Supporting information for:

Structure Activity Relationship of (N)-Methanocarba Phosphonate Analogues of 5'-AMP as Cardioprotective Agents Acting Through a Cardiac P2X Receptor

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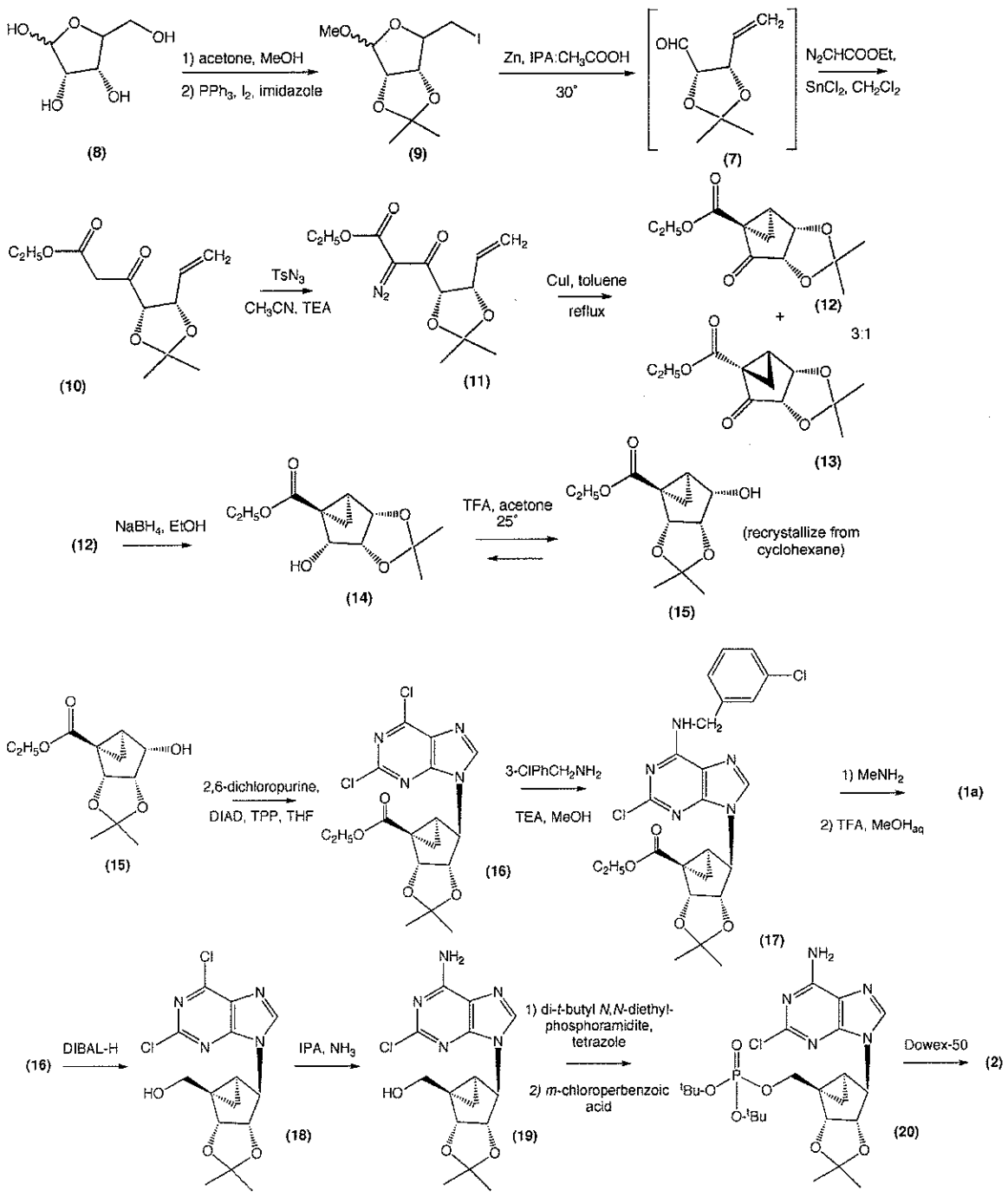
Ramachandran Balasubrimanian, Nielle Marshall, Bruce T. Liang,

Kenneth A. Jacobson

Contents:

I.



Compound numbers correspond to: Joshi, B.V.; Melman, A.; Mackman, R.L.; Jacobson, K.A. Synthesis of ethyl (1S,2R,3S,4S,5S)-2,3-O-(isopropylidene)-4-hydroxy-bicyclo[3.1.0]hexane-carboxylate from L-ribose: A versatile chiral synthon for preparation of adenosine and P2 receptor ligands. *Nucleos. Nucleot. Nucleic Acids* **2008**, *27*, 279-291.

Ethyl-(1S,2R,3S,4S,5S)-2,3-O-(isopropylidene)-4-hydroxybicyclo[3.1.0]hexanecarboxylate (15).

Alcohol **14** (1.2 g, 4.95 mmol) was dissolved in anhydrous CH_2Cl_2 (13 mL). Trifluoromethane sulfonic acid (0.1 mL, 1.09 mmol) was added, and the reaction mixture was allowed stir at room temperature for 5 h. This reaction was monited by NMR, indicating that after 5 h there was no improvement in the ratio of isomerized to non-isomerized product. The reaction mixture was neutralized with Et_3N and diluted with CH_2Cl_2 (50 mL), and the organic layer was washed with H_2O (1 × 25 mL). The aqueous phase was back-extracted with CH_2Cl_2 (3 × 35 mL). The combined organic phase was evaporated to dryness, and the resulting crude residue was filtered using a short silica pad (50-80% EtOAc in petroleum ether, v/v) to afford a mixture of isomerized and non-isomerized alcohols (1.1 g) as a colorless oil. The mixture of isomers was further purified by crystallization using cyclohexane (~12 mL) using mild warming for dissolution, in which the majority of non-isomerized alcohol **14** separated out as crystals and was collected at 2 h, along with minor amount of isomerized alcohol **15**. Ratio of isomerized to non-isomerized alcohols varied but was most frequently ~ 3:2, respectively. The mother liquor was concentrated and crystallization in cyclohexane was repeated twice (sometimes up to four successive crystallizations were required) to get isomerized alcohol **15** (650 mg, 55%) as white long needles with purity >97%. The waiting period for the crystallization of **15** was the same 2 h. Most of the remaining 45% was non-isomerized alcohol **14**, which could be resubjected to the isomerization. Isomerization on a large scale (5 to 10 g) gave considerably lower yields (~25- 30 %). The ideal batch size was ≤3 g.

Ethyl-(1'S,2'R,3'S,4'R,5'S)-4'-(2,6-dichloropurin-9-yl]-2',3'-O-(isopropylidene)-bicyclo[3.1.0]hexanecarboxylate (16).

The alcohol **15** (350 mg, 1.44 mmol) was coevaporated with anhydrous toluene (3 × 15 mL) and dried under high vacuum. Triphenyl phosphine (757 mg, 2.88 mmol) and 2,6-dichloropurine (545 mg, 2.88 mmol) were dried under high vacuum. To a mixture of triphenyl phosphine and 2,6-dichloropurine in anhydrous THF (5 mL, < 5 ppm H_2O) was added freshly opened diisopropyl azodicarboxylate (97 µL, 0.49 mmol) at rt. After stirring for 45 min, a solution of compound **15** in THF (5 mL) was added. After stirring for 18 h, the reaction mixture was evaporated to dryness. The resulting residue was purified by silica gel column chromatography (0-50% EtOAc in n-hexane in increments of 5%, v/v) to afford nucleoside **16** (405 mg, 68%) as a white solid material. The chromatography gave DIAD impurities during the elution with 0-40% EtOAc, and at 45-50% EtOAc the desired compound could be collected.

(1'S,2'R,3'S,4'R,5'S)-4-(2,6-Dichloro-purin-9-yl)-1-[hydroxymethyl]bicyclo-[3.1.0]-hexane-2,3-(O-isopropylidine) (18).

Nucleoside **16** (200 mg, 0.48 mmol) was coevaporated with anhydrous toluene (3 × 10 mL), dissolved in anhydrous THF (10 mL) and cooled to -70 °C. 1.5 M Diisobutylaluminium hydride in toluene (3.2 mL, 4.8 mmol) was added dropwise for 15 min and the temperature allowed to warm to -40 °C. After stirring for 6 h at -40 °C, the reaction mixture was cooled to -70 °C again and quenched very slowly with MeOH until the cessation of air bubbles was observed. The reaction mixture was allowed to warm to rt, treated with EtOAc (25 mL) and a saturated aqueous solution of potassium sodium tartrate (20 mL), and allowed to stir at rt. After stirring for 1 h, the phases were separated; the aqueous phase was back-extracted with EtOAc (2 × 10 mL) and CH_2Cl_2 (2 × 10 mL). The combined organic phase was evaporated to dryness, and the resulting residue was purified by silica gel column chromatography (0-80% EtOAc in n-hexane in increments of 10%, v/v) to afford nucleoside

18 (100 mg, 87%) as a white solid material. The compound was collected at 70-80% EtOAc. This reaction works best on a scale of <800 mg.

(1'S,2'R,3'S,4'R,5'S)-4-(6-Amino-2-chloro-purin-9-yl)-1-[hydroxymethyl]bicyclo-[3.1.0]hexane-2,3-(O-isopropylidine) (19).

Nucleoside **18** (216 mg, 0.58 mmol) was treated with 2 M NH_3 in *i*-PrOH (5 mL) and heated to 70 °C. After stirring the reaction for 16 h, reaction mixture evaporated to dryness. The resulting residue purified by silica gel column chromatography (0-8% MeOH in CH_2Cl_2, v/v) to afford nucleoside **19** (165 mg, 80%) as a white solid material.

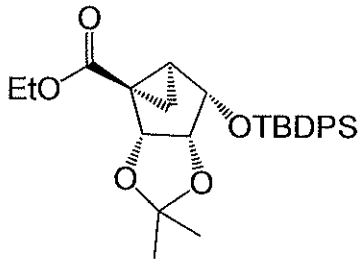
(1'S,2'R,3'S,4'R,5'S)-4-(6-Amino-2-chloro-9H-purin-9-yl)-1-[(di-tert-butylphosphate)methyl]bicyclo[3.1.0]hexane-2,3-(O-isopropylidene) (20).

Nucleoside **16** (125 mg, 0.36 mmol) was coevaporated with anhydrous toluene (3 × 10 mL), dissolved in anhydrous CH_3CN (10 mL). Di-*t*-butyl*N,N*-diethylphosphoramidite (0.3 mL, 1.07 mmol) and tetrazole (220 mg, 3.2 mmol) were added. After stirring at rt for 4h, the reaction mixture was cooled to -70 °C followed by the addition of *m*-chloroperbenzoic acid (50 mg, 77%). The reaction mixture was warmed to 0 °C and allowed to stir for 15 min followed by the addition of triethylamine (0.5 mL). The reaction mixture was evaporated to dryness, and the resulting crude residue was purified by silica gel column chromatography (0-100% EtOAc in CH_2Cl_2 in increments of ~15%, v/v) to afford nucleoside **20** (120 mg, 62%) as a white solid material. The product eluted at 100% EtOAc. If residual *m*-CPBA was present, the product was rechromatographed until ≥98% purity.

