Supplementary Information

Design, synthesis and evaluation of pyrimidinobenzylamide and pyrimidinothiophenamide derivatives as inhibitors of DOT1L and related epigenetic targets DNMT3a, PRMT4 and other HMTs

Oscar Omar Castillo-Aguilera,[a, b] Patrick Depreux,[a, b] Ludovic Halby,[c] Christian Bailly,[d] Lenin Domínguez-Ramírez,[e] Sheraz Gul,[f, g] Paola B. Arimondo,[c] and Laurence Goossens[a, b]

[a] Univ. Lille, CHU Lille, ULR 7365 - GRITA - Groupe de Recherche sur les formes Injectables et les Technologies Associées, 3 rue du Pr. Laguesse, F-59000 Lille, France.

[b] Institut de Chimie Pharmaceutique Albert Lespagnol, 3 rue du Pr. Laguesse, F-59000 Lille, France.

[c] Epigenetic Chemical Biology, Department of Structural Biology and Chemistry, Institut Pasteur, Université Paris Cité, CNRS UMR3523 Chem4Life, F-75015 Paris, France.

[d] CNRS, Inserm, CHU Lille, UMR9020-U1277-CANTHER, OncoLille Institut, Univ. Lille, Lille, France.

[e] Population Health and Metadynamics Lab, Centro de Investigación Biomédica de Oriente, Instituto Mexicano del Seguro Social, Atlixco 74360, Puebla, Mexico.

[f] Fraunhofer Institute for Translational Medicine and Pharmacology ITMP, Discovery Research ScreeningPort, Schnackenburgallee 114, D-22525 Hamburg, Germany.

[g] Fraunhofer Cluster of Excellence for Immune-Mediated Diseases (CIMD), Schnackenburgallee 114, D-22525 Hamburg, Germany.

Content

Docking

The structure of DOT1L and PRMT4 were taken from Protein Data Bank (PDB-DOT1L: 4ER5 and 4EKI; PDB-PRMT4: 5IH3). The ligands were docked using Autodock vina 1.1.2 with a box centred at X=37, y=53, z=-0.2, with size x=81.3, y=65.5, z=49.2 and exhaustiveness of 1000 under UCSF Dock and AMBER parameters. The images were prepared with PyMOL (DeLanoScientific, 2009). All docking were run at the computer cluster from the Laboratorio Nacional de Supercómputo del Sureste de México.

Pharmacological Evaluation

DOT1L activity

Tests of DOT1L activity of the synthesized compounds were performed by Eurofins Cerep, France, with **SAH** (IC_{50} = 240±26 nM) as the reference compound. The test compound, reference compound, or water (control) was incubated for 15 min at 22°C with 60 ng DOT1L enzyme (human recombinant, *E. coli*), 100 nM [^{3}H]-SAM and 0.125 µg polynucleosome (from HeLa cells, 2,5 µg/mL) in a buffer containing 18 mM Tris-HCl (pH 8), 90 mM NaCl and 1.8 mM dithiothreitol DTT (to prevent inter- and intramolecular disulfide bridges in the nucleosome proteins). For basal control measurements, the enzyme was absent from the reaction mixture. After incubation, the reaction was stopped by adding 33 mM citric acid and the samples were filtered under vacuum through glass fiber filters (GF/B, Packard) presoaked and rinsed several times with ice-cold 33 mM citric acid using a 96-sample cell harvester (Unifilter, Packard). The filters were dried then radioactivity was measured on scintillation counter (Topcount, Packard) using a scintillation cocktail (Microscint 0, Packard).[1,2]

DNMT3a activity

Tests of DNMT3a activity of the synthesized compounds were performed in ETaC, Toulouse, with **SGI-1027** (IC_{50} = 0.96±0.2 µM) as the reference compound. A 5'-strand oligonucleotide labelled with biotin was hybridized to its complementary 3'-strand labelled with 6-carboxyfluorescein (FAM) (incubation for 2 h at 37 °C) into a 384-well microplate (black Optiplates; PerkinElmer) previously precoated with avidin. The duplex has a unique CpG site containing a restriction site of a methylation-sensitive restriction enzyme (HpyCH4IV). The human C-terminal DNMT3A (a.a. 623-908) was added to each well (200 ng/well) and mixed with the tested compound at the desired concentration (in DMSO) and SAM (10 mM final concentration) to start the reaction in a total volume of 40 µL. After incubation for 1 h at 37 °C, each well was washed three times with phosphate-buffered saline (PBS) containing 0.05% Tween-20 and NaCl (500 mM), and three more times with phosphate-buffered saline Tween-20 (PBST). The methylation-sensitive restriction enzyme HpyCH4IV (New England Biolabs, Ipswich, MA, USA) was added to the wells. After incubation for 1 h at 37 °C, each well was washed three times with phosphate-buffered saline (PBS) containing 0.05% Tween-20 and NaCl (500 mM), and three more times with phosphate-buffered saline Tween-20 (PBST). Specific fluorescence signals were measured on a PerkinElmer Envision detector.[3,4]

Tests of Specificity on PRC2, G9a, PRMT1, PRMT4 and PRMT5

Tests of activity on PRC2, G9a, PRMT1, PRMT4 and PRMT5 were performed by Eurofins Cerep, France, with SAH as the reference compound. The general conditions were summarized in **table SI-1**.

Table S1. General Conditions of Enzymatic Assays on a Panel of HMTs

Test	Protein (source)	Substrate, stimulus and/or tracer	Incubation	Component measured	Detection method	Reference SAH, IC_{50}	Biblography
EZH2/EED/SUZ12 (*h*)(PRC2 complex)	recombinant human(Sf9 cells)	Histone 3 (50 nM) / [^{3}H]SAM (350 nM)	120 min TA	Methylated H3	Scintillation	44 μM	5
G9a (*h*)	recombinant human(*E. coli*)	Histone 3 (5 nM) / [^{3}H]SAM (25 nM)	120 min TA	Methylated H3	Scintillation	2.4 μM	1
PRMT1 (*h*)	recombinant human(*E. coli*)	Histone 4 (50 nM) / [^{3}H]SAM (700 nM)	20 min TA	Methylated H4	Scintillation	0.46 μM	6
PRMT4 (*h*)	recombinant human(Sf9 cells)	Histone 3 (25 nM) / [^{3}H]SAM (60 nM)	60 min TA	Methylated H3	Scintillation	60 nM	7
complex PRMT5 (*h*)	recombinant human(Sf9 cells)	Histone 4 (250 nM) / [^{3}H]SAM (600 nM)	120 min TA	Methylated H4	Scintillation	460 nM	1

h: human.

Figure S1. Activities of 1n, 1p and 19g on PRMT4 and Activity of 19d on DNMT3a

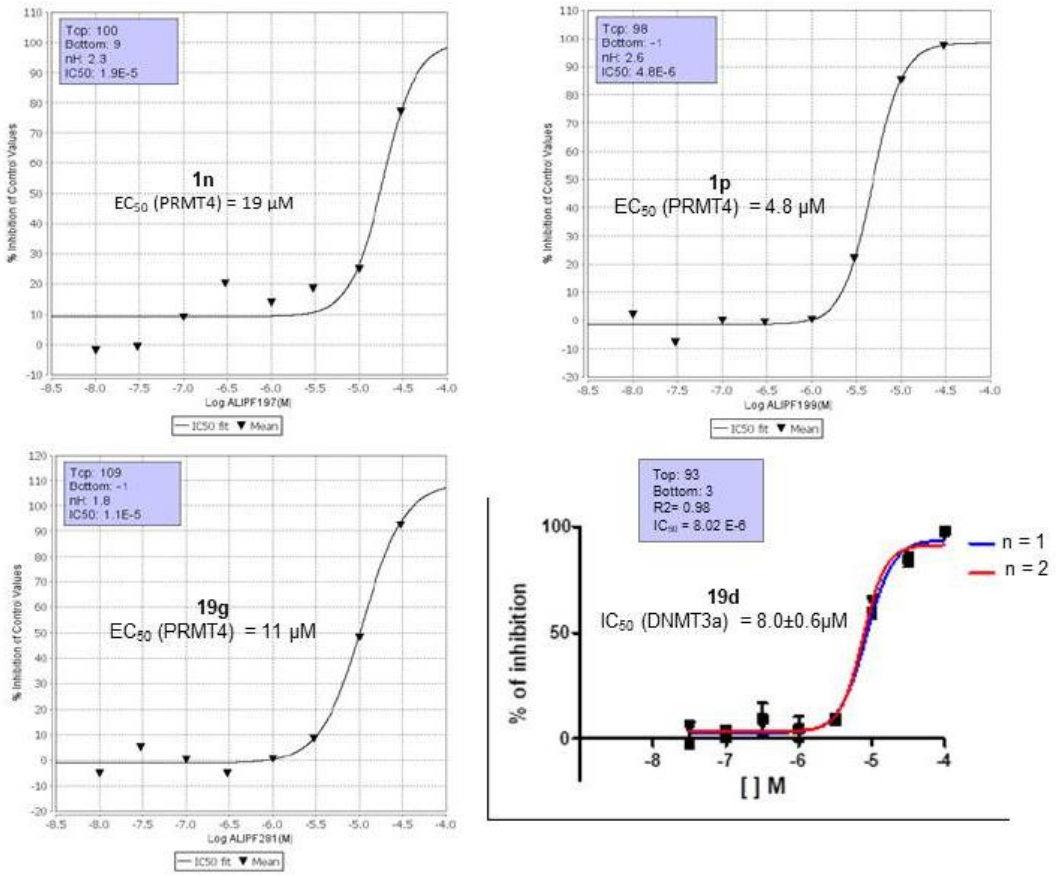


Table S2. Physicochemical Properties of Final Compounds

Compound	Molecular Formula	Molecular Weight	HBA[a]	HBD[a]	S[a]	cLogP[b]	SD LogP	cLogD[c]	SD LogD	TPSA[a]
1a	$C_{26}H_{31}N_7O$	457.57	6	4	-7.78	4.84	0.18	4.12	0.43	116.2
1b	$C_{27}H_{33}N_7O$	471.60	6	4	-8.26	5.17	0.15	4.39	0.56	116.2
1c	$C_{28}H_{35}N_7O$	485.62	6	4	-8.77	5.60	0.16	4.84	0.38	116.2
1d	$C_{30}H_{39}N_7O$	513.68	6	3	-8.70	6.06	0.21	5.19	0.35	107.4
1e	$C_{23}H_{25}N_7O$	415.49	6	4	-6.36	3.62	0.24	2.95	0.40	116.2
1f	$C_{24}H_{24}F_3N_7O$	483.49	6	4	-7.48	4.33	0.30	3.58	0.21	116.2
1g	$C_{24}H_{27}N_7O_2$	445.52	7	4	-6.45	3.52	0.42	2.82	0.70	125.4
1h	$C_{23}H_{24}ClN_7O$	449.94	6	4	-7.12	4.07	0.28	3.49	0.41	116.2
1i	$C_{25}H_{30}N_8O$	458.56	7	5	-7.01	4.27	0.29	3.85	0.21	128.2
1j	$C_{26}H_{32}N_8O$	472.59	7	5	-7.44	4.35	0.65	3.72	0.52	128.2
1k	$C_{26}H_{31}N_7O_2$	473.57	7	5	-7.17	5.03	0.60	4.56	0.84	136.4
1l	$C_{27}H_{33}N_7O_2$	487.60	7	5	-7.64	5.36	0.55	4.89	0.93	136.4
1m	$C_{25}H_{31}N_7O_2$	461.56	6	5	-6.92	4.02	0.33	3.52	0.47	133
1n	$C_{26}H_{33}N_7O_2$	475.59	6	5	-7.29	4.24	0.37	3.63	0.57	133
1o	$C_{28}H_{37}N_7O_2$	503.64	6	4	-7.38	4.86	0.23	4.25	0.37	124.2
1p	$C_{29}H_{39}N_7O_2$	517.67	6	4	-7.73	5.11	0.35	4.38	0.38	124.2
1q	$C_{25}H_{31}N_7O_3$	477.56	7	6	-6.37	4.12	0.75	3.69	1.11	153.2
1r	$C_{26}H_{33}N_7O_3$	491.59	7	6	-6.72	4.40	0.78	3.87	1.11	153.2
1s	$C_{28}H_{37}N_7O_3$	519.64	7	5	-6.84	4.81	0.41	4.29	0.72	144.4
1t	$C_{29}H_{39}N_7O_3$	533.67	7	5	-7.17	5.10	0.48	4.49	0.68	144.44
1u	$C_{24}H_{31}N_9O_2$	477.56	8	6	-6.27	2.69	0.76	2.28	0.64	171.3
1v	$C_{19}H_{26}N_6O_3$	386.45	7	4	-4.56	2.39	0.20	1.89	0.59	130.2
1w	$C_{14}H_{18}N_6O$	286.33	6	4	-3.01	0.53	0.15	-1.48	1.18	117.8
15a	$C_{25}H_{33}N_7O$	447.58	6	5	-7.29	4.21	0.41	2.44	0.71	115.93
15b	$C_{26}H_{35}N_7O$	461.60	6	5	-7.74	4.42	0.34	2.31	0.67	115.9
15c	$C_{27}H_{35}N_7$	457.61	6	4	-8.85	5.33	0.22	3.13	1.11	99.2
19a	$C_{24}H_{29}N_7OS$	463.60	6	4	-7.72	4.72	0.66	4.58	0.50	116.2
19b	$C_{25}H_{31}N_7OS$	477.62	6	4	-8.22	5.00	0.71	4.86	0.57	116.2
19c	$C_{26}H_{33}N_7OS$	491.65	6	4	-8.72	5.38	0.67	5.24	0.52	116.2
19d	$C_{24}H_{30}N_8OS$	478.61	7	5	-7.38	4.46	ND	4.46	ND	ND
19e	$C_{23}H_{29}N_7O_2S$	467.59	6	5	-6.84	3.80	0.74	3.83	0.62	133
19f	$C_{24}H_{31}N_7O_2S$	481.61	6	5	-7.22	3.71	ND	3.71	ND	ND
19g	$C_{27}H_{37}N_7O_2S$	523.69	6	4	-7.67	5.13	0.71	5.08	0.52	124.2
19h	$C_{22}H_{29}N_9O_2S$	483.59	8	6	-6.17	3.08	ND	3.07	ND	ND

[a] Hydrogen-bond acceptors (HBA), hydrogen-bond donors (HBD), molecular solubility (S) in mol/L, TPSA in $Å^2$, calculated by PipelinePilot (PP).

[b] The values of cLogP correspond to the average values determined by PipelinePilot (PP), Data Warrior (DW), Pharma Algorithms ADME Boxes v2.0 (AB/LogP 2.0), ACD Labs, XLogP.

[c] The values of cLogD correspond to the average values determined by PipelinePilot (PP), ACD Labs (pH=7.4).

ND: Not Determined.

Table S3. Screening of Final Compounds over a Panel of HDAC

Compound	HDAC1 (% inhibition)[a]	HDAC3 (% inhibition)[a]	HDAC6 (% inhibition)[a]
1a	6±1	4±1	0±18
1b	11±2	13±3	0±26
1c	1±1	0±0	0±17
1f	1±3	1±1	0±11
1g	8±2	21±1	21±19
1h	3±2	40±51	8±18
1i	0±3	1±2	0±9
1j	2±1	7±2	8±9
1k	13±0	13±2	0±19
1l	9±1	7±2	0±17
1m	0±2	0±2	0±1
1n	0±3	0±1	0±0
1o	0±5	0±2	0±4
1p	0±2	0±2	0±4
1q	0±1	0±2	0±11
1r	0±5	0±2	0±33
1s	0±1	0±2	0±14
1t	0±1	0±1	1±13
1u	0±3	0±6	0±8
1w	0±4	0±1	0±11
1x	0±4	0±1	0±62
15a	0±4	0±1	0±11
19a	10±3	3±1	45±15
19d	8±2	5±2	2±13
19e	9±0	5±2	0±13
19f	5±1	0±2	0±10

[a] Percentage inhibition at a concentration of 10 μM of the tested compound. Tests performed in triplicate according to reported methods.[8]

Table S4. ADMET Profile of Final Compounds

Compound	786-O cell-line (% cytotoxicity)	A549 cell-line (% cytotoxicity)	CYP3A4 (% inhibition)	*h*ERG (% inhibition)
1a	0±8	0±15	77±3	32±2
1b	0±8	4±16	67±2	0±9
1c	0±2	0±15	53±12	0±19
1f	1±3	4±7	52±2	11±16
1g	0±18	0±17	49±3	68±4
1h	0±11	0±18	48±5	41±11
1i	0±8	0±15	21±2	76±15
1j	3±3	0±16	31±9	51±6
1k	15±10	6±16	46±1	47±13
1l	10±13	0±14	47±10	46±11
1m	0±12	0±14	45±2	64±20
1n	0±8	0±12	28±5	20±10
1o	24±14	11±14	53±2	32±1
1p	15±12	20±12	50±2	15±3
1q	0±12	0±9	26±1	63±23
1r	0±2	0±13	16±2	34±7
1s	0±10	0±1	48±4	78±20
1t	0±12	0±8	49±4	67±9
1u	0±4	0±11	10±5	44±9
1w	0±8	0±11	0±4	73±14
1x	0±6	0±11	0±2	48±13
15a	0±2	0±15	46±3	76±18
19a	9±10	0±18	43±2	63±11
19d	12±10	0±21	48±3	49±5
19e	4±14	0±13	16±8	45±7
19f	8±9	0±20	25±19	7±13

[a] Percentage inhibition at a concentration of 10 μM of the tested compound. Tests performed in triplicate according to reported methods.[8] The drugability of compounds is colour coded: safe profile (green), moderately safe (yellow), undesirable (red).

Table S5. Smiles codes for final compounds 1a-w, 15a-c, 19a-h

Compound	Smiles
1a	n1c(N)nc(Nc2cccc(C(=O)NCCCc3[nH]c(cc(C(C)(C)C)cc4)c4n3)c2)cc1C
1b	n1c(N)nc(Nc2cccc(C(=O)NCCCCc3[nH]c(cc(C(C)(C)C)cc4)c4n3)c2)cc1C
1c	n1c(N)nc(Nc2cccc(C(NCCCCCc3[nH]c(cc(C(C)(C)C)cc4)c4n3)=O)c2)cc1C
1d	n1c(N)nc(Nc2cccc(C(=O)N(C(C)C)CCCCc3[nH]c(cc(C(C)(C)C)cc4)c4n3)c2)cc1C
1e	n1c(N)nc(Nc2cccc(C(=O)NCCCCc3[nH]c(cccc4)c4n3)c2)cc1C
1f	C(Cc1[nH]c(cc(C(F)(F)F)cc2)c2n1)CCNC(c(cc3Nc(nc(n4)N)cc4C)ccc3)=O
1g	C(Cc1[nH]c(cc(OC)cc2)c2n1)CCNC(c(cc3Nc(nc(n4)N)cc4C)ccc3)=O
1h	C(Cc1[nH]c(cc(Cl)cc2)c2n1)CCNC(c(cc3Nc(nc(n4)N)cc4C)ccc3)=O
1i	n1c(N)nc(Nc2cccc(C(=O)NCCNc3[nH]c(cc(C(C)(C)C)cc4)c4n3)c2)cc1C
1j	C(Nc1[nH]c(cc(C(C)(C)C)cc2)c2n1)CCNC(c(cc3Nc(nc(n4)N)cc4C)ccc3)=O
1k	n1c(N)nc(Nc2ccc(O)c(C(=O)NCCCc3[nH]c(cc(C(C)(C)C)cc4)c4n3)c2)cc1C
1l	n1c(N)nc(Nc2ccc(O)c(C(=O)NCCCCc3[nH]c(cc(C(C)(C)C)cc4)c4n3)c2)cc1C
1m	n1c(N)nc(Nc2cccc(C(=O)NCCNC(=O)Nc3ccc(C(C)(C)C)cc3)c2)cc1C
1n	n1c(N)nc(Nc2cccc(C(=O)NCCCNC(=O)Nc3ccc(C(C)(C)C)cc3)c2)cc1C
1o	n1c(N)nc(Nc2cccc(C(=O)N(C(C)C)CCNC(=O)Nc3ccc(C(C)(C)C)cc3)c2)cc1C
1p	n1c(N)nc(Nc2cccc(C(=O)N(C(C)C)CCCNC(=O)Nc3ccc(C(C)(C)C)cc3)c2)cc1C
1q	n1c(N)nc(Nc2ccc(O)c(C(=O)NCCNC(=O)Nc3ccc(C(C)(C)C)cc3)c2)cc1C
1r	n1c(N)nc(Nc2ccc(O)c(C(=O)NCCCNC(=O)Nc3ccc(C(C)(C)C)cc3)c2)cc1C
1s	n1c(N)nc(Nc2ccc(O)c(C(=O)N(C(C)C)CCNC(=O)Nc3ccc(C(C)(C)C)cc3)c2)cc1C
1t	n1c(N)nc(Nc2ccc(O)c(C(=O)N(C(C)C)CCCNC(=O)Nc3ccc(C(C)(C)C)cc3)c2)cc1C
1u	n1c(N)nc(Nc2cccc(C(=O)NCCCNC(=O)Nc3ccc(C(C)(C)C)cc3)c2)nc1N
1v	n1c(N)nc(Nc2cccc(C(=O)NCCNC(OC(C)(C)C)=O)c2)cc1C
1w	n1c(N)nc(Nc2cccc(C(=O)NCCN)c2)cc1C
15a	n1c(N)nc(Nc2cccc(CNCCNC(=O)Nc3ccc(C(C)(C)C)cc3)c2)cc1C
15b	n1c(N)nc(Nc2cccc(CNCCCNC(=O)Nc3ccc(C(C)(C)C)cc3)c2)cc1C
15c	n1c(N)nc(Nc2cccc(CNCCCCc3[nH]c(cc(C(C)(C)C)cc4)c4n3)c2)cc1C
19a	N(c1cc(C)nc(N)n1)c(sc2C(NCCCc3[nH]c(cc(C(C)(C)C)cc4)c4n3)=O)cc2
19b	N(c1cc(C)nc(N)n1)c(sc2C(NCCCCc3[nH]c(cc(C(C)(C)C)cc4)c4n3)=O)cc2
19c	N(c1nc(N)nc(C)c1)c(sc2C(=O)NCCCCCc3[nH]c(cc(C(C)(C)C)cc4)c4n3)cc2
19d	N(c1cc(C)nc(N)n1)c(sc2C(=O)NCCCNc3[nH]c(cc(C(C)(C)C)cc4)c4n3)cc2
19e	c1(C(NCCNC(Nc(c2)ccc(C(C)(C)C)c2)=O)=O)sc(Nc(nc3N)cc(C)n3)cc1
19f	c1(C(=O)NCCCNC(=O)Nc2ccc(C(C)(C)C)cc2)sc(Nc(nc3N)cc(C)n3)cc1
19g	c1(C(=O)N(CCCNC(=O)Nc2ccc(C(C)(C)C)cc2)C(C)C)sc(Nc(nc3N)cc(C)n3)cc1
19h	c1(C(=O)NCCCNC(=O)Nc2ccc(C(C)(C)C)cc2)sc(Nc(nc3N)nc(N)n3)cc1

Chemistry

Materials

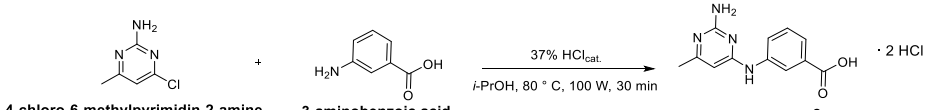
- Reagents, chemicals and (anhydrous) solvents were purchased from commercial suppliers and used without further purification.
- Sensitive reactions were performed under a nitrogen atmosphere.
- Reactions were monitored by Thin-Layer Chromatography (TLC), carried out on precoated silica gel Polygram SIL G/UV_{254} plates (Macherey-Nagel). Spots were visualized using an UV lamp (λ = 254 and 366 nm).
- Preparative-TLC were carried out on SIL G-200/UV_{254}, 20x20 cm plates (Macherey – Nagel), and spots were visualized using an UV lamp (λ = 254 and 366 nm).
- Microwave-Assisted Chemistry was performed on a DiscoverSP apparatus (CEM) driven by Synergy software.
- Flash chromatography was performed on a spot flash type apparatus (Armen) using silica gel prepacked Chromabond® Flash RS cartridges (Macherey-Nagel).
- Liquid Chromatography-Mass Spectrometry (LC-MS) experiments were performed on a Q Exactive Benchtop LC-MS (Thermo Scientific) using Atmospheric-Pressure Chemical Ionisation (APCI +/-).
- LC-MS was also performed on a HPLC Alliance (Waters) - MS Micromass ZQ2000 (Waters) using ElectroSpray Ionisation (ESI+/−). Data were analyzed on Mass Lynx software. A prepacked C_{18} reverse-phase column (Waters XBridge, 50x4.6 mm, 5 µm) at 35 °C was used for analytical HPLC with a binary gradient elution (mobile phase A: water + buffer formate 5 mM, pH 3.8; mobile phase B: acetonitrile and buffer formate 5 mM, pH 3.8 (5% water); gradient 0-0.25 min = 2% B, 0.25-4.0 min = 100% B, 4.0-5.0 min = 2% B) and at a flow rate of 2 mL/min.
- Purity of final compounds was controlled by an analytical HPLC performed on a WATERS 600^{E} chromatographer, with detection on a UV WATERS 2487 dual absorbance detector (254 and 366 nm). A Chromasile C18 column (Macherey Nagel, 4.6x150 mm, 5 µm, 100 Å) was used with a binary gradient elution (mobile phase: acetonitrile – water (8:2), 0.08% formic acid) and at a flow rate of 1 mL/min/20 min.
- NMR spectra were recorded on a Bruker AC 300P spectrometer (300 MHz for ^{1}H and 75 MHz for ^{13}C). Spectra were performed at room temperature with tetramethylsilane (TMS) as intern reference, and they were treated using TopSpin (Bruker) software.
- High-Resolution Mass Spectrometry (HRMS) was performed on a Q Exactive Benchtop LC-MS/MS (Thermo Scientific) using electrospray ionization (ESI+/-).
- Melting points were determined on a Büchi B-530 apparatus. The values were not corrected.
- Infrared spectra (IR) were performed on a Bruker Alpha-P spectrometer.

Experimental procedures

<u>General procedure 1:</u> Synthesis of precursors 2a-c

37% HCl (10 uL) was added to a mixture of the corresponding chlorinated pyrimidine (1-1.5 eq.), the corresponding amine (1 eq.) in isopropanol (qsp 1 M solution) were stirred for 30 minutes (15 min. for **2c**) in a microwave reactor, at 80 °C, at 100 W. The mixture was filtrated to recover a solid which was washed with ethanol. The resulting filtrate was concentrated *in vacuo*, and ethanol was added to the residue to recover by filtration a second solid. Both solids were gathered together, and dried over P_2O_5.

3-((2-Amino-6-methylpyrimidin-4-yl)amino)benzoic acid dihydrochloride, **2a**



4-chloro-6-methylpyrimidin-2-amine
1.5 eq., 1 g, 6.97 mmol

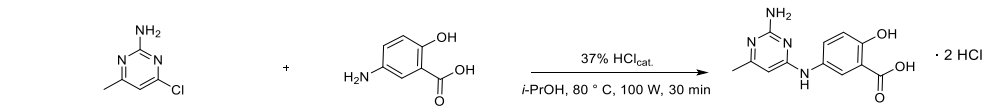
3-aminobenzoic acid
1 eq., 0.637 g, 4.64 mmol

86 %

2a
Formula: $C_{12}H_{14}Cl_2N_4O_2$
MW (dihydrochloride): 317.17
MW (base): 244.25

Appearance: White powder. **mp** >265 °C **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 2.28 (s, 3H, CH_3), 6.24 (s, 1H, ArH), 7.49 (m, 1H, ArH), 7.71 (m, 1H, ArH), 7.88 (m, 1H, NH), 8.04 (m, 1H, ArH), 8.25 (m, 1H, ArH), 10.93 (br s, 1H, COOH), 13.05 (br s, 2H, NH_2). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 18.8, 97.7, 122.6, 125.5, 126.1, 129.6, 131.9, 138.7, 153.2, 156.2, 162.2, 167.3. **LC-MS** (ESI^+): m/z 245 $[M+H]^+$. **HRMS** (ESI^+): m/z calculated $C_{12}H_{13}N_4O_2$ $[M+H]^+$ 245.1033, found 245.1023. **IR**, υ' (cm^{-1}): 1650 (C=O).

5-((2-Amino-6-methylpyrimidin-4-yl)amino)-2-hydroxybenzoic acid dihydrochloride, **2b**



4-chloro-6-methylpyrimidin-2-amine
1 eq., 2.5 g, 16.9 mmol

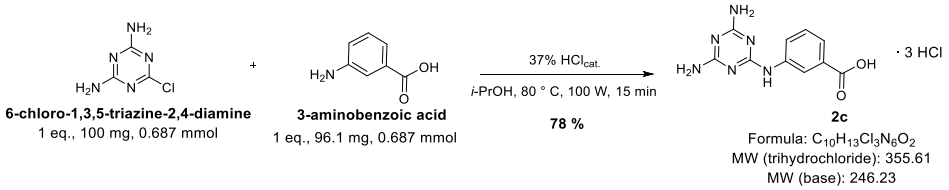
5-amino-2-hydroxybenzoic acid
1 eq., 2.59 g, 16.9 mmol

98 %

2b
Formula: $C_{12}H_{14}Cl_2N_4O_3$
MW (dihydrochloride): 333.17
MW (base): 260.25

Appearance: white powder. **mp** = 240-242 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 2.25 (s, 3H, CH_3), 6.02 (s, 1H, ArH), 6.85 (m, 1H, ArH), 7.74 (m, 2H, ArH), 7.81 (br s, 2H, NH_2), 10.29 (br s, 1H, OH), 13.10 (br s, 1H, COOH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 18.3, 97.0, 115.1, 117.0, 124.0, 129.3, 131.4, 152.7, 155.7, 158.9, 162.5, 171.3. **LC-MS** (ESI^+): m/z 261 $[M+H]^+$. **HRMS** (ESI^+): m/z calculated $C_{12}H_{13}N_4O_3$ $[M+H]^+$ 261.0982, found 261.0969. **IR**, υ' (cm^{-1}): 1662 (C=O).

3-((4,6-Diamino-1,3,5-triazin-2-yl)amino)benzoic acid trihydrochloride, **2c**



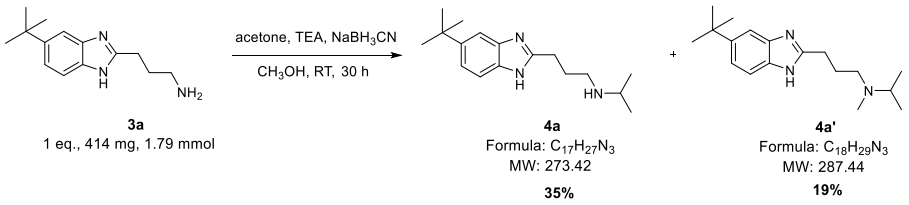
Appearance: white powder. **Mp** >265 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 6.30 (s, 4H, NH_2), 7.32 (m, 1H, ArH), 7.47 (m, 1H, ArH), 8.04 (m, 1H, ArH), 8.28 (m, 1H, ArH), 9.02 (s, 1H, NH),12.75 (br s, 1H, COOH). **LC-MS** (ESI$^-$): m/z 245 [M-H]$^-$. **IR**, υ (cm^{-1}): 1644 (C=O).

<u>General procedure 2:</u> Synthesis of precursors 4a-b

Primary amines 3a-g were obtained according to the procedure previously described. [9]

TEA (1 eq.) and acetone (1.1 eq.) were subsequently added to a solution of the corresponding amine **3a-b** (1 eq.) in CH_3OH (qsp 0.1 M solution) under nitrogen in a round bottom flask connected to a 10% NaOH trap. After 30 minutes of stirring at room temperature, sodium cyanoborohydride (1.1 eq.) was added to the mixture and it was stirred 24 h at rt. For **4a** acetone (0.5 eq.) and sodium cyanoborohydride (0.5 eq.) were added twice (24 and 27 h later) and the mixture was stirred 6 h more. 10% NaOH was added to the mixture and it was stirred 20 minutes. After concentration *in vacuo*, cold water was added to the residue, and the aqueous layer was extracted three times with EtOAc. The organic layers were gathered together, washed with water, brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography over silica gel (CH_2Cl_2:CH_3OH/ammonia, 9:1). The oil obtained was dried over P_2O_5.

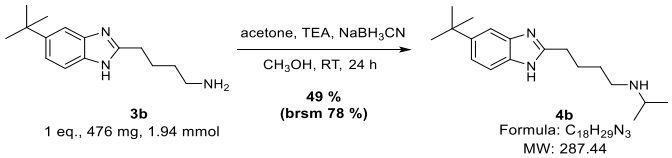
3-(5-(*Tert*-butyl)-1*H*-benzo[d]imidazole-2-yl)-*N*-isopropylpropan-1-amine, **4a**



Appearance: brown oil. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.23 (m, 6H, *i*-Pr), 1.31 (s, 9H, *t*-Bu), 2.11 (m, 2H, CH_2), 2.93 (m, 2H, CH_2-Ar), 3.01 (m, 2H, CH_2-NH), 3.31 (m, 1H, CH), 7.20 (m, 1H, ArH), 7.38 (m, 2H, ArH). **LC-MS** (APCI$^+$): m/z 274 [M+H]$^+$.

Characterization of 3-(5-(*tert*-butyl)-1*H*-benzo[d]imidazol-2-yl)-*N*-isopropyl-*N*-methylpropan-1-amine, **4a'**: **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.22 (m, 6H, *i*-Pr), 1.31 (s, 9H, *t*-Bu), 2.17 (m, 2H, CH_2), 2.63 (s, 3H, CH_3), 2.93 (m, 2H, CH_2), 3.10 (m, 2H, CH_2), 3.51 (m, 1H, CH), 7.22 (m, 1H, ArH), 7.42 (m, 2H, ArH), 11.55 (m, 1H, NH). **LC-MS** (APCI$^+$): m/z 288.2 [M+H]$^+$.

4-(5-(*Tert*-butyl)-1*H*-benzo[d]imidazol-2-yl)-*N*-isopropylbutan-1-amine, **4b**



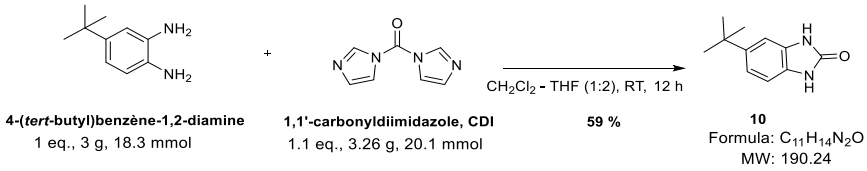
Appearance: brown oil. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.05 (d, ^3J = 6.4Hz, 6H, *i*-Pr), 1.38 (s, 9H, *t*-Bu), 1.50 (tt, ^3J = 7.9, 7.8 Hz, 2H, CH_2), 1.80 (tt, ^3J = 7.9 Hz, 7.7 Hz, 2H, CH_2), 2.66 (t, ^3J = 7.8 Hz, 2H, CH_2), 2.80 (t, ^3J = 7.7 Hz, 2H, CH_2), 2.91 (sp, ^3J = 6.4Hz, 1H, CH), 7.18 (m, 1H, ArH), 7.38 (m, 2H, ArH). **LC-MS** (ESI^+): m/z 288 $[M+H]^+$.

<u>General procedure 3:</u> Synthesis of precursors 5a-b

Synthesis of benzimidazolone, 10

A solution of 4-(*tert*-butyl)benzene-1.2-diamine (1 eq., 3 g, 18.3 mmol) in anhydrous THF (10 mL) was added to a solution of 1,1'-carbonyldiimidazole (1.1 eq., 3.26 g, 20.1 mmol) in a mixture of CH_2Cl_2:THF (1:2, 60 mL), and it was stirred overnight at room temperature under nitrogen. After removing the solvent *in vacuo*, CH_2Cl_2 was added to the dry residue to give a white powder recovered by filtration. The filtrate was concentrated *in vacuo*, and last procedure was performed again to give a second precipitate. Both precipitates were gathered together and dried over P_2O_5.

5-(*Tert*-butyl)-1*H*-benzo[*d*]imidazol-2(3*H*)-one, **10**

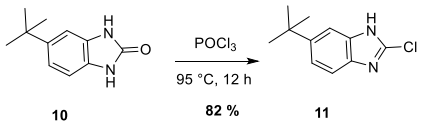


Appearance: white powder. **mp** >265 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.25 (s, 9H, *t*-Bu), 6.81 (m, 1H, ArH), 6.89 (m, 1H, ArH), 6.95 (m, 1H, ArH), 10.43 (s, 2H, NH). **LC-MS** (ESI^+): m/z 191 $[M+H]^+$. **IR**, υ' (cm^{-1}): 1701 (C=O).

Synthesis of benzimidazole 11

A solution of **10** (1 eq., 2.05 g, 10.8 mmol) in $POCl_3$ (30 mL) was stirred overnight at 95 °C. Toluene was added to the reaction mixture and it was concentrated *in vacuo*. Water was added to the resulting oil, and the suspension was extracted with EtOAc. The organic layers were gathered together, washed with 10% K_2CO_3, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Diethyl ether was added to the residue to yield a powder which was recovered by filtration and dried over P_2O_5.

5-(*Tert*-butyl)-2-chloro-2,3-dihydro-1*H*-benzo[*d*]imidazole, **11**



10
1 eq., 3 g, 18.3 mmol

82 %

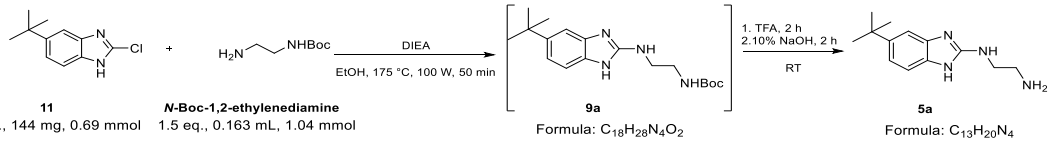
11
Formula: $C_{11}H_{13}ClN_2$
MW: 208.69

Appearance: white powder. **mp** = 196-198 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.32 (s, 9H, *t*-Bu), 7.29 (m, 1H, ArH), 7.41 (m, 2H, ArH), 13.04 (br s, 1H, NH). **LC-MS** (ESI$^+$): m/z 210 $[M+H]^+$.

Synthesis of amines 5a, 5b

A mixture of **11** (1 eq.), the corresponding amine (1.5 eq.) and DIPEA (2 eq.) in EtOH (qsp 0.5 M) was stirred for 50 minutes in a microwave reactor, at 175 °C, at 100 W. The reaction mixture was concentrated *in vacuo* and the residue was suspended in TFA (30 eq.). After stirring at room temperature for 4 h, the solvent was removed *in vacuo*. 10% NaOH was added to the residue at 0 °C and the mixture was stirred for 2 h. The aqueous layer was extracted three times with CH_2Cl_2. The organic layers were gathered together, dried over $CaCl_2$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography over silica gel (CH_2Cl_2:CH_3OH/ammonia, 9:1). The oil obtained was dried over P_2O_5.

N^1-(5-(*Tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)ethane-1,2-diamine, **5a**



11
1 eq., 144 mg, 0.69 mmol

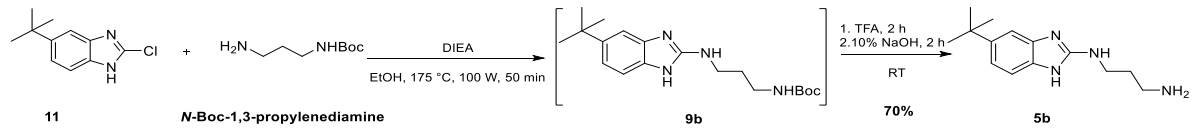
***N*-Boc-1,2-ethylenediamine**
1.5 eq., 0.163 mL, 1.04 mmol

9a
Formula: $C_{18}H_{28}N_4O_2$
MW: 332.44

5a
Formula: $C_{13}H_{20}N_4$
MW: 232.32
44% over all

Appearance: brown oil. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.28 (s, 9H, *t*-Bu), 3.10 (m, 2H, CH_2), 3.31 (m, 2H, CH_2), 6.72 (m, 1H, ArH), 7.05 (m, 1H, ArH), 7.16 (m, 1H, ArH). **LC-MS** (ESI$^+$): m/z 233 $[M+H]^+$.

N^1-(5-(*Tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)propane-1,3-diamine, **5b**



11
1 eq., 532 mg, 2.55 mmol

***N*-Boc-1,3-propylenediamine**
2 eq., 890 µL, 5.1 mmol

9b

70%

5b
Formula: $C_{14}H_{22}N_4$
MW: 246.35

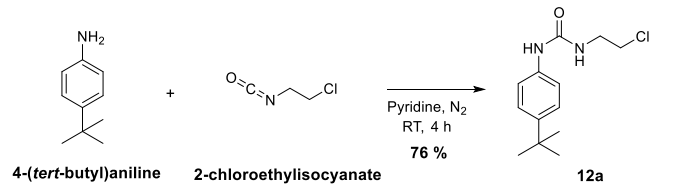
Appearance: brown oil. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.28 (s, 9H, *t*-Bu), 1.62 (m, 2H, CH_2), 2.61 (m, 2H, CH_2-NH_2), 3.31 (m, 2H, CH_2-NH), 6.88 (m, 1H, ArH), 6.99 (m, 1H, ArH), 7.11 (m,1H, ArH). **LC-MS** (ESI$^+$): m/z 247 $[M+H]^+$. **IR**, υ' (cm^{-1}): 3235 (NH).

<u>General procedure 4:</u> Synthesis of precursors 6a-b

Synthesis of amines 12a-b, 14

A solution of 4-(*tert*-butyl)aniline (1 eq.) in pyridine (5 mL) was added to a solution of the corresponding isocyanate (1 eq.) in pyridine (to give a 0.4 M solution), and the mixture was stirred under nitrogen, at room temperature for 4 h (24 h for **12b**). The mixture was concentrated *in vacuo* and 1N HCl was added to the residue. The visible solid was recovered by filtration, washed with cyclohexane, and dried over P_2O_5.

1-(4-(*Tert*-butyl)phenyl)-3-(2-chloroethyl)urea, **12a**



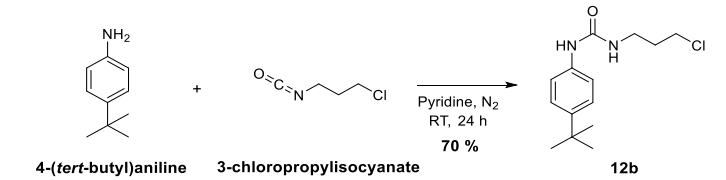
4-(*tert*-butyl)aniline 1 eq., 4 mL, 25.2 mmol

2-chloroethylisocyanate 1 eq., 2.15 mL, 25.2 mmol

12a
Formula: $C_{13}H_{21}Cl_3N_2O$
MW: 254.76

Appearance: white powder. **mp** = 125-127 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.23 (s, 9H, *t*-Bu), 3.40 ppm (td, ^3J = 6.1 Hz; ^3J = 6.2 Hz, 2H, CH_2-NH), 3.64 (t, ^3J = 6,1 Hz, 2H, CH_2-Cl), 6.32 (t, ^3J = 6.2 Hz, 1H, NH), 7.25 (m, 4H, ArH), 8.59 ppm (s, 1H, NH). **LC-MS** (APCI$^+$): m/z 255 $[M+H]^+$ for ^{35}Cl and 257 $[M+H]^+$ for ^{37}Cl. **IR**, υ' (cm^{-1}): 1644 (C=O), 3301(NH).

1-(4-(*Tert*-butyl)phenyl)-3-(3-chloropropyl)urea, **12b**



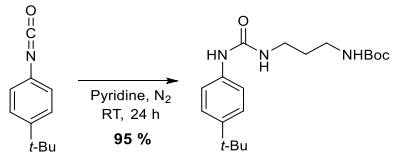
4-(*tert*-butyl)aniline 1 eq., 0.663 mL, 4.18 mmol

3-chloropropylisocyanate 1 eq., 0.342 mL, 4.18 mmol

12b
Formula: $C_{14}H_{23}Cl_3N_2O$
MW: 268.78

Appearance: white powder. **mp** = 140-142 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.23 (s, 9H, *t*-Bu), 1.87 (tt, ^3J = 6.4 Hz, ^3J = 6.6 Hz, 2H, CH_2), 3.18 (td, ^3J = 5.8 Hz, ^3J = 6.6 Hz, 2H, CH_2-NH), 3.66 (t, ^3J = 6.4 Hz, 2H, CH_2-Cl), 6.16 (t, ^3J = 5.8 Hz, 1H, NH), 7.25 (m, 4H, ArH), 8.30 (s, 1H, NH). **LC-MS** (APCI$^+$): m/z 269 $[M+H]^+$ for ^{35}Cl and 271 $[M+H]^+$ for ^{37}Cl. **IR**, υ' (cm^{-1}): 1642 (C=O), 3344 and 3297 (NH).

Tert-butyl (3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)carbamate, **14**



1-(*tert*-butyl)-*4*-isocyanatobenzene
1 eq., 0.500 mL, 2.9 mmol

14
Chemical Formula: $C_{19}H_{31}N_3O_3$
Molecular Weight: 349,48

Appearance: white powder. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.23 (s, 9H, *t*-Bu), 1.38 (s, 9H, *t*-Bu), 1.50 (t, *J*=6 Hz, 2H, CH_2), 2.95 (q, *J*=6 Hz, 2H, CH_2-NHBoc), 3.04 (q, *J*=6 Hz, 2H, CH_2-urea), 6.05 (t, *J*=6 Hz, 1H, NH), 7.24 (q, *J*=9 Hz, 4H, ArH), 8.36 (m, 1H, NH). **LC-MS** ($APCI^+$): m/z 350 $[M+H]^+$.

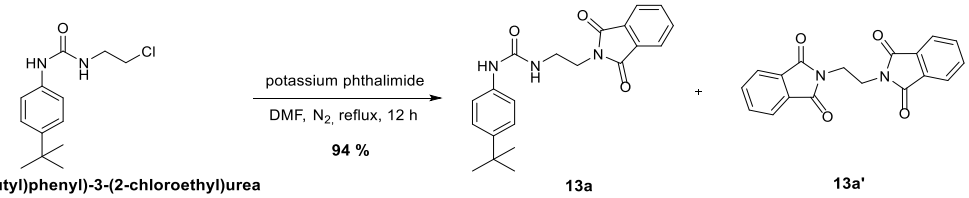
Synthesis of phthalimido derivatives 13a-b

Potassium phthalimide (2 eq.) was added to a solution of the corresponding halide **12a-b** (1 eq.) in anhydrous DMF (qsp 0.3 M) under nitrogen, and the mixture was refluxed 4 h (overnight for **13a**). After cooling to room temperature, cold water was added to the mixture.

For **13a** cold water was added to the mixture, and the visible solid was recovered by filtration. The powder was recrystallized from acetonitrile. The powder was dried over P_2O_5.

For **14a** the aqueous layer was extracted three times with EtOAc. The organic layers were gathered together, washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was suspended in CH_2Cl_2, and petroleum ether was added until precipitation. The powder was recovered by filtration, washed subsequently with water, 10% K_2CO_3, and cyclohexane. The solid was dried over P_2O_5.

1-(4-(*tert*-butyl)phenyl)-3-(2-(1,3-dioxoisoindolin-2-yl)ethyl)urea, **13a**



1-(4-(*tert*-butyl)phenyl)-3-(2-chloroethyl)urea
1 eq., 200 mg, 0.785 mmol

13a
Formula: $C_{21}H_{23}N_3O_3$
MW: 365.43

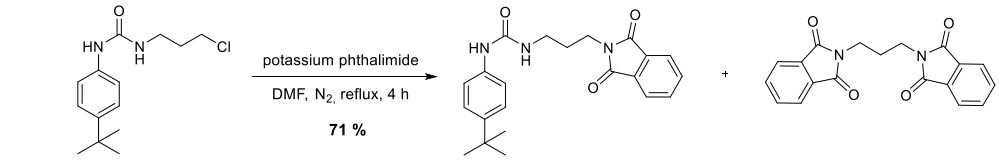
13a'
Formula: $C_{18}H_{12}N_2O_4$
MW: 320.30

Appearance: white powder. **mp** = 168-170 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.22 (s, 9H, *t*-Bu)**,** 3.34 (m, 2H, CH_2-NH), 3.67 (m, 2H, CH_2-N), 6.19 (m, 1H, NH), 7.18 (m, 4H, ArH), 7.84 (m, 4H, ArH), 8.31 (s, 1H, NH). **LC-MS** ($APCI^+$): m/z 366 $[M+H]^+$. **IR**, υ' (cm^{-1}): 1648 (C=O, urea), 1775 and 1709 (C=O, imide), 3400 and 3960 (NH).

Characterization of 2,2'-(ethane-1,2-diyl)bis(isoindoline-1,3-dione), **13a':**

Appearance: white powder. **mp** = 233-235 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 3.84 ppm (s, 4H, CH_2), 7.82 (m, 8H, ArH). **LC-MS** (ESI^+): m/z 321 $[M+H]^+$. **IR**, υ' (cm^{-1}): 1697 (C=O).

1-(4-(*Tert*-butyl)phenyl)-3-(3-(1,3-dioxoisoindolin-2-yl)propyl)urea, **13b**



12b
1 eq., 390 mg, 1.45 mmol

13b
Formula: $C_{22}H_{25}N_3O_3$
MW: 379.45

13b'
Formula: $C_{19}H_{14}N_2O_4$
MW: 334.33

Appearance: white powder. **mp** = 95-97 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.22 (s, 9H, *t*-Bu), 1.74 (m, 2H, CH_2), 3.08 (m, 2H, CH_2-NH), 3.60 (m, 2H, CH_2-N), 6.20 (m, 1H, NH), 7.22 (m, 4H, ArH), 7.85 (m, 4H, ArH), 8.50 (s, 1H, NH). **LC-MS** (APCI$^+$): m/z 380 $[M+H]^+$. **IR**, υ' (cm^{-1}): 1642 (C=O, urea), 1774 and 1708 (C=O, imide), 3314 (NH).

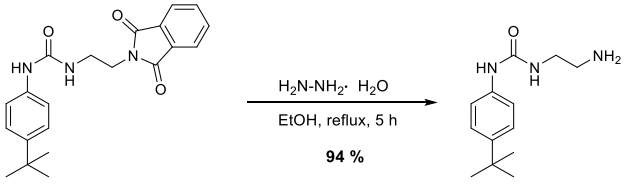
Characterization of 2,2'-(propane-1,3-diyl)bis(isoindoline-1,3-dione), **13b':**

Appearance: white powder. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.97 ppm (tt, ^3J = 7.1 Hz, 2H, CH_2), 3.61 (t, ^3J = 7.1 Hz, 4H, CH_2), 7.82 (m, 8H, ArH). **LC-MS** (ESI$^+$): m/z 335 $[M+H]^+$. **IR**, υ' (cm^{-1}): 1701 (C=O).

Synthesis of primary amines 6a, 6b

Hydrazine monohydrate (5 eq.) was added to a solution of the corresponding phthalimido-urea **13a-b** (1 eq.) in ethanol (to give a 0.2 M solution) and it was refluxed for 5 h. After cooling at room temperature, the white solid (phthalhydrazide) was discarded by filtration. The filtrate was concentrated *in vacuo*, and the residue was purified by column chromatography over silica gel (CH_2Cl_2:CH_3OH/ammonia, 9:1). The oil obtained was dried over P_2O_5.

1-(2-Aminoethyl)-3-(4-(*tert*-butyl)phenyl)urea, **6a**

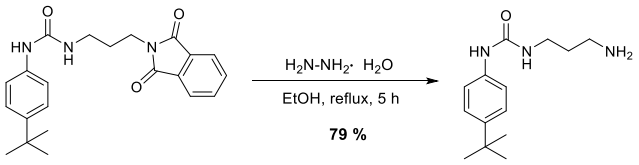


13a
1 eq., 2.09 g, 5.72 mmol

6a
Formula: $C_{13}H_{21}N_3O$
MW: 235.33

Appearance: brown oil. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.23 (s, 9H, *t*-Bu), 2.61 (t, *J*=6 Hz, 2H, CH_2-NH_2), 3.08 (dt, *J*=6 Hz, 12Hz, 2H, CH_2-NH), 6.19 (t, *J*=6 Hz, 1H, NH), 7.19 (dd, *J*=3 Hz, *J*=12 Hz, 4H, ArH), 8.47 (s, 1H, NH). **LC-MS** (APCI$^+$): m/z 236 $[M+H]^+$. **IR**, υ' (cm^{-1}): 1652 (C=O), 3299 (NH).

1-(3-Aminopropyl)-3-(4-(*tert*-butyl)phenyl)urea, **6b**



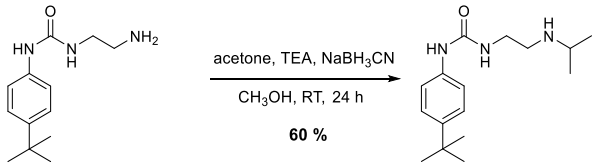
13b
1 eq., 4.7 g, 12.4 mmol

6b
Formula: $C_{14}H_{23}N_3O$
MW: 249.35

Appearance: brown oil. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.23 (s, 9H, *t*-Bu), 1.49 (m, 2H, CH_2), 2.58 (m, 2H, CH_2-NH_2), 3.12 (m, 2H, CH_2-NH), 3.31 (m, 2H, NH_2), 6.11 (m, 1H, NH), 7.25 (m, 4H, ArH), 8.37 (s, 1H, NH). **LC-MS** (APCI$^+$): m/z 250 $[M+H]^+$. **IR**, υ' (cm^{-1}): 1650 (C=O), 3303 (NH).

