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Synthesis and Biological Evaluation of a Conformationally Free *seco*-Analogue of the Immunosuppressant FR901483

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Abstract—The synthesis of an azaspirocyclic analogue of FR901483, phosphate **2**, is described based on the implementation of a key 5-endo aminocyclization promoted by iodine for direct functionalization of the 1-azaspiro[4.5]decane ring at the C-3 atom. Compound **2** has no inhibitory activity in the cell proliferation assays reported.

Introduction

FR901483 (**1**) is a novel immunosuppressant recently isolated from the fermentation broth of *Cladobotryum* sp. No. 11231 and characterized by the Fujisawa Laboratories.[1] Immunosupressive agents display a wide variety of structures,[2,3] but none are related to that of **1**. This compound has a tricyclic ring system unprecedented among natural products, consisting of a 2-azabicyclo[3.3.1]nonane nucleus fused to a pyrrolidine moiety, resulting in a spiro framework. Additionally, **1** has a phosphate unit, which plays an important role in this compound's immunosuppressive activity. Recently, the unique structure of FR901483 has been the subject of two synthetic approaches directed to its tricyclic ring system,[4,5] the Snider approach[5] reaching the demethylamino derivative of **1**.

Part of our ongoing research project towards the synthesis of FR901483 (**1**) is concerned with simplifying the structure and increasing the flexibility of the molecule; this has led to the consideration of the spiro derivative **2** (R=Me, Fig. 1). The *seco*-analogue **2** has a conformational mobility that **1** lacks, and constitutes an interesting target for pharmacological evaluation. Considering the chair axial–axial conformation for compound **2**, a good overlap between pyrrolidine and methylamino nitrogen, as well as for the phosphate unit, can be seen in the superimposition showed in Figure 2. However, it is worth pointing out the absence in **2** of the hydroxyl group present in FR901483 and the slight separation of the methoxyphenyl moieties.

In this paper, we describe the synthesis and biological evaluation of the *seco*-analogue **2** (R=Bn),[6] introducing a new synthetic procedure to obtain its azabicyclyc framework.

Results and Discussion

Synthesis

Our procedure for the synthesis of 1-azaspiro-[4.5]decanes (for other approaches leading to this azabicyclic system by N(1)–C(2) bond formation in the last step, see refs 7–12; for other synthetic entries, starting from carbocyclic compounds, see inter alia, refs 13–20) consists of the treatment of a homoallylamine (i.e. **3**) with iodine (for successful examples of the scarce reactions of amino cyclization promoted by iodine, see refs 21 and 22; for iodine initiated cyclization of unsaturated nitrogen containing compounds other than amines, see refs 23–25), which promotes the electrophilic cyclofunctionalization of the unsaturated amine to give a pyrrolidine ring.[26] The required homoallylamine was prepared in a one-pot procedure involving the formation of an imine from the monoethylene acetal of 1,4-cyclohexanedione and 4-methoxyphenylethylamine followed by the addition of allylmagnesium bromide (Scheme 1).[27] Treatment of **3** with iodine promoted the

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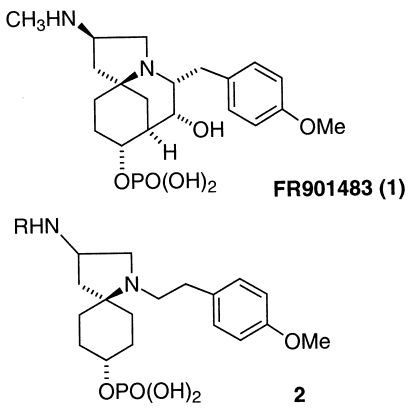


Figure 1.

iodoaminocyclization process to give **4**. Thus, in only two separate steps we gained access to the suitably functionalized azabicyclic system. The oxidation of the iodo derivative **4** to ketone **5** was effected using DMSO and silver tetrafluoroborate.[28] The overall sequence worked well and we obtained azaspiro **5** in 30% overall yield. Compound **5** was analyzed spectroscopically by means of NOESY experiments in order to elucidate its preferred conformation, which proved to be that where the amino group was disposed equatorially from the carbocyclic ring.

Having established a simple and efficient synthetic entry to 3,8-functionalized 1-azaspiro[4.5]decanes,[29] we next turned our attention to the preparation of the *seco*-analogue of FR901483 **2** (R=Bn). The subsequent steps involved several functional transformations: (i) reductive amination to introduce the amino group at C-3; (ii) deprotection of the acetal group in **6**, followed by reduction of the resulting ketone **7** to stereoselectively afford the alcohol **8**; (iii) protection of the secondary amino group, in order to avoid an oxidative process in later steps; (iv) assembly of the phosphomonoester group, which was achieved by phosphorylation of the hydroxyl group of **9** with *O,O*-bisbenzyl-*N,N*-diisopropyl phosphoramidate in the presence of tetrazole followed by MCPBA oxidation;[30] (v) finally, the benzyl phosphates of **10** were hydrogenolyzed and the resulting monoester **11** was treated with TFA to afford the diamino derivative **2**, which was isolated as its dipotassium salt. The relative configuration at C-5 and C-8, which was generated in the ketone reduction step, was inferred taking into account the preferred conformation of ketone **7** (the same as that of the corresponding related acetal **5**), and the fact that the NaBH4 reduction furnished an equatorial alcohol, as expected. Additionally, NOESY experiments upon **2** agreed with the configuration and conformation depicted in Scheme 1.

