
Supporting Information

An Efficient Synthesis of Tenofovir (PMPA): A Key Intermediate Leading to Tenofovir-based HIV Medicines

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General Remarks

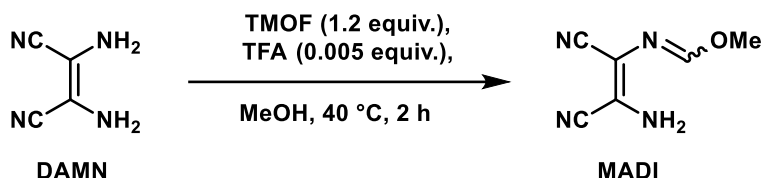
Instrumentation. For all compounds, ^1H , ^{13}C and ^{31}P NMR spectra were recorded on a Bruker Avance III 600 MHz spectrometer. Chemical shifts were measured relative to the residual solvent resonance for ^1H and ^{13}C NMR (CDCl_3 = 7.26 ppm for ^1H and 77.2 ppm for ^{13}C , $\text{DMSO}-d_6$ = 2.50 ppm for ^1H and 39.52 ppm for ^{13}C). Coupling constants J are reported in hertz (Hz). The following abbreviations were used to designate signal multiplicity: s, singlet; d, doublet; t, triplet; q, quartet, p, pentet; dd, doublet of doublet; ddd, doublet of doublet of doublet; dt, double of triplet; ddt, doublet of doublet of triplet; m, multiplet; br, broad. Reactions were monitored by GC-MS or HPLC using the methods indicated. Formation of MADI, including the amidine intermediate was monitored via GC-MS. The column used was an Agilent J&W GC column, type HP-1 (30 m x 0.320 mm, 5 μm film). The oven was maintained at 200 $^\circ\text{C}$ and the inlet at 225 $^\circ\text{C}$ for the duration of the method (20 minutes). A split ratio of 50:1 was used. The flow rate was 0.8 mL/min with helium as the carrier gas. HPI, HPA and PMPA were monitored using identical HPLC methods. The mobile phase was a mixture of 15% MeOH and 85% aqueous potassium phosphate buffer (pH 7.6, 10 mM). The chromatograms were acquired on an Agilent 1100 system using an Agilent Extend C18 column (5 μm , 4.6 mm x 250 mm) and were monitored at 245 (HPI) and 260 nm (HPA and PMPA). Exact mass measurements were obtained on a Thermo Scientific LTQ Orbitrap Velos. Glassware was oven-dried at 120 $^\circ\text{C}$, assembled while hot, and cooled to ambient temperature under an inert atmosphere. Unless noted otherwise, reactions involving air sensitive reagents and/or requiring anhydrous conditions were performed under a nitrogen atmosphere.

Reagents and solvents. Reagents and solvents were purchased from Aldrich Chemical Company, Fisher Scientific, Alfa Aesar, Acros Organics, Oakwood, or TCI. Liquid reagents were purified by distillation when necessary. Unless otherwise noted, solid reagents were used without further purification. Methylene chloride (DCM) and dimethylformamide (DMF) taken from a solid-sorbant Solvent Dispensing System purchased from Pure Process Technologies or distilled as described in the literature.

Synthetic Procedures

Synthesis of MADI (13)

PRECAUTIONS: DAMN, MADI, and HPI should all be handled in a fumehood for safety due to the potential liberation of HCN gas. Additionally, waste from these reactions should be separated from other organic and solid waste – consult with your safety team to determine best authorized practices for decomposing HCN.



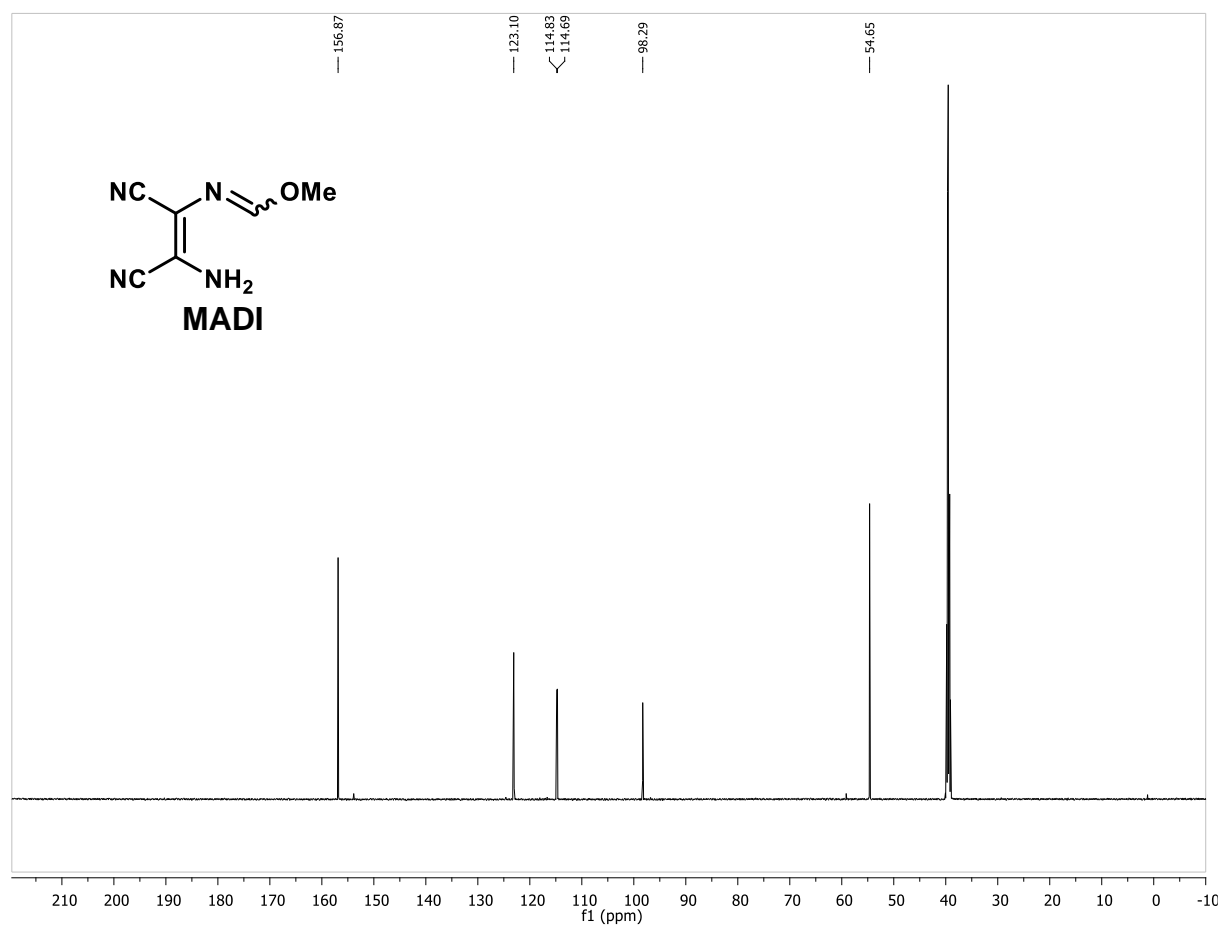
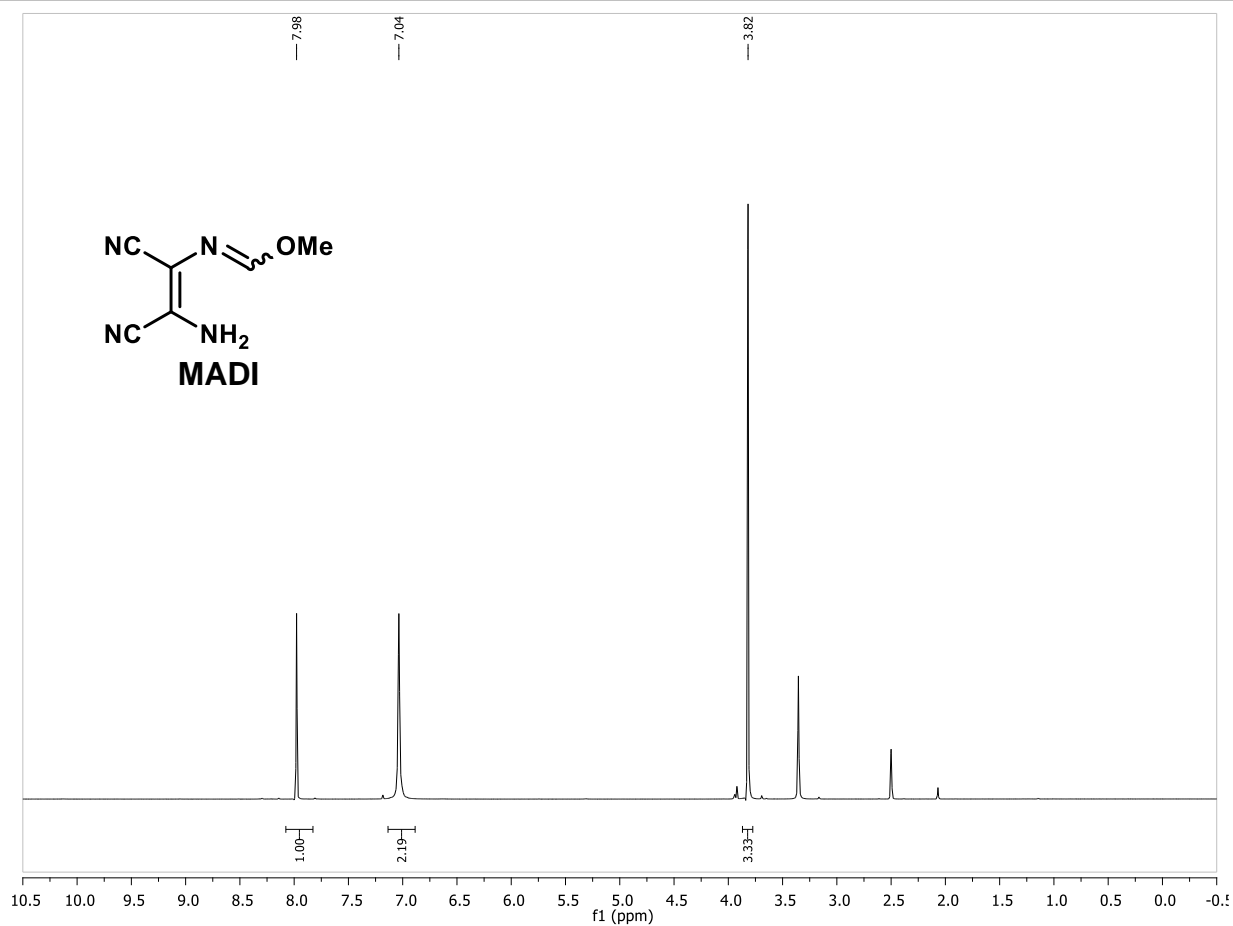
Methyl N-2-amino-1,2-dicyanovinylformimidate (MADI)¹: To a 2-L three-neck round bottom flask equipped with an overhead stirrer and thermocouple was charged 2,3-diaminomaleonitrile (DAMN) (100 g, 1.0 equiv., 926 mmol) and reagent grade MeOH (400 mL). The mixture was stirred at room temperature for 5 minutes. Trimethyl orthoformate (TMOF) (120 mL, 1.2 equiv., 1.11 mol) was charged to the reaction mixture in one portion. Afterwards, trifluoroacetic acid (TFA) (50 μ L, 0.005 equiv, 4.63 mmol) was charged to the reaction in one portion. (*This reaction is rapid and results in a thick slurry within 5 minutes, therefore, overhead stirring is required. Additionally, care should be taken to avoid adding excess TFA as this causes the reaction mixture to thicken to a viscosity where stirring is challenging. Poor mixing results in lower isolated yields.*) The reaction was heated to an internal temperature of 40 °C and stirred for 2 hours. After consumption of the starting materials as determined by GC-MS, reagent grade hexanes (600 mL) was charged to the reaction mixture in one portion. The suspension was allowed to slowly cool to room temperature over the course of approximately 20 minutes, then cooled to an internal temperature 0 °C and stirred an additional hour. The solids were isolated by vacuum filtration and dried under vacuum to afford a pale-yellow solid in a 93% yield (128.9 g), with an GC-MS assay purity of 98%.

¹H-NMR (600 MHz, DMSO- d_6): δ = 7.98 (s, 1H), 7.04 (bs, 2H), 3.82 (s, 3H) ppm.

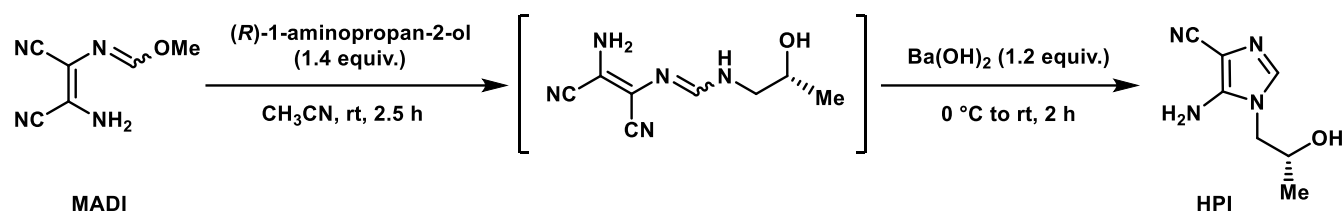
¹³C-NMR (150 MHz, DMSO- d_6): δ = 156.8, 123.0, 114.8, 114.6, 98.2, 54.6 ppm.

FTIR (ATR, neat) 3466, 3356, 2238, 2196, 1636, 1606, 1369, 1271, 912, 799, 501 cm^{-1} .

HRMS (ESI) m/z calculated for $\text{C}_6\text{H}_6\text{N}_4\text{OH}$ $[\text{M} + \text{H}]^+$ 151.0620, found 151.0614.



Synthesis of HPI (15)



(R)-5-amino-1-(2-hydroxypropyl)-1H-imidazole-4-carbonitrile (HPI)¹: To a 500-mL three-neck round bottom flask fitted with an internal temperature probe was added (*R*)-1-aminopropan-2-ol (18.2 mL, 231 mmol, 1.4 equiv.) and MeCN (25 mL) at room temperature. The solution was stirred for 5 minutes. MADI (25.0 g, 167 mmol, 1.0 equiv.) was suspended in MeCN (175 mL) and charged to the reaction mixture. Additional MeCN (15 mL) was used to rinse the reactor walls to rinse down any adhering solid. After addition of MADI, the reaction mixture turned dark brown and was stirred at room temperature for 2.5 hours. Conversion to the desired amino-alcohol intermediate was monitored by HPLC while the consumption of MADI was monitored by GC-MS. After complete conversion, the reaction mixture was cooled to an internal temperature of 0 – 5 °C using an ice bath. Solid Ba(OH)₂ monohydrate (37.9 g, 200 mmol, 1.2 equiv.) was charged with deionized H₂O (113.7 mL). The reaction was warmed to room temperature. As the reaction proceeded, the color turned from bright red to dark brown with visible precipitate stirring at the bottom of the flask. Complete conversion to the desired HPI product occurred at 1 hour 15 min as tracked by HPLC.

After 1 hour 15 min, the reaction mixture was filtered on a Büchner funnel and the round bottom flask was rinsed with MeCN (3 x 25 mL) to give a total volume of approximately 300 mL. This was concentrated to approximately 200 mL via rotary evaporation under reduced pressure at 40 °C. The solution was then heated to 50 °C at which time the mixture turned dark brown. After stirring in a 40 °C oil bath for 10 minutes, the solution was charged with DCM (200 mL) in one portion and allowed to cool to room temperature over the course of 1 hour. The mixture became biphasic with a black oil visible in the bottom of the flask. The solution was then cooled to an internal temperature of 0 – 5 °C and stirred for an additional 1 hour. Upon cooling, precipitation was observed and the mixture was filtered on a Büchner funnel with the black oil remaining in the filtrate to afford HPI as a pale grey solid. The solid was dried under vacuum to yield 23.7 g of product in an 82% yield with a 96% HPLC purity based on peak area.

