Investigation of the *l*-Phenylacetylcarbinol Process to Substituted Benzaldehydes of Interest

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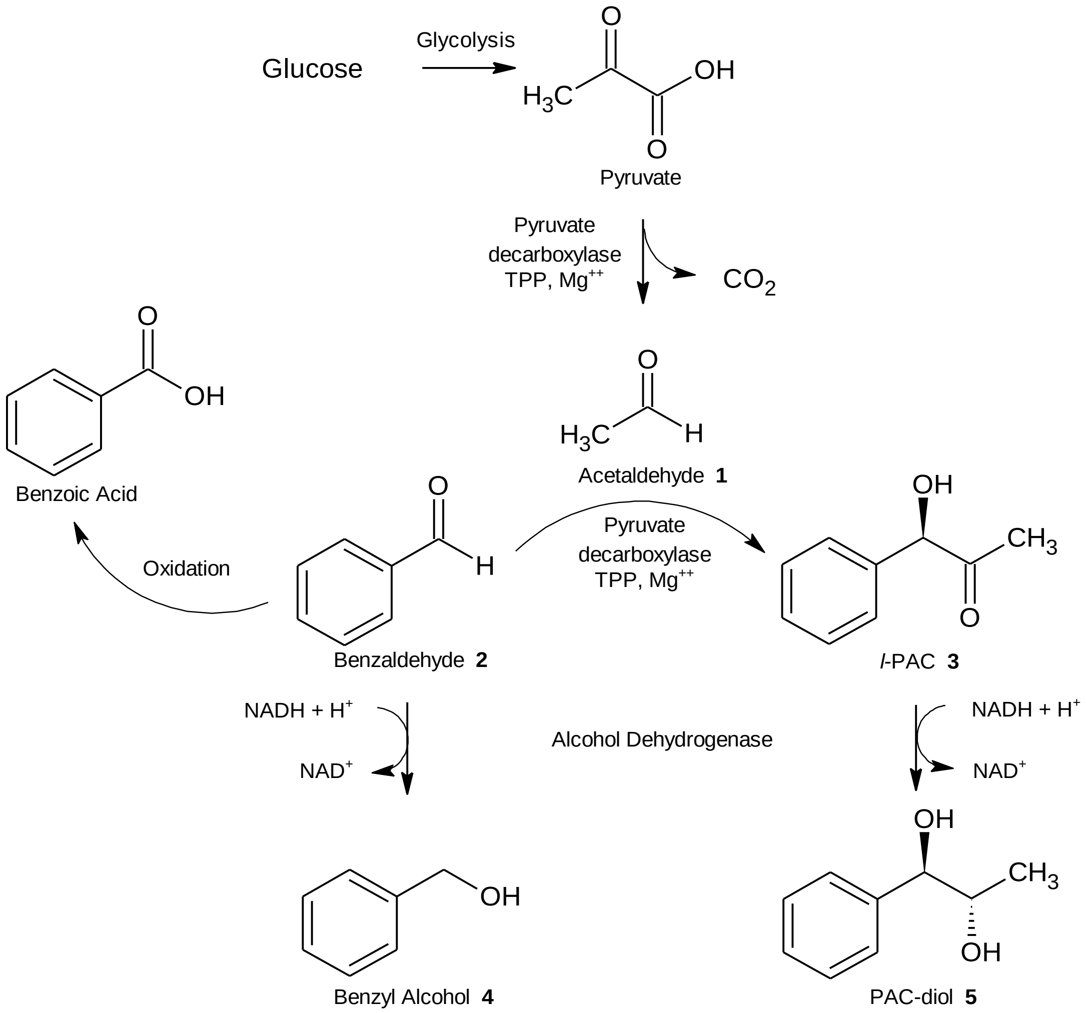
Abstract

The large scale industrial manufacture of the nasal decongestant pseudoephedrine is typically carried out by the reductive amination of *l*-phenylacetylcarbinol *(l*-PAC), which in turn is produced *via* the biotransformation of benzaldehyde using yeast. In recent years there has been increasing legislative control of the supply of pseudoephedrine due to it being diverted for the clandestine production of methylamphetamine and there is some evidence that a number of clandestine drug laboratory chemists have considered the application of the *l*-PAC process to manufacture their own pseudoephedrine. This work examined the use of a number of substituted benzaldehydes for the manufacture of the corresponding substituted *l*-PAC analogue followed by reductive amination to the corresponding substituted pseudoephedrine/ephedrine analogues. These substituted pseudoephedrine/ephedrine analogues were either reduced or oxidised to determine the feasibility of producing the corresponding methylamphetamine or methcathinone analogues. As a result, the *l*-PAC process was identified as a viable route for synthesis of substituted methylamphetamines and methcathinones.

Keywords *l*-PAC, Biotransformation, Methamphetamine, Clandestine, Ephedrine

Introduction

The *l*-Phenylacetylcarbinol (*l*-PAC) process is a biotransformation process using the fungus yeast. The mechanism, which was initially investigated by Neuberg *et al.*[1,2], involves glycolysis of glucose to produce pyruvic acid which is then decarboxylated by pyruvate decarboxylase to produce acetaldehyde. The resulting acetaldehyde then undergoes a condensation reaction with benzaldehyde to produce *l*-PAC [(*R*)-1-hydroxy-1-phenylpropan-2-one]. During this process, by-products are formed due to the action of alcohol dehydrogenase on benzaldehyde and *l*-PAC resulting in benzyl alcohol and (1*R*,2*S*)-1-phenylpropan-1,2-diol (PAC-diol) respectively.[3,4] (Refer to Scheme 1)



Scheme 1: Mechanism of the fermentation of benzaldehyde to produce *l*-PAC and various by-products[3,4]

The *l*-PAC process has previously been employed by the pharmaceutical industry due to the ease with which *l*-PAC can be chemically converted to pseudoephedrine/ephedrine. In recent years, a number of clandestine drug laboratories have been located which have exploited the commercial process for the production of pseudoephedrine/ephedrine for use in the manufacture of methylamphetamine[3].

Global controls over the supply of pseudoephedrine as a potential illicit drug precursor has heightened the possibility that this process will gain increased importance in illicit drug production. This process has dual applicability in that the pseudoephedrine/ephedrine produced by this process can be employed for both methylamphetamine and methcathinone production.

The work presented here aimed to investigate the *l*-PAC process as a viable manufacturing pathway to produce a variety of substituted methylamphetamines and methcathinones and hence identify the potential emerging precursor chemicals for substituted methylamphetamine and methcathinone analogues.

