INTERNATIONAL ATOMIC ENERGY AGENCY
UNITED NATIONS EDUCATIONAL, SCIENTIFIC AND CULTURAL ORGANIZATION

INTERNATIONAL CENTRE FOR THEORETICAL PHYSICS
34100 TRIESTE (ITALY) - P.O.B. 586 - MIRAMARE - STRADA COSTIERA 11 - TELEPHONES: 224281/2/3/4/5/6
CABLE: CENTRATOM - TELEX 460392-1

SMR/111 - 19

SECOND SUMMER COLLEGE IN BIOPHYSICS

30 July - 7 September 1984

Lecture 1. DNA Synthesis in Solution

Lecture 2. RNA Synthesis and the Introduction of Terminal Phosphates

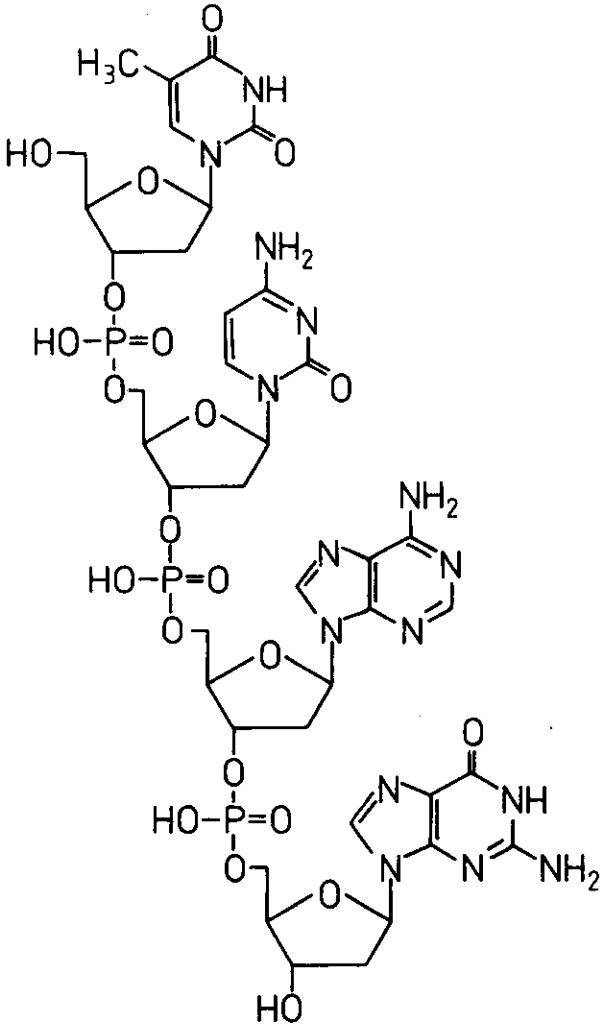
Lecture 3. DNA Synthesis on a Solid Support

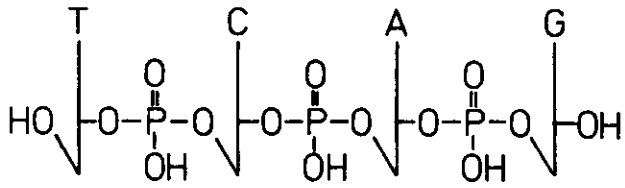
G.A. VAN DER MAREL
Gorlaeus Laboratories
Department of Chemistry
University of Leiden
2300 RA Leiden
The Netherlands

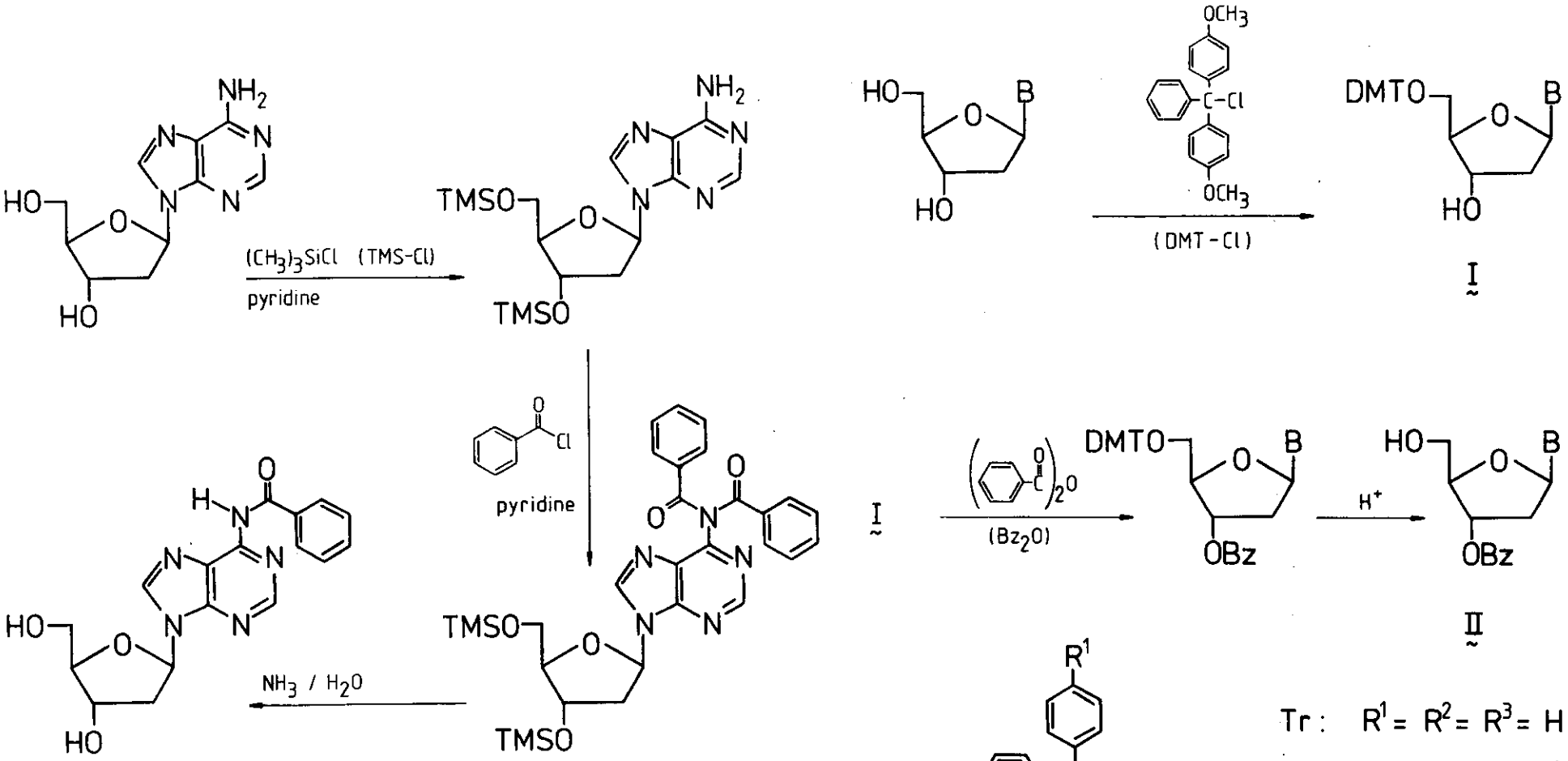
REVIEWS Nucleic Acid Chemistry

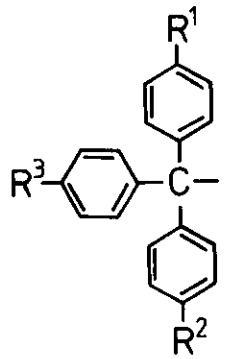
1. J.H. VAN BOOM, Heterocycles, 7, 1197 (1977).
2. C.B. Reese, Tetrahedron, 34 3143 (1978).
3. S.A. Narang, R. Brousseau, H.M. Hsiung, J.J. Michniewicz, Methods Enzymology 65 610 (1980).
4. E. Ontsuka, M. Ikehara, D. Söll, Nucleic Acids Res. 10 6553 (1982)
5. Chemical and Enzymatic Synthesis of gene fragments Edited by H.G. Gassen, A Lang. Verlag Chemie Weinheim (1982)
6. Recent aspects of chemistry of nucleosides, nucleotides, and nucleic acids Edited by C.B. Reese Tetrahedron symposia-in-print number 13 Tetrahedron 40 3-163 (1984)