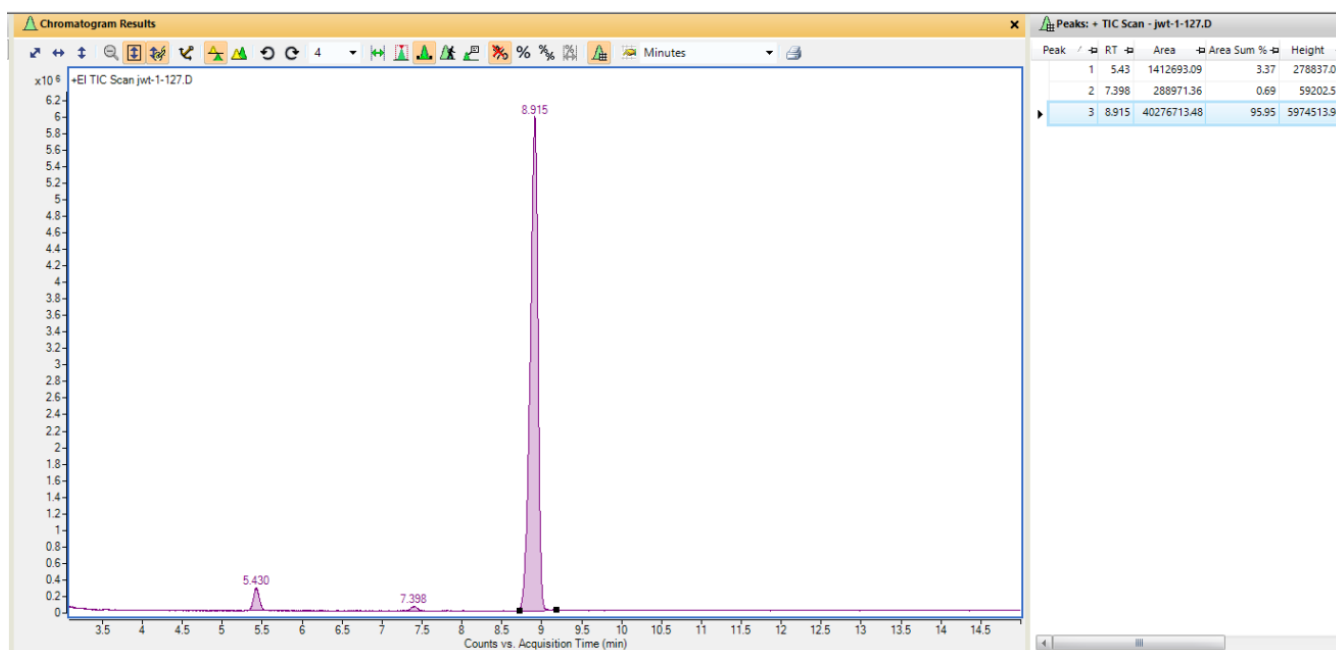
¹H-NMR (600 MHz, DMSO-*d*₆): δ = 7.11 (s, 1H), 6.07 (s, 2H), 5.06 (s, 1H), 3.85 – 3.69 (m, 2H), 3.65 – 3.50 (m, 1H), 1.03 (d, *J* = 3.9 Hz, 3H) ppm.

¹³C-NMR (150 MHz, DMSO-*d*₆): δ = 147.9, 133.5, 117.6, 90.1, 64.8, 50.1, 20.6 ppm.

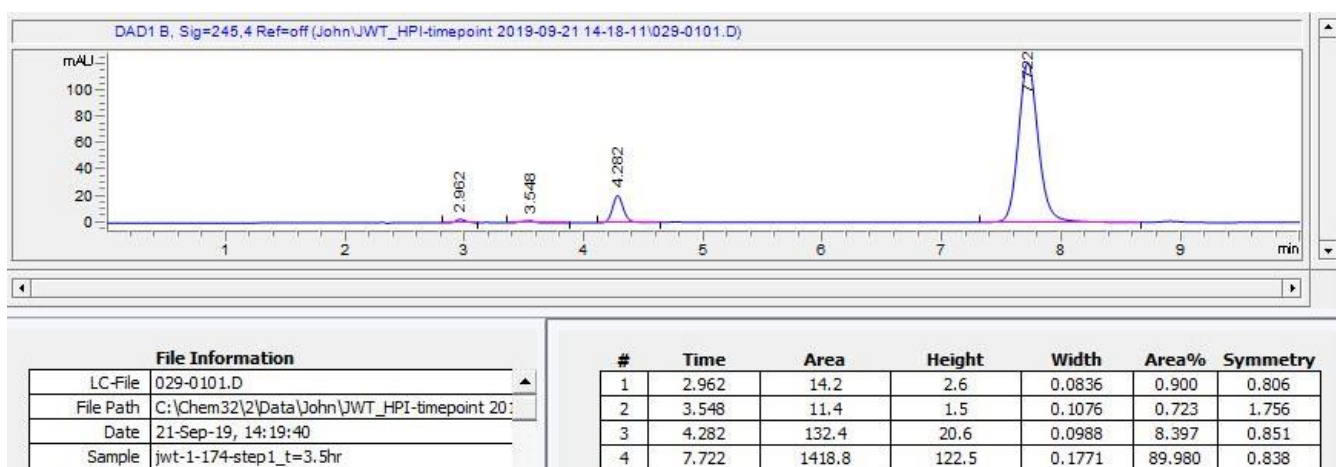
FTIR (ATR, neat) 3159, 2208, 1570, 1174, 1090 cm⁻¹.

HRMS (ESI) *m/z* calculated for C₇H₁₀N₄OH [M + H]⁺ 167.0933, found 167.0926.

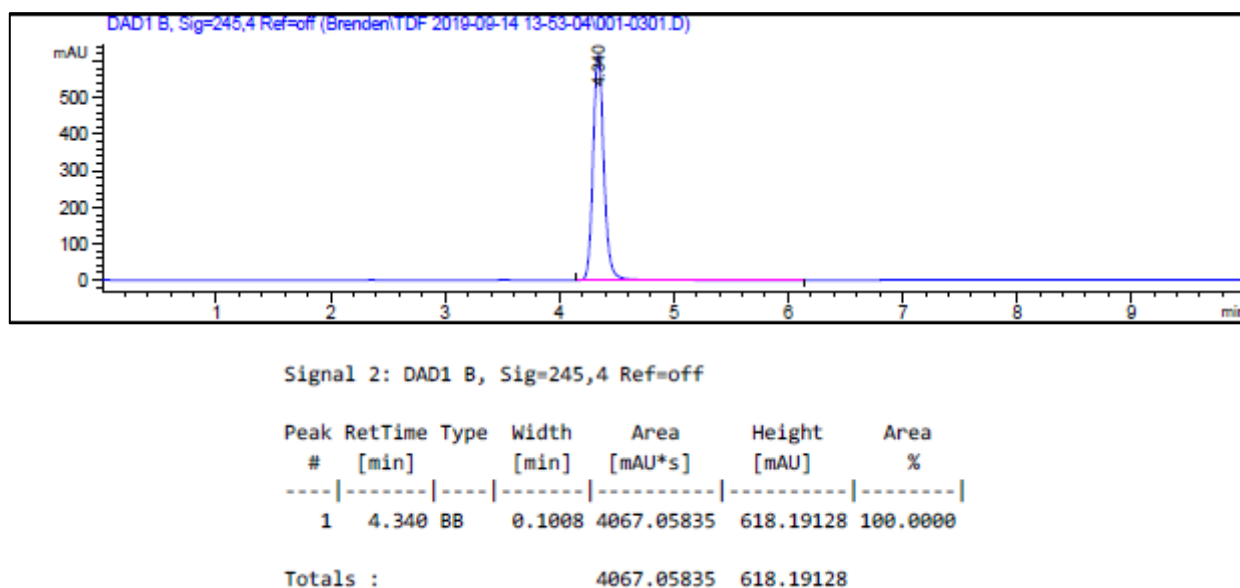
MADI GC-MS Chromatogram (MADI elution times (*E*- and *Z*- isomers): 5.430 and 8.915 min; DAMN elution time: 7.398)



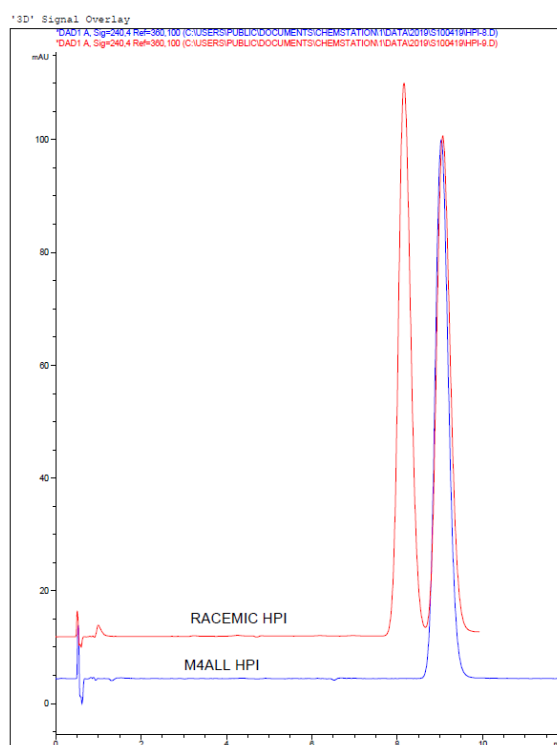
Amidine Intermediate HPLC Chromatogram (HPI elution time: 4.282 min; amidine intermediate 7.772 min)

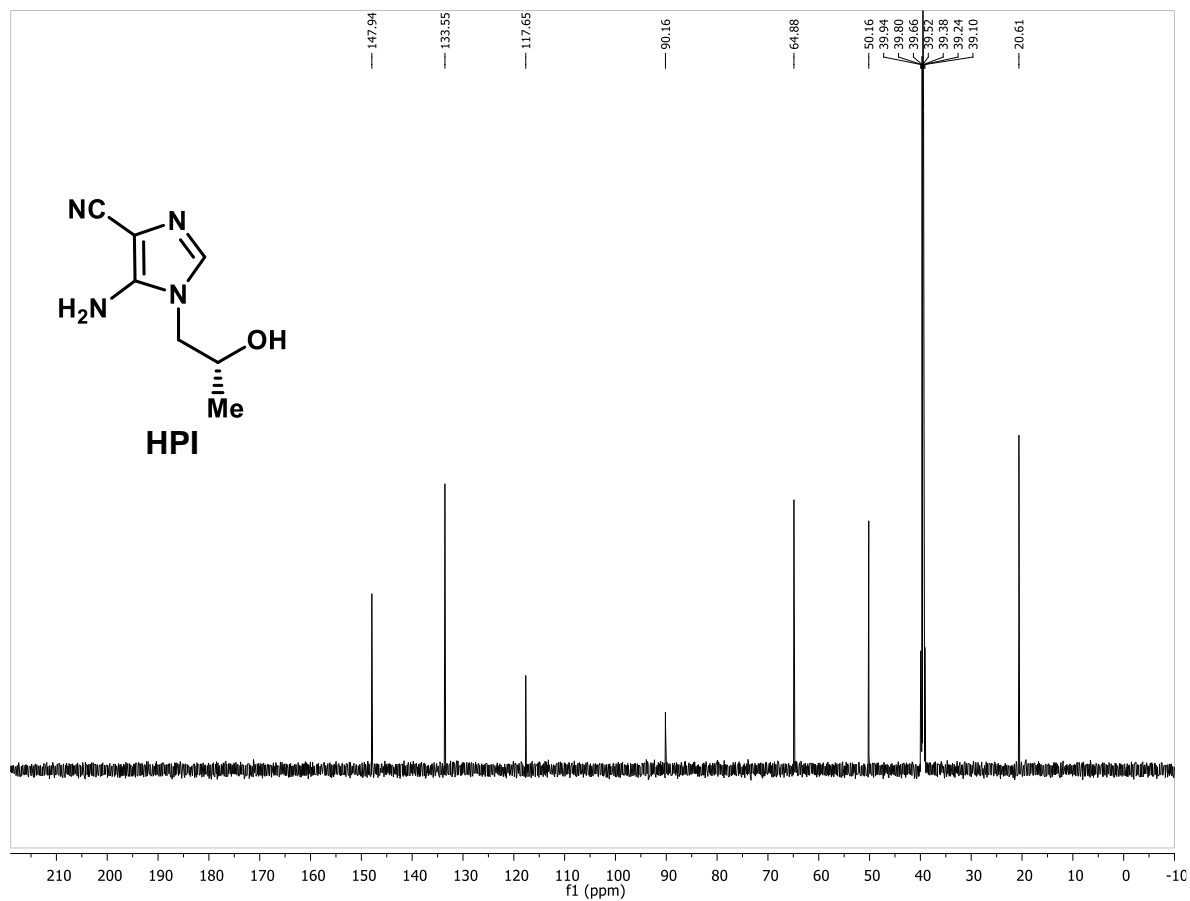
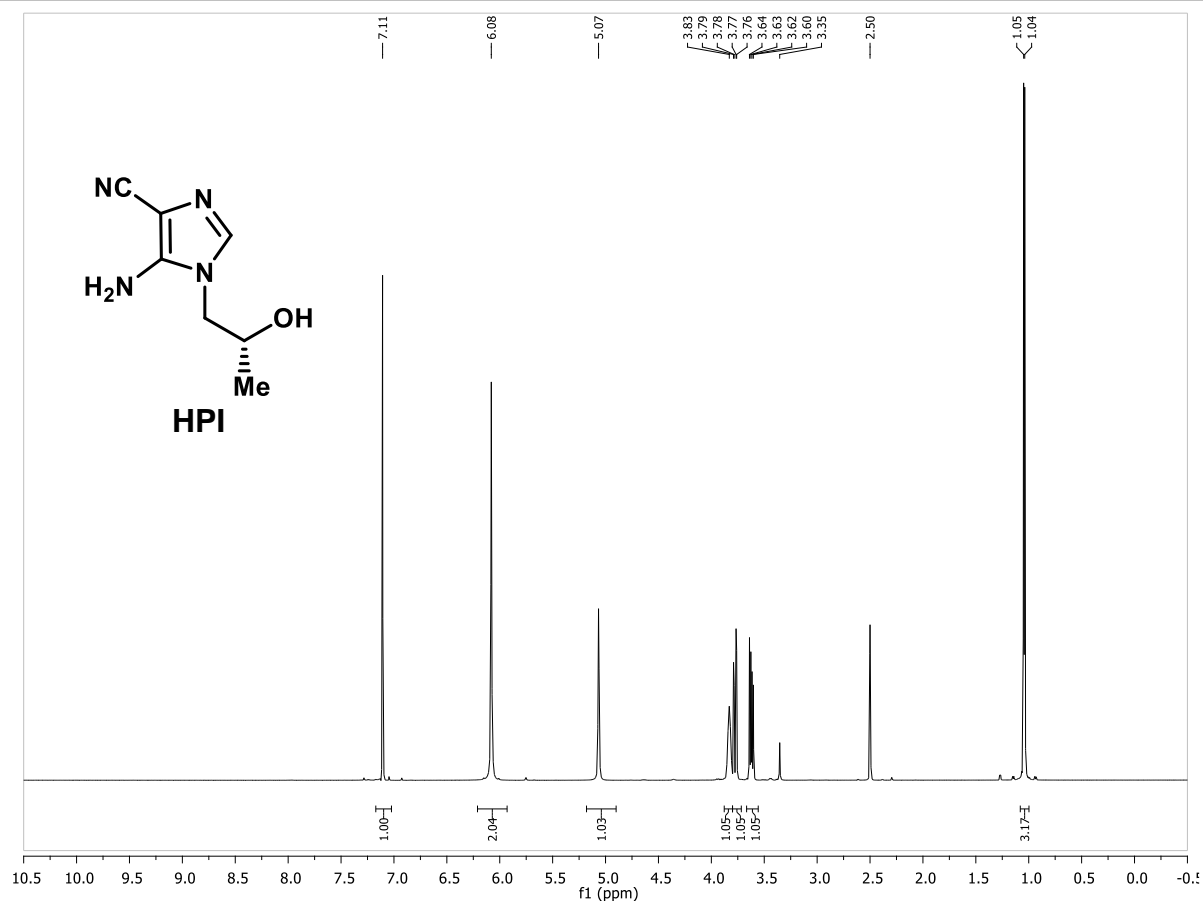


HPI HPLC Chromatogram (HPI elution time: 4.340 min)

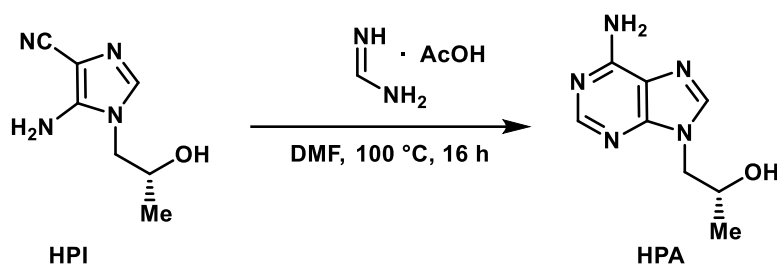


The chiral purity of HPI were established via supercritical fluid chromatography. A ChiralPak IC column (10 cm X 0.46 cm ID) was used with a mobile phase consisting of 10% ethanol: 90% CO₂ pumped at 2 mL/min. Racemates were well-resolved under these conditions. Both materials produced using this chemistry showed 100% chiral purity.





