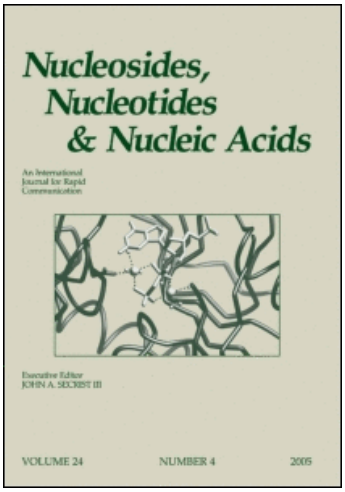

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Online publication date: 31 March 2001

To cite this Article Chapman, H. , Kernan, M. , Prisbe, E. , Rohloff, J. , Sparacino, M. , Terhorst, T. and Yu, R.(2001) 'PRACTICAL SYNTHESIS, SEPARATION, AND STEREOCHEMICAL ASSIGNMENT OF THE PMPA PRO-DRUG GS-7340', Nucleosides, Nucleotides and Nucleic Acids, 20: 4, 621 — 628
To link to this Article: DOI: 10.1081/NCN-100002338
URL: http://dx.doi.org/10.1081/NCN-100002338

NUCLEOSIDES, NUCLEOTIDES & NUCLEIC ACIDS, 20(4–7), 621–628 (2001)

PRACTICAL SYNTHESIS, SEPARATION, AND STEREOCHEMICAL ASSIGNMENT OF THE PMPA PRO-DRUG GS-7340

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ABSTRACT

The practical synthesis of a mixed phenoxy-amidate derivative of PMPA with high oral bioavailability and favorable pharmacokinetics is described. The non-stereoselective synthetic route produces a 1:1 mixture of two diastereomers at phosphorous. Simulated moving bed chromatography using Chiralpak AS enabled kilo-scale isolation of the more potent diastereomer (GS-7340). The GS-7340 phosphorous chiral center was found to be (*S*) by X-ray crystallography.

The nucleotide analog, 9-[2-(*R*)-phosphonomethoxypropyl]adenine (PMPA, **1**) (1) has shown potent activity against human immunodeficiency virus *in vitro* (2). The lipophilic diester pro-drug, tenofovir disoproxil fumarate (**2**), is currently in advanced clinical evaluation as an oral AIDS therapy (3). Continuing research into novel PMPA pro-moieties has recently led to the identification of the mixed phenoxy-amidate derivative of PMPA (**3**) which was designated as GS-7171 (Fig. 1). Due to the asymmetric center at phosphorous and non-stereoselective synthetic route, GS-7171 was composed of a 1:1 mixture of two diastereomers (the (*R*)-PMPA side-chain and L-amino acid ester were homochiral starting materials). The high oral bioavailability and favorable tissue-selective distribution of GS-7171 made it a promising candidate for further development. To ascertain the properties of the individual diastereomers, a method to separate them was needed.

