

Supplementary File

Identification and preclinical development of kinetin as a safe error-prone SARS-CoV-2 antiviral and anti-inflammatory therapy

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#These authors contributed equally to this work

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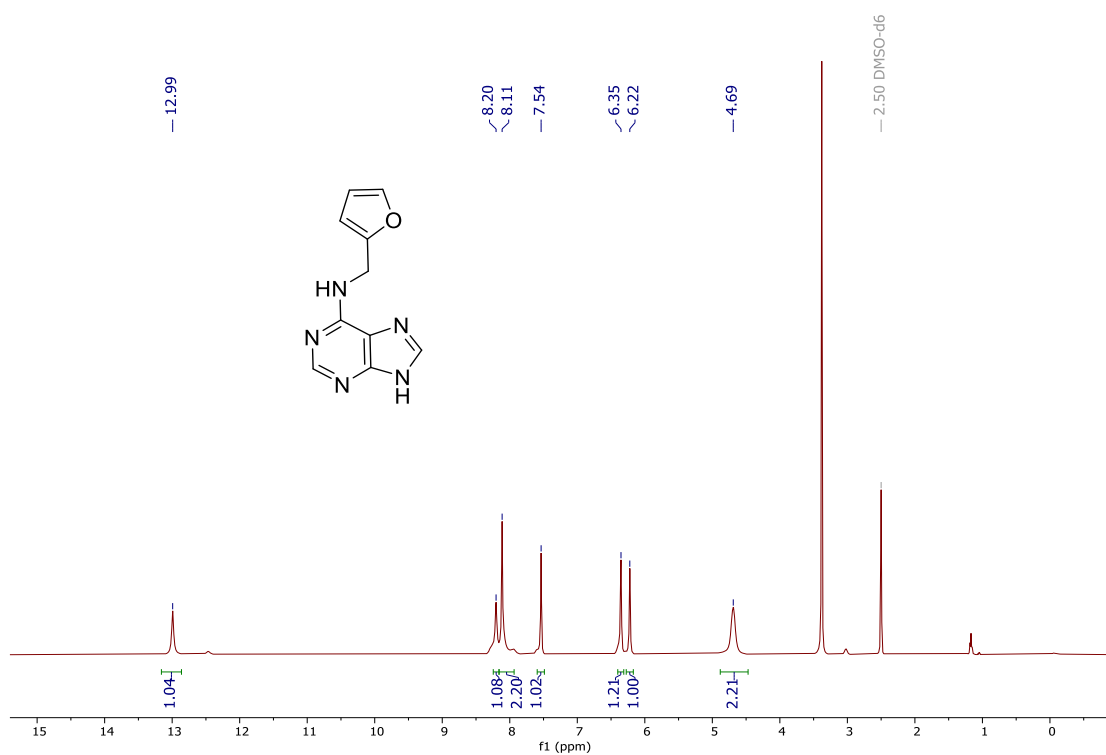
6 – Laboratory on Thymus Research, Oswaldo Cruz Institute, Fiocruz, Rio de Janeiro, RJ, Brazil.

7 - Universidade Iguaçu, Nova Iguaçu, RJ, Brazil.

