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Graphical Abstract

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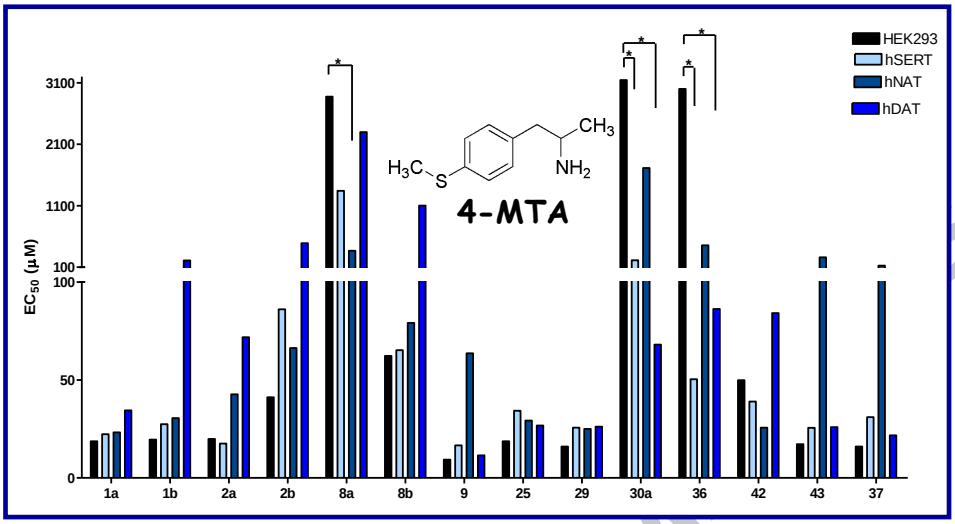

Impurities isolated from clandestine 4-MTA synthesis show cytotoxicity to cells expressing the human serotonin, noradrenaline and dopamine transporters.

Synthesis and *in vitro* toxicity of 4-MTA, its characteristic clandestine synthesis byproducts and related sulphur substituted α-alkylthioamphetamines.

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Abstract

4-Methylthioamphetamine (4-MTA) is recognised as a 3,4-methylenedioxymethamphetamine (MDMA)-like drug of abuse. Such amphetamine-type drugs often contain byproducts of uncontrolled, illegal clandestine synthetic processes. We report the isolation and structural identification of a number of novel pyridines, dihydropyridone and N,N-di(1-aryl-2-propyl) amines as route-specific byproducts associated with clandestine synthesis of 4-MTA and related amphetamines. We report the *in vitro* cytotoxicity of 4-MTA, its synthesis byproducts together with some structurally related sulphur substituted α-alkyl phenethylamines in cell lines overexpressing human monoamine transporters as well as in a primary neuronal cell line model and a dopaminergic neuroblastoma cell line. 4-MTA along with a number of other structurally related amphetamine derivatives and synthetic impurities were found to be cytotoxic to these cells within pharmacologically defined concentrations implying that 4-MTA is a cytotoxic agent *in vitro* and therefore might have the potential to be a neurotoxic agent *in vivo*.

Keywords

4-Methylthioamphetamine (4-MTA)

Leuckart Reaction

Clandestine synthesises

Impurity profiling

Toxicity profiling

Abbreviations

4-MTA	4-Methylthioamphetamine
5-HT	5-Hydroxytryptamine
ALEPH-2	1-(2, 5-Dimethoxy-4-ethylthiophenyl)-2-aminopropane
CNS	Central Nervous System
DAT	Dopamine Reuptake Transporter
DEPT	Distortionless Enhancement by Polarization Transfer
EI	Electron Impact
FBS	Fetal Bovine Serum
He	Helium
HEK	Human Embryonic Kidney Cells
HMQC	Heteronuclear Multiple Quantum Coherence
HRMS	High Resolution Molecular Ion Determination
IR	Infra Red
LRMS	Low Resolution Mass Spectra
MBDB	N-methyl-1- (3, 4-methylenedioxyphenyl)-2-butanamine
MDA	3, 4-methylenedioxyamphetamine
MDEA	Methylenedioxyethamphetamine
MDMA	3, 4 Methylenedioxymethamphetamine
NAT	Noradrenaline Reuptake Transporter
NGF	Nerve Growth Factor
NMR	Nuclear Magnetic Resonance
NOE	Nuclear Overhauser effect

NR	Neutral Red
PCA	*p*-Chloroamphetamine
PMA	*p*-Methoxyamphetamine
SAR	Structure-Activity Relationship
SEM	Standard Error of the Mean
SERT	Serotonin Reuptake Transporter
SSRI	Selective Serotonin Reuptake Inhibitors
TCA	Tricyclic Antidepressant
TMS	Tetramethylsilane
TOCSY	Total Correlation Spectroscopy

1. Introduction

Amphetamine misuse is a significant problem in Europe with new ring-substituted amphetamines such as methylenedioxyethamphetamine (MDEA), N-methyl-1- (3, 4-methylenedioxyphenyl)-2-butanamine (MBDB) and 4-methylthioamphetamine (4-MTA), **(1a)** (Figure 1) constantly appearing on the illicit drug market. As these drugs arise, new drug legislation appears simultaneously in an attempt to halt the manufacture, trafficking and sale of these drugs. 4-MTA was first synthesised in 1963 as an anorectic agent [1] and became the subject of abuse when, in 1997, the first underground laboratory involved in its manufacture was identified in the Netherlands. It is recognized as a 3, 4-methylenedioxymethamphetamine (MDMA)-like drug of abuse [2] and is classified as a Schedule-1 controlled substance [3]. 4-MTA is commonly sold as tablets marked as "Ecstasy" or "flatliners" [4].

Amphetamine samples often contain byproducts of uncontrolled, illegal and clandestine synthetic processes [5-7]. Detailed knowledge of the synthetic methods employed by clandestine chemists towards the manufacture of illegal drugs such as 4-MTA provides valuable information to drug enforcement agencies and may assist in the detection and seizure of illegal underground laboratories with subsequent reduction in supply. Further knowledge of the impurities that are formed as byproducts of illicit drug manufacture enhances the ability of determining the synthetic route employed. Impurities are present in variable quantities, depending on the reaction conditions and purification procedure used (if any). Most amphetamines abused in Europe are believed to be synthesised by the Leukart route [5, 8] which involves the condensation of formamide with the benzylketone precursor (Scheme 1) at elevated temperatures, resulting in the formation of an intermediate N-formyl amphetamine derivative. Subsequent hydrolysis of the N-formyl group affords the desired primary amphetamines. In the present study, 4-MTA and its C-4 analogue are synthesised by a number of routes including the Leuckart-Wallach, reductive amination and oxime and nitrostyrene reduction routes. A comprehensive group of synthesis impurities specific to these routes are isolated and identified.

Recent surveys show that there is an increase in the use of illegal amphetamines with 5.4% of 15-34 year old Europeans admitting to haven taken Ecstasy at some stage in their lives [3]. In the UK, from 1996-2006, there was an average of around 50 drug-related deaths per year involving Ecstasy and it was the sole drug implicated in around 10 cases per year. There have been six reported fatalities associated with 4-MTA in the UK and Netherlands together with one

death and seven non-fatal cases of 4-MTA intoxication in Belgium. Such fatalities are thought to be associated with large doses (21.3-9800mg/kg), the use of other drugs and to inter-individual differences in metabolism [9-11]. As 4-MTA is a relatively new amphetamine derivative and the number of reported poisonings is limited, the pathology findings and the mechanism of death due to this drug have not yet been fully evaluated.

4-MTA has previously been shown to be toxic to rat hypothalamic cultures [12] and to CYP2D6 expressing cells [11] but has been shown to be devoid of serotonin neurotoxic effects in rat models [13]. MDMA and its related metabolites, including 3,4-methylenedioxyamphetamine (MDA) have been found to be toxic to numerous cell lines [14-18] while there is little information on the *in vitro* cellular toxicity of *p*-methoxyamphetamine (PMA) or *p*-chloroamphetamine (PCA). MDMA synthesis byproducts have also been shown to interact with monoamine transporters at a similar potency to MDMA without displaying any neurotoxic effects [19]. To the best of our knowledge, there have been no reports on the identification and potential toxicity of byproducts associated with 4-MTA synthesis. In the second part of this study, we therefore wish to evaluate the *in vitro* monoamine transporter toxicity of 4-MTA along with a representative group of synthetic impurities which may arise from clandestine synthesis of 4-MTA together with some related sulphur substituted α-alkylthioamphetamines. This will be carried out using an *in vitro* cytotoxicity assay using Human Embryonic Kidney (HEK) cells expressing the serotonin, noradrenaline and dopamine transporters, in a primary neuronal cell line model (PC-12) and in a dopaminergic neuroblastoma cell line (SHSY-5Y). Any concentration-dependent *in vitro* cytotoxic effects exerted by these compounds in the cell lines used in this study will provide an insight into the potential of these compounds to behave as neurotoxic agents *in vivo*. Such revelations could have implications for social amphetamine abuse, potentially identifying neurotoxic effects of 4-MTA related compounds present in street drugs.

2. Results and Discussion

2.1 Chemistry

In the present study, 4-MTA (**1a**), the N-methyl analogue **2a**, 2-amino-1- (4-methylthiophenyl) butane (**1b)** and corresponding N-methyl analogue **2b** were synthesised by a number of routes e.g. Leuckart-Wallach and reductive amination, together with oxime and nitrostyrene reduction

routes, as illustrated in Scheme 1. The major synthesis impurities specific to these routes are now isolated and identified.

2.1.1 Route A: Leuckart – Wallach reaction

Most of the amphetamines abused in Europe are believed to be synthesised by the Leuckart and reductive amination routes [5, 8] due to the comparative ease of these synthetic processes. It would be expected that similar routes used in the Leuckart manufacture of amphetamine and the MDA series of amphetamines would also be employed in the synthesis of 4-MTA (**1a**) and related derivative **2a**. The β-nitrostyrene **4a** was prepared *via* a Henry reaction of 4-methylthiobenzaldehyde (**5)**, with an appropriate nitroalkane and cyclohexylamine in a modification of the reported procedure.[20] The nitrostyrene **4a** was converted to the corresponding alkanone **3a** using Fe powder as the reducing agent in glacial acetic acid. Propanone **3a** was also synthesised from phenylacetic acid **6** by refluxing in acetic anhydride and pyridine, with the ketone **7** isolated as a minor product (2%). The Leuckart reaction involves two synthetic steps from the benzylketone **3a**. In the first step, condensation of formamide with the benzylketone **3a** at elevated temperatures results in the formation of an intermediate *N*-formyl amphetamine derivative **8a**. Subsequent hydrolysis of the *N*-formyl group affords the desired primary amine **1a**, (Route A, Scheme 1).

Due to the many possibilities for condensation of the precursor ketone with formamide, a large number of additional compounds could be potentially formed in the first step of the Leuckart synthesis. Van der Ark *et al* have reported the isolation and identification of a series of highly characteristic, route specific impurities which arise from this synthesis of amphetamine[21-24]. Route-specific pyridines have been identified from the Leuckart MDMA syntheses[6, 22]. The tentative presence of pyridines in PMA was reported as detected by GC-MS[25]. Although the presence of two pyrimidines was reported by Kirkbride [20], the comprehensive structural characterisation of the forensically significant signature 4-MTA Leuckart impurities and synthesis byproducts has not yet been reported.

In the present work, we have isolated from the Leuckart reaction five novel pyridines **11-15,** a pyridone **16**, the 2-N-formylamino-1-(4-methylthiophenyl)propane **8a,** N-formyl-N,N-di (1-(4-methylthiophenyl)-2-propyl) amine **17** together with the previously reported pyrimidines **9, 10**[20] as route specific impurities. The structures and isolated amount of the various Leuckart

impurities for the 4-MTA-synthesis route are illustrated in Table 1. The mechanism of formation of the 4-methylthio-pyrimidine and pyridine impurities **11-15** would be similar to that reported for corresponding PMA and MDMA analogues[25]. The compounds **11-17** are previously unreported as 4-MTA impurities and are novel entities.

Identification of the isomeric pyridines **11-15** was achieved by interpretation of the high and low resolution mass spectra, and also by interpretation of the ^{1}H and ^{13}C NMR spectra. Due to the polyaromatic nature of the isolated pyridines, their low resolution mass spectra are dominated by a very intense molecular ion, while the intensities of the fragment ions are significantly lower by comparison. The pyridines **11-15** can be considered as condensation products of two molecules of ketone and one molecule of formamide. The mechanism of formation of pyridines in Leuckart syntheses of amphetamine has been proposed[23, 25]. Orientation of the ketones upon ring cyclisation determines to a large extent the structures of the resulting pyridines.

As an example of the identification of the novel pyridines **11-15**, the ^{1}H NMR spectrum of **13** displays two singlets at δ6.99 and δ8.34, revealing that the pyridine ring is trisubstituted at positions 2, 4 and 5. C-2 is substituted by a 4-methylthiobenzyl group as indicated by the benzylic methylene singlet at δ4.09. A series of NOE experiments provided the evidence for the proposed substitution pattern. Irradiation of the C-2 benzyl singlet resulted in a positive NOE to the pyridine proton singlet, located at δ6.99, which corresponds to H-3. In addition, the irradiation also produces a partial enhancement of the aromatic multiplet, centered at δ7.23 and represents the H-3' and H-5' protons of the C-2 aryl ring system. Irradiation of H-3 afforded the expected reciprocal positive NOE to the benzyl singlet in addition to the methyl singlet at δ2.21, which corresponds to the C-4 methyl group. Finally, when both the C-4 methyl and H-6 proton signals were irradiated, an enhancement of the aromatic doublet, centered at δ7.20 was found. By elimination, the doublet must represent H-3'' and H-5'' of the C-5 aryl system.

Perhaps the most interesting of the characteristic byproducts isolated from the Leuckart synthesis is the novel dihydropyridone **16** (obtained in 0.7% yield) which is formed from the reaction of two molecules of propanone **3a** and one molecule of formamide. The ^{1}H NMR spectrum of **16** exhibits three upfield signals at δ1.26, δ2.46 and δ2.47 corresponding to the methyl group at C-2 and both SCH_3 moieties respectively. The methylene benzyl group at C-2 resonates at δ2.60 as a singlet. Both H-3 protons are diastereotopic and so are represented as two

distinct doublets at δ2.73 and δ3.00 exhibiting geminal coupling constants of J=13.0Hz and J=13.5Hz respectively. A broad doublet centered further downfield at δ4.90 (J = 6.5Hz) is assigned to the amino proton. A total correlation spectroscopy experiment (TOCSY) shows coupling of this proton to the deshielded doublet (J = 3.5Hz) at δ7.25 which corresponds to the vinylic H-6 proton. Addition of D_2O to the NMR sample collapses this signal to a singlet with the concomitant disappearance of the amino proton resonance. The series of four doublets, each integrating for two protons, at δ7.07 (J = 8.0Hz), δ 7.21 (J = 7.5Hz), δ 7.23 (J = 8.0 Hz), and δ 7.32 (J = 8.5Hz) correspond to both A_2B_2 *para* substituted aromatic ring systems. The TOCSY experiment confirmed that protons represented by the doublets at δ7.07 and δ 7.21 are on the same aromatic ring with the more downfield doublets corresponding to protons of the second ring.

A HMQC experiment proved helpful in assigning the ^{13}C NMR spectrum. The C-2 methyl group resonates at 24.86ppm while the signal at 43.36ppm, which is inverted in the DEPT 135^o spectrum, corresponds to C-3. The signal at 56.77 ppm disappears in the DEPT 135^o spectrum and is identified as the aliphatic quaternary C-2 carbon. A further quaternary signal at 110.55ppm is assigned to the vinylic C-5 of the dihydropyridine ring, while the resonance at 147.34 ppm, which remains in the DEPT 135^o spectrum is assigned to the C-6 vinyl carbon. Finally, the conjugated C-4 carbonyl carbon is identified at 189.57ppm. The IR spectrum of **16**, displays the NH absorbance at 3238cm^{-1}, together with a strong stretch at 1567cm^{-1}, which is due to carbonyl stretching involved in hydrogen bonding to the NH proton of a second molecule of **16**.

The most likely mechanism of formation of the pyridone **16** is outlined in Scheme 2, where R represents the 4-methylthiophenyl substituent group. Base catalysed enolisation of propanone **3a** by ammonia (formed by partial hydrolysis of formamide under the reaction conditions) affords a resonance stabilised enolate, which nucleophilically attacks a second molecule of **3a** at the carbonyl carbon. The resulting alkoxide anion is protonated by water or formic acid. The aldol product **A** then undergoes dehydration, giving rise to the conjugated enone **B** which undergoes a conjugate Michael-type addition of formamide , followed by proton abstraction to afford amidoketone **C**. A further base catalysed enolisation can occur, this time with participation of the carbon α to the 4-methylthiophenyl ring. Nucleophilic attack by the enolate on the formyl amide carbon results in the formation of cyclic aldol **D**. A further

dehydration then affords dihydropyridone **17**. A similar route specific impurity has been identified in the synthesis of amphetamine[22]. 2,3-Dihydro-4-pyridones are usually obtained by hetero-Diels-Alder reactions or from cyclisations of enamines derived from α,β-unsaturated 1,3-diketones. [26]

The origin of route specific tertiary amide **17** (obtained in 2% yield) is most likely from a further Leuckart reaction between the initially formed *N*-formyl-4-MTA (**8a**) and propanone **3a**. The compound was isolated as an inseparable 65:35 mixture of diastereomers. 4-Methylthiotoluene was also isolated as a route specific impurity in this synthesis (3.5%). The presence of the route specific Leuckart impurities in the above formamide Leuckart reaction mixture before hydrolysis was confirmed by GC-MS analysis where the impurities were identified at the following retention times **8a** (m/z 209, 10.57min), **9** (m/z 216, 10.38min), **10** (m/z 216, 10.27min), **11**(m/z 351, 27.98min), **12**(m/z 351, 27.97min), **13**(m/z 351, 30.67min), **14**(m/z 365, 28.39min), **15**(m/z 351, 28.61min), **16**(m/z 373, 29.84min), **17**(m/z 369, 9.60min) and 4-methylthiotoluene (m/z 138, 4.18min) by comparison with reference isolated samples of these compounds.

Leuckart-Wallach reaction of 1-(4-methylthiophenyl)-2-alkanone **3a** and *N*-methylformamide results in formation of the 2-(*N*-formyl-*N*-methyl)aminoalkane **18**. Subsequent hydrolysis of the *N*-formyl bond from 2-*N*-formylaminoalkane **8a** and 2-(*N*-formyl-*N*-methyl)aminoalkane **18** afforded the amine products **1a** and **2a** respectively (Scheme 1).

A similar study of the Leuckart-Wallach synthesis of the homologous amine **1b** was investigated with the ketone **3b,** (Scheme 1). This reaction also afforded a signature group of two purines **20** and **21**, five pyridine reaction byproducts **22-26** (listed in Table 2), together with amines **27** and **28**. The symmetrical amine **27** is diastereomeric and it was possible to isolate each diastereomer by flash chromatography. The formation of **27** can be rationalised by two successive reductive aminations between ammonia (formed from initial partial decomposition of formamide to formic acid and ammonia) and two molecules of ketone **3b**, with formic acid acting as the reducing agent. Analogous amines has been detected in the Leuckart reactions of 4-methoxyphenylacetone [25] and 1-(3,4-methylenedioxyphenyl)-2-propanone[27]. The origin of the tertiary amine **28** is most likely from a further Leuckart reaction between **1b** and ketone **3b**. The compound was isolated as an inseparable 60:40 mixture of diastereomers. Leuckart-Wallach reaction of 1-(4-methylthiophenyl)-2-alkanone **3b** and *N*-methylformamide results in formation

of the 2-(*N*-formyl-*N*-methyl)aminoalkane **19**. Subsequent hydrolysis of the *N*-formyl bond from 2-*N*-formylaminoalkane **8b** and 2-(*N*-formyl-*N*-methyl)aminoalkane **19** afforded the amine products **1b** and **2b** respectively (Scheme 1).

2.1.2 Route B: Reductive amination

Ketone **3a** was subjected to reductive amination conditions, with either ammonium acetate or methylamine HCl as nitrogen sources and $NaCNBH_3$ or aluminium amalgam as reducing agents to afford the amines **1a** and **2a** respectively. The novel symmetrical secondary amine **29** was isolated as a significant reaction byproduct in 19% overall yield as an inseparable mixture of diastereomers (in a ratio of 75:25) from the $NaCNBH_3$ reduction of **3a** with ammonium acetate, (Table 1) and was identified by ^{1}H and ^{13}C spectra and by comparison with a similar amine previously identified as a Leuckart derived MDMA impurity [6]. The formation of this product is explained by condensation of the initially formed amine **1a** with propanone **3a**, followed by subsequent reduction of the imine under the reaction conditions. A similar product **27** was isolated as a minor reaction byproduct (0.2% yield) in the reductive amination of the ketone **3b** (Table 2).

2.1.3 Route C: Oxime reduction

The production of primary amines such as amphetamine and MDA is also possible by a two-step synthesis from an appropriate ketone *via* an oxime intermediate.[28, 29] The oximes **30a, 30b** were prepared from ketones **3a, 3b** respectively by reaction with hydroxylamine hydrochloride (Scheme 1). Oxime **30a** was obtained as a 1/3 *syn*/*anti* isomer mixture, whereas oxime **30b** was obtained as a 3/2 *syn/anti* isomer mixture as determined from the chemical shifts of the H-1' and C-1' atoms. In the ^{1}H and ^{13}C NMR spectra of *syn* oximes, benzylic protons resonate at further downfield and carbons further upfield than corresponding resonances of their *anti* oxime isomers. Oxime **30a** was reduced with $LiAlH_4$ to afford 4-MTA (**1a**) in 50% yield, together with unreacted oxime **30a** (18%), while reduction of **30b** afforded the amine **1b** (30%) together with unreacted oxime (1%) (Tables 1 & 2).

