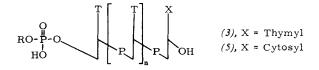
We have now achieved a stepwise synthesis of oligodeoxynucleotides with a terminal 5'-phosphate group.

Condensation of thymidine-5'-(2,2,2-trichloroethyl hydrogen phosphate)  $(1)^{[2]}$  with 3'-O-acetylthymidine-5'-phosphoric acid (2) by means of dicyclohexylcarbodiimide in pyridine and subsequent removal of the acetyl group and separation on a DEAE-cellulose column give the dinucleotide (3a). This can be converted by condensation with (2) into the trinucleotide (3b). We have carried out this condensation up to the tetranucleotide (3c). N<sup>6</sup>,3'-O-Diacetyldeoxycytidine-5'-phosphoric acid (4) can be used in place of (2) in the last condensation, and then the oligonucleotides (5a) to (5c) are obtained.

After conversion of these compounds into the pyridine salts, the 2,2,2-trichloroethyl group can be removed from all of them with activated Cu/Zn<sup>[3]</sup> in dimethylformamide (50 °C, 1 hr)<sup>[2]</sup>. The yields of oligonucleotide in these processes amount to 85-90% for (3a)-(3c), 65% for (5a) and (5c), and 90% for (5b).



After conversion into the ammonium salts the oligonucleotides can be cleaved to mononucleotides by snake-venom phosphodiesterase.

	R	n	Yield (%) [a]	Rf in system [b]		
				Α	в	C
(3a)	CCl <sub>3</sub> -CH <sub>2</sub>	0	53	0.57	0.61	
(36)	CCl <sub>3</sub> -CH <sub>2</sub>	1	56	0.39	0.57	}
(3c)	CCl <sub>3</sub> -CH <sub>2</sub>	2	43	0.20	0.42	
(3d)	н	0				0.35
(3e)	н	1				0.25
(3f)	н	2				0.19
5a)	CCl <sub>3</sub> -CH <sub>2</sub>	0	42	0.53	0.66	
5b)	CCl <sub>3</sub> -CH <sub>2</sub>	1	40	0.31	0.55	
5c)	CCl <sub>3</sub> -CH <sub>2</sub>	2	43	0.18	0.45	
(5d)	н	0				0.33
(5e)	н	1		1		0.27
(5f)	н	2				0.23

[a] Calculated on the total oligonucleotide used.

[b] A: Ethanol/I M ammonium acetate (7:3).

B: 2-Propanol/conc. NH<sub>3</sub>/water (7:1:2).

C: 1-Propanol/conc. NH<sub>3</sub>/water (55:10:35).

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 [Z 250 IE]

 German version: Angew. Chem. 78, 682 (1966)

[1] H. G. Khorana, T. M. Jacob, M. W. Moon, S. A. Narang, and E. Ohtsuka, J. Amer. chem. Soc. 87, 2954 (1965).

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[3] E. LeGoff, J. org. Chemistry 29, 2048 (1964).

## Osazone Formation by D-Glucosamine

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The literature and textbooks express the view, based on experiments by F. Tiemann<sup>[1]</sup>, that D-glucosamine gives D-phenylglucosazone analogously to D-glucose. As there is a close relation between the mechanism of the Amadori rearrangement and formation of osazones by aldose we have re-examined the behavior of D-glucosamine towards phenyl-hydrazine.

We have established that on complete exclusion of atmospheric oxygen D-glucosamine does not form D-glucosazone, but only a phenylhydrazone (m.p. 118-120 °C,  $[\alpha]_D^{25} = -13.2$ ° (after 10 min)  $\rightarrow -72.3$ ° (c = 1, water)]. This hitherto unknown substance was separated by way of the N-tosyl derivative. It can be obtained from the components in good yield without addition of acid. The N-formyl, N-acetyl, N-benzyl-oxycarbonyl, and N-tosyl derivatives of D-glucosamine also form the phenylhydrazone, with loss of the acyl group attached to the N atom. From the N-tosyl derivative, N-tosyl-derivative, N-tosyl-derivative, N-tosyl-derivative, N-tosyl-derivative, N-tosyl-derivative, N-tosyl-derivative, N-tosyl-derivative, N-tosyl-derivative.

