Supporting Information

Pre-clinical efficacy and characterization of candidate vaccines for treatment of opioid use disorders using clinically viable carrier proteins

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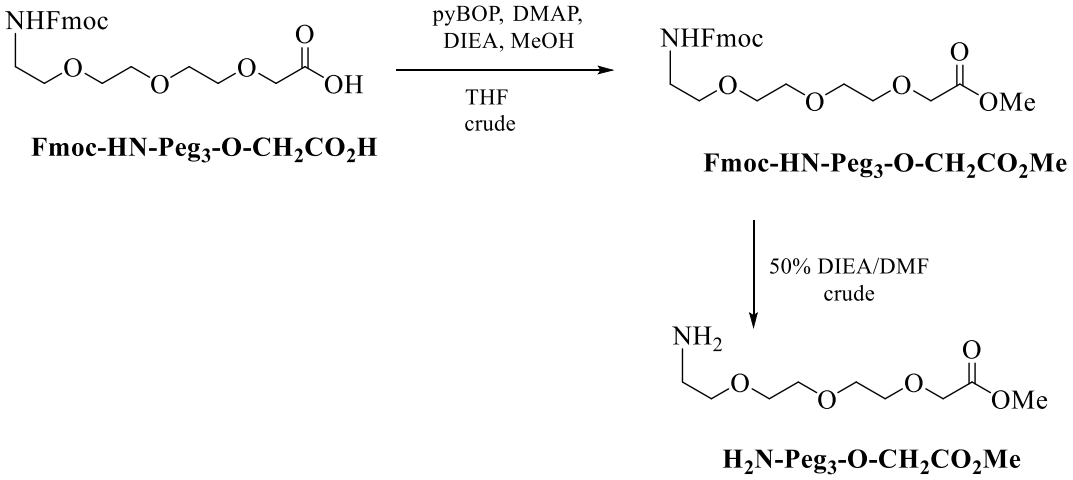
S1. Synthesis of Oxycodone-based hapten with PEG linker [OXY(PEG)$_3$]

Synthesis of Oxy-carboxymethoxyloxime-Peg$_3$OCH$_2$CO$_2$ Lithium salt (Oxy-Glyoxime-Peg$_3$-OCH$_2$CO$_2$Li, OXY(Peg)$_3$OH)

Scheme 1: Synthesis of H$_2$NPeg$_3$OGly-OMe

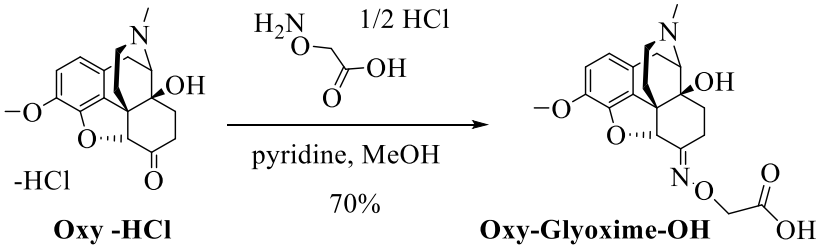


Fmoc-HN-Peg$_3$-O-CH$_2$CO$_2$H

Fmoc-HN-Peg$_3$-O-CH$_2$CO$_2$Me

H$_2$N-Peg$_3$-O-CH$_2$CO$_2$Me

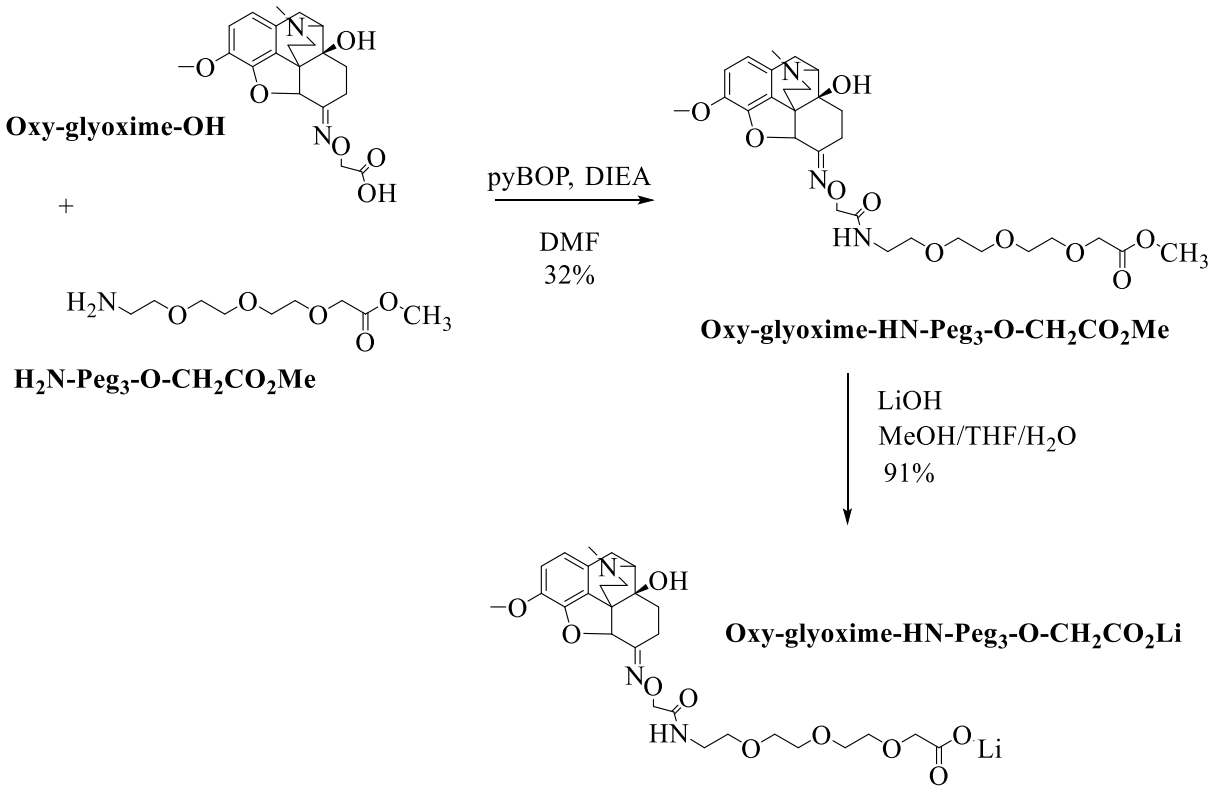
Scheme 2: Synthesis of Oxy-Glyoxime-OH



Oxy -HCl

Oxy-Glyoxime-OH

Scheme 3: Synthesis of Oxy-Glyoxime-NH-Peg$_3$-O-CH$_2$CO$_2$Li



Experimental details:

Chemistry General: All standard reagents were commercially available. Fmoc-HN-Peg$_3$-O-CH$_2$CO$_2$H was purchased from ChemPep Inc. (Wellington, Florida). Intermediates were purified by column chromatography on a Teledyne ISCO Rf chromatography unit unless otherwise indicated. The purity of intermediates and final compounds was determined using an Agilent-Varian HPLC system equipped with Prostar 210 dual pumps, a Prostar 335 Diode UV detector and a SEDEX75 (SEDERE, Olivet, France) ELSD detector and an analytical Synergy Hydro® RP80A C18 (4 µm particle size, 250 x 4.60 mm column; Phenomenex) with a linear gradient of 5%-95% solvent B over 20 min at a flow rate of 1 mL/min. Absorbance was monitored at 220 nm. The HPLC solvent system was binary: water containing 0.1% trifluoroacetic acid (TFA) and solvent B (acetonitrile containing 5% water and 0.1% TFA). The molecular ion of intermediates and final compounds was determined using a PE Sciex API 150 EX LC/MS system from Perkin Elmer (San Jose, California). Reactions were monitored by thin-layer

chromatography (TLC) carried out on pre-coated 60 Å 250 mm silica gel TLC plates with F-254 indicator visualized under UV light. ^{1}H NMR spectra were recorded at 300 MHz on a Bruker Avance 300 Spectrospin instrument and are reported as follows: chemical shift δ in ppm (multiplicity, coupling constant (Hz), and integration. The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, br = broad, dd = doublet of doublets.