2.1.4 Route D: Reduction of nitrostyrenes

Reduction of nitrostyrene **4a** with $LiAlH_4$ afforded 4-MTA **1a** in 80% yield with the oxime **30a** (0.6%) and aziridine **31** (3%) identified as the major impurities, (Table 1). A similar reduction of the nitrostyrene **4b** afforded the amine **2a** (74%) together with the aziridine **32** (7%)[30] . The structures of the aziridines **31** and **32** (which were found to be unstable and decomposed rapidly) were confirmed to be the *cis* configuration from ^{1}H NMR spectrum in which the H-2 proton was observed as a doublet, δ3.22, $J_{2,3}$ = 5.5Hz.

2.1.5 Synthesis of structurally related 1,3-bis(4-methylthiophenyl)-2-propanamines

The synthesis of a number structurally related 1,3-bis(4-methylthiophenyl)-2-propanamines for investigation in the toxicity study was also explored as illustrated in Scheme 3. These amines are derived from the diaryl propanone compound **7**, already isolated as a minor product in the preparation of the propanone **3** from 4-methylthiophenylacetic acid[3], and therefore could be regarded as a possible source of further route specific impurities in the synthesis of 4-MTA[19]. The related MDMA 1,3-bis(3,4-methylenedioxyphenyl)-2-propanamines were reported to be weakly active at monoamine transporters, with uptake inhibitory potencies similar to MDMA in low micromolar range[19]. Reduction of nitroethene **33**, followed by Henry reaction of **34** with 4-methylthiobenzaldehyde **5** gave the corresponding 2-nitro-1,3-diphenylprop-1-ene **35** which was treated with Fe/AcOH to afford the propanone **7**. Subsequent reaction of ketone **7** with formamide gave the **36** which on hydrolysis afforded primary amine **37** in 84% yield. $LiAlH_4$ reduction of the oxime **38** also afforded the primary amine **37** in 17% yield together with the novel aziridine **39** as the major reaction product in 61% yield. The aziridine **39** was isolated as the *cis* isomer determined by the H-2 and H-3 ^{1}HNMR coupling constant of 6.5Hz. Hydrolysis of **40** (produced from **7** on treatment with N-methylformamide) afforded the corresponding N-methyl product **41**. **37** and **41** are analogous in structure to previously reported Leuckart derived MDA[6] and MDMA impurities[24]. The N-ethylamine analogue **43** was obtained by $LiAlH_4$ reduction of the N-acetyl compound **42**.

2.2 Biological Activity

Using an *in vitro* screen for cytotoxicity on HEK293 cells stably expressing each of the human monoamine transporters, SERT (serotonin transporter), NAT (noradrenaline transporter)

and DAT (dopamine transporter) (Supplemental Figure 1), on a dopaminergic cell line model (SHSY-5Y) as well as on a primary differentiated neuronal cell line (PC-12) model provides a quick and economical method for assessing some aspects of the potential neurotoxicity of amphetamines and their related synthetic derivatives and impurities. To classify a drug as 'neurotoxic' requires further experimentation such as the use of animal models or glutamate and serotonin release experiments. However, any evidence of selective *in vitro* cytotoxicity toward either one or more of the monoamine transporters compared to the control HEK cell line implies that such a chemical may have an *in vivo* serotonergic, dopaminergic or noradrenergic toxic effect and provides useful information for studying further the possible mechanism of action of such compounds.

2.2.1 *In vitro* cytotoxicity to monoamine transporters.

It has been difficult to determine if amphetamines are genuine substrates of the monoamine transporters, due to their lipophilic nature and to the lack of a co-crystallised structural determination with human SERT, NAT or DAT. However, amphetamines are thought to release stores of catecholamines from nerve endings by converting the respective molecular transporters into open channels They are thought to compete with substrate for the transporters, reversing the transport of monoamines by either binding to the transporter as a substrate or binding without being transported [31]. Amphetamine analogues such as 4-MTA, MDMA, MDA and PCA have all been previously shown to inhibit SERT, NAT and DAT activities with IC_{50} values of 74nM, 2,375nM, 3,073nM (4-MTA) , 425nM, 405nM, 1,442nM (MDMA), 478nM, 266nM, 890nM (MDA) and 182nM 207nM, 424nM (PCA) for SERT/NAT/DAT respectively[32]. PMA is another common substituted amphetamine that is thought to act in a similar way to MDMA, with evidence of PMA decreasing synaptosomal 5-HT uptake and content[32].

Toxicity of 4-MTA in animal studies is thought to be related to its ability to induce a hyperserotonergic state known as serotonin syndrome which is characterised by a number of symptoms including fever, confusion, shivering, diaphoresis, ataxia, hyperreflexia and diarrhoea[32]. In humans adverse effects upon 4-MTA ingestion include nausea, hyperthermia, memory loss, nystagmus, thirst, shivering, confusion, sweating and amnesia. Metabolic pathways for 4-MTA have only been demonstrated *in vivo* in mice and *in vitro* in primary hepatocytes[32]. In humans, metabolism of 4-MTA is speculated to involve the following: (i) oxidative

deamination to a ketone metabolite which can be further reduced to the corresponding alcohol or suffer degradation of the side chain to produce the 4-methylthiobenzoic acid metabolite, (ii) ring hydroxylation to a phenolic structure (iii) β-hydroxylation of the side chain to 4-methylthioephedrine or (iv) oxidation of the thioether[32].

The compounds selected for this study of 4-MTA(**1a**) were the related amphetamines **1b**, **2a** and **2b** together with some representative synthesis related byproducts **8a**, **8b**, **9**, **25**, **29** and **30a** selected from the Leuckart, reductive amination and oxime routes. The structurally related 1, 3-bis (4-methylthiophenyl)-2-propanamines **36**, **37**, **42** and **43** were also included in the study. The cytotoxicity was assessed in HEK293 cells stably expressing the human monoamine transporters SERT, NAT and DAT as well as in a dopaminergic (SHSY-5Y) and a primary neuronal cell line model (PC-12). The results are displayed in Tables 3 and 4. As EC_{50} values were estimated from Log-concentration sigmoidal dose response curves, the cytotoxic potency of each compound was quantified by a pEC_{50} value, where pEC_{50} is $-[-LogEC_{50}] \pm S.E$ (log EC_{50} is the log [Dose] when response is equal to 50% cell viability). To determine if any of the derivatives had a selective cytotoxic effect to any of the cells lines, the effects of each derivative in each of the cell lines was compared in a two-way ANOVA test (GRAPH pad Prism 4) with no matching followed by a Bonferroni Post Test. P values of <0.05 were considered to reflect a significant difference.

2.2.1.1 4-MTA and a number of related compounds showed no transporter-selective cytotoxic effects

In this study, 4-MTA (**1a**) was found to be toxic to all of the human monoamine transporter expressing cell lines as well as to the control HEK293 cell line with EC_{50} values in the low micromolar range (10-40μM) (Table 3 and Figure 2). 4-MTA (pEC_{50} values from 4.38-4.73) was also found to be more toxic than MDMA (pEC_{50} values from 2.71-3.61) and MDA (pEC_{50} values from 2.23-3.49) in these cell lines ($P<0.05$) (Table 3).

Many of the compounds presented in Table 3 were found by this study to have a similar effect to 4-MTA. One such compound, the secondary amine (**29**) is the most abundant synthetic byproduct from the reductive amination route for synthesis of 4-MTA, (obtained in 19% yield) which also displayed a general toxic effect on all of the catecholamine expressing cell lines as well as the control HEK293 cell line. As the amine **29** occurs in significant amount in 4-MTA

obtained by the reductive amination route, it would be interesting to investigate the levels of this compound in seized 4-MTA street samples.

The 4-MTA analogue (**2a**), two representative heterocyclic Leuckart-type impurities including the pyrimidine (**9**) and the pyridine (**25**), as well as the N-acetylamine derivative (**42**) all had a similar general toxic effect to 4-MTA (Table 3 and Figure.2). Although no extensive SAR (Structure-Activity-Relationship) analysis was carried out, it appears that the introduction of a methylthio group at position 4 on the amphetamine aryl ring as in 4-MTA and **1b** could be responsible for the increased cytotoxic effects of these compounds, when compared to MDMA which contains the 3, 4-methylenedioxy substitution in aryl ring.

4-MTA is known to be highly selective for SERT compared to DAT (ratio of IC_{50} value for serotonin versus dopamine: $3.07x10^3$) [13] and NAT (ratio IC_{50} value for serotonin versus noradrenaline: $2.37x10^3$) [13]. Although the SERT, NAT and DAT binding activities of these compounds were not determined by this study, it appears that SERT, NAT or DAT expression alone may not be sufficient to confer 4-MTA- (and the above associated compounds) toxicity to HEK cell lines. This is consistent with previous reports of 4-MTA having a SERT-independent *in vitro* cytotoxic effect[32]. To the best of our knowledge this study is the first report comparing the cytotoxic effects of 4-MTA as well as the cytotoxicities of a range of different 4-MTA synthetic derivatives and sulphur substituted α-alkylthioamphetamines on cell lines overexpressing human monoamine transporters.

2.2.1.2 Compounds not toxic to any of the monoamine transporter-expressing cell lines

In this study, the only amphetamine-type compound investigated that had no cytotoxic effect to any of the cell lines was **8a**, which is the N-formyl derivative of 4-MTA. **8a** is one of the major impurities of 4-MTA from the Leuckart route (A), and is obtained in 3.2% yield. Analysis of the toxicity of this impurity revealed that it had little effect on all of the monoamine transporter-expressing cell lines with approximate EC_{50} values of between 500μM and 1mM (Figure 2). **8a** features the N-formyl group rather than the primary amine which is found in the other members of the 4-thiomethylamphetamine series e.g. **1a** that were found by this study to be cytotoxic. Further SAR analysis is needed to confirm if the primary or secondary amine is a pre-requisite for toxic activity in these compounds as the observation that compound **8b** has a similar cytotoxic effect to 4-MTA and **1b**, **2a**, **2b,** despite the presence of the N-formyl group rather than

a primary or secondary amine. The N-formyl compounds **8a** and **8b**, because of amide resonance effects have reduced availability of the lone pair of electrons on the nitrogen and may have altered target binding and reactivity when compared with parent amine **1a**.

2.2.1.3 Derivative selectively cytotoxic to monoamine transporters only

Compound **36** is an N-formyl amine derivative of 1, 3-bis (4-methylthiophenyl)-2-propanamine, and is structurally related to 4-MTA. **36** was the only impurity found to be selectively toxic to all of the human monoamine transporter-expressing cell lines ($P<0.05$) with an average estimated EC_{50} of between 10μM and 50μM (Table 3 and Figure 2). The activity of **36** therefore differs from **8a** (N-formyl derivative of 4-MTA) which was not toxic to all cell lines.

2.2.1.4 Compounds not cytotoxic to the hDAT cell line

A number of compounds presented in Table 3 were found by this study to have little effect on the dopamine transporter-expressing cell line ($P<0.05$). This cell line was found to be insensitive to the toxic effects of the N-methyl analogue of 4-MTA (**1b**), the N-methyl-1-(4-methylthiophenyl) butane (**2b**) and 2-N-formylamino-1-(4-methylthiophenyl) butane (**8b**),which is a significant synthetic byproduct of the Leuckart synthetic route for the butane analogue **2a**. Each of these derivatives had a cytotoxic effect on the SERT, NAT and control HEK cell lines with EC_{50} values in the low micromolar range (Figure 2). The above data suggests that hDAT may confer a protective effect to HEK293 cells against the above compounds. This maybe a direct result of these compounds preferentially binding to hDAT and consequently sequestering the cytotoxic activity. As these compounds appear to be having an equipotent effect on HEK control cells and on HEK cells expressing SERT and NAT, it is unlikely that these compounds elicit their toxic effects through SERT or NAT. Investigating the SERT/NAT/DAT binding of these compounds will provide further information into the mechanism of action of these toxic compounds.

Interestingly, both MDMA and MDA were also less toxic to the hDAT cell line compared to the other cell lines ($P<0.05$), with pEC_{50} values of 2.71 ± 0.15 and 2.23 ± 0.38 respectively (Table 3). MDMA and MDA have a lower binding affinity for hDAT, binding to SERT/NAT/DAT with IC_{50} values of 425nM, 405nM, 1,442nM (MDMA) [33] and 478nM,

266nM, 890nM (MDA) [13] respectively. Therefore, hDAT expression may confer a protective effect to HEK cells upon MDMA or MDA treatment, perhaps due to the lack of MDMA/MDA binding to these cells or through a yet undefined mechanism involving another target.

It is also of interest that the oxime compound **30a** which is a significant synthesis impurity in 4-MTA derived from the oxime reduction route**,** had the opposite effect on the hDAT cell line. This compound was found to have little effect on the hSERT, hNAT and HEK293 cell lines (EC50 value of 500µM-1mM) but displayed a selective cytotoxic effect to the hDAT (EC_{50} value of 50-100µM) cell line ($P<0.05$). This result may imply that this oxime impurity is selectively neurotoxic to dopaminergic cells.

2.2.1.5 Compounds not toxic to hNAT

The primary amine **37**, which is structurally related to 4-MTA, together with the related N-ethylamine **43** were found to be less toxic to the hNAT cell line (pEC_{50} values of 3.90 ± 0.18 (**37**) and 3.58 ± 0.16 (**43**)) compared to the hSERT, HEK293 and hDAT cell lines ($P<0.05$). The above data suggests that hNAT may confer a protective effect to HEK293 cells against the above compounds. This maybe a direct result of these compounds preferentially binding to hNAT and consequently sequestering the cytotoxic activity. As these compounds appear to be having an equipotent effect on HEK control cells and on HEK cells expressing SERT and DAT, it is unlikely that these compounds elicit their toxic effects through SERT or DAT. Again, investigating the SERT/NAT/DAT binding of these compounds will provide further information into the mechanism of action of these toxic compounds.

2.2.1.6 The effects of representative compounds on the PC-12 cell line

4-MTA together with some relevant synthesis byproducts and structurally related amines were then tested on the nerve growth factor (NGF) differentiated PC-12 cell line, (Table 4, Figure 3). The PC-12 cell line is derived from a transplantable rat pheochomocytoma [34] that when supplemented with NGF displays a differentiated morphology from a proliferating cell to a post-mitotic, neurite bearing neuron. The PC-12 cell line is a classical *in vitro* neuroendocrine cell line model, [34] that has been previously used to test the potential neurotoxicity of a range of drugs [35]. Using this model, 4-MTA (**1a**) was found to be cytotoxic to the PC-12 cell line at high concentrations (Figure 3) with an approximate pEC_{50} value of 3.53 ± 0.13 (corresponds to an

approximate EC_{50} range of between 160 and 600μM) (Table 4). This data is consistent with previous reports showing that 4-MTA is neurotoxic particularly at high concentrations [11, 12, 36-39]. These results also show that 4-MTA is more toxic to PC-12 cells than MDA and MDMA which have been previously shown to have little effect on the PC-12 cell line (IC_{50} values of 3365 μM (MDA) and 4464 μM (MDMA) respectively) [15].

The N-methyl-4-MTA derivative **1b**, together with significant reductive amination synthesis byproduct **29** were also found to behave in a similar way to 4-MTA having a potent cytotoxic effect at high concentrations (Table 4 and Figure 3). Derivative **29** was found to have a greater pEC_{50} value than 4-MTA (4.56 versus 3.53) ($P<005$) implying that this derivative is more cytotoxic to the PC-12 cell line than 4-MTA itself. The pyridine **25** and the N-ethylamine **43** also demonstrated a similar toxicity profile, showing more toxicity than 4-MTA at 40μM. Derivative **25** was also found to have a significantly greater pEC_{50} value than 4-MTA ($P<0.005$) implying it is more toxic to the PC-12 cell line than 4-MTA (Table 4). These compounds were also found to have a general toxic effect to HEK293 and to HEK cells overexpressing hSERT, hNAT and hDAT (Section 2.2.1.1 above). Compound **36** was also found to have a significantly greater ($p<0.05$) pEC_{50} value than 4-MTA in the PC-12 cell line, having a cytotoxic effect at 40μM (Figure 3) again implying this derivative is more toxic to the PC-12 cells than 4-MTA (Figure 3 and Table 4). This derivative also had an effect against the hSERT, hNAT and hDAT overexpressing cell lines but had little effect on the HEK293 cell line (Table 3).

Of the other compounds screened in the PC-12 cell line, the majority also displayed cytotoxic activity in the PC-12 cell line with pEC_{50} values in the range of 2.38-4 (corresponding to an approximate EC_{50} range of between 200μM-1mM). Compounds **8b**, **30a**, **37** and **43** all had a potent cytotoxic effect in the PC-12 cell line at 1mM (Figure 3), comparable to the effect induced by 4-MTA at 1mM. Compounds **8a** and **42** were found to have no effect on the PC-12 cell line.

2.2.1.7 The effects of representative compounds on the SHSY-5Y cell line

The SHSY-5Y cell line SH-SY5Y is a thrice cloned (SK-N-SH -> SH-SY -> SH-SY5 -> SH-SY5Y) subline of the human neuroblastoma cell line SK-N-SH established from a metastatic bone tumour [40]. This cell line was chosen for use in this screen as is widely used as a model for dopaminergic cells and it has been shown not to express SERT [40]. This line has been previously

used to test the potential neurotoxicity of a range of drugs[41] including a number of catecholamine derivatives.[42]. We have previously reported that 4-MTA demonstrated cytotoxicity for the SHSY-5Y cell line [32] (pEC_{50} of 3.29) . In the present study a number of the compounds in particular **25**, **29**, **36**, **37** and **43** showed significant cytotoxicity to the SHSY-5Y cell line with pEC_{50} values in the range 4.65, 4.49, 4.73 and 4.86 respectively, correlating to an EC_{50} range of approximately 10-33μM. (Table 4 and Figure 3). These compounds were also significantly more cytotoxic to the SHSY-5Y cells than 4-MTA ($p<0.05$). This result is in contrast to the effects obtained for MDMA and MDA which were shown not to have any significant effect on SHSY-5Y cell line ($_pEC_{50}<2$) . Compounds **1b**, **30a** and **42** were found to behave in a similar way to 4-MTA having a potent cytotoxic effect at high concentrations (Table 4 and Figure 3). These compounds were also found to have a general toxic effect to HEK293 and to HEK cells overexpressing hSERT, hNAT and hDAT (Section 2.2.1.1). Compounds **8a, 8b and 9** were found to have no effect on the SHSY-5Y cell line. Although many of the compounds did not appear to be selective in toxicity for the monoamine transporters, there appears to be some correlation of activity between HEK293hDAT cytotoxic effects and SHSY-5Y cytotoxicity for compounds **29**, **37** and **43.**

3. Conclusion

4-MTA is known to be more dangerous than other designer drugs of abuse because of its slow onset of action encouraging abusers to administer further doses assuming that the first dose was inadequate thus leading to fatally high concentrations of 4-MTA and its derivatives *in vivo* [3]. In this study we report for the first time that 4-MTA is toxic to monoamine transporter-expressing cells *in vitro* having a more potent effect than MDA and MDMA. Such concentrations are pharmacologically comparable to *in vivo* reported fatal 4-MTA intoxications, which have consisted of blood concentrations of 4-MTA between 7.7 and 29.6μM [9, 38, 39, 43] and tissue (brain or liver) concentrations of 170-201.6μM [38, 44].

In this study, the synthetic methods employed by clandestine chemists towards the manufacture of ring-substituted amphetamines were used to create a library of 4-MTA-like-compounds. This study also reports, for the first time the cytotoxic activities of some representative 4-MTA synthetic byproducts and derivatives on the human monoamine transporters, SERT, NAT and DAT and on the PC-12 and SHSY-5Y neuronal cell line models.

Out of 14 representative derivatives investigated by this study, the majority had a similar effect to 4-MTA displaying cytotoxic EC_{50} values in the low micromolar range (1μM-50μM). A number of the derivatives used in this study, including 4-MTA itself were found to be more toxic than MDA and MDMA to the primary neuronal PC-12 cell line model and to the dopaminergic neuroblastoma SHSY-5Y cell line. Such a concentration dependent effect by 4-MTA and its related synthetic derivatives in these cell lines implies that these derivatives are cytotoxic agents *in vitro* and therefore might have the potential to act as neurotoxic agents *in vivo*. Future investigations utilising a variety of *in vitro* and *in vivo* models will be required to gain a better understanding of their possible *in vivo* neurotoxic potential and mechanism of action.

4. Experimental

4.1. Chemistry

Uncorrected melting points were measured on a Gallenkamp apparatus. Infra-red (IR) spectra were recorded on a Perkin Elmer FT-IR Paragon 1000 spectrometer. ^{1}H, ^{13}C and ^{19}F nuclear magnetic resonance (NMR) spectra were recorded at 27°C on a Brucker DPX 400 spectrometer (400.13MHz, ^{1}H; 100.61MHz, ^{13}C; 376.47MHz, ^{19}F) in either $CDCl_3$ (internal standard tetramethylsilane (TMS)) or CD_3OD. Low resolution mass spectra (LRMS) were acquired on a Hewlett-Packard 5973 MSD GC-MS system in electron impact (EI) mode. GC-MS analysis was performed using an Agilent 6890 gas chromatograph with split-splitless injection (2 ml injected) and a HP-5MS column (30 m x 0.25 mm, 0.25 mm film thickness). Helium (He) was used as the carrier gas at a flow rate of 1.0ml/minute. The GC was coupled to an Agilent 5973 MSD (EI, 70eV, TIC mode scanning m/z 50-800). The following temperature program was used: 90^{o}C for 0.1 minutes, 15^{o}C/minute to 250^{o}C, 250^{o}C for 1 minute, 2^{o}C/minute to 300^{o}C, 300^{o}C for 5 minutes, injector port 270^{o}C, transfer line, 280^{o}C. High resolution molecular ion determinations (HRMS) were acquired on either a Kratos Profile HV-4 mass spectrometer using a direct insertion probe and EI mode (Department of Chemistry, University College Cork) or a Micromass mass spectrometer (EI mode) at the Department of Chemistry, Trinity College, Dublin. Elemental analyses were performed on an Exetor Analytical CE4400 CHN analyser in the microanalysis laboratory, Department of Chemistry, University College Dublin. R_f values are quoted for thin layer chromatography on silica gel Merck F-254 plates, unless otherwise stated. Flash column chromatography was carried out on Merck Kieselgel 60 (particle size 0.040-

0.063mm), Aldrich aluminium oxide, (activated, neutral, Brockmann I, 50 mesh) or Aldrich aluminium oxide, (activated, acidic, Brockmann I, 50 mesh).

