SUPPORTING INFORMATION

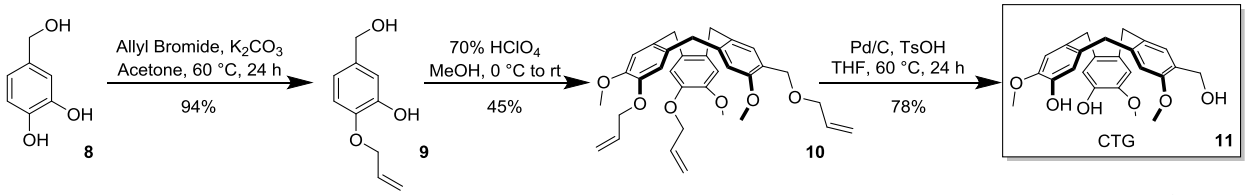
A Cryptophane-Folate Biosensor for ^{129}Xe NMR

Najat S. Khan, Brittany A. Riggle, Garry K. Seward, Yubin Bai, and Ivan J. Dmochowski*

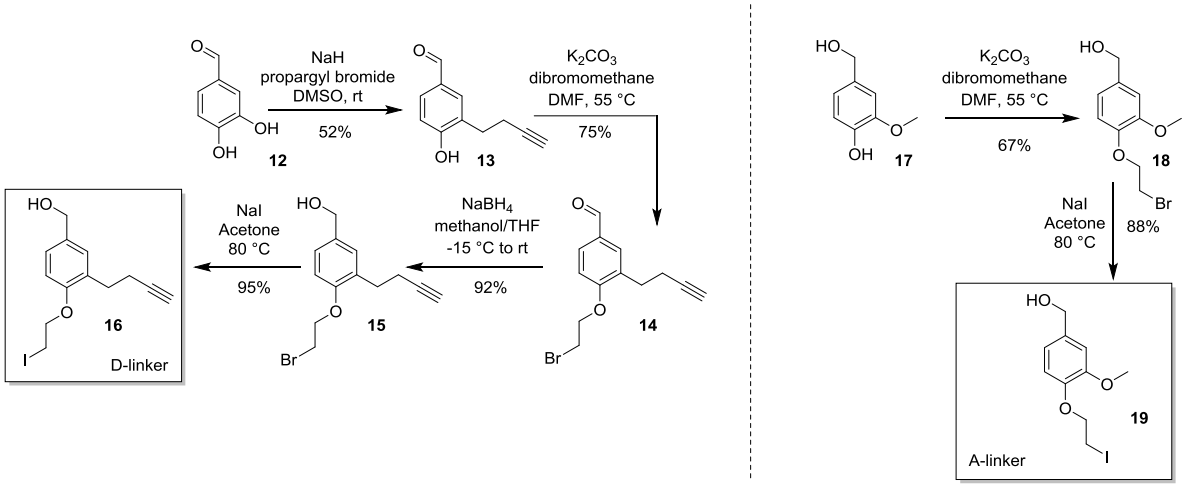
Department of Chemistry, University of Pennsylvania
231 S. 34th Street, Philadelphia PA 19104, USA

*To whom correspondence should be addressed:
E-mail: ivandmo@sas.upenn.edu
Phone: 215-898-6459

