Supporting Information

Large-Scale Screening of Foods for Glucose-Derived *β*-Carboline Alkaloids by Stable Isotope Dilution LC-MS/MS

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Supporting Information Table S1. Instrument and method parameters for the chromatographic separation of the investigated β-carboline alkaloids by semipreparative LC with ultraviolet detection. ACN: acetonitrile, id: inner diameter, NH_4OAc: ammonium acetate.

LC system	Jasco (Jasco, Groß-Umstadt, Germany)		
column	Eclipse XDB-C18 (250 x 9.4 mm id, particle size 5 µm; Agilent, Waldbronn, Germany)		
flow rate	6.25 mL/min		
detection wavelength	250 nm		
mobile phase	ACN/H_2O 95/5 (V/V) + 5 mM NH_4OAc (**A**), H_2O/ACN 95/5 (V/V) + 5 mM NH_4OAc (**B**)		
gradient	time [min]	**A** [%]	**B** [%]
	00.0	0	100
	18.0	17	83
	24.0	17	83
	29.0	22	78
	29.1	100	0
	33.0	100	0
	33.1	0	100
	38.0	0	100

Supporting Information Table S2. LC-MS/HRMS conditions applied for the analysis of the synthesized β-carboline alkaloids. FA: formic acid, HCD: higher-energy collision induced dissociation, HESI: heated electrospray ionization.

LC system	Nexera XR LC system (Shimadzu, Duisburg, Germany)		
column	Reprosil-Gold C18 (150 x 2 mm id, particle size 3 µm; Dr. Maisch GmbH, Ammerbuch, Germany) equipped with a 5 x 2 mm guard column of the same material		
flow rate	0.35 mL/min		
mobile phase	ACN + 0.1% FA (**A**), H_2O + 0.1% FA (**B**)		
gradient	time [min]	**A** [%]	**B** [%]
	00.00	5	95
	11.00	50	50
	13.00	95	5
	15.00	95	5
	15.01	5	95
	20.00	5	95
ionization type (polarity)	HESI (positive)		
resolution	30 000		
heater temperature	350 °C		
capillary temperature	350 °C		
sheath gas	40 arbitrary units		
aux gas	20 arbitrary units		
sweep gas	10 arbitrary units		
spray voltage	4000 V		
capillary voltage	20 V		
tube lens voltage	125 V		
fragmentation type	HCD (35-55% relative normalized collision energy)		
activation time	30 ms		
isolation width	1.7 *m/z*		

Supporting Information Table S3. Concentrations of the investigated β-carboline alkaloids and their stable isotope-labeled analogues in the calibration solutions.

solution	c(Tan E) [ng/mL]	c(Tan F) [ng/mL]	c(DH-Tan E/F) [ng/mL]	c($^{13}C_6$-Tan E) [ng/mL]	c($^{13}C_6$-Tan F) [ng/mL]	c($^{13}C_6$-DH-Tan E/F) [ng/mL]
1	0.05	0.05	0.05	25.00	25.00	25.00
2	0.10	0.10	0.10	25.00	25.00	25.00
3	0.25	0.25	0.25	25.00	25.00	25.00
4	0.50	0.50	0.50	25.00	25.00	25.00
5	0.75	0.75	0.75	25.00	25.00	25.00
6	1.00	1.00	1.00	25.00	25.00	25.00
7	2.50	2.50	2.50	25.00	25.00	25.00
8	5.00	5.00	5.00	25.00	25.00	25.00
9	7.50	7.50	7.50	25.00	25.00	25.00
10	10.00	10.00	10.00	25.00	25.00	25.00
11	25.00	25.00	25.00	25.00	25.00	25.00
12	50.00	50.00	50.00	25.00	25.00	25.00
13	65.00	65.00	65.00	25.00	25.00	25.00

Supporting Information Table S4. Detailed list of food items analyzed for β-carboline alkaloids Tan E, Tan F and DH-Tan E/F as well as corresponding analyte levels.

sample number	sample name	mean (range) [mg/kg]					
		Tan E		Tan F		DH-Tan E/F	
1	apple pink lady	<LOD		<LOD		<LOD	
2	apple golden delicious	<LOD		<LOD		<LOD	
5	beer 1	0.02	(<0.01)	0.02	(<0.01)	<LOQ	
6	beer 2	0.01	(<0.01)	0.01	(<0.01)	0.01	(<0.01)
7	tea infusion Assam	<LOD		<LOD		<LOD	
8	tea infusion Darjeeling	<LOD		<LOD		<LOD	
9	chicken filet strips	<LOD		<LOQ		<LOD	
10	chicken nuggets	<LOQ		0.01	(<0.01)	0.04	(<0.01)
11	chunky canned tomatoes 1	0.07	(<0.01)	0.05	(<0.01)	0.01	(<0.01)
12	chunky canned tomatoes 2	0.09	(<0.01)	0.06	(<0.01)	0.02	(<0.01)
13	pasta durum wheat fusilli	<LOD		<LOQ		<LOQ	
14	pasta egg spaetzle	<LOD		<LOD		<LOD	
15	cheese cheddar	<LOD		<LOD		<LOD	
16	cheese gouda	<LOD		<LOD		<LOD	
17	dried chili	0.53	(0.02)	0.49	(0.01)	0.14	(<0.01)
18	peperoni	<LOD		<LOD		<LOD	
19	coffee beverage 1	0.01	(<0.01)	0.01	(<0.01)	0.01	(<0.01)
20	coffee beverage 2	<LOD		<LOQ		<LOQ	
21	semi-skimmed milk 1	<LOD		<LOD		<LOD	
22	semi-skimmed milk 2	<LOD		<LOD		<LOD	
25	hard-boiled egg	<LOD		<LOD		<LOD	
26	fried egg	<LOD		<LOD		<LOD	
27	fried eggplant 1	0.02	(<0.01)	0.03	(<0.01)	0.01	(<0.01)
28	fried eggplant 2	0.03	(<0.01)	0.03	(<0.01)	0.01	(<0.01)
29	soy yoghurt 1	<LOD		<LOD		<LOD	
30	soy yoghurt 2	<LOD		<LOD		<LOD	

