Supplementary Information

Improvement of *N*-benzoylation catalysis driven by amyloid-substrate complex

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1. Supplementary Table and Figures

Table S1 Data of ThT assay

amyloid	trial 1	trial 2	trial 3	mean
Ac-NFAAIL-NH_2 (**3a**)	10176	8158	8410	8914
Ac-NFFAIL-NH_2 (**3b**)	11258	10465	10133	10618
Ac-NFLAIL-NH_2 (**3c**)	9911	12876	11578	11455
Ac-NFIAIL-NH_2 (**3d**)	13762	11488	11837	12362
US-amyloid	4757	4500	3194	4150
His-amyloid	4305	4439	4092	4279

Aliquot sample (12.5 μL) of peptide stock solution (4 mM) was added to 5 μM ThT solution (987.5 μL) in phosphate buffered saline (pH 7.4). The fluorescence intensity of the solution was measured with 440 nm of an excitation wavelength and 480 nm of an emission wavelength at room temperature.

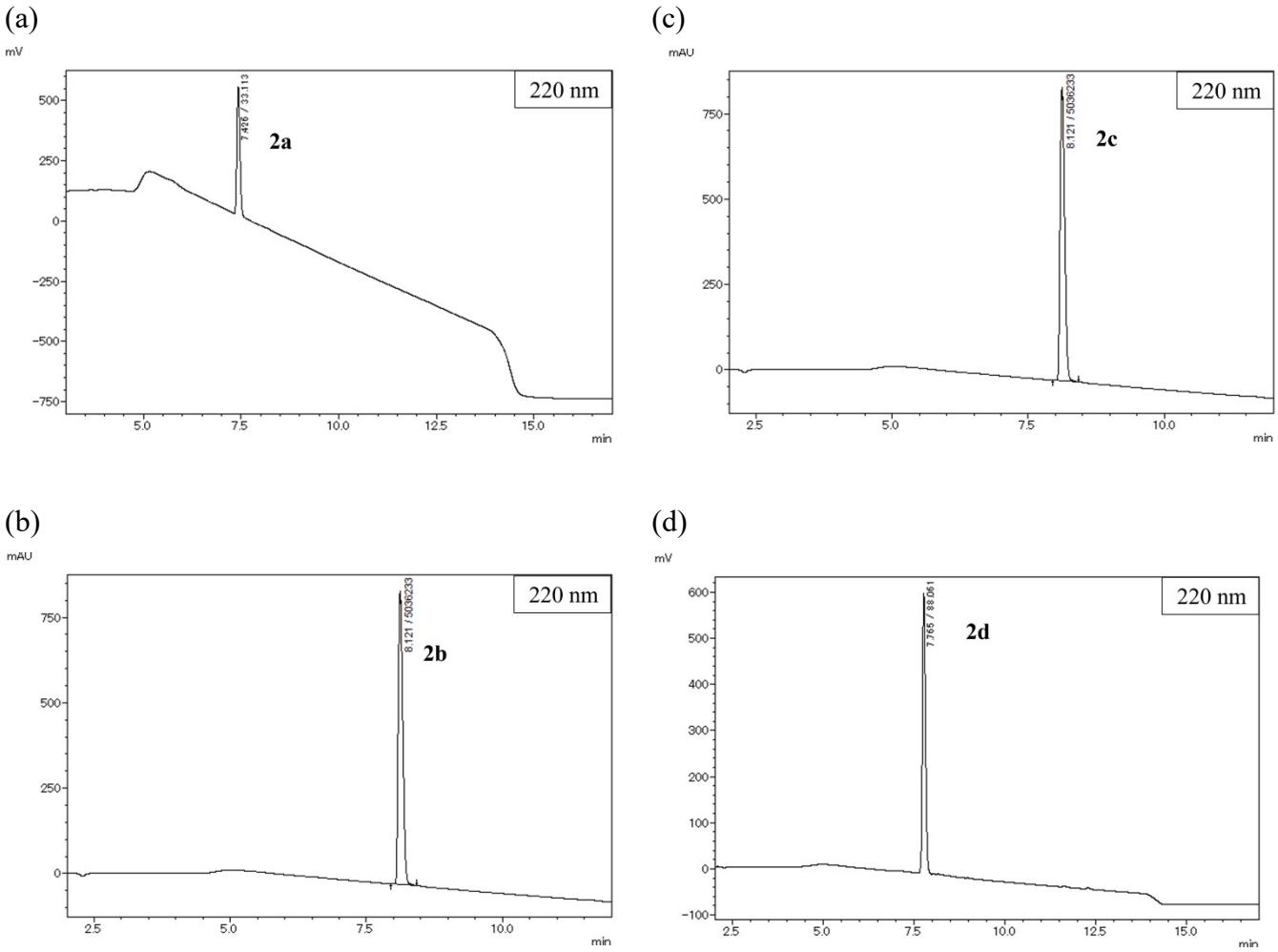


Fig. S1 Purity of **2a**-**2d** verified using HPLC. (a) **2a**, (b) **2b**, (c) **2c**, and (d) **2d**.

