5-chloro-[1,2,4]triazolo[4,3-a]pyrazine series 4 of Open Source Malaria synthesis, purification and characterisation

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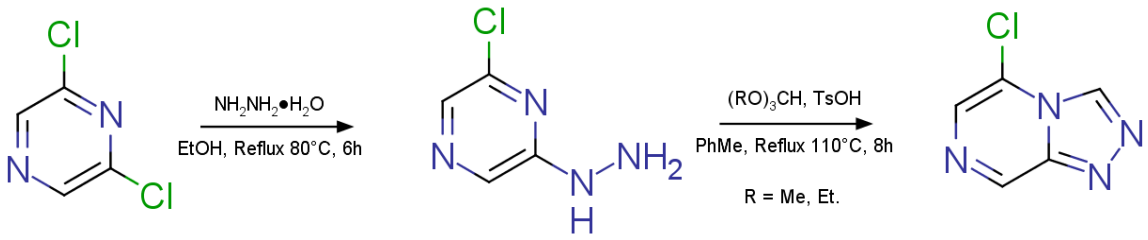
1.0 Abstract:

Recent efforts for combating malaria via drug discovery have increased within the last seven years and are focusing on various stages of the disease one being the parasite. An open-source science approach through Open Source Malaria (OSM) has been working towards antimalarial drug discovery allowing members throughout university around the world to share information and research increasing the productivity in antimalarials. The efforts of this group are focused around a series of triazolopyrazines which require the precursor 3-bromo-5-chloro-[1,2,4]triazolo[4,3-a]pyrazine synthesised from 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine. The current method is inefficient with a final yield of 34.6% (after three steps) and therefore was investigated in this report. Optimisation through divergent synthesis was explored. Purification methods such as liquid/liquid and semi-preparative HPLC was also investigated. Monitoring and characterisation techniques were trialled and development. A new method using trimethyl orthoformate was determined to have a 91.5% purity via GC-FID compared to the previous 4.2% crude product. Methods for characterisation and preparation were explored and resulted in new techniques including GC characterisation and HPLC Semi-preparation purification. These methods will provide members of OSM a useful basis and needed improvements to the OSM efforts in antimalarial research allowing a greater number of options for characterisation and monitoring of this common synthetic steps.



2.0 Introduction

Open Source Malaria is a project started by the University of Sydney students and headed by Mat Todd (University of Sydney). It takes an open source approach to drug design and discovery focusing around the 13 500 compounds released by GlaxoSmithKlein to the public that were found to be active against the malaria parasite. Malaria is still one of the deadliest and widely occurring diseases throughout Africa and has accounted for more than 660, 000 in 2010 according to the WHO.[6] Drug research in Malaria has be directed towards targeting various stages of the disease (Liver, Blood, Transmission and Mosquito). However, this research is still in early development and requires collaborative efforts to become viable and cost effective.[6]

The project encourages anyone to become involved and has common principles in sharing data and findings through research under the OSM banner to the public so that others may benefit.[8] There have been arguments put forward which suggests that open sourced science can improve the productivity of drug discovery.[2] The reduction of duplicated work and overarching scale of new drug research lends itself to a creative commons model which in turn allows shared resources between industry and scientists.[2] The Open Source Malaria project is currently on their forth series of potential lead compounds known as the Triazolopyrazines. The open source research done by OSM is used to further understanding and willingness for malaria drug development despite the attitude of large pharmaceutical companies regrading profitability.[8]

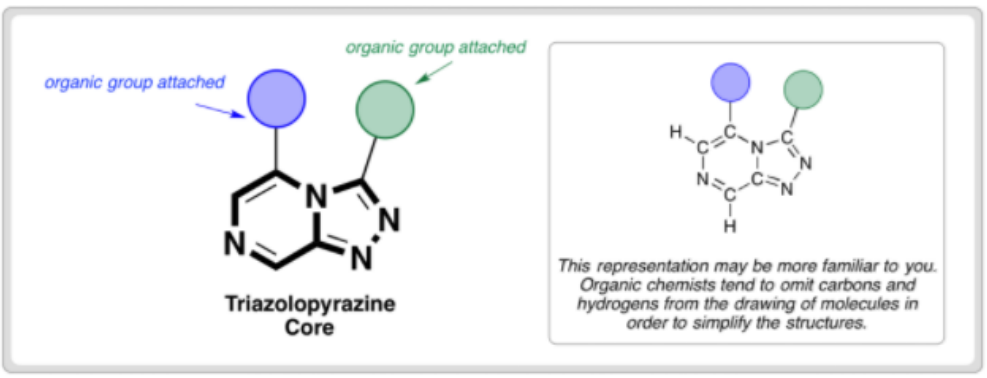


Figure X: The core structure of the triazolopyrazine, sourced: http://malaria.ourexperiment.org/the_osm_blog

The 3-bromo-5-chloro-[1,2,4]triazolo[4,3-a]pyrazine compound is widely used as a precursor to many triazolopyrazine lead drugs working to improve and optimise the characteristics of drug development. However, the current method for synthesis and purification in inefficient with yields approximately 34.6%, in 3 steps from the previous methods, making the process costly. Work in this area has been pioneered by OSM members such as Thomas MacDonald and Jasper Tyler.[4, 5, 8, 10, 11]

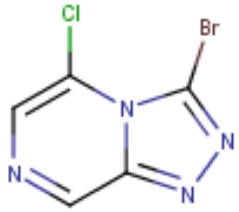


Figure X: 3-bromo-5-chloro-[1,2,4]triazolo[4,3-a]pyrazine

Due to the techniques being used throughout the OSM project focus has been placed on diverse synthesis complex compounds for activity analysis to determine SAR and lead towards a more effective drug applicate.[8] However, the synthetic steps used to produce the precursors for these compounds is largely left alone. Addition of hydrazine is a common protocol in the reprocess of cyclisation and is followed with a orthoformate reagent to complete the formation of the heterocyclic structure. These methods, mainly for the creation of similar triazole groups, are present in synthesis of many other potential drugs. [1, 3, 4, 5, 7, 9, 10, 11] The mechanism of the synthetic steps in this report are shown below in figures X

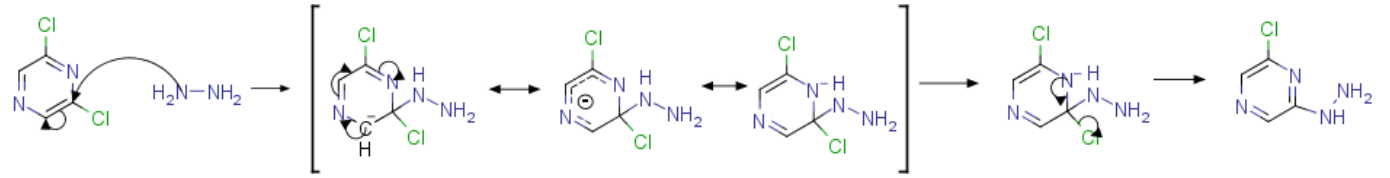


Figure X: Mechanism of reaction, 2-6dicholorpyrazine to 2-chloro-6-hydrazinylpyrazine