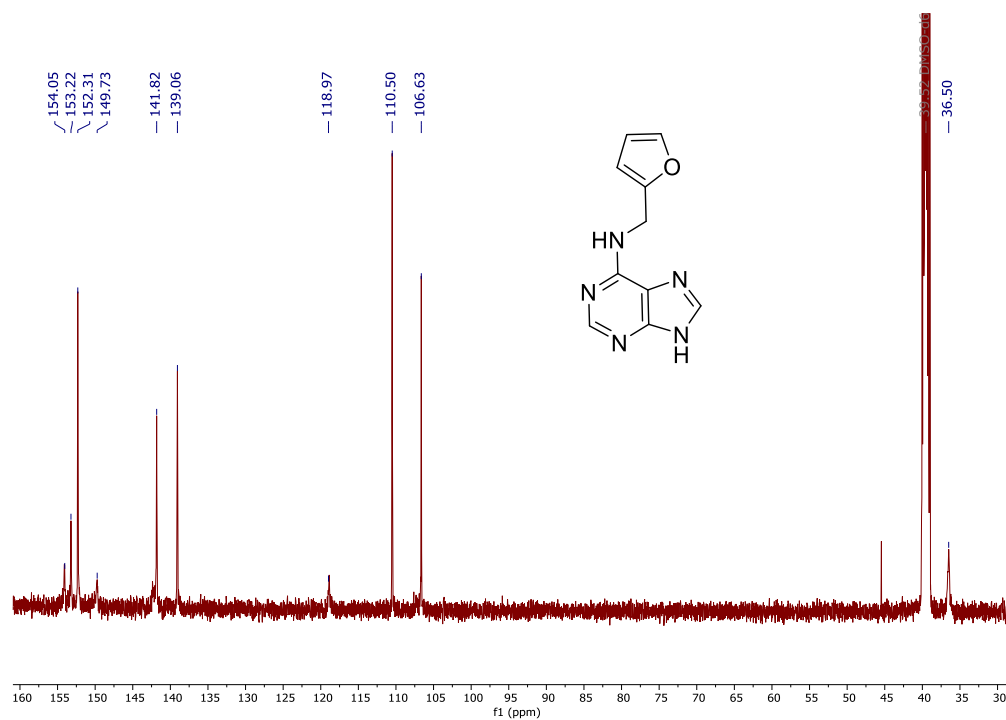
8 - Program of Immunology and Tumor Biology, Brazilian National Cancer Institute (INCA), Rua André Cavalcanti 37, 5th floor, Centro, Rio de Janeiro, Brazil;

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Kinetin, MB-905 (A)¹

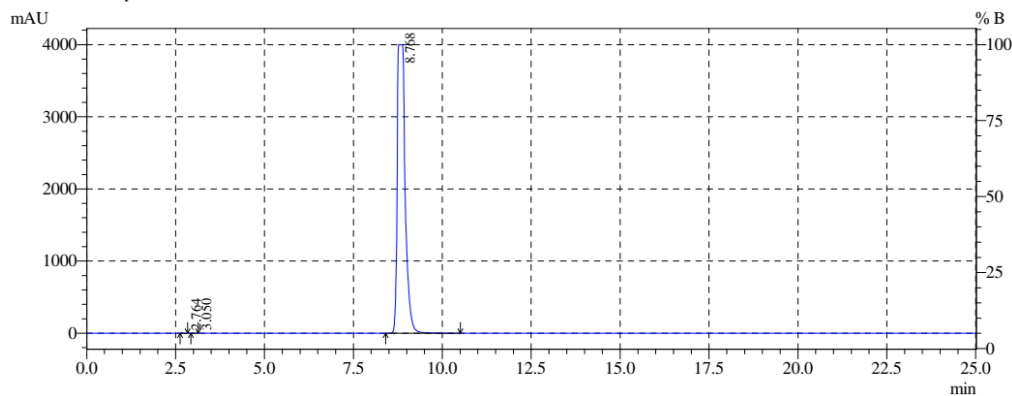


¹H NMR (500 MHz, CDCl₃) of compound MB-905 (A)



¹³C NMR (126 MHz, CDCl₃) of compound MB-905 (A)

D:\Dados\Cinetina\Desenvolvimento\Métodos\Cinetina_XTerra RP18_CRM 007.lcm
D:\Dados\Cinetina\Desenvolvimento\2021\09-Set\Cinetina_XTerra RP18_TFA_CRM 007_080921_2.lcd
Acquired: 08/09/2021 09:50:27 Date Process: 08/09/2021 10:40:33
Sample ID: Cinetina
Data Description: CN.01/21S1
Diluição: 25,1mg + 20mL NaOH 0,1N/50mL
Fase Móvel: Iso 10% Fase B [MeOH:ACN (1:1)] : 90% Fase A [3,4g/L KH2PO4 pH2,54 (H3PO4)]
Temp. Forno: 30°C
Coluna: X Terra RP18 250 x 4,6mm 5um (02593832313854)
Fluxo: 1,0mL/min Pressão Inicial: 107kgf/cm2
Vol. de injeção: 20uL
User Name: Pesquisa e Desenvolvimento