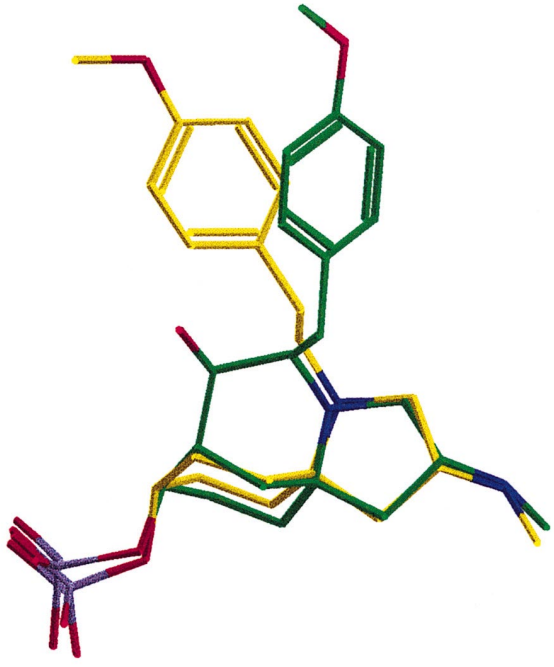


Figure 2. Molecular fitting of FR901483 (green) and **2** (yellow).

Biological evaluation

The synthesized simplified analogues of **1** (compounds **2**, **7** and **8**) were tested in the three lymphocyte in vitro proliferation assays indicated in Experimental (TPA plus IL-2, Tetanus toxoid or Phytohemegglutinin). None of these three compounds showed inhibitory activity at the range dose of 40 ng/mL to 4 mg/mL. The reference compound cyclosporin A showed good activity at 1 mg/mL.

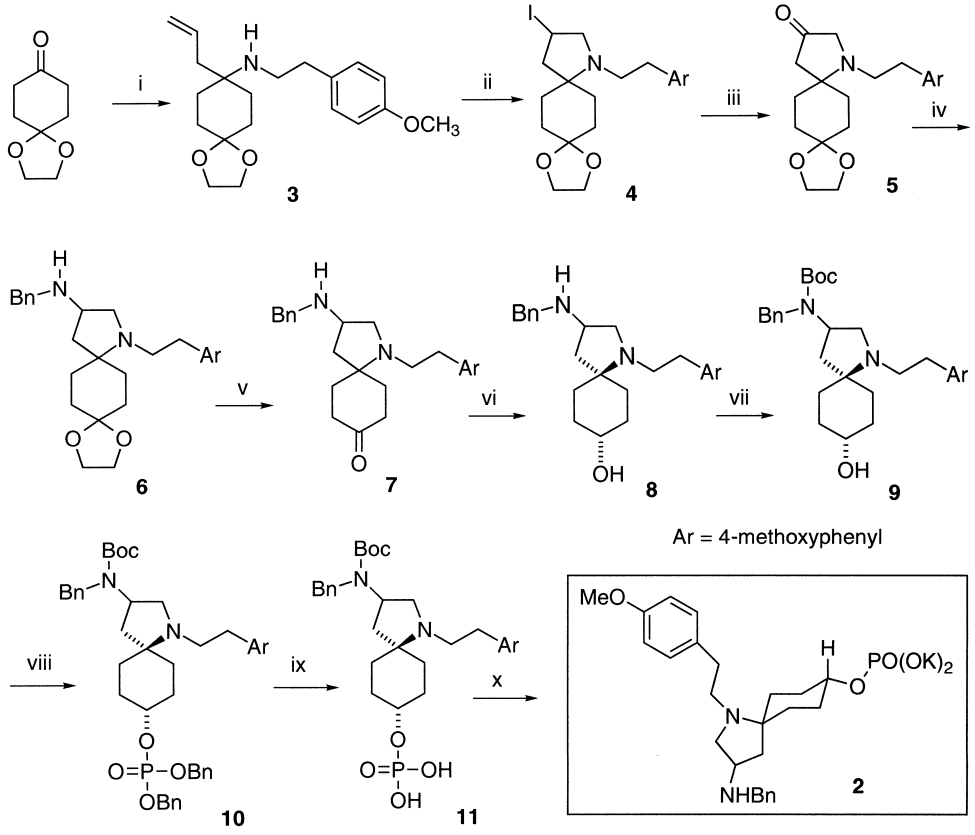
Conformational studies

This lack of activity in the proliferation assay studies, particularly for compound **2** which is the closest analogue of **1**, may be due to either the differences in functional groups between the compounds (absence of OH, benzyl in place of methyl) or an unsuitable conformation of **2**, as suggested by the NMR experiments.

For a better understanding of this second possibility, a conformational analysis of the cyclohexane ring in the spiro moiety was carried out. We elected to evaluate this point by calculating the differences in the heat of formation for the two chair conformations. Comparisons of the resultant energy minima revealed that the conformation **2-I** is indeed favored thermodynamically vis-à-vis the conformer **2-II**; the corresponding heats of formation differed by 3.2 kcal/mol (Fig. 3). Thus, as suggested by the NMR experiments, the only conformation detected for compound **2** was that corresponding to a chair equatorial–equatorial (conformation **2-I**) since the equilibrium constant between the two conformers is about 1000 at room temperature.[31] This may suggest that one of the reasons for the lack of activity for compound **2** is the inaccessibility of the correct chair conformation.