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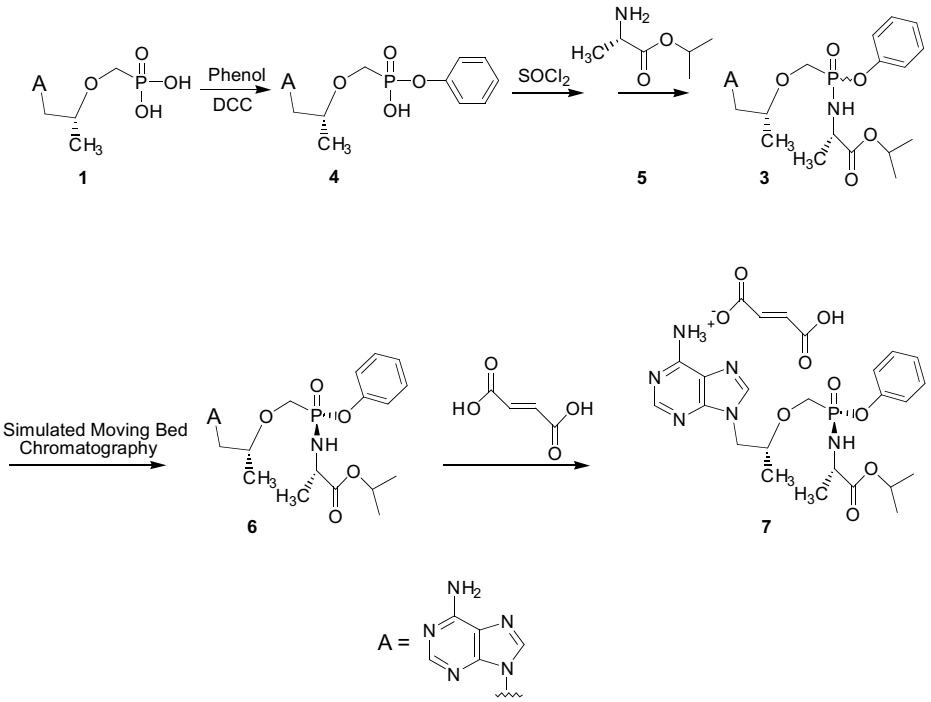

Scheme 1.

McGuigan and coworkers have recently reported related phenoxy-amidate pro-drugs of the monophosphates of antiviral nucleoside analogs (4). Although these compounds are also P-chiral diastereomeric mixtures, the issues of diastereomer separation and differential activity have not been addressed. This communication discloses a solution to the diastereomer "problem" that may be generally applicable.

The additional pre-clinical testing required a kilogram-scale preparation of the pure diastereomers. Esterification of the soluble triethylamine salt of PMPA (**1**) with phenol using dicyclohexylcarbodiimide (DCC) in hot 1-methyl-2-pyrrolidinone (NMP) afforded a 51% yield of PMPA monophenyl ester (**4**). Activation of (**4**) with thionyl chloride in dichloromethane gave the phosphonochloridate, which smoothly coupled with an excess of isopropyl L-alanine (**5**) to give the GS-7171 mixture (**3**) in 47% yield (unoptimized). Both steps were readily performed on multi-kilogram scale in standard pilot plant equipment.

Initially, the component diastereomers of amidate prodrug analogs were separated with repeated HPLC purifications on a preparative C18 column. The marginal resolution of the diastereomers on this system (Fig. 2) necessitated multiple passes but culminated in isolation of ~100 mg of each isomer enriched to >95:5 purity. *In vitro* HIV assay showed that the less retained isomer (GS-7340, **6**) was more potent by a factor of ~10 and was selected as the candidate for additional testing.

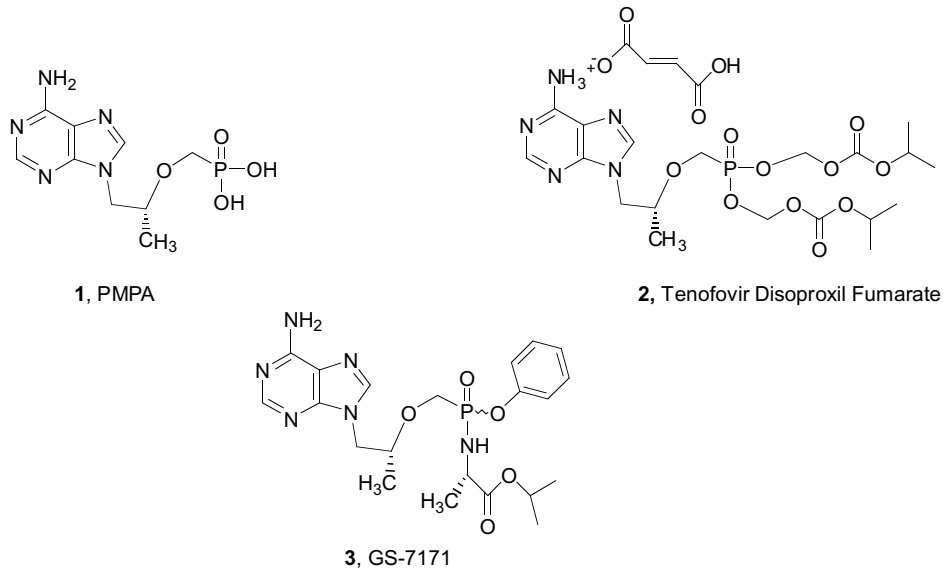

Figure 1.

