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First Scale-Up Synthesis of FU-23, a Novel Water-Soluble Pleuromutilin-Derived Antibiotic

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Abstract:

A first scale-up synthesis of FU-23 (1), a potent and effective water-soluble pleuromutilin-derived antibiotic, is described. The original synthesis from the medicinal chemistry group provided 1 in seven steps and 10.9% overall yield, required four chromatographies and employed expensive reagents such as AgOCN and 4-acetylsalicoyl chloride. The optimized synthetic route for the preparation of phosphate salt 1 consists of seven linear steps with a 42.8% overall yield. Significant cost reduction and more robust reaction conditions have been developed with no chromatography required at any stage.

Introduction

Owing to the rapid emergence of multidrug-resistant Gram-positive bacteria, there is an urgent need to identify and develop new antibacterial agents with novel mechanisms of action against drug-resistant bacterial strains. Pleuromutilin was first isolated in 1951 from basidiomycetes *Pleurotus* and *P. passeckerianus* and displayed modest activity against Gram-positive organisms *in vitro*.[1] Further studies have shown that this class of antibiotics interferes with bacterial protein synthesis via a specific interaction with the 23S rRNA of the 50S bacterial ribosome subunit.[2] These compounds display a distinct antibacterial profile and show no cross-resistance with any other class of antibiotics.[3,4] In 2007, GlaxoSmithKline's novel pleuromutilin analogue retapamulin, with excellent activity *in vitro*, was first approved for human use as a topical antimicrobial agent to treat skin infections.[5,6] The emergence of FU-23 (**1**) as a promising water-soluble pleuromutilin-derived preclinical candidate within our lab discovery program led us to seek a practical and scalable route to synthesize mutigram quantities required for regulatory toxicology studies and preclinical evaluation.[7] Herein is described the first scale-up synthesis of FU-23 (**1**).

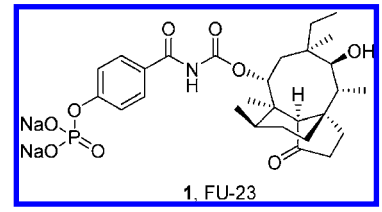

Figure 1. **Chemical structure of FU-23 (1).**

Results and Discussion

The initial medicinal chemistry approach to the synthesis of the title compound **1** is depicted in Scheme 1, which provided **1** in 10.9% overall yield. The key intermediate **7** was prepared successfully, following the method reported by Jeremy D. Hinks.[8] The synthesis of **7** started with commercially available pleuromutilin **2**, which was treated with trimethyl orthoformate and concd H_2SO_4 in methanol at 35 °C to give **3**, followed by the subsequent hydrolysis to afford **4**. AgOCN was suspended in a solution of 4-acetoxybenzoyl chloride **5** in dry dichloromethane under an atmosphere of argon and stirred at reflux for 4 h; then, **4** was added at room temperature for 1 h to give compound **6**, which was obtained in high yield after chromatographic isolation. Then, **6** in dioxane was treated with a saturated solution of zinc chloride in concd HCl to afford the intermediate **7** in 27% yield after chromatographic purification.[8] The regioselective phosphorylation of the phenolic hydroxyl group of **7**[9,10] and hydrogenolysis of the benzyl and vinyl moieties of **8** provided the free acid of **1**. Methanol and water were then added to the free acid of **1**, and the pH was adjusted to 9 by addition of 10% Na_2CO_3 solution. The salt was purified by chromatography (C18), eluting with a gradient of 0−100% methanol in water to give **1** in about 92% yield for two steps.[7]

As noted above, the original synthesis was judged not suitable for scale-up, as it involved several chromatographic purifications and provided very low overall yield (10.9%). Moreover, it also required the use of 4-acetoxybenzoyl chloride and AgOCN which are expensive and have processing issues.

Alternative Approaches to 1. We first looked at the possibility of a more efficient process for the synthesis of **1**. As a result, the alternative approach shown in Scheme 2 was implemented.

