Supporting Information

Binding position analysis of target proteins with the use of amidopyrene probes as LA-LDI enhancing tags

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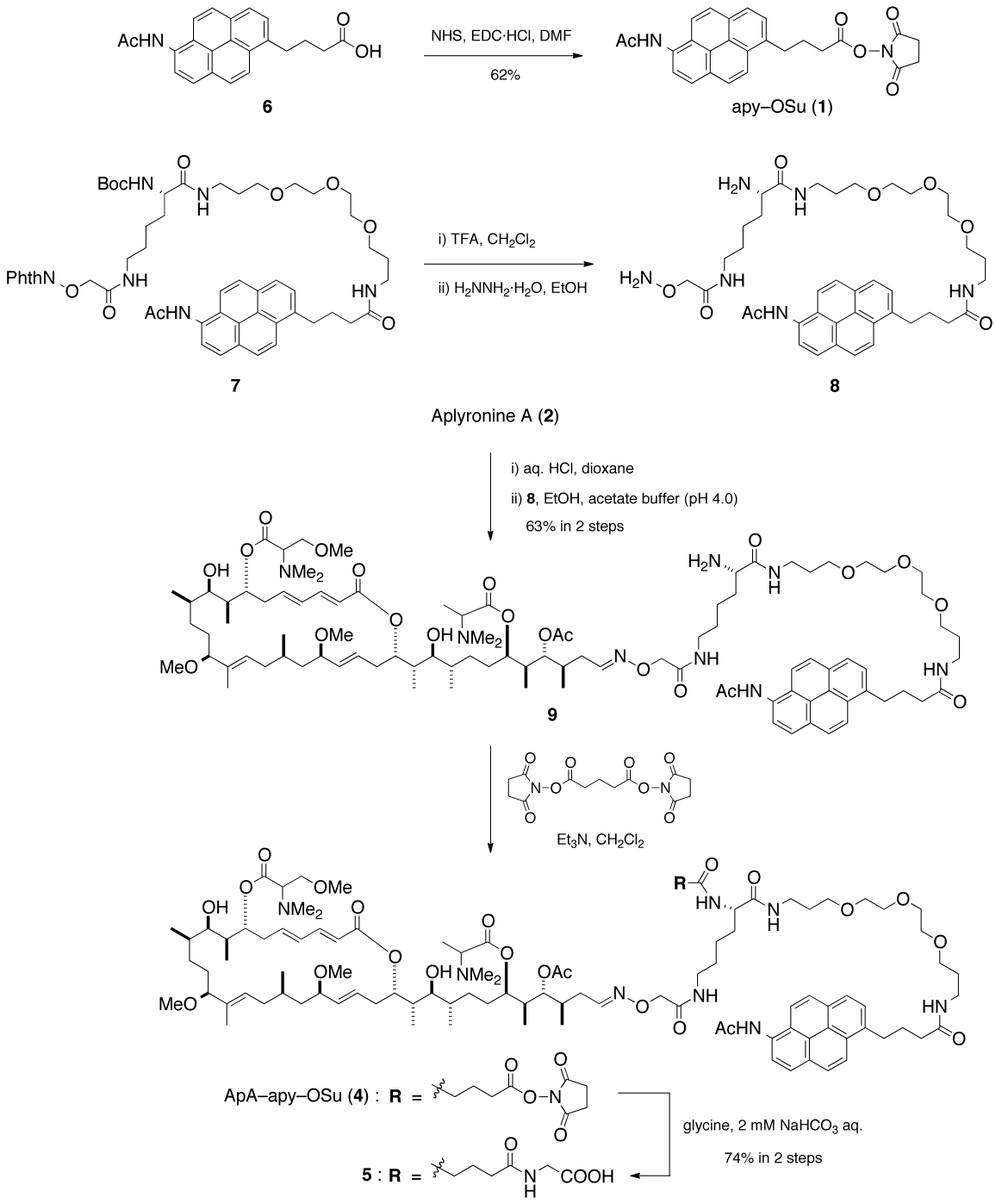
(12 pages)



Scheme S1. Synthesis of apy–OSu (**1**) and ApA–apy–OSu (**4**).

Table S1. Tryptic peptides of the actin labeled with Apy–OSu (**1**) detected by MALDI and LA-LDI MS.

No.	Observed (*m/z*) [a]		Calculated (*m/z*) [a]		Start/end	Sequence [e]
	MALDI	LA-LDI	peptide	apy-peptide		
1	1086.6	1044.5 [c] 1086.6	759.5	1086.6	191 / 196	**K**ILTER
2	1246.6		919.5	1246.6	285 / 291	CDIDIR**K**
3	1272.6 [b]	1230.6 [b, c] 1272.6 [b]	923.6	1250.7	329 / 336	IIAPPER**K**
4	1363.7	1363.7 1385.7 [b]	1036.7	1363.8	327 / 335	I**K**IIAPPER
5	1130.5 1457.6	1130.5 1457.6	1130.5	1457.6	197 / 206	G**YS**FVTTAER [f]
6	1187.5		1187.6	1514.7	40 / 50	HQGVmVGMGQ**K**
7	1198.7		1198.7	1525.8	29 / 39	AVFPSIVGRPR
8	1681.7	1681.7	1354.6	1681.7	51 / 62	DSYVGDEAQS**K**R
9	1483.6 [d] 1500.7		1500.7	1827.8	360 / 372	QEYDEAGPSIVHR
10	1515.7	1515.7	1515.7	1842.8	85 / 95	IWHHTFYNELR
11		1895.9 [c] 1937.9 1959.9 [b]	1610.8	1937.9	184 / 196	DLTDYLM**K**ILTER
12	1790.8		1790.9	2118.0	239 / 254	SYELPDGQVITIGNER
13	1956.0		1956.0	2283.1	96 / 113	VAPEEHPTLLTEAPLNPK
14	2246.0		2246.0	2573.1	292 / 312	DLYANNVMSGGTTMYPGIADR
15	2624.2	2624.2 2646.2 [b]	2297.2	2624.3	96 / 116	VAPEEHPTLLTEAPLNP**K**ANR
16	2374.0 2701.1	2701.1	2374.1	2701.2	291 / 312	**K**DLYANNVMSGGTTMYPGIADR
17	2681.3		2681.2	3008.3	292 / 315	DLYANNVmSGGTTmYPGIADRmQK
18	3196.5		3196.6	3523.7	148 / 177	TTGIVLDSGDGVTHNVPIYEGYALPHAIMR

[a] The data represent the monoisotopic ion peaks $(M+H)^+$ values.

