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First asymmetric synthesis of chiral analogues of the novel immunosuppressant FTY720

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Abstract—FTY720 is an immunosuppressant with a novel mode of action and is highly effective in animal models of transplantation and autoimmunity. Herein we describe the first asymmetric synthesis of chiral FTY720 analogues using the Schöllkopf-protocol. We also describe a practical synthesis of the corresponding phosphates, which are essential tools for elucidation of FTY720's mechanism of action.

FTY720 **1** (Fig. 1) is a novel immunosuppressant which is highly effective in animal models of transplantation and autoimmunity.[1] Additionally, in a recently completed Phase II trial, the drug has proven efficacious in preventing kidney allograft rejection in humans.[2] Unlike any other immunosuppressant currently on the market, FTY720 does not inhibit T- and B-cell proliferation and activation at therapeutically relevant concentrations in vitro; instead it leads to a sequestration of lymphocytes from the periphery into secondary lymphoid organs.[3] According to our current understanding, the phosphorylated molecule FTY720-phosphate **2** (Fig. 1), which is generated in vivo via a sphingosine-kinase, signals as an agonist through four of five sphingosine-1-phosphate (S1P) receptors (formerly known as EDG-receptors).[4]

Using chiral FTY720 analogues (*R*)-**3** and (*S*)-**3** and their corresponding phosphates (*R*)-**4** and (*S*)-**4** (Fig. 1) revealed that only *R*-enantiomer (*R*)-**3** is biologically active in vivo and only phosphate (*R*)-**4** has low nanomolar affinities on S1P-receptors.[4] Chiral analogues and phosphates are therefore invaluable tools to differentiate biological effects and to further elucidate FTY720's mechanism of action.

We herein describe a practical and versatile stereoselective synthesis of chiral FTY720 analogues **3** and their corresponding phosphates **4**. This synthetic protocol is also amenable to the preparation of additional structural analogues.

The Schöllkopf-protocol[5] is the centerpiece of our synthesis. It was chosen because both Gly- and Ala-derived auxiliaries are commercially available in either enantiomeric form.[6] In addition, the protocol allows for broad structural variations at the quaternary center, which was considered to be advantageous for the gener-

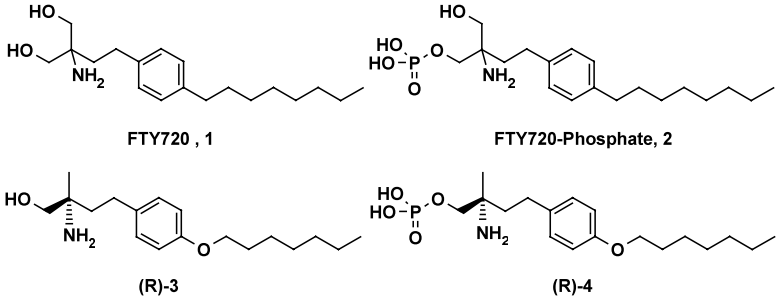

Figure 1. Structures of immunosuppressant FTY720 and chiral analogues.

Keywords: immunosuppressant; FTY720; Schoellkopf-auxiliary; phosphates.

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ation of a larger collection of chiral FTY720 analogues. As shown in Scheme 1 for the synthesis of the biologically active *R*-enantiomer, D-*cyclo*-Val-Gly-OEt **5**[7] was lithiated and treated with iodide **6**[8] to generate the monoalkylated Schöllkopf adduct as pure diastereomer in 71% yield.[9] This intermediate was subsequently lithiated and methylated to generate the quaternary center in **7** stereoselectively with 65% yield.[10] Hydrolysis of the bislactimether proceeded uneventfully to produce the aminoacid ester (58% yield), which was reduced to (*R*)-**3** in 95% yield.[11]

The (*S*)-enantiomer was synthesized in an analogous manner starting from L-*cyclo*-Val-Gly-OEt.[12] The enantiomeric purity of (*R*)-**3** and (*S*)-**3** was confirmed by HPLC analysis of the corresponding dinitrobenzamide derivatives.[13] As described earlier, only the *R*-enantiomer was biologically active and induced lymphocyte depletion after oral dosing in rats.[4]

