Development of Alternative BCDP Reagents for the Synthesis of 5'-Terminal Phosphate Oligonucleotides: Application in the Synthesis of SmartCap™, a Novel mRNA Capping Reagent

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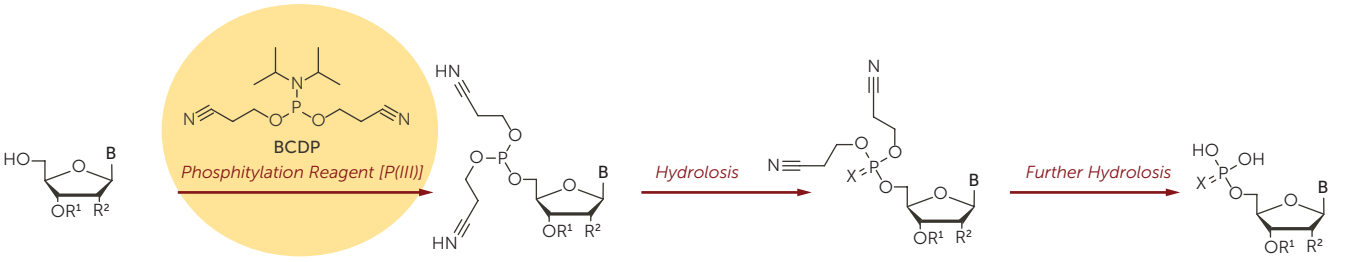
ABSTRACT

BCDP (Bis(2-cyanoethyl) diisopropylphosphoramidite) is a phosphitylating agent that facilitates the formation of phosphite triester bonds between nucleosides. These bonds can be subsequently oxidized or sulfurized to produce a terminal phosphate or thiophosphate at the 3' or 5' hydroxyl group of various oligonucleotides. Terminal phosphate or thiophosphate oligonucleotides serve as versatile building blocks for chemical or enzymatic conjugation reactions, enabling the synthesis of longer nucleotide sequences.

In this technical note, we will address the safety concerns associated with the use of BCDP reagent and explore the development of alternative phosphitylating reagents. Additionally, we will discuss its application in the synthesis of SmartCap™, a novel mRNA capping reagent.

INTRODUCTION

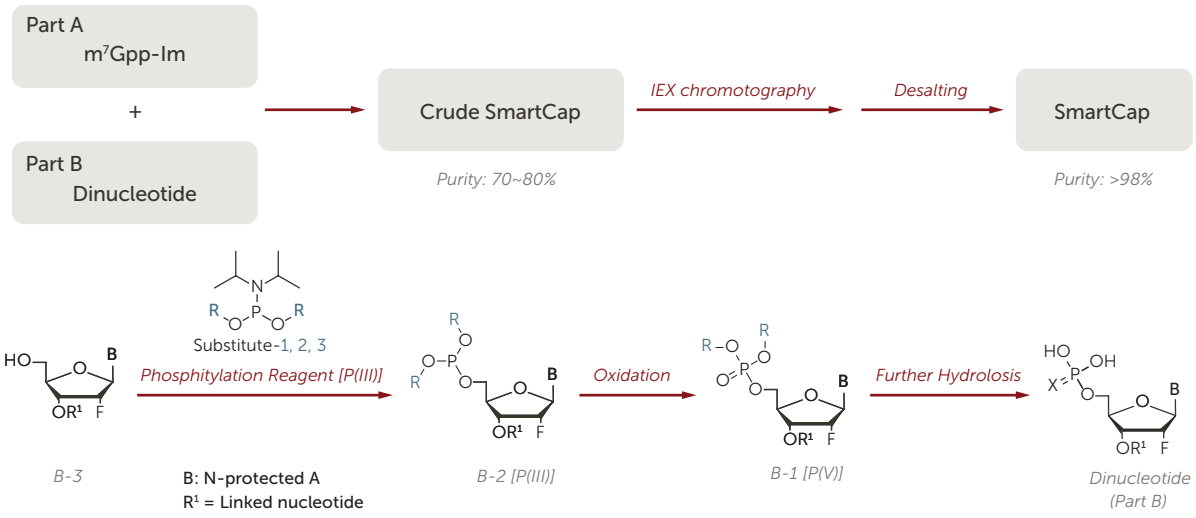
A General Approach to Terminal Modification via BCDP Phosphitylation



BCDP is a phosphoramidite commonly used in oligonucleotide synthesis, but it requires careful handling due to concerns related to stability, exothermicity, and toxicity.

