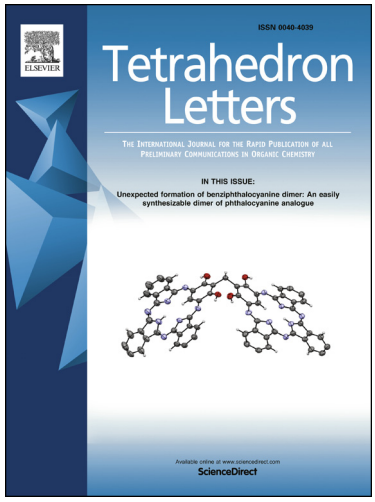

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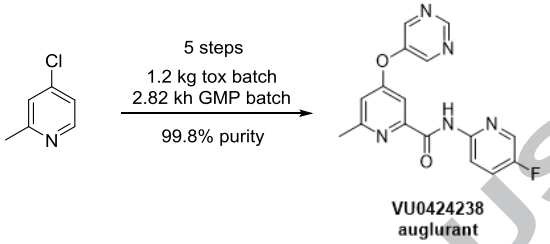

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ABSTRACT

This communication details the kilogram-scale synthesis of *N*-(5-fluoropyridin-2-yl)-6-methyl-4-(pyrimidin-5-yloxy)picolinamide (VU0424238, auglurant), a novel $mGlu_5$ negative allosteric modulator (NAM) developed as an alternative treatment for depression. The process highlights a challenging pyridine *N*-oxidation sequence, an S_NAr reaction, and the elimination of all chromatography steps (required in the medicinal chemistry route) with replacement by highly efficient recrystallizations (save one silica plug). The improved process was utilized for the preparation of a 1.2 kg toxicology batch, as well as a 2.82 kg GMP batch to support the Phase I trial, in very high purity (99.8%).

The class C G protein-coupled receptor (GPCR) metabotropic glutamate receptor subtype 5 ($mGlu_5$) is one of eight mGluRs activated by glutamate (L-glutamic acid).[1] Due to high evolutionary conservation of the orthosteric glutamate binding site and the physiochemical challenges with glutamate analogs, drug discovery efforts have largely focused on allosteric modulation (negative allosteric modulation (NAM) or positive allosteric modulation (PAM)) as the desired mode of pharmacology for these GPCRs.[2-5] Decades of preclinical evidence provide strong support for the therapeutic utility of $mGlu_5$ NAMs for the potential treatment of major depressive disorder, anxiety, fragile X syndrome, Parkinson's disease, autism and Alzheimer's disease.[6-16] Derived from the first $mGlu_5$ NAM MPEP (**1**), several $mGlu_5$ NAMs **2**-**4**, based on a conserved phenyl/heteroaryl acetylene chemotype, have entered Phase II clinical trials, but none have made it to market launch (**Figure 1**).[18-26]

Our $mGlu_5$ NAM effort departed from the prototypical phenyl/heteroaryl acetylene chemotype by virtue of a high-throughput screen that identified fundamentally new chemical matter devoid of the prototypical aryl/heteroaryl acetylene motif.[27-30] During the course of our discovery effort, we identified VU0424238 (**5**), later named auglurant, as a clinical candidate with all of the requisite pharmacological and DMPK properties for advancement into IND-enabling studies.[30] Herein, we report on the development of an efficient process route for the multi-kilogram production of VU0424238 to support both GLP toxicology studies as well as drug product for a human Phase I clinical trial.

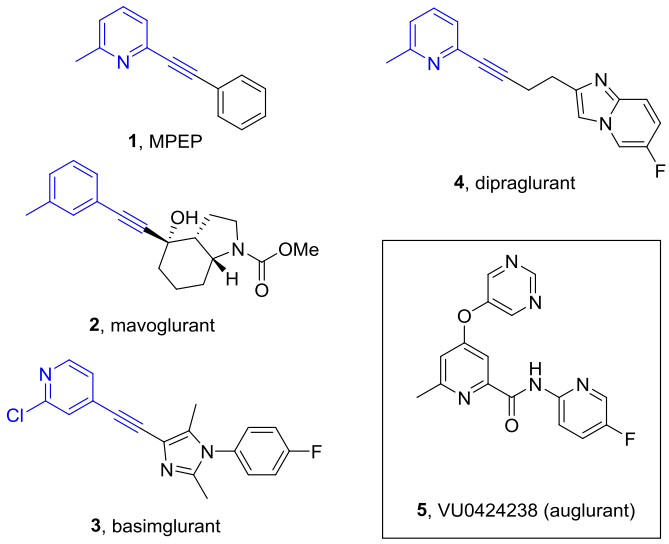
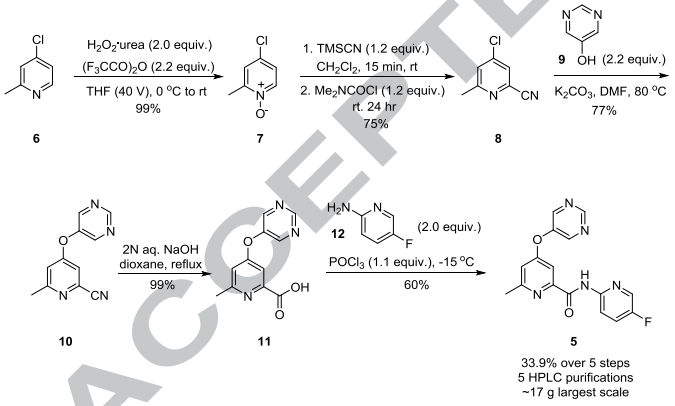