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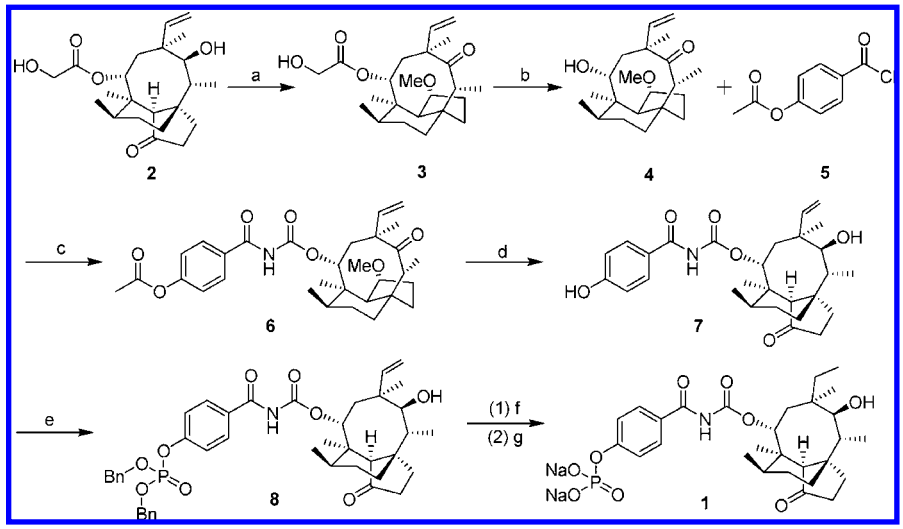
Scheme 1. **Medicinal chemistry synthesis of compound 1**[a]



[a] Reagents and conditions: (a) $(CH_3O)_3CH$, MeOH, H_2SO_4, 75%; (b) NaOH, MeOH, H_2O, chromatography, 90%; (c) CH_2Cl_2, AgOCN (4.6 equiv), chromatography, 80%; (d) dioxane, concd HCl, $ZnCl_2$, 27%; (e) $(BnO)_2POH$, DIPEA, DMAP, CCl_4, CH_3CN, chromatography, 82%; (f) 10%Pd/C, MeOH; (g) Na_2CO_3, 92% for 2 steps.

As reported by Joseph Sisko,[11] there were clear benefits in the conversion of **2** to **4** without isolation of **3** with good overall yields. However, intermediate **4** was difficult to crystallize, probably owing to impurities derived from fermentation product **2**. Despite this, we refocused our efforts on the purification of intermediate **4** by recrystallization and found that **4** could be crystallized in petroleum ether at 0 °C. After a wash with cold petroleum ether, compound **4** was isolated as a fine crystalline solid in high purity (98%, HPLC). This method avoids the need for isolating the intermediate **3** and offers advantages in terms of practicality.

To avoid 4-acetoxybenzoyl chloride and AgOCN owing to cost and processing issues, we turned our attention to 4-benzyloxybenzamide **9** as an alternative starting material. This compound is readily available in bulk quantities.[12] 4-Benzyloxybenzoyl isocyanate **10** was prepared by treating amide **9** with oxalyl chloride in dichloromethane at 50 °C in almost quantitative yield.[13] Without any isolation, slow addition of **4** in dichloromethane to the solution of **10** generated intermediate **11** in high yield (92%) and high purity (>98%).

The skeletal rearrangement of intermediate **11** to generate **12** proved to be more difficult than expected. When a solution of **11** in dioxane was treated with a saturated solution of zinc chloride in concd HCl, up to 10% of impurities formation was observed, and the major one is amide **9** as identified by HPLC and NMR. We speculated that the presence of zinc chloride accelerated the decomposition, perhaps owing to the high concentration of nucleophilic chloride ion. However, when concd HCl without zinc chloride was used, a longer reaction time was needed, and the reaction stalled at 90−95% conversion. When a temperature greater than 30 °C was employed, more than 10% of impurity formation was observed, and further recrystallization from a variety of solvents did not remove the impurity. Finally, we found that a half-saturated solution of zinc chloride in concd HCl (250 g $ZnCl_2$ in 500 mL concd HCl) at room temperature effected the conversion of **11** to **12** in good yield with low levels of impurity formation (Table 1). Furthermore, under these reaction conditions the poor solubility of **12** could be used advantageously in the isolation. In fact **12** was obtained directly from the reaction mixture by simple addition of a 1/3 equivalent volume of water followed by filtration and washing with water and ethanol, giving **12** as a colourless solid in high yield (93.4%) and high purity (>99.0%). Hydrogenation of the double bond and debenzylation to deprotect the hydroxy group could be done simultaneously to afford alcohol **13** in near quantitative yield.

