

Synthesis and ¹⁸F-radiolabeling of thymidine AMBF₃ conjugates

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Supplementary Information

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Supplemental Synthetic Schemes and NMR Spectra
5-hydroxymethyl-2'-deoxyuridine
Spectral data agrees with previous literature¹².

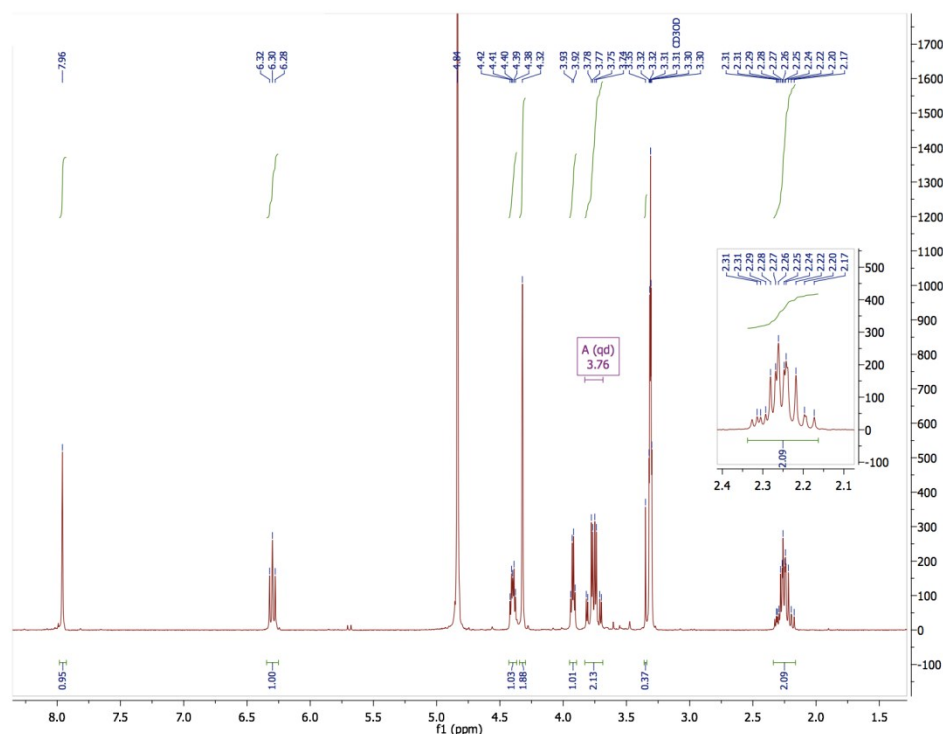


Figure S1. ¹H NMR (300 MHz) of 5-hydroxymethyl-2'-deoxyuridine.

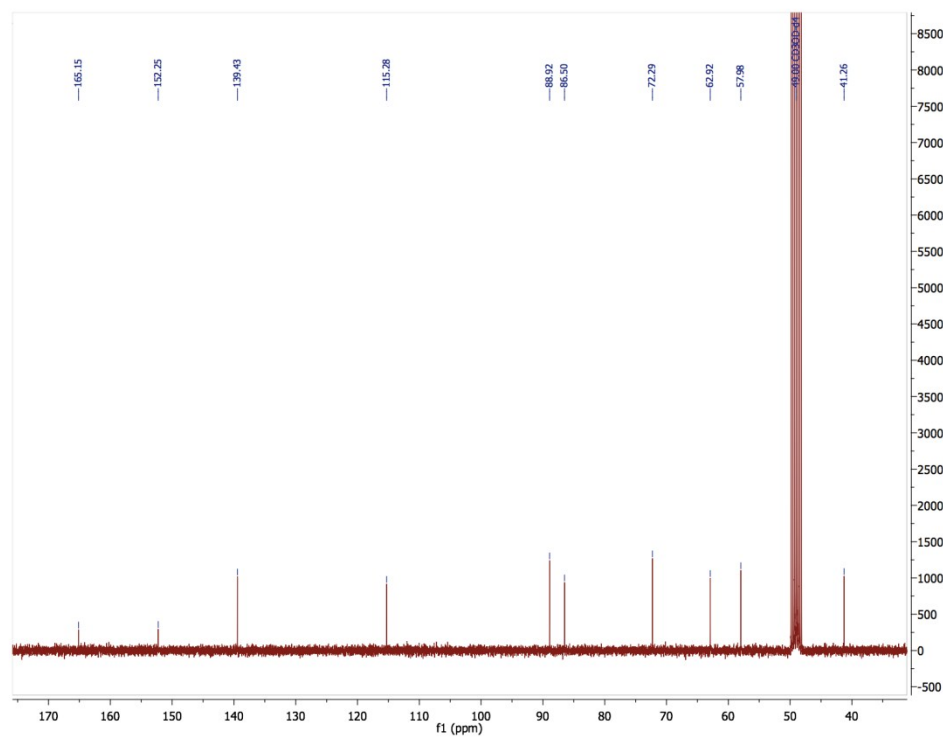


Figure S2. ^{13}C NMR (75 MHz) of 5-hydroxymethyl-2'-deoxyuridine.

5-azidomethyl-2'-deoxyuridine, **5**
Spectral data agrees with previous literature ²⁷.

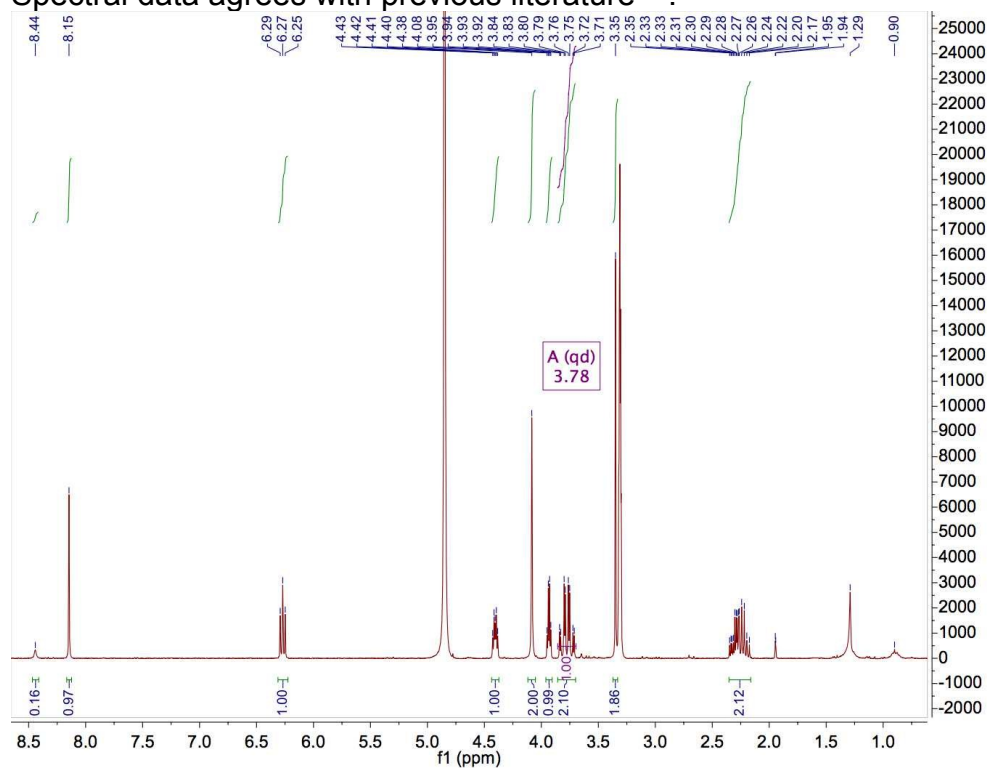


Figure S3. ^1H NMR (300 MHz) of **5**.

