

Supporting information

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

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^cLaboratory of Molecular Chemistry, Faculty of Basic Pharmaceutical Sciences, School of Pharmacy, Nihon University, 7-7-1, Narashinodai, Funabashi, Chiba, 274-8555, Japan.

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HPLC chromatogram profile

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High-Resolution ESI-TOF MS

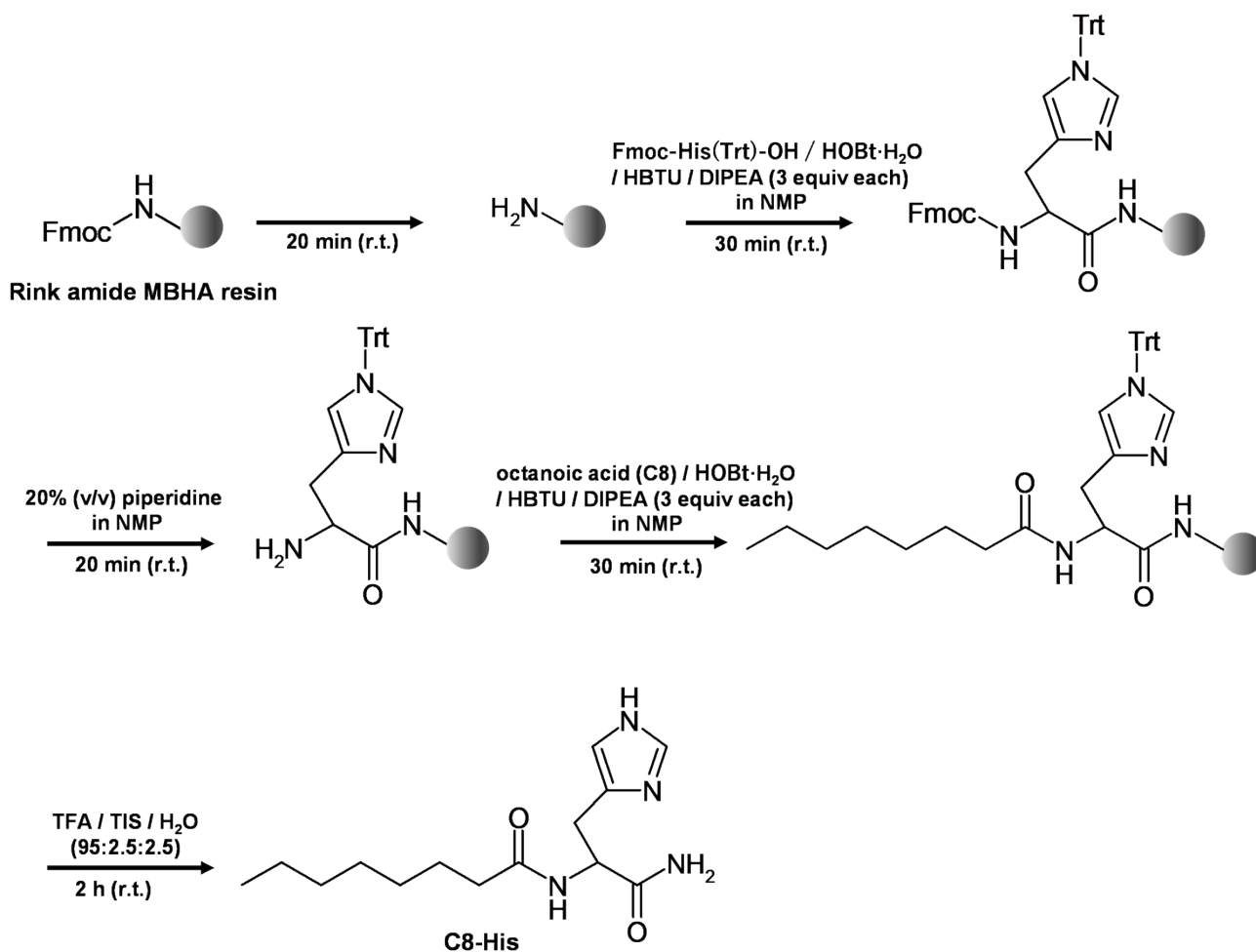
- Characterization of Cn-His incorporated liposomes

Hydrodynamic diameter (Dh) and ζ-potential values (Table S1)