The regioselective phosphorylation of the phenolic hydroxyl group of **13** at the C-14 side chain without protecting the C-11 skeleton hydroxyl group relied on the higher acidity of the phenolic proton and the steric hindrance of the C-11 hydroxyl group.[9] Treatment of **13** with *N*,*N*-dimethylaminopyridine (DMAP), carbon tetrachloride, *N*,*N*-diisopropylethylamine (DIPEA) and dibenzyl phosphite $(BnO)_2P(O)H$ successfully produced the phosphoric acid dibenzyl ester compound **14** in high yield and high purity after recrystallization from hexane and ethyl acetate. Various conditions were tested before the suitable reaction conditions were found. The results from the screening studies are shown in Table 2. A critical parameter in the reaction is to control the reaction temperature between −20 and −25 °C (entries 5−9, Table 2). At this low temperature, impurity formation was greatly reduced. The number of equivalents of $(BnO)_2P(O)H$ is also very important. The reaction was best run using 1.15 equiv of $(BnO)_2P(O)H$ at −25 °C, under which conditions the byproducts were minimised (entry 7, Table 2).

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(12) Compound **9** was readily prepared in high yield from commercially available 4-benzyloxybenzoic acid by sequential treatment with oxalyl chloride and concentrated aqueous ammonia.

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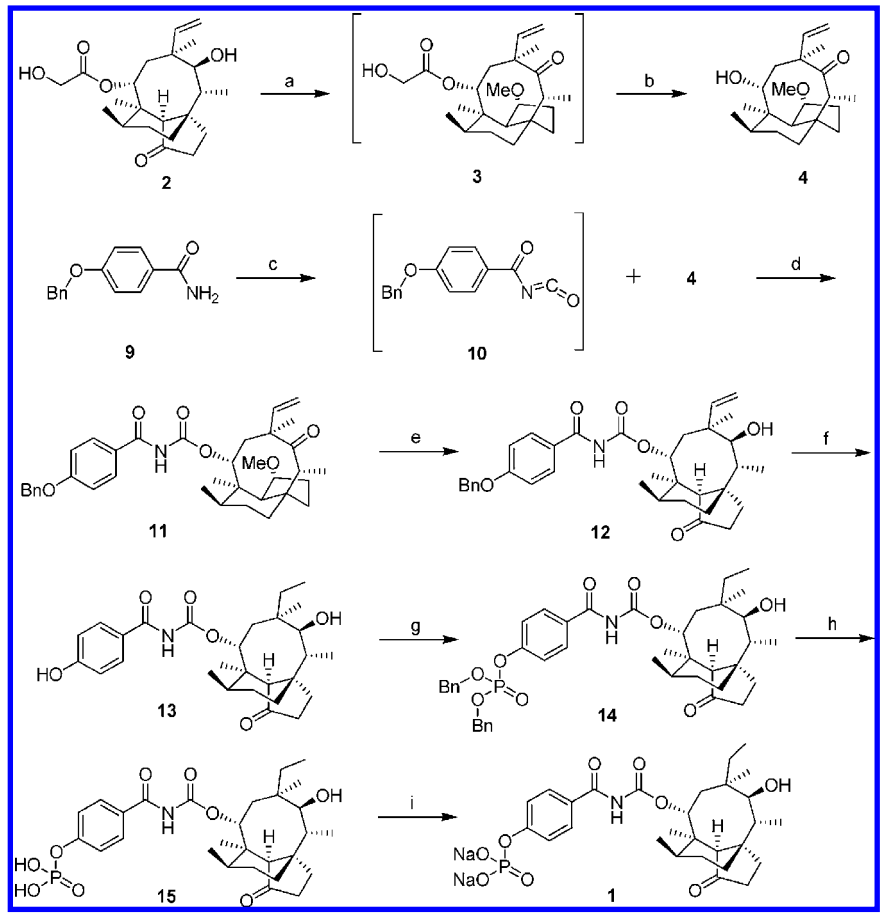
Scheme 2. **First scale-up synthesis of compound 1**[a]