Figure 1. Structures of prototypical and clinical $mGlu_5$ NAMs: MPEP (**1**), mavoglurant (**2**), basimglurant (**3**), dipraglurant (**4**) and the atypical, non-acetylenic VU0424238/auglurant (**5**).

The medicinal chemistry route developed to prepare *N*-(5-fluoropyridin-2-yl)-6-methyl-4-(pyrimidin-5-yloxy)picolinamide (**5**, VU0424238, auglurant) was a five step linear sequence (**Scheme 1**) that proceeded in 33.9% overall yield, and involved five HPLC purification steps.[30] Moreover, this route was adapted from 10 mg scale to a maximum of ~17 g in the medicinal chemistry laboratory. In short, treatment of pyridine **6** with peroxide-urea complex in trifluoroacetic anhydride in dilute THF (40 V), while maintained at 0 °C, delivered the pyridine *N*-oxide **7** in 99% yield. Note, due to potential thermal stability concerns of the crude pyridine *N*-oxide, the temperature was maintained at 0 °C for these scale reactions.[30] The pyridine *N*-oxide **7** is also highly water soluble; thus, we were concerned about both the isolation of **7** and the thermal stability of pyridine *N*-oxide generation on kilo scale. Cyanation proceeded smoothly affording **8**, which then underwent an S_NAr reaction with 5-hydroxypyrimidine to deliver **10** in 77% yield after HLPC purification. Hydrolysis of the nitrile provided acid **11** in near quantitative yield, and a subsequent $POCl_3$-mediated amide coupling with 2-amino-5-fluoropyridine **12** gave the candidate **5** in 60% yield after HLPC purification. While this route provided the quantity of material needed to evaluate and select **5** as a preclinical development candidate, there were a number of issues and concerns to be addressed prior to efficient scale-up of toxicology and GMP batches in the pilot plant: (a) Step 1, the pyridine *N*-oxidation sequence (exotherm risk) and water solubility of the product; (b) Step 3, the S_NAr reaction; (d) the final amide coupling reaction (only 60% on small scale), and (c) the elimination of all HPLC purification steps and replacement with high yielding recrystallization protocols. Moreover, pilot dose efficacy and toxicology escalation studies required extensive pharmaceutical sciences intervention and formulation studies. Ultimately, a spray-dried dispersion (SDD) formulation was developed (12.5% VU0424238

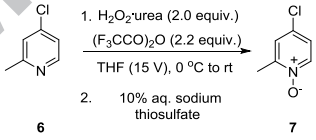
Scheme 1. Original Medicinal Chemistry Route to **5** (VU0424238).



Pilot chemistry was performed at multiple scales (10 g, 25 g, 100g and 500 g) to assess and optimize reaction conditions prior to committing to the 1.2 kg IND-toxicology and 2.82 kg GMP batch. The initial focus was on safety assessment and reaction optimization of step 1, the formation of the pyridine *N*-oxide **7**. Due to the potential for thermal instability of both crude and purified pyridine *N*-oxide **7**, a differential scanning calorimetry (DSC) study and safety evaluation was performed on both crude and purified *N*-oxide **7**. In the event, DSC thermogram of purified **7** displayed a strong exotherm at 121 °C, with heat evolution of 1,495J/g, and the crude **7** lesser so, at 431 J/g! Based on the DSC data, the temperature of the reaction, as well as of the jacket/heating media during reaction concentration, must not exceed 30 °C at any point to maintain a safety margin. Following these temperature guidelines, the process resulted in no thermal stability/safety issues in the subsequent IND-toxicology and GMP batch. However, this was a major concern for the large scale production of **5**, and the concern was realized.