In the presence of atmospheric oxygen, however, p-glucosazone is formed, the yield depending on the amount of oxygen admitted. Our maximal yield was 61%. Clearly a dehydrogenation by the oxygen, and probably also loss of ammonia from an imino group, must occur before entry of the second phenylhydrazine residue.

The fact that D-glucosamine does not form an osazone in the absence of oxygen is in agreement with the behavior of its *N*-glycosyl derivatives. In no case is it possible to induce the *N*-glycosyl derivative to undergo the Amadori rearrangement <sup>[2]</sup>. Since osazone formation is preceded by an Amadori rearrangement it is understandable that there can be no osazone formation from D-glucosamine in the absence of oxygen. Received: May 27th, 1966 [Z 251 IE]

German version: Angew. Chem. 78, 681 (1966)

[1] F. Tiemann, Ber. dtsch. chem. Ges. 19, 50 (1886).

[2] K. H. Heinemann, Dissertation, Universität Münster, 1966; K. H. Schwieger, Diploma Thesis, Universität Münster, 1959.

## Reactions of N-Alkyl- and N-Aryl-S-chloroisothiocarbamoyl Chlorides with Isocyanates; Synthesis of 1,2,4-Thiadiazolidine-3,5-diones<sup>[1]</sup>

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*N*-Alkyl and *N*-aryl-*S*-chloroisothiocarbamoyl chlorides(1)<sup>[2]</sup> react with aliphatic and aromatic isocyanates to form *S*-(chlorocarbonylamino)isothiocarbamoyl chlorides (2). The weakly exothermic reaction proceeds quantitatively below 60 °C according to IR-spectroscopy, but is reversible at elevated temperatures.

$$R-N=C \begin{pmatrix} SC1 \\ C1 \end{pmatrix} + \begin{pmatrix} N-R' \\ C=O \end{pmatrix} & \hline S0^{\circ}C \end{pmatrix} \qquad R-N=C \begin{pmatrix} S-N-R' \\ O \\ C1 \end{pmatrix} & \hline C1 \end{pmatrix}$$

(2), R	R'	M.p. [ °C]	Yield [%]
C <sub>2</sub> H <sub>5</sub>	n-C <sub>8</sub> H <sub>17</sub>	132-133	77
n-C4H9	n-C4H9	133-134	85
n-C₄H9	n-C <sub>8</sub> H <sub>17</sub>	114	65
$C_6H_5$	C <sub>2</sub> H <sub>5</sub>	108-109	90
$C_6H_5$	i-C <sub>3</sub> H <sub>7</sub>	101-102	78
C <sub>6</sub> H <sub>5</sub>	n-C <sub>4</sub> H <sub>9</sub>	97—99	86
$C_6H_5$	n-C <sub>8</sub> H <sub>17</sub>	92-93	68
C <sub>6</sub> H <sub>5</sub>	C <sub>6</sub> H <sub>5</sub>	7980	97
C <sub>6</sub> H <sub>5</sub>	CH3·C6H4	95-96	91
$C_6H_5$	p-Cl·C <sub>6</sub> H <sub>4</sub>	72-73	95
$C_6H_5$	p-CH <sub>3</sub> OC <sub>6</sub> H <sub>4</sub>	85-92	98
$C_{6}H_{11}$	i-C <sub>3</sub> H <sub>7</sub>	131-132	55

The structure of the products (2) is supported by elemental analysis, molecular-weight determination, IR spectra, and their reactions. The infrared spectrum is void of the characteristic isocyanate absorption around 2270 cm<sup>-1</sup> but features absorptions at 1760 cm<sup>-1</sup> and 1670 cm<sup>-1</sup>, which indicate the presence of a C=O group and a C=N linkage.