[a] Reagents and conditions: (a) $(CH_3O)_3CH$, MeOH, H_2SO_4, 35 °C; (b) NaOH, MeOH, H_2O, 65 °C, 81% for 2 steps; (c) ClCOCOCl, CH_2Cl_2, rt to 50 °C; (d) CH_2Cl_2, rt, 92% for 2 steps; (e) dioxane, concd HCl, $ZnCl_2$, 25 °C, 93%; (f) H_2, 10% Pd/C, THF, rt, 95%; (g) $(BnO)_2P(O)H$, DIPEA, DMAP, CCl_4, CH_3CN, −25 °C, 82%; (h) H_2, 5% Pd/C, THF, rt, 96%; (i) H_2O, MeOH, Na_2CO_3, i-PrOH, 82%.

Table 1. **Optimization of skeletal rearrangement conditions**

		reaction	mixture	composition (%)	
entry	reaction conditions	**12**	impurity **9**	other impurities	yield (%)
1	concd HCl, 10 h, 35 °C	85.0	10.0	5.0	73.9
2	saturated $ZnCl_2$−concd HCl, 2.5 h, 30 °C	86.2	8.0	5.8	81.5
3	half-saturated $ZnCl_2$−concd HCl, 3 h, 30 °C	93.0	4.3	2.7	86.8
4	half-saturated $ZnCl_2$−concd HCl, 5 h, 25 °C	96.5	2.0	1.5	93.4

Table 2. **Effects of $(BnO)_2P(O)H$ and temperature on formation of phosphate 14**

	reaction condition				
entry	*T* (°C)	$(BnO)_2P(O)H$ (equiv)	impurity (%)	yield (%)	purity[a] (%)
1	−5	1.5	15.0	55.2	90.0
2	−10	1.5	13.2	66.1	93.0
3	−15	1.5	10.3	72.8	93.8
4	−20	1.5	9.1	75.0	95.0
5	−20	1.2	8.0	75.1	97.1
6	−25	1.2	6.0	77.5	98.0
7	−25	1.15	4.2	82.0	98.8
8	−25	1.10	7.8	76.5	98.0
9	−40	1.15	10.6[b]	73.6	96.5

[a] The single recrystallization purity by HPLC. [b] Including unreacted starting material **13** (4.0%).

Hydrogenolysis of the benzyl moieties of **14** smoothly gave **15** in excellent yield. As a part of our optimization study, we screened various solvent systems such as methanol, methanol/THF, methanol/ethyl acetate, and THF for the hydrogenolysis of **14**. In methanol the rate of reaction was fast; however, the purity of product **15** was less than 98.0%. Ultimately, it was determined that the preferred reaction solvent was THF, and further recrystallization of **15** from water and CH_3CN gave a purity over 99.2%.

For salt formation, **15** was dissolved in methanol and water, and a solution of Na_2CO_3 (1.05 equiv) was added. After a small quantity of insoluble material was removed by filtration, the filtrate was concentrated under reduced pressure, and the residue was recrystallized from methanol/isopropanol to give **1** in 82% yield with >99.5% purity and no single impurity >0.1%.

Conclusion

In conclusion, we have developed an improved and more efficient process for the synthesis of **1**, which offers distinctive advantages over the published procedures.[7,8] The present

synthesis is free of column chromatography in all stages and greatly improves the overall yield from 10.9% to 42.8%. This process is amenable for the large-scale laboratory production of FU-23 (**1**).

Experimental Section

General Methods. All reagents and solvents were commercially available and were used without further purification unless otherwise stated. NMR spectra of the intermediates were recorded on a Bruker 300 NMR spectrometer using TMS as an internal standard. EI-MS spectra were obtained on a Finnigan MAT 95 mass spectrometer and ESI-MS spectra were obtained on a Kratos MS 80 mass spectrometer. Elemental analysis was obtained using a vario EL spectrometer. HPLC analysis of the intermediates and reaction monitoring was performed on an Agilent 1100 liquid chromatograph equipped with a Dikma Technologies ODS 250 mm ×4.6 mm 5 μM column; flow rate 1 mL/min, wavelength: 210 nm, temperature: 25 °C. Standard mobile phase composition: 30:70 to 10:90 gradient of water/acetonitrile. Product **1** (**FU-23**) mobile phase composition: 30% water with 0.1% TFA, 70% methanol, flow rate 1 mL/min.

