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Towards a Scalable Synthesis and Process for EMA401. Part I: Late Stage Process Development, Route Scouting and ICH M7 Assessment

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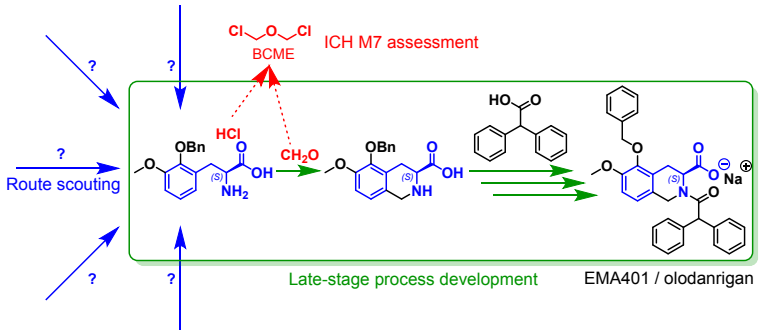
TOC


ICH M7 assessment
BCME
Route scouting
Late-stage process development
EMA401 / olodanrigan

ABSTRACT We present the enantioselective synthesis of sodium (3*S*)-5-(benzyloxy)-2-(diphenylacetyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (EMA401, olodanrigan), an angiotensin II type 2 antagonist. The manuscript features the process optimizations of the end game used for late phase clinical supplies; an overview of synthetic strategies identified in a route scouting exercise to a key intermediate phenylalanine derivative; and the analytical control strategy of the potentially formed highly toxic impurity bis(chloromethyl)ether (BCME). Starting from the phenylalanine derivative, we describe the optimizations of the end game from early phase to late phase processes, with consequent improvements in the PMI factor. This sequence includes a Pictet-Spengler cyclization and an amide coupling as the last bond-forming steps, and the manufacturing process was successfully implemented on a 175 kg scale in a pilot plant setup. The modified process conditions eliminated one step by *in situ* activation of the carboxylic acid, avoided the REACH listed solvent DMF, and resulted in a PMI improvement by a factor of 3. In the final crystallization, a new, thermodynamically more stable, modification of the drug substance was found in the complex solid-state landscape of EMA401 during an extensive polymorph screening. A

process suitable for large-scale production was developed to prepare the new polymorph, avoiding the need of any special equipment such as fluidized-bed drying required in the early phase process. In the second section, some of the synthetic approaches investigated for the route scouting of the phenylalanine derivative key intermediate are presented. To conclude, we discuss the analytical control strategy for BCME, the formation of which, due to the simultaneous presence of HCl and CH_2O in the Pictet-Spengler cyclization, could not be ruled out. The BCME purge factor calculations using the tools of ICH M7 control option 4 are compared to actual results from spiking experiments.

KEYWORDS EMA401, olodanrigan, phenylalanine derivatives, scale-up, route scouting, ICH M7 control option 4, BCME, bis(chloromethyl)ether

Introduction

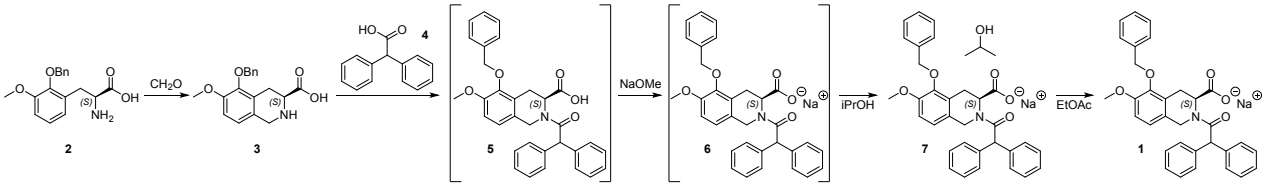
The first report of EMA401 (**1**; olodanrigan) dates back to the early 1990s,[1,2] when the structure of EMA401 (**1**) was identified in a program aiming at the development of

angiotensin II type 2 (AT_2R) antagonists. Twenty years after its first disclosure, the potential of EMA401 as a AT_2R antagonist in the treatment for neuropathic pain was recognized and the results of clinical studies were published,[3–6] and in 2015 the compound was in-licensed to Novartis. When we started process development work on EMA401 (**1**) as its sodium salt, we performed both i) an extensive route scouting exercise to find an enantioselective, environmentally-friendly and cost-effective synthesis of EMA401, and ii) ran a complete program to find a new, more stable polymorph of the drug substance. Herein, we report our results from the process optimizations for the synthetic end game and the final crystallization, evaluate different synthetic strategies to the phenylalanine key derivative, and discuss the comparison between ICH M7 control option 4 with spiking experiments for bis(chloromethyl)ether (BCME), a highly toxic, potential impurity of the penultimate bond-forming step of the EMA401 synthesis.

Results and Discussion

Late stage synthesis of EMA401 (**1**)

The retrosynthetic analysis of EMA401 (**1**) provides a number of potential disconnections as late stage conversions. Based on chemical and technical feasibility, strategic considerations, health, safety and environment (HSE) assessments, and cost calculations, the synthetic end game depicted in Scheme 1 for EMA401 (**1**) was selected. The same synthetic route had already been used for early phase development and in all syntheses published on EMA401 (**1**),[1–3,6,7] which allowed us to freeze the impurity profile on the drug substance. Starting from phenylalanine derivative **2**, tetrahydroisoquinoline **3** was synthesized in a Pictet-Spengler cyclization. Tetrahydroisoquinoline **3** was coupled in the last bond-forming step to diphenylacetic acid (**4**) to give carboxylic acid [**5**].



Scheme 1. Manufacturing route of the synthetic end game of EMA401 (**1**). Starting from phenylalanine derivative **2**, EMA401 (**1**) was synthesized in a Pictet-Spengler cyclization

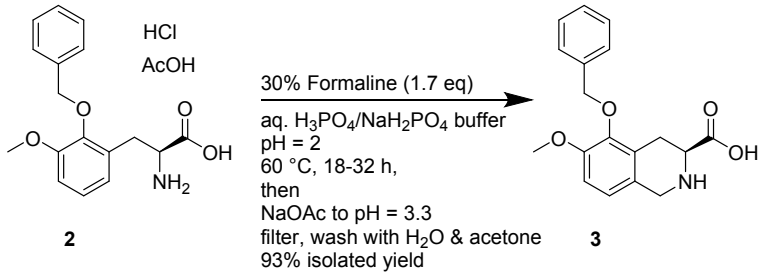
to afford tetrahydroisoquinoline **3**, and subsequently coupled with diphenylacetic acid (**4**).

Addition of NaOMe to carboxylic acid [**5**] gave carboxylate [**6**], which was isolated as its *i*PrOH solvate **7**. The final crystallization afforded EMA401 drug substance (**1**) as a sodium salt. An in-depth route scouting exercise conducted for the synthetic approach to the key intermediate phenylalanine derivative **2** is presented in a separate Section in this manuscript (*vide infra*).

*Process Development of the Pictet-Spengler Cyclization to Tetrahydroisoquinoline **3***

The early phase process for the Pictet-Spengler cyclization from phenylalanine derivative **2**, used as its hydrochloride salt and acetic acid solvate, to tetrahydroisoquinoline **3** is given in Scheme 2.[6] The reaction was run in an aqueous phosphate buffer system at pH 2. An initial assessment of the process identified the following challenges: i) long reaction times of 18–32 h, ii) a low concentration (0.1 M), iii)

a slow filtration during the isolation of tetrahydroisoquinoline **3**, iv) the product had a tendency to float on the aqueous layer on scale, causing IPCs to fail due to non-representative sampling, and v) an assessment of the commercial production capabilities identified this step as the bottle-neck defining the cycle time of the entire drug substance production.



Scheme 2. Early phase process for the Pictet-Spengler reaction to tetrahydroisoquinoline **3** in an aqueous buffer.