[b] $(M+Na)^+$ values.

[c] Fragment ion by losing ketene (CH_2=C=O) from amidopyrene.

[d] *N*-terminus glutamine was converted into pyroglutamic acid.

[e] "**K**" means apy-labeled lysine resudue. Cysteine residues are carbamidomethylated. "m" means oxidized methyonine residue.

[f] Either tyrosine or serine residue was labeled with amidopyrene.

Table S2. Digested peptides of the actin labeled with ApA–apy–OSu (**4**) detected by MALDI and LA-LDI MS.

No.	Observed (*m/z*) [a]			Calculated (*m/z*) [a]	Start/end	Sequence [d]
	Actin + probe **4**		Actin			
	MALDI (Fig. 4a)	LA-LDI (Fig. 4b)	LA-LDI (Fig. 4c)			
1		531.2 [b]		531.2	–	probe **4** (fragment)
2			589.4	589.3	58 / 62	AQSKR
3	631.3	631.3	631.4	631.4	192 / 196	ILTER
4	644.3	644.3	644.4	644.4	178 / 183	LDLAGR (or ^{63}GILTLK^{68})
5	791.3	791.3		791.4	285 / 290	CDIDIR
6	795.4	795.4	795.4	795.5	329 / 335	IIAPPER
7	836.4	836.4	836.4	836.5	365 / 372	AGPSIVHR
8			851.2	851.4	4 / 11	ETTALVCD
9		880.2	880.2	880.6	327 / 334	IKIIAPPE
10			899.5	899.4	119 / 125	MTQIMFE (or ^{185}LTDYLMK^{191})
11			955.2	955.5	73 / 80	HGIITNWD
12	976.4	976.4	976.4	976.4	19 / 28	AGFAGDDAPR
13		998.4		998.5	184 / 191	DLTDYLMK
14	1086.5	1086.6	1086.6	1086.6	245 / 254	GQVITIGNER
15	1128.6	1128.6		1128.6	158 / 167	GVTHNVPIYE (or ^{168}GYALPHAIMR^{177})
16	1130.5	1130.5	1130.5	1130.5	196 / 205	RGYSFVTTAE (or ^{197}GYSFVTTAER^{206})
17			1198.7	1198.7	29 / 39	AVFPSIVGRPR (or ^{227}mATAASSSSLEK^{238})
18	1246.5	1246.5		1246.6	85 / 93	IWHHTFYNE
19	1790.9	1790.9	1790.9	1790.9	239 / 254	SYELPDGQVITIGNER
20		2130.9		2131.0	293 / 312	LYANNVMSGGTTMYPGIADR
21	2246.0	2245.9	2246.0	2246.0	292 / 312	DLYANNVMSGGTTMYPGIADR
22	2371.2	2371.2	2371.2	2371.2	126 / 147	TFNVPAMYVAIQAVLSLYASGR
23			2387.2	2387.2		TFNVPAmYVAIQAVLSLYASGR
24		2799.5 [c]		2799.6 [c]	108 / 116	APLNPKANR + probe **4**
25	2841.6	2841.5		2841.6		
26			3251.5	3251.6	119 / 147	MTQIMFETFNVPAMYVAIQAVLSLYASGR

[a] The data represent the monoisotopic ion peaks $(M+H)^+$ values.

[b] Cleaved at the C–C bond of Lys carbonyl α position from parent ion (No. 25).

[c] Fragment ion by losing ketene (CH_2=C=O) from amidopyrene.

[d] Cysteine residues are carbamidomethylated. "m" means oxidized methyonine residue.