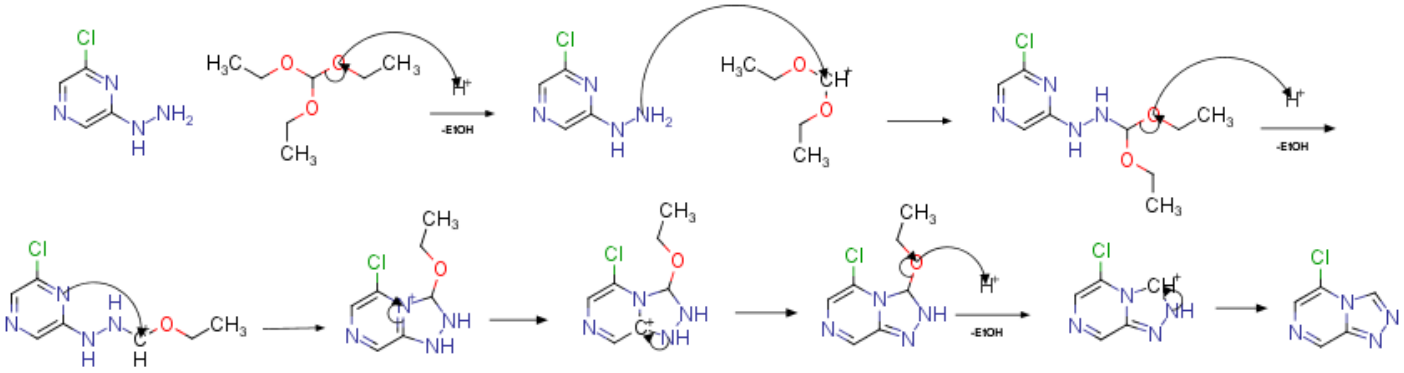


Figure X: : Mechanism of reaction, 2-chloro-6-hydrazinylpyrazine to 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine

The nature of the open source project allows groups and individuals to work within their field and contribute to others, moving closer to a common goal. The investigation of reactions, characterisation techniques and synthetic pathways is required to help understand and improve the effectiveness of research, even more so in an open source setting.[2, 8] The focus of this report is to explore some of the most common and highly used reactions from OSM to gain important insight and information for other research to use as a backbone for further improvement and efficiency of this synthetic method.

3.0 Experimental Methods

All reagents were used as purchased from Sigma Aldrich unless otherwise stated and temperatures recorded from oil bath. Reactions were analysed via gas chromatography on a Shimadzu© GC-2010 Plus using Labsolutions and Rxi-1MS Column 30m, 0.25mmID, 0.25µm df, with either method below (Table X). All liquid chromatography UV/Vis and Semi-preparative work occurred on a Shimadzu© LC-10ADVP using Labsolutions and analytic column Alltech Platinum (C18) 100A, 5µ and Semi-preparative column Supelco Ascentis C18, 15cm, 21.2mm, 5µm, according to the methods below (Table X). Gas Chromatography – Mass Spectrum was obtained using Shimadzu© GC-2010 Plus and Shimadzu© GCMS-TQ8030 with Agilent GC Column 122-5532UI 30m, 0.25mm ID and 0.25 µm df. Mass Spectrum data was acquired via WATERS Micromass ZQ using MassLynx and MS-TOF Perkin Elmer AxION 2 TOF using TOF MS Driver. NMR data was acquired on either 400MHz XXXXX or XXXMhz, XXXXX with d^6-dmso acquired from Cambridge Isotope Laboratories Inc. Full Spectrum UV/Vis data was obtained via Agilent 8453.

Table X GC Methods:

	Method 1	Method 2
Injection Volume	1.0µL	1.0µL
Injection Temperature	230.0C	230.0C
Split	25:1	25:1
Column Flow	0.93mL/min	0.93mL/min
Initial Temperature	80.0C for 2 mins	100.0C for 2 mins
Final Temperature	230.0C for 4 mins	230.0C for 4 mins
FID Temperature	250.0 C	250.0C
Run Time	12.00 min	11.20 min

Table X LC Methods:

	Analytical Method	Semi-Prep Method
Injection volume	10µL	2mL
Total Flow	1.0mL/min	8.0mL/min
Detector wavelengths	210nm, 254nm	210nm, 254nm
Initial MeOH Composition	35%	30% until 2 mins
Isocratic Region		33% for 6 mins (finishing at 8 mins)
Final MeOH Composition	90% for 10 mins	90% for 7 min
Run Time	50 min	20 min

Table X: GC/MS Method:

	Method
Injection Volume	1.0µL
Injection Temperature	230.0C
Split	5:1
Column Flow	0.93mL/min
Initial Temperature	100.0C for 2 mins
Final Temperature	230.0C for 4 mins
FID Temperature	250.0 C
Run Time	12.00 min
MS Scanning region	43 to 600 m/z

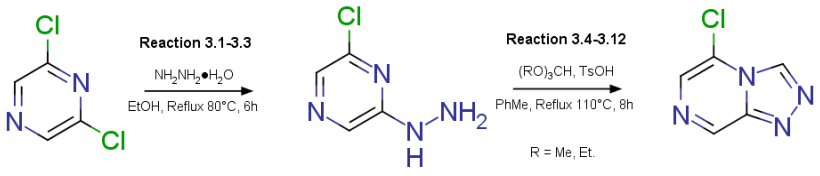


Figure X: Reaction Scheme of 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine

3.1-3.3 2,6-dichloropyrazine (1) (1eq) was dissolved in ethanol (20-50ml) and hydrazine hydrate (2eq) was added. The solution was heated and stirred to reflux at 80℃ for 6 hours (5.16-6h). The solvent was removed via rotatory evaporation and was then redissolved in ethyl acetate (40-90ml). Supersaturated brine solution (30-90ml) was used to wash the mixture (3 times) and the organic layer was kept. Ethyl Acetate was removed via reduced pressure and to produce a yellow solid, which was flaky in nature. The above method occurred 3 times with reference to table X. TLC was used to monitor the reactions and determine completion via loss of starting material. The spectroscopic data matched that of previously reported.[10] ^{1}H NMR (400 MHz, dmso) δ 8.44 (s, 1H, H7), 8.05 (s, 1H, H5), 7.70 (s, 1H, NH), 4.38 (s, 2H, NH_2).

3.4-3.7 2-chloro-6-hydrazinylpyrazine (1eq) was dissolved in toluene (13-80ml), Triethyl orthoformate (2eq) and toluene sulfonic acid was added (.05-.5eq). The reaction mixture was stirred and heated to reflux at 110℃ for 6 hours (6-40h). The mixture turned a dark red which throughout the reaction. The solvent was removed via reduced pressure. TLC was used to monitor the reaction in process and determine the completion. Multiple reactions occurred following table X below.

The solid product (3.4-3.6) was made up in dichloromethane. Silica (X) was added to the solution and was removed via a combination of reduced pressure and nitrogen gas. Flash Column Chromatography was performed (1:1 ethyl acetate:hexane) and the fractions were identified via TLC and NMR. The product containing fractions were pooled according to TLC and the solvent was removed via reduced pressure and nitrogen gas. The spectroscopic results matched those of previously reported.[10] ^{1}H NMR (400 MHz, dmso) δ 9.69 (s, 1H, H3), 9.43 (s, 1H, H8), 8.16 (s, 1H, H6). ^{13}C NMR (101 MHz, dmso) δ 145.64, 141.74, 135.97, 127.74, 121.19.

3.8-3.12 Based off previous methods [1, 3, 7], the ring closure reaction step (3.4-3.7) was repeated with differing reagents, trimethyl orthoformate both neat and in 2eq as well as the exclusion of the pTsOH catalysis. The parameters of the reactions are listed below in Table X. The resulting mixtures had the solvents removed under reduced pressure and nitrogen gas. The reactions were monitored via TLC and analysed with 1H NMR, GC (appendix). The trimethyl orthoformate product was purified via liquid chromatography semi-prep.

3.14-3.15 Solid crude product was removed of reaction 3.7 and was dissolved in an organic solvent (hexane or ethyl acetate) and water added to it. The resulting mixture was placed in a sonicating bath for 2 minutes, was removed to allow the layers to separate. The solvent layer and aqueous were isolated and analysed via GC-FID and TOF-MS. The procedure followed table X.

3.16 Solid crude product was removed from reaction 3.7 (5.3mg) and was added to 2ml of water. The mixture was placed in a sonicating bath for 2 minutes. The mixture (which contained particles) was filtered through a cellulose filter (Kinesis 25mm, 0.45µm, Mixed Cellulose). The filter was then washed with methanol (1ml). The resulting samples were analysed via GC-FID and TOF-MS 155 m/z pos. ion.

