Supporting information

Weakly acidic pH-responsive liposomal content release induced by histidine-modified agents

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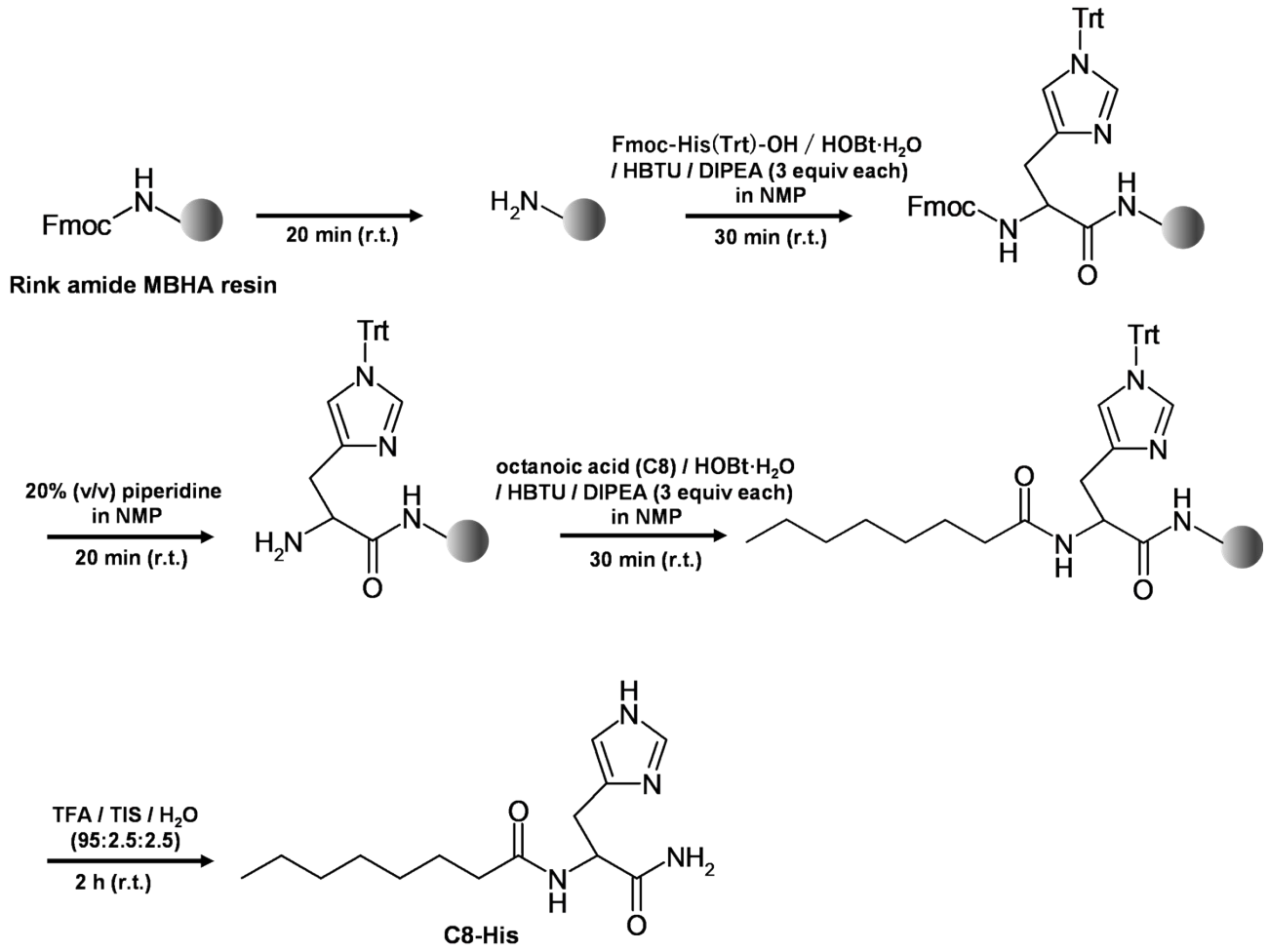
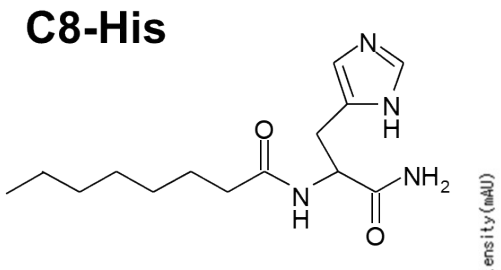
Contents:

