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An investigation by ^{1}H NMR spectroscopy into the factors determining the β:α ratio of the product in 2'-deoxynucleoside synthesis

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ABSTRACT

By following the course of the reaction between a suitably-protected base and a chlorosugar in an NMR tube at 250 MHz, it has been shown that the products are consistent with those expected from an SN2 mechanism with inversion of configuration at the anomeric carbon of chlorosugar. In order to achieve high yields of β-2'-deoxynucleoside, the crystalline α-chlorosugar used must react swiftly so that anomerization of the sugar moiety is kept to a minimum. If the base is sufficiently reactive (e.g. 5-methyluracil, uracil), then no catalyst is required and chloroform is the preferred solvent. Using equimolar quantities of the reactants, almost quantitative yields of nucleoside can be obtained in one hour with a β:α ratio >4. With an excess of base, the β:α ratio can be increased even further. With less reactive bases (e.g. 5-nitrouracil, 5-acetyluracil), addition of catalyst can increase the rate of condensation more than the rate of anomerization or decomposition of the sugar. $ZnCl_2$ (0.1 equivalents) has been found to give satisfactory results, although the slower the reaction, inevitably the more α-2'-deoxynucleoside is formed. Essentially pure α-2'-deoxynucleoside can be isolated in high yield by allowing chlorosugar to anomerize by letting it stand in a polar solvent (acetonitrile) before addition of the base.

INTRODUCTION

Most nucleosides currently commercially available in large quantities are produced by enzymatic and/or chemical degradation of RNA or DNA. In the case of RNA, this presents few problems as large quantities of yeast are available and the phosphodiester bond in RNA is so much more easily hydrolysed than in DNA. In any case, ribonucleoside synthesis, starting from a suitably protected base and a commercially-available stable sugar derivative, is straightforward giving high yields of exclusively β-nucleoside due to the participation of the 2-acyl substituent on the sugar moiety.[1]

Recently, the need for substantial quantities of deoxynucleosides for gene synthesis and also for potential therapeutic use has increased and has meant that a method that could give high yields of β-2'-deoxynucleoside might be competitive with material obtained from natural polymers. In addition, the synthesis of analogues of thymidine in the hope of producing a compound

with interesting therapeutic properties, has meant that current methods for chemical deoxynucleoside synthesis which usually lead to the production of both α- and β-anomers, are not satisfactory. The yield of required product (β-anomer) is lowered and procedures for separating the anomers can be time-consuming and costly.

With the introduction of the use of alkoxypyrimidines by Hilbert and Johnson,[2] modified by Wittenberg[3] to use the silyl group and by Vorbrüggen[4] to use a peracylated sugar and a Friedel-Craft's catalyst, ribonucleosides can be made in high yield. However, little systematic work has been reported on deoxynucleoside synthesis and there appears to be no work dealing with what is actually produced in such condensations rather than what is isolated. The normal starting material for the sugar moiety is 2-deoxy-3,5-di-O-p-toluoyl-ribofuranosyl chloride[5] which crystallises out when prepared, in the form of the α-anomer.[6] Thus, despite the lack of steric control caused by the absence of an acyl group at the 2-position, if the condensation can be performed under conditions which prevent sugar anomerization and encourage SN2-type reaction, it should be possible to obtain high yields of β-2'-deoxynucleoside.

We thus decided to follow the condensation of suitably-protected 5-methyluracil derivatives with α-2-deoxy-3,5-di-O-p-toluoylribofuranosyl chloride (chlorosugar) under a variety of conditions in the NMR tube. The advantage of this approach is that the concentrations of reactants necessary are similar to those used on a preparative scale and by following the 5-CH_3 signal at 250 MHz, one can distinguish between all the possible 5-methyluracil-containing compounds in solution at all times. We have shown that by a rational choice of solvent, catalyst and other conditions, one can produce very high yields of either almost exclusively β- or α-nucleoside. The principles learned have been applied to the synthesis of the previously difficult-to-prepare 2'-deoxynucleosides, 5-nitro-2'-deoxyuridine[7] and 5-acetyl-2'-deoxyuridine.[8]

