Electronic Supplementary Material (ESI) for RSC Advances

Amination of Benzylic and Cinnamic Alcohols via a Biocatalytic Aerobic Oxidation-Transamination-Cascade

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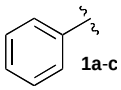
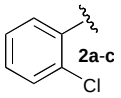
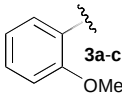
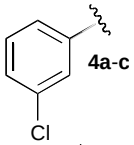
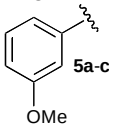
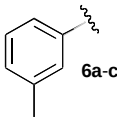
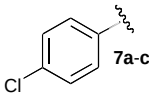
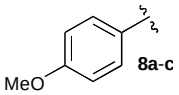
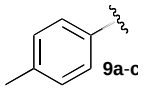
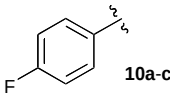
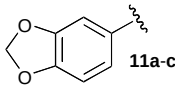
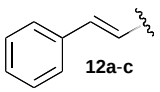
Supporting Information

1. Additional Data

Table S01. Additional Data from Oxidation-Transamination Cascade

R	ω-TA	Conversion [%][a]		
		a	**b**	**c**
 **1a-c**	Vf-ω-TA[c]	<1 (<1)	<1 (<1)	>99 (>99)
	Paracoccus denitrificans ω-TA	<1	<1	>99
 **2a-c** Cl	Vf-ω-TA	99	<1	trace[b]
	Paracoccus denitrificans ω-TA	99	<1	trace[b]
 **3a-c** OMe	Vf-ω-TA	41	59	<1
	Paracoccus denitrificans ω-TA	33	49	18
 **4a-c** Cl	Vf-ω-TA	< 1	4	96
	pCR6	< 1	7	93
 **5a-c** OMe	Vf-ω-TA	<1	19	81
	Paracoccus denitrificans ω-TA	<1	50	50
 **6a-c**	Vf-ω-TA	<1	2	98
	Paracoccus denitrificans ω-TA	<1	<1	>99
 **7a-c** Cl	Vf-ω-TA	18	38	44
	Paracoccus denitrificans ω-TA	35	26	39
 **8a-c** MeO	Vf-ω-TA	<1	50	50
	Paracoccus denitrificans ω-TA	<1	32	68
 **9a-c**	Vf-ω-TA	38	24	38
	Paracoccus denitrificans ω-TA	16	2	82
 **10a-c** F	Vf-ω-TA	21	4	75
	Paracoccus denitrificans ω-TA	40	2	48
 **11a-c** O O	Vf-ω-TA	<1	65	35
	Paracoccus denitrificans ω-TA	<1	64	36
 **12a-c**	Vf-ω-TA	12	85	3
	Paracoccus denitrificans ω-TA[c]	8 (9)	<1 (<1)	92 (91)

Reaction conditions: Substrate (50 mM), galactose oxidase [20 mg/mL, lyophilized whole cells of *E. coli* BL21(DE3) host containing overexpressed galactose oxidase], horseradish peroxidase (HRP, 0.075 mg/mL), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, 0.075 mg/mL), ω-TA [20 mg, lyophilized whole cells of *E. coli* BL21(DE3) host containing overexpressed ω-TA], P_i buffer (100 mM, pH 7.0, 1 mM PLP, 10 mM $CuSO_4$),

alanine dehydrogenase (Ala-DH) from *Bacillus subtilis* (7.5 µg, 0.013 units), glucose dehydrogenase (GDH, 20 U/mL reaction mixture), NH_4Cl (3.5 equiv.), glucose (3 equiv.); a) amine formation was determined using GC-MS analysis; b) only trace amounts of products were observed; c) conversions in brackets indicate the results obtained when HRP/ABTS was replaced by catalase from *Micrococcus lysodeikticus* (10 µL, 1500 U).

2. Experimental Procedures

2.1. General

All chemicals were purchased from Sigma Aldrich or Acros Organics and used as received, solvents were obtained from Roth. Rehydration of enzymes and biocatalytic reactions were performed in a HT Infors Unitron AJ 260 incubator at 120 rpm shaking (horizontal position) and 30°C or in an oxygen pressure chamber apparatus as previously described.[5] Centrifugation was done at 13000 rpm in a Heraeus Biofuge pico or at 4000 rpm in a Heraeus Biofuge primo. NMR-spectra were recorded on a Bruker NMR at 300 (^{1}H) and 75 (^{13}C) MHz, shifts are given in ppm and coupling constants (*J*) are given in Hz. Conversions were determined by gas chromatography using either a Varian GC3900, equipped with Varian CP8400 autosampler and an Agilent Technologies HP-5 column (30 m x 0.32 mm x 0.25 µm film). GC program parameters: injector 220 °C; 14.5 psi N_2; temperature program 70 °C/hold 4 min, 10 °C/min 180 °C/hold 15 min, 20 °C/min 280 °C/hold 1 min, or via GC-MS measurements on an Agilent 7890A GC system, equipped with an Agilent 5975C mass-selective detector (EI 70 eV) and a HP-5-MS column (30 m x 0.25 mm x 0.25 µm film) using He at a flow rate of 0.55 mL/min. Temperature program: 100 °C, hold 0.5 min, 10°C/min 300 °C, inlet temperature 250°C. Compounds **13** and **14** were measured using the following temperature program: 200 °C, hold 0.5 min, 5°C/min 300 °C, hold 2 min, inlet temperature: 300°C. Flash chromatography was performed using Merck silica gel 60 (mesh size 40-63 µm). Petroleum ether had a boiling range from 60 to 95°C. Plasmids of pCR6, pCR7, pCR8, Vf-ωTA and of alanine dehydrogenase from *Bacillus subtilis* were kind gifts of Arne Skerra (TU Munich). The activity of the alanine dehydrogenase (measured by transformation of L-alanine to pyruvate) was 12.6 U/mL. For plasmid preparation and handling of ωTAs from *Bacillus megaterium* SC6394, *Chromobacterium violaceum* DSM 30191, *Alcaligenes denitrificans* Y2k-2 see previously published procedures.[1] All plasmids were transformed into *E. coli* BL21(DE3) expression hosts (Invitrogen) according to manufactor's manual. Experiments under oxygen pressure were conducted in a plexiglass cylinder (27 cm length x 10 cm diameter, for detailed description see supporting information of ref. 5).[5] Formate dehydrogenase (FDH) was obtained from Codexis (# 24.11, H62411.01, EC 1.2.1.2, 220

U/mL), horseradish peroxidase (HRP, # P8125, EC 1.11.1.7) and catalase from *Micrococcus lysodeikticus* (# 60634, EC 1.11.1.6) were purchased from Sigma Aldrich and glucose dehydrogenase (GDH) was obtained from X-zymes (# B4A). The configuration of the C=C bond of product **12c** was determined via co-injection with independent reference materials on GC-MS retention [(*E*)-**11c**: t_{ret} 6.71 min, (*Z*)-**11c**: t_{ret} 6.22 min], which were obtained commerically [(*Z*)-isomer] or synthesised [(*E*)-isomer] via reduction of the acorresponding azide,[2] which was prepared from (*E*)-3-phenylallyl chloride.[3]

2.2. pET16b + Galactose Oxidase insert

The insert was ligated into the pET16b vector via NdeI- (*N*-terminal) and XhoI- (*C*-terminal) restriction sites using a T4 ligase (Fermentas #EL0011). The ligation was conducted according to the manufactor's manual. The enzyme was expressed and purified according to a literature protocol[4] with the following modifications: $CuSO_4.5H_2O$ (250 mg/L) was added to the LB medium for the whole cell preparations in order to enhance enzyme activity and viable *E. coli* cells were induced by Isopropyl-β-D-thiogalactopyranoside (IPTG) at an OD_{600} of 0.6.

2.3. Preparation of Whole Cell Systems.

20 mL of LB/Amp medium were inoculated with a freshly picked colony (LB-agar plate with 100 µg/mL Ampicillin) and were grown at 30°C and 120 rpm (horizontal position) overnight. 0.6 L LB/ampicillin (100 µg/mL) were inoculated with 4 mL of the previously prepared overnight culture and shaken at 37°C and 120 rpm until the cell density reached an OD_{600} of 0.6. At this point expression was induced with the appropriate inducer [pet16b and pet21a: isopropyl-β-D-thiogalactopyranoside (600 µL, 238 mg/ml $H_2O_{dist.}$), pASK-IBA3+ and pASK-IBA5+: anhydrotetracycline (60 µL, 2 mg/mL in EtOH)]. The cultures were grown at 20°C, 120 rpm overnight in the case of pET-vector systems, or at 30°C and 120 rpm for 3 h in the case of the pASK-IBA-vector systems. Subsequent centrifugation (8000 rpm, 20 min, 4°C), washing with physiological NaCl solution, centrifugtion (8000 rpm, 20 min, 4°C) and lyophilisation of the cell pellet from sodium phosphate buffer (20 mL, pH 7.0, 100 mM) gave a whole-cell preparation which was used for all experiments.

2.4. Aminoacid Sequences of Enzymes

For sequences of ω-transaminases from *Bacillus megaterium* SC6394 (BM-ωTA), *Chromobacterium violaceum* DSM 30191 (CV-ωTA) and *Alcaligenes denitrificans* Y2k-2 (AD-ωTA) see the supporting information of ref. 1.[1]

ω-Transaminase from *Pseudomonas putida* KT2440 (Gen PP5182)[6]

MASWSHPQFEKGASVNNPQTREWQTLSGEHHLAPFSDYKQLKEKGPRIITKAQGVHLWDS
EGHKILDGMAGLWCVAVGYGREELVQAAEKQMRELPYYNLFFQTAHPPALELAKAITDVA
PEGMTHVFFTGSGSEGNDTVLRMVRHYWALKGKPHKQTIIGRINGYHGSTFAGACLGGMS
GMHEQGGLPIPGIVHIPQPYWFGEGGDMTPDAFGIWAAEQLEKKILEVGEDNVAAFIAEP
IQGAGGVIIPPETYWPKVKEILAKYDILFVADEVICGFGRTGEWFGSDYYDLKPDLMTIA
KGLTSGYIPMGGVIVRDKVAKVISEGGDFNHGFTYSGHPVAAAVGLENLRILRDEQIVEK
ARTEAAPYLQKRLRELQDHPLVGEVRGLGMLGAIELVKDKATRSRYEGKGVGMICRTFCF
ENGLIMRAVGDTMIIAPPLVISHAEIDELVEKARKCLDLTLEAIR-

ω-Transaminase from *Pseudomonas putida* KT2440 (Gen Gen PP2180)[6]

MASWSHPQFEKGASEQNSQTLAWQSMSRDHHLAPFSDVKQLAEKGPRIITSAKGVYLWDS
EGNKILDGMAGLWCVAVGYGRDELAEVASQQMKQLPYYNLFFQTAHPPALELAKAIADVA
PQGMNHVFFTGSGSEGNDTVLRMVRHYWALKGKKNKNVIIGRINGYHGSTVAGAALGGMS
GMHQQGGVIPDIVHIPQPYWFGEGGDMTEADFGVWAAEQLEKKILEVGVDNVAAFIAEPI
QGAGGVIIPPQTYWPKVKEILARYDILFVADEVICGFGRTGEWFGTDYYDLKPDLMTIAK
GLTSGYIPMGGVIVRDEVAKVISEGGDFNHGFTYSGHPVAAAVGLENLRILRDEQIIQQV
HDKTAPYLQQRLRELADHPLVGEVRGLGMLGAIELVKDKATRARYEGKGVGMICRQHCFD
NGLIMRAVGDTMIIAPPLVISIEEIDELVEKARKCLDLTYEAVR-

ω-Transaminase from *Paracoccus denitrificans*[6, 7]

MASWSHPQFEKGANQPQSWEARAETYSLYGFTDMPSVHQRGTVVVTHGEGPYIVDVHGRR
YLDANSGLWNMVAGFDHKGLIEAAKAQYDRFPGYHAFFGRMSDQTVMLSEKLVEVSPFDN
GRVFYTNSGSEANDTMVKMLWFLHAAEGKPQKRKILTRWNAYHGVTAVSASMTGKPYNSV
FGLPLPGFIHLTCPHYWRYGEEGETEAQFVARLARELEDTITREGADTIAGFFAEPVMGA
GGVIPPAKGYFQAILPILRKYDIPMISDEVICGFGRTGNTWGCLTYDFMPDAIISSKNLT
AGFFPMGAVILGPDLAKRVEAAVEAIEEFPHGFTASGHPVGCAIALKAIDVVMNEGLAEN
VRRLAPRFEAGLKRIADRPNIGEYRGIGFMWALEAVKDKPTKTPFDANLSVSERIANTCT
DLGLICRPLGQSIVLCPPFILTEAQMDEMFEKLEKALDKVFAEVA-

ω-Transaminase from *Vibrio fluvialis* (Vf-ωTA)[6]