Schematic representation of Cn-His solid phase synthesis (example for C8-His)

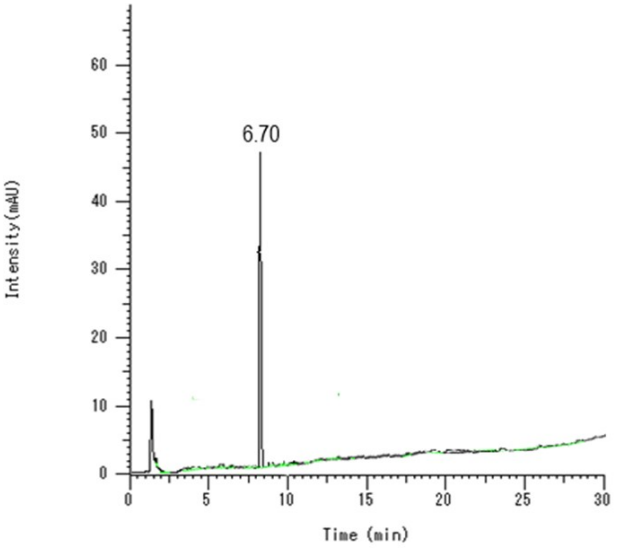


Identification of C8-His

HPLC chromatogram profile of purified C8-His

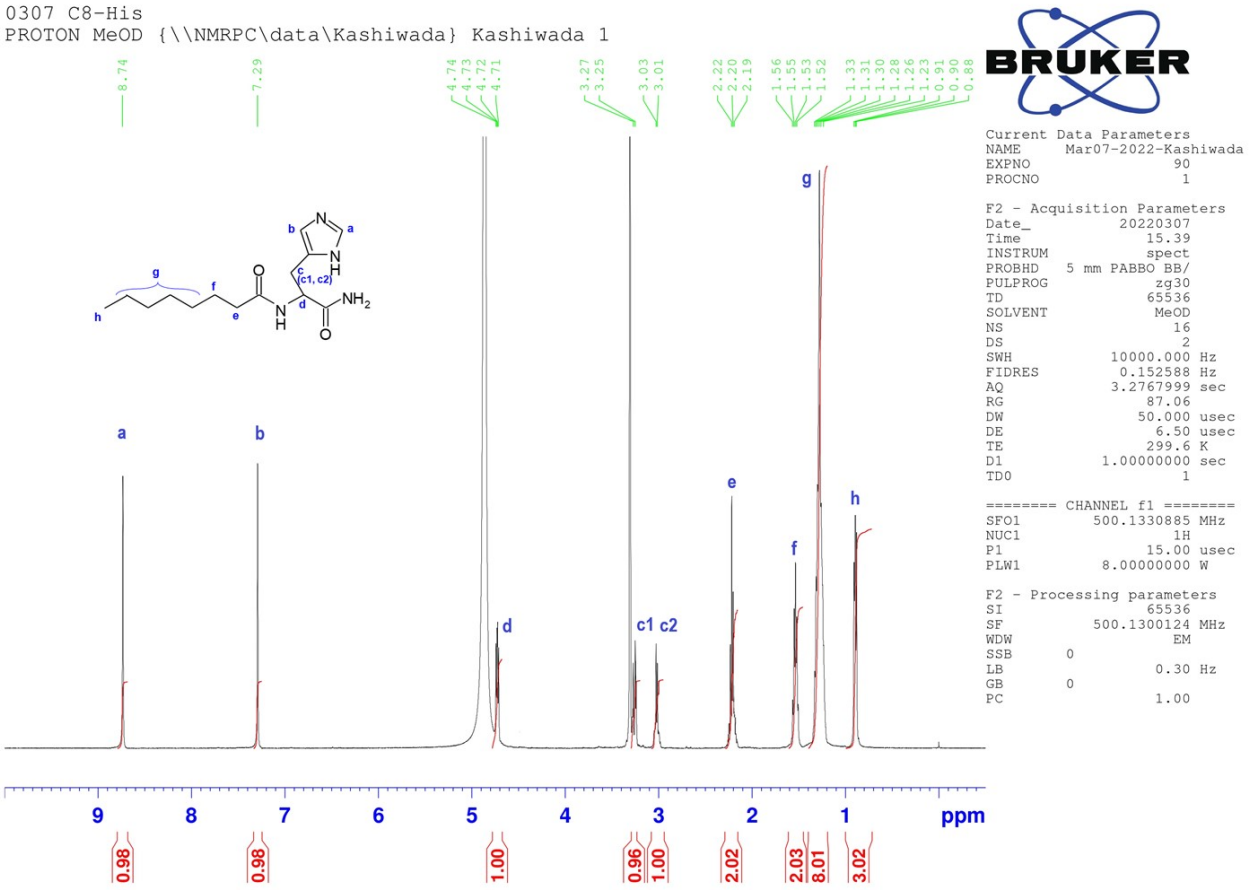


Column	Inertsil ODS-3 (5 µm, 250 × 4.6 mm i.d.)
Eluent	A: 0.1% TFA / H_2O B: 0.1% TFA / acetonitrile Linear gradient of 5–95% (v/v) eluent B over 30 min
Flow rate	1.0 mL/min
Temp.	25℃
Detection	UV at 230 nm



^{1}H NMR (500 MHz, MeOD, δ in ppm)