4.0 Results and Discussion

The procedure originally followed was based on MacDonald's previous work for the OSM and has been repeated multiple times by other members of OSM exploring this field. The reaction itself is common and other than the 6-hour reflux time, optimisation was not required. The yields produced are similar to predicted. [4, 9]

Table 4.1: Reaction mixture for synthesis of 2-chloro-6-hydrazinylpyrazine.

Reaction	2,6-dichloropyrazine	EtOH	Hydrazine Hydrate	Reflux Time	Crude Yield
3.1†	0.0273mol, 1eq	50ml	0.0547 mol, 2eq	6h	17.4%*
3.2	0.0274mol, 1eq	20ml	0.0548 mol, 2eq	5.16h	71.9%
3.3	0.0405 mol, 1eq	35ml	0.0811 mol, 2eq	6h	103%‡

* Yields reduced due to manual handling error

† No brine washes

‡ Most likely due to scale error

The quality of 2,6-dichloropyrazine should be considered. Though the products were not affected, predicted yields will be if the starting material is not pure and the production of brown solid occurred based on the purity of the material. This was observed in reaction 3.3. The procedure was repeated 3 times to replenish material for later reactions.

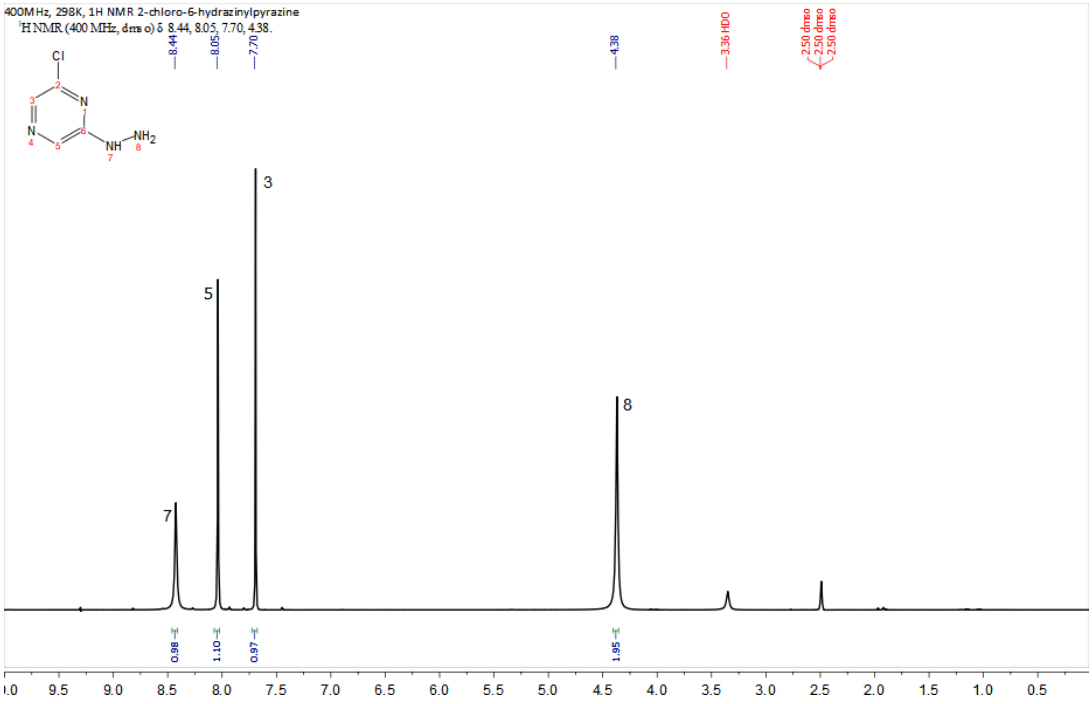


Figure X: *1H NMR of 2-chloro-6-hydrazinylpyrazine 400MHz, 298K*

The purity based on the 1H NMR (Figure X) showed that further purification of the product was not required and therefore could be used to continue the project. Efforts were therefore not used to improve the synthesis of 2-chloro-6-hydrazinyl pyrazine and focus was placed on the second step.

Working from previous literature the synthesis of 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine shows a yield of 42% and was investigated to find an alternate route of synthesis or purification to reduce the impact of the inefficiency to the overall synthesis. [9] The reaction mixture turns red during reflux however the resulting pure solid was yellow and flaky. Purification of the product was difficult due to a red oil-like by product that remained after evaporation of the solvent. TLC showed the production of other products through the reaction and then loss of those products as the reaction continued into the later hours of the reflux. Closure monitoring of the reaction (via NMR or GC) could provide insight into the reaction profile and may suggest the formation of intermediates. Reaction 3.4 was purified via flash column chromatography (~10ml per fraction) and the fractions were identified with TLC. Fractions were pooled based on retention times (TLC appendix) NMR and MS were used to identified the compounds and it was deemed that fractions 14-24 contained the desired product, all pooled fractions were tested (appendix).

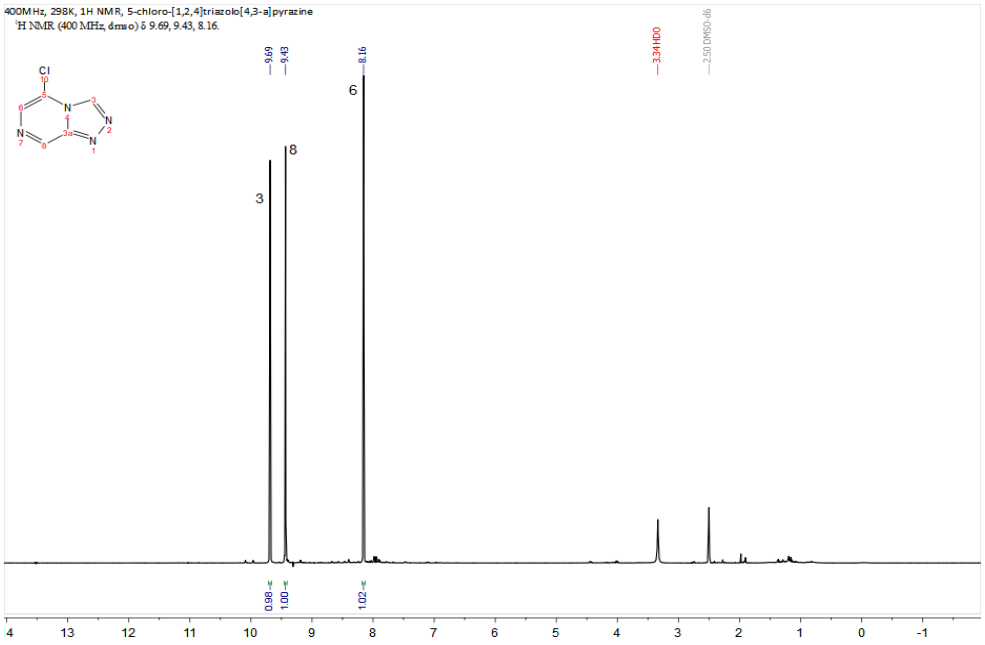


Figure X: 1H NMR of 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine 400MHz, 298K

Based on the 1H NMR purity of the product with FCC purification was high (99.5% via GC-FID, Figure X). Mass spectrum reported a m/z of 155 in ES+ which coincides with the MW of 154 g/mol. However, the yields after column purification were less than 1%. This also occurred with chromatography of reaction 3.5 which had extremely low yields post FCC and was not used due to an unrelated issue of contamination. The reaction was repeated two more times which yielded crude product for purification experiments.

Due to the low yields of the flash column chromatography it was determine that the silica used for said procedure contained a majority of the triazolopyrazine compound. More material was required for trial runs of purification techniques so 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine and its by products were extracted from the column and underwent preliminary liquid/liquid extraction and LC-semi preparation work up (appendix). This was then applied to the crude product (3.7).

Table X: Reaction mixture for synthesis of 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine.