Secondary amines 7a-b were synthesized following General procedure 2.

1-(4-(*Tert*-butyl)phenyl)-3-(2-(isopropylamino)ethyl)urea, **7a**

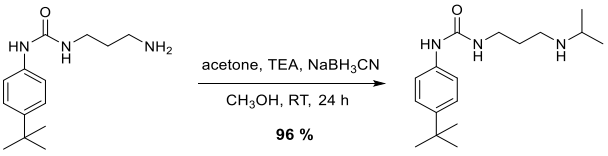


6a
1 eq., 150 mg, 0.637 mmol

7a
Formula: $C_{16}H_{27}N_3O$
MW: 277.41

Appearance: brown oil. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.00 (d, ^3J = 6.3 Hz, 6H, *i*-Pr), 1.23 (s, 9H, *t*-Bu), 2.62 (m, 2H, CH_2-NH), 2.78 (m, 1H, CH), 3.15 (m, 2H, CH_2-NHCO), 3.31 (m, 1H, NH), 6.11 (m, 1H, NH), 7.24 (m, 4H, ArH), 8.50 (s, 1H, NH). **LC-MS** (APCI$^+$): m/z 278 $[M+H]^+$. **IR**, υ' (cm^{-1}): 1650 (C=O), 3304 (NH).

1-(4-(*Tert*-butyl)phenyl)-3-(3-(isopropylamino)propyl)urea, **7b**



6b
1 eq., 170 mg, 0.682 mmol

7b
Formula: $C_{17}H_{29}N_3O$
MW: 291.43

Appearance: brown oil. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.01 (d, ^3J = 6.2 Hz, 6H, *i*-Pr), 1.23 (s, 9H, *t*-Bu), 1.56 (m, 2H, CH_2), 2.58 (m, 2H, CH_2-NH), 2.78 (m, 1H, CH), 3.10 (m, 2H, CH_2-NHCO), 3.31 (m, 1H, NH), 6.10 (m, 1H, NH), 7.24 (m, 4H, ArH), 8.34 (s, 1H, NH). **LC-MS** (APCI$^+$): m/z 291 $[M+H]^+$. **IR**, υ' (cm^{-1}): 1650 (C=O), 3324 (NH).

<u>General procedure 5:</u> Synthesis of benzimidazolophenylamides and ureophenylamides

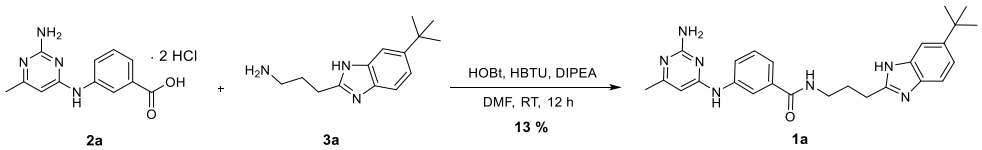
Synthesis of amides 1a-w

A solution of the corresponding amine (**3**-**7,** 1-1.6 eq.) in DMF was added to a mixture of the corresponding carboxylic acid (**2a**-**c**, 1 eq), DIPEA (4 eq.), coupling agents (see details below), in DMF, previously stirred for 20 minutes. After stirring overnight at RT, the reaction mixture was concentrated *in vacuo*. 10% NaOH was added to the residue, and the aqueous layer was extracted three times with EtOAc. The organic layers were gathered together, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography over silica gel (CH_2Cl_2:CH_3OH/ammonia, 95:5). The corresponding fraction was concentrated *in vacuo*, and diethyl ether was added to the dry residue to yield a powder (except for **20f** that is an oil) which was recovered by filtration and dried over P_2O_5.

Coupling reagents: HOBt (0.5 eq.), and HBTU (1 eq.); EDCI (2.5 eq.) and HOBt (1.5 eq.); or PYBOP (1.2 eq.).

For amides 1l, 1r: A solution of **2b** (1 eq.) in $SOCl_2$ (40 eq.) was refluxed overnight. The solvent was removed *in vacuo*. Triethylamine (1.5 eq.) and dry CH_2Cl_2 (to give a 0.1 M solution) were added to the residue under nitrogen. The corresponding amine suspended in dry CH_2Cl_2 was added to the previous mixture, and it was stirred overnight at room temperature. After removing the solvent *in vacuo*, water was added to the residue, and the aqueous layer was extracted three times with EtOAc. The organic layers were gathered together, washed with 10% K_2CO_3, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography over silica gel (CH_2Cl_2 - CH_3OH/ammonia, 95:5). The corresponding fraction was concentrated *in vacuo*, and diethyl ether was added to the dry residue to yield a powder which was recovered by filtration and dried over P_2O_5.

3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(3-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)propyl)benzamide, **1a**



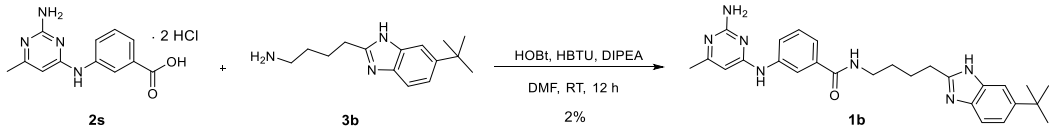
2a
1 eq., 329 mg, 1.04 mmol

3a
1 eq., 240 mg, 1.04 mmol

1a
Formula: $C_{26}H_{31}N_7O$
MW: 457.57

Appearance: white powder. **mp** = 201-203 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.31 (s, 9H, *t*-Bu), 2.01 (t,^3J= 6.8 MHz, 2H, CH_2), 2.08 (s, 3H, CH_3), 2.85 (t, ^3J= 6.8 MHz, 2H, CH_2-Ar), 3.26-3.37 (m, 2H, CH_2-N), 5.88 (s, 1H, ArH), 6.16 (br s, 2H, NH_2), 7.7 (d, ^3J= 7.6 MHz, 1H, ArH), 7.28-7.40 (m, 4H, ArH), 7.88 (m, 1H, ArH), 8.08 (d, ^3J= 7.6 MHz, 1H, ArH), 8.56 (t, ^3J= 5.1 MHz, 1H, NH), 9.14 (br s, 1H, NH), 12.04 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 23.4, 26.3, 27.5, 31.7, 34.4, 94.9, 118.4, 118.9, 119.8, 121.9, 128.4, 135.3, 140.8, 143.9, 154.6, 161.3, 162.7, 165.0, 166.4, 179.5. **LC-MS** (APCI$^+$): m/z 458 $[M+H]^+$. **HRMS** (ESI$^+$): m/z calculated $C_{26}H_{32}N_7O$ $[M+H]^+$ 458.2662, found 458.2636. **IR**, υ' (cm^{-1}): 1572 (C=O).

3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(4-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)butyl)benzamide, **1b**



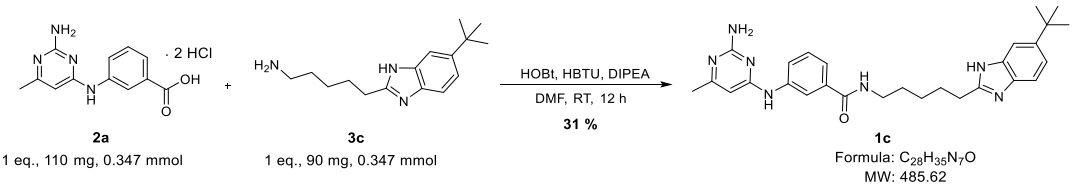
2s
1 eq., 500 mg, 1.58 mmol

3b
1 eq., 386 mg, 1.58 mmol

1b
Formula: $C_{27}H_{33}N_7O$
MW: 471.60

Appearance: white powder. **mp** = 137-139 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.33 (s, 9H, *t*-Bu), 1.54-1.63 (m, 2H, CH_2), 1.76-1.86 (m, 2H, CH_2), 2.10 (s, 3H, CH_3), 2.83 (t, ^3J=7.1 MHz, 2H, CH_2-Ar), 3.26-3.35 (m, 2H, CH_2-N), 5.89 (s, 1H, ArH), 6.18 (br s, 2H, NH_2), 7.18 (t, ^3J=7.1 MHz, 1H, ArH), 7.29-7.40 (m, 4H, ArH), 7.85 (s, 1H, ArH), 8.10 (t, ^3J=7.1 MHz, 1H, ArH), 8.39 (t, ^3J=4.5 MHz, 1H, NH), 9.14 (br s, 1H, NH), 12.01 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 23.8, 25.5, 28.6, 29.2, 32.2, 34.8, 95.4, 118.8, 119.3, 120.2, 122.4, 128.9, 135.8, 141.2, 144.2, 155.2, 161.8, 163.1, 165.4, 166.7. **LC-MS** (APCI$^+$): m/z 472 $[M+H]^+$. **HRMS** (ESI$^+$): m/z calculated $C_{27}H_{34}N_7O$ $[M+H]^+$ 472.2819, found 472.2798. **IR**, υ' (cm^{-1}): 1571 (C=O).

3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(5-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)pentyl)benzamide, **1c**



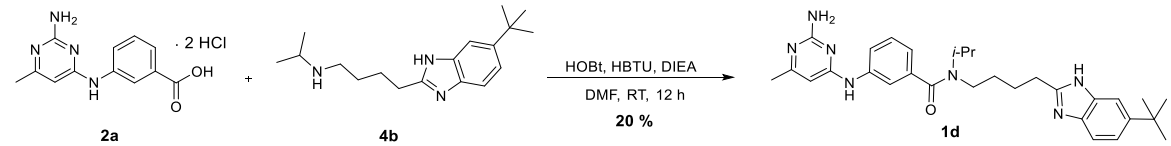
2a
1 eq., 110 mg, 0.347 mmol

3c
1 eq., 90 mg, 0.347 mmol

1c
Formula: $C_{28}H_{35}N_7O$
MW: 485.62

Appearance: white powder. **mp** = 150-152 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.31 (s, 9H, *t*-Bu), 1.34-1.41 (m, 2H, CH_2), 1.57 (tt, ^3J=7.5, 6.7 MHz, 2H, CH_2), 1.78 (tt, ^3J=7.5, 6.7 MHz, 2H, CH_2), 2.09 (s, 3H, CH_3), 2.78 (t, ^3J=6.7 MHz, 2H, CH_2-Ar), 3.21-3.28 (m, 2H, CH_2-NH), 5.89 (s, 1H, ArH), 6.23 (br s, 2H, NH_2), 7.16 (d, ^3J=8.1 MHz, 1H, ArH), 7.28-7.39 (m, 4H, ArH), 7.85 (s, 1H, ArH), 8.06 (d, ^3J=8.1 MHz, 1H, ArH), 8.35 (t, ^3J=5.0 MHz, 1H, NH), 9.19 (br s, 1H, NH), 11.92 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 23.6, 26.5, 27.8, 28.9, 29.3, 32.2, 34.8, 95.4, 118.9, 119.3, 120.3, 122.4, 128.9, 135.8, 141.1, 144.2, 155.3, 161.8, 162.8, 164.9, 166.7. **LC-MS** (ESI^-): m/z 484 $[M-H]^-$. **HRMS** (ESI^+): m/z calculated $C_{28}H_{36}N_7O$ $[M+H]^+$ 486.2975, found 486.2955. **IR**, υ' (cm^{-1}): 1572 (C=O).

3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(4-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)butyl)-*N*-isopropylbenzamide, **1d**



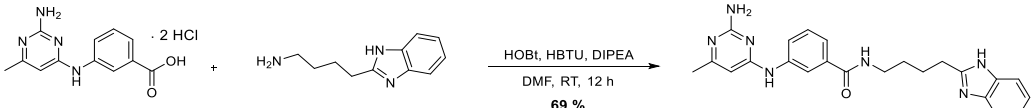
2a
1 eq., 275 mg, 0.87 mmol

4b
1 eq., 250 mg, 0.87 mmol

1d
Formula: $C_{30}H_{39}N_7O$
MW: 513.68

Appearance: white powder. **mp** = 130-132 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.31 (m, 6H, *i*-Pr), 1.31 (s, 9H, *t*-Bu), 1.65 (m, 2H, CH_2), 1.80 (m, 2H, CH_2), 2.10 (s, 3H, CH_3), 2.83 (m, 2H, CH_2-Ar), 2.97 (m, 2H, CH_2-N), 3.85 (m, 1H, CH), 5.91 (s, 1H, ArH), 6.34 (br s, 2H, NH_2), 6.85 (m, 1H, ArH), 7.38 (m, 1H, ArH), 7.65 (m, 4H, ArH), 7.94 (m, 1H, ArH), 9.30 (br s, 1H, NH), 12.10 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 15.63, 21.2, 23.2, 26.0, 28.8, 29.0, 32.2, 34.8, 50.2, 65.3, 95.7, 117.0, 118.2, 119.3, 120.2, 129.2, 137.3, 138.5, 141.0, 144.3, 155.2, 161.8, 162.3, 164.1, 170.5. **LC-MS** (ESI^-): m/z 512 $[M-H]^-$. **HRMS** (ESI^+): m/z calculated $C_{30}H_{40}N_7O$ $[M+H]^+$ 514.2388, found 514.3265. **IR**, υ' (cm^{-1}): 1571 (C=O).

N-(4-(1*H*-benzo[*d*]imidazol-2-yl)butyl)-3-((2-amino-6-methylpyrimidin-4-yl)amino)benzamide, **1e**



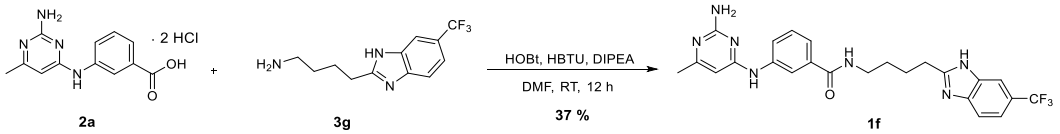
2a
1.2 eq., 402 mg, 1.27 mmol

3d
1 eq., 200 mg, 1.06 mmol

1e
Formula: $C_{23}H_{25}N_7O$
MW: 415.49

Appearance: white powder. **mp** = 245-247 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.59 (tt, ^3J=6.6 MHz, 2H, CH_2), 1.83 (^3J=6.6 MHz, 2H, CH_2), 2.09 (s, 3H, CH_3), 2.85 (t, ^3J=6.6 MHz, 2H, CH_2-Ar), 3.27-3.31 (m, 2H, CH_2-N), 5.88 (s, 1H, ArH), 6.14 (br s, 2H, NH_2), 7.06-7.13 (m, 2H, ArH), 7.28-7.41 (m, 3H, ArH), 7.47-7.53 (m, 1H, ArH), 7.84 (s, 1H, ArH), 8.10 (d, ^3J=8.3 MHz, 1H, ArH), 8.39 (t, ^3J=5.5 MHz, 1H, NH), 9.11 (s, 1H, NH), 12.16 (s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 23.9, 25.5, 28.6, 29.2, 39.9, 95.3, 118.5, 118.8, 120.1, 121.2, 121.7, 122.3, 128.9, 135.7, 141.3, 155.4, 161.8, 163.3, 165.7, 166.7. **LC-MS** (ESI$^+$): m/z 416 $[M+H]^+$. **HRMS** (ESI$^+$): m/z calculated $C_{23}H_{26}N_7O$ $[M+H]^+$ 416.2193, found 416.2164. **IR**, υ' (cm^{-1}): 1569 (C=O).

3-((2-amino-6-methylpyrimidin-4-yl)amino)-N-(4-(6-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)butyl)benzamide, **1f**



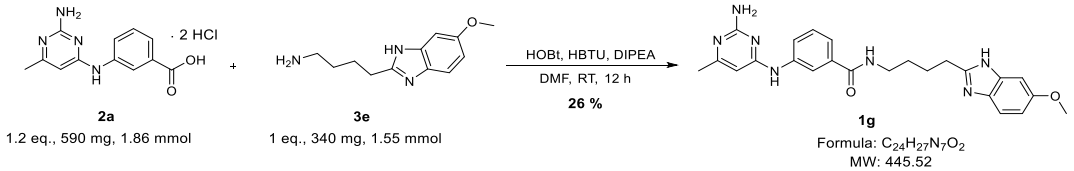
2a
1.2 eq., 295 mg, 0.933 mmol

3g
1 eq., 200 mg, 0.777 mmol

1f
Formula: $C_{24}H_{24}F_3N_7O$
MW: 483.49

Appearance: white powder. **mp** = 176-178 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.59 (tt, ^3J=7.4 MHz, 2H, CH_2), 1.84 (tt, ^3J=7.4 MHz, 2H, CH_2), 2.10 (s, 3H, CH_3), 2.90 (t, ^3J=6.8 MHz, 2H, CH_2-Ar), 3.26-3.34 (m, 2H, CH_2-NH), 5.90 (s, 1H, ArH), 6.33 (br s, 2H, NH_2), 7.29-7.37 (m, 2H, ArH), 7.40-7.45 (m, 1H, ArH), 7.58-7.61 (m, 1H, ArH), 7.69.7.74 (m, 1H, ArH), 7.82-7.86 (m, 1H, ArH), 8.06-8.08 (m, 1H, ArH), 8.39 (t, ^3J=5.7 MHz, 1H, NH), 9.27 (br s, 1H, NH), 12.63 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 23.2, 25.3, 28.6, 29.2, 95.6, 112.0, 115.7, 118.6, 119.1, 120.6, 122.6, 128.9, 135.8, 140.9, 161.8, 162.3, 164.1, 166.7. **LC-MS** (ESI$^+$): m/z 484 $[M+H]^+$. **HRMS** (ESI$^-$): m/z calculated $C_{24}H_{23}F_3N_7O$ $[M-H]^-$ 482.1910, found 482.1886. **IR**, υ' (cm^{-1}): 1572 (C=O).

3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(4-(6-methoxy-1*H*-benzo[*d*]imidazol-2-yl)butyl)benzamide, **1g**



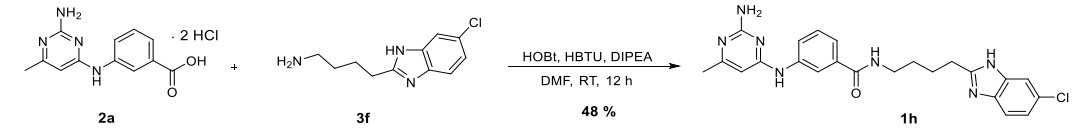
2a
1.2 eq., 590 mg, 1.86 mmol

3e
1 eq., 340 mg, 1.55 mmol

1g
Formula: $C_{24}H_{27}N_7O_2$
MW: 445.52

Appearance: white powder. **mp** = 142-144 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.52-1.62 (m, 2H, CH_2), 1.74-1.84 (m, 2H, CH_2), 2.09 (s, 3H, CH_3), 2.75-2.84 (m, 2H, CH_2-Ar), 3.21-3.29 (m, 2H, CH_2-NH), 3.74 (s, 3H, OCH_3), 5.88 (s, 1H, ArH), 6.19 (br s, 2H, NH_2), 6.69-6.75 (m, 1H, ArH), 6.92-6.99 (m, 1H, ArH), 7.26-7.40 (m, 3H, ArH), 7.81-7.87 (m, 1H, ArH), 8.04-8.11 (m, 1H, ArH), 8.39 (br s, 1H, NH), 9.16 (br s, 1H, NH), 12.00 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 23.8, 25.5, 28.6, 29.2, 55.8, 95.4, 110.6, 112.8, 118.8, 120.3, 122.4, 124.5, 128.9, 135.7, 141.2, 155.5, 161.8, 163.0, 165.3, 166.7. **LC-MS** (ESI^+): m/z 446 $[M+H]^+$. **HRMS** (ESI^-): m/z calculated $C_{24}H_{26}N_7O_2$ $[M-H]^-$ 444.2142, found 444.2125. **IR**, υ' (cm^{-1}): 1572 (C=O).

3-((2-amino-6-methylpyrimidin-4-yl)amino)-N-(4-(6-chloro-1*H*-benzo[d]imidazol-2-yl)butyl)benzamide, **1h**



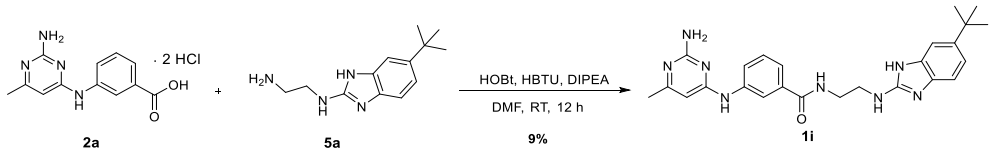
2a
1.2 eq., 255 mg, 0.805 mmo

3f
1 eq., 150 mg, 0.671 mmol

1h
Formula: $C_{23}H_{24}ClN_7O$
MW: 449.94

Appearance: white powder. **mp** = 143-145 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.58 (tt, ^3J=6.4 MHz, 2H, CH_2), 1.81 (tt, ^3J=6.4 MHz, 2H, CH_2), 2.09 (s, 3H, CH_3), 2.84 (t, ^3J=7.1 MHz, 2H, CH_2-Ar), 3.25-3.30 (m, 2H, CH_2-NH), 5.88 (s, 1H, ArH), 6.21 (br s, 2H, NH_2), 7.10-7.14 (m, 1H, ArH), 7.28-7.38 (m, 2H, ArH), 7.41-7.55 (m, 2H, ArH), 7.84 (m, 1H, ArH), 8.06-8-10 (m, 1H, ArH), 8.38 (t, ^3J=5.7 MHz, 1H, CONH), 9.17 (s, 1H, NH), 12.37 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 23.7, 25.3, 28.6, 29.2, 95.4, 118.0, 118.9, 120.3, 121.7, 122.4, 125.8, 128.9, 135.7, 141.1, 157.2, 161.8, 162.9, 165.1, 166.7. **LC-MS** (ESI^+): m/z 450 $[M+H]^+$ for ^{35}Cl and 452 $[M+H]^+$ for ^{37}Cl. **HRMS** (ESI^+): m/z calculated $C_{23}H_{23}ClN_7O$ $[M+H]^+$ 448.1647, found 448.1633. **IR**, υ' (cm^{-1}): 1621 (C=O).

3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(2-((6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)amino)ethyl)benzamide, **1i**.



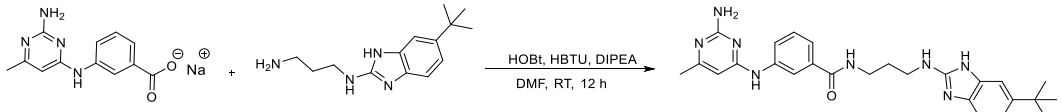
2a
1 eq., 95.6 mg, 0.301 mmol

5a
1 eq., 70 mg, 0.301 mmol

1i
Formula: $C_{25}H_{30}N_8O$
MW: 458.56

Appearance: white powder. **mp**= 182-184 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.28 (s, 9H, *t*-Bu), 2.08 (s, 3H, CH_3), 3.46-3.50 (m, 4H, CH_2), 5.88 (s, 1H, ArH), 6.13 (br s, 2H, NH_2), 6.67-6.71 (m, 1H, NH), 6.89-6.93 (m, 1H, ArH), 7.02-7.06 (m, 1H, ArH), 7.16 (br s, 1H, ArH), 7.30 (t, ^3J=7.8 MHz, 1H, ArH), 7.41-7.43 (m, 1H, ArH), 7.88-7.90 (m, 1H, ArH), 8.10-8.13 (m, 1H, ArH), 8.95-9.00 (m, 1H, NH), 9.13 (s, 1H, NH), 10.70 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 23.9, 29.4, 32.3, 34.6, 41.2, 42.0, 95.4, 118.7, 118.8, 120.1, 122.3, 128.9, 135.5, 141.3, 141.4, 156.2, 161.7, 161.8, 163.2, 165.6, 166.9, 166.9. **LC-MS** (ESI$^-$): m/z 457 [M-H]$^-$. **HRMS** (ESI$^+$): m/z calculated $C_{25}H_{31}N_8O$ [M+H]$^+$ 459.2615, found 459.2598. **IR**, υ' (cm^{-1}): 1571 (C=O).

3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(3-((6-(tert-butyl)-1*H*-benzo[*d*]imidazol-2-yl)amino)propyl)benzamide, **1j**



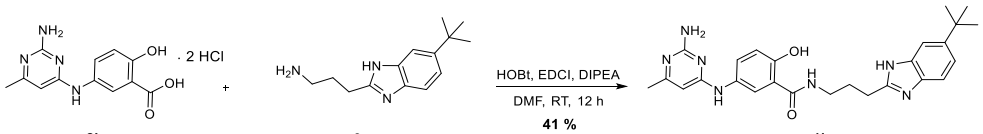
2a
1.2 eq., 216 mg, 0.682 mmol

5b
1 eq., 140 mg, 0.568 mmol

1j
Formula: $C_{26}H_{32}N_8O$
MW: 472.59

Appearance: yellowish powder. **mp** = 160-162 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.27 (s, 9H, *t*-Bu), 1.79 (t, ^3J=6.3 MHz, 2H, CH_2), 2.09 (s, 3H, CH_3), 3.30-3.39 (m, 4H, CH_2), 5.90 (s, 1H, ArH), 6.19 (br s, 2H, NH_2), 6.79 (m, 1H, NH), 6.94 (dd, ^3J=1.8, 8.0 MHz, 1H, ArH), 7.04 (d, ^3J=8.0 MHz 1H, ArH), 7.14 (d, ^3J=1.8 MHz, 1H, ArH), 7.31.7.38 (m, 1H, ArH), 7.43-7.47 (m, 1H, ArH), 7.92-7.94 (m, 1H, ArH), 8.09-8.11 (m, 1H, ArH), 8.71 (t, ^3J=5.3 MHz, 1H, NH), 9.18 (br s, 1H, NH), 11.02 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 23.7, 30.0, 32.0, 32.1, 32.2, 34.6, 36.8, 95.4, 117.2, 118.9, 120.2, 122.5, 128.9, 135.7, 141.3, 142.6, 155.7, 161.8, 163.0, 165.2, 166.8. **LC-MS** (ESI$^+$): m/z 473 [M+H]$^+$. **HRMS** (ESI$^-$): m/z calculated $C_{26}H_{31}N_8O$ [M-H]$^-$ 471.2615, found 471.2594. **IR**, υ' (cm^{-1}): 1570 (C=O).

5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(3-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)propyl)-2-hydroxybenzamide, **1k**



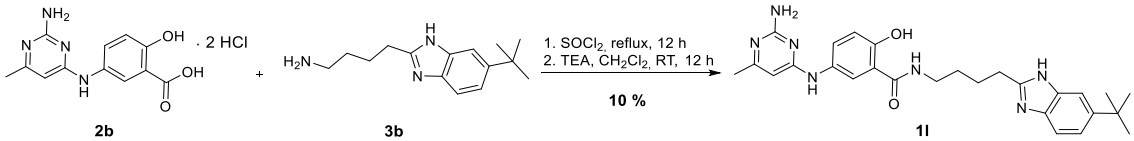
2b
1 eq., 151 mg, 0.454 mmol

3a
1 eq., 105 mg, 0,454 mmol

1k
Formula: $C_{26}H_{31}N_7O_2$
MW: 473.57

Appearance: white powder. **mp** = 178-180 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.30 (s, 9H, *t*-Bu), 1.91 (dd, ^3J=5.8 MHz, 2H, CH_2), 2.06 (s, 3H, CH_3), 2.86 (t, ^3J=7.5 MHz, 2H, CH_2-Ar), 3.39 (t, ^3J=5.8 MHz, 2H, CH_2-NH), 5.76 (s, 1H, ArH), 6.10 (br s, 2H, NH_2), 6.86 (d, ^3J=8.6 MHz, 1H, ArH), 7.19 (dd, J=1.6, 8.3 MHz, 1H, ArH), 7.35-7.42 (m, 2H, ArH), 7.60 (dd, J=2.6, 8.9 MHz, 1H, ArH), 7.92 (d, ^3J=2.5 MHz, 1H, ArH), 8.80 (br s, 1H, OH), 8.85-8.89 (m, 1H, NH), 12.09 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 23.6, 26.6, 27.7, 32.1, 34.8, 94.3, 116.0, 117.7, 119.3, 121.4, 128.3, 131.8, 144.3, 154.8, 155.6, 162.4, 163.0, 164.8, 168.8. **LC-MS** (APCI$^+$): m/z 474 $[M+H]^+$. **HRMS** (ESI$^+$): m/z calculated $C_{26}H_{32}N_7O_2$ $[M+H]^+$ 474.2612 found 474.2586. **IR**, υ' (cm^{-1}): 1584 (C=O).

5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(4-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)butyl)-2-hydroxybenzamide, **1l**



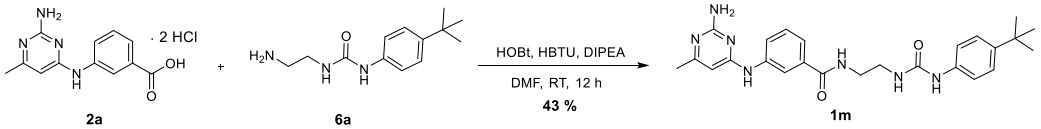
2b
1 eq., 300 mg, 0,9 mmol

3b
1 eq., 221 mg, 0.9 mmol

1l
Formula: $C_{27}H_{33}N_7O_2$
MW: 487.60

Appearance: beige powder. **mp** = 171-173 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.31 (m, 9H, *t*-Bu), 1.60 (m, 2H, CH_2), 1.80 (m, 2H, CH_2), 2.08 (s, 3H, CH_3), 2.82 (m, 2H, CH_2-Ar), 3.34 (m, 2H, CH_2-N), 5.81 (s, 1H, ArH), 6.43 (br s, 2H, NH_2), 6.86 (m, 1H, ArH), 7.16 (m, 1H, ArH), 7.36 (m, 2H, ArH), 7.55 (m, 1H, ArH), 7.88 (s,1H, ArH), 8.74 (m, 1H, NH), 9.13 (br s, 1H, NH), 12.18 (m, 2H, NH, OH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 22.6, 25.5, 28.6, 28.95 32.2, 34.8, 39.9, 94.8, 116.1, 117.8, 119.3, 121.8, 128.6, 131.2, 144.2, 155.2, 156.0, 161.5, 162.4, 168.6. **LC-MS** (ESI$^-$): m/z 486 $[M-H]^-$. **HRMS** (ESI$^+$): m/z calculated $C_{27}H_{34}N_7O_2$ $[M+H]^+$ 488.2768, found 488.2755. **IR**, υ' (cm^{-1}): 1653 (C=O).

3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(2-(3-(4-(*tert*-butyl)phenyl)ureido)ethyl)benzamide, **1m**



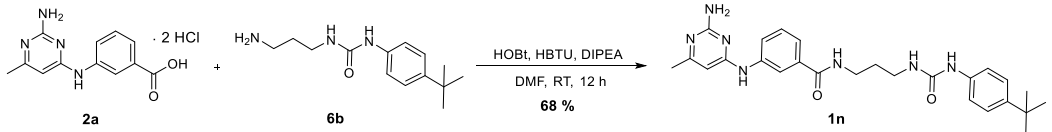
2a
1 eq., 500 mg, 1.58 mmol

6a
1 eq., 370 mg, 1.58 mmol

1m
Formula: $C_{25}H_{31}N_7O_2$
MW: 461.56

Appearance: white powder. **mp** = 177-179 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.22 (s, 9H, *t*-Bu), 2.13 (s, 3H, CH_3), 3.24-3.28 (m, 2H, CH_2), 3.34 (m, 2H, CH_2), 4.69-4.71 (m, 2H, NH_2), 5.97 (s, 1H, ArH), 6.18-6.22 (m, 1H, NH), 7.20 (d, ^3J=8.0 MHz, 2H, ArH), 7.27 (d, ^3J=8.0 MHz, 2H, ArH), 7.33-7.36 (m, 1H, ArH), 7.40-7.42 (m, 1H, ArH), 7.55-7.61 (m, 1H, NH), 7.85-7.91 (m, 1H, NH), 8.39-8.42 (m, 2H, ArH), 9.28 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 21.9, 31.7, 34.2, 96.2, 118.0, 119.8, 121.5, 123.3, 125.6, 129.0, 135.6, 138.3, 140.2, 143.7, 156.0, 160.4, 161.9, 166.8. **LC-MS** (APCI$^+$): m/z 462 $[M+H]^+$. **HRMS** (ESI$^+$): m/z calculated $C_{25}H_{32}N_7O_2$ $[M+H]^+$ 462.2612, found 462.2594. **IR**, υ' (cm^{-1}): 1632 (C=O), 1651 (C=O).

3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)benzamide, **1n**



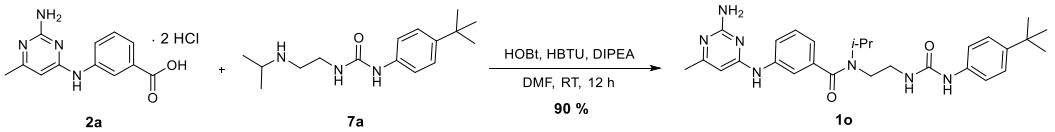
2a
1 eq., 286 mg, 0.902 mmol

6b
1.2 eq., 270 mg, 1.08 mmol

1n
Formula: $C_{26}H_{33}N_7O_2$
MW: 475.59

Appearance: white powder. **mp** = 220-222 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.23 (s, 9H, *t*-Bu), 1.65 (tt, ^3J=6.4 MHz, 2H, CH_2), 2.10 (s, 3H, CH_3), 3.12 (tt, ^3J=6.4 MHz, 2H, CH_2), 3.28-3.36 (m, 2H, CH_2), 5.90 (s, 1H, ArH), 6.15 (t, ^3J=5.6 MHz, 1H, NH), 6.29 (br s, 2H, NH_2), 7.19-7.22 (d, ^3J=8.7 MHz, 2H, ArH), 7.26-7.29 (d, ^3J=8.7 MHz, 2H, ArH), 7.34 (d, ^3J=7.8 MHz, 1H, ArH), 7.39 (d, ^3J=7.8 MHz, 1H, ArH), 7.87-7.89 (m, 1H, NH), 8.06 (d, ^3J=8.2 MHz, 1H, ArH), 8.37-8.43 (m, 2H, ArH, NH), 9.26 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 23.3, 30.5, 31.7, 34.2, 37.2, 95.6, 119.0, 120.5, 122.6, 125.6, 129.0, 135.7, 138.3, 141.0, 143.6, 155.9, 161.8, 162.4, 164.4, 166.8, 180.0. **LC-MS** (APCI$^+$): m/z 476 $[M+H]^+$. **HRMS** (ESI): m/z calculated $C_{26}H_{34}N_7O_2$ $[M+H]^+$ 476.2768, found 476.2770. **IR**, υ' (cm^{-1}): 1625 (C=O).

3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(2-(3-(4-(*tert*-butyl)phenyl)ureido)ethyl)-*N*-isopropylbenzamide, **1o**



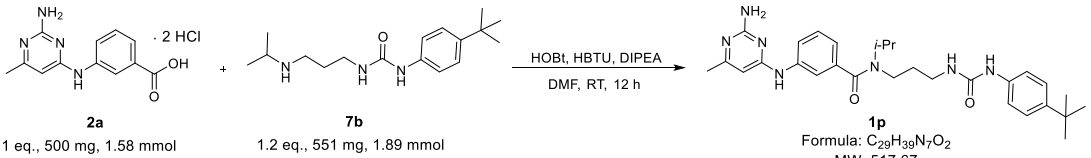
2a
1 eq., 500 mg, 1.58 mmol

7a
1.2 eq., 524 mg, 1.89 mmol

1o
Formula: $C_{28}H_{37}N_7O_2$
MW: 503.64

Appearance: white powder. **mp** = 208-210 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.11 (m, 6H, *i*-Pr), 1.22 (s, 9H, *t*-Bu), 2.12 (s, 3H, CH_3), 3.27-3.39 (m, 4H, CH_2), 3.87 (m, 1H, CH), 5.92 (s, 1H, ArH), 6.23-6.32 (m, 1H, NH), 6.46 (br s, 2H, NH_2), 6.94 (d, ^3J=6.8 MHz, 1H, ArH), 7.20 (d, ^3J=7.8 MHz, 2H, ArH), 7.26-7.36 (m, 3H, ArH), 7.55 (br s, 1H, ArH), 7.85 (d, ^3J=8.7 MHz, 1H, ArH), 8.44 (br s, 1H, NH), 9.38 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 20.6, 22.4, 31.3, 33.8, 49.8, 95.4, 116.9, 117.7, 119.4, 120.2, 125.1, 128.8, 137.8, 140.3, 143.3, 155.5, 161.4, 162.9, 170.6, 179.7. **LC-MS** (APCI$^+$): m/z 504 $[M+H]^+$. **HRMS** (ESI$^+$): m/z calculated $C_{28}H_{38}N_7O_2$ $[M+H]^+$ 504.3078, found 504.3078. **IR**, υ' (cm^{-1}): 1660 (C=O).

3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)-*N*-isopropylbenzamide, **1p**



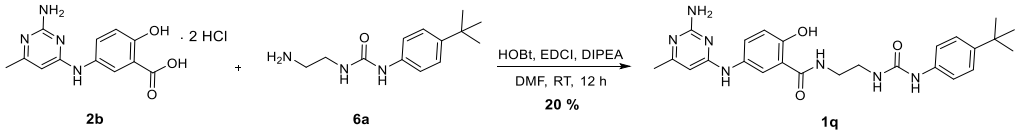
2a
1 eq., 500 mg, 1.58 mmol

7b
1.2 eq., 551 mg, 1.89 mmol

1p
Formula: $C_{29}H_{39}N_7O_2$
MW: 517.67

Appearance: white powder. **mp** = 154-156 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.06-1-17 (m, 15H, *i*-Pr, *t*-Bu), 1.72-1.80 (m, 2H, CH_2), 2.11 (s, 3H, CH_3), 3.24.3.32 (m, 2H, CH_2-NH), 3.14-3.17 (m, 2H, CH_2-N), 3.83-3.93 (m, 1H, CH), 5.90 (s, 1H, ArH), 6.12-6.18 (m, 1H, NH), 6.29 (br s, 2H, NH_2), 6.88 (d, *J*=7.5 MHz, 1H, ArH), 7.20-7.23 (m, 2H, ArH), 7.27-7.34 (m, 3H), 7.47 (br s, 1H, ArH), 7.90 (d, *J*=8.4 MHz,1H, ArH), 8.39 (br s, 1H, NH), 9.22 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 21.2, 23.4, 30.4, 31.7, 34.2, 37.6, 95.7, 116.9, 118.0, 119.4, 120.2, 125.6, 129.3, 138.4, 141.1, 143.6, 155.8, 161.8, 162.5, 164.5, 170.7. **LC-MS** (APCI$^+$): m/z 518 $[M+H]^+$. **HRMS** (ESI$^+$): m/z calculated $C_{29}H_{40}N_7O_2$ $[M+H]^+$ 518.3238, found 518.3237. **IR**, υ' (cm^{-1}): 1659 (C=O).

5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(2-(3-(4-(*tert*-butyl)phenyl)ureido)ethyl)-2-hydroxybenzamide, **1q**



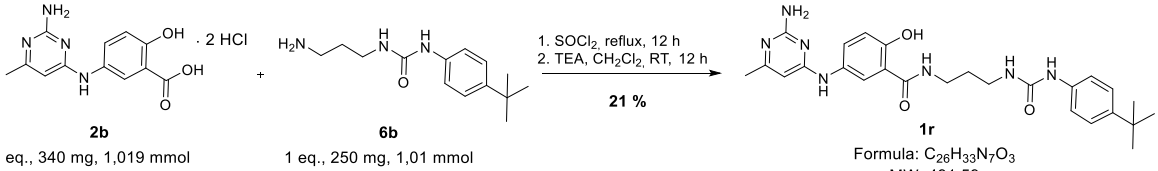
2b
1 eq., 492 mg, 1.48 mmol

6a
1 eq., 348 mg, 1.48 mmol

1q
Formula: $C_{25}H_{31}N_7O_3$
MW: 477.56

Appearance: white powder. **mp** = 228-230 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.22 (s, 9H, *t*-Bu), 2.12 (s, 3H, CH_3), 3.25-3-33 (m, 2H, CH_2), 3.33-3-47 (m, 2H, CH_2), 5.87 (s, 1H, ArH), 6.34 (t, *J*=5.4 MH, 1H, NH), 6.58 (br s, 2H, NH_2), 6.90 (d, *J*=9.1 MH, 1H, ArH), 7.18 (d, *J*=9.1 MH, 2H, ArH), 7.27 (d, *J*=9.1 MHz, 2H, ArH), 7.56-7.59 (m, 1H, ArH), 7.95-7.96 (m, 1H, ArH), 8.54 (br s, 1H, NH), 8.78-8.82 (m, 1H, NH), 9.31 (br s, 1H, NH), 12.19 (br s, 1H, OH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 22.2, 31.7, 34.2, 95.1, 116.1, 117.9, 118.1, 122.0, 125.6, 128.6, 131.1, 138.2, 143.8, 156.2, 161.0, 161.3, 161.4, 162.4, 168.8. **LC-MS** (APCI$^+$): m/z 478 $[M+H]^+$. **HRMS** (ESI$^+$): m/z calculated $C_{25}H_{32}N_70_3$ $[M+H]^+$ 478.2561, found 478.2545. **IR**, υ' (cm^{-1}): 1651 (C=O).

5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)-2-hydroxybenzamide, **1r**



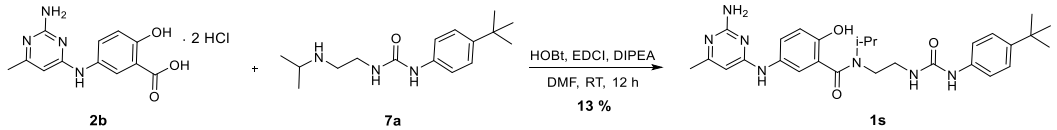
2b
1 eq., 340 mg, 1,019 mmol

6b
1 eq., 250 mg, 1,01 mmol

1r
Formula: $C_{26}H_{33}N_7O_3$
MW: 491.59

Appearance: white powder. **mp** = 227-229 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.23 (s, 9H, *t*-Bu), 1.63-1.74 (m, 2H, CH_2), 2.06 (s, 3H, CH_3), 3.13-3.17 (m, 2H, CH_2), 3.32-3-38 (m, 2H, CH_2), 5.77 (s, 1H, ArH), 6.08-6.19 (m, 3H, NH_2, NH), 6.86 (d, *J*= 7.0 Hz,1H, ArH), 7.18-7.56 (m, 4H, ArH), 7.54-7.59 (m, 1H, ArH), 7.92 (br s, 1H, ArH), 8.43 (br s, 1H, NH), 8.73 (br s, 1H, NH), 8.83 (br s, 1H, NH), 12.08 (br s, 1H, OH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 23.6, 30.3, 31.7, 34.2, 37.0, 94.3, 116.0, 117.7, 118.0, 121.3, 125.6, 128.2, 131.9, 138.3, 143.6, 155.6, 155.9, 162.4, 162.9, 164.6, 168.7. **LC-MS** (ESI$^+$): m/z 492 $[M+H]^+$. **HRMS** (ESI$^+$): m/z calculated $C_{26}H_{34}N_7O_3$ $[M+H]^+$ 492.2717, found 492.2722. **IR**, υ' (cm^{-1}): 1644 (C=O), 1585 (C=O).

5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(2-(3-(4-(*tert*-butyl)phenyl)ureido)ethyl)-2-hydroxy-*N*-isopropylbenzamide, **1s**



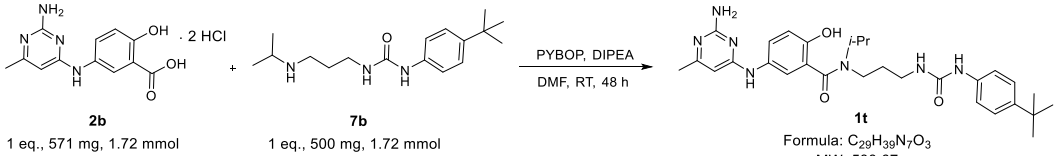
2b
1 eq., 228 mg, 0.685 mmol

7a
1 eq., 190 mg, 0.685 mmol

1s
Formula: $C_{28}H_{37}N_7O_3$
MW: 519.64

Appearance: white powder. **mp** = 166-168 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.15 (m, 6H, *i*-Pr), 1.24 (s, 9H, *t*-Bu), 2.04 (s, 3H, CH_3), 3.21-3.43 (m, 4H, CH_2), 3.73-3.83 (m, 1H, CH), 5.75 (s, 1H, ArH), 5.97 (br s, 2H, NH_2), 6.26-6.32 (m, 1H, NH), 6.78 (d, *J*=8.0 MHz, 1H, ArH), 7.11-7.16 (m, 1H, ArH), 7.18-7.25 (m, 2H, ArH), 7.27-7.35 (m, 2H, ArH), 7.52 (d, *J*=9.4 MHz, 1H, ArH), 8.45-8.50 (m, 1H, NH), 8.67 (br s, 1H, OH), 9.38 (m, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 21.3, 23.9, 31.7, 34.2, 50.0, 94.3, 116.2, 118.1, 119.9, 122.9, 125.6, 132.7, 138.3, 143.8, 155.9, 162.1, 163.4, 165.3. **LC-MS** (APCI$^+$): m/z 520 $[M+H]^+$. **HRMS** (ESI$^+$): m/z calculated $C_{28}H_{38}N_7O_3$ $[M+H]^+$ 520.3030, found 520.3034. **IR**, υ' (cm^{-1}): 1582 (C=O), 1649 (C=O).

5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)-2-hydroxy-*N*-isopropylbenzamide, **1t**



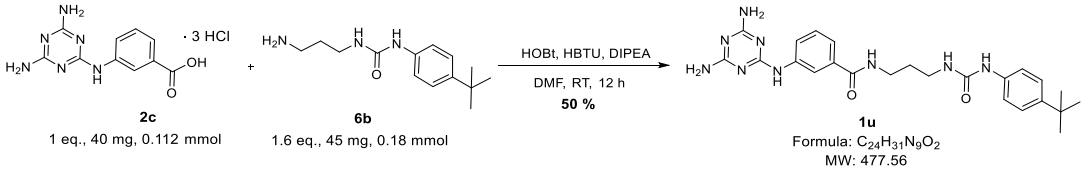
2b
1 eq., 571 mg, 1.72 mmol

7b
1 eq., 500 mg, 1.72 mmol

1t
Formula: $C_{29}H_{39}N_7O_3$
MW: 533.67

Appearance: white powder. **mp** = 179-181 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.05-1.14 (m, 6H, *i*-Pr), 1.23 (s, 9H, *t*-Bu), 1.67-1.80 (m, 2H, CH_2), 2.18 (s, 3H, CH_3), 3.07-3.18 (m, 2H, CH_2-NH), 3.33-3.38 (m, 2H, CH_2-N), 3.68-3.78 (m, 1H, CH), 5.92 (s, 1H, ArH), 6.17 (br s, 1H, NH), 6.84 (d, *J*=8.2 MHz, 1H, ArH), 7.09-7.13 (m, 2H, NH_2), 7.17-7.24 (m, 3H, ArH), 7.25-7.30 (m, 2H, ArH), 7.56 (d, *J*=8.2 MHz, 1H, ArH), 8.40 (br s, 1H, NH), 9.67 (br s, 1H, OH), 9.80 (br s, 1H, NH), 12.01 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 20.2, 21.3, 31.7, 34.2, 37.6, 37.9, 50.1, 96.1, 116.2, 117.9, 121.2, 124.1, 125.6, 130.5, 138.4, 143.6, 150.3, 155.8, 157.9, 162.1, 168.1. **LC-MS** (APCI$^+$): m/z 534 $[M+H]^+$. **HRMS** (ESI$^+$): m/z calculated $C_{29}H_{40}N_70_3$ $[M+H]^+$ 534.3187, found 534.3163. **IR**, υ' (cm^{-1}): 1657 (C=O).

N-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)-3-((4,6-diamino-1,3,5-triazin-2-yl)amino)benzamide, **1u**



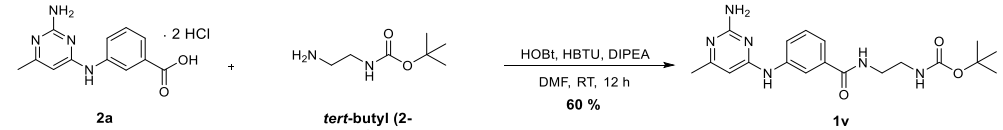
2c
1 eq., 40 mg, 0.112 mmol

6b
1.6 eq., 45 mg, 0.18 mmol

1u
Formula: $C_{24}H_{31}N_9O_2$
MW: 477.56

Appearance: beige powder. **mp** = 140-142 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.23 (s, 9H, *t*-Bu), 1.65 (dd, *J*=6.7, 6.1 MHz, 2H, CH_2), 3.13 (dd, *J*=6.1MHz, 2H, CH_2), 3.28-3.40 (m, 2H, CH_2), 6.15 (t, *J*=5.5 MHz, 1H, NH), 6.52 (br s, 4H, NH_2), 7.20 (d, *J*=8.5 MHz, 2H, ArH), 7.28 (dd, *J*=8.2, 7.5 MHz, 3H, ArH), 7.37 (d, *J*=7.5 MHz, 1H, ArH), 7.97-8.01 (m, 2H, ArH), 8.34 (t, *J*=5.8 MHz, 1H, NH), 8.43 (br s, 1H, NH), 9.13 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 19.2, 30.5, 31.7, 34.2, 37.2, 46.7, 118.0, 119.6, 120.6, 123.1, 125.6, 128.6, 135.6, 138.3, 140.7, 143.6, 155.9, 164.8, 166.0, 167.0. **LC-MS** (ESI^-): m/z 476 $[M-H]^-$. **HRMS** (ESI^+): m/z calculated $C_{24}H_{32}N_9O_2$ $[M+H]^+$ 478.2673, found 478.2662. **IR**, υ' (cm^{-1}): 1536 (C=O), 1594 (C=O).

Tert-butyl (2-(3-((2-amino-6-methylpyrimidin-4-yl)amino)benzamido)ethyl)carbamate, **1v**



2a
1 eq., 700 mg, 2.21 mmol

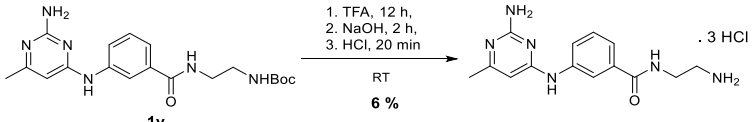
***tert*-butyl (2-aminoethyl)carbamate**
1 eq., 346 µL, 2.21 mmol

1v
Formula: $C_{19}H_{26}N_6O_3$
MW: 386.45

Appearance: beige powder. **mp** = 163-165 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.36 (s, 9H, *t*-Bu), 2.09 (s, 3H, CH_3), 3.09 (dd, *J*=4.9, 6.6 MHz, 2H, CH_2), 3.25-3.30 (m, 2H, CH_2), 5.88 (s, 1H, ArH), 6.17 (br s, 2H, NH_2), 6.90 (t, *J*=5.2 MHz, 1H, NH), 7.28-7.38 (m, 2H, ArH), 7.88 (br s, 1H, ArH), 8.05-8.07 (m, 1H, ArH), 8.33 (t, *J*=6.1 MHz, 1H, NH), 9.14 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 23.8, 28.7, 78.1, 95.3, 118.8, 120.2, 122.4, 128.8, 135.5, 141.3, 156.2, 161.8, 163.1, 165.4, 166.9. **LC-MS** (ESI^+): m/z 387 $[M+H]^+$. **HRMS** (ESI^+): m/z calculated $C_{19}H_{27}N_6O_3$ $[M+H]^+$ 387.2139, found 387.2124. **IR**, υ' (cm^{-1}): 1642 (C=O), 1687 (C=O).

For 1w: 1v (1 eq., 465 mg, 1.2 mmol) was suspended in TFA (50.7 eq., 4.53 mL, 61 mmol) and the mixture was stirred overnight at room temperature. The solvent was removed *in vacuo*, 10 % NaOH was added to the residue, and it stirred for 2 h. The aqueous layer was extracted three times with EtOAc. The organic layers were gathered together, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography over silica gel (CH_2Cl_2:CH_3OH/ammonia, 9:1). The corresponding fraction was concentrated *in vacuo*, and the residue was suspended in diethyl ether, to which 50 µL of 5-6N HCl in isopropanol were added dropwise. After stirring for 20 minutes, the visible solid was recovered by filtration, washed with diethyl ether, and dried over P_2O_5.