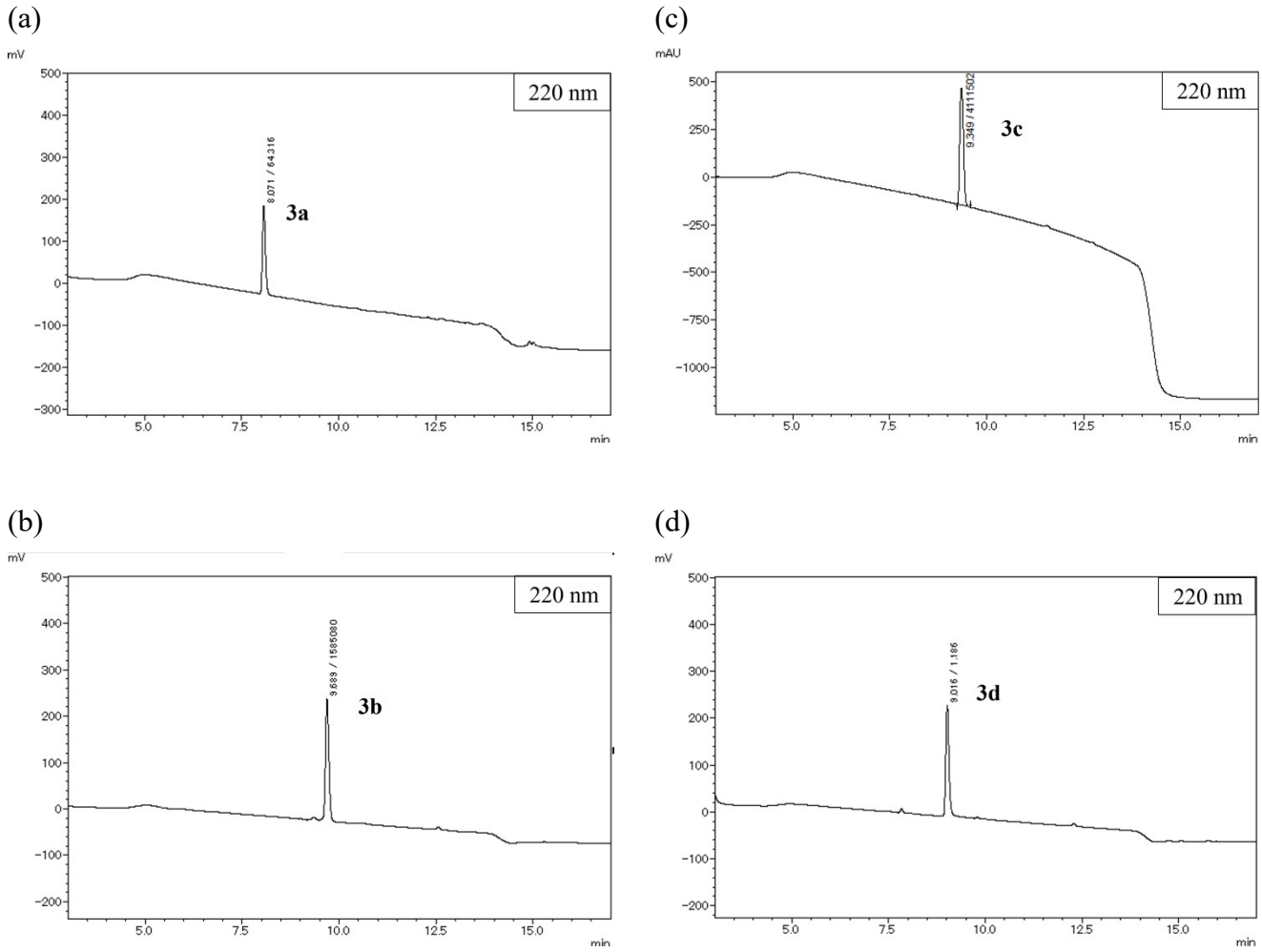


Fig. S2 Purity of **3a**-**3d** verified using HPLC. (a) **3a**, (b) **3b**, (c) **3c**, and (d) **3d**.