MASRGSHHHHHHGANKPQSWEARAETYSLYGFTDMPSLHQRGTVVVTHGEGPYIVDVNGR
RYLDANSGLWNMVAGFDHKGLIDAAKAQYERFPGYHAFFGRMSDQTVMLSEKLVEVSPFD
SGRVFYTNSGSEANDTMVKMLWFLHAAEGKPQKRKILTRWNAYHGVTAVSASMTGKPYNS
VFGLPLPGFVHLTCPHYWRYGEEGETEEQFVARLARELEETIQREGADTIAGFFAEPVMG
AGGVIPPAKGYFQAILPILRKYDIPVISDEVICGFGRTGNTWGCVTYDFTPDAIISSKNL
TAGFFPMGAVILGPELSKRLETAIEAIEEFPHGFTASGHPVGCAIALKAIDVVMNEGLAE
NVRRLAPRFEERLKHIAERPNIGEYRGIGFMWALEAVKDKASKTPFDGNLSVSERIANTC
TDLGLICRPLGQSVVLCPPFILTEAQMDEMFDKLEKALDKVFAEVA-

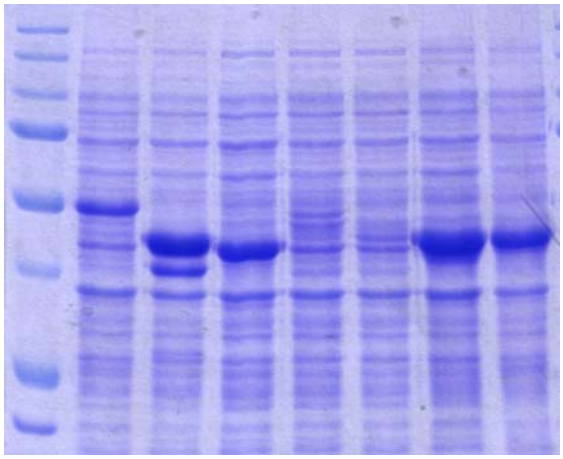
Alanine dehydrogenase from *Bacillus subtilis* (Ala-DH)[8]

MASRGSHHHHHHGAIIGVPKEIKNNENRVALTPGGVSQLISNGHRVLVETGAGLGSGFEN
EAYESAGAEIIADPKQVWDAEMVMKVKEPLPEEYVYFRKGLVLFTYLHLAAEPELAQALK

DKGVTAIAYETVSEGRTLPLLTPMSEVAGRMAAQIGAQFLEKPKGGKGILLAGVPGVSRG
KVTIIGGGVVGTNAAKMAVGLGADVTIIDLNADRLRQLDDIFGHQIKTLISNPVNIADAV
AEADLLICAVLIPGAKAPTLVTEEMVKQMKPGSVIVDVAIDQGGIVETVDHITTHDQPTY
EKHGVVHYAVANMPGAVPRTSTIALTNVTVPYALQIANKGAVKALADNTALRAGLNTANG
HVTYEAVARDLGYEYVPAEKALQDESSVAGA-

Galactose oxidase from *Fusarium* NRRL 2903 M1-variant (GOX-M1)[9]
MGHHHHHHHHHHSSGHIEGRHMASAPIGSAIPRNNWAVTCDSAQSGNECNKAIDGNKDTF
WHTFYGANGDPKPPHTYTIDMKTTQNVNGLSVLPRQDGNQNGWIGRHEVYLSSDGTNWGS
PVASGSWFADSTTKYSNFETRPARYVRLVAITEANGQPWTSIAEINVFQASSYTAPQPGL
GRWGPTIDLPIVPAAAAIEPTSGRVLMWSSYRNDAFEGSPGGITLTSSWDPSTGIVSDRT
VTVTKHDMFCPGISMDGNGQIVVTGGNDAKKTSLYDSSSDSWIPGPDMQVARGYQSSATM
SDGRVFTIGGSWSGGVFEKNGEVYSPSSKTWTSLPNAKVNPMLTADKQGLYRSDNHAWLF
GWKKGSVFQAGPSTAMNWYYTSGSGDVKSAGKRQSNRGVAPDAMCGNAVMYDAVKGKILT
FGGSPDYQDSDATTNAHIITLGEPGTSPNTVFASNGLYFARTFHTSVVLPDGSTFITGGQ
RRGIPFEDSTPVFTPEIYVPEQDTFYKQNPNSIVRAYHSISLLLPDGRVFNGGGGLCGDC
TTNHFDAQIFTPNYLYDSNGNLATRPKITRTSTQSVKVGGRITISTDSSISKASLIRYGT
ATHTVNTDQRRIPLTLTNNGGNSYSFQVPSDSGVALPGYWMLFVMNSAGVPSVASTIRVT
Q-

SDS-Gel showing expression level of ω-transaminases



1 2 3 4 5 6 7 8

Lanes: 1 Standard, 2 BM-ωTA, 3 CV-ωTA, 4 AD-ωTA, 5 Pp1-ωTA, 6 Pp2-ωTA, 7 Pd-ωTA, 8 Vf-ωTA.

2.5. Additional Synthetic Procedures

Cascade process using catalase for the removal of hydrogen peroxide. The cascade was performed as described, by replacing HRP and ABTS with catalase from *Micrococcus lysodeikticus* (10 µL, 1500 U).

Representative upscaling-procedure for the preparation of (*E*)-3-phenylallylamine (12c). pCR6 transaminase (200 mg dry weight, lyophilized whole cells) and galactose oxidase from *Fusarium* NRRL 2903 (200 mg dry weight, lyophilized whole cells) were rehydrated in sodium phosphate buffer (5 mL, 100 mM, pH 7.0, 2 mM PLP, 6 mg/mL $CuSO_4.5H_2O$) at 30°C with shaking at 120 rpm for 30 min (horizontal position). A solution of L-alanine (125 mg, 1.4 mmol), glucose (216 mg, 1.2 mmol), ammonium chloride (53 mg, 1.0 mmol) and nicotinamide adenine dinucleotide sodium salt (3 mg, 0.005 mmol) in sodium phosphate buffer (5 mL, 100 mM, pH 7.0) was added, followed by horseradish peroxidase (600 µL, 10 mg/mL stock solution), ABTS (600 µL, 10 mg/mL stock solution), glucose dehydrogenase (200 µL, 20 mg/mL stock solution) and alanine dehydrogenase from *Bacillus subtilis* (100 µL, 7.5 mg protein/mL stock solution, 0.13 U). Substrate **12a** (67 mg, 0.5 mmol) was added, the reaction mixture was divided up into two 10 mL round bottom flasks, which were transferred to the plastic plates for the oxygen apparatus and placed into the apparatus, which was primed with oxygen (technical grade) for about 1 min and subsequently pressurized to 4 bar. The presurized apparatus was shaken at room temperature and 170 rpm for 24 h. The obtained mixture was acidified with $HCl_{aq.}$ (6 M) to pH <2, extracted with EtOAc (1 x 10 mL) to remove remaining starting material, basified with $NaOH_{aq.}$ (10 M) and extracted with EtOAc (3 x 10 mL). The combined organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure to give compound **12c** (colorless oil, 47 mg, 0.35 mmol, 71%) with the physical properties described in the analytical data section.

Preparation of *N*-[(1*E*)-1-naphthalenylmethylene]-3-phenyl-2-propen-1-amine. Molecular sieve (1g, 3Å) was covered with toluene (ca. 10 mL) and activated via microwave irradiation in a domestic microwave oven at 800 W for 1 min. (*E*)-3-Phenylallylamine (**12c**, 47 mg, 0.35 mmol) and 1-naphthaldehyde (55 mg, 48 µL, 0.35 mmol) were added. The flask was closed with a plastic stopper and kept at r.t. for 16.5 h. The mixture was filtered to remove the molecular sieve and the solvent was evaporated under reduced pressure to give ***N***-[(1*E*)-1-naphthalenylmethylene]-3-phenyl-2-propen-1-amine (71 mg, 0.26 mmol, 73%). Since the product decomposed in NMR solvents ($CDCl_3$, DMSO) due to traces of water, it was characterized by GC-EI-MS: t_{ret} 13.75 min, m/z (relative intensity [%]): 271 (99), 228 (7), 194 (3), 180 (6), 167 (51), 154 (20), 139 (22),128 (20), 117 (100), 91 (22), 77 (6).

Preparation of (*E*)-*N*-(1-Naphthylmethyl)-3-phenylallylamine (13). *N*-[(1*E*)-1-naphthalenylmethylene]-3-phenyl-2-propen-1-amine (28 mg, 0.10 mmol) was dissolved in

MeOH (10 mL). Sodium borohydride (20 mg, 0.53 mmol) was added and the reaction mixture was stirred at room temperature for 4 h. The solvent was removed under reduced pressure, the remaining white solid was treated with $HCl_{aq.}$ (2 M) to remove the excess of reagent, basified to pH >10 by addition of $NaOH_{aq.}$ (10 M) and extracted with EtOAc (3 x 20 mL). The combined organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure to give compound **13** (pale yellow oil, 27 mg, 0.10 mmol, 99%) with following physical properties: δ_H ($CDCl_3$) 8.17 (d, 1H, J = 8.1), 7.92-7.81 (m, 2H), 7.60-7.24 (m, 9H), 6.62 (d, 1H, J = 15.9), 6.40 (td, 1H, J_1 = 15.9, J_2 = 6.3), 4.32 (s, 2H), 3.57 (d, 2H, J = 6.3), 1.88 (bs, 2H); δ_C ($CDCl_3$) 137.1, 135.7, 133.9, 131.8, 131.7, 128.8, 128.6, 128.4, 127.9, 127.4, 126.3, 126.2, 125.7, 125.4, 123.6, 51.7, 50.9; GC-EI-MS: t_{ret} 13.22 min, m/z (relative intensity [%]): 273 (19), 182 (24), 168 (9), 154 (5), 141 (100), 128 (22), 115 (33), 91 (7), 77 (4).

Preparation of (*E*)-*N*-(Methyl)-*N*-(1-Naphthylmethyl)-3-phenylallylamine (Naftifine, 14). (*E*)-*N*-(1-Naphthylmethyl)-3-phenylallylamine (**13**, 27 mg, 0.10 mmol) was dissolved in MeOH (20 mL). Formaldehyde (13 µL of a 37% aqueous solution, 5 mg, 0.16 mmol), sodium sulfate (7 mg, 0.05) and sodium triacetoxyborohydride (72 mg, 0.34) were added and the reaction mixture was stirred for additional 2h. The solvent was removed under reduced pressure, the remaining white solid was treated with $HCl_{aq.}$ (2 M) to remove excess of reagent, basified to pH >10 by addition of $NaOH_{aq.}$ (10 M) and extracted with EtOAc (3 x 20 mL). The combined organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure to give compound **14** (pale yellow oil, 28 mg, 0.10 mmol, >99%) with the following physical properties: d_H ($CDCl_3$) 8.37 (d, 1H, J = 8.4), 7.92-7.83 (m, 2H), 7.62-7.26 (m, 9H), 6.64 (d, 1H, J = 15.9), 6.64 (d, 1H, J = 15.9), 6.44 (td, 1H, J_1 = 15.9, J_2 = 6.7), 4.01 (s, 2H), 3.34 (d, 2H, J = 6.7), 2.34 (s, 3H); δ_C (DMSO) 137.2, 134.9, 134.0, 132.8, 132.6, 128.6, 128.5, 128.0, 127.6, 127.6, 127.4, 126.4, 126.0, 125.7, 125.2, 124.7, 60.5, 60.1, 42.5; GC-EI-MS: t_{ret} 12.08 min, m/z (relative intensity [%]): 287 (34), 196 (41), 182 (12), 141 (100), 115 (33), 91 (8), 77 (2).

3. Analytical Data

Benzylamine (1c). d_H (DMSO) 7.34-7.26 (m, 4H), 7.21-7.16 (m, 1H), 3.69 (s, 2H); δ_C (DMSO) 144.4, 128.6, 127.5, 126.7, 46.0; GC-EI-MS: t_{ret} 3.79 min, m/z (relative intensity [%]): 107 (58), 106 (100), 91 (12), 77 (22).

2-Methoxybenzylamine (3c). δ_H (DMSO) 7.30-7.27 (m, 1H), 7.22-7.16 (m, 1H), 6.93-6.86 (m, 2H), 3.78 (s, 3H), 3.64 (s, 2H); δ_C (DMSO) 157.1, 132.0, 128.2, 128.0, 120.6, 110.6, 55.5, 41.2; GC-EI-MS: t_{ret} 6.17 min, m/z (relative intensity [%]): 137 (65), 136 (100), 122 (21), 106 (66), 91 (57), 77 (55).

3-Chlorobenzylamine (4c). δ_H (DMSO) 7.39 (bs, 1H), 7.33-7.20 (m, 3H), 3.69 (s, 2H); δ_C (DMSO) 147.1, 133.3, 130.4, 127.3, 126.5, 126.1, 45.4; GC-EI-MS: t_{ret} 5.88 min, m/z (relative intensity [%]): 141 (11), 140 (47), 125 (7), 111 (3), 106 (100),77 (27).

3-Methoxybenzylamine (5c). δ_H (DMSO) 7.19 (t, 1H, J = 7.8), 6.91-6.86 (m, 2H), 6.76-6.73 (m, 1H), 3.72 (s, 3H), 3.66 (s, 2H); δ_C (DMSO) 159.7, 146.1, 129.6, 119.7, 112.9, 112.2, 55.3, 46.0; GC-EI-MS: t_{ret} 6.49 min, m/z (relative intensity [%]): 137 (58), 136 (100), 122 (10), 106 (44), 94 (20), 77 (22).

