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NEW CONDENSING REAGENTS FOR STEREOSPECIFIC SYNTHESIS OF DINUCLEOSIDE MONOPHOSPHATE ARYL ESTERS

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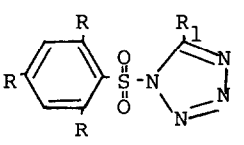
Summary. Condensation of 5'-O-dimethoxytritylnucleoside 3'-O-(o-chlorophenyl) phosphates and 3'-O-benzoylnucleosides with a new condensing reagent, 2,4,6-triisopropylbenzenesulfonyl 5-(pyridin-2-yl) tetrazolide gave o-chlorophenyl ester of protected dinucleoside monophosphates which had a stereospecific configuration. The corresponding mesitylenesulfonyl derivative gave similar results.

Various approaches for the synthesis of oligonucleotides by the phosphotriester method have been developed by several investigators.[1] Powerful activating reagents for phosphodiesters such as mesitylenesulfonyl tetrazolide[2] and mesitylenesulfonyl nitrotriazolide (MSNT)[3] facilitated the condensation of diesterified nucleotides. Triester intermediates thus obtained can be purified by chromatography on silica gel. However, the diastereoisomers on each phosphate disturb the separation of products in some cases.[4] We wish to report in this communication that new condensing reagents mesitylenesulfonyl 5-(pyridin-2-yl) tetrazolide (MSPy)(1a)(Chart 1) and 2,4,6-triisopropylbenzenesulfonyl 5-(pyridin-2-yl) tetrazolide (TPSPy)(1b)yield stereospecific products in the synthesis of phosphotriesters. MSPy and TPSPy were prepared by treatment of the arenesulfonyl chloride with 5-(pyridin-2-yl)tetrazole[5] in the presence of triethylamine in dioxane at room temperature for 1 hr. Mesitylenesulfonyl 5-phenyltetrazolide (MSPh)(1c) was prepared similarly. Condensation of $(MeO)_2$TrTp(o-ClC_6H_4)(2a)[6] and T(Bz)(3a)[7] with TPSPy gave a single spot in thin layer chromatography (TLC) on silica gel, while the same condensation using MSNT gave two diastereoisomers which were separated by TLC. These results are summarized in Table I together with a few other reactions involving TPSPy and MSPy. Condensations of diesterified nucleotides (2) and two diastereoisomers of protected nucleotides (5) with MSPy or TPSPy yielded protected dGpGp or dCpCp (6) which migrated as two spots in TLC. These results are also included in Table I. All dimers obtained by using MSPy or TPSPy except for protected dGpG showed slower mobility than the other diastereoisomer. The mechanism of stereospecific formation of the triesters (4 and 6) is to be investigated and absolute configuration[8] of diastereomers has to be determined by NMR spectroscopy. It is assumed that the pyridinium cation on the tetrazole interacts with the phosphate anion of phosphodiesters (2). Effects of bulkiness of the pyridine ring on the tetrazole in the stereospecific synthesis can be excluded by the fact that MSPh (1c) gave two diastereoisomers in the synthesis of protected GpG (4).



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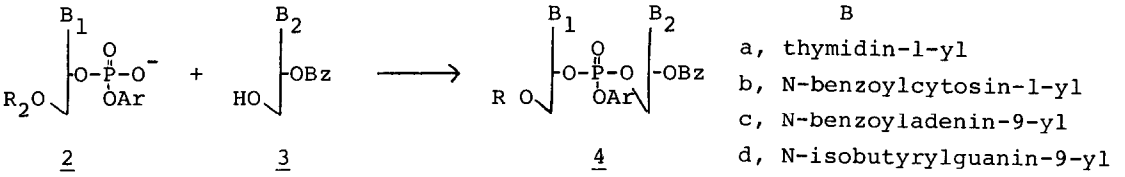
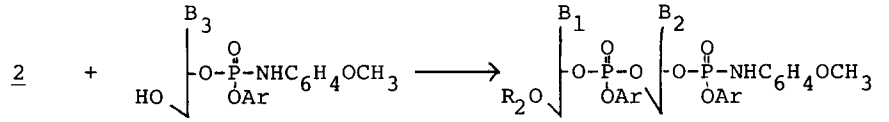
	R	R_1
a,	Me	pyridin-2-yl (MSPy)
b,	Ip	pyridin-2-yl (TPSPy)
c,	Me	phenyl (MSPh)



2 3 4

B
a, thymidin-1-yl
b, N-benzoylcytosin-1-yl
c, N-benzoyladenin-9-yl
d, N-isobutyrylguanin-9-yl
R_2 = dimethoxytrityl
Ar = o-chlorophenyl