8.74 (1 H, s), 7.29 (1 H, s), 4.73 (1 H, m), 3.26 (1 H, d), 3.02 (1 H, d), 2.20 (2 H, t), 1.54 (2 H, q), 1.33 – 1.23 (8 H, m), 0.90 (3 H, t).

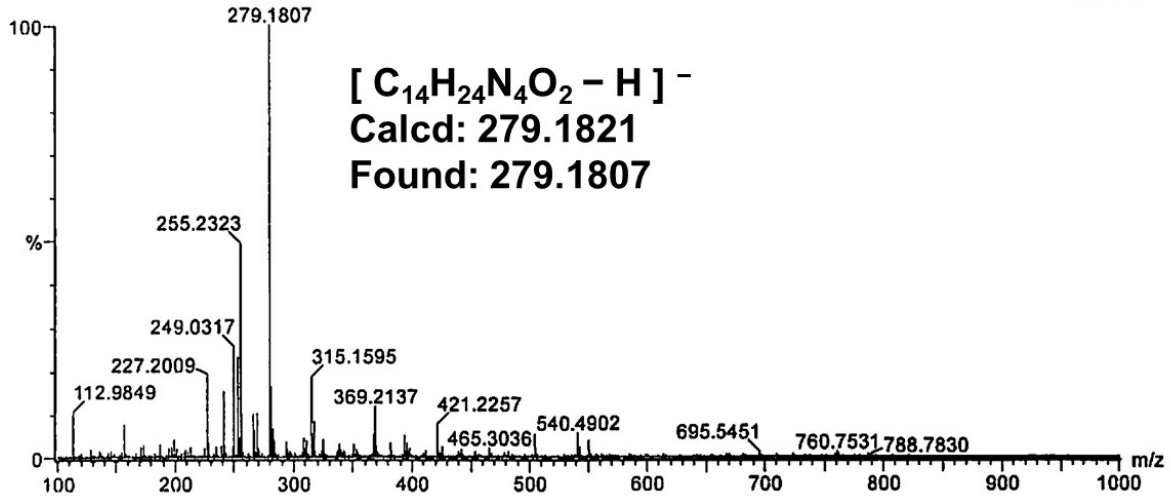


High-Resolution ESI-TOF MS

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

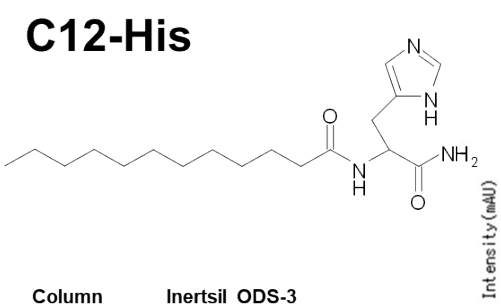
Monoisotopic Mass, Even Electron Ions
888 formula(e) evaluated with 8 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-500 H: 0-1000 N: 0-200 O: 0-200 Na: 0-1

25-Jan-2024 18:03:10
C8-His 2ppm nega
XEVO-G2SQTOF#YDA064
2024_0125_01 3 (0.129) AM2 (Ar,30000.0,0.00,0.00); Cm (2:15)
1: TOF MS ES-
6.88e+007