(1'S,2'R,3'S,4'R,5'S)-4-(6-Amino-2-chloro-9H-purin-9-yl)-1-[phosphoryloxymethyl] bicyclo[3.1.0]hexane-2,3-diol (2, MRS2339).

To a solution containing highly purified nucleoside **20** (97 g, 0.18 mmol) in MeOH (8 mL) and water (8 mL) was added Dowex-50 resin (50 mg). The mixture was stirred for 3 h at 70

°C and the resin removed by filtration. The filtration was then treated with 1 M triethylammonium bicarbonate buffer (1 mL) and evaporated to dryness. The resulted mixture was dissolved in H_2O (50 mL) and lyophilized. Lyophilization was repeated 7 times to give pure **2** (57 mg, 80%) as a white solid. The product was 98 to 99% pure, as determined by HPLC using two different eluent systems.



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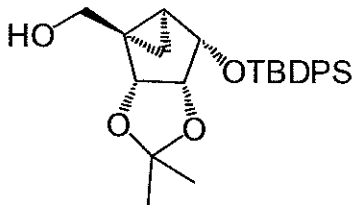

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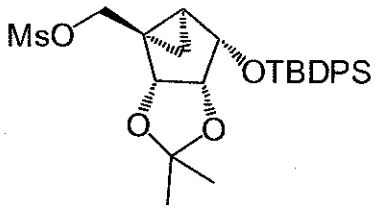

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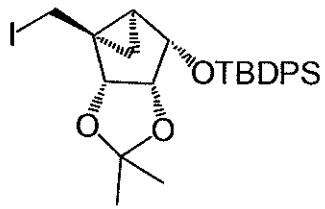

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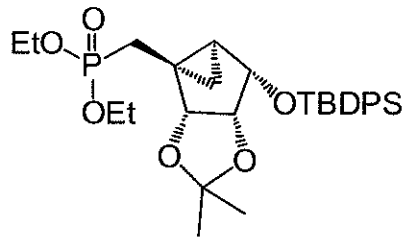

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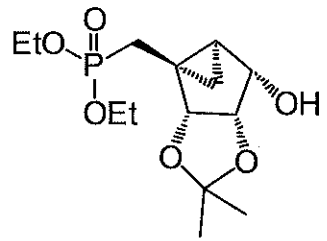

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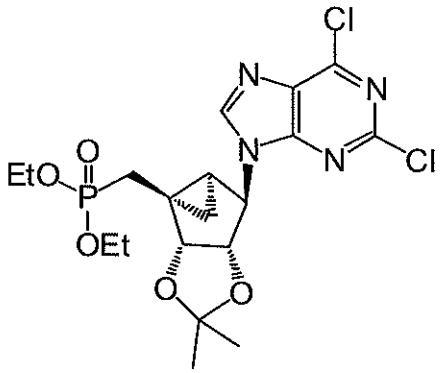
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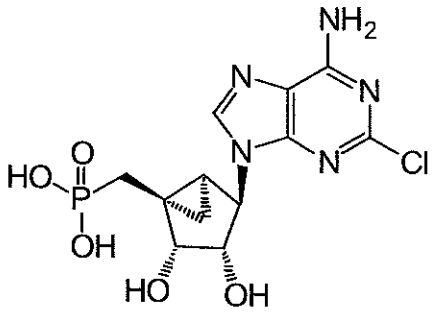
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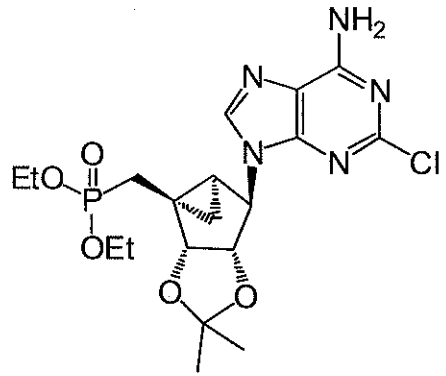
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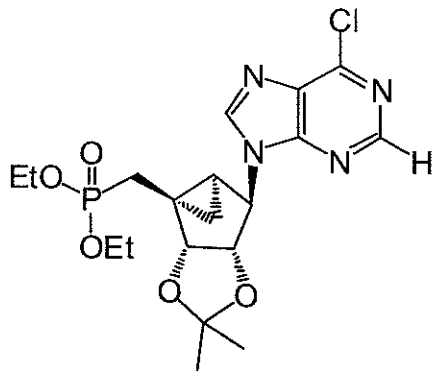
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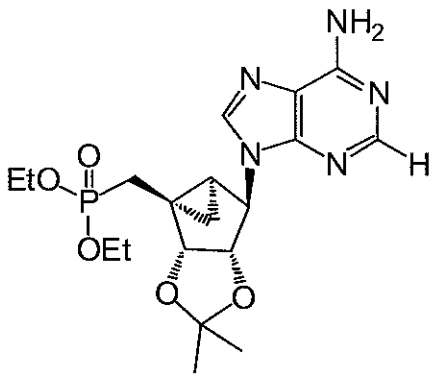
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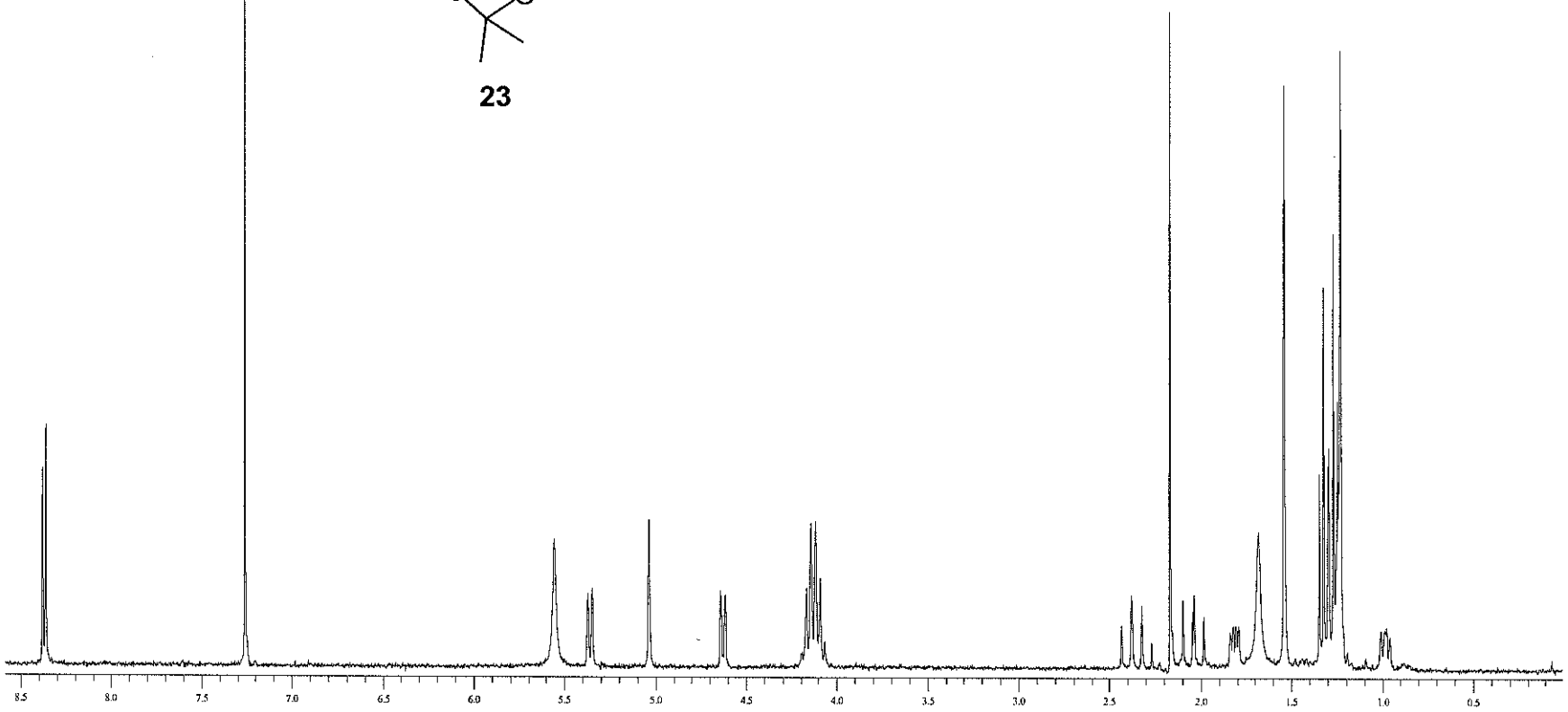
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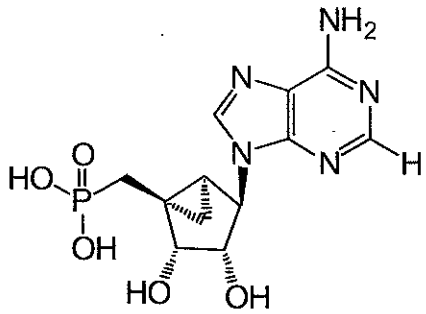
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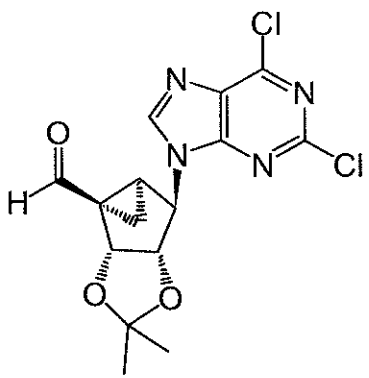
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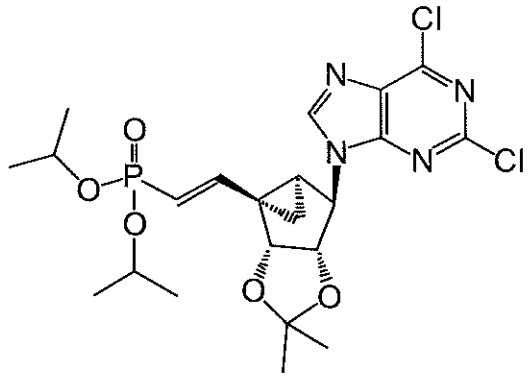

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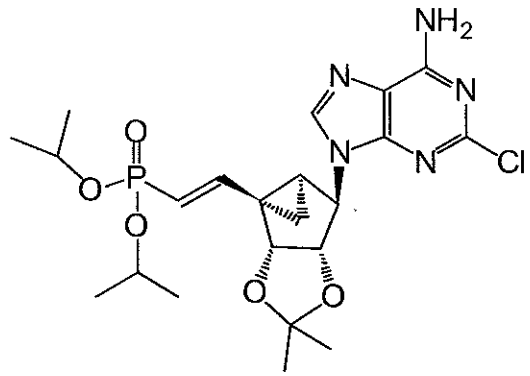