2. Materials and Methods

2.1 Materials

Piperonal (3,4-methylenedioxybenzaldehyde), acetaldehyde, benzaldehyde, hypophosphorous acid, 50% w/w, sodium bicarbonate, sodium carbonate, sodium chloride and trisodium citrate were purchased from Sigma-Aldrich. 4-(Methylthio)benzaldehyde, 4-fluorobenzaldehyde and sodium borohydride were purchased from Alfa Aesar. 4-Methylbenzaldehyde, 4-anisaldehyde (4-methoxybenzaldehyde), Celite 545 (particle size 0.02-0.1 mm) and analytical grade ethanol were purchased from Merck. Citric Acid Monohydrate, ethyl acetate, hydrochloric acid 32% and sodium hydroxide were purchased from Ajax Fine Chemicals. Chloroform, dichloromethane and diethyl ether were purchased from Macron Fine Chemicals, J.T.Baker and BioLab respectively. Iodine, methylamine aqueous solution 40%, sodium dichromate dihydrate and sulphuric acid were purchased from Asia Pacific Specialty Chemicals LTD, Prolabo, Fluka and BDH respectively. Sodium hydrosulfite and sodium sulphate anhydrous granular were purchased from Chem-Supply. All reagents and solvents purchased were of analytical grade and not further purified before use. Dextose (glucose), manufactured by Brigalow Natural Products and Instant Dried, Premium Bakers' Yeast (species *Saccharomyces cerevisiae)*, manufactured by Lowan Whole Foods was purchased from a local supermarket.

2.2 Instrumentation

Gas Chromatography-Mass Spectrometry (GC-MS) analyses were performed on an Agilent HP-6890N Network GC System using an Agilent HP-5MS capillary column (30 m x 0.25 µm) fitted with an Agilent HP-5973 mass selective detector. The carrier gas was helium at a constant flow of 1.0 mL/min and a split ratio of 25:1 with an injection volume of 0.2 µL. The injector temperature was set to 100^{o}C, with an initial oven temperature of 100^{o}C held for 1 min, then ramped at 30^{o}C/min to 280^{o}C and held there for 10 mins. The mass selective detector operated between *m/z*= 40 and 450 in electron impact mode with an ionization energy of 70eV.

Gas Chromatography-Vapour Phase Infrared (GC-IRD) analyses were performed on an Agilent HP-7890A GC System using an Agilent HP-5MS capillary column (30 m x 0.25 µm) fitted with an ASAP IRD II Infrared Detector. The carrier gas was helium at a constant flow of 4.2 mL/min. The injector was set at a pulsed split of 24 psi for 2 mins with a split ratio of 1:1 and an injection volume of 2 µL. The injector temperature was set to 100^{o}C, with an initial oven temperature of 100^{o}C held for 2 mins, then ramped at 20^{o}C/min to 280^{o}C and held there for 8 mins. The temperature of the transfer lines and light pipe were kept at 250^{o}C and the IRD II's scanning range was set to 4000–500 cm^{-1}.

Nuclear Magnetic Resonance (NMR) analysis was carried out using a Varian 400 MHz Unity INOVA spectrometer operating at 400 MHz (^{1}H) and 100 MHz (^{13}C). Samples were run in a mixed solvent system of d^{6}DMSO and $CDCl_3$ at 298 K. ^{1}H spectra were referenced to the d^{6}DMSO solvent residual taken as 2.49 ppm and ^{13}C spectra were referenced to the d^{6}DMSO solvent residual taken at 39.50 ppm at the temperature quoted.

2.4 General Fermentation Procedure

Yeast (44 g) and glucose (310 mmol) were added to a beaker and placed in a water bath heated to 30^{o}C. To this, 500 mL of a 0.1 M citrate buffer (pH 5) consisting of 7.35 g trisodium citrate and 5.25 g citric acid in deionised water was added. The broth was stirred for 40 mins before a substituted benzaldehyde (25 mmol) in 5 mL ethanol and acetaldehyde (30 mmol) was added to the broth. After being left to ferment for approximately 60 mins, the broth was filtered through Celite and extracted with ethyl acetate (3 x 100mL). Emulsions in the extract were broken through the use of a saturated sodium chloride solution, dried over anhydrous sodium sulphate, filtered and evaporated. The crude reaction product was dissolved in chloroform and analysed using GC-MS and GC-IRD. Specific quantities of each reagent and fermentation times can be found in Table 1. Samples were deliberately not purified between each reaction stage to allow reaction specific by-products as well as the product of interest to carry through to the next stage. This would most likely mimic those conditions found in a clandestine drug laboratory where precursor materials from each stage are not purified greatly from one step to the next.

Table 1: Reagents and fermentation times used in each substituted benzaldehyde fermentation

Starting Material		Acetaldehyde (g)	Yeast (g)	Glucose (g)	Filtered After (mins)
Substituted Benzaldehyde	Grams				
Benzaldehyde	3.218 (30 mmol)	2.706 (61 mmol)	44.750	55.308 (307 mmol)	80
3,4-Methylenedioxy-benzaldehyde	3.159 (21 mmol)	1.039 (24 mmol)	44.325	55.821 (310 mmol)	30
4-Methoxybenzaldehyde	3.259 (24 mmol)	1.201 (27 mmol)	44.427	55.845 (310 mmol)	60
4-Fluorobenzaldehyde	3.403 (27 mmol)	1.268 (29 mmol)	44.729	55.431 (308 mmol)	60
4-Methylbenzaldehyde	3.347 (28 mmol)	1.287 (29 mmol)	44.564	55.713 (309 mmol)	50
4-(Methylthio)-benzaldehyde	3.111 (20 mmol)	1.863 (42 mmol)	44.212	55.913 (310 mmol)	40

2.5 Bisulfite Adduct Purification prior to NMR Analysis

Whilst our intent was to only perform minimal purification in order to observe reaction marker by-products at each stage, the corresponding *l*-PAC products were purified prior to NMR analysis by the bisulfite adduct purification technique previously employed by Neuberg and Ohle[5]. Each crude sample was taken up in 10 mL diethyl ether and extracted with saturated sodium carbonate solution (2 x 5 mL) to remove excess benzoic acid. The diethyl ether layer was then washed with deionised water (2 x 5 mL), the aqueous extracts combined and further extracted with diethyl ether (2 x 5 mL). The diethyl ether extracts were then combined and 15 mL of a freshly made saturated sodium hydrosulfite solution was added. The solution was left to react for 60 mins, being shaken every few minutes. Any benzyl alcohol in the sample was then removed through extraction with diethyl ether (3 x 10 mL). To the retained aqueous layer, solid sodium bicarbonate was added until the evolution of carbon dioxide ceased. This aqueous layer was then extracted with diethyl ether (3 x 10 mL), dried over anhydrous sodium sulphate, filtered and evaporated on a rotary evaporator. A sample of each product was dissolved in chloroform and assessed for purity by GC-MS prior to NMR analysis.

