

**Supporting Information**

**Design, Synthesis and Pharmacological Characterization of  
Heterobivalent Ligands for the Putative 5-HT<sub>2A</sub>/mGlu<sub>2</sub> Receptor  
Complex**

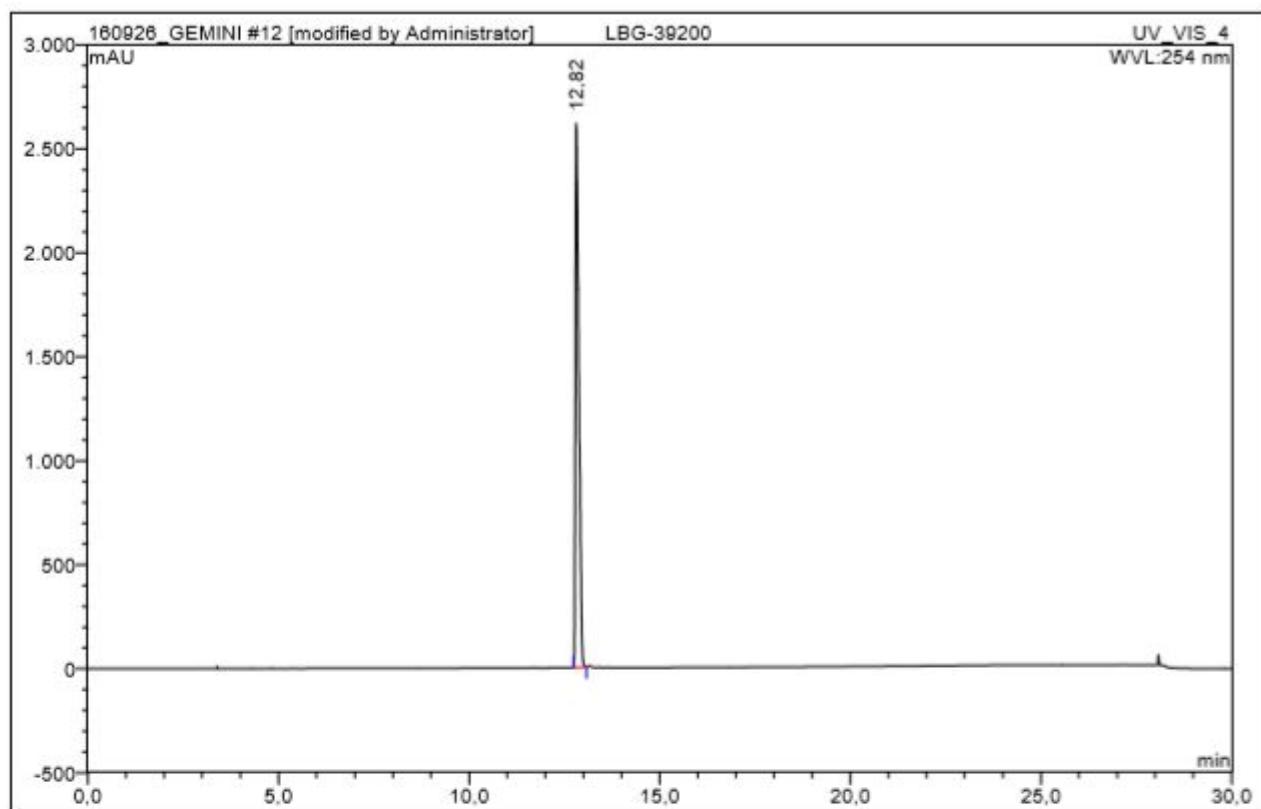
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University of Copenhagen, 2100 Copenhagen ØE, Denmark*

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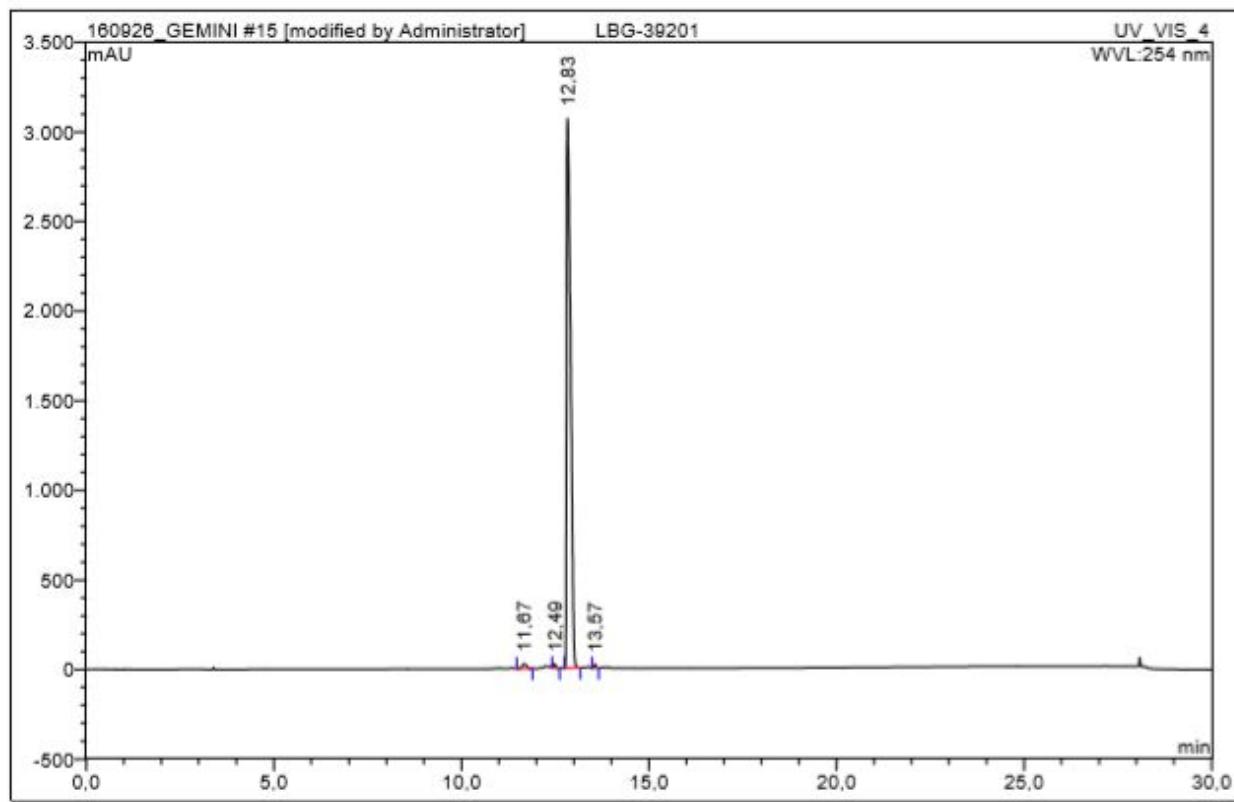
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## Analytical HPLC traces.