Screening of commercially available chiral stationary phases revealed that Diacel's Chiralpak AS with a mobile phase of 30% methanol in acetonitrile was a markedly more efficient chromatographic system (5) (Fig. 3). The remarkable resolution of the diastereomers ($\alpha = \sim 9$) and the high solubility of GS-7171 in the mobile phase (>300 g/L) allowed facile diastereomer separation. Using a

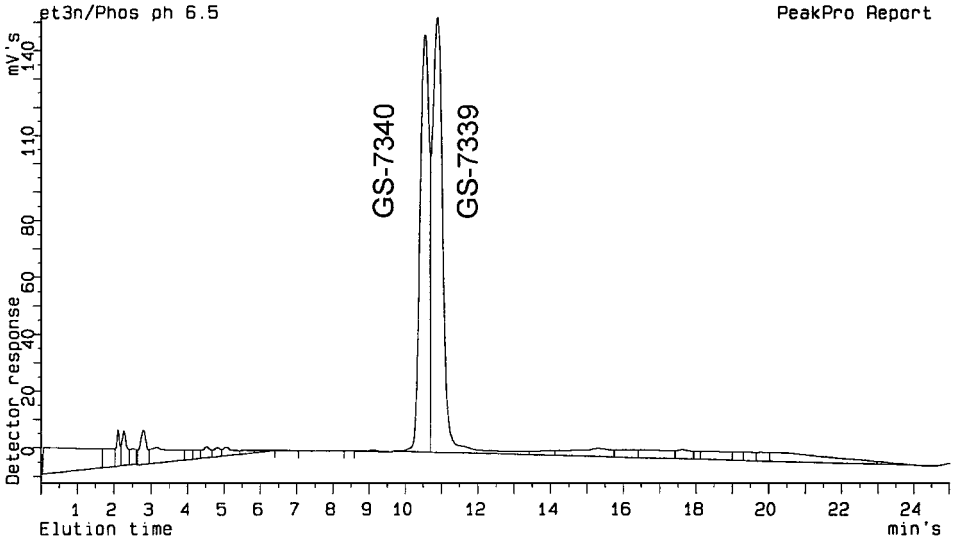

Figure 2. HPLC separation of the GS-7171 component diastereomers on C18 column packing.

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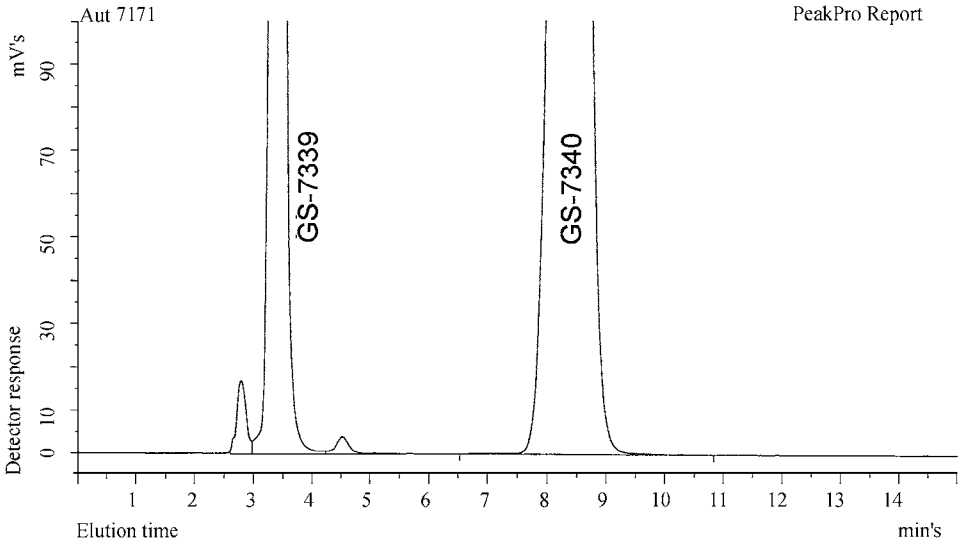