2.6 Reductive Amination Procedure

Using quantities as outlined in Table 2, ethanol and methylamine (40% in water) was added to each sample of crude *l*-PAC analogue and left to react at room temperature for 30 mins, to allow imine formation. The solution was cooled in an ice bath and reduced using sodium borohydride for 90 mins. The solution was then basified with a 10% sodium hydroxide solution (approximately 10 mL) and extracted with dichloromethane (3 x 10 mL). The combined extracts were dried over anhydrous sodium sulphate, filtered and evaporated. A sample of each product was dissolved in chloroform and analysed by GC-MS and GC-IRD.

Excess benzyl alcohol that had formed as a by-product was then removed through an acid-base extraction. The sample was acidified using 10 mL of 10% hydrochloric acid, shaken briefly and extracted with dichloromethane (3 x 10 mL) to remove the neutral components. The aqueous layer was then basified with a 10% solution of sodium hydroxide in water and extracted with dichloromethane (3 x 10 mL). The combined extracts were dried over anhydrous sodium sulphate, filtered and evaporated on a rotary evaporator. A sample of each product was collected, dissolved in chloroform and analysed by GC-MS and GC-IRD. Specific quantities of each reagent can be found in Table 2.

Table 2: Reagents used in the reductive amination of each *l*-PAC analogue.

Starting Material		Methylamine (mL)	Ethanol (mL)	Sodium Borohydride (g)
Crude *l*-PAC analogues	Grams (% *l*-PAC Analogue)			
l-PAC	6.672 (21%) (9 mmol)	5.6 (65 mmol)	8.4	3.934 (104 mmol)
3,4-Methylenedioxy-*l*-PAC	15.773 (10%) (8 mmol)	2.5 (29 mmol)	15	2.045 (54 mmol)
4-Methoxy-*l*-PAC	10.769 (19%) (11 mmol)	1.5 (17 mmol)	15	0.900 (24 mmol)
4-Fluoro-*l*-PAC	10.473 (23%) (14 mmol)	2.5 (29 mmol)	15	1.577 (42 mmol)
4-Methyl-*l*-PAC	2.025 (14%) (2 mmol)	2 (23 mmol)	6	1.726 (46 mmol)
4-Methylthio-*l*-PAC	11.526 (14%) (8 mmol)	3 (35 mmol)	20	3.185 (84 mmol)

2.7 Reduction Procedure

To each of the pseudoephedrine/ephedrine analogues a mixture of iodine in hypophosphorous acid was added. Each solution was refluxed for 120 mins and allowed to cool to room temperature. Each solution was basified with a saturated sodium carbonate solution and extracted with chloroform (3 x 10 mL). The combined extracts were dried over anhydrous sodium sulphate, filtered and evaporated on a rotary evaporator. A sample of each product was dissolved in chloroform and analysed by GC-MS and GC-IRD. Specific quantities of each reagent can be found in Table 3.

Table 3: Reagents used in the reduction of each pseudoephedrine/ephedrine analogue.

Starting Material		Iodine (g)	Hypophosphorous Acid (mL)
Crude Pseudoephedrine/ephedrine Analogue	Grams (% Pseudoephedrine/ephedrine Analogue)		
Pseudoephedrine/ephedrine	0.497 (88%) (3 mmol)	0.496 (2 mol)	2 (37 mmol)
3,4-Methylenedioxy-α-[1-(methylamino)ethyl]-benzenemethanol	0.531 (29%) (0.7 mmol)	0.456 (2 mol)	2 (37 mmol)
4-Methoxy-α-[1-(methylamino)ethyl]-benzenemethanol	0.201 (23%) (0.2 mmol)	0.507 (2 mol)	2 (37 mmol)
4-Fluoro-α-[1-(methylamino)ethyl]-benzenemethanol	0.83 (62 %) (3 mmol)	0.959 (4 mol)	2 (37 mmol)
4-Methyl-α-[1-(methylamino)ethyl]-benzenemethanol	0.192 (32%) (0.3 mmol)	0.103 (0.4 mmol)	2 (37 mmol)
4-Methylthio-α-[1-(methylamino)ethyl]-benzenemethanol	0.061 (45%) (0.1 mmol)	0.188 (0.7 mmol)	2 (37 mmol)

2.8 Oxidation Procedure

Each of the crude pseudoephedrine/ephedrine analogues was dissolved in 10mL of 10% sulphuric acid and placed in an ice bath. To each solution, a chilled solution of sodium dichromate in 10 mL of 10% sulphuric acid was added drop-wise (approximately 1 drop per 15 secs). The reaction mixture was stirred and left to react in the ice bath. After 180 mins, the reaction mixture was washed with cold chloroform to remove any aldehyde analogues which may have formed. This solution was basified with a saturated sodium carbonate solution (approximately 10 mL) before being extracted with chloroform (3 x 10 mL). The extracts were dried, filtered and evaporated. A sample of each product was collected, dissolved in chloroform and analysed by GC-MS and GC-IRD. Specific quantities of each reagent can be found in Table 4.

Methcathinone has a tendency to form a pyrazine dimer when in the free base form[6]. For stability purposes the analogues were converted to the corresponding hydrochloride salt by the addition of hydrogen chloride gas to each of the substituted methcathinone samples in chilled isopropyl alcohol. Once the pH of the solution had reached pH 3 or lower, it was placed on a heater block and the solvent evaporated under a stream of nitrogen.