RESULTS AND DISCUSSION

A prerequisite for the successful formation of high yields of β-2'-deoxynucleoside, assuming that an SN2 reaction mechanism can be invoked, is the prevention of anomerization of the α-chlorosugar. Thus we first investigated the stability of this compound in solvents having a range of dielectric constants, with and without the presence of catalysts and halide ions. By NMR we could only follow accurately the disappearance of α-chlorosugar but we had evidence that under most conditions the only significant product was β-

chlorosugar and that there was little general decomposition. Thus an NMR spectrum of such a reaction mixture of the chlorosugar "aged" for 3 hours in dichloromethane from which the spectrum of the α-chlorosugar had been subtracted by computer, left a spectrum consistent with that for β-chlorosugar. Also, even after considerable amounts of α-chlorosugar had disappeared from solution, it is still possible to obtain essentially quantitative yields of nucleoside (mainly α-) from such a reaction mixture.

Anomerization of halopyranoses has been studied extensively and the preference for the equilibrium mixture to contain a majority of the α-anomer is explained by the anomeric effect.[9] In more polar solvents, the constraints on the sugar for the more polar groups to be axial are not so great for pyranoses, so that more β-anomer is present at equilibrium.[10] Little work has been reported on equivalent studies in the furanose system but one might expect the results to be less clear cut and the equilibrium more easily perturbed by outside influences. Also, we are more interested in the initial rate of anomerization compared with the rate of nucleoside synthesis rather than the position of the final equilibrium. Previous evidence has indicated[11] and results here confirm, that β-chlorosugar reacts to produce α-nucleoside at a faster rate than does α-chlorosugar give β-nucleoside. Thus unless the rate of equilibration of the sugar anomers is very slow compared with the rate of nucleoside formation, α-nucleoside may always predominate no matter where the equilibrium position between the two sugar anomers lies.

The results (Fig. 1) show that as expected, much more β-chlorosugar is formed in polar solvents like acetonitrile than is formed in dichloromethane. In benzene, no anomerization is detectable at all. Contrary to previous reports,[11] the addition of trimethylsilyl chloride causes little increase in the rate of anomerization, and as reported for the halopyranoses,[12] bromide ion is a more effective anomerizing agent than is chloride ion. We cannot provide quantitative data for the effect of the addition of catalysts to chlorosugar because even within a few minutes, the α-anomer spectrum had almost completely vanished and quite clearly decomposition products predominated. However when a nucleoside condensation was attempted in the presence of fluoride or bromide ion, predominantly α-nucleoside was obtained under conditions in which, in the absence of halide ion, the β-anomer would have been the major product.

With this evidence concerning chlorosugar anomerization, we turned to nucleoside synthesis and preliminary experiments showed that benzene was quite unsuitable as a solvent due to solubility problems and in the absence of

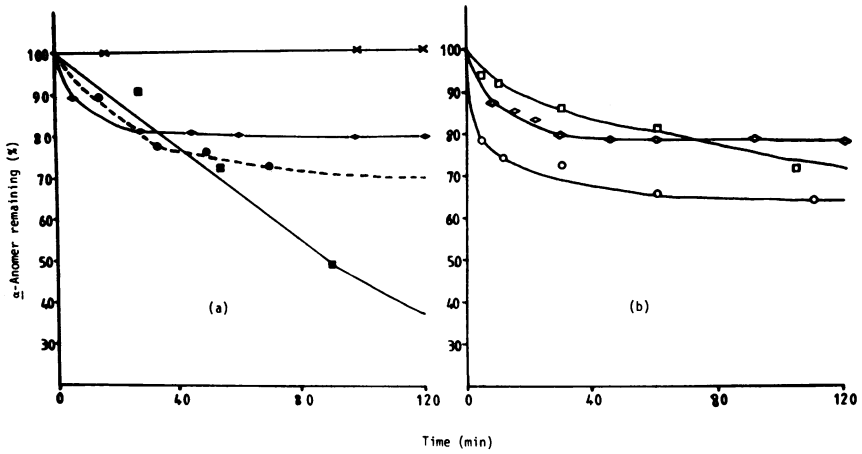

Fig. 1. Anomerization of chlorosugar in various solvents (a), and with addition of various ions (b). Benzene or pyridine (x), acetonitrile (■), chloroform (◆), dichloromethane (●), chloroform + 1 eq. tetrabutylammonium bromide (○), chloroform + 1 eq. trimethylsilyl chloride (◇), dichloromethane + 1 eq. trimethylsilyl chloride (□).