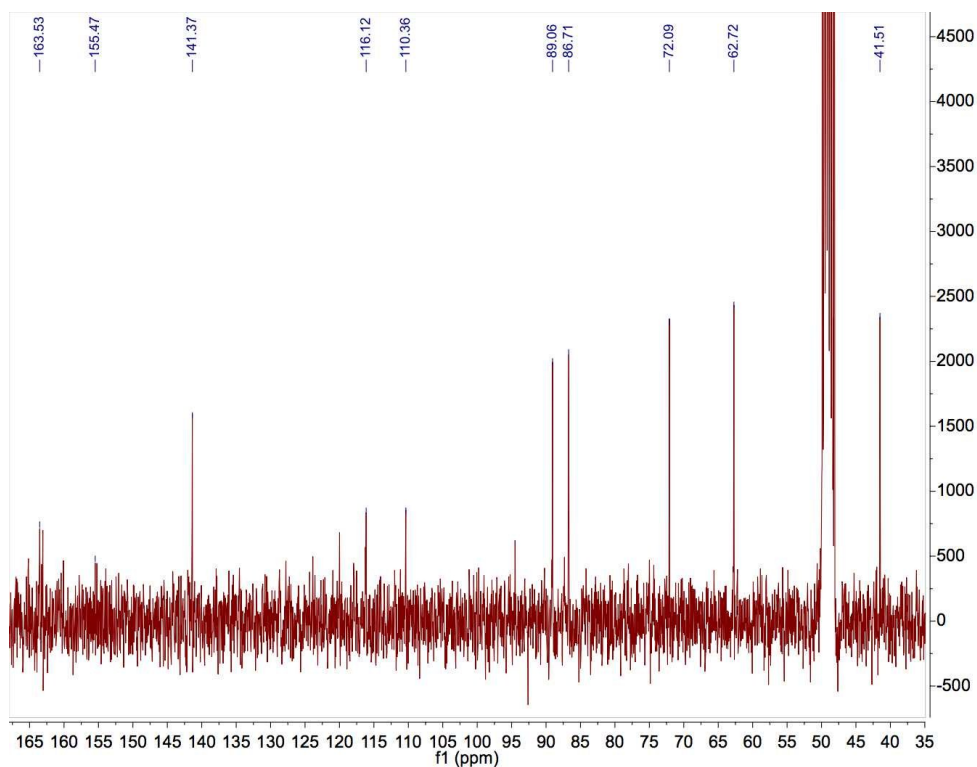


Figure S4. ^{13}C NMR (75 MHz) of **5**.

N-((difluoroboraneyl)methyl)-N,N-dimethylprop-2-yn-1-aminium fluoride, **4**

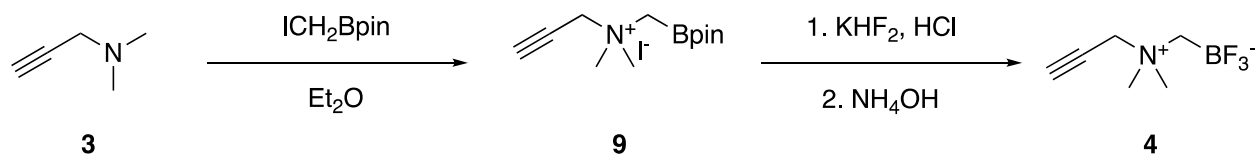


Figure S5. Synthesis of **4**.

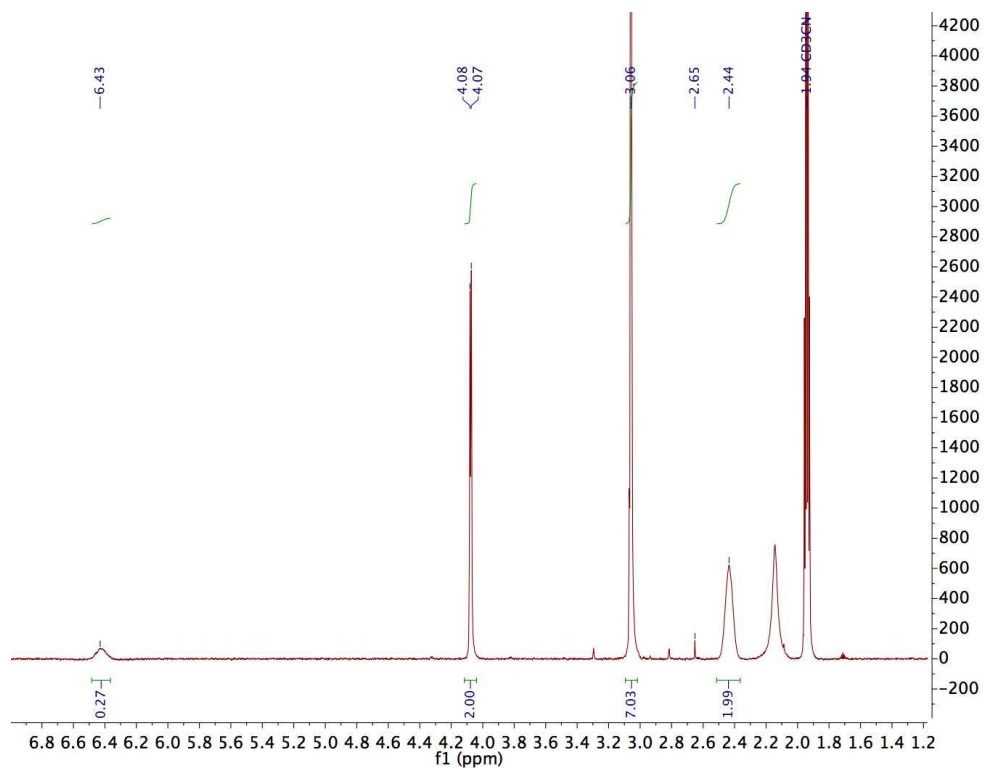


Figure S6. ^1H NMR (300 MHz) of 4.

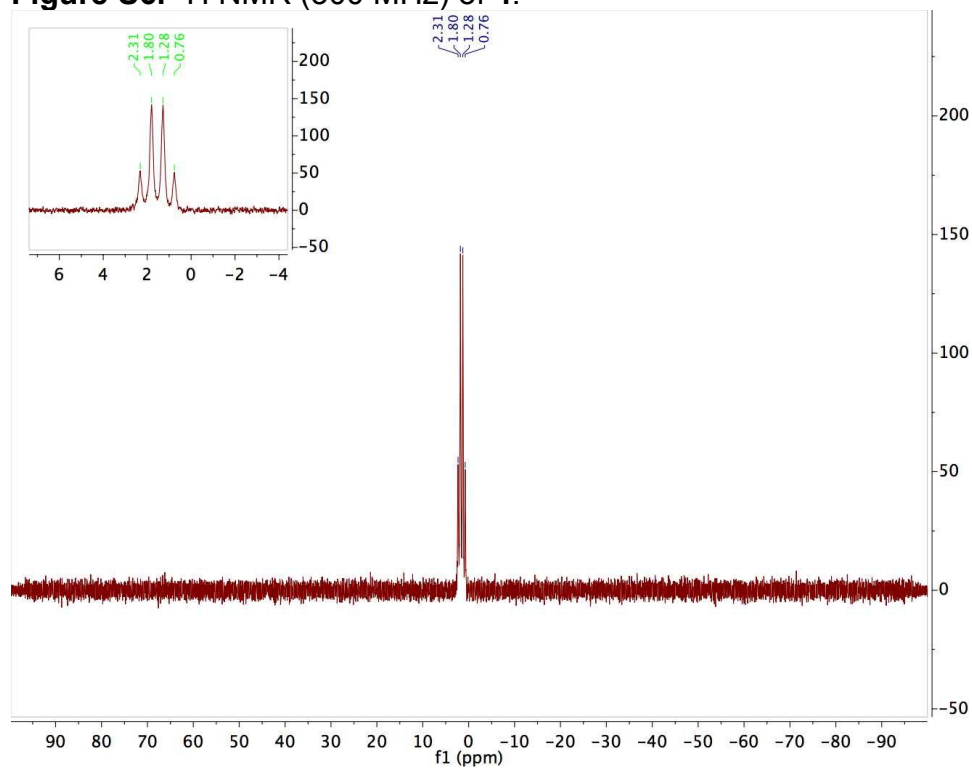


Figure S7. ^{11}B NMR (96 MHz) of 4.