Figure 3. HPLC separation of the GS-7171 component diastereomers on Chiracel AS column packing.

laboratory-scale simulated moving bed chromatography (6) system composed of ten Chiralpak AS (7) columns in series allowed separation of >1 kg/day of mixture (8). The desired isomer, GS-7340, (**6**), was recovered in nearly quantitative yield and >98% diastereomeric purity.

After chromatographic purification, GS-7340 was readily crystallized as the free base or as the fumarate salt (**7**). Needles of GS-7340 free base were grown from water.

Structure determination by X-ray crystallography (9) allowed definitive assignment of the phosphorous chiral center as (*S*) (Fig. 4) in the more active isomer, GS-7340.

In summary, a practical kilo-scale process for synthesis and purification of the phenoxy-amidate pro-drug GS-7340 has been developed. The first correlation of activity with phosphorous absolute configuration in a phenoxy-amidate pro-drug has been made. Research into a diastereoselective synthetic process for GS-7340 is ongoing.

EXPERIMENTAL

[(*R*)-2-(Phenylphosphonomethoxy)propyl]adenine **4**

A glass-lined reactor was charged with anhydrous PMPA, (**1**) (14.6 kg, 50.8 mol), phenol (9.6 kg, 102 mol), and 1-methyl-2-pyrrolidinone (39 kg). The mixture was heated to 85°C and triethylamine (6.3 kg, 62.3 mol) added. A solution of 1,3-dicyclohexylcarbodiimide (17.1 kg, 82.9 mol) in 1-methyl-2-pyrrolidinone (1.6 kg) was then added over 6 hours at 100°C. Heating was continued for 16 hours.

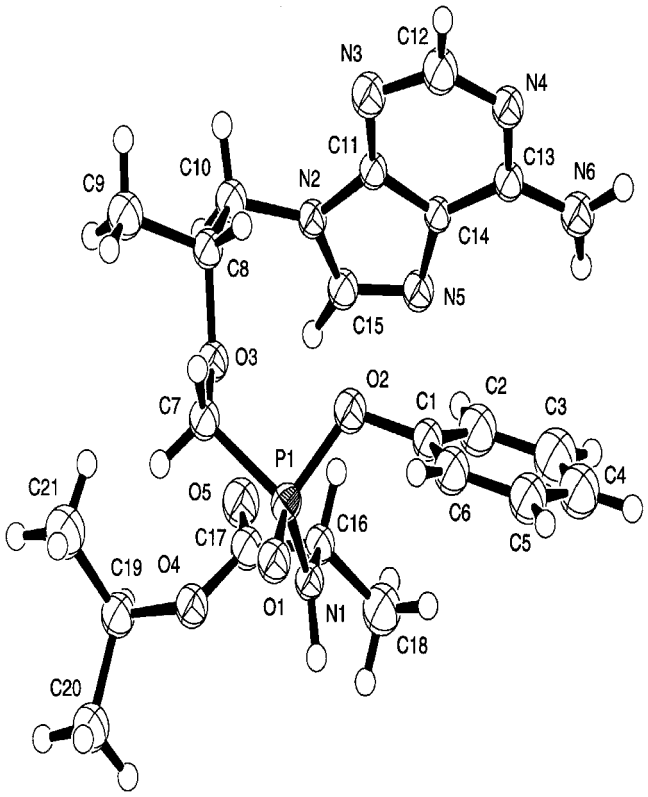

Figure 4. Crystal Structure of GS-7340, (6).

