ABSTRACT: The α-aminophosphonate UAMC-00050, a newly developed trypsin-like serine protease inhibitor, is a lead compound for the treatment of dry eye syndrome and ocular inflammation. The medicinal chemistry route developed at the University of Antwerp possessed several problems hampering the scale-up such as poor yields for some of the steps, hazardous reagents, and environmental footprint. Herein, we report an optimized route for the UAMC-00050, in which environmental unfriendly solvents were excluded, hazardous reagents were replaced with safer alternatives, and are more efficient in terms of atom economy. Every reaction step was optimized to reach a higher yield, and design of experiment was used to find the optimum conditions in the last step. Furthermore, all the flash chromatography purifications of intermediates were replaced with plug filtration, slurry purifications, or crystallization. The overall yield was increased from 3% in the medicinal chemistry route to 22% in the process development route.

KEYWORDS: α-aminophosphonate, design of experiment, dry eye disease, yttrium catalysis, uPA

INTRODUCTION

Dry eye disease (DED), also known as keratoconjunctivitis sicca, is a multifactorial disease of the ocular surface that affects hundreds of millions of people worldwide. The disease is characterized by a dry, gritty, or burning feeling in the eye and excessive tearing and photosensitivity. Recently, a new pharmacologically active molecule, UAMC-00050 (Figure 1), was developed at the University of Antwerp (UA) for the treatment of DED. This compound is based on an α-aminophosphonate substructure mimicking amino acids where the carboxylic acid is swapped with a phosphonate ester. Compound 1 has shown good inhibitory potency against a urokinase plasminogen activator (uPA) and other trypsin-like serine proteases, which play a role in eye diseases. To continue pre-clinical investigation, we needed rapid access to reproducible multigram quantities (10–20 g per year) of compound 1. We optimized the discovery route to one suitable for a multigram scale with a potential for large-scale application.

RESULT AND DISCUSSION

Route Selection. The medicinal chemistry route started from the commercially available 4-aminophenethyl alcohol (4). After protection of the amine with Boc₂O in the presence of triethylamine, alcohol 5 was oxidized to aldehyde 6 with Dess–Martin periodinane (DMP). The aminophosphonate intermediate 8 was assembled by the one-pot three-component Birum–Oleksyszyn reaction between aldehyde 6, benzyl carbamate (7), and phosphite 3, using copper triflate as the catalyst. Triarylphosphite 3 was prepared from paracetamol (2) and used as a crude reagent. Then, the Boc group in aminophosphonate 8 was removed with TFA in DCM (1:1 v/v) to generate salt 9. The guanidine moiety was inserted using N,N′-di-Boc-1H-pyrazole-1-carboxamidine (10), the two Boc groups were removed with TFA in DCM (1:1 v/v), and the trifluoroacetate counterion was exchanged with chloride after stirring compound 12 with a DOWEX 1X8 Cl resin to get 1 (Scheme 1).

When performing the original process on a multigram scale, we noted reproducibility issues. In particular, the Birum–Oleksyszyn reaction represented a bottleneck for the overall...
yield and purity of the final material since the majority of side products were formed in this step. In previous studies, we optimized the preparation of 8, improving the yield and purity profile and finding yttrium triflate as an optimal catalyst. Further optimizations were necessary to prepare phosphite 3 due to its particular instability in the presence of oxygen and water. The purity of 3 was important to curb the generation of impurities in the Birum–Oleksyszyn reaction since the diarylphosphite can cleave the Boc group and lead to the formation of side products. Notably, purification by chromatography led to almost complete decomposition of the phosphite 3. This led us to remove the chromatographic separation from the preparation of phosphite 3 and focus on a careful synthetic protocol that yielded 3 with a purity above 90%.

Preparation of Phosphite 3. For the preparation of compound 3, the original conditions were successfully upscaled with minor modifications (Scheme 2). The reaction time was decreased from 105 to 60 min as longer times led to reduced product purity. Separating the triarylphosphite 3 from the main impurities (diarylphosphite 13 and paracetamol (2)) via chromatographic separation, precipitation, or crystallization proved challenging. Therefore, particular attention was paid to optimizing the reaction conditions leading to a minimum amount of side products.

The presence of water in the starting material was the main reason for the reduced purity of the triarylphosphite 3 as water can decompose PCl₃ to H₃PO₃ and HCl. This changes the ratio of the reagents, increasing the amount of 2 and 13 in the crude product. Moreover, water can also decompose the triarylphosphite 3 generating paracetamol (2) and diarylphosphite 13 (Scheme 2).

