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Emile Morin April 12th, 2022

Synthesis of Novel EP2 Prostaglandin Antagonists for Optimal Blood-Brain Barrier Permeation as Radioligands

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Abstract

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Contents

Abstract

The neuroinflammatory pathway regulated by the EP2 prostaglandin receptor is a key target in pharmacological studies. In order to demonstrate the mechanism of action in novel EP2 antagonist compounds, PET studies are utilized to showcase the binding to the target organ regions. Novel EP2 antagonists were synthesized to provide an optimal partition coefficient to permeate across the blood-brain barrier while containing fluorine that are accessible for PET studies. The synthesis of four derivative compounds based upon a model compound were developed in this study. The derivative synthesis was centered around the modification of key target function groups in locations that would minimally affect the reactivity and selectivity. The target compounds synthesized and partially synthesized were aimed with modifying functional groups to lower the Log P for optimal values in relation to membrane permeation. Resulting in two successfully synthesized possible EP2 receptor antagonists compounds with an experimental LogP value below 2.0.

Introduction

The development of pharmacological agents that target biochemical pathways involved with inflammation provide promising avenues in novel medical treatment protocols. Specifically in targeting neuroinflammation pathways that are critical towards developing methods for treatments towards seizures, traumatic brain injuries, cerebrovascular accidents, and sepsis.[1] These medical problems have been reported to have neuroinflammation as a key component in their residual long term damage to the brain. Compounding the initial health complications of trauma with cognitive decline that critically impact long term patient health.[2] Previous studies performed by Dr. Ganesh and Dr. Dingledine show the promising potential behind the relationship of blocking particular branches in inflammation pathways involved by targeting key receptors involved in known neuroinflammation biochemical pathways.[3,4]

Building upon previous studies, the target of the EP2 prostaglandin receptor has been determined to be critical in biochemical pathways that are pro-inflammatory in chronic peripheral, central nervous system disease and cancer models.[4-6] The EP2 receptor is a G-protein-coupled plasma membrane receptor for PGE2 which is part of the cyclooxygenase-2 (COX-2)/ PGE2/EP2 pathway.[7] Pharmacological studies have targeted other proteins and receptors of this inflammation pathway, but have proven to lead to excessive detrimental side effects including gastric ulcers and myocardial infarctions.

Therefore, the choice in targeting specifically the EP2 receptor, although it is lower in the signaling pathway, is due to the toxicity that accrues from inhabiting the COX enzyme higher up

in the pathways.[8] Focus within the field has shifted primarily towards the biological effects of PGE2 as its activity is mediated by signaling through four distinct E-type prostanoid (EP) receptors - EP1 , EP2 , EP3 and EP4. However, the EP2 subunit is an important regulator in numerous physiological and pathological process that are particularly linked to cancers as it is involved in significant inflammation pathways. The continued expression of these subunits has been found to be generate pro-inflammatory factors, including cytokines, chemotactic factors, nitric oxide synthase (iNOS) and even COX-2. These factors can promote cell proliferation, survival, angiogenesis, invasion, migration and metabolism.[5,6]

Such factors cause the EP2 receptor to be a key target in the development of pharmacological agents within the domaine of pharmacology and chemical biology. Already novel compounds synthesized within the Ganesh Lab have had proven reactivity with the target EP2 receptor in microbiology studies.[4] An essential component to the development of compounds towards pharmaceutical purposes is the demonstration of effective inhibition of the receptor in a model system that would simulate the conditions relevant to that of neuroinflammation with seizures, brain injuries and sepsis.

Such model systems have been tested through *in vivo* studies conducted in rodent models utilizing lipopolysaccharide (LPS), an endotoxin produced by gram-negative bacteria to produce a short-lived neuroinflammation.[9] This allows for the controlled and systematic methodology of inducing a inflamed state that will cause lasting damage to the brains of the tested specimen. The results from a study of Jiang et Al demonstrate that the inhibition of the EP2 receptor prevents long-term cognitive impairment in the LPS mouse model of sepsis-associated encephalopathy (SAE).[4] Several compounds of interest developed within the Ganesh Lab have been the critical

components to developing this link between decreased detrimental affects from the induced status epilepticus and that of the inhibition of the EP2 prostaglandin receptor. With the inhibition of the EP2 receptor proving to be an effective means for reducing neuroinflammation, the subsequent steps in the development pipeline is ensuring that the compounds are effectively accumulating in the correct region and demonstrating the mechanism of action.

PET Studies to Result the Mechanism of Action

Positron emission tomography (PET) studies are a powerful and critical tool in the development pipeline of pharmaceutical agents. As an essential metabolic imaging technique within pharmacological studies, developing compounds that can be utilized in PET studies allow for the observation and analysis of substrate absorption within a model system. The value of such studies are extensive in the identification and characterization of pathophysiology in diseased states.[10] Such as in the case of studies conducted with EP2, where neuroinflammated states must be induced to asses the effect of the antagonist compound. PET studies in the study of the EP2 receptor and neuroinflammation would allow for the determination of where the neuroinflammation is occurring along with whether it is being reduced in the target region. However, PET studies also allow for the measurement of the blood-brain barrier penetration and the cerebral occupation of pharmacological compounds that are exposed to the specimen.[11] Providing a vast wealth of critical information regarding the pharmaceutical implications of novel compounds in the setting of relevant pathophysiology.

The major challenge that remains in progressing the pharmacological development for novel compounds targeting the EP2 receptor is to demonstrate the mechanism of action that the compound is targeting specifically the EP2 receptors in the target region of the brain. The latest studies done in the Ganesh lab have been in the synthesis and assessment of novel antagonist compounds through PET studies. Through the development of synthesis that coordinate a fluorine atom to be efficiently substituted onto the final compound would permit radioactive labeling with ^{18}F. Essentially creating a radio tracer that is unique to the EP2 receptor that would demonstrate the location of the compound after absorption in the model system.

The synthesis of radio tracers present a unique set of challenges due to the nature of radioactive compounds. Primarily in the inherent danger in handling of radioactive compounds. Secondly, in the narrow timing window from the reaction, purification, and injection into the specimen. Therefore, in the consideration of the reaction pathways used for the synthesis of novel antagonists, the ease of adding a fluorine within the final reactions must be interwoven within the synthesis roadmap. The development of specialized radioligands has been established as a practical biomarker. Other neuroinflammatory markers such as the translocator protein of 18kDa (TSPO) has been used in such a PET study to identify glial activation and neuroinflammation within LPS-injected mouse models.[12] This study seeks to achieve a similar finding through the development of a radioligand specific to the EP2 receptor. The development of such a radioligand would allow for the specific quantification of the area of activity in which the antagonist is binding to EP2 receptors. Which overall would conclude whether or not the antagonist compound is in fact reducing neuroinflammation specifically in the brain.

The critical step within radio-synthesis procedures is the addition or substitution of the radioactive isotope onto the target compound. Due to this processes' time sensitive nature it is essential that the synthesis of the target compound is developed with this factor in mind. As the current methods of radio-synthesis may result in low yields and high manufacturing costs. Therefore, the implementation of a simple nucleophilic substitution for final reaction of the ^{18}F compound would be the optimal scheme to create a radioligand. The common approach for this compounds is through the use of a tosylate or mesylate as a leaving group.[12,] The advantage of using such groups is that the substitutions reactions are not under the strongly acidic conditions. The importance of this final substitution was a center point in the reaction scheme as to keep the potential for novel developments such as "In Loop" fluorination methods applicable to the compounds developed.[13] The "In Loop" fluorination would allow for the reaction and purification of the final substitution reaction to all take place within a single loop, reducing time spent with the radioactive compounds, increasing yields, and lowering costs.

Membrane Permeation

The blood-brain barrier is a major obstacle in the development of most pharmaceutical compounds. If the developed compound is unable to cross this membrane effectively then no matter the reactivity and selectivity the compounds effectiveness will be drastically reduced. This due to the properties of the tight epithelial junction of the brain capillary endothelium.[14] The current compounds that are the center of investigation within the Ganesh Lab are showing sub-optimal permeation into the brain. The PET studies done on the current antagonist compounds of interest indicate that the sublate is accumulating in organs and joints besides the brain. To solve

this challenge, the chemical property that is involved in the permeation between membranes is the subject of investigation and is measured through the partition coefficient of Octanol and water. The use of this partition coefficient is based upon Lipinskis rule of 5, which are pharmacological principles used to direct the synthesis of medicinal compounds. It states that compounds must have a molecular mass less than 500 Da, no more than 5 hydrogen bond donors, no more than 10 hydrogen bond acceptors, and an octanol–water partition coefficient log *P* no greater than 5. The rule of 5 serves as a basic foundational guideline, however as the receptor of interest is in regards to brain neuroinflammation the optimal LogP values for the blood-brain barrier permeation are in the range of 1.5-2.7.[15] The overall acceptable range for the blood and intestinal permeability is greater than 0 and less than 3.[16]

Derivative analogues of the current effective compounds with computed logP value lower then 3.0 are the goal of this study to further promote the permeation across the blood brain membranes. This will be pursued by specifically modifying the functional groups located on the 5’ indole position of the compound. This one region is isolated for the derivative compounds to simultaneously maintain the potential for selectivity and reactivity with the EP2 receptor that is present in current working compounds. The particular functional groups that have been pursued in the synthesis of several derivatives have been focused on the addition of an amine or a hydroxy group to the 5’ position of the indole starting material. This is also with the consideration of changing the position in which the coupling of additional ring structures are added onto the indole. In this study the synthesis of four unique derivatives on the antagonist to the EP2 receptor were pursued to produce a compound with an optimal partition coefficient

along with a readily accessible and substitutional position for fluorination to create an effective EP2 radioligand.