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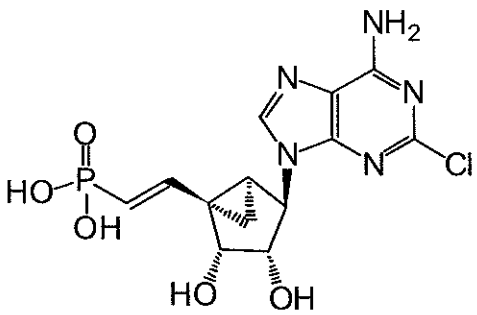
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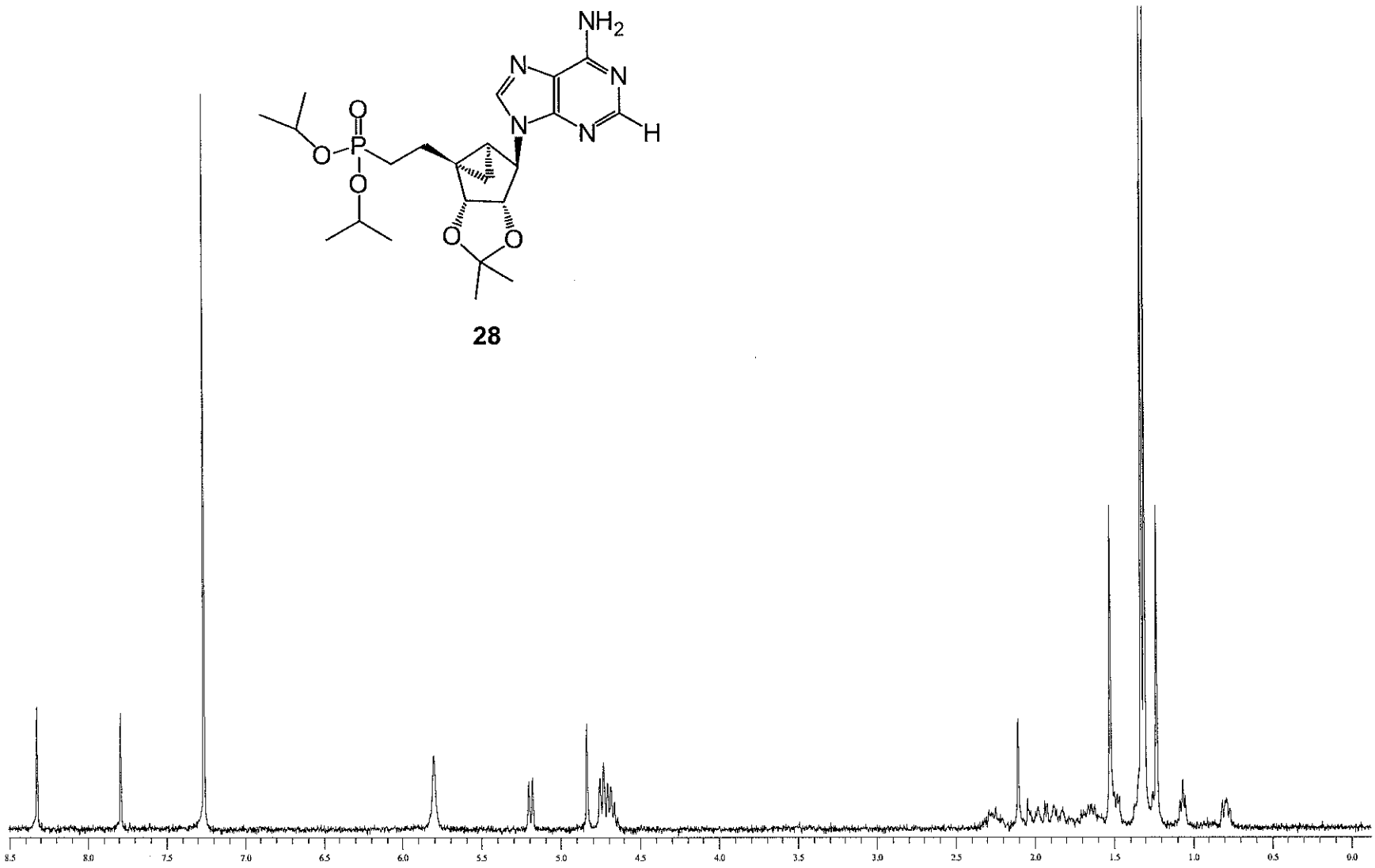
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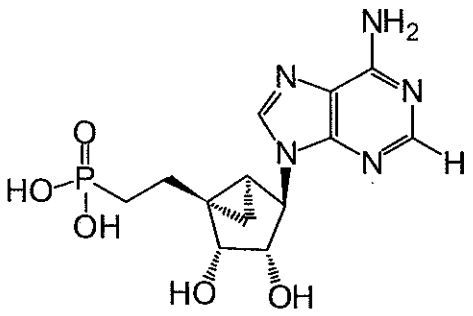
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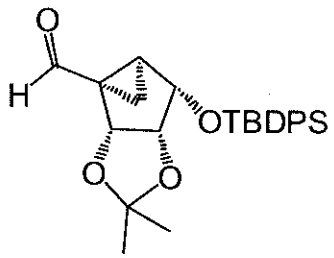

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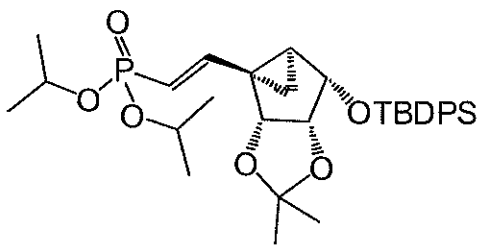

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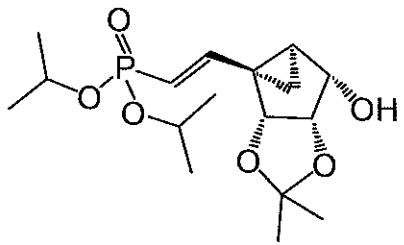

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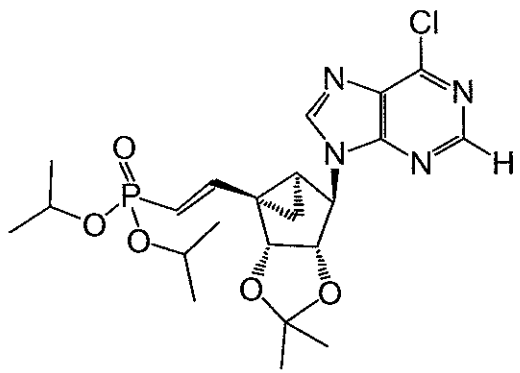

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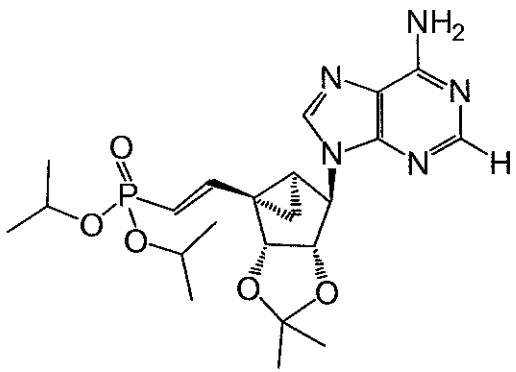
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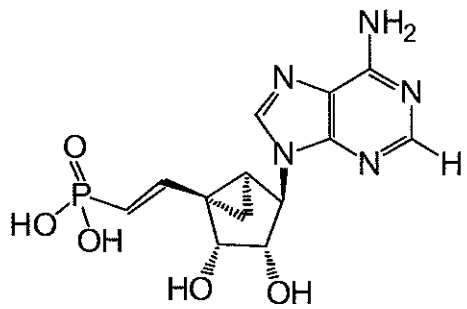
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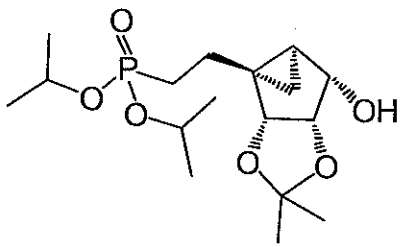

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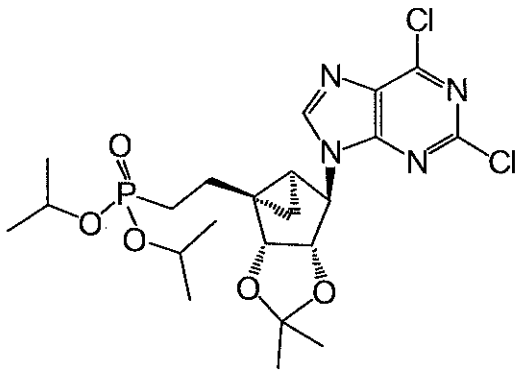

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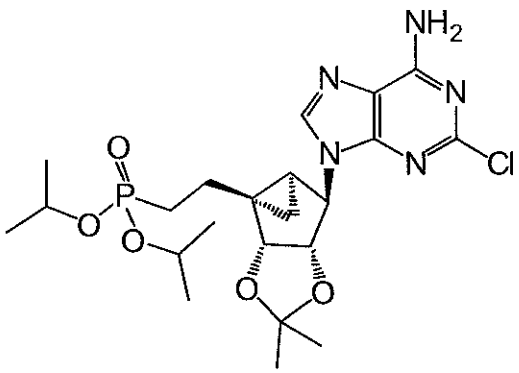

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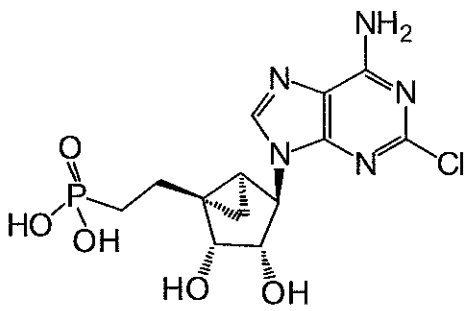
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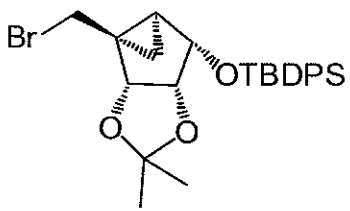

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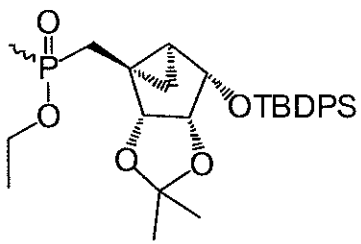

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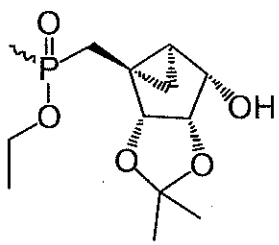

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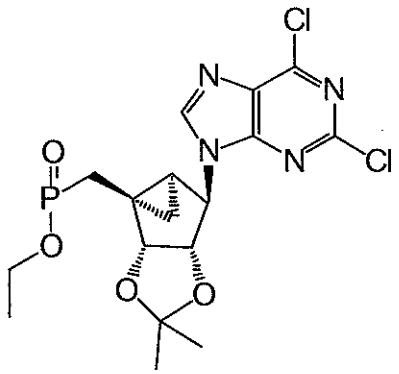

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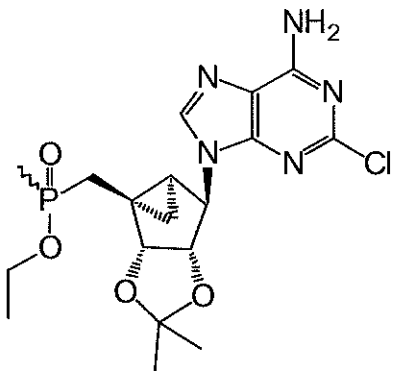