Conclusions

Although compound **2** could in principle adopt a conformation that matches to a great extention that of



Scheme 1. Reagents and conditions: (i) 4-methoxyphenethylamine, molecular sieves; then allylmagnesium bromide, Et_2O:CH_2Cl_2, 71%; (ii) I_2, $NaHCO_3$, CH_2Cl_2:H_2O, 51%; (iii) $AgBF_4$, DMSO, 80%; (iv) benzylamine, molecular sieves; then $NaBH_4$ (85%); (v) HCl, THF:H_2O, 79%; (vi) $NaBH_4$, MeOH, 75%; (vii) $(Boc)_2O$. MeOH:Et_3N, 76%; (viii) $(BnO)_2PN(iPr)_2$, tetrazole; then *m*-CPBA, CH_2Cl_2, −50°C, 73%; (ix), H_2, Pd/C, MeOH, 92%; (x) TFA, CH_2Cl_2; then K_2CO_3.

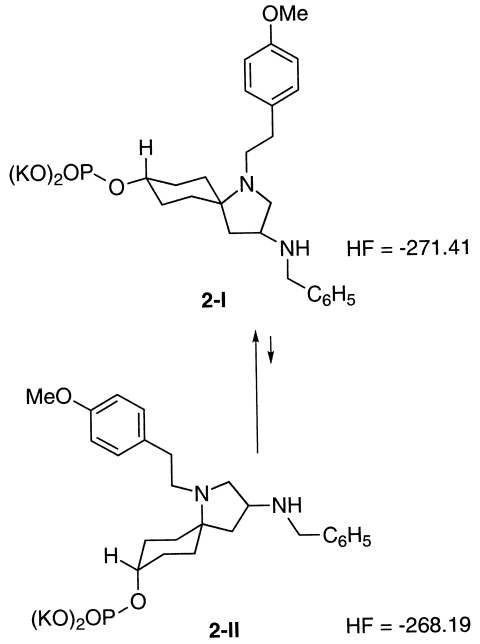


Figure 3. Conformational analysis of cyclohexane ring of compound **2** (HF = heat of formation in Kcal/mol, MOPAC/AM1).

structure **1** (see Fig. 1), in fact **2** adopts a *trans*-diequatorial conformational relationship between the phosphate group and the nitrogen atom, which is non-accessible to the natural product **1** in which both substituents of the cyclohexane ring must be *trans*-diaxial for the additional ring linkage. As a consequence, the different distances between the various functional groups in **2** with respect to those in **1** may explain the lack of activity of the *seco*-analogue **2**.

From a synthetic standpoint, we report a new synthetic entry to the azaspiro[4,5]decane scaffold that might allow us to access the hitherto unprepared natural product FR901483, using an adequate amine (i.e. tryrosine derivative) as starting material which could allow the elaboration of the additional ring.

Experimental

Computational procedures

Structures were built with standard bond lengths and angles using the Chem-X[32,33] molecular modelling package. All structures were initially optimised using the steepest descents and conjugate gradient methods. After semiempirical optimization using the MOPAC program,[34,35] charge distributions were calculated by means of the AM1 method. The PULAY keyword and the eigenvector following the (EF) routine for minimum search with GNORM = 0.1 were used in the optimisation step. Conformation analysis was performed with fully AM1 optimization. All calculations were performed on a Digital Alpha Station 3000.

Biology

Assays were performed with freshly isolated human peripheral blood mononuclear cells isolated from blood by density gradient centrifugation. Lymphocyte proliferation induced by different stimulators (TPA plus IL-2, Tetanus toxoid or Phytohemagglutinin) were assessed by tritiated thymidine uptake. In all the cases 1×10^5 cells were placed in flat bottom wells from a microtiter plate in triplicates in a final volume of 200 mL of RPMI 1640 complete medium with or without test compound. For the TPA plus IL-2 induced proliferation assay nylon wool purified T cells were used. Cell proliferation was assessed on day 1 for TPA plus IL-2 and Phytohemagglutinin stimulation, whereas for Tetanus toxoid, induced proliferation was tested on day 6. Cyclosporin A was used as a control.

Chemistry

All reactions were carried out under an argon atmosphere with dry, freshly distilled solvents under anhydrous conditions. TLC was carried out on SiO_2 (silica gel 60 F_{254}, Merck), and the spots were located with UV light, iodoplatinate reagent or 1% aqueous $KMnO_4$. Chromatography refers to flash chromatography and was carried out on SiO_2 (silica gel, SDS, 230–400 mesh ASTM). Unless otherwise noted, drying of organic extracts during work up of reactions was performed over anhydrous Na_2SO_4. Evaporation of solvents was accomplished with a rotatory evaporator. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian 300 or a Varian VXR-500 instrument. Chemical shifts are reported in ppm downfield from Me_4Si. IR spectra were recorded on a Nicolet 205 FT–IR spectrophotometer, and only noteworthy absorptions are listed. Microanalyses were performed by the Centro de Investigación y Desarrollo (CSIC), Barcelona.