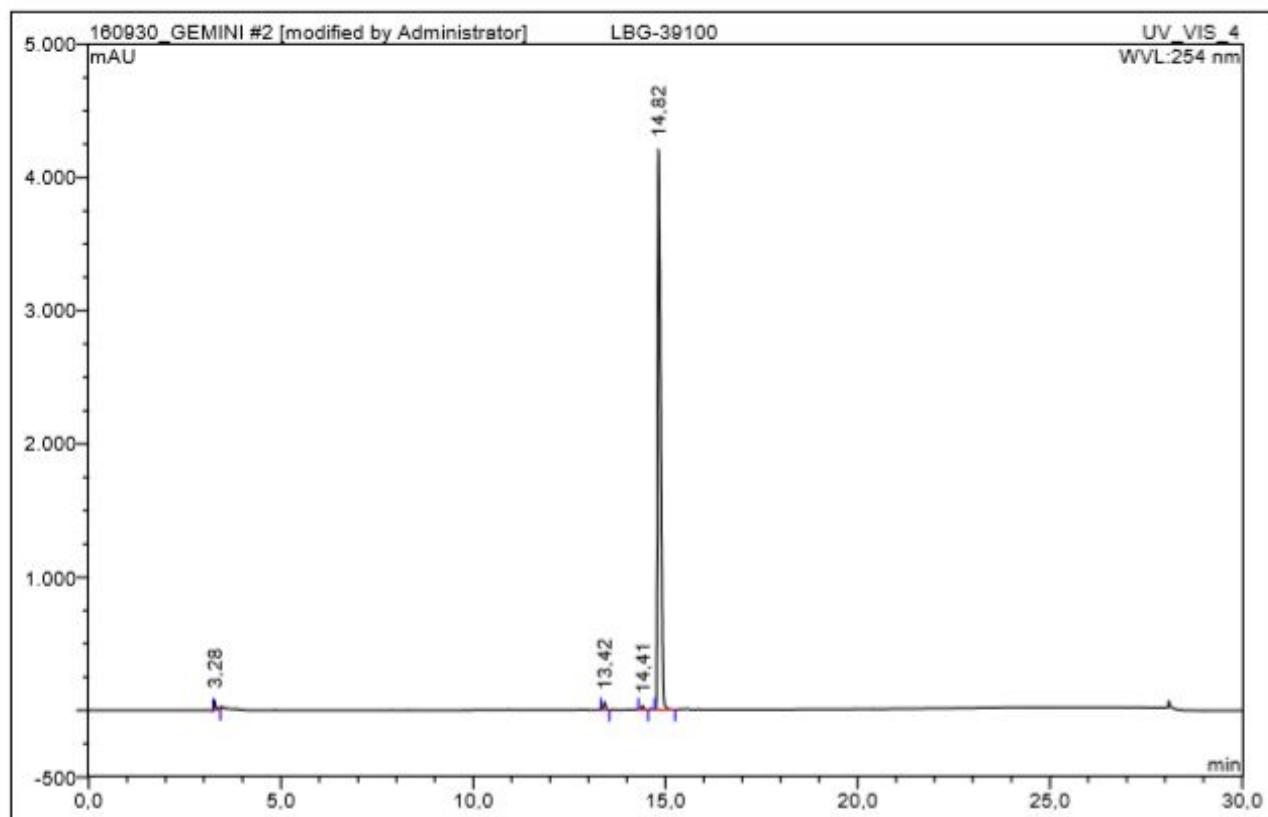
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Resolution(EP)
1	12,82	n.a.	2618,964	271,678	100,00	n.a.	n.a.
Total:			2618,964	271,678	100,00	0,000	

SI Figure 1. Analytical HPLC trace of 9



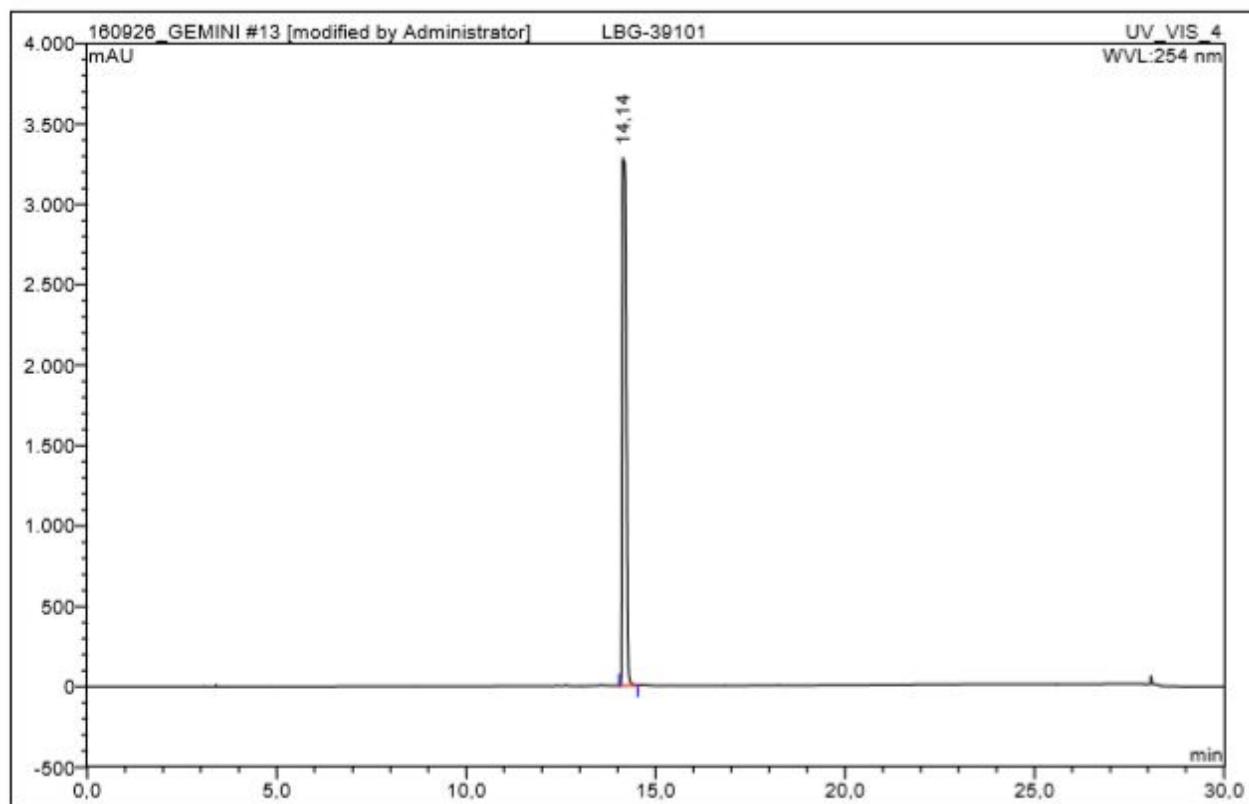
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Resolution(EP)
1	11,67	n.a.	27,138	3,574	0,95	n.a.	5,10
2	12,49	n.a.	23,225	1,560	0,41	n.a.	2,24
3	12,83	n.a.	3068,630	370,887	98,25	n.a.	4,75
4	13,57	n.a.	22,047	1,473	0,39	n.a.	n.a.
<b>Total:</b>			3141,039	377,494	100,00	0,000	

**SI Figure 2.** Analytical HPLC trace of **10**



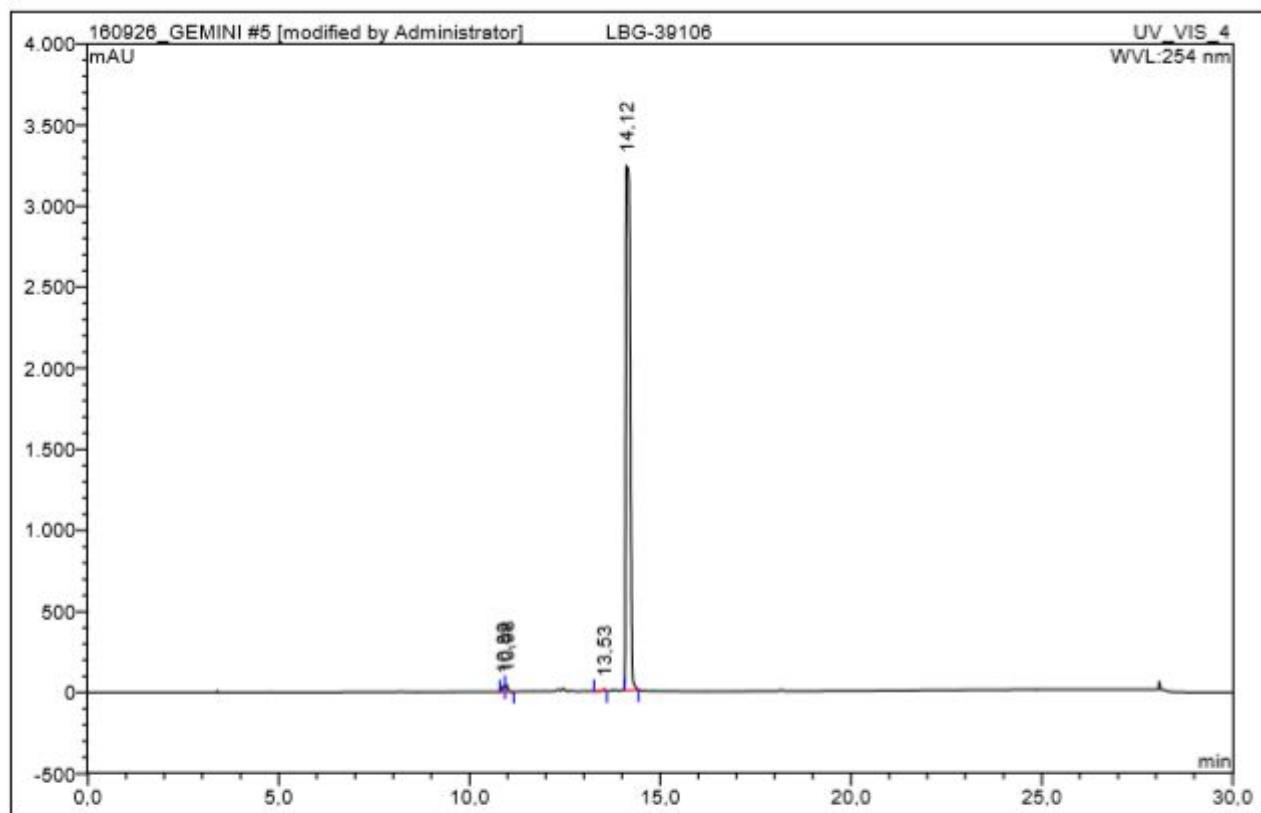
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Resolution(EP)
1	3.28	n.a.	72,804	3,719	0,98	n.a.	119,10
2	13.42	n.a.	56,405	3,694	0,97	n.a.	9,65
3	14.41	n.a.	28,266	1,957	0,52	n.a.	3,38
4	14.82	n.a.	4207,486	370,506	97,53	n.a.	n.a.
<b>Total:</b>			<b>4364,961</b>	<b>379,877</b>	<b>100,00</b>	<b>0,000</b>	

