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# Large Scale Synthesis of an Ampakine-Type API Based on a Telescoped Regioselective Double Amidation Reaction

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[†] These authors developed the one-pot procedure involving the diazotization and annulation sequence to synthesize the final product.

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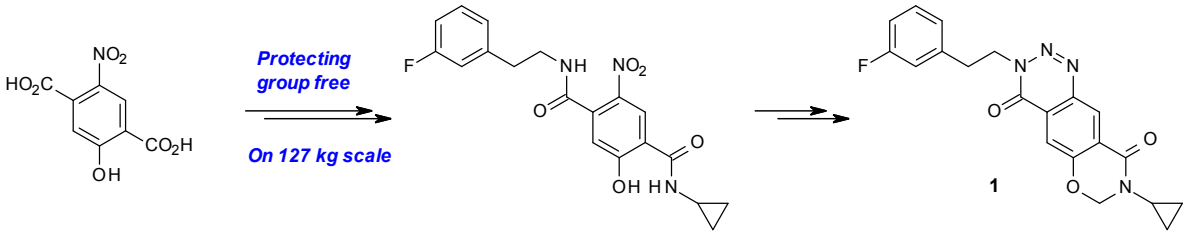


**ABSTRACT:** This work describes the process development and manufacture of an ampakine-type API in clinical trials. A regioselective CDI-mediated amidation process was optimized for the subsequent couplings of two distinctive amines with a terephthalic acid substrate. Choice of the synthetic route, key scale up issues, safety calorimetry and optimization of all steps for multi kg production are discussed.

**KEYWORDS:** regioselective CDI-mediated amidation, protecting-group-free synthesis, telescoped process, AMPA receptors.

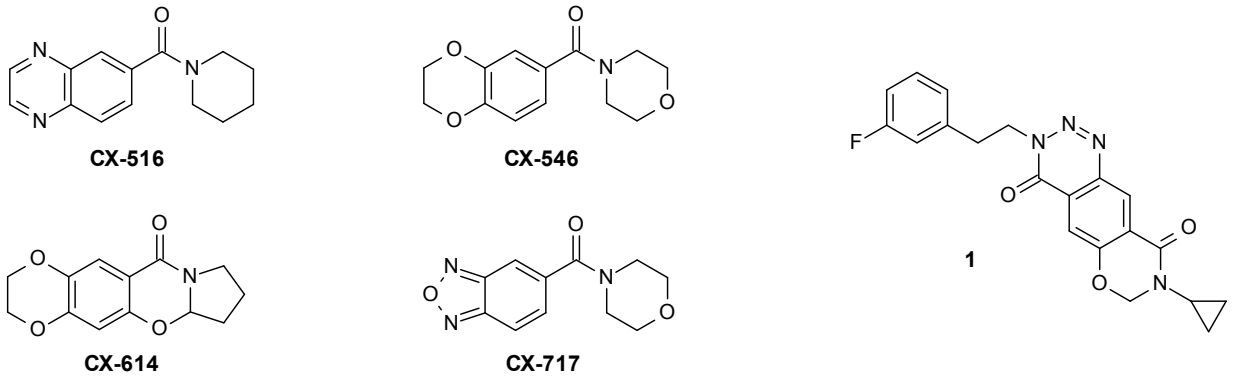
## Introduction

Glutamate (Glu) is the major excitatory neurotransmitter in the central nervous system (CNS) and is involved in the physiological regulation of processes such as synaptic plasticity, learning and memory.[1] Glutamate activates specific receptors that belong to the classes of metabotropic receptors (coupled to G-proteins) and ionotropic receptors (ligand-gated ion channel), the latter consisting of two primary families: *N*-methyl-D-aspartic acid (NMDA) receptor family and non-NMDA family including the kainic acid (KA) receptor and the (*R,S*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) receptor.[2,3] AMPA-type receptors are found in high concentration in the superficial layers of the neocortex, in each of the major synaptic zones of hippocampus and in the striatal complex.[4] They mediate fast excitatory postsynaptic currents at the great majority of synapses and are involved in the expression of long-term potentiation, an increase in the strength of synaptic contacts that follows repetitive physiological activity of a type known to occur in the brain during learning.[5,6] Thus a promising therapy under investigation based on restoring diminished glutamatergic neurotransmission is the administration of AMPA receptor potentiators for the treatment of Alzheimer's disease, cognitive disorders, schizophrenia or depression.[7,8,9] Considerable advances have been made in solving the three-dimensional structure of the AMPA receptor and X-ray structures have been reported.[10,11] Based on strong association with neurological disorders, extensive studies have been pursued to identify AMPA receptor positive modulators as cognition enhancers.[12] Among several distinct classes of compounds investigated, Ampakines such as CX-516,[13] CX-546,[14] CX-614[15] or CX-717[16] (Figure 1) have shown to have very high activity in *in vitro* and *in vivo* models, acting as positive allosteric modulators (PAM). After extensive research, compounds bearing a triazone moiety increasing metabolic stability were disclosed and were considered as potential drugs to cure neurodegenerated diseases by modulating AMPA receptors at nanomolar concentrations. A wide

range of Ampakines have been synthesized leading to **1** which entered in clinical trials.[17,18] Unlike "low-impact" AMPA receptor potentiators such as CX-516, **1** is able to increase AMPA receptor activation and is therefore considered as a "high-impact" AMPA receptor potentiator.[19] Compound **1** has also been found to enhance cognition and memory in animals, to produce antidepressant-, antianhedonic-, and anxiolytic-like effects, and to have neurotrophic and neuroplasticity-promoting activities.[20,21] Moreover, it has been found to increase levels of brain-derived neurotrophic factor (BDNF) in the hippocampus and to stimulate hippocampal neurogenesis.[20,22]

As part of a program aimed at identifying compounds that increase AMPA receptor-mediated responses, **1** was identified as a good candidate to enter in clinical trials. In order to deliver large quantities of **1**, we embarked upon an effort to develop an optimal route for its long-term manufacture. The successful completion of this goal is the subject of this paper.

**Figure 1.** Chemical structures of Ampakines



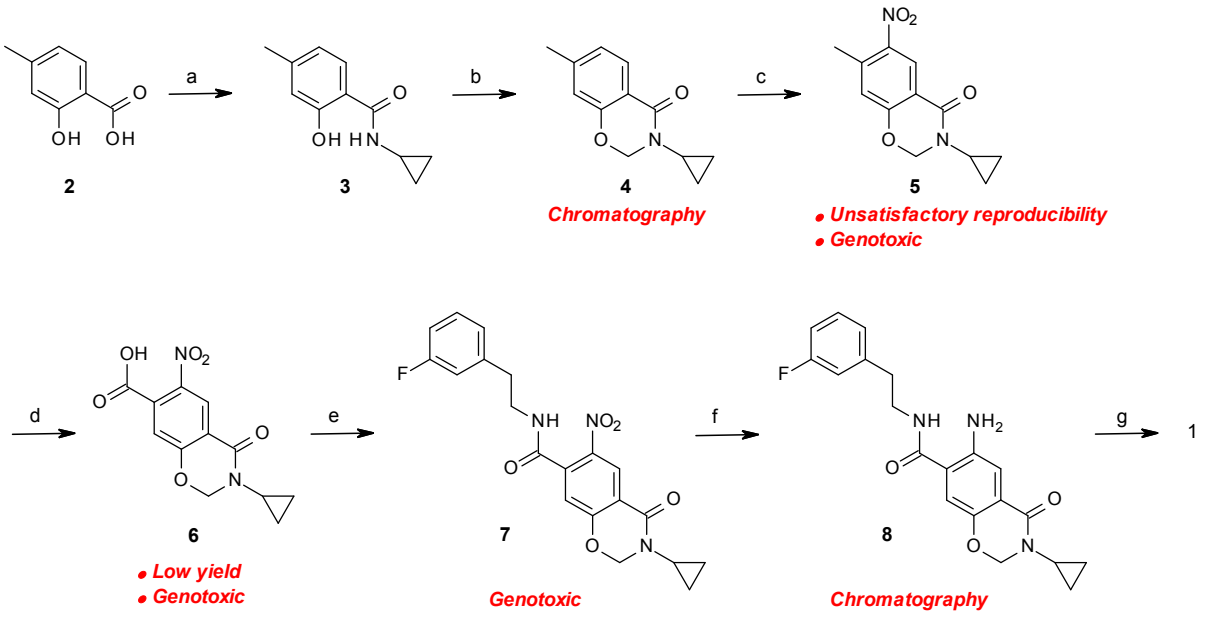

## Results and Discussion

The original synthesis of **1** is illustrated in Scheme 1 and starts with 2-hydroxy-4-methylbenzoic acid **2**.[18] Amide formation with cyclopropylamine mediated with CDI and $Et_3N$ in methylene chloride provided intermediate **3** as a solid. Insertion of the formaldehyde synthon was carried out with trioxane and sulfuric acid in refluxed chloroform in the presence of sodium sulfate. Chromatography of the crude on silica gel was required to obtain a satisfactory chemical purity. Compound **4** was then subjected to nitric acid in a mixture of methylene chloride and acetic acid to provide the desired 6-nitro isomer **5**. The methyl group was then oxidized in a two-step sequence: treatment with N,N-dimethylformamide dimethyl acetal in DMF at 125 °C during 16 hours followed by concentration to dryness and addition of sodium periodate in a mixture of THF and water to provide the nitro aldehyde intermediate, which was smoothly converted into acid **6** with oxone in DMF. Coupling of 2-(3-fluorophenyl)ethanamine to the nitro acid derivative was accomplished in methylene chloride using thionyl chloride and a catalytic amount of DMF. Reduction of the nitro moiety was performed with a freshly prepared Zn/Cu reagent[23] in a mixture of THF, MeOH and formic acid. Filtration on silica gel followed by concentration to dryness provided aniline **8** which was treated with an excess of isoamyl nitrite in DMF to generate the triazinone ring. Addition of diethyl ether caused precipitation of the final product **1**.

Although this route produced successfully **1** on gram scale, several issues had to be addressed to manufacture larger quantities for clinical trials. Our main concern was the lack of reproducibility of the nitration step and also the instability of the reaction mixture. Other drivers for the development of a new route for the synthesis of **1** focused on operational issues. Besides some low-yielding steps, eight isolations by concentration to dryness were necessary and are unsuitable for a pilot-plant environment combined with a reprocess of the mother liquors after the nitration step to isolate the remaining nitro intermediate **5**. Chromatography on silica gel of **4** and **8** was also required to obtain a satisfactory chemical purity which is a burden on scale. Finally

quantitative structure-activity relationship (QSAR) predictions (DEREK and Leadscope) showed genotoxic alert for compounds **5**, **6** and **7** that was confirmed since all intermediates gave a positive Ames II test. Therefore, we were mindful of the potential difficulties engendered by the presence of impurities in the active pharmaceutical ingredient (API). This latter driver prompted us to reinvestigate the entire synthesis.

**Scheme 1.** Discovery route of **1**[a]



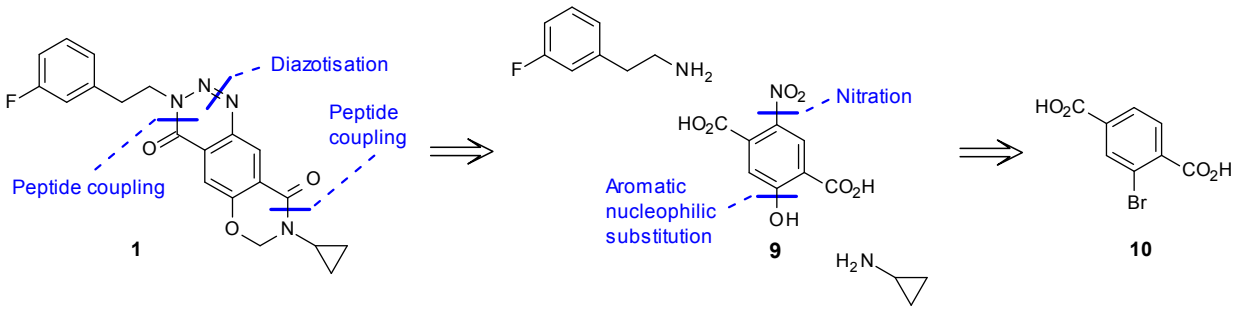

[a]Reagents and conditions: (a) CDI, $CH_2Cl_2$, 24 h at 20 °C then cyclopropylamine, $Et_3N$, 3 days, 86%; (b) trioxane, $CHCl_3$, conc. $H_2SO_4$, $Na_2SO_4$, 2 h at reflux, 83% after chromatography; (c) conc. $HNO_3$, AcOH, $CH_2Cl_2$, 0 °C, 41%; (d) *N,N*-Dimethylformamide dimethyl acetal, DMF, 125 °C, 16 h, concentration then $NaIO_4$, THF, water, 20 °C; oxone, DMF, 20 °C overnight, 38% over 2 steps; (e) $SOCl_2$, $CH_2Cl_2$, DMF cat., 20 °C overnight then 2-(3-fluorophenyl)ethanamine, $Et_3N$, 68%; (f) Zn/Cu, THF, MeOH, $HCO_2H$, 20 °C overnight, chromatography; (g) isoamyl nitrite, DMF, 2 h at 20 °C, 59% over 2 steps.

## Early development synthesis of 1

One of our major goals was to define a robust and efficient method to synthesize **1**. We reasoned that desired product **1** could be disconnected into three components that required two distinctive

amide bond formations (see Figure 2). Our strategy relied on taking advantage of the non-symmetrical terephtalic acid to selectively functionalize one carboxylic acid moiety over the other one without any protecting group. This approach could be valuable in terms of both step and atom economy.[24] Therefore compound **9** was selected as key intermediate of the synthesis and could be manufactured starting from brominated terephthalic acid **10**.