Synthesis of HPA



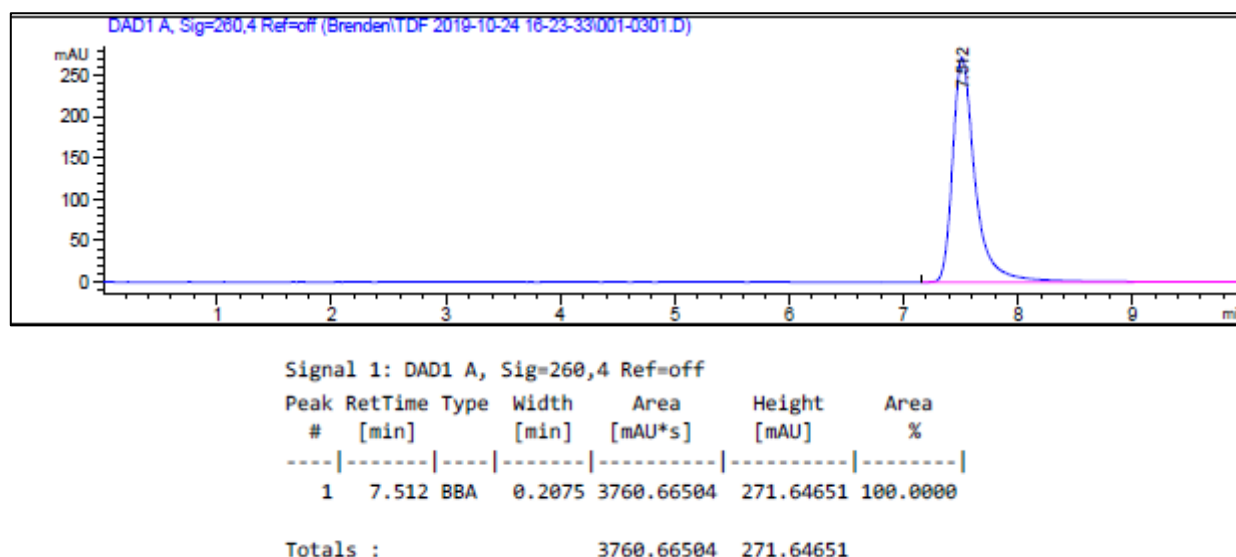
(*R*)-1-(6-Amino-9*H*-purin-9-yl)propan-2-ol (HPA)^{1,2}: To a 500-mL 3-necked round bottom flask, the mixture of (*R*)-5-amino-1-(2-hydroxypropyl)-1*H*-imidazole-4-carbonitrile (HPI, 97.8% purity) (25.0 g, 150 mmol, 1.0 equiv.) and formamidine acetate (26.6 g, 256 mmol, 1.7 equiv.) was added in DMF (50 mL). The reaction vessel was equipped with a J-KEM internal temperature probe and the mixture was heated to an internal temperature of 100 °C for 16 hours. After completion of the reaction as monitored by HPLC, the heating was turned off and isopropyl alcohol (150 mL) was added. The reaction mixture was cooled down to room temperature (25 °C). The reaction mixture was cooled to an internal temperature of -15 °C and stirred for three hours at same temperature. The suspension was filtered and washed with cold isopropanol (38 mL + 37 mL). The isolated solid was dried in vacuum oven at 65 °C for two hours to afford (*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-ol (HPA) as a white solid, (26.67 g, 93% adjusted with a purity of 99% by HPLC). The spectroscopic data are in accordance with reported values.

¹H NMR (600 MHz, DMSO) δ = 8.13 (s, 1H), 8.05 (s, 1H), 7.18 (s, 2H), 5.04 (bs, 1H), 4.18 – 4.05 (m, 1H), 4.05 – 3.94 (m, 2H), 1.06 (d, J = 5.9, 3H) ppm.

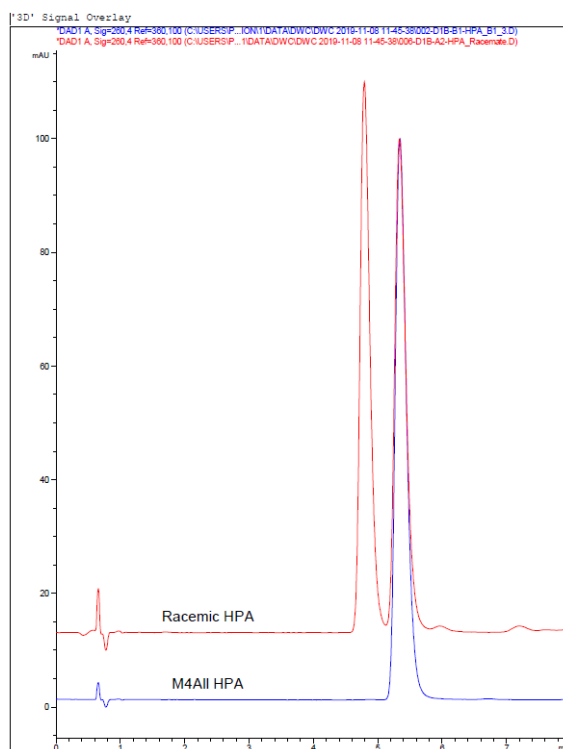
¹³C NMR (150 MHz, DMSO) δ = 155.9, 152.2, 149.7, 141.5, 118.5, 64.6, 50.1, 20.9 ppm.

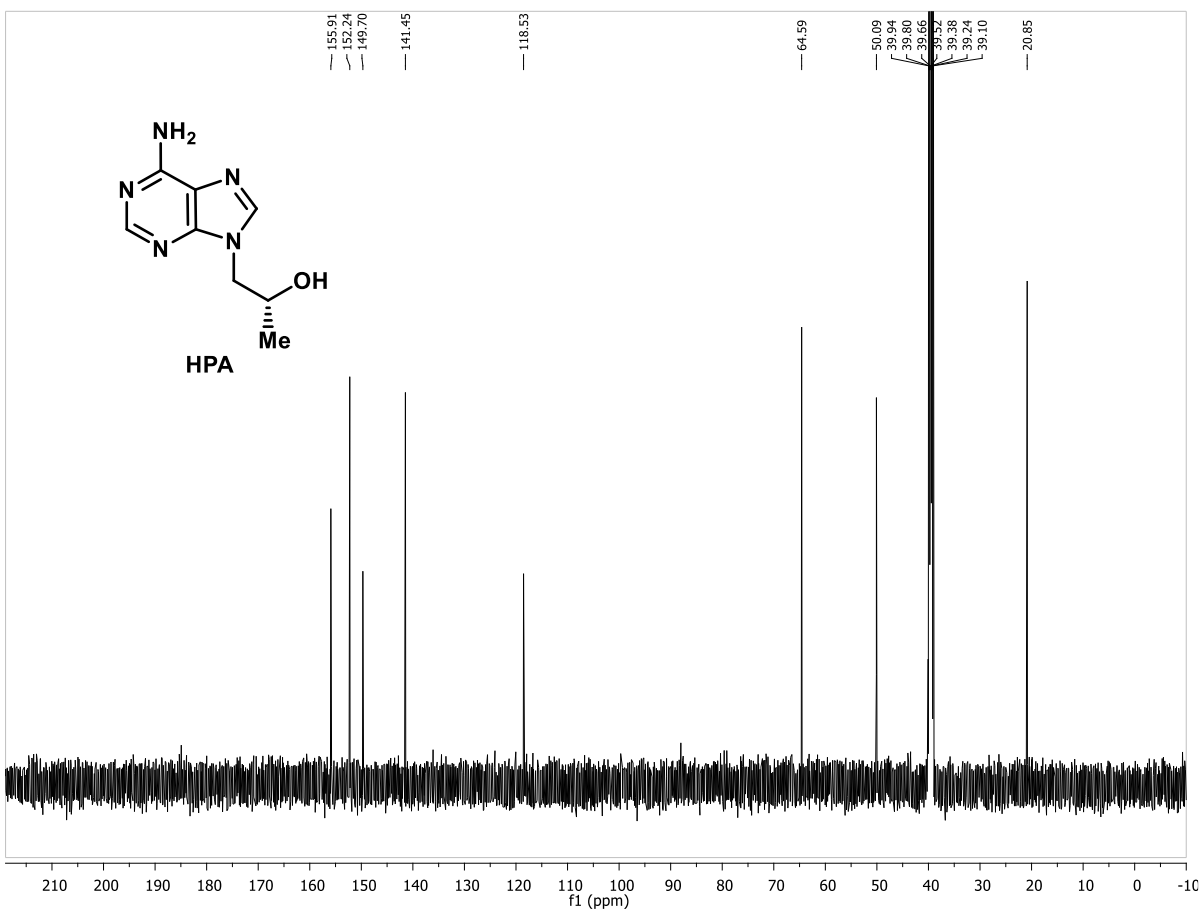
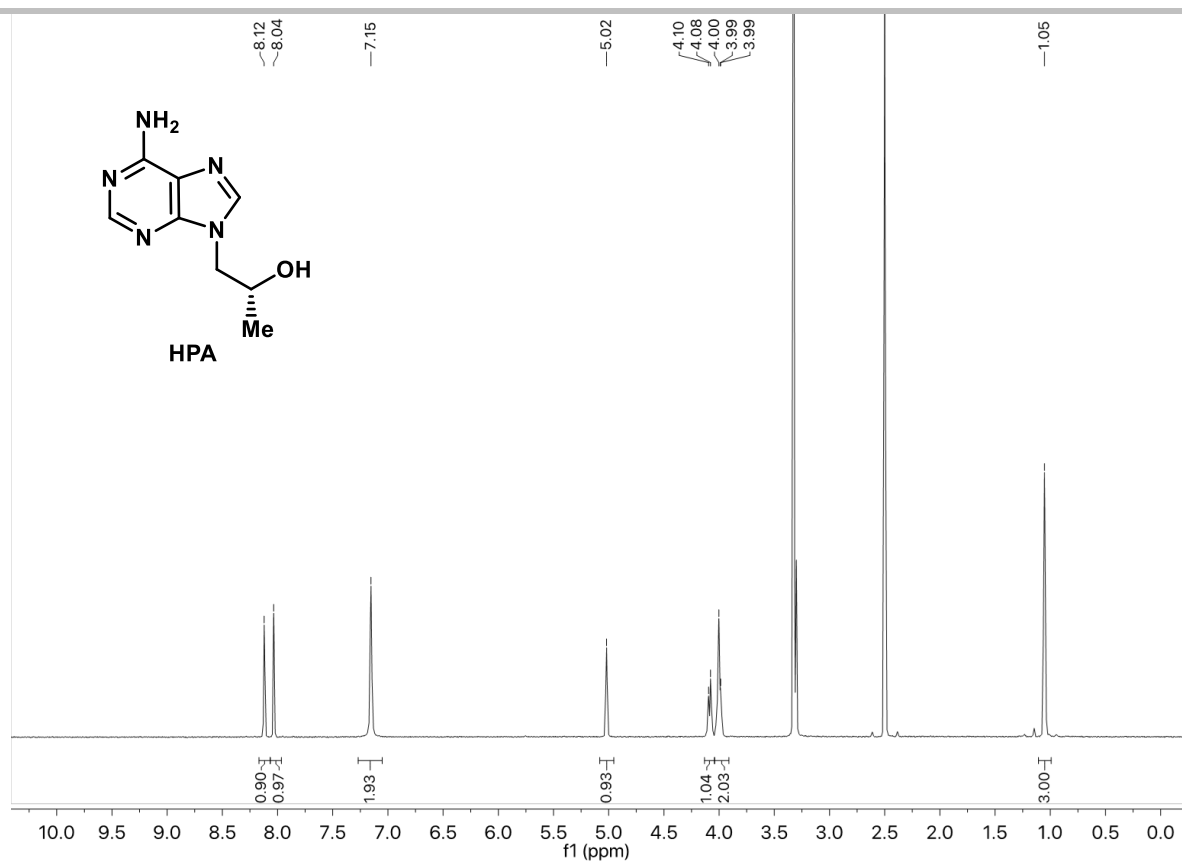
HRMS (ESI) m/z calculated for C₈H₁₁N₄OH [M + H]⁺ 194.1042, found 194.1040.

HPA HPLC Chromatogram (HPA elution time: 7.512 min)

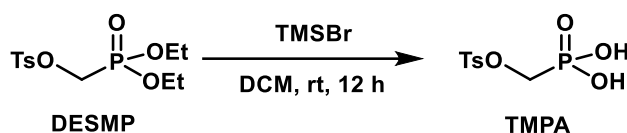


The chiral purity of HPI were established via supercritical fluid chromatography. A ChiralPak IC column (10 cm X 0.46 cm ID) was used with a mobile phase consisting of 10% ethanol: 90% CO₂ pumped at 2 mL/min. Racemates were well-resolved under these conditions. Both materials produced using this chemistry showed 100% chiral purity.





Synthesis of TMPA



((tosyloxy)methyl)phosphonic acid (TMPA)³: To a 250-mL round bottom flask was added (diethoxyphosphoryl)methyl 4-methylbenzenesulfonate (25.3 g, 78.8 mmol, 1.0 equiv.) and dissolved in DCM (100 mL). With stirring, TMSBr (30.1 g, 197 mmol, 2.1 equiv.) was added in one portion, no exothermic reaction was observed on this scale. The reaction mixture was stirred overnight at room temperature. The solvent was removed via rotary evaporation under reduced pressure and the residue was dissolved in 1:1 ethanol/water (50 mL) to cleave the silyl ether bond to give the free phosphonic acid and the solvent was immediately removed via rotary evaporation under reduced pressure. The residue was re-dissolved in ethanol (25 mL) then azeotropically dried with toluene (2 x 50 mL). The toluene was removed via rotary evaporation under reduced pressure to yield TMPA as a colorless oil which, upon standing, crystallized as a white solid. This solid was further dried under vacuum at 50 °C to give pure product (20.4 g, 97%).

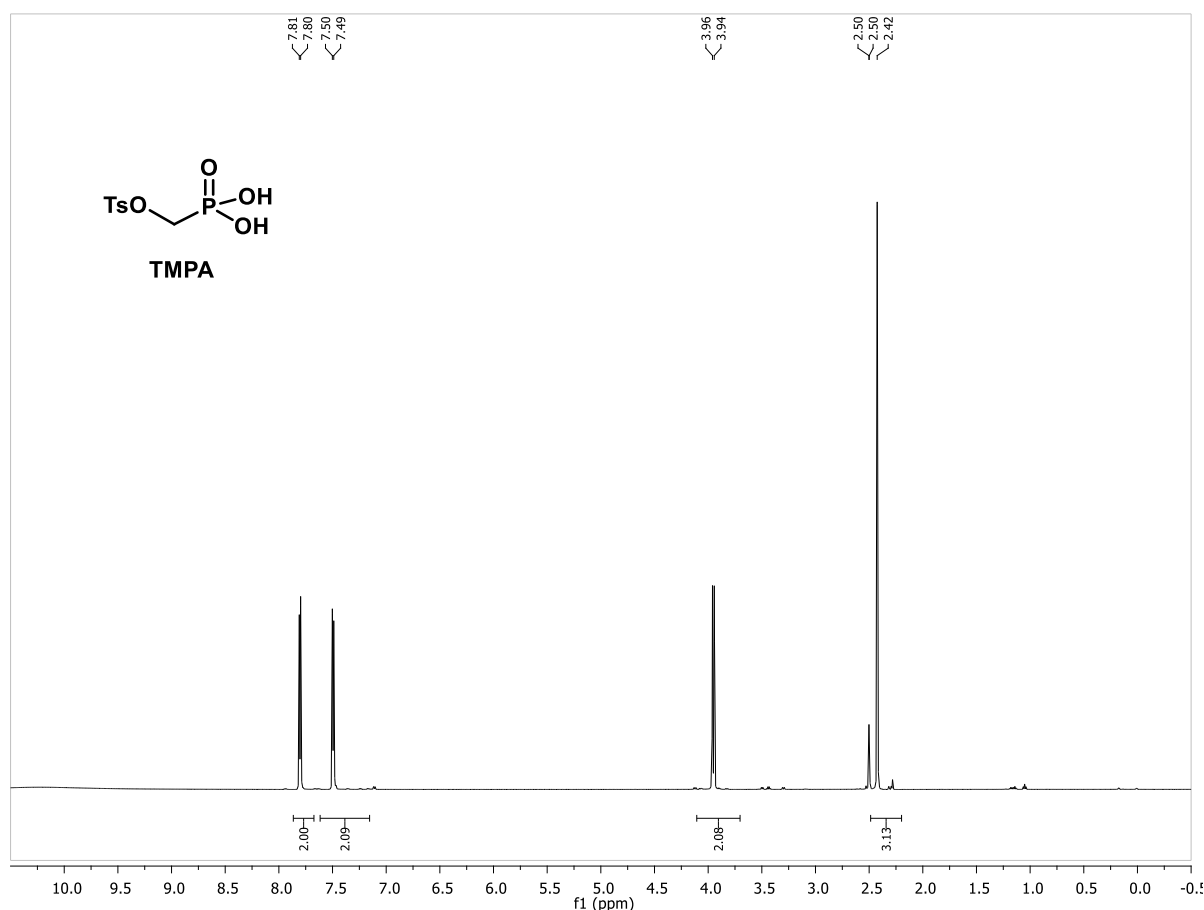
¹H NMR (600 MHz, DMSO) δ = 7.80 (d, J = 7.6, 2H), 7.50 (d, J = 7.9, 2H), 3.95 (d, J = 10.0, 2H), 2.42 (s, 3H) ppm.

