Supporting Information

Type I toxin-antitoxin systems as unprecedented targets for RNA ligands: toward new antimicrobial strategies

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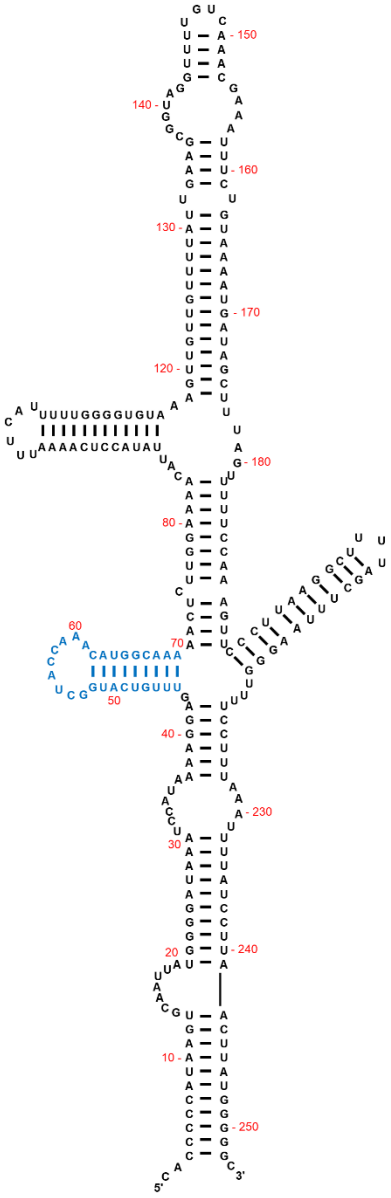
Figure S1. Primary and secondary structures of the full-length *H. pylori* IsoA1 toxin and AapA1 antitoxin. In blue are indicated the short RNA constructs employed for anisotropy and affinity studies allowing for the evaluation of IC_{50} and K_D *in vitro* and for these experiments each oligonucleotide was 5'-labeled with fluorescein. 5'-FAM labeled sequences were also employed for PAGE analysis and DSF. The full-length sequences were employed unlabeled for PAGE analysis and DSF.

IsoA1



AapA1

Figure S2. PAGE analysis of the formation of an extended duplex between AapA antitoxin RNA labeled in 5'-position with a fluorophore (fluorescein) and IsoA toxin RNA. This was performed on a 20% acrylamide/bis-acrylamide 19:1 gel in TBE 1x) using the truncated sequences (blue sequences in Figure S1). Lane 1 corresponds to 5'-FAM-AapA1 alone, lane 2 corresponds to 5'-FAM-AapA1-IsoA1 preformed duplex (structured as described in the experimental protocol), lane 3 corresponds to a mixture of structured 5'-FAM-AapA1 and IsoA1 stem loops at time 0, lanes 4-8 correspond to a mixture of structured 5'-FAM-AapA1 and IsoA1 stem loops incubated 2 hours at 4°C, 2 hours at 25°C, 15 minutes at 37°C, 30 minutes at 37°C and two hours at 37°C, respectively. Migration was performed at 4°C. All RNAs were structured and incubated in 20 mM Hepes, pH 7.4 containing 20 mM NaCl, 140 mM KCl, and 3 mM $MgCl_2$.

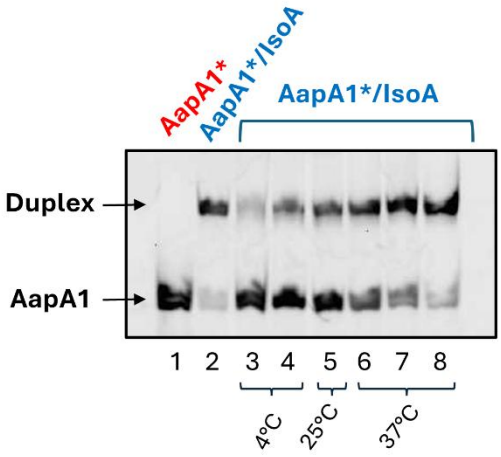


Figure S3. Anisotropy assay results. Inhibition curves for the compounds showing inhibition activity on the IsoA/AapA interaction (compounds **1a-c**) in comparison with neomycin obtained by fluorescence anisotropy. Each curve allowed for the calculation of IC_{50} values that are reported above each graphic. The anisotropy experiments were performed in 20 mM Hepes, pH 7.4 containing 20 mM NaCl, 140 mM KCl, and 3 mM $MgCl_2$ using 5'-FAM-AapA1 truncated sequence and unlabeled IsoA1 truncated sequence after incubation at 37°C for 2 hours in the absence or in the presence of increasing concentrations of compounds **1a**, **1b**, **1c** and neomycin, used as a control.

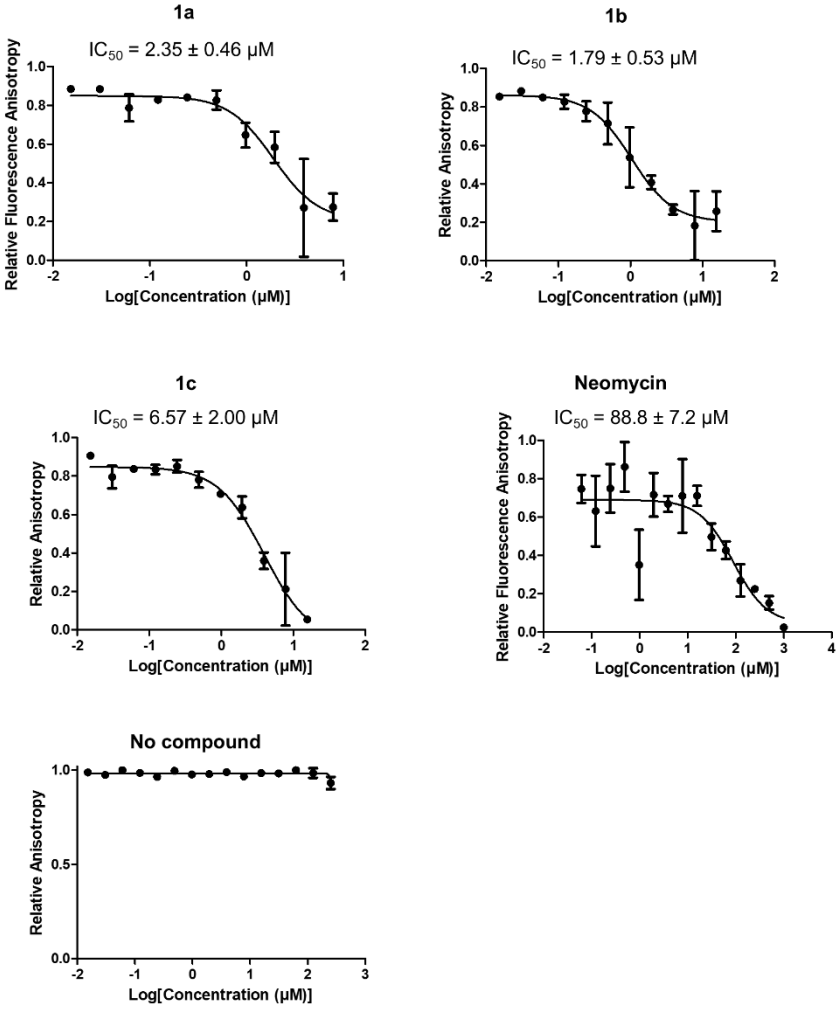


Figure S4. PAGE analysis of the inhibition of the formation of the extended duplex between truncated sequences of AapA1 antitoxin RNA and IsoA1 toxin RNA in the presence of compounds **1a-c** and neomycin. This was performed on a 20% acrylamide/bis-acrylamide 19:1 gel in TBE 1x). Lane 1 corresponds to IsoA1 alone, lane 2 corresponds to AapA1 alone, lane 3 corresponds to a mixture of pre-structured AapA1 and IsoA1 stem loops incubated two hours at 37°C and lanes 4-7 correspond to increasing concentrations (from 1 to 25 µM) of compounds **1a** (A), **1b** (B), **1c** (C) and neomycin (D) after two hours incubation at 37°C. Migration was performed at 4°C. All mixtures were structured and incubated in 20 mM Hepes, pH 7.4 containing 20 mM NaCl, 140 mM KCl, and 3 mM $MgCl_2$. The RNA sequences used are the ones highlighted in blue in figure S1. When the two RNA sequences are mixed together it is possible to detect the kissing complex intermediate between the the two separate stem-loops and the full-length duplex as previously reported for DIS RNA.[1]

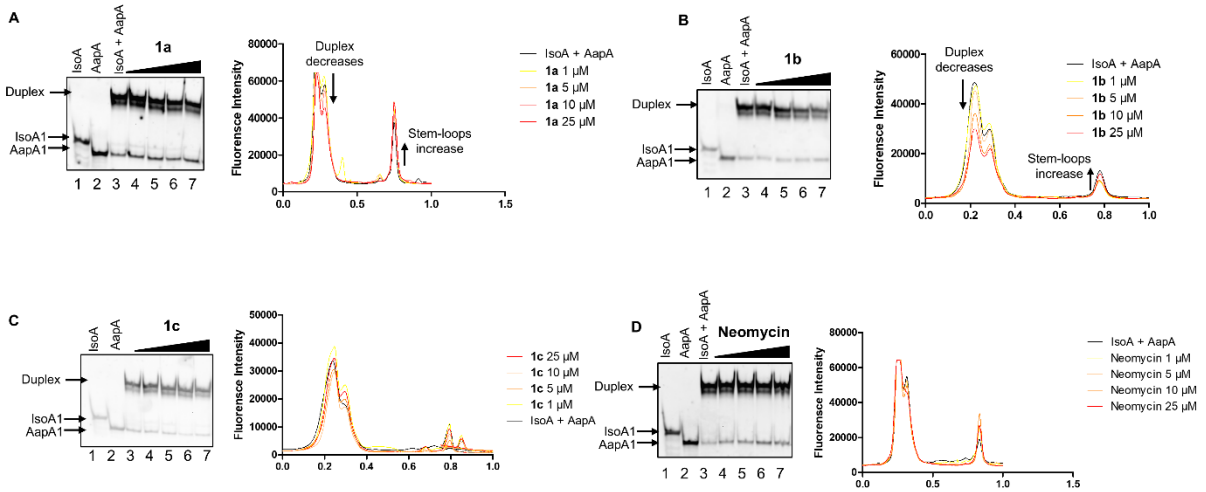


Figure S5. Binding curves allowing for the calculation of dissociation constants (K_D, µM). The fluorescence-based assays were performed in 20 mM Hepes, pH 7.4 containing 20 mM NaCl, 140 mM KCl, and 3 mM $MgCl_2$ using 5'-FAM-AapA1 truncated sequence and unlabeled IsoA1 truncated sequence after incubation at 37°C for 30 minutes in the absence or in the presence of increasing concentrations of compounds **1a-2a, 4a, 6a-7a**, **1b-2b**, **1c-2c**, **7c-9c** and **2d**.

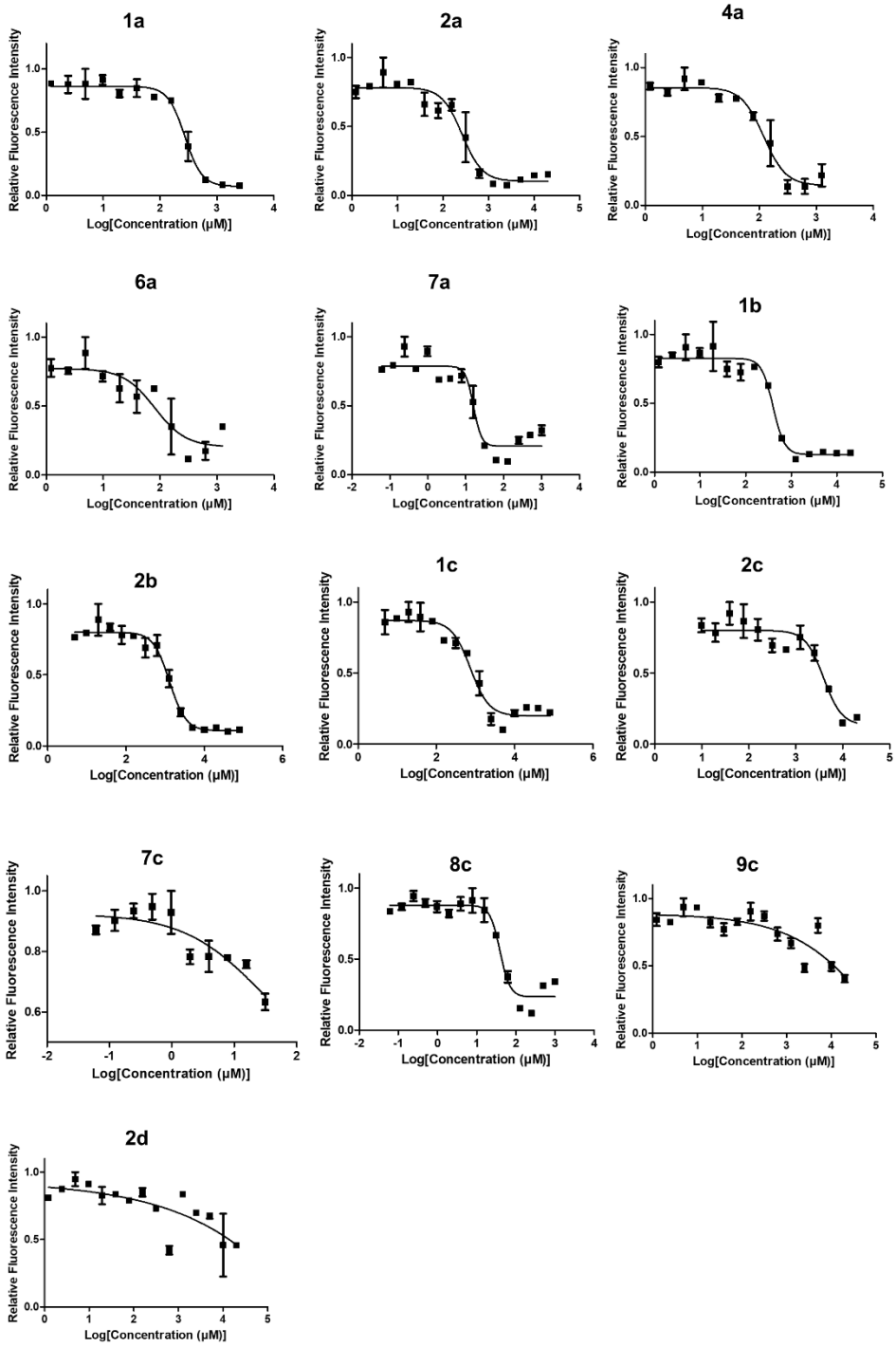


Figure S6. PAGE analysis of the inhibition of the formation of the extended duplex between AapA antitoxin RNA and IsoA toxin RNA in the presence of compounds **1a** and neomycin. This was performed on a 8% acrylamide/bis-acrylamide 19:1 gel in TBE 1x) using the full-length sequences illustrated in Figure S1. Lanes 1 correspond to IsoA alone, lane 2 corresponds to AapA alone, lane 3 corresponds to a mixture of structured AapA and IsoA stem loops incubated two hours at 37°C and lanes 4-7 correspond to increasing concentrations (from 1 to 25 µM) of compounds **1a** (A and B), **1b** (C and D) and neomycin (E and F) after two hours incubation at 37°C. Migration was performed at 4°C. All mixtures were structured and incubated in 20 mM Hepes, pH 7.4 containing 20 mM NaCl, 140 mM KCl, and 3 mM $MgCl_2$. AapA1 RNA forms the main secondary structure illustrated in Figure S1 as well as a second isoform present in a lower amount, as often observed for structured RNAs.[2] The presence of a second isoform for the 252-mer AapA1 RNA is not surprising as these long sequences can be present as a dynamic ensemble in solution.[2] The RNA sequences used are the ones highlighted in blue in figure S1. Labeling was obtained after a 20 minutes staining in a SybrGreen II stain.

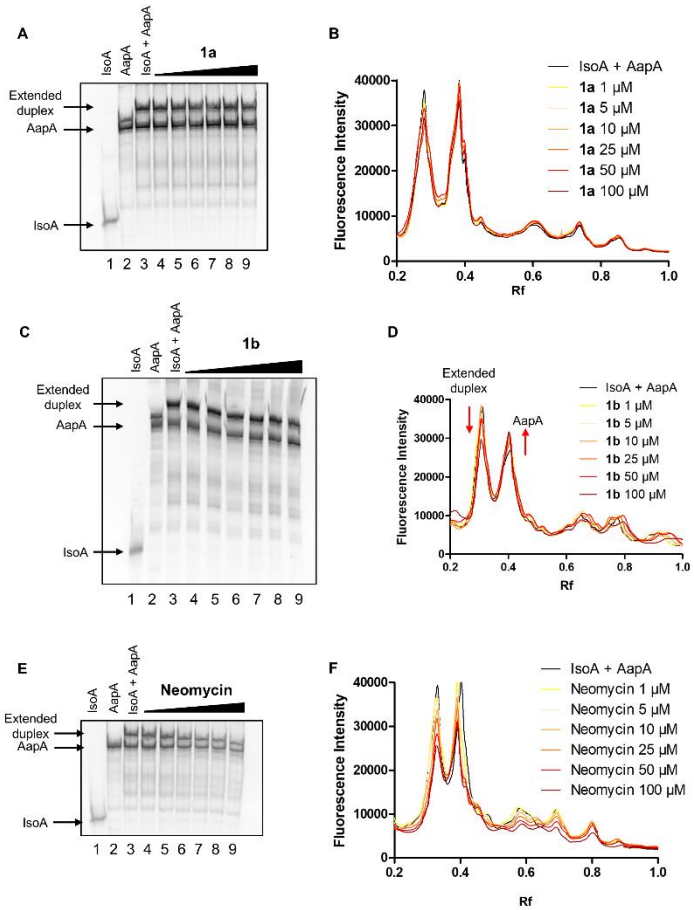


Figure S7. Differential scanning fluorimetry study using AapA1 and IsoA1 full-length sequences. (A) DSF profile performed on IsoA1 alone (blue line), AapA1 alone (red line) and on a mixture of IsoA1 and AapA1 (green line). (B) DSF profile of IsoA1/AapA1 mixture alone (dark green line) and in the presence of increasing concentrations of neomycin (from dark green to light green lines). (C) DSF profile of IsoA1/AapA1 mixture alone (dark green line) and in the presence of increasing concentrations of compound **1b** (from dark green to light green lines).

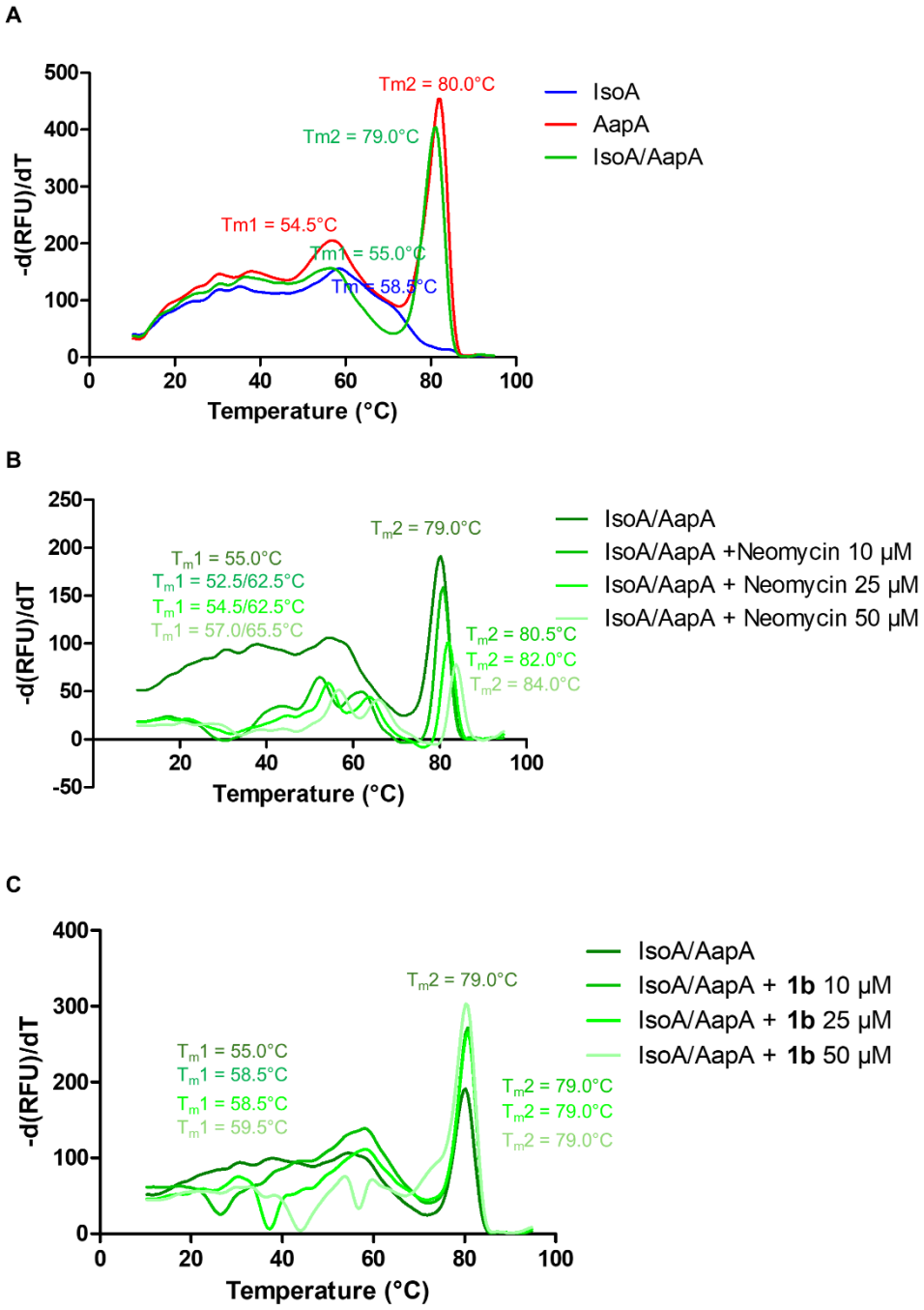


Figure S8. Differential scanning fluorimetry study using AapA1 and IsoA1 truncated sequences. DSF profile of IsoA1 (blue line), AapA1 (red line) and the mixture IsoA1/AapA1 (green line). Tm values are indicated using the same color code.

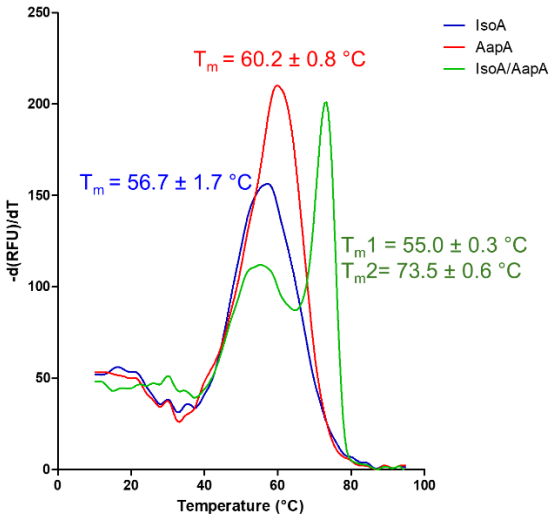


Figure S9. Differential scanning fluorimetry study using AapA1 and IsoA1 truncated sequences in the presence of neomycin. (A) IsoA1 truncated sequence alone and in the presence of increasing concentrations of neomycin. (B) AapA1 truncated sequence alone and in the presence of increasing concentrations of neomycin. (C) IsoA1/AapA1 mixture alone and in the presence of increasing concentrations of neomycin.

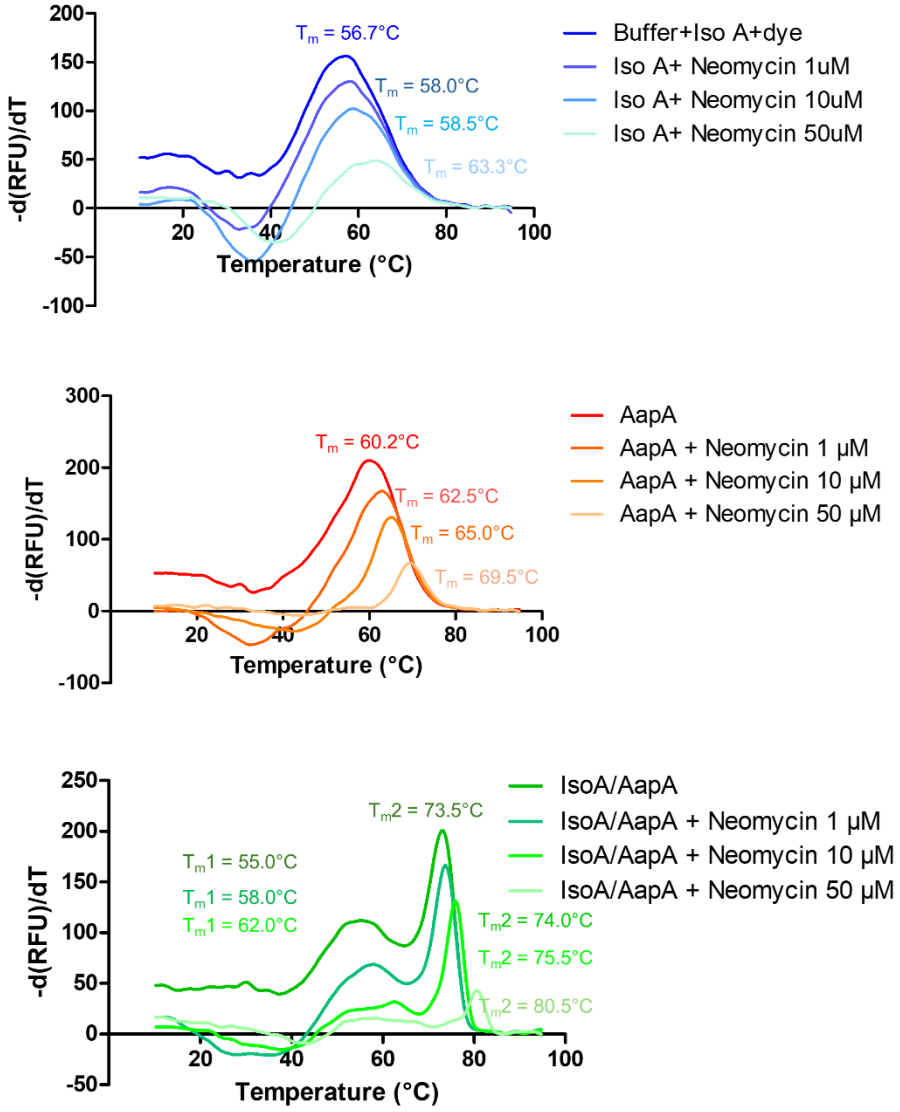
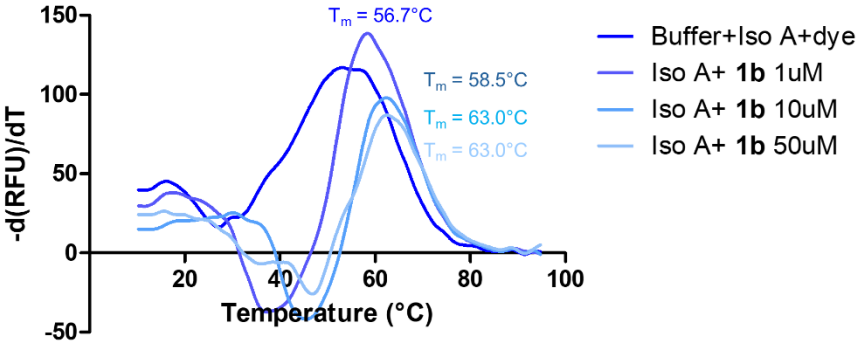
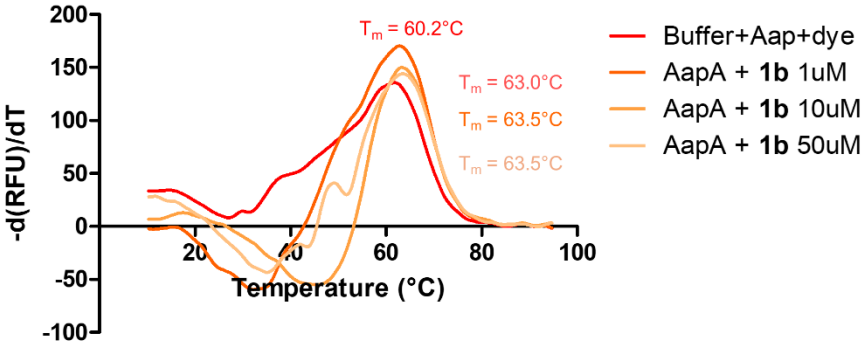


Figure S10. Differential scanning fluorimetry study using AapA1 and IsoA1 truncated sequences in the presence of compound **1b**. (A) IsoA1 truncated sequence alone and in the presence of increasing concentrations of **1b**. (B) AapA1 truncated sequence alone and in the presence of increasing concentrations of **1b**. (C) IsoA1/AapA1 mixture alone and in the presence of increasing concentrations of **1b**.





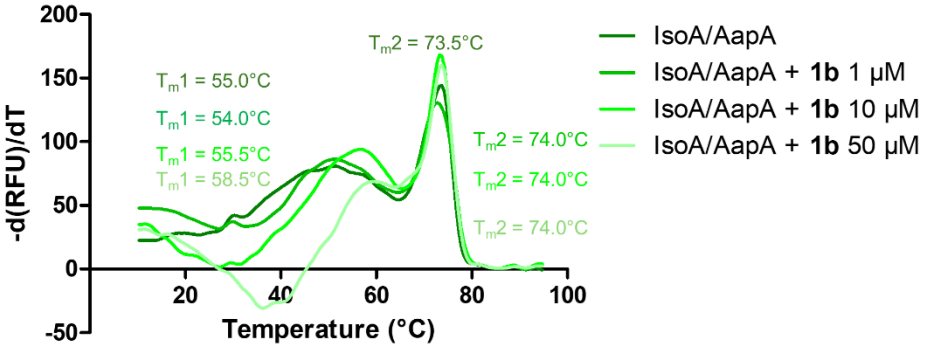


Table S1. Dissociation constants measured in the presence of AapA1 stem-loop RNA (K_D, µM) alone or in competition with 100 eq. of tRNA (K'_D, µM) or DNA (K''_D) and in the presence of IsoA1 stem-loop.

ID	K_D AapA1	K'_D[a]	K'_D/K_D	K''_D[b]	K''_D/K_D	K_D IsoA1
1a	0.301 ± 0.073	0.216 ± 0.096	0.7	0.737 ± 0.290	2.4	0.640 ± 0.077
2a	0.407 ± 0.130	0.513 ± 0.067	1.3	1.50 ± 0.39	3.7	1.33 ± 0.29
3a	not tested	not tested	-	not tested	-	not tested
4a	0.136 ± 0.015	0.250 ± 0.068	1.8	0.555 ± 0.025	4.1	0.773 ± 0.044
5a	not tested	not tested	-	not tested	-	not tested
6a	0.152 ± 0.084	0.172 ± 0.099	1.1	0.383 ± 0.041	2.5	0.316 ± 0.020
7a	18.4 ± 0.2	not tested	-	not tested	-	13.7 ± 1.8
1b	0.428 ± 0.028	0.790 ± 0.290	1.8	1.17 ± 0.05	2.7	1.17 ± 0.39
2b	1.24 ± 0.06	6.78 ± 0.55	5.5	1.90 ± 0.15	1.5	5.02 ± 0.63
3b	not tested	not tested	-	not tested	-	not tested
1c	0.870 ± 0.098	0.779 ± 0.110	0.9	4.02 ± 0.01	4.6	4.73 ± 0.39
2c	3.96 ± 0.04	2.90 ± 0.28	0.7	16.4 ± 2.4	4.1	> 50 µM
3c	not tested	not tested	-	not tested	-	not tested
7c	> 50 µM	not tested	-	not tested	-	> 50 µM
8c	47.7 ± 5.2	not tested	-	not tested	-	> 50 µM
9c	no binding	not tested	-	not tested	-	not tested
2d	> 50 µM	not tested	-	not tested	-	> 50 µM
3d	not tested	not tested	-	not tested	-	not tested
Neomycin	4.35 ± 0.45	30.4 ± 0.3	7.0	45.0 ± 4.2	10	3.04 ± 0.25

All K_D are expressed in µM and were measured in 20 mM HEPES buffer, pH 7.4, 20 mM NaCl, 140 mM KCl, 3 mM $MgCl_2$. Values represent the mean and standard deviation of 3 independent experiments performed in duplicates. [a] K'_D has been measured in the presence of 100 eq. of tRNA. [b] K''_D has been measured in the presence of 100 eq. of duplex DNA.

Table S2. Activity of synthetized compounds against *H. pylori* .

ID	*H. pylori* ATCC43504 MIC (µM)	*H. pylori* ATCC43504 MBC (µM)	CMB/CMI
1a	200	Not tested	-
2a	> 400	Not tested	-
4a	100	Not tested	-
6a	6.25	6.25	1
7a	> 400	Not tested	-
1b	6.25	12.5	2
2b	200	Not tested	-
1c	400	Not tested	-
2c	> 400	Not tested	-
7c	> 400	Not tested	-
8c	> 400	Not tested	-
2d	> 400	Not tested	-
Neomycin	0.200	Not tested	-

Minimal inhibitory concentrations (MIC) and minimal bactericidal concentrations (MBC) given in µM were determined in Müller Hinton II medium on 96 well plates incubated overnight (18-22 hours) in microaerobic conditions at 37°C. Maximal measured concentration is 400 µM. Experiments were done in triplicate.

Table S3. Percentage growth inhibition of *H. pylori* and other bacterial strains by synthesized compounds at 10 µM

ID	***H. pylori*** **ATCC43504**	***K. pneumoniae*** **CIP 82.91**	***E. coli*** **CIP 54.81**	***S. aureus*** **SA009**	***P. aeruginosa*** **CIP100720**	***A. baumannii*** **ATCC 19606**	***K. aerogenes*** **ATCC 13048**
1a	0.0 ± 0.0	0.0 ± 0.0	9.10 ± 0.10	0.0 ± 0.0	0.0 ± 0.0	7.10 ± 2.50	0.800 ± 0.100
2a	0.0 ± 0.0	7.00 ± 4.50	0.0 ± 0.0	0.100 ± 0.100	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
4a	12.8 ± 5.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
6a	93.0 ± 1.2	99.6 ± 0.5	99.5 ± 0.0	59.5 ± 27.4	0.0 ± 0.0	17.1 ± 6.1	8.40 ± 5.40
7a	0.0 ± 0.0	0.0 ± 0.0	9.10 ± 2.50	7.20 ± 1.80	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1b	70.0 ± 2.8	0.0 ± 0.0	1.9 ± 0.01	17.0 ± 4.6	0.0 ± 0.0	26.3 ± 2.8	0.0 ± 0.0
2b	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1c	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	3.2 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
2c	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
7c	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.60 ± 0.50	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
8c	0.0 ± 0.0	0.0 ± 0.0	8.10 ± 0.30	0.0 ±0.0	0.0 ± 0.0	8.40 ± 3.30	0.0 ± 0.0
2d	0.0 ± 0.0	1.5 ± 0.01	0.0 ± 0.0	0.0 ± 0.0	0.400 ± 0.001	0.0 ± 0.0	0.0 ± 0.0

Bacterial growth was monitored by measuring suspension absorbance at 600 nm after 18-22 hours of incubation in presence of synthetized compounds at 10 µM. The percentage of growth inhibition was obtained by the equation: 100- [(OD of sample–OD of negative control) × 100 / (OD of positive control – OD of negative control)]. Data shown are mean ± SD of triplicate values of independent experiments.

Experimental procedures

Chemistry

General procedures and Instrumentation:

Reagents and solvents were purchased from Aldrich, Alfa Aesar, Fluorochem, Carlo Erba, Acros, Novabiochem and used without further purification. All reactions involving air- or moisture-sensitive reagents or intermediates were performed under an argon atmosphere. Flash column chromatography was carried out on silica gel columns (Interchim Puriflash silica HP 15 to 50 µm) on a Puriflash® XS420 system (Interchim). Analytical thin-layer chromatography (TLC) was conducted on Sigma Aldrich precoated silica gel and compounds were visualized by irradiation (254 nm) and/or by staining with ninhydrin or vanillin stains.

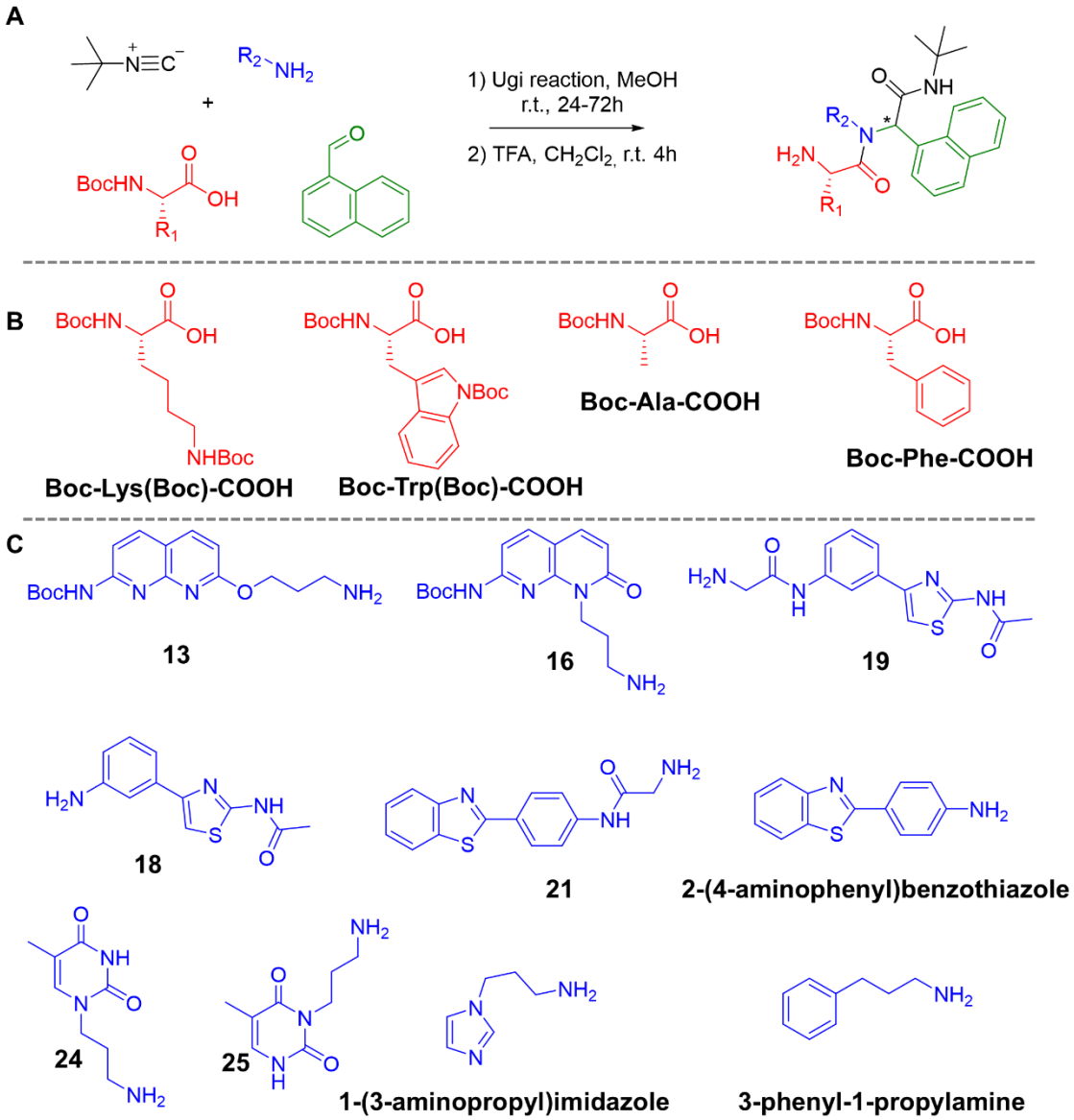
HPLC analyses were performed using a Water Alliance 2695 pump coupled to a Water 996 photodiode array detector and two different column were used. Method A: Phenomenex Synergi^{TM} Fusion RP 80Å reverse-phase C18 column (250 x 4.6 mm, 5 µm) for analytical HPLC analysis and reverse-phase C18 column (250 x 10 mm, 5 µm) for semipreparative HPLC. Method B: X-Select CSH^{TM} fluorophenyl reverse-phase C18 column (50 x 4.6 mm, 5 µm) for analytical HPLC analysis and reverse-phase C18 column (250 x 10 mm, 5 µm) for semipreparative HPLC. All HPLC analyses were run at room temperature using a gradient of CH_3CN containing 0.1% TFA (eluent B) and water containing 0.1% TFA (eluent A) from 5% to 100% of B in 30 minutes at a flow rate of 1 mL/min for the analytical column and 3.5 mL/min for the semipreparative column.

^1H and ^{13}C NMR spectra were recorded on Bruker AC 200 and 400 MHz spectrometers. Chemical shifts are reported in parts per million (ppm, δ) referenced to the residual ^1H resonance of the solvent (D_2O, δ 4.79; $CDCl_3$, δ 7.26; CD_3OD, δ 3.31 and DMSO-d_6, δ 2.50) and referenced to the residual ^{13}C resonance of the solvent ($CDCl_3$, δ 77.2; CD_3OD, δ 49.0 and DMSO-d_6, δ 39.52). Splitting patterns are designed as follows: s (singlet), d (doublet), t (triplet), q (quadruplet) p (pentuplet), m (multiplet), and br (broad). Coupling constant (*J*) are listed in hertz (Hz).

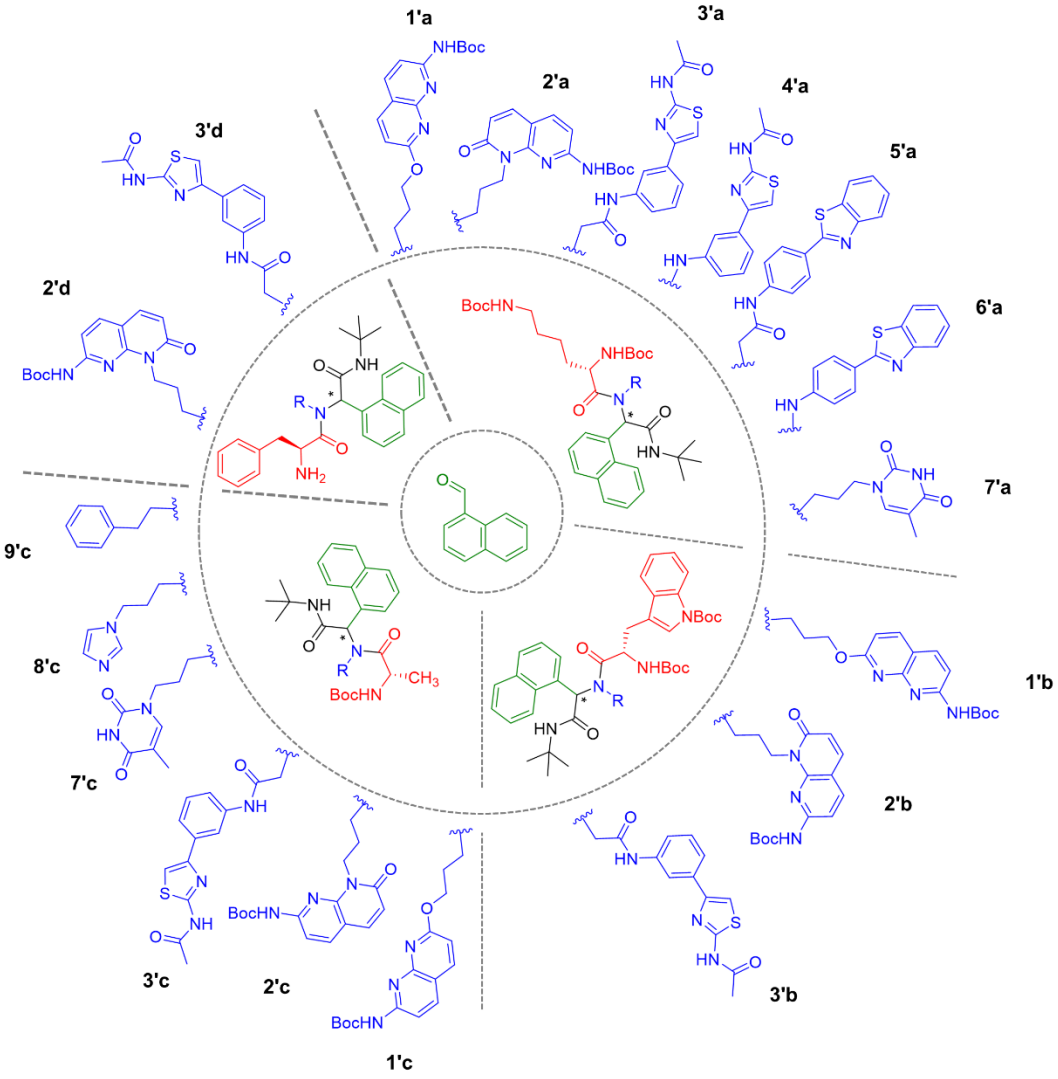
Low resolution mass spectra (MS) were obtained with a Bruker Daltonics Esquire 3000+ electrospray spectrometer equipped with API ionization source. High-resolution mass spectrometry (HRMS) was carried out on a LTQ Orbitrap hybrid mass spectrometer with an electrospray ionization probe (Thermoscientific, San Jose, CA) by direct infusion from a pump syringe, to confirm the correct molar mass and high purity of the compounds.