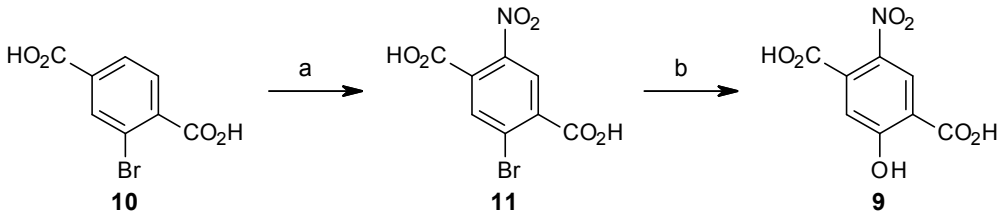
**Figure 2.** Proposed retrosynthesis to access **1**




The synthesis of **9** is depicted in Scheme 2. The first step of the sequence consisted of a regioselective nitration performed on **10** in neat sulfuric acid at 40 °C by adding sequentially a 1:2 v/v mixture of nitric acid and sulfuric acid. For safety reasons this process was evaluated in a reaction calorimeter (RC1). As shown in Figure 3, a steady heat flow of 15-20 W/kg was observed over the entire course of the addition of $HNO_3$ except when the product precipitates.[25] The spike in heat flow was attributed to the onset of crystallization that continued throughout addition of $HNO_3$. The total amount of heat generated was 94 kJ/kg of reaction mass that corresponded to a $\Delta T_{ad}$ of 54 °C. Despite some thermal accumulation (23% at stoichiometry), the main concern was the ability to stir the thick reaction mixture. DSC (Differential scanning calorimetry) showed an event with moderate energy ($\Delta T_{ad}$ of 36 °C) between 86 and 166 °C (see Figure 4). Associated with a $TMR_{ad}$ of 3 days at 40 °C, the probability to reach 166 °C where an

exothermic decomposition occurred was low. Hydrolysis was also evaluated in a RC1 prior to the pilot-plant batch. To ensure a safe control of the heat release (the total heat output was 312 kJ/kg of reaction mass corresponding to a $\Delta T_{ad}$ of 90 °C), it was decided to add slowly 20% of the total amount of water. In that case, the average heat release varied between 20 and 31 W/kg (Figure 5). The remaining amount of water was then added and the average heat release was 10 W/kg as shown on Figure 6. It is worth mentioning that the reaction of water with both sulfuric and nitric acids was responsible for most of the heat release. This safety assessment study was also consolidated by DSC where an estimated $TMR_{ad}$ of 24 hours at 47 °C was associated to a moderate event between 80 and 340 °C ($\Delta T_{ad}$ of 152 °C) including an exothermic decomposition at 200 °C (see Figure 7). Following those observations, compound **11** was successfully synthesized on 100 kg scale with 85% yield after precipitating out of the reaction mixture.

**Scheme 2.** Synthesis of **9**[a]




[a]Reagents and conditions: (a) conc. $H_2SO_4$ (1 equiv), 65% aq. $HNO_3$ (1.2 equiv), 3 h at 65 °C, 85%; (b) aq. NaOH (10 equiv), 12 h at reflux, 75%.

**Figure 3.** Heatflow for nitration of **10** with a mixture $HNO_3$ / $H_2SO_4$

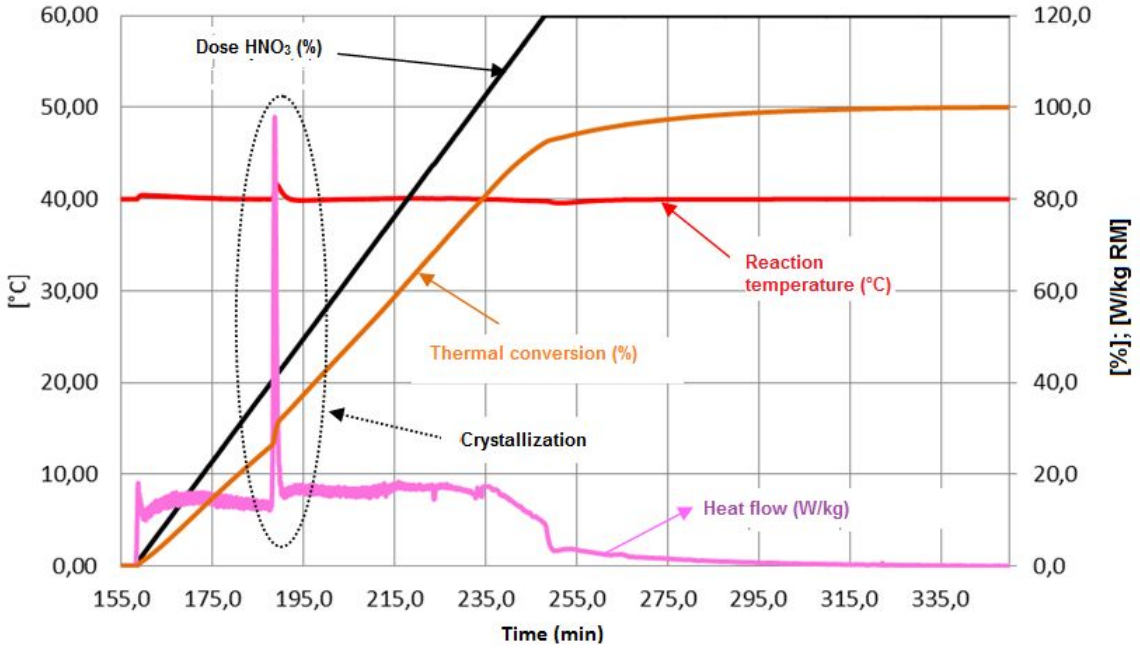
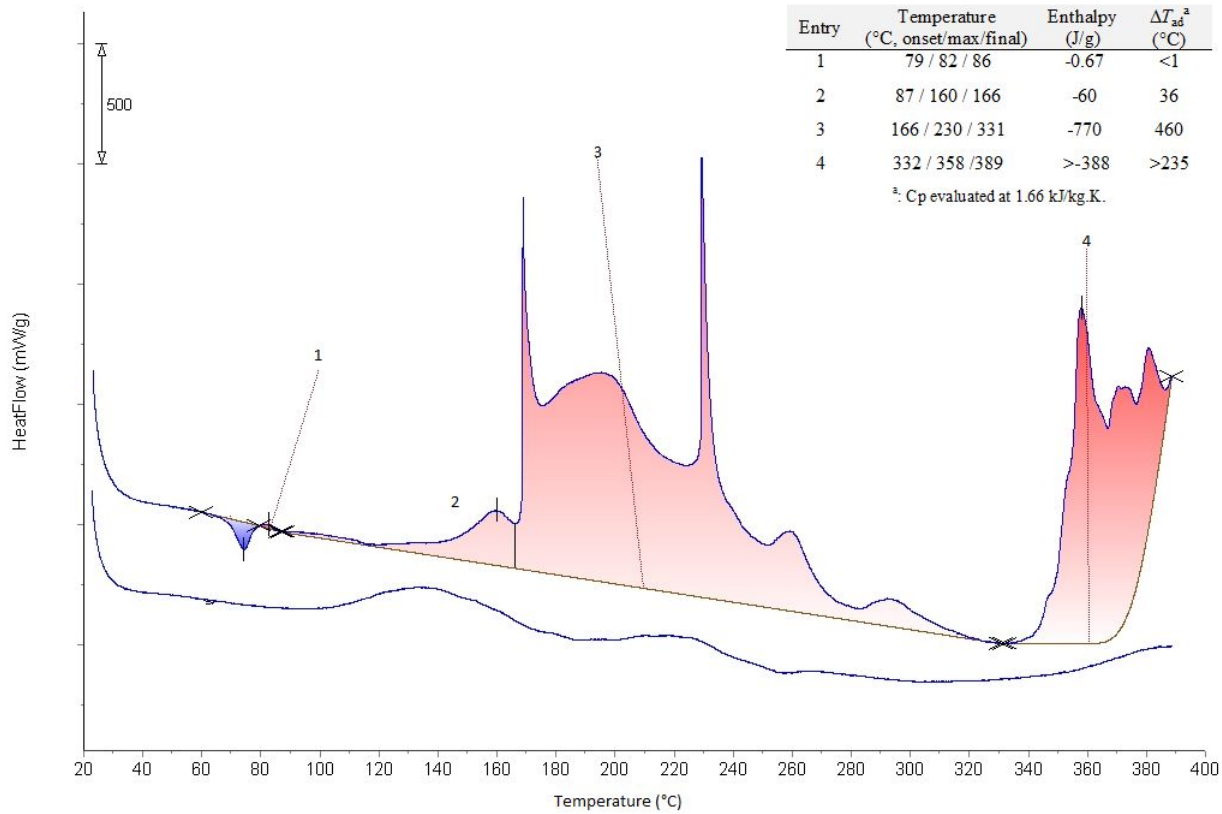



**Figure 4.** DSC thermogram for nitration of **10** with a mixture $HNO_3$ / $H_2SO_4$




| Entry | Temperature (°C, onset/max/final) | Enthalpy (J/g) | $\Delta T_{ad}$[a] (°C) |
|---|---|---|---|
| 1 | 79 / 82 / 86 | -0.67 | <1 |
| 2 | 87 / 160 / 166 | -60 | 36 |
| 3 | 166 / 230 / 331 | -770 | 460 |
| 4 | 332 / 358 /389 | >-388 | >235 |

[a]: Cp evaluated at 1.66 kJ/kg.K.

**Figure 5 .** Heatflow for hydrolysis with 20% of total amount of water

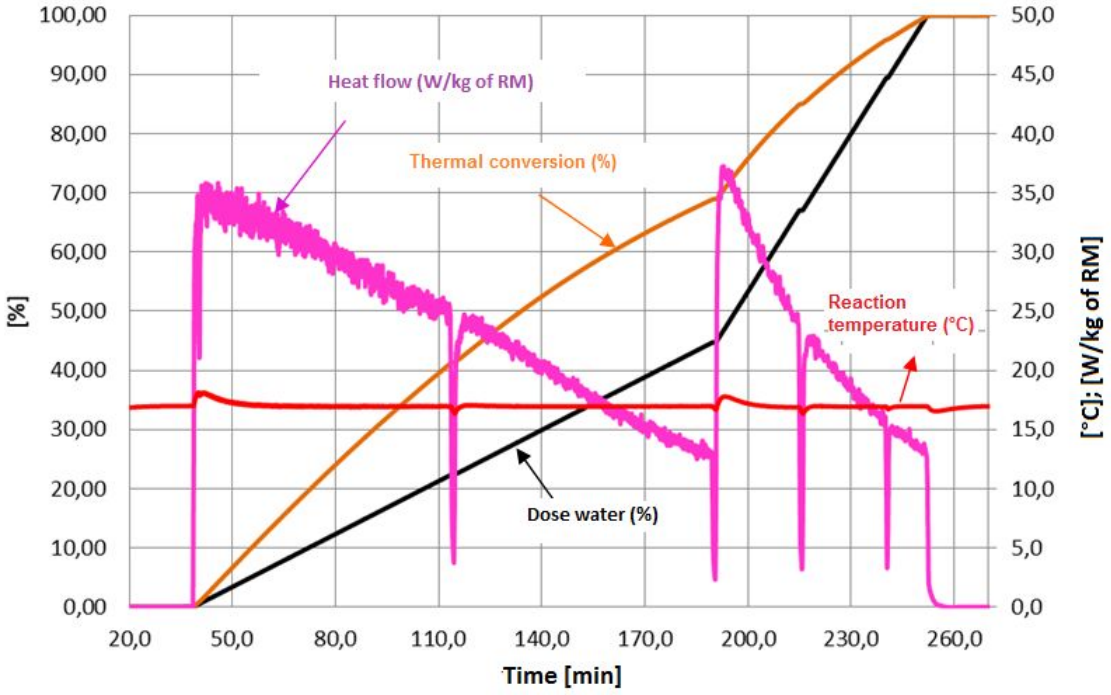



**Figure 6.** Heatflow for hydrolysis with the 80% of remaining water

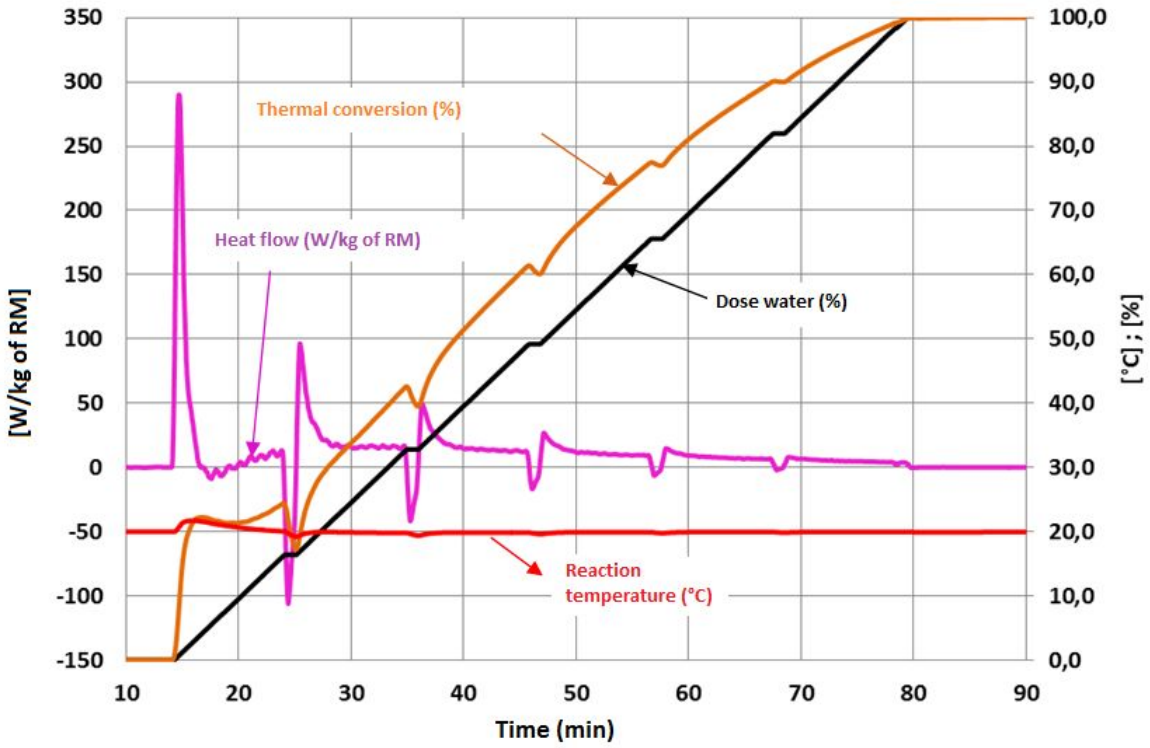
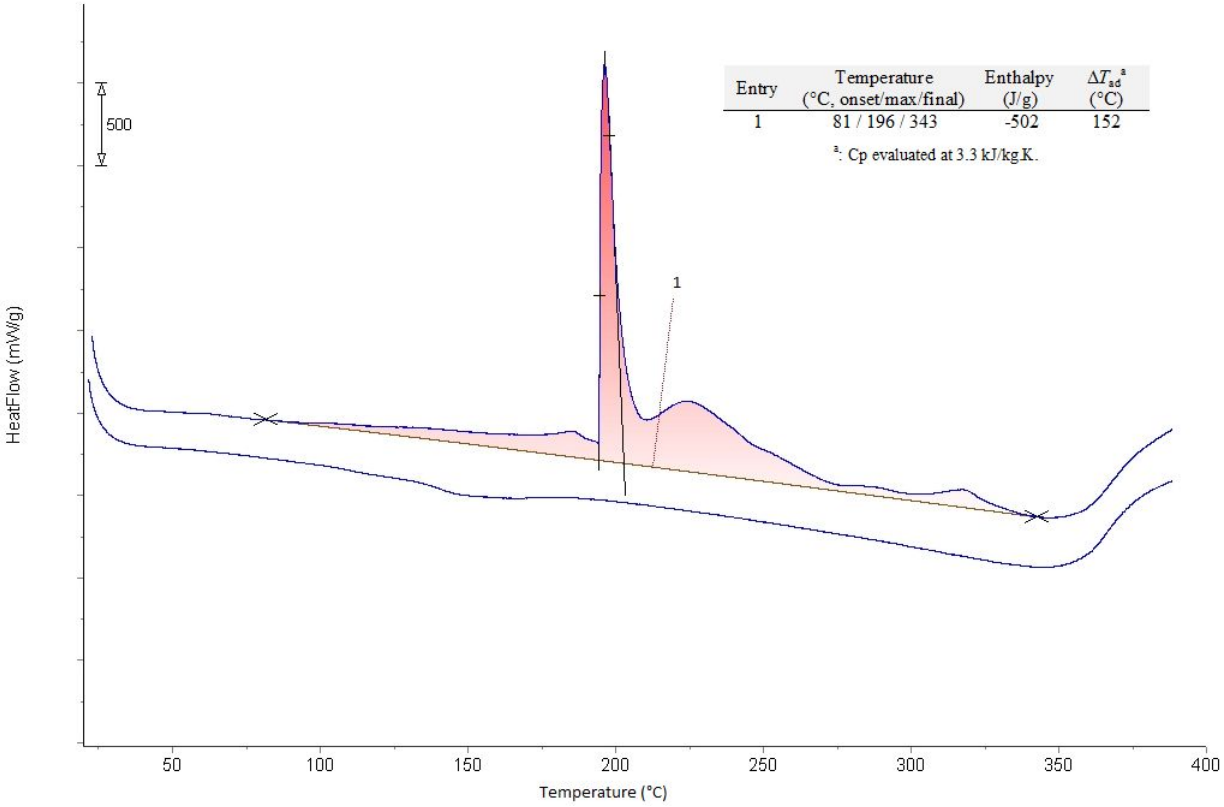