catalyst at 21°C, little (<5%) reaction occurred. Dichloromethane and acetonitrile were thus used as representatives of a less-polar and polar solvent which have previously been used extensively in nucleoside syntheses. Our results using the standard Hilbert-Johnson 2,4-dialkoxypyrimidines showed that 2,4-dimethoxy-5-methyluracil was far too unreactive in either solvent, gave low yields (30-40%) of predominantly α-nucleoside and as the reaction is so slow, the addition of catalyst served only to increase the rate of decomposition of the chlorosugar. 2,4-Di-t-butoxy-5-methylpyrimidine reacted much faster (data not shown) but yields were still low, were mainly α-nucleoside and insoluble precipitates formed due to the lability of the t-butoxy groups which caused problems in following the reaction by NMR spectroscopy.

2,4-Dibenzyloxy-5-methyluracil reacts much faster. In dichloromethane, a 55% isolated yield was obtained after 24 hours, but this was exclusively α-nucleoside. In chloroform (Fig. 2) the nucleoside yield rose to 80% with a β:α ratio of 0.6 which decreases as the reaction proceeds. Addition of catalyst reduces the yield but gives a higher β:α ratio (0.72). The yield is lower because the nucleoside formation reaction is still fairly slow and decomposition of chlorosugar occurs. It is apparent that a derivative of the pyrimidine which is much more reactive, is required.

2,4-Bis-trimethylsilyl-5-methylpyrimidine is far more active than the

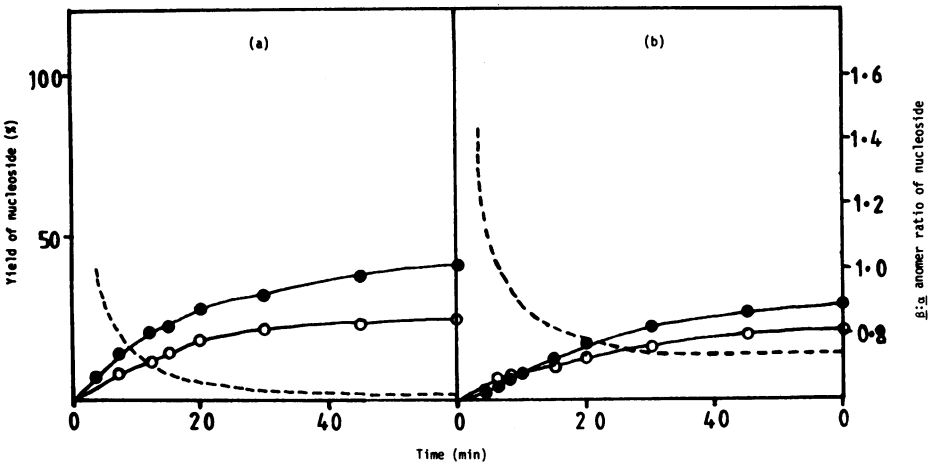

Fig. 2. Condensation of 2,4-dibenzyloxy-5-methyluracil with chlorosugar (a) in chloroform, (b) in chloroform + 0.1 eq. $ZnCl_2$. α-nucleoside formed (●), β-nucleoside formed (O), β:α ratio (----).

alkoxypyrimidines and thus the nucleoside yields in all solvents are correspondingly higher except in benzene where no significant reaction occurs. The results (Fig. 3) follow the predictable pattern in that in acetonitrile, reaction is rather slow, yields are high but are predominantly α-nucleoside. Indeed when chlorosugar is allowed to anomerize in the solvent before addition of the base, the subsequent reaction is much faster and almost exclusively α-nucleoside is produced. The speed of this reaction is further evidence for the theory that β-chlorosugar is more reactive than the α-anomer. [In all the reactions described here, there is no evidence for any equilibrium between α- and β-nucleosides].

