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Proceeding Paper

Development and Optimization of the Multi-Gram Synthesis of the Antiviral 18-(Phthalimide-2-yl)ferruginol †

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Abstract: Virus-induced diseases are very common in our society, and we continuously need new treatments for these challenging infections. We discovered by serendipity some years ago that the molecule 18-(Phthalimide-2-yl)ferruginol, an analogue of the natural diterpenoid (+)-ferruginol, a pharmacologically active molecule, was able to inhibit the spread of dengue virus type-2 (DENV-2) and human herpes virus 1 and 2 (HHV-1 and HHV-2). During the development and further study of the above-mentioned analogue, we required the scaling-up of the semisynthesis of the target molecule. The synthesis was already reported by Waldvogel and co-workers in 2007, starting from the commercially available ca. 60% (+)-dehydroabietylamine. In this communication, we describe the several issues that we faced and propose an optimized experimental procedure in order to obtain this broad-spectrum antiviral, which we found is even active against several strains of Zika virus.

Keywords: antiviral; semisynthesis; ferruginol; dehydroabietylamine6

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1. Introduction

Dengue disease is the most prevalent mosquito-borne infection around the world, however, at present, drugs are not available for its treatment. Dengue virus (DENV) is a main human pathogen. It infects as many as 400 million people every year, with ~100 million showing symptoms including fever, headache, rash, conjunctivitis, and pain in muscles and joints [1]. However, ~500,000 cases/year develop grave and possibly life-threatening dengue hemorrhagic fever (DHF) or dengue shock syndrome (DSS), with symptoms including bleeding, severe vomiting with blood, black stools, and drowsiness. Moreover, ~22,000 people (mostly children) die of DENV per year.

While in search of new antiviral alternatives to control dengue virus infection, the so-called host-targeted antivirals (HTAs) have become highly relevant and the research that includes them is flourishing because these compounds do not induce drug-resistant mutant selection and they may show broad-spectrum antiviral activity and would be complementary to direct-acting antivirals [2,3]. In this line of study, we have previously reported in 2016 that an analogue of the bioactive abietane diterpenoid (+)-ferruginol (**1**) (Figure 1) [4], the semi-synthetic compound 18-(Phthalimide-2-yl)ferruginol (**2**) (Figure 1), has relevant and selective anti-dengue activity in post-infective stages, showing a dramatic reduction in viral plaque size as well as some anti-herpetic activities [5].

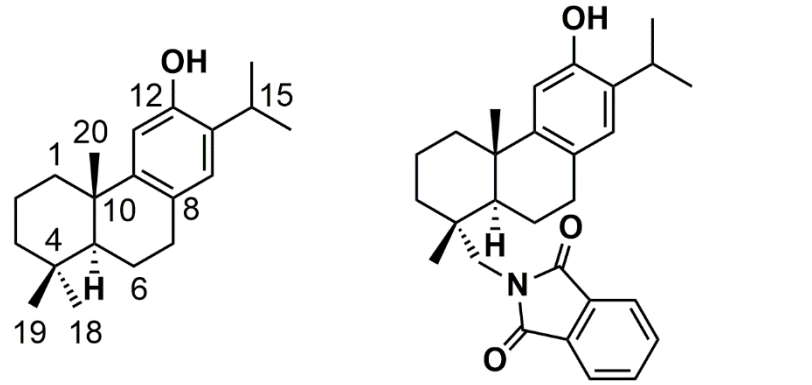
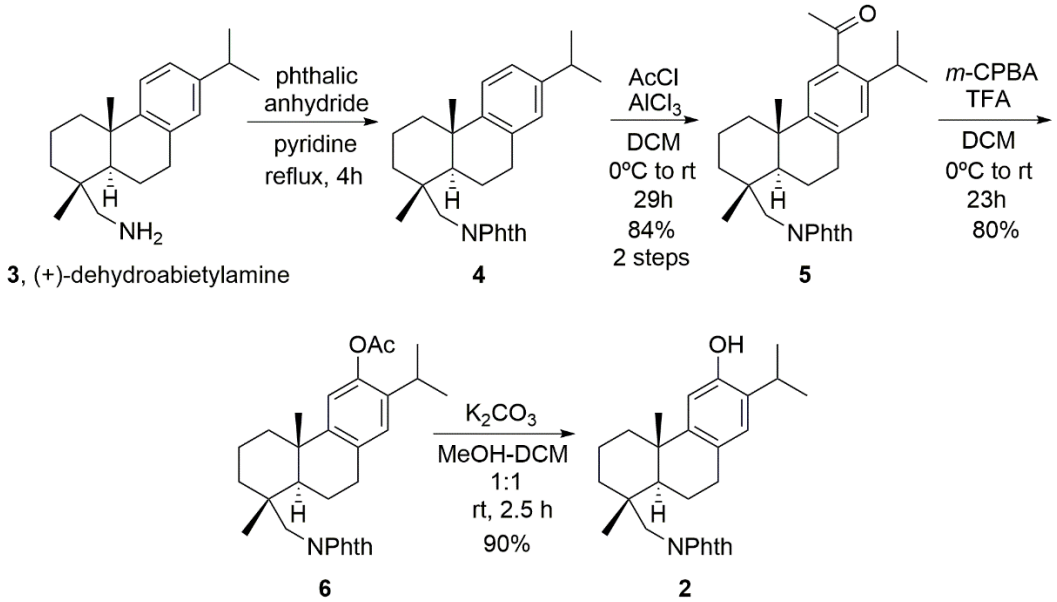