The synthesis of all conjugates was performed as illustrated in Scheme S1 and Scheme S2.

Scheme S1. Synthesis of the new series of RNA ligands using the Ugi multicomponent reaction. (A) General synthetic procedure including a Ugi 4-component reaction as the first step and a deprotection of the Boc groups in acidic conditions. (B) Commercially available protected amino acids employed in the synthesis of the new series of RNA binders as the carboxylic acid partner. (C) Heteroaromatic compounds employed in the Ugi reaction as the amine partner. Compounds **13**, **16**, **18**, **19**, **21** and **24-25** have been prepared accordingly to the procedure described below while 2-(4-aminophenyl)benzothiazole, 1-(3-aminopropyl)imidazole and 3-phenyl-1-propylamine were commercially available.



Scheme S2. Boc-protected intermediates obtained after the Ugi reactions constituting the first step illustrated in Scheme S1A.

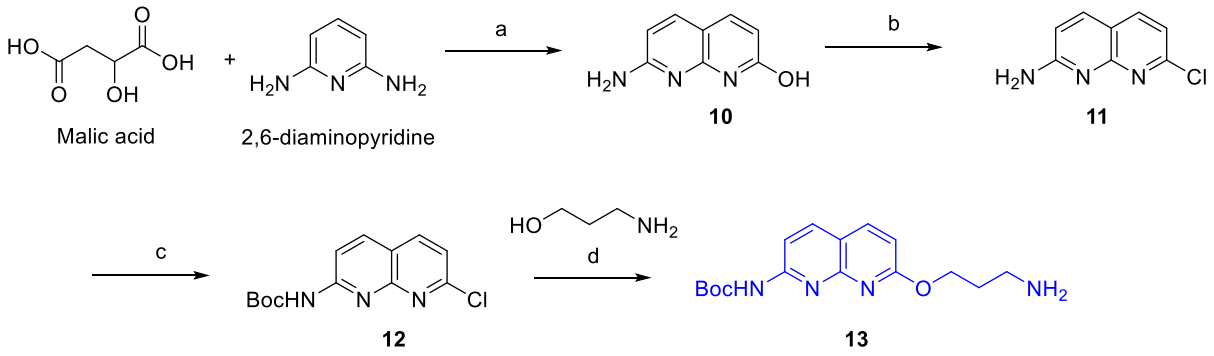


General procedures.

General procedure A - Synthesis of UGI products. A mixture of the chosen amine **13**, **16**, **19**, **18**, **21**, **24**, 2-(4-aminophenyl)benzothiazole, 1-(3-aminopropyl)imidazole or 2-phenethylamine (1 eq.) and 1-naphthaldehyde (1 eq.) in MeOH or CH_2Cl_2/MeOH was stirred at room temperature for 2h. When the amine was purchased or obtained as a salt, a solution of KOH (1 eq.) in MeOH (0.1 mL) was added as well. Carboxylic acids Boc-Lys(Boc)-OH, Boc-Trp(Boc)-OH, Boc-Phe-OH and Boc-Ala-OH (1 eq.) and tert-butylisocyanide (1 eq.) were added, and the reaction mixture was stirred at room temperature for 24 to 72 hours and monitored by TLC. Once the rection is complete, the crude mixture was concentrated under reduced pressure. A saturated aqueous solution of $NaHCO_3$ was added, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated under reduced pressure. The crude residue was purified by flash chromatography (CH_2Cl_2/MeOH or CHX/EtOAc) to afford a mixture of diastereomers for compounds **1'a-7'a**, **1'b-3'b**, **1'c-3'c**, **7'c-9'c**, **2'd-3'd**.

General procedure B - Boc group removal. To a solution of protected compounds in CH_2Cl_2 were added 50 eq. of TFA. The reaction mixture was stirred at room temperature for 4h. The solvent and the remaining TFA were then removed under reduced pressure by co-evaporating twice with toluene, then the crude mixture was triturated in Et_2O. After filtration, mixtures of diastereomers were obtained as TFA salts **1a-7a**, **1b-3b**, **1c-3c**, **7c-9c**, **2d-3d**.

Scheme S3. Synthesis of tert-butyl (7-(3-aminopropoxy)-1,8-naphthyridin-2-yl)carbamate (**13**). Reagents: a) H_2SO_4, 110°C, 3h; b) $POCl_3$, toluene, reflux, 3 days; c) Boc_2O, THF, r.t., 16h; d) 3-amino-1-propanol, NaH, THF, 60°C, 16h.



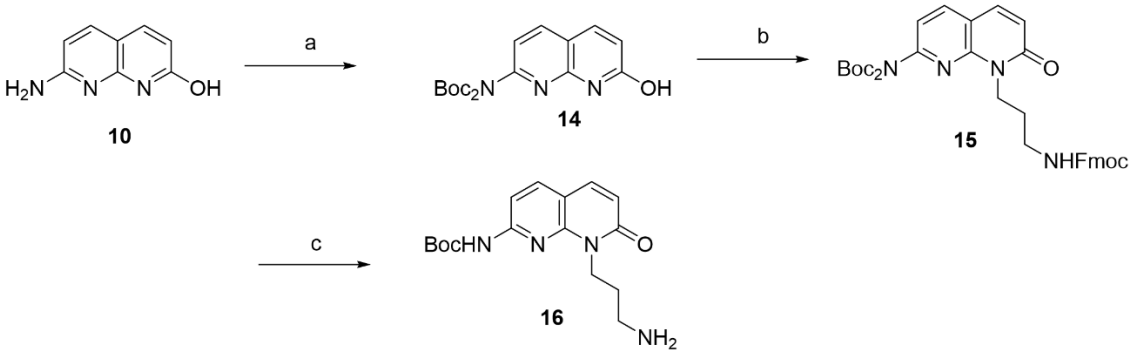
2-Amino-7-chloro-1,8-naphthyridine (11). 2-Amino-7-hydroxy-1,8-naphthyridine (**10**)[3] (2.65 g, 1.86 mmol, 1 eq.) was suspended in anhydrous toluene (18 mL) and 18 mL of $POCl_3$ were added. The reaction mixture was stirred at reflux for 3 days. After cooling, the reaction solution was neutralized with NH_4OH at 0°C and filtered. The aqueous phase was extracted three times with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4 and then concentrated under reduced pressure to give the crude product **11** as a yellow solid (2.65 g, 80%). R_f = 0.39 (CH_2Cl_2/MeOH 95:5); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.08 (d, J = 8.2 Hz, 1H), 7.96 (d, J = 8.8 Hz, 1H), 7.18 (d, J = 8.2 Hz, 1H), 7.04 (s, 2H), 6.82 (d, J = 8.8 Hz, 1H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 161.5, 156.5, 151.0, 139.6, 137.4, 117.2, 115.7, 113.5; MS (ESI): *m/z* 180.4 $[M+H]^+$ (theoretical *m/z* 180.03).

***tert*-butyl (7-chloro-1,8-naphthyridin-2-yl)carbamate (12).** To a suspension of compound **11** (2.65 g, 16.8 mmol, 1 eq.) in anhydrous THF (140 mL) at 0°C, Boc_2O (18.0 g, 84.6 mmol, 5 eq.) was added. The reaction mixture was stirred at reflux for 3 days then concentrated under reduced pressure. Water was added, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4 and then concentrated under reduced pressure. The crude residue was purified by flash chromatography (CHX/EtOAc gradient 100:0 to 50:50, v/v) to afford compound **12** (3.78 g, 80%) as a pale-yellow solid. R_f = 0.55 (CHX-EtOAc 6:4); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 10.51 (s, 1H), 8.43 (d, J = 9.0 Hz, 1H), 8.41 (d, J = 8.4 Hz, 1H), 8.19 (d, J = 9.0 Hz, 1H), 7.54 (d, J = 8.4 Hz, 1H), 1.50 (s, 9H); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 155.6, 154.3, 152.7, 152.5, 140.4, 139.3, 121.1, 118.4, 114.3, 80.4, 27.9; MS (ESI): *m/z* 279.73 $[M+H]^+$ (theoretical *m/z* 280.08).

***tert*-Butyl (7-(3-aminopropoxy)-1,8-naphthyridin-2-yl)carbamate (13).** To a solution of 3-amino-1-propanol (1.64 mL, 21.5 mmol, 3 eq.) in THF (75 mL) was added NaH (1.00 g, 43.0 mmol, 6 eq.) and the reaction was stirred for 30 min before to adding compound **12** (2.00 g, 7.17 mmol, 1 eq.). The reaction mixture was stirred overnight at 60°C, then concentrated under reduced pressure. Water

was added, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4 and then concentrated under reduced pressure. The crude residue was purified by flash chromatography (CH_2Cl_2/MeOH [10% NH_4OH] gradient 100:0 to 90:10, v/v) to afford compound **13** (1.67 g, 73%) as a pale-yellow solid. R_f = 0.18 (CH_2Cl_2/MeOH [10% NH_4OH] 93:7); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.10 (d, *J* = 8.7 Hz, 1H), 8.01 (d, *J* = 8.7 Hz, 1H), 7.90 (d, *J* = 8.7 Hz, 1H), 7.58 (s, 1H), 6.83 (d, *J* = 8.7 Hz, 1H), 4.59 (t, *J* = 6.4 Hz, 2H), 2.90 (t, *J* = 6.9 Hz, 2H), 2.04 – 1.89 (m, 2H), 1.53 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 165.4, 154.5, 153.7, 152.4, 138.8, 138.7, 116.1, 112.3, 110.6, 81.6, 64.6, 39.4, 33.1, 28.4; MS (ESI): *m/z* = 319.00 $[M+H]^+$ (theoretical *m/z* 319.10).

Scheme S4. Synthesis of *tert*-butyl (8-(3-aminopropyl)-7-oxo-7,8-dihydro-1,8-naphthyridin-2-yl)carbamate (**16**). Reagents: a) Boc_2O, DMAP, THF, r.t. 16h; 3-(Fmoc-amino)propyl bromide, K_2CO_3, acetone, 56°C, 12h; c) piperidine, DMF, r.t., 2h.



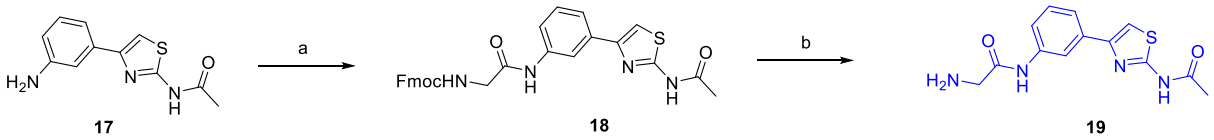
***N*^{2},*N*^{2}-Bis(*tert*-butyl)-(7-oxo-7,8-dihydro[1,8]naphthyridin-2-yl)carbamate (14).** To a suspension of compound **10** (3.00 g, 18.6 mmol, 1 eq.) in THF (120 mL) at 0°C, DMAP (1.00 g, 8.20 mmol, 0.4 eq.) and Boc_2O (15.0 g, 68.8 mmol, 3.7 eq.) were added. The reaction mixture was stirred overnight at room temperature, then concentrated under reduced pressure. Water was added, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and then concentrated under reduced pressure. The crude residue was purified by flash chromatography (CHX/EtOAc gradient 90:10 to 40:60, v/v) to afford compound **14** (5.64 g, 84%) as a brown solid.
R_f = 0.29 (CHX/EtOAc 4:6); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.78 (s, 1H), 7.87 (d, *J* = 8.3 Hz, 1H), 7.68 (d, *J* = 9.5 Hz, 1H), 7.30 (d, *J* = 8.3 Hz, 1H), 6.65 (d, *J* = 9.5 Hz, 1H), 1.49 (s, 18H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 163.4, 152.7, 150.9, 148.3, 138.6, 137.8, 122.7, 114.8, 112.6, 84.0, 28.0; MS (ESI): *m/z* 361.93 $[M+H]^+$ (theoretical *m/z* 362.12).

***N*^{2}-*N*^{2}-(*tert*-butyl)-(8-(*N'*-Fmoc-3-aminopropyl)-7-oxo-7,8-dihydro[1,8]naphthyridin-2-yl)carbamate (15).** To a solution of compound **14** (200 mg, 0.553 mmol, 1 eq.) in acetone (8 ml), K_2CO_3 (305 mg, 2.21 mmol, 4 eq.) was added and the reaction was stirred for 30 min before addition of commercially available 3-(Fmoc-amino)propyl bromide (399 mg, 1.11 mmol, 2 eq.). The reaction

mixture was stirred overnight at reflux, then the solvent was concentrated under reduced pressure. Water was added, and the aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4 and then concentrated under reduced pressure. The crude residue was purified by flash chromatography (CHX/EtOAc gradient 100:0 to 50:50, v/v) to afford compound **15** (254 mg, 72%) as a pale-yellow solid. R_f = 0.34 (CHX/EtOAc 5:5); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.89 (d, *J* = 8.3 Hz, , 1H), 7.75 (d, *J* = 7.5 Hz, 2H), 7.69 – 7.54 (m, 3H), 7.42 – 7.24 (m, 5H), 6.74 (d, *J* = 9.4 Hz, 1H), 6.13 (t, *J* = 6.3 Hz, 1H), 4.54 (t, *J* = 6.0 Hz, 2H), 4.34 (d, *J* = 7.4 Hz, 2H), 4.25 (t, *J* = 7.3 Hz, 1H), 3.16 – 3.07 (m, 2H), 2.04 – 1.92 (m, 2H), 1.52 (s, 18H); MS (ESI): *m/z* 640.67 $[M+H]^+$ (theoretical *m/z* 641.30).

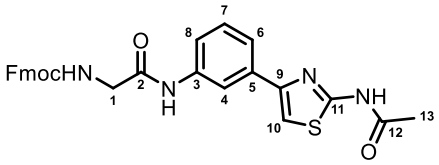
***tert*-Butyl (8-(3-aminopropyl)-7-oxo-7,8-dihydro-1,8-naphthyridin-2-yl)carbamate (16).** To a solution of compound **15** (393 mg, 0.610 mmol, 1 eq.) in DMF (1.6 ml) was added piperidine (400 µL, 4.05 mmol, 6.6 eq.). The reaction mixture was stirred at room temperature for 3h, then the solvent was concentrated under reduced pressure. Water was added, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4 and then concentrated under reduced pressure. The crude residue was purified by flash chromatography (CH_2Cl_2/MeOH [10% NH_4OH] gradient 100:0 to 92:8, v/v) to afford compound **16** (140 mg, 72%) as a pale-yellow solid. R_f = 0.40 (CH_2Cl_2/MeOH [10% NH_4OH] 90/10); ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.85 (d, *J* = 8.5 Hz, 1H), 7.81 (d, *J* = 8.5 Hz, 1H), 7.56 (d, *J* = 9.4 Hz, 1H), 7.40 (s, 1H), 6.60 (d, *J* = 9.4 Hz, 1H), 4.48 (t, *J* = 6.9 Hz, 2H), 2.73 (t, *J* = 6.6 Hz, 2H), 1.91 (p, *J* = 6.8 Hz, 2H), 1.55 (s, 9H); ^{13}C NMR (101 MHz, $CDCl_3$) δ = 163.4, 151.9, 151.91, 148.8, 138.8, 137.0, 120.2, 111.6, 106.7, 81.8, 39.3, 38.5, 31.7, 28.3; MS (ESI): *m/z* 319.00 $[M+H]^+$ (theoretical *m/z* 319.18).

Scheme S5. Synthesis of *N*-(3-(2-acetamidothiazol-4-yl)phenyl)-2-aminoacetamide (**19**). Reagents: a) Fmoc-Gly-COOH, EDC/HOSu, CH_2Cl_2, DMF, r.t., 16h; b) piperidine, DMF, r.t., 16h.



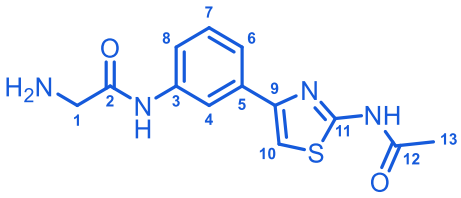
N-(3-(2-acetamidothiazol-4-yl)phenyl)-2-aminoacetamide (TFA salt) **19** was obtained according to previously published procedure[4] in 38% yield using compound commercially available 2-bromo-3'-nitroacetophenone as starting material.

***Nα*-Fluorenylmethyloxycarbonyl-[*N*-(2-*N*-acetylamino-4-(3-aminophenyl)-thiazole)]-*tert*-butoxyglycinamide (18).**



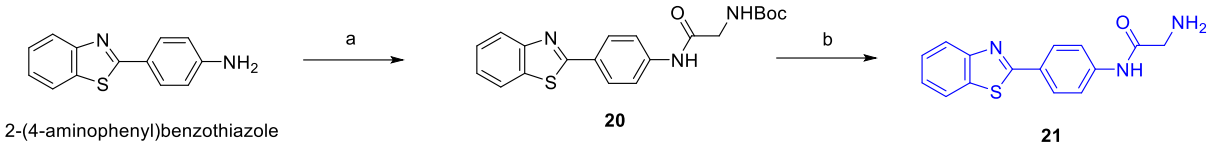
To a solution of Fmoc-Gly-OH (765 mg, 2.58 mmol, 1eq.) in CH_2Cl_2/DMF (4 mL/0.5 mL) was added 1- ethyl-3-(3-dimethylaminopropyl)carbodiimide (690 mg, 3.60 mmol, 1.4 eq.) and *N*-hydroxysuccinimide (414 mg, 3.60 mmol, 1.4 eq.) and the mixture was stirred 30 min at room temperature. The compound **17** (600 mg, 2.58 mmol, 1 eq.) was added and the reaction mixture was stirred overnight at room temperature, then the solvent was concentrated under reduced pressure. After solvent removal, the crude was precipitated in CH_2Cl_2 to afford compound **18** (926 mg, 70%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 10,04 (s, NH), 8.28 (s, 1H, H_4), 7.90, 7.73 (2d, J = 7.4 Hz, 4H, 4C$\underline{H}_{Fmoc}$), 7.66 – 7.54 (m, 1H, H_6), 7.51 (s, 1H, H_{10}), 7.46 – 7.30 (m, 6H, 4C$\underline{H}_{Fmoc}$ + $H_{7\text{-}8}$), 4.32 (d, J = 6.8 Hz, 2H, C$\underline{H}_{2Fmoc}$), 4.28 – 4.18 (m, 1H, C$\underline{H}_{Fmoc}$), 3.82 (d, J = 6.1 Hz, 2H, H_1), 2.15 (s, 3H, H_{13}); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 168.8, 168.6 (C_2 + C_{12}), 158.0 (C_{11}), 148.7 (C_9), 142.6 (2$C_{IV.Fmoc}$), 139.5 ($C_{IV.Fmoc}$), 139.2 (C_3), 137.5 ($C_{IV.Fmoc}$), 134.9 (C_5), 129.2 (C_7), 129.0, 127.4, 121.4 (6$\underline{C}H_{Fmoc}$), 120.7 (C_6), 120.1 (2$\underline{C}H_{Fmoc}$), 118.7 (C_8), 117.0 (C_4), 108.1 (C_{10}), 66.1 ($\underline{C}H_{2.Fmoc}$),45.3 ($\underline{C}H_{Fmoc}$), 44.3 (C_1), 22.5 (C_{13}); MS (ESI): *m/z* 513.5 $[M+H]^+$ (theoretical *m/z* 513.16).

S-Gly-NH$_2$ = *N*-(2-*N*-acetylamino-4-(3-aminophenyl)-thiazole)]-*tert*-butoxyglycinamide (19).



To a solution of compound **18** (800 mg, 1.56 mmol, 1 eq.) in DMF (4 ml) was added piperidine (1.00 mL, 10.1 mmol, 6.5 eq.). The reaction mixture was stirred overnight at room temperature, then the solvent was concentrated under reduced pressure. Water was added, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4 and then concentrated under reduced pressure. The crude residue was purified by flash chromatography (CH_2Cl_2/MeOH [10% NH_4OH] gradient 100:0 to 93:7, v/v) to afford compound **19** (389 mg, 86%) as a white solid. R_f = 0.44 (CH_2Cl_2/MeOH [10% NH_4OH] 93/7); ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.27 (s, 1H, H_4), 7.60 – 7.55 (m, 1H, H_6), 7.52 (s, 1H, H_{10}), 7.53 – 7.47 (m, 1H, H_8), 7.35 (t, J = 7.8 Hz, 1H, H_7), H_1 (signal under the D_2O peak), 2.16 (s, 1H, H_{13}); ^{13}C NMR (101 MHz, DMSO-d_6) δ = 170.8 (C_2), 168.7 (C_{12}), 158.0 (C_{11}), 148.6 (C_9), 139.1 (C_3), 134.9 (C_5), 129.1 (C_7), 120.7 (C_6), 118.5 (C_8), 116.6 (C_4), 108.0 (C_{10}), 44.7 (C_1), 22.5 (C_{13}); MS (ESI): *m/z* 291.4 $[M+H]^+$ (theoretical *m/z* 291.09).

Scheme S6. Synthesis of 2-amino-N-(4-(benzo[d]thiazol-2-yl)phenyl)acetamide (**21**). Reagents: a) Boc-Gly-OH, HBTU, Et_3N, CH_2Cl_2, r.t;, 16h; b) TFA, CH_2Cl_2, r.t., 4h.

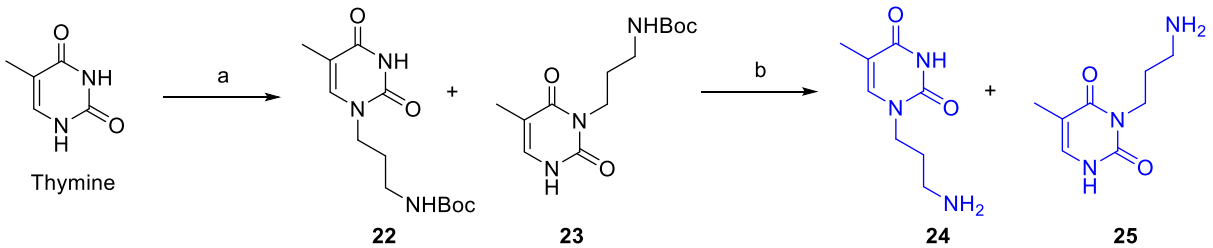


2-(4-aminophenyl)benzothiazole **20** **21**

2-(*N*-*tert*-butoxycarbonyl)amino-*N*'-[4-(benzothiazol-2-yl)phenyl]acetamide (20). To a solution of Boc-Gly-OH (354 mg, 2.02 mmol, 1 eq.) in CH_2Cl_2 (5.5 mL) was added triethylamine (560 µL, 4.04 mmol, 2 eq.) and HBTU (918 mg, 2.42 mmol, 1.2 eq.) and the mixture was stirred 30 minutes at room temperature. Commercially available 2-(4-aminophenyl)benzothiazole (548 mg, 2.42 mmol, 1.2 eq.) was added and the reaction mixture was stirred overnight at room temperature, then the solvent was concentrated under reduced pressure. After solvent removal, the crude was precipitated in CH_2Cl_2 and washed with EtOH (3 × 2 mL) to afford compound **20** (513 mg, 66%) as a colorless solid. R_f = 0.40 (CHX/EtOAc 5:5); ^{1}H NMR (200 MHz, DMSO-d_6) δ = 10.30 (s, 1H, 1H), 8.20 – 7.92 (m, 4H), 7.88 – 7.72 (m, 2H), 7.60 – 7.38 (m, 2H), 7.14 (t, J = 4.6 Hz, 1H), 3.77 (d, J = 4.6 Hz, 2H), 1.40 (s, 9H); ^{13}C NMR (50 MHz, DMSO- d_6) δ = 168.8, 167.0, 156.0, 153.7, 141.8, 134.3, 128.1, 127.5, 126.6, 125.2, 122.6, 122.2, 119.3, 78.1, 43.9, 28.2; MS (ESI): m/z = 384.1 $[M+H]^+$ (theoretical m/z 384.1).

2-Amino-*N*-[4-(benzothiazol-2-yl)phenyl]acetamide (21). To a solution of protected compound **20** (319 mg, 0.83 mmol) in CH_2Cl_2 was added 50 eq. of TFA. The reaction mixture was stirred at room temperature for 4h. The solvent and the remaining TFA were then removed under reduced pressure by co-evaporating twice with toluene, then the crude mixture was triturated in Et_2O. After filtration, compound **21** (241 mg, 73%) as a colorless solid was obtained. R_f = 0.47 (CH_2Cl_2/MeOH 80:20); ^{1}H NMR (200 MHz, DMSO-d_6) δ = 10.91 (s, 1H), 8.27 (s br, 2H), 8.17 – 7.98 (m, 4H), 7.81 (d, J = 8.7 Hz, 2H), 7.59 – 7.38 (m, 2H), 3.87 (s, 2H); ^{13}C NMR (50 MHz, DMSO- d_6) δ = 166.8, 165.4, 153.6, 141.0, 134.4, 128.3, 128.2, 126.7, 125.4, 122.7, 122.3, 119.5, 41.2; MS (ESI): m/z = 284.1 $[M+H]^+$ (theoretical m/z 284.1).

Scheme S7. Synthesis of *N*-(3-aminopropyl)thymine (**24**). Reagents: a) *N*-Boc-(3-aminopropyl) bromide, K_2CO_3, DMF, 65°C, 7h; b) TFA, CH_2Cl_2, r.t., 4h.



22 **23** **24** **25**

***N*^{1}-(3-(*N*'-tert-butoxycarbonyl)aminopropyl)-thymine (22).** To a solution of thymine (780 mg, 6.20 mmol, 1.2 eq.) in DMF (37 mL) was added K_2CO_3 (932 mg, 6.72 mmol, 1.3 eq.), then 3-(Boc-amino)propyl bromide (1.23 g, 5.17 mmol, 1 eq.) was added under argon and the mixture was stirred at 60°C overnight. The mixture was concentrated under vacuum and the residual crude sample was dissolved in CH_2Cl_2. The mixture was filtered off, then the filtrate was concentrated under reduced pressure. The crude residue was purified by flash chromatography (CH_2Cl_2/acetone gradient 100:0 to 80:20, v/v) to provide **22** (370 mg, 25%) as a colourless solid. R_f = 0.58 (CH_2Cl_2/MeOH 9:1); ^{1}H NMR (200 MHz, $CDCl_3$) δ = 9.51 (s, 1H), 7.05 (s, 1H), 5.10 (br t, 1H), 3.76 (t, *J* = 6.5 Hz, 2H), 3.14 (dd, *J* = 11.6, 5.6 Hz, 2H), 1.91 (s, 3H), 1.93 – 1.68 (m, 2H), 1.42 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$) δ = 164.6, 156.2, 151.5, 140.6, 111.2, 79.6, 45.8, 37.1, 29.6, 28.5, 12.5; MS (ESI): *m/z* 589.1 $[2M+Na]^+$ (theoretical *m/z* 589.3).

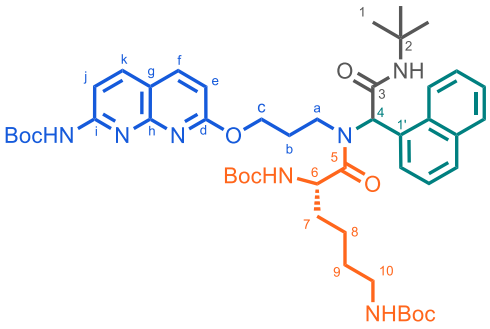
***N*^{3}-(3-(*N*'-*tert*-butoxycarbonyl)aminopropyl)-thymine (23).** Compound **23** was isolated together with compound **22** during the same purification step. Compound **23** (160 mg, 11%) was obtained as a colourless solid. R_f = 0.58 (CH_2Cl_2-MeOH 9:1); ^{1}H NMR (200 MHz, $CDCl_3$) δ = 10.18 (s, 1H), 7.07 (d, *J* = 5.2 Hz, 1H), 5.31 (br t, 1H), 4.01 (t, *J* = 6.3 Hz, 2H), 3.09 (dd, *J* = 11.0, 5.5 Hz, 2H), 1.92 (s, 3H), 1.99 – 1.32 (m, 2H), 1.43 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$) δ = 164.6, 156.9, 153.3, 134.9, 110.1, 79.2, 38.2, 37.3, 28.6, 28.1, 13.1; MS (ESI): *m/z* 283.9 $[M+H]^+$ (theoretical *m/z* 284.2).

***N*^{1}-(3-aminopropyl)thymine (24).** To a solution of protected compound **22** (74.0 mg, 0.26 mmol) in CH_2Cl_2 was added 50 eq. of TFA. The reaction mixture was stirred at room temperature for 4h. The solvent and the remaining TFA were then removed under reduced pressure by co-evaporating twice with toluene, then the crude mixture was triturated in Et_2O. After filtration, compound **24** (78.0 mg, 100%) as a colourless solid was obtained. R_f = 0.12 (CH_2Cl_2-MeOH 8:2); ^{1}H NMR (200 MHz, CD_3OD) δ = 7.46 (s, 1H), 3.84 (t, *J* = 6.6 Hz, 2H), 2.99 (t, *J* = 7.3 Hz, 2H), 2.19 – 1.83 (m, 2H), 1.87 (s, 3H); ^{13}C NMR (50 MHz, CD_3OD) δ = 166.8, 153.4, 142.7, 111.8, 46.1, 37.8, 28.1, 12.2; MS (ESI): *m/z* 184.1 $[M+H]^+$ (theoretical *m/z* 184.1).

***N*^{3}-(3-aminopropyl)thymine (25**). To a solution of protected compound **23** (172 mg, 0.61 mmol) in CH_2Cl_2 was added 50 eq. of TFA. The reaction mixture was stirred at room temperature for 4h. The solvent and the remaining TFA were then removed under reduced pressure by co-evaporating twice with toluene, then the crude mixture was triturated in Et_2O. After filtration, compound **25** (170 mg, 94%) as a colourless solid was obtained. R_f = 0.33 (CH_2Cl_2/MeOH 8:2); ^{1}H NMR (200 MHz, CD_3OD) δ = 7.28 (s, 1H), 4.03 (t, *J* = 6.3 Hz, 2H), 2.96 (t, *J* = 6.9 Hz, 2H), 2.13 – 1.86 (m, 2H), 1.87 (s, 3H); ^{13}C NMR (50 MHz, CD_3OD) δ = 166.6, 153.6, 137.8, 109.9, 38.3, 26.9, 12.8; MS (ESI): *m/z* 184.1 $[M+H]^+$ (theoretical *m/z* 184.1).

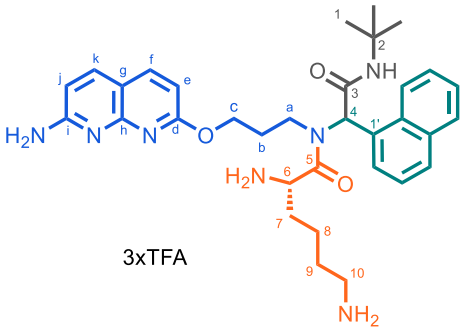
Synthesis of the Ugi products and deprotection

Ugi product 1'a.



Compound **1'a** was prepared following general procedure A using *tert*-butyl (7-(3-aminopropoxy)-1,8-naphthyridin-2-yl)carbamate (**13**) (31.8 mg, 0.10 mmol) as the amine, 1-naphthaldehyde (13.6 µL, 0.10 mmol) as aldehyde, Boc-L-Lys(Boc)-OH (34.6 mg, 0.10 mmol) as the acid and *tert*-butyl isocyanide (11.3 µl, 0.10 mmol) in MeOH (0.2 mL). After purification by flash chromatography (CH_2Cl_2/MeOH gradient 100:0 to 97:3, v/v), a mixture of diastereomers **1'a** was obtained as a white solid (36.6 mg, 41%). $R_{f.dia1+2}$ = 0.20 (CH_2Cl_2-MeOH 98:2); NMR data as a mixture of 2 diastereomers and conformers; ^{1}H NMR (400 MHz, CD_3OD) δ = 8.20 – 8.13 , 8.12 – 7.97 (2m, 3H, H_f + $H_{j\text{-}k}$), 8.33 – 8.26, 7.92 – 7.43, 7.42 – 7.29 (3m, 7H, 7C$\underline{H}_{Ar}$), 6.91, 6.77, 6.59 (3s, 1H, H_4), 6.69, 6.75, 6.61 (2d, J = 8.7 Hz, 1H, H_e), 4.63 – 4.46 (2m, 1H, H_6), 4.12 – 3.85 (2m, 2H, H_c), 3.79 – 3.63, 3.62 – 3.45 (2m, 2H, H_a), 3.12 – 3.02, 3.01 – 2.92 (2m, 2H, H_{10}), 1.91 – 1.80, 1.63 – 1.23, 0.90 – 0.84, 0.67 – 0.53 (4m, 44H, 9C$\underline{H}_{3.Boc}$ + H_b + H_1 + $H_{7\text{-}9}$); ^{13}C NMR (101 MHz, CD_3OD) δ = 175.9, 175.8 (C_5), 171.9, 171.5 (C_3), 166.0, 165.9 (C_d), 158.4, 157.6, 155.5 (3CO_{Boc}), 155.1 (C_i), 154.2 (C_h), 140.3, 140.0 (C_k + C_f), 135.0, 134.9, 134.0, 133.7, 133.1 (3$C_{IV.Ar}$), 130.4, 130.0, 129.7, 128.5, 128.4, 127.9, 127.2, 127.1, 126.2, 126.0, 124.7, 123.9 (7C$\underline{H}_{Ar}$), 117.2, 117.1 (C_g), 113.3, 113.2 (C_e), 112.1, 112.0 (C_j), 82.0, 80.7, 80.4, 80.2, 79.7 (3$C_{IV.Boc}$), 65.3, 64.7, 64.5 (C_c), 62.3, 60.0, 59.2 (C_4), 52.9, 52.5, 52.4, 52.2 (C_2 + C_6), 44.6, 44.4, 44.3 (C_a), 41.1, 41.0 (C_{10}), 33.9, 33.1 (C_7), 30.8, 30.6, 30.2 (C_b + C_9), 28.9, 28.8, 28.8, 28.7, 28.5 (9C$\underline{H}_{3.Boc}$ + C_1), 24.3, 24.2 (C_8); MS (ESI): *m/z* 886.13 $[M+H]^+$ (theoretical *m/z* 886.51).

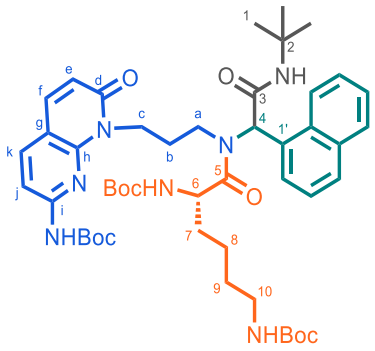
Deprotected Ugi product 1a.



General procedure B was employed for the deprotection of compound **1'a** (30.0 mg, 0.037 mmol) in 1 mL of CH_2Cl_2 and in the presence of 50 equiv. of TFA. After filtration, a mixture of diastereomers **1a**

was isolated as a yellow-pale powder (23.9 mg, 70%). HPLC: rt_{dia1} = 9.1 min and rt_{dia2} = 9.7 min (method A); NMR data as a mixture of 2 diastereomers and conformers; ^{1}H NMR (400 MHz, D_2O) δ = 8.33, 8.28 (2d, J = 9.3 Hz, 1H, H_k), 8.08, 8.06 (2d, J = 8.7 Hz, 1H, H_f), 7.80 – 7.21, 7.15 – 6.98 (2m, 7H, 7C<u>H</u>$_{Ar}$), 7.15 – 6.98 (m, 1H, H_j), 6.65 – 6.59 (m, 1H, H_e), 6.63, 6.40, 6.22 (3s, 1H, H_4), 4.99, 4.57, 4.52 (3t, J = 6.2 Hz, 1H, H_6), 4.37 – 4.22, 4.04 – 3.94, 3.88 – 3.79 (3m, 2H, H_c), 3.59 – 3.49, 3.41 – 3.28 (2m, 2H, H_a), 3.12 – 2.99 (m, 2H, H_{10}), 2.17 – 2.00 (m, 2H, H_7), 1.87 – 1.43 (m, 4H, $H_{8\text{-}9}$), 1.33, 1.31, 1.28 (s, 9H, H_1), 1.87 – 1.75, 1.75 – 1.43, 1.09 – 0.92 (m, 2H, H_b); ^{13}C NMR (101 MHz, D_2O) δ = 171.0, 170.9 (C_3), 170.3, 170.2 (C_5), 164.5, 164.1 (C_d), 155.0 (C_i), 145.3 (C_h), 143.5, 143.4 (C_k), 140.1 (C_f), 132.8, 132.6, 131.7, 131.4 (2$C_{IV.Ar}$), 129.6, 129.3, 128.7, 128.3, 128.1, 127.6, 127.4 ($C_{IV.Ar}$ + 4<u>C</u>H$_{Ar}$), 126.2, 126.0, 125.1, 122.6, 121.9 (3<u>C</u>H$_{Ar}$), 117.7, 114.8 (C_g), 111.9, 111.5, 111.2, 111.0 (C_e), 110.4 (C_j), 64.1, 63.9 (C_c), 60.6, 58.9 (C_4), 52.0, 51.8, 51.6 (C_2), 51.3, 51.1, 50.8 (C_6), 43.7, 42.9 (C_a), 39.0 (C_{10}), 30.9, 30.5 (C_7), 29.2, 28.5 (C_b), 27.5, 27.5, 27.4 (C_1), 26.6 (C_9), 21.7, 21.0 (C_8); HRMS (ESI): *m/z* 586.35065 $[M+H]^+$ ($C_{33}H_{44}N_7O_3$ requires 586.35001).

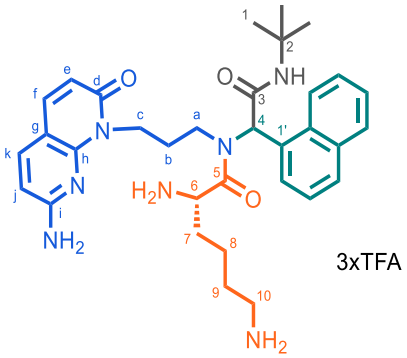
Ugi product 2'a.



Compound **2'a** was prepared following general procedure A using *tert*-butyl (8-(3-aminopropyl)-7-oxo-7,8-dihydro-1,8-naphthyridin-2-yl)carbamate (**16**) (31.8 mg, 0.10 mmol) as the amine, 1-naphthaldehyde (13.6 µL, 0.10 mmol) as aldehyde, Boc-L-Lys(Boc)-OH (34.6 mg, 0.10 mmol) as the acid and *tert*-butyl isocyanide (11.3 µl, 0.10 mmol) in MeOH (0.2 mL). After purification by flash chromatography (CH_2Cl_2/MeOH gradient 100:0 to 96:4, v/v), a mixture of diastereomers **2'a** was obtained as a pale-white solid (52.1 mg, 59%). $R_{f.dia1}$ = 0.32 and $R_{f.dia2}$ = 0.35 (CH_2Cl_2-MeOH 96:4); NMR data as a mixture of 2 diastereomers and conformers; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.99 – 7.81 (m, 2H, H_j + H_k), 8.20 – 8.13, 7.81 – 7.57 (2m, 3H, 2C<u>H</u>$_{Ar}$ + H_f), 7.57 – 7.33 (m, 3H, 3C<u>H</u>$_{Ar}$), 7.33 – 7.13 (m, 1H, C<u>H</u>$_{Ar}$), 7.05 – 6.84 (m, 1H, C<u>H</u>$_{Ar}$), 6.70 ,6.65 (2s, 1H, H_4), 6.49 – 6.32 (m, 1H, H_e), 4.79 – 4.70, 4.50 – 4.22 (2m, 1H, H_6), 4.17 – 3.93, 3.92 – 3.77 (2m, 2H, H_c), 3.61 – 3.29 (m, 2H, H_a), 3.13 – 2.95 (m, 2H, H_{10}), 1.99 – 1.15, 0.72 – 0.47 (2m, 44H, 9C<u>H</u>$_{3.Boc}$ + H_b + H_1 + $H_{7\text{-}9}$); ^{13}C NMR (101 MHz, CD_3OD) δ = 175.7, 175.7, 175.6 (C_5), 171.7, 171.5, 171.4 (C_3), 165.2, 165.1, 165.0 (C_d), 158.5, 158.2, 157.9, 157.8, 154.3, 154.2, 154.1 (3CO_{Boc} + C_i), 149.2, 149.0 (C_h), 140.1, 139.9, 139.8, 139.5, 139.4, 139.3 (C_f + C_k), 134.7, 134.5, 133.8, 133.7, 132.8, 132.5 (3$C_{IV.Ar}$), 130.6, 130.1, 130.0, 129.6, 128.1, 127. 9, 127.8, 127.0, 126.9, 125.7, 125.3, 124.6, 123.8 (7<u>C</u>H$_{Ar}$), 119.5, 119.4 (C_e), 112.9, 112.8 (C_g),

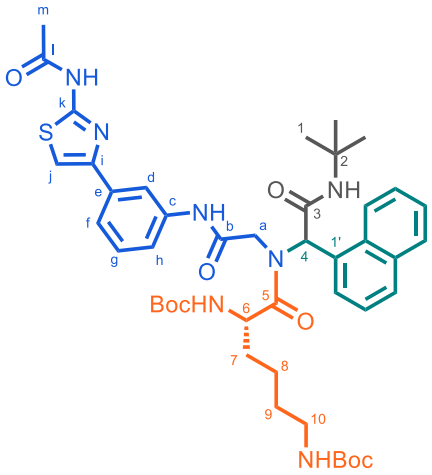
108.8, 108.7 (C_j), 82.0, 82.0, 80.7, 80.5, 80.3, 79.8 ($3C_{IV.Boc}$), 59.9, 59.2, 59.2 (C_4), 53.0, 52.7, 52.6, 52.3, 52.3, 52.1 (C_2 + C_6), 45.3, 44.9, 44.9 (C_a), 41.1, 41.0 (C_{10}), 39.4, 39.0, 39.0 (C_c), 33.7, 33.1, 32.8 (C_7), 30.9, 30.6, 30.5, 30.1 (C_b + C_9), 28.9, 28.9, 28.8, 28.7 ($9\underline{C}H_{3.Boc}$ + C_1), 24.5, 24.3, 24.2 (C_8); MS **(ESI):** *m/z* 885.93 $[M+H]^+$ (theoretical m/z 886.51).

Deprotected Ugi product 2a.



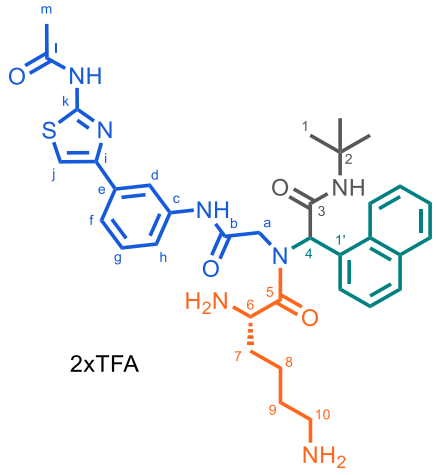
General procedure B was employed for the deprotection of compound **2'a** (35.2 mg, 0.040 mmol) in 1 mL of CH_2Cl_2 and in the presence of 50 eq. of TFA. After filtration, a mixture of diastereomers **2a** was isolated as a white powder (32.2 mg, 88%). HPLC: rt_{dia1} = 10.6 min and rt_{dia2} = 10.9 min (method A); NMR data as a mixture of 2 diastereomers; ^{1}H NMR (400 MHz, D_2O) δ = 7.75 – 7.41 (m, 6H, $4C\underline{H}_{Ar}$ + H_f + H_k), 7.34, 7.26 (2d, *J* = 8.2 Hz, 1H, $C\underline{H}_{Ar}$), 7.07, 7.03 (2d, *J* = 7.2 Hz, 1H, $C\underline{H}_{Ar}$), 6.87 – 6.76 (m, 1H, $C\underline{H}_{Ar}$), 6.54, 6.49 (2d, *J* = 8.4 Hz, 1H, H_j), 6.52, 6.29 (2s, 1H, H_4), 6.23, 6.13 (2d, *J* = 9.3 Hz, 1H, H_e), 4.53, 4.48 (2t, *J* = 5.9 Hz, 1H, H_6), 4.01 – 3.82, 3.81 – 3.51 (2m, 2H, H_c), 3.50 – 3.32, 3.28 – 2.96, 2.94 – 2.75 (3m, 2H, H_a), 3.13 – 2.95 (m, 2H, H_{10}), 2.17 – 1.88 (m, 2H, H_7), 1.88 – 1.43 (m, 4H, $H_{8\text{-}9}$), 1.88 – 1.43, 1.11 – 0.96, 0.57 – 0.41 (3m, 2H, H_b), 1.29, 1.25 (2s, 9H, H_1); ^{13}C NMR (101 MHz, D_2O) δ = 170.9, 170.9 (C_5), 170.1, 170.0 (C_3), 164.6, 164.6 (C_d), 159.3, 159.2 (C_i), 147.6, 147.1 (C_h), 140.3, 140.2 (C_f), 139.2, 139.1 (C_k), 132.6, 132.4, 131.4, 131.1 ($2C_{IV.Ar}$), 130.2, 129.9, 129.3, 129.0 ($2\underline{C}H_{Ar}$), 128.2, 128.1 ($C_{IV.Ar}$), 127.2, 126.9, 126.2, 126.2, 124.4, 122.1, 121.5 ($5\underline{C}H_{Ar}$), 113.5 (C_e), 108.6, 108.5 (C_g), 107.1, 106.8 (C_j), 60.3, 58.9 (C_4), 51.8, 51.5 (C_2), 51.0, 50.8 (C_6), 43.9, 43.6 (C_a), 39.0 (C_{10}), 37.6, 37.5 (C_c), 31.1, 30.6 (C_7), 29.1, 29.1 (C_b), 27.5, 27.5 (C_1), 26.6, 26.5 (C_9), 21.8, 21.0 (C_8); HRMS (ESI): *m/z* 586.35059 $[M+H]^+$ ($C_{33}H_{44}N_7O_3$ requires 586.35001).

