Practical Phosphorylation Methods for α,α-Disubstituted α-Amino Alcohol Derivatives

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Abstract: We report herein practical phosphorylation methods for α,α-disubstituted α-amino alcohol derivatives which act as $S1P_1$ receptor agonists. A novel direct phosphorylation method for α,α-disubstituted α-amino alcohol derivatives by biotransformation using *Circinella muscae*, *Circinella minor*, *Circinella mucoroides*, and *Circinella umbellate* was developed. We applied the present method to the synthesis of phosphates of α,α-disubstituted α-amino alcohol derivatives.

Key words: amino alcohols, bioorganic chemistry, biotransfomation, $S1P_1$ receptor agonist, phosphorylation

FTY720 (**1**), a synthetic analogue of ISP-1 (myriocin) derived from the fungus *Isaria sinclairii*, is an orally active immunomodulator under development by Mitsubishi Tanabe Pharma and Novartis for potential use in autoimmune diseases.[1] The systemic administration of FTY720 induces a dose-responsive lowering of circulating lymphocytes and the efficacy of FTY720 has been attributed to arise from this pharmacodynamic phenomenon. According to recent information, the active phosphorylated form of FTY720 (*S*)-**2** (Figure 1), which is generated in vivo via a sphingosine kinase, acts as a sphingosine 1-phosphate-1 ($S1P_1$) receptor agonist. It has recently been shown to induce internalization of the $S1P_1$ receptor, rendering lymphocytes unresponsive to S1P present in the blood, and thus depriving T and B cells of an obligatory signal to exit from lymphoid organs.[2] On the other hand, Hinterding et al. also reported the first asymmetric synthesis of chiral FTY720 analogue **3** and its phosphate **4**, and revealed that only the phosphate of *R*-enantiomer (*R*)-**4** has strong binding affinity on S1P receptors.[3] Therefore, chiral analogues of FTY720, such as (*S*)-**2** and (*R*)-**4**, are invaluable tools to differentiate biological effects and to further elucidate FTY720's mechanism of action.

To date, several synthetic methods for the preparation of **2** and **4** have been developed.[4–7] In general, chemical synthetic methods are based on phosphorylation with a trivalent phosphorylating agent followed by hydrogenation. Recently, Takeda et al. reported a novel method for the direct phosphorylation of various 1,3-diols using silver(I) oxide, tetrabenzyl pyrophosphate, and tetrahexylammonium iodide, and they applied this to the synthesis of FTY720-phosphate.[6] However, the synthetic efficiency was still unsatisfactory, mainly because of tedious protection–deprotection procedures. To overcome this drawback, practical and straightforward reactions are required.

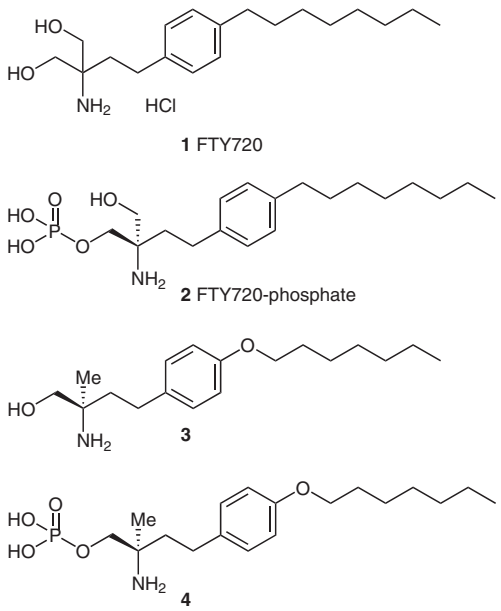

Figure 1

Our preliminary studies on $S1P_1$ receptor agonists indicated that compounds possessing pyrrole, thiophene, and furan analogues exhibited equal or more potent immunosuppressive efficacy than FTY720 in vivo. Among the series of pyrrole analogues, compounds such as **8** brought us to realize a wide range of structure–activity relationships. In this context, and as a part of our research program, we first focused our attention on the synthesis of the phosphate of **8**. The synthetic route of **8** is shown in Scheme 1. We already reported on the practical method used for the preparation of optically active (2*R*)-amino-2-methyl-4-(1-methylpyrrol-2-yl)butan-1-ol (**5**) via enzymatic desymmetrization of 2-*tert*-butoxycarbonylamino-2-methylpropane-1,3-diol.[8] Treatment of **5** with Ac_2O and Et_3N resulted in providing the diacetate **6** in good yield. Then, acylation at the 5-position on the pyrrole ring was carried out in the presence of an excess amount of 5-phenylpentanoyl chloride and 4-(dimethylamino)pyridine (DMAP) in toluene at 110 °C to afford