4.1.1 1-(4-Methylthiophenyl)-2-propanone (3a).

A suspension of iron powder (573 mmol, 32.0 g) in glacial acetic acid (150 mL) was heated on a steam bath for 20min, stirring occasionally. To this mixture, a solution of nitrostyrene **4a** (126.0 mmol) in glacial acetic acid (150 mL) was added over 20 min, while stirring the reaction occasionally. The reaction was heated for a further 2 h and the added to a mixture of ice/water (1200 mL). The product was extracted with dichloromethane (4x100 mL) and organic extracts washed with 15% aq. NaOH (3x100 mL). The organic layer was then dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and the resulting residue vacuum distilled to afford the product as a colourless oil (82%) (lit.[20] bp 133ºC/0.4mmHg).

4.1.2 1-(4-Methylthiophenyl)-2-butanone (3b).

A suspension of iron powder (57.3 mmol, 3.20 g) in glacial acetic acid (15 mL) was heated on a steam bath for 20 min, stirring occasionally. To this mixture, a solution of the nitrostyrene **4b**[45] (3.19 g, 14.30 mmol) in glacial acetic acid (15 mL) was added over 20 min, while stirring the reaction occasionally. The reaction was heated for a further 2 h when the mixture became a grey-white colour. The reaction was allowed to cool to ambient temperature and added to a mixture of ice/water (120 mL). The product was extracted with dichloromethane (4x100 mL) and organic extracts washed with 15% aq. NaOH (3x100 mL). The organic layer was then dried over anhydrous Na_2SO_4, concentrated *in vacuo*, and the resulting residue vacuum distilled, producing the desired ketone as a colourless solid (96%), mp 38-39ºC, lit. bp 116 °C/0.25mm Hg[46]. IR ν_{max} (KBr) 1711 (C=O) cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.02 (3H, t, $J_{4',3'}$=7.3Hz, H-4'), 2.26 (2H, q, $J_{3',4'}$=7.3Hz, H-3'), 2.46 (3H, s, SCH_3), 3.63 (2H, s, H-1'), 7.12, 7.22 (4H, 2xd, J=8.5, J=8.5Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 7.73, 15.95, 35.18, 49.11, 127.05, 129.81, 131.29, 137.04, 208.70. MS *m/z* 194 (M^+). Anal. Calculated for $C_{11}H_{14}OS$; C, 68.00; H, 7.26. Found: C, 68.24; H, 7.40%.

4.1.3 1-(4-Methylthiophenyl)-2-nitro-1-propene (**4a**).

A solution of 4-methylthio benzaldehyde (**5**) (300 mmol, 45.66 g), nitroethane (600 mmol, 45.04 g, 43.10 mL), cyclohexylamine (300 mmol, 29.76 g, 34.30 mL) in glacial acetic acid (240 mL) was heated on a steam bath for 6 h. Water (125 mL) was then added and the mixture was then allowed to stand at room temperature overnight. The resulting crystalline precipitate was isolated by vacuum filtration and washed with water (250 mL). Recrystallisation from ethanol afforded the pure product as yellow crystals (53.79 g, 257.04 mmol, 86%), mp 72-73ºC (ethanol), (lit mp. 71-73ºC[20]). R_f 0.53 (hexane/diethylether : 80/20). IR ν_{max} (KBr) 1648, 1509, 1312 cm^{-1}. ^1H NMR δ ($CDCl_3$) 2.46 (3H, s, H-3'), 2.52 (3H, s, SCH_3), 7.29, 7.37 (4H, 2xd, J=8.5Hz, J=8.5Hz, H-2, H-3, H-5, H-6), 8.04 (1H, s, H-1'). ^{13}C NMR ppm ($CDCl_3$) 14.11, 15.00, 125.89, 130.46, 128.58, 133.15, 142.14, 146.87. MS *m/z* 209 (M^+).

4.1.4 1-(4-Methylthiophenyl)-2-propanone (3a) *via* 4-methylthiophenylacetic acid (6).

A stirred solution of 4-methylthiophenylacetic acid (**6**) (8.23 mmol, 1.50 g), pyridine (4.15 mL) and acetic anhydride (4.15 mL) was refluxed for 7hr. After cooling the reaction was diluted with 10% aq. HCl (100 mL) and extracted with diethylether (3x50 mL). The organic phases were combined, dried over anhydrous Na_2SO_4 and solvent removed *in vacuo*. The residue was purified by flash chromatography, providing the desired ketone (20%) (**3a**), and 1,3-di(4-methylthiophenyl)-2-propanone (**7**) as colourless needles (2%), mp 80-81ºC (diethylether/hexane), (lit. mp 79-80ºC[47]).

4.1.5 1-(4-Methylthiophenyl)-2-nitro-1-butene (4b).

Compound **4b** was prepared from 4-methylthiobenzaldehyde (**5**), (40 mmol, 6.09 g) and nitropropane (80 mmol, 7.12 g, 7.13 mL) according to general procedure for **4a**. The crude product was purified by flash chromatography on silica (eluent : hexane/diethylether : 80/20) and obtained as a yellow oil (82%)[45]. R_f 0.56 (hexane/diethylether : 70/30). IR ν_{max} (film) 1647, 1508, 1324 cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.28 (3H, t, $J_{4',3'}$=7.5Hz, H-4'), 2.52 (3H, s, SCH_3), 2.88 (2H, q, $J_{3',4'}$=7.5Hz, H-3'), 7.29, 7.36 (4H, 2d, J=8.5Hz, J=8.0Hz, H-2, H-3, H-5, H-6), 7.98 (1H,

s, H-1'). ^{13}C NMR ppm ($CDCl_3$) 12.34, 15.00, 20.81, 125.99, 130.17, 128.51, 132.73, 142.21, 152.47. MS *m/z* 223 (M^+).

4.1.6 Preparation of 4-methylthioamphetamine (1a) via Leuckart-Wallach reaction

A mixture of formamide (70g) and the ketone (**3a**) (117.88 mmoL, 21.01 g) was heated at 190ºC for 5 h. After cooling the reaction was diluted with water (150 mL) and extracted with ethylacetate (3x75 mL). The extracts were combined, washed with water (3x50 mL) and dried over anhydrous Na_2SO_4. After removing all volatiles *in vacuo* the resulting dark brown oil was dissolved in methanol (30 mL) and 30% aq. HCl (150 mL) an the mixture was heated under reflux for 7 h. Upon cooling, the reaction was diluted with water (100 mL), made basic (pH 14), with 15% aq. NaOH and extracted with dichloromethane (4x100 mL). The extracts were combined and dried over anhydrous Na_2SO_4. All volatiles were removed *in vacuo*, affording a dark brown oil. Isolation of the desired product **1a**, together with the following reaction byproducts was performed by flash column chromatography over silica gel.

4.1.7 2-Amino-1-(4-methylthiophenyl)propane, 4-MTA(1a)

(4-MTA) (**1a**) was obtained as an amber oil (48%) (lit. bp 108-118°C/0.6mm Hg[48]). IR ν_{max} (film) 3359, 3285 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.10 (3H, d, $J_{3',2'}$=6.5Hz, H-3'), 1.25 (2H, br s, NH_2), 2.46 (3H, s, SCH_3), 2.48 (1H, dd, J_{gem}=13.3Hz, $J_{1',2'}$=8.0Hz, H-1'), 2.67 (1H, dd, J_{gem}=13.3Hz, $J_{1',2'}$=5.0Hz, H-1'), 3.13 (1H, m, H-2'), 7.10, 7.21 (4H, 2xd, J=8.0Hz, J=8.0Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 16.13, 23.48, 46.04, 48.36, 127.01, 129.68, 135.76, 136.71. **HCl salt**. Colourless solid. mp 187-189ºC (ethanol/hexane), (lit. mp 190-191°C[49]). MS *m/z* 181 (M^+) The following synthesis impurities were also isolated:

4.1.8 2-*N*-Formylamino-1-(4-methylthiophenyl)propane (8a)

2-*N*-Formylamino-1-(4-methylthiophenyl)propane (**8a**) was isolated by flash chromatography on silica gel (eluent : ethyl acetate). Pale brown solid (3.2%), mp 70-72°C. IR ν_{max} (KBr) 3333, 1660 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.11 (2.4H, d, $J_{3',2'}$=6.5Hz, H-3'), 1.23 (0.6H, d, $J_{3',2'}$=6.5Hz, H-3'), 2.43 (3H, s, SCH_3), 2.60-2.81 (0.4H, m, H-1'), 2.66 (0.8H, dd, J_{gem}=13.6Hz,

$J_{1',2'}$=7.0Hz, H-1'), 2.79 (0.8H, dd, J_{gem}=13.6Hz, $J_{1',2'}$=6.5Hz, H-1'), 3.64 (0.2H, m, H-2'), 4.26 (0.8H, m, H-2'), 6.30 (0.8H, d, J=7.6Hz, NH), 6.35 (0.2H, d, J=10.6Hz, NH), 7.05, 7.18 (0.8H, 2d, J=8.0Hz, J=8.6Hz, H-2, H-3, H-5, H-6), 7.09, 7.17 (3.2H, 2xd, J=8.5Hz, J=8.5Hz, H-2, H-3, H-5, H-6), 7.73 (0.2H, d, J=11.5Hz, CHO), 8.00 (0.8H, s, CHO). ^{13}C NMR ppm ($CDCl_3$) 15.58, 15.66*, 19.66*, 21.57, 41.48*, 43.52, 44.80*, 49.74, 126.52*, 126.62, 129.58*, 129.64, 134.12, 134.57*, 135.98, 136.43*, 160.42*, 163.52. MS *m/z* 209 (M^+), Anal. Calculated for $C_{11}H_{15}NOS$: C, 63.12; H, 7.22; N, 6.69. Found: C, 63.13; H 7.09; N, 6.58%.

4.1.9 4-(4-Methylthiobenzyl)pyrimidine (9)

4-(4-Methylthiobenzyl)pyrimidine (**9**) was isolated by flash chromatography on silica gel (eluent : diethylether/ethylacetate 50/50) as an amber oil (0.5%). [20] IR ν_{max} (film) 2856, 1577 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 2.46 (3H, s, SCH_3), 4.07 (2H, s, CH_2), 7.10 (1H, dd, $J_{5,6}$=5.0Hz, H-5), 7.18, 7.23 (4H, 2xd, J=8.0Hz, J=8.0Hz, H-2', H-3', H-5', H-6'), 8.58 (1H, d, $J_{6,5}$=5.0Hz, H-6), 9.13 (1H, s, H-2). ^{13}C NMR ppm ($CDCl_3$) 15.92, 43.58, 127.15, 129.67, 134.14, 137.16, 157.00, 158.73, 169.22. MS *m/z* 216 (M^+).

4.1.10 4-Methyl-5-(3,4-methylthiophenyl)pyrimidine (10)

4-Methyl-5-(3,4-methylthiophenyl)pyrimidine (**10**) was isolated by flash chromatography on silica gel (eluent : diethylether/hexane 80/20) as an amber oil (2%).[20] IR ν_{max} (film) 2856, 1598 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 2.52 (3H, s, CH_3), 2.54 (3H, s, SCH_3), 7.25, 7.36 (4H, 2xd, J=8.0Hz, J=8.5Hz, H-2', H-3', H-5', H-6'), 8.51 (1H, s, H-6), 9.06 (1H, s, H-2). ^{13}C NMR ppm ($CDCl_3$) 15.44, 22.78, 126.40, 129.28, 132.18, 134.23, 139.32, 156.18, 157.02, 164.32. MS *m/z* 216 (M^+).

4.1.11 2,4-Dimethyl-3,5-di(4-methylthiophenyl)pyridine (11)

2,4-Dimethyl-3,5-di(4-methylthiophenyl)pyridine (**11**) was isolated by flash chromatography on silica gel (eluent : diethylether/hexane : 65/35). Amber oil (0.3%). IR ν_{max} (film) 2851, 1597 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.94 (3H, s, CH_3), 2.31 (3H, s, CH_3), 2.52 (3H, s, SCH_3), 2.54 (3H, s, SCH_3), 7.11, 7.25, 7.32, 7.35 (8H, 4xd, J=8.5Hz, J=8.5Hz, J=8.5Hz, J=8.0Hz, H-2', H-2", H-3', H-3", H-5', H-5", H-6', H-6"), 8.31 (1H, s, H-6). ^{13}C NMR ppm

($CDCl_3$) 15.62, 15.72, 18.09, 23.71, 126.40, 126.73, 129.40, 129.90, 134.97, 135.18, 135.80, 136.39, 137.66, 137.90, 142.94, 147.96, 155.10. MS *m/z* 351 (M^+), HRMS Calculated for $C_{21}H_{22}NS_2$: (M^++H) 352.1194; Found: 352.1174.

4.1.12 2,6-Dimethyl-3,5-di(4-methylthiophenyl)pyridine (12)

2,6-Dimethyl-3,5-di(4-methylthiophenyl)pyridine (**12**) was isolated by flash chromatography on silica gel (eluent : diethylether/hexane : 60/40) followed by flash chromatography on silica gel (eluent : hexane/ethylacetate : 88/12). Amber oil (0.4%). IRν_{max} (film) 2852, 1596 cm^{-1}. ^1H NMR δ ($CDCl_3$) 2.52 (6H, s, SCH_3), 2.53 (6H, s, CH_3), 7.24-7.35 (8H, m, H-2', H-3', H-5', H-6'), 7.30 (1H, s, H-4). ^{13}C NMR ppm ($CDCl_3$) 15.74, 22.99, 126.42, 129.50, 133.74, 136.43, 137.79, 138.31, 153.75. MS *m/z* 351 (M^+). HRMS Calculated for $C_{21}H_{22}NS_4$: (M^++1) 352.1194, Found: 352.1173.

4.1.13 4-Methyl-2-(4-methylthiobenzyl)-5-(4-methylthiophenyl)pyridine (13)

4-Methyl-2-(4-methylthiobenzyl)-5-(4-methylthiophenyl)pyridine (**13**) was isolated by flash chromatography on silica gel (eluent : diethylether/hexane : 60/40). Amber oil, (0.5%). IR ν_{max} (film) 2853, 1594 cm^{-1}. ^1H NMR δ ($CDCl_3$) 2.21 (3H, s, CH_3), 2.45 (3H, s, SCH_3), 2.51 (3H, s, SCH_3), 4.09 (2H, s, CH_2), 6.99 (1H, s, H-3), 7.20 (2H, d, J=8.5Hz, ArH), 7.21-7.23 (4H, m, ArH), 7.31 (2H, d, J=8.5Hz, ArH), 8.34 (1H, s, H-6). ^{13}C NMR ppm ($CDCl_3$) 15.66, 16.10, 19.83, 43.69, 124.32, 126.35, 127.16, 129.55, 129.65, 134.51, 134.82, 136.13, 136.61, 137.94, 145.00, 149.41, 159.32. MS *m/z* 351 (M^+). HRMS Calculated for $C_{21}H_{22}NS_2$: (M^++H) 352.1194; Found: 352.1179.

4.1.14 2,4-Dimethyl-6-(4-methylthiobenzyl)-3-(4-methylthiophenyl)pyridine (14)

2,4-Dimethyl-6-(4-methylthiobenzyl)-3-(4-methylthiophenyl)pyridine (**14**) was isolated by flash chromatography on silica gel (eluent : diethylether/hexane : 60/40). Amber oil (0.3%). IR ν_{max} (film) 2853, 1587 cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.95 (3H, s, CH_3), 2.27 (3H, s, CH_3), 2.46 (3H, s, SCH_3), 2.51 (3H, s, SCH_3), 4.07 (2H, s, CH_2), 6.79 (1H, s, H-5), 7.04 (2H, d, J=8.0Hz, ArH), 7.21-7.24 (4H, m, ArH), 7.30 (2H, d, J=8.6Hz, ArH). ^{13}C NMR ppm ($CDCl_3$) 15.63, 16.10, 20.24, 23.56, 43.81, 121.67, 126.58, 127.09, 129.51, 129.66, 133.96, 135.56, 135.99,

136.74, 137.35, 145.86, 155.57, 158.48. MS *m/z* 365 (M^+). HRMS Calculated for $C_{22}H_{24}NS_4$: (M^++H) 366.1350; Found: 366.1346.

4.1.15 2-Methyl-6-(4-methylthiobenzyl)-3-(4-methylthiophenyl)pyridine (15)

2-Methyl-6-(4-methylthiobenzyl)-3-(4-methylthiophenyl)pyridine (**15**) was isolated by flash chromatography on silica gel (eluent : diethylether/hexane : 60/40) followed by an additional flash chromatography on silica gel (eluent : hexane/ethylacetate 90/10). Amber oil (0.4%). IR ν_{max} (film) 2851, 1585 cm^{-1}. ^1H NMR δ ($CDCl_3$) 2.46 (3H, s, SCH_3), 2.50 (3H, s, SCH_3), 2.52 (3H, s, CH_3), 4.12 (2H, s, CH_2), 6.93 (1H, d, $J_{5,4}$=7.5Hz, H-5), 7.21 (2H, d, J=8.5Hz, ArH), 7.23 (4H, s, ArH), 7.30 (2H, d, J=8.5Hz, ArH), 7.37 (1H, d, $J_{4,5}$=8.0Hz, H-4). ^{13}C NMR ppm ($CDCl_3$) 15.76, 16.16, 23.43, 43.92, 120.20, 126.41, 127.18, 129.50, 129.70, 133.91, 136.16, 136.63, 136.73, 137.69, 137.75, 155.22, 159.06. MS *m/z* 351 (M^+). HRMS Calculated for $C_{21}H_{22}NS_2$: (M^++H) 352.1194; Found: 352.1200.

4.1.16 2-Methyl-2-methylthiobenzyl)-5-(4-methylthiophenyl)-2,3-dihydropyrid-4-one (16)

2-Methyl-2--methylthiobenzyl)-5-(4-methylthiophenyl)-2,3-dihydropyrid-4-one (**16**) was isolated by flash chromatography on silica gel (eluent : diethylether). Colourless solid (0.7%) (ethylacetate/hexane), mp 148.5-149.5°C. IR ν_{max} (KBr) 3238, 1567 cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.26 (3H, s, CH_3), 2.46 (3H, s, SCH_3), 2.47 (3H, s, SCH_3), 2.60 (2H, s, CH_2), 2.73 (1H, d, J_{gem}=13.0Hz, H-3), 3.00 (1H, d, J_{gem}=13.5Hz, H-3), 4.90 (1H, d, J=6.5Hz, NH), 7.07, 7.21, 7.23, 7.32 (8H, 4xd, J=8.0Hz, J=7.5Hz, J=8.0Hz, J=8.5Hz, H-2', H-3', H-5', H-6'), 7.25 (1H, d, J=3.5Hz, H-6). ^{13}C NMR ppm ($CDCl_3$) 15.85 (SCH_3), 16.46, 24.86, 43.36, 48.78, 56.77, 110.55, 126.66, 127.18, 128.03, 130.97, 132.61, 133.09, 135.22, 137.31, 147.34, 189.57. MS *m/z* 369 (M^+). Anal. Calculated for $C_{21}H_{23}NOS_2$: C, 68.25; H, 6.27; N, 3.79; S, 17.35. Found: C, 68.08; H, 6.19; N, 3.67; S, 17.67. HRMS Calculated for $C_{21}H_{23}NOS_2$: (M^+) 369.12210; Found: 369.12215.

4.1.17 N-Formyl-N,N-di(1-(4-methylthiophenyl)-2-propyl)amine (17)

N-Formyl-N,N-di(1-(4-methylthiophenyl)-2-propyl)amine (**17**) was isolated by flash chromatography on silica gel as a 65/35 mixture of diastereomers (eluent : diethylether/hexane : 75/25). Amber oil (2%). IR ν_{max} (film) 1664, 1599 cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.00 (1.05H, d,

$J_{3',2'}$=7.0Hz, H-3'), 1.16 (1.95H, d, $J_{3',2'}$=6.5Hz, H-3'), 1.21 (1.05H, d, $J_{3',2'}$=7.0Hz, H-3'), 1.24 (1.95H, d, $J_{3',2'}$=7.0Hz, H-3'), 2.44 (1.95H, s, SCH_3), 2.45 (4.05H, s, SCH_3), 2.59 (1.3H, d, $J_{1',2'}$=7.5Hz, H-1'), 2.68 (0.35H, dd, J_{gem}=13.6Hz, $J_{1',2'}$=8.0Hz, H-1'), 2.80 (0.35H, dd, J_{gem}=13.6Hz, $J_{1',2'}$=7.0Hz, H-1'), 2.83 (0.65H, dd, J_{gem}=13.8Hz, $J_{1',2'}$=7.8Hz, H-1'), 2.87 (0.35H, dd, J_{gem}=14.0Hz, $J_{1',2'}$=8.0Hz, H-1'), 2.95 (0.65H, dd, J_{gem}=13.6Hz, $J_{1',2'}$=7.0Hz, H-1'), 3.06 (0.35H, dd, J_{gem}=13.8Hz, $J_{1',2'}$=7.3Hz, H-1'), 3.52 (1H, m, H-2'), 3.98 (1H, m, H-2'), 7.00-7.21 (8H, m, H-2, H-3, H-5, H-6), 8.16 (0.35H, s, CHO), 8.18 (0.65H, s, CHO). ^{13}C NMR ppm ($CDCl_3$) 15.76*, 15.93, 16.05, 16.10*, 17.69, 17.81*, 20.68*, 20.93, 39.50*, 39.80, 42.51*, 42.80*, 51.47, 52.00*, 54.39, 54.59*, 126.91, 126.95, 126.99*, 127.02*, 129.47*, 129.60, 129.67, 134.78, 134.82*, 136.10, 136.16*, 136.18*, 136.76*, 162.17*, 162.23. MS *m/z* 373 (M^+); HRMS Calculated for $C_{21}H_{28}NOS_2$: (M^++H) 374.1612; Found: 374.1611.