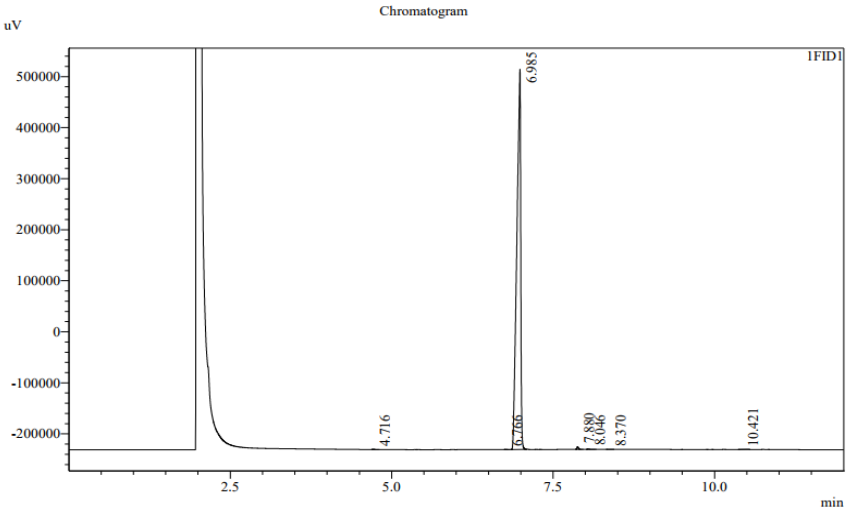
Reaction	2-chloro-6-hydrazinylpyrazine	From reaction	Triethyl orthoformate	TsOH	PhMe	Time	Crude Yield
3.4	0.0031 mol, 1eq	3.2	0.0062 mol, 2eq	0.05eq*	13ml	25h	NA**
3.5	0.0071 mol, 1eq	3.2	0.0141 mol, 2eq	0.1eq	20ml	6h	113%
3.6	0.0056 mol, 1eq	3.1/3.2	0.0113 mol, 2eq	0.5eq†	20ml	40h‡	152%
3.7	0.0251 mol, 1eq	3.3	0.0500 mol, 2eq	0.1eq	80ml	7.2h	112%

* The Thomas MacDonald method stated 0.1eq but used 0.05eq which was followed in 3.4 but changed to 0.1eq hereafter.

** Crude yield was not recorded

† A higher catalyst ratio was used to increase the rate of the reaction to be completed in 4 hours, however it did not do so.

‡ Due to the time the reaction started it was force to continue for 40h which was not recommended and produced poor results.



Peak Table CH_OSM_170912_1_Pure, MeOH_01.gcd

FID1

Peak#	Ret. Time	Height	Height%	Area	Area%	Area/Height	Name
1	4.716	677	0.091	1196	0.039	1.766	
2	6.766	428	0.058	757	0.025	1.767	
3	6.985	736586	99.006	3063224	99.547	4.159	TA-P
4	7.880	4805	0.646	7973	0.259	1.659	
5	8.046	866	0.116	1797	0.058	2.074	
6	8.370	244	0.033	637	0.021	2.613	
7	10.421	371	0.050	1579	0.051	4.258	
Total		743978	100.000	3077163	100.000		

Figure X: Synthesis of 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine, purified via FCC

Other purification methods were attempted in an effort to reduce the dependency on FCC and allow for simpler and more efficient purification of the reactions mixture. Liquid/liquid extraction was explored due to the nature of the mixture to have a solid present when water was added. The mixtures in table X were followed and the resulting layers GC results were obtained (figure X, appendix). Two other samples were used to determine the effectiveness of the extractions, these were methanol and water. The water sample required filtering before GC was obtained, which removed by products and the desired compound from the solution (figures X).

Table X: Liquid/Liquid Extraction on Crude Reaction Mixture (3.7)

Sample	Mass of Crude Product	Volume of Water	Solvent	Volume of solvent
3.14	22mg	2ml	Ethyl acetate	2ml
3.15	16mg	2ml	Hexane	2ml
3.16	11.2mg	NA	Methanol	2ml
3.17	5.3mg	2ml	NA	

Table X:

	Peak (Ret. Time)	Area	Percentage Area	Identification
Crude in MeOH				
	6.786	225882	46.305%	
	9.915	20819	4.268%	Triazolopyrazine
	8.399	127839	26.206%	
Crude in Water (Filtered)				
	6.780	75439	9.270%	
	6.897	6195	0.761%	Triazolopyrazine
	8.366	63201	7.766%	
Crude in Ethyl Acetate/Water Ethyl Acetate Sample				
	6.778	150848	29.440%	
	6.905	8095	1.580%	Triazolopyrazine
	8.416	262721	51.274%	
Crude in Ethyl Acetate/Water Water Sample				
	6.796	352501	66.812%	
	6.928	41298	7.827%	Triazolopyrazine
	8.382	99453	18.850%	
Crude in Hexane/Water Hexane Sample				
	6.887	4309	20.229	Triazolopyrazine

Assuming the FID intensity between compounds is equal and the retention time of 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine is consistent, large amount of by product are present in the crude at R_t 6.786 and 8.399 mins. These species are also present in the filtered water sample (to a lesser extent) and the ethyl acetate sample which suggests affinity to polar solvents. There is a decrease in percentage purity of the 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine in water and ethyl acetate sample (similar to the large impurities). However, the hexane extraction shows an increase, but is most likely not accurate due to the solvent drift (see appendix) for the presence of water in the sample. The water layer from the ethyl acetate/water extraction shows a large decrease in impurities. The percentage area of the triazolopyrazine compound increased, however one larger impurities mentioned previously (R_t 6.786 mins) increased from a composition percentage of 46.3% to 66.8%. The purification via ethyl acetate/water liquid/liquid extraction is not significant enough to deem a viable method for purification.

Future work into liquid/liquid extraction or including the ethyl acetate/water extracting in a larger purification method may be useful, but was not investigated in this report. All liquid/liquid extraction samples and water-filter samples were analysed via GC-FID however results were similar to the above discussed (see appendix). Note, the reaction for formation of crude may not have gone to completion or other variables may have effected it's overall purity which is different to the reported values of 42%.[9] It was suspected that the retention time of the desired product may have shifted due to the lower purity of the crude and was confirmed to not be the case via GCMS of the crude sample, pure sample and trimethyl orthoformate sample (discussed later) Figure X.

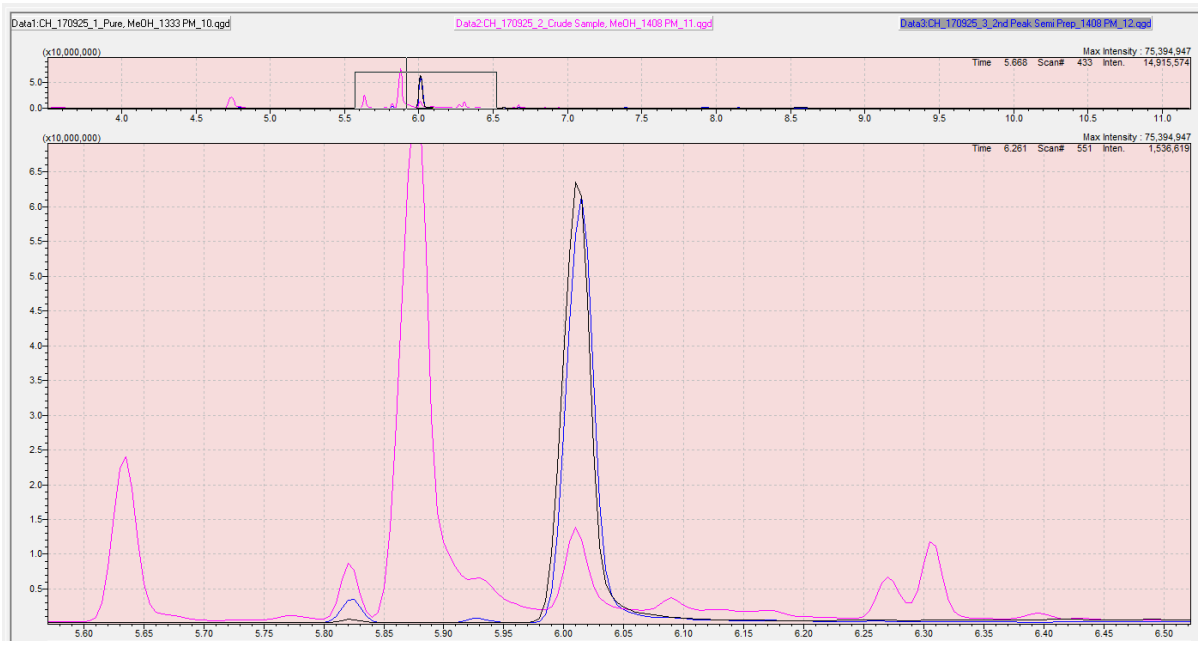


Figure X: GC-MS of Crude reaction mixture (Lowest at R_t 6.02 mins), Pure sample (Highest at R_t 6.02 mins) and MTp sample (Middle at R_t 6.02 mins) layered

