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A Different Approach to the EGFR Inhibitor Gefitinib Involving Solid-Phase Synthesis

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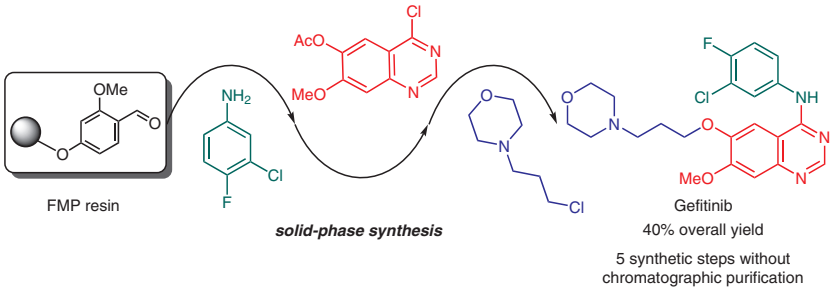

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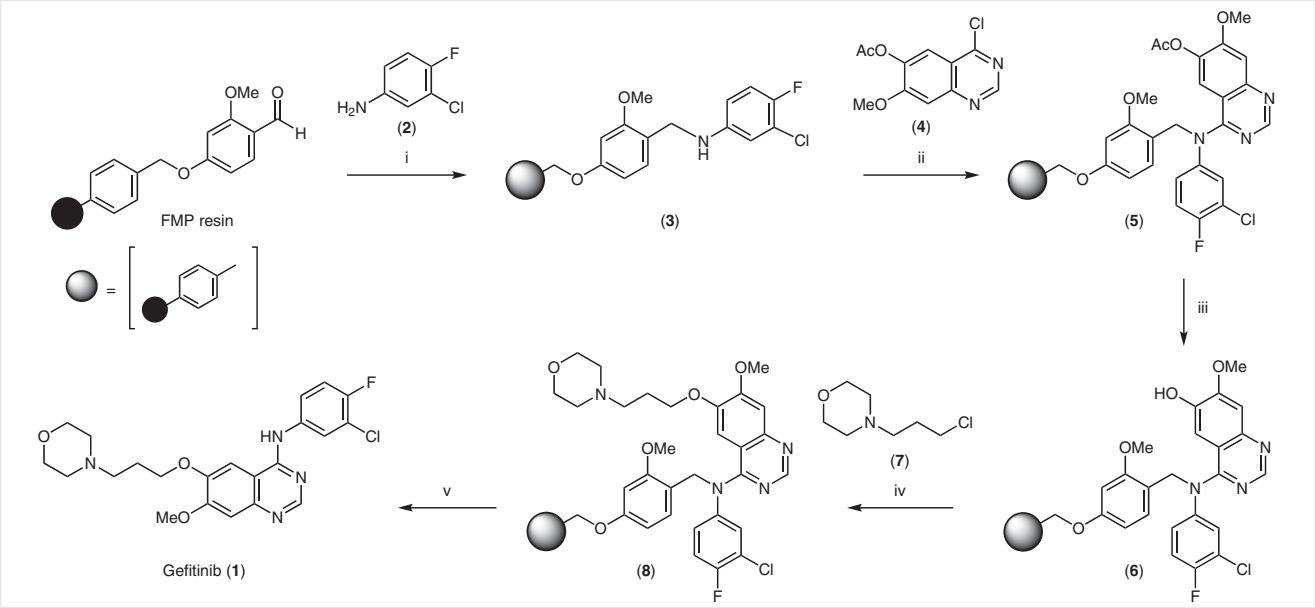
Abstract An efficient solid-phase synthesis approach is here reported for the first time to prepare the EGFR inhibitor Gefitinib. The five-step synthetic strategy used FMP resin as the solid support, and FTIR and colorimetric assays were used to track the reaction's progress. Gefitinib was obtained with an overall yield of 40%.

