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SOLUTION SYNTHESIS OF FULLY PROTECTED THYMIDINE DIMERS USING VARIOUS PHOSPHORAMIDITES

MICHAEL W. SCHWARZ AND WOLFGANG PFLEIDERER *
Fakultät für Chemie, Universität Konstanz, Postfach 5560, D-7750 Konstanz/W. Germany

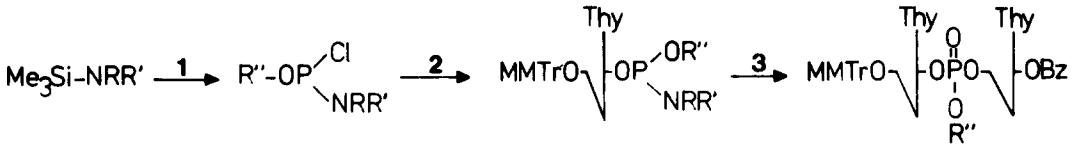
The synthesis of thymidine-3'-phosphoramidites of various amines and their use in the solution synthesis of fully protected thymidine dimers in high yields is described.

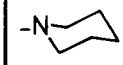
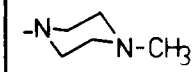
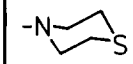
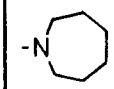
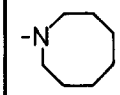
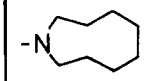
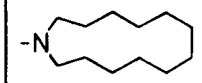
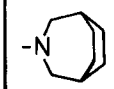
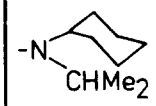
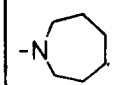
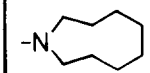
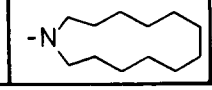
Since M.H. Caruthers [1] has reported the use of methyl-N,N-dimethylamino-phosphoramidites in oligodeoxynucleotide chemistry, several publications have broadened the scope of this method. Although these compounds have been used with good results in oligodeoxynucleotide [2-4] and oligoribonucleotide chemistry [5-7], their general instability led to investigations of derivatives with modified amidite moieties. Independently, M.H. Caruthers and S.P. Adams reported the methyl-N-morpholino-phosphoramidites [8] and methyl-N,N-diisopropyl-phosphoramidites [8,9] of the common base-protected deoxynucleosides as the components of choice applied recently more general in oligodeoxynucleotide chemistry [3, 10-15].

Several investigators also changed the ester moiety of the phosphoramidites in order to improve deblocking of the P-O protecting group. Until now there have been used the o-chlorophenyl [16], the 2-cyanoethyl [17], the 2-methylsulfonylethyl [18] and the 1,1-dimethyl-2-cyanoethyl-group [19]. Although all these compounds, when used in solid support synthesis, led to condensation yields exceeding 95 %, the few examples applied to solution chemistry resulted in unsufficient low internucleotidic bond formation of 60 % [7] and 65 % [18], respectively.

The good features of p-nitrophenylethyl-N-morpholino-phosphoramidites in solution synthesis [20] prompted us to improve the condensation yields comparable to those in solid support synthesis in using preferentially p-nitro-phenylethyl-phosphoramidites of higher membered cyclic amines.

The synthesis of the deoxynucleoside phosphoramidites 27-39 afforded first the preparation of various silylamines 4-13, of which 4 [21], 5 [21], 6 [22] and 7 [21] are known already. Since other amines do not react easily with trimethylchlorosilane at r.t. the method of L. Tansjö [23] was applied treating first with methylmagnesiumiodide and followed by trimethylchlorosilane. After reflux for 48 hours the trimethylsilylamines 8-11, 13 were isolated in good yields by distillation. 3-Azabicyclo(3.2.2.)nonane was silylated by refluxing in an excess of hexamethyldisilazane, using a catalytic amount of ammonium



