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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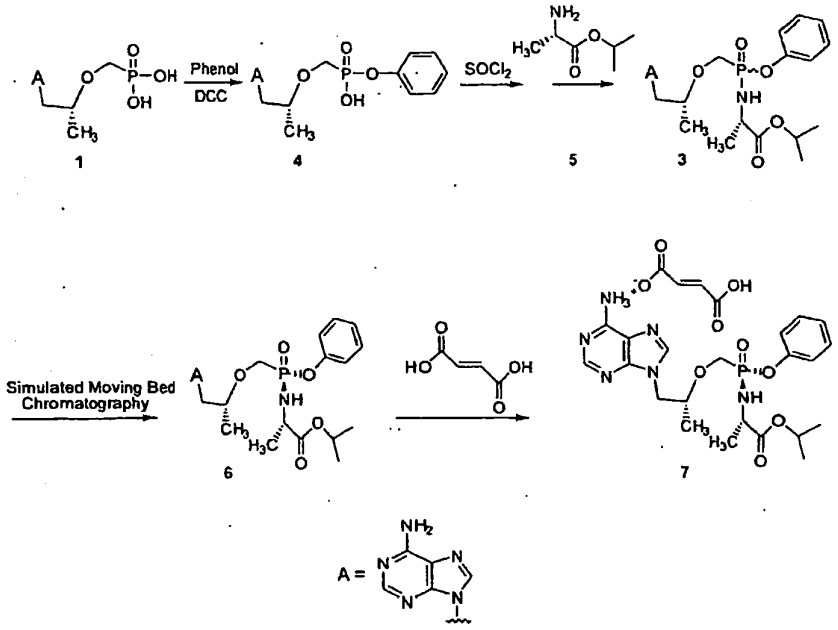

Scheme 1.

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.

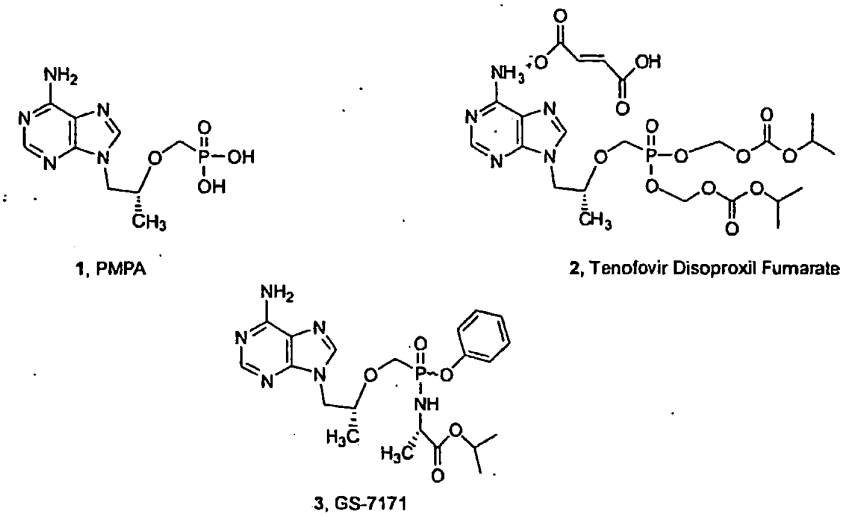

Figure 1.