**Figure 7.** DSC thermogram of the reaction mixture after hydrolysis




Substitution of the bromide was performed with an excess of sodium hydroxide at reflux. After treatment with an aqueous solution of HCl and filtration of the reaction mixture, the remaining NaCl was removed with several aqueous washes to provide anhydrous **9** (Kf < 0.4 m/m) with 75% yield and high purity (HPLC: > 99.0 area %) on 113 kg scale.

To overcome the issues associated with the medicinal route, we first developed an approach based on the selective formation of the 1,3-benzodioxin-4-one intermediate **12** (Scheme 3). Treatment of **9** with trioxane in a mixture of water and sulfuric acid provided **12** with 75% yield. The remaining carboxylic acid function was then reacted with 2-(3-fluorophenyl)ethanamine in acetonitrile in the presence of $Et_3N$ and T3P to provide amide **13**. The nitro group was easily

reduced with hydrogen and a catalytic amount of Pd/C in THF yielding aminobenzamide **14** (88% yield and 98.6% purity). Diazotization was initially performed with *tert*-butyl nitrite in THF. The conversion was complete after 1 hour at 10 °C but the filtration of the slurry was extremely slow after acidic hydrolysis. DSC showed that even if an estimated $TMR_{ad}$ of 5 days at 20 °C was attributed for *tert*-butyl nitrite (Table 1, entry 1), a couple of highly exothermic decompositions with onsets at respectively 94 and 195 °C prompted us to evaluate alternative conditions in terms of process safety. The use of isoamyl nitrite in THF was rapidly eliminated because of a decomposition exotherm with an onset at 74 °C and a $TMR_{ad}$ of only 24 hours at 20 °C (entry 2). The results depicted in Table 1 showed that sodium nitrite in a mixture of water and acetic acid was the best candidate (entry 3). It was selected because its degradation was moderate and spread on a wide window of temperature associated with a $TMR_{ad}$ > 7 days at 20 °C (see Supporting Information).

**Table 1.** Evaluation of thermal stability of three nitrite derivatives

| Entry | Nitrite | Temperature (°C, onset/max/final) | Enthalpy (J/g) | $\Delta T_{ad}$ (°C) | $TMR_{ad}$ at 20 °C |
|---|---|---|---|---|---|
| 1[a] | *tert*-Butyl | 94/182/195 | -234 | 117[d] | 5 days |
| | | 195/230/384 | -2455 | 1228 | |
| 2[b] | Isoamyl | 74/166/222 | -1000 | 590[e] | 24 h |
| 3[c] | Sodium | 108/269/287 | -296 | 115[f] | >7 days |

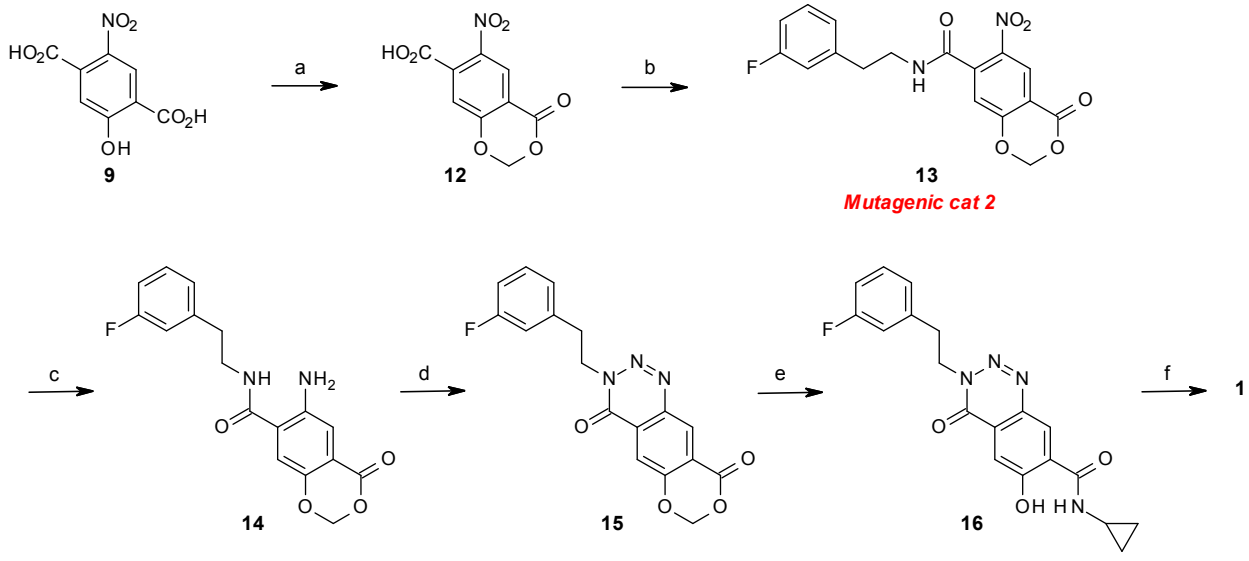
[a]: neat. [b]: 1.4 g in 1.5 g of THF. [c]: 1 g in 5.26 g of water and 12.5 g of acetic acid. [d]: Cp evaluated at 2 kJ/kg.K. [e]: Cp = 1.7 kJ/kg.K based on THF. [f]: Cp = 2.6 kJ/kg.K based on water and acetic acid.

When an aqueous solution of sodium nitrite was added on a solution of **14** in acetic acid, triazinone **15** was obtained with 90% yield and 99.0% chemical purity. Although some accumulation occurred, the heat release was easily manageable ($\Delta T_{ad}$ of 27 °C) and heat flow in

accordance with our cooling capacities.[26] Some brown smokes were observed five minutes after the end of the nitrite addition. It reflected the release of $NO_2$ resulting from decomposition of $N_2O_3$, the latter being generated from $HNO_2$. Few minutes seemed to be necessary to initiate and to go through the whole process of $NO_2$ evolution.

Formation of the second amide bond was achieved by adding cyclopropanamine on **15** in THF to afford intermediate **16**, which was re-slurred in a mixture of acetonitrile and DMSO to improve the purity (63% overall yield). The desired product was finally obtained after reacting **16** with trioxane in $CH_2Cl_2$ under acidic conditions. Crude **1** was recrystallized in a mixture of EtOAc and DMSO and was isolated in 77% yield with a satisfactory purity of 99.8%.

**Scheme 3.** Early development synthesis of **1**[a]




[a]Reagents and conditions: (a) trioxane, conc. $H_2SO_4$, water, 75%; (b) 2-(3-fluorophenyl)ethanamine, T3P, $Et_3N$, MeCN, 71%; (c) $H_2$, THF, Pd/C, 88%; (d) $NaNO_2$, AcOH, water, 90%; (e) cyclopropanamine, EtOAc, $iPr_2O$, then reslurry in a 9:1 MeCN/DMSO mixture, 63%; (f) trioxane, $CH_2Cl_2$, $Na_2SO_4$, conc. $H_2SO_4$, then recrystallization in a 8:2 DMSO/AcOEt mixture, 77%.

At this stage, we had developed from **12** a 5 isolated step synthesis with 26% overall yield including two recrystallizations that allowed us to manufacture batches of up to 30 kg of API. However, the use of trioxane, considered as CMR (carcinogenic, mutagenic, reprotoxic) reagent, in first and last steps was not ideal. Besides, intermediates **12**, **13**, **14** and **15** were positive in the Ames assay (**13** identified as mutagenic, category 2). We assumed it was linked to the relative labile nature of the 1,3-benzodioxin-4-one moiety releasing formaldehyde after ring opening. It is worth mentioning that during the synthesis we observed the corresponding hydroxy-benzoic acid derivatives of compounds **12**, **13**, **14** and **15** at levels of up to few %. Since we anticipated that dealing with genotoxic impurities could be a burden for the development of the API, our strategy was to avoid the formation of the 1,3-benzodioxin-4-one moiety.

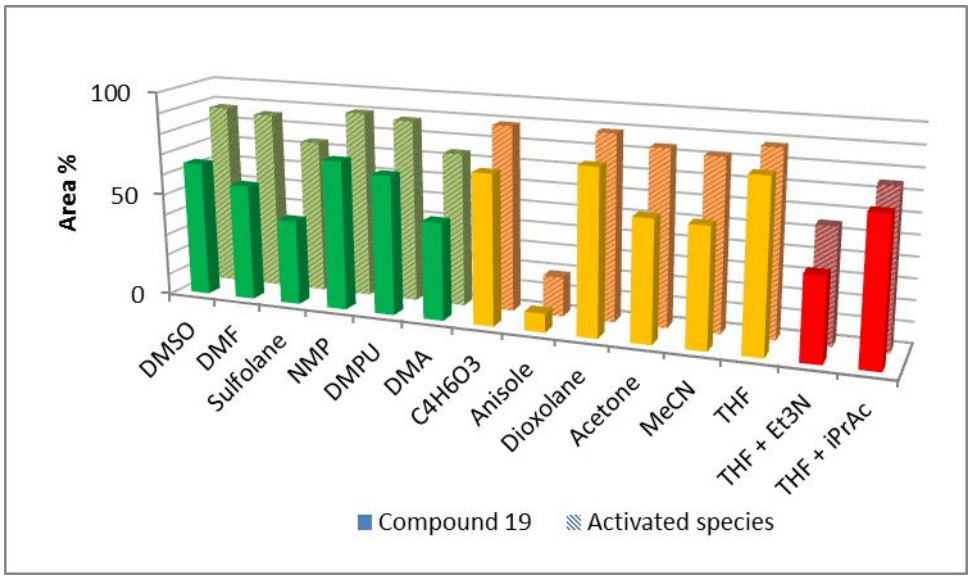
**Final process development**

Peptide couplings are one of the most common chemical reactions performed in the pharmaceutical industry.[27] Our goal was to generate selectively two amide bonds early in the synthesis taking advantage of the difference in terms of reactivity of the carboxylic acid functions. Acid activation by coupling agents was required to promote the condensation with an amine.[28] We selected several coupling agents described in the literature such as T3P, ethyl chloroformate, oxalyl chloride, thionyl chloride, EDCI, HBTU, TBTU and CDI. After screening the aforementioned coupling agents, we were pleased to discover that a stepwise process reacting first CDI followed by addition of 2-(3-fluorophenyl)ethanamine in refluxing EtOAc provided selectively amide **19** with 83% yield and 85% purity on lab scale (see Scheme 4).[29] This superb regioselectivity was attributed to the distinctive pKa between the two acidic functions (pKa of respectively 1.1 and 2.2 for acids in ortho- and meta-position of the nitro group). The order of

addition was also crucial to get a satisfactory yield. Addition of **9** to a solution of 1.1 equiv of CDI furnished up to 20% of impurity **31** (See structure on Scheme 9).

Gum formation after addition of CDI was the major issue and was attributed to intermediates **17** and/or **18**. Since attempts to filter and purify **19** failed, significant effort was put into finding alternate conditions to improve both process and purity.[30] To identify scalable conditions, we conducted a solvent survey and the results are illustrated in Figure 8. Ability to stir the reaction mixture was the major driver: when CDI was dissolved in DMSO, DMF, sulfolane, NMP, DMPU or DMA and added to the starting material in EtOAc solution, no gum formation was observed. DMSO was finally selected because of its lower toxicity. Solubility of CDI in DMSO was also evaluated for concentrations ranging from 5% w/w up to 43.8% w/w in conjunction with DSC (see Supporting Information). For safety reasons, a solution of 28% w/w was finally selected (heating the mixture at 37 °C was required to solubilize CDI instead 75 °C for a 43.8% w/w solution).

**Figure 8.** Coupling of **9** and 2-(3-fluorophenyl)ethanamine in EtOAc with CDI in additional solvents (in red, no ability to stir; in yellow, formation of more or less gums; in green, solution).