Due to the difficult physicochemical properties of phosphates **4**, in particular their low solubility, and due to lack of availability of sphingosine kinases, biochemical synthesis and isolation is tedious. We therefore describe here a straightforward chemical approach for the preparation of phosphates (*R*)- and (*S*)-**4** (Scheme 2). Boc-protected aminoalcohols **8**[14] were phosphorylated with tri-valent phosphorylating agent **9**[15] and oxidized in situ using H_2O_2. This sequence produced fully protected phosphates **10** in 85% yield. At this stage, the compounds were purified by chromatography and then subjected to two deprotection steps without any further purification. This protocol delivered phosphates **4**[16] from Boc-protected aminoalcohols with an overall yield of 75%. Again as described earlier, only phosphate (*R*)-**4** had low nanomolar affinities to S1P-receptors.[4]

In summary, we described a highly practical asymmetric synthesis of chiral FTY720 analogues and an efficient procedure to generate the corresponding phosphates. These compounds as well as additional structural analogues accessible via the synthetic procedures described here will be essential tools to further elucidate the mechanism of action of the novel immunosuppressant FTY720.

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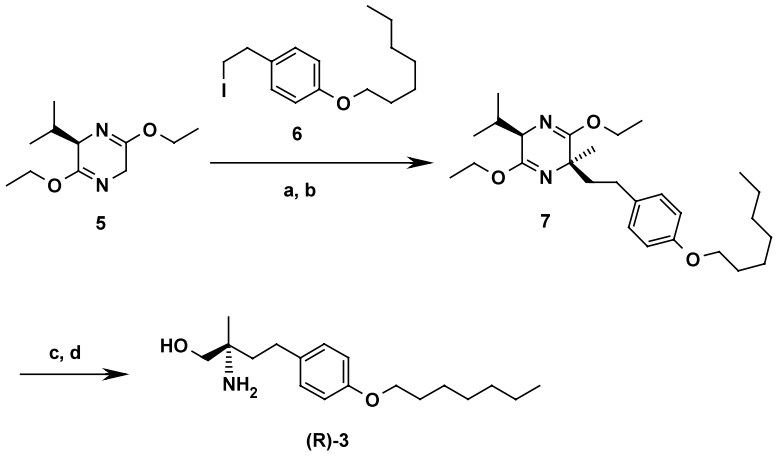
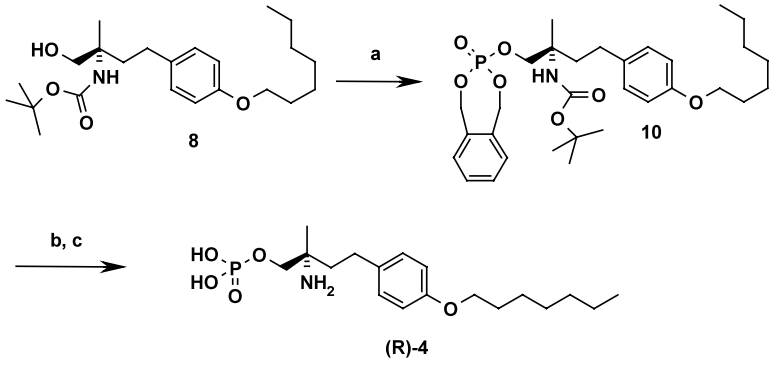


Scheme 1. (a) BuLi, THF, −78°C to 0°C; then **6**, −78°C to 0°C, 71%; (b) BuLi, THF, −78°C to 0°C, then MeI, −78°C to 0°C, 65%; (c) 0.5N HCl, dioxane, rt, 16 h, 58%; (d) $LiAlH_4$, THF, 65°C, 2 h, 95%.



Scheme 2. (a) (1,5-Dihydro-benzo[e][1,3,2]dioxaphosphepin-3-yl)-diethylamine **9**, tetrazole, THF, rt, 2 h; then 30% H_2O_2, rt, 1 h, 85%; (b) 1 bar H_2, Pd/C, MeOH, rt, 1 h, 88%; (c) conc. HCl, HOAc, rt, 16 h, quant.