3-Methylbenzylamine (6c). δ_H (DMSO) 7.20-7.09 (m, 3H), 7.01-6.99 (m, 1H), 3.67 (s, 2H), 2.29 (s, 3H); δ_C (DMSO) 144.7, 137.5, 128.4, 128.1, 127.2, 124.5, 46.1, 21.5; GC-EI-MS: t_{ret} 4.75 min, m/z (relative intensity [%]): 121 (30), 120 (73), 106 (57), 104 (100), 91 (47), 77 (28).

4-Chlorobenzylamine (7c). δ_H (DMSO) 7.32 (bs, 4H), 3.66 (s, 2H); δ_C (DMSO) 143.4, 131.1, 129.4, 128.4, 45.2; GC-EI-MS: t_{ret} 5.90 min, m/z (relative intensity [%]): 141 (14), 140 (54), 125 (12), 111 (5), 106 (100),77 (30).

4-Methoxybenzylamine (8c). δ_H (DMSO) 7.23 (td, 2H, J_1 = 8.7, J_2 = 2.1), 6.86 (td, 2H, J_1 = 8.7, J_2 = 2.1), 3.72 (s, 3H), 3.64 (s, 2H); δ_C (DMSO) 158.2, 136.8, 128.6, 113.9, 55.4, 45.6; GC-EI-MS: t_{ret} 6.51 min, m/z (relative intensity [%]): 137 (51), 136 (100), 122 (10), 106 (37), 94 (20), 77 (21).

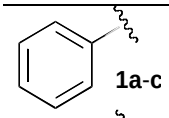
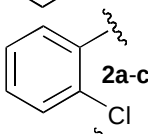
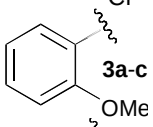
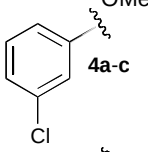
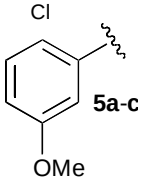
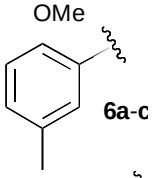
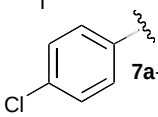
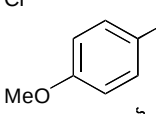
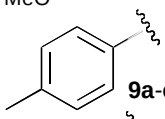
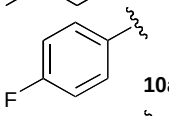
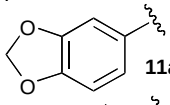
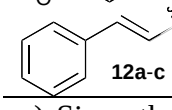
4-Methylbenzylamine (9c). δ_H (DMSO) 7.21 (d, 2H, J = 7.9), 7.10 (d, 2H, J = 7.9), 3.66 (s, 2H), 2.27 (s, 3H); δ_C (DMSO) 141.7, 135.4, 1291, 127.4, 45.9, 21.1; GC-EI-MS: t_{ret} 4.77 min, m/z (relative intensity [%]): 121 (31), 120 (77), 106 (62), 104 (100), 91 (48), 77 (29).

4-Fluorobenzylamine (10c). δ_H (DMSO) 7.38-7.33 (m, 2H), 7.13-7.07 (m, 2H), 3.69 (s, 2H); δ_C (DMSO) 161.3 (d, J = 239.5), 140.9 (d, J = 2.9), 129.2 (d, J = 7.9), 115.1 (d, J = 20.9), 45.4; GC-EI-MS: t_{ret} 3.88 min, m/z (relative intensity [%]): 125 (30), 124 (100), 110 (24), 105 (44), 97 (32), 75 (11).

Piperonyl amine (11c). δ_H (DMSO) 6.92-6.91 (m, 1H), 6.82-6.77 (m, 2H), 5.96 (s, 2H), 3.61 (s, 2H); δ_C 147.6, 146.0, 139.0, 120.2, 108.2, 108.1, 101.0, 46.0; GC-EI-MS: t_{ret} 7.71 min, m/z (relative intensity [%]): 151 (92), 150 (100), 135 (38), 121 (72), 105 (7), 93 (66), 77 (38).

(*E*)-3-Phenylallylamine (12c). δ_H ($CDCl_3$) 7.40-7.21 (m, 5H), 6.52 (d, 1H, J = 15.7), 6.33 (td, 1H, J_1 = 15.7, J_2 = 6.0), 3.49 (dd, 2H, J_1 = 5.7, J_2 = 1.2), 1.76 (s, 2 H); δ_C ($CDCl_3$) 137.2, 131.1, 129.5, 128.6, 127.3, 126.3, 126.2, 44.3; GC-EI-MS: t_{ret} 6.71 min, m/z (relative intensity [%]): 133 (98), 132 (100), 117 (25), 103 (11), 91 (19), 77 (20).

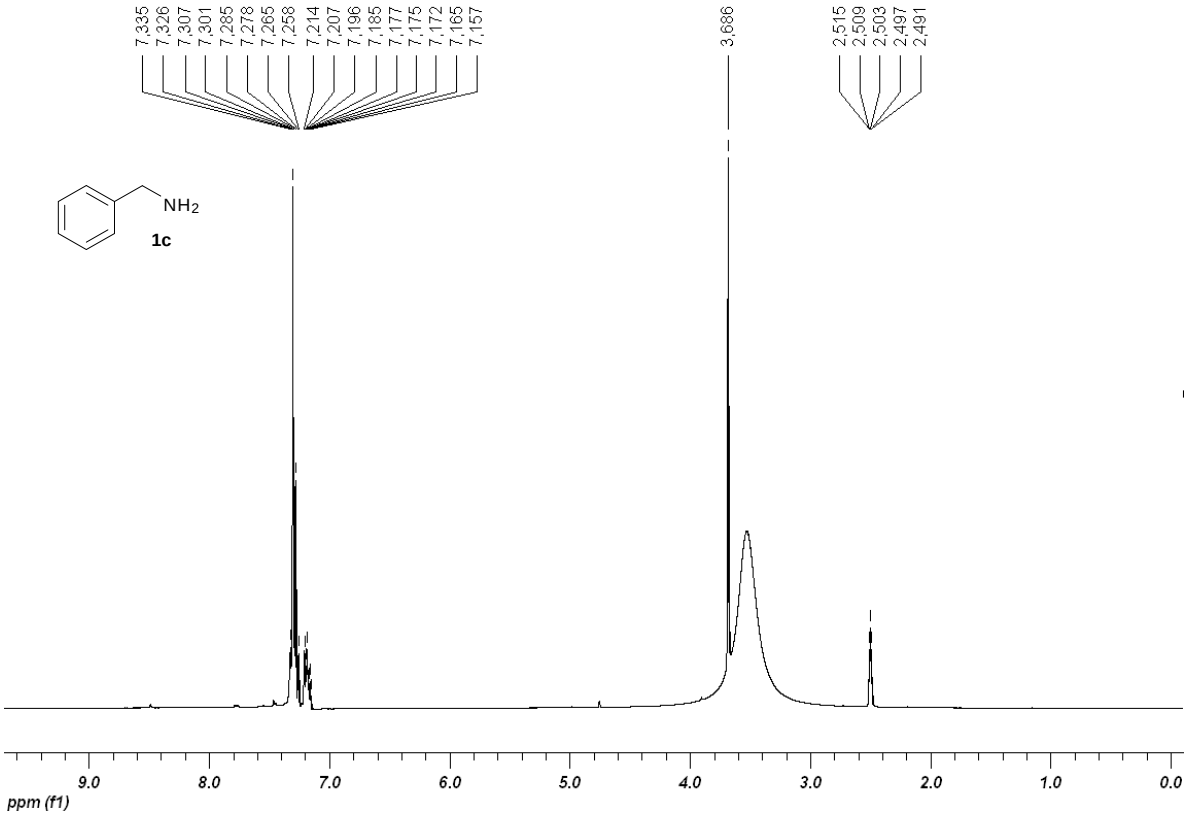
3.1. Retention times – GC-MS

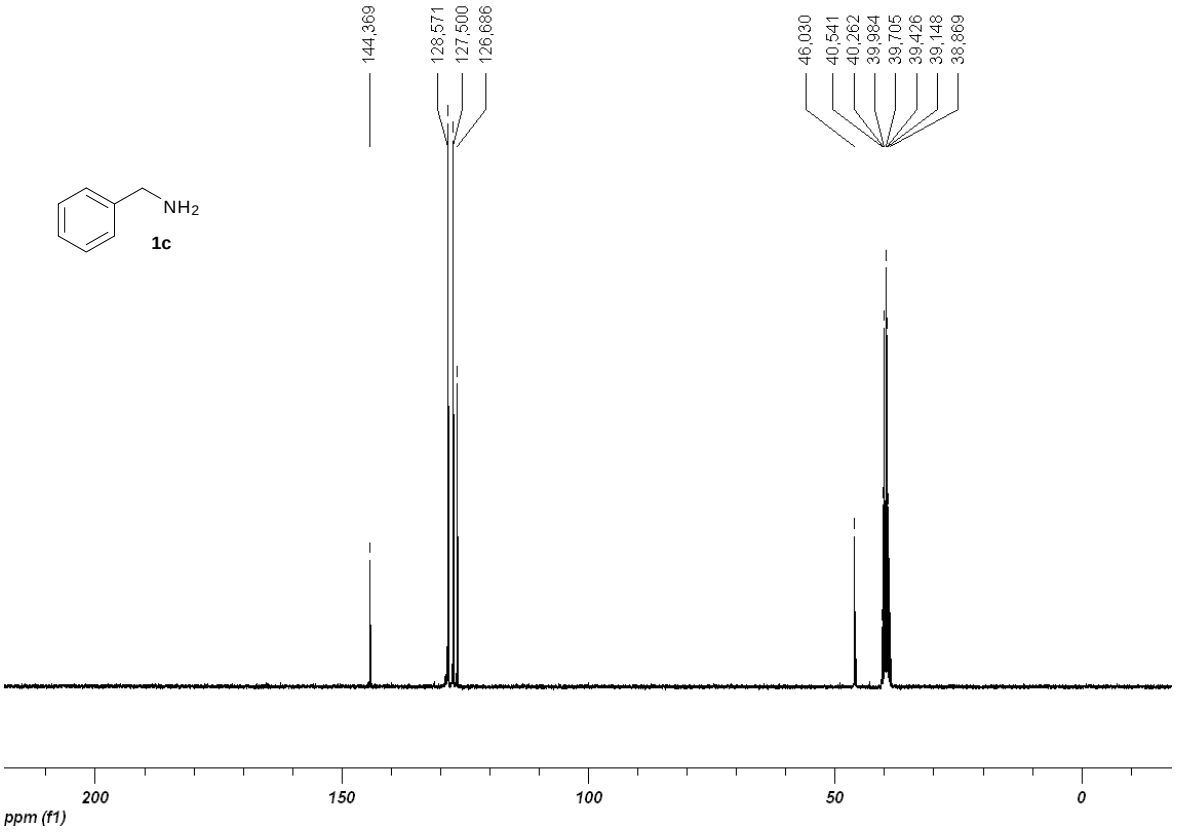
R	Retention time [min]		
	a	**b**	**c**
 **1a-c**	3.92	3.39	3.79
 **2a-c**	5.84	4.84[a]	5.60[a]
 **3a-c**	6.37	6.21	6.17
 **4a-c**	6.16	4.88	5.88
 **5a-c**	6.65	5.72	6.49
 **6a-c**	4.92	4.46	4.75
 **7a-c**	6.13	4.93	5.90
 **8a-c**	6.66	6.38	6.51
 **9a-c**	4.92	4.45	4.77
 **10a-c**	4.04	3.32	3.88
 **11a-c**	7.87	7.37	7.71
 **12a-c**	6.95	6.57	6.71

a) Since the products were only observed in trace amounts, their isolation was not possible and retention times were confirmed by comparison with commercially available reference material.

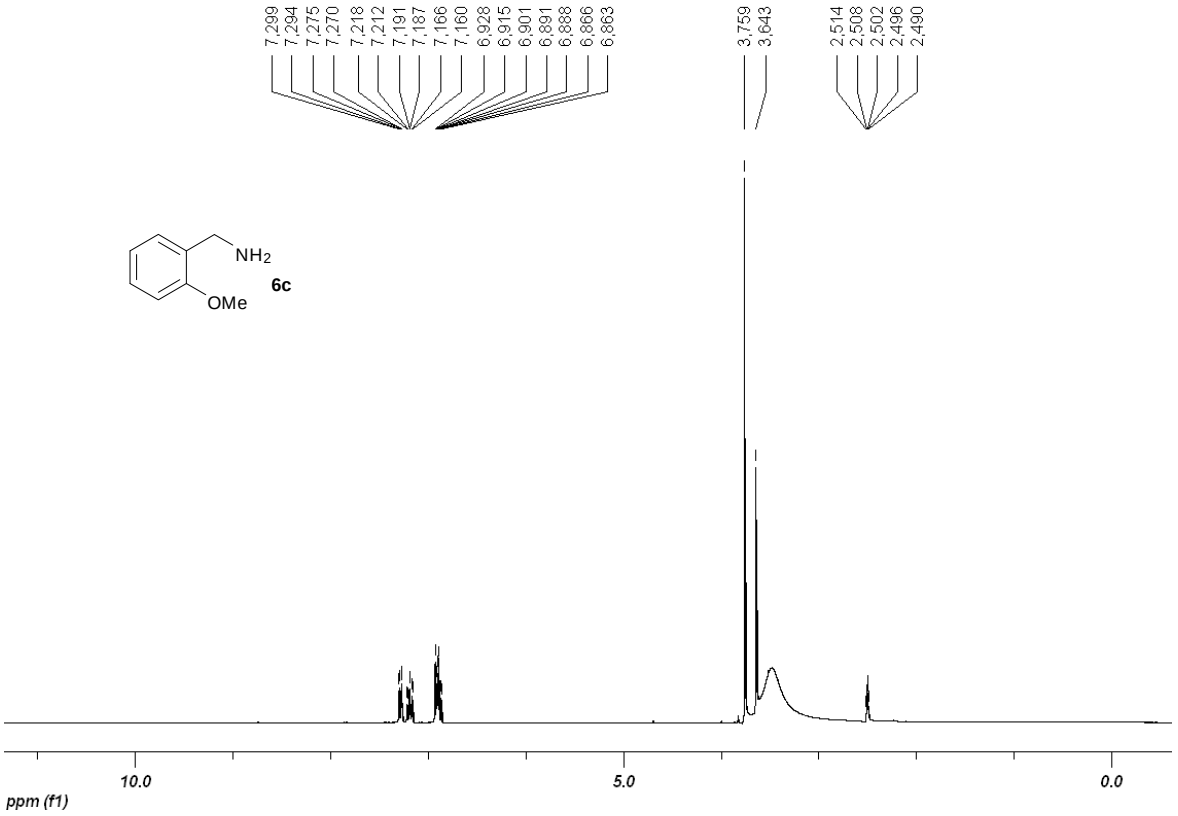
3.2. ^{1}H- and ^{13}C-NMR spectra.