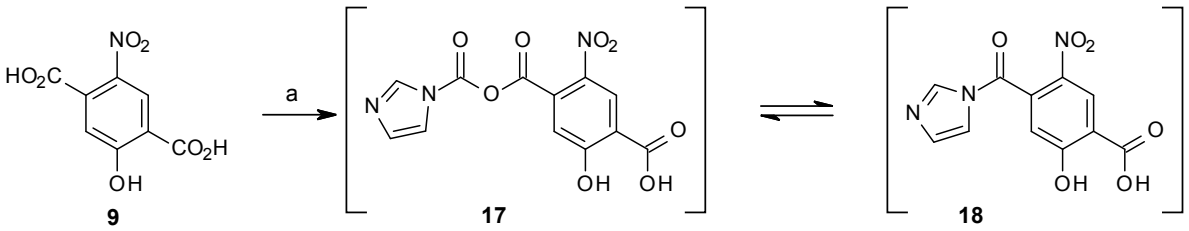

$C_4H_6O_3$: propylene carbonate

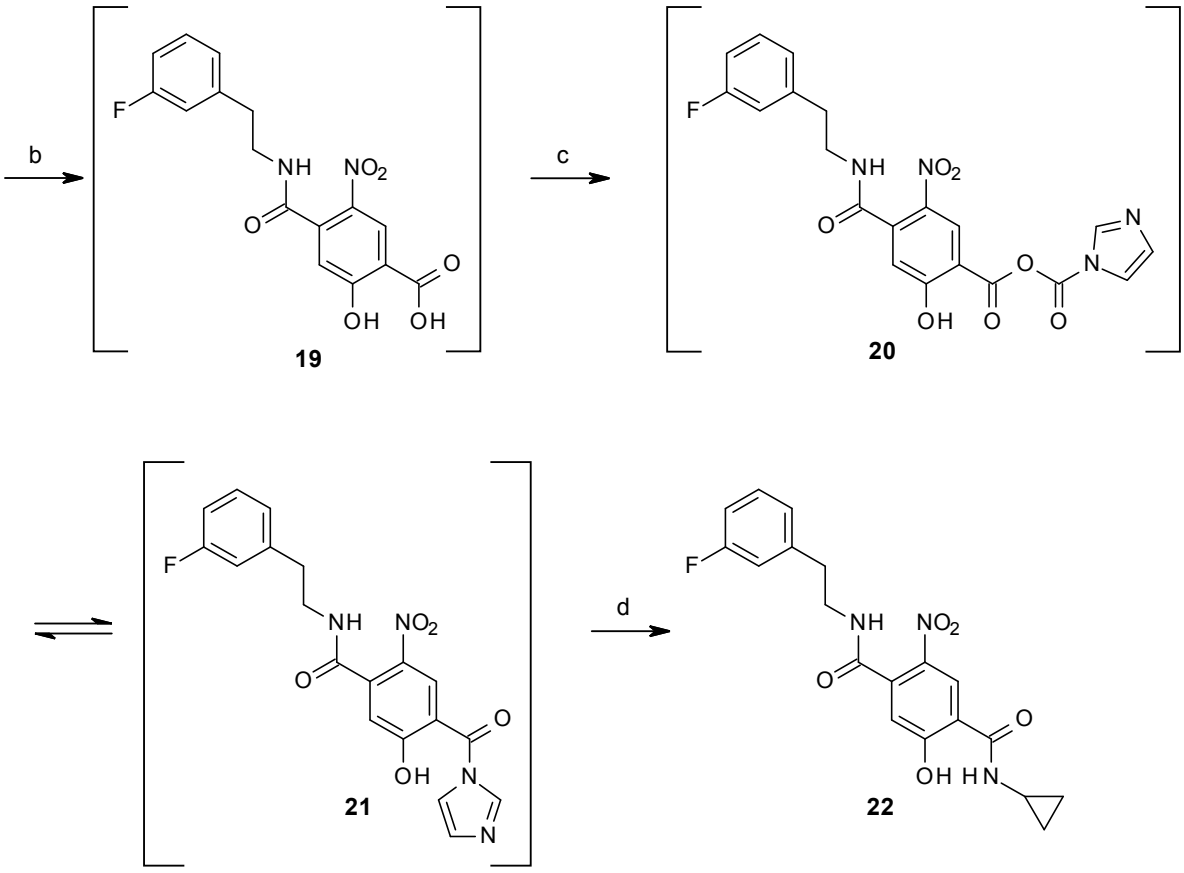
Failure to efficiently isolate amide **19** prompted us to envision a telescoped process to install the second amide bond. CDI was kept as coupling agent and scouting experiments were initiated on a pure sample of **19**. Among the solvents or mixtures of solvents we evaluated, MeTHF provided the best results and advantageously dissolved CDI. When a solution of **19** in a mixture of EtOAc and DMSO was sequentially reacted with CDI in MeTHF followed by a solution of cyclopropanamine in MeTHF at 20 °C, desired **22** was obtained. Room temperature was preferred because of the low boiling point of cyclopropanamine. It is noteworthy to point out that this order of addition was crucial to get the best selectivity.[31] Concentration followed by acidic treatment allowed **22** to crystallize. After filtration, the wet cake was washed with MeCN to provide **22** with 50-60% overall yield and a satisfactory 98% chemical purity on lab scale. However, when this sequence was scaled up, we experienced a significant drop in terms of yield (only 30 to 50% at best). Investigations showed that stirring the solution of CDI in DMSO at 40 °C for few hours before addition to terephthalic acid **9** was deleterious. Degradation of CDI was

suspected since the level of impurities in the reaction mixture increased resulting in a lower yield of isolated **22**.

In order to improve the robustness of this telescoped sequence, CDI was finally dissolved in MeCN and added on **9** in DMSO. Implementing this procedure on large scale allowed us to address the issue associated with the instability of CDI and we were pleased to isolate **22** with 65% yield on 127 kg scale with an adequate quality to be processed to the next step without additional purification.

**Scheme 4.** Sequential regioselective amidation[a]

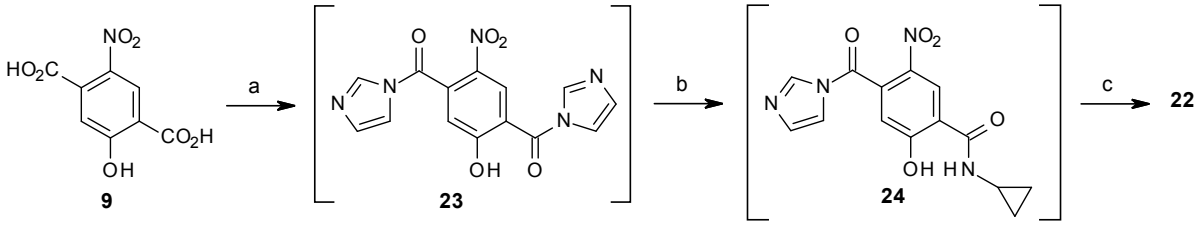





[a]Reagents and conditions: (a) **9** in DMSO/MeCN, then CDI (1.1 equiv) in MeCN, 4 h at 60 °C; (b) 2-(3-fluorophenyl)ethanamine in MeCN, 18 h at 80 °C; (c) CDI (1.1 equiv) in MeCN, 9 h at 25 °C; (d) cyclopropanamine (1.1 equiv) in MeCN, 1 h at 25 °C; 5 N HCl, 65% overall yield.

It should be mentioned that addition of 2.3 equivalents of CDI to diacid **9** yielded to intermediate **23**. In that case, the regioselectivity of the peptide couplings was inverted. Treatment with cyclopropanamine at room temperature provided amide **24** that was directly reacted at 80 °C with 2-(3-fluorophenyl)ethanamine to give desired compound **22** (see Scheme 5). However, since the rate conversion was too slow (more than 48 hours for the second peptide coupling) and the overall yield less than 30%, this approach was not further explored.

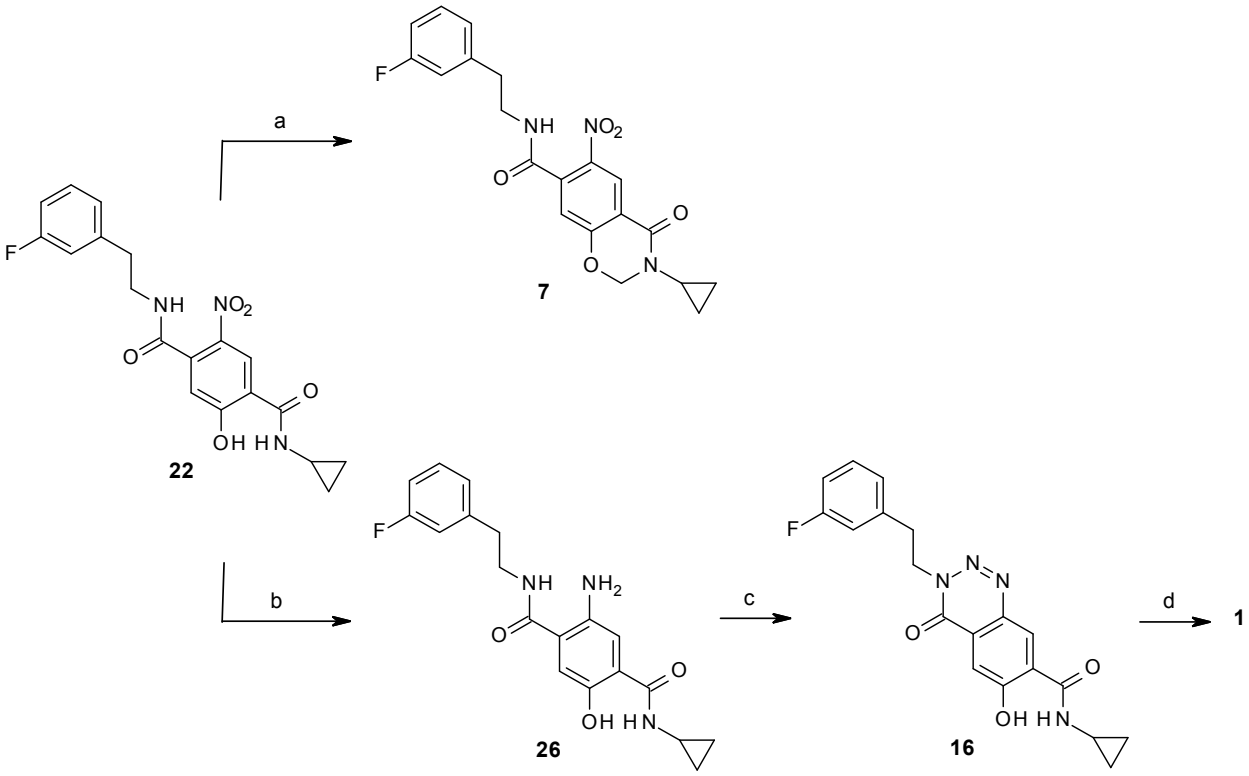
**Scheme 5.** Regioselective amidation with 2.3 equivalents of CDI[a]




[a]Reagents and conditions: (a) CDI (2.3 equiv) in DMSO, EtOAc, 25 °C, 1 h; (b) cyclopropanamine (1.2 equiv), 18 h; (c) 2-(3-fluorophenyl)ethanamine (1.05 equiv), 65 °C, 48 h, 24% overall yield.

At this stage, two different routes could be envisioned to access **1** (see Scheme 6). The first one consisted in reacting **22** with formaldehyde to generate **7**. Since the reaction stalled at 50% conversion in preliminary trials, we decided to reduce the nitro moiety first before conducting the diazotization with sodium nitrite. Reduction in THF with Pd/C under 1 bar of hydrogen smoothly provided compound **26** which was crystallized by addition of water after solvent switch from THF to DMSO. 2-Aminobenzamide **26** was then re-slurred with 20v of EtOAc to obtain a satisfactory purity but with inconsistent yields ranging from 50 to 70%. To improve both efficiency and robustness, a solvent switch directly from THF to EtOAc (10 L/kg) resulted in a smooth crystallization of **26** with 82% yield and with a quality greater than 99%.

**Scheme 6.** Envisioned routes and completion of the synthesis of **1**[a]




[a]Reagents and conditions: (a) Formaldehyde 37 wt % in water (2 equiv), 2 h at 90 °C, 40% after isolation; (b) Pd/C, THF, $H_2$, 1 bar, 2 h at 25 °C, 82%; (c) $NaNO_2$ (1.3 equiv), AcOH, $H_2O$, 3 h at 50 °C, 89%; (d) 1,3,5-trioxane (0.5 equiv), conc. $H_2SO_4$, EtOAc, AcOH, 92% then recrystallization in anisole/EtOH, 86%.

The triazinone ring was installed by reacting sodium nitrite in a mixture of AcOH and water. As shown on Figure 9, safety aspect of this step was evaluated in a RC1 experiment. The heat output was moderate (37 kJ/kg of reaction mass) but thermal accumulation not negligible. The former was linked to a limited mass transfer because of the thickness of the reaction mixture. However with an associated $\Delta T_{ad}$ of 12 °C, the average heat flow of 16 W/kg was in accordance with our cooling capacities. Thermal stability of the reaction mixture at the end of the addition was also evaluated by DSC and didn't show any major issue (Figure 10). This is why we estimated that 50 °C was reasonable to run the reaction in safe conditions. Concerning intermediate **16**, an exothermic decomposition with an onset at 128 °C and an associated $\Delta T_{ad}$ of 563 °C (see Figure

11) prompted us to dry the powder at 45 °C (estimated $TMR_{ad}$ of 8 days instead of 24 hours at 75 °C). Taking in consideration that efficiency of stirring was a key factor, the reaction proceeded well in the pilot-plant and intermediate **16** was isolated with 89% yield and >98% purity on 24 kg scale.