Careful drying of the starting material 2 in a vacuum (5 mbar) for at least 24 h significantly improved the conversion and the purity of triarylphosphite 3. The water content in paracetamol (2) after drying was 0.030% (Karl Fischer titration). Once the reaction was completed, the product was separated from triethylammonium chloride by filtration of the reaction mixture under argon flow. To prevent thermal decomposition of 3, THF was then removed under reduced pressure at 20–25 °C. Phosphite 3 was obtained with yields of 98 and 92.3% area normalized (AN) by HPLC on a 44 mmol scale.

Boc-Protection of the Amino Group. To enable the oxidation of alcohol 5, protection of the amino group in 4 was required. In the medicinal chemistry procedure, this was achieved using 1.1 equiv of di-tert-butyl dicarbonate in the presence of 1.0 equiv of triethylamine in dioxane (Table 1, entry 1). The standard reaction protocol reported in the literature (which does not require triethylamine) was successfully used in our process development. Compound 4 was treated with 1.1 equiv of di-tert-butyl dicarbonate in

Scheme 1. Medicinal Chemistry Route to UAMC-00050

Scheme 2. Synthesis of Compound 3 from Paracetamol (2)
EtOAc for 16 h (Table 1, entry 2). The purified product 5 was obtained from the reaction mixture after silica pad filtration (Table 1, entry 3). The amount of silica required in the filter pad was then reduced from 15.0 to 8.3 w/w, obtaining intermediate 5 (99% AN by HPLC) on a 73 mmol scale (Table 1, entry 4). In a later improvement, after the reaction, crude 5 was purified by crystallization. Among seven different conditions (see the Supporting Information), the mixture of MeCN/MTBE 1:1 v/v (solvent/S = 1.5:1 v/w) was found able to provide the product with 99% AN by HPLC and 98% yield.

**Preparation of Aldehyde 6.** The medicinal chemistry synthesis of aldehyde 6 used 1.5 equiv of DMP in DCM at −78 to 23 °C, and the crude product was purified by flash chromatography. An attempt to apply the (bpy)CuI/TEMPO-catalyzed aerobic oxidation failed to provide conversion of substrate 5. Hydrogen peroxide with AlCl₃ or KBr/TEMPO, and the reaction time to 15 min, which allowed us to get aldehyde 6 in 66% yield (Table 2, entry 7).

To avoid the chromatographic column purification, several methods for the isolation of aldehyde 6 were investigated. An attempt to use a silica pad to purify the crude product failed to provide 6 with an acceptable purity (52% AN by HPLC %). On the other hand, the bisulfite adduct protocol provided 6 with 99% AN by HPLC. The crude material was reacted with NaHSO₃, and the bisulfite adduct 15 was separated by filtration (Table 2). The aldehyde 6 was regenerated in good purity when treating 15 with aqueous Na₂CO₃ followed by extraction with EtOAc. When the bisulfite adduct purification method was applied to a 73 mmol scale preparation of 6, a decrease in yield (59%) was observed (Table 2, entry 8). Examination of the mother liquid indicated that it was caused by a problem with the formation of adduct 15 rather than with the catalytic oxidation. Extending the reaction time of the crude aldehyde with NaHSO₃ from 2 to 16 h allowed the complete conversion of aldehyde 6 to the bisulfite adduct 15. The bisulfite derivative was then converted back to the aldehyde, providing 6 in 71% yield from 5 with 99.2% AN by HPLC (Table 2, entry 9).

**Birum–Oleksyszyn Reaction.** The one-pot three-component reaction between the aldehyde 6, the phosphite 3, and the benzyl carbamate (7) is a key step for the synthetic process of catalytic amount of TEMPO, KBr and nBuBr provided a 69% conversion of substrate 5 (Table 2, entry 1). Gratifyingly, raising the equivalents of sodium hypochlorite to 1.8, we obtained a complete conversion of alcohol 5, after 15 min (Table 2, entry 2). Despite the complete consumption of 5, a poor isolated yield (31%) was obtained (Table 2, entry 3). Carboxylic acid 14 was noted as one of the main impurities of the desired compound 6. As reported by Lucio Anelli et al., the presence of nBu₄NBr catalyzes the oxidation of the aldehyde to carboxylic acid 14. Removal of the quaternary salt allowed us to increase the yield of aldehyde 6 to 59% (Table 2, entry 4). The yield was further increased to 71% when the amount of TEMPO was reduced from 0.05 to 0.01 equiv, and the reaction time was reduced from 60 to 30 min (Table 2, entry 5). However, upscaling the reaction to 37 mmol resulted in a decrease in the yield to 60% (Table 2, entry 6). This was fixed by further reducing the amount of NaClO at 1.6 equiv and the reaction time to 15 min, which allowed us to get aldehyde 6 in 66% yield (Table 2, entry 7).