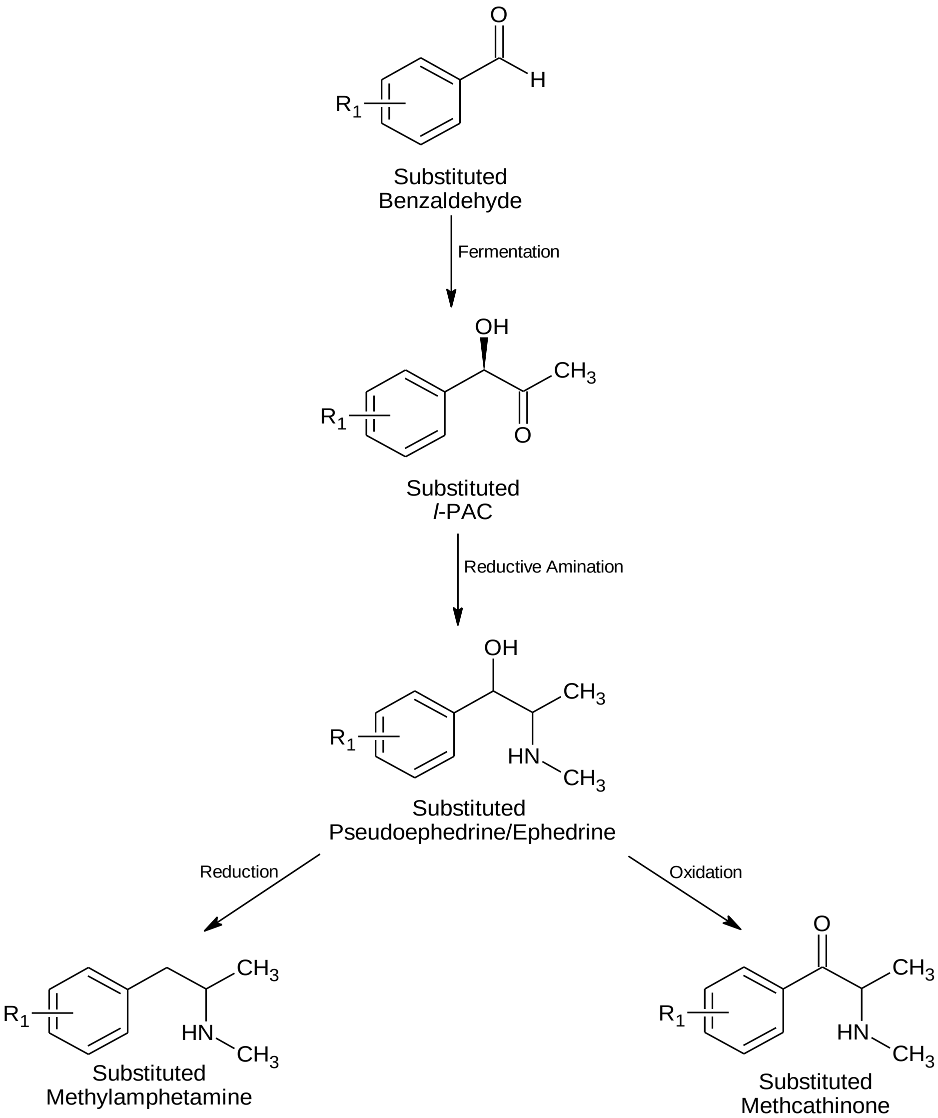
Table 4: Reagents used in the oxidation of each pseudoephedrine/ephedrine analogue.

Starting Material		Sodium Dichromate (g)
Crude Pseudoephedrine/ ephedrine Analogue	Grams (% Pseudoephedrine/ ephedrine Analogue)	
Pseudoephedrine/ephedrine	0.450 (88%) (2 mmol)	0.613 (2 mmol)
3,4-Methylenedioxy-α-[1-(methylamino)ethyl]-benzenemethanol	0.843 (29%) (1 mmol)	1.032 (3 mmol)
4-Methoxy-α-[1-(methylamino)ethyl]-benzenemethanol	0.257 (23%) (0.3 mmol)	0.673 (2 mmol)
4-Fluoro-α-[1-(methylamino)ethyl]-benzenemethanol	0.167 (55%) (0.5 mmol)	0.526 (2 mmol)
4-Methyl-α-[1-(methylamino)ethyl]-benzenemethanol	0.220 (16%) (0.2 mmol)	0.297 (1 mmol)
4-Methylthio-α-[1-(methylamino)ethyl]-benzenemethanol	0.106 (45%) (0.2 mmol)	0.516 (2 mmol)

3.0 Results and Discussion

The synthetic pathway employed to obtain the respective analogues is outlined in Scheme 2. Products formed during fermentation, reductive amination, reduction and oxidation of the various precursors are outlined in Tables 3, 4, 5, and 6 respectively.

Substituted Benzaldehydes of Interest	**R_1**
Benzaldehyde	4-H
3,4-Methylenedioxybenzaldehyde	3,4-methylenedioxy
4-Methoxybenzaldehyde	4-methoxy
4-Fluorobenzaldehyde	4-F
4-Methylbenzaldehyde	4- CH_3
4-(Methylthio)benzaldehyde	4-CH_3S



Scheme 2: The substituted benzaldehydes of interest investigated and the pathway followed.

3.1 Fermentation

Initially, a number of small scale fermentations were conducted to examine the *l*-PAC process parameters using benzaldehyde. The parameters of buffer, buffer volume, temperature and acetaldehyde addition were examined. It was found that a pH 5 0.1 M citrate buffer made from dissolving trisodium citrate and citric acid in deionised water gave a higher yield of *l*-PAC than when a deionised water buffer was employed. The buffer volumes were then altered to produce a benzaldehyde concentration of 12 g/L and 6 g/L with the 6 g/L fermentation broth found to produce the highest *l*-PAC yield. It was also found that heating the fermentation broth to 30^{o}C in a water bath as opposed to being left at room temperature contributed to an increased *l*-PAC yield. Acetaldehyde addition at various ratios of 1:1 benzaldehyde to acetaldehyde and 1:2 benzaldehyde to acetaldehyde also produced an increased *l*-PAC yield with each substituted benzaldehyde's preferred ratio noted.

Using the devised fermentation procedure each of the substituted benzaldehydes of interest underwent fermentation. The results of the fermentations are listed in Table 5. Note: Calculated percentage yields (calc'd % yield) were found by comparing the theoretical yield of each compound with the compound's actual yield (calculated using the crude product weight and the relative (area %) of the compound, reported during GC-MS analysis).

Table 5: The *l*-PAC analogues formed following fermentation of the corresponding substituted benzaldehyde.