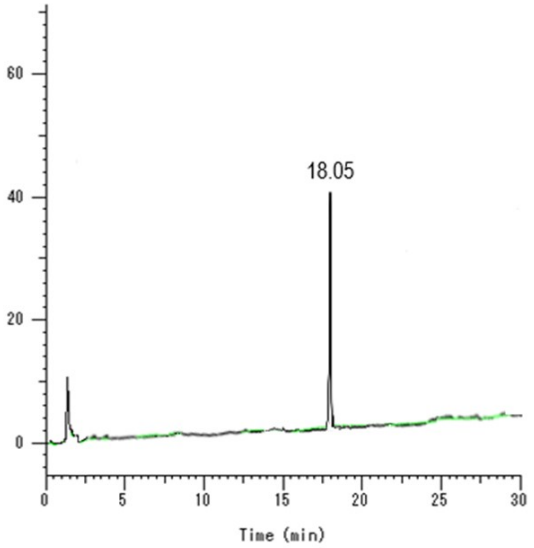


Identification of C12-His

HPLC chromatogram profile of purified C12-His

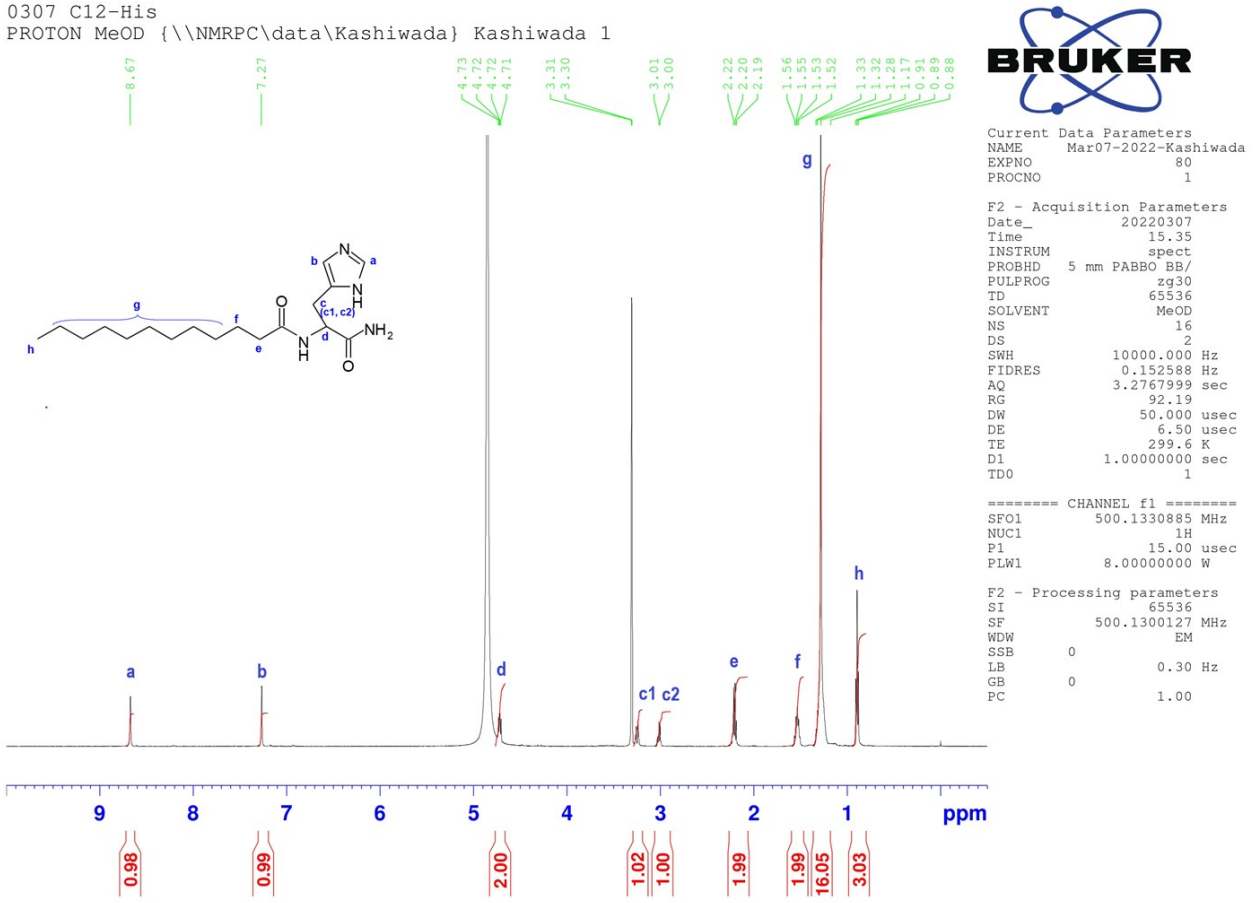


Column	Inertsil ODS-3 (5 μm, 250 × 4.6 mm i.d.)
Eluent	A: 0.1% TFA / H_2O
	B: 0.1% TFA / acetonitrile
	Linear gradient of 5–95% (v/v) eluent B over 30 min
Flow rate	1.0 mL/min
Temp.	25℃
Detection	UV at 230 nm