**Table 1. Optimization of the Purification Process of Alcohol 5**

| entry | solvent | scale (mmol) | additive | method of purification | yield (%) | purity (%) |
|-------|---------|--------------|----------|-------------------------|-----------|-----------|
| 1     | dioxane | 37.00        | TEA      | flash chromatography    | 97        | 99        |
| 2     | EtOAc   | 37.00        |          | flash chromatography    | 99        | 99        |
| 3     | EtOAc   | 37.00        |          | pad of silica (SiO₂/5 = 15:1 w/w) | 95 | 99 |
| 4     | EtOAc   | 73.00        |          | pad of silica (SiO₂/5 = 8:3:1 w/w) | 99 | 99 |
| 5     | EtOAc   | 73.00        |          | crystallization (MeCN/MTBE 1:1 v/v) | 98 | 99 |

| entry | alcohol (mmol) | equiv NaClO | equiv KBr | equiv nBu₄NBr | equiv TEMPO | time (min) | bisulfite extraction | conversion (%) | yield (%) |
|-------|----------------|-------------|-----------|---------------|-------------|------------|----------------------|----------------|----------|
| 1     | 0.42           | 1.5         | 0.1       | 0.05          | 0.05        | 120        | 69                   | 69             | ND       |
| 2     | 0.42           | 1.8         | 0.1       | 0.05          | 0.05        | 15         | 100                  | 100            | ND       |
| 3     | 4.22           | 1.0         | 0.1       | 0.05          | 0.05        | 15         | 2 h r.t.             | 100            | 31       |
| 4     | 4.22           | 1.0         | 0.1       | 0.05          | 0.05        | 60         | 2 h r.t.             | 100            | 59       |
| 5     | 4.22           | 1.0         | 0.1       | 0.05          | 0.05        | 30         | 2 h r.t.             | 100            | 71       |
| 6     | 37.00          | 1.7         | 0.1       | 0.01          | 0.1         | 30         | 2 h r.t.             | 100            | 60       |
| 7     | 37.00          | 1.7         | 0.1       | 0.1           | 15          | 15         | 2 h r.t.             | 100            | 66       |
| 8     | 73.00          | 1.7         | 0.1       | 0.1           | 15          | 15         | 2 h r.t.             | 100            | 59       |
| 9     | 73.00          | 1.7         | 0.1       | 0.1           | 15          | 16         | 16 h r.t.            | 100            | 71       |

*aNaClO concentration (11–15%). bStirred for 1 h at 0 °C before filtration. cIsolated yield.
compound 1. Unfortunately, this reaction step suffered from a poor yield (11%) and a low selectivity toward the product even on a 1 g scale. Moreover, the impurities in the crude material made purification challenging. In a separate study, we investigated the role of the catalyst and found Y(OTf)$_3$ as the most efficient in providing $\alpha$-aminophosphonate 8 in an improved 42% yield (Table 3, entry 1).

With a good catalyst in hand, our attention moved to solvent selection. While running the reaction in acetonitrile, we noted the formation of a precipitate, identified as aminal 16. We then started to investigate a new medium for the $\alpha$-aminophosphonate 8 preparation. Screening of seven anhydrous solvents and one solvent combination (see the Supporting Information) revealed the mixture THF/MeCN (1:1 v/v) as the most appropriate to improve the yield (44%) of aminophosphonate 8 (Table 3, entry 2). The higher yield, obtained with the mixture of THF/MeCN (1:1 v/v), is likely due to the capability of THF to solubilize aminal 16 and, therefore, increase the reaction rate. In addition, the yield was slightly raised to 45% when the Birum−Oleksyszyn reaction was run in THF/MeCN (1:1 v/v) with a concentration of 0.17 M (Table 3, entry 3).

A range of anhydrides was then screened as additives as these are known in literature to promote the reaction between intermediate aminals and alkylphosphonous acids. Equimolar amounts of both of acetic and trifluoroacetic anhydride were able to increase the yields of $\alpha$-aminophosphonate 8 to 50 and 52% (Table 3, entries 4 and 5, respectively).