4-Allyl-4-[2-(4-methoxyphenyl)ethylamino]cyclohexanone ethylene acetal (3). To a solution of 1,4-cyclohexanedione monoethylene acetal (8 g, 51.2 mmol) in CH_2Cl_2 (160 mL) were added 2-(4-methoxyphenyl)-ethylamine (9.8 mL, 66.6 mmol) and 4 Å molecular sieves (16 g). After stirring at room temperature for 4 h, the suspension was filtered through Celite, and the filtrate was concentrated to give the corresponding imine (^{13}C NMR (74.5 MHz, $CDCl_3$) δ 24.3 (CH_2), 33.6 (CH_2), 34.5 (CH_2), 35.9 (CH_2), 36.0 (CH_2), 52.2 (CH_2N), 54.8 (CH_3O), 64.0 (CH_2O), 107.5 (C-1), 113.3 (CH), 129.4 (CH), 131.8 (C), 157.6 (C), 170.6 (CN)). To a solution of this imine in CH_2Cl_2 (80 mL) was added dropwise a solution of allyl magnesium bromide (102.4 mmol) in Et_2O (120 mL). The mixture was stirred at room temperature for 4 h, poured into saturated aqueous NH_4Cl, and extracted with CH_2Cl_2. The organic extracts were washed with brine, dried, and concentrated. Chromatography (from CH_2Cl_2 to 9:1 CH_2Cl_2:MeOH) yielded amine **3** (12 g, 71%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.75 (br, 1H, NH), 1.40–1.58 (m, 6H), 1.70–1.80 (m, 2H), 2.10 (d, $J=7.5$ Hz, 2H, CH_2C=), 2.66 (m, 4H, CH_2CH_2), 3.77 (s, 3H, OCH_3), 3.90 (m, 4H, CH_2O), 4.96 (ddt, $J=17$, 2 and 1.5 Hz, 1H, H-*cis*), 5.00 (ddt, $J=10$, 2.5 and 1.5 Hz, 1H, H-*trans*), 5.66 (ddt, $J=17$, 10 and 7.5 Hz, 1H, H-*gem*), 6.81 (d, $J=8.5$ Hz, 2H, 3′-H), 7.10 (d, $J=8.5$ Hz, 2H, 2′-H); ^{13}C NMR (74.5 MHz, $CDCl_3$) δ 30.1 and 32.5 (C-2 and C-3), 36.2 (CH_2Ar), 41.4 (CH_2CH), 42.8 (CH_2N), 52.7 (C-4), 55.1 (CH_3O), 64.0 (CH_2O), 108.9 (C-1), 113.6 (C-3′), 117.5 (=CH_2), 129.5 (C-2′), 132.3 (C-1′), 134.0 (=CH), 157.8 (C-4′). Anal. calcd for $C_{20}H_{29}NO_3$: C, 72.47; H, 8.82; N, 4.23. Found: C, 72.26; H, 9.05; N, 4.30.

1-[2-(4-Methoxyphenyl)ethyl]-3-iodo-1-azaspiro[4.5]decan-8-one ethylene acetal (4). To a solution of amine **3** (2 g, 6 mmol) in CH_2Cl_2 (60 mL) and 5% aqueous $NaHCO_3$ (60 mL) was added dropwise a solution of I_2 (2.28 g, 9 mmol) in CH_2Cl_2 (60 mL). After stirring at room temperature for 12 h, saturated aqueous sodium thiosulfate was added. The mixture was extracted with CH_2Cl_2. The dried organic extracts were concentrated and chromatographed (99.5:0.5 CH_2Cl_2:MeOH) to give compound **4** (1.41 g, 51%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36 (m, 1H), 1.45–1.85 (m, 7H), 2.26 (dd, $J=14$ and 6.6 Hz, 1H, H-4), 2.50 (dd, $J=14$ and 8.2 Hz, 1H, H-4), 2.69 (m, 4H, CH_2CH_2), 3.23 (dd, $J=10.1$ and 6.9 Hz, 1H, H-2), 3.39 (dd, $J=10.1$ and 7.4 Hz, 1H, H-2), 3.79 (s, 3H, CH_3O), 3.91 (m, 4H, CH_2O), 4.27 (dddd, $J=8.2$, 7.4, 6.9 and 6.6 Hz, 1H, H-3), 6.82 (d, $J=8.6$ Hz, 2H, 3′-H), 7.12 (d, $J=8.6$ Hz, 2H, 2′-H); ^{13}C NMR (74.5 MHz, $CDCl_3$) δ 15.0 (C-3), 28.9 and 29.9 (C-6 and C-10), 31.9 and 32.4 (C-7 and C-9), 34.5 (CH_2Ar), 47.0 (C-4), 49.3 (CH_2N), 55.1 (CH_3O), 61.9 (C-2), 64.0 and 64.2 (CH_2O), 64.6 (C-5), 107.8 (C-8), 113.6 (C-3′), 129.6 (C-2′), 131.8 (C-1′), 157.8 (C-4′). Anal. calcd for $C_{20}H_{28}INO_3$: C, 52.52; H, 6.17; N, 3.06. Found: C, 52.76; H, 6.25; N, 3.02.

