A Thermal Stability Study of Phosphoramidites Employed in Oligonucleotide Synthesis

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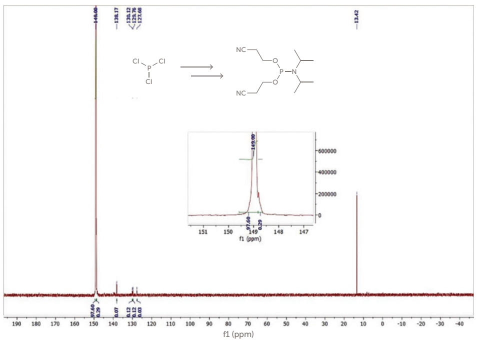
INTRODUCTION

Phosphoramidites have played a pivotal role in development of RNA- and DNA-oligonucleotide drug design via *"Phosphoramidite Method of DNA Synthesis."* The synthesis of phosphoramidites have been described in literature; however, little has been reported on the thermal stability of phosphoramidites. Process safety is an important mission when carrying out chemistry, not only in the manufacturing scale but also on bench and kilo scale. One of the common and detrimental industrial accidents is thermal runaway reaction. The impact of thermal runaway is very high because it could lead to catastrophe along with business interruption. In this work, we performed series of tests on phosphoramidites with various protecting groups to identify chemically unstable and high energy reactions. These tests identify sufficient safety margin about undesired and potential thermal decomposition.

METHODS

- Entegris proprietary route
- Non-distilled product isolated in 97.6% purities
- Stability issues – purity decreases upon standing at room temperature
- Initiated accelerating rate calorimetry (ARC) and differential scanning calorimetry (DSC) testing

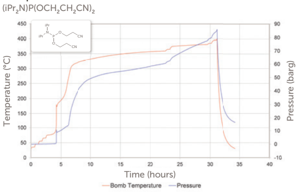
^{31}P NMR of Non-Distilled $(iPr_2N)P(OCH_2CH_2CN)_2$



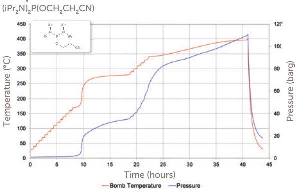
RESULTS

- Onset temperature of exotherm is 75.9°C, temperature rise to 179.6°C with **-473.0 J/g** of heat
- Product has low initiation temperature and high energy of decomposition
- Total heat released during ARC study is **-1271.3 J/g**
- Time to maximum rate (TMR):
  - 0.9 hr at 70°C
  - 24 hr at 47.7°C
  - 69.6 hr at 41°C
- Product is a candidate for classification as a UN Class 1 explosive substance

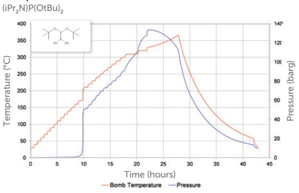
Temperature and Pressure vs. Time
$(iPr_2N)P(OCH_2CH_2CN)_2$



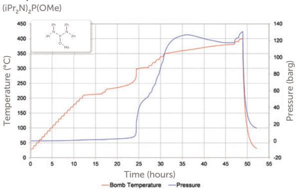
Temperature and Pressure vs. Time
$(iPr_2N)_2P(OCH_2CH_2CN)$



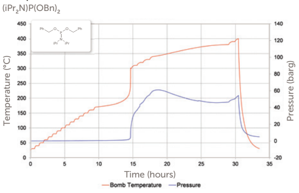
Temperature and Pressure vs. Time
$(iPr_2N)P(OtBu)_2$



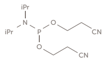
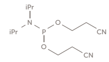
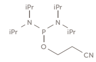
Temperature and Pressure vs. Time
$(iPr_2N)_2P(OMe)$



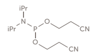
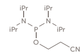
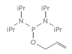
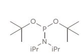
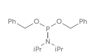
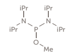
Temperature and Pressure vs. Time
$(iPr_2N)P(OBn)_2$



