An Efficient Method for the Synthesis of Laquinimod

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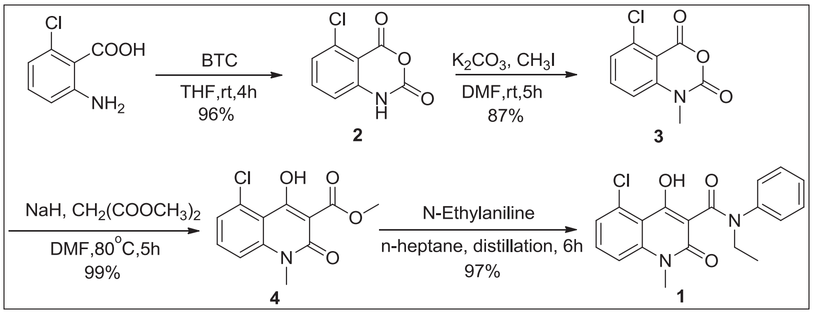

Laquinimod, 5-chloro-1,2-dihydro-*N*-ethyl-4-hydroxy-1-methyl-2-oxo-*N*- phenyl-3-quinoline carboxamide, is an oral drug in clinical trials for the treatment of multiple sclerosis. An efficient synthetic method for laquinimod from 2-amino-6-chlorobenzoic acid via four steps was established. The overall yield of laquinimod is up to 82% as compared with 70% reported in literature. It has also been demonstrated that green reagent dimethyl carbonate is not suitable for the *N*-methylation of 5-chloroisatoic anhydride owing to the ring-cleavage reaction induced by the generated methanol. The ring-cleavage by-products were isolated and characterized by ^{1}H-NMR and ^{13}C-NMR. In addition, in the study of laquinimod derivatives, we found that 5-chloro-1,2-dihydro-*N*-ethyl-4-hydroxy-1-methyl-2-oxo-*N*-phenyl-3-quinoline carboxamide (laquinimod) was obtained in much higher yield than 7-chloro-1,2-dihydro-*N*-ethyl-4-hydroxy-1-methyl-2-oxo-*N*-phenyl-3-quinoline carboxamide under the same reaction conditions, and it is possibly attributed to a neighboring group effect.

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INTRODUCTION

Laquinimod, 5-chloro-1,2-dihydro-*N*-ethyl-4-hydroxy-1-methyl-2-oxo-*N*-phenyl-3-quinoline carboxamide (Fig. 1), also known as ABR-215062, is a novel oral immunomodulator for the treatment of multiple sclerosis. Compared with its lead compound Linomide (roquinimex), which had been terminated in the phase III trial because of infrequent and important toxicities [1,2]; laquinimod demonstrated high potency and superior toxicological profile [3–6]. Now, laquinimod is just under phase III clinical trial, so development of an efficient synthetic method for laquinimod is urgent.

Laquinimod was first synthesized at laboratory scale via a three-step procedure [7]. However, this process suffered from several major disadvantages from an industrial perspective, including high cost, tedious workup procedures, low yield, and the use of phosgene. In 2007, another synthetic route of laquinimod was reported by Johan Wennerberg (Scheme 1) [7].

Although this route was more feasible for large-scale production than the previous method, the usage of unfriendly ethyl chloroformate and expensive methyl iodide makes it industrially unattractive. Furthermore, the undesirable side reaction might happen owing to the cascade reaction nature of the first step, resulting in the hard purification of compound **2**. As described in Scheme 2, intermediate **2a** formed in the first step may react with ethyl chloroformate before the addition of acetyl chloride, which leads to the generation of by-product **2b**. Therefore, this synthetic route was modified in this paper; a more efficient synthesis of laquinimod was established. Under optimized reaction conditions, laquinimod was obtained in 82% overall yield.

