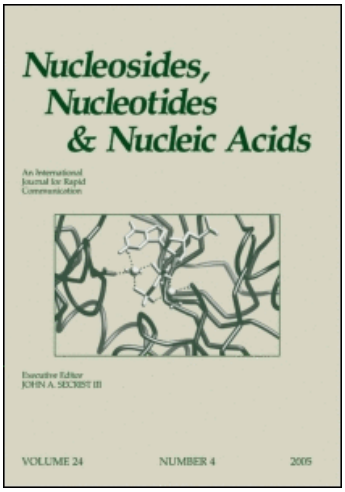

Nucleosides, Nucleotides and Nucleic Acids

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Synthesis of (−)-DAPD

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ABSTRACT

A new synthesis of (−)-DAPD, suitable for large scale development, is described.

Key Words: DAPD; HIV; HBV; Dioxolane nucleosides.

INTRODUCTION

(−)-DAPD is a potent and selective inhibitor of HIV in vitro and in vivo[1] and HBV replication in vitro.[1] Structurally it belongs to a class of compounds known as the dioxolane nucleosides.

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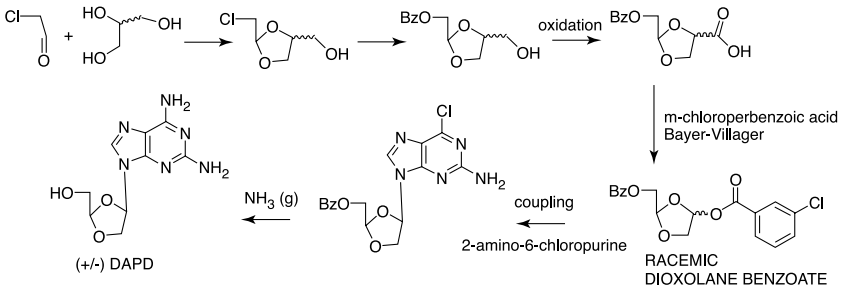
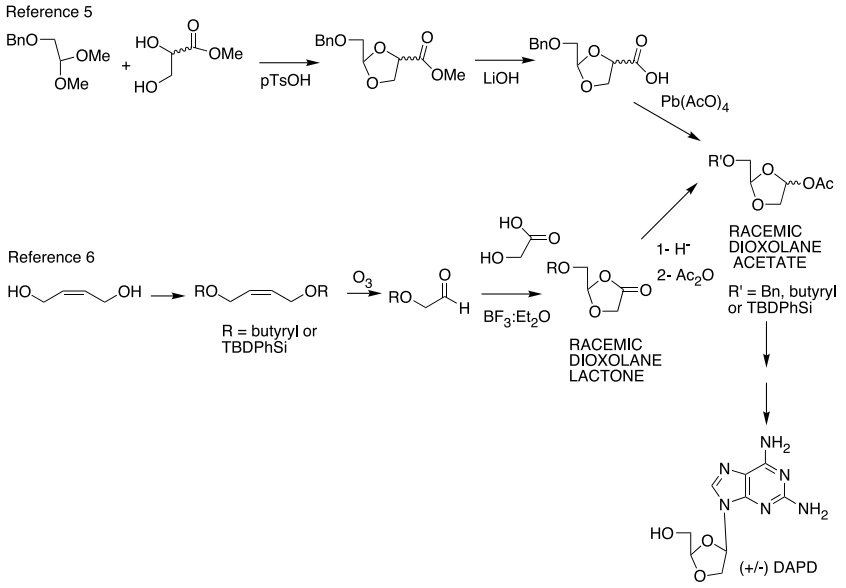


Scheme 1. Synthesis of (+/−) DAPD by Belleau et al.

In 1988 Belleau et al. filed patent application U.S. Patent No. 5,041,449[2] which disclosed the generic group of racemic 1,3-dioxolane nucleosides for the treatment of HIV and portions of this work were presented in 1989 at the Fifth International Conference on AIDS.[3] Racemic DAPD was synthesized as depicted in Scheme 1. Belleau et al. reacted glycerol and chloroacetaldehyde to generate a dioxolane intermediate. After chlorine displacement with a benzoic acid salt, oxidation of the primary alcohol to a carboxylic acid and Baeyer-Villiger rearrangement with *m*-chloroperbenzoic acid, the corresponding racemic dioxolane benzoate was obtained. This compound was then coupled with 2-amino-6-chloropurine and the resulting nucleoside intermediate was reacted with ammonia under pressure to afford racemic DAPD. In 1989, Norbeck et al.[4] concurrently published an article which described the synthesis of racemic cis-1,3-dioxolane thymidine which also had anti-HIV activity in vitro. Norbeck et al.[4] condensed (±)-methyl glycerate and benzyloxyacetaldehyde dimethylacetal, which after saponification and oxidative decarboxylation afforded a racemic dioxolane acetate. The same racemic dioxolane acetate intermediate was synthesized by Liotta et al.[5,6] starting from *cis*-2-buten-1,4-diol (Scheme 2). After protection and ozonolysis of the double bond an aldehyde was isolated which, due to its unstable nature, was immediately used in the next step. Condensation with glycolic acid afforded a racemic dioxolane lactone, which was reduced and acetylated to afford the racemic dioxolane acetate. The drawback of these procedures, shown in Scheme 2, is that they involve the synthesis of an unstable aldehyde and/or a tedious oxidative step(s).