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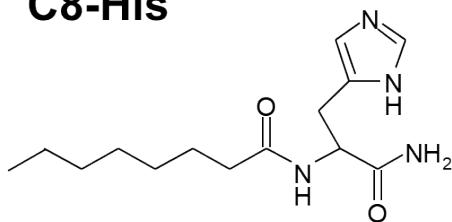
Schematic representation of Cn-His solid phase synthesis (example for C8-His)



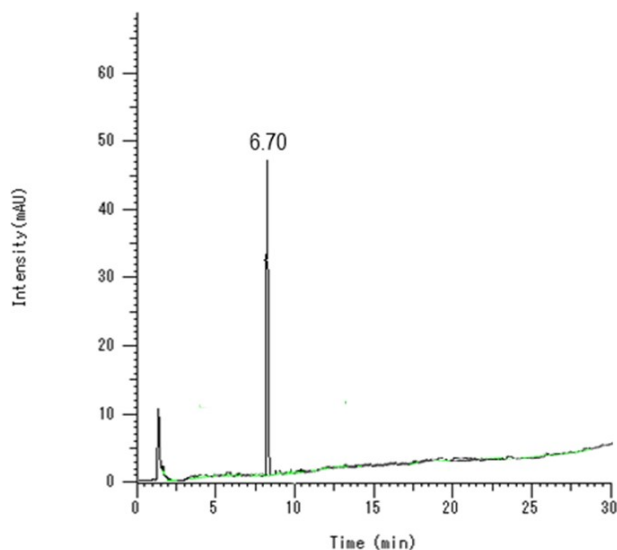
Identification of C8-His

HPLC chromatogram profile of purified C8-His

C8-His



Column Inertsil ODS-3
(5 μ m, 250 $\times$ 4.6 mm i.d.)
Eluent A: 0.1% TFA / H₂O
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°C
Detection UV at 230 nm

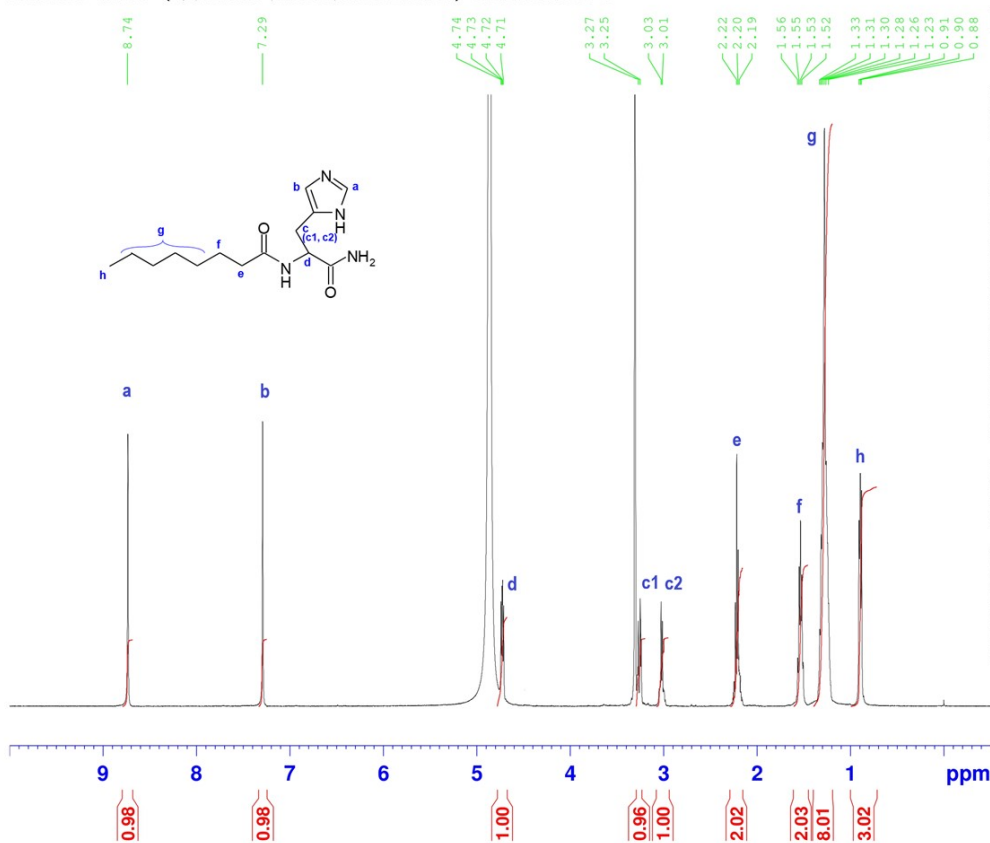


¹H NMR (500 MHz, MeOD, δ in ppm)

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).