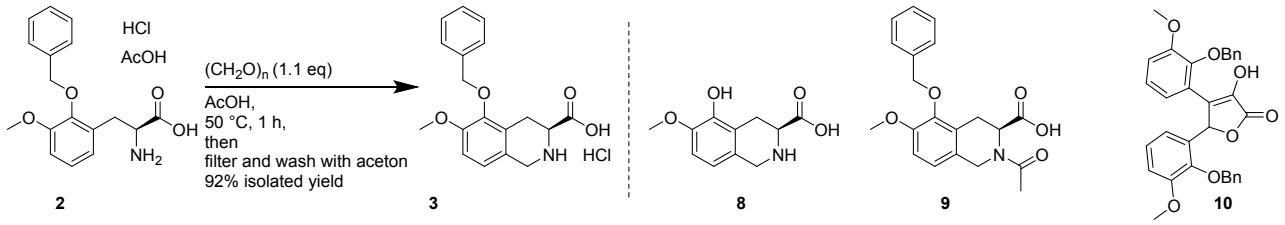
Attempts to decrease the reaction time by increasing the temperature or by decreasing the pH led to the partial cleavage of the benzyl group.[2] While the thus formed phenol **8** (Scheme 3) could be purged in the isolation of **3** as its zwitter ion, its formation led to a significant reduction of the yield. We therefore turned our attention to the addition of co-solvents aiming to increase the concentration and to decrease the

reaction time. However, none of the co-solvents screened showed an improvement in terms of space-time-yield. Gratifyingly when we ran the cyclization entirely in acetic acid as solvent, full conversion was observed at 50 °C within 1 h in a slurry-to-slurry process (Scheme 3). In addition, the concentration could be increased to 0.5 M (5 v/w with respect to phenylalanine **2**) and the equivalents of *para*-formaldehyde could be reduced to 1.1 eq. As phenylalanine **2** was isolated as its hydrochloride salt, tetrahydroisoquinoline **3** was also isolated as its hydrochloride salt after cooling to 20 °C, with much improved filtration properties as compared to the zwitter ion of **3**. The filter cake was then washed with acetone to purge acetic acid, residual phenylalanine **2**, and the three main impurities (Scheme 3). The de-benzylated phenol **8** originated mostly from the synthesis of phenylalanine derivative **2**, and had to be analytically controlled by specification on phenylalanine **2**. While phenol **8** was purged in the isolation of tetrahydroisoquinoline **3** as the zwitter ion, purging of de-benzylated impurity **8** in the isolation of **3** as the hydrochloride salt and in the subsequent steps to the drug substance was low. The *N*-acetyl byproduct **9** (Scheme 3) was observed at levels up to 0.38a% in stress tests and after spiking with acetic anhydride, and was purged in the

isolation of **3**·HCl. Lactone byproduct **10** was observed at less than 1a% in IPCs and purged to below limit of detection (<LOD) in the isolation of **3**·HCl. Finally, the water content in the reaction was found to be critical and the addition of 2wt% H_2O with respect to phenylalanine **2** caused the loss of more than 10% tetrahydroisoquinoline **3** in the mother liquor, due to the increased solubility of **3** in aqueous acidic conditions.

The residual formaldehyde content in the isolated **3**·HCl was determined to be 77 ppm, *i.e.* already by a factor of more than 200 below the permitted content in the drug substance for oral administration (oral limit for formaldehyde : maximum daily dose of EMA401; 10'000 μg/d : 0.6 g/d = 16'666 ppm).[8] None of the safety measurements indicated a critical exotherm for the reaction, reagents or product. In summary, the space-time-yield could be improved by a factor of 20, the number of unit operations as well as the number of discrete raw materials required was significantly reduced as compared to the early phase process, which led to a reduction of the PMI by a factor of 5 (Table 1). The modified process was successfully implemented on 1 kg of phenylalanine derivative **2**·HCl. It is noted that the process in aqueous medium as well

as the process in acetic acid could potentially lead to the formation of the theoretical impurity bis(chloromethyl)ether (BCME), a highly toxic by-product (*vide infra*).



Scheme 3. Modified process of the Pictet-Spengler reaction to tetrahydroisoquinoline **3**·HCl in acetic acid. By-products phenol **8**, *N*-acetyl derivative **9** and lactone **10** were observed in this step in IPCs and the isolated product.

Table 1. Comparison of the original Pictet-Spengler processes to tetrahydroisoquinoline **3** in aqueous buffer and optimized process to **3**·HCl in acetic acid.

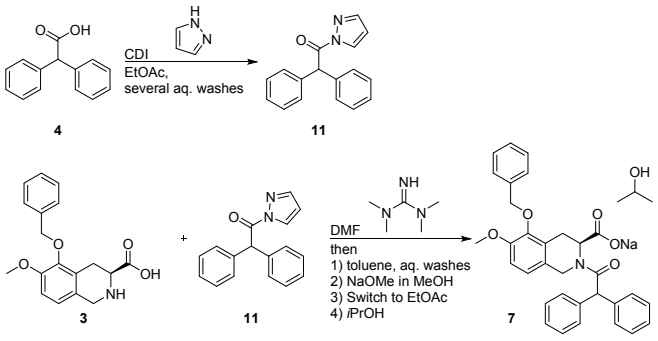
Early phase process in aqueous buffer	Modified process in acetic acid
Long reaction times: 18–32 h	Short reaction times: 1 h
Low concentration: 0.1 M	High concentration: 0.5 M
Slow filtration of **3**	Fast filtration of **3**·HCl

Floating of **3** on aqueous mixture on scale	No floating observed, slurry to slurry reaction
Increasing temperature or lowering pH led to the formation of by-products	Extended reaction time led to by-products
PMI = 55	PMI = 11

*Amide coupling and salt formation to intermediate **7***

The early phase process for the amide coupling to intermediate [**5**] from **3** and **4** is depicted in Scheme 4.[3] Diphenylacetic acid (**4**) was activated as its acyl pyrrazole derivative **11**, a stable compound isolated after several washing steps. Pyrrazole **11** was then added to a mixture of **3** and tetramethylguanidine in DMF. After complete conversion to amide [**5**], toluene was added and the mixture was subjected to an aqueous acidic quench and aqueous washings, followed by the addition of NaOMe to obtain carboxylate [**6**], a solvent switch to EtOAc, and finally to the crystallization as the *i*PrOH solvate **7**. We aimed at streamlining this sequence of processes to i) reduce the amount and number of chemicals used, and hence the amount of waste created, ii) reduce the number of operations in each process, iii) avoid the isolation of pyrrazole **11**,

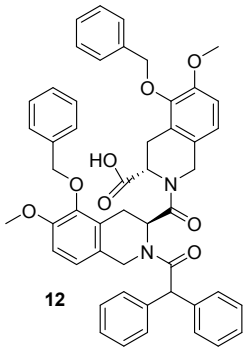
and iv) replace the REACH listed solvent DMF to isolate the isopropanol solvate **7** in high yield with a comparable impurity profile.



Scheme 4. Early phase synthesis of *i*PrOH solvate **7** from tetrahydroisoquinoline **3** and pyrrazole derivative **11**.[3]

We aimed at the *in-situ* activation of diphenylacetic acid (**4**) to avoid the isolation of pyrrazole derivative **11**. Since both, **3** and **4** bear a carboxylic acid functionality, it was of obvious importance to activate only the carboxylic acid on diphenylacetic acid (**4**). Activation of the carboxylic acid on tetrahydroisoquinoline **3** led to the erosion of the chiral purity and to the formation of coupling products **12** and **13** (Figure 1). These

impurities could be purged in the crystallization of **7**, albeit at the cost of a significantly reduced yield.[9]



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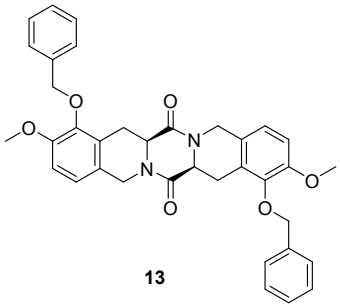

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Figure 1. Impurities **12** and **13** were observed when the carboxylic acid on tetrahydroisoquinoline **3** was inadvertently activated by an excess of activating reagent.

Since EMA401 was predicted to be a multi ton project, we initiated a screening for amide coupling conditions with a special focus on the large scale availability and price of the amide coupling reagent as recently discussed in this journal.[10] The obvious choice would have been Schotten-Baumann conditions employing, for example, the acid chloride of diphenylacetic acid. In initial screenings, those conditions led to an increased number of impurities, and to an erosion of the chiral purity as noted in earlier reports.[2] We therefore decided to look for alternative conditions to deliver material for

clinical studies and focused our screening on surfactants, which had shown to be an excellent alternative to dipolar aprotic solvents for amide coupling reactions.[11] To our dismay, none of the conditions screened gave full and clean conversion, most likely due to solubility issues observed with tetrahydroisoquinoline **3**. In a next phase, we screened amide coupling reagents in organic solvents. Of all reagents tested, 1,2-carbonyldiimidazole (CDI) showed the most promising results. Most other reagents tested (PivCl, isobutyl chloroformate, T3P, EDC, DCC and triazine-based reagents) suffered from poor conversion, formation of impurities, or required the use of HSE critical additives such as HOBt for EDC and DCC.