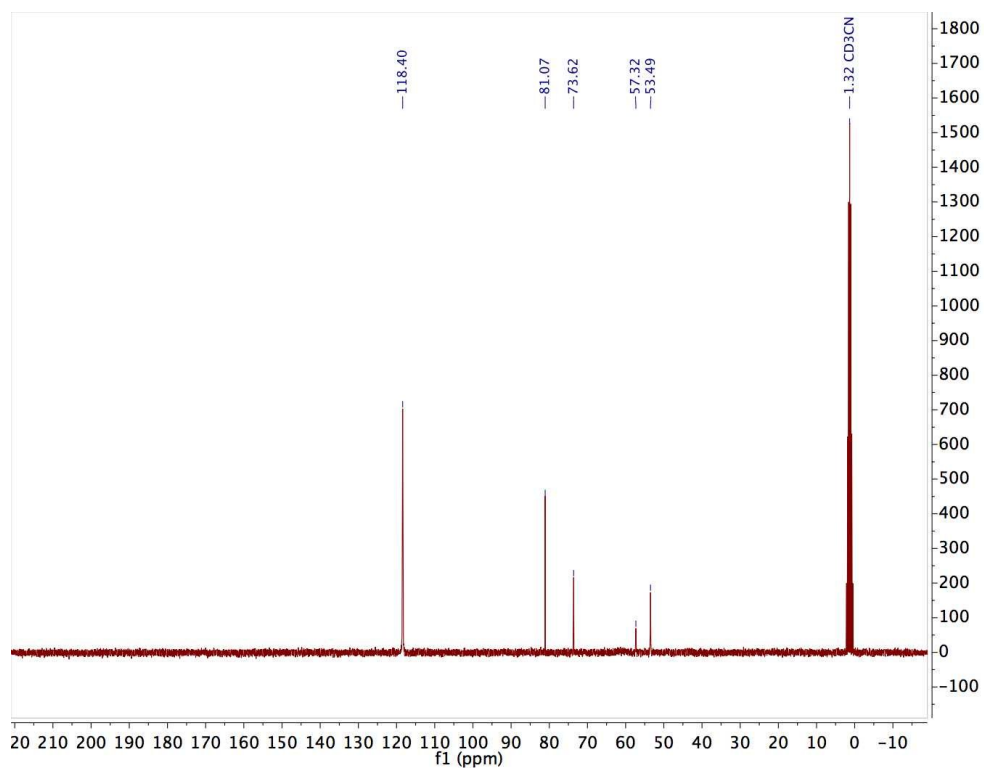


Figure S8. ^{13}C NMR (75 MHz) of 4.

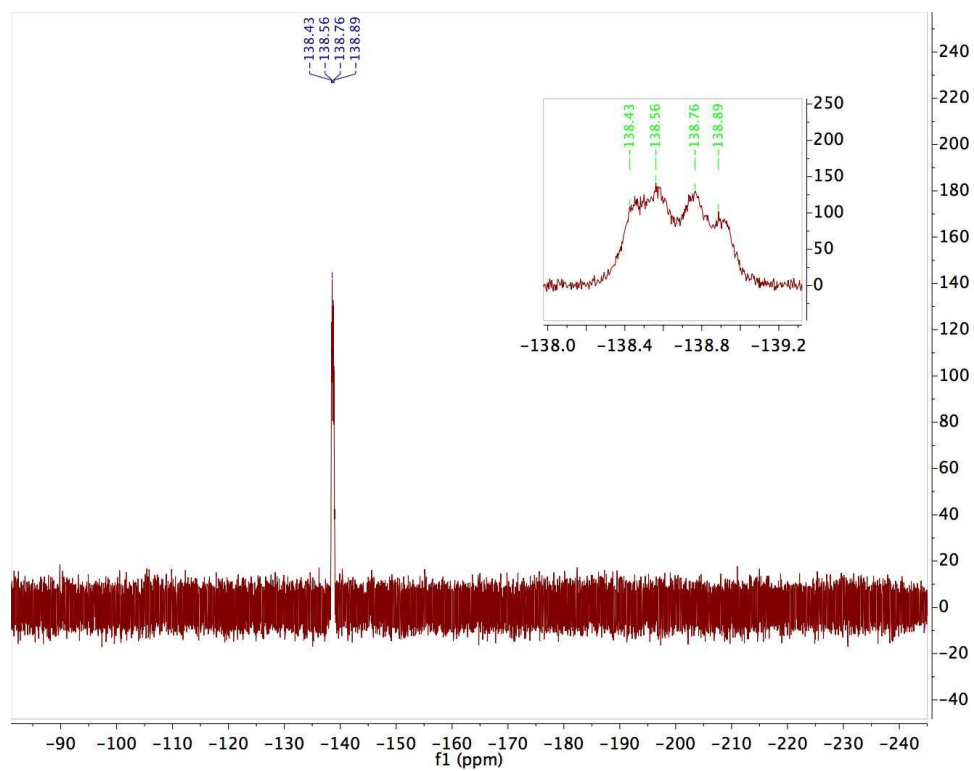


Figure S9. ^{19}F NMR (282 MHz) of 4.

(((3-chloropropyl)dimethylammonio)methyl)trifluoroborate, **7**

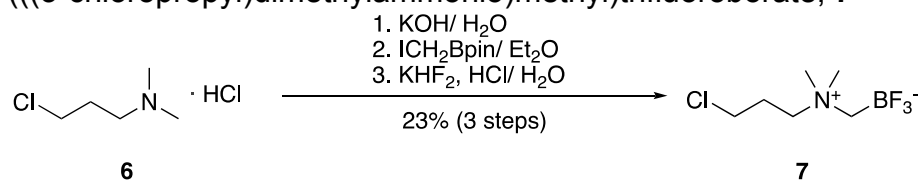


Figure S10. Synthesis of **7**.

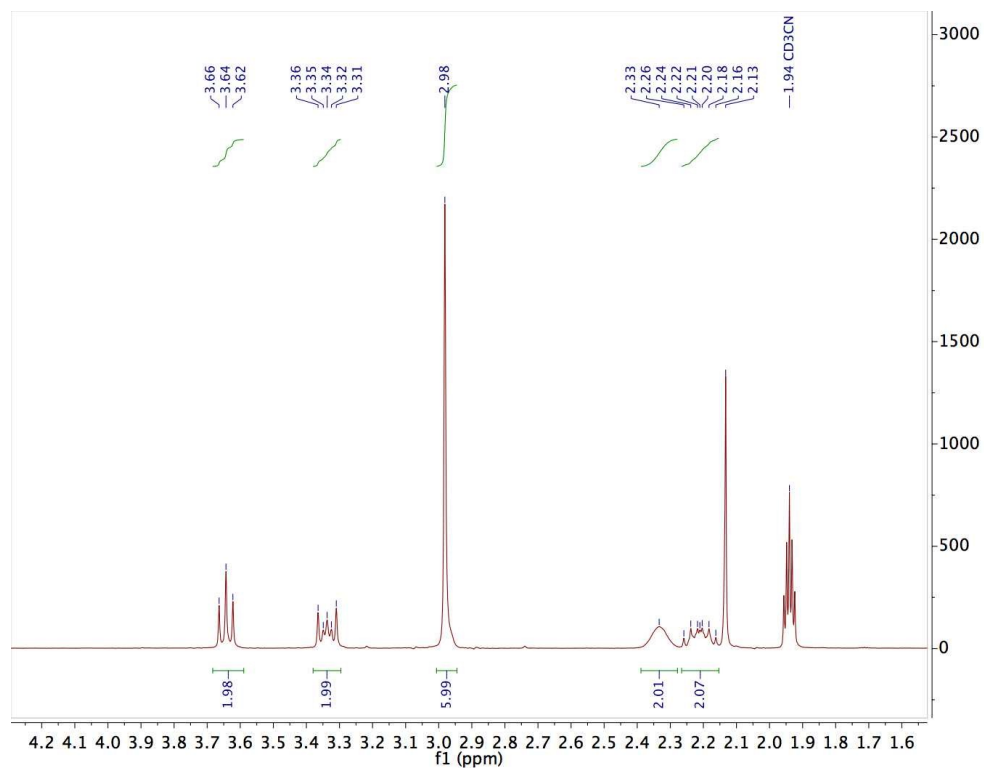


Figure S11. ¹H NMR (300 MHz) of **7**.