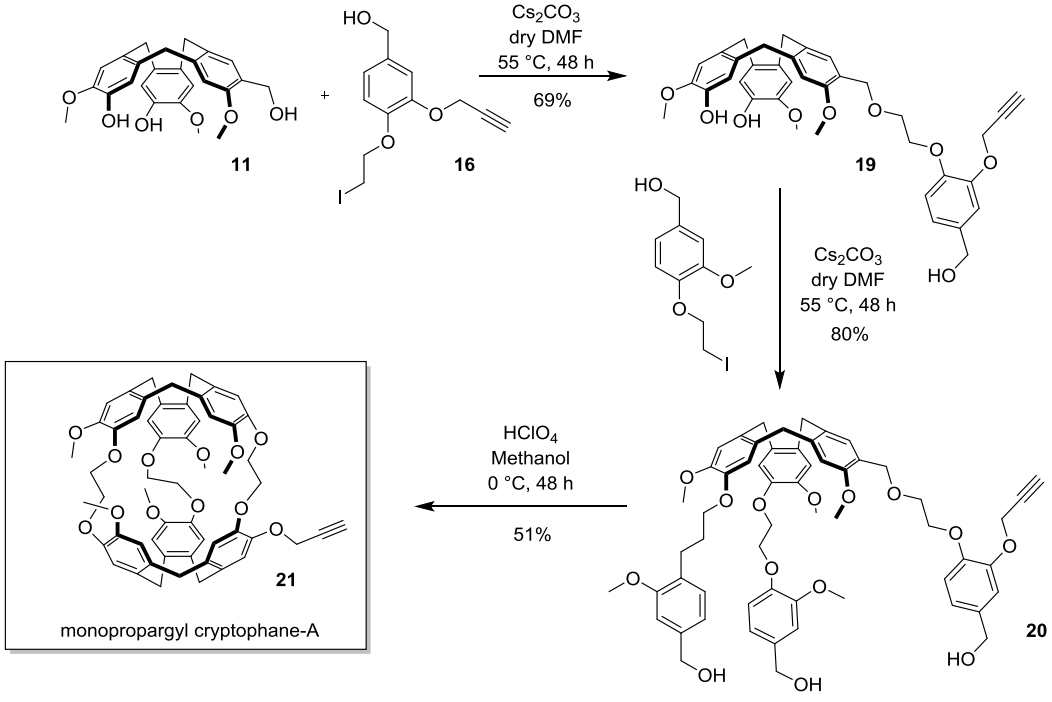
CONTENTS:



Scheme S1: 3-step synthesis of cyclotriguaiacylene (CTG). Figure adapted from Wei *et al.*[1]



Scheme S2: Synthesis of [3-propargyloxy-4-(2-iodoethoxy)phenyl]methanol (D-linker) and [4-(2-iodoethoxy)-3-methoxyphenyl]methanol (A-linker). Figure adapted from Wei *et al.*[1]



Scheme S3: Final 3 steps in synthesis of monopropargyl derivative of cryptophane-A. Figure adapted from Wei *et al.*[1]

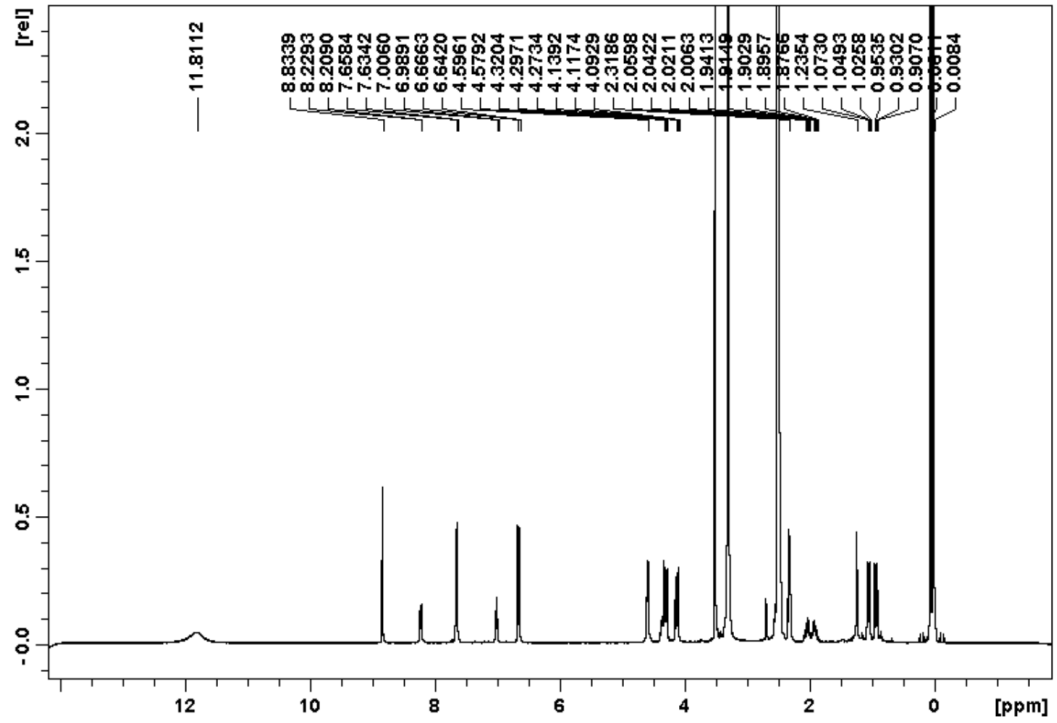


Figure S1: ^{1}H NMR spectrum of folate recognition moiety **7** in DMSO-d_6 at 298 K.