In dichloromethane, the overall reaction is quite fast giving a high yield of α- and β-nucleoside, the β-anomer predominating. Under these conditions, the rate of anomerization of the sugar must be substantially slower than the reaction of α-chlorosugar with the base to give β-nucleoside. Only as the concentration of reactants falls and the rate of reaction slows, does anomerization become significant and the β:α ratio of nucleoside produced decreases. Prior anomerization of the sugar, by allowing it to stand in solution before base addition, once again produces a high yield of nucleoside but with a decreased β:α ratio. In this and other cases of anomerization of chlorosugar prior to reaction, the β:α ratio actually increases as the experiment continues because the initial β-chlorosugar formed, reacts rapidly.

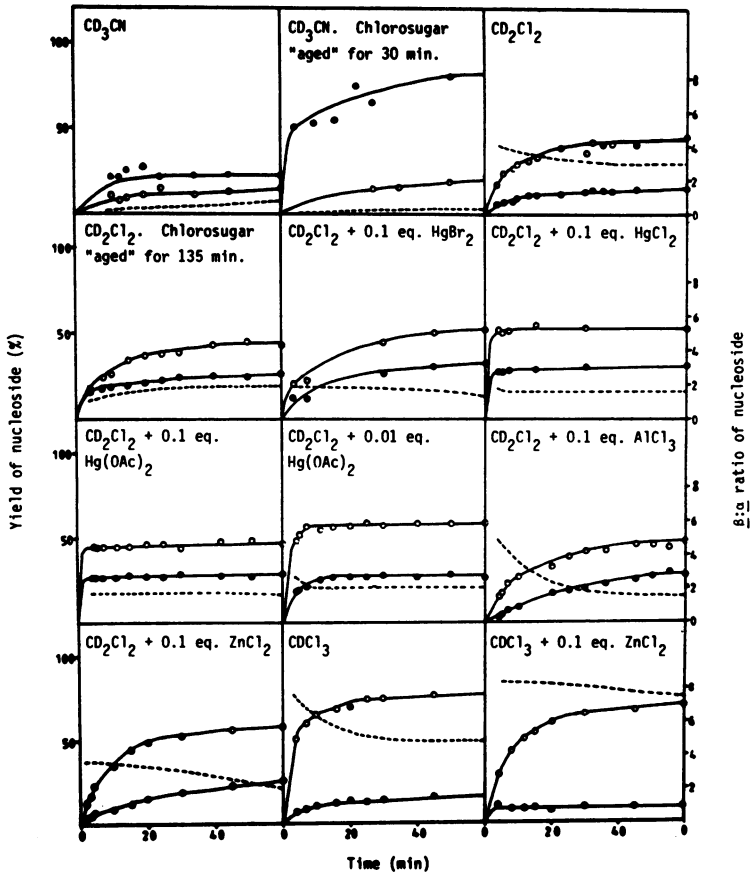

Fig. 3. Condensation of 2,4-bis-trimethylsilyloxy-5-methyluracil with chlorosugar under various conditions. α-nucleoside formed (●), β-nucleoside formed (○), β:α ratio (----).

The addition of catalysts increases the rate of nucleoside formation but also increases the rate of chlorosugar anomerization and decomposition. Thus with $HgBr_2$ and $HgCl_2$ (0.1 equivalents), the condensation reaction is complete in a few minutes. The yield is high but the β:α ratio is lower than for the uncatalysed reaction. Mercuric acetate is an even better catalyst such that the quantity used can be reduced to 0.01 equivalents and still a very high yield of nucleoside is obtainable in a few minutes. However, once again the β:α ratio is lower than in the non-catalysed reaction. $AlCl_3$ seems to offer no advantages, but for a catalysed reaction, the use of $ZnCl_2$ appears to favour nucleoside synthesis rather than chlorosugar anomerization but even

this is not as good as in the non-catalysed reaction. In our hands, $SnCl_4$ is very difficult to work with, particularly on a small scale and it offers no advantages over other catalysts. In fact is appears to favour α-nucleoside production and can also cause the production of emulsions on work-up. Trimethylsilyl triflate appeared to cause rapid decomposition of the chlorosugar and gave very low yields.