**Figure 9.** Heat flow for triazinone **16** formation

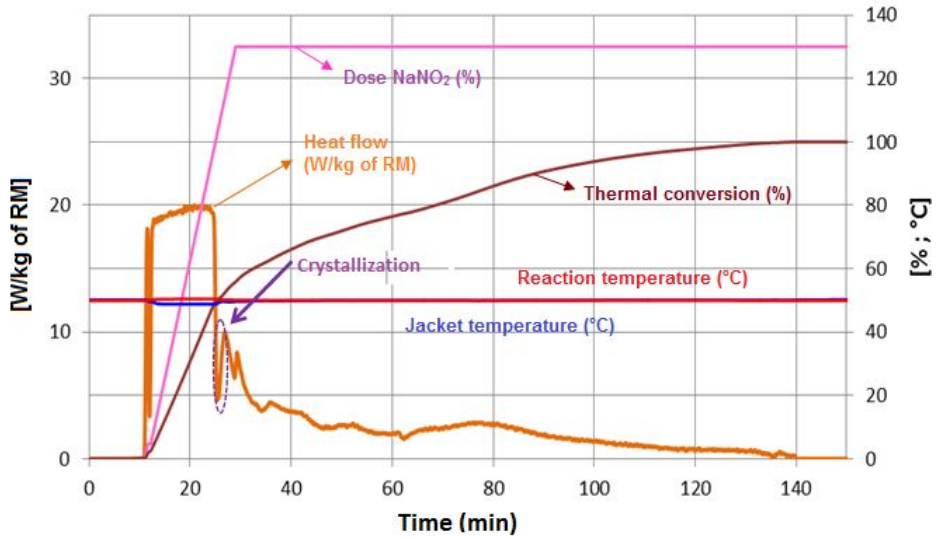



**Figure 10.** DSC thermogram of the reaction mixture for diazotization step

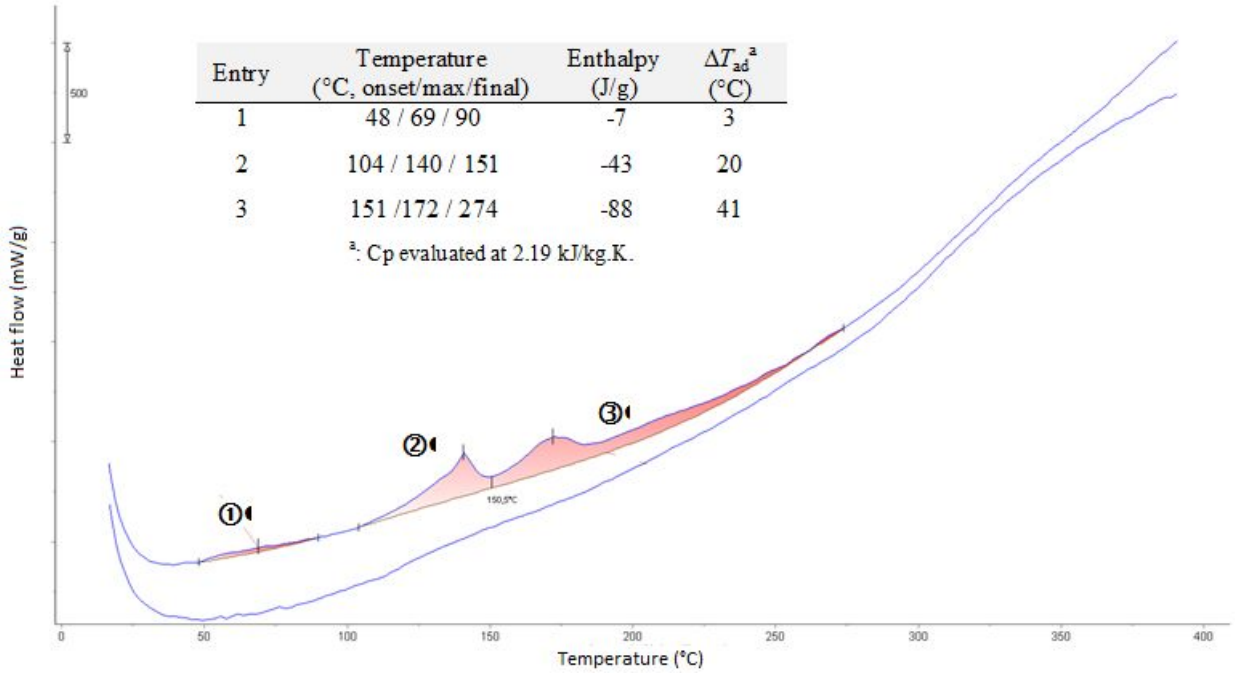
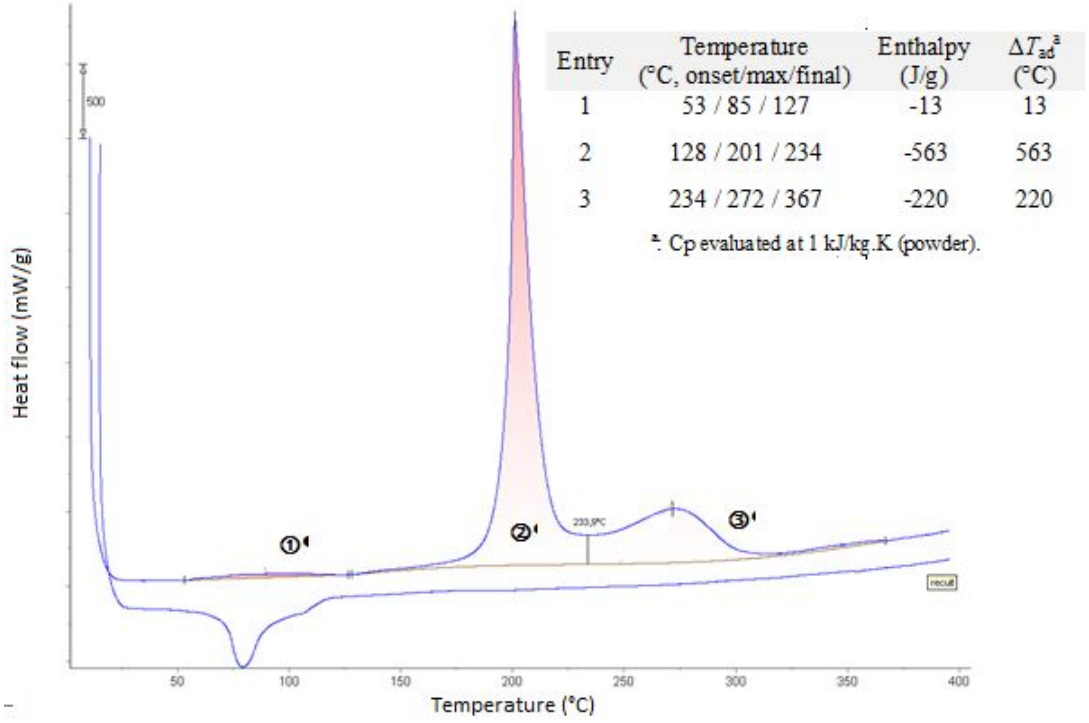



**Figure 11.** DSC profile of intermediate **16**



In the early development synthesis, cyclisation was performed reacting 1,3,5-trioxane in a mixture of concentrated $H_2SO_4$ and dichloromethane with $Na_2SO_4$ used as dehydrating agent. However we encountered few incomplete conversions during the manufacturing campaigns without any root cause clearly identified. In order to circumvent this lack of robustness, we embarked into a survey of alternative conditions and the results are depicted in Table 2. We focused on a mixture of AcOH and concentrated $H_2SO_4$ in $CH_2Cl_2$ that enabled us to generate the desired **1** (entry 2).[32] Even if the amount of trioxane was lowered, the conversion proceeded faster at room temperature than in the original procedure (entry 1). Addition of water resulted in precipitating **1** which was easily isolated by filtration. Removal of $CH_2Cl_2$ allowed us to get a greener synthesis while improving the yield (entry 3). Replacement of AcOH with formic acid proved to be deleterious with the formation of an unidentified impurity (entry 5). Reducing the amount of trioxane to 0.3 equiv led to a slow conversion rate even at higher temperature (entry 6) whereas 0.5 equiv seemed to be enough to get a full conversion (entry 7 and 8). Addition of EtOAc was evaluated and proved to be beneficial in terms of mass transfer (entry 9). In that case the reaction mixture turned out to be a solution and the rate conversion was improved. Further experiments showed that the amount of trioxane could be lowered to 0.5 equiv. The combination of $H_2SO_4$ and AcOH was the key factor to obtain a satisfactory outcome since the reaction stalled in a EtOAc/$H_2SO_4$ mixture (entry 10) and degradation was observed in neat $H_2SO_4$ (entry 4).

**Table 2**. Initial studies to generate **1** in acidic conditions

| Entry | Trioxane (equiv) | Conditions | Conv. (%) | Yield (%)[a] | Purity (%)[b] |
|---|---|---|---|---|---|
| 1[c] | 3 | $H_2SO_4$ (1 equiv), $Na_2SO_4$ (5 equiv), $CH_2Cl_2$, 6 h at reflux | 98 | 70 | 98 |
| 2 | 1 | $H_2SO_4$ (3.5 equiv), AcOH (2.5 mL/g), | 54 | 51 | 78 |

| | | | | | |
|---|---|---|---|---|---|
| | | $CH_2Cl_2$ (2.5 mL/g), 3 h at 20 °C | | | |
| 3 | 1 | $H_2SO_4$ (3.5 equiv), AcOH (5 mL/g), 2 h at 20 °C | 86 | 74 | 97 |
| 4 | 1 | $H_2SO_4$ (35 equiv), 1 h at 20 °C | 0 | - | - |
| 5 | 1 | $H_2SO_4$ (3.5 equiv), $HCO_2H$ (5 mL/g), 1 h at 20 °C | 40 | 32 | 59 |
| 6 | 0.3 | $H_2SO_4$ (3.5 equiv), AcOH (5 mL/g), 2 h at 50 °C then 1 h at 90 °C | 18 | - | - |
| 7 | 0.3+0.2 | $H_2SO_4$ (3.5 equiv), AcOH (5 mL/g), 22 h at 20 °C then 2 h at 50 °C | 14 | 58 | 97 |
| 8 | 0.5 | $H_2SO_4$ (3.5 equiv), AcOH (5 mL/g), 2 h at 50 °C | 46 | 57 | 98 |
| 9 | 1 | $H_2SO_4$ (3.5 equiv), AcOH (2.5 mL/g), EtOAc (2.5 mL/g), 2 h at 20 °C | 87 | 74 | 98 |
| 10 | 0.5 | $H_2SO_4$ (3.5 equiv), EtOAc (5 mL/g), 2 h at 20 °C then 4 h at 40 °C | 1 | - | - |

[a]Isolated yields. [b] Determined by HPLC. [c] Solvent switch from $CH_2Cl_2$ to isopropyl ether and filtration.

The scouting experiments suggested that temperature, amount of $H_2SO_4$, ratio of EtOAc and AcOH were variables potentially influencing both yield and purity. In order to map the reaction parameters in terms of robustness and to identify the best reaction conditions for scale-up, we initiated a Design of Experiment (DoE) optimization.[33] The parameters chosen for this study and their settings are shown in Table 3. The amount of trioxane (0.5 equiv) and the dilution (5 mL/g) were held constant.

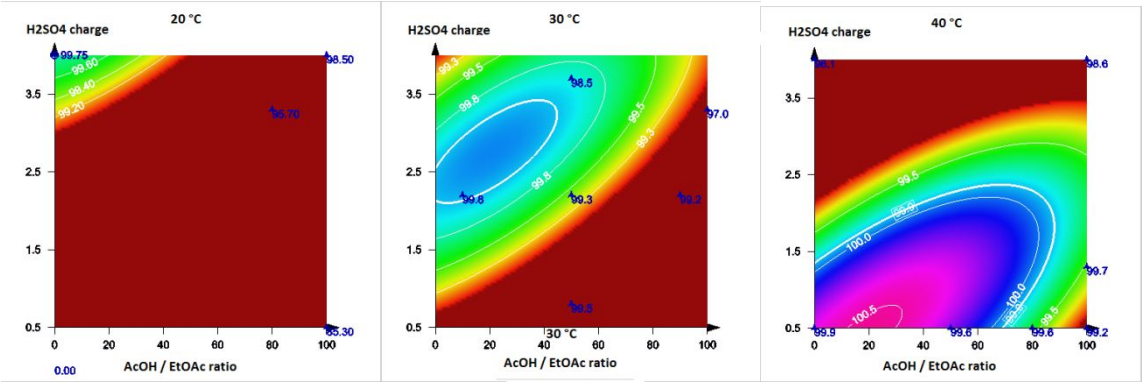
**Table 3**. Overview of DoE parameters and responses

| Parameter studied (unit) | Range | Responses measured |
|---|---|---|
| AcOH/EtOAc ratio (%) | 0 – 100 | Yield (%, from w/w assay) |

| | | |
|---|---|---|
| $H_2SO_4$ charge (equiv) | 0.5 – 4.0 | Conversion (% area) |
| Temperature (°C) | 20 – 40 | Purity (% area) |

A $2^4$ full factorial screening design was selected and 20 reactions were launched on 1 g scale. **1** was isolated by filtration after adding water to the reaction mixture. Analysis of the data revealed that running the reaction at 40 °C gave better purity (see Figure 12). At the same time, the amount of $H_2SO_4$ has to be lowered to avoid the formation of impurities. This was also consistent with the lower yield of isolated product.

**Figure 12.** Contour plots for purity as a function of AcOH/EtOAc ratio and $H_2SO_4$ charge at 20, 30 and 40 °C



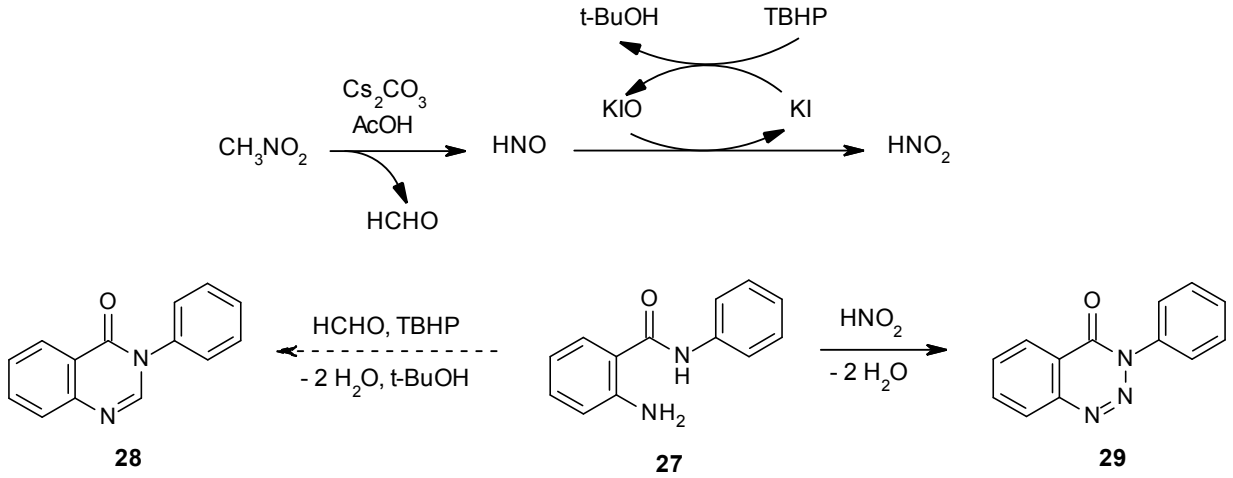

On the basis of these data, we opted to run the reaction at 40 °C and finally adjusted the amount of $H_2SO_4$ to 1.5 equiv and the ratio of AcOH/EtOAc to 1:1 (v/v). Following those conditions, intermediate **16** was converted into desired **1** after 5 hours with a purity of 93.7 area % in the reaction mixture. Water was used as anti-solvent and its amount had a dramatic impact on the yield. A 7.5 mL/g dilution was selected to furnish **1** with 94% yield and with an excellent >99 area % purity by HPLC (identical purity with 5 and 10 mL/g with respectively 73 and 93%

yield). Upon scale-up to 48 kg, we were pleased to see that the reaction proceeded well and provided crude **1** with 92% yield and >98% purity.

