Supporting Information

Synthesis of Cyclic Py-Im Polyamide Libraries

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Synthesis of hairpin polyamide **17**:

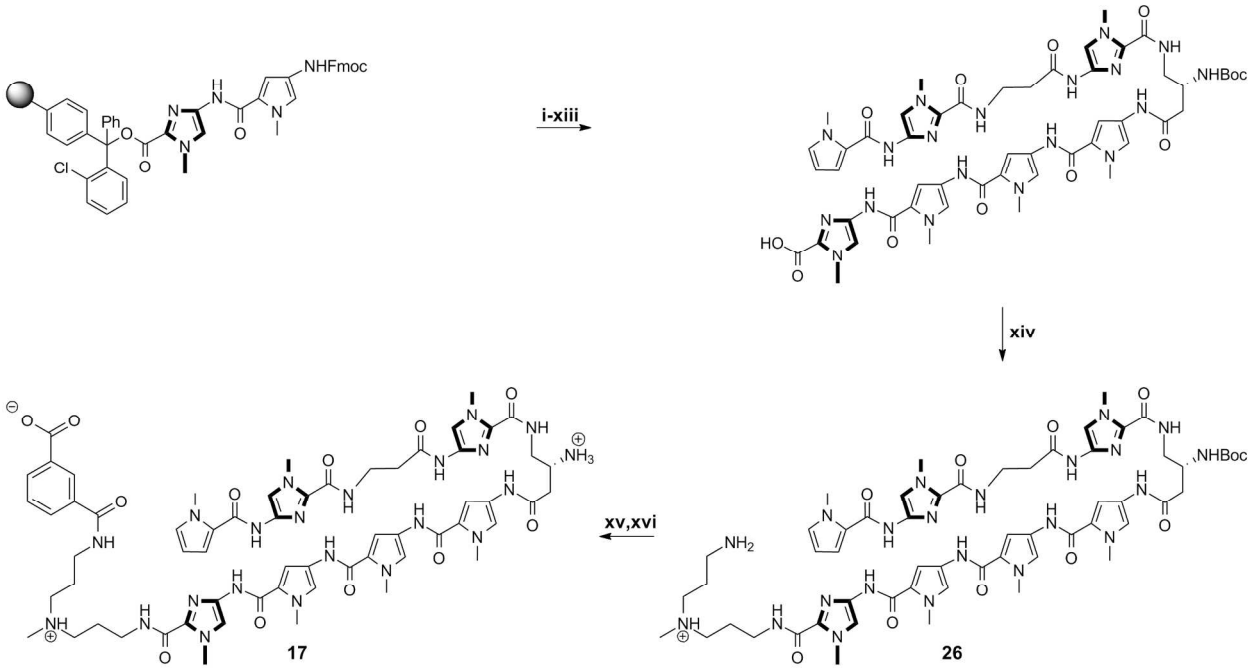
Fmoc-Py-Im-(2-Cl-Trt) resin, obtained via the same procedure as for **8**, was subjected to the previously described microwave-assisted solid-phase synthesis conditions to build the corresponding polyamide sequence with an N-terminal PyImOH cap. The resin (70 mg, 0.039 mmol) was then suspended in 2 mL 30% HFIP in DCM, stirred for 1 h, filtered, washed, and concentrated to afford the crude C-terminal free acid. The residue was then dissolved in DMF (1.8 mL) and added dropwise to a pre-activated solution of 3,3'-diamino-N-methyldipropylamine (252 μL, 1.56 mmol, 40 eq.) and PyBOP (40 mg, 0.078 mmol, 2 eq.) in DMF (6.0 mL). After stirring for 1 h and confirmation of complete conjuation by HPLC, the crude product was isolated by Et_2O precipitation, air dried, redissolved in 8 mL 15% AcN : 0.1% aqueous TFA, and purified by reverse-phase HPLC to yield polyamide intermediate **26** as an off-white powder (11.8 μmol, 30% yield).

The isolated **26** (3.1 μmol) was dissolved in DMF (200 μL) and added dropwise to a pre-activated solution of isophthalic acid (12 mg, 0.072 mmol) and PyBOP (5 mg, 9 μmol) in DMF (800 μL) and DIEA (13 μL). After stirring for 1 h and confirmation of complete conjuation by HPLC, the reaction mixture was then precipitated in Et_2O, isolated by centrifugation, and air dried. The remaining residue was then subjected to 500 μL neat TFA, stirred for 15 min, frozen in LN_2, thawed by addition of 1 mL DMF, diluted with 6 mL 0.1% aqueous TFA, and purified by reverse-phase HPLC to afford cyclic polyamide **8** (3.1 nmol, 86% yield).

[Note: Extinction coefficient for compounds **26** and **17** were established as 40,000 M^{-1} cm^{-1} at λmax = 302nm.]

(26): MALDI-TOF $[M + H]^+$ calcd for $C_{58}H_{79}N_{22}O_{11}^+$ = 1259.6, observed = 1260.1.
(17): MALDI-TOF $[M + H]^+$ calcd for $C_{61}H_{75}N_{22}O_{12}^+$ = 1307.6, observed = 1307.8.

Scheme S1. Synthesis of hairpin polyamide **17**



[1]All PyBOP-mediated coupling conditions were performed under microwave-assisted conditions (see Table 1).
[2]Reagents and conditions: (i) 50% piperidine, DMF; (ii) FmocPyOH, PyBOP, DIEA, DMF; (iii) 50% piperidine, DMF; (iv) FmocPyOH, PyBOP, DIEA, DMF; (v) 50% piperidine, DMF; (vi) Boc-β-Dab(Fmoc)-OH, PyBOP, DIEA, DMF; (vii) 50% piperidine, DMF; (viii) FmocImOH, PyBOP, DIEA, DMF; (ix) 50% piperidine, DMF; (x) Fmoc-β-Ala-OH, PyBOP, DIEA, DMF; (xi) 50% piperidine, DMF; (xii) PyImOH, PyBOP, DIEA, DMF; (xiii) 30% HFIP, DCM; (xiv) 3,3'-diamino-N-methyldipropylamine, PyBOP, DMF; (xv) Isophthalic acid, PyBOP, DIEA, DMF; (xvi) TFA.