0307 C8-His

PROTON MeOD {\\NMRPC\data\Kashiwada} Kashiwada 1



Current Data Parameters
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EXPNO 90
PROCNO 1

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PULPROG zg30
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DS 2
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FIDRES 0.152588 Hz
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RG 87.06
DW 50.000 usec
DE 6.50 usec
TE 299.6 K
D1 1.00000000 sec
TD0 1

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NUC1 1H
P1 15.00 usec
PLW1 8.00000000 W

F2 - Processing parameters
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SF 500.1300124 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

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

C8-His 2ppm nega

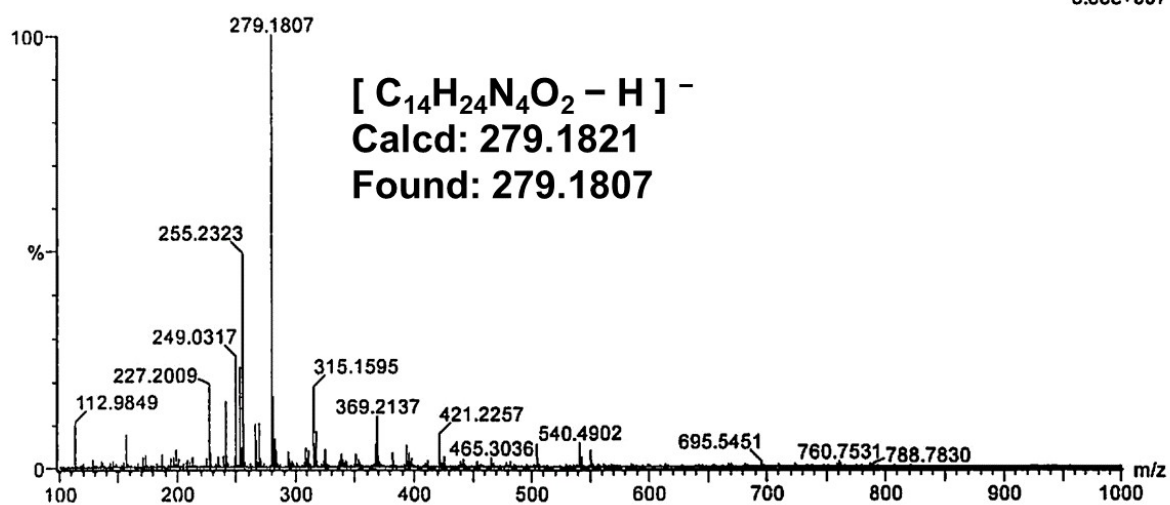
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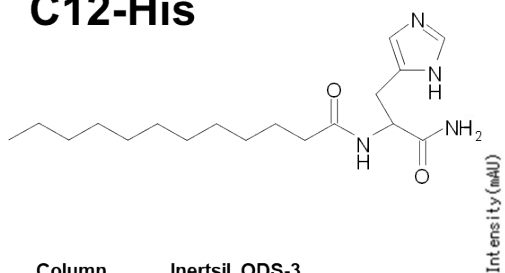
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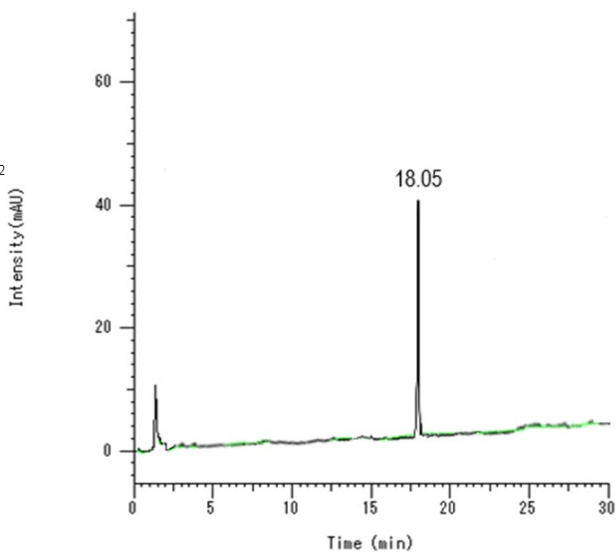
Identification of C12-His