Risk information about Bis(2-cyanoethyl)-N,N-diisopropylphosphoramidite. 'BCDP'

Exothermicity	• UN class 1 explosive • Flammable liquid and vapor
Stability	• Recommended storage temperature -20°C • Handle and store under inert gas • At room temperature, the purity decreased from 96.88% to 8.22% within 30 days
Toxicity	• Class 6.1 in DOT • May cause an allergic skin reaction



Alternative P-reagents were introduced at this stage of the process.

THERMAL STABILITY (ARC AND DSC) OF P-REAGENT SUBSTITUTES

ARC Testing Comparison of Phosphoramidites

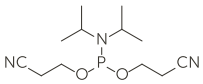
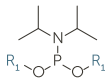
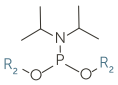
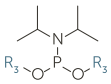
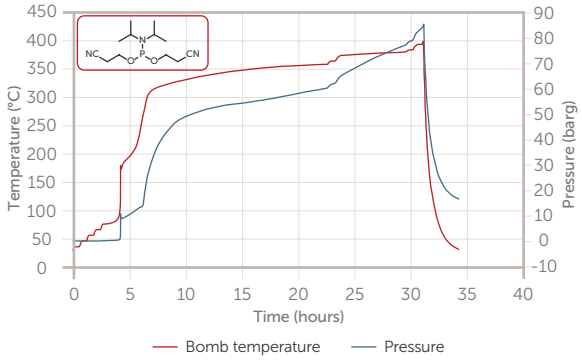
Structure				
Compound	BCDP ~98% Purity	Substitute-1 96% purity	Substitute-2 ~99% purity	Substitute-3
Earliest onset temperature	75.9°C	170.9°C	170.5°C	130.7°C
Earliest onset energy	-473.0 J/g	-490.5 J/g	-142.9 J/g	-224.4 J/g
Number of additional onsets	3	2	2	2

Table continues on next page

Highest energy onset	-772.3 J/g (190°C)	-409.5 J/g (170.9°C)	>-158.5 J/g (330.1°C)	-224.4 J/g (130.7°C)
Time to maximum rate (TMR) estimates	0.9 hr at 70°C 24 hr at 48°C 70 hr at 41°C	8 hr at 156°C 24 hr at 143°C 48 hr at 128°C	0.5 hr at 176°C 24 hr at 170°C 48 hr at 169°C	0.8 hr at 130°C 24 hr at 118.5°C 71 hr at 115°C
Total heat released	-1,271.3 J/g	-616.6 J/g	-301.4 J/g	-348.4 J/g

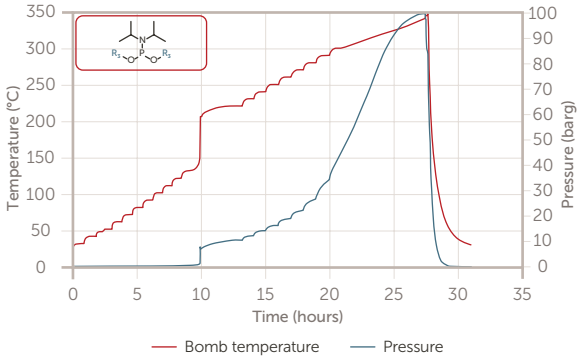
Temperature and Pressure vs. Time BCDP

Accelerating Rate Calorimetry



Temperature and Pressure vs. Time of Substitute-3

Accelerating Rate Calorimetry



DSC Testing Comparison of Phosphoramidites

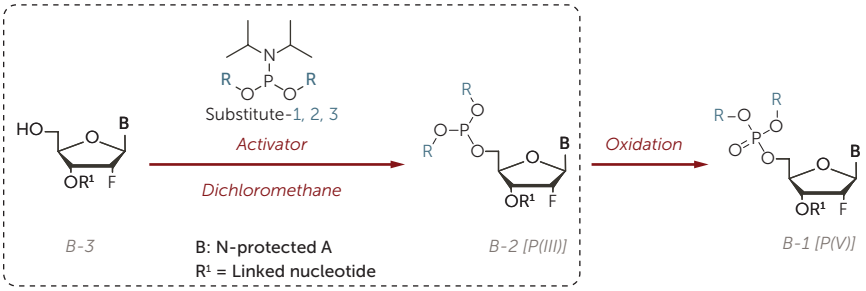
SAMPLE	EVENT	START TEMP.	PEAK TEMP.	END TEMP.	ENERGY
BCDP	Exotherm	66.7°C	129.4°C	194.0°C	-542.7 J/g
	Exotherm	194.1°C	284.6°C	382.6°C	-165.4 J/g
Substitute-3	Endotherm	35.3°C	47.2°C	55.6°C	97.74 J/g
	Exotherm	190.6°C	206.6°C	217.5°C	-232.45 J/g
	Endotherm	323.3°C	362.6°C	390.0°C	317.53 J/g

BCDP is a candidate for classification as a UN Class 1 explosive substance.

