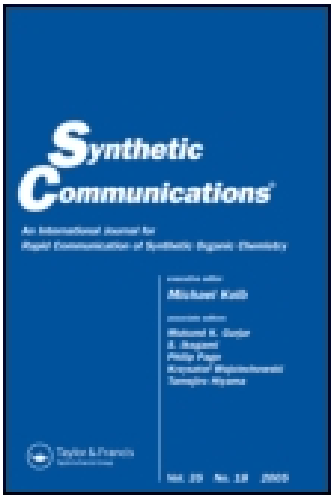

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Improved Experimental Procedure for the Synthesis of the Potent MEK Inhibitor PD184352

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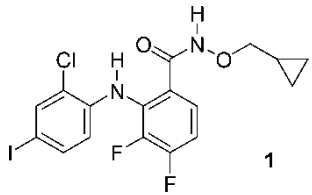
Abstract: An improved synthesis of the potent MEK inhibitor PD184352 (2-(2-Chloro-4-iodo-phenylamino)-N-cyclopropylmethoxy-3,4-difluoro-benzamide)) is herein reported. This new and reproducible protocol provides a simple and efficient way of generating gram quantities of PD184352 with minimal purification.

Keywords: Inhibitor, Hydroxylamines, PD184352

As part of our efforts to study the selectivity and efficacy of some known kinase inhibitors, we became interested in the MEK inhibitor PD184352. PD184352, or 2-(2-chloro-4-iodo-phenylamino)-*N*-cyclopropylmethoxy-3,4-difluoro-benzamide, was originally reported by Warner Lambert in 1998 as a potent MEK/ERK kinase cascade inhibitor that could potentially be used as a treatment for septic shock.[1]



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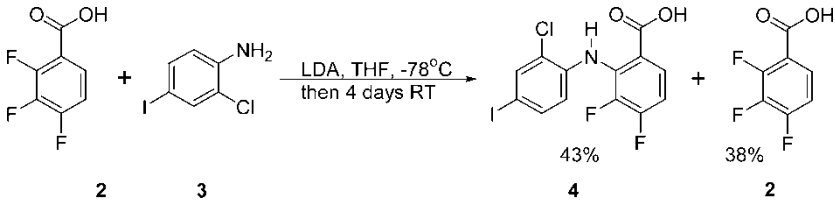
Unfortunately, the reported synthesis of PD184352 requires a number of nontrivial low-yielding steps, which can seriously limit its availability for biological studies. Thus, we now report an improved practical procedure that can generate the desired benzamide **1** in increased yields compared with those reported thus far.[2]

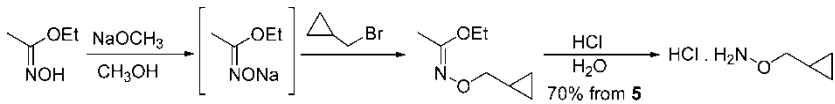
In our modified procedure, 2,3,4-trifluoro benzoic acid **2** is treated with 4-iodo-2-chloro-aniline **3** under lithium diisopropyl amide (LDA)-promoted conditions to generate the desired benzoic acid **4** in reasonable yield. Interestingly, despite various procedural modifications, conversion of the triflurobenzoic acid **2** to the corresponding methyl benzoate failed to improve the coupling yield.



2 **3** **4** **2**

Once the desired carboxylic acid unit **4** was available, we were faced with the challenge of introducing the required cyclopropylmethyl hydroxylamine unit. After a considerable amount of experimentation in which a variety of approaches were considered, the most promising one appeared to involve the direct peptide coupling of a cyclopropylmethyl hydroxylamine unit to carboxylic acid **4**.

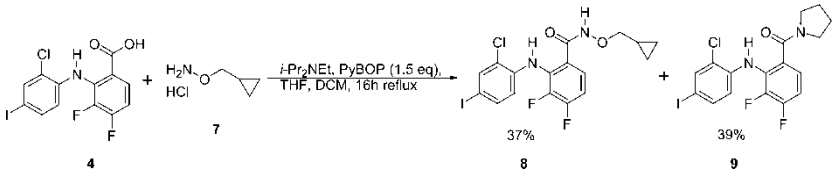
Generation of the required noncommercially available cyclopropylmethyl hydroxylamine hydrochloride unit began with ethyl N-hydroxyacetimidate **5**, which was efficiently deprotonated and alkylated to generate the desired alkylated N-hydroxyacetimidate **6**.[3] Acid hydrolysis of the crude acetamidate **6** then cleanly generated the desired cyclopropyl hydroxylamine hydrochloride **7** and ethyl acetate in good yield for the whole sequence.