4.1.18 4-Methylthiotoluene

4-Methylthiotoluene was isolated as an impurity from the preparation of (**8a**) by flash chromatography on silica gel (eluent : hexane)as an amber oil (3.5%) (bp 52-54°C/1mmHg), identified by comparison with authentic sample purchased from Sigma-Aldrich. R_f 0.96 (diethylether/hexane : 80/20). IR ν_{max} (film) 2854, 1598 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 2.35 (3H, s, CH_3), 2.49 (3H, s, SCH_3), 7.13, 7.22 (4H, 2xd, H-2, H-3, H-5, H-6). δ^{13}C NMR ppm ($CDCl_3$) 16.56, 20.87, 127.37, 129.58, 134.71, 135.06. MS *m/z* 138 (M^+, 100%).

4.1.19 2-(*N*-Formyl-*N*-methyl)amino-1-(4-methylthiophenyl)propane (18)

A solution of the ketone (**3a**) (25.32 mmol) in N-methylformamide (64.41 mmol, 3.81 g) and 96% formic acid (1.84 g) was stirred and refluxed at 150ºC for 7h. After cooling the reaction was diluted with water (50 mL) and extracted with dichloromethane (3x25 mL). The extracts were combined, washed with water (2x50 mL) and satd. aq. $NaCHO_3$ (2x25 mL), followed by drying over anhydrous Na_2SO_4. The volatiles were removed *in vacuo*, yielding an oil which was purified by flash chromatography over silica gel (eluent : diethylether). Pale amber oil (73%). IR ν_{max} (film) 2858, 1667 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.16 (0.75H, d, $J_{3',2'}$=6.5Hz, H-3'), 1.29 (2.25H, d, $J_{3',2'}$=6.5Hz, H-3'), 2.45 (3H, s, SCH_3), 2.69-2.79 (2H, m, H-1'), 2.77 (0.75H, s, NCH_3), 2.79 (2.25H, s, NCH_3), 3.75 (0.75H, m, H-2'), 4.73 (0.25H, m, H-2'),

7.00, 7.18 (3H, 2xd, J=8.0Hz, J=8.5Hz, H-2, H-3, H-5, H-6), 7.12, 7.18 (1H, 2xd, J=8.0Hz, J=8.5Hz, H-2, H-3, H-5, H-6), 7.80 (0.75H, s, CHO), 7.94 (0.25H, s, CHO). ^{13}C NMR ppm ($CDCl_3$) 15.85*, 15.94, 16.74, 18.91*, 24.85*, 29.69, 39.01, 40.07*, 47.83, 55.79*, 126.83, 127.02*, 129.19*, 129.30, 134.58*, 134.96, 136.15, 136.73*, 162.37*, 162.56. MS *m/z* 223 (M^+); HRMS Calculated for $C_{12}H_{18}NOS$: (M^++H) 224.1109; Found: 224.1098.

4.1.20 2-(*N*-Formyl-*N*-methyl)amino-1-(4-methylthiophenyl)butane (19)

2-(*N*-Formyl-*N*-methyl)amino-1-(4-methylthiophenyl)butane (**19**) was prepared from (**3b**) (2.00 g, 10.29 mmol scale) according to general procedure for **18** above and isolated by flash chromatography on silica gel (eluent : diethylether). Pale amber oil (67%). R_f 0.52 (diethylether). IR ν_{max} (film) 2874, 1671 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.87 (3H, t, $J_{4',3'}$=7.3Hz, H-4'), 1.57 (0.4H, m, H-3'), 1.64 (1.6H, m, H-3'), 2.45 (3H, s, SCH_3), 2.72-2.83 (2H, m, H-1'), 2.72 (2.4H, s, NCH_3), 2.75 (0.6H, s, NCH_3), 3.40 (0.8H, m, H-2'), 4.55 (0.2H, m, H-2'), 7.00, 7.18 (3.2H, 2xd, J=8.0Hz, J=8.6Hz, H-6), 7.12, 7.19 (0.8H, 2xd, J=8.0Hz, J=8.6Hz, H-6), 7.74 (0.8H, s, CHO), 8.00 (0.2H, s, CHO). ^{13}C NMR ppm ($CDCl_3$) 10.62, 10.69*, 15.87*, 15.94, 23.89, 24.67*, 24.98*, 29.72, 37.66, 38.65*, 54.83, 62.24*, 126.83, 127.03*, 129.17*, 129.30, 134.66*, 134.96, 136.05, 136.64*, 163.02*, 163.24. MS *m/z* 237 (M^+). HRMS Calculated for $C_{13}H_{19}NOSNa$: (M^++Na) 260.1085, Found: 260.1109.

4.1.21 2-(*N*-Formyl)amino-1-(4-methylthiophenyl)propane (8a).

A mixture of formamide (70 g) and the ketone **3a** (117.88 mmol, 21.01 g) was heated at 190ºC for 5h. After cooling the reaction was diluted with water (150 mL) and extracted with ethylacetate (3x75 mL). The extracts were combined, washed with water (3x50 mL) and dried over anhydrous Na_2SO_4. After removing all volatiles *in vacuo* the resulting black oil was purified by flash chromatography (eluent: ethyl acetate), providing the expected product **8a**, as a pale brown solid (52%) identified by comparison with authentic sample, together with the impurities and reaction byproducts **9-17** as reported above for direct preparation of **1a** by Leuckart route.

4.1.22 22 2-(*N*-Formyl)amino-1-(4-methylthiophenyl)butane (8b)

Compound **8b** was prepared from **3b** (2.00 g, 10.29 mmol scale) according to general procedure for compound **8a** above and isolated by flash chromatography on silica gel (eluent : diethylether). Colourless needles (37%), mp 67-69°C (diethylether/hexane). R_f 0.05 (diethylether/hexane : 50/50). IR ν_{max} (KBr) 3300, 1656 cm^{-1}. ^1H NMR δ ($CDCl_3$) 0.93 (2.25H, t, $J_{4',3'}$=7.5Hz, H-4'), 0.96 (0.75H, t, $J_{4',3'}$=7.5Hz, H-4'), 1.39 (1H, m, H-3'), 1.62 (1H, m, H-3'), 2.45 (3H, s, SCH_3), 2.62 (0.25H, dd, J_{gem}=13.8Hz, $J_{1',2'}$=8.3Hz, H-1'), 2.74 (0.75H, dd, J_{gem}=15.0Hz, $J_{1',2'}$=6.5Hz, H-1'), 2.77 (0.75H, dd, J_{gem}=15.0Hz, $J_{1',2'}$=6.5Hz, H-1'), 2.80 (0.25H, dd, J_{gem}=13.5Hz, $J_{1',2'}$=5.0Hz, H-1'), 3.38 (0.25H, m, H-2'), 4.14 (0.75H, m, H-2'), 5.93 (0.25H, d, J=10.5Hz, NH), 5.60 (0.75H, d, J=7.5Hz, NH), 5.93 (0.25H, t, J=10.5Hz, NH), 7.05, 7.19 (1H, 2xd, J=8.0Hz, J=8.0Hz, H-2, H-3, H-5, H-6), 7.10, 7.18 (3H, 2xd, J=8.5Hz, J=8.5Hz, H-2, H-3, H-5, H-6), 7.72 (0.25H, d, J=11.5Hz, CHO), 8.10 (0.75H, s, CHO). ^{13}C NMR ppm ($CDCl_3$) 10.24*, 10.37, 15.84, 15.91*, 26.71*, 28.26, 39.74*, 41.94, 50.38*, 55.97, 126.78*, 126.91, 129.78*, 129.81, 134.26, 134.66*, 136.20*, 136.64, 160.73*, 164.07. MS *m/z* 223 (M^+). Anal. Calculated for $C_{12}H_{17}NOS$: C, 64.53; H, 7.67; N, 6.27. Found: C, 64.51; H, 7.58; N, 6.20%.

4.1.23 Preparation of 2-amino-1-(4-methylthiophenyl)propane (4-MTA) (1a) *via N*-formyl hydrolysis of 8a

To a stirred solution of an appropriate *N*-formylaminoalkane (**8a**) (11.68 mmol) in methanol (15 mL) was added 30% aq. HCl (50 mL) and the solution was refluxed for 7h. After cooling the reaction was diluted with water (100 mL) and washed with dichloromethane (3x30 mL). The aqueous phase was basified with 15% aq. NaOH and extracted with dichloromethane (3x50 mL). The organic phases were combined, dried over anhydrous Na_2SO_4 and solvent removed *in vacuo*, yielding the product **1a** as an amber oil,(85%), confirmed by comparison with an authentic sample.

4.1.24 Preparation of 2-*N*-methylamino-1-(4-methylthiophenyl)propane (2a) *via N*-formyl hydrolysis of 18

To a stirred solution of *N*-formyl-*N*-methylaminoalkane (**18**) (11.68 mmol) in methanol (15 mL) was added 30% aq. HCl (50 mL) and the solution was refluxed for 7h. After cooling

the reaction was diluted with water (100 mL) and washed with dichloromethane (3x30 mL). The aqueous phase was basified with 15% aq. NaOH and extracted with dichloromethane (3x50 mL). The organic phases were combined, dried over anhydrous Na_2SO_4 and solvent removed *in vacuo*, yielding the product **2a** as a pale amber oil (66%). IR ν_{max} (film) 3321, 2788 cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.04 (3H, d, $J_{3',2'}$=6.0Hz, H-3'), 1.35 (1H, br s, NH), 2.39 (3H, s, NCH_3), 2.47 (3H, s, SCH_3), 2.57 (1H, dd, J_{gem}=13.3Hz, $J_{1',2'}$=6.5Hz, H-1'), 2.67 (1H, dd, J_{gem}=13.0Hz, $J_{1',2'}$=7.0Hz, H-1'), 2.76 (1H, m, H-2'), 7.11, 7.20 (4H, 2xd, J=8.6Hz, J=8.6Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 16.13, 19.64, 33.94, 42.83, 56.26, 127.02, 129.76, 135.76, 136.50. MS *m/z* 195 (M^++1). HCl salt. Colourless solid. M.p. 161-162ºC (ethanol/hexane)[12, 49]. IRν_{max} (KBr) 2449 (NH^+) cm^{-1}. Anal. Calculated for $C_{11}H_{18}ClNS$: C, 57.00; H, 7.83; N, 6.04; S 13.83. Found: C ,56.81; H ,7.79; N ,6.05; S, 13.43%.

4.1.25 2-Amino-1-(4-methylthiophenyl)butane (1b)

2-Amino-1-(4-methylthiophenyl)butane (**1b**) was prepared from (**8b**) (0.54 g, 2.42 mmol scale) by hydrolysis in HCl according to general procedure for hydrolysis of **8a** above. Colourless oil (53%). R_f 0.26 (methanol). IR ν_{max} (film) 3360, 3292 cm^{-1}. ^1H NMR δ ($CDCl_3$) 0.97 (3H, t, $J_{4',3'}$=7.5Hz, H-4'), 1.17 (2H, br s, NH_2), 1.36 (1H, m, H-3'), 1.52 (1H, m, H-3'), 2.41 (1H, dd, J_{gem}=13.6Hz, $J_{1',2'}$=8.5Hz, H-1'), 2.47 (3H, s, SCH_3), 2.75 (1H, dd, J_{gem}=13.5Hz, $J_{1',2'}$=4.5Hz, H-1'), 2.88 (1H, m, H-2'), 7.12, 7.21 (4H, 2xd, J=8.0Hz, J=8.5Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 10.54, 16.21, 30.30, 43.69, 54.18, 127.11, 129.78, 135.75, 136.85. MS *m/z* 195 (M^+). **HCl salt**. Colourless solid, mp 154-155ºC (ethanol/hexane)[49]. IR ν_{max} (KBr) 2600, 2531, 2449, 2362 cm^{-1}. Anal. Calculated for $C_{11}H_{18}ClNS$: C, 57.00; H, 7.83; N, 6.04. Found: C, 56.90; H, 7.69; N, 5.78%.

4.1.26 2-*N*-Methylamino-1-(4-methylthiophenyl)butane (2b).

Compound **2b** was prepared from **19** (1.50 g, 6.32 mmol scale) by hydrolysis in HCl according to general procedure for the hydrolysis of **18** above. The product was obtained as a pale amber oil (77%). R_f 0.16 (methanol), together with unreacted **19** (5%). IR ν_{max} (film) 3324, 2789 cm^{-1}. ^1H NMR δ ($CDCl_3$) 0.93 (3H, t, $J_{4',3'}$=7.5Hz, H-4'), 1.36-1.52 (2H, m, H-3'), 1.55

(1H, br s, NH), 2.37 (3H, s, NCH_3), 2.47 (3H, s, SCH_3), 2.53-2.72 (3H, m, H-1', H-2'), 7.11, 7.20 (4H, 2xd, J=8.0Hz, J=8.0Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 9.75, 16.14, 25.48, 33.69, 39.23, 62.05, 127.04, 129.74, 135.67, 136.70. MS *m/z* 209 (M^++1). **HCl salt**. Colourless solid, mp 128-129ºC (ethanol/hexane). IR ν_{max} (KBr) 2505, 2462 cm^{-1}. Anal. Calculated for $C_{12}H_{20}ClNS$: C, 58.63; H, 8.20; N 5.70; S, 13.04. Found: C, 58.50; H, 8.22; N, 5.78; S, 12.75%.

4.1.27 Preparation of 2-N-methylamino-1-(4-methylthiophenyl)butane (2b) via Leuckart-Wallach reaction

A mixture of formamide (60 g) and the ketone **3b** (16.74 g, 86.16 mmol scale) was heated at 190^o for 5h according to the procedure used in the Leuckart –Wallach preparation of **1a** above. After cooling the reaction was diluted with water (150 mL) and extracted with ethylacetate (3x75 mL). The extracts were combined, washed with water (3x50 mL) and dried over anhydrous Na_2SO_4. After removing all volatiles *in vacuo* the resulting dark brown oil was dissolved in methanol (30 mL) and 30% aq. HCl (150 mL) an the mixture was heated under reflux for 7h. Upon cooling, the reaction was diluted with water (100 mL), made basic (pH 14), with 15% aq. NaOH and extracted with dichloromethane (4x100 mL). The extracts were combined and dried over anhydrous Na_2SO_4. All volatiles were removed *in vacuo*, affording a dark brown oil. Isolation of the desired product **2b**, together with the following reaction byproducts was performed by flash column chromatography over silica gel.

4.1.28 2-*N*-Formylamino-1-(4-methylthiophenyl)butane (8b)

2-*N*-Formylamino-1-(4-methylthiophenyl)butane (**8b**) was isolated by flash chromatography on silica gel (eluent : ethyl acetate/diethylether : 50/50). Colourless needles (5%), mp 67-69°C (diethylether/hexane). R_f 0.05 (diethylether/hexane : 50/50); identified by comparison with an authentic sample.

4.1.29 5-Methyl-4-(4-methylthiobenzyl)pyrimidine (20)

5-Methyl-4-(4-methylthiobenzyl)pyrimidine (**20**) was isolated as an impurity from the preparation of (**1b**) by flash chromatography on silica gel (eluent : diethylether). Amber oil

(1%). R_f 0.13 (diethylether/hexane : 50/50). IR ν_{max} (film) 2858, 1576 cm^{-1}. ^1H NMR δ ($CDCl_3$) 2.24 (3H, s, CH_3), 2.44 (3H, s, SCH_3), 4.08 (2H, s, CH_2), 7.13, 7.18 (4H, 2xd, J=8.0Hz, J=8.5Hz, H-2', H-3', H-5', H-6'), 8.42 (1H, s, H-6), 9.00 (1H, s, H-2). ^{13}C NMR ppm ($CDCl_3$) 15.62, 15.94, 40.97, 127.02, 129.29, 129.04, 133.83, 136.72, 156.63, 157.53, 166.71. MS *m/z* 230 (M^+). HRMS Calculated for $C_{13}H_{15}N_2S$: (M^++H) 231.0956, Found: 231.0948.

4.1.30 4-Ethyl-5-(3,4-methylthiophenyl)pyrimidine (21)

4-Ethyl-5-(3,4-methylthiophenyl)pyrimidine (**21**) was isolated as an impurity from the preparation of (**1b**) by flash chromatography on silica gel (eluent : diethylether/hexane 70/30) followed by flash chromatography on neutral alumina (eluent : ethylacetate). Amber oil (2%). R_f 0.34 (diethylether/hexane : 50/50). IR ν_{max} (film) 2858, 1576 cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.24 (3H, t, J=7.5Hz, CH_2CH_3), 2.54 (3H, s, SCH_3), 2.78 (2H, q, J=7.5Hz, CH_2CH_3), 7.23, 7.35 (4H, 2xd, J=8.5Hz, J=8.0Hz, H-2', H-3', H-5', H-6'), 8.50 (1H, s, H-6), 9.11 (1H, s, H-2). ^{13}C NMR ppm ($CDCl_3$) 15.86, 15.52, 28.18, 126.45, 129.39, 132.30, 133.94, 139.26, 156.47, 157.40, 168.92. MS *m/z* 230 (M^+–1). HRMS Calculated for $C_{13}H_{15}N_2S$: (M^++H) 231.0956, Found: 231.0958.

4.1.31 2,4-Diethyl-3,5-di(4-methylthiophenyl)pyridine (22)

2,4-Diethyl-3,5-di(4-methylthiophenyl)pyridine (**22**) was isolated as an impurity from the preparation of (**1b**) by flash chromatography on silica gel (eluent : hexane/diethylether : 40/60) followed by flash chromatography on neutral alumina (eluent : hexane/ethylacetate : 80/20). Amber oil (0.2%). R_f 0.52 on neutral alumina (diethylether/hexane : 50/50). IR ν_{max} (film) 2853, 1598 cm^{-1}. ^1H NMR δ ($CDCl_3$) 0.71 (3H, t, J=7.5Hz, CH_2CH_3), 1.15 (3H, t, J=7.5Hz, CH_2CH_3), 2.35 (2H, q, J=7.5Hz, CH_2CH_3), 2.52 (2H, q, J=7.5Hz, CH_2CH_3), 2.53 (3H, s, SCH_3), 2.55 (3H, s, SCH_3), 7.15, 7.26, 7.32, 7.33 (8H, 4xd, J=8.0Hz, J=8.0Hz, J=8.0Hz, J=8.5Hz, H-2', H-2'', H-3', H-3'', H-5', H-5'', H-6', H-6''), 8.32 (1H, s, H-6). ^{13}C NMR ppm ($CDCl_3$) 13.89, 14.59, 15.60, 15.73, 23.28, 29.35, 126.24, 126.32, 129.93, 129.95, 134.53, 135.12, 135.24, 135.39, 137.61, 137.80, 148.90, 149.13. MS *m/z* 379 (M^+–1). HRMS Calculated for $C_{23}H_{26}NS_2$: (M^++H) 380.1507, Found: 380.1499.

4.1.32 2,6-Diethyl-3,5-di(4-methylthiophenyl)pyridine (**23**)

2,6-Diethyl-3,5-di(4-methylthiophenyl)pyridine (**23**) was isolated as an impurity from the preparation of (**1b**) by flash chromatography on silica gel (eluent : hexane/diethylether : 70/30) followed by flash chromatography on acidic alumina (eluent : diethylether). Amber oil (0.2%). R_f 0.88 (diethylether/hexane : 50/50). IR ν_{max} (film) 2852, 1597 cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.24 (6H, t, J=7.5Hz, CH_2CH_3), 2.52 (6H, s, SCH_3), 2.81 (4H, q, J=7.5Hz, CH_2CH_3), 7.26, 7.31 (8H, 2xd, J=8.5Hz, J=8.0Hz, H-2', H-3', H-5', H-6'), 7.29 (1H, s, H-4). ^{13}C NMR ppm ($CDCl_3$) 14.23, 15.78, 28.45, 126.39, 129.57, 133.02, 136.66, 137.60, 138.72, 158.91. MS *m/z* 379 (M^+-1). HRMS Calculated for $C_{23}H_{26}NS_2$: (M^++H) 380.1507, Found: 380.1496.

4.1.33 4-Ethyl-3-methyl-2-(4-methylthiobenzyl)-5-(4-methylthiophenyl)pyridine (**24**)

4-Ethyl-3-methyl-2-(4-methylthiobenzyl)-5-(4-methylthiophenyl)pyridine (**24**) was isolated as an impurity from the preparation of (**1b**) by flash chromatography on silica gel (eluent : hexane/diethylether : 60/40) followed by flash chromatography on neutral alumina (eluent : hexane/ethylacetate : 80/20). Amber oil (0.2%). R_f 0.46 on neutral alumina (diethylether/hexane : 50/50). IR ν_{max} (film) 2853, 1599 cm^{-1}. ^1H NMR δ ($CDCl_3$) 0.92 (3H, t, J=7.5Hz, CH_2CH_3), 2.26 (3H, s, CH_3), 2.45 (3H, s, SCH_3), 2.53 (3H, s, SCH_3), 2.57 (2H, q, J=7.5Hz, CH_2CH_3), 4.20 (2H, s, CH_2), 7.17-7.19 (4H, m, ArH), 7.20, 7.31 (4H, 2xd, J=8.5Hz, J=8.5Hz, ArH), 8.20 (1H, s, H-6). ^{13}C NMR ppm ($CDCl_3$) 13.63, 14.08, 15.28, 15.74, 23.32, 41.74, 125.81, 126.67, 128.71, 129.42 ,135.01, 135.19, 135.29, 136.00, 137.29, 138.80, 145.00, 146.48, 157.24. MS *m/z* 379 (M^+-1). HRMS Calculated for $C_{23}H_{26}NS_2$: (M^++H) 380.1507, Found: 380.1474.