HPLC chromatogram profile of purified C12-His

C12-His



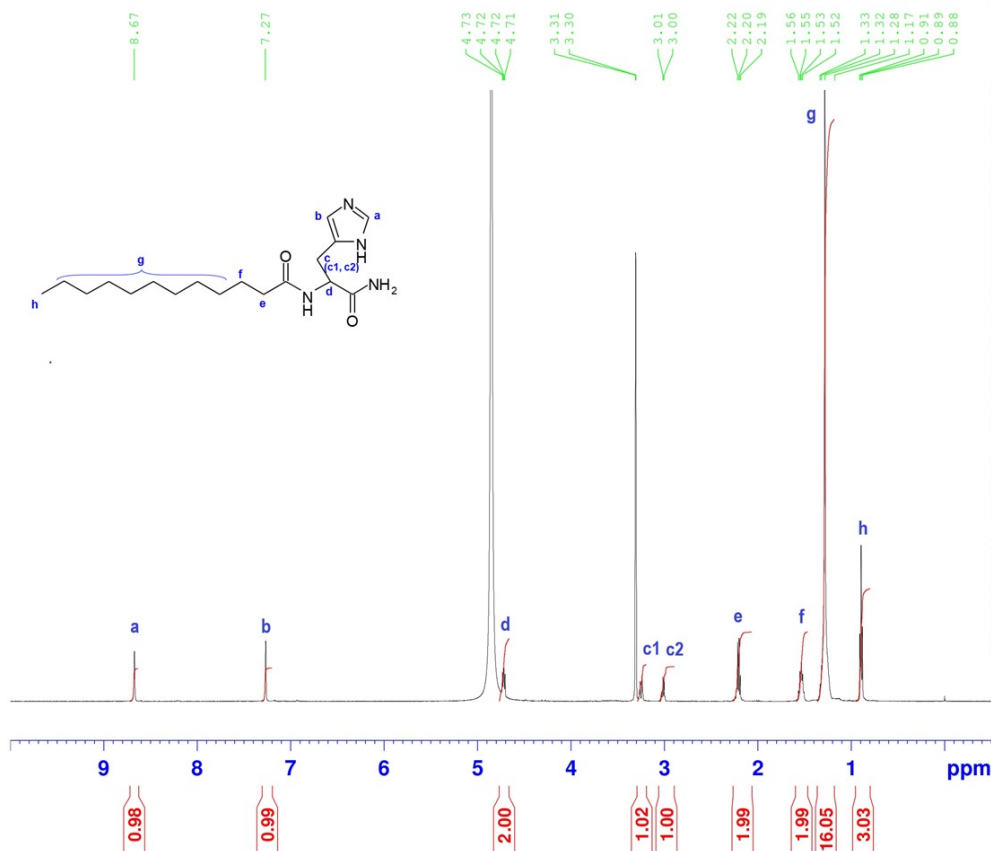
Column Inertsil ODS-3
(5 μ m, 250 $\times$ 4.6 mm i.d.)
Eluent A: 0.1% TFA / H₂O
B: 0.1% TFA / acetonitrile
Linear gradient of 5–95% (v/v)
eluent B over 30 min
Flow rate 1.0 mL/min
Temp. 25°C
Detection UV at 230 nm



¹H 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).

0307 C12-His
PROTON MeOD {\\NMRPC\data\Kashiwada} Kashiwada 1



Current Data Parameters
NAME Mar07-2022-Kashiwada
EXPNO 80
PROCNO 1

F2 - Acquisition Parameters
Date_ 20220307
Time 15.35
INSTRUM spect
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PULPROG zg30
TD 65536
SOLVENT MeOD
NS 16
DS 2
SWH 10000.000 Hz
FIDRES 0.152588 Hz
AQ 3.2767999 sec
RG 92.19
DW 50.000 usec
DE 6.50 usec
TE 299.6 K
D1 1.00000000 sec
TD0 1

===== CHANNEL f1 =====
SFO1 500.130885 MHz
NUC1 1H
P1 15.00 usec
PLW1 8.00000000 W

F2 - Processing parameters
SI 65536
SF 500.1300127 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