Supporting Information Table S4. Continued from previous page.

sample number	sample name	mean (range) [mg/kg]					
		Tan E		Tan F		DH-Tan E/F	
33	strawberry jam 1	0.02	(<0.01)	0.02	(<0.01)	<LOQ	
34	strawberry jam 2	0.01	(<0.01)	0.01	(<0.01)	<LOD	
35	apple juice 1	<LOQ		<LOQ		<LOD	
36	apple juice 2	<LOD		<LOD		<LOD	
41	tomato juice seasoned salted 1	0.70	(0.01)	0.49	(<0.01)	0.06	(<0.01)
42	tomato juice spicy carrots 2	0.56	(0.05)	0.39	(0.04)	0.06	(<0.01)
43	tomato ketchup 1	1.81	(0.17)	1.35	(0.06)	0.19	(0.02)
44	tomato ketchup 2	2.16	(0.28)	1.53	(0.09)	0.16	(0.03)
45	whole-grain bread	0.02	(<0.01)	0.02	(<0.01)	0.10	(<0.01)
46	whole-grain bread pumpernickel	0.33	(0.04)	0.28	(0.02)	0.09	(<0.01)
47	red pepper	<LOD		<LOD		<LOD	
48	yellow pepper	<LOD		<LOD		<LOD	
51	pork cutlet 1	0.01	(<0.01)	0.01	(<0.01)	0.01	(<0.01)
52	pork cutlet 2	<LOQ		<LOQ		0.02	(<0.01)
53	potato boiled 1	<LOD		<LOD		<LOD	
54	potato boiled 2	<LOD		<LOD		<LOD	
55	fries 1	<LOD		<LOD		<LOD	
56	fries 2	<LOD		<LOQ		<LOQ	
57	oven-baked crisps	0.04	(<0.01)	0.03	(<0.01)	0.01	(<0.01)
58	crisps fried	<LOQ		<LOQ		<LOQ	
59	cornflakes cereal	0.05	(<0.01)	0.04	(<0.01)	0.02	(<0.01)
60	honey wheat cereal	0.25	(0.01)	0.24	(0.01)	0.06	(<0.01)
61	pizza salami 1	0.01	(<0.01)	0.01	(<0.01)	0.01	(<0.01)
62	pizza salami 2	0.05	(<0.01)	0.04	(<0.01)	0.01	(<0.01)
63	lasagne 1	0.25	(0.02)	0.20	(0.01)	0.03	(<0.01)
64	lasagne 2	0.06	(<0.01)	0.05	(<0.01)	<LOQ	

Supporting Information Table S4. Continued from previous page.

sample number	sample name	mean (range) [mg/kg]					
		Tan E		Tan F		DH-Tan E/F	
65	packet soup chicken	<LOD		<LOD		<LOD	
66	packet soup mushrooms	<LOD		<LOD		<LOD	
67	sesame seeds hulled	<LOD		<LOD		<LOD	
68	sesame seeds unpeeled	<LOD		<LOD		<LOD	
69	soy sauce 1	0.45	(0.02)	0.41	(<0.01)	0.06	(<0.01)
70	soy sauce 2	0.82	(0.14)	0.81	(0.12)	<LOQ	
71	pickled dried tomato	0.70	(0.02)	0.52	(0.05)	0.02	(<0.01)
72	dried tomato	1.51	(0.12)	1.43	(0.09)	0.01	(<0.01)
73	chili sauce 1	1.81	(<0.01)	1.35	(0.04)	0.20	(0.02)
74	chili sauce 2	0.54	(<0.01)	0.43	(0.03)	0.04	(<0.01)
75	tomato paste 1	6.75	(0.54)	5.07	(0.18)	0.75	(0.02)
76	tomato paste 2	5.63	(0.47)	4.61	(0.09)	0.74	(0.09)
77	tomato sauce 1	0.22	(0.02)	0.16	(0.01)	0.01	(<0.01)
78	tomato sauce 2	0.25	(0.01)	0.19	(<0.01)	0.02	(<0.01)
81	wheat bread white bread rolls	<LOQ		0.01	(<0.01)	0.02	(<0.01)
82	wheat bread white baguette	<LOD		<LOQ		0.05	(<0.01)
83	tea infusion organic herbal tea 1	<LOD		<LOD		<LOD	
84	tea infusion herbal tea 2	<LOD		<LOD		<LOD	
85	cola 1	<LOD		<LOD		<LOD	
86	cola 2	<LOD		<LOD		<LOD	
87	orange juice 1	0.01	(<0.01)	0.01	(<0.01)	<LOD	
88	orange juice 2	<LOQ		0.01	(<0.01)	<LOD	
89	red wine temperonillo	<LOD		<LOD		<LOD	
90	red wine dornfelder	<LOD		<LOD		<LOD	
91	pickled grilled peppers 1	0.03	(<0.01)	0.03	(<0.01)	<LOD	
92	pickled grilled peppers 2	<LOD		<LOD		<LOD	
93	liquid seasoning 1	0.49	(0.04)	0.46	(0.07)	0.10	(<0.01)
94	liquid seasoning 2	<LOD		<LOD		<LOD	