Figure X: Mass spectra of Crude reaction mixture, Pure sample and MTp Sample at R_t 6.02 mins peak

It was determined that the retention time of the product peak did not drift and the identification method of the product was sound which concludes that the most likely cause of the low purity from the crude sample was the reaction. The same tests were run on the original GC method to extremely similar results (see appendix). The largest peak at R_t 5.87 mins was also analysed via MS (Figure X). The data obtained was inconclusive to the structure but may represent a chlorined impurity, by-product or intermediate.

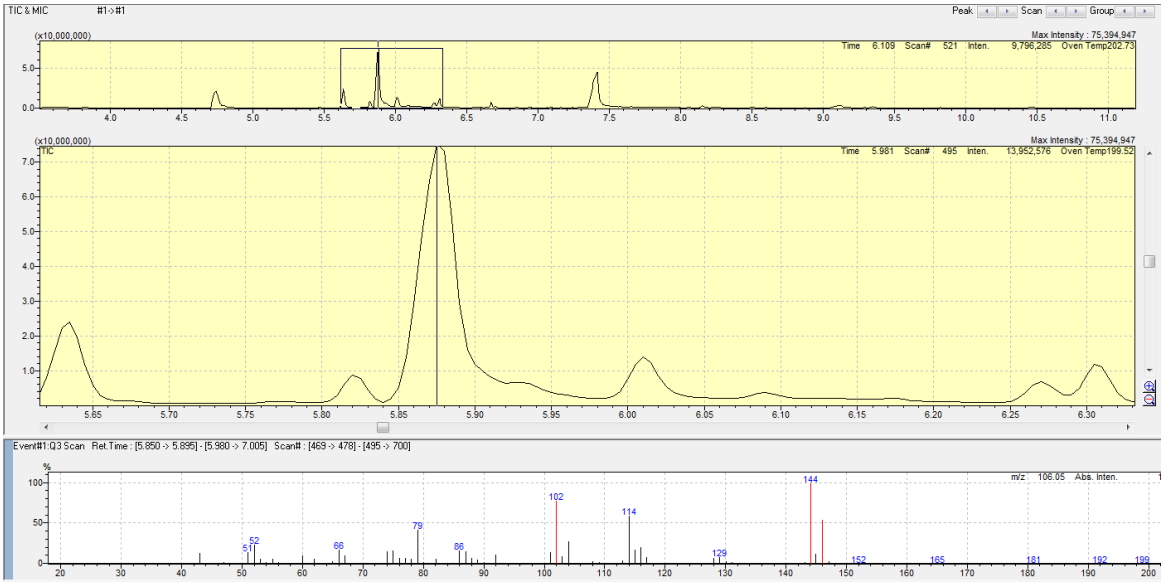


Figure X: Gas Chromatography of Crude reaction mixture and MS of peak at R_t 5.87 mins

Based off the work by Dooseop & Co and Hong-Jian % Co, reaction using trimethyl orthoformate were examined for the potential of decreasing impurities and increase yields for the 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine synthesis. The following reactions tableted in table X were run simultaneously and monitored via TLC. The TLC showed no desired species in reactions 3.8, 3.9 3.11 and 3.12 however reaction 3.10 showed a singular compound in the region expected for 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine. Based on purely the results of the TLC it can be assumed that pTsOH is required for the reaction to reach completion and a trimethyl orthoformate neat, with pTsOH should be tested in the future.

Table X: Reaction mixtures of differing orthoformate substituents and catalysis.

Reaction	Sample Tag	Chloro-hydrazinylpyrazine	Orthoformate*	Orthoformate Volume	Solvent	pTsOH	Crude Mass
3.8	1ET	1.38mmol	Triethyl	2eq	Toluene (4ml)	NA	0.2479g
3.9	2EN	1.39mmol	Triethyl	Neat (2ml)	NA	NA	0.2263g
3.10	3MTp	1.38mmol	Trimethyl	2eq	Toluene (4ml)	0.1eq	0.1791g
3.11	4MT	1.38mmol	Trimethyl	2eq	NA	NA	0.2440g
3.12	5MN	1.38mmol	Trimethyl	Neat (2ml)	NA	NA	0.2407g

*Triethyl orthoformate or trimethyl orthoformate were used.

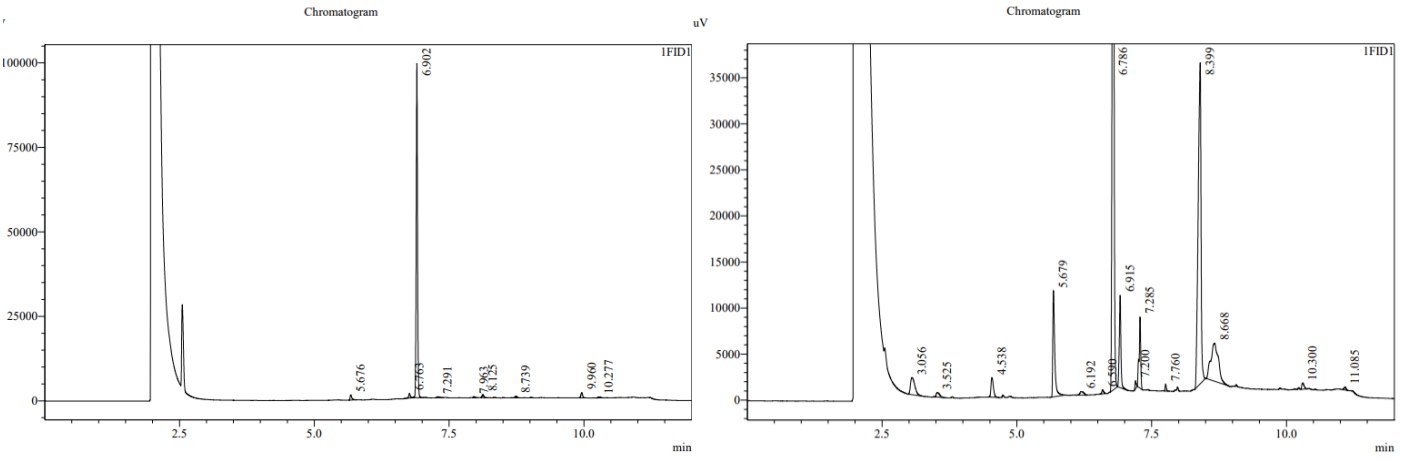


Figure X: Chromatogram of Crude 3MTp (3.10 left) vs Crude Reaction of 3.7 (right)

Peak Table CH_OSM_170913_3_3, MV, MTp_03.gcd

FID1

Peak#	Ret. Time	Height	Height%	Area	Area%	Area/Height	Name
1	5.676	1518	1.484	3425	1.871	2.256	
2	6.763	1366	1.336	3049	1.666	2.231	
3	6.902	95590	93.461	167556	91.566	1.753	TA-P
4	7.291	302	0.295	1232	0.673	4.079	
5	7.963	325	0.318	626	0.342	1.926	
6	8.125	908	0.888	1932	1.056	2.127	
7	8.739	475	0.465	953	0.521	2.005	
8	9.960	1506	1.472	3239	1.770	2.151	
9	10.277	287	0.281	978	0.534	3.403	
Total		102278	100.000	182989	100.000		