Methyl 1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13-tetraoxa-4-azapentadecan-15-oate, Fmoc-HN-Peg$_3$-O-CH$_2$CO$_2$Me: To a solution of 9H-fluoren-9-yl)-3-oxo-2,7,10,13-tetraoxa-4-azapentadecan-15-oic acid (**Fmoc-HN- Peg$_3$-O-CH$_2$CO$_2$H**, 500 g, 1.16 mmol) in methanol/THF (1:5, 12 mL) was added pyBOP (905 mg, 1.74 mmol), DIEA (808 uL, 4.64 mmol), and DMAP (14 mg, 0.12 mmol). The reaction mixture was stirred at room temperature for 12 hr. The mixture was concentrated *in vacuo* to provide methyl 1-(9H-fluoren-9-yl)-3-oxo-2,7,10,13-tetraoxa-4-azapentadecan-15-oate (**Fmoc-HN-Peg$_3$-O- CH$_2$CO$_2$Me**, 514 mg, quant.) as a crude mixture. 1H NMR (300 MHz, DMSO-d6) δ ppm 3.24 - 3.59 (m, 13 H) 3.63 (s, 3 H) 4.12 (s, 1 H) 4.18 - 4.24 (m, 1 H) 4.28 (s, 1 H) 4.36 (s, 1 H) 7.25 - 7.46 (m, 5 H) 7.59 (d, J=8.29 Hz, 1 H) 7.69 (d, J=6.97 Hz, 1 H) 7.82 - 7.93 (m, 2 H); ESI MS m/z: calculated for $C_{24}H_{29}NO_7$ 443.5, found 466.7 (M+Na)+.

Methyl {2-[2-(2-aminoethoxy)ethoxy]ethoxy}acetate, H$_2$N-Peg$_3$-O- CH$_2$CO$_2$Me: To a solution of crude methyl 1-(9*H*-fluoren-9-yl)-3-oxo-2,7,10,13-tetraoxa-4-azapentadecan-15-oate (**Fmoc-HN-Peg$_3$-O-CH$_2$CO$_2$Me**) in methanol (15 mL) was added piperidine (3 mL), and the reaction mixture stirred at room temperature for 6 hr. The reaction mixture was concentrated and dried *in vacuo* to provide methyl {2-[2-(2-aminoethoxy)ethoxy]ethoxy}acetate (**H$_2$N-Peg$_3$-O-CH$_2$CO$_2$Me**) as a crude mixture. ESI MS *m/z*: calculated for $C_9H_{19}NO_5$ 221.2, found 222.2 $(M+H)^+$.

({[(6*E*)-14-hydroxy-3-methoxy-17-methyl-4,5-epoxymorphinan-6-ylidene]amino}oxy)acetic acid, Oxy-Glyoxime-OH: To a solution of 14-hydroxy-3-methoxy-17-methyl-4,5-epoxymorphinan-6-one hydrochloride salt (**Oxycodone HCl**, 2.0 g, 6.34 mmol) in MeOH (10 mL) was added 1M NaOH (6.6 mL, 1.05 equiv). The reaction mixture was stirred for 15 min, then concentrated *in vacuo*, and dried *in vacuo* for 2 h. The residue was re-dissolved in anhydrous MeOH (200 mL) and carboxylmethoxylamine (1.04 mg, 9.5 mmol) and pyridine (1.52 mL, 3.0 equiv) were added. The mixture was stirred at reflux temperature for 16 hr, then cooled to room temperature and concentrated *in vacuo*. The residue was re-dissolved in acetone (10 mL) and precipitated with ether (120 mL). The precipitate was collected, re-precipitated and dried *in vacuo* to provide ({[(6*E*)-14-hydroxy-3-methoxy-17-methyl-4,5-epoxymorphinan-6-ylidene]amino}oxy)acetic acid (**Oxy-Glyoxime-OH**) as a white powder (2.6 g, 70% yield). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.08 - 1.25 (m, 2 H) 1.35 - 1.62 (m, 3 H) 1.81 (d, *J*=9.23 Hz, 1 H) 2.30 (br. s., 2 H) 2.41 - 2.46 (m, 3 H) 2.63 (br. s., 2 H) 3.05 (br. s., 2 H) 3.14 - 3.23 (m, 1 H) 3.71 - 3.80 (m, 3 H) 4.55 (s, 2 H) 4.94 (br. s., 1 H) 6.69 (d, *J*=7.72 Hz, 1 H) 6.81 (d, *J*=8.10 Hz, 1 H); ESI MS *m/z*: calculated for $C_{20}H_{24}N_2O_6$ 388.4, found 389.7 $(M+H)^+$; HPLC (Synergy Hydro, 20 min) t_R =11.32 min (>99%).

Methyl 14-({[(6E)-14-hydroxy-3-methoxy-17-methyl-4,5-epoxymorphinan-6-ylidene]amino}oxy)-13-oxo-3,6,9-trioxa-12-azatetradecan-1-oate, Oxy-glyoxime-HN-Peg3-O- CH_2CO_2Me: To a clear solution of ({[(6*E*)-14-hydroxy-3-methoxy-17-methyl-4,5-epoxymorphinan-6-ylidene]amino}oxy)acetic acid (**Oxy-glyoxime-OH**, 186 mg, 0.48 mmol) in DMF (20 mL) was added pyBOP (328 mg, 0.63 mmol) and methyl {2-[2-(2-aminoethoxy)ethoxy]ethoxy}acetate (**H_2N-Peg$_3$-O-CH_2CO_2Me**) (128 mg, 0.58 mmol), then DIEA (418 µL, 2.4 mmol). The reaction mixture was stirred at room temperature for 6 hr under nitrogen atmosphere, then concentrated to dryness *in vacuo*. The residue was purified by column chromatography [20 g SiO_2, eluent DCM 100% to 50% CMA 80 (Chloroform/MeOH/NH_4OH 80/19.8/0.2)] to provide methyl 14-({[(6E)-14-hydroxy-3-methoxy-17-methyl-4,5-epoxymorphinan-6-ylidene]amino}oxy)-13-oxo-3,6,9-trioxa-12-azatetradecan-1-oate (**Oxy-glyoxime-HN-Peg$_3$-O-Gly-**

OMe) as a white powder (94 mg. 32%). ESI MS *m/z*: calculated for $C_{29}H_{41}N_3O_{10}$ 591.7, found 592.2 $(M+H)^+$; HPLC (Synergy Hydro, 20 min) t_R =12.37 min (95%).