Supporting Information Table S5. Individual chronic daily intake values of the β-carboline alkaloids Tan E, Tan F and DH-Tan E/F corresponding to consumption of the analyzed foods by consideration of weighed-mean chronic consumption data for adults (EFSA Comprehensive European Food Consumption Database).[23]

sample number	FoodEx Level 4 category	calculated chronic daily intake [ng/day]		
		Tan E	Tan F	DH-Tan E/F
1/2	apple (Malus domesticus)	54.6	63.0	33.9
5/6	beer, regular	1593.5	1296.7	750.6
7/8	black tea, infusion	100.2	115.6	62.3
9/10	chicken meat (Gallus domesticus)	50.8	148.6	385.2
11/12	tomato chutney	5.0	3.6	0.9
13	pasta, wheat flour, without eggs	7.1	29.9	16.1
14	pasta, wheat flour, with eggs	2.9	3.3	1.8
15	cheese, Cheddar	1.7	2.0	1.1
16	cheese, Gouda	4.5	5.1	2.8
17	chili powder	1.2	1.1	0.3
18	chilli pepper (Capsicum frutescens)	0.1	0.1	0.1
19/20	coffee (beverage)	648.4	980.8	642.6
21/22	cow milk, 1 - 2.9% fat (semi-skimmed milk)	69.0	79.6	42.9
25	whole egg, chicken	13.2	15.3	8.2
26	fried eggs	2.9	3.3	1.8
27/28	aubergines (egg plants) (Solanum melongena)	43.1	43.6	17.2
29/30	soy yoghurt	0.3	0.4	0.2
33/34	jam, strawberries (Fragaria × ananassa)	3.6	4.5	0.4
35/36	juice, apple	109.7	126.6	29.3
41/42	juice, tomato	601.7	420.7	58.9
43/44	tomato ketchup	2885.3	2093.5	252.7
45	wheat bread, brown	302.0	305.9	1268.5
46	rye bread, wholemeal	1850.9	1571.3	490.0
51/52	pork / piglet meat (Sus scrofa)	143.3	162.2	364.8
53/54	potato boiled	36.8	42.5	22.9
55/56	french fries	11.1	29.9	16.1

Supporting Information Table S5. Continued from previous page.

sample number	FoodEx Level 4 category	calculated chronic daily intake [ng/day]		
		Tan E	Tan F	DH-Tan E/F
57/58	potato crisps	49.1	45.5	14.5
59	corn flakes	48.9	41.9	25.0
60	wheat flakes	269.4	251.4	68.3
61/62	pizza and pizza-like pies	68.0	53.8	18.7
65/66	ready to eat soups	11.7	13.5	7.3
67/68	sesame seed (Sesamum indicum syn. S. orientale)	0.0	0.0	0.0
69/70	soy sauce	128.8	123.9	6.0
71/72	sun-dried tomatoes	67.4	59.6	1.0
73/74	savoury sauces	3325.7	2510.0	343.2
75/76	tomato puree	17329.8	13539.1	2086.1
77/78	vegetable sauce	628.3	479.7	44.6
81	wheat rolls, white	46.4	96.1	256.4
82	wheat bread, white	39.8	167.7	1813.7
83/84	herbal tea, infusion	52.6	60.7	32.7
85/86	cola beverages, caffeinic	44.1	50.9	27.4
87/88	juice, orange	238.3	274.1	18.5
89/90	wine, red	28.3	32.6	17.6
91/92	peppers, paprika (Capsicum annuum)	105.2	80.5	3.8
93/94	seasoning or extracts	22.6	21.5	4.8
63/64	lasagne	no data		

Supporting Information Equation S1. Equation used for the determination of the limit of detection based on matrix calibration in the course of LC-MS/MS analysis of β-carboline alkaloids in the sample blank matrix.

$$x_{LOD} = \frac{s_y}{m} \cdot t \cdot \sqrt{\frac{1}{\hat{N}} + \frac{1}{N} + \frac{\bar{x}^2}{Q_{xx}}}$$

t: t-value: one-sided, f = N - 2, p = 0.95
f: degrees of freedom
p: significance level
s_y: residual standard deviation
N: number of calibrants
$\hat{N}$: number of replicates
m: slope of linear regression
Q_{xx}: sum of average square deviation, $Q_{xx} = \sum (x_i - \bar{x})^2$

x_i: calibrant concentration
$\bar{x}$: mean concentration

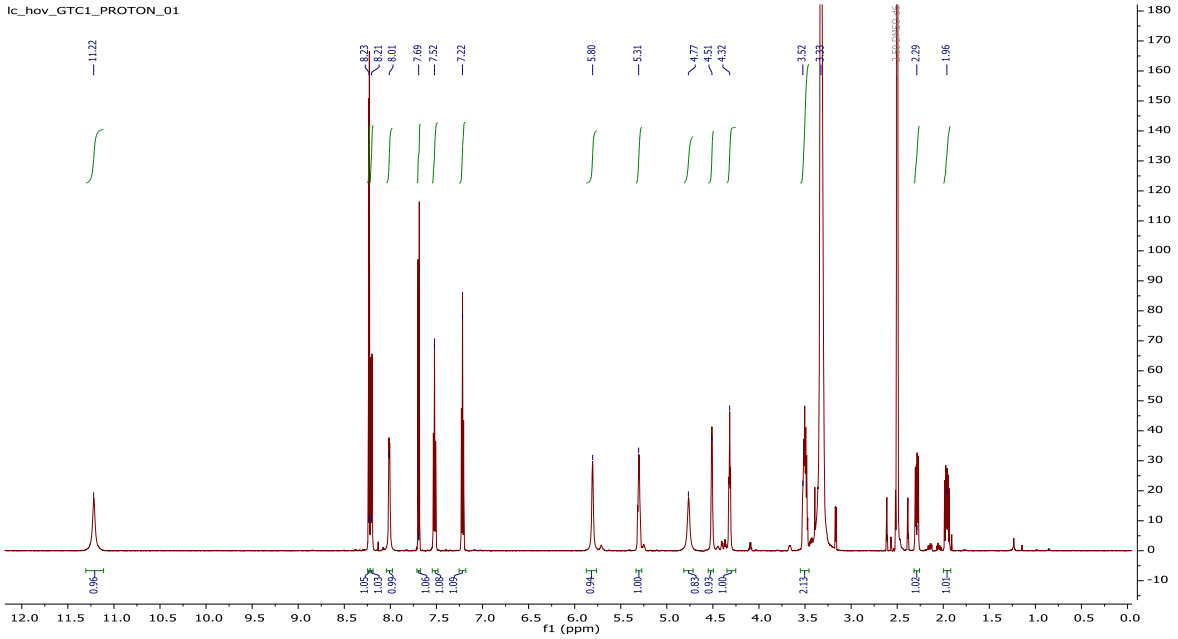
Supporting Information Equation S2. Equation used for the determination of the limit of quantitation based on matrix calibration in the course of LC-MS/MS analysis of β-carboline alkaloids in the sample blank matrix.