Figure 1. Antiviral abietane diterpenoids **1** and **2** and carbon skeleton numbering.

Antiviral compound **2** was synthesized after our work in 2012 on the synthesis of (+)-ferruginol (**1**) itself, starting from the commercially available (+)-dehydroabietylamine (**3**) [6]. We essentially followed the work of the group of Waldvogel and co-workers, who, in 2007, described the synthesis of compound **2** starting from ca. 65% (+)-dehydroabietylamine (**3**) in four synthetic steps in multi-gram scale [7]. However, during the development of further studies of molecule **2**, we found several problems in the reported sequence and, therefore, we have optimized this four-step sequence (Scheme 1). In this communication, we describe the several issues that we faced and propose an optimized experimental procedure in order to obtain this broad-spectrum antiviral.



Scheme 1. Optimized synthesis of antiviral **2** from (+)-dehydroabietylamine (**3**).

2. Materials and Methods

2.1. General Experimental Procedures

NMR spectra were recorded on a 300 MHz spectrometer (^{1}H: 300 MHz, ^{13}C: 75 MHz) or 400 MHz (^{1}H: 400 MHz, ^{13}C: 100 MHz) and referenced to the solvent peak at 7.26 ppm (^{1}H) and 77.00 ppm (^{13}C) for $CDCl_3$. All spectra were recorded in $CDCl_3$ as solvent. Reactions were monitored by TLC using Merck silica gel 60 F_{254} (0.25 mm thick) plates. Compounds on TLC plates were detected under UV light at 254 nm and visualized by immersion in a 10% sulfuric acid solution and heating with a heat gun. Purifications were performed

by flash chromatography on Merck silica gel (230–400 mesh). Commercial reagent grade solvents and chemicals were used as purchased unless otherwise noted. Combined organic extracts were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The starting material, (+)-dehydroabietylamine, was purchased from Aldrich (Saint Louis, MI, USA) ca. 60% purity and from TCI Europe (Zwijndrecht, Belgium) ca >90% purity. The carbon numbering of all synthetic compounds corresponds to that of natural products.

2.2. Chemistry

Materials. All compounds prepared in this work display spectroscopic data in agreement with the reported data [7]. Purity of the final compound was 95% or higher.