**Toward a telescoped process to generate the triazinone moiety and to access 1**

In our quest for reducing batch cycle times, workup and purification unit operations as well, process intensification *via* reaction telescoping was explored to synthesize **1**. Recently, a method to prepare 1,2,3-benzotriazine-4(*3H*)-ones was developed using the Nef reaction.[34] In the presence of $Cs_2CO_3$ and AcOH, nitromethane could generate HCHO and HNO. Then, HNO could be further oxidized to $HNO_2$ by KIO generated in situ from KI and TBHP. Finally, the direct condensation of **27** with $HNO_2$ affords the desired product **29** by removal of two molecules of water (Scheme 7). As a minor pathway, **28** can be obtained via a tandem condensation-addition-oxidation process of **27** with HCHO.

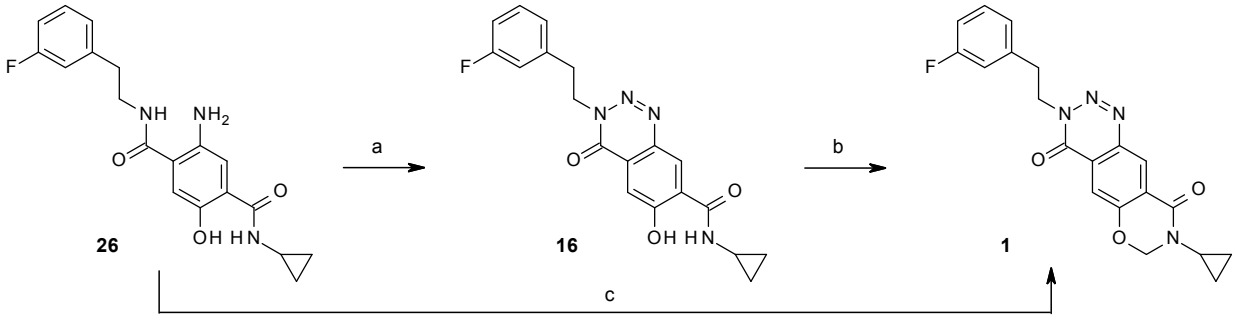
**Scheme 7.** In situ generating of $HNO_2$ and HCHO by Nef reaction.




According to the authors, $HNO_2$ demonstrated more active reactivity than HCHO when subjected to compound **27**. On the basis of this study, we envisioned a one-pot reaction to access to **1**. In

the scouting experiments, we initiated an experiment by treating **26** with $NaNO_2$, paraformaldehyde and AcOH under catalysis of concentrated $H_2SO_4$ to depolymerize paraformaldehyde into formaldehyde. As outlined in Scheme 8, the reaction was initially performed at room temperature and starting material **26** was fully converted to benzotriazinone **16**. Increasing the reaction temperature to 80 °C provided desired product **1**. We therefore envisioned that this one-pot reaction could be completed if the initial reaction temperature was set at 80 °C. This assumption was confirmed on 2 g scale and desired product **1** was isolated with 99% yield and 95% purity starting from **26**. These new conditions could therefore be an opportunity to improve the cost of the synthesis.

**Scheme 8.** One-pot synthesis of **1**




[a]Reagents and conditions: (a) $NaNO_2$ (1.2 equiv), paraformaldehyde excess, AcOH, $H_2SO_4$, 20 °C; (b) 80 °C; (c) $NaNO_2$ (1.2 equiv), paraformaldehyde excess, AcOH, $H_2SO_4$, 80 °C, 99%.

## Control of impurities

In order to insure the safety of the patient, control of impurities generated in the steps discussed before was carefully monitored. As shown on Scheme 9, compounds **30**, **31**, and **32** were identified as the most prevalent impurities generated during the regioselective double amidation step. Compound **30** arises from **22** undergoing a second peptide coupling with 2-(3-fluorophenyl)ethanamine mediated with CDI. Compounds **31** and **32** arise respectively from

condensation of CDI on **30** and **22**. We assumed that condensation of acetic acid with **26** was responsible of the formation of compound **39**. Detailed studies revealed that the abovementioned impurities reacted in the downstream process and were purged except compound **35** with a level below 0.1%.

To ensure that none of the impurities introduced in the process could impact the purity profile of the drug substance, **1** was initially recrystallized in a mixture of EtOAc and DMSO (8:2, 11 L/kg) with a moderate 77% yield. In order to improve this result, further studies were initiated and the solubility profile of **1** was evaluated in various solvent systems. A mixture of EtOH and anisole (3:1, 4.5v) heated at 80 °C was identified in which the polish filtered solution of **1** was stirred, cooled at 10 °C and filtered. Gratifyingly, this new process produced **1** in 86% yield with a satisfactory chemical purity of 99.8%. Levels of all the impurities were below 0.10% thanks to the efficient control offered by the recrystallization. Comparison of the powder X-ray diffraction (PXRD) traces of **1** revealed that the form was identical as the one obtained in the EtOAc/DMSO mixture (see Figure 13).

**Scheme 9.** Fate of process related impurities **30**, **31** and **32** found in intermediate **22** after hydrogenation, sodium nitrite treatment and annulation mediated with paraformaldehyde

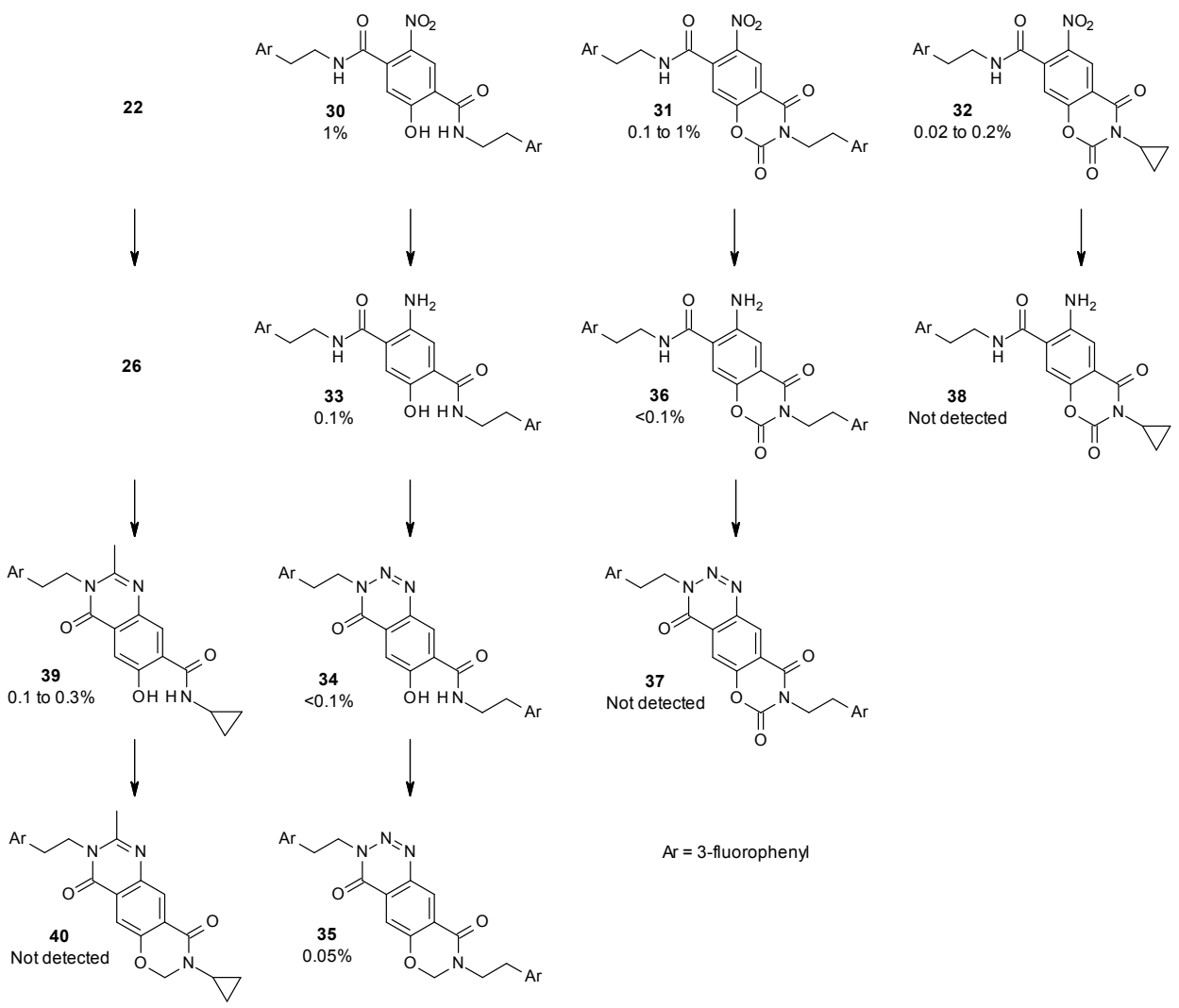
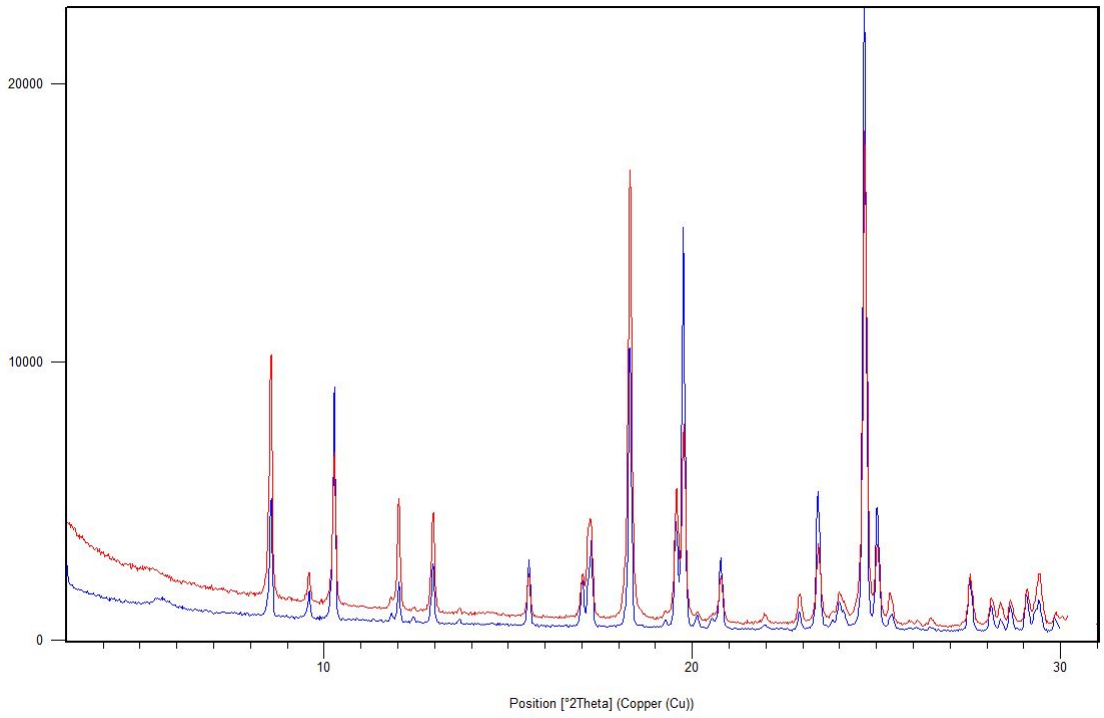



**Figure 13.** Representative PXRD traces of **1** in DMSO/EtOAc (blue trace) and in anisole/EtOH (red trace).




## Conclusion

To manufacture **1**, we developed an efficient synthetic process on large scale with 7 isolated steps including a telescoped protecting-group-free regioselective double amidation (Scheme 10). Morever a promising access to **1** via a one-pot diazotization-annulation sequence was also performed on small scale. Although there is still some room for productivity improvement, this new approach compares favorably in terms of key performance indicators, process safety, environment and patient safety (see Tables 4-7). Limiting the use of CMR solvents and CMR reagents along with isolations by crystallization provided each intermediate with 98-99% chemical purity. The scalable procedures enabled us to successfully support needs of **1** for clinical studies.

**Scheme 10.** Optimized manufacturing route for **1**

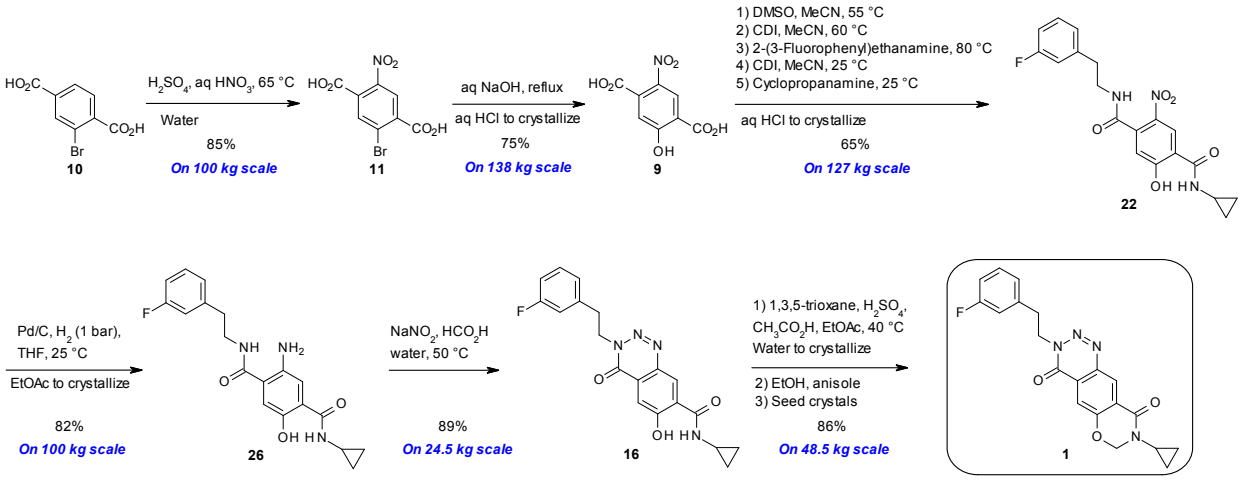



**Table 4.** Key performance indicators for routes of **1** on large scale

| Criteria | Discovery route | Optimized route | Indicator |
|---|---|---|---|
| Overall yield | 4.8% | 12.8% | + |
| Number of chemical steps | 8 | 7 | + |
| Reprocess | 1 | 0 | + |
| Flash chromatography | 2 | 0 | + |
| Evaporation to dryness | 8 | 0 | + |
| Crystallization | 3 | 7 | + |
| Lack of robustness | Nitration | None | + |

**Table 5.** Comparison of process safety for routes of **1**

| Criteria | Discovery route | Optimized route | Indicator |
|---|---|---|---|
| Nitro reduction | Zn/Cu + $HCO_2H$ | $H_2$ + Pd/C | + |
| Hazardous reaction | Nitration | Nitration | - |

**Table 6.** Comparison of environment and safety for routes of **1**

| Criteria | Discovery route | Optimized route | Indicator |
|---|---|---|---|
| CMR solvents | THF, $CH_2Cl_2$, $CHCl_3$, DMAc, DMF, MeOH, hexane | THF | + |
| CMR reagents | CDI, trioxane, $NaIO_4$, isoamyl nitrite | CDI, trioxane | + |
| Environment | Zn/Cu (waste) | Pd (recycled) | + |
| PMI[a] | 3025 | 346 | + |

[a]: Product Mass Intensity [total raw material input (kg) / quantity of bulk API (kg)]. See Ref. 35.