5 **6** **7**

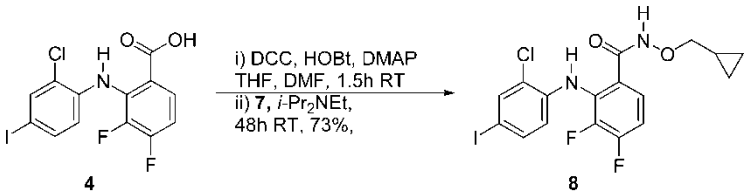
However, peptide coupling of the newly generated cyclopropylmethyl hydroxylamine **7** to benzoic acid **4** under the conditions originally reported by Bridges, namely, through the use of Benzotriazolyloxy tripyrrolidinophosphonium hexafluorophosphate PyBOP and diisopropyl ethyl amine (DIPEA), failed to afford the desired amide **8** in greater than 37%

yield. A greater amount of the resulting product was composed of the undesired and difficult to separate pyrrolidine amide **9** (39%). Interestingly, biological testing of this pyrrolidine amide side product showed much lower Mitogen activated protein kinase kinase/extracellular signal regulated kinase (MEK) inhibitory activity when compared to PD184352.[4]



4 7 8 9

Faced with this disappointing yield, a significant amount of time and effort was spent on the optimization of the coupling conditions of cyclopropylmethyl hydroxylamine **7** to the difluoro carboxylic acid **4**. Finally, coupling under similar conditions to those reported by Tripathy and Georg significantly increased the yield as to afford the desired MEK inhibitor PD184352 in 73% yield.[5]



4 8

This reproducible, optimized process is amenable to scaling and has finally allowed us to generate multigram quantities of PD184352 for all of our testing needs.

In conclusion, we have managed to modify and improve the yields for the synthesis of the potent kinase inhibitor PD184352. This reliable protocol should be able to provide most researchers with a simple way of generating gram quantities of PD184352 for biological research.

EXPERIMENTAL PROCEDURES

Synthesis of 2-(2-Chloro-4-iodo-phenylamino)-3,4-difluoro-benzoic Acid, 4

A -78°C solution of commercially available 4-iodo-2-chloro aniline **3** (1.58 g, 6.25 mmol) in THF (10 mL) was treated with a 1.8-M solution of lithium diisopropyl amide in heptane/tetrahydrofuran/ethylbenzene (6.63 mL, 11.93 mmol). The resulting suspension was stirred at -78°C for 15 min, at which point a solution of 2,3,4-trifluorobenzoic acid **2** (1 g, 5.68 mmol) in THF (15 mL)

was added over 15 min. The reaction was then allowed to warm up to room temperature over 4 h and subsequently stirred at room temperature for 4 days.

The reaction was then diluted with diethyl ether (100 mL) and acidified with 1M HCl, until the aqueous phase reached a pH of 1. The aqueous layer was then extracted with diethyl ether (3 × 15 mL), and the combined organic extracts dried over sodium sulphate. Solvent removal under vacuum afforded a crude solid, which was recrystallized from dichloromethane to afford 994 mg (43%) of the pure carboxylic acid **4** and 381 mg of recovered starting acid (38%). δ_H (300 MHz, DMSO-d_6) 6.73 (1H, dd, $J = 8.5$, 7.0 Hz), 7.18 (1H, td, $J = 9.4$, 7.3 Hz), 7.55 (1H, dd, $J = 8.6$, 1.9 Hz), 7.88–7.82 (2H, m), 9.27 (1H, bs), 13.9 (1H, bs).

Synthesis of *O*-cyclopropylmethyl-hydroxylamine Hydrochloride, 7

Sodium metal (670 mg, 29.09 mmol) was added to anhydrous methanol (45 mL), and the resulting suspension stirred at room temperature until the sodium was completely dissolved. The resulting anhydrous solution of sodium methoxide was then treated with ethyl *N*-hydroxyacetamidate (3 g, 29.09 mmol) in small portions. The resulting solution was then allowed to stir at room temperature for 1 h before the solvent was removed under vacuum to generate a white solid. The crude white residue was then suspended in ether (20 mL), filtered under argon, and subsequently dried under vacuum to afford ethyl N-hydroxy acetamidate sodium salt as a hygroscopic white solid, which was used without any further purification.

