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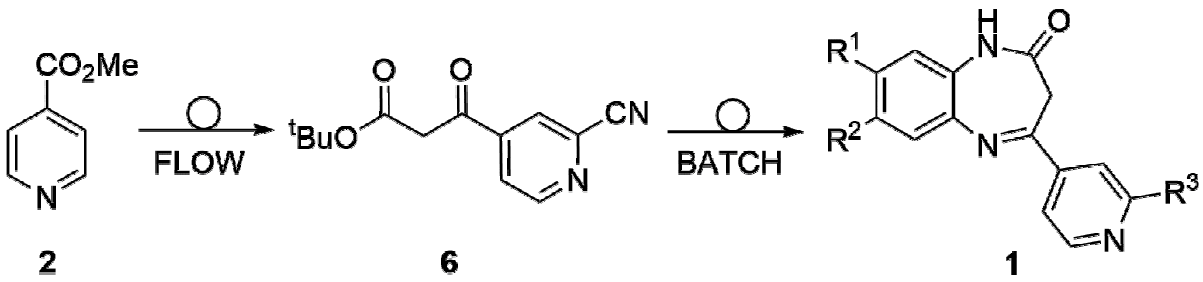

2 **6** **1**

A Combination of Flow and Batch Mode Processes for the Efficient Preparation of $mGlu_{2/3}$ Receptor Negative Allosteric Modulators (NAMs)

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ABSTRACT

Benzodiazepinones are privileged scaffolds with activity against multiple therapeutically relevant biological targets. In support of our ongoing studies around allosteric modulators of metabotropic glutamate receptors (mGlus) we required the multigram synthesis of a β-ketoester key intermediate. We report the continuous flow synthesis of *tert*-butyl 3-(2-cyanopyridin-4-yl)-3-oxopropanoate and its transformation to potent $mGlu_{2/3}$ negative allosteric modulators (NAMs) in batch mode.

Keywords: flow chemistry, microreactor, multistep synthesis, β-ketoester, metabotropic glutamate receptor, benzodiazepinone.

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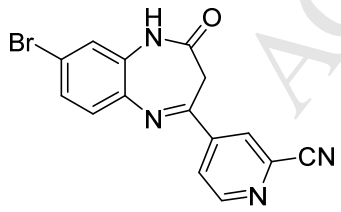
The amino acid glutamate is the predominant excitatory neurotransmitter, acting at neurons both peripherally as well as in the central nervous system (CNS). Glutamate mediates fast synaptic transmission through ionotropic glutamate receptors, ligand-gated ion channels that include α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate, and *N*-methyl-D-aspartate (NMDA) receptor subtypes.[1] It also exerts a modulatory role via the family of G protein-coupled receptors known as metabotropic glutamate (mGlu) receptors. There are eight mGlu receptor subtypes that are grouped according to their respective second messenger systems and sequence homology. The Group I mGlus ($mGlu_1$ and $mGlu_5$) are positively coupled to Ca^{2+} signaling, while Groups II ($mGlu_2$, $mGlu_3$) and Group III ($mGlu_4$, $mGlu_6$, $mGlu_7$ and $mGlu_8$) negatively regulate the activity of adenylyl cyclase via coupling to $G_{i/o}$ proteins.[2] Several orthosteric (competitive) agonists and antagonists that are selective for Group II mGlu receptors have been reported. These compounds are analogues of glutamate that bind to the endogenous glutamate binding site in the extracellular "venus fly trap" (VFT) domain of the receptor.[3, 4] On the other hand, efforts by our group and others have focused on the discovery and optimization of small molecule allosteric modulators that bind non-competitively to mGlu receptors. Over the past several years selective positive allosteric modulators (PAMs) and negative allosteric modulators (NAMs) of $mGlu_2$ and/or $mGlu_3$ have been reported.[5-7] This interest was prompted by the concept that such compounds may have improved therapeutic properties by subtly modulating the activity of malfunctioning receptor signaling pathways in concert with the endogenous neurotransmitter (glutamate). Furthermore, high sequence homology in the $mGlu_2$ and $mGlu_3$ glutamate binding sites limits the possibility of selective orthosteric ligands. Thus, the improved selectivity of allosteric modulators may offer the benefit of an improved side-effect profile, as well as superior drug-like properties compared with competitive agonists and antagonists. Importantly, allosteric modulators of $mGlu_2$ and/or $mGlu_3$ receptors have significant potential as drugs for the treatment of various CNS disorders such as schizophrenia, anxiety, drug dependence, cognitive dysfunction or depression that are caused by aberrant glutamatergic transmission [6-13].

We recently initiated a programme focused on the design, synthesis and *in vitro* and *in vivo* evaluation of $mGlu_{2/3}$ negative allosteric modulators (NAMs). One component of our research involved the investigation of a series of $mGlu_{2/3}$ NAMs exemplified by the benzodiazepine derivative MNI-137 (**1a**).[14] In order to investigate the structure-activity relationships (SAR) around this compound, we required large amounts of a common intermediate that could be converted to multiple analogues. We identified *tert*-butyl 3-(2-cyanopyridin-4-yl)-3-oxopropanoate (**6**) as such an intermediate that would provide access to analogues in which various substituents could be introduced into the fused aryl ring. Keeping in mind the objective of generating multi-gram amounts of intermediate, we elected to investigate the continuous flow (microfluidic) synthesis of this key heterocyclic β-ketoester derivative.