Beyond safety assessment concerns, initial development work echoed earlier considerations on the high aqueous solubility and polarity of **7**, rendering product extraction and isolation tedious. Pilot chemistry indicated that the equivalents of H_2O_2-urea adduct (2.0 equiv.) and TFAA (2.2 equiv.), relative to 1.0 equivalents of **6** were indeed optimal; however, reduction of the solvent volume of THF proved fruitful. In the medicinal chemistry route, 40 V of THF was employed, but this complicated product extraction on large scales. We were able to progressively lower the amount of THF to only 15 V (~63% reduction), which greatly enhanced product extraction. Beyond the reduction in THF, the reaction was quenched with 10% sodium thiosulfate, the aqueous layer saturated with NaCl and extracted with CH_2Cl_2, followed by washing with saturated potassium carbonate to remove excess TFAA and TFA. These modifications provided the desired *N*-oxide **7** in average purity of 97.6% (plus 1.2% **6**) reproducibly from 25 g to 4.25 kg scale batches, and avoided the need for HPLC purification. For both the toxicology batch and GMP batches, **7** was advanced without additional purification (>97.6% purity, with <1.5% of **6**), and the residual **6** would be removed at a later purification step (**Scheme 2**).

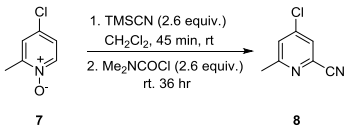
Scheme 2. Synthesis of pyridine *N*-oxide **7.**



Scale	Yield	Purity
25g	84.6%	97.5%
100g	93.3%	97.4%
500 g	94.1%	96.3%
2.5 kg	95.9%	96.4%
4.3 kg	96.0%	97.6%

The cyanation of **7** to provide **8** also required revision of the original medicinal chemistry route.[30] Here, the equivalents of both TMSCN and dimethylcarbamoyl chloride (DMCC) were increased from 1.2 equivalents to 2.6 equivalents, while reducing the DCM solvent volume to 20 V and lowering the reaction temperature to 0 °C. On scale, the optimal reaction time for >80% conversion, as judged by HPLC, was 36 hours. For the pilot chemistry (10-500 g scales) as well as the toxicology and GMP batches, filtration through a silica plug and recrystalization from *n*-heptane afforded 50-52% isolated yields of **8** with HPLC purities >99.75%. Moreover, these conditions afforded no trace of the *N*-oxide **7** and DMCC content was <110 ppm (**Scheme 3**). While finding an alternative to DCM would ideally be environmentally attractive, project timelines required pushing forward.

Scheme 3. Synthesis of cyanide **8.**

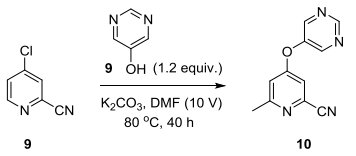


Scale	Yield	Purity
50g	52.8%	99.8%
100g	56.0%	99.9%
2.5 kg	56.2%	99.9%
4.3 kg	51.0%	99.7%

The S_NAr reaction between **8** and 5-hydroxypryimidine **9** was also revised.[30] We were pleased to find that we could reduce the equivalents of 5-hydroxypryimidine **9** from 2.2 to 1.2 equivalents with no diminution in yields. Moreover, chromatography was also avoided. In the event, 5-hydroxypryimidine (1.2 equiv.) and potassium carbonate (3.0

equiv.) in DMF (10V) was treated with **8** at room temperature and subsequently heated to 80 °C for 40 h (>94% conversion by HPLC). At this point, the reaction was cooled to room temperature, and the solid was filtered and washed with DMF. The filtrate was then cooled to 0 °C, and purified water was added dropwise over 1 hour, forming a white precipitate. The precipitate was washed with purified water (10 V) and dried to afford 80-85% isolated yield of **10** in >99.7% purity, without the need for chromatography (**Scheme 4**).

Scheme 4. S_NAr reaction to afford **10.**



Scale	Yield	Purity
50g	78.0%	99.7%
300g	86.4%	99.9%
1.48 kg	85.3%	99.9%
2.3 kg	82.1%	99.8%

The hydrolysis of nitrile **10** to the carboxylic acid **11** afforded three impurities not observed via the reaction scales and conditions of the medicinal chemistry route (**Scheme1**).[30] Pilot scale-up chemistry noted the formation of the primary carboxamide **13** (~1%) and two acrolein impurities **14** and **15** (6-9% combined by HPLC) due to the harsh 90 °C hydrolysis conditions (**Figure 2**); however, all three Impurities could be eliminated or greatly reduced in the work-up procedure (note, **15** is present in final **5** at ~0.05%).

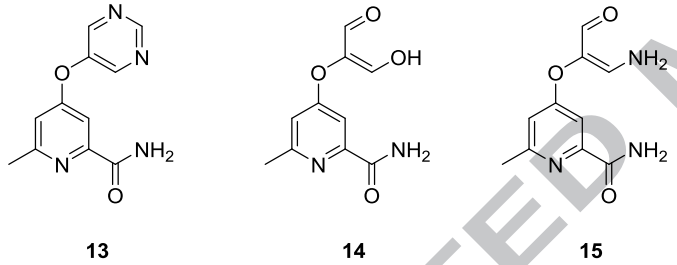
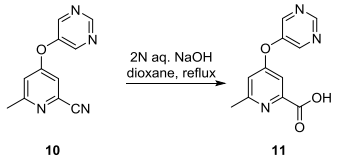


Figure 2. Impurities **13-15** generated under the harsh amide hydrolysis conditions.