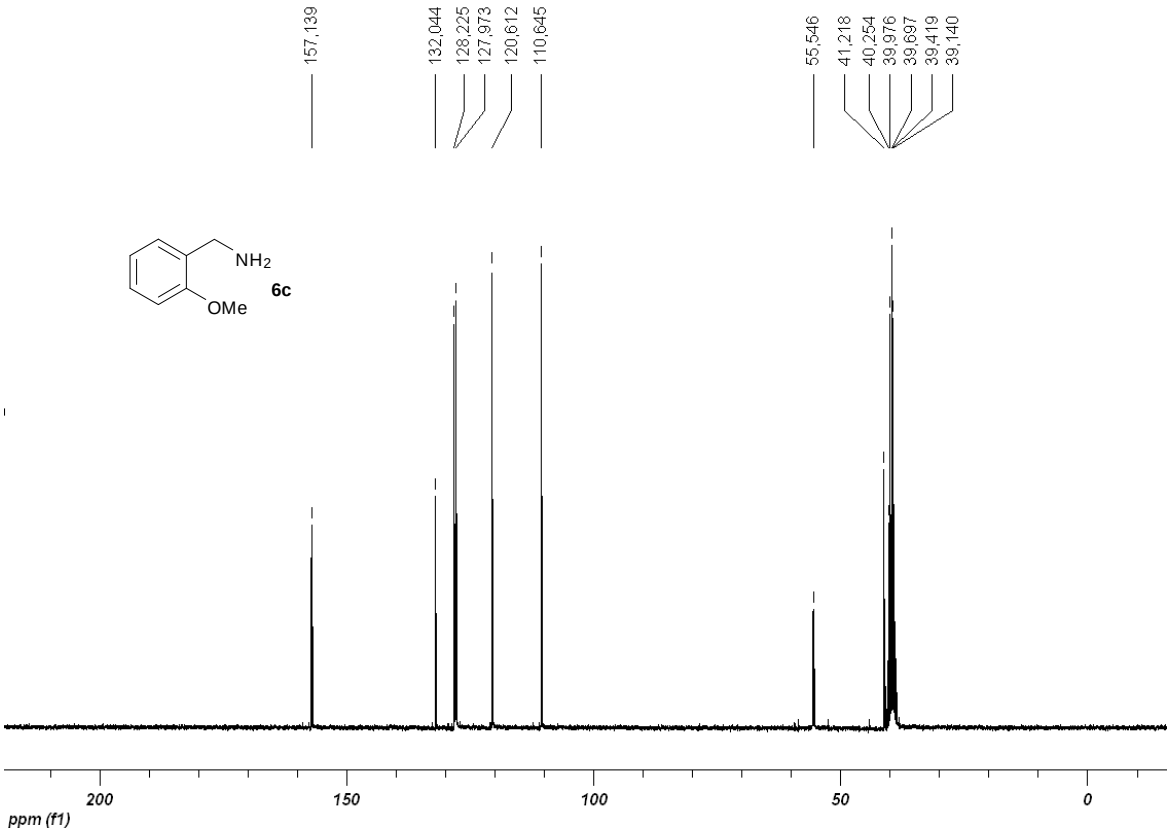
Benzylamine (1c).



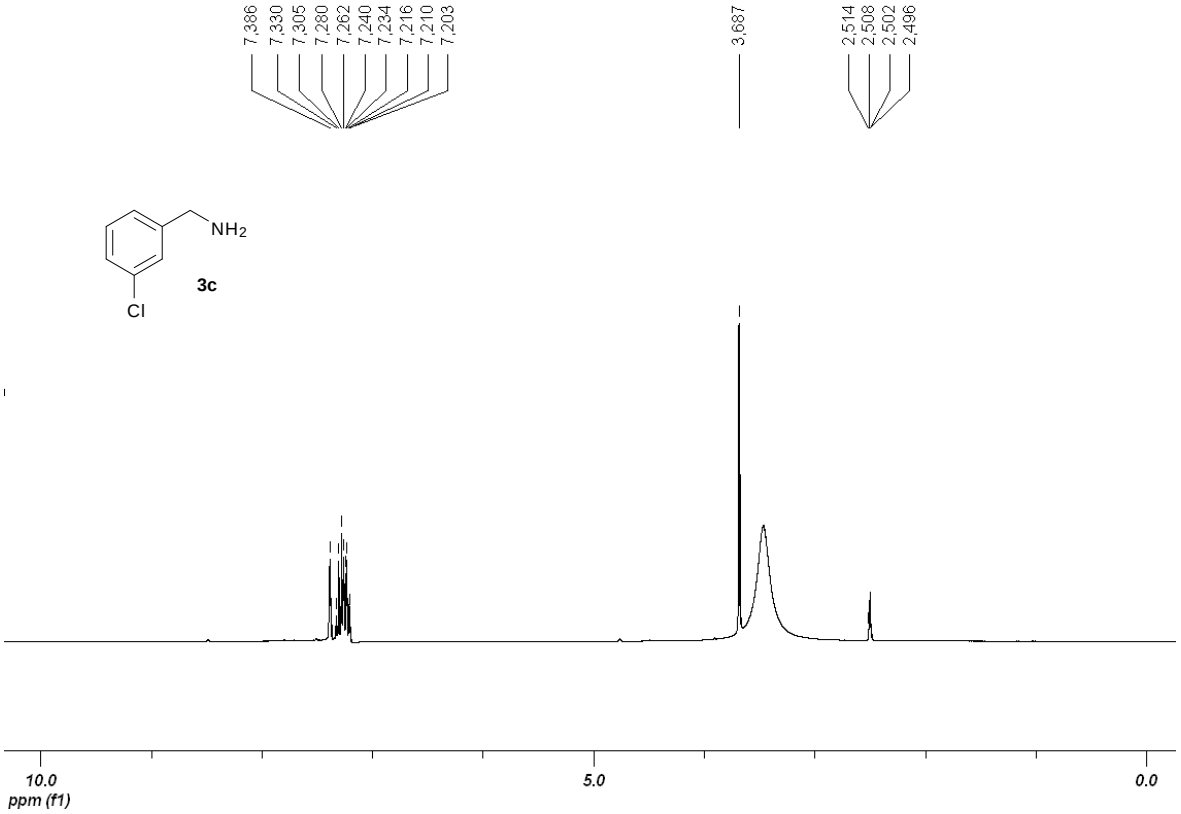


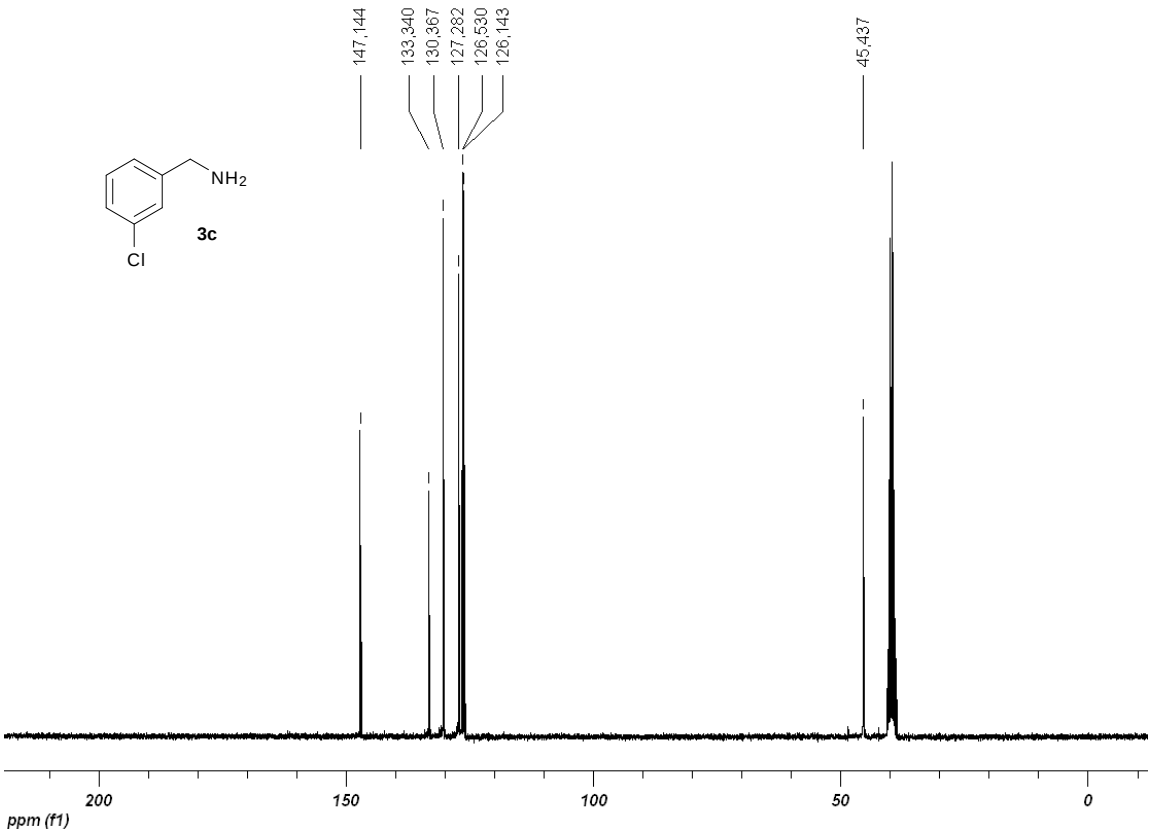
2-Methoxybenzylamine (3c).