3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(2-aminoethyl)benzamide trihydrochloride, **1w**



1v
1 eq., 465 mg, 1.2 mmol

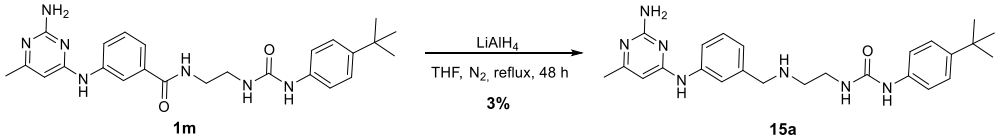
1w
Formula: $C_{14}H_{21}Cl_3N_6O$
MW (chloride): 395.72
MW (base): 286.33

Appearance: beige powder. **mp** = 160-162 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 2.20 (s, 3H, CH_3), 2.95-3.02 (m, 2H, CH_2), 3.49-3.55 (m, 2H, CH_2), 6.06 (s, 1H, ArH), 7.15 (br s, 3H, NH_3^+), 7.41 (t, *J*=7.7 Hz, 1H, ArH), 7.58 (d, *J*=9.0 Hz, 1H, ArH), 7.96-8.06 (m, 5H, ArH, NH_3^+), 8.65-8.72 (m, 1H, CONH), 10.07 (br s, 2H, NH_2^+). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 21.0, 37.6, 95.5, 120.2, 122.3, 123.9, 129.1, 135.2, 139.8, 162.0, 167.1. **LC-MS** (ESI^+): m/z 287 $[M+H]^+$. **HRMS** (ESI^+): m/z calculated $C_{14}H_{19}N_6O$ $[M+H]^+$ 287.1614, found 287.1651. **IR**, υ' (cm^{-1}): 1653 (C=O).

<u>General procedure 6:</u> Synthesis of amines 15a-c

For 15a: 1m (1 eq., 259 mg, 0.561 mmol) suspended in THF (5 mL) was added dropwise via an addition funnel to a solution of $LiAlH_4$ (4 eq., 85.2 mg, 2.24 mmol) in THF (10 mL) at 0 °C under nitrogen, and the mixture was refluxed during 48 h. After cooling to room temperature, the reaction was quenched with 0.3 mL of water, then with 0.3 mL of 2N NaOH, and with 0.9 mL of water, and it was stirred for 2 h. The precipitate observed was filtered and washed with THF. The resulting filtrate was extracted three times with EtOAc. The organic layers were gathered together, washed with brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography over silica gel (gradient CH_2Cl_2:CH_3OH/ammonia, 95:5 to 90:10). The corresponding fraction was concentrated *in vacuo*, and diethyl ether was added to the dry residue to yield a powder which was recovered by filtration and dried over P_2O_5.

1-(2-((3-((2-amino-6-methylpyrimidin-4-yl)amino)benzyl)amino)ethyl)-3-(4-(*tert*-butyl)phenyl)urea, **15a**



1m
1 eq., 152 mg, 0.329 mmol

15a
Formula: $C_{25}H_{33}N_7O$
MW: 447.58

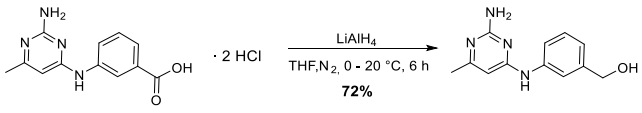
Appearance: white powder. **mp** = 141-143 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.23 (s, 3H, CH_3), 1.73 (s, 9H, *t*-Bu), 2.03 (m, 2H, CH_2), 2.10 (s, 2H, CH_2), 3.47 (m, 2H, CH_2), 5.91 (s, 1H, ArH), 6.20 (br s, 3H, NH, NH_2), 7.17-7.20 (m, 1H), 7.30-7.37 (m, 2H, ArH), 7.43-7.47 (m, 2H, ArH), 8.07 (t, J= 2.3 Hz, 1H, ArH), 8.10 (t, J= 2.3 Hz, 1H, ArH), 8.25 (br s, 1H, ArH), 9.33 (s, 1H, NH), 9.98 (s, 1H, NH). **LC-MS** ($APCI^+$): m/z 448 $[M+H]^+$. **HRMS** (ESI^+): m/z calculated $C_{25}H_{34}N_7O$ $[M+H]^+$ 448.2819, found 448.2872. **IR**, υ' (cm^{-1}): 1576 (C=O).

For 15b-c

Synthesis of primary alcohol 16

2a (1 eq.) suspended in THF was added dropwise via an addition funnel to a solution of $LiAlH_4$ (4 eq.) in THF at 0 °C under nitrogen, and the mixture was stirred 6 h at room temperature. The reaction was quenched with a volume of water, then with a volume of 2N NaOH, and with 3 volumes of water, and it was stirred for 30 minutes. The precipitate observed was filtered and washed with THF. The resulting filtrate was concentrated *in vacuo* and the residue was purified by column chromatography over silica gel (CH_2Cl_2:CH_3OH, 95:5). The corresponding fraction was concentrated *in vacuo*, and diethyl ether was added to the dry residue to yield a powder which was recovered by filtration and dried over P_2O_5.

(3-((2-amino-6-methylpyrimidin-4-yl)amino)phenyl)methanol, **16**



2a
1 eq., 3 g, 9.46 mmol

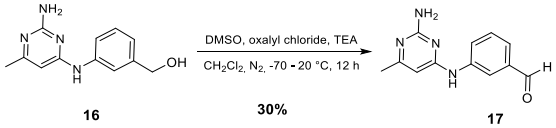
16
Formula: $C_{12}H_{14}N_4O$
MW: 230.27

Appearance: white powder. **mp** = 159-161 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 2.07(s, 3H, CH_3), 4.46 (d, ^3J = 5.4 Hz, 2H, CH_2), 5.13 (t, ^3J = 5.4 Hz, 1H, OH), 5.87 (s, 1H, ArH), 6.10 (br s, 2H, NH_2), 6.89 (d, ^3J = 7.5 Hz, 1H, ArH), 6.89 (dd, ^3J = 7.5 Hz, 1H, ArH), 7.50 (s, 1H, ArH), 7.66 (d, ^3J = 7.5 Hz, 1H, ArH), 8.94 (s, 1H, NH). **LC-MS** (ESI^-): m/z 229 $[M-H]^-$. **HRMS** (ESI^+): m/z calculated $C_{12}H_{15}N_4O$ $[M+H]^+$ 231.1240, found 231.1226. **IR**, υ' (cm^{-1}): 3305 (OH).

Synthesis of aldehyde 17

DMSO (4.3 eq., 729 mg, 0.663 mL, 9.34 mmol) suspended in CH_2Cl_2 (10 mL) was added dropwise to a solution of oxalyl chloride (2.3 eq., 2 M in CH_2Cl_2, 2.5 mL, 4.99 mmol) in CH_2Cl_2 (10 mL) and the solution was stirred for 5 min at −70 °C under nitrogen. **16** (1 eq., 500 mg, 2.17 mmol) suspended in CH_2Cl_2 (10 mL) was added dropwise to the first solution and the mixture was stirred for 25 min at −70 °C. TEA (20 eq., 4394 mg, 6.04 mL, 43.4 mmol) was added and the mixture was stirred to RT for 12 h. The reaction was quenched with water, and CH_2Cl_2 was removed *in vacuo*. The aqueous residue was extracted three times with EtOAc. The resulting aqueous phase was adjusted to pH 10 with 10% K_2CO_3, then extracted three times with EtOAc. The organic layers were gathered together, washed with water, brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography over silica gel (CH_2Cl_2:CH_3OH, 9:1). The corresponding fraction was concentrated *in vacuo*, and diethyl ether was added to the dry residue to yield a powder which was recovered by filtration and dried over P_2O_5.

3-((2-amino-6-methylpyrimidin-4-yl)amino)benzaldehyde, **17**



16
1 eq., 500 mg, 2.17 mmol

30%

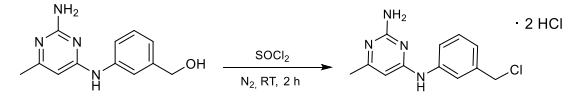
17
Formula: $C_{12}H_{12}N_4O$
MW: 228.25

Appearance: white powder. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 2.11 (s, 3H, CH_3), 5.90 (s, 1H, ArH), 6.21 (br s, 2H, NH_2), 7.45-7.48 (m, 2H, ArH), 8.07-8.11 (m, 1H, ArH), 8.24-8.26 (m, 1H, ArH), 9.29 (br s, 1H, NH), 9.98 (s, 1H, CHO). **LC-MS** (ESI^-): m/z 227 $[M-H]^-$. **IR**, υ' (cm^{-1}): 1690 (C=O).

Synthesis of chloride derivative 18a

16 (1 eq., 250 mg, 1.09 mmol) was suspended in $SOCl_2$ (3 mL) and the mixture was stirred for 2 h at room temperature under nitrogen. The mixture was concentrated *in vacuo* and water was added to the dry residue. The white precipitate observed was recovered by filtration and dried over P_2O_5.

N^4-(3-(chloromethyl)phenyl)-6-methylpyrimidine-2,4-diamine, **18a**



16
1 eq., 250 mg, 1.09 mmol

79%

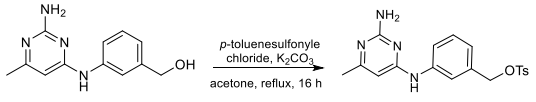
18a
Formula: $C_{12}H_{13}ClN_4$
MW (chloride): 321,63
MW (base): 248.71

Appearance: white powder. **mp** >250 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 2.24 (s, 3H, CH_3), 4.77 (s, 2H, CH_2), 6.10 (s, 1H, ArH), 7.18 (d, J=9.1 Hz, 1H, ArH), 7.36 (t, J=7.5 Hz, 1H, ArH), 7.44 (br s, 2H, NH), 7.73 (d, J= 7.5 Hz, 1H, ArH), 7.80 (s, 1H, ArH), 10.26 (br s, 2H, NH), 12.69 (m, 1H, NH). **LC-MS** (ESI^+): m/z 249 $[M+H]^+$ for ^{35}Cl, 251 $[M+H]^+$ for ^{37}Cl . **IR**, υ' (cm^{-1}): 3304 (NH).

Synthesis of tosyl derivative 18b

16 (1 eq., 500 mg, 2.17 mmol), *p*-toluenesulfonyl chloride (1.1 eq., 455 mg, 2.39 mmol) and K_2CO_3 (1 eq., 300 mg, 2.17 mmol) were suspended in acetone (30 mL) and the mixture was refluxed overnight. *p*-toluenesulfonyl chloride (1.1 eq., 455 mg, 2.39 mmol) was added and the reflux was kept for 4 more h. The solvent was removed *in vacuo* and the residue was extracted three times with EtOAc. The organic layers were gathered together, washed with 10% NaOH, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography over silica gel (CH_2Cl_2:CH_3OH, 9:1). The corresponding fraction was concentrated *in vacuo*, and diethyl ether was added to the dry residue to yield a powder which was recovered by filtration and dried over P_2O_5.

3-((2-amino-6-methylpyrimidin-4-yl)amino)benzyl 4-methylbenzenesulfonate, **18b**



16
1 eq., 500 mg, 2.17 mmol

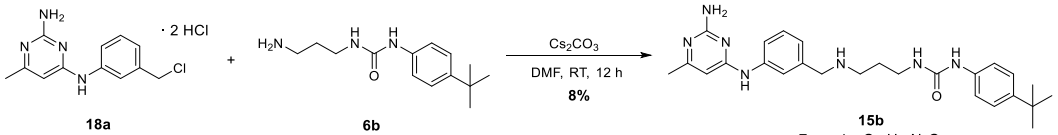
18b
Formula: $C_{19}H_{20}N_4O_3S$
MW: 384.45

Appearance: white powder. **mp** = 109-111 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 2.29 (s, 6H, CH_3), 4.78 (s, 2H, CH_2), 6.14 (s, 1H, ArH), 7.10 (m, 1H, ArH), 7.23 (m, 1H, ArH), 7.39 (m, 2H, ArH), 7.47 (m, 1H, ArH), 7.80 (m, 3H, ArH), 10.53 (br s, 1H, NH), 12.45 (m, 2H, NH). **LC-MS** (ESI^-): m/z 383 $[M-H]^-$. **IR**, υ' (cm^{-1}): 1655 (C=O).

Synthesis of 15b-c

A mixture of **18a** (1 eq.), the corresponding amine (1-1.5 eq.), and Cs_2CO_3 (1.5 eq.) (and KI (1 eq.) for **15c**) in DMF was stirred overnight at room temperature. The reaction mixture was concentrated *in vacuo*, and 10% K_2CO_3 was added to the residue, and the aqueous layer was extracted three times with EtOAc. The organic layers were gathered together, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography over silica gel (CH_2Cl_2:CH_3OH/ammonia, 9:1). The corresponding fraction was concentrated *in vacuo*, and diethyl ether was added to the dry residue to yield a powder which was recovered by filtration and dried over P_2O_5.

1-(3-((3-((2-amino-6-methylpyrimidin-4-yl)amino)benzyl)amino)propyl)-3-(4-(*tert*-butyl)phenyl)urea, **15b**



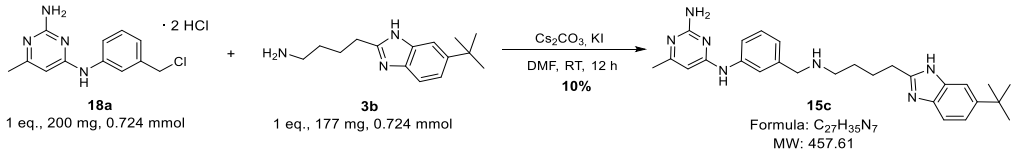
18a
1 eq., 40 mg, 0.124 mmol

6b
1.5 eq., 46,5 mg, 0,186 mmol

15b
Formula: $C_{26}H_{35}N_7O$
MW: 461.60

Appearance: beige powder. **mp** = 101-103 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.23 (s, 9H, *t*-Bu), 1.72-1.82 (m, 2H, CH_2), 2.08 (s, 3H, CH_3), 2.82-2.90 (m, 2H, CH_2-NH), 3.12-3.19 (m, 2H, CH_2-NHCO), 3.50 (s, 1H, NH), 4.01 (br s, 2H, CH_2-NH), 5.90 (s, 1H, ArH), 6.17 (br s, 2H, NH_2), 6.27-6.32 (m, 1H, NH), 7.01-7.04 (m, 1H, ArH), 7.19-7.29 (m, 5H, ArH), 7.62-7.65 (m, 1H, ArH), 7.85-7.89 (m, 1H, ArH), 8.52 (br s, 1H, NH), 9.10 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 23.9, 28.0, 31.7, 34.4, 36.8, 38.2, 57.5, 70.2, 95.3, 118.1, 119.6, 120.8, 122.6, 125.6, 129.2, 134, 138.2, 144., 145.1, 156.2, 161.9. **LC-MS** (ESI^+): m/z 462 $[M+H]^+$. **HRMS** (ESI^+): m/z calculated $C_{26}H_{36}N_7O$ $[M+H]^+$ 462.2975, found 462.2948. **IR**, υ' (cm^{-1}): 1574 (C=O).

N^4-(3-(((4-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)butyl)amino)methyl)phenyl)-6-methylpyrimidine-2,4-diamine, **15c**



18a
1 eq., 200 mg, 0.724 mmol

3b
1 eq., 177 mg, 0.724 mmol

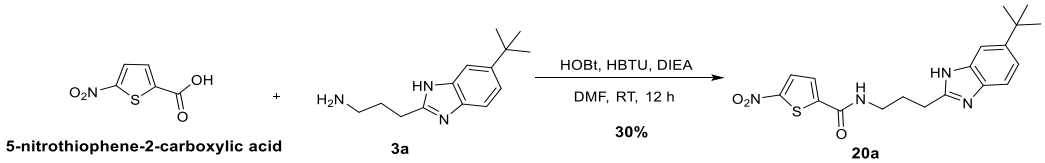
15c
Formula: $C_{27}H_{35}N_7$
MW: 457.61

Appearance: white powder. **mp** = 104-106 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.32 (s, 9H, *t*-Bu), 1.46-1.50 (m, 2H, CH_2), 1.78 (dd, J=7.0 Hz, 2H, CH_2), 2.06 (s, 3H, CH_3), 2.22 (t, J=5.4 Hz, 1H, NH), 2.77 (mt J=7.5 Hz, 2H, CH_2-Ar), 3.55 (t, J=5.3 Hz, 2H, CH_2-N), 3.64 (s, 2H, CH_2-N), 5.86 (s, 1H, ArH), 6.08 (br s, 2H, NH_2), 6.88 (d, J= 7.4 Hz, 1H, ArH), 7.14-7.18 (m, 2H, Ar), 7.33-7.35 (m, 1H, ArH), 7.37-7.39 (m, 1H, ArH), 7.48 (s, 1H, ArH), 7.66-7.68 (m, 1H, ArH), 8.92 (br s, 1H, NH). **LC-MS** (ESI^+): m/z 458 $[M+H]^+$. **HRMS** (ESI^+): m/z calculated $C_{27}H_{36}N_7$ $[M+H]^+$ 458.3026, found 458.3020.

<u>General procedure 7:</u> Synthesis of thiophenecarboxamides 19a-g

The amines 20a-g were synthesized according to General procedure 5.

N-(3-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)propyl)-5-nitrothiophene-2-carboxamide, **20a**



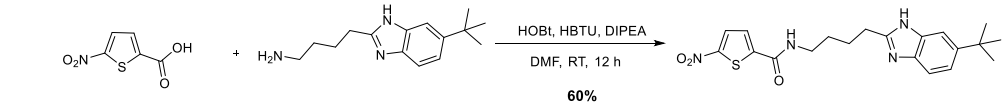
5-nitrothiophene-2-carboxylic acid
1.2 eq., 277 mg, 1.6 mmol

3a
1 eq., 309 mg, 1.34 mmol

20a
Formula: $C_{19}H_{22}N_4O_3S$
MW: 386.47

Appearance: yellow powder. **mp** = 187-189 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.31 (s, 9H, *t*-Bu), 2.03 (tt, ^3J = 6.8, 7.3 Hz, 2H, CH_2), 2.85 (t, ^3J = 7.3 Hz, 2H, CH_2-Ar), 3.35 (td, ^3J = 5.4, 6.8 Hz, 2H, CH_2-NH), 7.17 (dd, J = 1.5, 8.5 Hz, 1H, ArH), 7.34-7.38 (m, 2H, ArH), 7.77 (d, ^3J = 4.3 Hz, 1H, ArH), 8.11 (d, ^3J = 4.3 Hz, 1H, ArH), 9.09 (t, ^3J = 5.4 Hz, 1H, NH_{amide}), 12.07 (br s, 1H, NH_{benz}). **LC-MS** (ESI^+): m/z 387 $[M+H]^+$. **IR**, υ' (cm^{-1}): 1633 (C=O), 3344 (CON-H).

N-(4-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)butyl)-5-nitrothiophene-2-carboxamide, **20b**



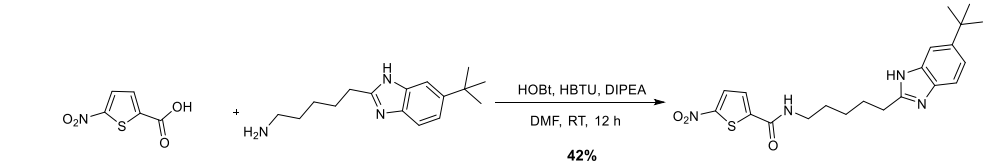
5-nitrothiophene-2-carboxylic acid
1.2 eq., 372 mg, 2.15 mmol

3b
1 eq., 440 mg, 1.79 mmol

20b
Formula: $C_{20}H_{24}N_4O_3S$
MW: 400.49

Appearance: orange powder. **mp** = 125-127 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.32 (s, 9H, *t*-Bu), 1.57 (tt, ^3J = 7.4 Hz, 2H, CH_2), 1.80 (tt, ^3J = 7.4 Hz, 2H, CH_2), 2.84 (t, ^3J = 7.4 Hz, 2H, CH_2-Ar), 3.30 (td, ^3J = 5.3, 7.4 Hz, 2H, CH_2-NH), 7.23 (m, 1H, ArH), 7.40 (m, 2H, ArH), 7.76 (d, ^3J = 4.4 Hz, 1H, ArH), 8.12 (d, ^3J = 4.4 Hz, 1H, ArH), 8.97 (t, ^3J = 5.3 Hz, 1H, NH_{amide}), 12.44 (br s, 1H, NH_{benz}). **LC-MS** (ESI^-): m/z 399 $[M-H]^-$. **IR**, υ' (cm^{-1}): 1631 (C=O).

N-(5-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)pentyl)-5-nitrothiophene-2-carboxamide, **20c**



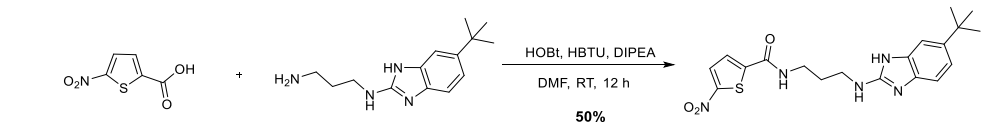
5-nitrothiophene-2-carboxylic acid
1.2 eq., 215 mg, 1.24 mmol

3c
1 eq., 269 mg, 1.04 mmol

20c
Formula: $C_{21}H_{26}N_4O_3S$
MW: 414.52

Appearance: orange powder. **mp** = 74-76 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.31 (s, 9H, *t*-Bu), 1.36 (tt, ^3J = 7.1 Hz, 2H, CH_2), 1.57 (tt, ^3J = 7.1 Hz, 2H, CH_2), 1.77 (tt, ^3J = 7.1 Hz, 2H, CH_2), 2.78 (t, ^3J = 7.1 Hz, 2H, CH_2-Ar), 3.26 (td, ^3J = 5.2, 7.1 Hz, 2H, CH_2-NH), 7.16 (dd, ^3J = 1.7, 6.7 Hz, 1H, ArH), 7.33-7.38 (m, 2H, ArH), 7.75 (d, ^3J = 4.3 Hz, 1H, ArH), 8.11 (d, ^3J = 4.3 Hz, 1H, ArH), 8.95 (t, ^3J = 5.2 Hz, 1H, NH_{amide}), 12.05 (br s, 1H, NH_{benz}). **LC-MS** (ESI^-): m/z 413 $[M-H]^-$. **IR**, υ' (cm^{-1}): 1632 (C=O).

N-(3-((6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)amino)propyl)-5-nitrothiophene-2-carboxamide, **20d**



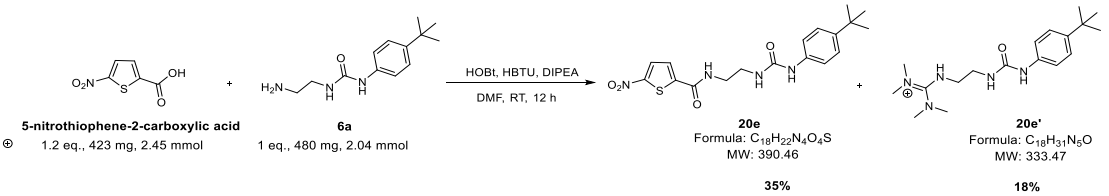
5-nitrothiophene-2-carboxylic acid
1.2 eq., 391 mg, 2.26 mmol

5b
1 eq., 464 mg, 1.88 mmol

20d
Formula: $C_{19}H_{23}N_5O_3S$
MW: 401.48

Appearance: brownish powder. **mp** = 181-183 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.30 (s, 9H, *t*-Bu), 1.88 (m, 2H, CH_2), 2.90 (m, 2H, CH_2-CONH), 3.34 (m, 2H, CH_2-NHAr), 7.25 (m, 3H, ArH), 7.81 (m, 1H, ArH), 8.14 (m, 1H, ArH), 8.55 (br s, 1H, NH-Ar), 9.11 (m, 1H, NH_{amide}), 12.29 (br s, 1H, NH_{benz}). **LC-MS** (ESI^+): m/z 402 $[M+H]^+$. **IR**, υ' (cm^{-1}): 1628 (C=O).

N-(2-(3-(4-(*tert*-butyl)phenyl)ureido)ethyl)-5-nitrothiophene-2-carboxamide, **20e**



5-nitrothiophene-2-carboxylic acid
1.2 eq., 423 mg, 2.45 mmol

6a
1 eq., 480 mg, 2.04 mmol

20e
Formula: $C_{18}H_{22}N_4O_4S$
MW: 390.46
35%

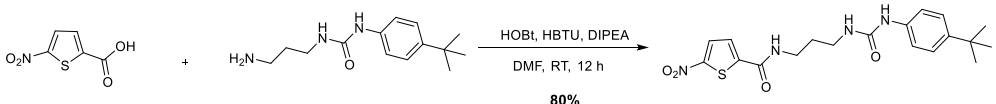
20e'
Formula: $C_{18}H_{31}N_5O$
MW: 333.47
18%

Appearance: yellow powder. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.23 (s, 9H, *t*-Bu), 3.28 (dd, *J*=5.8, 6.2 Hz, 2H, CH_2), 3.36 (m, 2H, CH_2), 6.20 (t, *J*=6.2Hz, 1H, NH_{urea}), 7.26 (dd, *J*= 9.0, 10.9 Hz, 4H, ArH), 7.78 (d, ^3J = 4.3 Hz, 1H, ArH), 8.14 (d, ^3J = 4.3 Hz, 1H, ArH), 8.42 (br s, 1H, NH_{urea}), 9.07 (t, *J*= 5.5 Hz, 1H, NH_{amide}). **LC-MS** (ESI^+): m/z 391 $[M+H]^+$. **IR**, υ' (cm^{-1}): 1649 (C=O), 1710 (C=O).

1-(2-((bis(dimethylamino)methylene)amino)ethyl)-3-(4-(*tert*-butyl)phenyl)urea, **20e'**

^{1}H NMR (300 MHz, DMSO-d_6), δ (ppm): 1.23 (s, 9H, *t*-Bu), 2.88 (br s, 12H, CH_3), 3.21-3.27 (m, 4H, CH_2), 6.18-6.22 (m, 1H, NH), 7.25 (dd, *J*=9.0, 6.7 Hz, 4H, ArH), 7.63-7.66 (m, 1H, NH), 8.52 (s, 1H, NH). **LC-MS** (ESI^+): m/z 334 $[M+H]^+$. **IR**, υ' (cm^{-1}): 1658 (C=O).

N-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)-5-nitrothiophene-2-carboxamide, **20f**



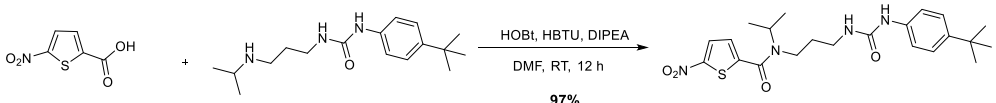
5-nitrothiophene-2-carboxylic acid
1.2 eq., 0.843 g, 4.87 mmol

6b
1 eq., 1.01 g, 4.06 mmol

20f
Formula: $C_{19}H_{24}N_4O_4S$
MW: 404.48

Appearance: yellow oil. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.23 (s, 9H, *t*-Bu), 1.68 (dd, J= 6.2 Hz, 2H, CH_2), 3.12 (dd, *J*=6.2 Hz, 2H, CH_2), 3.27-3.33 (m, 2H, CH_2), 6.12 (t, *J*= 4.8Hz, 1H, NH_{urea}), 7.24 (dd, *J*=8.8, 11.7 Hz, 4H, ArH), 7.77 (d, ^3J = 4.3 Hz, 1H, ArH), 8.12 (d, ^3J = 4.3 Hz, 1H, ArH), 8.39 (br s, 1H, NH_{urea}), 8.99 (t, *J*= 5.4Hz, 1H, NH_{amide}). **LC-MS** (ESI^+): m/z 405 $[M+H]^+$. **IR**, υ' (cm^{-1}): 1598 (C=O), 1650 (C=O), 3311 (N-H).

N-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)-*N*-isopropyl-5-nitrothiophene-2-carboxamide, **20g**



5-nitrothiophene-2-carboxylic acid
1.2 eq., 224 mg, 1.3 mmol

7b
1 eq., 315 mg, 1.08 mmol

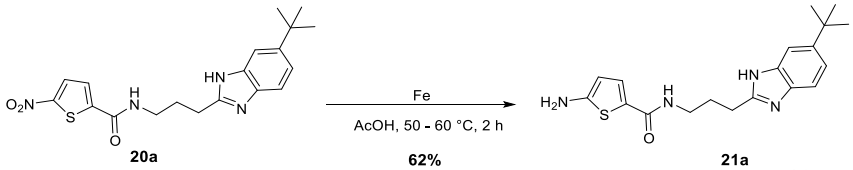
20g
Formula: $C_{22}H_{30}N_4O_4S$
MW: 446.56

Appearance: yellow powder. **mp** = 84 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.19 (d, *J*=6.5 Hz, 6H, *i*-Pr), 1.23 (s, 9H, *t*-Bu), 1.73 (dd, *J*=7.1 Hz, 2H, CH_2), 3.06-3.12 (m, 2H, CH_2), 3.32-3-37 (m, 2H, CH_2), 4.24 (br s, 1H, CH), 6.08-6.13 (m, 1H, NH_{urea}), 7.23 (dd, *J*=8.6, 9.8Hz, 4H, ArH), 7.40 (d, *J*=4.0 Hz, 1H, ArH), 8.02 (br s, 1H, ArH), 8.34 (br s, 1H, NH_{urea}). **LC-MS** (ESI^+): m/z 447 $[M+H]^+$. **IR**, υ' (cm^{-1}): 1653 (C=O), 1597 (C=O), 3340 (N-H).

Synthesis of amines 21a-g

A solution of the corresponding nitro compound (1 eq.) in AcOH was added dropwise via an addition funnel to a mixture of iron (5 eq.) in AcOH (qsp 0.1 M) at 50-60 °C, and it was stirred until the starting product disappeared (around 2 h). The reaction mixture was cooled to room temperature, then diluted with EtOAc, and adjusted to pH 11 with 6N NaOH. The mixture was filtered over celite, and the layers were separated. The organic layer was washed with H_2O and brine, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. Diethyl ether was added to the dry residue to give a powder which was recovered by filtration and dried over P_2O_5. If necessary, the residue was purified by column chromatography over silica gel (CH_2Cl_2:CH_3OH/ammonia, 95:5). The corresponding fraction was concentrated *in vacuo*, and diethyl ether was added to the dry residue to yield a powder which was recovered by filtration and dried over P_2O_5.

5-amino-*N*-(3-(6-(*tert*-butyl)-1*H*-benzo[d]imidazol-2-yl)propyl)thiophene-2-carboxamide, **21a**



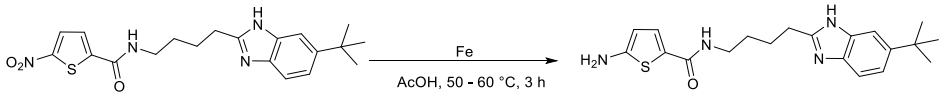
20a
1 eq., 157 mg, 0.406 mmol

62%

21a
Formula: $C_{19}H_{24}N_4OS$
MW: 356.49

Appearance: white powder. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.32 (s, 9H, *t*-Bu), 1.95 (tt, ^3J = 6.8, 7.2 Hz, 2H, CH_2), 2.80 (t, ^3J = 7.2 Hz, 2H, CH_2-Ar), 3.24 (td, ^3J = 5.7, 6.8 Hz, 2H, CH_2-NH), 5.80 (d, ^3J = 4.0 Hz, 1H, ArH), 6.17 (br s, 2H, NH_2), 7.18 (m, 1H, ArH), 7.25 (d, ^3J = 4.0 Hz, 1H, ArH), 7.30-7.34 (m, 1H, ArH), 7.38-7.43 (m, 1H, ArH), 8.00-8.06 (m, 1H, NH_{amide}), 12.02 (br s, 1H, NH_{benz}). **LC-MS** (ESI^+): m/z 357 $[M+H]^+$.

5-amino-*N*-(4-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)butyl)thiophene-2-carboxamide, **21b**



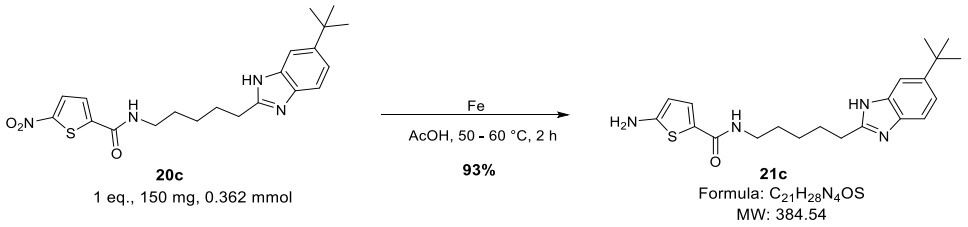
20b
1 eq., 370 mg, 0.924 mmol

53%

21b
Formula: $C_{20}H_{26}N_4OS$
MW: 370.51

Appearance: orange powder. **mp** = 119-121 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.31 (s, 9H, *t*-Bu), 1.45-1.54 (m, 2H, CH_2), 1.70-1.80 (m, 2H, CH_2), 2.78 (t, J=6.4 Hz, 2H, CH_2-Ar), 3.13-3.19 (m, 2H, CH_2-NH), 5.78 (d, ^3J = 4.0 Hz, 1H, ArH), 6.14 (br s, 2H, NH_2), 7.17 (dd, J= 1.8, 8.8 Hz, 1H, ArH), 7.24 (d, ^3J = 4.0 Hz, 1H, ArH), 7.33-7.40 (m, 2H, ArH), 7.87 (t, J=5.2 Hz, 1H, NH_{amide}), 11.99 (br s, 1H, NH_{benz}). **LC-MS** (ESI^+): m/z 371 $[M+H]^+$. **IR**, υ' (cm^{-1}): 1606 (C=O).

5-amino-*N*-(5-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)pentyl)thiophene-2-carboxamide, **21c**

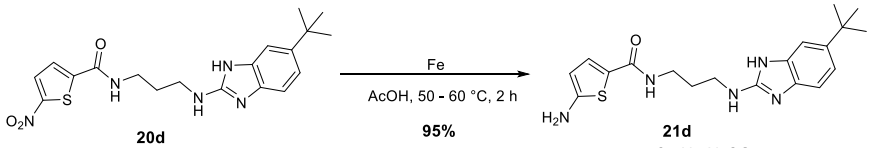


20c
1 eq., 150 mg, 0.362 mmol

21c
Formula: $C_{21}H_{28}N_4OS$
MW: 384.54

Appearance: brown powder. **mp** = 80-82 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.22-1.28 (m, 2H, CH_2), 1.31 (s, 9H, *t*-Bu), 1.44-1.54 (m, 2H, CH_2), 1.71-1.79 (m, 2H, CH_2), 2.76 (t, J=7.5 Hz, 2H, CH_2-Ar), 3.10-3.17 (m, 2H, CH_2-NH), 5.77 (d, ^3J = 4.0 Hz, 1H, ArH), 6.14 (br s, 2H, NH_2), 7.15-7.18 (m, 1H, ArH), 7.23 (d, ^3J = 4.0 Hz, 1H, ArH), 7.36 (m, 2H, ArH), 7.85 (t, J=4.5 Hz, 1H, NH_{amide}), 11.96 (br s, 1H, NH_{benz}). **LC-MS** (ESI$^+$): m/z 385 $[M+H]^+$. **IR**, υ' (cm^{-1}): 1606 (C=O).

5-amino-*N*-(3-((6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)amino)propyl)thiophene-2-carboxamide, **21d**

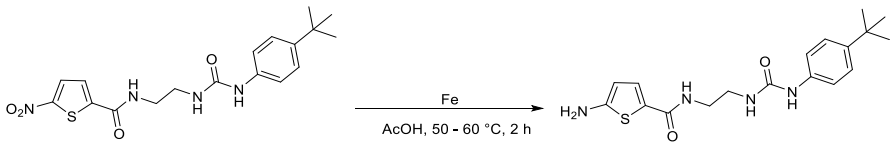


20d
1 eq., 1.32 g, 3.29 mmol

21d
Formula: $C_{19}H_{25}N_5OS$
MW: 371.50

Appearance: orange oil. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.28 (s, 9H, *t*-Bu), 1.64-1.74 (m, 2H, CH_2), 3.18-3.22 (m, 2H, CH_2-CONH), 3.29-3.33 (m, 2H, CH_2-NHAr), 5.82 (d, ^3J = 3.9 Hz, 1H, ArH), 6.18 (br s, 2H, NH_2), 6.59-6.61 (m, 1H, NH-Ar), 6.89-6.92 (m, 1H, ArH), 7.00-7.03 (m, 1H, ArH), 7.11-7.14 (m, 1H, ArH), 7.31 (d, ^3J = 3.9 Hz, 1H, ArH), 8.14 (t, J=6.8 Hz, 1H, NH_{amide}), 10.71 (br s, 1H, NH_{benz}). **LC-MS** (ESI$^+$): m/z 372 $[M+H]^+$. **IR**, υ' (cm^{-1}): 1671 (C=O), 3241 (NH).

5-amino-*N*-(2-(3-(4-(*tert*-butyl)phenyl)ureido)ethyl)thiophene-2-carboxamide, **21e**

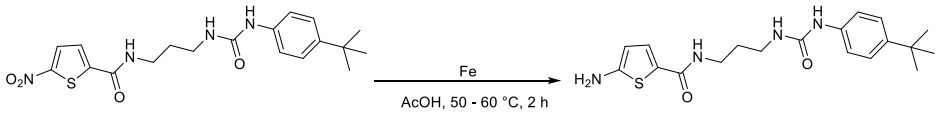


20e
1 eq., 280 mg, 0.717 mmol

21e
Formula: $C_{18}H_{24}N_4O_2S$
MW: 360.47

Appearance: orange powder. **mp** = 103-105 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.23 (s, 9H, *t*-Bu), 3.15-3.25 (m, 4H, CH_2), 5.80 (d, ^3J = 4.0 Hz, 1H, ArH), 6.10-6.23 (m, 3H, NH_{urea}, NH_2), 7.19-7.29 (m, 5H, ArH), 7.95-8.0 (m, 1H, NH_{amide}), 8.43 (br s, NH_{urea}). **LC-MS** (ESI$^+$): m/z 361 $[M+H]^+$. **IR**, υ' (cm^{-1}): 1651 (C=O), 1722 (C=O).

5-amino-*N*-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)thiophene-2-carboxamide, **21f**



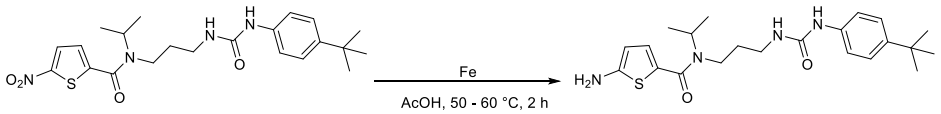
20f
1 eq., 1.32 g, 3.26 mmo

48%

21f
Formula: $C_{19}H_{26}N_4O_2S$
MW: 374.50

Appearance: yellowish oil. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.23 (s, 9H, *t*-Bu), 1.62 (tt, ^3J = 6.3 Hz, 2H, CH_2), 3.08 (td, ^3J = 5.5, 6.3 Hz, 2H, CH_2-CONH), 3.23 (td, ^3J = 5.5, 6.3 Hz, 2H, CH_2-NH), 6.13 (t, ^3J = 5.5 Hz, 1H, NH_{urea}), 6.58 (d, J=4.1 Hz, 1H, ArH), 7.19-7.30 (m, 4H, ArH), 7.47 (d, ^3J = 4.1 Hz, 1H, ArH), 8.26 (t, J=4.8 Hz, 1H, NH), 8.41 (br s, 1H, NH_{urea}), 11.35 (s, 1H, NH). **LC-MS** (ESI^+): m/z 375 $[M+H]^+$. **IR**, υ' (cm^{-1}): 1655 (C=O), 3315 (NH).

5-amino-*N*-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)-*N*-isopropylthiophene-2-carboxamide, **21g**



20g
1 eq., 200 mg, 0.448 mmol

38%

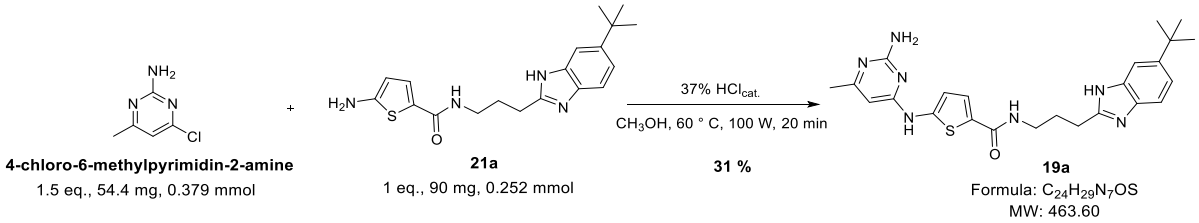
21g
Formula: $C_{22}H_{32}N_4O_2S$
MW: 416.58

Appearance: orange powder. **mp** = 96-98 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.17 (d, ^3J = 6.6 Hz, 6H, *i*-Pr), 1.23 (s, 9H, *t*-Bu), 1.65-1.74 (m, 2H, CH_2), 3.04-3.11 (m, 2H, CH_2-N), 3.25-3.30 (m, 2H, CH_2-NH), 4.51 (sept, ^3J = 6.6 Hz, 1H, CH), 5.80 (d, ^3J = 4.1 Hz, 1H, ArH), 6.12 (t, J= 5.5Hz, 1H, NH_{urea}), 6.17 (br s, 2H, NH_2), 6.95 (d, ^3J = 4.1 Hz, 1H, ArH), 7.19-7.30 (m, 4H, ArH), 8.35 (s, 1H, NH_{urea}). **LC-MS** (ESI^+): m/z 417 $[M+H]^+$. **IR**, υ' (cm^{-1}): 1650 (C=O), 1657 (C=O).

Synthesis of amines 19a-h

A mixture of 2-amino-4-chloro-6-methylpyrimidine (1.2-1.5 eq.), the corresponding amine (1 eq.), and 5 uL of 37% HCl in CH_3OH were stirred for 20 min in a microwave reactor, at 60 ° C, at 100 W. The solvent was removed *in vacuo*, 10% K_2CO_3 was added to the residue, and the aqueous layer was extracted three times with EtOAc. The organic layers were gathered together, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography over silica gel (CH_2Cl_2:CH_3OH/ammonia, 95:5). The corresponding fraction was concentrated *in vacuo*, and diethyl ether was added to the dry residue to yield a powder which was recovered by filtration and dried over P_2O_5.

5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(3-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)propyl)thiophene-2-carboxamide, **19a**



4-chloro-6-methylpyrimidin-2-amine
1.5 eq., 54.4 mg, 0.379 mmol

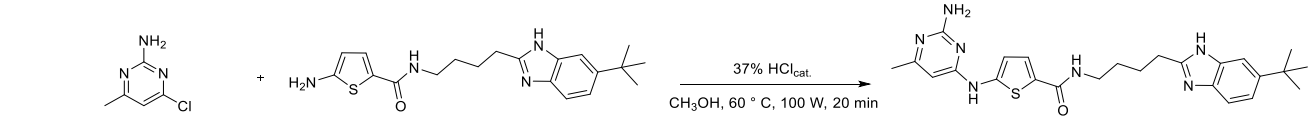
21a
1 eq., 90 mg, 0.252 mmol

31 %

19a
Formula: $C_{24}H_{29}N_7OS$
MW: 463.60

Appearance: yellowish powder. **mp** = 179-181 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.32 (s, 9H, *t*-Bu), 1.93-2.01 (m, 2H, CH_2), 2.11 (s, 3H, CH_3), 2.83 (t, J=6.4 Hz, 2H, CH_2), 3.25-3.32 (m, 2H, CH_2), 5.88 (s, 1H, ArH), 6.27 (br s, 2H, NH_2), 6.57 (d, ^3J = 3.7 Hz, 1H, ArH), 7.17-7.20 (m, 1H, ArH), 7.35-7.40 (m, 2H, ArH), 7.48 (d, ^3J = 3.7 Hz, 1H, ArH), 8.24-8.29 (m, 1H, NH), 10.40 (br s, 1H, NH), 12.07 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 23.8, 26.6, 28.2, 32.2, 34.8, 39.9, 94.8, 110.2, 119.3, 126.4, 128.5, 144.3, 146.8, 155.0, 158.8, 165.6, 162.7, 165.8. **LC-MS** (ESI$^+$): m/z 464 $[M+H]^+$. **HRMS** (ESI$^-$): m/z calculated $C_{24}H_{28}N_7OS$ $[M-H]^-$ 462.2070, found 462.2062. **IR**, υ' (cm^{-1}): 1538 (C=O).

5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(4-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)butyl)thiophene-2-carboxamide, **19b**



4-chloro-6-methylpyrimidin-2-amine
1.5 eq., 104 mg, 0.729 mmol

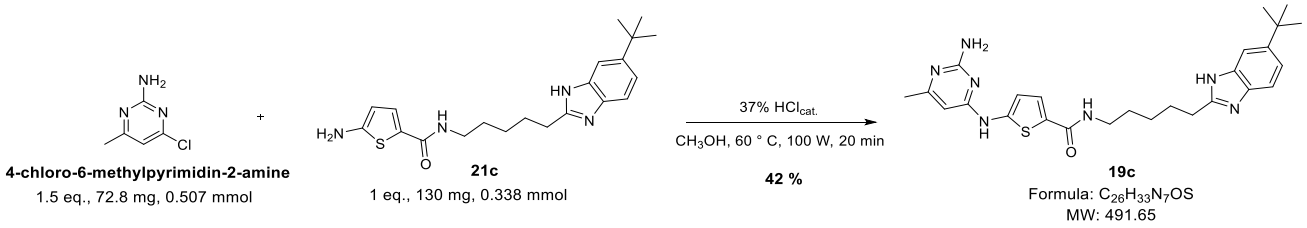
21b
1 eq., 180 mg, 0.486 mmol

86 %

19b
Formula: $C_{25}H_{31}N_7OS$
MW: 477.62

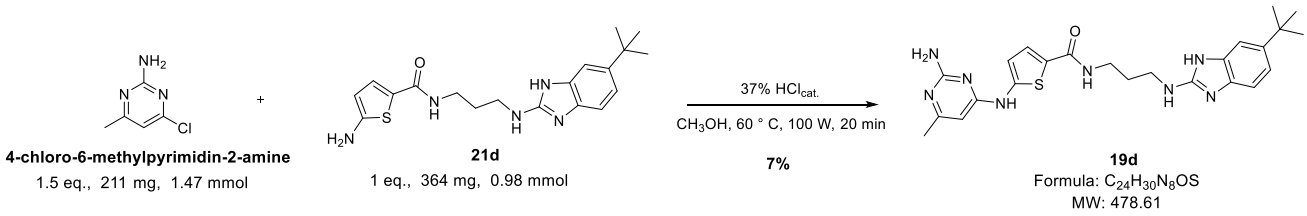
Appearance: yellowish powder. **mp** =174-176 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.31 (s, 9H, *t*-Bu), 1.48-1.58 (m, 2H, CH_2), 1.72-1.82 (m, 2H, CH_2), 2.11 (s, 3H, CH_3), 2.81 (t, J=6.6 Hz, 2H, CH_2), 3.19-3.25 (m, 2H, CH_2), 5.88 (s, 1H, ArH), 6.28 (br s, 2H, NH_2), 6.56 (d, ^3J = 3.8 Hz, 1H, ArH), 7.17 (dd, J= 1.8, 8.2 Hz, 1H, ArH), 7.34-7.39 (m, 2H, ArH), 7.46 (d, ^3J = 3.8 Hz, 1H, ArH), 8.11 (t, J= 4.8 Hz, 1H, NH), 10.39 (br s, 1H, NH), 12.03 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 23.7, 25.6, 28.6, 29.5, 32.2, 34.8, 39.9, 94.8, 110.2, 119.3, 126.3, 128.7, 144.2, 146.6, 155.2, 158.8, 162.5, 165.7. **LC-MS** (ESI$^+$): m/z 478 $[M+H]^+$. **HRMS** (ESI$^+$): m/z calculated $C_{25}H_{32}N_7OS$ $[M+H]^+$ 478.2383, found 478.2361. **IR**, υ' (cm^{-1}): 1584 (C=O).

5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(5-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)pentyl)thiophene-2-carboxamide, **19c**



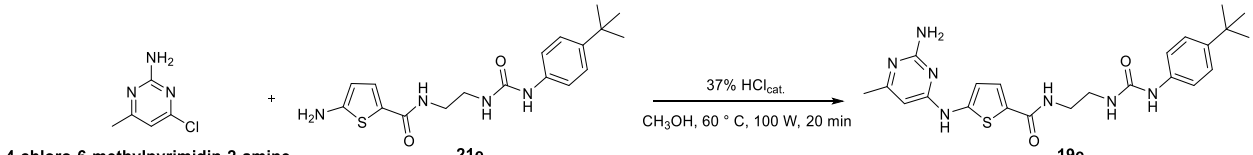
Appearance: yellowish powder. **mp** = 171-173 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.31 (s, 9H, *t*-Bu), 1.48-1.57 (m, 2H, CH_2), 1.72-1.82 (m, 2H, CH_2), 2.13 (s, 3H, CH_3), 2.78 (t, J=7.1 Hz, 2H, CH_2), 3.19 (t, J=7.8 Hz, 2H, CH_2), 3.33 (m, 2H, CH_2), 5.90 (s, 1H, ArH), 6.28-6.41 (m, 2H, NH_2), 6.56-6.59 (m, 1H, ArH), 7.15-7.19 (m, 1H, ArH), 7.4-7.39 (m, 2H, ArH), 7.46 (d, J=4.0 Hz, 1H, ArH), 8.07-8.12 (m, 1H, NH), 10.44-10-48 (m, 1H, NH), 12.06 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 23.7, 26.5, 27.8, 28.9, 29.6, 32.2, 34.8, 94.9, 110.5, 113.5, 119.3, 126.3, 144.4, 155.4, 156.6, 158.9, 162.5. **LC-MS** (ESI^+): m/z 492 $[M+H]^+$. **HRMS** (ESI^-): m/z calculated $C_{26}H_{32}N_7OS$ $[M-H]^-$ 490.2383, found 490.2370. **IR**, υ' (cm^{-1}): 1586 (C=O).

5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(3-((6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)amino)propyl)thiophene-2-carboxamide, **19d**



Appearance: beige powder. **mp** = 193-195 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.01-1.08 (m, 2H, CH_2), 1.28 (s, 9H, *t*-Bu), 1.72-1.79 (m, 2H, CH_2-CONH), 2.11 (s, 3H, CH_3), 3.25-3.33 (m, 2H, CH_2-NHAr), 5.88 (s, 1H, ArH), 6.23 (br s, 2H, NH_2), 6.53 (t, J=5.6 Hz, 1H, NHAr), 6.59 (d, ^3J = 4.0 Hz, 1H, ArH), 6.91 (dd, J=1.2, 8.2 Hz, 1H, ArH), 7.02 (d, J=8.2 Hz, 1H, ArH), 7.14 (d, J=1.2 Hz, 1H, ArH), 7.53 (d, ^3J = 4.0 Hz, 1H, ArH), 8.37 (t, J=5.9 Hz, 1H, NH_{amide}), 10.40 (br s, 1H, NH), 10.60 (m, 1H, NH_{benz}). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 23.9, 30.3, 32.3, 34.6, 36.6, 46.1, 94.8, 110.0, 116.8, 126.4, 128.5, 142.2, 146.8, 156.2, 158.8, 162.7, 162.8, 166.1. **LC-MS** (ESI^+): m/z 479 $[M+H]^+$. **HRMS** (ESI^+): m/z calculated $C_{24}H_{31}N_8OS$ $[M+H]^+$ 479.2336, found 479.2301. **IR**, υ' (cm^{-1}): 1635 (C=O).

5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(2-(3-(4-(*tert*-butyl)phenyl)ureido)ethyl)thiophene-2-carboxamide, **19e**



4-chloro-6-methylpyrimidin-2-amine
1.2 eq., 109 mg, 0.766 mmol

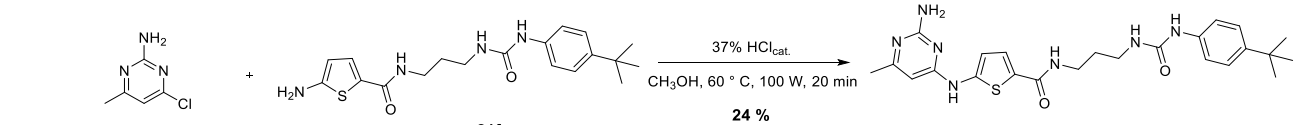
21e
1 eq., 230 mg, 0.638 mmol

44 %

19e
Formula: $C_{23}H_{29}N_7O_2S$
MW: 467.59

Appearance: yellowish powder. **mp** = 248-250 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.23 (s, 9H, *t*-Bu), 2.10 (m, 3H, CH_3), 3.16-3.19 (m, 4H, CH_2), 5.87 (s, 1H, ArH), 6.19 (br s, 2H, NH_2), 6.43-6.49 (m, 1H, NH), 6.53-6.57 (m, 1H, ArH), 7.19-7.31(m, 4H, ArH), 7.47-7.50 (m, 1H, ArH), 8.19-8.21 (m, 1H, NH), 8.64 (br s, 1H, NH), 10.41 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 22.4, 31.7, 34.2, 95.5, 111.5, 118.0, 125.6, 126.6, 129.4, 138.3, 139.2, 141.4, 143.7, 146.0, 155.9, 158.8, 160.8, 162.8. **LC-MS** (ESI$^+$): m/z 468 $[M+H]^+$. **HRMS** (ESI$^+$): m/z calculated $C_{23}H_{30}N_7O_2S$ $[M+H]^+$ 468.2176, found 468.2163. **IR**, υ' (cm^{-1}): 1624 (C=O), 1660 (C=O).