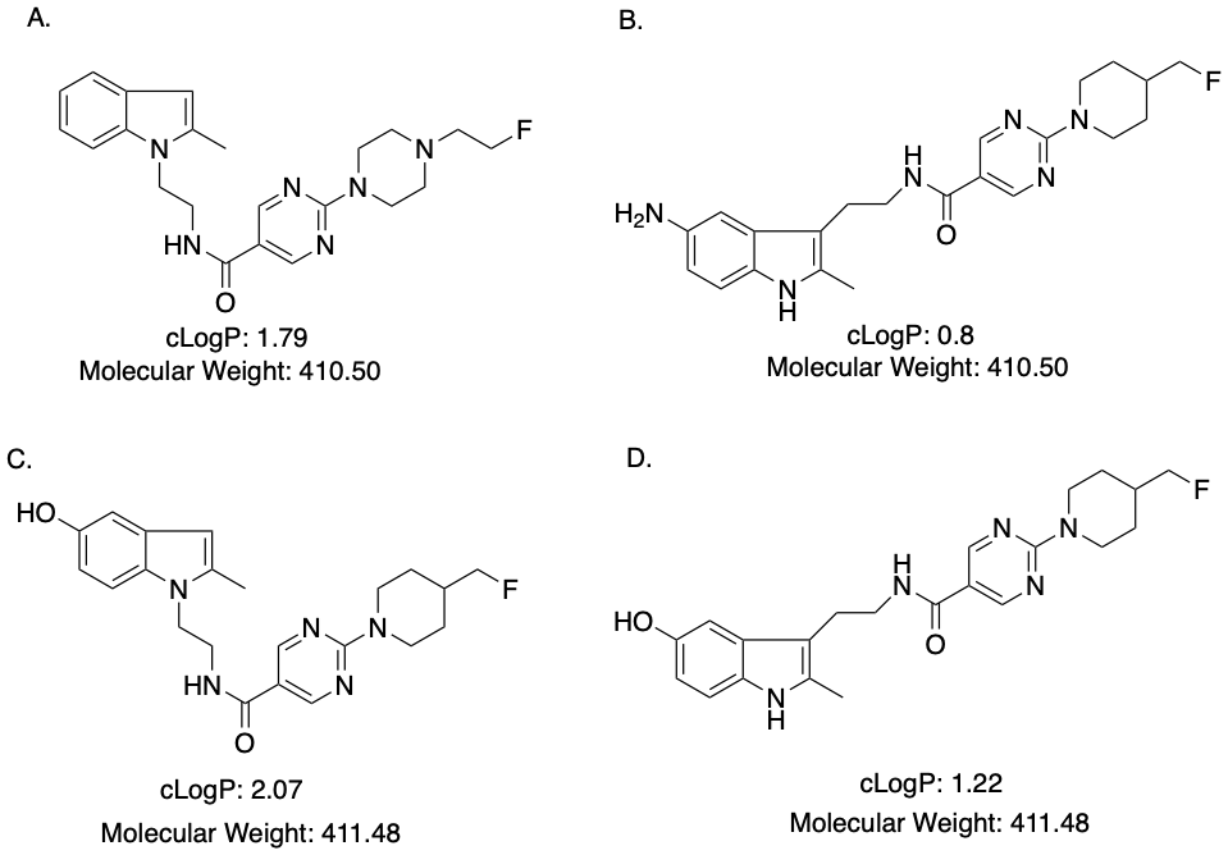
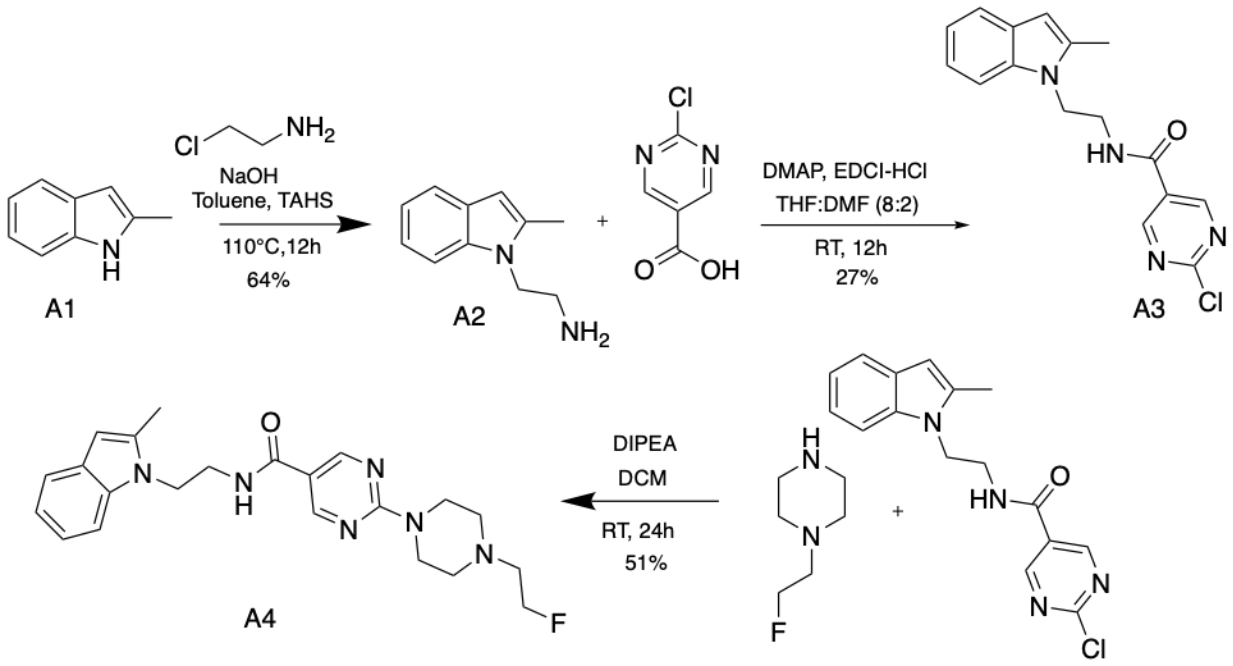


Figure 1. Target compounds for EP2 receptor antagonists along with their calculated partition coefficients values (cLogP). The calculated partition coefficients were performed on ChemDraw utilizing BioByte software.

Methods

Synthesis of TG-154 - 2-methyl-indole to 2-(4-(2-fluoroethyl)piperazin-1-yl)-*N*-(2-(2-methyl-1*H*-indol-1-yl)ethyl)pyrimidine-5-carboxamide



Scheme 1. Compound A

Addition of ethylenamine to 2-methyl-indole - A2 - TG13-126

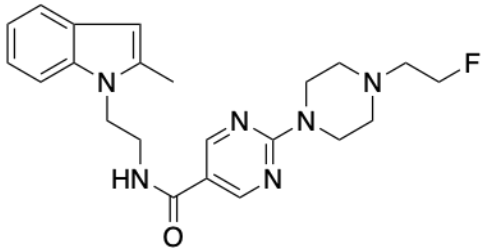
In a round bottom flask 2.633 grams of the starting compound, 2-methyl-indole, (20.07 mmol, 1 equiv) was dissolved in 30ml of toluene. Subsequently 2-chloroethylamine hydrochloride (40.14 mmols, 2 equiv) was added followed by NaOH (140.5 mmol, 7 equiv). Next tetrabutylammonium hydrogen sulfate (2.01 mmol, 0.1 equiv) was added to the solution. The reaction vessel was refluxed under a nitrogen balloon at 100°C for 20 hours. The reaction was then worked up with via a liquid liquid extraction of water and ethyl acetate, then water and

10%MeOH/Chloroform. The organic layer was purified on a HPLC combi-flash with 0.1% NH3OH MeOH/ DCM.

Coupling Reaction of 2-Chloropyrimidine-5-carboxylic acid with A3 - 130/152

2-Chloropyrimidine-5-carboxylic acid (2.85 mmol, 1 equiv) was dissolved in 10ml of DMF and then under nitrogen gas DMAP (0.571mmol, 0.2 equiv) was added into the round bottom flask and stirred till it was dissolved. This was then followed by the addition of EDCI (3.42 mmol, 1.2 equiv). In a separate round bottom flask the 1' amine indole compound, A1, was dissolved in 15ml of DMF and then added to the reaction vessel. The reaction vessel was purged with nitrogen gas. The reaction was left to stir at room temperature for 12 hours. The reaction solution was then worked up via a liquid-liquid extraction with ethyl acetate and H_2O, then with 10% MeOH/DCM. When the organic layer was run through the HPLC combiflash for purification there was poor separation due to remnants of DMF in the organic layer, multiple columns were performed before adequate purity was obtained.

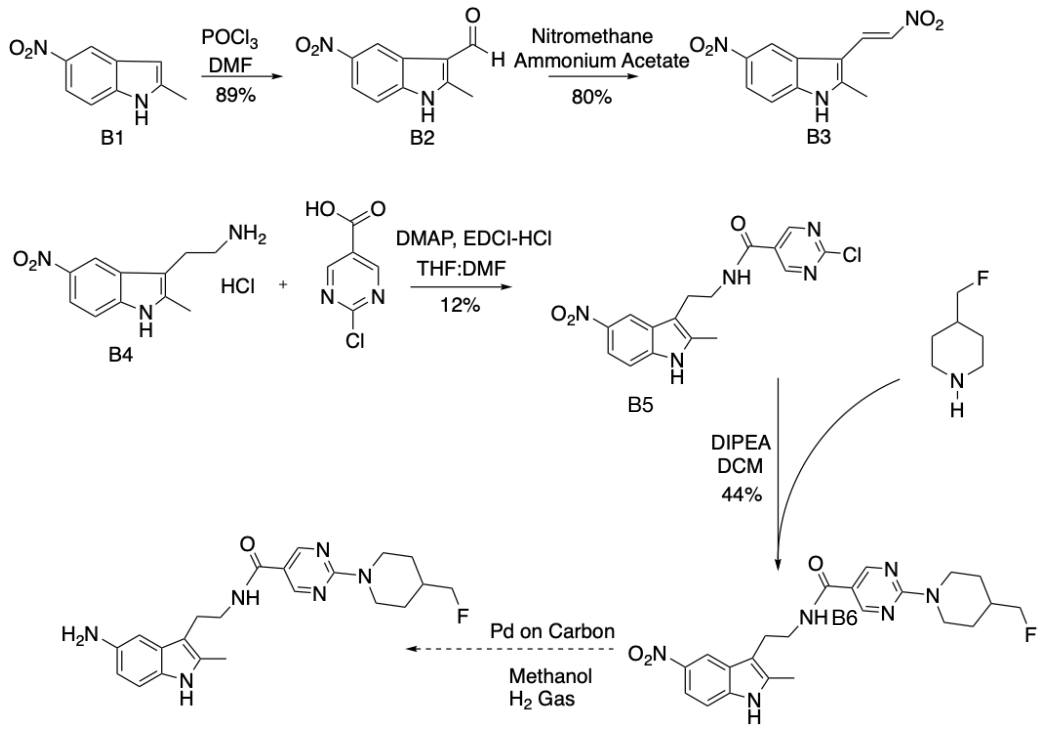
Coupling Reaction with 4(fluoroethyl)piperazine to Produce Compound A - TG13-154



A4 - TG13-154

Compound A3 (0.756 mmol, 1 equiv) are placed in a round bottom flask and dissolved in 1.5ml of DCM followed by 0.12ml of DIPEA (2.27 mmol, 3 equiv), all under a nitrogen gas environment. Then the fluoroethyl-piperizine (0.756 mmol, 1 equiv) is dissolved in 11.5ml of DMC and added to the initial reaction vessel and stirred for 24 hours at room temperature. The reaction vessel was then neutralized with NH_3Cl and the reaction was separated with a liquid liquid extraction of 10% MeOH/DCM and H_2O. The product solution was then purified through on a combiflash purification column.

2-Methyl-5-Nitroindole to *N*-(2-(5-amino-2-methyl-1*H*-indol-3-yl)ethyl)-2-(4-(fluoromethyl)piperidin-1-yl)pyrimidine-5-carboxamide.



Scheme 2. Compound B

Vilsmeir Reaction with 2-Methyl-5-Nitroindole - B2 - 85/97

In a 25ml round bottom flask 30ml of DMF was chilled to 0°C in an ice bath. The reaction vessel was purged with nitrogen and kept under a nitrogen gas environment. Then 1.75ml [18.73 mmol, 1.1 equivalents] of $POCl_3$ was added drips over 10 minutes to the reaction vessel. This was left to stir for 30 minutes to allow for adequate cooling. Then 3.00 grams of the 2-methyl-5-nitroindole, B1, (17.03 mmol, 1 equiv) was added to a separate round bottom flask and dissolved in 30ml of DMF. This solution of the nitroindole was added to the vessel with the $POCl_3$ drop-wise over 5 minutes. An additional 1ml of DMF was added into the vessel used to dissolve the nitroindole to wash any remaining compound and added to the reaction vessel. The reaction was left to stir for 2 hours at room temperature.

Henry's Reaction - Aldehyde to Ethylene-amine - B3 - 86

The B2 compound was dissolved into 10ml of nitromethane and then 0.480g of ammonium acetate (5.98 mmol, 3 equiv) was added to the solution. The reaction vessel was sited for 2 hours at 90°C. At the 2 hour mark a thin layer chromatography sample was taken and it was determined to let the reaction run for an additional 1 hour as starting material was till present. The reaction mixture was then concentrated, dried, and washed with 20% ethyl acetate/hexane followed by DCM. Both fraction were checked by TLC. The DCM fraction showed to contain the product.

Sodium Borohydride Reduction of alkene into alkane with $NiCl_2$ - 110

In a round bottom flask, 0.102g of compound B3 (1.42 mmol, 1 equiv) was added. Under a nitrogen gas environment 3ml of dry methanol was added into the round bottom flask and dissolved the starting material. The reaction vessel was chilled to 0°C and then 0.030g of NaBH4 (2.83 mmol, 2 equiv) was added along with 0.168 g of $NiCl_2 \bullet H_2O$ (0.709 mmol, 0.5 equiv). The vessel was removed from ice and the solution was left to stir for 1 hour before checking a TLC to determine the progress of the reaction. The reaction was then worked up with a liquid liquid extraction with H_2O and ethyl acetate, and then with 10% MeOH/DCM and H_2O. However, from mass and NMR spectroscopy it was evident that the reduction of the nitro group into an amine was not occurring.

Later reassessment of the sodium borohydride demonstrated that the NaBH4 that was being used had been incorrectly stored and had lost its reactivity. This was tested by dropping some NaBH4 onto ice to test its reactivity with moisture.

