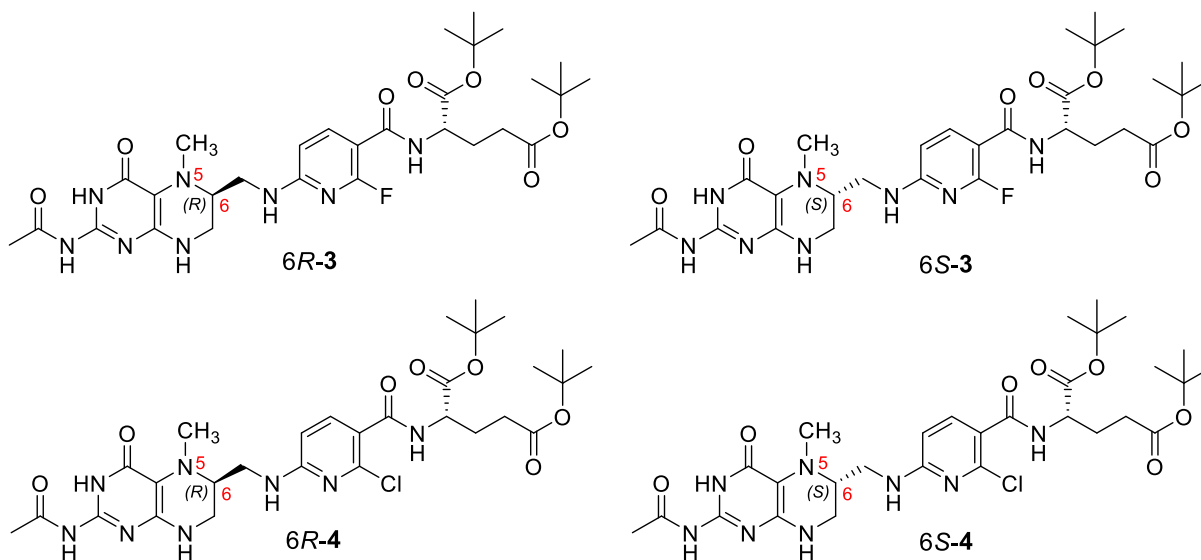
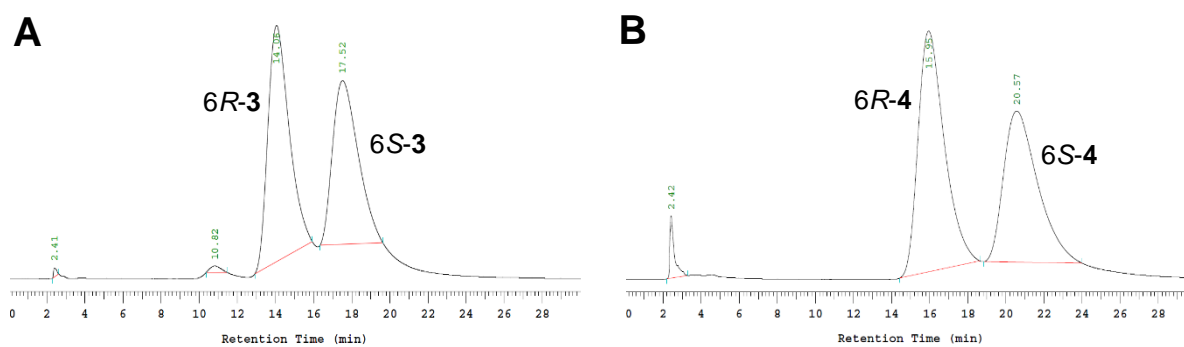