(3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-epi-mutilin (4). Pleuromutilin (400 g, 1.06 mol) was dissolved in methanol (1.5 L) and trimethyl orthoformate (504 g, 520 mL, 4.75 mol) at 20 °C. Concentrated sulphuric acid (198 g, 100 mL, 2.06 mol) was added over 10 min at <30 °C. The mixture was heated to 30 °C and stirred for 8 h. A solution of NaOH (320 g, 8.0 mol) in water (350 mL) was added to the methanol mixture, and the contents were heated to 60−63 °C for 2 h. The mixture was cooled to 40 °C, and methanol was removed by vacuum distillation to a final volume of 500 mL. After cooling to room temperature, water (350 mL) was added, and the reaction mixture was extracted with ethyl acetate (1000 mL). The aqueous layer was further extracted with ethyl acetate (500 mL), and the combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to give the crude oily mixture (300 mL). Petroleum ether (500 mL) was added to the crude oily mixture at 0 °C, and a thick, white slurry resulted within 10 min. The slurry was stirred for 1 h. The white precipitate was filtered and washed with cold petroleum ether to give 285.6 g (81.0%) of compound **4** as a crystalline solid, mp 80−81 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.05 (1H, dd, J = 11.0, 10.8 Hz), 5.26 (2H, m), 4.61 (1H, d, J = 9.2 Hz), 3.48 (1H, m), 3.23 (3H, s), 2.92 (1H, q, J = 6.6 Hz), 2.45 (1H, dd, J = 15.3, 10.0 Hz), 2.18 (1H, m), 2.01 (2H, m), 1.86 (1H, d, J = 15.4 Hz), 1.73 (1H, d, J = 11.4 Hz), 1.58 (2H, s), 1.46 (1H, m), 1.32 (1H, m), 1.18 (3H, s), 1.67 (3H, s), 1.16 (1H, m), 1.09 (1H, m), 1.01 (3H, d, J = 6.4 Hz), 0.95 (3H, d, J = 6.6 Hz). Anal. Calcd for $C_{20}H_{34}O_3$: C, 75.41; H, 10.25. Found: C, 75.38; H, 10.16. MS: 235 (M + 1).

(3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-epi-mutilin 14-[*N*-(4- Benzyloxybenzoyl)carbamate] (11). To a well-stirred suspension of dry 4-benzyloxybenzamide (181.6 g, 0.80 mol) in dry dichloromethane (1.2 L), freshly distilled oxalyl chloride (83.7 mL, 111.7 g, 0.88 mol) was added in dropwise fashion at 25 °C in 15 min. The mixtures agitated for a further 30 min at 25 °C, then were heated at 65 °C for 1 h, and the suspended solid disappears completely. Extreme care was taken to prevent the introduction of moisture into the sample during these manipulations. Heating was discontinued after 2 h, and the mixture allowed to cool to room temperature. **4** (267.5 g, 0.8 mol) dissolved in dichloromethane (500 mL) was added dropwise to the above mixture, resulting in a mild exotherm. The mixture was stirred for 2 h at room temperature before quenching with saturated brine (600 mL). The aqueous layer was washed with dichloromethane (500 mL), and the combined organic extracts were dried over anhydrous Na_2SO_4. Dichloromethane was removed by vacuum distillation to a final volume of 300 mL. Acetonitrile (2.0 L) was added, and the resulting solution was heated to 60 °C for 1 h, and then the mixture was cooled to 25 °C. The white precipitated crystals were filtered off, washed with cold acetonitrile, and air dried to afford 423 g (92.2%) of the title product, mp 80−81 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.87 (1H, s), 7.79 (2H, d, J = 8.8 Hz), 7.28−7.42 (5H, m), 7.05 (2H, d, J = 8.8 Hz), 6.74 (1H, dd, J = 11.0, 10.8 Hz), 5.84 (1H, d, J = 9.8 Hz), 5.30 (1H, d, J = 10.8 Hz), 5.13 (2H, s), 5.05 (1H, d, J = 17.1 Hz), 3.46 (1H, m), 3.23 (3H, s), 2.90 (1H, q, J = 6.6 Hz), 2.51 (1H, m), 2.20 (1H, m), 1.90−2.05 (2H, m), 1.74 (2H, dd, J = 5.8, 9.7 Hz), 1.4−1.6 (3H, m), 1.35 (1H, m), 1.30 (3H, s), 1.25 (1H, m), 1.20 (3H, s), 1.05 (1H, m), 1.02 (3H, d, J = 6.4 Hz), 0.95 (3H, d, J = 6.6 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 214.3, 165.0, 161.8, 151.3, 140.5, 136.5, 130.6, 128.5, 127.9, 127.8, 125.6, 117.8, 114.6, 114.4, 82.3, 72.2, 69.4, 63.1, 56.3, 53.3, 47.0, 44.7, 44.2, 43.5, 42.9, 30.1, 28.9, 28.3, 25.3, 20.5, 16.3, 15.4. Anal. Calcd for $C_{36}H_{45}NO_6$: C, 73.57; H, 7.72; N, 2.38. Found: C, 73.66; H, 7.70; N, 2.41. MS (ESI) m/z 610.1 $(M + Na)^+$; 586.3 $(M - H)^-$.