However, based on the thermograms for 'substitute-3', this chemical is not a candidate for classification as a UN Class 1 explosive substance or a UN Class 4, Division 4.1 self-reactive substance.

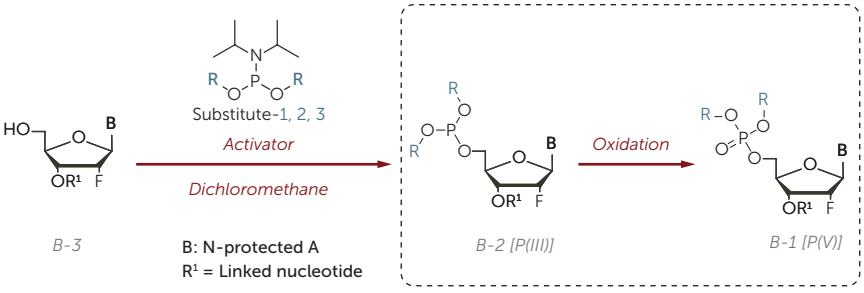
APPLICATION OF ALTERNATIVE P-REAGENTS IN THE SYNTHESIS OF SMARTCAP

Step 1 – Comparative Phosphitylation Results Using Various Phosphoramidites



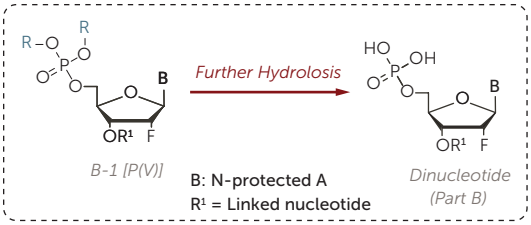
PHOSPHORAMIDITES	PHOSPHINE ACTIVATOR	IPC RESULT (HPLC AREA %)	
		RESIDUAL B-3	RXN TIME (HR)
BCDP	1H-Tetrazole	1.40 ~ 4.50%	1
Substitute-1	1H-Tetrazole	0.42%	1
Substitute-2	1H-Tetrazole	~ 0.50%	~ 2
Substitute-3	1H-Tetrazole	~ 0.04%	~ 3
	5-(Benzylthio)-1H-tetrazole	0.06%	1
	5-(Ethylthio)-1H-tetrazole	0.17%	1
	4,5-dicyanoimidazole	0.45%	1

Step 2 – Comparative Oxidation Results Using Various Phosphoramidites



PHOSPHORAMIDITES	OXIDATION REAGENT	IPC RESULT (HPLC AREA (%)		ISOLATION YIELD
		RESIDUAL B-2	RXN TIME (HR)	
BCDP	I_2, (THF : PW : Py = 66 : 33 : 1)	~ 0.50%	~ 1	63 ~ 72%
Substitute-1	H_2O_2	0.37%	~ 1	68%
Substitute-2	H_2O_2	0.35%	~ 1	30%
Substitute-3	I_2, (THF : PW : Py = 66 : 33 : 1)	N.D	~ 1	67%

Step 3 – Comparative Deprotection Results Using Various Phosphoramidites



PHOSPHORAMIDITES	IPC RESULT (HPLC AREA %)		ISOLATION YIELD
	RESIDUAL B-1	RXN TIME (HR)	
BCDP	N.D ~ 1.3%	24	43 ~ 53%
Substitute-3	1.4%	48	67%

Using the same process, deprotection with Substitute-3 enabled the synthesis of the dinucleotide with a higher yield compared to BCDP.

CONCLUSION

In the manufacturing process of dinucleotides for SmartCap synthesis, BCDP is used as the phosphitylating reagent. However, BCDP is susceptible to oxidation to P(V) during storage and inherently exhibits explosive properties.

In contrast, ARC and DSC analyses confirmed that the three alternative reagents exhibit reduced hazardous characteristics. Furthermore, it was experimentally demonstrated that all three alternatives can effectively replace BCDP in the synthesis of dinucleotides required for SmartCap production.

ACKNOWLEDGEMENTS

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