$$x_{LOQ} = k \cdot \frac{s_y}{m} \cdot t \cdot \sqrt{\frac{1}{\hat{N}} + \frac{1}{N} + \frac{(k \cdot x_{LOD} - \bar{x})^2}{Q_{xx}}}$$

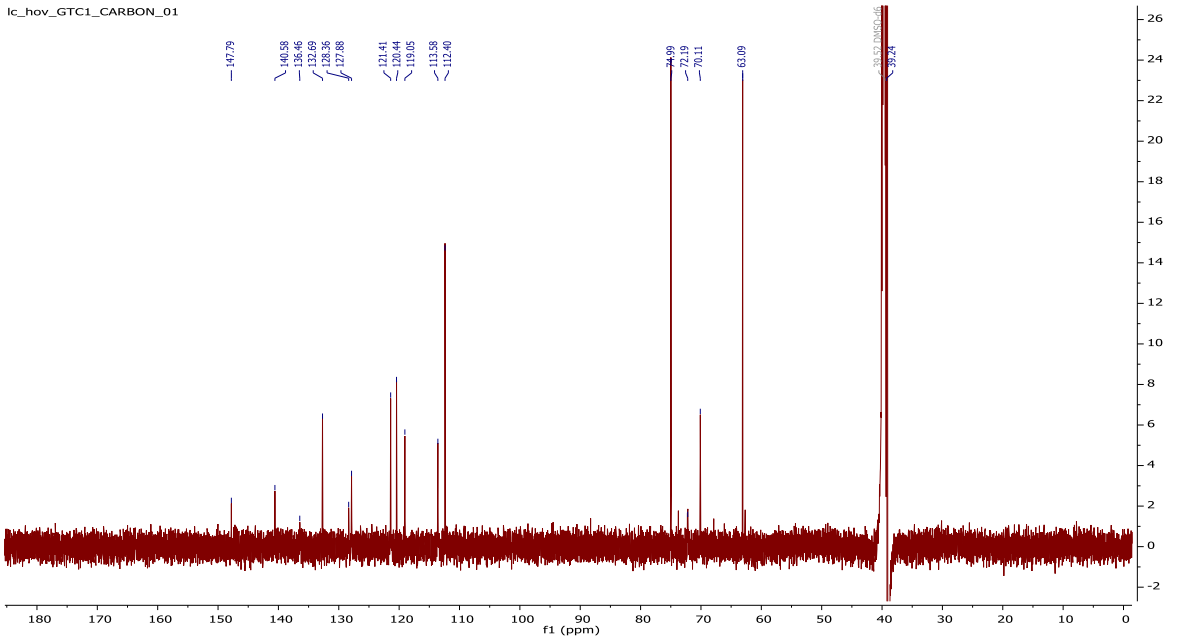
k = 3, corresponding to a maximum accepted relative uncertainty of 33.3%
t: t-value: two-sided, f = N - 2, p = 0.95
f: degrees of freedom
p: significance level
s_y: residual standard deviation
N: number of calibrants
$\hat{N}$: number of replicates
m: slope of linear regression
Q_{xx}: sum of average square deviation, $Q_{xx} = \sum (x_i - \bar{x})^2$

x_i: calibrant concentration
$\bar{x}$: mean concentration

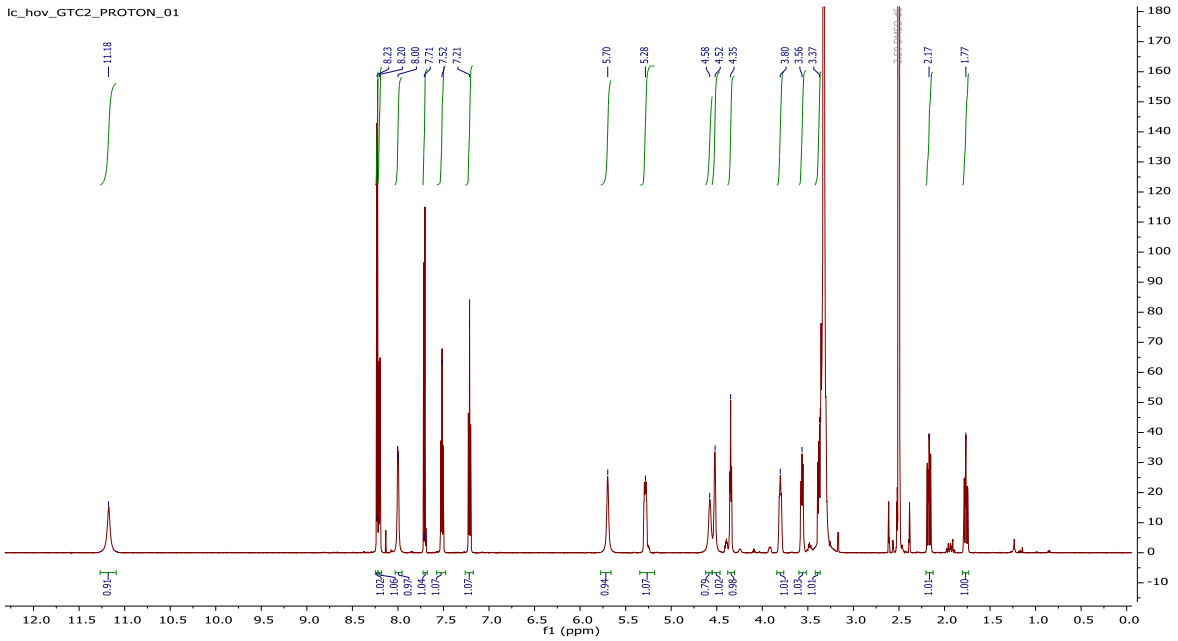
Supporting Information Figure S1. ^{1}H-NMR spectrum of Tan E obtained in DMSO-d_6 at 600 MHz.