The low solubility of tetrahydroisoquinoline **3** in most organic solvents mandated the use of a base to increase its solubility. Various bases were screened (DBU, Na_2CO_3, Na imidazolate, NMM, TMG, DIPEA) and out of those only DBU was capable of forming a stirrable suspension of tetrahydroisoquinoline **3** in organic solvents, resulting in full conversion to [**5**] with a low by-product profile. Since DBU is a strong base, which potentially could also lead to E_1cb elimination of the activated diphenylacetic acid (**4**), the equivalents were kept at below 1.1 with respect to tetrahydroisoquinoline **3**.

Less polar solvents such as THF, MeTHF, *i*PrOAc, acetone, or toluene led to the formation of gels or unstirrable suspensions of **3**, even when DBU was used as a base. From the existing process, we knew that DMF afforded a well-stirrable suspension of **3**. This behavior was also found with 1-methylpyrrolidin-2-one (NMP, CAS# 872-50-4), 1-butylpyrrolidin-2-one (NBP, CAS# 3470-98-2), 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU, CAS# 7226-23-5) and 4-formylmorpholine (NFM, CAS# 4394-85-8). Combined with the fact that the sodium salt [**6**] was soluble in toluene, we focused our attention to solvent mixtures of dipolar aprotic solvents with toluene. From the four dipolar aprotic solvents above, NFM was found to be the most benign option in terms of HSE and compatibility with downstream processing, and further optimization was continued with 4:1 (v/v) mixtures of toluene/NFM as the solvent. The ratio was later optimized to 3.25:1 (w/w). Using less NFM led to the formation of biphasic mixtures resulting in slower conversion and an increased impurity profile.

As discussed above, the stoichiometry of CDI was critical to avoid the formation of impurities. Additional CDI could not be dosed once the solution of imidazole derivative [**14**] had been added to tetrahydroisoquinoline **3** (Scheme 5), therefore, specifications

for CDI and the implementation of an IPC for the activation of diphenylacetic acid (**4**) were crucial for the entire process. Hence, a solution of **4** (1.20 eq, *i.e.* an excess as compared to CDI) in toluene/NFM 4:1 was added to a suspension of CDI (1.15 eq) in toluene/NFM 4:1. At the end of the addition, a slightly yellow, clear solution of imidazole derivative [**14**] was obtained, which was added to a suspension of **3** in toluene/NFM 4:1 with 1.06 eq (2.06 eq) of DBU when starting from the zwitter ion of **3** (the hydrochloride **3**·HCl). Complete conversion of tetrahydroisoquinoline **3** was achieved within 3 h at 30 °C, indicated by the change in aspect from a suspension to a solution.

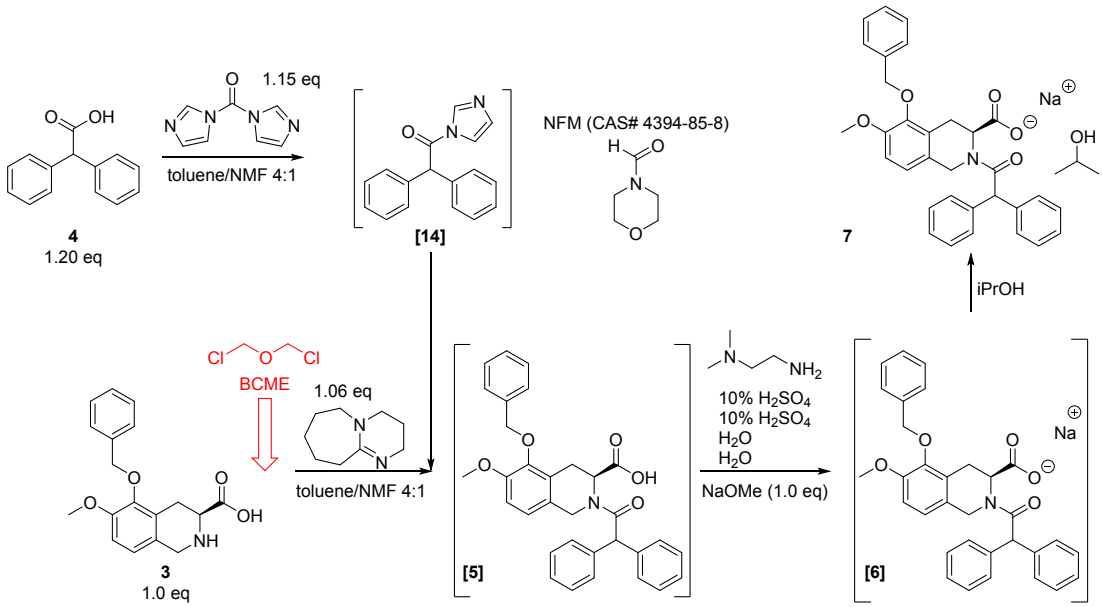
In the early phase process, the post-reaction quench of an excess of pyrrazole **11** with dimethylethylenediamine (CAS# 108-00-9) was routinely done in all GMP campaigns. Lab results indicated that with the change from pyrrazole **11** to the less stable imidazole [**14**] in the late phase process, the addition of dimethylethylenediamine might not be required for the removal of by-products originating from diphenylacetic acid, but that it may act as a buffer in the subsequent quench with H_2SO_4 to maintain the chiral purity. Indeed this first H_2SO_4 quench proved to be pivotal for the chiral purity, with stirring speed, addition time and temperature all having an influence on the chiral purity of [**5**].[12]

To extract all amines present in the mixture, a total of two acidic and two neutral washes were implemented.

Lab experiments and IPCs on scale indicated that NFM mostly hydrolyzed under the aqueous acidic conditions with $\tau_{1/2}$ = 25 min at 25 °C (see Supporting Information (SI)) and only small amounts of NFM remained in the toluene layer after four aqueous washes. The toluene solution of carboxylic acid [**5**] was treated with a solution of NaOMe (1.0 eq) in MeOH to obtain sodium salt [**6**]. When more than 1.2 eq. of NaOMe were used, the excess of NaOMe could react with traces of NFM and water in the toluene phase, leading to the formation of sodium formate, which was not purged and carried through as an impurity to the drug substance.

Azeotropic removal of H_2O and MeOH from the solution of [**6**] in toluene, and subsequent dilution with toluene afforded a solution of [**6**], which was then added to *i*PrOH at 50 °C, resulting in the formation of the desired *i*PrOH solvate **7**. Addition at lower temperatures did not afford the *i*PrOH solvate, but upon heating the mixtures to above 45 °C, the formation of **7** as a white precipitate was observed.

Both addition orders, addition of the toluene solution of [**6**] to *i*PrOH and *i*PrOH to the toluene solution of [**6**], were suitable. When the solution of [**6**] in toluene was added to *i*PrOH, a suspension with superior stirring properties and a more controllable crystallization was achieved. Finally, the suspension of **7** was cooled, filtered and washed with *i*PrOH to afford intermediate **7** in around 90% yield, and a purity greater than 99.6a%, with diphenylacetic acid (**4**) being the only impurity detected. Experiments indicated that in the subsequent step to the drug substance **1**, diphenylacetic acid (**4**) could be depleted from 1.2wt% in intermediate **7** to <LOD in the drug substance. The modified process was successfully run at a 175 kg scale of tetrahydroisoquinoline **3**.



Scheme 5. Process to *i*PrOH solvate **7** with activation of diphenylacetic acid (**4**) with CDI and telescoping of the corresponding imidazole derivative [**14**] into the amide coupling step to carboxylic acid [**5**]. For BCME spiking, see the last Section of this manuscript.

Table 2. Comparison of the early and late phase processes to *i*PrOH solvate **7**.

Early phase process to **7**	Late phase process to **7**
Two isolated steps	One isolated step
Combined reaction time: 21 h	Combined reaction time: 4 h
Many aqueous washes in the isolation of pyrrazole derivative **11**	Telescoping of imidazole derivative [**14**]
DMF (ICH class 2 solvent) used in penultimate step	DMF replaced by 4-formylmorpholine (NFM)
PMI = 89	PMI = 32

*Crystallization of EMA401 (**1**) drug substance*

EMA401 as its sodium salt has a highly complex solid-state landscape (Figure SI2). The sodium salt of EMA401 (**1**) can crystallize in different solid states as an isopropanol solvate, a mixed solvate MeOH-EtOAc, a mesomorph produced *via* desolvation of the

isopropanol solvate, several hydrates, amorphous and as an anhydrous phase. The latter was our target phase, which was stable up to 65% relative humidity at 25 °C, a limit applied to all the steps from production to downstream processing.

A targeted solvent screen was carried out for EMA401 (**1**) in order to obtain the anhydrous phase (modification B, Figure SI2). The solvent system selected was water/EtOAc, even though several hydrates were found. The rationale behind this decision was that due to the existence of several hydrate phases of EMA401 (**1**), the addition of water to EtOAc would lead to an increase in solubility. Figure 2 shows the temperature-dependent solubility of EMA401 in mixtures of water and EtOAc.