Lecture 1





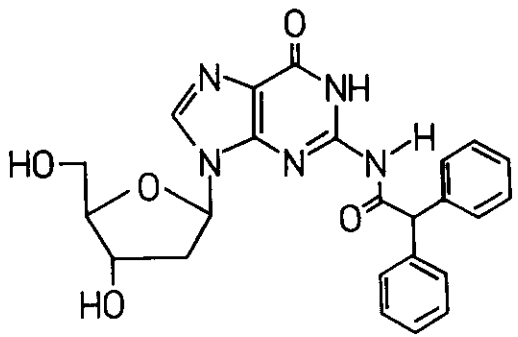


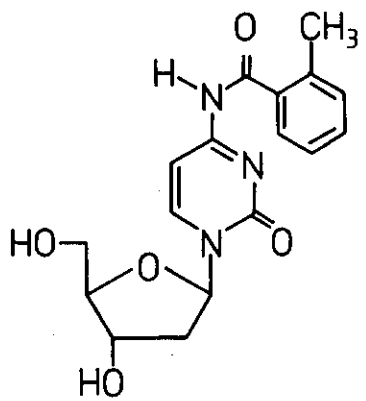


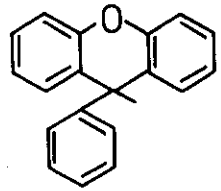
Tr : $R^1 = R^2 = R^3 = H$

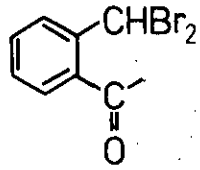
MMT : $R^1 = OCH_3$ $R^2 = R^3 = H$

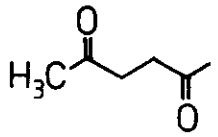
DMT : $R^1 = R^2 = OCH_3$ $R^3 = H$

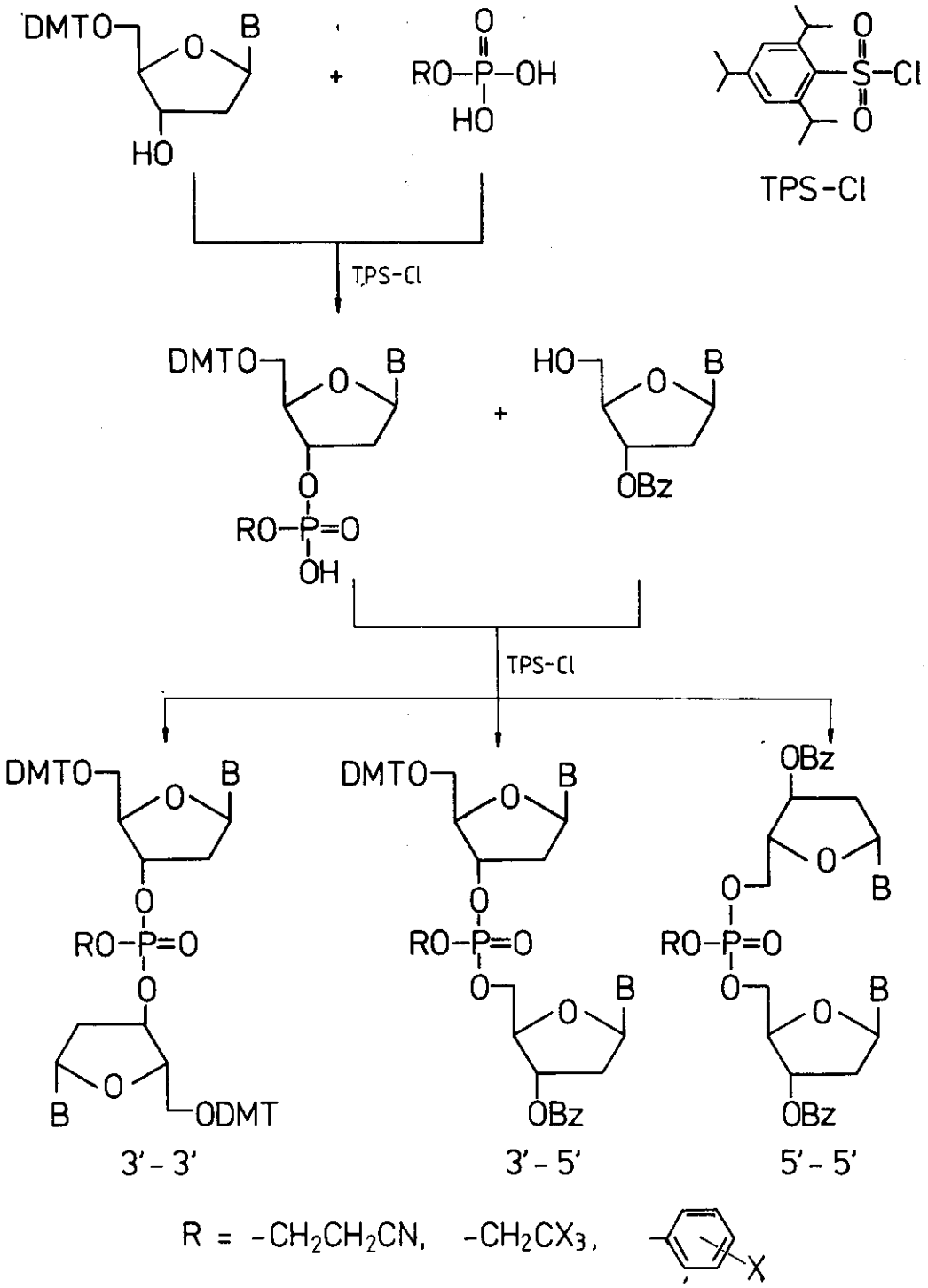




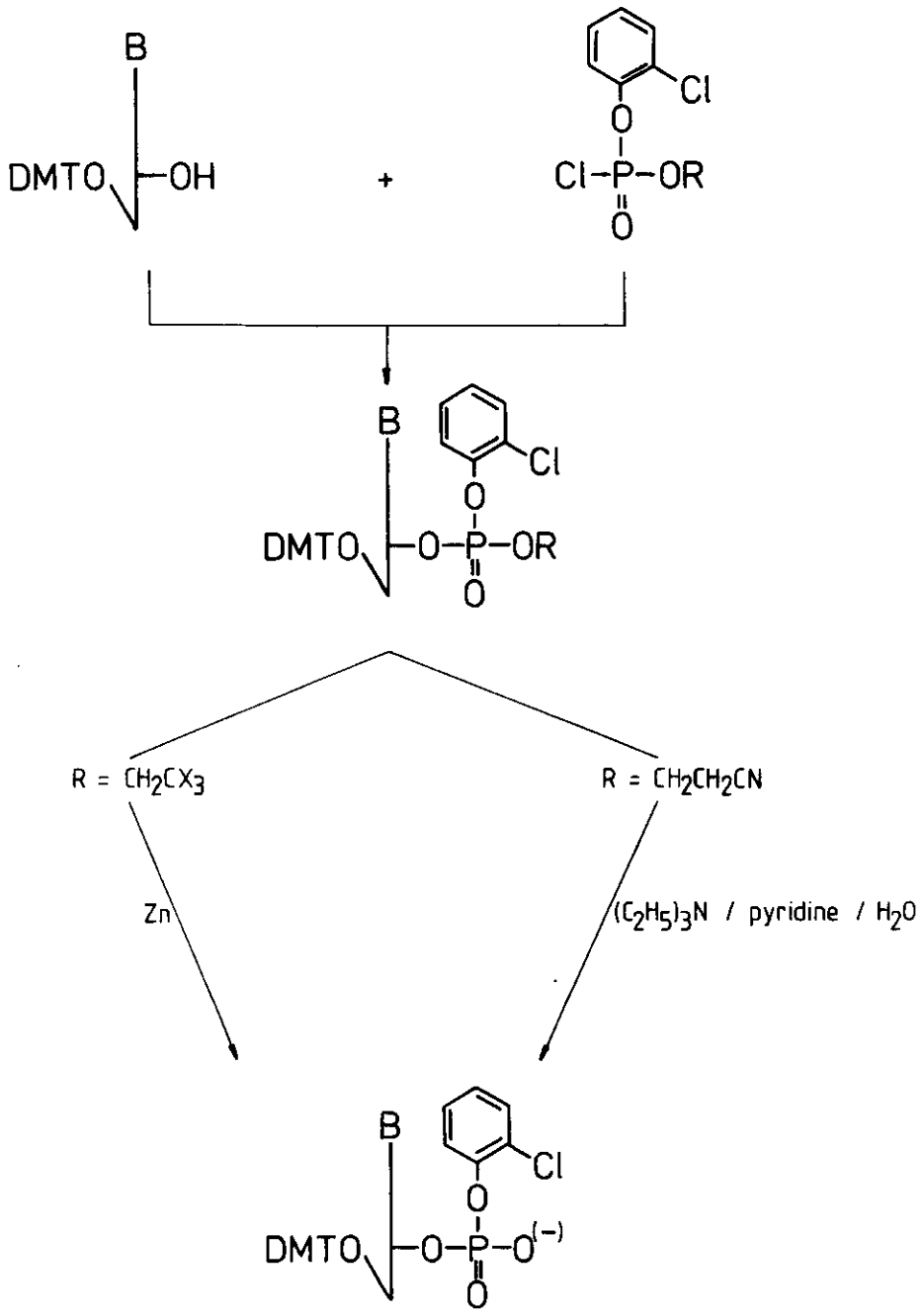




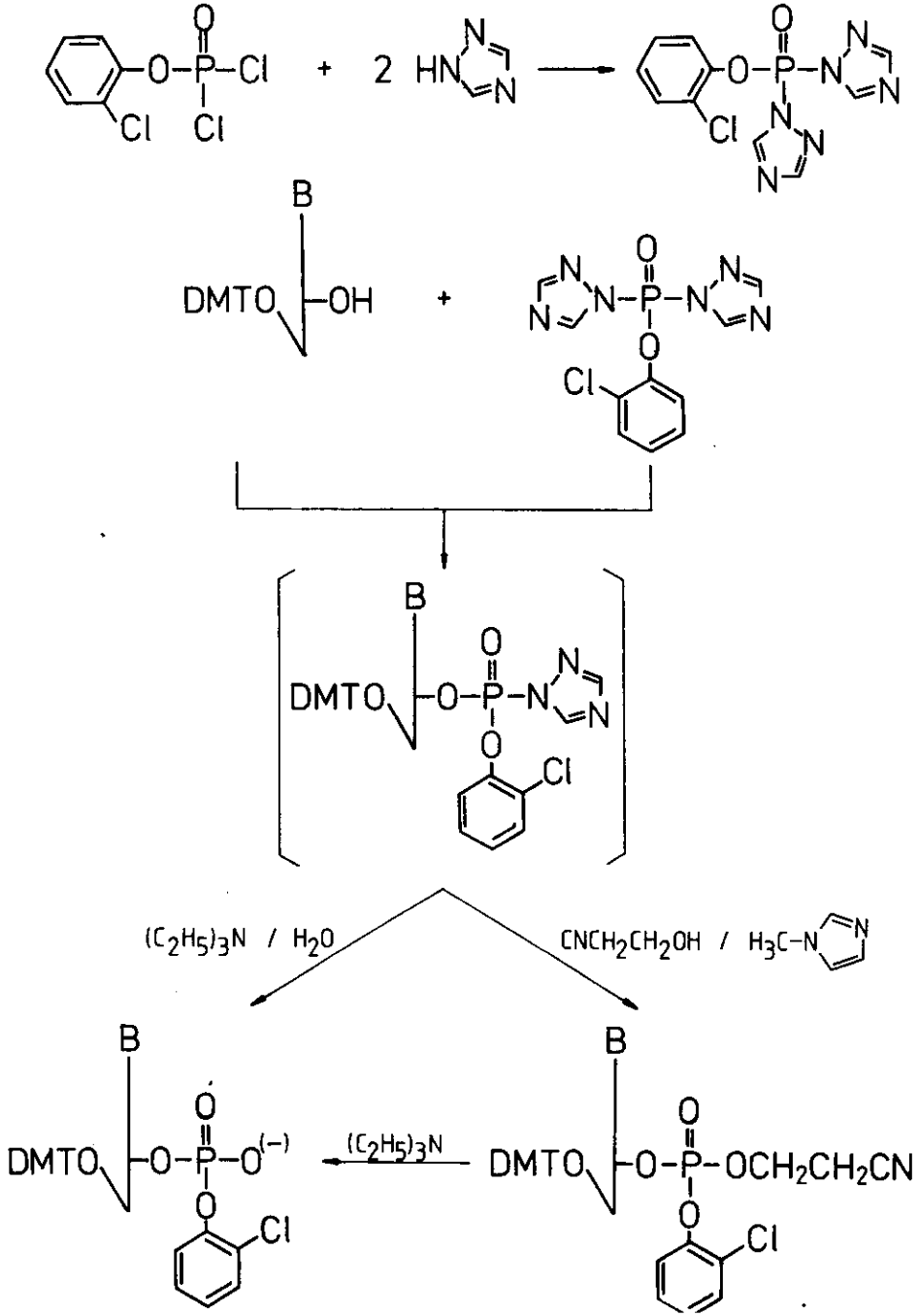




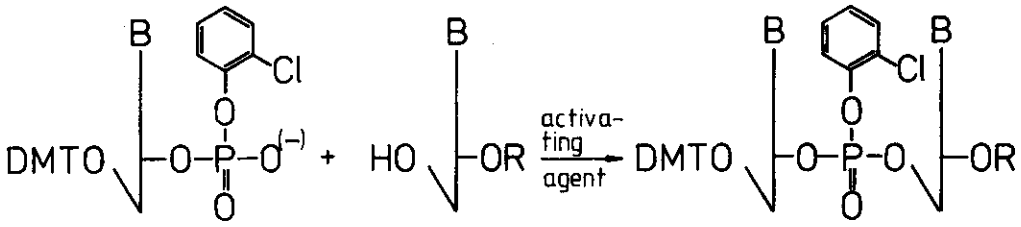
Monofunctional phosphorylating agents

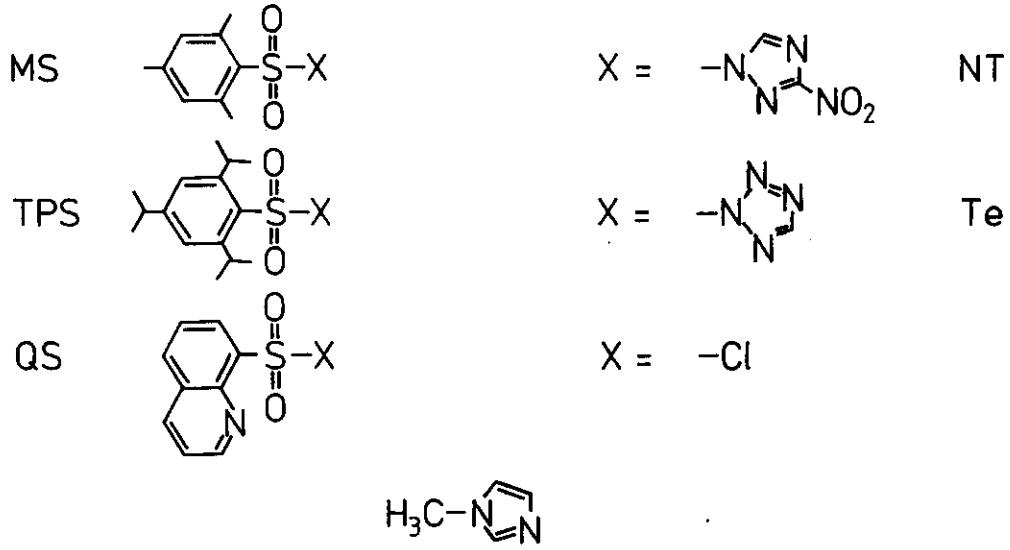


Ditriazolide method

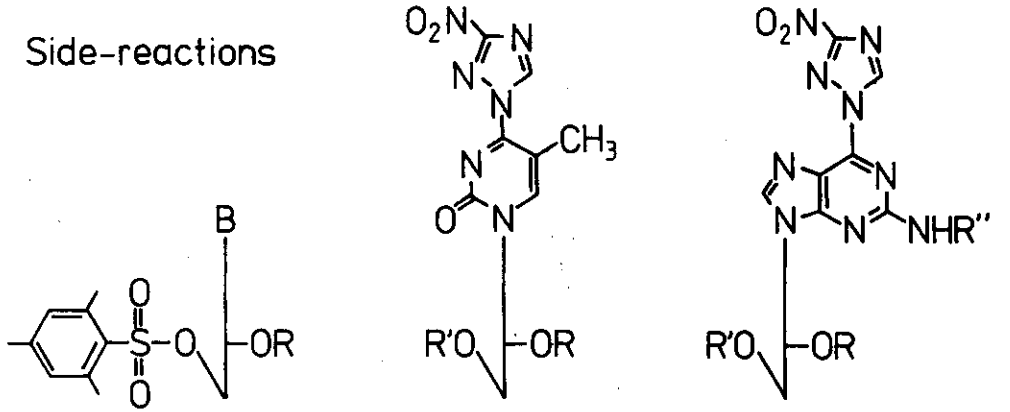