4.1.34 5-Methyl-2-ethyl-6-(4-methylthiobenzyl)-3-(4-methylthiophenyl)pyridine (**25**)

5-Methyl-2-ethyl-6-(4-methylthiobenzyl)-3-(4-methylthiophenyl)pyridine (**25**) was isolated as an impurity from the preparation of (**1b**) by flash chromatography on silica gel (eluent : hexane/diethylether : 70/30) followed by flash chromatography on silica gel (eluent : hexane/acetone 90/10). Amber oil (0.6%). R_f 0.84 (diethylether/hexane : 50/50). IR ν_{max} (film) 2869, 1594 cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.19 (3H, t, J=7.3Hz, CH_2CH_3), 2.20 (3H, s, CH_3), 2.44 (3H, s, SCH_3), 2.51 (3H, s, SCH_3), 2.76 (2H, q, J=7.5Hz, CH_2CH_3), 4.15 (2H, s, CH_2), 7.18 (1H, s, H-4), 7.21 (2H, d, J=8.5Hz, ArH), 7.18-7.20 (4H, m, ArH), 7.29 (2H, d, J=8.5Hz, ArH). ^{13}C NMR ppm ($CDCl_3$) 14.38, 15.74, 16.15, 18.24, 28.15, 41.44, 126.31, 126.98, 129.21, 129.48,

128.32, 133.81, 135.60, 136.37, 136.80, 137.45, 139.50, 156.85, 157.35. MS *m/z* 379 (M^+). HRMS Calculated for $C_{23}H_{26}NS_2$: (M^++H) 380.1507, Found: 380.1525.

4.1.35 3,5-Dimethyl-2,6-di(4-methylthiobenzyl)pyridine (**26**)

3,5-Dimethyl-2,6-di(4-methylthiobenzyl)pyridine (**26**) was isolated as an impurity from the preparation of (**1b**) by flash chromatography on silica gel (eluent : hexane/diethylether : 70/30) followed by flash chromatography on silica gel (eluent : hexane/acetone : 90/10). Amber oil (0.2%). R_f 0.75 (diethylether/hexane : 50/50). IR ν_{max} (film) 2854, 1596 cm^{-1}. ^1H NMR δ ($CDCl_3$) 2.16 (6H, s, CH_3), 2.50 (6H, s, SCH_3), 4.11 (4H, s, CH_2), 7.11, 7.15 (8H, 2xd, J=8.0Hz, J=8.5Hz, H-2', H-3', H-5', H-6'), 7.13 (1H, s, H-4). ^{13}C NMR ppm ($CDCl_3$) 16.25, 18.21, 41.12, 127.03, 129.14, 129.31, 135.45, 136.72, 140.34, 155.30. MS *m/z* 379 (M^+). HRMS Calculated for $C_{23}H_{26}NS_2$: (M^++H) 380.1507, Found: 380.1522.

4.1.36 N,N-Di(1-(4-methylthiophenyl)-2-butyl)amine (**27a**)

N,N-Di(1-(4-methylthiophenyl)-2-butyl)amine (**27a**) was isolated as an impurity from the preparation of (**1b**) by flash chromatography on silica gel as a single diastereomer (eluent : diethylether/hexane : 80/20). Amber oil (0.2%). R_f 0.18 (diethylether/hexane : 50/50). IR ν_{max} (film) 3317, 1598 cm^{-1}. ^1H NMR δ ($CDCl_3$) 0.75 (6H, t, $J_{4',3'}$=7.3Hz, H-4'), 1.28-1.37 (5H, m, H-3', NH), 2.45 (8H, m, SCH_3, H-1'), 2.52 (2H, dd, J_{gem}=13.5Hz, $J_{1',2'}$=5.0Hz, H-1'), 2.72 (2H, m, H-2'), 7.08, 7.18 (8H, 2xd, J=8.5Hz, J=8.0Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 9.40, 16.29, 26.17, 40.57, 57.61, 127.02, 129.81, 135.48, 137.12. MS *m/z* 373 (M^+–1). HRMS Calculated for $C_{22}H_{32}NS_2$: (M^++H) 374.1976, Found: 374.1964.

4.1.37 N,N-Di(1-(4-methylthiophenyl)-2-butyl)amine (**27b**)

N,N-Di(1-(4-methylthiophenyl)-2-butyl)amine (**27b**) was isolated from the preparation of (**1b**) by flash chromatography on silica gel as a single diastereomer (eluent : diethylether/hexane : 80/20). Amber oil (0.2%). R_f 0.27 (diethylether/hexane : 50/50). IR ν_{max} (film) 3317, 1598 cm^{-1}. ^1H NMR δ ($CDCl_3$) 0.91 (6H, t, $J_{4',3'}$=7.5Hz, H-4'), 1.29-1.43 (5H, m, H-3', NH), 2.46 (2H, dd, J_{gem}=13.5Hz, $J_{1',2'}$=6.5Hz, H-1'), 2.47 (6H, s, SCH_3), 2.54 (2H, dd, J_{gem}=13.5Hz, $J_{1',2'}$=6.0Hz, H-1'), 2.70 (2H, m, H-2'), 6.93, 7.13 (8H, 2xd, J=8.0Hz, J=8.5Hz, H-2, H-3, H-5, H-6). ^{13}C

NMR ppm ($CDCl_3$) 9.86, 16.16, 27.04, 39.79, 57.54, 126.89, 129.81, 135.49, 136.71. *m/z* 373 (M^+–1). HRMS Calculated for $C_{22}H_{32}NS_2$: (M^++H) 374.1976; Found: 374.1979.

4.1.38 N-Methyl-N,N-di(1-(4-methylthiophenyl)-2-butyl)amine (28)

N-Methyl-N,N-di(1-(4-methylthiophenyl)-2-butyl)amine (**28**) was isolated as an impurity from the preparation of (**1b**) by flash chromatography on silica gel (eluent : hexane/acetone 85/15) as a 60/40 mixture of diastereomers. Amber oil (1%). R_f 0.67 (diethylether/hexane : 50/50). IR ν_{max} (film) 1664, 1599 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.75 (2.4H, t, $J_{4',3'}$=7.3Hz, H-4'), 0.78 (3.6H, t, $J_{4',3'}$=7.3Hz, H-4'), 1.20-1.43 (4H, m, H-3'), 2.28 (1.2H, s, NCH_3), 2.29 (1.8H, s, NCH_3), 2.45 (6H, s, SCH_3), 2.47 (0.8H, m, H-1'), 2.49 (1.2H, dd, J_{gem}=14.5Hz, $J_{1',2'}$=6.5Hz, H-1'), 2.69 (2H, m, H-2'), 2.80 (0.8H, dd, J_{gem}=13.1Hz, $J_{1',2'}$=5.5Hz, H-1'), 2.86 (1.2H, dd, J_{gem}=13.3Hz, $J_{1',2'}$=4.8Hz, H-1'), 7.05, 7.16 (8H, 2xd, J=8.0Hz, J=8.0Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 11.41* , 16.40*, 16.45, 24.32, 24.42*, 29.50*, 30.08, 37.74, 37.81*, 65.67, 66.21*, 127.11*, 129.72*, 129.74, 134.90, 134.98*, 138.58*, 138.64. *m/z* 387 (60% diastereomer M^+–1), MS *m/z* 387 (40% diastereomer M^+–1). HRMS Calculated for $C_{23}H_{34}NS_2$: (M^++H) 388.2133, Found: 388.2147.

4.1.39 Preparation of 2-amino- and 2-*N*-alkylamino-1-(4-methylthiophenyl) alkanes (1a,1b) and (2a,2b) *via* reductive amination (Route B)

To a stirred mixture of the appropriate ketone (**3a, 3b**) (22.45 mmol) in dry methanol (100 mL) there was added either ammonium acetate or methylamine HCl salt (179.60 mmol) and sodium cyanoborohydride (31.84 mmol, 2.00 g) and the reaction was stirred at ambient temperature for 72h. The pH of the reaction was occasionally adjusted to pH 5-6 by the addition of 4M methanolic HCl as determined by damp universal pH paper. Excess hydride was decomposed by the addition of 10% aq. HCl (150 mL) and resulting aqueous phase washed with dichloromethane (3x50 mL). The aqueous phase basified with 15% aq. NaOH solution and extracted with dichloromethane (3x50 mL). The organic phases were combined, dried over anhydrous Na_2SO_4, and volatiles removed *in vacuo* leaving the product as an oil. The acidic phase organic washes were each combined, washed with 15% aq. NaOH (5 mL) and the residue chromatographed to afford synthesis related impurities.

4.1.40 2-Amino-1-(4-methylthiophenyl)propane (**1a**)

2-Amino-1-(4-methylthiophenyl)propane (**1a**) was obtained as a colourless oil (80%), which was identified by comparison with an authentic sample.

4.1.41 N,N-Di(1-(4-methylthiophenyl)-2-propyl)amine (29)

N,N-Di(1-(4-methylthiophenyl)-2-propyl)amine **(29)** was isolated as an impurity by flash chromatography on silica gel as a 75/25 mixture of diastereomers (eluent : ethylacetate). Amber oil (19%). IR ν_{max} (film) 3284 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 0.93 (3H, d, $J_{3',2'}$=6.0Hz, H-3'), 1.02 (1H, d, $J_{3',2'}$=6.5Hz, H-3'), 1.12 (1H, br s, NH), 2.45 (4H, s, SCH_3), 2.46 (1H, s, SCH_3), 2.45-2.52 (2H, m, H-1'), 2.58 (0.5H, dd, J_{gem}=13.3Hz, $J_{1',2'}$=6.8Hz, H-1'), 2.71 (1.5H, dd, J_{gem}=13.6Hz, $J_{1',2'}$=6.0Hz, H-1'), 2.96 (2H, m, H-2'), 6.94, 7.13 (2H, 2xd, J=8.0Hz, J=8.0Hz, H-2, H-3, H-5, H-6), 7.06, 7.17 (6H, 2xd, J=8.6Hz, J=8.5Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 16.01, 16.11*, 20.07*, 21.28, 42.65, 43.54*, 51.10*, 51.55, 126.78, 126.86*, 129.68, 129.75*, 135.62*, 135.63, 136.63, 136.48. MS *m/z* 346 (M^+). HRMS Calculated for $C_{20}H_{27}NS_2$: (M^+) 345.15849; Found: 345.15922.

4.1.42 Preparation of 2-Amino-1-(4-methylthiophenyl)butane (1b) by reductive amination (route B)

2-Amino-1-(4-methylthiophenyl)butane **(1b)** was prepared from **3b** and ammonium acetate as described for **1a** above. Colourless solid (53%), mp 109-110 ºC. **HCl salt**. Colourless solid. mp 154-155 ºC (ethanol/hexane)[49], identified by comparison with an authentic sample

4.1.43 N,N-Di(1-(4-methylthiophenyl)-2-butyl)amine (**27a**)

N,N-Di(1-(4-methylthiophenyl)-2-butyl)amine (**27a**) was isolated by flash chromatography on silica gel as a single diastereomer (eluent : diethylether/hexane : 80/20) as an impurity from the preparation of **1b** by reductive amination of ketone **3b** with ammonium acetate. The product was isolated as an amber oil (0.2%), and identified by comparison with an authentic sample.

4.1.44 2-*N*-Methylamino-1-(4-methylthiophenyl)propane (2a)

2-N-Methylamino-1-(4-methylthiophenyl)propane (**2a**) was prepared from **3a** and methylamine. HCl as described for **1a** above and obtained as a colourless oil, (39%), which was identified by comparison with an authentic sample.

4.1.45 2-*N*-Methylamino-1-(4-methylthiophenyl)butane (2b)

2-*N*-Methylamino-1-(4-methylthiophenyl)butane **(2b)** was prepared from **3b** and N-methylamine HCl as described for **1a** above. The product was isolated as a colourless oil (77%) and identified by comparison with an authentic sample.

4.1.46 1-(4-Methylthiophenyl)-2-propanone oxime (30a)

A stirred solution of the ketone **(3a)** (15.23 mmol) and hydroxylamine HCl (36.00 mmol, 2.51 g) in pyridine (12.5 mL) and ethanol (12.5 mL) was refluxed for 2h. After cooling the reaction was acidified with 10% aq. HCl and extracted with dichloromethane (3x50 mL). The organic phases were combined, dried over anhydrous Na_2SO_4, and solvent removed *in vacuo*. The crude product was purified by flash chromatography on silica gel (eluent : diethylether/hexane : 35/65), providing a 25/75 mixture of *syn*/*anti* isomers. Colourless solid (85%), mp 57-59ºC (ethanol/water). IR ν_{max} (KBr) 3260, 1668 cm^{-1}. ***Anti*-(30a)**. ^{1}H NMR δ ($CDCl_3$) 1.81 (2.25H, s, H-3'), 2.46 (3H, s, SCH_3), 3.45 (1.5H, s, H-1'), 7.14, 7.20 (3H, 2xd, J=8.0Hz, J=8.6Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 16.02, 19.61, 41.51, 127.05, 129.45, 133.61, 136.72, 157.49. ***Syn*-(30a)**. ^{1}H NMR δ ($CDCl_3$) 1.80 (0.75H, s, H-3'), SCH_3 signal overlapping with *anti*-(**30a**), 3.70 (0.5H, s, H-1'), 7.15, 7.19 (1H, 2xd, J=8.5Hz, 9.5Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 16.06, 19.61, 34.25, 127.11, 129.62, 133.42, 136.34, 156.75. MS *m/z* 195 (*syn*/*anti* isomer mixture, M^+). Anal. Calculated for $C_{10}H_{13}NOS$: C, 61.50; H, 6.71; N, 7.17. Found: C, 61.40; H, 6.71; N, 7.08%.

4.1.47 1-(4-Methylthiophenyl)-2-butanone oxime (30b)

1-(4-Methylthiophenyl)-2-butanone oxime (**30b**) was prepared from (**3b**) (2.50 g, 12.87 mmol scale) according to general procedure for **30a** above without the need for flash

chromatography, generating a 60/40 mixture of *syn*/*anti* isomers. Colourless solid (86%), mp 77-79ºC (ethanol/water). R_f 0.36 (dichloromethane/hexane : 80/20). IR ν_{max} (KBr) 3220, 1669 cm^{-1}. ***Syn*-(30b)**. ^{1}H NMR δ ($CDCl_3$) 1.04 (1.8H, t, $J_{4',3'}$=7.5Hz, H-4'), 2.18 (1.2H, q, $J_{3',4'}$=7.5Hz, H-3'), 2.45 (3H, s, SCH_3), 3.70 (1.2H, s, H-1'), 7.13-7.21 (4H, m, H-2, H-3, H-5, H-6), OH signal not observed. ^{13}C NMR ppm ($CDCl_3$) 10.62, 16.04, 27.00, 32.90, 127.10, 128.54, 133.62, 136.19, 160.42. ***Anti*-(30b)**. ^{1}H NMR δ ($CDCl_3$) 0.99 (1.2H, t, $J_{4',3'}$=7.5Hz, H-4'), 2.31 (0.8H, q, $J_{3',4'}$=7.7Hz, H-3'), SCH_3 signal overlapping with *syn*-(**30b**), 3.46 (0.8H, s, H-1'), H-2, H-3, H-5, H-6 signals overlapping with *syn*-(**30b**), OH signal not observed. ^{13}C NMR ppm ($CDCl_3$) 10.00, 16.08, 20.49, 39.43, 127.03, 129.51, 133.69, 136.62, 161.78. MS *m/z* (*syn*/*anti* isomer mixture) 209 (M^+). Anal. Calculated for $C_{11}H_{15}NOS$: C, 63.12; H, 7.22; N, 6.69. Found: C, 63.46; H, 7.11; N, 6.50%.

4.1.48 Preparation of 2-amino-1-(4-methylthiophenyl)alkanes (1a and 1b) *via* oxime reduction (Route C)

To a stirred and refluxing suspension of $LiAlH_4$ under nitrogen (52.70 mmol, 2.00 g) in the dry THF (100 mL) there was added dropwise over 20 min a solution of the appropriate 1-(4-methylthiophenyl)-2-alkanone oxime (**30a**) (10.24 mmol) in dry THF (50 mL). The reaction was maintained at the specified temperature for 3h. After cooling in an ice bath the excess $LiAlH_4$ was quenched by the successive dropwise additions of propan-2-ol (2 mL), 15% aq. NaOH (2 mL) and water (15 mL). The aluminium salts were removed by filtration and washed with THF (100 mL). All organic phases were combined and volatiles removed *in vacuo*, leaving a residue which was diluted with dichloromethane (100 mL). This was dried over anhydrous Na_2SO_4 and volatiles again removed *in vacuo* leaving an oil which was purified by flash chromatography over silica gel (diethylether/hexane:50/50) to afford the amine products: **2-Amino-1-(4-methylthiophenyl)propane** (**1a**) was isolated by flash chromatography on silica gel (eluent : methanol) and was obtained as an amber oil(50%), identified by comparison with an authentic sample. **1-(4-Methylthiophenyl)-2-propanone oxime** (**30a**) was also recovered by flash chromatography on silica gel (eluent : diethylether/hexane : 50/50) as an amber solid, 18%.

4.1.49 Preparation of 2-Amino-1-(4-methylthiophenyl)butane (1b) via oxime reduction (Route C)

2-Amino-1-(4-methylthiophenyl)butane (**1b**) was obtained from oxime **(30b)** following the method above and was isolated by flash chromatography over silica gel 30% yield and identified by comparison with an authentic sample, together with unreacted oxime **30b**, (1%).

4.1.50 Preparation of 2-amino-1-(4-methylthiophenyl)alkane 4-MTA (1a) *via* reduction of nitrostyrene 4a (Route D)

To a stirred solution of $LiAlH_4$ (184.45 mmol, 7.50 g) in dry THF (150 mL) under nitrogen there was added dropwise over 30 min, a solution of **4a**, (37.78 mmol) in dry THF (100 mL). The reaction was then refluxed for 8h. After cooling in an ice bath the excess $LiAlH_4$ was quenched by the successive dropwise additions of propanol-2-ol (7.5 mL), 15% aq. NaOH (7.5 mL) and water (20 mL). The aluminium salts were removed by filtration and washed with THF (100 mL). All organic phases were combined and reduced *in vacuo*. The resulting residue was dissolved in dichloromethane (100 mL), dried over anhydrous Na_2SO_4 and solvent removed *in vacuo*, leaving an oil. The residue was purified by flash chromatography on silica gel (eluent : ethylacetate) providing the product **1a**, (80%), identified by comparison with an authentic sample. The following synthesis byproducts were also obtained: **1-(4-Methylthiophenyl)-2-propanone oxime** (**30a**), isolated by flash chromatography on silica gel (eluent : ethylacetate) as a colourless solid (0.6%) and identified by comparison with an authentic sample. **3-Methyl-2-(4-methylthiophenyl)aziridine** (**31**) isolated by flash chromatography on silica gel (eluent : ethylacetate) as an amber oil (3%). R_f 0.21 (diethylether/hexane : 80/20). IR ν_{max} (KBr) 3302, 2869 cm^{-1}. ^1H NMR δ ($CDCl_3$) 0.89 (3H, d, J=6.0Hz, CH_3), 1.06 (1H, br s, NH), 2.38 (1H, m, H-3), 2.46 (3H, s, SCH_3), 3.18 (1H, m, H-2), 7.21, 7.25 (4H, 2xd, J=8.5Hz, J=8.0Hz, H-2', H-3', H-5', H-6'). ^{13}C NMR ppm ($CDCl_3$) 13.48, 15.97, 32.11, 36.60, 126.34, 128.21, 134.69, 136.23. *m/z* 179 (M^+-1). HRMS Calculated for $C_{10}H_{14}NS$: (M^++H) 180.0847; Found: 180.0856.

4.1.51 Preparation of 2-amino-1-(4-methylthiophenyl)butane (1b) *via* reduction of nitrostyrene 4b (Route D)

Compound **1b** was prepared from **4a** as described for **1a** above. Purification of the crude product by flash chromatography over silica gel (eluent : ethylacetate) provided the product **2a**,

(74%), identified by comparison with an authentic sample. The following synthesis byproducts was also obtained: **3-Ethyl-2-(4-methylthiophenyl) aziridine (32)** was isolated by flash chromatography on silica gel (eluent : diethylether/hexane : 80/20) as a colourless oil, (7%). R_f 0.51 (diethylether/hexane : 80/20). IR ν_{max} (film) 3308, 2871 cm^{-1}. ^1H NMR δ ($CDCl_3$) 0.85 (3H, t, J=7.3Hz, CH_2CH_3), 1.17 (1H, m, CH_2CH_3), 1.21 (1H, br s, NH), 1.24 (1H, m, CH_2CH_3), 2.24 (1H, m, H-3), 2.47 (3H, s, SCH_3), 3.22 (1H, d, $J_{2,3}$=5.5Hz, H-2), 7.20, 7.26 (4H, 2xd, J=8.0Hz, J=8.5Hz, H-2', H-3', H-5', H-6'). ^{13}C NMR ppm ($CDCl_3$) 11.32, 16.04, 21.43, 36.59, 38.97, 126.37, 128.18, 134.89, 136.22. MS *m/z* 193 (diastereomer M^+).

