

# Supporting information

## **Optimized Opioid-Neurotensin Multitarget Peptides: From Design to Structure-Activity Relationship Studies**

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## 1. Analytical characterization of the OP-NT hybrids

Peptide number	Sequence	Yield	Purity	HPLC rt (min)	Formula	HRMS [M+H] <sup>+</sup> (Da)	
						Calculated	Found
3	<i>H-Dmt-DArg-Aba-βAla-Arg-Arg-Pro-Tyr-Tle-Leu-OH</i>	19% <sup>b</sup>	> 97%	1.77	C <sub>68</sub> H <sub>103</sub> N <sub>19</sub> O <sub>13</sub>	1394.8055	1394.8020
4	<i>H-Dmt-DArg-Aba-βAla-Lys-Lys-Pro-Tyr-Tle-Leu-OH</i>	18% <sup>b</sup>	> 97%	1.88	C <sub>68</sub> H <sub>103</sub> N <sub>15</sub> O <sub>13</sub>	1338.7932	1338.7921
5	<i>H-Dmt-DArg-Aba-βAla-Arg-Arg-Pro-Dmt-Tle-Leu-OH</i>	26% <sup>b</sup>	> 97%	1.98	C <sub>70</sub> H <sub>107</sub> N <sub>19</sub> O <sub>13</sub>	1422.8368	1422.8341
6	<i>H-Dmt-DArg-Aba-βAla-Lys-Lys-Pro-Dmt-Tle-Leu-OH</i>	2.5% <sup>a</sup>	> 95%	1.78	C <sub>70</sub> H <sub>107</sub> N <sub>15</sub> O <sub>13</sub>	1366.8246	1366.8253
7	<i>H-Dmt-DArg-Aba-βAla-β3hArg-Arg-Pro-Dmt-Tle-Leu-OH</i>	21% <sup>b</sup>	> 97%	1.97	C <sub>71</sub> H <sub>109</sub> N <sub>19</sub> O <sub>13</sub>	1436.8525	1436.8553
8	<i>H-Dmt-DArg-Aba-βAla-β3hLys-Lys-Pro-Dmt-Tle-Leu-OH</i>	13% <sup>b</sup>	> 97%	1.95	C <sub>71</sub> H <sub>109</sub> N <sub>15</sub> O <sub>13</sub>	1380.8402	1380.8401
9	<i>H-Dmt-DArg-Aba-βAla-Arg-Arg-Pro-(6-OH)Tic-Tle-Leu-OH</i>	31% <sup>b</sup>	> 97%	1.78	C <sub>69</sub> H <sub>103</sub> N <sub>19</sub> O <sub>13</sub>	1406.8062	1406.8007
10	<i>H-Dmt-DArg-Aba-βAla-Lys-Lys-Pro-(6-OH)Tic-Tle-Leu-OH</i>	3.5% <sup>a</sup>	> 95%	1.85	C <sub>69</sub> H <sub>103</sub> N <sub>15</sub> O <sub>13</sub>	1350.7932	1350.7971
11	<i>H-Dmt-DArg-Aba-βAla-Arg-Arg-Pro-mTyr-Tle-Leu-OH</i>	12% <sup>b</sup>	> 97%	1.81	C <sub>68</sub> H <sub>103</sub> N <sub>19</sub> O <sub>13</sub>	1394.8062	1394.8090

<b>12</b>	<i>H-Dmt-DArg-Aba-βAla-Lys-Lys-Pro-mTyr-Tle-Leu-OH</i>	15% <sup>b</sup>	> 97%	1.89	C <sub>68</sub> H <sub>103</sub> N <sub>15</sub> O <sub>13</sub>	1338.793 2	1338.7910
<b>PK20</b>	<i>H-Dmt-DLys-Phe-Phe-Lys-Lys-Pro-Phe-Tle-Leu-OH</i>	46% <sup>b</sup>	> 97%	3.01 <sup>c</sup>	C <sub>73</sub> H <sub>107</sub> N <sub>13</sub> O <sub>12</sub>	1358.823 5	1358.8241