Dry acetonitrile (100 mL) was added to the crude ethyl *N*-hydroxy-acetamidate sodium salt, and the resulting suspension was then treated with neat cyclopropyl methyl bromide (2.15 mL, 22 mmol). The reaction was then stirred under argon at room temperature for 24 h and then refluxed for an additional 3 h. The reaction mixture was then allowed to cool down to room temperature and diluted with diethyl ether (100 mL), causing a white precipitate to form. The solid was removed through filtration, and the resulting filtrate concentrated under vacuum to generate a yellowish residue. The crude residue was then dissolved in 2-M HCl (50 mL), and the resulting solution refluxed for 1 h. The reaction was then cooled to room temperature and concentrated under vaccum to afford an amorphous solid. The resulting solid was then dissolved in methanol (30 mL) and slowly reconcentrated under vaccum as to induce precipitation of a white solid. Removal of the last traces of solvent under vacuum finally afforded 1.89 g (70%) of the desired hydrochloride salt as a white solid. Mp 114–117°C.

Synthesis of PD184352, 8

A solution of benzoic acid **4** (2.01 g, 4.91 mmol) in a 1 : 1 mixture of anhydrous THF and anhydrous DMF (40 mL total volume) was treated with 1,3-

dicyclohexylcarbodiimide (DCC) (1.52 g, 7.36 mmol), which after stirring for 15 min caused a white precipitate to form. The suspension was then treated with 1-hydroxybenzotriazole hydrate (HOBT) (0.99 g, 7.36 mmol) and 4-dimethylamino pyridine (DMAP) (120 mg, 0.98 mmol). The resulting mixture was then stirred at room temperature for 1.5 h, after which freshly prepared cyclopropyl methyl hydroxylamine hydrochloride **7** (1.21 g, 9.81 mmol) and diisopropyl ethyl amine (2.56 mL, 14.72 mmol) were sequentially added. The complete reaction mixture was then stirred at room temperature for 48 h.

Solvent removal under vacuum afforded a crude residue, which was purified by flash column chromatography (silica gel, 50% diethyl ether in 40–60 petroleum ether) to produce a whitish solid, which was subsequently recrystallized from ethanol and methanol respectively to provide 1.72 g (73%) of PD184352, **8**, as a white solid. δ_H (300 MHz, $CDCl_3$) 0.34–0.28 (2H, m), 0.65–0.59 (2H, m), 1.28–1.11 (1H, m), 3.78 (2H, d, $J = 7.3$ Hz), 6.48 (1H, dd, $J = 8.5$, 5.5 Hz), 6.96 (1H, appq, $J = 8.9$ Hz), 7.44–7.38 (2H, m), 7.70 (1H, d, $J = 1.9$ Hz), 8.17 (1H, bs), 8.87 (1H, bs). Mp 175.5–176.5°C (lit. 172.5–173.5°C).

NMR Data for [2-(2-Chloro-4-iodo-phenylamino)-3,4-difluoro-phenyl]-pyrrolidin-1-yl-methanone, 9

δ_H (300 MHz, $CDCl_3$) 1.95–1.82 (4H, m), 3.40 (2H, appt, $J = 6.3$ Hz), 3.55 (2H, appt, $J = 6.8$ Hz), 6.52 (1H, dd, $J = 8.6$, 5.8 Hz), 6.94 (1H, td, $J = 9.0$, 7.0 Hz), 7.17 (1H, ddd, $J = 8.6$, 5.4, 2.0 Hz), 7.40 (1H, dd, $J = 8.6$, 1.9 Hz), 7.64 (1H, d, $J = 2.0$ Hz), 7.72 (1H, bs). δ_C (75 MHz, $CDCl_3$) 24.72, 26.61, 46.61, 49.91, 81.61, 111.46 (d, $J = 17.8$ Hz), 117.82 (d, $J = 5.3$ Hz), 123.36, 123.88, 126.43, 129.99 (d, $J = 6.9$ Hz), 136.32, 137.64, 139.60, 144.70 (dd, $J = 250.9$, 14.5 Hz), 152.36 (dd, $J = 250.6$, 11.5 Hz), 167.02. IR (*V*max) film/cm^{-1} 3344, 2924, 1630, 1464, 1105, 912, 743, 650, 503. m/z (CI) 463.0 (100%, MH+), 465.0 (27).

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