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

C12-His 2ppm nega

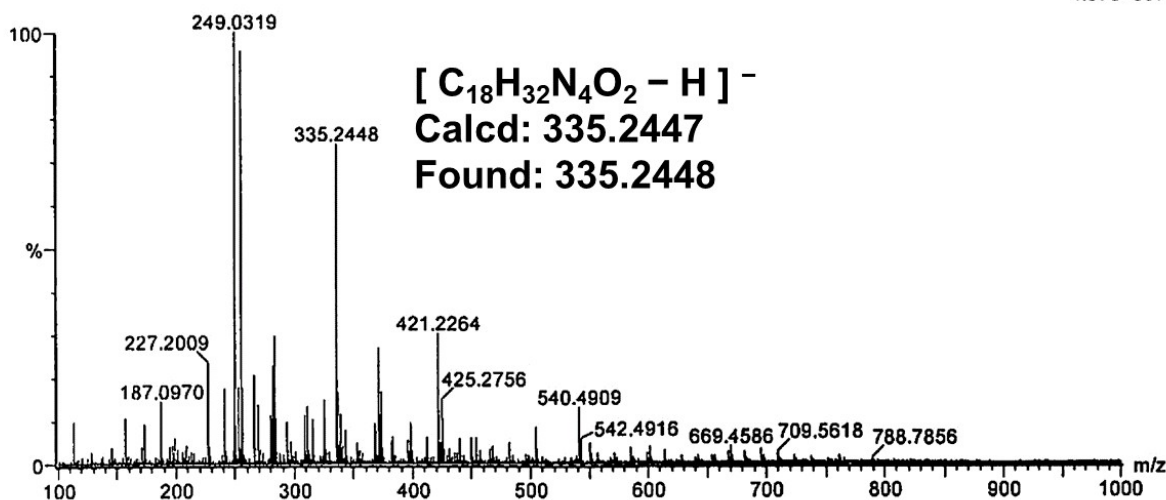
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15:18:03