1-[2-(4-Methoxyphenyl)ethyl]-1-azaspiro[4.5]decan-3,8-dione 8-monoethylene acetal (5). To a solution of $AgBF_4$ (0.68 g, 3.44 mmol) in DMSO (40 mL) was added dropwise a solution of **4** (1.64 g, 3.6 mmol) in DMSO (30 mL). After stirring at room temperature for 12 h, the suspension was filtered through Celite, and the filtrate partitioned between Et_2O and water. The organic extracts were washed with brine, dried, and concentrated. Chromatography (CH_2Cl_2:MeOH, 99:1) afforded ketone **5** (0.99 g, 80%): IR (film) 1758; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.37 (dm, $J=13.5$ Hz, 2H, H-6eq and H-10eq), 1.50 (td, $J=13.5$ and 4 Hz, 2H, H-7ax and H-9ax), 1.73 (dm, $J=13.5$ Hz, 2H, H-7eq and H-9eq), 1.88 (td, $J=13.5$ and 4 Hz, 2H, H-6ax and H-10ax), 2.37 (s, 2H, H-4), 2.65 (dd, $J=9$ and 6.5 Hz, 2H, CH_2Ar), 2.77 (dd, $J=9$ and 6.5 Hz, 2H, CH_2N), 3.24 (s, 2H, H-2), 3.77 (s, 3H, CH_3O), 3.90 (s, 4H, CH_2O), 6.81 (d, $J=9$ Hz, 2H, 3′-H), 7.09 (d, $J=9$ Hz, 2H, 2′-H); ^{13}C NMR (74.5 MHz, $CDCl_3$) δ 28.3 (C-6 and C-10), 32.4 (C-7 and C-9), 35.0 (CH_2Ar), 47.7 (C-4), 49.9 (CH_2N), 55.2 (CH_3O), 58.9 (C-2), 62.1 (C-5), 64.2 and 64.3 (CH_2O), 107.9 (C-8), 113.6 (C-3′), 129.6 (C-2′), 132.1 (C-1′), 157.9 (C-4′), 214.0 (C-3). Anal. calcd for $C_{20}H_{27}NO_4$: C, 69.54; H, 7.88; N, 4.05. Found: C, 69.59; H, 7.88; N, 4.24.

3-Benzylamino-1-[2-(4-methoxyphenyl)ethyl]-1-azaspiro-[4.5]decan-8-one ethylene acetal (6). To a solution of

ketone **5** (0.55 g, 1.6 mmol) in CH_2Cl_2 (2.8 mL) were added benzylamine (0.34 mL, 3.2 mmol) and 4 Å molecular sieves (0.55 g). After stirring at room temperature for 4 h, the suspension was filtered through Celite, and the filtrate was concentrated to give the corresponding imine. To a cooled (0°C) solution of this imine in MeOH (14 mL) was added $NaBH_4$ (121 mg, 3.2 mmol). After 2 h at 0°C and 2 h at room temperature, the reaction mixture was poured into saturated aqueous NH_4Cl and extracted with CH_2Cl_2 (5×50 mL). The organic extracts were dried and concentrated. Chromatography (CH_2Cl_2 to 9:1 CH_2Cl_2:MeOH) yielded amine **6** (0.59 g, 85%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.20 (d, J=9.5 Hz, 1H, H-6eq), 1.30 (d, J=12.5 Hz, 1H, H-10eq), 1.46 (dd, J=13 and 6 Hz, 1H, H-4), 1.49–1.66 (m, 5H, H-6ax, H-7 and H-9), 1.69 (td, J=12.5 and 4 Hz, 1H, H-10ax), 2.12 (dd, J=13 and 8.5 Hz, 1H, H-4), 2.51 (m, 1H, NCH_2), 2.56–2.68 (m, 3H, NCH_2 and CH_2Ar), 2.77 (dd, J=9.5 and 4.5 Hz, 1H, H-2), 2.93 (dd, J=9.5 and 7 Hz, 1H, H-2), 3.24 (m, 1H, H-3), 3.68 (s, 2H, NCH_2Ar), 3.70 (s, 3H, CH_3O), 3.82 (m, 4H, OCH_2-CH_2O), 6.72 (d, J=8.5 Hz, 2H, 3′-H), 7.03 (d, J=8.5 Hz, 2H, 2′-H), 7.17 (m, 1H), 7.21–7.28 (m, 4H); ^{13}C NMR (74.5 MHz, $CDCl_3$) δ 28.0 (C-6), 30.8 (C-10), 32.4 (C-7), 32.6 (C-9), 35.3 (CH_2Ar), 42.0 (C-4), 50.0 (NCH_2), 52.4 (NCH_2Ar), 54.5 (C-3), 55.0 (CH_3O), 57.2 (C-2), 62.5 (C-5), 64.1 and 64.2 (OCH_2CH_2O), 108.5 (C-8), 113.6 (C-3′), 126.9 (*p*-C), 128.2 and 128.4 (*o*-C and *m*-C), 129.6 (C-2′), 132.8 (C-1′), 140.2 (*ipso*-C), 157.8 (C-4′). Anal. calcd for $C_{27}H_{36}N_2O_3.1/2H_2O$: C, 72.77; H, 8.37; N, 6.29. Found: C, 73.08; H, 8.20; N, 6.69.

