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TOWARDS UNDERSTANDING THE MECHANISM OF ACTION OF ABUSED
CATHINONES

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science
at Virginia Commonwealth University.

by

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List of Abbreviations

6-AB	6-Amino-6,7,8,9-tetrahydro-5H-benzocycloheptene
7-AB	7-Amino-6,7,8,9-tetrahydro-5H-benzocycloheptene
2-AT	2-Aminotetralin
2-AI	2-Aminoindane
5-HT	Serotonin
1-NAP	1-Naphthyl analog of amphetamine
2-NAP	2-Naphthyl analog of amphetamine
AAA	Arylalkylamine
AlCl ₃	Aluminum chloride
AMPH	Amphetamine
CATH	Cathinone
CH ₂ Cl ₂	Dichloromethane
CHCl ₃	Chloroform
CNS	Central nervous system
DA	Dopamine
DAT	Dopamine transporter
DEA	Drug Enforcement Administration
DMA	Dimethoxyamphetamine
DMSO	Dimethylsulfoxide
DOM	2,5-Dimethoxy-4-methylamphetamine
EC ₅₀	Effective concentration (half-maximal effect)
Et ₂ O	Diethylether
EtOAc	Ethyl acetate
EtOH	Ethanol
GABA	γ-Aminobutyric acid
GST	Glutathione S-transferase
HEPES	4-(2-Hydroxyethyl)-1-piperazineethanesulfonic acid
IAA	Indolealkylamine
<i>i</i> -PrOH	Isopropanol
K ₂ CO ₃	Potassium carbonate
KO	Knock-out
LiAlH ₄	Lithium aluminum hydride
MAO	Monoamineoxidase
MAPK	Mitogen-activated protein kinase
MBDB	1,3-Benzodioxolyl- <i>N</i> -methylbutanamine

MCAT	Methcathinone
MDC	3,4-Methylenedioxcathinone
MDEA	3,4-Methylenedioxyethylamphetamine
MDMA	3,4-Methylenedioxymethamphetamine
MDMC	3,4-Methylenedioxymethcathinone
MDPV	Methylenedioxypropylamphetamine
MeNH ₂	Methylamine
MeOH	Methanol
METH	Methamphetamine
MPD	Methylphenidate
MPP ⁺	1-Methyl-4-phenylpyridium
Na ₂ SO ₄	Sodium sulfate
NaBH ₄	Sodium borohydride
NaHCO ₃	Sodium bicarbonate
NaOH	Sodium hydroxide
NE	Norepinephrine
NET	Norepinephrine transporter
PAA	Phenylalkylamine
PD	Parkinson's disease
PDZ	PSD-95/Discs-large/ZO-1
PEA	Phenylethylamine
PET	Positron emission tomography
PICK1	Protein interacting with C-kinase
PKC	Protein kinase C
PMA	<i>p</i> -Methoxyamphetamine
PMMA	<i>p</i> -Methoxymethamphetamine
PSD	Postsynaptic density protein
ROS	Reactive oxygen species
SAR	Structure-activity relationship
SERT	Serotonin transporter
TAP	Tolylaminopropane
TH	Tyrosine hydroxylase
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TMA	Trimethoxyamphetamine
TMS	Tetramethylsilane
VMAT-2	Vesicular monoamine Transporter-2
ZO	Zonula occludens

Abstract

TOWARDS UNDERSTANDING THE MECHANISM OF ACTION OF ABUSED CATHINONES

By Rakesh Harsukhlal Vekariya, M. S.

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

Virginia Commonwealth University, 2012.

Major Director: Dr. Richard A. Glennon
Professor and Chairman, Department of Medicinal Chemistry

The dopamine transporter (DAT) mediates reuptake of dopamine from the synaptic cleft into the presynaptic terminus and plays a critical role in maintaining the normal function of dopaminergic neurons. DAT is the major target of widely abused psychostimulant drugs, including cocaine and amphetamine. DAT also figures into disease states, and it is a target for therapeutic drugs. It is known that cathinone and methcathinone, β -keto analogs of amphetamine and methamphetamine, respectively, produce pharmacological actions similar to amphetamine.

Cathinone and methcathinone analogs are recently gaining in popularity on the clandestine market (e.g. 'bath salts'). Cathinone and methcathinone analogs as well as their

amphetamine and methamphetamine counterparts were synthesized and examined at the hDAT expressed in *Xenopus* oocytes. One of the two major constituents of ‘bath salts’ (i.e., mephedrone) produced an electrophysiological signature similar to the dopamine releasing agent *S(+)*-amphetamine while the other major constituent (i.e., MDPV) produced an electrophysiological signature similar to the dopamine re-uptake inhibitor cocaine.

I. Introduction

Khat (*Catha edulis*, Celastraceae) is a plant, indigenous to the Arabian Peninsula and tropical East Africa. The fresh leaves of the khat plant have been brewed as a 'tea' or chewed for their central stimulant properties in the Arabian Peninsula and in certain regions of eastern Africa. Cathinone was determined to be an active constituent of khat. Racemic cathinone and its individual optical isomers were found to have pharmacological actions similar to amphetamine. Methcathinone, the *N*-methyl analog of cathinone, was investigated by Glennon and co-workers. It was found that cathinone and methcathinone produced discriminative stimulus effects similar to *S*(+)-amphetamine in rats. The studies also showed that amphetamine, methamphetamine, cathinone and methcathinone produced similar locomotor stimulation in mice.

There are number of new synthetic analogs of cathinone and methcathinone gaining in popularity on the clandestine market and have created considerable attention. Although, cathinone and methcathinone are controlled substances, most of their analogs are not. One of the more popular synthetic cathinones is a combination known as 'bath salts' which contains mephedrone and methylenedioxypropylone. The major constituents of 'bath salts' were recently scheduled (Schedule I). However, very limited data are available regarding the pharmacology and mechanism of action of cathinone and methcathinone analogs. Therefore, there is need for investigation in this area

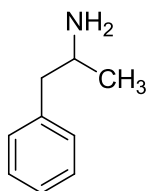
Dopamine (DA) is involved in the control of numerous functions including locomotor activity, reward mechanisms, cognition and neuroendocrine functions. In addition, the dysfunction of DA system in CNS is related to a broad spectrum of neuropsychiatric disorders, such as Parkinson's disease, schizophrenia, Tourette's syndrome, attention-deficit hyperactivity disorder, and drug addiction. The dopamine transporter (DAT) mediates reuptake of dopamine from the synaptic cleft into the pre-synaptic nerve terminus and thereby plays a critical role in terminating dopaminergic signaling and in maintaining a releasable pool of dopamine. Amphetamine-like psychostimulant drugs cause a drastic increase in synaptic DA levels by reverse transport and/or channel-like activity of the DAT.

II. Background

A. Amphetamine-like CNS Stimulants:

1. Overview:

Simple arylalkylamines (AAAs) are known to have widespread abuse potential. Arylalkylamines are further subdivided into the indolealkylamine (IAAs) and the phenylalkylamines (PAAs).¹ The phenylalkylamines can be further subdivided into the phenylethylamines and phenylisopropylamines. Amphetamine (AMPH, **1**) is the prototypical central stimulant of the phenylisopropylamine class.¹



1

The synthesis of amphetamine was first reported in 1931 and 1932 by Hartung and Munch² and Alles,³ respectively. The pressor effects of amphetamine were explained by Piness and coworkers.⁴ Amphetamine, due to its ability to promote wakefulness and vigilance, was used in the treatment of narcolepsy.⁵ After some time, a study demonstrated that benzedrine (racemic or

dl-AMPH) administration could improve the academic performance of children with behavioral disorder.⁶

This created a foundation for the usefulness of psychostimulants to treat attention-deficit hyperactivity disorder.⁷ Amphetamine has also been used to treat fatigue, obesity, Parkinsonism and for the reversal of CNS depressant toxicity.⁸ Amphetamine has both peripheral and central effects.⁸ Oral administration of amphetamine increases systolic and diastolic blood pressure in humans and animals.⁸ It leads to decreased heart rate, and cardiac arrhythmias may result after large doses.⁸ As with other sympathomimetic agents, smooth muscle reacts to amphetamine.⁸ Amphetamine causes relaxation of bronchial muscle, while it contracts the urinary bladder sphincter.⁸ In the periphery, the (-)-isomer of amphetamine is equiactive or slightly more potent than its enantiomer.⁸ Peripheral effects of amphetamine include mydriasis, tremor, sweating, jaw clenching, dry mouth and restlessness.⁹ These actions may be mediated through the release of norepinephrine, causing indirect sympathomimetic stimulation.⁹

Amphetamine, a psychostimulant, causes increased alertness, wakefulness, insomnia, energy and self-confidence in addition to decreased fatigue and appetite, as well as also enhancing mood, well-being and producing euphoria.^{10,11} High doses lead to convulsions, stereotypic movements and psychosis.^{10,11} When the effect of amphetamine fades, fatigue, anxiety and tiredness can be seen.^{10,11} These undesirable symptoms ('crash') are seen more when high or repeated doses are administered, and depression and lethargy can occur.⁹ Long term amphetamine use may lead to development of a so called 'amphetamine psychosis' characterized

by psychotic reactions, hallucinations and paranoia.⁹ Amphetamine has high abuse potential and can induce dependence, tolerance and withdrawal symptoms.⁹

Amphetamine has been used as anorectic drug, but it appears to cause unacceptable tachycardia and hypertension.¹² Because amphetamine has high abuse potential, it does not have US Food and Drug administration indication for the treatment of obesity.¹² It was found that in humans weight loss is due to decreased food intake and not to increased metabolism.⁸ Drug induced acute loss of smell and taste have been described; however, dietary restriction is important for successful weight loss.⁸ The anorectic action of amphetamine has been reported due to the activation of dopaminergic and/or β -adrenergic receptors within the perifornical hypothalamus.¹³

Additional physiological responses of amphetamine in humans and animals have been reported as amphetamine-induced hypothermia due to a decrease in metabolic heat production.¹⁴ However, at high ambient temperatures, amphetamine induces hyperthermia, which is through the increase in metabolic rate due to behavioral excitation and cutaneous vasoconstriction.^{8,14}

Amphetamine-induced acute toxic effects are related to its pharmacological actions.¹⁵ Amphetamine anorectic activity has been related to an increased risk of pulmonary hypertension.¹⁶ Symptoms of mild toxicity include nausea, vomiting, mydriasis, dry mouth, sweating, hyperreflexia, bruxism, trismus and palpitations.¹⁷ Moderate intoxication by amphetamine can include hyperactivity, anxiety, confusion, panic attack, psychosis with hallucinations, tachycardia, hypertension and increased body temperature.¹⁷ Sometimes suicidal and homicidal tendencies can occur.¹⁷ Severe intoxication by amphetamine includes delirium,

coma, seizures, hypertension, dysrhythmia, hyperpyrexia, and renal failure associated with rhabdomyolysis. Additionally, amphetamine can induce acute ischaemia and haemorrhagic stroke.¹⁷

2. General Structure-activity relationship (SAR):

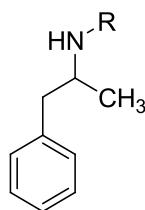
Most phenylisopropylamine derivatives lack central stimulant activity.¹⁸ In general, there are more “non-amphetamine like” derivatives of amphetamine than “amphetamine like” derivatives of amphetamine.¹⁸ That is, comparatively few amphetamine derivatives retain the central stimulant action of amphetamine (**1**), still fewer retain the potency of amphetamine.¹⁸

The central stimulant action of amphetamine and amphetamine-related agents is commonly assessed by measuring their ability to increase the locomotor activity of rodents. That is, these agents are locomotor stimulants and produce hyperlocomotion. Another means of measuring the “amphetamine-like” nature of central stimulants is to examine their stimulus properties in animals trained to discriminate amphetamine, an amphetamine isomer, or a related agent, from saline vehicle. In this procedure, animals are generally trained to distinguished (i.e., discriminate) among the effects produced by one drug to those produced by another. Another drug, which compares to test drug, may be a different drug, a different dose of the test drug or vehicle. Studies can be done, when the animals learned to discriminate the training drug from, for example, vehicle (saline).

These two measures provide comparable results and offer a convenient approach to formulation of structure-activity relationships. Using data from such assays, it is relatively easy to determine the effect of structure modification on amphetamine-like activity.

a. *N*-Alkylated substituents:

The primary aim of structure-activity studies are to identify those structural features of an agent that are necessary for or that contribute to activity. It was reported by Woolverton in self-administration studies of test drugs in cocaine-maintained animals that *N*-alkylated amphetamine having substituent groups larger than ethyl are less potent behaviorally than *N*-methyl (i.e., **2**) and *N*-ethyl (i.e., **3**) substituted amphetamine derivatives and it may be due to decreased ability of those compounds to release catecholamines centrally.¹⁹ It was reported that methamphetamine (**2**) produces stimulus generalization to (+)-amphetamine.²⁰ Van der Schoot et al.²¹ found that homologation of the *N*-methyl group of (±)-methamphetamine to ethyl, n-propyl, and n-butyl, resulted in a rapid decrease in a mouse locomotor activity assay.



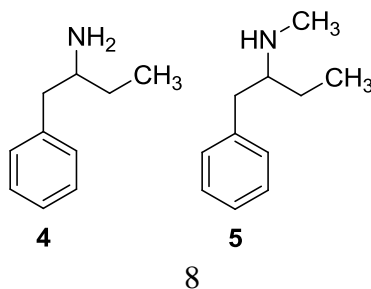
2, R = -CH₃
3, R = -CH₂CH₃

b. α -Alkyl substituents:

The methyl group present alpha to the amino group in amphetamine has been previously established to hinder metabolism by monoamine oxidase by a steric effect.⁸ The methyl group

also makes amphetamine optically active.²² The both enantiomers of amphetamine have been examined.²² As behavioral stimulants and as releasers of striatal DA, the (+)-isomer of amphetamine is 5-7 times more potent than the (-)-isomer; however, the (-)-isomer was found to be equipotent to the (+)-isomer for the release of NE and is similar in potency for the development of acute psychotic symptoms in humans.²² It is reported that with respect to peripheral actions, both enantiomers of amphetamine are essentially equivalent in potency, while the (+)-isomer of amphetamine is seven-fold more active than the (-)-isomer in producing central effects.²³ As both optical isomers of amphetamine produce a similar discriminative stimulus effect but that one isomer is fairly more potent than the other, Young et al.²³ referred to amphetamine as being stereoselective rather than stereospecific. The removal of the α -methyl group results in a compound (PEA, phenylethylamine) which does not produce amphetamine-stimulus generalization in animals.²³

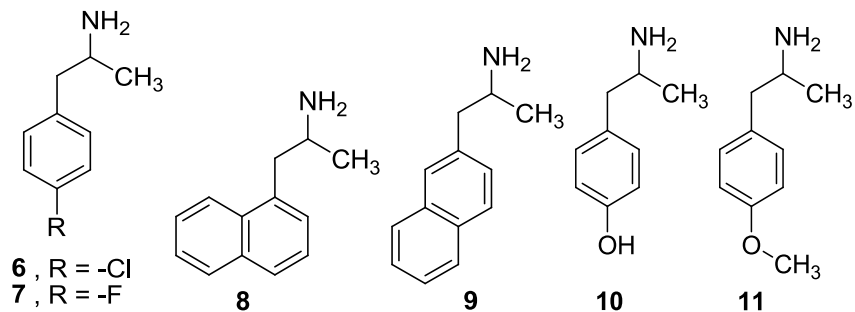
At the α -position of amphetamine, extension of the methyl to an ethyl group dramatically reduces amphetamine-like activity.²⁴ It has been reported that the (+)- α -ethyl homolog of amphetamine (i.e., **4**) failed to fully substitute for 1 mg/kg of amphetamine in drug discrimination studies in rats.²⁴ In the same study, the (\pm)- α -ethyl homolog of *N*-methylamphetamine (i.e., **5**) was able to substitute to amphetamine but showed one-tenth the potency of amphetamine.²⁴



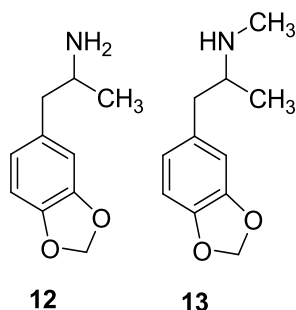
c. Aromatic substituents:

The introduction of para-chloro substitution (i.e., **6**) in the aromatic portion of amphetamine failed to produce stimulus generalization in drug discrimination studies in rats trained to discriminate amphetamine from saline, while in the same studies para-fluoro substitution (i.e., **7**) produced stimulus generalization.²⁵ The benzene ring fusion of the b-face (i.e., 1-NAP, 1-naphthyl analog of amphetamine, **8**) or the c-face (i.e., 2-NAP, 2-naphthyl analog of amphetamine, **9**) of racemic amphetamine failed to produce stimulus generalization in drug discrimination studies in rats trained to discriminate amphetamine from saline.²³ These two naphthyl analogs were inactive as locomotor stimulants in mice.²¹

Amphetamine analogs resulting from aromatic substitution are, in general, not amphetamine-like.²³ It was demonstrated by several groups that the 4-hydroxy analog of racemic amphetamine (i.e., 4-OH PIA, **10**) does not produce amphetamine-appropriate responding,²⁶⁻²⁸ and was inactive in mouse locomotor assays.²¹ Probably, this is because of the inability of 4-OH PIA to penetrate the blood-brain barrier.²³ O-Methylation of 4-OH PIA results in a less polar compound, i.e., 4-methoxyamphetamine (PMA, **11**).^{28,29} In two separate studies, it was found that PMA produces amphetamine-stimulus generalization, but is less potent than amphetamine.^{28,29} PMA was only a weak locomotor stimulant in mice.^{21,30}



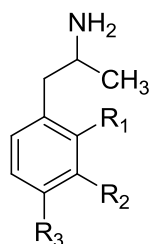
The 3,4-methylenedioxy analogs of amphetamine and methamphetamine (MDA, **12** and MDMA, **13**, respectively) have been studied.³¹ It was found that racemic MDA and MDMA produce amphetamine-like stimulus effect in rats trained to discriminate amphetamine from saline.³¹ However, in the same study, *S*(+)-MDA produced amphetamine-like effects while *R*(-)-MDA failed to do so.³¹



The six possible dimethoxy analogs (DMAs) of amphetamine have been evaluated in amphetamine trained animals.²⁸ It was found that none of these analogs produced complete amphetamine-stimulus generalization.²⁸ Five possible trimethoxy analogs (TMA's) of amphetamine (i.e. 2,3,4-TMA, 2,3,5-TMA, 2,4,5-TMA, 2,4,6-TMA, 3,4,5-TMA) have been studied.²⁸ 2,3,4-TMA and 2,3,5-TMA produced saline-like effects, while the other three analogs produced disruption of behavior.²³ Two of the DMA (i.e., 2,4-DMA and 2,5-DMA) and all TMA

derivatives of amphetamine were found to produce DOM like hallucinogenic effect in drug discrimination study.³²

Methyl group substitution on the aromatic ring portion of amphetamine results in three possible methylamphetamines (or tolylaminopropanes; TAPs); i.e., oTAP, mTAP, and pTAP (**14**, **15** and **16**, respectively). Only oTAP produced amphetamine-like stimulus effects in rats trained to discriminate (+)-amphetamine from saline, while mTAP and pTAP produced partial amphetamine-like stimulus effect in the same studies.³³ Compounds **14** and **15** were found to be weak locomotor stimulants in the mouse.²¹



14, R₁ = -CH₃, R₂ = -H, R₃ = -H

15, R₁ = -H, R₂ = -CH₃, R₃ = -H

16, R₁ = -H, R₂ = -H, R₃ = -CH₃

Wee et al.³⁴ reported the *in-vitro* potency of *p*-methylamphetamine (pTAP, **16**) and *p*-fluoroamphetamine (**7**) as releasers of monoamine neurotransmitters (**Table 1**). They also reported that as these compounds have the reinforcing effects consistent with full or partial amphetamine-like discriminative stimulus effects, pTAP and *p*-fluoroamphetamine have amphetamine type abuse potential.³⁴ It has been also reported that *p*-methoxyamphetamine (PMA, **11**) releases dopamine and norepinephrine (**Table 1**).³⁵ PMA has been known to be used illicitly in Australia since 1994 and is also becoming popular at rave parties in the United States.³⁶

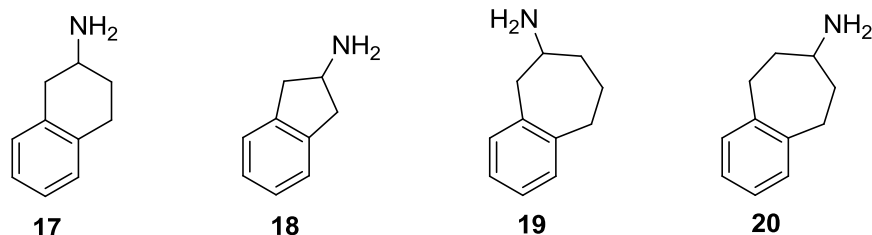
Table 1. In vitro potency of 4-substituted amphetamine analogs as releasers of monoamine neurotransmitters.^{34,35}

Drug	[³ H] NE EC ₅₀ (nM)	[³ H] DA EC ₅₀ (nM)	[³ H] 5-HT EC ₅₀ (nM)
<i>p</i> -Methylamphetamine (pTAP; 16)	22.2	44.1	53.4
<i>p</i> -Fluoroamphetamine (7)	28.0	51.5	939
<i>p</i> -Methoxyamphetamine (11)	166	867	–

d. Conformational constraint:

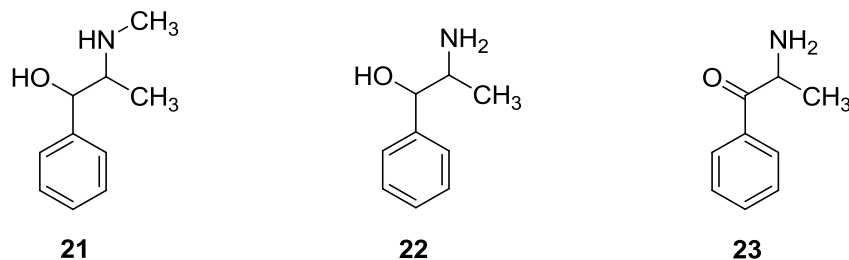
The side chain conformations of various phenylisopropylamines have been studied by nuclear magnetic resonance, and suggest that in solution, an extended trans-phenylamino arrangement is preferred.²⁹ Some of the conformationally restricted analogs of phenylalkylamines mimic this conformation.²⁹ For example 2-aminotetralin (2-AT, **17**) mimics this to some extent, while 2-aminoindane (2-AI, **18**) to a lesser extent. It was found that 2-AI (**18**) and in particular 2-AT (**17**) are capable of producing various amphetamine-like effects, including anorexia and locomotor stimulation in animals.²⁹ Four conformationally restricted analogs, 2-AI (**18**), 2-AT (**17**), 6-amino- and 7-amino-6,7,8,9-tetrahydro-5H-benzocycloheptene (6-AB, **19** and 7-AB, **20**, respectively) were studied and it was found that 2-AT (**17**) is most similar to racemic amphetamine in potency and may be the conformation that best mimics amphetamine necessary for producing amphetamine-like stimulant effects, however, compounds **19** and **20** failed to produce amphetamine-like stimulant effect.²⁹ The racemic aminotetralin **17**

produced 10% the locomotor stimulant action of amphetamine in mice, whereas **18** was inactive at the highest doses tested.²¹



e. β -Substituents:

Substituents β to the amine have not been well explored.²⁰ Ephedrine (**21**), an agent that possesses a β -hydroxy group was found to produce amphetamine-stimulus generalization.²⁰ In animals, administration of norephedrine (**22**) produced 70-75% amphetamine-appropriate responding.²⁰



The β -keto analog of amphetamine, i.e., cathinone (**23**), is a central stimulant that occurs naturally.^{29,37} There is little effect of this carbonyl group on potency.^{29,37} Cathinone was found to produce amphetamine-like responding and also like amphetamine, the *S*-isomer of cathinone is more potent than the *R*-isomer.^{29,37} Cathinone and its derivatives are discussed in more detail in a later section.

The general structure-activity relationships for amphetamine-like action are summarized in

Figure 1.

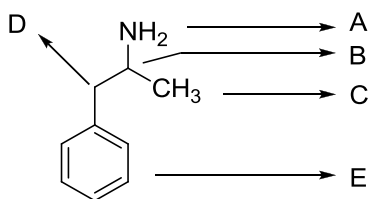
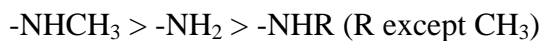


Figure 1. General structure-activity requirements for producing amphetamine-like central stimulant and/or discriminative stimulus effects

A. N-alkyl substituents:



B. Chiral center:

S(+)-isomer is more potent than *R*(-)-isomer

C. α -Methyl substituent:

Optimal; removal or extension from methyl reduces potency

D. β -substituents:



E. Aromatic substituents:

Generally reduce potency

3. Mechanism of Action:

Amphetamine acts as an indirect monoamine agonist, producing release of norepinephrine, dopamine and serotonin from presynaptic terminals in the CNS and at the peripheral levels.^{38,39} Similar results have been reported by Rothman et al.⁴⁰ in invitro studies (**Table 2**).