General Coupling Reaction - B5 - 138,156

The starting material B4 (13.69 mmol, 1 equiv), which was obtained through commercial means, was dissolved in 35ml of DCM and was basified using ammonium hydroxide (54.76 mmol, 4 equivalents) and was left to stir for 30 minutes in a round bottom flask. The neutralized product was then extracted using DCM and H_2O.

See Coupling Reaction of 2-Chloropyrimidine-5-carboxylic acid with A2.

Coupling Reaction with Fluoropiperidine with B6 - 170,146

Compound B5 (0.756 mmol, 1 equiv) are placed in a round bottom flask and dissolved in 1.5ml of DCM followed by 0.12ml of DIPEA (2.27 mmol, 3 equiv), all under a nitrogen gas environment. Then the fluoromethyl piperidine, FE, (0.756 mmol, 1 equiv) is dissolved in 11.5ml of DMC and added to the initial reaction vessel and stirred for 24 hours at room temperature. The reaction vessel was then neutralized with NH_3Cl and the reaction was separated with a liquid liquid extraction of 10% MeOH/DCM and H_2O. The product solution was then purified through on a combiflash purification system.

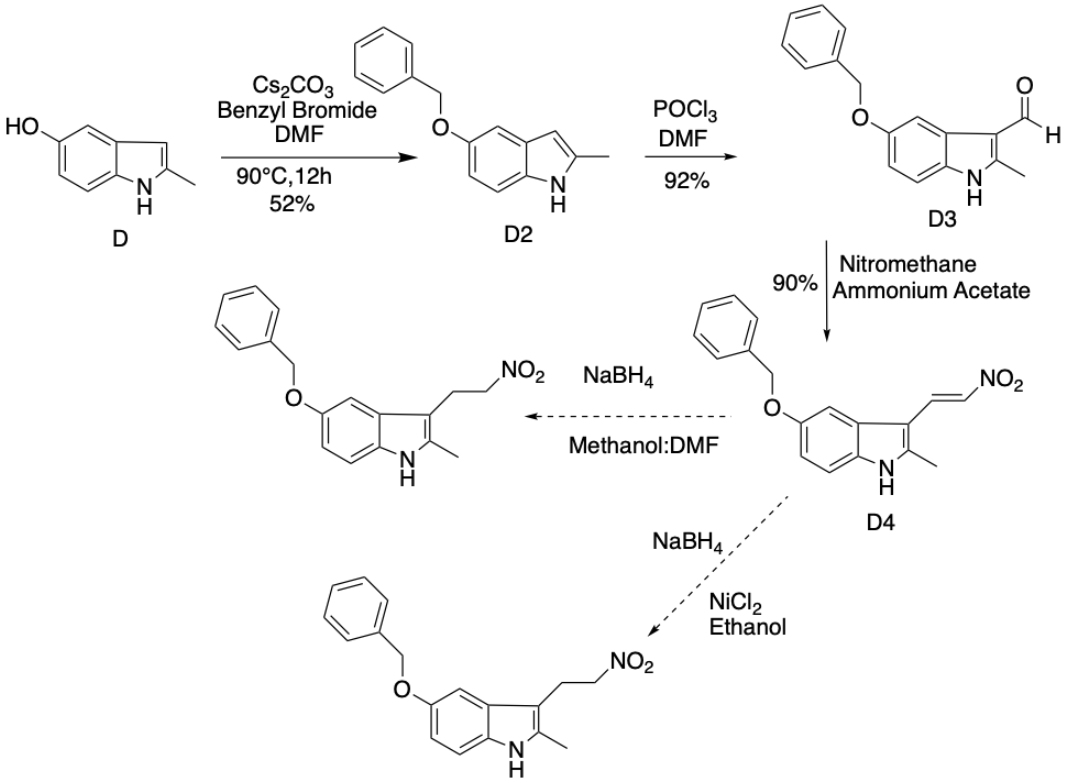
Reduction of Nitro to Amine via Zinc Power in Acidic Conditions - Unsuccessful

50mg of starting material B6 (0.114 mmol, 1 equiv) was suspended in a round bottom flask with anhydrous methanol and zinc powder (0.375 mmol, 3.3 equiv) was subsequently added. Then 6.6 ml of 2N HCl. The reaction vessel was refluxed at 65°C for 2 hours.

Reduction of Nitro to Amine via Hydrogenation of Pd on Carbon - Unsuccessful

50mg of the staring material B6 (0.114 mmol, 1 equiv) was added into a round bottom flask and 10ml of anhydrous methanol was added. Then with additional precautions taken within the hood and with technique approximately 10mg of palladium on carbon was added into the reaction vessel and kept under hydrogen gas at 2 atmospheres for 24 hours.

Synthesis of 2-Methyl-5-hydroxyindole to 2-(4-(fluoromethyl)piperidin-1-yl)-*N*-(2-(5-hydroxy-2-methyl-1*H*-indol-3-yl)ethyl)pyrimidine-5-carboxamide



Scheme 3 - Compound D

Addition of Boc as a Protecting Group - D2 - 93/97

In a 50ml round bottom flask 0.50 grams of 2-Methyl-5-hydroxyindole (3.4 mmol, 1 equiv) and under a nitrogen gas environment 7ml of DMF was added to dissolve the compound. Following this 1.28 grams of Cs_2CO_3 (3.74 mmol, 1.1 equiv) was added to the reaction vessel followed by 0.30ml of benzyl bromide (2.72 mmol, 0.8 equiv). Extra precautions were taken

with the addition of benzyl bromide due to its high potential for toxicity. The reaction was then refluxed at 90°C for 12 hours.

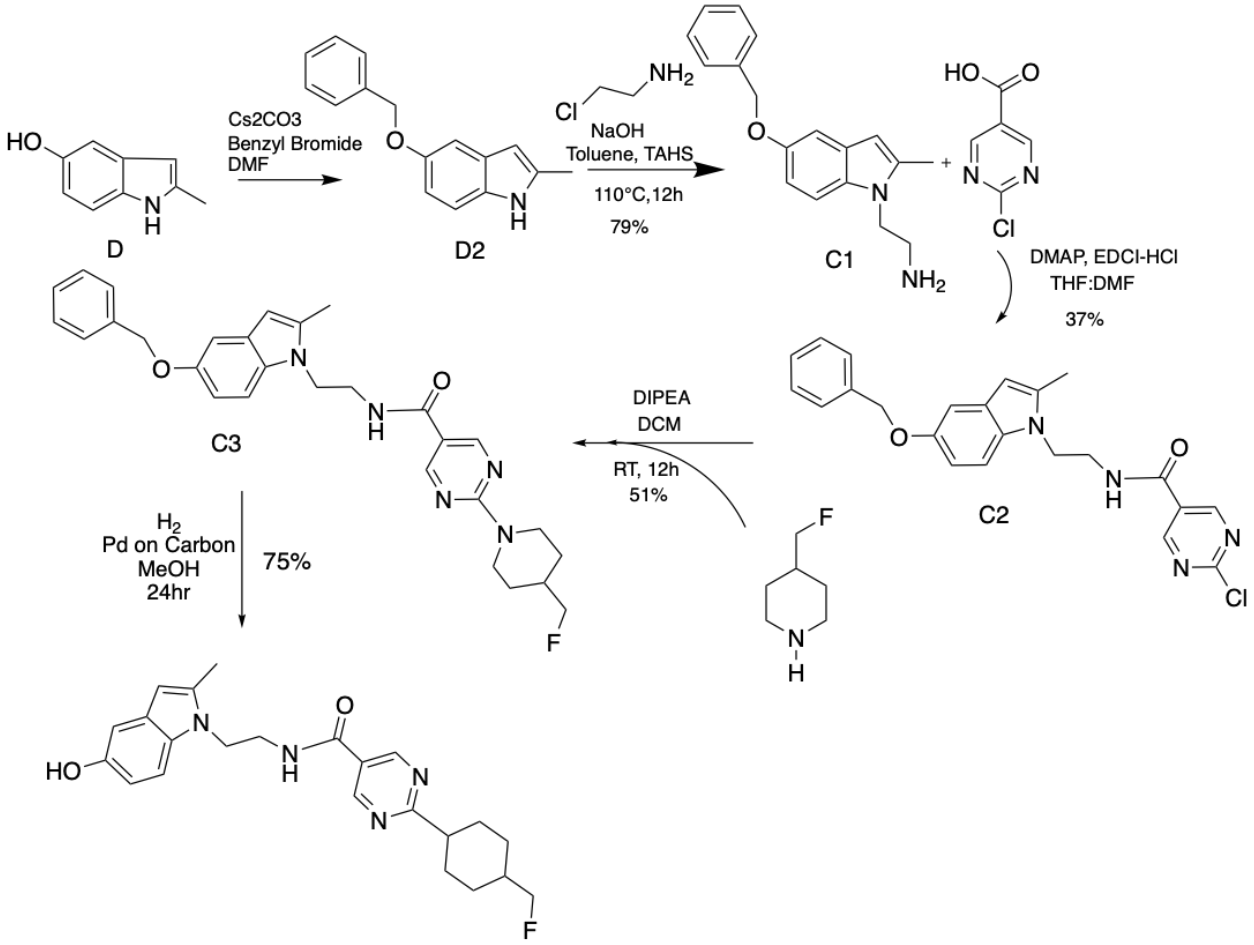
Vilsmeir Reaction - D3 - 101

See Vilsmeir Reaction with 2-Methyl-5-Nitroindole

Henry's Reaction - D4- 108/138

See Henry's Reaction - Aldehyde to Ethyleneamine

Synthesis of 2-Methyl-5-hydroxyindole to 2-(4-(fluoromethyl)piperidin-1-yl)-*N*-(2-(5-hydroxy-2-methyl-1*H*-indol-1-yl)ethyl)pyrimidine-5-carboxamide



Scheme 4. Compound C

Ethylenamine Addition to C1 - 162

See Addition of Ethylenamine to 2-methyl-indole

Coupling Reaction - C2 - 200

See Coupling Reaction of 2-Chloropyrimidine-5-carboxylic acid with A2

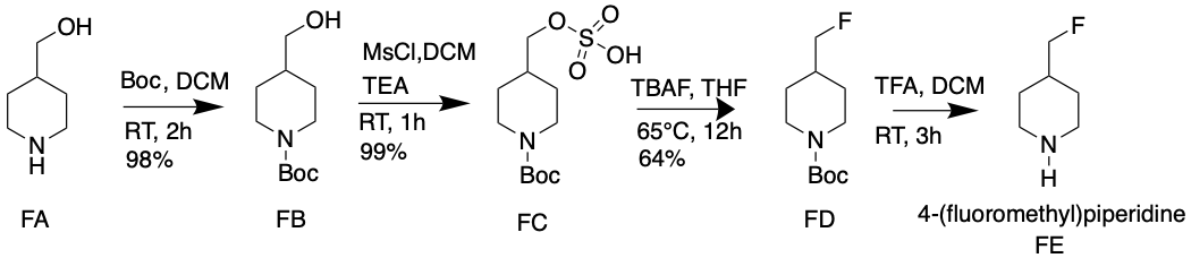
Coupling Reaction with 4(fluoromethyl)piperidine - C3 - 206

With the fluoro compound FE adequately basified to a pH of 8 with DIPEA 5ml of solution (1.15 mmol, 1.2 equiv) of the compound were added to a round bottom flask containing C2 (0.95 mmol, 1 equiv) in 2ml of DCM. An additional 0.26ml of DIPEA (1.5 equiv) were added to the reaction vessel under a nitrogen gas environment. The reaction was left to stir for 12 hours at room temperature. The final reaction was then separated using DCM and chilled water in a liquid liquid separation and subsequently condensed and purified on a silica column.

Deprotection of the Benzyl Protecting Group back to a Hydroxyl group - C4 - 210

50mg of the staring material B6 (0.114 mmol, 1 equiv) was added into a round bottom flask and 10ml of anhydrous methanol was added and stirred to dissolve the starting compound. With additional precautions taken within the hood and with careful technique, approximately 10mg of palladium on carbon was added into the reaction vessel and kept under a hydrogen gas balloon set up for 24 hours. After the reaction was completed a TLC was taken to confirm no starting material was present. The palladium on carbon was filtered out using a center funnel with Cellite and the RB and funnel were washed with 20ml of MeOH three times. The filtered solution was then concentrated and purified.