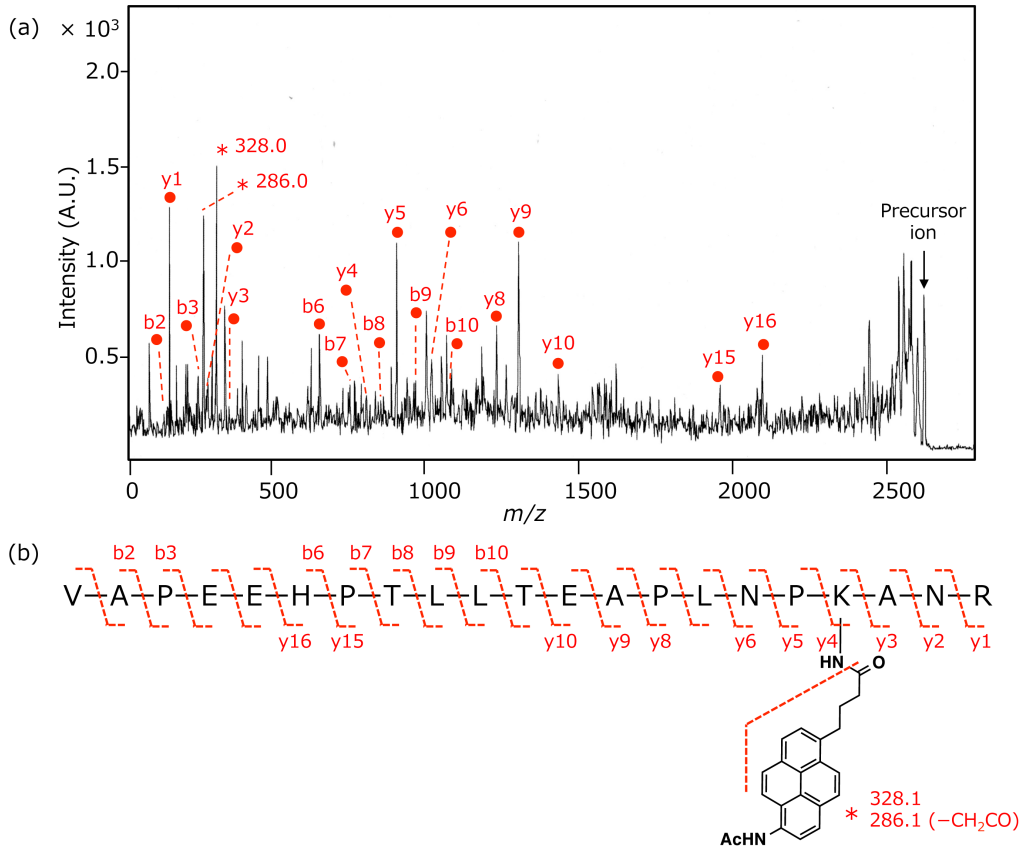


Figure S1. LA-LDI MS/MS analysis of an amidopyrene-labeled peptide (No. 15, precursor ion: *m/z* 2624.3). (a) LA-LDI MS/MS. Red circles show the peaks that correspond to the b or y fragment ions. (b) Structure of an amidopyrene-labeled peptide (*m/z* 2624.3 for $[M+H]^+$). Red asterisk shows the fragment ion cleaved at the amide bond between the lysine residue and amidopyrene moiety.

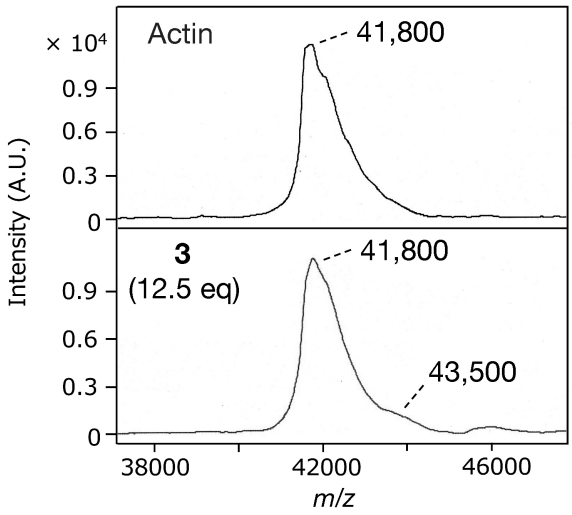
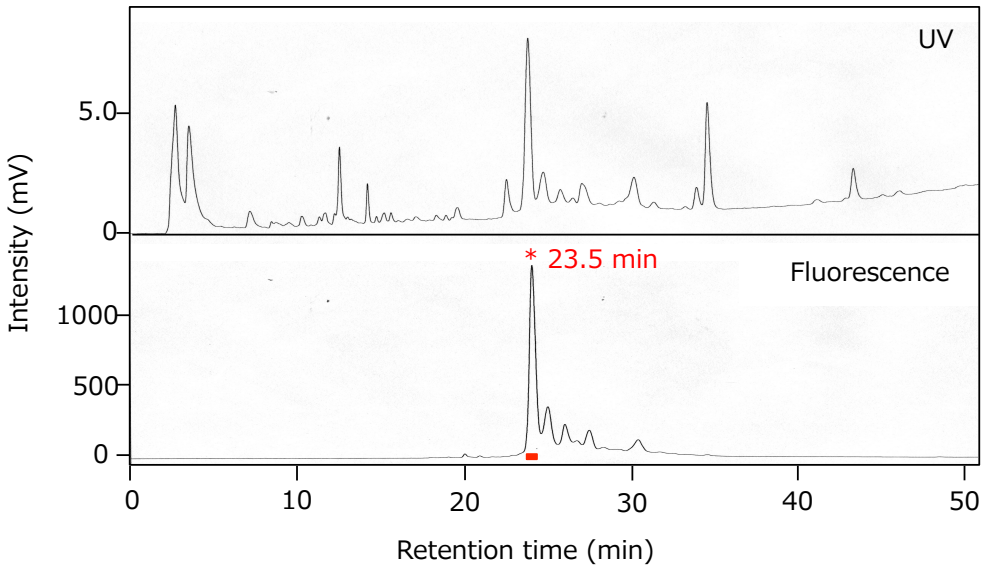


Figure S2. MALDI mass spectra of the untreated actin (top) and the photoreacted actin with ApA-PaP (**3**) (bottom).

(a)



(b)