5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)thiophene-2-carboxamide, **19f**



4-chloro-6-methylpyrimidin-2-amine
1.2 eq., 115 mg, 0.801 mmol

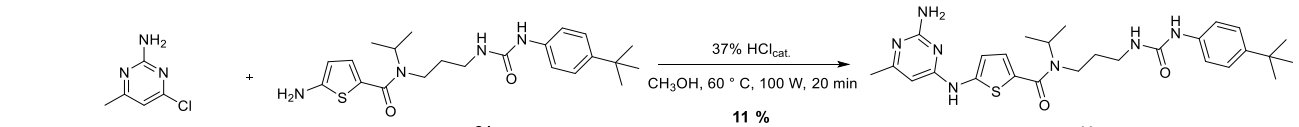
21f
1 eq., 250 mg, 0.668 mmol

24 %

19f
Formula: $C_{24}H_{31}N_7O_2S$
MW: 481.61

Appearance: white powder. **mp** = 155-157 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.23 (s, 9H, *t*-Bu), 1.59-1.66 (m, 2H, CH_2), 2.11 (m, 3H, CH_3), 3.07-3.14 (m, 2H, CH_2-NHCO), 3.21-3.28 (m, 2H, CH_2-NH_{urea}), 5.88 (s, 1H, ArH), 6.10-6.16 (m, 1H, NH_{urea}), 6.23 (br s, 2H, NH_2), 6.57 (d, ^3J = 4.0 Hz,1H, ArH), 7.27 (dd, J=8.2, 13.3 Hz, 4H, ArH), 7.46 (d, ^3J = 4.0 Hz, 1H, ArH), 8.13 (t, J=4.8 Hz, 1H, NH_{amide}), 8.42 (br s, 1H, NH_{urea}), 10.37 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 23.9, 30.8, 31.7, 34.2, 36.9, 37.2, 94.8, 110.1, 117.9, 125.6, 126.4, 128.4, 138.4, 143.6, 146.8, 155.8, 158.8, 162.8, 166.1. **LC-MS** (ESI$^+$): m/z 482 $[M+H]^+$. **HRMS** (ESI$^-$): m/z calculated $C_{24}H_{30}N_7O_2S$ $[M-H]^-$ 480.2176, found 480.2139. **IR**, υ' (cm^{-1}): 1587 (C=O), 1630 (C=O).

5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)-N-isopropylthiophene-2-carboxamide, **19g**



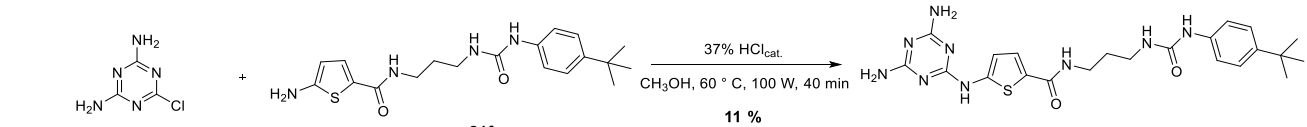
4-chloro-6-methylpyrimidin-2-amine
1.5 eq., 36.2 mg, 0.252 mmol

21g
1 eq., 70 mg, 0.168 mmol

19g
Formula: $C_{27}H_{37}N_7O_2S$
MW: 523.69

Appearance: yellowish powder. **mp** = 145-147 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.19 (d, ^3J = 6.6 Hz, 6H, *i*-Pr), 1.23 (s, 9H, *t*-Bu), 1.68-1.78 (m, 2H, CH_2), 2.12 (s, 3H, CH_3), 3.06-3.13 (m, 2H, CH_2), 3.33 (m, 2H, CH_2), 4.49 (q, J=6.6 Hz, 1H, CH), 5.88 (s, 1H, ArH), 6.13 (t, J=5.2 Hz, 1H, NH), 6.30 (br s, 2H, NH_2), 6.53 (d, ^3J = 4.0 Hz, 1H, ArH), 7.10 (d, ^3J = 4.0 Hz, 1H, ArH), 7.25 (dd, J=8.7, 12.3 Hz, 4H, ArH), 8.36 (s, 1H, NH), 10.43 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 21.3, 23.7, 30.9, 31.7, 34.2, 37.5, 39.9, 40.4, 94.8, 109.4, 118.0, 125.6, 126.4, 128.0, 135.1, 138.3, 143.6, 145.5, 155.8, 158.7, 162.5, 164.3. **LC-MS** (ESI^+): m/z 524 $[M+H]^+$. **HRMS** (ESI^+): m/z calculated $C_{27}H_{38}N_7O_2S$ $[M+H]^+$ 524.2802, found 524.2782. **IR**, υ' (cm^{-1}): 1542 (C=O), 1586 (C=O).

N-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)-5-((4,6-diamino-1,3,5-triazin-2-yl)amino)thiophene-2-carboxamide, **19h**



6-chloro-1,3,5-triazine-2,4-diamine
1.7 eq., 106 mg, 0.74 mmoll

21f
1 eq., 163 mg, 0.435 mmol

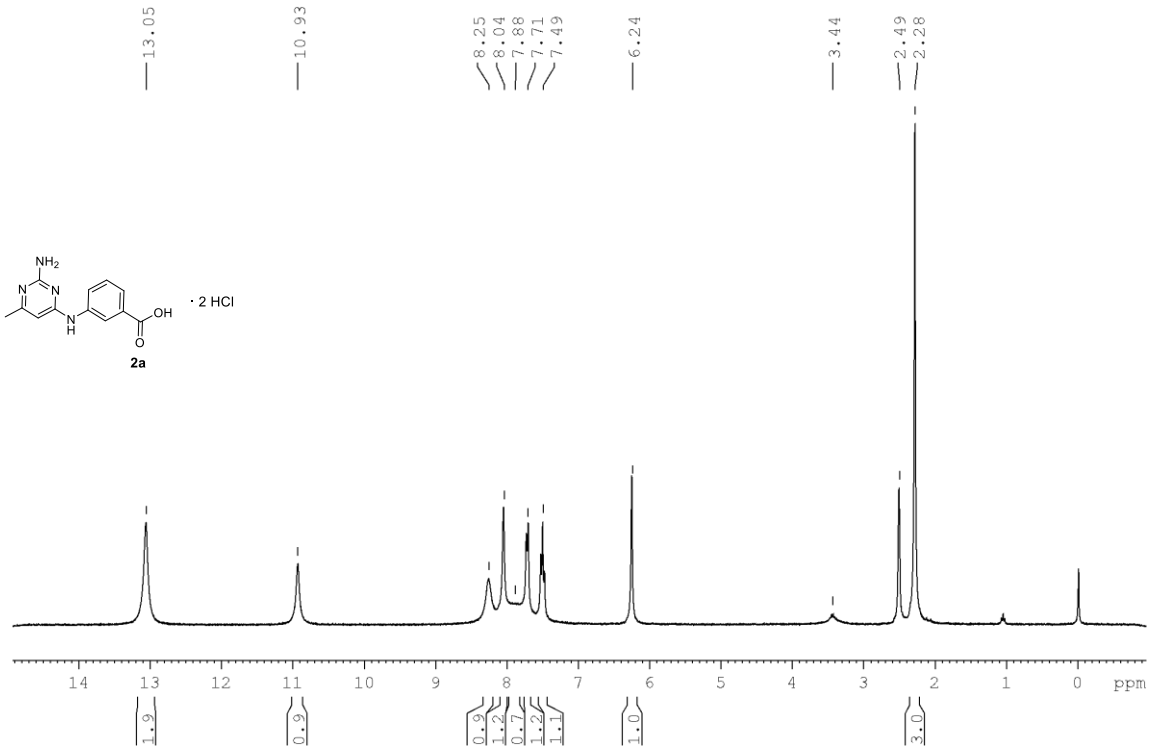
19h
Formula: $C_{22}H_{29}N_9O_2S$
MW: 483.59

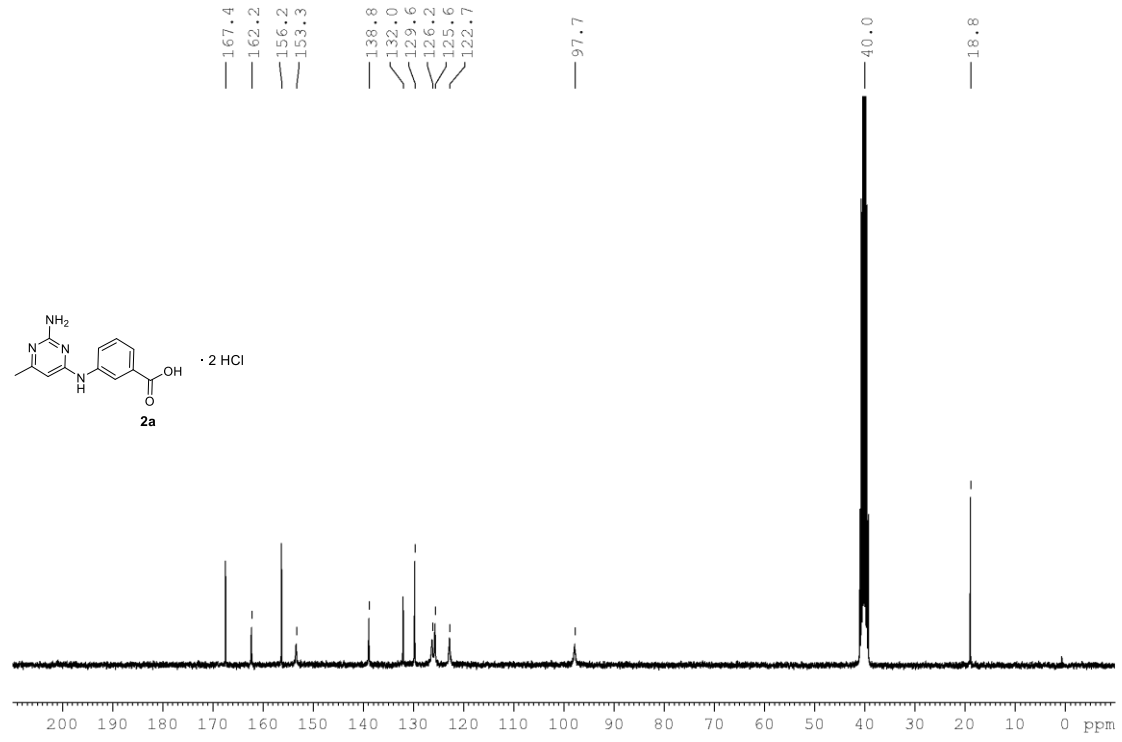
Appearance: white powder. **mp** = 172-174 °C. **^{1}H NMR** (300 MHz, DMSO-d_6), δ (ppm): 1.23 (s, 9H, *t*-Bu), 1.56-1.67 (m, 2H, CH_2), 3.06-3.12 (m, 2H, CH_2-NHCO), 3.18-3.27 (m, 2H, CH_2-NH_{urea}), 6.34 (br s, 4H, NH_2), 6.40-6.46 (m, 1H, NH_{urea}), 6.49-6.56 (m, 1H, ArH), 7.21 (d, J=8.8 Hz, 2H, ArH), 7.30 (d, J= 7.8 Hz, 2H, ArH), 7.42 (d, J= 4.4 Hz, 1H, ArH), 8.04-8.11 (m, 1H, NH_{amide}), 8.66 (br s, 1H, NH_{urea}), 10.23 (br s, 1H, NH). **^{13}C NMR** (75 MHz, DMSO-d_6), δ (ppm): 31.1, 32.1, 34.6, 37.3, 37.6, 110.6, 118.3, 126.0, 126.8, 139.0, 143.8, 156.3, 163.3, 167.7. **LC-MS** (ESI^+): m/z 484 $[MH]^+$. **HRMS** (ESI^+): m/z calculated $C_{22}H_{30}N_9O_2S$ $[M+H]^+$ 484.2237, found 484.2313. **IR**, υ' (cm^{-1}): 1596 (C=O), 1635 (C=O).

NMR spectra

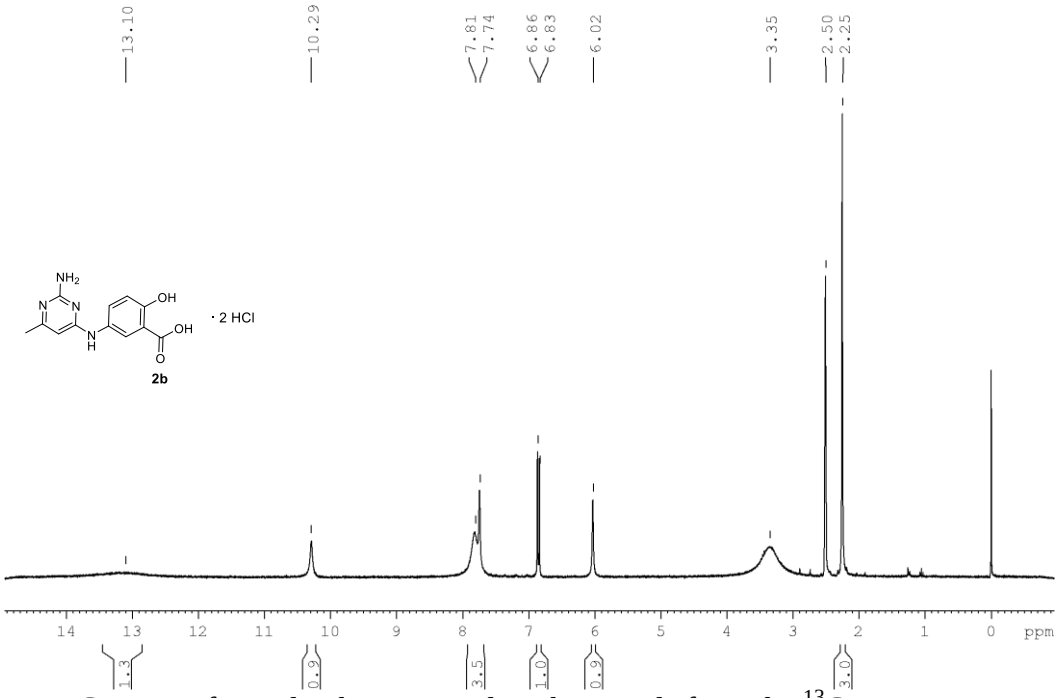
Intermediate compounds

3-((2-Amino-6-methylpyrimidin-4-yl)amino)benzoic acid dihydrochloride, **2a**

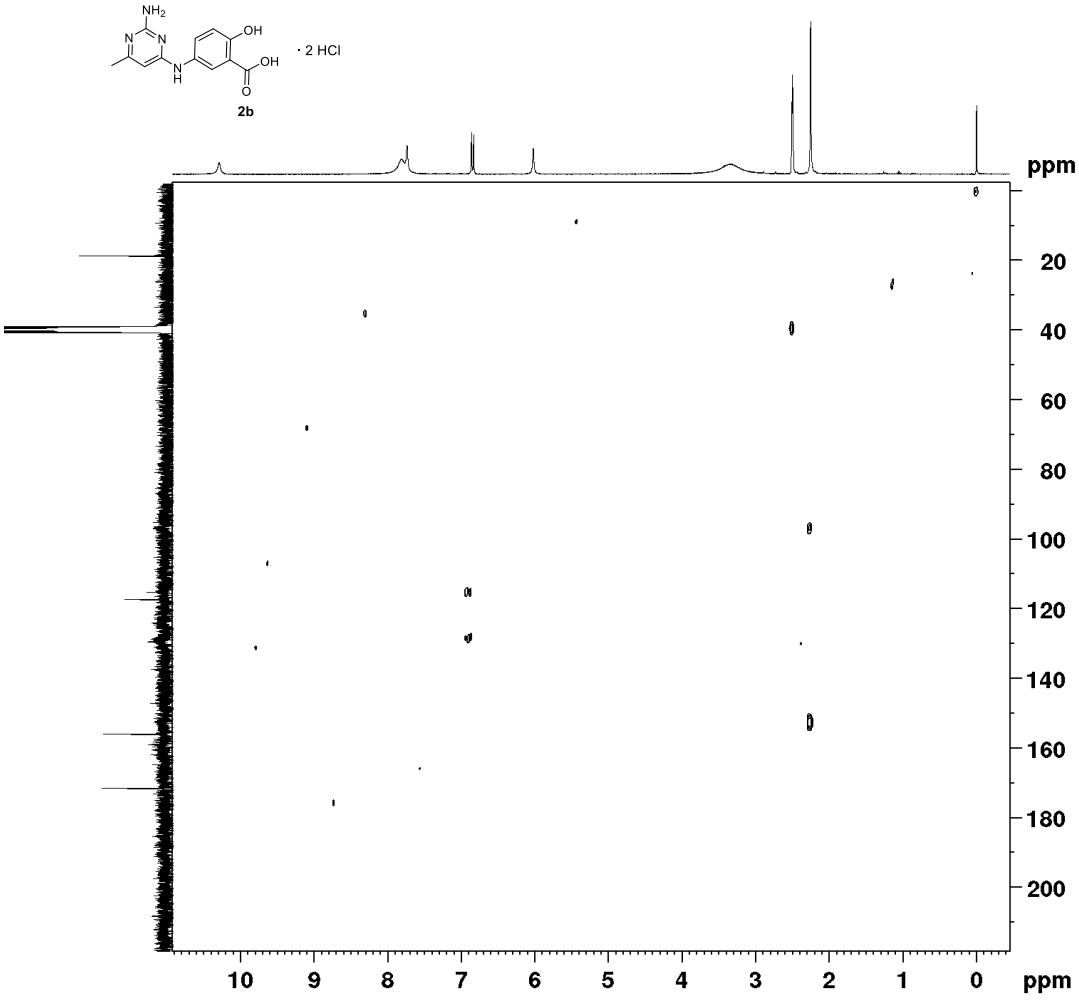




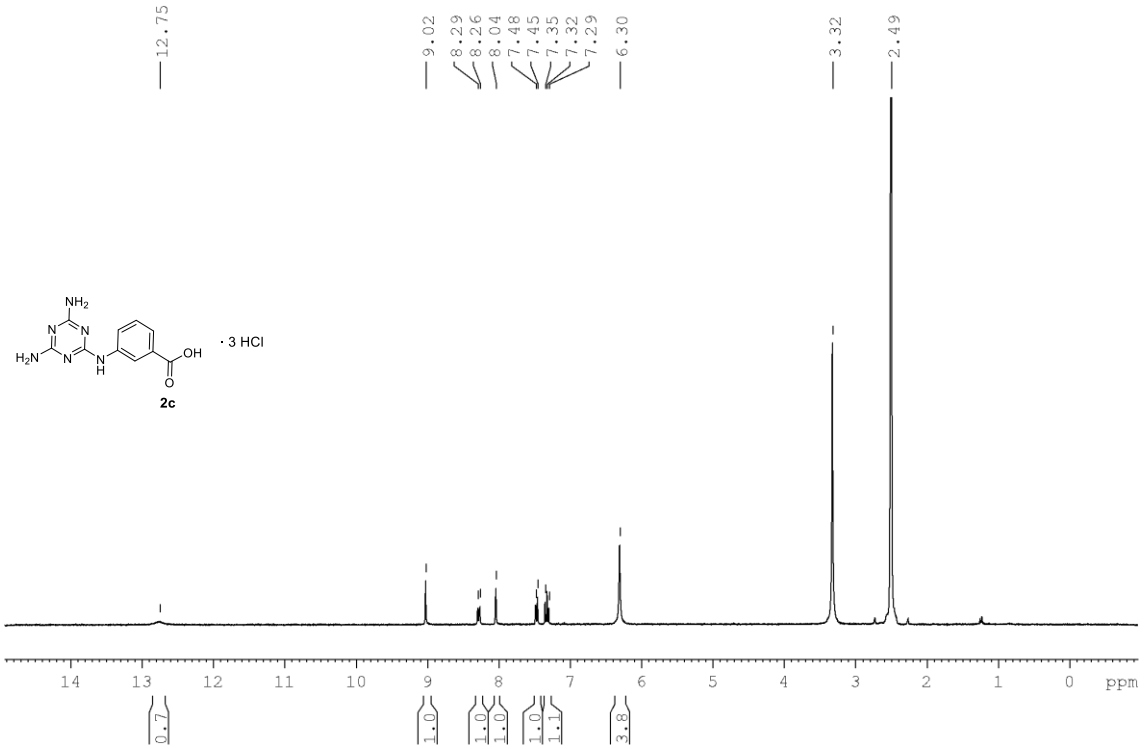
5-((2-Amino-6-methylpyrimidin-4-yl)amino)-2-hydroxybenzoic acid dihydrochloride, **2b**



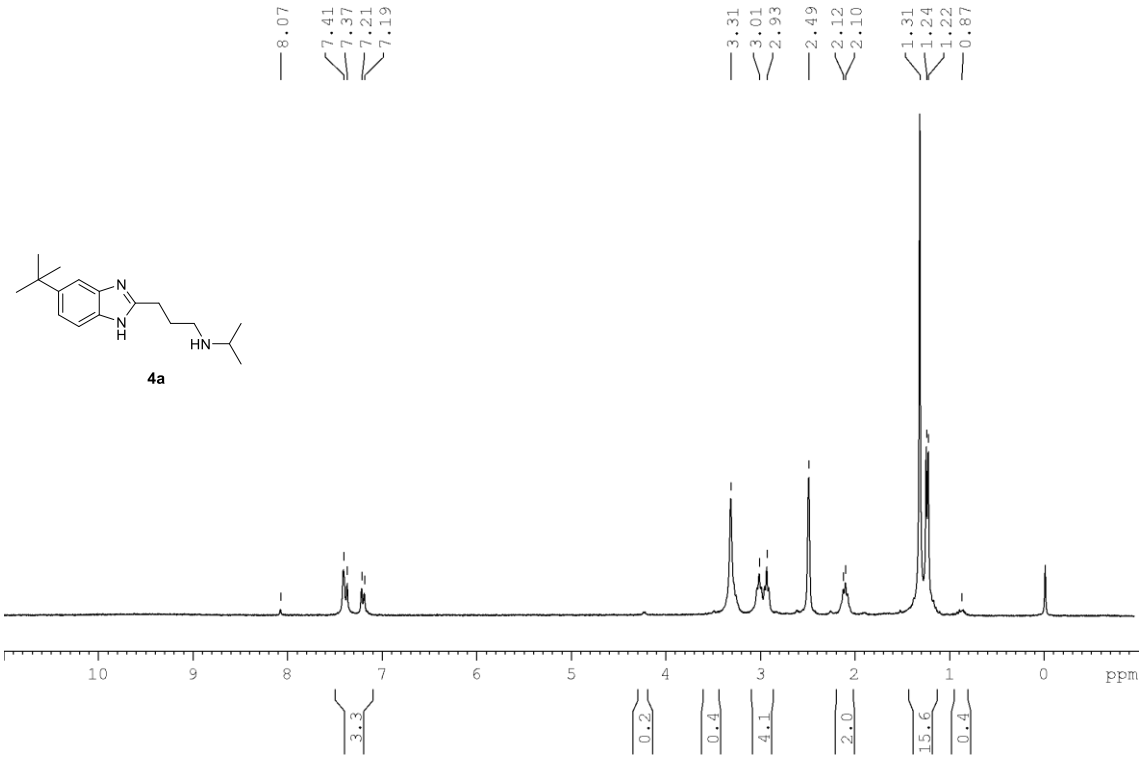
HMBC was performed to better visualize the signals from the ^{13}C spectrum.



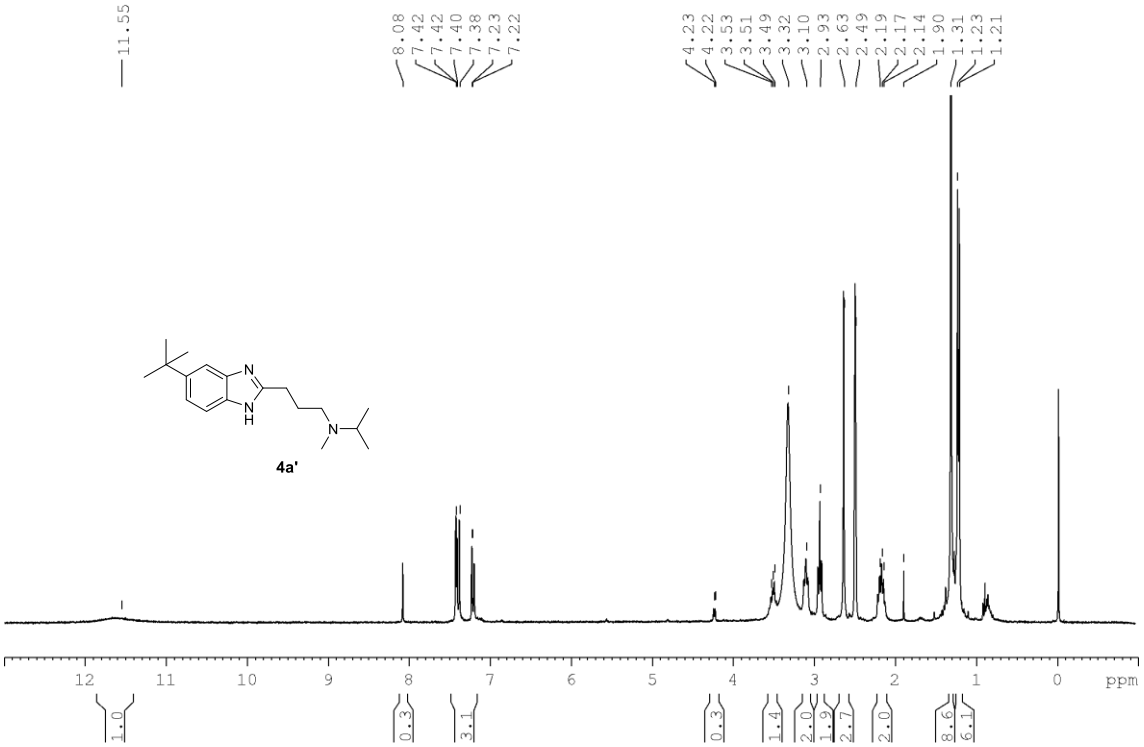
3-((4,6-Diamino-1,3,5-triazin-2-yl)amino)benzoic acid trihydrochloride, **2c**



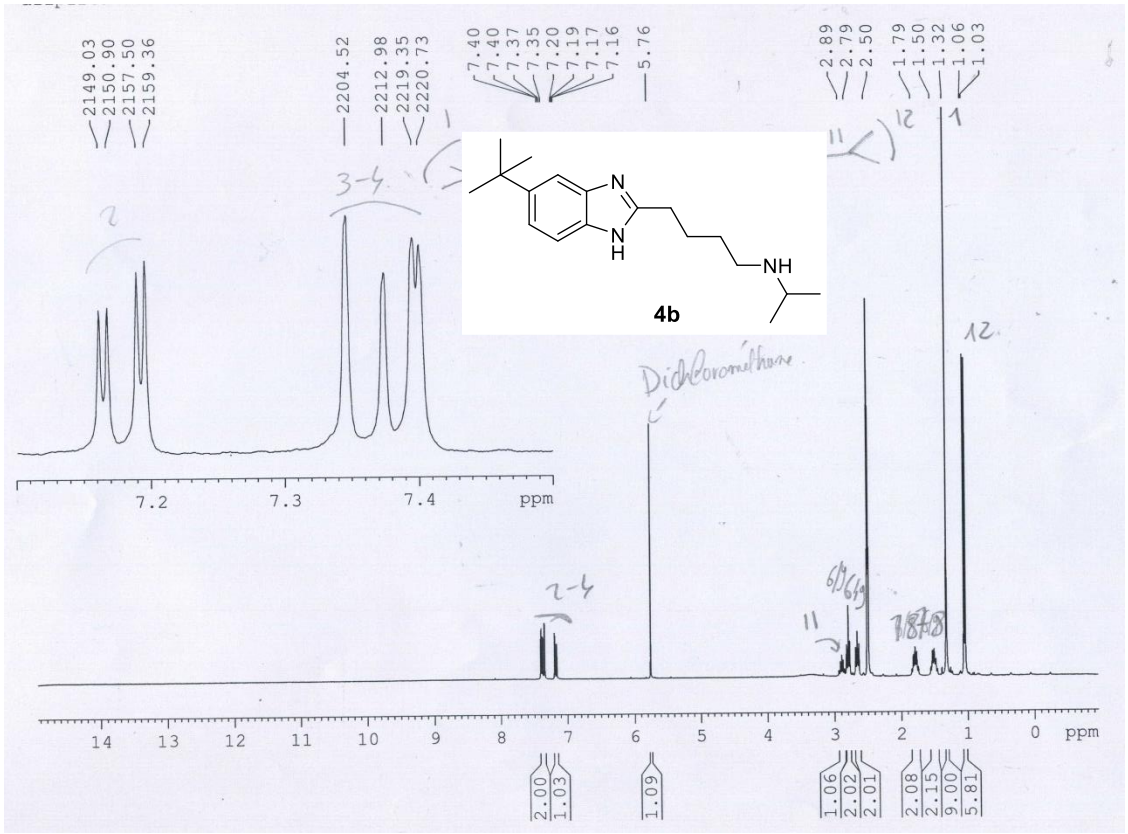
3-(5-(*Tert*-butyl)-1*H*-benzo[d]imidazol-2-yl)-*N*-isopropylpropan-1-amine, **4a**



3-(5-(*Tert*-butyl)-1*H*-benzo[d]imidazol-2-yl)-*N*-isopropyl-*N*-methylpropan-1-amine, **4a'**

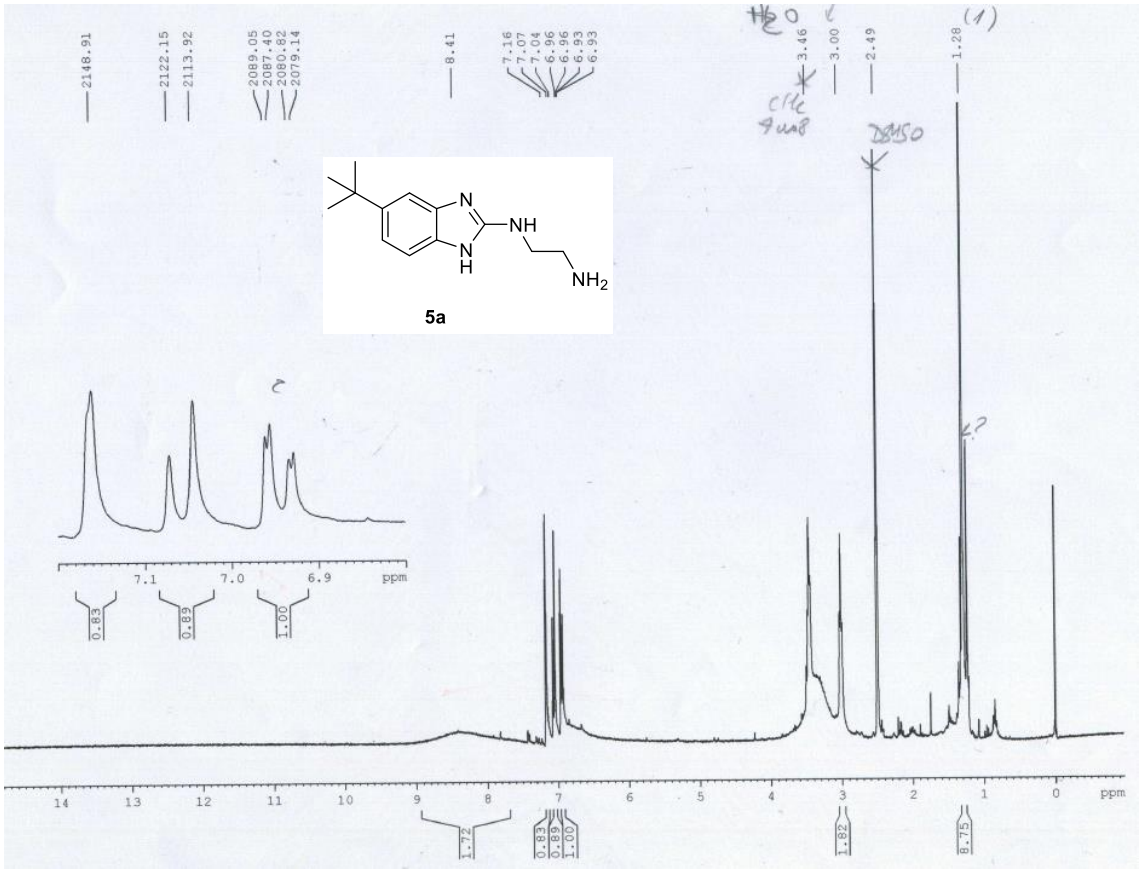


4-(5-(*Tert*-butyl)-1*H*-benzo[d]imidazol-2-yl)-*N*-isopropylbutan-1-amine, **4b**

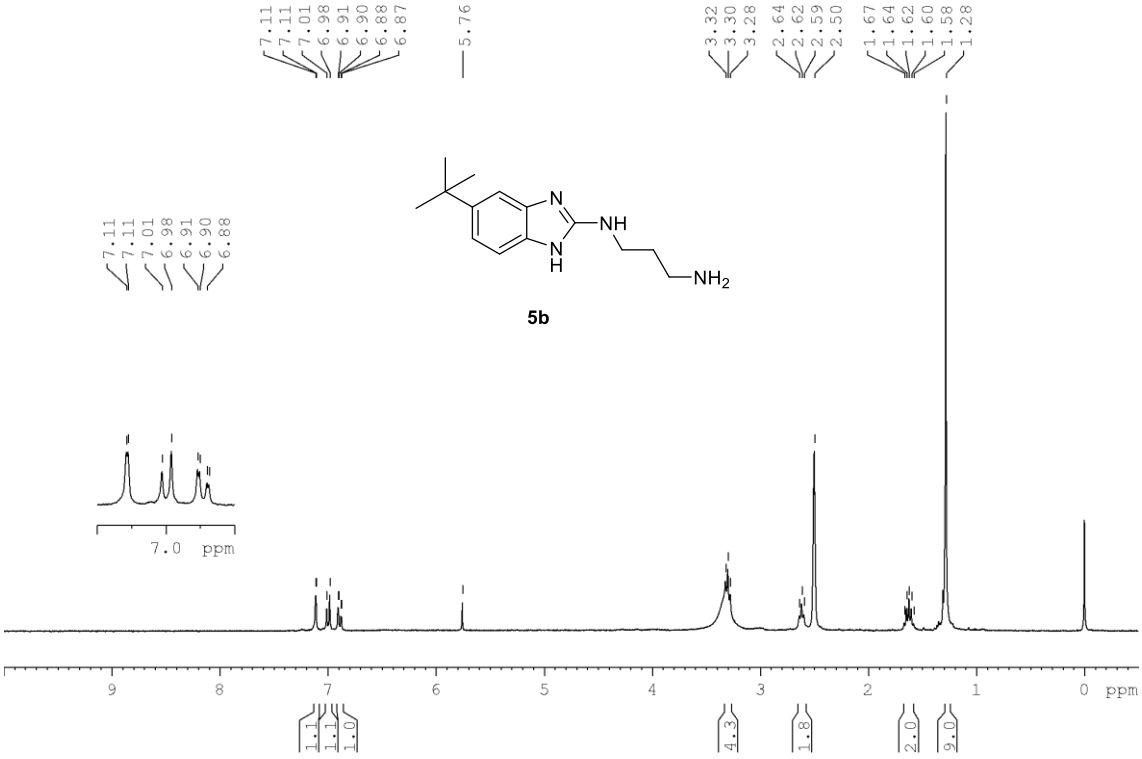


Traces of CH_2Cl_2 are observed.

N^1-(5-(*Tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)ethane-1,2-diamine, **5a**

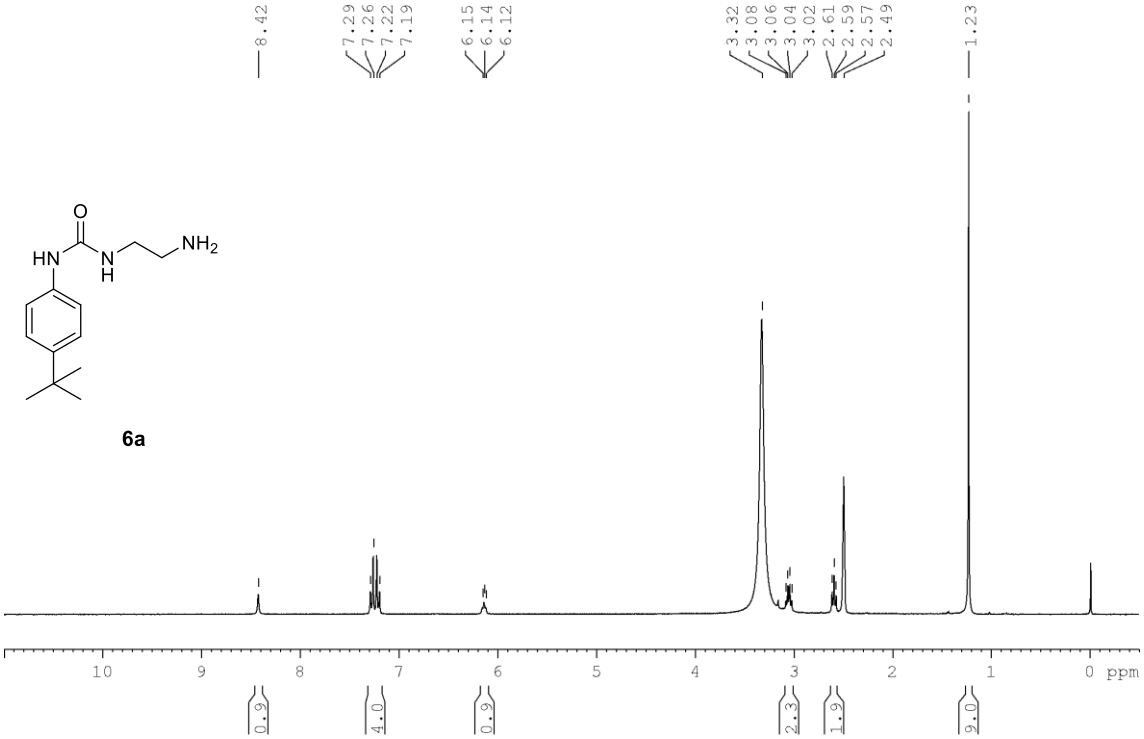


N^1-(5-(*Tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)propane-1,3-diamine, **5b**



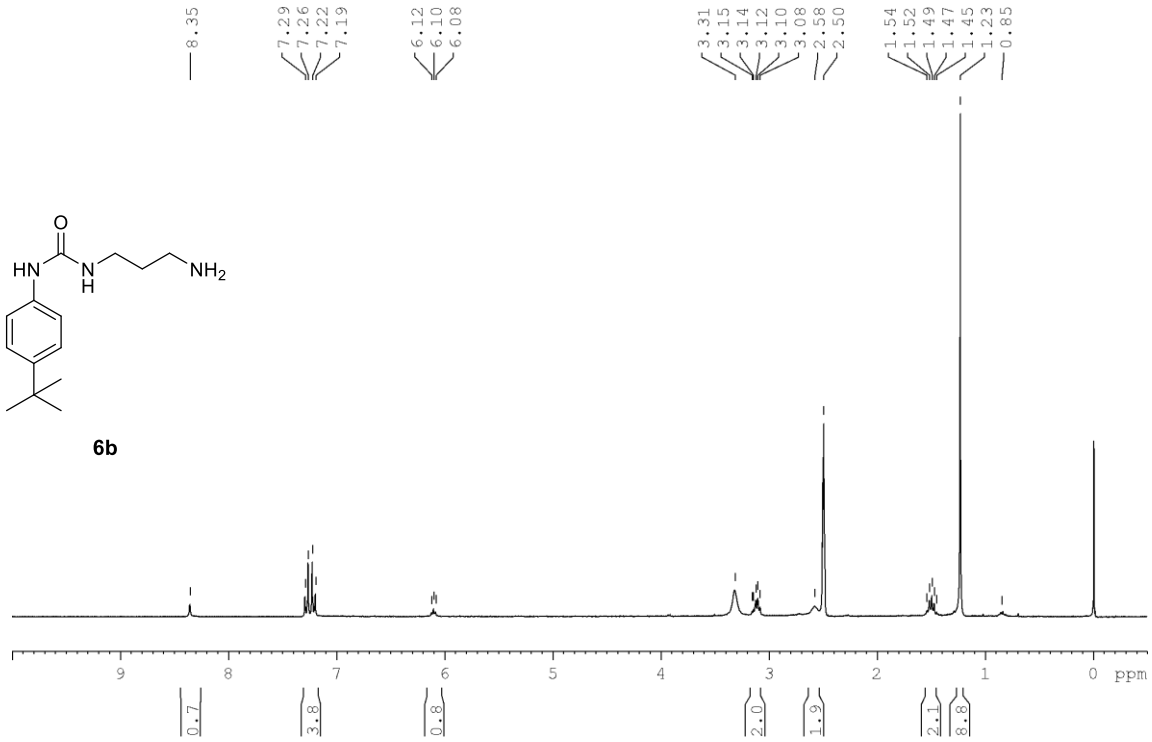
Traces of CH_2Cl_2 are observed.

1-(2-Aminoethyl)-3-(4-(*tert*-butyl)phenyl)urea, **6a**



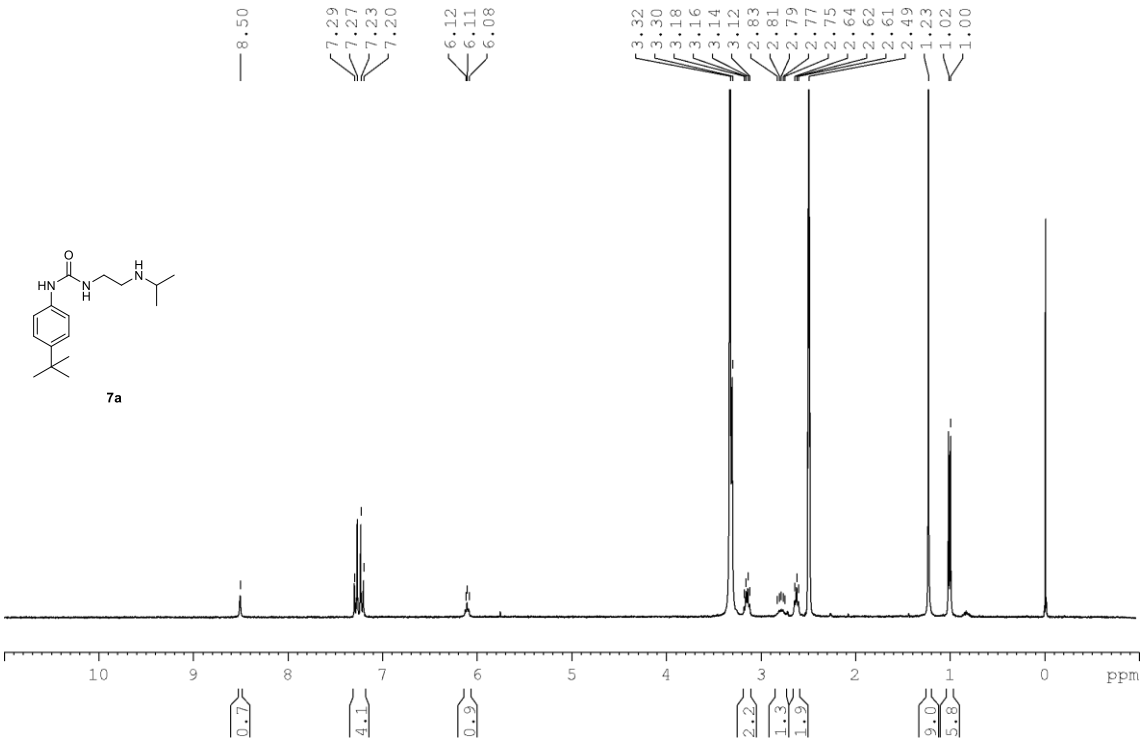
6a

1-(3-Aminopropyl)-3-(4-(*tert*-butyl)phenyl)urea, **6b**

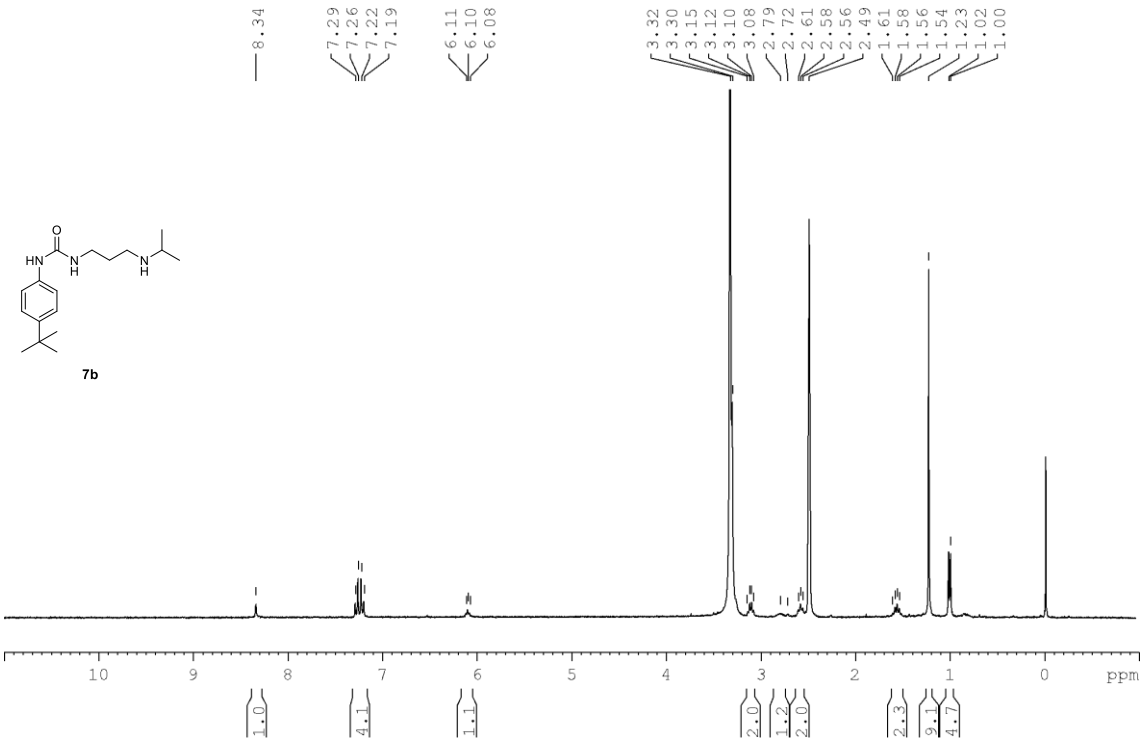


6b

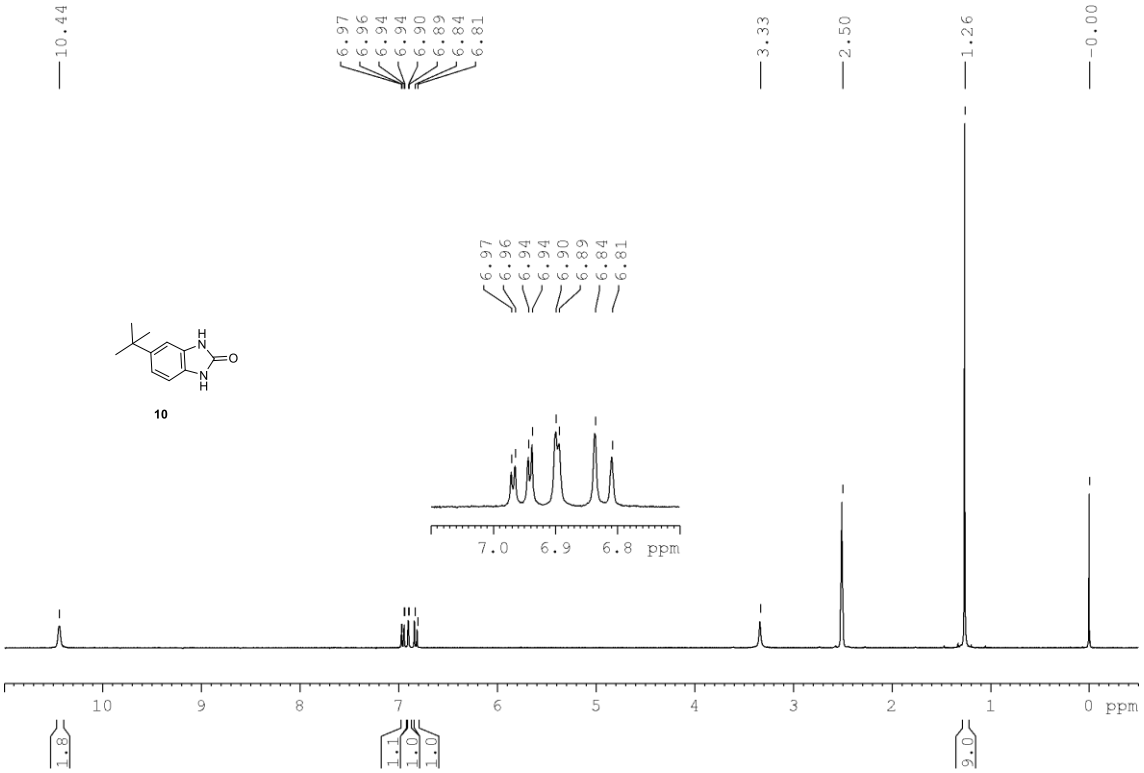
1-(4-(*Tert*-butyl)phenyl)-3-(2-(isopropylamino)ethyl)urea, **7a**



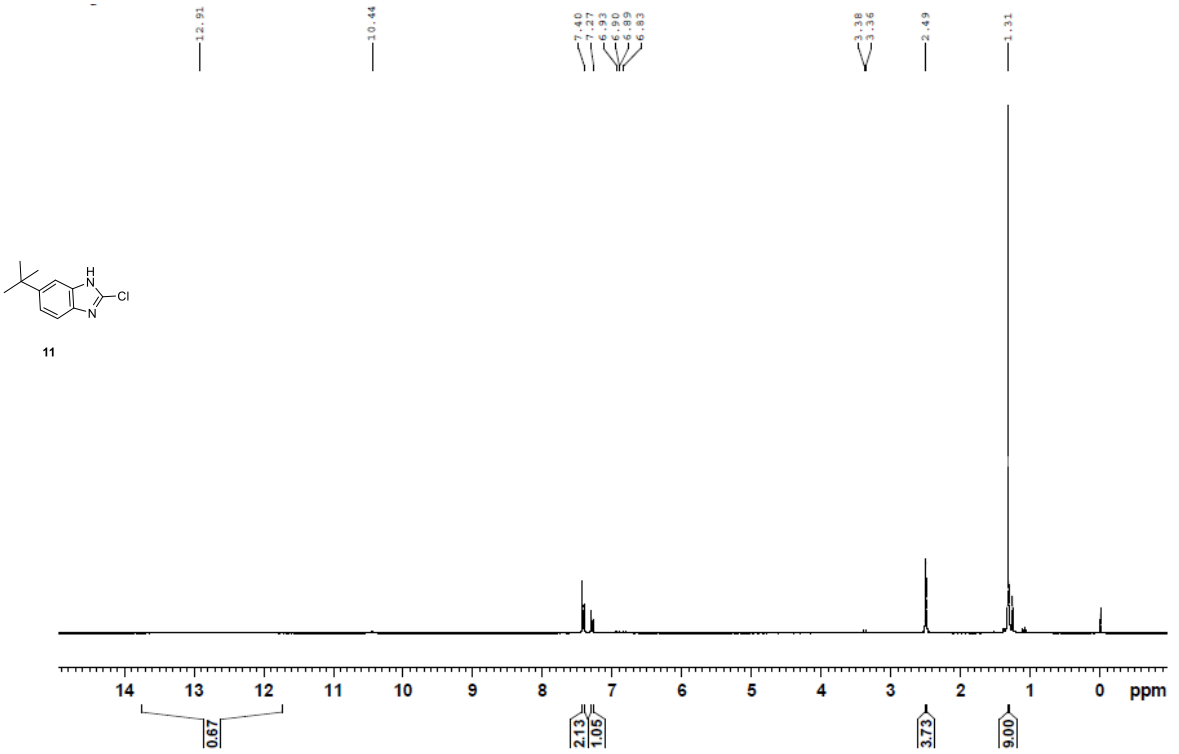
1-(4-(*Tert*-butyl)phenyl)-3-(3-(isopropylamino)propyl)urea, **7b**