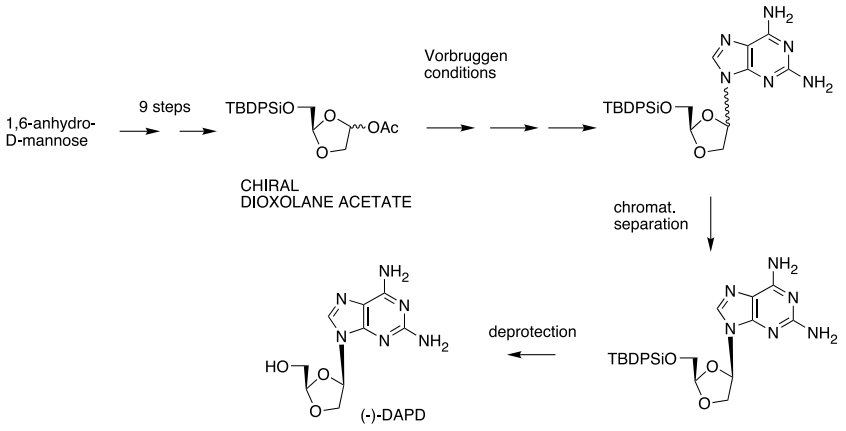
In 1990, Chu and Shinazi filed U.S. Patent No. 5,179,104 which disclosed a method to obtain enantiomerically pure β-D-1,3-dioxolane nucleosides via a stereospecific synthesis and Kim et al.[7,8] subsequently published a paper entitled ''1,3-Dioxolanylpurine Nucleosides (2R,4R) and (2R,4S) with Selective Anti-HIV-1 Activity in Human Lymphocytes''. The synthesis of (−)-DAPD was described as a thirteen-step process from 1,6-anhydro-D-mannose. 1,6-Anhydro-D-mannose was converted to a chiral dioxolane acetate in nine steps (Scheme 3). After coupling of the acetate under Vorbruggen conditions, several purification and deprotection steps, (−)-DAPD was obtained in modest yield. While this is an asymmetric synthesis, it is too long and involves difficult oxidation steps that make the process unsuitable for commercial development.



Scheme 2. Synthesis of (+/−) DAPD by Norbeck et al.

In 1992 Belleau et al.[9] published a synthesis of enantiomerically pure 2′,3′-dideoxy-3-oxacytidine stereoisomers. The derivatives were synthesized in eight steps from L-ascorbic acid. While this route was stereospecific, the use of lead tetraacetate made the route unsuitable for scale-up.

Relevant chemistry was found in a patent filed by Liotta and Shinazi, WO 92/14729[10] and in a publication by Jin et al.[11] Liotta and Shinazi found that racemic



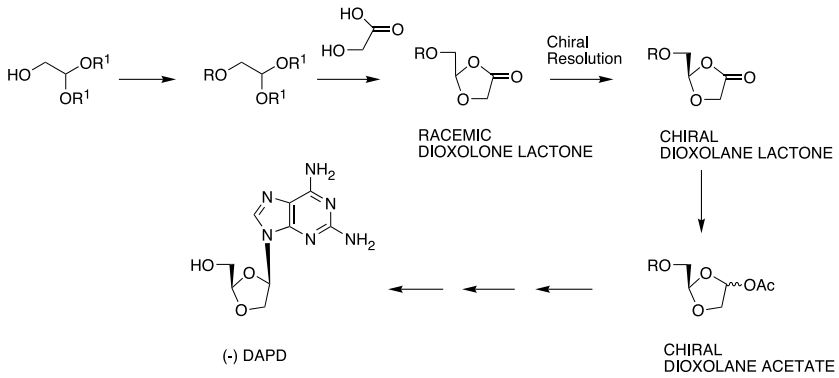
Scheme 3. Synthesis of (−) DAPD by Kim et al.

2-O-protected-5-O-acylated-1,3-dioxolanes could be coupled with purine or pyrimidine bases in the presence of a titanium containing Lewis acid to predominately generate the racemic β-isomers. In addition, Jin et al. published that titanium tetrachloride and tin tetrachloride promoted the racemization of enantiomerically pure 2′-deoxy-3′-oxaribosides during coupling reactions but that the use of the Lewis acids trimethylsilyltriflate, trimethylsilyl iodide and $TiCl_2(Oi\text{-}Pr)_2$ provided enantiomerically pure cytosine dioxolane nucleosides in low diastereoselectivity.