Substituted Benzaldehyde Fermented	*l*-PAC Analogue Produced	R_1	Calc'd % Yield	MS	IR
Benzaldehyde	*l*-PAC	4-H	41.5%	150, 107, 79, 77, 51, 43	2866 (vC-H al), 1365 (δC-H al), 3035 (vC-H ar), 3074 (vC-H ar), 1730 (vC=O), 3442 (2vC=O), 3508 (vO-H), 1176 (vC-OH), 3651 (vOH:O=C)
3,4-Methylenedioxy	1-Hydroxy-1-[3,4-(methylenedioxy)phenyl]-2-propanone*	3,4-CH_2OCH_2	12.8%	194, 151, 123, 93, 65, 43	2877 (vC-H al), 2918 (vC-H al), 2981 (vC-H al), 1362 (δC-H al), 3018 (vC-H ar), 3074 (vC-H ar), 1730 (vC=O), 3426 (2vC=O), 3506 (vO-H), 1174 (vC-OH), 3654 (vOH:O=C), 1049 (vC-O-C of methylenedioxy group), 2777 (vC-H of methylenedioxy group)
4-Methoxy	1-Hydroxy-1-(4-methoxyphenyl)-2-propanone*	4-OCH_3	20.4%	180, 137, 109, 94, 77, 43	2949 (vC-H al), 1363 (δC-H al), 3008 (vC-H ar), 3074 (vC-H ar), 1728 (vC=O), 3437 (2vC=O), 3508 (vO-H), 3653 (vOH:O=C), 1174 (vC-OH), 1252 (vAryl-O), 1041 (vO-CH_3), 2920 (vC-H of OCH_3), 2846 (2δC-H of OCH_3)
4-Fluoro	1-(4-Fluorophenyl)-1-hydroxy-2-propanone	4-F	29.2%	168, 125, 97, 95, 77, 43	2868 (vC-H al), 2927 (vC-H al), 1365 (δC-H al), 3014 (vC-H ar), 3047 (vC-H ar), 1730 (vC=O), 3446 (2vC=O), 3505 (vO-H), 3651 (vOH:O=C), 1161 (vC-OH), 1178 (vC-F)
4-Methyl	1-Hydroxy-1-(4-methylphenyl)-2-propanone	4- CH_3	21.3%	164, 121, 93, 91, 77, 65, 43	2872 (vC-H al), 2933 (vC-H al), 1363 (δC-H al), 3028 (vC-H ar), 1730 (vC=O), 3446 (2vC=O), 3508 (vO-H), 3651 (vOH:O=C), 1176 (vC-OH)
4-Methylthio	1-Hydroxy-1-(4-(methylthio)-phenyl)-2-propanone	4-SCH_3	29.8%	196, 153, 151, 109, 77, 43	2933 (vC-H al), 2995(vC-H al), 1365 (δC-H al), 3026 (vC-H ar), 3080 (vC-H ar), 1730 (vC=O), 3442 (2vC=O), 3506 (vO-H), 3651 (vOH:O=C), 1176 (vC-OH), 2866 (vS-CH_3), 1325 (δS-CH_3)

* Structure(s) assigned by GC-MS

The fermentation product compound assignments were confirmed through ^{1}H and ^{13}C NMR analysis:

3.1.2. 4-Fluoro Analogue of *l*-PAC

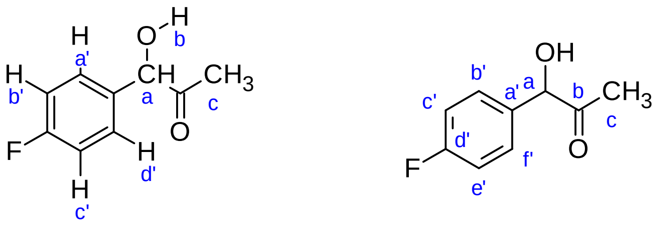


Figure 1: The protons (left) and the carbons (right) of the 4-fluoro analogue of *l*-PAC.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.38-7.28 (m, 2H, Ha', Hd'), 7.05-6.91 (m, 2H, Hb', Hc'), 5.63 (d, J= 4.3 Hz, 1H, Hb), 4.99 (d, J= 4.2 Hz, 1H, Ha), 3.19 (s, H_2O), 2.00 (s, 3H, Hc);

^{13}C NMR (100 MHz, DMSO-d_6) δ 207.88 (Cb), 161.87 (d, J= 245.6, Cd'), 134.81 (Ca'), 128.30 (Cb', Cf'), 115.06 (Cc', Ce'), 78.43 (Ca), 24.7 (Cc) (refer to Figure 1 for proton and carbon labels).

3.1.3. 4-Methyl Analogue of *l*-PAC

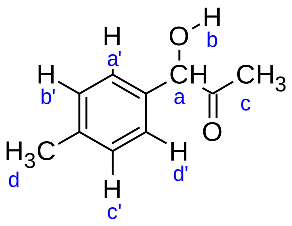


Figure 2: The protons of the 4-methyl analogue of l-PAC.

To confirm the production of 4-methyl-PAC **22**, the sample which was purified via the bisulfite adduct method was analysed by ^{1}H NMR; ^{1}H NMR (400 MHz, DMSO-d_6) δ 7.20-7.11 (m, 2H, Ha', Hd'), 7.11-6.99 (m, 2H, Hb', Hc'), 5.14 (d, J= 4.3 Hz, 1H, Hb), 4.95 (d, J= 4.2 Hz, 1H, Ha), 2.25 (s, 3H, Hd), 1.98 (s, 3H, Hc) (refer to Figure 2 for proton labels). A gCOSY (gradient correlation spectroscopy) spectrum was also obtained to confirm the assignment of each proton signal.

3.1.4. 4-Methylthio Analogue of *l*-PAC

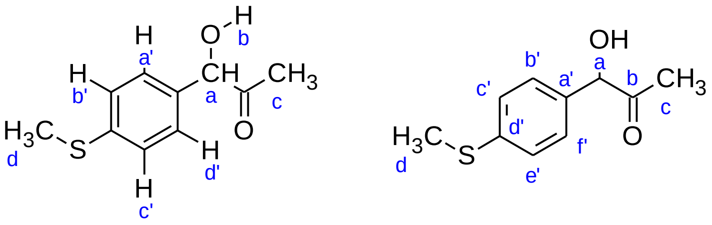


Figure 3: The protons (left) and the carbons (right) of the 4-methylthio analogue of *l*-PAC.

^{1}H NMR (400 MHz, DMSO-d_6) δ 7.24-7.20 (m, 2H, Ha', Hd'), 7.18-7.08 (m, 2H, Hb', Hc'), 5.28 (d, J= 4.2, 1H, Hb), 4.95 (d, J= 4.0 Hz, 1H, Ha), 2.39 (s, 3H, Hd), 1.99 (s, 3H, Hc);
^{13}C NMR (100 MHz, DMSO-d_6) δ 138.02 (Cd'), 134.94 (Ca'), 126.83 (Cb', Cf'), 125.84 (Cc', Ce'), 78.93 (Ca), 24.58 (Cc), 14.91 (Cd) (refer to Figure 3 for proton and carbon labels).