The reaction was cooled to 45°C, diluted with water (29 kg), and cooled to 25°C. Solids were removed by filtration and rinsed with water (15.3 kg). The combined filtrate and rinse was concentrated to a tan slurry under reduced pressure, water (24.6 kg) was added, and adjusted to pH 11 with NaOH (25% in water). Suspended solids were removed by filtration through diatomaceous earth (2 kg) followed by a water (4.4 kg) rinse. The combined filtrate and rinse was extracted with ethyl acetate (28 kg). The aqueous solution was adjusted to pH 3.1 with HCl (37% in water) (4 kg), precipitating crude **4** which was isolated by filtration and washed with methanol (12.7 kg). The wet product was slurried in methanol (58 kg), isolated by filtration, washed with methanol (8.5 kg), and dried under reduced pressure to yield **4** as a white powder (9.33 kg, 51% yield). ^{1}H NMR (300 MHz, D_2O, δ): 1.2 (d, 3H), 3.45 (q, 2H), 3.7 (q, 2H), 4 (m, 2H), 4.2 (q, 2H), 4.35 (dd, 2H), 6.6 (d, 2H), 7 (t, 1H), 7.15 (t, 2H), 8.15 (s, 1H), 8.2 (s, 1H); ^{31}P NMR (72 MHz, D_2O, δ):15.0 (decoupled).

Isopropyl L-alanine **5**

A glass-lined reactor was charged with L-alanine (7.1 kg, 80 mol) and isopropanol (35.6 kg). The slurry was heated to reflux and chlorotrimethylsilane

(14.6 kg, 134 mol) added over 69 minutes. Reflux was continued for 3 hours at which time the reaction was homogeneous. Volatiles were removed by distillation at atmospheric pressure until the pot temperature reached 125°C. The pot residue was cooled to 34°C and diethyl ether (50 kg) added with vigorous stirring and further cooling to 21°C. Vigorous agitation was continued until crystals formed. The slurry was cooled to about 0°C. The solid isopropyl L-alanine hydrochloride was isolated by filtration, rinsed with diethyl ether (5 kg), and dried to 12.35 kg off-white deliquescent crystals. ^{1}H NMR (300 MHz, $CDCl_3$, δ) :1.2 (m, 6H), 1.7 (d, 3H), 4.2 (m, 1H), 5.05 (m, 1H), 8.6 (m, 3H). The free base isopropyl L-alanine was prepared by combining a solution of isopropyl L-alanine hydrochloride, (9.12 kg, 54 mol), in tetrahydrofuran (34 kg), with a solution of 1,4-diazabicyclo[2.2.2]octane (5.48 kg, 49 mol) in tetrahydrofuran (15.5 kg) in a glass-lined reactor. Solid 1,4-diazabicyclo[2.2.2]octane hydrochloride precipitated and was removed by filtration. Tetrahydrofuran was removed from the isopropyl L-alanine in the filtrate by careful distillation under reduced pressure. The product isopropyl L-alanine (**5**) was isolated as an oil for use immediately in the next step without further processing or characterization.