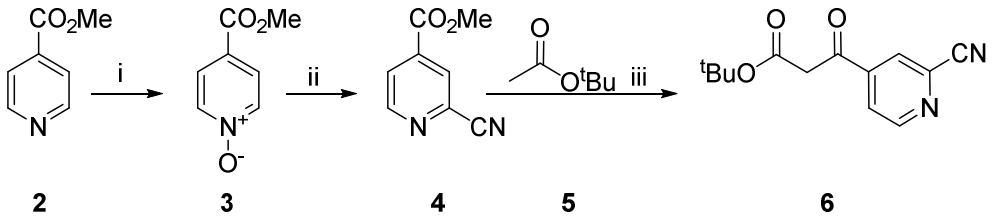
Over the past few years, multistep microfluidic chip-based processes have emerged as attractive methods for the large scale preparation of numerous organic molecules.[15, 16] Advantages include enhanced reagent mixing, optimal heat transfer, small reaction volumes, precise reaction times, and the ability to conduct multistep reactions in a single, unbroken microreactor sequence. We have previously described the development of automated flow chemistry methods to rapidly access complex, drug-like heterocycles from readily available precursors. Thus, we have reported the continuous flow syntheses of bis-substituted 1,2,4-oxadiazoles,[17] functionalized imidazo[1,2-*a*] heterocycles,[18, 19] pyrrole-3-carboxylic acid derivatives,[20] 2-(1*H*-indol-3-yl)thiazoles[21] and 5-(thiazol-2-yl)-3,4-dihydropyrimidin-2(1*H*)-one derivatives.[22, 23] Herein we describe the flow synthesis of the key intermediate *tert*-butyl 3-(2-cyanopyridin-4-yl)-3-oxopropanoate and its utility in the preparation of $mGlu_{2/3}$ receptor NAMs that are analogues of compound **1a**.[14]



1a: MNI-137

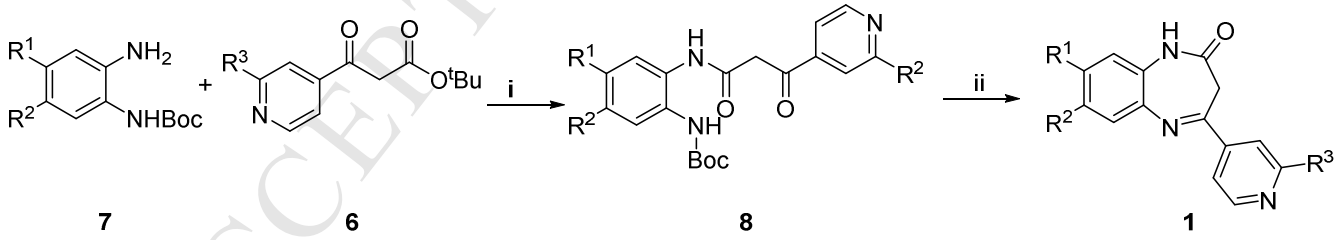
Figure 1. The prototypical benzodiazepine-derived $mGlu_{2/3}$ receptor negative allosteric modulator MNI-137 (**1a**).

RESULTS AND DISCUSSION



Scheme 1. The batch synthesis of 2-cyanoisonicotinate (**4**) and *tert*-butyl 3-(2-cyanopyridin-4-yl)-3-oxopropanoate (**6**). i) mCPBA, CH_2Cl_2, 25 ºC, 16 h; ii) TMSCN, Et_3N, MeCN, 80 ºC, 2 h; iii) LDA, THF, -78 ºC, 4 h.

As noted above, the key intermediate required for the preparation of the target benzodiazepinone derivatives is a heterocyclic β-ketoester derivative with potentially sensitive functionality. The reported batch mode synthesis of *tert*-butyl 3-(2-cyanopyridin-4-yl)-3-oxopropanoate (**6**) is shown in Scheme 1. The procedure involves an initial synthesis of methyl 2-cyanoisonicotinate (**4**), followed by the use of this intermediate in the preparation of *tert*-butyl 3-(2-cyanopyridin-4-yl)-3-oxopropanoate (**6**). Thus, methyl 4-carboxypyridine (**2**) is treated with mCPBA in methylene chloride to provide methyl 4-carboxypyridine *N*-oxide (**3**). This material is then treated with cyanotrimethylsilane in acetonitrile to afford methyl 2-cyanoisonicotinate (**4**). Intermediate **4** is then subjected to base-mediated condensation with *tert*-butylacetate (**5**) to furnish *tert*-butyl 3-(2-cyanopyridin-4-yl)-3-oxopropanoate (**6**).