^{1}H NMR (500 MHz, MeOD, δ in ppm)

8.67 (1 H, s), 7.27 (1 H, s), 4.72 (1 H, m), 3.31 (1 H, d), 3.01 (1 H, d), 2.21 (2 H, t), 1.54 (2 H, q), 1.33 – 1.17 (16 H, m), 0.89 (3 H, t).

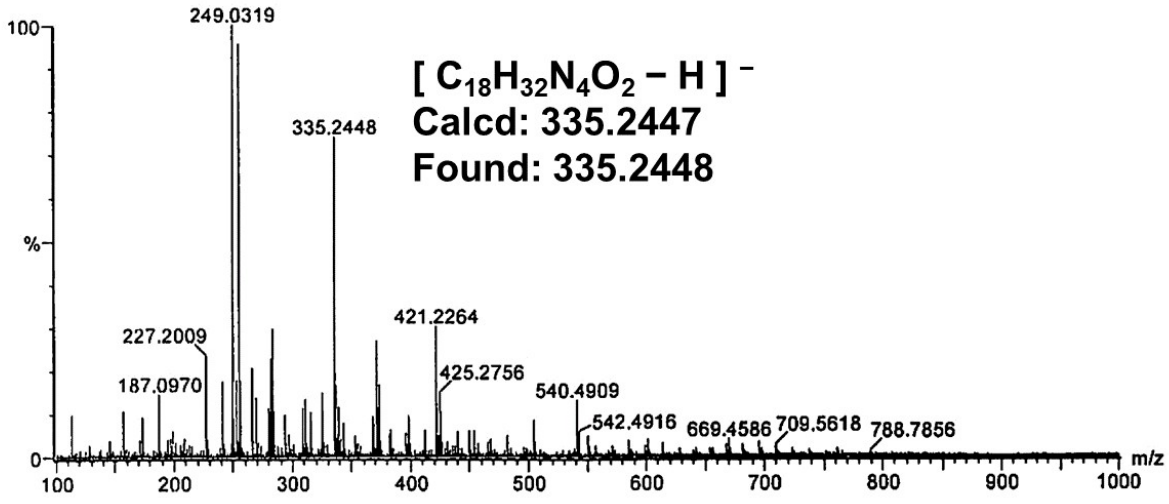


High-Resolution ESI-TOF MS

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

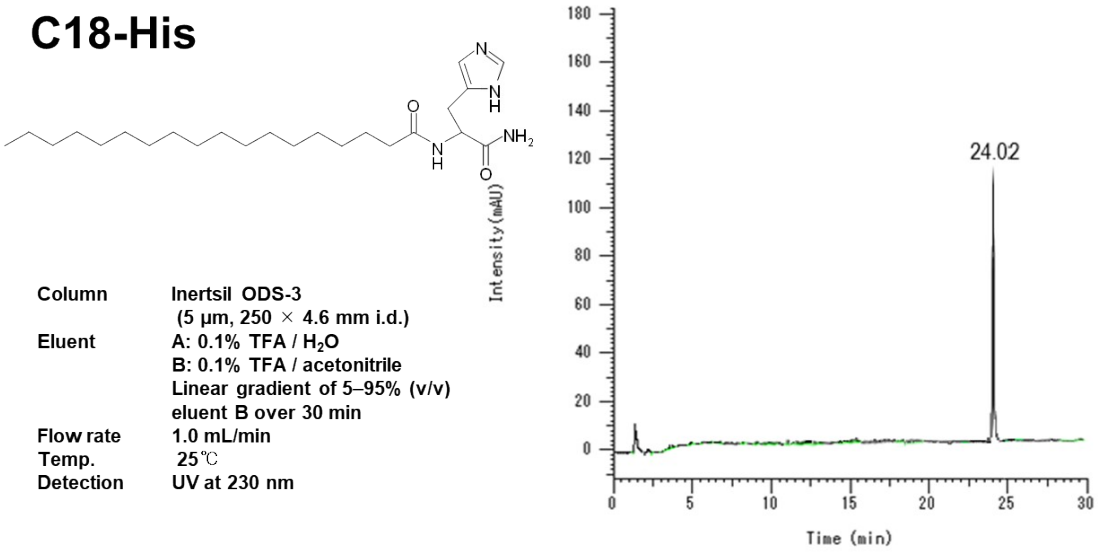
Monoisotopic Mass, Even Electron Ions
1423 formula(e) evaluated with 11 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-500 H: 0-1000 N: 0-200 O: 0-200 Na: 0-1