Lithium14-({[(6E)-14-hydroxy-3-methoxy-17-methyl-4,5-epoxymorphinan-6-ylidene]amino}oxy)-13-oxo-3,6,9-trioxa-12-azatetradecan-1-oate, Oxy-glyoxime-HN-Peg$_3$-O-CH$_2$CO$_2$Li: To a solution of methyl 14-({[(6E)-14-hydroxy-3-methoxy-17-methyl-4,5-epoxymorphinan-6-ylidene]amino}oxy)-13-oxo-3,6,9-trioxa-12-azatetradecan-1-oate (**Oxy-glyoxime-HN-Peg$_3$-O-CH$_2$CO$_2$Me**, 32 mg, 0.054 mmol) in MeOH/THF (1:1, 4 mL) was added H_2O (0.5 mL), then LiOH (1.3 mg, 0.054 mmol). The reaction mixture was stirred at room temperature for 6 hr and concentrated to dryness *in vacuo*. The residue was re-dissolved in minimal MeOH and precipitated with Et_2O to provide lithium14-({[(6E)-14-hydroxy-3-methoxy-17-methyl-4,5-epoxymorphinan-6-ylidene]amino}oxy)-13-oxo-3,6,9-trioxa-12-azatetradecan-1-oate (**Oxy-glyoxime-HN-Peg$_3$-O-CH$_2$CO$_2$Li)** as a white powder (12 mg, 38%). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.14 - 1.27 (m, 1 H) 1.29 - 1.59 (m, 3 H) 1.99 - 2.26 (m, 3 H) 2.31 (s, 4 H) 2.34 - 2.47 (m, 2 H) 2.79 (d, *J*=6.03 Hz, 1 H) 3.13 (d, *J*=18.65 Hz, 1 H) 3.19 - 3.29 (m, 3 H) 3.37 - 3.58 (m, 9 H) 3.69 - 3.80 (m, 4 H) 4.44 (s, 2 H) 4.79 - 4.89 (m, 2 H) 6.60 - 6.70 (m, 1 H) 6.72 - 6.80 (m, 1 H) 7.83 (t, *J*=5.56 Hz, 1 H); ESI MS *m/z*: calculated for $C_{28}H_{38}LiN_3O_{10}$ 583.6, found 584.9 $(M+H)^+$; HPLC (Synergy Hydro, 20 min) t_R =11.31 min (95%).

HNMR Spectrum of Oxy-glyoxime-HN-Peg$_3$-O-Gly-OLi

13835-118 recrystallized

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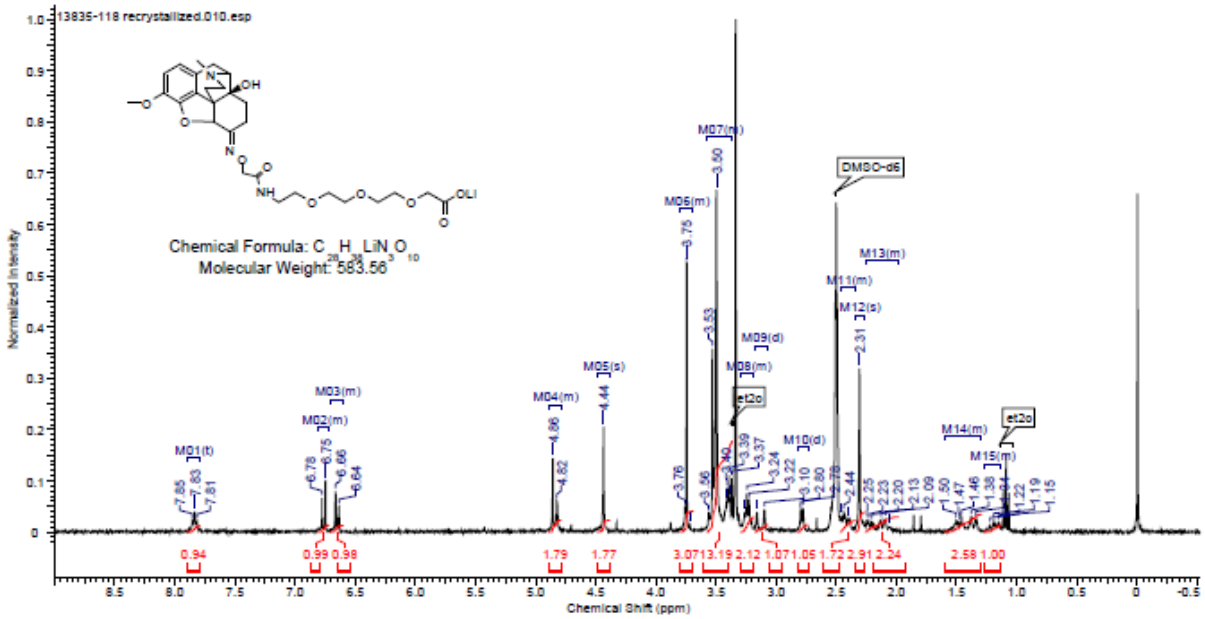
Spectrum Type	STANDARD	*Sweep Width (Hz)*	6172.65	*Temperature (degree C)*	27.000		

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.14 - 1.27 (m, 1 H) 1.29 - 1.59 (m, 3 H) 1.99 - 2.26 (m, 3 H) 2.31 (s, 4 H) 2.34 - 2.47 (m, 2 H) 2.79 (d, *J*=6.03 Hz, 1 H) 3.13 (d, *J*=18.65 Hz, 1 H) 3.19 - 3.29 (m, 3 H) 3.37 - 3.58 (m, 9 H) 3.69 - 3.80 (m, 4 H) 4.44 (s, 2 H) 4.79 - 4.89 (m, 2 H) 6.60 - 6.70 (m, 1 H) 6.72 - 6.80 (m, 1 H) 7.83 (t, *J*=5.56 Hz, 1 H)



HPLC Chromatogram of Oxy-glyoxime-HN-Peg$_3$-O-Gly-OLi

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System : System_1
Method : auto5-95,20min
synergy hydro analytical

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Processed : 2/23/2016 9:32:04 AM
Printed : 9/16/2016 1:03:01 PM

13835-118 after Et2O recryst 2_23_2016 9_04_18 AM1.DATA - Prostar 335 Absorbance Analog Channel 1 192.168.230.141
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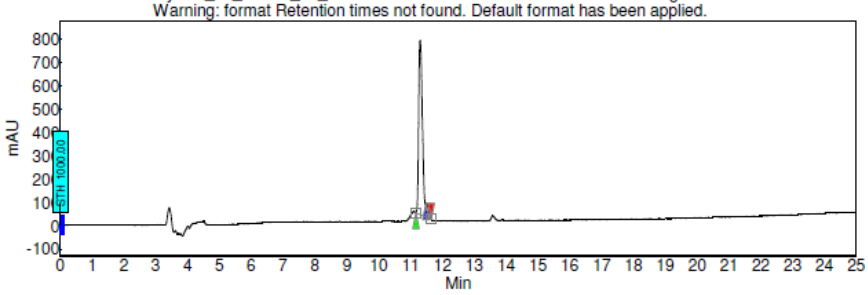

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HNMR Spectrum of Oxy-Glyoxime-OH