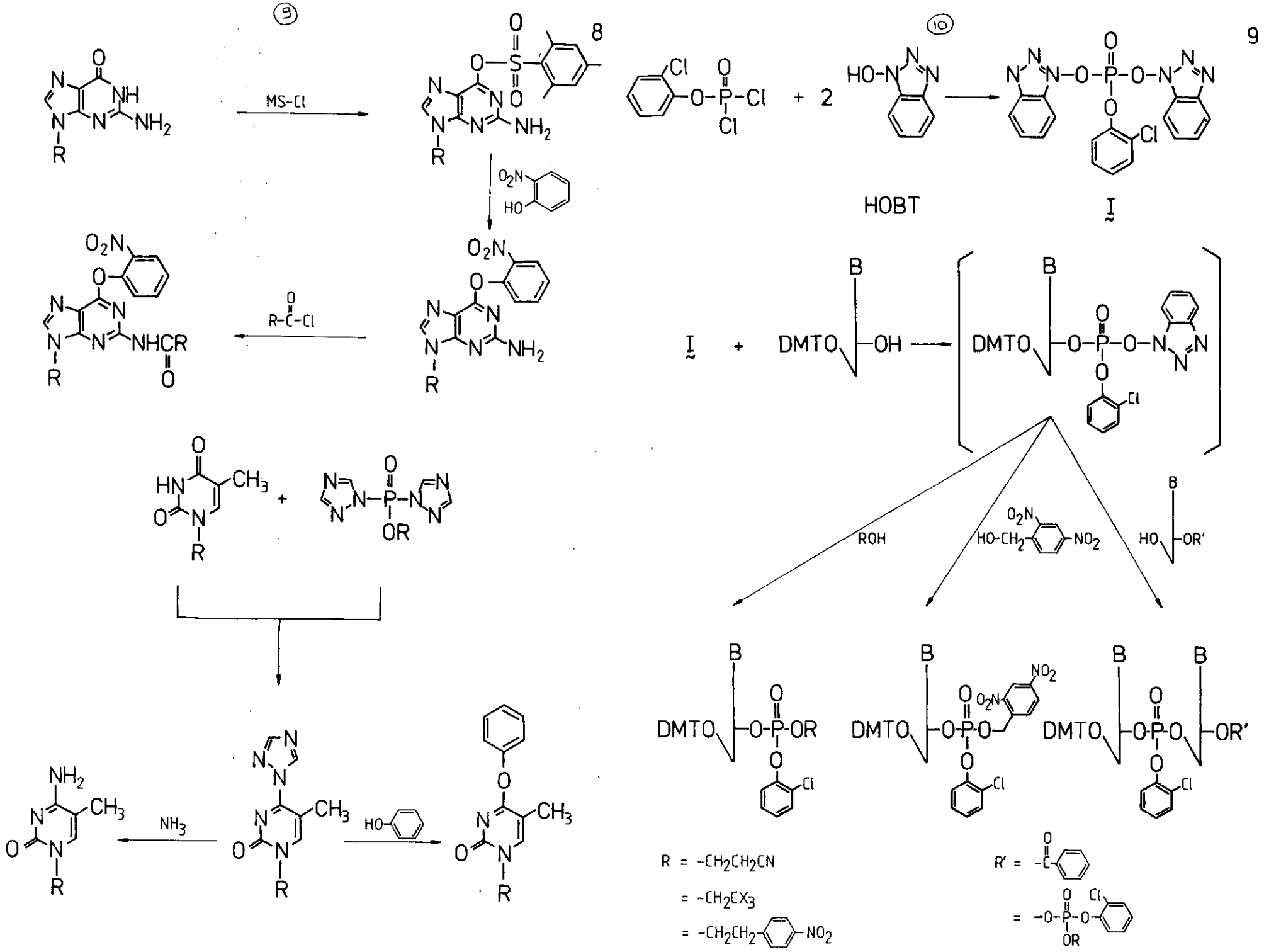
Condensation

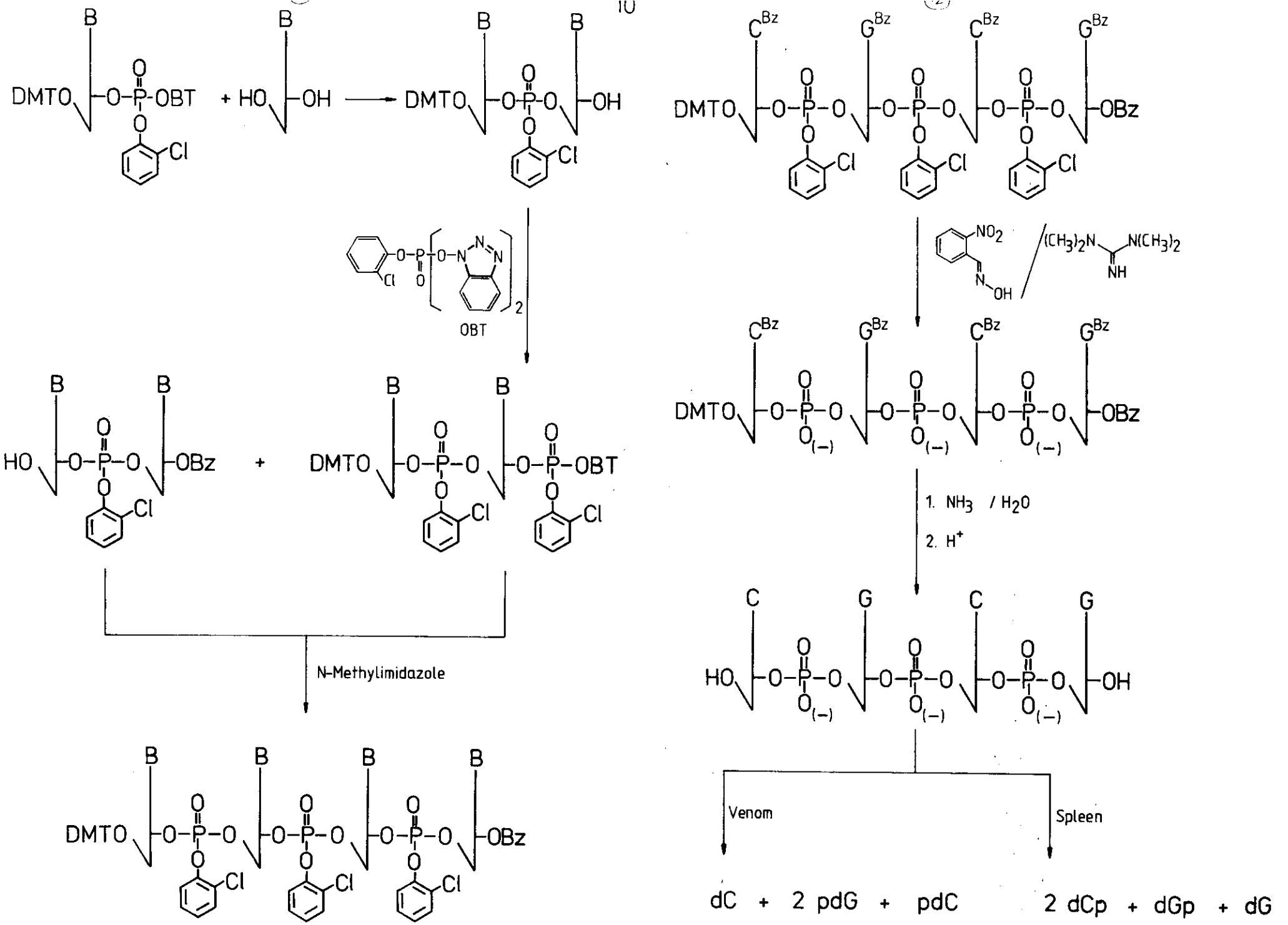




Side-reactions

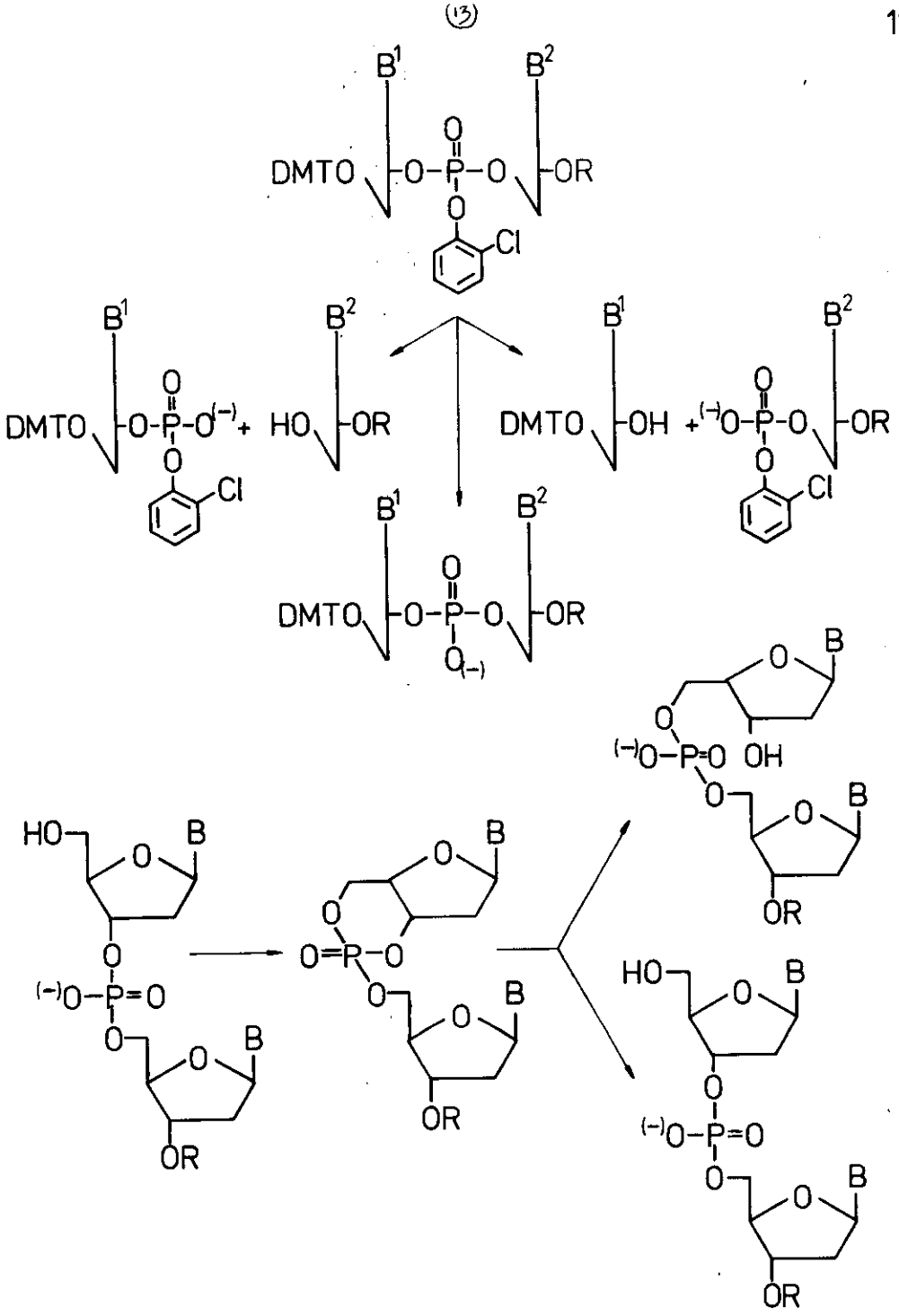



⑨
MS-Cl
R-C-Cl
NH3
HO
⑩
HOBT
I
ROH
DMTO
R = -CH2CH2CN
= -CH2CX3
R' =


DMTO
OBT
HO
OH
Cl
OBT
N-Methylimidazole
OBz

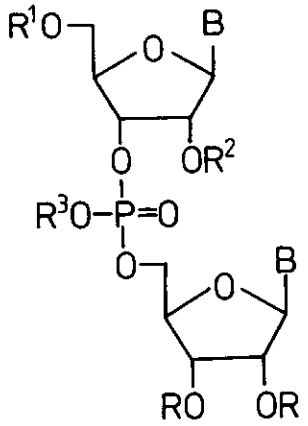
DMTO
OBz
NO2
OH
(CH3)2N
N(CH3)2
NH
1. NH3 / H2O
2. H+
HO
OH
Venom
Spleen
dC + 2 pdG + pdC
2 dCp + dGp + dG

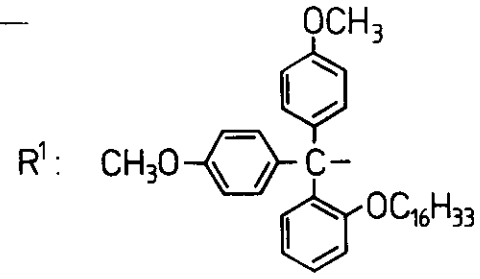
(13)

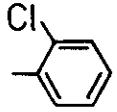


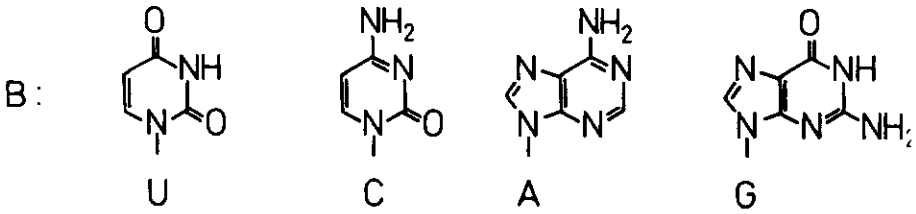
Lecture 2

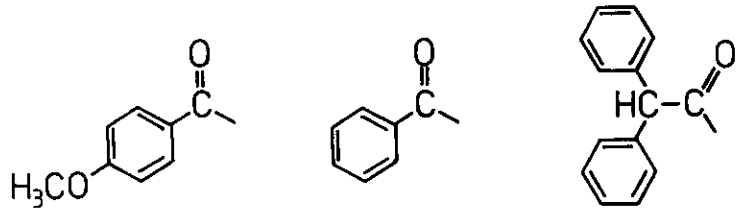
(14)