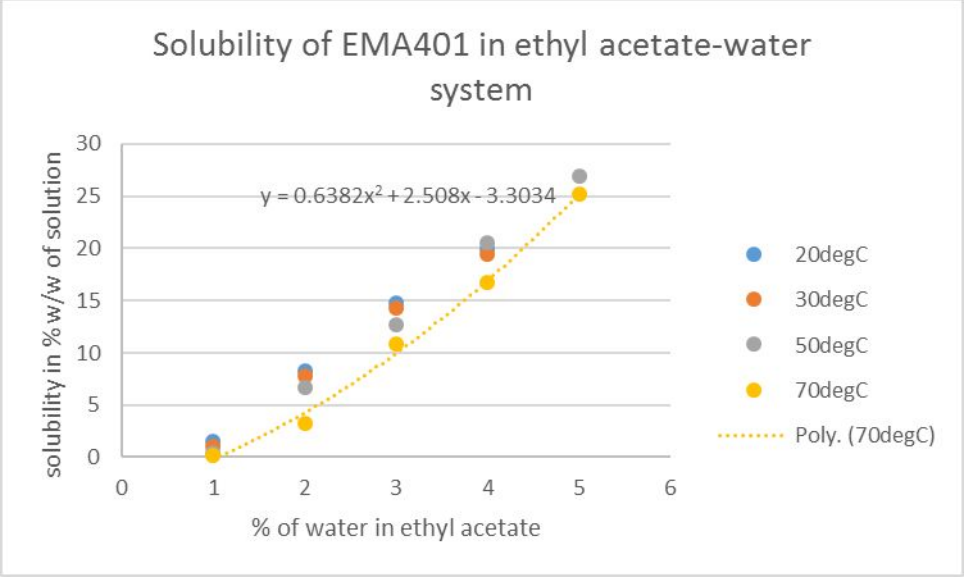


Figure 2. Solubility of EMA401 (**1**) sodium salt as a function of temperature and water content in EtOAc. Solubility values were determined gravimetrically and are given in w/w of solution.

EMA401 exhibits a retrograde solubility, *i.e.* the solubility decreases at higher temperature. The temperature-dependent solubility was found to be less pronounced than the change with the content of water added to the solvent system. Hence, the crystallization procedure was developed as a seeded crystallization by antisolvent addition, *i.e.* EtOAc was added to reduce the water concentration to less than 1wt% as the final concentration. Following this crystallization procedure, EMA401 (**1**) was obtained as crystalline needles (see Experimental Details and Figure SI3).

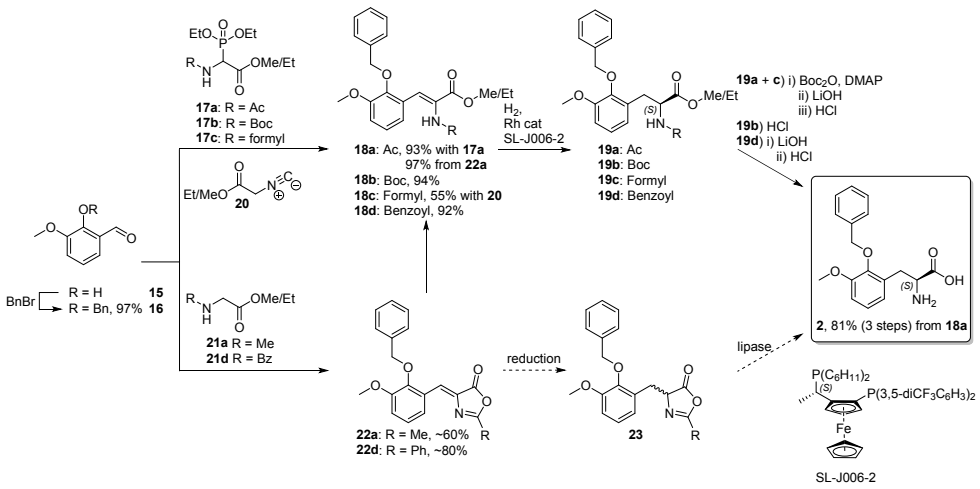
The crystallization procedure was successfully scaled up to 90 kg scale. As the procedure was an antisolvent crystallization, the final volume was high and an alternative procedure including a constant volume distillation was developed in order to increase the productivity and successfully scaled up to 90 kg. For both procedures, a final wash with heptane was implemented, as the filter cake was hygroscopic when

wetted with EtOAc, and therefore would have induced issues when unloading the wet cake from the filter to the tray dryer, a common equipment in pilot plants. This additional wash was not required when working with a filter dryer, as the product would remain under dry nitrogen. The XRPD (Figure SI4) showed the evolution of a wet cake of EMA401 (**1**) under ambient conditions. One of the hydrates was detected after a few hours and led to the formation of a sticky material (hygroscopicity of the solid) and concomitant loss of crystallinity, if water-content was not completely controlled. Importantly, the modified process to the new polymorph no longer required special equipment such as fluidized-bed drying required for the early phase process.

Route scouting for the key intermediate phenylalanine derivative **2**

The synthesis of optically pure non-natural amino acid derivatives remains a synthetic challenge, in particular for process chemistry. For the synthetic access to the key intermediate phenylalanine derivative **2**, we grouped the most promising approaches for scale-up identified in an internal route scouting exercise as shown in Scheme 6–8. The synthetic route used for the delivery of drug substance for early phase clinical trials

relied on the asymmetric hydrogenation approach developed by Burk *et al.* for *N*-acetyl protected derivatives (Scheme 6).[6,13] *ortho*-Vanillin (**15**), which is available on bulk scale at high purity and low price, was treated with BnBr to afford benzylated *ortho*-vanillin **16**, which was subjected to a Horner-Wadsworth-Emmons reaction with phosphonate **17a**, to deliver enamide **18a** in good yield and excellent selectivity. The key step of this route was the asymmetric hydrogenation of enamide **18a** to establish the chiral center in amide **19a**, and a screening of ligands, precatalysts and solvents indicated $[Rh(COD)_2]OTf$ and the Josiphos ligand SL-J006-2 in THF or MeOH to deliver **19a** with full conversion, high selectivity and excellent chiral purity (Scheme 6). In our hands, MeOH as solvent delivered a more robust reaction than in THF. The catalyst loading could be reduced to 1:2'000 (w/w, C/S) and the asymmetric hydrogenation step was scaled up to a maximum batch size of 110 kg of enamide **18a**.



Scheme 6. Routes to phenylalanine derivative **2** *via* asymmetric hydrogenation as the key step.

In order to cleave the *N*-acetyl group under mild conditions, a Boc-group had to be introduced (Boc_2O, DMAP) on the nitrogen atom, followed by the cleavage of the acetyl group and concomitant saponification of the ester under basic conditions (LiOH), and subsequent carbamate deprotection under acidic conditions (HCl, AcOH) to obtain phenylalanine derivative **2** as the HCl salt and AcOH solvate.[6] *N*-acetyl deprotection under acidic conditions at elevated temperature was not practical in our case due the lability of the benzyl group under these conditions.[2]

Alternative approaches were investigated to avoid the functional group interconversions, for example the use of Boc-protected didehydroamino acid derivative **18b** (Scheme 6). However, the asymmetric hydrogenation with *N*-Boc derivative **18b** was not found to be robust, requiring higher catalyst loading, leading to stalled reactions as well as incomplete conversions. The *N*-Boc derivative **18b** was synthesized in a Horner-Wadsworth-Emmons reaction from benzylated *ortho*-vanillin **16** and HWE reagent **17b**, which itself was prepared from the corresponding Cbz derivative.[14] This led to the conclusion that the overall gain in protecting group efficiency was negligible.