Ugi product 3'a.



Compound **3'a** was prepared following general procedure A using *N*-(3-(2-acetamidothiazol-4-yl)phenyl)-2-aminoacetamide (**19**) (43.5 mg, 0.15 mmol) as the amine, 1-naphthaldehyde (20.4 µL, 0.15 mmol) as aldehyde, Boc-L-Lys(Boc)-OH (51.9 mg, 0.15 mmol) as the acid and *tert*-butyl isocyanide (17.0 µl, 0.15 mmol) in MeOH (0.3 mL). The reaction mixture was stirring at 50°C. After purification by flash chromatography (CH_2Cl_2/MeOH gradient 100:0 to 97:3, v/v), the diastereomers **3'a,d1** (47.9 mg, 37%) and **3'a,d2** (47.0 mg, 37%) were separated as white solids. $R_{f.dia1}$ = 0.57 and $R_{f.dia2}$ = 0.45 (CH_2Cl_2-MeOH 97:3); **3'a,d1** NMR data as a single conformer; ^{1}H NMR (400 MHz, CD_3OD) δ = 8.13 – 7.72 (m, 4H, 3C$\underline{H}_{Ar}$ + H_d), 7.72 – 7.40 (m, 4H, 3C$\underline{H}_{Ar}$ + H_f), 7.40 – 7.16 (m, 4H, CH_{Ar} + $H_{g\text{-}h}$ + H_j), 6.69 (s, 1H, H_4), 4.48, 3.77 – 3,52 (d and m, J = 18.5 Hz, 2H, H_a), 4.27 (dd, J = 4.3, 8.8 Hz, 1H, H_6), 2.96 – 2.73 (m, 2H, H_{10}), 2.21 (s, 3H, H_m), 1.86 – 1.59 (m, 2H, H_7), 1.57 – 1.23 (m, 31H, 6C$\underline{H}_{3.Boc}$ + H_1 + $H_{8\text{-}9}$); ^{13}C NMR (101 MHz, CD_3OD) δ = 177.0 (C_5), 172.6 (C_3), 170.9 (C_l), 170.1 (C_b), 159.4, 158.4, 158.2 (2CO_{Boc} + C_k), 150.8 (C_i), 139.4, 136.5 (C_c + C_e), 135.4, 133.8, 131.8 (3$C_{IV.Ar}$), 131.2, 130.0, 129.9, 128.9, 128.6, 127.4, 126.4, 124.3 (7$\underline{C}H_{Ar}$ + C_g), 123.2 (C_f), 120.5 (C_h), 118.6 (C_d), 108.8 (C_j), 80.7, 79.8 (2$C_{IV.Boc}$), 61.9 (C_4), 54.8, 53.2, 52.8, 52.7 (C_2 + C_6), 50.3 (C_a), 40.8 (C_{10}), 32.0 (C_7), 30.6 (C_9), 28.9, 28.8 (6$\underline{C}H_{3.Boc}$ + C_1), 24.2 (C_8), 22.6 (C_m); MS (ESI): *m/z* 858.07 $[M+H]^+$ (theoretical *m/z* 858.42). **3'a,d2** NMR data as a mixture of 2 conformers; ^{1}H NMR (400 MHz, CD_3OD) δ = 8.40 – 8.25, 8.02 – 7.83, 7.82 – 7.73 (3m, 3H, 3C$\underline{H}_{Ar}$), 8.06, 7.89 (2s, 1H, H_d), 7.65 – 7.43 (m, 5H, 4C$\underline{H}_{Ar}$ + H_f), 7.43 – 7.21 (m, 3H, $H_{g\text{-}h}$ + H_j), 6.91, 6.58 (2s, 1H, H_4), 4.73, 4.52 (2dd, $J_{conf.1}$ = 4.1, 9.5 Hz and $J_{conf.2}$ = 4.6, 8.7 Hz, 1H, H_6), 4.10, 3.84 – 3.64, 3.25 (2d and m, $J_{conf.1}$ = 18.2 Hz and $J_{conf.2}$ = 16.5 Hz, 2H, H_a), 3.11 – 2.92 (m, 2H, H_{10}), 2.22, 2.21 (2s, 3H, H_m), 1.90 – 1.64 (m, 2H, H_7), 1.64 – 1.22 (m, 31H, 6C$\underline{H}_{3.Boc}$ + H_1 + $H_{8\text{-}9}$); ^{13}C NMR (101 MHz, CD_3OD) δ = 177.3, 176.2 (C_5), 173.1, 172.3 (C_3), 170.8 (C_l), 170.3, 169.1 (C_b), 159.4, 159.3, 158.5, 158.4, 157.5 (2CO_{Boc} + C_k), 153.4, 150.9 (C_i), 139.8, 139.6, 136.6, 136.4 (C_c + C_e), 135.5, 135.4, 133.7, 133.5, 132.1, 131.7 (3$C_{IV.Ar}$), 131.5, 131.2, 130.1, 130.0, 129.8, 128.9, 128.7, 128.4, 127.7, 127.5, 126.4, 126.4, 124.8, 124.1 (7$\underline{C}H_{Ar}$ + C_g), 123.1, 123.0 (C_f), 121.0, 120.2 (C_h), 119.1, 118.4 (C_d), 108.8 (C_j), 80.9, 80.5, 79.8 (2$C_{IV.Boc}$), 63.6, 62.0 (C_4), 53.3, 52.9, 52.7, 52.6 (C_2 + C_6), 49.9 (C_a), 41.0, 41.0 (C_{10}), 33.4, 32.5 (C_7), 31.0, 30.6 (C_9), 29.0, 28.9, 28.8, 28.8, 28.7 (6$\underline{C}H_{3.Boc}$ + C_1), 24.6, 24.0 (C_8), 22.6 (C_m); MS (ESI): *m/z* 858.00 $[M+H]^+$ (theoretical *m/z* 858.42).

Deprotected Ugi product 3a, d1.

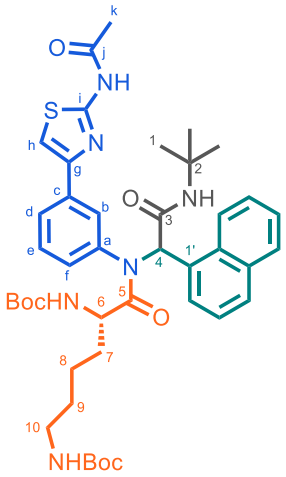


General procedure B was employed for the deprotection of compound **3'a (d1)** (30.0 mg, 0.035 mmol) in 1 mL of CH_2Cl_2 and in the presence of 50 equiv. of TFA. After filtration, diastereomer **3a,d1** was isolated as a white powder (24.0 mg, 77%). HPLC: rt_{dia1} = 12.3 min (method B); ^{1}H NMR (400 MHz, D_2O) δ = 7.80 (d, J = 8.6 Hz, 1H, C$\underline{H}_{Ar}$), 7.64 – 7.24 (m, 5H, 5C$\underline{H}_{Ar}$), 7.24 – 7.12 (m, 2H, C$\underline{H}_{Ar}$ + H_f), 7.11 – 7.00 (m, 1H, H_g), 6.92 – 6.79 (m, 2H, H_j + H_4), 6.68 (br, 1H, H_d), 6.56 (d, J = 7.3 Hz, 1H, H_h), 4.64 – 4.50 (m, 1H, H_6), 4.34 – 4.12 (m, 2H, H_a), 3.02 (t, J = 7.5 Hz, 2H, H_{10}), 2.17 (s, 3H, H_m), 2.10 – 1.93 (m, 2H, H_7), 1.81 – 1.68 (m, 2H, H_9), 1.66 – 1.44 (m, 2H, H_8), 1.33 (s, 9H, H_1); ^{13}C NMR (101 MHz, D_2O) δ = 171.4, 171.0, 170.1 (C_l + C_3 + C_5), 166.5 (C_b), 158.7 (C_k), 147.3 (C_i), 136.0, 133.4 (C_c + C_e), 133.3, 131.7 (2$C_{IV.Ar}$), 130.7 (C$\underline{H}_{Ar}$), 129.1, 128.9, 128.6, 127.2, 126.4, 125.3 ($C_{IV.Ar}$ + 5C$\underline{H}_{Ar}$ + C_g), 122.9 (C$\underline{H}_{Ar}$), 122.6 (C_f), 120.4 (C_h), 118.2 (C_d), 109.2 (C_j), 58.4 (C_4), 51.8 (C_2), 51.3 (C_6), 48.6 (C_a), 39.0 (C_{10}), 29.8 (C_7), 27.6 (C_1), 26.5 (C_9), 22.1 (C_m), 20.9 (C_8); MS (ESI): *m/z* 658.13 $[M+H]^+$ (theoretical *m/z* 658.32).

General procedure B was employed for the deprotection of compound **3'a,d2** (30.0 mg, 0.035 mmol) in 1 mL of CH_2Cl_2 and in the presence of 50 equiv. of TFA. After filtration, a diastereomer was isolated as a white powder (21.9 mg, 71%). HPLC: rt_{dia2} = 12.7 min (method B); ^{1}H NMR (400 MHz, D_2O) δ = 7.89 – 7.72 (m, 1H, C$\underline{H}_{Ar}$), 7.72 – 7.24 (m, 6H, 6C$\underline{H}_{Ar}$), 7.23 – 6.99 (m, 2H, H_f + H_g), 6.98 – 6.85 (m, 1H, H_j), 6.80 – 6.61 (m, 3H, H_d + H_h + H_4), 4.53 (br s, 1H, H_6), 4.46 – 4.10 (m, 2H, H_a), 2.96 (t, J = 7.5 Hz, 2H, H_{10}), 2.30 – 1.91 (m, 5H, H_m + H_7), 1.78 – 1.38 (m, 4H, $H_{8\text{-}9}$), 1.30 (s, 9H, H_1); ^{13}C NMR (101 MHz, D_2O) δ = 171.7, 171.4, 170.8 (C_l + C_3 + C_5), 166.3 (C_b), 158.8 (C_k), 147.4 (C_i), 136.4, 133.4, 133.3, 131.7 (2$C_{IV.Ar}$ + C_c + C_e), 130.7 (C$\underline{H}_{Ar}$), 129.2, 129.0, 128.8, 127.3, 126.5, 125.2 ($C_{IV.Ar}$ + 5C$\underline{H}_{Ar}$ + C_g), 122.7, 122.6 (C$\underline{H}_{Ar}$ + C_f), 120.4 (C_h), 118.3 (C_d), 109.3 (C_j), 59.3 (C_4), 51.9 (C_2), 51.3 (C_6), 49.3 (C_a), 38.9 (C_{10}), 30.3 (C_7), 27.6 (C_1), 26.3 (C_9), 22.1 (C_m), 21.3 (C_8); MS (ESI): *m/z* 658.13 $[M+H]^+$ (theoretical *m/z* 658.32).

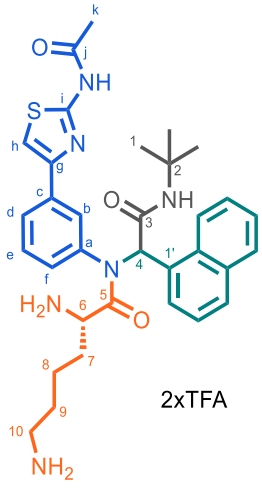
The compounds resulted not sufficiently stable for full characterization and evaluation.

Ugi product 4'a.



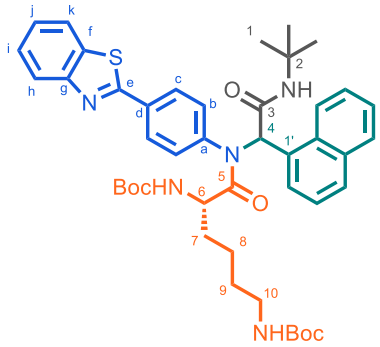
Compound **4'a** was prepared following general procedure A using *N*-(3-(2-acetamidothiazol-4-yl)phenyl)-2-amine (**17**) (17.0 mg, 0.073 mmol) as the amine, 1-naphthaldehyde (9.90 µL, 0.073 mmol) as aldehyde, Boc-L-Lys(Boc)-OH (25.3 mg, 0.073 mmol) as the acid and *tert*-butyl isocyanide (8.2 µl, 0.073 mmol) in MeOH (0.2 mL). The reaction mixture was stirred at 50°C. After purification by flash chromatography (CH_2Cl_2/MeOH gradient 100:0 to 96:4, v/v), a mixture of diastereomers **4'a** was obtained as a white solid (30.0 mg, 51%). $R_{f.dia1+2}$ = 0.25 (CH_2Cl_2-MeOH 96:4); NMR data as a mixture of 2 diastereomers and conformers; ^{1}H NMR (400 MHz, CD_3OD) δ = 8.55 – 8.34, 8.16 – 7.96, 7.94 – 6.80 (3m, 10H, 9C<u>H</u>$_{Ar}$ + H$_4$), 6.73 – 5.86 (3m, 3H, C<u>H</u>$_{Ar}$), 4.28 – 4.03 (m, 1H, H$_6$), 2.95 – 2.69 (m, 2H, H$_{10}$), 2.22, 2.17, 2.16 (3s, 3H, H$_k$), 1.84 – 0.78 (m, 33H, 6C<u>H</u>$_{3.Boc}$ + H$_1$ + H$_{7\text{-}9}$); ^{13}C NMR (101 MHz, CD_3OD) δ = 175.3, 174.8 (C$_5$), 171.7, 170.9, 170.8 (C$_j$ + C$_3$), 159.2, 158.4, 158.0 2CO$_{Boc}$ + C$_i$), 150.3, 149.5 (C$_g$), 139.8, 139.7, 139.7, 136.8, 136.1, 135.5, 135.2, 135.1, 134.9, 133.8, 133.7, 132.8, 132.2, 131.1 (5C$_{IV.Ar}$), 130.3, 130.2, 130.1, 129.7, 129.5, 129.3, 129.0, 128.9, 128.2, 127.8, 127.1, 126.9, 126.5, 126.1, 126.1, 125.7, 125.1, 125.0, 124.3, 124.2 (11C<u>H</u>$_{Ar}$), 109.6, 109.2, 108.8, 108.3 (C$_h$), 80.3, 79.8 (2C$_{IV.Boc}$), 62.4, 62.0 (C$_4$), 53.4, 52.4, 52.3 (C$_2$ + C$_6$), 40.9 (C$_{10}$), 33.6, 32.1 (C$_7$), 30.8, 30.1 (C$_9$), 28.9, 28.8, 23.9 (6C<u>H</u>$_{3.Boc}$ + C$_1$), 23.8, 23.8 (C$_8$), 22.6, 22.6, 22.6 (C$_k$); MS (ESI): *m/z* 801.00 [M+H]$^+$ (theoretical *m/z* 801.40).

Deprotected Ugi product 4a.



General procedure B was employed for the deprotection of compound **4'a** (24,4 mg, 0.031 mmol) in 1 mL of CH_2Cl_2 and in the presence of 50 eq. of TFA. After filtration, a mixture of diastereomers **4a** was isolated as a white powder (24.0 mg, 94%). HPLC: rt_{dia1} = 11.6 min and rt_{dia2} = 12.0 min (method A); NMR data as a mixture of 2 diastereomers and conformers; ^{1}H NMR (400 MHz, CD_3OD) δ = 8.43, 8.18 – 8.03, 7.89 – 7.45 (d and 3m, J = 8.0 Hz, 7H, 7C<u>H</u>$_{Ar}$), 7.43 – 7.08 (m, 2H, 2C<u>H</u>$_{Ar}$), 7.43 – 7.08, 6.77, 6.71 (m and 2t, J = 7.8 Hz, 1H, H_e), 7.43 – 7.08, 6.42, 5.96 (m and 2s, 1H, H_h), 7.03, 6.96 (2d, J_{dia1} = 8.9 Hz and J_{dia2} = 4.9 Hz, 1H, H_4), 6.50, 6.34, 5.91 (s and 2d, J = 7.8 Hz and J = 8.2 Hz, 1H, C<u>H</u>$_{Ar}$), 4.08 – 4.02, 3.88 – 3.77 (2m, 1H, H_6), 2.94 – 2.63 (m, 2H, H_{10}), 2.23, 2.17 (2s, 3H, H_k), 1.87 – 1.63 (m, 2H, H_7), 1.63 – 1.18 (m, 13H, H_1 + H_{8-9}); ^{13}C NMR (101 MHz, CD_3OD) δ = 171.9, 171.7, 171.5, 171.4 (C_5), 171.0, 170.9, 170.5, 169.9, 169.8 (C_j + C_3), 159.8, 159.5 (C_i), 150.0, 149.7, 149.2, 149.1 (C_g), 138.8, 138.7, 137.9, 137.3, 136.5, 136.2, 135.9, 135.2, 135.2, 134.9, 133.7, 133.7, 133.5 (4$C_{IV.Ar}$), 132.1, 131.9, 131.6, 131.4, 131.3, 131.0, 130.7, 130.7, 130.5, 130.4, 130.4, 130.2, 129.9, 129.8, 129.7, 129.6, 129.3, 128.3, 128.1, 127.8, 127.8, 127.6, 127.5, 127.3, 127.3, 127.2, 127.0, 126.2, 126.1, 125.7 ($C_{IV.Ar}$ + 11C<u>H</u>$_{Ar}$), 124.7, 124.5, 124.2, 124.0 (C_h), 62.9, 62.3 (C_4), 52.8, 52.6, 52.5 (C_2 + C_6), 40.1, 40.1 (C_{10}), 31.6, 31.6, 30.7, 30.6 (C_7), 28.9, 28.9, 28.8 (C_1), 27.8, 27.8 (C_9), 23.0, 22.6, 22.5, 22.0, 21.8 (C_k + C_8); HRMS (ESI): *m/z* 601.29529 $[M+H]^+$ ($C_{33}H_{41}N_6O_3S$ requires 601.29554).

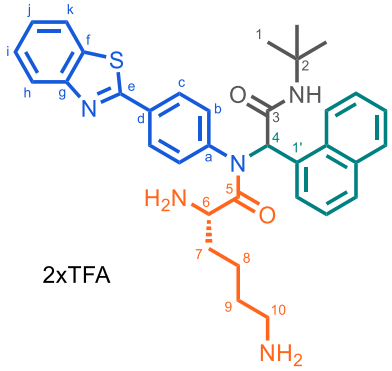
Ugi product 6'a.



Compound **6'a** was prepared following general procedure A using 2-(4-aminophenyl)benzothiazole (34.0 mg, 0.15 mmol) as the amine, 1-naphthaldehyde (20.4 µL, 0.15 mmol) as aldehyde, Boc-L-Lys(Boc)-OH (51.9 mg, 0.15 mmol) as the acid and *tert*-butyl isocyanide (17.0 µl, 0.15 mmol) in MeOH (0.3 mL). The reaction mixture was stirred at 50°C. After purification by flash chromatography (CH_2Cl_2/MeOH gradient 100:0 to 97:3, v/v), a mixture of diastereomers **6'a** was obtained as a yellow-pale solid (68.1 mg, 58%). $R_{f.dia1+2}$ = 0.44 (CH_2Cl_2-MeOH 97:3); NMR data as a mixture of 2 diastereomers; ^{1}H NMR (400 MHz, CD_3OD) δ = 8.25 – 7.87, 7.86 – 7.32 (2m, 13H, 13C<u>H</u>$_{Ar}$), 7.30 – 7.12 (m, 2H, 2C<u>H</u>$_{Ar}$), 7.01, 6.98 (2s, 1H, H_4), 6.89 – 6.80, 6.73 – 6.64, 6.43 – 6.31, 6.16 – 5.96 (4m, 2NH), 4.22 – 4.03 (m, 1H, H_6), 2.96 – 2.75 (m, 2H, H_{10}), 1.82 – 1.01 (m, 33H, 6C<u>H</u>$_{3.Boc}$ + H_1 + H_{7-9}); ^{13}C NMR (101 MHz, CD_3OD) δ = 175.2, 174.8 (C_5), 171.6, 171.5 (C_3), 168.5, 168.4 (C_e), 158.4, 158.0, 157.4 (2CO_{Boc}), 155.0, 154.9 (C_g), 142.4 (C_a), 136.2, 136.2 (C_f), 135.2, 135.0, 134.2, 133.8, 133.7, 132.5, 132.4, 132.1 (3$C_{IV.Ar}$ + C_d), 130.4, 130.2, 130.2, 129.8, 129.5, 128.2, 127.9, 127.7, 127.2, 127.1, 126.8, 126.1, 125.8, 124.9, 124.2, 123.9, 123.0 (15<u>C</u>H$_{Ar}$), 80.4, 79.7 (2$C_{IV.Boc}$), 62.5, 61.9 (C_4), 53.5, 53.3 (C_6),

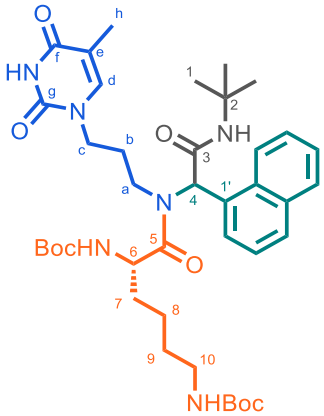
52.5, 52.3 (C_2), 41.0 (C_{10}), 33.4, 31.9 (C_7), 30.3, 30.1 (C_9), 28.9, 28.9, 28.8, 28.8, 28.7 (6$\underline{C}H_{3.Boc}$ + H_1), 23.9, 23.8 (C_8); MS (ESI): *m/z* 794.07 $[M+H]^+$ (theoretical *m/z* 794.39).

Deprotected Ugi product 6a.



General procedure B was employed for the deprotection of compound **6'a** (53.2 mg, 0.067 mmol) in 1 mL of CH_2Cl_2 and in the presence of 50 equiv. of TFA. After filtration, a mixture of diastereomers **6a** was isolated as a yellow-pale powder (48.2 mg, 88%). HPLC: rt_{dia1} = 13.8 min and rt_{dia2} = 14.3 min (method A); NMR data as a mixture of 2 diastereomers; ^{1}H NMR (400 MHz, CD_3OD) δ = 8.19 – 7.99, 7.98 – 7.84, 7.79 – 7.57, 7.54 – 7.30, 7.27 – 7.13 (5m, 14H, 14$\underline{C}H_{Ar}$), 7.09, 7.02 (2s, 1H, H_4), 6.79, 6.10 (d and br s, *J* = 8.04 Hz, 1H, $C\underline{H}_{Ar}$), 4.13, 3.85 (2t, J_{dia1} = 5.2 Hz and J_{dia2} = 6.0 Hz, 1H, H_6), 2.90, 2.81 (2t, J_{dia1} = 7.3 Hz and J_{dia2} = 7.5 Hz, 2H, H_{10}), 1.89 – 1.63 (m, 2H, H_7), 1.63 – 1.22 (m, 4H, $H_{8\text{-}9}$), 1.43, 1.38 (2s, 9H, H_1); ^{13}C NMR (101 MHz, CD_3OD) δ = 171.6, 171.3 (C_3), 170.4, 169.8 (C_5), 168.0, 167.9 (C_e), 154.9, 154.8 (C_g), 141.1, 141.1 (C_a), 136.2, 136.1 (C_f), 135.2, 134.9, 134.4, 133.6, 133.4, 132.8, 131.8, 131.5, 131.3 (3$C_{IV.Ar}$ + $\underline{C}H_{Ar}$ + C_d), 130.8, 130.6, 130.3, 129.9, 129.8, 129.7, 129.3, 128.4, 128.3, 128.0, 127.9, 127.8, 127.4, 127.2, 127.0, 126.9, 126.1, 125.8, 124.5, 124.0, 123.9, 123.9, 123.0, 122.9 (14$\underline{C}H_{Ar}$), 63.1, 62.3 (C_4), 52.8 (C_2), 52.6, 52.5 (C_6), 40.1 (C_{10}), 31.6, 30.5 (C_7), 28.9, 28.8 (C_1), 27.8, 27.8 (C_9), 22.9, 21.8 (C_8); HRMS (ESI): *m/z* 594.28992 $[M+H]^+$ ($C_{35}H_{40}N_5O_2S$ requires 594.28972).

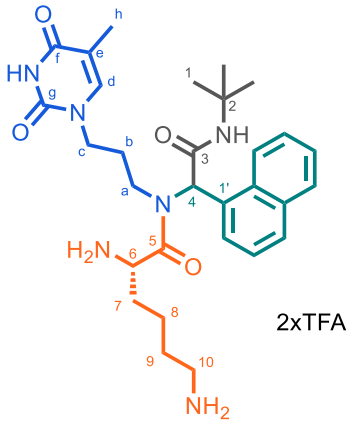
Ugi product 7'a.



Compound **7'a** was prepared following general A procedure using the TFA salt of N^1-(3-aminopropyl)thymine (**24**) (53.5 mg, 0.18 mmol) as the amine, 1-naphthaldehyde (24.7 µL, 0.18 mmol)

as aldehyde, Boc-L-Lys(Boc)-OH (62.3 mg, 0.18 mmol) as the acid and *tert*-butyl isocyanide (20.4 µl, 0.18 mmol) in MeOH (1.0 mL). After purification by flash chromatography (CHX/EtOAc gradient 60:40 to 10:90, v/v), a mixture of diastereomers **7'a** was obtained as a white solid (45.0 mg, 33%). $R_{f.dia1+2}$ = 0.36 (cHex-EtOAc 20:80); NMR data as a mixture of 2 diastereomers and conformers; ^{1}H NMR (400 MHz, CD_3OD) δ = 8.41 – 8.28, 8.01 – 7.77, 7.75 – 7.34 (3m, 7H, 7C<u>H</u>$_{Ar}$), 6.95, 6.77, 6.65 (3s, 1H, H_d), 6.81, 6.63, 6.60 (3s, 1H, H_4), 4.83 – 4.72, 4.52 – 4.20 (2m, 1H, H_6), 3.60 – 3.12 (m, 4H, H_a + H_c), 3.11 – 2.92 (m, 2H, H_{10}), 1.88 – 1.59 (m, 5H, H_h + H_7), 1.59 – 1.27 (m, 31H, 6C<u>H</u>$_{3.Boc}$ + H_1 + $H_{8\text{-}9}$), 1.88 – 1.59, 1.04 – 0.64, 0.57 – 0.39 (3m, 2H, H_b); ^{13}C NMR (101 MHz, CD_3OD) δ = 176.2, 175.8 (C_5), 171.8, 171.7, 171.4 (C_3), 166.8, 166.8 (C_f), 158.5, 158.4, 157.8, 157.7 (2CO_{Boc}), 152.5, 152.4, 152.3 (C_g), 142.7, 142.5, 142.5 (C_d), 135.1, 135.1, 135.0, 134.0, 133.8, 133.7, 133.0, 132.8, 132.4 (3$C_{IV.Ar}$), 131.1, 130.7, 130.6, 130.2, 130.0, 129.9, 128.5, 128.5, 128.3, 128.2, 128.1, 127.5, 127.4, 127.4, 126.3, 126.1, 125.3, 124.7, 123.9 (7C<u>H</u>$_{Ar}$), 111.1, 111.0, 110.8 (C_e), 80.8, 80.5, 80.4, 79.8 (2$C_{IV.Boc}$), 62.3, 60.4, 59.4 (C_4), 53.0, 52.6, 52.4, 52.3 (C_2 + C_6), 46.8, 46.4, 45.9, 44.0, 43.8 (C_a + C_c), 41.1 (C_{10}), 33.9, 32.9 (C_7), 30.9, 30.7, 30.6 (C_b + C_9), 28.8, 28.8 (6C<u>H</u>$_{3.Boc}$ + C_1), 24.6, 24.3, 24.2 (C_8), 12.3, 12.3 (C_h); MS (ESI): *m/z* 751.5 $[M+H]^+$ (theoretical m/z 751.44).

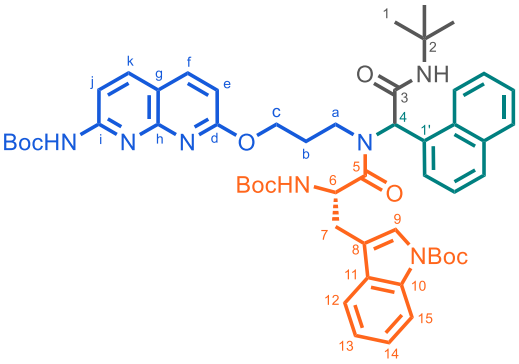
Deprotected Ugi product 7a.



General procedure B was employed for the deprotection of compound **7'a** (22.0 mg, 0.029 mmol) in 1 mL of CH_2Cl_2 and in the presence of 50 equiv. of TFA. After filtration, a mixture of diastereomers **7a** was isolated as a white powder (20.7 mg, 91%). HPLC: rt_{dia1} = 8.5 min and rt_{dia2} = 8.7 min (method A); NMR data as a mixture of 2 diastereomers; ^{1}H NMR (400 MHz, D_2O) δ = 7.99 – 7.89 (m, 1H, C<u>H</u>$_{Ar}$), 7.89 – 7.82 (m, 1H, C<u>H</u>$_{Ar}$), 7.81 – 7.72, 7.69 – 7.54 (m, 3H, 3C<u>H</u>$_{Ar}$), 7.44 – 7.32 (m, 2H, 2C<u>H</u>$_{Ar}$), 6.74, 6.59 (2s, 1H, H_d), 6.55, 6.31 (2s, 1H, H_4), 4.54 – 4.44 (2m, 1H, H_6), 3.43 – 3.34, 3.33 – 3.12, 2.70 – 2.58 (3m, 4H, H_a + H_c), 3.08 – 2.98 (m, 2H, H_{10}), 2.09 – 1.95 (m, 2H, H_7), 1.82 – 1.67 (m, 2H, H_9), 1.69, 1.65 (2s, 3H, H_h), 1.68 – 1.45 (m, 2H, H_8), 1.34, 1.29 (2s, 9H, H_1), 1.83 – 1.47, 1.39 – 1.22, 0.77 - 0.60 (2m, 2H, H_b); ^{13}C NMR (101 MHz, D_2O) δ = 170.8, 170.8, 170.3, 170.0 (C_3 +C_5), 166.6, 166.5 (C_f), 151.2, 151.1 (C_g), 142.3, 142.2 (C_d), 133.2, 133.1, 131.7, 131.4 (2$C_{IV.Ar}$), 130.1, 130.0, 129.3, 129.1, 128.7, 128.5 ($C_{IV.Ar}$ + 2C<u>H</u>$_{Ar}$), 128.2, 127.8, 127.8, 127.5, 126.8, 126.8, 125.2, 125.1, 122.5, 121.7 (5C<u>H</u>$_{Ar}$),

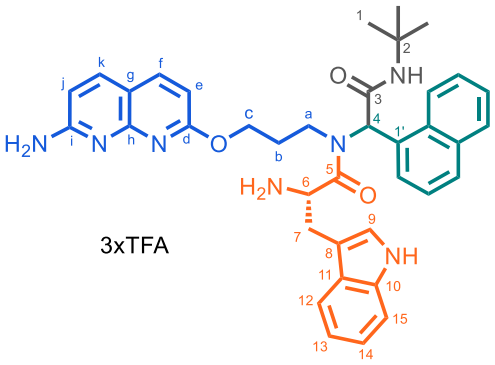
110.6 (C_e), 61.1, 59.9 (C_4), 51.9, 51.6 (C_2), 51.0, 50.9 (C_6), 45.3, 45.1, 42.6, 42.5 (C_a + C_c), 39.0, 39.0 (C_{10}), 30.8, 30.5 (C_7), 29.4, 29.2 (C_b), 27.6, 27.6 (C_1), 26.5 (C_9), 21.6, 20.8 (C_8), 11.2, 11.2 (C_h); HRMS (ESI): *m/z* 551.33453 $[M+H]^+$ ($C_{30}H_{43}N_6O_4$ requires 551.33403).

Ugi product 1'b



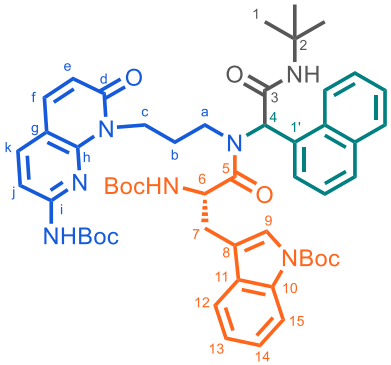
Compound **1'b** was prepared following general procedure A using Boc-naphthyridine(*O*-alkyl)-NH_2 **(13)** (31.8 mg, 0.10 mmol) as the amine, 1-naphthaldehyde (13.6 µL, 0.10 mmol) as aldehyde, Boc-L-Trp(Boc)-OH (40.4 mg, 0.10 mmol) as the acid and *tert*-butyl isocyanide (11.3 µl, 0.10 mmol) in MeOH (0.2 mL). After purification by flash chromatography (CHX/EtOAc gradient 100:0 to 75:25, v/v), a mixture of diastereomers **1'b** was obtained as a white solid (84.5 mg, 90%).$R_{f,dia1+2}$ = 0.26 (CHX-EtOAc 70:30); NMR data as a mixture of 2 diastereomers and conformers; ^{1}H NMR (400 MHz, CD_3OD) δ = 8.19 – 7.98, 7.97 – 7.81, 7.80 – 7.59 (3m, 7H, 4C$\underline{H}_{Ar}$ + H_f + $H_{j\text{-}k}$), 7.58 – 6.95 (m, 8H, 8C$\underline{H}_{Ar}$), 6.90, 6.62, 6.56 (3s, 1H, H_4), 6.69, 6.57, 6.32 (3d, *J* = 8.7 Hz, 1H, H_e), 5.24 – 5.18, 5.10 – 4.99, 4.96 – 4.77 (m, 1H, H_6), 4.19 – 4.12, 4.06 – 3.97, 3.93 – 3.83, 3.82 – 3.62 (4m, 2H, H_c), 3.82 – 3.62, 3.63 – 3.36 (2m, 2H, H_a), 3.35 – 3.26, 3.19 – 2.94 (2m, 2H, H_7), 1.76 – 1.11, 1.10 – 0.84 (m, 36H, 9C$\underline{H}_{3.Boc}$ + H_1), 1.76 – 1.11, 0.68 – 0.51, 0.50 – 0.35 (3m, 2H, H_b); ^{13}C NMR (101 MHz, CD_3OD) δ = 175.1, 174.6, 173.9, 172.9, 171.9, 171.8 (C_3 + C_5), 166.0, 165.9 (C_d), 158.1, 157.3, 157.3, 155.4, 155.4, 155.0, 155.0, 154.2, 154.2, 150.9 (3CO_{Boc}, C_h + C_i), 139.9, 139.9 (C_f + C_k), 136.7, 134.9, 134.8, 134.0, 133.7, 133.1, 132.7, 131.9, 131.7 (5$C_{IV.Ar}$), 130.5, 130.5, 130.4 129.9, 129.8, 128.5, 128.4, 128.2, 128.0, 127.8, 127.2, 127.2, 127.0, 126.1, 126.0, 125.4, 125.4, 125.2, 124.6, 123.7, 123.7, 123.6 (10$\underline{C}H_{Ar}$), 120.3, 120.0, 117.7 ($\underline{C}H_{Ar}$), 117.7, 117.5, 117.2, 117.1 (C_g + C_8), 116.2 (C_{15}), 113.3, 113.2, 113.0 (C_e), 111.9, 111.8 (C_j), 83.4, 83.2, 80.6, 79.6, 79.2, 78.9 (3$C_{IV.Boc}$), 65.3, 64.6, 64.4 (C_c), 59.8, 59.5 (C_4), 52.8, 52.6, 52.4, 52.3, 51.9 (C_2 + C_6), 44.5, 44.1 (C_a), 30.7, 30.4, 29.7 (C_b), 28.9, 28.9, 28.8, 28.7, 28.6, 28.5, 28.5, 27.9 (9$\underline{C}H_{3.Boc}$ + C_1 + C_7) ; MS (ESI): *m/z* 944.00 $[M+H]^+$ (theoretical *m/z* 944.49).

Deprotected Ugi product 1b



General procedure B was employed for the deprotection of compound **1'b** (50.0 mg, 0.053 mmol) in 1 mL of CH_2Cl_2 and in the presence of 50 equiv. of TFA. After filtration, a mixture of diastereomers **1b** was isolated as a brown-pale powder (42.5 mg, 82%). HPLC: rt_{dia1} = 12.7 min and rt_{dia2} = 12.9 min (method A); NMR data as a mixture of 2 diastereomers; ^{1}H NMR (400 MHz, CD_3OD) δ = 8.23, 8.21 (2d, J = 9.2 Hz, 1H, H_k), 8.03, 7.98 (2d, J = 8.7 Hz, 1H, H_f), 8.00 – 7.95, 7.76 – 7.68, 7.63 – 7.48, 7.42 – 7.36, 7.33 – 7.26 (9m, 5H, 5C$\underline{H}_{Ar}$), 7.19 – 7.12, 7.10 – 7.00, 6.98 – 6.90 (3m, 4H, 2C$\underline{H}_{Ar}$ + H_j + H_9), 6.56, 6.50 (2d, 1H, H_e), 7.28, 6.50 (2s, 1H, H_4), 4.67, 4.56 (2dd, J_{dia1} = 9.2, 5.6 Hz and J_{dia2} = 8.2, 5.0 Hz, 1H, H_6), 4.06 – 3.99, 3.79 – 3.70 (m, 2H, H_c), 3.78 – 3.33, 3.32 – 3.12, 3.06 – 2.94 (3m, 4H, H_a + H_7), 1.38, 1.35 (2s, 9H, H_1), 1.43 – 1.26, 1.29 – 1.27, 1.01 – 0.84, 0.82 – 0.67 (3m, 2H, H_b); ^{13}C NMR (101 MHz, CD_3OD) δ = 172.5, 171.5, 171.5, 171.0 (C_3 + C_5), 166.6, 166.5 (C_d), 157.3, 157.2 (C_h), 147.5, 147.5 (C_i), 144.3, 144.3 (C_k), 140.8 (C_f), 138.3, 138.1 (C_{10}), 134.9, 134.7, 133.9, 133.7, 132.3, 131.7 (3$C_{IV.Ar}$), 130.5, 129.7, 129.6 (2C$\underline{H}_{Ar}$), 128.9, 128.7, 128.6, 128.3, 128.2, 127.4, 127.4 ($C_{IV.Ar}$ + 3C$\underline{H}_{Ar}$), 126.2, 126.1, 125.9, 125.6 (C$\underline{H}_{Ar}$ + C_9), 124.5 , 123.9, 122.9, 122.9, 120.4, 118.8, 118.6 (4C$\underline{H}_{Ar}$), 112.8, 112.8 (C_{15}), 112.6, 112.5 (C_g), 112.3, 112.1 (C_e), 111.6, 111.6 (C_j,), 108.0, 107.6 (C_8), 65.4, 65.4 (C_c), 61.0, 59.4 (C_4), 53.2, 52.6, 52.4, 52.2 (C_2 + C_6), 44.1, 43.9 (C_a), 30.4, 30.1 (C_b), 29.2, 28.8, 28.7, 28.5 (C_1 + C_7); HRMS (ESI): *m/z* 644.33453 $[M+H]^+$ ($C_{38}H_{42}N_7O_3$ requires 644.33436).

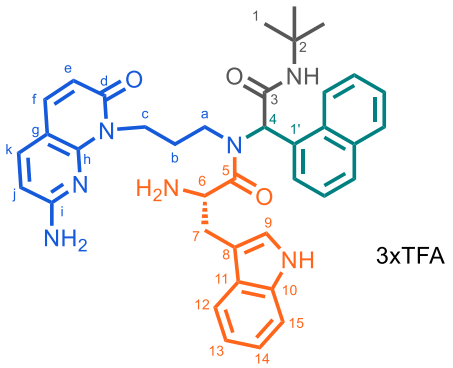
Ugi product 2'b



Compound **2'b** was prepared following general procedure A using compound **(16)** (31.8 mg, 0.10 mmol) as the amine, 1-naphthaldehyde (13.6 µL, 0.10 mmol) as aldehyde, Boc-L-Trp(Boc)-OH (40.5 mg, 0.10 mmol) as the acid and *tert*-butyl isocyanide (11.3 µl, 0.10 mmol) in MeOH (0.2 mL). After purification by flash chromatography (CH_2Cl_2/MeOH gradient 100:0 to 97:3, v/v), a mixture of diastereomers **2'b**

was obtained as a white solid (59.6 mg, 63%). $R_{f.dia1}$ = 0.41 and $R_{f.dia2}$ = 0.46 (CH_2Cl_2-MeOH 96:4); NMR data as a mixture of 2 diastereomers and conformers; ^{1}H NMR (400 MHz, CD_3OD) δ = 8.18 – 8.01 (m, 1H, H_{15}), 7.94 – 7.54 (m, 4H, H_f + $H_{j\text{-}k}$), 7.93 – 6.75 (m, 11H, 11C$\underline{H}_{Ar}$), 6.79, 6.73, 6.44, 6.46 (4s, 1H, H_4), 6.43– 6.24 (m, 1H, H_e), 5.19 – 5.08, 5.05 – 4.93, 5.92 – 4.71 (3m, 1H, H_6), 4.30 – 3.95, 3.90 – 3.69 (2m, 2H, H_c), 3.68 – 3.22 (m, 2H, H_a), 3.52 – 3.22, 3.19 – 2.84 (2m, 2H, H_7), 1.95 – 0.81, 0.74 – 0.50, 0.39 – 0.22 (3m, 2H, H_b), 1.77 – 0.81 (m, 36H, 9C$\underline{H}_{3.Boc}$ + H_1); ^{13}C NMR (101 MHz, CD_3OD) δ = 174.8, 174.5, 174.3, 173.3 (C_5), 171.9, 171.7, 171.6 (C_3), 165.2, 165.1, 164.7 (C_d), 158.1, 157.6, 157.6, 157.6, 154.1, 154.1, 154.0 (2CO_{Boc} + C_i), 151.0, 149.0, 148.9 (CO_{Boc} + C_h), 140.0, 139.8 (C_k), 139.3, 139.2 (C_f), 136.8, 134.5, 134.4, 133.8, 133.5, 132.5, 132.3, 131.8 (5$C_{IV.Ar}$), 130.4, 130.1, 129.7, 128.1, 128.0, 127.8, 127.6, 126.9, 126.8, 125.6, 125.4, 125.3, 124.4, 123.8, 123.6, 123.5 (10C$\underline{H}_{Ar}$), 120.3, 120.2 (C_{12}), 119.4, 119.3 (C_e), 117.8, 117.7 (C_8), 116.1 (C_{15}), 112.8 (C_g), 108.7, 108.6 (C_j), 84.7, 84.7, 82.1, 82.0, 81.9, 80.9, 80.4 (3$C_{IV.Boc}$), 59.9, 59.4 (C_4), 53.2, 52.8, 52.6, 52.5, 52.3, 51.8 (C_2 + C_6), 45.2, 44.7 (C_a), 39.1, 39.1 (C_c), 30.8, 30.2, 29.9 (C_b), 29.4, 28.9, 28.8, 28.7, 28.7, 28.5, 28.4, 28.1 (9C$\underline{H}_{3.Boc}$ + C_1 + C_7); MS (ESI): *m/z* 944.07 $[M+H]^+$ (theoretical *m/z* 944.49).