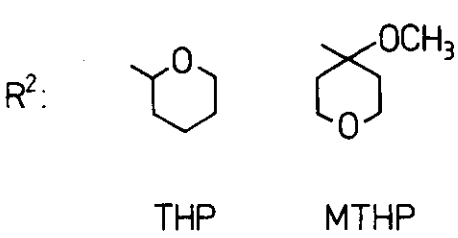


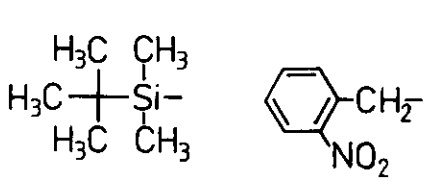


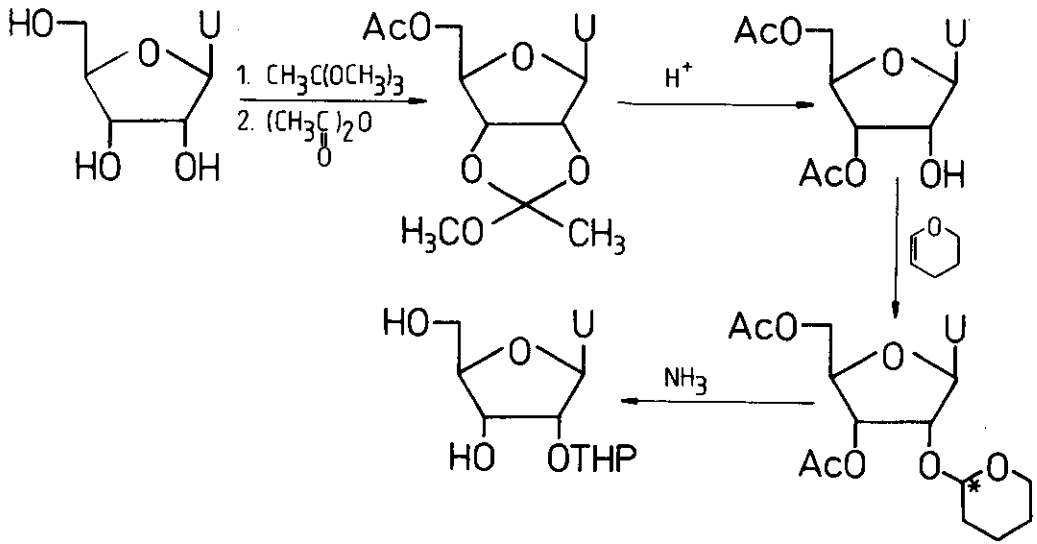


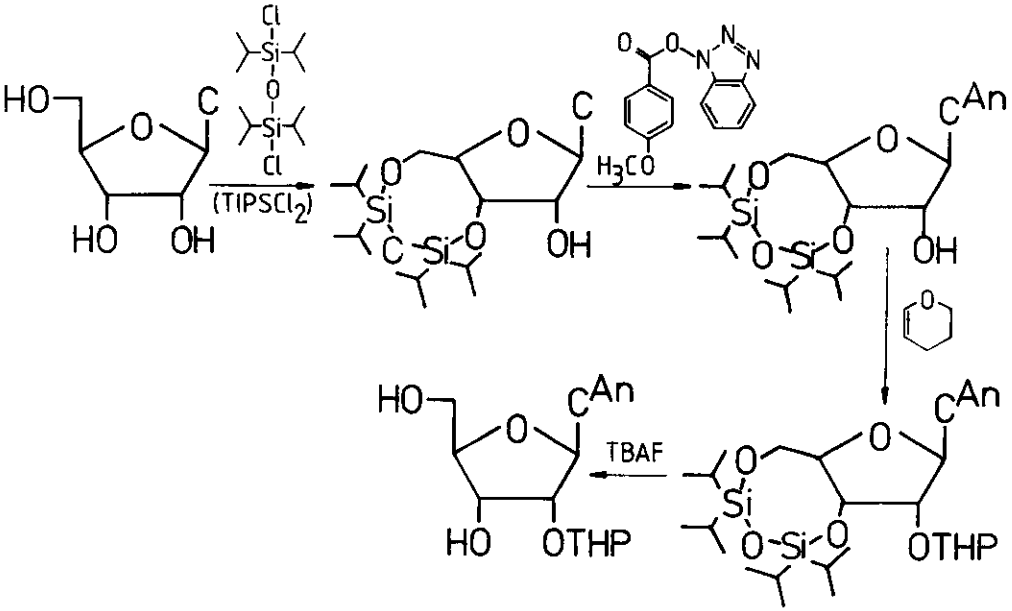


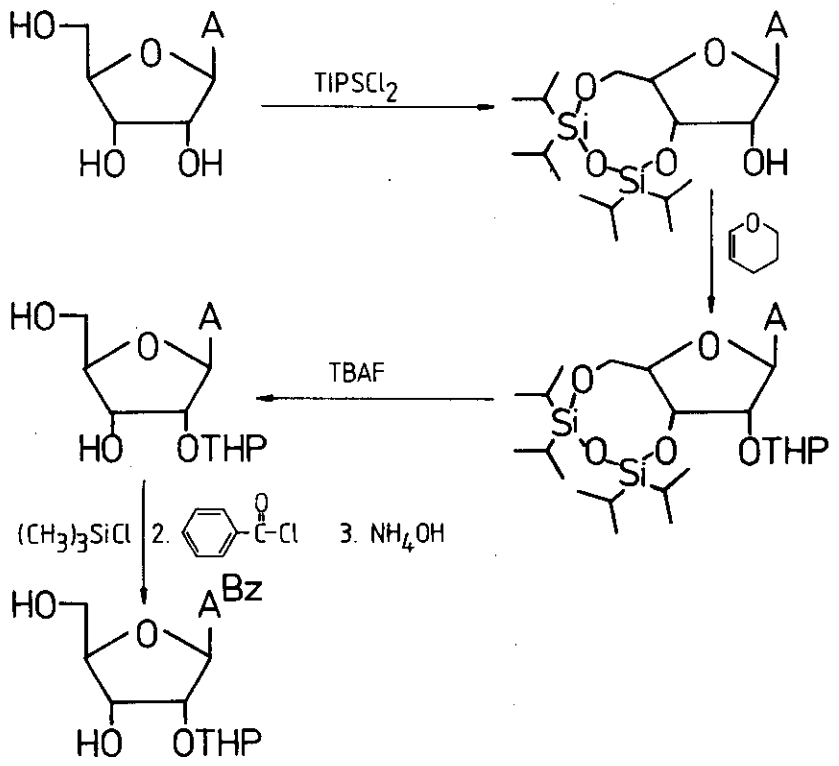


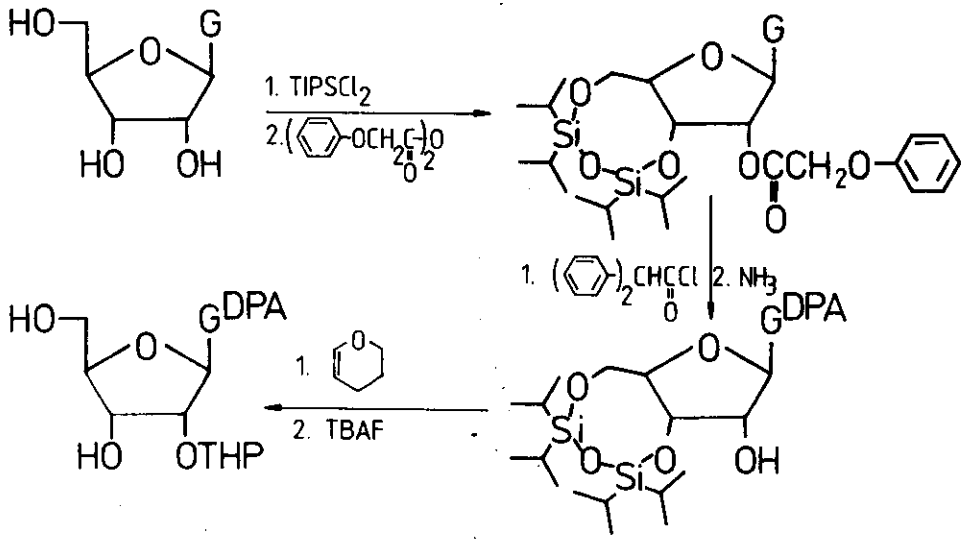


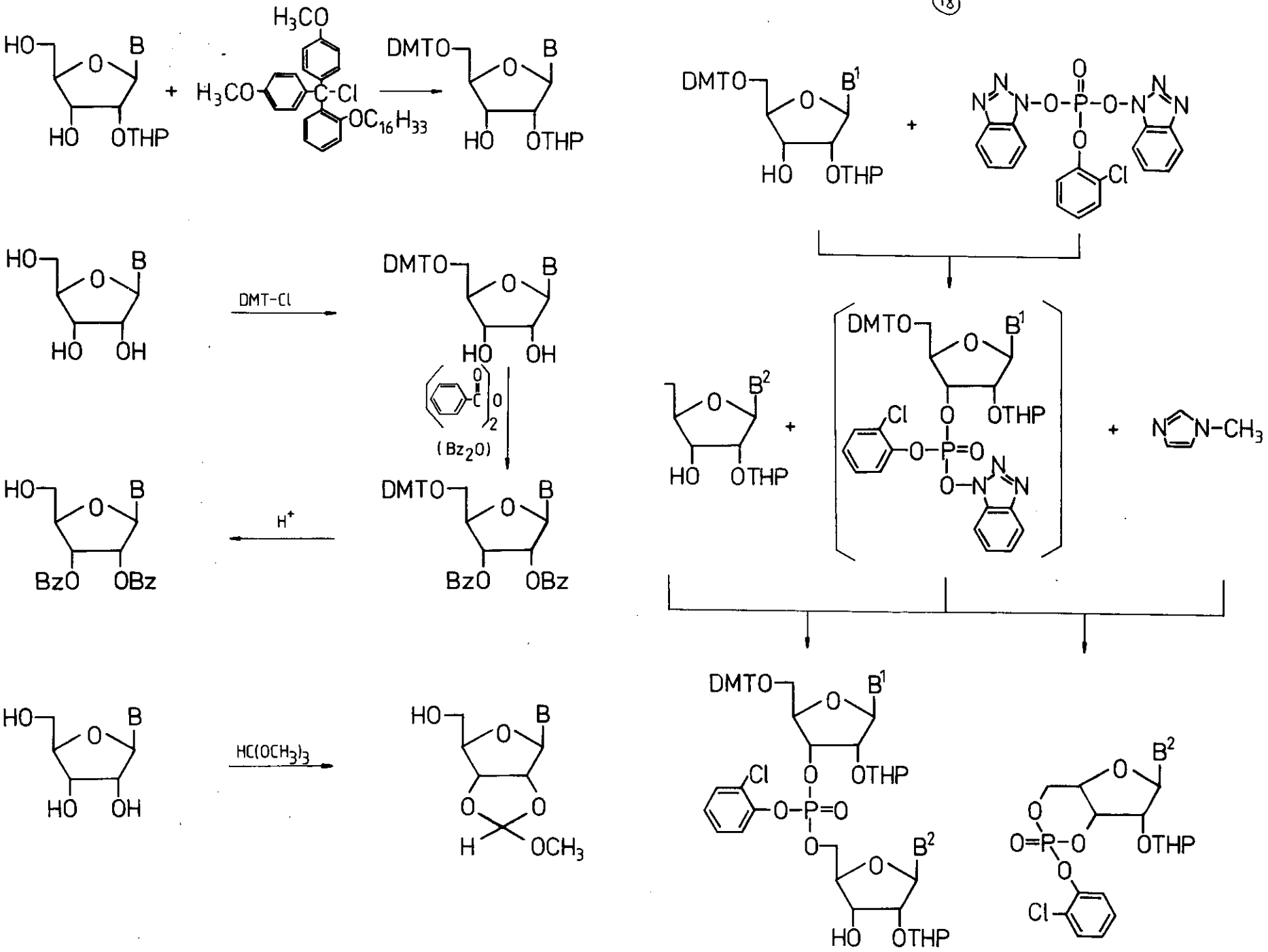


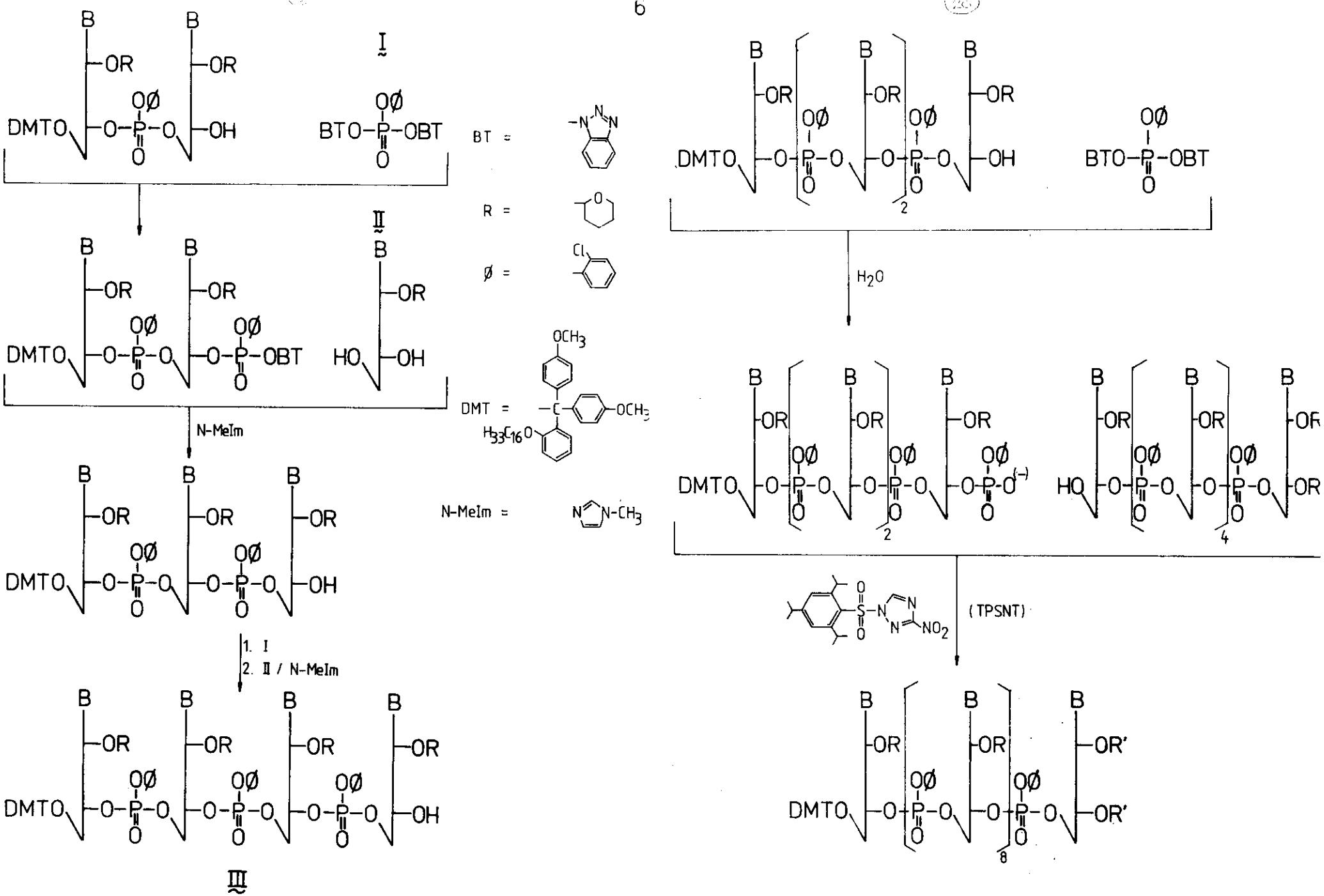

HO
U
O
HO
OH
1. $CH_3C(OCH_3)_3$
2. $(CH_3C(=O))_2O$
AcO
H_3CO
CH_3
H^+
THP
NH_3
OTHP
*


HO
C
O
HO
OH
Cl
Si
O
(TIPSCl2)
H_3CO
N
An
TBAF
OTHP

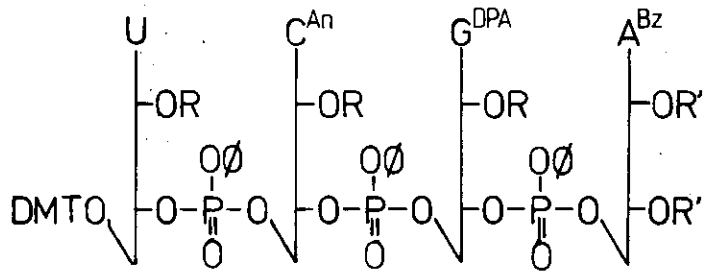

HO
A
O
HO
OH
$TIPSCl_2$
Si
THP
TBAF
OTHP
1. $(CH_3)_3SiCl$
2.
C(=O)Cl
3. NH_4OH
Bz

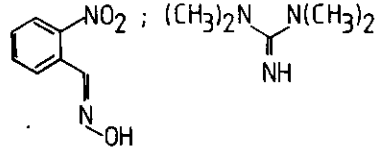

HO
G
O
HO
OH
1. $TIPSCl_2$
2.
$OCH_2C(=O)O$
$OCCH_2O$
1.
$CHCCl$
2. NH_3
DPA
THP
TBAF
OTHP


HO
B
O
HO
OTHP
H3CO
H3CO
C-Cl
OC16H33
DMTO
DMT-Cl
OH
(Bz2O)
H+
BzO
OBz
HC(OCH3)3
H
OCH3
B1
B2
N
N-O-P-O-N
Cl
N-CH3


I
II
III
N-MeIm
1. I
2. II / N-MeIm
H2O
(TPSNT)
BT =
R =
Ø =
DMT =
N-MeIm =

(21)



1. 