Among the range of side products, anilines resulting from Boc cleavage of the group were also observed during the reaction. We hypothesized that anilines get oxidized to form colored impurities, in which separation proved to be challenging. Based on these considerations, we decided to investigate the use of different aldehydes as intermediates. In the first case, the amino group in 4 was protected with a Fmoc group, and then, the alcohol 18 was converted to aldehyde 19 by oxidation with DMP. In the second case, 4-nitrophenethyl alcohol (21) was oxidized to the corresponding aldehyde 22 with DMP (Scheme 3). Unfortunately, Fmoc-protected aldehyde 19 failed to provide a good yield and a good purity profile to give aminophosphonate 20 in the Birum−Oleksyszyn reaction. 2-(4-Nitrophenyl)acetaldehyde (22) rapidly decomposed when in contact with air, a Lewis acid, or a base like Na$_2$CO$_3$, rendering it inappropriate for the synthesis of aminophosphonate 23.

The unsuccessful performance of aldehydes 19 and 22 prompted us to focus on the purification of aminophosphonate

### Table 3. Screening of Birum−Oleksyszyn Conditions

| entry$^a$ | solvent | additive | time (h) | conc (M) | yield of 8 (%)$^b$ |
|-----------|---------|----------|----------|----------|------------------|
| 1         | MeCN    |          | 4        | 0.07     | 42$^c$          |
| 2         | MeCN/THF 1:1 |        | 4        | 0.07     | 44              |
| 3         | MeCN/THF 1:1 |        | 4        | 0.17     | 45              |
| 4         | MeCN/THF 1:1 | 1.0 equiv Ac$_2$O | 4    | 0.17     | 50              |
| 5         | MeCN/THF 1:1 | 1.0 equiv TFAA | 4       | 0.17     | 52              |

$^a$All reactions were carried out with 4.3 mmol of aldehyde 6. $^b$Isolated yield after flash chromatography. $^c$From ref 10.

### Scheme 3. Alternative Aldehydes and Their Performance in Birum−Oleksyszyn Reaction

\[\text{Scheme 3. Alternative Aldehydes and Their Performance in Birum−Oleksyszyn Reaction}\]
8 derived from Boc-protected substrate 6. At the end of the reaction, the HPLC chromatogram of the reaction mixture showed paracetamol (2), dialkylphosphonate 13, monoaryl phosphonate 17, and aminal 16 as major impurities. The acidic impurities such as paracetamol (2), monoaryl phosphonate 17, and dialkylphosphonate 13 were almost completely removed after washing the organic phase with an 0.5 M aqueous NaOH. Most of the aminal 19 and other lipophilic impurities were separated from product 8 with silica pad filtration. These procedures provided the α-amino-phosphonate 8 with an HPLC purity of 64.2%. After the basic wash, an 8.3% relative area percentage of paracetamol α-phosphonate lipophilic impurities were separated from product 8. Most of the aminal completely removed after washing the organic phase with an acidic impurities such as paracetamol (2) showed paracetamol (Scheme 4). The use of 4 N HCl in acid at room temperature. HCl was investigated as a more protecting group was carried out with TFA in DCM (1:1 v/v) medicinal chemistry procedure, the removal of the Boc-α-aminophosphonate 8 was collected with an HPLC purity of 74.1%. THF was also able to dissolve the crude compound 8; however, once the solution was added to aqueous NaHCO₃ an oil was formed. Under the same conditions, acetone, as a solvent for crude compound 8, provided the precipitate as very fine particles that clogged the filter. Changing the mode of addition (i.e., adding the bicarbonate solution to the acetone solution) made compound 8 a better filterable solid with an HPLC purity of 82.3%

Next, we focused on the removal of the yellow color. charcoal was first tested as a standard treatment for the removal of colored impurities. A total of 11 different charcoal batches were tested (see the Supporting Information), but none of them were able to remove the yellow color from the crude material. Shurry conditions were also screened as a purification method. The crude material was stirred in EtOAc for 16 h at r.t., and then, the solid was filtered obtaining an off-white product with 92% HPLC purity. We were pleased to find that the target purity (98%) was reached after using a solution of EtOAc/aceton (19:1 v/v) instead of pure EtOAc. After 16 h of stirring, compound 8 was isolated from the mother liquor with an HPLC purity of 98%. The reaction and the purification protocols were then tested with 10.00 g (43 mmol) of aldehyde 6 as a starting material, providing the α-amino-phosphonate 8 in 44% yield with a 98.2% AN by HPLC.

Boc-Cleavage for the Preparation of Aniline 24. In the medicinal chemistry procedure, the removal of the Boc-protecting group was carried out with TFA in DCM (1:1 v/v) at room temperature. HCl was investigated as a more economical alternative to TFA and also leading to less hygroscopic HCl salt (Scheme 4). The use of 4 N HCl in dioxane enabled the complete cleavage of the Boc group within 3 h despite the fact that the starting material 8 is poorly soluble in dioxane.