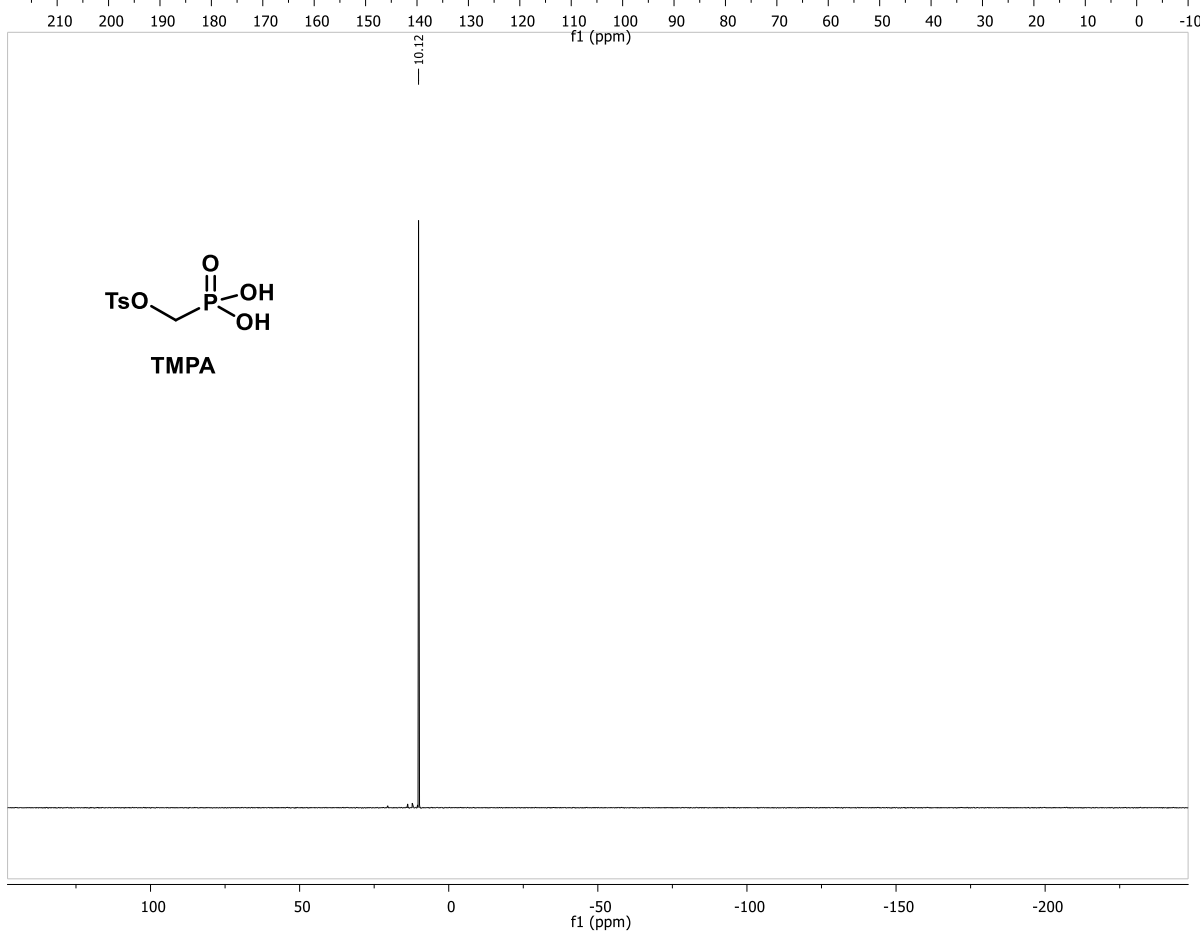
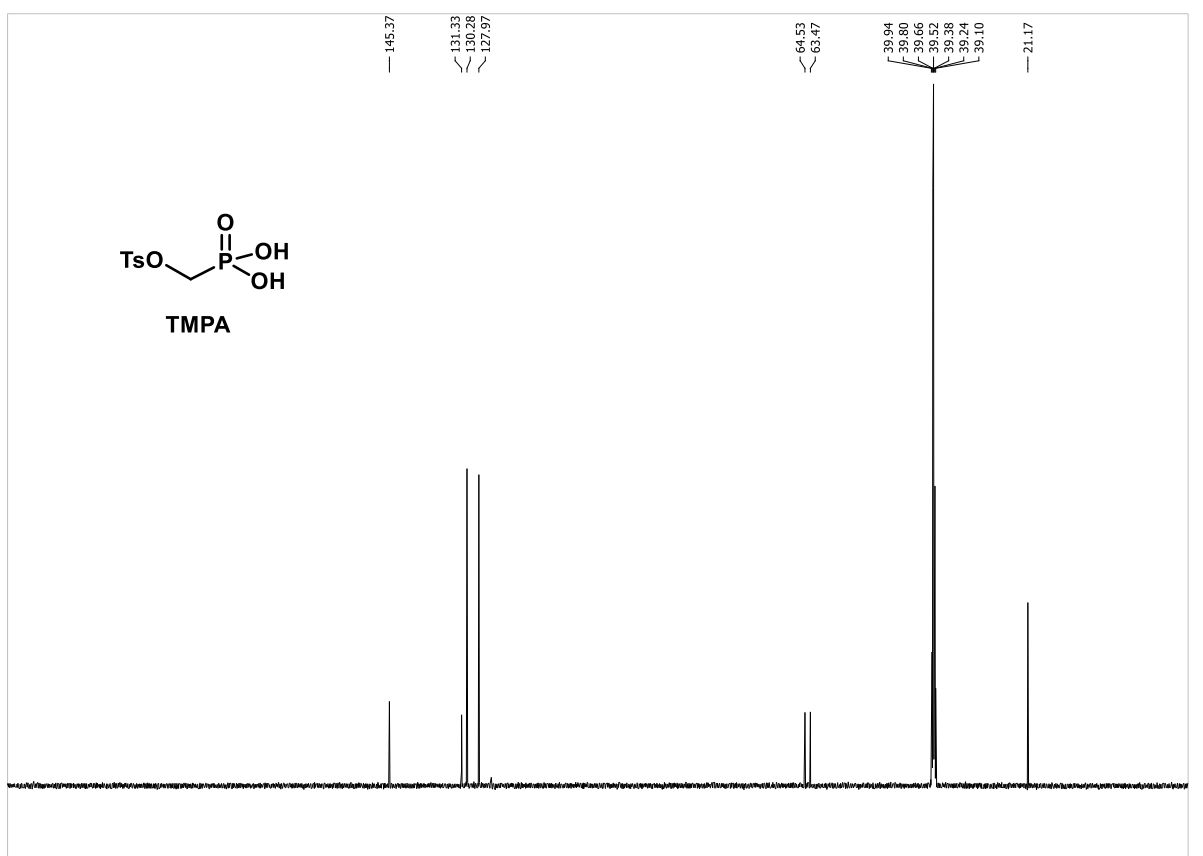
¹³C NMR (150 MHz, DMSO) δ = 145.4, 131.3, 130.3, 127.9, 64.0 (d, J = 159.2), 21.2 ppm.

³¹P NMR (243 MHz, DMSO) δ = 10.1 ppm.

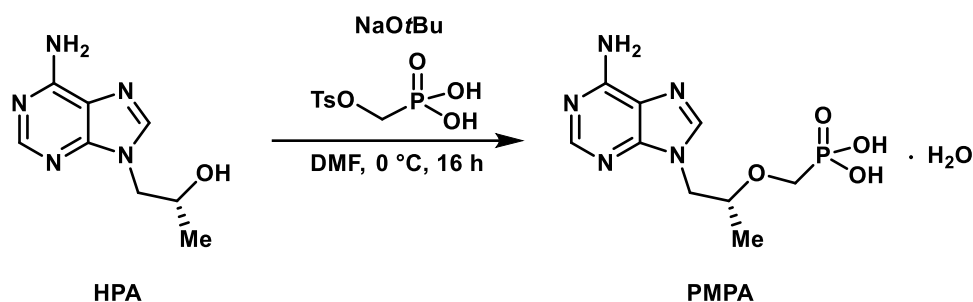
FTIR (ATR, neat) 2731, 2315, 1595, 1363, 1175, 1024, 1008, 936, 659, 551, 456 cm⁻¹.

HRMS (ESI) m/z calculated for C₈H₁₁O₆PSH [M + H]⁺ 267.0092, found 267.0091.





Synthesis of PMPA (1)



(R)-(((1-(6-amino-9H-purin-9-yl)propan-2-yl)oxy)methyl)phosphonic acid (PMPA)²: A 500-mL three necked round bottom flask was equipped with a J-KEM internal temperature probe, overhead stirrer and nitrogen line. The flask was charged with anhydrous DMF (400 mL) and cooled to an internal temperature of 2 – 5 °C under an atmosphere of nitrogen. With stirring (300 RPM), sodium *tert*-butoxide (74.5 g, 776 mmol, 6.0 equiv.) was charged to the reaction vessel to afford a clear solution and a temperature increase of 12 °C was observed. To the clear solution was added (*R*)-1-(6-amino-9H-purin-9-yl)propan-2-ol (HPA, 92% purity) (25.0 g, 129.4 mmol, 1.0 equiv.) over 10 minutes with stirring (5 x 5 g portions). The yellow solution was stirred at an internal temperature of 5 °C for 30 minutes. Solid ((tosyloxy)methyl)phosphonic acid (58.5 g, 220 mmol, 1.7 equiv.) was added over 10 minutes in four portions (~14.6 g portions), the internal temperature was not allowed to exceed 15 °C. After the addition was complete, the reaction mixture was warmed to room temperature overnight (16 hours). After complete consumption of the starting material, as indicated by HPLC, the solvent was removed via rotary evaporation under reduced pressure. The residue was dissolved in 250 mL of water and concentrated hydrochloric acid (approx. 40 mL) was added until pH = 2.8 – 3.0. A precipitate formed and the mixture was stirred at an internal temperature of 5 °C for 3 hours then collected by vacuum filtration. The resulting off white solid was washed with cold H₂O (25 mL), a cold 1:1 mixture H₂O/acetone (25 mL) and ice-cold acetone (25 mL). The solid was dried *in vacuo* at 75 °C overnight to yield PMPA as a white solid (26.2 g, 70% adjusted for KF). KF Titration: 2.4%. The spectroscopic data are in accordance with reported values.

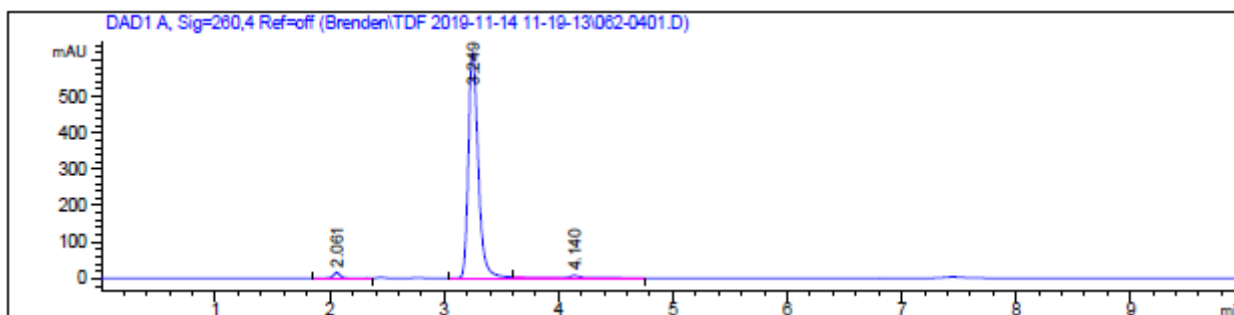
¹H NMR (600 MHz, DMSO) δ = 8.15 (s, 2H), 7.25 (s, 2H), 4.28 (dd, J = 14.4, 3.7, 1H), 4.17 (dd, J = 14.4, 5.6, 1H), 3.91 (dd, J = 10.7, 5.3, 1H), 3.59 (p, J = 13.2, 2H), 1.03 (d, J = 6.2, 3H) ppm.

¹³C NMR (150 MHz, DMSO) δ = 155.8, 152.2, 149.7, 141.6, 118.2, 75.3 (d, J = 12.1), 64.4 (d, J = 161.7), 46.4, 16.9 ppm.

³¹P NMR (243 MHz, DMSO) δ = 16.6 ppm. cm^{-1} .

HRMS (ESI) m/z calculated for C₉H₁₄N₅O₄PH [M + H]⁺ 288.0862, found 288.0853.

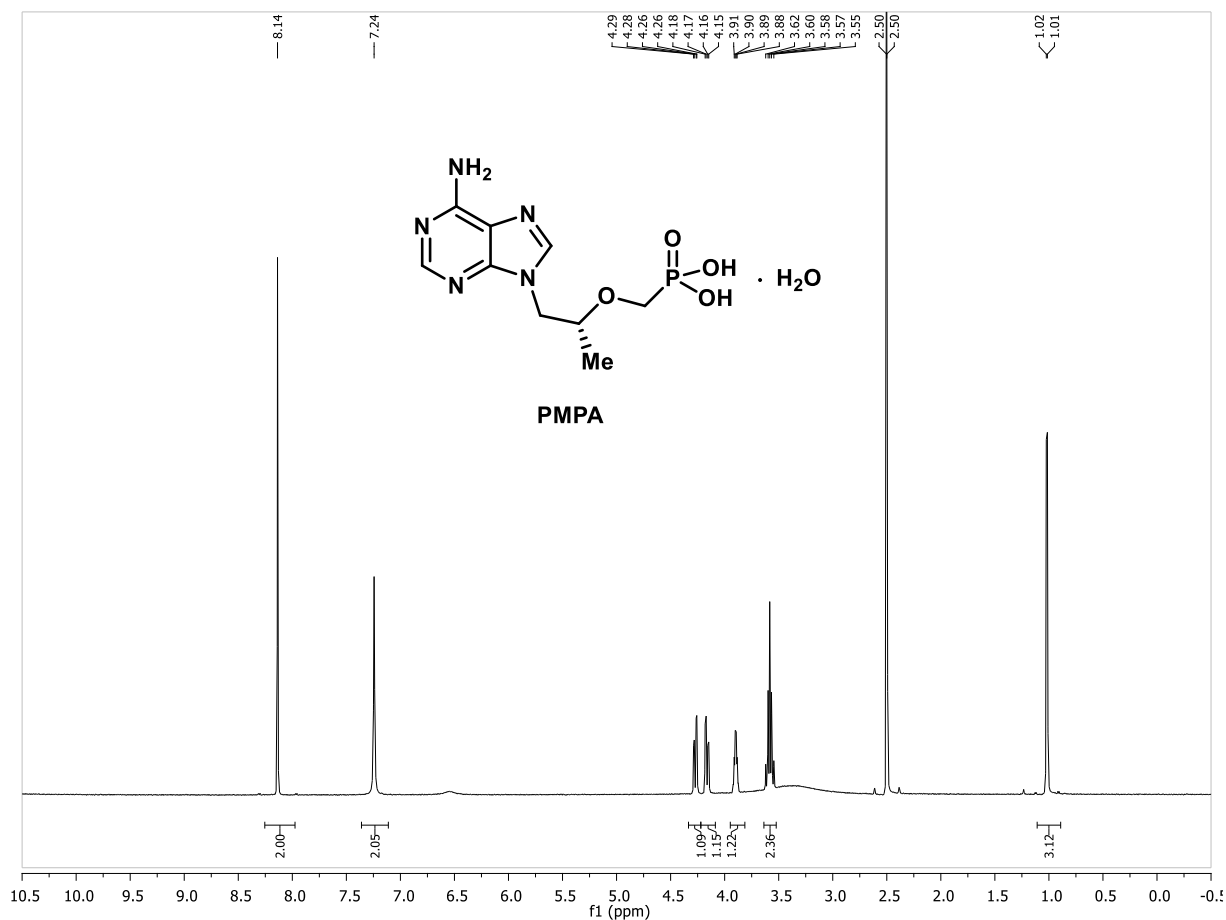
PMPA HPLC Chromatogram (*bis*-alkylated HPA elution time: 2.061 min; PMPA elution time: 3.249 min)