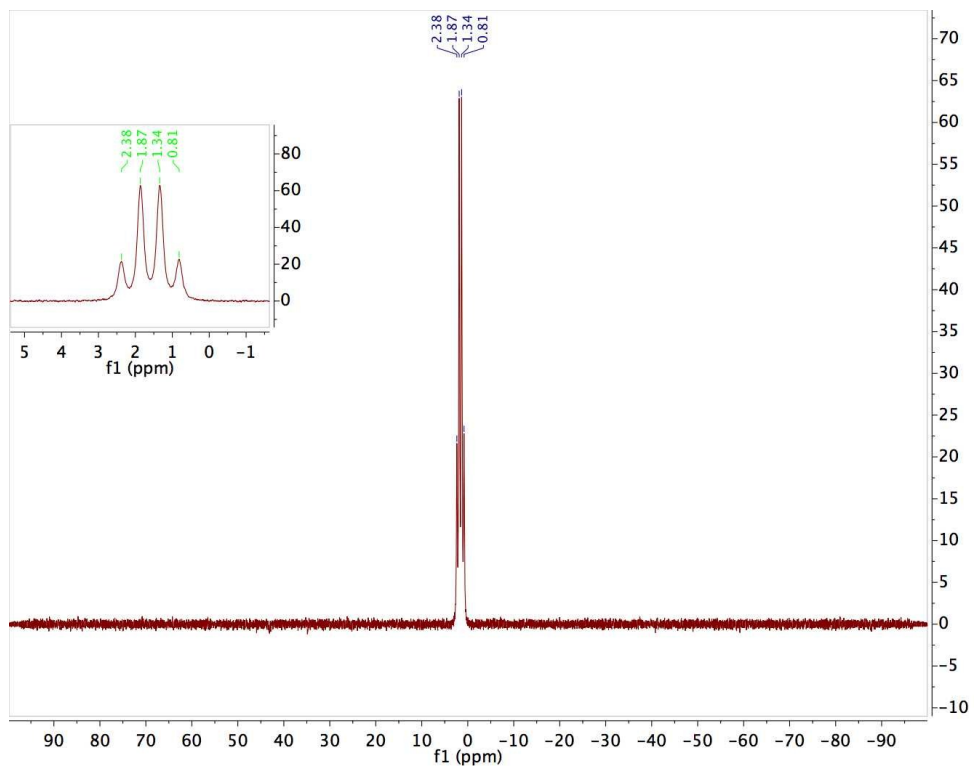


Figure S12. ^{11}B NMR (96 MHz) of 7.

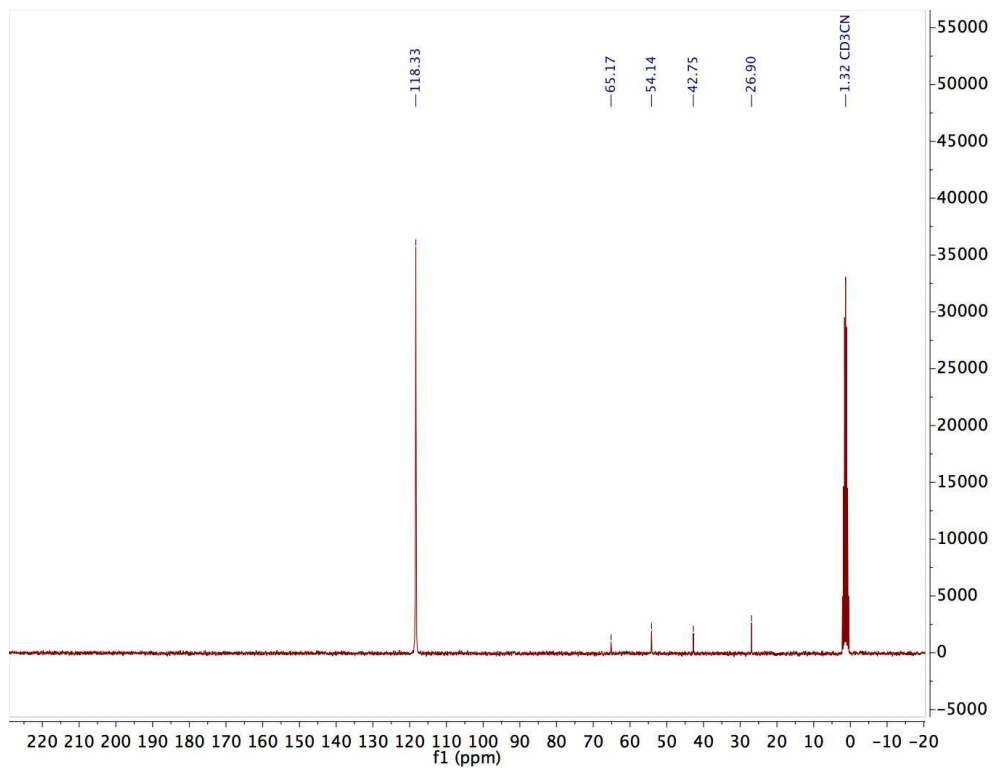


Figure S13. ^{13}C NMR (75 MHz) of 7.

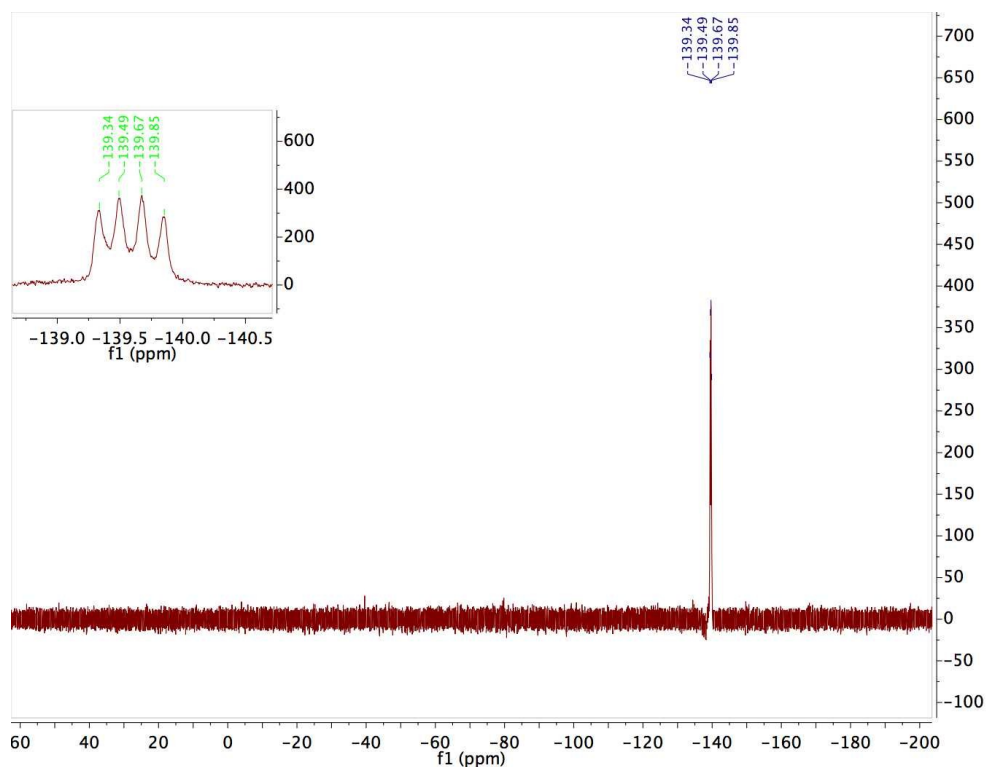


Figure S14. ^{19}F NMR (282 MHz) of **7**.