Chiral HPLC Separation

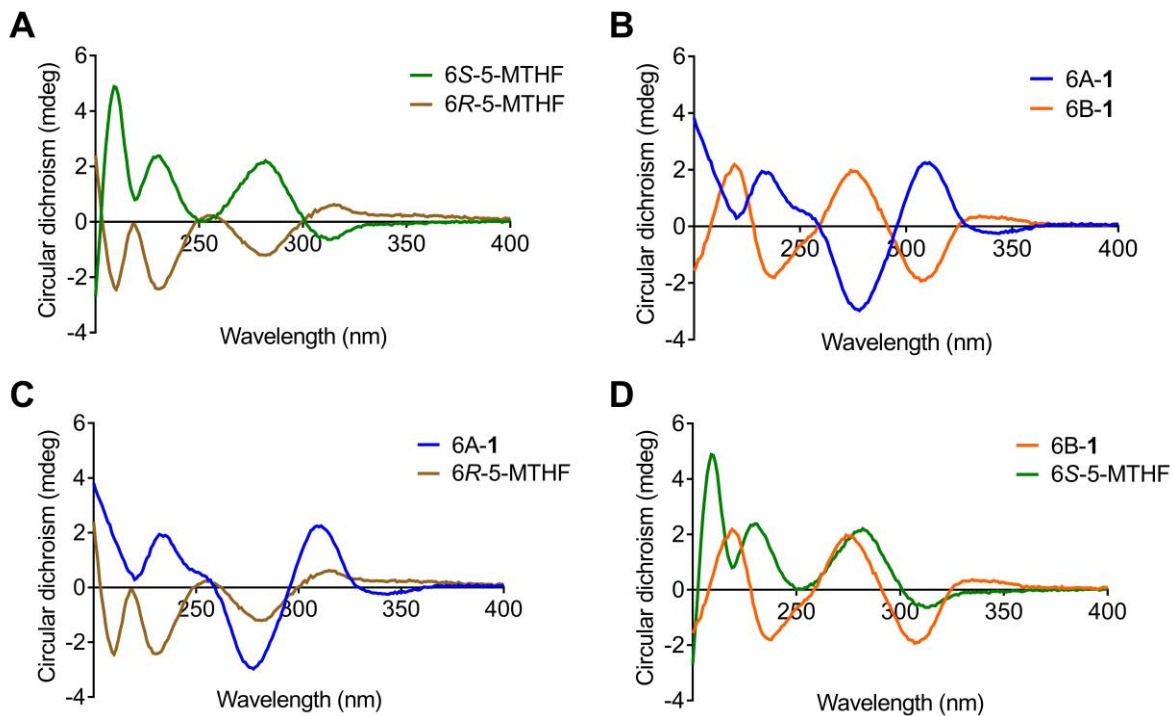


Supplemental Figure 1: Chemical structures of the two protected reference diastereoisomers **6R-3** and **6S-3** and the two precursor diastereoisomers **6R-4** and **6S-4**.



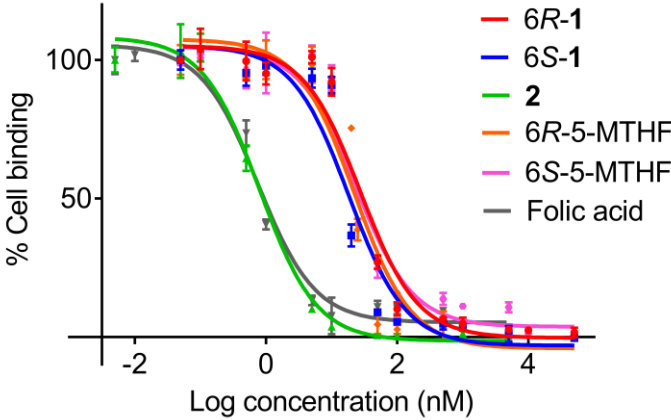
Supplemental Figure 2: HPLC chromatograms of the separation of **6R-3** and **6S-3** (A) and **6R-4** and **6S-4** (B) using the chiral column.

Circular Dichroism



Supplemental Figure 3: Comparison of the CD spectra of 6R- and 6S-5-MTHF (A), 1st peak and 2nd peak of chiral HPLC separation after the hydrolysis reaction (B), 6A-1 and 6R-5-MTHF (C) and 6B-1 and 6S-5-MTHF (D) measured from 200 nm to 400 nm.

Binding Curves



Supplemental Figure 4: Binding curves of 6R-1, 6S-1, 2, 6R-5-MTHF, 6S-5-MTHF and folic acid to the FR- α .

Biodistribution

Supplemental Table 1: Biodistribution of 6R-3'-aza-2'-¹⁸F-fluoro-5-MTHF (6R-¹⁸F-1) in KB tumor-bearing mice at 0.5, 1, 1.5, 2 and 3 h p.i.^a