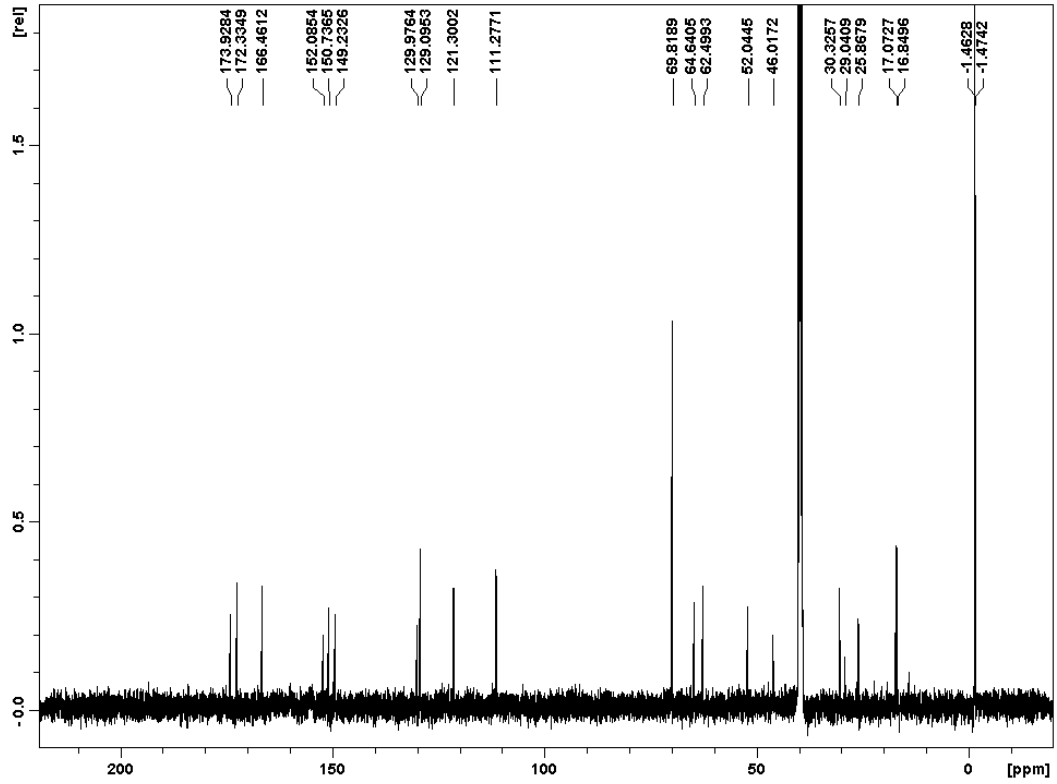


Figure S2: ^{13}C NMR spectrum of folate recognition moiety **7** in DMSO-d_6 at 298 K.

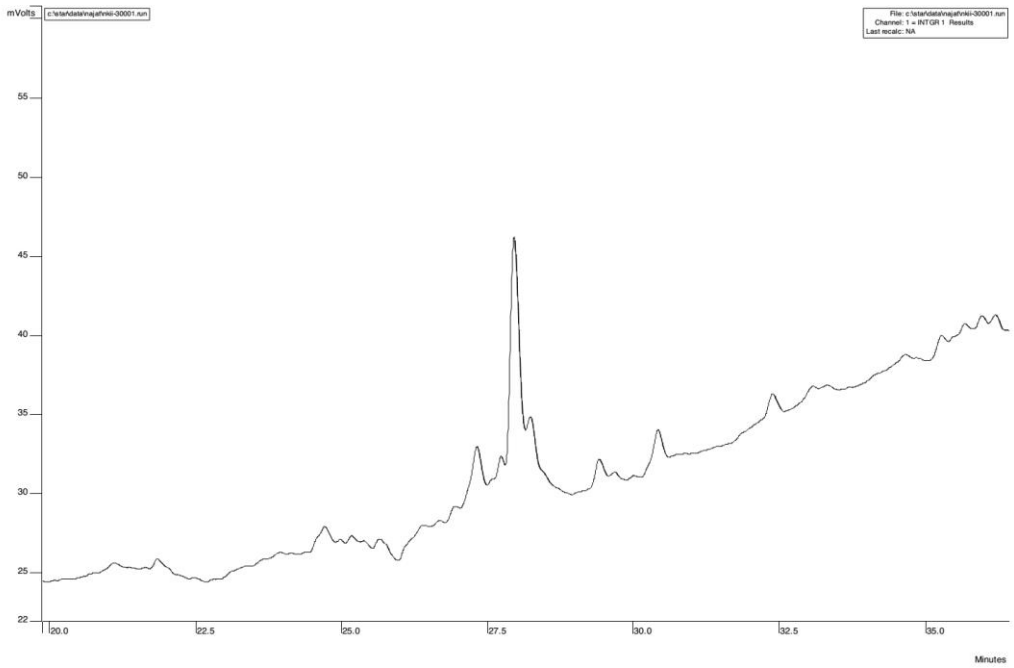


Figure S3: HPLC purification trace for azido-peptide **22**.