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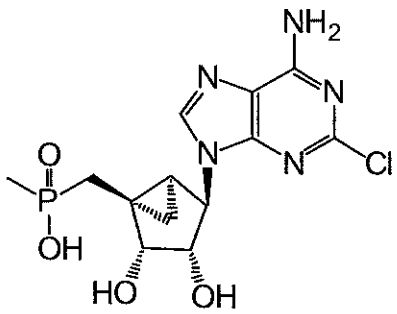

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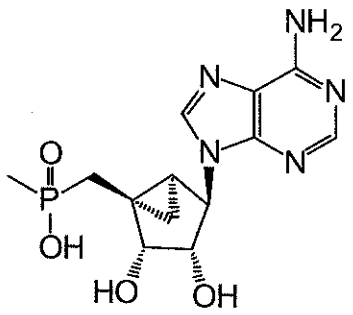

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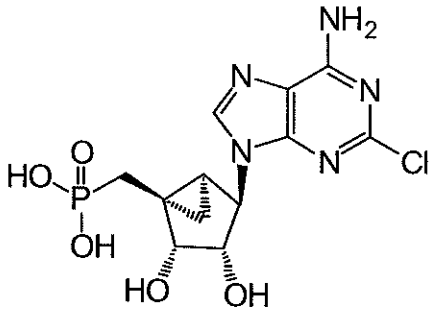
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Cl
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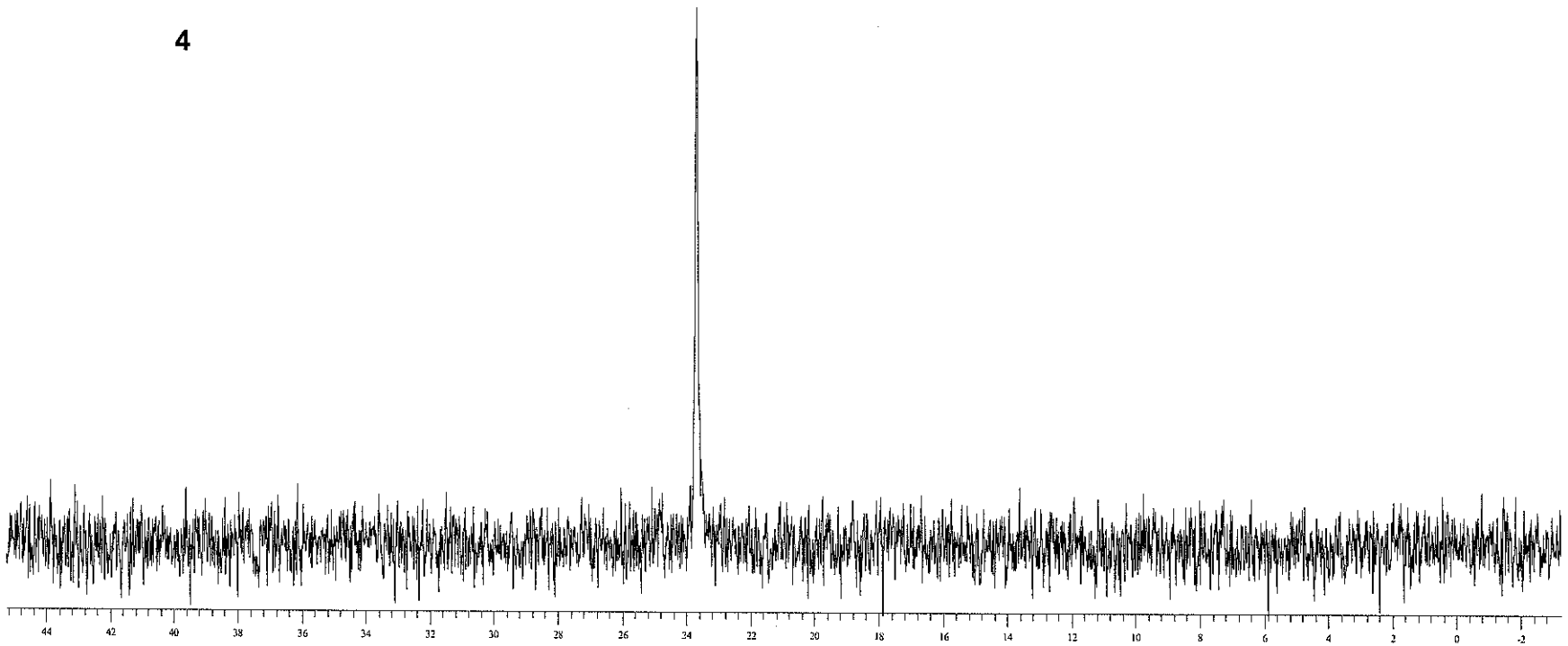
HO
OH

4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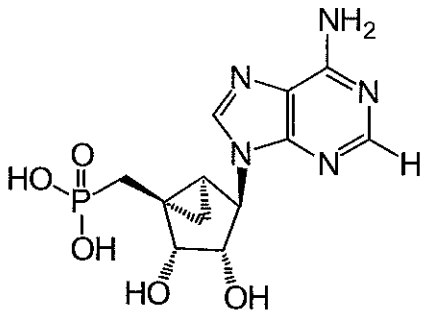
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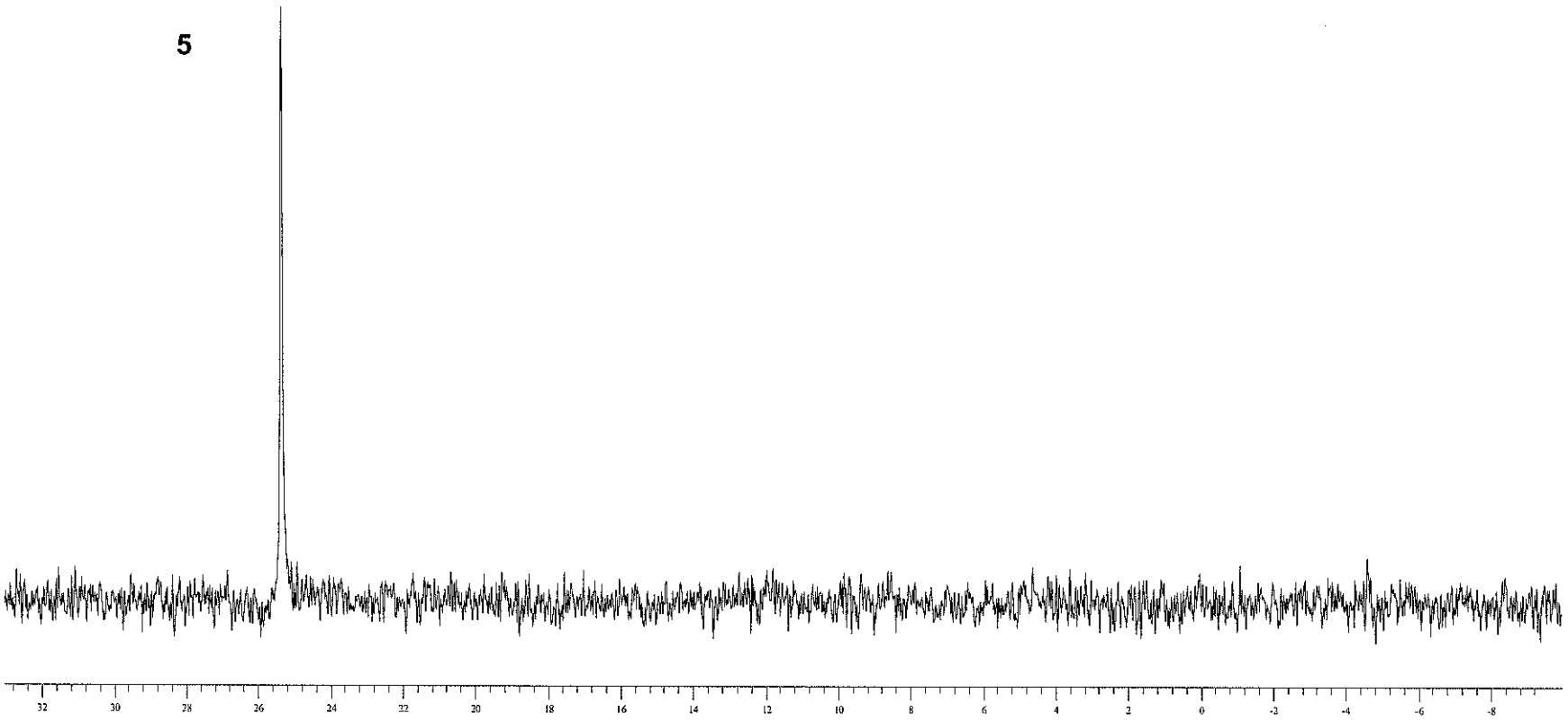
N
N
H
O
HO
P
OH
HO
OH

5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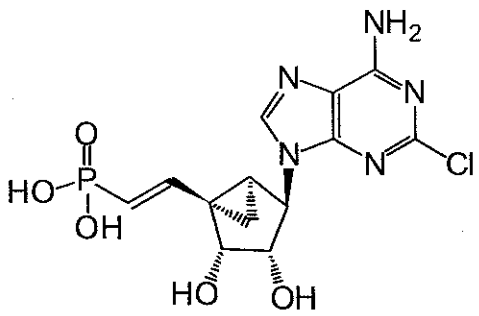
-6
-8



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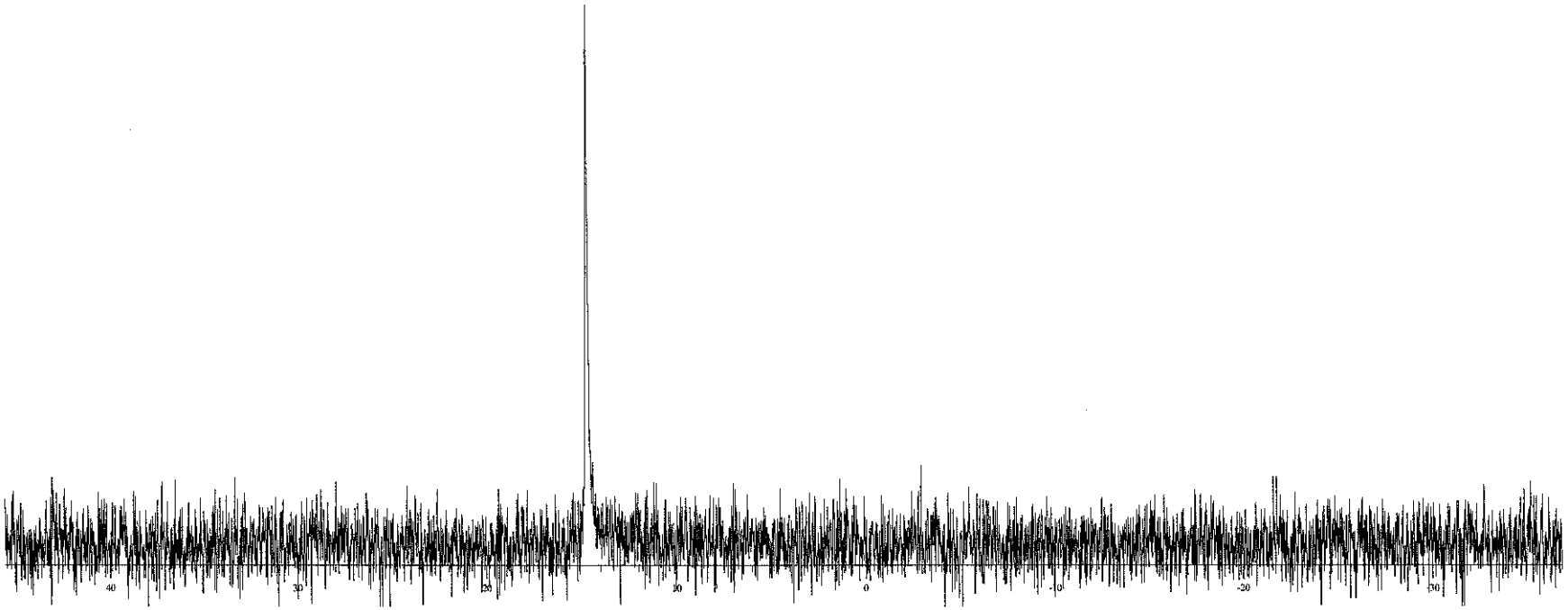