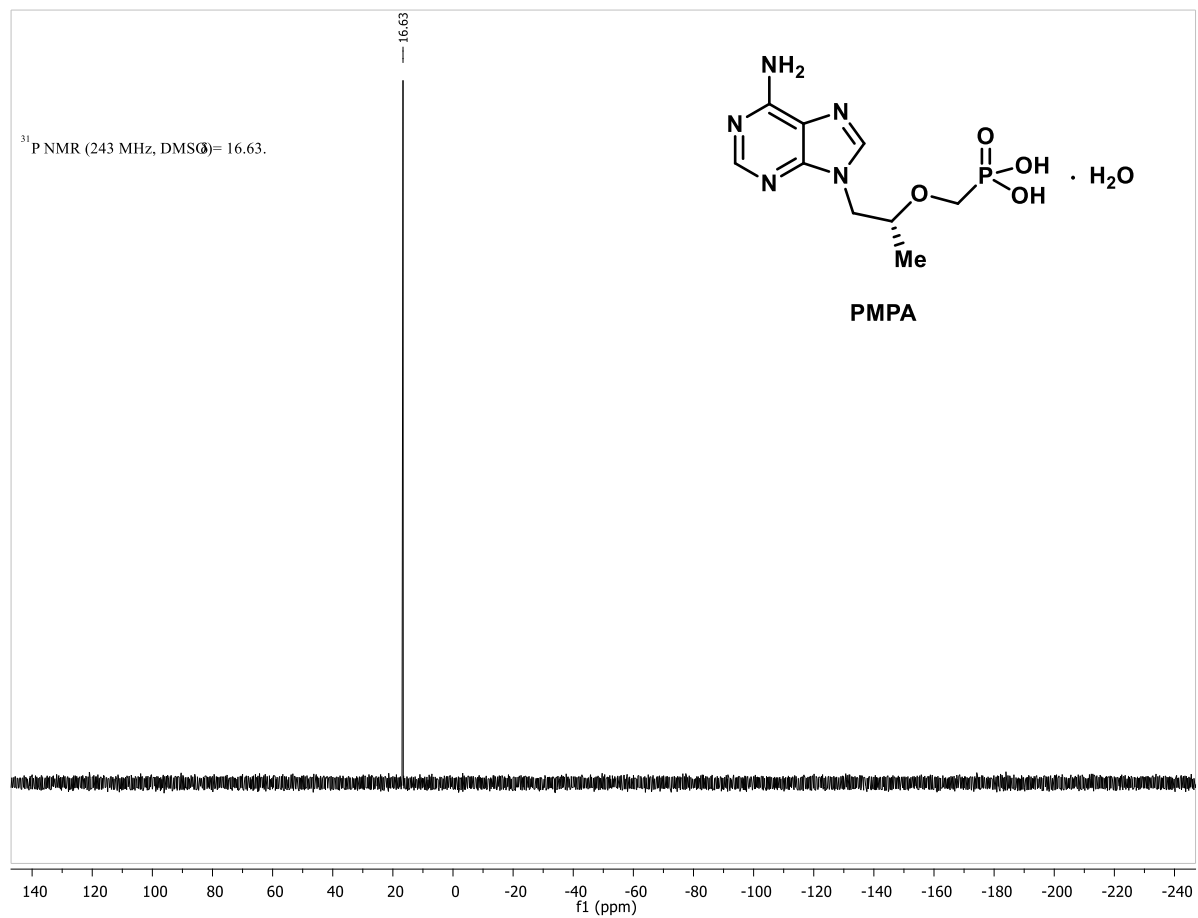
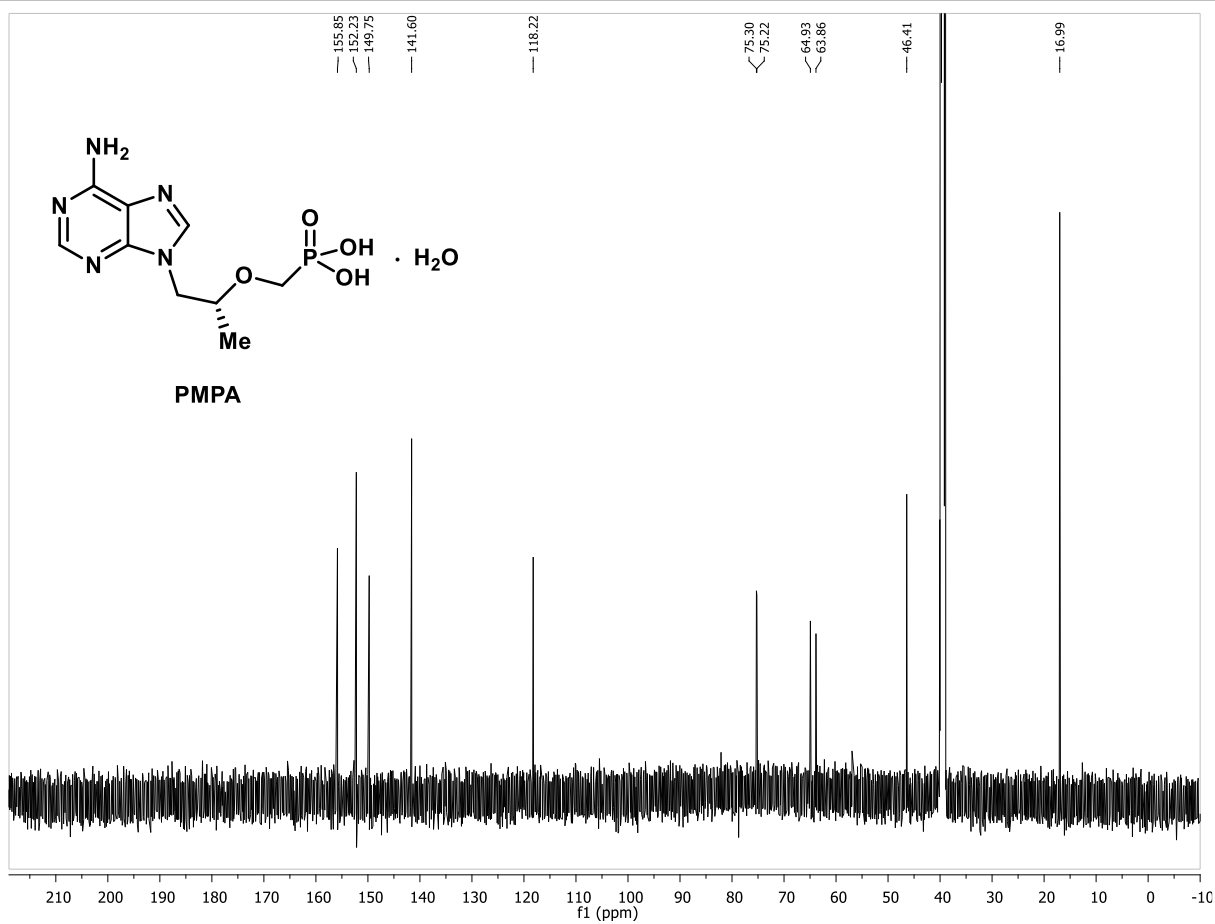


Signal 1: DAD1 A, Sig=260,4 Ref=off

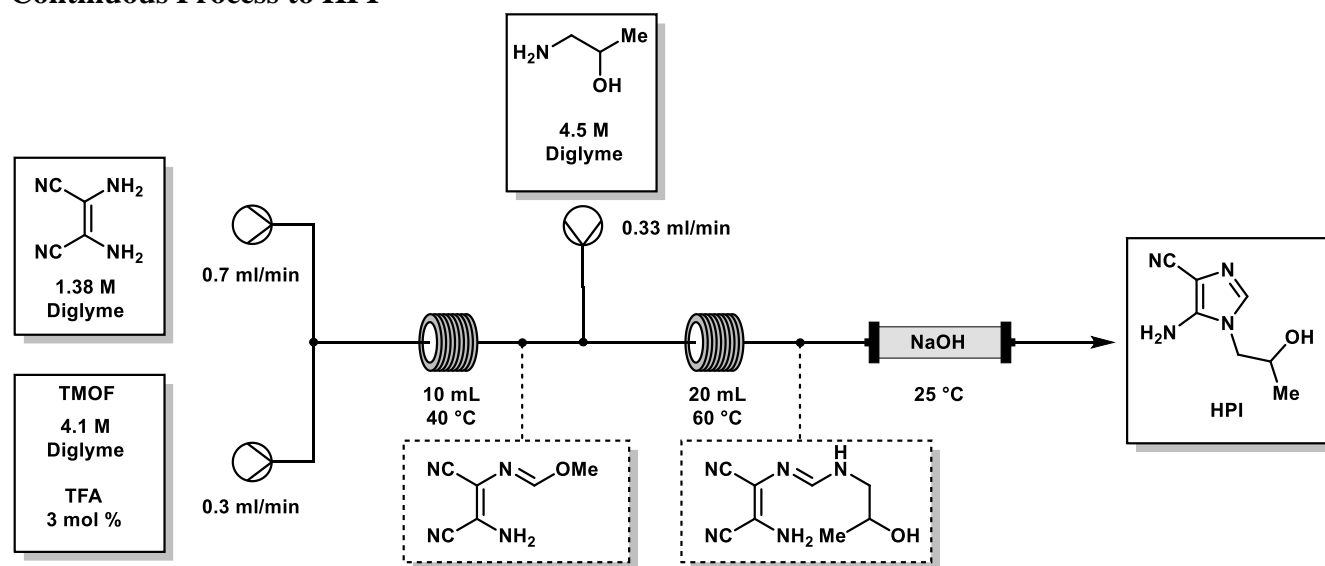
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.061	BV R	0.0657	72.41965	16.34308	1.8549
2	3.249	BV R	0.0931	3768.59546	618.38422	96.5247
3	4.140	VB E	0.1162	63.26757	7.67407	1.6205

Totals : 3904.28268 642.40136





Continuous Process to HPI



5-amino-1-(2-hydroxypropyl)-1H-imidazole-4-carbonitrile (HPI): A 1.38 M stock solution of diaminomaleonitrile (DAMN) (37.3 g, 344 mmol) was prepared in diglyme (250 mL) at room temperature, and the molarity was confirmed by quantitative NMR. A 4.1 M solution of trimethylorthoformate (44.0 g, 414 mmol) in diglyme (100 mL) with TFA (3 mol %) was prepared and the molarity was confirmed by quantitative NMR. The DAMN solution was pumped at a rate of 0.7 mL/min and the TMOF/TFA solution at a rate of 0.3 mL/min using a commercial, meso-scale (either fitted with HPLC or peristaltic pumps) reactor set up using 0.125 in diameter FEP tubing for all reactor setups. The two solutions were connected via a T-union and pumped through a 10 mL reactor at 40 °C. The solution of the amino-alcohol (4.5 M in diglyme) was pumped at a rate of 0.33 mL/min and connected via T-union to the previous reactor setup. The resulting mixture was passed through a 20 mL FEP loop at 60 °C. When the system had reached steady state, as indicated by HPLC, a packed bed of sodium hydroxide (18.1 g, 450 mmol) was attached to the previous reactor setup. Initially, the solution containing the desired product was yellow/orange which over 15 minutes became dark brown/black. Upon collection, the reaction mixture was assayed by HPLC and quantitative NMR theoretically providing 7.28 g/hour of HPI (75 wt% assay yield). A packed bed of K₃PO₄ (18.6 g, 87.6 mmol) was attached to the previous reactor setup. The solution was passed through the packed bed at a rate of 1.33 mL/min. Upon contact with the basic material the solution began to darken which would slowly leach through the packed bed. Upon collection, the reaction mixture was assayed by HPLC and quantitative NMR theoretically providing 8.39 g/hour of HPI (87 wt% assay yield).



Packed bed of K_3PO_4 after use.



Packed bed of NaOH after use.

The above pictures show the channeling of the tar-like materials produced during the cyclization to HPI. This material prevents clean isolation if converted to HPA and therefore removal is prudent to ensure quality material is recoverable.

Initial Results for Process Development of HPA

We have further developed the initial research route described in the manuscript. Herein is reported the initial process development, these conditions have been vetted for manufacturability and reproducibility.

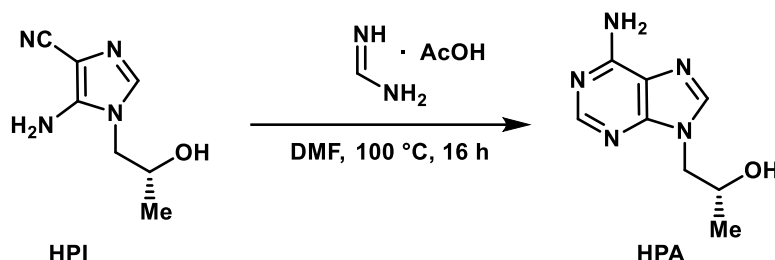


Table S1: Material balance sheet for synthesis of HPA from HPI.

Material	Mol. Wt. (g/mol)	mmol	Equiv.	Amount	Density (g/mL)
HPI	166.8	120.4	1.0	20.0 g	---
Formamidinium Acetate	104.1	204.6	1.7	21.30 g	---
DMF	72.1	---	---	40 mL	0.944
<i>i</i> PrOH	60.1	---	---	180 mL	0.786

Safety Precautions

1. HPI is a fine powder that can release cyanide when exposed to nucleophiles. Take precautions while handling HPI.
2. The anti-solvent (IPA) is added to the system at a temperature that is higher than the boiling point.

Production of (*R*)-1-(6-amino-9*H*-purin-9-yl)propan-2-ol (HPA)

1. A 250 mL three neck round bottom flask was equipped with an over-head stirrer, J-KEM temperature probe to check internal temperature, rubber septum and condenser (optional).
2. The flask was charged with HPI (20.0 g, 120 mmol, 1.0 equiv.), formamidinium acetate (21.3 g, 205 mmol, 1.7 equiv.) and DMF (40 mL).
3. The reaction mixture was stirred at 130 RPM.
4. The reaction mixture was heated to 110 °C (external temperature) using heating block (internal temperature around 100 °C).
5. The reaction was stirred at 100 °C (internal temperature) for 16 hours.
6. Reaction progress was monitored by HPLC (consumed HPI > 97%)
7. After complete consumption of starting material as indicated by HPLC, heating was turned off and reaction mixture was allowed to cool to 75 °C (internal temperature).

8. The isopropanol (120 mL) was added to reaction mixture with rate of 15 mL/min. (In the initial batches, IPA was added 100 °C (internal temperature); the condensation of IPA through condenser was not observed.
9. The reaction mixture cooled to room temperature (25 °C) over a period of time.
10. The reaction mixture was cooled to -15 °C (internal temperature) by using a NaCl salt/ice bath (1:3 ratio) and stirred for another 3 hours.
11. The suspension was filtered through a Büchner funnel.
12. The solid cake was washed with cold (-15 °C) isopropanol (30 mL + 30 mL).
13. The residue was dried for half an hour by passing air through the Büchner funnel.
14. The solid was transferred to tared glass tray and dried in vacuum oven at 65 °C, until loss of mass was less than 1%.
15. The HPA was obtained as a white to off-white solid (21.18 g, 92% assay by HPLC, 98 area% at 210 nm, 100% chiral purity, $\leq 0.10\%$ isopropanol; isolated adjusted yield = 88%).

Sensitivity Assessment

Though the reaction proceeds in excellent yield, variability was found in ability to consistently isolate high quantities of high purity product. Depending on nature of the experimental run, the following factor was found to have high impact on the reaction outcome as judged by purity and yield.

Keeping all of the reaction materials in solution was found to be an important factor in successful reproduction of results. On occasion, precipitation of colorless crystals above the reaction media was observed (Figure S1), and when this occurred, purity of the isolated product dropped sharply (Table S2). Based on ^{13}C NMR, this crystal is believed to be ammonium acetate.

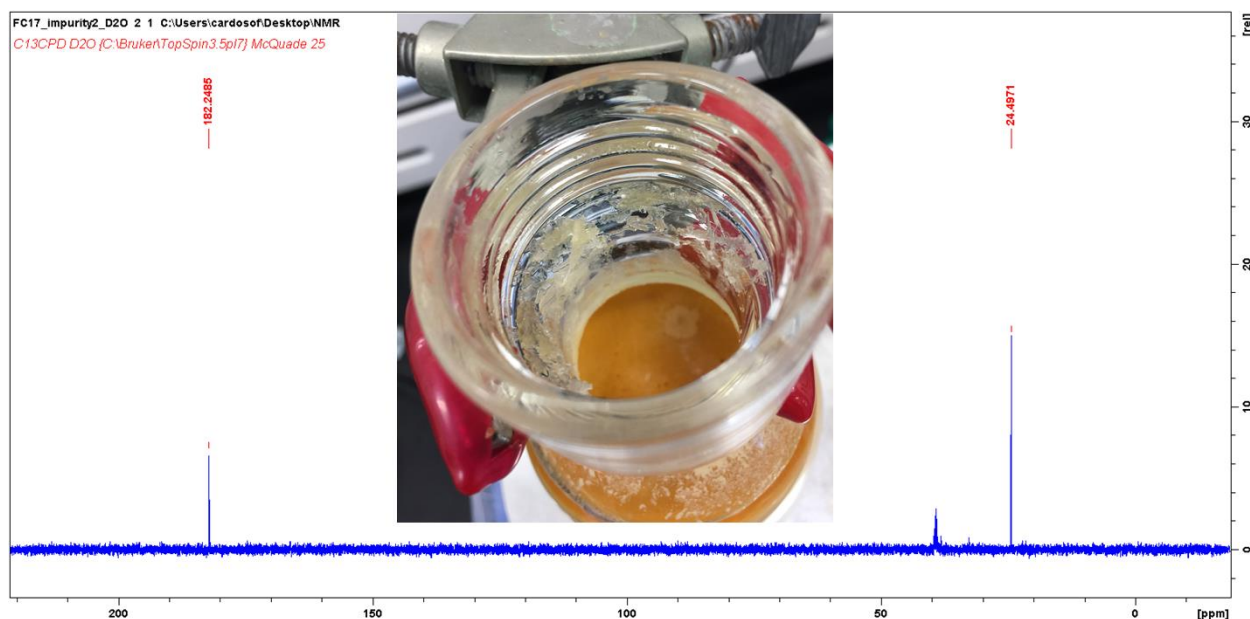


Figure S1: Crystals precipitated out of reaction media leading to depressed purity of final product.