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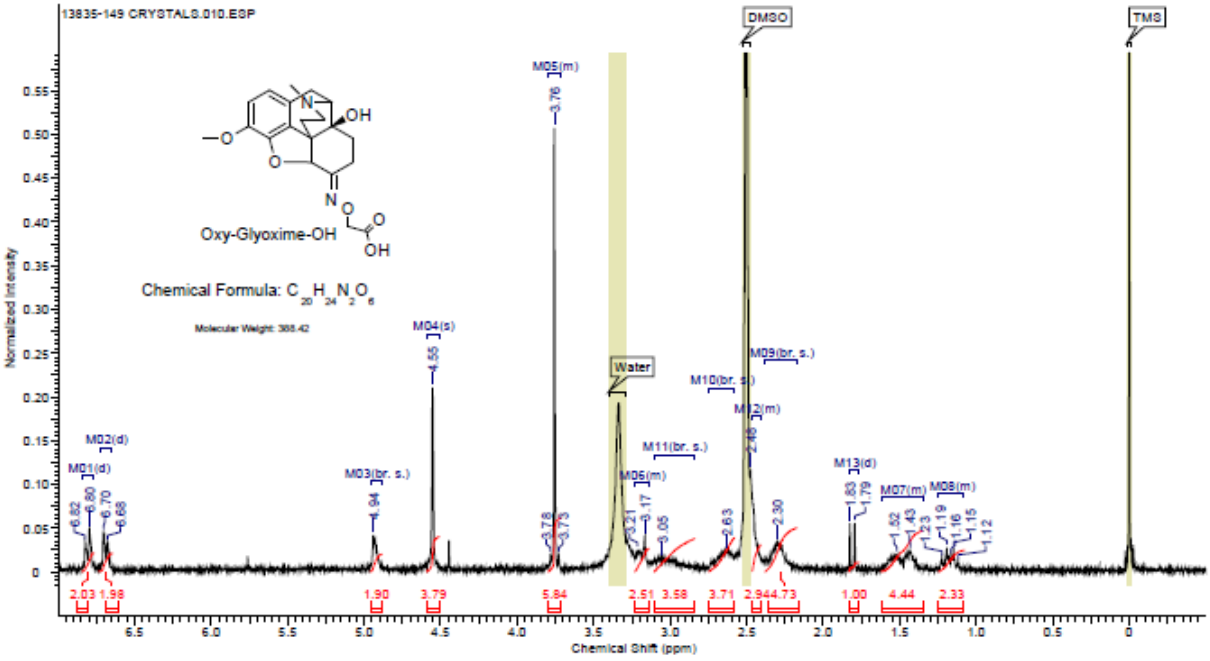
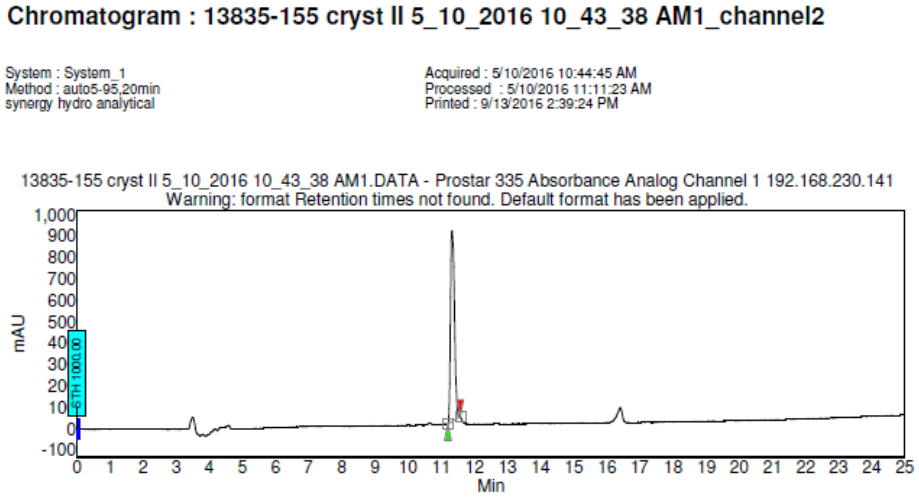
Spectrum Type	STANDARD	Sweep Width (Hz)	6172.65	Temperature (degree C)	27.000		

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.08 - 1.25 (m, 2 H) 1.35 - 1.62 (m, 3 H) 1.81 (d, *J*=9.23 Hz, 1 H) 2.30 (br. s., 2 H) 2.41 - 2.46 (m, 3 H) 2.63 (br. s., 2 H) 3.05 (br. s., 2 H) 3.14 - 3.23 (m, 1 H) 3.71 - 3.80 (m, 3 H) 4.55 (s, 2 H) 4.94 (br. s., 1 H) 6.69 (d, *J*=7.72 Hz, 1 H) 6.81 (d, *J*=8.10 Hz, 1 H)



HPLC Chromatogram of Oxy-GlyoximeOH



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HNMR of Fmoc-HN-Peg$_3$-O-Gly-OMe

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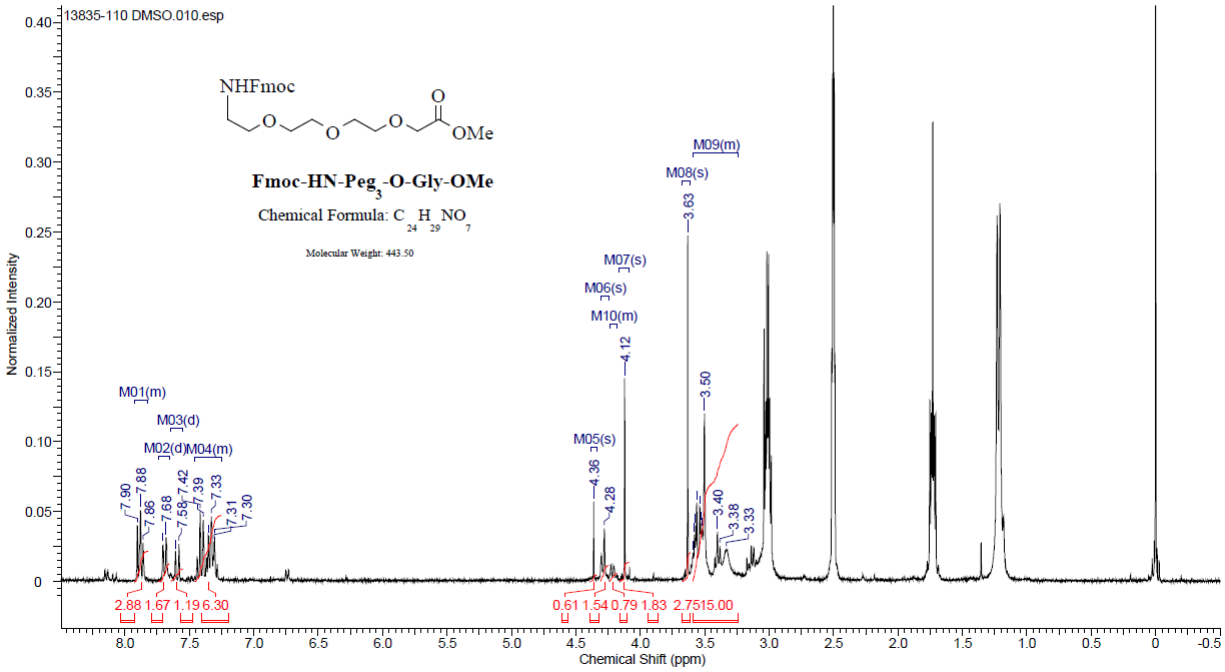
Receiver Gain	912.30	***SW(cyclical) (Hz)***	6172.84	***Solvent***	DMSO-d6	***Spectrum Offset (Hz)***	1852.6998
Spectrum Type	STANDARD	***Sweep Width (Hz)***	6172.65	***Temperature (degree C)***	27.000		