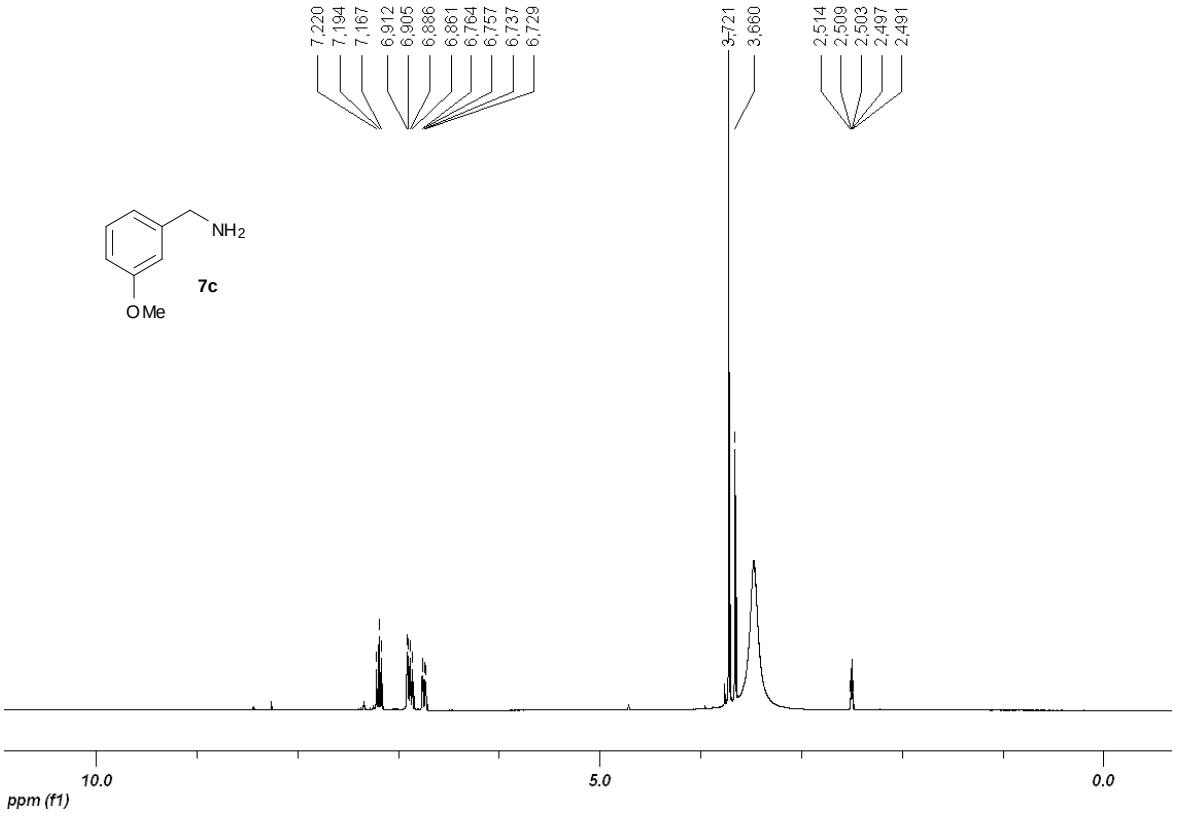


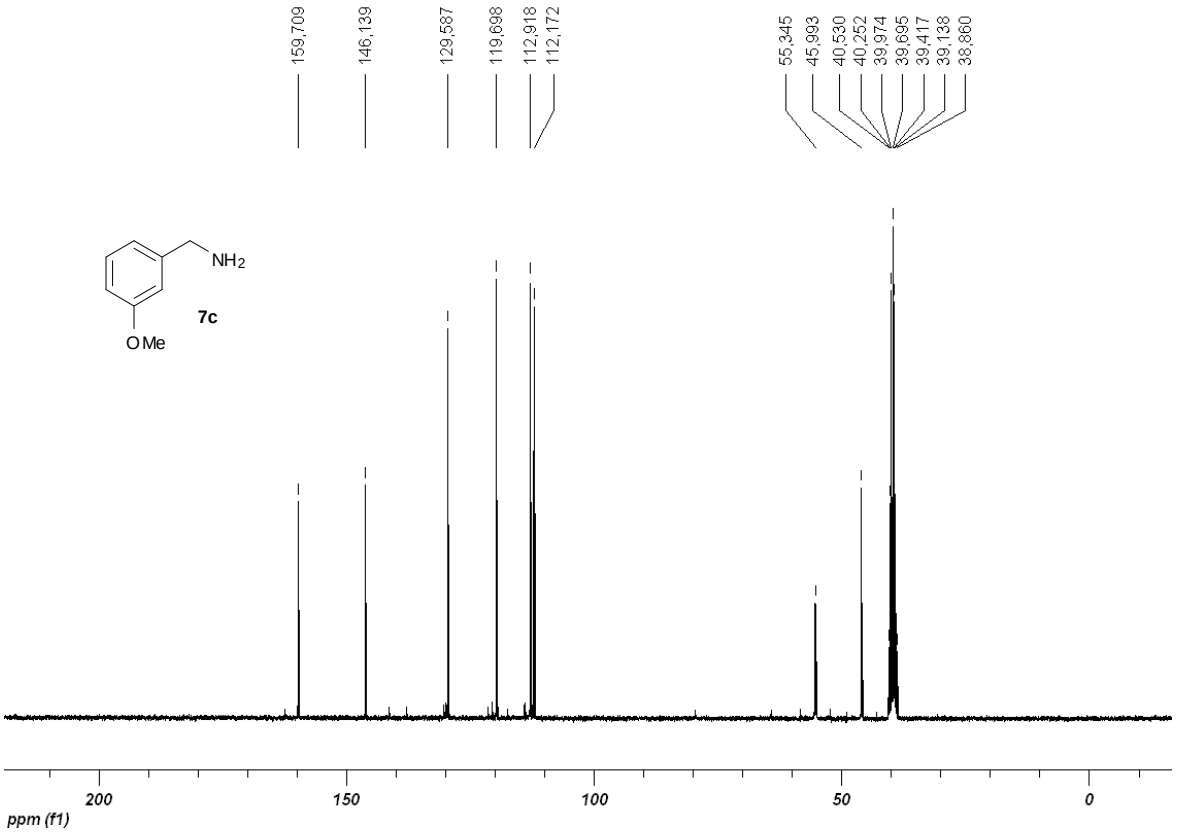
3-Chlorobenzylamine (4c).



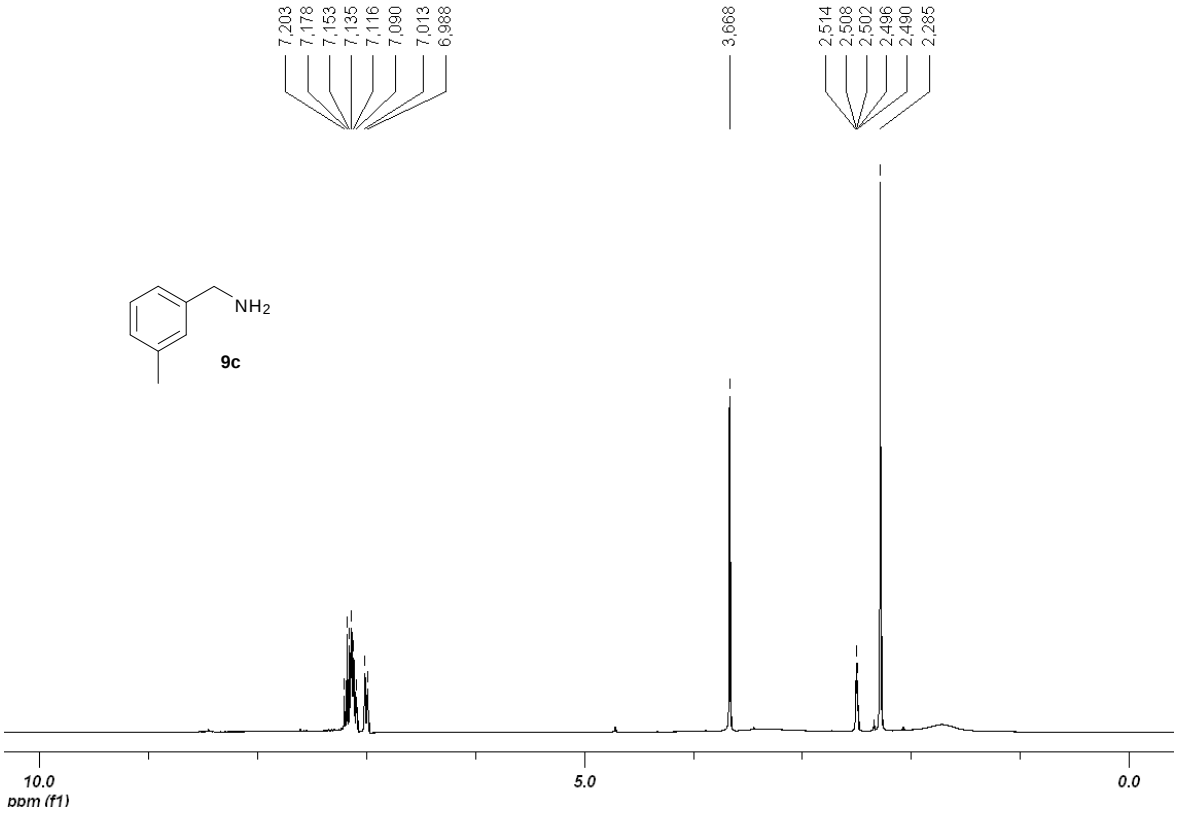


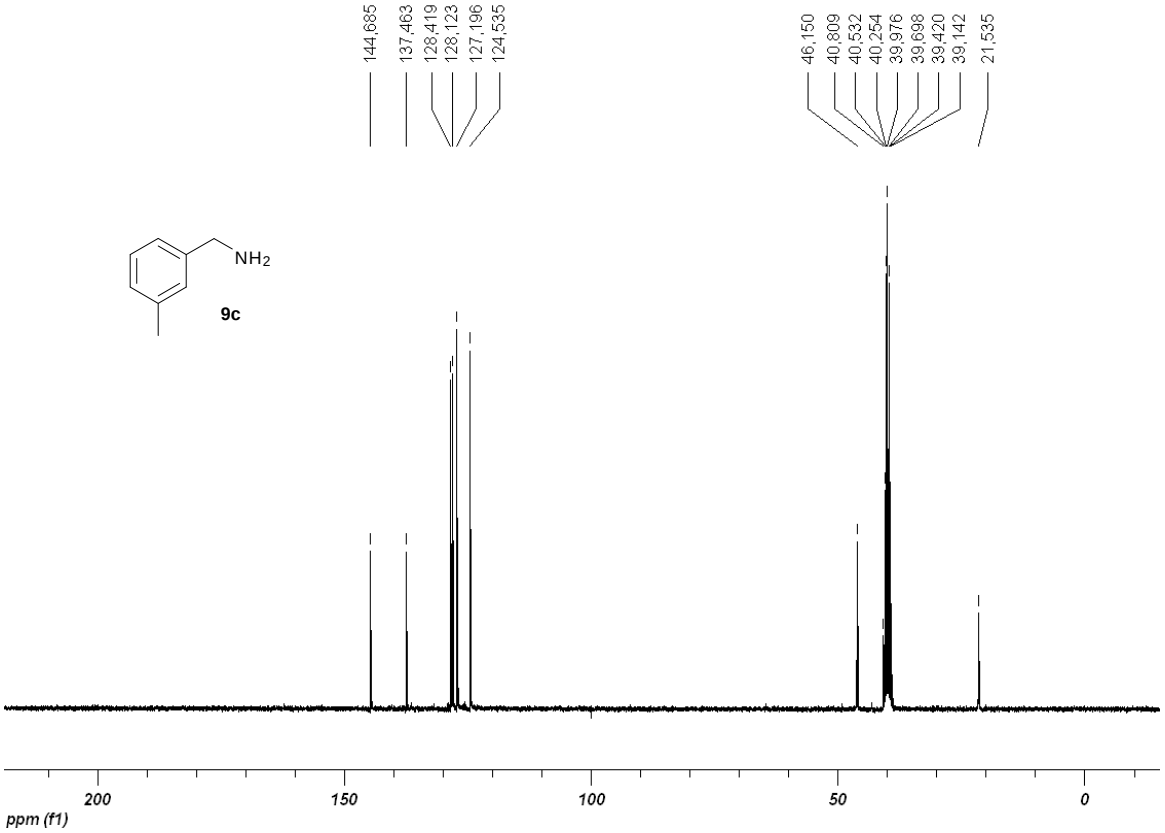
3-Methoxybenzylamine (5c).