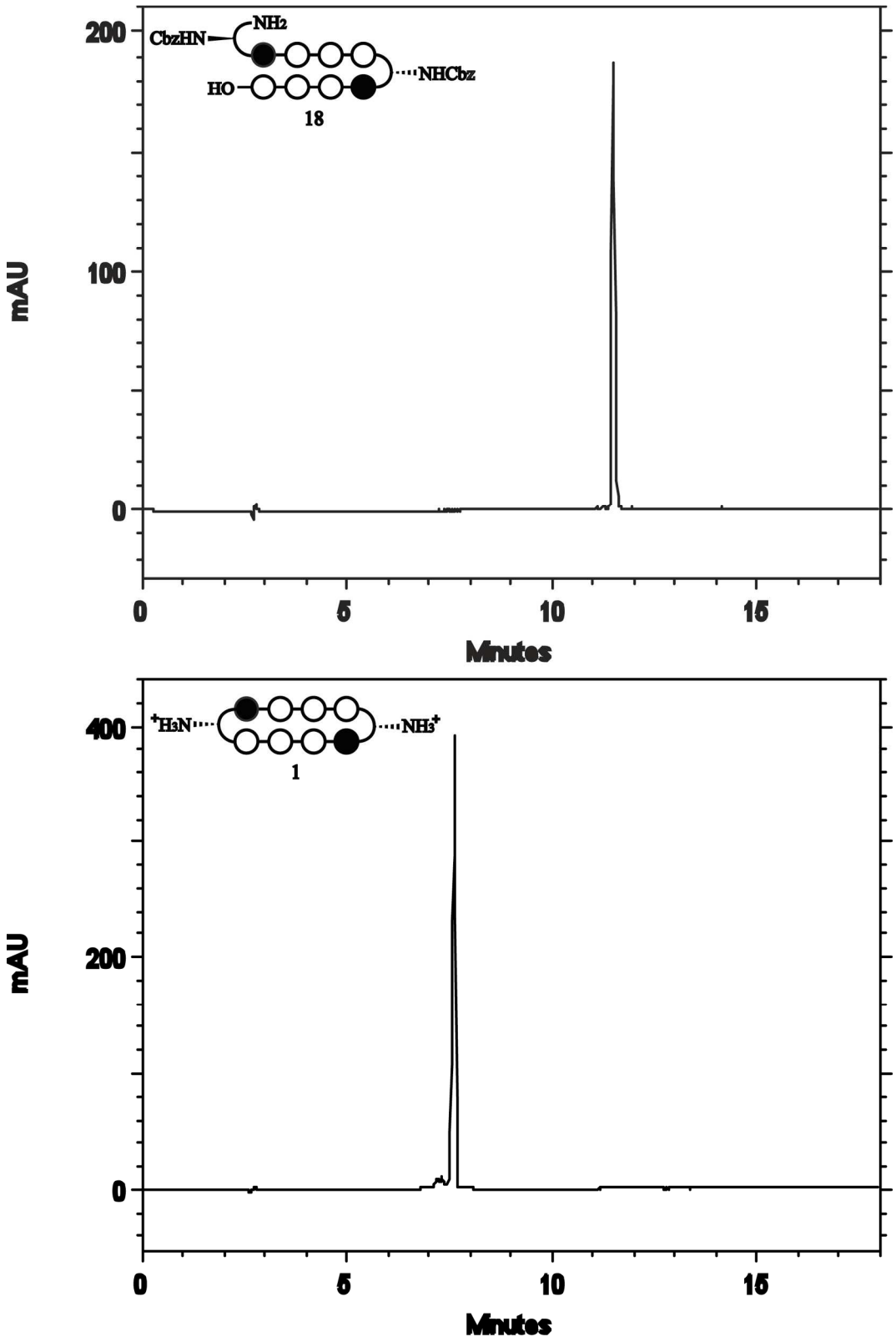


Figure S1. HPLC Spectrum of **18** and **1.**

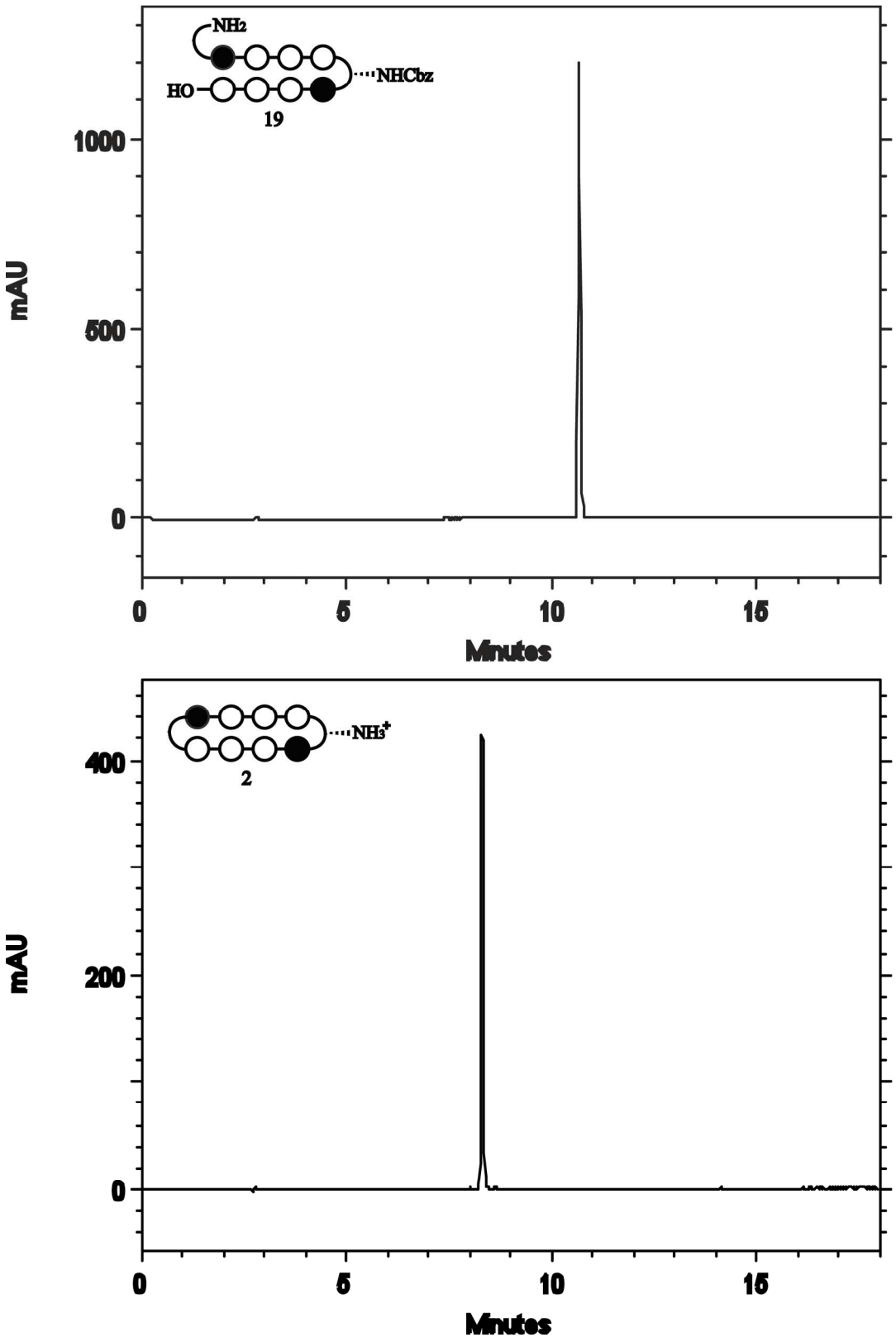


Figure S2. HPLC Spectrum of **19** and **2.**

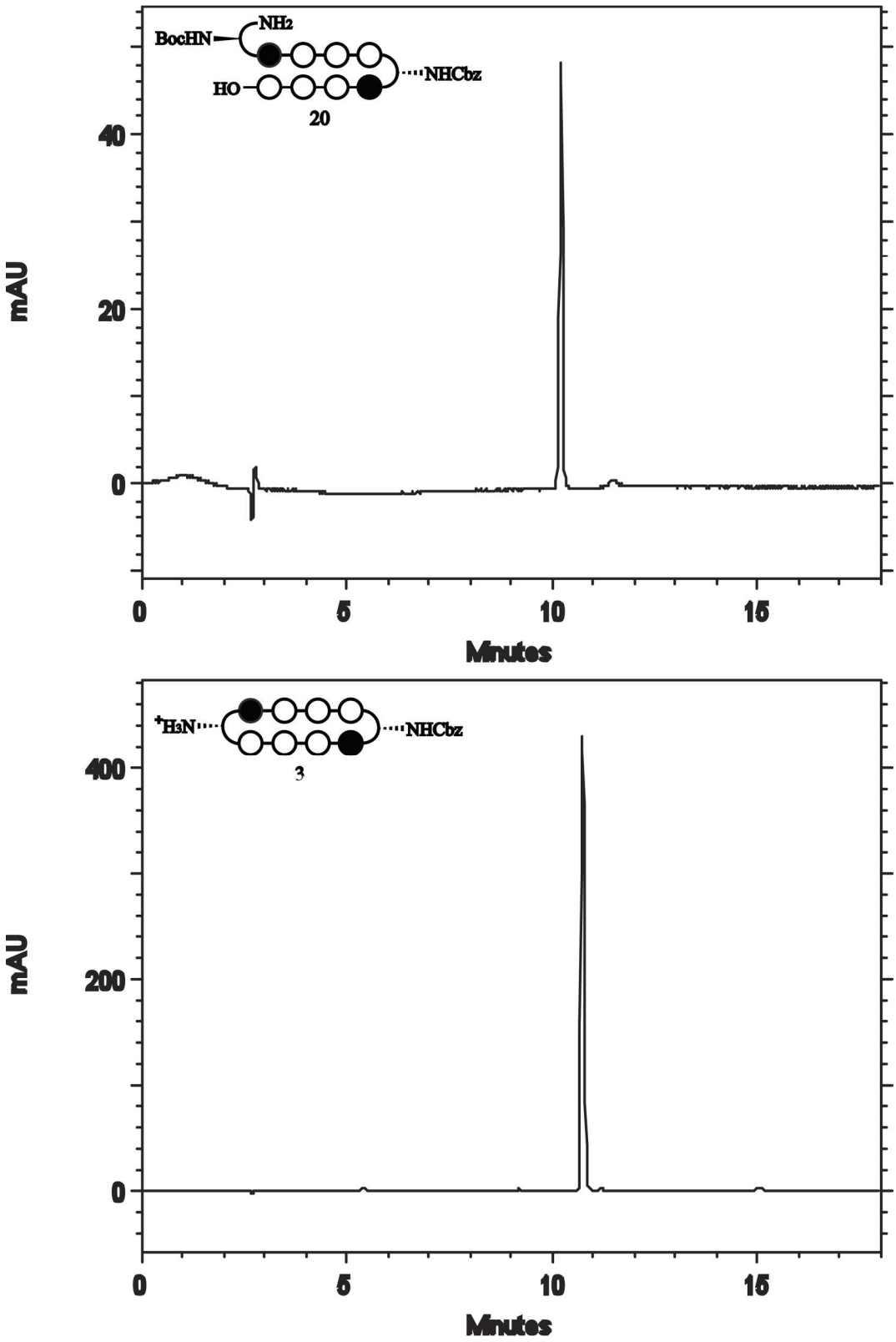
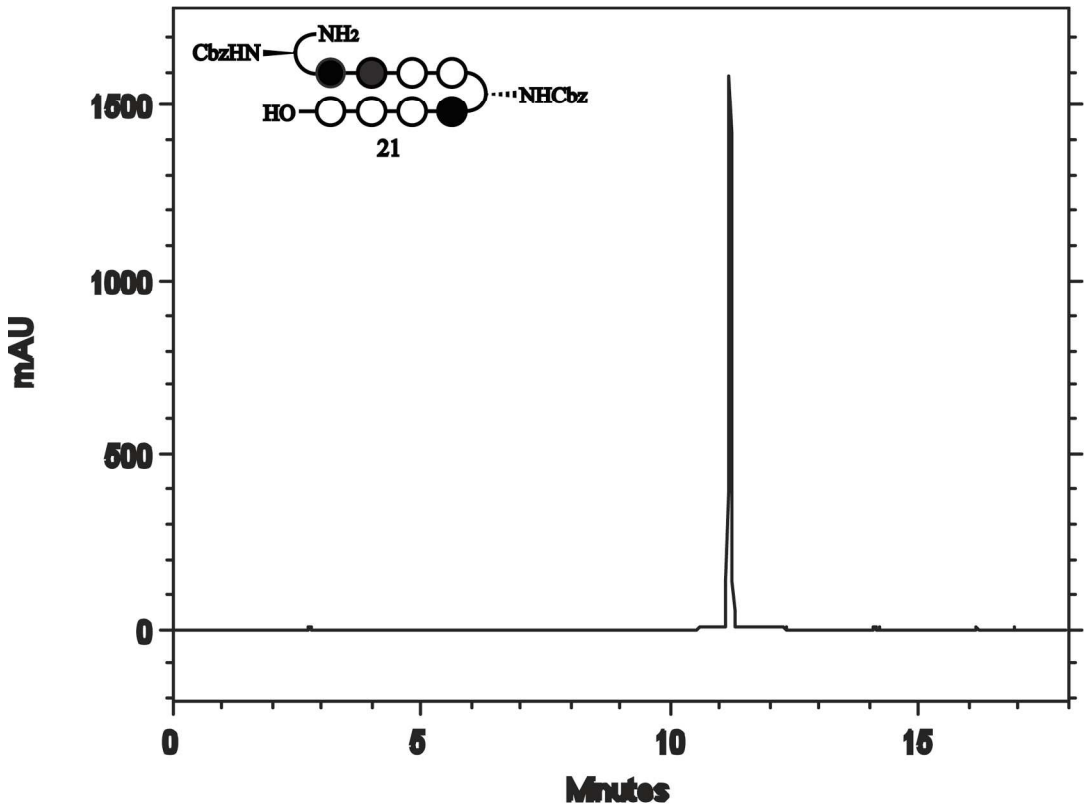