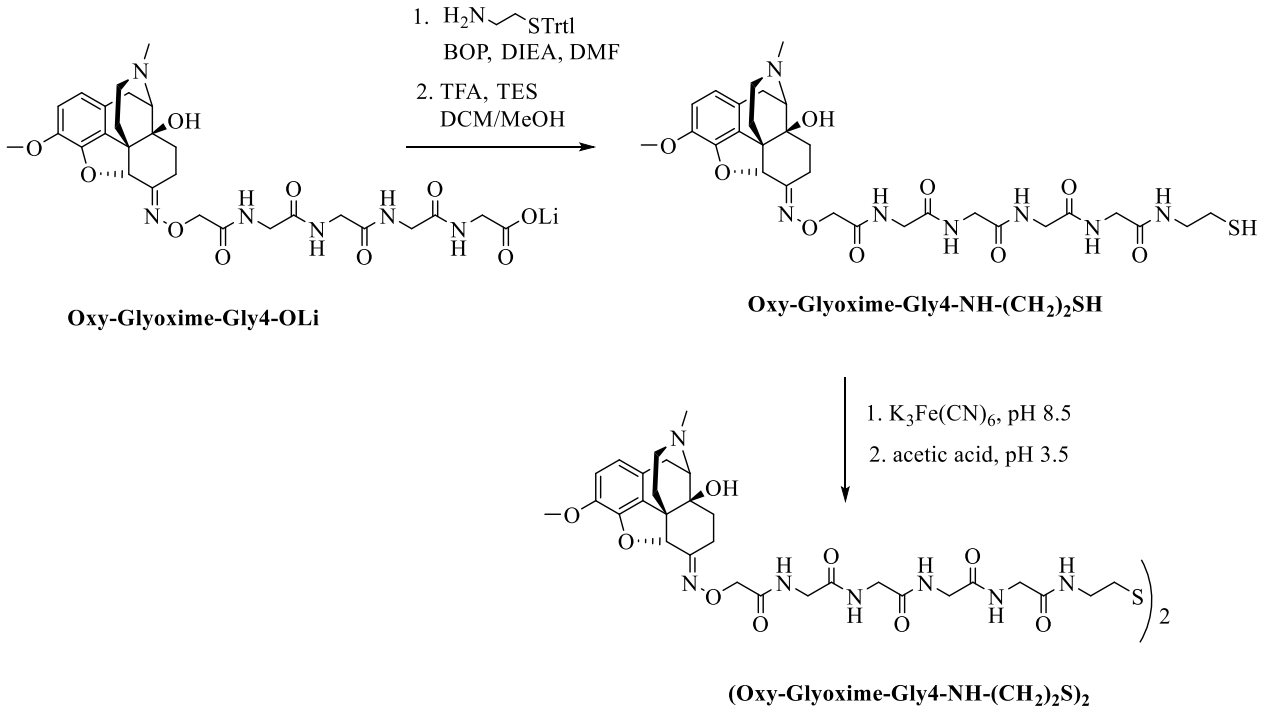
^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 3.24 - 3.59 (m, 13 H) 3.41 - 3.41 (m, 1 H) 3.63 (s, 3 H) 4.12 (s, 1 H) 4.18 - 4.24 (m, 1 H) 4.28 (s, 1 H) 4.36 (s, 1 H) 7.25 - 7.46 (m, 5 H) 7.59 (d, *J*=8.29 Hz, 1 H) 7.69 (d, *J*=6.97 Hz, 1 H) 7.82 - 7.93 (m, 2 H)



S2. Synthesis of Oxycodone-based hapten with thiol linker [OXY(Gly)$_4$-SH hapten]

Synthesis of Oxy-carboxymethoxyloxime-Gly$_4$-NH-cysteaminesulfide dimer (Oxy-Glyoxime-Gly$_4$-NH-$(CH_2)_2$-S)$_2$)

Scheme 1: Synthesis of (Oxy-Glyoxime-Gly4-NH-$(CH_2)_2$-S)$_2$



Oxy-Glyoxime-Gly4-OLi

Oxy-Glyoxime-Gly4-NH-$(CH_2)_2$SH

(Oxy-Glyoxime-Gly4-NH-$(CH_2)_2$S)$_2$

Experimental details:

Chemistry General: All standard reagents were commercially available. Intermediates were purified by column chromatography on a Teledyne ISCO Rf chromatography unit unless otherwise indicated. The purity of intermediates and final compounds was determined using an Agilent-Varian HPLC system equipped with Prostar 210 dual pumps, a Prostar 335 Diode UV detector and a SEDEX75 (SEDERE, Olivet, France) ELSD detector and an analytical Synergy Hydro® RP80A C18 (4 µm particle size, 250 x 4.60 mm column; Phenomenex) with a linear gradient of 5%-95% solvent B over 20 min at a flow rate of 1 mL/min. Absorbance was monitored at 220 nm. The HPLC solvent system was binary: water containing

0.1% trifluoroacetic acid (TFA) and solvent B (acetonitrile containing 5% water and 0.1% TFA). The molecular ion of intermediates and final compounds was determined using a PE Sciex API 150 EX LC/MS system from Perkin Elmer (San Jose, California). ESI-HRMS were obtained on a Waters – SYNAPT –G2 mass spectrometer. ^{1}H NMR spectra were recorded at 300 MHz on a Bruker Avance 300 Spectrospin instrument and at 700 MHz on a Bruker Avance Neo. Chemical shifts are reported as follows: δ in ppm (multiplicity, coupling constant (Hz), and integration. The following abbreviations explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, br = broad, dd = doublet of doublets.

***N*-[({[(5α,6*E*)-14-hydroxy-3-methoxy-17-methyl-4,5-epoxymorphinan-6-ylidene]amino}oxy)acetyl]glycylglycylglycyl-*N*-(2-sulfanylethyl)glycinamide (Oxy-Glyoxime-Gly$_4$-NH-(CH$_2$)$_2$SH):** To a clear solution of Lithium *N*-[({[(6E)-14-hydroxy-3-methoxy-17-methyl-4,5-epoxymorphinan-6-ylidene]amino}oxy)acetyl]glycylglycyl-N-(carboxylatomethyl)glycinamide (**Oxy-glyoxime-Gly$_4$-OLi** 200 mg, 0.32 mmol) in DMF (30 mL) was added BOP (284 mg, 0.643 mmol) and 2-(tritylthio)ethanamine (123 mg, 0.386 mmol), then diisopropylethylamine (280 µL, 1.61 mmol). The reaction mixture was stirred at room temperature for 16 hr under nitrogen atmosphere, then concentrated to dryness *in vacuo*. The residue was purified by column chromatography [24 g SiO_2, eluent 0% to 40% CMA 80 (Chloroform/MeOH/NH_4OH 80/19.8/0.2) in DCM] to provide *N*-[({[(5α,6*E*)-14-hydroxy-3-methoxy-17-methyl-4,5-epoxymorphinan-6-ylidene]amino}oxy)acetyl]glycylglycylglycyl-*N*-[2-(tritylsulfanyl)ethyl]glycinamide (**Oxy-Glyoxime-Gly$_4$-NH-(CH$_2$)$_2$STrtl**) as a yellow powder (192 mg. 65%). To a solution of this material in DCM/MeOH (10 mL/4 mL) was added TFA (6 mL) and triethylsilane (0.3 mL), and the solution stirred at room temperature for 1 hr. The mixture was concentrated to dryness and purified by column chromatography [12 g SiO_2, eluent 0% to 40% CMA 80 (Chloroform/MeOH/NH_4OH 80/19.8/0.2) in DCM] to provide **Oxy-Glyoxime-Gly$_4$-NH-(CH$_2$)$_2$SH (**91 mg, 64%) as a white powder. ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.12 - 1.31 (m, 1 H) 1.39 - 1.69 (m, 2 H) 2.25 - 2.43 (m, 2 H) 2.55 - 3.03 (m, 10 H) 3.09 - 3.27 (m, 4 H) 3.56 - 4.03 (m, 12 H) 4.45 - 4.63 (m,