Table 2. Pharmacological profile of amphetamine in DA, NE, and 5-HT release and uptake inhibition assays.⁴⁰

Drug	NE Release EC ₅₀ (nM)	NE Uptake K _i (nM)	5-HT Release EC ₅₀ (nM)	5-HT Uptake K _i (nM)	DA Release EC ₅₀ (nM)	DA Uptake K _i (nM)
S(+)- Amphetamine	7.07	38.9	1765	3830	24.8	34
S(+)- Methamphetamine	12.3	48.0	736	2137	24.5	114

Amphetamine interacts with the membrane transporters responsible for neurotransmitter reuptake and vesicular storage systems.¹⁷ It looks like amphetamine is able to enter the nerve terminal through passive transport or through a reuptake transporter, thus inhibiting the reuptake of monoamines.¹⁷ Once transported inside the neuron, amphetamine reverses the direction of the transporter causing it to release norepinephrine, dopamine and serotonin to the synaptic cleft.¹⁷ Its exact mechanism for producing these effects is unknown. However, an alternative mechanism explained by De Felice and co-workers, once amphetamine is inside the terminal.⁴¹ In addition, amphetamine is also found to act as a mild inhibitor of the enzymes monoamine oxidase A and B.¹⁷ However, there does not seem to be a relationship between the locomotor actions of these agents and inhibition of MAO.²¹

The anorectic effect, alerting effect and a part of the locomotor-stimulating action of amphetamine are presumably through release of NE and DA from noradrenergic nerve terminals.⁸ Treatment of the animals with α -methyltyrosine, an inhibitor of tyrosine hydroxylase, prevents all these effects of amphetamine by inhibiting catecholamine synthesis.⁸ Particularly in the neostriatum, release of DA from dopaminergic nerve terminals by amphetamine has been linked to certain aspects of locomotor activity and stereotyped behavior.⁸ These behavioral effects are seen at higher doses, and it can be understood by the need of higher concentration of amphetamine to release DA from brain slices or synaptosomes in vitro.⁸

Depletion of serotonin by pretreatment of animals with para-chlorophenylalanine (oral or intraperitoneal administration) trained to discriminate amphetamine from saline had no significant effect on amphetamine-appropriate responding.⁴² Similarly, there was no effect when animals pre-treated with disulfiram,⁴² phenoxybenzamine,⁴² phentolamine,⁴³ atropine⁴³ and propranolol.^{42,44,45} Ho and Huang,⁴⁴ based on the results gained with α -methyl-para-tyrosine suggested that dopamine might play dominant role in the discriminative stimulus produced by amphetamine; they also suggested that the stimulus generated by amphetamine might be more dependent on newly formed dopamine rather than direct interaction of amphetamine with dopamine receptors. It was found that the dopamine precursor L-DOPA (in combination with a decarboxylase inhibitor) produced amphetamine-stimulus generalization.²³ From the majority of studies, it was found that apomorphine can produce an effect that is somewhat similar in nature to that produced by amphetamine.²³ This result supports the suggestion made by Ho and Huang, that a direct dopamine interaction may not be as important as the release of newly synthesized

dopamine in producing amphetamine stimulus.^{23,44} Further, D'Mello has suggested that mesolimbic dopamine system may play a role in the amphetamine discriminative stimulus, based on electrical brain-stimulation experiments.⁴⁶

It has been suggested by McMillen that behavioral stimulants can be sub-divided into two classes of drugs: 1. amphetamine-like direct releasers, and 2. up-take blockers, of dopamine and norepinephrine.²³ Amphetamine-like agents release directly, as well as inhibit reuptake of dopamine and norepinephrine in both *in vivo* and *in vitro* studies.²³ Additional studies were done with antagonists of particular neurotransmitters to study discriminative stimuli mechanism.²³ All attempts to abolish or attenuate the amphetamine stimulus effects by pretreatment of animals with serotonin antagonists have been unsuccessful.²³ Similarly, tricyclic antidepressants, e.g. imipramine, nortryptiline, desipramine, failed to block an amphetamine stimulus.⁴⁷ Supporting the belief that the stimulus effects of amphetamine are mediated through a dopaminergic mechanism, certain dopamine antagonists have been found to attenuate amphetamine appropriate responding; e.g. chlorpromazine,^{44,47} clozapine,⁴⁸ pimozide,⁴⁴ trifluperazine,^{47,49} thioridazine,⁴⁷ fluphenazine,⁴⁹ and haloperidol.^{42,45,49-51} Thus, it appears that amphetamine is producing discriminative stimulus, most likely centrally mediated, through a mechanism that involves dopamine and to a lesser extent norepinephrine.²³

It has been reported that chronic misuse of amphetamine may result in long-lasting impairment of brain function.⁵² Neurochemical and morphological changes in dopamine or serotonin neurons in animal studies with response to administration of amphetamine have been

partially confirmed with brain imaging studies in humans (reduction in dopamine/serotonin transporters).⁵³⁻⁶⁰

Methamphetamine produces euphoria by elevating synaptic dopamine.^{61,62} Methamphetamine, being lipophilic, may enter nerve terminals by diffusing across the plasma membrane.^{61,62} Inside the terminal, methamphetamine binds to the dopamine transporter (see **Table 2**) to prevent reuptake and also induces the release of dopamine into the synapse.^{61,62} Methamphetamine increases dopamine in the cytoplasm which causes neurotoxicity.^{61,62} Methamphetamine produces these effects via increasing cytoplasmic dopamine through promoting the activity of tyrosine hydroxylase (which increases dopamine production) and inhibiting monoamine oxidase (which metabolizes dopamine), while the dominant mechanism is the effect of methamphetamine on the dopamine transporter, VMAT-2. The combined action of methamphetamine leads to increased concentration of cytoplasmic and synaptic dopamine.⁶³⁻⁷¹

Methamphetamine, apart from binding to the dopamine transporter and preventing reuptake of dopamine from the synapse, also reverses the dopamine transporter direction causing the transporter to release dopamine from the cytoplasm into the synapse.⁷² The mechanism of this phenomenon is unknown.⁷² After 1 h post ingestion, methamphetamine decreases the function of the vesicular dopamine reuptake transporter.⁶¹ The vesicular dopamine transporter normalizes within 24 h following the ingestion of a single dose of methamphetamine, but after multiple high doses of methamphetamine, vesicular dopamine transporters only normalize partially.⁶¹

In postmortem studies using positron emission tomography (PET), the chronic methamphetamine use decreases dopamine transporter density in certain regions of brain

associated with motor and cognitive impairment.⁷² Though after prolonged drug abstinence, dopamine transporter density may slowly return to normal, implying that the decrease in transporter density at the beginning is a neuroadaptive response to the increased synaptic dopamine.⁷² While, even if dopamine transporter density returns to normal after drug abstinence, cognitive deficits may still persist.⁷²

In human methamphetamine users, PET studies show decreased D₂ receptor density that may be due to down-regulation from exposure to increased synaptic dopamine concentrations.⁷³ A redistribution of VMAT-2 due to methamphetamine within the nerve terminal is seen, which makes the transporter less available to the dopamine molecule, reducing the ability of cytoplasmic dopamine to move into the protective vesicle.⁷² In addition, methamphetamine also leads to release of dopamine from the vesicle into the cytoplasm by two methods.⁶⁶⁻⁶⁹ First, binding of methamphetamine to VMAT-2 causes vesicular dopamine efflux into the cytoplasm.⁶⁶⁻⁶⁹ Second, amphetamine, being a weak base, moves across the vesicular membrane in its unchanged form and accumulates in the acidic vesicle in its charged form (now less able to penetrate the vesicle membrane).⁶⁶⁻⁶⁹ The acidic pH gradient inside the vesicle provides the energy for amphetamine accumulation in the vesicle against its concentration gradient.⁶⁶⁻⁶⁹ As more and more basic amphetamine accumulates into vesicles, the interior of the vesicle becomes more alkaline.⁶⁶⁻⁶⁹ Due to this alkalinization, the vesicle collapses releasing dopamine into the cytoplasm.⁶⁶⁻⁶⁹

As vesicular dopamine decreases, it also causes a decrease in dopamine release into the synapse following depolarization.⁶¹ However, the overall concentration of synaptic dopamine

depends on the action of methamphetamine on the dopamine transporter.⁶¹ Methamphetamine produces selective degeneration of dopamine neuron terminals without cell body loss in neuronal cell cultures.⁷⁴ Methamphetamine acidotropic uptake causes osmotic swelling of vacuoles.⁷⁴ Hyperthermia and oxidative stress may be seen at the initial stage of methamphetamine neurotoxicity.⁷⁴

Acidic organelles, like synaptic vesicles, are collapsed by methamphetamine-induced release of dopamine into the cytoplasm.⁷² In the cytoplasm, dopamine reacts with molecular oxygen to form reactive oxygen species (ROS) such as superoxide- and hydroxyl-free radicals and hydrogen peroxide.⁷² This whole process is known as intracellular oxidative stress.⁷² These ROS lead to damage all cellular biomacromolecules (lipids, sugar, proteins, polynucleotides) and can also form secondary products that cause damage as well.⁷² The CNS is more susceptible to oxidative insult due to high concentration of polysaturated lipids and redox-active transition metals, as well as poor concentration of antioxidant and high rates of oxygen utilization.⁷² The neurotoxic effects seen in animals after methamphetamine administration might be due to oxidative stress.⁷²

The original dopamine hypothesis of schizophrenia proposed that there is overactivity of the striatal dopamine systems.⁷⁵ Additionally, antipsychotic drugs function by blocking dopamine D₂ receptors; also, chronic use of psychomotor stimulants can induce psychotic symptoms and this supports the hyperdopaminergic basis for schizophrenia.⁷⁵ Strong evidence supporting the increased dopaminergic activity in schizophrenia has come from imaging studies showing that the binding of radiolabelled dopamine D₂ receptor ligands to D₂ receptors is displaced by

amphetamine-induced dopamine release, and this effect is increased in schizophrenia.⁷⁵ One study showed that a low dose of amphetamine worsens psychosis in patients with schizophrenia, and the severity of this response was correlated to the estimated release of dopamine.⁷⁵

The presence of amphetamine sensitization in humans has been obtained indirectly from observing behavioral and psychological changes in chronic amphetamine abusers.⁷⁵ The limitation of this approach is that it is primarily a correlation and it is not possible to rule out that the observed behavioral changes preceded the start of amphetamine abuse.⁷⁵ There are some studies which provide direct evidence for amphetamine sensitization in drug-naïve human subjects.⁷⁵ In one study, subjects were exposed to a single dose of amphetamine at three different time points, with certain pre-selected behaviors being recorded during the first and third amphetamine exposure.⁷⁵ After full amphetamine treatment, subjects showed an increased rate of eye-blink responses and increased motor activity following the third amphetamine exposure, as compared to their response following the first or second exposures.⁷⁵ Different studies conducted by another group provide additional evidence for amphetamine-induced behavioral changes and, further, showed that amphetamine exposure was associated with a decrease in D₂ receptor radioligand binding ([¹¹C]raclopride) in the ventral striatum following re-exposure to amphetamine, which indicates enhanced mesolimbic dopamine activity.⁷⁵

B) Dopamine Transporter (DAT):

Dopamine (DA) is involved in the control of numerous functions including locomotor activity, reward mechanisms, cognition and neuroendocrine functions.⁷⁶ In addition, the

importance of the dopamine system in the CNS has been established based on the finding that dysfunction of this system is related to a broad spectrum of neuropsychiatric disorders, such as Parkinson's disease (PD), schizophrenia, Tourette's syndrome, attention-deficit hyperactivity disorder, and drug addiction.⁷⁶ Even though, there are many important physiological and pathophysiological functions, dopamine is synthesized and released only from a relatively discrete number of neurons.⁷⁷ These dopaminergic neurons are primarily located in the ventral tegmental area and the substantia nigra from where they extend to areas in the striatum, the limbic system, and the cortex;⁷⁷ consequently, it is important to study regulatory mechanisms relevant to the functioning of DA systems to understand the etiology of various disorders associated with it and to develop effective therapeutics.⁷⁸

The DAT mediates reuptake of dopamine from the synaptic cleft into the pre-synaptic nerve terminus and thereby plays a critical role in terminating dopaminergic signaling and in maintaining a releasable pool of dopamine.⁷⁶ The DAT, just like transporters for serotonin (SERT), norepinephrine (NET), GABA, glycine, creatine, taurine, and proline, is a member of Na⁺/Cl⁻-dependent transporter family. The DAT contains 12 transmembrane domains having both amino- and carboxy-termini projected into the cytoplasm.⁷⁹ DATs transport DA through sequential binding and cotransport of two Na⁺ ions and one Cl⁻ ion in association with one molecule of DA.⁷⁹ Expression of DAT is exclusive to the dopaminergic nerve bodies and terminals and can serve as a selective marker of these dopaminergic neurons.⁷⁹ In the brain, DAT is expressed highest in the striatum and nucleus accumbens followed by the olfactory tubercle, hypothalamic nuclei, and pre-frontal cortex.⁷⁹ DAT expresses in peripheral areas including the

retina, gastrointestinal tract, lung, kidney, pancreas, and lymphocytes.⁷⁹ DAT is mostly localized perisynaptically rather than in the synaptic compartment based on ultrastructural analysis which supports the previous estimations that reuptake of dopamine occurs at a distance from release site.⁷⁹

DAT-KO mice are hyperactive, dwarf, and display cognitive and sensorimotor gating deficits, and sleep dysregulation.⁸⁰ Normal social interaction has been seen in the mutant mice, but DAT-lacking females show an impaired capability to care for their offspring, most probably due to anterior pituitary hypoplasia-related hormonal dysregulation.⁸⁰ DAT is the major target of the widely abused psychostimulant drugs cocaine and amphetamine.⁷⁶ But, these drugs act through different mechanisms.⁷⁶ Cocaine binds to the DAT substrate binding site and blocks transporter activity as a competitive inhibitor, while amphetamine is a transporter substrate able to promote DAT-mediated dopamine release.⁷⁶

In DAT-KO mice, due to disruption of clearance of the released DA, there is about a 300-fold increase in the lifetime of DA in the extracellular space, as shown by cyclic voltametry measurements, and in vivo microdialysis at least five-fold elevation in the basal extracellular DA levels.⁷⁹ In addition, a profound depletion of intraneuronal dopamine stores (20-fold) and an attenuated level of evoked dopamine release (4-fold) was found in DAT-KO mice. Due to lack of dopamine-uptake-mediated recycling, the amount of dopamine in the striatum depends on the rate of its ongoing synthesis in these mice.⁷⁹ Inhibition of tyrosine hydroxylase (TH), the rate-limiting enzyme in DA synthesis, essentially eliminates dopamine in the striatum of mutant mice.⁷⁹ Therefore, in DAT-KO mice, the DA levels are represented basically by a newly

synthesized pool.⁷⁹ Thus, in the normal situation, major DA storage pools in the presynaptic striatal terminals must be regulated by DAT-mediated DA recycling based on these observations.⁷⁹

Dopamine receptors undergo regulation due to the persistent increased dopaminergic tone.⁸¹ Due to a marked desensitization of the major autoreceptor functions, there is loss of functional activity of autoreceptors observed as response to regulation of neuronal firing rate and DA release and synthesis.⁸¹ In DAT-KO mice, D₁ DA receptors are down-regulated by approximately 50% in the striatum, but paradoxically, the postsynaptic DA receptors belong to certain populations that appear to be supersensitive.⁷⁹

In addition to DAT function in the regulation of efficacy of DA transmission, it plays a major role in neurotoxic reactions induced by large doses of amphetamine derivatives and dopaminergic neurotoxins.⁷⁹ In experimental animals Parkinson's disease (PD) can be modeled by toxic lesions of dopaminergic neurons using MPTP.⁷⁹ MPTP-induced death of dopaminergic neurons is due to its reactive metabolite 1-methyl-4-phenylpyridium (MPP⁺) which is known to transport into dopaminergic terminals through the DAT.⁸² As per prediction, a lack of MPTP neurotoxicity was found in DAT-KO mice.⁷⁹ In DAT-KO mice, a significant reduction of dopaminergic neurotoxicity and lethality was observed even after administration of a neurotoxic regimen of methamphetamine-related compounds.⁷⁹ Thus, it is clear that the DAT is critical for the degeneration of presynaptic DA neurons primarily by allowing entry of toxic compounds into the dopaminergic neurons.⁷⁹

C. Regulation of the Dopamine Transporter:

Numerous studies have been conducted to understand the cellular mechanisms responsible for regulating the availability and activity of the DAT in the presynaptic membrane.⁷⁶ Several proteins have been identified, including kinases, receptors, and scaffolding protein, that modulate the catalytic activity of the DAT or its trafficking by their interaction with the DAT.⁷⁶

DAT is exposed to dynamic regulation in the plasma membrane.⁷⁶ This regulation may be important in the sense that it provides the strength to dopaminergic signaling which can be either attenuated or intensified.⁷⁶ The regulatory effect of protein kinase C (PKC) activation has been studied.⁷⁶ It has been shown in various studies involving several heterologous cell lines transfected with DAT that activation of PKC by phorbol esters, like phorbol 12-myristate 13-acetate, down-regulate DAT capacity.⁸³⁻⁸⁸ The sustained DAT down-regulation due to PKC activation results most likely from DAT endocytosis.⁷⁶ The PKC-induced inactivation of DAT is independent of DAT phosphorylation by PKC.⁷⁶ In PKC-activated DAT down-regulation, involvement of another post-translational modification, ubiquitination, has been seen in recent studies.⁷⁶ Ubiquitination regulating protein homeostasis is a widespread post-translational modification.⁷⁶ In studies conducted by Miranda et al.,⁸⁹ it was shown that DAT is ubiquitinated and this is augmented upon phorbol 12-myristate 13-acetate stimulation. The ubiquitination was dependent on the presence of three lysines at the intracellular N-terminus (lysine 19, lysine 27, and lysine 35) of DAT and mutation of these residues to arginine residues essentially diminished DAT down-regulation.⁷⁶

In the DAT C-terminus, a motif has also been shown to be essential for DAT internalization.⁹⁰ The motif consist of a stretch of 10 residues of amino acids (587-596 in hDAT) which upon mutation to alanines caused impairment of both constitutive and PKC mediated DAT internalization.⁹⁰ Additionally, it was reported that substitution of only 587-590 residues with alanine was sufficient to diminish PKC-associated DAT down-regulation and increase constitutive DAT internalization.⁹¹ This study suggested that the stretch of four residues is part of an endocytosis braking mechanism, which is relieved upon PKC stimulation.⁹¹

MAPK has been found to regulate DAT; for example, in transfected HEK293 cells and in striatal synaptosomes MAPK inhibitors were shown to decrease dopamine uptake.⁹² This might be due to alteration in DAT transport capacity and redistribution of DAT from the plasma membrane to the cytosol.⁹² Moreover, DAT regulation might be subject to regulation by phosphatases, as it has been reported that DAT exists in a complex with protein phosphatase 2A.⁹³ DAT substrates and inhibitors are also involved in regulation of DAT surface levels.⁷⁶ Both amphetamine and cocaine promote internalization of DAT whereas cocaine increases DAT surface levels.⁷⁶ The mechanism behind this and responsible protein-protein interactions are still poorly understood.⁷⁶

Lewy bodies, aggregation of α -synuclein in protein inclusions, are characteristic for the pathology of Parkinson's disease (PD).⁷⁶ In PD pathogenesis a role for synuclein is supported by the observation that point mutations in the α -synuclein gene as well as multiplications of the wild type gene have been identified in a rare familial form of PD.⁷⁶ It has been reported that α -synuclein binds directly to the C-terminal tail of DAT and was shown to involve the last 22

amino acids of DAT and the non-amyloid beta component domain of α -synuclein.⁹⁴ An increase in dopamine-uptake was observed in Ltk-mouse fibroblasts in cells co-expressing α -synuclein and DAT compared to cells expressing α -synuclein and DAT alone and dopamine-induced cellular apoptosis was also observed.⁹⁴ The coupling of α -synuclein to DAT was confirmed by Wersinger and Sidhu;⁹⁵ however, they observed a reduction in dopamine uptake upon over-expression of α -synuclein in the Ltk-cells. These controversial results might reflect differences in the level of α -synuclein over-expression, similar results were obtained in the regulation of NET by α -synuclein.⁹⁶ So far no alteration in DAT function has been observed in α -synuclein knock-out mice.⁷⁶

Two studies have given evidence that the dopamine D₂ receptor short variant (D₂Rs), presynaptic autoinhibitory receptor expressed in dopaminergic neurons is likely to regulate DAT function.^{97,98} D₂Rs directly interacts with DAT; this has been seen in co-immunoprecipitation and GST fusion protein pull-down experiments in striatal tissue extracts.⁹⁸ The evidence suggests that the interaction depends on residues 1-33 in the DAT N-terminus and residues 311-344 in the D₂Rs third intracellular loop.⁹⁸ DAT with over-expressed D₂Rs in a cell line increases dopamine uptake by 30-60%, mostly through an increased DAT surface expression and independent of the presence of D₂R ligands.⁹⁸ In addition, the dopamine D₃ receptor, another D₂-class receptor, was shown to up-regulate DAT surface expression in transfected HEK293 cells upon activation; however, the interaction of DAT and the D₃ receptor was not investigated.⁹⁹

The orphan receptor GPR37 has also been recently suggested to interact with DAT.¹⁰⁰ DAT function was increased in GPR37 knock-out mice through an increased DAT expression, and

was suggested to involve an interaction between DAT and GPR37.¹⁰⁰ In transfected HEK293 cells, the putative physical interaction was only supported by co-immunoprecipitation experiments and immunofluorescence co-localization.¹⁰⁰ Thus, additional studies have to be conducted to explore the significant of a putative DAT/GPR37 interaction.⁷⁶

The scaffolding proteins are multiple protein interaction domains, serving as assembly modules and glue together the proper interaction partners.⁷⁶ This includes proteins connecting membrane to their downstream signaling partners or anchoring them in the right cellular microdomains.⁷⁶ Various studies have been done to investigate putative proteins and protein domains involved in DAT scaffolding.⁷⁶ The most widespread protein domains known as PSD-95/Discs-large/ZO-1 homology (PDZ) domains in cellular scaffolding processes have been investigated.⁷⁶ The C-terminus of DAT has a canonical PDZ-binding sequence and in a yeast two-hybrid screen the C-kinase 1 (PICK 1) was discovered as a DAT interaction partner.¹⁰¹ Co-immunoprecipitation experiments in brain tissue extracts suggested that this interaction promotes DAT surface expression and induces a clustering phenotype in transfected cells.¹⁰¹ However, this finding was challenged by Bjerggaard et al.,¹⁰² who showed that although PICK 1 binds the extreme DAT C-terminus, the interaction does not play a role in ER export and surface targeting of the transporter. C-Terminal residues of DAT are important for proper membrane targeting of DAT, however, mutations in DAT were identified, which was shown to disrupt PDZ domain interactions without affecting surface targeting, and mutations were recognized that disrupted surface targeting without affecting PICK 1 binding.¹⁰² Thus, the functional significance of the DAT-PICK 1 interactions still remained to identify.⁷⁶

D. Classes of Drugs acting through DAT:

Psychostimulants are agents which enhance extracellular DA concentration. There are two classes of psychostimulants based on their mechanism by which they affect the DAT:⁷⁸ 1) uptake blockers and 2) releasers.