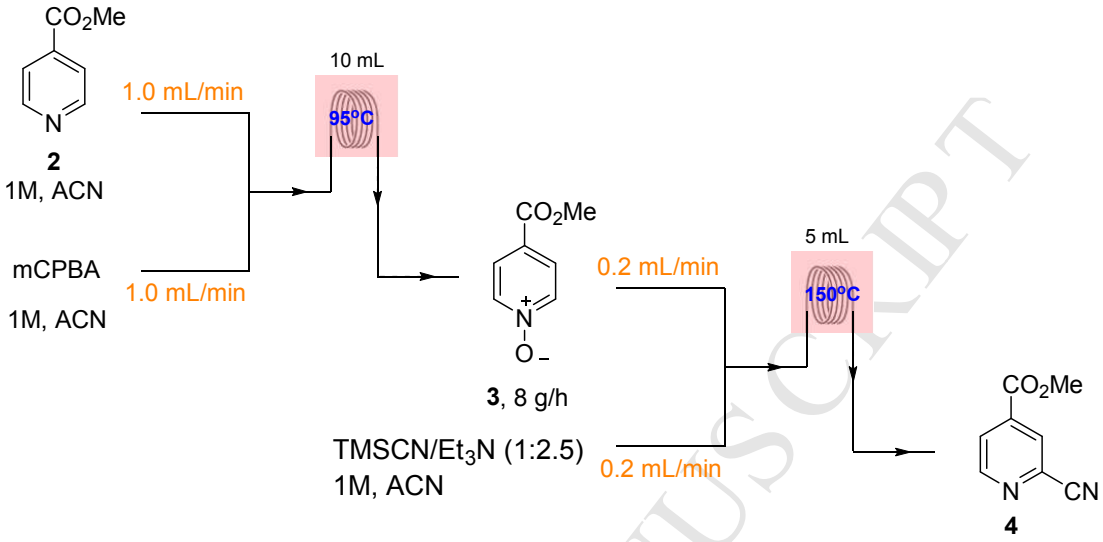


Scheme 2. The batch synthesis of 2-oxo-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepin-4-yl derivatives. i) Toluene, Δ, 1 h; ii) TFA, CH_2Cl_2, 0 ºC, 7 h.

The batch mode preparation of benzodiazepinone derivatives is performed as shown in Scheme 2. Thus, condensation of mono *N*-Boc protected *o*-phenelenediamines **7** with *tert*-butyl β-ketoesters **6** in boiling toluene provides the corresponding β-ketoamide derivatives **8**. Upon TFA-mediated Boc-

deprotection the intermediate β-ketoamides undergo in-situ cyclization to yield the desired 1,3-dihydro-benzo[b][1,4]diazepin-2-ones derivatives **1**.

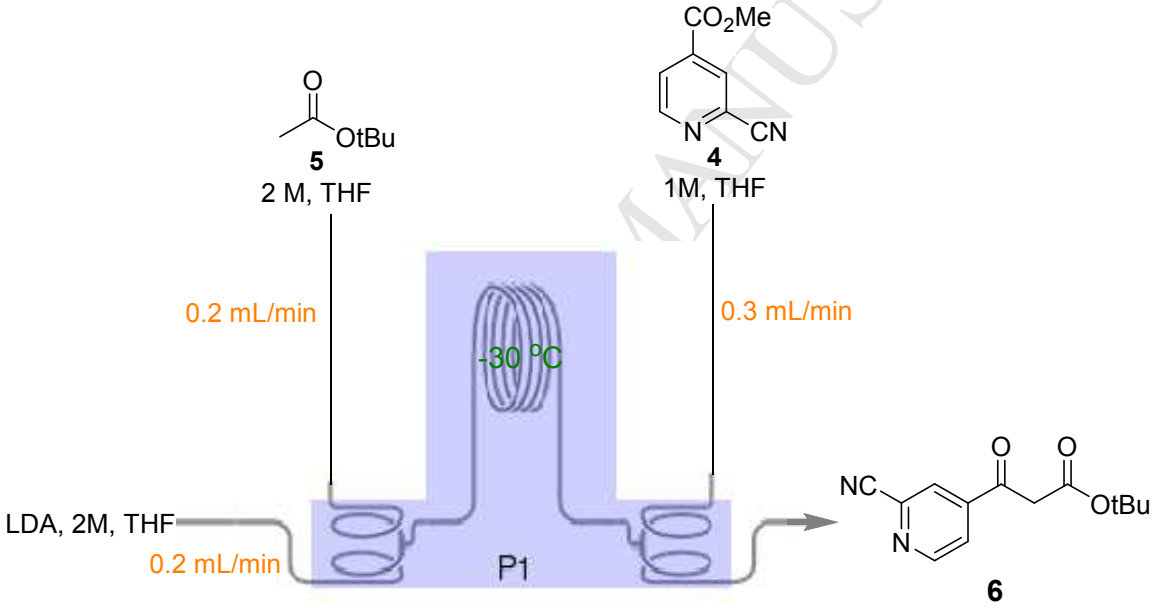
Scheme 3. Microfluidic synthesis of methyl 2-cyanoisonicotinate (**4**).



The batch synthesis of methyl 2-cyanoisonicotinate (**4**) involves two different solvents, a non-polar haloalkane (dichloromethane) in the first step and a polar aprotic solvent (acetonitrile) in the second step (Scheme 1). For the flow synthesis we determined that it was possible for the N-oxide formation to proceed in acetonitrile, thus avoiding the need for solvent change part way through the process. Thus, as shown in Scheme 3, methyl 4-carboxypyridine (**2**) was dissolved in MeCN as a 1 M solution. Similarly, mCPBA was also dissolved in MeCN at a concentration of 1M, and each of these solutions was pumped at a rate of 1 mL/minute initially through a T-mixer at ambient temperature and subsequently into a 10 mL flow reactor held at 95 ^{o}C. All high temperature flow reactions were carried out under an optimal pressure of 8 bar controlled by the back pressure regulator (BPR). The acetonitrile solution exiting the reactor contained the methyl 4-carboxypyridine *N*-oxide (**3**) generated during the flow process at a rate of 8g/h. This material was pumped into a second T-mixer and combined with a mixture of cyanotrimethylsilane and triethylamine (1:2.5) as a 1M solution in acetonitrile with both streams flowing into the T-mixer at a rate of 0.2 mL/minute. The combined reaction mixture was then pumped through a 5 mL reactor held at 150 ^{o}C to form the desired methyl 2-cyanoisonicotinate (**4**). This material was collected and used in a second flow process as shown in Scheme **4**. For the next step we established that THF was a suitable solvent to use in