Key words solid-phase synthesis, Gefitinib, quinazoline, FMP resin, colorimetric assays

Gefitinib (**1**) is an orally administered drug indicated for the treatment of adults with locally advanced or metastatic non-small cell lung cancer.[1,2] It is responsible for the selective and reversible inhibition of the epidermal growth factor receptor (EGFR),[1,2] which is overexpressed in many epithelial carcinomas[3] and is involved in several cellular responses, such as proliferation, invasion and metastasis of cancer cells, and inhibition of its apoptosis.[4] Gefitinib is a quinazoline derivative and various methods to efficiently synthesize this compound were previously developed. Gibson *et al.* in 1996, patented the first synthetic strategy with six steps and an overall yield of approximately 20%, using a quinazolinone derivative as starting material.[5] Ten years later, Knesl *et al.* employed the same synthetic strategy, improving the yield to 33%.[6] In 2005, Gilday *et al.* patented a five-step synthetic method to obtain Gefitinib using isovanillin, avoiding chromatographic procedures to isolate the intermediates, in order to increase the overall yield, which was achieved in 51%.[7] Aggarwal *et al.* in 2010 (13%, eight steps) and Zhang *et al.* in 2015 (56%, five steps) developed synthetic strategies using the methyl ester derivative of isovanillin and 3-hydroxy-4-methoxybenzonitrile as starting materials, respectively.[8,9] Most recently, in 2016, it was reported a one-pot synthetic strategy by Chao *et al.*[10] using a quinazolinone derivative, with an overall yield of 86% for the quinazolinone deprotection and alkylation with *N*-(3-chloropropyl) morpholine. The several strategies that emerged after the first patented synthesis of Gefitinib (**1**), allowed to obtain a pure product with a considerable increase of the overall yield. However, the synthetic process must be also applicable to industry. The use of large excess of reagents, the presence of unexpected impurities, and some reaction steps which are not applicable and economically sustainable in a scale-up (industrial) process may become obstacles for the use of certain strategies.

Solid-phase synthesis (SPS)[11] provides several advantages, such as efficient and simple workup procedures, not requiring purification of the intermediates/final product. The large excess of reagents that can be used in each step may be recovered, and most important, is a process which can be applied industrially, since it is easily adapted to an automated process[11,12] leading to faster and safer reactions.[13–15] Methods to monitor solid-phase reactions, such as FTIR analysis,[16] high resolution magic-angle spinning (HRMAS) NMR spectroscopy,[17,18] and colorimetric assays,[19] allows SPS to be a good alternative to solution-phase synthesis. Historically, the industrial application of SPS methods is concerned mainly with the manufacture of peptide-based active pharmaceutical ingredient (APIs).[20,21] However, other molecules with pharmaceutical interest, such as oligonucleotides precursors[22] and aromatic amides[23,24] are also synthesized using a solid-phase strategy. Solid-phase strategies were also frequently used for the design, synthesis, and screening of libraries based on natural product templates.[25,26] Here we report an innovative and efficient solid-phase approach to the synthesis of Gefitinib using FTIR spectroscopy and colorimetric assays to track the reaction's sequence.



Scheme 1 Proposed solid-phase synthetic route for Gefitinib (**1**). *Reagents and conditions*: i) $NaBH(OAc)_3$, AcOH 2% in DCM/DCE (1:1), 3-chloro-4-fluoraniline (2), RT; ii) 4-chloro-6-acetoxy-7-methoxyquinazoline (**4**), DMF, Na_2CO_3, 100 °C; iii) NH_4OH/DMF (1:1), RT; iv) 4-(3-chloropropyl)morpholine (**7**), NaOH, DMSO, 85 °C; v) TFA 10% in DCM, RT.

The proposed solid-phase strategy for the synthesis of Gefitinib is shown in Scheme 1. The solid support chosen for this synthesis was the 4-(4-formyl-3-methoxyphenoxy)ethyl (FMP) resin, which possesses a terminal aldehyde functionality.