SI Figure 3. Analytical HPLC trace of **31**



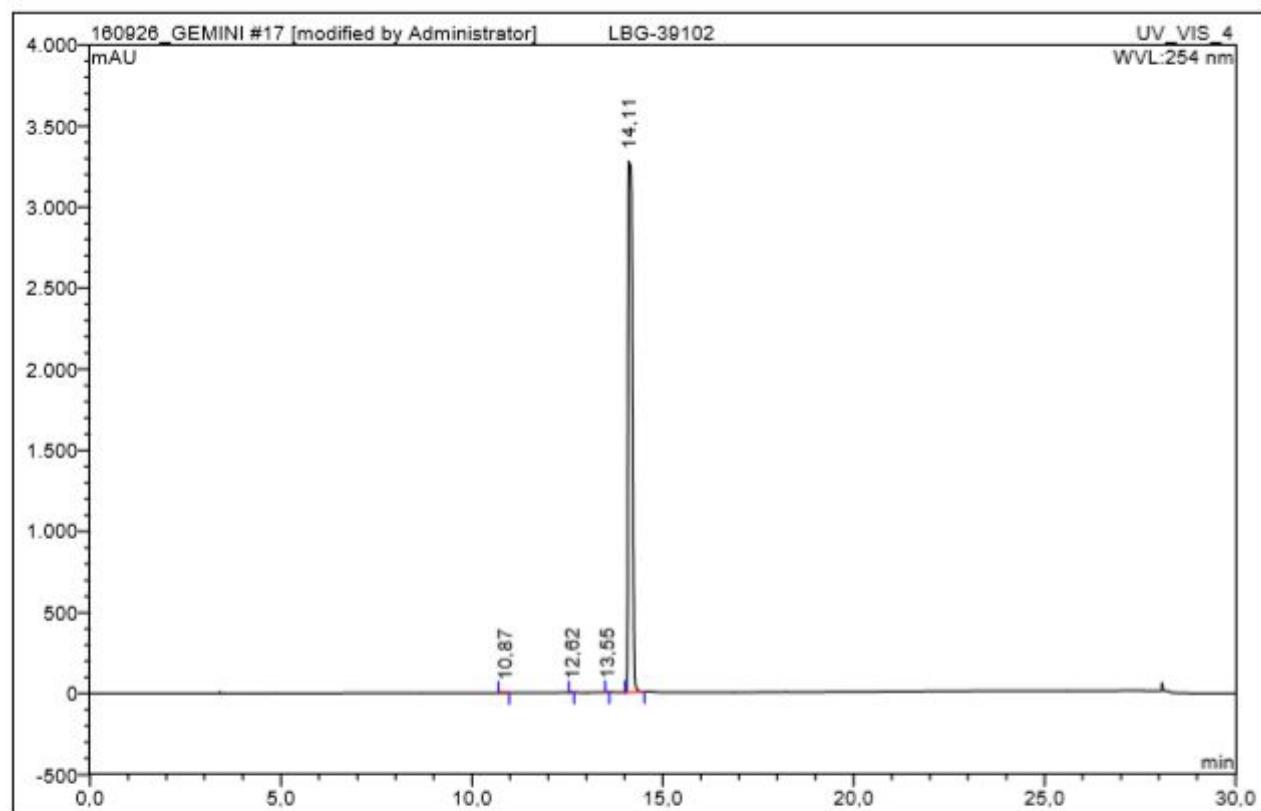
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Resolution(EP)
1	14.14	n.a.	3285,915	410,129	100,00	n.a.	n.a.
<b>Total:</b>			3285,915	410,129	100,00	0,000	

SI Figure 4. Analytical HPLC trace of 32



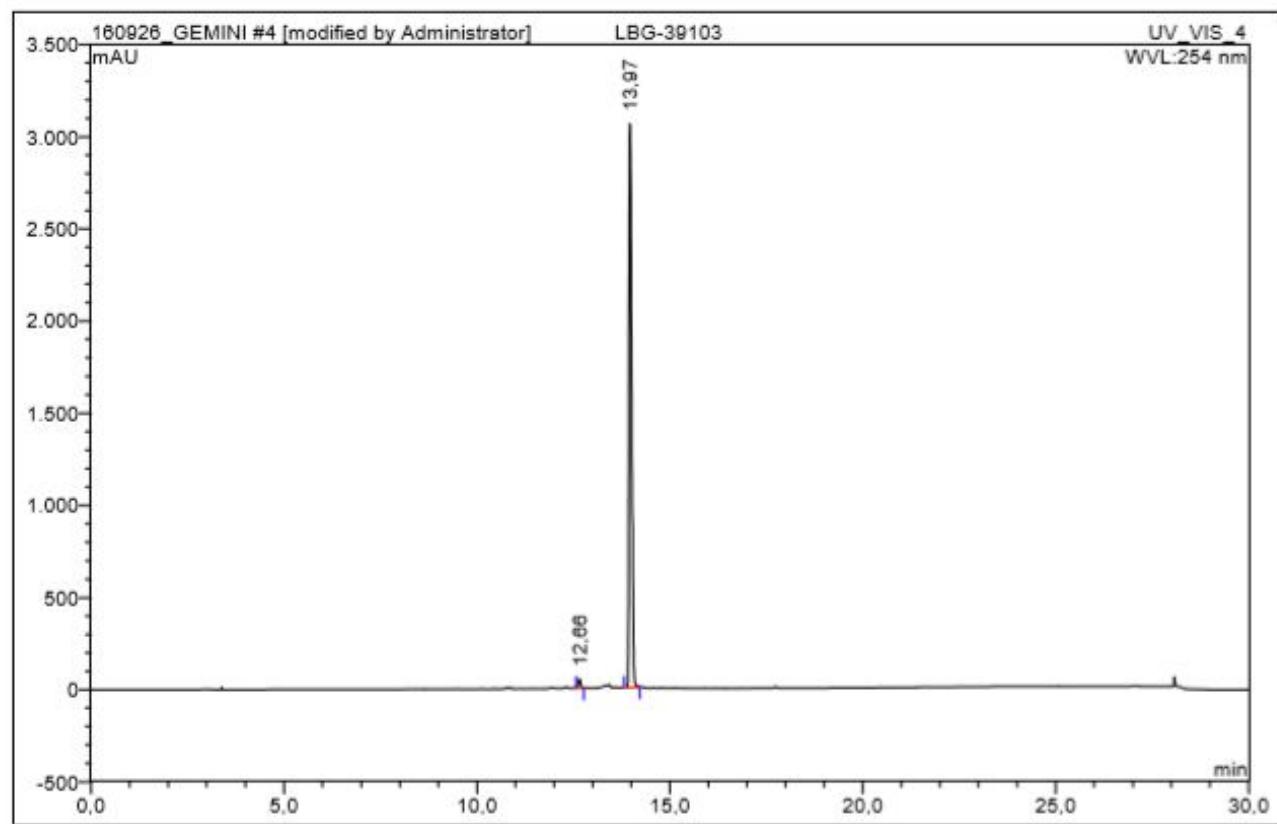
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount %	Resolution(EP)
1	10,89	n.a.	36,050	2,539	0,58	n.a.	n.a.
2	10,98	n.a.	44,619	3,199	0,72	n.a.	n.a.
3	13,53	n.a.	12,089	1,134	0,26	n.a.	3,57
4	14,12	n.a.	3240,407	434,492	98,44	n.a.	n.a.
<b>Total:</b>			<b>3333,165</b>	<b>441,364</b>	<b>100,00</b>	<b>0,000</b>	