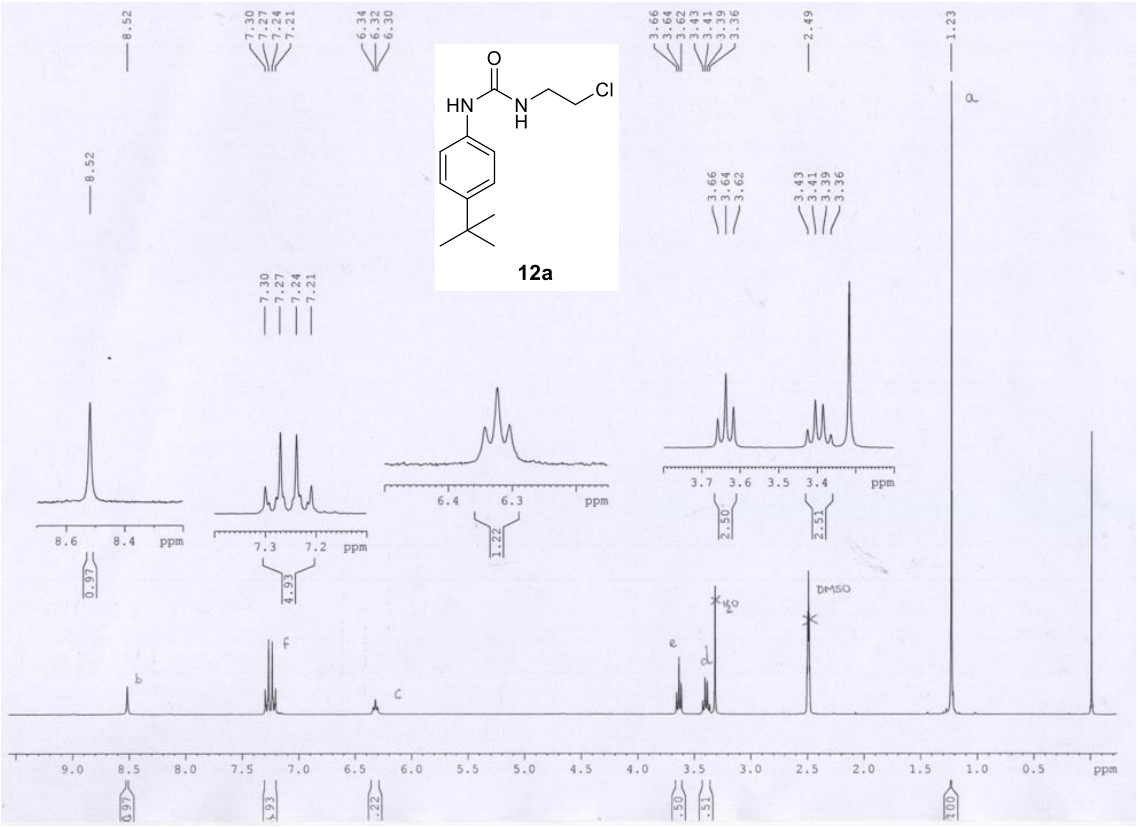
5-(*Tert*-butyl)-1*H*-benzo[*d*]imidazol-2(3*H*)-one, **10**



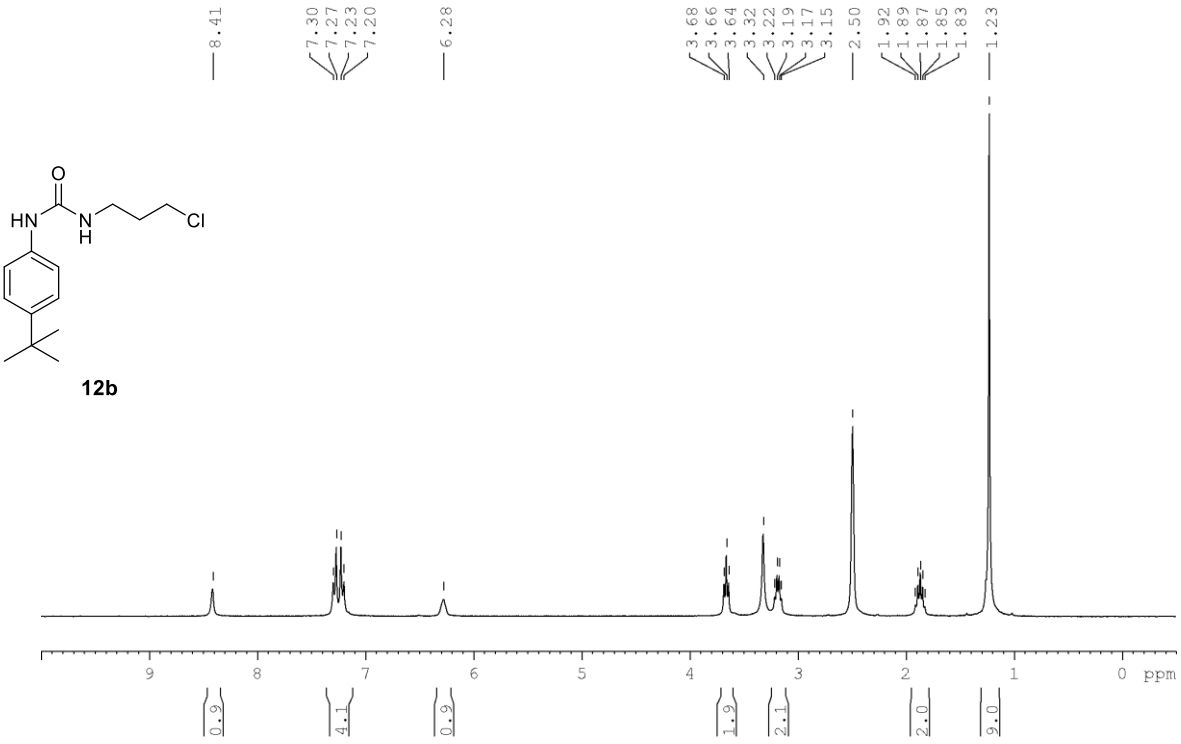
5-(*Tert*-butyl)-2-chloro-2,3-dihydro-1*H*-benzo[*d*]imidazole, **11**



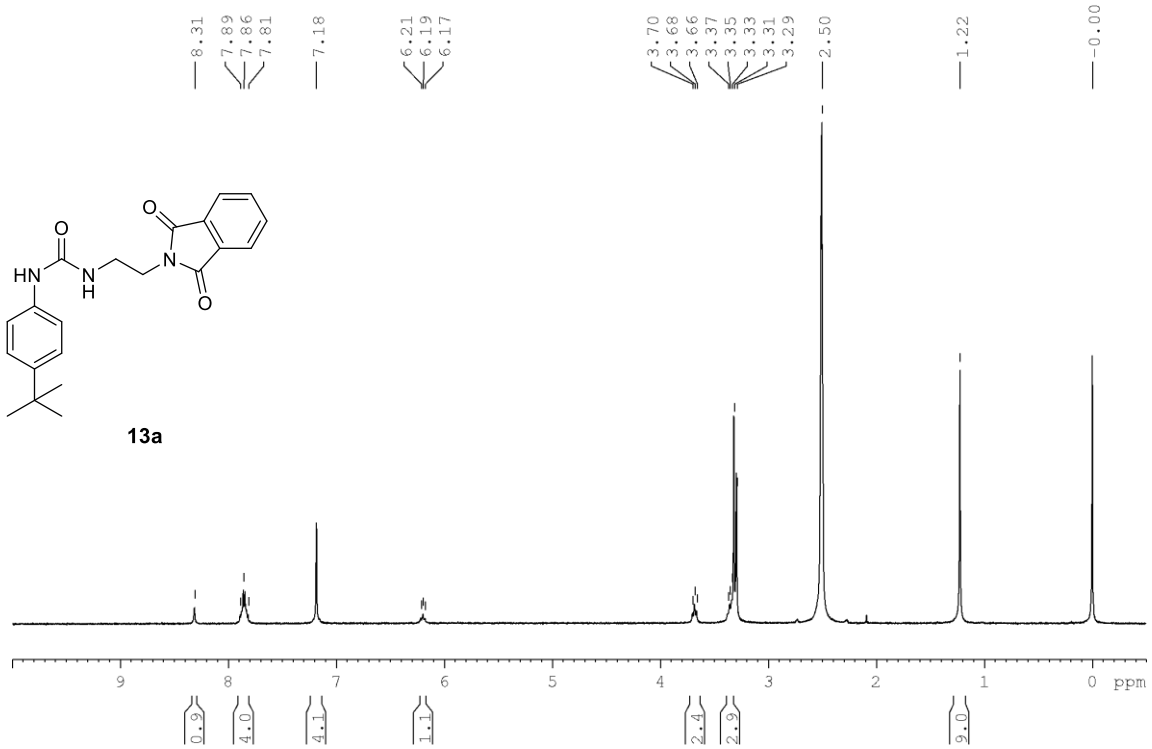
1-(4-(*Tert*-butyl)phenyl)-3-(2-chloroethyl)urea, **12a**



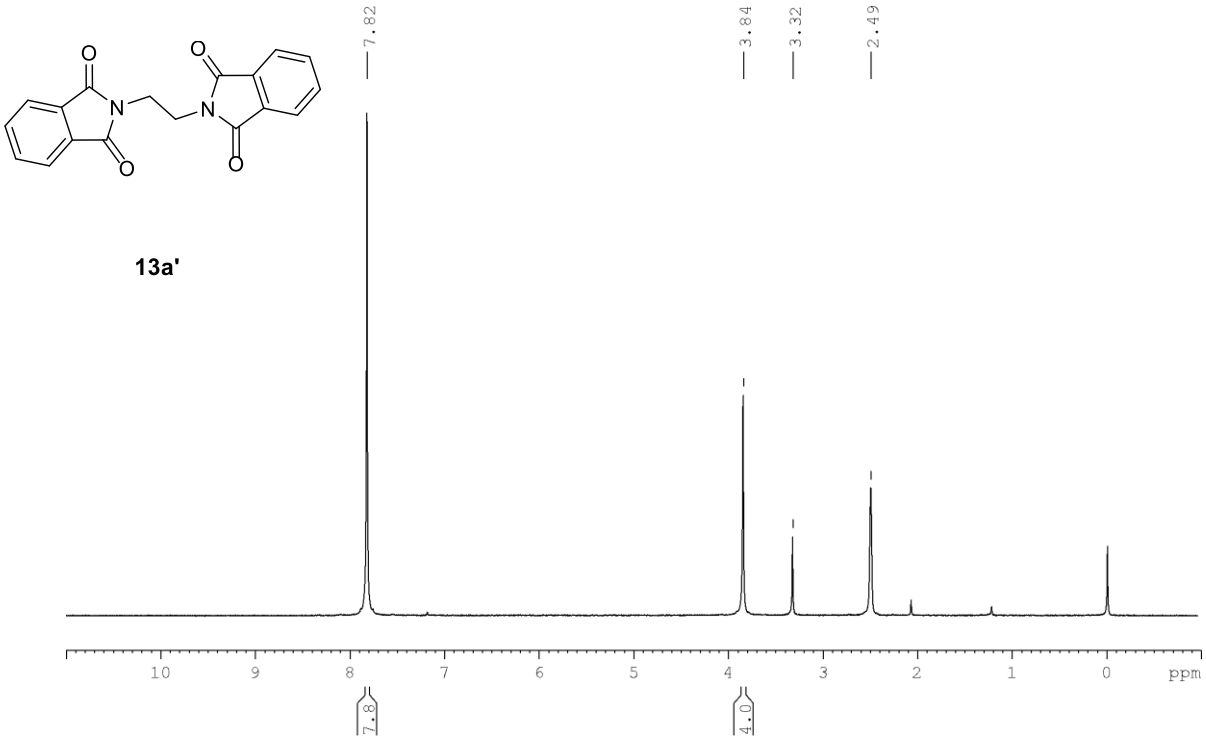
1-(4-(*Tert*-butyl)phenyl)-3-(3-chloropropyl)urea, **12b**



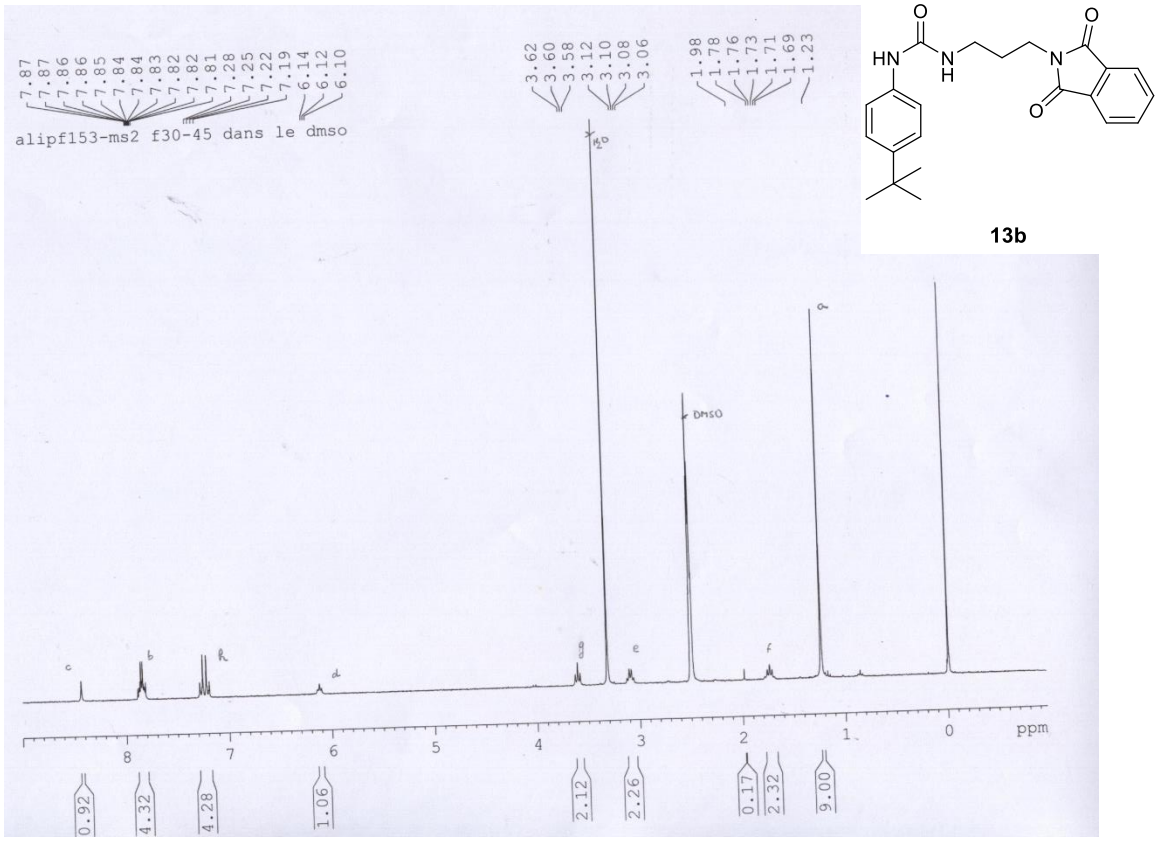
1-(4-(*Tert*-butyl)phenyl)-3-(2-(1,3-dioxoisoindolin-2-yl)ethyl)urea, **13a**



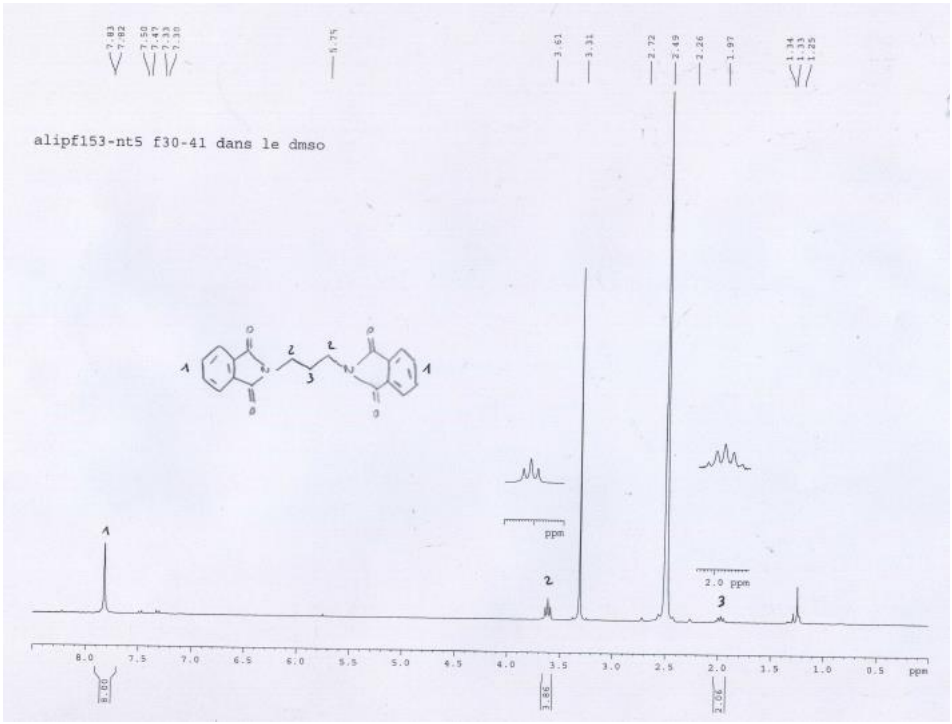
Characterization of 2,2'-(ethane-1,2-diyl)bis(isoindoline-1,3-dione), **13a'**



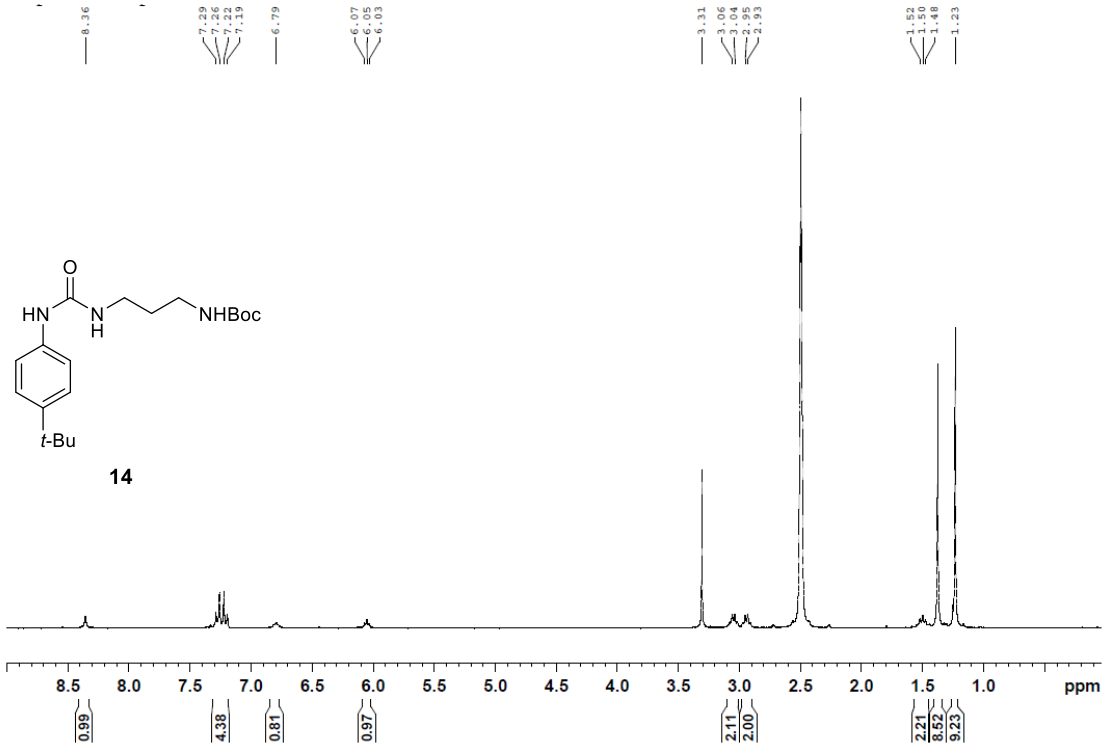
1-(4-(*Tert*-butyl)phenyl)-3-(3-(1,3-dioxoisoindolin-2-yl)propyl)urea, **13b**



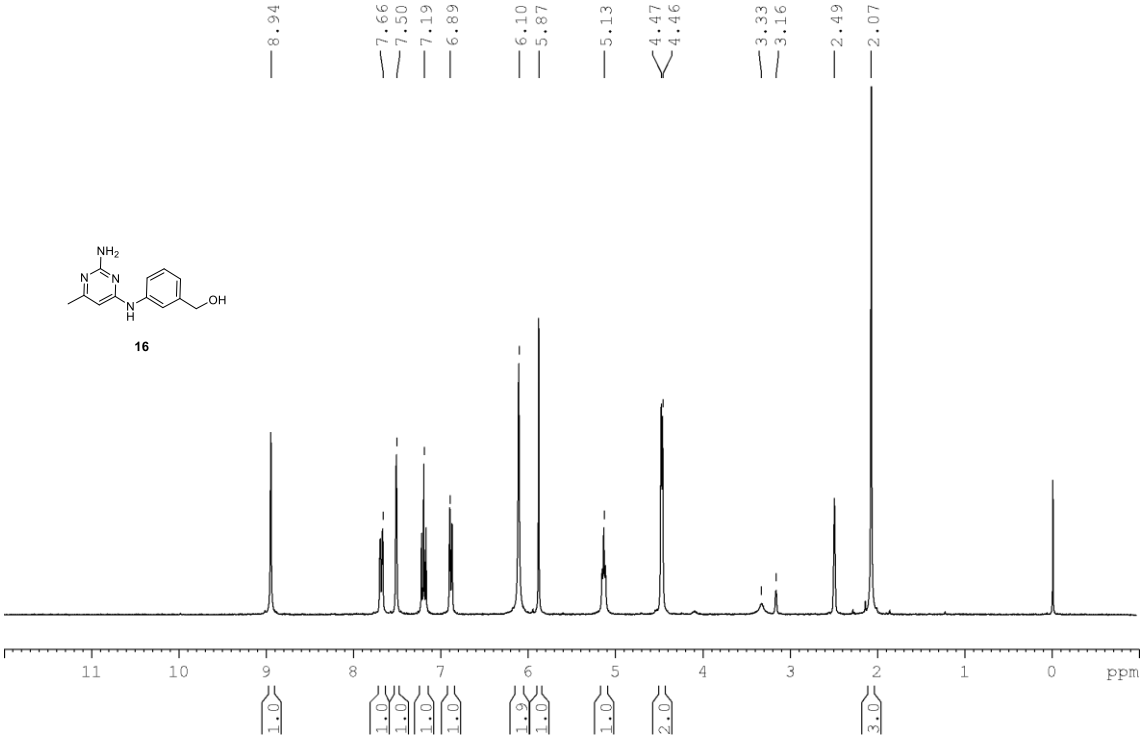
2,2'-(propane-1,3-diyl)bis(isoindoline-1,3-dione), **13b'**



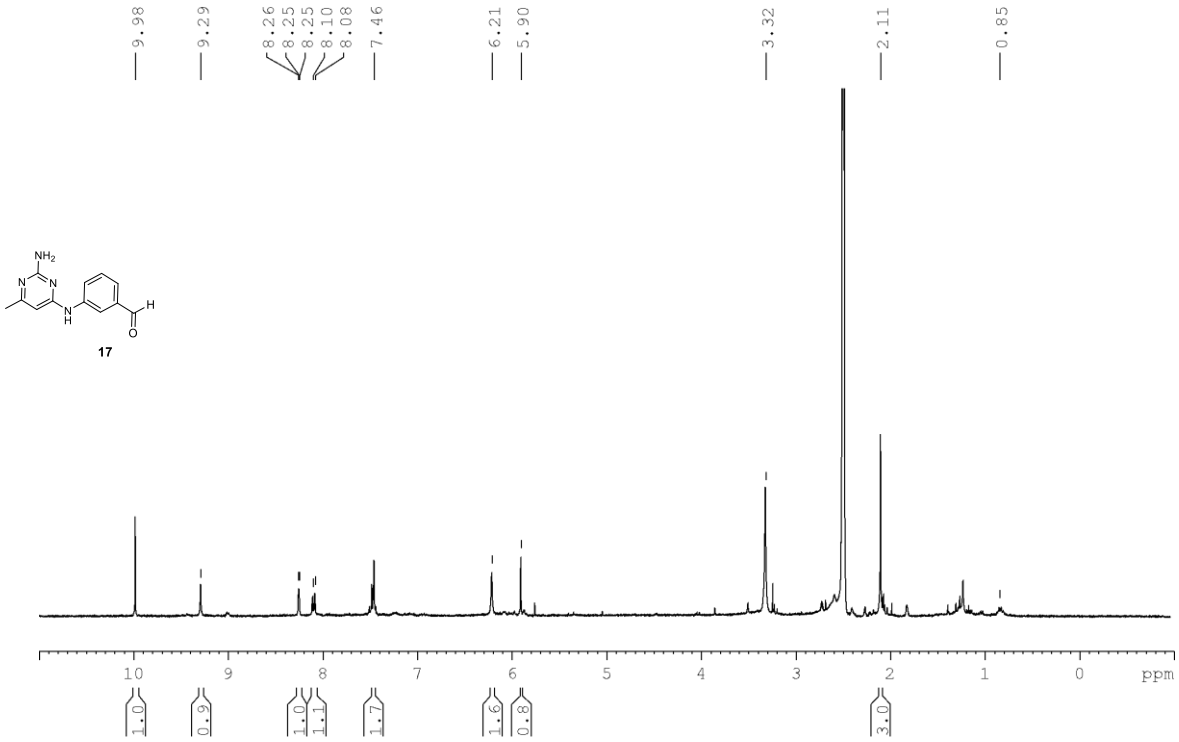
Tert-butyl (3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)carbamate, **14**



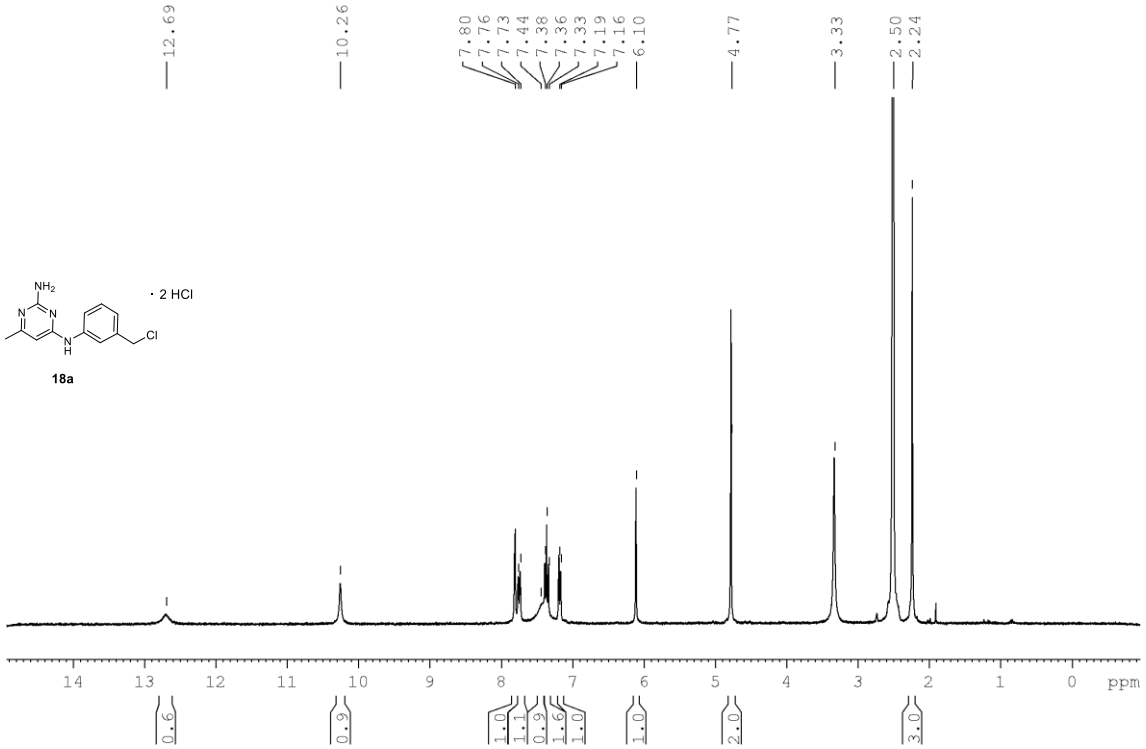
(3-((2-amino-6-methylpyrimidin-4-yl)amino)phenyl)methanol, **16**



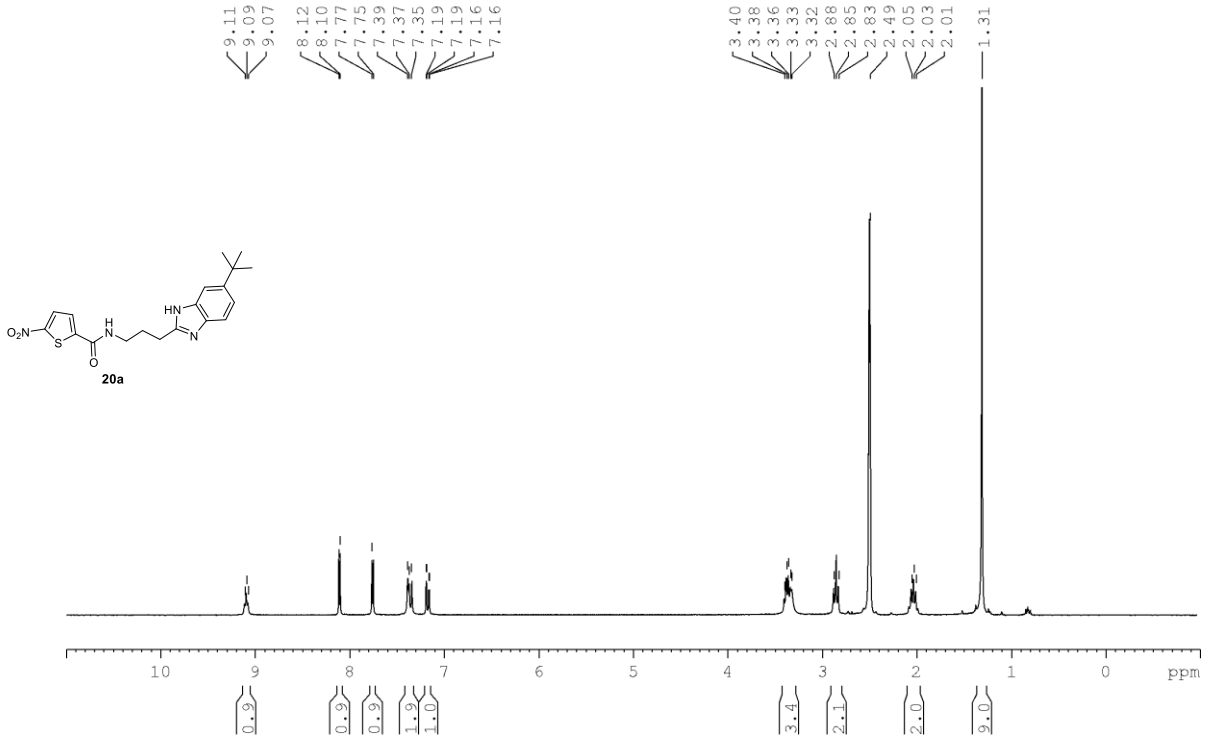
3-((2-amino-6-methylpyrimidin-4-yl)amino)benzaldehyde, **17**



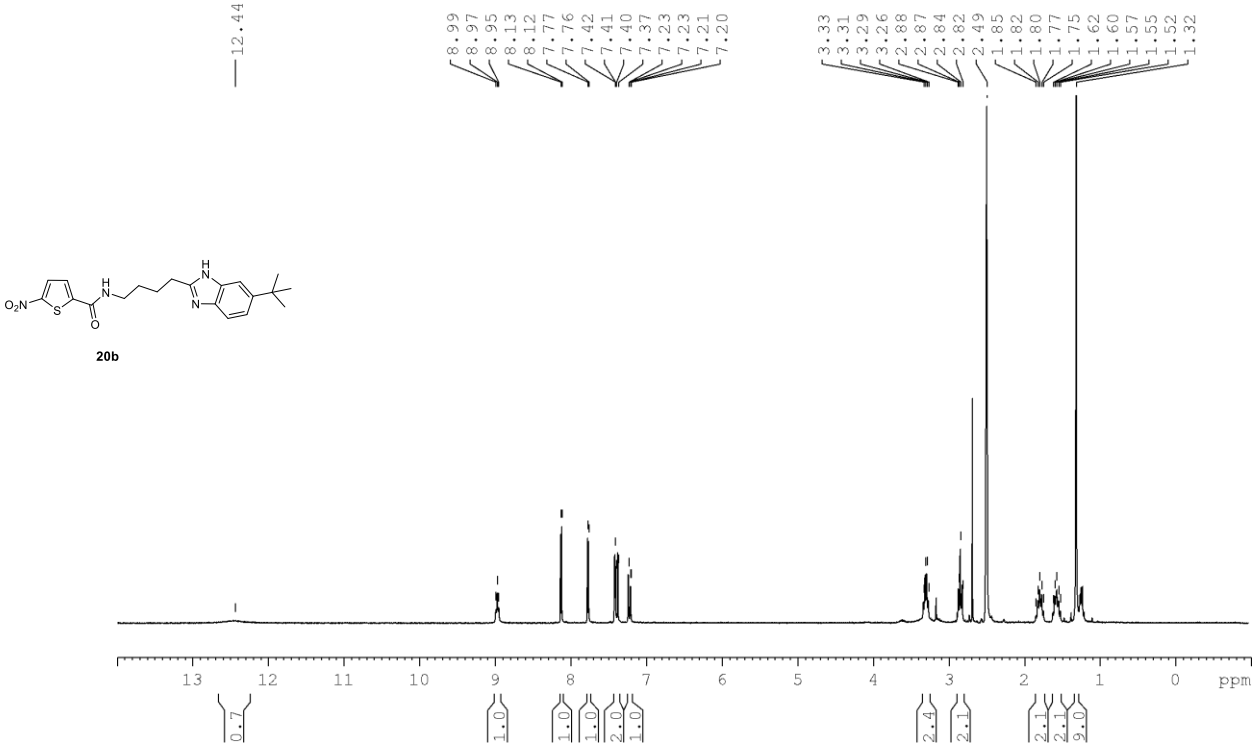
N^4-(3-(chloromethyl)phenyl)-6-methylpyrimidine-2,4-diamine, **18a**



N-(3-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)propyl)-5-nitrothiophene-2-carboxamide, **20a**

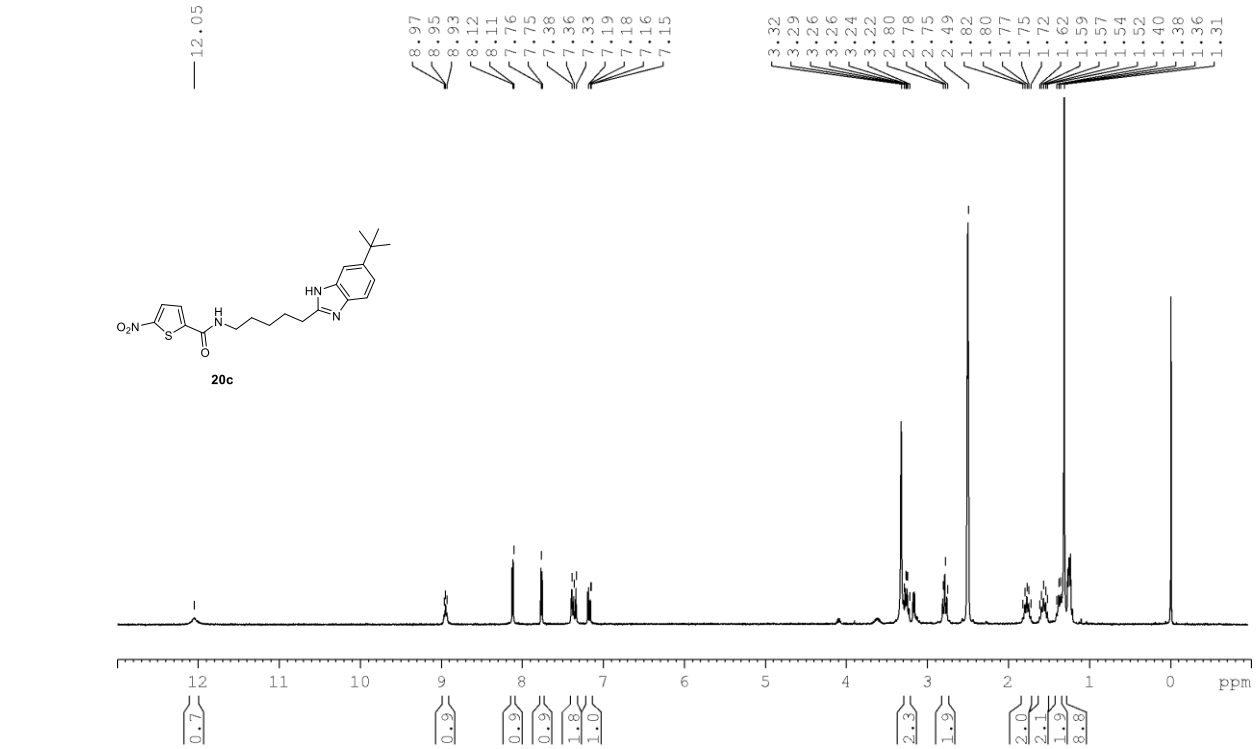


N-(4-(6-(tert-butyl)-1*H*-benzo[*d*]imidazol-2-yl)butyl)-5-nitrothiophene-2-carboxamide, **20b**



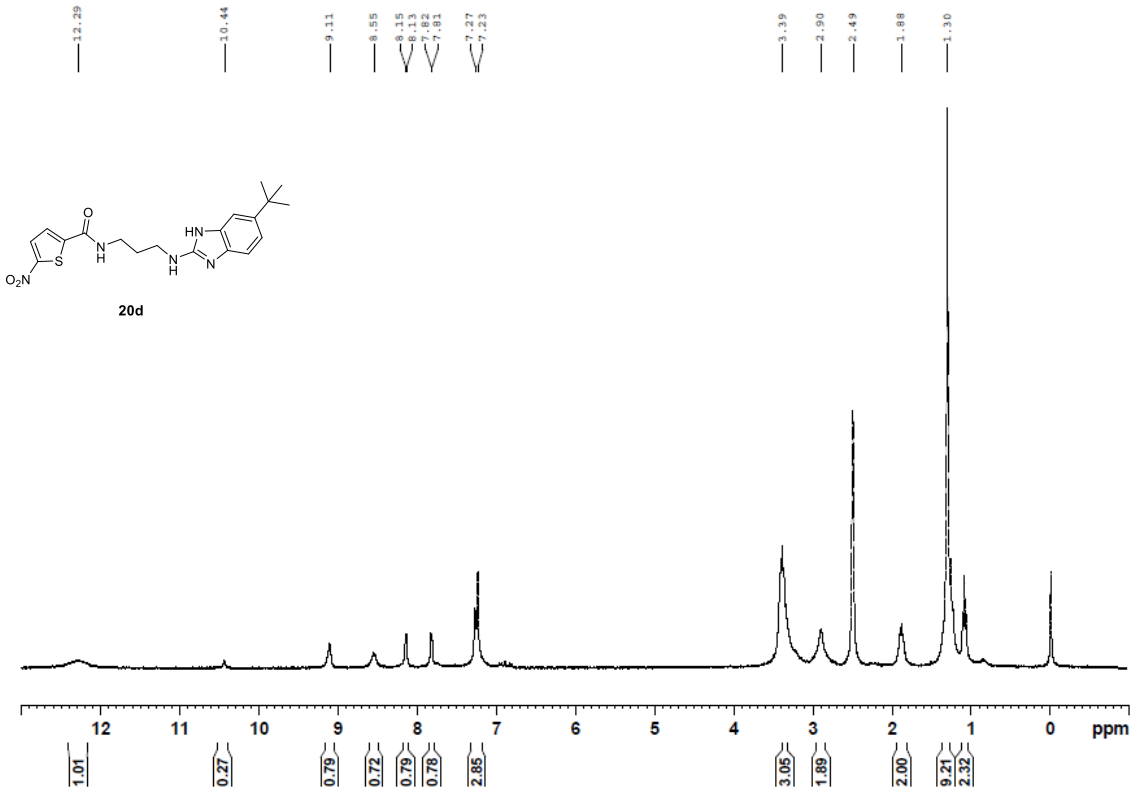
It shows impurity not characterized.

N-(5-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)pentyl)-5-nitrothiophene-2-carboxamide, **20c**

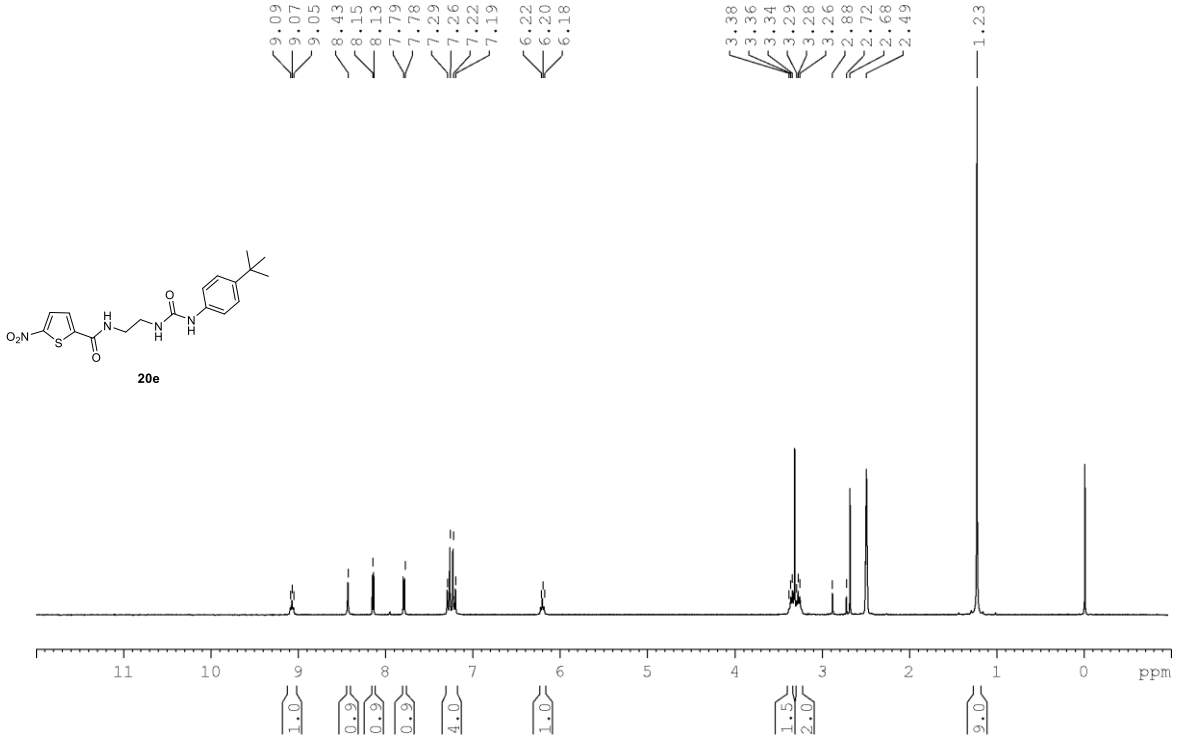


It shows impurity not characterized.

N-(3-((6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)amino)propyl)-5-nitrothiophene-2-carboxamide, **20d**

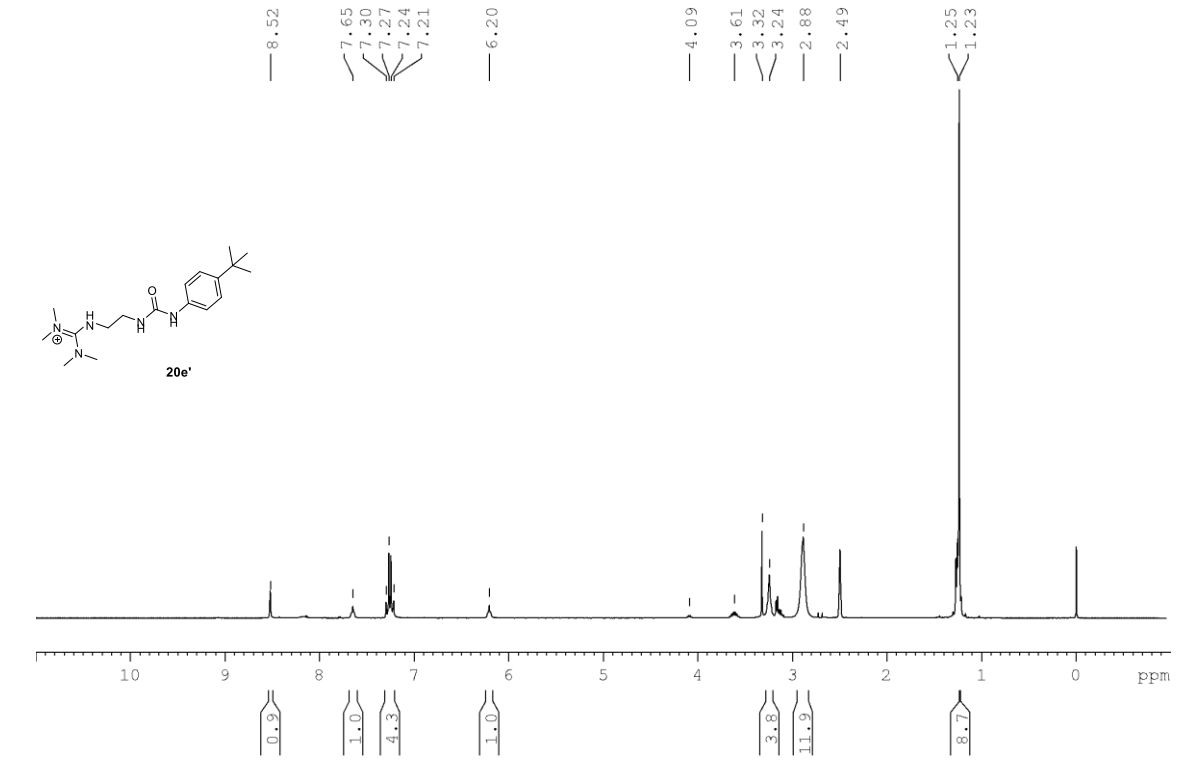


N-(2-(3-(4-(*tert*-butyl)phenyl)ureido)ethyl)-5-nitrothiophene-2-carboxamide, **20e**

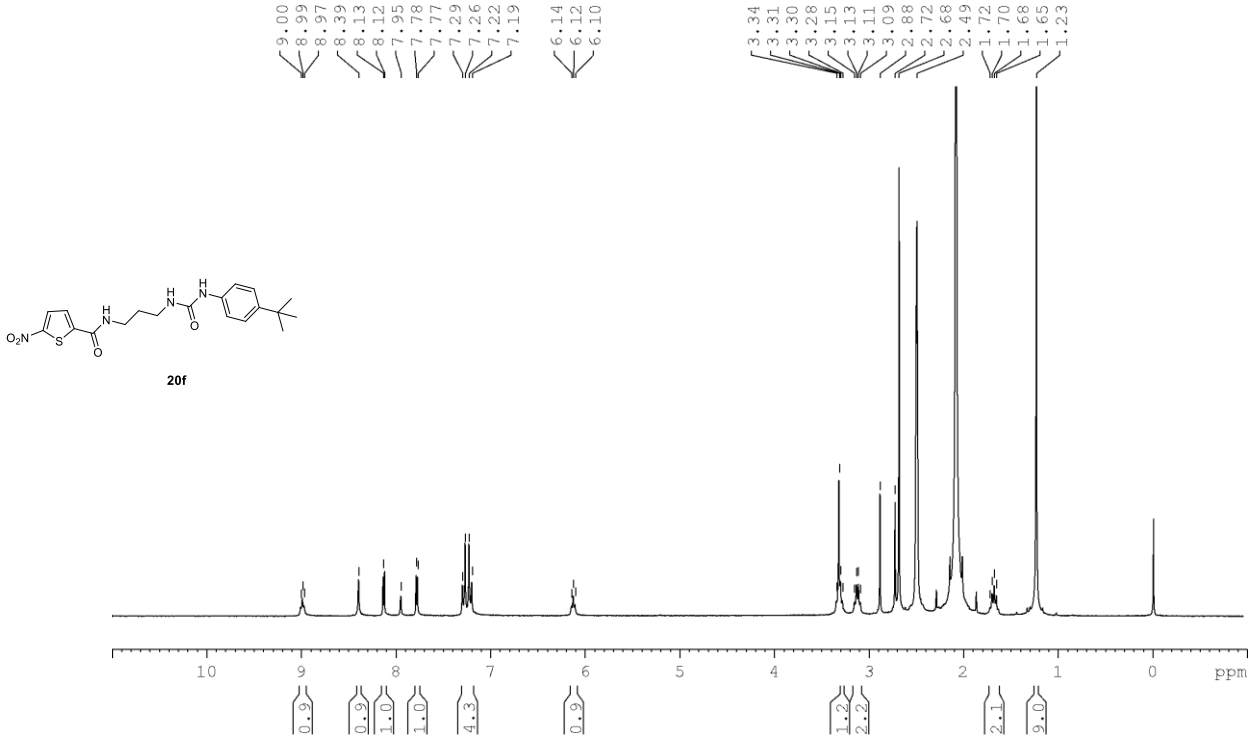


It shows impurity not characterized.