These classes of psychostimulants are based on their effects on acute neurotransmitter flux through the DAT.⁷⁸ However, releasers may have some ability to act as uptake blockers and uptake blockers can have some ability of releasing neurotransmitter, but this general separation of drugs into two classes helps to distinguish the pharmacological profiles of the most commonly used psychostimulants.⁷⁸

1. Uptake blockers:

Based on their effect on DAT, cocaine and methylphenidate (MPD) are the best-characterized uptake blockers.⁷⁸ Cocaine and MPD share common binding on the DAT system¹⁰² and their mechanism of action on the DA systems are similar.⁷⁸

The primary mechanism of action of cocaine and MPD is to bind directly and inhibit the transport of DA through the DAT.¹⁰⁴ There is an increase in extracellular DA levels due to blockade of DAT activity and is not related with selective longterm toxicity to the nigrostriatal DA pathway.¹⁰⁵ It has been seen that there is an increase in DA uptake in synaptosomes prepared from treated rats, a preparation from which the drug has been presumably washed out, due to blockade of DAT by cocaine.¹⁰⁶ This may be due to the increased recruitment of DATs to the plasma membrane.⁷⁸ After cocaine administration in rodents and cell lines, respectively, this

acute increase in DA uptake and plasmalemmal surface expression was observed, likely due to maintain normal synaptic DA function.⁷⁸

In humans, those who have acutely enhanced synaptic DA levels through the use of cocaine, enhanced DAT function is observed in synaptosomes from cryoprotected human brain.¹⁰⁷ The development and expression of cocaine addiction is most likely based on the combination of an initial DAT blockade and a subsequent increase in DA uptake.⁷⁸ The drug dependence, perhaps developed by an overabundance of extracellular DA due to DAT blockade which initiates a compensatory increase in DAT activity, leads to a deficit of extracellular DA.⁷⁸

2. Releasers:

Amphetamine-like psychostimulant drugs that are classified as “releasers” include amphetamine (**1**), methamphetamine (METH, **2**), and 3,4-methylenedioxymethamphetamine (MDMA, **13**).^{108,109} These releaser drugs increase DA release by disrupting vesicular pH gradients allowing vesicular DA to redistribute into the cytoplasm.^{108,109} As cytoplasmic DA levels rise, DA leaves the neuron through reverse transporter and/or channel-like activity of the DAT,^{110,111} which causes a drastic increase in synaptic DA levels.⁷⁸

In rats, injection of METH (**2**) in a single high-dose (10 mg/kg) rapidly (within an hour) and reversibly decreases the amount of DA taken up into synaptosomes developed from treated rodents.¹¹² Rapid exposure to amphetamine reduces plasma membrane-associated DAT demonstrated by data from cell lines expressing the DAT, most likely representing a significant shift of the DAT protein to the cytosolic fraction.¹¹³ It is difficult to extrapolate the time course

of DA release via the DAT and a reduction of DAT on the cell surface in vivo, while, most probably, releasing drugs enhance initial DA release followed by a removal of DAT from the cell surface.⁷⁸

Table 3. Pharmacological profile of selected agents in dopamine, norepinephrine and 5-HT release assays.¹¹⁴

Drug	Release NET EC₅₀(nM)	Release DAT EC₅₀ (nM)	Release SERT EC₅₀ (nM)
<i>S</i> (+)-Amphetamine	7.07	24.8	1765
<i>S</i> (+)-Methamphetamine	12.3	24.5	736
<i>S</i> (-)-Methamphetamine	28.5	416	4640
<i>S</i> (+)-MDMA	136	142	74
<i>S</i> (-)-MDMA	560	3700	340

With higher doses of releasers, the effects become more complicated and cause persistent deficits in striatal DA systems (such as 4x10 mg/kg/injection of METH at 2-hour intervals).¹¹⁵ As compared to single injection of METH, multiple high-dose administration leads to a rapid (within an hour after final METH injection) decrease in DAT activity; however, this reduction in DAT is substantially greater and may be associated to persistent dopaminergic deficits.¹¹⁵ The mechanism behind this releaser-induced toxicity is not completely understood, but most likely increased DA, hyperthermia and oxygen radicals contribute to this phenomenon.¹¹⁵

In addition to changes in DAT activity induced by releasers, it has been demonstrated that higher doses of these drugs cause physical alterations in DAT, most likely a neurotoxic regimen of METH induces DAT complex formation.¹¹⁶ Whether these protein complexes at the site of

production are homomeric or heteromeric are not clear, however, when neurotoxic regimens of METH are administered.⁷⁸ These complexes are seen to be associated with toxicity as their production is dependent on DA, hyperthermia, and reactive species,¹¹⁷ which are requisite factors for the METH-induced persistent DA deficits in the striatum. The functional influence of METH-induced DAT complex formation still needs to be determined.⁷⁸

E. Cathinone:

1. Historical Background:

“Khat (Catha edulis, Celastraceae) is a flowering plant, indigenous to tropical East Africa and the Arabian Peninsula. The origins of the plant are often argued. Many believe its origins are Ethiopian, others state that khat originated in Yemen before spreading to Ethiopia and the nearby countries Arabia, Kenya, Somalia, Uganda, Tanzania, Malawi, Congo, Zambia, Zimbabwe and South Africa; it has also been found in Afghanistan and Turkestan. The ancient Ethiopians considered the plant a “divine food”, while the Egyptians used the plant for more than its stimulant effects. They used it in a metamorphic process to transcend into “apotheosis”, thus the human being was made “god-like”. The earliest documented description of khat dates back to the Kitab al-Saidana fi al-Tibb, an 11th century work on pharmacy and material medica, written by Abu Rayhan al-Biruni, a Persian scientist.”¹¹⁸

“The name Catha edulis was first given to the plant by Forsskal in 1775, and this name has since been used by most authors. (other locally used names are: qut, q’ut, kat, kath, gat, chat,

tschat, miraa, and murungu; the dried leaves of the plant are known as Abyssinian tea, Arabian tea or Bushman tea). Catha edulis is a shrub or decorative tree growing 1-25 m tall and is widely distributed in Africa. The leaves are elliptic to oblong, pendulous, leathery, bright green and shiny above, paler below with an evenly toothed margin. They are 5-10 cm long and 1-4 cm wide. Khat grows in habitats varying from evergreen submontane forest to deciduous at 800-2000 m altitude and is now indigenous in Ethiopia, Kenya, Uganda and Tanzania, and from East Congo (formerly Zaire) southward to South Africa. Very recently it has been introduced to Somalia."¹¹⁸

Chewing leaves of the khat plant, in areas in which the plant is indigenous, is a habit due to its pleasurable stimulant effect.¹¹⁹⁻¹²¹ It has been estimated that about 5-10 million people chew the leaves every day.¹¹⁹⁻¹²¹ For example, in Yemen 60% of the males and 35% of the females chewed khat leaves for long periods of their lives.¹¹⁹⁻¹²¹ Khat leaf chewing induces stimulant effects and produces a certain degree of euphoric effect.¹¹⁹⁻¹²¹ It was reported by Alles et al.¹²² that quantitative comparisons in man of the central stimulant aspects of khat plant material, its aqueous extracts, and its detannated extracts, gave results that corresponded to the amount of *dextro*-norpseudoephedrine isolated. These desirable effects are only produced by fresh leaves, so that until the present time the chewing habit has remained in those areas where the plant is indigenous.¹¹⁹⁻¹²¹ After harvesting, khat is sold as a bundle of twigs, stems and leaves, and is wrapped in banana leaves to preserve freshness.¹¹⁸ During the past few years because of rapid and relatively inexpensive transportation, the drug has been reported in Great Britain, Italy, The Netherlands, Canada, Australia, New Zealand, the USA, and Hungary.¹¹⁸

Khat has been traditionally used as a socializing drug and this is still the case.¹²³ In the countries where it grows, it is used as a recreational drug, also it may be used by farmers and agricultural and other laborers for decreasing physical fatigue and by drivers and students for increasing attention.¹²³ At the age of 10, children often start chewing khat.¹²³ At present, khat is so popular in Yemen that about 40% of the country's water supply goes towards irrigation of khat plants.¹²³

In the USA a kilo of khat is being sold for \$300-500 and a bundle of leaves sold for \$30-50.¹²⁴ It has been seen that there is an increase in use of khat in the upstate New York area.¹²⁴ The USA Drug Enforcement Administration (DEA) executed operation Somalia Express in July 2006, an 18-month investigation that resulted in the coordinated takedown of a 44-member international trafficking organization that was responsible for smuggling 25 tons of khat from the Horn of Africa to the USA, which was worth more than \$10 million according to DEA estimation.¹²⁴

It is reviewed that in 1887, Flücklger and Gerock first attempted to isolate the active principle of the plant.¹¹⁹ It is reviewed that Wolfes identified norpseudoephedrine in khat leaves in 1930 and in 1941, Brücke stated that the amount of norpseudoephedrine in khat was insufficient to account for the symptoms produced.¹¹⁸ Due to this statement, the plant was reinvestigated and studies resulted in isolation of the keto-analog of norpseudoephedrine from khat leaves, and cathinone (β -keto-amphetamine; **23**) was suggested as the name for this alkaloid.¹²⁵⁻¹²⁹ The khat plant contains the phenylalkylamine cathinone ((-)-cathinone) and the diastereoisomers cathine (1*S*,2*S*-(+)-norpseudoephedrine or (+)-norpseudoephedrine) and

norephedrine (1*R*,2*S*-(-)-norephedrine).¹³⁰ These phenylalkylamines are structurally similar to amphetamine and noradrenaline.¹³⁰ The khat plant contains the (-)-enantiomer, but not the (+)-enantiomer of cathinone.¹³⁰

Cathinone is chemically unstable, undergoes decomposition reactions after harvesting and during drying or extraction of the plant material.¹¹⁸ Cathinone generally decomposes to a dimer (3,6-dimethyl-2,5-diphenylpyrazine) and most likely to some small fragments.¹³¹ This is the reason why users prefer the fresh leaves as cathinone is the psychoactive component of khat.¹¹⁸ The content of phenylalkylamines in khat leaves varies within wide limits.¹¹⁸ A 100 g sample khat of leaves contains, on average, 36-114 mg cathinone, 83-120 mg cathine and 8-47 mg norephedrine.¹³²⁻¹³⁴

2. Pharmacology:

(-)-Cathinone has a positive inotropic and chronotropic effect in isolated guinea pig atria.¹³⁵ In whole animal, (-)-cathinone and (+)-amphetamine were found equipotent in increasing the heart rate when injected i.v. at a dose of 1 mg/kg.¹³⁵ It was reported that (-)-cathinone has a pressor effect in anaesthetized cats; when administered i.v. 1 mg/kg resulted in a transient rise in the blood pressure by 30 to 35 mmHg.¹³⁰

As like (+)-amphetamine, (-)-cathinone produces hyperthermia in rabbits after its injection and reduces the body temperature of rats previously exposed to a cold temperature.¹³⁵ (-)-Cathinone produced long lasting analgesia in rats using the tail-flick test, and the duration of analgesic effect was dose related.¹³⁰

It has been reported that s.c. administration of cathinone in rats increases the locomotor activity of the animals, and that (±)-cathinone had a potency approaching that of (+)-amphetamine.¹³⁵ Van der Schoot et al.²¹ found (±)-cathinone to produce half the maximal locomotor effect of (+)-amphetamine in mice, but specific doses were not provided. Quantitatively, in another study using mice, the locomotor activity of (-)-cathinone was one-seventh of the potency of (+)-amphetamine.¹³⁶ The dose-response curve of cathinone's effect on locomotor activity was observed to be an inverted-U shape, which is typical of stimulants of the amphetamine type.¹³⁰ Reserpization only partially antagonized the locomotor response of mice, which is similar to that for (+)-amphetamine hypermobility.¹³⁷ In order to find out whether the stimulation of locomotor activity involves activation of dopamine receptors as in the case of (+)-amphetamine, the effect of dopamine receptor antagonists, like haloperidol, spiroperidol and pimozide were investigated.¹³⁷ It was seen that dopamine receptor antagonists blocked the locomotor response to (-)-cathinone; this finding is in agreement with those for (+)-amphetamine.¹³⁷

Pretreatment of the animals with the catecholamine synthesis blocker α -methylparatyrosine, completely blocked the induction of stereotyped behavior by (-)-cathinone.¹³⁵ However, pretreatment of animals with the dopamine receptor antagonist haloperidol reduced biting and licking movements induced by cathinone.¹³⁸

(-)-Cathinone has been reported to act as anorectic compound in behavioral experiments with monkeys.¹³¹ In rats, intracerebroventricular injection of (-)-cathinone inhibits food intake to a greater extent than amphetamine.¹³¹ In rats, it has been reported that i.p. injection of racemic

cathinone resulted in reduced food intake, and that chronic administration led to a decrease in body weight.¹³⁹ In this study, (+)-amphetamine was seen more potent than cathinone.¹³⁹

The similarity of cathinone to amphetamine was shown by Rosecrans et al.³⁷ who reported that racemic cathinone could be substituted for (+)-amphetamine in rats trained to distinguish between a placebo and (+)-amphetamine. When administered cathinone, the animals responded the same as if they had been given (+)-amphetamine, and this response was dose related.³⁷ It has been seen that cathinone and (+)-amphetamine produced the same response pattern and were equipotent in drug-discrimination studies in rats trained to discriminate (+)-amphetamine from vehicle.²⁹ Cathinone has a more rapid onset of action compared to amphetamine based on drug discrimination experiments.¹⁴⁰ (-)-Cathinone (i.e., **S(-)23**) is several times more potent compared to (+)-cathinone (i.e., **R(+)**23) in producing central stimulant and drug discriminative stimulus effects, while (+)-amphetamine (i.e., **S(+)**1) is more potent than (-)-amphetamine (i.e., **R(-)**1).¹⁴¹ However, (-)-cathinone (**S(-)23**) and (+)-amphetamine (**S(+)**1) have the same absolute stereochemistry (i.e., *S*), so that *S*(-)-cathinone (**S(-)23**) structurally resembles *S*(+)-amphetamine more than *R*(-)-amphetamine (**R(-)**1).¹⁴¹ Cathinone's discriminative stimulus effects were not blocked by the serotonin antagonist BC105/B.¹²⁶ It was reported by Glennon et al.¹⁴² that rats trained in a two-lever drug-discrimination procedure were less likely to distinguish between (-)-cathinone (**S(-)23**) and quipazine, a serotonin receptor agonist, than between (+)-amphetamine (**S(+)**1) and quipazine. However, it was found that chronic treatment of rats with racemic cathinone reduces the level of dopamine in several brain areas but does not affect the level of serotonin.¹²⁵

In monkeys trained to press a lever for cocaine injection, the animals continue to respond at high rates when the training drug was replaced with (-)-cathinone.¹⁴³ In this study, the reinforcing effect of (-)-cathinone was reported to be greater than (+)-amphetamine.¹⁴³ Cathinone may produce rates of responding higher than amphetamine based on self-administration experiments with monkeys.¹²⁹

It has been reported that cathinone modified brain catecholamine turnover, but to a lesser extent than (+)-amphetamine.³⁷ In mice, pretreated with (-)-cathinone, the turnover of dopamine increased by 32%, but that of norepinephrine was practically unaffected.¹⁴⁴ In rats, repeated administration of racemic cathinone produced a long-lasting depletion of dopamine in several brain regions, with no effect on the level of norepinephrine.¹⁴⁴ It has been found in an assay system involving beef monoamine oxidase and benzylamine as a substrate, that (-)-cathinone was considered more potent in inhibition of monoamine oxidase than racemic amphetamine.¹⁴⁵

There are two possible mechanism of cathinone action: that its effects may be produced by a blocking of the reuptake of, primarily, physiologically released dopamine, and another possibility would be that cathinone acts by inducing the release of, primarily, presynaptic storage dopamine, a mechanism considered of importance for amphetamine on dopaminergic transmission.¹⁴⁶ Therefore, the efflux of radioactivity from rabbit caudate nucleus prelabeled with ³H-dopamine induced by (-)-cathinone was studied.¹⁴⁷ It was observed that superfusion of the tissue with 4 μM (-)-cathinone resulted in a rapid and reversible increase of efflux of radioactivity which was comparable to that produced by the same concentration of (+)-

amphetamine.¹⁴⁷ A releasing effect for racemic cathinone also was found in ³H-dopamine-preloaded synaptosomes obtained from rat neostriatum.¹⁴⁴

In conclusion, based on various studies, it is known that cathinone is in a real sense a natural amphetamine while being the major psychostimulant constituent of khat.¹²⁹ See **Figure 2** for a structural comparison of these and related agents. It might be noted that there are some discrepancies in the studies that have used cathinone and these can probably be attributed to species differences, or the use by various investigators of either (±)- or (-)-cathinone. For example, whereas (-)-cathinone is a locomotor stimulant, (+)-cathinone decreases the locomotor action of mice up to a dose of 100 μmoles/kg; (±)-cathinone produces intermediate results.¹³⁶ Nevertheless, cathinone has a pharmacological profile same as that of amphetamine: cathinone shows the same actions of amphetamine on the CNS as well as its sympathomimetic effects.¹²⁹ The major difference among the two drugs is the shorter duration of the action of cathinone; its reduced stability promotes a more rapid inactivation.¹²⁹

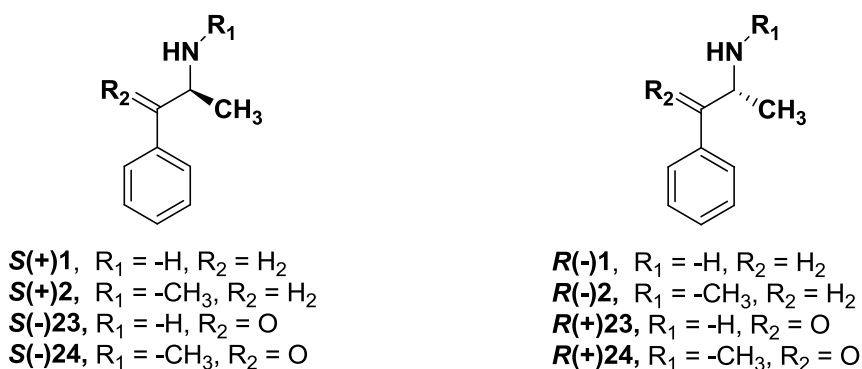


Figure 2. Stereochemistry of amphetamine (1), methamphetamine (2), cathinone (23) and methcathinone (24) isomers.

3. Cathinone Analogs:

Cathinone/methcathinone analogs are structurally-related to amphetamine/methamphetamine derivatives but bear an additional β -keto group. The structural relationships among representative examples of these agents are shown in **Figure 3**.

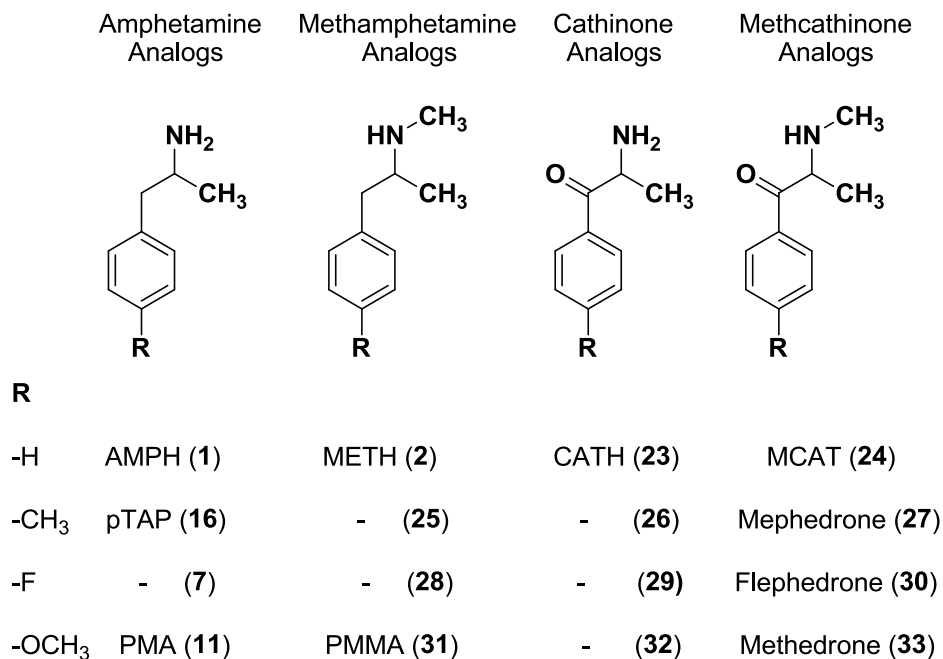
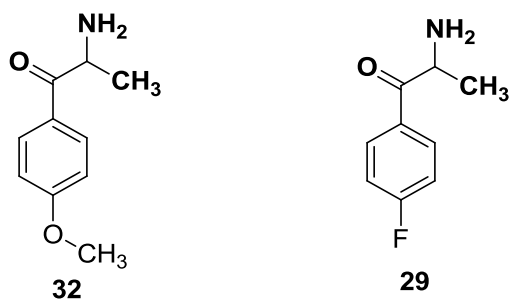


Figure 3. Structural relationship between amphetamine (AMPH), methamphetamine (METH) and their β -keto or cathinone (CATH) or methcathinone (MCAT) counterparts.

Glennon et al.¹³⁶ examined the effects of various substituent groups on racemic cathinone on locomotor activity. They found that 2-methoxy, 4-methoxy (i.e., **32**), 2,4-dimethoxy and 4-fluoro (i.e., **29**) derivatives of racemic cathinone failed to produce locomotor stimulant activity.¹³⁶ They also found that the α -desmethyl analog of cathinone had no significant effect on locomotor activity.¹³⁶ Furthermore, it was reported that stimulus generalization occurs between

(+)-amphetamine and cathinone regardless which drug is used as the training drug.¹⁴⁸ 2-Aminotetralone, a conformationally restricted cathinone (ringcathinone), produced saline-appropriate responding in rats trained to discriminate (+)-amphetamine from saline.¹⁴⁸ In the same studies, *N,N*-dimethylaminopropiophenone and α -desmethylcathinone failed to produce (+)-amphetamine-like effects.¹⁴⁸



Cathinone is a naturally occurring amphetamine-like substance and both share similar pharmacological effects.¹⁴¹ If parallel structural modification results in parallel changes in action and potency, *N*-monomethylation of amphetamine should enhance potency. That is, *N*-monomethylamphetamine (methamphetamine) is twice as potent as amphetamine as central stimulant. Hence, *N*-monomethylation of cathinone, not surprisingly, should be more potent than cathinone both as locomotor stimulant in mice and in tests of stimulus generalization in rats trained to discriminate (+)-amphetamine from saline vehicle. This was found to be the case.¹⁴⁹ Glennon et al.¹⁴⁹ termed this substance methcathinone (**24**).

N-Methylcathinone was first synthesized by the Germans^{150,151,152} and the French¹⁵³ as well as Adams¹⁵⁴ and co-workers in the late 1920's as an intermediate in the synthesis of ephedrine and was first mentioned by Chen et al.¹⁵⁵ in 1926. The two isomers of *N*-methylcathinone were

first reported in 1936¹⁵⁶ and the (-)-isomer was thereafter patented as an analeptic.^{157,158} It was found that methcathinone is more potent than cathinone both as a locomotor stimulant and in test of stimulus generalization using rats trained to discriminate (+)-amphetamine from saline.¹⁴¹ In a locomotor stimulant test in mice, *S*(-)-methcathinone (i.e., ***S*(-)**24****) was five times more potent than its optical isomer.¹⁴⁹ *S*(-)-Methcathinone (***S*(-)**24****) was nearly three times more potent than *R*(+)-methcathinone (i.e., ***R*(+)**24****) with racemic methcathinone potency falling between the potencies of the two isomers in drug discrimination studies using cocaine-trained rats, and *S*(-)-methcathinone (***S*(-)**24****) was more potent than *R*(+)-methcathinone (***R*(+)**24****) in same test using *S*(+)-amphetamine-trained animals.¹⁴¹ Thus, all three results are in agreement that *S*(-)-cathinone (***S*(-)**23****) is more potent than *R*(+)-cathinone (***R*(+)**23****) where the *S*-isomer of amphetamine is more potent than *R*-isomer of amphetamine.

In 1997, Glennon¹⁵⁹ and co-workers wished to determine whether structural modification of cathinone paralleled the effects observed upon structural modification of amphetamine. They tested several *N*-alkylated and methylenedioxy-substituted analogs of cathinone and compared them with amphetamine analogs. Similar to amphetamine, *N*-monomethylation of cathinone was found to retain potency, while any further increase in alkyl chain length was found to decrease potency.¹⁵⁹ It was surprising for them that (+)-*N,N*-dimethylamphetamine resulted in a 7-fold decrease in potency over (+)-methamphetamine (i.e., ***S*(+)**2****) in producing (+)-amphetamine appropriate responding in rats trained to discriminate (+)-amphetamine from saline, while (±)-*N,N*-dimethylcathinone (**36**; see **Table 5**) was found only slightly (1.6 fold) less potent than racemic methcathinone.¹⁵⁹ Based on the knowledge that incorporation of a 3,4-methylenedioxy

group can change amphetamine from a CNS stimulant to a combination of CNS stimulant, hallucinogenic (DOM-like) and empathogenic (MDMA-like) agent (i.e. MDA, **12**), Glennon and colleagues studied the 3,4-methylenedioxy derivatives of cathinone and methcathinone.¹⁵⁹ It was found that the 3,4-methylenedioxy analog of cathinone (i.e. MDC, **34**), failed to completely substitute for (+)-amphetamine or DOM, so introduction of a carbonyl group resulted in an agent which no longer acts like its parent compound (MDA, **12**).¹⁵⁹ The 3,4-methylenedioxy analog of methamphetamine, MDMA (**13**) shows amphetamine-like effect but lacks DOM-like character.¹⁵⁹ *N*-Monomethylation of MDC (**34**) results in an agent (i.e. MDMC, **35**) which behaves similar to MDMA (**13**).¹⁵⁹ It was interesting that, both MDC (**27**) and MDMC (**35**) show MDMA-like properties in MDMA-trained rats.¹⁵⁹ It was found that with MDMA (**13**), introduction of carbonyl group resulted in a compound (i.e. MDMC, **35**) which is less potent (about two-fold).