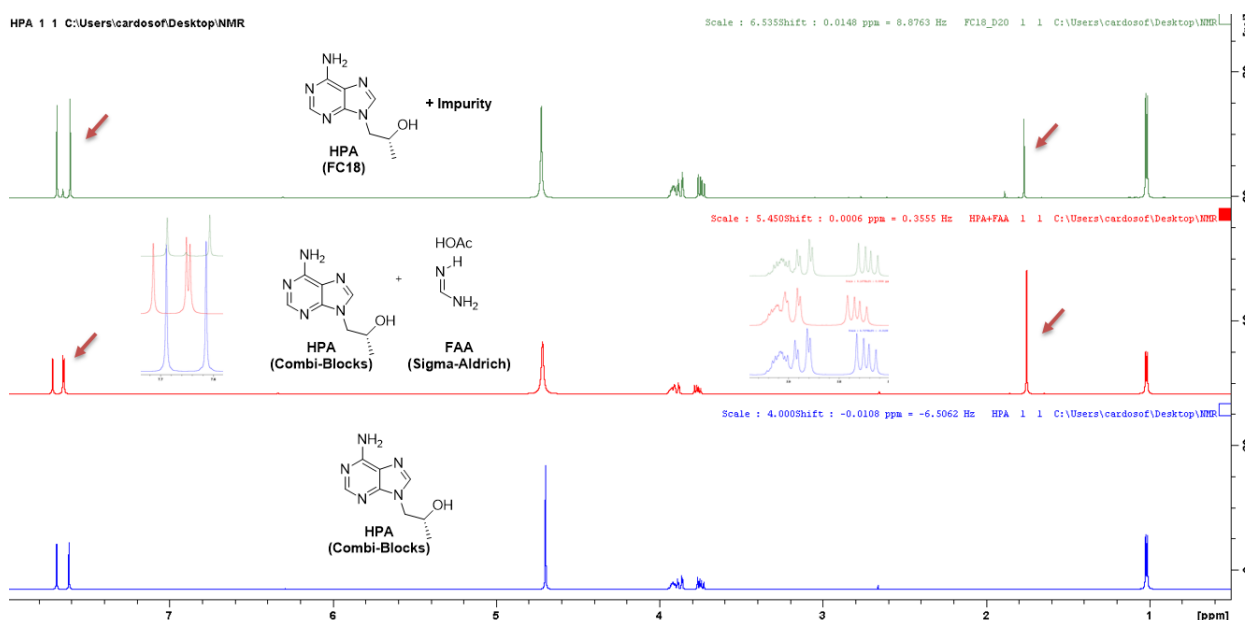
Table S2: Impact of crystal precipitation on purity of final isolated product.

Entry	Scale (g)	Anti-solvent	Purity (%)	Yield (%)	Crystal Precipitation
1	1	MeCN	80	86.9	No
2	1	MeCN	66.46	90	Yes
3	5	MeCN	86	91	No
4	5	MeCN	68	85	Yes
5	1	IPA	98	79	No
6	1	IPA	79.28	81.9	Yes

Impurity Profile

Formamidineum acetate was quickly selected as a C₁ source to form the adenine ring. This greatly improves the yield as compared to formamide. Formamidineum possesses not just the additional carbon required to reach adenine but also the prerequisite nitrogen. Presumably, the increased nucleophilicity of formamidineum enhances rate of adenine formation.

While the reaction clearly proceeded with excellent assay yield, isolated yield and purity varied, and the challenge became to reliably purification of reaction material. On crystallization, the major remaining impurity appears to be formamidineum acetate when acetonitrile was used as anti-solvent. The ¹H and ¹³C NMR showed additional peaks (sample FC18, Figure S2) compared with the commercially available HPA. By doping pure HPA with formamidineum acetate, it suggests that it is the same impurity observed in isolated product (Figure S2).



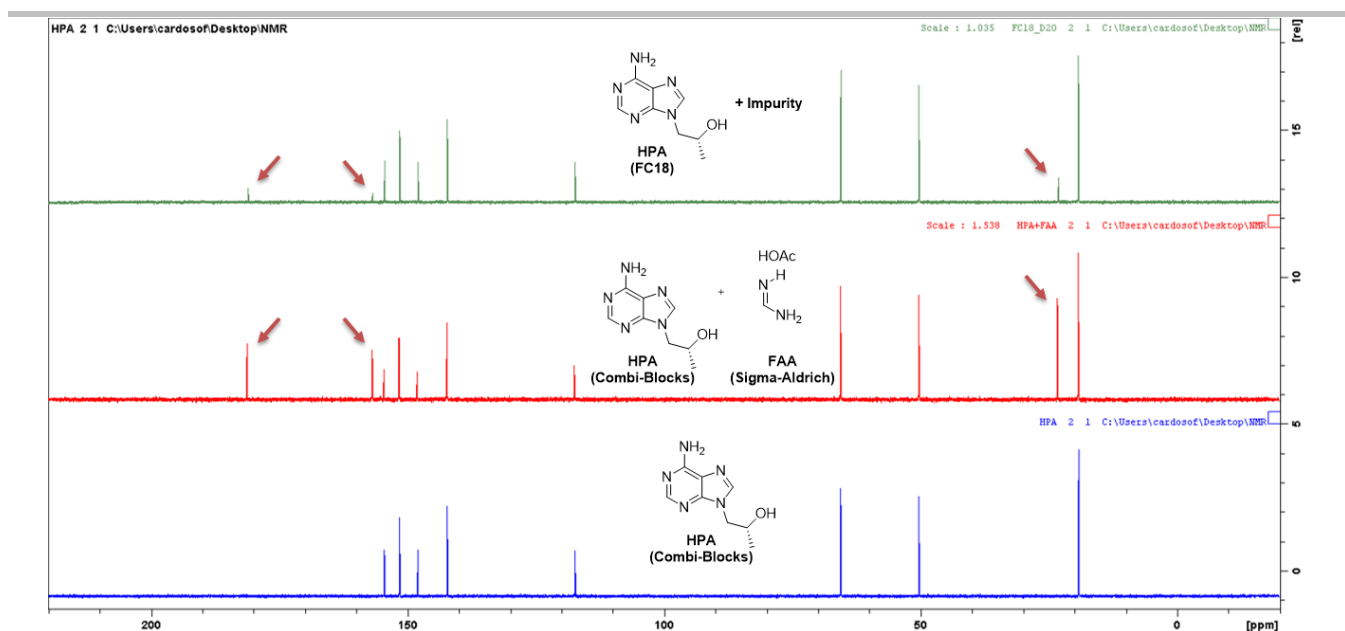


Figure S2: Analysis of isolated product by ^1H and ^{13}C NMR showing formamidine acetate as major.

Reaction Solvent

The reaction was screened with various solvents and equivalents of FAA to yield HPA. Several solvents were chosen to screen at various temperatures (Table S3). It was found that DMF provided the optimal results at 110 °C with lower loadings of FAA where 1.7 equivalents was found to be the best.

Table S3: Screening solvent and equivalents of FAA at various temperatures.

Equivalents	1.2	1.4	1.6	1.8	2	2.2	Temp
DMF	11%	13%	15%	20%	21%	24%	70 °C
ACN	35%	39%	37%	43%	37%	20%	
CPME	12%	13%	27%	23%	13%	14%	
Butanol	28%	39%	27%	30%	35%	27%	
DMF	14%	20%	21%	34%	17%	44%	90 °C
ACN	25%	63%	23%	50%	30%	58%	
CPME	43%	33%	34%	44%	34%	32%	
Butanol	27%	51%	42%	60%	39%	49%	
DMF	85%	89%	93%	95%	96%	98%	110 °C
ACN	46%	59%	65%	79%	76%	85%	
Ethyl Acetate	51%	53%	57%	66%	75%	73%	
Butanol	39%	53%	54%	61%	68%	78%	

The reaction was known to function well in polar amide solvents. However, HPA was found to be significantly less soluble in DMF than NMP when mixed with a variety of anti-solvents, making DMF a desirable reaction solvent for improvements in isolated yield. Antisolvent combinations were screened in a similar manner, and based on difference in solubility between HPA and formamidine acetate,

alcohols and acetonitrile with small amounts of water emerged as lead candidates (Figure S3). Many solvent combinations did not show selective crystallization. Based on the obtained data, DMF was selected as the reaction solvent.

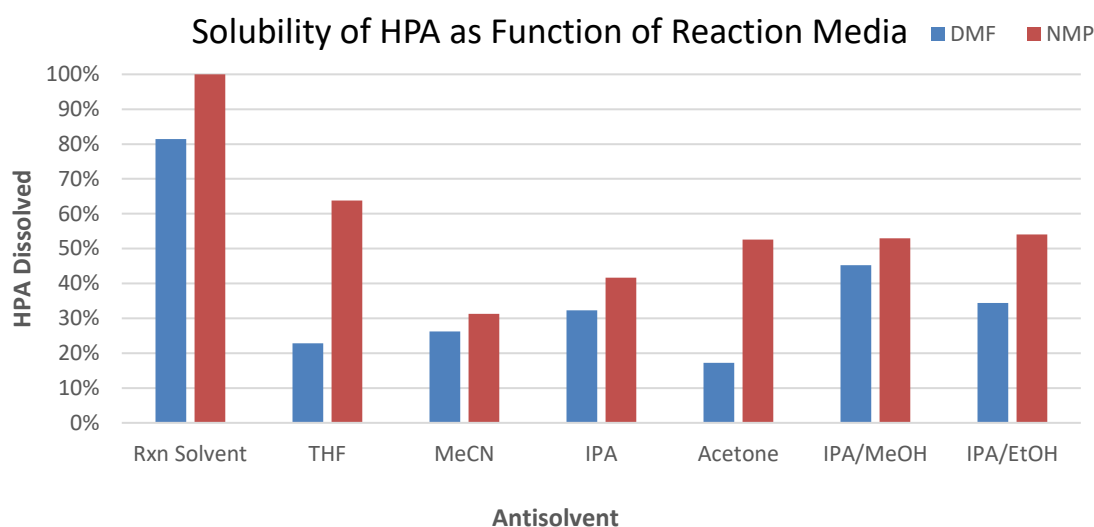


Figure S3: HPA has lower solubility in DMF systems than NMP.

Formamidinium acetate equivalents and reaction concentration

Next, the amount of formamidinium acetate for the reaction and the volume of DMF as the reaction solvent were optimized (Figure S4). It was hypothesized that excess FAA would lead to lower purity of the final product. A set of experiments clearly showed that decreasing quantity of FAA led to a large gain in purity (10%) while maintaining an equivalent yield. Decreasing the amount of DMF led to a small increase in yield (3%) without impacting purity (Figure S5).

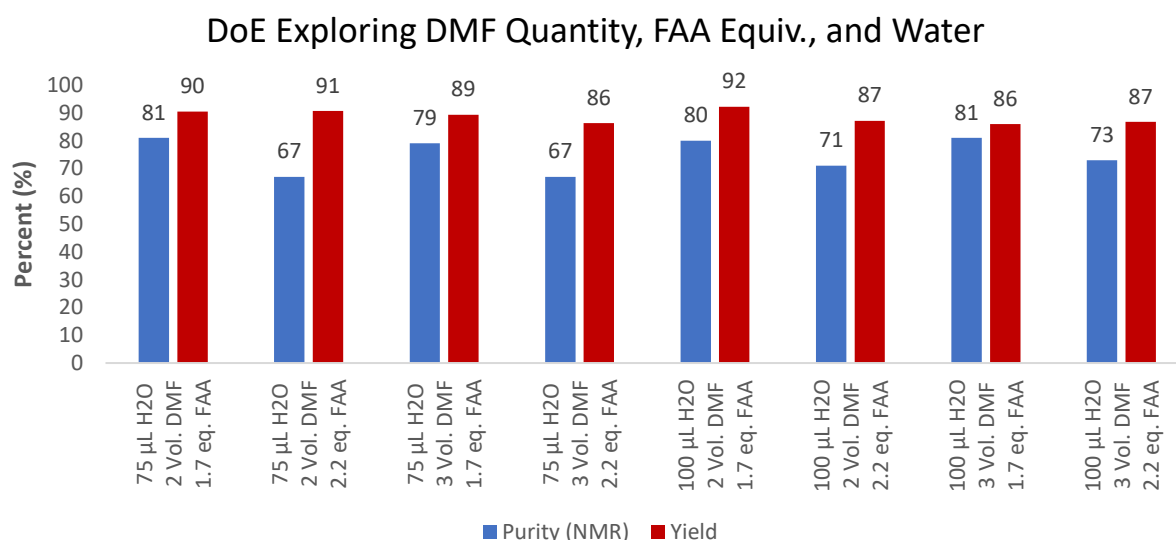


Figure S4: HPA purity and yield based on equiv. FAA and added water.

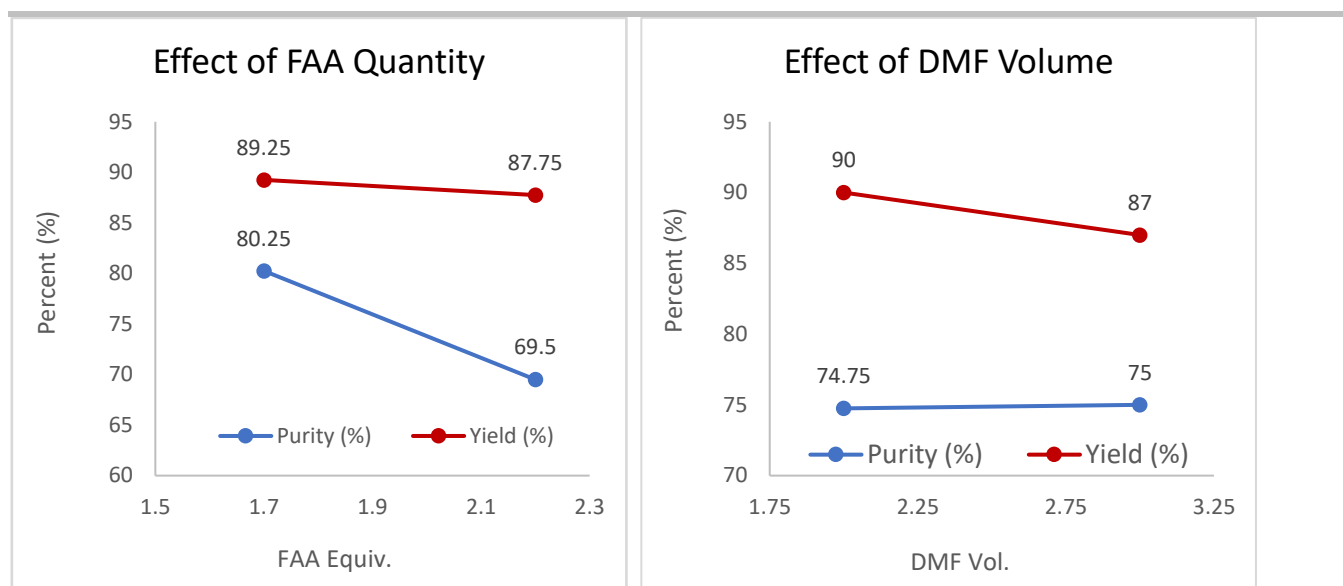


Figure S5: Study on impact of formamidine acetate equivalents and volume of DMF solvent. Using less FAA improves purity significantly without decreasing the yield. Use of smaller volumes of DMF slightly improves the yield.

Antisolvent Selection

Antisolvent combinations were screened in combination with DMF. Based on differences in solubility between HPA and formamidine acetate, alcohols and acetonitrile with small amounts of water emerged as lead candidates (Figure S6). Anti-solvent was screened with 0.5 g reactions. While the presence of MeOH and H₂O aided in provision of high purity product, there was a corresponding detrimental impact on yield. Unfortunately, purity and yield were inversely related. Leads were found in other systems. Acetonitrile without any water or methanol gave the highest isolated yield (92%), and purity was near 90%. Use of isopropanol as anti-solvent afforded the highest purity (100%) and moderate yield (81%). These solvent combinations were selected for further optimization.

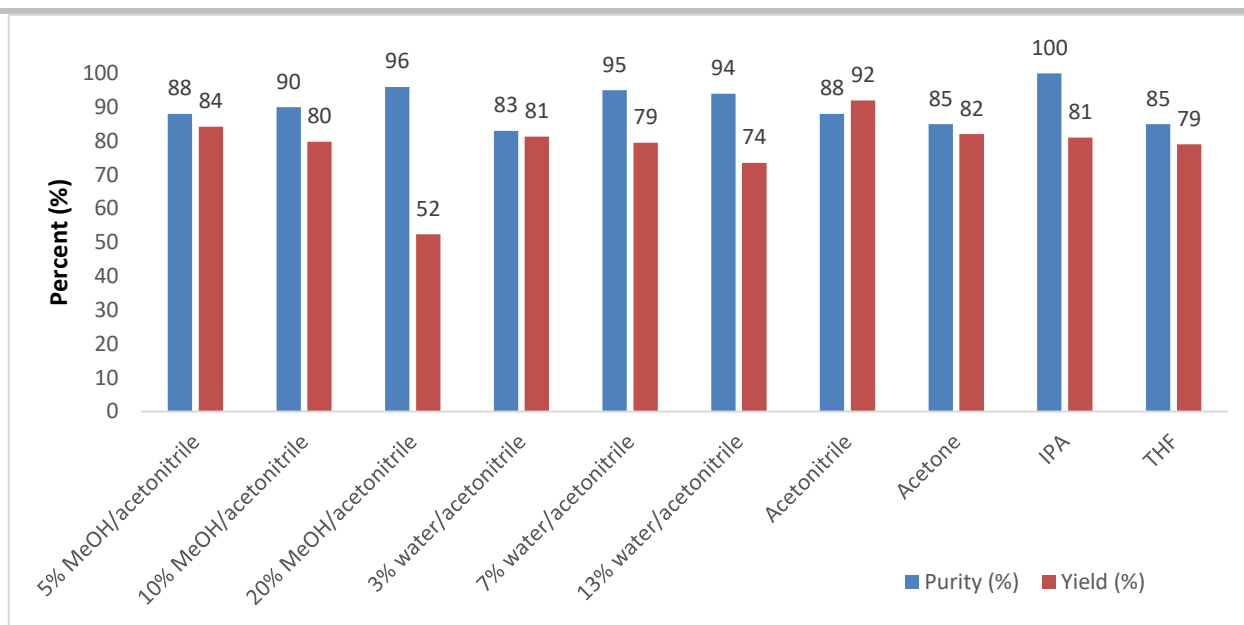


Figure S6: Screen of anti-solvents in 0.5 g reactions. Highest yield obtained with acetonitrile and highest purity with isopropanol.