Figure S3. HPLC Spectrum of **20** and **3.**


CbzHN
NH2
HO
NHCbz
21
1500
1000
500
0
mAU
0
5
10
15
Minutes

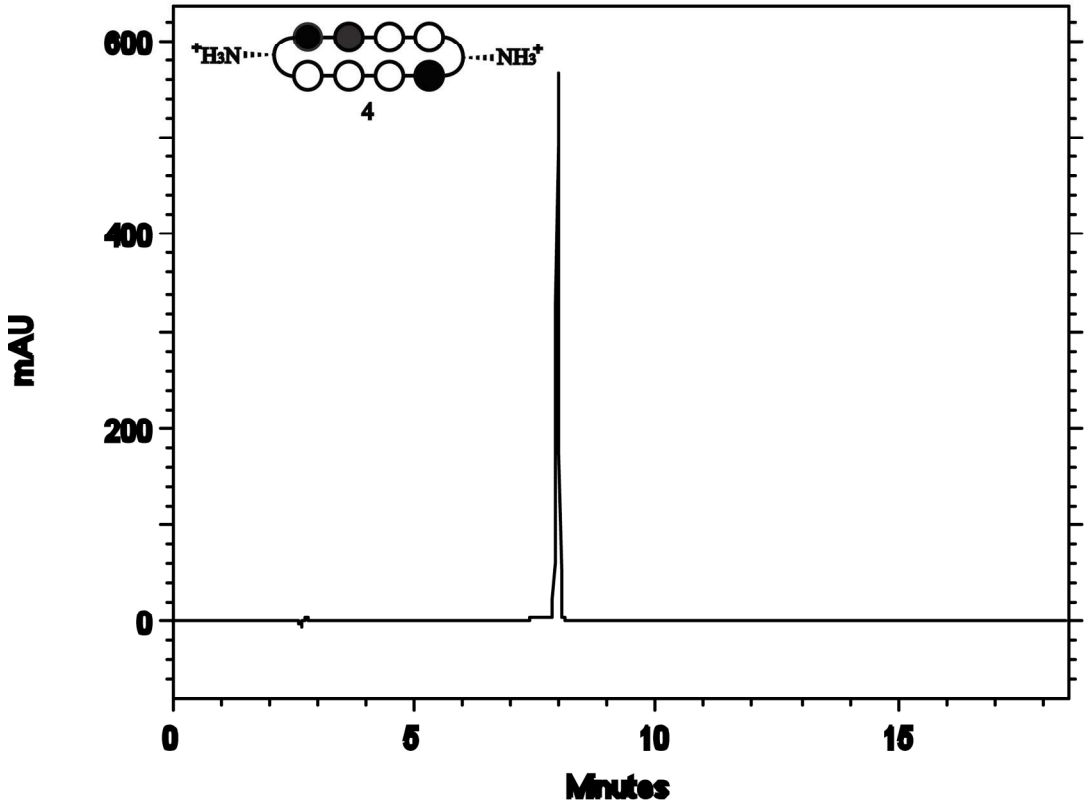

+H3N
NH3+
4
600
400
200
0
mAU
0
5
10
15
Minutes

Figure S4. HPLC Spectrum of **21** and **4.**

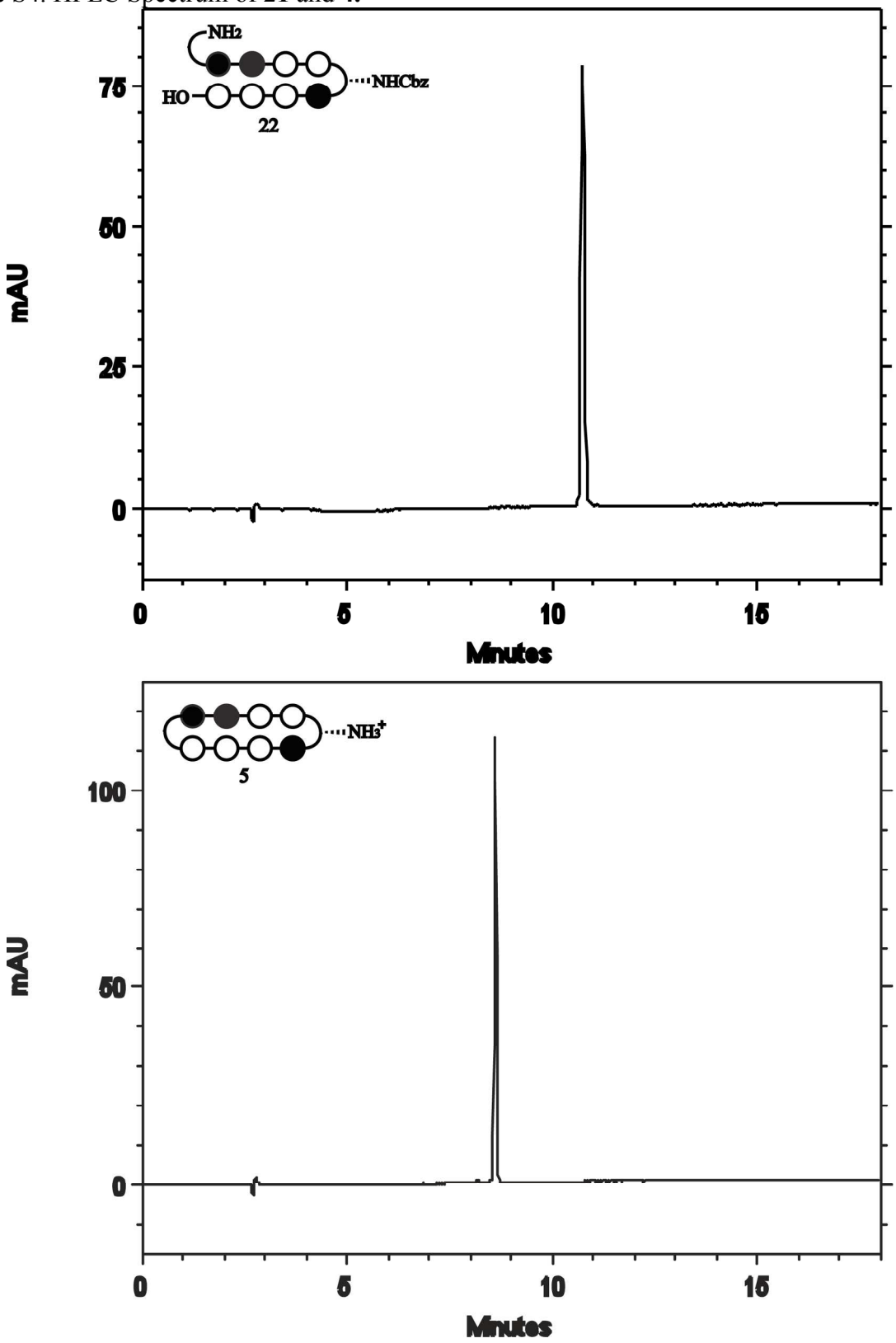
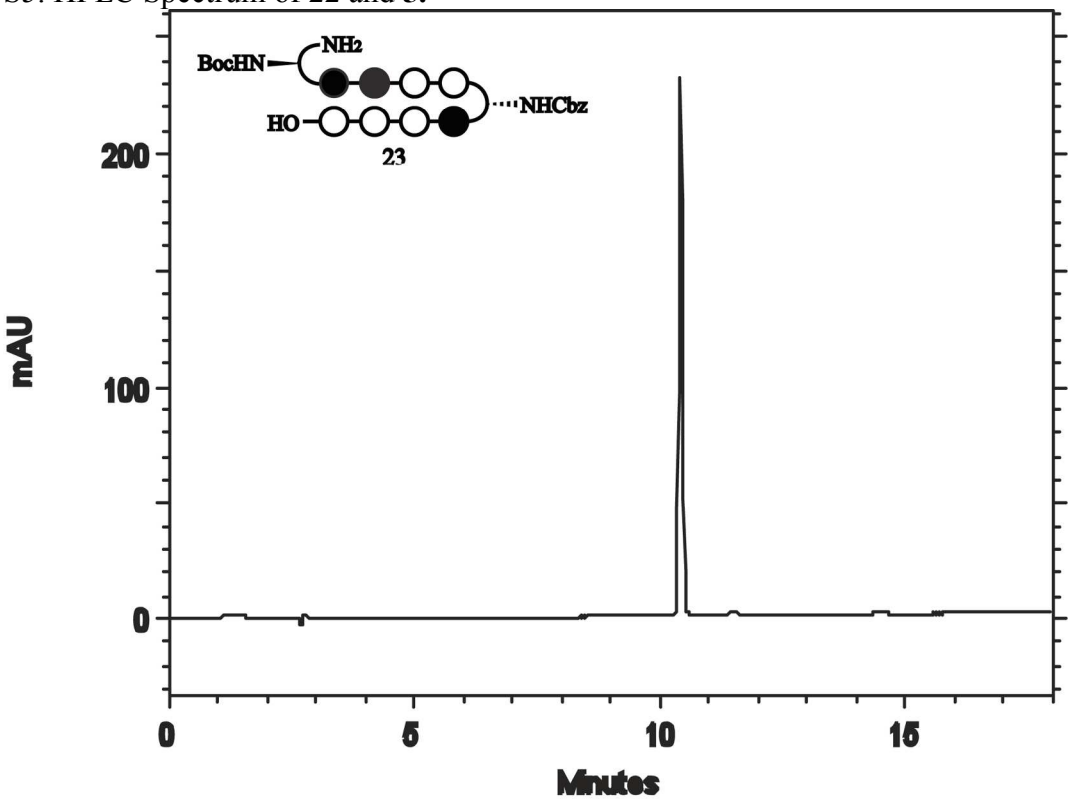