In the event, hydrolysis of **10** with 2 N NaOH (10 V) in 1,4-dioxane (15 V) at 90 °C for 6 hours afforded conversion to **11** (with low levels of **13-15**). Dilution with DCM and subsequent washing of the aqueous layer with DCM, afforded an aqueous solution that was acidified to pH 3-4 using 6 N HCl. The aqueous layer was then evaporated, resuspended in 10% MeOH/DCM (50 V) and stirred at 50-55 °C for 1 h. The undissolved solid was filtered and the solvent evaporated to afford acid **11** in 75-80% isolated yield and in purities ranging from 94.4 to 99.7% (**Scheme 5**).

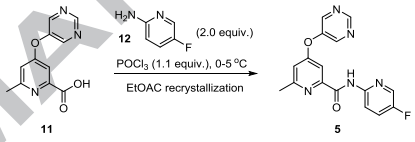
Scheme 5. Nitrile hydrolysis to afford **11.**



Scale	Yield	Purity
50g	86.4%	95.1%
200g	87.0%	95.6%
1.71 kg	77.3%	94.8%
2.45 kg	78.4%	94.7%

For the final amide coupling step between **11** and **12**, reaction conditions were once again revised from the original medicinal chemistry route.[30] Here, the equivalents of **12** was reduced from 2.0 to 1.3 equivalents, and the temperature was maintained between 0-15 °C with only 15 V of pyridine. In addition, the final HPLC purification employed by the medicinal chemistry team was replaced by a highly efficient recrystallization from ethyl acetate (**Scheme 6**). This protocol afforded **5**, *N*-(5-fluoropyridin-2-yl)-6-methyl-4-(pyrimidin-5-yloxy)picolinamide (VU0424238, auglurant), in yields averaging 50% and with high purities >99.5% by HPLC (no single impurity was >0.05% and total impurities 0.19%).[31] Release testing of the GMP batch by validated analytical methods revealed a purity of the API of 99.8%, Total impurities of 0.19% with individual impurities at 0.02-0.07%, 0.08% residue on ignition, 0.52% water content by KF and a clean melt at 167.25° (by DSC). Residual solvents, metals and microbial analysis met specified guidelines. A portion of the GMP batch was spray dried with HPMCAS-M yielding a stable, amorphous 12.5% SDD that was then incorporated into a blend formulation and encapsulated in 5 and 20mg capsule strengths. Stability studies of the VU0424238 API and SDD Tox batches at 6 months under normal and forced/accelerated conditions show very stable products with no change in quality attributes. Overall, the process route (here highlighting the final GMP process and data) proceeded in five steps with an overall yield of 17.5% (cf with 33.9% via the medicinal chemistry scales), eliminated all HPLC chromatography purifications (save one slicia plug), and is a scalable process of high purity (99.8%).

Scheme 6. Synthesis of 5 (VU0424238, auglurant)**.**



Scale	Yield	Purity
50g	68.2%	99.4%
200g	64.0%	99.2%
1.90 kg	56.1%	99.3%
2.20 kg	58.2%	99.5%

A scalable approach to the novel $mGlu_5$ NAM **5** (VU0424238, auglurant) has been successfully developed and adapted to multi-kilo scale. The developed process is scalable and provided kilogram quantities of material for GLP tox studies formulated as a 12.5% SDD, and GMP material for drug product manufacture of clinical studies.. Notably, we found an initial safety concern with *N*-oxide production **7**, and observed a large exotherm at 121 °C, with heat evolution of 1,495J/g, requiring stringent control of temperature. The yields achieved with this optimized GMP route are comparable to the original medicinal chemistry route, but all HPLC purification has been replaced with aqueous work-ups and highly efficient recrystallization procedures affording product of high purity.

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References and notes

1. Niswender,C.M.,and Conn,P.J. *Annu.Rev.Pharmacol. Toxicol* **2010**, *50*, 295−322.
2. Golubeva, A. V.; Moloney, R.D.; O'Connor, R. M.; Dinan, T. G.; Cryan, J. F. *Curr. Drug Targets* **2016**, *17*, 538-616.
3. Lindsley, C. W.; Emmitte, K. A.; Hopkins, C. R.; Bridges, T. M.; Gregory, K. J.; Niswender, C. M.; Conn, P. J. *Chem. Rev.* **2016**, *116*, 6707-6741.
4. Lindsley, C.W. *J. Med. Chem.* **2014**, *57*, 7485-7498. Conn, P.J.; Lindsley, C.W.; Meiler, J.; Niswender, C.M. *Nat. Rev. Drug Discov.* **2014**, *13*, 692-708.
5. Wenthur, C.J.; Gentry, P.R.; Mathews, T.P.; Lindsley, C.W. *Annu. Rev. Pharmacol. Toxicol.* **2014**, 54, 165-184.
6. Emmitte, K. A. *Expert Opin. Ther. Pat.* **2017**, *27*, 691-706..