The aldehyde group allows the easy attachment of 3-chloro-4-fluoroaniline (**2**), and the removal of intermediates and the final product under mild conditions.[27] In parallel, our attempts to assemble the desired compound using Merrifield resin were unsuccessful, due to the difficulty to remove the synthetic products from this resin. FTIR analysis and colorimetric assays were used to monitor the reactions outcome.[19] Moreover, stepwise cleavage of intermediates was performed to track the evolution of the reaction by confirming the presence of intermediate products and the absence of undesired impurities.

The first step of the synthesis consisted on a reductive amination between the resin-bound aldehyde with 3-chloro-4-fluoroaniline (**2**) followed by addition of a proper reducing agent. Initially, the reaction was conducted using $NaBH_3CN$ in DMF. However, it was observed that three additions of $NaBH_3CN$ (3 × 5 equiv) were required along 96 hours to completely functionalize the resin (tracking by colorimetric test). With this prolonged reaction time, the direct reduction of the aldehyde group to the alcohol was observed by FTIR spectroscopy. Using a reported procedure,[28] which employs $NaBH(OAc)_3$ in DCM/DCE, the reaction was complete in 17 hours with only one addition of 2 equiv of the reducing agent. The colorimetric assays confirmed the absence of the free aldehyde (negative result to 2-4-dinitrophenylhydrazine (DNP) test)[29] and the presence of the desired product **3** (positive result with chloranil/acetaldehyde in DMF, test for secondary amines).[30] FTIR analysis also confirmed the presence of a secondary amine due to a broad band with a maximum at 3411 cm^{-1} (Figure 1).

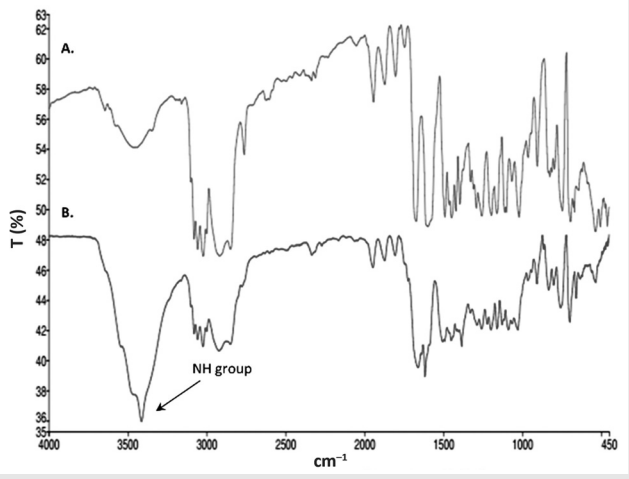


Figure 1 FTIR spectrum of: **A.** FMP-resin; **B.** compound **3**.

To perform the second step, 6-acetoxy-7-methoxy-4-chloro-quinazoline (**4**) was synthesized following a reported procedure.[6] The solution-phase reaction between **2** and **4** use conditions[6] which cannot be applied in solid-phase synthesis, e.g., the use of polar protic solvents.[31] Several conditions were tested, such as different solvents, bases, and reaction temperatures (Table 1). Dioxane was shown to be a poor solvent yielding an incomplete reaction (Table 1,

Table 1 Reactions Conditions for the Preparation of **5**

Entry	Compound **4** (equiv)	Base (equiv)	Solvent	Temp. (°C)	Reaction time (h)	Observations
1	5	Na_2CO_3(5)	Dioxane	85	144	incomplete
2	5	Na_2CO_3 (5)	DMF	85	144	complete
3	5	Na_2CO_3(5)	DMF	100	96	complete
4	5	K_2CO_3(5)	DMF	85	144	complete
5	10	Cs_2CO_3(10)	DMF	85	144	formation of compound **9**
6	5	DBU (5)	DMF	100	144	no reaction

entry 1). Using DMF and independently of the base used, all the reactions were completed probably due to the better solubility of compound **4** in this solvent (Table 1, entries 2–5). Moreover, the best conditions, which correspond to the shorter reaction time (96 h), were achieved with a reaction temperature of 100 °C (Table 1, entry 3). No reaction was observed when DBU was used as base (Table 1, entry 6).