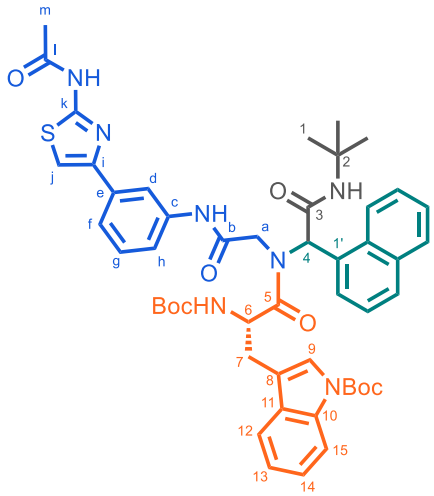
Deprotected Ugi product 2b



General procedure B was employed for the deprotection of compound **2'b** (40.7 mg, 0.043 mmol) in 1 mL of CH_2Cl_2 and in the presence of 50 equiv. of TFA. After filtration, a mixture of diastereomers **2b** was isolated as a pale-white powder (34.3 mg, 81%). HPLC: rt_{dia1} = 14.9 min and rt_{dia2} = 15.2 min (method A); NMR data as a mixture of 2 diastereomers; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.92 – 7.86, 7.80 – 7.71, 7.70 – 7.52 (3m, 3H, 3C$\underline{H}_{Ar}$), 7.64 – 7.52 (m, 2H, H_f + H_k), 7.51 – 7.33 (m, 4H, 4C$\underline{H}_{Ar}$), 7.28 (s, 1H, C_9), 7.22 – 7.03, 7.01 – 6.95 (2m, 4H, 4C$\underline{H}_{Ar}$), 6.85, 6.31 (2s, 1H, H_4), 6.44, 6.37 (2d, J = 8.5 Hz, 1H, H_j), 6.19, 6.16 (2d, J = 9.2 Hz, 1H, H_e), 4.61, 4.44 (2dd, J_{dia1} = 6.5, 8.4 Hz and J_{dia2} = 3.9, 9.2 Hz, 1H, H_6), 4.14 – 4.04, 3.90 – 3.77, 3.74 – 3.60 (3m, 2H, H_c), 3.74 – 3.60, 3.51 – 3.43, 3.40 – 3.18 (3m, 2H, H_7), 3.60 – 3.52, 3.13 – 3.00, 2.89 – 2.76 (3m, 2H, H_a), 1.55 – 1.41, 1.40 – 1.14, 0.95 – 0.80, 0.65 – 0.51 (4m, 2H, H_b), 1.34, 1.30 (2s, 9H, H_1); ^{13}C NMR (101 MHz, CD_3OD) δ = 172.4, 171.4 (C_5), 171.2, 170.6 (C_3), 165.5 (C_d), 161.5, 161.2 (C_i), 150.4, 150.3 (C_h), 140.2 (C_f), 139.2, 139.0 (C_k), 138.4, 138.2 (C_{10}), 134.7, 134.5, 133.8, 133.4, 131.8, 131.1 (3$C_{IV.Ar}$), 130.8, 130.8, 130.0, 129.9 (2C$\underline{H}_{Ar}$), 128.6, 128.4, 128.3, 128.0, 127.1, 127.1 ($C_{IV.Ar}$ + 3C$\underline{H}_{Ar}$), 126.1 (C_9), 125.7, 125.4, 124.2, 123.5, 123.0, 120.6, 120.5 (4C$\underline{H}_{Ar}$), 119.0, 118.9 (C_{12}), 114.6 (C_e), 112.8 (C_{15}), 108.6, 108.6 (C_g), 108.0, 107.7 (C_8), 106.6,

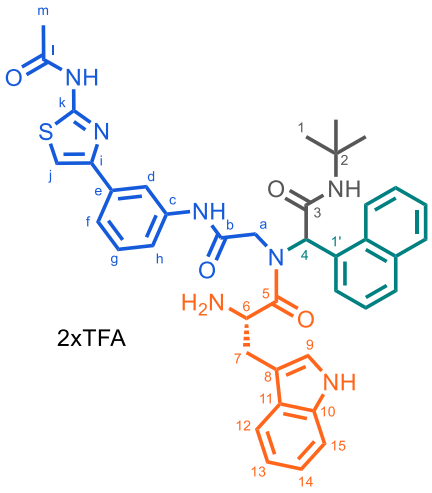
106.5 (C_j), 61.2, 59.4 (C_4), 53.5, 52.5, 52.4, 52.4 (C_2 + C_6), 45.0, 44.8 (C_a), 38.7, 38.6 (C_c), 30.7, 30.4 (C_b), 29.1, 28.8, 28.7, 28.7 (C_1 + C_7); HRMS (ESI): *m/z* 644.33478 $[M+H]^+$ ($C_{38}H_{42}N_7O_3$ requires 644.33436).

Ugi product 3'b



Compound **3'b** was prepared following general procedure A using compound (**19**) (29.1 mg, 0.10 mmol) as the amine, 1-naphthaldehyde (13.6 µL, 0.10 mmol) as aldehyde, Boc-L-Trp(Boc)-OH (40.5 mg, 0.10 mmol) as the acid and *tert*-butyl isocyanide (11.3 µl, 0.10 mmol) in MeOH (0.2 mL). After purification by flash chromatography (CH_2Cl_2/MeOH gradient 100:0 to 96:4, v/v), a mixture of diastereomers **3'b** was obtained as a white solid (66.8 mg, 73%).$R_{f,dia1+2}$ = 0.25 (CH_2Cl_2-MeOH 96:4); NMR data as a mixture of 2 diastereomers and conformers; ^{1}H NMR (400 MHz, CD_3OD) δ = 8.25 – 6.88 (m, 17H, 17C$\underline{H}_{Ar}$), 6.87, 6.61, 6.41 (3s, 1H, H_4), 5.23 – 5.15, 5.01 – 4.84, 4.72 – 4.62 (3m, 1H, H_6), 4.12 – 3.80, 3.52 – 3.38 (d and 2m, *J* = 18.7 Hz, 2H, H_a), 3.37 – 3.01 (m, 2H, H_7), 2.19 (s, 3H, H_m), 1.74 – 1.07, 0.94 – 0.78 (2m, 21H, 6C$\underline{H}_{3.Boc}$ + H_1); ^{13}C NMR (101 MHz, CD_3OD) δ = 176.0, 175.3 (C_5), 172.9, 172.8, 172.4 (C_3), 170.9 (C_l), 170.1 (C_b), 159.4, 159.2, 158.7, 157.2 (2CO_{Boc} + C_k), 150.7 (C_i), 139.7, 139.5, 136.8, 136.5, 136.4, 136.3, 135.6, 135.4, 135.3, 133.7 (3$C_{IV.Ar}$ + C_c + C_e), 131.9, 131.8, 131.6, 131.5, 131.3, 131.2 (2$C_{IV.Ar}$ + $\underline{C}H_{Ar}$), 130.1, 130.0, 129.8, 128.8, 128.7, 128.5, 127.7, 127.5, 127.3, 126.5, 126.3, 125.6, 125.5, 125.2, 124.0, 123.8, 123.7, 123.6, 123.2, 123.2, 123.1, 120.8, 120.4, 120.3, 120.3, 120.3, 120.2, 119.9 (9$\underline{C}H_{Ar}$ + C_{f-h}), 118.9, 118.4, 117.8, 117.4, 117.2, 116.1, 116.1, 114.6, 111.8 (C_d + C_8 + C_{12} + C_{15}), 108.8, 108.8 (C_j), 84.8, 84.7, 84.5, 81.1, 80.8, 80.6 (2$C_{IV.Boc}$), 63.7, 62.8, 62.3 (C_4), 53.2, 52.8, 52.7, 52.5, 52.0 (C_2 + C_6), 49.9, 49.9, 49.3, 49.2 (C_a), 29.6, 29.0, 28.9, 28.8, 28.7, 28.7, 28.5, 28.4, 27.8 (6$\underline{C}H_{3.Boc}$ + C_1 + C_7), 22.6 (C_m); MS (ESI): *m/z* 914.07 $[M+H]^-$ (theoretical *m/z* 914,39).

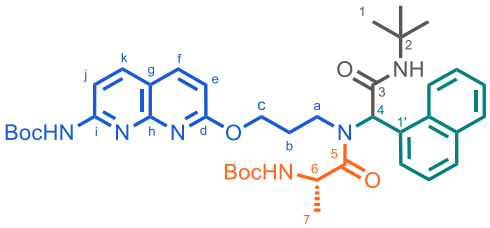
Deprotected Ugi product 3b



General procedure B was employed for the deprotection of compound **3'b** (38.7 mg, 0.042 mmol) in 1 mL of CH_2Cl_2 and in the presence of 50 equiv. of TFA. After filtration, the crude mixture was purified by semipreparative HPLC (method B) leading to the mixture of diastereomers **3b** as a white powder (7.1 mg, 18%)HPLC: rt_{dia1} = 16.39 min and rt_{dia2} = 16.57 min (method B); NMR data as a mixture of 2 diastereomers; ^{1}H NMR (400 MHz, CD_3OD) δ = 8.30 7.10 (m, 16H, 16C$\underline{H}_{Ar}$), 6.96 – 6.82, 6.78, 6.49 (m and 2s, 2H, 1CH_{AR}, H_4), 4.54 – 4.45, 4.41 – 4.36 (2m, 1H, H_6), 4.34 – 4.26, 4.24 – 4.09, 4.06 – 3.92, 3.79 – 3.72 (4m, 2H, H_a), 3.84 – 3.65, 3,36 – 3.23 (2m, 2H, H_7), 2.22,(1s, 3H, H_m), 1.45, 1.40 (2s, 9H, H_1); ^{13}C NMR (101 MHz, CD_3OD) δ = 172.4, 172.4, 172.0, 171.9, 170.9, 168.4 (C_b + C_l + C_3 + C_5), 159.5, 159.5 (C_k), 151.7, 150.8 (C_i), 139.5, 138.3, 136.6, 135.4, 134.2, 133.8, 133.5, 131.5, 131.4, 131.0, 130.0, 130.0, 129.8, 129.7, 129.3, 128.9, 128.5, 128.3, 128.2, 128.2, 128.1, 127.6, 127.5, 127.4, 126.3, 126.2, 126.2, 125.9, 125.8, 124.1, 123.4, 123.3, 123.1, 122.9, 120.7, 120.6, 120.4, 119.7, 119.3, 119.0, 118.7, 118.6 (7$C_{IV.Ar}$ + 15$\underline{C}H_{Ar}$), 112.8, 112.8, 108.9, 108.8, 107.8, 107.3 (C_j + C_8 + C_{15}), 61.8, 61.4 (C_4), 53.2, 53.1, 52.8, 52.6 (C_2 + C_6), 49.0 (C_a) 28.9, 28.8 (C_1 + C_7), 22.6 (C_m); MS (ESI): *m/z* 716.07 $[M+H]^+$ (theoretical *m/z* 716.30).

The compound resulted not sufficiently stable for full characterization and evaluation.

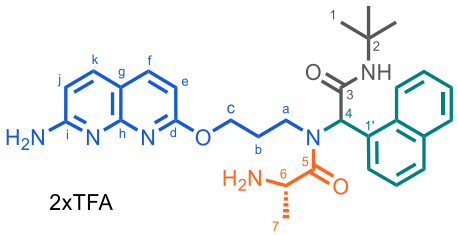
Ugi product 1'c



Compound **1'c** was prepared following general procedure A using Boc-naphthyridine(*O*-alkyl)-NH_2 (**16**) (31.8 mg, 0.10 mmol) as the amine, 1-naphthaldehyde (13.6 µL, 0.10 mmol) as aldehyde, Boc-L-Ala-OH (18.9 mg, 0.10 mmol) as the acid and *tert*-butyl isocyanide (11.3 µl, 0.10 mmol) in MeOH (0.2 mL). After purification by flash chromatography (CH_2Cl_2/MeOH gradient 100:0 to 98:2, v/v), a mixture of diastereomers **1'c** was obtained as a yellowish solid (54.7 mg, 75%).$R_{f.dia1+2}$ = 0.22 (CH_2Cl_2-MeOH

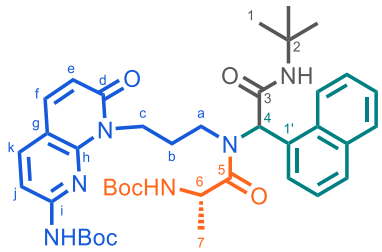
98:2); NMR data as a mixture of 2 diastereomers and conformers; ^{1}H NMR (400 MHz, CD_3OD) δ = 8.40 – 8.30, 7.91 – 7.84, 7.82 – 7.40, 7.39 – 7.25 (4m, 7H, 7C$\underline{H}_{Ar}$), 8.15 – 8.08, 8.07 – 8.03, 8.02 – 7.92 (3m, 3H, H_f + $H_{j\text{-}k}$), 6.88, 6.77, 6.62 (3s, 1H, H_4), 6.72, 6.69, 6.55 (3d, *J* = 8.7 Hz, 1H, H_e), 4.97 – 4.87, 4.67 – 4.45 (2m, 1H, H_6), 4.10 – 3.81 (m, 2H, H_c), 3.74 – 3.58, 3.57 – 3.27 (2m, 2H, H_a), 1.90 – 1.72, 1.66 – 1.26, 0.95 – 0.79, 0.65 – 0.44 (4m, 2H, H_b), 1.68 – 1.20 (m, 30H, 6C$\underline{H}_{3.Boc}$ + H_1 + H_7); ^{13}C NMR (101 MHz, CD_3OD) δ = 176.8, 176.7, 176.4 (C_5), 171.9, 171.4 (C_3), 166.0, 165.9 (C_d), 157.8, 157.4, 157.3, 155.6, 155.5 (2CO_{Boc}), 155.1, 155.1, 154.2, 154.2 (C_{h+i}), 140.3, 140.3 (C_f), 140.0, 140.0, 139.9 (C_k), 135.0, 134.9, 134.0, 133.8, 133.1, 133.0, 132.3 (3$C_{IV.Ar}$), 130.0, 130.4, 130.1, 129.7, 128.7, 128.5, 128.4, 128.1, 128.0, 127.9, 127.2, 127.2, 126.2, 126.0, 125.3, 124.9, 123.8 (7$\underline{C}H_{Ar}$), 117.2, 117.1 (C_g), 113.1, 113.1, 113.0, 112.9 (C_e), 112.0, 112.0, 111.8 (C_j), 82.1, 82.1, 82.0, 80.7, 80.4, 80.2 (2$C_{IV.Boc}$), 65.3, 64.7, 64.5 (C_c), 62.5, 60.2, 59.2 (C_4), 52.6, 52.5, 52.3 (C_2), 48.2, 47.7 (C_6), 44.5, 44.5, 44.2 (C_a), 30.7, 30.5, 30.4, 30.2 (C_b), 28.9, 28.8, 28.8, 28.6 (6$\underline{C}H_{3.Boc}$ + C_1), 19.0, 18.4, 18,1 (C_7); MS (ESI): *m/z* 729.00 $[M+H]^+$ (theoretical *m/z* 729.88).

Deprotected Ugi product 1c



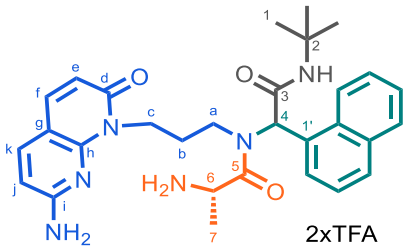
General procedure B was employed for the deprotection of compound **1'c** (38.0 mg, 0.052 mmol) in 1 mL of CH_2Cl_2 and in the presence of 50 equiv. of TFA. After filtration, a mixture of diastereomers **1c** was isolated as a yellow-pale powder (34.8 mg, 88%).HPLC: rt_{dia1} = 10.3 min and rt_{dia2} = 10.6 min (method A); NMR data as a mixture of 2 diastereomers and conformers; ^{1}H NMR (400 MHz, D_2O) δ = 8.23, 8.18 (2d, *J* = 9.2 Hz, 1H, H_k), 7.97, 7.93, 7.92, 7.87 (4d, *J* = 8.7 Hz, 1H, H_f), 7.76 – 7.68, 7.63 – 7.52, 7.51 – 7.25, 7.23 – 7.15 (4m, 6H, 6C$\underline{H}_{Ar}$), 7.10 – 6.91 (m, 2H, C$\underline{H}_{Ar}$ + H_j), 6.57, 6.34, 6.21, 6.11 (4s, 1H, H_4), 6.49, 6.46, 6.40, 6.31 (4d, *J* = 8.7 Hz,1H, H_e), 4.95, 4.59, 4.49 (3q, *J* = 6.8 Hz, 1H, H_6), 4.29 – 4.07, 3.97 – 3.84, 3.83 – 3.63 (3m, 2H, H_c), 3.56 – 3.34, 3.33 – 3.14 (2m, 2H, H_a), 1.85 – 1.72, 1.52 – 1.35, 1.02 – 0.86 (3m, 2H, H_b), 1.79, 1.67, 1.56 (3d, *J* = 6.8 Hz, 3H, H_7), 1.30, 1.30, 1.26 (3m, 9H, H_1); ^{13}C NMR (101 MHz, D_2O) δ = 172.0, 171.4 (C_3), 170.9, 170.4 (C_5), 164.4, 164.1 (C_d), 154.9 (C_i), 145.2, 145.1 (C_h), 143.3, 143.1 (C_k), 139.8, 139.8 (C_f), 132.6, 132.6, 131.7, 131.4 (2$C_{IV.Ar}$), 129.6, 129.3, 128.8, 128.7, 128.5, 128.3, 128.1, 127.4, 127.4 ($C_{IV.Ar}$ + 4$\underline{C}H_{Ar}$), 126.1, 126.0, 125.0, 124.9, 122.5, 121.9 (3$\underline{C}H_{Ar}$), 111.3, 111.3 (C_g), 111.1, 111.0 (C_e), 110.4 (C_j), 64.1, 63.9 (C_c), 60.7, 58.9 (C_4), 52.0, 51.8, 51.7 (C_2), 47.5, 47.2 (C_6), 43.4, 42.8 (C_a), 29.2, 28.4 (C_b), 27.6, 27.6, 27.4 (C_1), 16.9, 16.4, 15.8 (C_7); HRMS (ESI): *m/z* 529.29272 $[M+H]^+$ ($C_{30}H_{37}N_6O_3$ requires 529.29217).

Ugi product 2'c



The two diastereoisomers of **2'c** were prepared following general procedure A using compound (**16**) (31.8 mg, 0.10 mmol) as the amine, 1-naphthaldehyde (13.6 µL, 0.10 mmol) as aldehyde, Boc-L-Ala-OH (18.9 mg, 0.10 mmol) as the acid and *tert*-butyl isocyanide (11.3 µl, 0.10 mmol) in MeOH (0.2 mL). After purification by flash chromatography (CH_2Cl_2/MeOH gradient 100:0 to 96:4, v/v), the diastereomers **2'c (dia1)** (22.7 mg, 31%) and **2'c (dia2)** (26.3 mg, 36%) were separated as white solids. $R_{f.dia1}$ = 0.31 and $R_{f.dia2}$ = 0.29 (CH_2Cl_2-MeOH 96:4);**2'c – Dia1:** NMR data as a mixture of 2 conformers; ^{1}H NMR (400 MHz, CD_3OD) δ = 8.27, 7.64 – 7.56 (d and m, J = 8.4 Hz, 1H, C$\underline{H}_{Ar}$), 7.97, 7.92 (2d, J = 8.6 Hz, 1H, H_k), 7.87, 7.83 (2d, J = 8.6 Hz, 1H, H_j), 7.76, 7.73 – 7.65 (d and m , J = 9.4 Hz, 1H, H_f), 7.73 – 7.65, 7.64 – 7.56 (2m, 1H, C$\underline{H}_{Ar}$), 7.49 – 7.24 (m, 3H, 3C$\underline{H}_{Ar}$), 7.20, 7.09 (2d, J = 7.1 Hz, 1H, C$\underline{H}_{Ar}$), 6.95, 6.90 (2t, J = 7.7 Hz, 1H, C$\underline{H}_{Ar}$), 6.64, 6.50 (2s, 1H, H_4), 6.43, 6.37 (2d, J = 9.4 Hz, 1H, H_e), 4.90 – 4.78, 4.46 (m and q, J = 6.6 Hz, 1H, H_6), 4.16 – 3.80 (m, 2H, H_c), 3.66 – 2.98 (m, 2H, H_a), 1.83 – 1.18, 0.77 – 0.54 (2m, 2H, H_b), 1.62, 1.60, 1.51, 1.47, 1.39, 1.32 (6s, 27H, 6C$\underline{H}_{3.Boc}$ + H_1), 1.33 – 1.26 (m, 3H, H_7); ^{13}C NMR (101 MHz, CD_3OD) δ = 176.8, 176.7 (C_5), 171.4, 171.3 (C_3), 165.1 (C_d), 157.8, 157.7, 154.3, 154.2, 154.2, 154.0 (2CO_{Boc} + C_i), 149.2, 149.0 (C_h), 140.1, 139.8 (C_k), 139.5, 139.3 (C_f), 134.6, 134.5, 133.7, 133.6, 132.6, 131.8 (3$C_{IV.Ar}$), 130.6, 130.1, 130.0, 129.6, 128.1, 127.9, 127.9, 127.0, 126.9, 125.6, 125.3, 125.2, 123.6 (7$\underline{C}H_{Ar}$), 119.5, 119.4 (C_e), 112.8 (C_g), 108.8, 108.7 (C_j), 82.0, 81.9, 80.6, 80.4 (2$C_{IV.Boc}$), 62.3, 60.1 (C_4), 52.8, 52.4 (C_2), 48.2, 47.7 (C_6), 45.3, 45.0 (C_a), 39.4, 39.0 (C_c), 30.2 (C_b), 28.9, 28.8, 28.7, 28.7 (6$\underline{C}H_{3.Boc}$ + C_1), 18.6, 18.3 (C_7); MS (ESI): *m/z* 728,87 $[M+H]^+$ (theoretical m/z 729.40).**2'c – Dia2:** NMR data as a single conformer; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.95 (d, J = 8.6 Hz, 1H, H_k), 7.86 (d, J = 8.6 Hz, 1H, H_j), 7.78 (d, J = 8.7 Hz, 1H, C$\underline{H}_{Ar}$), 7.73 (d, J = 9.3 Hz, 1H, H_f), 7.65 – 7.57, 7.48 – 7.38 (m, 3H, 3C$\underline{H}_{Ar}$), 7.34 (d, J = 8.1 Hz, 1H, C$\underline{H}_{Ar}$), 7.14 (d, J = 7.0 Hz, 1H, C$\underline{H}_{Ar}$), 6.85 (t, J = 7.7 Hz, 1H, C$\underline{H}_{Ar}$), 6.69 (s, 1H, H_4), 6.44 (d, J = 9.3 Hz, 1H, H_e), 4.57 – 4.47 (m, 1H, H_6), 4.15 – 4.04, 3.93 – 3.81 (2m, 2H, H_c), 3.48 – 3.33 (m, 2H, H_a), 1.99 – 1.84, 0.69 – 0.47 (2m, 1H, H_b), 1.61 , 1.47, 1.27 (3s, 27H, 6C$\underline{H}_{3.Boc}$ + H_1), 1.38 (d, J = 6.9 Hz, 3H, H_7); ^{13}C NMR (101 MHz, CD_3OD) δ = 176.3 (C_5), 171.8 (C_3), 165.2 (C_d), 157.5, 154.2, 154.1 (2CO_{Boc} + C_i), 149.0 (C_h), 139.9 (C_k), 139.4 (C_f), 134.4, 133.9, 132.5 (3$C_{IV.Ar}$), 130.1, 129.6, 127.9, 127.8, 126.9, 125.3, 124.7 (7$\underline{C}H_{Ar}$), 119.4 (C_e), 112.9 (C_g), 108.8 (C_j), 82.0, 80.3 (2$C_{IV.Boc}$), 59.2 (C_4), 52.3 (C_2), 48.4 (C_6), 44.9 (C_a), 39.0 (C_c), 30.1 (C_b), 28.8, 28.8, 28.7 (6$\underline{C}H_{3.Boc}$ + C_1), 18.2 (C_7); **MS (ESI):** *m/z* 728,93 $[M+H]^+$ (theoretical m/z 729.40).

Deprotected Ugi product 2c

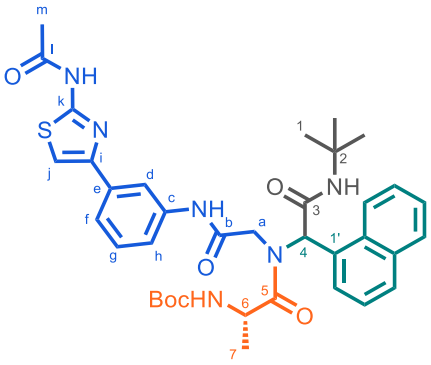


General procedure B was employed for the deprotection of compound **2'c** (16.5 mg, 0.023 mmol) in 1 mL of CH_2Cl_2 and in the presence of 50 equiv. of TFA. After filtration, the two diastereomers of **2c** were isolated as a white powder (12.6 mg, 74% for dia1) and (16.3 mg, 77% for Dia2)..

HPLC: rt_{dia1} = 12.1 min (method A); NMR data of **2c, d1** as a mixture of 2 conformers; ^{1}H NMR (400 MHz, D_2O) δ = 7.80 – 7.62 (m, 3H, C<u>H</u>$_{Ar}$ + H$_f$ + H$_k$), 7.59 – 7.44 (m, 3H, 3C<u>H</u>$_{Ar}$), 7.33 (d, *J* = 7.2 Hz, 1H, C<u>H</u>$_{Ar}$), 7.07 – 7.00 (m, 1H, C<u>H</u>$_{Ar}$), 6.91 – 6.81 (m, 1H, C<u>H</u>$_{Ar}$), 6.60, 6.53 (2d, *J* = 8.6 Hz, 1H, H$_j$), 6.30, 6.28 (2s, 1H, H$_4$), 6.24 – 6.15 (m, 1H, H$_e$), 4.91, 4.60 (2q, *J* = 6.8 Hz, 1H, H$_6$), 4.09 – 3.89, 3.80 – 3.65 (2m, 2H, H$_c$), 3.25 – 3.13, 3.06 – 2.93, 2.89 – 2.78 (3m, 2H, H$_a$), 1.82 – 1.66, 1.10 – 0.95 (2m, 1H, H$_b$), 1.63, 1.53 (2d, *J* = 6.8 Hz, 3H, H$_7$), 1.28 (s, 9H, H$_1$); ^{13}C NMR (101 MHz, D_2O) δ = 172.0 (C$_5$), 171.0 (C$_3$), 164.6 (C$_d$), 159.0 (C$_i$), 147.1 (C$_h$), 140.3 (C$_f$), 139.4 (C$_k$), 132.5, 131.2 (2C$_{IV.Ar}$), 130.1, 129.2 (2C<u>H</u>$_{Ar}$), 128.2 (C$_{IV.Ar}$), 127.4, 126.8, 126.2, 124.3, 121.5 (5C<u>H</u>$_{Ar}$), 113.7 (C$_e$), 108.5 (C$_g$), 107.0 (C$_j$), 60.4 (C$_4$), 51.8 (C$_2$), 47.6 (C$_6$), 43.8 (C$_a$), 37.8 (C$_c$), 28.9 (C$_b$), 27.6 (C$_1$), 17.0 (C$_7$); HRMS (ESI): *m/z* 529.29266 $[M+H]^+$ ($C_{30}H_{37}N_6O_3$ requires 529.29217).

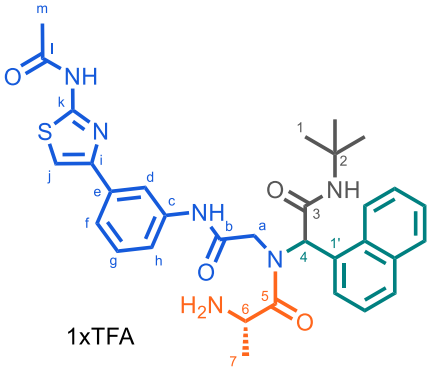
HPLC: rt_{dia2} = 12.6 min (method A); NMR data for **2c,d2** as a single conformer**; ^{1}H NMR** (400 MHz, D_2O) δ = 7.71 (d, *J* = 8.6 Hz, 1H, H$_k$), 7.67 – 7.57 (m, 3H, 2C<u>H</u>$_{Ar}$ + H$_f$), 7.56 – 7.54 (m, 2H, 2C<u>H</u>$_{Ar}$), 7.26 (d, *J* = 7.7 Hz, 1H, C<u>H</u>$_{Ar}$), 7.05 (d, *J* = 7.7 Hz, 1H, C<u>H</u>$_{Ar}$), 6.82 (t, *J* = 7.7 Hz, 1H, C<u>H</u>$_{Ar}$), 6.56 (d, *J* = 8.6 Hz, 1H, H$_j$), 6.49 (s, 1H, H$_4$), 6.21 (d, *J* = 9.2 Hz, 1H, H$_e$), 4.47 (q, *J* = 6.7 Hz, 1H, H$_6$), 3.99 – 3.88, 3.58 – 3.48 (2m, 2H, H$_c$), 3.42 – 3.29, 3.17 – 3.06 (2m, 2H, H$_a$), 1.69 – 1.52, 0.52 – 0.37 (2m, 2H, H$_b$), 1.64 (d, *J* = 6.7 Hz, 3H, H$_7$), 1.24 (s, 9H, H$_1$); ^{13}C NMR (101 MHz, D_2O) δ = 171.2 (C$_5$), 170.3 (C$_3$), 164.4 (C$_d$), 158.8 (C$_i$), 146.7 (C$_h$), 140.2 (C$_f$), 139.5 (C$_k$), 132.4, 131.4 (2C$_{IV.Ar}$), 129.9, 129.0 (2C<u>H</u>$_{Ar}$), 128.3 (C$_{IV.Ar}$), 127.4, 127.2, 126.2, 124.4, 122.1 (5C<u>H</u>$_{Ar}$), 113.8 (C$_e$), 108.4 (C$_g$), 107.0 (C$_j$), 58.9 (C$_4$), 51.6 (C$_2$), 47.6 (C$_6$), 43.5 (C$_a$), 37.6 (C$_c$), 29.1 (C$_b$), 27.6 (C$_1$), 16.4 (C$_7$); HRMS (ESI): *m/z* 529.29248 $[M+H]^+$ ($C_{30}H_{37}N_6O_3$ requires 529.29217).

Ugi product 3'c



Compound **3'c** was prepared following general procedure A procedure using the TFA salt of compound **19** (43.5 mg, 0.15 mmol) as the amine, 1-naphthaldehyde (20.4 µL, 0.15 mmol) as aldehyde, Boc-L-Ala-OH (28.4 mg, 0.15 mmol) as the acid and *tert*-butyl isocyanide (17.0 µl, 0.15 mmol) in MeOH (0.3 mL). After purification by flash chromatography (CH_2Cl_2/MeOH gradient 100:0 to 98:2, v/v), a mixture of diastereomers **3'c** was obtained as a white solid (48.5 mg, 46%).$R_{f.dia1+2}$ = 0.29 (CH_2Cl_2-MeOH 98:2); NMR data as a mixture of 2 diastereomers and conformers; ^{1}H NMR (400 MHz, CD_3OD) δ = 8.48, 8.13 – 7.69 (d and m, J = 8.6 Hz, 4H, 3C$\underline{H}_{Ar}$ + H_d), 7.64 – 7.34 (m, 5H, 4C$\underline{H}_{Ar}$ + H_f), 7.64 – 7.34, 7.35 – 7.12 (2m, 1H, H_h), 7.35 – 7.12 (m, 2H, H_g + H_j), 6.97, 6.75, 6.58 (3s, 1H, H_4), 4.85 – 4.76, 4.68 – 4.57, 4.41 – 4.30 (3m, 1H, H_6), 4.50, 4.10, 3.86, 3.20 (4d, J_{dia1} = 18.6 Hz and J_{dia2} = 16.6 Hz, 2H, H_a), 2.19, 2.18 (s, 3H, H_m), 1.54 – 1.29 (m, 21H, 3C$\underline{H}_{3.Boc}$ + H_1 + H_7); ^{13}C NMR (101 MHz, CD_3OD) δ = 178.4, 177.7 (C_5), 173.2, 172.4 (C_3), 170.8, 170.3, 169.1 (C_l + C_b), 159.3, 158.0 (CO_{Boc} + C_k), 150.9, 150.8 (C_i), 139.8, 139.6, 136.5, 136.3 (C_c + C_e), 135.5, 135.3, 133.8, 133.6, 131.9, 131.8, 131.5, 131.2 (3$C_{IV.Ar}$ + $\underline{C}H_{Ar}$), 130.0, 129.9, 129.7, 128.9, 128.6, 128.5, 127.7, 127.3, 126.4, 126.3, 125.1, 124.4, 124.0 (6$\underline{C}H_{Ar}$ + C_g), 123.2, 123.0 (C_f), 120.9, 120.6, 120.3 (C_h), 119.0, 118.7, 118.5 (C_d), 108.8 (C_j), 80.8, 80.7, 80.5 ($C_{IV.Boc}$), 63.7, 62.0 (C_4), 53.3, 53.2, 52.7, 52.6 (C_2), 50.4, 49.9, 49.7, 49.3, 49.3, 49.1 (C_a + C_6), 28.9, 28.8, 28.8, 28.7 (3$\underline{C}H_{3.Boc}$ + C_1), 22.6 (C_m), 18.6, 17.8, 17.2 (C_7); MS (ESI): *m/z* 701.00 $[M+H]^+$ (theoretical *m/z* 701.31).

Deprotected Ugi product 3c

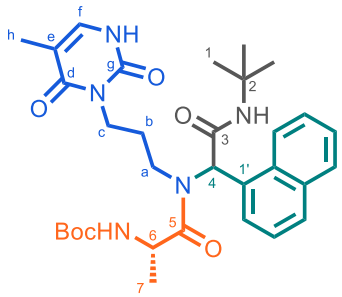


General procedure B was employed for the deprotection of compound **3'c** (30.0 mg, 0.043 mmol) in 1 mL of CH_2Cl_2 and in the presence of 50 equiv. of TFA. After filtration, a mixture of diastereomers **3c** was isolated as a yellow-pale powder (28.2 mg, 92%).HPLC: rt_{dia1} = 14.15 min and rt_{dia2} = 14.65 min

(method B); NMR data as a mixture of 2 diastereomers; ^{1}H NMR (400 MHz, CD_3OD) δ = 8.00 – 7.71, 7.69 – 7.35 (2m, 10H, 7C<u>H</u>$_{Ar}$ + H$_d$ + H$_{f\text{-}g}$), 7.32 – 7.09, 6.92 – 6.81 (2m, 1H, H$_h$), 7.22, 7.14 (2s, 1H, H$_j$), 6.87, 6.78 (2s, 1H, H$_4$), 4.50, 4.36 (2q, J = 6.8 Hz, 1H, H$_6$), 4.31, 4.03 – 3.96 (d and m, J = 17,9 Hz, 2H, H$_a$) 2.22, 2.22 (2s, 3H, H$_m$), 1.58, 1.57 (d, J = 6.8 Hz, 3H, H$_7$), 1.42, 1.41 (2s, 9H, H$_1$); ^{13}C NMR (101 MHz, CD_3OD) δ = 173.1, 172.6 (C$_5$), 171.9, 171.8 (C$_3$), 170.9 (C$_l$), 168.3 (C$_b$), 159.4 (C$_k$), 150.8, 150.7 (C$_i$), 139.5, 138.8, 136.5, 136.3 (C$_c$ + C$_e$), 135.5, 135.3, 133.9, 133.5 (2C$_{IV.Ar}$), 131.8, 131.5, 131.4 (C$_{IV.Ar}$ + <u>C</u>H$_{Ar}$), 130.1, 129.9, 129.8, 129.7, 129.3, 129.1, 128.3, 128.1, 127.5, 127.4, 126.4, 126.2, 124.8, 124.1 (6<u>C</u>H$_{Ar}$ + C$_g$), 123.3, 123.2 (C$_f$), 120.7, 120.6 (C$_h$), 118.7, 118.6 (C$_d$), 108.8, 108.8 (C$_j$), 61.1 (C$_4$), 52.7, 52.6 (C$_2$), 49.5, 49.9 (C$_a$), 49.1, 48.9 (C$_6$), 28.8, 28.7 (C$_1$), 22.6 (C$_m$), 17.4, 16.9 (C$_7$); MS (ESI): *m/z* 600.87 [M+H]$^+$ (theoretical *m/z* 601.26).

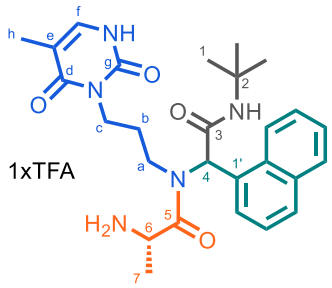
The compound resulted not sufficiently stable for full characterization and evaluation.

Ugi product 7'c



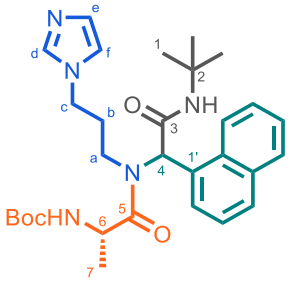
Compound **7'c** was prepared following general A procedure using the TFA salt of N^3-thymine-NH$_2$ (**23**) (54.0 mg, 0.18 mmol) as the amine, 1-naphthaldehyde (24.7 µL, 0.18 mmol) as aldehyde, Boc-Ala-OH (34.1 mg, 0.18 mmol) as the acid and *tert*-butyl isocyanide (20.4 µl, 0.18 mmol) in MeOH (0.6 mL). After purification by flash chromatography (CHX/EtOAc gradient 65:35 to 15:85, v/v), a mixture of diastereomers **7'c** was obtained as a white solid (49.0 mg, 46%).R$_{f.dia1+2}$ = 0.47 (CHX-EtOAc 10:90); NMR data as a mixture of 2 diastereomers and conformers; ^{1}H NMR (400 MHz, CD_3OD) δ = 8.34 – 8.28, 7.99 – 7.77, 7.76 – 7.63, 7.60 – 7.29 (4m, 7H, 7C<u>H</u>$_{Ar}$), 7.14, 7.12, 7.09, 7.08 (3d and br d, J_{dia1} = 1.0 Hz and J_{dia2} = 0.7 Hz, 1H, H$_f$), 6.81, 6.73, 6.66, 6.59 (4s, 1H, H$_4$), 4.90 – 4.74, 4.54 – 4.32 (2m, 1H, H$_6$), 3.63 – 2.96 (m, 4H, H$_a$ + H$_c$), 1.89 -1.57, 0.79 – 0.40 (2m, 2H, H$_b$), 1.79, 1.79, 1.78, 1.74 (4s, 3H, H$_h$), 1.56 – 1.22 (m, 21H, 3C<u>H</u>$_{3.Boc}$ + H$_1$ + H$_7$); ^{13}C NMR (101 MHz, CD_3OD) δ = 176.9, 176.7, 176.4 (C$_5$), 171.8, 171.4 (C$_3$), 166.1, 165.9 (C$_d$), 157.6, 157.4 (CO$_{Boc}$), 153.1, 153.0 (C$_g$), 137.4, 137.2 (C$_f$), 135.1, 134.0, 133.9, 132.9, 132.8 (3C$_{IV.Ar}$), 131.1, 130.7, 130.6, 130.2, 129.8, 128.6, 128.4, 128.3, 128.1, 128.0, 127.3, 127.3, 127.2, 126.1, 125.9, 125.3, 124.6, 123.7 (7<u>C</u>H$_{Ar}$), 109.8, 109.7 (C$_e$), 80.7, 80.4, 80.3 (C$_{IV.Boc}$), 62.7, 60.4, 59.7 (C$_4$), 52.8, 52.3, 52.2 (C$_2$), C$_6$ (signal under the CD_3OD peak), 44.9 (C$_a$), 38.9, 38.5 (C$_c$), 29.8 (C$_b$), 28.9, 28.8, 28.8, 28.8 (3<u>C</u>H$_{3.Boc}$ + C$_1$), 18.6, 18.3 , 18.2 (C$_7$), 12.9 (C$_h$); MS (ESI): *m/z* 594.3 M+H]$^+$ (theoretical *m/z* 594.33).

Deprotected Ugi product 7c



General procedure B was employed for the deprotection of compound **7'c (**33.0 mg, 0.056 mmol) in 1 mL of CH_2Cl_2 and in the presence of 50 equiv. of TFA. After filtration, a mixture of diastereomers **7c** was isolated as a white solid (30.0 mg, 89%).HPLC: rt_{dia1} = 14.9 min and rt_{dia2} = 15.2 min (method A); NMR data as a mixture of 2 diastereomers; ^{1}H NMR (400 MHz, D_2O) δ = 7.98 – 7.89, 7.86 – 7.78 (m, 2H, 2C$\underline{H}_{Ar}$), 7.76 – 7.67, 7.64 – 7.51 (2m, 3H, 3C$\underline{H}_{Ar}$), 7.39 – 7.28 (m, 2H, 2C$\underline{H}_{Ar}$), 7.18, 7.15 (2d, J_{dia1} = 1.0 Hz and J_{dia2} = 0.7 Hz, 1H, H_f), 6.49, 6.25 (2s, 1H, H_4), 4.57, 4.53 (2q, J = 6.8 Hz, 1H, H_6), 3.49 – 3.28, 3.24 – 3.03 (2m, 2H, H_c), 3.24 – 3.03, 2.67 – 2.51 (2m, 2H, H_a), 1.79 – 1.46 (m, 6H, H_h + H_7), 1.79 – 1.50, 1.37 – 1.09, 0.64 – 0.45 (3m, 2H, H_b), 1.32, 1.28 (2s, 9H, H_1); ^{13}C NMR (101 MHz, D_2O) δ = 171.8, 171.4, 170.9, 170.2 (C_3 + C_5), 165.7, 165.6 (C_d), 151.9, 151.9 (C_g), 137.0 (C_f), 133.0, 132.9, 131.7, 131.5 (2$C_{IV.Ar}$), 130.7, 130.6, 129.2, 129.1 (2$\underline{C}H_{Ar}$), 128.7, 128.5 ($C_{IV.Ar}$), 128.1, 127.9, 127.7, 127.3, 126.7, 126.6, 124.9, 124.8, 122.4, 121.7 (5$\underline{C}H_{Ar}$), 109.4, 109.3 (C_e), 61.0, 59.7 (C_4), 51.9, 51.7 (C_2), 47.5, 47.5 (C_6), 43.5, 43.4 (C_a), 37.5, 37.3 (C_c), 28.8, 28.46 (C_b), 27.6, 27.6 (C_1), 16.7, 16.5 (C_7), 12.0, 12.0 (C_h); HRMS (ESI): *m/z* 494.27649 $[M+H]^+$ ($C_{27}H_{36}N_5O_4$ requires 494.27618).

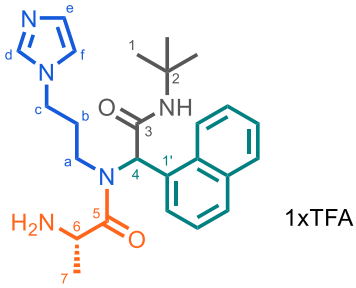
Ugi product 8'c



Compound **8'c** was prepared following general procedure A using 1-(3-aminopropyl)imidazole (21.0 µL, 0.18 mmol) as the amine, 1-naphthaldehyde (24.7 µL, 0.18 mmol) as aldehyde, Boc-L-Ala-OH (34.1 mg, 0.18 mmol) as the acid and *tert*-butyl isocyanide (20.4 µl, 0.18 mmol) in MeOH (0.7 mL). After purification by flash chromatography (CH_2Cl_2/MeOH gradient 100:0 to 97:3, v/v), a mixture of diastereomers **8'c** was obtained as a white solid (78.0 mg, 81%).$R_{f.dia1+2}$ = 0.36 (CH_2Cl_2-MeOH 97:3); NMR data as a mixture of 2 diastereomers and conformers; ^{1}H NMR (400 MHz, CD_3OD) δ = 8.60 – 8.49, 8.09 – 7.81, 7.76 – 7.37 (3m, 7H, C$\underline{H}_{Ar}$), 7.25, 7.18, 7.07 (3s, 1H, H_d), 6.90, 6.75, 6.69 (3s, 1H, H_4), 6.84, 6.82, 6.74 (3br s, 1H, H_e), 6.67, 6.58, 6.45 (3br s, 1H, H_f), 4.92, 4.45, 4.29 (3q, J = 6.7 Hz, 1H, H_6), 3.74 – 3.13 (m, 4H, H_a + H_c), 1.90 – 1.57, 1.41 – 1.11, 1.06 – 0 .84, 0.55 – 0.37 (4m, 2H, H_b), 1.51, 1.47, 1.39, 1.33 (4s, 18H, 3C$\underline{H}_{3.Boc}$ + H_1), 1.41 – 1.13 (m, 3H, H_7); ^{13}C NMR (101 MHz, CD_3OD)

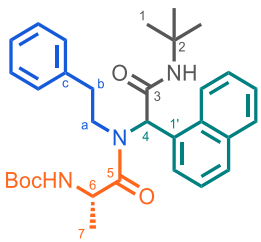
δ = 177.3, 176.5, 176.3 (C_5), 171.8, 171.8, 171.2 (C_3), 157.9, 157.5, 157.4 (CO_{Boc}), 138.0, 137.7 (C_d), 135.1, 135.0, 134.0, 133.9, 133.7, 133.3, 133.0, 132.4 ($3C_{IV.Ar}$), 131.0, 130.6, 130.5, 130.1, 129.9, 129.8, 128.9, 128.8, 128.6, 128.5, 128.5, 128.1, 128.2, 128.1, 128.0, 127.5, 127.4, 126.6, 126.4, 125.5, 125.0, 123.9 ($7\underline{C}H_{Ar}$ +H_e), 120.4, 120.0 (C_f), 80.6, 80.5, 80.3 ($C_{IV.Boc}$), 62.2, 60.1, 58.9 (C_4), 52.5, 52.4, 52.3 (C_2), 48.2, 47.9 (C_6), 45.6, 45.1, 44.0, 43.8, 43.7 (C_a + C_c), 32.4, 31.7, 31.3 (C_b), 28.9, 28.8, 28.8 ($3\underline{C}H_{3.Boc}$ + C_1), 19.0, 18.1, 17.8 (C_7); MS (ESI): *m/z* 536.6 $[M+H]^+$ (theoretical *m/z* 536.32).