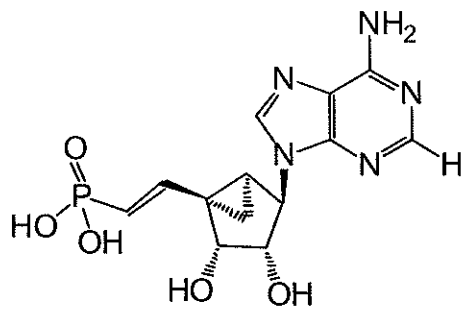

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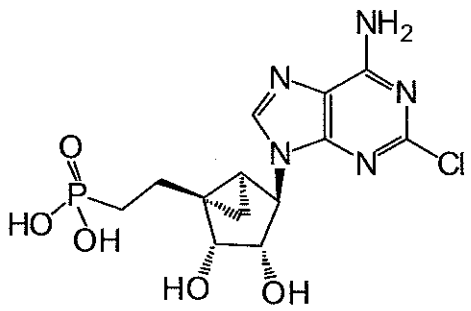

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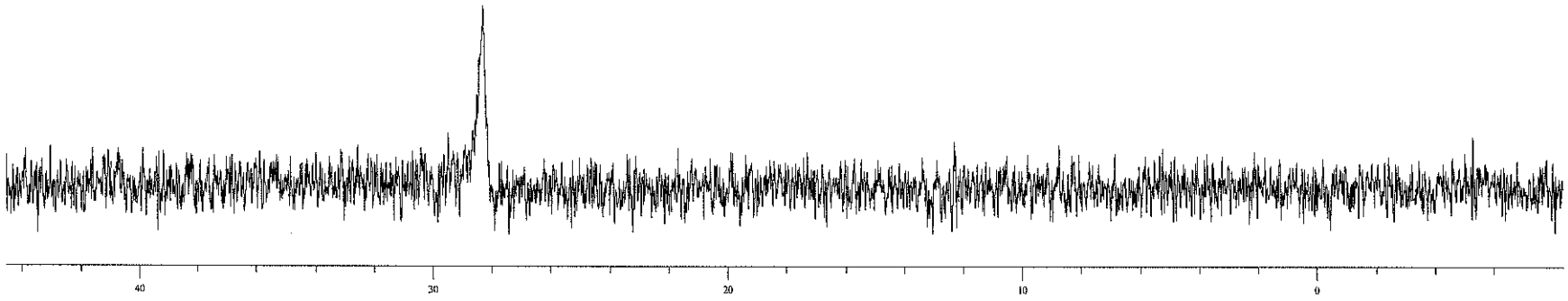

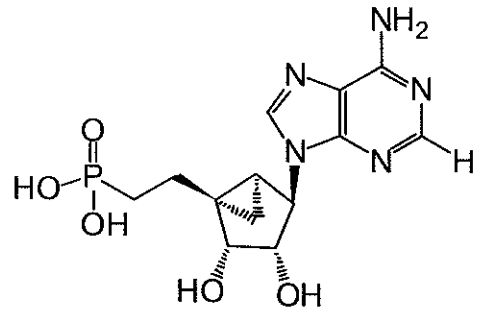

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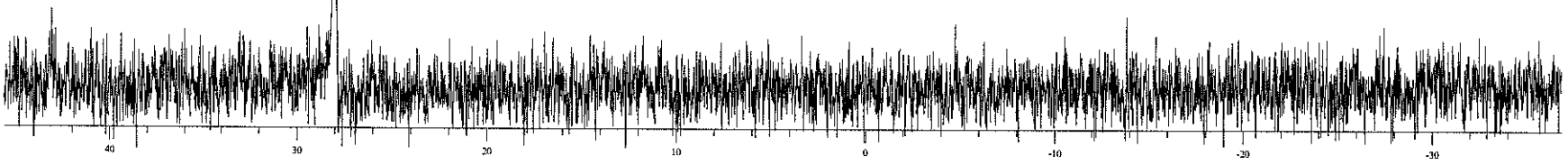

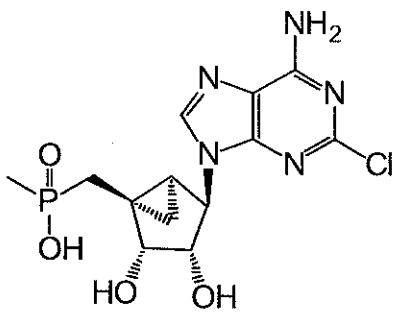

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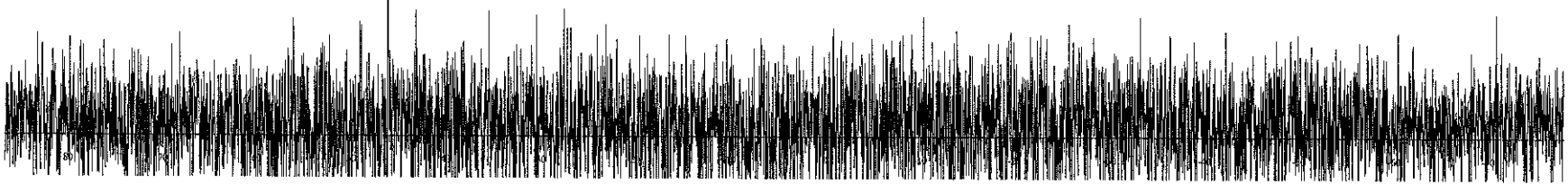

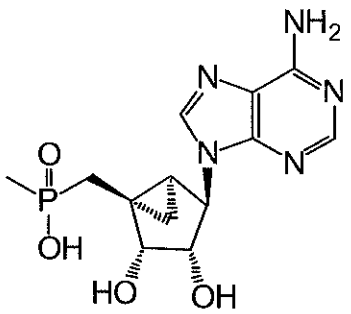

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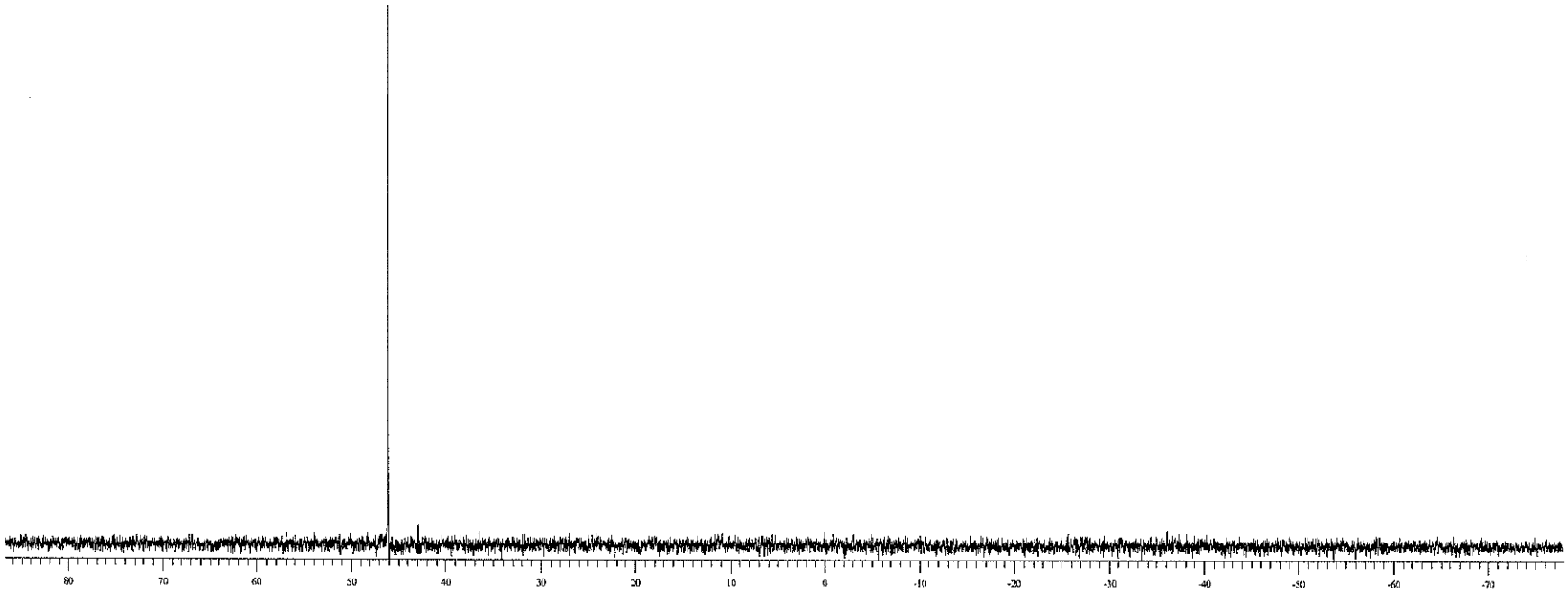
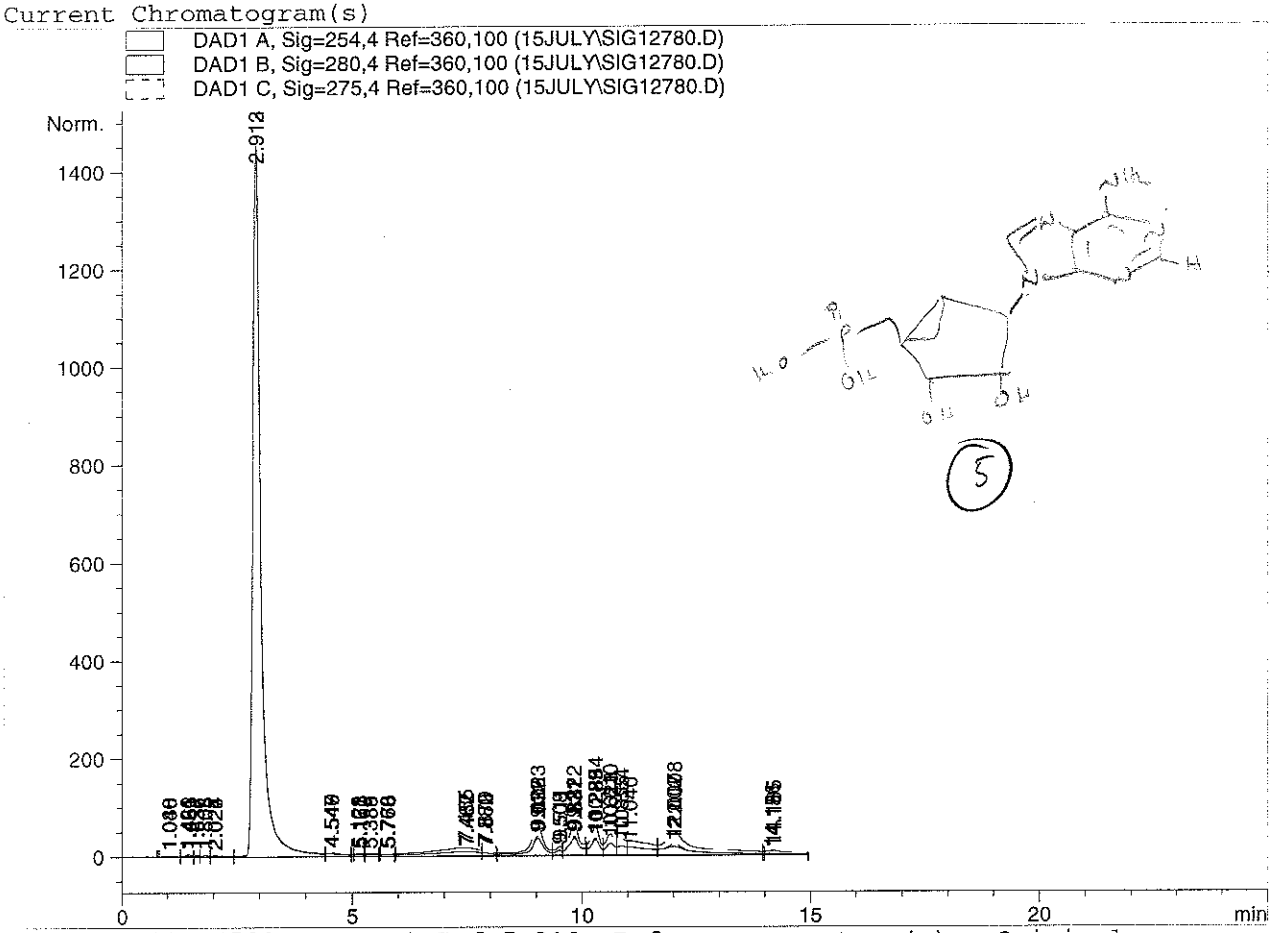