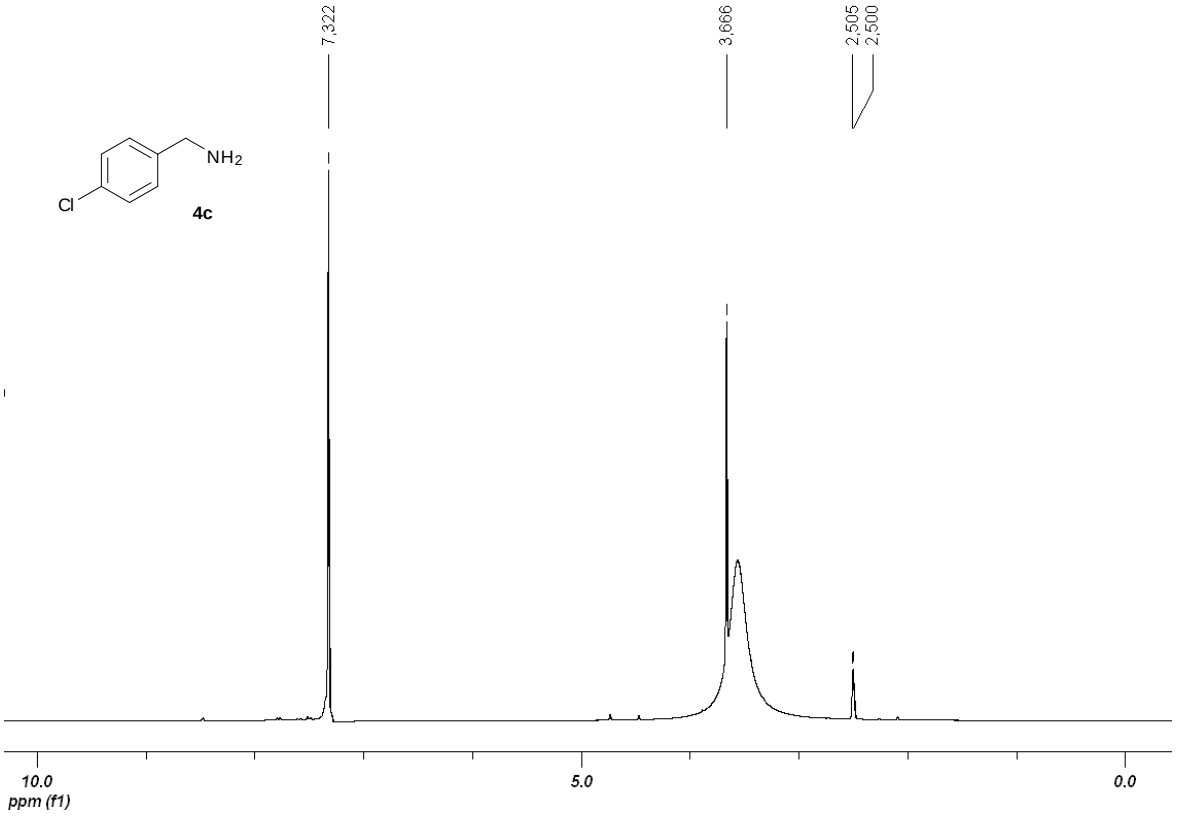


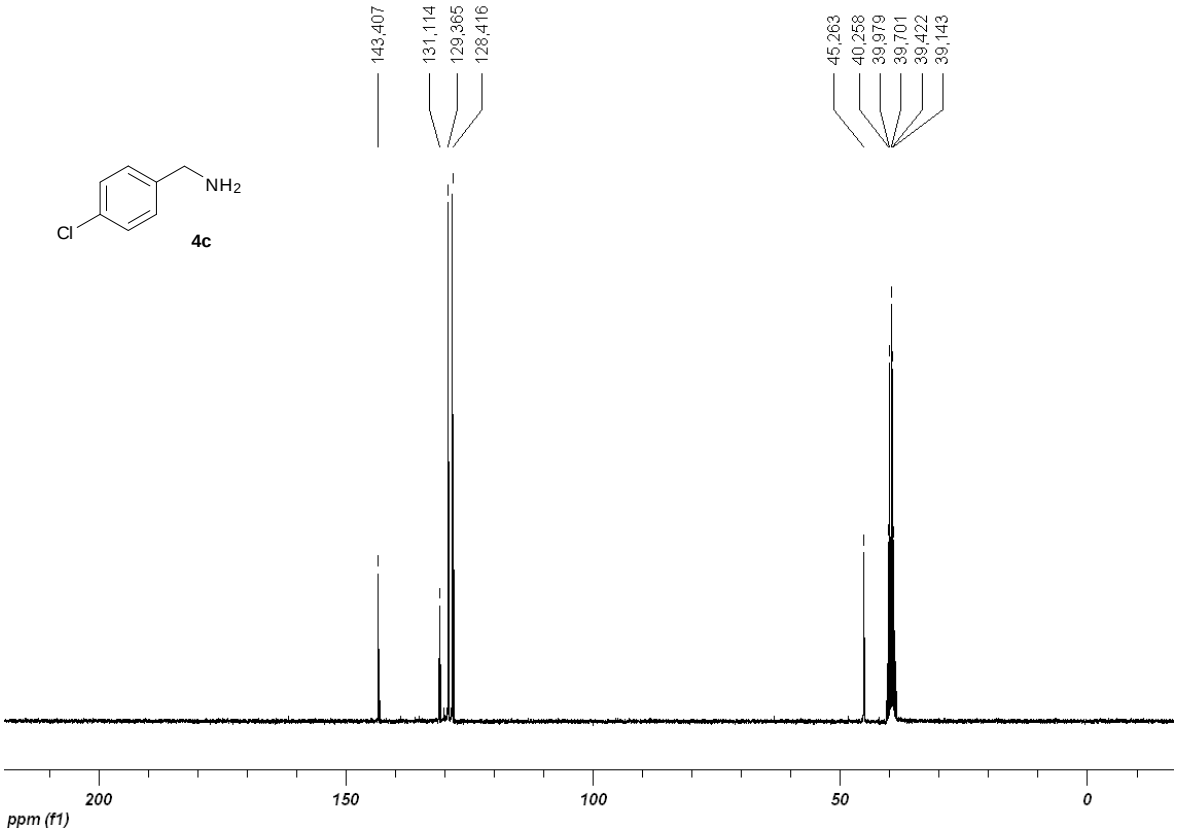
3-Methylbenzylamine (6c).



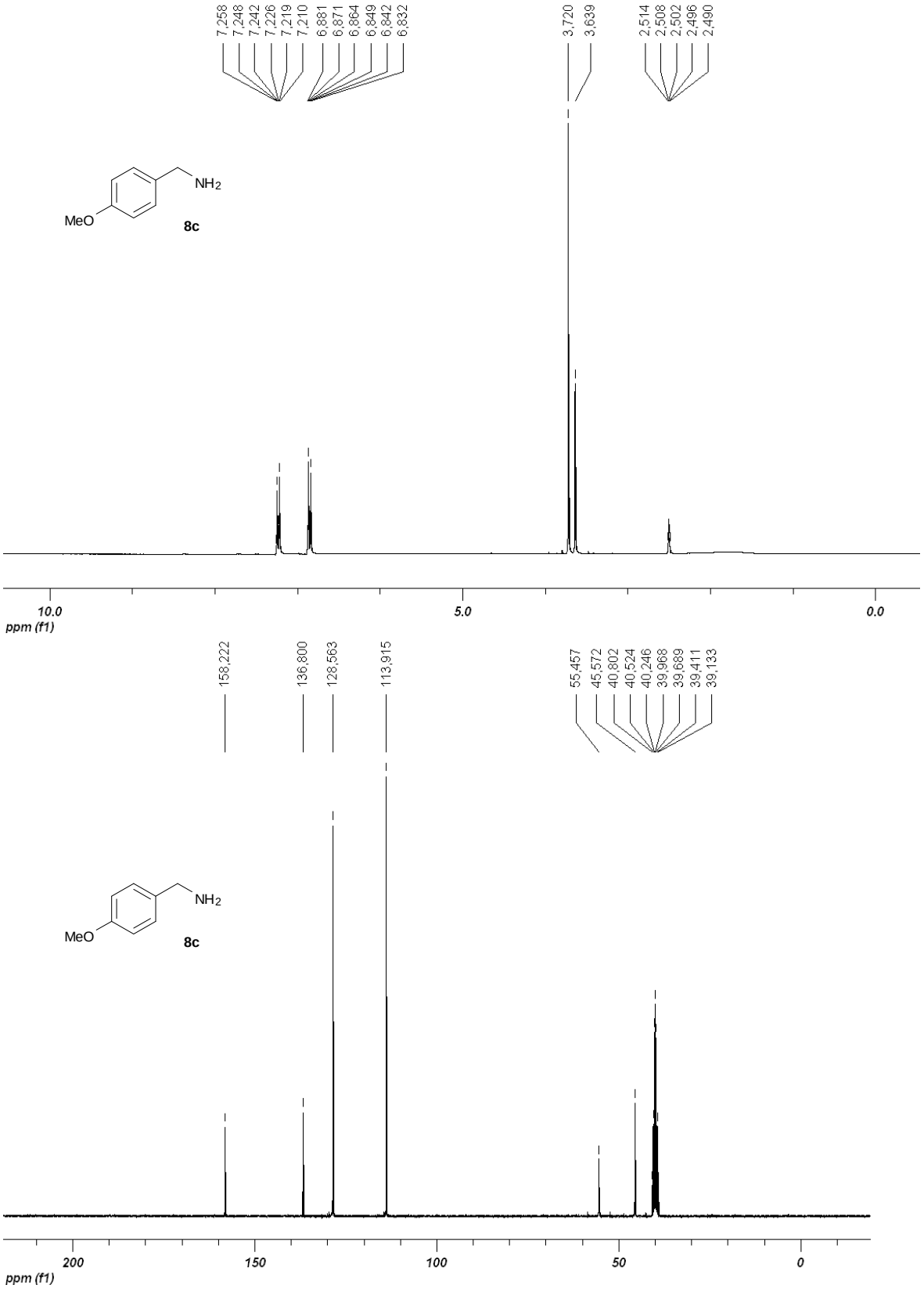


4-Chlorobenzylamine (7c).

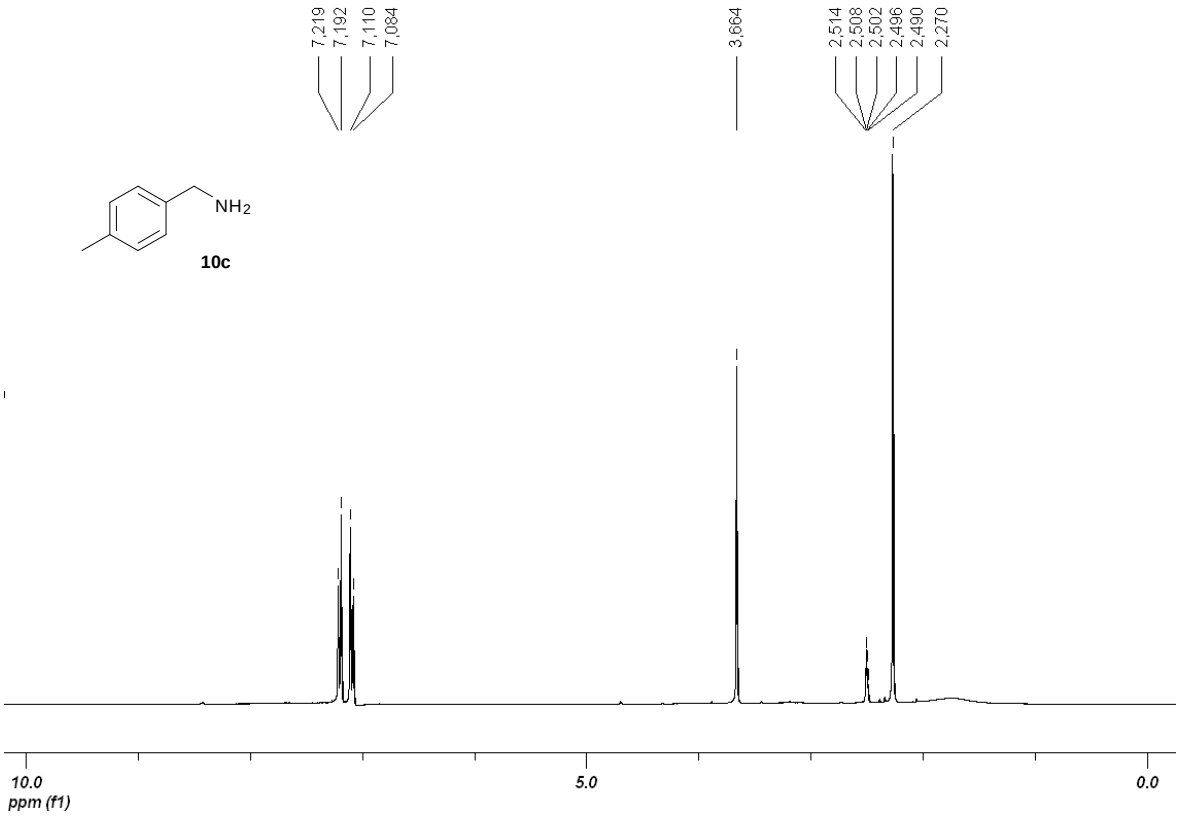


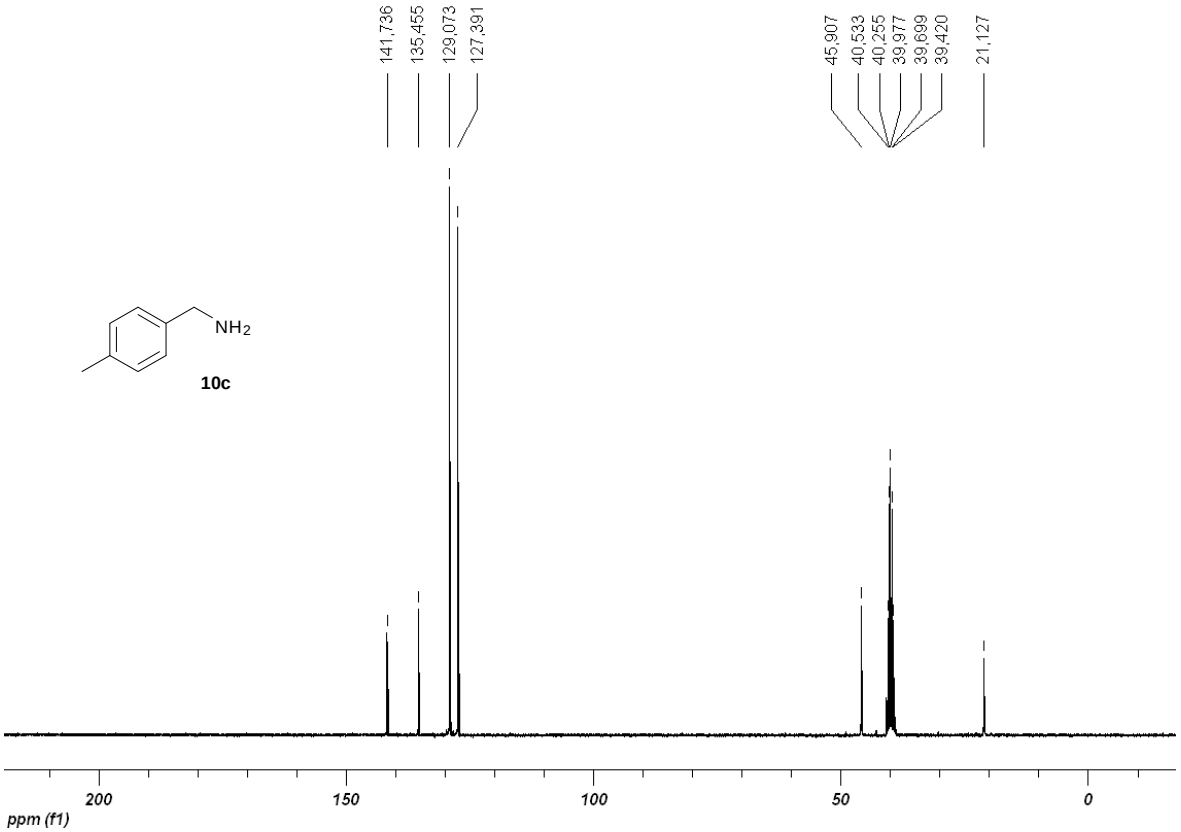


4-Methoxybenzylamine (8c).