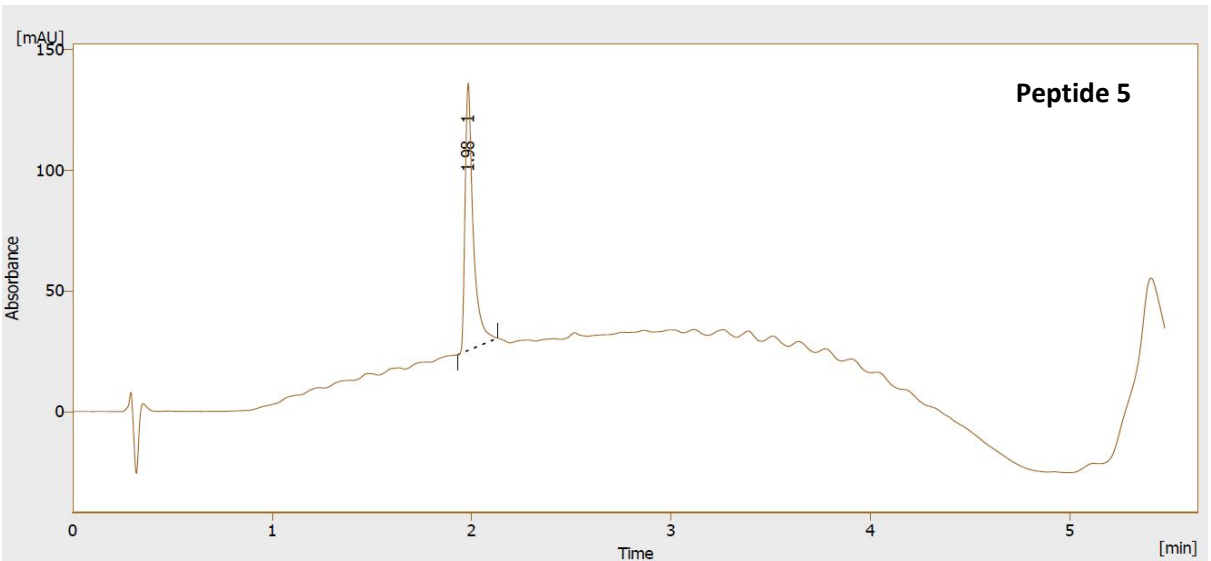
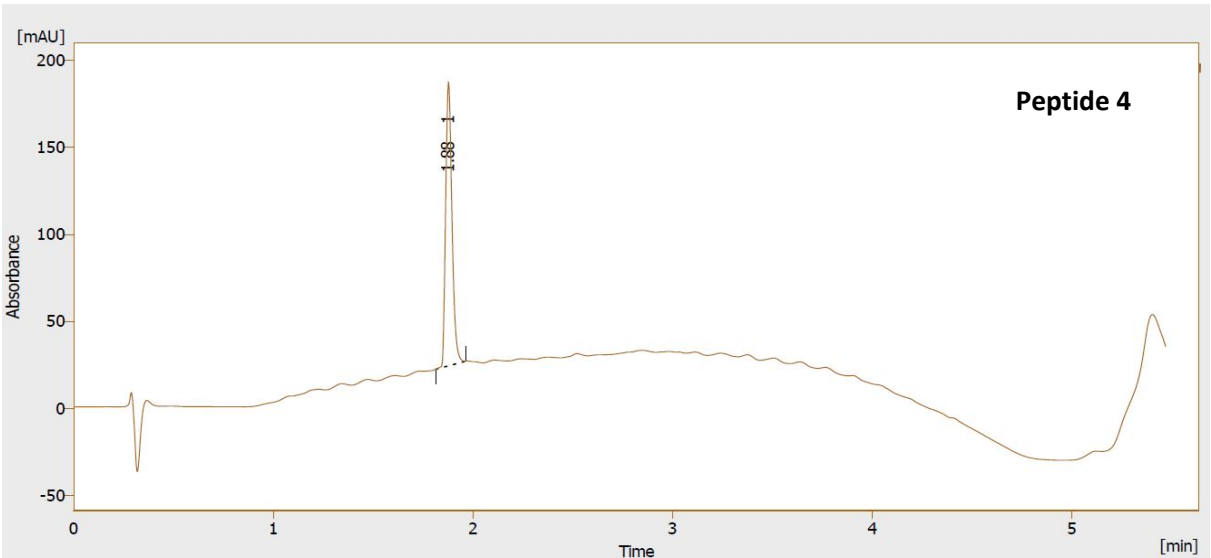
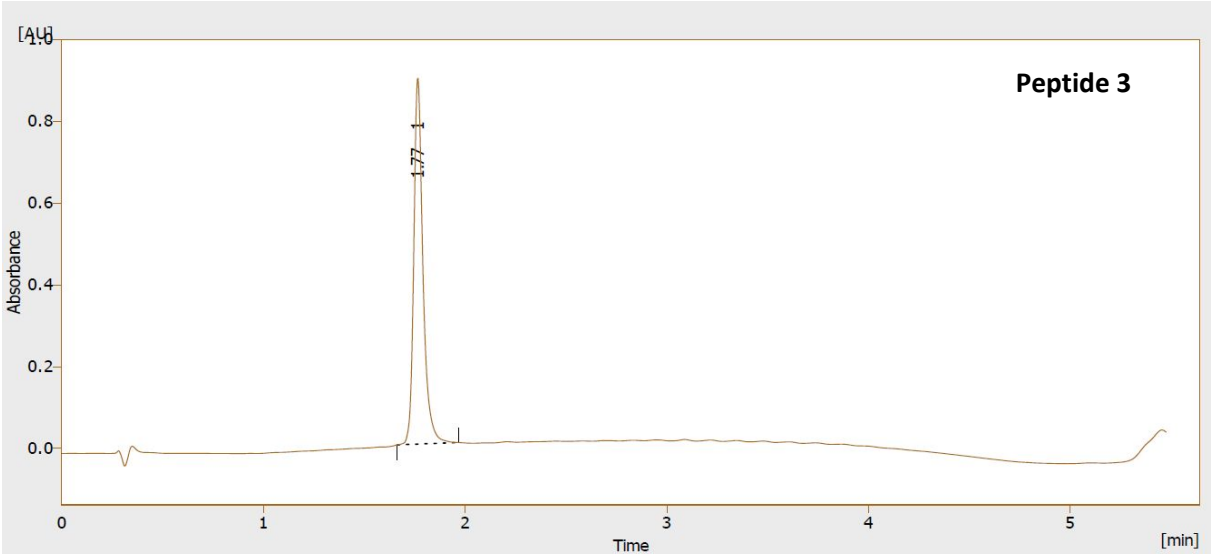
<sup>a</sup>Synthesized via Method A (see experimental section) using Fmoc-Leu Wang resin (0.827 mmol/g, 121 mg).

