Supplementary Information

De novo design of protein kinase inhibitors by *in silico* identification of hinge region-binding fragments

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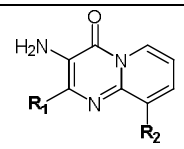
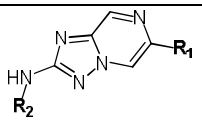
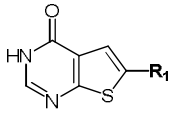
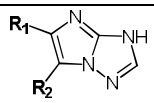
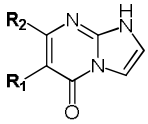
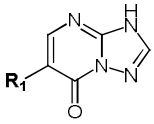
Table S 1: Names and PDB codes of protein kinases which were available for compound profiling in the MRC Protein Phosphorylation Unit in Dundee and for which a crystal structure was available in the public domain at the time of the study.

Name	PDB-Code
ABL	3cs9
Aurora A	1mq4
Aurora B	2vgp
BTK	1k2p
CAMK1	2jam
CAMKKβ	3bhh
CDK2	2w05
CHK1	1zys
CHK2	2cn5
CK1	1csn
CK2	1lp4
CSK	1byg
DYRK1A	2vx3
EPH-A2	1mqb
EPH-B4	2vwx
ERK2	2ojg

Name	PDB-Code
FGF-R1	1agw
GSK3β	1q5k
IGF-1R	2oj9
JNK1	1uki
JNK2	3e7o
Lck	1qpc
MAPKAP-K2	1nxk
MARK3	2qnj
MKK1	1s9j
MNK1	2hw6
MNK2	2ac3
NEK2a	2w5a
p38α MAPK	1yqj
p38δ MAPK	3coi
p38γ MAPK	1cm8
PAK4	2cdz

Name	PDB-Code
PAK5	2f57
PAK6	2c30
PDK1	1uu3
PIM1	1xws
PKBα	3cqw
PKBβ	1o6l
PLK1	2rku
ROCK 2	2f2u
RSK1	2z7r
RSK2	2qr8
SGK1	2r5t
SRPK1	1wbp
SYK	1xbb
VEG-FR	1ywn

Table S 2: Enumeration of core fragments **A-F** using commercially available building blocks.

Core fragment		Number of chosen building blocks for R1 substitution	Number of chosen building blocks for R2 substitution	R_1 and R_2 variation	Number of successfully synthesized compounds
A		50	20	respectively	64
B		8	12	simultaneously	56
C		20	-	N/A*	17
D		17	2	simultaneously	17
E		20	5	respectively	24
F		20	-	N/A	13

* Not applicable

Table S 3: Percent inhibition data for 15 compounds tested at 100 μM against a panel of 117 protein kinases. The kinases are ranked by the number of hits per kinase with the most frequently inhibited kinase at the top of the table. The compounds are sorted from left to right by decreasing number of inhibited kinases. Percent inhibition values ≥ 75% are marked green, between 40 and 75% yellow and below 40% red.

Kinase	Compound														
	B1	A2	C1	C2	B3	F3	A1	B2	D2	E2	D1	F1	B4	E1	F2
CLK2	74	91	46	50	79	48	57	33	43	1	34	0	28	0	3
GSK3b	72	51	70	62	73	44	64	27	38	2	24	9	8	0	13
RSK2	51	52	39	52	9	25	84	42	48	47	13	10	23	11	20
FGF-R1	79	88	57	32	45	41	34	42	40	25	65	14	3	0	6
MAPKAP-K3	40	54	61	56	2	15	70	33	38	37	10	33	74	0	21
BTK	67	67	46	29	28	49	1	44	51	25	6	14	0	17	16
CAMK1	41	60	75	66	17	9	35	52	83	0	22	11	35	6	6
VEG-FR	82	72	74	39	69	39	54	22	0	38	12	31	9	0	28
MINK1	68	75	40	60	58	28	2	45	3	16	0	25	0	0	0
PKBb	32	68	47	47	22	16	24	37	39	41	31	14	43	15	31
ERK8	74	36	72	18	88	18	0	25	1	8	60	7	7	4	3
JAK2	76	89	75	60	25	25	35	16	11	23	3	9	9	0	0
DYRK3	76	12	47	36	69	30	49	30	17	20	7	18	12	1	10
HIPK2	57	18	38	58	49	61	0	8	18	1	22	7	18	0	0
PIM3	55	35	51	61	30	83	1	26	18	0	0	0	1	0	0
HER4	83	65	33	17	19	48	28	28	53	31	19	25	9	21	34
YES1	89	57	0	0	35	33	57	42	4	30	0	11	17	0	0
CK2	55	94	41	31	11	20	97	17	5	28	9	11	0	0	3
TrkA	61	88	41	15	28	50	37	8	6	0	27	39	0	0	0
RIPK2	56	3	12	65	29	57	28	43	13	0	0	16	5	0	0
DYRK1A	77	55	52	36	43	31	36	19	30	10	0	0	0	0	5
TAK1	86	80	78	45	30	37	0	3	31	2	3	26	0	15	20
EPH-B3	54	42	6	0	32	0	0	35	90	8	68	5	4	0	0
SRPK1	41	58	19	36	25	0	74	31	3	42	0	14	10	0	9
PHK	83	38	9	13	68	8	3	41	10	30	6	44	0	28	24
PAK4	89	50	34	30	47	11	0	0	0	15	0	20	0	3	18
IRR	71	51	0	0	60	6	0	10	0	0	1	0	8	0	0
GCK	93	95	7	0	39	29	0	59	0	0	8	6	0	0	0
IR	97	88	0	0	60	6	1	24	4	0	0	11	0	0	0
DYRK2	63	20	53	56	30	37	40	21	6	27	4	19	0	27	13
Aurora B	76	81	6	19	61	0	32	29	12	0	34	0	0	0	0
BRK	53	67	50	16	1	30	21	15	21	9	25	30	0	19	31
MLK3	89	63	23	8	17	7	0	0	0	0	0	40	0	0	11
MKK1	37	72	31	23	34	43	7	22	0	11	0	24	0	20	57
ABL	72	40	15	34	48	3	2	14	11	5	0	4	18	3	0
MLK1	81	79	0	4	43	0	0	18	14	0	0	2	0	0	0
PRK2	44	73	0	0	13	2	0	93	16	1	17	0	0	0	0
MARK4	44	62	0	23	0	0	56	24	22	26	15	13	17	0	21
PRAK	34	83	0	5	6	13	75	26	44	3	13	17	17	0	0
MNK1	71	1	0	5	53	31	0	24	0	3	0	0	0	0	0
IGF-1R	91	85	0	0	11	28	0	5	0	0	0	0	0	0	0
ROCK 2	52	76	39	19	25	29	17	19	0	7	2	11	0	0	13
ASK1	32	86	54	19	25	8	4	15	2	16	4	0	0	9	0
RSK1	34	21	47	59	24	16	0	21	3	19	3	23	0	15	17
Aurora A	52	89	21	1	13	17	2	12	5	23	16	6	10	0	8
PKD1	6	57	53	25	13	15	26	13	8	10	11	4	0	0	0
Src	48	35	33	0	18	11	73	29	12	38	0	8	0	0	0
MARK3	59	86	29	24	38	11	25	0	13	17	0	12	0	5	9
CDK2	54	88	10	0	34	21	8	17	14	0	33	0	0	0	0
NUAK1	73	76	0	0	33	6	3	28	20	0	24	0	0	0	0
PIM1	30	31	61	38	13	67	7	18	30	0	11	0	9	0	0
NEK6	1	0	44	60	21	7	0	3	0	3	0	15	0	0	6
MST4	41	77	12	16	16	13	2	21	0	0	0	7	0	0	0
IRAK4	80	31	28	9	39	40	3	18	0	0	0	19	0	0	6
Lck	75	39	40	29	29	23	2	37	5	0	0	23	0	18	0
PAK2	31	41	0	0	0	0	3	56	14	17	10	12	0	0	5
CAMKKb	54	0	0	0	41	0	0	21	28	0	34	0	18	0	0
BRSK1	27	22	38	45	23	44	12	14	0	10	0	14	0	2	2
PAK5	79	35	0	0	20	0	0	13	0	0	18	3	5	0	0

Kinase	Compound														
	B1	A2	C1	C2	B3	F3	A1	B2	D2	E2	D1	F1	B4	E1	F2
CHK1	45	0	0	0	18	4	0	9	0	0	0	2	0	0	0
MST2	6	64	27	9	0	27	0	9	0	0	0	21	0	0	3
SGK1	34	0	14	63	16	18	0	19	0	0	0	1	0	0	0
EPH-B1	48	0	0	4	22	20	0	22	0	1	0	17	0	1	3
ERK2	47	13	1	0	7	8	12	14	0	13	0	10	0	4	0
TAO1	32	82	3	28	0	3	0	12	0	0	0	0	1	0	0
LKB1	2	62	0	0	0	6	0	0	0	0	0	0	0	0	0
JNK3	47	33	7	3	11	0	9	17	0	0	1	0	0	0	0
MARK1	0	49	31	27	6	5	26	15	0	32	0	16	0	0	13
CSK	71	11	10	14	29	8	0	24	0	20	0	23	0	8	26
MEKK1	34	21	0	0	9	53	0	17	0	0	0	3	0	6	0
MNK2	66	6	30	21	35	26	0	30	0	0	9	25	0	10	13
CK1	73	6	4	17	28	24	28	26	0	0	0	2	0	0	0
MSK1	19	33	24	22	11	21	0	6	0	28	0	4	0	52	0
MAPKAP-K2	56	20	15	8	7	22	0	14	2	26	0	29	0	17	23
NEK2a	77	0	2	0	25	29	0	38	6	0	0	6	0	0	4
IKKb	27	66	37	29	10	15	22	0	9	0	11	0	0	0	0
TBK1	72	23	17	3	31	6	0	21	9	7	1	14	15	24	0
MKK2	35	71	8	0	6	18	0	28	11	0	0	0	0	0	0
SYK	84	30	0	0	31	20	13	26	12	9	7	22	10	0	12
EPH-B4	56	39	0	0	11	6	12	14	13	1	19	5	27	6	9
MARK2	2	53	0	0	18	0	13	0	13	0	0	0	0	0	0
AMPK	7	40	50	0	14	0	2	12	18	0	5	0	0	0	0
CHK2	81	25	34	38	25	19	0	13	19	14	0	32	0	0	20
PKA	9	8	42	34	0	0	15	19	0	0	5	0	1	0	0
TIE2	39	42	0	0	11	0	5	6	0	0	9	0	0	0	0
JNK1	30	41	8	8	2	0	14	7	2	2	0	0	0	0	0
EPH-A4	42	25	0	12	19	16	16	38	4	22	9	16	10	11	15
TTK	34	45	0	25	4	11	3	20	7	0	10	0	0	0	0
PDK1	35	20	16	41	15	2	31	15	13	29	3	14	0	1	0
MELK	13	43	25	22	17	1	7	8	16	0	0	2	0	0	0
BRSK2	28	43	30	9	20	24	32	18	22	34	26	27	11	22	18
PKCz	13	0	0	0	11	0	0	6	0	0	0	3	0	0	0
EPH-A2	28	33	37	37	0	9	0	11	0	0	0	31	0	0	12
p38g MAPK	1	17	0	0	24	6	1	0	0	0	0	0	0	0	0
MPSK1	7	3	4	12	0	15	6	6	0	3	0	21	0	10	0
p38d MAPK	18	0	0	0	1	0	0	15	0	0	0	0	19	0	0
ZAP70	0	1	0	0	16	0	38	0	0	23	1	9	9	0	0
STK33	20	4	0	0	5	12	8	12	0	15	0	21	0	0	13
IKKe	39	13	36	32	23	22	0	28	0	0	0	3	0	0	0
HIPK1	28	11	10	13	16	30	0	16	0	0	6	24	0	0	0
PLK1	18	0	0	0	4	8	0	8	0	8	0	10	0	0	0
S6K1	9	0	34	30	5	8	0	8	0	0	0	4	0	0	9
PKCa	2	36	21	18	0	2	15	9	0	23	0	2	0	0	2
EF2K	36	16	39	2	13	7	35	13	0	10	18	11	7	6	6
EPH-B2	30	29	27	17	4	0	20	0	1	22	0	14	0	6	3
MKK6	3	10	2	18	0	0	1	8	1	0	3	0	0	3	0
HIPK3	16	23	0	0	0	0	0	7	1	0	0	0	0	0	0
ERK1	31	23	19	26	13	3	0	31	1	0	0	10	10	13	7
p38b MAPK	34	13	15	15	22	23	0	20	3	0	0	23	0	2	7
p38a MAPK	27	0	0	0	10	21	0	35	5	0	0	2	0	0	0
PKCy	24	25	16	0	2	7	0	6	8	0	9	0	12	2	0
JNK2	26	17	0	8	1	10	6	0	10	5	3	11	0	3	7
DAPK1	31	0	0	0	12	5	0	0	14	5	0	16	0	0	0
PIM2	16	21	13	25	11	17	19	2	16	0	15	9	5	0	4
PAK6	36	10	0	0	18	3	4	11	20	0	0	6	0	0	0
PKBa	9	12	17	38	0	11	0	0	21	14	0	2	0	0	0
SmMLCK	19	25	8	0	0	13	0	12	24	0	14	36	0	0	0

Table S 4: Hit rates (number of hits*100 / number of assayed compounds) obtained in different screening exercises. The total number of compounds that were tested is given in brackets. "-" indicates that no data was available. Kinase names in brackets indicate different abbreviations used in different publications.

Kinase	Hit rate (%) (number of compounds tested)				
	This work (15)	Anastassiadis *et al.*(*30*) (178)	Davis *et al.*(*47*) (72)	Bamborough *et al.* (*29*) (577)	Posy *et al.*(*27*) (21851)
ABL1	20.0	11.8	50.0	1.9	4.7
AMPK	13.3	-	23.6	0.3	2.8
ASK1	13.3	2.8	2.8	0.0	0.2
Aurora A (AURKA)	13.3	14.0	22.2	2.6	2.9
Aurora B (AURKB)	20.0	18.0	34.7	6.4	4.9
BRK	20.0	12.4	22.2	4.5	2.7
BRSK1	13.3	12.9	9.7	-	0.3
BRSK2	6.7	11.2	11.1	1.2	0.9
BTK	40.0	12.9	23.6	0.7	7.2
CAMK1α	40.0	1.1	18.1	0.3	0.9
CAMKKb	13.3	8.4	26.4	1.7	2.1
CDK2	13.3	14.0	11.1	7.1	2.5
CHK1 (CHEK1)	6.7	9.6	16.7	-	1.2
CHK2 (CHEK2)	6.7	15.7	19.4	-	0.7
CK1	6.7	2.2	8.3	0.9	1.3
CK2α	26.7	5.1	15.3	8.1	4.5
CLK2	53.3	13.5	43.1	17.7	8.9
CSK	6.7	5.6	22.2	4.7	2.7
DAPK1	0.0	4.5	22.2	2.3	5.0
DYRK1A	26.7	7.3	31.9	-	5.3
DYRK2	26.7	6.2	20.8	-	3.6
DYRK3	26.7	2.8	-	-	-
EF2K	0.0	-	-	-	-
EPH-A2	0.0	6.2	27.8	1.2	2.6
EPH-A4	6.7	4.5	20.8	2.4	2.1
EPH-B1	6.7	5.6	33.3	11.1	3.8
EPH-B2	0.0	4.5	20.8	3.8	1.7
EPH-B3	26.7	2.2	9.7	2.8	1.2

EPH-B4	6.7	5.1	30.6	2.3	2.9
ERK1	0.0	1.1	1.4	2.4	0.1
ERK2	6.7	1.1	1.4	3.8	0.2
ERK8 (ERK7)	26.7	-	25.0	-	-
FGF-R1	53.3	9.0	31.9	1.7	3.0
GCK	20.0	14.6	-	-	4.0
GSK3β	46.7	15.7	22.2	-	3.9
HER4	26.7	7.3	27.8	2.6	1.7
HIPK1	0.0	0.0	29.2	-	4.3
HIPK2	26.7	1.7	33.3	-	3.2
HIPK3	0.0	1.7	30.6	-	3.3
IGF-1R	13.3	3.9	16.7	1.6	5.7
IKKβ	6.7	1.7	12.5	-	3.1
IKKε	0.0	6.7	25.0	-	1.3
IR (INSR)	20.0	5.1	22.2	1.4	5.4
IRAK4	13.3	6.7	26.4	-	-
IRR (INSRR)	20.0	5.6	19.4	1.6	6.7
JAK2	26.7	8.4	33.3	3.3	10.3
JNK1	6.7	1.1	22.2	9.4	1.9
JNK2	0.0	6.2	26.4	3.3	0.9
JNK3	6.7	0.0	30.6	21.3	4.0
Lck	13.3	16.9	62.5	7.8	10.9
LKB1	6.7	8.4	13.9	12.7	1.8
MAPKAP-K2	6.7	1.1	2.8	-	0.8
MAPKAP-K3	40.0	0.0	-	-	-
MARK1	6.7	8.4	15.3	1.4	0.7
MARK2	6.7	9.0	19.4	0.9	1.7
MARK3	13.3	7.3	16.7	-	0.9
MARK4	20.0	11.2	13.9	1.2	0.8
MEKK1	6.7	-	8.3	-	0.2
MELK	6.7	13.5	27.8	-	1.0
MINK1	40.0	14.0	44.4	-	5.1
MKK1 (MEK1)	20.0	2.2	37.5	-	4.5
MKK2 (MEK2)	6.7	3.4	34.7	-	4.6
MKK6 (MEK6)	0.0	2.2	9.7	-	0.3
MLK1	20.0	20.2	20.8	-	4.0

MLK3	20.0	20.2	20.8	0.5	2.4
MNK1 (MKNK1)	13.3	6.2	16.7	-	4.7
MNK2	6.7	10.1	31.9	14.4	5.0
MPSK1 (STK16)	0.0	7.9	29.2	11.3	9.4
MSK1	6.7	6.2	-	0.5	0.3
MST2	6.7	15.2	29.2	9.5	4.7
MST4	13.3	6.7	18.1	2.3	1.8
NEK2a	6.7	0.6	19.4	5.9	0.3
NEK6	13.3	0.0	9.7	3.1	1.7
NUAK1 (ARK5)	13.3	24.7	22.2	-	6.1
p38α MAPK	0.0	6.2	13.9	2.9	3.9
p38β MAPK	0.0	3.4	15.3	4.9	3.0
p38δ MAPK	0.0	0.0	6.9	-	0.5
p38γ MAPK	0.0	0.0	11.1	0.2	0.5
PAK2	13.3	2.8	11.1	2.3	0.3
PAK4	20.0	2.2	16.7	1.6	2.5
PAK5 (PAK6)	6.7	3.4	12.5	2.4	3.6
PAK6	0.0	1.7	12.5	1.2	1.2
PDK1	6.7	3.9	-	0.2	0.2
PHKγ1	26.7	14.0	26.4	0.7	-
PIM1	13.3	10.7	16.7	3.6	1.4
PIM2	0.0	3.4	12.5	2.8	1.7
PIM3	26.7	11.8	18.1	-	2.1
PKA	6.7	3.4	9.7	-	-
PKBα	0.0	1.1	5.6	-	0.3
PKBβ(AKT2)	33.3	1.1	8.3	0.5	0.1
PKCγ	0.0	3.4	-	-	-
PKCα	0.0	6.7	-	5.5	-
PKCζ	0.0	1.1	-	-	-
PKD1 (PRKD1)	13.3	8.4	20.8	-	1.1
PLK1	0.0	3.4	13.9	3.1	0.6
PRAK	20.0	1.1	5.6	-	0.2
PRK2 (PKN2)	20.0	3.4	18.1	0.7	1.3
RIPK2	26.7	12.9	34.7	5.9	2.8
ROCK 2	13.3	7.9	25.0	-	2.7

RSK1	13.3	14.6	22.2	0.7	2.3
RSK2	46.7	14.6	20.8	1.2	0.3
S6K1 (RPS6KB1)	0.0	9.6	26.4	-	-
SGK1	6.7	-	-	-	1.2
SmMLCK	0.0	9.6	22.2	4.5	-
Src	13.3	14.0	43.1	9.0	11.6
SRPK1	26.7	1.1	23.6	3.1	3.7
STK33	0.0	9.0	33.3	1.0	2.4
SYK	6.7	7.3	19.4	1.0	1.3
TAK1	26.7	9.0	37.5	-	6.2
TAO1	6.7	5.6	37.5	-	3.4
TBK1	6.7	9.0	27.8	-	0.9
TIE2	6.7	2.2	34.7	2.9	3.0
TrkA	26.7	12.4	31.9	2.8	5.5
TTK	6.7	2.8	26.4	9.0	1.6
VEG-FR (KDR)	33.3	11.2	41.7	2.6	3.5
YES1	26.7	24.2	45.8	5.2	7.8
ZAP70	0.0	1.1	9.7	0.0	0.5

Table S 5: Data Collection and Refinement Statistics for cSrc with **B1**.

	cSrc with **B1** (4fic)
Data collection	
Wavelength (Å)	0.979400
Temperature	90 K
X-ray source	SLS X10SA
α, β, γ (°)	79.63, 88.12, 90.17
Resolution (Å)	50.0–2.5 (2.60–2.50)[a]
R_{sym} or R_{merge} (%)	6.1 (34.7)
I / σI	14.2 (4.1)
Completeness (%)	97.8 (97.1)
Redundancy	3.5 (3.6)
Refinement	
Resolution (Å)	43.4–2.50
No. reflections	25142
R_{work} / R_{free}	19.8 / 24.4
No. atoms	
Protein	3913
Ligand/ion	32
Water	97
B-factors	41.9
Protein	42.0
Ligand/ion	45.1
Water	37.3
R.m.s. deviations	
Bond lengths (Å)	0.013
Bond angles (°)	1.473
Ramachandran Plot:	
Residues in	
most favoured regions	87.9%
additional allowed regions	11.4%
generously allowed regions	0.7%
disallowed regions	0.0%

[a]Diffraction data from one crystal was used to determine the complex structure. Values in parenthesis are referring to the highest resolution shell.