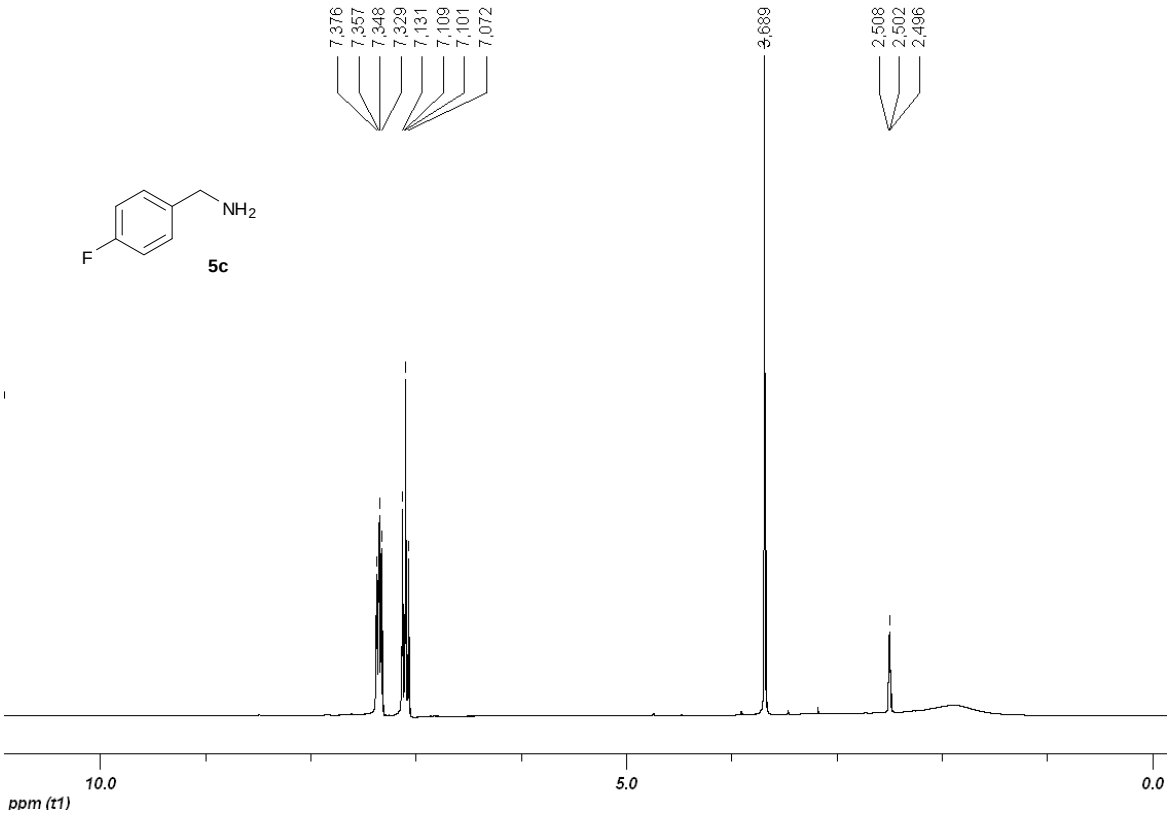


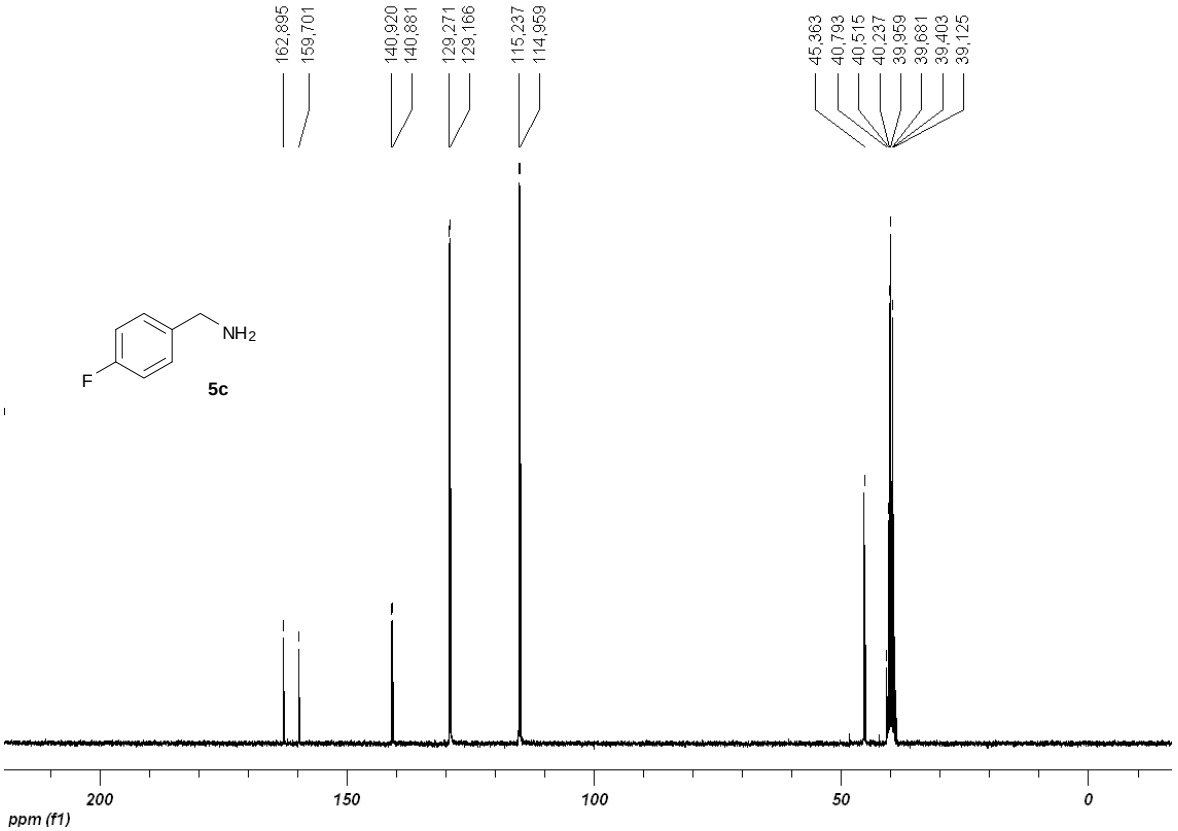
4-Methylbenzylamine (9c).



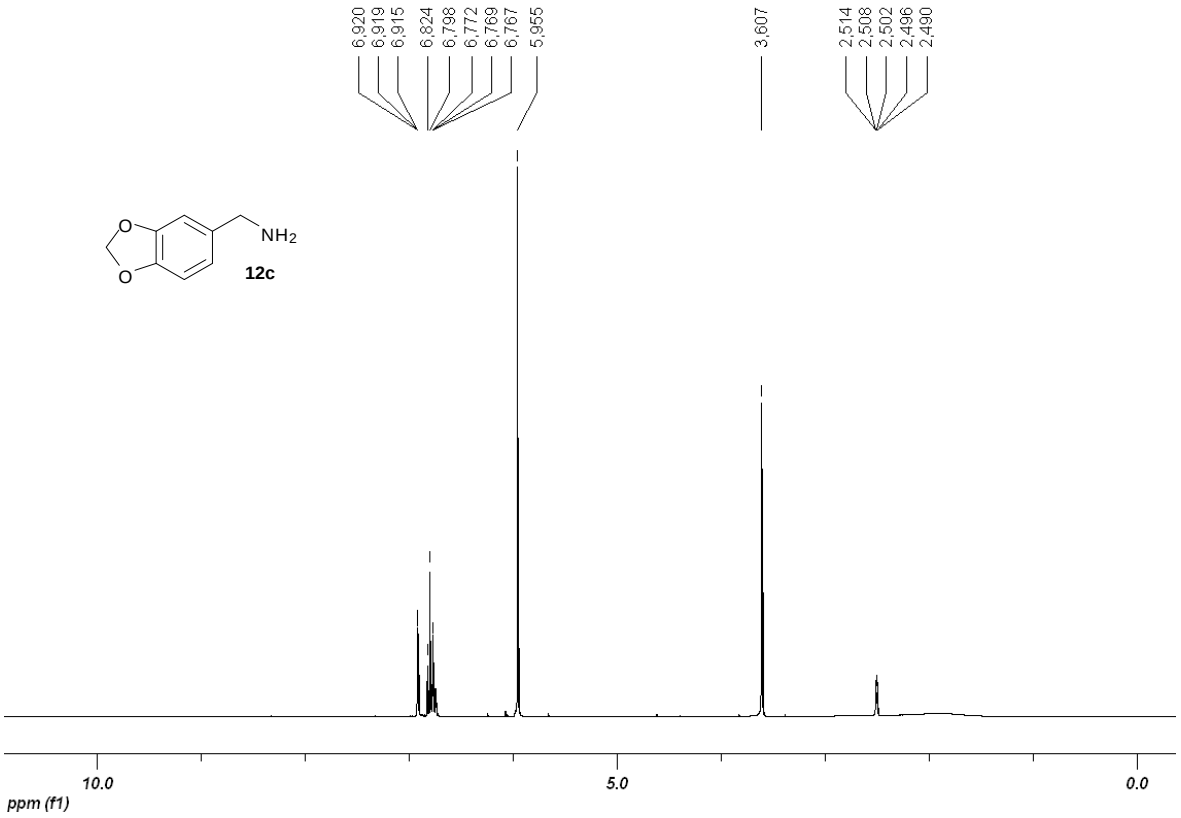


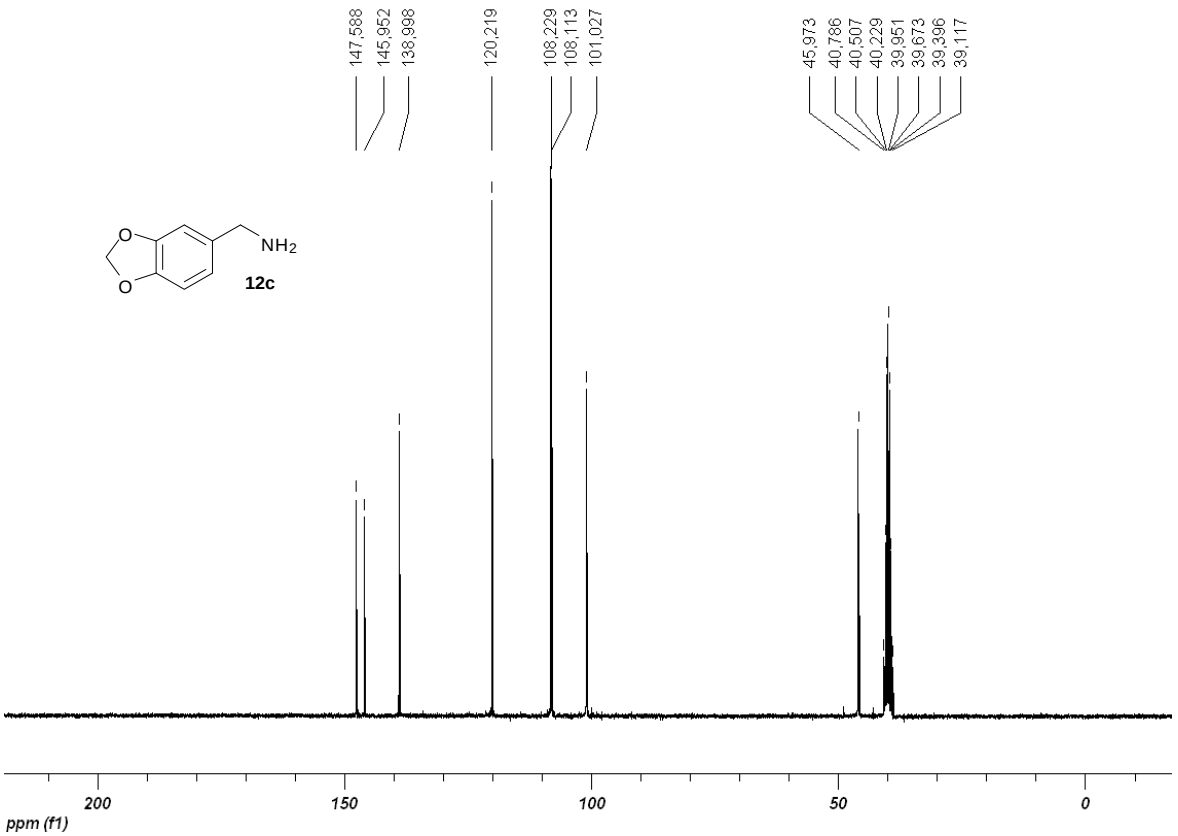
4-Fluorobenzylamine (10c).