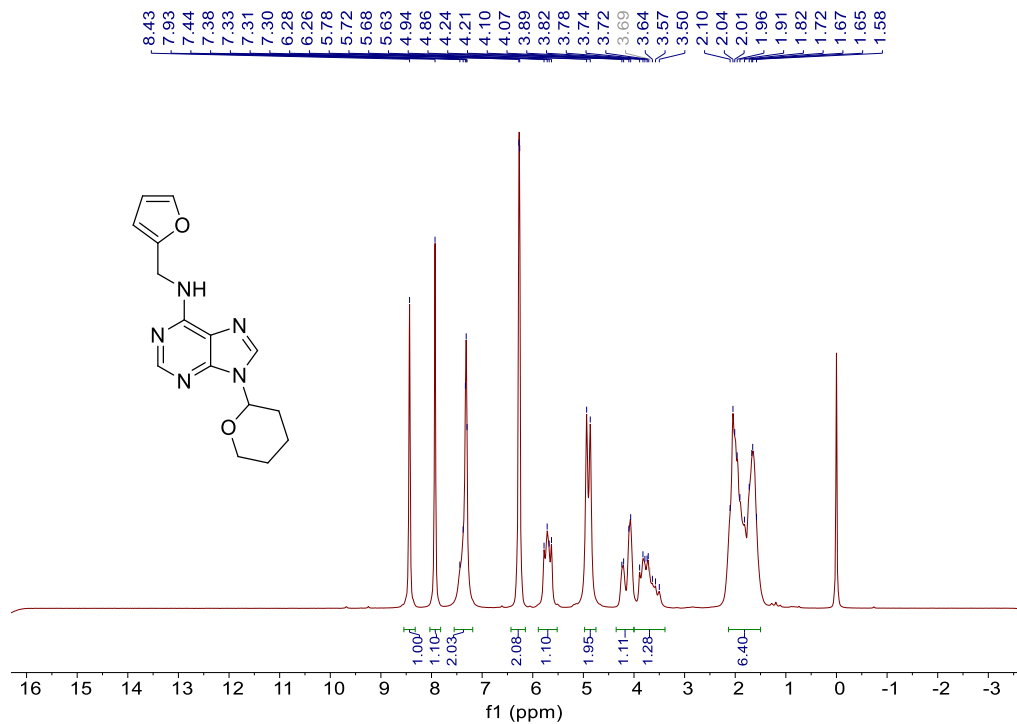
1 PDA Multi 1 / 210nm 4nm

PDA Ch1 210nm 4nm

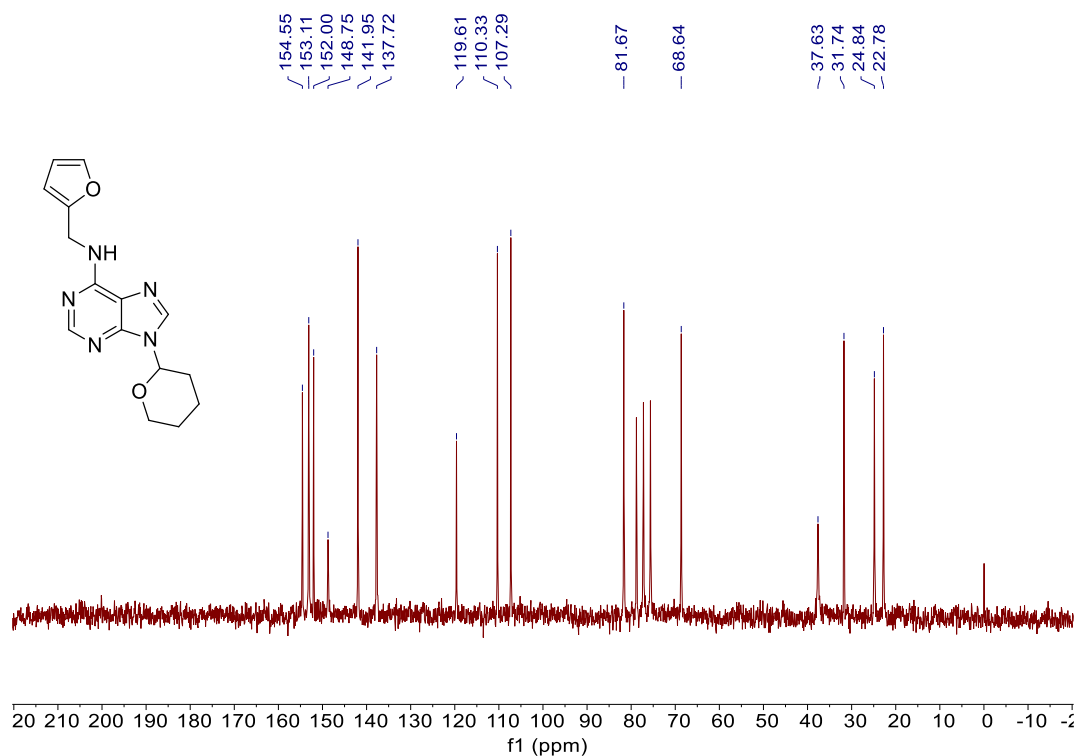
Peak#	Ret. Time	Area	Area %	Theoretical Plate#	Resolution	Tailing Factor	Lambda max
1	2.764	13410	0.021	3034.506	0.000	0.000	663/674/772/583/546
2	3.050	8391	0.013	4347.799	1.480	0.000	795/771/743/583/549
3	8.768	62444202	99.965	11616.785	22.406	2.229	205/272/657/674/631
Total		62466002	100.000				