Table S 6: Lowest docking rank of an inhibitor (defined as ≥ 75% inhibition at 100 µM) when all 15 compounds for which percent inhibition data was obtained were docked compared to the number of identified inhibitors at the same cut-off for all kinases in the panel for which crystal structures were available at the start of the study.

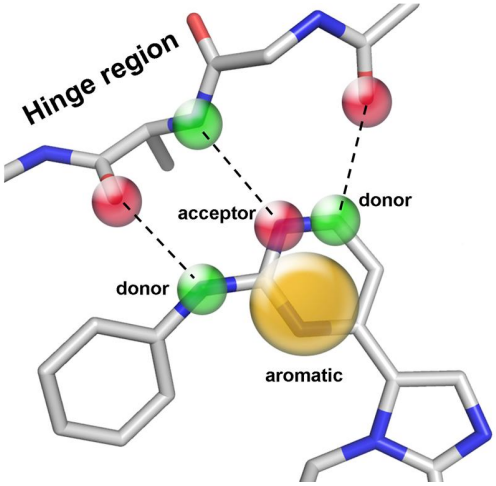
Kinase	Worst rank for an inhibitor	Number of identified inhibitors
Aurora A	1	1
Aurora B	3	2
CAMK1	2	2
CDK2	1	1
CHK2	2	1
CK2	12	2
DYRK1A	8	1
FGF-R1	11	2
IGF-1R	7	2
Lck	8	1
MARK3	8	1
NEK2a	7	1
PAK4	6	1
PAK5	9	1
ROCK 2	2	1
RSK2	4	1
SYK	2	1
VEG-FR	2	1

Table S 7: Comparison of experimentally detected kinase inhibitors with docking predictions. For tested compounds, percent inhibition (PI) at 100 µM is given. Cells containing PI values ≥ 40% are marked in orange. For docked compounds the numbers indicate the normalised total score. Cells with a normalised score ≥ 1.0 before rounding are marked in blue.

	ABL	Aurora A	Aurora B	BTK	CAMK1	CAMKKb	CDK2	CHK1	CHK2	CK1	CK2	CSK	DYRK1A	EPH-A2	EPH-B4	ERK2	FGF-R1	GSK3b
A1 (experimental)	2	2	32	1	35	0	8	0	0	28	97	0	36	0	12	12	34	64
A1 (docked)	1.0	0.0	0.7	ND	0.6	0.5	0.7	0.7	0.2	0.7	0.4	0.9	0.7	0.6	0.6	-3.7	0.9	0.6
A2 (experimental)	40	89	81	67	60	0	88	0	25	6	94	11	55	33	39	13	88	51
A2 (docked)	-3.5	1.0	1.1	ND	0.8	1.0	1.0	1.1	0.9	1.0	1.0	1.0	0.9	1.0	0.9	0.9	0.9	1.1
B1 (experimental)	72	52	76	67	41	54	54	45	81	73	55	71	77	28	56	47	79	72
B1 (docked)	1.0	0.9	1.0	-0.7	0.7	0.9	0.8	0.9	0.9	1.0	0.7	1.0	0.9	1.0	1.1	0.5	0.9	0.8
B2 (experimental)	14	12	29	44	52	21	17	9	13	26	17	24	19	11	14	14	42	27
B2 (docked)	0.2	-0.5	0.1	-45.8	0.0	0.6	0.1	0.5	0.4	0.5	-1.5	0.7	0.5	0.6	0.9	-1.1	0.6	0.6
B3 (experimental)	48	13	61	28	17	41	34	18	25	28	11	29	43	0	11	7	45	73
B3 (docked)	1.0	-1.5	0.6	-1.5	0.6	0.9	0.6	0.7	0.2	0.8	0.2	1.0	0.5	-0.9	0.9	-0.8	0.7	0.6
B4 (experimental)	18	10	0	0	35	18	0	0	0	0	0	0	0	0	27	0	3	8
B4 (docked)	-0.4	-1.2	0.3	ND	-1.0	0.6	-0.6	0.6	-4.7	-0.8	-51.5	0.6	0.4	0.4	0.9	-38.3	0.5	0.6
C1 (experimental)	15	21	6	46	75	0	10	0	34	4	41	10	52	37	0	1	57	70
C1 (docked)	0.9	0.8	1.0	-2.7	0.9	0.9	0.9	0.9	0.7	1.0	1.0	0.9	0.9	1.0	1.0	0.0	0.9	1.0
C2 (experimental)	34	1	19	29	66	0	0	0	38	17	31	14	36	37	0	0	32	62
C2 (docked)	0.9	0.8	1.0	-2.8	0.8	0.8	0.9	0.9	0.7	0.9	0.9	0.9	0.9	0.9	1.0	0.4	0.9	1.0
D1 (experimental)	0	16	34	6	22	34	33	0	0	0	9	0	0	0	19	0	65	24
D1 (docked)	-0.1	0.9	0.7	ND	0.8	0.7	0.9	0.9	0.6	0.8	0.5	0.6	0.7	0.7	0.6	0.5	0.9	1.0
D2 (experimental)	11	5	12	51	83	28	14	0	19	0	5	0	30	0	13	0	40	38
D2 (docked)	-0.2	0.9	1.0	-10.0	1.1	0.7	0.9	1.1	0.8	1.1	0.8	0.9	0.9	1.0	0.9	0.7	1.0	1.1
E1 (experimental)	3	0	0	17	6	0	0	0	0	0	0	8	0	0	6	4	0	0
E1 (docked)	1.1	0.8	0.9	-3.9	0.8	0.8	0.8	1.0	0.8	0.8	0.8	1.1	0.9	1.0	0.9	0.7	1.0	1.0
E2 (experimental)	5	23	0	25	0	0	0	0	14	0	28	20	10	0	1	13	25	2
E2 (docked)	1.0	0.7	1.0	-2.8	0.8	0.9	0.9	1.0	0.6	0.8	0.7	1.0	0.8	0.9	1.1	0.8	0.9	0.9
F1 (experimental)	4	6	0	14	11	0	0	2	32	2	11	23	0	31	5	10	14	9
F1 (docked)	1.1	0.8	0.8	ND	0.8	0.9	0.9	1.0	0.8	0.9	0.8	1.0	0.9	0.7	0.9	0.6	1.0	0.9
F2 (experimental)	0	8	0	16	6	0	0	0	20	0	3	26	5	12	9	0	6	13
F2 (docked)	1.1	0.9	1.1	-3.1	0.9	1.1	0.9	1.1	1.1	0.9	1.0	1.2	1.0	0.9	1.3	0.4	1.2	1.1
F3 (experimental)	3	17	0	49	9	0	21	4	19	24	20	8	31	9	6	8	41	44
F3 (docked)	0.8	0.6	0.9	-7.0	0.7	0.7	0.7	0.9	0.8	0.9	0.9	0.9	0.8	0.7	0.9	0.6	0.9	0.8

	[illegible]	JNK2	Lck	MAPKAP-K2	MARK3	MKK1	MNK1	MNK2	NEK2a	p38a MAPK	p38d MAPK	p38g MAPK	PAK4	PAK5	PAK6	PDK1	PIM1	PKA	PKBa	PLK1	ROCK 2	RSK1	RSK2	SGK1	SRPK1	SYK	VEG-FR
A1 (experimental)	4	6	2	0	25	7	0	0	0	0	0	1	0	0	4	31	7	15	0	0	17	0	84	0	74	13	54
A1 (docked)	0	0.7	1.2	0.8	0.9	0.4	-1.6	0.6	0.5	-1.1	0.5	-1.5	0.9	0.6	0.6	0.7	0.7	-1.2	0.6	0.9	0.3	0.8	0.9	0.5	0.7	0.5	0.8
A2 (experimental)	1	17	39	20	86	72	1	6	0	0	0	17	50	35	10	20	31	8	12	0	76	21	52	0	58	30	72
A2 (docked)	1	0.9	1.0	1.0	0.8	0.5	-11.4	0.5	0.9	0.7	0.7	0.8	0.9	-3.8	0.7	1.0	0.9	0.7	0.7	0.9	0.7	0.9	0.8	1.0	0.8	1.0	0.8
B1 (experimental)	0	26	75	56	59	37	71	66	77	27	18	1	89	79	36	35	30	9	9	18	52	34	51	34	41	84	82
B1 (docked)	0	1.0	1.0	0.9	0.8	1.0	0.7	1.1	0.8	0.9	0.7	0.5	0.9	0.6	0.8	0.7	1.0	0.9	0.8	1.0	-4.1	0.9	1.0	0.8	0.7	1.1	1.1
B2 (experimental)		0	37	14	0	22	24	30	38	35	15	0	0	13	11	15	18	19	0	8	19	21	42	19	31	26	22
B2 (docked)	8	0.5	0.7	0.6	0.7	-6.4	-18.2	-1.8	0.7	0.4	0.5	-2.3	0.5	0.6	0.7	-1.8	-4.1	ND	0.0	0.8	ND	0.5	0.6	0.3	0.8	0.5	0.6
B3 (experimental)	:	1	29	7	38	34	53	35	25	10	1	24	47	20	18	15	13	0	0	4	25	24	9	16	25	31	69
B3 (docked)	7	0.7	0.9	1.0	0.7	ND	ND	-166.0	0.4	0.5	0.6	ND	0.1	ND	ND	-74.7	0.7	ND	ND	0.8	ND	0.7	0.8	0.5	0.7	0.8	1.0
B4 (experimental)		0	0	0	0	0	0	0	0	0	19	0	0	5	0	0	9	1	0	0	0	0	23	0	10	10	9
B4 (docked)	5	0.2	0.8	0.7	0.5	-11.3	-13.6	-14.0	0.0	-0.4	0.5	ND	-1.0	0.6	0.6	-3.1	ND	ND	-0.2	0.5	ND	0.6	0.6	-0.9	0.5	0.4	0.7
C1 (experimental)	:	0	40	15	29	31	0	30	2	0	0	0	34	0	0	16	61	42	17	0	39	47	39	14	19	0	74
C1 (docked)	0	0.8	1.1	0.9	0.9	0.8	0.1	0.8	1.0	0.6	0.8	0.2	1.1	0.4	0.7	0.6	0.7	0.7	0.6	0.7	-0.6	0.9	0.9	0.9	0.9	0.9	0.8
C2 (experimental)	:	8	29	8	24	23	5	21	0	0	0	0	30	0	0	41	38	34	38	0	19	59	52	63	36	0	39
C2 (docked)	9	0.9	1.0	1.0	0.8	0.6	-0.9	0.7	1.0	0.4	0.8	0.0	0.9	-2.3	0.7	0.7	0.9	0.6	0.4	0.9	0.6	0.8	0.7	0.8	0.8	0.9	0.8
D1 (experimental)		3	0	0	0	0	0	9	0	0	0	0	0	18	0	3	11	5	0	0	2	3	13	0	0	7	12
D1 (docked)	8	1.0	0.7	0.6	0.8	0.5	-0.8	-0.2	0.6	0.6	0.6	0.6	0.7	0.6	0.8	0.5	1.0	0.1	0.5	1.0	0.5	0.8	0.8	0.7	0.8	0.8	0.8
D2 (experimental)	:	10	5	2	13	0	0	0	6	5	0	0	0	0	20	13	30	0	21	0	0	3	48	0	3	12	0
D2 (docked)	1	1.1	1.0	1.0	1.0	0.7	0.6	-0.3	0.8	0.8	0.8	0.3	0.8	1.0	1.0	1.0	1.1	0.3	0.7	1.2	0.7	1.0	1.0	0.8	0.9	1.1	1.1
E1 (experimental)		3	18	17	5	20	0	10	0	0	0	0	3	0	0	1	0	0	0	0	0	15	11	0	0	0	0
E1 (docked)	0	0.9	1.1	0.9	1.0	0.6	0.1	0.6	0.9	0.8	0.7	0.3	0.9	0.8	0.6	1.0	0.8	0.8	0.7	0.9	0.6	0.9	0.7	0.8	0.8	1.0	1.0
E2 (experimental)	:	5	0	26	17	11	3	0	0	0	0	0	15	0	0	29	0	0	14	8	7	19	47	0	42	9	38
E2 (docked)	0	0.9	1.0	0.9	1.0	0.6	0.3	0.7	1.0	0.5	0.6	0.4	0.9	0.6	0.9	0.8	1.2	0.7	0.7	1.0	0.5	0.9	0.8	0.7	0.8	1.0	0.9
F1 (experimental)		11	23	29	12	24	0	25	6	2	0	0	20	3	6	14	0	0	2	10	11	23	10	1	14	22	31
F1 (docked)	0	0.9	1.0	0.9	0.9	0.6	-0.3	0.6	0.8	0.6	0.7	0.5	0.9	0.7	0.7	1.0	0.8	0.7	0.7	1.0	0.4	0.9	0.7	0.8	0.8	1.0	0.8
F2 (experimental)		7	0	23	9	57	0	13	4	0	0	0	18	0	0	0	0	0	0	0	13	17	20	0	9	12	28
F2 (docked)	1	0.9	1.2	1.0	1.1	0.7	-0.4	0.6	0.9	0.7	0.8	0.5	1.0	0.9	0.8	1.0	1.0	0.7	0.7	1.2	0.6	1.0	0.9	0.8	1.0	1.1	1.0
F3 (experimental)		10	23	22	11	43	31	26	29	21	0	6	11	0	3	2	67	0	11	8	29	16	25	18	0	20	39
F3 (docked)	8	0.9	1.0	0.9	0.7	0.6	-0.4	0.6	0.6	0.6	0.6	0.4	0.8	0.4	0.4	0.8	0.8	0.6	0.6	0.8	0.5	0.8	0.7	0.7	0.7	0.8	0.7

a)



b)

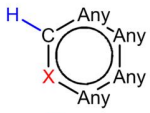
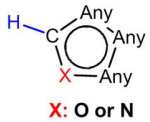
 **X: O or N**	**Six-membered aromatic system** **SLN:**Any[1]:C[IS=CH]:Any[IS=N,O]:Any:Any:Any:@1; Label:_DL_6b
 **X: O or N**	**Five-membered aromatic system** **SLN:**Any[1]:C[IS=CH]:Any[IS=N,O]:Any:Any:@1; Label:_DL_6a

Figure S 1: a) Pharmacophore model for fragments binding to the hinge region of protein kinases. Hydrogen-bond donor (green spheres) and acceptor functionalities (red spheres) were linked to the corresponding atoms in the receptor to consider the directionality of the hydrogen bonds. To fulfill the pharmacophore, all hits were required to contain the aromatic feature (orange sphere) and two out of the possible three hydrogen-bonding features. b) To account for CH-hydrogen bonds as often observed in protein kinases, aromatic CH groups being part of a heterocycle were also considered as hydrogen-bond donors. (Definition is given in SYBYL line notation (SLN)).

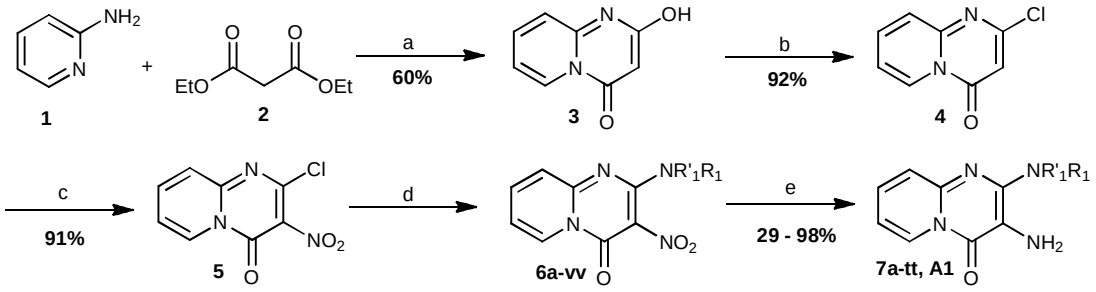
Synthesis of chemical libraries

^{1}H- and ^{13}C-NMR spectra were recorded on either a Bruker Avance DPX 300 MHz or 500 MHz spectrometer. Chemical shifts (δ) are expressed in parts per million (ppm) and coupling constants (J) are in Hertz (Hz). Signal splitting patterns are described as singlet (s), broad singlet (br s), doublet (d), triplet (t), quartet (q), quintuplet (quin), sextuplet (sex), septet (sept), multiplet (m) or combinations thereof. LCMS (liquid chromatography mass spectrometry) analyses were performed with either an Agilent HPLC 1100 series connected to a Bruker Daltonics MicrOTOF, or an Agilent Technologies 1200 series HPLC connected to an Agilent Technologies 6130 quadrupole LCMS, both instruments were connected to an Agilent diode array detector. LCMS chromatographic separations were conducted with a Phenomenex Gemini C18 column, 50 x 3.0 mm, 5 µm particle size; mobile phase / acetonitrile + 0.1% HCOOH 80:20 to 5:95 over 3.5 min, and then held for 1.5 min; flow rate 0.5 ml min^{-1}. High resolution electrospray measurements were performed on a Bruker Daltonics micrOTOF Mass Spectrometer. Thin layer chromatography (TLC) was carried out on Merck silica gel 60 F254 plates using UV light and/or $KMnO_4$ for visualization. Column chromatography was performed using RediSep® 4 or 12 g silica pre-packed columns. When applicable, all glassware was oven-dried overnight and all reactions were carried out under dry and inert conditions (argon atmosphere).