6R-3'-aza-2'- ¹⁸ F-fluoro-5-MTHF (6R- ¹⁸ F-1) (% IA/g)						
	0.5 h p.i.	1 h p.i.	1.5 h p.i.	2 h p.i.	3 h p.i.	1 h p.i. & Folic Acid
	n = 4	n = 4	n = 4	n = 4	n = 4	n = 3
Blood	3.13 ± 0.31	2.16 ± 0.25	1.92 ± 0.19	1.47 ± 0.09	1.21 ± 0.07	1.52 ± 0.43
Heart	2.75 ± 0.33	2.38 ± 0.18	2.39 ± 0.47	1.74 ± 0.10	1.69 ± 0.20	1.60 ± 0.46
Lung	3.37 ± 0.32	2.80 ± 0.29	2.92 ± 0.53	2.22 ± 0.15	2.20 ± 0.27	1.73 ± 0.43
Spleen	8.25 ± 0.96	8.25 ± 0.88	13.0 ± 4.14	9.54 ± 1.54	13.7 ± 2.79	3.89 ± 0.40
Liver	14.3 ± 1.36	14.1 ± 0.80	16.8 ± 3.58	14.0 ± 0.71	14.4 ± 1.27	9.64 ± 1.55
Gall bladder	37.5 ± 11.7	39.0 ± 22.2	56.7 ± 13.2	108 ± 32.8	93.1 ± 88.4	26.5 ± 16.6
Stomach	2.20 ± 0.23	2.35 ± 0.49	2.51 ± 0.42	2.16 ± 0.35	2.28 ± 1.07	2.02 ± 0.52
Intestines	7.68 ± 1.28	6.93 ± 1.06	8.01 ± 2.72	7.57 ± 1.73	7.32 ± 2.16	5.23 ± 0.73
Feces	12.2 ± 3.54	17.8 ± 5.09	13.1 ± 6.07	38.7 ± 8.88	16.2 ± 8.65	15.2 ± 1.75
Salivary glands	7.16 ± 0.51	6.49 ± 0.93	7.31 ± 1.17	5.17 ± 0.73	5.64 ± 0.84	2.61 ± 0.70
Muscle	1.54 ± 0.13	1.36 ± 0.15	1.48 ± 0.31	1.12 ± 0.11	1.09 ± 0.11	1.05 ± 0.42
Bone	2.73 ± 0.14	2.43 ± 0.10	3.53 ± 0.90	2.23 ± 0.23	2.79 ± 0.39	1.51 ± 0.34
Kidneys	34.5 ± 2.45	27.3 ± 3.07	29.8 ± 7.46	21.6 ± 3.27	19.7 ± 0.80	4.80 ± 1.94
KB tumor	13.3 ± 1.80	18.1 ± 2.86	23.9 ± 2.74	25.4 ± 2.95	32.2 ± 6.10	3.41 ± 0.88
Tu-to-blood	4.27 ± 0.61	8.47 ± 1.62	12.5 ± 1.46	17.3 ± 1.82	27.0 ± 6.90	
Tu-to-liver	0.94 ± 0.14	1.29 ± 0.18	1.46 ± 0.26	1.82 ± 0.15	2.22 ± 0.28	
Tu-to-kidney	0.39 ± 0.05	0.67 ± 0.14	0.83 ± 0.17	1.19 ± 0.11	1.63 ± 0.32	

^avalues shown represent the mean ± S.D. of data from three to four animals

Supplemental Table 2: Biodistribution of 6*S*-3'-aza-2'-¹⁸F-fluoro-5-MTHF (6*S*-¹⁸F-1) in KB tumor-bearing mice at 0.5, 1, 1.5, 2 and 3 h p.i.^a