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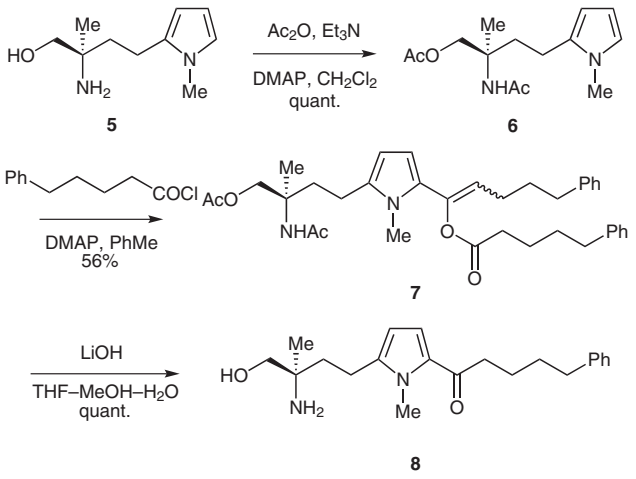
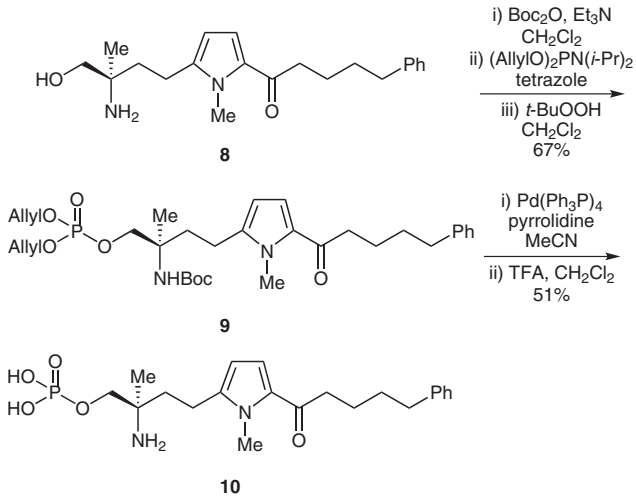


Scheme 1

enol ester **7**. The two acetyl groups and the enol ester moiety were removed by saponification with aqueous LiOH in THF–MeOH to afford **8** in good yield.

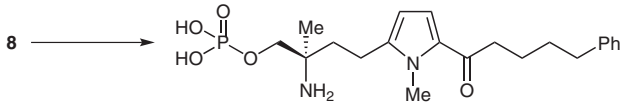
The chemical synthesis of the phosphate of **8** was carried out as shown in Scheme 2. After protection of the amino group of **8** with Boc_2O and Et_3N in CH_2Cl_2, phosphorylation using $(AllylO)_2PN(i\text{-}Pr)_2$ was performed in the presence of tetrazole, and subsequent treatment with *t*-BuOOH in CH_2Cl_2 cleanly provided **9** in moderate yield. Both the allyl and Boc groups of **9** were deprotected to give the desired **10**. But this synthetic route was still unsatisfactory because of the low yield and multistep reactions.



Scheme 2

Due to the difficult physicochemical properties of amino phosphates, in particular their low solubility, and due to the lack of availability of sphingosine kinases, their biochemical synthesis and isolation seems to be tedious. In order to circumvent these issues, we sought to develop one-step biotransformation of α,α-disubstituted α-amino alcohol derivatives. Although many successful examples of phosphorylation methods using microorganisms have already been reported,[9] to the best of our knowledge, there are no examples of the phosphorylation of α,α-disubstituted α-amino alcohol derivatives. Screenings for the organisms to provide the biotransformation of **8** was performed using 106 strains consisting of 84 actinomycetes, 33 fungi, and 25 bacteria from our microbial library. Among them, strains belonging to *Circinella muscae*, which was reported to have microbial phosphorylation activity of ML-236B,[10] were found to convert **8** to phosphate **10** in good yield. In particular, a culture of the strain NBRC 4457 exhibited significant conversion.[11] As shown in Table 1, from among the numerous organisms screened, *Circinella muscae*, *Circinella minor*, *Circinella mucoroides*, and *Circinella umbellate* clearly showed good results.[12]

Table 1 Conversions of Microorganisms



Run	Microorganisms[a]	Conversion (%)
1	*Circinella muscae* (NBRC 4457)	82
2	*Circinella minor* (NBRC 6448)	75
3	*Circinella mucoroides* (NBRC 4453)	57
4	*Circinella umbellate* (NBRC 5842)	92

[a] Each microorganism was cultivated at 23 °C for 2 d in 100 mL Erlenmeyer flasks containing 30 mL of the medium consisting of 0.1% malt extract, 0.3% corn steep liquor, 1.0% glucose, 0.1% polypepton, 0.1% yeast extract, and 0.1% NaH_2PO_4, and the fermentation broth (1 mL) was transferred to 24-well microplate. To each well of 24-well microplate, 0.8 mL of **8** (40 mg, 1.1×10^{-4} mol) dissolved in 0.01% aq formic acid (10 mL) was added. The reaction was carried out at 23 °C for 2 d on a rotary shaker at 250 rpm.