HPLC of compound MB-905 (A)

6-furfurylamino-9-(tetrahydropyran-2-yl)-9H-purine, MB-906 (B)²

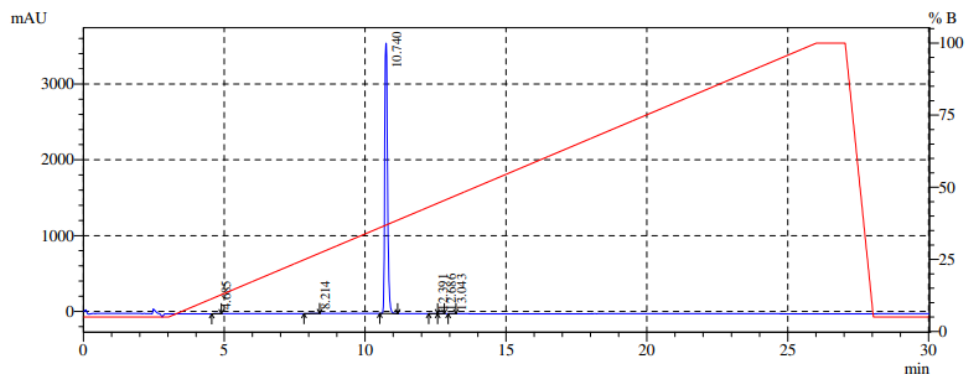


¹H NMR (80 MHz, CDCl₃) of compound MB-906 (B)



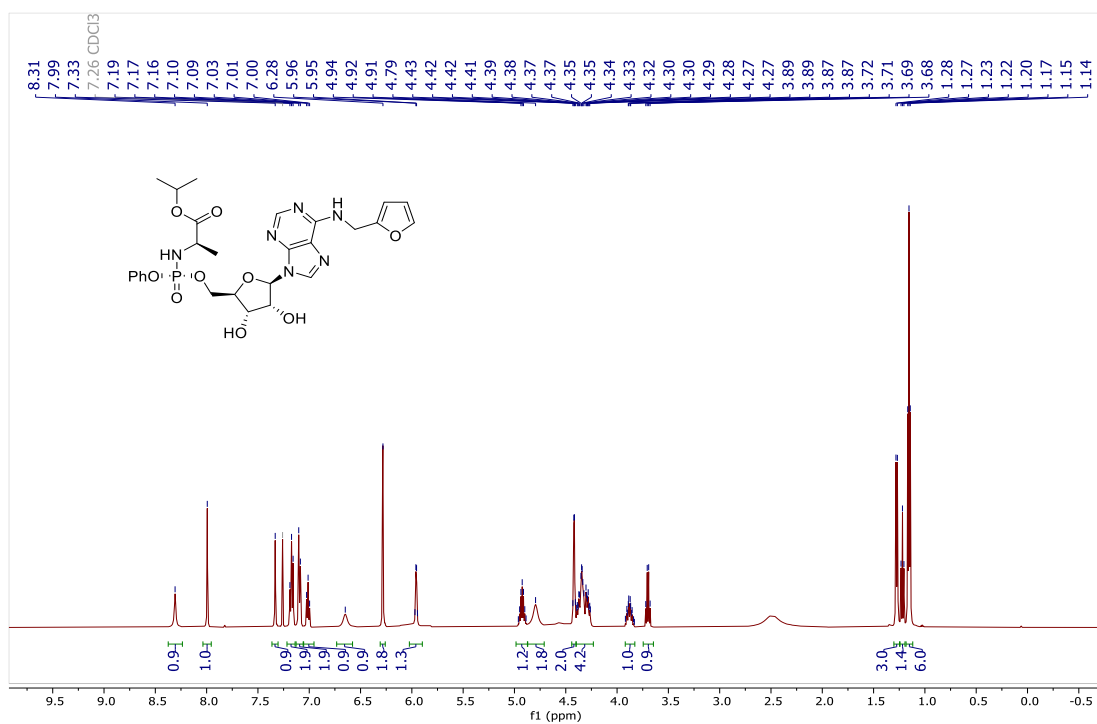
¹³C NMR (20 MHz, CDCl₃) of compound MB-906 (B)