RESULTS AND DISCUSSION

As shown in Scheme 3, in order to avoid the use of poisonous ethyl chloroformate, triphosgene (bis(trichloromethyl) carbonate or BTC) was chosen as a carbonyl equivalent in the present study. As is well known, BTC, which is a stable solid and also an active acylation agent, can release phosgene quantitatively in the presence of nucleophiles [8–10]. Thus, the cyclization reaction of 2-amino-6-chlorobenzoic acid with BTC proceeds smoothly in tetrahydrofuran (THF) at room temperature to afford compound **2** in 96% yield. A large amount of hydrogen chloride was generated, so an effective absorption apparatus of hydrogen chloride was required. Moreover, the posttreatment of the cyclization was quite simple, quenching the reaction and washing the obtained precipitates just by water. Fortunately, no impurities such as **2a** and **2b** were detected in the reaction mixture. Compared with the cyclization described in Scheme 1, shorter reaction

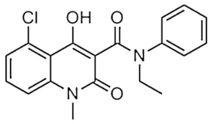


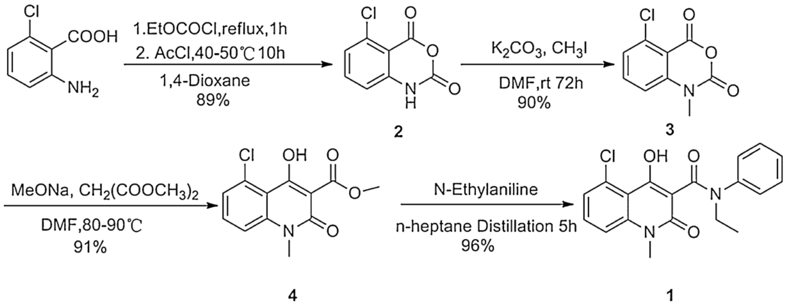
Figure 1. Chemical structure of laquinimod.

time, milder reaction conditions, better yield, and easier post-treatment made it more competitive in industrial production.

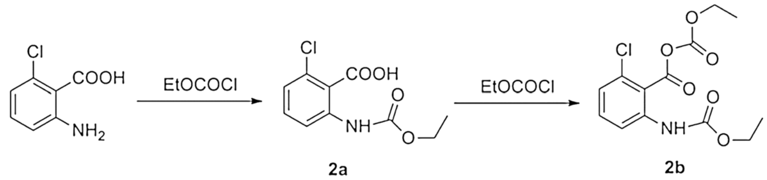
As we all know, methyl iodide and dimethyl sulfate (DMS) are conventional methylating agents. However, the genotoxicity of DMS and its derived alkyl sulfates prevent it from being used in pharmaceutical production. With consideration of the cost and toxicity of methyl iodide, we tried to use green reagent dimethyl carbonate (DMC) as the *N*-methylating agent in the second step. In order to increase the activity of compound **2** and enhance the *N*-methylation, the reaction with DMC as the *N*-methylating agent was conducted in the presence of catalytic amounts of base at high temperature (over 120°C). Unfortunately, the results were not encouraging. It was found that CH_3O^- was generated during the process [11]. As a strong nucleophile, it possibly attacks the carbonyl carbon of 5-chloroisatoic anhydride and leads to ring-cleavage reaction (Scheme 4). As a consequence, by-products **3a** and **3b** were isolated and confirmed by ^{1}H-NMR and ^{13}C-NMR. Besides, **3a** or **3b** was hydrolyzed at reflux temperature (150°C) in the presence of water to yield the corresponding aminobenzoic acid. Owing to so many side reactions, the yield of the target compound was really poor. No choice, methyl iodide was employed as a methylating agent in this reaction to afford compound **3** in 87% yield.

The enolate of dimethyl malonate was formed by the treatment of dimethyl malonate with sodium methoxide in Scheme 1. However, sodium methoxide and generated methanol probably induce the ring-cleavage side reaction of compound **3** according to the mechanism shown in Scheme 4. Thus, we employed sodium hydride instead of sodium methoxide to generate the enolate of dimethyl

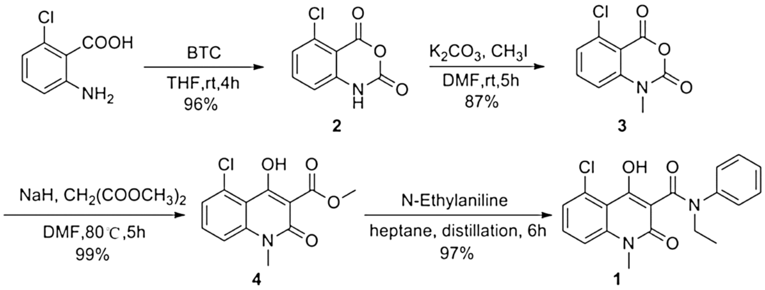
Scheme 1. The synthetic route of laquinimod reported by Johan Wennerberg.



