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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.

1. Introduction

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] (see Fig. 1).

We recently initiated a programme focused on the design, synthesis and in vitro and *in vivo* evaluation of $mGlu_{2/3}$ negative

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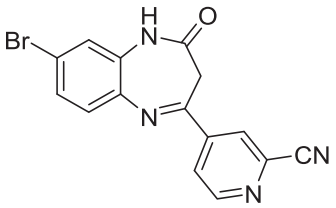


1a: MNI-137

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

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.

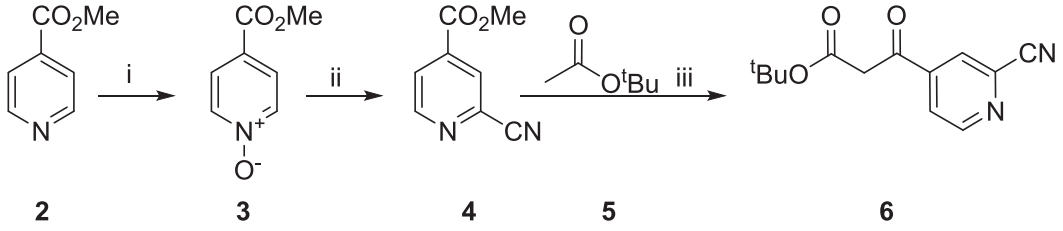
Over the past few years, multistep microfluidic chip-based processes have emerged as attractive methods for the large scale preparation of numerous organic molecules.[15,16] Advantages include enhanced reagent mixing, optimal heat transfer, small reaction volumes, precise reaction times, and the ability 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]

2. Results and discussion

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**).

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**.

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 1 M, and each of these solutions was pumped at a rate of 1 mL/min initially through a T-mixer at ambient temperature and subsequently into a 10 mL flow reactor held at 95 °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 8 g/h. This material was pumped into a second T-mixer and combined with a mixture of cyanotrimethylsilane and triethylamine (1:2.5) as a 1 M solution in acetonitrile with both streams flowing into the T-mixer at a rate of 0.2 mL/min. The combined reaction mixture was then pumped through a 5 mL reactor held at 150 °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 °C through 0 °C to establish that −30 °C was ideal for the flow process. As illustrated in Scheme 4, a 2 M solution of *tert*-butylacetate (**5**) and a 2 M solution of LDA are both pumped at a rate of 0.2 mL/min into a T-mixer that then flows the reaction mixture into a cooler module held at −30 °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**)



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 °C, 16 h; ii) TMSCN, Et_3N, MeCN, 80 °C, 2 h; iii) LDA, THF, −78 °C, 4 h.