Semi-preparative HPLC purification of **22** was accomplished using RP-HPLC with 0.1% TFA in ddH_2O (A) and 0.1% TFA in HPLC-grade acetonitrile (B) using a gradient method and monitoring at 280 nm. Time 0 to 45 min, A/B = 95/5 to 50/50; 45 to 57 min, A/B = 50/50 to 20/80. Retention time: 27.96 min.

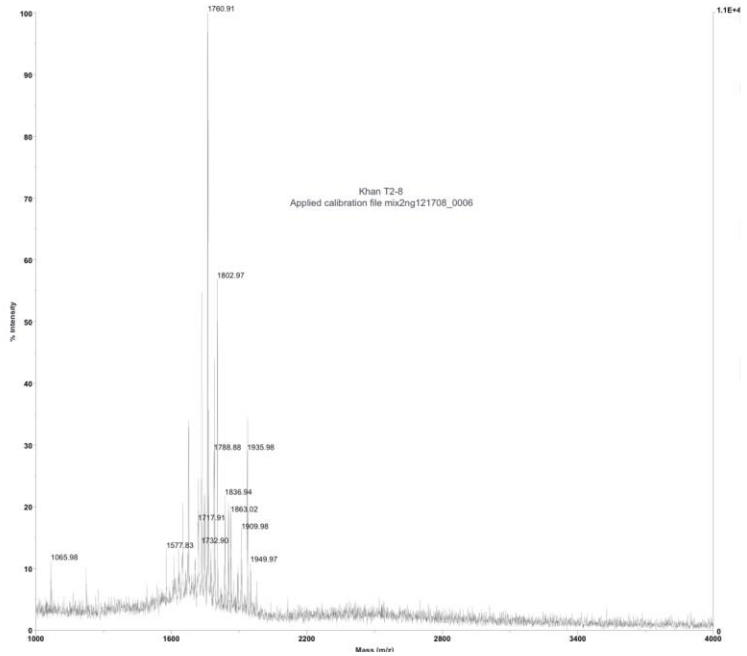


Figure S4: MALDI-MS for azido-peptide **22** from microcleavage and analytical HPLC. $C_{72}H_{141}N_{31}O_{16}S_2$ ($M + H^+$) 1761.06; found 1760.91. Note, laser power was attenuated to mitigate photodissociation of the azide.