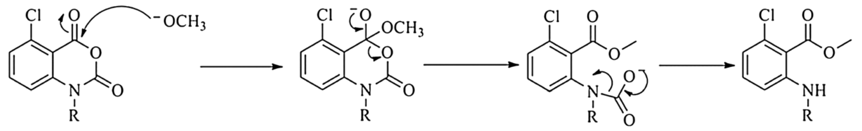
Scheme 2. The generation of by-product **2b**.



Scheme 3. The efficient synthetic route of laquinimod.



Scheme 4. The mechanism of ring-cleavage reaction.



3a,R=H
3b,R=CH$_3$

malonate, which condensed with compound **3** to yield the desired compound **4**. As an inorganic alkaline, sodium hydride can acquire the proton of dimethyl malonate at room temperature quickly and release hydrogen that will not induce side reactions. In fact, the condensation of compound **3** and dimethyl malonate consists of four elementary reactions, including nucleophilic substitution, nucleophilic substitution, hydrolysis, and decarboxylation. So, the choice of base is very important to this condensation. Under the optimized conditions, compound **4** was finally gained in 97% yield and 98.9% purity.

The last step was the *N*-acylation of *N*-ethylaniline with ester **4** to the amide **1**. Esters are normally taken as weak acylating agents. An equilibrium exists between compound **4** and compound **1** [12]. The removal of formed methanol during the reaction will force the equilibrium shift to the right and lead to high yield of compound **1**. However, in the study of laquinimod derivatives, we accidentally found that the *N*-acylation of compound **5** with *N*-ethylaniline (Scheme 4) proceeded much slower than that of compound **4** at the same reaction conditions. The structures of compounds **4** and **5** are similar except the position of the substituted chlorine, but the conversion of compound **4** was obviously higher than that of compound **5** (Table 1). As a result, the yield of compound **1** was also higher than that of compound **6** (Table 1). The different behaviors may be attributed to the neighboring group effect. The situation of chlorine at the C5 position of the quinoline ring is crowded to the hydroxyl group, which disturbs the conjugated system, resulting in the increase of the positive charge of the carbonyl group. Nevertheless, when chlorine substituted at the C7 position of the quinoline ring, far away from the hydroxyl group, it does not destroy the planarity of compound **5** (Scheme 5), and the positive charge of the carbonyl group is dispersed on the whole conjugated system. Hence, the activity of compound **5** is much lower than that of compound **4**.

Table 1

Reaction of esters with *N*-ethylaniline.

Ester	Product	Conversion	Yield
4	**1**	99%	97%
5	**6**	60%	56%

CONCLUSION

In conclusion, we have established an efficient synthetic method for laquinimod from 2-amino-6-chlorobenzoic acid via four steps. Laquinimod was finally obtained in 82% total yield and 99.5% purity. It was found that the ring-cleavage side reaction of compound **2** was induced by the generated methanol. The ring-cleavage by-products were isolated and characterized by ^{1}H-NMR and ^{13}C-NMR. Moreover, in the study of laquinimod derivatives, we found that the *N*-acylation of compound **4** with *N*-ethylaniline exhibited higher reactivity than that of compound **5**, and it is probably caused by the neighboring group effect.

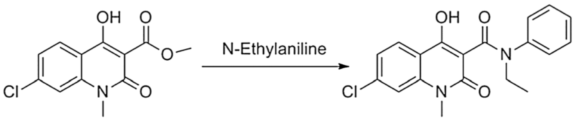
EXPERIMENTAL

Reagents and solvents were obtained from commercial suppliers. All reactions were monitored by thin-layer chromatography using commercial silica gel plates. The purity of products was detected by HPLC on Agilent 1100 series. Melting points were observed on YRT-3 Melting Point Tester and uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on Bruker AVANCE III 600 MHz. High-resolution mass spectrometry (HRMS) was measured on MicrOTOF-Q II.

5-Chloroisatoic anhydride (2). 2-Amino-6-chlorobenzoic acid (10.00 g, 58.5 mmol) was suspended in THF (60 mL) at 0°C. Triphosgene (6.93 g, 23.4 mmol) was added, and the mixture was stirred for 3 h at room temperature. Water (50 mL) was added. The precipitate was collected by filtration, washed with water (2 × 20 mL), and dried at 50°C under vacuum over P_2O_5 to give product **2** as a white solid (10.97 g, yield 97%). mp 271.9–272.5°C ([5] 272.5–272.9°C); ^{1}H-NMR [hexadeuterated dimethyl sulfoxide (DMSO-d_6), 600 MHz] δ: 7.10 (d, J = 8.4 Hz, 1H), 7.31 (d, J = 7.8 Hz, 1H), 7.66 (t, J = 8.1 Hz, 1H), 11.86 (s, 1H).^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 108.00, 114.50, 125.64, 134.90, 136.49, 143.85, 146.61, 156.43.