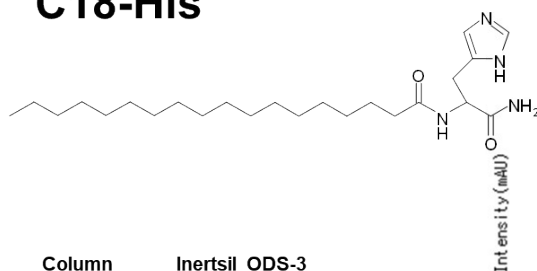
4.87e+007



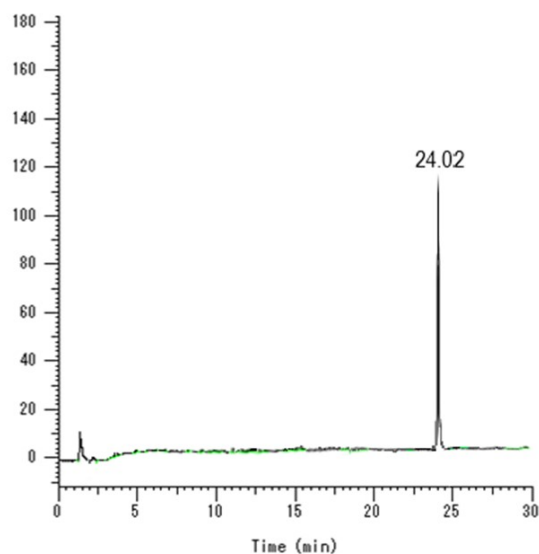
Identification of C18-His

HPLC chromatogram profile of purified C18-His

C18-His



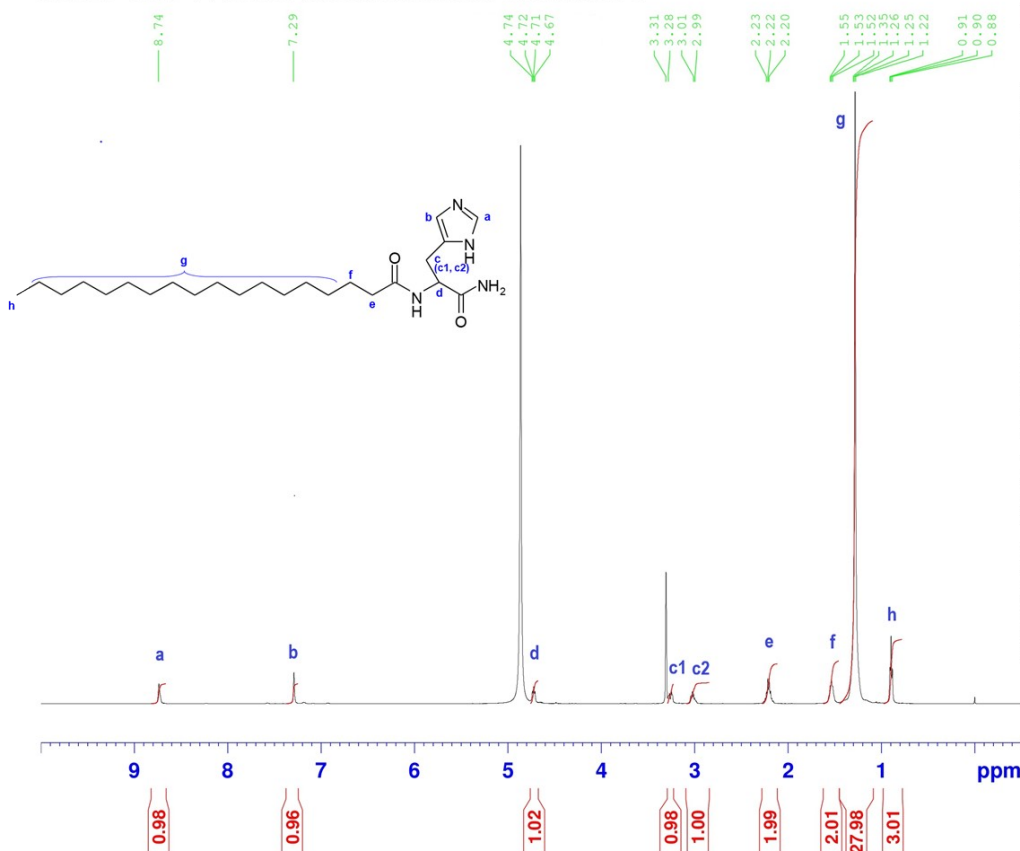
Column Inertsil ODS-3
(5 μ m, 250 $\times$ 4.6 mm i.d.)
Eluent A: 0.1% TFA / H₂O
B: 0.1% TFA / acetonitrile
Linear gradient of 5–95% (v/v)
eluent B over 30 min
Flow rate 1.0 mL/min
Temp. 25°C
Detection UV at 230 nm



¹H 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).

0310 C18-His
PROTON MeOD {\\NMRPC\data\Kashiwada} Kashiwada 1



Current Data Parameters
NAME Mar10-2022-Kashiwada
EXPNO 10
PROCNO 1

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Time 11.22
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PULPROG zg30
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NS 16
DS 2
SWH 10000.000 Hz
FIDRES 0.152588 Hz
AQ 3.2767999 sec
RG 87.06
DW 50.000 usec
DE 6.50 usec
TE 299.5 K
D1 1.00000000 sec
TD0 1

===== CHANNEL f1 =====
SFO1 500.1330885 MHz
NUC1 1H
P1 15.00 usec
PLW1 8.00000000 W

F2 - Processing parameters
SI 65536
SF 500.1300127 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

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

C18-His 2ppm nega HFIP/AN/MeOH

XEVO-G2SQTOF#YDA064

2024_0130_02 7 (0.286) AM2 (Ar,30000.0,0.00,0.00); Cm (2:13)