SI Figure 5. Analytical HPLC trace of 33



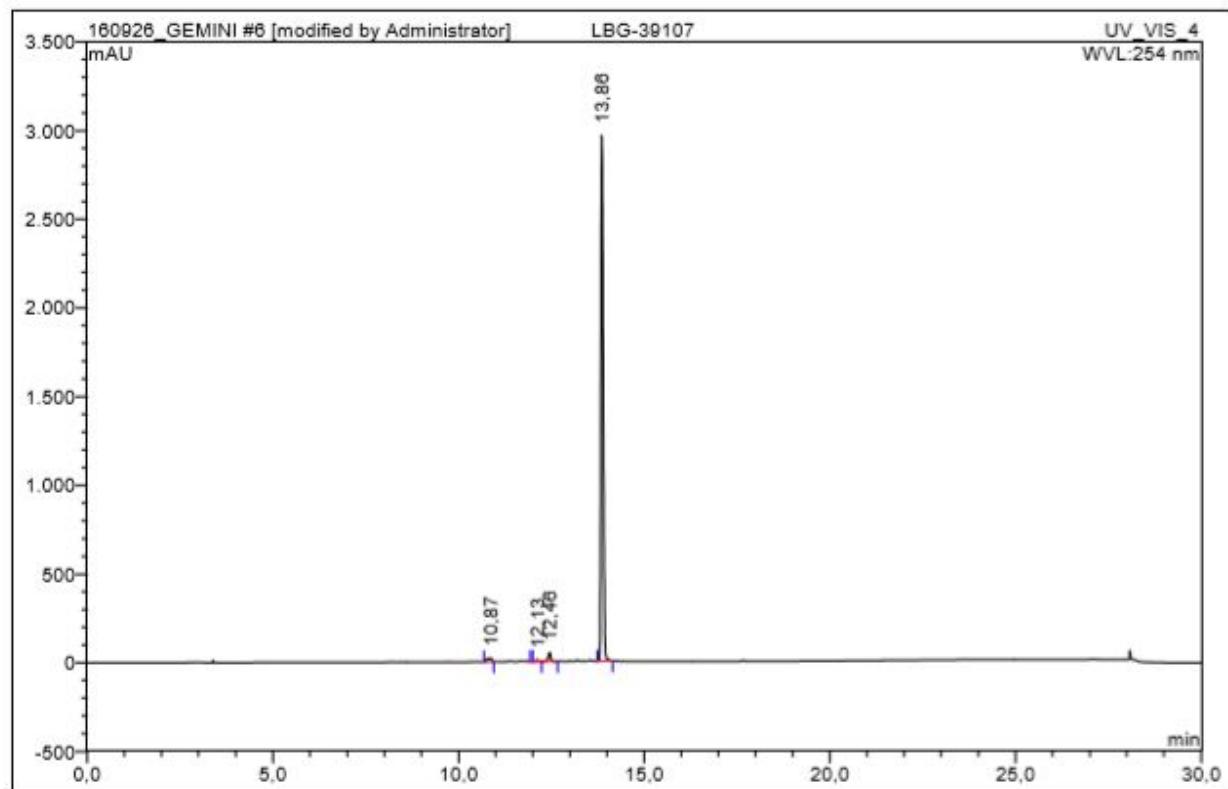
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Resolution(EP)
1	10.87	n.a.	1,558	0,204	0,05	n.a.	9,29
2	12.62	n.a.	5,242	0,362	0,08	n.a.	9,45
3	13.55	n.a.	5,578	0,289	0,07	n.a.	3,71
4	14.11	n.a.	3275,869	430,512	99,80	n.a.	n.a.
<b>Total:</b>			<b>3288,247</b>	<b>431,367</b>	<b>100,00</b>	<b>0,000</b>	

SI Figure 6. Analytical HPLC trace of 34



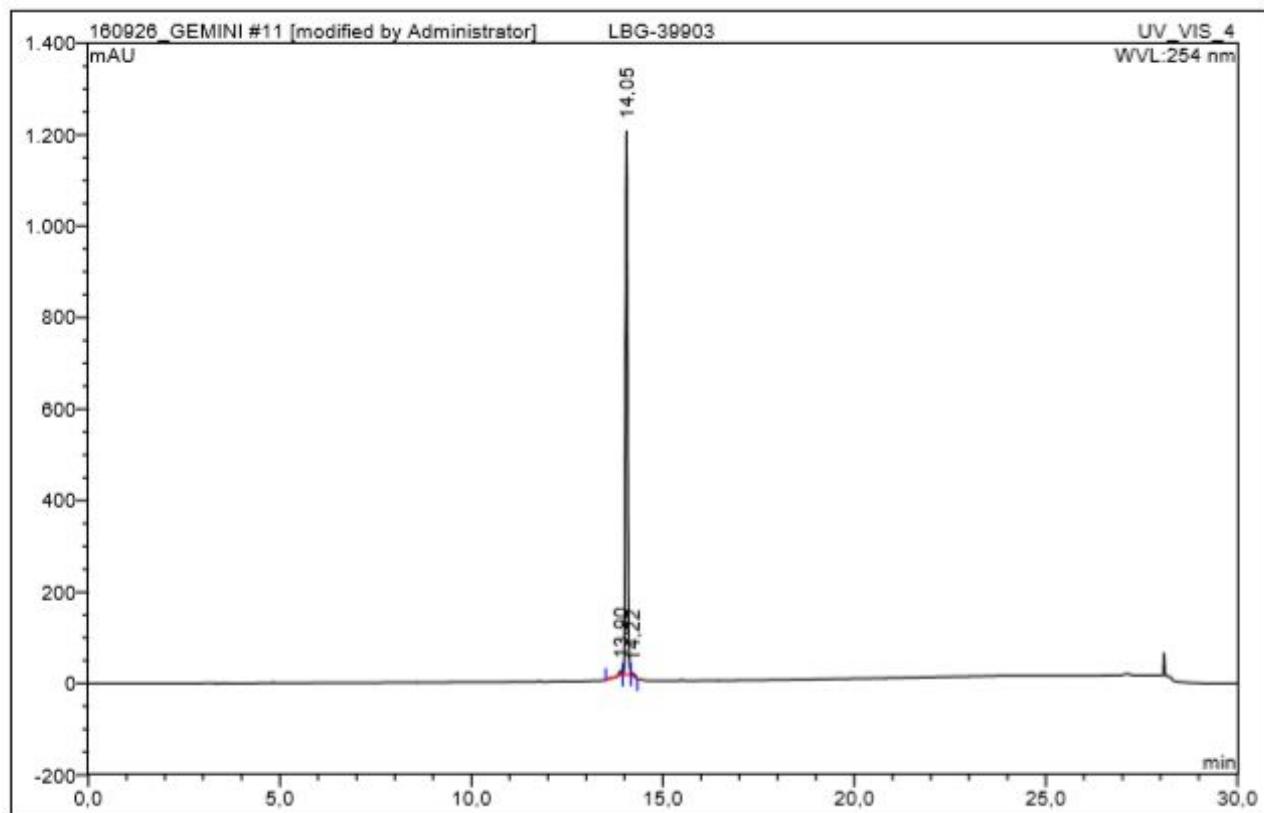
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Resolution(EP)
1	12,66	n.a.	49,037	3,549	1,46	n.a.	10,86
2	13,97	n.a.	3061,579	239,077	98,54	n.a.	n.a.
<b>Total:</b>			<b>3110,616</b>	<b>242,626</b>	<b>100,00</b>	<b>0,000</b>	