the flow process and that a 2 M solution was a viable concentration for both the reactants (**4** and **5**) and the lithium amide base (LDA). Optimization of the LDA step involved testing multiple temperatures from -70 ^{o}C through 0 ^{o}C to establish that -30 ^{o}C was ideal for the flow process. As illustrated in Scheme **4**, a 2M solution of *tert*-butylacetate (**5**) and a 2M solution of LDA are both pumped at a rate of 0.2 mL/minute into a T-mixer that then flows the reaction mixture into a cooler module held at –30 ^{o}C. A 1 M solution of methyl 2-cyanoisonicotinate (**4**) in THF is pumped (0.3 mL/min) via a T-mixer to combine with the solution of lithium 1-(tert-butoxy)ethen-1-olate exiting the cooler module. A solution of the key intermediate *tert*-butyl 3-(2-cyanopyridin-4-yl)-3-oxopropanoate (**6**) is collected and for use in the preparation of the target benzodaizepinone derivatives and described in Scheme 2 and the Experimental.

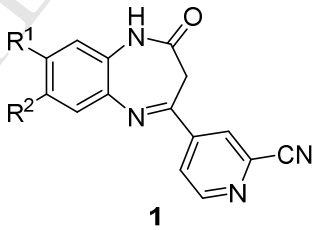
Scheme 4. Microfluidic synthesis of *tert*-butyl 3-(2-cyanopyridin-4-yl)-3-oxopropanoate (**6**).



To illustrate the utility of the flow/batch synthetic procedure we prepared a series of benzodiazepinone analogues using the optimized chemistry and evaluated them in vitro against $mGlu_2$ or $mGlu_3$ receptors. The cellular assay used for in vitro testing employs HEK cells that express either $mGlu_2$ or $mGlu_3$ in addition to G protein-coupled inwardly rectifiying potassium (GIRK) channels. These specifically transfected cells are employed in an assay that utilizes thallium flux, as previously described,[24] and the results for compounds **1a-1g** are shown in Table 1. Thus, the prototypical benzodiazepinone $mGlu_{2/3}$ NAM **1a** (MNI-137) was determined to have IC_{50} values in this assay of 84 nM ($mGlu_2$) and 135 nM ($mGlu_3$), respectively (Table 1, entry 1). Replacement of the bromine atom at R^1 in **1a** with fluorine, as

in **1b**, resulted in an approximate 3-fold loss of potency at $mGlu_2$ and a >20-fold loss of potency at $mGlu_3$ (Table 1, entry 2). On the other hand, replacement of bromine with trifluoromethyl, as in **1c**, led to retension of potency at $mGlu_2$ (IC_{50} = 74 nM) and a 3-fold loss of potency at $mGlu_3$ (IC_{50} = 388 nM) compared with **1a** (Table 1, entry 3). Interestingly, replacement of bromine with the much larger halogen iodine, as in **1d**, resulted in a 2-fold increase in potency at $mGlu_2$ (IC_{50} = 35 nM) and retension of potency at $mGlu_3$ (IC_{50} = 159 nM) compared with **1a** (Table 1, entry 4). Another way to improve potency was noted when the hydrogen at R^2 was replaced with a methyl substituent, as in compound **1e**. As shown in Table 1, entry 5, addition of the R^2 methyl in **1e** increases potency at both $mGlu_2$ (IC_{50} = 33 nM) and at $mGlu_3$ (IC_{50} = 173 nM) compared with **1c** (entry 3). Introduction of the highly electron withdrawing nitrile substituent at R^1, as in **1f**, resulted in a 5-fold loss of potency at $mGlu_2$ (IC_{50} = 467 nM) and a 24-fold loss of potency at $mGlu_3$ (IC_{50} = 3314 nM) compared with **1a** (Table 1, entry 6). Finally, replacement of the bromine atom at R^1 in **1a** with a bulky arylalkynyl substituent, as in **1g**, resulted in largest loss of potency at $mGlu_2$ (14-fold) and a 9-fold loss of potency at $mGlu_3$ (Table 1, entry 7). Overall, even within this relatively small library of analogues, a distinctive and useful structure-activity relationship (SAR) emerged to form the basis for the design of additional potent analogues.

Table 1. *In vitro* on-target potency data for $mGlu_{2/3}$ receptor NAMs[a]



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Entry	ID	R^1	R^2	$mGlu_2$ IC_{50} µM	$mGlu_3$ IC_{50} µM
1	**1a**	Br	H	0.084 ± 0.012	0.135 ± 0.043
2	**1b**	F	H	0.310 ± 0.081	2.915 ± 1.286
3	**1c**	CF_3	H	0.074 ± 0.011	0.388 ± 0.113
4	**1d**	I	H	0.035 ± 0.008	0.159 ± 0.050
5	**1e**	CF_3	CH_3	0.033 ± 0.007	0.173 ± 0.029
6	**1f**	CN	H	0.467 ± 0.041	3.314 ± 1.382

7	**1g**		H	1.200 ± 0.256	1.265 ± 0.409

[a]$mGlu_2$ and $mGlu_3$ NAM IC_{50} μM data represent the ± SEM for at least three independent experiments performed in triplicate in either $mGlu_2$ or $mGlu_3$ GIRK thallium flux assays as previously described[24] and utilizing a 1 hour incubation with test compound prior to assay.