**Table 7.** Impact on patient safety

| Criteria | Discovery route | Optimized route | Indicator |
|---|---|---|---|
| Genotoxic intermediates (Derek / Leadscope) | **5**, **6** and **7** | **9** and **22**[a] | + |
| Positive Ames II testing | For all | None | + |
| Purification step | 3 precipitations and 2 chromatographies | 7 crystallizations | + |
| API characterization | No crystallization | Crystallization | + |

[a]: the nitro group induced the positive result (see Ref. 36).

## EXPERIMENTAL SECTION

**General.** All reagents and solvents were purchased from commercial suppliers and used without further purification. Nuclear magnetic resonance (NMR) spectra were obtained using a Bruker 400 MHz spectrometer in the indicated solvents. Chemical shifts are expressed in parts per

million (ppm) and coupling constants are reported in Hertz (Hz). Splitting patterns are indicated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad.

**2-Bromo-5-nitro-terephthalic acid (11).** Sulfuric acid (920 kg) and 2-bromoterephthalic acid **10** (100 kg, 408 mol 1 equiv) were charged to a 6 $m^3$ reactor. A 65% aqueous solution of nitric acid (47.5 kg, 490 mol, 1.2 equiv) was added and the reaction mixture was heated to 65 °C for 3 h. It was cooled down to 30 °C, diluted with water (3000 L) and the suspension was filtered. The cake was slurred with water (300 L). Crude **11** (dry basis 101 kg, 85% yield) was obtained and used in the next step without drying. $^1$H NMR (DMSO-$d_6$, 400 MHz): δ (ppm) 8.34 (s, 1 H), 8.17 (s, 1 H). $^{13}$C NMR (DMSO-$d_6$, 100 MHz): δ (ppm) 165.2 (s, 1 C), 164.3 (s, 1 C), 146.4 (s, 1 C), 136.8 (s, 1 C), 134.8 (s, 1 C), 130.4 (s, 1 C), 125.7 (s, 1 C), 125.0 (s, 1 C).

Column: Ascentis Express C18 2.7 μm, 150 mm x 4.6 mm; flow rate 1 mL/min; temperature 40 °C; UV detection 210 nm; solvent system: water + 0.11% v/v AMS (A) and acetonitrile (B). Gradient: t=0 95A/5B; t=30 5A/95B; t=31 95A/5B; t=45 95A/5B. Solution of 15 mg of **11** in 100 mL of (A). Amount injected: 5 μL. Retention time: 7.14 min.

**2-Hydroxy-5-nitro-terephthalic acid (9).** 2-Bromo-5-nitro-terephthalic acid **11** (113 kg, 348 mol, 1 equiv) was added to a solution of sodium hydroxide (138 kg, 3623 mol, 10.4 equiv) in water (565 L). The reaction was stirred for one day at reflux. The mixture was diluted with water (450 L) and adjusted to pH 2 with a hydrochloric acid solution. The suspension was filtered, slurred with water (220 L) and dried under vacuum between 70 – 80 °C to afford **9** (59 kg, 75%) as a white solid. $^1$H NMR (DMSO-$d_6$, 400 MHz): δ (ppm) 11.81 (br s, 3 H), 8.40 (s, 1 H), 7.18 (s, 1 H). $^{13}$C NMR (DMSO-$d_6$, 100 MHz): δ (ppm) 169.1 (s, 1 C), 166.2 (s, 1 C), 164.9 (s, 1 C),

137.1 (s, 1 C), 135.9 (s, 1 C), 127.6 (s, 1 C), 117.5 (s, 1 C), 115.4 (s, 1 C). HRMS ESI (*m*/*z*) calcd for $C_8H_4NO_7$ $[M - H]^-$ 225.9993 found 225.9986.

Column: Ascentis Express C18 2.7 µm, 100 mm x 3 mm; flow rate 0.9 mL/min; temperature 40 °C; UV detection 210 nm; solvent system: water + 0.11% v/v AMS (A) and acetonitrile + 0.1% v/v AMS (B). Gradient: t=0 92A/8B; t=10 76A/24B; t=12 20A/80B; t=12.5 92A/8B; t=18 92A/8B. Solution of 70 mg of **9** in 100 mL of a 95:5 v/v mixture of water/acetonitrile. Amount injected: 0.5 µL. Retention time: 1.76 min.

**N-1-cyclopropyl-N-4-[2-(3-fluorophenyl)ethyl]-2-hydroxy-5-nitro-terephthalamide (22)**. To a solution at 60 °C of 2-hydroxy-5-nitro-terephthalic acid **9** (127 kg, 560 mol, 1 equiv) in a mixture of acetonitrile (380 L) and DMSO (250 L) was added a solution at 55 °C of 1,1'-carbonyldiimidazole (100 kg, 617 mol, 1.1 equiv) in acetonitrile (570 L) for 30 minutes. The mixture was stirred at 60 °C for 4 hours and the conversion was monitored by LC analysis. Then, the reaction mixture was heated to 80 °C and a solution of 2-(3-fluorophenyl)ethanamine (85.6 kg, 615 mol, 1.1 equiv) in acetonitrile (40 L) was added. After stirring for 18 hours, the mixture was concentrated under reduced pressure to 1150 L and cooled to 25 °C. This solution was added to another reactor containing a suspension of 1,1'-carbonyldiimidazole (127 kg, 783 mol, 1.4 equiv) in acetonitrile (570 L) at 25 °C over 2 hours. The reaction was stirred during 9 hours and conversion was monitored by LC analysis. The mixture was degassed for 1 hour under reduced pressure (100 mbar) at 25 °C and a solution of cyclopropanamine (35 kg, 614 mol, 1.1 equiv) in acetonitrile (130 L) was introduced over 1 hour to control the heat release. After stirring for 1 hour, the resulting solution was concentrated to 900 L under reduced pressure. A 5 N hydrochloric acid solution (890 L) was then added for 2.5 hours at 20 °C to precipitate the

product. The suspension was cooled to 5 °C and then filtered. The cake was successively washed with a 1 N hydrochloric acid solution (510 L), water (1000 L) and acetonitrile (1000 L). The wet cake was dried at 40 °C under reduced pressure to afford **22** as an off white solid (141 kg, 65%). $^{1}$H NMR (DMSO-$d_6$, 400 MHz): δ (ppm) 13.79 (br s, 1 H), 9.13 (d, $J$ = 3.9 Hz, 1 H), 8.78−8.54 (m, 2 H), 7.40−7.30 (m, 1 H), 7.17−6.98 (m, 3 H), 6.87 (s, 1 H), 3.47 (q, $J$ = 6.8 Hz, 2 H), 2.98−2.80 (m, 3 H), 0.87−0.60 (m, 4 H). $^{13}$C NMR (DMSO-$d_6$, 100 MHz): δ (ppm) 168.2 (s, 1 C), 164.9 (s, 1 C), 164.0 (s, 1 C), 162.2 (d, $^{1}J$ = 242.5 Hz, 1 C), 142.2 (d, $^{3}J$ = 7.4 Hz, 1 C), 138.2 (s, 1 C), 137.3 (s, 1 C), 130.1 (d, $^{3}J$ = 8.8 Hz, 1 C), 125.5 (s, 1 C), 124.9 (d, $^{4}J$ = 2.2 Hz, 1 C), 117.6 (s, 1 C), 115.5 (s, 1 C), 115.4 (d, $^{2}J$ = 20.6 Hz, 1 C), 112.9 (d, $^{2}J$ = 21.4 Hz, 1 C), 40.1 (s, 1 C), 34.2 (s, 1 C), 23.0 (s, 1 C), 5.6 (s, 2 C). HRMS ESI ($m/z$) calcd for $C_{19}H_{19}FN_3O_5$ $[M + H]^+$ 388.1309 found 388.1310.

Column: Ascentis Express C18 2.7 µm, 150 mm x 4.6 mm; flow rate 1.0 mL/min; temperature 60 °C; UV detection 210 nm; solvent system: water + 0.11% v/v AMS (A) and acetonitrile (B). Gradient: t=0 70A/30B; t=25 2A/98B; t=25.01 70A/30B; t=35 70A/30. Concentration of **22** in MeOH: 0.080 mg/mL. Amount injected: 10 µL. Retention time: 8.01 min.

**2-Amino-N-4-cyclopropyl-N-1-[2-(3-fluorophenyl)ethyl]-5-hydroxy-terephthalamide 26.** In an hydrogenation reactor was introduced a solution of N-1-cyclopropyl-N-4-[2-(3-fluorophenyl)ethyl]-2-hydroxy-5-nitro-terephthalamide **22** (100 kg, 258 mol, 1 equiv) in THF (600 L). Palladium (10 kg, 10 wt % on activated carbon, 50% wet) was added to the solution at 25 °C and the reaction mixture was stirred for 2 hours under 1 bar of hydrogen. Completion of the reaction was monitored by LC analysis. The catalyst was eliminated by filtration and washed with THF (800 L). The solution was concentrated to 1 m$^3$ and the product was crystallized by

solvent switch from THF to ethyl acetate under atmospheric pressure. The solution was cooled to 20 °C and the product was filtered, washed with 500 L of ethyl acetate and dried at 45 °C under reduced pressure. Desired **26** (75 kg) was isolated with 82% yield as a yellow solid in high purity (≥99 area %). $^{1}$H NMR (DMSO-$d_6$, 400 MHz): δ (ppm) 11.24 (s, 1 H), 8.72 (d, $^{3}J$ = 4.4 Hz, 1 H), 8.46 (t, $^{3}J$ = 5.4 Hz, 1 H), 7.38−7.26 (m, 1 H), 7.11−7.05 (m, 3 H), 7.05−6.98 (m, 1 H), 6.95 (s, 1 H), 5.57 (s, 2 H), 3.50−3.39 (m, 2 H), 2. 94−2.77 (m, 3 H), 0.79−0.50 (m, 4 H). $^{13}$C NMR (DMSO- $d_6$, 100 MHz): δ (ppm) 169.6 (s, 1 C), 167.9 (s, 1 C), 162.2 (d, $^{1}J$ = 242.5 Hz, 1 C), 148.8 (s, 1 C), 142.5 (d, $^{3}J$ = 7.4 Hz, 1 C), 140.8 (s, 1 C), 130.1 (d, $^{3}J$ = 8.8 Hz, 1 C), 124.9 (d, $^{4}J$ = 3,0 Hz, 1 C), 121.1 (s, 1 C), 118.9 (s, 1 C), 115.8 (s, 1 C), 115.4 (d, $^{2}J$ = 20.6 Hz, 1 C), 115.1 (s, 1 C), 112.9 (d, $^{2}J$ = 20.6 Hz, 1 C), 40.1 (s, 1 C), 34.5 (s, 1 C), 22.8 (s, 1 C), 5.8 (s, 2 C). HRMS ESI (*m*/*z*) calcd for $C_{19}H_{21}FN_3O_3$ $[M + H]^+$ 358.1567 found 358.1564.

Column: Ascentis Express C18 2.7 µm, 150 mm x 4.6 mm; flow rate 1.0 mL/min; temperature 60 °C; UV detection 210 nm; solvent system: water + 0.11% v/v AMS (A) and acetonitrile/MeOH (50v/50v) (B). Gradient: t=0 70A/30B; t=25 2A/98B; t=25.01 70A/30B; t=35 70A/30. Concentration of **26** in MeOH: 0.40 mg/mL. Amount injected: 1 µL. Retention time: 4.99 min.

**N-cyclopropyl-3-[2-(3-fluorophenyl)ethyl]-6-hydroxy-4-oxo-1,2,3-benzotriazine-7-carboxamide (16)**. To a solution at 50 °C of 2-amino-N-4-cyclopropyl-N-1-[2-(3-fluorophenyl)ethyl]-5-hydroxy-terephthalamide **26** (24.55 kg, 68.7 mol, 1 equiv) in a mixture of acetic acid (368 L) and water (49 L) was added over 90 minutes a solution of sodium nitrite (6.16 kg, 89.3 mol, 1.3 equiv) in water (37 L). The reaction was stirred at 50 °C for 3 hours. The resulting suspension was cooled to 25 °C, water (196 L) was added and reaction mixture was stirred for 6 hours at 20 °C before filtration. The cake was washed with water (370 L) and dried

at 50 °C in ventilated oven to afford **16** as a brown solid (22.5 kg, 89%) with >98% HPLC purity. $^1$H NMR (Pyr-$d_5$, 400 MHz): δ (ppm) 10.76 (br s, 1 H), 10.00−9.70 (m, 1 H), 9.11 (s, 1 H), 7.99 (s, 1 H), 7.26−7.16 (m, 1 H), 7.12 (dt, $^3J$ = 10.0 Hz, $^4J$ = 2.0 Hz, 1 H), 7.07−6.93 (m, 2 H), 4.77−4.66 (m, 2 H), 3.30−3.21 (m, 2 H), 3.20−3.09 (m, 1 H), 0.88−0.70 (m, 4 H). $^{13}$C NMR (Pyr-$d_5$, 100 MHz): δ (ppm) 169.5 (s, 1 C), 163.6 (d, $^1J$ = 242.5 Hz, 1 C), 163.6 (s, 1 C), 155.3 (s, 1 C), 141.7 (d, $^3J$ = 7.3 Hz, 1 C), 138.3 (s, 1 C), 131.3 (s, 1 C), 131.0 (d, $^3J$ = 8.1 Hz, 1 C), 125.5 (d, $^4J$ = 2.9 Hz, 1 C), 125.1 (s, 1 C), 124.5 (s, 1 C), 116.5 (d, $^2J$ = 20.5 Hz, 1 C), 114.1 (d, $^2J$ = 20.5 Hz, 1 C), 111.4 (s, 1 C), 50.9 (s, 1 C), 35.0 (s, 1 C), 24.1 (s, 1 C), 7.1 (s, 2 C). HRMS ESI (*m*/*z*) calcd for $C_{19}H_{18}FN_4O_3$ $[M + H]^+$ 369.1363 found 369.1320.