Deprotected Ugi product 8c



General procedure B was employed for the deprotection of compound **8'c** (60.0 mg, 0.112 mmol) in 1 mL of CH_2Cl_2 and in the presence of 50 equiv. of TFA. After filtration, a mixture of diastereomers **8c** was isolated as a white powder (50.9 mg, 83%).HPLC: rt_{dia1} = 8.0 min and rt_{dia2} = 8.4 min (method A); NMR data as a mixture of 2 diastereomers and conformers; ^{1}H NMR (400 MHz, D_2O) δ = 8.05 – 7.85 (m, 3H, $2C\underline{H}_{Ar}$ + H_d), 7.84 – 7.73, 7.70 – 7.56 (m, 3H, $3C\underline{H}_{Ar}$), 7.50 – 7.37, 7.36 – 7.26 (2m, 2H, $2C\underline{H}_{Ar}$), 7.20, 7.17, 7.14 (3d, *J* = 1.5 Hz, 1H, H_f), 6.78, 6.76, 6.67, 6.64, (4br s, 1H, H_e), 6.64, 6.40, 6.32, 6.18 (4s, 1H, H_4), 4.95, 4.48, 4.36, 4.25, (4q, *J* = 6.8 Hz, 1H, H_6), 3.91 – 3.53 (m, 2H, H_c), 3.34 – 3.09, 3.06 – 2.90, 2.82 – 2.66 (2m, 2H, H_a), 1.99 – 1.84, 1.78 – 1.47, 1.42 – 1.18, 0.74 – 0.43 (4m, 2H, H_b), 1.59, 1.58, 1.52 (3d, *J* = 6.8 Hz, 3H, H_7), 1.31, 1.27 (2s, 9H, H_1); ^{13}C NMR (101 MHz, D_2O) δ = 172.0, 171.4, 171.1, 170.8, 170.3, 170.2 (C_3 + C_5), 134.2, 134.1 (C_d), 133.3, 133.2, 131.7, 131.5, 131.3 ($2C_{IV.Ar}$), 130.6, 130.4, 130.3, 129.4, 129.2, 129.1, 129.1, 128.8 ($C_{IV.Ar}$ + $2\underline{C}H_{Ar}$), 128.1, 128.0, 127.8, 127.4, 126.9, 125.5, 125.4, 122.5, 122.3, 121.9 ($5\underline{C}H_{Ar}$), 121.1, 121.0, 120.9, 120.7 (C_e + C_f), 60.8, 60.6, 59.0 (C_4), 52.1, 51.8, 51.7 (C_2), 47.6, 47.4 (C_6), 46.1, 45.7, 45.5(C_c), 42.2, 42.2 (C_a), 30.0, 29.9 (C_b), 27.6, 27.6, 27.4 (C_1), 16.7, 16.4, 15.9 (C_7); HRMS (ESI): *m/z* 436.27100 $[M+H]^+$ ($C_{25}H_{34}N_5O_2$ requires 436.27100).

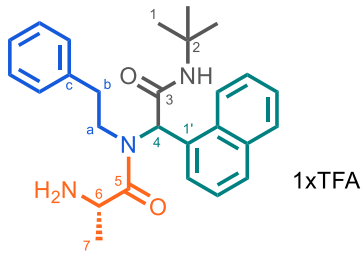
Ugi product 9'c



Compound **9'c** was prepared following general procedure A using 2-phenylethylamine (22.3 mg, 0.184 mmol) as the amine, 1-naphthaldehyde (25.0 µL, 0.184 mmol) as aldehyde, Boc-L-Ala-OH (34.8 mg, 0.184 mmol) as the acid and *tert*-butyl isocyanide (20.8 µl, 0.184 mmol) in MeOH (1.0 mL). After

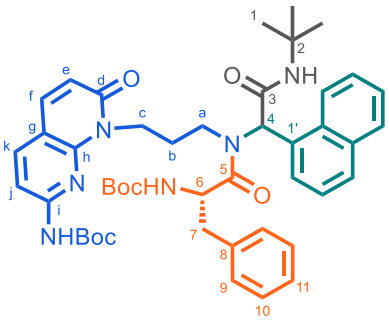
purification by flash chromatography (CHX/EtOAc gradient 85:15 to 65:35, v/v), a mixture of diastereomers **9'c** was obtained as a white solid (48.0 mg, 49%). $R_{f.dia1+2}$ = 0.56 (CHX-EtOAc 7:3); NMR data as a mixture of 2 diastereomers and conformers; ^{1}H NMR (400 MHz, CD_3OD) δ = 8.53 – 8.45, 8.12 – 7.79, 7.71 – 7.50 (3m, 7H, 7C<u>H</u>$_{Ar}$), 7.14 – 6.97, 6.62 – 4.40 (2m, 5H, 5C<u>H</u>$_{phenyl}$), 6.98, 6.95, 6.84, 6.72 (4s, 1H, H_4), 5.02 – 4.92, 4.73 – 4.50 (2m, 1H, H_6), 3.74 – 3.33, 3.22 – 3.11 (m, 2H, H_a), 2.70 – 2.27, 1.64 – 1.10 (2m, 2H, H_b), 1.64 – 1.10 (m, 21H, 3C<u>H</u>$_{3.Boc}$ + H_1 + H_7); ^{13}C NMR (101 MHz, CD_3OD) δ = 176.7, 176.5, 176.2 (C_5), 172.0, 171.6, 171.5 (C_3), 157.9, 157.6, 157.5 (CO_{Boc}), 140.5, 139.6, 139.1 (C_c), 135.3, 135.3, 135.2, 134.3, 134.1, 134.1, 133.5, 132.7 (3$C_{IV.Ar}$), 131.1, 130.8, 130.7, 130.2, 129.9, 129.4, 129.4, 129.4, 129.3, 129.2, 129.1, 128.9, 128.7, 128.3, 128.2, 128.2, 127.5, 127.5, 127.4, 127.3, 126.9, 126.4, 126.2, 125.5, 125.0, 124.1 (12<u>C</u>H$_{Ar}$), 80.7, 80.6, 80.4 ($C_{IV.Boc}$), 62.5, 60.0, 59.2 (C_4), 52.7, 52.6, 52.5, 52.3 (C_2), C_a + C_6 (signal under the CD_3OD peak), 47.8 (C_6), 37.5, 37.1, 35.6 (C_b), 28.9, 28.9, 28.8, 28.8, 28.8, 28.8 (3<u>C</u>H$_{3.Boc}$ + C_1), 19.1, 18.4, 18.2 (C_7); MS (ESI): *m/z* 532.2 $[M+H]^+$ (theoretical 532.32).

Deprotected Ugi product 9c



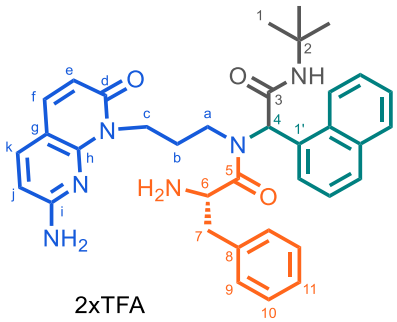
General procedure B was employed for the deprotection of compound **9'c** (22.7 mg, 0.043 mmol) in 1 mL of CH_2Cl_2 and in the presence of 50 equiv. of TFA. After filtration, a mixture of diastereomers **9c** was isolated as a white powder (12.2 mg, 53%).HPLC: rt_{dia1} = 15.3 min and rt_{dia2} = 15.7 min (method A); NMR data as a mixture of 2 diastereomers; ^{1}H NMR (400 MHz, D_2O) δ = 8.11 – 7.86, 7.76 – 7.54 (2m, 7H, 7C<u>H</u>$_{Ar}$), 7.17 – 7.06 (m, 3H, 3C<u>H</u>$_{Phenyl}$), 6.83, 6.58 (2s, 1H, H_4), 6.60 – 6.46 (m, 2H, 2C<u>H</u>$_{Phenyl}$), 4.51, 4.18 (2q, *J* = 6.8 Hz, 1H, H_6), 3.56 – 3.28, 3.21 – 3.07 (2m, 2H, H_a), 2.60 – 2.47, 2.14 – 2.04, 2.01 – 1.89, 1.51 – 1.41 (4m, 2H, H_b), 1.58, 1.55 (2d, *J* = 6.9 Hz, 3H, H_7), 1.34, 1.31 (2s, 9H, H_1); ^{13}C NMR (101 MHz, D_2O) δ = 171.9, 171.2 (C_5), 170.8, 170.3 (C_3), 137.6, 137.5 (C_c), 133.5, 133.5, 132.2, 131.9 (2$C_{IV.Ar}$), 130.4, 129.8, 129.6, 129.3, 129.2 ($C_{IV.Ar}$ + 2C<u>H</u>$_{Ar}$), 128.7, 128.6, 128.6, 128.2, 127.9, 127.9, 127.8, 126.8, 126.7, 126.7, 125.6, 122.7, 122.1 (10<u>C</u>H$_{Ar}$), 60.4, 58.8 (C_4), 51.9, 51.8 (C_2), 47.7, 47.5 (C_6), 47.2, 47.1 (C_a), 35.5, 35.3 (C_b), 27.7, 27.6 (C_1), 16.7, 16.2 (C_7); HRMS (ESI): *m/z* 432.26480 $[M+H]^+$ ($C_{27}H_{34}N_3O_2$ requires 432.26455).

Deprotected Ugi product 2'd



Compound **2'd** was prepared following general procedure A using compound **16** (31.8 mg, 0.10 mmol) as the amine, 1-naphthaldehyde (13.6 µL, 0.10 mmol) as aldehyde, Boc-L-Phe-OH (26.5 mg, 0.10 mmol) as the acid and *tert*-butyl isocyanide (11.3 µl, 0.10 mmol) in MeOH (0.2 mL). After purification by flash chromatography (CH_2Cl_2/MeOH gradient 100:0 to 97:3, v/v), a mixture of diastereomers **2'd** was obtained as a white solid (53.5 mg, 67%). $R_{f.dia1+2}$ = 0.38 (CH_2Cl_2-MeOH 96:4); NMR data as a mixture of 2 diastereomers and conformers; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.98 – 7.81 (m, 2H, H_j + H_k), 7.74 – 7.58 (m, 2H, C$\underline{H}_{Ar}$ + H_f), 8.13 – 8.08, 7.80 – 7.74, 7.56 – 7.11 (3m, 10H, 10C$\underline{H}_{Ar}$), 7.11 – 7.03, 7.00 – 6.84 (2m, 1H, C$\underline{H}_{Ar}$), 6.79, 6.72, 6.50, 6.48 (4s, 1H, H_4), 6.44, 6.38, 6.32 (3d, J = 9.4 Hz, 1H, H_e), 5.12 – 5.05, 4.94 – 4.62 (2m, 1H, H_6), 4.19 – 3.76 (m, 2H, H_c), 3.67 – 3.29 (m, 2H, H_a), 3.27 – 2.75 (m, 2H, H_7), 1.96 – 1.67, 0.67 – 0.34 (2m, 1H, H_b), 1.67 – 1.08 (m, 27H, 6C$\underline{H}_{3.Boc}$ + H_1); ^{13}C NMR (101 MHz, CD_3OD) δ = 174.8, 173.9 (C_5), 171.7, 171.5 (C_3), 165.1, 165.1, 164.7 (C_d), 157.5, 154.2, 154.1 (2CO_{Boc} + C_i), 149.0, 148.9 (C_h), 140.0, 139.9 (C_k), 139.4, 139.2 (C_f), 138.5, 138.4 (C_8), 134.5, 134.5, 133.8, 133.6, 132.5, 132.4 (3$C_{IV.Ar}$), 130.5, 130.5, 130.4, 130.1, 129.7, 129.7, 129.4, 129.4, 129.3, 128.2, 128.0, 127.8, 127.8, 127.6, 126.9, 126.8, 125.7, 125.4, 124.4, 123.8 (12$\underline{C}H_{Ar}$), 119.5, 119.4 (C_e), 112.9, 112.7 (C_g), 108.8, 108.6 (C_j), 82.0, 82.0, 80.8, 80.7, 80.4 (2$C_{IV.Boc}$), 62.1, 59.9, 59.3 (C_4), 54.6, 54.2, 53.2 (C_6), 52.5, 52.4, 52.2 (C_2), 45.4, 45.0, 44.7 (C_a), 40.1, 39.2, 39.0, 39.0 (C_c + C_7), 30.1 (C_b), 28.9, 28.8, 28.7, 28.7, 28.7, 28.1 (6$\underline{C}H_{3.Boc}$ + C_1); MS (ESI): *m/z* 804.93 $[M+H]^+$ (theoretical *m/z* 805.43).

Deprotected Ugi product 2d

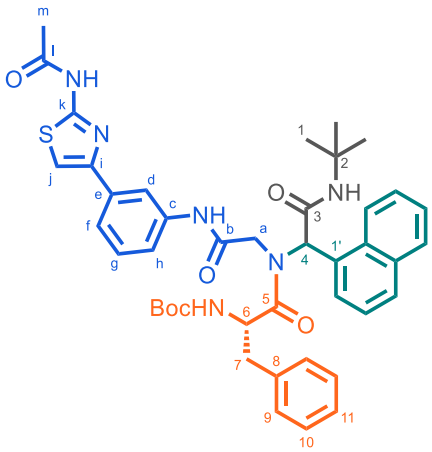


2xTFA

General procedure B was employed for the deprotection of compound **2'd** (39.7 mg, 0.049 mmol) in 1 mL of CH_2Cl_2 and in the presence of 50 equiv. of TFA. After filtration, a mixture of diastereomers **2d** was isolated as a white powder (38.1 mg, 93%).HPLC: rt_{dia1+2} = 19.81 min (method A); NMR data as a mixture of 2 diastereomers; ^{1}H NMR (400 MHz, CD_3OD) δ = 7.91 – 7.73, 7.54 – 7.43 (2m, 4H, 4C$\underline{H}_{Ar}$), 7.69 – 7.54 (m, 3H, C$\underline{H}_{Ar}$ + H_f + H_k), 7.43 – 7.07 (m, 7H, 7C$\underline{H}_{Ar}$), 6.84, 6.47 (2s, 1H, H_4), 6.47, 6.43 (2d,

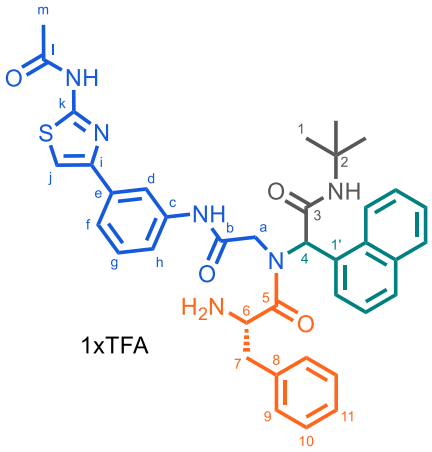
J = 8.5 Hz, 1H, H_j), 6.22, 6.18 (2d, J = 9.2 Hz, 1H, H_e), 4.48, 4.39 (2dd, J = 8.3, 6.4 Hz and J = 7.7, 4.3 Hz, 1H, H_6), 4.13 – 3.78 (m, 2H, H_c), 3.64 – 2.88 (m, 4H, H_a + H_7), 1.58 – 1.45, 1.44 – 1.11, 0.95 – 0.71, 0.65 – 0.45 (4m, 2H, H_b), 1.35, 1.31 (2s, 9H, H_1); ^{13}C NMR (101 MHz, CD_3OD) δ = 172.3, 171.4 (C_5), 170.7, 169.9 (C_3), 165.6, 165.5 (C_d), 161.5, 161.3 (C_i), 150.5, 150.4 (C_h), 140.3, 140.2 (C_f), 139.2, 139.2 (C_k), 135.4, 134.9, 134.7, 134.7, 133.8, 133.5, 131.9, 131.3 ($3C_{IV.Ar}$ + C_8), 130.9, 130.8, 130.6, 130.2, 130.1, 123.0, 128.9, 128.6, 128.5, 128.3, 128.0, 127.1, 125.7, 124.2, 123.9 ($12\underline{C}H_{Ar}$), 114.6 (C_e), 108.6 (C_g), 106.7, 106.6 (C_j), 60.8, 59.3 (C_4), 53.9, 53.2 (C_6), 52.5, 52.4 (C_2), 44.9, 44.8 (C_a), 39.0, 38.8, 38.6, 38.1 (C_c + C_7), 30.7, 30.2 (C_b), 28.8, 28.7 (C_1); HRMS (ESI): *m/z* 605.32404 $[M+H]^+$ ($C_{36}H_{41}N_6O_3$ requires 605.32347).

Ugi product 3'd



Compound **3'd** was prepared following general procedure A procedure using the TFA salt of compound **19** (29.1 mg, 0.10 mmol) as the amine, 1-naphthaldehyde (13.6 µL, 0.10 mmol) as aldehyde, Boc-L-Phe-OH (26.5 mg, 0.10 mmol) as the acid and *tert*-butyl isocyanide (11.3 µl, 0.10 mmol) in MeOH (0.2 mL). After purification by flash chromatography (CH_2Cl_2/MeOH gradient 100:0 to 97:3, v/v), a mixture of diastereomers **3'd** was obtained as a yellow solid (39.1 mg, 51%).$R_{f.dia1+2}$ = 0.36 (CH_2Cl_2-MeOH 96:4); NMR data as a mixture of 2 diastereomers and conformers; ^{1}H NMR (400 MHz, CD_3OD) δ = 8.32 – 7.74 (m, 4H, $3C\underline{H}_{Ar}$ + H_d), 7.74 – 6.96 (m, 13H, $9C\underline{H}_{Ar}$ + H_{f-h} + H_j), 6.89, 6.67, 6.41 (3s, 1H, H_4), 5.19 – 5.07, 4.91 – 4.74, 4.61 – 4.44 (3m, 1H, H_6), 3.97 – 3.70, 3.67 – 3.34 (2m, 2H, H_a), 3.38 – 2.81 (m, 2H, H_7), 2.20, 2.19 (2s, 3H, H_m), 1.68 – 0.94 (m, 18H, $3C\underline{H}_{3.Boc}$ + H_1); ^{13}C NMR (101 MHz, CD_3OD) δ = 176.4, 176.2, 175.4 (C_5), 172.3 (C_3), 170.9 (C_l), 170.3, 169.1 (C_b), 164.8, 159.4, 159.3, 158.1, 157.9, 157.0 (CO_{Boc} + C_k), 150.8 (C_i), 139.9, 139.7, 139.5, 138.4, 138.3, 138.2 (C_c + C_e), 136.6, 136.5, 136.4, 135.5, 135.4, 135.3, 133.7, 133.5, 131.8, 131.6, 131.3, 131.2 ($4C_{IV.Ar}$ + $\underline{C}H_{Ar}$), 130.8, 130.6, 130.4, 130.4, 130.1, 130.0, 129.9, 129.8, 129.6, 129.4, 129.4, 128.9, 128.7, 128.6, 127.9, 127.7, 127.6, 127.5, 126.6, 126.5, 126.3 ($10\underline{C}H_{Ar}$ + C_g), 124.5, 124.1 ($\underline{C}H_{Ar}$), 123.3, 123.1 (C_f), 120.9, 120.6, 120.4 (C_h), 119.0, 118.7, 118.5 (C_d), 108.8, 108.7 (C_j), 81.0, 80.8, 80.5 ($C_{IV.Boc}$), 62.3 (C_4), 54.8, 54.8, 54.2, 53.4, 53.2, 52.7, 52.6 (C_2 + C_6), C_a (signal under the CD_3OD peak), 40.3, 38.5, 38.4 (C_7), 28.9, 28.9, 28.7, 28.7, 28.6, 28.1 ($3\underline{C}H_{3.Boc}$ + C_1), 22.60 (C_m); MS (ESI): *m/z* 777.00 $[M+H]^+$ (theoretical *m/z* 777.34).

Deprotected Ugi product 3d



General procedure B was employed for the deprotection of compound **3'd** (30.3 mg, 0.039 mmol) in 1 mL of CH_2Cl_2 and in the presence of 50 equiv. of TFA. After filtration, the crude mixture was purified by semipreparative HPLC (method B) leading to the mixture of diastereomers **3d** as a white powder (10.6 mg, 34%).

HPLC: rt_{dia1} = 15.92 min and rt_{dia2} = 16.23 min (method B); NMR data as a mixture of 2 diastereomers; ^{1}H NMR (400 MHz, CD_3OD) δ = 8.03 – 7.40, 7.39 – 7.10 (2m, 17H, 17C$\underline{H}_{Ar}$), 6.98, 6.79 (2s, 1H, H_4), 4.65, 4.59 – 4.49 (t and m, J = 6.4 Hz, 1H, H_6), 4.32, 4.09 – 3.73 (d and m, J = 17.8 Hz, 2H, H_a), 3.53 – 3.08 (m, 2H, H_7), 2.22, 2.22 (2s, 3H, H_m), 1.44, 1.40 (2s, 9H, H_1); ^{13}C NMR (101 MHz, CD_3OD) δ = 172.0, 171.0, 170.9 (C_3 + C_5), 168.7, 168.7, 168.3, 168.3 (C_b +C_l), 159.5 (C_k), 150.7 (C_i), 139.5, 138.2 (C_c + C_e), 136.5, 135.6, 134.9, 133.5, 131.5, 131.5, 131.4 (4$C_{IV.Ar}$ + $\underline{C}H_{Ar}$), 131.1, 131.0, 130.9, 130.6, 130.2, 130.1, 130.0, 129.8, 129.7, 129.3, 128.9, 128.3, 128.2, 128.0, 127.5, 127.1, 126.3, 126.1, 125.0 (10$\underline{C}H_{Ar}$ + C_g), 124.8, 124.5 ($\underline{C}H_{Ar}$), 123.3, 123.2 (C_f), 120.8, 120.6 (C_h), 118.7, 118.6 (C_d), 110.4 (C_j), 57.9, 57.6 (C_4), 54.2, 52.7, 52.5 (C_2 + C_6), 49.78 (C_a), 38.8, 37.7 (C_7), 28.8, 28.8 (C_1), 22.7 (C_m); MS (ESI): *m/z* 677.00 $[M+H]^+$ (theoretical *m/z* 677.29).

The compound resulted not sufficiently stable for full characterization and evaluation.

Biochemistry and Biology

Materials

All buffers and solutions employed in fluorescence-based assays were filtered through 0.22 μm Millipore filters (GP ExpressPLUS® membrane). Tris·HCl 20 mM, pH 7.2 containing 12 mM NaCl, 3 mM $MgCl_2$ and 1 mM DTT (Buffer A) was used for K_D determination. HEPES 20 mM, pH 7.4, 20 mM NaCl, 140 mM KCl and 3 mM $MgCl_2$ (Buffer B) was used for inhibition assays.

RNA and DNA oligonucleotides were purchased from Eurogentec (Liege, Belgium). For competition experiments in the presence of tRNA, a mixture of pre- and mature yeast tRNAs (from *Escherichia coli*, Sigma) was added to buffer A to obtain a 100-fold nucleotide excess regarding RNA *AapA1*. For competition experiments in the presence of a dsDNA, a 15-mer sequence (5'-CGTTTTTATTTTTGC-3') and its complementary oligonucleotide, annealed beforehand, were added to buffer A to obtain a 100-fold nucleotide excess regarding RNA *AapA1*.

Oligonucleotides are as follows: *AapA*1 (RNA messenger) and *IsoA1* (RNA antitoxin)

For K_D determination: *AapA1*-FAM: 5'-FAM-UUU-GUC-AUG-GCU-ACC-AAA-CAU-GGC-AAA-3'

For inhibition assays:

AapA1-FAM: 5'-FAM-UUU-GUC-AUG-GCU-ACC-AAA-CAU-GGC-AAA-3'

IsoA1: FAM-5'-UUU-GCC-AUG-UUU-GGU-AGC-CAU-GAC-AAA-3'

IsoA1: 5'-UUU-GCC-AUG-UUU-GGU-AGC-CAU-GAC-AAA-3'

Full length IsoA1 and AapA1 sequences were produced as previously reported.[5]

For DSF assay:

AapA1 (truncated sequence): UUU GUC AUG GCU ACC AAA CAU GGC AAA

IsoA1 (truncated sequence): UUU GCC AUG UUU GGU AGC CAU GAC AAA

AapA1 (full-length sequence): CAC CCC CAU AAG UGC AAU UAU GGG GAU AAA UCC AUA AAA GGA GUU UGU CAU GGC UAC CAA ACA UGG CAA AAA CUC UUG GAA AAC AUU AUA CCU CAA AAU UUC AUU UUU GGG GUG UAA AGU UGU UGU UUU AUU GAA GCG GUA GUU UUG UCA AAC GAA AUU UUG UAA AAU GAU AGC UUU AGU UUU UCC AAA GUU CCC UUA AGG CUU UUA GCU UUA AGG GUU UUC CUU UAA AUU UUA UCC UUA ACU UAU GGG GGC

IsoA1 (full-length sequence): CAA GAG UUU UUG CUA UGU UUG GUA GCC AUG ACA AAC UCC UUU UAU GGA UUU AUC CCC AUA AUU GCA CUU AUG GGG GUG UUU

Procedure of anisotropy assay

Inhibition assays were performed in 384-well black plates (Greiner bio-one) in a final volume of 70 µL using a 5070 EpMotion automated pipetting system (Eppendorf). Each experiment was performed in duplicate and repeated three times. Refolding of the RNA was performed using a thermocycler (ThermoStatPlus Eppendorf) as follows: the RNA 5'-FAM-*AapA1* or RNA *IsoA1*, was diluted in 1 mL of buffer B, was first denatured by heating to 90°C for 2 min and then cooled to 4°C for 10 min, followed by incubation at 25°C for 15 min. After refolding, the RNA was diluted to a working concentration of 11.7 nM for *AapA1* and 35 nM for *IsoA1* through addition of the appropriate amount of buffer B.

During inhibition assays, 30 µL of RNA *AapA1* solution was added in each well containing 30 µL of each desired ligand concentration. Each ligand was added in 15 dilutions (from 61 pM to 1 mM). After incubating the plate at 4°C overnight, 10 µL of RNA *IsoA1* solution was added to each well (final volume 70 µL). The plate was incubated at 37°C for 2h then at 4°C for 20 min. The anisotropy was measured on a SpectraMax (Molecular Devices).

Procedure of fluorescence-based assays

K_D determinations were performed in 384-well black plates (Greiner bio-one) in a final volume of 60 µL, respectively, using a 5070 EpMotion automated pipetting system (Eppendorf). Each experiment was performed in duplicate and repeated three times. Refolding of the RNA was performed using a thermocycler (ThermoStatPlus Eppendorf) as follows: the RNA (5'-FAM-*AapA1*), was diluted in 1 mL of buffer A, was first denatured by heating to 90°C for 2 min and then cooled to 4°C for 10 min, followed by incubation at 25°C for 15 min. After refolding, the RNA was diluted to a working concentration of 10 nM through addition of the appropriate amount of buffer A.

During binding assays, 30 µL of RNA solution were added in each well containing 30 µL of each desired ligand concentration (final volume 60 µL). Each ligand was added in 15 dilutions (from 61 µM to 1 mM) and fluorescence was measured after incubating the plate at 4°C overnight. The fluorescence was measured at various temperatures from 5°C to 35°C on a GeniosPro (Tecan).

Data analysis

Inhibition data were analyzed using GraphPad Prism 5 software using a nonlinear regression following the equation:

$$Y = Bottom + \frac{(Top - Bottom)}{(1 + 10^{(\log KD - X) \times hills\ slope})}$$

With bottom = Minimal value for Y; Top = Maximal value for Y; K_D = IC_{50} for the calculation of IC_{50}.

Binding profiles were well modelled using a simple model assuming a one-to-one stoichiometry.

Procedure of electrophoretic mobility shift assay

The RNAs (0.2 nmol /100 µL of buffer B) AapA1 and IsoA1 were incubated separately for 2 min at 90°C, 10 min at 4°C and 15 min at 25°C in buffer B. Once mixed, they were incubated for 2h at 37°C, 25°C or 4°C adding RNA IsoA1 (10µL) and *IsoA1* (10 µL).

The samples were then charged on a 20% of 19:1 acrylamide/bis-acrylamide in TBE buffer 1x gel after addition of 10 µL of a 50% sucrose solution. Migration was performed at 4°C.

For full length RNA sequences, we employed the same protocol but performed a 8% acrylamide/bis-acrylamide 19:1.

Procedure of differential scanning fluorimetry assay

DSF experiments were performed in 96-well plates (Bio-Rad, MLL9601) in a final volume of 50 µL using a CFX96 Touch Real-Time PCR System (Bio-Rad).

Refolding of the unlabeled RNA was performed using a ThermoStat Plus thermocycler (Eppendorf) as follow: RNA was diluted in 50mM cacodylate sodium buffer (to a working concentration of 150 nM for short RNA and 75 nM for long ones), denatured by heating to 90°C for 2 min, cooled to 4°C for 10 min and incubation at 25°C for 15 min. SYBR Green (Thermo Fisher Scientific) was solubilized at a concentration seventeen times higher than the desired final concentration on plate, while compounds ten times higher, in order to allow for the subsequent dilution while adding the RNA and dye solution to the plate.

42 µL of folded pre-miRNA (150 nM final) and 5 µL of each desired compound were added subsequently to the plate, which was then incubated at 25°C for 1h. 3 µL of the previously prepared dye solution (0.5X final concentration for short RNA, 0.25X for long ones) were added to wells and the plate sealed with an optically clear foil (Bio-Rad, MSB1001).

The plate was placed in the instrument and fluorescence intensity measured using the SYBR Green channel and a temperature gradient from 10°C to 95°C, with a ramp rate of 0.1 °C/sec.

The fluorescence raw data were plotted on GraphPad Prism 9.0.0 software, followed by calculation of the first derivative and smoothing of the curves to obtain melting temperature values.

Bacterial strains, Cultural conditions, and Reagents

Antimicrobial activity of synthetized compounds was assayed against *Helicobacter pylori* (ATCC 43504), *Staphylococcus aureus* (SA009), *Escherichia coli* (CIP 54.8), *Klebsiella pneumoniae* (CIP 82.91), *Klebsiella aerogenes* (ATCC 13048), *Acinetobacter baumannii* (ATCC 19606) and *Pseudomonas aeruginosa* (CIP 1001720). Bacteria were routinely maintained on cation-adjusted Mueller-Hinton (MHII) agar plates and grown in MHII broth at 37°C. *H. pylori* was grown in microaerophilic chamber (6% O_2, 10% CO_2, and 84% N_2; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Neomycin and DMSO were purchased from Sigma-Aldrich (St Quentin Fallavier, France)

Antibacterial activity

Synthetized compounds were screened at a 10 µM final concentration on the BAC-SCREEN platform, with a Freedom EVO 150 liquid handling system (Tecan, Lyon, France). Assays were carried out in 96-well microtiter plates in MHII broth with a final volume of 200 µl. The bacterial inoculum was adjusted to 5×10^5 CFU/ml. Microtiter plates were read at 600 nm on an Infinite M200 Pro plate reader (Tecan, Lyon, France) after 18-22 h incubation at 37°C. MHII broth with bacterial inoculum was considered as the positive control and MHII broth alone was considered as the negative control. The percentage growth inhibition was determined by the following formula: 100- [(OD of sample–OD of negative control) × 100 / (OD of positive control – OD of negative control)]. Assays were performed in three independent biological experiments.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations (MIC) were determined by the microdilution broth method according to the CLSI guidelines.[6] Two-fold serial dilutions of the compounds ranging from 400 to 6.25 µM were made in a 100 µL volume of MHII broth and 100 µL of a *H. pylori* inoculum of 10^6 CFU/mL were added to achieve 5×10^5 CFU/mL final concentration per well. The plates were then incubated for 18-22 h at 37°C in a microaerophilic environment. MICs were defined as the lowest concentration of drug that completely inhibited visible growth. To determine MICs, the measurements were independently repeated at least three times.

Determination of minimum bactericidal concentration (MBC)

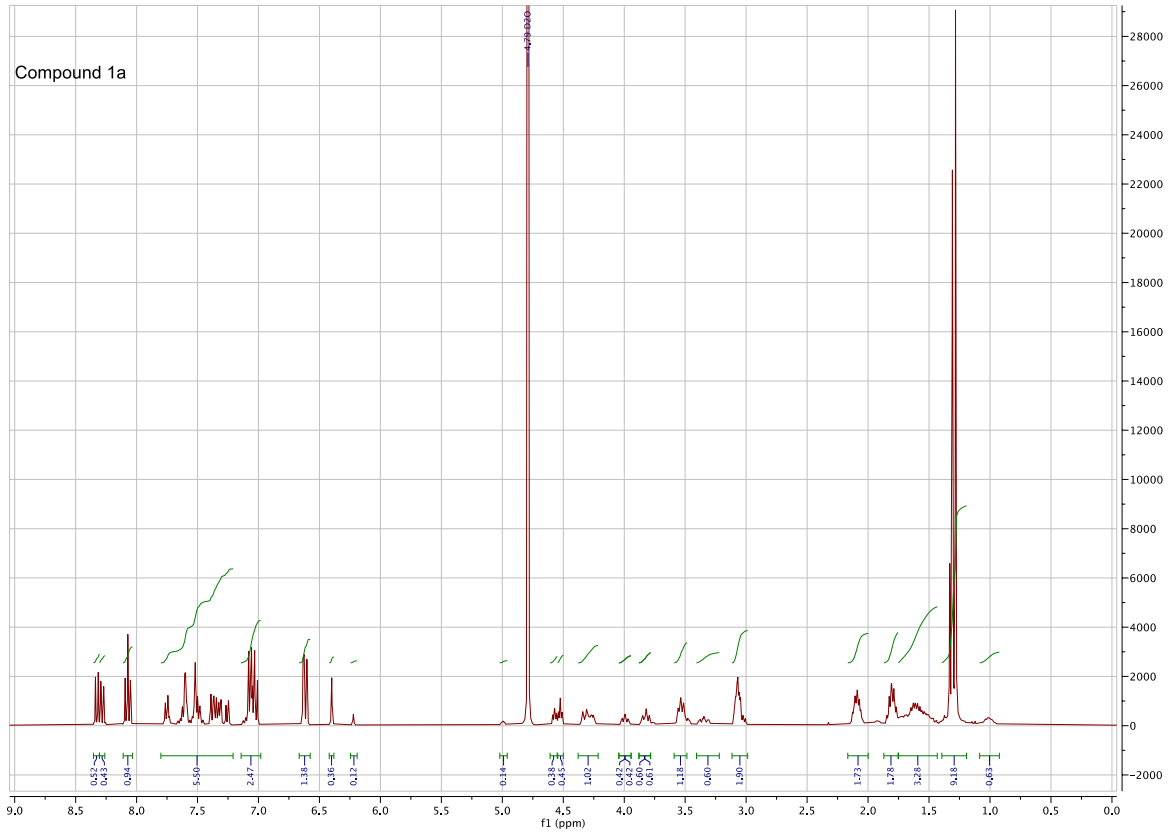
The *in vitro* activity of compounds **1b** and **6a** was assessed using the reference broth microdilution method in Mueller-Hinton II (MH2) medium, following the CLSI guidelines. Bacterial suspensions were prepared at a final inoculum of 5×10^5 CFU/mL. The compounds were tested over a concentration range of 100 to 1.56 µM, prepared from a 100 mM stock solution in DMSO.

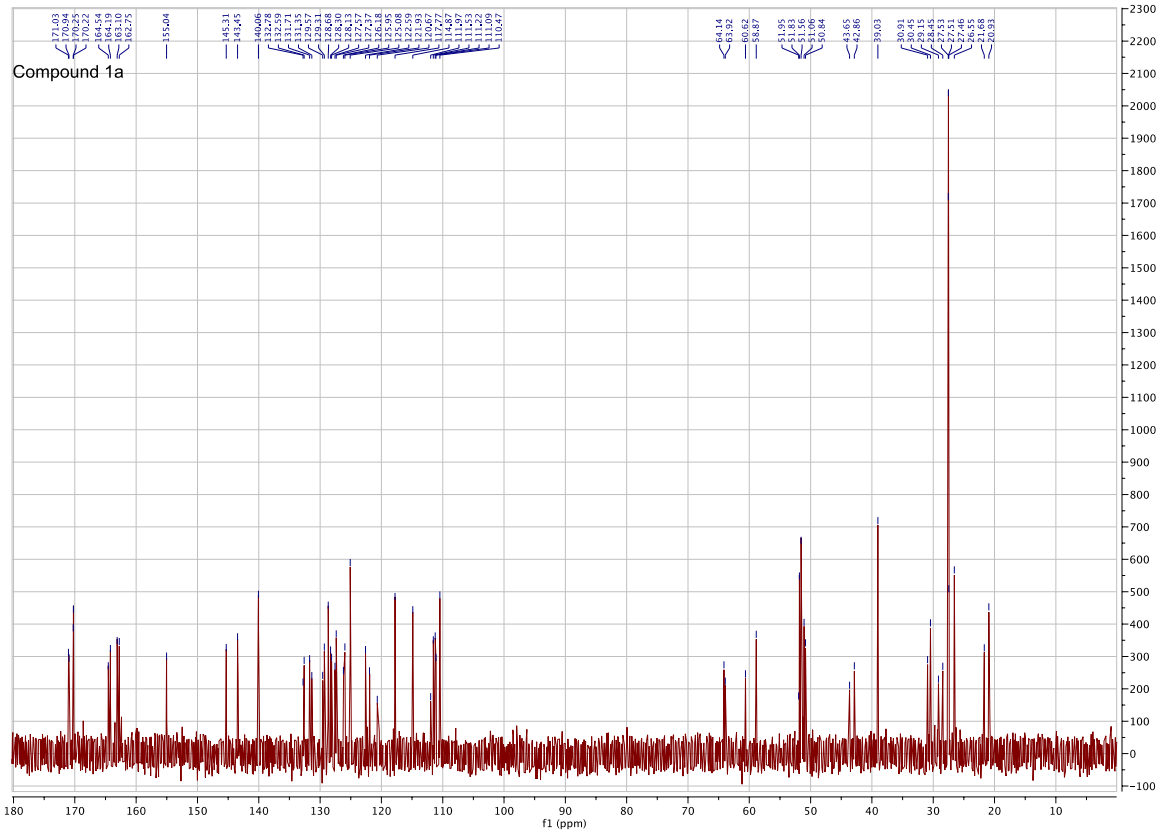
At the end of incubation, 5 µL of supernatant was taken from wells containing compound concentrations between 50 and 3.125 µM. These samples were spotted onto MH2 agar plates and incubated at 37 °C for 18 hours. Colony formation was then assessed to determine the MBC.

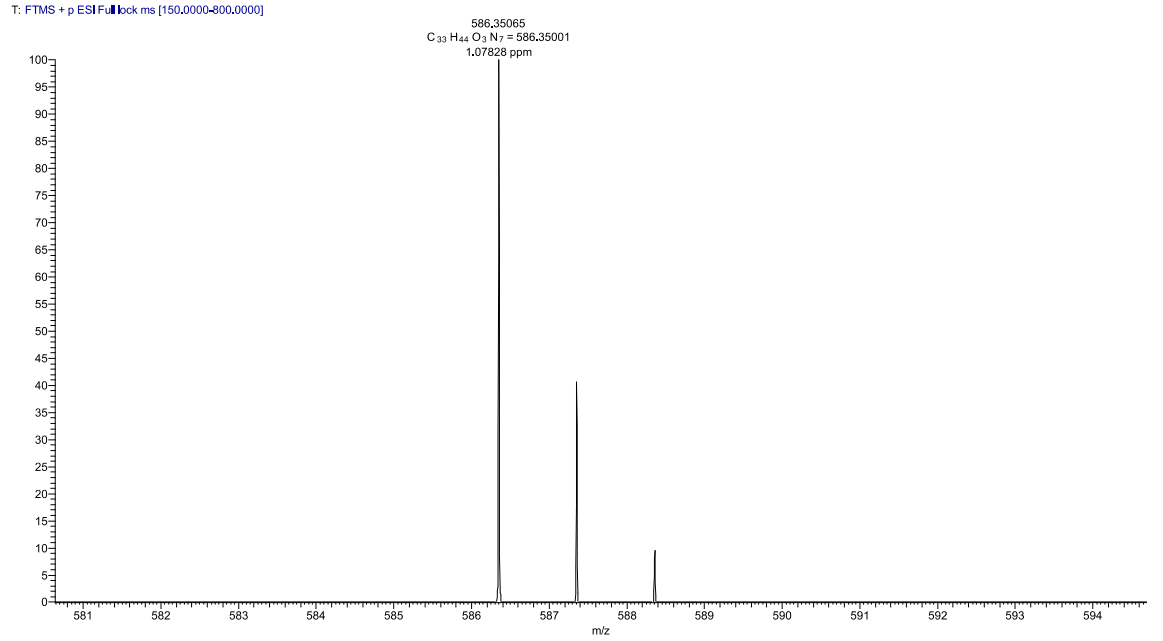
The nature of the antibacterial activity was determined based on the MBC/MIC ratio[7]:

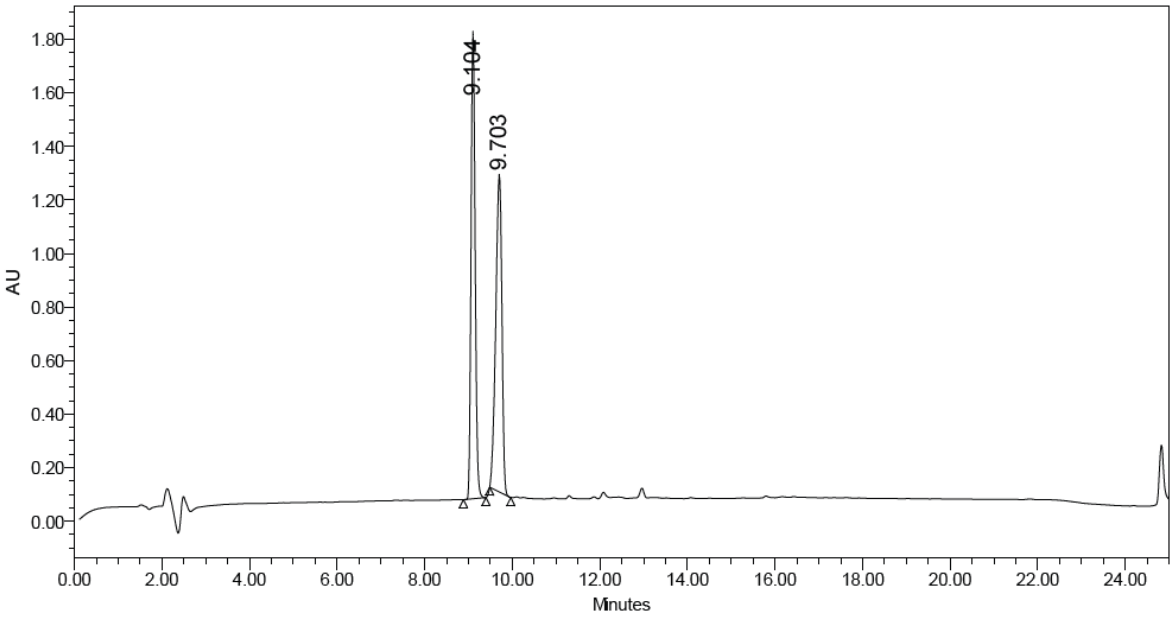
- MBC/MIC < 4: bactericidal effect,
- 4 ≤ MBC/MIC ≤ 32: bacteriostatic effect,
- MBC/MIC > 32: tolerance.

Figure S11. ^{1}H NMR, ^{13}C NMR, HRMS spectra and HPLC profile of compound **1a**.