Trifluoro(((1-(((1-((2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-yl)methyl)-1H-1,2,3-triazol-4-yl)methyl)dimethylammonio)methyl)borate, dT-C⁵-AMBF₃, **1**

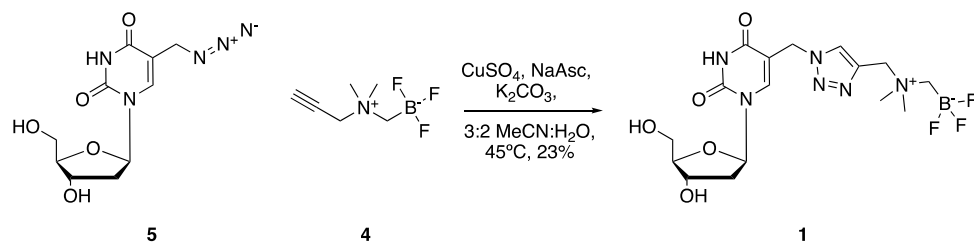


Figure S15. Synthesis of **1**.

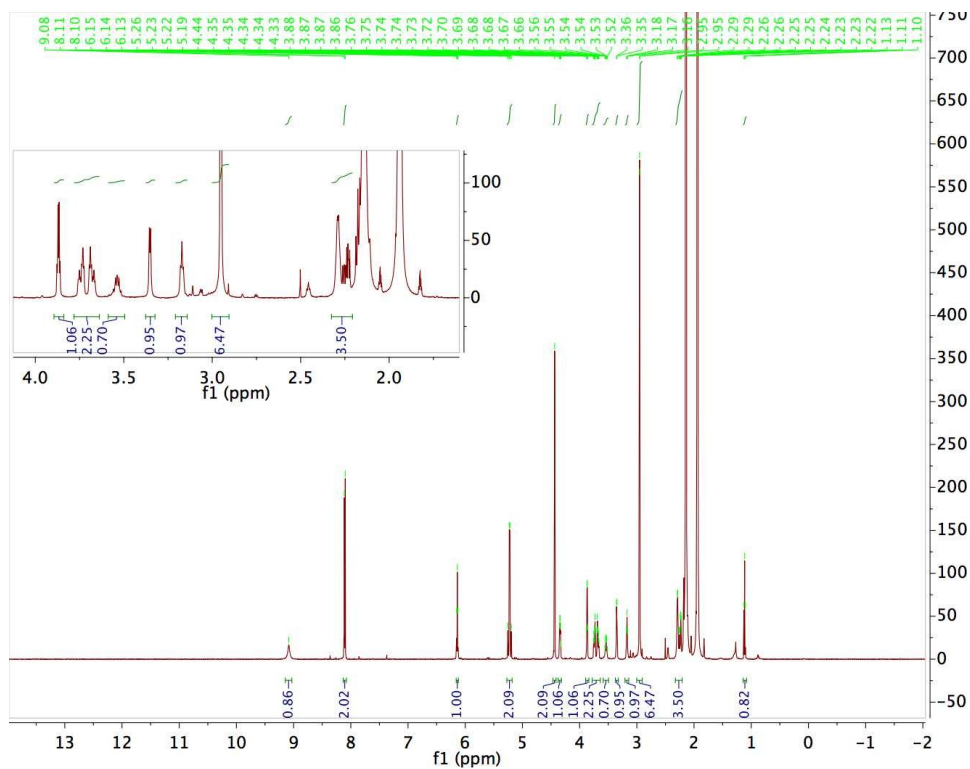


Figure S16. ^1H NMR (600 MHz) of **1**.

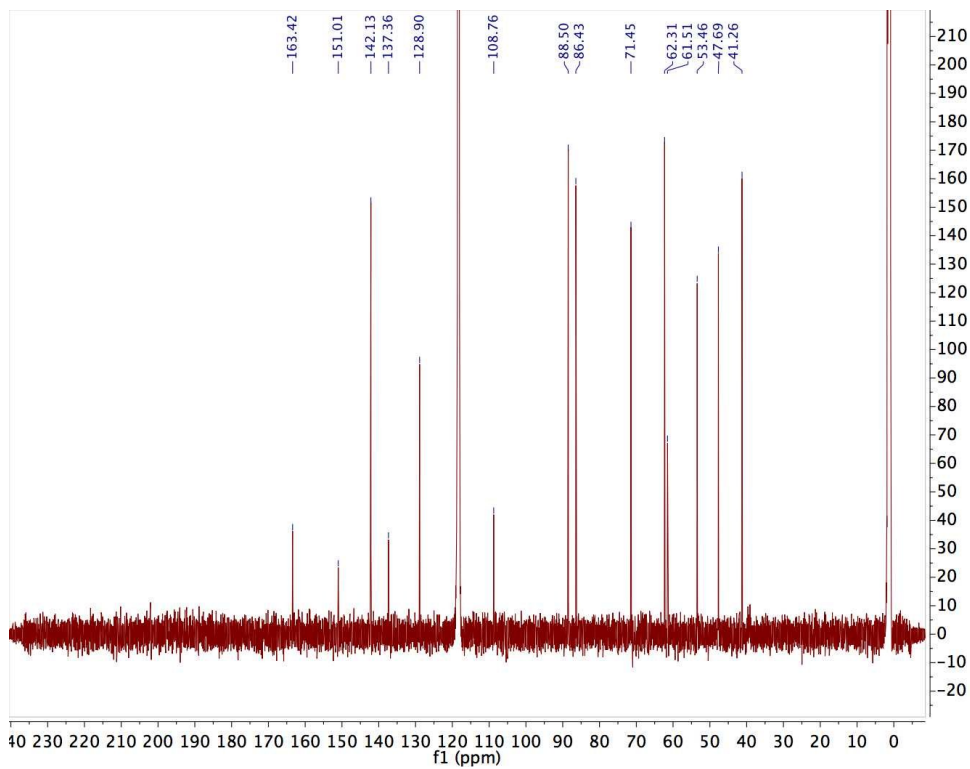


Figure S17. ^{13}C NMR (150 MHz) of **1**.

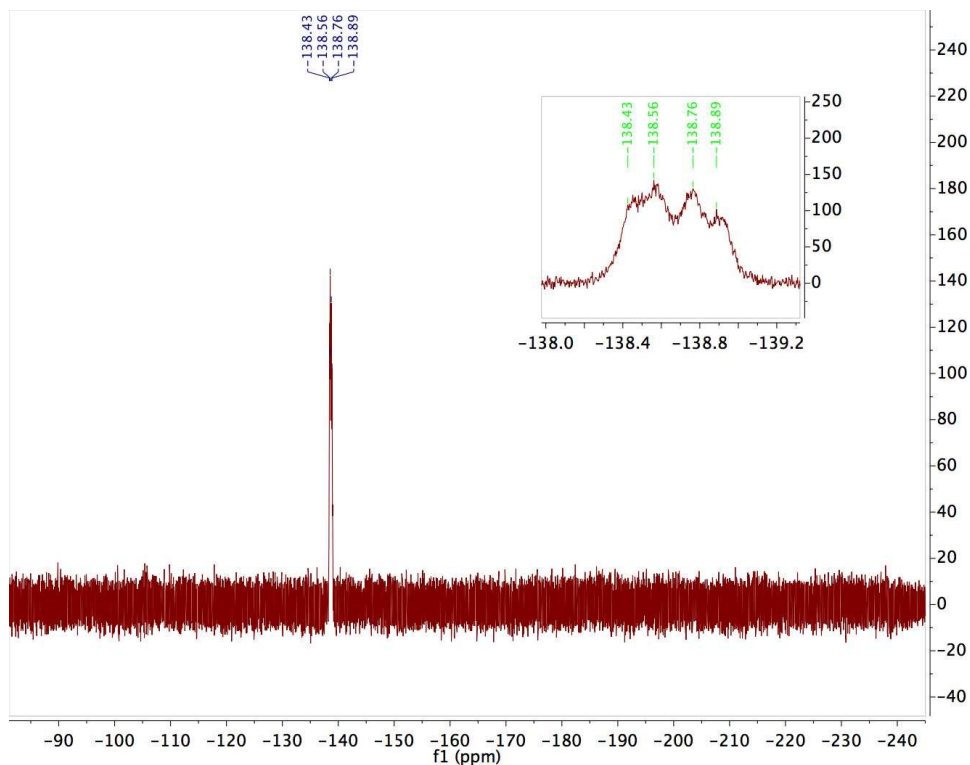


Figure S18. ^{19}F NMR (282 MHz) of **1**.