4.1.52 1-(4-Methylthiophenyl)-1-nitroethane (34)

To a vigorously stirred mixture of 1-(4-methylthiophenyl)-1-nitroethene (**33**)[50] (15.00 mmol), silica gel (30 g) and propan-2-ol (45 mL) in dichloromethane (240 mL) at room temperature there was added, over a period of 5 min, $NaBH_4$ (61.50 mmol, 2.38 g). The mixture was stirred for an additional 15min or until the reaction has turned from yellow to colourless. Excess $NaBH_4$ was quenched by the addition of dilute HCl (50 mL). The mixture was filtered and silica gel washed with dichloromethane (100 mL). All organic phases were combined and washed successively with brine (3x50 mL) and water (3x50 mL). The organic phase was dried over anhydrous Na_2SO_4 and solvent removed *in vacuo*, leaving a brown oil. This was purified by flash chromatography on silica gel. (2.96 g, 15.00 mmol scale) and chromatographed on silica gel (eluent : hexane/diethylether : 65/35). Amber oil (89%). IR ν_{max} (film) 1549, 1378 cm^{-1}. ^1H NMR δ ($CDCl_3$) 2.46 (3H, s, SCH_3), 3.27 (2H, t, $J_{1',2'}$=7.3Hz, H-1'), 4.57 (1H, t, $J_{2',1'}$=7.3Hz, H-2'). 7.12, 7.21 (4H, 2d, J=8.0Hz, J=8.0Hz, H-2, H-3, H-5, H-6). ^{13}C NMR ppm ($CDCl_3$) 15.81, 32.85, 76.16, 127.12, 128.99, 132.38, 137.73. MS *m/z* 197 (M^+). HRMS Calculated for $C_9H_{11}NO_2S$: (M^+) 197.05105; Found: 197.05167.

4.1.53 1,3-Di(4-methylthiophenyl)-2-nitro-1-propene (35)

A mixture 4-methylthiobenzaldehyde (**5**) (3.20 g, 21.00 mmol) the nitroalkane (**34**) (21 mmol), dimethylamine HCl (44.36 mmol, 3.61 g) and potassium fluoride (3.66 mmol, 0.19 g) in toluene (30 mL) was refluxed with a Dean-Stark trap for 24h. The reaction was then diluted with toluene (150 mL) and the organic phase washed with 10% aq. HCl (3x75 mL). The organic phase was dried over anhydrous Na_2SO_4, and volatiles were removed *in vacuo* leaving an orange

oil which was chromatographed on silica gel (eluent : hexane/diethylether : 70/30). Yellow crystals (73%), mp 81-82ºC (diethylether/hexane). IR ν_{max} (KBr) 1636, 1506, 1312 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 2.46 (3H, s, SCH_3), 2.49 (3H, s, SCH_3), 4.22 (2H, s, H-3), 7.12, 7.22, 7.24, 7.35 (8H, 4xd, J=8.6Hz, J=8.6Hz, J=8.5Hz, J=8.0Hz, H-2', H-2", H-3', H-3", H-5', H-5", H-6', H-6"), 8.25 (1H, s, H-1). ^{13}C NMR ppm ($CDCl_3$) 14.92, 15.97, 32.58, 125.98, 127.33, 128.08, 130.24, 127.98, 133.50, 137.07, 148.52, 143.02. MS *m/z* 331 (M^+). Anal. Calculated for $C_{17}H_{17}NO_2S_2$: C, 61.60; H, 5.17; N, 4.23. Found: C, 61.56; H, 5.13; N, 4.13%.

4.1.54 1,3-Di(4-methylthiophenyl)-2-propanone (7)

1,3-Di(4-methylthiophenyl)-2-propanone **(7)** was prepared from **35** (2.38 g, 7.54 mmol scale) as described for (**3a**) above. The product was obtained as colourless needles (72%) following purification by flash chromatography on silica gel (eluent : hexane/diethylether : 90/10). mp 80-81ºC (diethylether/hexane) (lit.[47] mp 79-80ºC). IR ν_{max} (KBr) 1698 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 2.46 (6H, s, SCH_3), 3.66 (4H, s, H-1), 7.06, 7.20 (8H, 2xd, J=8.5Hz, H-2', H-3', H-5', H-6'). ^{13}C NMR ppm ($CDCl_3$) 15.91, 48.46, 127.00, 129.90, 130.69, 137.25, 205.31. MS *m/z* 302 (M^+).

4.1.55 2-*N*-Formylamino-1,3-di(4-methylthiophenyl)propane (36)

2-*N*-Formylamino-1,3-di(4-methylthiophenyl)propane **(36)** was prepared from **7** (1.50 g, 4.96 mmol scale) according to the procedure for compound (**8a**) above following chromatography on silica gel (eluent : diethylether) as colourless needles (15%), mp 132-133ºC (diethylether/hexane). IR ν_{max} (KBr) 3326, 1658 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 2.46 (6H, s, SCH_3), 2.66 (0.5H, dd, J_{gem}=14.0Hz, $J_{1,2}$=8.5Hz, H-1), 2.74 (1.5H, dd, J_{gem}=14.1Hz, $J_{1,2}$=7.0Hz, H-1), 2.82 (1.5H, dd, J_{gem}=13.8Hz, $J_{1,2}$=6.5Hz, H-1), 2.87 (0.5H, dd, J_{gem}=13.8Hz, $J_{1,2}$=4.5Hz, H-1), 3.70 (0.25H, m, H-2), 4.48 (0.75H, m, H-2), 5.35 (0.75H, d, J=8.0Hz, NH), 5.60 (0.25H, t, J=10.8Hz, NH), 7.05, 7.20 (2H, 2xd, J=8.5Hz, J=8.5Hz, H-2', H-3', H-5', H-6'), 7.10, 7.19 (6H, 2xd, J=8.5Hz, J=8.0Hz, H-2', H-3', H-5', H-6'), 7.48 (0.25H, d, J=12.0Hz, CHO), 8.01 (0.75H, s, CHO). ^{13}C NMR ppm ($CDCl_3$) 15.87, 15.95*, 39.23*, 41.64, 50.16*, 55.68, 126.92*, 127.04, 129.76*, 129.83, 133.82, 134.38*, 137.06*, 160.60*, 163.68. MS *m/z* 331 (M^+). Anal. Calculated for $C_{18}H_{21}NOS_2$: C, 65.22; H, 6.39; N, 4.23. Found: C, 65.29; H, 6.34; N, 4.15%. HRMS Calculated for $C_{19}H_{24}NOS_2$: (M^++H) 346.1299; Found: 346.1314.

4.1.56 2-(*N*-Formyl-*N*-methyl)amino-1,3-di(4-methylthiophenyl)propane (40)

2-(*N*-Formyl-*N*-methyl)amino-1,3-di(4-methylthiophenyl)propane **(40)** was prepared from **7** (1.00 g, 3.31 mmol scale) according to procedure for compound **19a** above and isolated as an amber oil (29%), which was used without further purification for the next reaction. IR ν_{max} (film) 1666 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 2.45 (6H, s, SCH_3), 2.63 (0.6H, s, NCH_3), 2.80 (2.4H, s, NCH_3), 2.83-2.94 (4H, m, H-1), 3.47 (0.8H, m, H-2), 4.75 (0.2H, m, H-2), 6.99, 7.12 (1.6H, 2xd, J=9.0Hz, J=8.5Hz, H-2', H-3', H-5', H-6'), 7.00, 7.18 (6.4H, 2xd, J=8.6Hz, J=8.6Hz, H-2', H-3', H-5', H-6'), 7.51 (0.8H, s, CHO), 7.85 (0.2H, s, CHO). ^{13}C NMR ppm ($CDCl_3$) 15.89, 15.90*, 25.44*, 30.31, 37.10, 38.26*, 62.37*, 126.93, 127.14*, 129.18*, 129.34, 134.29*, 134.83, 136.34, 137.00*, 162.88*, 163.03. MS *m/z* 345 (M^+).

4.1.57 2-Amino-1,3-di(4-methylthiophenyl)propane *via N*-formyl hydrolysis (37)

Compound **37** was prepared from **36** as described above for **1a** and obtained as a pale amber solid (84%), mp 52-53°C. IR ν_{max} (KBr) 3340 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 1.45 (2H, s, NH_2), 2.47 (6H, s, SCH_3), 2.51 (2H, dd, J_{gem}=13.5Hz, $J_{1,2}$=8.5Hz, H-1), 2.79 (2H, dd, J_{gem}=13.3Hz, $J_{1,2}$=5.0Hz, H-1), 3.22 (1H, m, H-2), 7.13, 7.21 (8H, 2xd, J=8.5Hz, J=8.5Hz, H-2', H-3', H-5', H-6'). ^{13}C NMR ppm ($CDCl_3$) 16.15, 43.59, 54.09, 127.12, 129.77, 136.06, 136.35. MS *m/z* 303 (M^+). Anal. Calculated for $C_{17}H_{21}NS_2$: C, 67.28; H, 6.97; N, 4.62. Found: C, 67.36; H, 7.02; N, 4.67. HCl salt. Colourless solid. M.p. 248-252ºC (dec.). IR ν_{max} (KBr) 2579, 2478 cm^{-1}. Anal. Calculated for $C_{17}H_{22}ClNS_2$: C, 60.06; H, 6.52; N, 4.12. Found: C, 59.98; H, 6.39; N, 4.11%.

4.1.58 1, 3-Di(4-methylthiophenyl)-2-propanone oxime (38)

1,3-Di(4-methylthiophenyl)-2-propanone oxime (**38**) was prepared from **7** (0.80 g, 2.64 mmol scale) according to general procedure for compound (**19a**) above, and was isolated as colourless needles (73%), mp 109-110ºC (ethanol/water). IR ν_{max} (KBr) 3229 cm^{-1}. ^{1}H NMR δ ($CDCl_3$) 2.50 (3H, s, SCH_3), 2.51 (3H, s, SCH_3), 3.42 (2H, s, H-1), 3.63 (2H, s, H-3), 7.05, 7.07, 7.17, 7.18 (8H, 4xd, J=8.0Hz, J=8.0Hz, J=8.0Hz, J=8.0Hz H-2', H-3', H-5', H-6'). ^{13}C NMR ppm ($CDCl_3$) 16.06, 16.07, 32.04, 39.06, 127.04, 127.09, 129.62, 129.70, 133.18, 133.36, 136.38, 136.78, 158.83. MS *m/z* 317 (M^+). Anal. Calculated for $C_{17}H_{19}NOS_2$: C, 64.32; H, 6.03; N, 4.41. Found: C, 64.33; H, 5.96; N, 4.42%.

4.1.59 Preparation of 1,3-di(phenyl substituted)-2-aminopropane (37) *via* oxime reduction

Compound **37** was prepared by $LiAlH_4$ reduction of **38** (0.45 g, 1.42 mmol scale) according to general procedure for reduction of compound **30a** above. The product was obtained as a pale amber solid (17%), mp 52-53°C. Spectroscopic analyses were comparable to an authentic sample.

4.1.60 2-(4-Methylthiobenzyl)-3-(4-methylthiophenyl)aziridine (39)

2-(4-Methylthiobenzyl)-3-(4-methylthiophenyl)aziridine (**39**) was isolated as an impurity by flash chromatography on silica gel (eluent : diethylether) from the preparation of **37** by $LiAlH_4$ reduction of **38**. Colourless needles (61%), mp 97-99ºC (diethylether/hexane). R_f 0.64 (methanol). IR ν_{max} (KBr) 3174 cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.13 (1H, s, NH), 2.35 (1H, dd, J_{gem}=14.3Hz, J=6.3Hz, CH_2), 2.44 (3H, s, SCH_3), 2.48 (1H, dd, J_{gem}=12.6Hz, J=1.5Hz, CH_2), 2.49 (3H, s, SCH_3), 2.52 (1H, m, H-2), 3.30 (1H, d, $J_{3,2}$=6.5Hz, H-3), 6.98, 7.15, 7.24, 7.31 (8H, 4xd, J=8.5Hz, J=8.5Hz, J=8.0Hz, J=8.0Hz, H-2', H-3', H-5', H-6'). ^{13}C NMR ppm ($CDCl_3$) 16.06, 16.26, 33.75, 36.77, 38.53, 126.46, 127.07, 128.30, 129.23, 134.50, 135.70, 136.69, 136.77. MS *m/z* 301 (M^+). Anal. Calculated for $C_{17}H_{19}NS_2$: C, 67.73; H, 6.35; N, 4.65. Found: C, 67.70; H, 6.30; N, 4.68%.

4.1.61 2-*N*-Methylamino-1,3-di(4-methylthiophenyl)propane (41)

Compound **41** was prepared from **40** as described above for **2a** and obtained as a pale amber oil (76%). IR ν_{max} (film) 3310, 2797 cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.68 (1H, br s, NH), 2.41 (3H, s, NCH_3), 2.49 (6H, s, SCH_3), 2.63 (2H, dd, J_{gem}=13.8Hz, $J_{1,2}$=6.3Hz, H-1), 2.70 (2H, dd, J_{gem}=13.8Hz, $J_{1,2}$=6.8Hz, H-1), 2.90 (1H, m, H-2), 7.12, 7.22 (8H, 2xd, J=8.0Hz, J=8.5Hz, H-2', H-3', H-5', H-6'). ^{13}C NMR ppm ($CDCl_3$) 16.12, 34.12, 39.56, 62.58, 127.04, 129.79, 135.86, 136.35. MS *m/z* 317 (M^+). HRMS Calculated for $C_{18}H_{24}NS_2$: (M^++H) 318.1350; Found: 318.1355.

4.1.62 2-*N*-Acetylamino-1,3-di(4-methylthiophenyl)- propane (42)

To a stirred solution of **37** (2.92 mmol) in pyridine (6 mL) was added acetic anhydride (5.84 mmol, 0.60 g, 0.55 mL) and the mixture was stirred at ambient temperature for 1h. The

reaction was acidified with 10% aq. HCl (100 mL) and extracted with dichloromethane (3x25 mL). The extracts were combined, dried over anhydrous Na_2SO_4 and volatiles removed *in vacuo*, generating an amber oil which slowly solidified. Recrystallisation provided the pure product as pale amber needles (64%),mp 137-138ºC (diethylether/dichloromethane). IR ν_{max} (KBr) 3294, 1647 cm^{-1}. ^1H NMR δ ($CDCl_3$) 1.85 (3H, s, $COCH_3$), 2.46 (6H, s, SCH_3), 2.70 (2H, dd, J_{gem}=14.0Hz, $J_{1',2'}$=7.0Hz, H-1), 2.79 (2H, dd, J_{gem}=14.0Hz, $J_{1',2'}$=6.5Hz, H-1), 4.41 (1H, m, H-2), 5.24 (1H, d, J=8.0Hz, NH), 7.09, 7.19 (8H, 2xd, J=8.0Hz, J=8.0Hz, H-2', H-3', H-5', H-6'). ^{13}C NMR ppm ($CDCl_3$) 15.98, 23.37, 39.14, 50.99, 126.87, 129.76, 136.35, 169.46. MS *m/z* 345 (M^+). Anal. Calculated for $C_{19}H_{23}NOS_2$: C, 66.05; H, 6.71; N, 4.05. Found: C, 65.75; H, 6.69; N, 4.01%.

4.1.63 2-*N*-Ethylamino-1,3-di(4-methylthiophenyl)propane (43)

To a stirred suspension of $LiAlH_4$ (32.94 mmol, 1.25 g) in dry THF (100 mL) under nitrogen was added dropwise over 15min a solution of **42** (7.50 mmol) in dry THF (100 mL) and the reaction was heated at reflux for 24 h. After cooling the reaction in an ice bath, excess $LiAlH_4$ was decomposed by the successive dropwise additions of propan-2-ol (1.5 mL), 15% aq. NaOH (1.5 mL) and water (12 mL). All organic phases were combined and volatiles removed *in vacuo*, leaving a residue that was dissolved in dichloromethane (100 mL). This was dried over anhydrous Na_2SO_4 and solvent again removed *in vacuo*, leaving a residue that was purified by flash chromatography on silica gel. (eluent : methanol). Pale amber oil (30%). IR ν_{max} (film) 3312 cm^{-1}. ^1H NMR δ ($CDCl_3$) 0.99 (3H, t, J=7.0Hz, NCH_2CH_3), 1.31 (1H, br s, NH), 2.46 (6H, s, SCH_3), 2.59-2.69 (6H, m, H-1, NCH_2CH_3), 2.98 (1H, m, H-2), 7.09, 7.19 (8H, 2xd, J=8.0Hz, J=8.0Hz, H-2', H-3', H-5', H-6'). ^{13}C NMR ppm ($CDCl_3$) 15.28, 16.07, 39.99, 41.53, 60.72, 126.96, 129.73, 135.78, 136.36. MS *m/z* 331 (M^++1). HCl salt. Colourless solid. mp 156-158°C (ethanol/hexane). IR ν_{max} (KBr) 2618, 2480, 2353 cm^{-1}. Anal. Calculated for $C_{19}H_{26}ClNS_2$: C, 62.01; H, 7.12; N, 3.81. Found: C, 62.05; H, 7.05; N, 3.80%.

4. 2 Biochemistry

4.2.1 Cell Culture

HEK293 cells lines stably overexpressing SERT, NAT and DAT were obtained from Dr. Patrick Schloss (Central Institute for Mental Health, Mannheim, Germany) and were cultured in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS), L-Glutamine (2mM), penicillin/streptomycin (100mg/ml) and Geneticin (500mg/ml). Stable expression was valid up to 30 passages. The PC-12 cell line was obtained from Dr. Gabriele Schmuck (Bayer HealthCare AG). PC-12 cells are derived from a transplantable rat pheochromocytoma. Undifferentiated PC-12 cells were cultured in DMEM/F12 (1:1) supplemented with 10% (v/v) horse serum, 5% (v/v) FBS, L-Glutamine (2mM) and penicillin/streptomycin (100μg/ml) and were grown on collagen coated plates. Cells were cultured for seven days in DMEM/F12 (1:1) supplemented with 1% (v/v) Horse serum, 5% (v/v) FBS, L-glutamine (2mM), penicillin/streptomycin (100μg/ml) supplemented with 100ng/ml of nerve growth factor (NGF) with media changed every two days. After seven days, all cells displayed a differentiated morphology and a static population was obtained for an additional seven days thereafter. SHSY-5Y cells were purchased from the European Collection of Cell Cultures (ECACC Lot04/c/011p17) and were cultured in DMEM/F12 (1:1) supplemented with 10% (v/v) FBS, sodium pyruvate (1mM), L-glutamine (2mM), non-essential amino acids (0.1mM) and penicillin/streptomycin (100μg/ml). Cells were maintained in a 72cm^2 tissue culture flasks at 37°C in a humidified atmosphere of 95% Oxygen, and 5% carbon dioxide.

4.2.2 Neutral Red Assay

5x 10^4 cells per well (200μl) were seeded in a 96 well plate until sub-confluent (24-36h) and treated with the appropriate compound for 48h. Following exposure of cells to drug the supernatant was removed and the cells incubated for 3 $\pm$ 1 h with 250μl neutral red (NR) dye solution under sterile conditions. NR solution was removed carefully and the cells washed before the addition of 100μl of NR assay Solubilisation solution (50% ethanol- 1% acetic acid solution in dH_20). Plates were left to incubate in the dark for 20-30 min at room temperature with gentle shaking. The absorbance of each plate was read at 540nm and at 690nm (background) within 1h. Relative cell viability was expressed as percent of vehicle treated cells. Sodium azide and Triton-X were used as positive controls for cytotoxicity, where 30mM sodium azide and 2% Triton-X

resulted in 80-90% cytotoxicity on all cell lines. Untreated cells represented 0% cytotoxicity (100% viability).

4.2.3 Statistical analysis

4.2.3.1 Non-linear regression analysis

Each compound was screened over a 1μM-1mM concentration range in triplicate on two independent days with activity expressed as percentage cell viability compared to vehicle treated controls. The cytotoxic potency of each compound was quantified by a pEC_{50} value determined by non-linear regression analysis of sigmoidal log concentration dependence curves whereby pEC_{50} is - [-logEC_{50}] ± SE (log EC_{50} is the log [Dose] when response is equal to 50% cell viability) (Tables 3-4). This was carried out as in order to express the EC_{50} values as $EC_{50} \pm$ SEM (standard error of the mean) as the $EC_{50} \pm$ SEM cannot be calculated from a Log scale. All data points (expressed as means ± S.E.M.) were analysed using GRAPHPAD Prism (version 4) software (Graphpad software Inc., San Diego, CA).

4.2.3.2 Comparison of pEC_{50} values in all cell lines

To determine if the pEC_{50} value calculated for each drug differed significantly in each cell line, statistical analysis was carried out using a one-way ANOVA Test comparing each pEC_{50} value. A P value of <0.05 was considered to reflect a significant difference. The means for different treatment groups were then compared using a two-way ANOVA test with no matching followed by a Bonferroni Post Test to compare replicate means by row to the control cell line HEK293. P values of <0.05 were considered to reflect a significant difference.

4.2.3.3 Comparison of pEC_{50} values to 4-MTA

To determine if the pEC_{50} value calculated for each drug differed significantly to 4-MTA, statistical analysis was carried out using an unpaired T-test, comparing each pEC_{50} value to the pEC_{50} value of 4-MTA. A P value of <0.05 was considered to reflect a significant difference.

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Table Legends

Table 1. 4-MTA synthesis related byproducts from Leuckart and related synthetic routes

Table 2. 2-Amino-1-(4-methylthiophenyl)butane synthesis related byproducts from Leuckart and related synthetic routes

Table 3. The effects of the 4-Methylthioamphetamine synthetic-derivatives, on hSERT, hNAT and hDAT expressing HEK cells (EC_{50})

$5x10^4$cells/well were seeded treated with for 48h. Supernatant was removed and the cells incubated for 3 $\pm$ 1 h with neutral red dye solution. NR solution was removed, washed before the addition NR assay solubilisation solution. Absorbance was read at 540nm (690nm, background) within 1h. Cell viability was expressed as percent of vehicle treated cells. The cytotoxic potency of each compound was quantified by a pEC_{50} value determined by non-linear regression analysis of sigmoidal log concentration dependence curves whereby pEC_{50} is - [-logEC_{50}] ± SE (log EC_{50} is the log [Dose] when response is equal to 50% cell viability). The effect of each compound on

the HEK293 cell line was compared to the effect of the compound on each of the transporter-overexpressing cell lines using Two-way ANOVA statistical analysis, where $p<0.05$ (*) represented a significant difference.