Synthesis of 4-fluoromethyl-piperidine



Scheme 5. Reaction scheme for the synthesis of 4-(fluoromethyl)piperidine

Boc Protecting of the Nitrogen on Piperidine - FB

A solution of 4-methylhydroxy piperidine, FA, (44.96 mmol, 1.00 equiv) in 50ml of DCM was chilled to 0°C in 150ml round bottom flask. Tert-Butyloxycarbonyl (49.46 mmol, 1.1 equiv) was added intermittently and stirred for 2 hours. Reaction was quenched with chilled H_2O and extracted with DCM and H_2O in a liquid liquid extraction.

Hydroxy to O-methylsulfate - FC

In a round bottom flask with 50ml of DCM 5.0 grams of FB (23.26 mmol, 1 equiv) was dissolved. TEA (34.89 mmol, 1.5 equiv) was added at -10°C, and stirred for 10 minutes. Then MsCl (27.91 mmol, 1.2 equiv) was added incremental and stirred for 1 hour at room temperature. Ammonium chloride was used to quenched the reaction and then was extracted with DCM and H_2O in a liquid liquid extraction.

Methylsulfate to Fluorine - FD

In a round bottom flask of FC (23.17mmols, 1 equiv) dissolved in TBAF in 1M THF (115.89 mmol, 10 equiv) the reaction vessel was then refluxed at 65°C, for 24 hours. The solution was extracted with H_2O and ethyl acetate in a liquid-liquid extraction.

F-Boc Deprotection - FE

The protocol used for deprotecting the Boc group was to dissolve the piperidine compound, FD (2.30 mmol, 1 equiv), in 10ml of DCM per 1 gram of starting material. Then in the round bottom flask was chilled to 0°C in an ice bath before adding trifluroacetic acid (8.06 mmol, 3.5 equiv). The reaction is checked every hour via a TLC with 30% ethyl acetate/hexane to determine if there is still starting material left over and the reaction was left to run for an additional hour, for a total of 3 hours. The solution was then neutralize to a pH above 7 with DIPEA and checked with pH strips. With only one TLC spot in 10% Ethyl Acetate / Hexane, with no evidence of starting material present when heated with ninhydrin it was assumed that all of the piperidine had been deprotected.

Calculated and Experimental Partition Coefficients

Compound	cLogP	Experimental LogP
A (TG13-154)	1.79±0.47	1.55±0.22
B	0.8±0.47	-
C (TG13-210)	2.07±0.47	0.75±0.03
D	1.22±0.47	-

Table 1. The partition coefficients of target compounds within octanol and water as LogP values. cLogP is determined through chemdraw estimation calculations. Experimental LogP was determined from the average of three ratios of peak areas from HPLC. No experimental data was gathered for B and D as they were not fully synthesized.

The initial Log P values of the compounds that are indicative of the membrane permeations were calculated from the calculations performed on Chemdraw. These calculations take into account numerous factors that contribute to the polarity and characteristics that play into the separation of the compound between Octanol and water. The methods are based upon 94 atomic contributions evaluated by a least squares analysis. This method works with a standard deviation of 0.47 LogP units and is able to calculate values for compounds containing hydrogen, oxygen, nitrogen, sulfur and halogen. However, to determine whether these estimations are accurate for the types of compounds produced the final compounds are also assessed for the experimental partition coefficient. This is performed by taking 6 milligrams of the final

compounds and dissolving it into 3ml of Octanol and 3ml of water to obtain a final total concentration of 1milligram per milliliter. This mixture was then left to shake over night to fully achieve equilibrium between the separation of the compound into the organic and aqueous layer. A 0.5ml sample from each layer is then taken and dissolved in 0.5ml of methanol. The samples are then processed via high pressure liquid chromatography to compare the ratios of the compound peak areas to produce the separation ratios. The use of a control of solely octanol and water was used to verify the impacts of the particular solvents on the area of the peaks produced. Three samples were taken from each layer and the average area of the compound peaks were taken to determine the ratio of the partitioning into each solvent layer. The total peak area was then also compared to an equal concentration solution of the compound in DMSO to ensure consistency in the total area of compound detected.

Discussion

The complete synthesis of compounds A (TG13-154) and C (TG13-210) were the two successfully created compounds out of the total of four target compounds. The major differences in the reaction schemes for all four compounds were in regards to the position in which the addition of the ethylene amine was added to on the starting indole, either the 1' position on the nitrogen or the 3' position. Due to the significant cost of starting materials reactions were kept in initially small batches and then upon confirmation of the desired product, through LCMS and $_1$H NMR spectroscopy, larger batches were subsequently followed up. The initial Henry's and Vilsmeir reactions used for compounds B and D proved to be a challenge as the purification of

the compounds after the workup required multiple silica columns to be run in order to achieve adequate purification. These procedures were optimized during the course of this study as to create an optimal solvent ratio of THF to DMF of 8:2. The first major hurdle that was encountered in the proposed synthetic pathway for the B and D target compounds was in the reduction reactions of the alkene into an alkane along with the reduction of a nitro group to an amine. This was present for both the 5'hydroxy and 5'amine, compounds D4 and B3, where ethylamine was synthesized on 3' position of the indole. A multitude of differing reaction schemes were utilized in order to reduce the nitros into amines. However, there was either little success or only partial reductions that did not yield any substantial amount of desired product to be isolated for the next step of the synthesis. The use of sodium borohydride and nickel catalyzed reactions in solvents such as ethanol and methanol:DMF did not work under the conditions set. The one reaction that might have proven to be the most successful in achieving this difficult reduction would have been a hydrogenation reaction with palladium on carbon, but due to the safety constraints of the lab facilities there were few opportunities to utilize the resources of a lab with such safety conditions necessary to maintain a hydrogen tank and pressure apparatus. It was theorized based upon the NMR spectroscopy data that the reactions were providing partially reduced nitro groups or that the reactions were solely reducing the alkene into an alkane with no affect on the nitro group.

However, the synthesis of the target compound D with the hydroxy functional group was halted at this step in order to prioritize alternative reaction schemes. For compound B however the synthesis of the ethylene amine was circumvented utilizing commercial available compounds. The subsequent reactions of the coupling of the 2-chloropyrimidine-5-carboxylic

acid and then the fluoromethyl piperidine proceeded with little complications. However, once again it was in the final step of the reduction of the 5' nitro group on the indole to an amine that was unsuccessful in multiple reaction conditions. Similar to the reductions attempted on the 3' ethylene nitro group into an alkane and amine, there was not a complete reduction present in the NMR and mass spectroscopy measurements taken from either of the purified reactions. Within the analyzed major spots separated on a silica column it was discerned that the starting material was still present in a major quantity along with a partial reduced nitro group into a hydroxyl amine. Future attempts at such a hydrogenation reaction for the reduction should be made in higher pressure conditions and the adequate purging procedures taken to ensure an anhydrous environment. The use of a Parr Shaker type hydrogenated would allow for the optimal conditions to be created, where the rigorous shaking under pressures up to 5 atmospheres are able to be obtained.

Where success was found was in the target compounds synthesized focusing on the 1' position of the indole. This was due to an alternative reaction pathway that was able to be utilized for the synthesis of the ethylamine on the nitrogen of the indole. The use of chloroethanamine allowed for the circumvention of the Vilsmeir and Henry's reaction to create the ethylenamine necessary for the subsequent coupling reactions. This reduction in reaction steps greatly minimized the complexity of the schemas for compounds A and C, allowing for their coupling reactions to be completed. The synthesis of the final coupling with a fluorine piperidine allowed for an effective confirmation of the final compound via ^{19}F NMR spectroscopy. The deprotection of the benzyl protecting group on compound C was successfully performed utilizing the hydrogenation reaction that was used unsuccessfully for the reduction of

the nitro in the amine. This is likely due to the stability of the aromaticity of the benzyl group promoting the leaving group substitution. The notable difference between compounds A and C that may likely impact both selectivity and their resulting permeability through the blood-brain barrier is the differences on the two ends of the compounds. On the indole section of the compounds the presence of the hydroxyl group in compound C will likely be a source of additional hydrogen bonding and polar interactions with proteins. The lack of this polar group on Compound A is likely the reason for its lower calculated Log P. The other difference being on the final ring coupled onto the compound that contains the fluorine. For compound A a 2-fluoroethyl-piperazine was added while for compound C a fluoromethyl-piperidine was utilized. This was to further assesses novel analogues as in previous studies done within the Ganesh lab the equivalent compound of Compound A with a fluoromethyl-piperidine had already been synthesized.

Despite the unsuccessful synthesis thus far for compounds B and D, the completed compounds of A and B demonstrate a noteworthy success of the addition of a fluorine onto a potential EP2 receptor antagonist. Presenting the potential to substitute a radioactive fluorine in its place to create a radioligand through methods of substitution of a tosylate or mesylate in the position of the fluorine. The desired property of the target compounds were to also have increased permeation across the blood brain barrier during the PET study and the calculated and experimental LogP's determined are promising indicators for their improved success. For compound A, the difference between the LogP that was calculated versus the experimental came out to a difference of 0.24. For compound C the difference was significantly higher at an order of 1.32, which is either an indicator for the lack of sophistication it the software models prediction

capabilities or to the methods utilized in the measurements of the experimental LogP. However, with both LogP indicating favorable partition coefficients that are within the target range of membrane permeation that is a green light towards their progression as potential radioligands for the detection of inhibiting EP2 receptors in neuroinflammatory studies.

Future Studies

The necessary steps in determine whether these target compounds are viable for further assessment is in determine several key biochemical properties that pertain to their desired effect within model systems and towards the EP2 receptor. With the completed target compounds and their experimental Log P values determined to be adequate the next step would be testing their reactivity with cells expressing the EP2 receptor. With biological cell assays to determine both their reactivity and selectivity it would all for the determination of whether the changes in the functional groups on the indole or the difference between a piperidine versus piperazine are major contributing factors to the inhibition of the EP2 receptor. This would then allow for the assessment of the compound as an actual radioligand within PET studies. As the Log P values in both the calculated and experimental show favorable partition coefficients the preparation of the compound as a radioligand should be examined. This would involve optimizing the conditions in which the fluorine can be substituted with a tosylate or mesylate group, then subsequently substituted back with another fluorine. An optimized procedure of the addition of a fluorine will need to be fully prepared in order for the compound to be passed along to a radio synthesis lab for injection into a live rodent model system. However, this would allow for the potential

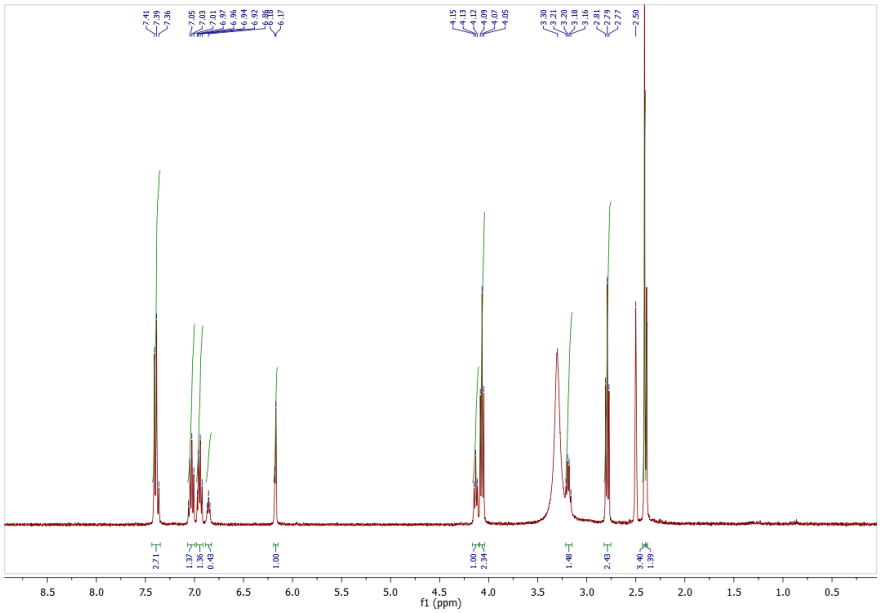
illumination of the mechanism of action for the inhibition of the EP2 prostaglandin receptor further advancing potential treatments in cases of neuroinflammation.