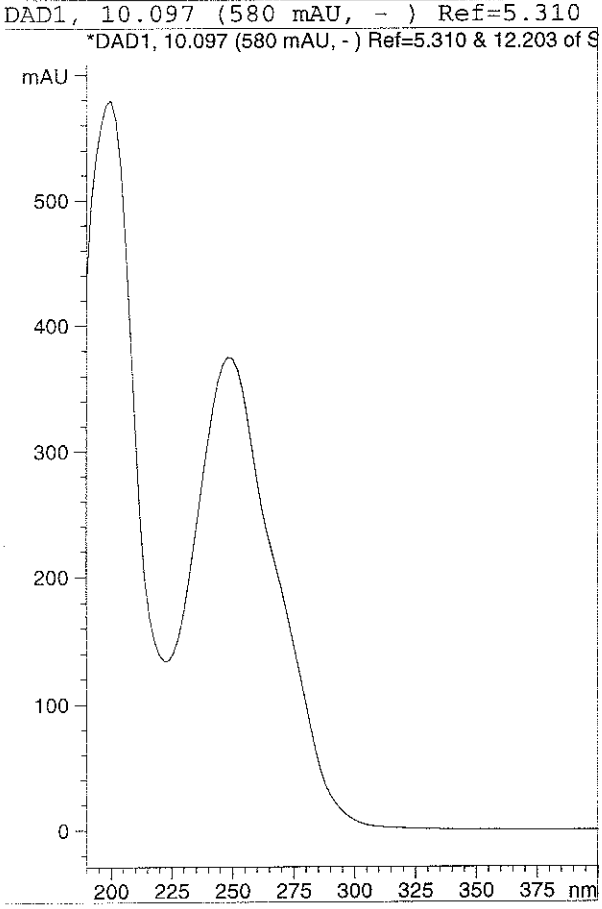
549

Current Chromatogram(s)

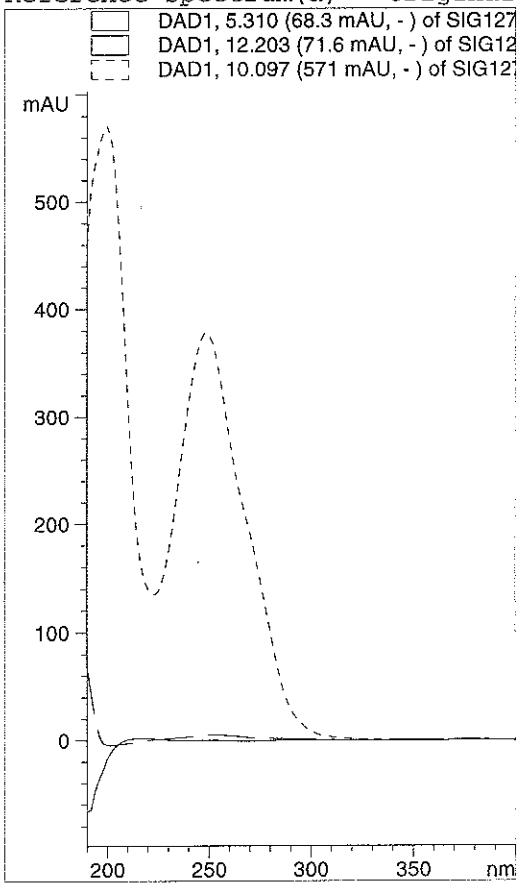


(5)

DAD1, 10.097 (580 mAU, -) Ref=5.310



Reference spectrum(a) + Original



Close all graphic windows

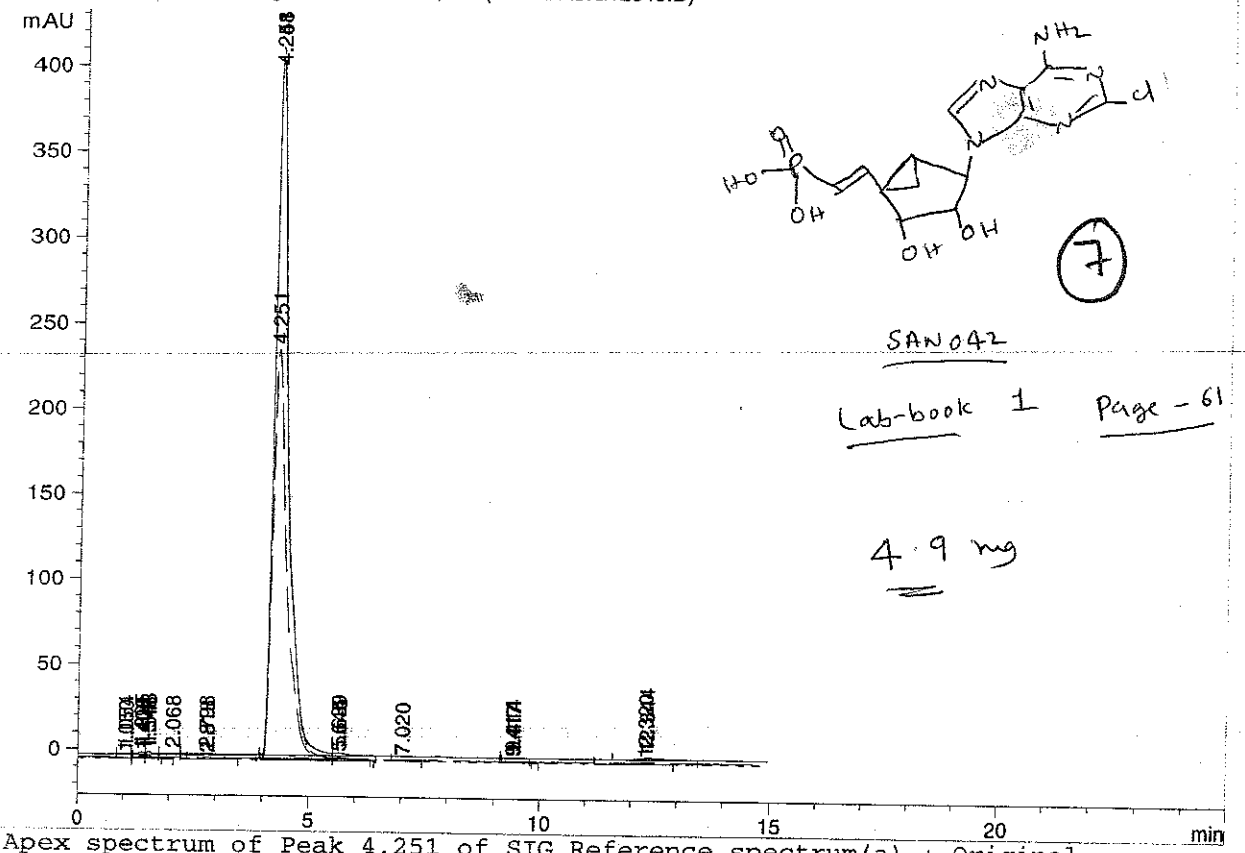
550

TBAP / $CH_3N\!\not\!O_2$ 80/20 → 40/60

13 mins

Current Chromatogram(s)

DAD1 A, Sig=254,4 Ref=360,100 (15JULY\SIG12345.D)
DAD1 B, Sig=280,4 Ref=360,100 (15JULY\SIG12345.D)
DAD1 C, Sig=275,4 Ref=360,100 (15JULY\SIG12345.D)