Synthesis of 2-substituted core fragment **A**

Derivatives of the 2-substituted core fragment **A** were synthesized following a five-step strategy (Scheme 2).(*48, 49*) Heating of 2-aminopyridine **1** with diethylmalonate **2** at 170 °C afforded the dioxo intermediate **3**. The hydroxyl group of intermediate **3** was replaced by chlorine using an excess of phosphoryl chloride, yielding the desired intermediate **4**.

The introduction of a nitro group in the 3-position of intermediate **4**, afforded intermediate **5,** which was subsequently reacted with various primary and secondary amines in order to substitute the chlorine. Finally, the reduction of the nitro group of all 2-substituted intermediates **6a-vv** using zinc and ammonium chloride gave the desired compounds **7a-tt** and **A1**. Using this synthetic pathway, a library of 2-substituted core fragment **A** consisting of 48 compounds was prepared.



Scheme 2: Reagents and conditions: (a) 170 °C; (b) $POCl_3$, reflux; (c) HNO_3, H_2SO_4, rt; (d) $R_1R'_1NH$, MeOH, MW, 140 °C; (e) Zn, NH_4Cl, MeOH, rt.

2-Hydroxy-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (3)

A mixture of 2-aminopyridine (1.54 g, 16.4 mmol) and freshly distilled dry diethyl malonate (6.56 g, 41mmol) was heated at 140 °C until complete precipitation for about 12 h. The mixture was then cooled, triturated with diethyl ether (50 mL), washed several times with diethyl ether to remove the non-reacted materials, and crystallized from water to give the desired product. **Yield:** 1.61 g, 61%; **^{1}H-NMR:** (d_6-DMSO) δ (ppm) 12.07 (s, 1H), 8.94 (m, 1H), 8.10 (m, 1H), 7.42 (m, 1H), 7.34 (m, 1H), 4.98 (s, 1H); **LRMS(ES$^+$):** m/z 163 $[M+H]^+$.

2-Chloro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (4)

2-Hydroxy-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (975 mg, 6.02 mmol) was cautiously dissolved in phosphorus(III) oxychloride (7.5 mL, 80.4 mmol) to give a solution that was heated at reflux for 6 h. After cooling, the reaction mixture was poured carefully into ice-cold water (100 mL) and the pH was adjusted to 7 by addition of a saturated solution of sodium carbonate. The aqueous layer was extracted with DCM. The combined organic layers were dried ($MgSO_4$) and the solvent was removed to yield a brown solid. The

crude product was purified by pressure chromatography using DCM as eluent to furnish the title compound as a white solid. **Yield:** 0.65 g, 60%; **^{1}H-NMR:** ($CDCl_3$) δ (ppm) 9.09 (d, J = 7.2 Hz, 1H), 7.90 (m, 1H), 7.71 (d, J = 9.0 Hz, 1H), 7.29 (m, 1H), 6.52 (s, 1H); **^{13}C-NMR:** ($CDCl_3$) δ (ppm) 158.47, 157.08, 150.44, 138.27, 127.76, 125.82, 116.46, 102.49; **LRMS(ES$^+$):** m/z 181 $[M+H]^+$.

2-Chloro-3-nitro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (5)

Nitric acid (100%, 5 mL) was placed in a cooled flask and concentrated sulfuric (7 mL) acid was added with stirring. After 5 minutes 2-chloro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (1 g, 5.5 mmol) was slowly added. The mixture was stirred at room temperatures for 4 h and then poured slowly with shaking into cracked ice. The resulting solid was filtered, washed with water, and dried at room temperature under house vacuum overnight. **Yield:** 1.14 g, 91%; **^{1}H-NMR:** (d_6-DMSO) δ (ppm) 9.12 (br s, 1H), 8.35 (br s, 1H), 7.94 (br s, 1H), 7.70 (br s, 1H); **^{13}C-NMR:** (d_6-DMSO) δ (ppm) 151.05, 149.11, 148.26, 142.62, 129.67, 125.84, 119.45; **LRMS(ES$^+$):** m/z 226 $[M+H]^+$.

General procedure for the preparation of 2-substituted 3-nitro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one derivatives (6a-vv)

2-Chloro-3-nitro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one was treated with various primary and secondary amines (2 eq.) in methanol (1 mL). The reaction mixtures were irradiated under microwave condition at 140 °C for 15 min to complete the reaction. The crude reaction mixtures were used without further purification for the next step (reduction of nitro group with Zn, and ammonium chloride).

General procedure for the preparation of 2-substituted 3-amino-4*H*-pyrido[1,2-*a*]pyrimidin-4-one derivatives (7a-tt)

To the crude solution of 2-substituted 3-nitro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one in methanol, Zn (0.5 g, 7.5 mmol, 30 eq.) and ammonium chloride (0.24 g, 3.75 mmol, 15.0 eq.) were added. After 1 h saturated NH_4OAc solution (15 eq.) was added and the reaction mixture was stirred for another 30 min. The crude reaction mixture was finally

filtered through Celite®, concentrated and subsequently purified using preparative HPLC.

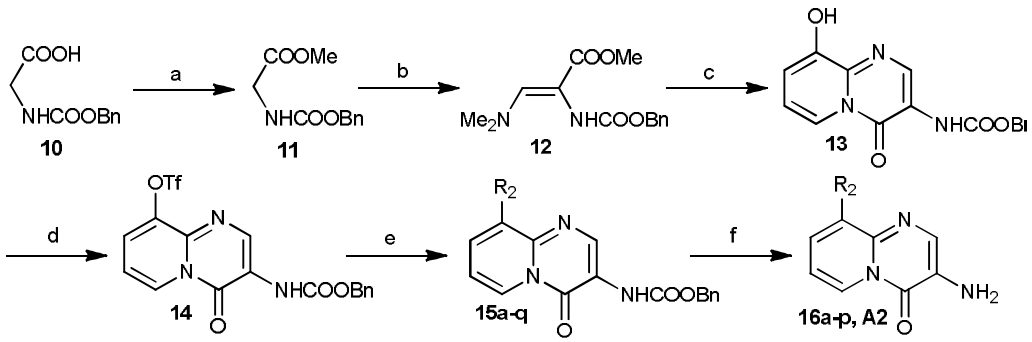
Cis/trans-(3-Amino-2-(2,6-dimethylmorpholino)-4*H*-pyrido[1,2-*a*]pyrimidin-4-one) (A1)

2-Chloro-3-nitro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (50 mg, 0.22 mmol) was treated with 2,6-dimethylmorpholine (51 mg, 0.44 mmol) in methanol (1 mL). The reaction mixture was irradiated under microwave condition at 140 °C for 15 min to complete the reaction. The crude reaction mixture was used without further purification for the next step. To the crude solution of 2-(2,6-dimethylmorpholino)-3-nitro-4*H*-pyrido[1,2-*a*]pyrimidin-4-one in methanol, Zn (0.5 g, 7.5 mmol) and ammonium chloride (0.24 g, 3.75 mmol) were added. After 1 h saturated NH_4OAc solution (15 eq.) was added and the reaction mixture was stirred for another 30 min. The crude reaction mixture was finally filtered through Celite®, concentrated and subsequently purified using preparative HPLC. **Yield:** 34 mg, 56%; **^{1}H-NMR:** (CD_3OD) δ (ppm) 8.78 (d, J = 7.1 Hz, 2H, *cis*/*trans*), 7.58 – 7.53 (m, 2H, *cis*/*trans*), 7.46 (d, J = 9 Hz, 2H, *cis*/*trans*), 7.12 (t, J = 7.1 Hz, 2H, *cis*/*trans*), 4.19 (m, 0.9H, *trans*), 3.95 (d, J = 12.6 Hz, 3.1H, *cis*), 3.84 (m, 3.1H, *cis*), 3.56 (dd, J = 12.7, 3.0 Hz, 0.9H, *trans*), 3.25 – 3.20 (m, 1H, *trans*), 2.56 (m, 3H, *cis*), 1.29 (d, J = 6.6 Hz, 2.7H, *trans*), 1.20 (d, J = 6.3 Hz, 9.3H, *cis*); **^{13}C-NMR:** (CD_3OD) δ (ppm) 156.56 (*cis*), 152.28 (*trans*), 151.51 (*cis/trans*), 143.49 (*trans*), 143.30 (*cis*), 133.01 (*trans*), 132.86 (*cis*), 126.80 (*trans*), 126.74 (*cis*), 126.00 (*cis/trans*), 115.93 (*cis/trans*), 115.27 (*cis*), 114.99 (*trans*), 73.07 (*cis*), 67.97 (*trans*), 53.62 (*cis*), 52.92 (*trans*), 19.11 (*cis*), 18.05 (*trans*); **LRMS(ES$^+$):** m/z 275 $[M+H]^+$; **HRMS (ES$^+$):** calcd for $C_{14}H_{18}N_4O_2$ $[M+H]^+$ 275.1503, found 275.1508.

Synthesis of 9-substituted core fragment **A**

9-substituted core fragment **A** derivatives were synthesized following Scheme 3. Using the methods previously described by Okano *et al.,* Simunek *et al.* and Čebašek *et al.,* it was possible to design a six-step synthesis. (*50-52*) In the first step, the carboxylic acid group of *N*-protected glycine **10** was esterified with methanol using thionyl chloride. The resulting ester **11** was then reacted with Bredereck's reagent to give propenoate **12**.

Subsequent heating of intermediate **12** with 2-amino-3-hydroxypyridine in acetic acid afforded the desired intermediate **13**, which was triflated and coupled (one-pot two-step reaction) without further purification using a selection of boronic acids. Finally, all resulting intermediates **15a-q** were deprotected using trimethylsilyl iodide (TMSI) to give the final compounds **16a-p** and **A2**. Using this synthetic pathway, a library of 9-substituted core fragment **A** consisting of 17 compounds was prepared.



Scheme 3: Reagents and conditions: (a) $SOCl_2$, MeOH, 0 °C-rt; (b) Bredereck's reagent, toluene, 115 °C; (c) 2-amino-3-hydroxy-pyridine, NaOAc, AcOH, 90 °C; (d) *N*-Phenylbis(trifluoromethane- sulfonimide), K_2CO_3, THF, MW, 120 °C; (e) $R_2B(OH)_2$, $Pd(PPh_3)_4$, 120 °C; (f) TMSI, DCM, rt.

2-(((Benzyloxy)carbonyl)amino)acetic acid (10)

A 300-mL three-necked round-bottomed flask equipped with a magnetic stirring bar and fitted with two dropping funnels was charged with glycine (5.0 g, 66.5 mmol) and 2 M aqueous sodium hydroxide (33.8 mL). The flask was then cooled to 0 °C. To the vigorously stirred solution CbzCl (11.4 mL, 80 mmol, 1.2 eq.) and 4 M sodium hydroxide (17 mL) were added simultaneously over a period of 10 min via each dropping funnel. The reaction mixture was stirred for an additional 40 min at 0 °C, after which time TLC indicated complete consumption of glycine. The aqueous solution was washed three times with diethyl ether and acidified with 6 M hydrochloric acid to pH 1. The resulting mixture was cooled at 0 °C to give a precipitate, which was collected by filtration, washed with small portions of cold water, and dried under reduced pressure to afford analytically pure colourless crystals of the desired product. **Yield:** 13.2 g, 95%;

^{1}H-NMR: (d_6-DMSO) δ (ppm) 12.58 (br s, 1H), 7.62-7.48 (br s, 1H), 7.44.7.25 (m, 5H), 5.05 (s, 2H), 3.70 (d, J = 5.6 Hz, 2H); **^{13}C-NMR:** (d_6-DMSO) δ (ppm) 171.70, 156.60, 137.10, 128.40, 127.90, 127.80, 65.60, 42.20; **LRMS(ES$^+$):** m/z 210 $[M+H]^+$.

Methyl 2-(((benzyloxy)carbonyl)amino)acetate (11)

A 300-mL three-necked round-bottomed flask equipped with a magnetic stirring bar and fitted with a dropping funnel was charged with 2-(((Benzyloxy)carbonyl)amino)acetic acid (2.50 g, 12.0 mmol) and methanol (60 mL). The flask was cooled to 0 °C, and thionyl chloride (1.20 mL, 16.5 mmol, 1.4 equiv) was added to the vigorously stirred solution over a period of 10 min. The mixture was stirred for an additional 2 h, after which time TLC (ethyl acetate) indicated complete consumption of the starting acid. The reaction mixture was concentrated under reduced pressure to give a crude material, which was purified by silica gel column chromatography (ethyl acetate). **Yield:** 2.66 g, 99%; **^{1}H-NMR:** ($CDCl_3$) δ (ppm) 7.32 - 7.18 (m, 5H), 5.58 (br s, 1H), 5.02 (s, 2H), 3.84 (d, J = 5.6 Hz, 2H), 3.62 (s, 3H); **^{13}C-NMR:** ($CDCl_3$) δ (ppm) 170.40, 156.20, 136.10, 128.20, 127.90, 127.80, 66.70, 51.90, 42.30; **LRMS(ES$^+$):** m/z 224 $[M+H]^+$.

(*Z*)-Methyl 2-(((benzyloxy)carbonyl)amino)-3-(dimethylamino)acrylate (12)

A mixture of methyl 2-(((benzyloxy)carbonyl)amino)acetate (2.118 g, 9.5 mmol), anhydrous toluene (8 mL), and bis(dimethylamino)-*tert*-butoxymethane (1.74 g, 10 mmol) was stirred under argon at the reflux temperature for 4 h. Volatile components were evaporated *in vacuo* and the oily residue was purified by flash chromatography (diethyl ether). Fractions containing the product were combined and volatile components were evaporated *in vacuo*. **Yield:** 2.58 g, 98%; **^{1}H-NMR:** ($CDCl_3$) δ (ppm) 7.41 - 7.26 (m, 6H), 5.57 and 5.35 (br 2s, 1H), 5.18 (s, 2H), 3.69 and 3.64 (2s, 3H), 3.04 and 2.98 (2s, 6H); **^{13}C-NMR:** ($CDCl_3$) δ (ppm) 168.34; 157.16; 146.81; 136.53; 128.49; 128.06; 94.09; 67.13; 51.28; 42.07; **LRMS(ES$^+$):** m/z 279 $[M+H]^+$.

Benzyl(9-hydroxy-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)carbamate (13)

A mixture of 2-aminopyridin-3-ol (165 mg, 1.5 mmol), anhydrous sodium acetate (123 mg, 1.5 mmol), acetic acid (3 mL), and (*Z*)-methyl 2-(((benzyloxy)carbonyl)amino)-3-(dimethylamino)acrylate (417 mg, 1.5 mmol) was stirred at 90 °C for 10 h and cooled to room temperature. Water (1 mL) was added, the suspension was stirred at 10 °C for 10h, and the precipitate was collected by filtration and washed with water (5 mL). The reaction vessel with product was put in a desiccator and dried *in vacuo* over P_4O_{10} at room temperature for 24 h. **Yield:** 331 mg, 71%; **^{1}H-NMR:** (d_6-DMSO) δ (ppm) 10.60 (br s, 1H), 9.04 (s, 1H), 8.74 (s, 1H), 8.46 (d, J = 7.4 Hz, 1H), 7.45 (d, J = 7.3 Hz, 2H), 7.40 (t, J = 7.3 Hz, 2H), 7.34 (t, J = 7.3 Hz, 1H), 7.22 (t, J = 7.4 Hz, 1H), 7.14 (d, J = 7.4 Hz, 1H), 5.18 (s, 2H), 3.36 (br s, 1H); **^{13}C-NMR:** (d_6-DMSO) δ (ppm) 154.22, 153.52, 150.34, 141.32, 136.56, 128.39, 127.92, 127.77, 117.20, 116.71, 113.42, 66.07; **LRMS(ES$^+$):** m/z 312 $[M+H]^+$.

Benzyl(9-(2,6-difluorophenyl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)carbamate (15q)

Benzyl(9-hydroxy-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)carbamate (70 mg, 0.23 mmol) was placed in a microwave vessel containing *N*-phenylbis(trifluoromethanesulfonimide) (80 mg, 0.23 mmol), K_2CO_3 (93 mg, 0.68 mmol) and THF (1 mL). The resulting solution was microwaved for 6 min at 120 °C. 2,6-Difluorophenylboronic acid (36 mg, 0.23 mmol) and $Pd(PPh_3)_4$ (33 mg, 0.029 mmol) were added and the reaction was microwaved for a further 10 min at 120 °C. The reaction mixture was concentrated and purified by medium pressure chromatography. **Yield:** 91.02 mg, 99%; **^{1}H-NMR:** ($CDCl_3$) δ (ppm) 9.21 (br s, 1H), 9.02 (dd, J = 7.5, 1.5 Hz, 1H), 7.60 (d, J = 6.6 Hz, 1H), 7.52 (s, 1H), 7.43 – 7.32 (m, 6H), 7.19 (t, J = 7.1 Hz, 1H), 7.07 – 7.03 (m, 2H), 5.23 (s, 2H); **LRMS(ES$^+$):** m/z 408 $[M+H]^+$.

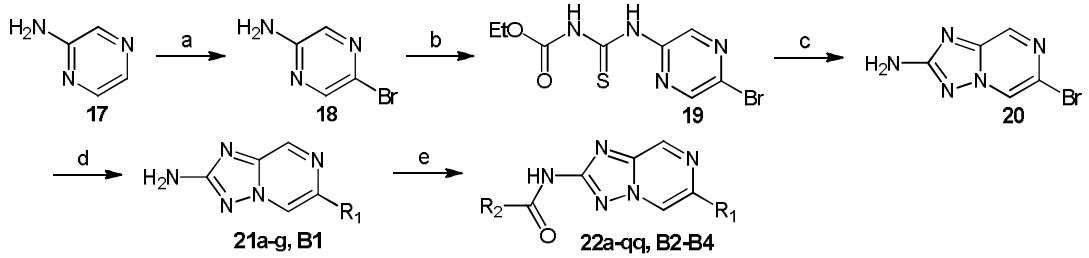
3-Amino-9-(2,6-difluorophenyl)-4*H*-pyrido[1,2-*a*]pyrimidin-4-one (A2)

A mixture of benzyl(9-(2,6-difluorophenyl)-4-oxo-4*H*-pyrido[1,2-*a*]pyrimidin-3-yl)carbamate (91.02 mg, 0.22 mmol), DCM (1 mL), and iodotrimethylsilane (53 µL, 0.37

mmol) was left to stir at room temperature for 1 h before quenching with MeOH. The final product was isolated from the reaction mixture by filtering through a short column of SCX under gravity. **Yield:** 36 g, 59%; **^{1}H-NMR:** (d_6-DMSO) δ (ppm) 8.86 (dd, J = 7.0, 1.5 Hz, 1H), 7.81 (s, 1H), 7.59 – 7.52 (m, 2H), 7.26 – 7.20 (m, 3H), 5.33 (s, 2H); **^{13}C-NMR:** (d_6-DMSO) δ (ppm) 159.82 (dd, J = 247.0, 7.1 Hz), 152.35, 139.46, 131.51, 130.79, 130.75 (t, J = 10.0 Hz), 129.35, 125.99, 125.68, 114.34, 113.89 (t, J = 20.8 Hz), 111.56 (dd, J = 19.3, 5.3 Hz); **LRMS(ES$^+$):** m/z 274 $[M+H]^+$; **HRMS (ES$^+$):** calcd for $C_{14}H_9F_2N_3OS$ $[M+H]^+$ 274.0786, found 274.0779.