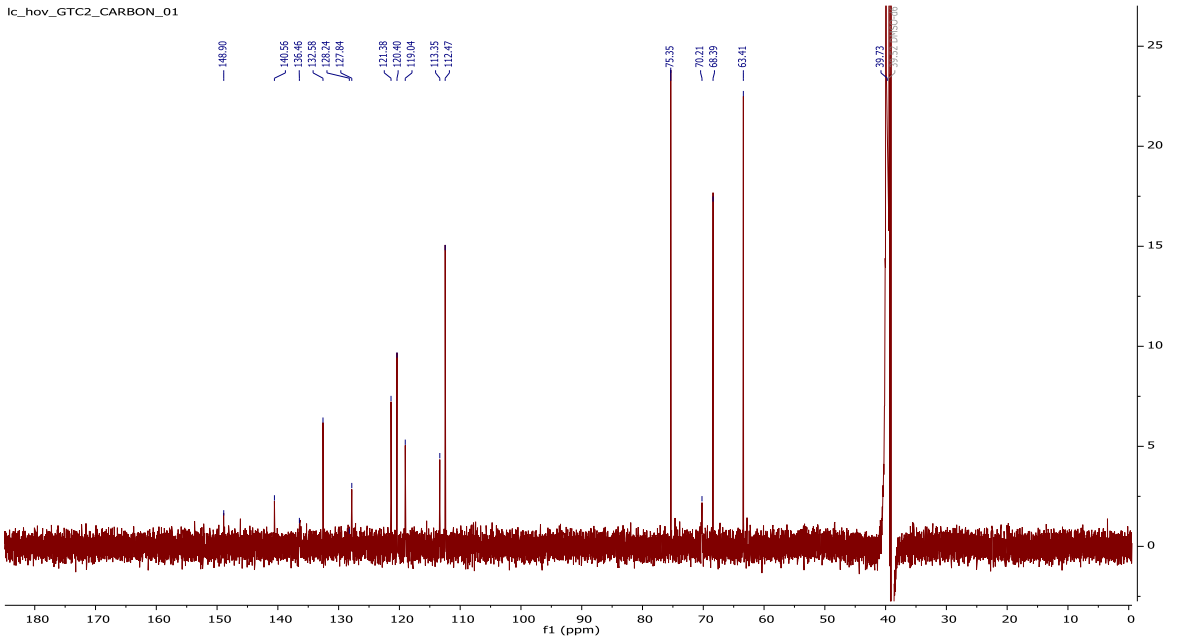
Supporting Information Figure S2. ^{13}C-NMR spectrum of Tan E obtained in DMSO-d_6 at 150 MHz.



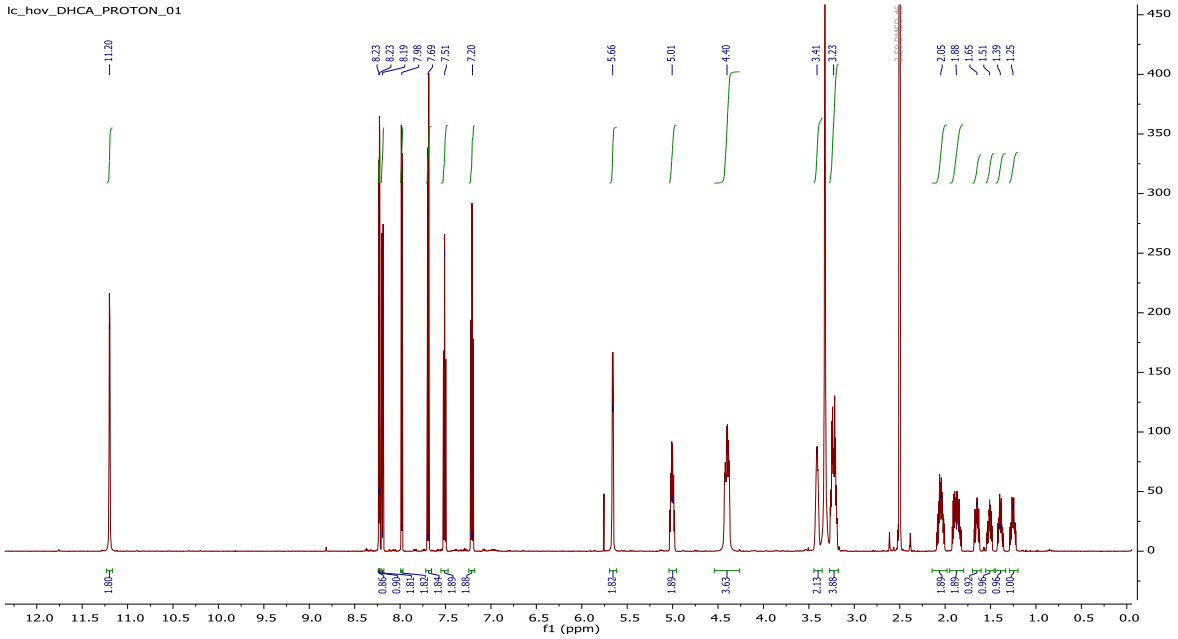
Supporting Information Figure S3. ^{1}H-NMR spectrum of Tan F obtained in DMSO-d_6 at 600 MHz.