Trifluoro(((3-(3-((2R,4S,5R)-4-hydroxy-5-(hydroxymethyl)tetrahydrofuran-2-yl)-5-methyl-2,6-dioxo-3,6-dihydropyrimidin-1(2H)-yl)propyl)dimethylammonio)methyl)borate, dT-N³-AMBF₃, **2**

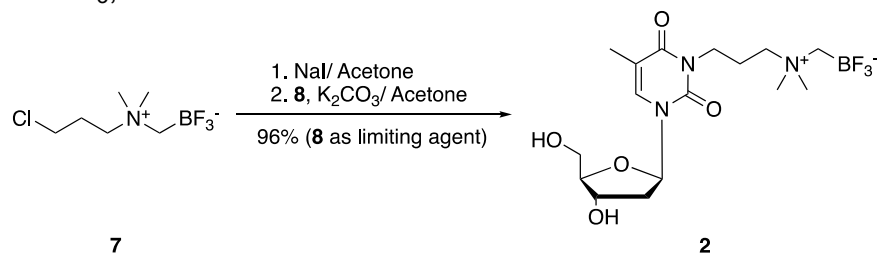


Figure S19. Synthesis of **2**.

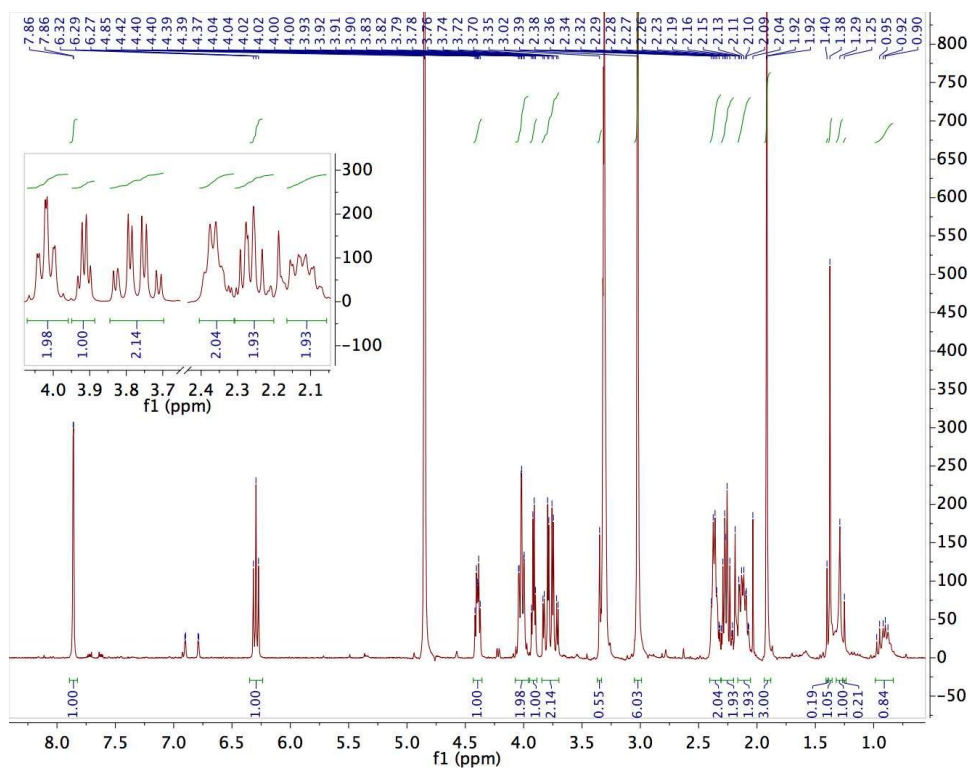


Figure S20. ^1H NMR (300 MHz) of **2**.

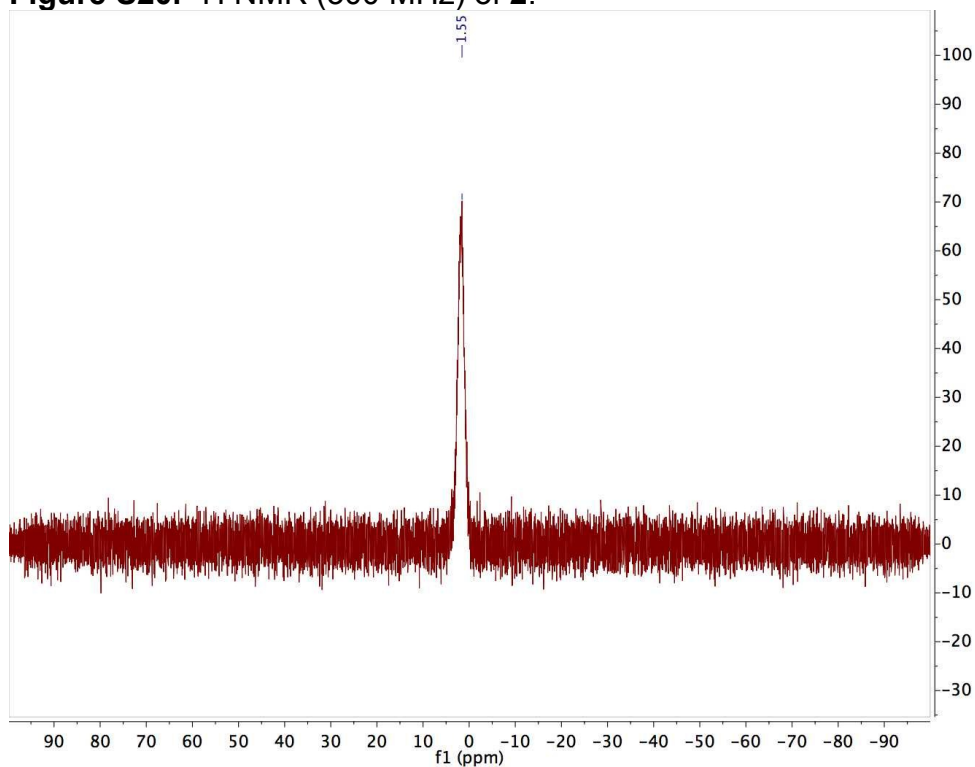


Figure S21. ^{11}B NMR (96 MHz) of **2**.