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6. Commercially available as OMe-ethers from Merck and Fluka.
7. Alternatively, D-*cyclo*-Val-Gly-OMe can be used.
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9. The diastereomeric ratio (>10:1) of this step was irrelevant, since the stereochemical outcome of the overall sequence was determined by the following methylation.
10. Isolated yield of pure diastereomer at ca. 70% conversion; other isomer not detected (d.r.>20:1). Experimental procedure: To a solution of monoalkylated Schöllkopf adduct (13.45 g, 31.3 mmol) in dry THF (75 ml) was added a solution of *n*-BuLi (21.38 ml, 1.6 M in hexanes, 34.2 mmol) at −78°C to −65°C under an Ar atmosphere. After stirring for 0.5 h at −78°C, methyliodide (4.05 g, 28.5 mmol) dissolved in dry THF (50 ml) was added dropwise below −65°C. The temperature was kept at −78°C for 0.5 h before the mixture was warmed to 0°C and stirred for an additional 5 h. Quenching with satd aqueous NH_4Cl (100 ml) was followed by extraction with ethylacetate (2×150 ml) and drying of the organic phase with $MgSO_4$. After evaporation of the solvent the crude oil was purified by chromatography (hexanes/ether = 9/1) to give the desired product as a slowly crystallizing oil (9.87 g, 22.2 mmol).
11. Analytical data: ^{1}H NMR (400 MHz, CD_3OD): $\delta = 0.71$ ppm (t, $J = 7$ Hz, 3H, CH_2CH_3), 0.88 (s, 3H, CCH_3), 1.05–1.21 (m, 6H, CH_2), 1.21–1.29 (m, 2H, CH_2), 1.38–1.47 (m, 1H, CCH_2), 1.47–1.56 (p, $J = 7$ Hz, 2H, OCH_2CH_2), 2.35 (t, $J = 7$ Hz, 1H, $PhCH_2$), 3.13 (d, $J^2 = 9$ Hz, 1H, OCH_2), 3.16 (d, $J^2 = 9$ Hz, 1H, OCH_2), 3.71 (t, $J = 7$ Hz, 2H, $PhOCH_2$), 6.58 (d, $J = 8$ Hz, 2H, arom. CH), 6.89 (d, $J = 8$ Hz, 2H, arom. CH); MS (EI): 294.5 (MH^+); α_D −14 (20°C; *c* 0.98, $CHCl_3$).
12. Alternatively, bis-alkylated Schöllkopf adducts **7** can be synthesized from L-*cyclo*-Val-Ala-OMe by alkylation with iodide **6**.
13. Column: Chiracel OD (25×0.46 cm); Eluent: EtOH/hexane = 1/1; UV detection at 210 nm; flow: 1 ml/min; injection: 20 mL 0.1% in EtOH; ee of (*R*)-3 = 95%.
14. Accessible from **3** in 85% yield by treatment with Boc_2O in CH_2Cl_2/THF.
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16. Analytical data: ^{1}H NMR (400 MHz, CD_3OD): $\delta = 0.94$ ppm (t, $J = 7$ Hz, 3H, CH_2CH_3), 1.30–1.45 (m, 6H, CH_2), 1.40 (s, 3H, CCH_3), 1.45–1.53 (m, 2H, CH_2), 1.73–1.82 (p, $J = 7$ Hz, 2H, OCH_2CH_2), 1.97–2.06 (dt, $J^2 = 12$ Hz, $J^3 = 7$ Hz, 1H, CCH_2), 2.57–2.64 (dt, $J^2 = 13$ Hz, $J^3 = 7$ Hz, 1H, $PhCH_2$), 2.66–2.73 (dt, $J^2 = 13$ Hz, $J^3 = 7$ Hz, 1H, $PhCH_2$), 3.86 (dd, $J_{P-H} = 11$ Hz, $J_{H-H} = 7$ Hz, 1H, $POCH_2$), 3.93–3.98 (m, 1H, $POCH_2$), 3.96 (t, $J = 7$ Hz, 2H, $PhOCH_2$), 6.84 (d, $J = 8$ Hz, 2H, arom. CH), 7.16 (d, $J = 8$ Hz, 2H, arom. CH); MS (EI): 745 $(2M-H)^-$, 372 $(M-H)^-$.