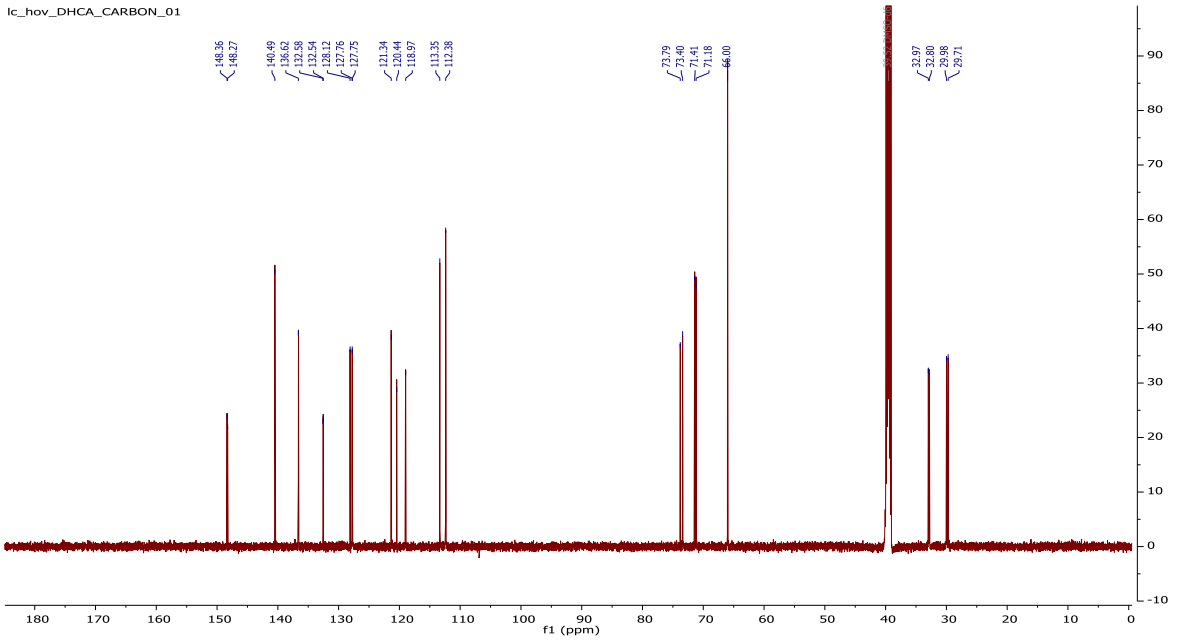
Supporting Information Figure S4. ^{13}C-NMR spectrum of Tan F obtained in DMSO-d_6 at 150 MHz.



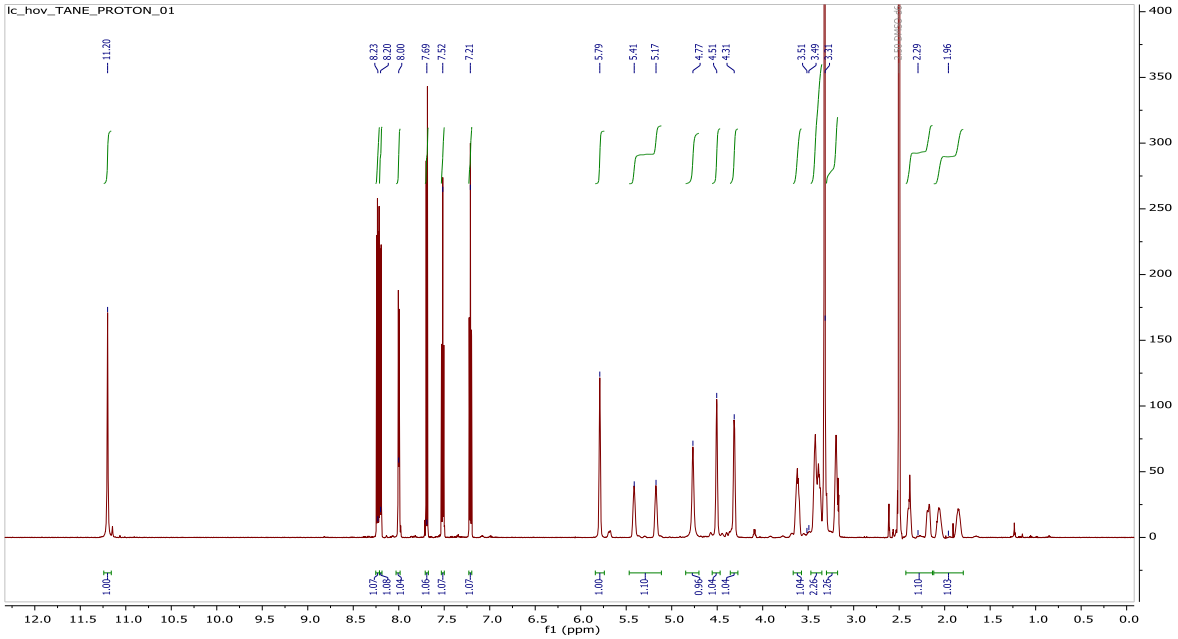
Supporting Information Figure S5. ^{1}H-NMR spectrum of DH-Tan E/F (diastereomeric mixture) obtained in DMSO-d_6 at 600 MHz.