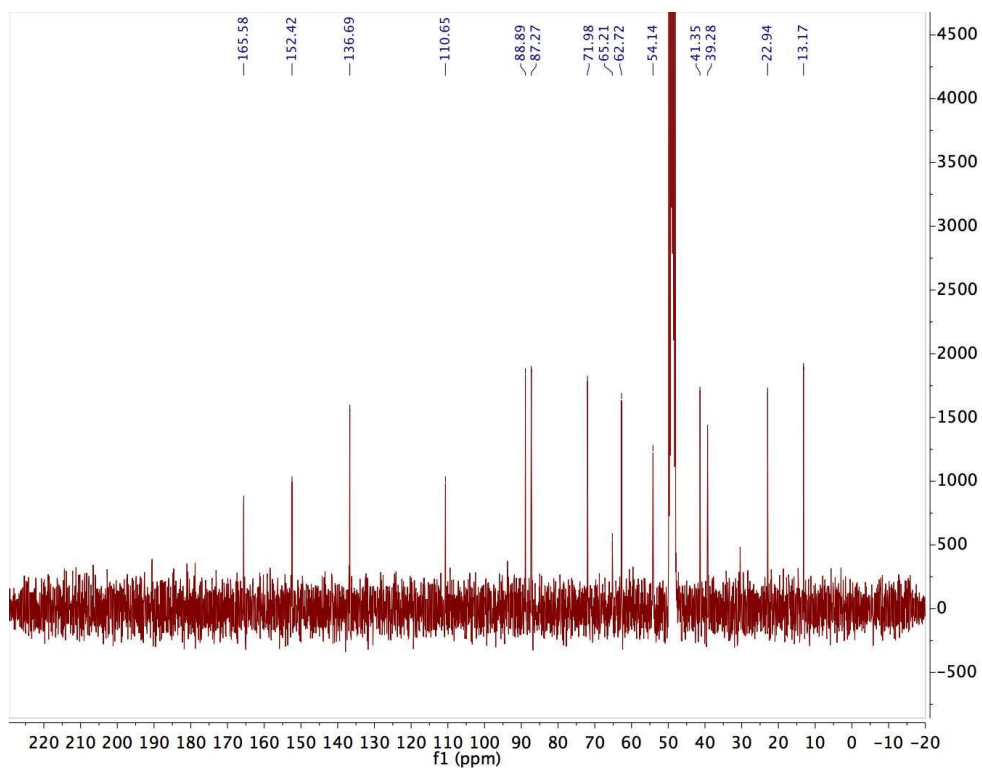


Figure S22. ^{13}C NMR (75 MHz) of **2**.

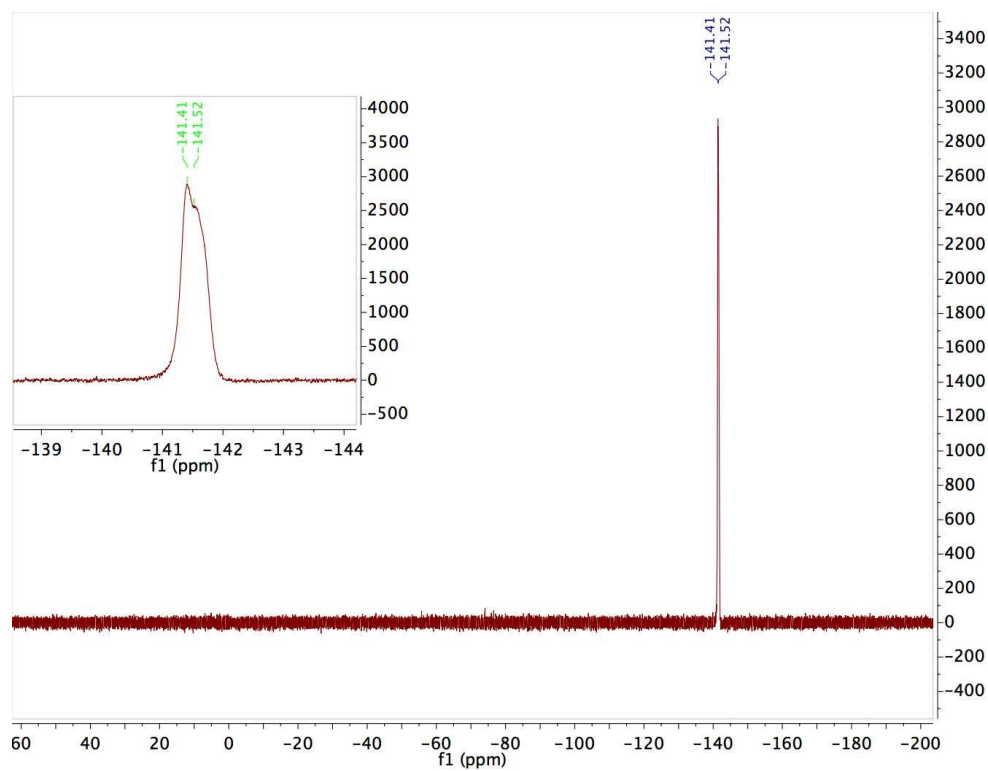


Figure S23. ^{19}F NMR (282 MHz) of **2**.

Radiochemistry and Imaging

Radiosynthesis

Lot numbers of [^{18}O]- H_2O used for [^{18}F]-fluoride generation were 18-0539, 18-0757, 19-0097, 19-0149 and 19-0285 in British Columbia Cancer Research Centre.

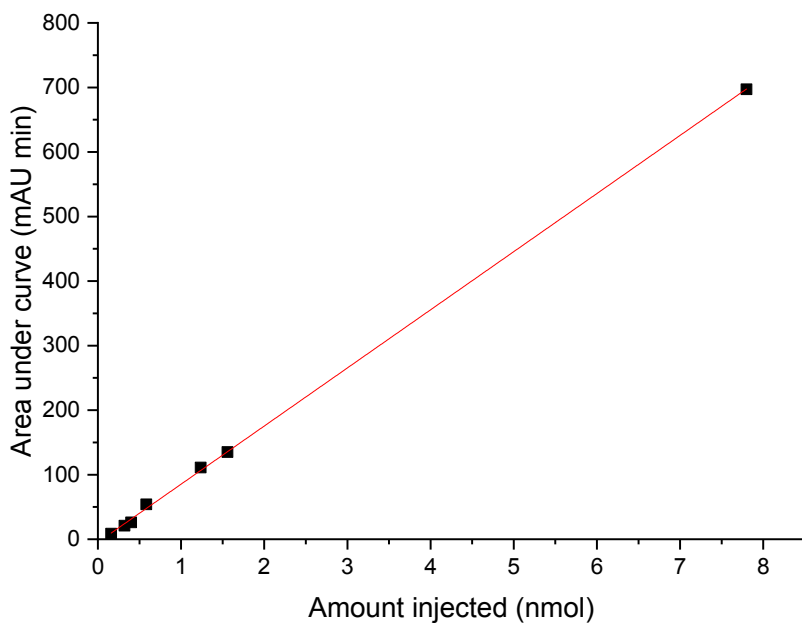


Figure S24. Standard curve for [^{18}F]**1**. $y = 90.1x - 4.76$, $R^2 = 0.9997$.

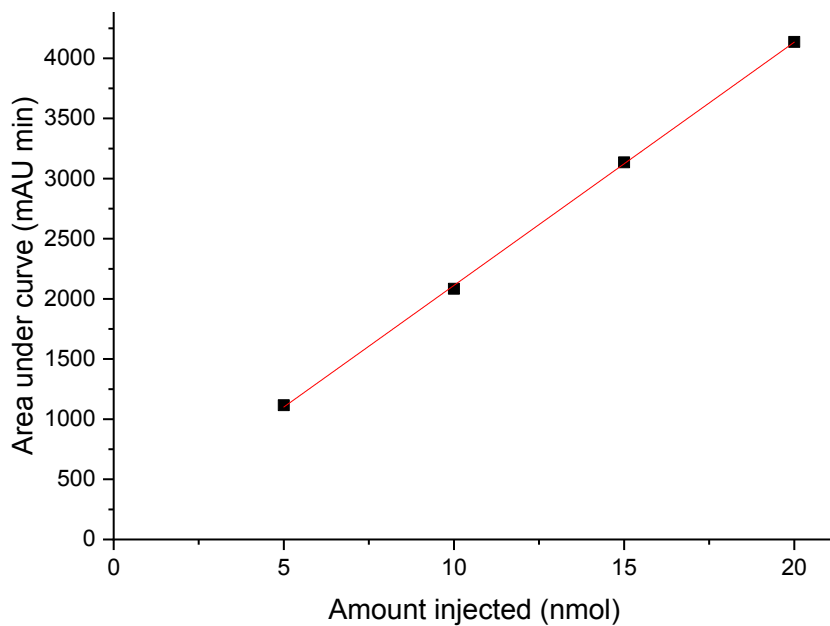


Figure S25. Standard curve for [^{18}F]**2**. $y = 202x - 90.2$, $R^2 = 0.9997$.