Table 4. *In vitro* cytotoxic effects (pEC_{50}) of the 4-MTA synthetic derivatives, on the primary neuronal PC-12 and dopaminergic, SHSY-5Y cell lines

$5x10^4$cells/well were seeded treated with for 48h. Supernatant was removed and the cells incubated for 3 ± 1 h with neutral red dye solution. NR solution was removed, washed before the addition NR assay solubilisation solution. Absorbance was read at 540nm (690nm, background) within 1h. Cell viability was expressed as percent of vehicle treated cells. The cytotoxic potency of each compound was quantified by a pEC_{50} value determined by non-linear regression analysis of sigmoidal log concentration dependence curves whereby pEC_{50} is $- [-\log EC_{50}] \pm SE$ ($\log EC_{50}$ is the log [Dose] when response is equal to 50% cell viability). pEC_{50} values were compared to the pEC_{50} value of 4-MTA using an unpaired T-test, where $p<0.05$ (*) represented a significant difference.

Figure Legends

Figure 1 MDMA, 4-MTA and related amphetamines

Figure2 The majority of representative 4-MTA synthetic impurities are cytotoxic to hSERT, hNAT, hDAT and wildtype HEK cells with EC_{50} values in the low micromolar range.

$5x\ 10^4$ cells were seeded and treated for 48h. Cells were incubated for 3 ± 1 h with neutral red dye solution. Absorbance was read at 540nM (690nm background). Relative cell viability was expressed as percent of vehicle treated cells. The cytotoxic potency of each compound was quantified by an EC_{50} value determined by non-linear regression analysis of sigmoidal log concentration dependence curves where EC_{50} is the dose at 50% cell viability. The effect of each compound on the HEK293 cell line was compared to the effect of the compound on each of the transporter-overexpressing cell lines using Two-way ANOVA statistical analysis, where $p<0.05$ (*) represented a significant difference.

Figure3. A number of impurities associated with clandestine 4-MTA synthesis are toxic to the primary neuronal PC-12 and dopaminergic SHSY-5Y cell lines at high concentrations. $5x\ 10^4$ undifferentiated PC-12 cells were seeded and cultured for seven days in media supplemented with 100ng/ml NGF. After seven days, all cells displayed a differentiated morphology from a proliferating cell to a post-mitotic, neurite bearing neuron. Cells (including $5x\ 10^4$ SHSY-5Y) were then treated for 48h with each impurity at the desired concentration before incubating for 3 ± 1 h with neutral red dye solution. Absorbance was read at 540nM (690nm background). Relative cell viability was expressed as percent of vehicle treated cells. The cytotoxic potency of each concentration of compound was compared to untreated control cells using One-Way ANOVA statistical analysis.

Schemes

Scheme 1: Synthetic routes for 2-amino and 2-N-methylamino-1-(4-methylthiophenyl)alkanes 1a, 1b, 2a, 2b.

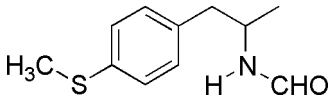
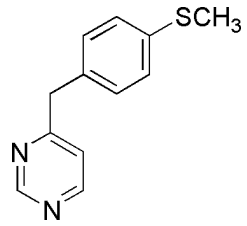
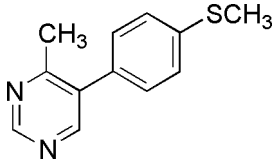
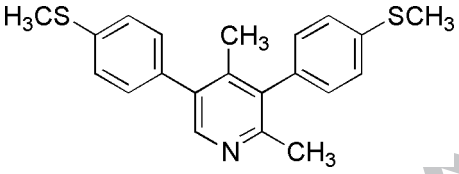
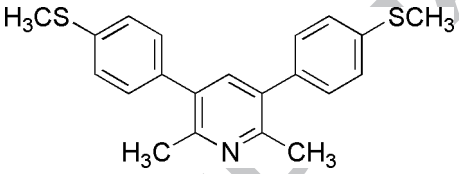
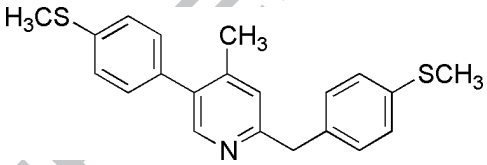
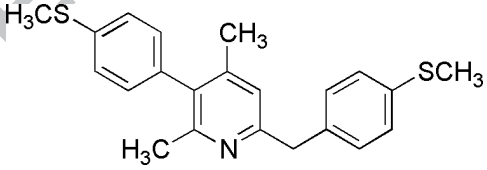
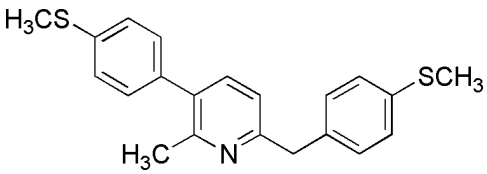
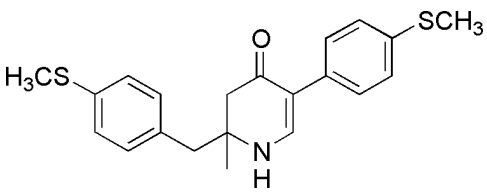
Scheme reagents and conditions: (a) $CH_3CH_2NO_2$ or $CH_3CH_2CH_2NO_2$, cyclohexylamine, CH_3CO_2H, reflux; (b) Fe, CH_3COOH, 100^o, 2h, (c) $NaCNBH_3$, NH_4OCOCH_3, CH_3OH, rt, 72h; (d) $NH_2OH.HCl$, Pyridine, EtOH, reflux, 2h; (e) $LiAlH_4$, THF, reflux, 3h; (f) Ac_2O, pyridine, reflux, 7h; (g) CH_3NHCHO, $HCOOH\ 150^o$,7h; (h) NH_2CHO,190^o, 5h; (i) 30%aq. HCl, MeOH, reflux, 7h; (j) $LiAlH_4$, THF, reflux, 8h; (k) $NaCNBH_3$, $CH_3NH_2.HCl$, CH_3OH, rt, 72h.

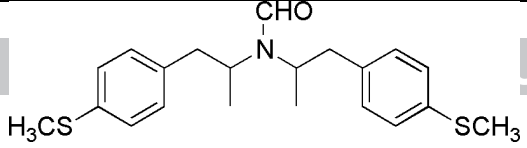
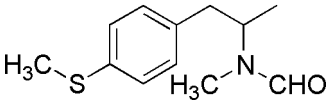
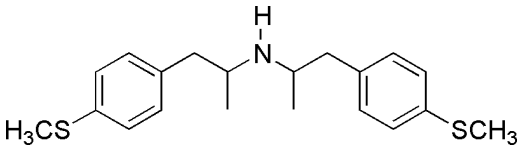
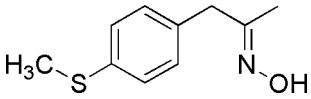
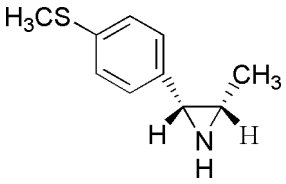
Scheme 2: Mechanism of formation of dihydropyridone 16 (R = 4-methylthiophenyl) from 1-(4-methylthiophenyl)-2-propanone (3a) and formamide

Scheme 3: Synthesis of 2-amino-1,3-diphenylpropanamines and related compounds

Scheme reagents and conditions : (a) CH_3NO_2, cyclohexylamine, AcOH ; (b) $NaBH_4$, IPA, $CHCl_3$, silca gel (c) **5**, $(CH_3)_2NH.HCl$, KF, toluene (d) Fe, CH_3COOH, 100^o, 2h, (e) NH_2CHO,190^o,5h; (f) CH_3NHCHO, $HCOOH\ 150^o$,7h; (g) 30%aq. HCl, MeOH, reflux, 7h; (h) $NH_2OH.HCl$, Pyridine, EtOH, reflux, 2h; (i) $LiAlH_4$, THF,reflux, 3h; (j) Ac_2O, pyridine, 20^o,1h, (k) $LiAlH_4$, THF, reflux, 24h.

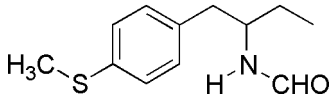
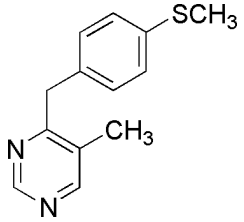
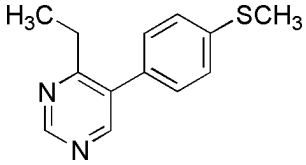
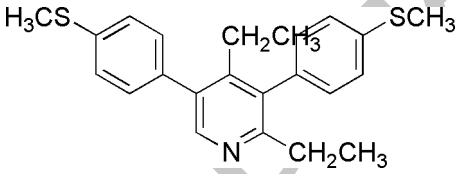
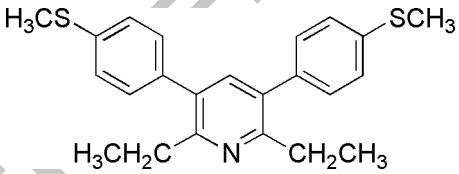
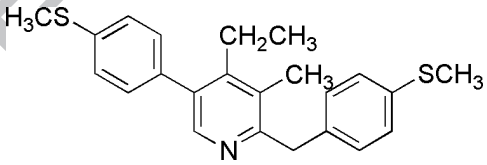
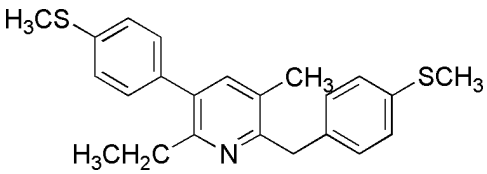
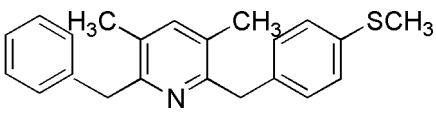
Table 1: 4-MTA synthesis related byproducts from Leuckart and related synthetic routes

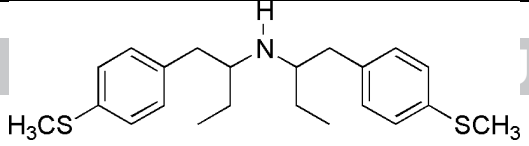
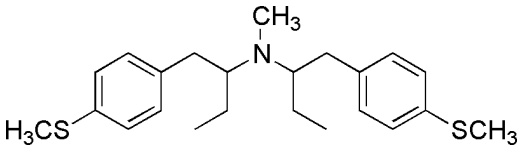
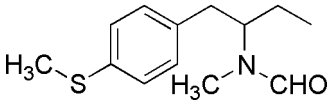
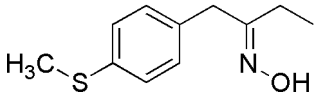
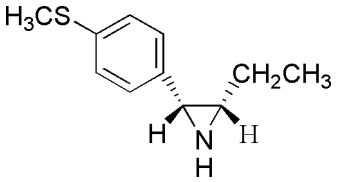
Compound	Structure	Synthesis route[a]	Yield (%)[b,c]
8a		A	3.2
9		A	0.5
10		A	2
11		A	0.3
12		A	0.4
13		A	0.5
14		A	0.3
15		A	0.4
16		A	0.7
17		A	2

			
18		A	>5
29		B	19
30a		C,D	18, 0.6
31		D	3

[a]Synthetic route A: Leuckart-Wallach reaction of propanone **3a** with formamide**,** Synthetic route A*: Leuckart-Wallach reaction of propanone **3a** with N-methylformamide . Synthetic route B: Reductive amination of propanone **3a** with sodium amalgam and sodium cyanoborohydride. Synthetic route C: Reduction of oxime with lithium aluminium hydride . Synthetic route D: Reduction of nitroalkane with lithium aluminium hydride. 4-Methylthiotoluene was also isolated as a route specific impurity in this synthesis (3.5%).
[b]Isolated yields [c]The presence of the route specific Leuckart impurities in the above formamide Leuckart reaction mixture before the hydrolysis step was confirmed by GC-MS analysis where the impurities were identified at the following retention times **8a** (m/z 209, 10.57min), **9** (m/z 216, 10.38min), **10** (m/z 216, 10.27min), **11**(m/z 351, 27.98min), **12**(m/z 351, 27.97min), **13**(m/z 351, 30.67min), **14**(m/z 365, 28.39min), **15**(m/z 351, 28.61min), **17**(m/z 373, 29.84min), **18**(m/z 369, 9.60min) and 4-methylthiotoluene (m/z 138, 4.18min) by comparison with reference isolated samples of these compounds.

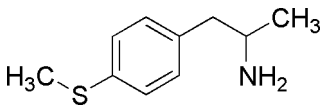
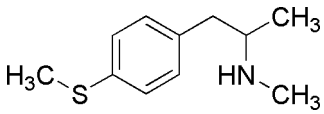
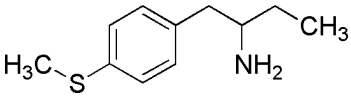
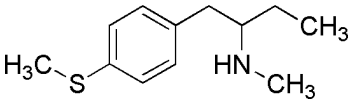
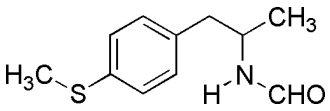
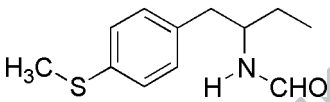
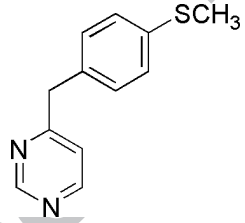
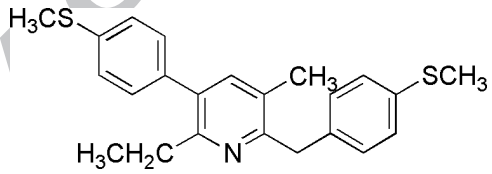
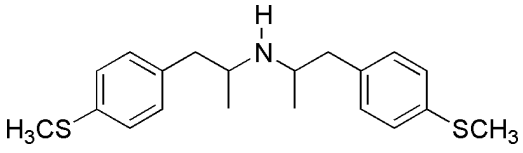
Table 2. 2-Amino-1-(4-methylthiophenyl)butane synthesis related byproducts from Leuckart and related synthetic routes

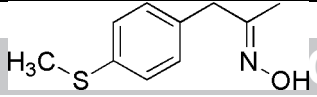
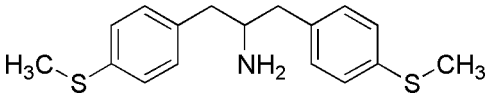
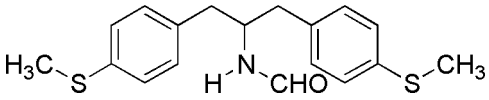
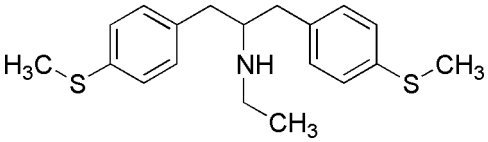
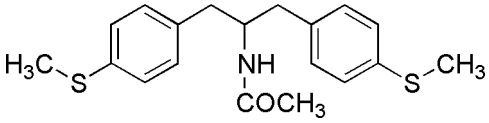
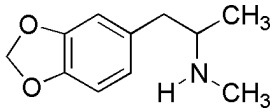
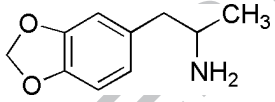
Compound	Structure	Synthesis route [a]	Yield (%)[b]
8b		A	>5
20		A	1
21		A	2
22		A	0.2
23		A	0.2
24		A	0.2
25		A	0.6
26		A	0.2
27		A,B	0.4, 0.2

			
28		A	1
19		A*	>5
30b		C	1
32		D	7

[a]Synthetic route A: Leuckart-Wallach reaction of propanone **3b** with formamide, Synthetic route A*: Leuckart-Wallach reaction of propanone **3a** with N-methylformamide ; Synthetic route B: Reductive amination of propanone **3b** with sodium amalgam and sodium cyanoborohydride; Synthetic route C: Reduction of oxime **19b** with lithium aluminium hydride ; Synthetic route D: Reduction of nitroalkene **4b** with lithium aluminium hydride [b] Isolated yield

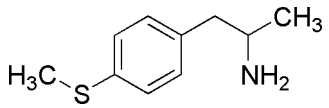
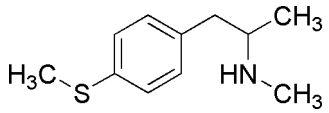
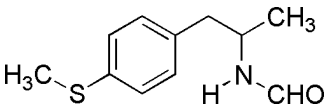
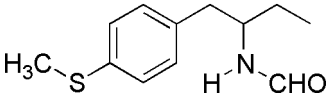
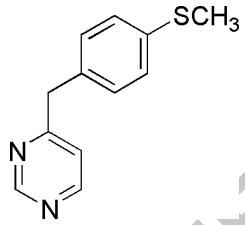
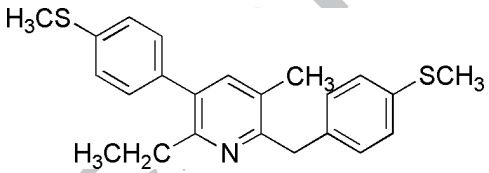
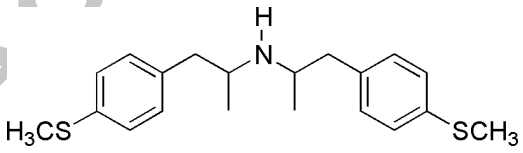
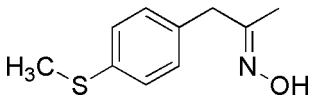
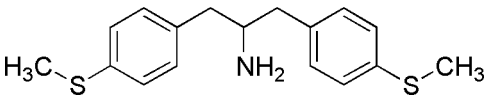
Table 3. ***In vitro*** **cytotoxic effects of the 4-MTA synthetic derivatives, on SERT, NAT and DAT expressing HEK cells (EC_{50})**

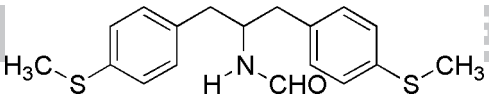
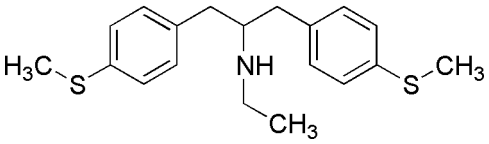
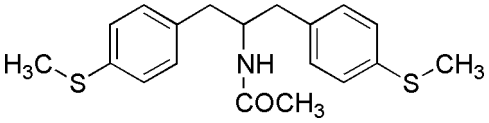
Compound structure	Compound Number	HEK293 pEC50[a] ±SE	HEK293hSERT pEC50[a] ± SE	HEK293 hNAT pEC50[a]± SE	HEK293 hDAT pEC50[a]± SE
	**1a 4-MTA**	4.73 ± 0.14	4.65 ± 0.15	4.65± 0.14	4.38 ± 0.17
	**1b**	4.71 ± 0.1	4.56 ± 0.22	4.52± 0.16	3.67 ± 0.22*
	**2a**	4.83 ± 0.11	4.75 ± 0.13	4.37 ± 0.13	4.14 ± 0.18
	**2b**	4.30 ± 0.15	4.26 ± 0.10	4.18 ± 0.21	3.38 ± 0.14*
	**8a**	2.54 ± 0.01	2.87 ± 0.19	3.43 ± 0.17	2.64 ± 0.18
	**8b**	4.21 ± 0.18	4.19 ± 0.21	4.10 ± 0.15	2.95 ± 0.16*
	**9**	5.03 ± 0.16	4.78 ± 0.17	4.20± 0.18	4.93 ± 0.19
	**25**	4.73 ± 0.1	4.46 ± 0.21	4.53 ± 0.20	4.57 ± 0.22
	**29**	4.79 ± 0.14	4.59 ± 0.19	4.60 ± 0.15	4.58 ± 0.17
	30a	2.52 ± 0.25	3.67 ± 0.17	2.76 ± 0.26	4.17 ± 0.15

					
	**37**	4.65 ± 0.20	4.61 ± 0.15	3.90 ± 0.18	4.60 ± 0.25
	**36**	< 1	4.30 ± 0.20	3.34 ± 0.17	4.06 ± 0.24
	**43**	4.77 ± 0.16	4.59 ± 0.17	3.58 ± 0.16	4.57 ± 0.19
	**42**	4.30 ± 0.18	4.41 ± 0.26	4.59 ± 0.23	4.08 ± 0.34
	**MDMA**	3.61 ± 0.19	3.31 ± 0.21	2.81 ± 0.23*	2.71 ± 0.15*
	**MDA**	3.49 ± 0.18	3.16 ± 0.34	3.23 ± 0.22*	2.23 ± 0.38*