6 <i>S</i> -3'-aza-2'- ¹⁸ F-fluoro-5-MTHF (6 <i>S</i> - ¹⁸ F-1) (% IA/g)						
	0.5 h p.i.	1 h p.i.	1.5 h p.i.	2 h p.i.	3 h p.i.	1 h p.i. & Folic Acid
	n = 4	n = 4	n = 4	n = 4	n = 4	n = 3
Blood	1.52 ± 0.31	1.26 ± 0.08	1.16 ± 0.20	1.37 ± 0.39	1.14 ± 0.20	1.09 ± 0.28
Heart	2.02 ± 0.22	1.53 ± 0.15	1.48 ± 0.17	1.40 ± 0.32	1.41 ± 0.16	0.50 ± 0.13
Lung	2.31 ± 0.42	2.04 ± 0.09	2.08 ± 0.35	2.19 ± 0.34	2.04 ± 0.38	0.84 ± 0.20
Spleen	1.90 ± 0.34	1.57 ± 0.16	1.82 ± 0.49	1.86 ± 0.46	1.98 ± 0.24	0.90 ± 0.24
Liver	8.10 ± 1.16	6.28 ± 0.66	5.86 ± 1.11	5.40 ± 0.60	5.29 ± 0.86	4.76 ± 0.87
Gall bladder	20.0 ± 9.00	16.6 ± 8.91	11.4 ± 7.18	56.9 ± 45.5	33.2 ± 13.8	5.87 ± 0.45
Stomach	2.54 ± 0.56	1.84 ± 0.33	1.69 ± 0.33	1.63 ± 0.30	1.36 ± 0.27	0.73 ± 0.32
Intestines	2.61 ± 0.65	2.58 ± 0.42	2.34 ± 0.39	2.74 ± 0.51	2.22 ± 0.38	4.34 ± 0.93
Feces	3.71 ± 2.34	4.85 ± 1.95	4.57 ± 1.98	7.68 ± 4.66	7.53 ± 4.53	8.42 ± 2.10
Salivary glands	13.8 ± 2.06	13.8 ± 1.28	21.4 ± 4.59	18.9 ± 4.87	25.9 ± 3.17	0.89 ± 0.26
Muscle	1.36 ± 0.25	1.29 ± 0.14	1.24 ± 0.14	1.37 ± 0.26	1.18 ± 0.20	0.26 ± 0.06
Bone	1.70 ± 0.13	1.38 ± 0.14	1.49 ± 0.30	1.37 ± 0.21	1.24 ± 0.16	0.49 ± 0.12
Kidneys	42.5 ± 2.73	37.5 ± 3.61	42.3 ± 7.05	47.0 ± 10.1	41.4 ± 1.27	2.28 ± 0.95
KB tumor	11.1 ± 0.90	14.2 ± 1.91	19.2 ± 4.16	32.2 ± 19.0	34.8 ± 5.97	2.11 ± 0.38
Tu-to-blood	7.41 ± 0.96	11.3 ± 1.13	16.5 ± 0.59	26.9 ± 21.5	30.9 ± 6.19	
Tu-to-liver	1.38 ± 0.16	1.87 ± 0.96	3.30 ± 0.59	5.93 ± 3.35	6.70 ± 1.48	
Tu-to-kidney	0.26 ± 0.01	0.38 ± 0.04	0.45 ± 0.07	0.75 ± 0.54	0.84 ± 0.12	

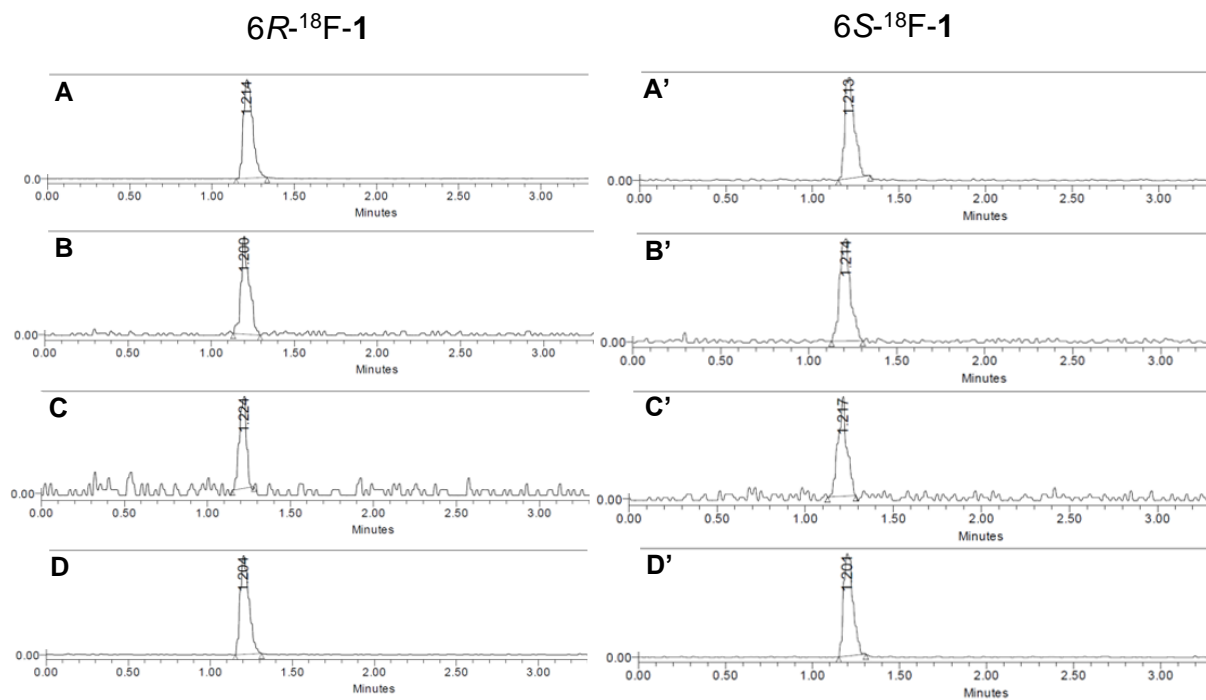
^avalues shown represent the mean ± S.D. of data from three to four animals.