7. Rocher, J. P.; Bonnet. B.; Boléa, C.; Lütjens, R.; Le Poul, E.; Poli, S.; Epping-Jordan, M.; Bessis, A. S.; Ludwig, B.; Mutel, V. *Curr. Top. Med. Chem.* **2011**, *11*, 680-695.
8. Mihov, Y.; Hasler, G. *Int. J. Neuropsychopharmacol.* **2016**, *19*, pii: pyw002.
9. Ribeiro, F. M.; Vieira, L. B.; Pires, R. G.; Olmo, R. P.; Ferguson, S. S. *Pharmacol Res.* **2017**, *115*, 179-191.
10. Busse, C. S.; Brodkin, J.; Tattersall, D.; Anderson, J. J.; Warren, N.; Tehrani, L.; Bristow, L. J.; Varney, M. A.; Cosford, N. D. P. *Neuropsychopharmacology* **2004**, *29*, 1971-1979.
11. Silverman, J. L.; Smith, D. G.; Rizzo, S. J.; Karras, M. N.; Turner, S. M.; Tolu, S. S.; Bryce, D. K.; Smith, D. L.; Fonseca, K.; Ring, R. H.; Crawley, J. N. *Sci. Transl. Med.* **2012**, *4*, 131ra51.
12. Pop, A. S.; Gomez-Mancilla, B.; Neri, G.; Willemsen, R.; Gasparini, F. *Psychopharmacology (Berl)* **2014**, *231*,1217-1226.
13. Litim, N.; Morissette, M.; Di Paolo, T. *Neuropharmacology* **2016**, *115*, 166-179.
14. Rascol, O.; Fox, S.; Gasparini, F.; Kenney, C.; Di Paolo, T.; Gomez-Mancilla, B. *Parkinsonism Relat. Disord.* **2014**, *20*, 947-956
15. Jaso, B. A.; Niciu, M. J.; Iadarola, N. D.; Lally, N.; Richards, E. M.; Park, M.; Ballard, E. D.; Nugent, A. C.; Machado-Vieira, R.; Zarate, C. A. *Curr. Neuropharmacol.* **2017**, *15*, 57-70.
16. Rutrick, D.; Stein, D. J.; Subramanian, G.; Smith, B.; Fava, M.; Hasler, G.; Cha, J.; Gasparini, F.; Donchev, T.; Ocwieja, M.; Johns, D.; Gomez-Mancilla, B. *Adv. Ther.* **2017**, *34*, 524-541.
17. Gasparini, F.; Lingenhöhl, K.; Stoehr, N.; Flor, P. J.; Heinrich, M.; Vranesic, I.; Biollaz, M.; Allgeier, H.; Heckendorn, R.; Urwyler, S.; Varney, M. A.; Johnson, E. C.; Hess, S. D.; Rao, S. P.; Sacaan, A. I.; Santori, E. M.; Veliocelebi, G.; Kuhn, R. Methyl-6-(phenylethynyl)-pyridine (MPEP), a potent, selective and systematically active $mGlu_5$ receptor antagonist. *Neuropharmacology* **1999**, *38*, 1493-1503.
18. Cosford, N. D.; Tehrani, L.; Roppe, J.; Schweiger, E.; Smith, N. D.; Anderson, J. *J. Med. Chem.* **2003**, *46*, 204-206.
19. Porter, R. H. P.; Jaeschke, G.; Spooren, W.; Ballard, T. M.; Büttelmann, B.; Kolczewski, S.; Peters, J. U.; Prinssen, E.; Wichmann, J.; Vieira, E.; Mühlemann, A.; Gatti, S.; Mutel, V.; Malherbe, P. *J. Pharmacol. Exp. Ther.* **2005**, *315*, 711-721.
20. Vranesic, I.; Ofner, S.; Flor, P. J.; Bilbe, G.; Bouhelal, R.; Enz, A.; Desrayaud, S.; McAllister, K.; Kuhn, R.; Gasparini, F. *Bioorg. Med. Chem.* **2014**, *22*, 5790-5803.