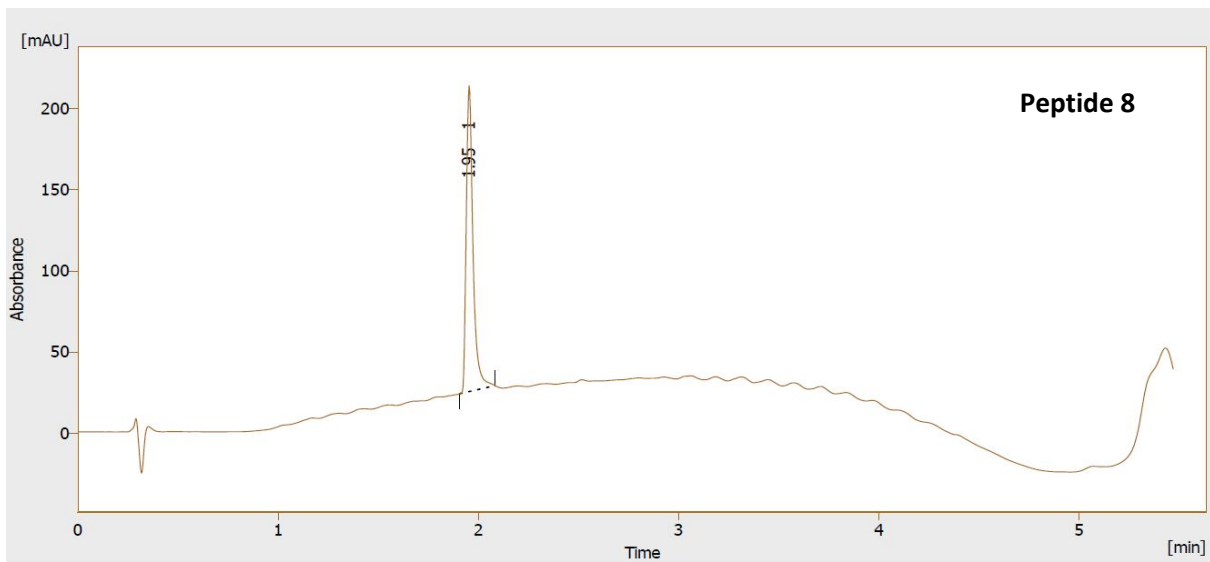
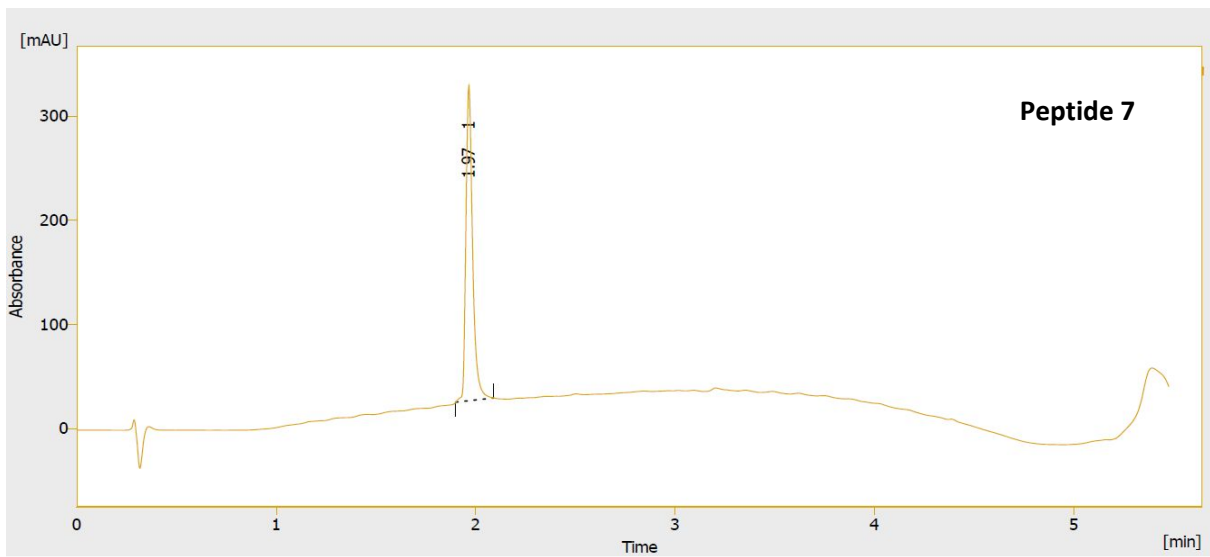
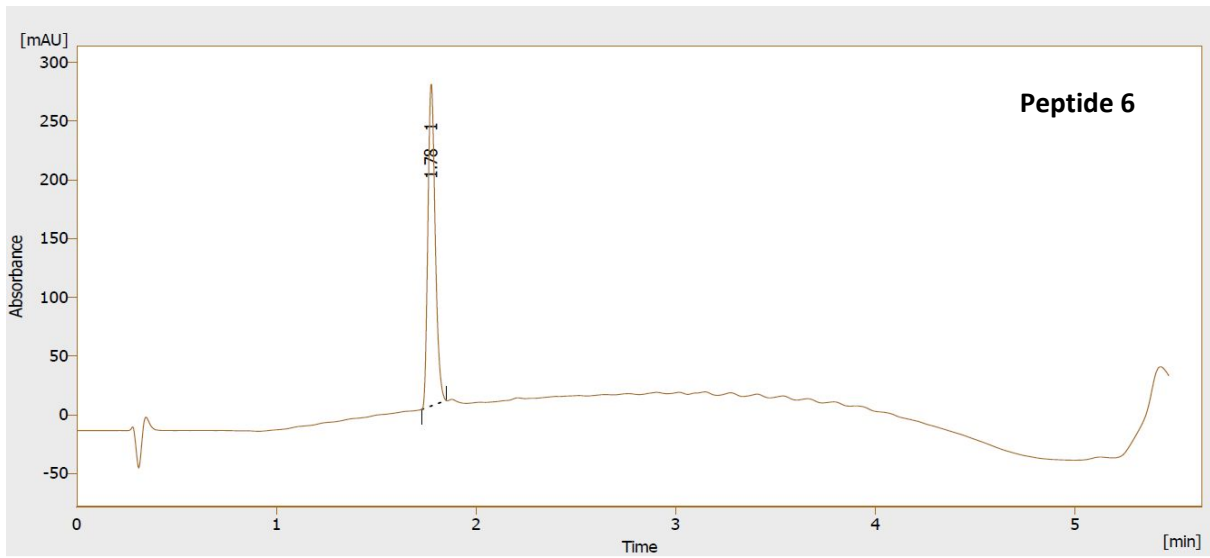
<sup>b</sup>Synthesized via Method B (see experimental section) using preloaded Fmoc-Leu Wang TG resin (0.25 mmol/g, 400 mg).

