and N^4-(2-phenyl)ethyl- cytidines although in latter cases rather drastic conditions (sealed tube, 100°C, 24 hrs) for the conversion of 4-thiouridine into corresponding N^4-alkyl cytidines were used. These experimental conditions may be a serious limitation when temperature sensitive substrates are used for synthesis. We have found much milder conditions are quite sufficient to quantitatively effect the above reaction. This allowed us to extend the method to the synthesis of various N^4-monoalkyl-2'-deoxycytidine derivatives even those bearing sterically hindered N^4-alkyl moiety as iso-propyl or cyclohexyl. Analogous experiments were performed with secondary amines and the unexpected results are briefly discussed later on in this communication.

3',5'-di-O-acetyl-2'-deoxyuridine obtained by acylation of 2'-deoxyuridine (I) [6] was treated with P_2S_5 [7] (1.05 eqv.) in dioxane [8] to yield 3',5'-di-O-acetyl-4-thio-2'-deoxyuridine (II) which was isolated using short column silica gel chromatography (yield 81 %). Compound II was the substrate for the synthesis of all N^4-alkylated derivatives of 2'-deoxycytidine III_{a-k}.

To substitute the 4-thiol group by an alkylamino one, compound II was treated with the appropriate mono- or dialkylamine in ethanol (33% solution, 1 ml/0.1 g) at room



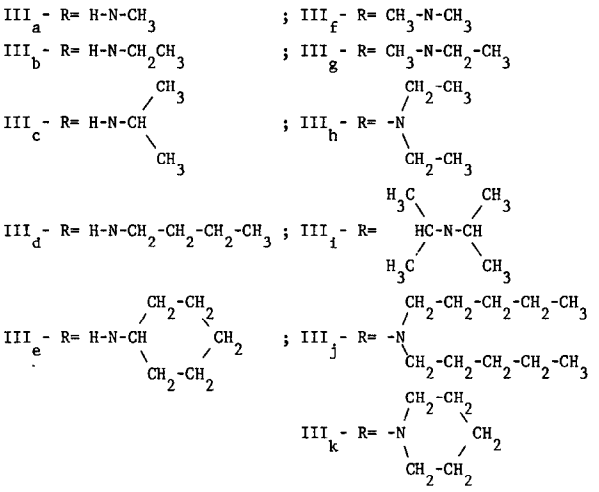


Fig. 1.

temperature or at 55°C. As shown in Table I, the quantitative conversion of II into the N^4-alkyl-2'-deoxycytidine derivatives III_{a-k} requires 1 hr to 48 hrs for primary amines and in the case of compounds III_{a-c} were the only nucleosidic products present in the reaction mixture.

Analogous reaction with secondary amines (dimethyl-, ethyl-methyl-, diethyl-, di-isopropyl-, di-n-butyl- and piperidine) only gave similar results in the preparations of N^4-dimethyl-2'-deoxycytidine (III_f), 4-piperidyl-pyrimidine nucleoside (III_k) and N^4-ethyl-N^4-methyl-2'-deoxycytidine (III_g) [9] although a longer reaction time (48 hrs, 48 hrs, 7 days respectively) was required to complete the substitution of the 4-thiol group. Under the same experimental conditions when diethyl-, di-n-butyl- and di-isopropylamine were used, a much longer period (18, 28, 20 days respectively) was needed for quantitative conversion of the 4-thiol group to the alkylamino one.

Table I

Product	Reaction time Room temper.	55°C	Yield isolated (%)	Rf value [a] A	B	UV max (nm)	max_1 max_2	min (nm)
III_a	24 hrs	1 hr	90	0.44	0.62	275;242	1.18	254
III_b	48 hrs	3.5 hrs	92	0.66	0.81	276;242	1.26	252
III_c	-	48 hrs	91	0.82	0.94	276;242	1.20	253
III_d	48 hrs	3.5 hrs	87	1.06	1.35	276;242	1.26	252
III_e	-	36 hrs	87	1.13	1.19	276;245	1.34	253
III_f	-	48 hrs	88	1.11	1.19	283	-	242
III_g	-	7 days	93 (2)[9]	1.32	1.44	284	-	243
III_h	-	18 days	58 (24)[b]	1.37	1.44	283	-	243
III_i	-	20 days	4 (93)[b]	1.84	1.60	284	-	243
III_j	-	28 days	56 (27)[b]	1.76	1.81	284	-	245
III_k	-	48 hrs	91	1.51	1.35	284	-	244

a Rf value were calculated relative to 2'-deoxyuridine :
A - chloroform/methanol 9:1 (v/v) ; B - chloroform/methanol 4:1 (v/v)
b in brackets isolated yield of N^4-monoalkyl derivative of 2'-deoxycytidine.

Surprisingly, for the diethyl- and di-n-butylamine reactions mixtures were composed of the desired N^4-di-alkyl derivatives $III_{h,j}$ and also a considerable amount of the N^4-mono-alkylated-2'-deoxycytidine derivatives $III_{b,d}$ (24 %, 27 %, respectively), while di-isopropylamine gives N^4-mono-isopropyl-2'-deoxycytidine almost exclusively (see Table I). To explain the mechanism of the reaction, further studies are needed.

From the reaction mixture, N^4-dialkyl-2'-deoxycytidines $III_{h,j}$ were isolated with reasonably high yields (Table I) making the procedure described herein an effective method for the synthesis of various N^4-dialkyl-cytidines.

Isolation of particular N^4-alkyl derivatives of 2'-deoxycytidine was performed by crystallisation (III_a and III_f) or by simple silica gel filtration using a gradient of methanol (5 - 15) in chloroform as the solvent system. The purity and structure of products III_{a-k} was confirmed by NMR, MS and UV spectroscopy, and t.l.c. and h.p.l.c. analysis [10].

Compared to methods currently available, this methods provides (i) a very simple and efficient procedure, (ii) easy isolation, (iii) high yields of products, and (iv) the possibility of synthesising a wide variety of N^4-mono- and dialkyl derivatives of 2'-deoxycytidine.

N^4-methyl-2'-deoxycytidine after protection of the 5'-OH function with a dimethoxytrityl [6] group and phosphorylation with p-chlorophenylphospho-di-triazolide was incorporated into the hexamer d(CGm^4CGCG) for further studies. The same will be done with other N^4-alkyl derivatives of 2'-deoxycytidine. After deblocking, the hexamer was purified by h.p.l.c. (SAX-10). The pure compound was characterised by pyrolysis mass spectrometry [11] analysis and standard DNA analytical procedures (32-P labelling, gel electrophoresis) [12].

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9 According to h.p.l.c. analysis, 2 % of N^4-methyl-2'-deoxycytidine was found in the reaction mixture.

10 Full NMR, MS and other data will be published elsewhere and are available upon request.

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