Another alternative target designed to overcome the functional group interconversion was enamide **18c**, the corresponding *N*-formyl protected derivative.[15] The HWE reagent **17c** required was prepared in analogy to similar reagents, but its isolation was complicated due to its physical properties as a viscous oil.[16] The alternative approach *via* isocyanate **20** afforded mixtures of the *E*- and *Z*-isomer, with the desired *E*-isomer isolated as a solid, while the *Z*-isomer was a viscous oil and purged to the mother liquor. This led to low isolated yields of *E*-isomer **18c**, while the mother liquor consistently contained a 1:1 mixture of the *E*- and *Z*-isomers of **18c**. In addition, isocyanates have

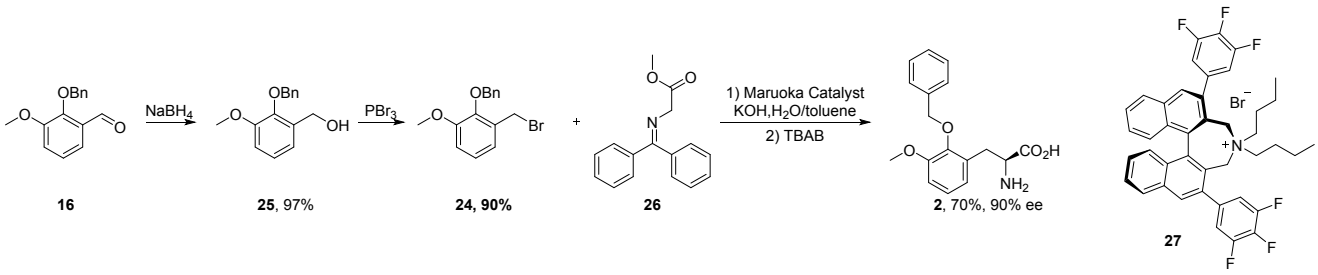
unfavorable health, safety and environment (HSE) properties and in our case required the removal of black impurities by distillation directly before their use in the subsequent condensation reaction.

The raw material costs of the HWE reagents **17a–c** contributed significantly to the overall drug substance costs, while only delivering five heavy atoms. In our attempts to avoid the HWE reagent, we investigated the approach *via* the Erlenmeyer-Plöchl azlactone condensation (Scheme 6).[17] When starting from aceturic acid (**21a**), isolated yields of azlactone **22a** were usually moderate (around 60%). The subsequent solvolysis to enamide **18a** could then be achieved in good yields. The yield of the Erlenmeyer-Plöchl condensation product azlactone **22d** was improved to approx. 80% when using hippuric acid (**21d**). However, the subsequent solvolysis to enamide **18d** was found to be capricious, and the removal of benzoic acid proved to be challenging. An alternative approach to phenylalanine **2** from azlactones **22** would have been the use of lipases after reduction to lactone **23**. However, first screening hits advised against this synthetic approach, also considering a maximum yield of 50% and the need for re-cycling of the undesired enantiomer. Preparation of amino acid **2** *via* the formation

of coumarins or a nitro derivative, as described in prior literature,[7] were not pursued, the latter due to safety concerns. An alternative approach *via* a hydantoin intermediate had also been disclosed previously.[1,2,6] Access to amino acid *via* hydantoin lyase has not been pursued in depth due to prior in-house knowledge advising against this enzyme class.

We also aimed at developing a synthetic approach relying on the substitution of bromide **24**, obtained from benzylic alcohol **25**, with protected glycine **26** (Scheme 7).[1,18] This step was run under phase-transfer catalysis conditions, using asymmetric or achiral phase-transfer catalysts. Various different cinchona-based and Maruoka-type phase-transfer catalysts were screened. The best results were obtained with 0.5 mol% of Maruoka-type phase-transfer catalyst **27** delivering amino acid **2** in 70% yield and 90% ee. Attempts to enrich the chiral purity by dynamic kinetic resolution of the racemic amino acid **2**, or ester and imine derivatives thereof, obtained by using an achiral phase-transfer catalyst in the conversion of derivatives of **24** and **26**, were not fruitful. Equally the application of conglomerate crystallization failed to meet the requirements. All these results let us to conclude that the synthetic approach depicted in Scheme 7

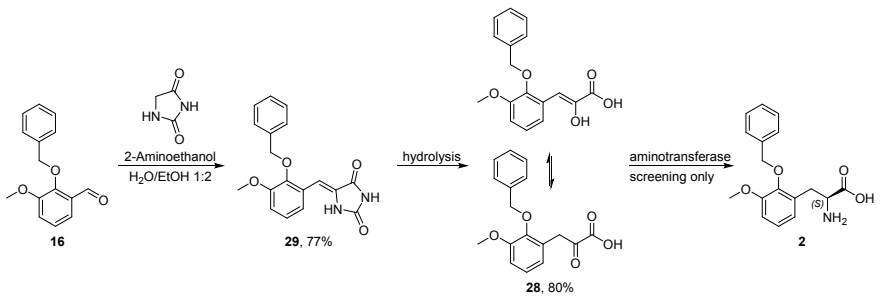
was not the most suitable strategy to phenylalanine derivative **2** for scientific and economic reasons.



Scheme 7. Synthetic approach to phenylalanine derivative **2** using phase-transfer catalysis

The aminotransferase-catalyzed amination of ketoacid **28** offered yet another alternative approach towards phenylalanine derivative **2** (Scheme 8).[1,2,6] Transaminases have become increasingly popular for large scale production as highlighted by recent publications.[19] We were pleased to find that in an initial screening with ketoacid **28**, we were able to detect conversion to phenylalanine **2**. Ketoacid **28** was prepared in two synthetic steps from benzylated *ortho*-vanillin **16** *via* its hydantoin derivative **29**.[1] The condensation with hydantoin could be achieved with 2-aminoethanol

in EtOH/H_2O, affording good conversion and an isolated yield of 77%. Hydantoin **29** was subsequently hydrolyzed to obtain ketoacid **28**. During development, we observed major challenges in the isolation of ketoacid **28**, due to slow tautomerization to the corresponding enol acid which was isolated as a white solid at pH below 3, while the keto form was a viscous oil. The tautomerization in toluene was found to be slow and took several hours. In addition, slow degradation of ketoacid **28** under exposure to light, as well as to air at ambient temperature was observed, which led to the formation of several impurities. Alternative approaches to the keto acid **28** *via* azlactone **22** (Scheme 6) were less preferred due to an increased by-product profile and the limited stability of the target compound under strongly acidic or basic conditions and elevated temperatures. Due to the issues observed in the isolation of ketoacid **28**, we turned our attention to an alternative biocatalytic system and identified the phenylalanine ammonia lyase (PAL) catalyzed synthesis of amino acid **2** as a very attractive alternative in terms of safety, ecology and economy. These findings are disclosed in part II and III of this publication series.[20,21]



Scheme 8. Synthesis of phenylalanine derivative **2** using aminotransferases

Investigations on the theoretical impurity bis(chloromethyl)ether

Bis(chloromethyl)ether (BCME; CAS# 542-888-1) is a highly toxic compound and known mutagen, classified as an ICH M7 class 1 impurity.[22] According to the ICH M7 guideline addendum, the permitted daily exposure *via* pharmaceuticals has been limited to 4 ng/day for lifetime exposures. Up to the early 1970s, BCME was produced on multi ton scale from CH_2O and HCl, until its high toxicity was discovered and production was ceased.[23] Due to the simultaneous presence of HCl (amino acid **2** was isolated as its HCl salt) and CH_2O in the process to tetrahydroisoquinoline **3**, BCME could theoretically be formed. With a maximum daily dose of 0.6 grams per day as projected for EMA401

(**1**), the maximum allowed concentration (MAC) for BCME for life-long treatment with EMA401 was calculated according to Equation 1.

4 ng/d / 0.6 g/d = 6.7 ppb. Equation 1

We therefore studied the literature as a basis for applying ICH M7 control option 4 according to the publication by Teasdale *et al,*[24] whereby scientifically justified, but theoretically selected purge factors for reactive chemicals, are considered *in-lieu* of direct detection.

Literature Review on the Formation and Degradation of BCME

According to Alvarez and Rosen, BCME can be formed in a chloromethylating medium (HCl-HCOH-$ZnCl_2$) in concentrations of up to 300–500 ppm.[25] While the authors found that BCME decomposed slowly under purely aqueous conditions, the decomposition of BCME in the presence of MeOH under basic conditions and in the presence of ammonia was significantly expedited (less than 5 min for the decomposition of 718 μg/mL). Tou, Westover and Sonnabend described the hydrolysis of BCME in water at 20 °C with half-life times, calculated by the authors of this manuscript based on

the published value for the rate constant *k* and assuming an unimolecular reaction for the data provided in the original publication, of 38.5 s (neutral), 28.9 s (2 N NaOH) and 63.0 s (3 N HCl).[26]

Frankel and co-workers investigated the formation of BCME from formaldehyde and HCl in moist air with a yield of 0.01mol%.[27] The amount of BCME formed was found to be relatively insensitive to moderate changes in temperature and humidity, but strongly dependent on the concentrations of the reactants. The maximum concentration of BCME formed was 5000 ppb at [CH_2O] = 0.4wt% (4'000 ppm) and [HCl] = 4wt% (40'000 ppm). The concentration dependence described by Frankel *et al.* was supported in a study by Kallos and Solomon, who did not find any detectable amounts of BCME in the vapor phase or aqueous phase at HCl and formaldehyde concentrations of up to 500 ppm.[28] A maximum of 48 ppb BCME was detected starting from concentrations as high as 3'000 ppm formaldehyde and 10'000 ppm HCl. Those findings were later corroborated by a computational study by Bock *et al.*, who did not find any "evidence that BCME forms spontaneously whenever formaldehyde and hydrogen chloride coexist in humid air."[29] Tou and Kallos studied the BCME formation at

HCl and formaldehyde concentrations of up to 2'000 ppm at ambient temperature. No BCME was detected in the aqueous layer or the head-space above the solution with a detection limit of 1 ppb.[30]

Applying ICH M7 Control Option 4: Purge Factor Tool according to Teasdale et al.[24]

In the synthesis of EMA401 (**1**), phenylalanine derivative **2** was isolated as a HCl salt, which was subjected to a Pictet-Spengler cyclization with formaldehyde to obtain tetrahydroisoquinoline **3**. This reaction was either run using 37% formalin solution (CAS# 50-00-0) in an aqueous medium isolating **3**, or *para*-formaldehyde (CAS# 30525-89-4) in acetic acid isolating **3**·HCl (*vide supra*). In both cases, due to the simultaneous presence of hydrochloric acid and formaldehyde in the reaction mixture, the formation of BCME cannot be excluded *a priori*.