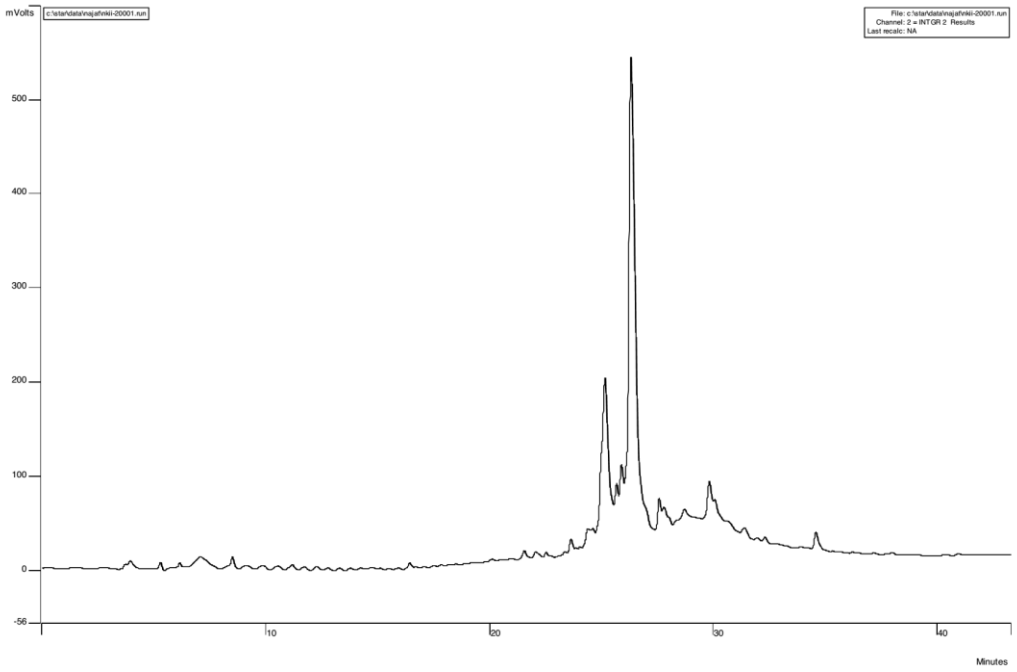


Figure S5: HPLC purification trace for peptide-folate conjugate **23**.

Semi-preparative HPLC purification of **23** was accomplished using RP-HPLC with 0.1% TFA in ddH_2O (A) and 0.1% TFA in HPLC-grade acetonitrile (B) using a gradient method and monitoring at 280 nm. Time 0 to 45 min, A/B = 95/5 to 50/50; 45 to 57 min, A/B = 50/50 to 20/80. Retention time: 26.31 min.

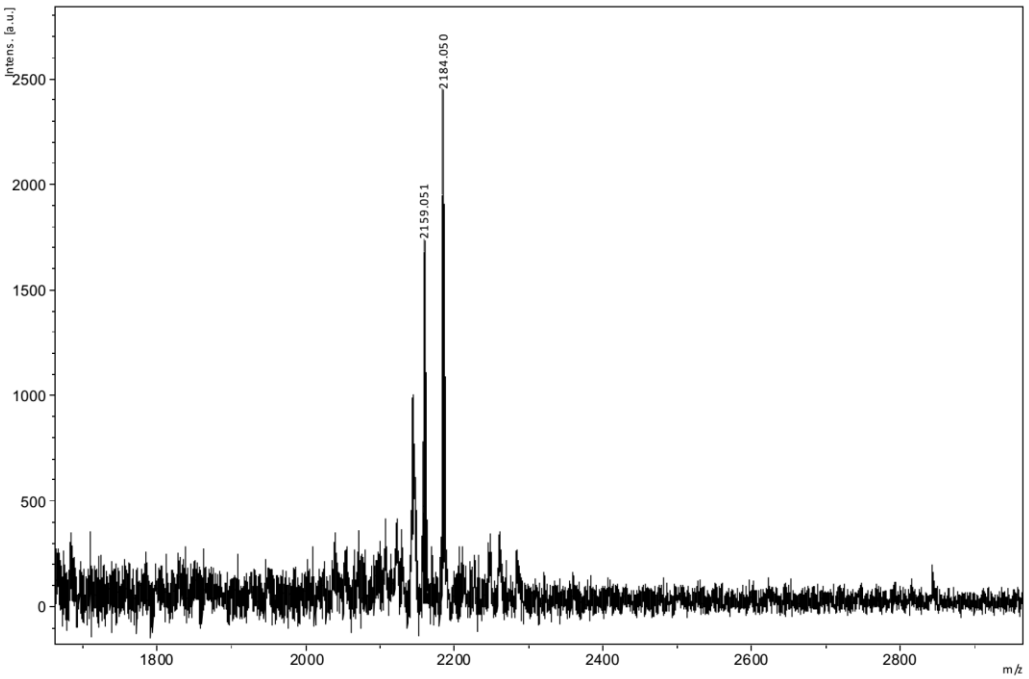


Figure S6: MALDI-MS for purified peptide-folate conjugate **23**. $C_{91}H_{158}N_{38}O_{21}S_2$ ($M + H^+$) 2184.19; found 2184.05