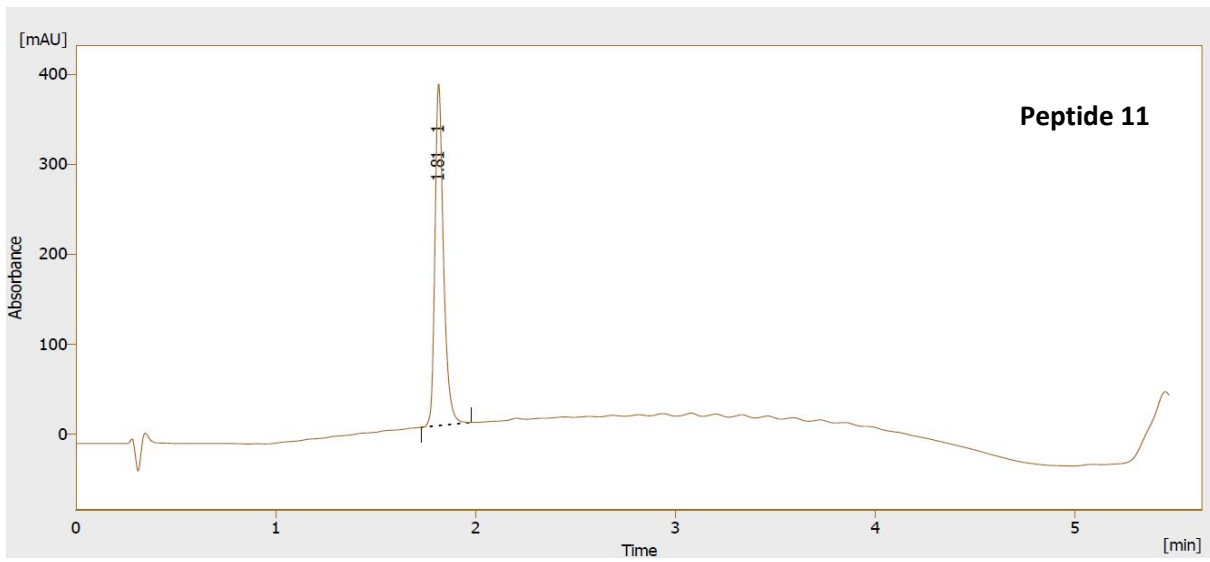
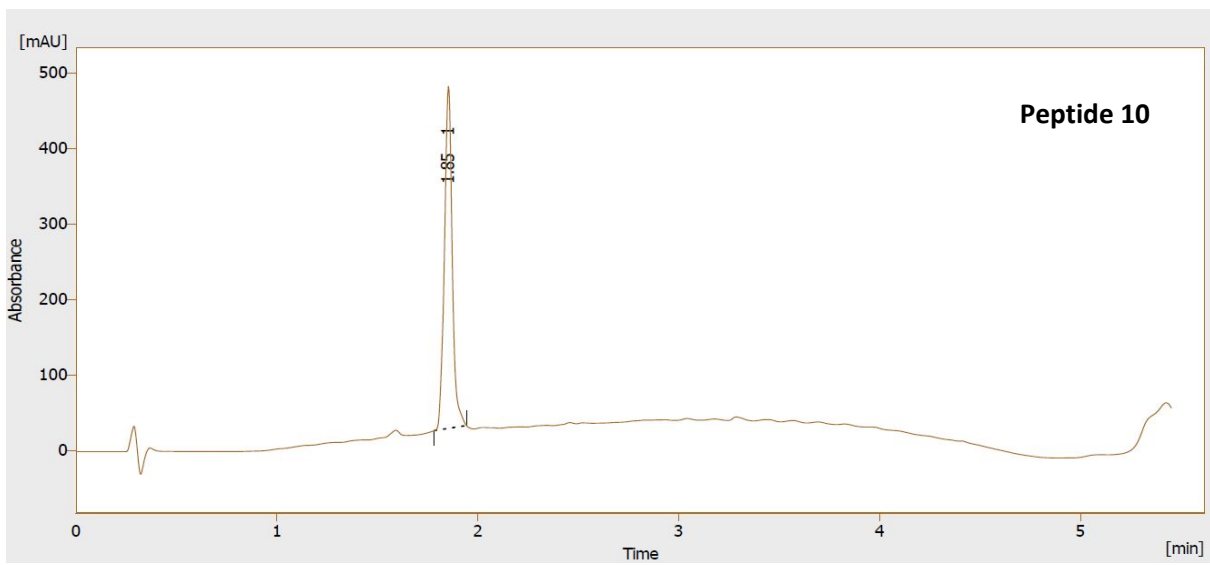
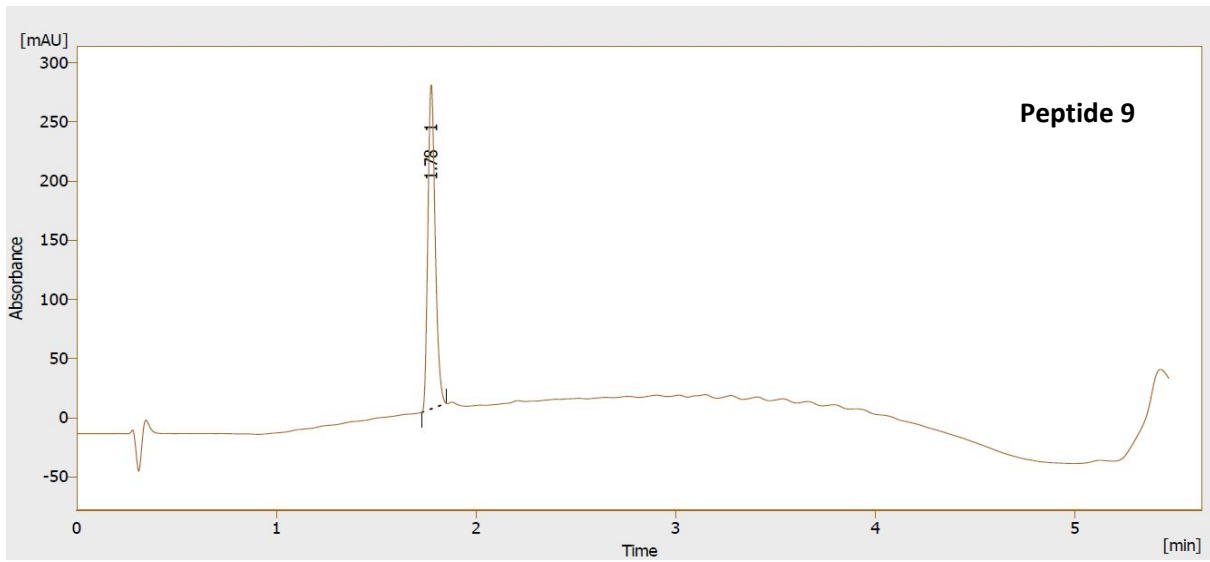
HPLC analyses were performed with a Chromolith HR C18 50x4.6 column using a gradient from 1% AcN + 0.1% TFA to 99% in mQ water + 0.1% TFA.