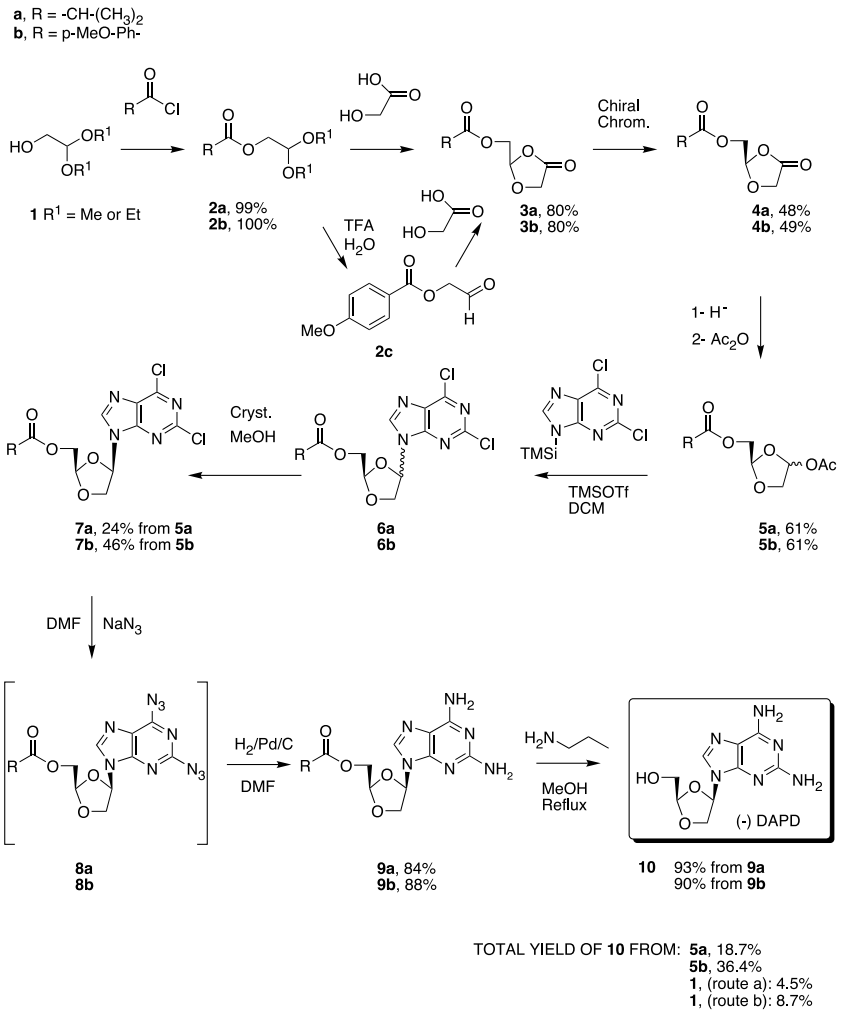
While there are other papers in the literature which involve the synthesis of dioxolane nucleosides,[12–14] the chemistry was not applicable to the synthesis of (−)-DAPD on large scale. For these reasons we carried out the following work.

RESULTS AND DISCUSSION

In pursuit of a facile and economical synthesis of (−)-DAPD we envisioned the following strategy: preliminary results showed that properly substituted racemic dioxolane lactones, like the ones shown in Scheme 2, could be resolved by chiral chromatography (unpublished results). As previously mentioned, one of our goals was to avoid oxidative steps and/or to avoid the production of unstable aldehydes as depicted in Scheme 2. For these reasons, we proposed a simple two-step synthesis of a racemic dioxolane lactone from commercially available 2,2-dialkoxy-ethanol (Scheme 4). After resolution and reductive acetylation chiral dioxolane acetates would be obtained. Any suitable R group should comply with all of the following conditions: 1) the corresponding lactone should be resolvable, 2) the corresponding ester should be stable under the reductive acetylation conditions, 3) after coupling, the corresponding anomers should be easily separated, preferentially by crystallization, 4) should be easy to remove at the end of the synthesis, 5) have a low molecular weight to avoid carrying large mass during the process and, 6) be commercially available and inexpensive. Two groups that satisfied all of these conditions were: *iso*-butyryl and p-methoxy benzoyl. The results are summarized in Scheme 5.



Scheme 4. Proposed synthesis of dioxolane lactone.



Scheme 5. Synthesis of (−) DAPD.

Initially a 2,2-dialkoxy-ethanol (**1**) was esterified with the corresponding acid chloride in quantitative yields. In the case of the *iso*-butyryl (**2a**) ester, cyclization to the corresponding dioxolane lactone (**3a**), was affected with glycolic acid in the presence of a Lewis acid ($BF_3 \cdot Et_2O$) in 80% yield. However, under the same conditions, the *p*-methoxy benzoate ester (**2b**) afforded the corresponding lactone in low yield and purity. Much better results were achieved in a two step procedure in which the acetal was first hydrolyzed to the corresponding aldehyde (**2c**), which then was subjected to similar cyclization conditions, affording the corresponding lactone (**3b**) in 80% yield as a solid. The lactones were resolved by chiral chromatography in

almost quantitative yield.[a] Selective reduction of the chiral lactones (**4a** and **4b**) with $LiAlH(OtBu)_3$ and subsequent treatment with acetic anhydride afforded the corresponding acetates (**5a** and **5b**) in reasonable yields, after purification by flash column chromatography. Coupling with 2,6-dichloropurine under Vobruggen conditions afforded crude mixtures of α:β anomers of the correponding nucleosides (**6a** and **6b**). The *iso*-butyrate ester was first purified by column chromatography and then crystallized from MeOH to afford the pure β anomer (**7a**) in a 24% yield. The *p*-methoxy benzoate ester nucleoside did not require chromatography, and was purified by crystallization from MeOH to afford pure β anomer (**7b**) in 46% yield. The chlorines were replaced with amino groups in a two-step procedure by treatment with sodium azide and then hydrogenation. The corresponding intermediates (**9a** and **9b**) were isolated and fully described. Removal of the protecting groups was achieved with *n*-butylamine in refluxing MeOH to afford (−)-DAPD (**10**) in 93% and 90% yield respectively.