CONCLUSION

In summary, we have developed an efficient procedure for the preparation of $mGlu_{2/3}$ NAMs (**1**) using a combination of flow and batch chemistry. These benzodiazepine derivatives are constructed rapidly and in high yield from inexpensive, readily available starting materials. The new process provides access to diversely substituted analogues in quantities (150-350 mg) sufficient for both initial in vitro testing, as well as further evaluation, including assessment in vivo. This is an important consideration when expanding a lead series using medicinal chemistry in an active drug discovery program. The further characterization of these and additional analogues in this series will be described in detail in future disclosures.

EXPERIMENTAL

Unless otherwise noted, all solvents and other reagents are commercially available and used without further purification. Purity and characterization of compounds were established by a combination of liquid chromatography-mass spectroscopy (LC-MS) and NMR analytical techniques and was >95% for all compounds. Silica gel column chromatography was carried out using prepacked silica cartridges from RediSep (ISCO Ltd.) and eluted using an Isco Companion system. Melting points were reordered on a MEL-TEMP® apparatus and are uncorrected. ^{1}H- and ^{13}C-NMR spectra were obtained on a Jeol 400 spectrometer at 400 MHz and 100 MHz, respectively. Chemical shifts are reported in δ (ppm) relative to residual solvent peaks or TMS as internal standards. Coupling constants are reported in Hz. High-resolution ESI-TOF mass spectra were acquired from the Mass Spectrometry Core at The Sanford-Burnham Medical Research Institute (Orlando, Florida). HPLC-MS analyses were performed on a Shimadzu 2010EV LCMS using the following conditions: Kromisil C18 column (reverse phase, 4.6 mm × 50 mm); a linear

gradient from 10% acetonitrile and 90% water to 95% acetonitrile and 5% water over 4.5 min; flow rate of 1 mL/min; UV photodiode array detection from 200 to 300 nm. Continuous flow (microreactor) experiments were carried out using a Vapourtec R Series Flow Chemistry System.

Flow synthesis of methyl 2-cyanoisonicotinate (4).

A solution of methyl 4-carboxypyridine (1M in MeCN) and a solution of mCPBA (1M in MeCN) were flowed via a T-mixer into a 10 mL flow reactor at 95 ^{o}C at a rate of 1.0 mL per minute (Scheme 3). The solution of methyl 4-carboxypyridine *N*-oxide exiting the first reactor was combined with TMSCN/NEt_3 (1:2.5, 1M in MeCN) via a second T-mixer. This solution was flowed into a 5 mL reactor at 150 ^{o}C at a rate of 0.2 mL per minute. The solution exiting the reactor was collected and the solvent evaporated to provide methyl 2-cyanoisonicotinate (**4**).

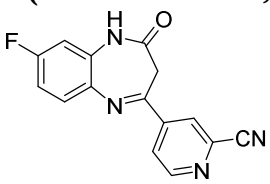
Flow synthesis of tert-butyl 3-(2-cyanopyridin-4-yl)-3-oxopropanoate (6).

A solution of *tert*-butyl acetate (2M in THF) was flowed into a cooled flow reactor at a rate of 0.2 mL per minute and a stream of LDA (2M in THF) is introduced to the same flow reactor, also at a rate of 0.2 mL per minute. During this process the temperature of the flow reactor is held at –30 ^{o}C to allow formation of the anion. A solution of methyl 2-cyanoisonicotinate (1M in THF) is flowed into the stream containing the *tert*-butyl acetate anion at a rate of 0.3 mL per minute. The solution exiting the reactor was collected and the solvent evaporated to furnish *tert*-butyl 3-(2-cyanopyridin-4-yl)-3-oxopropanoate (**6**). The residue was mixed with excess water and extracted three times with dichloromethane. The combined organic layers were washed with brine, dried over magnesium sulfate, filtered, and concentrated and residue was purified via automated flash chromatography (SiO_2) to afford the desired product (CombiFlash® Rf column). The solvent gradient was 90% hexane to 50% ethyl acetate over 15 min at a flow rate of 15 mL/min. The purified *tert*-butyl 3-(2-cyanopyridin-4-yl)-3-

oxopropanoate (**6**) appeared as a mixture of keto-enol forms in a 1:3 ratio as a pale yellow solid (8 g, 66 % yield). ^{1}H NMR keto form ($CDCl_3$): δ 8.96 (d, J = 5.0 Hz, 1H), 8.18 (s, 1H), 7.82 (m, 1H), 3.90 (s, 2H), 1.41 (s, 9H). ^{13}C NMR keto form (100 MHz, $CDCl_3$): δ 190.5, 165.2, 152.5, 143.2, 135.4, 126.2, 122.6, 116.6, 83.4, 47.2, 27.9. ^{1}H NMR enol form ($CDCl_3$): δ 8.77 (d, J = 4.6 Hz, 1H), 7.96 (s, 1H), 7.78 (m, 1H), 5.71 (s, 1H), 1.59 (s, 9H). ^{13}C NMR keto form (100 MHz, $CDCl_3$): δ 172.0, 165.1, 151.8, 142.8, 134.8, 124.6, 122.7, 116.9, 93.1, 82.8, 28.3. LCMS ESI *m/z*: 247.00 $[M + H]^+$. HRMS (ESI) *m/z* calcd for $C_{13}H_{14}N_2O_3$ $[M + H]^+$: 247.1001. Found: 247.1072.

General procedure for the batch synthesis of benzodiazepinones.