20-Jan-2024 15:18:03
C12-His 2ppm nega
XEVO-G2SQTOF#YDA064
2024_0120_05 4 (0.174) AM2 (Ar,30000.0,0.00,0.00); Cm (2:14)
1: TOF MS ES- 4.87e+007



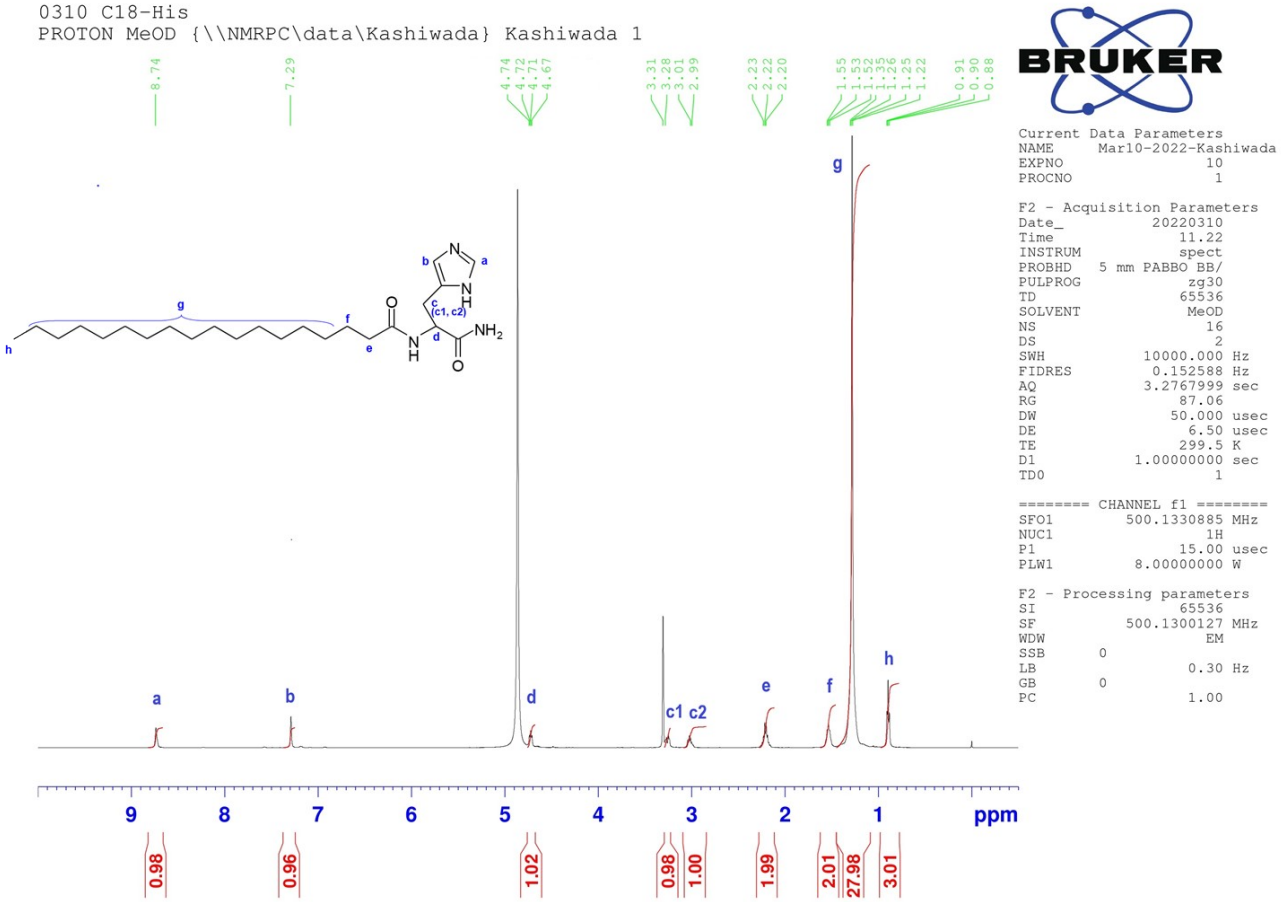
Identification of C18-His

HPLC chromatogram profile of purified C18-His



^{1}H NMR (500 MHz, MeOD, δ in ppm)

8.74 (1 H, s), 7.29 (1 H, s), 4.71 (1 H, m), 3.30 (1 H, d), 3.00 (1 H, d), 2.22 (2 H, t), 1.53 (2 H, q), 1.35 – 1.22 (28 H, m), 0.90 (3 H, t).