The total overall yields from the corresponding chiral acetates (**5a** and **5b**) were: 18.7% for the *iso*-butyrate and 36.4 % for the *p*-methoxy benzoate. Much better than the overall 10.6% yield previously reported from the *t*-butyl-di-phenyl-silyl chiral acetate.[7,8] The total overall yield from **1** was 4.5% using route a, and 8.7% using route b, in 8 steps, using one chiral resolution and one regular column chromatography in the case of the *p*-methoxy benzoate protecting group. The overall yield of (−)-DAPD previously described in the literature[7,8] was 1.1% in 14 steps from D-Mannose, using six chromatographic separations. Preliminary results in our laboratory showed that this synthesis could be successfully repeated in a kilogram scale, without using any chromatographic separation.[b]

EXPERIMENTAL SECTION

General methods. Melting points were determined in open glass capillaries by use of a Melt-Temp II apparatus with a digital thermometer. ^{1}H-NMR spectra were recorded at 400 MHz with a Varian XL-400 spectrometer. Evaporations were

[a]These are typical conditions for the chiral resolution of 5 grams of either **3a** or **3b**: Column: Chiralpak Semi-prep. AD-RH, 250 × 10 mm. Mobile phase: Methanol. Gradient: Isocratic. Detection: UV at 280 nm. The recovery of both enantiomers of **3a** and **3b** was almost quantitative (close to 50% for each enantiomer for both compounds).

[b]In an exploratory large scale run racemic *iso*-butyrate lactone **3a** (3.6 kg, 19 mol) was obtained in 80% yield from **1**. The lactone was resolved enzymaticaly using Chirazyme-L2 in isopropanol: water as a solvent at 0°C. Chiral lactone **4a** (1.4 kg, 7.4 mol) was obtained in 40% yield. This lactone was converted to the crude acetate **5a**, which without any further purification was coupled with 2,6-dichloroputine to afford **7a** (432 g, 1.2 mol) as almost pure β anomer in 16% yield from **4a**. This material was carried through the next steps as described before to afford the final product: (−)-DAPD (**10**, 217 g, 0.86 mol) in 72% yield from **7a**. The total overall yield for this procedure from **1** was 3.7%, a little bit lower than the 4.5% we reported in this manuscript for the smaller scale, but no chromatographic resolution of any type was required and, since this was our first run, further improvements can be made to improve the yield. We also believe that further improvements will also be made by using *p*-methoxybenzoate as the protecting group that gave much better yields and purity than the *iso*-butyrate.

performed under diminished pressure using a Buchi rotatory evaporator at 40°C unless otherwise indicated. Solutions were dried over anhydrous Na_2SO_4. TLC was performed on precoated glass plates (0.25 mm) with Silica Gel $60F_{254}$ (E. Merck, Darmstad). Flash column chromatography was performed with Silica Gel 60 (230–400 mesh, E. Merck, Darmstad). Elemental analyses were performed by Atlantic Microlab (Atlanta, GA). High Resolution Mass Spectra (HRMS) were performed by Analytical Instrument Group Inc. (Raleigh, NC).

Gas chromatography analysis. Samples were prepared by dissolving 1 mg sample per 1 mL THF, or other suitable solvent. A 1 μl injection volume was used. Samples were separated using a 30 m HP-5 (crosslinked 5% PH ME siloxane) capillary column, Hewlett Packard part #19091J-413. The inlet temperature was set at 200°C, and the oven temperature as follows: 35°C for 1 minute, ramp to 250°C at 12.5°C/minute, hold at 250°C for 1.8 minutes. The flame ionization detector temperature was set at 250°C. The carrier gas was nitrogen set at a nominal flow of 8.0 mL/minute.

HPLC analysis.

Method A. Column: Chiralpak AD-RH, 150 × 4.6 mm. Mobile phase: Methanol. Gradient: Isocratic. Flow rate: 0.5 mL/min. Run time: 30 min. Detection: UV at 280 nm.

Method B. Column: Aquasil C18, 150 × 4.6 mm. Mobile phase: solvent A: acetonitrile, solvent B: 50 mmol NH_4OAc, 0.1% AcOH in water. Gradient: time: 0 min., A: 1%, B: 99%; time: 17 min., A: 50%, B: 50%, then isocratic. Flow rate: 1.0 mL/min. Run time: 30 min. Detection: UV at 290 nm.

Method C. Column: Chiralpak AD, 250 × 4.6 mm. Mobile phase: Methanol. Gradient: Isocratic. Flow rate: 0.8 mL/min. Run time: 20 min. Detection: UV at 254 nm.

Isobutyric acid 2,2-dimethoxy-ethyl ester (2a).

4-Methoxy-benzoic acid 2,2-diethoxy-ethyl ester (2b). To a well-stirred solution of 2,2-diethoxy or 2,2-dimethoxy-ethanol (**1**, 100 mmol), DMAP (61 mg, 0.5 mmol) and Et_3N (16 mL, 11.64 g, 115 mmol) in EtOAc or *tert*-butylmethyl ether (50 mL) at 0°C was slowly added the corresponding acid chloride (105 mmol). After stirring for 16 h at room temperature the reaction mixture was diluted with EtOAc (50 mL), and successively washed with: (c) $NaHCO_3$ (2 × 100 mL), brine (2 × 100 mL), dried, filtered and evaporated to afford:

Isobutyric acid 2,2-dimethoxy-ethyl ester (**2a**, 99%) as a yellow liquid that was used in the next step without any further purification. GC (R_t = 5.24 min., 98%); ^{1}H-NMR ($CDCl_3$) δ: 4.57 (1H, t, J = 5.2, $(MeO)_2CHCH_2-$), 4.11 (2H, d, J = 5.2, $(MeO)_2CHCH_2-$), 3.40 (6H, s, CH_3O-), 2.60 (1H, m, $OCOCH(CH_3)_2$), 1.54 (6H, d, J = 6.8, $OCOCH(CH_3)_2$).