SI Figure 7. Analytical HPLC trace of **35**



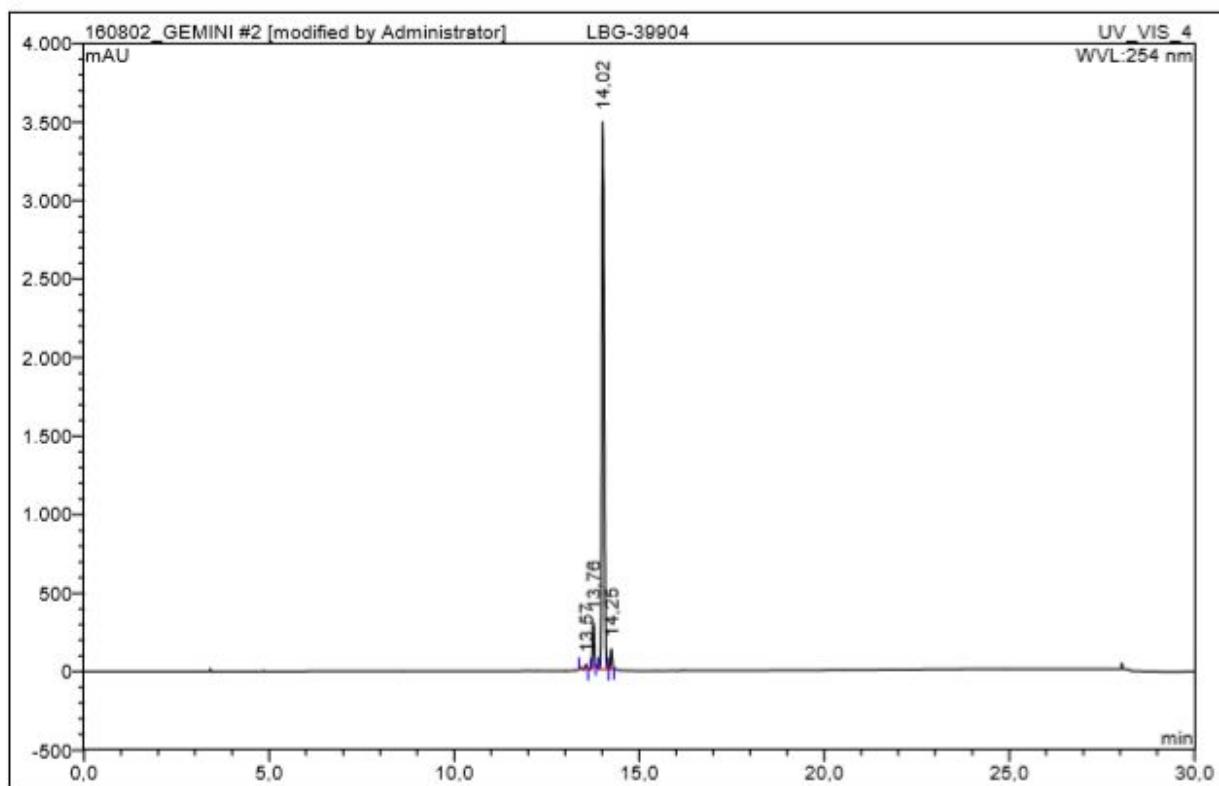
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Resolution(EP)
1	10,87	n.a.	21,254	2,806	1,26	n.a.	8,24
2	12,13	n.a.	10,038	0,858	0,38	n.a.	n.a.
3	12,46	n.a.	50,778	4,423	1,98	n.a.	11,69
4	13,86	n.a.	2966,399	215,507	96,38	n.a.	n.a.
<b>Total:</b>			<b>3048,469</b>	<b>223,594</b>	<b>100,00</b>	<b>0,000</b>	

SI Figure 8. Analytical HPLC trace of 36



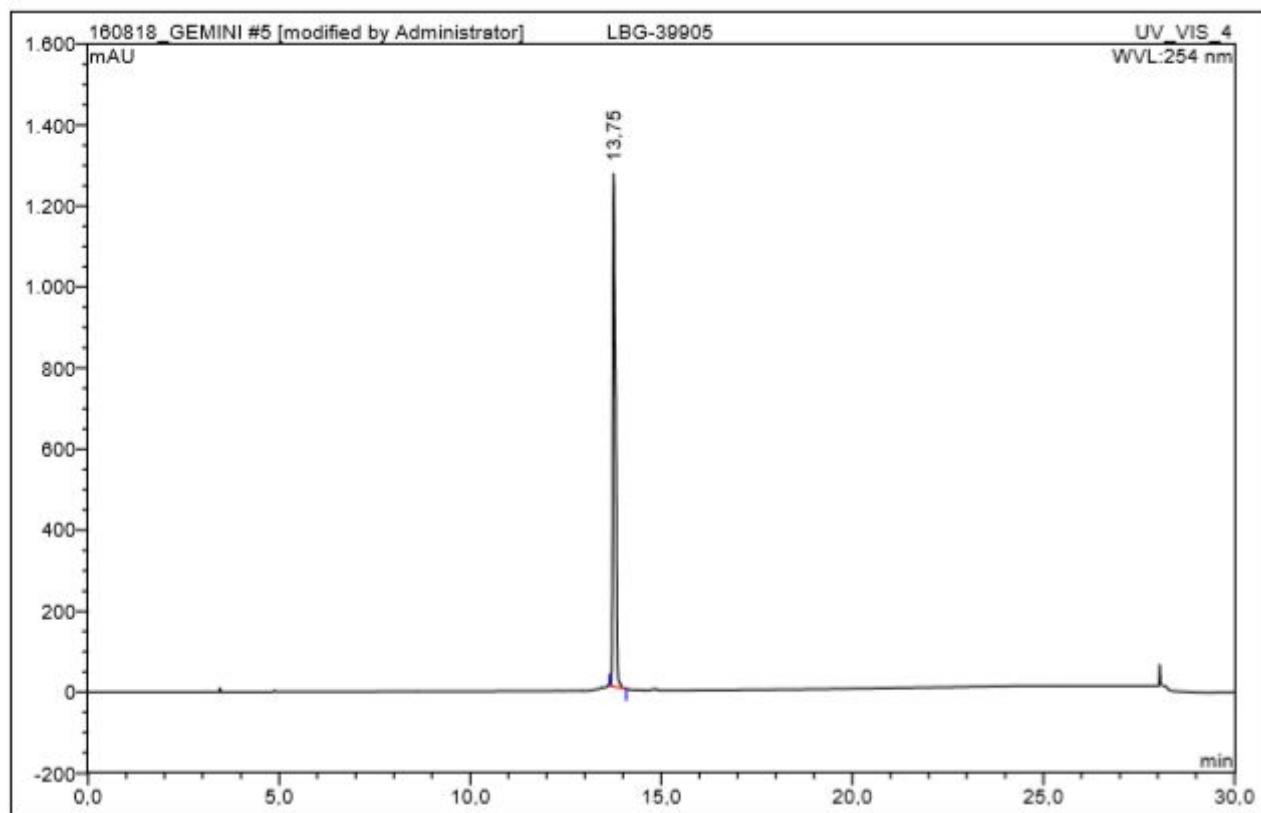
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Resolution(EP)
1	13,90	n.a.	9,798	0,848	1,23	n.a.	1,39
2	14,05	n.a.	1188,884	67,332	98,06	n.a.	1,25
3	14,22	n.a.	4,992	0,481	0,70	n.a.	n.a.
<b>Total:</b>				<b>1203,674</b>	<b>68,660</b>	<b>100,00</b>	<b>0,000</b>