Peak Table CH_OSM_170912_2.2_Crude, F, MeOH_02.gcd

FID1

Peak#	Ret. Time	Height	Height%	Area	Area%	Area/Height	Name
1	3.056	1854	0.998	10508	2.154	5.668	
2	3.525	501	0.270	2196	0.450	4.384	
3	4.538	2108	1.135	6683	1.370	3.171	
4	5.679	11417	6.146	28859	5.916	2.528	
5	6.192	375	0.202	1897	0.389	5.056	
6	6.590	447	0.240	1036	0.212	2.319	
7	6.786	110021	59.224	225882	46.305	2.053	
8	6.915	9968	5.366	20819	4.268	2.089	TA-P
9	7.200	887	0.477	1437	0.295	1.620	
10	7.285	7573	4.077	16125	3.306	2.129	
11	7.760	720	0.388	1135	0.233	1.576	
12	8.399	34774	18.719	127839	26.206	3.676	
13	8.668	4122	2.219	40500	8.302	9.825	
14	10.300	683	0.368	2060	0.422	3.017	
15	11.085	320	0.172	841	0.172	2.631	
Total		185769	100.000	487815	100.000		

Figure X: Crude 3MTp (top) vs Crude Reaction of 3.7 (bottom) peak tables

According to the GC-FID the purity of 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine is much better when the reaction occurs with trimethyl orthoformate compared to triethyl orthoformate. Increasing from 4.2% to 91.5% purity via GC. Therefore, making the reaction much for efficient and reducing the cost of synthesis. Having a high purity may allow for little to no purification before moving onto bromination (not investigated in this report) which is commonly followed by a recrystallisation.[9] However, purification of the reaction was still investigated using the crude mixture from 3.10.

Analytic high-pressure liquid chromatography was used for preparation and development of a semi-preparative method for the crude material (3.10). The resulting run (figure X) was shown to have a large peak at R_t 3.302 (210nm) and approximately 40% MeOH (in water) which was concluded to be 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine. Due to the separation acquired via the analytic run, no further modification was needed to the method. The resulting process allow for a good standard of purification testing when evaluating reaction mixtures. However, the information gained was applied to fraction collecting and preparative work.

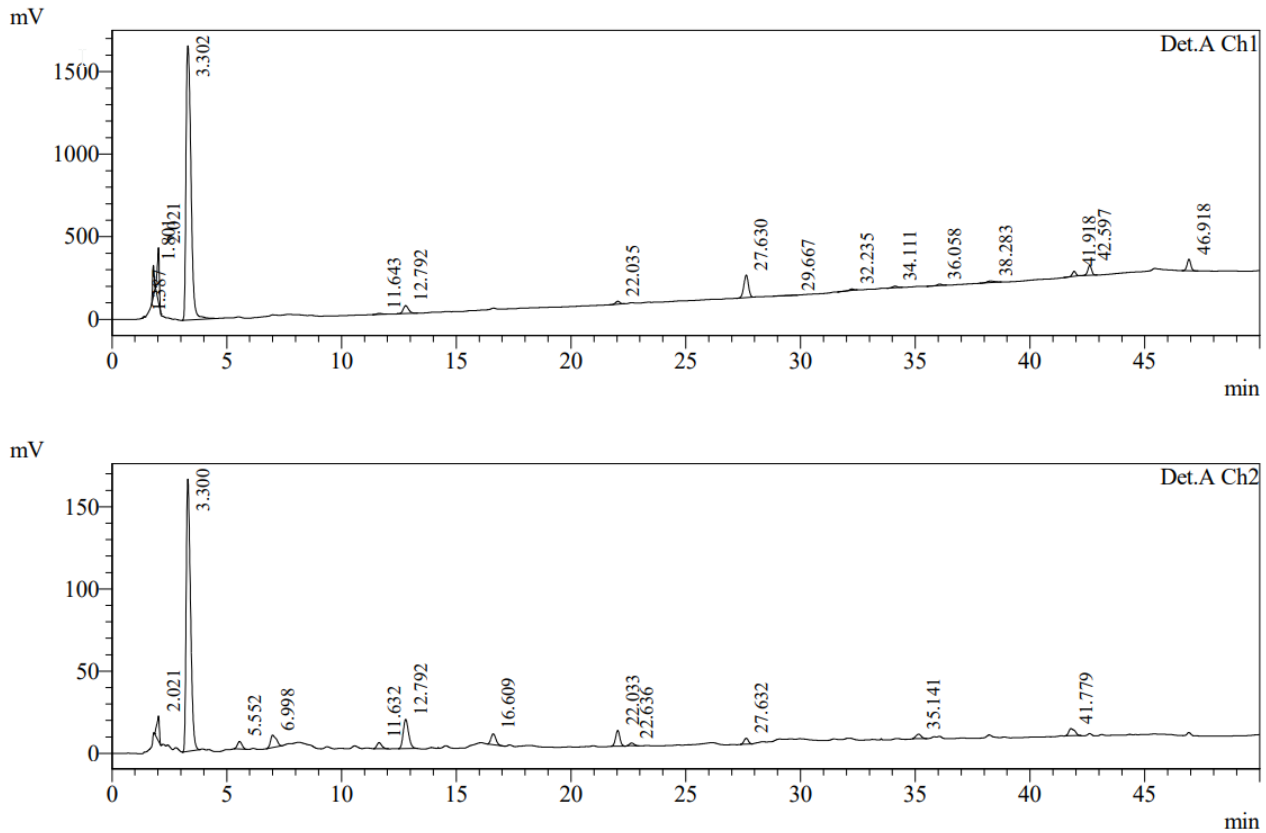


Figure X: Analytic HPLC of reaction mixture 3.10, at 210nm and 254nm. Peak table appendix X

The method was hence scaled up to account for a 5000ppm, 2ml sample of the crude (containing 10mg) loaded on a cotton plug. The sample was run on a modified method (appendix) and over loaded the column which can be seen on the chromatogram (appendix). The same method was applied to a 2500ppm sample which resulted in a similar problem (appendix). The sample was diluted again to 1250ppm, 2ml, the method modified (methods X) and was introduced via direct injection to the column. The final method included an isocratic region at 2-8 mins at 33% MeOH to provide greater separation of the R_t 3.922 and 6.861 (210nm) mins peaks.