Mutilin 14-[*N*-(4-Benzyloxybenzoyl)carbamate] (12). A solution of **11** (364 g, 0.62 mol) in dioxane (2.1 L) was cooled to 10 °C and treated with a half-saturated solution of zinc chloride in concd HCl (910 mL, 450 g $ZnCl_2$) keeping the temperature <15 °C. Then the mixture was heated at 25 °C for 5 h, and water (600 mL) was added slowly. Prior to completion of the addition of water, a white precipitate began to form. After completion of the addition, the heterogeneous mixture was stirred at room temperature for 1 h. The white precipitate was filtered, washed with water (2 × 300 mL) and ethanol (300 mL), then dried under vacuum to afford 335 g (93.4%) of **12** as a white solid, mp 186−187 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.83 (1H, s), 7.75 (2H, d, J = 8.81), 7.28−7.43 (5H, m), 7.03 (2H, d, J = 8.81), 6.55 (1H, dd, J = 10.9, 10.8 Hz), 5.83 (1H, d, J = 8.5 Hz), 5.38 (1H, d, J = 11.0 Hz), 5.20 (1H, d, J = 17.3 Hz), 5.12 (2H, s), 3.37 (1H, d, J = 6.4 Hz), 2.35 (1H, t, J = 6.1 Hz), 2.30 (2H, m), 2.10 (2H, m), 1.60−1.80 (4H, m), 1.50 (3H, s), 1.35−1.45 (3H, m), 1.20 (3H, s), 1.05 (1H, m), 0.93 (3H, d, J = 6.4 Hz), 0.90 (3H, d, J = 6.6 Hz). ^{13}C NMR (100 MHz, DMSO-d_6): δ 217.2, 164.9, 161.7, 151.0, 140.9, 136.5, 130.5, 128.5, 127.9, 127.8, 125.6, 115.2, 114.4, 72.7, 70.1, 69.4, 66.3, 57.4, 44.9, 43.9, 41.6, 36.4, 34.0, 30.1, 28.3, 26.6, 24.5, 16.0, 14.9, 11.5. Anal. Calcd for $C_{35}H_{43}NO_6$: C, 73.27; H, 7.55; N, 2.44. Found: C, 73.19; H, 7.70; N, 2.41. MS (ESI) m/z 572.6 $(M - H)^-$.

19,20-Dihydromutilin 14-[*N*-(4-Hydroxybenzoyl)carbamate] (13). To a solution of compound **12** (270 g, 0.47 mol) in THF (2.0 L) was added 10% Pd−C (30 g, 50% water) and the mixture stirred under H_2 atmosphere employing 3 bar of