Figure S5. HPLC Spectrum of **22** and **5.**



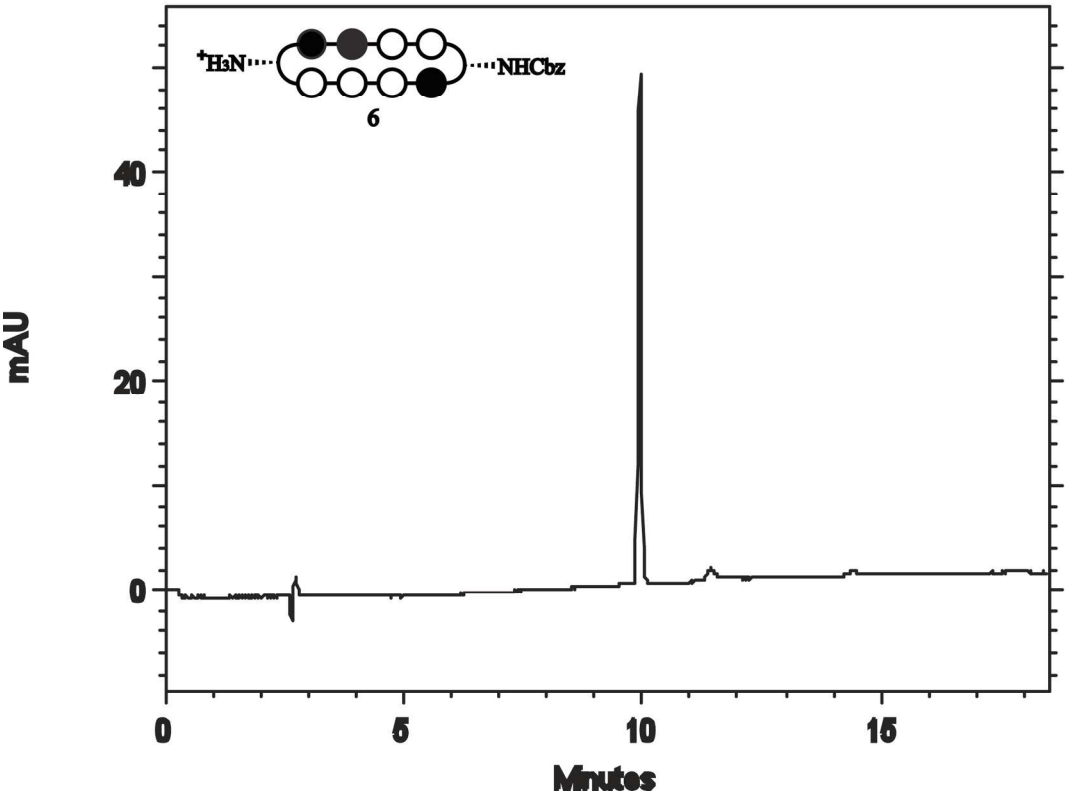


Figure S6. HPLC Spectrum of **23** and **6.**

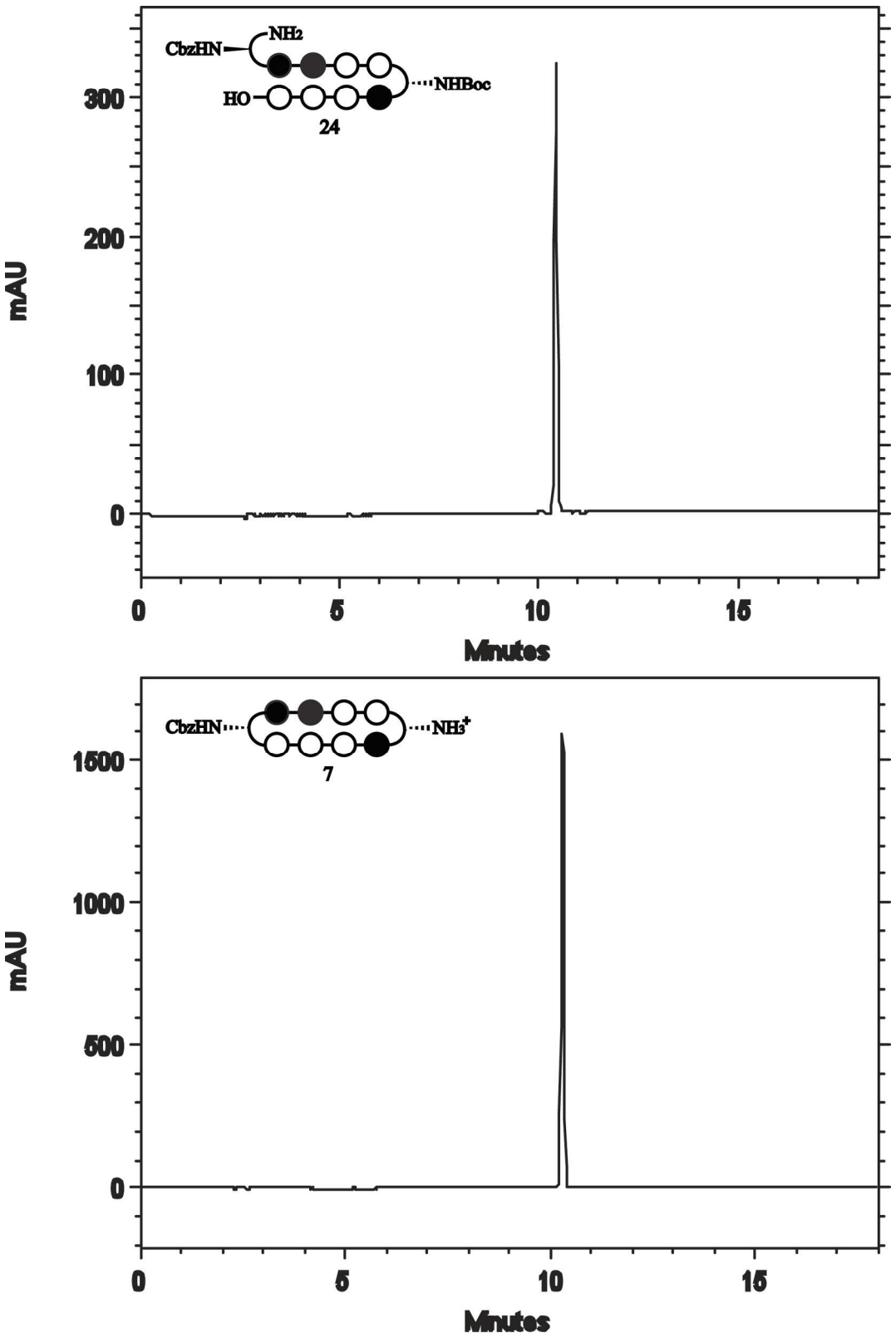


Figure S7. HPLC Spectrum of **24** and **7.**