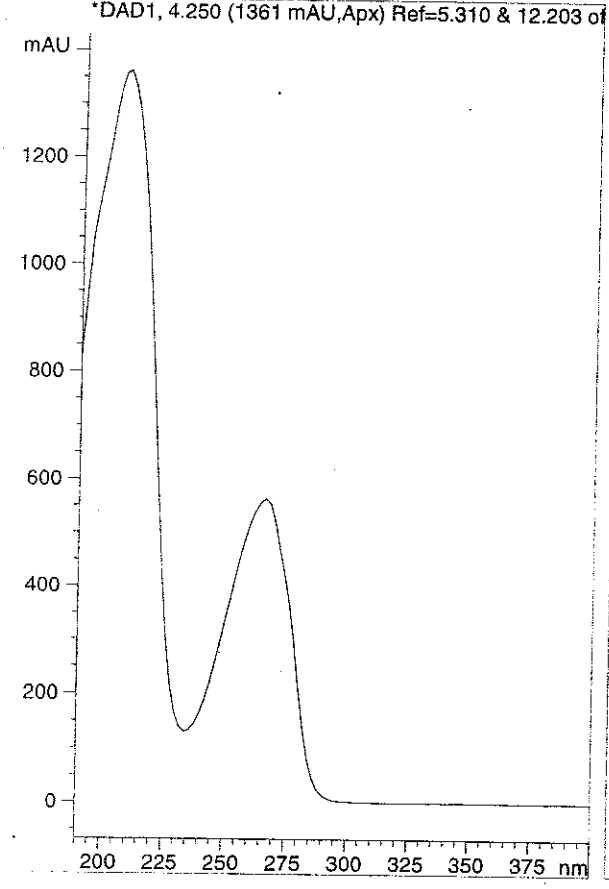
7

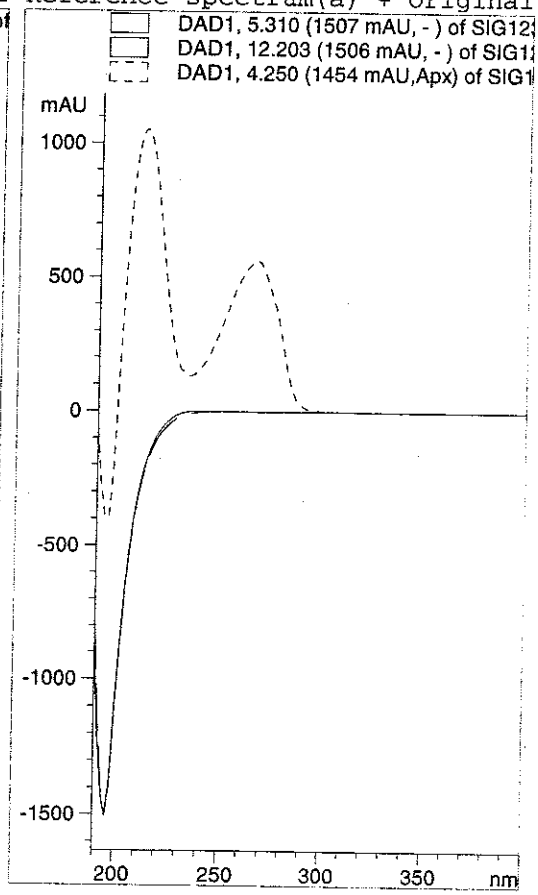
SAN042

Lab-book 1 Page - 61

4.9 mg

UV Apex spectrum of Peak 4.251 of SIG Reference spectrum(a) + Original



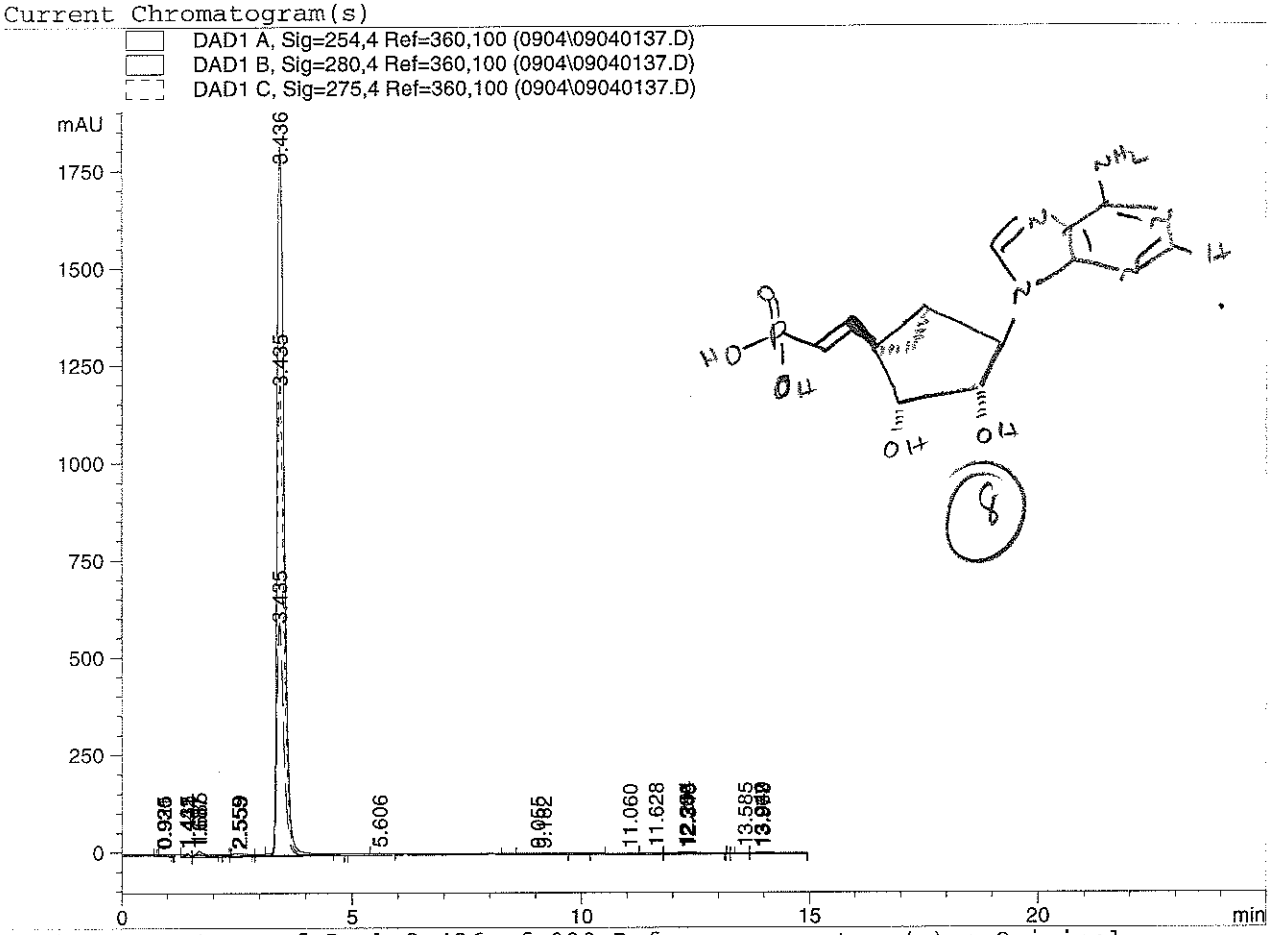


Instrument 1 1/28/2009 1:06:56 PM

wt. of the empty vial (without Cap) :- 1.09702 gm

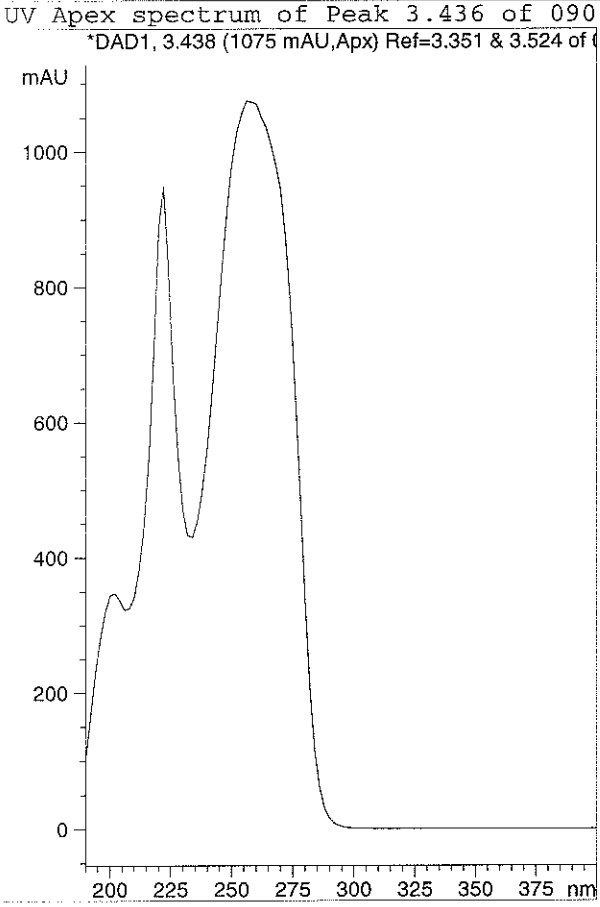
(3) fraction (2) 551 (8)

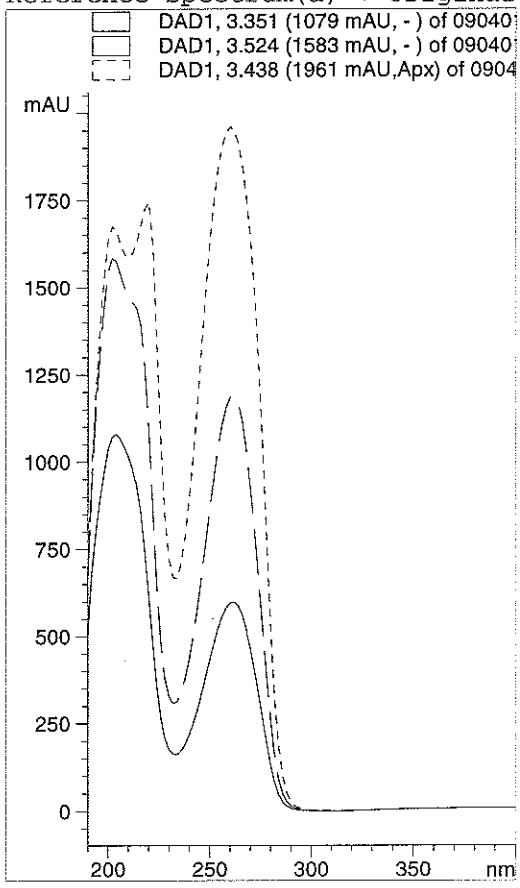
Current Chromatogram(s)



(8)

UV Apex spectrum of Peak 3.436 of 090 Reference spectrum(a) + Original

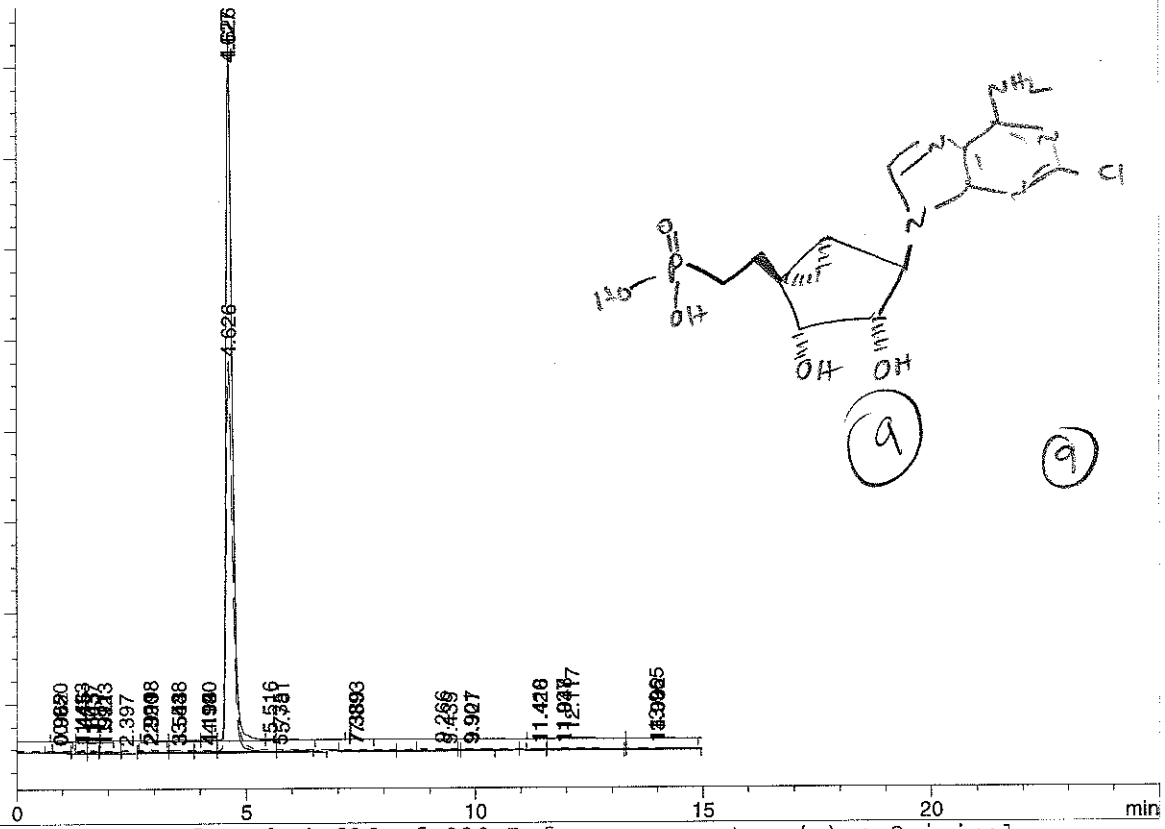




(4) fraction (8) 552

Current Chromatogram(s)