pressure at 25 °C. The reaction mixture was hydrogenated until no further H_2 was consumed. The reaction mixture was filtered, and the filtrate was concentrated to give crude solid **13**. Acetone (200 mL) was added to the crude solid, and the slurry was stirred for 1 h. The white precipitate was filtered and washed with ethanol (300 mL) to afford 217.0 g (95.2%) of compound **13** as a white solid, mp 187−189 °C; ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.47 (1H, s), 10.25 (1H, s), 7.74 (2H, d, J = 8.8 Hz), 6.81 (2H, d, J = 8.8 Hz), 5.53 (1H, d, J = 7.9 Hz), 4.40 (1H, d, J = 6.4 Hz), 2.35 (1H, t, J = 6.1 Hz), 2.0−2.30 (3H, m), 1.45−1.80 (6H, m), 1.42 (3H, s), 1.20−1.40 (3H, m), 1.05 (1H, m), 0.95 (3H, s), 0.90 (3H, d, J = 6.4 Hz), 0.60−0.80 (6H, m). ^{13}C NMR (100 MHz, DMSO-d_6): δ 217.3, 164.9, 161.5, 151.4, 130.7, 123.8, 114.9, 73.7, 69.6, 57.5, 44.9, 40.3, 36.4, 36.3, 34.4, 33.9, 30.2, 26.7, 26.4, 24.4, 20.2, 16.3, 14.9, 11.5, 8.1. Anal. Calcd for $C_{28}H_{39}NO_6$: C, 69.25; H, 8.09; N, 2.88. Found: C, 69.31; H, 8.00; N, 2.78. MS (ESI) m/z 508.1 $(M + Na)^+$; 484.2 $(M - H)^-$.

19,20-Dihydromutilin 14-[*N*-(4-Phosphoric Acid Dibenzyl Ester Benzoyl)carbamate] (14). **13** (172.8 g, 0.36 mol) and dimethylaminopyridine (4.4 g, 0.036 mol) were dissolved in anhydrous CH_3CN (2.0 L) under argon. The reaction mixture was cooled to −25 °C, and CCl_4 (160 mL, 1.66 mol) and diisopropylethylamine (134.5 mL, 0.77 mol) were added. After 10 min, dibenzylphosphite (94.4 mL, 0.43 mol) was added over a 30 min period. The mixture was then stirred at −20 °C for 2 h. During this time, the starting material dissolved. KH_2PO_4 (0.5 M, 600 mL) was added, and the solution was allowed to warm to room temperature and stirred for 1 h. The aqueous mixture was extracted with ethyl acetate (3 × 350 mL) and then washed with water (2 × 150 mL). The organic layer was dried over Na_2SO_4 and concentrated *in vacuo* to a volume of 500 mL. Hexane (750 mL) was added. Solvent (500 mL) was removed *in vacuo*, and the solid precipitated during this process. Hexane (500 mL) and ethyl acetate (300 mL) were added, and the mixture was heated to reflux until all solid dissolved. The solution was cooled to room temperature and then to 0 °C for 2 h. A white solid was collected, washed twice with cold hexane, and dried *in vacuo* to yield 220.0 g (82.0%) of **14**, mp 88−90 °C; HPLC t_R = 12.8 min; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.81 (3H, d, J = 6.6 Hz), 0.88 (3H, d, J = 7.0 Hz), 1.10−1.81 (12H, m), 1.15 (3H, s), 1.51 (3H, s), 2.09−2.26 (2H, m), 2.38 (1H, bs), 2.40 (1H, quintet J = 6.9 Hz), 3.41 (1H, d), 5.10−5.16 (4H, d, J = 8.8 Hz), 5.57 (1H, dd, J = 11.0, 1.5 Hz), 7.20 (2H, d, J = 8.8 Hz), 7.30−7.40 (10H, m), 7.90 (2H, d, J = 8.8 Hz), 10.80 (1H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 217.2, 163.9, 153.9, 151.1, 135.0, 134.9, 129.8 129.7, 128.8, 128.6, 128.0, 120.0, 77.3, 77.0, 76.7, 71.6, 70.3, 70.2, 58.4, 45.5, 41.9, 40.8, 36.6, 34.4, 34.3, 30.2, 26.8, 26.3, 24.8, 20.6, 16.5, 15.0, 14.1, 11.0, 8.2. Anal. Calcd for $C_{42}H_{52}NO_9P$: C, 67.64; H, 7.03; N, 1.88. Found: C, 67.84; H, 6.87; N, 1.81. MS (ESI) m/z 768.80 $(M + Na)^+$.