2.2.1. Synthesis of N-Phthaloyldehydroabietylamine (**4**)

Adapted from Malkowsky and co-workers [7]. (+)-Dehydroabietylamine **3** (ca. 60% aldrich, 20 g, ca. 42 mmol) was dissolved in pyridine (90 mL), and phthalic anhydride (24.88 g, 168 mmol, 4 equiv.) was added at rt. The reaction mixture was heated at reflux (in a heating block with hot plate at 135 °C) and stirred at 400 rpm for 4 h. After cooling at rt, the mixture was poured into a beaker with cold water (300 mL) and was extracted with diethyl ether (100 mL and 2 × 80 mL). The combined organic phases were washed with 10% HCl (2 × 80 mL), H_2O (2 × 50 mL), and brine (50 mL), and dried ($MgSO_4$) under stirring overnight. The next day, the extract was filtered and concentrated to give 31.5 g of pale yellow oil, which could not be induced to crystallize with absolute EtOH. Then, the crude was chromatographed on silica (ca. 200 g) eluting with *n*-hexane-EtOAc (9:1) to give 24.9 g of phthalimide **4** as a yellowish semisolid, which had ^{1}H NMR data in agreement with those reported [7] and showing some unidentified minor impurities, which did not affect the next step.

2.2.2. Synthesis of 12-Acetyl-N-Phthaloyldehydroabietylamine (**5**)

Adapted from Malkowsky and co-workers [7]. A solution of compound **4** (24.9 g, ca. 42 mmol) in DCM (300 mL) was cooled in an ice-bath and AcCl (10.45 mL, 11.53 g, 147 mmol, 3.5 equiv.) was added followed by $AlCl_3$ (16.8 g, 126 mmol, 3.0 equiv.). The reaction mixture turned from yellowish to dark brown and was stirred for 20 min. Then, the ice-bath was removed and the reaction was stirred for 29 h at rt. After this time, the resulting brownish-red solution was cooled in an ice-bath and quenched dropwise in a beaker with saturated aq. $NaHCO_3$ (100 mL) (gas evolution!). The mixture was poured onto saturated aq. $NaHCO_3$ (200 mL) in a 1 L separation funnel and the phases were separated. The aqueous phase was extracted with DCM (2 × 100 mL). The combined organic phases were washed with H_2O (100 mL) and brine (50 mL), and dried ($MgSO_4$) under stirring overnight. The next day, the extract was filtered and concentrated to give 29.2 g of yellowish brown semi-solid, which was crystallized with EtOH (90 mL) overnight. The resulting greenish solid was filtered off under vacuum and washed with cold EtOH (60 mL) and dried under vacuum to give 16.1 g of acetyl derivative **5** as a pale greenish solid (84%, two steps), which had ^{1}H NMR data in agreement with those reported [7]. From the mother liquor was recovered, after chromatography on silica eluting with *n*-hexane-EtOAc (8:2), an additional 4.8 g of product as a yellow solid.

2.2.3. Synthesis of 12-Acetoxy-N-Phthaloyldehydroabietylamine (**6**)

Adapted from Malkowsky and co-workers [7]. Compound **5** (20.7 g, 45.7 mmol) and *meta*-chloroperbenzoic acid (MCPBA, 27.3 g, 118.9 mmol, 2.6 equiv.) were dissolved in DCM (125 mL) and cooled in an ice-bath. Then, trifluoroacetic acid (3.5 mL, 5.2 g, 45.7 mmol, 1.0 equiv.) was added dropwise and the mixture was stirred for 20 min before being allowed to warm to rt, and stirring continued for 23 h. The next day, the reaction mixture was diluted with DCM (80 mL) and quenched with aqueous 10% Na_2SO_3 (120 mL). Phases were separated in a 1 L separation funnel and the aqueous phase was extracted with

DCM (60 mL). The combined organic phases were washed with H_2O (100 mL + 10 mL of brine), 50% saturated aq. $NaHCO_3$ (2 × 125 mL), and brine (100 mL), dried over $MgSO_4$ under stirring for 30 min, filtered, and concentrated to give a crude of 23 g as a pale oil. The crude was chromatographed on silica eluting with *n*-hexane-EtOAc (7:3) to give 16.9 g (80%) of acetate **6** as a yellow foam, which had ^{1}H NMR data in agreement with those reported [7].