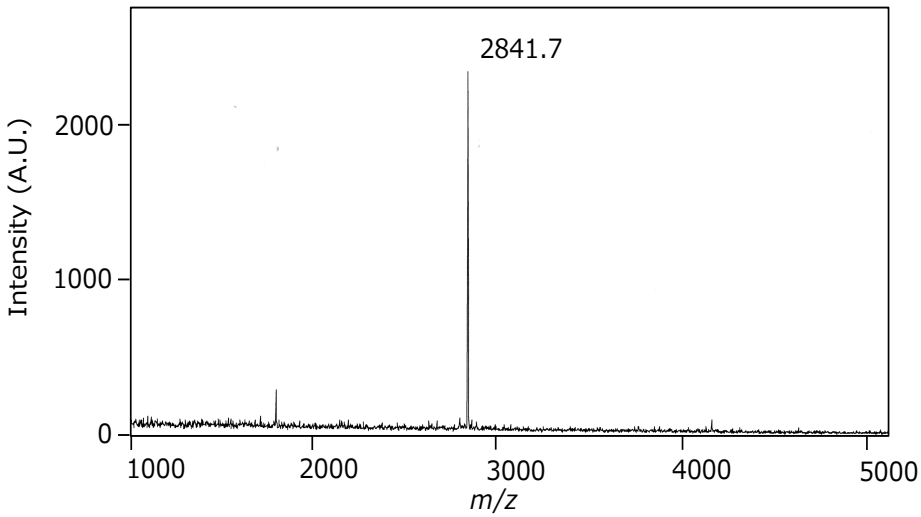


Figure S3. (a) HPLC analysis of the digested peptides of actin labeled with probe **4**. Column: Develosil RP-AQUEOUS AR-5 (1.5 mm I.D. × 150 mm), buffer: a linear gradient of 10% to 100% aq. MeCN containing 0.05 % TFA for 60 min, temperature: 25 °C, flow rate: 100 μL/min, detection: UV 254 nm (top), fluorescence λ_{ex} 337 nm and λ_{em} 409 nm (bottom). Collected area is shown in red. (b) MALDI mass spectrum of the purified peptide labeled with probe **4** (retention time: 23.5 min in (a)).

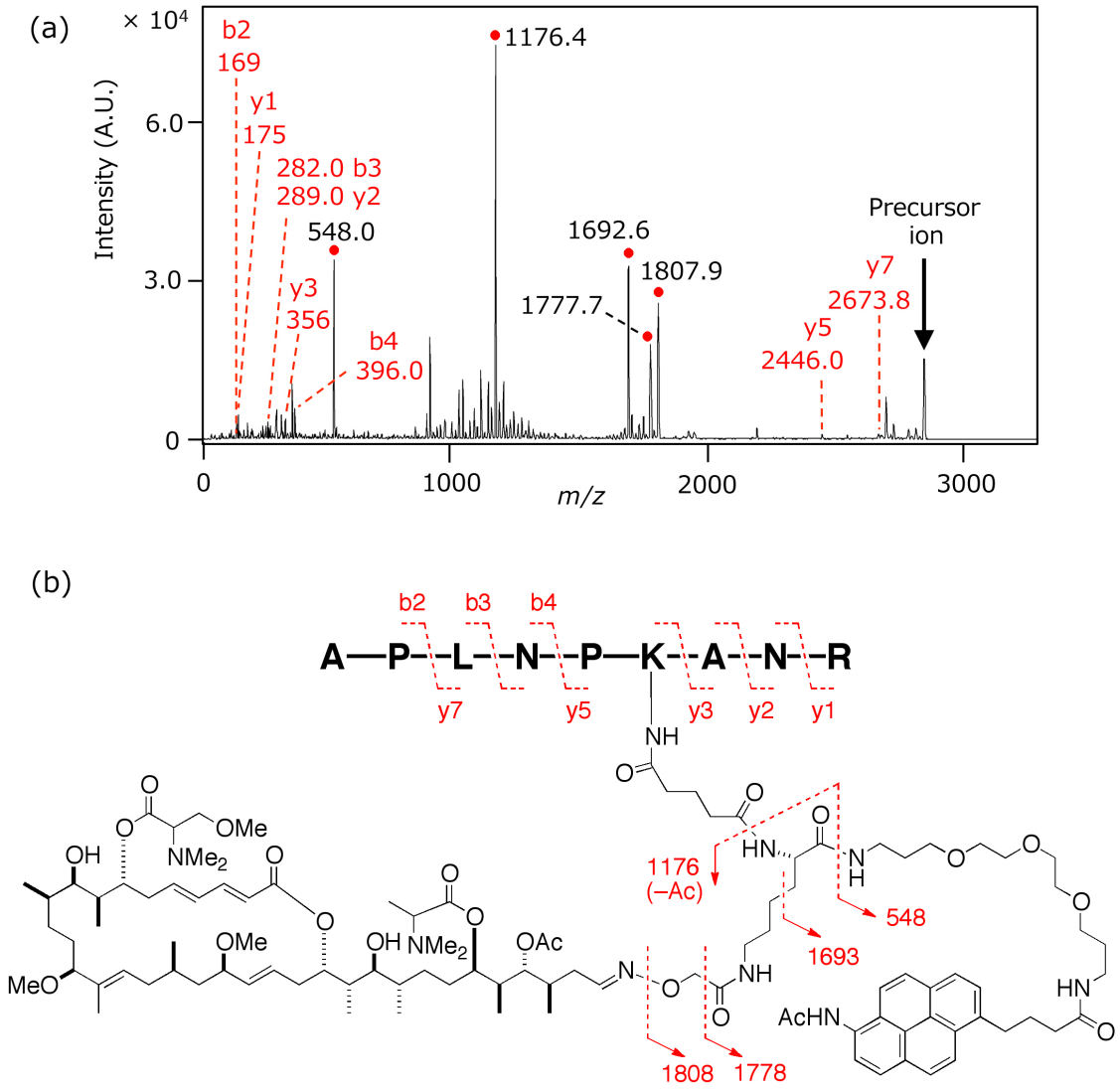


Figure S4. MALDI MS/MS analysis of the nonapeptide labeled with probe **4**. (a) MS/MS analysis of an amidopyrene-labeled peptide (precursor ion: *m/z* 2841.7, see Figure S3). Assigned b and y fragment ion peaks are shown in red. (b) Structure of an amidopyrene-labeled peptide (*m/z* 2841.7 for $[M+H]^+$).

Synthesis and spectroscopic data of amidopyrene derivatives.