Z:\HPLC\P&D\Tritil-Zeatina\Tritil-Zeatina_XTerra RP18_TFA_CRM 007.lcm
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 Acquired: 29/11/2021 20:53:31 Date Process: 29/11/2021 21:23:41
 Sample ID: Amostra
 Data Description: MB-CN-THP001.2016
 Diluição: Am + ACN/10mL 95% H₂O/ACN
 Fase Móvel: Grad 5-100% Fase B [90% ACN/10% H₂O (0,1% TFA)] : Fase A [90% H₂O (0,1% TFA)/10% ACN]
 Temp. Forno: 30°C
 Coluna: X Terra RP18 250 x 4,6mm 5um (02593832313854)
 Fluxo: 1,2mL/min Pressão Inicial: 129kgf/cm²
 Vol. de injeção: 10uL
 User Name: lara

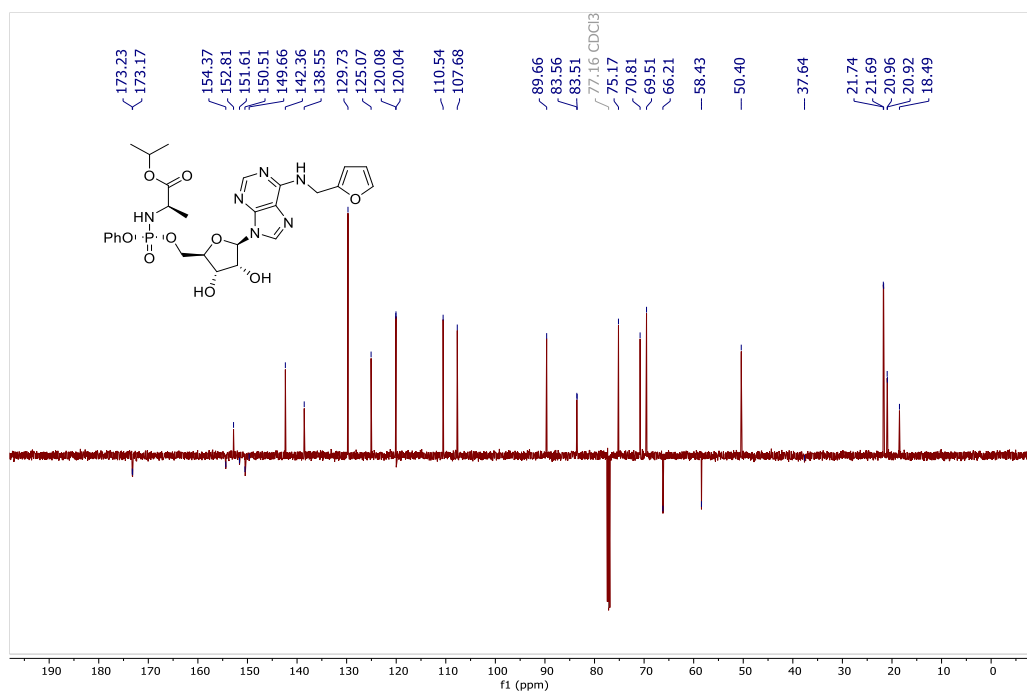


Peak#	Ret. Time	Area	Area %	Theoretical Plate#	Resolution	Tailing Factor	Lambda max
1	4.685	29547	0.120	6261.508	0.000	1.346	275/774/704/688/485
2	8.214	11846	0.048	17558.058	14.556	0.926	642/272/704/774/485
3	10.740	24470202	99.708	56232.770	11.775	1.307	213/263/667/641/485
4	12.391	12294	0.050	69530.741	8.945	1.392	667/270/485/704/569
5	12.686	5397	0.022	97718.246	1.686	1.182	668/641/270/485/711
6	13.043	12457	0.051	93410.744	2.141	1.135	642/274/485/774/687
Total		24541742	100.000				