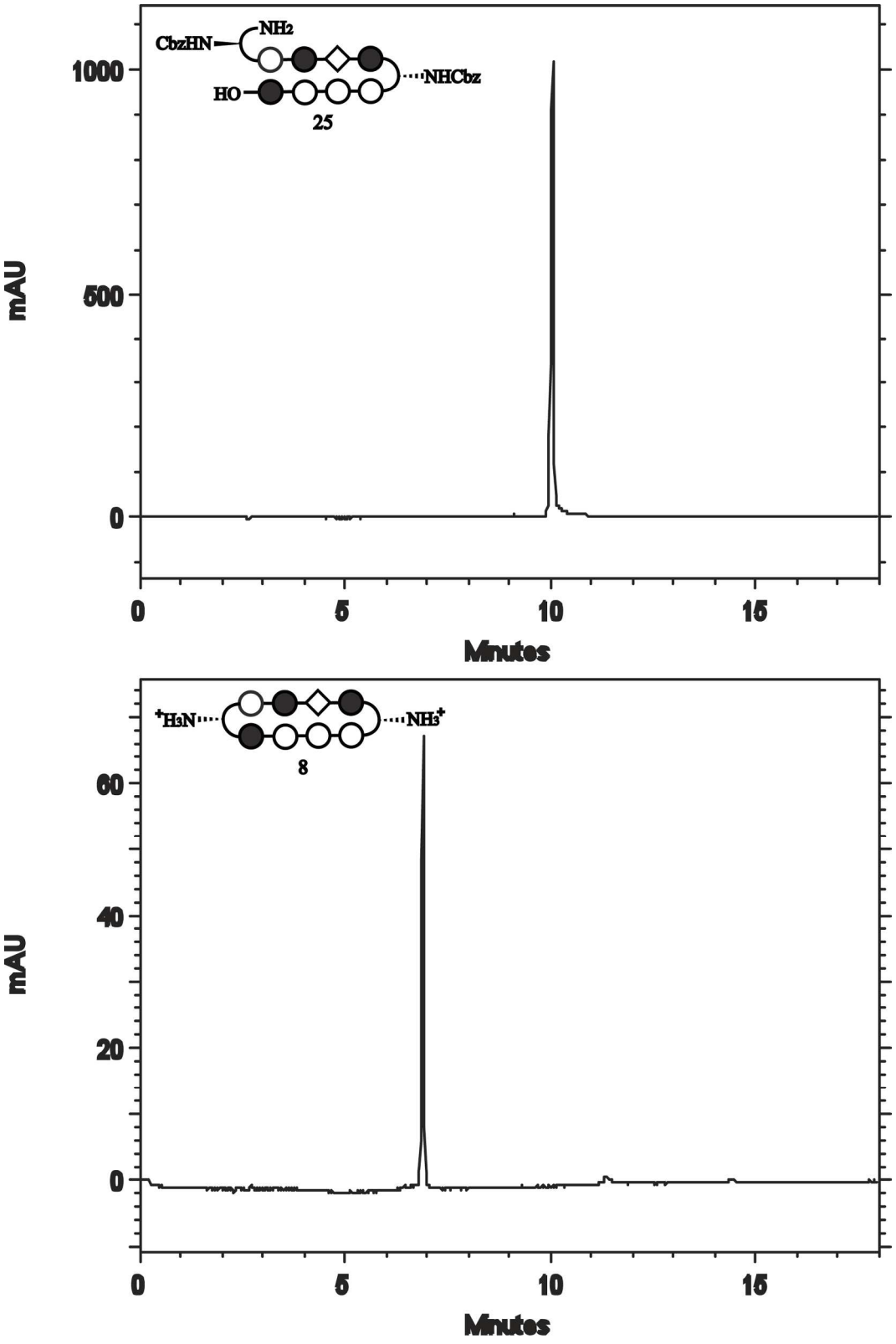


Figure S8. HPLC Spectrum of **25** and **8.**

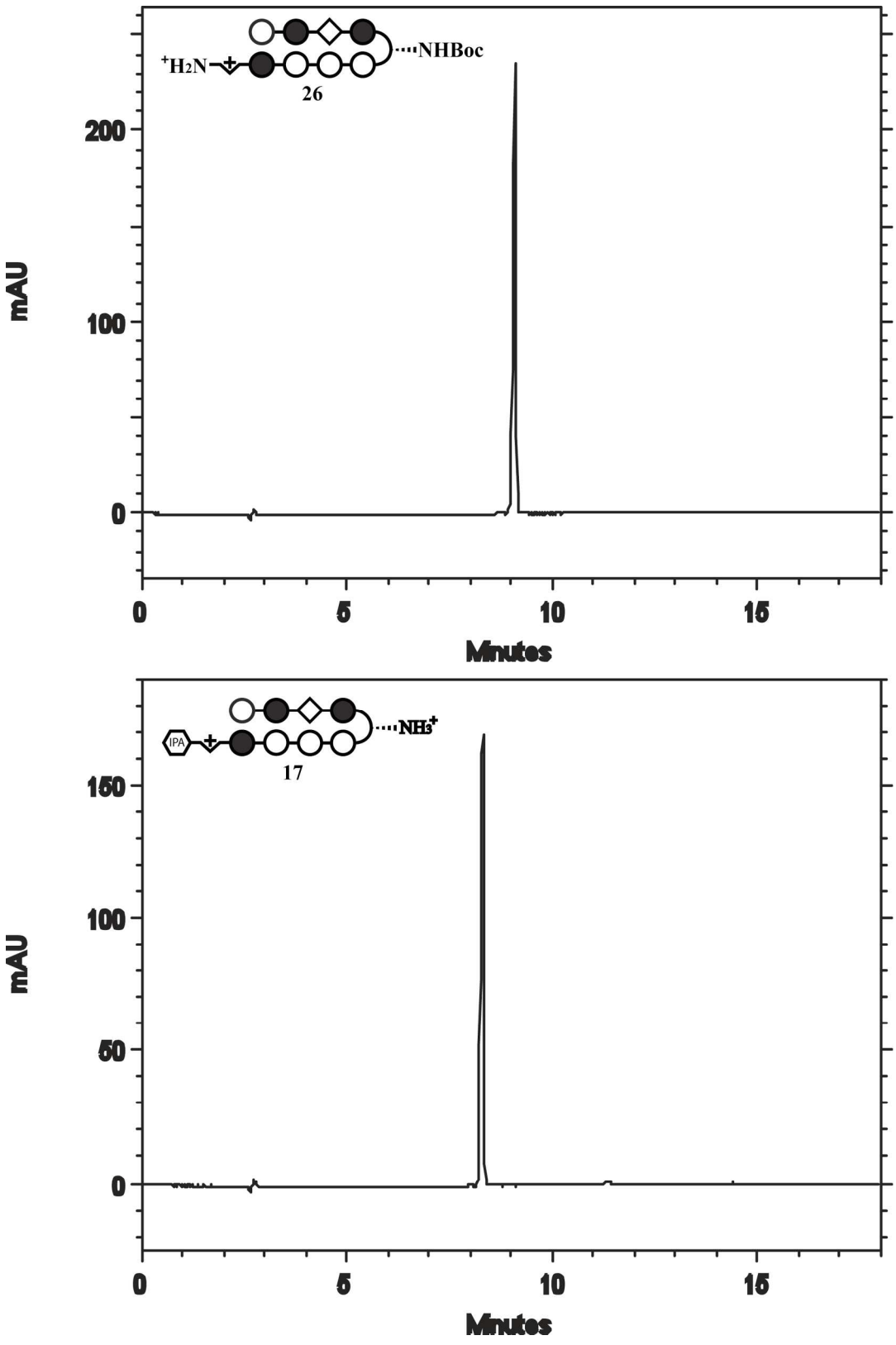


Figure S9. HPLC Spectrum of **26** and **17.**

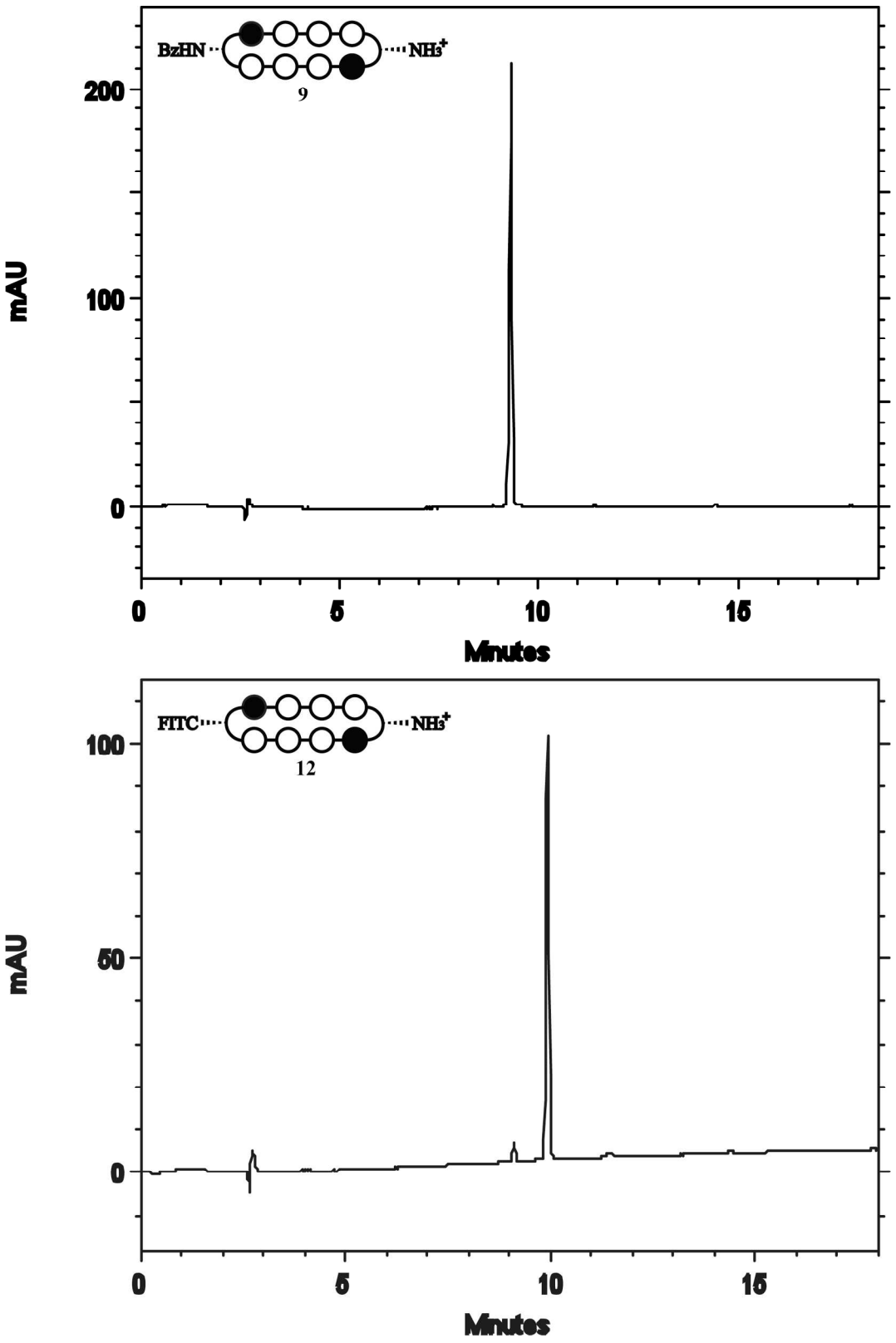