	-NRR'		R'' = CH_3						
			Yield	^{31}P-NMR::		Yield	^{31}P-NMR::	Ratio:::	Yield
4		14	76	182.34	27	95	143.30/143.99	6/2/1	48
5		15	55	172.2	28	94	143.88/144.53	6/2/1	37
6		16	57	177.27	29	96	143.55/144.49	4/2/1	37
7		17	47	177.65	30	92	145.72/146.05	4/2/1	44
8		18	84	186.62	31	84	149.07/149.6	5/1.5/1	82
9		19	76	184.9	32	87	151.11/151.85	5/1.5/1	80
10		20	89	188.01	33	82	152.95/153.73	5/1.4/1	81
11		21	83	183.67	34	76	150.09/150.79	5/1.4/1	79
12		22	73	177.65	35	74	146.77/147.49	5/1.4/1	78
13		23	78	184.54	36	85	148.78/149.36	5/1.4/1	84
			R''=CH_2CH_2-Ø-NO_2						
8		24	92	177.71	37	74	147.60/148.05	5/1.5/1	92
10		25	89	184.46	38	72	151.44/151.89	5/1.4/1	93
11		26	82	182.14	39	70	148.75/149.28	5/1.4/1	93

MMTr = Monomethoxytrityl; Bz = Benzoyl; Thy = Thymine. : = ^{31}P-NMR spectra were recorded on a Bruker HX-90 and Bruker WP-80 spectrometer in $CDCl_3$ or $CHCl_2$; values in ppm.
::: = Molar ratio of tetrazole/thymidine-3'-phosphoramidite/3'-O-benzoyl-thymidine.

sulfate to yield 73 % of 22.

The trimethylsilylamines 8-13 were added dropwise under N_2 at 0°C to methoxydichlorophosphine (1) [24] (1:3 mol ratio) and then the mixture slowly warmed up to r.t.. After 2 hours of additional stirring all volatile components were evaporated and the residue distilled in high vacuum in a Kugelrohr apparatus obtaining the methylphosphoramidites 14-23 in good yields. 14 gave only satisfactory results after prolonged reaction times. The same scheme led to the p-nitrophenylethyl-phosphoramidites 24-26 starting from p-nitrophenylethoxy-dichlorophosphine [25]. These products could, however, not be distilled without decomposition but were ^{31}P NMR spectroscopically pure simply after high vacuum evaporation of all volatile components.

The 5'-O-monomethoxytritylthymidine-3'-phosphoramidites 27-39 were prepared according to the literature [8,10]. The various phosphoramidites 14-26 were added in about 1.6 molar excess to a solution of 5'-O-monomethoxytritylthymidine (2) in dry methylene chloride via a syringe under N_2. As acid scavenger a fourfold molar excess of N,N,N-diisopropyl-ethylamine was used. After work-up the 5'-O-monomethoxytritylthymidine-3'-phosphoramidites 27-30 were precipitated from toluene solution into pentane and collected by filtration. The derivatives 31-39 can be purified chromatographically on silica gel using ethyl acetate/triethylamine (95/5) and yielded on evaporation colourless foams which are stable to normal laboratory conditions for months and appear to be essentially homogenous based on ^{31}P NMR data.

In order to synthesize the thymidine dimers, 3'-O-benzoylthymidine (3) and one of the 5'-O-monomethoxytritylthymidine-3'-phosphoramidites 27-39 (1:1.4-2 mol ratio) are combined in a serum capped 5 ml flask and dried in high vacuum, usually over night. The same procedure is appliedto a fivefold molar excess of 1H-tetrazole. The tetrazole is then dissolved in freshly distilled dry acetonitrile and added to the reactants via a syringe under N_2. After ten minutes stirring at r.t. the resulting phosphitetriester is oxidized with iodine in the usual manner [1]. The solution is then transferred with 10 ml of chloroform to a separatory funnel and washed twice with 40 ml of a saturated sodium thiosulfate solution to reduce excess iodine. The organic layer is dried over anhydrous $MgSO_4$ and coevaporated twice with 15 ml toluene. The product is isolated by preparative thin layer chromatography on a silica gel plate (40x20x0.2 cm) and developed with chloroform/methanol (95:5 v/v).

The results show that the condensations in solution work especially well and exceed a 90 % yield when the p-nitrophenylethyl-phosphoramidites of hexahydroazepine(24), octahydroazonine(25), and azacyclotridecane (26) respectively have been used. These compounds also can be purified by silica gel chromatography and can be stored as stable foams at r.t. for months. We believe these compounds to be rather useful synthons in large scale synthesis of oligodeoxyribonucleotides, where low excesses of phosphoramidites are desirable. The extension of this method to the other deoxynucleosides is now under investigation.

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