3-Benzylamino-1-[2-(4-methoxyphenyl)ethyl]-1-azaspiro-[4.5]decan-8-one (7). To a solution of acetal **6** (1.65 g, 3.8 mmol) in THF (30 mL) was added hydrochloric acid (10%, 83 mL). After 12 h at room temperature, the reaction mixture was poured into 5% aqueous $NaHCO_3$ and extracted with CH_2Cl_2 (5×50 mL). The organic extracts were dried and concentrated. Chromatography (CH_2Cl_2 to 94:6 CH_2Cl_2:MeOH) afforded ketone **7** (1.18 g, 79%): IR (film) 1714; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.60 (br, 1H, H-6eq), 1.75 (m, 3H, H-4, H-6ax and H-10eq), 1.86 (td, J=13 and 5.5 Hz, 1H, H-10ax), 2.30–2.42 (m, 5H, H-4, H-7 and H-9), 2.56 (m, 1H, CH_2N), 2.62–2.72 (m, 3H, CH_2N and CH_2Ar), 2.89 (dd, J=9.5 and 4.5 Hz, 1H, H-2), 3.06 (dd, J=9.5 and 7.5 Hz, 1H, H-2), 3.39 (m, 1H, H-3), 3.75 (s, 3H, CH_3O), 3.78 (s, 2H, NCH_2Ar), 6.78 (d, J=8.5 Hz, 2H, 3′-H), 7.07 (d, J=8.5 Hz, 2H, 2′-H), 7.25 (m, 1H), 7.30–7.34 (m, 4H); ^{13}C NMR (74.5 MHz, $CDCl_3$) δ 30.5 (C-6), 32.9 (C-10), 35.1 (CH_2Ar), 38.6 (C-7), 38.9 (C-9), 42.1 (C-4), 49.9 (NCH_2), 52.4 (NCH_2Ar), 54.4 (C-3), 55.2 (CH_3O), 57.2 (C-2), 61.9 (C-5), 113.6 (C-3′), 127.0 (*p*-C), 128.2 and 128.4 (*o*-C and *m*-C), 129.5 (C-2′), 132.4 (C-1′), 139.9 (*ipso*-C), 157.8 (C-4′), 211.2 (C-8). Anal. calcd for $C_{25}H_{32}N_2O_3.H_2O$: C, 73.14; H, 8.34; N, 6.82. Found: C, 73.41; H, 8.08; N, 6.94.

***trans*-3-Benzylamino-1-[2-(4-methoxyphenyl)ethyl]-1-azaspiro[4.5]decan-8-ol (8).** To a cooled (0°C) solution of ketone **7** (0.58 g, 1.5 mmol) in MeOH (20 mL) was added $NaBH_4$ (112 mg, 3 mmol). After 30 min at 0°C and 1.5 h at room temperature, the reaction mixture was poured into saturated aqueous NH_4Cl and extracted with CH_2Cl_2 (4×25 mL). The organic extracts were dried and concentrated. Chromatography (CH_2Cl_2 to 88:12 CH_2Cl_2:MeOH) yielded alcohol **8** (0.44 g, 75%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20–1.33 (m, 4H, H-6 and H-9), 1.35 (br d, J=13.5 Hz, 1H, H-10eq), 1.42 (td, J=13.5 and 3.2 Hz, 1H, H-10ax), 1.46 (dd, J=13 and 6 Hz, 1H, H-4), 1.57 (br s, 1H, NH), 1.83 (m, 2H, H-7), 2.11 (dd, J=13 and 8.7 Hz, 1H, H-4), 2.48 (m, 1H, CH_2N), 2.54–2.65 (m, 3H, CH_2N and CH_2Ar), 2.77 (dd, J=9.5 and 4.5 Hz, 1H, H-2), 2.94 (dd, J=9.5 and 7 Hz, 1H, H-2), 3.23 (m, 1H, H-3), 3.41 (m, 1H, H-8), 3.69 (s, 2H, NCH_2Ar), 3.71 (s, 3H, CH_3O), 6.74 (d, J=8.5 Hz, 2H, 3′-H), 7.04 (d, J=8.5 Hz, 2H, 2′-H), 7.18 (m, 1H), 7.24–7.27 (m, 4H); ^{13}C NMR (74.5 MHz, $CDCl_3$) δ 28.8 (C-6) 31.6 (C-10), 32.9 (C-9), 33.1 (C-7), 35.3 (CH_2Ar), 42.7 (C-4), 50.1 (NCH_2), 52.4 (NCH_2Ar), 54.5 (C-3), 55.2 (CH_3O), 57.2 (C-2), 62.6 (C-5), 70.6 (C-8), 113.6 (C-3′), 126.9 (*p*-C), 128.2 and 128.4 (*o*-C and *m*-C), 129.6 (C-2′), 132.8 (C-1′), 140.2 (*ipso*-C), 157.8 (C-4′).