Apy–OSu (1). A solution of *N*-hydroxysuccinimide (NHS) (7.5 mg, 65 μmol) and EDC·HCl (13.8 mg, 72.0 μmol) in dry DMF (0.5 mL) was added to carboxylic acid **6** [4] (18.1 mg, 52.4 μmol). After being stirred for 11.5 h, the resulting mixture was diluted with $CHCl_3$ (4 mL) and MeOH (0.5 mL), and washed with sat. NH_4Cl aq. (1 mL × 4) and brine (5 mL), dried with Na_2SO_4, and concentrated *in vacuo*. The crude material was purified by recrystalization ($CHCl_3$/MeOH/hexane) to give apy–OSu (**1**) (15.4 mg, 62%) as a colorless amorphous solid. Compound **1**: R_f = 0.56 ($CHCl_3$/acetone = 1/1); mp. 213.8–214.2 °C; ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.32 (s, 1H), 8.36 (d, *J* = 9.2 Hz, 1H), 8.27–8.24 (m, 3H), 8.22 (d, *J* = 7.8 Hz, 1H), 8.18 (d, *J* = 8.9 Hz, 1H), 8.17 (d, *J* = 8.9 Hz, 1H), 7.93 (d, *J* = 7.8 Hz, 1H), 3.42 (t, *J* = 7.9 Hz, 2H), 2.87 (t, *J* = 7.1 Hz, 2H), 2.85 (s, 4H), 2.28 (s, 3H), 2.12 (tt, *J* = 7.9, 7.1 Hz, 2H); ^{13}C NMR (150 MHz, DMSO-d_6) δ 170.4 (2C), 169.1, 169.0, 135.7, 131.9, 129.4, 128.4, 127.9, 127.7, 127.4, 127.1, 124.8, 124.7, 124.7, 124.4, 124.0, 123.4, 122.5, 121.6, 31.6, 30.2, 26.7, 25.5 (2C), 23.6; IR (KBr) 3250, 3041, 2983, 2945, 1671, 1645, 1602, 1546, 1531, 1496, 847, 722, 684 cm^{-1}; HRMS (ESI) *m/z* 465.1421 (calcd for $C_{26}H_{22}N_2NaO_5$ $[M+Na]^+$, Δ ±0.0 mmu).

Alkoxyamine 8. A solution of amide **7** [7c] (2.4 mg, 2.5 μmol) in a 1:1 mixture of dry CH_2Cl_2 and trifluoroacetic acid (1 mL) was stirred for 30 min at room temperature, and the resulting mixture was azeotropically concentrated with toluene *in vacuo* to give an amine TFA salt. This amine was dissolved in a 21 mM solution of hydrazine monohydrate in EtOH (2.0 mL, 42 μmol). After being stirred at room temperature for 30 min, the resulting mixture was azeotropically concentrated with toluene *in vacuo* to give alkoxyamine **8** (2.1 mg, quant. monitored by TLC analysis), which was immediately used for the next step without further purification. Compound **8**: R_f = 0.11 ($CHCl_3$/MeOH = 2/1).