19,20-Dihydromutilin 14-[*N*-(4-Phosphoric acid benzoyl)-carbamate] (15). To a solution of **14** (200 g, 26.82 mmol) in THF (1.5 L) was added 5% Pd−C (45 g), and the mixture was hydrogenated at ambient temperature and 15 psig hydrogen for 5 h. The mixture was filtered through Celite-521 and a 0.45 μm membrane and was rinsed with THF. The solvent was evaporated and *tert*-butyl methyl ether was added. A white solid was collected by filtration and washed twice with *tert*-butyl methyl ether. The crude product was dissolved in CH_3CN (1.6 L), and water (32 mL) was added slowly into the solution at ambient temperature. The solution was then cooled to 0 °C and was seeded while cooling. After 2 h, the white crystals were collected by filtration and washed with CH_3CN (500 mL). The solid was dried under high vacuum at 35 °C to yield 145.0 g (95.6%) of **15**. ^{1}H NMR (300 MHz, CD_3OD): δ 0.81 (3H, d, J = 6.6 Hz), 0.88 (3H, d, J = 7.0 Hz), 1.10−1.81 (12H, m), 1.15 (3H, s), 1.51 (3H, s), 2.09−2.26 (2H, m), 2.38 (1H, bs), 2.40 (1H, q, J = 6.9 Hz), 3.45 (1H, d), 5.78 (1H, d, J = 8.5 Hz), 7.30 (2H, d, J = 8.8 Hz), 7.90 (2H, d, J = 8.8 Hz). ^{13}C NMR (100 MHz, CD_3OD): δ 220.4, 168.2, 157.0, 156.9, 153.6, 131.8, 131.2, 121.8, 121.7, 77.2, 72.5, 60.0, 47.3, 43.7, 42.3, 42.0, 38.6, 36.5, 35.8, 32.0, 28.6, 27.3, 26.3, 21.9, 17.4, 16.0, 12.3, 9.1. Anal. Calcd for $C_{28}H_{40}NO_9P$: C, 59.46; H, 7.13; N, 2.48. Found: C, 59.41; H, 7.15; N, 2.46. MS (ESI) m/z 565.2 $(M - H)^-$.

Disodium 19,20-Dihydromutilin 14-[*N*-(4-Phosphoric acid benzoyl)carbamate] (1). The acid **15** (120 g, 0.21 mol) was dissolved in methanol (200 mL) and cooled to 0 °C. A solution of Na_2CO_3 (23.3 g, 0.22 mol) in distilled water (50 mL) was added dropwise to the methanol solution over 10 min. After the addition was complete, the clear reaction mixture was stirred for an additional 2 h at room temperature. After filtration to remove some unsoluble material, the solution volume was reduced by 50% (100 mL) on a rotary evaporator, and the residue was recrystallised from methanol/isopropanol (600 mL, 1:2) giving **1** as a white precipitate which was filtered off and washed with cold isopropanol (100 mL). The filter cake was dried under vacuum at 35 °C to afford 111.5 g (82.3%) of **1** as white solid, mp 200−202 °C. HPLC t_R = 6.58 min, >99.5% purity. ^{1}H NMR (300 MHz, D_2O): δ 0.79 (6H, m), 0.88 (5H, m), 1.12 (1H, t), 1.40−1.71 (12H, m), 1.75−1.90 (3H, m), 2.20−2.36 (2H, m), 2.40 (1H, q, J = 6.9 Hz), 2.50 (1H, bs), 3.54 (1H, d), 5.55 (1H, d, J = 8.5 Hz), 7.32 (2H, d, J = 8.8 Hz), 7.80 (2H, d, J = 8.8 Hz). ^{13}C NMR (100 MHz, D_2O): δ 224.0, 169.2, 161.8, 158.4, 152.3, 129.8, 126.0, 119.9, 119.8, 76.0, 71.4, 58.4, 45.5, 41.6, 40.1, 39.3, 36.7, 34.5, 34.1, 29.8, 26.3, 25.5, 24.2, 20.0, 15.7, 14.5, 10.7, 7.3. Anal. Calcd for $C_{28}H_{38}NNa_2O_9P$: C, 55.17; H, 6.28; N, 2.30. Found: C, 55.16; H, 6.32; N, 2.31.

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Supporting Information Available

Copies of the NMR spectra of all intermediates and final product as well as HPLC conditions and retention times. This material is available free of charge via the Internet at http://pubs.acs.org.

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