Figure S10. HPLC Spectrum of **9** and **12.**

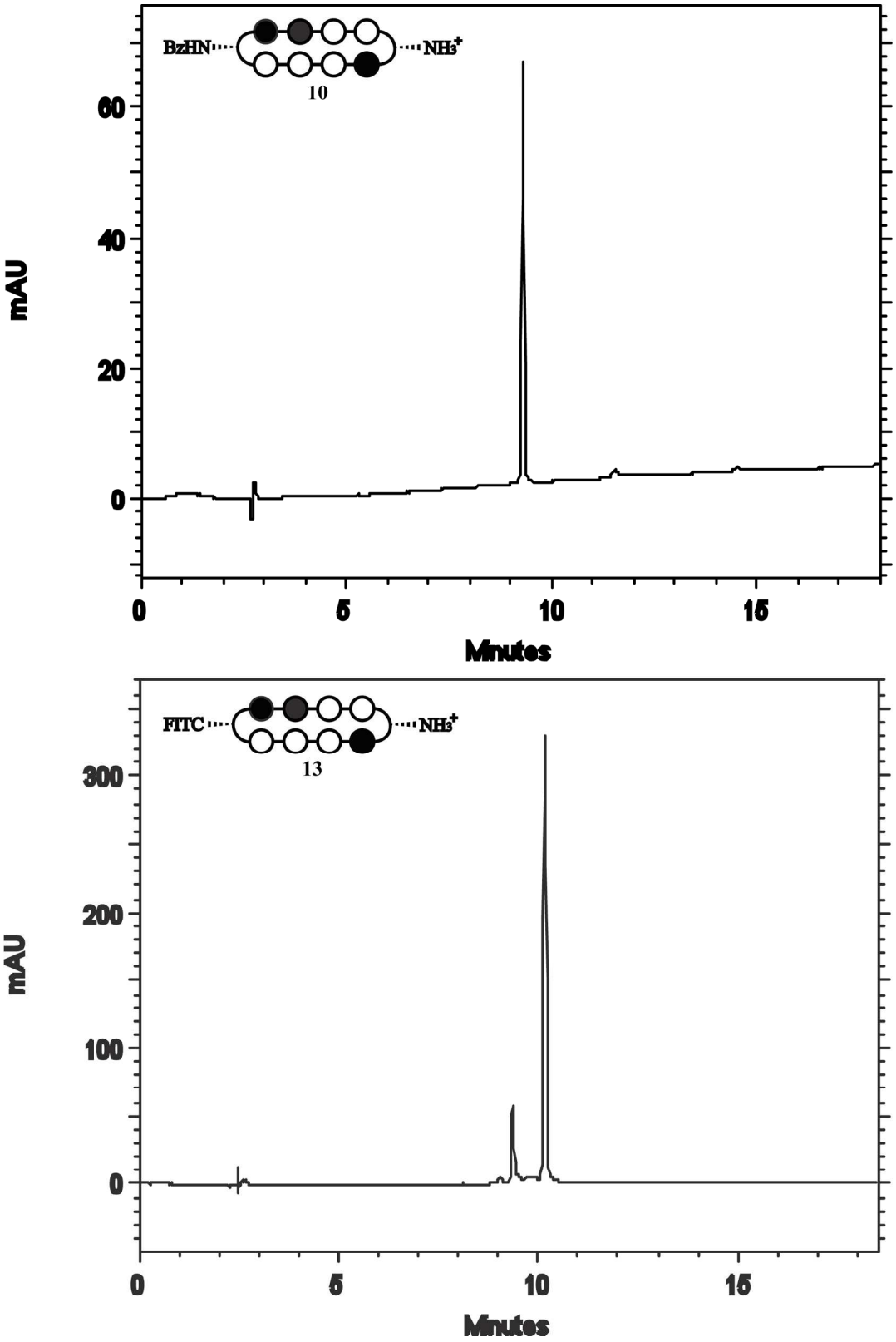


Figure S11. HPLC Spectrum of **10** and **13.**

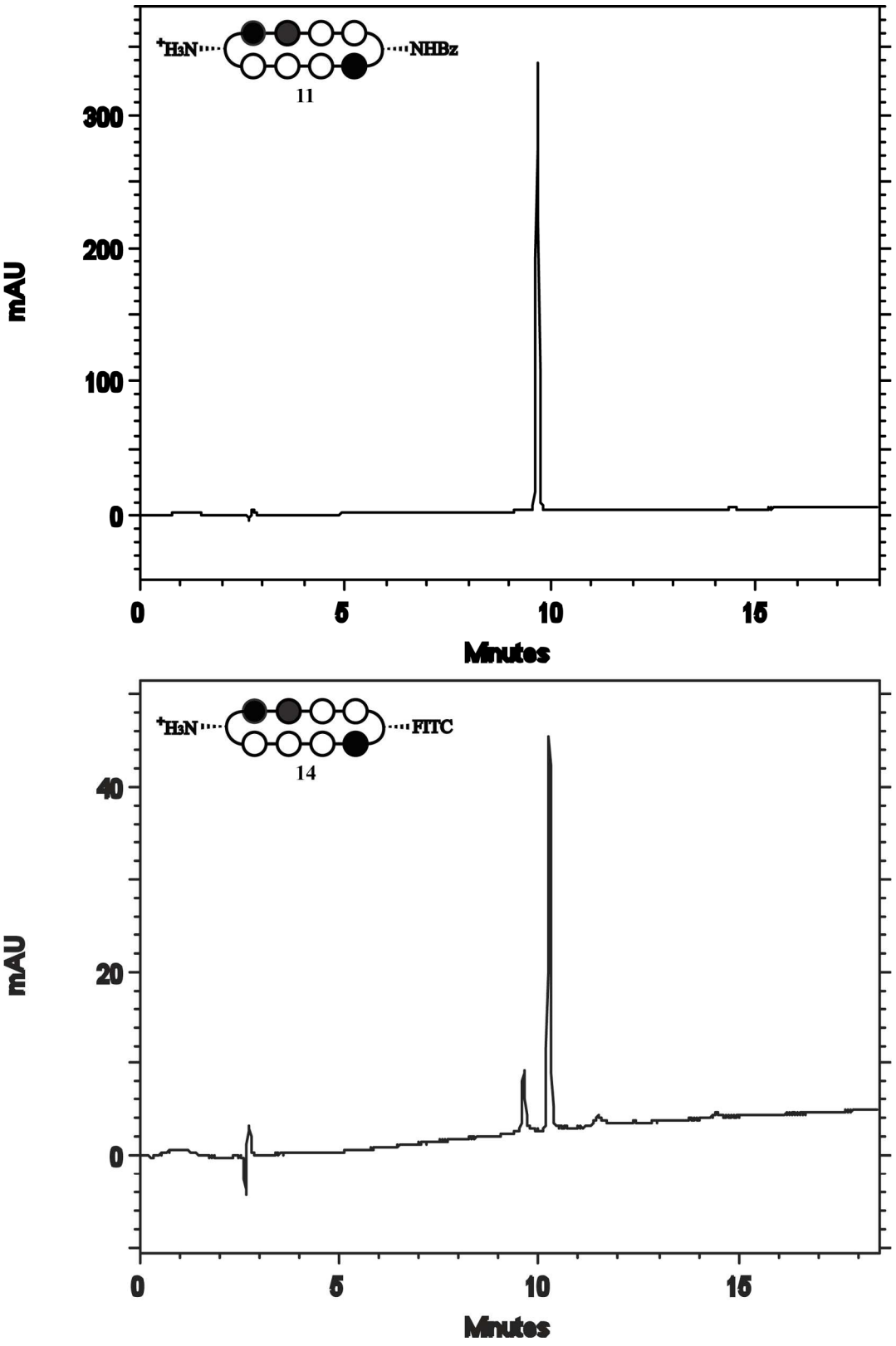


Figure S12. HPLC Spectrum of **11** and **14.**