PET/CT imaging and biodistribution studies

Table S1. Biodistribution of [¹⁸F]1 in NSG mice bearing U87 tumor.

1h p.i.	Subject 1	Subject 2	Subject 3	Mean (%ID/g)	Standard deviation
Tumor/bone	1.57	2.77	1.73	2.02	0.65
Tumor/muscle	4.57	4.72	3.10	4.13	0.90
Tumor/blood	1.23	1.16	0.93	1.11	0.16
Tumor/kidney	0.33	0.30	0.26	0.30	0.04

Table S2. Imaging data of [¹⁸F]1 in NSG mice bearing U87 tumor.

1h p.i.	Subject 1	Subject 2	Subject 3	Mean (%ID/g)	Standard deviation
Blood	0.07	0.14	0.07	0.09	0.04
Urine	53.49	70.23	119.60	81.11	34.37
Fat	0.01	0.01	0.01	0.01	0.00
Intestine	0.47	0.48	0.37	0.44	0.06
Spleen	0.02	0.02	0.01	0.02	0.01
Pancreas	0.03	0.06	0.02	0.04	0.02
Liver	0.37	0.51	0.14	0.34	0.19
Kidney	0.26	0.52	0.25	0.35	0.15
Heart	0.02	0.04	0.01	0.03	0.01
Lungs	0.06	0.11	0.04	0.07	0.03
U87 Tumor	0.09	0.16	0.07	0.10	0.05
Bone	0.06	0.06	0.04	0.05	0.01
Muscle	0.02	0.03	0.02	0.02	0.01
Brain	0.00	0.01	0.00	0.00	0.00
Tail	0.25	0.26	0.10	0.21	0.09

Table S3. Biodistribution of [¹⁸F]1 in unblocked NSG mice bearing U87 tumor.

1h p.i.	Subject 4	Subject 5	Subject 6	Subject 7	Mean (%ID/g)	Standard deviation
Tumor:bone	0.66	0.71	2.19	0.66	1.05	0.76
Tumor:muscle	2.97	1.18	7.58	1.07	3.20	3.05
Tumor:blood	0.96	0.19	2.03	1.29	1.12	0.77
Tumor:kidney	0.18	0.25	0.34	0.22	0.24	0.07

Table S4. Imaging data of [¹⁸F]1 in unblocked NSG mice bearing U87 tumor.

1h p.i.	Subject 4	Subject 5	Subject 6	Subject 7	Mean (%ID/g)	Standard deviation
Blood	0.06	0.32	0.06	0.05	0.13	0.13
Urine	397.79	269.12	270.64	176.45	278.50	90.91
Fat	0.11	0.01	0.00	0.01	0.03	0.05
Intestine	0.39	0.49	0.51	0.55	0.48	0.07
Spleen	0.02	0.04	0.03	0.03	0.03	0.01
Pancreas	0.03	0.03	0.02	0.03	0.03	0.01
Liver	0.21	0.33	0.30	0.37	0.30	0.07
Kidney	0.35	0.24	0.39	0.32	0.33	0.06
Heart	0.03	0.03	0.02	0.02	0.02	0.01
Lungs	0.06	0.07	0.06	0.06	0.06	0.00
U87 Tumor	0.06	0.06	0.13	0.07	0.08	0.03
Bone	0.09	0.09	0.06	0.11	0.09	0.02
Muscle	0.02	0.05	0.02	0.07	0.04	0.02
Brain	0.00	0.01	0.00	0.00	0.00	0.00
Tail	0.18	0.34	0.24	0.11	0.22	0.09

Table S5. Biodistribution of [¹⁸F]1 in blocked NSG mice bearing U87 tumor.

1h p.i.	Subject 8	Subject 9	Subject 10	Subject 11	Mean (%ID/g)	Standard deviation
Tumor:bone	2.01	0.23	1.35	0.62	1.05	0.79
Tumor:muscle	3.27	2.93	3.05	2.50	2.94	0.33
Tumor:blood	0.77	0.71	0.83	0.83	0.78	0.06
Tumor:kidney	0.17	0.15	0.26	0.29	0.22	0.07

Table S6. Imaging data of [¹⁸F]1 in blocked NSG mice bearing U87 tumor.

1h p.i.	Subject 8	Subject 9	Subject 10	Subject 11	Mean (%ID/g)	Standard deviation
Blood	0.21	0.05	0.30	0.10	0.17	0.11
Urine	268.64	282.13	252.92	240.07	260.94	18.33
Fat	0.01	0.01	0.03	0.01	0.01	0.01
Intestine	0.51	0.47	0.61	0.67	0.57	0.09
Spleen	0.07	0.02	0.12	0.04	0.06	0.04
Pancreas	0.07	0.02	0.09	0.06	0.06	0.03
Liver	0.57	0.27	1.11	0.67	0.66	0.35
Kidney	0.99	0.21	0.95	0.29	0.61	0.42
Heart	0.07	0.02	0.09	0.04	0.05	0.03
Lungs	0.18	0.05	0.22	0.08	0.13	0.08
U87 Tumor	0.17	0.03	0.25	0.08	0.13	0.09
Bone	0.08	0.14	0.18	0.14	0.14	0.04
Muscle	0.05	0.01	0.08	0.03	0.04	0.03
Brain	0.01	0.00	0.01	0.01	0.01	0.00

Table S7. Biodistribution of [¹⁸F]2 in blocked NSG mice bearing U87 tumor.

1h p.i.	Subject 12	Subject 13	Subject 14	Mean (%ID/g)	Standard deviation
Tumor/bone	2.23	1.38	1.93	1.84	0.43
Tumor/muscle	3.84	3.44	4.01	3.76	0.29
Tumor/blood	0.86	0.72	0.75	0.78	0.07
Tumor/kidney	0.27	0.15	0.15	0.19	0.07

Table S8. Imaging data of [¹⁸F]2 in blocked NSG mice bearing U87 tumor.

1h p.i.	Subject 12	Subject 13	Subject 14	Mean (%ID/g)	Standard deviation
Blood	0.11	0.10	0.14	0.11	0.02
Urine	256.42	177.58	54.19	162.73	101.93
Fat	0.01	0.00	0.01	0.01	0.01
Intestine	1.45	1.78	1.62	1.62	0.17
Spleen	0.07	0.07	0.09	0.08	0.02
Pancreas	0.05	0.05	0.07	0.06	0.01
Liver	1.32	1.54	2.17	1.68	0.44
Kidney	0.35	0.48	0.70	0.51	0.18
Heart	0.04	0.04	0.05	0.04	0.01
Lungs	0.09	0.08	0.12	0.10	0.02
U87 Tumor	0.09	0.07	0.10	0.09	0.02
Bone	0.04	0.05	0.05	0.05	0.01
Muscle	0.02	0.02	0.03	0.02	0.00
Brain	0.01	0.00	0.00	0.00	0.00
Tail	0.10	0.19	0.10	0.13	0.05

$\log P_{7.4}$

Table S9. $\log P_{7.4}$ study of [^{18}F]1. All activities are corrected to the time of quench.

	Volume (μL)	Sample 1	Sample 2	Sample 3
Radioactivity, <i>n</i> -octanol layer (cpm)	2000	28741	30570	30634
Radioactivity, PBS layer (cpm)	50	789897	815208	703487
$P_{7.4}$, volume adjusted		0.0009096	0.0009375	0.0010886
$\log P_{7.4}$		-3.041	-3.028	-2.963

Table S10. $\log P_{7.4}$ study of [^{18}F]2. All activities are corrected to the time of quench.

	Volume (μL)	Sample 1	Sample 2	Sample 3
Radioactivity, <i>n</i> -octanol layer (cpm)	1000	600	436	468
Radioactivity, PBS layer (cpm)	100	926664	967277	989905
$P_{7.4}$, volume adjusted		0.0000647	0.0000451	0.0000473
$\log P_{7.4}$		-4.189	-4.346	-4.325