SI Figure 9. Analytical HPLC trace of 45



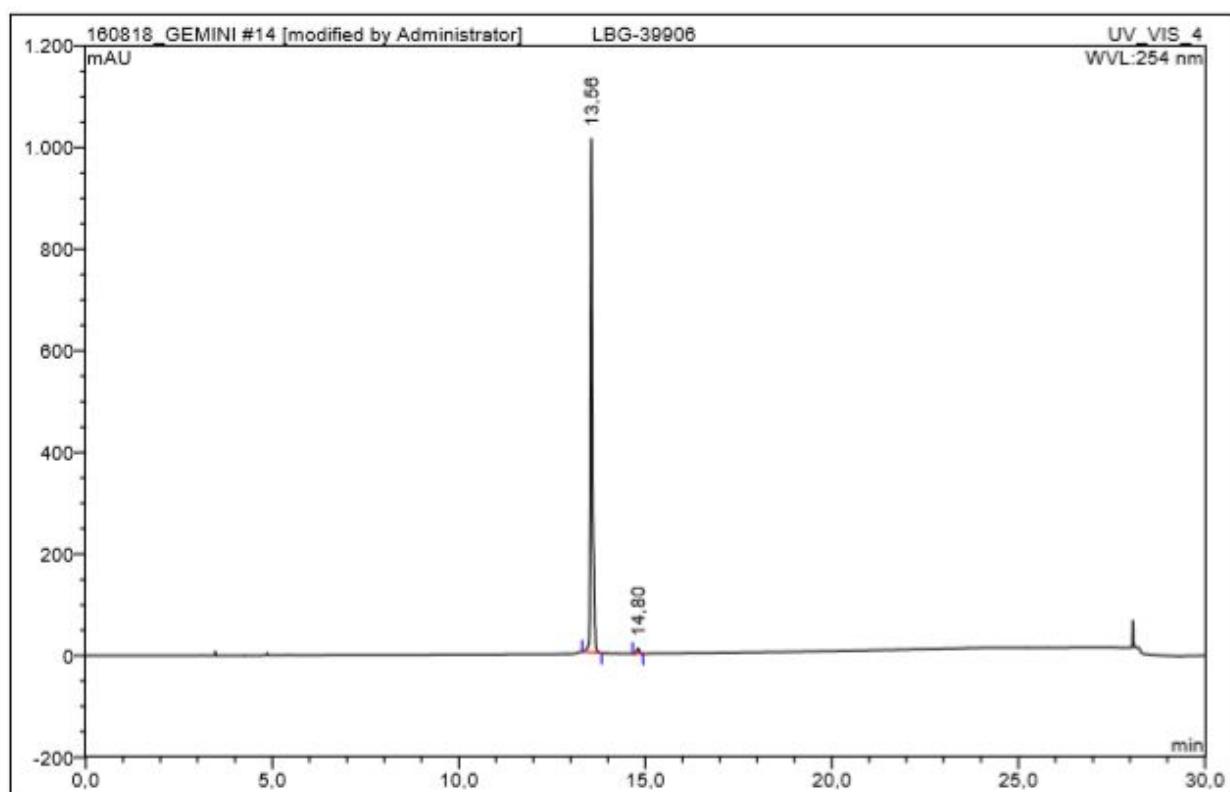
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Resolution(EP)
1	13,57	n.a.	27,553	1,638	0,65	n.a.	2,17
2	13,76	n.a.	282,173	15,313	6,09	n.a.	2,68
3	14,02	n.a.	3485,093	227,480	90,45	n.a.	2,37
4	14,25	n.a.	125,628	7,075	2,81	n.a.	n.a.
<b>Total:</b>			<b>3920,447</b>	<b>251,506</b>	<b>100,00</b>	<b>0,000</b>	

**SI Figure 10.** Analytical HPLC trace of **46**



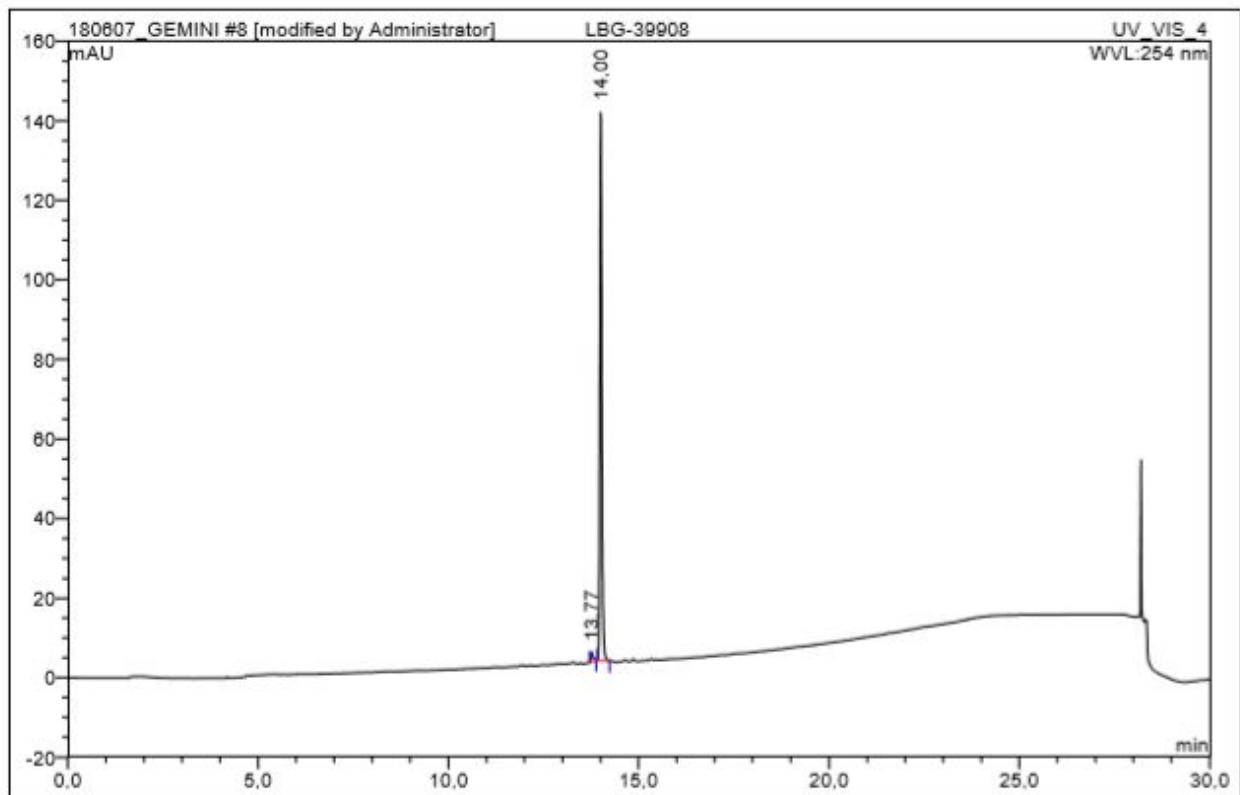
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Resolution(EP)
1	13,75	n.a.	1266,137	104,729	100,00	n.a.	n.a.
Total:			1266,137	104,729	100,00	0,000	

SI Figure 11. Analytical HPLC trace of 47



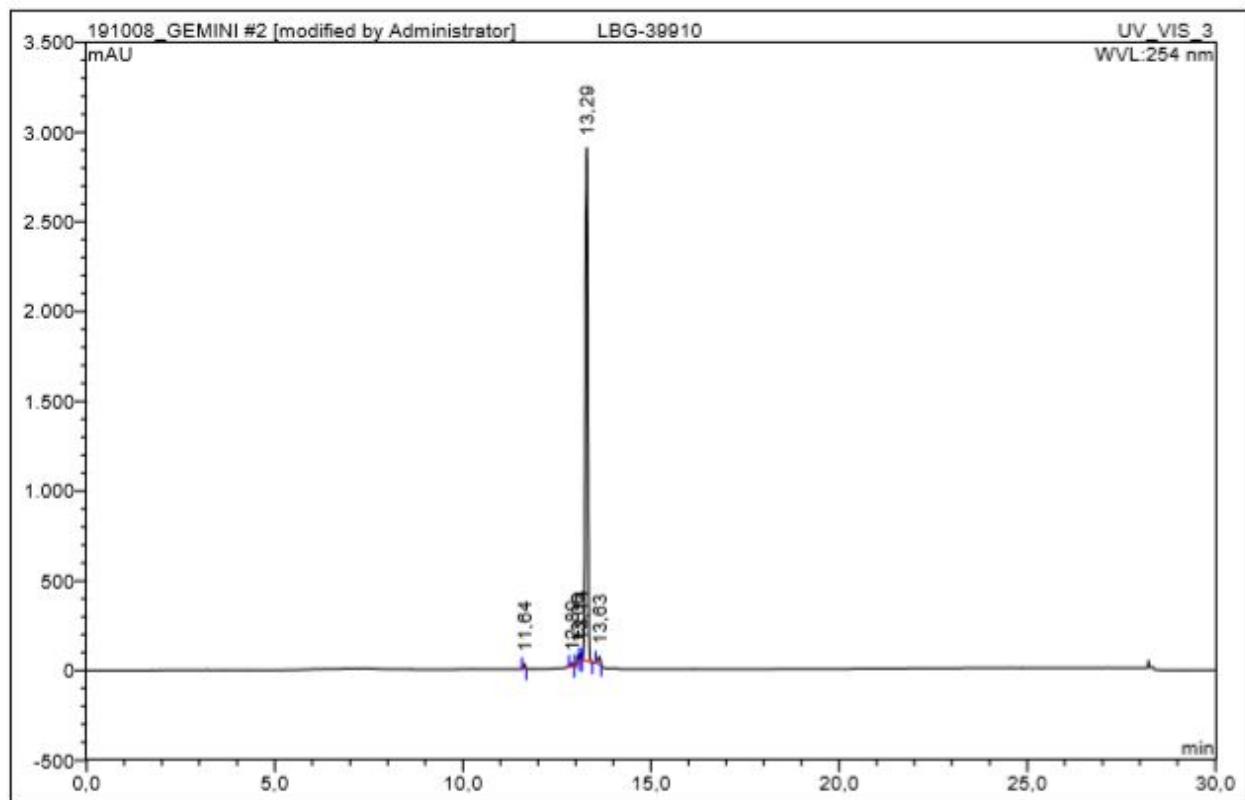
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Resolution(EP)
1	13,56	n.a.	1011,356	63,462	98,96	n.a.	13,45
2	14,80	n.a.	10,318	0,667	1,04	n.a.	n.a.
<b>Total:</b>			<b>1021,674</b>	<b>64,129</b>	<b>100,00</b>	<b>0,000</b>	