An additional peak at δ 207 was assigned to the carbon of the aldehyde group of 4-(methylthio)benzaldehyde but was suspected to also be masking Cb of the 4-methylthio analogue of *l*-PAC. This assignment was based upon the chemical shifts that Cb was found at in the previous ^{13}C NMR's of *l*-PAC and the 4-fluoro analogue of *l*-PAC. Additional peaks were also encountered in the ^{1}H NMR at δ 9.82, 7.69, 7.26 and 2.46 and the ^{13}C NMR at δ 132.25, 129.37, 147.31, 124.57 and 13.98 which were attributed to remaining 4-(methylthio)benzaldehyde in the sample.

A NOESY spectrum provided the through-space proton interactions of the sample and was also employed to confirm the assignment of each proton signal. This therefore enabled confirmation of the successful production of the 4-methylthio analogue of *l*-PAC from the biotransformation of 4-(methylthio)benzaldehyde.

The bisulfite adduct purification technique employed for the purification of the fermentation products was accompanied by a number of problems such as loss of large amounts of starting material and carryover of substituted benzaldehyde. As a result, samples pure enough for NMR analysis could not be obtained for the 3,4-methylenedioxybenzaldehyde and 4-methoxybenzaldehyde fermentation products.

The assignment of these compounds was therefore made using GC-MS and GC-IRD analysis.

Throughout the fermentations, full conversion of the substituted benzaldehydes to the corresponding *l*-PAC analogue proved difficult due to competing side reactions. Unlike benzaldehyde, the substituted benzaldehydes all produced fermentation products with anywhere between 5-30% (GC-MS relative yield) substituted benzaldehyde.

Shin and Rogers[7] had previously reported that a 60-70% conversion of benzaldehyde to *l*-PAC could be obtained when using a sophisticated fermentation set-up employing partially purified pyruvate decarboxylase and immobilized yeast cells. Using the basic fermentation method employed during this project, which was designed to mimic a clandestine process, the fermentation of benzaldehyde, 4-methoxybenzaldehyde, 4-fluorobenzaldehyde, 4-methylbenzaldehyde, 4-(methylthio)benzaldehyde appeared to be reasonable yielding reactions with conversion rates of 42%, 20%, 29%, 21% and 30% respectively. Conversely, the fermentation of 3,4-methylenedioxybenzaldehyde was only a low yielding reaction (13%), attributed to an increased sensitivity of the yeast to 3,4-methylenedioxybenzaldehyde. However, the activity of alcohol dehydrogenase, demonstrated by the production of 3,4-methylenedioxybenzyl alcohol implies that the yeast was not killed during the fermentations. This would indicate that rather than poisoning the yeast, 3,4-methylenedioxybenzaldehyde could not be utilized as efficiently as the other substituted benzaldehydes.

3.2. Reductive Amination

The results of the reductive amination of each of the crude *l*-PAC analogue samples appear in Table 6.

Table 6: The pseudoephedrine/ephedrine analogues formed following reductive amination of the corresponding *l*-PAC analogue.

l-PAC Analogue Reductively Aminated	Pseudoephedrine/ ephedrine Analogue Produced	R_1	Calc'd % Yield	MS	IR
l-PAC	Pseudoephedrine/ ephedrine	4-H	53.7%	164, 105, 77, 58, 42	2884 (vC-H al), 2974 (vC-H al), 1381 (δC-H al), 3033 (vC-H ar), 3070 (vC-H ar), 1196 (vC-N), 3649 (vO-H), 3498 (vOH:NH), 1068 (vC-OH)
3,4-Methylenedioxy	α-[1-(Methylamino)ethyl]-1,3-benzo-dioxole-5-methanol	3,4-CH_2OCH_2	17.5%	209, 149, 121, 93, 58, 42	2879 (vC-H al), 2972 (vC-H al), 1381 (δC-H al), 3018 (vC-H ar), 3078 (vC-H ar), 1188 (vC-N), 3649 (vO-H), 3498 (vOH:NH), 1093 (vC-OH), 1049 (vC-O-C of MD), 2777 (vC-H of methylenedioxy group)
4-Methoxy	4-Methoxy-α-[1-(methylamino)ethyl]-benzenemethanol*	4-OCH_3	4.5%	195, 137, 109, 107, 77, 58, 51	2804 (vC-H al), 2964 (vC-H al), 1379 (δC-H al), 3005 (vC-H ar), 3072 (vC-H ar), 1173 (vC-N), 3649 (vO-H), 3502 (vOH:NH), 1041 (vC-OH), 1248 (vAryl-O), 1041 (vO-CH_3), 2914 (vC-H of OCH_3), 2846 (2δC-H of OCH_3)
4-Fluoro	4-Fluoro-α-[1-(methylamino)ethyl]-benzenemethanol*	4-F	53.2%	184, 164, 125, 123, 97, 95, 77, 58, 51	2893 (vC-H al), 2978 (vC-H al), 1383 (δC-H al), 3043 (vC-H ar), 3078 (vC-H ar), 3651 (vO-H), 3496 (vOH:NH), 1080 (vC-OH), 1122 (vC-N), 1157 (vC-F)
4-Methyl	4-Methyl-α-[1-(methylamino)ethyl]-benzenemethanol*	4- CH_3	60.3%	180, 162, 146, 121, 119, 93, 91, 77, 65, 58, 51	2879 (vC-H), 2935 (vC-H), 1381 (δC-H al), 2974 (vC-H ar), 3024 (vC-H ar), 3649 (vO-H), 1178 (vC-N), 3500 (vOH:NH), 1078 (vC-OH), 2803 (vC-H of Aryl-CH_3)

4-Methylthio	4-Met-ylthio-α-[1-(methylamino)ethyl]-benzenemethanol*	4-SCH_3	10.8%	211, 194, 178, 153, 151, 131, 125, 123, 109, 77, 58, 51	2933 (vC-H al), 2976 (vC-H al), 1383 (δC-H al), 3024 (vC-H ar), 3078 (vC-H ar), 1176 (vC-N), 3649 (vO-H), 1092 (vC-OH), 3496 (vOH:NH), 2885 (vS-CH_3), 1317 (δS-CH_3), 2804 (vC-H of SCH_3)
* Structure(s) assigned by GC-MS					

In each reductive amination where the crude *l*-PAC analogue used contained substituted benzaldehyde, the corresponding *N*-methyl-benzylamine analogue was produced, formed through the amination of residual benzyl alcohol. The presence of *N*-methyl-benzylamine analogues can be used as an indicative marker that the product was synthesised from an initial substituted benzaldehyde fermentation followed by reductive amination.