***trans*-3-[*N*-Benzyl-*N*-(*tert*-butoxycarbonyl)amino]-1-[2-(4-methoxyphenyl)-ethyl]-1-azaspiro[4.5]decan-8-ol (9).** To a solution of alcohol **8** (430 mg, 1.1 mmol) in 9:1 MeOH:Et_3N (7 mL) was added di*tert*-butyl dicarbonate (476 mg, 2.2 mmol), and the mixture was heated at 50°C for 5 h. The solvent was removed in vacuo, and the residue was purified by chromatography (CH_2Cl_2 to 88:12 CH_2Cl_2:MeOH) to give carbamate **9** (0.41 g, 76%): IR ($CHCl_3$) 1681; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15–1.75 (m, 17H), 1.80–1.96 (m, 2H), 2.22–2.44 (m, 2H), 2.45–2.64 (m, 2H), 2.72 (m, 1H), 2.82 (m, 1H), 2.94 (m, 1H), 3.46 (m, 1H, H-8), 3.78 (s, 3H, CH_3O), 4.43 (br s, 2H, NCH_2Ar), 4.85 (br s, 1H, OH), 6.78 (d, J=8.7 Hz, 2H, 3′-H), 7.04 (d, J=8.7 Hz, 2H, 2′-H), 7.13–7.34 (m, 5H); ^{13}C NMR (74.5 MHz, $CDCl_3$) δ 26.1 (CH_2), 28.2 (CH_3), 32.6 (CH_2), 32.9 (CH_2), 34.8 (CH_2Ar), 39.5 (C-4), 46.6 (NCH_2Ar), 49.6 (NCH_2), 52.3 (C-3), 54.1 (C-2), 55.1 (CH_3O), 62.8 (C-5), 70.3 (C-8), 79.7 (C), 113.5 (C-3′), 126.3 (*p*-C), 125.9 and 128.1 (*o*-C and *m*-C), 129.4 (C-2′), 132.4 (C-1′), 140.2 (*ipso*-C), 155.9 (CO), 157.7 (C-4′). Anal. calcd for $C_{30}H_{42}N_2O_4.H_2O$: C, 70.28; H, 8.65; N, 5.46. Found: C, 70.37; H, 8.33; N, 5.46.

***trans*-3-[*N*-Benzyl-*N*-(*tert*-butoxycarbonyl)amino]-1-[2-(4-methoxyphenyl)ethyl]-1-azaspiro[4.5]dec-8-yl dibenzyl phosphate (10).** To a solution of alcohol **9** (370 mg, 0.75 mmol) in CH_2Cl_2 (37 mL) were added 1-*H*-tetrazole (158 mg, 2.25 mmol) and N,N-di*iso*propyl dibenzyl phosphoramidite (0.38 mL, 1.12 mmol). The mixture was stirred at room temperature for 5 h and then cooled to −50°C. MCPBA (98%, 197 mg, 1.12 mmol) in CH_2Cl_2 (10 mL) was added. The resulting solution was stirred at 0°C for 2 h, diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ (4×50 mL). The organic extracts were dried and concentrated. Chromatography (CH_2Cl_2 to 95:5 CH_2Cl_2:MeOH) yielded phosphate **10** (0.41 g, 73%): IR ($CHCl_3$) 1681; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.10–1.54 (m, 16H), 1.93 (m, 2H), 2.25 (m, 1H), 2.33 (m, 1H), 2.54 (m, 2H), 2.65 (m, 1H), 2.79 (m, 1H), 2.92 (m, 1H), 3.78 (s, 3H, CH_3O), 4.10 (br s, 1H), 4.40 (br s, 2H, NCH_2Ar), 4.70 (br s, 1H), 4.98 (s,

2H, OCH_2Ar), 5.01 (s, 2H, OCH_2Ar), 6.78 (d, $J=8.7$ Hz, 2H, 3′-H), 7.01 (d, $J=8.7$ Hz, 2H, 2′-H), 7.10–7.40 (m, 15H); ^{13}C NMR (74.5 MHz, $CDCl_3$) δ 25.9 (CH_2), 28.2 (CH_3), 30.5 (CH_2), 31.0 (CH_2), 32.8 (CH_2), 34.9 (CH_2Ar), 39.7 (C-4), 46.6 (NCH_2Ar), 49.4 (NCH_2), 52.3 (C-3), 54.1 (C-2), 55.1 (CH_3O), 62.0 (C-5), 68.9 and 69.0 (OCH_2Ar), 77.5 (C-8), 79.7 (C), 113.5 (C-3′), 126.3 (*p*-C), 125.9 and 128.1 (*o*-C and *m*-C), 127.8 (CH), 128.3 and 128.4 (CH), 129.4 (C-2′), 132.5 (C-1′), 135.8 and 135.9 (C), 140.3 (*ipso*-C), 155.9 (CO), 157.7 (C-4′); ^{31}P NMR (121.5 MHz, $CDCl_3$)δ −5.44. Anal. calcd for $C_{44}H_{55}N_2O_7P.1/8H_2O$: C, 69.80; H, 7.36; N, 3.70. Found: C, 69.40; H, 7.31; N, 3.73.

In some runs, the corresponding *N*-oxide was formed as a minor byproduct:

***trans*-3-[*N*-Benzyl-*N*-(*tert*-butoxycarbonyl)amino]-8-(dibenzylphosphoroxy)-1-[2-(4-methoxyphenyl)ethyl]-1-azaspiro[4.5]decane 1-oxide.** ^1H NMR (300 MHz, $CDCl_3$) δ 1.20–1.65 (m, 15H), 1.90–2.10 (m, 2H), 2.28 (m, 1H), 2.32–2.48 (m, 2H), 2.87 (m, 1H), 3.13 (m, 2H), 3.44–3.62 (m, 2H), 3.78 (s, 3H, CH_3O), 3.88 (m, 1H), 4.05 (m, 1H), 4.80–5.20 (m, 6H), 6.83 (d, $J=8.6$ Hz, 2H, 3′-H), 7.13 (d, $J=8.6$ Hz, 2H, 2′-H), 7.18–7.37 (m, 15H); ^{13}C NMR (74.5 MHz, $CDCl_3$) δ 28.1 (CH_3), 29.3 (CH_2), 30.2 (CH_2), 30.3 (CH_2), 33.8 (CH_2), 46.5 (NCH_2Ar), 50.4 (C-3), 55.2 (CH_3O), 61.7 (CH_2N), 68.9 and 69.0 (C-5), 69.2 and 69.3 (OCH_2Ar), 69.8 (C-2), 76.0 and 76.1 (C-8), 80.4 and 81.3 (C), 114.1 (C-3′), 126.3 (*p*-C), 125.9 and 128.2 (*o*-C and *m*-C), 127.9 (CH), 128.5 and 128.6 (CH), 129.8 (C-2′), 135.6 and 135.7 (C), 140.6 (*ipso*-C), 156.5 (CO), 158.3 (C-4′).