HPLC of compound MB-906 (B)

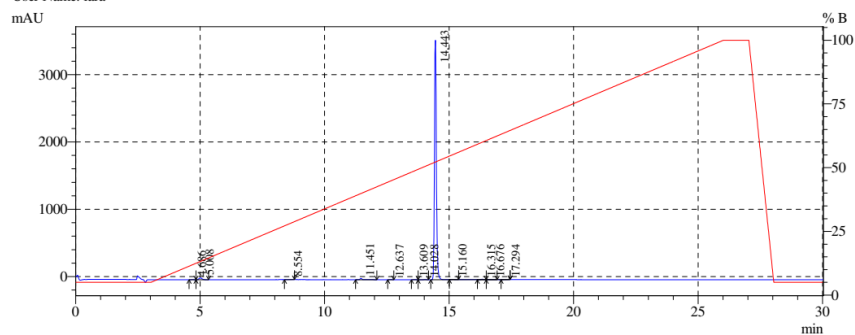
Phenyl (Isopropoxy-L-alaninyl) Kinetin Riboside Phosphoramidate, MB-711 (C)³

¹H NMR (500 MHz, CDCl₃) of compound MB-711 (C)



¹³C (Dept-135) NMR (126 MHz, CDCl₃) of compound MB-711 (C)

Z:\HPLC\P&D\Tritil-Zeatina\Tritil-Zeatina_XTerra RP18_TFA_CRM 007.lcm
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 Acquired: 29/11/2021 15:47:33 Date Process: 29/11/2021 16:17:45
 Sample ID: Amostra
 Data Description: MB-711
 Diluição: Am + ACN/10mL 95% H2O/ACN
 Fase Móvel: Grad 5-100% Fase B [90% ACN/10% H2O (0,1% TFA)] : Fase A [90% H2O (0,1% TFA)/10% ACN]
 Temp. Forno: 30°C
 Coluna: X Terra RP18 250 x 4,6mm 5um (02593832313854)
 Fluxo: 1,2mL/min Pressão Inicial: 129kgf/cm2
 Vol. de injeção: 10uL
 User Name: Iara



