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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 continuously, we 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 studies of the above-mentioned analogue, we required scaling-up 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 that 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 there are not drugs 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 muscle 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. ~22,000 people (mostly children) die of DENV per year.

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 among others because these compounds do not induce drug-resistant mutant selection, they may show a 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].

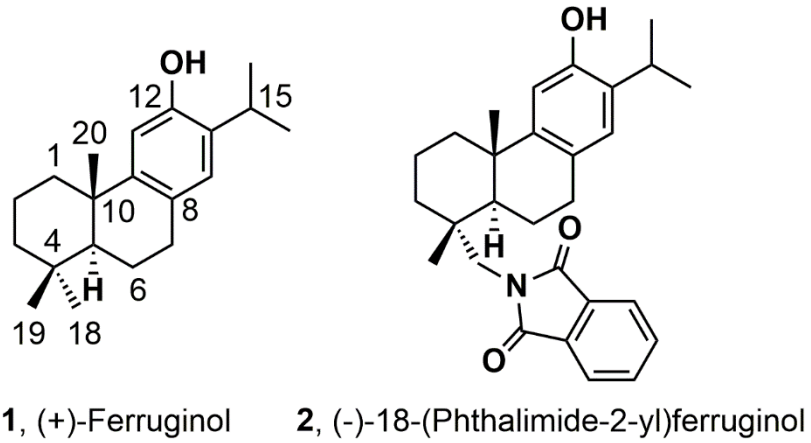
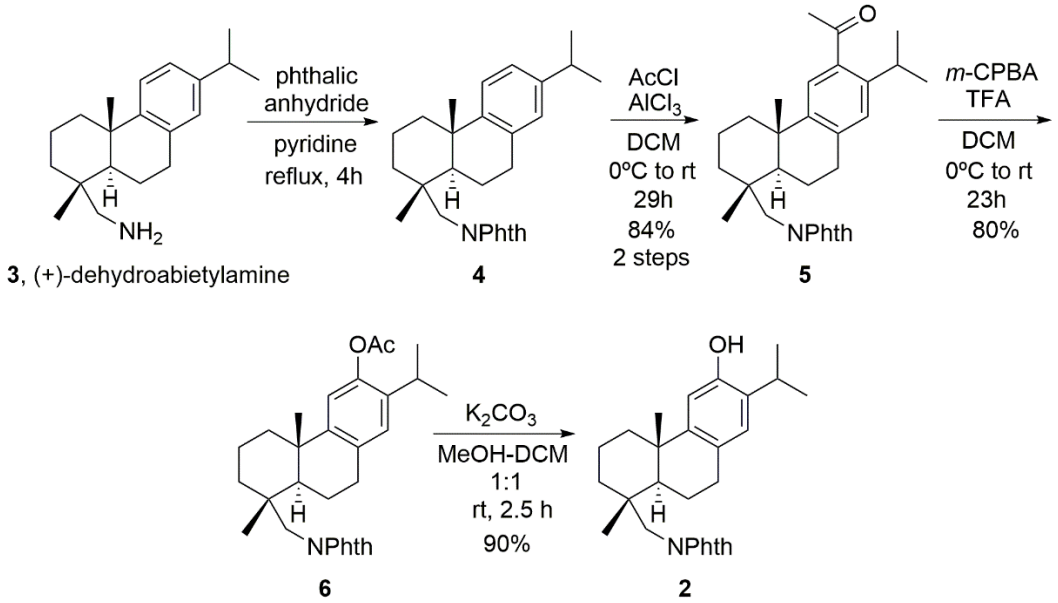


Figure 1. Antiviral abietane diterpenoids **1** and **2** and carbon skeleton numbering.

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 immer-

sion 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 ca. 60% purity and from TCI Europe 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 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 stirring at 400 rpm for 4 h. After cooling at rt, the mixture was poured onto 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), dried ($MgSO_4$) under stirring overnight. 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 do not affect 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 became from yellowish to dark brown and 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), dried ($MgSO_4$) under stirring overnight. Next day, the extract was filtered and concentrated to give 29.2 g of yellowish brown semisolid 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 were recovered, after chromatography on silica eluting with *n*-hexane-EtOAc (8:2), 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 allowed to warm to rt and stirring continued for 23 h. 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 washed with DCM (60 mL + 20 mL). Then, the filtrate was acidified with 10% HCl (ca. 10 mL) until a colour change to yellow was observed. The solution was washed with brine (40 mL) which was reextracted with additional 20 mL of DCM. The combined organic phases were dried over $MgSO_4$ under stirring overnight. 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 ($[\alpha]^{23}_D$ –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) reported initially 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 do not considered for scale-up because the use of expensive hexafluoro-2-propanol as solvent and need of synthesizing the peroxide, as well as the safety of working in large scale with peroxides. In 2017, the group of Csuk and co-workers did some modifications in the original procedure but working also in small scale [9]. For example, the 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. Also, 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 solvent using as starting material (+)-dehydroabietylamine **3** (>90%, TCI Europe) in 2-g scale and the resulting yield was lower (74%). For this reason, we maintained the original conditions for 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 high yield by crystallization in EtOH as reported by Malkowsky et al. [7] but by column chromatography leaving minor less polar impurities which did not affect the next step. If we use (+)-dehydroabietylamine **3** (12 g, >90%, TCI Europe) as 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 is time-consuming as well as needs 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 solvent giving a 88% yield [7]. We maintained essentially the same conditions for the reaction changing the solvent by DCM and work-up was done differently quenching with saturated aqueous $NaHCO_3$ instead of 6N HCl and extraction was done 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 the double of 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 solvent giving a 85% yield [7]. We maintained essentially the same conditions for the reaction changing the work-up adding further washing with 50% saturated aqueous $NaHCO_3$ as we found that the by-product *meta*-chlorobenzoic acid remains in the crude and 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** in 80% yield, while the reaction of **5** prepared from (+)-dehydroabietylamine **3** (>90%, TCI Europe) afforded acetate **6** in 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 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 adding more water to the reaction media as Csuk and co-workers did and extended reaction time [9]. The starting material is difficult to dissolve in MeOH so we choose 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 quenching with 10% HCl instead of 0.12 M H_2SO_4, and extracting with DCM instead of methyl *tert*-butyl ether. Under this new conditions, the reaction was completed in about 3 h giving 90–93% yield of pure phenol after chromatography with *n*-hexane-EtOAc (7:3) instead of original eluent mixture cyclohexane-EtOAc (90:10 to 85:15).

This molecule proved to have 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 starting from commercially available (+)-dehydroabietylamine **3** (ca. 60%, Aldrich, 100 g, ca. 100 euro) the sequence can be performed successfully in a cheaper way 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 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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