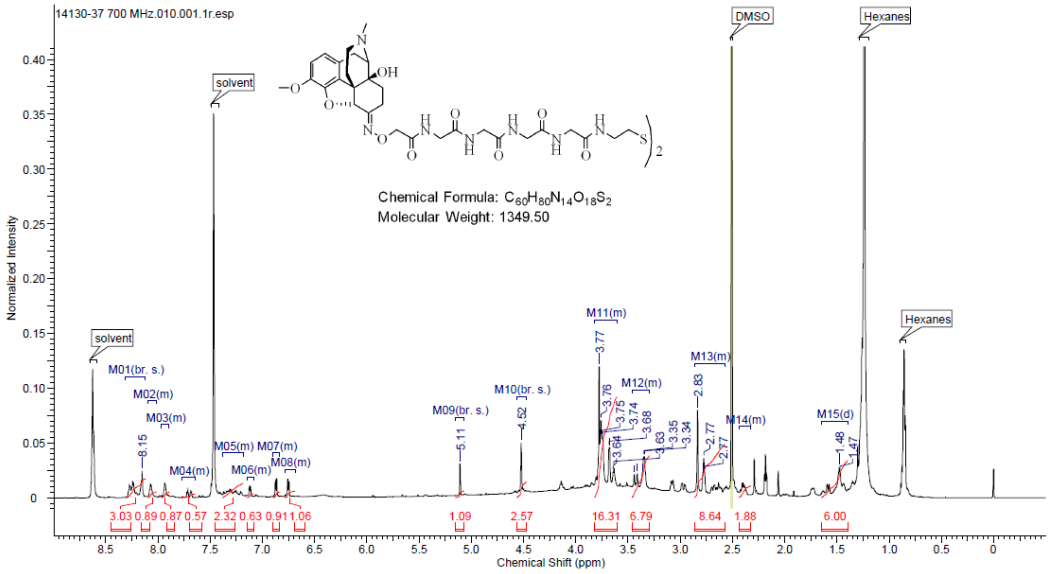
2 H) 5.01 (br. s., 1 H) 6.62 - 6.87 (m, 2 H) 7.79 - 7.97 (m, 2 H) 8.06 - 8.28 (m, 3 H); ESI MS *m/z*: calculated for $C_{30}H_{41}N_7O_9S$ 675.76, found 676.6 $(M+H)^+$; HPLC (Synergy Hydro, 20 min) t_R =10.96 min (>95%).

(Oxy-Glyoxime-Gly$_4$-NH-(CH$_2$)$_2$S)$_2$: To a solution of **Oxy-Glyoxime-Gly$_4$-NH-(CH$_2$)$_2$SH** (87 mg, 0.129 mmol) in water/acetonitrile/methanol (40 mL, ratio 2/1/1) was added pH 8.5 NH_4OAc buffer solution (2.5 mL) and the reaction mixture saturated with nitrogen. Potassium ferricyanide (125 mg, 0.380 mmol) was added and the reaction mixture stirred at room temperature for 16 hr. The solution was acidified to pH 3.5 with glacial acetic acid, then stirred over Amberlite IRA-68 resin for 20 min and filtered. The filtrate was concentrated to dryness and purified by column chromatography [130 g C18, eluent 0% to 100% MeOH in water] to provide **Oxy-Glyoxime-Gly$_4$-NH-(CH$_2$)$_2$S)$_2$** (79mg, 91%) as a white solid. ^{1}H NMR (700 MHz, DMSO-d_6) δ ppm 1.47 (d, *J*=5.72 Hz, 6 H) 2.33 - 2.44 (m, 3 H) 2.57 - 2.86 (m, 14 H) 3.29 - 3.46 (m, 11 H) 3.61 - 3.82 (m, 26 H) 4.52 (br. s., 4 H) 5.11 (br. s., 2 H) 6.68 - 6.78 (m, 1 H) 6.83 - 6.90 (m, 1 H) 7.09 - 7.15 (m, 1 H) 7.19 - 7.38 (m, 4 H) 7.65 - 7.77 (m, 1 H) 7.89 - 7.97 (m, 1 H) 8.01 - 8.09 (m, 1 H) 8.15 (br. s., 4 H); ESI HRMS *m/z*: calculated for $C_{60}H_{80}N14O_{18}S$ 1349.50, found 1349.53 $(M)^+$, 675.27 $(M)^{2+}$. HPLC (Synergy Hydro, 20 min) t_R =11.59 min (>95%).

HNMR Spectrum of (Oxy-Glyoxime-Gly$_4$-NH-(CH$_2$)$_2$S)$_2$

Acquisition Time (sec)	2.3593	*Date*	06 Feb 2018 19:29:04			*Date Stamp*	06 Feb 2018 19:29:04
File Name	C:\Users\chassler\Documents\NMR\14130-37 700 MHz\10\pdata\1\1r					*Frequency (MHz)*	700.20
Nucleus	1H	*Number of Transients*	256	*Origin*	spect	*Original Points Count*	32768
Owner	nmrsu	*Points Count*	65536	*Pulse Sequence*	zg30	*Receiver Gain*	32.00
SW(cyclical) (Hz)	13888.89	*Solvent*	DMSO-d6	*Spectrum Offset (Hz)*	4322.6074	*Spectrum Type*	STANDARD
Sweep Width (Hz)	13888.68	*Temperature (degree C)*	25.000				

^{1}H NMR (700 MHz, DMSO-d_6) δ ppm 1.47 (d, *J*=5.72 Hz, 6 H) 2.33 - 2.44 (m, 3 H) 2.57 - 2.86 (m, 14 H) 3.29 - 3.46 (m, 11 H) 3.61 - 3.82 (m, 26 H) 4.52 (br. s., 4 H) 5.11 (br. s., 2 H) 6.68 - 6.78 (m, 1 H) 6.83 - 6.90 (m, 1 H) 7.09 - 7.15 (m, 1 H) 7.19 - 7.38 (m, 4 H) 7.65 - 7.77 (m, 1 H) 7.89 - 7.97 (m, 1 H) 8.01 - 8.09 (m, 1 H) 8.15 (br. s., 4 H)