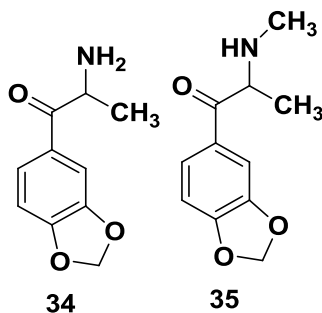
MDMC (**35**) was first patented by Jacob III et al.¹⁶⁰ and they called this substance methylone. Cozzi et al.¹⁶¹ have compared methcathinone (**24**) and methylone (MDMC, **35**) to methamphetamine (**2**) and MDMA (**13**) for their abilities to inhibit ³H-serotonin, ³H-dopamine, and ³H-norepinephrine uptake via the plasma membrane uptake transporters and they also tested inhibition of ³H-serotonin uptake by the vesicular monoamine transporter, VMAT-2 (**Table 4**). They found that methcathinone (**24**) and methylone (**35**) were as potent as the respective methamphetamine (**2**) and MDMA (**13**) at inhibiting monoamine accumulation, and all of the test drugs were more potent at the dopamine transporter than at the norepinephrine transporter.¹⁶¹ At the serotonin uptake carrier, methcathinone (**24**) and methylone (**28**) were one-third as potent as

methamphetamine (**2**) and MDMA (**13**), respectively.¹⁶¹ They found that methcathinone (**24**) and methylone (**35**) are highly selective for the plasma membrane catecholamine transporters and show decreased potency at VMAT-2 compared to methamphetamine (**2**) and MDMA (**13**), respectively.¹⁶¹

Table 4. IC₅₀ values (μM) for drug inhibition of monoamine uptake.¹⁶¹

Drug	[³ H] 5-HT	[³ H] DA	[³ H] NE	VMAT2 ([³ H] 5-HT)
(±)-Methcathinone (24)	34.6	0.356	0.511	112.1
(±)-Methamphetamine (2)	11.6	0.467	0.647	10.9
(±)-Methylone (35)	5.75	0.819	1.220	165.6
(±)-MDMA (13)	2.14	0.478	1.380	12.7

Methylone (**35**) abuse was first reported in 2004 as a liquid solution sold as a vanilla-scented odorizer.¹⁶² Recently, it has been found that methylone is sold in plastic tubes containing 5 mL of liquid called Explosion via the internet and in head shops.¹⁶³ There is no significant clinical literature on the effects of methylone (**35**).¹⁶⁴



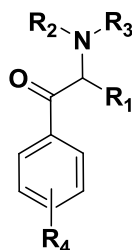
Mephedrone (4-methylmethcathinone; **27**) is a cathinone derivative, which elicits a stimulant effect like amphetamine (**1**), methamphetamine (**2**), cocaine and MDMA (**13**).¹⁶⁵ Recently, it has drawn media attention due to its link to a number of fatalities.¹⁶⁶ Saez described the first synthesis of mephedrone (**27**) in 1929.¹⁶⁷ Due to the cathinone (**23**) ban, chemist started altering the structure of cathinone (**23**) to produce related unscheduled agents.¹⁶⁶ In May 2003, the first online report on mephedrone (**27**) appeared, however, the online availability and related popularity of mephedrone (**27**) started in 2007.¹⁶⁶ The national Addiction Centre in London conducted research involving 2,295 readers of the dance magazine 'Mixmag' and reported that 41.7% of surveyed people had tried mephedrone (**27**) and 33.2% had used it during the previous month, showing its popularity among 'clubbers' and making it the sixth most popular drug, after tobacco, alcohol, cannabis, ecstasy and cocaine.¹⁶⁸ In the UK, the Advisory Council on the Misuse of Drugs recommended inclusion of mephedrone (**27**) in the Misuse of Drugs Act 1971 under class B and as a result, it was made a controlled drug (class B) on the 16th of April 2010.¹⁶⁹

Mephedrone (**27**) is the *N*-methyl cathinone analog of pTAP (**16**). It has been reported that pTAP produces partial stimulus generalization in rats trained to discriminate (+)-amphetamine from saline.³³ **Table 1** shows the potency of pTAP (**16**) as a releaser of monoamine neurotransmitters.³⁴ pTAP (**16**) was found to produce positive reinforcing effects in monkeys.³⁴ In 2010, pTAP (**16**) was detected in seized amphetamine mixture containing amphetamine, caffeine, di-(phenylisopropyl)amine (DPIA) and some by products.¹⁷⁰ There is not much known

about its *N*-methyl analogs 4-methylmethamphetamine (**25**) and 4-methylcathinone (**26**) which are, respectively, methamphetamine and cathinone counterpart of mephedrone (**27**).

Since 2006, an additional 10 cathinones have been reported in the European Union (shown in **Table 5**).¹⁶⁹

Table 5. List of cathinones reported in European Union.¹⁶⁹



Name	Common Name	R ¹	R ²	R ³	R ⁴
N,N-dimethylcathinone (36)		Me	Me	Me	H
Ethcathinone (37)		Me	Et	H	H
4-Methylmethcathinone (27)	Mephedrone	Me	Me	H	4-Me
bk [*] -PMMA (33)	Methedrone	Me	Me	H	4-OMe
4-Fluoromethcathinone (30)	Flephedrone	Me	Me	H	4-F
3-Fluoromethcathinone (38)		Me	Me	H	3-F
bk [*] -MDMA (35)	Methylone; MDMC	Me	Me	H	3,4-methylenedioxy
bk [*] -MDEA (39)	Ethylone	Me	Et	H	3,4-methylenedioxy
bk [*] -MBDB (40)	Butylone	Et	Me	H	3,4-methylenedioxy
MDPV (41)	Methylenedioxypropylone	<i>n</i> -Pr	pyrrolidinyl		3,4-methylenedioxy

*bk = beta keto.

Online purchase of mephedrone (**27**) is claimed to be ‘plant feeders’, ‘bath salts’, and ‘not for human consumption’ and prosecution as such may be difficult. Mephedrone (**27**) is most commonly administered by insufflation (snorting) and oral ingestion.¹⁷¹ Also, because mephedrone (**27**) is soluble in water, it is used by rectal administration (dissolved in an enema or within gelatin capsules), or injected intravenously.¹⁷¹ Mephedrone (**27**) produces its effects within a few minutes after being snorted, with the peak effects reached in <30 mins leading to a rapid comedown.¹⁷¹ Snorted doses of mephedrone (**27**) range between 25 and 75 mg, with a threshold dose being 5-15 mg; 90 mg is considered a high dosage.¹⁷¹ Most commonly, oral dosages are, on average, higher than snorted doses, usually in a range between 150 and 250 mg, and the onset of action may be of 45 min to 2h.¹⁷¹

Self reported subjective effects of mephedrone (**27**) have been described, and include intense stimulation, alertness, euphoria, empathy/feeling of closeness, sociability, talkativeness, intensification of sensory experiences, moderate sexual arousal and perceptual distortions (only with higher doses).^{168,172} There are many unwanted effects associated with mephedrone (**27**) that have been reported: adverse effects related to the gastrointestinal system, central nervous system – neurological and psychiatric, cardiovascular system and renal/urinary excretory system.¹⁶⁶ These adverse effects are very similar to those already reported for amphetamine (**1**), methamphetamine (**2**) and MDMA (**13**), and support a sympathomimetic action by mephedrone (**27**).¹⁶⁶

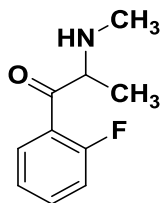
The first death related to mephedrone (**27**) appeared in Sweden in December 2008; only mephedrone (**27**) was identified by the toxicological screenings.¹⁷³ The first mephedrone-related

death in the USA involved the combined use of mephedrone (**27**) and heroin.¹⁷⁴ Based on the data obtained from the National Programme on Substance Abuse Deaths report, there have been 45 suspected deaths in England associated with mephedrone (**27**), 12 in Scotland, 1 in Wales, 1 in Northern Ireland and 1 in Guernsey, by the beginning of October 2010.¹⁶⁶ Out of these 60 cases, 48 provided positive results for the existence of mephedrone (**27**), while other cases need to be further investigated.¹⁶⁶

Mephedrone (**27**), due to its popularity as a legal high, is now a substance controlled by legislation in the United Kingdom, Germany, Norway, Sweden, The Netherlands, Finland, Romania, Republic of Ireland, Denmark, Canada and Israel,¹⁷⁵ as well as in US. Prevalence of cathinone (**23**) derivatives has given rise to both legal and analytical challenges in the identification of these substances.¹⁷⁵ Thus, it is required to develop robust analytical profiling and validated methods of testing.¹⁷⁵ Therefore, recently, many publications have reported the synthesis of mephedrone (**27**) and methods for its identification.¹⁷⁵⁻¹⁷⁸

It has been found that bath salts contains methylenedioxypropylone (i.e., MDPV; **41**) in addition to mephedrone (**27**).¹⁷⁹ Recently, The New York Times published an article showing the growing popularity of bath salts in the USA and discussed its danger among people using it.¹⁸⁰ Regarding bath salts, Karen E. Simone, director of the Northern New England Poison Center, says, "If you gave me a list of drugs that I wouldn't want to touch, this would be at the top."¹⁸⁰ Bath salts have been banned in 28 US states,¹⁸⁰ including Virginia. Westphal et al.¹⁸¹ identified a compound which was seized as a powder in Germany in 2007 as MDPV (**41**), a propylone carrying a methylenedioxy moiety. It has been reported that besides in Germany, MDPV (**41**) has

appeared in many countries in Europe and Asia.¹⁸² In June 2007 a customs officer in Germany seized MDPV (**41**) as a nearly pure substance while investigating a person who was the addressee of a drug mail shipment from China.¹⁸¹ In mice, MDPV (**41**) was found to have a milder effect on the increase of dopamine levels than methamphetamine (**2**) and MDMA (**13**), and showed no significant influence on serotonin levels.¹⁸³ It has been seen that in locomotor activity MDPV (**41**) has a shorter duration of action compared to MDMA (**13**) and methamphetamine (**2**).¹⁸³ MDPV (**41**) has gained popularity for claimed sex-enhancing properties.¹⁸⁴ However, in the study of Ojanpera et al.¹⁸⁵ the reputation of MDPV (**41**) as a sex drug was found less important; rather, a clear stimulation effect induced by MDPV (**41**) was seen in some patients. It was assumed that MDPV (**41**) is taken orally.¹⁸² Ojanpera et al.¹⁸⁵ reported a GCMS method for the detection of MDPV (**41**) in urine together with the stimulants amphetamine, methamphetamine (**2**), and MDMA (**13**). In Japan, Uchiyama et al.¹⁸⁶ found seven designer drugs in fifteen confiscated products, including: MDPV (**41**), bk-MBDB (**40**), bk-MDEA (**39**), *N*-hydroxy-1-(3,4-methylenedioxyphenyl)-2-aminopropane (*N*-OH MDMA), *N*-methyl-1-(4-fluorophenyl)propan-2-amine (*N*-Me-4-FMP; **30**), and 5-methoxy-*N*-ethyl-*N*-isopropyltryptamine (5-Meo-EIPT). In the United Kingdom, MDPV (**41**) was banned in 2010 by way of a generic definition.¹⁸⁷ It has been reported that mephedrone (**27**), methylone (**35**) and MDPV (**41**) seizures collectively represented over 97% of the synthetic cathinone seizures.¹⁸⁸



42

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Archer¹⁸⁹ has reported that internet-based companies are known to sell 4-fluoromethcathinone (flephedrone; **30**), the N-methyl analog of 4-fluorocathinone (**29**), and he reported a method for the synthesis and identification of various fluoromethcathinones (includes: 2-fluoromethcathinone (**42**), 3-fluoromethcathinone (**38**), 4-fluoromethcathinone (**30**)). There have been no animal studies reported using flephedrone (**30**).

Flephedrone (**30**) is the N-methyl cathinone analog of *p*-fluoroamphetamine (**7**). **Table 1** shows the *invitro* potency of *p*-fluoroamphetamine (**7**) as a releaser of monoamine neurotransmitters.³⁴ *p*-Fluoroamphetamine (**7**) produced stimulus generalization in rats trained to discriminate (+)-amphetamine from saline.²⁵ There is little known about *p*-fluoromethamphetamine (**28**) and *p*-fluorocathinone (**29**) which are, respectively, methamphetamine and cathinone counterparts of flephedrone (**30**) but (±)-4-fluorocathinone (**29**) failed to produce hypermotor activity in mice.¹³⁶ In 2003, a series of clandestinely prepared phenylalkylamines was seized in the federal state of Sachsen-Anhalt (Germany), which contained 4-fluoroamphetamine (**7**) as well as 4-fluoromethamphetamine (**28**).¹⁹⁰ It has been reported that since 2008, larger quantities of drug preparations containing 4-fluoroamphetamine have been seized in several German federal states and in Switzerland.¹⁹¹

Methedrone (**33**), the N-methyl analog of 4-methoxycathinone (**32**), was reported as an abused substance for the first time in October 2009 and two deaths were partly attributed to methedrone (**33**) in Sweden.^{192,193} Wilkstrom et al.¹⁹⁴ reported two deaths related to methedrone (**33**) due to its toxic properties and they found that blood concentrations in the two cases are close to those seen in subjects who abused the drug, suggesting that a rather narrow “therapeutic”

window exists for methedrone (**33**). This emphasizes the risks associated in taking this kind of drug for recreational purposes.¹⁹⁴ Methedrone (**33**) is controlled in Sweden and Romania.¹⁶⁴ There are no animal or pharmacological studies on methedrone (**33**).

Camilleri et al.¹⁷⁶ reported the results of chemical analysis of four capsules delivered to the Royal Adelaide Hospital (Australia), which originated from an Israel-based internet company, “Neorganics”. They found that capsule 1, which was marketed as “Spirit”, contained 4-methylmethcathinone (mephedrone; **27**); capsule 2, which was marketed as “Sub Coca 2”, contained α -phthalimidopropiophenone and 2-fluoromethcathinone (**42**); capsule 3 and capsule 4, which were marketed as “Neo dove” and “Sub Coca”, respectively, both contained caffeine, 4-methylmethcathinone (mephedrone, **27**), *N*-ethylcathinone (**37**) and α -phthalimidopropiophenone.¹⁷⁶ Jankovics et al.¹⁹⁵ developed a “screening method” to provide a preferably simple and fast analytical procedure for the detection of methcathinone-derived designer drugs, including: mephedrone (**27**), methedrone (**33**), flephedrone (**30**), MDPV (**37**), methylone (MDMC; **35**), butylone (i.e., bk-MBDB; **40**) and 4-methylethcathinone (4-MEC).

Methedrone (**33**) is the *N*-methyl cathinone analog of 4-methoxyamphetamine (PMA, **11**). In two separate studies, it has been observed that PMA (**11**) results in amphetamine stimulus generalization, but is less potent than amphetamine.^{28,29} **Table 1** shows the *invitro* monoamine transporter release potency of PMA (**11**). PMMA (*p*-methoxymethamphetamine, **31**), a methamphetamine counterpart of methedrone, failed to produce stimulus generalization in rats trained to discriminate (+)-amphetamine from saline.³⁰ However, PMMA (**31**) produced complete stimulus generalization in rats trained to discriminate MDMA (**13**) from saline and was

three times more potent than MDMA (**13**).¹⁹⁶ PMA (**11**) produced partial stimulus generalization in rats trained to discriminate PMMA (**31**) from saline.¹⁹⁷ Shulgin has called PMMA (**31**) “DOONE”.¹⁹⁸ PMA (**11**) has been found a potent hallucinogen.¹⁹⁹ (+)-PMA and (-)-PMA failed to produce stimulus generalization in rats trained to discriminate (+)-amphetamine from saline, however (-)-PMA, but not (+)-PMA, substituted for PMMA (**31**) in PMMA (**31**) trained rats.²⁰⁰ PMA (**11**) is classified as a Schedule I controlled substance.²⁰¹ (+)-PMMA completely, while (-)-PMMA partially, substituted for (±)-PMMA (**31**) in rats trained to discriminate (±)-PMMA from saline.²⁰² **Table 6** shows the potency of (+)-PMMA and (-)-PMMA as releasers of neurotransmitters.²⁰³ It has been reported that PMA (**11**) produces little locomotor stimulation in mice at doses below 30 mg/kg and that PMMA (**31**) looks even less potent than PMA (**11**) at doses of up to 30 mg/kg.³⁰ PMA (**11**), compared to (+)-amphetamine, is more effective in increasing the release and blocking the uptake of ³H-serotonin, while less effective in increasing the release and blocking the uptake of ³H-norepinephrine and ³H-dopamine.²⁰⁴ PMA (**11**) has been used illicitly in Australia since 1994 and later became popular at rave parties in the US.³⁶ In 2000, three fatal cases were reported involving PMA (**11**) and PMMA (**31**) abuse in Denmark.²⁰⁵ 4-Methoxycathinone (**32**), which is the cathinone counterpart of methedrone (**33**), failed to produce stimulus generalization in rats trained to discriminate cathinone from saline.²⁰⁶ It has been also reported that 4-methoxycathinone (**32**) failed to produce locomotor activity in mice.¹³⁶

Table 6. In vitro potency as releasers of neurotransmitters.²⁰³

Drug	Release NET EC₅₀ nM	Release DAT EC₅₀ nM	Release SERT EC₅₀ nM
S(+)-PMMA	147	1000	41
R(-)-PMMA	1600	> 14000	134

In conclusion, β -keto amphetamines, including: mephedrone (**27**), methedrone (**33**), flephedrone (**30**), MDPV (**41**), methylone (MDMC; **35**) and many others, have recently become popular on the illicit drug market and, as discussed above, there are many reports regarding their abuse. Furthermore, although cathinone (**23**) and methcathinone (**24**) are controlled substances, their analogs are not. These drugs are a growing threat for society they financially, socially, as well as producing detrimental effects on health among their users. There is essentially nothing known about their pharmacology (except cathinone (**23**) and methcathinone (**24**)). Based on the structural similarity of these drugs to cathinone (**23**) and methcathinone (**24**), one might assume that their pharmacology and mechanism of action could be similar to cathinone (**23**) and methcathinone (**24**), but little is known based on current scientific data. One of the difficulties in studying these drugs is to obtain them in pure form. Bath salts (containing primarily methedrone (**27**) and MDPV (**41**)), as mentioned above, is recently gaining more and more in popularity on the illicit market. Certainly, based on increasing interest of these cathinone derivatives among abusers, there is a need for more research to determine how these drugs are producing their effect and also a need for validated techniques to screen potential candidates related to the cathinones which might become a future threat.

II. Specific Aims

The overall goal of the present project is to synthesize and initiate an examination of the mechanism(s) of action of a new class of abused substances known as “cathinones”, “synthetic cathinones”, or “ β -keto amphetamines” and, more specifically, the constituents of “bath salts” and several structurally related agents. These substances represent a relatively new and fast-growing class of designer drugs (**Table 5**). Although the first members of this class, cathinone (**23**) and methcathinone (**24**), were identified more than 30 years ago (see Introduction), it is only within the last few years that they have been acknowledged as representing the first members of an entire class of agents. These agents are, structurally, β -keto analogs of amphetamine and might be referred to as “amphetamones”. That is, the amphetamone counterpart of amphetamine (**1**) is cathinone (**23**), whereas that of methamphetamine is methcathinone (**24**). Although cathinone and methcathinone are Schedule 1 substances,²⁰⁷ analogs of these agents are essentially unregulated. It might be noted that certain states have controlled various specific β -keto amphetamines, but they have not been regulated at the federal level. (“Bath salts”, itself, was placed in US Schedule 1²⁰⁸ only after the synthetic and pharmacological studies described below were completed.)

“Bath salts” is a combination of two cathinone or ‘synthetic cathinone’ analogs: mephedrone (**27**) and MDPV (**41**). Mephedrone is the amphetamone analog of *N*-methyl pTAP (i.e., **25**). MDPV (**41**) is a co-constituent of “bath salts”; one explanation for its presence in the mixture is that it is a contaminant (i.e., a synthetic precursor of mephedrone).

However, it is difficult (if not impossible) to understand how mephedrone could be prepared from MDPV, or how MDPV could be a by-product of mephedrone synthesis. Another possibility is that MDPV is simply a “filler”. But, why go to the trouble of preparing this compound when much simpler “fillers” (e.g. lactose) could be used. A third possibility is that MDPV is behaviorally active. Yet, being a tertiary amine with a homologated α -methyl group, current amphetamine-like SAR would suggest that this compound should be inactive. So, why is MDPV present in the “bath salts” mixture?

One, relatively obscure, study found that a 20 mg/kg dose of MDPV (**41**) (i.e., the only dose examined) increased the locomotor activity of mice.¹⁸³ The same study also found that MDPV can increase striatal levels of DA.¹⁸³ Hence, the possibility exists that MDPV (**41**) might act at the level of the dopamine transporter. This needs to be further examined. Nevertheless, although this is a clue that MDPV might be psychoactive, there is certainly no reason to suspect (from a structure-activity perspective) that MDPV would ever become a component of a widely used drug of abuse (i.e., ‘bath salts’). Given its seemingly low potency (i.e., it was evaluated at a dose of 10 times that of methamphetamine),¹⁸³ MDPV might not seem attractive (relative to the potency of other central stimulants) for distribution. Certainly, then, there was no *obvious* reason why it should be included with mephedrone as a component of bath salts.

Flephedrone (**30**) is the *N*-methyl cathinone analog of *p*-fluoroamphetamine (**7**). Recently, flephedrone abuse has been on an increase^{190,191} and, because it is an analog of methcathinone, an agent that acts at the dopamine transporter, it is essential to examine its activity at the dopamine transporter as well.

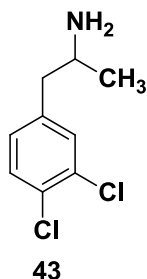
Methedrone (**33**) is the *N*-methyl analog of 4-methoxycathinone (**32**). There are some deaths reported related to abuse of methedrone (see Introduction). Methedrone is the *N*-methyl

cathinone analog of the Schedule 1 drug 4-methoxyamphetamine (PMA, **11**).²⁰¹ Methedrone is also the methcathinone analog of *p*-methoxymethamphetamine (PMMA, **31**), which is also a drug of abuse. Based on this knowledge regarding methedrone, it is important to study its mechanism of action.

A major goal of the present investigation will be to prepare mephedrone (**27**), methedrone (**33**), and flephedrone (**30**) so that their actions at the hDAT can be evaluated and compared with that of methamphetamine (**2**) and methcathinone (**24**). Structurally-related amphetamine and cathinone analogs (see **Figure 3**) not currently on-hand will also be synthesized.

A related goal is to prepare at least one example of the optical isomers of a cathinone and/or methcathinone analog to determine the effect of stereochemistry.

Other proposed synthetic targets are (\pm)amphetamine (**1**), the individual optical isomers of 3,4-dichloroamphetamine (**43**), and *S*(+)-*N*-ethylamphetamine.



Krasnodara Cameron, a graduate student in the De Felice laboratory, obtained the response as shown in **Figure 4** using different combinations of (+)-amphetamine and (-)-amphetamine. The response curve appears to show an anomaly for the 5:5 mixture (i.e., the ‘synthetic’ racemate). To resolve the problem, authentic (\pm)-amphetamine (**1**) will be synthesized and evaluated.

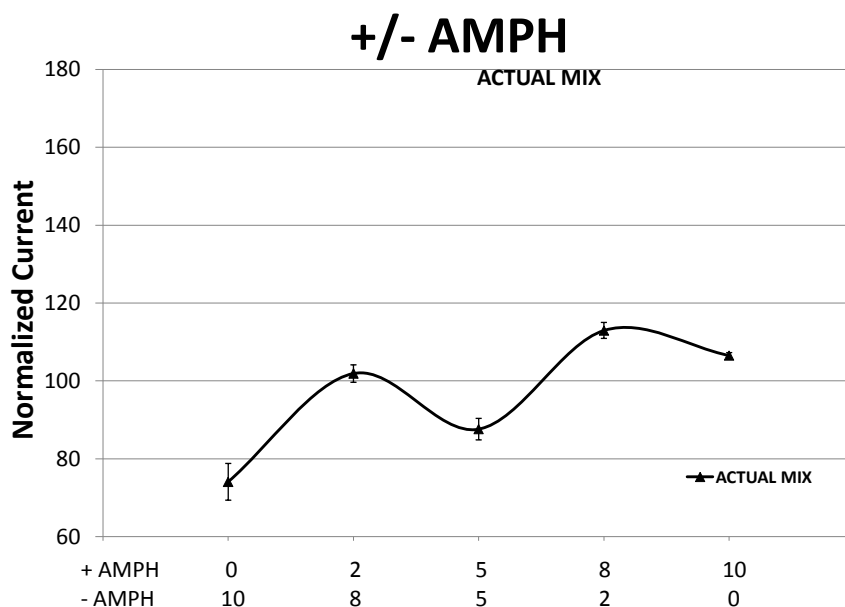
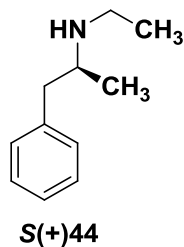


Figure 4. Response (normalized current) curve of isomers of amphetamine (**1**) at different ratios generated at the hDAT expressed in frog oocytes.

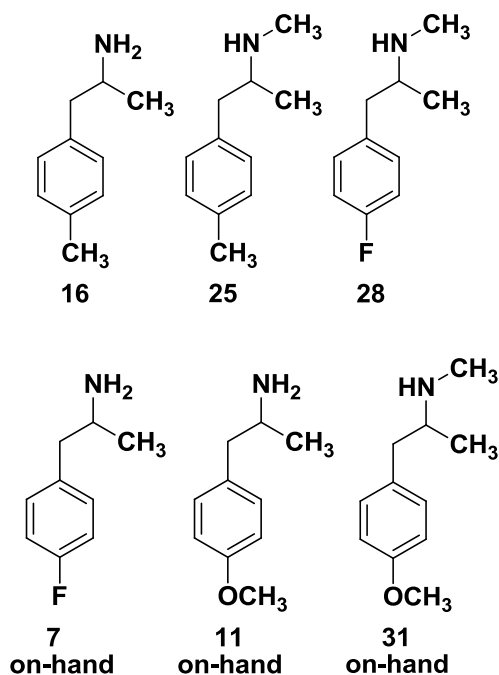
The individual optical isomers of 3,4-dichloroamphetamine will be synthesized as a precursor for its eventual reduction with tritium gas to obtain tritiated isomers of amphetamine. These will be utilized for studying transport mechanisms at the DAT.