Crude aniline salt 24 was dissolved in 96% EtOH and was added dropwise to EtOAc while stirring at r.t. Unfortunately, the product formed clots that stuck to the walls of the flask. Using absolute ethanol instead of 96% ethanol prevented the formation of clots, and the solid was obtained as off-white flakes with an HPLC purity of 96.9%. The conditions of Boc-cleavage and work-up were applied for the upscale. Aniline 24 was obtained with yields of 99 and 97.9% AN by HPLC, when using 10.00 g (14 mmol) of α-aminophosphonate 8 as a starting material.

Preparation of Product 1. In the medicinal chemistry route, the final product 1 was prepared from aniline TFA salt 9 by inserting the guanyl group using N,N′-di-Boc-1H-pyrazole-1-carboxamide (10). This was followed by removal of the Boc groups with TFA in DCM and salt exchange with DOWEX 1X8 Cl resin to convert intermediate 12 to product 1.

We investigated a direct way to convert aniline HCl salt 24 to product 1, reducing the step count, increasing the overall yield, and cutting the cost. A range of literature methods is available for the direct transformation of aniline to aryl guanidine. From these, guanylation with cyanamide was selected to develop the protocol with the best atom economy and costs. However, heating aniline HCl salt 24 with cyanamide in a protic solvent in the presence of a Bronsted or a Lewis acid led to a decomposition of the α-amino-phosphonate. Therefore, we investigated the guanylation of aniline salt 24 with 1.2 equiv of cyanamide in the presence of 0.1 equiv of Sc(OTf)₃ in a panel of solvents and solvent mixtures at room temperature (see the Supporting Information).

These studies revealed MeCN/iPrOH (1:1 v/v) as the most optimal reaction media to give 38% conversion of aniline salt 24 in 72 h (Table 4, entry 1). Then, we moved our focus to the reaction’s catalyst. Lewis acids like Bi(OTf)₃ and Y(OTf)₃ (Table 4, entries 2 and 3) and Bronsted acids like HCl, HNO₃ and AcOH (Table 4, entries 4–6) failed to provide improved conversion compared to Sc(OTf)₃.

Before further optimization efforts, an isolation/purification method for product 1 was developed. The reaction mixture was first concentrated, and the residue was dissolved in abs-EtOH. Then, HCl 2.5 N in EtOH (HCl 2.5 N/1 = 1.2 v/v) was added to form a guanidine HCl salt, and the ethanol solution was dropped to an antisolvent (see the Supporting Information). iPrOAc was found to be the antisolvent of choice providing a solid that was easily filtrated.

To increase the conversion, three variables were investigated: concentration, reaction time, and equivalents of cyanamide. A design of experiment (DoE) approach was selected to explore all the three variables at the same time and eventually identify any interaction between them. At first, we set the limits of the three variables: 0.1–2.0 M for the concentration, 1.2–10.0 for the cyanamide equivalents, and 48–96 h for the reaction time. The DoE was performed with the support of the artificial intelligence web-based software xT SAAM. The program uses stochastic optimization techniques to produce suggestions for the next experiments until an objective is satisfied. The objective was to maximize the purity and the yield of the final product as well as to create models for predicting purity and yield. Within this study, four consecutive iterations of parallel experiments were carried out, with a total of 22 experiments (see the Supporting Information). The results on purity and yield were collected, and the xT SAAM
software uses an automated mechanism to produce a cross-validated ensemble modeling to create the final model. Ensemble modeling is a type of modeling that combines the results of multiple individual models to produce a more accurate final model. A multitude of non-linear features is produced from the input parameters and iteratively tested within the ensemble model using cross-validation; only parts of randomly selected data points are used at a time for training and fitting the model. Then, the average test-data R² score is reported and the appropriate final model is selected. In our case, we used ensemble modeling to generate the response surface model (RSM). From the RSM (Figure 2), it was observed that the best yield and purity could be obtained when the concentration was 0.5 M with NH₂CN equivalents and time maximized.

With a concentration of starting material 24 0.5 M, 10.0 equiv of cyanamide, and 96 h of reaction time, the conversion was improved to 95%, and the final product 1 was obtained on a small scale (0.08 mmol) with 86% yield and 89% HPLC purity (Table 5, entry 1). Upscaling the reaction to a 0.77 mmol scale, we noticed a drop in conversion and therefore in yield and purity. An increase in the equivalents of cyanamide to 15.0 was necessary to maintain 95% conversion of starting material 24 and a purity of final product 1 around 87−88% (Table 5, entries 2 and 3). Further optimization of the reaction conditions identified that the mixture of THF/EtOH (2:1 v/v) was also able to provide a 95% conversion when using 10 equiv of cyanamide (see the Supporting Information). Gratifyingly, when the reaction in THF/EtOH (2:1 v/v) was upscaled from 0.77 to 7.7 mmol, the conversion of aniline 24 to guanidine 1 was kept above 95% without needing to increase the equivalents of cyanamide (Table 5, entries 4 and 5).