***trans*-3-[*N*-Benzyl-*N*-(*tert*-butoxycarbonyl)amino]-1-[2-(4-methoxyphenyl)ethyl]-1-azaspiro[4.5]dec-8-yl dihydrogenphosphate (11).** A mixture of phosphate **10** (280 mg, 0.37 mmol) and 10% Pd/C (140 mg) in MeOH (40 mL) was stirred under 1 atm H_2 for 12 h. The suspension was filtered through Celite, and the filtrate concentrated to give phosphate **11** (195 mg, 92%): IR (film) 3420, 1690, 1661; ^1H NMR (200 MHz, CD_3OD) δ 1.10–2.20 (m, 21H), 2.42 (br s, 1H), 2.80 (br s, 2H), 3.32 (m, 1H), 3.67 (s, 3H), 3.85 (br s, 1H), 4.32 (br s, 1H), 4.38 (s, 2H), 6.76 (d, $J=8.4$ Hz, 2H), 6.98–7.35 (m, 7H); ^{13}C NMR (74.5 MHz, CD_3CN) δ 25.6 (CH_2), 28.5 (CH_3), 30.0 (CH_2), 31.2 (CH_2), 36.5 (CH_2), 38.2 (CH_2), 50.1 (CH_2), 51.0 (CH_2), 53.7 (CH), 55.9 (CH_3), 73.1 (CH), 74.1 (C), 81.4 and 82.0 (C), 115.0 (CH), 127.8 (CH), 129.4 (CH), 131.1 (CH), 139.9 (C), 156.9 (C), 159.6 (C). ISMS: 576 (M + H).

***trans*-3-(Benzylamino)-1-[2-(4-methoxyphenyl)ethyl]-1-azaspiro[4.5]dec-8-yl dihydrogenphosphate (2).** To a solution of carbamate **11** (165 mg, 0.29 mmol) in CH_2Cl_2 (13 mL) was added at 0°C TFA (3.3 mL, 43 mmol). After 12 h at room temperature, the solvent and the excess of acid were removed in vacuo to give the trifluoroacetic acid salt of **2** (170 mg, quantitative): IR (film) 2800 (br), 1668; ^1H NMR (200 MHz, CD_3OD) δ 1.40–2.30 (m, 10H), 2.80–3.20 (m, 3H), 3.40 (m, 1H), 3.67 (s, 3H), 3.60–4.00 (m, 2H), 4.00–4.30 (br, 4H), 6.79 (d, $J=8.8$ Hz, 2H), 7.13 (d, $J=8.8$ Hz, 2H), 7.30–7.50 (m, 5H); ^{13}C NMR (74.5 MHz, DMSO-d_6) δ 29.3 (CH_2), 29.7 (CH_2), 30.3 (CH_2), 30.7 (CH_2), 35.8 (CH_2), 48.8 (CH_2), 49.3 (CH_2), 52.1 (CH), 55.3 (CH_3), 60.5 (CH_2), 70.9 (C), 72.3 (CH), 114.2 (CH), 117.1 (q, C) 129.0 (CH), 129.1 (CH), 129.3 (CH), 130.1 (CH), 132.1 (C), 158.4 (C), 159.1 (q, C). ISMS: 475 (M + H). This trifluoroacetate (50 mg) was dissolved in MeOH (12 mL) containing K_2CO_3 (30 mg). The solvent was removed to give the dipotassium salt of **2**: ^1H NMR (300 MHz, CD_3OD) δ 1.26–1.66 (m, 8H), 2.13 (br, 2H), 2.23 (dd, $J=13$ and 8.6 Hz, 1H), 2.56 (m, 1H), 2.60–2.72 (m, 3H), 2.78 (dd, $J=9.5$ and 5.7 Hz, 1H), 3.01 (dd, $J=9.5$ and 7.4 Hz, 1H), 3.25 (m, 1H), 3.70–3.75 (m, 5H), 3.90 (br, 1H), 6.79 (d, $J=8.7$ Hz, 2H), 7.08 (d, $J=8.7$ Hz, 2H), 7.20–7.40 (m, 5H); ^{13}C NMR (74.5 MHz, CD_3OD) δ 30.5 (CH_2), 32.6 (CH_2), 32.9 (CH_2), 32.9 (CH_2), 36.0 (CH_2Ar), 43.1 (C-4), 51.4 (NCH_2), 53.1 (NCH_2Ar), 55.3 (OMe), 55.7 (C-3), 57.8 (C-2), 64.3 (C-5), 73.7 (d, $J_{C,P}=4.5$ Hz, C-8), 114.7 (C-3′), 128.2 (*p*-C), 129.5 and 129.6 (*o*-C and *m*-C), 130.6 (C-2′), 133.8 (C-1′), 140.2 (*ipso*-C), 157.8 (C-4′).

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