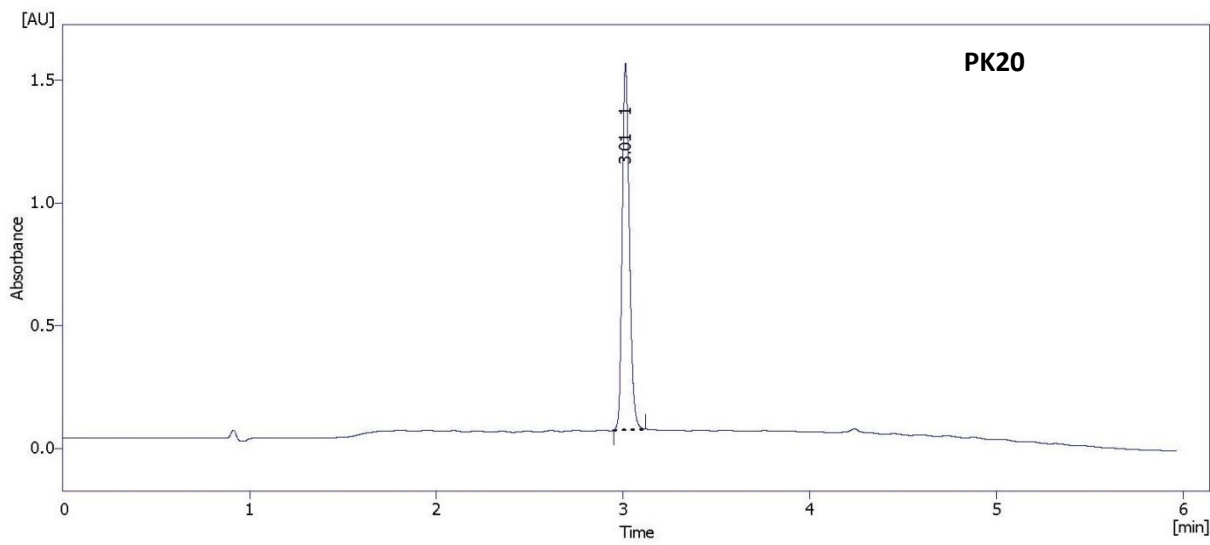
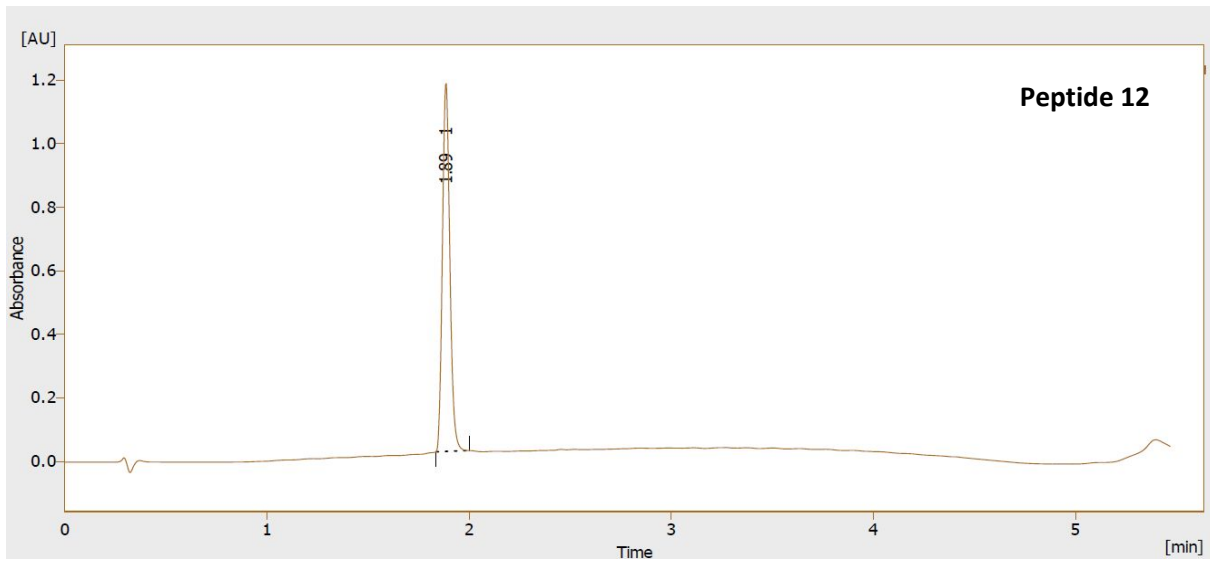
<sup>c</sup>HPLC analysis performed with a Chromolith HR C18 150x4.6 column using a gradient from 1% AcN + 0.1% TFA to 99% in mQ water + 0.1% TFA.