21. Berry-Kravis, E.; Des Portes, V.; Hagerman, R.; Jacquemont, S.; Charles, P.; Visootsak, J.; Brinkman, M.; Rerat, K.; Koumaras, B.; Zhu, L.; Barth, G. M.; Jaecklin, T.; Apostol, G.; von Raison, F. *Sci. Transl. Med.* **2016**, *8*, 321ra5.
22. Jaeschke, G.; Kolczewski, S.; Spooren, W.; Vieira, E.; Bitter-Stoll, N.; Boissin, P.; Borroni, E.; Büttelmann, B.; Ceccarelli, S.; Clemann, N.; David, B.; Funk, C.; Guba, W.; Harrison, A.; Hartung, T.; Honer, M.; Huwyler, J.; Kuratli, M.; Niederhauser, U.; Pähler, A.; Peters, J. U.; Petersen, A.; Prinssen, E.; Ricci, A.; Rueher, D.; Rueher, M.; Schneider, M.; Spurr, P.; Stoll, T.; Tännler, D.; Wichmann, J.; Porter, R. H.; Wettstein, J. G.; Lindemann, L. *J. Med. Chem.* **2015**, *58*, 1358-1371.
23. Guerini, E.; Schadt, S.; Greig, G.; Haas, R.; Husser, C.; Zell, M.; Funk, C.; Hartung, T.; Gloge, A.; Mallalieu, N. L. *Xenobiotica* **2017**, *47*, 144-153.
24. Quiroz, J. A.; Tamburri, P.; Deptula, D.; Banken, L.; Beyer, U.; Rabbia, M.; Parkar, N.; Fontoura, P.; Santarelli, L. *JAMA Psychiatry* **2016**, *73*, 675-684.
25. Bezard E.; Pioli, E. Y.; Li, Q.; Girard, F.; Mutel, V.; Keywood, C.; Tison, F.; Rascol, O.; Poli, S. M. *Mov. Disord.* **2014**, *29*, 1074-1079.
26. Tison, F.; Keywood, C.; Wakefield, M.; Durif, F.; Corvol, J. C.; Eggert, K.; Lew, M.; Isaacson, S.; Bezard, E.; Poli, S. M.; Goetz, C. G.; Trenkwalder, C.; Rascol, O. *Mov. Disord.* **2016**, *31*, 1373-1380.
27. Palanisamy, G. S.; Marcek, J. M.; Cappon, G. D.; Whritenour, J.; Shaffer, C. L.; Brady, J. T.; Houle, C. *Toxicol. Pathol.* **2015**, *43*, 995-1003.
28. Zhang, L.; Balan, G.; Barreiro, G.; Boscoe, B. P.; Chenard, L. K.; Cianfrogna, J.; Claffey, M. M.; Chen, L.; Coffman, K. J.; Drozda, S. E.; Dunetz, J. R.; Fonseca, K. R.; Galatsis, P.; Grimwood, S.; Lazzaro, J. T.; Mancuso, J. Y.; Miller, E. L.; Reese, M. R.; Rogers, B. N.; Sakurada, I.; Skaddan, M.; Smith, D. L.; Stepan, A. F.; Trapa, P.; Tuttle, J. B.; Verhoest, P. R.; Walker, D. P.; Wright, A. S.; Zaleska, M. M.; Zasadny, K.; Shaffer, C. L. *J. Med. Chem.* **2014**, *57*, 861-877.
29. Felts, A.S.; Rodriguez, A.L.; Morrison, R.D.; Venable, D.F.; Bates, B.S.; Manka, J.T.; Blobaum, A.L.; Byers, F.W.; Santomango, T.S.; Daniels, J.S.; Jones, C.K.; Niswender, C.M.; Conn, P.J.; Lindsley, C.W.; Emmitte, K.A. *Bioorg. Med.Chem. Lett.* **2013**, *23*, 5779-5785.
30. Felts, A.S.; Rodriguez, A.L.; Blobaum, A.L.; Morrison, R.D.; Bates, B.S.; Thompson, A.D.; Rook, J.M.; Byers, F.W.; Chang, S.; Venable, D.F.; Luscombe, V.B.; Tamagnan, G.D.; Bridges, T.M.; Niswender, C.M.; Daniels, J.S.; Jones, C.K.; Conn, P.J.; Lindsley, C.W.; Emmitte, K.A. *J. Med. Chem.* **2017**, *60*, 5072-5085.