Results

NMR Data

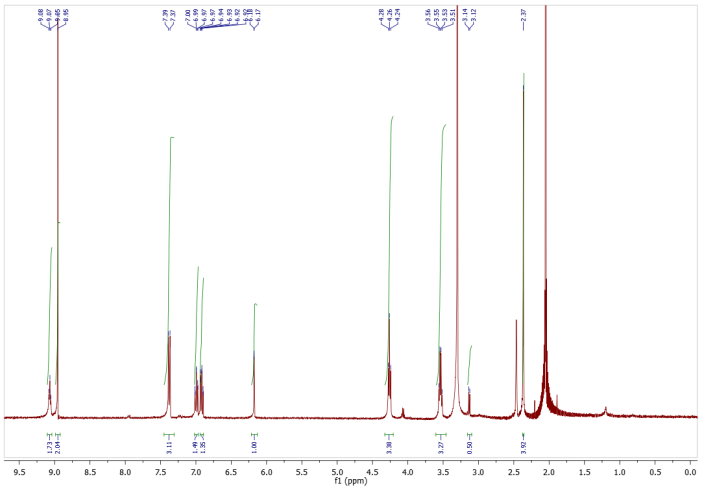
TG13-126-A2

^{1}H NMR (400 MHz, dmso) δ 7.39 (t, *J* = 8.9 Hz, 6H), 7.03 (dd, *J* = 14.6, 7.2 Hz, 3H), 6.95 (dd, *J* = 13.8, 6.0 Hz, 3H), 6.86 (t, *J* = 5.7 Hz, 1H), 6.17 (d, *J* = 4.3 Hz, 2H), 4.13 (t, *J* = 6.8 Hz, 2H), 4.07 (t, *J* = 7.0 Hz, 5H), 3.19 (dd, *J* = 13.2, 6.8 Hz, 3H), 2.79 (t, *J* = 7.0 Hz, 5H), 2.41 (s, 7H), 2.39 (s, 3H).



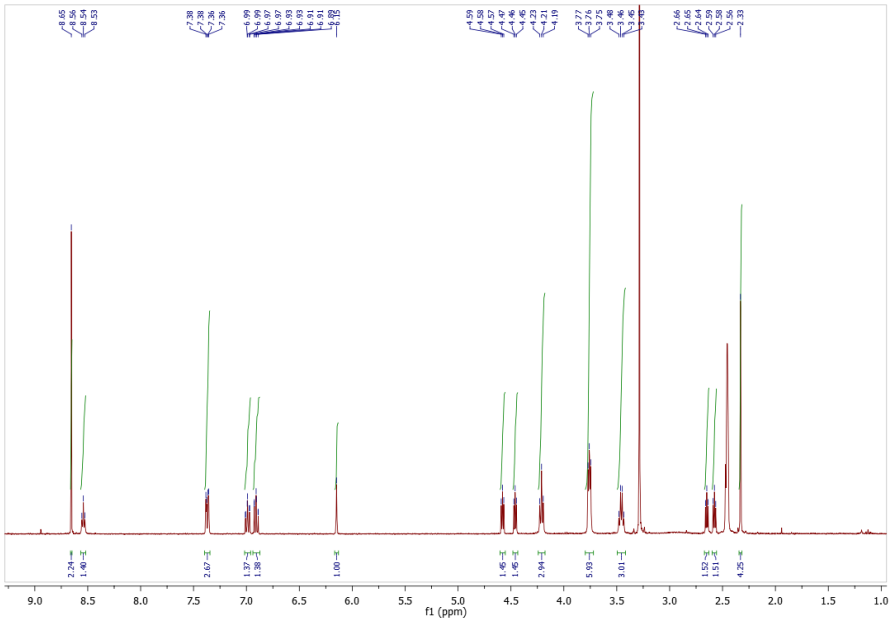
TG13-152-A3

^{1}H NMR (400 MHz, dmso) δ 9.07 (t, *J* = 5.8 Hz, 10H), 8.95 (s, 12H), 7.38 (d, *J* = 8.9 Hz, 18H), 7.02 – 6.97 (m, 9H), 6.94 – 6.89 (m, 8H), 6.18 (d, *J* = 0.8 Hz, 6H), 4.26 (t, *J* = 6.6 Hz, 20H), 3.54 (q, *J* = 6.4 Hz, 19H), 3.13 (d, *J* = 5.2 Hz, 3H), 2.37 (s, 23H).



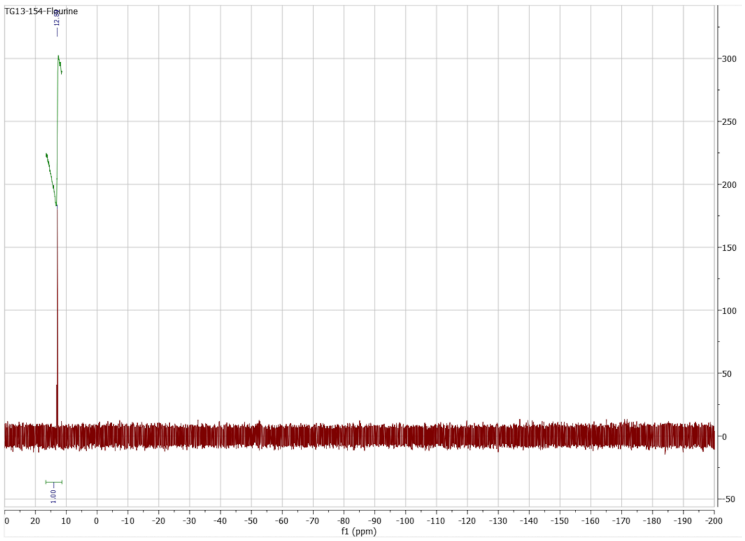
TG13-154 - A4- Final Product

^{1}H NMR (400 MHz, dmso) δ 8.65 (s, 2H), 8.54 (t, *J* = 5.7 Hz, 1H), 7.37 (dd, *J* = 7.7, 3.0 Hz, 2H), 7.02 – 6.96 (m, 1H), 6.91 (td, *J* = 7.5, 0.9 Hz, 1H), 6.15 (s, 1H), 4.60 – 4.55 (m, 1H), 4.48 –



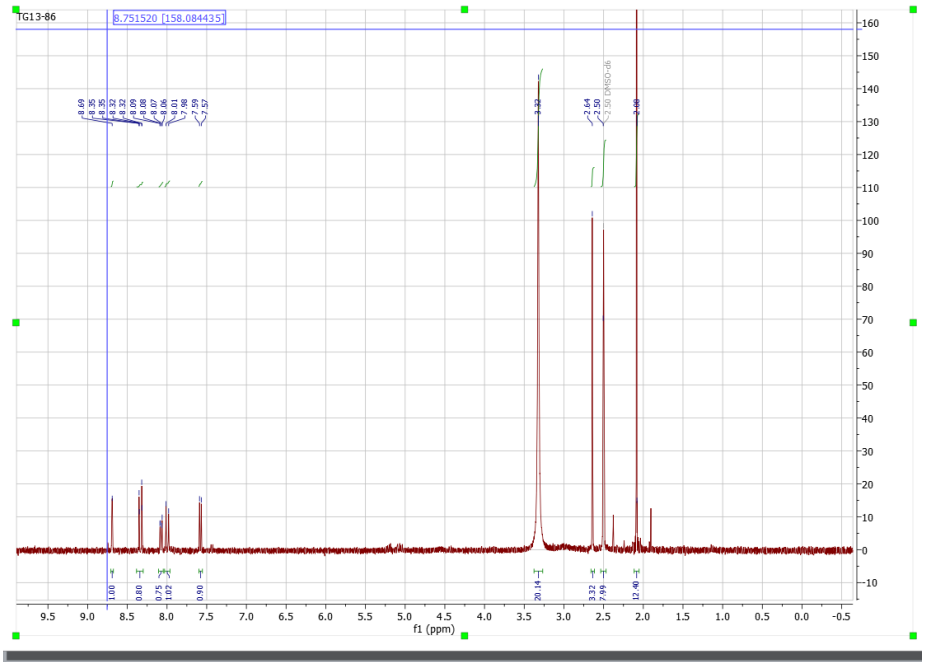
4.44 (m, 1H), 4.21 (t, *J* = 6.6 Hz, 2H), 3.80 – 3.72 (m, 4H), 3.46 (q, *J* = 6.4 Hz, 2H), 2.65 (t, *J* = 4.9 Hz, 1H), 2.58 (t, *J* = 4.9 Hz, 1H), 2.33 (s, 3H).

^{19}F NMR (376 MHz, dmso) δ 12.89.



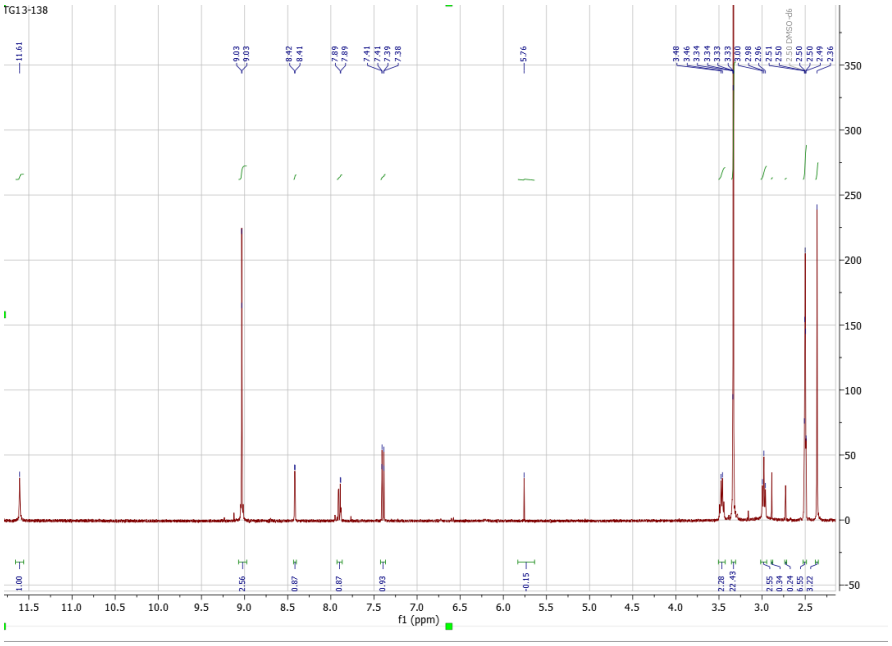
TG13-86 -

^{1}H NMR (400 MHz, dmso) δ 8.69 (s, 1H), 8.33 (dd, *J* = 13.3, 0.8 Hz, 1H), 8.08 (dd, *J* = 9.1, 2.0 Hz, 1H), 8.00 (d, *J* = 13.4 Hz, 1H), 7.58 (d, *J* = 8.9 Hz, 1H), 3.32 (H2O), 2.64 (s, 3H), 2.56 – 2.44 (m, 7H. DMSO), 2.08 (d, *J* = 0.7 Hz, 11H, Acetone).