5-Chloro-*N*-methylisatoic anhydride (3). Compound **2** (10.00 g, 52.0 mmol) was dissolved in dimethylformamide (DMF; 60 mL). Potassium carbonate (9.32 g, 67.5 mmol) and iodomethane (9.54 g, 67.5 mmol) were added, and the mixture was stirred for 5 h at room temperature. Then, 5% hydrochloric

Scheme 5. Synthesis of compound **5**.



5 **6**

acid was added dropwise until no gas was emitted. The mixture was stirred for an additional 10 min. The precipitate was filtered, washed with water (2 × 20 mL), and dried to constant weight at 50°C under vacuum over P_2O_5 to give the title compound **3** (9.55 g, yield 87%) as a white solid. mp 217.8–218.5°C ([5] 217.9–218.2°C). ^{1}H-NMR (DMSO-d_6, 600 MHz) δ: 3.46 (s, 3H), 7.41–7.43 (m, 2H), 7.78 (t, $J = 8.4$ Hz, 1H). ^{13}C-NMR (DMSO-d_6, 150 MHz) δ: 32.37, 109.33, 114.07, 125.99, 135.37, 136.54, 144.56, 147.38, 155.46.

A mixture of compound **2** (10.00 g, 52.0 mmol), sodium hydride (60% in mineral oil, 2.70 g, 67.6 mmol), DMC (9.36 g, 104.0 mmol), and DMF (30 mL) was stirred and heated to reflux for 5 h under N_2 atmosphere. After that, the solvent was evaporated under reduced pressure, and the title compound was purified by silica gel column (petroleum ether: ethyl acetate (PE:EA) = 2.5:1). Besides, **3a** and **3b** were also isolated during the process.

Methyl 2-amino-6-chlorobenzoate (3a). Yellow oil [13]. ^{1}H-NMR (CD$_3$OD, 600 MHz) δ: 3.86 (s, 3H), 6.63–6.65 (m, 2H), 7.04 (t, $J = 8.1$ Hz, 1H). ^{13}C-NMR (CD$_3$OD, 150 MHz) δ: 52.81, 116.14, 116.38, 119.58, 133.15, 134.32, 150.50, 169.12.

Methyl 2-chloro-6-(methylamino)benzoate (3b). Yellow oil [14]. ^{1}H-NMR (CDCl$_3$, 600 MHz) δ: 2.81 (s, 3H), 3.90 (s, 3H), 6.52 (d, $J = 8.4$ Hz, 1H), 6.67 (dd, $J = 7.8$ and 0.6 Hz, 1H), 7.16 (t, $J = 8.1$ Hz, 1H). ^{13}C-NMR (CDCl$_3$, 150 MHz) δ: 30.00, 51.90, 109.08, 114.01, 117.73, 132.39, 133.92, 150.48, 168.00.

Methyl 5-chloro-1,2-dihydro-4-hydroxy-1-methyl-2-oxo-3-quinolinecarboxylate (4). Dimethyl malonate (12.30 g, 94.8 mmol) was added slowly to a suspension of sodium hydride (60% in mineral oil, 3.83 g, 94.8 mmol) in DMF (30 mL) under N_2 atmosphere. The mixture was stirred at room temperature until the evolution of hydrogen gas ceased. A solution of compound **3** (10.00 g, 47.4 mmol) in DMF (100 mL) was added slowly, and the mixture was heated to 80°C for 5 h. The mixture was cooled to room temperature, poured into ice water (100 mL), and acidified by cold 5% hydrochloride acid (90 mL). The precipitate was filtered, washed with water (2 × 20 mL), and dried at 50°C under vacuum to give the title compound (12.65 g, yield 99%) as a white solid in 98.9% purity. mp 158.8–159.7°C. ([5] 159.1–160.1°C). ^{1}H-NMR (CDCl$_3$, 600 MHz) δ: 3.66 (s, 3H), 4.05 (s, 3H), 7.25–7.30 (m, 2H), 7.53 (t, $J = 8.1$ Hz, 3H), 14.96(s, 1H). ^{13}C-NMR (CDCl$_3$, 150 MHz) δ: 30.10, 53.25, 97.92, 112.28, 113.33, 126.00, 134.38, 134.45, 143.44, 158.71, 172.59, 173.31.