1-(2-((bis(dimethylamino)methylene)amino)ethyl)-3-(4-(*tert*-butyl)phenyl)urea, **20e'**

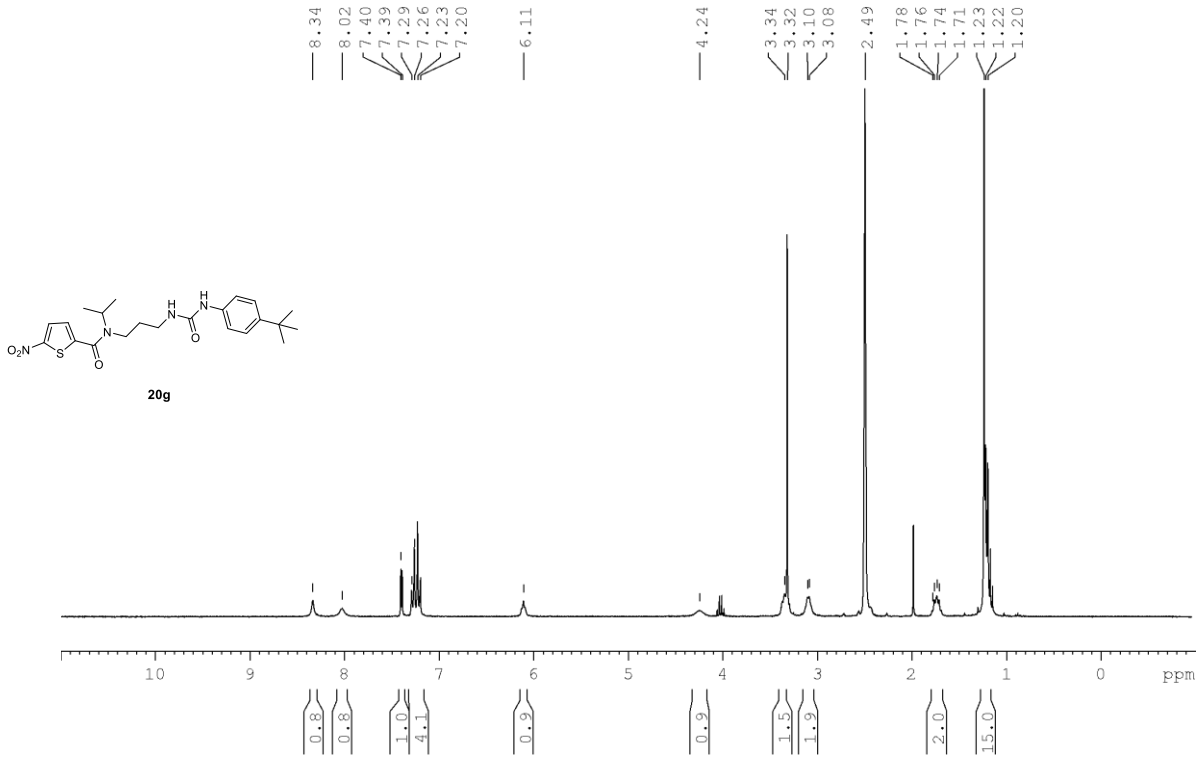


N-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)-5-nitrothiophene-2-carboxamide, **20f**



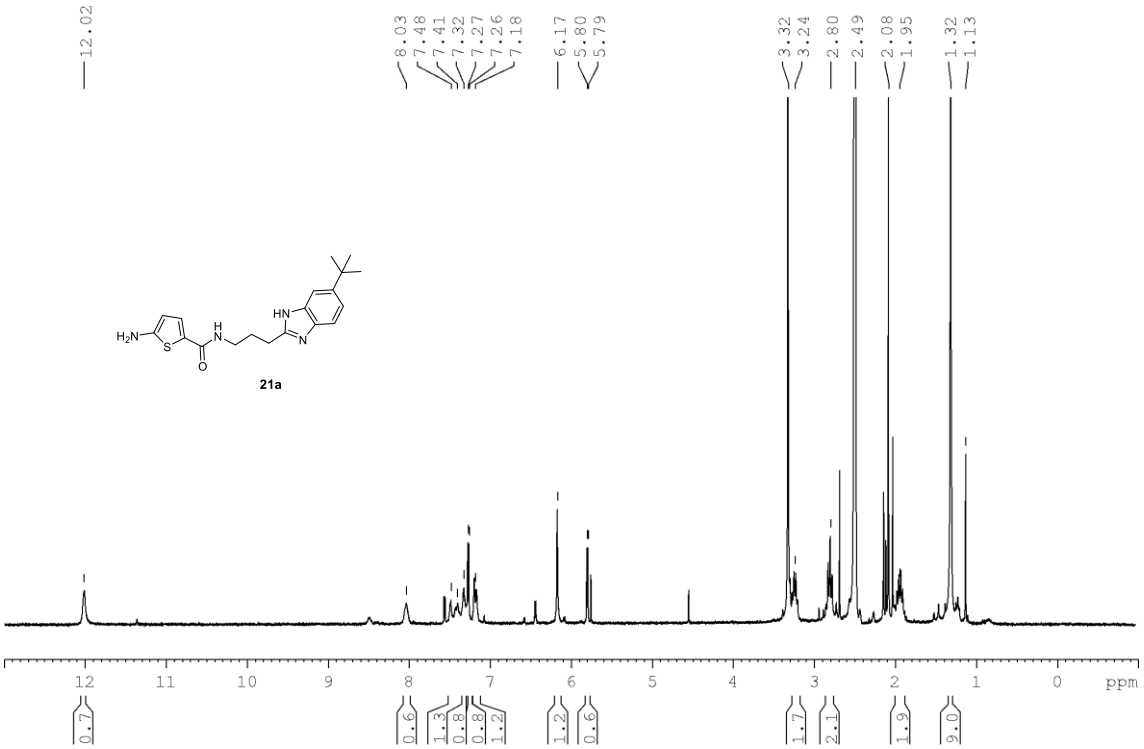
20f

N-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)-*N*-isopropyl-5-nitrothiophene-2-carboxamide, **20g**

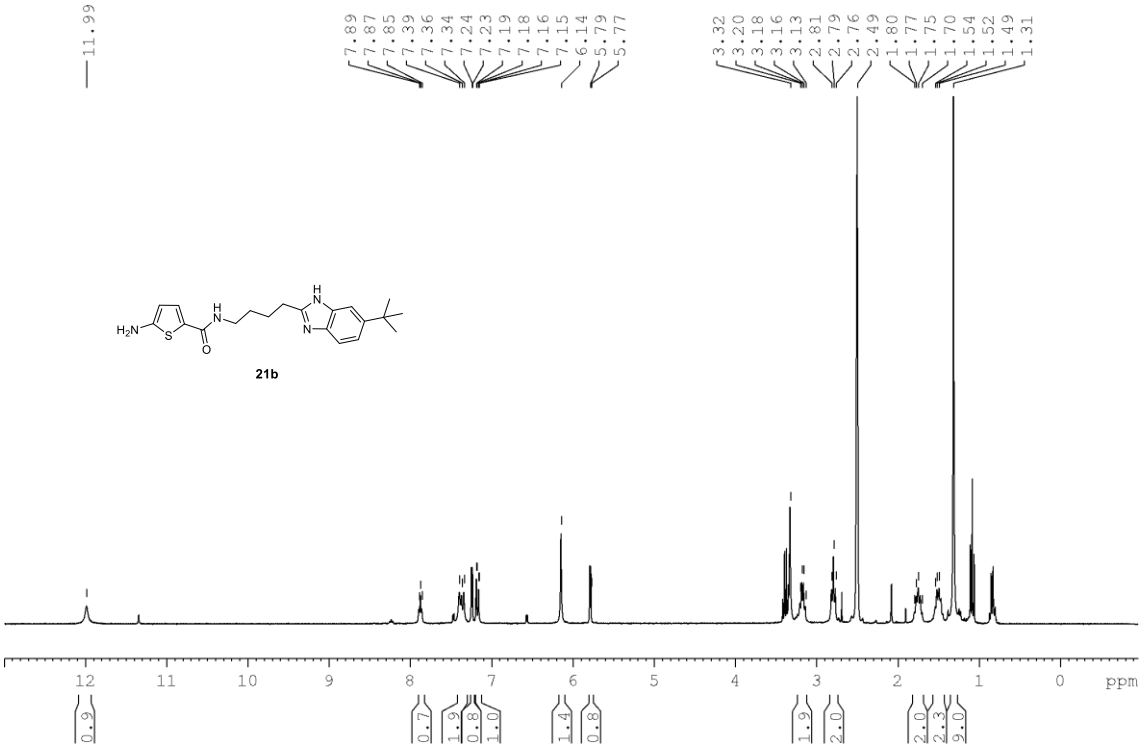


20g

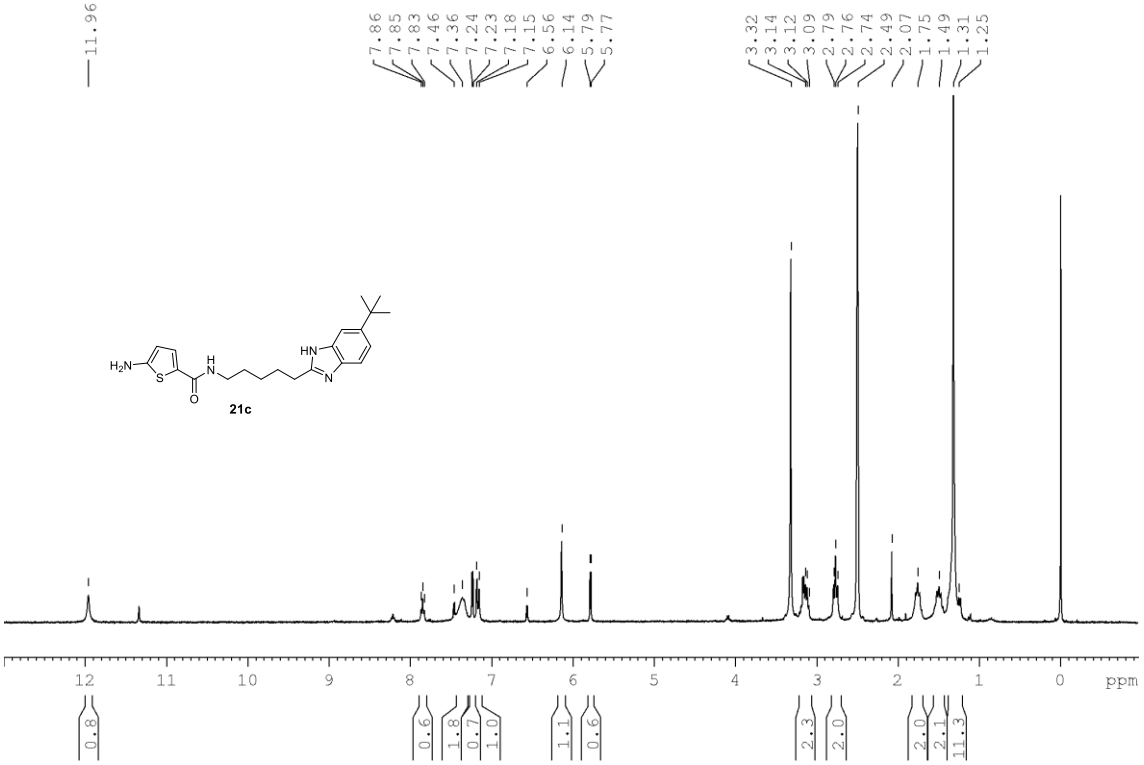
5-amino-*N*-(3-(6-(*tert*-butyl)-1*H*-benzo[d]imidazol-2-yl)propyl)thiophene-2-carboxamide, **21a**



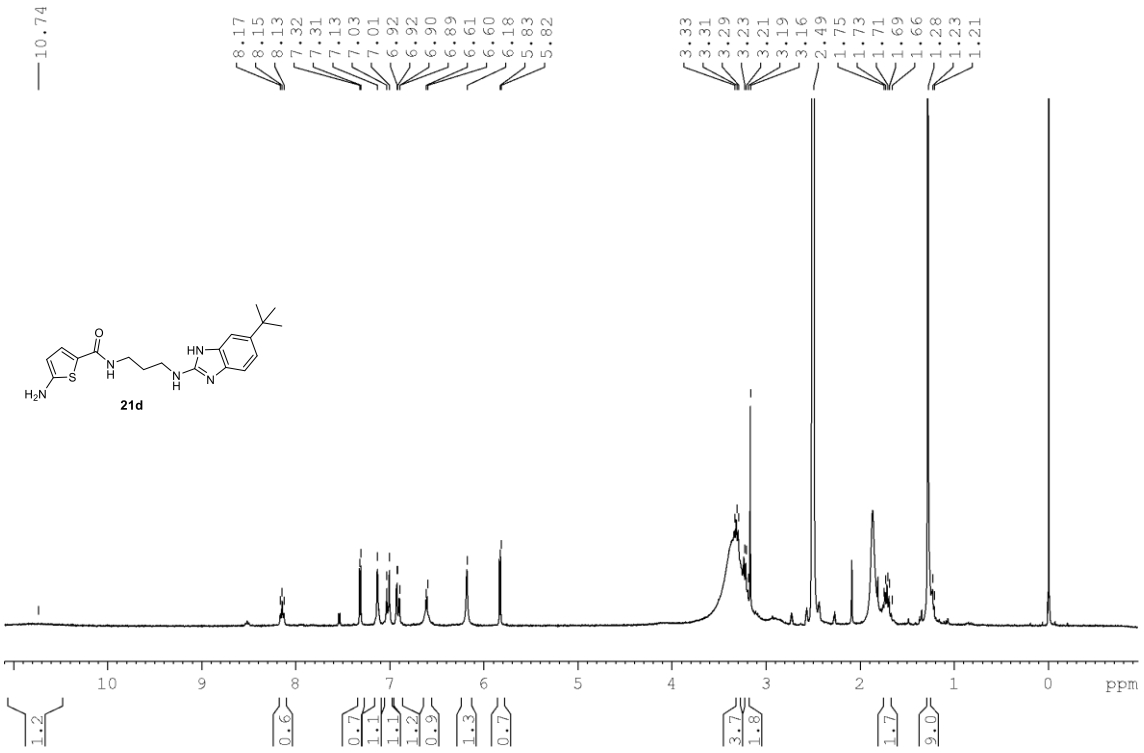
5-amino-*N*-(4-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)butyl)thiophene-2-carboxamide, **21b**



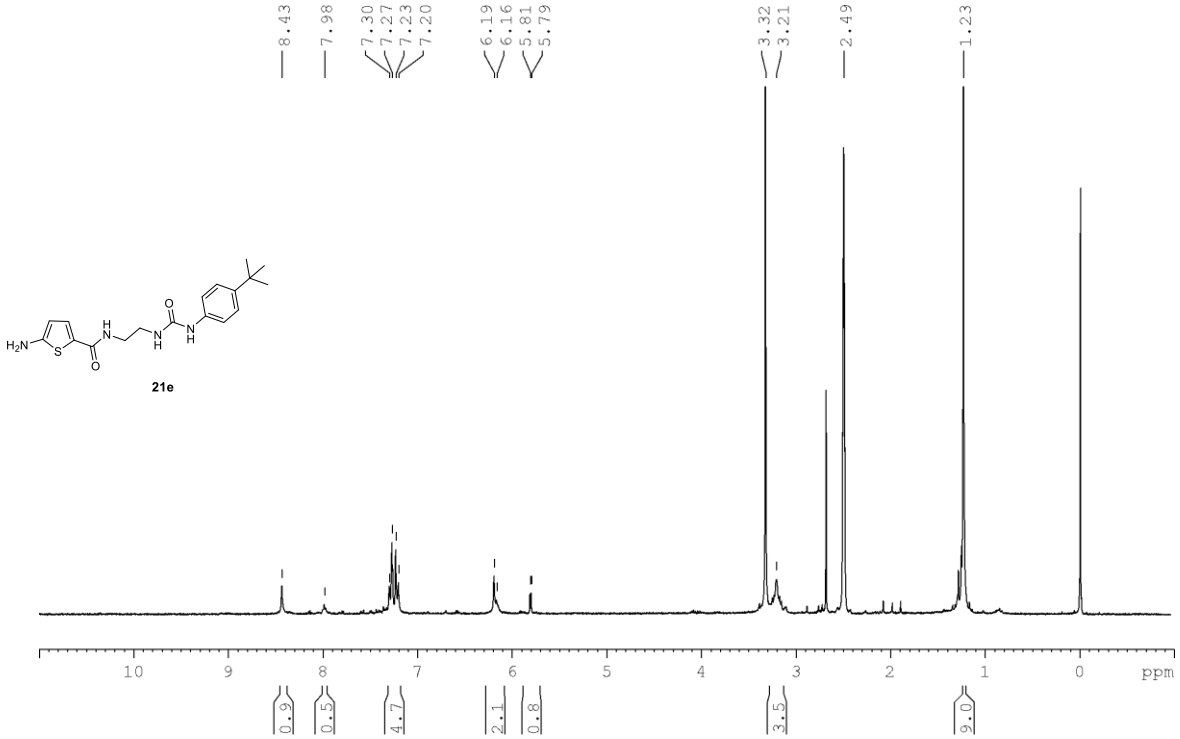
5-amino-*N*-(5-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)pentyl)thiophene-2-carboxamide, **21c**



5-amino-*N*-(3-((6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)amino)propyl)thiophene-2-carboxamide, **21d**

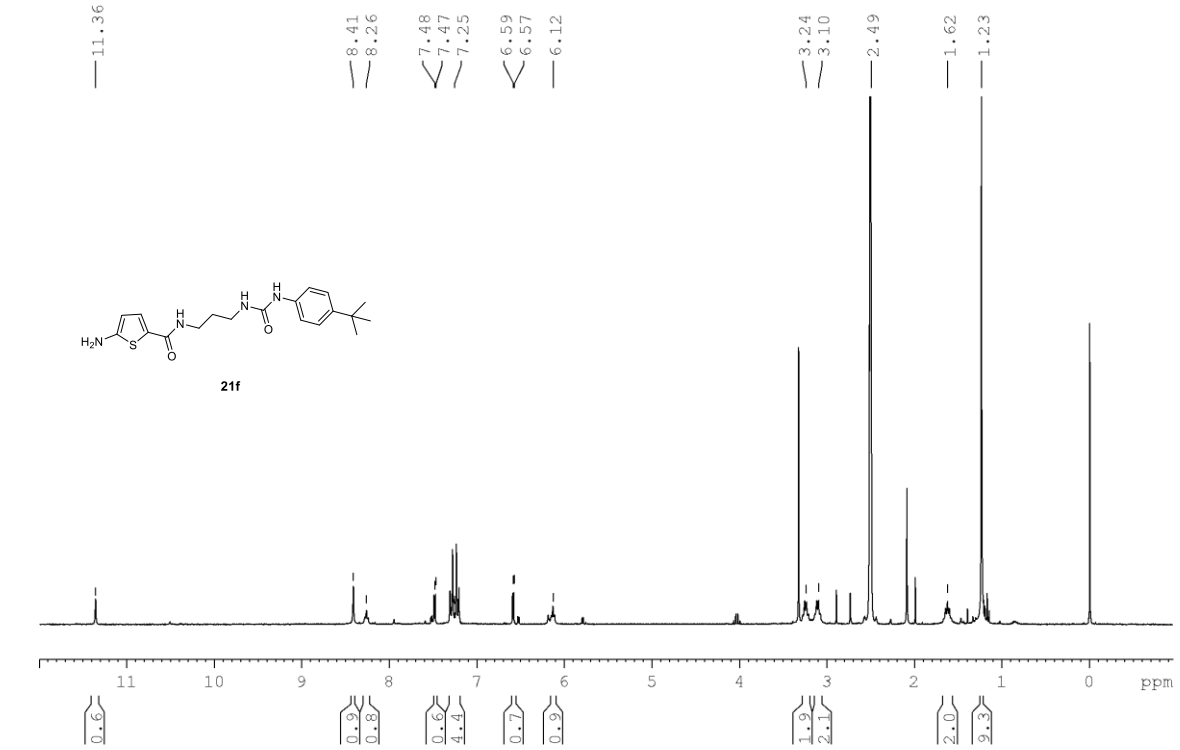


5-amino-*N*-(2-(3-(4-(*tert*-butyl)phenyl)ureido)ethyl)thiophene-2-carboxamide, **21e**



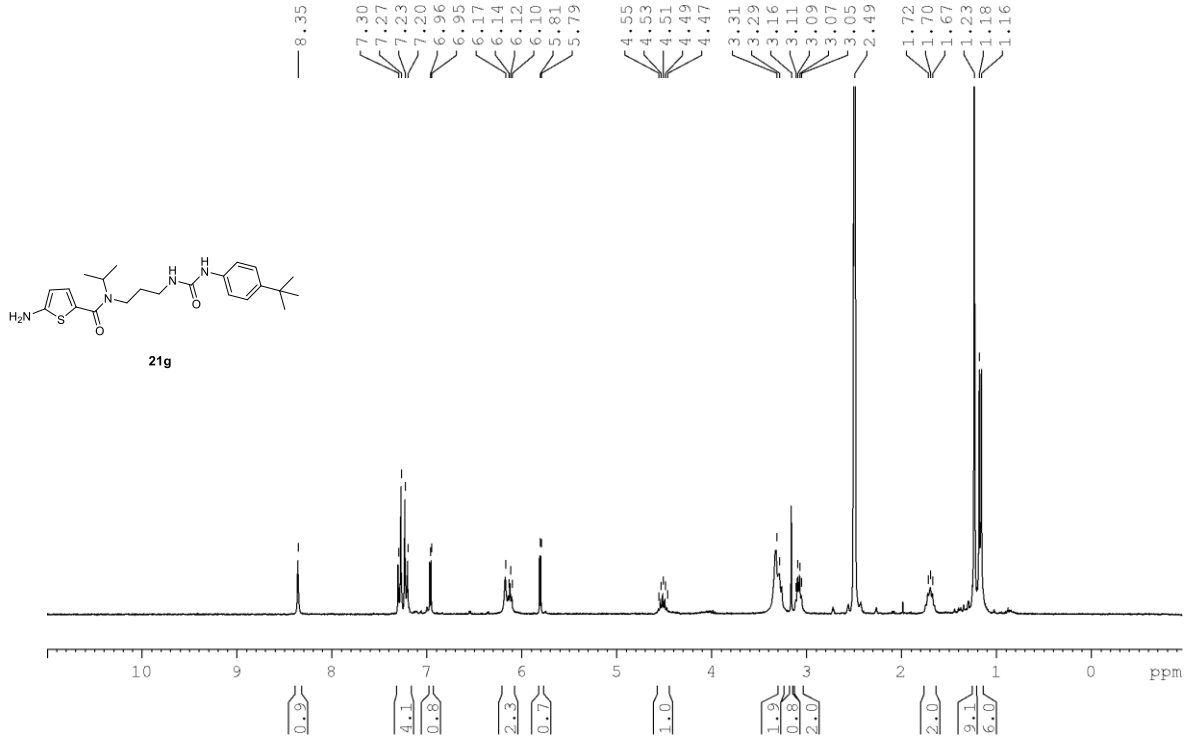
21e

5-amino-*N*-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)thiophene-2-carboxamide, **21f**



21f

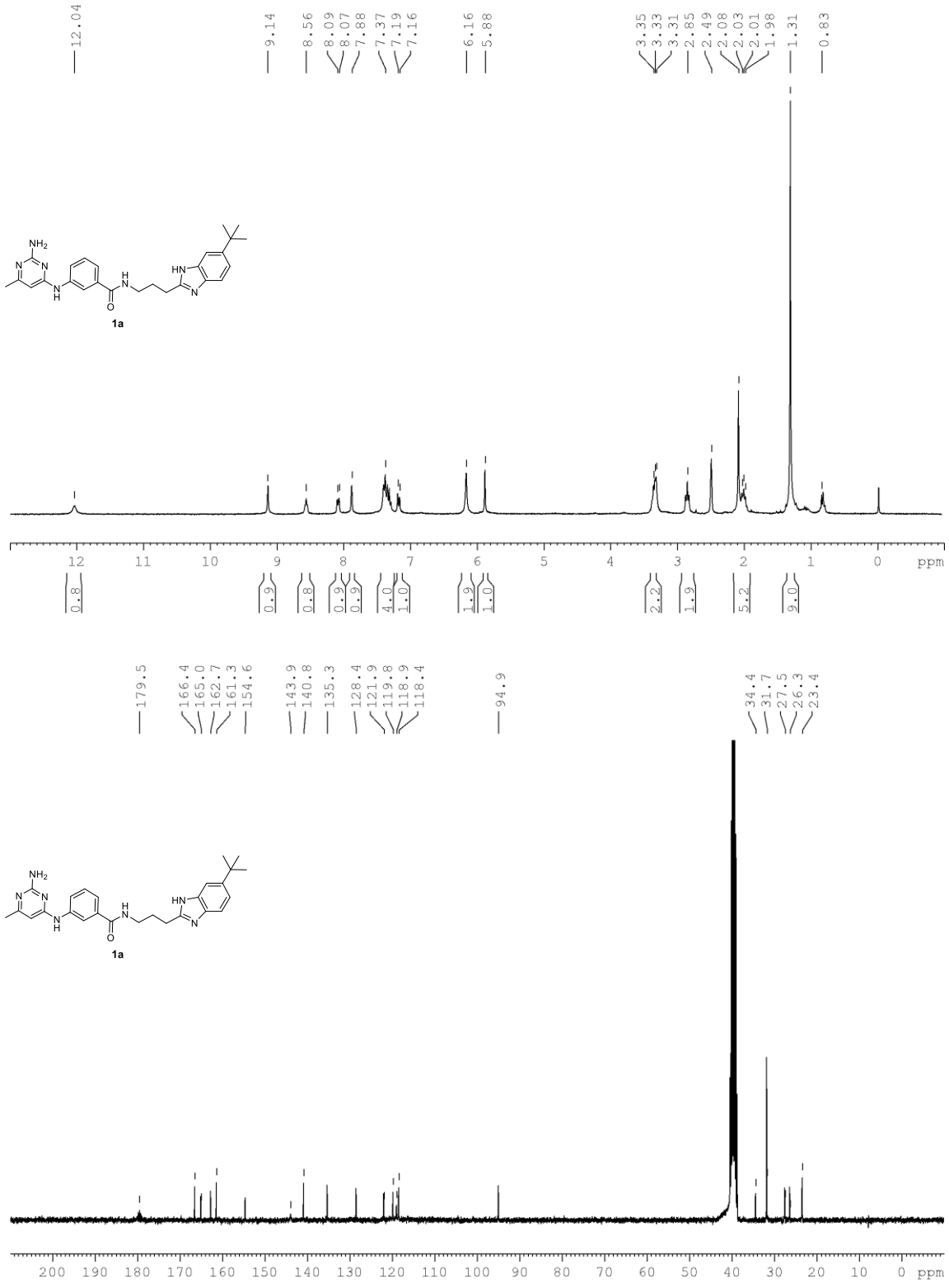
5-amino-*N*-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)-*N*-isopropylthiophene-2-carboxamide, **21g**



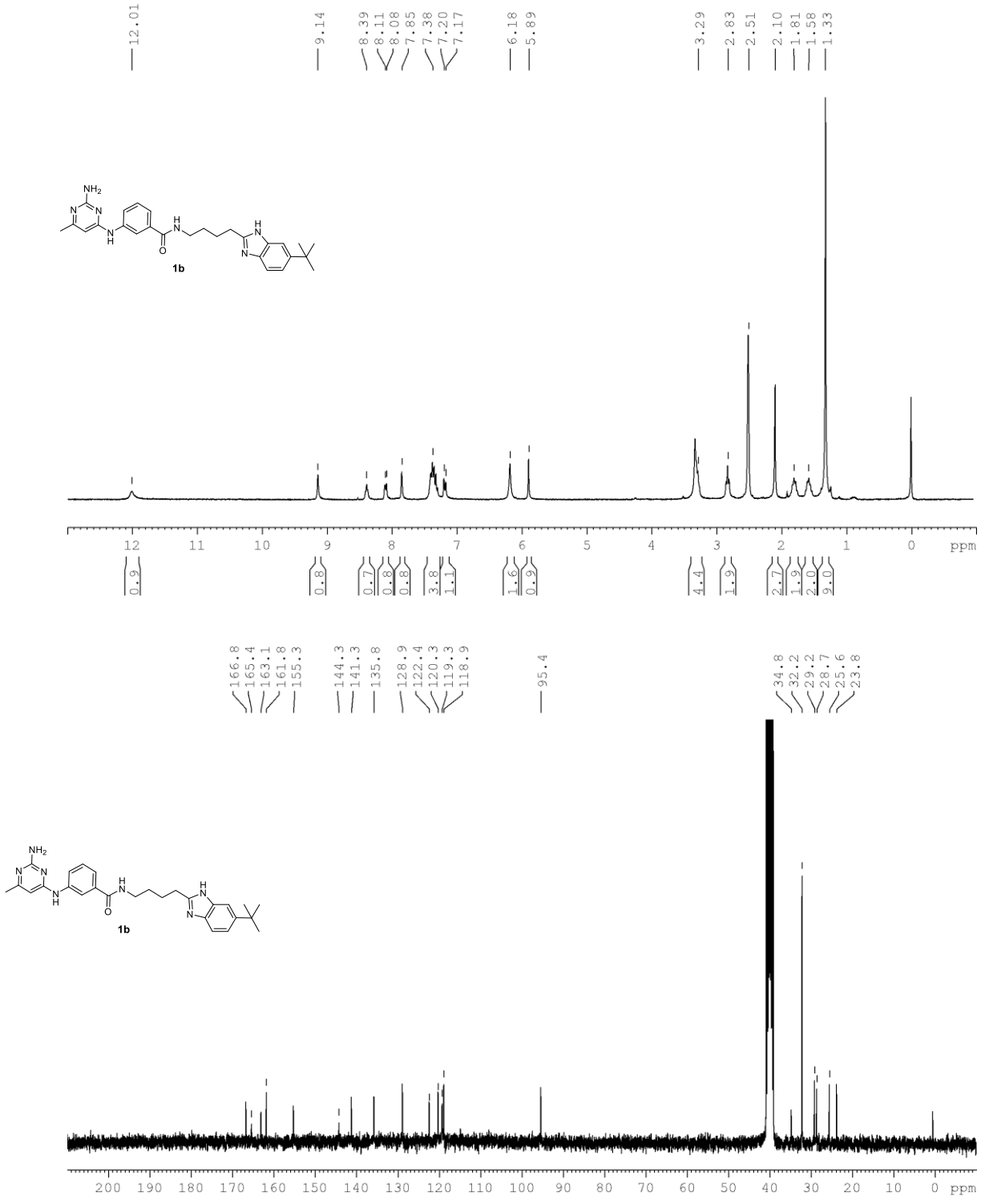
21g

Final compounds

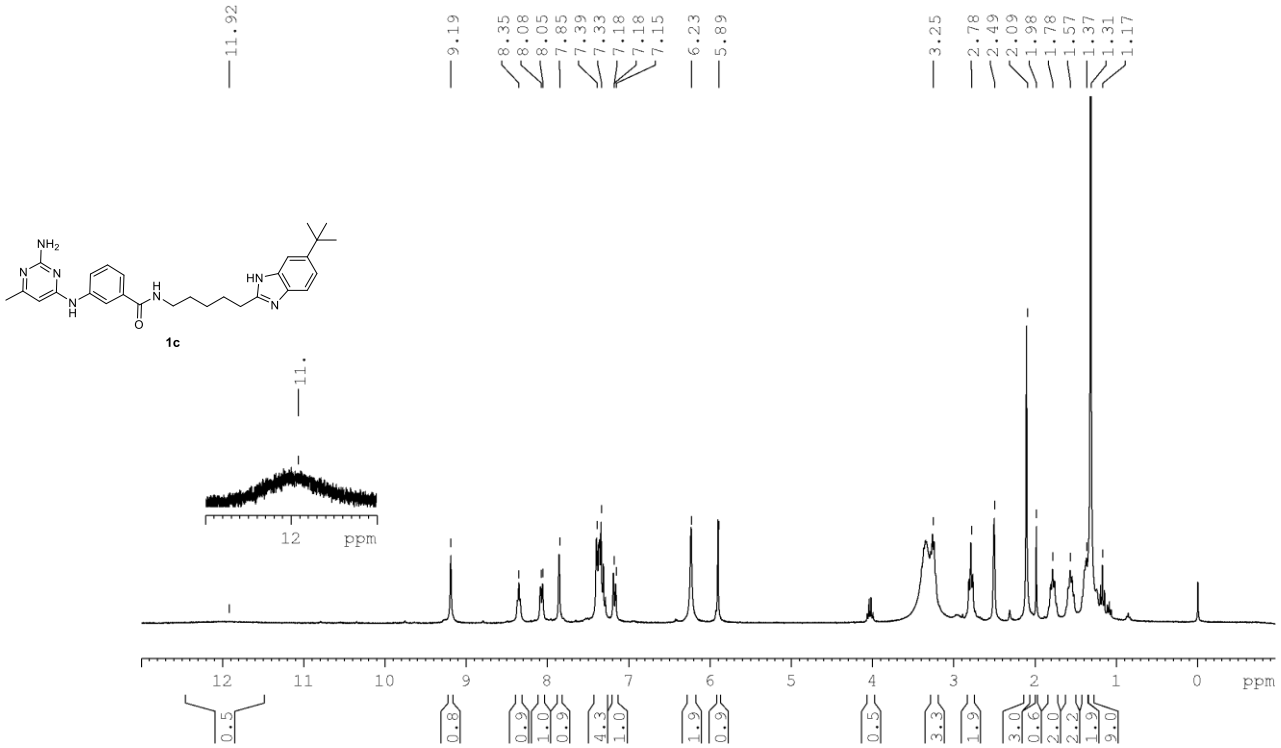
3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(3-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)propyl)benzamide, **1a**

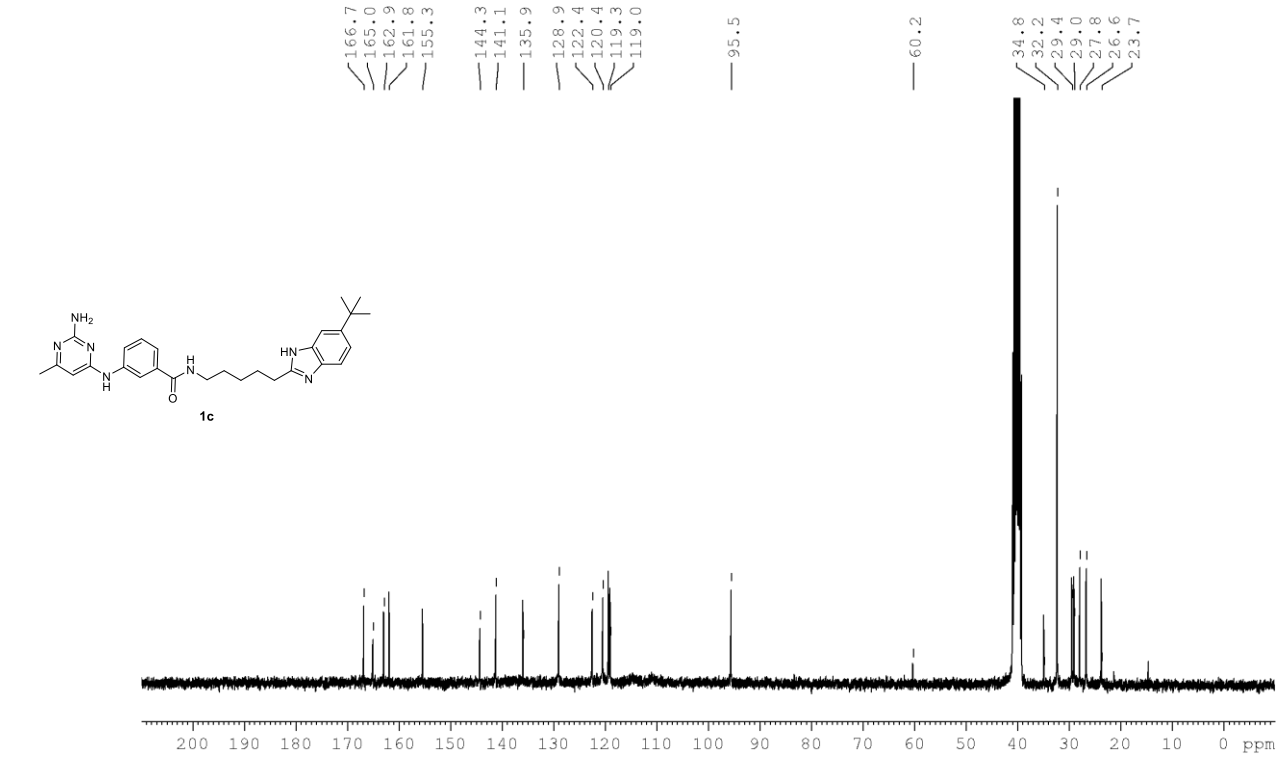


3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(4-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)butyl)benzamide, **1b**

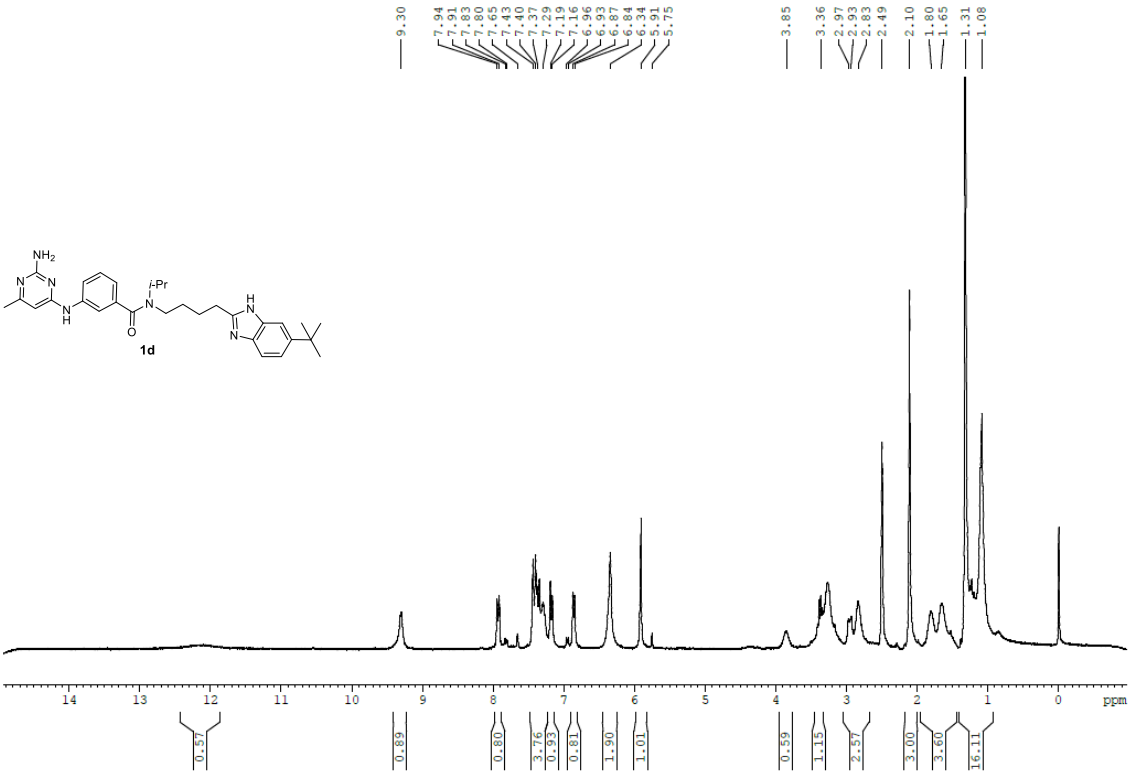


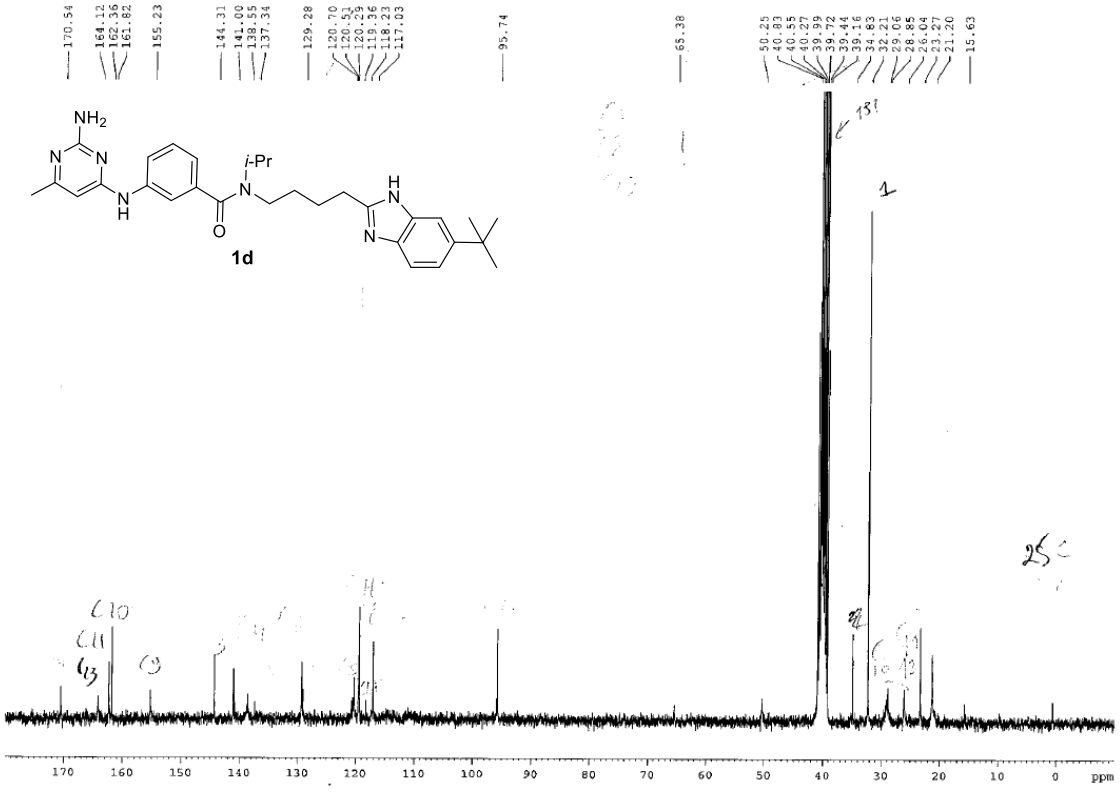
3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(5-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)pentyl)benzamide, **1c**



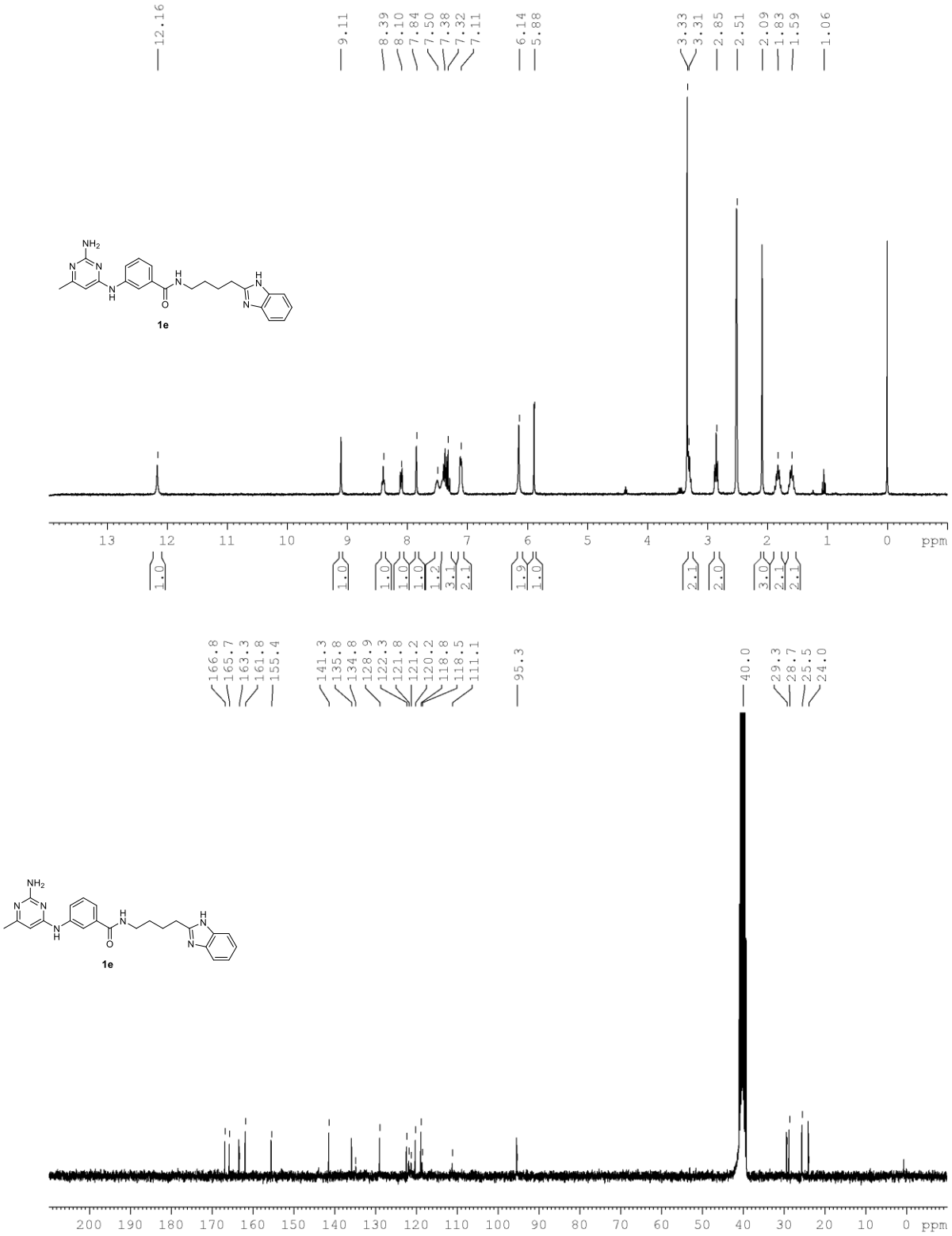


3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(4-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)butyl)-*N*-isopropylbenzamide, **1d**

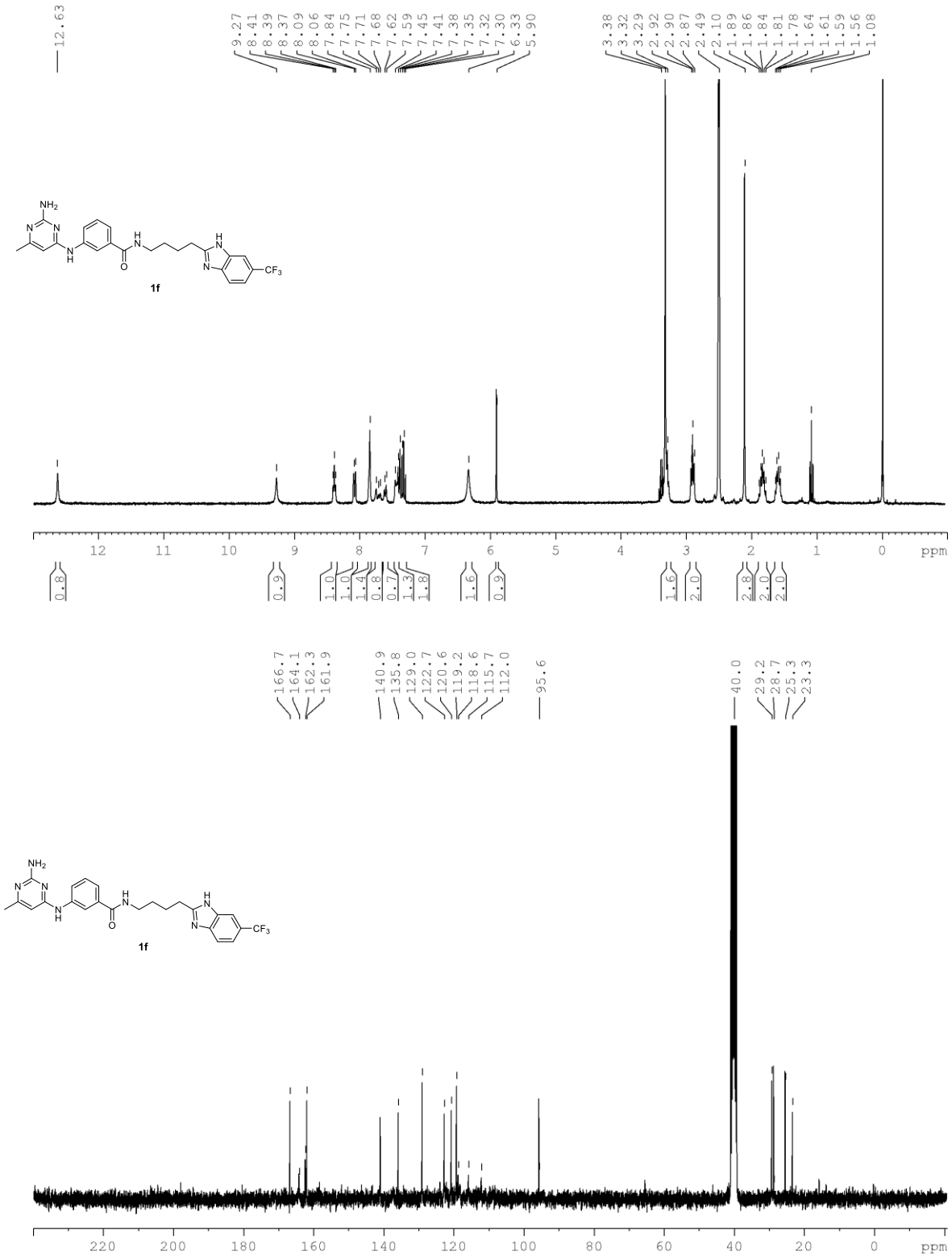




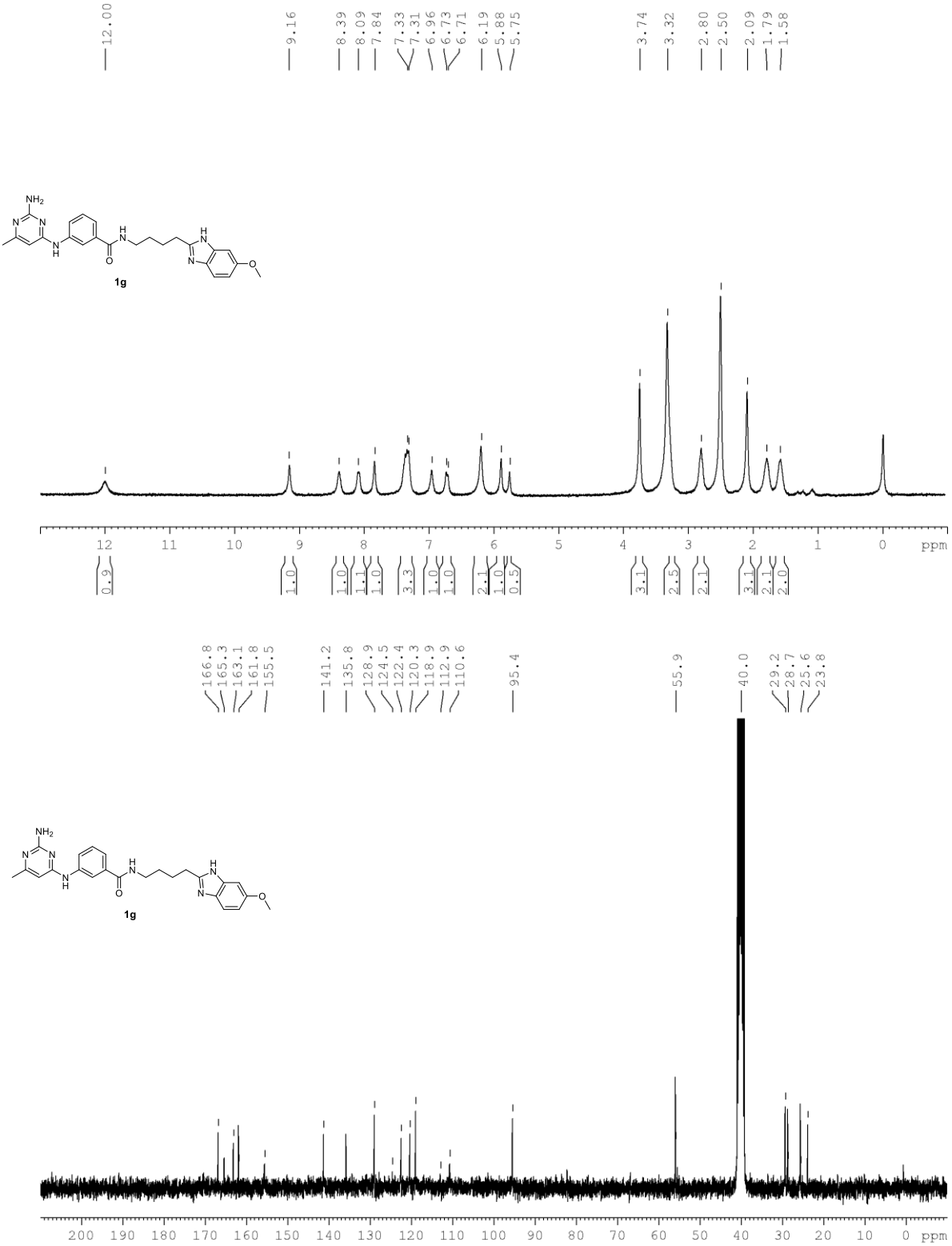
N-(4-(1*H*-benzo[*d*]imidazol-2-yl)butyl)-3-((2-amino-6-methylpyrimidin-4-yl)amino)benzamide, **1e**



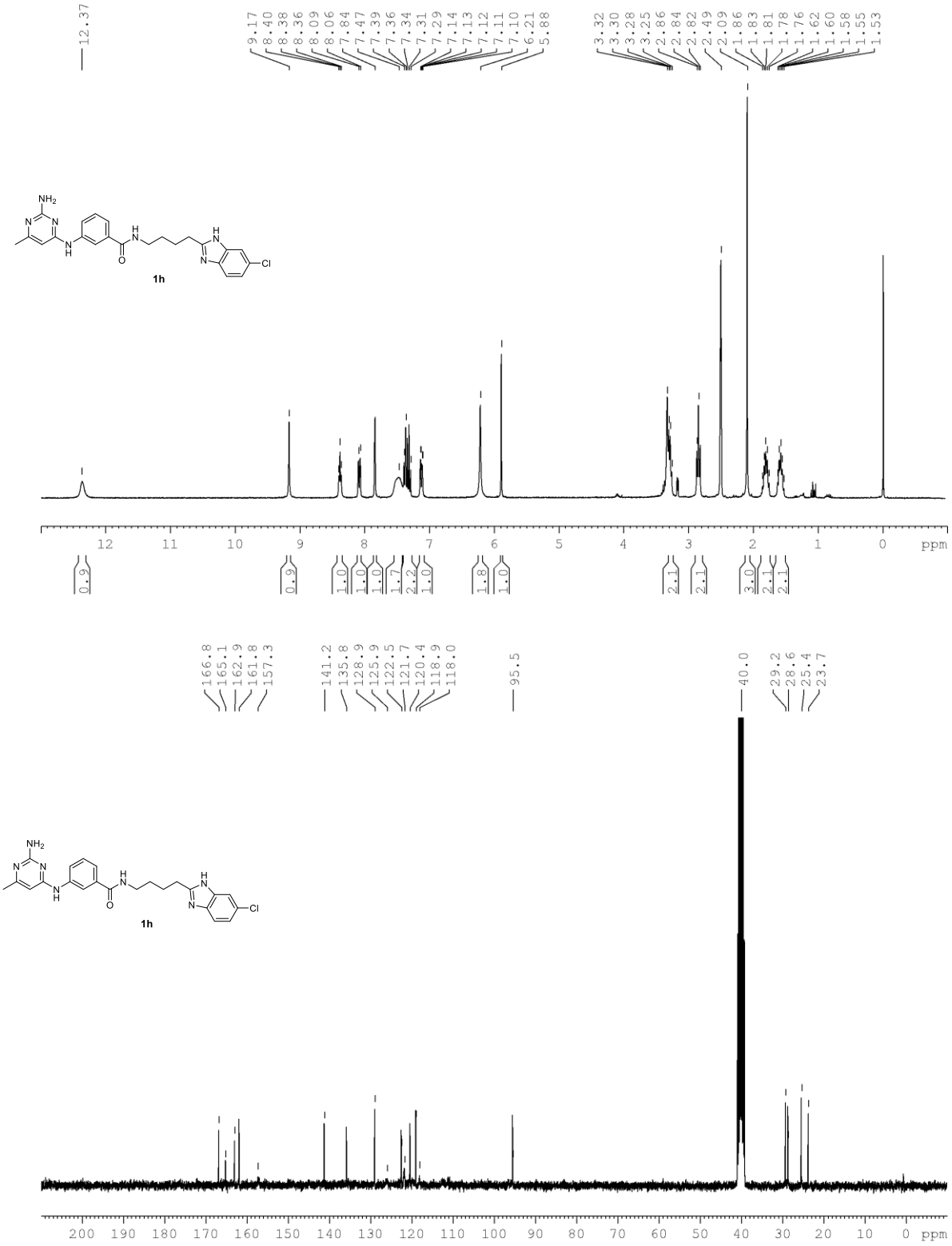
3-((2-amino-6-methylpyrimidin-4-yl)amino)-N-(4-(6-(trifluoromethyl)-1*H*-benzo[*d*]imidazol-2-yl)butyl)benzamide, **1f**