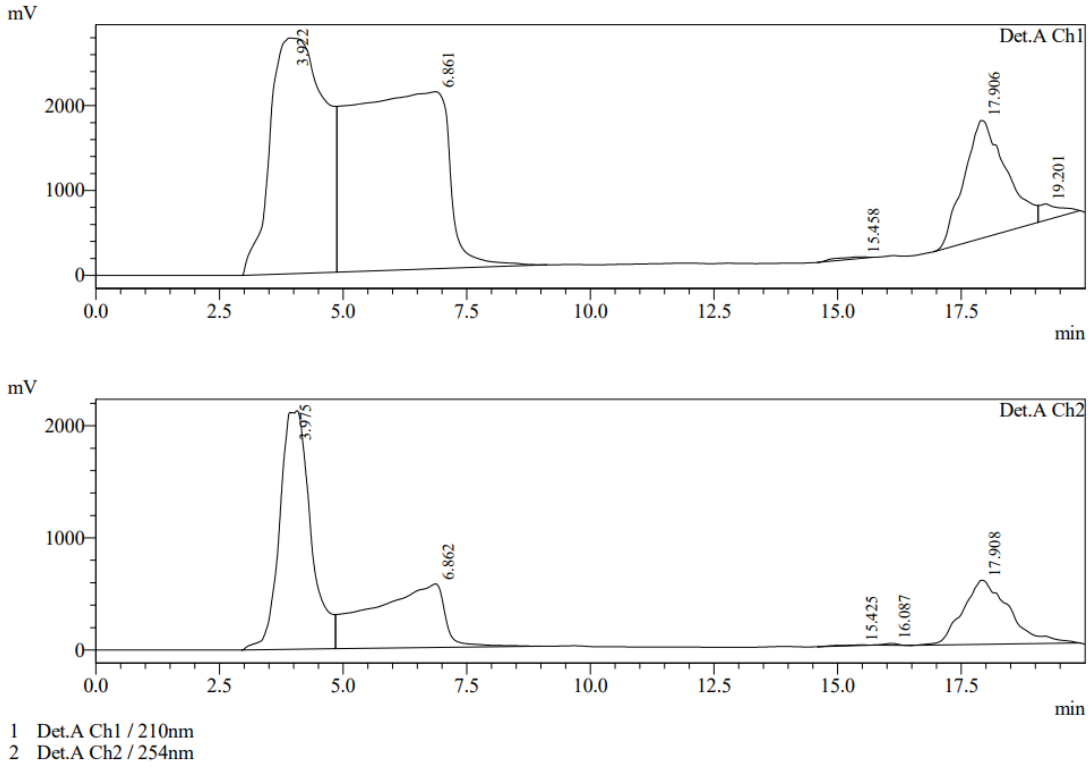
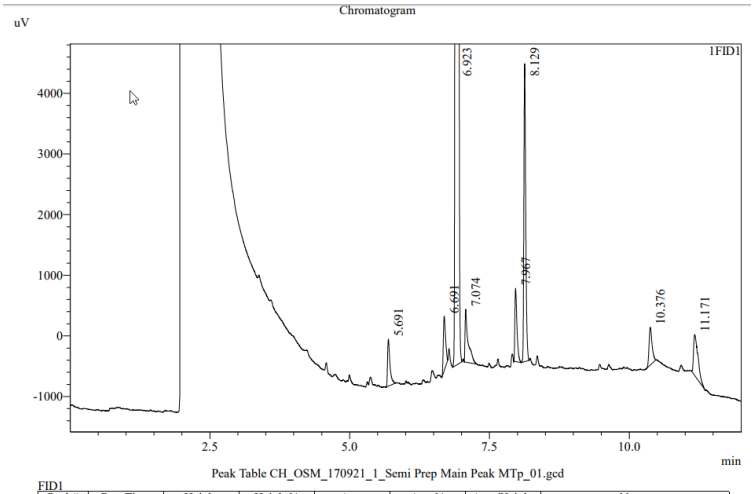


Figure X: Semi-preparative HPLC of reaction mixture 3.10 at 210nm and 254nm. Peak table appendix X

The peaks were separated physically via a fraction collector and were pooled according to their retention times and to avoid side peaks. The resulted samples were remade and analysed via GC, NMR and MS for purity and identification. The effectiveness of the semi-preparative run is determined by the concentration of the reaction mixture. Even at low concentrations and high flow rate separation of the desired compound is difficult. Therefore, at least in this case, multiple runs were required to gain enough material for testing (2 runs). A larger column (for mainly preparative work) or pre-injection purification, maybe via liquid/liquid extraction, could be used to reduce the load on the column for efficient separation and resolution. Other solvents may result in for effective separation such as acetonitrile, which was used in washing the column after preparative runs, could be a successful elution tool. Future work in purification may not be needed due to the new synthesis method, nevertheless the investigation into semi-preparative work is useful for the OSM project and would be worth an extended look. It was identified that the purest sample (96.7% via GC-FID) elutes at R_t 6.861 mins (Figure X) which is an improvement of the crude sample (91.6% via GC-FID, Figure X).

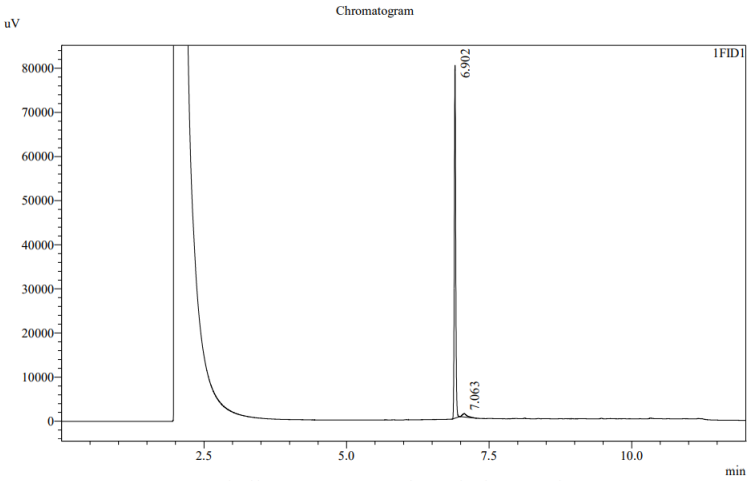


Peak Table CH_OSM_170921_1_Semi Prep Main Peak MTp_01.gcd

FID1

Peak#	Ret. Time	Height	Height%	Area	Area%	Area/Height	Name
1	5.691	768	0.326	2318	0.464	3.018	
2	6.691	893	0.379	2527	0.506	2.828	
3	6.923	225677	95.798	470490	94.233	2.085	TA-P
4	7.074	866	0.367	3317	0.664	3.832	
5	7.967	1188	0.504	3149	0.631	2.652	
6	8.129	4891	2.076	11410	2.285	2.333	
7	10.376	620	0.263	2349	0.470	3.790	
8	11.171	673	0.286	3724	0.746	5.532	
Total		235576	100.000	499285	100.000		

Figure X: GC-FID of pool fraction at R_t 3.922 min (210nm)



Peak Table CH_OSM_170921_1_Semi Prep 2nd Peak MTp_02.gcd

FID1

Peak#	Ret. Time	Height	Height%	Area	Area%	Area/Height	Name
1	6.902	78257	98.993	135227	96.707	1.728	TA-P
2	7.063	796	1.007	4604	3.293	5.786	
Total		79053	100.000	139831	100.000		

Figure X: GC-FID of pool fraction at R_t 6.861 min (210nm)

The same samples were run on MS-TOF and produced the expected 155 m/z signal (Figure X) when looking at positive ions. A represent signal of 156, 157 and 158 m/z occurred which was predicted to be the result of the isotopic ratio of the 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine. This was seen in both samples which matches the GC-FID data obtained. Also matching the previous results is the purity of the compound which can be seen in the full spectrum mass spectra (Figure X) with the R_t 3.922 min (210nm) peak having multiple impurities at fall within the 150-300 m/z range and few impurities in the 450-690 m/z range. In the secondary peak sample less impurities are seen at the lower ranges and more impurities are seen at the higher ones (450-690 m/z). However, in both samples a large section of the spectra (310-360 m/z) have highly occurring signals, namely 331 m/z. It can be assumed that 331 m/z is highly ionizable due to its prevalence in mass spectrum though lack of presence in the GC-FID and does not follow the noticeable isotopic ratios of a chlorine containing compound (appendix). This rules out multiple coordination to a sodium ion.

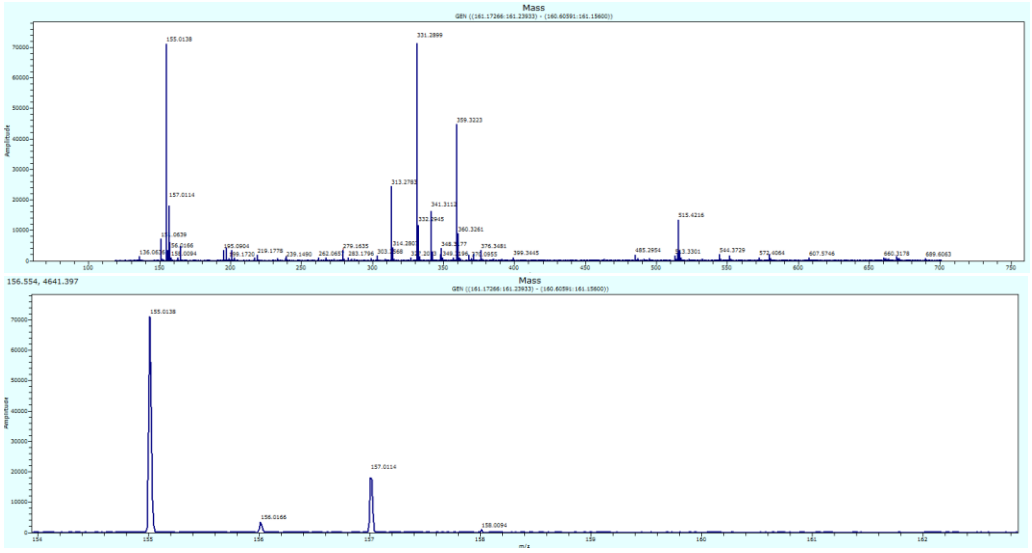


Figure X: TOF-MS of 3.10 Reaction mixture, semi-prep R_t 3.922 min (210nm). Full spectrum (top) and Product (bottom).