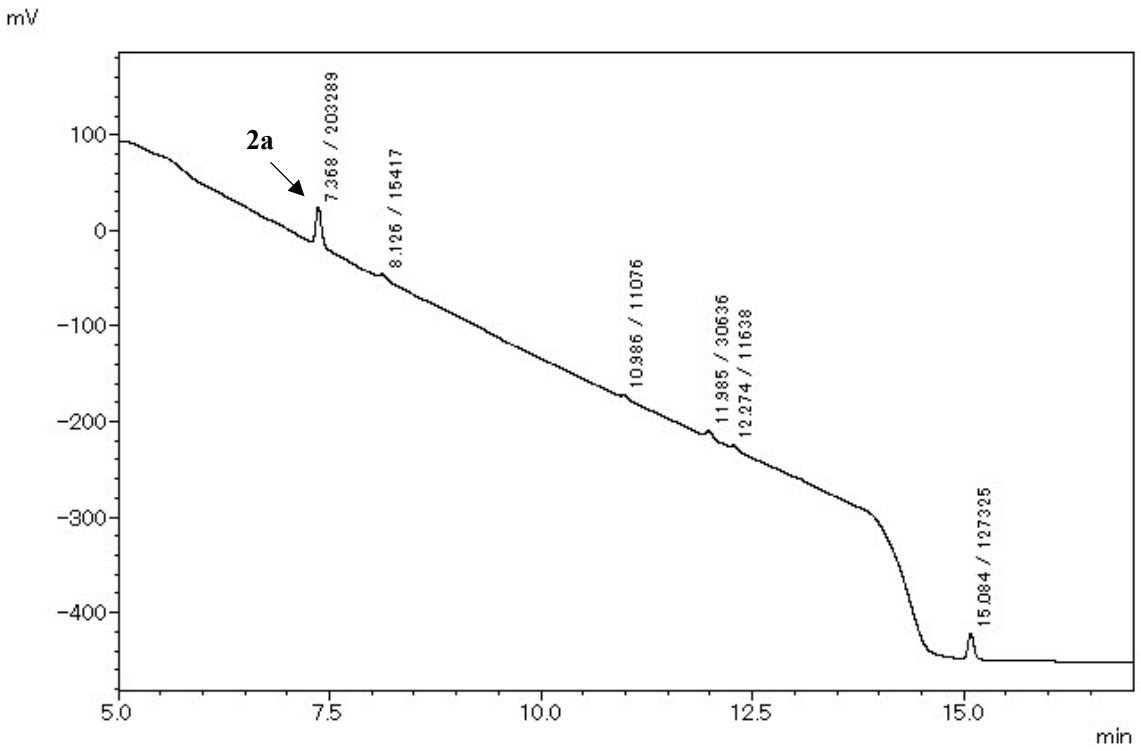
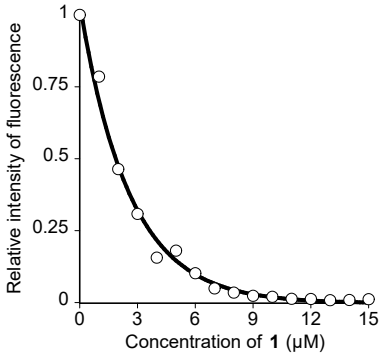


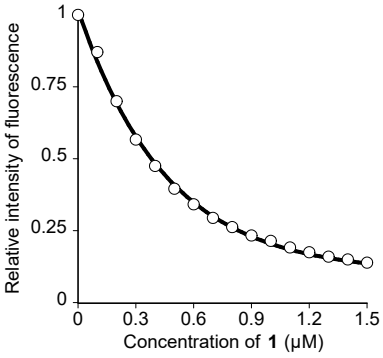
Fig. S3 Analytical HPLC trace of crude **2a** before preparative HPLC purification.

(a)



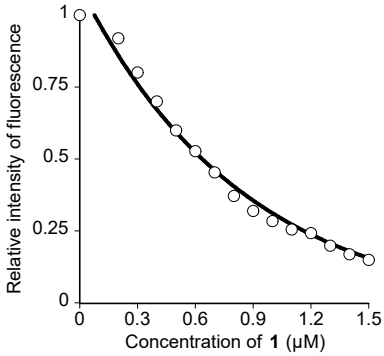
m1	-0.00266	m3	0.389
m2	1.041	R	0.995

(b)



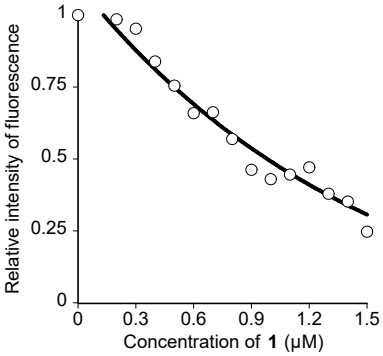
m1	0.1015	m3	2.186
m2	0.9166	R	0.999

(c)



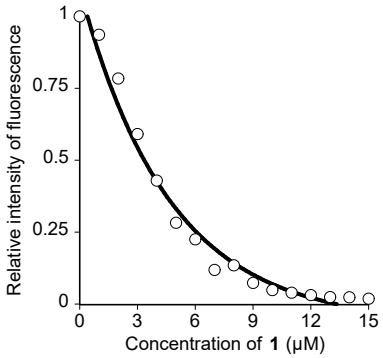
m1	-0.04234	m3	1.170
m2	1.141	R	0.994

(d)



m1	-0.1704	m3	0.6561
m2	1.277	R	0.987

(e)