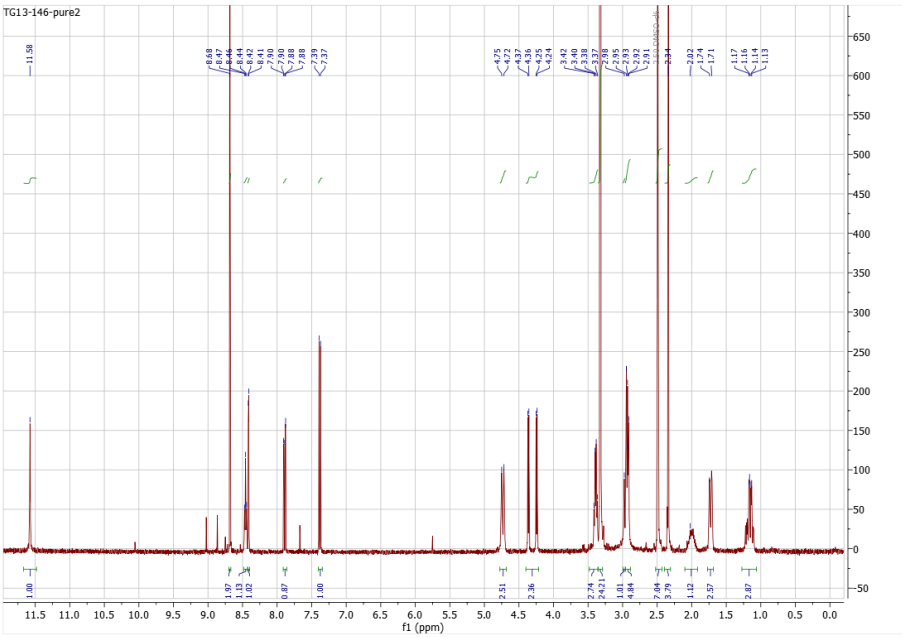
TG-138

^{1}H NMR (400 MHz, dmso) δ 11.61 (s, 1H), 9.03 (d, *J* = 0.5 Hz, 2H), 8.42 (d, *J* = 2.3 Hz, 1H), 7.89 (d, *J* = 2.4 Hz, 1H), 7.40 (dd, *J* = 8.9, 0.6 Hz, 1H), 3.47 (d, *J* = 6.5 Hz, 2H), 3.33 (H2O), 2.98 (t, *J* = 7.1 Hz, 2H), 2.50 (p, *J* = 1.9 H, DMSO), 2.36 (s, 3H).

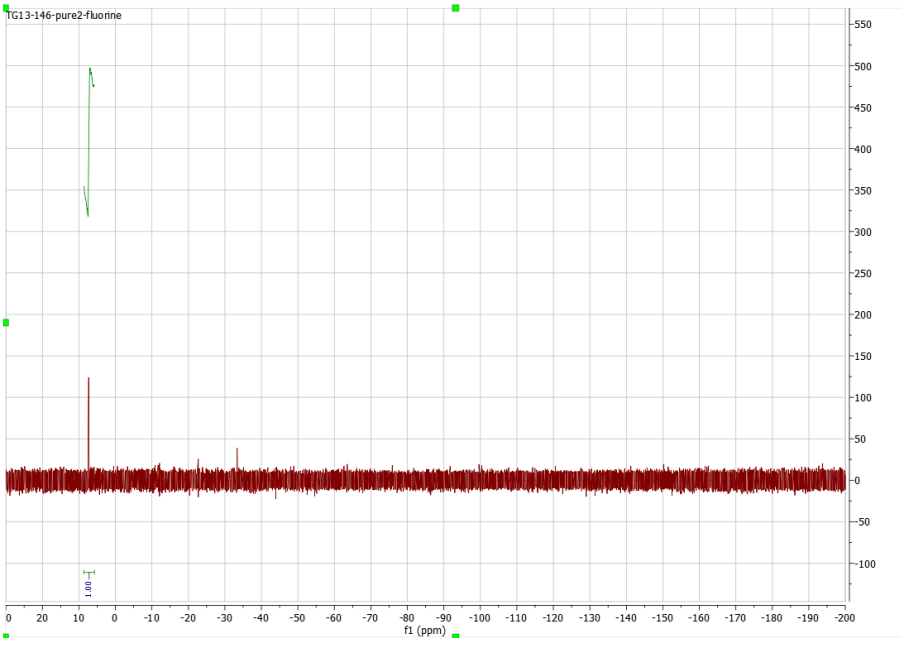


TG13-146/170

^{1}H NMR (400 MHz, dmso) δ 11.58 (s, 1H), 8.68 (s, 2H), 8.46 (t, *J* = 5.8 Hz, 1H), 8.41 (d, *J* = 2.3 Hz, 1H), 7.89 (dd, *J* = 8.9, 2.3 Hz, 1H), 7.38 (d, *J* = 8.9 Hz, 1H), 4.73 (d, *J* = 13.3 Hz, 3H), 4.40 – 4.22 (m, 2H), 3.39 (q, *J* = 6.7 Hz, 3H), 3.32 (s, H2O), 2.98 (s, 1H), 2.96 – 2.89 (m, 5H), 2.49 (p, *DMSO*), 2.34 (s, 4H), 2.02 (s, 1H), 1.73 (d, *J* = 13.0 Hz, 3H), 1.15 (dd, *J* = 12.4, 4.1 Hz, 3H).

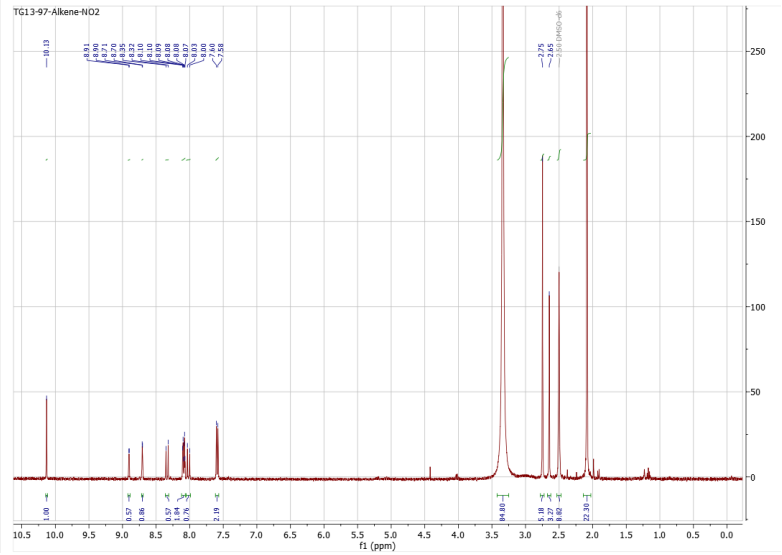


^{19}F NMR (376 MHz, dmso) δ 7.30 (d, *J* = 17.2 Hz).



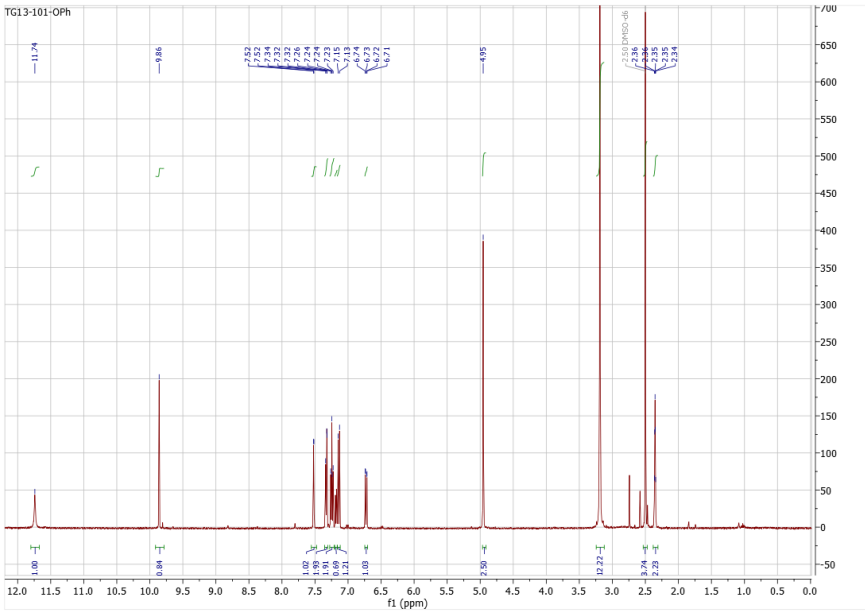
TG13-97/93

^{1}H NMR (400 MHz, dmso) δ 10.13 (s, 1H), 8.90 (d, *J* = 2.4 Hz, 1H), 8.70 (d, *J* = 2.2 Hz, 1H), 8.34 (d, *J* = 13.4 Hz, 1H), 8.09 (dt, *J* = 8.9, 2.5 Hz, 2H), 8.02 (d, *J* = 13.4 Hz, 1H), 7.59 (d, *J* = 8.9 Hz, 2H), 3.34 (s, 85H, H2O), 2.75 (s, 5H), 2.65 (s, 3H), 2.50 (p, *J* = 1.9 Hz, 9H, DMSO), 2.08 (s, 22H, Acetone).



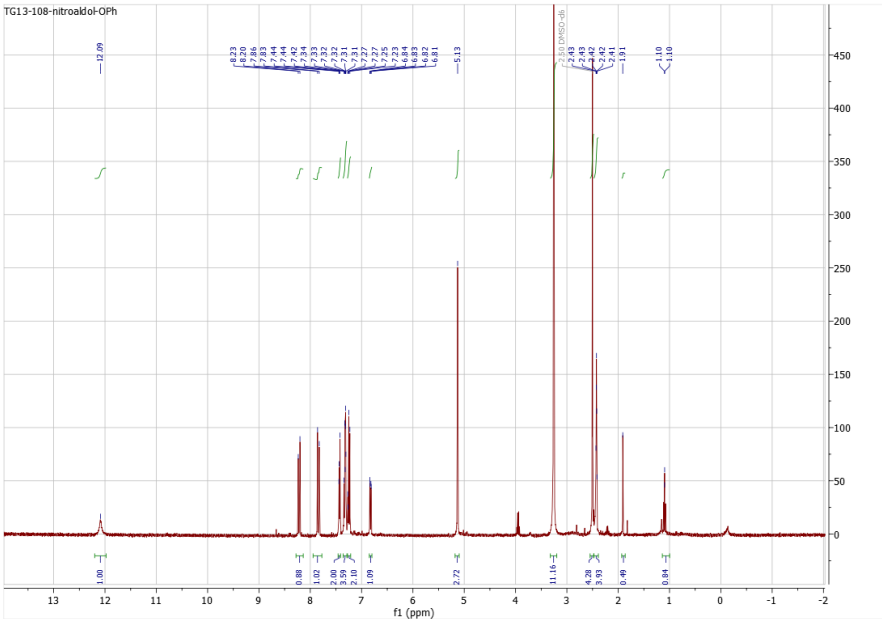
TG13-101

^{1}H NMR (400 MHz, dmso) δ 11.74 (s, 1H), 9.86 (s, 1H), 7.52 (d, *J* = 2.5 Hz, 1H), 7.35 – 7.31 (m, 2H), 7.24 (t, *J* = 7.4 Hz, 2H), 7.18 (d, *J* = 7.3 Hz, 1H), 7.14 (d, *J* = 8.8 Hz, 1H), 6.73 (dd, *J* = 8.7, 2.5 Hz, 1H), 4.95 (s, 3H), 3.19 (s, H2O), 2.50 (s, DMSO), 2.35 (p, *J* = 1.8 Hz, 2H).



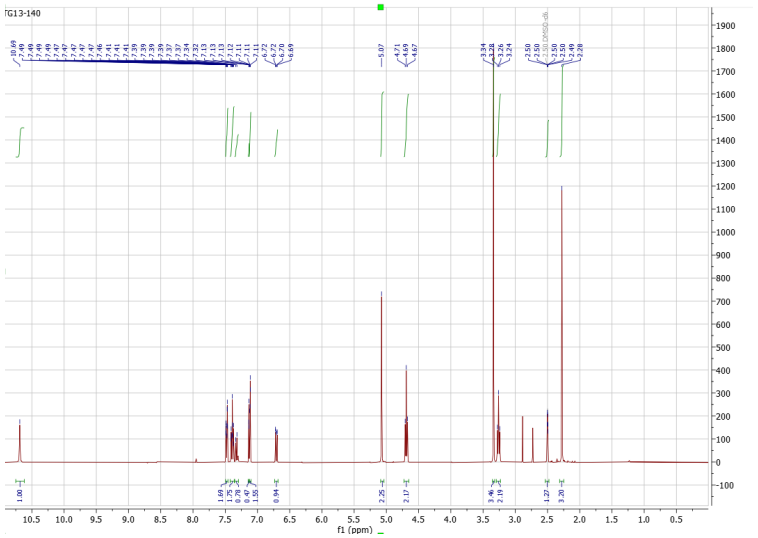
TG13-108

^{1}H NMR (400 MHz, dmso) δ 12.09 (s, 1H), 8.22 (d, *J* = 13.2 Hz, 1H), 7.84 (d, *J* = 13.2 Hz, 1H), 7.43 (d, *J* = 7.1 Hz, 2H), 7.36 – 7.29 (m, 4H), 7.25 (t, *J* = 8.2 Hz, 2H), 6.83 (dd, *J* = 8.7, 2.3 Hz, 1H), 5.13 (s, 3H), 3.26 (s, 11H, H2O), 2.50 (s, 4H, DMSO), 2.42 (p, *J* = 1.9 Hz, 4H), 1.10 (d, *J* = 0.6 Hz, 1H).