Synthesis of 2,6-disubstituted core fragment **B**

The synthetic route for the 2,6-disubstituted core fragment **B** consisted of 5 steps (Scheme 4). In the first step, bromopyrazine **18** was prepared from commercially available pyrazine **17**, which was selectively brominated using *N*-bromosuccinimide (NBS). Next, bromopyrazine **18** was reacted with ethoxycarbonylisothiocyanate to give the intermediate **19** which was subsequently subjected to a cyclisation procedure employing hydroxylamine to yield intermediate **20**.(*53*) In the second last step, the Suzuki cross-coupling of the bromo-intermediate **20** with eight boronic acids led to the corresponding 6-substituted core fragment B intermediates **21a-g** and **B1**. Finally, treatment of intermediates **21a-g** and **B1** with a selection of carboxylic acids under amide coupling conditions yielded 2,6-disubstituted core fragment **B** derivatives **22a-qq** and **B2-B4**. Using this synthetic pathway, a library consisting of 47 compounds was prepared.



Scheme 4: Reagents and conditions: (a) NBS, DCM, 0°C; (b) Ethoxycarbonylisothiocyanate, dioxane, rt; (c) NH_2OH•HCl, DIPEA, MeOH/EtOH, 60°C; (d) $R_1B(OH)_2$, K_3PO_4, PCy_3, $Pd_2(dba)_3$, dioxane/water, MW, 130°C; (e) R_2COOH, PCl_3, CH_3CN, MW, 150°C.

5-Bromopyrazin-2-amine (18)

A solution of pyrazin-2-ylamine (6.66 g, 70 mmol) in DCM (200 mL) was cooled to 0 °C, treated with *N*-bromosuccinamide (12.5 g, 70 mmol) and allowed to warm to room temperature. The resulting reaction mixture was stirred overnight, then diluted with additional DCM (200 mL) and washed with 10% aqueous Na_2CO_3 solution. The layers were separated, and the organic layer washed with sat. aqueous NaCl solution, then dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. The residue was taken up in EtOAc (50 mL) and the product was precipitated by the addition of hexane (300 mL). The precipitate was dried under vacuum. **Yield:** 5.57 g, 46%; **^{1}H-NMR:** (d_6-DMSO) δ (ppm) 8.04 (d, J = 1.4 Hz, 1H), 7.68 (d, J = 1.4 Hz, 1H), 6.67 (br s, 2H); **^{13}C-NMR:** (d_6-DMSO) δ (ppm) 155.28, 143.55, 132.08, 123.59; **LRMS(ES$^+$):** m/z 175 $[M+H]^+$.

Ethyl *N*-[(5-bromopyrazin-2-yl)carbamothioyl]carbamate (19)

5-Bromopyrazin-2-amine (120 mg, 0.69 mmol) was dissolved in dioxane (7 mL), cooled to 10 °C, treated with ethoxycarbonyl isothiocyanate (90 mg, 0.69 mmol) and allowed to warm to room temperature. The resulting reaction mixture was stirred overnight, concentrated under reduced pressure and purified by flash chromatography. **Yield:** 180 mg, 86%; **^{1}H-NMR:** (d_6-DMSO) δ (ppm) 12.14 (br s, 1H), 11.81 (br s, 1H), 9.56 (s, 1H), 8.73 (d, J = 1.4 Hz, 1H), 4.25 (q, J = 7.1 Hz, 2H), 1.27 (t, J = 7.1 Hz, 3H); **^{13}C-NMR:** (d_6-DMSO) δ (ppm) 178.36, 153.52, 147.25, 145.17, 138.82, 134.62, 62.52, 14.04; **LRMS(ES$^+$):** m/z 306 $[M+H]^+$.

6-Bromo-[1,2,4]triazolo[1,5-a]pyrazin-2-amine (20)

Hydroxylamine hydrochloride (9.6 g, 138 mmol) and diisopropylethylamine (14.4 mL, 10.7 g, 83 mmol) were mixed with ethanol (300 mL) for a few minutes and then ethyl *N*-[(5-bromopyrazin-2-yl)carbamothioyl]carbamate (8.36 g, 27.5 mmol) was added with stirring. The resulting mixture was stirred at room temperature for 20 min and then heated to reflux for 3 hours. The volatile components were removed by evaporation and the residue obtained was mixed with water. The resulting slurry was filtered and the solids collected were washed with further water. **Yield:** 5.23 g, 89%; **^{1}H-NMR:** (d_6-DMSO) δ

(ppm) 9.13 (d, J = 1.1 Hz, 1H), 8.68 (d, J = 1.1 Hz, 1H), 6.66 (br s, 2H); **^{13}C-NMR:** (d_6-DMSO) δ (ppm) 167.16, 146.09, 136.04, 122.18, 120.88; **LRMS(ES$^+$):** m/z 215 $[M+H]^+$; **HRMS (ES$^+$):** calcd for $C_5H_5BrN_5$ $[M+H]^+$ 213.9723, found 213.9716.

6-Phenyl-[1,2,4]triazolo[1,5-*a*]pyrazin-2-amine (B1)

Phenylboronic acid (410 mg, 3.36 mmol), potassium phosphate (1,01 g, 4.77 mmol), 6-bromo-[1,2,4]triazolo[1,5-*a*]pyrazin-2-amine (600 mg, 2.8 mmol), $Pd_2(dba)_3$ (128 mg, 0.14 mmol) and tricyclohexylphosphine (94 mg, 0.34 mmol) were dispersed in dioxane (7 mL) and water (7 mL). The mixture was heated by microwave irradiation for 10 min at 130 °C, then quenched with DCM and filtered through a phase separator. The crude product was finally purified by flash chromatography. **Yield:** 527 mg, 89%; **^{1}H-NMR:** (d_6-DMSO) δ (ppm) 9.31 (d, J = 1.3 Hz, 1H), 8.92 (d, J = 1.3 Hz, 1H), 8.09 (d, J = 8.0 Hz, 2H), 7.49 (t, J = 8.0 Hz, 2H), 7.41 (t, J = 7.3 Hz, 1H), 6.55 (s, 2H); **^{13}C-NMR:** (d_6-DMSO) δ (ppm) 166.90, 145.40, 138.58, 136.31, 135.79, 128.71, 128.38, 125.64, 117.48; **LRMS(ES$^+$):** m/z 212 $[M+H]^+$; **HRMS (ES$^+$):** calcd for $C_{11}H_9N_5$ $[M+H]^+$ 212.0931, found 212.0929.

6-(2,6-Dimethylphenyl)-[1,2,4]triazolo[1,5-*a*]pyrazin-2-amine (B2)

Following a procedure similar to the preparation of **B1, B2** was obtained from **20** and the appropriate boronic acid in good yield. **Yield:** 626 mg, 93%; **^{1}H-NMR:** (CD_3OD) δ (ppm) 8.88 (s, 1H), 8.50 (s, 1H), 7.25 (t, J = 7.6 Hz, 1H), 7.15 (d, J = 7.6 Hz, 2H), 2.09 (s, 6H); **^{13}C-NMR:** (CD_3OD) δ (ppm) 168.40, 146.84, 142.31, 138.38, 137.87, 137.22, 129.99, 128.77, 122.45, 20.43; **LRMS(ES$^+$):** m/z 240 $[M+H]^+$; **HRMS (ES$^+$):** calcd for $C_{13}H_{13}N_5$ $[M+H]^+$ 240.1244, found 240.1233.

N-(6-phenyl-[1,2,4]triazolo[1,5-*a*]pyrazin-2-yl)acetamide (B3)

6-Phenyl-[1,2,4]triazolo[1,5-*a*]pyrazin-2-amine (55 mg, 0.26 mmol) was treated with acetic acid (30 mg, 0.5 mmol) and PCl_3 (68 mg, 0.5 mmol) in acetonitrile under microwave irradiation (150 °C). After 15 min of microwave irradiation and subsequent purification *N*-(6-phenyl-[1,2,4]triazolo[1,5-*a*]pyrazin-2-yl)acetamide was obtained in 9% (6 mg) yield**. ^{1}H-NMR:** (d_6-DMSO) δ (ppm) 11.12 (br s, 1H), 9.65 (d, J = 1.3 Hz, 1H), 9.29 (d, J = 1.3 Hz, 1H), 8.15 (d, J = 7.9 Hz, 2H), 7.53 (t, J = 7.7 Hz, 2H), 7.45 (t, J

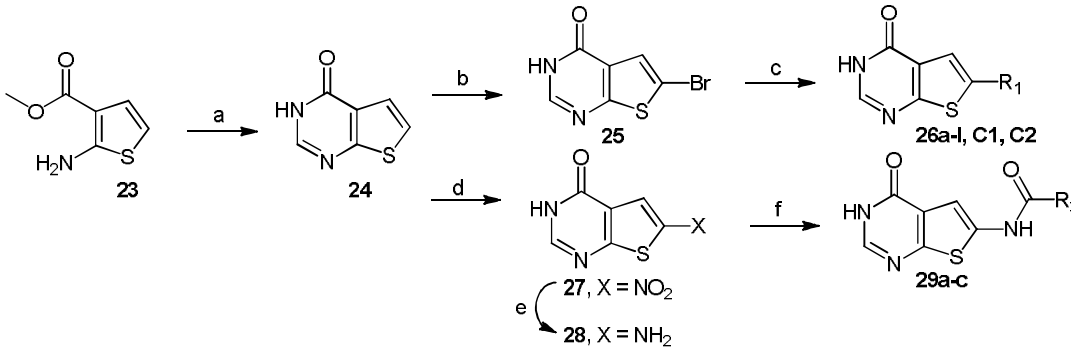
= 7.1 Hz, 1H), 2.19 (br s, 3H); **^{13}C-NMR:** (d_6-DMSO) δ (ppm) 159.40, 144.10, 140.14, 139.44, 135.44, 128.91, 126.04, 118.51, 23.72; **LRMS(ES$^+$):** m/z 254 $[M+H]^+$; **HRMS (ES$^+$):** calcd for $C_{13}H_{11}N_5O$ $[M+H]^+$ 254.1036, found 254.1030.

N-(6-(2,6-dimethylphenyl)-[1,2,4]triazolo[1,5-*a*]pyrazin-2-yl)acetamide (B4)

Following a procedure similar to the preparation of **B3, B4** was obtained from **B2** and acetic acid in low yield. **Yield:** 8 mg, 11%; **^{1}H-NMR:** (d_6-DMSO) δ (ppm) 11.12 (br s, 1H), 9.31 (d, J = 1.4 Hz. 1H), 9.07 (d, J = 1.4 Hz, 1H), 7.27 (t, J = 7.5 Hz, 1H), 7.18 (d, J = 7.5 Hz, 2H), 2.19 (br s, 3H), 2.07 (s, 6H); **^{13}C-NMR:** (d_6-DMSO) δ (ppm) 159.23, 143.75, 140.96, 139.91, 136.68, 136.15, 128.39, 127.49, 121.71, 23.68, 20.02; **LRMS(ES$^+$):** m/z 282 $[M+H]^+$; **HRMS (ES$^+$):** calcd for $C_{15}H_{15}N_5O$ $[M+Na]^+$ 304.1169, found 304.1194.

Synthesis of 6-substituted core fragment **C**

The synthesis of 6-substituted core fragment **C** followed Scheme 6. Starting from the commercially available thiophene **23**, the thienopyrimidine core fragment **24** was prepared according to Hesse *et al.* and Jung *et al.*(*54, 55*) Two different ways were used to introduce substituents on core fragment **24**. Compounds **26a-l, C1** and **C2** were prepared by the regioselective bromination of **24** at the 6-position to yield the bromo intermediate **25**, which was used for Suzuki cross-coupling with boronic acids. Compounds **29a-c** were obtained by the regioselective nitration of **24** at position six and subsequent reduction of the nitro group of intermediate **27** to give amino intermediate **28**, which was reacted with acid chlorides.



Scheme 5: Reagents and conditions: (a) Formamide, 200 °C; (b) Br_2, AcOH, rt; (c) $R_1B(OH)_2$,

K_3PO_4, PCy_3, $Pd_2(dba)_3$, dioxane/water, MW, 100 °C; (d) H_2SO_4, HNO_3, rt; (e) Pd on C 10%, H_2, MeOH, rt; (f) R_2COCl, MeOH, MW, 100 °C.

Thieno[2,3-*d*]pyrimidin-4(3*H*)-one (24)

Methyl 2-amino-thiophene-3-carboxylate (6.54 g, 41.6 mmol) was refluxed (200 °C) with formamide (5 eq.) for 3 h and left to stir overnight at room temperature. **Yield:** 3.1 g, 49%; **^{1}H-NMR:** (d_6-DMSO) δ (ppm) 12.01 (br s, 1H), 8.14 (s, 1H), 7.58 (d, J = 5.8 Hz, 1H), 7.40 (d, J = 5.8 Hz, 1H); **^{13}C-NMR:** (d_6-DMSO) δ (ppm) 164.21, 157.58, 145.71, 124.57, 123.71, 121.60; **LRMS(ES$^+$):** m/z 153 $[M+H]^+$.

6-Bromothieno[2,3-*d*]pyrimidin-4(3*H*)-one (25)

A mixture of thieno[2,3-*d*]pyrimidin-4(3*H*)-one (50 mg, 0.33 mmol), bromine (0.11 mL, 2 mmol) and acetic acid (1.5 mL) was stirred at room temperature for 2 h. The reaction mixture was concentrated under reduced pressure. The obtained residue was washed with water and dried under reduced pressure. **Yield:** 75 mg, 99%; **^{1}H-NMR:** (d_6-DMSO) δ (ppm) 12.67 (br s, 1H), 8.16 (s, 1H), 7.56 (s, 1H); **^{13}C-NMR:** (d_6-DMSO) δ (ppm) 164.93, 156.02, 146.47, 124.54, 110.27; **LRMS(ES$^+$):** m/z 232 $[M+H]^+$.

6-(3-(Morpholine-4-carbonyl)phenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (C1)

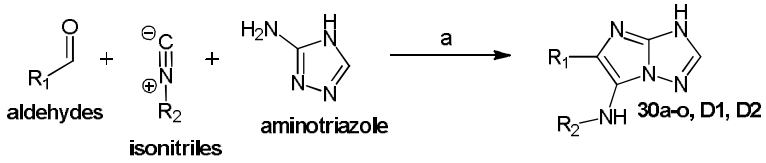
A mixture of $Pd_2(dba)_3$ (9.9 mg, 0.05 eq.), potassium phosphate (115 mg, 0.54 mmol, 2.5 eq.), tricyclohexylphosphine (7 mg, 0.12 eq.), 6-bromothien[2,3-*d*]pyrimidin-4(3*H*)-one (50 mg, 0.22 mmol, 1 eq.), (3-(morpholine-4-carbonyl)phenyl)boronic acid (61 mg, 0.26 mmol, 1.2 eq.), water (0.6 mL) and dioxane (0.6 mL) was stirred at 100 °C for 2 h. The reaction mixture was filtered through Celite, concentrated under reduced pressure and DCM was added to the obtained residue. The organic layer was washed with water, dried ($MgSO_4$) and concentrated. The crude material was purified by column chromatography on silica gel. **Yield:** 40 mg, 54%; **^{1}H-NMR:** (d_6-DMSO) δ (ppm) 12.60 (br s, 1H), 8.17 (s, 1H), 7.92 (s, 1H), 7.86 (d, J = 8.1 Hz, 1H), 7.80 (m, 1H), 7.54 (t, J = 7.7 Hz, 1H), 7.40 (d, J = 7.7 Hz, 1H), 3.66 – 3.38 (m, 8H); **^{13}C-NMR:** (d_6-DMSO) δ (ppm) 168.31, 163.66, 157.13, 146.19, 138.58, 136.73, 132.92, 129.48, 126.90, 126.75, 126.09, 124.01, 118.20,

65.99, 47.71, 42.02; **LRMS(ES$^+$):** m/z 342 $[M+H]^+$; **HRMS (ES$^+$):** calcd for $C_{17}H_{15}N_3O_3S$ $[M+H]^+$ 342.0907, found 342.0914.

6-(3,4-Dimethoxyphenyl)thieno[2,3-*d*]pyrimidin-4(3*H*)-one (C2)

Following a procedure similar to the preparation of **C1, C2** was obtained from **25** and the appropriate boronic acid in acceptable yield. **Yield:** 19 mg, 39%; **^{1}H-NMR:** (d_6-DMSO) δ (ppm) 12.52 (br s, 1H), 8.12 (s, 1H), 7.78 (s, 1H), 7.38 (d, J = 2.1 Hz, 1H), 7.24 (dd, J = 8.3, 2.1 Hz, 1H), 7.02 (d, J = 8.4 Hz, 1H), 3.87 (s, 3H), 3.80 (s, 3H); **^{13}C-NMR:** (d_6-DMSO) δ (ppm) 162.79, 157.10, 149.36, 149.22, 145.55, 139.94, 126.13, 125.55, 118.50, 116.25, 112.09, 109.26, 55.70, 55.59; **LRMS(ES$^+$):** m/z 289 $[M+H]^+$; **HRMS (ES$^+$):** calcd for $C_{14}H_{12}N_2O_3S$ $[M+H]^+$ 289.0641, found 289.0643.

Synthesis of 5,6-disubstituted core fragment **D** derivatives



Scheme 6: Reagents and conditions: (a) $HClO_4$, MeOH, rt

Synthesis of 5- and 6-substituted imidazotriazoles (core fragment **D**) was achieved by a very efficient three-component coupling reaction in which aminotriazole was reacted with commercially available aldehydes and isonitriles in the presence of catalytic amount of protic perchloric acid (Scheme 6).(*56*, *57*) Using this synthetic strategy, a library consisting of 17 compounds was prepared.