m1	-0.06631	m3	0.2145
m2	1.162	R	0.989

Fig. S4 Binding affinities between **1** and amyloids verified by ThT displacement assay. Substrate concentration-dependent changes in thioflavin-T (ThT, a gold standard binder to amyloid)-derived fluorescence intensities upon binding with the amyloids are shown. The displacement assay was performed in a similar manner to that previously reported (T. Sawazaki, *et al.*, *Proc. Natl. Acad. Sci. U. S. A.* 2024, **121**, e2314704121). (a) Affinity of **1** with **3a**. The relative intensity of ThT fluorescence was halved at 1.9 μM of **1**. (b) Affinity of **1** with **3b**. The relative intensity of ThT fluorescence was halved at 0.38 μM of **1**. (c) Affinity of **1** with **3c**. The relative intensity of ThT fluorescence was halved at 0.64 μM of **1**. (d) Affinity of **1** with **3d**. The relative intensity of ThT fluorescence was halved at 0.98 μM of **1**. (e) Affinity of **1** with **NL6**. The relative intensity of ThT fluorescence was halved at 3.3 μM of **1**.

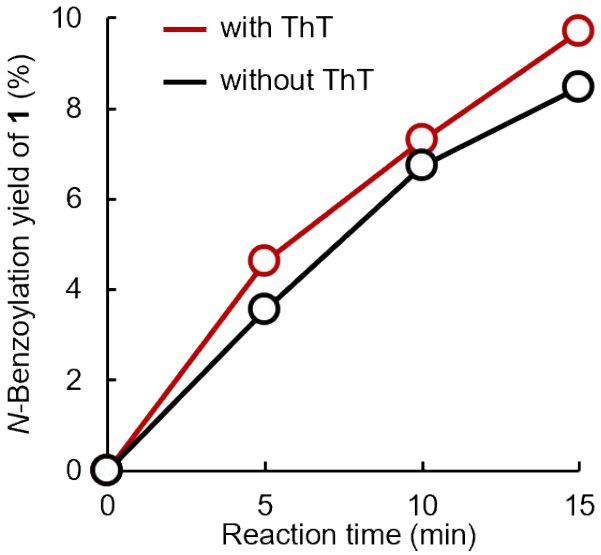


Fig. S5 Time-course study of *N*-benzoylation of **1** in shorter time-points (within 15 min). Conditions: substrate **1** (25 μM), 3-benzoylthiazolidine-2-thione (Bz donor, 100 μM), **3d** amyloid (200 μM), ThT (0 or 250 μM) at 37˚C in acetate buffer (pH 6.0).

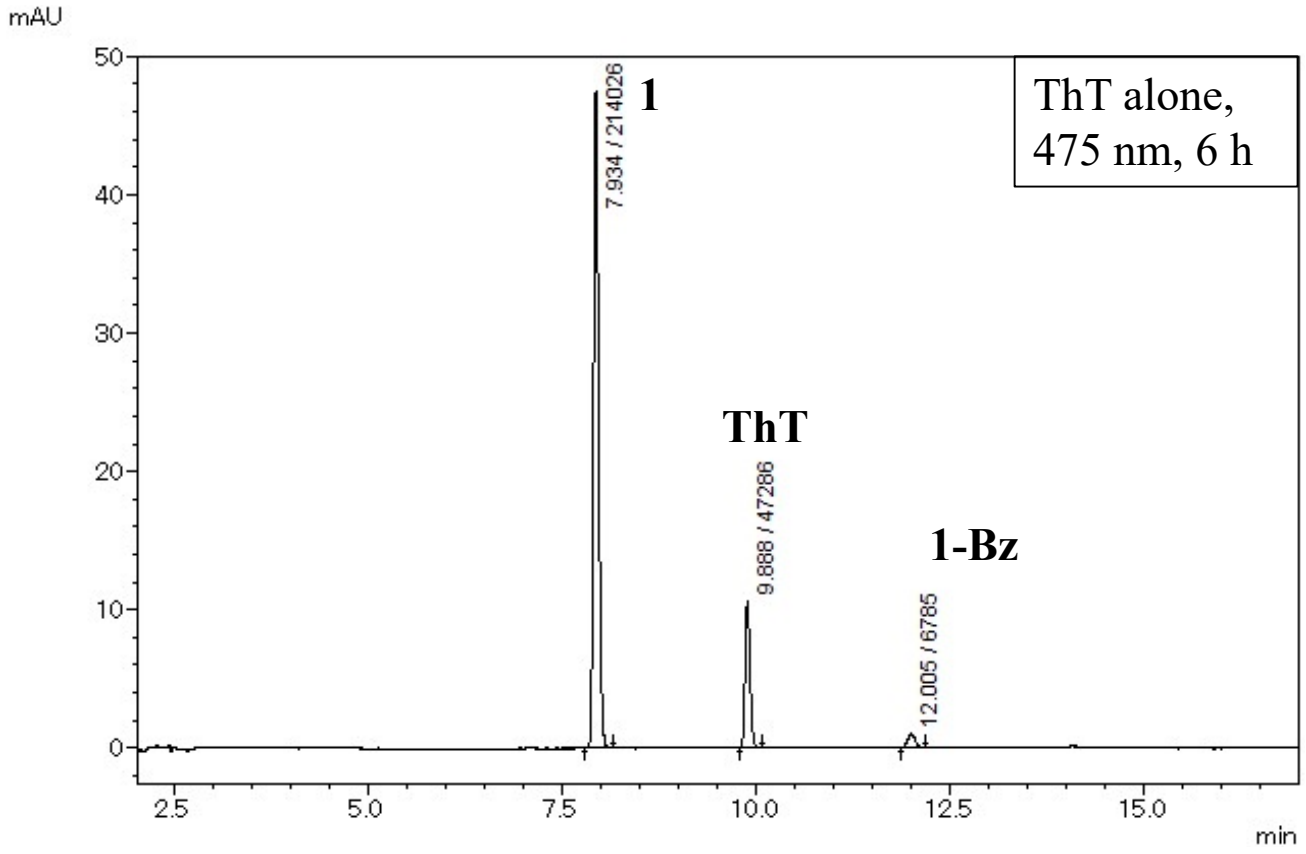
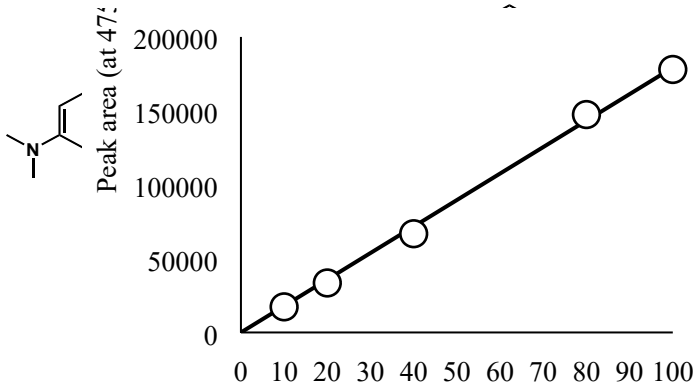