2.2.4. Synthesis of 12-Hydroxy-N-Phthaloyldehydroabietylamine or 18-(Phthalimide-2-yl)ferruginol (**2**)

Compound **6** (10.8 g, 22.8 mmol) was dissolved in DCM (80 mL) and absolute MeOH (80 mL). Then, K_2CO_3 (15.8 g, 114.2 mmol, 5.0 equiv.) was added in portions under continuous stirring at rt and the heterogeneous yellow mixture became reddish-brown. After 2.5 h, monitored by TLC (eluted twice with *n*-hexane-EtOAc (8:2)), the mixture was filtered under vacuum in a sintered or Büchner funnel and the solid was washed with DCM (60 mL + 20 mL). Then, the filtrate was acidified with 10% HCl (ca. 10 mL) until a color change to yellow was observed. The solution was washed with brine (40 mL), which was re-extracted with an additional 20 mL of DCM. The combined organic phases were dried over $MgSO_4$ under stirring overnight. The next day, it was filtered and concentrated to give a crude of 9.6 g as a yellow solid. The crude was chromatographed on silica eluting with *n*-hexane-EtOAc (7:3) to give 8.94 g (90%) of phenol **2** as a yellow foam, which had ^{1}H and ^{13}C NMR and specific optical rotation ($[\alpha]_D^{23}$—31.4 (c 0.7, DCM) data in agreement with those reported [7]. Anal. calcd. for $C_{28}H_{33}NO_3$: C, 77.9; H, 7.7; N, 3.2. Found: C, 77.6; H, 7.8; N, 3.1.

3. Results and Discussion

The current procedure in multi-gram scale for the synthesis of antiviral compound **2** involves four synthetic steps (Scheme 1) initially reported by Malkowsky et al. in 2007 [7]: (a) synthesis of intermediate phthalimide **4**; (b) synthesis of acetyl derivative **5** by Friedel–Crafts reaction; (c) synthesis of acetate derivative **6** by Baeyer–Villiger oxidation; (d) synthesis of phenol **2** by methanolysis. After this report, Siegel and co-workers reported in 2013 a shorter route with similar yields but in small scale [8] using phthaloyl peroxide for the direct hydroxylation of **4**, which we did not consider for scaling-up because of the use of expensive hexafluoro-2-propanol as a solvent and the need for synthesizing the peroxide, as well as the safety of working on a large scale with peroxides. In 2017, the group of Csuk and co-workers made some modifications to the original procedure but also worked in small scale [9]. For example, they introduced the use of DCM for the Friedel–Crafts reaction instead of 1,2-dichloroethane and increased the equivalents of reagents to reduce the reaction time. Moreover, they changed the conditions of the methanolysis using a higher proportion of water as solvent, making more hydrolytic conditions.

*3.1. Formation of Intermediate Phthalimide **4***

In the previously reported condensation step of amine **3** with phthalic anhydride (4 equiv.), pyridine is used as solvent [7]. We tried the use of glacial acetic acid, more benign than pyridine, as a solvent, using as starting material (+)-dehydroabietylamine **3** (>90%, TCI Europe) on a 2 g scale, and the resulting yield was lower (74%). For this reason, we maintained the original conditions for a higher yield, originally 96% starting from (+)-dehydroabietylamine **3** (30 g, ca. 65%) [7]. In our hands, starting with (+)-dehydroabietylamine **3** (20 g, ca. 60%, Aldrich) we could not purify the product obtained in a high yield by crystallization in EtOH as reported by Malkowsky et al. [7] but by column chromatography, leaving minor, fewer polar impurities, which did not affect the next step. If we use (+)-dehydroabietylamine **3** (12 g, >90%, TCI Europe) as the starting material, a yield of 86–89% is obtained after chromatography. Additionally, we also tried the purification of the starting material (+)-dehydroabietylamine **3** (ca. 60%, Aldrich) by crystallization of the corresponding acetate salt in toluene [10], but the process was time-

consuming and needed further chemicals to give 14.9 g of pure amine starting from 35 g of **3** (ca. 60%, Aldrich), though the crude product of condensation with purified amine **3** can be used directly in the next step.