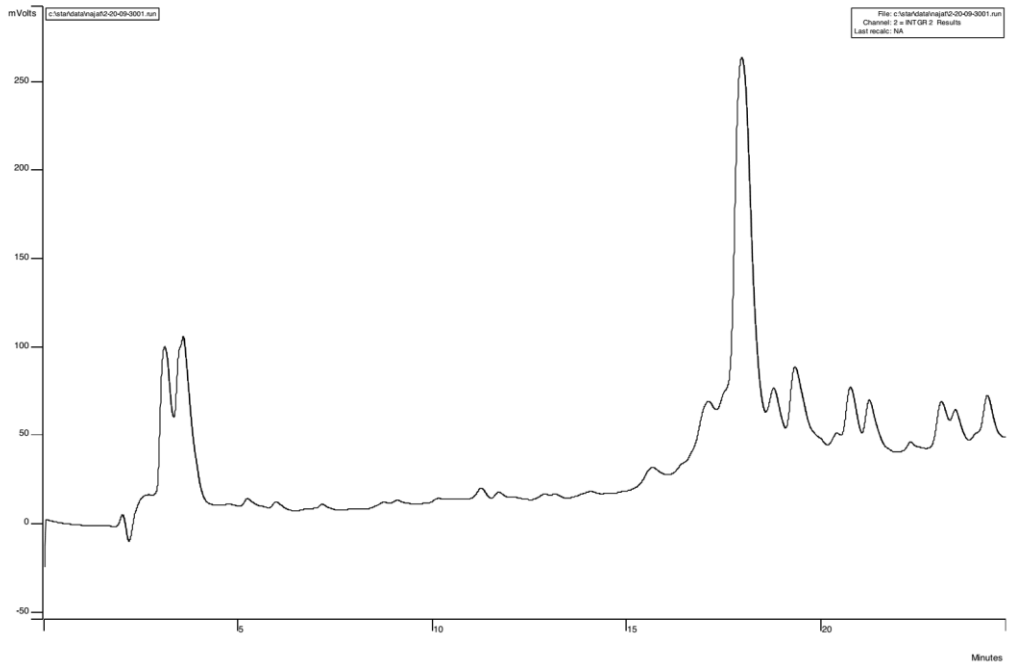


Figure S7: HPLC trace for peptide-folate-conjugate **24**.

Semi-preparative HPLC purification of **24** was accomplished using RP-HPLC with 0.1% TFA in ddH_2O (A) and 0.1% TFA in HPLC-grade acetonitrile (B) using a gradient method and monitoring at 280 nm. Time 0 to 65 min, A/B = 95/5 to 30/70; 65 to 68 min, A/B = 30/70 to 20/80. Retention time: 18.73 min.

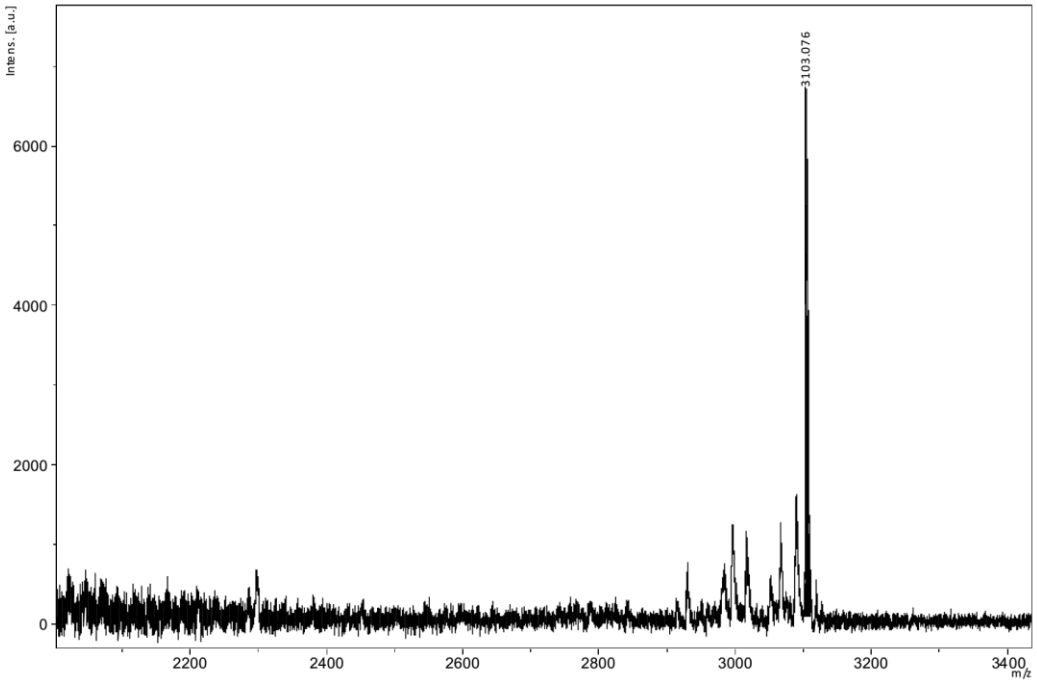


Figure S8: MALDI-MS for purified peptide-folate-cryptophane conjugate **24**. $C_{147}H_{211}N_{38}O_{32}S_2$ ($M + H^+$) 3102.61; found 3103.08