On the 7.7 mmol scale, a direct guanylation of aniline 24 in THF/EtOH (2:1 v/v) with 10 equiv of NH₂CN provided 1 in

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**Table 4. Catalyst Optimization for the Direct Guanylation of 24**

| entry | catalyst | equiv catalyst | conversion (%) |
|-------|----------|----------------|----------------|
| 1     | Sc(OTf)₃ | 0.1            | 38             |
| 2     | Bi(OTf)₃ | 0.1            | 34             |
| 3     | Y(OTf)₃  | 0.1            | 25             |
| 4     | HCl      | 1.0            | 8              |
| 5     | HNO₃     | 1.0            | 40             |
| 6     | AcOH     | 1.0            | 11             |

Aniline 28 (0.08 mmol), 1.2 equiv of NH₂CN, 1.0 M, 72 h, MeCN/iPrOH 1:1 v/v.

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**Table 5. Medium Optimization for the Direct Guanylation of 24**

| entry | scale (mmol) | solvent     | equiv NH₂CN | yield (%) | purity (%) |
|-------|--------------|-------------|-------------|-----------|------------|
| 1     | 0.08         | MeCN/iPrOH  | 10          | 86        | 89         |
| 2     | 0.77         | MeCN/iPrOH  | 10          | 77        | 85         |
| 3     | 0.77         | MeCN/iPrOH  | 15          | 83        | 88         |
| 4     | 0.77         | THF/EtOH 2:1| 10          | 89        | 91         |
| 5     | 7.7          | THF/EtOH 2:1| 10          | 90        | 91         |

Sc(OTf)₃ (0.1 equiv) as the catalyst, 96 h, 0.5 M. †Isolated yield.

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**Figure 2.** Predicted yield RSM for the cyanamide guanylation of 1 in iPrOH/MeCN 1:1: (A) when cyanamide equivalents are fixed at 10 and (B) when time is fixed at 96 h. Yellow regions indicate the maximum predicted yield.
90.0% yield with 91.0% AN by HPLC. Among the impurities in the final material, we noticed a small presence of monoarylguanidine 25 amounting to 0.4–1.1% RAP by HPLC.

**Purification of the Final Compound 1.** The first attempt was to crystallize the crude product 1; however, none of the 17 solvents screened were able to yield a pure 1 (see the Supporting Information).

With these results in hand, we focused on different methods of purification. In the work-up of 1, we noted that the antisolvent precipitation in iPrOAc was able to remove part of the impurities generated in the guanylation reaction. We decided to test precipitation with a series of antisolvents to see if it was possible to increase the purity. Crude 1 was dissolved in absolute ethanol, and the solution was added to eight different antisolvents (see the Supporting Information).

Among five organic solvents and two aqueous solutions, only iPrOAc and EtOAc were able to slightly increase the HPLC purity by 0.9 and 1.5%, respectively, but not in a sufficient way to reach the 98% purity target.

Last, we investigated the reverse phase chromatography (RP) for the purification of final product 1. After a range of eluents screening, a gradient of premixed MeCN/EtOH (9:1 v/v) water was selected. Compound 1 was successfully isolated with a C-18 RP column. The purification was tested on a 3.75 g scale obtaining 1 in two fractions, S1 with 98.1% AN by HPLC and S2 with 99.4% AN by HPLC. The pure material was recovered with 79% yield from the crude product, with a total yield of α-aminophosphonate 1 from aniline 24 of 72%.