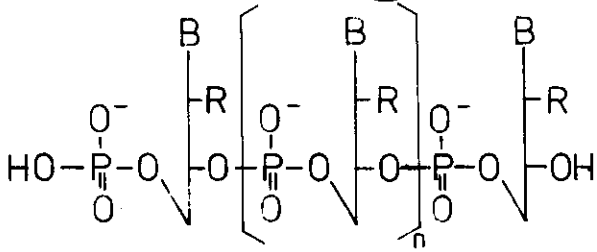
2. NH_3

3. H^+

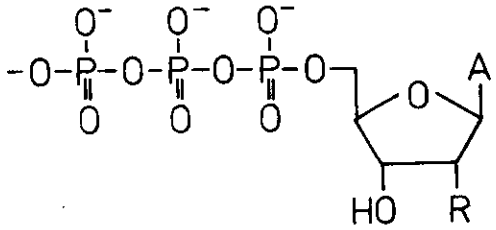
UpCpGpA

Venom	Spleen	Takadiastase T1	RNAse	Takadiastase T2
U + pC + pG + pA	Up + Cp + Gp + A	UpCpGp + A	Up + Cp + GpA	Up + Cp + Gp + A

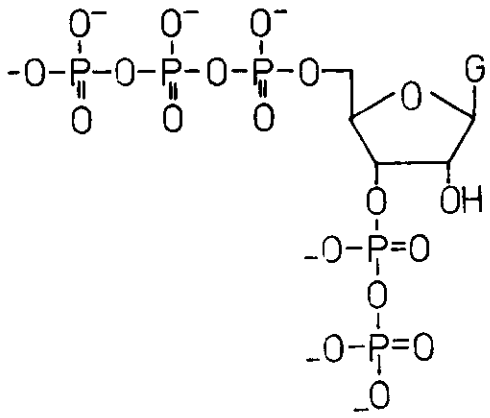
(22)

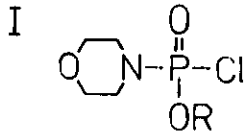


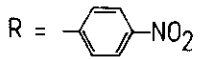
R = H
R = OH

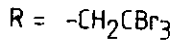


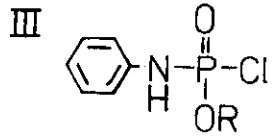
R = OH
R = NH_2

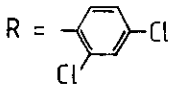


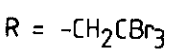


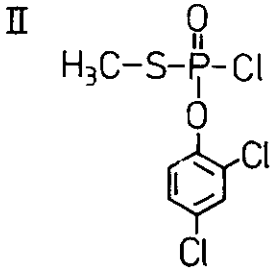


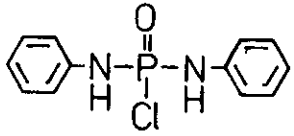


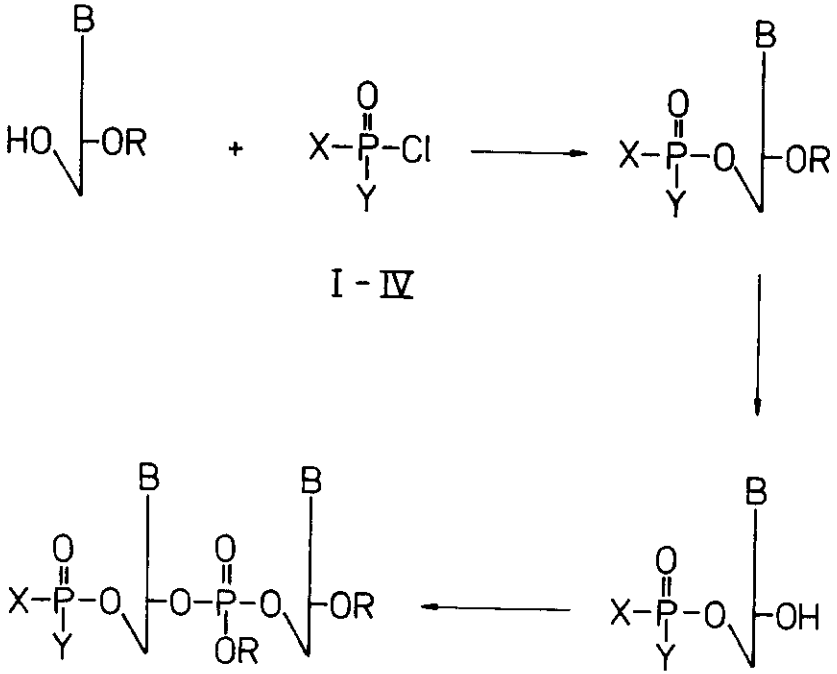


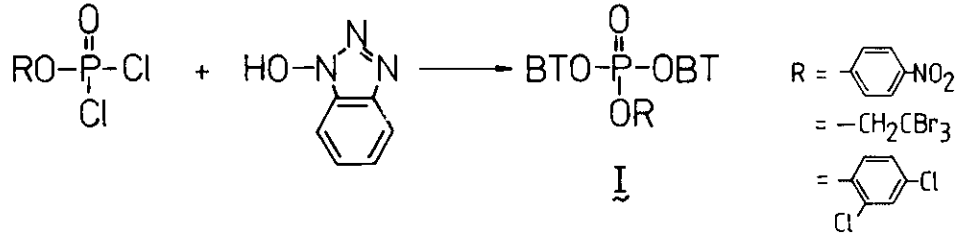


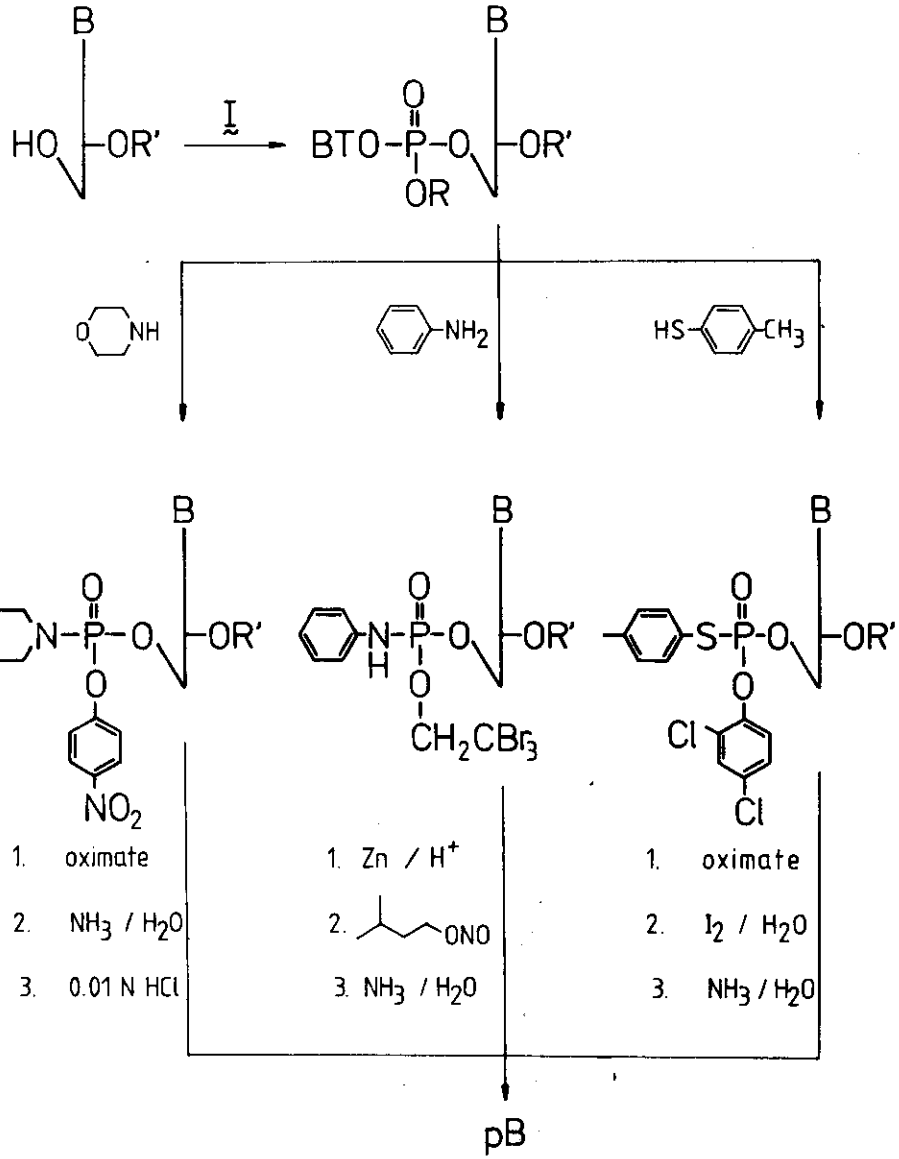


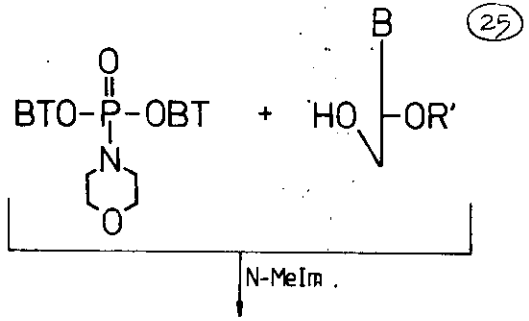




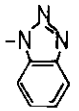




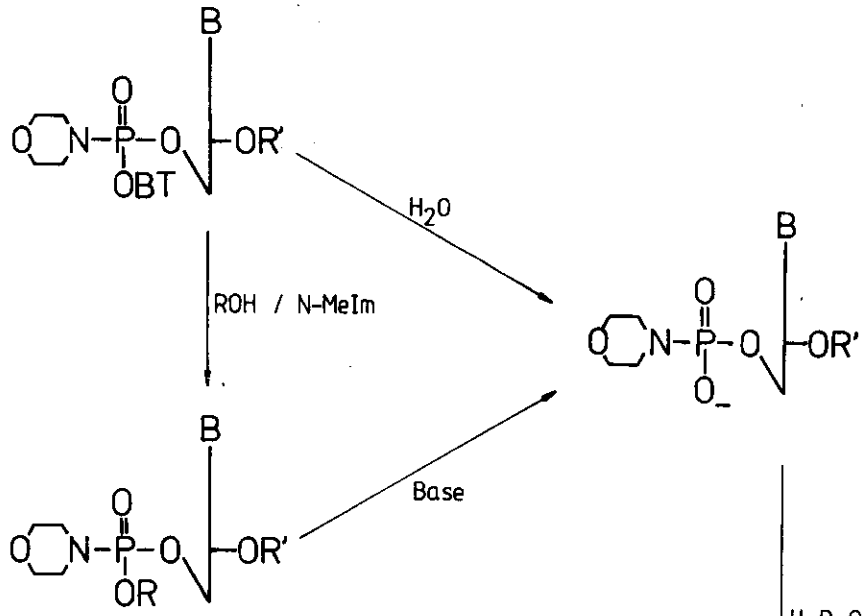




(25)