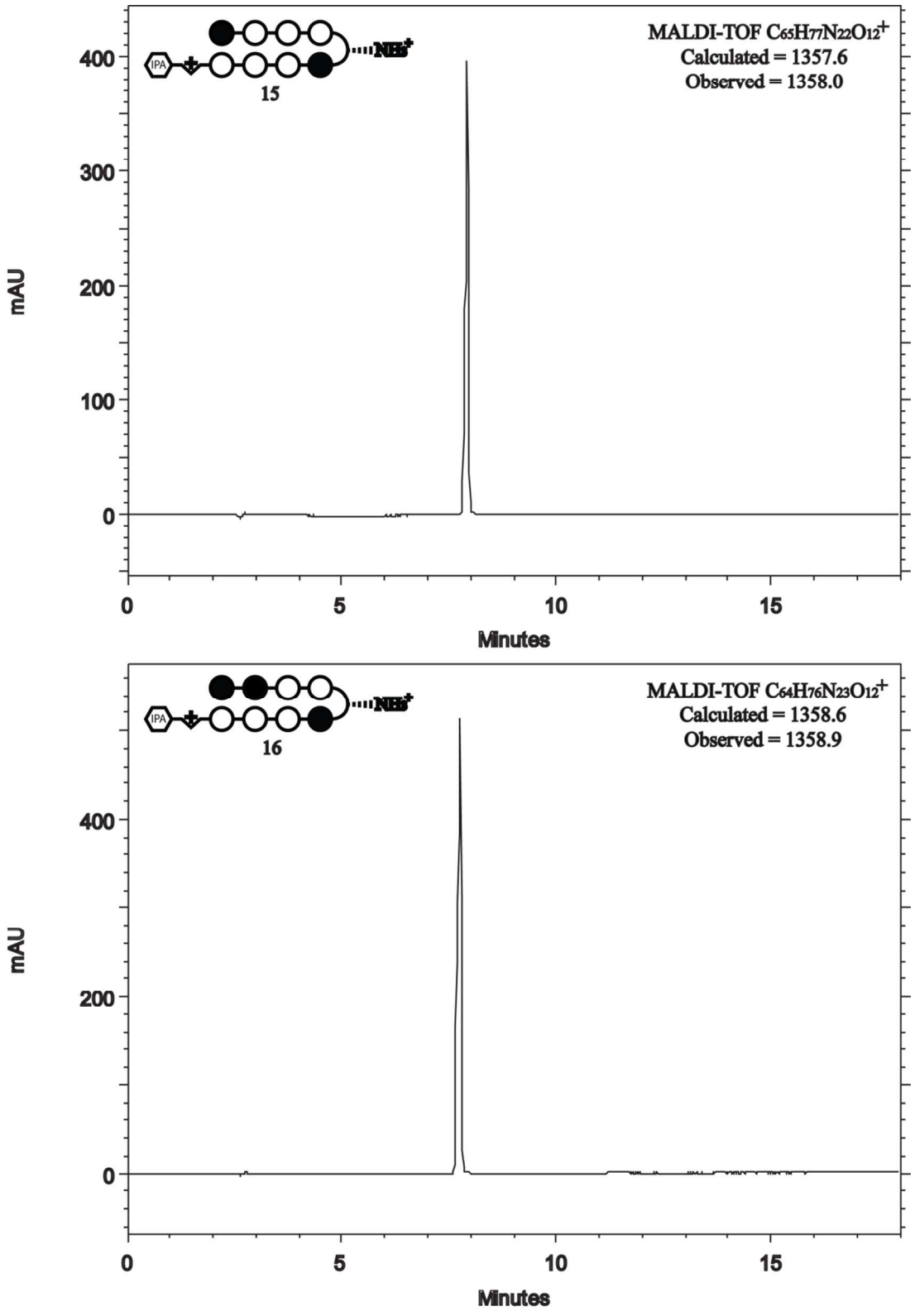


Figure S13. HPLC Spectrum of **15** and **16.**

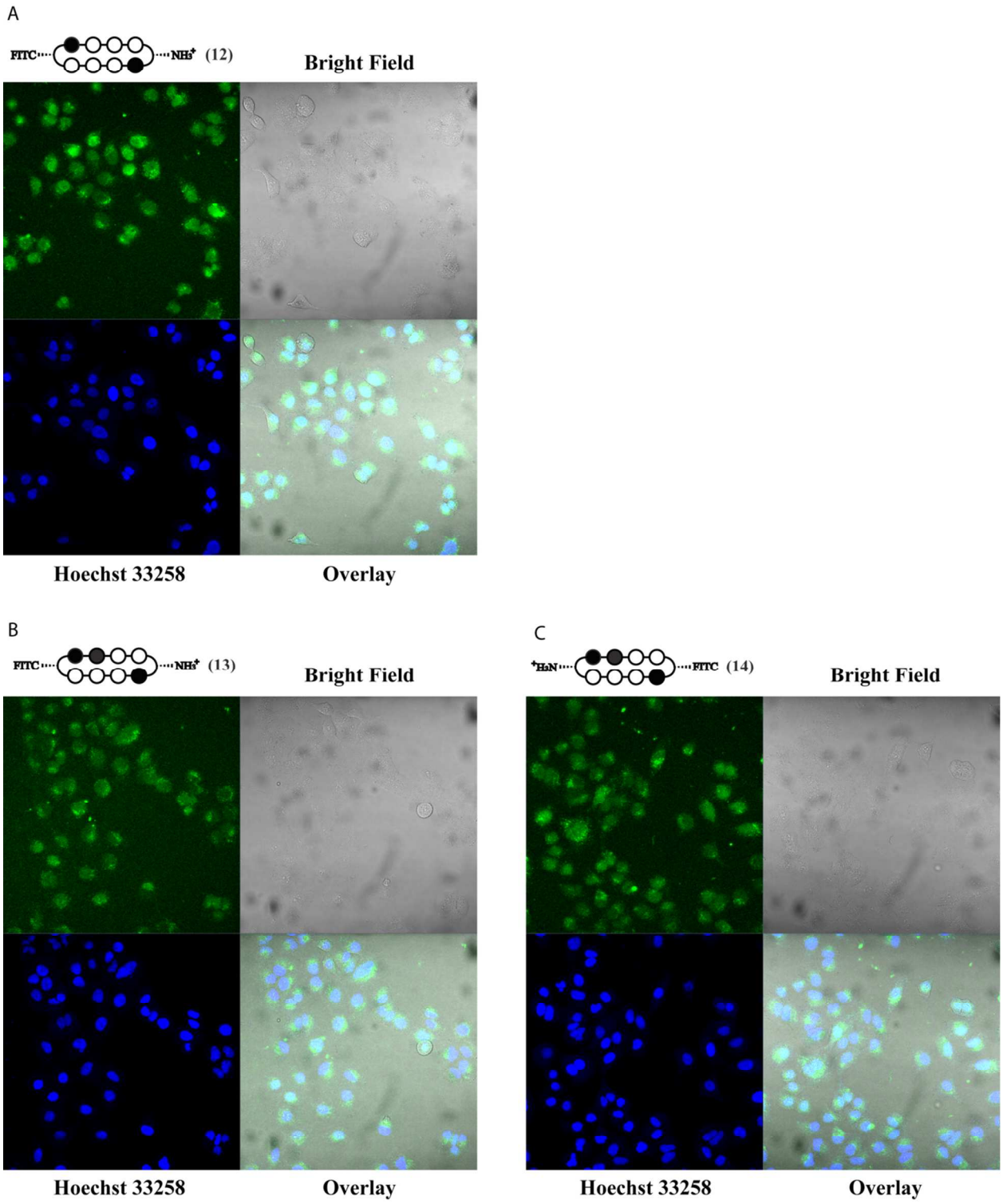


Figure S14. Confocal microscopy analysis of **12**-**14** in A549 cells.