SI Figure 12. Analytical HPLC trace of **48**



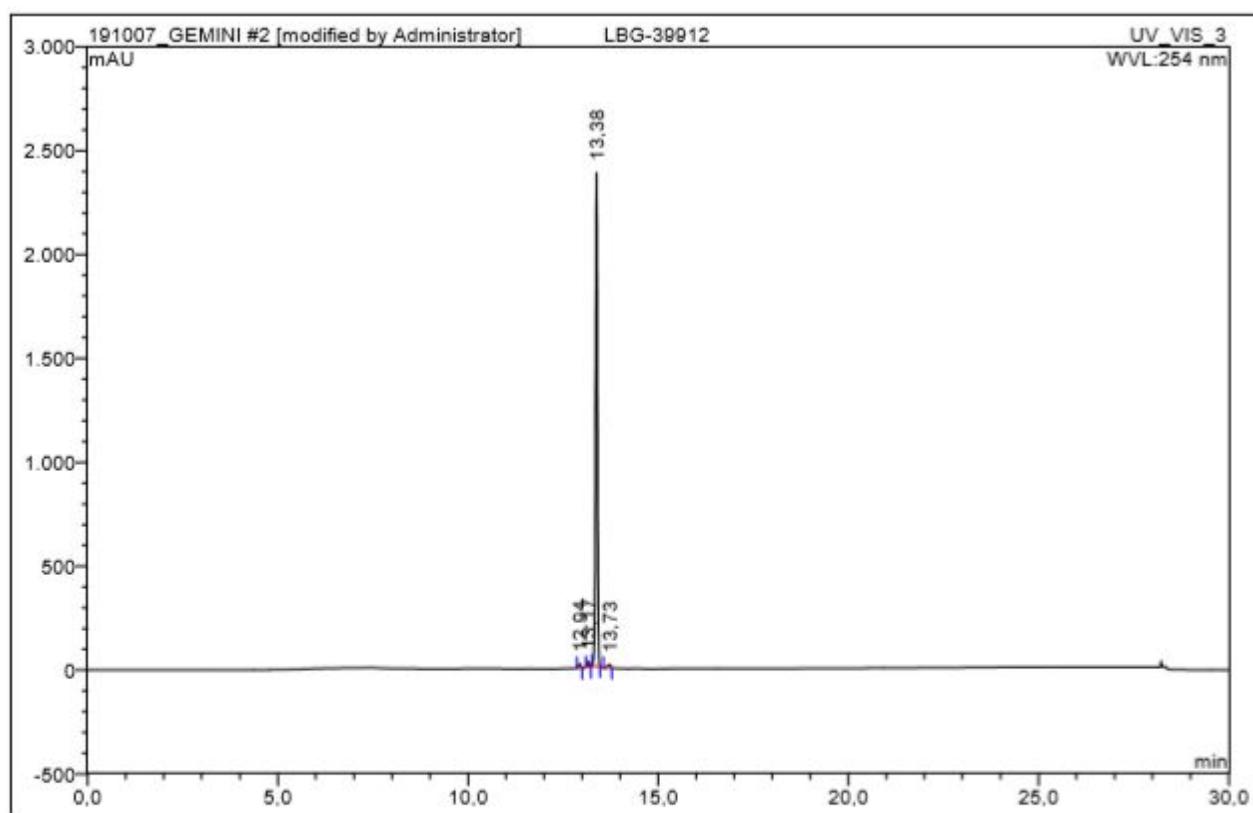
No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Resolution(EP)
1	13.77	n.a.	2,331	0,165	1,93	n.a.	2,54
2	14.00	n.a.	137,869	8,421	98,07	n.a.	n.a.
<b>Total:</b>			140,201	8,586	100,00	0,000	

SI Figure 13. Analytical HPLC trace of **49**



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Resolution(EP)
1	11,64	n.a.	28,128	1,562	0,73	n.a.	13,15
2	12,89	n.a.	18,138	1,126	0,52	n.a.	1,75
3	13,06	n.a.	44,650	2,393	1,11	n.a.	0,85
4	13,14	n.a.	46,877	2,263	1,05	n.a.	1,64
5	13,29	n.a.	2860,076	204,555	95,13	n.a.	3,09
6	13,63	n.a.	43,120	3,116	1,45	n.a.	n.a.
<b>Total:</b>			<b>3040,989</b>	<b>215,017</b>	<b>100,00</b>	<b>0,000</b>	

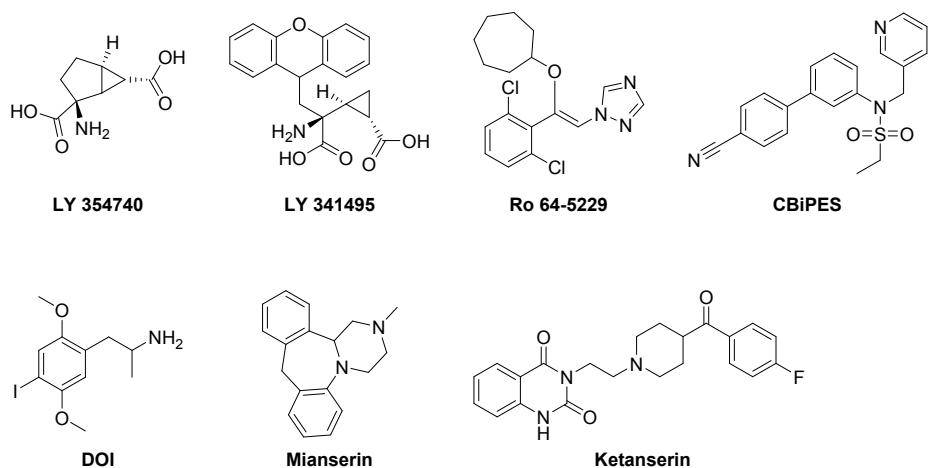
SI Figure 14. Analytical HPLC trace of **50**



No.	Ret.Time min	Peak Name	Height mAU	Area mAU*min	Rel.Area %	Amount	Resolution(EP)
1	12,94	n.a.	19,633	1,062	0,75	n.a.	2,47
2	13,17	n.a.	27,728	1,756	1,24	n.a.	2,19
3	13,38	n.a.	2377,157	137,668	96,96	n.a.	2,76
4	13,73	n.a.	15,507	1,498	1,05	n.a.	n.a.
<b>Total:</b>			<b>2440,024</b>	<b>141,983</b>	<b>100,00</b>	<b>0,000</b>	