31. Large scale synthesis of **5**. **Synthesis of 4-chloro-2-methylpyridine-*N*-oxide (7).** To a solution of 4-chloro-2-methyl pyridine (4.0 kg, 31.32 mol) in tetrahydrofuran (40.0 L, 10 V), hydrogen peroxide-urea adduct (588 kg, 62.7 mol) was added lot wise at 25 to 30 ℃. The reaction mass was stirred at 25-30℃ for about 10-15 minutes and cooled the reaction mass to 0-5℃. A solution of trifluoroacetic anhydride (14.48 kg, 68.9 mol) in THF (20.0 L, 5 V) was added drop wise to the reaction mass over a period of 2-3 h maintaining the temperature between 0-5℃. The reaction was slowly allowed to attain 25-30℃ and stirred for 18 h. The reaction was monitored by HPLC (**Int 1** NMT 5.0 % by HPLC). Reaction mass was cooled to 0-5℃ and quenched with 10% sodium thiosulphate solution (40.0 L, 10 V) over a period of 1 h maintaining temperature between 0-5℃. The reaction mass was diluted with dichloromethane (8.0 L, 2 V) and the temperature was slowly allowed to attain 25-30℃ and stirred for 10 minutes at 25 to 30℃. The layers were separated; aqueous layer was saturated with sodium chloride (14.0 kg, 3.5 w/w on SM) [M R Fine chem, Assay by titrimetry - 99.9 % w/w] and extracted with dichloromethane (20.0 L, 5 V x 3). To the combined organic layer was added aqueous saturated potassium carbonate solution (20.0 L, 5.0 V) [Chemielink, Assay by titrimetry - 99.8 % w/w] at 25-30℃ and stirred for 15 minutes. The layers were separated; aqueous layer was extracted with dichloromethane (20.0 L, 5 V x 2). The combined organic layer was dried over sodium sulfate (0.8 kg, 0.2 w/w) and concentrated under vacuum to obtain crude **Int 2** (4.32 kg, 96%) as pale yellow oil which was taken as such for next step without purification. ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.23 (d, J = 7.0 Hz, 1H), 7.66 (d, J = 3.0 Hz, 1H), 7.40 (dd, J = 7.0, 3.0 Hz, 1H), 2.32 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ 149.53, 139.78, 128.73, 126.41, 124.17, 17.09 ppm; LCMS (Method 1): R_T = 0.302 min, m/z = 144.2 $[M+H]^+$; HRMS, calc'd for C_6H_6ClNO [M], 143.0138; found 143.0139.**Synthesis of 4-chloro-6-methylpicolinonitrile (8).** To a solution of 4-chloro-2-methylpyridine-*N*-oxide (**7**, 4.3 kg, 29.94 mol) in dichloromethane (64.5 L, 15 V) was added trimethyl silyl cyanide (9.76 L, 77.83 mol) at 25-30 ℃ over a period of 30 minutes and further stirred for 15 minutes. Reaction mass was cooled to 0-5 ℃. *N*,*N*-Dimethylcarbamoyl chloride (7.17 L, 77.83 mol) was added drop wise over a period of 1h. The reaction mass was slowly warmed to 25-30 ℃ and stirred for 36 h. Reaction was monitored by HPLC [**7** NMT 1.0 %]. The reaction mass was cooled to 0-5 ℃. The reaction was quenched with 10% aqueous potassium carbonate solution until the pH of aqueous layer was about 9-10. The temperature of reaction mass was allowed to attain 25-30 ℃ and the layers were separated. The aqueous layer was extracted with dichloromethane (8.6 L, 2 V). The combined organic layer was washed with 0.5 N HCl solution (21.5 L, 5 V) followed by brine solution (21.5 L, 5 V), dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was chased twice with n-heptane (2 x 17.2 L). The crude product was diluted with 2 % ethyl acetate [Ashok alco-chem limited, Purity (area %) by GC - 100 %] in hexanes (43 L, 10 V) and filtered through silica plug (21.5 kg, 5 % w/w on SM, 230-400 mesh) and silica plug was washed with 10% ethyl acetate in hexanes till the product was completely eluted (300 L, 69 V). Combined fractions were concentrated and residue was chased twice with n-heptane (2 x 17.2 L). Resulting mass was stirred in n-heptane (21.5 L, 5 V) at 0-5 ℃ for 1h and filtered. Solid was washed with cold *n*-heptane (4.3 L, 1 V) and dried to get **8** as off white solid (2.32 kg, 51 %). . ^{1}H NMR (400 MHz, DMSO-d_6) δ 8.13 (d, J = 1.6 Hz, 1H), 7.82 (d, J = 1.7 Hz, 1H), 2.52 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 161.99, 144.13, 133.01, 127.65, 126.40, 116.60, 23.57 ppm; LCMS (Method 1): R_T = 0.715 min, m/z = 153.2 $[M+H]^+$; HRMS, calc'd for $C_7H_5ClN_2$ [M], 152.0141; found 152.0139. **Synthesis of 6-methyl-4-(pyrimidin-5-yloxy)picolinonitrile (10).** To a solution of 5-hydroxypyrimidine (**9**, 1.73 kg, 18.08 mol) in DMF (23.0 L, 10 V), potassium carbonate (6.25 kg, 45.21 mol) was added followed by 4-chloro-6-methylpicolinonitrile (**8**, 2.3 kg, 15.07 mol) at 25-30℃. The reaction mass was heated at 75-80 ℃ for 36 h. The reaction was monitored by HPLC **8** NMT 2.0 % by HPLC). Reaction mass was cooled to 25-30 ℃. The solid was filtered and washed the solid with DMF (4.6 L, 2.0 V). The filtrate was cooled to 0-5 ℃ and purified water (27.6 L, 12 V) was added drop wise over a period of 1 h. The solid precipitated was filtered and washed with purified water (2.3 L, 10 V) to afford **10** as off white solid (2.62 kg, 82.1%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.17 (s, 1H), 8.85 (s, 2H), 7.74 (d, J = 2.4 Hz, 1H), 7.32 (d, J = 2.3 Hz, 1H), 2.48 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ =