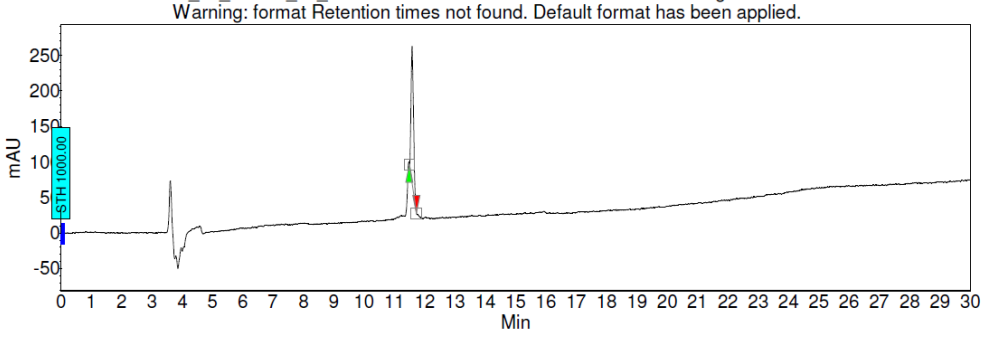
HPLC Chromatogram of (Oxy-Glyoxime-Gly$_4$-NH-(CH$_2$)$_2$S)$_2$

Chromatogram : 14130-27-1 fr15-23 5_25_2017 2_07_31 PM1_channel2

System : System_1
Method : auto5-95,20min
synergy hydro analytical

Acquired : 5/25/2017 2:08:39 PM
Processed : 5/25/2017 2:40:31 PM
Printed : 2/5/2018 5:24:45 PM

14130-27-1 fr15-23 5_25_2017 2_07_31 PM1.DATA - Prostar 335 Absorbance Analog Channel 1 192.168.230.141
Warning: format Retention times not found. Default format has been applied.



Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
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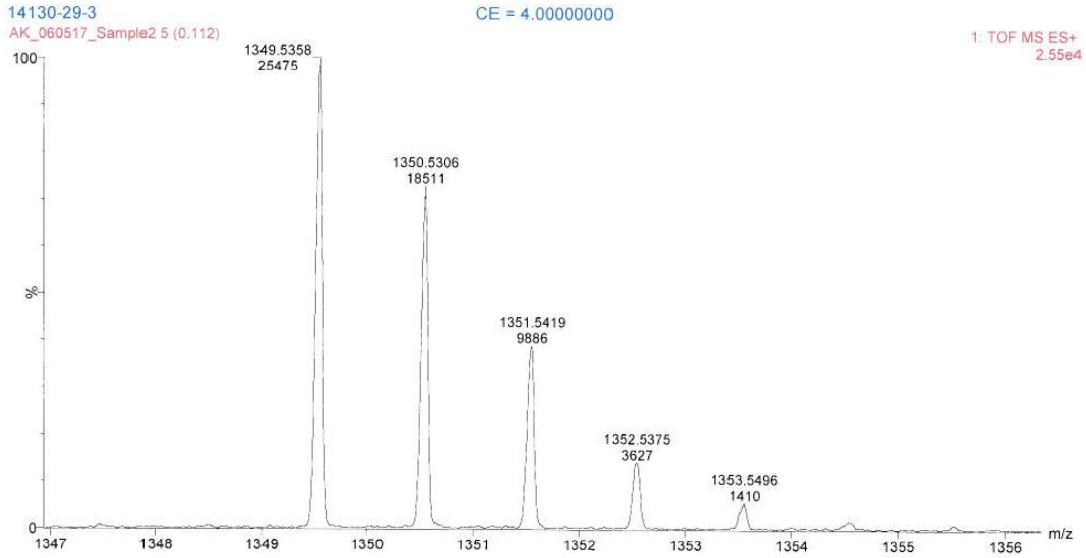
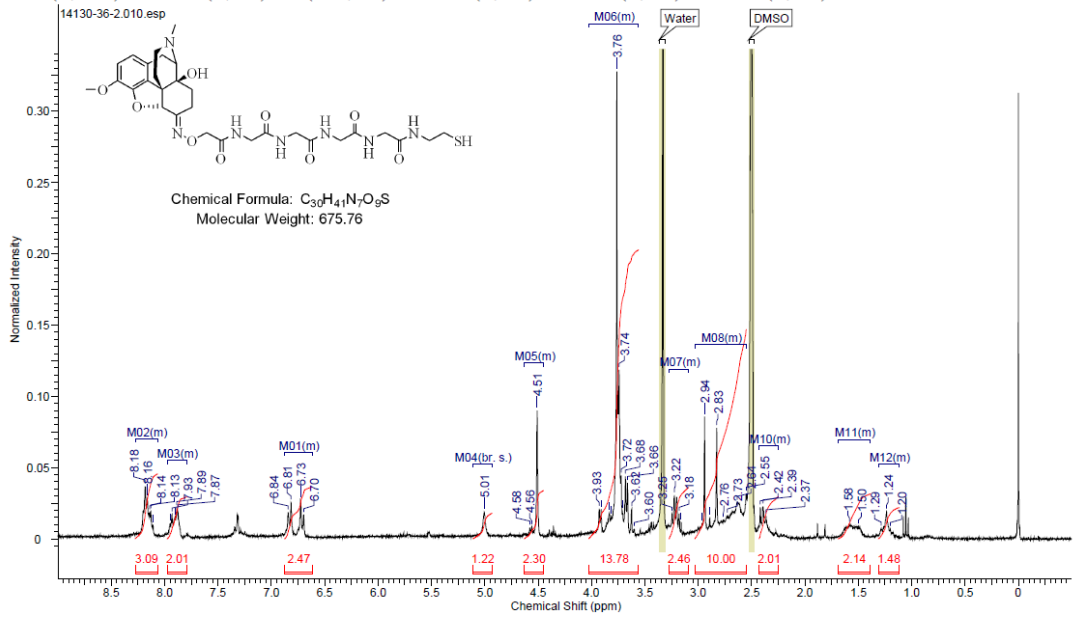
HRMS of (Oxy-Glyoxime-Gly$_4$-NH-(CH$_2$)$_2$S)$_2$



HNMR Spectrum of Oxy-Glyoxime-Gly$_4$-NH-(CH$_2$)$_2$SH

Acquisition Time (sec)	5.3084	*Comment*	14130-36-2	*Date*	19 Jun 2017 11:49:20		
Date Stamp	19 Jun 2017 11:49:20			*File Name*	C:\Users\chassler\Documents\NMR\14130-36-2\10\fid		
Frequency (MHz)	300.13	*Nucleus*	1H	*Number of Transients*	256	*Origin*	spect
Original Points Count	32768	*Owner*	nmruser	*Points Count*	32768	*Pulse Sequence*	zg30
Receiver Gain	1024.00	*SW(cyclical) (Hz)*	6172.84	*Solvent*	DMSO-d6	*Spectrum Offset (Hz)*	1852.9191
Spectrum Type	STANDARD	*Sweep Width (Hz)*	6172.65	*Temperature (degree C)*	27.000		

^{1}H NMR (300 MHz, DMSO-d_6) δ ppm 1.12 - 1.31 (m, 1 H) 1.39 - 1.69 (m, 2 H) 2.25 - 2.43 (m, 2 H) 2.55 - 3.03 (m, 10 H) 3.09 - 3.27 (m, 4 H) 3.56 - 4.03 (m, 12 H) 4.45 - 4.63 (m, 2 H) 5.01 (br. s., 1 H) 6.62 - 6.87 (m, 2 H) 7.79 - 7.97 (m, 2 H) 8.06 - 8.28 (m, 3 H)