N-(*tert* -butyl)-5-cyclopropyl-3*H*-imidazo[1,2-*b*][1,2,4]triazol-6-amine (D1)

1,2,4-Triazol-3-amine (322 mg, 3.84 mmol) was dissolved in methanol (8 mL). Cyclopropanecarbaldehyde (405 mg, 5.79 mmol) and *tert*-butylisonitrile (0.50 mL, 4.42 mmol) were added at room temperature. One drop of perchloric acid was added, and the formation of the strongly UV-active adduct was followed by TLC. After 18 h at room temperature the crude reaction mixture was purified by medium pressure chromatography. **Yield:** 139 mg, 76%; **^{1}H-NMR:** (d_6-DMSO) δ (ppm) 11.02 (br s, 1H), 7.70 (s, 1H), 4.11 (s, 1H), 2.05 (m, 1H), 1.18 (s, 9H), 0.90 (m, 2H), 0.81 (m, 2H); **^{13}C-**

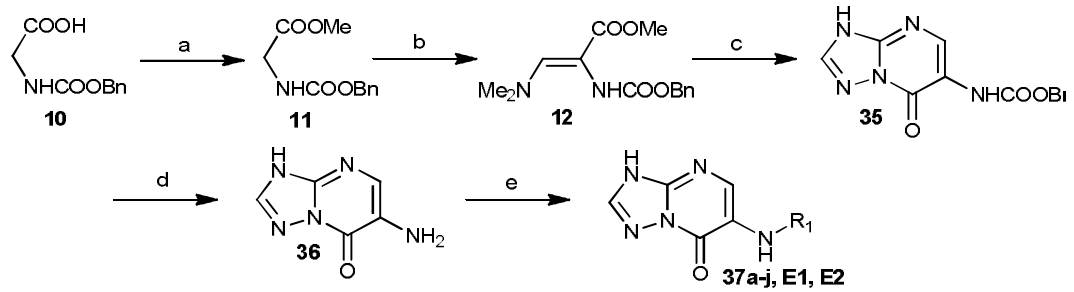
NMR: (d_6-DMSO) δ (ppm) 151.96, 147.15, 125.82, 120.35, 53.88, 29.96, 6.76, 6.50; **LRMS(ES$^+$):** m/z 220 $[M+H]^+$; **HRMS (ES$^+$):** calcd for $C_{11}H_{17}N_5$ $[M+H]^+$ 220.1557, found 220.1559.

5-((Benzyloxy)methyl)-*N*-(*tert*-butyl)-3*H*-imidazo[1,2-*b*][1,2,4]triazol-6-amine (D2)

Following a procedure similar to the preparation of **D1, D2** was obtained from 1,2,4-triazol-3-amine and the appropriate aldehyde and isonitrile in good yield. **Yield:** 177 mg, 71%; **^{1}H-NMR:** (CD_3OD) δ (ppm) 7.84 (s, 1H), 7.38 – 7.26 (m, 5H), 4.58 (s, 2H), 4.55 (s, 2H), 1.18 (s, 9H); **^{13}C-NMR:** (CD_3OD) δ (ppm) 153.59, 149.11, 139.23, 129.42, 129.16, 128.87, 123.81, 123.49, 73.54, 62.91, 55.28, 30.32; **LRMS(ES$^+$):** m/z 300 $[M+H]^+$; **HRMS (ES$^+$):** calcd for $C_{16}H_{22}N_5O$ $[M+H]^+$ 300.1819, found 300.1821.

Synthesis of 6-substituted core fragment **E** derivatives

The overall synthetic strategy for the synthesis of 6-substituted core fragment **E** derivatives **37a-j, E1** and **E2** consisted of five steps (Scheme 7).(*50*, *52*) In the first step, the carboxylic acid group of *N*-protected glycine **10** was esterified with methanol by using thionyl chloride. The resulting glycine ester **11** was then reacted with Bredereck's reagent to give propenoate **12**. Subsequent heating of intermediate **12** with 1,2,4-triazol-3-amine in acetic acid afforded the desired intermediate **35**. In the following step, intermediate **35** was deprotected with 33% HBr in acetic acid mixture to give the intermediate **32.** Finally, compounds **37a-j, E1** and **E2** obtained by reaction with the corresponding and commercially available sulfonyl chlorides, acid chlorides, isocyanates and isothiocyanates.



Scheme 7: Reagents and conditions: (a) $SOCl_2$, MeOH, 0 °C-rt; (b) Bredereck's reagent, toluene,

115 °C; (c) 1,2,4-triazol-3-amine, NaOAc, AcOH, 90 °C; (d) 33% HBr-AcOH, 50 °C; (e) R_1SO_2Cl or R_1COCl or R_1NCO or R_1NCS, DCM, MW, 110 °C.

Benzyl (7-oxo-3,7-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-6-yl)carbamate (35)

A mixture of 1,2,4-triazol-3-amine (126 mg, 1.5 mmol), anhydrous sodium acetate (123 mg, 1.5 mmol), acetic acid (3 mL), and (*Z*)-methyl 2-(((benzyloxy)carbonyl)amino)-3-(dimethylamino)acrylate (417 mg, 1.5 mmol) was stirred at 90 °C for 10 h before cooling to room temperature. Water (1 mL) was added, the suspension was stirred at 10 °C for 10 h, and the precipitate was collected by filtration and washed with water (5 mL). The reaction vessel with product was put in a desiccator and dried *in vacuo* over P_4O_{10} at room temperature for 24 h. **Yield:** 389 mg, 91%; **^{1}H-NMR:** (d_6-DMSO) δ (ppm) 8.95 (br s, 1H), 8.30 (s, 1H), 8.26 (s, 1H), 7.43 – 7.34 (m, 5H), 5.14 (s, 2H); **^{13}C-NMR:** (d_6-DMSO) δ (ppm) 155.19, 154.34, 152.38, 150.48, 137.23, 136.69, 128.36, 127.88, 127.82, 111.20; **LRMS(ES$^+$):** m/z 286 $[M+H]^+$; **HRMS (ES$^+$):** calcd for $C_{13}H_{11}N_5O_3$ $[M+H]^+$ 286.0935, found 286.0933.

6-Amino-[1,2,4]triazolo[1,5-*a*]pyrimidin-7(3*H*)-one (36)

A mixture of benzyl (7-oxo-3,7-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-6-yl)carbamate (5 g, 17.5 mmol) and hydrogen bromide in acetic acid (33%) was heated at 50 °C for 3 h. The reaction mixture was cooled to 20 °C, the precipitate collected by filtration, washed with ethyl acetate, and dried *in vacuo* over sodium hydroxide pellets at room temperature for 24 h to give fused heteroarylamine hydrobromide. **Yield:** 3.174 g, 78%; **^{1}H-NMR:** (d_6-DMSO) δ (ppm) 7.88 (s, 1H), 7.53 (s, 1H); **^{13}C-NMR:** (d_6-DMSO) δ (ppm) 153.63, 152.97, 151.99, 131.65, 119.98; **LRMS(ES$^+$):** m/z 152 $[M+H]^+$; **HRMS (ES$^+$):** calcd for $C_5H_5N_5O$ $[M+H]^+$ 152.0567, found 152.0573.

N-(7-oxo-3,7-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-6-yl)benzamide (E1)

A mixture of benzoyl chloride (50 mg, 0.36 mmol, 1.1 eq.), triethylamine (37 mg, 0.36 mmol, 1.1 eq.), 6-amino-[1,2,4]triazolo[1,5-*a*]pyrimidin-7(3H)-one (50 mg, 0.33 mmol, 1.0 eq.) and DCM (2 mL) was heated by microwave irradiation for 15 min at 110 °C. The

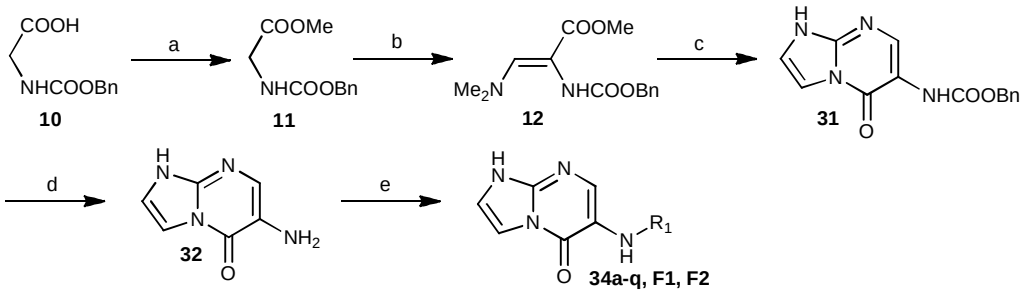
reaction mixture was concentrated and purified by medium pressure chromatography. **Yield:** 58 mg, 69%; **^{1}H-NMR:** (d_6-DMSO) δ (ppm) 13.56 (br s, 1H), 9.75 (s, 1H), 8.46 (s, 1H), 8.33 (s, 1H), 8.00 (d, J = 7.3 Hz, 2H), 7.62 (t, J = 7.3 Hz, 1H), 7.55 (t, J = 7.3 Hz, 2H); **^{13}C-NMR:** (d_6-DMSO) δ (ppm) 166.11, 154.09, 152.48, 149.60, 136.17, 133.59, 131.85, 128.46, 127.64, 111.53; **LRMS(ES$^+$):** m/z 256 $[M+H]^+$; **HRMS (ES$^+$):** calcd for $C_{12}H_9N_5O_2$ $[M+H]^+$ 256.0829, found 256.0834.

1-(6-Methoxypyridin-3-yl)-3-(7-oxo-3,7-dihydro-[1,2,4]triazolo[1,5-*a*]pyrimidin-6-yl)thiourea (E2)

Following a procedure similar to the preparation of **E1, E2** was obtained from **36** and the appropriate acid chloride in acceptable yield. **Yield:** 46 mg, 44%; **^{1}H-NMR:** (CD_3OD) δ (ppm) 8.20 (s, 1H), 8.12-8.11 (m, 2H), 7.81 (d, J = 8.8 Hz, 1H), 6.78 (d, J = 8.8 Hz, 1H), 3.91 (s, 3H); **^{13}C-NMR:** (CD_3OD) δ (ppm) 184.99, 163.53, 157.95, 153.50, 152.38, 145.26, 139.40, 131.73, 110.91, 54.28; **LRMS(ES$^+$):** m/z 318 $[M+H]^+$; **HRMS (ES$^+$):** calcd for $C_{12}H_{11}N_7O_2S$ $[M+H]^+$ 318.0768, found 318.0752.

Synthesis of 6-substituted core fragment **F** derivatives

The overall synthetic strategy for the synthesis of 6-substituted core fragment **F** derivatives **34a-q, F1** and **F2** consisted of five steps (Scheme 8).(*50*, *52*) In the first step, the carboxylic acid group of *N*-protected glycine **10** was esterified with methanol by using thionyl chloride. The resulting glycine ester **11** was then reacted with Bredereck's reagent to give propenoate **12**. Subsequent heating of intermediate **12** with 2-aminoimidazole in acetic acid afforded the desired intermediate **31**. In the following step, intermediate **31** was hydrogenolysed on Pd/C to give the intermediate **32.** Finally, compounds **34a-q**, **F1** and **F2** obtained by reaction with the corresponding and commercially available sulfonyl chlorides, acid chlorides, isocyanates and isothiocyanates.



Scheme 8: Reagents and conditions: (a) $SOCl_2$, MeOH, 0 °C-rt; (b) Bredereck's reagent, toluene, 115 °C; (c) 2-aminoimidazole, NaOAc, AcOH, 90 °C; (d) H_2, 10% Pd/C, EtOH, H-Cube®; (e) R_1SO_2Cl or R_1COCl or R_1NCO or R_1NCS, DCM, MW, 110 °C.

Benzyl (5-oxo-1,5-dihydroimidazo[1,2-*a*]pyrimidin-6-yl)carbamate (31)

Following a procedure similar to the preparation of **35, 31** was obtained from **12** and 1*H*-imidazol-2-amine in good yield. **Yield:** 4.03 g, 75%; **^{1}H-NMR:** (d_6-DMSO) δ (ppm) 12.68 (br s, 1H), 8.56 (br s, 1H), 8.05 (br s, 1H), 7.65 (d, J = 2.5 Hz, 1H), 7.55 (d, J = 2.5 Hz, 1H), 7.41 – 7.34 (m, 5H), 5.11 (s, 2H); **^{13}C -NMR:** (d_6-DMSO) δ (ppm) 171.95, 155.43, 154.17, 148.71, 145.67, 136.91, 128.33, 127.78, 119.39, 110.43, 107.18, 65.66; **LRMS(ES$^+$):** m/z 285 $[M+H]^+$; **HRMS (ES$^+$):** calcd for $C_{14}H_{12}N_4O_3$ $[M+H]^+$ 285.0982, found 285.0975.

6-Aminoimidazo[1,2-*a*]pyrimidin-5(1*H*)-one (32)

Using 10% Pd/C as catalyst, benzyl (5-oxo-1,5-dihydroimidazo[1,2-*a*]pyrimidin-6-yl)carbamate (2.00 g, 7 mmol) in methanol (200 mL) was pumped through the H-Cube® at a 1 mL/min flow rate. The applied temperature was 60 °C. The product mixture was analysed by LCMS. **Yield:** 4.03 g, 75%; **^{1}H-NMR:** (d_6-DMSO) δ (ppm) 7.53 (s, 1H), 7.47 (d, J = 2.4 Hz, 1H), 7.39 (d, J = 2.4 Hz, 1H); **^{13}C -NMR:** (d_6-DMSO) δ (ppm) 152.68, 142.57, 131.97, 121.68, 120.10, 105.25; **LRMS(ES$^+$):** m/z 151 $[M+H]^+$; **HRMS (ES$^+$):** calcd for $C_6H_6N_4O$ $[M+H]^+$ 151.0614, found 151.0628.

N-(5-oxo-1,5-dihydroimidazo[1,2-*a*]pyrimidin-6-yl)benzamide (F1)

Following a procedure similar to the preparation of **E1, F1** was obtained from **32** and the appropriate acid chloride in good yield. **Yield:** 38 mg, 89%; **^{1}H-NMR:** (d_6-DMSO) δ (ppm) 12.90 (br s, 1H), 9.54 (s, 1H), 8.22 (s, 1H), 8.00 (d, J = 7.0 Hz, 2H), 7.69 (d, J =

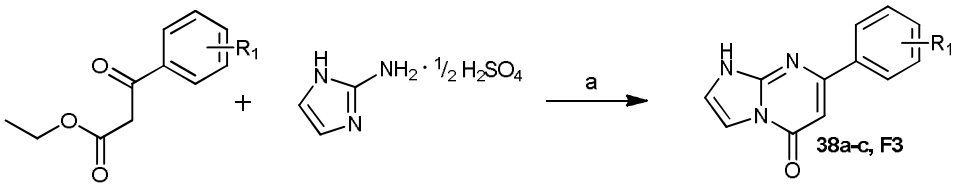
2.4 Hz, 1H), 7.62 – 7.50 (m, 4H); **^{13}C -NMR:** (d_6-DMSO) δ (ppm) 165.92, 153.87, 148.68, 145.71, 134.19, 131.49, 128.36, 127.53, 119.56, 110.50, 107.21; **LRMS(ES$^+$):** m/z 255 $[M+H]^+$; **HRMS (ES$^+$):** calcd for $C_{13}H_{10}N_4O_2$ $[M+H]^+$ 255.0877, found 255.0865.

1-Benzyl-3-(5-oxo-1,5-dihydroimidazo[1,2-a]pyrimidin-6-yl)thiourea (F2)

Following a procedure similar to the preparation of **E1, F2** was obtained from **32** and the appropriate acid chloride. **Yield:** 19 mg, 39%; **^{1}H-NMR:** (d_6-DMSO) δ (ppm) 12.92 (br s, 1H), 8.88 (s, 1H), 8.08 (br s, 1H), 7.99 (br s, 1H), 7.70 (d, J = 2.4 Hz, 1H), 7.55 (d, J = 2.4 Hz, 1H), 7.31 – 3.29 (m, 4H), 7.24 – 7.20 (m, 1H), 4.71 (d, J = 5.8 Hz, 2H); **^{13}C -NMR:** (d_6-DMSO) δ (ppm) 182.54, 154.44, 151.16, 146.31, 139.48, 132.79, 128.04, 126.96, 126.49, 119.31, 107.66, 47.32; **LRMS(ES$^+$):** m/z 300 $[M+H]^+$; **HRMS (ES$^+$):** calcd for $C_{14}H_{13}N_5OS$ $[M+H]^+$ 300.0914, found 300.0916.

Synthesis of 7-substituted core fragment **F** derivatives

The overall synthetic strategy for the synthesis of the 5-substituted core fragment **F** derivatives consists of one step (Scheme 9). It involves the condensation of 2-aminoimidazole with a set of β-ketoesters.(*58*) Due to the limited number of readily available starting materials (β-ketoesters) to elaborate this scaffold, only a small set of compounds was synthesized.

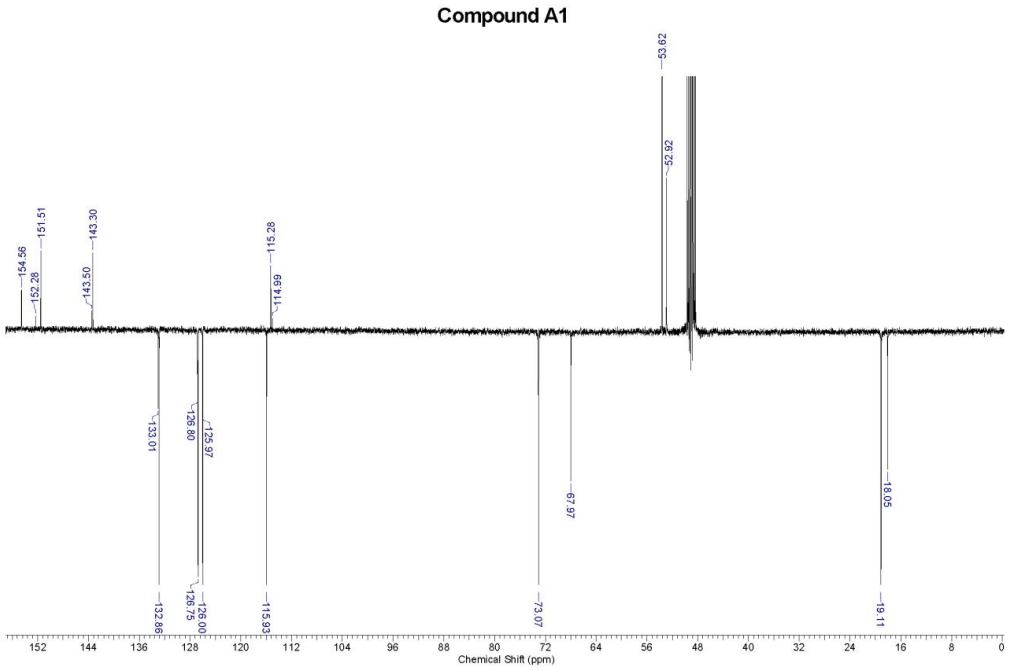


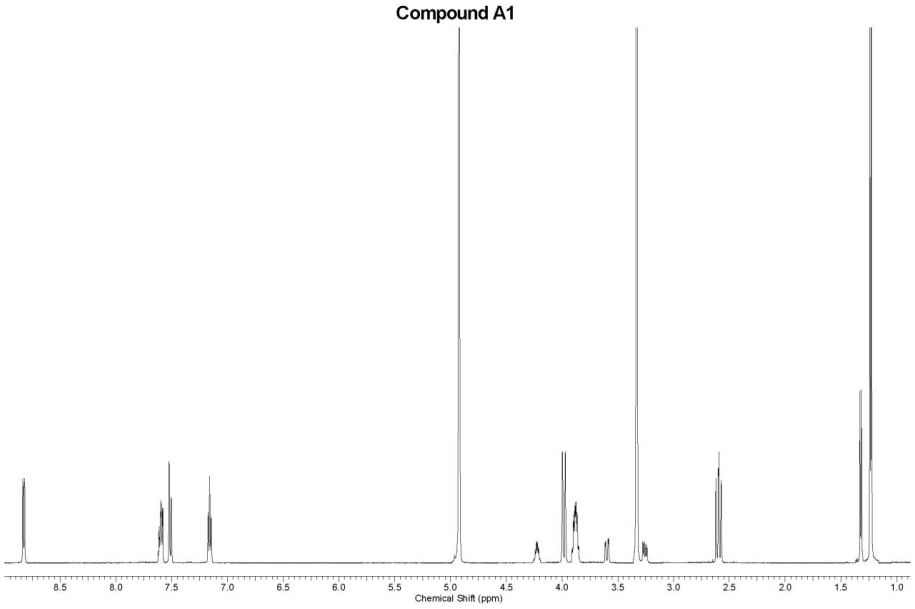
Scheme 9: Reagents and conditions: (a) PPA, 120°C