Acetonitrile and isopropanol were taken forward as anti-solvent for 1g and 5g scale batches (Table S4). The isopropanol as an antisolvent provided an excellent purity (98 to 100%), however the yields were lower compare with acetonitrile.

Table S4: Further optimization of anti-solvents on 1 g and 5 g scale.

Entry	Scale	Antisolvent	Purity (%)	Yield (%)
1	1 g	MeCN	80	87
2	1 g	IPA	98	79
3	5 g	MeCN	86	91
4	5 g	IPA	100	80

Crystallization Temperature

Crystallization temperature had a measurable effect on the isolated yield (Table S5). Lowering the crystallization temperature was found helpful to recover maximum amount of product. The isolated yield of HPA increased by ~10% while maintaining high purity.

Table S5: Impact of crystallization temperature on yield of final isolated product.

Entry	Anti-solvent	Crystallization Temp.	Purity (%)	Yield (%)
1	IPA	0 °C	98	80
2	IPA	-15 °C	99	88
3	IPA	-15 °C	100	89
4	IPA	-15 °C	100	91

Water content within the system

We observed that wet solvent would negatively impact yields. Solubility of HPA is very high in water and if wet solvent is used correspondingly lower isolated yields are obtained.

Results

These optimal parameters were scaled to 20-25 g batches. The results are shown in Table S6 below. At larger scale crystallization, stirring the suspension becomes difficult with a magnetic stirrer, an overhead stirrer was used for following batches. Another scale-up concern was regarding the addition of isopropanol (anti-solvent) at 100 °C, above its boiling point. To address this point, batch 14 was carried adding isopropanol at 75 °C, reproducing the isolated yield, although with a small decrease in purity. Batch sheets are available upon request.

Table S6: HPI to HPA Process Summary.

Batch #	HPI (g)	HPI purity (%)	Type of stirrer	Rate of stirring	Internal Temperature while adding IPA	HPA cake (g)	Weight % purity	Yield adjusted with purity (%)
1	25.00	97.8	Magnetic	350	100 °C	26.62	99.0	93.1
3	25.02	99.8	Magnetic	350	100 °C	27.45	98.5	93.5
4	25.02	99.0	Over-head	80	100 °C	26.87	95.9	89.8
14	20.05	94.7	Over-head	130	75 °C	21.18	91.7	88.0
15	20.05	94.7	Over-head	130	100 °C	20.06	95.1	86.5

Conclusions

This report details M4ALL's process for the synthesis of HPA, an intermediate in the synthesis of TDF. The process has been repeated by three different chemists at 25 g scale. Excellent yields and purity results were obtained. While direct comparisons to current market prices cannot be made since HPA is not an article of commerce we can estimate that the reported process could result in meaningful decreases in the prices of TDF and its intermediates.

Initial Results for the Process Development of PMPA:

Initial development of the reaction conditions, and reported above, used DMF as the reaction solvent. Although DMF was a suitable solvent for the conversion of HPA to PMPA, challenges remained in isolation of the product from the reaction mixture. In order to encourage adoption in the marketplace by manufacturers we have attempted to further develop the initial reactions reported in the manuscript.

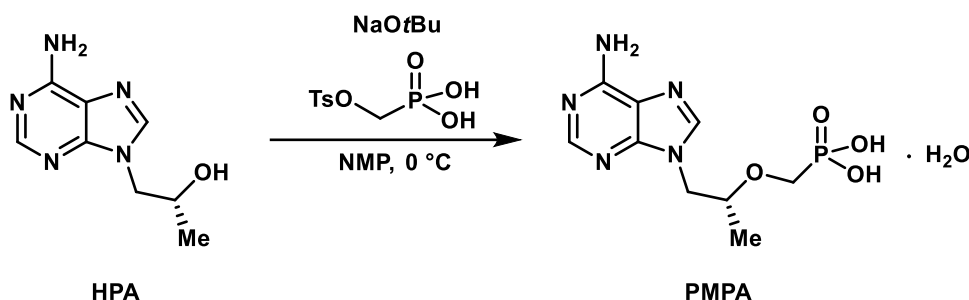


Table S7. Reagents for PMPA synthesis

Material	Mol. Wt. (g/mol)	mmol	Equiv.	Amount	Density (g/mL)
(R)-1-(6-amino-9H-purin-9-yl)propan-2-ol	193.5	129	1.0	25.0 g	---
Sodium <i>tert</i> -Butoxide	96.1	775	6.0	74.4 g	---
((tosyloxy)methyl)phosphonic acid	266.2	219	1.7	58.2 g	---
N-Methylpyrrolidone	99.1	---	---	10V	1.03
Hexanes	88.2	---	---	10V	0.655
Hexanes	88.2	---	---	10V	0.655
Water	18	---	---	10V	0.997
Hydrochloric acid (37%)	36.5	---	---	2V*	---
Water	18	---	---	1.5V	0.997
Acetone	58.1	---	---	1.5V	0.784

Safety Precautions

1. Sodium *tert*-butoxide is a strong base and is caustic, avoid contact and inhalation.
2. Hydrochloric acid is extremely corrosive, care should be taken while handling this reagent.
3. The tosyloxy methylphosphonic acid (TMPA) is an unknown chemical and care should be taken while handling it. Biological effects are not documented, treat as toxic.
4. PMPA is an active pharmaceutical ingredient (TDF is the pro-drug) care should be taken to avoid ingestion or inhalation.

Procedure for the synthesis of PMPA:

1. Assemble the reactor vessel. A jacketed reactor (500 mL) was fitted with an overhead stirrer and paddle blade. A stream of nitrogen was introduced to inert the atmosphere of the reactor. A J-KEM temperature probe was introduced.
2. The reactor was charged with NMP (7.5V).

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3. The stirrer was set to 300 RPM.
 4. The temperature of the jacket was reduced to 0 °C.
 5. The reactor was charged with sodium *tert*-butoxide (74.6 g, 776 mmol, 6.0 equiv.). The temperature increased approximately 10 °C. The internal temperature was reduced to 5 °C. The solution turned pink/red. There were small amounts of solids still undissolved.
 6. The reactor was charged with HPA (25.0 g, 129 mmol, 1 equiv.).
 7. The reactor was charged with NMP (2.5V) to wash any solids into the mixture.
 8. The reaction was stirred for 15 minutes at 2 °C.
 9. The reactor was charged with the phosphonic acid (14.6 g, 55 mmol, 0.43 equiv.), the temperature did not increase above 5 °C.
 10. The reactor was charged with the phosphonic acid (14.6 g, 55 mmol, 0.43 equiv.), the temperature did not increase above 5 °C.
 11. The reactor was charged with the phosphonic acid (14.6 g, 55 mmol, 0.43 equiv.), the temperature did not increase above 5 °C.
 12. The reactor was charged with the phosphonic acid (14.6 g, 55 mmol, 0.43 equiv.), the temperature did not increase above 5 °C.
 13. The temperature was maintained at 2 °C for 16 hours.
 14. The internal temperature was increased to 40 °C.
 15. The reactor was charged with hexanes (10V).
 16. The temperature was maintained for 1 hour at 40 °C.
 17. The suspension was filtered and the solids collected.
 18. The reactor was washed with hexanes (10V) and this was used to wash the previously collected solids. NMP was largely removed through this method.
 19. The solids were charged into the reactor.
 20. The reactor was charged with water (10V).
 21. Stirred at 100 RPM.
 22. The pH of the solution was adjusted to pH = 2.9 with concentrated hydrochloric acid (~44 mL).
 23. The temperature was reduced to 5 °C.
 24. The temperature was maintained at 5 °C for 2 hours.
 25. The white suspension was filtered (sufficient rinsing is required to wash the salts away).
 26. The product was washed with 5 °C water (1V).
 27. The product was washed with a 1:1 mixture of 5 °C water/acetone (1V).
 28. The product was washed with 5 °C acetone (1V).

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29. The product was air dried for 30 minutes before drying under vacuum at 75 °C until less than 5 wt% loss on drying.
 30. The product was weighed and assayed by HPLC and NMR.

Sensitivity Assessment

Several variables have been tested to determine the sensitivity of the above reaction:

1. **Solvent volume:** *The reaction was screened in variable solvent amounts from 5V to 20V. The minimum amount of solvent found suitable for our reactors was 10V. Lowering solvent volume resulted in a mixture that became extremely viscous and difficult to stir.*
2. **Tosyl Phosphonic acid purity:** *Optimal purity of the tosyl phosphonic acid is 97 wt% or greater. The presence of protic impurities results in a lower purity material at 75 wt%. This material resulted in a reaction that only proceeded to 60 A% PMPA with 30 A% HPA remaining. When the corrected amount of material was added, the reaction was complete, although assay yields were lower.*
3. **Temperature:** *Elevation of the reaction temperature above 5 °C during the addition of the base can cause the reaction mixture to become viscous. The viscosity of the reaction mixture was observed to be acceptable if the reaction temperature was maintained below 10 °C. The conversion to PMPA was consistent at 0 – 5 °C with temperatures of -10 °C at 25 °C the yields of PMPA dropped. At 70 °C the conversion was higher but the reaction viscosity became too viscous to stir. Maintaining the reaction temperature at 2 – 5 °C reduces total impurities*
4. **Equivalents of *t*-BuONa:** *The equivalents of base have been screened from 3 equiv. to 7.5 equiv. The optimal equivalents of base have been found to be 6.0 equiv. for both reaction yield and economic analysis. Lower equivalents led to incomplete consumption of the starting material. Higher equivalents resulted in complete consumption of the starting material but added additional material costs. **It should be noted that aged samples of base resulted in lower yields. Anhydrous conditions are crucial for high conversions.***

Solvent and Base Selection:

Table S8: Solvent and base screen.

Entry	Solvent	Base	<i>N, N</i> -O Alkylated (A%)	PMPA (A%)	HPA (A%)
1	DMPU	LiOtBu	8.4	4.4	83.0
2	DMPU	NaOtBu	15.4	4.1	75.0
3	DMPU	KOtBu	47.8	12.6	35.9
4	DMPU	LiHMDS	41.5	6.8	43.8
5	DMPU	NaHMDS	26.2	6.2	63.4
6	DMPU	LDA	4.3	24.7	57.2
7	NMP	LiOtBu	7.5	13.0	71.9
8	NMP	NaOtBu	31.1	24.9	37.9
9	NMP	KOtBu	27.5	5.6	58.9
10	NMP	LiHMDS	8.0	4.1	81.7
11	NMP	NaHMDS	33.9	2.3	58.7
12	NMP	LDA	7.9	3.7	83.5
13	DMF	LiOtBu	9.0	5.6	83.9
14	DMF	NaOtBu	27.3	20.1	48.6
15	DMF	KOtBu	24.3	2.9	67.8
16	DMF	LiHMDS	5.1	9.9	80.1
17	DMF	NaHMDS	37.7	7.8	52.0
18	DMF	LDA	6.6	-	89.1

*All reactions were conducted on 10 mg scale and 10V of solvent with 3 equivalents of base and 1.1 equivalents of the tosylated phosphonic acid pyridinium salt at 70 °C.

The results from Table S8 above suggest that alkoxide bases yielded the highest A% of the desired PMPA. Stronger bases resulted in more of the undesired alkylated products forming in higher A%. In addition, incomplete conversion suggested that more base and electrophile were required as demonstrated in later experiments. NMP and DMF were chosen as potential reaction solvent mixtures to move to larger scale, DMPU was eliminated due to cost. Stronger bases such as LiHMDS, NaHMDS and LDA were avoided due to a larger A% of the undersired alkylated products forming. The exception being, LDA and DMPU (Table S8, entry 6) where almost no undersired product formed. Further development was done with NaOtBu with the goal to decrease impurity formation and increase PMPA formation.

Slow addition of the phosphonic acid, as a solution, to the reaction mixture was done over one, two and four hours (Figure S7). Albeit only a minor difference, one hour addition was found to result in the best conversion to PMPA with the least amount of unwanted by-products formed. Changing the order of addition as shown in the inverse addition plot resulted in the lowest amount of PMPA and highest amount of by-products.

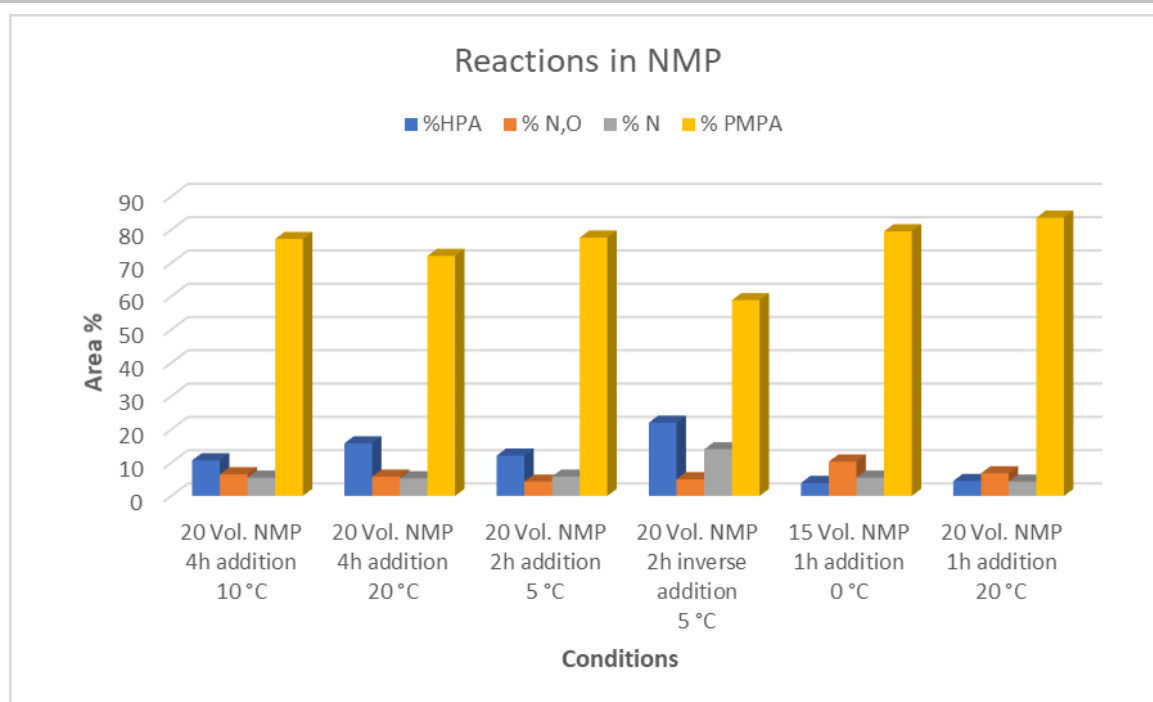


Figure S7: Area % of PMPA reactions in NMP under various conditions. All reactions used a solution of the phosphonic acid. All reactions run on 3 g scale.

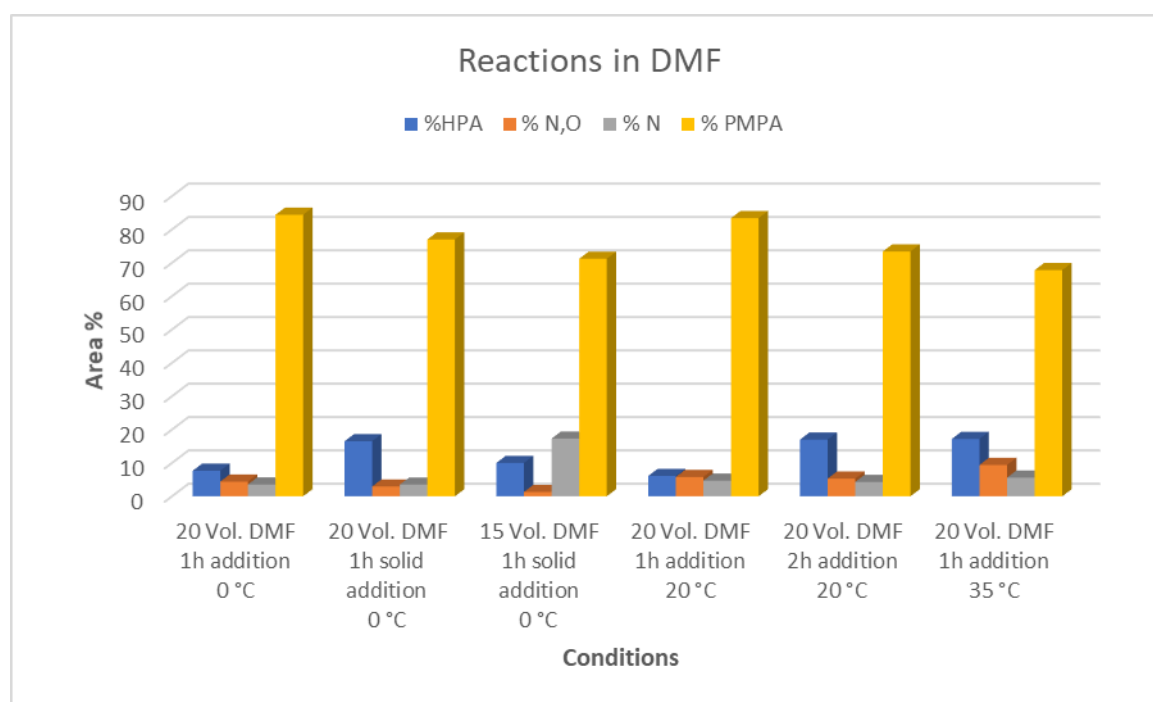


Figure S8: Area % of PMPA reactions in DMF under various conditions. Unless otherwise noted a solution of the phosphonic acid was used. All reactions run on 3 g scale.