5 6

Chart 1

Products (4, 6) were deblocked by treatment with concentrated ammonia followed by treatment with 80% acetic acid. The structure of the two diastereoisomers of protected TpT and that of the product obtained by using TPSPy were confirmed by characterizing the deblocked product. All products from 2a and 3a gave TpT after deblocking, which was hydrolyzed with nuclease P1 to give thymidine and thymidine 5'-phosphate. Rf values and relative mobilities in paper chromatography and paper electrophoresis of these compounds are listed in Table II. Protected dGpG obtained either by using MSPy or MSPh under the conditions shown in Table I gave dGpG after deblocking. Protected dGpGp and dCpCp (6) were characterized by the similar procedure: treatment with ammonia followed by treatment with 80% acetic acid yielded 3'-phosphoro-p-anisidate of dGpG and that of dCpC. These dinucleotides were also hydrolyzed with nuclease P1 to give the nucleoside and 3'-phosphoro-p-anisidate of nucleoside 5'-phosphate. A part of the anisidate of dinucleotides was hydrolyzed during treatment with 80% acetic acid. dGpGp and dCpCp thus obtained were also characterized by the enzymatic hydrolysis.

Table I. Reaction conditions for condensations

3'-Diester component (mmol)	5'-Hydroxyl component (mmol)	Reagent (mmol)	Pyridine (ml)	Reaction time (min)	Product[a] (mmol)	Yield[b] (%)	Rf, TLC ($CHCl_3$:MeOH)
2a	3a	TPSPy	1	30	TpT	93	0.42
(0.283)	(0.257)	(0.573)			(0.240)		(20:1)
2a	3a	MSNT	2	30	TpT	92	0.42, 0.47
(0.424)	(0.386)	(0.857)			(0.354)		
2a	3d	TPSPy	1	10	dTpG	86	0.38
(0.044)	(0.040)	(0.086)			(0.034)		(20:1)
2c	3d	TPSPy	1	10	dApG	92	0.49
(0.044)	(0.040)	(0.089)			(0.037)		(10:1)
2d	3d	MSPy	2	90	dGpG	59	0.26
(0.50)	(0.42)	(1.00)			(0.246)		(15:1)
2d	3d	MSPh	2	90	dGpG	64	0.21, 0.26
(0.50	(0.42)	(1.00)			(0.267)		
2d	5d	MSPy	1	5	dGpGp	74	0.29, 0.42
(0.050)	(0.045)	(0.100)			(0.033)		(10:1)
2d	5d	MSNT	2	30	dGpGp	80	0.29, 0.33
(1.00)	(0.899)	(2.00)			(0.715)		0.42, 0.48
2b	5b	TPSPy	1	15	dCpCp	93	0.29, 0.42
(0.112)	(0.094)	(0.209)			(0.087)		(20:1)
2b	5b	MSNT	2	15	dCpCp	84	0.29, 0.42
(0.498)	(0.415)	(1.11)			(0.349)		0.37, 0.40

a) Protecting groups of dimers (4, 6) are omitted.

b) Yields were estimated by measuring the weight of precipitated products.

Table II. Paper chromatography and paper electrophoresis

Compound	Paper chromatography ($iPrOH-NH_4OH-H_2O$, 7:1:2)	Paper electrophoresis (Triethylammonium bicarbonate, 50 mM, pH 7.5)
	Rf	Rm
T	0.65	0.02
dC	0.60	-0.14
dA	0.63	-0.26
dG	0.45	0.00
pT	0.21	0.98
dpC	0.16	1.18
dpA	0.15	0.88
dpG	0.09	1.00
dTpT	0.45	0.43
dTpG	0.42	0.41
dGpG	0.16	0.32
dApG	0.51	0.41
dCpCpanisidate	0.13	0.94
dCpCp	0.06	1.11
dGpGpanisidate	0.16	0.61
dGpGp	0.03	0.99
dpGp		1.27
dpGpanisidate		1.26

References

1. C.B. Reese, Tetrahedron, 34, 3143 (1978); M. Ikehara, E. Ohtsuka and A.F. Markham, Adv. Carbhyd. Chem. Biochem., 36, 135 (1979)
2. J. Stawinski, T. Hozumi, S.A. Narang, C.P. Bahl and R. Wu, Nucleic Acids Res., 4, 353 (1977)
3. C.B. Reese, R.C. Titmas and L. Yau, Tetrahedron Lett. 2727 (1978)
4. E. Ohtsuka, K. Fujiyama and M. Ikehara, Nucleic Acids Res., 9, 3503 (1981)
5. B. Brauwer-van Straaten, D. Solinger, C. van de Westeringh and H. Veldstrc, Rec. Trav. Chim., 77, 1129 (1958)
6. E. Ohtsuka, S. Shibahara, T. Ono, T. Fukui and M. Ikehara, Heterocycles, 15, 395 (1981)
7. H. Schaller, G. Weimann, B. Lerch and H.G. Khorana, J. Am. Chem. Soc., 85, 3821 (1963)
8. P.M.J. Burgers and F. Eckstein, Biochemistry, 18, 592 (1979)

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