The colorimetric assays confirmed the absence of the secondary amine **3** (negative result for secondary amines test[30]). FTIR analysis confirmed the presence of an acetyl group at 1739 cm^{-1} and a hydroxyl group with a broad band with a maximum at 3490 cm^{-1}(Figure 2, spectrum B), resulting from the deacetylation of **5** to give the resin-bound intermediate **6**.

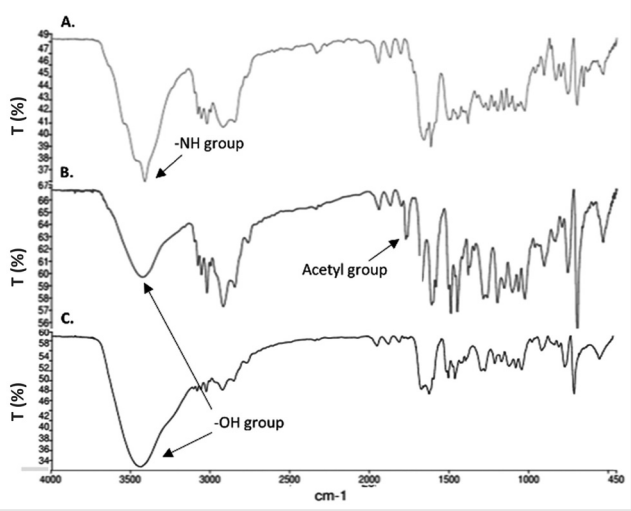


Figure 2 Comparison between FTIR spectra of: **A.** compound **3**; **B.** mixture of compounds **5** and **6**; **C.** compound **6**.

The intermediate cleavage from a resin aliquot confirmed the presence of the two products, the acetylated and deacetylated intermediates. It is noteworthy that in all cases the hydrolysis of compound **4** to the corresponding quinazolinone was observed during the reaction. This degradation was more evident in the reaction using cesium carbonate (Table 1, entry 5), where an extra amount of **4** was added. When attempting to scale-up the reaction using 1.5 g of **3**, and 10 equiv of **4**, the formation of acetylated compound **9** (isolated at the end of the synthesis and after cleavage from the resin) was observed (Figure 3) resulting from acetylation of **3** in the presence of excess of **4**.

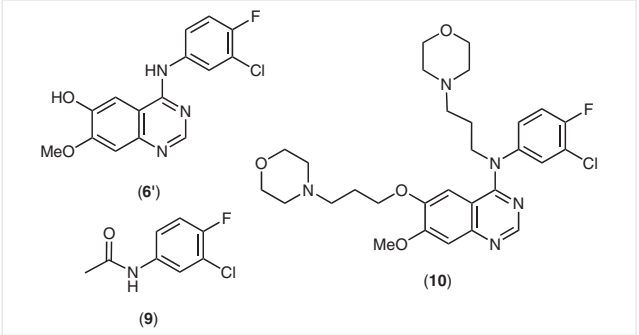


Figure 3 Byproducts found in the solid-phase approach to the synthesis of Gefitinib (**1**)

The third step involving the deacetylation of **5** to **6** was performed using a mixture of ammonia hydroxide solution and DMF at room temperature. It was observed that DMF was necessary to maintain the resin swelling after the addition of the aqueous solution. FTIR analysis confirmed the absence of the acetyl group and the increase of hydroxyl group band (Figure 2, spectrum C). Moreover, cleavage of resin-bound intermediate **6** confirmed the presence of the secondary amine (**6'**).

The fourth step comprised the insertion of the propyl morpholine side chain to attain **8** and was assayed using different bases and solvents (Table 2). The reaction was firstly attempted using potassium carbonate in DMF at 80 °C (Table 2, entry 1) employing a reported procedure for solution phase.[6] However, after 144 hours the reaction was not completed. The use of DMSO and NaOH allowed a complete reaction after 72 hours (Table 2, entry 2). DMSO was easily removed from resin by several washing cycles with water. An attempt to decrease the reaction time was performed under neat conditions, but without success, due to the formation of a secondary product – the dialkylated product **10** (Figure 3, Table 2, entry 3).