Similar conditions were screened with DMF as the reaction solvent mixture (Figure S8). As shown, 20 volumes of DMF demonstrated the highest conversion to PMPA. Higher temperature (20 °C compared to 0 °C) lead to slightly more consumption of starting material. However, slightly more bis-alkylated HPA was formed over time. Reducing the volumes of DMF and increasing the temperature beyond 20 °C both resulted in a lower conversion to PMPA as indicated by HPLC. Smaller volumes of solvent and

increased temperatures resulted in viscous reaction mixtures that became difficult to stir, even with overhead stirring.

Table S9: Optimization of PMPA Conversion.

Entry	Additive	Bath Temp. (° C)	Solvent	Vol (V)	PMPA (%)	N-Alkylated Product (%)	N,O-Alkylated Product (%)
1	-	RT	DMF	10	66	9	8
2	TMPA ⁻ Na ⁺	RT	DMF	10	62	7	5
3	1.7 equiv. LiH	RT	DMF	10	-	-	-
4	-	RT	DMF	25	64	11	8
5	3 equiv. Mg(OtBu) ₂	RT	DMF	20	41	8	1
6	4 Å M.S.	-20 to RT	DMF	20	66	8	4
7	4 Å M.S.	-20 to RT	NMP	20	82	12	4
8	4 Å M.S.	0 to RT	NMP	20	79	11	4
9	0.4 equiv. MgCl ₂	-20 to RT	NMP	20	69	4	6
10	4 Å M.S.	0 to RT	NMP	20	81	8	5
11	4 Å M.S.	0 to RT	DMF	20	66	9	7
12	0.4 equiv. KBr	0 to RT	NMP	20	73	15	6
13	0.4 equiv. NaI	0 to RT	NMP	20	70	15	7
14	6 equiv. NaOtBu ^a	0 to RT	NMP	74.1	77	10	5

^a Used freshly purchased NaOtBu stored and weighed in dry box

Attempts to increase the conversion of PMPA centered largely around improving the electrophilicity of the tosyl phosphonic acid, coordinating metals, and minimizing water content in the reaction. Based on observations when varying conditions between NMP and DMF, it was found that NMP consistently gave lower levels of conversion towards the undesired side products of PMPA, so further optimization of the reaction was carried out in NMP. In particular it was found that leaving the reaction in DMF for prolonged periods of time produced further impurities, while NMP did not produce such results. Our understanding of the base-mediated alkylation reaction led us to believe that the presence of water would lower the yield of the reaction by consuming excess base, so we began to investigate the water content of our starting materials through KF titration. However, after steps were taken to lower the water content in all starting materials and solvents, the reaction continued to stall at ~65% conversion towards PMPA. Tracking the water content in the reaction through aliquots revealed that the reaction was producing water *in situ*. Presumably this is due to the presence of NaOH in commercial sources of NaOtBu. Introducing molecular sieves to the reaction as a drying agent allowed the reaction to proceed towards ~80% conversion of PMPA regardless of the source or age of commercial NaOtBu.

Isolation of PMPA from aqueous mixtures:

In attempts to optimize the isolation of PMPA various conditions were screened in efforts to maximize the amount of PMPA out of solution. After completion of the reaction, significant quantities of caustic salts are present in the reaction mixture, pH adjustment in aqueous solution was required to isolate PMPA cleanly. Adjustment of the caustic PMPA solution with various acids (HCl, HBr, H₂SO₄ etc.) would create the corresponding sodium salts that have been known to have a salting in or salting out effect on organic compounds. The results of these studies with various salts, water and organic compositions are summarized below, Tables S10 – S13. General trends indicate that organic solvent such as acetonitrile and methanol decrease PMPA solubility and that aqueous salt solutions increase solubility of PMPA. The solubility of PMPA in aqueous media follows the general trend, distilled H₂O < NaCl_(sat) < Na₂SO_{4(sat)} < NaBr_(sat). The presence of organics may decrease solubility of PMPA but may also cause significant quantities of salts to co-precipitate during isolation.

Table S10: Solubility of PMPA in DMF/MeOH and Aqueous Solutions.

Entry	PMPA (mg)	Temp (° C)	Vol. DMF	Vol. MeOH	Vol. H ₂ O	mg dissolved	% Soluble
1	50.6	rt	20	0	1	0.55	1.1
2	50.5	rt	20	5	1	0.69	1.4
3	50	rt	20	10	1	0.71	1.4
4	50.4	rt	20	0	5	0.65	1.3
5	50.8	rt	20	5	5	0.66	1.3
6	49.7	rt	20	10	5	0.67	1.4
7	49.1	rt	20	0	10	0.87	1.8
8	51.4	rt	20	5	10	0.90	1.8
9	54	rt	20	10	10	0.94	1.7
10	46.9	rt	20	0	20	1.55	3.3
11	51.3	rt	20	5	20	1.57	3.1
12	51.7	rt	20	10	20	1.69	3.3

Table S11: Solubility of PMPA in DMF/ACN and Saturated NaCl and NaBr solutions.

Entry	PMPA (mg)	Temp (° C)	Vol. DMF	Vol. ACN	Vol. NaCl	Vol. NaBr	mg dissolved	% Soluble
1	52.6	rt	20	-	20	-	3.8	7.2
2	50.6	rt	20	-	30	-	6.3	12.5
3	54.1	rt	20	-	40	-	9.0	16.6
4	54.2	rt	20	-	-	20	25.4	46.8
5	53.2	rt	20	-	-	30	40.2	75.6
6	53.3	rt	20	-	-	40	49.0	91.8
7	50.8	rt	20	10	-	-	0.1	0.2
8	50.1	rt	20	20	-	-	0.0	0.1
9	50.5	rt	20	30	-	-	0.0	0.0
10	54.5	rt	20	10	10	-	0.9	1.6
11	49.3	rt	20	10	20	-	3.3	6.7
12	51.1	rt	20	10	30	-	6.8	13.3
13	49.3	rt	20	10	-	10	12.3	25.0
14	49	rt	20	10	-	20	26.6	54.2
15	51.4	rt	20	10	-	30	40.3	78.4

16	51.8	rt	20	20	-	20	26.8	51.8
17	54.3	rt	20	20	20	-	2.9	5.3
18	54.2	rt	10	10	10	-	1.4	2.6
19	51.2	rt	10	10	-	10	12.4	24.2
20	52.6	rt	10	10	20	-	4.9	9.3
21	50.9	rt	10	20	10	-	0.9	1.7
22	51.9	rt	10	20	20	-	3.8	7.3
23	51.1	rt	10	20	-	10	12.1	23.6
24	52.9	rt	10	20	-	20	29.1	54.9

Table S12: Solubility of PMPA in DMF/Aqueous Sodium Sulfate.

Entry	PMPA (mg)	Temp (° C)	Vol. DMF	Vol. Na ₂ SO ₄	mg dissolved	% Soluble
1	50.9	rt	20	10	1.5	3.0
2	48	rt	20	20	5.0	10.3
3	50.1	rt	20	30	7.2	14.4
4	51.6	rt	0	20	5.0	9.7
5	49.4	rt	0	40	10.2	20.7
6	55.1	rt	0	60	15.6	28.4

Table S13: Solubility of PMPA in DMF/Aqueous solutions.

Entry	PMPA (mg)	Temp (° C)	Vol. DMF	Vol. NaCl	Vol. H ₂ O	mg dissolved	% Soluble
1	49.9	rt	-	5	10	4.6	9.2
2	57.5	rt	-	5	20	8.0	13.9
3	47.9	rt	-	10	10	4.5	9.4
4	49.5	rt	-	10	20	9.3	18.7
5	49.4	rt	-	20	-	4.0	8.0
6	55.5	rt	-	20	10	7.8	14.0
7	56.2	rt	0.5	20	-	4.6	8.1
8	48.4	rt	1	20	-	4.9	10.2
9	49.6	rt	1.5	20	-	5.2	10.5
10	48.6	rt	2	20	-	5.3	10.9
11	50.9	rt	2.5	20	-	5.3	10.5
12	51.2	rt	0.5	10	10	5.9	11.6
13	49.5	rt	1	10	10	6.1	12.2
14	52.2	rt	1.5	10	10	6.1	11.6
15	58.1	rt	2	10	10	5.9	10.1
16	55.1	rt	2.5	10	10	5.7	10.4
17	47.2	rt	0.5	5	20	6.4	13.5
18	51	rt	1	5	20	7.7	15.1
19	56.9	rt	1.5	5	20	7.4	13.1
20	55.3	rt	2	5	20	7.7	13.9
21	48.5	rt	2.5	5	20	7.3	15.0
22	50.8	rt	0.5	10	20	8.9	17.6
23	52.2	rt	1	10	20	9.2	17.6
24	50.5	rt	1.5	10	20	8.6	16.9

25	52.8	rt	2	10	20	9.1	17.2
26	55.2	rt	2.5	10	20	8.9	16.2
27	48.4	rt	0.5	10	10	5.9	12.3
28	52.6	rt	1	10	10	5.8	11.0
29	45.8	rt	1.5	10	10	6.0	13.0
30	51.4	rt	0.5	20	-	4.6	8.9
31	49.5	rt	1	20	-	5.0	10.1
32	48	rt	1.5	20	-	5.1	10.5

Adjustment of PMPA precipitation parameters:

Table S14: PMPA precipitation at varying bath temperatures and concentrations.^a

Entry	Bath Temp. (° C)	Vol. (V)	Purity (%) ^b	Yield (%) ^c	Peak Area		Ratio PMPA:Impurities
					N- Alkylated Product (%)	N,O-Alkylated Product (%)	
1	rt	15	77.8	51.6	0.44	1.43	96.6 : 3.4
2	rt	20	78.6	43.9	0.25	1.88	97.7 : 2.3
3	10	15	80.5	54.1	0.46	1.46	96.6 : 3.4
4	10	20	91.5	57.1	0.11	2.11	97.7 : 2.3
5	0	5	61.2	62.9	1.14	2.72	94.5 : 5.5
6	0	10	69.6	62.9	0.59	1.68	95.9 : 4.1
7	0	15	81.9	64.8	0.09	2.28	97.9 : 3.1
8	0	20	90.1	62.4	0.12	2.30	97.5 : 2.5
9	-9	5	68.0	59.4	0.84	2.13	95.2 : 4.8
10	-9	10	75.5	61.3	0.41	1.44	94.9 : 5.1
11	-9	15	81.8	62.7	0.21	2.48	96.4 : 3.6
12	-15	5	69.7	56.3	0.85	1.92	95.1 : 4.9
13	-15	10	76.4	58.1	0.70	1.73	95.1 : 4.9
14	-15	15	74.1	54.8	0.53	1.60	96.0 : 4.0

^a All samples contained approximately an 80:20 H₂O:NMP content, ^bPurity is determined by HPLC, ^c Yield is adjusted for purity

Additional efforts were made to optimize the isolation of PMPA from the aqueous solution. Adjustment of the PMPA solution parameters (bath temperature, concentration, pH adjustment, and NMP content) was suspected to lead to a potential salting in or salting out effect and increase the yield of PMPA. Tables S11-S12 depict the results from these studies. The general trend indicates that lower volumes of H₂O results in decreased purity. Additionally, bath temperatures above room temperature and below -9 °C lead to decreased purity and/or yield. A greater effect in the yield and purity was further noticed when the pH adjustment occurred above ambient temperatures and contained larger concentrations of NMP.

Increased PMPA purity through a second purification:

A second purification can result in the increase the purity of PMPA with potentially minimal product loss. The results of the temperature cycles and pH cycles are summarized below in Tables S15-S16. As shown, while the pH cycle with aqueous PMPA solutions can increase the purity to >92% in most cases, a poor recovery of the product is observed in every attempt. Alternatively, the temperature cycle at low bath temperatures resulted in increased purity and excellent PMPA recovery (Table S17, entries 1-3). Increasing the volume of solvent beyond 15V had no noticeable effect on the yield.

Table S15: Effect of pH adjustment temperature and NMP content on PMPA crystallization.

Entry	pH Adjustment Temp. (° C)	Ratio H ₂ O:NMP	Yield (%) ^b	Purity (%) ^a	Peak Area		
					N-Alkylated Product (%)	N,O-Alkylated Product (%)	Ratio PMPA:Impurities
1 ^c	rt	98:2	55.9	89.0	0.20	1.95	97.6 : 2.4
2 ^c	rt	80:20	61.9	82.8	0.45	2.82	95.1 : 4.9
3	rt	60:40	62.4	79.1	0.08	2.58	94.6 : 5.4
4	0	98:2	56.8	89.1	0.22	2.56	96.5 : 3.5
5 ^c	0	80:20	54.3	83.6	0.39	3.62	95.5 : 4.5
6	0	60:40	53.2	69.0	0.61	6.53	92.0 : 8.0
7	40	98:2	50.6	87.9	0.34	2.30	96.6 : 3.4
8	40	80:20	58.2	76.7	0.52	3.59	95.4 : 4.6
9	40	60:40	31.8	37.2	0.08	2.94	94.0 : 6.0

^a Purity determined by HPLC, ^b Yield is adjusted for the purity of PMPA, ^c Results are the average of 2 or more experiments

Table S16: PMPA purification using pH cycling^a

Entry	Bath Temp. (° C)	Vol. (V) ^b	Purity (%) ^c	Recovery (%) ^d	Peak Area		
					N- Alkylated Product (%)	N,O-Alkylated Product (%)	Ratio PMPA:Impurities
Original Sample	-	-	79.9	-	0.35	2.90	95.9 : 4.1
1	rt	15	93.3	52.5	0	0.18	99.7 : 0.3
2	rt	20	94.2	46.7	0	0.20	99.6 : 0.4
3	10	15	96.4	52.0	0	0.16	99.7 : 0.3
4	10	20	86.8	62.7	0	0.22	99.6 : 0.4
5	0	15	96.4	48.1	0	0.15	99.7 : 0.3
6	0	20	91.9	77.0	0	0.28	99.6 : 0.4
7 ^f	0	15	99.8	47.4	0	0.19	99.6 : 0.4

^a All samples were adjusted 6 M NaOH to a pH of 8 then readjusted to pH 3.2-2.8 with 1 M HCl unless noted, ^b Volume H₂O based on amount of PMPA in sample, ^c Recovery is adjusted based on purity ^d Recovery is adjusted for purity, ^e 12 M HCl used to adjust pH

Table S17: PMPA purification using temperature cycling.^a

Entry	Bath Temp. (° C)	Vol. (V) ^b	Purity (%) ^c	Recovery (%) ^d	Peak Area		
					N- Alkylated Product (%)	N,O-Alkylated Product (%)	Ratio PMPA:Impurities
Original Sample	-	-	79.9	-	0.35	2.90	95.9 : 4.1
1	0	15	95.8	95.8	0	1.1	98.4 : 1.6
2	0	20	94.8	96.2	0	0.93	98.6 : 1.4
3	0	25	95.1	94.4	0	0.8	98.7 : 1.3
4	8	15	88.4	88.6	0.03	0.57	98.3 : 1.7
5	8	20	97.3	92.9	0	0.68	98.2 : 1.8
6	8	25	93.1	89.8	0	0.71	98.4 : 1.6

^a All samples were heated to 100 °C in H₂O then allowed to cool to the designated bath temperature, ^b Volume H₂O based on amount of PMPA in sample, ^c Recovery is adjusted based on purity ^d Recovery is adjusted for purity

References:

1. Takase, M.; Ookuchi, T.; Hatano, M. *Method for Producing Adenine Derivative*. WO 2005 051952, June 9th 2005.
2. Brown Ripin, D. H.; Teager, D. S.; Fortunak, J.; Basha, S. M.; Bivins, N.; Boddy, C. N.; Byrn, S.; Catlin, K. K.; Houghton, S. R.; Jagadeesh, S. T.; et al. Process Improvements for the Manufacture of Tenofovir Disoproxil Fumarate at Commercial Scale. *Org. Process Res. Dev.* **2010**, *14*, 1194–1201.
3. Vrbková, S.; Dračinský, M.; Holý, A.; Synthesis of phosphonomethoxyethyl or 1,3-bis(phosphonomethoxy)propan-2-yl lipophilic esters of acyclic nucleoside phosphonates. *Tetrahedron*, **2007**, *63*, 11391 – 11398.