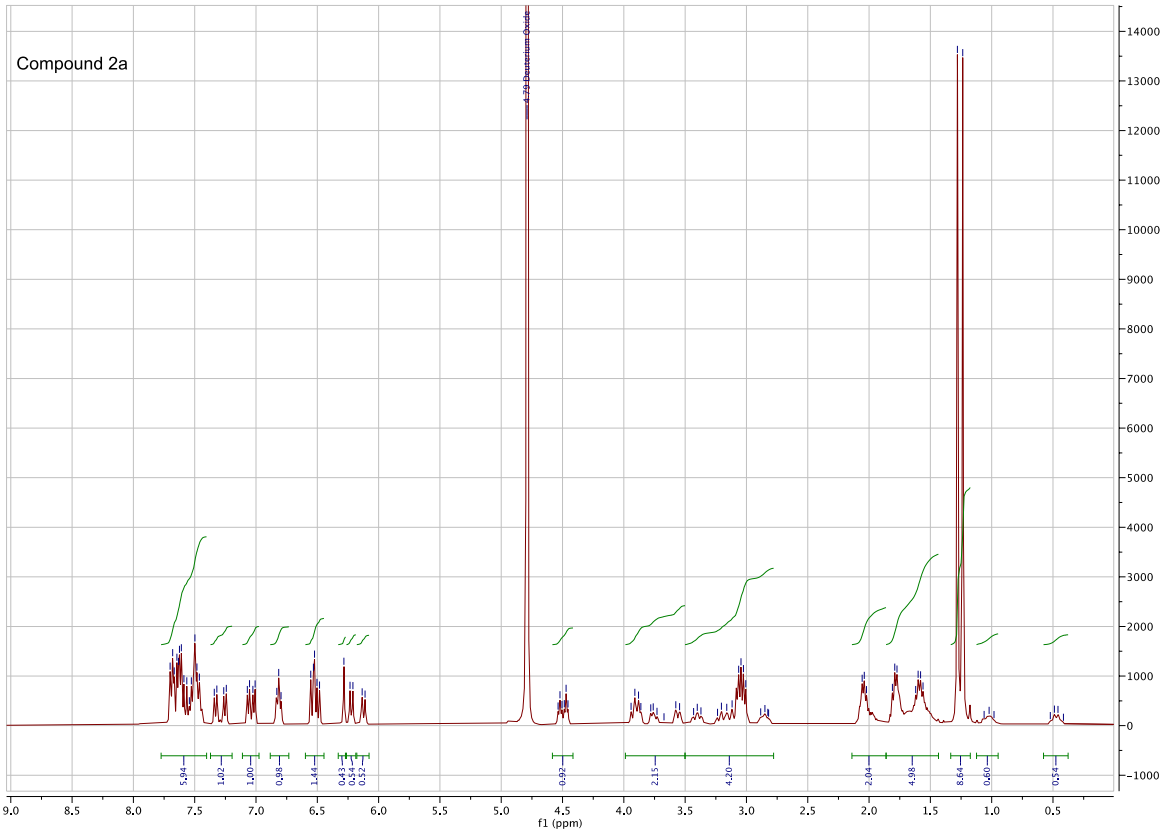


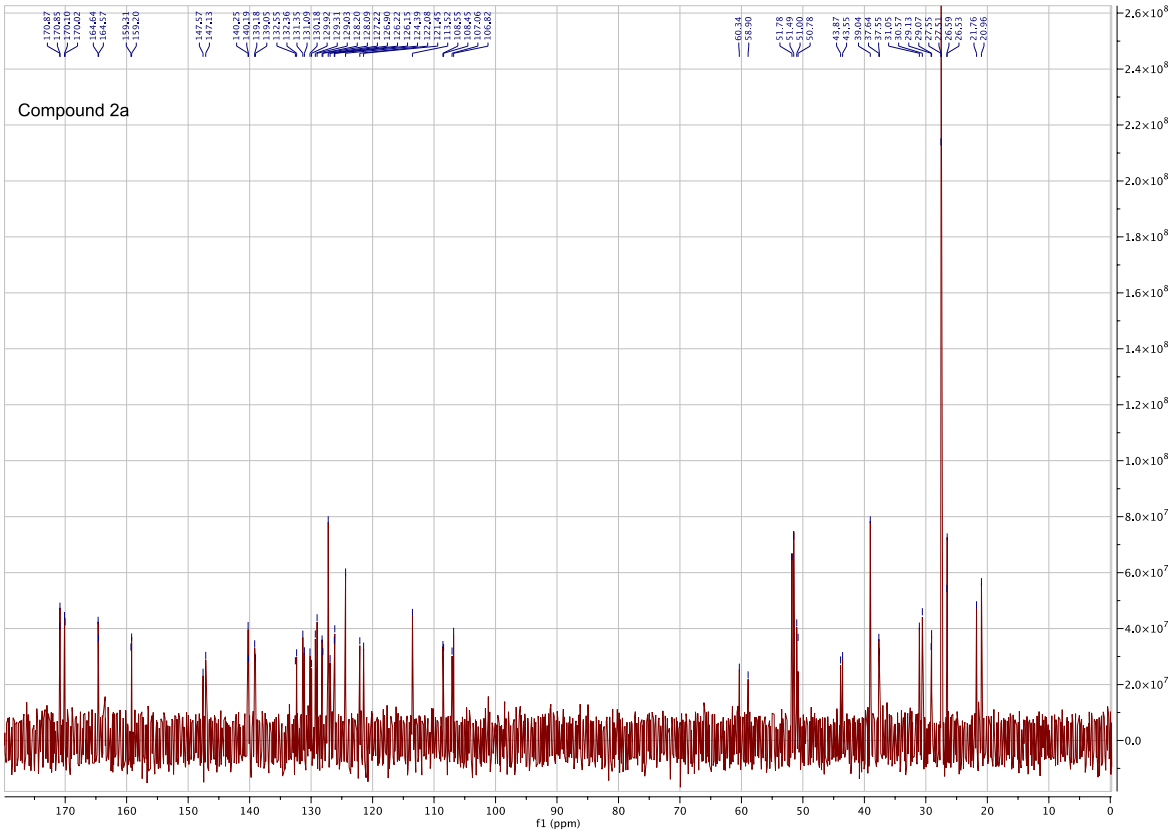


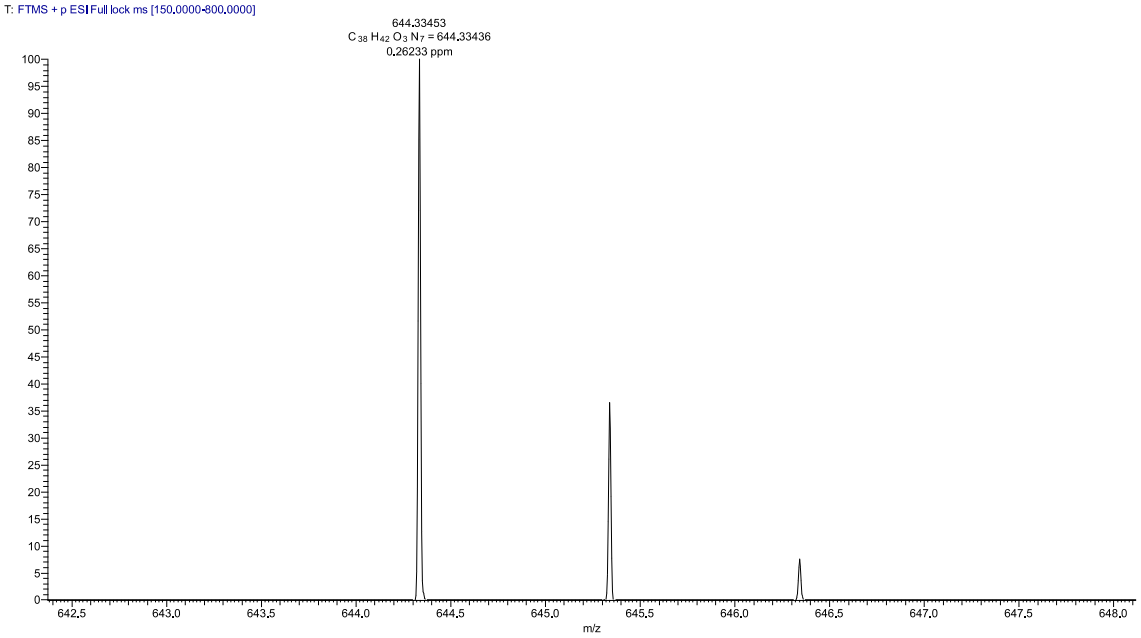


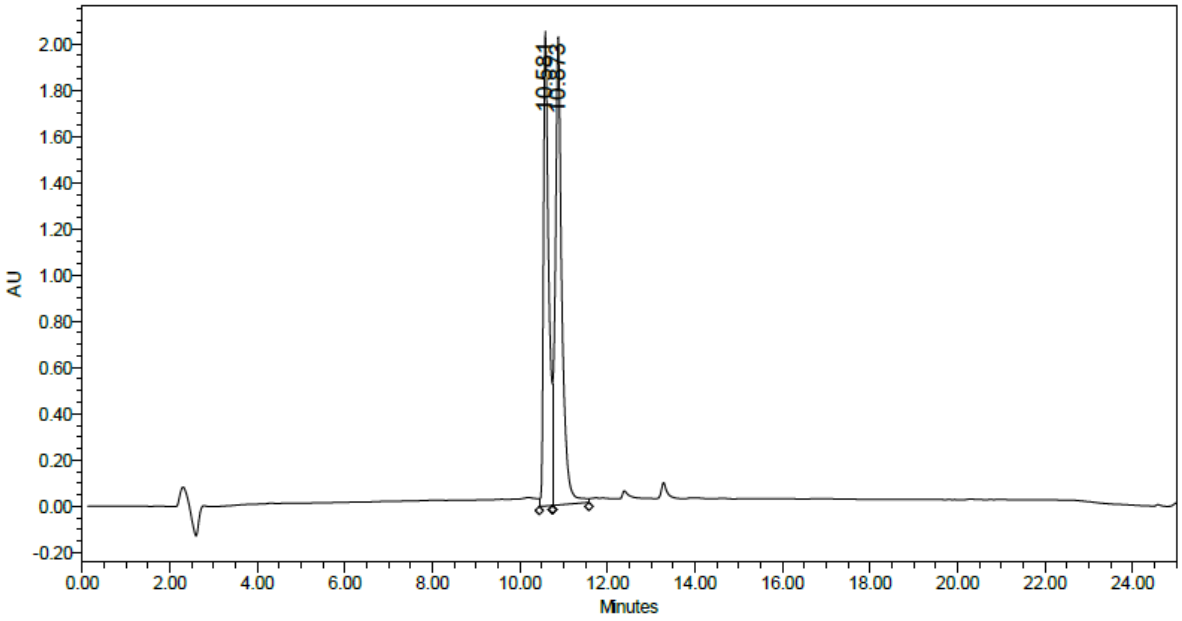
	RT	Area	% Area	Height
1	9.104	11477220	49.83	1740384
2	9.703	11556645	50.17	1185601

Figure S12. ^{1}H NMR, ^{13}C NMR, HRMS spectra and HPLC profile of compound **2a**.



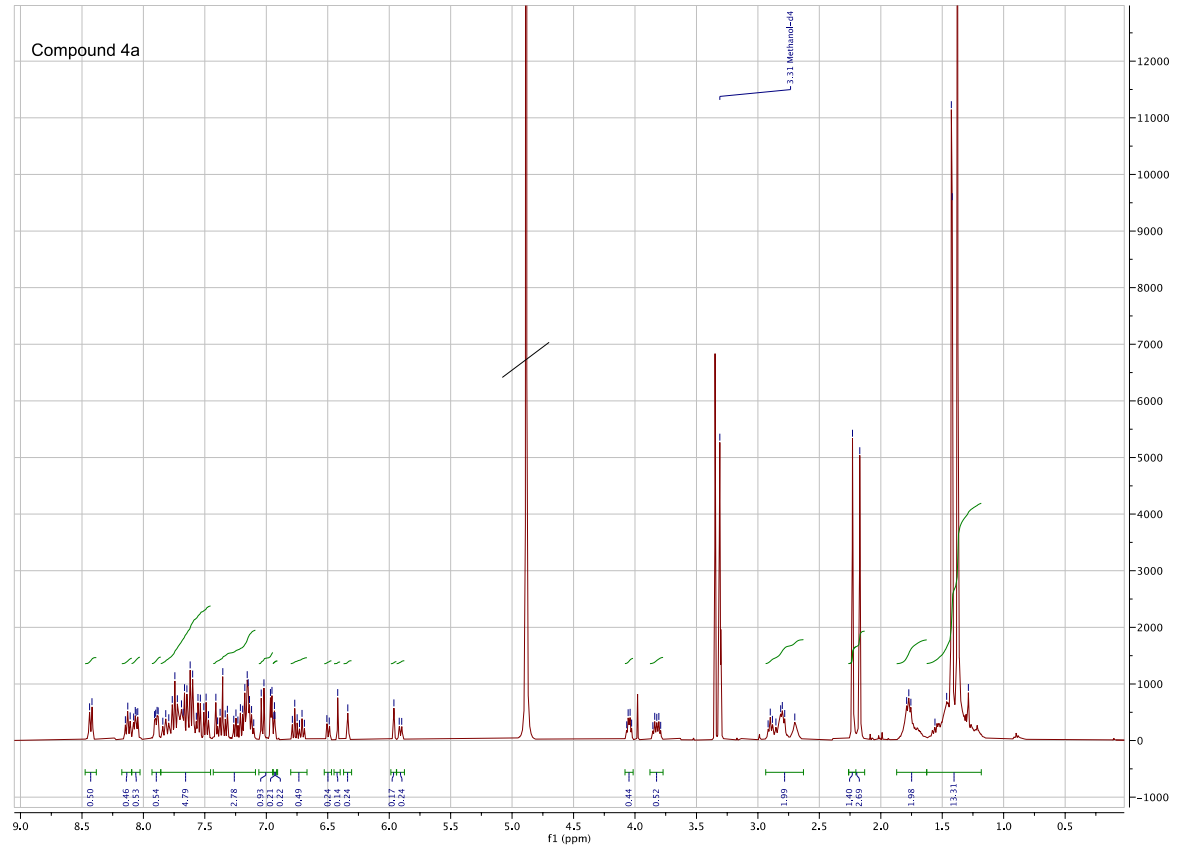


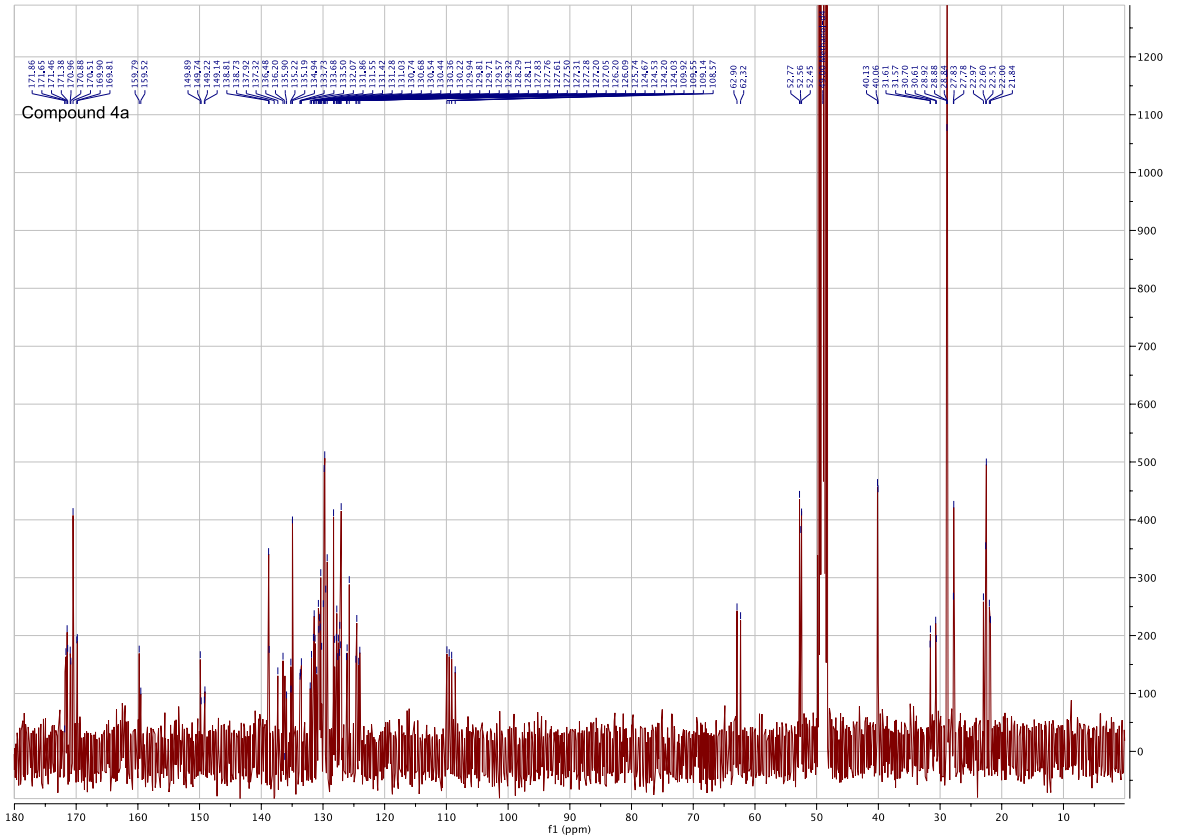


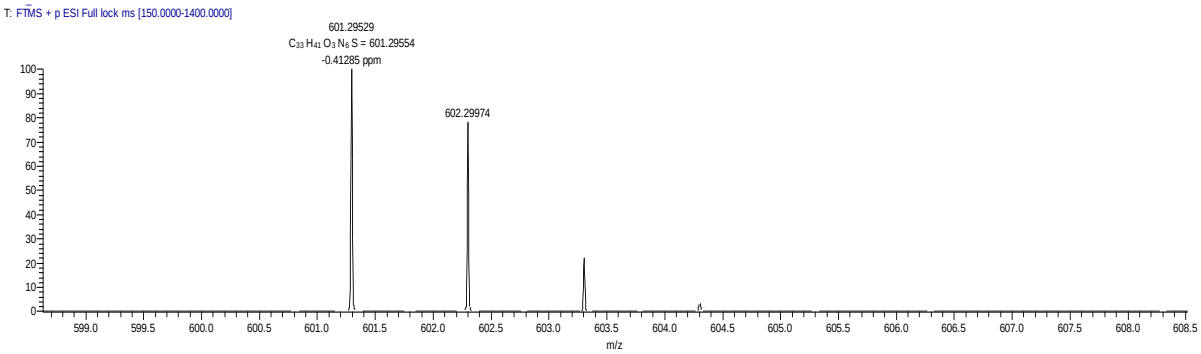


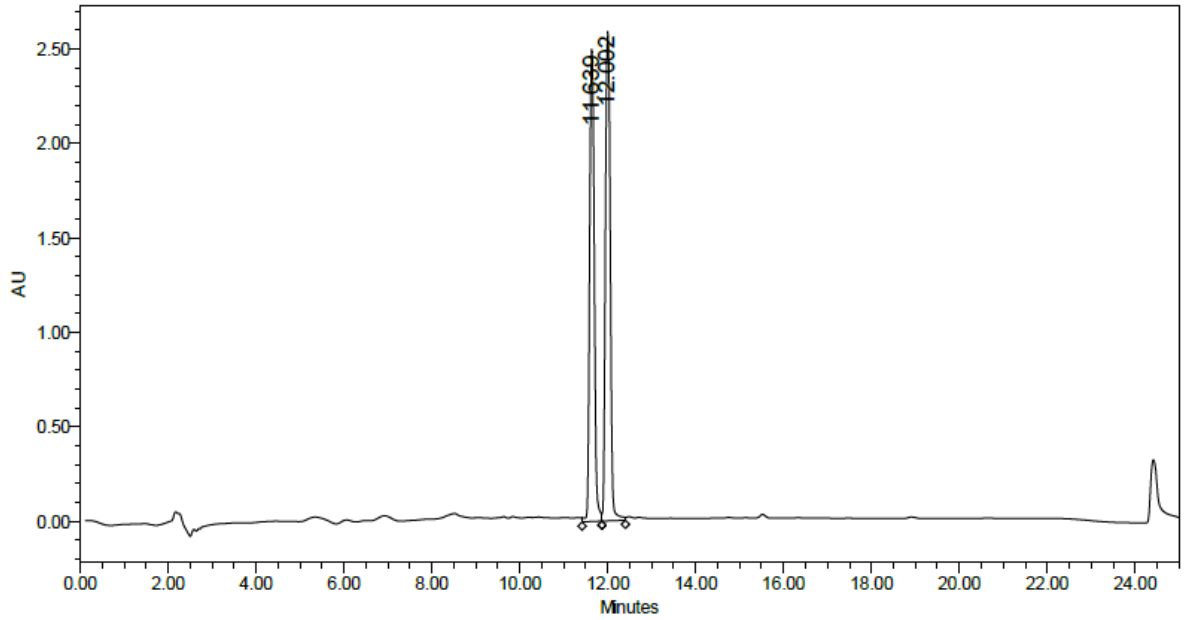
	RT	Area	% Area	Height
1	10.581	16786615	44.06	2058292
2	10.873	21311883	55.94	2019742

Figure S13. ^{1}H NMR, ^{13}C NMR, HRMS spectra and HPLC profile of compound **4a**.



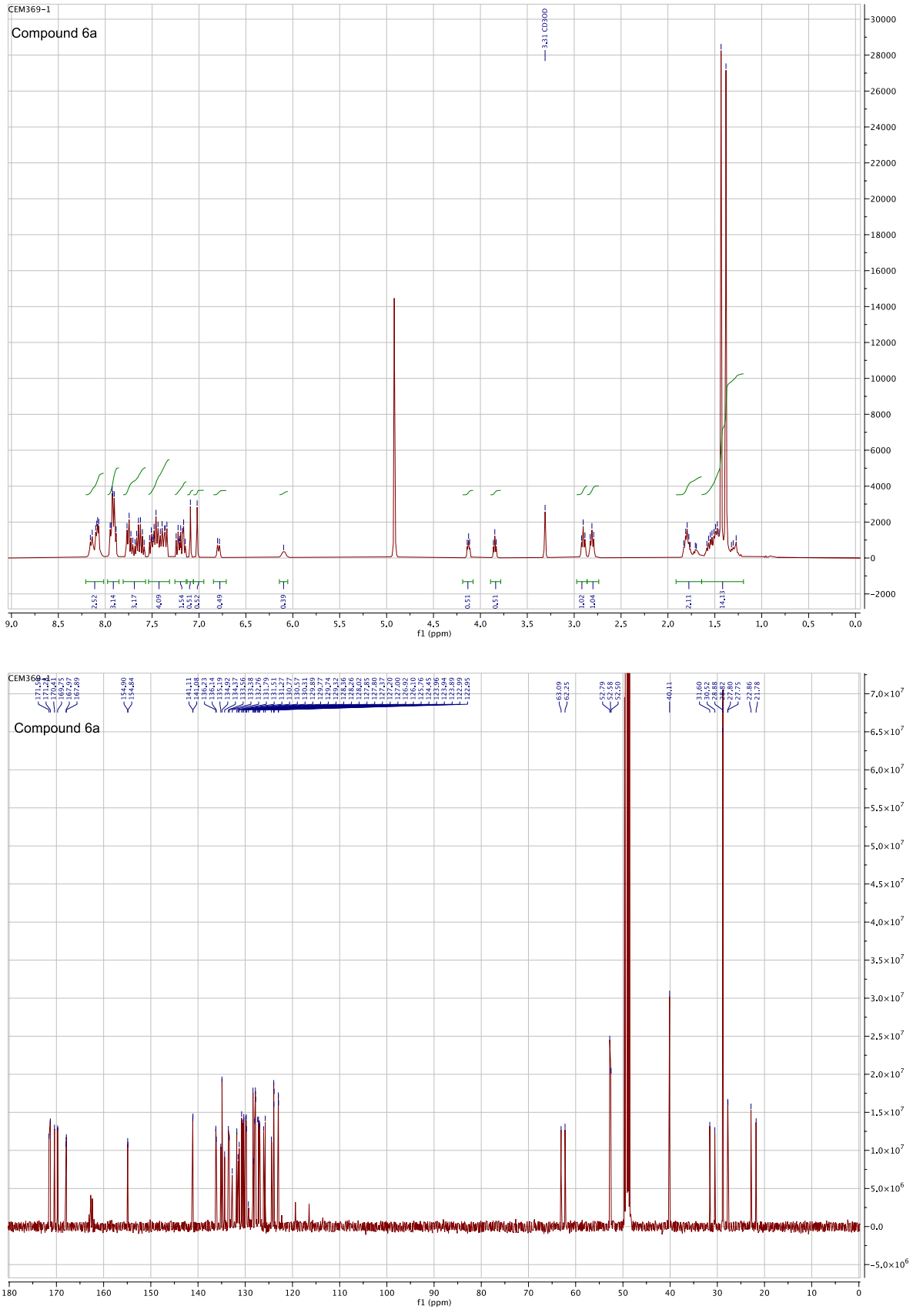


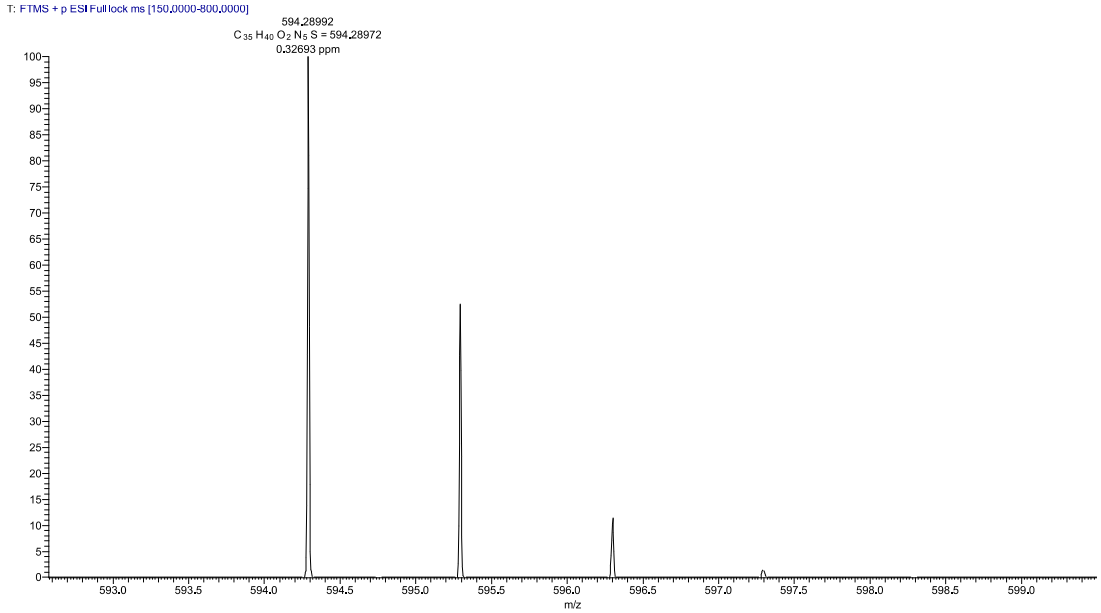


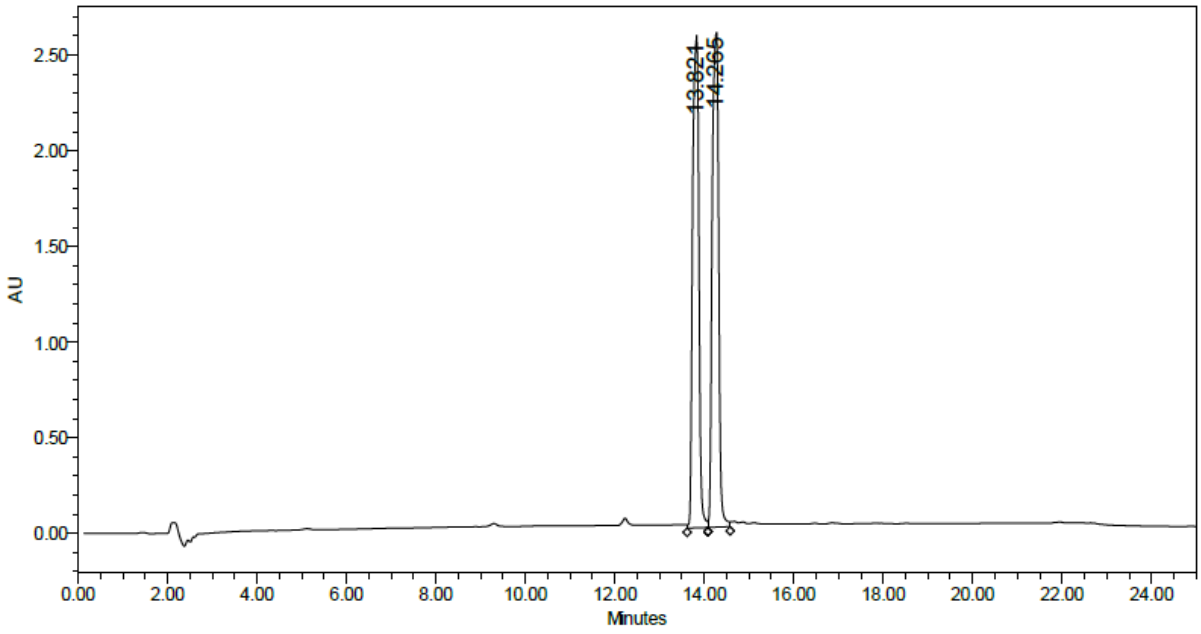


	RT	Area	% Area	Height
1	11.639	18594254	48.29	2514861
2	12.002	19909919	51.71	2601211

Figure S14. ^{1}H NMR, ^{13}C NMR, HRMS spectra and HPLC profile of compound **6a**.

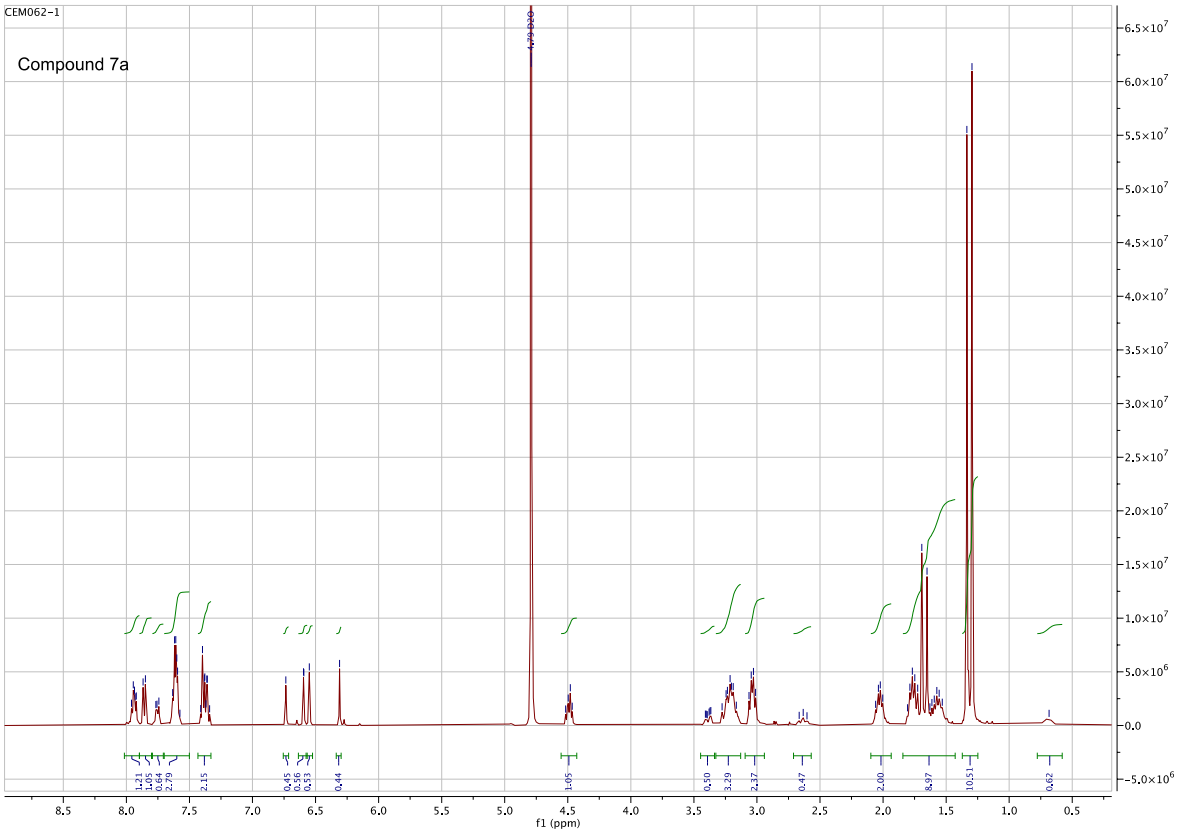


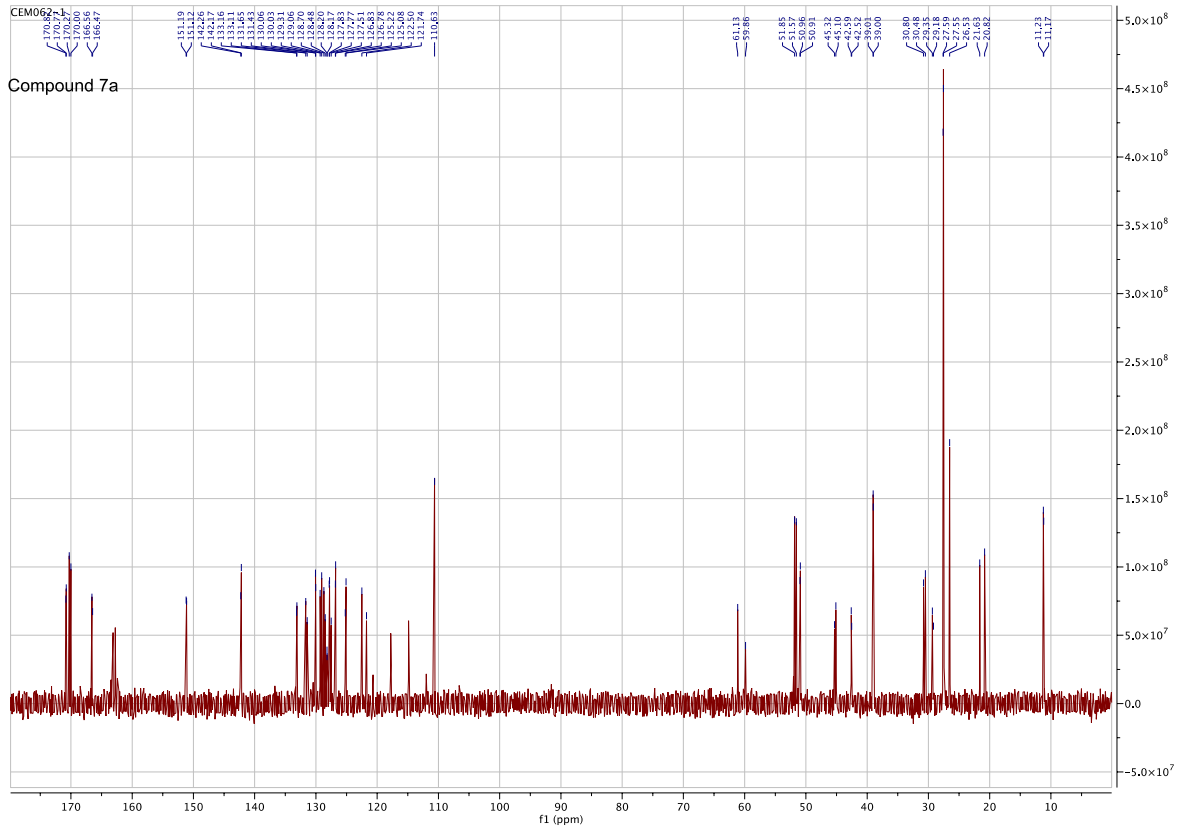


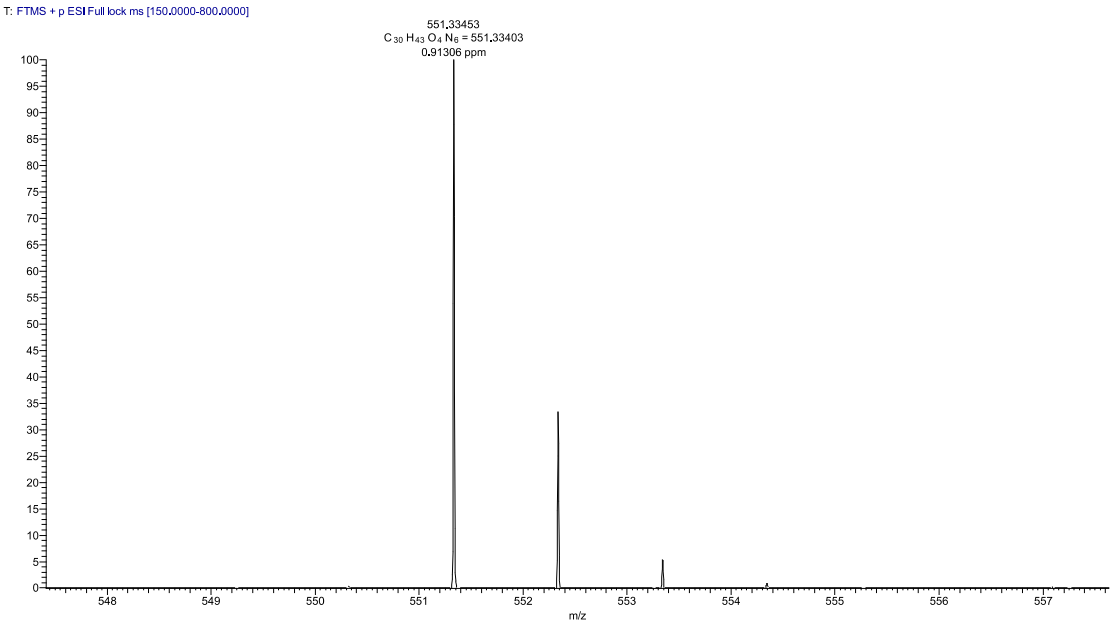


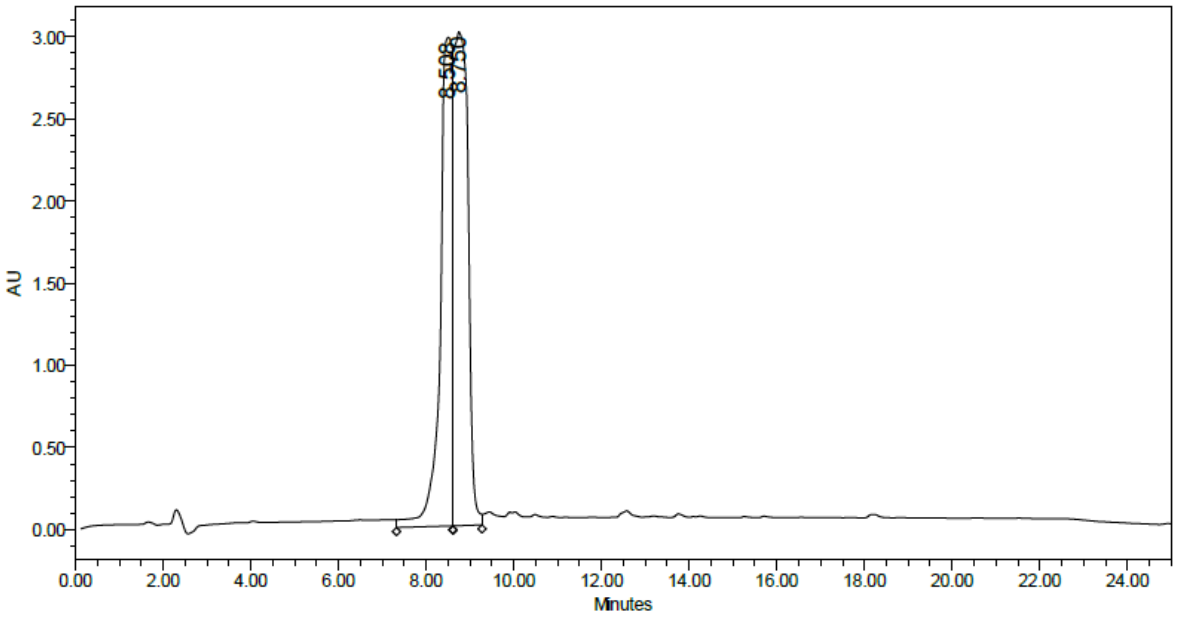
	RT	Area	% Area	Height
1	13.821	24976854	49.05	2592803
2	14.265	25939874	50.95	2633690

Figure S15. ^{1}H NMR, ^{13}C NMR, HRMS spectra and HPLC profile of compound **7a**.



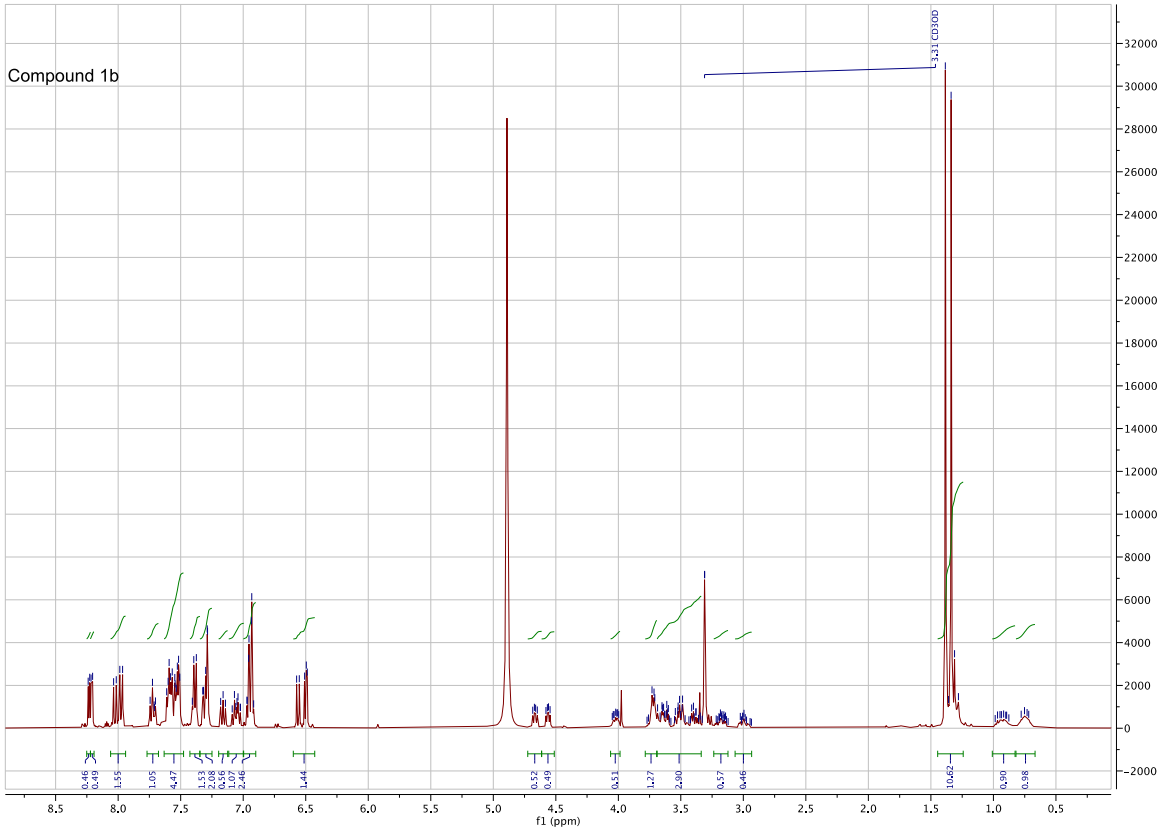


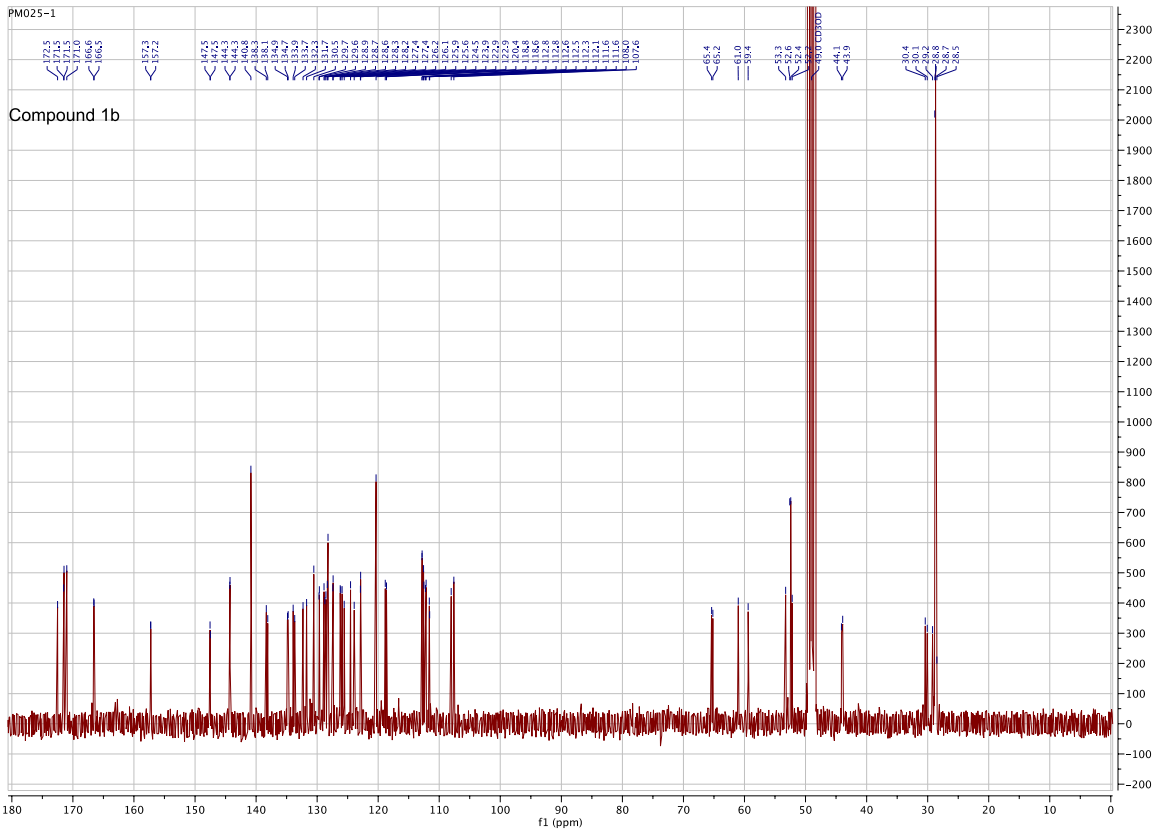


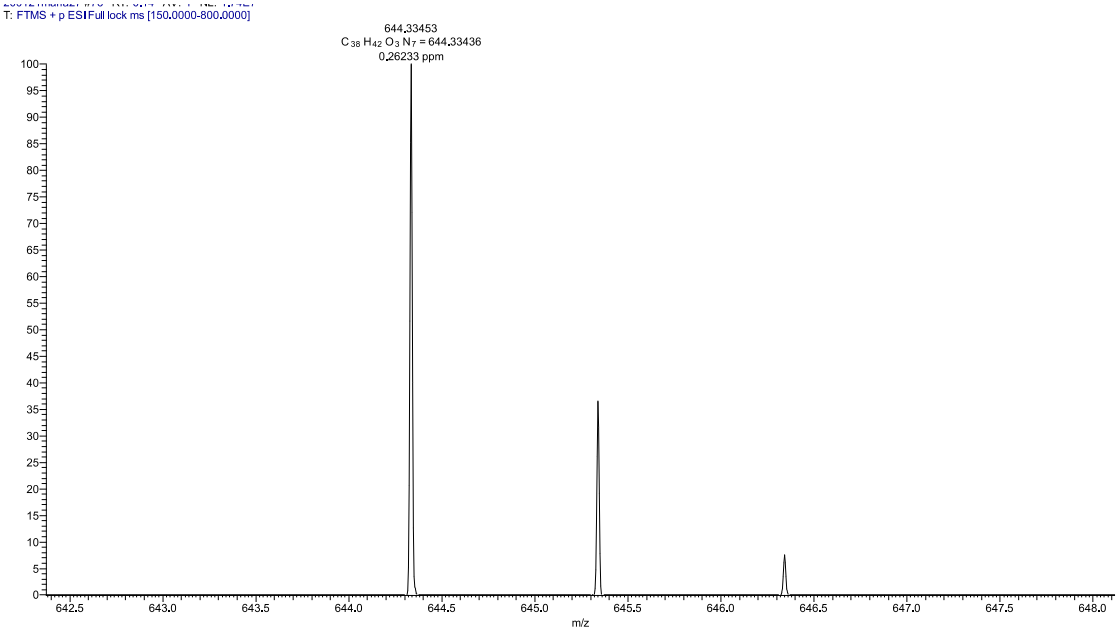


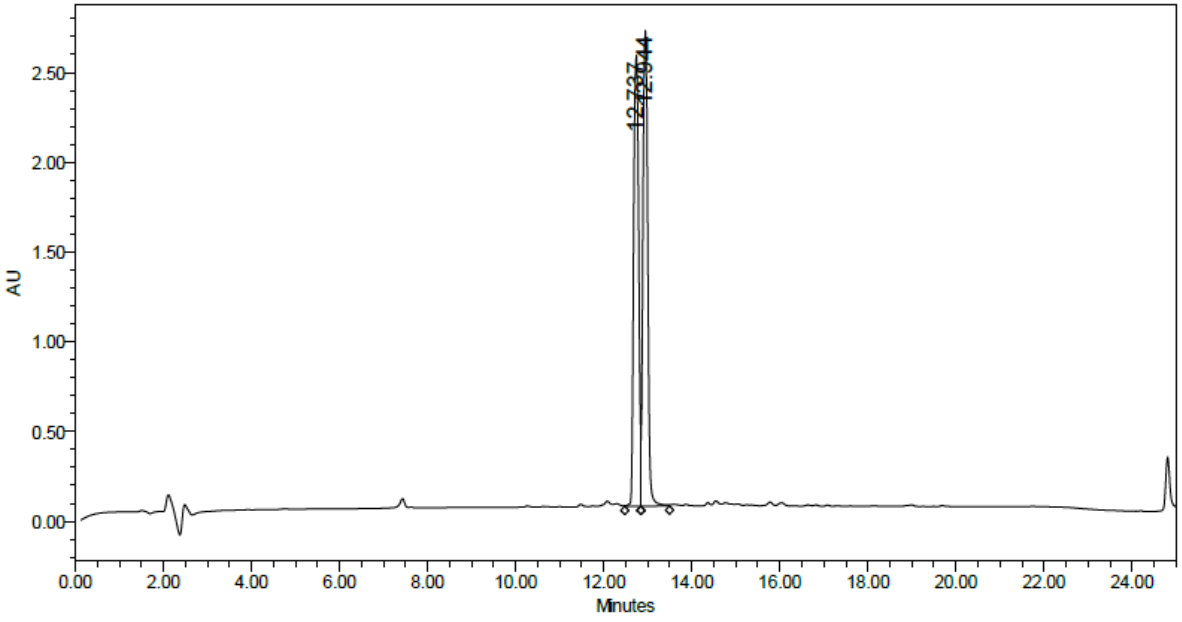
	RT	Area	% Area	Height
1	8.508	56481093	45.14	2972973
2	8.750	68656328	54.86	3003884

Figure S16. ^{1}H NMR, ^{13}C NMR, HRMS spectra and HPLC profile of compound **1b**.