7-(2-Fluorophenyl)imidazo[1,2-*a*]pyrimidin-5(1*H*)-one (F3)

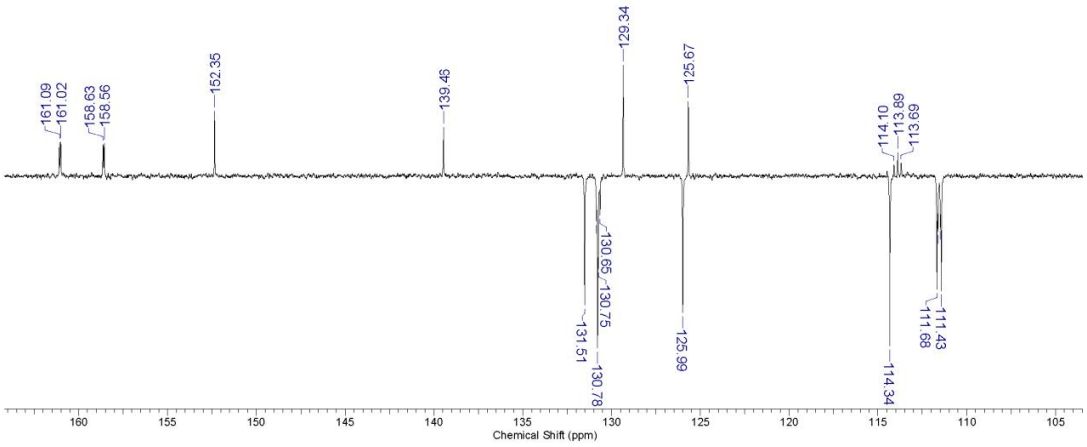
A stirred solution of 2-aminoimidazole sulfate (75 mg, 0.57 mmol) and ethyl 3-(2-fluorophenyl)-3-oxopropanoate (119 mg, 0.57 mmol) in polyphosphoric acid (PPA, 10 mL) was heated under reflux conditions (120 - 130 °C) for 3-4 h. The mixture was then cooled to 50 °C, and poured onto cold water (60 mL) with vigorous stirring. The precipitated light brown product was collected by suction filtration, washed with water (2 x 10 mL) and dried. **Yield:** 58 mg, 45%; **^{1}H-NMR:** (d_6-DMSO) δ (ppm) 12.92 (br s, 1H), 7.92 (t, J = 7.8 Hz, 1H), 7.66 (d, J = 2.4 Hz, 1H), 7.61 (d, J = 2.4 Hz, 1H), 7.51 (m, 2H), 7.35 – 7.31 (m, 2H), 6.26 (s, 1H); **^{13}C -NMR:** (d_6-DMSO) δ (ppm) 159.82 (d, J = 250.8 Hz), 156.82, 156.58, 147.25, 131.42 (d, J = 7.5 Hz), 130.52, 126.14 (d, J = 11.0 Hz), 124.59, 118.00, 116.35 (d, J = 21.8 Hz), 106.83, 98.09 (d, J = 7.3 Hz); **LRMS(ES^+):** m/z 230 $[M+H]^+$; **HRMS (ES^+):** calcd for $C_{12}H_8FN_3O$ $[M+H]^+$ 230.0724, found 230.0718.

NMR spectra of key compounds





Compound A2



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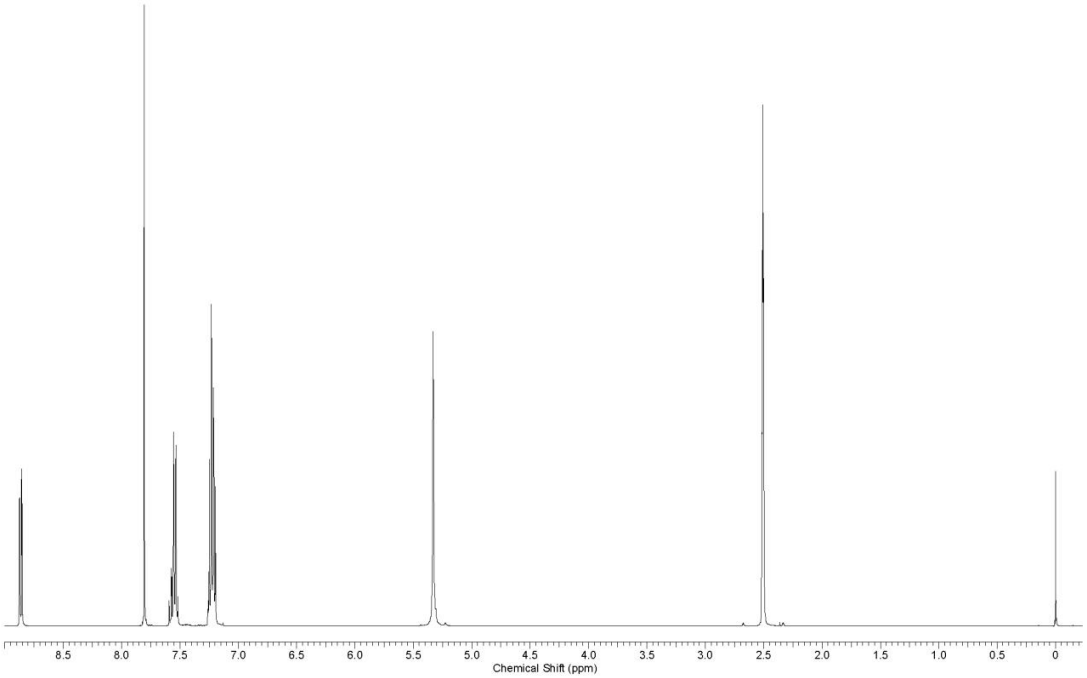
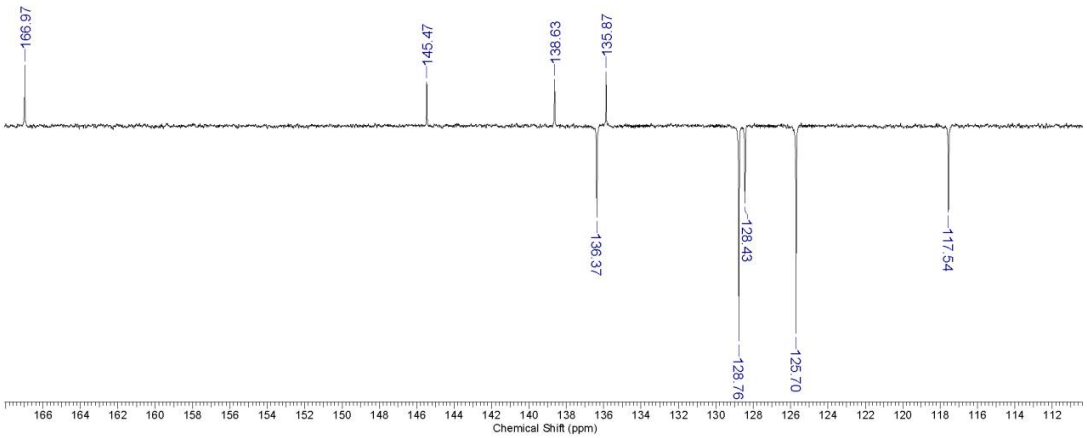
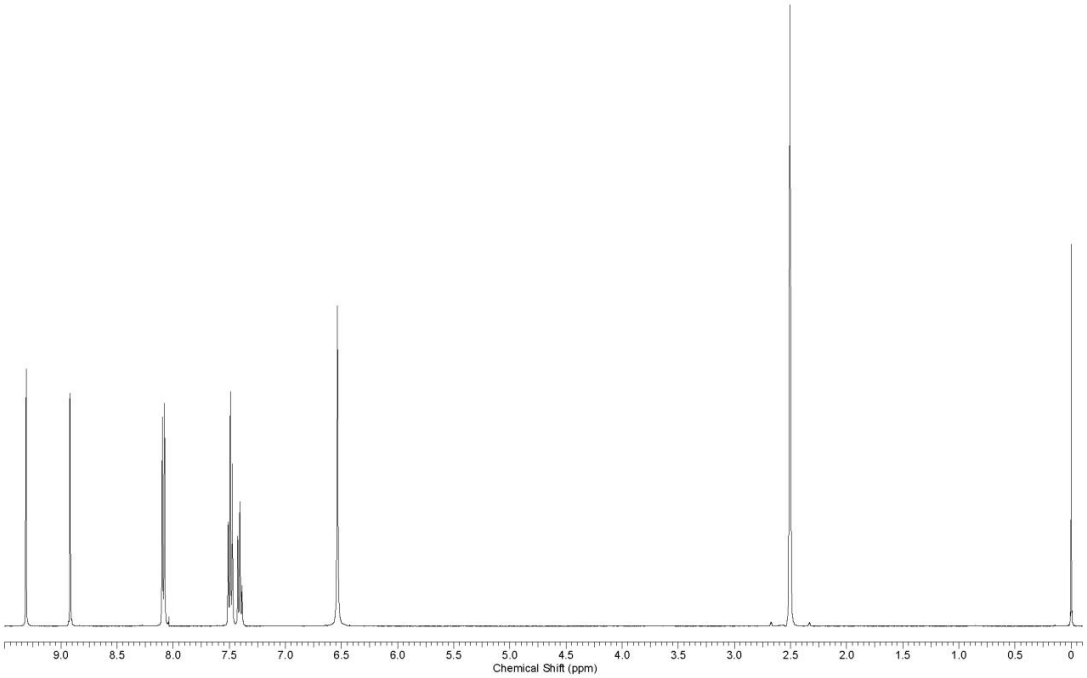


Compound B1



Compound B1



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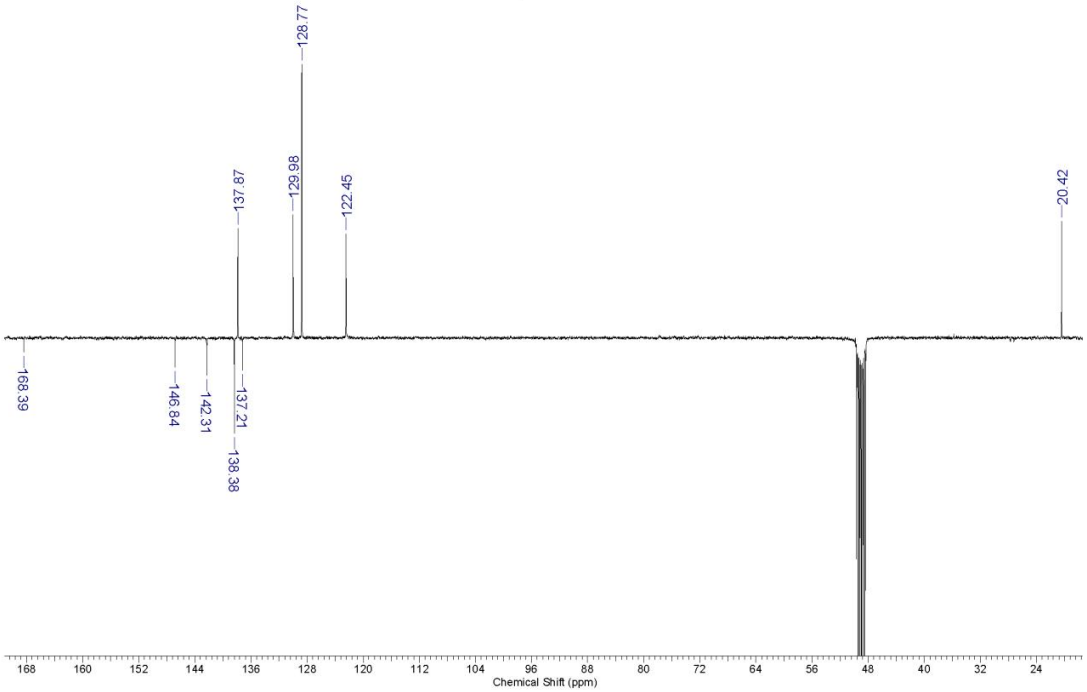
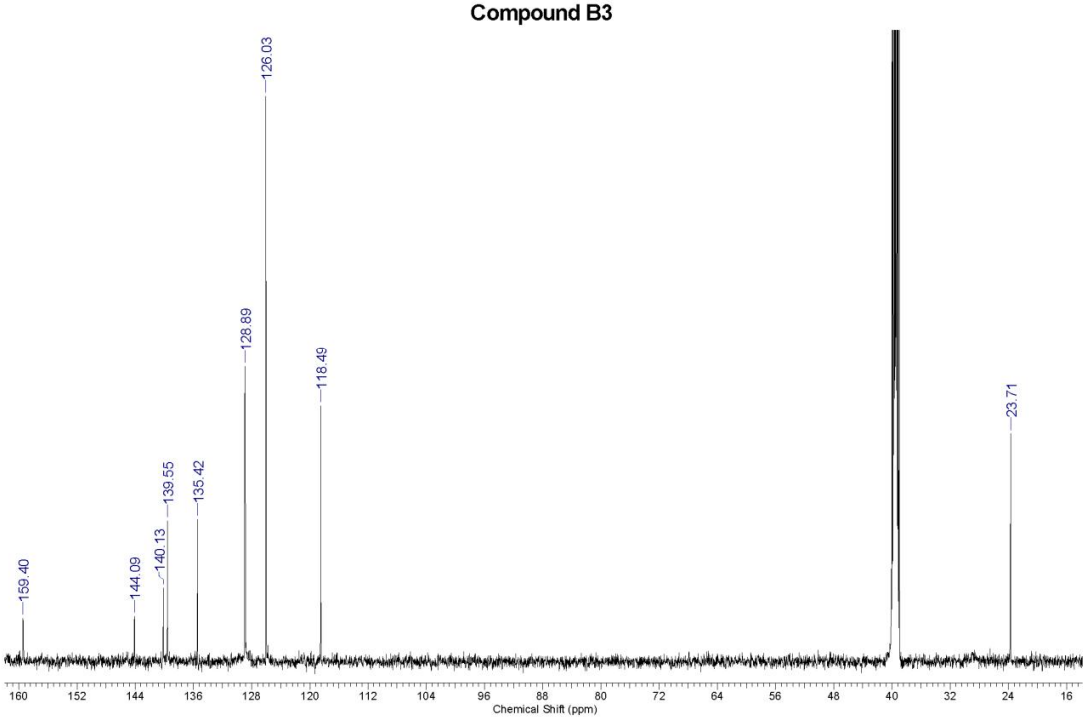
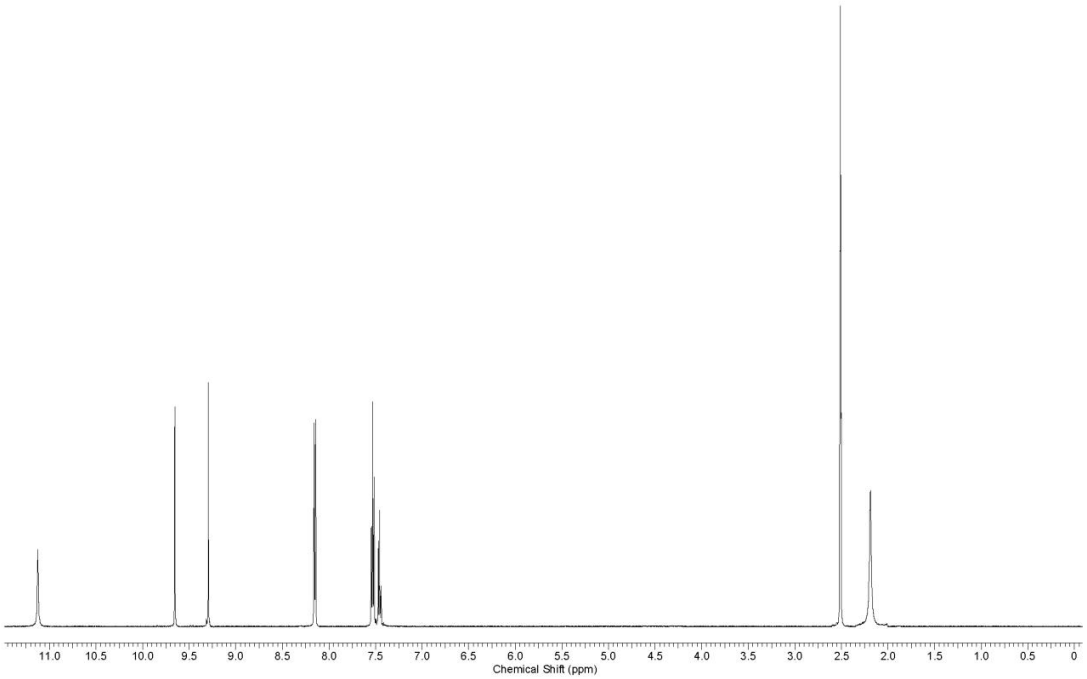