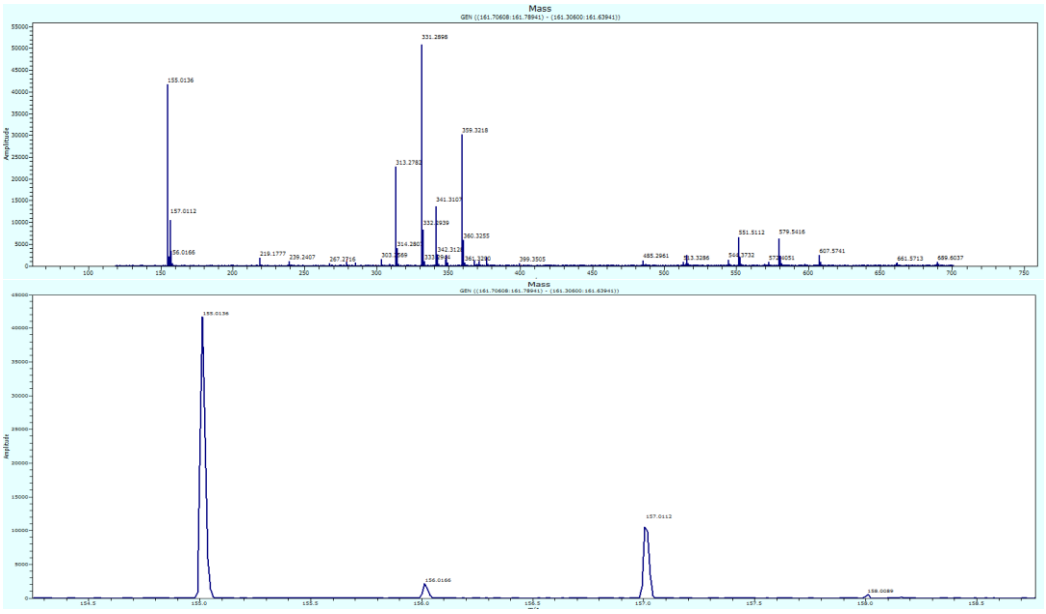
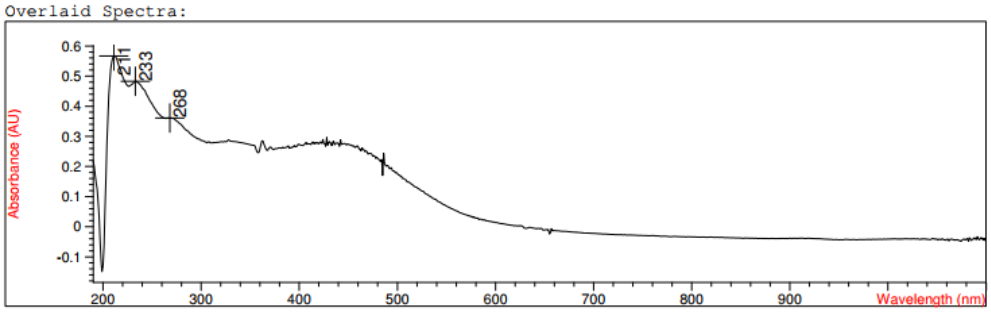


Figure X: TOF-MS of 3.10 Reaction mixture, semi-prep R_t 6.861 min (210nm). Full spectrum (top) and Product (bottom).

Data was collected for UV/vis spectrum and FTIR for the purpose of providing the OSM community with identification data. The UV/Vis spectra are shown below (Figure X) and the FTIR spectra was inconclusive due to the presense solvent.



#	Name	Peaks(nm)	Abs(AU)
1		211.0	0.56652
1		233.0	0.48271

#	Name	Peaks(nm)	Abs(AU)
1		268.0	0.36126

Figure X: UV and visible spectra of 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine

5.0 Conclusion

The aim of this project was to optimise and investigate the route of a common precursor 5-chloro-[1,2,4]triazolo[4,3-a]pyrazine used to synthesis antimalarial lead compounds. The current method for the synthesis of this compound is low yielding due to the purity and prevalence of by-products. Using the trimethyl orthoformate reaction (3.10) will provide a more efficient way for the Open Source Malaria community to synthesis future desired compounds. Methods for GC-FID analysis and HLPC preparative work will also provide a starting point for identification and purification to a larger suite of chemists entering the OSM project. In future, a complete work up of the trimethyl orthoformate reaction is required to obtain true yields and assess the overall position the step can fill in the larger scheme for OSM. Furthermore, future investigation of the reaction and purification will be needed before a complete adoption could take place. The project will likely have an impact on the Open Source Malaria members and help lower the bar of entry for these synthetic steps.

6.0 Acknowledgements and References

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7.0 Appendix

1ST LC ANALYSIS MTP

min

1 Det.A Ch1 / 210nm
2 Det.A Ch2 / 254nm

Detector A Ch1 210nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	1.387	22538	6628	0.068	0.256
2	1.801	888336	186918	2.666	7.208
3	2.021	2030338	330416	6.093	12.741
4	3.302	24871769	1660914	74.646	64.046
5	11.643	91520	6305	0.275	0.243
6	12.792	784053	47965	2.353	1.850
7	22.035	241711	18336	0.725	0.707
8	27.630	1902296	137055	5.709	5.285
9	29.667	26145	340	0.078	0.013
10	32.235	120649	8847	0.362	0.341
11	34.111	142815	9872	0.429	0.381
12	36.058	134325	9549	0.403	0.368
13	38.283	221715	9618	0.665	0.371
14	41.918	339138	30926	1.018	1.193
15	42.597	708441	59714	2.126	2.303
16	46.918	794045	69930	2.383	2.697
Total		33319833	2593331	100.000	100.000

PeakTable

Detector A Ch2 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	2.021	113158	15179	3.494	6.248
2	3.300	2176292	165675	67.194	68.201
3	5.552	62354	4488	1.925	1.847
4	6.998	132165	7573	4.081	3.118
5	11.632	53476	3724	1.651	1.533
6	12.792	287594	17746	8.880	7.305
7	16.609	97960	6601	3.025	2.717
8	22.033	123965	9594	3.828	3.949
9	22.636	21973	1648	0.678	0.678
10	27.632	47077	3615	1.454	1.488
11	35.141	43123	2702	1.331	1.112
12	41.779	79670	4378	2.460	1.802
Total		3238807	242923	100.000	100.000

FRACTION:

PeakTable

Detector A Ch1 210nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.922	212330769	2774870	36.124	42.985
2	6.861	286818853	2082903	48.797	32.266
3	15.458	1213616	19139	0.206	0.296
4	17.906	81867550	1386827	13.928	21.483
5	19.201	5545072	191654	0.943	2.969
Total		587775860	6455394	100.000	100.000

PeakTable

Detector A Ch2 254nm

Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.975	93409704	2109525	49.274	64.573
2	6.862	58213900	566296	30.708	17.334
3	15.425	277286	4676	0.146	0.143
4	16.087	278180	15497	0.147	0.474
5	17.908	37394403	570907	19.726	17.476
Total		189573474	3266901	100.000	100.000