5-Chloro-1,2-dihydro-*N*-ethyl-4-hydroxy-1-methyl-2-oxo-*N*-phenyl-3-quinoline carboxamide (1). A mixture of compound **4** (10.00 g, 37.5 mmol) and *N*-ethylaniline (10 mL, 75.0 mmol) in heptane (200 mL) was heated to reflux. The methanol formed during the reaction was distilled off together with some heptane (120 mL) for 6 h. After cooling to room temperature, the precipitate was filtered, washed with heptane (2 × 20 mL), and dried at 50°C under vacuum to give the crude title compound (13.35 g, yield 97%) as a white crystal in 99.5% purity. mp 200.8–201.5 ([5] 201°C). ^{1}H-NMR (CDCl$_3$, 600 MHz) δ: 1.21 (t, $J = 6.9$ Hz, 3H), 3.29 (s, 3H), 3.99 (q, $J = 6.9$ Hz, 2H), 7.12–7.27 (m, 2H), 7.41 (t, $J = 8.1$ Hz, 1H), 12.64 (br, 1H). ^{13}C-NMR (CDCl$_3$, 150 MHz) δ: 12.91, 29.82, 45.68, 105.37, 112.72, 113.28, 125.40, 126.71, 126.94, 128.54, 131.64, 132.67, 142.05, 142.57, 157.99, 165.26, 168.69.

Methyl 7-chloro-1,2-dihydro-4-hydroxy-1-methyl-2-oxo-3-quinolinecarboxylate (5). Compound **5** was similarly synthesized as compound **4** in total yield of 76%. mp 201.9–202.5°C. ^{1}H-NMR (CDCl$_3$, 600 MHz) δ: 3.66 (s, 3H), 4.05 (s, 3H), 7.23 (dd, $J = 12.6$ and 1.8 Hz, 1H), 7.32 (d, 1.8 Hz, 1H), 8.12 (d, $J = 13.2$ Hz, 1H), 14.11 (s, 1H). ^{13}C-NMR (CDCl$_3$, 150 MHz) δ: 172.73, 170.97, 159.24, 141.90, 140.84, 126.99, 122.41, 114.09, 113.14, 97.66, 53.04, 29.26. HRMS [electrospray ionization (ESI)], calcd for $C_{12}H_{10}ClNO_4$ $[M + Na]^+$ m/z: 290.0118; found: 290.0190.

7-Chloro-1,2-dihydro-*N*-ethyl-4-hydroxy-1-methyl-2-oxo-*N*-phenyl-3-quinoline carboxamide (6). A mixture of compound **5** (10.00 g, 37.5 mmol) and *N*-ethylaniline (10 mL, 75.0 mmol) in heptane (200 mL) was heated to reflux. The methanol formed during the reaction was distilled off together with some heptane (120 mL) for 6 h. After cooling to room temperature, the precipitate was filtered. Sodium hydroxide solution (5%; 50 mL) was added to the filtrate and stirred for 15 min. After filtering, the filtrate was acidified by hydrochloric acid. The mixture was centrifuged, and the residue was dried at 50°C under vacuum to give the crude title compound (5.34 g, yield 56%) as a white solid (assay 97.7%). mp 143.8–146.5°C. ^{1}H-NMR (CDCl$_3$, 600 MHz) δ: 1.22 (t, $J = 7.2$ Hz, 3H), 3.20 (s, 3H), 3.99 (q, $J = 7.2$ Hz, 2H), 7.12–7.27 (m, 7H), 8.00 (d, $J = 8.4$ Hz, 1H), 12.48 (br, 1H). ^{13}C-NMR (CDCl$_3$, 150 MHz) δ: 12.90, 28.93, 45.55, 106.07, 113.49, 114.21, 121.75, 126.03, 126.67, 127.27, 128.62, 138.38, 140.72, 141.66, 159.00, 162.61, 168.64. HRMS (ESI), calcd for $C_{19}H_{17}ClN_2O_3$ $[M + Na]^+$ m/z: 379.0747; found: 379.0822.

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