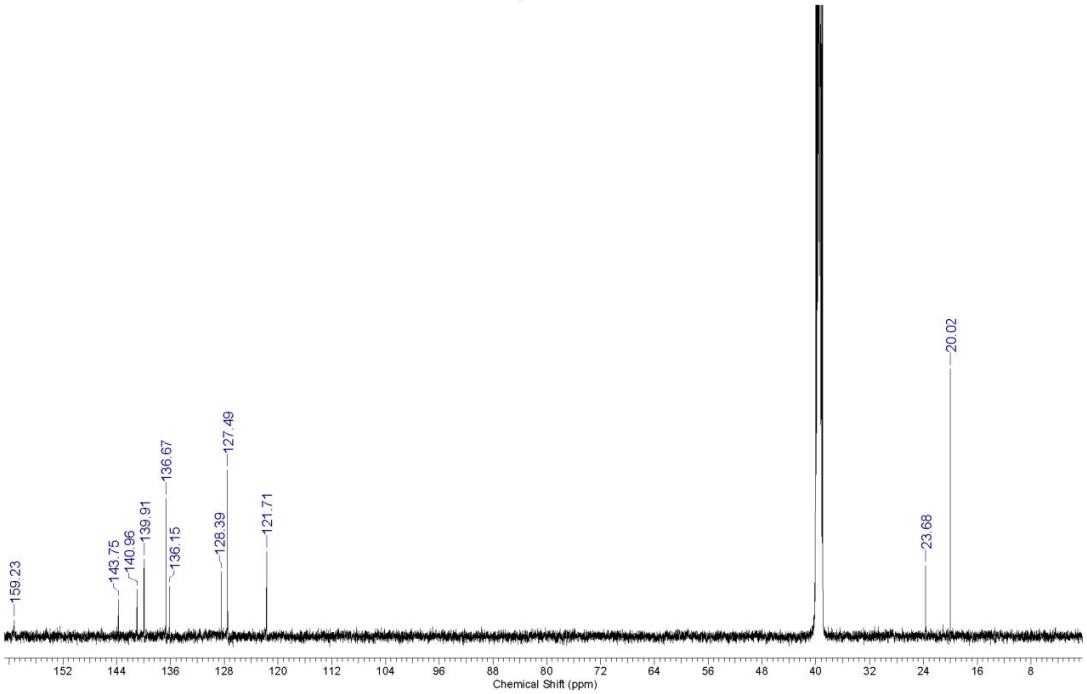
Compound B3



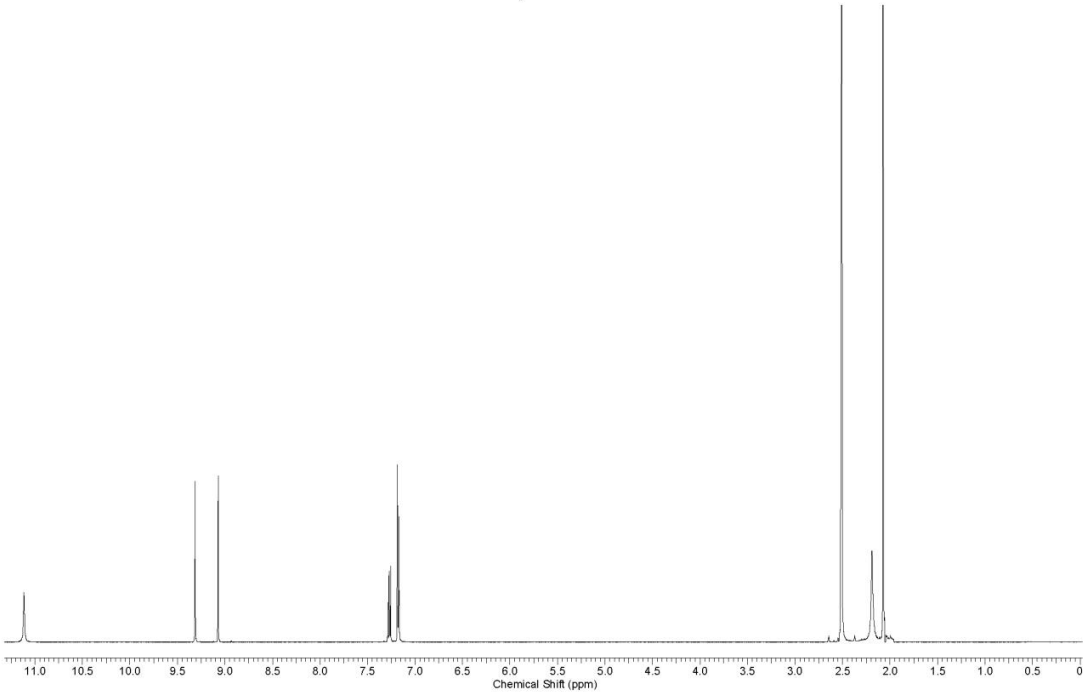
Compound B3



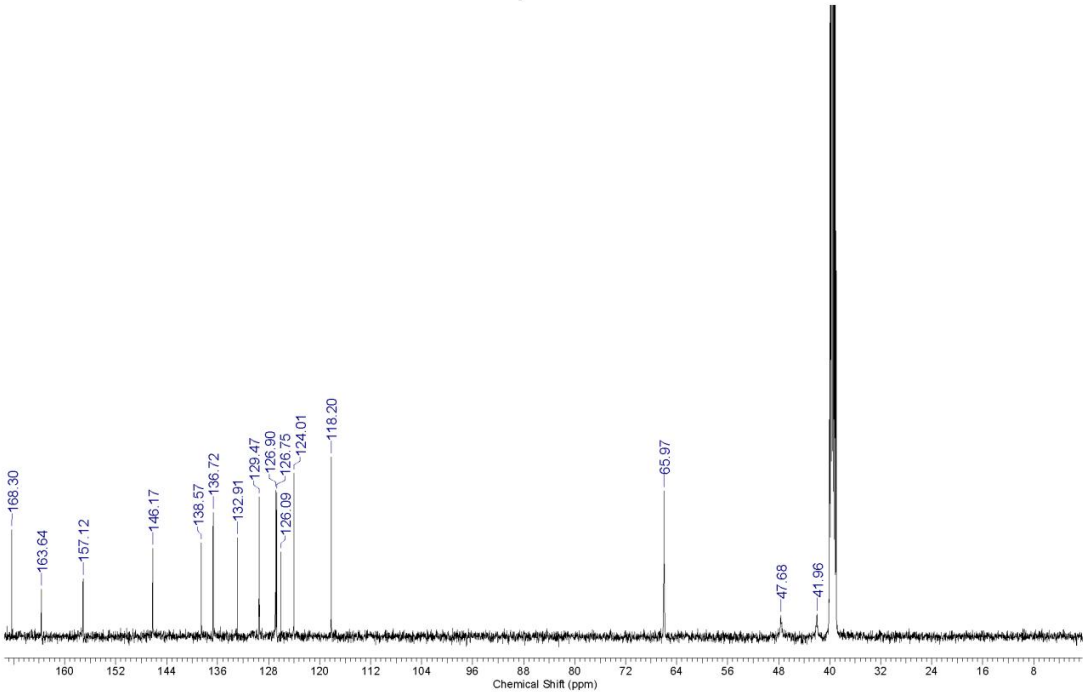
Compound B4



Compound B4



Compound C1



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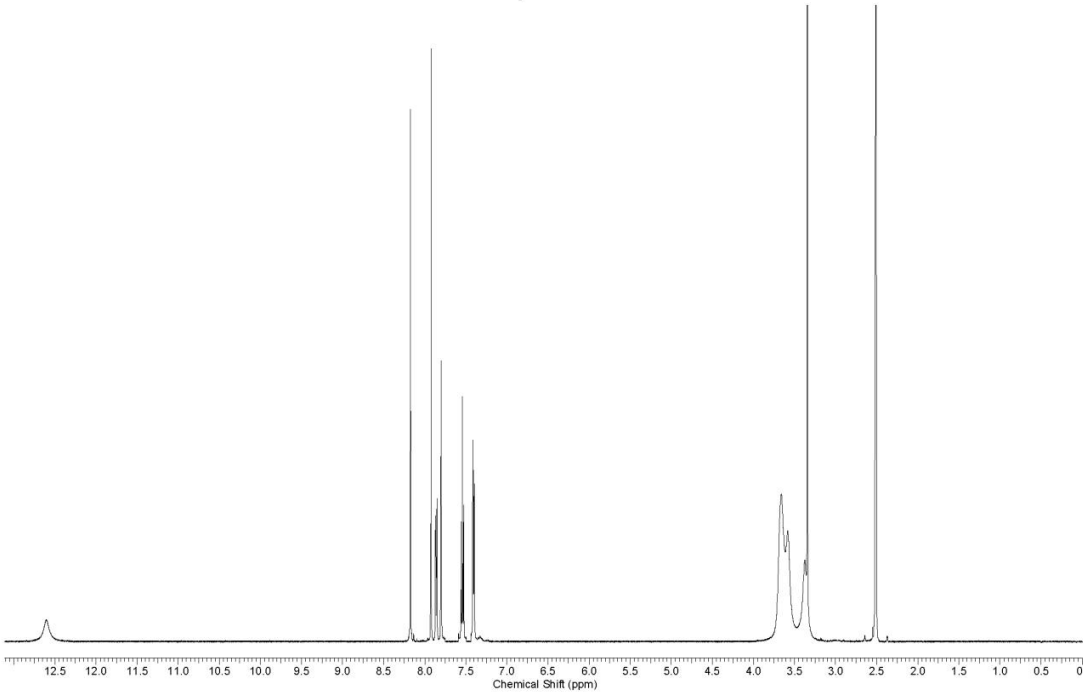

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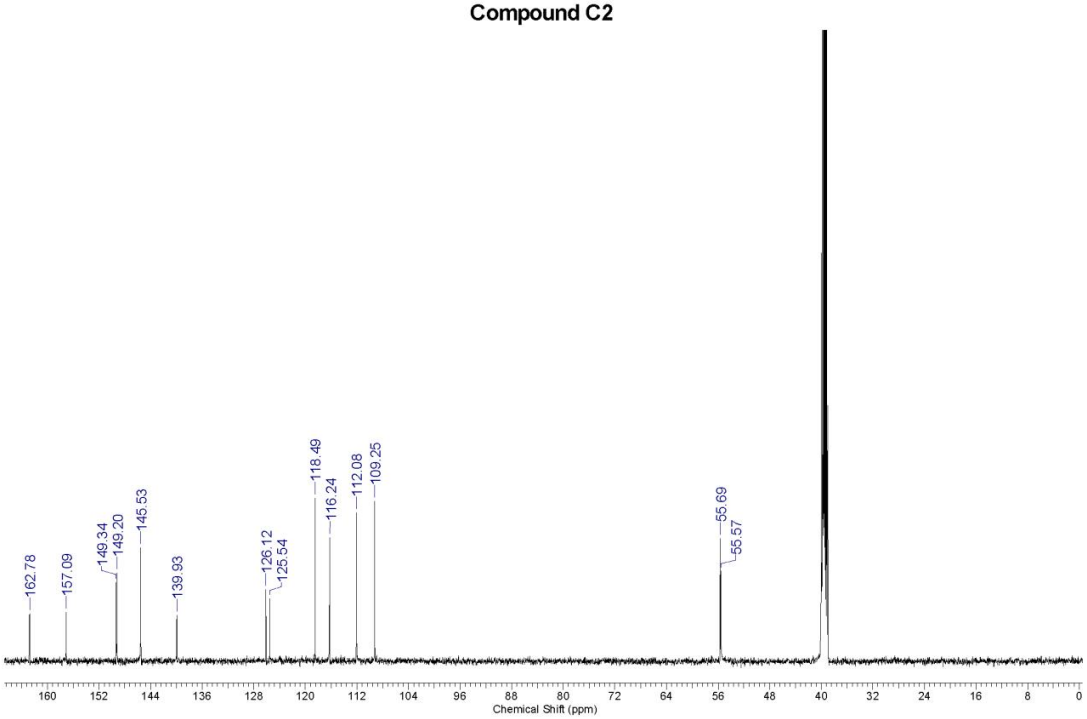

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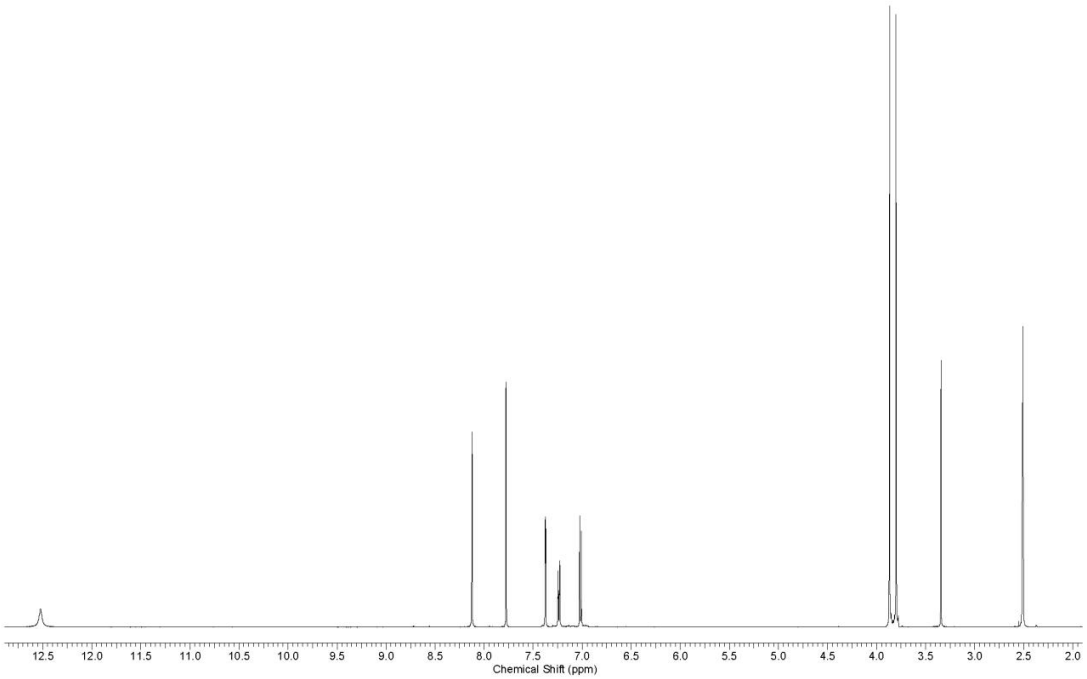

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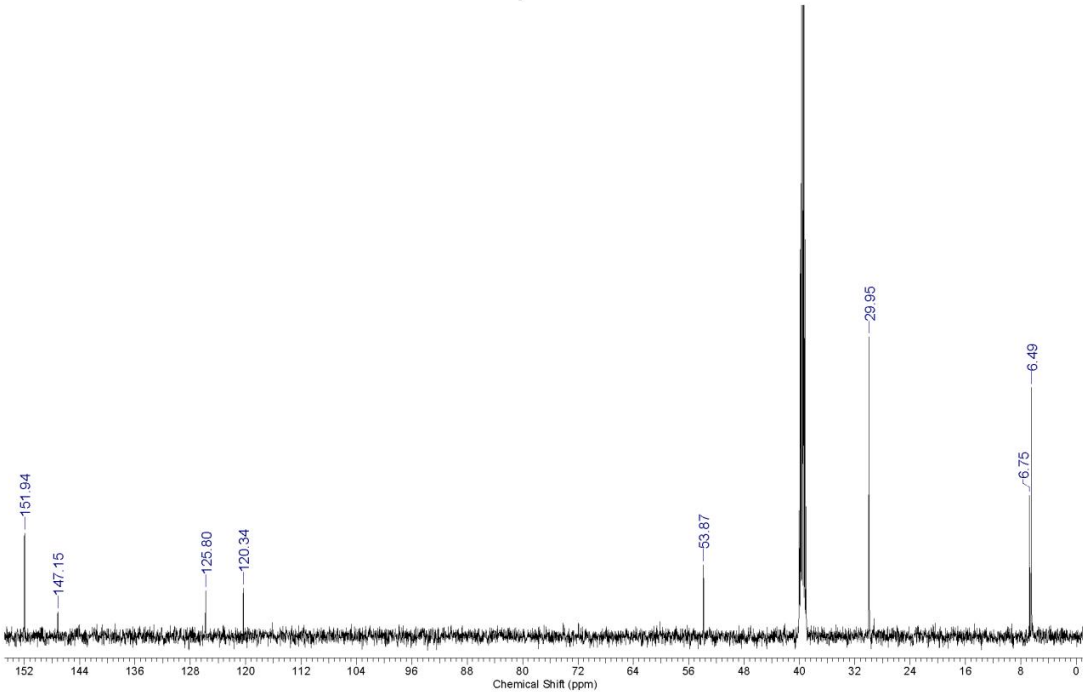

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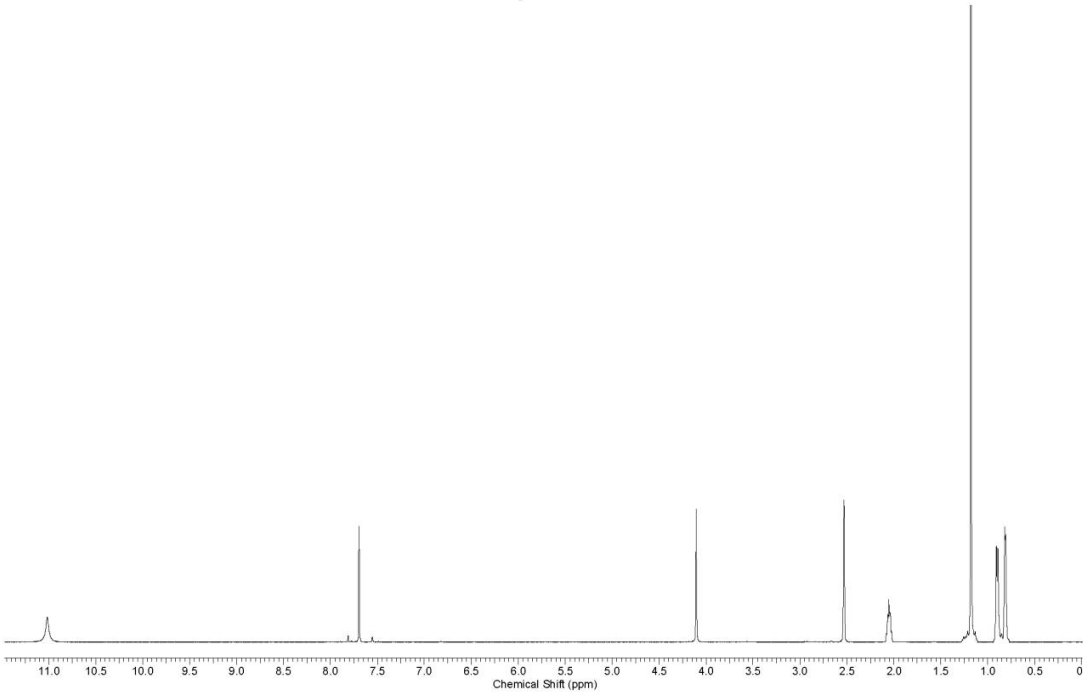

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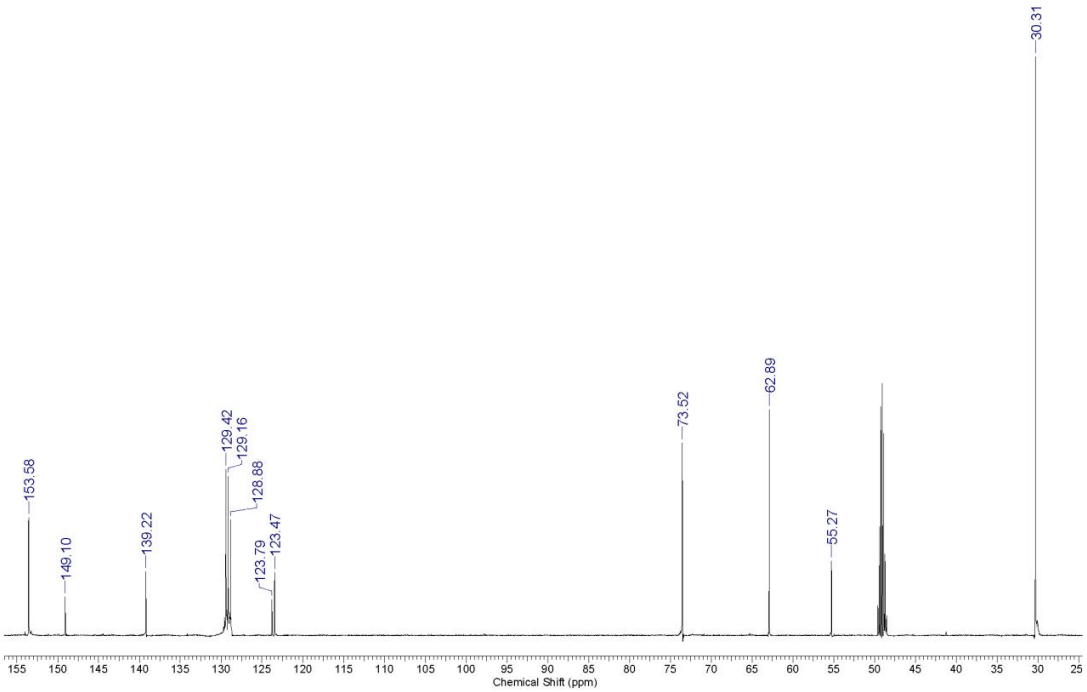