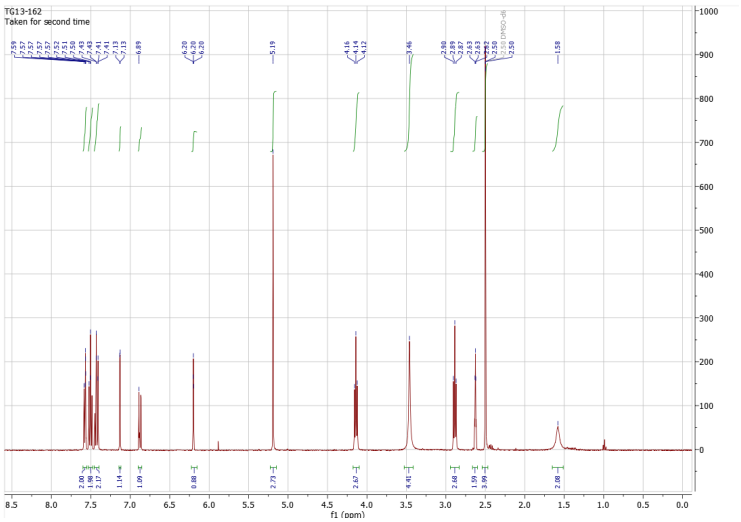
TG13-140

^{1}H NMR (400 MHz, dmso) δ 10.69 (s, 1H), 7.49 – 7.46 (m, 2H), 7.42 – 7.36 (m, 2H), 7.33 (d, *J* = 7.3 Hz, 1H), 7.13 – 7.10 (m, 2H), 6.71 (dd, *J* = 8.7, 2.3 Hz, 1H), 5.07 (s, 2H), 4.69 (t, *J* = 7.0 Hz, 2H), 3.34 (s, 3H), 3.26 (t, *J* = 7.0 Hz, 2H), 2.53 – 2.48 (m, 1H), 2.28 (s, 3H).



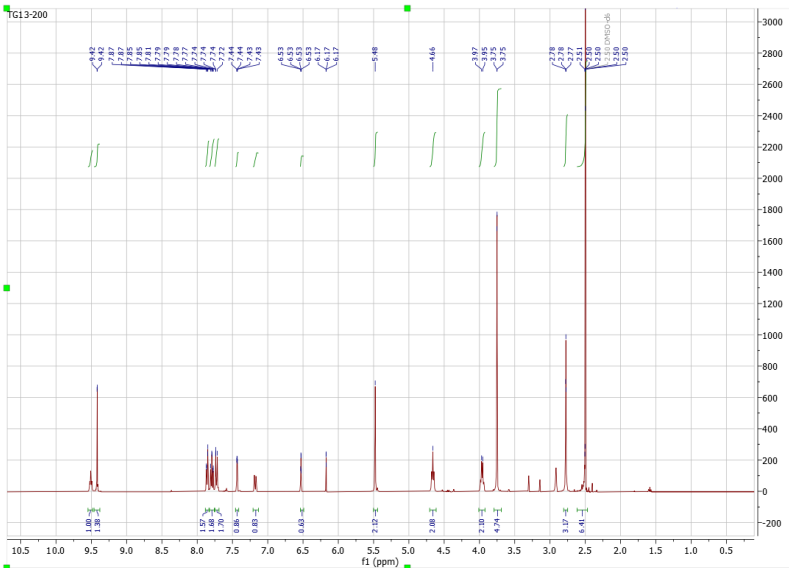
TG13-162

^{1}H NMR (400 MHz, dmso) δ 7.60 – 7.55 (m, 2H), 7.53 – 7.48 (m, 2H), 7.42 (dd, *J* = 8.8, 0.7 Hz, 2H), 7.13 (d, *J* = 2.4 Hz, 1H), 6.89 (s, 1H), 6.23 – 6.15 (m, 1H), 5.19 (s, 3H), 4.14 (t, *J* = 6.9 Hz, 3H), 3.46 (s, 4H), 2.89 (t, *J* = 6.9 Hz, 3H), 2.63 (t, *J* = 1.9 Hz, 2H), 2.50 (d, *J* = 0.9 Hz, 4H), 1.58 (s, 2H).



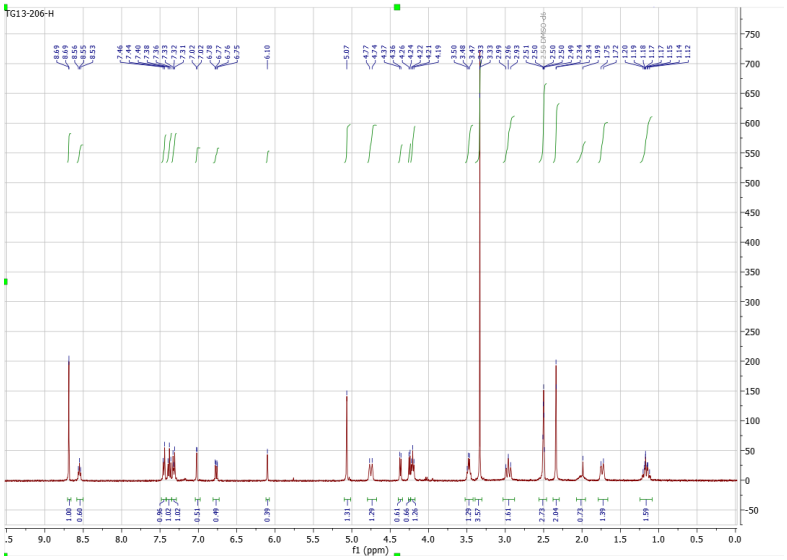
TG13-200

^{1}H NMR (400 MHz, dmso) δ 9.51 (t, *J* = 5.8 Hz, 1H), 9.42 (d, *J* = 1.0 Hz, 1H), 7.86 (dd, *J* = 7.0, 1.3 Hz, 2H), 7.83 – 7.76 (m, 2H), 7.75 – 7.69 (m, 2H), 7.43 (dd, *J* = 2.4, 0.9 Hz, 1H), 7.18 (ddd, *J* = 8.7, 2.5, 1.0 Hz, 1H), 6.53 (q, *J* = 1.0 Hz, 1H), 5.48 (s, 2H), 4.66 (s, 2H), 3.96 (d, *J* = 6.2 Hz, 2H), 3.75 (d, *J* = 1.0 Hz, 5H), 2.78 (d, *J* = 0.9 Hz, 3H), 2.62 – 2.47 (m, 6H).

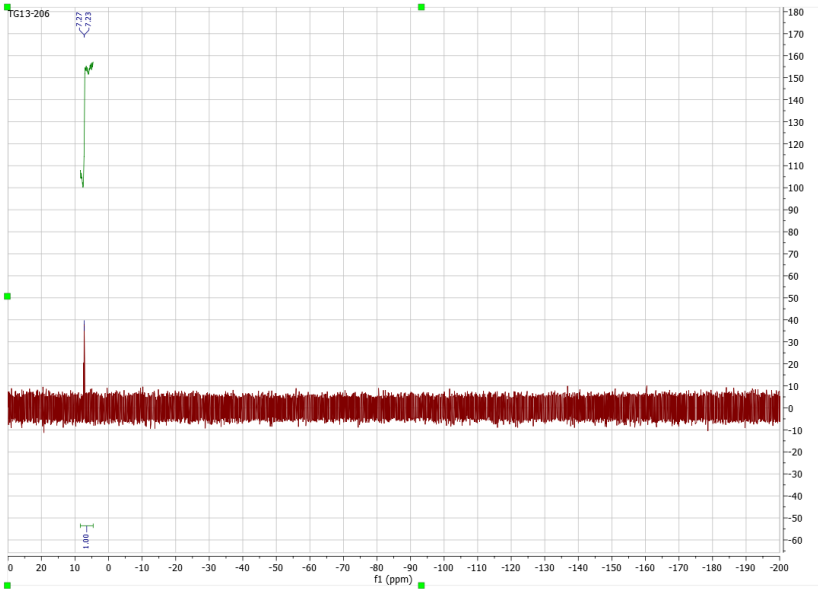


TG13-206

^{1}H NMR (400 MHz, dmso) δ 8.69 (d, *J* = 0.5 Hz, 1H), 8.55 (t, *J* = 5.7 Hz, 1H), 7.45 (d, *J* = 7.6 Hz, 1H), 7.38 (t, *J* = 7.4 Hz, 1H), 7.35 – 7.29 (m, 1H), 7.02 (d, *J* = 2.4 Hz, 1H), 5.07 (s, 1H), 4.75 (d, *J* = 13.2 Hz, 1H), 4.37 (d, *J* = 6.0 Hz, 1H), 4.25 (d, *J* = 6.1 Hz, 1H), 4.21 (t, *J* = 6.5 Hz, 1H), 3.52 – 3.42 (m, 1H), 3.33 (d, *J* = 0.5 Hz, 4H), 2.96 (t, *J* = 12.4 Hz, 2H), 2.50 (p, *J* = 1.9 Hz, 3H), 2.34 (s, 2H), 1.99 (s, 1H), 1.74 (d, *J* = 13.0 Hz, 1H), 1.24 – 1.08 (m, 2H).

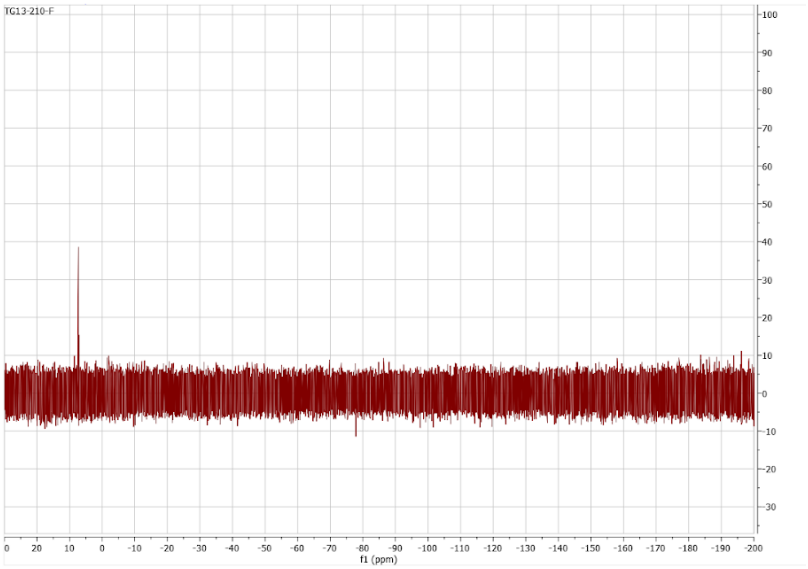


^{19}F NMR (376 MHz, dmso) δ 7.25 (d, *J* = 17.0 Hz).

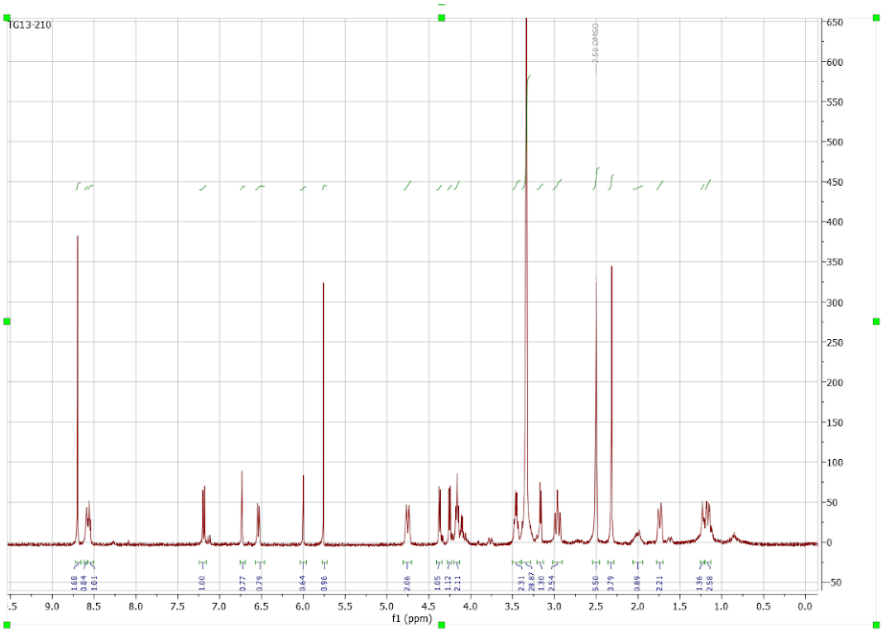


TG13-210

^{19}F NMR (376 MHz, dmso) δ 7.26 (d, *J* = 17.5 Hz).