4-Methoxybenzoic acid 2,2-diethoxy-ethyl ester (**2b**, 100%) as a syrup that was used in the next step without any further purification. GC (R_t = 13.7 min, 99%); ^{1}H-NMR ($CDCl_3$) δ: 7.98 (2H, d, J = 9.0, ArH), 6.89 (2H, d, J = 9.0, ArH), 4.79 (1H, t, J = 5.6, $(EtO)_2CHCH_2-$), 4.28 (2H, d, J = 5.6, $(EtO)_2CHCH_2-$), 3.82 (3H, s,

CH_3O-), 3.73 (2H, m, CH_3CH_2O-), 3.59 (2H, m, CH_3CH_2O-), 1.22 (6H, t, J = 6.9, CH_3CH_2O-).

Isobutyric acid 4-oxo-[1,3]-dioxolan-2-yl methyl ester (3a). To a well stirred solution of the corresponding acetal (**2a**, 30 mmol) and α-hydroxy acetic acid (3.42 g, 45 mmol) in acetonitrile (30 mL) at 0°C was slowly added $BF_3 \cdot Et_2O$ (6.38 g, 5.70 mL, 45 mmol). The solution was left at room temperature overnight with stirring. The solution was partitioned between EtOAc (150 mL) and (c) $NaHCO_3$ (150 mL). The organic solution was successively washed with (c) $NaHCO_3$ (150 mL), brine (2 × 150 mL), dried, filtered and evaporated to afford:

Isobutyric acid 4-oxo-[1,3]-dioxolan-2-yl methyl ester (**3a**, 80%) as a colorless syrup. GC (R_t = 7.89 min., 95%); ^{1}H-NMR ($CDCl_3$) δ: 5.83 (1H, s, H-2), 4.35–4.20 (4H, m, H-5, H-5′ and $-CH_2OCO-$), 2.62 (1H, m, $(CH_3)_2CHCOO-$), 1.19 (6H, d, J = 7.0, $(CH_3)_2CHCOO-$).

Calculated mass for $C_8H_{13}O_5$ $(M + 1)^+$: 189.0763. *Found*: (H.R.F.A.B.M.S.): 189.0763.

4-Methoxybenzoic acid 4-oxo-[1,3]-dioxolan-2-yl methyl ester (3b). A solution of 4-methoxybenzoic acid 2,2-diethoxy-ethyl ester (**2b**, 7.5 g, 28 mmol) in Cl_2CH_2 (75 mL) was treated with TFA (16.7 g, 11.3 mL, 140 mmol) and water (7.5 g, 7.5 mL, 28 mmol). The homogeneous solution was stirred for 3.5 hours at room temperature until GC showed complete reaction. The solution was concentrated in vacuo at 40°C and then diluted with hexane and concentrated in vacuo several times to remove traces of TFA. The product, 4-methoxybenzoic acid 2-oxo-ethyl ester (**2c**, 5.9 g, 28 mmol, 100%) was isolated as an amorphous white solid and was used in the next step without any further purification. GC (R_t = 11.0 min, 95%); ^{1}H-NMR ($CDCl_3$) δ: 9.72 (1H, s, HCO–), 8.06 (2H, d, J = 8.8, ArH), 6.95 (2H, d, J = 8.8, ArH), 4.87 (2H, s, $HCOCH_2O-$), 3.87 (3H, s, OCH_3).

To a well stirred solution of the crude aldehyde (**2c**, 5.9 g, 28 mmol) and α-hydroxy acetic acid (5.2 g, 68 mmol) in DME (100 mL) at 0°C was slowly added $BF_3 \cdot Et_2O$ (12.3 g, 11.0 mL, 85 mmol). The solution was left at room temperature overnight with stirring. The solution was partitioned between EtOAc (150 mL) and (c) $NaHCO_3$ (150 mL). The organic solution was successively washed with (c) $NaHCO_3$ (150 mL), brine (2 × 150 mL), dried, filtered and evaporated to afford a syrup. The syrup was treated with DME (15 mL) and a solid precipitated. After stirring for 30 minutes, the solid was filtered to afford 4-methoxybenzoic acid 4-oxo-[1,3]-dioxolan-2-yl methyl ester (**3b**, 5.7 g, 23 mmol, 80%) as a white granular solid; GC (R_t = 14.7 min, 99%); mp: 61–63°C; ^{1}H-NMR ($CDCl_3$) δ: 7.97 (2H, d, J = 9.2, ArH), 6.91 (2H, d, J = 9.2, ArH), 5.95 (1H, t, J = 3.0, H-2), 4.57 (1H, dd, J = 3.0 and J = 12.6, $-CH_2OCO-$), 4.50 (1H, dd, J = 3.0 and J = 12.6, $-CH_2OCO-$), 4.41 (1H, d, J = 15.0, H-5), 4.31 (1H, d, J = 15.0, H-5), 3.87 (3H, s, OCH_3).