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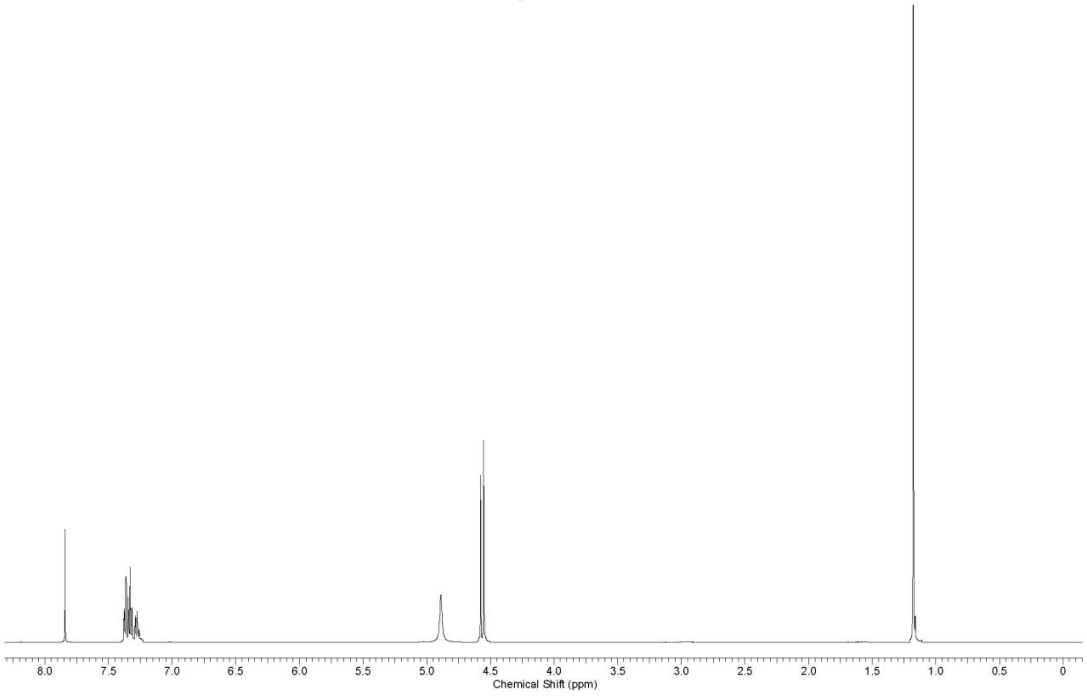

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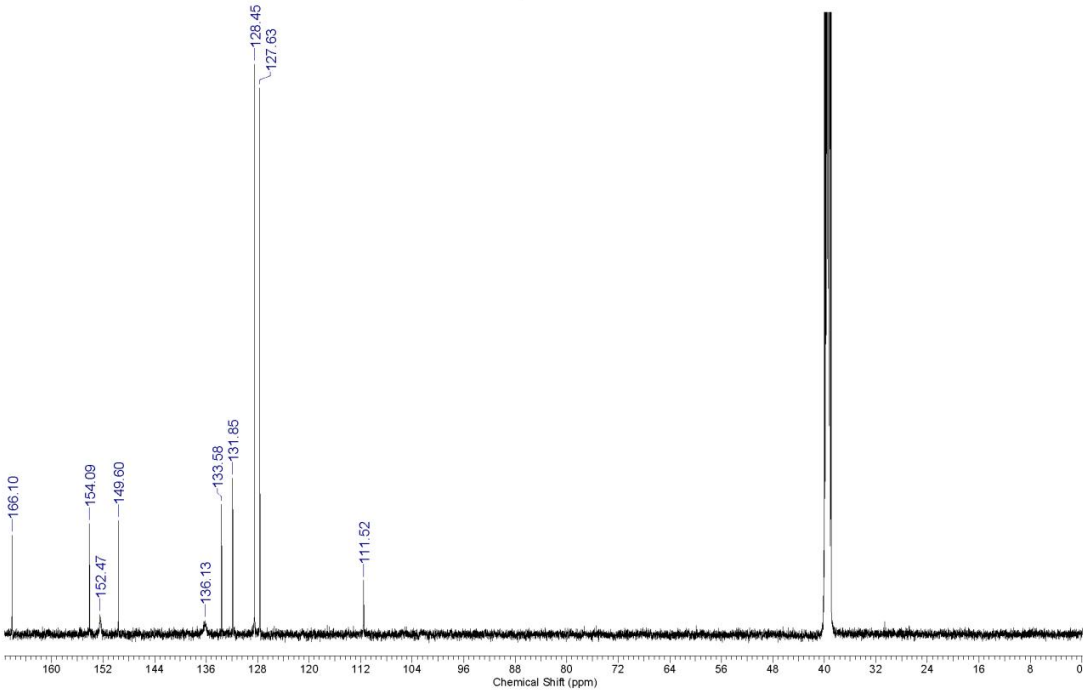

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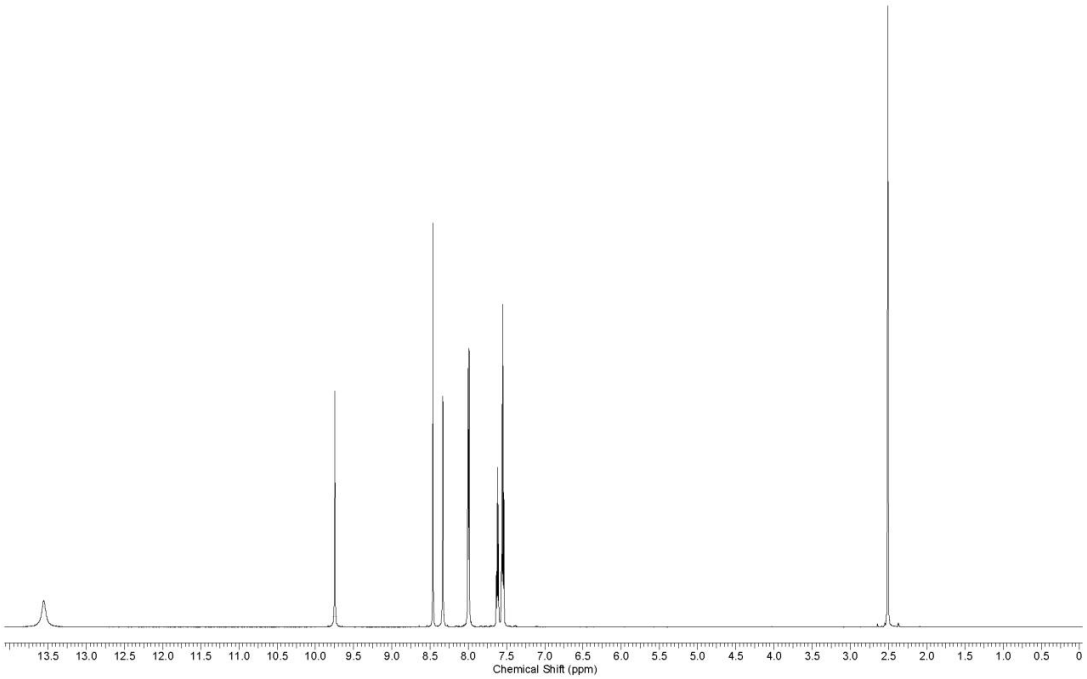

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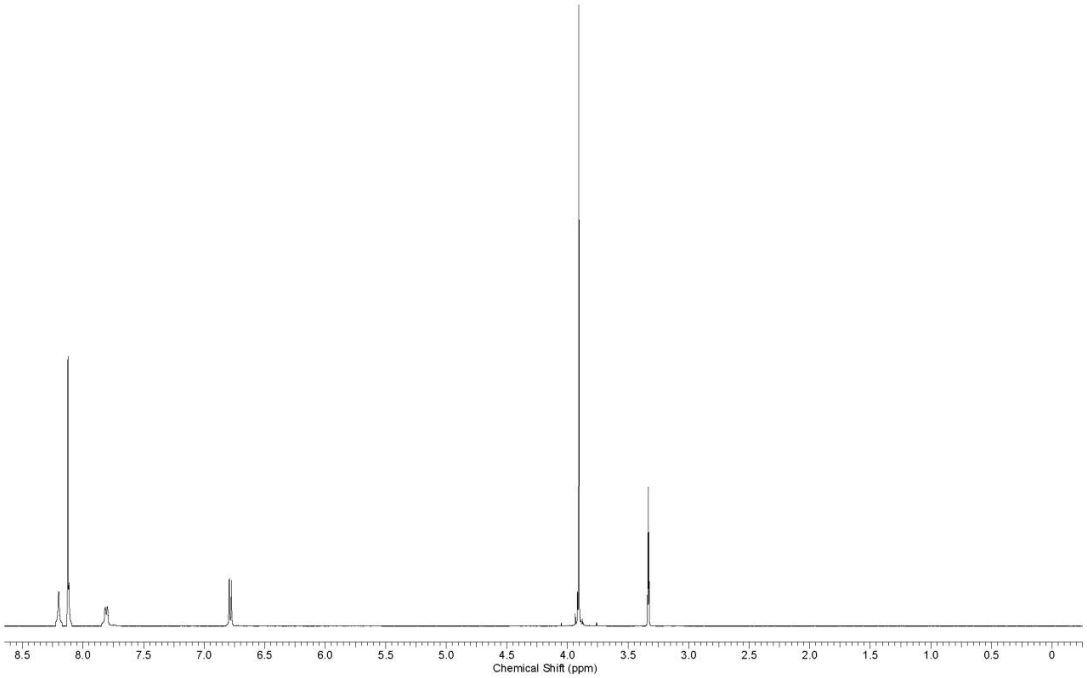

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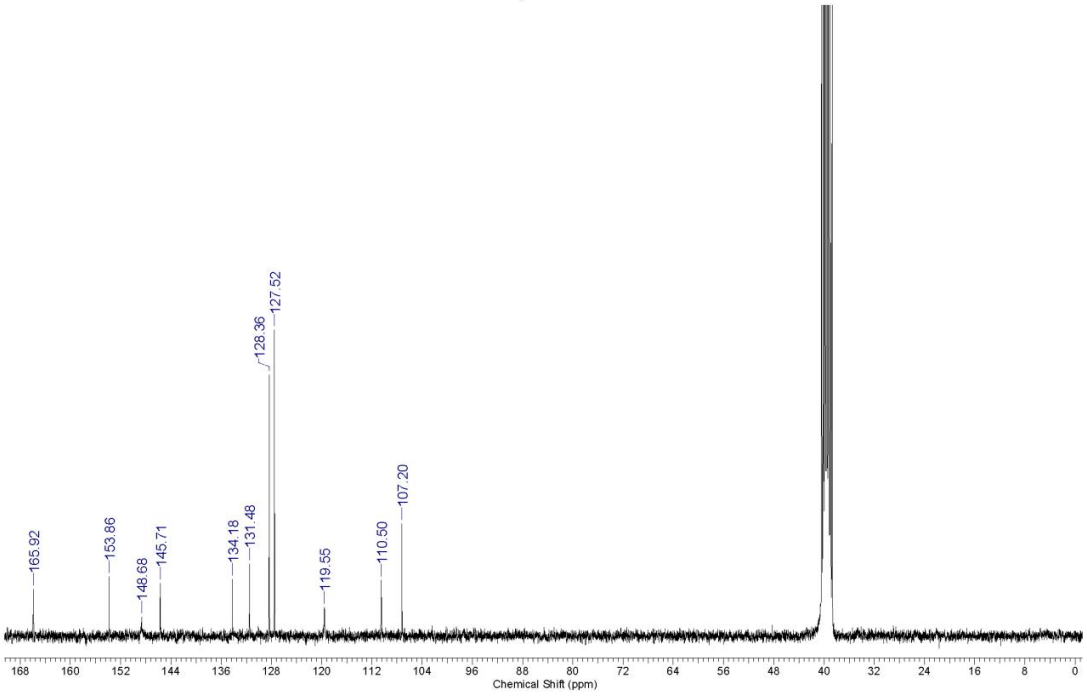

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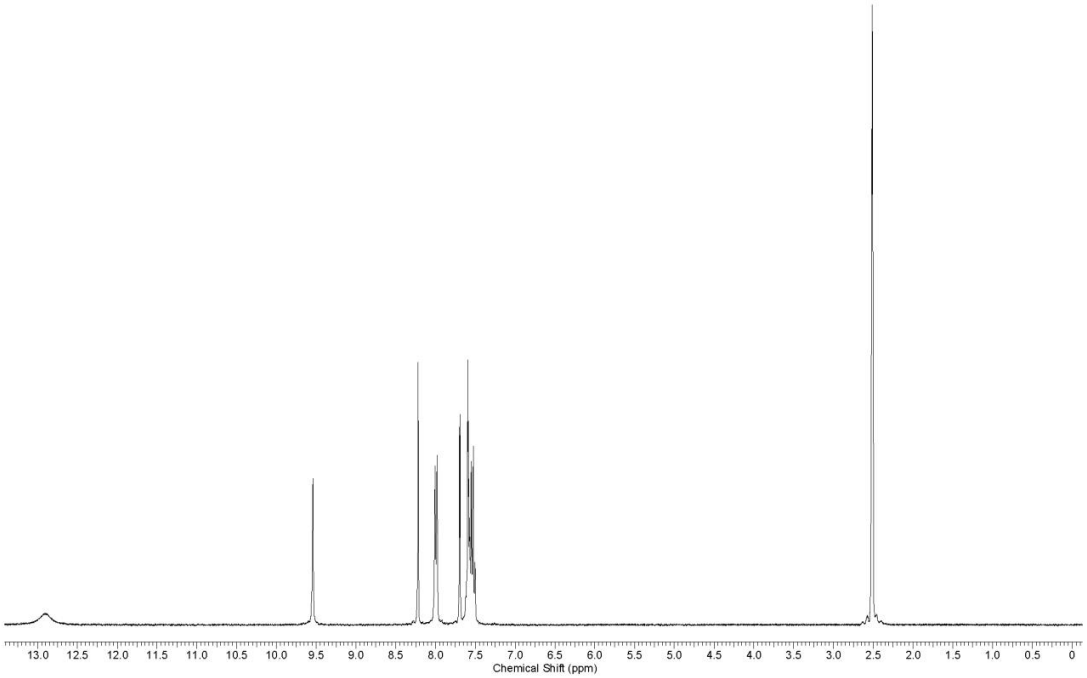

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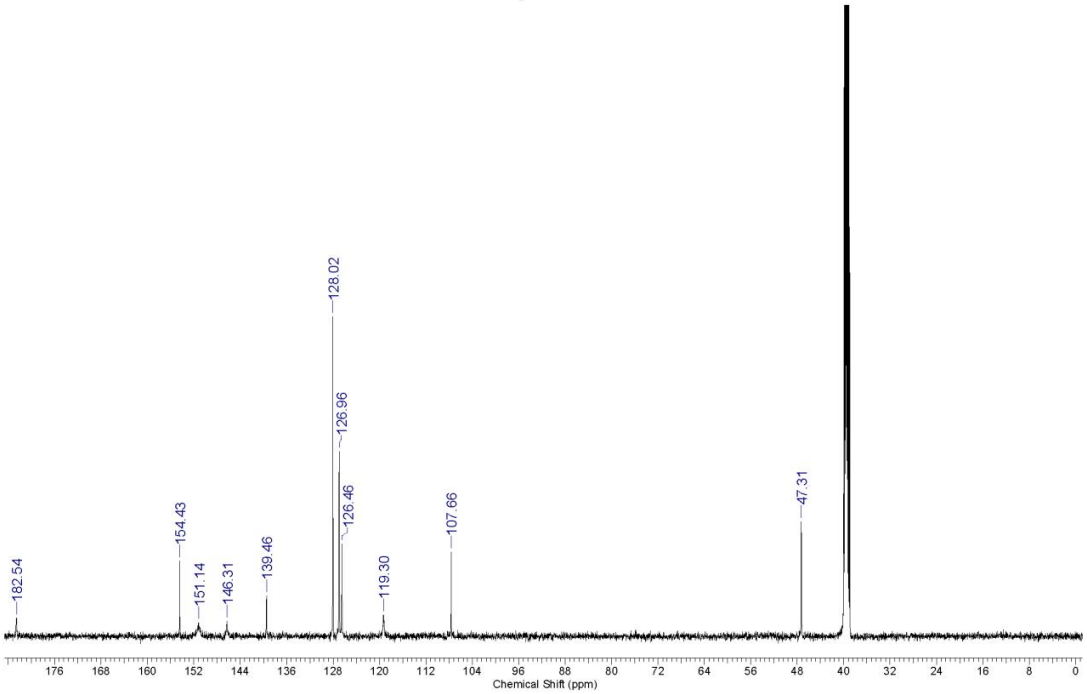

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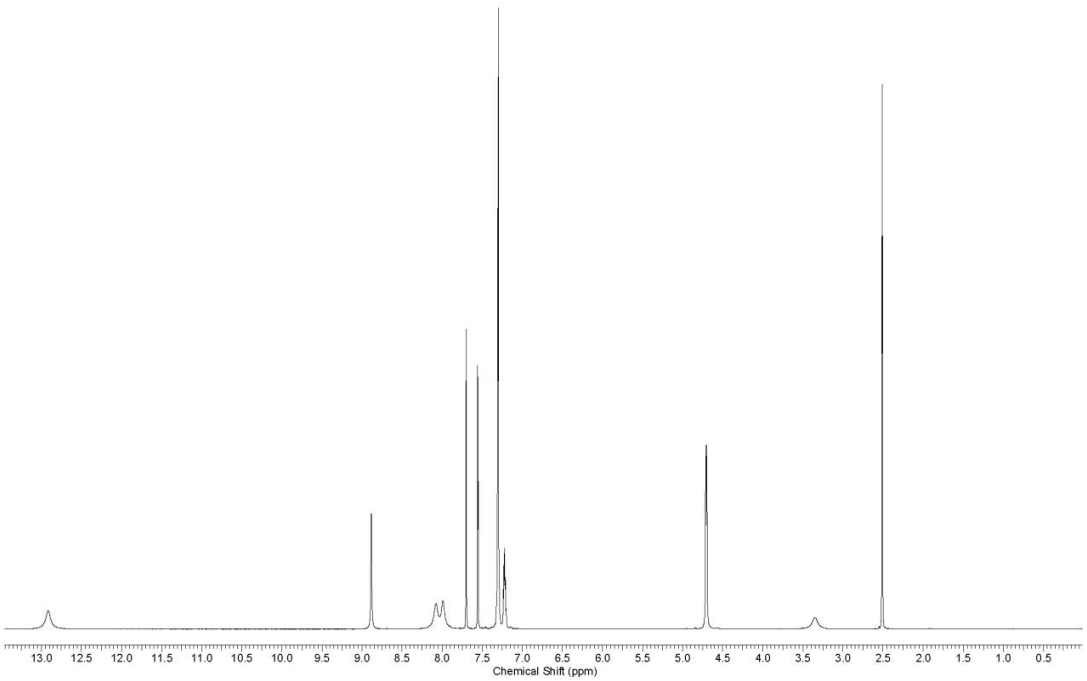

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