HPLC Chromatogram of Oxy-Glyoxime-Gly_4-NH-$(CH_2)_2$SH

Chromatogram : 14130-22 fr 35-45 4_28_2017 3_36_03 PM1_channel1

System : System_2
Method : 5to95%B20 min
synergy hydro analytical

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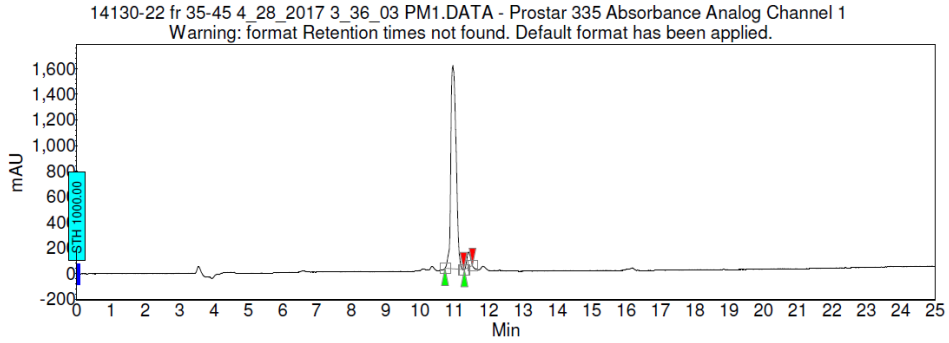
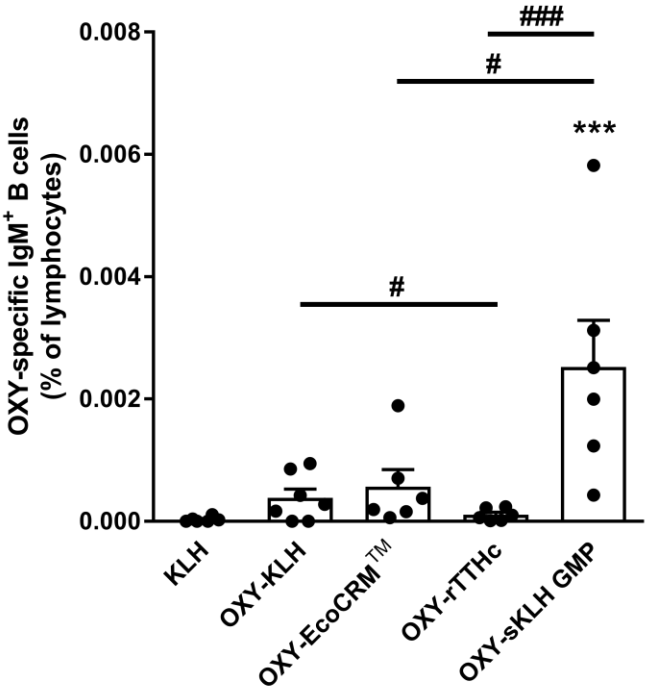
Processed : 4/28/2017 4:03:35 PM
Printed : 2/5/2018 5:22:37 PM



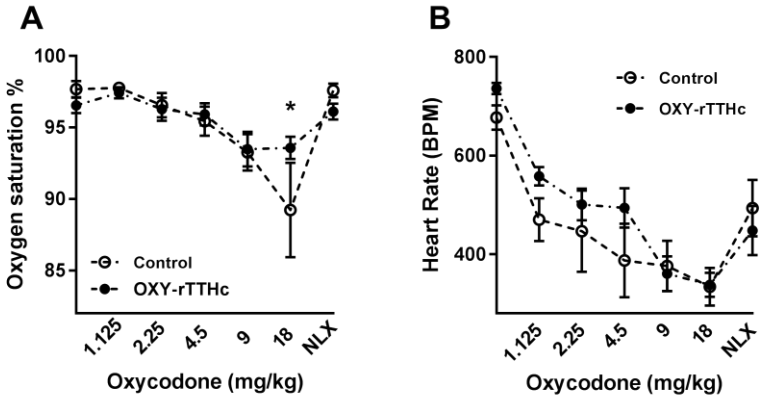
Index	Name	Time [Min]	Quantity [% Area]	Height [mAU]	Area [mAU.Min]	Area % [%]
1	UNKNOWN	10.96	95.23	1585.5	283.1	95.228
2	UNKNOWN	11.41	4.77	120.6	14.2	4.772
Total			100.00	1706.1	297.3	100.000

S3. Expansion of the early hapten-specific B cell population in mice immunized with oxycodone conjugate vaccines containing different carrier proteins.



BALB/c mice (n ≥ 4/group) were immunized IM with 100 µg unconjugated KLH, OXY-KLH, OXY-EcoCRM™, OXY-rTTHc, or OXY-sKLH, formulated with 70 µg of alum. At 7 days post-immunization, hapten-specific B cell populations from spleen and lymph nodes were analyzed by antigen-based magnetic enrichment paired with flow cytometry. OXY-specific IgM^+ B cells are expressed as percentage (%) of the total lymphocyte population after magnetic enrichment. P value symbols: asterisk (*) represents experimental groups compared to KLH, pound (#) represents inter-experimental group comparisons. #, $p \leq 0.05$, ***, $p \leq 0.001$, ###, $p \leq 0.001$ (by one-way ANOVA followed by Tukey's multiple comparison post hoc test). Line and error bar represent mean ± SEM.

S4. Efficacy of OXY-rTTHc in attenuating oxycodone-induced respiratory depression and bradycardia in mice.



Oxycodone-induced respiratory depression and bradycardia were tested by oximetry one week after the last immunization as previously described [1, 2]. A mouseOx (STARR life sciences corp., Oakmont, PA) was placed via neck collar to measure oxygen saturation and heart rate. After baseline recording, mice were challenged every 15 min with increasing oxycodone sc doses of 1.125, 2.25, 4.5, 9.0 mg/kg for a cumulative dose of 18 mg/kg, followed by administration of 0.1 mg/kg naloxone to reverse the effects of oxycodone. Respiratory activity and heart rate were measured 15 min after each oxycodone dose. In both panels, the X-axis shows the oxycodone cumulative dose administered over time. **A)** Percentage (%) oxygen saturation, and **B)** heart rate expressed in beat per minute (BPM). Statistical significance was assessed by two-way ANOVA followed by Tukey post hoc test. * $p \leq 0.05$. There were no differences between the two groups following naloxone treatment. Data are expressed as mean ± SEM, control (n = 4), and OXY-rTTHc (n=5).

Supplemental Material References:

1. Raleigh, M. D.; Laudenbach, M.; Baruffaldi, F.; Peterson, S. J.; Roslawski, M. J.; Birnbaum, A. K.; Carroll, F. I.; Runyon, S. P.; Winston, S.; Pentel, P. R.; Pravetoni, M. Opioid dose- and route-dependent efficacy of oxycodone and heroin vaccines in rats. *Journal of Pharmacology and Experimental Therapeutics* **2018**.
2. Laudenbach, M.; Baruffaldi, F.; Robinson, C.; Carter, P.; Seelig, D.; Baehr, C.; Pravetoni, M. Blocking interleukin-4 enhances efficacy of vaccines for treatment of opioid abuse and prevention of opioid overdose. *Scientific Reports* **2018,** *8*, (1), 5508.