BT = 

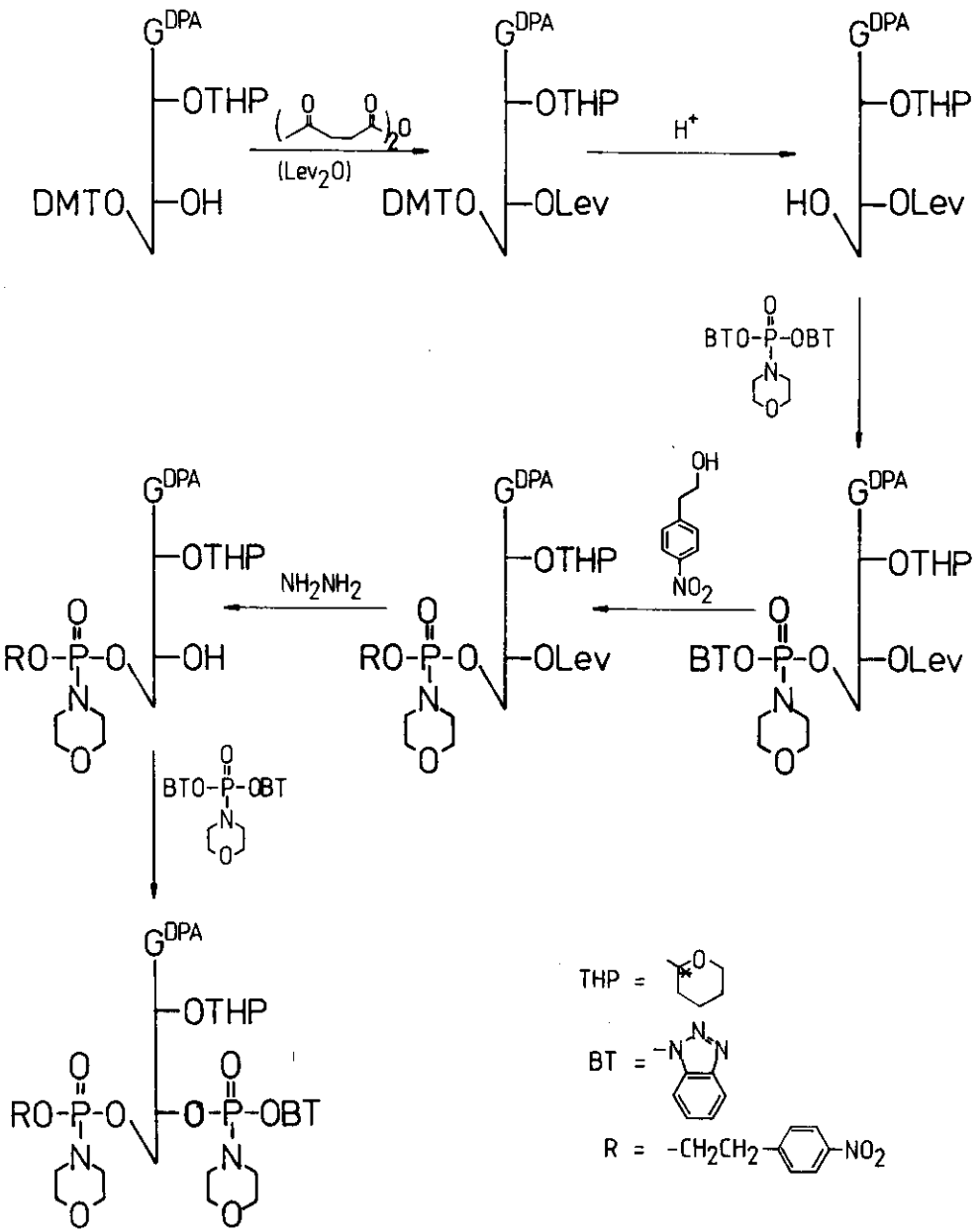
N-MeIm = 



R = $-CH_2CH_2CN$

R = $-CH_2CH_2-C_6H_4-NO_2$

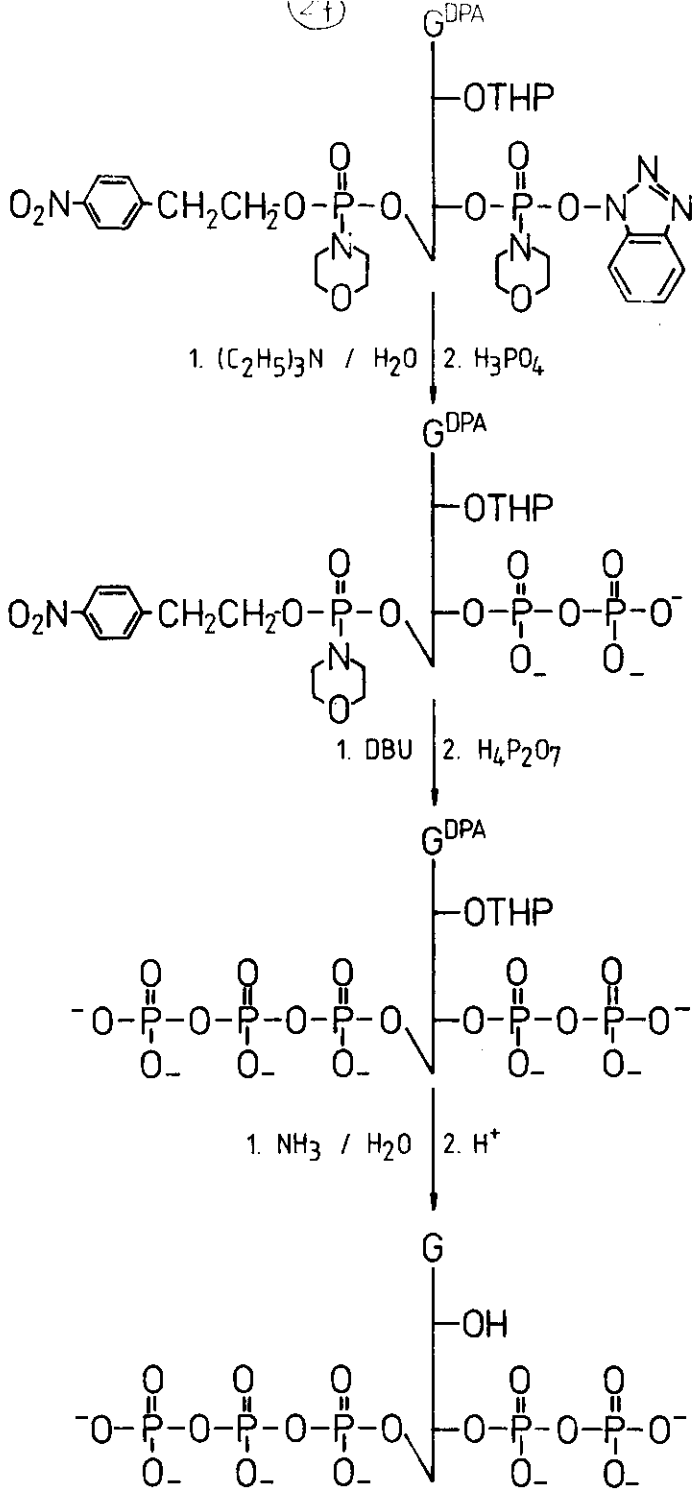
(26)



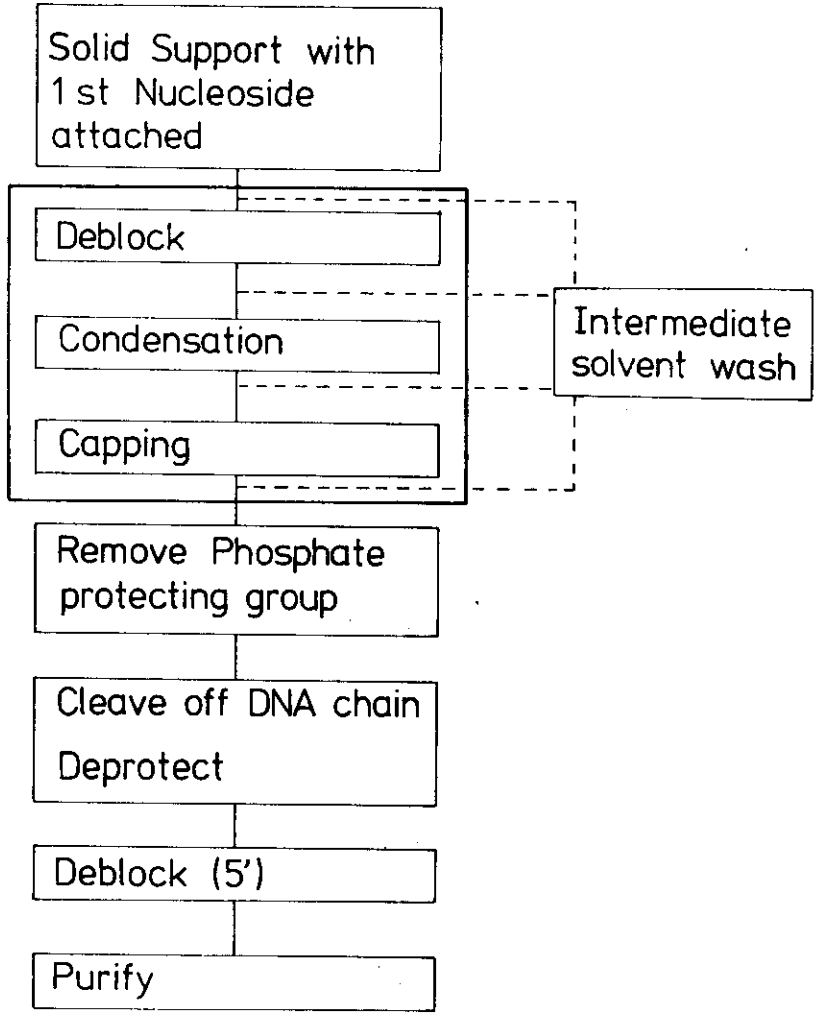
THP =

BT =

R = $-CH_2CH_2-C_6H_4-NO_2$

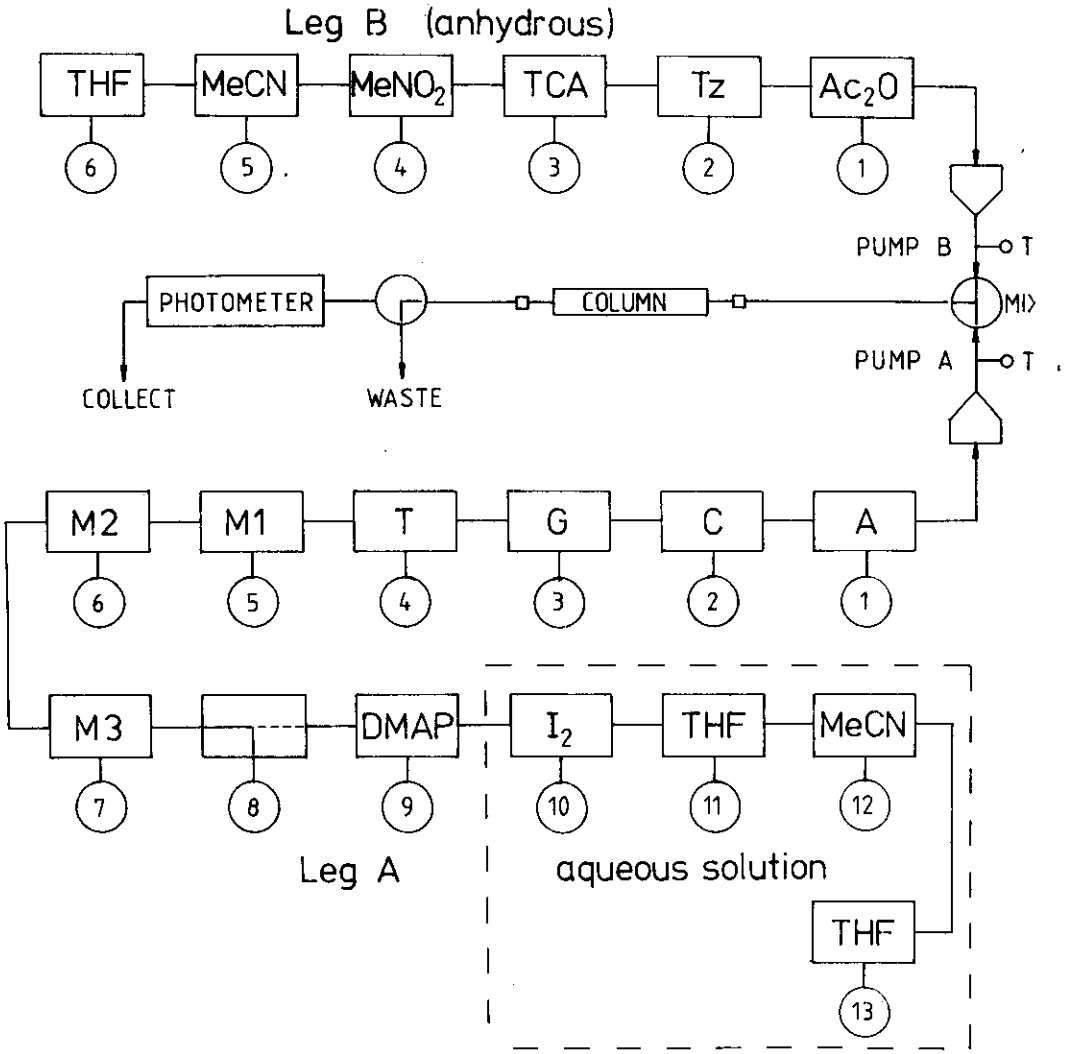

G^DPA
OTHP
1. (C2H5)3N / H2O 2. H3PO4
1. DBU 2. H4P2O7
1. NH3 / H2O 2. H+
G
OH

Lecture 3


Solid Support with 1st Nucleoside attached
Deblock
Condensation
Capping
Intermediate solvent wash
Remove Phosphate protecting group
Cleave off DNA chain
Deprotect
Deblock (5')
Purify