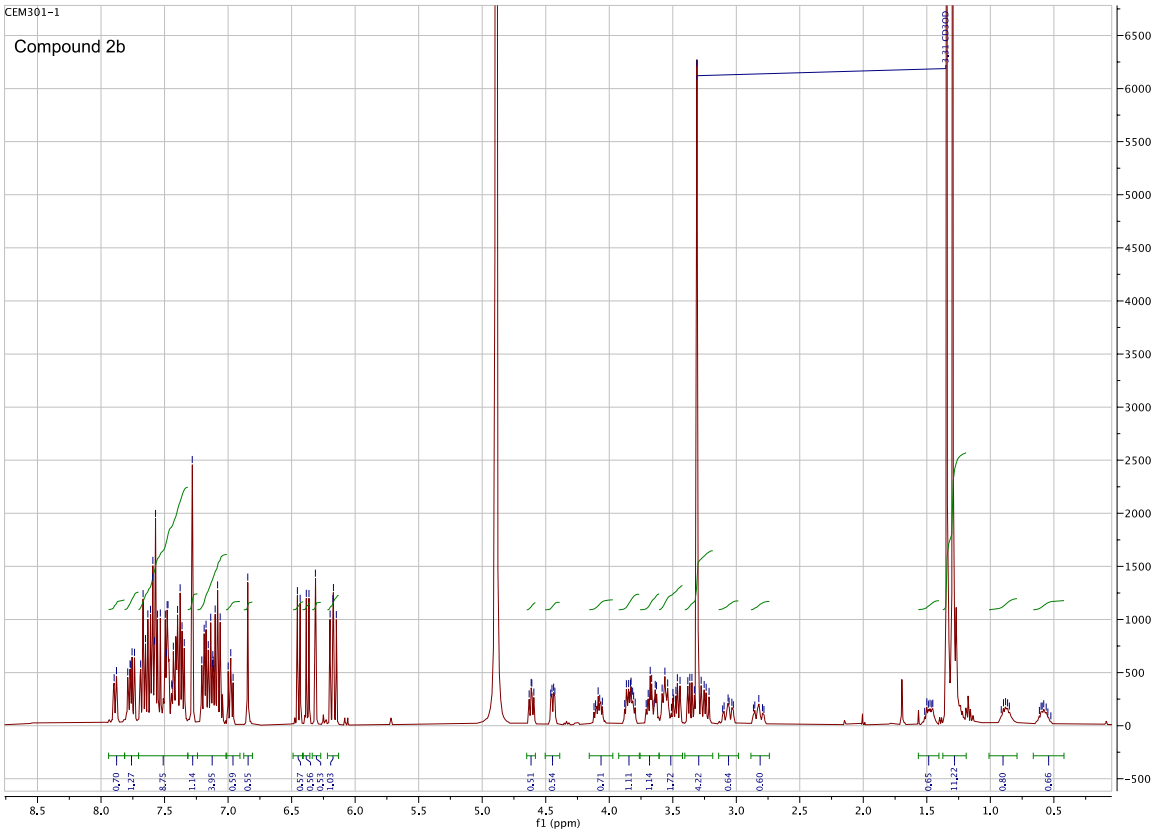


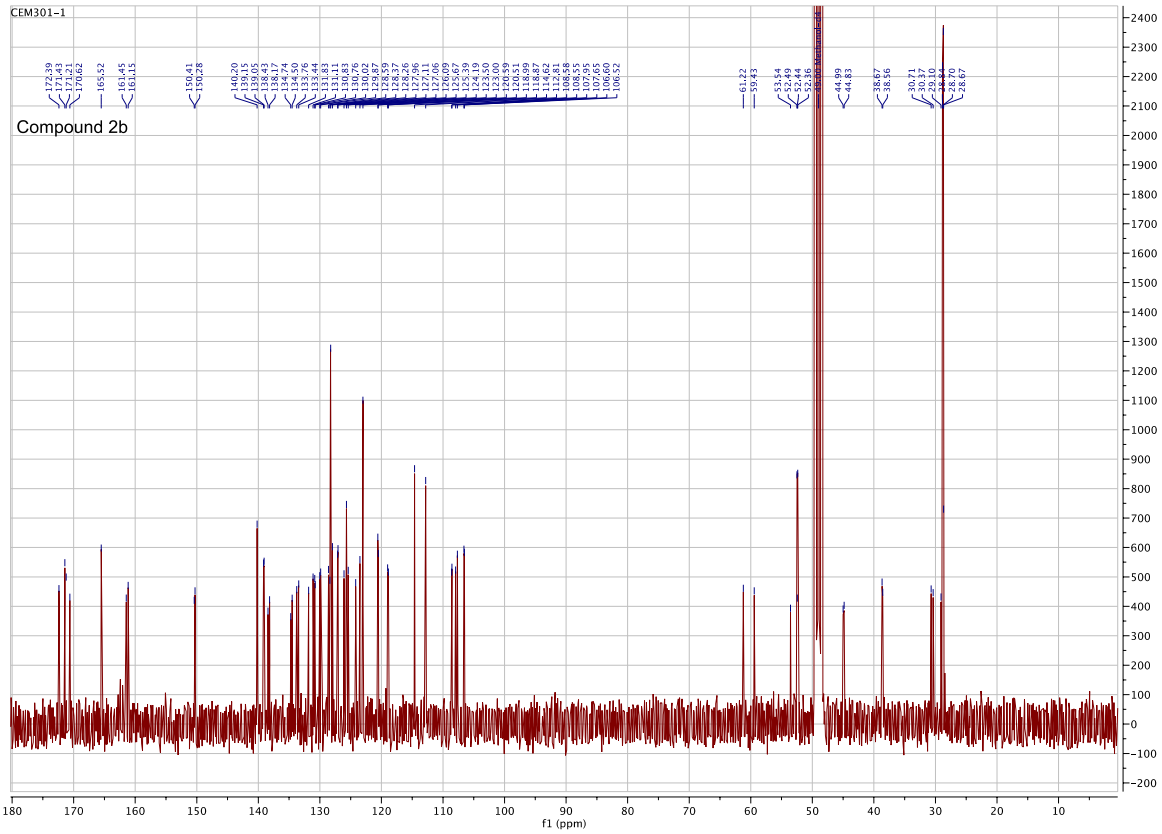


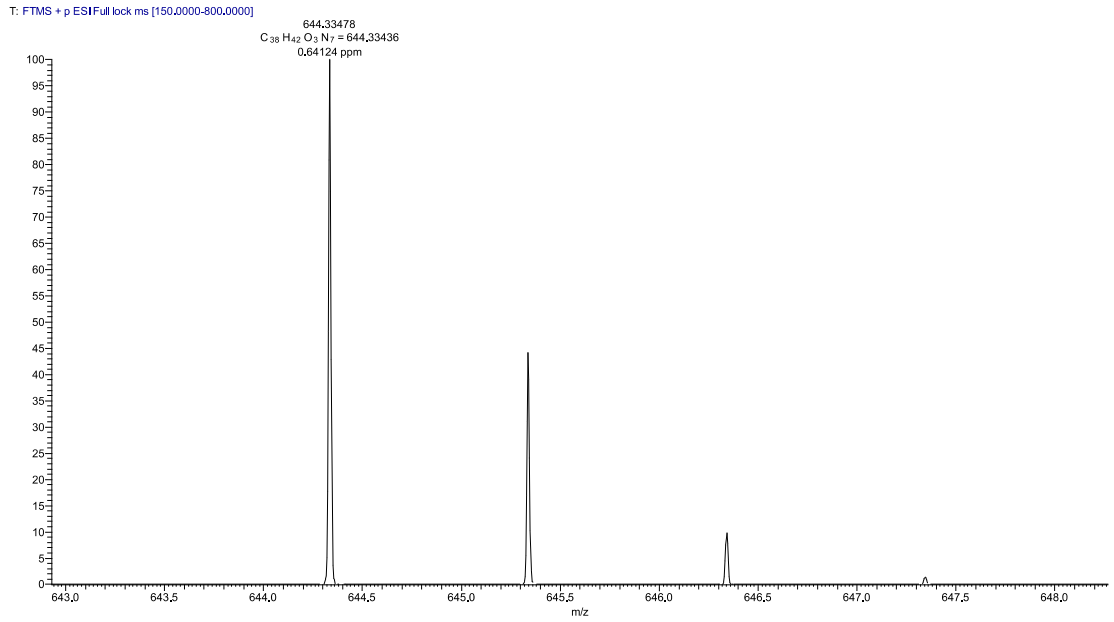


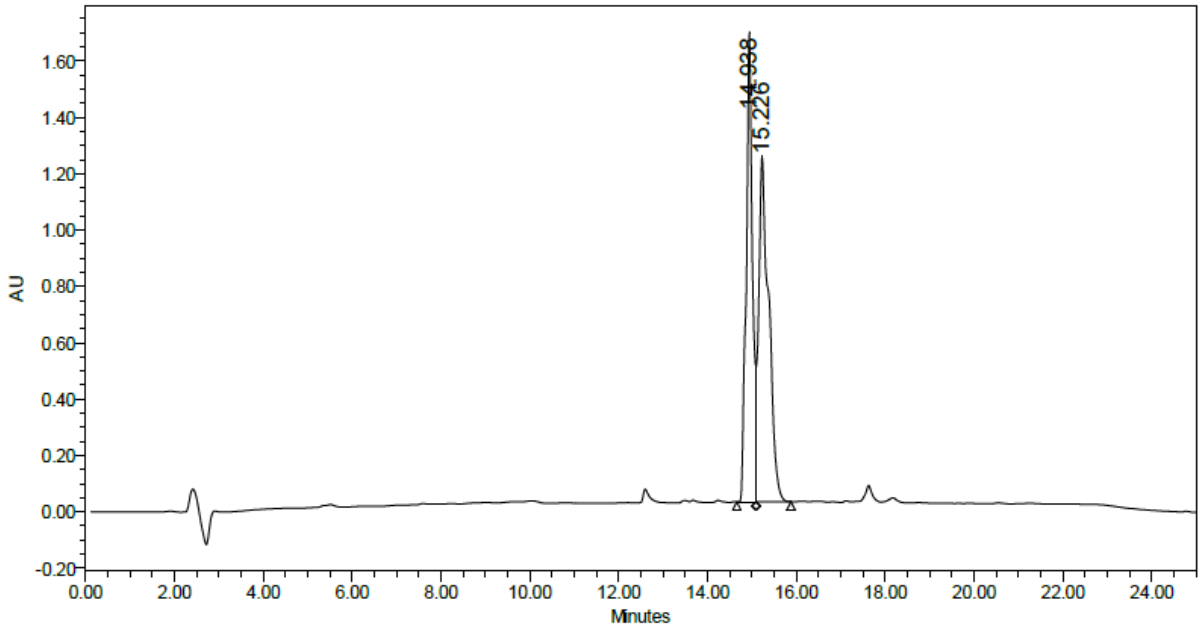
	RT	Area	% Area	Height
1	12.737	19708385	49.32	2533369
2	12.944	20254084	50.68	2661662

Figure S17. ^{1}H NMR, ^{13}C NMR, HRMS spectra and HPLC profile of compound **2b**.



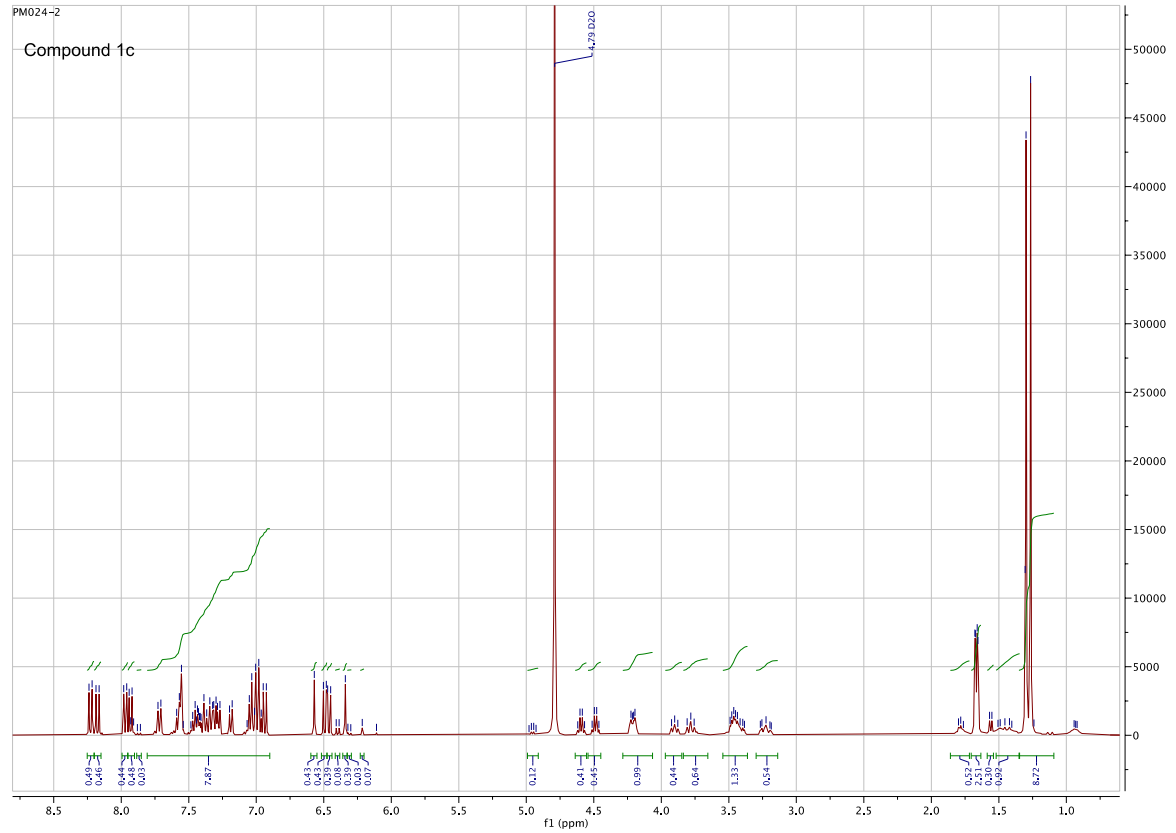


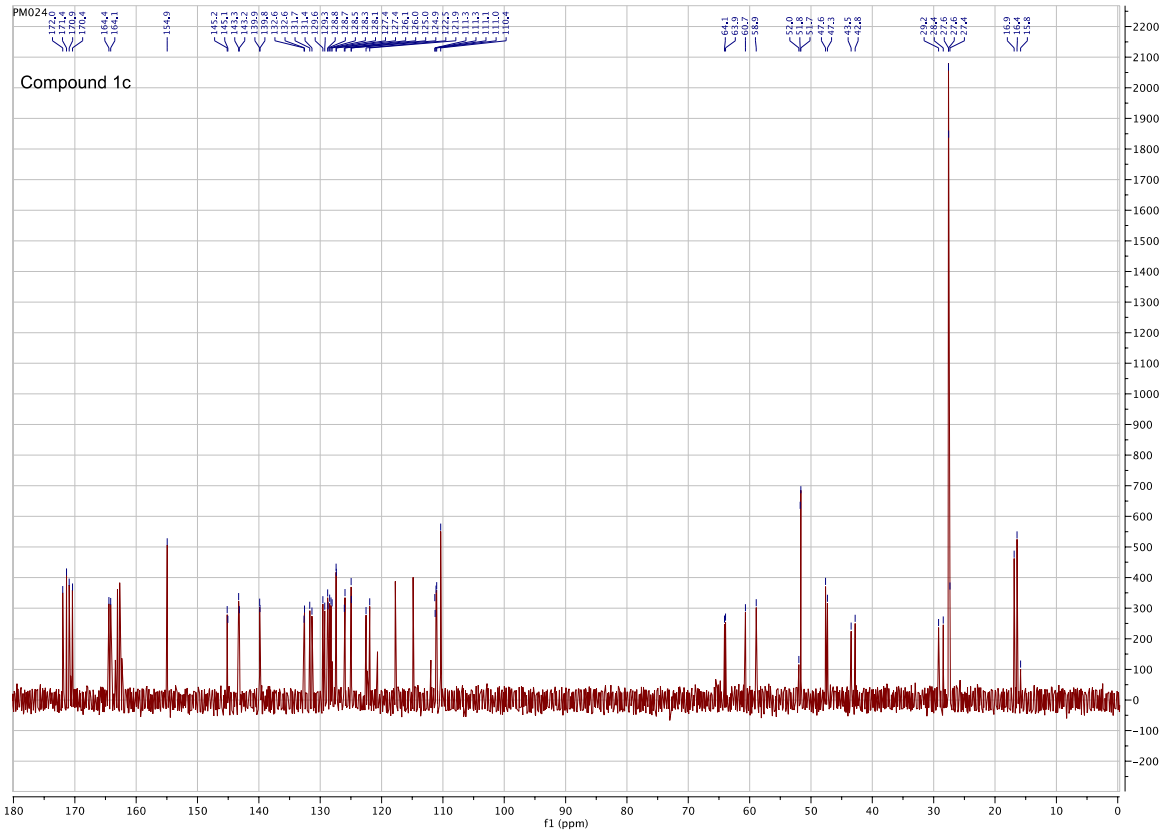


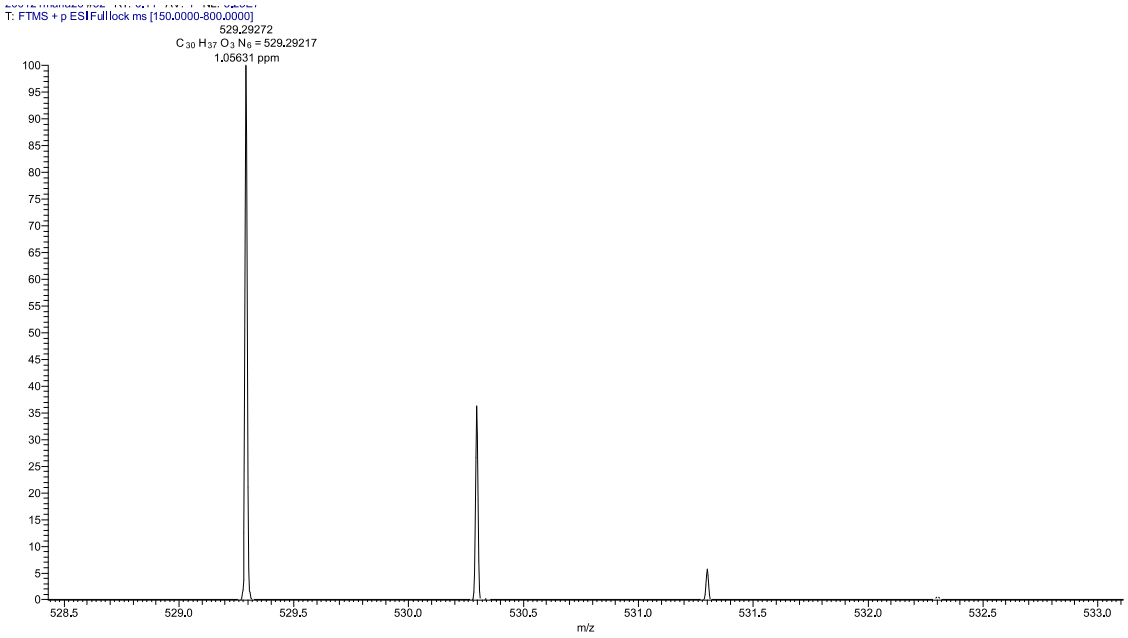


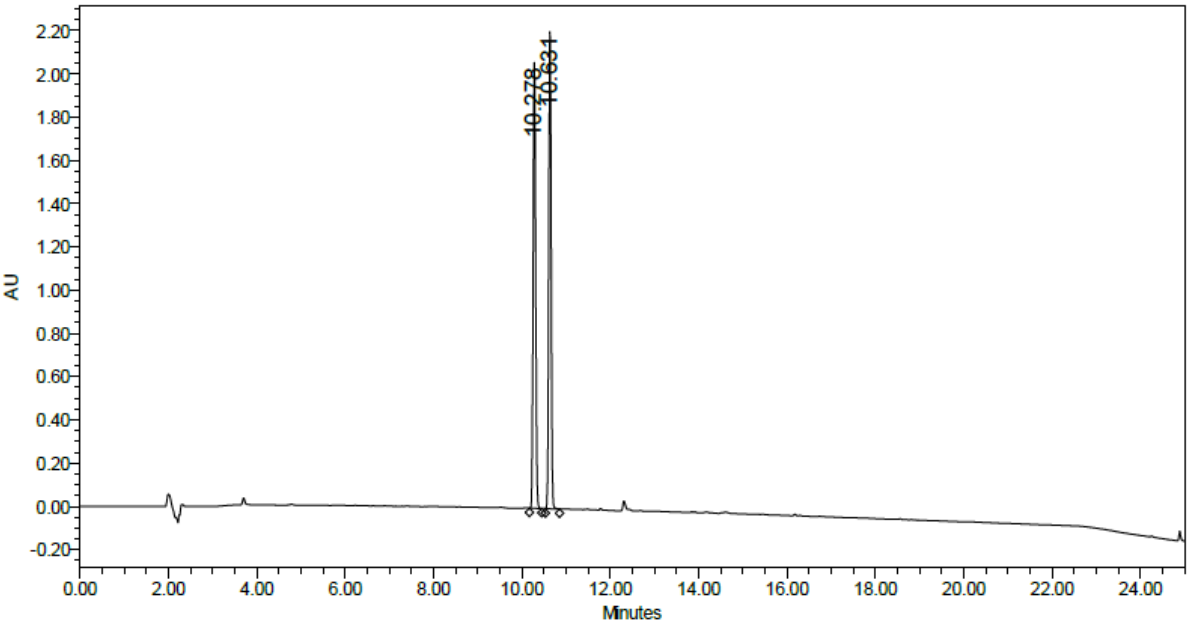
	RT	Area	% Area	Height
1	14.938	16247237	45.53	1658911
2	15.226	19438285	54.47	1226552

Figure S18. ^{1}H NMR, ^{13}C NMR, HRMS spectra and HPLC profile of compound **1c**.



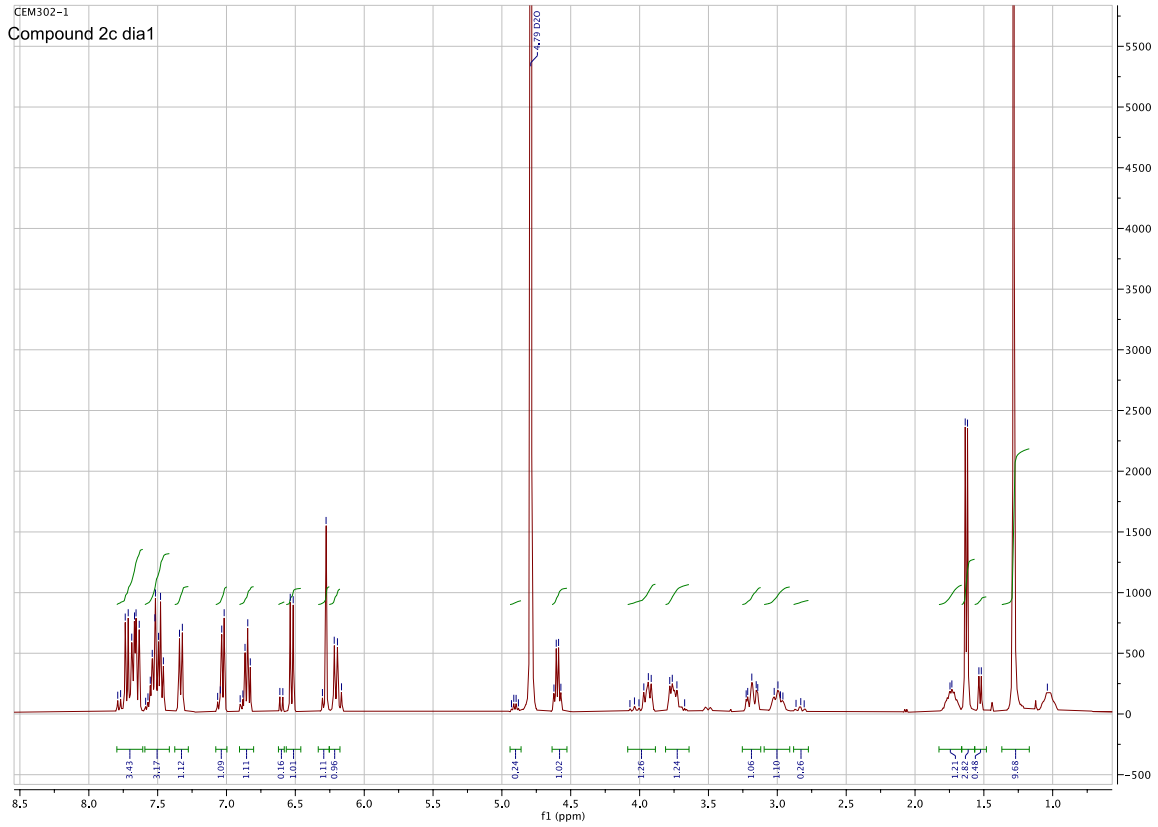


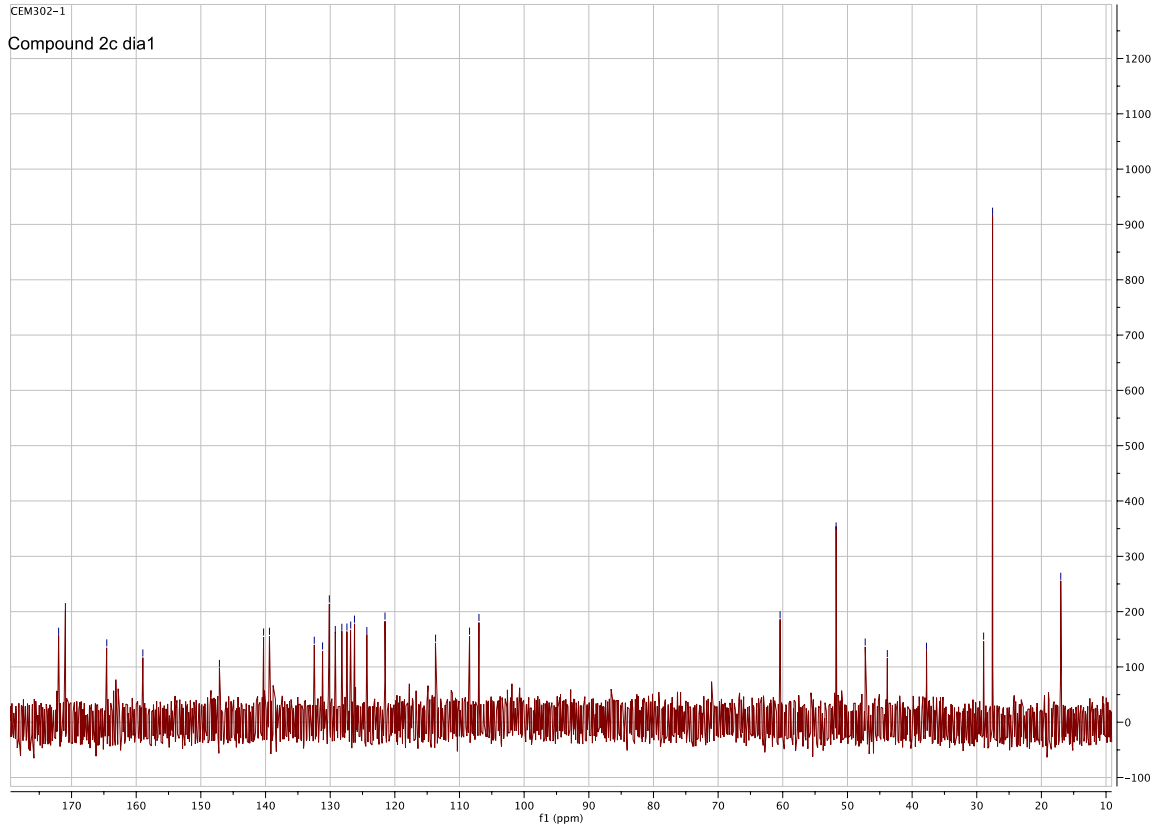


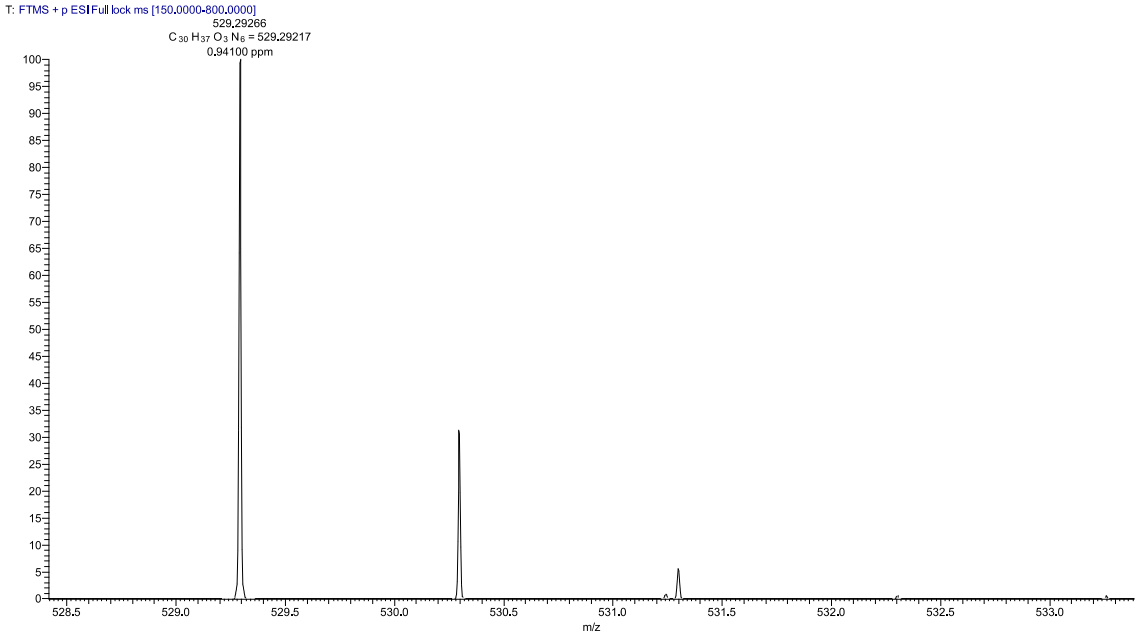


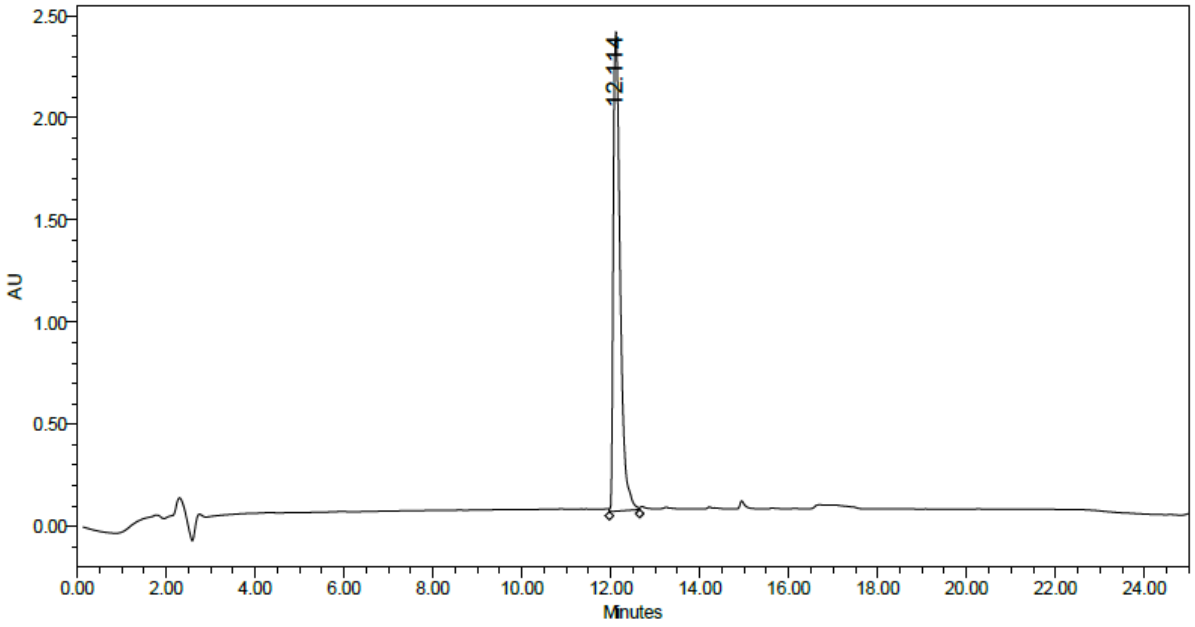
	RT	Area	% Area	Height
1	10.278	8120911	49.61	2062198
2	10.631	8247768	50.39	2205368

Figure S19. ^{1}H NMR, ^{13}C NMR, HRMS spectra and HPLC profile of compound **2c(dia1)**.



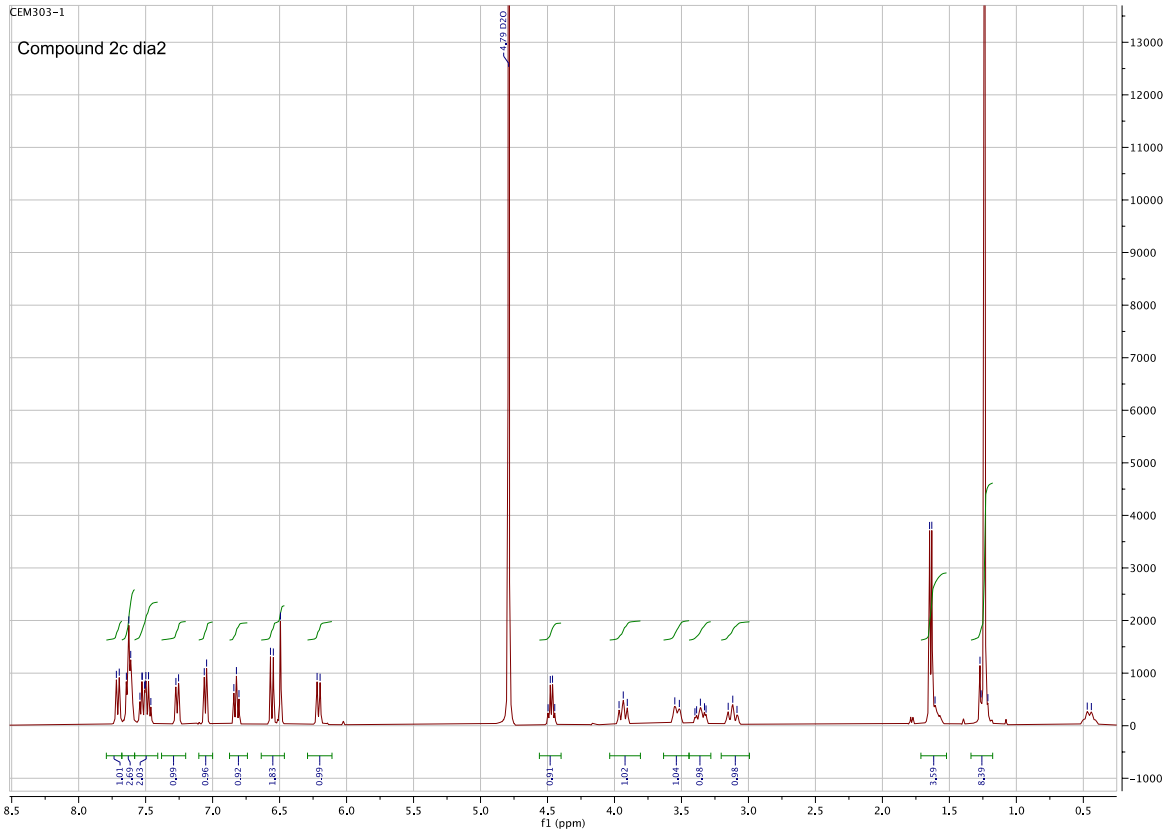


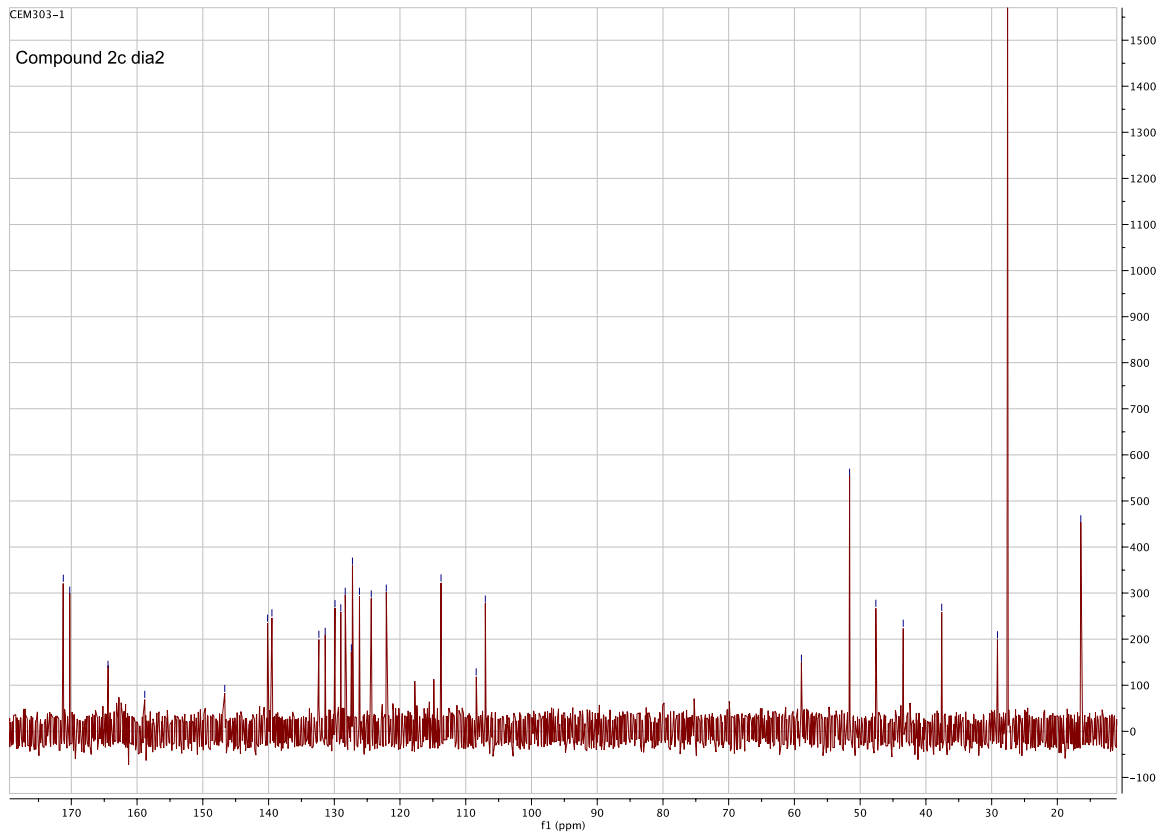


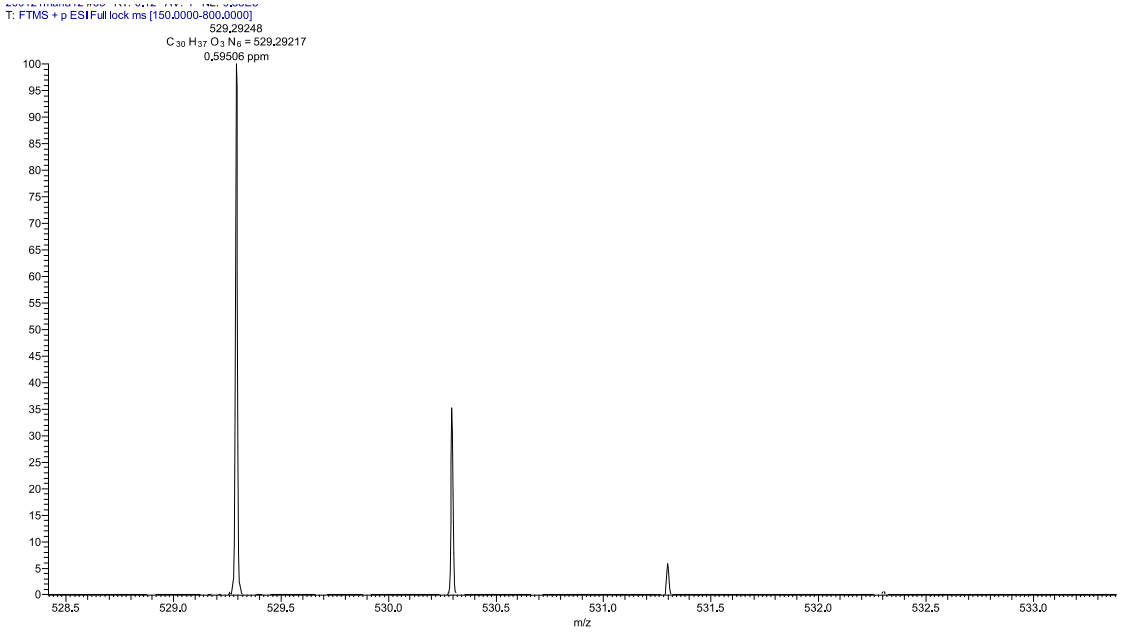


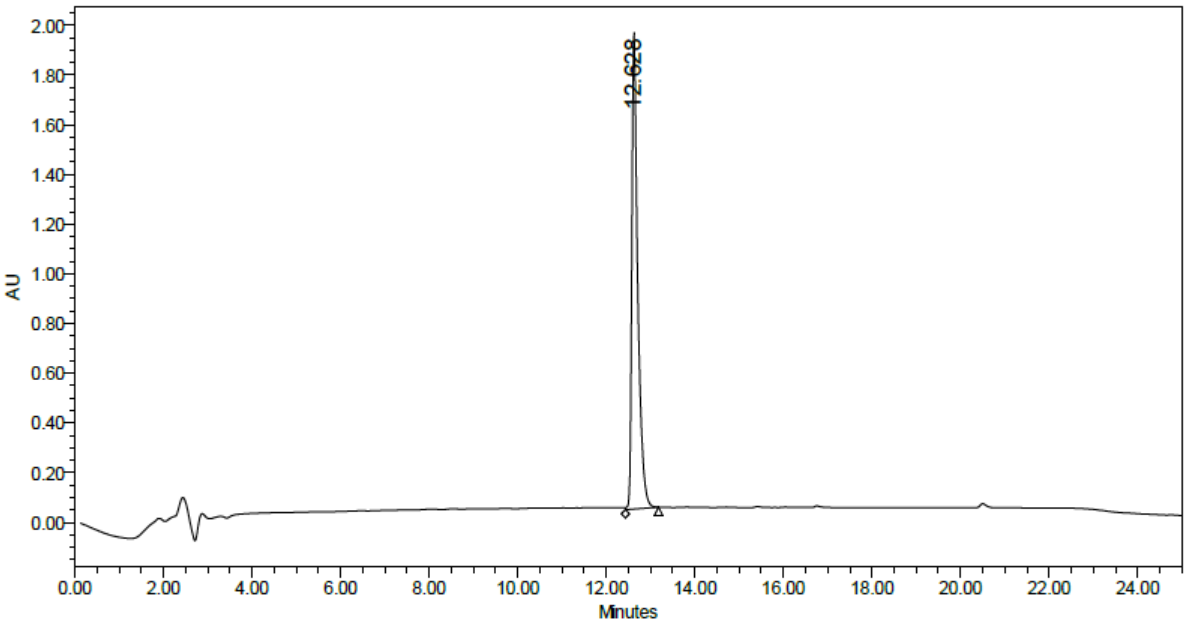
	RT	Area	% Area	Height
1	12.114	25201239	100.00	2348480

Figure S20. ^{1}H NMR, ^{13}C NMR, HRMS spectra and HPLC profile of compound **2c(dia2)**.