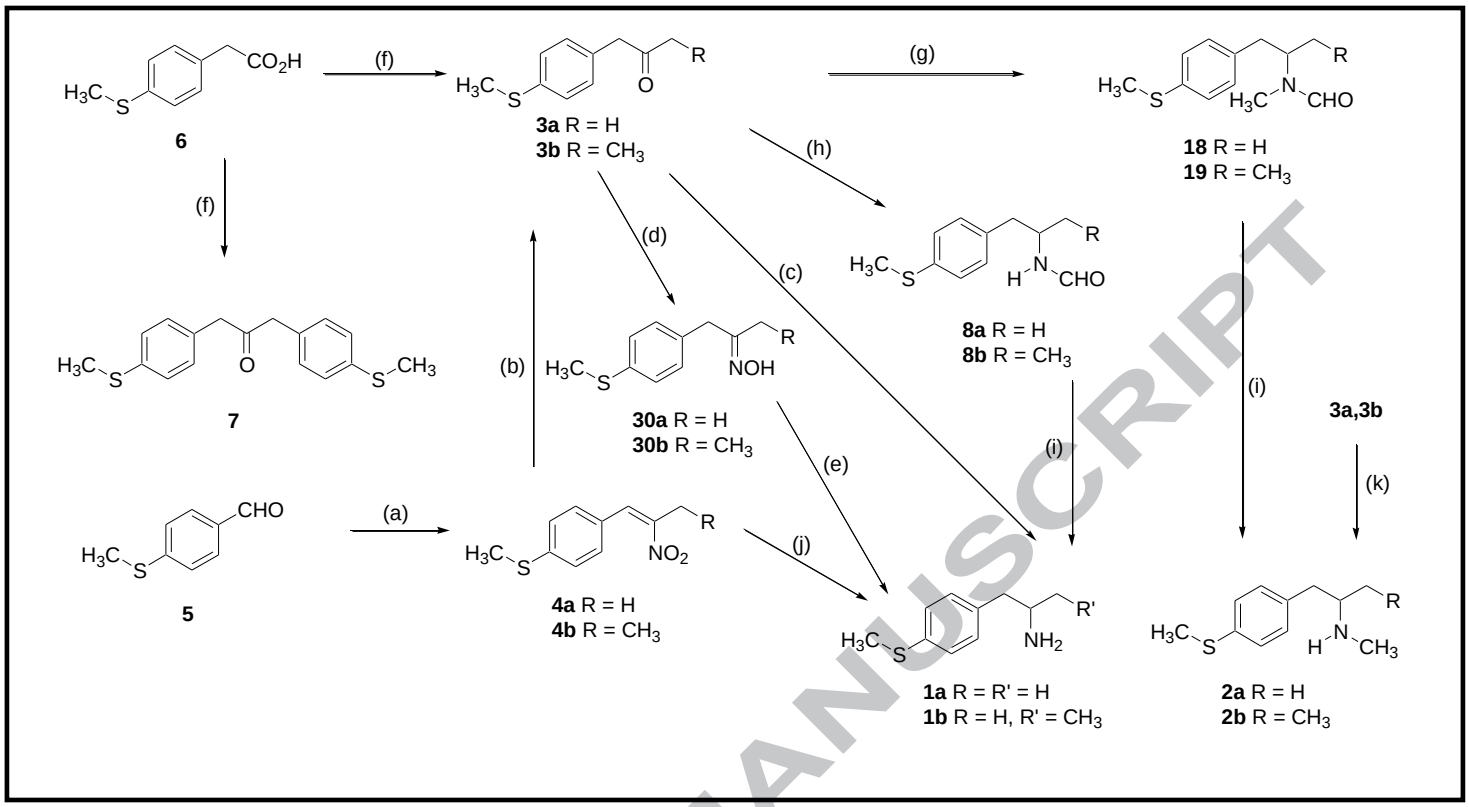
[a] **pEC50** (-(-LogEC50 is the log[Dose] when response=50%)) values were calculated from % Cell Viability versus –log concentration curves, using 4 concentrations in triplicate on two independent days. Data was subjected to non-linear regression analysis using a sigmoidal dose response (Hill slope=1) using GRAPHPAD Prism4 software (Graphpad software Inc., San Diego, CA).
Sodium azide (30mM) and Triton-X (2%) acted as positive controls for cytotoxicity resulting in 80-90% cytotoxicity to all cells.
* P<0.05. The effect of each compound in the HEK293 cell line was compared to the effect of each compound in all other cell lines using a two-way ANOVA test (GRAPH pad Prism 4) with no matching followed by a Bonferroni Multiple comparison Post test, comparing all cell lines to the HEK293 cell line.

Table 4. ***In vitro*** **cytotoxic effects (pEC_{50}) of the 4-MTA synthetic derivatives, on the primary neuronal PC-12 and dopaminergic, SHSY-5Y cell lines**

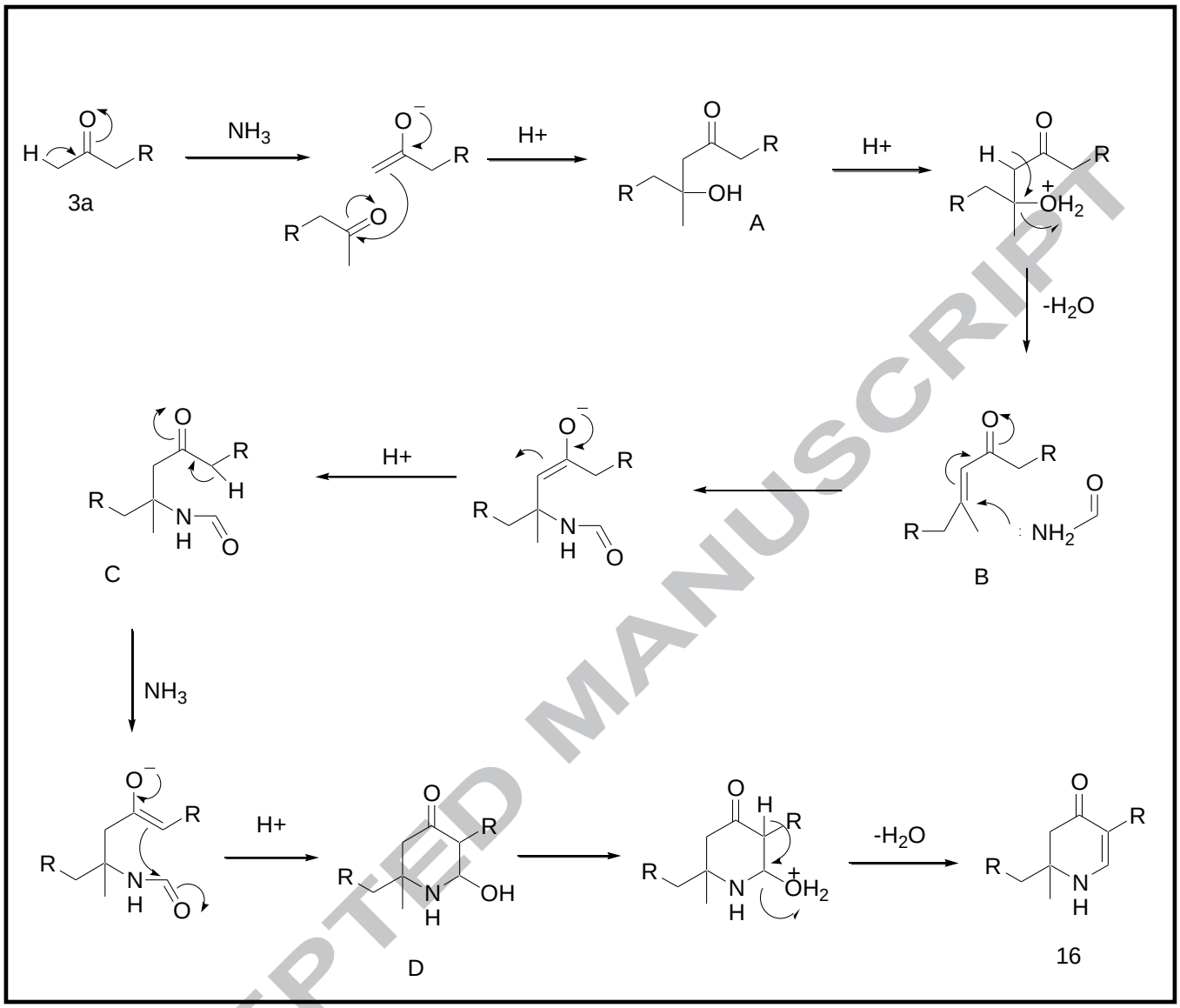
Compound structure	Compound Number	PC-12 pEC50[a] ±SE	SHSY-5Y pEC50[a] ± SE
	**1a 4-MTA**	3.53 ± 0.13	3.29 ± 0.16
	**1b**	3.26 ± 0.15	2.81 ± 0.21
	**8a**	<2	<2
	**8b**	3.13± 0.16	<2
	**9**	4.17 ± 0.25*	2.53 ± 0.11
	**25**	4.53 ± 0.15*	3.75 ± 0.09*
	**29**	4.56 ± 0.16*	4.65 ± 0.14*
	**30a**	3.11 ± 0.20	3.47 ± 0.06
	**37**	3.20 ± 0.22	4.73 ± 0.12*

	**36**	4.42 ± 0.20*	4.49 ± 0.15*
	**43**	3.64 ± 0.15	4.86 ± 0.13*
	**42**	2.38 ± 0.30	3.22 ± 0.12

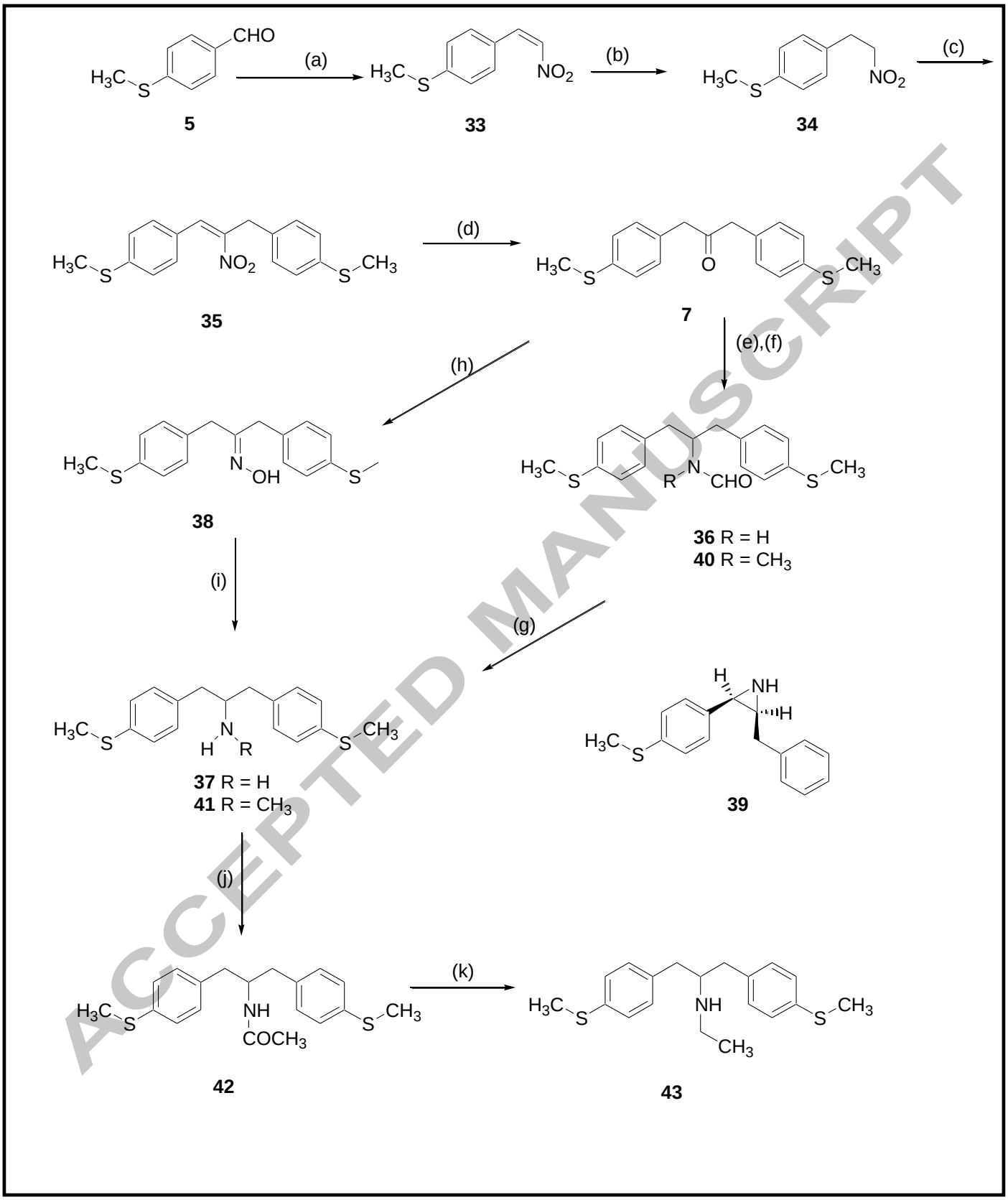
[a] **pEC50** (-(-LogEC50 is the log[Dose] when response=50%)) values were calculated from % Cell Viability versus –log concentration curves, using 4 concentrations in triplicate on two independent days. Data was subjected to non-linear regression analysis using a sigmoidal dose response (Hill slope=1) using GRAPHPAD Prism4 software (Graphpad software Inc., San Diego, CA). Sodium azide (30mM) and Triton-X (2%) acted as positive controls for cytotoxicity resulting in 80-90% cytotoxicity to all cells. * $P<0.05$. The effect of each compound was compared to the effect of 4-MTA in each cell line using an unpaired T-test (GRAPH pad Prism 4).



Scheme 1



Scheme 2



Scheme 3

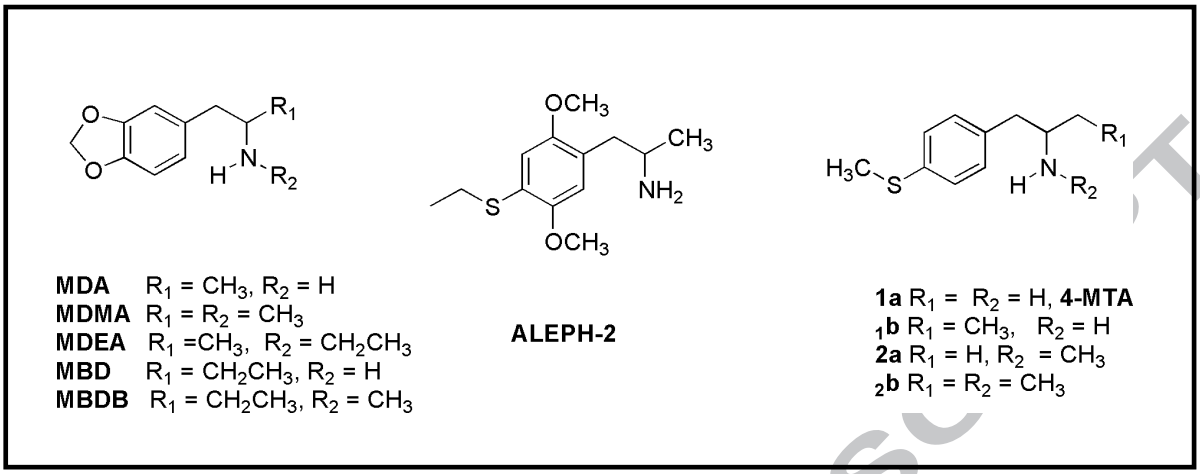


Fig. 1

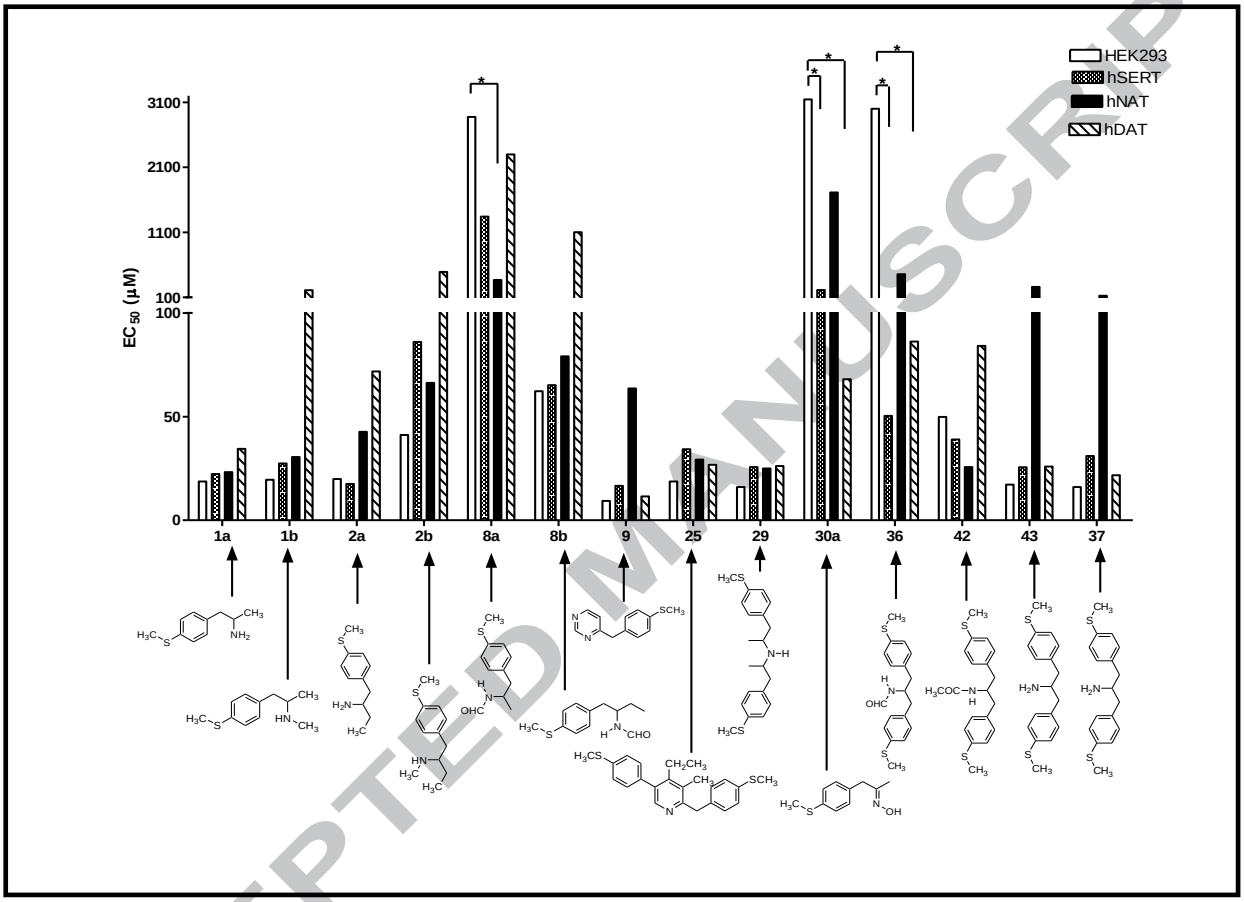


Fig.2

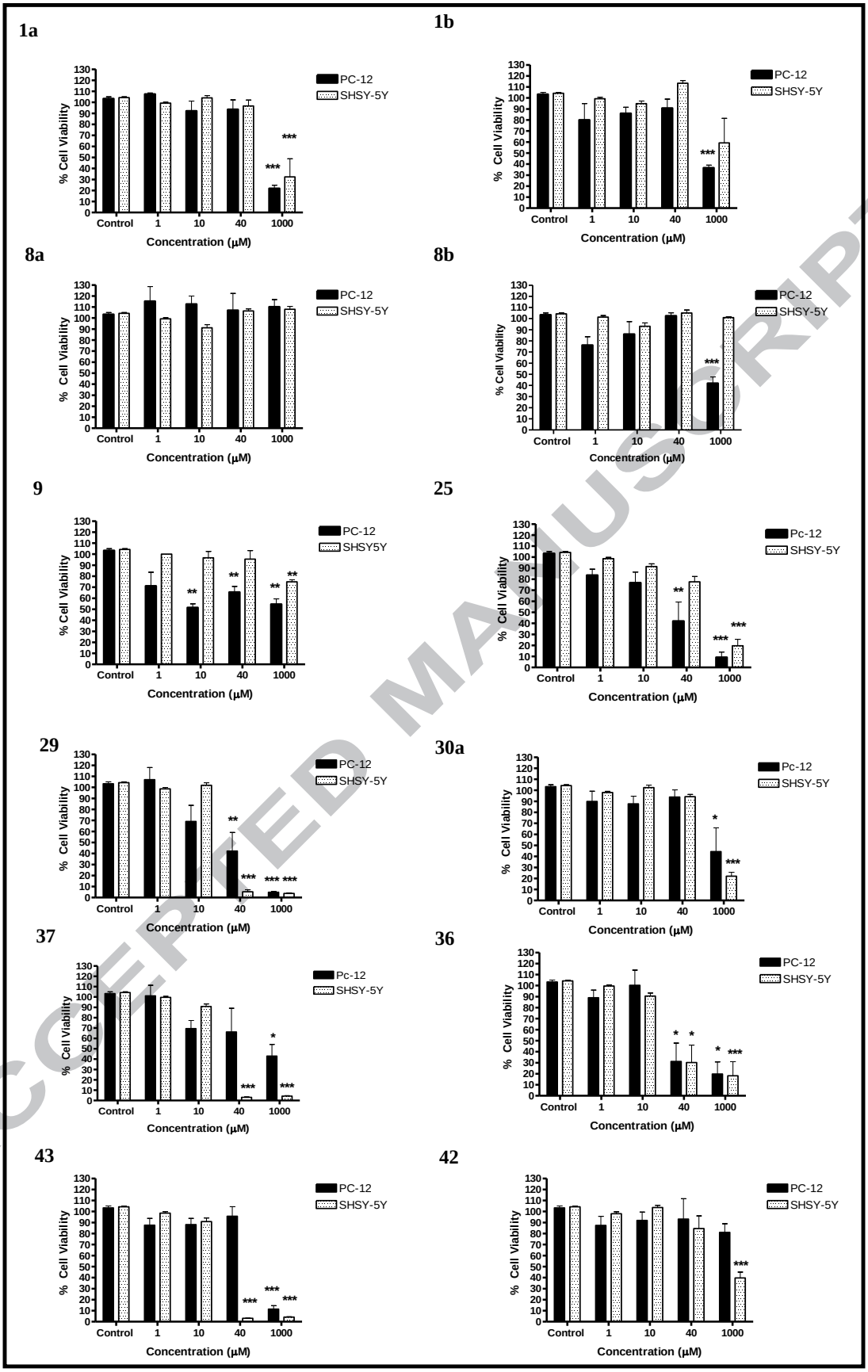


Fig.3