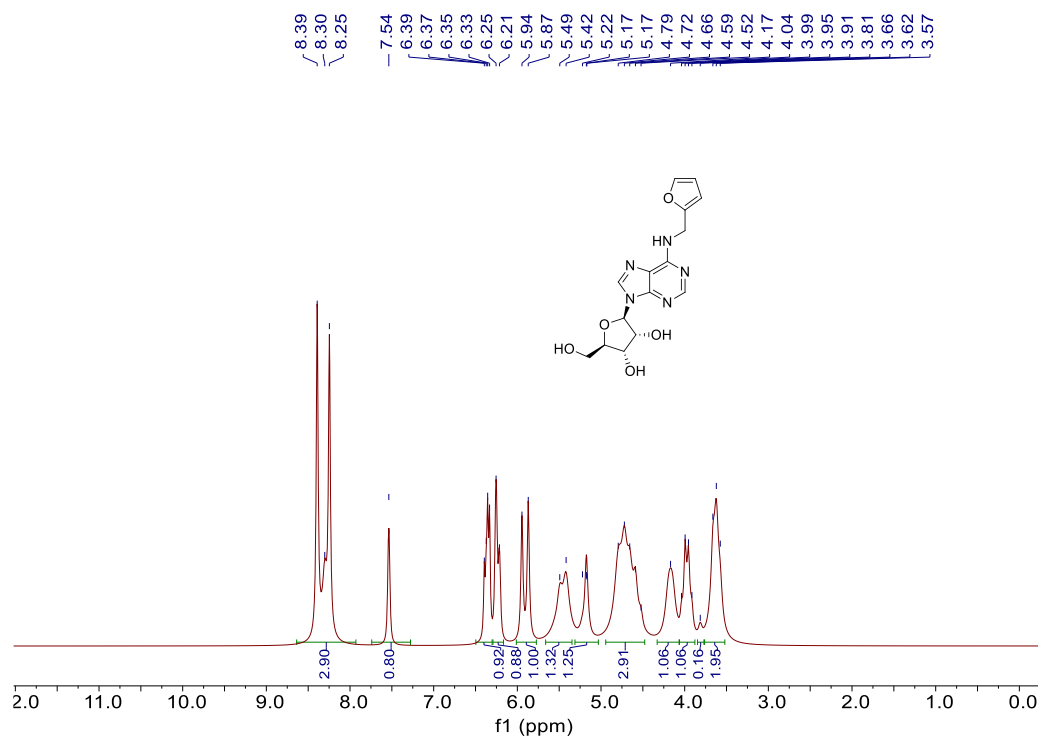
1 PDA Multi 1 / 210nm 4nm

PDA Ch1 210nm 4nm

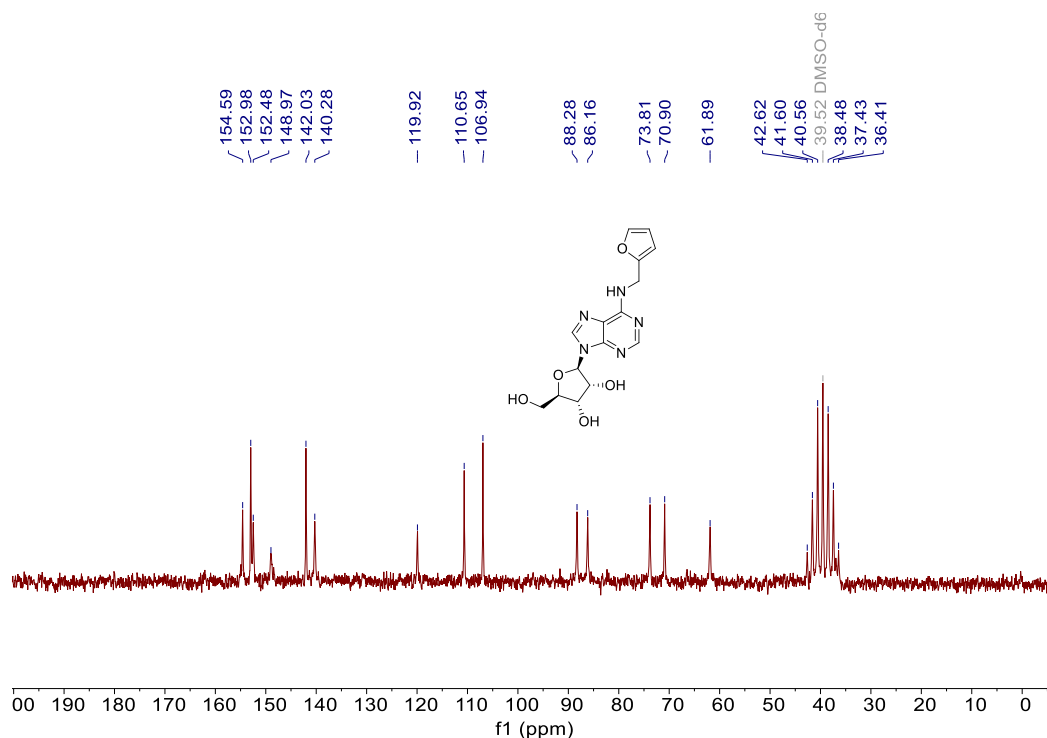
Peak#	Ret. Time	Area	Area %	Theoretical Plate#	Resolution	Tailing Factor	Lambda max
1	4.686	15813	0.080	7337.973	0.000	0.000	663/644/273/775/539
2	5.008	223687	1.127	6859.670	1.399	1.181	267/667/643/694/765
3	8.554	11101	0.056	18907.199	14.452	1.421	668/643/485/270/711
4	11.451	100112	0.504	81736.627	14.163	1.169	258/667/641/774/330
5	12.637	11079	0.056	102416.749	7.453	1.024	667/269/630/765/330
6	13.609	14899	0.075	95189.445	5.811	1.039	662/267/485/772/330
7	14.028	7662	0.039	86053.100	2.282	0.000	644/287/485/774/689
8	14.443	19362954	97.575	140090.443	2.399	1.278	212/264/662/485/688
9	15.160	26115	0.132	124928.393	4.402	1.236	667/269/694/773/782
10	16.315	36814	0.186	143403.289	6.717	1.305	667/643/269/694/774
11	16.676	19158	0.097	112904.350	1.947	0.000	667/641/273/485/774
12	17.294	14789	0.075	46659.238	2.383	0.865	662/273/705/774/741
Total		19844181	100.000				