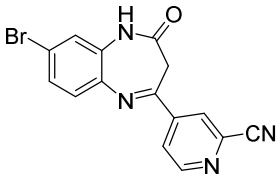
4-(8-Fluoro-2-oxo-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepin-4-yl)picolinonitrile (1b).



tert-Butyl (2-amino-4-fluorophenyl)carbamate[25] (0.226 g, 1 mmol) and tert-butyl 3-(2-cyanopyridin-4-yl)-3-oxopropanoate (0.320 g, 1.3 mmol) were dissolved in toluene (5 mL) and heated at reflux for 1h. The reaction mixture was cooled and the excess solvent was removed under reduced pressure. The crude intermediate was taken up in CH_2Cl_2 and TFA (1.14 g, 10 mmol) was added dropwise to it at 0 ºC. The resulting reaction mixture was stirred at rt for 7 h. The crude mixture was washed with sodium bicarbonate solution and the aqueous layer extracted with CH_2Cl_2. The combined organic layers were washed with water, then brine, and dried over Na_2SO_4. Evaporation of the solvent followed by column chromatography using hexanes:ethyl acetate (60:40) yielded the title compound. Yellow solid (0.186 g, 66%). ^{1}H NMR (DMSO-d_6): δ 10.74 (s, 1H), 8.91 (d, J = 5.0 Hz, 1H), 8.55 (s, 1H), 8.26-8.24 (m, 1H), 7.30-7.23 (m, 3H), 3.62 (s, 2H). ^{13}C NMR(, DMSO-d_6): δ 165.7, 159.3, 156.4, 152.2, 145.1, 139.6, 133.6, 127.0, 126.5,

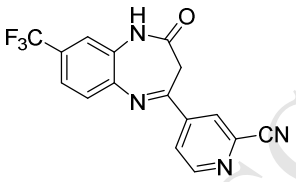
123.8, 117.2, 115.1 (d, *J* = 23.0 Hz), 113.4 (d, *J* = 23.0 Hz), 40.1. LCMS ESI *m/z*: 281.00 [M + H]$^+$. HRMS (ESI) *m/z* calcd for $C_{15}H_9FN_4O$ [M + H]$^+$: 281.0762. Found: 281.0834.

4-(8-Bromo-2-oxo-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepin-4-yl)picolinonitrile (1a).



tert-Butyl (2-amino-4-bromophenyl)carbamate[25] (0.286 g, 1 mmol), tert-butyl 3-(2-cyanopyridin-4-yl)-3-oxopropanoate (0.320 g, 1.3 mmol) and TFA (1.14 g, 10 mmol) were processed according to the general procedure. Yellow solid (0.267 g, 78%), mp: 238-240 ºC. ^{1}H NMR (DMSO-d_6) δ 10.77 (s, 1H), 8.89 (d, *J* = 5.0 Hz, 1H), 8.51 (s, 1H), 8.24 (dd, *J* = 1.0 Hz, 5.0 Hz, 1H), 7.41-7.36 (m, 3H), 3.61 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 165.7, 155.6, 152.2, 145.2, 137.7, 133.5, 131.7, 130.0, 127.1, 126.5, 125.1, 124.2, 119.5, 117.3, 40.2. LCMS ESI *m/z*: 341.00 [M + H]$^+$. HRMS (ESI) *m/z* calcd for $C_{15}H_9BrN_4O$ [M+H]$^+$ 340.9959, found: 341.0092.

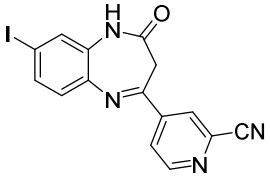
4-(2-Oxo-8-(trifluoromethyl)-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepin-4-yl)picolinonitrile (1c).



tert-Butyl (2-amino-4-(trifluoromethyl)phenyl)carbamate[25] (0.276 g, 1 mmol), tert-butyl 3-(2-cyanopyridin-4-yl)-3-oxopropanoate (0.320 g, 1.3 mmol) and TFA (1.14 g, 10 mmol) were processed according to the general procedure. Yellow solid (0.261 g, 79%), mp: 196-198 ºC. ^{1}H NMR (DMSO-d_6) *δ* 10.93 (s, 1H), 8.95 (d, *J* = 5.5 Hz, 1H), 8.53 (s, 1H), 8.26-8.24 (m, 1H), 7.64-7.53 (m, 3H), 3.71 (s, 2H); ^{13}C NMR (DMSO-d_6) *δ* 166.0, 157.6, 152.3, 145.0, 141.2,

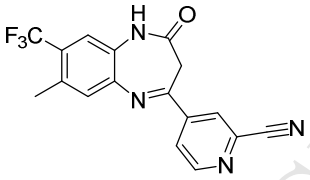
133.6, 130.6, 129.2, 126.6, 125.3, 122.5, 119.2, 117.2, 39.5; LCMS ESI *m/z*: 332.00 $[M + H]^+$. HRMS (ESI) *m/z* calcd for $C_{16}H_9F_3N_4O$ $[M+H]^+$ 332.0729, found 332.0801.

4-(8-Iodo-2-oxo-2,3-dihydro-1*H*-benzo[*b*][1,4]diazepin-4-yl)picolinonitrile (1d).



tert-Butyl (2-amino-4-iodophenyl)carbamate[25] (0.334 g, 1 mmol), tert-butyl 3-(2-cyanopyridin-4-yl)-3-oxopropanoate (0.320 g, 1.3 mmol) and TFA (1.14 g, 10 mmol) were processed according to the general procedure. White solid (0.352 g, 90%), mp: 253-255 ºC. ^{1}H NMR (DMSO-d_6): δ 10.74 (s, 1H), 8.92 (d, *J*= 5.0 Hz, 1H), 8.54 (s, 1H), 8.25-8.23 (m, 1H), 7.58-7.56 (m, 2H), 7.24 (d, *J* = 8.2 Hz, 1H), 3.62 (s, 2H); ^{13}C NMR (DMSO-d_6): δ 165.7, 155.6, 152.2, 145.2, 138.1, 133.5, 132.8, 131.6, 130.1, 129.9, 126.5, 125.0, 117.2, 92.5, 40.1. LCMS ESI *m/z*: 389.00$[M + H]^+$. HRMS (ESI) *m/z* calcd for $C_{15}H_9IN_4O$ $[M+H]^+$ 388.9818, found 388.9892.