In the process used for the synthesis of **3** or **3**·HCl, both CH_2O and HCl were used at roughly 0.6wt% (6'000 ppm) or 1.4wt% (14'000 ppm), respectively. For the reaction to **3** in an aqueous medium, a worst-case scenario of 0.01mol% of BCME was assumed as reported for gas phase reactions.[27] Due to the lack of literature precedence for the

formation of BCME in acetic acid, for the process in acetic acid to **3**·HCl, a worst-case scenario with 5mol% of BCME formed was assumed. This assumption was based on a yield of 90% for the isolated **3**·HCl salt, leaving 10mol% of chloride ions for the formation of 5mol% of BCME (one BCME requires two chloride ions), which corresponded to 2wt% BCME in **3**·HCl. No data could be found for the formation of BCME in acetic acid conditions and hence, to ensure maximum patient safety, full conversion of Cl ions to BCME is assumed. As this second - worst-case - scenario for the formation of BCME also covered the process in an aqueous medium to **3**, the formation of 5mol% (corresponding to 2wt%) BCME was assumed in the following calculations. Thus, the required purge factor was calculated as shown in Equation 2:

Required Purge factor = worst-case amount / MAC = 0.02 / 6.67 ppb = 3.0×10^6.

Equation 2

Values are assigned to physical properties with purge potential for each step, and cumulated in an overall purge factor for all stages from the introduction or formation of a mutagenic impurity to the isolation of the drug substance.[24] Table 3 summarizes the purge-factor calculations for the sequence of steps from **2** to **7** considering the

reactivity, volatility and solubility of BCME. Since BCME is not ionisable and no physical processing was performed as described by Teasdale *et al.*, ionisability and physical processing are not further discussed.[24] We could not find any quantitative data on solubility or miscibility of BCME with common solvents, and due to its high toxicity we refrained from determining those values ourselves. Based on the structural similarity of BCME to Et_2O we used solubility/miscibility data of Et_2O for our assessment of the solubility of BCME. Relying on the data in Table 3 and applying Equation 3, a calculated to required purge ratio of 9'000 was determined for BCME in the processes of the synthetic end game of EMA401 (**1**),[31] and it can be expected that the potential impurity BCME would be completely removed to levels significantly below the threshold of toxicological concern (TTC) even for the worst-case scenario with 5mol% formation of BCME. Because the potential impurity BCME was formed only two isolated steps from the drug substance, we performed spiking experiments with BCME to further support the value calculated using the purge factor tool.

Table 3. Calculation of the purge factor for the highly reactive potential impurity BCME based on the process in AcOH to **3**·HCl.

Intermediate	Reactivity (100, 10, 1)	Solubility (10, 3, 1)	Volatility (10, 3, 1)	Purge factor/step	Justification
3	100	10	3	3000	R: Hydrolysis of BCME according to literature[26] S: AcOH and Et_2O are miscible, the same is expected for BCME V: Drying for H_2O which has a boiling point ±20 °C of BCME (106 °C)
[**5**]	100	1	1	100	R: High reactivity with nitrogen containing compounds and hydrolysis in aqueous acidic quench[26]
[**6**]	100	1	3	300	R: Solvolysis with NaOMe/MeOH expected to be high based on literature[25,26] V: Distillation with toluene, which has a boiling point ±20 °C of BCME (106 °C)

Intermediate	Reactivity (100, 10, 1)	Solubility (10, 3, 1)	Volatility (10, 3, 1)	Purge factor/step	Justification
7	1	10	3	30	S: *i*PrOH and toluene are miscible with Et_2O, the same is expected for BCME V: Drying for toluene, which has a boiling point ±20 °C of BCME (106 °C)
1	1	10	1	10	S: EtOAc and Et_2O are miscible, the same is expected for BCME
Total				2.7×10^{10}	

Calculated to required purge farctor ratio: (=Calculated purge factor / required purge factor): $2.7\times10^{10}:3.0\times10^{6} = 9.0\times10^{3}$

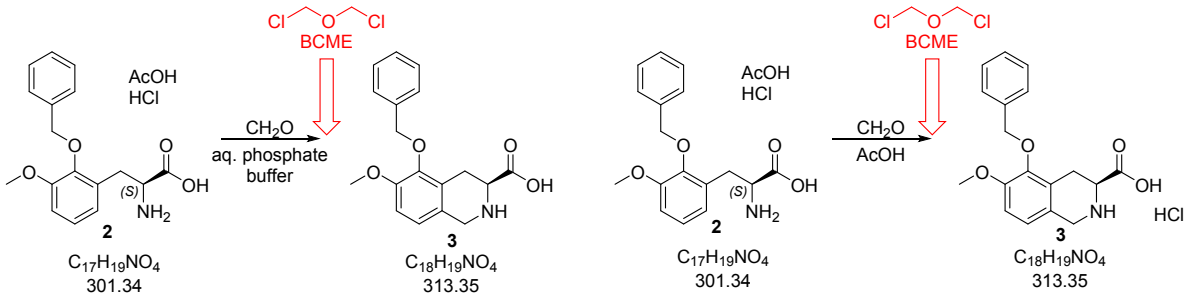
Equation 3

Spiking experiments with BCME

*Spiking experiments for processes to intermediate **3** and **3**·HCl*

In the case of the reaction **2** to **3**·HCl, a purge factor of 3'000 was calculated. Based on a limit of quantification (LOQ) of 100 ppb (see Section 5 in the SI) in the **3**·HCl matrix, BCME was spiked at ca. 600'000 ppb (100 ppb × 3'000 × 2) into the processes

in aqueous phosphate buffer conditions or in AcOH at the end of the reaction, before cooling, quenching (in the case of the process to **3** in an aqueous medium) and isolation of tetrahydroisoquinoline **3** (Scheme 9),[32] increasing chances for detection of BCME by applying an additional factor of 2 in the calculation of the concentration of BCME to be spiked into the reaction. This meant that reaction mixtures of 4.6 g or 4.1 g **2** in ca. 100 g H_2O solution or 20 mL AcOH solution were treated with a solution of BCME (2 mg) in hexane (1 mL) at the end of the conversion from **2** to **3** (or **3**·HCl). Spiking was done at this time point, as this allowed studying the elimination of BCME by the work-up procedure, assuming that a maximum of BCME was formed at the end of the conversion to tetrahydroisoquinoline **3**. The results of the BCME analysis in isolated **3** and **3**·HCl are highlighted in Table 4. No BCME could be detected at levels above the limit of quantification (>LOQ) and it can be concluded that the measured purge factor for BCME in both processes is therefore larger than 6'000, *i.e.* also larger than the calculated purge factors.



Scheme 9. Spiking of the aqueous buffer and acetic acid processes to **3** or **3**·HCl with BCME. The amount of BCME found by LCMS in both, **3** and **3**·HCl was <LOQ of 100 ppb.

Table 4. Conditions and results from BCME spiking experiments for the steps to **3** or **3**·HCl.

Conditions	Isolated yield of **3** or **3**·HCl	HPLC purity / a%	BCME Spiked based on product	BCME measured in **3** (or **3**·HCl)	Calculated purge factor	Measured purge factor (LOQ 100 ppb)
Aqueous buffer (to **3**)	3.38 g 92.1%	100%	2.0 mg / 3.38 g 592 ppm	<100 ppb	300*	>5‘917
Acetic acid (to **3**·HCl)	3.25 g 90.9%	100%	2.0 mg / 3.25 g 615 ppm	<100 ppb	3‘000	>6‘154

*) Solubility of BCME was assumed to be low (a rating of 1) in H_2O, as Et_2O and H_2O are not miscible, hence the reduction of the purge-factor by a factor of 10.