Calculated for $C_{12}H_{12}O_6$, C, 57.14; H, 4.80. *Found*: C, 57.40; H, 4.93.

Isobutyric acid 4-oxo-[1,3]-dioxolan-2(R)-yl methyl ester (4a). Racemic isobutyric acid 4-oxo-[1,3]-dioxolan-2-yl methyl ester (**3a**) was resolved by chiral chromatography[a] to afford two fractions corresponding to each one of the enantiomers. Both fractions were colorless syrups. The first fraction corresponded to the R

enantiomer (**4a**, isobutyric acid 4-oxo-[1,3]-dioxolan-2(R)-yl methyl ester); HPLC (Method A, R_t = 7.80 min., 100%); $[\alpha]_D^{20}$ = 20.20° (c 0.25, MeOH). The second fraction corresponded to the S enantiomer (isobutyric acid 4-oxo-[1,3]-dioxolan-2(S)-yl methyl ester); HPLC (Method A, R_t = 9.30 min., 100%); $[\alpha]_D^{20}$ = − 14.40° (c 0.25, MeOH).

4-Methoxybenzoic acid 4-oxo-[1,3]-dioxolan-2(R)-yl methyl ester (4b). Racemic 4-methoxybenzoic acid 4-oxo-[1,3]-dioxolan-2-yl methyl ester (**3b**) was resolved by chiral chromatography[a] to afford two fractions corresponding to each one of the enantiomers. Both fractions were white solids. The first fraction corresponded to the R enantiomer (**4b**, 4-methoxybenzoic acid 4-oxo-[1,3]-dioxolan-2(R)-yl methyl ester); mp: 76−78°C; HPLC (Method A, R_t = 13.59 min., 99%); $[\alpha]_D^{20}$ = 12.20° (c 0.25, MeOH). The second fraction corresponded to the S enantiomer (4-methoxybenzoic acid 4-oxo-[1,3]-dioxolan-2(S)-yl methyl ester); mp: 76−78°C; HPLC (Method A, R_t = 20.76 min., 99%); $[\alpha]_D^{20}$ = −13.50° (c 0.25, MeOH).

Isobutyric acid 4-acetoxy-[1,3]-dioxolan-2(R)-yl methyl ester (5a).

4-Methoxybenzoic acid 4-acetoxy-[1,3]-dioxolan-2(R)-yl methyl ester (5b). To a well stirred solution of the corresponding lactone (**4a** or **4b**, 15 mmol) in dry THF (45 mL) at −10°C, was slowly added a 1.0 M solution of $LiAlH(OtBu)_3$ in THF (19.5 mL, 19.5 mmol) over a period of 20 minutes, maintaining the temperature between −15°C and −10°C. The reaction was followed by GC and was stirred for 30 minutes at room temperature (complete disappearance of starting material). The solution was again cooled to −10°C and DMAP (0.92 g, 7.50 mmol) was added in one portion followed by the dropwise addition of Ac_2O (15.3 g, 14.20 mL, 150 mmol). The reaction was further stirred for 1 h at −15°C, and then overnight at room temperature. The solution was cooled to −15°C and quenched with MeOH (40 mL). After stirring for 20 minutes at room temperature, the reaction was concentrated in vacuo to a red syrup that was purified by flash column chromatography (250 g silica, hexane:EtOAc 3:1) to afford:

Isobutyric acid 4-acetoxy-[1,3]-dioxolan-2(R)-yl methyl ester (**5a**, 61%) as a yellow syrup. ^{1}H-NMR ($CDCl_3$) shows almost a 1:1 mixture of anomers, δ: 6.40 (d, J = 3.8) and 6.37 (d, J = 3.8) corresponding to H-4 α and H-4 β; 5.41 (t, J = 3.7) and 5.32 (t, J = 3.7) corresponding to H-2 α and H-2 β.

4-Methoxybenzoic acid 4-acetoxy-[1,3]-dioxolan-2(R)-yl methyl ester (**5b**, 61%) as a yellow syrup. ^{1}H-NMR ($CDCl_3$) shows almost a 1:1 mixture of anomers, δ: 6.44 (dd, J = 2.1 and J = 4.2) and 6.37 (d, J = 4.0) corresponding to H-4 α and H-4 β; 5.54 (t, J = 3.6) and 5.45 (t, J = 4.0) corresponding to H-2 α and H-2 β.

Isobutyric acid 4(R)-(2,6-dichloro-purin-9-yl)-[1,3]-dioxolan-2(R)-yl methyl ester (7a).