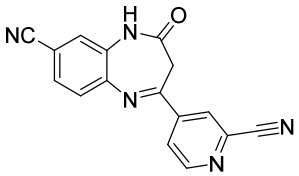
4-(7-Methyl-2-oxo-8-(trifluoromethyl)-2,3-dihydro-1H-benzo[b][1,4]diazepin-4-yl)picolinonitrile (1e).



tert-Butyl (2-amino-5-methyl-4-(trifluoromethyl)phenyl)carbamate[26] (0.290 g, 1 mmol), tert-butyl 3-(2-cyanopyridin-4-yl)-3-oxopropanoate (0.320 g, 1.3 mmol) and TFA (1.14 g, 10 mmol) were processed according to the general procedure. Yellow solid (0.248 g, 72%), mp: 228-230 ºC. ^{1}H NMR (DMSO-d_6) δ 10.83 (s, 1H), 8.93 (d, *J* = 5.0 Hz, 1H), 8.55 (s, 1H), 8.26-8.24 (m, 1H), 7.52 (brs, 2H), 3.75 (s, 2H), 2.45 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 165.8, 157.4, 152.2,

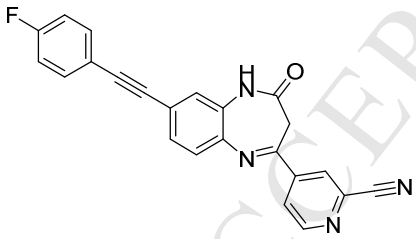
145.0, 140.8, 137.7, 133.6, 131.1, 128.2, 126.5, 125.6, 119.9, 117.2, 40.1, 17.9. LCMS ESI *m/z*: 345.00 $[M + H]^+$. HRMS (ESI) *m/z* calcd for $C_{17}H_{11}F_3N_4O$ $[M+H]^+$ 345.0881; found 345.0956.

4-(2-Cyanopyridin-4-yl)-2-oxo-2,3-dihydro-1H-benzo[b][1,4]diazepine-8-carbonitrile (1f).



tert-Butyl (2-amino-4-cyanophenyl)carbamate[27] (0.233 g, 1 mmol), and tert-butyl 3-(2-cyanopyridin-4-yl)-3-oxopropanoate (0.320 g, 1.3 mmol) and TFA (1.14 g, 10 mmol) were processed according to the general procedure. Yellow solid (0.227 g, 79%), mp: 213-215 ºC. ^{1}H NMR (DMSO-d_6) *δ:* 10.83 (s, 1H), 8.93 (d, *J* = 5.0 Hz, 1H), 8.54 (s, 1H), 8.27-8.25 (m, 1H), 7.52-7.51 (m, 3H), 3.76 (s, 2H). ^{13}C NMR (DMSO-d_6) *δ* 165.9, 158.0, 152.3, 144.9, 141.8, 133.6, 131.0, 129.2, 127.6, 126.3, 123.7, 121.9, 118.2, 117.2, 109.3, 40.1. LCMS ESI *m/z*: 288.00 $[M + H]^+$. HRMS (ESI) *m/z* calcd for $C_{16}H_9N_5O$ $[M+H]^+$ 288.0821; found 288.0878.

4-(8-((4-Fluorophenyl)ethynyl)-2-oxo-2,3-dihydro-1H-benzo[b][1,4]diazepin-4-yl)picolinonitrile (1g).



tert-Butyl (2-amino-4-((4-fluorophenyl)ethynyl)phenyl)carbamate[28] (0.326 g, 1 mmol), tert-butyl 3-(2-cyanopyridin-4-yl)-3-oxopropanoate (0.320 g, 1.3 mmol) and TFA (1.14 g, 10 mmol) were processed according to the general procedure. Yellow solid (0.258 g, 68%), mp: 229-231 ºC. ^{1}H NMR (DMSO-d_6) *δ* 10.83 (s, 1H), 8.92 (d, *J* = 5.0 Hz, 1H), 8.56 (s, 1H), 8.26-8.25 (m, 1H), 7.65-7.61 (m, 2H), 7.50-7.25 (m, 5H), 3.64 (s, 2H); ^{13}C NMR (DMSO-d_6) *δ* 165.7, 155.7,

152.3, 152.0, 145.2, 138.6, 133.9, 133.8, 130.7, 128.7, 127.2, 126.0, 125.2, 124.6, 121.0, 118.5, 117.3, 116.3, 115.0, 89.4, 88.3, 40.1. LCMS ESI *m/z*: 381.00 $[M + H]^+$. HRMS (ESI) *m/z* calcd for $C_{23}H_{13}FN_4O$ $[M+H]^+$ 381.1065; found 381.1144.