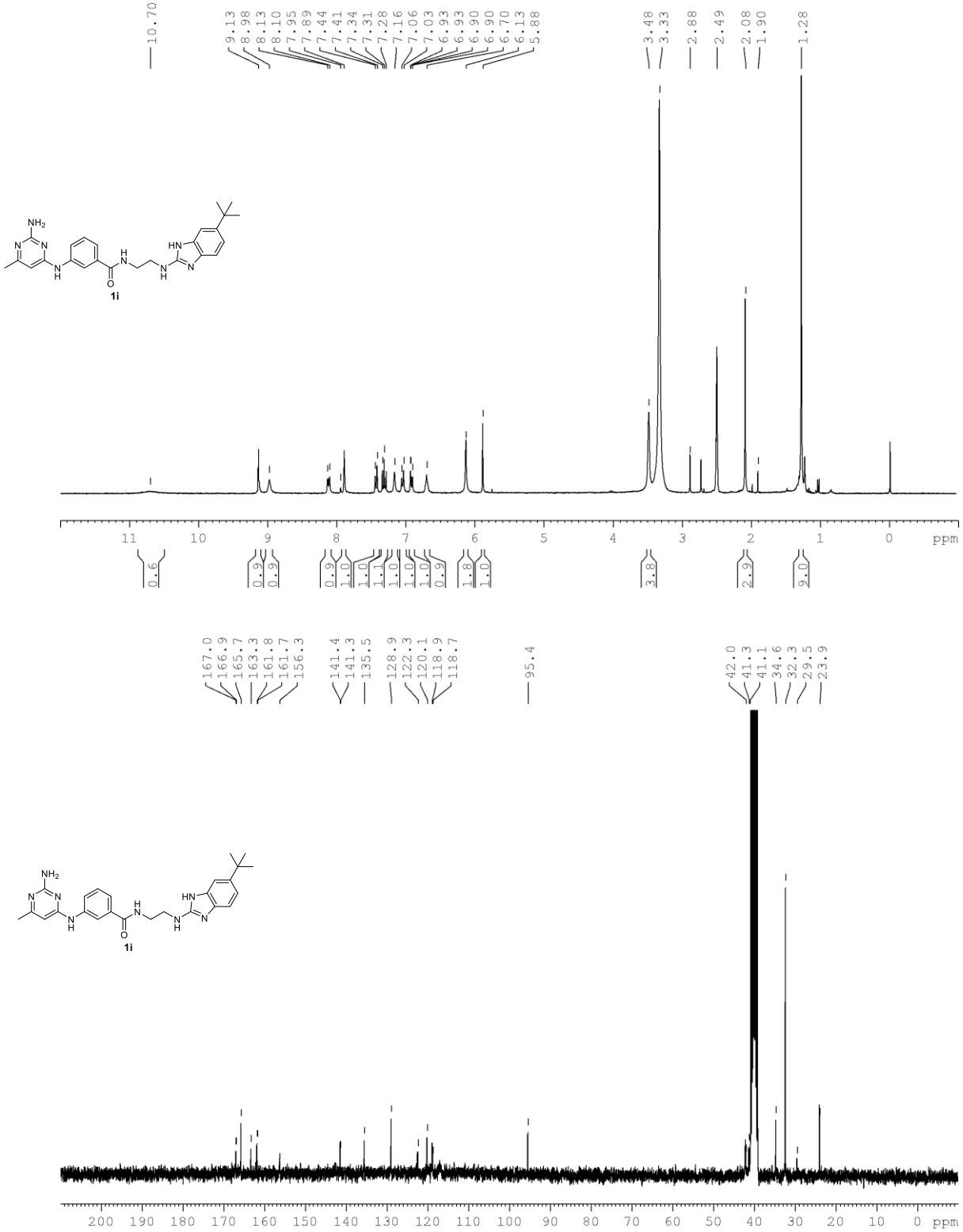
3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(4-(6-methoxy-1*H*-benzo[*d*]imidazol-2-yl)butyl)benzamide, **1g**



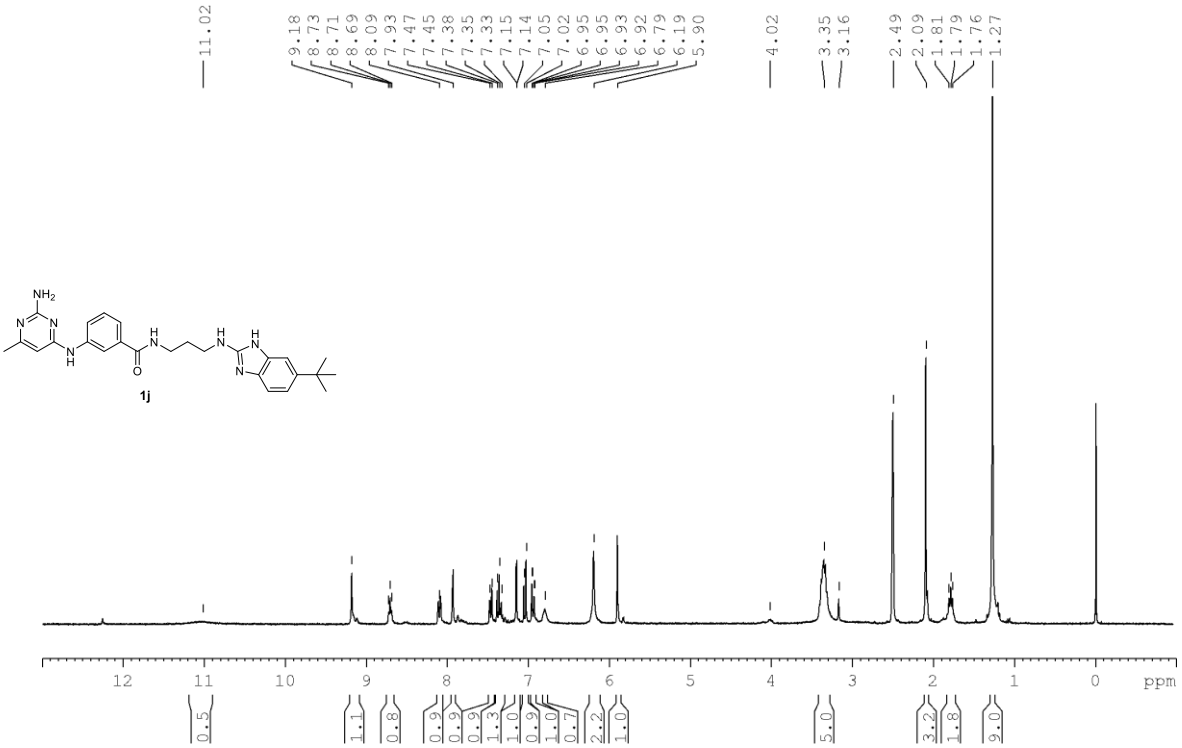
3-((2-amino-6-methylpyrimidin-4-yl)amino)-N-(4-(6-chloro-1*H*-benzo[d]imidazol-2-yl)butyl)benzamide, **1h**

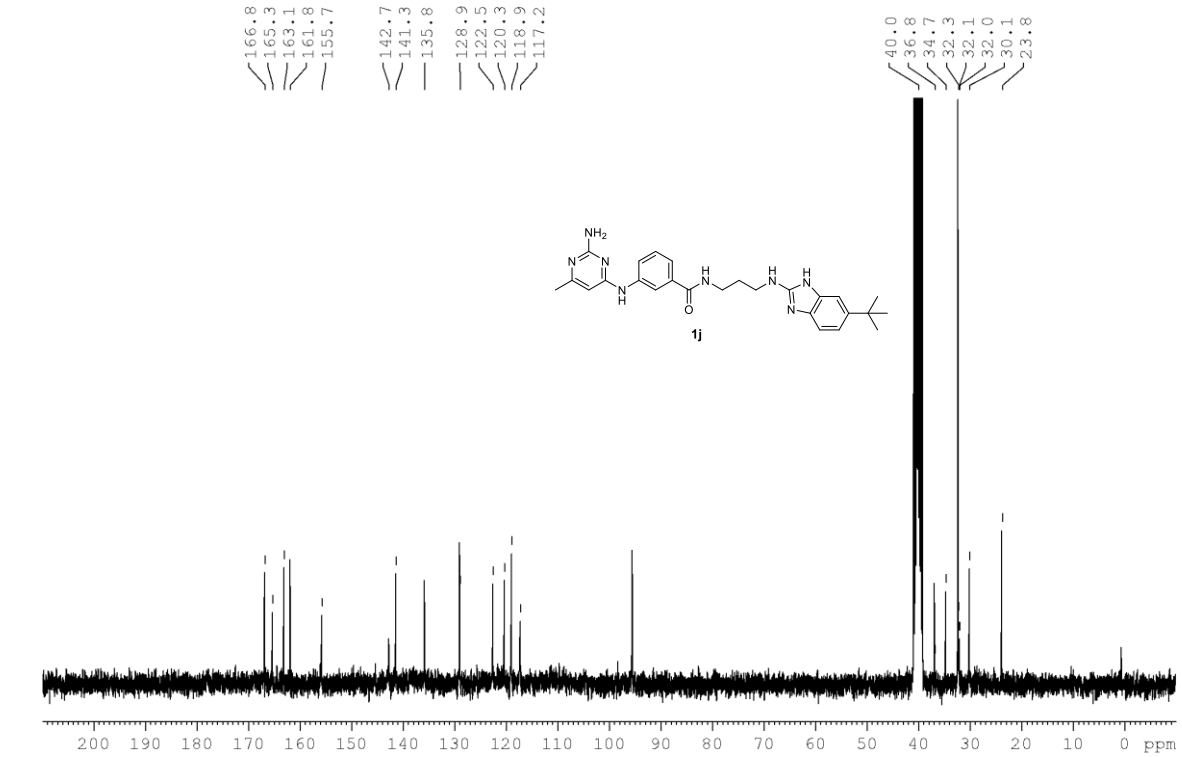


3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(2-((6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)amino)ethyl)benzamide, **1i**

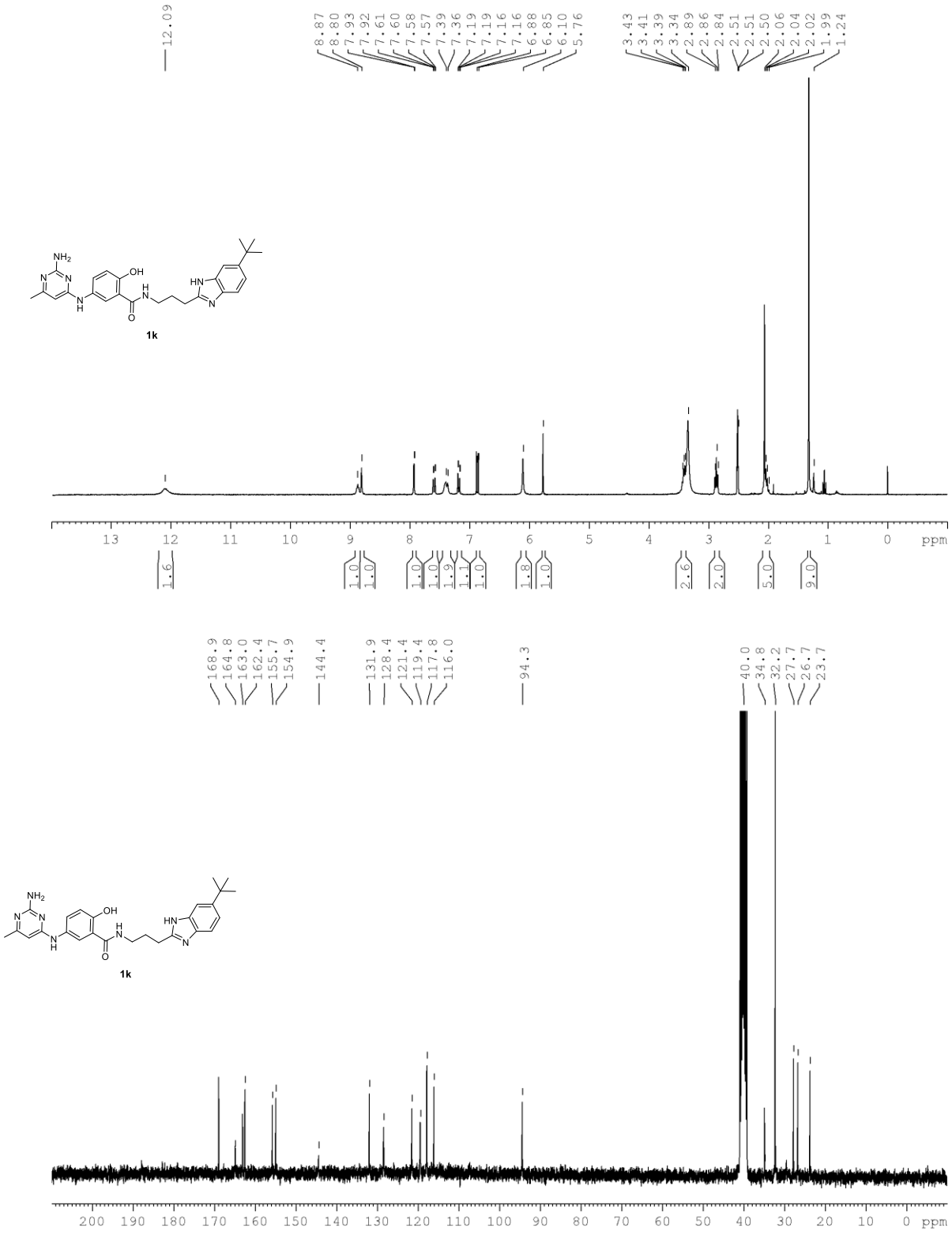


3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(3-((6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)amino)propyl)benzamide, **1j**

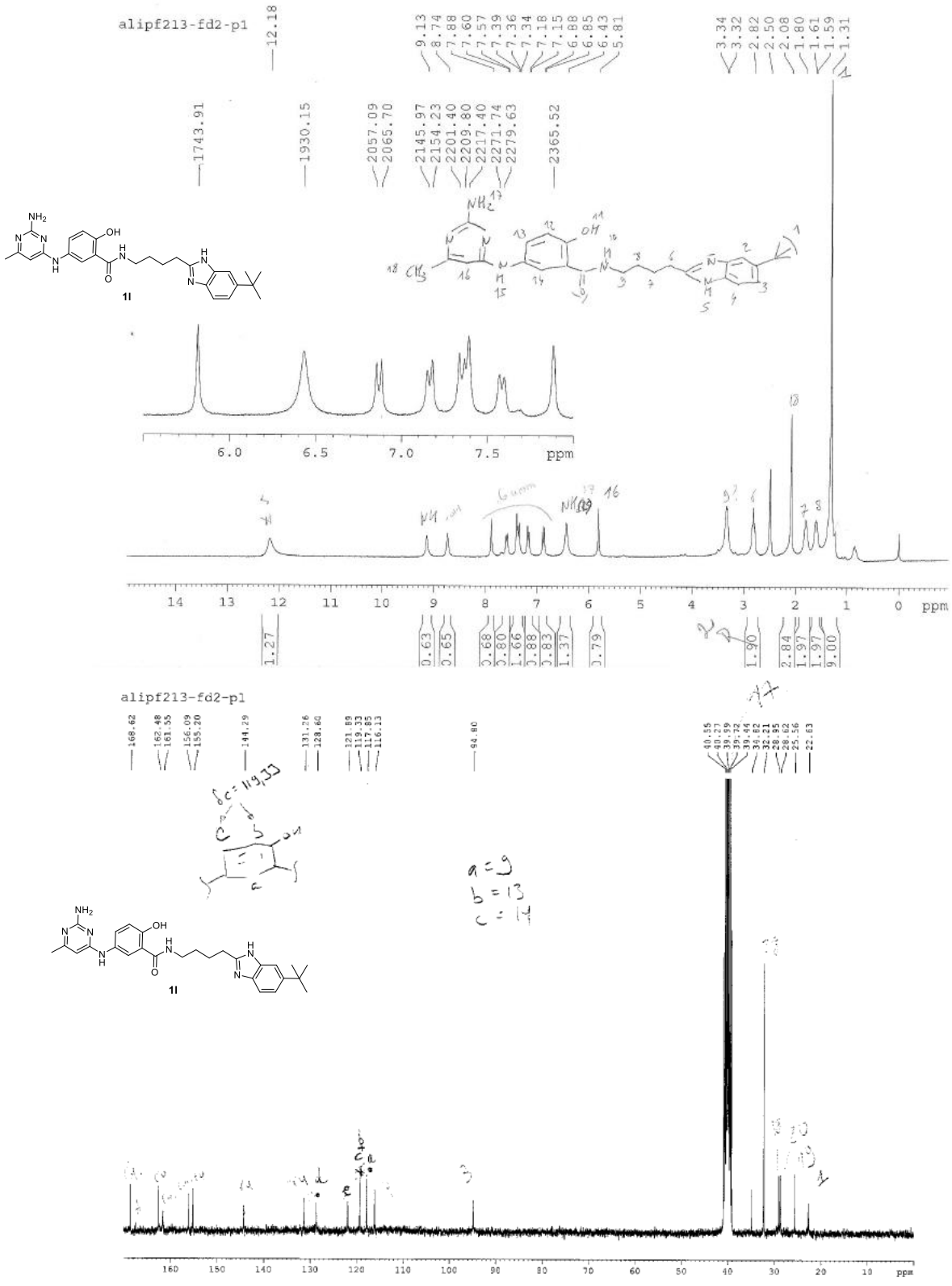




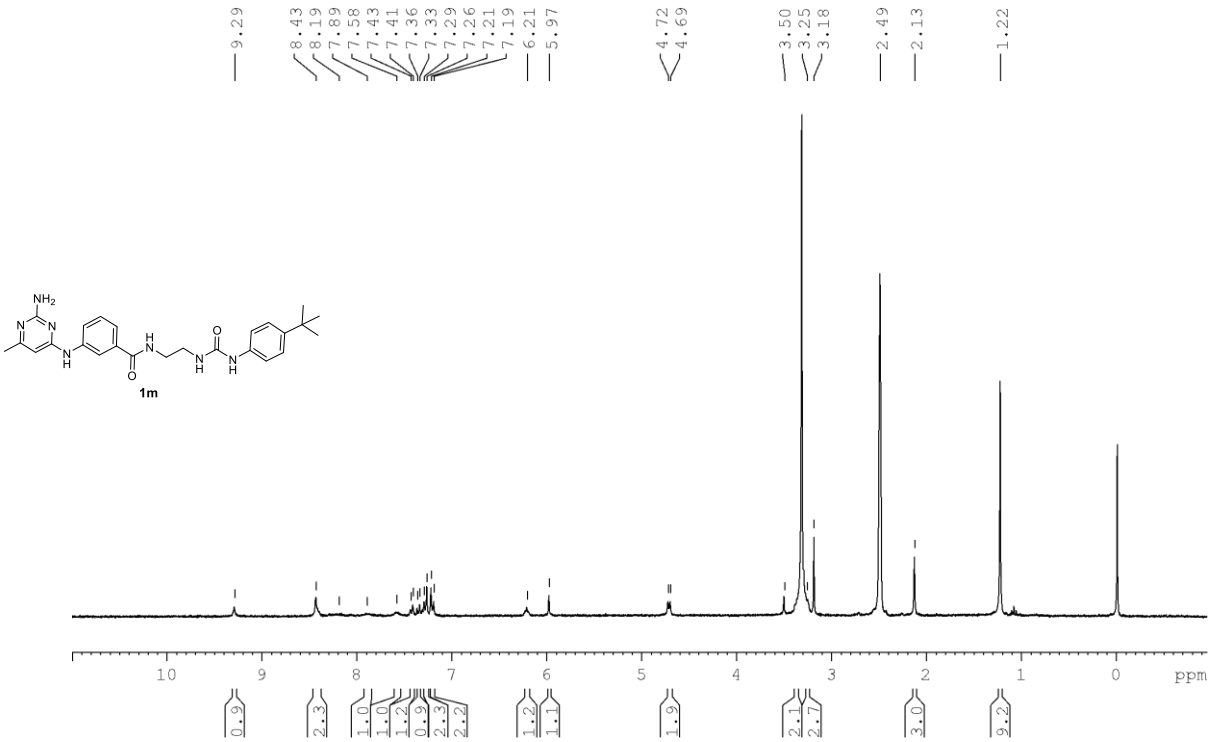
5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(3-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)propyl)-2-hydroxybenzamide, **1k**

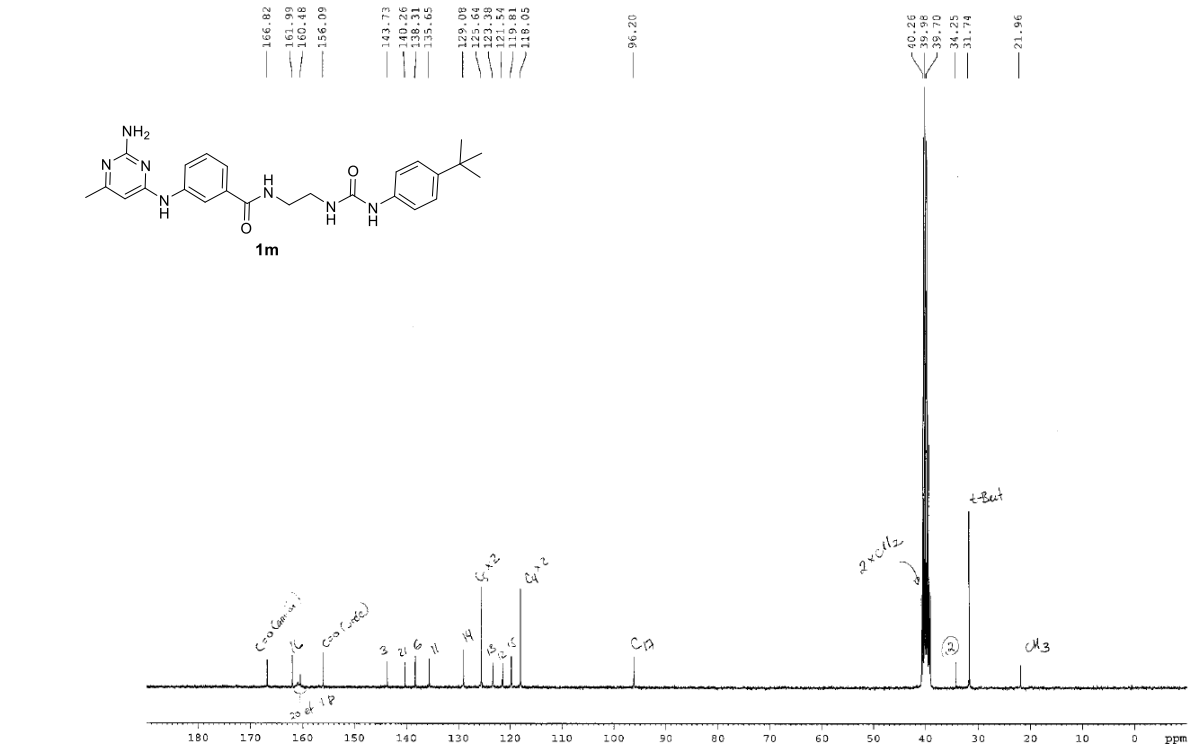


5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(4-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)butyl)-2-hydroxybenzamide, **1l**

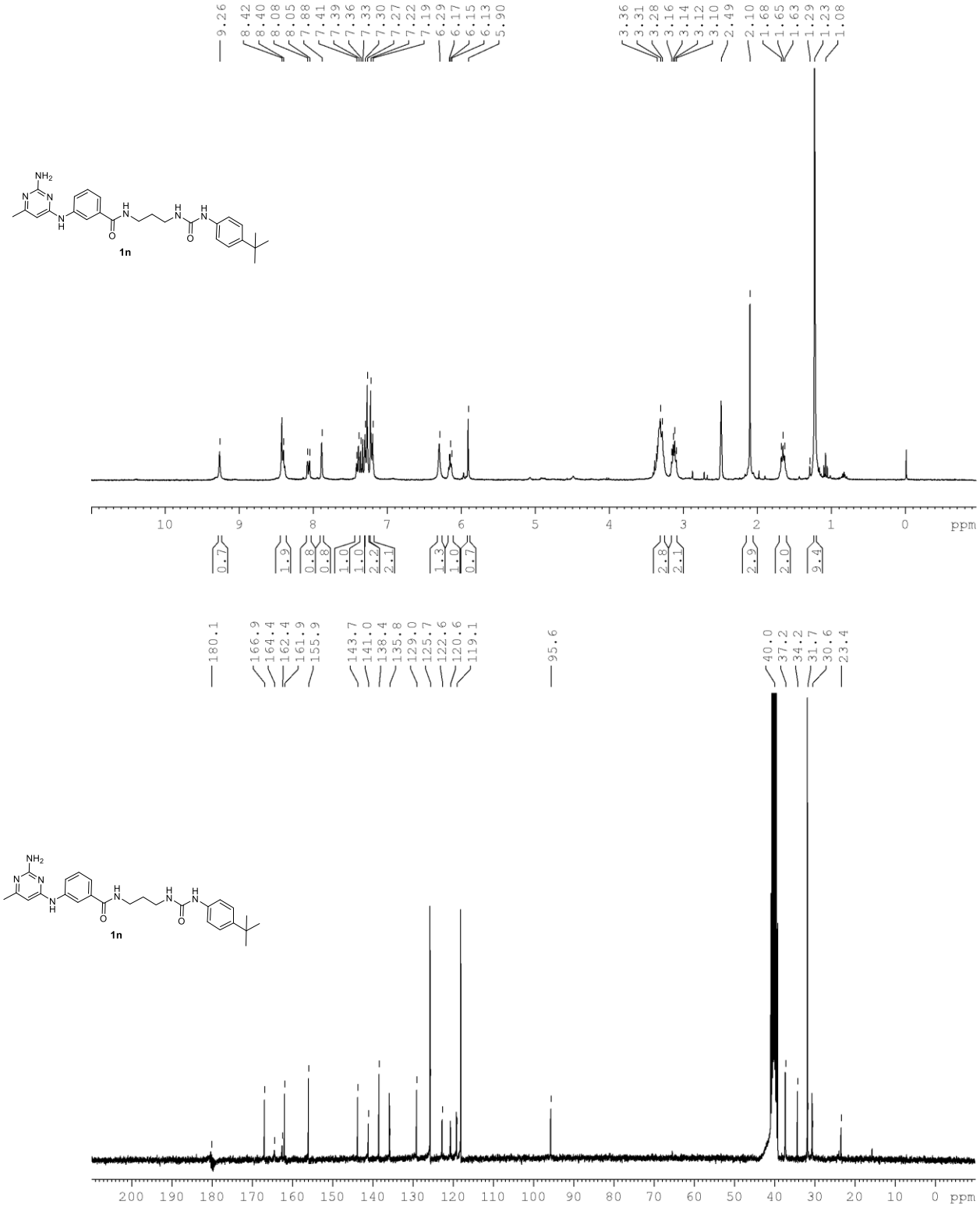


3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(2-(3-(4-(*tert*-butyl)phenyl)ureido)ethyl)benzamide, **1m**

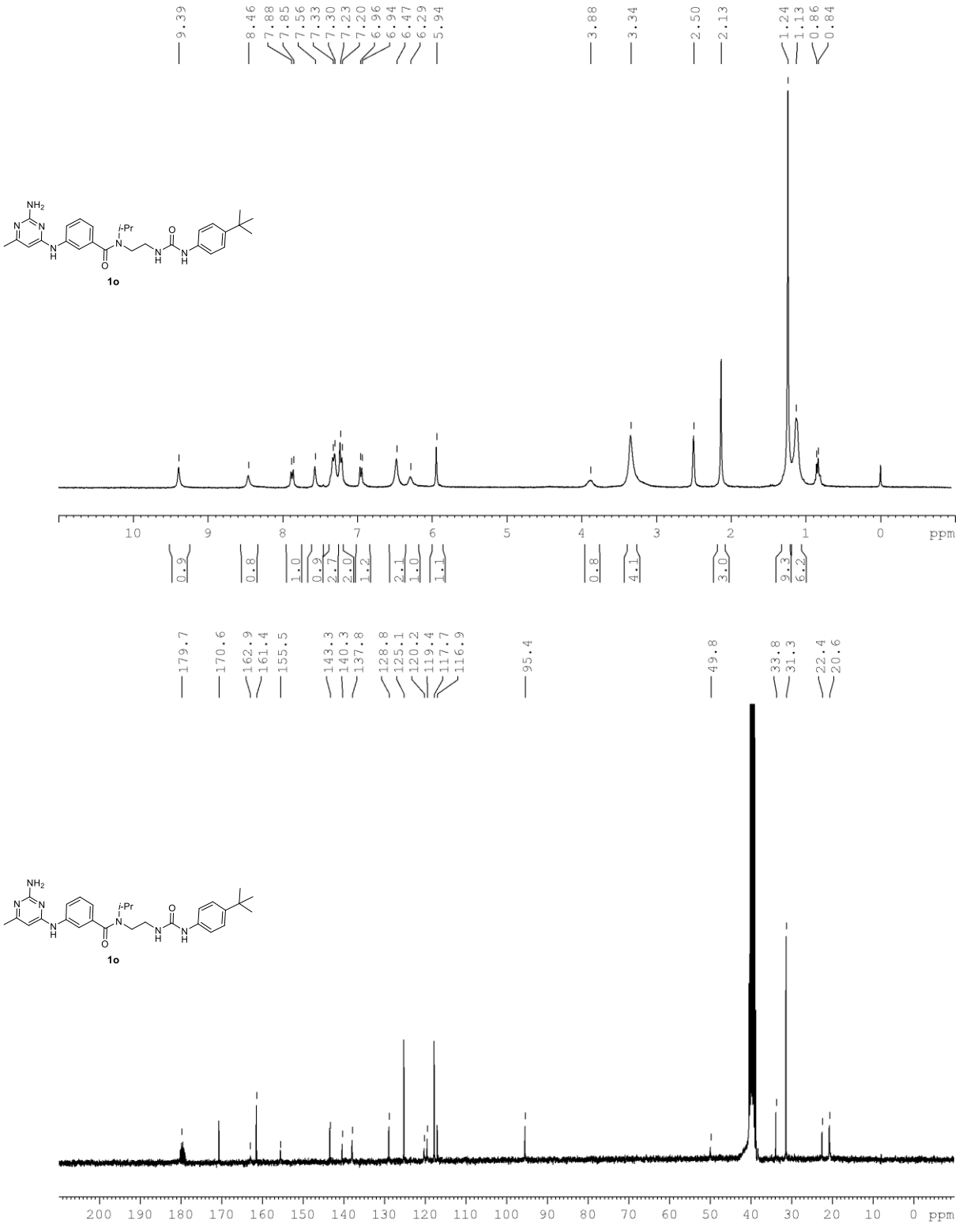




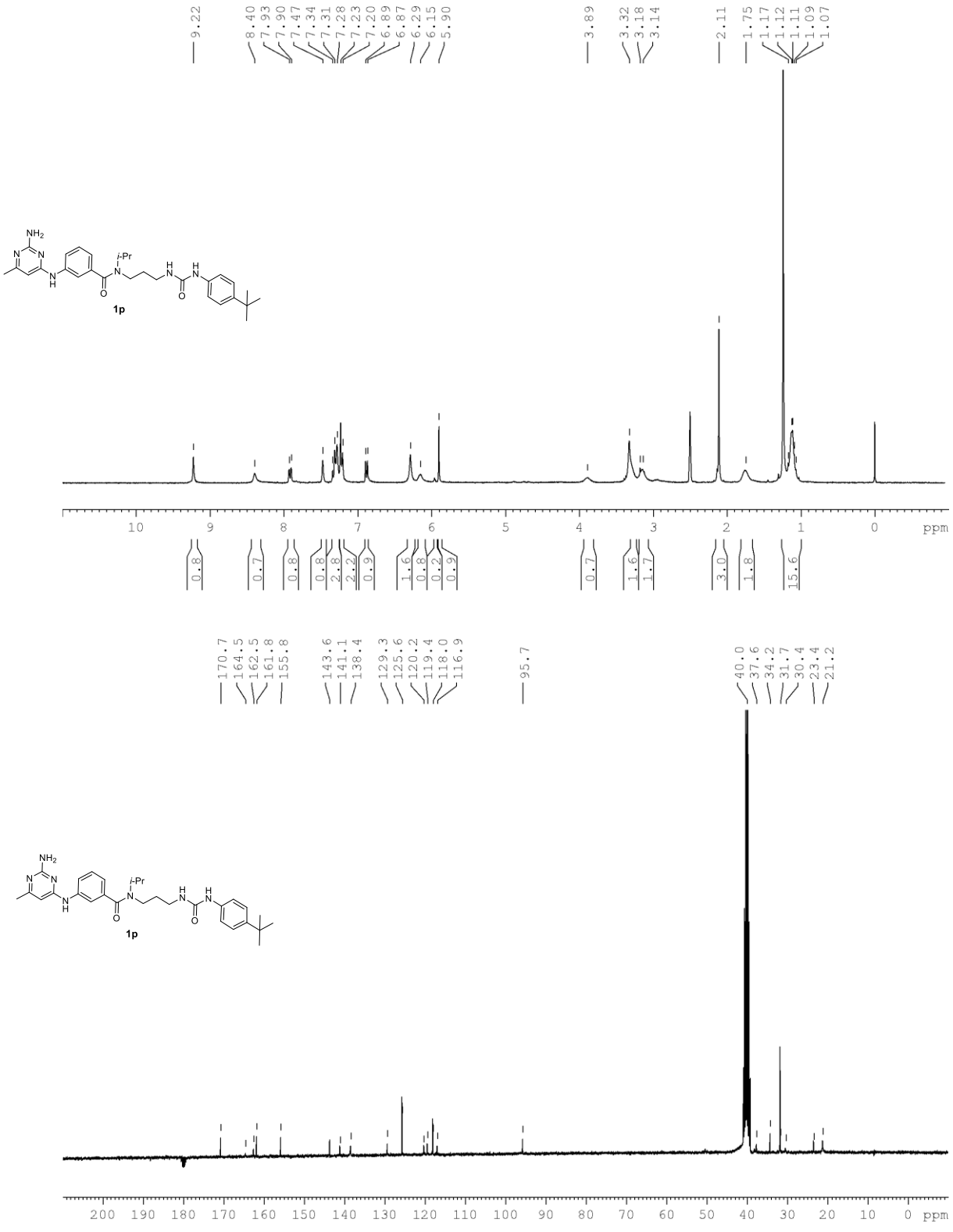
3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)benzamide, **1n**



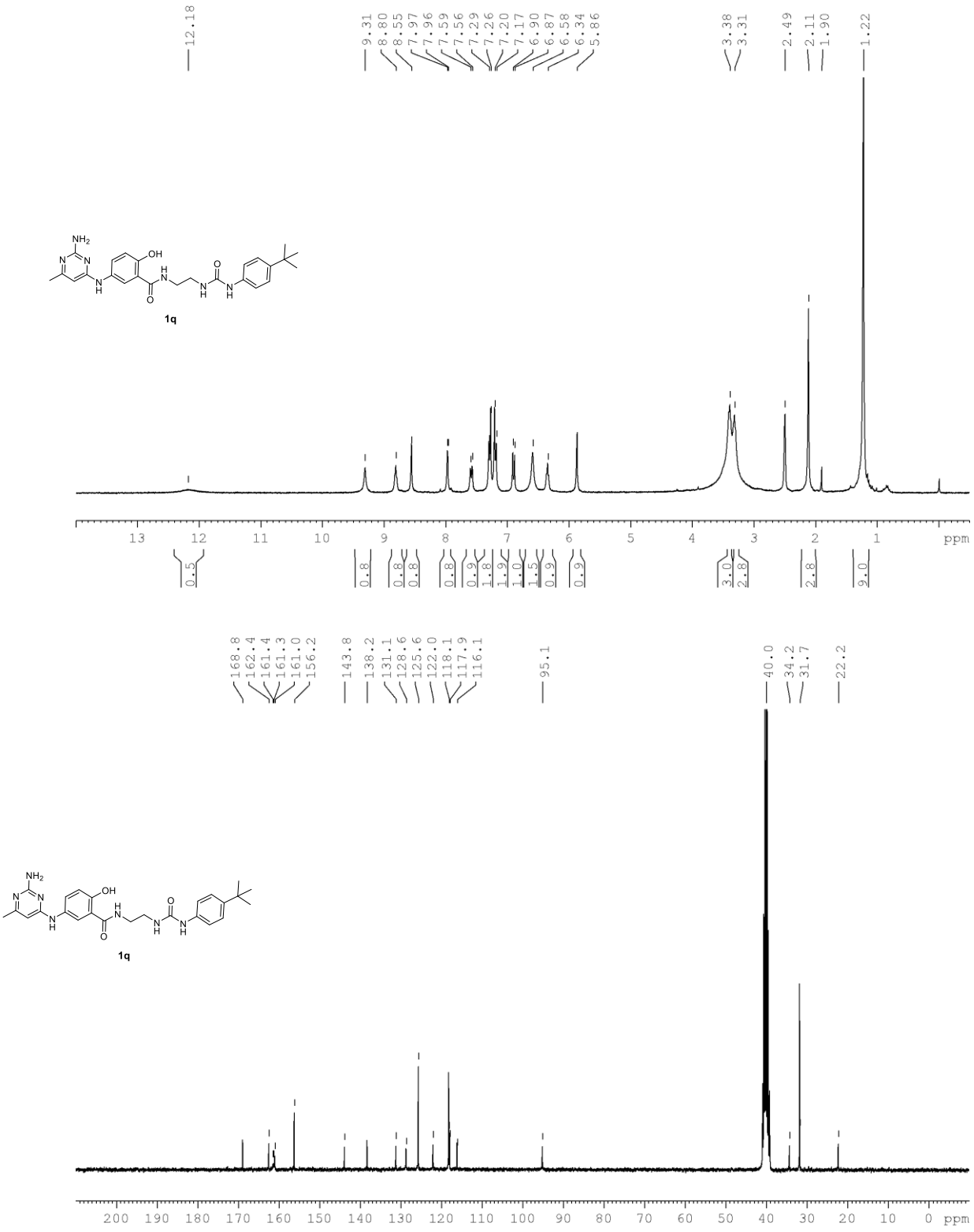
3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(2-(3-(4-(*tert*-butyl)phenyl)ureido)ethyl)-*N*-isopropylbenzamide, **1o**



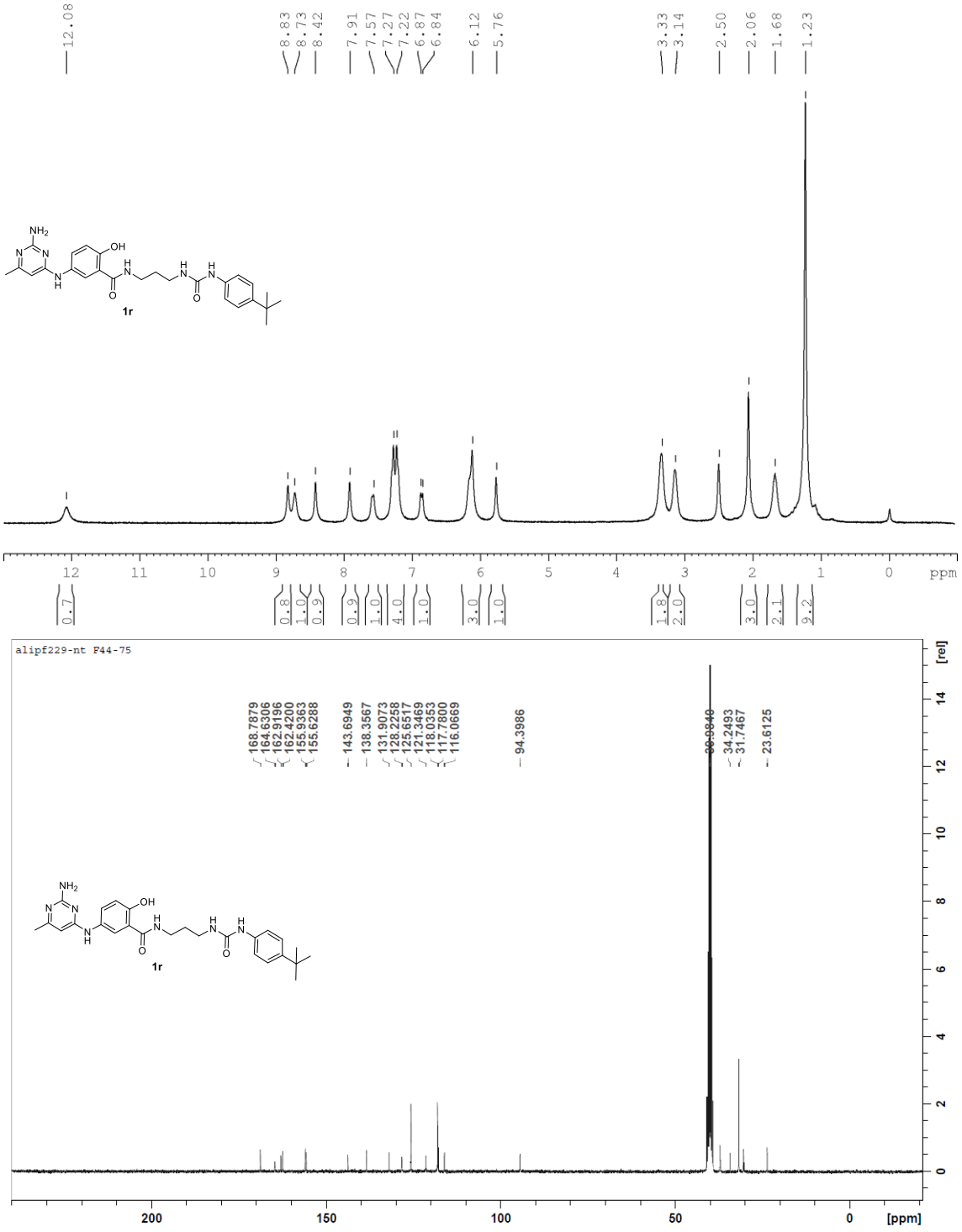
3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)-*N*-isopropylbenzamide, **1p**



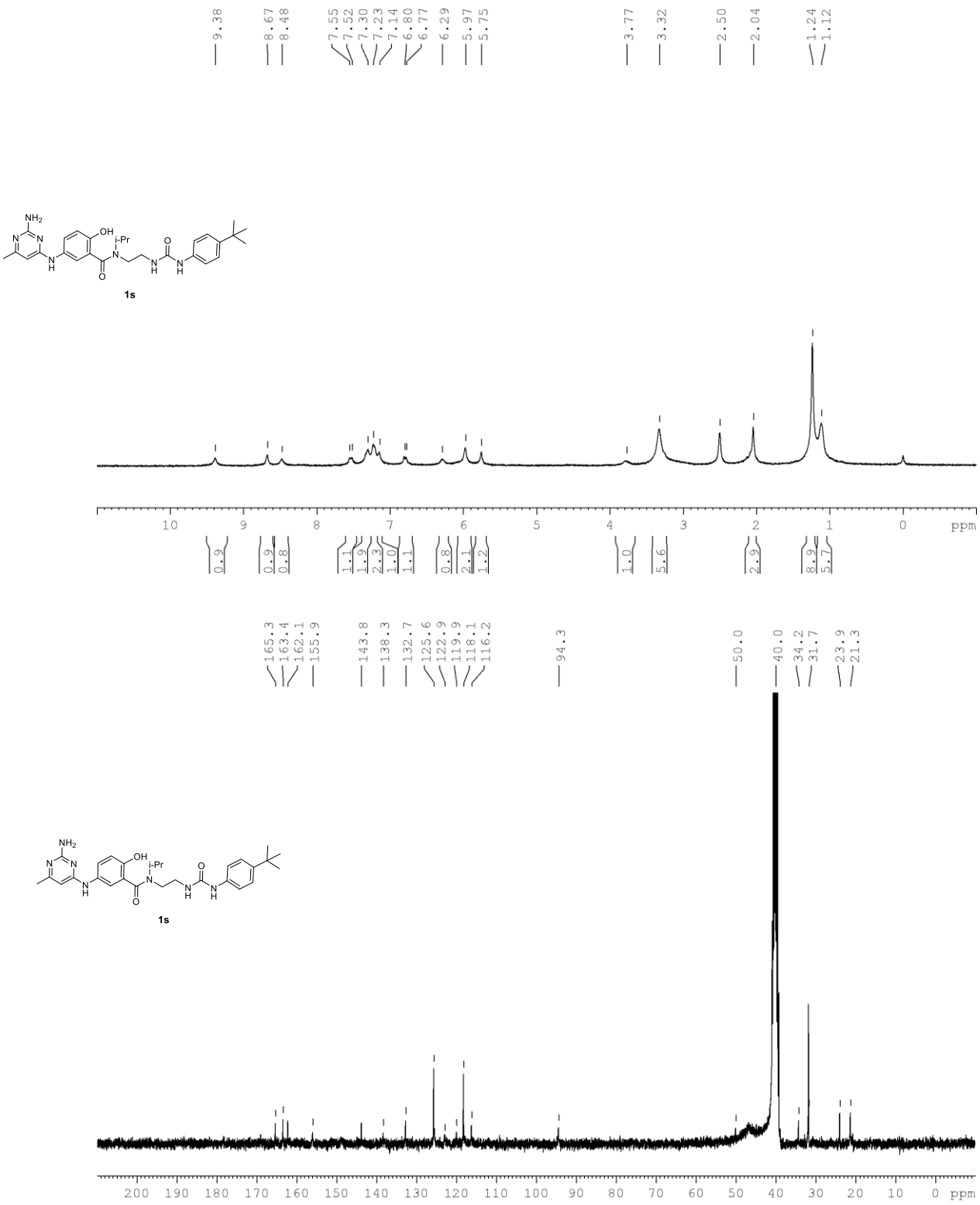
5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(2-(3-(4-(*tert*-butyl)phenyl)ureido)ethyl)-2-hydroxybenzamide, **1q**



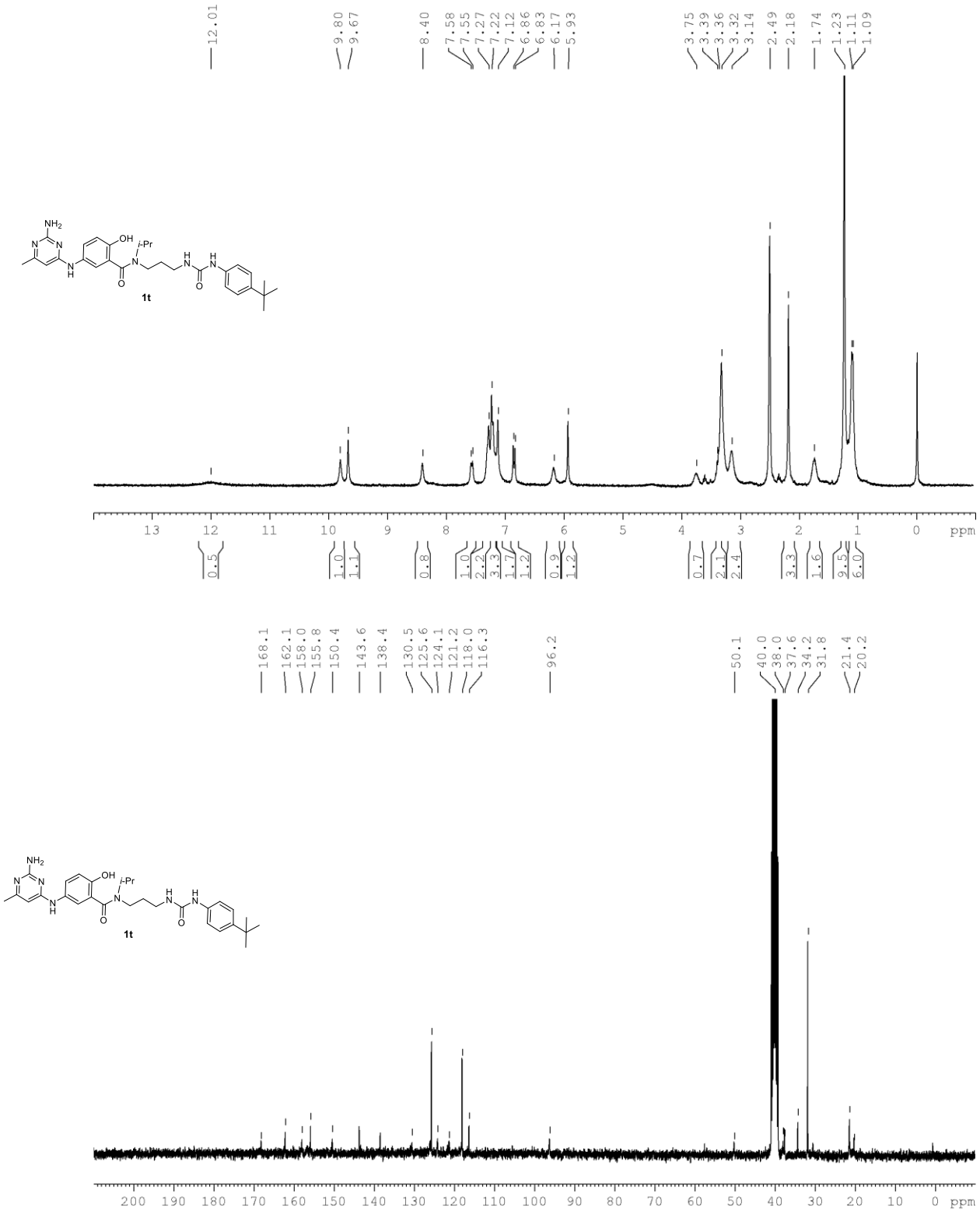
5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)-2-hydroxybenzamide, **1r**



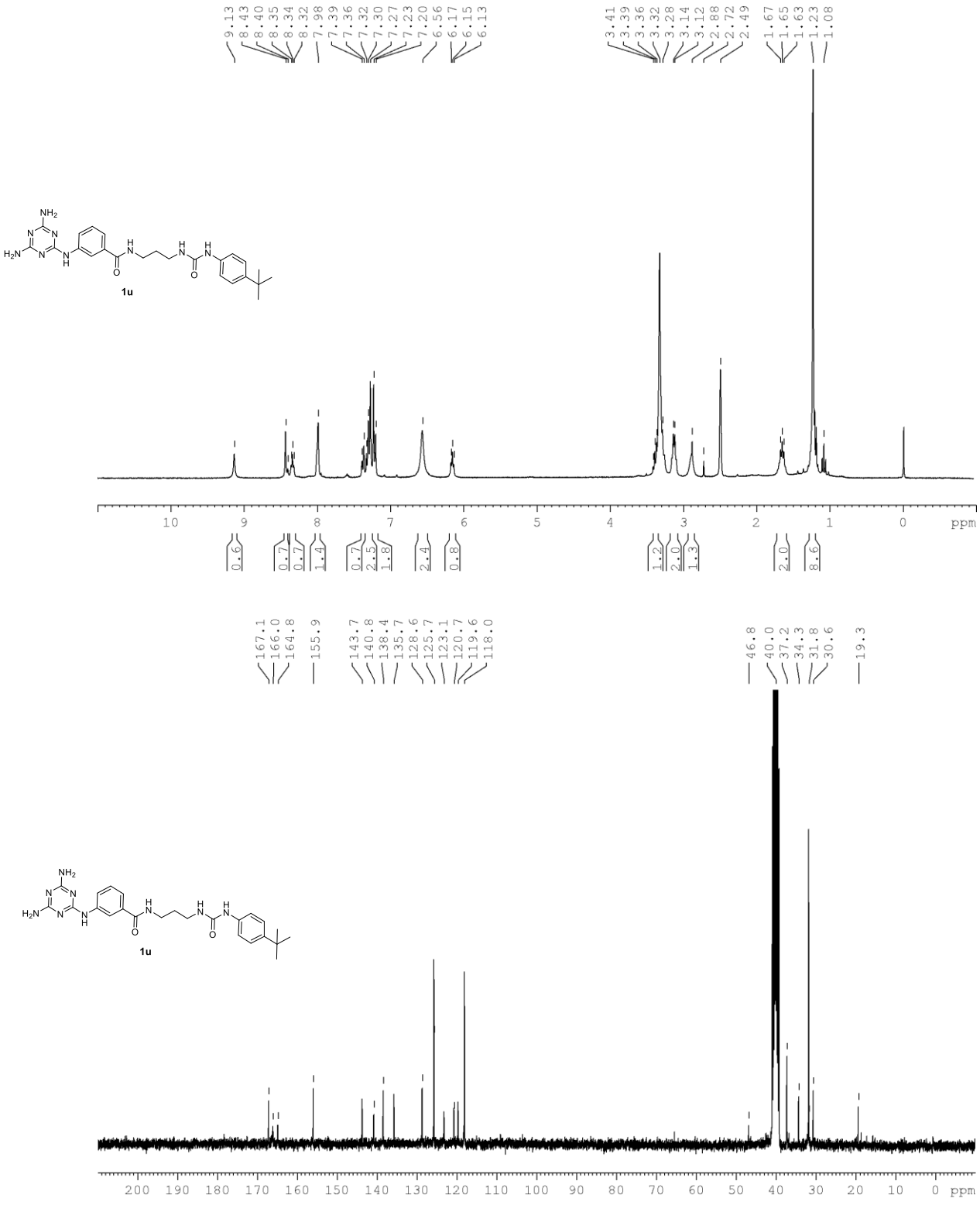
5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(2-(3-(4-(*tert*-butyl)phenyl)ureido)ethyl)-2-hydroxy-*N*-isopropylbenzamide, **1s**