High-Resolution ESI-TOF MS

Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
3003 formula(e) evaluated with 16 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-500 H: 0-1000 N: 0-200 O: 0-200 Na: 0-1

30-Jan-2024 posi
C18-His 2ppm ~~nega~~ HFIP/AN/MeOH 10:04:50
XEVO-G2SQTOF#YDA064
2024_0130_02 7 (0.286) AM2 (Ar,30000.0,0.00,0.00); Cm (2:13)
1: TOF MS ES+
2.59e+008

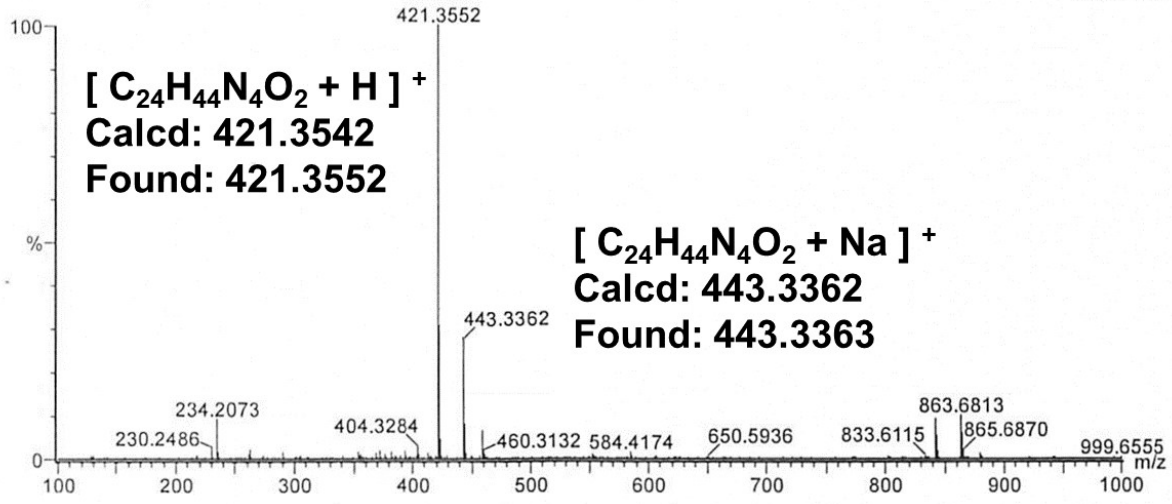


Table S1 Hydrodynamic diameter (Dh) from DLS measurements and ζ-potential values of bare DOPC liposomes and 1.0% of Cn-His incorporated liposomes