## 2. HPLC chromatograms of all synthesized OP-NT hybrids



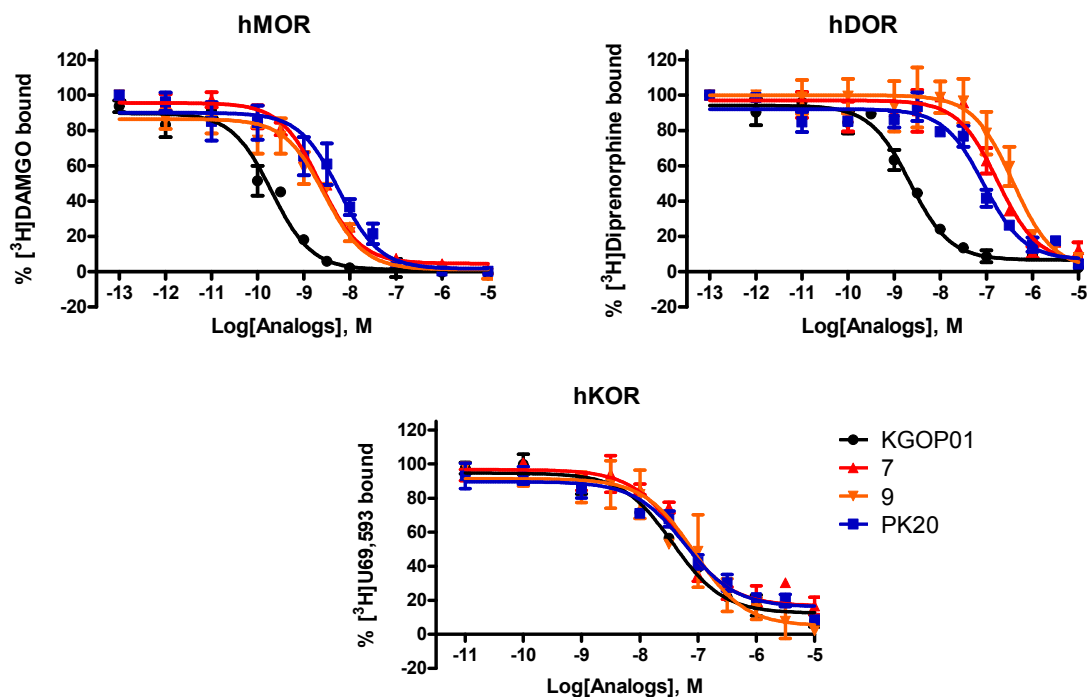




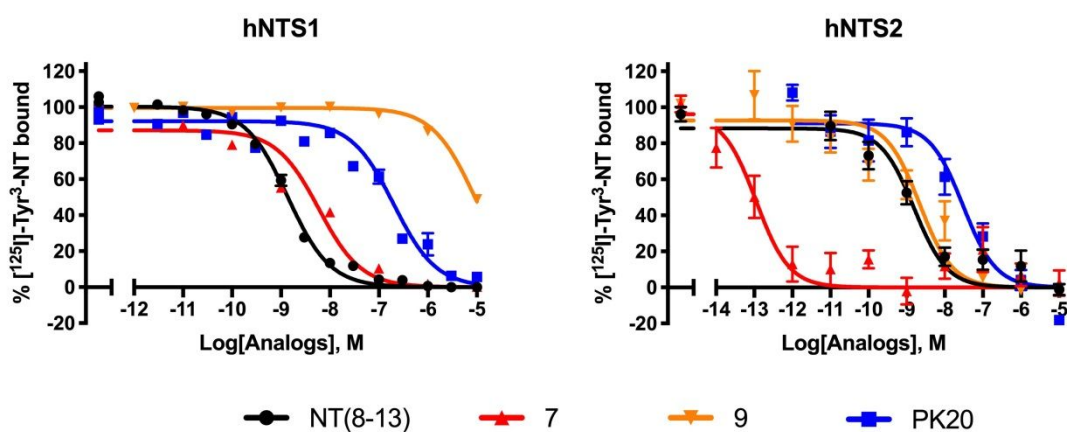




### 3. Competition binding curves



**Figure S1.** Competition binding curves of **KGOP01**, **PK20** and OP-NT hybrid peptides **7** and **9** to human opioid receptors. Concentration-dependent inhibition by test compounds of [<sup>3</sup>H]DAMGO (MOR), [<sup>3</sup>H]diprenorphine (DOR) and [<sup>3</sup>H]U69,593 (KOR) binding to membranes from CHO cells stably expressing hMOR, hDOR or hKOR, determined in radioligand binding assays. Data are means  $\pm$  SEM of three independent experiments performed in duplicate.



**Figure S2.** Competition binding curves of **NT(8-13)**, **PK20** and OP-NT hybrid peptides **7** and **9** to human neurotensin receptors. Concentration-dependent inhibition by test compounds of [<sup>125</sup>I]-NT binding to membranes from CHO cells stably expressing hNTS1 or membranes from 1321N1 cells stably expressing the hNTS2, determined in radioligand binding assays. Data are means  $\pm$  SEM of three independent experiments performed in triplicate.