One of the cathinones reported in **Table 5** is ethcathinone (**37**), which is the *N*-ethyl homolog of cathinone (**23**). To determine whether the amphetamine analog of ethcathinone (**37**) acts at the DAT, *S*(+)-*N*-ethylamphetamine (**S**(+)**44**) will be synthesized.



In summary, then, the specific aims of the present study are:

- To prepare mephedrone (**27**), methedrone (**33**), and flephedrone (**30**), compounds identified in what have been termed ‘bath salts’ for examination at the DAT
- To prepare, where necessary, amphetamine and/or methamphetamine analogs related to the above compounds for comparison with their cathinone or methcathinone counterparts at the DAT. Specifically, the following compounds are considered:



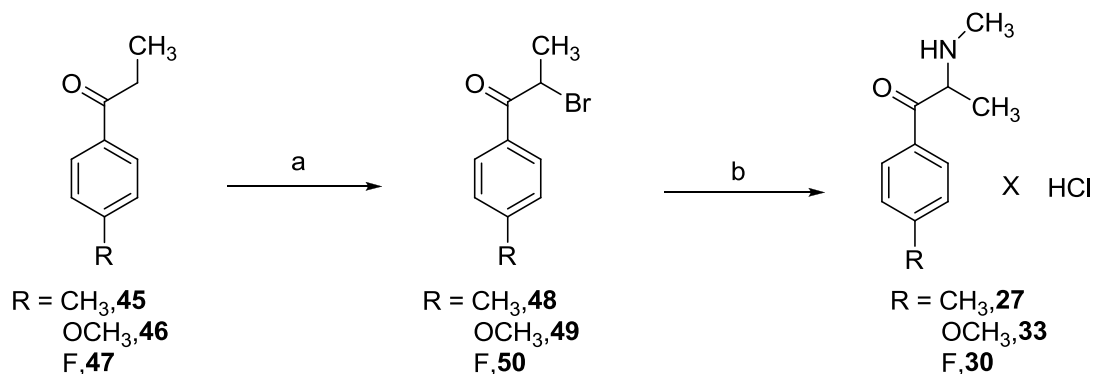
Compounds **7**, **11**, and **31** are already on-hand, so compounds **16**, **25**, and **28** will be synthesized.

- To prepare a pair of optical isomers of a cathinone or methcathinone analogs for examination at the DAT to determine the role of stereochemistry.
- To examine the effect of MDPV at the DAT.
- To prepare an authentic sample of racemic amphetamine (**1**), the individual optical isomers of its 3,4-dichloro counterpart (i.e., **43**), and *S*(+)-*N*-ethylamphetamine (*S*(+)**44**).

IV. Results and Discussion

A. SYNTHESIS

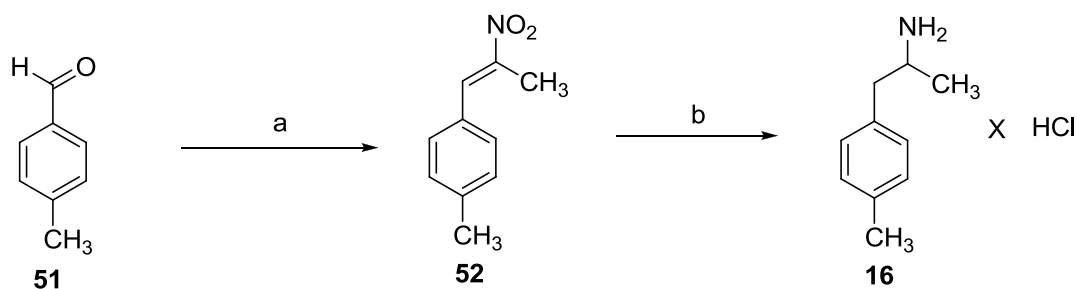
The various amphetamine and cathinone analogs required for this study were prepared in our laboratory. For example, the synthesis of mephedrone (**27**), methedrone (**33**) and flephedrone (**30**) are shown in **Scheme 1**.



Scheme 1. a: Br₂, CH₂Cl₂, N₂, rt; b: i) MeNH₂ (in 33% ethanol), absolute EtOH 0 °C; ii) concentrated HCl

Compounds **27**, **33**, and **30** were prepared based on published procedures for similar compounds.^{209,210} 4-Methylpropiophenone (**45**), 4-methoxypropiophenone (**46**) and 4-fluoropropiophenone (**47**) were dissolved in CH₂Cl₂ and allowed to react with bromine individually to afford white solid compounds **48**, **49**, and **50**, respectively. These intermediates were treated individually with MeNH₂ at 0 °C in absolute EtOH to afford the free base of compounds **27**, **33**, and **30** which, upon treatment with concentrated HCl, resulted in their hydrochloride salts. The melting points of compounds **27**, **33**, and **30** were consistent with their reported melting points.^{167,211,212}

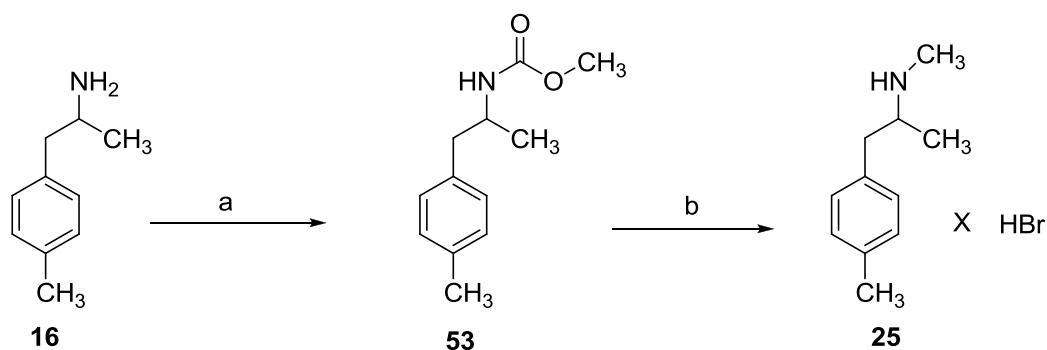
p-Methylamphetamine (**16**) was prepared following a published procedure for a similar compound (i.e., (±)-amphetamine) (**Scheme 2**).^{213,214}



Scheme 2. a: CH₃CH₂NO₂, *n*-butylamine, reflux; b: i) LiAlH₄, reflux; ii) HCl gas

p-Methylbenzaldehyde (**51**) was allowed to react with nitroethane in the presence of *n*-butylamine under reflux for 9 h to afford yellow crystals of *p*-methylnitrostyrene (**52**). Compound **52** was reduced with LiAlH₄ to give the free base of *p*-methylamphetamine (**16**) which upon treatment with HCl gas, resulted in the hydrochloride salt **16**. The melting point of compound **16** was consistent with the literature melting point for this compound.²¹⁵

p-Methylmethamphetamine (**25**) was prepared from *p*-methylamphetamine (**16**) as shown in **Scheme 3** based on the reported procedure for a similar compound (i.e., (*R*)-2-methylamino-1-phenylpropane).²¹⁶

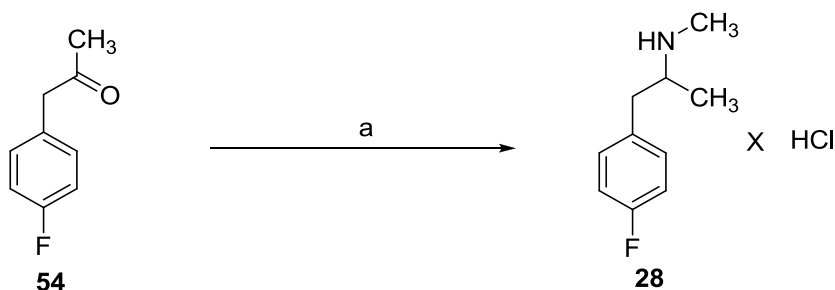


Scheme 3. a: ClCOOCH₃, K₂CO₃, rt; b: i) LiAlH₄, reflux; ii) aqueous HBr

p-Methylamphetamine (**16**) was treated with methyl chloroformate in the presence of K₂CO₃ for 1 h at room temperature to give **53**. Compound **53** was reduced with LiAlH₄ to afford

p-methylmethamphetamine as the free base, which was treated with aqueous HBr to afford the hydrobromide salt **25**. The melting point of compound **25** (mp = 125-128 °C) was not consistent with that in the literature (mp = 159 °C);²¹⁷ therefore, the product was further characterized by elemental microanalysis for C, H, and N, and instrumental analysis which supported the structure of compound **25**.

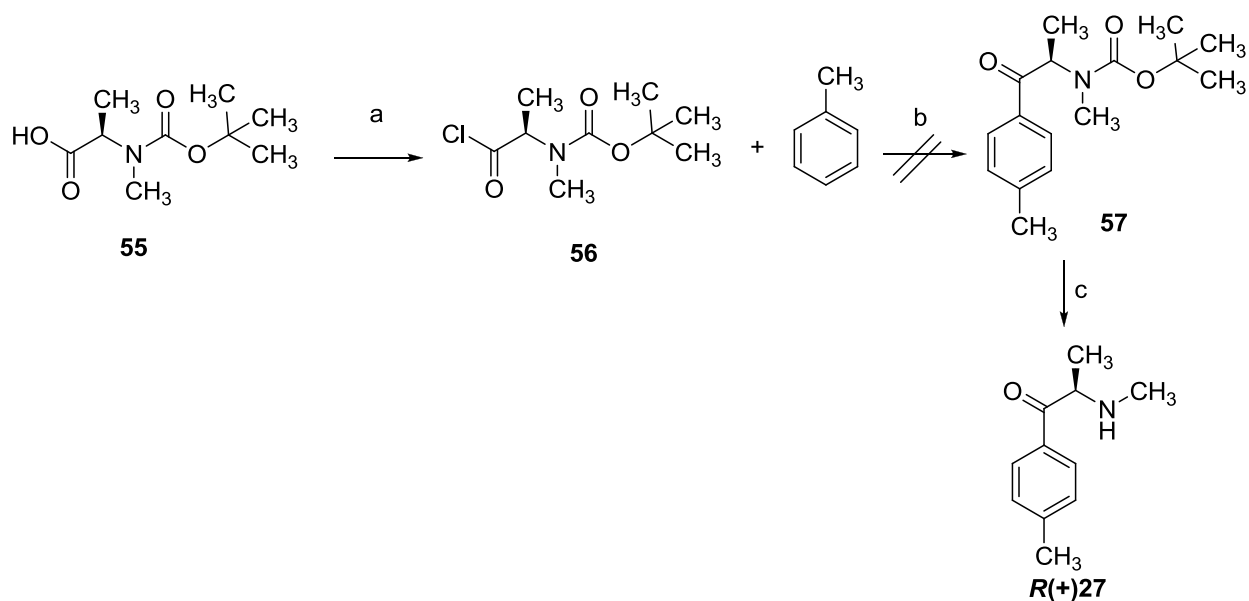
p-Fluoromethamphetamine (**28**) was prepared based on a reported procedure by Fotsch et al. (Scheme 4).²¹⁸



Scheme 4. a: i) CH₃NH₂·HCl, NaBH₄, Ti(IV)[OCH(CH₃)₂]₄; ii) HCl gas

4-Fluorophenylacetone (**54**) was reacted with methylamine HCl and titanium (IV) isopropoxide in the presence of trimethylamine for 3 h at room temperature to afford the free base of *p*-fluoromethamphetamine (**28**). The free base was treated with HCl gas to obtain the hydrochloride salt **28**. The final product, **28**, was characterized by elemental microanalysis for C, H, and N.

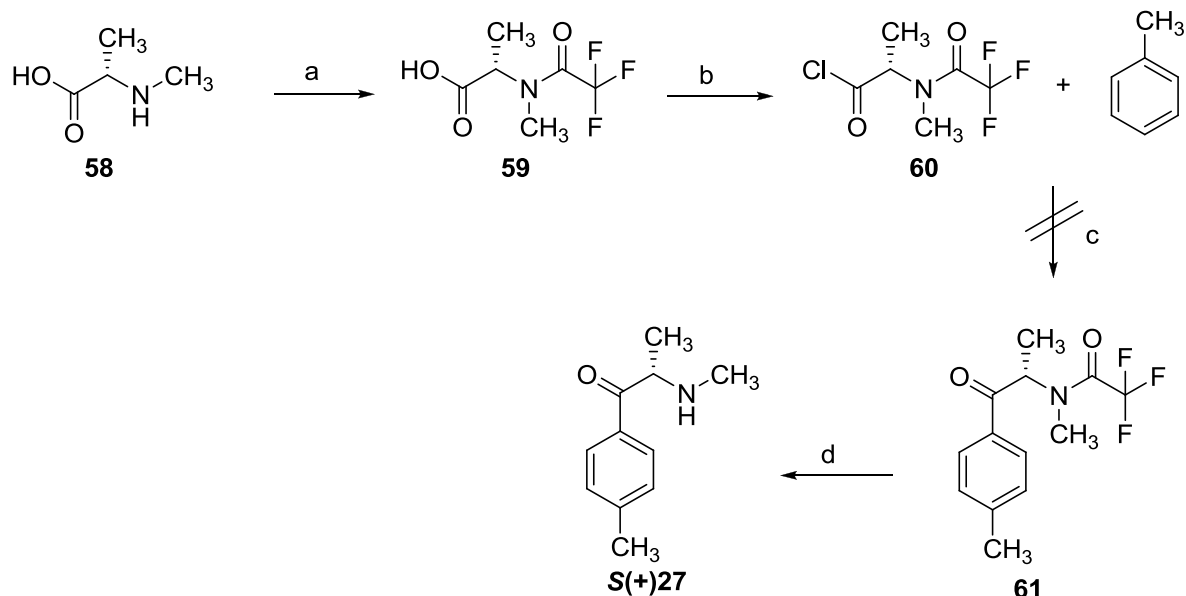
Attempts were made to prepare the optical isomers of mephedrone (**27**). One of the routes, shown below in **Scheme 5**, was based on a published procedure for a similar compound (i.e., (*S*)-2-amino-1-(4-methylphenyl)-1-propanone).²¹⁹



Scheme 5. a: oxalyl chloride; b: AlCl₃; c: CF₃COOH

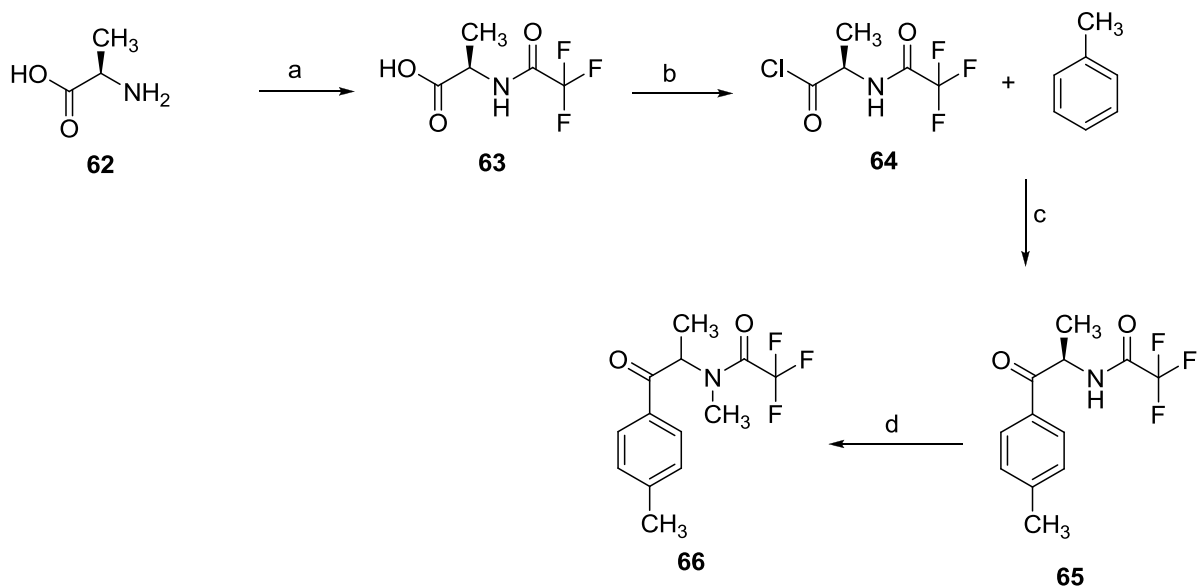
Compound **56** was prepared by treating Boc-*N*-methyl-D-alanine (**55**) with oxalyl chloride. Then compound **56** was reacted with toluene using a Lewis acid, AlCl₃, as catalyst in an attempt to obtain compound **57**, but this reaction did not work. A possible explanation for this might be that in the presence of the Lewis acid the Boc-protecting group is not stable. So, a different route was explored with a different protecting group to overcome this problem. This route (**Scheme 6**) was based on the published procedure for a similar compound (i.e., (*S*)-2-amino-1-(4-methylphenyl)-1-propanone).²¹⁹

Compound **59** was prepared by treating *N*-methyl-L-alanine (**58**) with ethyl trifluoroacetate in the presence of 1,1,3,3-tetramethylguanidine. Compound **59** was treated with oxalyl chloride to obtain compound **60**, which upon treatment with toluene in the presence of AlCl₃ should give compound **61**. But, unfortunately, this reaction did not work. This result was not expected and no possible explanation can be offered.



Scheme 6. a: $\text{CF}_3\text{COOC}_2\text{H}_5$, 1,1,3,3-tetramethylguanidine; b: oxalyl chloride; c: AlCl_3

Another attempt is shown in **Scheme 7**.

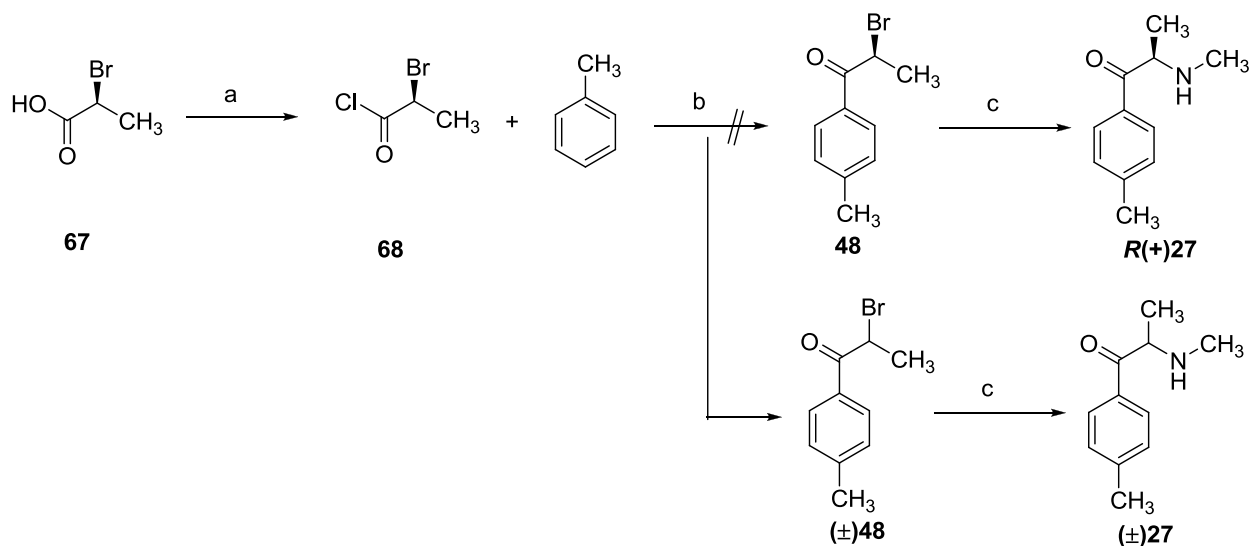


Scheme 7. a: $\text{CF}_3\text{COOC}_2\text{H}_5$, 1,1,3,3-tetramethylguanidine; b: oxalyl chloride; c: AlCl_3 ; d: K_2CO_3 , CH_3I

Compound **63** was prepared by treating D-alanine with ethyl trifluoroacetate in the presence of 1,1,3,3-tetramethylguanidine.²¹⁹ The compound **64** was made by reacting compound **63** with

oxalyl chloride.²¹⁹ Friedel Crafts acylation was accomplished using AlCl₃ to afford compound **65** from compound **64** and toluene.²¹⁹ Then compound **65** was treated with CH₃I in the presence of K₂CO₃ to obtain compound **66**.²²⁰ Here, optical activity was lost and compound **66** was obtained as a racemic mixture. A possible explanation is due to the presence of base the carbonyl group in compound **65** undergoes tautomerism which results in the racemic product.

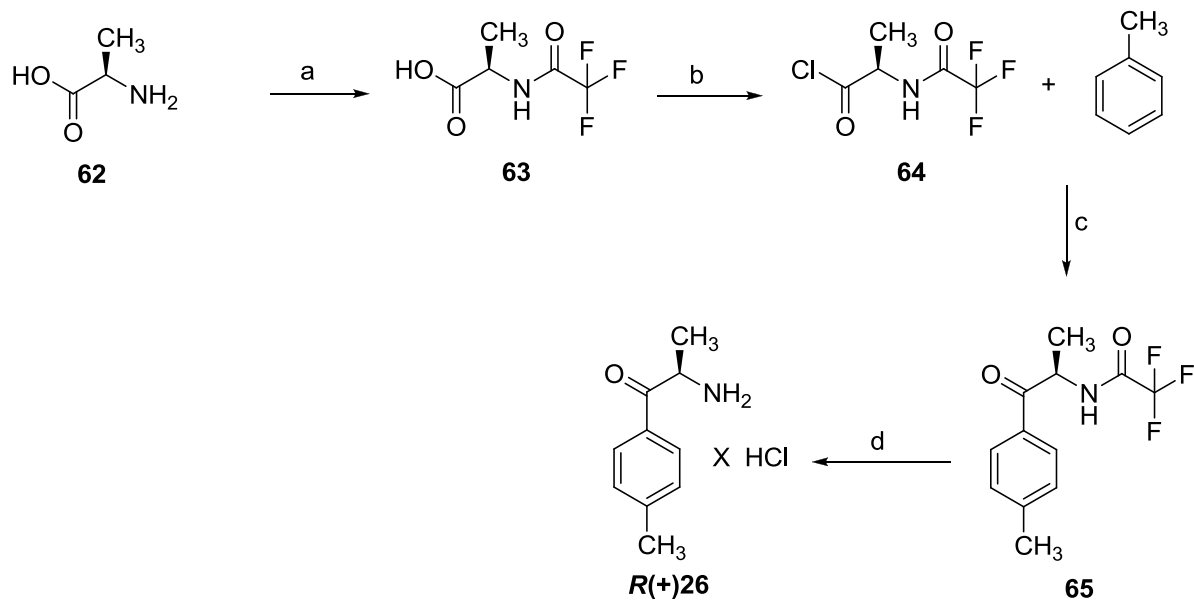
A totally different route to prepare the isomers of mephedrone (**27**) is shown in **Scheme 8**.



Scheme 8. a: SOCl₂; b: AlCl₃; c: CH₃NH₂

Compound **68** was prepared by treating *S*(-)-2-bromopropionic acid with thionyl chloride.²²¹ Compound **48** was then obtained by reacting compound **68** with toluene in the presence of AlCl₃.²¹⁹ Compound **48** was not optically active. A possible explanation for this racemization is the presence of the Lewis acid which promotes tautomerism which ultimately gives rise to racemic product.

Because many of the problems encountered in the above reactions seem to be related to the Friedel-Crafts acylation step, an attempt was made to prepare a known compound using a published procedure. Specifically, we focused on the preparation of optical isomers of *p*-methylcathinone (**26**) using a published route.²¹⁹

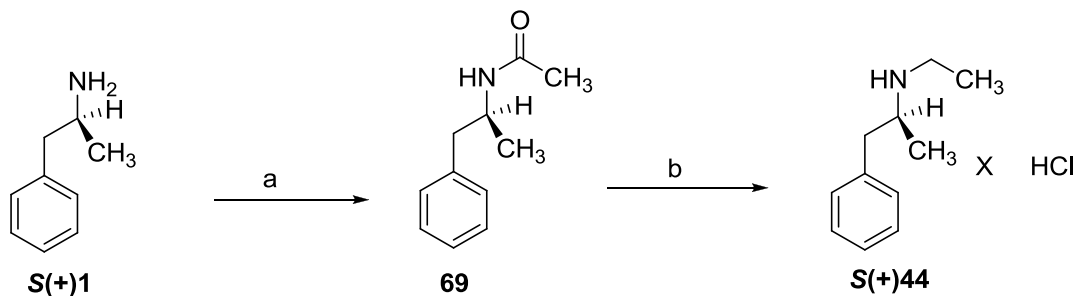


Scheme 9. a: $\text{CF}_3\text{COOC}_2\text{H}_5$, 1,1,3,3-tetramethylguanidine; b: oxalyl chloride; c: AlCl_3 ; d: concentrated HCl, *i*-PrOH

Compound **65** was prepared as mentioned in **Scheme 7**. Compound **65** was treated with concentrated HCl and *i*-PrOH to give one of the optical isomer of *p*-methylcathinone (i.e. *R*(+)**26**). The other isomer of *p*-methylcathinone (i.e. *S*(-)**26**) was obtained by the same synthetic scheme. Both optical isomers were characterized by microanalysis of C, H, and N, which supported the structure of the products, and optical rotations for the isomers were comparable with literature rotations.