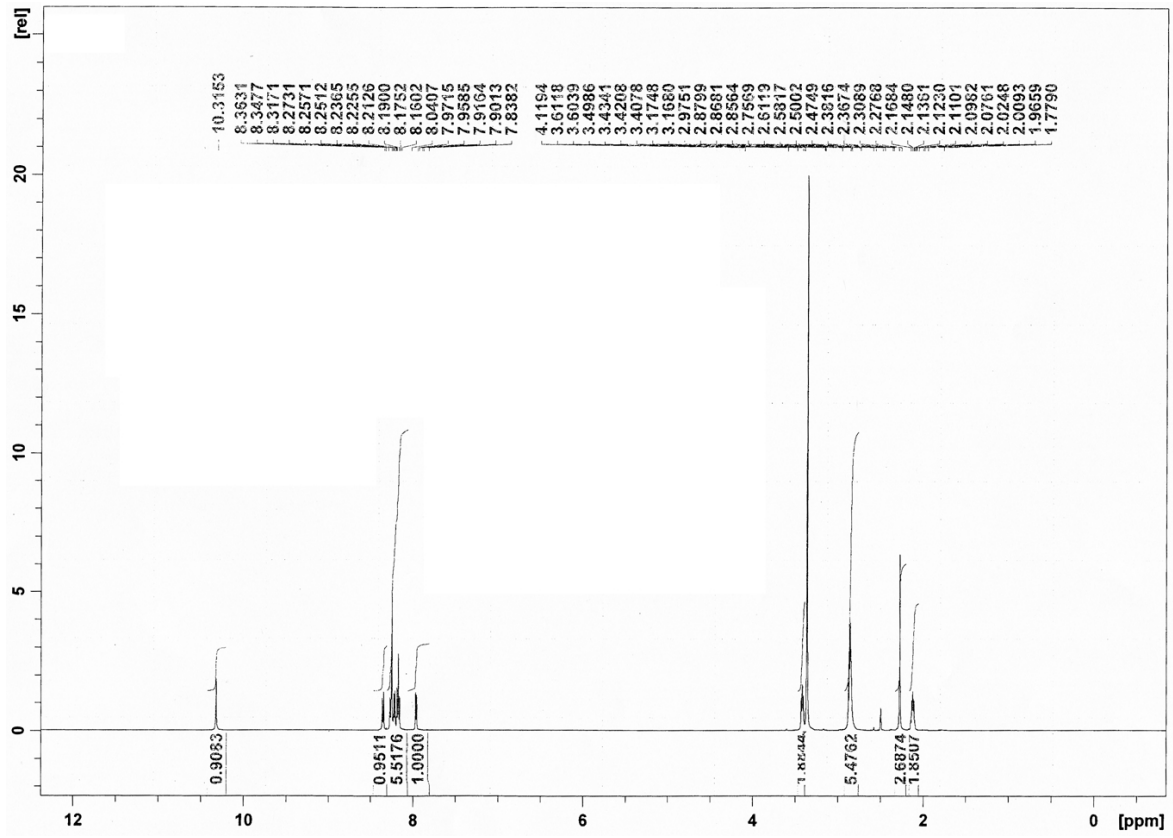
Amine 9. Alkoxyamine **8** (2.1 mg, 2.5 μmol) was added to a solution of aplyronine A C34 aldehyde [8], prepared from ApA (**2**) (466 μg, 433 nmol), in a 3:1 mixture of EtOH and 50 mM acetate buffer (pH 4.0) (0.4 mL). After being stirred at room temperature for 24.5 h, the reaction mixture was concentrated and applied to a Develosil ODS-HG-5 HPLC column (20 mm I.D. × 250 mm). Samples were eluted with MeOH / 20 mM NH_4OAc (80:20) aq. at a flow rate of 5 mL/min and with monitoring at 254 nm to give amine **9** (271 nmol, 63% in 2 steps, based on NMR quantification, t_R = 31–37 min, *E*/*Z* = 7/3 for the C34 stereoisomers). Desalting was conducted by the repeating lyophilization in water. Compound **9**: t_R = 9.0 min [Develosil ODS-HG-5 (4.6 mm I.D. × 250 mm), MeOH / 20 mM NH_4OAc aq. (80:20), 1 mL/min, λ_{ex} 337 nm, λ_{em} 409 nm]; ^{1}H NMR (600 MHz, CD_3OD) δ 8.35 (d, *J* = 9.3 Hz, 1H), 8.21 (d, *J* = 8.2 Hz, 1H), 8.17 (d, *J* = 9.0 Hz, 1H), 8.17 (d, *J* = 7.8 Hz, 1H), 8.15 (d, *J* = 9.3 Hz, 1H), 8.12 (d, *J* = 9.0 Hz, 1H), 8.12 (d, *J* = 8.2 Hz, 1H), 7.94 (d, *J* = 7.8 Hz, 1H), 7.55 [6.85]^1 (dd, *J* = 6.4, 6.4 Hz, 1H), 7.19 (dd, *J* = 15.1, 10.9 Hz, 1H), 6.35 (m, 1H), 6.21 (m, 1H), 5.96 (d, *J* = 15.1 Hz, 1H), 5.61 (ddd, *J* = 15.0, 10.7, 3.8 Hz, 1H), 5.54 (br d, *J* = 11.8 Hz, 1H), 5.09 (m, 1H), 4.97 (m, 1H), 4.88−4.77 (m, 2H), 4.67 (m, 1H), 4.40 [4.45]^1 (s, 2H), 3.98 (m, 1H), 3.68–3.64 (m, 2H), 3.56–3.48 (m, 7H), 3.50 (t, *J* = 6.0 Hz, 2H), 3.45 (m, 2H), 3.43–3.36 (m, 4H), 3.37 [3.38]^2 (s, 3H), 3.34–3.17 (m, 9H), 3.18 (s, 3H), 3.14 [3.14]^2 (s, 3H), 3.08 (dd, *J* = 9.2, 2.2 Hz,1H), 2.60–2.40 (m, 2H), 2.37 [2.38]^2 (s, 3H), 2.37 [2.38]^2 (s, 3H), 2.34 [2.32]^1 [2.34]^3 [2.33]^{1,3} (s, 6H), 2.38–2.22 (m, 5H), 2.19−2.13 (m, 3H), 2.06–1.96 (m, 4H), 2.04 [2.02]^1 [2.04]^3 [2.04]^{1,3} (s, 3H), 2.04 (s, 3H), 1.90−1.85 (m, 2H), 1.79–1.66 (m, 5H), 1.76 (tt, *J* = 6.5, 6.5 Hz, 2H), 1.69 (tt, *J* = 6.4, 6.4 Hz, 2H), 1.64–1.58 (m, 2H), 1.55–1.46 (m, 5H), 1.51 [1.52]^2 (s, 3H), 1.46–1.22 (m, 14H), 1.16–1.09 (m, 3H), 1.03 (d, *J* = 6.7 Hz, 3H), 1.00 (d, *J* = 6.2 Hz, 3H), 0.97 (d, *J* = 6.2 Hz, 3H), 0.91 [0.89]^2 (d, *J* = 7.0 Hz, 3H), 0.77−0.74 (m, 3H) Chemical shifts of the minor isomers are within parentheses as follows: []^1, 7:3 at C34 stereoisomers; []^2, 3:1 at C7 trimethylserine moiety; []^3, 1.4:1 at C29 dimethylalanine moiety; HRMS (ESI) *m/z* 883.5625 (calcd for

($C_{97}H_{154}N_8O_{21}$)/2 $[M+2H]^{2+}$, Δ +1.6 mmu).