Liposome	pH	Dh / nm	PDI	ζ-potential / mV
DOPC (bare)	5.5	119.8 ± 9.6	0.101 ± 0.027	−0.48 ± 0.40
	6.0	121.4 ± 1.2	0.144 ± 0.018	−2.12 ± 0.81
	6.5	123.2 ± 0.9	0.102 ± 0.004	−3.32 ± 0.66
	7.0	121.7 ± 2.8	0.150 ± 0.031	−4.45 ± 0.77
	7.5	124.2 ± 1.0	0.164 ± 0.025	−5.60 ± 0.76
	8.0	121.4 ± 1.8	0.163 ± 0.025	−8.55 ± 0.46
C8-His / DOPC	5.5	122.6 ± 5.9	0.106 ± 0.043	+3.06 ± 0.72
	6.0	110.0 ± 2.7	0.155 ± 0.034	+0.42 ± 0.28
	6.5	113.8 ± 1.1	0.125 ± 0.022	−1.19 ± 0.39
	7.0	116.5 ± 1.0	0.062 ± 0.018	−2.47 ± 0.56
	7.5	116.6 ± 4.6	0.126 ± 0.022	−4.57 ± 0.57
	8.0	122.4 ± 8.7	0.138 ± 0.051	−8.28 ± 1.04
C12-His / DOPC	5.5	119.6 ± 1.5	0.088 ± 0.027	+4.83 ± 0.73
	6.0	116.3 ± 7.1	0.120 ± 0.034	+1.76 ± 0.85
	6.5	115.0 ± 1.2	0.152 ± 0.033	+0.16 ± 0.88
	7.0	113.9 ± 1.5	0.170 ± 0.023	−1.51 ± 0.48
	7.5	115.7 ± 1.0	0.150 ± 0.061	−2.79 ± 0.62
	8.0	115.3 ± 0.5	0.115 ± 0.006	−7.59 ± 0.84
C18-His / DOPC	5.5	110.4 ± 1.3	0.126 ± 0.040	+8.24 ± 0.84
	6.0	105.7 ± 1.7	0.098 ± 0.015	+5.43 ± 0.61
	6.5	108.8 ± 0.6	0.109 ± 0.041	+0.48 ± 0.62
	7.0	113.8 ± 2.1	0.122 ± 0.026	−0.75 ± 0.23
	7.5	113.4 ± 1.3	0.145 ± 0.037	−2.30 ± 0.33
	8.0	113.8 ± 3.2	0.133 ± 0.051	−6.24 ± 0.51

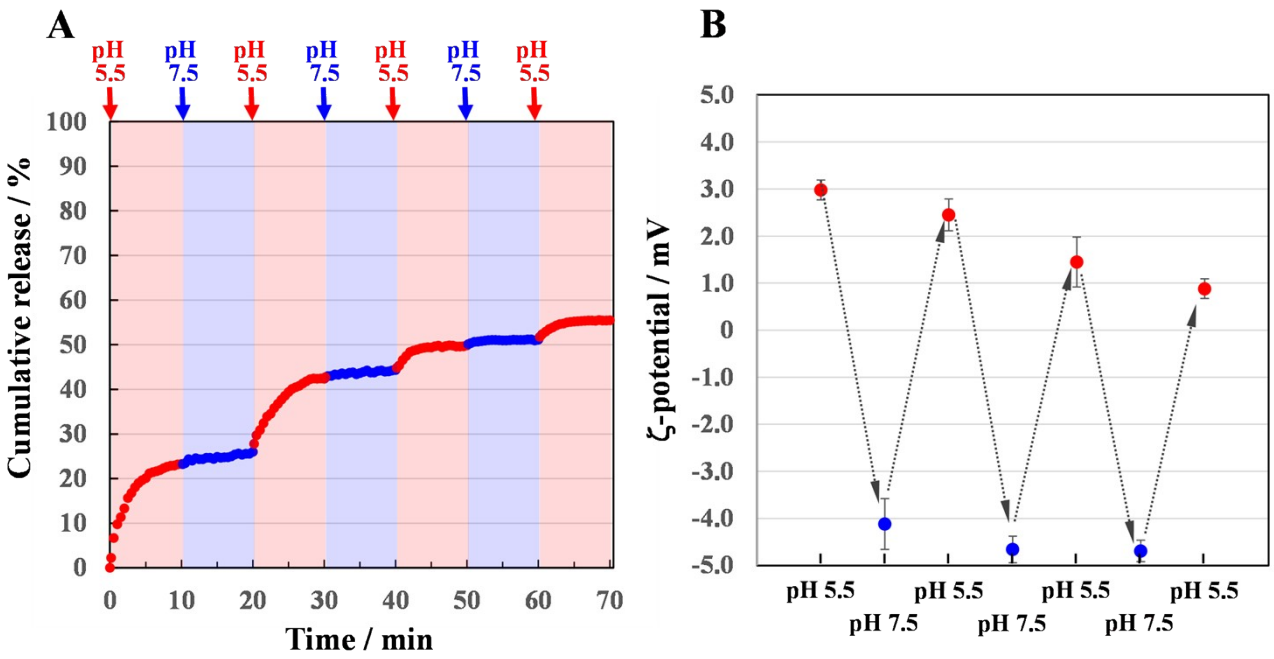


Fig. S1 A: Release profiles of CF from 1.0% of C8-His incorporated liposomes under intermittent pH between 5.5 and 7.5 (Red area: pH 5.5; Blue area: pH 7.5). B: The ζ-potential values of 1.0% of C8-His incorporated liposomes under intermittent pH between 5.5 and 7.5. Error bars indicate standard error over three measurements.