After attempting many routes to obtain optical isomers of mephedrone (**27**), finally, we succeeded in making the optical isomers of *p*-methylcathinone (**26**) which are cathinone analogs of mephedrone (**27**, *p*-methylmethcathinone).

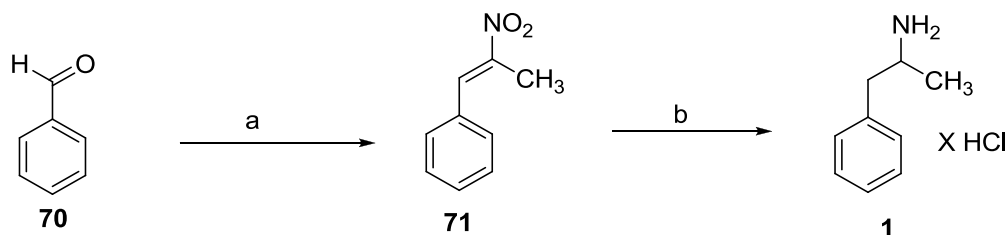
S(+)-*N*-Ethylamphetamine (*S*(+)**44**) was prepared based on a published procedure (**Scheme 10**).²²²



Scheme 10. a: (AcO)₂O, Na₂CO₃; b: i) LiAlH₄, THF, reflux; ii) HCl gas

Compound **69** was obtained by treatment of *S*(+)-amphetamine (**S(+))1**) with acetic anhydride in the presence of Na₂CO₃. Compound **69** was reduced with LiAlH₄ in THF to give *S*(+)-*N*-ethylamphetamine which upon treatment with HCl gas gave a yellow solid. The melting point of the compound **S(+))44** matches the reported melting point,²²³ and the optical rotation is consistent with the literature.²²⁴

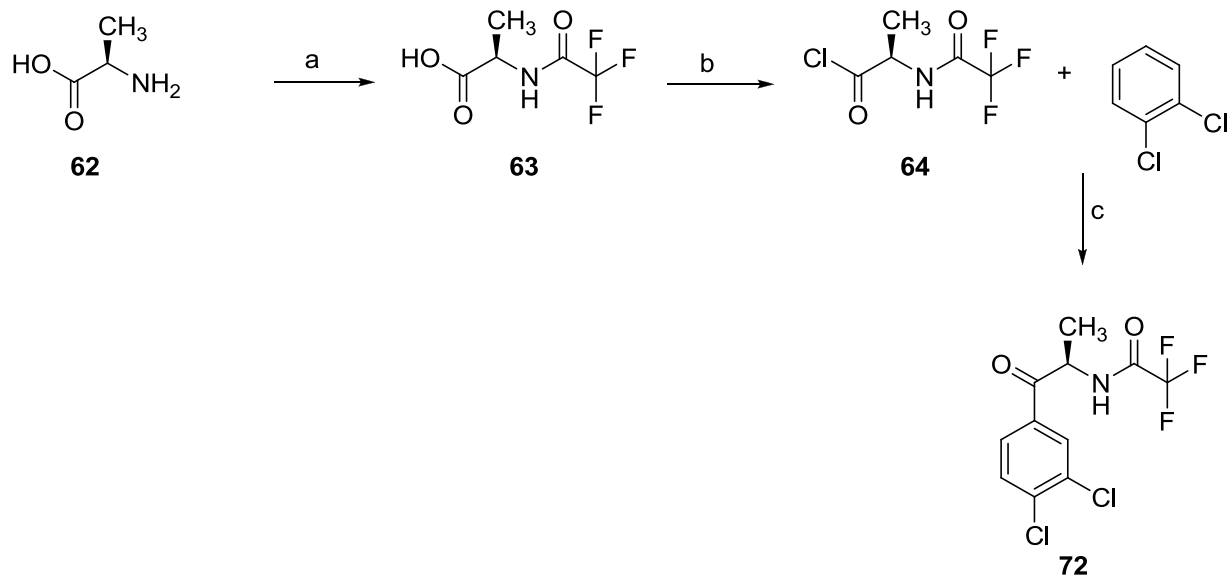
Racemic amphetamine (**1**) was prepared by same procedure mentioned in **Scheme 2**. The only change was that benzaldehyde was used instead of *p*-tolualdehyde (**Scheme 11**).^{213,214}



Scheme 11. a: CH₃CH₂NO₂, *n*-butylamine, reflux; b: i) LiAlH₄, reflux; ii) HCl gas

Racemic amphetamine (**1**) was obtained as its free base which upon treatment with HCl gas gave a white solid. The melting point of racemic amphetamine hydrochloride (**1**) matched the literature melting point.²²⁵

Attempted preparation of the individual optical isomers of 3,4-dichloroamphetamine, using the same procedure as in **Scheme 7**,²¹⁹ is shown in **Scheme 12**.

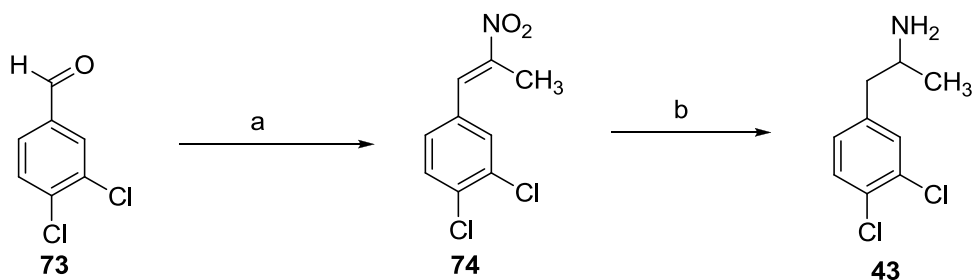


Scheme 12. a: $\text{CF}_3\text{COOC}_2\text{H}_5$, 1,1,3,3-tetramethylguanidine; b: oxalyl chloride; c: AlCl_3

Compound **64** was prepared as described in **Scheme 7**. Compound **64** was treated with 3,4-dichlorobenzene in the presence of AlCl_3 to give compound **72**. The reaction gave a product, but the yield was very low. Compound **72** was characterized by microanalysis of C, H, and N, which supported its structure.

The low yield may be due to the presence of two halogen groups on the benzene ring which might deactivate the aromatic ring to acylation.

As the above route was not very efficient, it was decided to synthesize racemic 3,4-dichloroamphetamine (**43**) using the same route shown in **Scheme 2**,^{213,214} and then resolve it.



Scheme 13. a: $\text{CH}_3\text{CH}_2\text{NO}_2$, n-butylamine, reflux; b: LiAlH_4 , reflux

The only difference in **Scheme 2** and **Scheme 13** is that **Scheme 2** uses *p*-methybenzaldehyde as starting material while 3,4-dichlorobenzaldehyde was used as starting material in **Scheme 13**. The melting point of 3,4-dichloroamphetamine matched the literature melting point. 3,4-Dichloroamphetamine (**43**) was reacted with *N*-acetyl-L-leucine to obtain a salt.²²⁶ The salt was recrystallized multiple times from H₂O.²²⁶ But, unfortunately, the isomers of 3,4-dichloroamphetamine were not obtained. Resolution of 3,4-dichloroamphetamine (**43**) with (-)-O-O'-dibenzoyl-L-tartaric acid,²²⁷ using MeOH as solvent was also not useful. Synthesis of the isomers was abandoned.

B. ELECTROPHYSIOLOGY:

Xenopus laevis oocytes were surgically harvested and injected with hDAT mRNA.^{228,229} Then, the injected oocytes were incubated for a period of 4-6 days in an incubation solution. Oocytes were held at -60 mV in a two-electrode voltage clamp system for all assays, and maintained in a bath with standard recording solution (120 mM NaCl, 5.4 mM K gluconate, 1.2 mM Ca gluconate, 15 mL of 0.5 M HEPES). All solutions were prepared in standard recording solutions and perfused over the oocytes using a gravity-fed perfusion system once a stable baseline was obtained. (**Note:** Electrophysiological studies were done by Krasnodara Cameron, a graduate student in Dr. De Felice Laboratory)

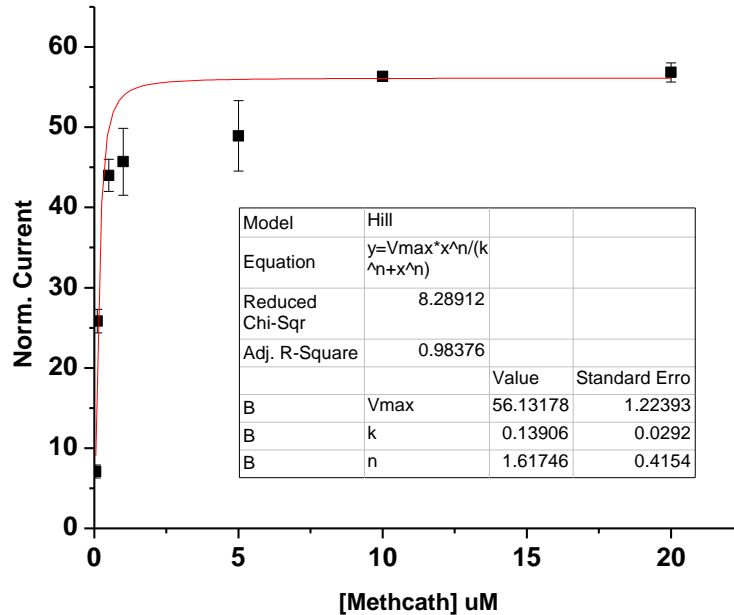


Figure 5. Dose-response curve for *S*(-)-methcathinone (*S*(-)**24**).

Figure 5 shows the dose-response curve of *S*(-)-methcathinone (*S*(-)**24**). Various data points were obtained by exposing hDAT-expressing oocytes to different concentrations of the *S*(-)-methcathinone (*S*(-)**24**) and measuring the peak current. The EC_{50} value for *S*(-)-methcathinone (*S*(-)**24**) was determined to be 0.14 μ M. The same method applied to *S*(+)-methamphetamine (*S*(+)**2**) provided an EC_{50} value of 0.56 (± 0.08) μ M (data not shown). These studies confirmed previous findings,^{147,230} using different methods, that *S*(-)-methcathinone is more potent than *S*(+)-methamphetamine. *S*(-)-Methcathinone (*S*(-)**24**) and *S*(+)-methamphetamine (*S*(+)**2**) will be used here as standards for comparing all compounds proposed in the Specific Aims.

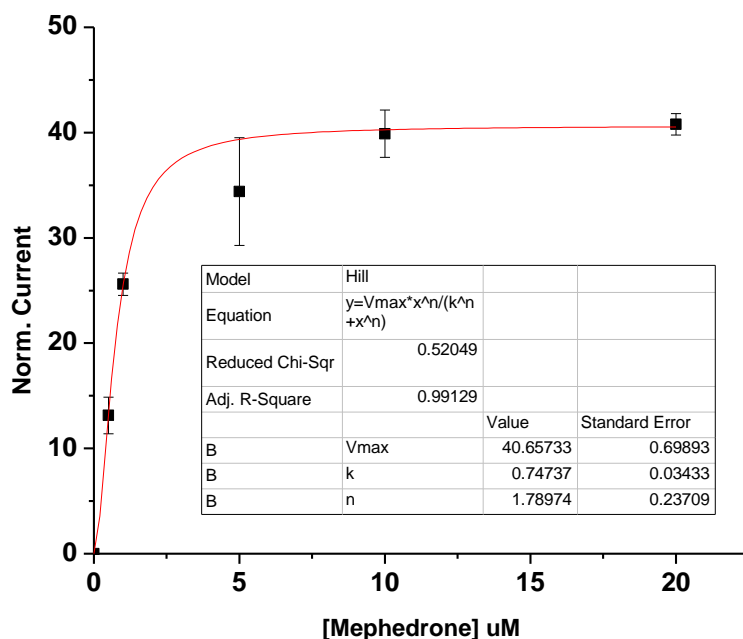


Figure 6. Dose-response curve for racemic mephedrone (**27**).

A dose-response curve was obtained for (\pm)-mephedrone (**27**) (**Figure 6**) and it was determined that the EC_{50} of (\pm)-mephedrone (**27**) is 0.75 μ M. It shows that (\pm)-mephedrone (**27**) has slightly lower potency (EC_{50} =0.75 μ M) than *S*(+)-methamphetamine (EC_{50} =0.56 μ M) and almost 6-fold lower potency than *S*(-)-methcathinone (EC_{50} =0.14 μ M). Although considering its EC_{50} value is for the racemate, the *S*-enantiomer of mephedrone might have a potency higher than that of *S*(+)-methamphetamine (**S(+)****2**). This remains to be determined. (\pm)-Mephedrone (**27**) showed notably lower efficacy (41%) than *S*(+)-methamphetamine (**S(+)****2**)(102%) but it showed comparable efficacy to *S*(-)-methcathinone (**S(-)****24**)(56%).

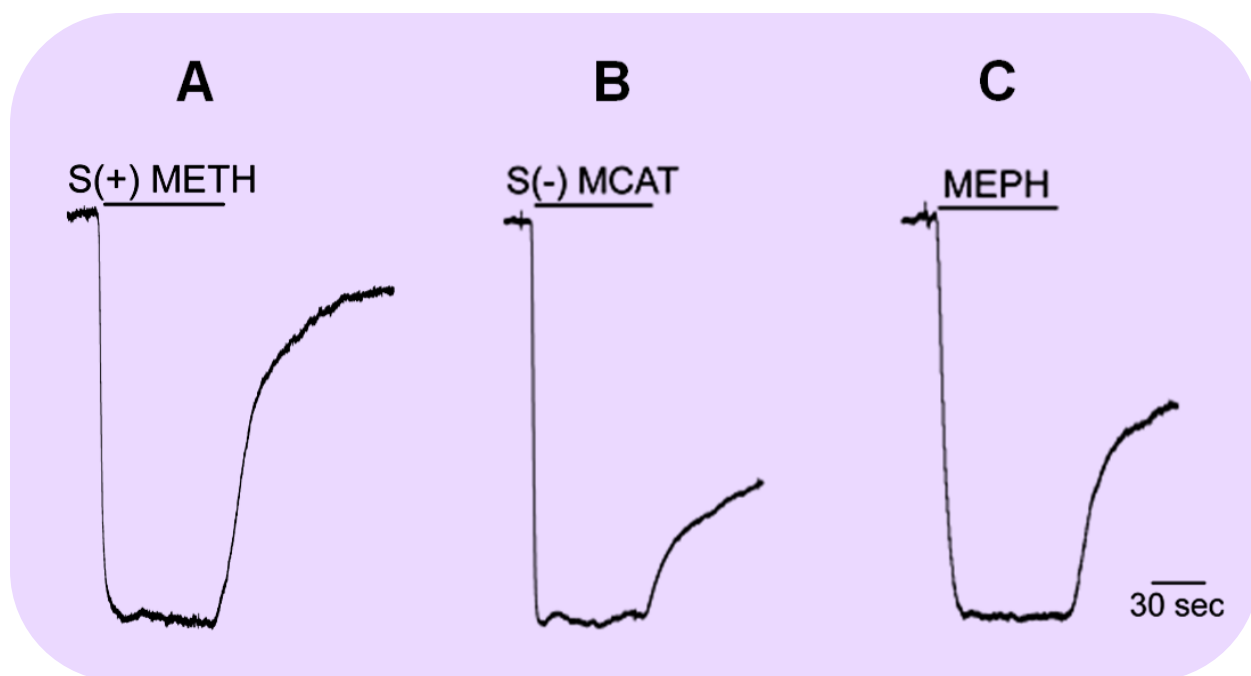


Figure 7. Current generated in hDAT by application of drugs (10 μ M) at -60 mV. All traces were normalized to the peak size of *S(-)*-MCAT (*S(-)*-**24**) and were in the range of 10-20 nA. A. *S(+)*-methamphetamine (*S(+)*-**2**); B. *S(-)*-methcathinone (*S(-)*-**24**); C. (\pm)-mephedrone (**27**).

As shown in **Figure 7**, (\pm)-mephedrone (**27**) as well as *S(-)*-methcathinone (*S(-)*-**24**) generated depolarizing currents with a sustained leak current (also called a ‘shelf’) that persisted even after the drug was removed. Multiple experiments showed that the size of shelf current was proportional to the time of exposure and the concentration of the drug (data not shown). It was found that the persistent depolarizing current caused by (\pm)-mephedrone (**27**) was proportionally larger than the shelf current induced by *S(+)*-methamphetamine (*S(+)*-**2**) but less pronounced than in the case of *S(-)*-methcathinone (*S(-)*-**24**). The similarity of the electrophysiological signature of (\pm)-mephedrone (**27**) to that of *S(+)*-methamphetamine (*S(+)*-**2**) suggests that (\pm)-mephedrone (**27**) shares dopamine-like releasing properties similar to *S(+)*-methamphetamine (*S(+)*-**2**).

As mentioned earlier (see Introduction), mephedrone (**27**) and MDPV (**41**) are constituents of “Bath salts”. It is worth discussing the electrophysiological results of MDPV (**41**) here. (**Note:** MDPV (**41**) was synthesized by Dr. R. Kolanos, in Dr. Glennon’s lab). It was found that (±)-MDPV (**41**) failed to produce a depolarizing effect similar to that of (±)-mephedrone (**27**). Unlike, (±)-mephedrone (**27**), (±)-MDPV (**41**) produced a hyperpolarizing current at hDAT similar to that produced by cocaine.

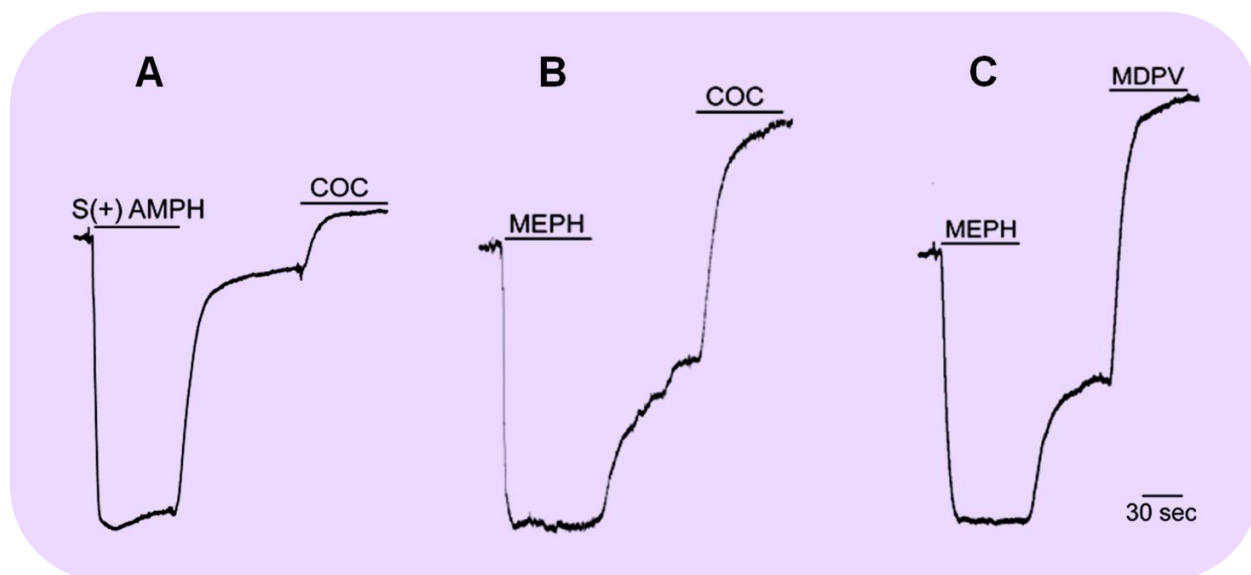


Figure 8. Blockade of hDAT-mediated currents at -60 mV. A) *S*(+)-amphetamine (*S*(+)**1**) is blocked by cocaine; B) (±)-mephedrone (**27**) blocked by cocaine; C) (±)-mephedrone blocked by (±)-MDPV (**41**). Traces were normalized to the peak size of *S*(-)-methcathinone (**Figure 6**) and were in the range of 10-20 nA.

The persistent shelf current produced by (±)-mephedrone (**27**) at hDAT is reversed, similarly to the *S*(+)-amphetamine (*S*(+)**1**) shelf reversal, by cocaine (a hDAT blocker) (**Figure 8**). The current generated by (±)-mephedrone (**27**) was also blocked by (±)-MDPV (**41**), suggesting that the (±)-MDPV (**41**), although structurally similar to other cathinones and very

different from cocaine, might represent a new class of cocaine-like hDAT blocker. Preliminary data indicated that (±)-MDPV (**41**) blocks hDAT-mediated current for a significantly longer time than cocaine. This action might be responsible for the “strong addicting” properties of (±)-MDPV (**41**) reported online by users.

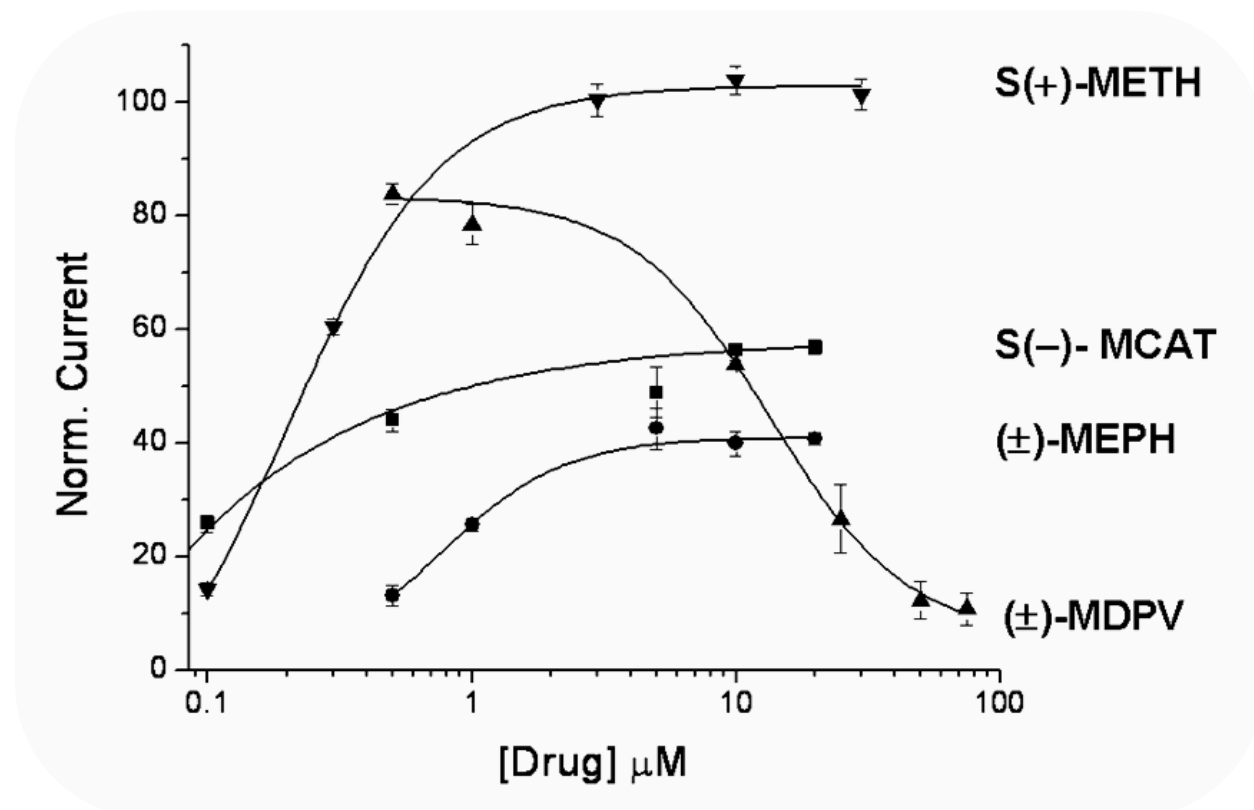


Figure 9. Dose-response curves for *S*(+)-methamphetamine (*S*(+)**2**), *S*(-)-methcathinone (*S*(-)**24**), (±)-mephedrone (**27**) and (±)-MDPV (**41**) in hDAT at -60 mV. In the case of (±)-MDPV (**41**) each drug concentration was applied in the presence of dopamine (5 μM).

Figure 9 shows the dose-response curves for *S*(+)-methamphetamine (*S*(+)**2**), *S*(-)-methcathinone (*S*(-)**24**), (±)-mephedrone (**27**) and (±)-MDPV (**41**). As mentioned earlier, it can be seen in **Figure 9** that (±)-mephedrone (**27**) has notably lower efficacy (41%) than *S*(+)-methamphetamine (*S*(+)**2**)(102%), but it has comparable efficacy to *S*(-)-methcathinone (*S*(-

)24)(56%). **Figure 9** also shows that (±)-MDPV (**41**) blocking the dopamine produced depolarization as mentioned earlier.

To conclude, the studies showed that structurally related synthetic cathinones can have dissimilar biophysical signatures depending on the feature added to the β-keto amphetamine template. “Bath salts” contains both (±)-mephedrone (**27**) and (±)-MDPV (**41**) as major ingredients. (±)-Mephedrone (**27**) has the biophysical signature of a dopamine releasing agent just like *S*(+)-methamphetamine (*S*(+)**2**) whereas the other synthetic cathinone, (±)-MDPV (**41**), appears to behave as a cocaine-like dopamine reuptake inhibitor. The combination of these two mechanisms may account for the severe behavioral toxicity of “bath salts” (see Introduction). “Bath salts” are relatively new products to the drug abuse market, hence there is limited information about their mechanism of action. The above-mentioned results might be useful in prediction of releasing or blocking properties of existing and novel psychoactive drugs as well as forecasting the action of next-generation drugs with abuse potential.