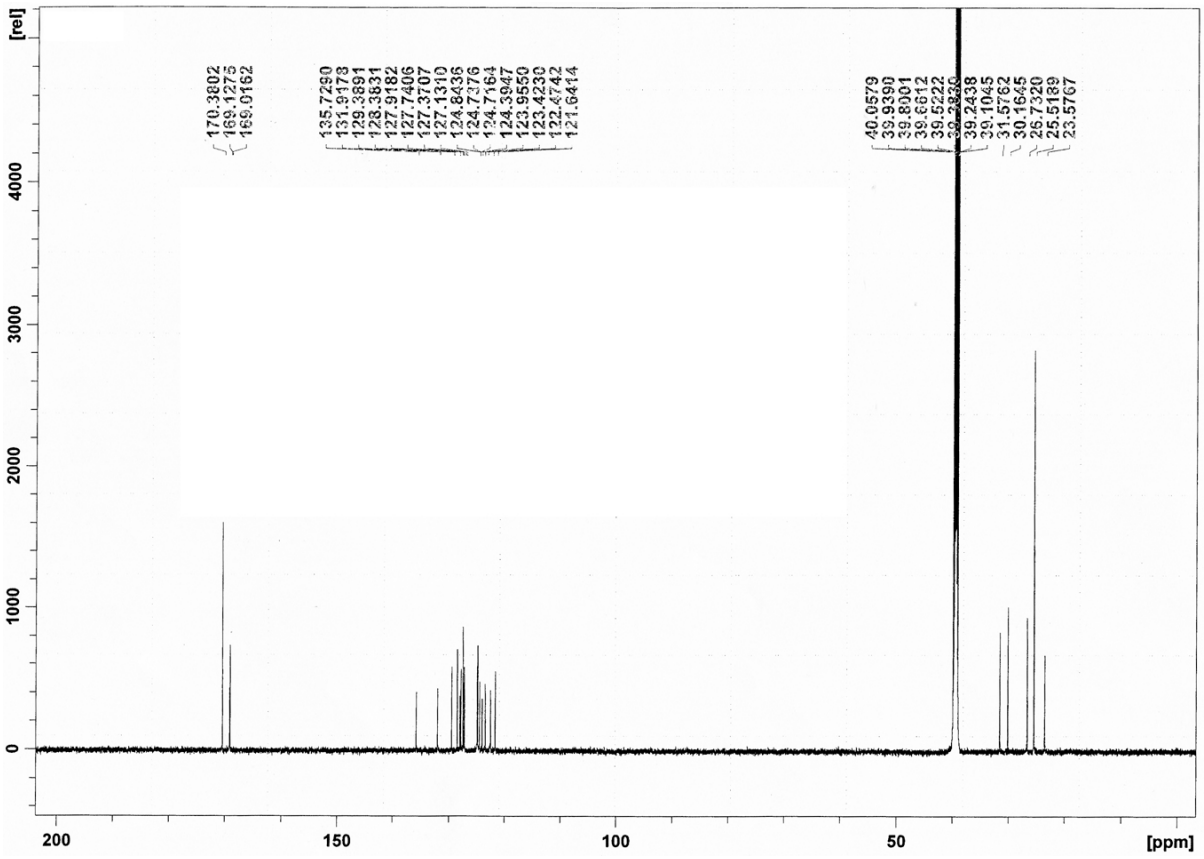
ApA-apy-OSu (4). To a solution of amine **9** (99 μg, 50 nmol) in dry CH_2Cl_2 (100 μL) were added 64 mM *N,N'*-disuccinimidyl glutarate solution in dry CH_2Cl_2 (1 μL, 64 nmol) and 54 mM triethylamine in dry CH_2Cl_2 (2 μL, 108 nmol). After being stirred at room temperature for 50 min, the reaction mixture was concentrated *in vacuo* to give ApA–apy–OSu (**4**) (quant., based on HPLC analysis), which was immediately used for the protein-labeling experiments without further purification. Compound **4**: t_R = 10.7 min [Develosil ODS-HG-5 (4.6 mm I.D. × 250 mm), MeCN / 20 mM NH_4OAc aq. (55:45), 1 mL/min, λ_{ex} 337 nm, λ_{em} 409 nm]; HRMS (ESI) *m/z* 989.0825 (calcd for $C_{106}H_{163}N_9O_{26}$ $[M+2H]^{2+}$, Δ +2.4 mmu).

Glycine adduct 5. To a 0.4 mM solution of glycine in water (50 μL, 20 nmol) was added 2 mM probe **4** in DMSO (1 μL, 2 nmol). After incubation for 25.5 h at room temperature, the resulting mixture was lyophilized and applied to a reversed-phase HPLC [Develosil ODS-HG-5 (4.6 mm I.D. × 250 mm), 1 mL/min, λ_{ex} 337 nm, λ_{em} 409 nm, MeCN / 20 mM NH_4OAc aq. (50:50), t_R = 5.4 min] to give glycine adduct **5** (68%, based on the fluorescence HPLC analysis) and carboxylic acid **11** (23%). Compound **5**: MS (MALDI) *m/z* 1959.2 $(M+Na)^+$.