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REFERENCES

1. Marmiroli, P. and G. Cavaletti, *The glutamatergic neurotransmission in the central nervous system.* Curr Med Chem, 2012. **19**(9): p. 1269-76.
2. Niswender, C.M. and P.J. Conn, *Metabotropic glutamate receptors: physiology, pharmacology, and disease.* Annu Rev Pharmacol Toxicol, 2010. **50**: p. 295-322.
3. Rondard, P. and J.P. Pin, *Dynamics and modulation of metabotropic glutamate receptors.* Curr Opin Pharmacol, 2015. **20**: p. 95-101.
4. Pin, J.P. and B. Bettler, *Organization and functions of mGlu and GABAB receptor complexes.* Nature, 2016. **540**(7631): p. 60-68.
5. Lundstrom, L., et al., *Reprint of Pharmacological and molecular characterization of the positive allosteric modulators of metabotropic glutamate receptor 2.* Neuropharmacology, 2017. **115**: p. 115-127.
6. Walker, A.G. and P.J. Conn, *Group I and group II metabotropic glutamate receptor allosteric modulators as novel potential antipsychotics.* Curr Opin Pharmacol, 2015. **20**: p. 40-5.
7. Sheffler, D.J., et al., *Recent progress in the synthesis and characterization of group II metabotropic glutamate receptor allosteric modulators.* ACS Chem Neurosci, 2011. **2**(8): p. 382-93.
8. Liechti, M.E. and A. Markou, *Role of the glutamatergic system in nicotine dependence : implications for the discovery and development of new pharmacological smoking cessation therapies.* CNS Drugs, 2008. **22**(9): p. 705-24.
9. Nickols, H.H. and P.J. Conn, *Development of allosteric modulators of GPCRs for treatment of CNS disorders.* Neurobiol Dis, 2014. **61**: p. 55-71.
10. Celanire, S., et al., *Novel metabotropic glutamate receptor 2/3 antagonists and their therapeutic applications: a patent review (2005 - present).* Expert Opin Ther Pat, 2015. **25**(1): p. 69-90.
11. Moussawi, K. and P.W. Kalivas, *Group II metabotropic glutamate receptors (mGlu2/3) in drug addiction.* Eur J Pharmacol, 2010. **639**(1-3): p. 115-22.

12. Hashimoto, K., et al., *Glutamate modulators as potential therapeutic drugs in schizophrenia and affective disorders.* Eur Arch Psychiatry Clin Neurosci, 2013. **263**(5): p. 367-77.
13. Chaki, S., et al., *mGlu2/3 and mGlu5 receptors: potential targets for novel antidepressants.* Neuropharmacology, 2013. **66**: p. 40-52.
14. Hemstapat, K., et al., *A novel family of potent negative allosteric modulators of group II metabotropic glutamate receptors.* J Pharmacol Exp Ther, 2007. **322**(1): p. 254-64.
15. Porta, R., M. Benaglia, and A. Puglisi, *Flow Chemistry: Recent Developments in the Synthesis of Pharmaceutical Products.* Organic Process Research & Development, 2016. **20**(1): p. 2-25.
16. Ingham, R.J., et al., *A systems approach towards an intelligent and self-controlling platform for integrated continuous reaction sequences.* Angew Chem Int Ed Engl, 2015. **54**(1): p. 144-8.
17. Grant, D., R. Dahl, and N.D. Cosford, *Rapid multistep synthesis of 1,2,4-oxadiazoles in a single continuous microreactor sequence.* J Org Chem, 2008. **73**(18): p. 7219-23.
18. Herath, A., R. Dahl, and N.D. Cosford, *Fully automated continuous flow synthesis of highly functionalized imidazo[1,2-a] heterocycles.* Org Lett, 2010. **12**(3): p. 412-5.
19. Herath, A. and N.D. Cosford, *Continuous-flow synthesis of highly functionalized imidazo-oxadiazoles facilitated by microfluidic extraction.* Beilstein J Org Chem, 2017. **13**: p. 239-246.
20. Herath, A. and N.D. Cosford, *One-step continuous flow synthesis of highly substituted pyrrole-3-carboxylic acid derivatives via in situ hydrolysis of tert-butyl esters.* Org Lett, 2010. **12**(22): p. 5182-5.
21. Pagano, N., M.L. Heil, and N.D. Cosford, *Automated Multistep Continuous Flow Synthesis of 2-(1H-Indol-3-yl)thiazole Derivatives.* Synthesis (Stuttg), 2012. **44**(16): p. 2537-2546.
22. Pagano, N., et al., *An integrated chemical biology approach reveals the mechanism of action of HIV replication inhibitors.* Bioorg Med Chem, 2017.
23. Pagano, N., A. Herath, and N.D. Cosford, *An Automated Process for a Sequential Heterocycle/Multicomponent Reaction: Multistep Continuous Flow Synthesis of 5-(Thiazol-2-yl)-3,4-Dihydropyrimidin-2(1H)-ones.* J Flow Chem, 2011. **1**(1): p. 28-31.
24. Dhanya, R.P., et al., *Design and synthesis of systemically active metabotropic glutamate subtype-2 and -3 (mGlu2/3) receptor positive allosteric modulators (PAMs): pharmacological characterization and assessment in a rat model of cocaine dependence.* J Med Chem, 2014. **57**(10): p. 4154-72.
25. Hutchinson, J.H., et al., *Novel Benzodiazepinones as modulators of metabotropic glutamate receptor functions and neurological uses therof. WO2013033246 (A2).* 2013.
26. Adam, G., et al., *Dihydrobenzodiazepin-2-one derivatives for the treatment of neurological disorders. WO03066623 (A1).* 2003.
27. Arndt, T., et al., *Substituted benzimidazolone derivatives, medicaments comprising them and their use. WO2008025736 (A1).* 2008.
28. Adam, G., et al., *Benzodiazepine derivatives. WO0129012 (A2).* 2001.