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2.59e+008

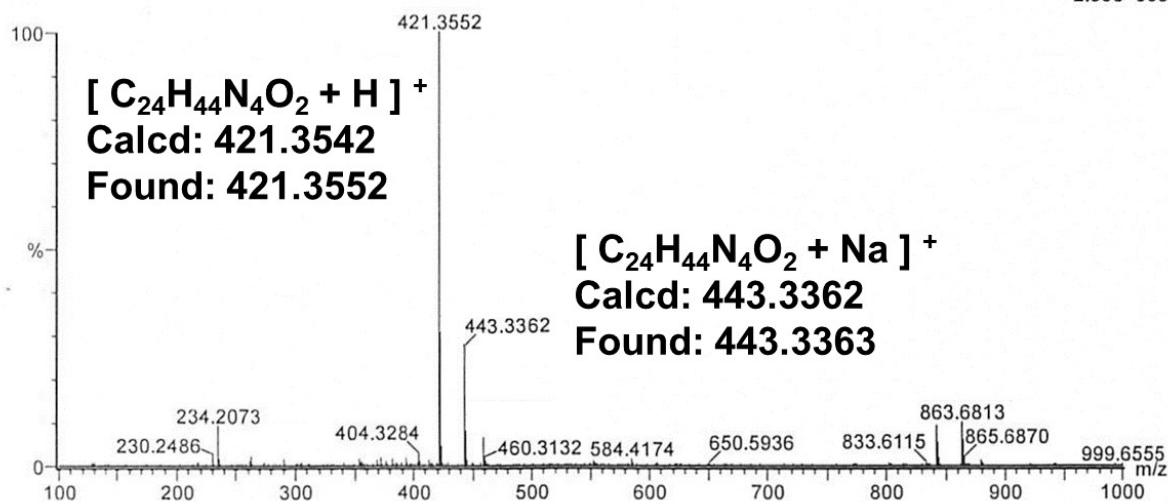


Table S1 Hydrodynamic diameter (Dh) from DLS measurements and ζ -potential values of bare DOPC liposomes and 1.0% of Cn-His incorporated liposomes

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

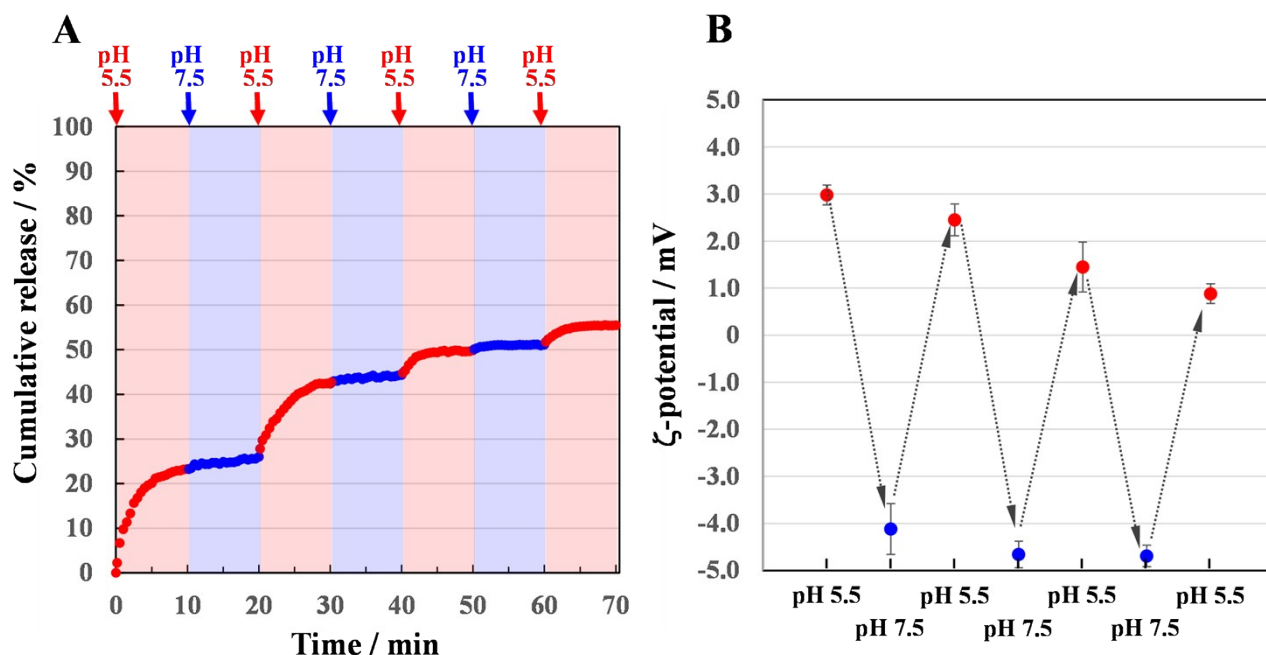


Fig. S1 A: Release profiles of CF from 1.0% of C8-His incorporated liposomes under intermittent pH between 5.5 and 7.5 (Red area: pH 5.5; Blue area: pH 7.5). B: The ζ -potential values of 1.0% of C8-His incorporated liposomes under intermittent pH between 5.5 and 7.5. Error bars indicate standard error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