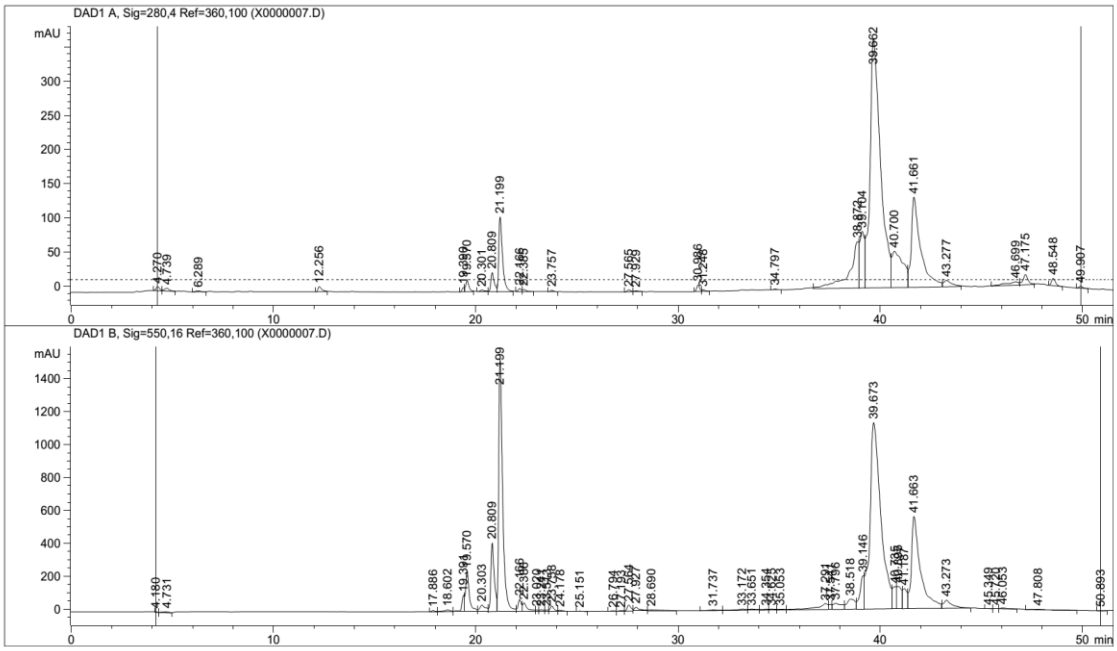


Figure S9: HPLC traces for Cy3-labeled peptide-folate-cryptophane conjugate **25**, monitored at 280 nm (above) and 550 nm (below).

Semi-preparative HPLC purification of **24** was accomplished using RP-HPLC with 0.1% TFA in ddH_2O (A) and 0.1% TFA in HPLC-grade acetonitrile (B) using a gradient method and monitoring at 280 and 550 nm. Time 0 to 65 min, A/B = 95/5 to 30/70; 65 to 68 min, A/B = 30/70 to 20/80. Retention time: 39.67 min.