Spiking experiments for processes to iPrOH solvate 7

Using *i*PrOH solvate **7** as the matrix, a limit of quantification of 27 ppb for BCME could be achieved (see Section 5 in the SI). For the reaction sequence from **3** to [**5**] to [**6**], and to **7**, a purge factor of 100 × 300 × 30 = 900'000 was calculated (Table 3). The purge factor was too high to be confirmed through spiking experiment with 2 mg BCME/mL solution.[33] Thus, it was decided to run the experiments at a similar scale as for the Pictet-Spengler cyclizations to tetrahydroisoquinoline **3**, and the process to **7** was set-up such that 2 mg BCME in 1 mL hexane were added in the process right after the addition of **3** or **3**·HCl, respectively (Scheme 5). This meant that 2.0 g **3** or 2.2 g **3**·HCl in approximately 17 mL of final reaction solvent were treated with 1 mL BCME solution (2 mg/mL). The results of the BCME analysis for the spiking experiments for the step from **3** (or **3**·HCl) to **7** are summarized in Table 5. Purge factors larger than 20'000 were experimentally determined starting from **3** or **3**·HCl.

Table 5. Conditions and results for the BCME spiking experiments on the step to *i*PrOH solvate **7**.

Conditions	Isolated yield of **7**	HPLC purity / a%	BCME Spiked based on product **7**	BCME measured in **7**	Calculated purge factor	Measured purge factor (LOQ 27 ppb)
Starting from **3**	3.15 g 85.1 %	99.85 %	2.0 mg / 3.15 g 635 ppm	<27 ppb	9'000'000	>23'518
Starting from **3**·HCl	3.41 g 82.3 %	100%	2.0 mg / 3.41 g 587 ppm	<27 ppb	9'000'000	>21'740

The combined experimentally determined purge factors for the steps from phenylalanine derivative **2** to *i*PrOH solvate **7** amount thus to more than 1.2×10^8 (=6'000 × 20'000) and are larger than the required purge factor of 3×10^6, even before the final crystallization to the drug substance **1**. It can, however, be assumed that a

further purging effect is achieved on this step. As the measured purge factor already exceeded the required purge factor, no spiking experiment was conducted on the step from **7** to **1**.

In summary of the BCME investigations, both the calculated and measured purge factors were larger than the purge factor needed for an absolute worst-case scenario for the formation of BCME (Table 6). Thus, it can be concluded that under the process conditions, any BCME potentially formed in the synthesis of tetrahydroisoquinoline **3** was purged below the TTC in the drug substance EMA401 (**1**).

Table 6. Comparison of ICH M7 control option 4 purge factor tool with spiking experiments

Step	Purge factor determined based on spiking experiments	Calculated purge factor according to ICH M7 control option 4
2→**3**	>5'917	300
2→**3**·HCl	>6'154	3'000
3→**7**	>23'516	9×10^5

3·HCl→**7**	>21'723	9×10^5
2→**7**	$>10^7$	

Experimental Details

(3*S*)-5-(Benzyloxy)-6-methoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid (**3**)

2 (HCl salt, AcOH solvate, 1 w/w) was dispersed in H_2O (15.83 w/w) and a buffered phosphate solution (2.73 w/w, prepared by mixing $NaH_2PO_4{\cdot}2\ H_2O$ (0.48 w/w), H_2O (2 w/w) and H_3PO_4 (85wt%, 0.25 w/w until pH= 2)) was added. A 37wt% formalin solution (0.35 w/w) was added at IT = 20–30 °C. The mixture was warmed to IT = 58–60 °C. The suspension was clearing up in the beginning and then the formation of a white thick suspension was observed. The mixture was maintained at IT = 58–60 °C for 60–72 h. The mixture was then cooled to IT = 20–25 °C, and the pH adjusted from 1.5 to 3–3.5 with NaOAc solution (1.58 w/w, prepared by dissolving NaOAc (0.5 w/w) in H_2O (1.75 w/w)). The resulting suspension was stirred for 2 h at 20–25 °C and filtered. The remaining solid in the reactor was slurried with a mixture of H_2O (7 w/w) and EtOH (2.7

w/w) and transferred to the filter. The filter cake was washed with acetone (2×2 w/w) and the wet product dried at 50 °C under full vacuum to obtain a white solid (93% yield).

^{1}H NMR (400 MHz, D_3CCO_2D): δ = 3.02 (dd, J = 17.6, 11.0 Hz, 1 H), 3.48 (dd, J = 17.6, 5.4 Hz, 1 H), 3.89 (s, 3 H), 4.16 (dd, J = 11.0, 5.3 Hz, 1 H), 4.33–4.51 (m, 2 H), 5.05 (d, J = 1.7 Hz, 2 H), 6.90–7.02 (m, 2 H), 7.26–7.40 (m, 3 H), 7.42–7.51 ppm (m, 2 H); ^{13}C NMR (101 MHz, D_3CCO_2D): δ = 25.05, 45.21, 56.40, 57.16, 75.26, 112.89, 121.68, 123.13, 127.27, 129.04, 129.34 (2 C), 129.40 (2 C), 138.62, 146.19, 153.39, 174.36 ppm.

(3*S*)-5-(Benzyloxy)-6-methoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid—hydrochloride (1/1) (**3**·HCl)

2 (HCl salt, AcOH solvate, 1.0 eq) was treated with *para*-formaldehyde (1.1 eq) and suspended in acetic acid (5 v/w wrt **2**). The mixture was heated to 50 °C and stirred for 2 h. IPC passed when (**2**/(**2**+**3**·HCl)) < 0.3a%. The mixture was cooled to 20 °C and stirred for 30 min, filtered and the filter cake washed with acetone (3×2 v/w wrt **2**). The

wet product was dried in the vacuum oven at 50 °C and 10–20 mbar to obtain **3**·HCl as a white solid (92% yield).

^{1}H NMR (400 MHz, $(CD_3)_2SO$): δ = 2.90 (dd, J = 17.3, 11.1 Hz, 1 H), 3.26 (dd, J = 17.3, 5.2 Hz, 1 H), 3.84 (s, 3 H), 4.16–4.35 (m, 3 H), 4.98 (d, J = 1.1 Hz, 2 H), 7.01 (d, J = 8.6 Hz, 1 H), 7.06 (d, J = 8.6 Hz, 1 H), 7.31–7.43 (m, 3 H), 7.43–7.48 (m, 2 H), 9.84 (s, 2 H), 14.10 ppm (s, 1 H); ^{13}C NMR (101 MHz, $(CD_3)_2SO$): δ = 23.60, 43.29, 52.95, 55.96, 73.60, 112.06, 121.10, 122.15, 125.02, 128.03 (2 C), 128.37 (2 C), 137.49, 144.40, 151.27, 169.87 ppm.

Sodium (3*S*)-5-(benzyloxy)-2-(diphenylacetyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate—propan-2-ol (1/1) (**7**)

CDI (1.15 eq) in a mixture of toluene/N-formylmorpholine (3.25:1 w/w, 2 v/w with respect to **3**) in reactor 1 under inert atmosphere at 25±3 °C was carefully treated with a solution of **4** (1.2 eq) in a mixture of toluene/N-formylmorpholine (3.25:1 w/w, 3 v/w with respect to **3**). Diphenylacetic acid (**4**) must be added carefully since CO_2 was produced upon addition. Furthermore, a weak exotherm was observed. Stopping the addition of **4**

does not lead to an immediate cease in CO_2 production. The mixture was stirred for 1 h at 25±3 °C and the consumption of CDI was checked by IPC1 (aliquot of the mixture was treated with an equal volume of benzyl amine, IPC pass when **4**/(**4**+activated-**4** derivative) < 9%).

3 (1.0 eq) was suspended in a mixture of toluene/N-formylmorpholine (3.25:1 w/w, 4.5 v/w with respect to **3**) in reactor 2 at 30±3 °C and treated with DBU (1.06 eq) affording a white suspension which was treated with the mixture from reactor 1 (no exotherm observed, dosed over 20 min) and stirred at 30±3 °C for 3 h, when all solids were dissolved. IPC2 (pass when **3**/(**3**+**5**) < 2%) showed complete conversion.