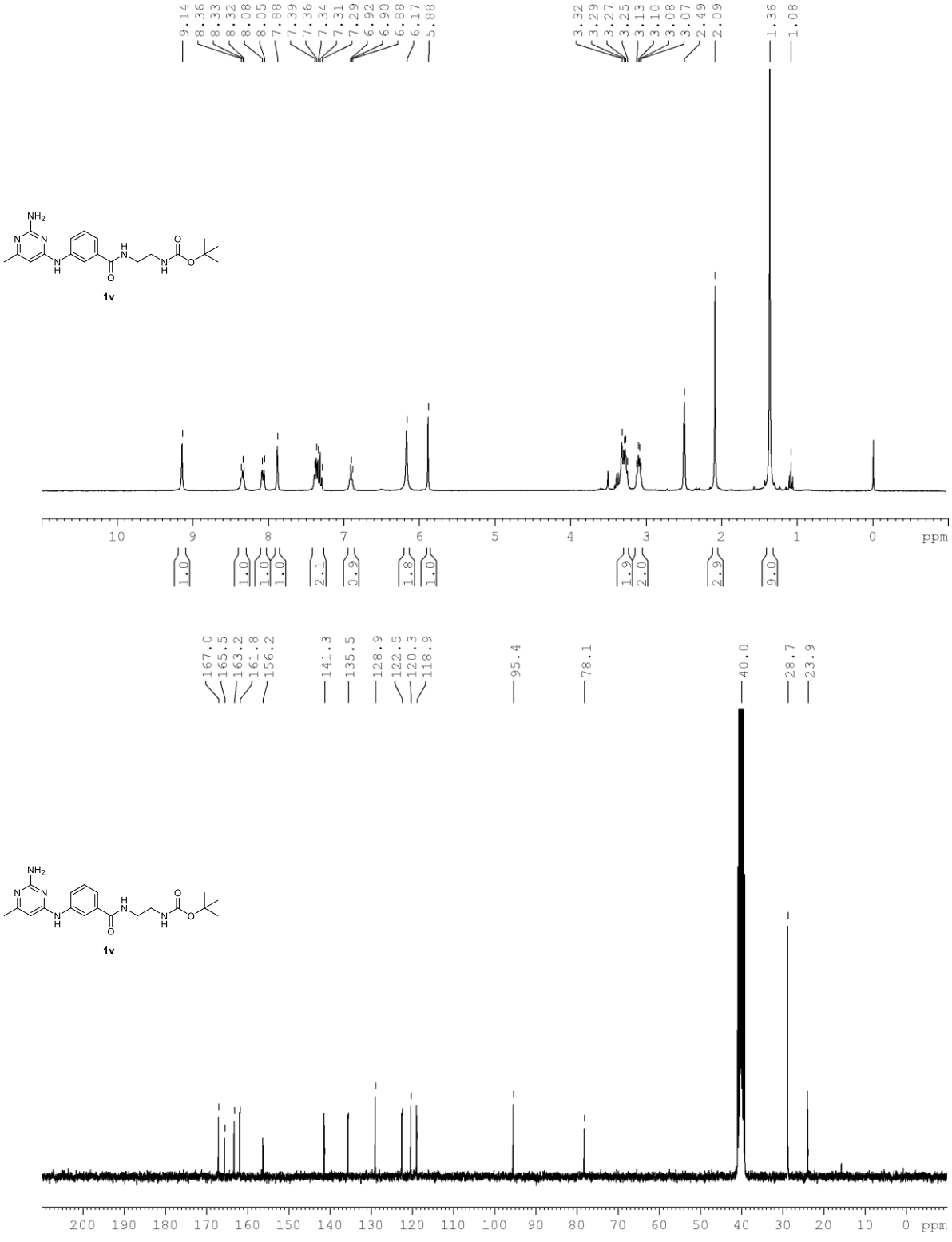
5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)-2-hydroxy-*N*-isopropylbenzamide, **1t**



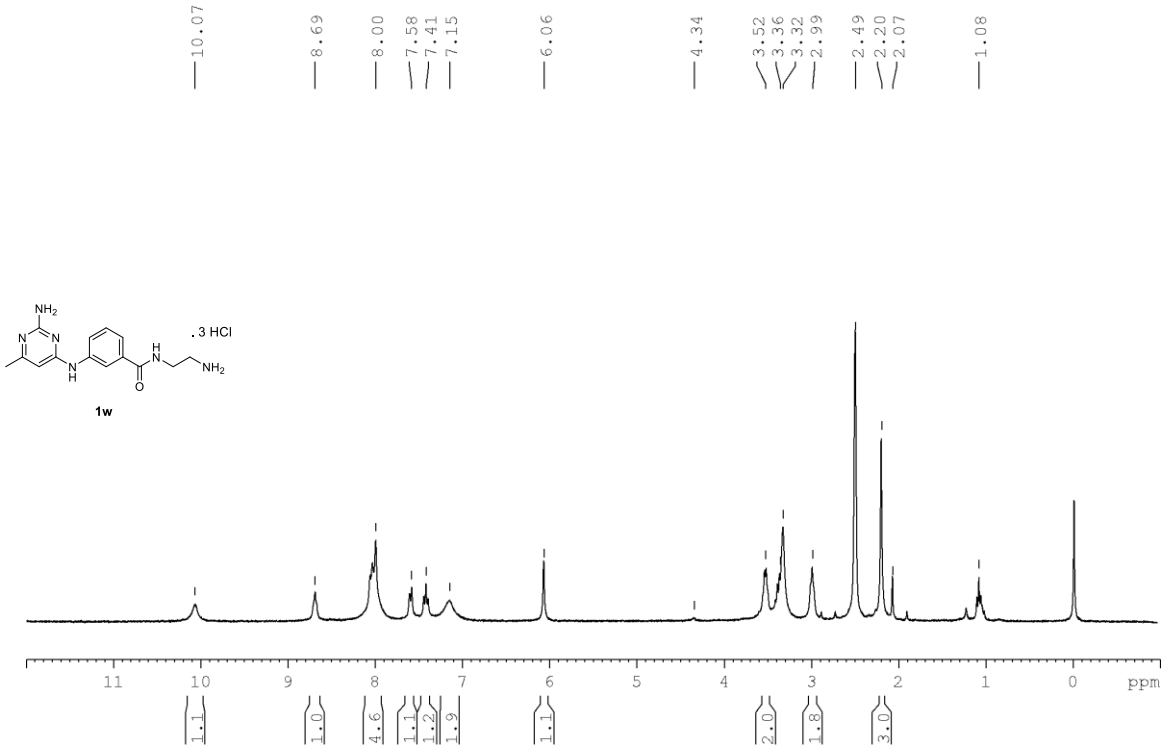
N-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)-3-((4,6-diamino-1,3,5-triazin-2-yl)amino)benzamide, **1u**

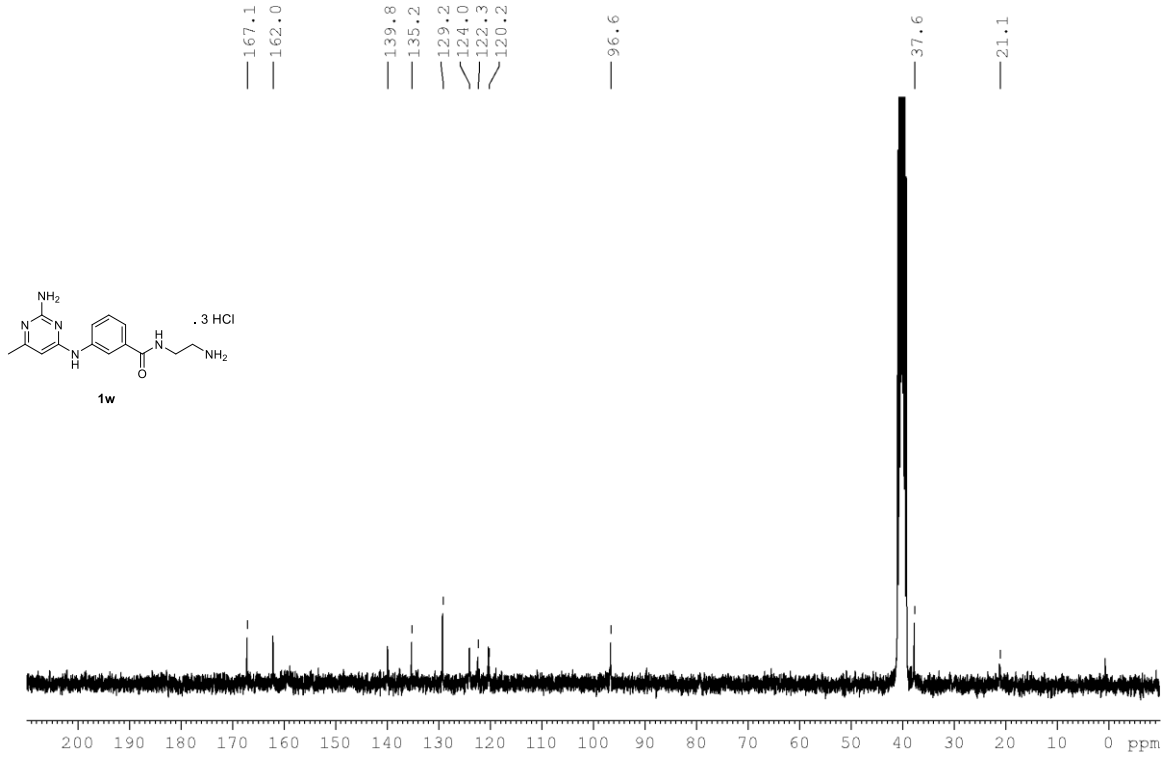


Tert-butyl (2-(3-((2-amino-6-methylpyrimidin-4-yl)amino)benzamido)ethyl)carbamate, **1v**

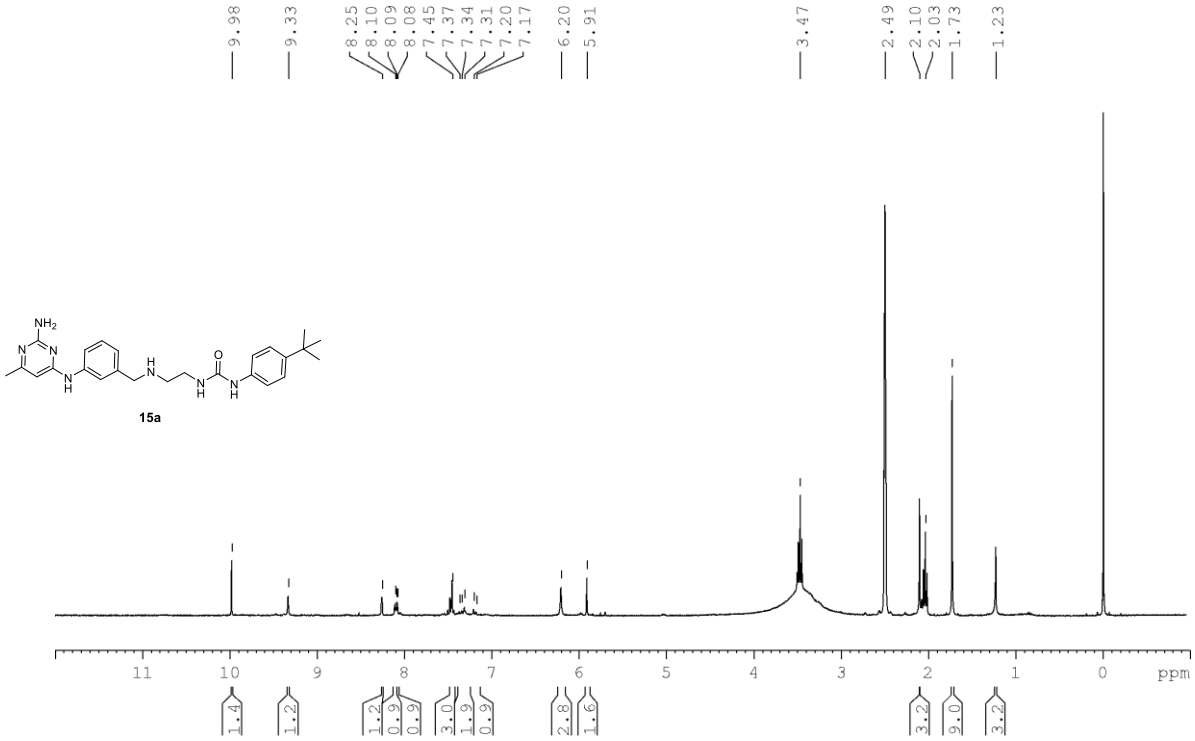


3-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(2-aminoethyl)benzamide trihydrochloride, **1w**

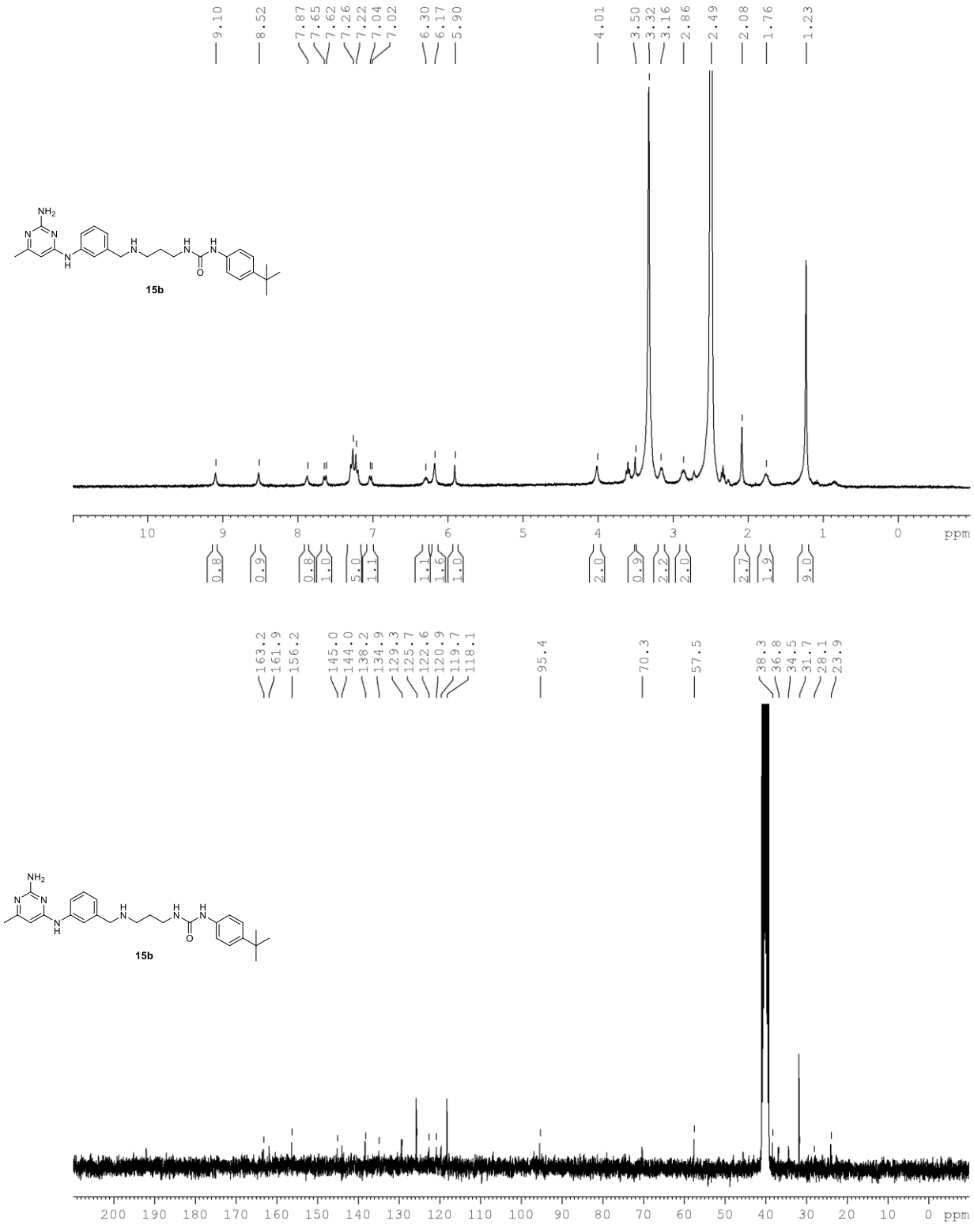




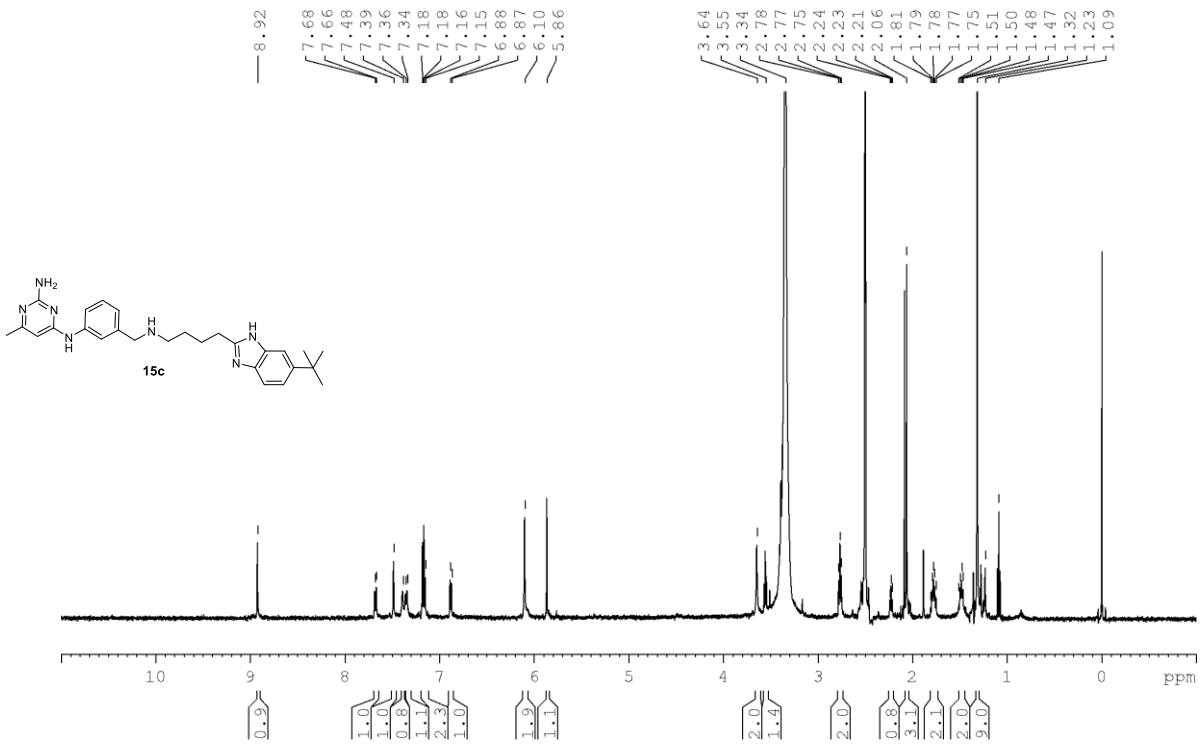
1-(2-((3-((2-amino-6-methylpyrimidin-4-yl)amino)benzyl)amino)ethyl)-3-(4-(*tert*-butyl)phenyl)urea, **15a**



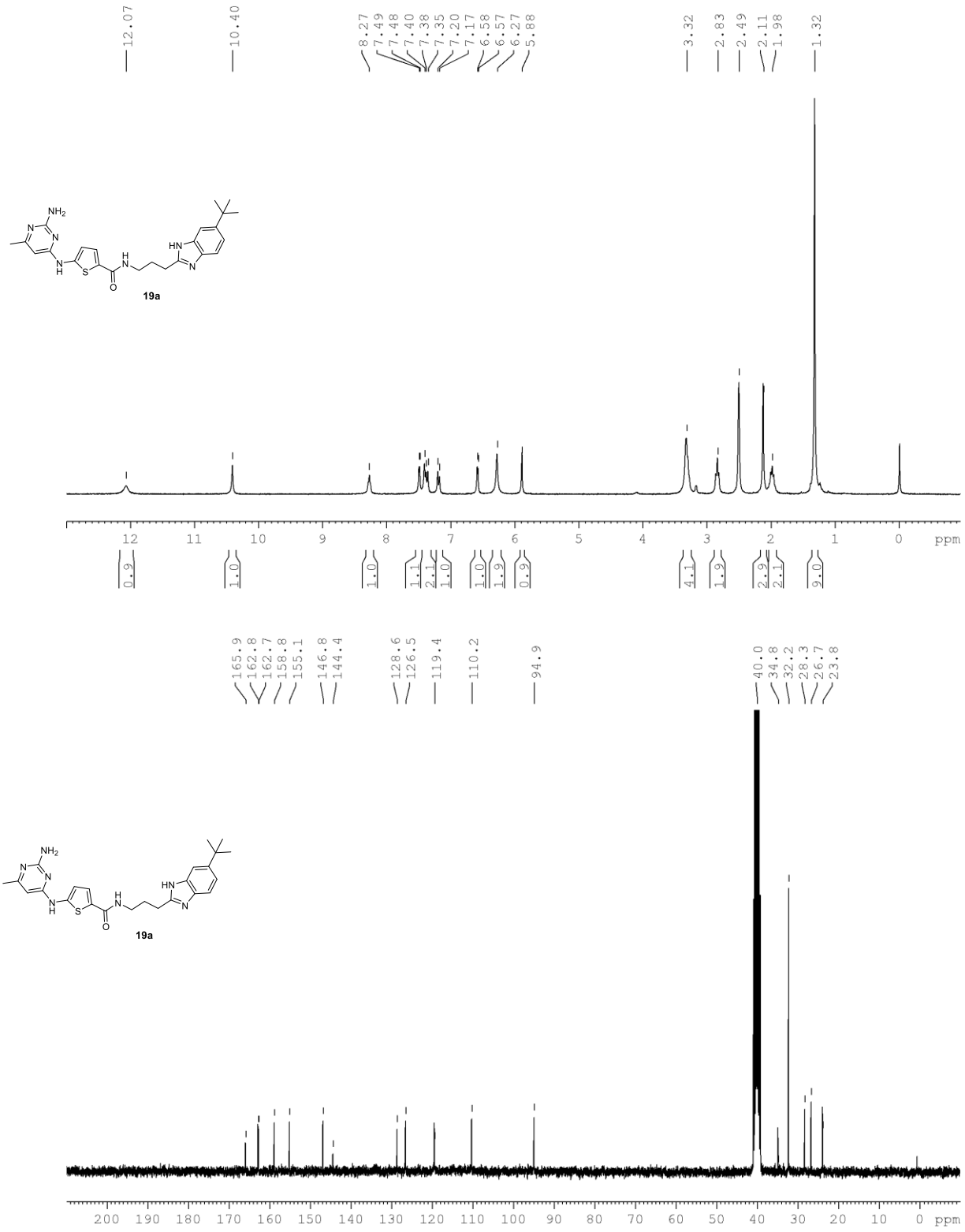
1-(3-((3-((2-amino-6-methylpyrimidin-4-yl)amino)benzyl)amino)propyl)-3-(4-(*tert*-butyl)phenyl)urea, **15b**



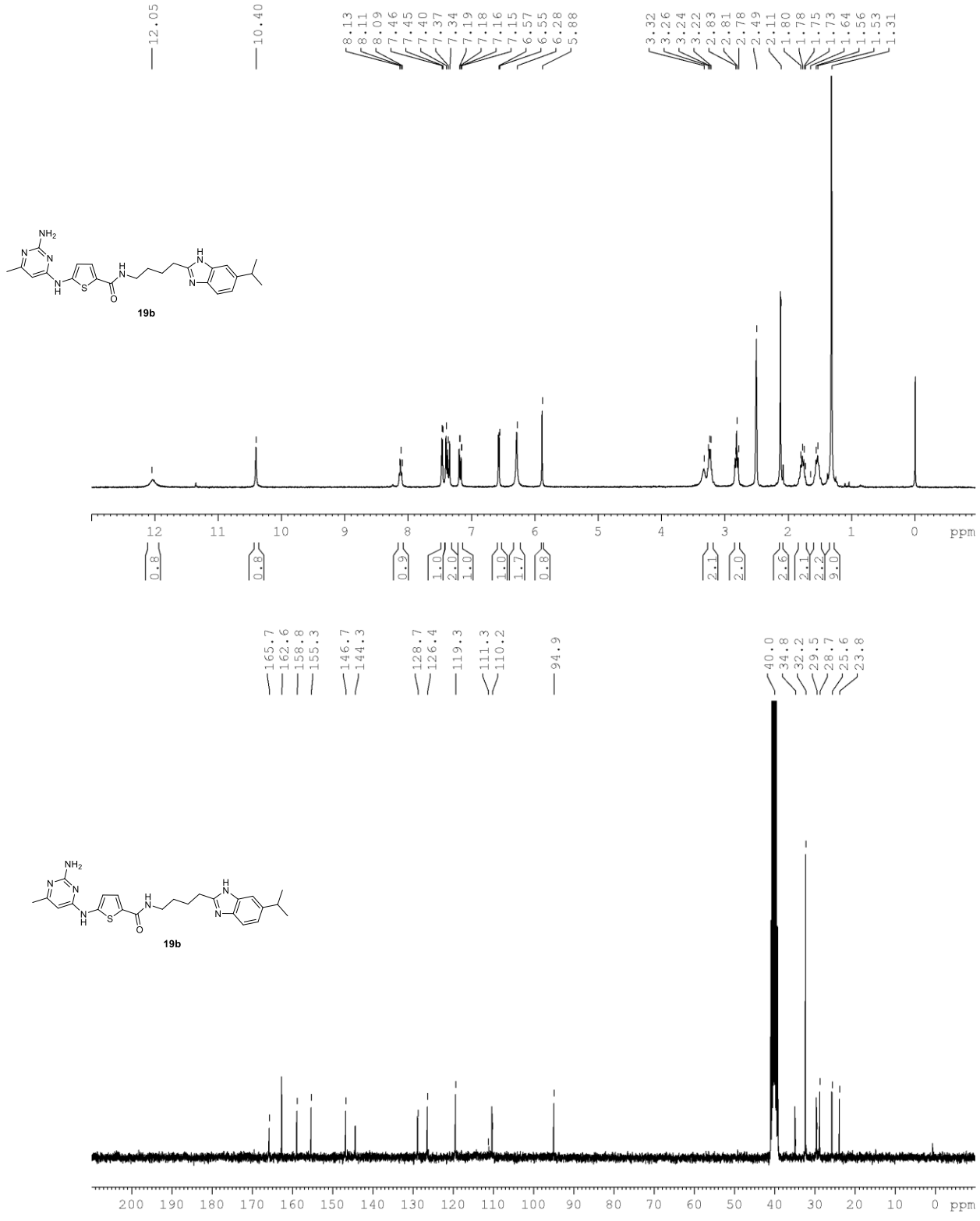
N^4-(3-(((4-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)butyl)amino)methyl)phenyl)-6-methylpyrimidine-2,4-diamine, **15c**



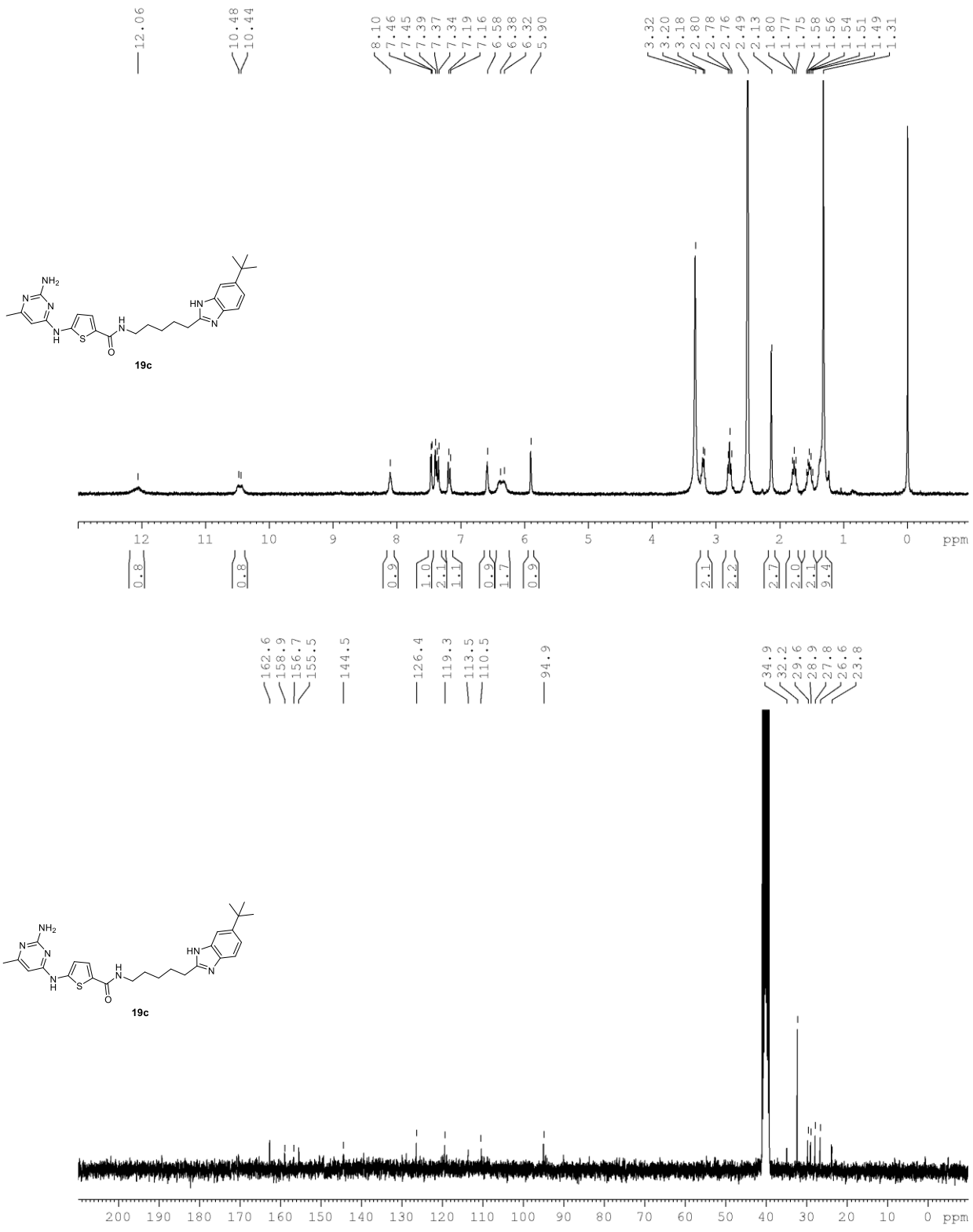
5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(3-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)propyl)thiophene-2-carboxamide, **19a**



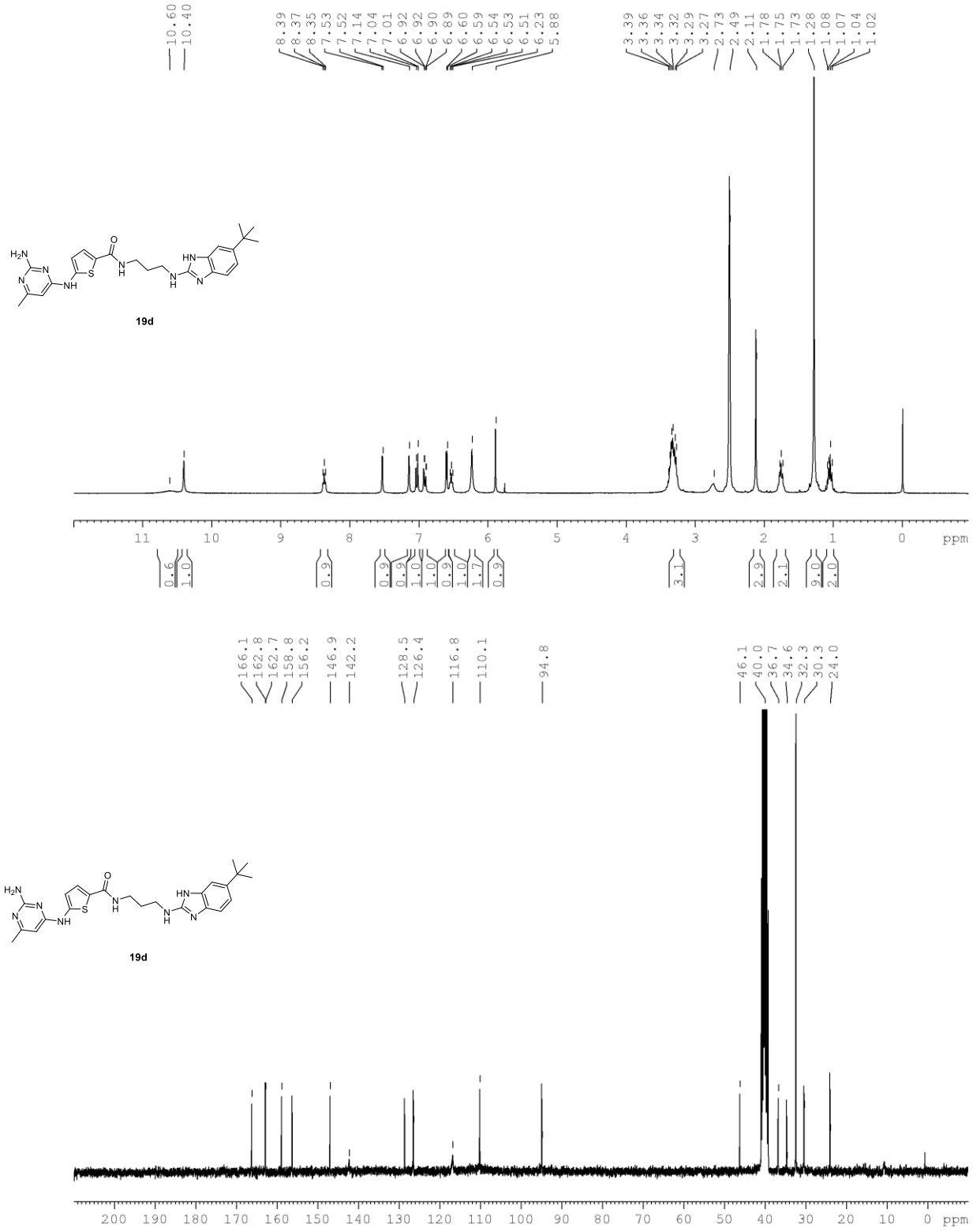
5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(4-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)butyl)thiophene-2-carboxamide, **19b**



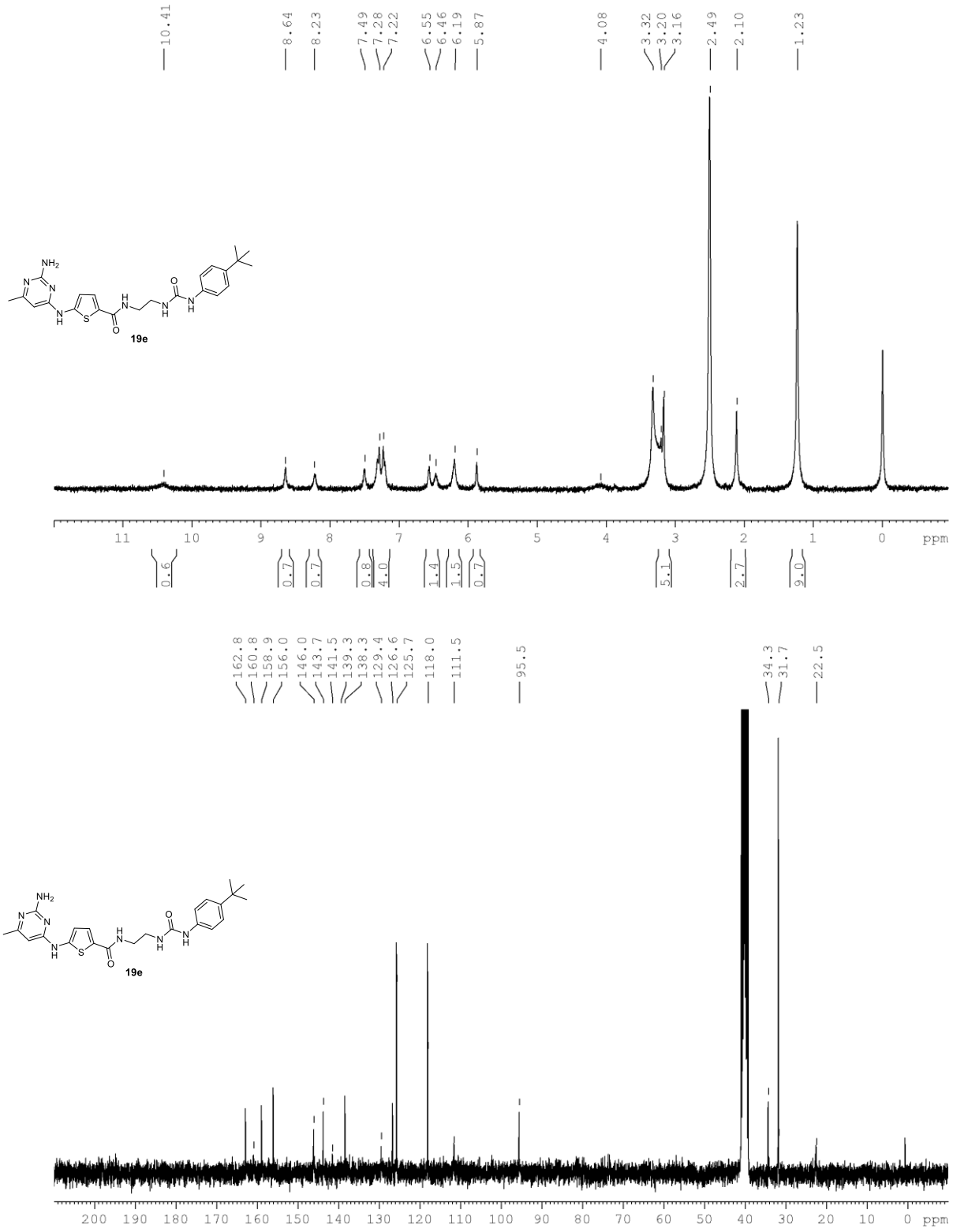
5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(5-(6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)pentyl)thiophene-2-carboxamide, **19c**



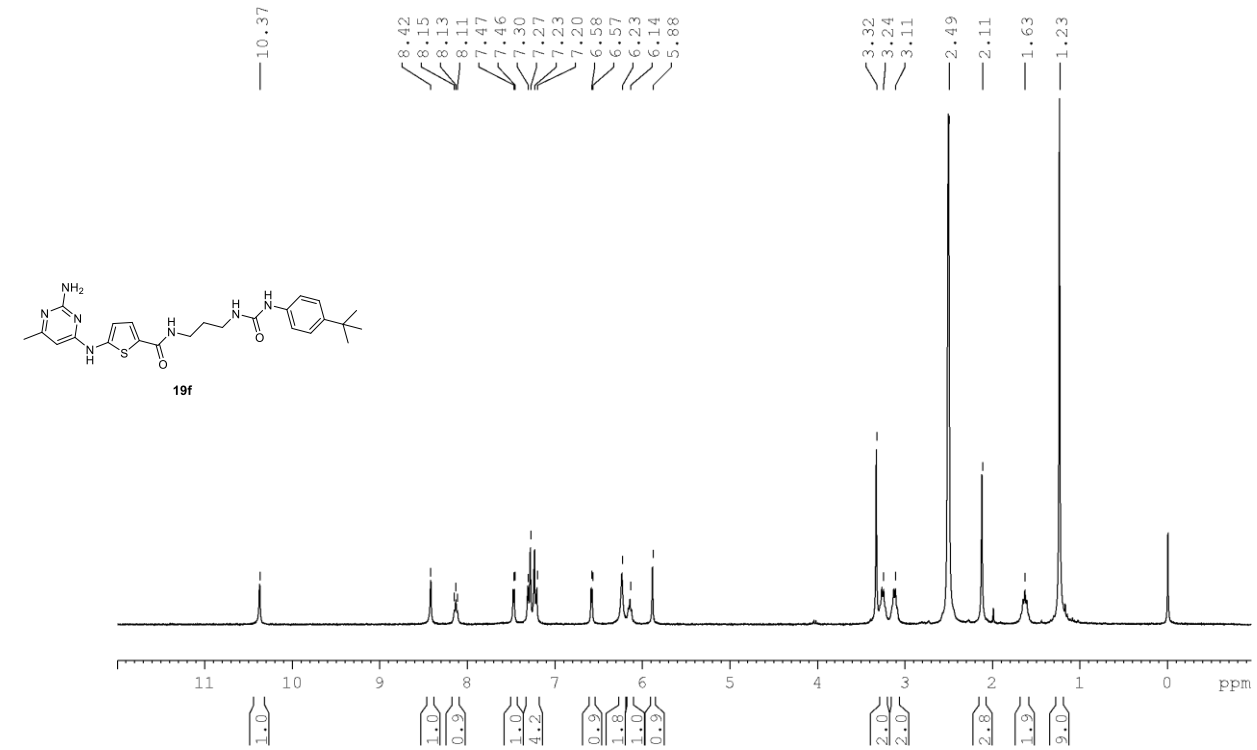
5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(3-((6-(*tert*-butyl)-1*H*-benzo[*d*]imidazol-2-yl)amino)propyl)thiophene-2-carboxamide, **19d**

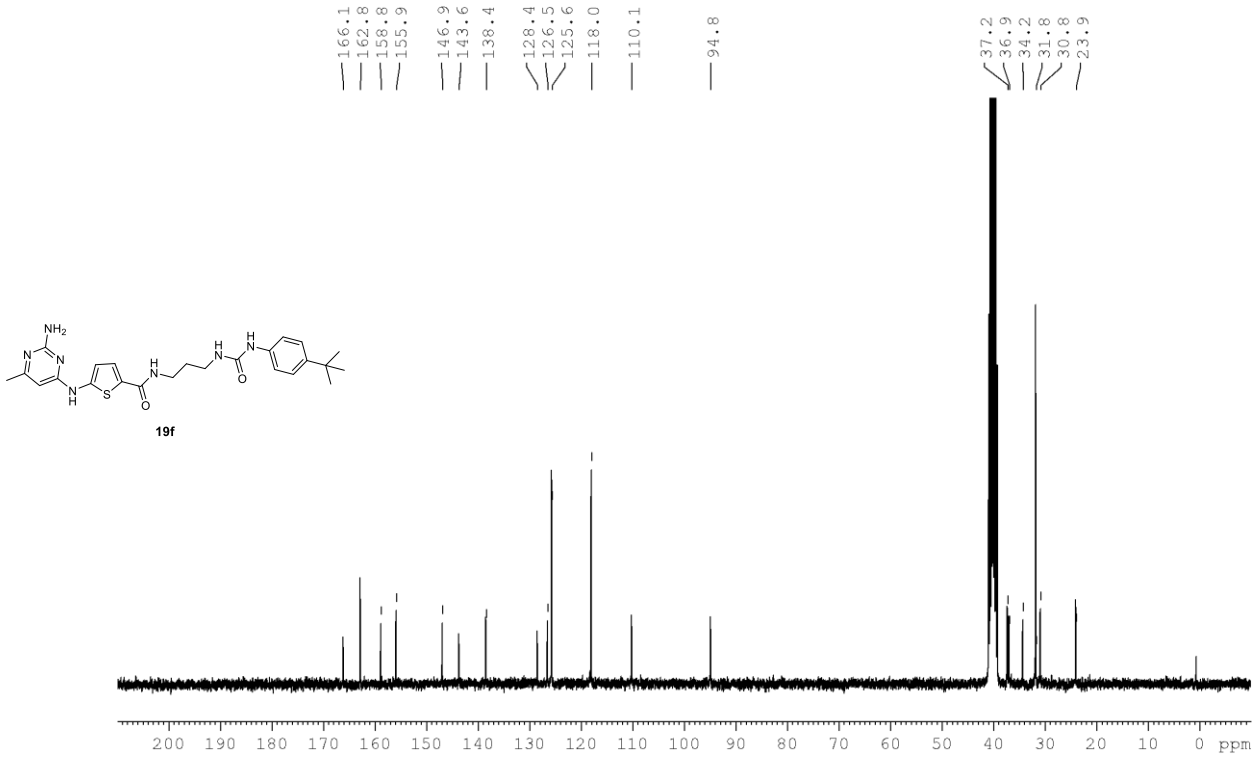


5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(2-(3-(4-(*tert*-butyl)phenyl)ureido)ethyl)thiophene-2-carboxamide, **19e**

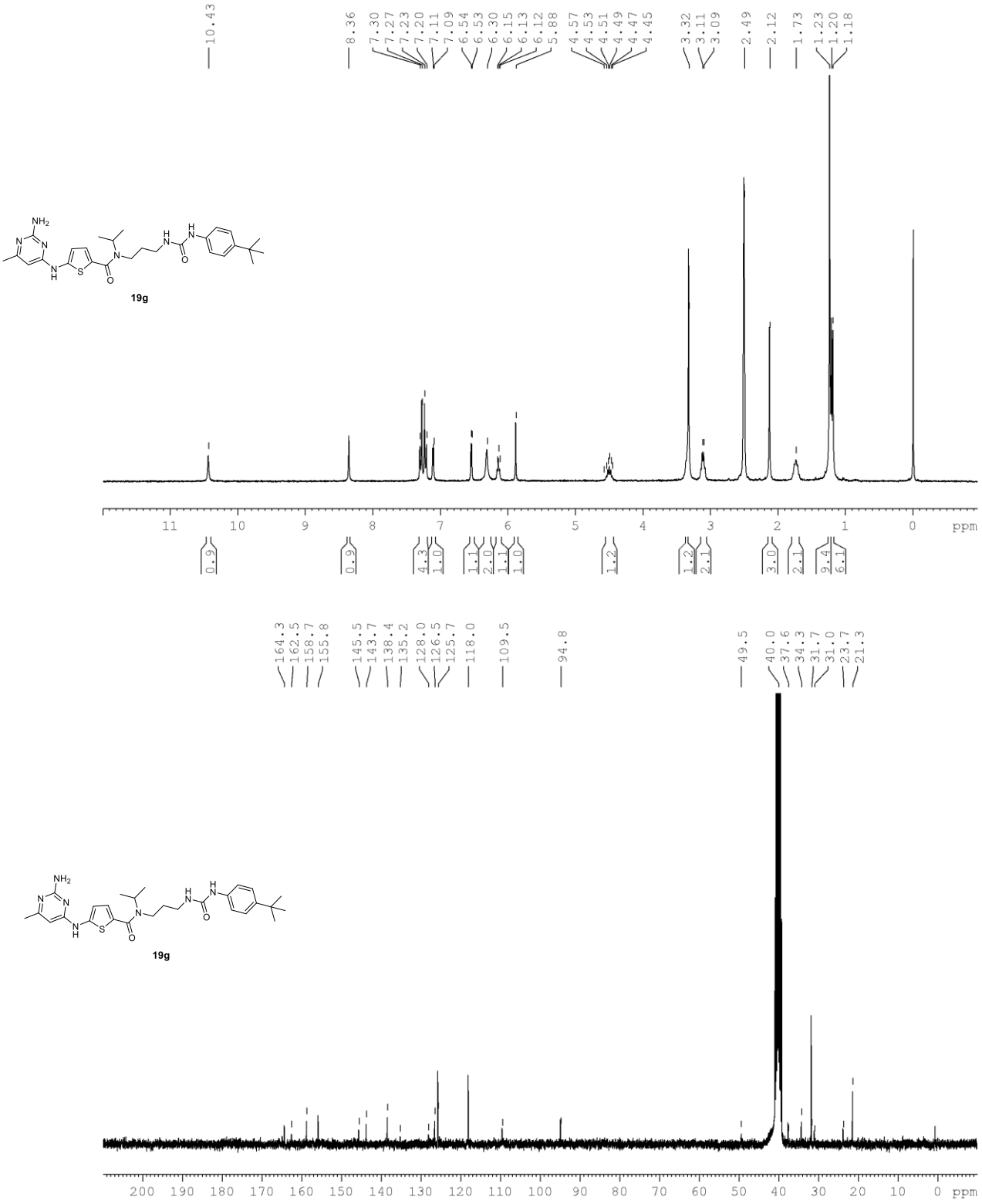


5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)thiophene-2-carboxamide, **19f**

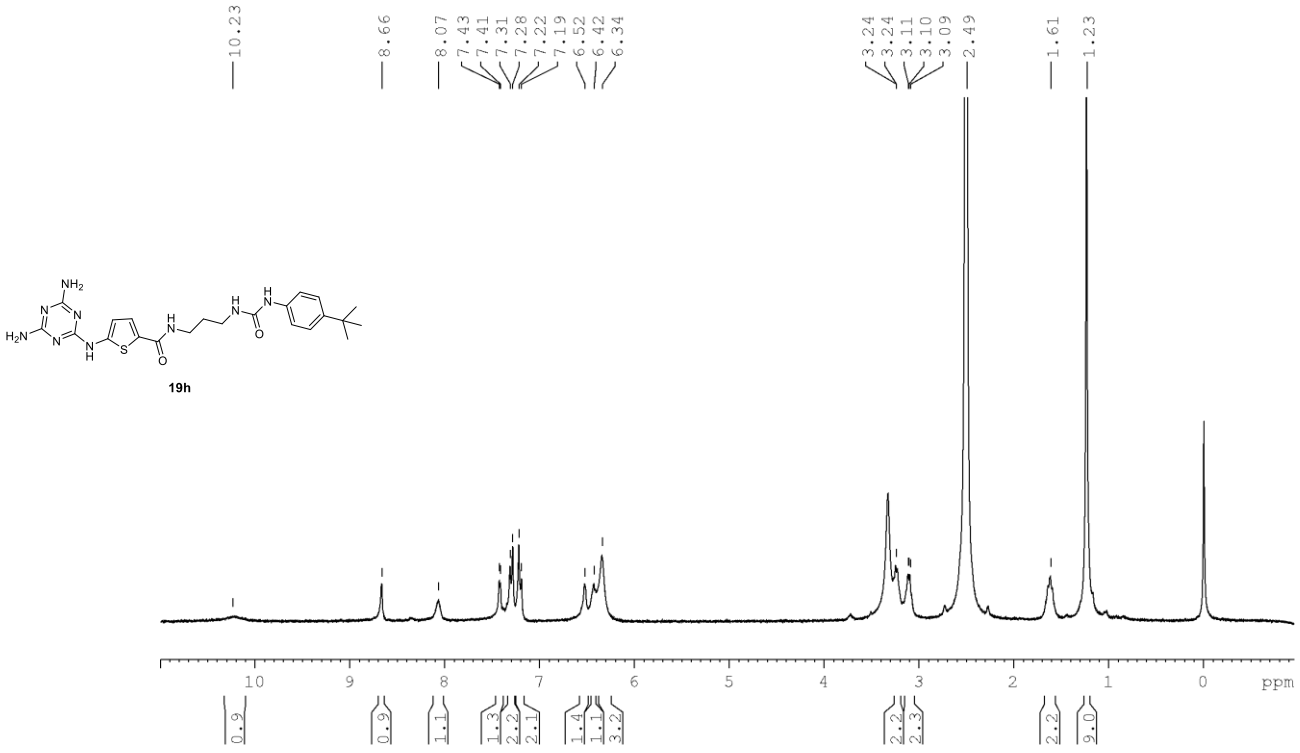


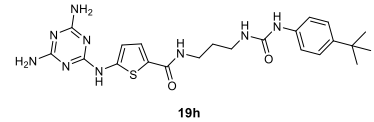


5-((2-amino-6-methylpyrimidin-4-yl)amino)-*N*-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)-N-isopropylthiophene-2-carboxamide, **19g**



N-(3-(3-(4-(*tert*-butyl)phenyl)ureido)propyl)-5-((4,6-diamino-1,3,5-triazin-2-yl)amino)thiophene-2-carboxamide, **19h**





Supplementary References

1 J. M. Yost, I. Korboukh, F. Liu, C. Gao and J. Jin, *Curr. Chem. Genomics*, 2011, **5**, 72–84.

2 K. Y. Horiuchi, *Drug Discov. Today Technol.*, 2015, **18**, 62–68.

3 A. Ceccaldi, A. Rajavelu, C. Champion, C. Rampon, R. Jurkowska, G. Jankevicius, C. Sénamaud-Beaufort, L. Ponger, N. Gagey, H. Dali Ali, J. Tost, S. Vriz, S. Ros, D. Dauzonne, A. Jeltsch, D. Guianvarc'h and P. B. Arimondo, *ChemBioChem*, 2011, **12**, 1337–1345.

4 R. Z. Jurkowska, A. Ceccaldi, Y. Zhang, P. B. Arimondo and A. Jeltsch, in *Epigenetics Protocols*, ed. T. O. Tollefsbol, Humana Press, Totowa, NJ, 2011, vol. 791, pp. 157–177.

5 V. Nayak, C. Xu and J. Min, *Nucleus*, 2011, **2**, 277–282.

6 D. Cheng, N. Yadav, R. W. King, M. S. Swanson, E. J. Weinstein and M. T. Bedford, *J. Biol. Chem.*, 2004, **279**, 23892–23899.

7 B. R. Selvi, K. Batta, A. H. Kishore, K. Mantelingu, R. A. Varier, K. Balasubramanyam, S. K. Pradhan, D. Dasgupta, S. Sriram, S. Agrawal and T. K. Kundu, *J. Biol. Chem.*, 2010, **285**, 7143–7152.

8 C. B. Moraes, G. Witt, M. Kuzikov, B. Ellinger, T. Calogeropoulou, K. C. Prousis, S. Mangani, F. Di Pisa, G. Landi, L. D. Iacono, C. Pozzi, L. H. Freitas-Junior, B. dos Santos Pascoalino, C. P. Bertolacini, B. Behrens, O. Keminer, J. Leu, M. Wolf, J. Reinshagen, A. Cordeiro-da-Silva, N. Santarem, A. Venturelli, S. Wrigley, D. Karunakaran, B. Kebede, I. Pöhner, W. Müller, J. Panecka-Hofman, R. C. Wade, M. Fenske, J. Clos, J. M. Alunda, M. J. Corral, E. Uliassi, M. L. Bolognesi, P. Linciano, A. Quotadamo, S. Ferrari, M. Santucci, C. Borsari, M. P. Costi and S. Gul, *Slas Discov.*, 2019, **24**, 346–361.

9 O. Castillo-Aguilera, P. Depreux, A. Ballée, F. Beaurain, P. B. Arimondo and L. Goossens, *Synlett*, 2020, 31 **12**, 1216-1220.