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

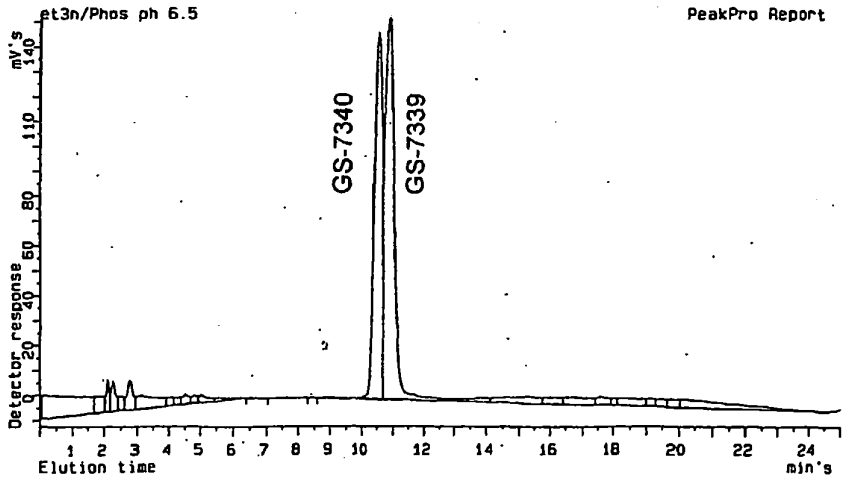

Figure 2. HPLC separation of the GS-7171 component diastereomers on C18 column packing.

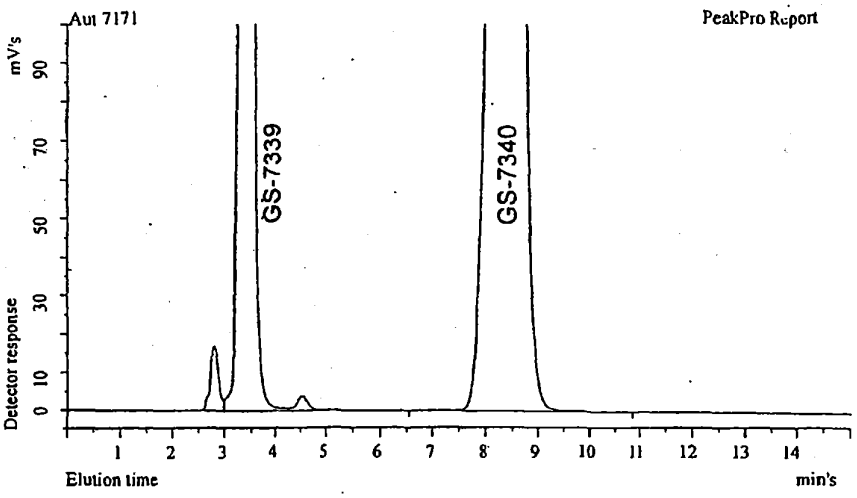

Figure 3. HPLC separation of the GS-7171 component diastereomers on Chiracel AS column packing.

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.

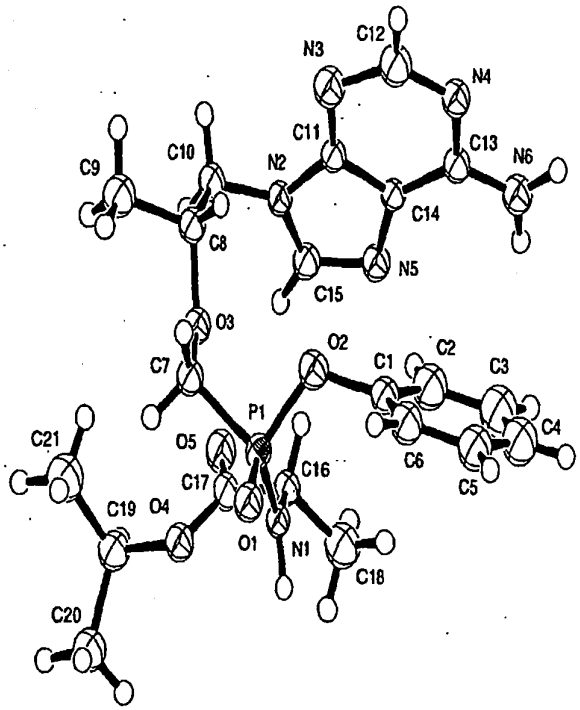

Figure 4. Crystal Structure of GS-7340, (6).

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

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