HPLC of compound MB-711 (C)

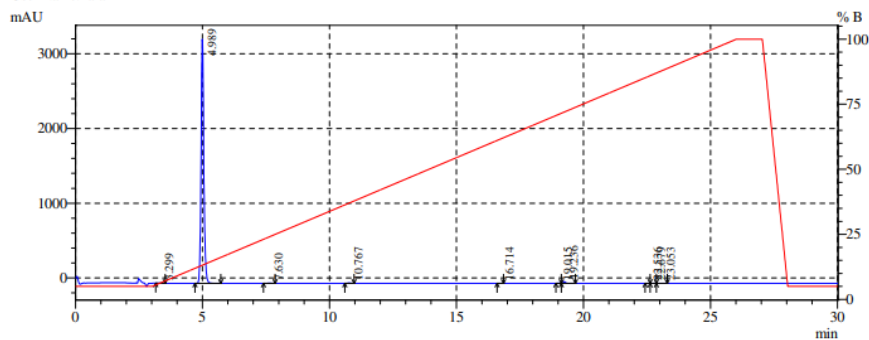
Kinetin riboside, MB-801 (D)³



¹H NMR (80 MHz, CDCl₃) of compound MB-801 (D)



Acquired: 29/11/2021 21:24:06 Date Process: 29/11/2021 21:54:17
 Sample ID: Amostra
 Data Description: MB-801
 Diluição: Am + ACN/10mL 95% H₂O/ACN
 Fase Móvel: Grad 5-100% Fase B [90% ACN/10% H₂O (0,1% TFA)] : Fase A [90% H₂O (0,1% TFA)/10% ACN]
 Temp. Forno: 30°C
 Coluna: X Terra RP18 250 x 4,6mm 5um (02593832313854)
 Fluxo: 1,2mL/min Pressão Inicial: 129kgf/cm²
 Vol. de injeção: 10uL
 User Name: Iara



Peak#	Ret. Time	Area	Area %	Theoretical Plate#	Resolution	Tailing Factor	Lambda max
1	3.299	10594	0.043	2593.918	0.000	1.069	667/643/485/271/711
2	4.989	24297617	98.914	9442.944	7.280	1.207	211/265/642/485/796
3	7.630	28425	0.116	12932.222	11.149	1.023	642/676/271/485/694
4	10.767	37750	0.154	53652.777	13.809	1.189	667/647/270/485/703
5	16.714	5987	0.024	146063.498	32.960	1.273	643/485/703/716/790
6	19.015	22043	0.090	189326.215	13.156	0.000	667/642/485/704/273
7	19.236	144458	0.588	202395.122	1.282	1.269	667/265/642/485/703
8	22.536	5107	0.021	138559.379	15.970	0.000	642/716/689/773/274
9	22.679	5936	0.024	170629.371	0.622	0.000	485/704/273/783/592
10	23.053	6492	0.026	157005.052	1.651	1.309	668/647/485/704/771
Total		24564409	100.000				

HPLC of compound MB-801 (D)

Literature where compounds are reported

1. Compound reported in Wang, X.; Han, C.; Wu, K.; Luo, L.; Wang, Y.; Du, X.; He, Q.; Ye, F. Design, Synthesis and Ability of Non-Gold Complexed Substituted Purine Derivatives to Inhibit LPS-Induced Inflammatory Response. *European Journal of Medicinal Chemistry* **2018**, *149*, 10–21.
2. Compound reported in Zahajská, L.; Nisler, J.; Voller, J.; Gucký, T.; Pospíšil, T.; Spíchal, L.; Strnad, M. Preparation, Characterization and Biological Activity of C8-Substituted Cytokinins. *Phytochemistry* **2017**, *135*, 115–127.
3. Compound reported in Osgerby, L.; Lai, Y.-C.; Thornton, P. J.; Amalfitano, J.; Le Duff, C. S.; Jabeen, I.; Kadri, H.; Miccoli, A.; Tucker, J. H. R.; Muqit, M. M. K.; Mehellou, Y. Kinetin Riboside and Its ProTides Activate the Parkinson's Disease Associated PTEN-Induced Putative Kinase 1 (PINK1) Independent of Mitochondrial Depolarization. *Journal of Medicinal Chemistry* **2017**, *60* (8), 3518–3524.