When chloroform is used however, the non-catalysed reaction is essentially complete in one hour and almost exclusively β-nucleoside is formed in high yield. Upon catalysis, the yield of nucleoside is slightly reduced but the β:α ratio is increased, presumably because in this solvent, decomposition of chlorosugar, rather than anomerization, occurs.

Thus using equimolar proportions of chlorosugar and base, the α-nucleoside of 5-methyluracil is best prepared by using an "aged" solution of chlorosugar in acetonitrile and the preferred conditions for the β-nucleoside synthesis are the use of 0.1 equivalents of $ZnCl_2$ in chloroform. The yield of the desired nucleoside is greater than 80% in the latter case and 60% in the former. Using an excess of chlorosugar, not unexpectedly, increases the proportion of α-nucleoside formed while an excess of base favours production of the β-nucleoside such that a two-fold excess of base results in a 30% decrease in the percentage of α-anomer produced.

In order to ascertain the generality of the predictions made here, we have proceeded to synthesise several 2'-deoxynucleosides on a preparative scale. The results (Table 1) show that scaling up the α- and β-thymidine preparations cause no problems. β-2'-Deoxyuridine and β-E-5-(2-bromovinyl)-2'-deoxyuridine[13] can be prepared under exactly analogous conditions.

When the relatively unreactive bases, 5-acetyluracil and 5-nitrouracil are used, although the same principles apply, some modification is required. Normally in dichloromethane, 5-acetyluracil gives a β:α ratio of less than 0.25.[8] This can only be increased to 0.53 by using chloroform as solvent because the rate of reaction of the base with α-halogenose is so much slower than the rate of anomerization of the sugar. However, upon catalysis with $ZnCl_2$, the ratio is further increased to 1.25 so that the β-nucleoside is the major product. Here, the catalyst is obviously catalysing the nucleoside condensation reaction more than the sugar anomerization reaction.

β-5-Nitro-2'-deoxyuridine has never previously been synthesised by a condensation reaction. However in chloroform with $ZnCl_2$ catalysis, although the reaction is rather slow, a high yield of nucleoside with the β-anomer predominating is produced. The yields quoted for these reactions are isolated yields

Table 1. Details of reaction conditions, isolated yields and β:α ratio of nucleosides from preparative scale reactions

Base	Catalyst	Solvent	Nucleoside Yield %	β:α
2,4-dibenzyloxy-5-methyluracil	None	CH_2Cl_2	55	Only α
2,4-bis-trimethylsilyloxy-5-methyluracil	None	CH_3CN	60	0.2
2,4-bis-trimethylsilyloxy-5-methyluracil	None	$CHCl_3$	83	Only β
2,4-bis-trimethylsilyloxy-uracil	None	$CHCl_3$	92	Only β
2,4-bis-trimethylsilyloxy-5-acetyluracil	0.1 eq. $ZnCl_2$	$CHCl_3$	76	1.23
2,4-bis-trimethylsilyloxy-5-nitrouracil	0.1 eq. $ZnCl_2$	$CHCl_3$	82	Mainly β
2,4-bis-trimethylsilyloxy-E-5-(2-bromovinyl)uracil	None	$CHCl_3$	72	Only β

and the β:α ratios are based on the signal for H-1' (and H-6 for 5-acetyl-2'-deoxyuridine).

In summary, all the eivdence is consistent with the view that nucleoside synthesis proceeds via an SN2 mechanism but in addition one has sugar anomerization and decomposition to take into account as well as the fact that β-chlorosugar reacts faster to give α-nucleoside than does α-chlorosugar to give β-nucleoside. Although the precise conditions will depend upon the base used, its reactivity and its complexing ability with any catalyst used, in general, chloroform seems to be the best solvent compatible with a fast reaction rate, solubility of reactants and only slow anomerization of chlorosugar. Catalyst (usually 0.1 equivalents) should only be used if the rate of reaction is so slow that chlorosugar anomerization occurs at a significant rate compared with nucleoside formation in the non-catalysed reaction.

EXPERIMENTAL

All ^1H NMR spectra were recorded on a Bruker Spectroscopic FT NMR spectrometer (250 MHz) at 21°C. One pulse was found to be sufficient to produce satisfactory spectra at the concentrations used. Deuterochloroform was used directly from the container as supplied. Chloroform was heated under reflux with P_2O_5, distilled and stored in a darkened bottle over molecular sieves (Type 4A).