### EXPERIMENTAL SECTION

**General.** Unless otherwise specified, all commercially available reagents were used as received. ^1^H-, ^13^C-, and ^31^P-NMR spectra were obtained on a 400 MHz Bruker Avance 400 spectrometer at ambient temperatures at 400, 101, and 162 MHz, respectively. Chemical shifts (δ) are reported in parts per million (ppm) relative to a residual DMSO peak (s, δ 2.50 for ^1^H and t, δ 39.53 for ^13^C); for ^31^P-NMR, it was calibrated with the use of an external standard (H_3PO_4). Multiplicities are given as s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Complex splittings are described by a combination of these abbreviations, i.e., dd (doublet of doublets). Reaction conversion was estimated by LC–MS on a Waters Acquity UPLC H-class instrument, column Waters Acquity UPLC BEH-C18, 2.1 × 50 mm, 1.7 μm, eluent 5–95% MeCN in 0.1% aq. HCOOH; flow rate: 0.8 mL/min; detection Waters PDA Detector (200–300 nm). HPLC was recorded on a Waters Alliance instrument equipped with a 2695 separations module, consisting of a quaternary pump, degasser, autosampler, and column heater, and a Waters 2489 dual wavelength absorbance detector was used for detection of analytes or Shimadzu Prominance-I LC-2030C, column prevail organic acid or Apollo C18-13, 4.6 × 150 mm, eluent 25–95% or 40–95% MeCN in 0.1% aq. H_3PO_4; flow rate: 1.0 mL/min, temperature = 40 °C, detector at 254 nm. HRMS spectra were acquired on an electrospray ionization mass spectrometer with a TOF analyzer using the following parameters: positive ionization mode, drying gas (10 mL/min), 325 °C, and fragment or ionization (100 V).

### CONCLUSIONS

In summary, an optimized process for the scalable preparation of the α-aminophosphonate UAMC-00050 has been developed (Scheme 5). The Anelli–Montanari protocol using TEMPO as the oxidation catalyst for the synthesis of aldehyde 6 proved to be superior to the DMP oxidation. The yield was increased from 65 to 71%, and the atom economy was improved from 33 to 66%. The key step of the route, the synthesis of α-aminophosphonate 8 by a three-component reaction between aldehyde 6, carbamate 7, and phosphite 3, was optimized. The use of Y(OTf)_3 as the catalyst, TFAA as the additive, and THF/MeCN (1:1 v/v) as the reaction medium provided the product 8 in increased yield. For the preparation of product 1, N,N′-di-Boc-1H-pyrazole-1-carboxamidine was substituted with considerably less expensive cyanamide to introduce a guanidine moiety. Smart DoE was used to optimize the conditions for the guanylation step. The use of chlorinated solvents and purification of intermediates by flash chromatography were removed from the process. The only chromatographic purification was done for the final product to reach the target purity >98%. The new process improved the overall yield of compound 1 from 3 to 22% with a total of six steps. The improved route was executed on a multigram scale and is suitable for preclinical batch preparation of UAMC-00050.

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**Scheme 5. Optimized Synthetic Route to UAMC-00050**

![Scheme 5](https://doi.org/10.1021/acs.oprd.2c00244)
To a 500 mL flask equipped with a magnetic stirrer were added, under argon, paracetamol (2) (10.0 g, 0.132 mol, 3.0 equiv) (water content <0.030%), previously dried in vacuum for 24 h, dry-THF (100 mL) (water content <0.005%), and dry-triethylamine (9.20 mL, 0.132 mol, 3.0 equiv) (water content <0.04%). The mixture was stirred for 1 h at 0 °C and then filtered under an argon flow to remove the solid byproduct formed during the reaction. The filtrate cake was washed with dry-THF (50 mL), the liquid was poured into a 500 mL flask containing the crude material was added 26 mL of THF (100 mL) (water content <0.005%), and dry-triethylamine (9.20 mL, 0.132 mol, 3.0 equiv) (water content <0.04%), the flask was placed in an ice bath, and after 10 min, phosphorus trichloride (1.92 mL, 0.044 mol, 1.0 equiv) was added. The solid mixture was placed on top of the filter and washed with dry-THF (50 mL), the liquid was poured into a 500 mL flask equipped with a magnetic stirrer. Sodium bisulfite (5.00 g, 0.073 mol, 1.0 equiv) dissolved in deionized water (260 mL), sodium carbonate (17.20 g, 0.162 mol, 2.2 equiv), and ethyl acetate (300 mL), and the mixture was stirred for 3 h at 20–25 °C. Then, the mixture was placed in a 1.0 L separation funnel and extracted with ethyl acetate (3 × 250 mL), the combined organic layers were washed with brine (400 mL) and dried on Na2SO4 (250 g), and the solvent was removed to get a pale yellow solid with a yield of 89%. 99.0% AN by HPLC. HRMS (ESI+) m/z calculated for C21H36N2O4Na [M + Na]+: 362.2156, found 362.2158. 1H-NMR (400 MHz, DMSO-d6) δ: 9.79 (s, 1H), 7.09 (d, J = 8 Hz, 6H), 2.03 (t, J = 16 Hz, 1H). 13C-NMR (101 MHz, DMSO-d6) δ: 153.34, 134.15, 130.32, 127.68, 117.93, 82.43, 79.59, 34.45, 23.89.