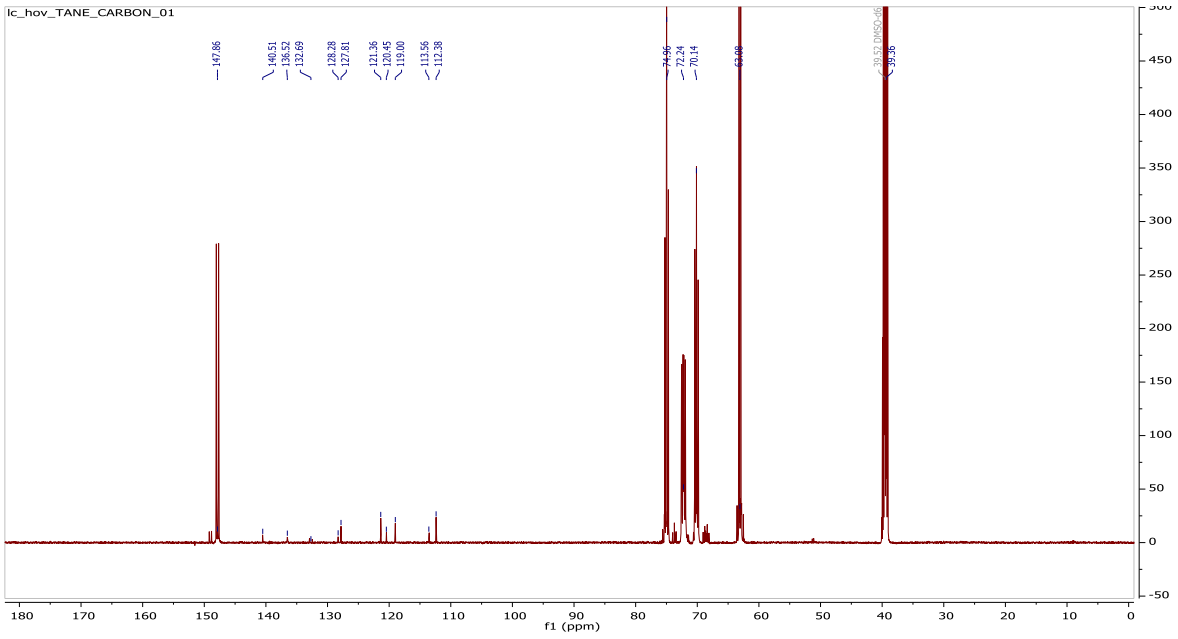
Supporting Information Figure S6. ^{13}C-NMR spectrum of DH-Tan E/F (diastereomeric mixture) obtained in DMSO-d_6 at 150 MHz.



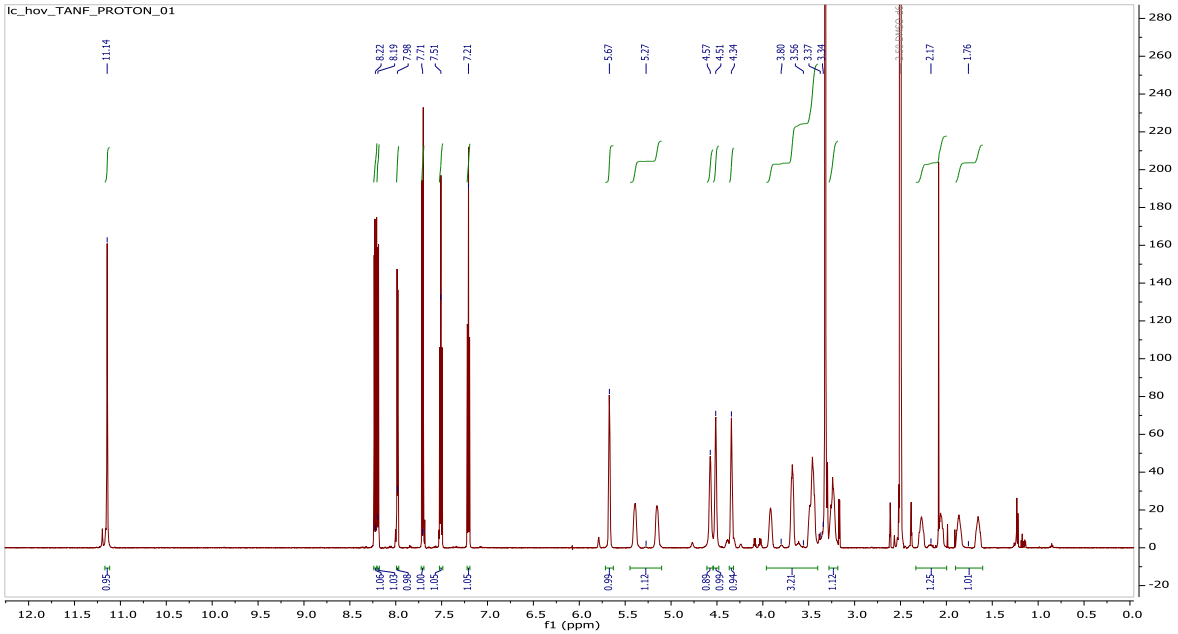
Supporting Information Figure S7. ^{1}H-NMR spectrum of $^{13}C_6$-Tan E obtained in DMSO-d_6 at 600 MHz.