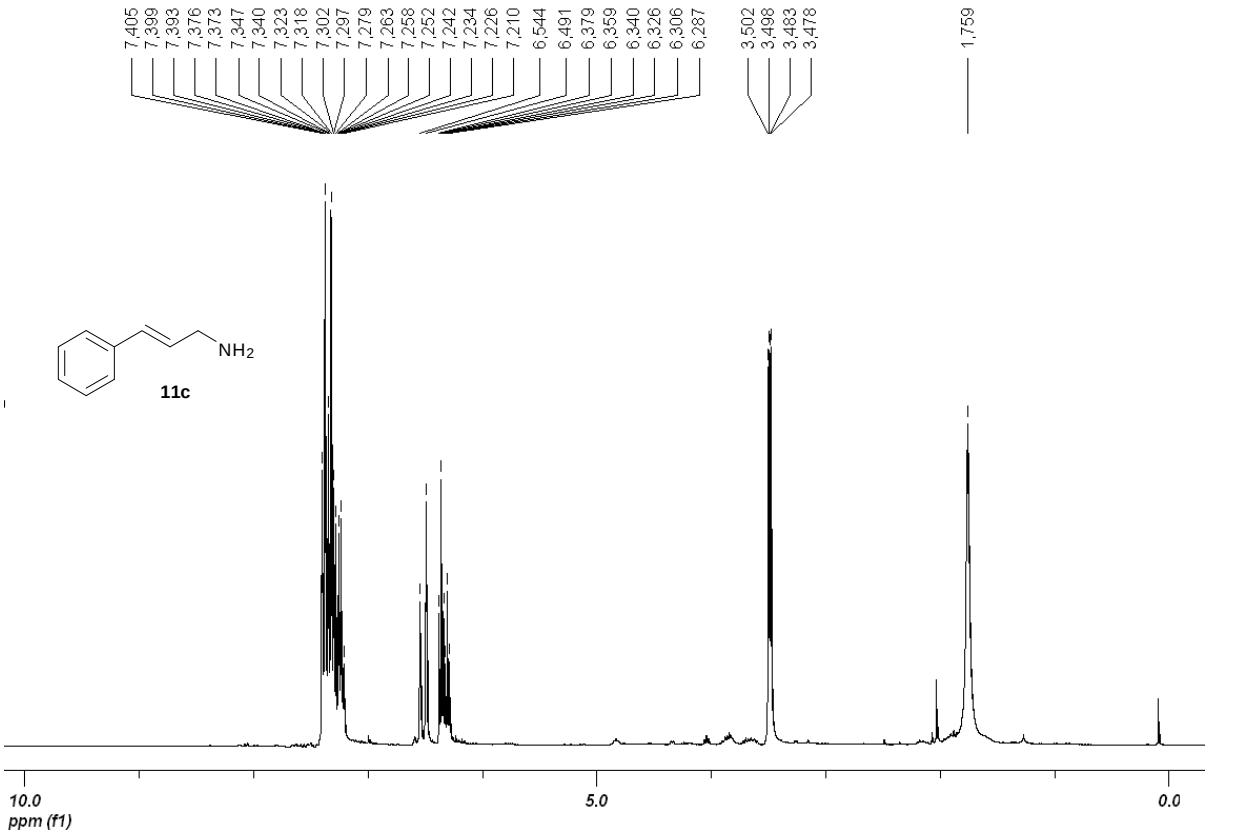


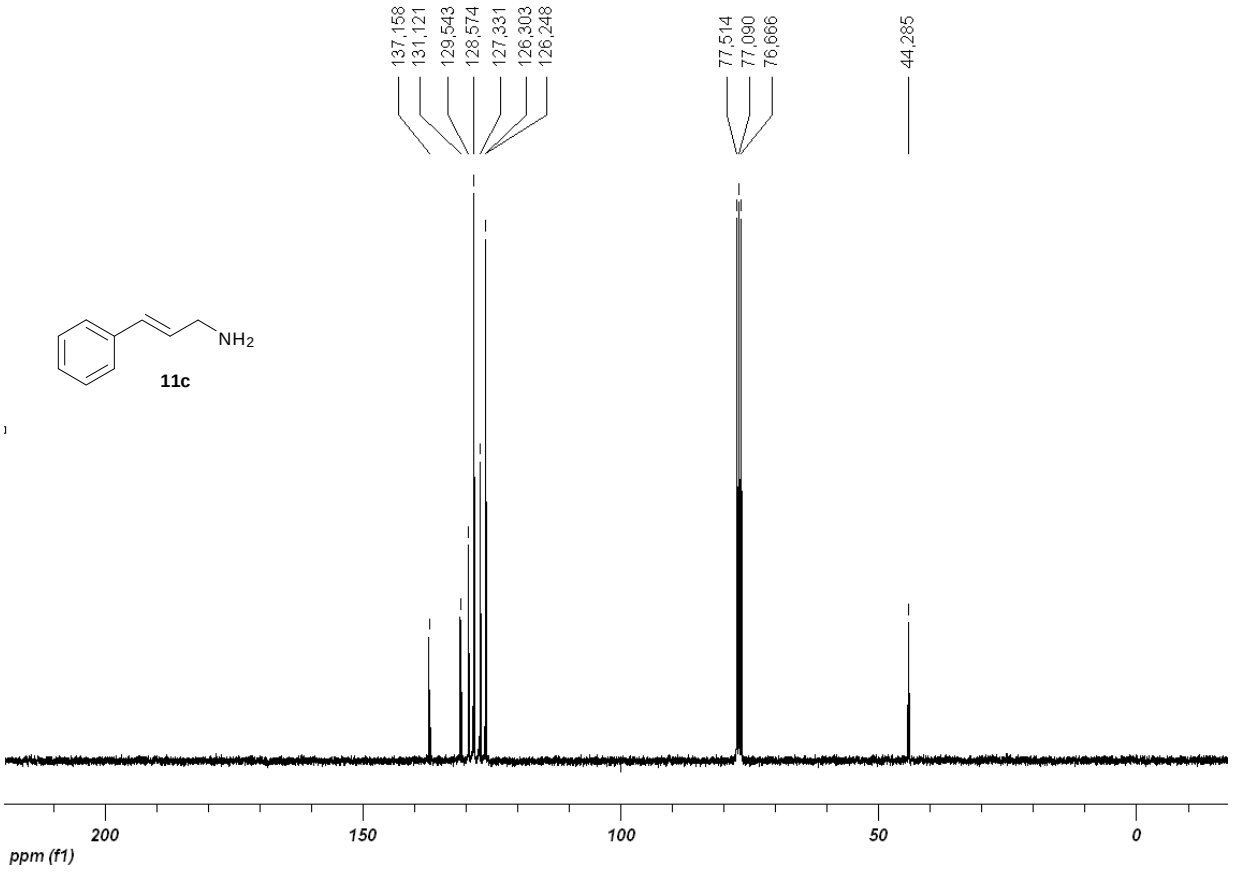
Piperonyl amine (11c).



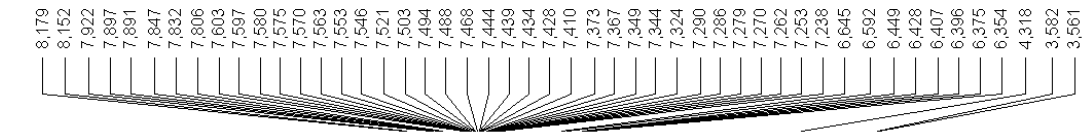


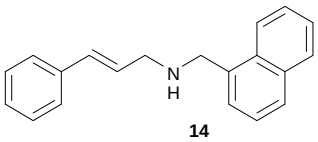
(*E*)-3-Phenylallylamine (12c).



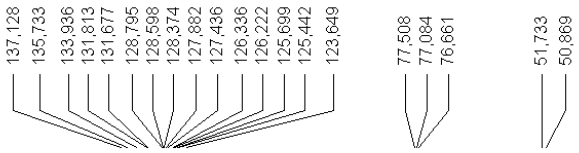


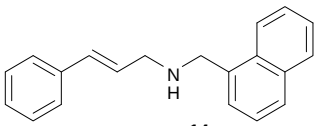
(*E*)-*N*-(1-Naphthylmethyl)-3-phenylallylamine (13).





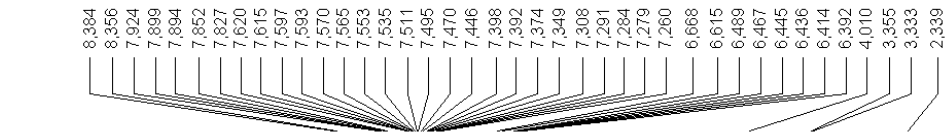
14

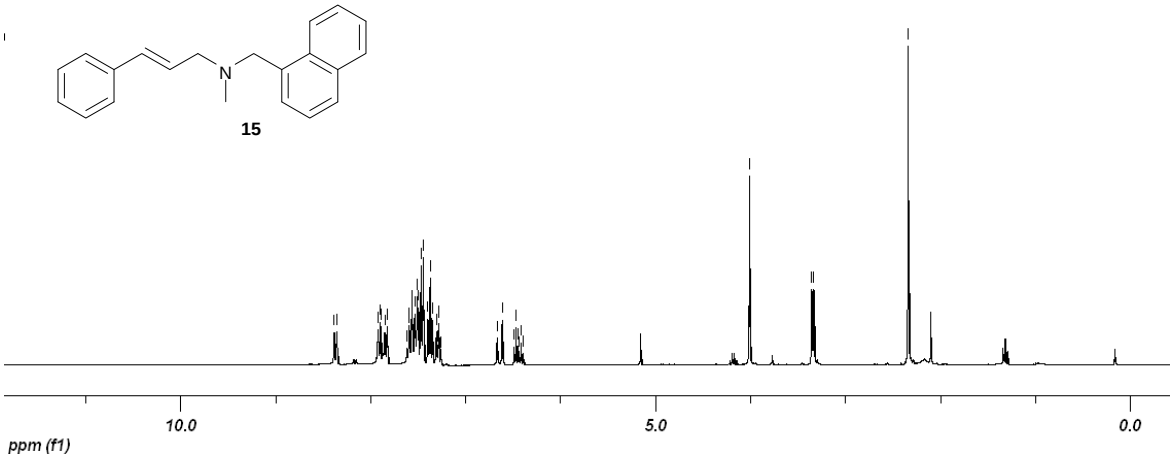


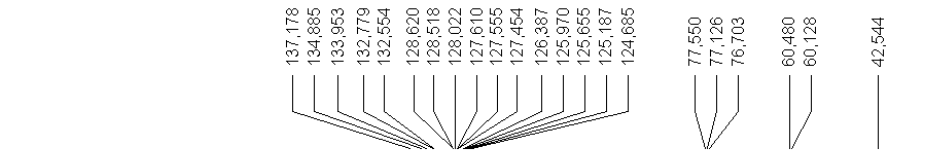


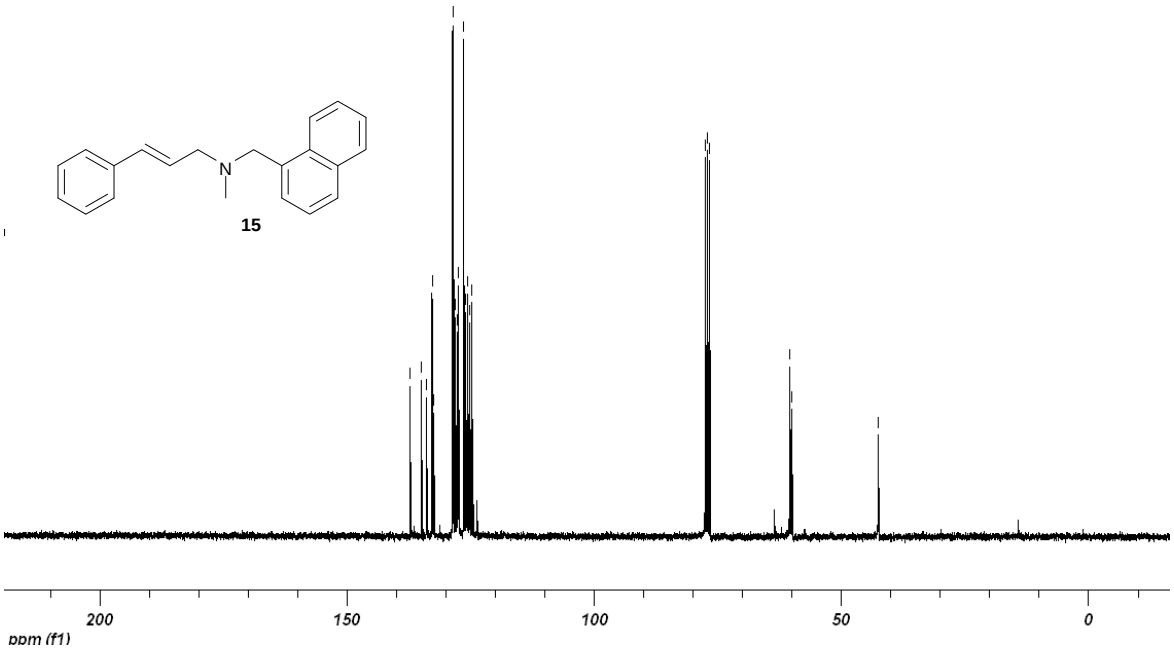
14

(*E*)-*N*-(Methyl)-*N*-(1-Naphthylmethyl)-3-phenylallylamine (Naftifine, 14).









4. References

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2. J. G. Lee, K. I. Choi, H. Y. Koh, Y. Kim, Y. Kang and S. C. Cho, *Synthesis*, 2001, 81-84.
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