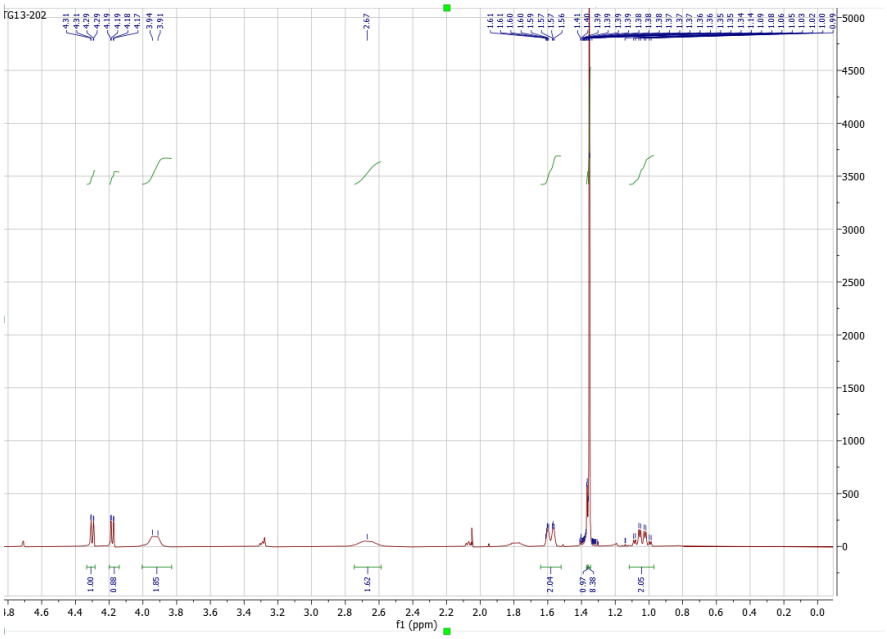


^{1}H NMR (400 MHz, dmso) δ 8.70 (d, *J* = 0.5 Hz, 1H), 8.57 (dd, *J* = 11.8, 6.3 Hz, 2H), 7.22 – 7.09 (m, 1H), 6.73 (d, *J* = 2.3 Hz, 1H), 6.53 (dd, *J* = 8.6, 2.4 Hz, 1H), 6.00 (s, 1H), 5.76 (d, *J* = 0.5 Hz, 1H), 4.75 (d, *J* = 13.4 Hz, 2H), 4.35 (dd, *J* = 13.0, 5.9 Hz, 1H), 4.28 – 4.20 (m, 1H), 4.16 (t, *J* = 6.6 Hz, 1H), 4.11 (q, *J* = 5.2 Hz, 0H), 3.46 (q, *J* = 6.3 Hz, 2H), 3.33 (s, 17H), 3.17 (d, *J* = 5.0 Hz, 1H), 2.96 (t, *J* = 12.5 Hz, 2H), 2.35 – 2.29 (m, 3H), 2.00 (s, 1H), 1.74 (d, *J* = 13.0 Hz, 2H), 1.23 (s, 0H), 1.17 (dd, *J* = 12.3, 4.2 Hz, 1H).



TG13-202

1H NMR (400 MHz, dmso) δ 4.30 (dd, J = 6.0, 1.1 Hz, 1H), 4.18 (dd, J = 6.0, 1.1 Hz, 1H), 3.93 (d, J = 13.1 Hz, 2H), 2.67 (s, 2H), 1.64 – 1.52 (m, 2H), 1.37 (d, J = 1.2 Hz, 1H), 1.36 – 1.35 (m, 8H), 1.04 (qd, J = 12.4, 4.4 Hz, 2H).



MASS from LCMS

Compound A TG13-154 - Molecular Weight 410.5

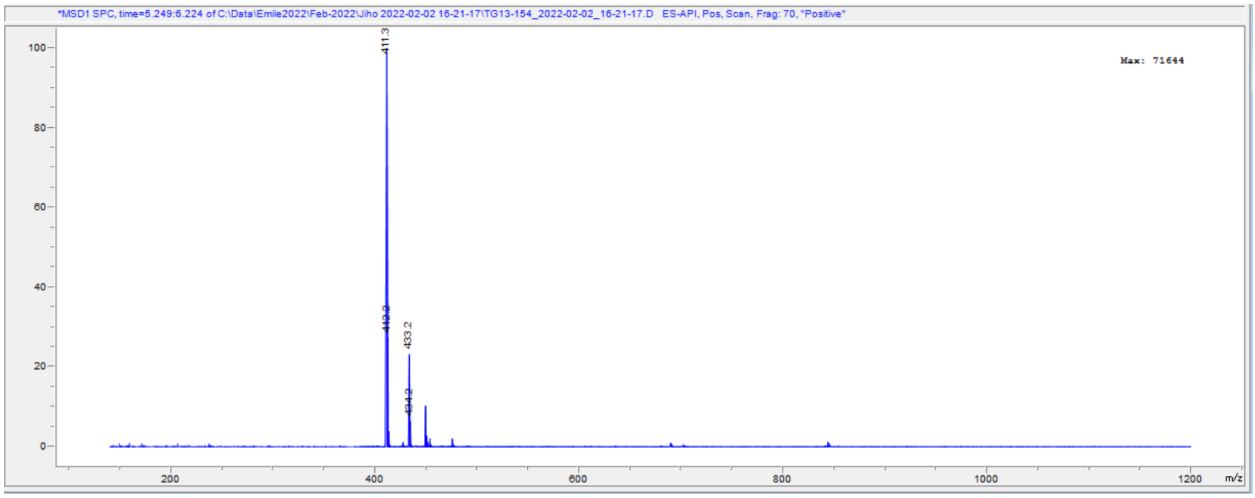


Figure 2. The positive scan from the LCSM data from the purified sample of TG13-154. With a molecular weight of 410.5, it is expected to have a mass of X+H in the positive scan. The 411.3 indicating the compound along with an additional proton species.

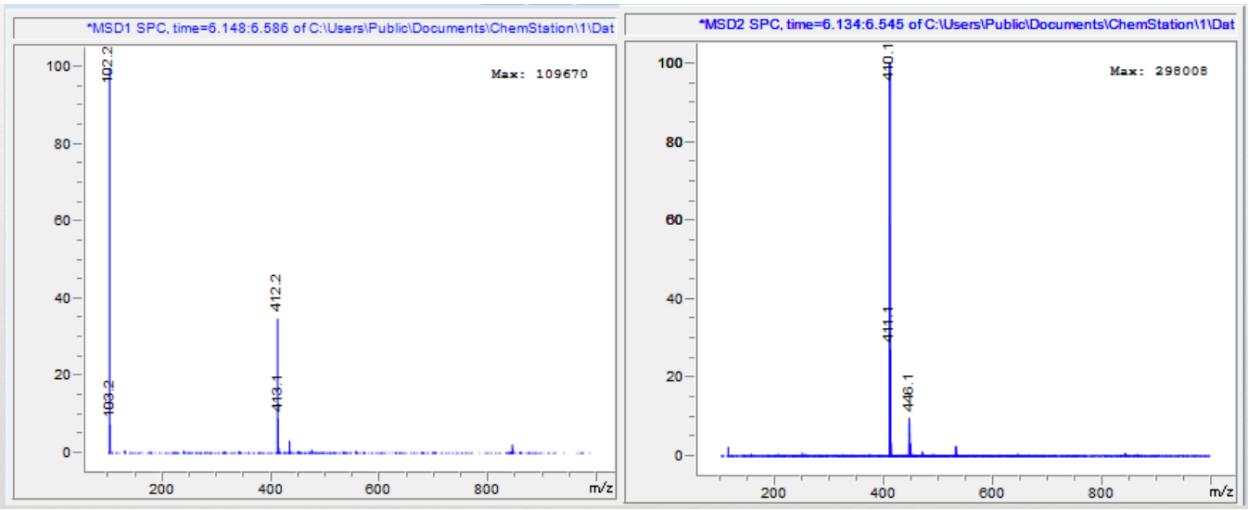


Figure 3. The positive (left) and negative scan (right) from the LCSM data from the purified sample of TG13-210. With a molecular weight of 411.5, it is expected to have a mass of X+H in the positive scan. The 412.2 m/z peak indicating the compound along with an additional proton species. The major 410 m/z peak of the negative scan would be indicative of the X-H ion present.

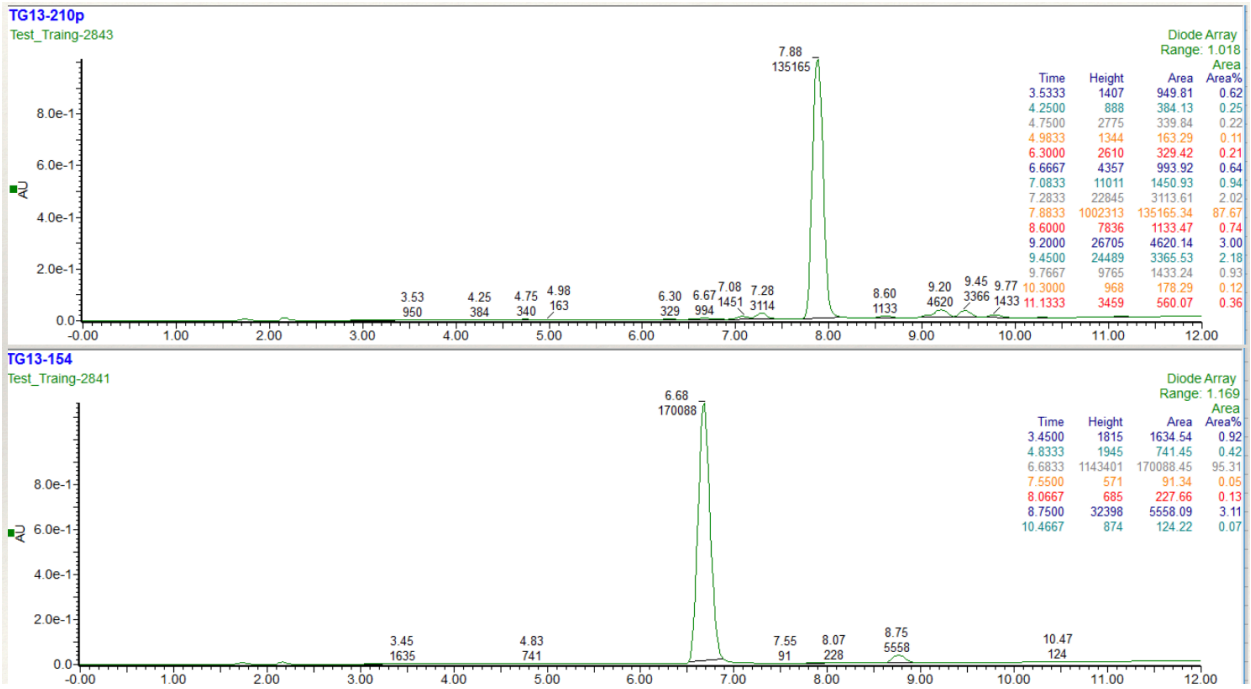


Figure 4. The HPLC data on analytic scans on purified final compounds. The initial solvent peaks around 2.00 minutes have been removed from the total integration analysis. Samples were run in a methanol sample and compared to a blank sample of solely methanol.

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