Fig. S6 Control experiment of *N*-benzoylation of **1** without amyloid. Conditions: substrate **1** (25 μM), 3-benzoylthiazolidine-2-thione (Bz donor, 100 μM), ThT (250 μM) at 37˚C in acetate buffer (pH 6.0).

2. Analytical HPLC charts for Table 1 and Figures 3e and 4

Calibration curve of **1-Bz**



Production yield of **1-Bz** **(%)** Table 1 entry 1

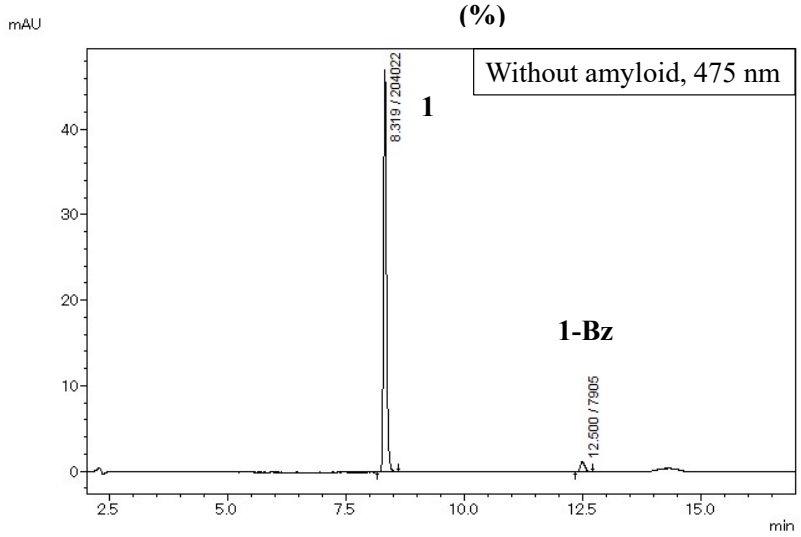


Table 1 entry 3