One variable which is difficult to control is the purity of the chlorosugar. The compound is not indefinitely stable and yet it is tedious and costly to have to prepare a new sample for each experiment. Traces of HCl left in the crysals cause decomposition and liberation of more HCl and it is generally found that chlorosugar which has been kept for some while reacts much faster than does newly-prepared material. In order to try and standardise this effect, all chlorosugar used was stored in vacuo over NaOH pellets for at least one week before use and used over a subsequent period of two weeks.

The preparation of 2,4-dimethoxy-5-methyluracil has been reported.[14] Bis-trimethylsilyoxy-5-methyluracil was prepared by heating 5-methyluracil under reflux in hexamethyldisilazane and trimethylsilyl chloride (7:3). When complete solution had been achieved, the excess of hexamethyldisilazane and trimethylsilyl chloride was removed by distillation in vacuo to yield a residual clear colourless oil which was stored over nitrogen in a flask equipped with a septum.

2,4-Di-t-butoxy-5-methyluracil. 2,4-Dichloro-5-methylpyrimidine (12 g, 74 mmol) in dry toluene (100 ml) was added dropwise with stirring to a suspension of potassium t-butoxide (25 g, 222 mmol) in toluene (250 ml) and the mixture heated under reflux for 2 h. After cooling, the solution was extracted with water (3 x 100 ml), the organic layer dried and the solvent removed by distillation in vacuo to give a dark brown gum. This was purified by distillation under high vacuum at 102-112°C to give a colourless oil which, on cooling, crystallised to give pure 2,4-di-t-butoxy-5-methyluracil (13.57 g, 77%); NMR [$(CD_3)_2SO$] δ 7.96 (1-H, s, H-6), 1.91 (3-H, s, 5-CH_3), 1.52 (18-H, d, t-butyl). (Found: C, 65.3; H, 9.6; N, 11.55. $C_{13}H_{22}N_2O_2$ requires C, 65.5; H, 9.3; N, 11.76%).

2,4-Dibenzyloxy-5-methyluracil. To a solution of benzyl alcohol (21.62 g, 200 mmol) in toluene (200 ml) was added sodium hydride (50% oil dispersion, 20 g, 417 mmol). The mixture was heated under reflux for 1 h and after cooling, a solution of 2,4-dichloro-5-methylpyrimidine (10 g, 61.3 mmol) in toluene (200 ml) was slowly added. The reaction mixture was heated under reflux for 2 h, allowed to cool and the toluene removed by distillation in vacuo. Water (125 ml) was added and the mixture extracted with ether (4 x 125 ml), dried and the solvent removed to yield a viscous yellow syrup (19 g). This was applied to a silica gel column which was eluted with chloroform-dichloromethane (1:4) to give 2,4-dibenzyloxy-5-methyluracil as a white waxy solid (12 g, 64%); NMR [$(CD_3)_2SO$] δ 8.05 (1-H, s, H-6), 7.5 (10-H, m, aromatic), 5.3.5 (4-H, d, CH_2), 2.0 (3-H, s, 5-CH_3). (Found: C, 73.9; H, 5.9; N, 9.1. $C_{19}H_{18}N_2O_2$ requires C, 74.49; H, 5.92; N, 9.15%).

"Aging" of 2-deoxy-3,5-di-O-(p-toluoyl)-α-D-erythro-pentofuranosyl chloride (chlorosugar) in different solvents. Chlorosugar (~30 mg) was dissolved in deuterated solvent (1-1.5 ml) (except in acetonitrile when <10 mg was used) in a dry NMR tube. The ^1H NMR spectrum of the reaction mixture was recorded at intervals. Anomerization of the chlorosugar was followed by noting the disappearance of the H-1 doublet assigned to the α-anomer and appearance of the corresponding upfield double doublet of the β-anomer. The effects on anomerization caused by addition of halide ion were followed using a similar procedure. Addition of halide ion was made as soon as solution of the chlorosugar had been effected.