Thus, the biotransformation method developed here is more practical and efficient than the previous published routes for the phosphorylation of α,α-disubstituted α-amino alcohol derivatives. We applied this method to the preparation of the phosphates of various kinds of α,α-disubstituted α-amino alcohol derivatives, such as thiophene and furan analogues **11**, **12** as shown in Figure 2. The desired phosphates of **11** and **12** could be

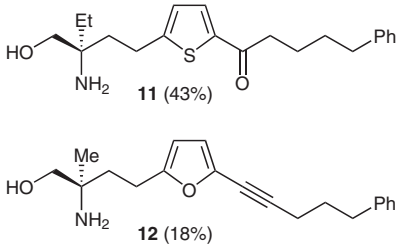


Figure 2

obtained with the same procedure using *Circinella muscae* (NBRC 4457) in one step.

In conclusion, this novel direct biotransformation has been shown to provide a useful procedure for the preparation of the phosphates of α,α-disubstituted α-amino alcohol derivatives using *Circinella muscae*, *Circinella minor*, *Circinella mucoroides*, and *Circinella umbellate*. We successfully applied this method to the preparation of the phosphates of various kinds of α,α-disubstituted α-amino alcohol derivatives. More detailed applications and further work in elucidating the biological activities will be described in future publications.

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(11) **Typical Biotransformation Procedure**
Circinella muscae was cultivated at 23 °C for 2 d in Erlenmeyer flasks containing 80 mL of the medium consisting of 0.1% malt extract, 0.3% corn steep liquor, 1.0% glucose, 0.1% polypepton, 0.1% yeast extract, and 0.1% NaH_2PO_4 after seed cultivation at 23 °C for 2 d. To each of five Erlenmeyer flasks containing the fermentation broth, 0.8 mL of **8** (40 mg, 1.1×10^{-4} mol) dissolved in 0.01% aq formic acid (4 mL) was added in order to start the biotransformation. The reaction was carried out at 23 °C for 3 d on a rotary shaker at 210 rpm. The fermentation broth (400 mL) was extracted with an equal volume of acetone with 40 μL of H_3PO_4, and the mixture was filtered. To the filtrate, an equal volume of distilled H_2O was added, and then the mixture was adsorbed onto a column (40 mL) of DIAION HP-20 packed with 0.1% aq H_3PO_4. The column was washed with H_2O (200 mL) and eluted with 30% and 50% acetone containing 10 mM $HCOONH_4$ (pH 8.0). Both fractions were combined and concentrated in vacuo and lyophilized to give crude powder (67.8 mg). The crude powder was purified by preparative HPLC using a Develosil ODS UG-5 (150 × 20 mm i.d., Nomura Chemical Co., Ltd., Japan) with 30% aq MeCN containing 10 mM $HCOONH_4$ (pH 8.0) as a mobile phase with a flow rate of 10 mL/min. The fractions were concentrated in vacuo and lyophilized to give **10** (34.7 mg, 70% yield) as a colorless powder. IR (KBr): 3429, 2934, 2857, 2717, 2603, 1639, 1557, 1480, 1455, 1378, 1182, 1056, 1041, 946, 915 cm^{-1}. ^1H NMR (400 MHz, CD_3CO_2D): δ = 7.25–7.22 (m, 2 H), 7.17–7.11 (m, 3 H), 7.07 (1 H, d, *J* = 4.4 Hz), 6.04 (1 H, d, *J* = 4.4 Hz), 4.17 (2 H, d, *J* = 10.3 Hz), 3.87 (s, 3 H), 2.82–2.71 (m, 4 H), 2.63 (2 H, t, *J* = 7.3 Hz), 2.20–2.01 (m, 2 H), 1.75–1.63 (m, 4 H), 1.46 (s, 3 H). MS–FAB: *m/z* = 421 $[M - H]^-$.

(12) The conversion ratio was determined by the peak-area ratios of alcohol **8** and phosphate **10** using RP-HPLC. The HPLC conditions were follows: mobile phase, 30% aq MeCN containing 10 mM $HCOONH_4$ (pH 4.0); column, Unison UK-C18 (75 × 4.6 mm i.d., Imtakt Corp., Japan); flow rate, 0.8 mL/min; detection, UV 280 nm. Compounds **8** and **10** were eluted at t_R = 6.7 and 3.2 min, respectively.