4-Methoxybenzoic acid 4(R)-(2,6-dichloro-purin-9-yl)-[1,3]-dioxolan-2(R)-yl methyl ester (7b). A suspension of 2,6-dichloropurine (1.27 g, 6.73 mmol), ammonium sulfate (38 mg, 0.29 mmol) and 1,1,1,3,3,3-hexamethyldisilazane (8.45 g, 11.04 mL, 52.3 mmol) was heated at reflux for 2.5 h. The resulting solution was cooled

to ambient temperature whereby a thick solid precipitated. The solids were redissolved through the addition of dry dichloromethane (14.9 mL). The solution was then cooled to −10°C, and a solution of the corresponding acetate (**5a** or **5b**, 8.6 mmol) in dry dichloromethane (10 mL) was slowly added over a period of 20 minutes, maintaining the temperature between −10°C and −5°C. Then, TMSOTf (2.5 g, 2.08 mL, 10.4 mmol) was slowly added over a period of 20 minutes. The reaction was left overnight with stirring at room temperature. The solution was diluted with dichloromethane (120 mL) and quenched with water (150 mL). The organic layer was separated and successively washed with water (150 mL), (c) $NaHCO_3$ (2 × 150 mL), water (2 × 150 mL), dried, filtered and evaporated to a syrup (crude **6a**) or a yellow solid (crude **6b**).

Crude **6a** was further purified by column chromatography (Hexane:AcOEt 4:1) to afford **6a** as a 1.2:1 β:α mixture of anomers, according to ^{1}H-NMR. The mixture was slowly crystallized from MeOH (10 mL) to afford isobutyric acid 4(R)-(2,6-dichloro-purin-9-yl)-[1,3]-dioxolan-2(R)-yl methyl ester (**7a**, 24%) as a white solid and characterized as the β anomer; mp: 145–147°C; $[\alpha]_D^{20} = -47.67°$ (c 0.25, MeOH); ^{1}H-NMR ($CDCl_3$) δ: 8.52 (1H, s, H-8), 6.55 (1H, d, J = 4.9, H-4), 5.34 (1H, t, J = 5.7, H-2), 4.58–4.30 (4H, m, $-CH_2COO-$, H-5 and H-5′), 2.61 (1H, m, $(CH_3)_2CHCOO-$), 1.19 (3H, d, J = 6.8, $(CH_3)_2CHCOO-$), 1.14 (3H, d, J = 6.8, $(CH_3)_2CHCOO-$).

Calculated for $C_{13}H_{14}Cl_2N_4O_4$: C, 43.23; H, 3.91; N, 15.51. *Found*: C, 43.39; H, 3.91; N, 15.58.

Crude **6b** was first washed with hot hexane to afford 4-methoxy-benzoic acid 4-(2,6-dichloro-purin-9-yl)-[1,3]-dioxolan-2(R)-yl methyl ester (**6b**) as a yellow solid. ^{1}H-NMR indicated a 2:1 mixture of β:α anomers. This mixture was slowly crystallized from MeOH (100 mL) to afford 4-methoxybenzoic acid 4(R)-(2,6-dichloro-purin-9-yl)-[1,3]-dioxolan-2(R)-yl methyl ester (**7b**, 46%) as a yellow solid and characterized as the β anomer; mp: 154–156°C; HPLC (Method B, R_t = 20.73 min.); $[\alpha]_D^{20} = -53.80°$ (c 0.25, MeOH); ^{1}H-NMR (Cl_3CD) δ: 8.41 (1H, s, H-8), 7.91 (2H, d, J = 8.5, ArH), 6.92 (2H, d, J = 8.5, ArH), 6.53 (1H, d, J = 4.8, H-4), 5.44 (1H, bs, H-2), 4.70–4.30 (4H, m, H-5, H-5′ and $-CH_2OCO-$), 3.85 (3H, s, OCH_3).

Calculated mass for $C_{17}H_{15}Cl_2N_4O_5$ $(M + 1)^+$: 425.0419. *Found* (H.R.F.A.B.M.S.): 425.0420.

Isobutyric acid 4(R)-(2,6-diazido-purin-9-yl)-[1,3]-dioxolan-2(R)-yl methyl ester (8a).

4-Methoxybenzoic acid 4(R)-(2,6-diazido-purin-9-yl)-[1,3]-dioxolan-2(R)-yl methyl ester (8b). To a well-stirred solution of the corresponding dichloropurine nucleoside (**7a** or **7b**, 2.9 mmol) in dry DMF (13.5 mL) was added NaN_3 (390 mg, 6.0 mmol). The reaction mixture was left at room temperature with stirring for 4 h. The mixture was filtered through celite to afford a solution of:

Isobutyric acid 4(R)-(2,6-diazido-purin-9-yl)-[1,3]-dioxolan-2(R)-yl methyl ester (**8a**) or 4-methoxybenzoic acid 4(R)-(2,6-diazido-purin-9-yl)-[1,3]-dioxolan-2(R)-yl methyl ester (**8b**) in DMF (70 mL) which was used in the next step without any further purification.

Isobutyric acid 4(R)-(2,6-diamino-purin-9-yl)-[1,3]-dioxolan-2(R)-yl methyl ester (9a).

4-Methoxybenzoic acid 4(R)-(2,6-diamino-purin-9-yl)-[1,3]-dioxolan-2(R)-yl methyl ester (9b). The solution from the previous reaction (**8a** or **8b**) was hydrogenated (Parr apparatus, 50 psi) at room temperature, in the presence of 10% Pd/C (200 mg) overnight. The mixture was filtered through Celite and the filter cake washed with additional DMF.