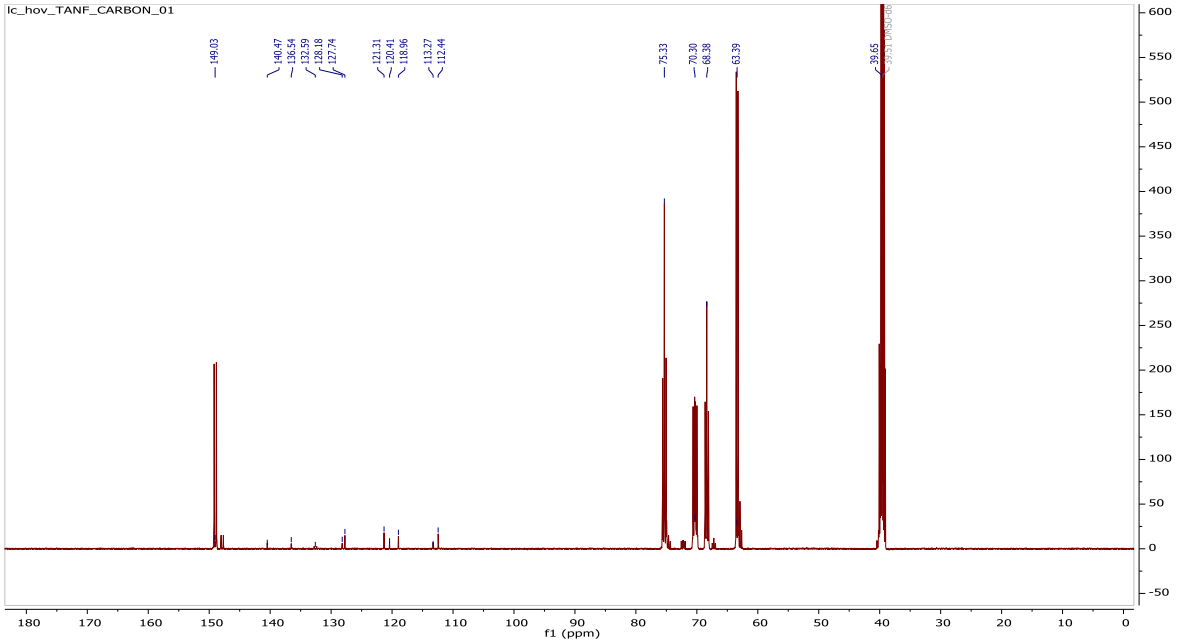
Supporting Information Figure S8. ^{13}C-NMR spectrum of $^{13}C_6$-Tan E obtained in DMSO-d_6 at 150 MHz.



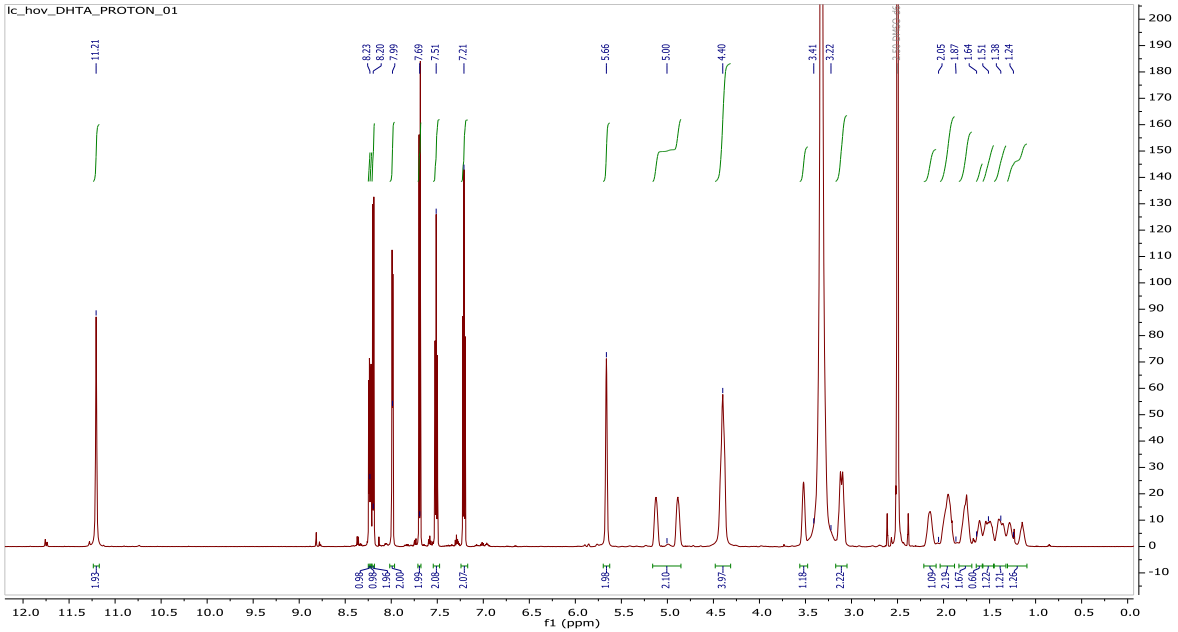
Supporting Information Figure S9. ^{1}H-NMR spectrum of $^{13}C_6$-Tan F obtained in DMSO-d_6 at 600 MHz.



Supporting Information Figure S10. ^{13}C-NMR spectrum of $^{13}C_6$-Tan F obtained in DMSO-d_6 at 150 MHz.



Supporting Information Figure S11. ^{1}H-NMR spectrum of $^{13}C_6$-DH-Tan E/F (diastereomeric mixture) obtained in DMSO-d_6 at 600 MHz.



Supporting Information Figure S12. ^{13}C-NMR spectrum of $^{13}C_6$-DH-Tan E/F (diastereomeric mixture) obtained in DMSO-d_6 at 150 MHz.