Just as with (±)-mephedrone (**27**), the EC₅₀ of (±)-flephedrone (**30**) was obtained (1.10 μM). Studies showed that flephedrone (data not shown) is half as potent as *S*(-)-methamphetamine (EC₅₀=0.56 μM) and 10-fold less potent than *S*(-)-methcathinone (EC₅₀=0.14 μM). Flephedrone (**30**) was found to produce 65% effect relative to DA. However, the similarity of the electrophysiological signature of (±)-flephedrone (**30**) and *S*(+)-methamphetamine (*S*(+)**2**) suggested that (±)-flephedrone (**30**) shares dopamine-like releasing properties similar to *S*(+)-methamphetamine (*S*(+)**2**).

Methedrone (**33**) and other amphetamine, methamphetamine, and cathinone analogs described in the Specific Aims (compounds: **7**, **11**, **16**, **25**, **28**, and **31**) are currently under

investigation. The pair of optical isomers of *p*-methylephedrine (i.e. *S*(-)**23**, and *R*(+)**23**) and *S*(+)-*N*-ethylamphetamine (*S*(+)**44**) are also under investigation.

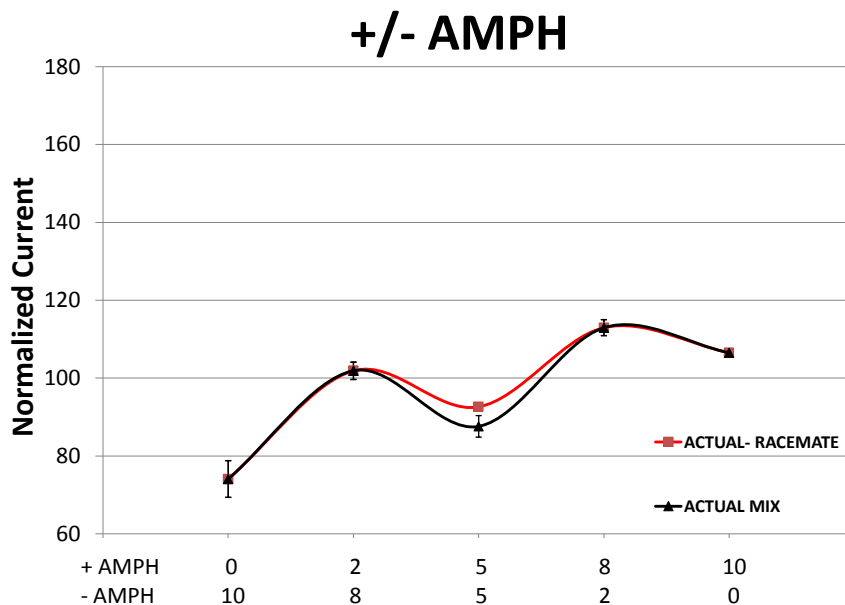


Figure 10. Response (normalized current) curve of isomers of amphetamine (**1**) at different ratios compared with the response curve of racemic amphetamine (**1**).

As mentioned in the Specific Aims, the De Felice lab obtained the response curves as shown in **Figure 4** using different combinations of *S*(+)-amphetamine (*S*(+)**1**) and *R*(-)-amphetamine (*R*(-)**1**). It appears that an equal mixture of the two isomers produced less of an effect than either an 8:2 mixture or a 2:8 mixture. This seemingly aberrant response might be the result of weighing error, or a problem associated with the optical purity of one of the isomers. (\pm)-Amphetamine (**1**) was synthesized and evaluated to resolve the problem. The experiment shown as **Figure 4** was repeated, but racemic amphetamine (**1**) was used in place of the 5:5 mixture (**Figure 10**). **Figure 10** shows that, in fact, the result in **Figure 4** is correct and that both the actual racemate of amphetamine and the mixture of two isomers gave the same results

(within experimental error). But, the question now is why the response of racemate amphetamine is less than that of the 8:2 or 2:8 mixtures. Further investigation is required to properly address this issue.

V. Conclusion

The most common constituents of ‘bath salts’, mephedrone (**27**) and MDPV (**41**), were prepared for electrophysiological examination at the hDAT. Methedrone (**33**) and flephedrone (**30**), which might sometimes appear (amongst other agents) in the ‘bath salts’ combination, were also prepared for evaluation. Amphetamine analogs (i.e., *p*-methylanphetamine (**25**), and *S*(+)-*N*-ethylanphetamine (**S(+)****44**)), and several methamphetamine analogs (i.e., *p*-methylethamphetamine (**25**), and *p*-fluoromethamphetamine (**28**)) were synthesized for electrophysiological comparison. Optical isomers of *p*-methylethamphetamine (**26**), which is the *N*-desmethyl counterpart of mephedrone (**27**, i.e., *p*-methylethamphetamine), were synthesized. Racemic amphetamine, although well known but not readily available, was synthesized. All compounds were synthesized for examination at hDAT.

‘Bath salts’ contains mephedrone (**27**) and MDPV (**41**) (see Introduction) as its most common constituents; sometimes, other constituents have been identified. Mephedrone was prepared and examined at the hDAT. (\pm)-Mephedrone (**27**; $EC_{50} = 0.75 \mu\text{M}$) was found to be slightly lower in potency than *S*(+)-methamphetamine ($EC_{50} = 0.56 \mu\text{M}$), and almost 6-fold lower in potency than *S*(-)-methamphetamine ($EC_{50} = 0.14 \mu\text{M}$). While (\pm)-mephedrone (**27**) displayed notably lower efficacy (41%) than *S*(+)-methamphetamine (**S(+)****2**; 102% relative to DA), it showed comparable efficacy to *S*(-)-methamphetamine (**S(-)****24**; 56%). (\pm)-Mephedrone (**27**) produced an electrophysiological signature similar to that of *S*(+)-methamphetamine

(*S*(+)2) suggesting that (±)-mephedrone (27) shares the dopamine-like releasing properties of *S*(+)-methamphetamine (*S*(+)2). Unlike, (±)-mephedrone (27), (±)-MDPV (41) produced a hyperpolarizing current at hDAT similar to that produced by cocaine.

The EC₅₀ value of (±)-flephedrone (30) was determined to be 1.1 μM. It was found that (±)-flephedrone (30) is half as potent as *S*(-)-methamphetamine (EC₅₀ = 0.56 μM) and 10-fold less potent than *S*(-)-methcathinone (EC₅₀ = 0.14 μM). As with (±)-mephedrone (27), (±)-flephedrone (30) produced an electrophysiological signature similar to *S*(+)-methamphetamine (*S*(+)2) suggesting that (±)-flephedrone (30) shares dopamine-like releasing properties of *S*(+)-methamphetamine (*S*(+)2).

Methedrone (33), and other amphetamine, methamphetamine, and cathinone analogs described in the Specific Aims (compounds: 7, 11, 16, 25, 28, and 31) are currently under investigation. The pair of optical isomers of *p*-methylcathinone (i.e. *S*(-)23, and *R*(+)23) and *S*(+)-*N*-ethylamphetamine (*S*(+)44) are also under investigation. Preliminary data (data not shown) already suggest that methedrone (33) is a dopamine-like releasing agent.

It was found that both racemic amphetamine (1) and the 5:5 mixture of the two individual optical isomers of amphetamine produce a response less than that of the 8:2 or 2:8 mixture of the two individual isomers. A possible explanation would be that the *R*(-)-amphetamine (*R*(-)1) might be competitively inhibiting the effect of *S*(+)-amphetamine (*S*(+)1) at certain concentrations. But, further investigation is required to properly address this issue.

The current studies provide the first information about the two major constituents of ‘bath salts’ on the hDAT expressed in *Xenopus* oocytes and set the stage for future investigations.

VI. Experimental

A. SYNTHESIS

Melting points were taken on a Thomas-Hoover melting point apparatus in glass capillary tubes and are uncorrected. ^1H NMR spectra were recorded with a Varian EM-390 spectrometer with tetramethylsilane (TMS) as an internal standard. Peak positions are given in parts per million (δ). Infrared spectra were obtained on a Nicolet iS10 FT-IR spectrometer. Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. Microanalyses were performed by Atlantic Microlab Inc. (Norcross, GA) for the indicated elements and results are within 0.4% of calculated values. Chromatographic separations were performed on silica gel columns (Silica Gel 60, 220-440 mesh, Sigma-Aldrich). Reactions were monitored by thin-layer chromatography (TLC) on silica gel GHLF plates (250 μ , 2.5 x 10 cm; Analtech Inc., Newark, DE).

Amphetamine Hydrochloride (1). Compound **1** was prepared according to a literature procedure.²¹⁴ A solution of 1-phenyl-2-nitropropene (**71**, 1.5 g, 9.2 mmol) in anhydrous THF (9 mL) was added in a dropwise manner to a suspension of LiAlH_4 (1.5 g, 40.4 mmol) in Et_2O at 0 °C (ice-bath). After completion of addition, the mixture was heated at reflux for 2 h and then quenched at 0 °C by the dropwise addition of absolute EtOH (1.5 mL), H_2O (1.5 mL), and 15% aqueous NaOH (1.5 mL). The mixture was filtered and the filtrate was dried (Na_2SO_4). The solvent was removed under reduced pressure to give an oily residue. The residue was dissolved in absolute EtOH and saturated with HCl gas to afford a

yellow solid. Recrystallization from absolute EtOH/anhydrous Et₂O gave 0.3 g (22%) of **1** as white crystals: mp 147-150 °C (lit.²²⁵ mp 147-149 °C); ¹H-NMR (DMSO-*d*₆: salt) δ 1.1(d, *J* = 6.5 Hz, 3H, CH₃), 2.65 (dd, *J* = 13.2, 9.2 Hz, 1H, CH₂), 3.05 (dd, *J* = 13.2, 5.0, 1H, CH₂), 3.35-3.40 (m, 1H, CH), 7.22-7.26 (m, 3H, ArH), 7.33 (t, *J* = 7.2 Hz, 2H, ArH), 8.17 (br s, 3H, NH₃⁺).

1-(4-Methylphenyl)-2-aminopropane Hydrochloride (16; *p*-Methylamphetamine. HCl).

Compound **16** was prepared using a literature procedure for a similar compound.²¹⁴ 1-(4-Methylphenyl)-2-nitropropene (**52**, 1.0 g, 5.6 mmol) in anhydrous THF (6 mL) was added in a dropwise manner to a stirred suspension of LiAlH₄ (0.9 g, 24.8 mmol) in Et₂O (14 mL) at 0 °C (ice-bath). After completion of the addition, the mixture was heated at reflux for 2 h and then quenched at 0 °C by the dropwise addition of absolute EtOH (0.9 mL), H₂O (0.9 mL), and 15% aqueous NaOH (0.9 mL). The mixture was filtered and the filtrate was dried (Na₂SO₄). The solvent was removed under reduced pressure to give an oily residue which was dissolved in absolute EtOH and saturated with HCl gas to afford a yellow solid. Recrystallization from absolute EtOH gave 0.3 g (22%) of **16** as white crystals: mp 157-159 °C (lit.²¹⁵ mp 158-159 °C); ¹H-NMR (DMSO-*d*₆: salt) δ 1.09 (d, *J* = 6.5 Hz, 3H, CH₃), 2.27 (s, 3H, CH₃), 2.61 (dd, *J* = 13.3, 9.2 Hz, 1H, CH₂), 2.99 (dd, *J* = 13.3, 5.0 Hz, 1H, CH₂), 3.32-3.37 (m, 1H, CH), 7.10-7.15 (m, 4H, ArH), 8.11 (s, 3H, NH₃⁺).

1-(4-Methylphenyl)-2-methylaminopropane Hydrobromide (25; *p*-Methylmethamphetamine. HBr).

Compound **25** was prepared using a literature procedure for a similar compound.²¹⁶ *N*-[1-Methyl-2-(4-methylphenyl)ethyl] methyl carbamate (**53**, 1.7 g, 8.3 mmol) in anhydrous THF (5 mL) was added to a cold (0 °C, ice-bath) suspension of LiAlH₄ (0.5 g, 12.4 mmol) in anhydrous THF (50 mL) at such a rate that the reaction remained under control. After addition of the carbamate the reaction mixture was heated at reflux under an N₂ atmosphere for 2

h. The reaction mixture was cooled to 0 °C and quenched with absolute EtOH (0.5 mL), H₂O (0.5 mL) and 15% NaOH (0.5 mL). The mixture was allowed to stir for 30 min, filtered, and the filtrate was dried (Na₂SO₄). The solvent was evaporated under reduced pressure to give an oily residue which was dissolved in absolute EtOH, and aqueous HBr (48%) was added to pH=1. The aqueous solvent was removed under reduced pressure to afford a white solid. Recrystallization from absolute EtOH gave 0.9 g (34%) of **25** as white crystals: mp 125-128 °C (lit.²¹⁷ mp 159 °C); ¹H NMR (DMSO-*d*₆) δ 1.07 (d, *J* = 6.5 Hz, 3H, CH₃), 2.28 (s, 3H, CH₃), 2.57 (dd, *J* = 13.1, 9.8 Hz, 1H, CH₂), 2.59 (s, 3H, CH₃), 3.08 (dd, *J* = 13.2, 4.3 Hz, 1H, CH₂), 3.34-3.45 (m, 1H, CH), 7.12-7.16 (m, 4H, ArH), 8.48 (br s, 2H, NH₂⁺). Anal. Calcd (C₁₁H₁₇N·HBr) C, 54.11; H, 7.43; N, 5.74. Found: C, 54.21; H, 7.44; N, 5.70.

R*(+)-1-(4-Methylphenyl)-2-aminopropan-1-one Hydrochloride (*R*(+)26; *R*(+)-*p*-Methylcathinone·HCl).** Compound ***R*(+)26** was prepared using a literature procedure for a similar compound.²¹⁹ (*R*)-*N*-[2-(4-Methylphenyl)-1-methyl-2-oxoethyl]-2,2,2-trifluoroacetamide (R*(65)**), 0.5 g, 2 mmol) was dissolved in *i*-PrOH (44 mL) and concentrated HCl (33 mL). The resulting solution was then stirred at 40 °C for 12 h. The solvent was evaporated under reduced pressure, followed by addition of Et₂O (15 mL) and *i*-PrOH (1 mL) to precipitate a white solid. Recrystallization from absolute EtOH gave 0.05 g (13%) of ***R*(+)26** as white crystals: mp 220-225 °C; IR (Diamond): 1682 cm⁻¹ (C=O); ¹H NMR (DMSO-*d*₆) δ 1.42 (d, *J* = 7.2 Hz, 3H, CH₃), 1.41 (s, 3H, CH₃), 5.04-5.09 (m, 1H, CH), 7.40 (d, *J* = 8.0 Hz, 2H, ArH), 7.96 (d, *J* = 8.2 Hz, 2H, ArH), 8.43 (br s, 3H, NH₃⁺). [α]_D²⁸ +34 °, *c* 1, MeOH. Anal. Calcd (C₁₀H₁₃NO·HCl·0.5H₂O) C, 57.55; H, 7.07; N, 6.71. Found: C, 57.62; H, 6.88; N, 6.58.

***S*(-)-1-(4-Methylphenyl)-2-aminopropan-1-one Hydrochloride (*S*(-)**26**; *S*(-)-*p*-Methylcathinone·HCl).** Compound *S*(-)**26** was prepared according to a literature procedure.²¹⁹

(*S*)-*N*-[2-(4-Methylphenyl)-1-methyl-2-oxoethyl]-2,2,2-trifluoroacetamide

(*S*)**65**, 0.5 g, 2 mmol) was dissolved in *i*-PrOH (44 mL) and concentrated HCl (33 mL). The resulting solution was then stirred at 40 °C for 12 h. The solvent was evaporated under reduced pressure, followed by addition of Et₂O (15 mL) and *i*-PrOH (1 mL) to precipitate a white solid. Recrystallization from absolute EtOH gave 0.1 g (21%) of *S*(-)**26** as white crystals: mp 220-225 °C (lit.²¹⁹ mp 192-193 °C); IR (Diamond): 1683 cm⁻¹ (C=O); ¹H NMR (DMSO-*d*₆) δ 1.42 (d, *J* = 7.2 Hz, 3H, CH₃), 1.41 (s, 3H, CH₃), 5.04-5.10 (m, 1H, CH), 7.41 (d, *J* = 8.0 Hz, 2H, ArH), 7.96 (d, *J* = 8.2 Hz, 2H, ArH), 8.44 (br s, 3H, NH₃⁺). [α]_D²⁸ -36.7 °, *c* 1, MeOH (lit.²²² [α]_D²² -32 °, *c* 1.06, MeOH).

1-(4-Methylphenyl)-2-methylaminopropan-1-one Hydrochloride (27**; Mephedrone·HCl).**

Compound **27** was prepared using a literature procedure for a similar compound.²¹⁰ A solution of 2-bromo-(4-methyl)propiophenone (**48**, 0.5 g, 2.2 mmol) in absolute EtOH (5 mL) was added in a dropwise manner to a 33% ethanolic solution of MeNH₂ at 0 °C (0.2 g, 5.9 mmol) and the reaction mixture was allowed to stir for 3 h. Cold, concentrated HCl was then added very slowly along with some finely cracked ice until the mixture became acidic (pH=0). The reaction mixture was extracted with Et₂O (3 x 15 mL) and **48** (0.3 g) was recovered. The aqueous portion was evaporated under reduced pressure to dryness. The residue was extracted several times with fresh portions of CHCl₃ (3 x 15 mL) and each time the insoluble MeNH₂·HCl was removed by filtration. The solvent was evaporated under reduced pressure to give a white solid. Recrystallization from absolute EtOH gave 0.05 g (27%) of **27** as white crystals: mp 230-232 °C (lit.¹⁶⁷ mp 232 °C); IR (Diamond) cm⁻¹: 1685 (C=O); ¹H NMR (DMSO-*d*₆) δ 1.44 (d, *J* = 7.2 Hz,

3H, CH₃), 1.41 (s, 3H, CH₃), 2.59 (s, 3H, CH₃), 5.09-5.14 (m, 1H, CH), 7.42 (d, $J = 8.0$ Hz, 2H, ArH), 7.94 (d, $J = 8.3$ Hz, 2H, ArH), 9.28 (br s, 2H, NH₂⁺).

1-(4-Fluorophenyl)-2-methylaminopropane Hydrochloride (28; *p*-Fluoromethamphetamine. HCl). Compound **28** was prepared according to a literature procedure.²¹⁸

Triethylamine (2.0 g, 20 mmol), MeNH₂·HCl (1.4 g, 20 mmol) and titanium(IV) isopropoxide (5.7 g, 20 mmol) were added to a solution of 4-fluorophenylacetone (**54**, 1.5 g, 10 mmol) in absolute EtOH (15 mL). The solution was allowed to stir for 3 h at room temperature. Then NaBH₄ (0.6 g, 16 mmol) was added to the reaction mixture and stirring was continued for an additional 3 h. Aqueous NH₃ was added and the white precipitate was removed by filtration. Water was added to the filtrate and the aqueous portion was extracted with CH₂Cl₂ (3 x 20 mL). The combined organic portion was washed with 1 N HCl (3 x 10 mL), the aqueous portions were combined and washed with CH₂Cl₂ (3 x 10 mL). The aqueous portion was basified (1N NaOH to bring the pH to 9) and the solution was extracted with CH₂Cl₂ (3 x 20 mL). The combined organic portion was dried (Na₂SO₄) and filtered. The solvent was evaporated under reduced pressure to give an oily residue which was dissolved in absolute EtOH and saturated with HCl gas to afford an off-white solid. Recrystallization from absolute EtOH gave 0.4 g (20%) of **28** as off-white crystals: mp 110-114 °C; ¹H NMR (DMSO-*d*₆) δ 1.09 (d, $J = 6.5$ Hz, 3H, CH₃), 2.55 (s, 3H, CH₃), 2.66 (dd, $J = 13.3, 9.9$ Hz, 1H, CH₂), 3.16 (dd, $J = 13.3, 4.2$ Hz, 1H, CH₂), 3.32-3.34 (m, 1H, CH), 7.14-7.19 (m, 2H, ArH), 7.29-7.32 (m, 2H, ArH), 9.03 (br s, 2H, NH₂⁺). Anal. Calcd (C₁₀H₁₄FN·HCl) C, 58.97; H, 7.42; N, 6.88. Found: C, 58.94; H, 7.36; N, 6.82.

1-(4-Fluorophenyl)-2-methylaminopropan-1-one Hydrochloride (30; Flephedrone. HCl).

Compound **30** was prepared using a literature procedure for a similar compound.²¹⁰ A solution of 2-bromo-(4-fluoro)propiophenone (**50**, 3.0 g, 13.0 mmol) in absolute EtOH (30 mL) was added

in a dropwise manner to a 33% ethanolic solution of MeNH₂ (1.0 g, 32.5 mmol) at 0 °C (ice-bath) and the reaction mixture was allowed to stir for 12 h. Cold, concentrated HCl was then added very slowly along with some finely cracked ice until the mixture was acidic (pH=1). The reaction mixture was extracted with Et₂O (3 x 15 mL). The aqueous portion was evaporated under reduced pressure to dryness. The residue was washed several times with fresh portions of CHCl₃ (3 x 10 mL). The resultant solid was dissolved in H₂O and 1N NaOH was added to the solution to pH=9. The solution was extracted with CH₂Cl₂ (3 x 15 mL). The combined organic portion was dried (Na₂SO₄) and the solvent was removed under reduced pressure to give a yellow solid. Recrystallization from absolute EtOH gave 0.05 g (2%) of **30** as yellow crystals: mp 225-227 °C (lit.²¹² mp 220-222 °C); IR (Diamond): 1686 cm⁻¹ (C=O); ¹H NMR (DMSO-*d*₆) δ 1.45 (d, *J* = 7.2 Hz, 3H, CH₃), 2.59 (s, 3H, CH₃), 5.14-5.19 (m, 1H, CH), 7.44-7.48 (m, 2H, ArH), 8.12-8.16 (m, 2H, ArH), 9.36 (s, 2H, NH₂⁺).

1-(4-Methoxyphenyl)-2-methylaminopropan-1-one Hydrochloride (33; Methedrone. HCl).

Compound **33** was prepared using a literature procedure for a similar compound.²¹⁰ A solution of 2-bromo-(4-methoxy)propiophenone (**49**, 3.0 g, 12.3 mmol) in absolute EtOH (30 mL) was added in a dropwise manner to a 33% ethanolic solution of MeNH₂ (1.0 g, 30.9 mmol) at 0 °C (ice-bath) and the reaction mixture was allowed to stir for 12 h. Cold, concentrated HCl was then added very slowly along with some finely cracked ice until the mixture was acidic (pH=1). The reaction mixture was extracted with Et₂O (3 x 15 mL). The aqueous portion was evaporated under reduced pressure to dryness. The residue was washed several times with fresh portions of CHCl₃ (3 x 10 mL). The resultant solid was dissolved in H₂O and 1N NaOH was added to the solution to pH=9. This solution was extracted with CH₂Cl₂ (3 x 15 mL). The combined organic portion was dried (Na₂SO₄) and the solvent was removed under reduced pressure to give a white

solid. Recrystallization from absolute EtOH gave 1.3 g (47%) of **33** as white crystals: mp 220-222 °C (lit.²¹¹ mp 216 °C); IR (Diamond): 1678 cm⁻¹ (C=O); ¹H NMR (DMSO-*d*₆) δ 1.44 (d, *J* = 7.1 Hz, 3H, CH₃), 2.57 (s, 3H, CH₃), 3.88 (s, 3H, CH₃), 5.07-5.13 (m, 1H, CH), 7.12 (d, *J* = 9.0 Hz, 2H, ArH), 8.02 (d, *J* = 9.0 Hz, 2H, ArH), 9.30 (s, 2H, NH₂⁺).

1-(3,4-Dichlorophenyl)-2-aminopropane Hydrochloride (43; 3,4-Dichloro-amphetamine.HCl). Compound **43** was prepared using a literature procedure for a similar compound.²¹⁴ 1-(3,4-Dichlorophenyl)-2-nitropropene (**74**, 6.1 g, 26.2 mmol) in anhydrous THF (5 mL) was added in a dropwise manner to a stirred suspension of LiAlH₄ (4.4 g, 115.4 mmol) in Et₂O (50 mL) at 0 °C (ice-bath). After completion of the addition, the mixture was heated at reflux for 2 h and then quenched at 0 °C by the dropwise addition of absolute EtOH (5 mL), H₂O (5 mL), and 15% aqueous NaOH (15 mL). The mixture was filtered and the filtrate was dried (Na₂SO₄). The solvent was evaporated under reduced pressure to give an oily residue which was dissolved in absolute EtOH and saturated with HCl gas to afford a yellow solid. Recrystallization from absolute EtOH gave 2.7 g (42%) of **43** as white crystals: mp 175-178 °C (lit.²³¹ mp 188-189 °C); ¹H-NMR (DMSO-*d*₆: salt) δ 1.16 (d, *J* = 6.5 Hz, 3H, CH₃), 2.77 (dd, *J* = 13.5, 8 Hz, 1H, CH₂), 3.04 (dd, *J* = 13.5, 5.9 Hz, 1H, CH₂), 3.34-3.46 (m, 1H, CH), 7.29 (dd, *J* = 8.2, 2 Hz, 1H, ArH), 7.58 (m, 2H, ArH), 8.26 (s, 3H, NH₃⁺).