*3.2. Friedel–Crafts Reaction (Intermediate **5**)*

In the previously reported Friedel–Crafts reaction of **4** with acetyl chloride and aluminum trichloride (AcCl: 3.5 equiv.; $AlCl_3$: 3 equiv.), 1,2-dichloroethane is used as a solvent giving an 88% yield [7]. We maintained essentially the same conditions for the reaction, changing the solvent by DCM, and the work-up was performed differently by quenching with saturated aqueous $NaHCO_3$ instead of 6N HCl and the extraction was conducted with DCM instead of diethyl ether. The reaction (AcCl: 3.5 equiv.; $AlCl_3$: 3 equiv.) during 1 day of **4** containing minor impurities from the previous step of condensation with (+)-dehydroabietylamine **3** (ca. 60%, Aldrich) gave acetyl derivative **5** (84%, two steps) after crystallization in EtOH. The reaction (AcCl: 5.5 equiv.; $AlCl_3$: 6.5 equiv.) of **4** (15 g) obtained from (+)-dehydroabietylamine **3** (>90%, TCI Europe) [9] finished in only two hours, giving a crude (15.7 g) pure enough for the next step, but obviously consuming double the reagents.

*3.3. Baeyer–Villiger Reaction (Intermediate **6**)*

In the previously reported Baeyer–Villiger reaction of **5** with *meta*-chloroperbenzoic acid and trifluoroacetic acid (MCPBA: 2.6 equiv.; TFA: 1 equiv.), DCM is used as a solvent, giving an 85% yield [7]. We maintained essentially the same conditions for the reaction, changing the work-up by adding further washing with 50% saturated aqueous $NaHCO_3$ as we found that the by-product *meta*-chlorobenzoic acid remains in the crude even after chromatography, and also avoiding potential basic hydrolysis of the acetate group. The reaction of **5** obtained from (+)-dehydroabietylamine **3** (ca. 60%, Aldrich) gave acetate **6** an 80% yield, while the reaction of **5** prepared from (+)-dehydroabietylamine **3** (>90%, TCI Europe) afforded acetate **6** an 83% yield, two steps, after chromatography with *n*-hexane-EtOAc (7:3) instead of original eluent mixture cyclohexane-EtOAc (9:1).

*3.4. Methanolysis (Compound **2**)*

In the previously reported acetate cleavage reaction of **6** with $NaHCO_3$ (4.3 equiv.) as a base, MeOH is used as solvent with a drop of water to give a 92% yield [7]. In our hands, this reaction was tricky, resulting mostly in recovering unreacted acetate even when adding more water to the reaction media than Csuk and co-workers did and extending the reaction time [9]. The starting material is difficult to dissolve in MeOH so we chose a solvent mixture with DCM, 1:1, and to force the reaction to proceed, we selected a stronger base as K_2CO_3 (5 equiv.). The work-up was also modified by quenching with 10% HCl instead of 0.12 M H_2SO_4 and extracting with DCM instead of methyl *tert*-butyl ether. Under these new conditions, the reaction was completed in about 3 h, giving a 90–93% yield of pure phenol after chromatography with *n*-hexane-EtOAc (7:3) instead of the original eluent mixture cyclohexane-EtOAc (90:10 to 85:15).

This molecule proved to have an antiviral effect in DENV-2 and herpes virus [5], as well as in Zika virus [11].

4. Conclusions

In summary, an optimized synthetic route in multi-gram scale for the preparation of antiviral ferruginol analogue **2** (60% overall yield, four steps), is described. We can conclude that by starting from commercially available (+)-dehydroabietylamine **3** (ca. 60%, Aldrich, 100 g, ca. 100 euro), the sequence can be performed successfully in a cheaper manner than using the more expensive (+)-dehydroabietylamine **3** (>90%, TCI Europe, 100 g, ca. 700 euro). The sequence has been optimized to use only three reaction solvents, pyridine, DCM, and MeOH, and work-up and purification methods, and even the reaction

conditions have been modified to overcome some problems found in the original procedure by Malkowsky et al. [7].

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Conflicts of Interest: The authors declare no conflict of interest.

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