Condensation reactions between a protected base and chlorosugar. Normally equivalent amounts of base and chlorosugar were used. In the case of the alkoxypyrimidines, base (~30 mg) was dissolved in deuterated solvent (0.5 ml) in a dry NMR tube which had been flushed with nitrogen. Catalyst (when used) was then added. Chlorosugar was added as a 'freshly' prepared solution in deuterated solvent (0.5 ml). The sealed NMR tube was inverted repeatedly and ^1H NMR spectra of the solution were recorded at intervals. In all solvents,

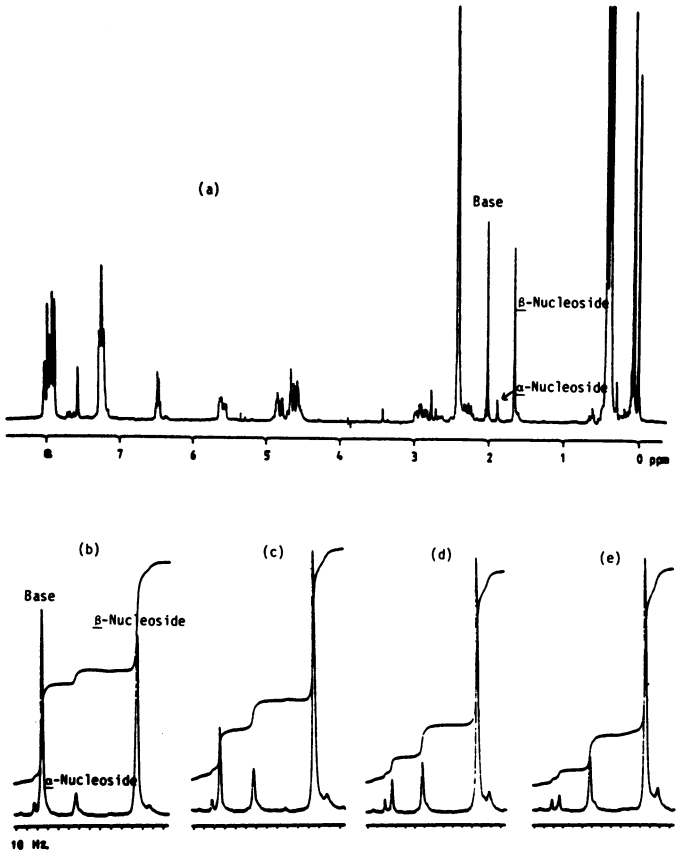

Fig. 4. ^{1}H NMR spectrum of bis-trimethylsilyloxy-5-methyluracil condensation with chlorosugar in $CDCl_3$ at 21°C after 4 min (a). Expansion of the region 1.5-2.1 ppm after 4 min (b), 16 min (c), 45 min (d) and 141 min (e).

the 5-CH_3 signals corresponding to the base and the nucleoside anomers were well separated and could be quantitated from the integration curve. Examples of expansions from the methyl region of spectra from one experiment are reproduced in Fig. 4. In all solvents, the base 5-CH_3 signal is downfield of the α-nucleoside signal which is downfield of the β-nucleoside signal.

Large scale preparations. To a suspension of the bis-trimethylsilyloxy base (~2 g) in chloroform (100 ml), was added chlorosugar (1 equivalent) and the reaction mixture stirred for 24 h at room temperature. Catalyst was added if indicated in Table 1. The solvent was then removed by distillation in vacuo. Apart from the nucleoside from 5-methyluracil which could be recrystalised directly from methanol, the residual oils were purified on silica gel using toluene-ethyl acetate (7:3). The yield of nucleoside isolated and its anomeric composition (no attempt was made to separate the anomers by chromatography) is given in Table 1. The anomeric ratio was determined from the NMR spectrum of the mixture.

In one case, bis-trimethylsilyloxy-5-methyluracil (2.0 g, 7.40 mmol) was suspended in dry acetonitrile (75 ml) and this was added to a solution of chlorosugar (2.9 g, 740 mmol) which had been allowed to stand ("aged") in acetonitrile (75 ml) for 30 min. The reaction mixture was stirred for 24 h and worked up in the usual way to yield nucleoside (2.1 g, 60%, $\underline{\beta}:\underline{\alpha}$ ratio = 0.2).

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