According to the literature[8] the reductive amination of *l*-PAC should produce a conversion of 79% *l*-PAC to pseudoephedrine/ephedrine. Therefore, the reductive amination of *l*-PAC and the 4-fluoro and 4-methyl analogues of *l*-PAC appeared to be reasonable yielding reactions with conversion rates of 54%, 53% and 60% respectively. Conversely, the reductive amination of the 3,4-methylenedioxy, 4-methoxy and 4-methylthio analogues of *l*-PAC were low yielding reactions (17%, 4.5% and 11% respectively).

3.3. Hypophosphorous Acid Reduction

The results of the hypophosphorous acid reduction of each of the crude pseudoephedrine/ephedrine analogue samples appear in Table 7.

Table 7: The methylamphetamine analogues formed following reduction of the corresponding pseudoephedrine/ephedrine analogue.

Pseudoephedrine/ ephedrine analogue reduced	Methylamphetamine Analogue Produced	R_1	Calc'd % Yield	MS	IR
Pseudoephedrine/ephedrine	Methylamphetamine	4-H	14.9%**	148, 134, 115, 91, 77, 58	2860 (vC-H al), 2933 (vC-H al), 2970 (vC-H al), 1377 (δC-H al), 3032 (vC-H ar), 3070 (vC-H ar), 1151 (vC-N)
3,4-Methylenedioxy	N,α-Dimethyl-1,3-benzodioxole-5-ethanamine*	3,4-CH_2OCH_2	1.4%	178, 160, 135, 105, 77, 58	Insufficient quantity
4-Methoxy	4-Methoxy-*N*,α-dimethylbenzeneethanamine*	4-OCH_3	4.8%	178, 148, 121, 103, 78, 58	Insufficient quantity
4-Fluoro	4-Fluoro-*N*,α-dimethyl-benzeneethanamine	4-F	57.7%	166, 152, 133, 109, 83, 58	2800 (vC-H al), 2860 (vC-H al), 2935 (vC-H al), 2971 (vC-H al), 1377 (δC-H al), 3043 (vC-H ar), 3074 (vC-H ar), 1155 (vC-F), vC-N masked by vC-F
4-Methyl	N,α,4-Trimethyl-benzeneethanamine	4-CH_3	33.3%	162, 148, 105, 77, 58	2881 (vC-H al), 2931 (vC-H al), 2972 (vC-H al), 1375 (δC-H al), 3022 (vC-H ar), 3049 (vC-H ar), 1149 (vC-N), 2798 (vC-H of Aryl-CH_3)
4-Methylthio	N,α-Dimethyl-4-(methylthio)-benzeneethanamine*	4-SCH_3	32.0%	194, 180, 164, 137, 121, 91, 77, 63, 58, 51	2931 (vC-H al), 2972 (vC-H al), 1377 (δC-H al), 3024 (vC-H ar), 3074 (vC-H ar), 1149 (vC-N), 2858 (vS-CH_3), 1342 (δS-CH_3), 2800 (vC-H of SCH_3)

* Structure(s) assigned by GC-MS

** Reaction used as a control and stopped before completion

Production of the 4-fluoro analogue of methylamphetamine was confirmed through ^{1}H and ^{13}C NMR analysis;

Figure 4: The protons (left) and the carbons (right) of the 4-fluoro analogue of methylamphetamine.

^{1}H NMR (400 MHz, DMSO-d_6) δ 3.14-3.05 (m, 1H, Hb), 3.03-2.55 (m, 2H, Ha), 2.48 (s, 3H, He), 1.07 (d, J= 6.3 Hz, 3H, Hc); ^{13}C NMR (100 MHz, DMSO-d_6) δ 162.76-159.75 (Cd'), 132.72 (Ca'), 130.39 (Cb', Cf'), 114.86 (Cc', Ce'), 55.69 (Cb), 31.13 (Cd), 16.18 (Cc) (refer to Figure 4 for proton and carbon labels).

A proton signal for proton d was not observed in the ^{1}H NMR spectrum. It is proposed that this signal would have been present at approximately 9-10 ppm, however, this cannot be confirmed as this region was not viewable in the ^{1}H NMR spectrum.

The shifts assigned to the aromatic proton signals would also have been contributed to by the aromatic protons of *N*-methyl-4-fluorobenzylamine. It was extremely difficult to separate the signals of these two compounds due to their overlapping nature. Therefore δ in the region of 7.37-6.83 were attributed to the aromatic H's of both 4-fluoromethamphetamine and *N*-methyl-4-fluorobenzylamine.

3.4. Acid Dichromate Oxidation

The results of the acid dichromate oxidation of each of the crude pseudoephedrine/ephedrine analogue samples appear in Table 8.

Table 8: The methcathinone analogues formed following oxidation of the corresponding pseudoephedrine/ephedrine analogue.

Pseudoephedrine/ ephedrine analogue reduced	Methcathinone Analogue Produced	R$_1$	Calc'd % Yield	MS	IR
Pseudoephedrine/ ephedrine	Methcathinone	4-H	5.4%**	163, 133, 105, 77, 58	2806 (vC-H al), 2898 (vC-H al), 2947 (vC-H al), 1375 (δC-H al), 2983 (vC-H ar), 3037 (vC-H ar), 3072 (vC-H ar), 1180 (vC-N), 1699 (vC=O)
3,4-Methylenedioxy	2-Methylamino-1-(3,4-methylenedioxyphenyl)-propan-1-one*	3,4-CH$_2$OCH$_2$	0.8%	207, 176, 149, 121, 91, 58	Insufficient quantity
4-Methoxy	1-(4-Methoxyphenyl)-2-(methylamino)-1-propanone	4-OCH$_3$	93.1%	135, 107, 92, 77, 64, 58	2806 (vC-H al), 2976 (vC-H al), 1373 (δC-H al), 3010 (vC-H ar), 3078 (vC-H ar), 1171 (vC-N), 1693 (vC=O), 3371 (2vC=O), 1255 (vAryl-O), 1039 (vO-CH$_3$), 2945 (vC-H of OCH$_3$), 2850 (2δC-H of OCH$_3$)
4-Fluoro	4-Fluoro-*N*,α-dimethyl-benzeneethanamine	4-F	80.2%	166, 146, 123, 95, 58	2881 (vC-H al), 2947 (vC-H al), 1265 (δC-H al), 2960 (vC-H ar), 2979 (vC-H ar), 1138 (vC-N), 1699 (vC=O), 1155 (vC-F)
4-Methyl	2-(Methylamino)-1-(4-methylphenyl)-1-propanone	4- CH$_3$	88.3%	162, 143, 119, 91, 58	2885 (vC-H al), 2937 (vC-H al), 1375 (δC-H al), 2978 (vC-H ar), 3033 (vC-H ar), 1178 (vC-N), 1697 (vC=O), 2806 (vC-H of Aryl-CH$_3$)
4-Methylthio	2-(Methylamino)-1-[4-(methylthio)phenyl]-1-propanone	4-SCH$_3$	-	-	-