Table 2 Reaction Conditions for the Alkylation Step with 4-(3-Chloropropyl)morpholine (**7**)[a]

Entry	Base (equiv)	Solvent	Reaction time (h)	Observations
1	K_2CO_3(5)	DMF	144	incomplete
2	NaOH (5)	DMSO	72	complete
3	K_2CO_3(5)	–	72	formation of dialkylated product **10**

[a] Reactions performed with 5 equiv of compound **7** at 80 °C.

The removal of Gefitinib (**1**) from the resin was performed using TFA 10% in DCM at room temperature.[28,24] Gefitinib was obtained as the major product over a five-step synthesis with a reasonable overall yield of 40% (in an optimized synthetic sequence which started with 400 mg of FMP resin).[32,33] In the steps where side products were observed, optimizations were performed to minimize or eliminate these compounds. The yield of each reaction was estimated by weighting the resin after each reaction step, showing that the removal of Gefitinib from the resin was the yield-determinant step (44% as a crude, HPLC purity of 99%). Since no significative degradation products were observed, it is proposed that the final cleavage of **1** with TFA is an incomplete process and possibly requires stronger acidic conditions.

In conclusion, we have developed an efficient SPS approach to produce Gefitinib (**1**) using FMP resin. Compound **1** was obtained in 40% overall yield, a result that overcomes the first patented synthesis of Gefitinib by Gibson *et al.* Tracking of the reaction intermediates was easily performed by stepwise removal and analysis of resin aliquots. This is a novel approach to obtain Gefitinib *via* solid-phase synthesis, with applicability to other quinazoline derivatives and potentially towards a continuous-flow process.

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Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0037-1610127.

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(33) ***N*-(3-Chloro-4-fluorophenyl)-7-methoxy-6-(3-morpholino-propoxy)quinazolin-4-amine, Gefitinib (1)**
A solution of TFA 10% in dry DCM was added to the functionalized FMP resin **8** (400 mg, 0.255 mmol). The mixture was stirred for 24 h at room temperature. The resin was filtered and washed with DCM, MeOH/THF (1:1) and H_2O/THF (1:1), and the combined phases were collected and evaporated. The crude was resuspended in DCM, and a 2 M NaOH solution was added to neutralize the removed compound. The organic phase was dried over Na_2SO_4 and evaporated to dryness. Gefitinib (**1**) was obtained as a yellow solid (50 mg, 0.112 mmol, 44%, crude, HPLC purity: >99%); mp 186–188 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.65 (s, 1 H, ArH-2), 7.92–7.91 (m, 1 H, ArH-13), 7.59–7.57 (m, 1 H, ArH-16), 7.53 (br s, 1 H, NH), 7.24 (s, 1 H, ArH-7), 7.18–7.13 (m, 2 H, ArH-10 and ArH-17), 4.20 (t, J = 6.5 Hz, 2 H, H-18), 3.99 (s, 3 H, OCH_3), 3.77 (m, 4 H, H-22 and H-22′), 2.65–2.55 (m, 6 H, H-21, H-21′ and H-20), 2.15–2.12 (m, 2 H, H-19). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 156.4 (C-4), 155.3 (C-8), 153.6 (C-2), 149.0 (C-9), 147.6 (C-5), 135.6 (C-12), 124.3 (C-16), 122.2 (C-17), 121.8 (C-15), 116.8 (C-13), 109.1 (C-6), 108.1 (C-10), 101.4 (C-7), 67.7 (C-18), 66.6 (C-22 and C-22′), 56.3 (OCH_3), 55.5 (C-20), 53.7 (C-21 and C-21′), 25.9 (C-19). FTIR: ν_{max} (KBr): 3400 (m, N–H stretch), 2957, 2809 (m, C–H stretch (aliphatic)), 1625, 1579 (w, C–C stretch (Ar)), 1501 (w, N–H bend), 1219 (m, C–F stretch (Ar)) cm^{-1}.