164.04, 162.28, 155.51, 150.02, 148.75, 133.54, 117.05, 115.59, 115.04, 23.82 ppm; LCMS (Method 1): R_T = 0.535 min, *m/z* = 213.2 $[M+H]^+$; HRMS, calc'd for $C_{11}H_8N_4O$ [M], 212.0698; found 212.0697. **Synthesis of 6-methyl-4-(pyrimidin-5-yloxy)picolinic acid (11).** To a solution of 6-methyl-4-(pyrimidin-5-yloxy) picolinonitrile (**10**, 2.6 kg, 12.25 mol) in 1,4-dioxane (39.0 L, 15 V) [Synergy chemicals, Purity (area %) by GC - 99.7 %], 2N aqueous NaOH solution (26.0 L, 10 V) was added at 25-30°C. The reaction mixture was heated at 85-90°C for 6 h. Reaction was monitored by HPLC, reaction mass was cooled to 25-30 °C and diluted with DCM (26.0 L, 10 V). The layers were separated, aqueous layer was washed with DCM (26.0 L, 10 V), cooled the aqueous layer to 0-5°C and adjusted the pH to 3-4 using 6N aqueous HCl solution. The aqueous layer was evaporated under vacuum, the crude product obtained was suspended in 10 % methanol in dichloromethane (130.0 L, 50 V) and stirred at 45-50 °C for 1 h. The un-dissolved solid was filtered and filtrate was evaporated under vacuum. Residue was chased with DCM (2 X 7 V), stirred with DCM: n-heptane (1:1, 10 V), filtered and dried to afford **11** as off white solid (2.22 kg, 78.4 %). **Synthesis of *N*-(5-Fluoropyridin-2-yl)-6-methyl-4-(pyrimidin-5-yloxy)picolinamide (5)**. A solution of 6-methyl-4-(pyrimidin-5-yloxy)pyridine-2-carboxylic acid (**11**, 2.2 kg, 9.51 mol) in pyridine (33.0 L, 15 V) was stirred at 25-30 °C for 15-20 minutes. 5-fluoro-2-aminopyridine (**12**, 1.386 kg, 12.36 mol) was added to the reaction mass at 25-30 °C and stirred at 25-30 °C for 45 minutes. Reaction mass was cooled to 0-5 °C, phosphorus oxychloride (1.33 L, 14.27 mol) was added drop wise over a period of 1 h by maintaining the temperature between 0-5 °C. The reaction mass was stirred at 10-15°C for 30 min. The reaction completion was monitored by HPLC. After reaction completion, reaction mass was quenched with water (44.0 L, 20 V) and neutralized with 10% potassium carbonate solution (22.0 L, 10 V) at 0-5 °C. The suspension was stirred at 0-5 °C for 1h. Solid was filtered, washed with water (22.0 L, 10 vol.) and dried under vacuum. Crude material was dissolved in DCM (22.0 L, 10 vol.) and washed with water (6 x 10 V) to remove pyridine traces. Organic layer was dried over sodium sulfate and evaporated under vacuum to get desired product as beige solid (1.8 kg, 58.2 %). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.48 (s, 1H), 9.18 (s, 1H), 8.88 (s, 2H), 8.40 (d, *J* = 3.0 Hz, 1H), 8.27 (dd, *J* = 9.2 Hz, 4.10 Hz, 1H), 7.86 (td, *J* = 8.6, 3.1 Hz, 1H), 7.55 (d, *J* = 2.3 Hz, 1H), 7.29 (d, *J* = 2.3 Hz, 1H), 2.58 (s, 3H); ^{13}C NMR (100 MHz, DMSO-d_6) δ = 165.15, 161.11, 160.57, 156.12 (d, *J*(C,F) = 249.0 Hz), 155.41, 150.29, 150.07, 149.01, 146.92 (d, *J*(C,F) = 2.1 Hz), 135.94 (d, *J*(C,F) = 25.5 Hz), 125.83 (d, *J*(C,F) = 19.9 Hz), 114.56, 114.27 (d, *J*(C,F) = 4.7 Hz), 108.01, 23.84 ppm; LCMS (Method 2): R_T = 0.727 min, *m/z* = 326.2 $[M+H]^+$; HRMS, calc'd for $C_{16}H_{12}FN_5O_2$ [M], 325.0975; found 325.0979.

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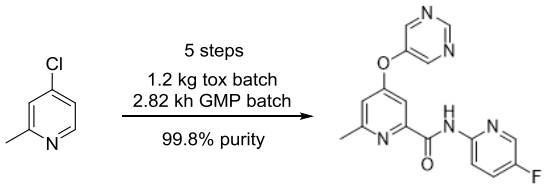
Highlights

- Scalable 5 step GMP synthesis of Auglurant in >99.8% purity
- Elimination of chromatography steps and replacement with recrystallizations
- Addressed thermal instability of pyridine *N*-oxide
- Efficient cyanation and S_NAr reactions



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