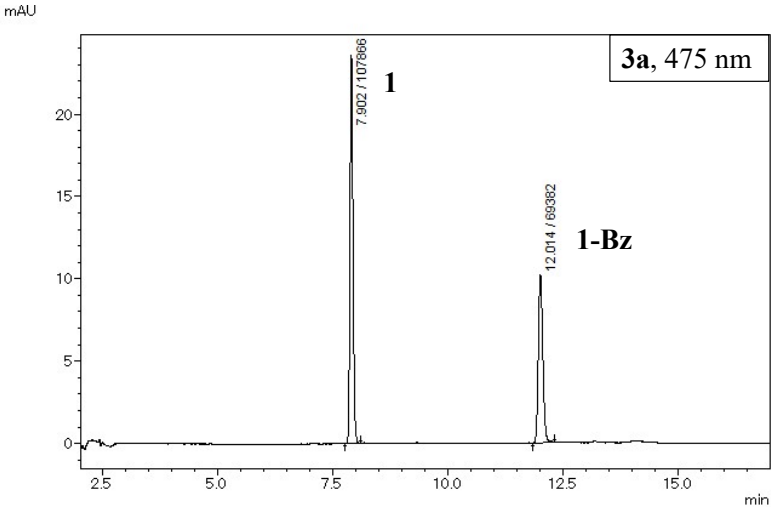


Table 1 entry 4

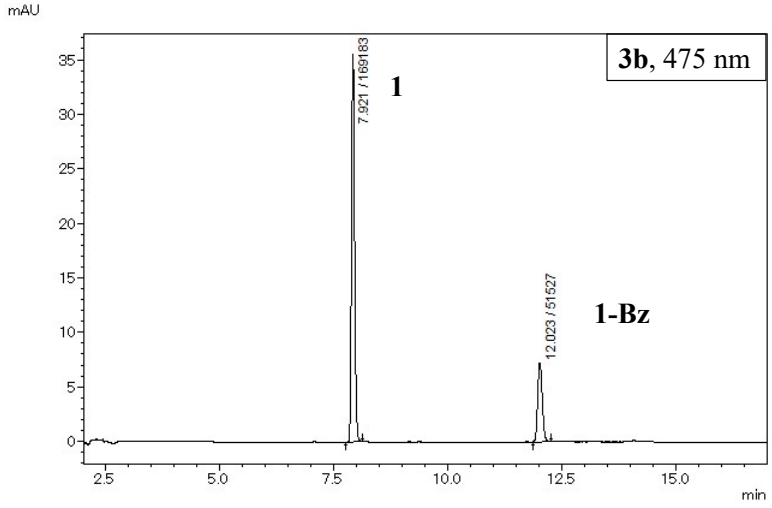


Table 1 entry 5

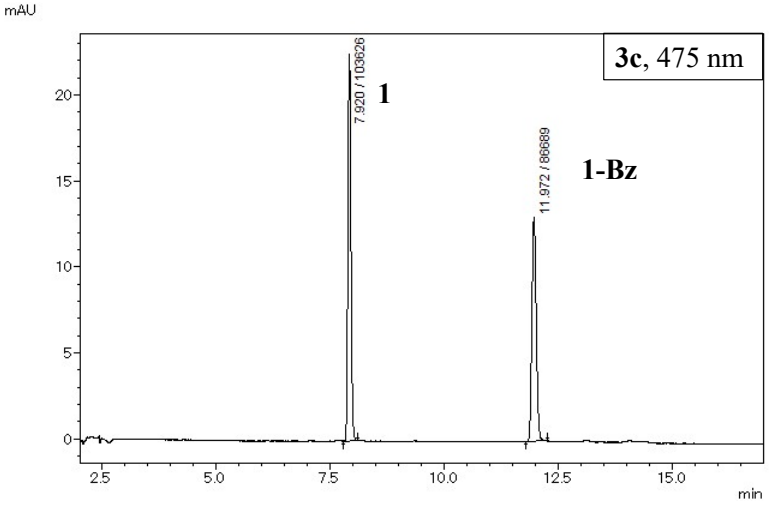


Table 1 entry 6

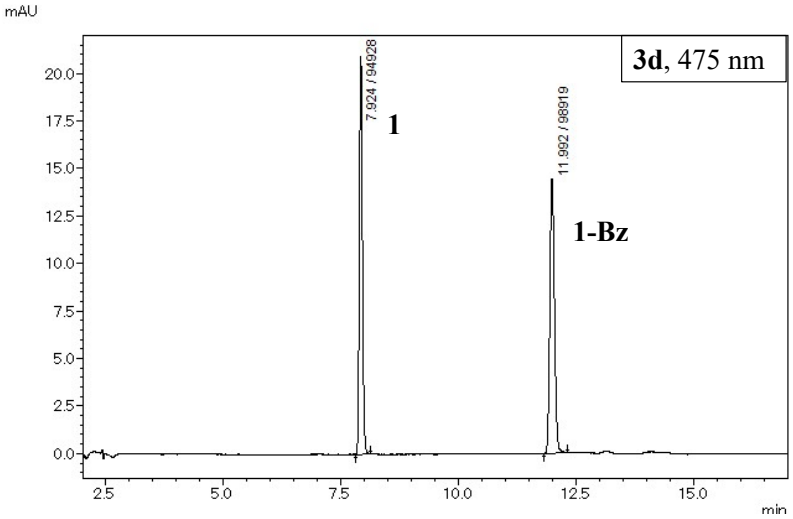


Fig. 3e entry 1

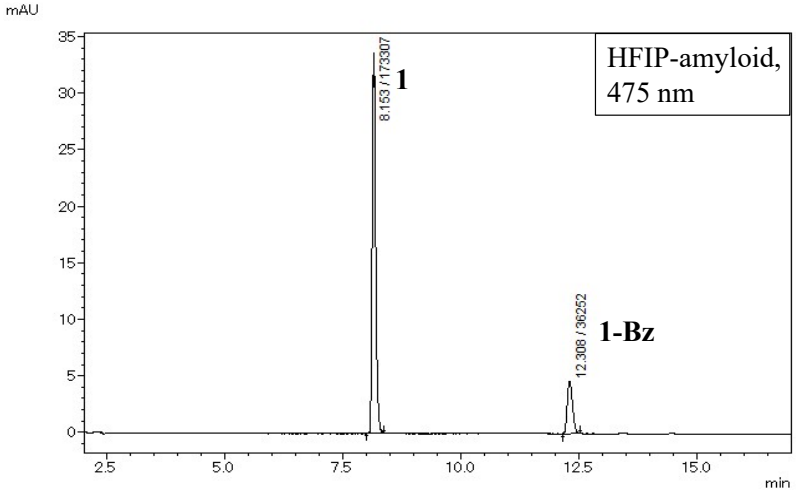


Fig. 3e entry 2

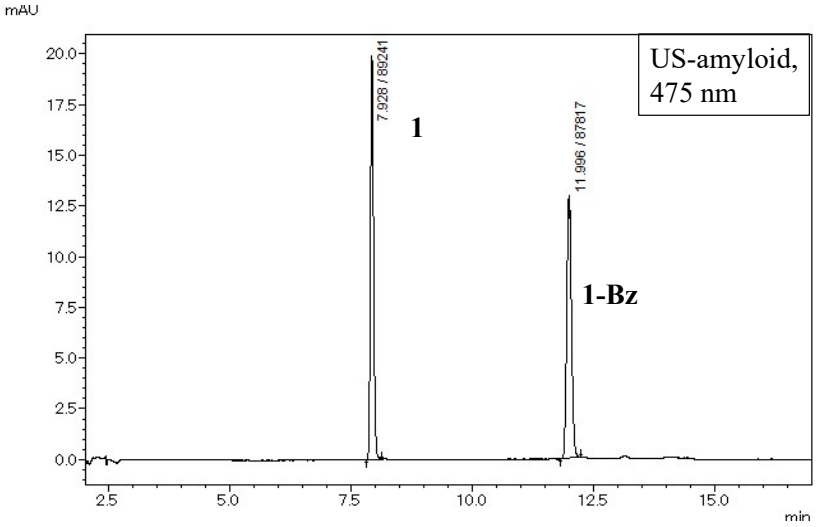
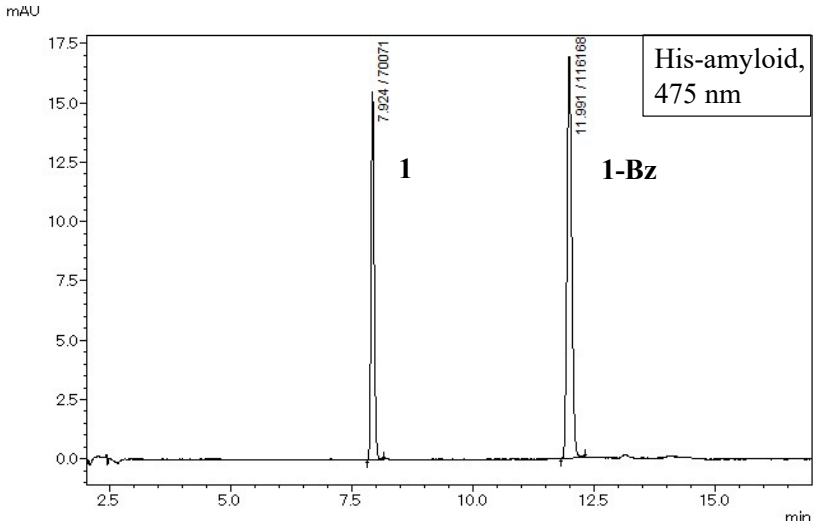


Fig. 3e entry 3



(NO prior co-incubation of **3d** and His)