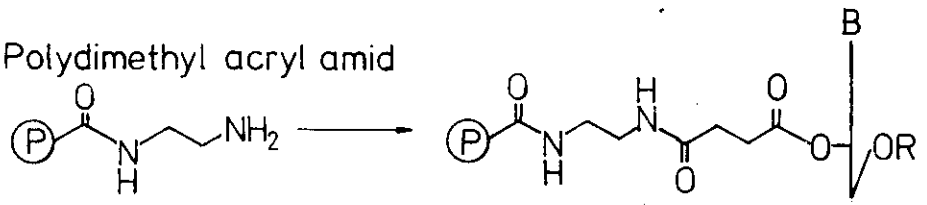
DNA Synthesizer

Flow Diagram

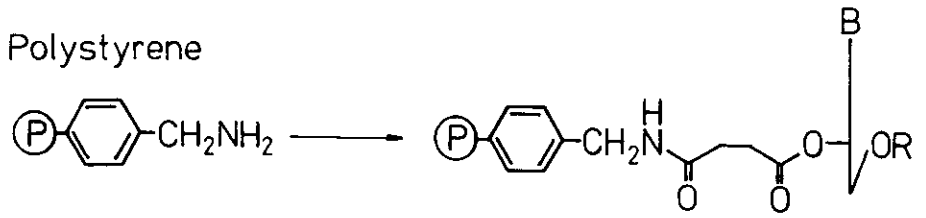


Swellable Solid Supports

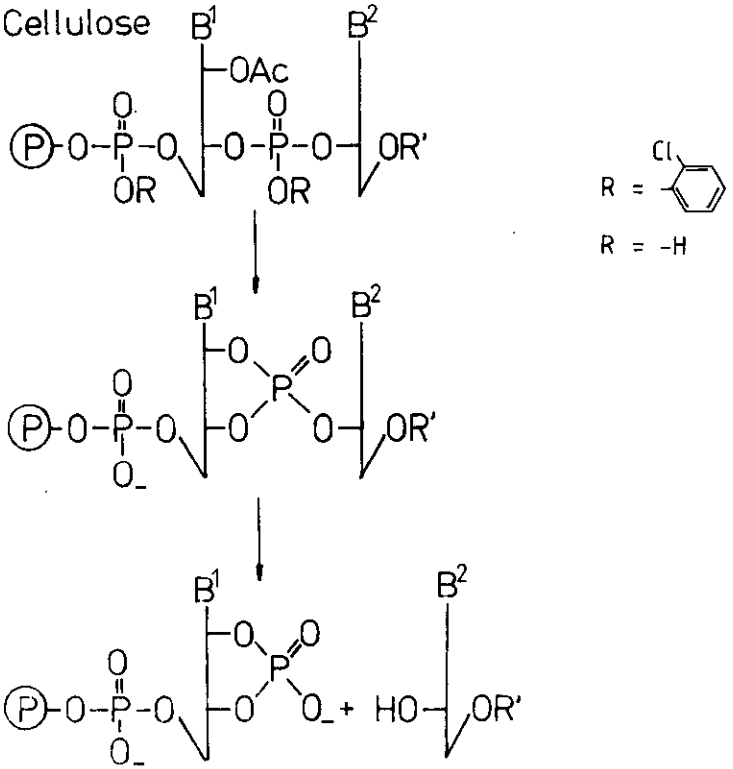
1. Polydimethyl acryl amid

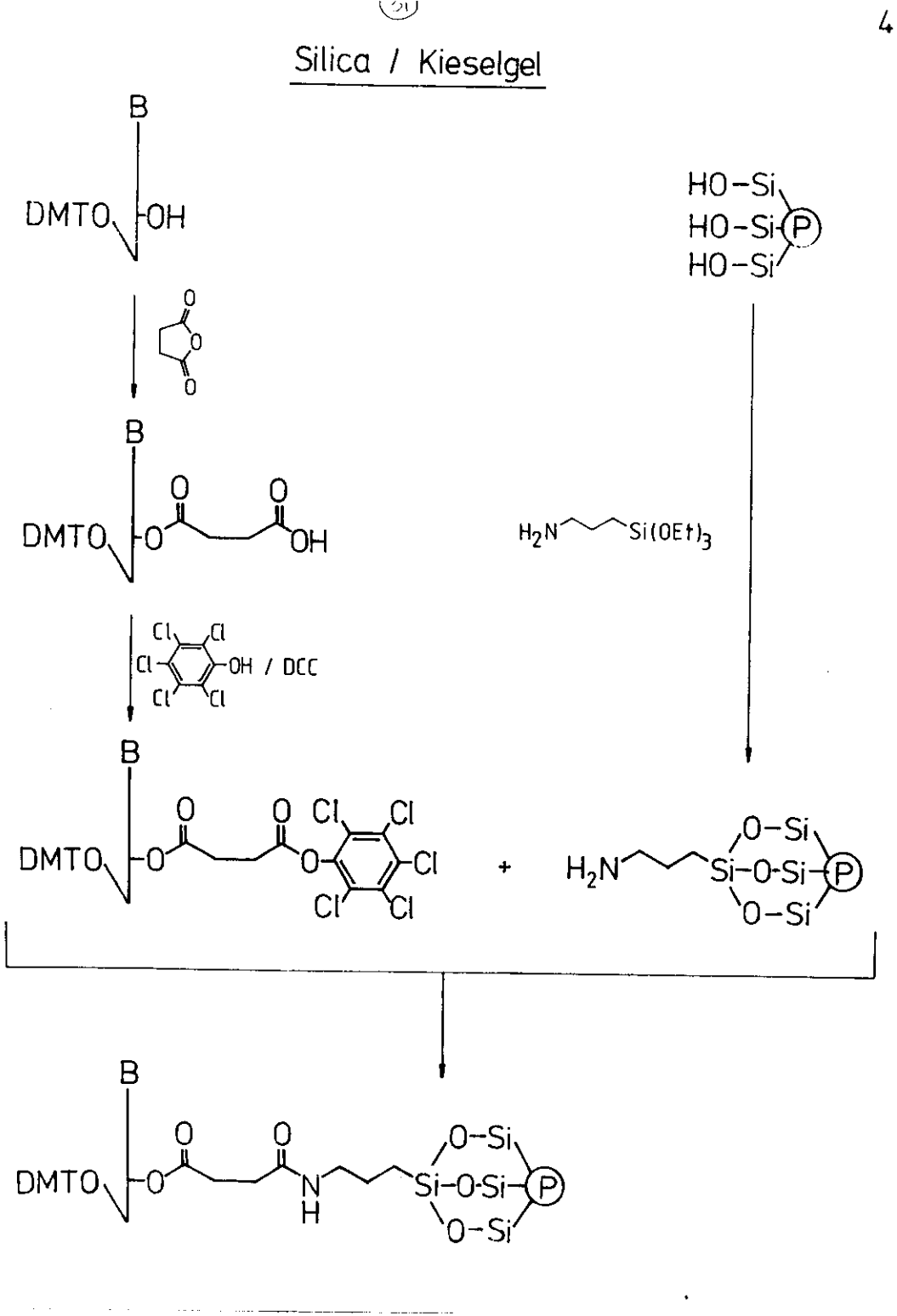


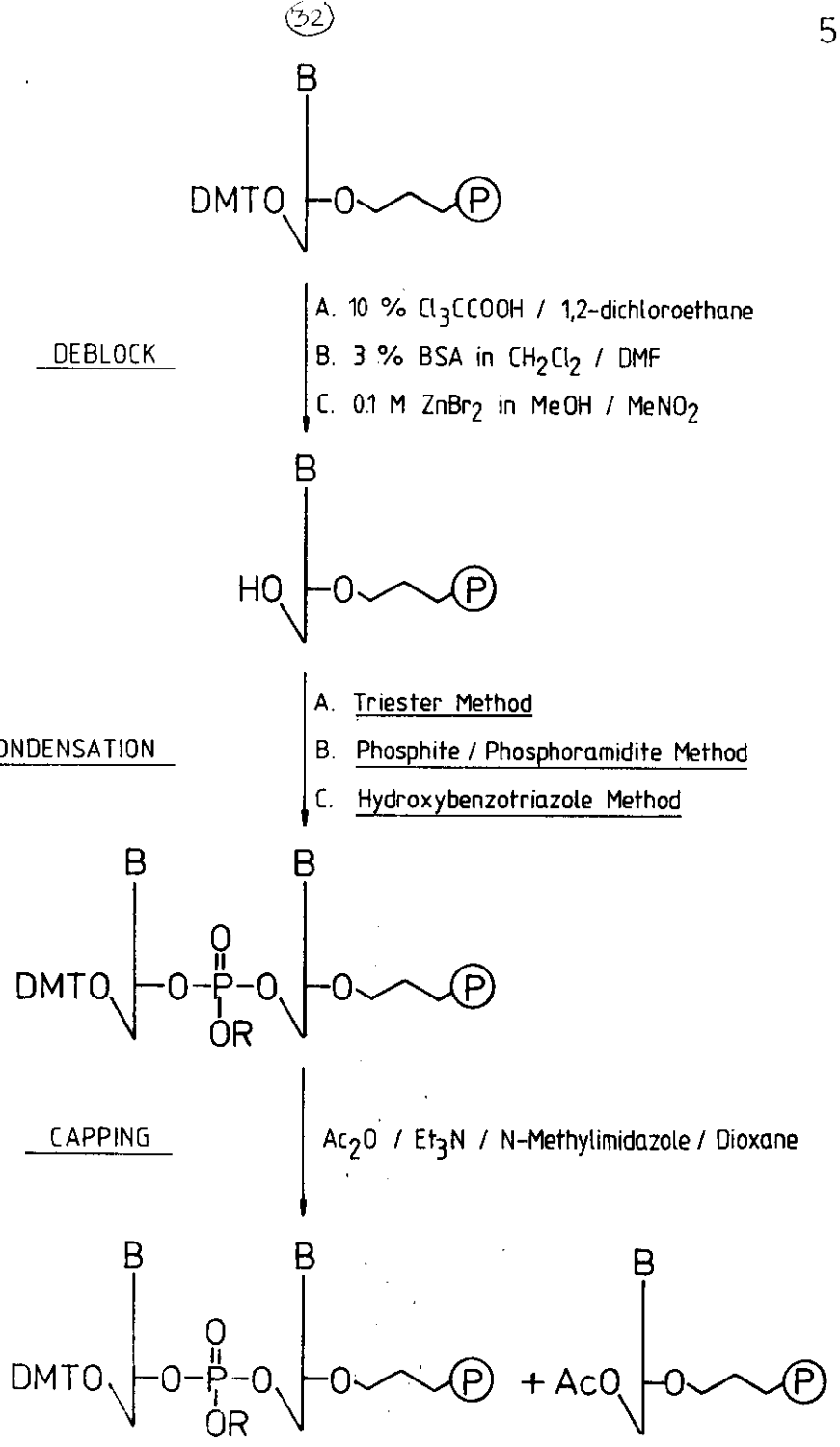
2. Polystyrene



3. Cellulose

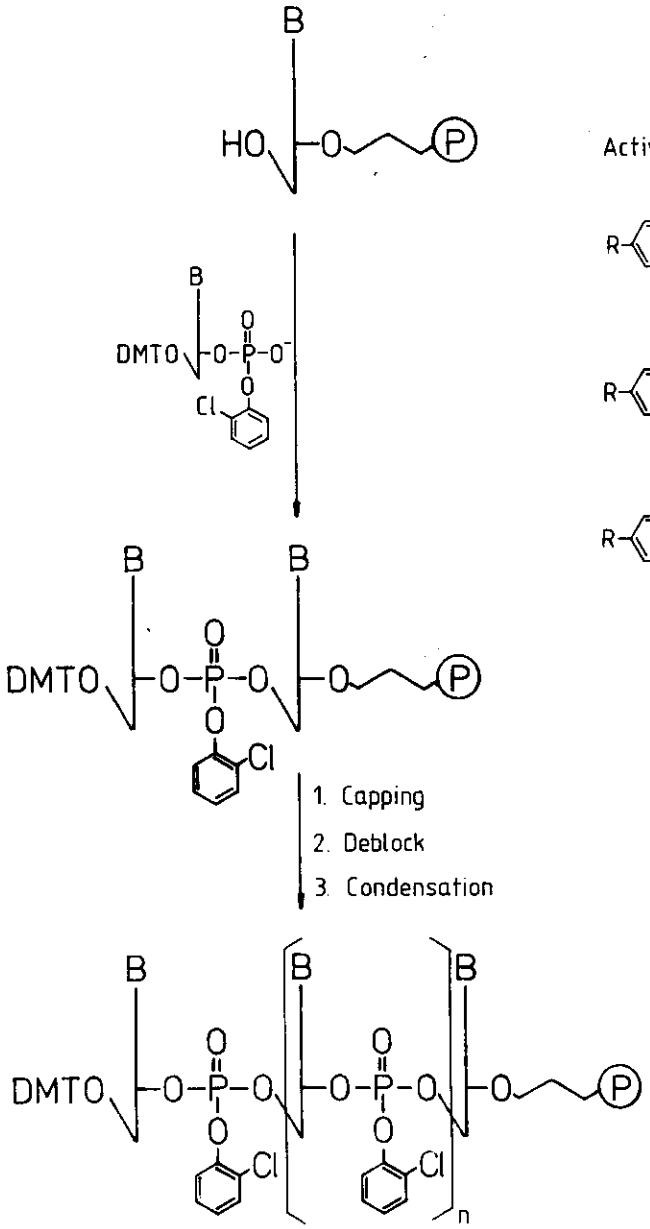



Silica / Kieselgel
B
DMTO
OH
HO-Si
HO-Si
HO-Si
P
O
O
O
OH
H_2N
$Si(OEt)_3$
Cl
OH / DCC
+
H_2N
O-Si
Si-O-Si
N
H

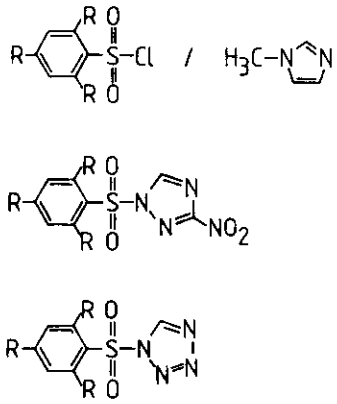

B
DMTO
O
P
DEBLOCK
A. 10 % Cl_3CCOOH / 1,2-dichloroethane
B. 3 % BSA in CH_2Cl_2 / DMF
C. 0.1 M $ZnBr_2$ in MeOH / $MeNO_2$
HO
CONDENSATION
A. Triester Method
B. Phosphite / Phosphoramidite Method
C. Hydroxybenzotriazole Method
OR
CAPPING
Ac_2O / Et_3N / N-Methylimidazole / Dioxane
AcO

(33)

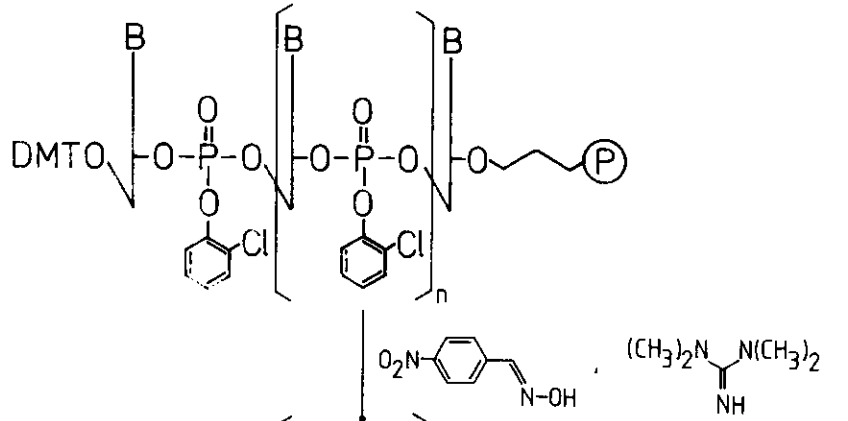
Triester Method

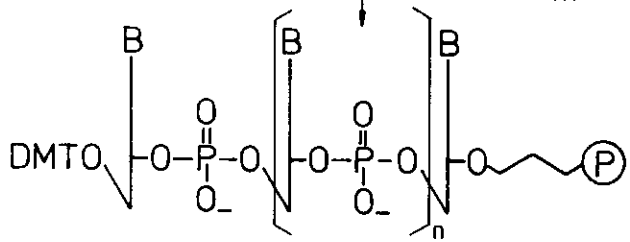


Activating Agent

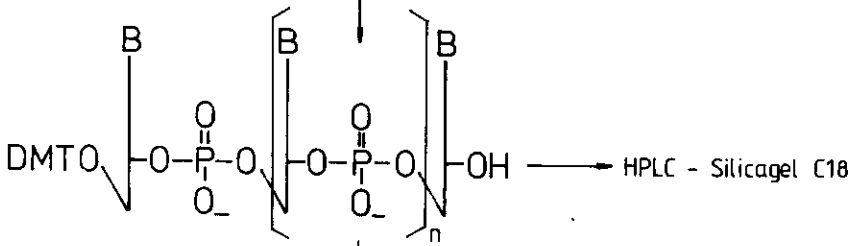


(34)