SI Figure 15. Analytical HPLC trace of **51**

## Chemical structures of the reference ligands.



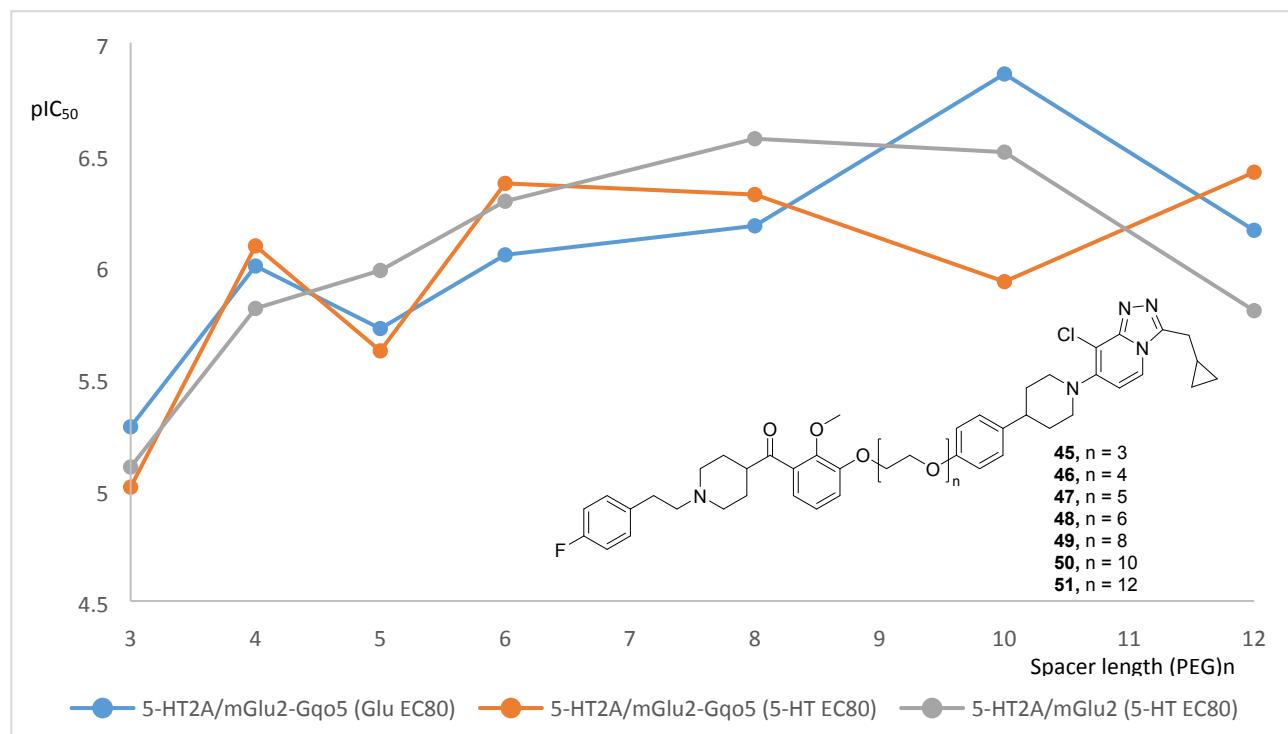
**SI Figure 16.** Chemical structure of the reference ligands.

## mGlu<sub>2</sub>-Gqo5 - Basal characterization.

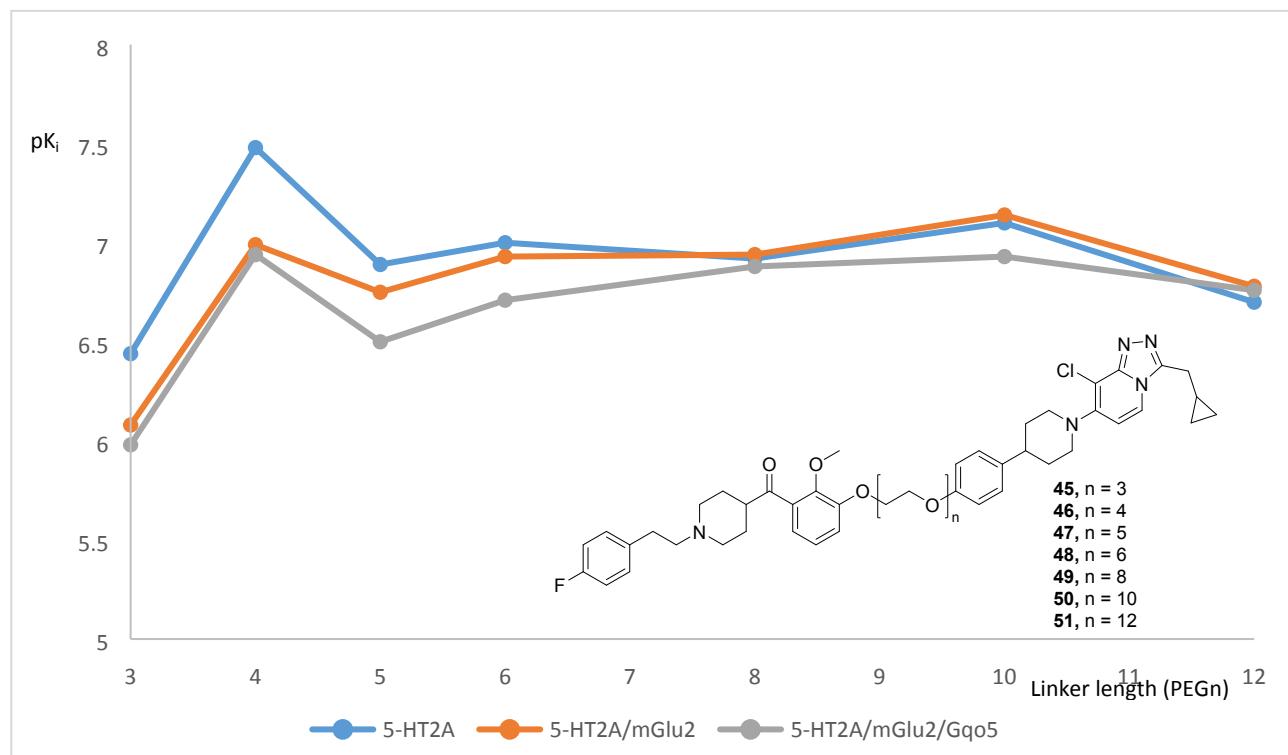
Agonists or ago-PAM	EC <sub>50</sub> (nM) [pEC <sub>50</sub> ± SEM]	R <sub>max</sub> ± SEM	n
Glu	3500 [5.45 ± 0.02]	100	17
LY 354740	24 [7.60 ± 0.09]	96 ± 3	5
CBiPES (tested as agonist)	1300 [5.87 ± 0.13]	90 ± 7	5
CBiPES (tested as PAM)	28 [7.54 ± 0.07]	93 ± 5	5
Comp. Antag. or NAM	IC <sub>50</sub> (nM) [pIC <sub>50</sub> ± SEM]		n
LY 341495	3.8 [8.42 ± 0.09]		4
Ro 64-5229	26 [7.58 ± 0.13]		4

**SI Table 1.** Functional properties of reference ligands at the mGlu<sub>2</sub>/Gqo5-HEK293 cell line in the Ca<sup>2+</sup>/Fluo-4 assay. EC<sub>50</sub> values for agonists and ago-PAMs and IC<sub>50</sub> values for competitive antagonists and NAMs are given in nM with pEC<sub>50</sub> ± S.E.M. and pIC<sub>50</sub> ± S.E.M. values in brackets, respectively. R<sub>max</sub> values for agonists and ago-PAMs are given in % of R<sub>max</sub> of Glu determined at the same plate. In the antagonist/NAM experiments, Glu EC<sub>80</sub> was used as agonist concentration. In the PAM experiments with CBiPES, the compound was co-applied with Glu EC<sub>10</sub>.

## Potency/Affinity-spacer length correlation.



**SI Figure 17.** Antagonist potencies of compounds **45-51** plotted against spacer length in (PEG)<sub>n</sub> 5-HT<sub>2A</sub>/mGlu<sub>2</sub>- and 5-HT<sub>2A</sub>/mGlu<sub>2</sub>/Gqo5-HEK293 cells in the Ca<sup>2+</sup>/Fluo-4 assay



**SI Figure 18.** Binding affinities of compounds **45-51** plotted against spacer length in (PEG)<sub>n</sub> at 5-HT<sub>2A</sub>-, 5-HT<sub>2A</sub>/mGlu2- and 5-HT<sub>2A</sub>/mGlu2/Gqo5-HEK293 cell membranes in the [<sup>3</sup>H]ketanserin binding assay