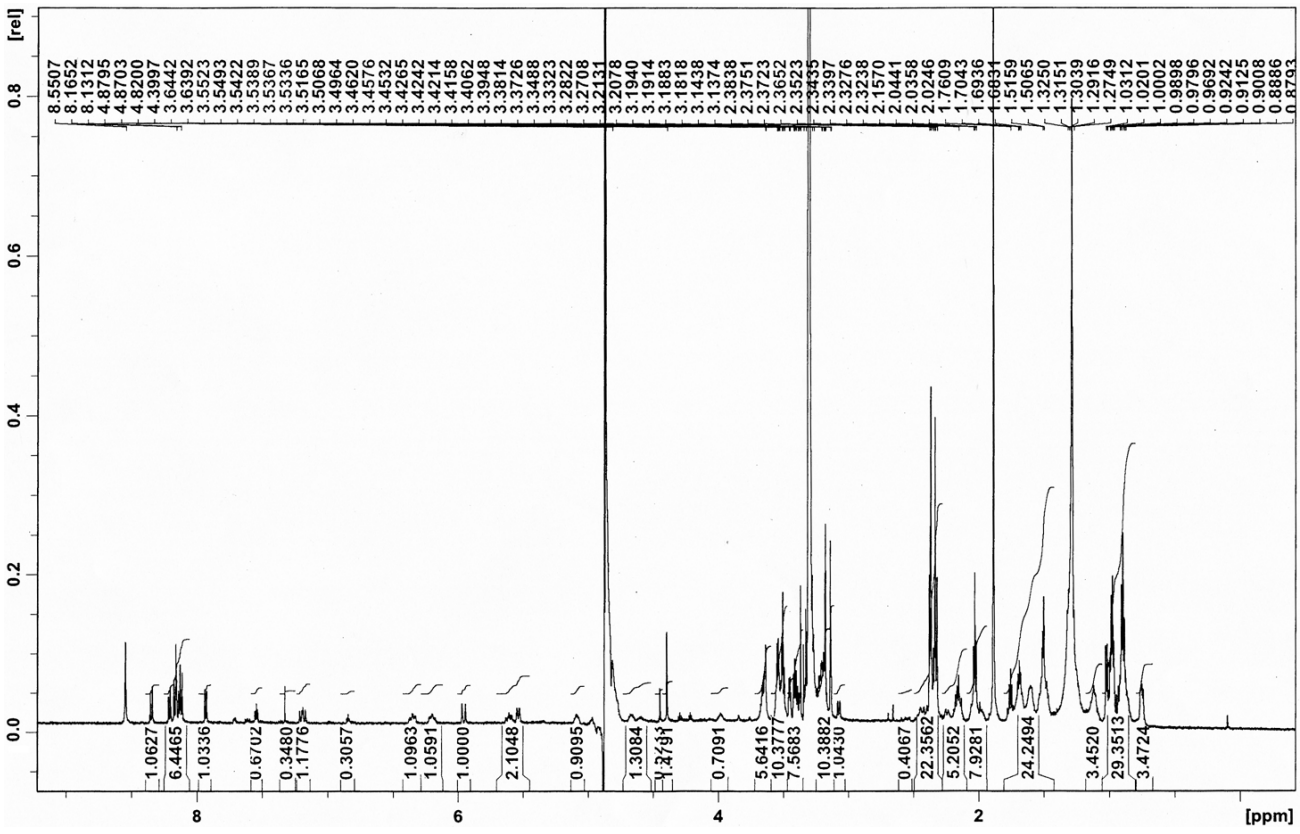
NMR charts



^{1}H NMR spectrum of Apy–OSu (**1**) (600 MHz, DMSO-d_6).



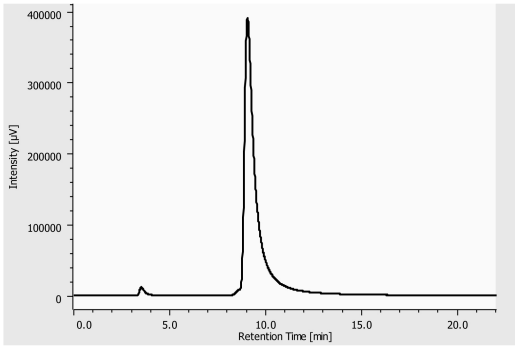
^{13}C NMR spectrum of Apy–OSu (**1**) (150 MHz, DMSO-d_6).



^{1}H NMR spectrum of amine **9** (600 MHz, CD_3OD).

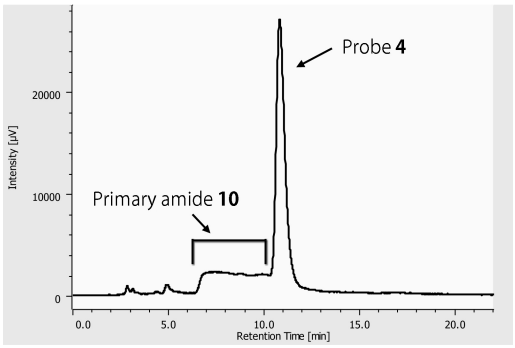
HPLC charts

Amine **9**

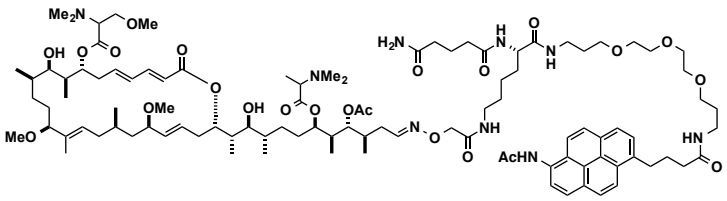


HPLC conditions: Column, Develosil ODS-HG-5 (4.6 mm I.D. × 250 mm); Eluate, MeOH / 20 mM NH_4OAc aq. = 80/20; Flow rate, 1 mL/min; Detection, fluorescence λ_{ex} 337 nm, λ_{em} 409 nm. Stereoisomers for the C34 oxime moiety in **9** were not separable. t_R = 9.0 min.

ApA-apy-OSu (**4**)

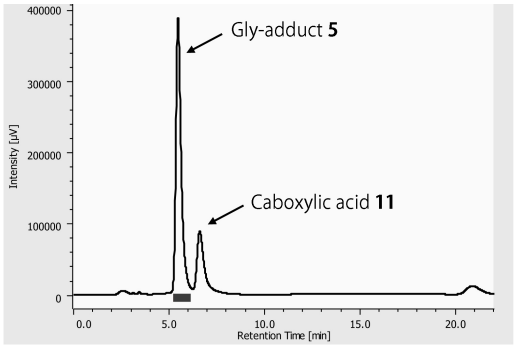


HPLC conditions: Column, Develosil ODS-HG-5 (4.6 mm I.D. × 250 mm); Eluate, MeCN / 20 mM NH_4OAc aq. = 55/45; Flow rate, 1 mL/min; Detection, fluorescence λ_{ex} 337 nm, λ_{em} 409 nm. Stereoisomers for the C34 oxime moiety in **9** were not separable. t_R = 10.7 min. ApA–apy–OSu (**4**) was unstable for the above HPLC condition, and primary amide **10** was formed during the HPLC analysis.

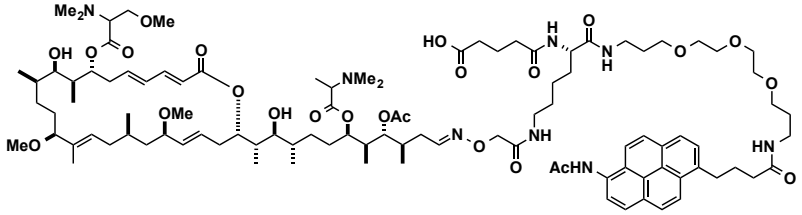


primary amide **10**

Glycine adduct **5**



HPLC conditions: Column, Develosil ODS-HG-5 (4.6 mm I.D. × 250 mm); Eluate, MeCN / 20 mM NH_4OAc aq. = 50/50; Flow rate, 1 mL/min; Detection, fluorescence λ_{ex} 337 nm, λ_{em} 409 nm. Stereoisomers for the C34 oxime moiety in **5** were not separable. t_R = 5.4 min. Carboxylic acid **11** (t_R = 6.8 min) was also formed by the hydrolysis of **4**.



carboxylic acid **11**