***S*(+)-1-Phenyl-2-ethylaminopropane Hydrochloride (*S*(+)44; *S*(+)-*N*-Ethyl-amphetamine.HCl).** Compound *S*(+)44 was prepared according to a literature procedure.²²² *S*(+)-*N*-(2-Phenyl-1-methylethyl)acetamide (*S*(+)69, 1.5 g, 8.5 mmol) in anhydrous THF (20 mL) was added to a cold (0 °C, ice-bath) suspension of LiAlH₄ (0.5 g, 12.6 mmol) in anhydrous THF (8 mL) at such a rate that the reaction remained under control. After addition of amide the reaction mixture was heated at reflux under an N₂ atmosphere for 15 h. The reaction mixture was

cooled at 0 °C and quenched with H₂O (0.5 mL), 15% NaOH (0.5 mL) and H₂O (1.4 mL). The mixture was allowed to stir for 30 min, filtered, and the filtrate was dried (Na₂SO₄). The solvent was evaporated under reduced pressure to give an oily residue, which was dissolved in absolute EtOH and saturated with HCl gas to afford a yellow solid. Recrystallization from absolute EtOH/anhydrous Et₂O gave 0.3 g (18%) of **S(+)**44 as yellow crystals: mp 147-150 °C (lit.²²³ mp 141-142 °C); ¹H NMR (DMSO-*d*₆) δ 1.09 (d, *J* = 6.6 Hz, 3H, CH₃), 1.24 (t, *J* = 7.2 Hz, 3H, CH₃), 2.62 (dd, *J* = 13.1, 10.4 Hz, 1H, CH₂), 2.95-3.04 (m, 2H, CH₂), 3.23 (dd, *J* = 13.1, 3.8 Hz, 1H, CH₂), 3.35-3.40 (m, 1H, CH), 7.24-7.27 (m, 3H, ArH), 7.34 (t, *J* = 7.4 Hz, 2H, ArH); [α]²⁴_D +14.8 °, *c* 2, H₂O (lit.²²⁴ [α]²⁵_D +17.3 °, *c* 2, H₂O).

2-Bromo-(4-methyl)propiophenone (48). Compound **48** was prepared according to a literature procedure.²⁰⁹ 4-Methylpropiophenone (**45**, 4.0 g, 27.0 mmol) and CH₂Cl₂ (100 mL) were placed in a 250 mL flask equipped with a magnetic stir bar. The solution was allowed to stir under an N₂ atmosphere and bromine (4.3 g, 27.0 mmol) was added to the flask. (**Note:** a small amount of bromine was added to initiate the reaction; the color dissipated as the reaction occurred. After the reaction initiated, the remaining bromine was added over 10 min). A needle was placed in the septa to allow the HBr gas that formed in the reaction to escape from the flask. After stirring the solution for 12 h, saturated NaHCO₃ was added to bring the pH of the mixture to 9. The aqueous layer was extracted with CH₂Cl₂ (3 x 15 mL). The combined organic portion was dried (Na₂SO₄) and the solvent was removed under reduced pressure to give a white solid. Recrystallization from absolute EtOH gave 2.7 g (44%) of **48** as white crystals: mp 75-78 °C (lit.²³² mp 76-77 °C); ¹H NMR (CDCl₃) δ 1.90 (d, *J* = 6.6 Hz, 3H, CH₃), 2.42 (s, 3H, CH₃), 5.25-5.30 (q, 1H, CH), 7.29 (d, *J* = 8.1 Hz, 2H, ArH), 7.92 (d, *J* = 8.2 Hz, 2H, ArH).

2-Bromo-(4-methoxy)propiophenone (49). Compound **49** was prepared using a literature procedure for a similar compound.²⁰⁹ 4-Methoxypropiophenone (**46**, 4.0 g, 24.4 mmol) and CH₂Cl₂ (100 mL) were placed in a 250 mL flask equipped with a magnetic stir bar. The solution was allowed to stir under an N₂ atmosphere and bromine (3.9 g, 24.4 mmol) was added to the flask. (**Note:** a small amount of bromine was added to initiate the reaction; the color dissipated as the reaction occurred. After the reaction initiated, the remaining bromine was added over 10 min.) A needle was placed in the septa to allow the HBr gas that formed in the reaction to escape from the flask. After stirring the solution for 12 h, saturated NaHCO₃ was added to bring the pH of the mixture to 9. The aqueous layer was extracted with CH₂Cl₂ (3 x 15 mL). The combined organic portion was dried (Na₂SO₄) and the solvent was removed under reduced pressure to give a white solid. Recrystallization from absolute EtOH gave 4.6 g (78%) of **49** as white crystals: mp 62-64 °C (lit.²³³ mp 66-69 °C); ¹H NMR (CDCl₃) δ 1.80 (d, *J* = 9.6 Hz, 3H, CH₃), 3.80 (s, 3H, CH₃), 5-16-5.21 (q, 1H, CH), 6.87 (d, *J* = 8.7 Hz, 2H, ArH), 7.93 (d, *J* = 8.8 Hz, 2H, ArH).

2-Bromo-(4-fluoro)propiophenone (50). Compound **50** was prepared using a literature procedure for a similar compound.²⁰⁹ 4-Fluoropropiophenone (**47**, 4.0 g, 26.3 mmol) and CH₂Cl₂ (100 mL) were placed in a 250 mL flask equipped with a magnetic stir bar. The solution was allowed to stir under an N₂ atmosphere and bromine (4.2 g, 26.3 mmol) was added to the flask. (**Note:** a small amount of bromine was added to initiate the reaction; the color dissipated as the reaction occurred. After the reaction initiated, the remaining bromine was added over 10 min.) A needle was placed in the septa to allow the HBr gas that formed in the reaction to escape from the flask. After 12 h, saturated NaHCO₃ was added to the stirred solution to pH=9. The aqueous layer was extracted with CH₂Cl₂ (3 x 15 mL). The combined organic portion was dried (Na₂SO₄) and the solvent was removed under reduced pressure to give white solid. Recrystallization from

absolute EtOH gave 4.3 g (71%) of **50** as white crystals: mp 33-35 °C (lit.²³⁴ mp 33-34 °C); ¹H NMR (CDCl₃) δ 1.89 (d, *J* = 6.6 Hz, 3H, CH₃), 3.80, 5.20-5.25 (q, 1H, CH), 7.13-7.17 (m, 2H, ArH), 8.04-8.07 (m, 2H, ArH).

1-(4-Methylphenyl)-2-nitropropene (52). Compound **52** was prepared using a literature procedure for a similar compound.²¹³ *p*-Methylbenzaldehyde (**51**, 5.0 g, 41.6 mmol), nitroethane (3.1 g, 41.6 mmol) and *n*-butylamine (0.2 mL) were added to absolute EtOH (4 mL). The solution was heated at reflux for 9 h. On cooling the reaction solution, a heavy, yellow and crystalline mass was formed. Recrystallization from absolute EtOH gave 2.1 g (29%) of **52** as yellow crystals: mp 45-48 °C (lit.²³⁵ mp 51.5-52.5 °C): ¹H NMR (CDCl₃) δ 2.41 (s, 3H, CH₃), 2.46 (d, *J* = 0.6 Hz, 3H, CH₃), 7.26 (d, *J* = 8 Hz, 2H, ArH), 7.35 (d, *J* = 8.1 Hz, 2H, Ar), 8.08 (s, 1H, CH).

***N*-[1-Methyl-2-(4-methylphenyl)ethyl] methyl carbamate (53).** Compound **53** was prepared using a literature procedure for a similar compound.²¹⁶ Methyl chloroformate (1.0 g, 10.86 mmol) was added to a solution of 1-(4-methylphenyl)-2-aminopropane (**16**, 1.3 g, 8.7 mmol) in CH₂Cl₂ (30 mL) with a vigorous stirring. Then K₂CO₃ (6.0 g, 43.6 mmol) in H₂O (30 mL) was added to the reaction mixture and stirring was continued for 1 h. The reaction mixture was extracted with CH₂Cl₂ (3 x 10 mL). The organic portion was combined, dried (Na₂SO₄) and filtered. The solvent was removed under reduced pressure to give 1.7 g (95%) of crude **53** as an oil, which was used without further purification for the next step. ¹H NMR (CDCl₃) δ 1.10 (d, *J* = 6.6 Hz, 3H, CH₃), 2.32 (s, 3H, CH₃), 2.65 (dd, *J* = 13.5, 7.1 Hz, 1H, CH₂), 2.79 (dd, *J* = 13.3, 5.3 Hz, 1H, CH₂), 3.64 (s, 3H, CH₃), 3.82-3.98 (m, 1H, CH), 4.51 (br s, 1H, NH), 7.05-7.13 (m, 4H, ArH).

(R)-N-Methyl-N-[2-chloro-1-methyl-2-oxoethyl]-1,1-dimethylethyl carbamate (56).

Compound **56** was prepared using a literature procedure for a similar compound.²¹⁹ Oxalyl chloride (1.5 g, 11.7 mmol) was added to a stirred suspension of Boc-*N*-methyl-D-alanine (**55**, 1.0 g, 4.9 mmol) in CH₂Cl₂ (18 mL), cooled to 0 °C (ice-bath) followed by addition of pyridine (3 drops). The reaction mixture was allowed to warm gradually to room temperature and was further stirred for 8 h. The solvent and excess oxalyl chloride were removed by rotary evaporation at 30 °C to afford 1.1 g (100%) of **56** as a colorless oil which was used without purification for subsequent reaction.

(S)-2-(N-Methyl-N-trifluoroacetyl)aminopropanoic Acid (59). Compound **59** was prepared using a literature procedure for a similar compound.²¹⁹ 1,1,3,3-Tetramethylguanidine (0.5 g, 5.0 mmol) was added to a suspension of *N*-methyl-L-alanine (**58**, 5.0 g, 55 mmol) in MeOH (3 mL). After 5 min, ethyl trifluoroacetate (0.9 g, 6.0 mmol) was added and the reaction mixture was allowed to stir for 6 h at room temperature. The solvent was evaporated under reduced pressure to give an oily residue which was dissolved in H₂O (8 mL) and acidified with concentrated HCl to pH=1. After stirring for 15 min, the mixture was extracted with EtOAc (3 x 20 mL). The combined organic portion was washed with brine (20 mL) and dried (Na₂SO₄). The solvent was evaporated under reduced pressure to give a white solid which was washed with *n*-hexane (20 mL) and dried to afford 0.9 g (94%) of **59** as an oil; IR (Diamond): 1682 cm⁻¹ (C=O).

(S)-2-(N-Methyl-N-trifluoroacetyl)aminopropanoyl Chloride (60). Compound **60** was prepared using a literature procedure for a similar compound.²¹⁹ Oxalyl chloride (1.4 g, 10.7 mmol) was added to a stirred suspension of (*S*)-2-(*N*-Methyl-*N*-trifluoroacetyl)aminopropanoic acid (**59**, 0.9 g, 4.5 mmol) in CH₂Cl₂ (17 mL), at 0 °C (ice-bath); this was followed by addition of pyridine (1 drop). The reaction mixture was allowed to warm gradually to room temperature

and was further stirred for 8 h. The solvent and excess oxalyl chloride were removed by rotary evaporation at 30 °C to afford 1.0 g (100%) of **60** as a colorless oil which was used without purification for subsequent reactions.

(R)-2-(N-Trifluoroacetyl)aminopropanoic Acid ((R)63). Compound **(R)63** was prepared using a literature procedure for a similar compound.²¹⁹ 1,1,3,3-Tetramethylguanidine (8.6 g, 75 mmol) was added to a suspension of D-alanine (**62**, 5.0 g, 55.0 mmol) in MeOH (28 mL). After 5 min, ethyl trifluoroacetate (9.9 g, 70.0 mmol) was added and the reaction mixture was allowed to stir for 6 h at room temperature. The solvent was evaporated under reduced pressure to give an oily residue which was dissolved in H₂O (70 mL) and acidified with concentrated HCl to pH=1. After stirring for 15 min, the mixture was extracted with EtOAc (3 x 20 mL). The combined organic portion was washed with brine (20 mL) and dried (Na₂SO₄). The solvent was evaporated under reduced pressure to give a white solid which was washed with *n*-hexane (20 mL) and dried to afford 9.8 g (94%) of **(R)63** as a white solid, sufficiently pure for subsequent use: mp 63-65 °C (lit.²³⁶ mp 70-71 °C); IR (Diamond): 1732 cm⁻¹ (C=O).

(S)-2-(N-Trifluoroacetyl)aminopropanoic Acid ((S)63). Compound **(S)63** was prepared according to a literature procedure.²¹⁹ 1,1,3,3-Tetramethylguanidine (8.6 g, 75.0 mmol) was added to a suspension of L-alanine (5.0 g, 55.0 mmol) in MeOH (28 mL). After 5 min, ethyl trifluoroacetate (9.9 g, 70.0 mmol) was added and the reaction mixture was allowed to stir for 6 h at room temperature. The solvent was evaporated under reduced pressure to give an oily residue which was dissolved in H₂O (70 mL) and acidified with concentrated HCl to pH=1. After stirring for 15 min, the mixture was extracted with EtOAc (3 x 20 mL). The combined organic portion was washed with brine (20 mL) and dried (Na₂SO₄). The solvent was evaporated under reduced pressure to give a white solid which was washed with *n*-hexane (20 mL) and dried to afford 10.0

g (96%) of **(S)63** as a white solid, sufficiently pure for subsequent use: mp 63-65 °C (lit.²¹⁹ mp 70-71 °C); IR (Diamond): 1731 cm⁻¹ (C=O).

(R)-2-(N-Trifluoroacetyl)aminopropanoyl Chloride ((R)64). Compound **(R)64** was prepared using a literature procedure for a similar compound.²¹⁹ Oxalyl chloride (6.4 g, 50.7 mmol) was added to a stirred suspension of **(R)-2-(N-trifluoroacetyl)aminopropanoic acid ((R)63**, 4.0 g, 21.6 mmol) in CH₂Cl₂ (80 mL) at 0 °C (ice-bath), followed by addition of pyridine (3 drops). The reaction mixture was allowed to warm gradually to room temperature and was further stirred for 8 h. The solvent and excess oxalyl chloride were removed by rotary evaporation at 30 °C afforded 4.4 g (100%) of **(R)64** as a colorless oil which was used without purification for subsequent reactions.

(S)-2-(N-Trifluoroacetyl)aminopropanoyl Chloride ((S)64). Compound **(S)64** was prepared according to a literature procedure.²¹⁹ Oxalyl chloride (4.8 g, 38.0 mmol) was added to a stirred suspension of **(S)-2-(N-trifluoroacetyl)aminopropanoic acid ((S)63**, 3.0 g, 16.2 mmol) in CH₂Cl₂ (60 mL) at 0 °C (ice-bath), followed by addition of pyridine (3 drops). The reaction mixture was allowed to warm gradually to room temperature and was further stirred for 8 h. The solvent and excess oxalyl chloride were removed by rotary evaporation at 30 °C to afford 3.3 g (100%) of **(S)64** as a colorless oil which was used without purification for subsequent reactions.

(R)-N-[2-(4-Methylphenyl)-1-methyl-2-oxoethyl]-2,2,2-trifluoroacetamide ((R)65). Compound **(R)65** was prepared using a literature procedure for a similar compound.²¹⁹ Toluene (23.0 g, 251.0 mmol) and AlCl₃ (5.8 g, 43.2 mmol) were added to **(R)-2-(N-trifluoroacetyl)aminopropanoyl chloride ((R)64**, 4.4 g, 21.6 mmol) at room temperature. The reaction mixture was allowed to stir for 18 h and then cooled in an ice-bath and slowly quenched with 1N HCl (80 mL). The aqueous layer was extracted with CH₂Cl₂ (3 x 20 mL) and the organic

portions were combined and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to give the crude product as an orange oil which was purified by column chromatography (silica gel; hexane/EtOAc; 9.7:0.3) to give 0.9 g (22%) of (**R**)**65** as a white solid: mp 77-78 °C; $^1\text{H-NMR}$ (CDCl_3) δ 1.52 (d, $J = 7.1$ Hz, 3H, CH_3), 2.45 (s, 3H, CH_3), 5.44-5.53 (m, 1H, CH), 7.33 (d, $J = 8.0$ Hz, 2H, ArH), 7.61 (s, 1H, NH), 7.88 (d, $J = 8.3$ Hz, 2H, ArH).

(S)-N-[2-(4-Methylphenyl)-1-methyl-2-oxoethyl]-2,2,2-trifluoroacetamide ((S)65).

Compound (**S**)**65** was prepared according to a literature procedure.²¹⁹ Toluene (17.3 g, 188.0 mmol) and AlCl_3 (4.3 g, 32.4 mmol) were added to (*S*)-2-(*N*-trifluoroacetyl)aminopropanoyl chloride ((**S**)**64**, 3.3 g, 16.2 mmol) at room temperature. The reaction mixture was allowed to stir for 18 h and, then cooled in an ice-bath and slowly quenched with 1N HCl (60 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL) and the organic portions were combined and dried (Na_2SO_4). The solvent was evaporated under reduced pressure to give the crude product as an orange oil which was purified by column chromatography (silica gel; hexane/EtOAc; 9.7:0.3) to give 0.7 g (17%) of (**S**)**65** as a white solid: mp 77-78 °C (lit.²¹⁹ mp 77-78 °C); $^1\text{H-NMR}$ (CDCl_3) δ 1.52 (d, $J = 7.2$ Hz, 3H, CH_3), 2.45 (s, 3H, CH_3), 5.46-5.53 (m, 1H, CH), 7.33 (d, $J = 8.0$ Hz, 2H, ArH), 7.61 (s, 1H, NH), 7.88 (d, $J = 8.3$ Hz, 2H, ArH).

N-Methyl-N-[2-(4-methylphenyl)-1-methyl-2-oxoethyl]-2,2,2-trifluoroacetamide (66).

Compound **66** was prepared using a literature procedure for a similar compound.²²⁰ (*S*)-*N*-[2-(4-Methylphenyl)-1-methyl-2-oxoethyl]-2,2,2-trifluoroacetamide (**65**, 1.4 g, 5.4 mmol) was dissolved in dry acetone (30 mL), and to that anhydrous K_2CO_3 (1.5 g, 10.9 mmol) and CH_3I (3.1 g, 21.8 mmol) were added. The reaction mixture was heated at reflux for 48 h. The solvent was removed under reduced pressure and the residue was dissolved in H_2O (3 mL). The aqueous layer was extracted with Et_2O (3 x 20 mL) and the organic portions were combined and dried

(Na₂SO₄). The solvent was evaporated under reduced pressure to give the crude product as an oil which was purified by column chromatography (silica gel; hexane/EtOAc; 9.9:0.1) to give 1.4 g (94%) of **66** as a colorless oil; ¹H-NMR (CDCl₃) δ 1.44 (d, *J* = 7 Hz, 3H, CH₃), 2.41 (s, 3H, CH₃), 2.96 (s, 3H, CH₃), 5.97-6.02 (m, 1H, CH), 7.27 (d, *J* = 7 Hz, 2H, ArH), 7.83 (d, *J* = 8.3 Hz, 2H, ArH).

(S)-N-(2-Phenyl-1-methylethyl)acetamide (69). Compound **69** was prepared according to a literature procedure.²²² Acetic anhydride (2.1 mL, 22.2 mmol) was added to a stirred suspension of Na₂CO₃ (7.8 g, 73.9 mmol) and *S*(+)-amphetamine hemisulfate (**S(+)****1**, 3.4 g, 9.3 mmol) in H₂O (22 mL) at 0 °C (ice-bath). The suspension was allowed to stir for 5 h at room temperature, and then extracted with CHCl₃ (3 x 20 mL); the combined organic portion was washed with H₂O (3 x 10 mL) and dried (Na₂SO₄). The solvent was evaporated under reduced pressure to give a white solid. Recrystallization from *i*-PrOH gave 2.5 g (76%) of **69** as white crystals: mp 121-123 °C (lit.²²² mp 121-124 °C); ¹H NMR (CDCl₃) δ 1.09 (d, *J* = 6.7 Hz, 3H, CH₃), 1.92 (s, 3H, CH₃), 2.71 (dd, *J* = 13.5, 7.1 Hz, 1H, CH₂), 2.82 (dd, *J* = 13.5, 5.7 Hz, 1H, CH₂), 4.22-4.29 (m, 1H, CH), 5.22 (br s, 1H, NH), 7.16 (d, *J* = 6.8 Hz, 2H, ArH), 7.17-7.29 (m, 3H, ArH).

1-Phenyl-2-nitropropene (71). Compound **71** was prepared according to a literature procedure.²¹³ Benzaldehyde (**70**, 2.0 g, 18.8 mmol), nitroethane (1.4 g, 18.8 mmol) and *n*-butylamine (0.1 mL) were added to absolute EtOH (1.9 mL). The solution was heated at reflux for 9 h. On cooling the reaction solution, a heavy, yellow and crystalline mass was formed. Recrystallization from absolute EtOH gave 1.1 g (36%) of **71** as yellow crystals: mp 61-62 °C (lit.¹¹³ mp 65 °C); ¹H NMR (CDCl₃) δ 2.27 (d, *J* = 1.0 Hz, 3H, CH₃), 7.23-7.29 (m, 5H, ArH), 7.90 (s, 1H, CH).

(R)-N-[2-(3,4-Dichlorophenyl)-1-methyl-2-oxoethyl]-2,2,2-trifluoroacetamide (72).

Compound **72** was prepared using a literature procedure for a similar compound.²¹⁹ 3,4-Dichlorobenzene (19.1 g, 129.6 mmol) and AlCl₃ (4.3 g, 32.4 mmol) were added to (R)-2-(N-trifluoroacetyl)aminopropanoyl chloride ((R)**64**, 3.3 g, 16.2 mmol) at room temperature. The reaction mixture was allowed to stir for 18 h and then cooled in an ice-bath and slowly quenched with 1N HCl (80 mL). The aqueous layer was extracted with CH₂Cl₂ (3 x 20 mL) and the organic portions were combined and dried (Na₂SO₄). The solvent was evaporated under reduced pressure to give the crude product as an oil which upon standing for 2 days gave 0.01 g (0.2%) of **72** as a white solid; ¹H-NMR (CDCl₃) δ 1.52 (d, 3H, CH₃), 5.30 (s, 1H, NH), 5.42-5.49 (q, 1H, CH), 7.79 (d, *J* = 2 Hz, 1H, ArH), 7.81 (d, *J* = 2 Hz, 1H, ArH), 8.07 (s, 1H, ArH). Anal. Calcd (C₁₁H₈Cl₂F₃NO₂·0.25H₂O) C, 41.47; H, 2.69; N, 4.40. Found: C, 41.06; H, 2.36; N, 4.27.

1-(3,4-Dichlorophenyl)-2-nitropropene (74). Compound **74** was prepared using a literature procedure for a similar compound.²¹³ 3,4-Dichlorobenzaldehyde (**73**, 5.0 g, 28.6 mmol), nitroethane (2.1 g, 28.6 mmol) and n-butylamine (0.1 mL) were added to absolute EtOH (3 mL). The solution was heated at reflux for 9 h. On cooling the reaction solution, a heavy, yellow and crystalline mass was formed. Recrystallization from absolute EtOH gave 4.0 g (61%) of **74** as yellow crystals: mp 70-72 °C (lit.²³⁷ mp 81 °C): ¹H NMR (CDCl₃) δ 0.07 (s, H, CH), 2.43 (d, 3H, CH₃), 7.96 (s, 1H, ArH), 7.51-7.54 (m, 1H, ArH), 7.24-7.27 (m, 1H, ArH).

B. ELECTROPHYSIOLOGY:

Xenopus laevis oocytes were harvested and prepared using *Xenopus laevis* females.^{228,229} Oocytes from stage V-VI were selected for cRNA injection within 24 hours of isolation. The pOTV vector was used to transcribe cRNA using mMessage Machine T7 kit (Ambion Inc.,

Austin, TX). Each oocyte was injected with 50 nL of 1 $\mu\text{g}/\mu\text{L}$ hDAT cRNA and was incubated in Ringers solution supplemented with Na^+ pyruvate (550 $\mu\text{g}/\text{mL}$), tetracycline (50 $\mu\text{g}/\text{mL}$), and 5% dialyzed horse serum. In all assays, oocytes were held at -60 mV in a two-electrode voltage system and maintained in a bath containing standard recording solution (120 mM NaCl, 5.4 mM K gluconate, 1.2 mM Ca gluconate, 15 mL of 0.5 M HEPES). In all assays measuring EC_{50} , compound's concentrations were varied depending on the response observed. Each concentration point was confirmed by at least three different oocytes measurements. In the recording, dopamine was perfused for 30 sec followed by the drug application which was applied for 1 min. The drug response was always represented as a percent of dopamine response as a normalization measure. Using Clampfit, raw traces were filtered and values for the dopamine and the drug induced responses were obtained for an analysis in Origin 8, $y = V_{\text{max}} * x^n / (k^n + x^n)$. (**Note:** Electrophysiological studies were done by Krasnodara Cameron, a graduate student in Dr. De Felice Laboratory)

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