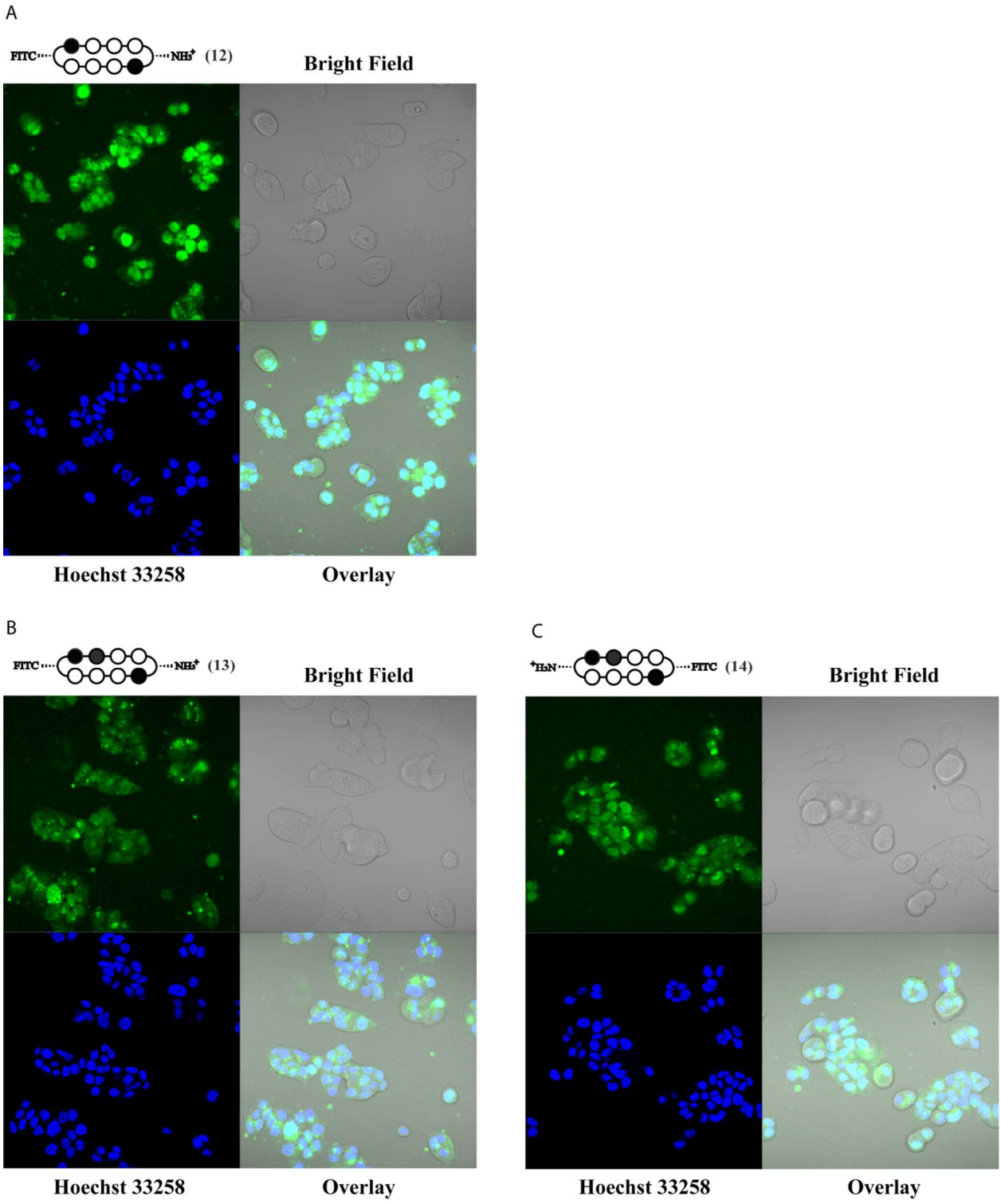
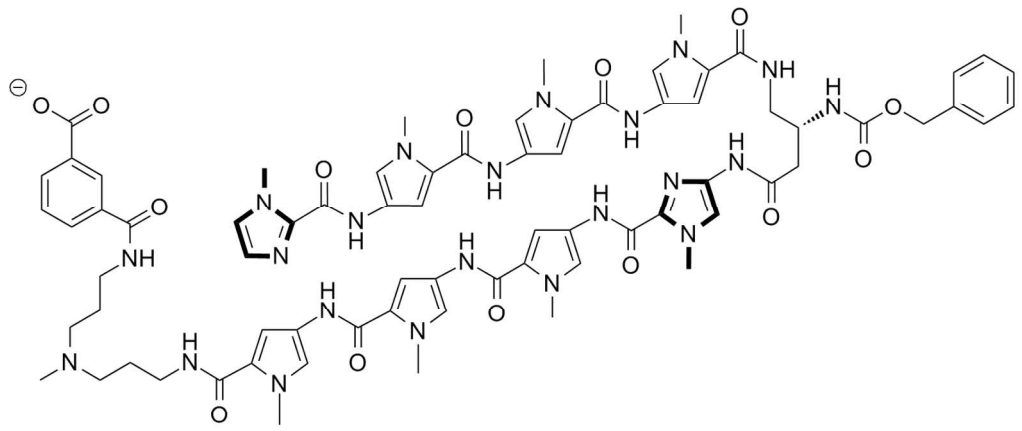


Figure S15. Confocal microscopy analysis of **12**-**14** in T47D cells.



S1

Figure S16. In previous unpublished results, the cytotoxicity of compound **S1** in A549 cells was examined using the same Sulforhodamine B assay described in the text. The IC_{50} was determined to be 0.003μM ± 0.002μM.

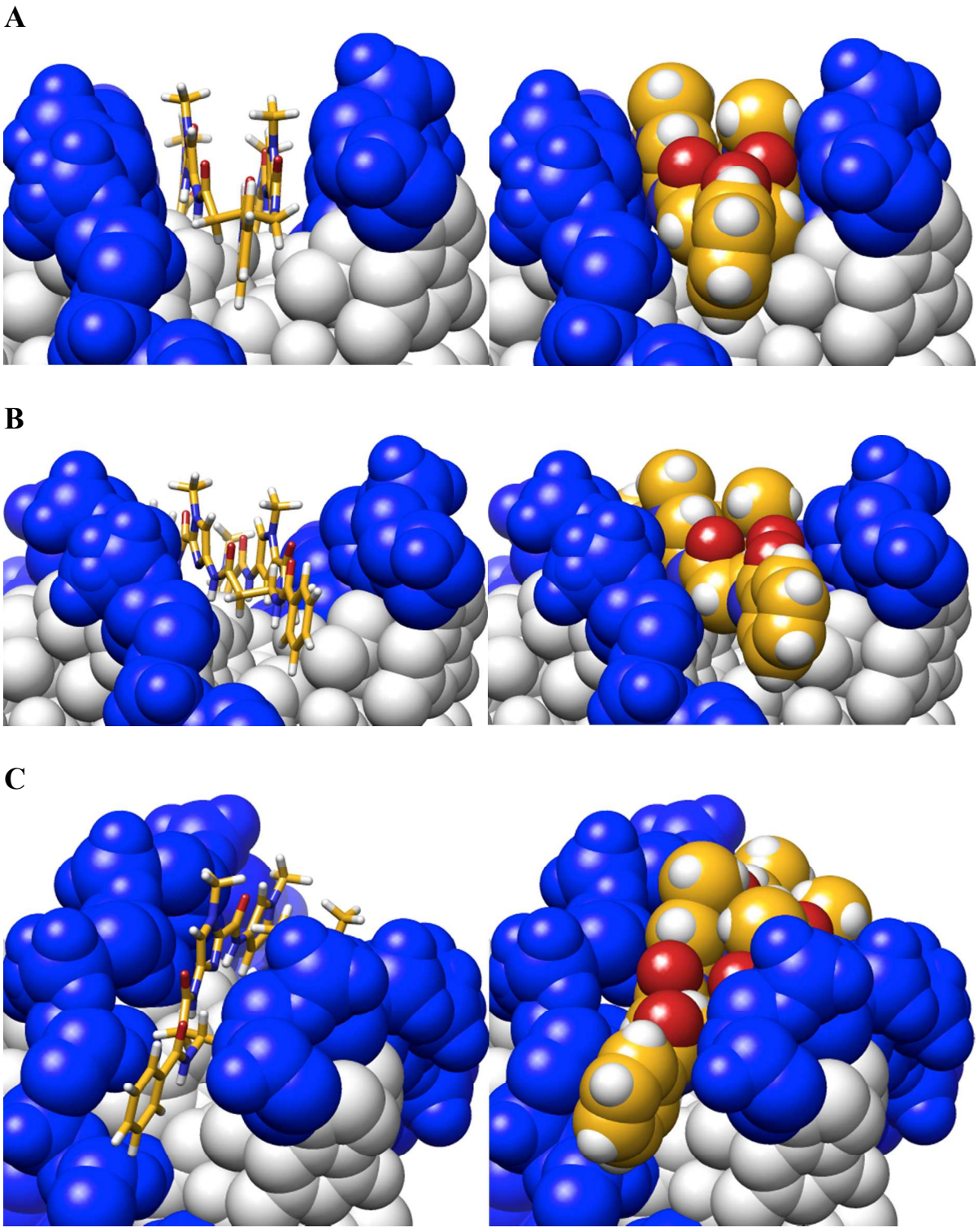


Figure S17. (Left) Stick model and (right) space-filling model of benzoyl substituted turn along the DNA minor groove from three different views. Based on published crystal structure by Chenoweth et al (PDB ID: 3OMJ).