* Structure(s) assigned by GC-MS

** Reaction used as a control and stopped before completion

Production of the 4-fluoro analogue of methcathinone was confirmed through ^{1}H and ^{13}C NMR analysis:

Figure 5: The protons (left) and the carbons (right) of the 4-fluoro analogue of methcathinone

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.69 (s, 1H, Hc), 8.07-7.97 (m, 2H, Ha', Hd'), 7.27-7.14 (m, 2H, Hb', Hc'), 5.05 (dt, J= 7.3, 5.9 Hz, 1H, Ha), 2.57 (q, J= 4.6, 3.9 Hz, 3H, Hd), 1.47 (d, J= 7.2 Hz, 3H, Hb);
^{13}C NMR (100 MHz, DMSO-d_6) δ 194.08 (Ca), 167.08 (Cd'), 131.43 (Cb', Cf'), 129.06 (Ca'), 115.89 (Cc', Ce'), 58.18 (Cb), 30.64 (Cd), 15.38 (Cc) (refer to Figure 5 for proton and carbon labels). A number of additional small peaks were also observed in the ^{1}H NMR and were attributed to impurities such as *N*-methyl-4-fluorobenzylamine.

Production of the 4-methyl analogue of methcathinone was confirmed through ^{1}H NMR analysis;

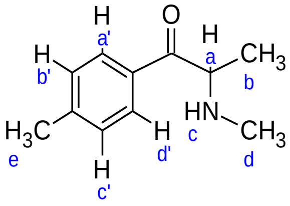


Figure 6: The protons of the 4-methyl analogue of methcathinone.

^{1}H NMR (400 MHz, DMSO-d_6) δ 9.32-9.22 (m, 1H, Hc), 7.79 (d, J= 8.2 Hz, 2H, either Ha' and Hd' or Hb' and Hc'), 7.22 (d, J= 8.0 Hz, 2H, either Ha' and Hd' or Hb' and Hc'), 4.99 (q, J= 6.5 Hz, 1H, Ha), 2.59 (q, J= 6.4, 5.8 Hz, 3H, Hd), 2.31 (s, 3H, He), 1.49 (d, J= 7.0 Hz, 3H, Hb) (refer to Figure 6 for proton labels).

Shifts at 6.80, 3.81 and 1.01, and 9.45, 7.34, 7.07, 3.95, 2.46 and 2.21 were also observed in the ^{1}H NMR, attributed to isopropyl alcohol and *N*-methyl-4-methylbenzylamine.

A United Nations Office on Drugs and Crime publication, *Amphetamines and Ecstasy: 2011 Global ATS Assessment*[9], reported that the hypophosphorous acid reduction of pseudoephedrine/ephedrine generally produces a percentage yield of 76% methylamphetamine whilst the oxidation of pseudoephedrine/ephedrine employing potassium permanganate generally produces a percentage yield of 50% methcathinone. In comparison, the reduction and oxidation of the 4-fluoro and 4-methyl analogues of pseudoephedrine/ephedrine appeared to be reasonable yielding reactions with conversion rates of 33% and higher. On the other hand, the reduction and oxidation of the 3,4-methylenedioxy analogue were poor yielding reactions with conversion rates of just 1% for each. In the case of the 4-methoxy pseudoephedrine/ephedrine analogue it was found to produce a reasonable yield of the 4-methoxy methcathinone analogue (93%) but was not suited for the reduction reaction (5%). Conversely, the 4-methylthio analogue produced a reasonable yield of the 4-methylthio methamphetamine analogue (32%) but the oxidation of which appeared to be unsuccessful.

It was anticipated that difficulties would be had during the reduction of the 3,4-methylenedioxy and 4-methoxy analogues of pseudoephedrine/ephedrine due to the tendency of hydriodic acid to cleave ethers and less commonly thioethers. An absence of the expected hydroxyl substituted methylamphetamine products was attributed due to deprotonation during basification and subsequent loss during extraction.

4.0. Conclusion

The fermentation of the substituted benzaldehydes was found to be a viable route for the production of the corresponding *l*-PAC analogues, except in the case of 3,4-methylenedioxybenzaldehyde. Reductive amination of these *l*-PAC analogues was able to generate viable amounts of the 4-fluoro and 4-methyl analogues of pseudoephedrine/ephedrine but was unsuccessful at producing useable quantities of the 3,4-methylenedioxy, 4-methoxy and 4-methylthio analogues.

Following reduction of the pseudoephedrine/ephedrine analogues, the hypophosphorous acid method was deemed practical for the manufacture of the 4-fluoro, 4-methyl and 4-methylthio analogues of methylamphetamine and unsuited for the reduction of the 3,4-methylenedioxy and 4-methoxy analogues. When oxidised, the acid dichromate method was capable of producing viable quantities of each of the corresponding methcathinone analogues apart from the 4-methylthio analogue.

In conclusion, this manufacturing pathway was found to be a viable route for the synthesis of the 4-fluoro and 4-methyl analogues of methylamphetamine and methcathinone. Whilst a reasonable yield of the 4-methylthio analogue of methylamphetamine could be produced following reduction of the corresponding pseudoephedrine/ephedrine analogue, synthesis of the pseudoephedrine/ephedrine analogue was difficult. Therefore, this manufacturing pathway was deemed inadequate for the production of the 4-methylthio analogue of methylamphetamine and methcathinone as well as for the manufacture of the 3,4-methylenedioxy and 4-methoxy analogues of methylamphetamine and methcathinone.

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