GS-7171 **3**

A glass-lined reactor was charged with [(*R*)-2-(phenylphosphonomethoxy)-propyl]adenine (**4**), (9.12 kg, 25.1 mol) and acetonitrile (30.7 kg). Thionyl chloride (6.57 kg, 56.7 mol) was added while maintaining the mixture below 50°C. The mixture was heated at 75°C until solids dissolved, then the reaction temperature was increased to 80°C and volatiles (11.4 kg) collected by atmospheric distillation under nitrogen. The pot residue was cooled to 25°C, diluted with dichloromethane (41 kg), and cooled to −29°C. A solution of isopropyl L-alanine (**5**) (ca. 7.1 kg, 54 mol) in dichloromethane (36 kg) was added over 60 minutes at −18°C followed by triethylamine (7.66 kg, 75.7 mol) over 30 minutes at −18 to −11°C. The reaction mixture was warmed to room temperature and washed with 10% aq. sodium dihydrogenphosphate solution (5 × 15.7 kg). The organic solution was dried with anhydrous sodium sulfate (18.2 kg), filtered, rinsed with dichloromethane (28 kg), and concentrated to an oil under reduced pressure. Acetone (20 kg) was charged to the oil and the mixture concentrated under reduced pressure. Acetone (18.8 kg) was again charged to the resulting oil. Half the product solution was purified by chromatography over a 38 × 38 cm bed of 22 kg silica gel 60 (230 to 400) mesh. The column was eluted with 480 kg acetone. The purification was repeated on the second half of the oil using fresh silica gel and acetone. Clean product bearing fractions were concentrated under reduced pressure to an oil. Acetonitrile (19.6 kg) was charged to the oil and the mixture concentrated under reduced pressure. Acetonitrile (66.4 kg) was again charged and the solution chilled to 0 to −5°C for 16 hours. Solids were removed by filtration and the filtrate concentrated under reduced pressure affording 5.6 kg of 3 as a dark oil. ^{1}H NMR (300 MHz, $CDCl_3$, δ):

1.1 (m, 12H), 3.7 (m, 1H), 4.0 (m, 5H), 4.2 (m, 1H), 5.0 (m, 1H), 6.2 (s, 2H), 7.05 (m, 5H), 8.0 (s, 1H), 8.25 (d, 1H); ^{31}P NMR (72 MHz, $CDCl_3$, δ): 21.0, 22.5 (decoupled).

9-[(R)-2-[[(S)-[[(S)-1-(Isopropoxycarbonyl)ethyl]amino]-phenoxyphosphinyl]methoxy]propyl]adenine **6**

GS-7171 (**3**), 2.8 kg, was purified by continuous simulated moving bed chromatography. Ten columns filled with 10 cm by 5 cm beds of packing, 20 micron Chiralpak AS (1.2 kg) were used. The columns were eluted with 30% methanol in acetonitrile. Product bearing fractions were concentrated to a solution of **6** in acetonitrile (2.48 kg). The solution solidified to a crystalline mass wet with acetonitrile on standing. The crystalline mass was dried under reduced pressure to a tan crystalline powder, 1.3 kg **6** , 98.7% diastereomeric purity by HPLC: mp 117–120°C; ^{1}H NMR (300 MHz, $CDCl_3$, δ): 1.15 (m, 12H), 3.7 (t, 1H), 4.0 (m, 5H), 4.2 (dd, 1H), 5.0 (m, 1H), 6.05 (s, 2H), 7.1 (m, 5H), 8.0 (s, 1H), 8.2 (s, 1H); ^{31}P NMR (72 MHz, $CDCl_3$, δ): 21.0 (decoupled).

9-[(R)-2-[[(S)-[[(S)-1-(Isopropoxycarbonyl)ethyl]amino]-phenoxyphosphinyl]methoxy]propyl]adenine Fumarate (**1:1**) **7**

A glass-lined reactor was charged with 9-[(R)-2-[[(S)-[[(S)-1-(isopropoxycarbonyl)ethyl]amino]phenoxyphosphinyl]methoxy]propyl] adenine (**6**), (1.29 kg, 2.71 mol), fumaric acid (284 g, 2.44 mol), and acetonitrile (24.6 kg). The mixture was heated to reflux to dissolve the solids, filtered while hot, and cooled to 5°C for 16 hours. The product was isolated by filtration, rinsed with acetonitrile (9.2 kg), and dried to 1.33 kg **7** as a white powder: mp 119.7–121.1°C; $[\alpha]_D^{20}$ −41.7° (*c* 1.0, acetic acid).

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