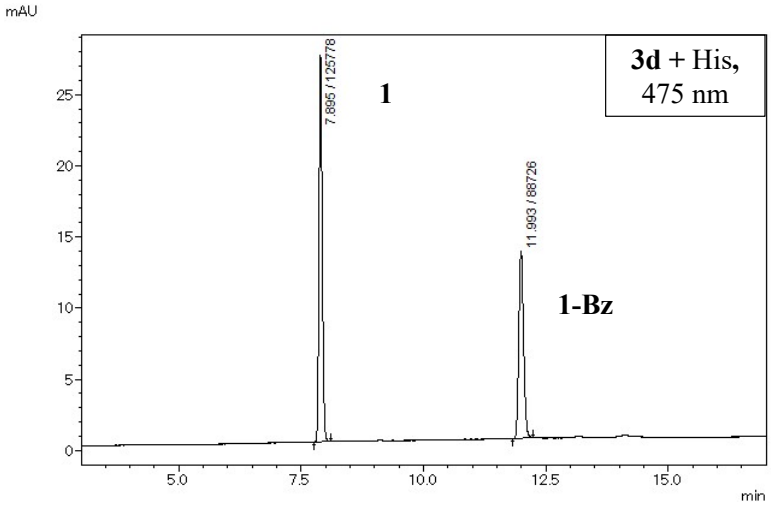


Fig. 3e entry 4

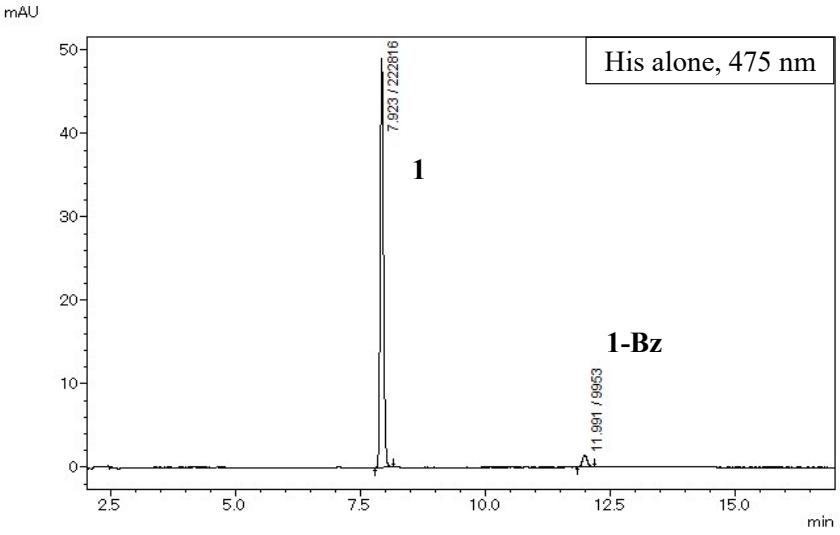


Fig. 4b

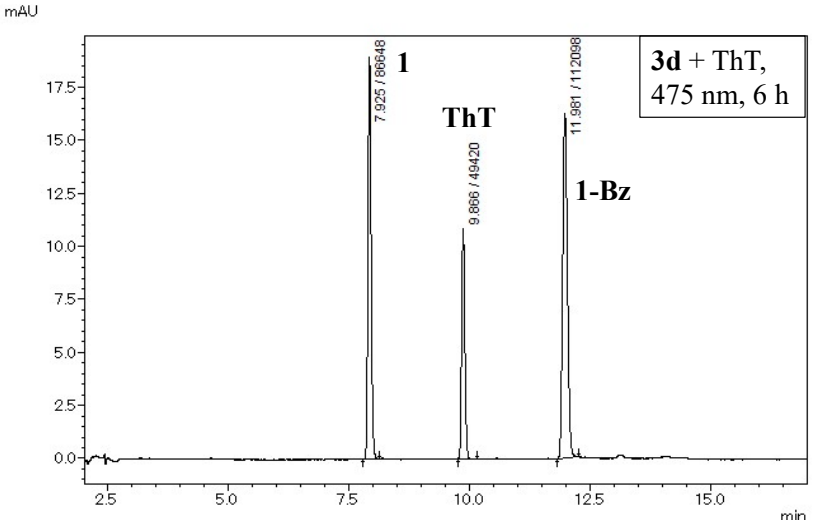
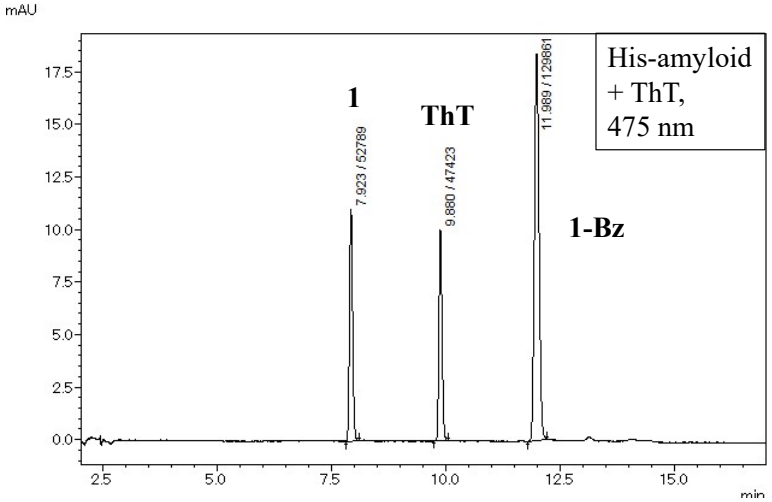

mAU
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
2.5
5.0
7.5
10.0
12.5
15.0
min
7.925 / 86648
9.886 / 49420
11.981 / 112098
1
ThT
1-Bz
3d + ThT,
475 nm, 6 h

Fig. 4c


mAU
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
2.5
5.0
7.5
10.0
12.5
15.0
min
7.923 / 52789
9.880 / 47423
11.989 / 129861
1
ThT
1-Bz
His-amyloid
+ ThT,
475 nm