The mixture was cooled to 10±3 °C and carefully treated with 10% aq. H_2SO_4 (6.6 v/w with respect to **3**). The addition was strongly exotherm, the inner temperature must not exceed 20 °C. After the end of the addition, the mixture was warmed to 20 °C and the phases were separated. The organic phase was washed once with 10% aq. H_2SO_4 (3.3 v/w with respect to **3**) and twice with water (3.3 v/w with respect to **3**). The organic phase was treated with 30% NaOMe (1.0 eq) in MeOH and stirred for 30 min. For azeotropic drying of the mixture, toluene/MeOH was evaporated at 40 °C to a minimal

volume and subsequently diluted with toluene to 4.5 v/w with respect to **3**. For crystallization, the toluene solution was added in 3 h to *i*PrOH (17.5 v/w with respect to **3**) in reactor 3 at 50±3 °C, yielding a white suspension. After the end of the addition, the mixture was stirred for 30 min at 50±3 °C, cooled to 20 +/-3°C in 2 h and stirred for 30 min. The suspension was filtered at 20 °C and rinsed with *i*PrOH (9 v/w with respect to **3**). The product was dried at 40 °C and 1–10 mbar for 12 h to obtain a white solid (90% yield).

Masses and volumes are adjusted accordingly for **3**·HCl, and the equivalents for DBU are increased from 1.06 to 2.06.

^{1}H NMR (400 MHz, $(CD_3)_2SO$, mixture of 2 rotamers): δ = 1.04 (d, J = 6.1 Hz, 6 H), 2.16 (dd, J = 15.9, 6.3 Hz, 0.8 H), 2.45 (d, J = 6.7 Hz, 0.2 H), 3.42 (d, J = 15.9 Hz, 0.8 H), 3.57 (d, J = 15.9 Hz, 0.5 H), 3.76 (d, J = 3.4 Hz, 4.4 H), 4.28–4.40 (m, 2.7 H), 4.72–4.87 (m, 3.8 H), 5.06 (d, J = 5.7 Hz, 0.3 H), 5.45 (s, 0.8 H), 5.56 (s, 0.3 H), 6.68 (d, J = 8.5 Hz, 0.3 H), 6.82 (d, J = 8.5 Hz, 0.3 H), 6.84 (s, 1.6 H), 7.11–7.51 ppm (m, 18 H); ^{13}C NMR (101 MHz, $(CD_3)_2SO$, mixture of 2 rotamers): δ = 25.49, 53.52, 53.54, 55.80, 55.83, 56.54, 62.00, 73.64, 110.62, 121.51, 125.95, 126.70, 127.46, 127.71, 127.83,

127.97, 128.10, 128.22, 128.22, 128.25, 128.56, 128.61, 129.10, 129.31, 129.52, 137.91, 139.83, 141.49, 144.60, 150.41, 171.08 ppm.

Sodium (3*S*)-5-(benzyloxy)-2-(diphenylacetyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline-3-carboxylate (**1**)

*i*PrOH solvate **7** (1 w/w) was dissolved in a mixture of EtOAc (4.02 w/w) and water (0.175 w/w). The suspension was warmed to 50–55 °C, then EtOAc (1.799 w/w) were added and followed by **1** seed crystals. The suspension was warmed up to 70–75 °C, and aged for 3 h. Then EtOAc (5.389 w/w) was added over 3 h, and the suspension aged for 1 h. The resulting mixture was distilled at constant volume by addition of EtOAc (7.211 w/w), in order to reach a water content lower than 0.5% w/w. The suspension was aged for 1 h and cooled to 50–55 °C over 1 h, aged for 1 h and filtered. The filter cake was washed twice EtOAc and once with n-heptane. The product was dried under full vacuum at 45 °C until dryness.

^{1}H NMR (400 MHz, $(CD_3)_2SO$, mixture of 2 rotamers): δ = 2.17 (dd, J = 15.9, 6.3 Hz, 0.7 H), 2.47 (d, J = 6.6 Hz, 0.3 H), 3.43 (dd, J = 15.9, 2.6 Hz, 0.7 H), 3.58 (dd, J = 16.2,

2.2 Hz, 0.3 H), 3.77 (d, J = 3.4 Hz, 3 H), 4.34 (d, J = 16.6 Hz, 0.7 H), 4.39 (dd, J = 6.4, 2.7 Hz, 0.7 H), 4.71–4.88 (m, 3.3 H), 5.09 (dd, J = 6.6, 2.2 Hz, 0.3 H), 5.45 (s, 0.6 H), 5.57 (s, 0.3 H), 6.68 (d, J = 8.5 Hz, 0.3 H), 6.82 (d, J = 8.5 Hz, 0.3 H), 6.85 (s, 1.3 H), 7.13–7.52 ppm (m, 15.3 H). ^{13}C NMR (101 MHz, $(CD_3)_2SO$, mixture of 2 rotamers): δ = 25.94, 26.17, 42.22, 44.54, 51.96, 53.32, 53.54, 55.79, 55.82, 56.41, 73.62, 73.65, 110.66, 110.74, 121.18, 121.53, 125.96, 126.15, 126.40, 126.43, 126.72, 127.01, 127.48, 127.73, 127.85, 127.98, 128.00, 128.11, 128.23, 128.26, 128.57, 128.58, 128.61, 128.65, 129.11, 129.29, 129.52, 137.89, 137.94, 139.80, 140.72, 140.89, 141.43, 144.61, 144.85, 150.31, 150.41, 170.05, 171.09, 172.14, 172.91 ppm.

Conclusion

We have presented the synthetic end game of EMA401, an angiotensin II type 2 antagonist which has been studied in clinical phases for the treatment of neuropathic pain and postherpetic neuralgia. While the synthetic route of the end game of the early phase synthesis was not modified, we were able to implement process optimizations and describe a more stable polymorph of the drug substance obtained by modifying the

final crystallization. For the key intermediate phenylalanine derivative **2**, we performed a route scouting exercise and present the conclusions on several alternative synthetic approaches. Out of the routes described in this publication, the asymmetric hydrogenation as published by Burk *et. al.* was the preferred option for early phase deliveries, while the biocatalytic approach using phenylalanine ammonia lyase (PAL) described in part III of this series of manuscripts would have been the preferred option for late phase clinical trials.[21] For the theoretical impurity BCME, potentially formed in the penultimate bond forming step, we analyzed its formation based on literature and compared the results from spiking experiments with the purge factor obtained by applying ICH M7 control option 4. Our results for both strategies showed that BCME was purged below levels of toxicological concern in the drug substance.

ASSOCIATED CONTENT

Supporting Information.

The following files are available free of charge.

Stability of NFM, solid state landscape of EMA401, SEM and XRPD of EMA401, GC-MS method for BCME detection, ^{1}H and ^{13}C NMR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS

BCME, bis(chloromethyl)ether; CDI, carbonyldiimidazole; DBU, 1,8-diazabicyclo[5.4.0]undec-7-en; DCC, *N,N*-dicyclohexylcarbodiimide; DIPEA, diisopropylethylamine; DMF, *N,N*-dimethylformamide; EDC, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt, hydroxybenzotriazole; HSE, health safety environment; IPC, in-process control; LOD, level of detection; LOQ, level of quantification; NBP, 1-butyl-pyrrolidin-2-one; NMM, *N*-methylmorpholine; NMP, 1-methyl-pyrrolidin-2-one; PMI, process mass intensity; T3P, n-propanephosphonic acid anhydride; wrt, with respect to.

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(32) For safety reasons, BCME can only be handled as a solution in hexane. A 2 mg BCME / 1 mL hexanes solution was used for spiking. It was decided to set the experiments up such that multiples of 2 mg of BCME were used, to avoid additional handling of the BCME solutions. Confirming the calculated purge factor (2.7×10^{10}) over the entire reaction sequence from phenylalanine **2** to drug substance, and assuming a detection limit of 27 ppb BCME on the drug substance would have meant that a total of 729 mg BCME (in 364.5 mL hexane) per mg of drug substance (corresponding to 0.6 mg **2**) need to be spiked after the end of the conversion from **2** to **3**. This would have resulted in the addition of

364.5 mL BCME solution to 0.5 mg of **3**·HCl in 2.5 μL of AcOH. Since this set-up was not deemed representative, and also for safety considerations, it was decided to confirm the purge factor on the first two isolated steps separately and in a step-wise fashion, namely on tetrahydroisoquinoline **3** and *i*PrOH solvate **7**, by spiking at the end of the reaction from **2** to **3** (or **3**·HCl), where the highest concentration of BCME would have been expected, and on **3** (or **3**·HCl) intermediate used in the step to intermediate **7**, respectively.

(33) This would have required the spiking of 243 mg of BCME in a total of 121.5 mL hexanes on 535 mg of **3**·HCl in a total of 4 mL reaction solvent. These conditions were deemed not representative and for the ease of handling and the safety of the personel, it was decided to run the reactions on a similar scale than those for **2** to **3** (or **3**·HCl).