Supplemental Table 3: Biodistribution of 3'-aza-2'-¹⁸F-fluorofolic acid (¹⁸F-2) in KB tumor-bearing mice at 0.5, 1, 1.5, 2 and 3 h p.i.. Data from 0.5, 1 and 1.5 h p.i. were taken from Betzel *et al.* (2013, *Bioconjug. Chem.* 24, 205-214)^a

3'-aza-2'- ¹⁸ F-fluorofolic acid (¹⁸ F-2) (% IA/g)						
	0.5 h p.i.	1 h p.i.	1.5 h p.i.	2 h p.i.	3 h p.i.	1 h p.i. & Folic Acid
	n = 4	n = 4	n = 4	n = 4	n = 4	n = 3
Blood	1.15 ± 0.33	0.55 ± 0.07	0.55 ± 0.06	0.53 ± 0.05	0.64 ± 0.04	0.82 ± 0.30
Heart	2.09 ± 0.21	1.89 ± 0.28	1.83 ± 0.10	1.54 ± 0.22	1.58 ± 0.21	0.39 ± 0.20
Lung	1.97 ± 0.31	1.52 ± 0.21	1.63 ± 0.27	1.91 ± 0.25	2.16 ± 0.39	0.72 ± 0.31
Spleen	1.25 ± 0.23	1.85 ± 0.14	2.20 ± 0.16	2.16 ± 0.39	3.44 ± 0.62	0.37 ± 0.15
Liver	13.7 ± 2.90	10.6 ± 0.89	10.3 ± 2.37	8.50 ± 1.45	10.3 ± 0.33	10.9 ± 3.39
Gall bladder	8.10 ± 2.90	8.40 ± 1.79	9.26 ± 0.59	6.98 ± 2.47	11.2 ± 5.87	15.8 ± 9.28
Stomach	2.76 ± 0.45	2.83 ± 0.86	2.74 ± 0.04	1.93 ± 0.30	2.17 ± 0.30	0.61 ± 0.26
Intestines	1.40 ± 0.14	1.90 ± 0.21	2.26 ± 0.40	1.75 ± 0.06	2.72 ± 0.26	1.93 ± 0.57
Feces	1.23 ± 0.22	3.03 ± 1.98	1.77 ± 0.25	2.73 ± 1.16	3.22 ± 2.62	5.98 ± 1.08
Salivary glands	9.00 ± 1.25	15.0 ± 6.09	14.1 ± 0.93	16.8 ± 2.64	14.7 ± 1.86	0.58 ± 0.21
Muscle	1.74 ± 0.38	1.24 ± 0.18	1.52 ± 0.38	1.48 ± 0.32	1.67 ± 0.09	0.33 ± 0.12
Bone	2.09 ± 0.32	1.57 ± 0.25	1.56 ± 0.32	1.56 ± 0.19	1.69 ± 0.10	0.57 ± 0.26
Kidneys	54.8 ± 6.10	53.6 ± 3.23	57.3 ± 8.40	51.0 ± 7.50	43.3 ± 5.14	5.89 ± 4.47
KB tumor	11.7 ± 0.87	11.9 ± 1.73	12.6 ± 1.77	12.9 ± 0.98	15.0 ± 2.32	1.74 ± 0.35
Tu-to-blood	11.1 ± 4.11	21.6 ± 2.33	23.8 ± 4.09	24.7 ± 2.85	23.4 ± 1.98	
Tu-to-liver	0.89 ± 0.24	1.13 ± 0.18	1.25 ± 0.35	1.55 ± 0.28	1.46 ± 0.24	
Tu-to-kidney	0.22 ± 0.03	0.22 ± 0.02	0.21 ± 0.04	0.26 ± 0.05	0.35 ± 0.05	

^avalues shown represent the mean ± S.D. of data from three to four animals.

Metabolite Studies.



Supplemental Figure 5: Radio-UPLC chromatograms of reference radiotracers $6R\text{-}^{18}\text{F}\text{-}1$ and $6S\text{-}^{18}\text{F}\text{-}1$ (A, A'), blood plasmas (B, B'), liver samples (C, C') and urine samples (D, D') 30 min p.i. of the radiofolates. Retention times are indicated above the peak.