tert-Butyl (4-(2-Amino)-phenethyl)carbamate (5). To a 500 mL flask equipped with a magnetic stirrer were added, under argon, compound 5 (17.30 g, 0.073 mol, 1.0 equiv) dissolved in ethyl acetate (400 mL), and dried in vacuum overnight to get a white solid with a yield of 98%. 99.6% AN by HPLC. HRMS (ESI+) m/z calculated for C24H34N2O5 [M + H]+: 388.5013, found 388.5016. 1H-NMR (400 MHz, DMSO-d6) δ: 9.99 (s, 3H), 7.58 (d, J = 8 Hz, 6H), 2.03 (t, J = 16 Hz, 1H). 13C-NMR (101 MHz, DMSO-d6) δ: 150.75, 135.29, 135.87, 133.49, 129.40, 118.59, 79.24, 62.83, 40.88, 28.61.

Sodium 2-(4-(tert-Butoxycarbonyl)amino)-1-hydroxyethane-1-sulfonate (15). To a 500 mL flask equipped with a magnetic stirrer were added in this sequence: compound 5 (17.30 g, 0.073 mol, 1.0 equiv) dissolved in ethyl acetate (90 mL), TEMPO (114 mg, 0.73 mmol, 0.01 equiv) dissolved in toluene (90 mL), and then potassium bromide (869 mg, 7.33 mmol, 0.1 equiv) dissolved in NaHCO3 sat. (67 mL). The mixture was vigorously stirred for 10 min in an ice bath, and then, sodium hypochlorite 11–15% (67 mL) was added dropwise in 5 min. The reaction was vigorously stirred for 10 min and then was quenched with sodium thiosulfate 10% (250 mL), the reaction mixture was washed with ethyl acetate (3 × 200 mL), the combined organic layers were then washed with brine (300 mL) and dried with Na2SO4 (250 g), and the solvent was removed by rotary evaporation. The crude aldehyde was then dissolved in ethanol 96% (340 mL) in a 500 mL flask equipped with a magnetic stirrer. Sodium bisulfite (11.71 g, 0.113 mol, 1.5 equiv) dissolved in 20 mL of deionized water was added dropwise in 5 min, and the mixture was stirred for 18 h at 20–25 °C and 1 h at 0 °C. The solid was filtered, washed with cold ethanol 96% (300 mL), and dried in a vacuum (5 mbar) for 16 h to give a white solid with a yield of 80%. 97.8% AN by HPLC. HRMS (ESI−) m/z calculated for C14H18N2O7PNa [M − Na]−: 328.0687, found 328.0688. 1H-NMR (400 MHz, D2O) δ: 4.61 (dd, J = 11, 3 Hz, 1H), 4.32 (dd, J = 16, 4 Hz, 1H), 2.88 (dd, J = 12, 12 Hz, 1H). 13C-NMR (100 MHz, D2O) δ: 153.34, 134.15, 130.32, 127.68, 117.93, 82.43, 79.59, 34.45, 23.89.
Preparation of aldehydes, supplementary screening, DoE experimental data, HPLC chromatograms, and $^1$H-, $^13$C-, and $^{31}$P-NMR spectra (PDF)

## AUTHOR INFORMATION

### Corresponding Author

Davide Ceradini — Latvian Institute of Organic Synthesis, Riga LV-1006, Latvia; orcid.org/0000-0001-9198-6714; Email: davide.ceradini@osi.lv

### Authors

Pavel Cacikins — Exponential Technologies Ltd., Riga LV-1006, Latvia

Alba Ramos-Llorca — University of Antwerp, Wilrijk 2610, Belgium; orcid.org/0000-0001-9523-2928

Kirill Shubin — Latvian Institute of Organic Synthesis, Riga LV-1006, Latvia

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.oprd.2c00244

### Author Contributions

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### Notes

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### ABBREVIATIONS

Boc, tert-butyloxycarbonyl protecting group; DED, dry eye disease; UA, University of Antwerp; DMP, Dess–Martin periodinane; Fmoc, fluorenylmethoxycarbonyl; RSM, response surface model; TEMPO, (2,2,6,6-tetramethylpiperidin-1-yl)-peroxid; UA, University of Antwerp; DMP, Dess–Martin periodinane; Fmoc, fluorenylmethoxycarbonyl; RSM, response surface model; TEMPO, (2,2,6,6-tetramethylpiperidin-1-yl)-peroxid; TFAA, trifluoroacetic anhydride; TFA, trifluoroacetic acid; uPA, urokinase plasminogen activator

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## ASSOCIATED CONTENT

### Supporting Information

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