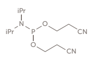
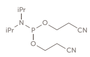
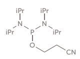
ARC Testing of $(iPr_2N)P(OCH_2CH_2CN)_2$ Purity Comparison

	 **COMMERCIAL SAMPLE**	 **ENTEGRIS 'PURIFIED' CRUDE SAMPLE**	 **PHOS REAGENT (DISTILLED)**
Earliest onset temperature	70.9°C	75.9°C	170.5°C
Earliest onset energy	-569.4 J/g	-473.0 J/g	-406.7 J/g
Number of additional onsets	10	3	2
Highest energy onset	-569.4 J/g (70.9°C)	-772.3 J/g (190°C)	-406.7 J/g (170.5°C)
Time to maximum rate (TMR) estimates	0.8 hr at 65°C 24 hr at 41°C 52 hr at 36°C	0.9 hr at 70°C 24 hr at 48°C 70 hr at 41°C	0.5 hr at 170°C 24 hr at 150°C 195 hr at 140°C
Total heat released	-1163.2 J/g	-1271.3 J/g	-616.6 J/g

ARC Testing Comparison of Common Phosphoramidites

	 **~98% PURITY**	 **>99% PURITY**	 **~94% PURITY**	 **~99% PURITY**	 **96% PURITY**	 **95% PURITY**
Earliest onset temperature	75.9°C	170.5°C	110.7°C	170.5°C	170.9°C	210.7°C
Earliest onset energy	-473.0 J/g	-406.7 J/g	-467.1 J/g	-142.9 J/g	-490.5 J/g	-15.3 J/g
Number of additional onsets	3	2	3	2	2	3
Highest energy onset	-772.3 J/g (190°C)	-406.7 J/g (170.5°C)	-467.1 J/g (110.7°C)	>-158.5 J/g (330.1°C)	-490.5 J/g (170.9°C)	-276.8 J/g (230.8°C)
Time to maximum rate (TMR) estimates	0.9 hr at 70°C 24 hr at 48°C 70 hr at 41°C	0.5 hr at 170°C 24 hr at 150°C 195 hr at 140°C	1 hr at 130°C 24 hr at 91°C 48 hr at 84°C	0.5 hr at 176°C 24 hr at 170°C 48 hr at 169°C	8 hr at 156°C 24 hr at 143°C 48 hr at 128°C	0.5 hr at 253°C 24 hr at 210°C 48 hr at 203°C
Total heat released	-1271.3 J/g	-616.6 J/g	-651.8 J/g	-301.4 J/g	-616.6 J/g	-417.3 J/g

DSC Testing of $(iPr_2N)P(OCH_2CH_2CN)_2$ Purity Comparison

		ONSET TEMPERATURE	**PEAK TEMPERATURE**	**END TEMPERATURE**	**ENERGY**
Commercial sample *90% purity*		40.5°C 81.8°C 194.9°C	67.9°C 131.4°C 287.7°C	77.3°C 191.1°C 382.1°C	-2.0 J/g -488.7 J/g -540.8 J/g
Entegris 'purified' crude sample *97.6% purity*		66.7°C 194.1°C	129.4°C 284.6°C	194.0°C 382.6°C	-542.7 J/g -615.4 J/g
Phos reagent (distilled) *99% purity*		110°C 209.5°C 269.0°C 339°C	166.9°C 236.6°C 286.6°C 363.4°C	197.2°C 268.0°C 320.5°C 375.9°C	-58.3 J/g -224.6 J/g -31.5 J/g -10.2 J/g

CONCLUSION

Protecting groups on phosphoramidites can facilitate instability, exothermic decomposition, and potential explosive behavior. It is strongly recommended to fully understand the thermal characteristics of the phosphoramidites from a synthetic and supply chain perspectives.

ACKNOWLEDGMENT

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