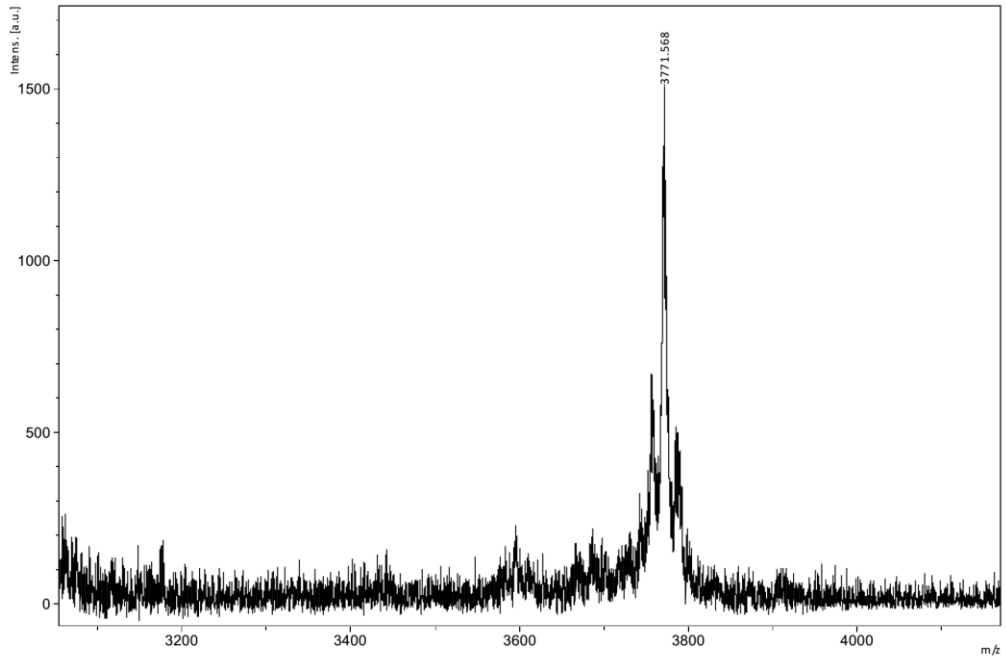


Figure S10: MALDI-MS for purified Cy3-labeled peptide-folate-cryptophane **25**. $C_{180}H_{253}N_{42}O_{42}S_3$ (Cy3-I) (M+ H^+) 3771.81; found 3771.59

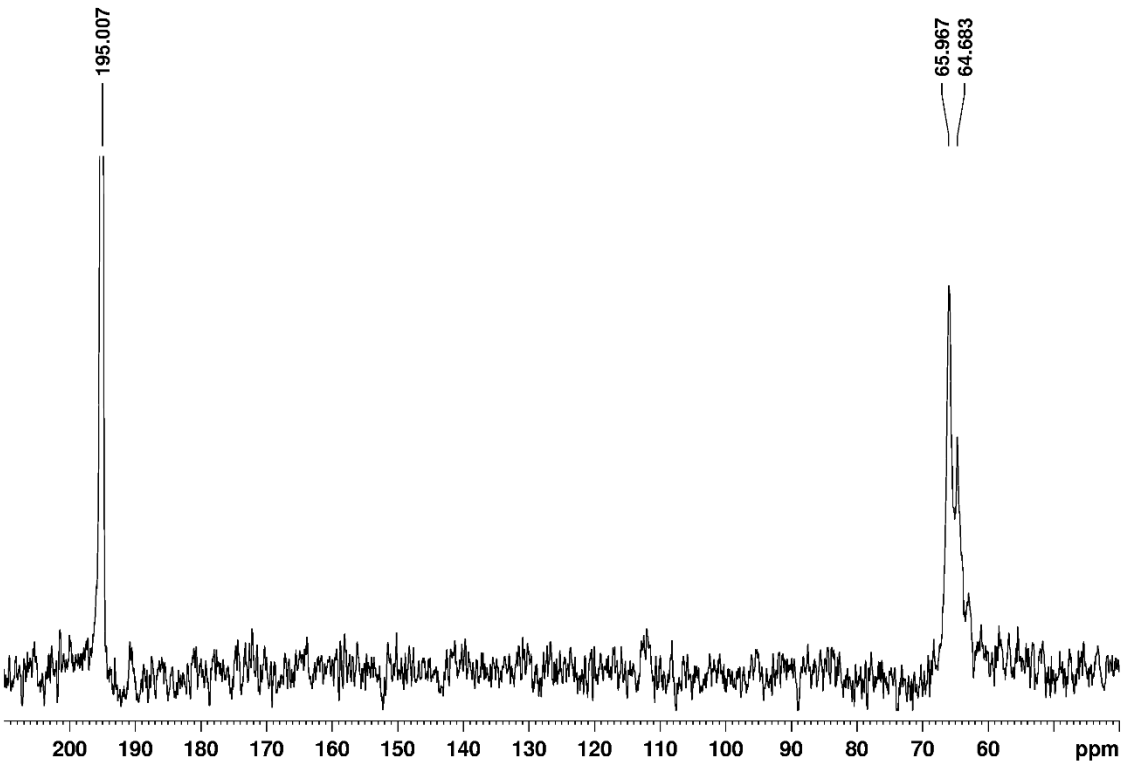


Figure S11: Hyperpolarized ^{129}Xe NMR spectrum of 60 µM biosensor **24** and 30 µM folate binding protein in acetate buffer at pH 5.0 (40 scans; S/N = 30:1 with 50 Hz line broadening).

Cited Reference

(1) Wei, Q.; Seward, G. K.; Hill, P. A.; Patton, B.; Dimitrov, I. E.; Kuzma, N. N.; Dmochowski, I. J., (2006) Designing ^{129}Xe NMR biosensors for matrix metalloproteinase detection. *J. Am. Chem. Soc. 128* (40), 13274-13283.