In the case of **8a**, the solution was concentrated to dryness and purified by column chromatography (Cl_3CH:MeOH 9:1) to afford a white solid that was crystallized from *iso*-propanol to afford isobutyric acid 4(R)-(2,6-diamino-purin-9-yl)-[1,3]-dioxolan-2(R)-yl methyl ester (**9a**, 84% after correction for the presence of one molecule of 2-propanol that co-crystallizes) as a white solid; mp: 143–145°C; $[\alpha]_D^{20} = -56.45°$ (c 0.25, MeOH); ^{1}H-NMR ($CDCl_3$) δ: 7.84 (1H, s, H-8), 6.33 (1H, dd, J = 1 and J = 5.1, H-4), 5.29 (3H, t, J = 3.2, H-2 and NH_2), 4.70 (2H, bs, NH_2), 4.50 (1H, dd, J = 1 and J = 9.0, H-5), 4.33 (2H, d, J = 3.2, $-CH_2COO-$), 4.23 (1H, dd, J = 5.1 and J = 9.0, H-5′), 2.60 (1H, m, $(CH_3)_2CHCOO-$), 1.18 (3H, d, J = 6.2, $(CH_3)_2CHCOO-$), 1.14 (3H, d, J = 7.2, $(CH_3)_2CHCOO-$).

Calculated for $C_{13}H_{18}N_6O_4 \cdot$ 2-propanol: C, 50.25; H, 6.85; N, 21.98. *Found*: C, 50.16; H, 6.84; N, 21.92.

In the case of **8b**, the solution was concentrated to a final volume of 15 mL in vacuo at 60°C. The solution was diluted with water (150 mL) and after a few minutes a solid precipitated. The product was filtered, washed with water, dried overnight at 50°C under vacuum to afford 4-methoxybenzoic acid 4(R)-(2,6-diamino-purin-9-yl)-[1,3]-dioxolan-2(R)-yl methyl ester (**9b**, 88%) as an amorphous solid; HPLC (Method B, R_t = 15.41 min.); $[\alpha]_D^{20} = -95.75°$ (c 0.25, MeOH); ^{1}H-NMR (DMSO-d_6) δ: 7.82 (2H, d, J = 8.8, ArH), 7.80 (1H, s, H-8), 7.02 (2H, d, J = 8.8, ArH), 6.77 (2H, bs, NH_2), 6.23 (1H, dd, J = 5.5 and J = 1.6, H-4), 5.85 (2H, bs, NH_2), 5.36 (1H, t, J = 3.3, H-2), 4.65 (1H, dd, J = 9.4 and J = 1.6, H-5), 4.46 (2H, d, J = 3.4, $-CH_2OCO-$), 4.26 (1H, dd, J = 9.4 and J = 5.5, H-5′), 3.84 (3H, s, OCH_3).

Calculated mass for $C_{17}H_{19}N_6O_5$: 387.1417. *Found* (H.R.F.A.B.M.S.): 387.1417.

4(R)-[-(2,6-Diamino-purin-9-yl)-[1,3]-dioxolan-2(R)-yl]-methanol, [10, (−)-DAPD] from 9a. A solution of isobutyric acid 4(R)-(2,6-diamino-purin-9-yl)-[1,3]-dioxolan-2(R)-yl methyl ester (**9a**, 100 mg, 0.31 mmol) and n-butylamine (0.45 g, 0.62 mL, 6.2 mmol) in MeOH (10 mL) was heated at reflux for 4 h. The reaction was cooled to ambient temperature and concentrated in vacuo to afford a solid that was triturated with *tert*-butyl methyl ether and filtered to afford a solid that was crystallized from EtOH:water (1:4.5 mL) to afford (−)-DAPD (**10**, 75 mg, 0.29 mmol, 93%) as a white solid; mp: 237–239°C (lit.[1] mp: 236–237°C); HPLC (Method C, R_t = 8.7 min., an authentic sample of (−)-DAPD showed R_t = 8.7 min. and a sample of (+) DAPD showed R_t = 6.0 min.); DAPD; ^{1}H-NMR (DMSO-d_6) δ: 7.80 (1H, s, H-8), 6.74 (2H, bs, NH_2), 6.20 (1H, d, J = 5.5, H-4), 5.84 (2H, bs, NH_2), 5.16 (1H, t, J = 6.3, OH), 5.03 (1H, t, J = 2.9, H-2), 4.42 (1H, d, J = 9.7, H-5), 4.18 (1H, dd, J = 9.7 and J = 5.5, H-5′), 3.58 (2H, dd, J = 6.3 and J = 2.9, $-CH_2OH$).

Calculated for $C_9H_{12}N_6O_3$: C, 42.86; H, 4.80; N, 33.32. *Found*: C, 42.88; H, 4.79; N, 33.32.

4(R)-[-(2,6-Diamino-purin-9-yl)-[1,3]-dioxolan-2(R)-yl]-methanol, [10, (−)-DAPD] from 9b. A suspension of 4-methoxybenzoic acid 4(R)-(2,6-diamino-purin-

9-yl)-[1,3]-dioxolan-2(R)-yl methyl ester (**9b**, 1.0 g, 2.6 mmol) and n-butylamine (3.7 g, 5.0 mL, 50 mmol) in MeOH (25 mL) was heated at reflux for 4 hours. The reaction was concentrated in vacuo to afford a yellow solid that was suspended in Cl_3CH at room temperature and filtered, to afford (−) DAPD (**10**, 590 mg, 2.3 mmol, 90%) as a white solid. Physical properties were identical to the sample of (−)-DAPD obtained above.

CONCLUSIONS

A new eight step scalable synthesis of (−)-DAPD was achieved in good overall yield, using inexpensive reagents and starting materials.

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