Column: Acquity UPLC BEH shield RP18 1.7 µm, 100 mm x 2.1 mm; flow rate 0.8 mL/min; temperature 50 °C; UV detection 236 nm; solvent system: water adjusted at pH 2.5 with TFA (A) and acetonitrile (B). Gradient: t=0 75A/25B; t=12 45A/55B; t=12.01 75A/25B; t=14 75A/25B. Concentration of **16** in A/B 50v/50v: 0.20 mg/mL. Amount injected: 2 µL. Retention time: 5.60 min.

Telescoped process to access **1** from **26**

**6-[[Cyclopropyl(methyl)amino]methoxy]-3-[2-(3-fluorophenyl)ethyl]-4-oxo-1,2,3-benzotriazine-7-carbaldehyde (1)**. A 100 mL three-necked round bottom flask was charged with **26** (1.79 g, 5 mmol, 1 equiv) and AcOH (20 mL). $NaNO_2$ (424 mg, 6.14 mmol, 1.2 equiv) and paraformaldehyde (4.50 g) were added sequentially and the mixture was stirred for 5 min at 20 °C. Concentrated $H_2SO_4$ (1 mL) was added dropwise and the mixture was stirred for 3 h at 80 °C. After completion of the reaction, the mixture was cooled to 20 °C and poured into ice water. Large amount of precipitate was formed and the mixture was filtered. The filter cake was washed

with water to give the crude product, which was then recrystallized from acetonitrile/$H_2O$ (3:1, 30 mL) to give desired product **1** as white solid with 99% yield and 95% purity.

**6-[[Cyclopropyl(methyl)amino]methoxy]-3-[2-(3-fluorophenyl)ethyl]-4-oxo-1,2,3-benzotriazine-7-carbaldehyde (1)**. A concentrated sulfuric acid solution (20.4 kg) was added over 1 hour to a solution of N-cyclopropyl-3-[2-(3-fluorophenyl)ethyl]-6-hydroxy-4-oxo-1,2,3-benzotriazine-7-carboxamide **16** (48.5 kg, 132 mol, 1 equiv) and 1.3.5-trioxane (5.97 kg, 66.3 mol, 0.5 equiv) in a mixture of ethyl acetate (120 L) and glacial acetic acid (120 L) at 40 °C. The reaction mixture was stirred for 5 hours at this temperature and was cooled to 20 °C before adding water (364 L) in 30 minutes. The suspension was filtered, washed with water (290 L) and isopropyl ether (145 L). Crude **1** was dried at 50 °C under reduced pressure to give a brown powder (48 kg, 92%) with >98% HPLC purity. To comply with cGMP requirements, **1** was then dissolved in mixture of ethanol (52 L) and anisole (166 L) heated at 90 °C. After filtration on activated charcoal followed by polish filtration on a 1 μm filter, the solution was cooled to 70 °C and seeded with **1** (1.2 kg). The suspension was cooled to 10 °C and then filtered. The wet cake was washed four times with ethanol (40 L) and dried at 50 °C under reduced pressure to afford **1** as a white solid (41 kg, 86%). $^1$H NMR (DMSO-$d_6$, 400 MHz): δ (ppm) 8.49 (s, 1 H), 7.71 (s, 1 H), 7.34−7.23 (m, 1 H), 7.11 (d, $^3J$ = 10.0 Hz, 1 H), 7.07−6.98 (m, 2 H), 5.45 (s, 2 H), 4.61 (t, $^3J$ = 7.3 Hz, 2 H), 3.17 (t, $^3J$ = 7.3 Hz, 2 H), 2.77 (m, 1 H), 0.94−0.82 (m, 2 H), 0.82−0.68 (m, 2 H). $^{13}$C NMR (DMSO-$d_6$, 100 MHz): δ (ppm) 163.4 (s, 1 C), 161.0 (d, $^1J$ = 240.3 Hz, 1 C), 159.1 (s, 1 C), 153.8 (s, 1 C), 140.9 (d, $^3J$ = 7.4 Hz 1 C), 139.1 (s, 1 C), 130.3 (d, $^3J$ = 8.1 Hz, 1 C), 128.6 (s, 1 C), 125.3 (s, 1 C), 124.9 (d, $^4J$ = 2.2 Hz, 1 C), 123.6 (s, 1 C), 115.5 (d, $^2J$ = 20.6 Hz, 1 C),

113.4 (d, $^{2}J$ = 21.4 Hz, 1 C), 110.8 (s, 1 C), 79.0 (s, 1 C), 50.0 (s, 1 C), 33.5 (s, 1 C), 26.9 (s, 1 C), 6.8 (s, 2 C). $^{19}$F NMR (DMSO-$d_6$, 376 MHz): δ (ppm) −113.01.

HRMS ESI (*m*/*z*) calcd for $C_{20}H_{18}FN_4O_3$ $[M + H]^+$ 381.1363 found 381.1321.

Column: Luna C18 (2) 3 µm, 150 mm x 4.6 mm; flow rate 1 mL/min; temperature 40 °C; UV detection 210 nm; solvent system: water + 0.01 M $KH_2PO_4$ (A) and acetonitrile (B). Gradient: t=0 80A/20B; t=30 20A/80B; t=45 20A/80B; t=46 80A/20B ; t=55 80A/20B. Concentration of **1** in B: 0.40 mg/mL. Amount injected: 5 µL. Retention time: 21.20 min.

## ASSOCIATED CONTENT

Supporting information

The Supporting Information is available free of charge on the ACS Publications website.

Copies of $^{1}$H and $^{13}$C spectra of compounds **9**, **22**, **26**, **16** and **1** (including $^{19}$F NMR for **1**).

DSC analyses of isoamyl nitrite, *tert*-butylnitrite and sodium nitrite.

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**Notes**

The authors declare no competing financial interest.

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## ABBREVIATIONS

AMPA, (*R,S*)-2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid; T3P, propanephosphonic acid anhydride; EDCI, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HBTU, O-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; TBTU, 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium tetrafluoroborate; CDI, carbonyl diimidazole; DMF, dimethylformamide; $Et_3N$, triethylamine; NMP, N-methyl-2-pyrrolidinone; DMPU, N,N′-dimethylpropylene urea; TBHP, *tert*-butyl hydroperoxide;

## REFERENCES

(1) Bräuner-Osborne, H.; Egebjerg, J.; Nielsen, E. Ø.; Madsen, U.; Krogsgaard-Larsen, P. Ligands for glutamate receptors: Design and therapeutic prospects. *J. Med. Chem*. **2000**, *43*, 2609−2645.

(2) Dingledine, R.; Borges, K.; Bowie, D.; Traynelis, S. F. The glutamate receptor ion channels. *Pharmacol. Rev*. **1999**, *51*, 2606−2645.

(3) Traynelis, S. F.; Wollmuth, L. P.; McBrain, C. J.; Menniti, F. S.; Vance, K. M.; Ogden, K. K.; Hansen, K. B.; Yuan, H.; Myers, S. J.; Dingledine, R. Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol. Rev*. **2010**, *62*, 405−496.

(4) Monaghan, D. T.; Yao, D.; Cotman, C. W. Distribution of [3H]AMPA binding sites in rat brain as determined by quantitative autoradiography. *Brain Research* **1984**, *324*, 160−164.

(5) Whitlock, J. R.; Heynen, A. J.; Shuler, M. G.; Bear, M. F. Learning induces long-term potentiation in the hippocampus. *Science* **2006**, *313*, 1093−1097.

(6) Pastalkova, E.; Serrano P.; Pinkhasova D.; Wallace E.; Fenton A. A.; Sacktor, T. C. Storage of spatial information by the maintenance mechanism of LTP. *Science* **2006**, *313*, 1141−1144.

(7) Marenco, S.; Weinberger, D. R. Therapeutic potential of positive AMPA receptor modulators in the treatment of neuropsychiatric disorders. *CNS Drugs* **2006**, *20*, 173−185.

(8) Zarate, J.; Manji, H. K. The role of AMPA receptor modulation in the treatment of neuropsychiatric diseases. *Exp. Neurol*. **2008**, *211*, 7−10.

(9) Yamada, K. A. modulating excitatory synaptic neurotransmission: potential treatment for neurological disease ? *Neurobiol. Disease* **1998**, *5*, 67−80.

(10) Armstrong, N.; Sun, Y.; Chen, G. Q.; Gouaux, E. Structure of a glutamate-receptor ligand-binding core in complex with kainate. *Nature* **1998**, *395*, 913−917.

(11) Sobolevsky, A. I.; Rosconi, M. P.; Gouaux, E. X-ray structure, symmetry and mechanism of an AMPA-subtype glutamate receptor. *Nature* **2009**, *462*, 745−756.

(12) Grove, S. J. A.; Jamieson, C.; Maclean, J. K. F.; Rankovic, Z. Positive Allosteric Modulators of the AMPA receptor. *J. Med. Chem.* **2010**, *52*, 7044−7053

(13) Arai, A. C.; Xia, Y. F.; Rogers, G.; Lynch, G.; Kessler, M. Benzamide-type AMPA receptor modulators from two subfamilies with distinct modes of action. *J. Pharm. Exp. Ther.* **2002**, *303*, 1075−1085.

(14) Arai, A. C.; Kessler, M. Pharmacology of Ampakines modulators: from AMPA receptors to synapses and behavior. *Curr. Drug Targets* **2007**, *8*, 583−602.

(15) Arai, A. C.; Kessler, M.; Rogers, G.; Lynch, G. Effects of the potent ampakine CX614 on hippocampal and recombinant AMPA receptors. *Mol. Pharmacol*. **2000**, *58*, 802−813.

(16) Hampson, R. E.; España, R. A.; Rogers, G. A.; Porrino, L. J.; Deadwyler, S. A. Mechanisms underlying cognitive enhancement and reversal of cognitive deficits in nonhuman primates by the ampakine CX717. *Psychopharmacology* **2009**, *202*, 355−370.

(17) Mueller, R.; Lee, S.; O'Hare, S.; Rogers, G.; Rachwal, S.; Street, L. 3-Substituted-[1,2,3]benzotriazinone compounds for enhancing glutamatergic synaptic responses. Int. Pat. Appl. WO2008/085505.

(18) Cordi, A. 3-Substituted-[1,2,3]benzotriazinone compound for enhancing glutamatergic synaptic responses. US. Pat. Appl. US2010/0137295.

(19) Roberts, B.M.; Holden, D. E.; Shaffer, C.L.; Seymour, P. A.; Menniti, F. S.; Schmidt, C. J.; Williams, G. V.; Castner, S. A. Prevention of ketamine-induced working memory impairments by AMPA potentiators in a nonhuman primate model of cognitive dysfunction. *Behav. Brain Res.* **2010**, *212*, 41−48.

(20) Mendez-David, I.; Guilloux, J. P.; Papp, M.; Tritschler, L.; Mocaer, E.; Gardier, A. M.; Bretin, S.; David, D. J. S 47445 produces antidepressant- and anxiolytic-like effects through neurogenesis dependent and independent mechanisms. *Front. Pharmacol.* **2017**, *8*, 462. doi: 10.3389/fphar.2017.00462.

(21) Giralt, A.; Gómez-Climent, M. Á.; Alcalá, R.; Bretin, S.; Bertrand, D.; María Delgado-García, J.; Pérez-Navarro, E.; Alberch, J.; Gruart, A. The AMPA receptor positive allosteric modulator S 47445 rescues in vivo CA3-CA1 long-term potentiation and structural synaptic changes in old mice. *Neuropharmacology* **2017**, *123*, 395−409.

(22) Calabrese, F.; Savino, E.; Mocaer, E.; Bretin, S.; Racagni, G.; Riva, M. A. Upregulation of neurotrophins by S 47445, a novel positive allosteric modulator of AMPA receptors in aged rats. *Pharmacol. Res*. **2017**, *121*, 59−69.

(23) For preparation, see ref. 18.

(24) Young, I. S.; Baran, P. S. Protecting-group-free synthesis as an opportunity for invention. *Nature Chemistry* **2009**, *1*, 193−205.

(25) Alternative solvents were evaluated such as MTBE, toluene, THF or ethyl acetate. MTBE gave the best results but we observed that this solvent reacted with $HNO_3$ to give isobutene. See Bretherick's Handbook of reactive Chemical Hazards.

(26) The process was evaluated in RC1.

(27) Dunetz, J. R.; Magano, J.; Weisenburger, G. A. Large-scale applications of amide coupling reagents for the synthesis of pharmaceuticals. *Org. Process Res. Dev.* **2016**, *20*, 140−177.

(28) Albericio, F.; El-Faham, A. Choosing the right coupling reagent for peptides: a twenty-five-year journey. *Org. Process Res. Dev.* **2018**, *22*, 1262−1275.

(29) THF, MeCN, MIBK were also tested but gave lower conversions.

(30) Filtration of intermediate **19** was extremely slow.

(31) Reacting cyclopropanamine first led to the formation of 4-(cyclopropylcarbamoyl)-2-hydroxy-5-nitro-benzoic acid.

(32) Ohnmacht, C. J.; Albert, J. S.; Bernstein, P. R.; Rumsey, W. L.; Masek, B. B.; Dembofsky, B. T.; Koether, G. M.; Donald, W.; Andisika, D. W.; Aharony, D. Naphtho[2,1-b][1,5] and [1,2-f][1,4]oxazocines as selective NK1 antagonists. *Bioorg. Med. Chem*. **2004**, *12*, 2653−2669.

(33) Carlson, R.; Carlson, J. E. Design and Optimization in Organic Synthesis, 2nd edition; Elsevier: Amsterdam, 2005.

(34) Yan, Y.; Niu, B.; Xu, K.; Yu, J.; Zhi, H.; Liu, Y. Potassium iodide / *tert*-butyl hydroperoxide-mediated oxidative annulation for the selective synthesis of N-substituted 1,2,3-Benzotriazine-4(3*H*)-ones using nitromethane as the nitrogen synthon. *Adv. Synth. Cat.* **2016**, *358*, 212−217.

(35) Jimenez-Gonzalez, C.; Ponder, C. S.; Broxterman, Q. B.; Julie B. Manley, J. B. Using the right green yardstick: why process mass intensity is used in the pharmaceutical industry to drive more sustainable processes. *Org. Process Res. Dev*. **2011**, *15*, 912–917.

(36) Elder, D. P.; Teasdale, A. Is avoidance of genotoxic intermediates/impurities tenable for complex, multistep syntheses? *Org. Process Res. Dev*. **2015**, *19*, 1437–1446.