25 % NH_3 / H_2O

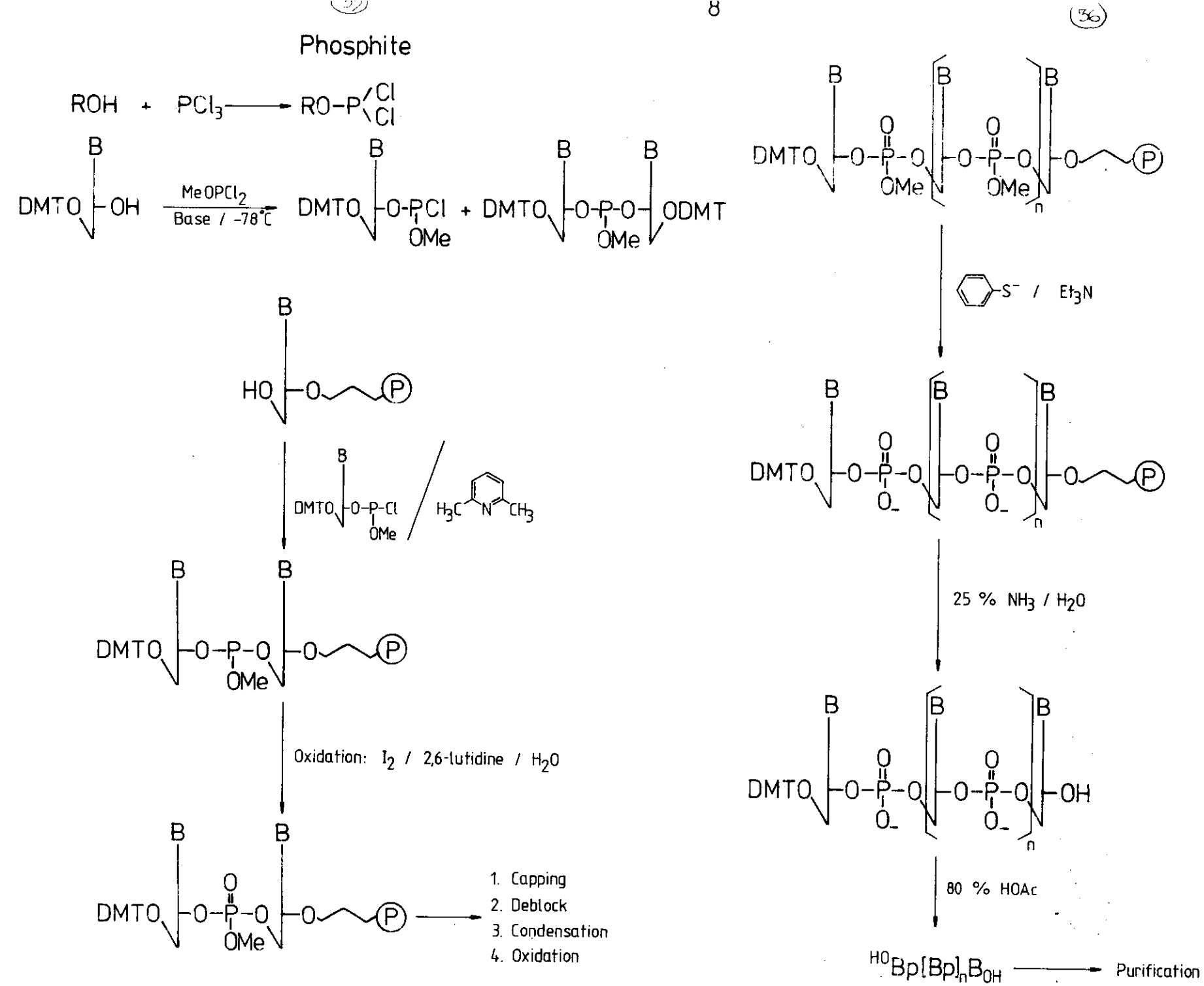


80 % HOAc

$^{HO}Bp[Bp]_n B_{OH}$

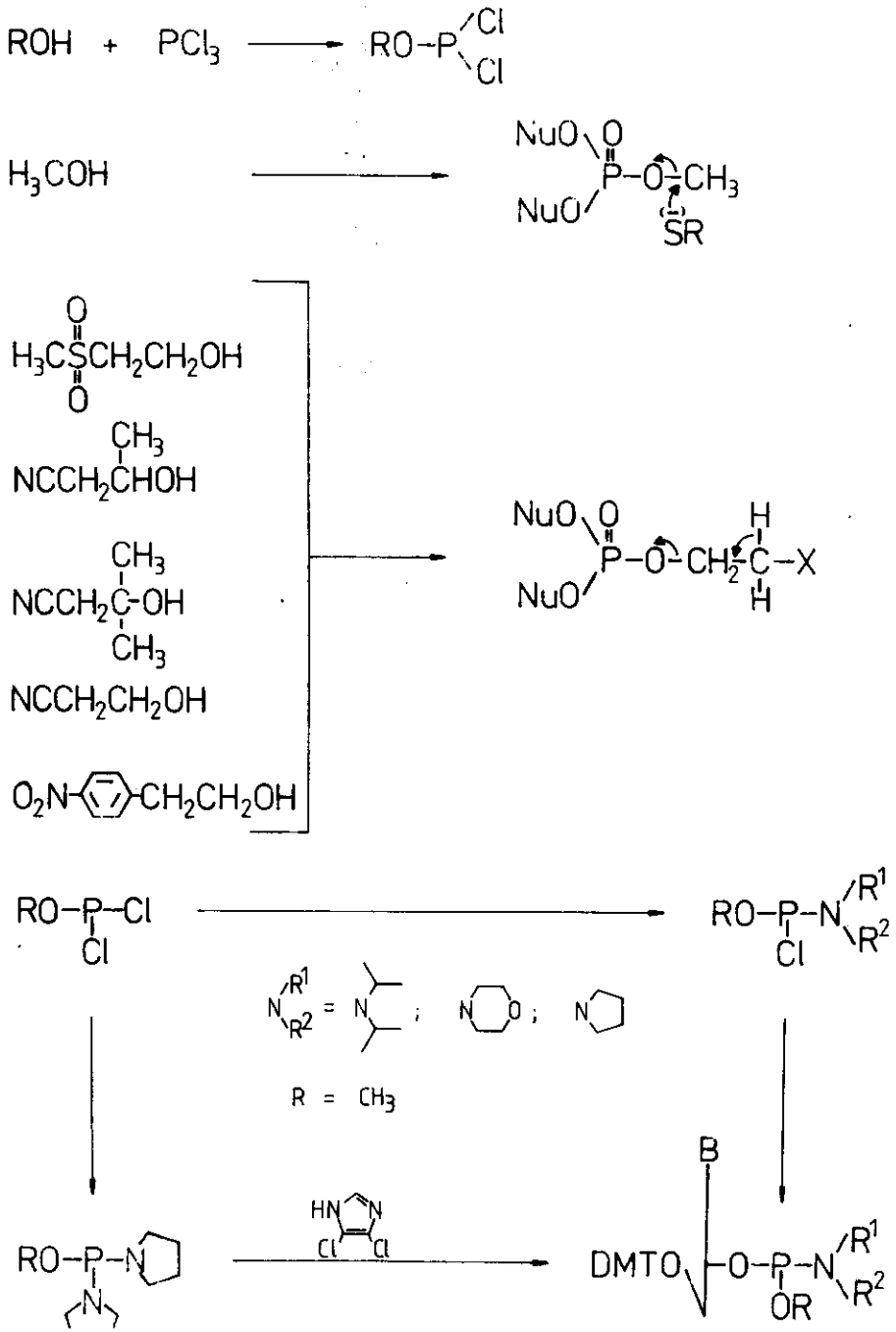
G50 Column : Kieselgel TLC

Gel Electrophoresis : HPLC

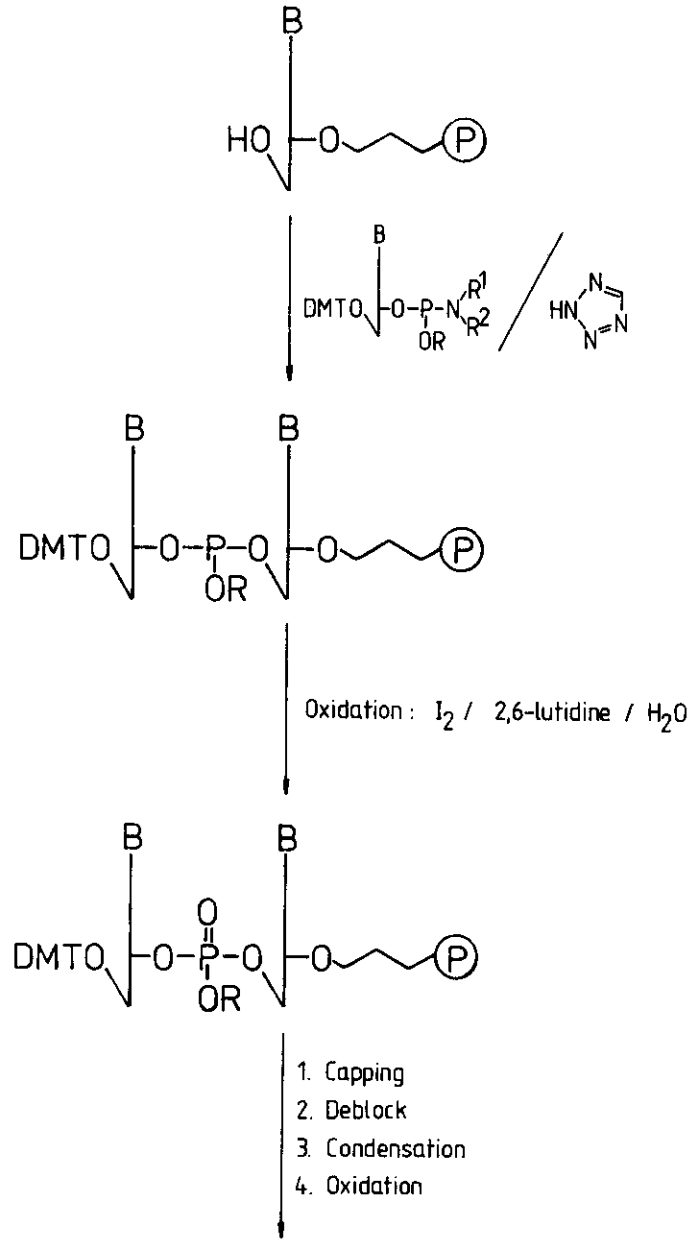

Phosphite
ROH + PCl3 → RO-P(Cl)Cl
DMTO-OH
MeOPCl2
Base / -78°C
DMTO-O-P(Cl)-OMe
DMTO-O-P(OMe)-O-ODMT
HO-P
DMTO-O-P(OMe)-Cl
H3C CH3
DMTO-O-P(OMe)-O-P
Oxidation: I2 / 2,6-lutidine / H2O
1. Capping
2. Deblock
3. Condensation
4. Oxidation
S⁻ / Et3N
25 % NH3 / H2O
80 % HOAc
HO Bp [Bp]n B OH
Purification

(37)

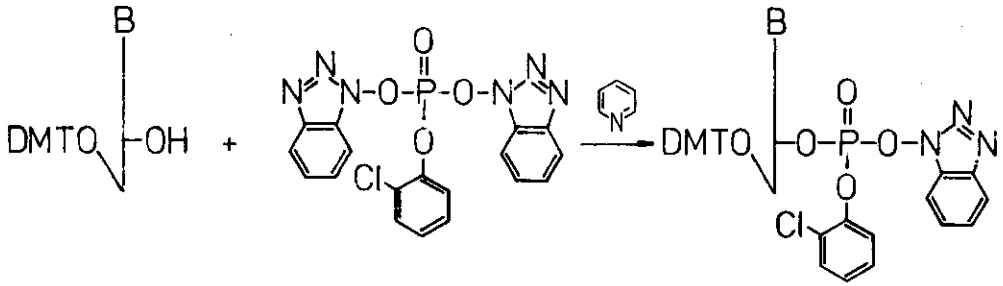
Phosphoramidite

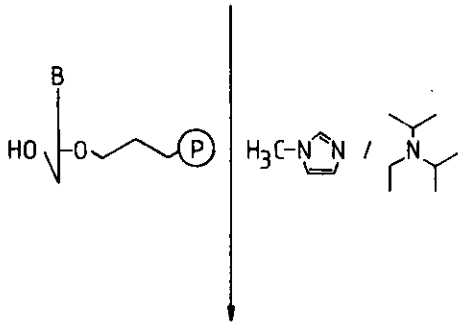


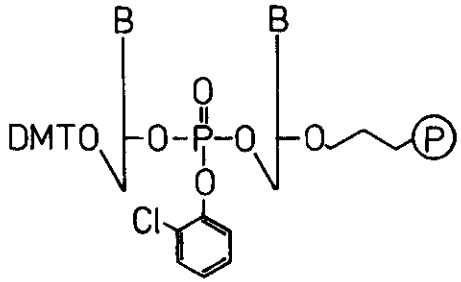
(38)



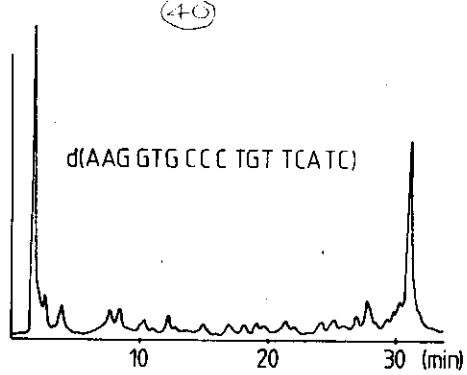
Hydroxybenzotriazole







1. Capping
2. Deblock
3. Condensation



	Phosphoramidite	Triester	HOBT
Deblock	3	3	3
Wash	5	5	5
Condensation	3 } 5	60	5
Oxidation	2		
Wash	2	2	2
Capping	5	5	5
Wash	5	5	5
Total (min.)	25	80	25