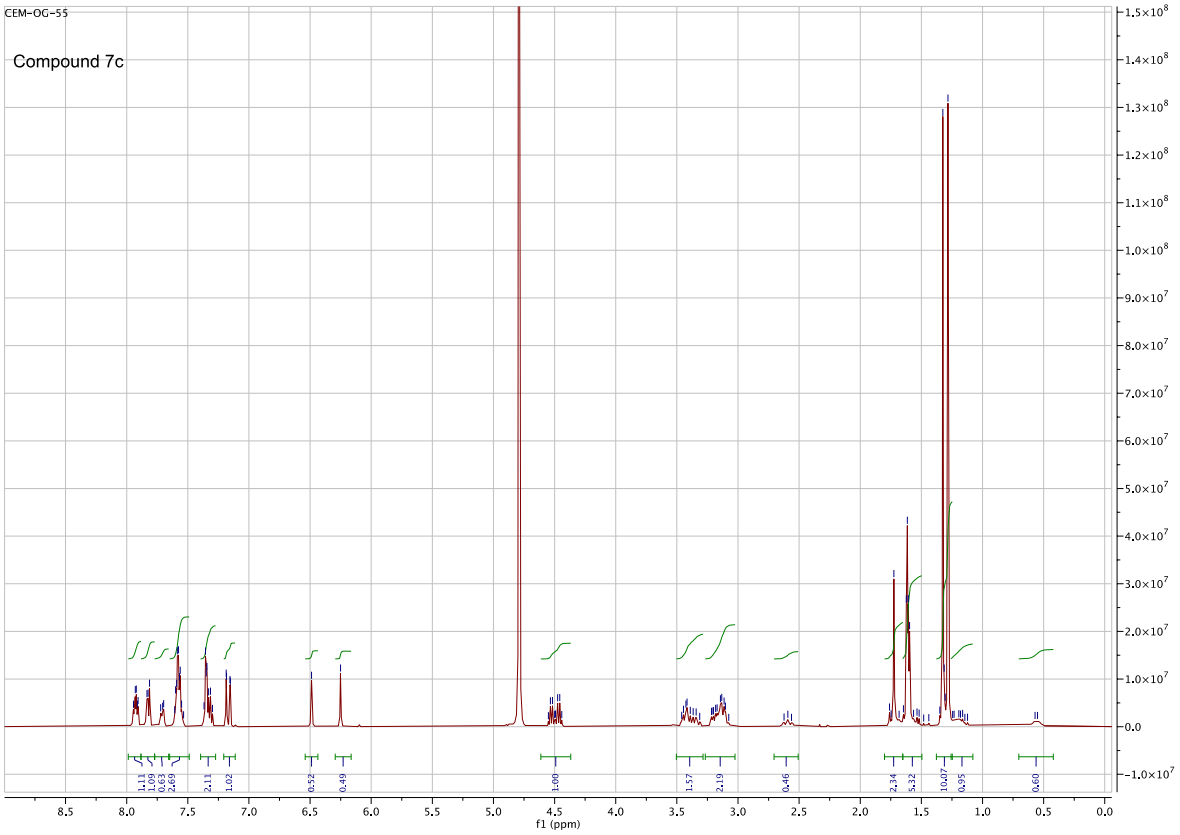


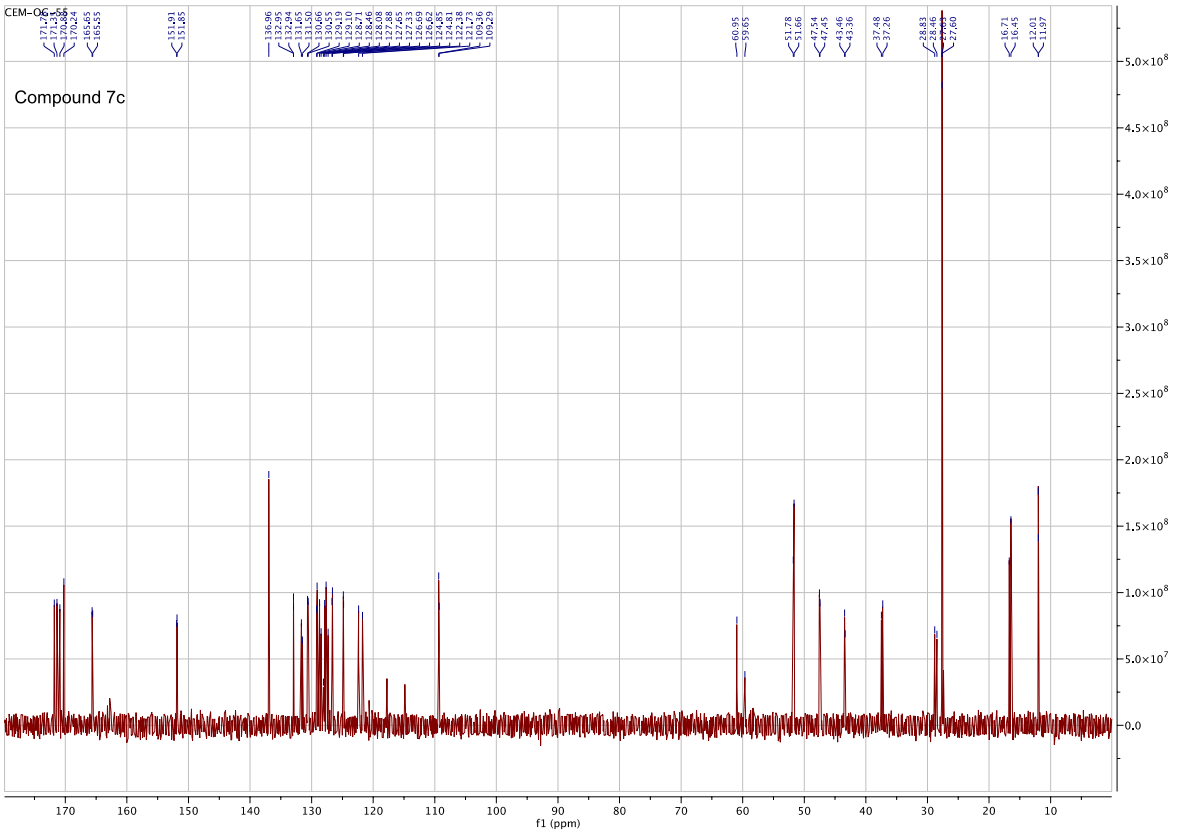


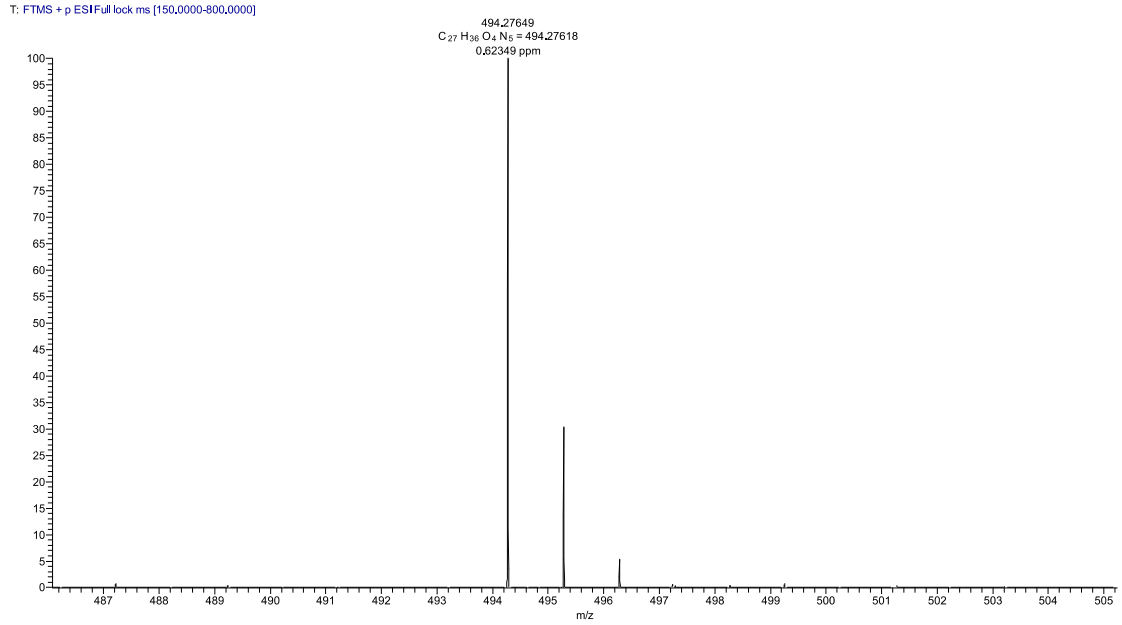


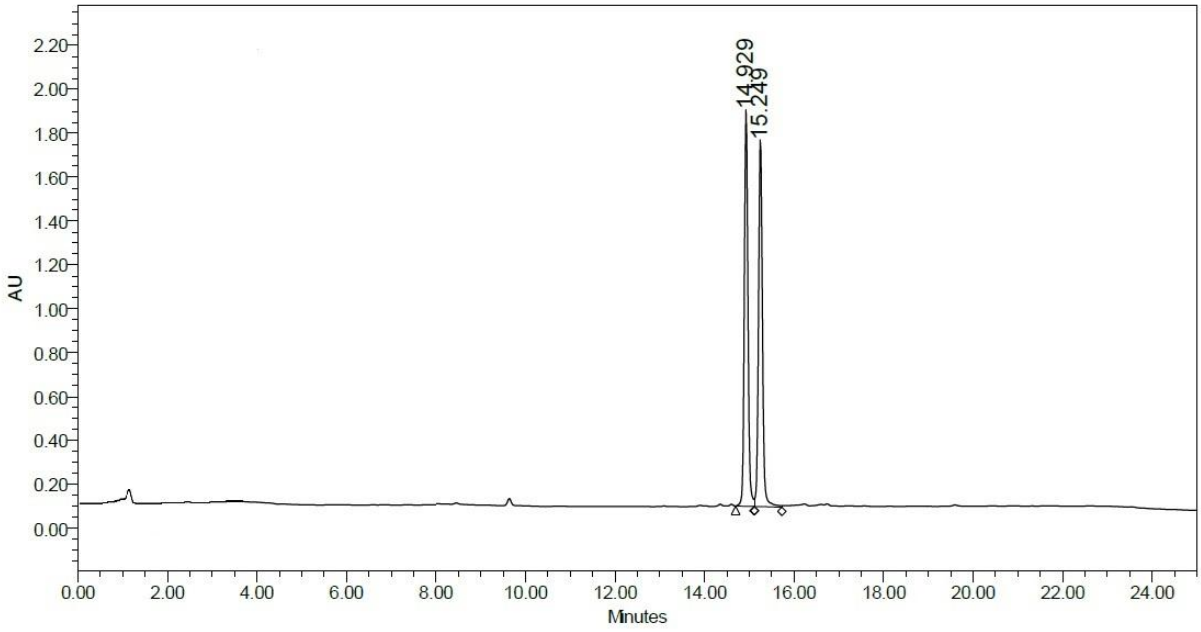
	RT	Area	% Area	Height
1	12.628	17729421	100.00	1913154

Figure S21. ^{1}H NMR, ^{13}C NMR, HRMS spectra and HPLC profile of compound **7c**.



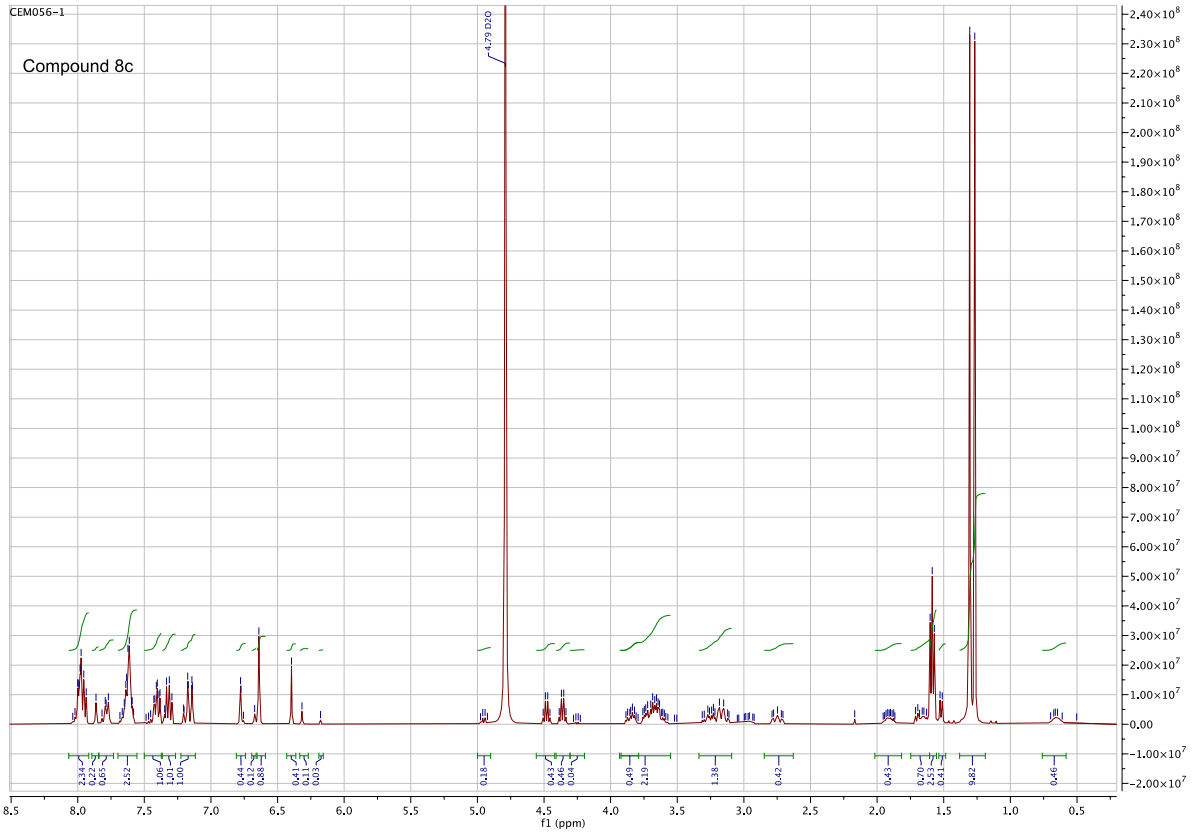


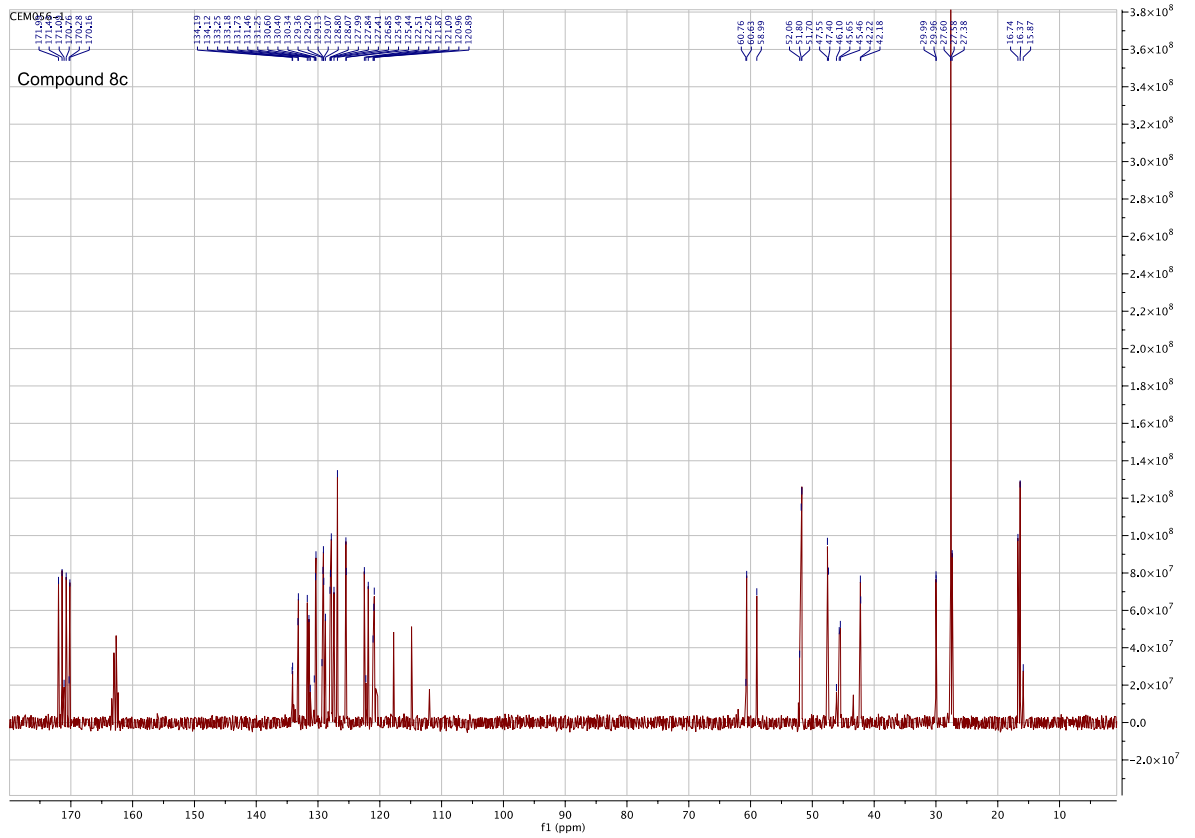


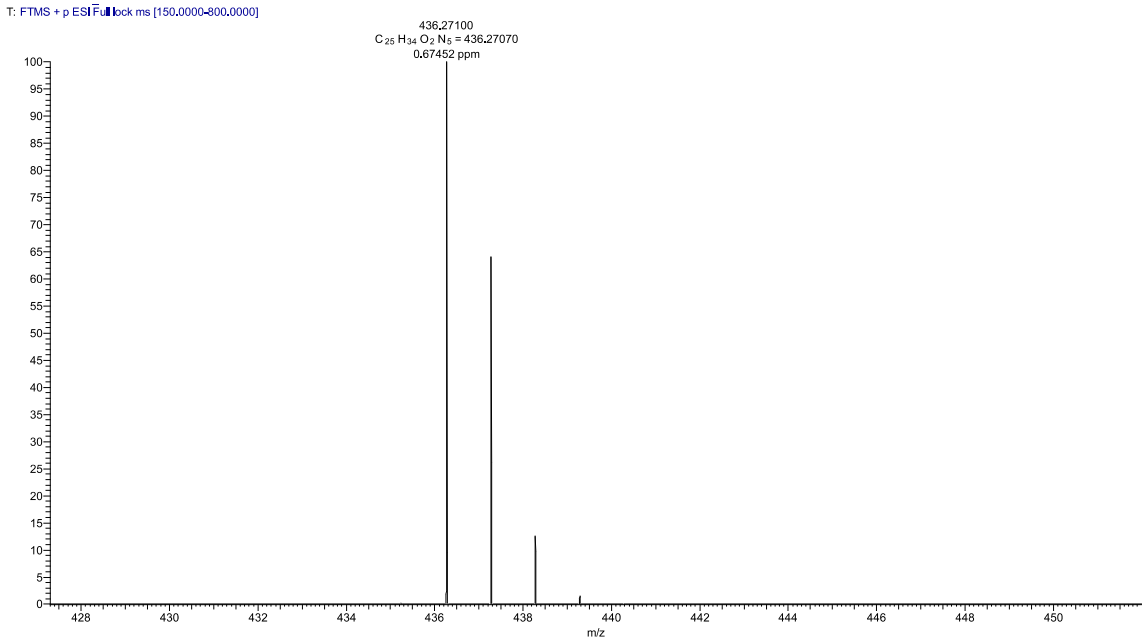


	RT	Area	% Area	Height
1	14.929	10181781	49.99	1790122
2	15.249	10186772	50.01	1669164

Figure S22. ^{1}H NMR, ^{13}C NMR, HRMS spectra and HPLC profile of compound **8c**.






T: FTMS + p ESI Full lock ms [150.0000-800.0000]
436.27100
C 25 H 34 O 2 N 5 = 436.27070
0.67452 ppm
m/z

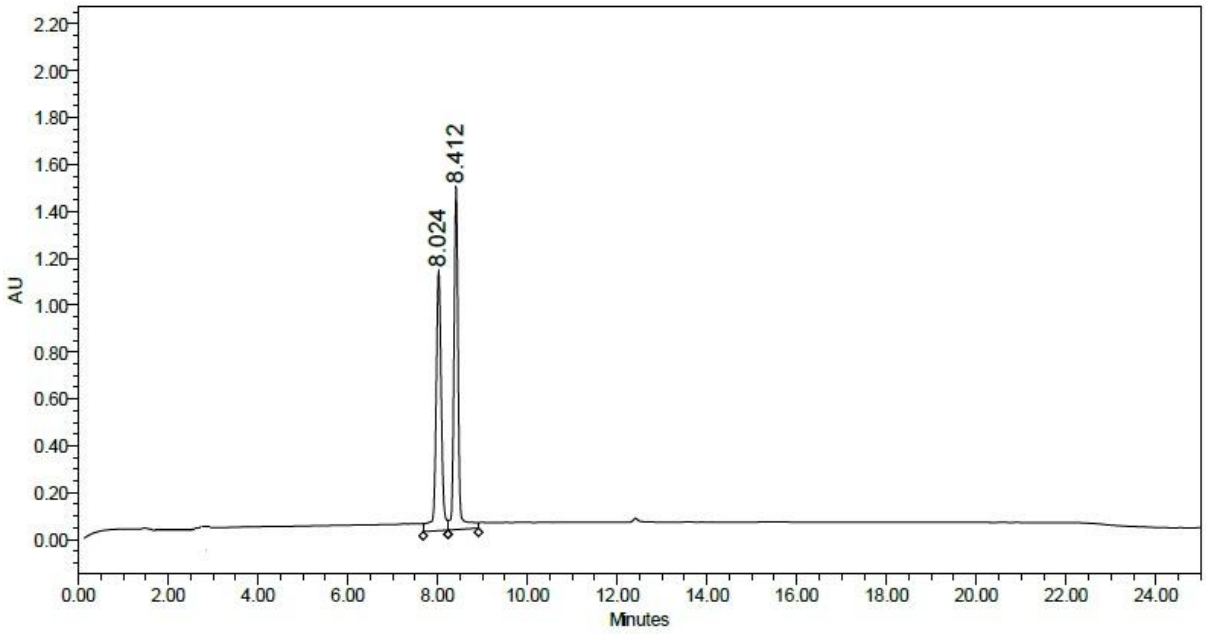
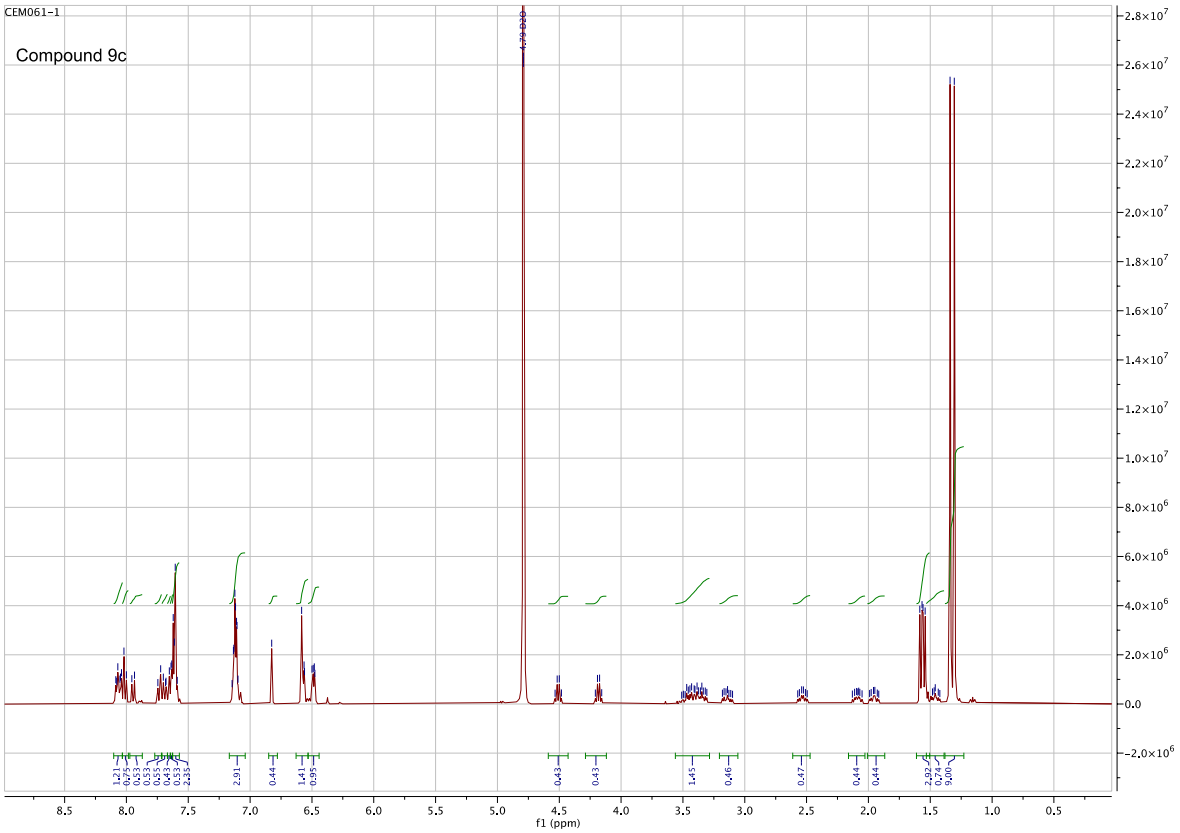
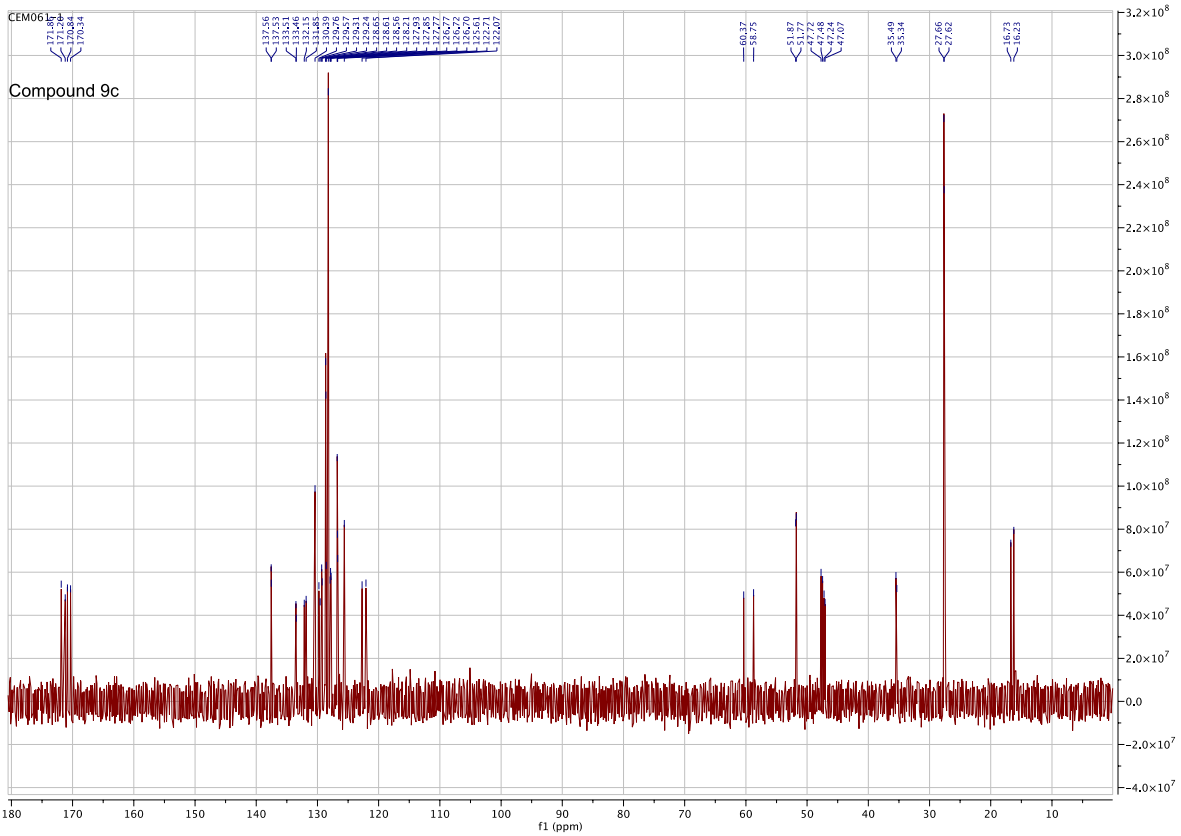
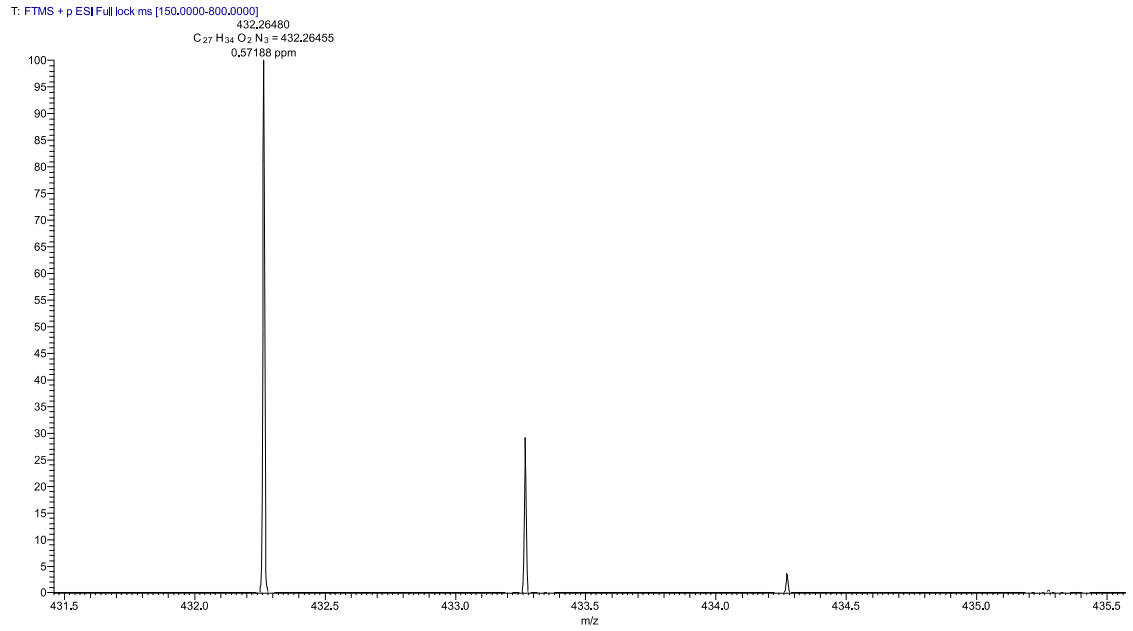

8.024
8.412
AU
Minutes

Figure S23. ^{1}H NMR, ^{13}C NMR, HRMS spectra and HPLC profile of compound **9c**.






T: FTMS + p ESI Full lock ms [150.0000-800.0000]
432.26480
C27 H34 O2 N3 = 432.26455
0.57188 ppm
m/z

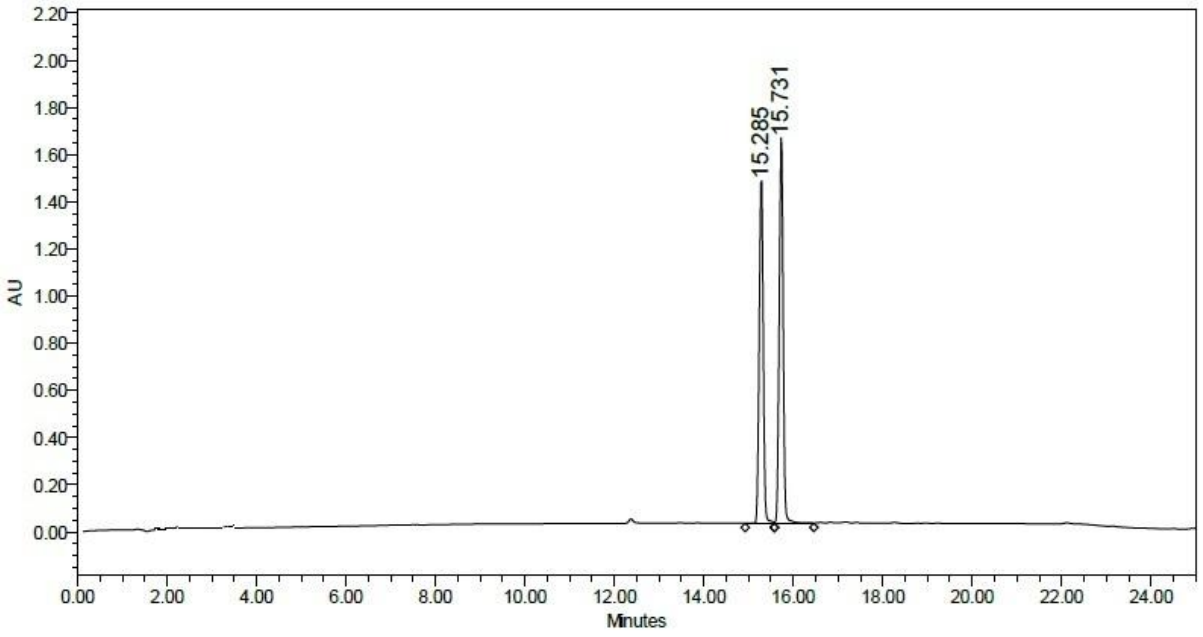
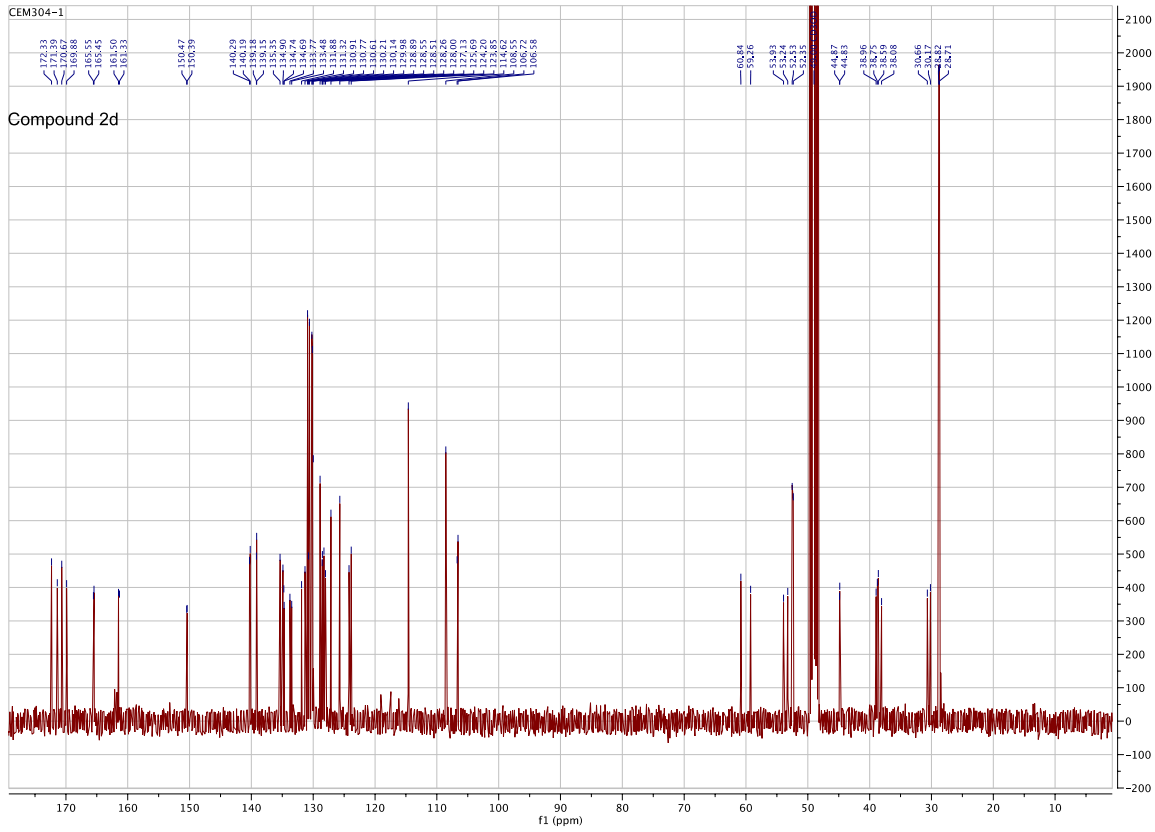
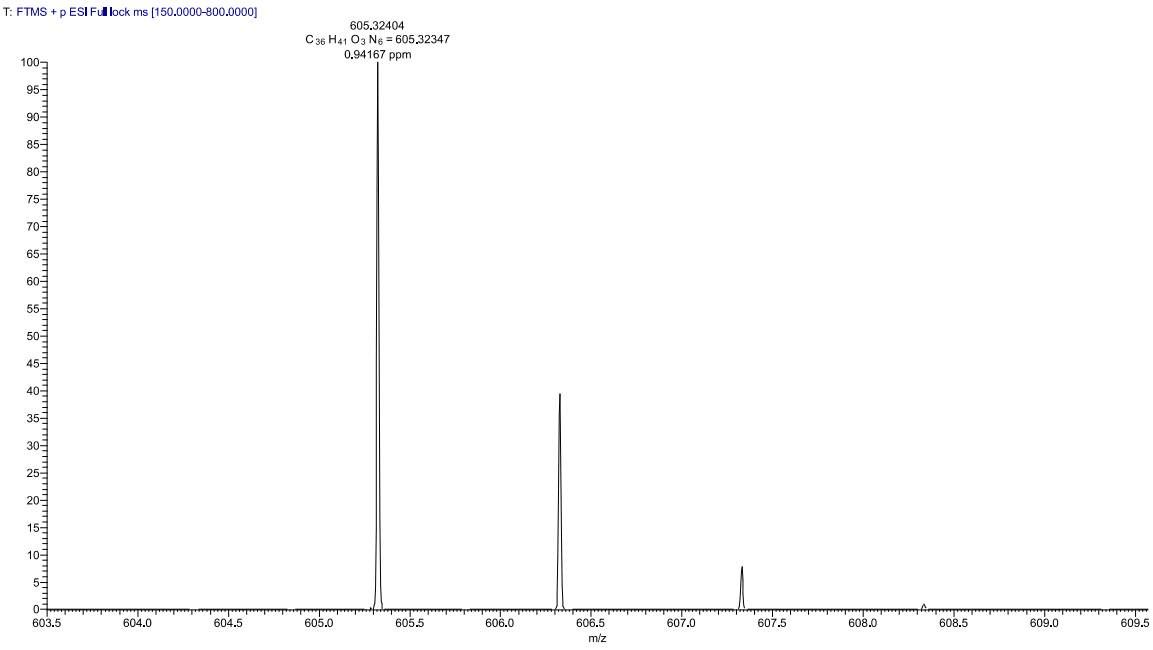
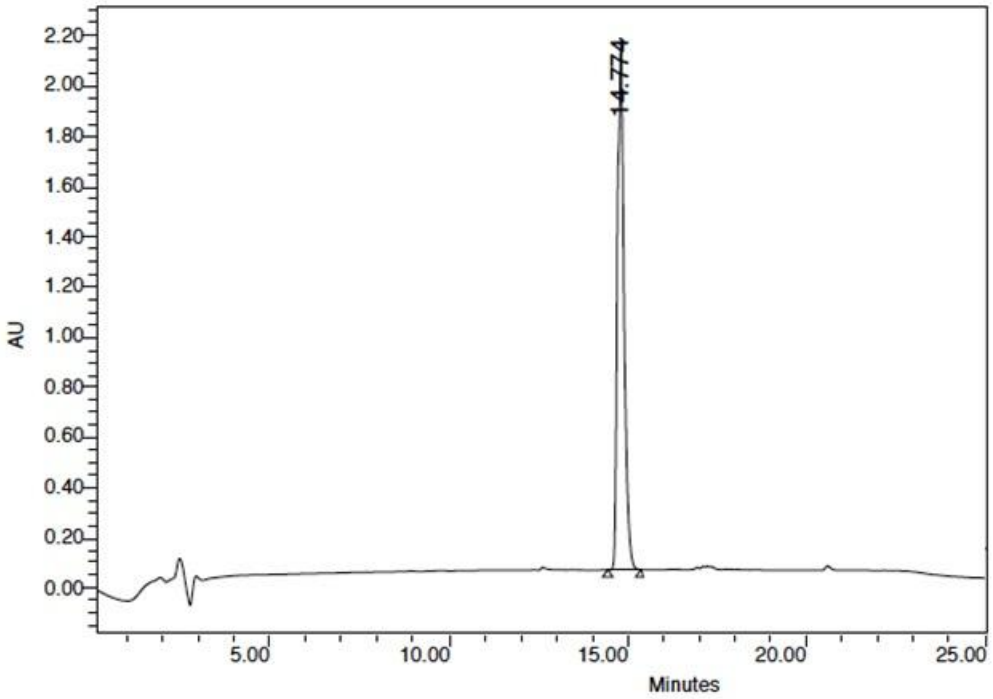

15.285
15.731
AU
Minutes

Figure S24. ^{1}H NMR, ^{13}C NMR, HRMS spectra and HPLC profile of compound **2d**.







	RT	Area	% Area	Height
1	14.774	27393654	100.00	2115482

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