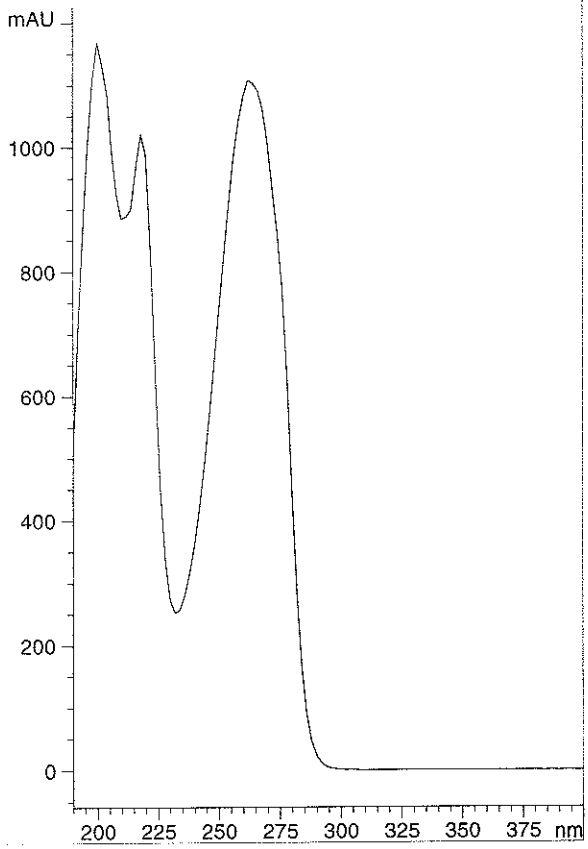
DAD1 A, Sig=254,4 Ref=360,100 (0904\09040114.D)
DAD1 B, Sig=280,4 Ref=360,100 (0904\09040114.D)
DAD1 C, Sig=275,4 Ref=360,100 (0904\09040114.D)



(9) (9)

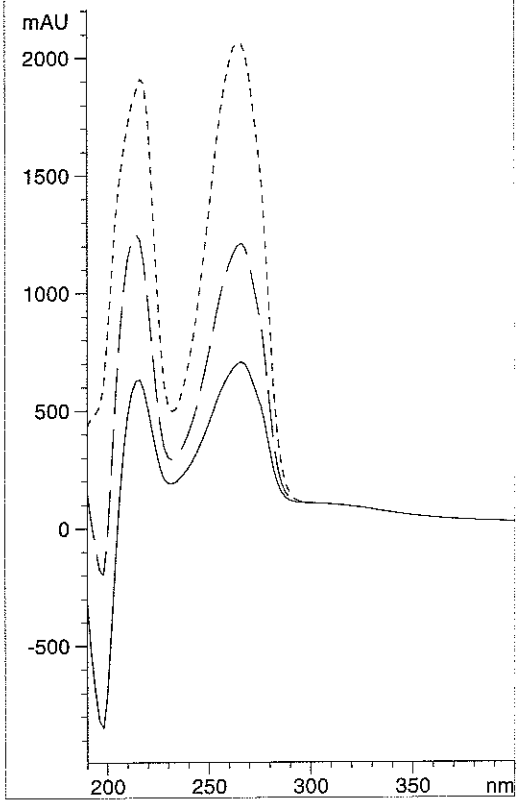
UV Apex spectrum of Peak 4.626 of 090

*DAD1, 4.629 (1168 mAU, -) Ref=4.549 & 4.702 of 09



Reference spectrum(a) + Original

DAD1, 4.549 (1555 mAU, -) of 09040
DAD1, 4.702 (1452 mAU, -) of 09040
DAD1, 4.629 (2032 mAU, -) of 09040

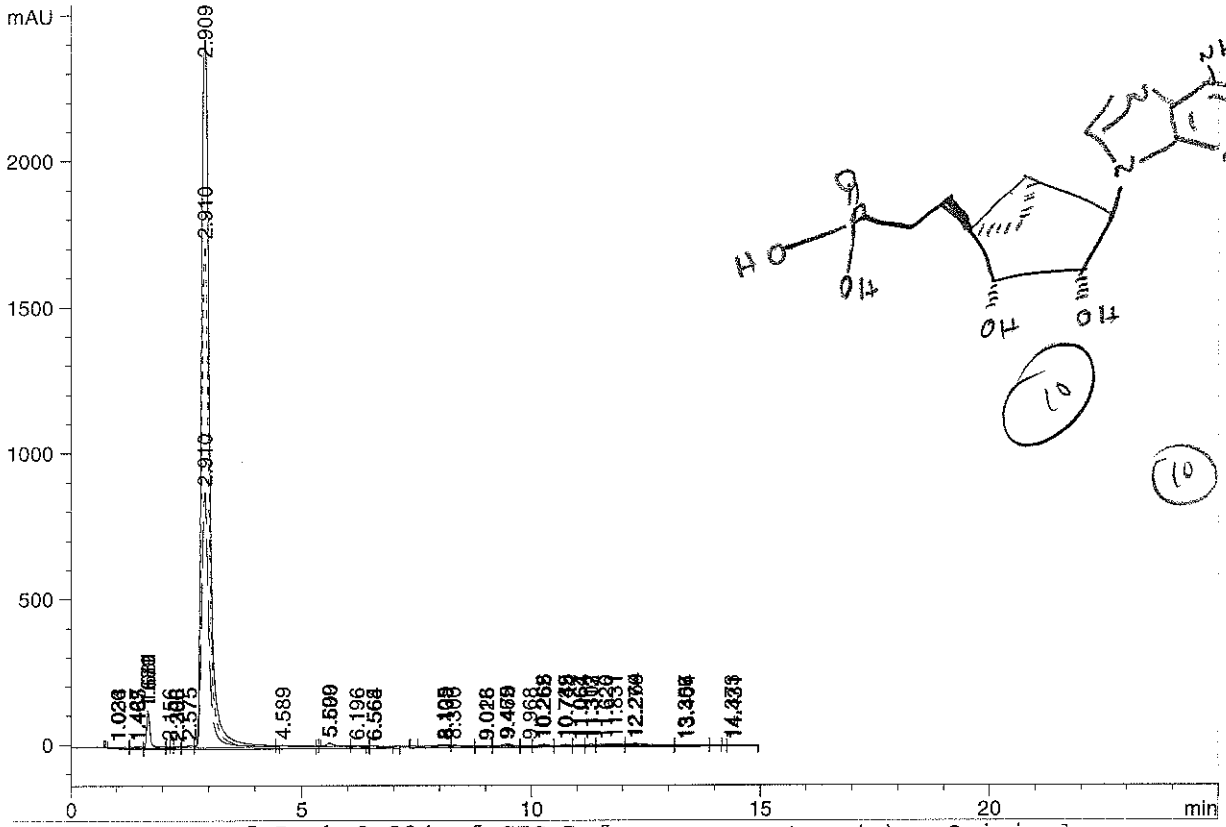


557

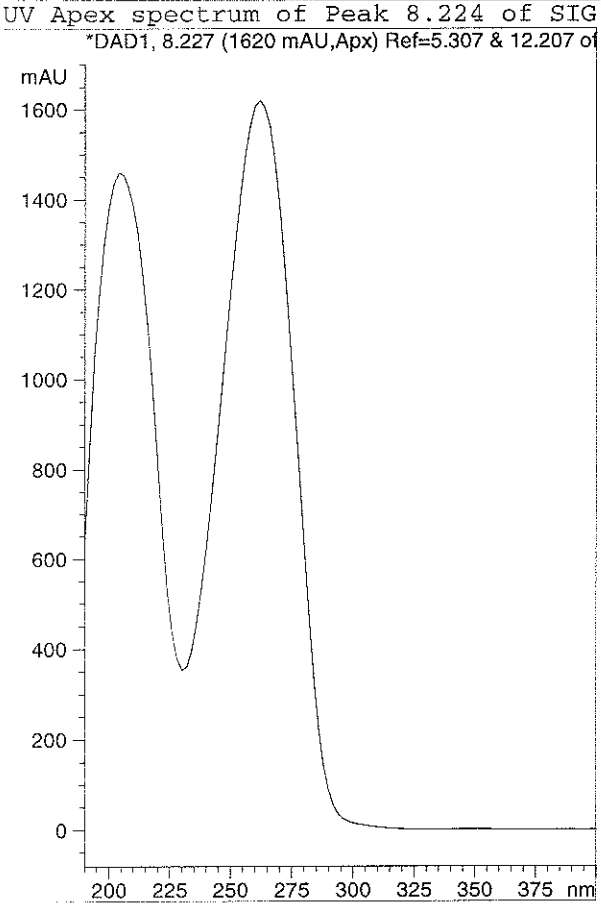
2nd fraction

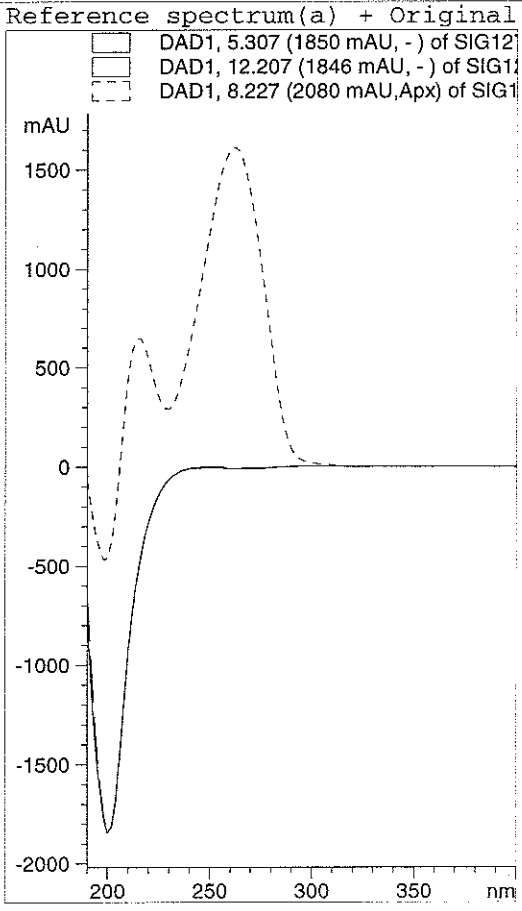
Current Chromatogram(s)

DAD1 A, Sig=254,4 Ref=360,100 (15JULY\SIG12770.D)
DAD1 B, Sig=280,4 Ref=360,100 (15JULY\SIG12770.D)
DAD1 C, Sig=275,4 Ref=360,100 (15JULY\SIG12770.D)



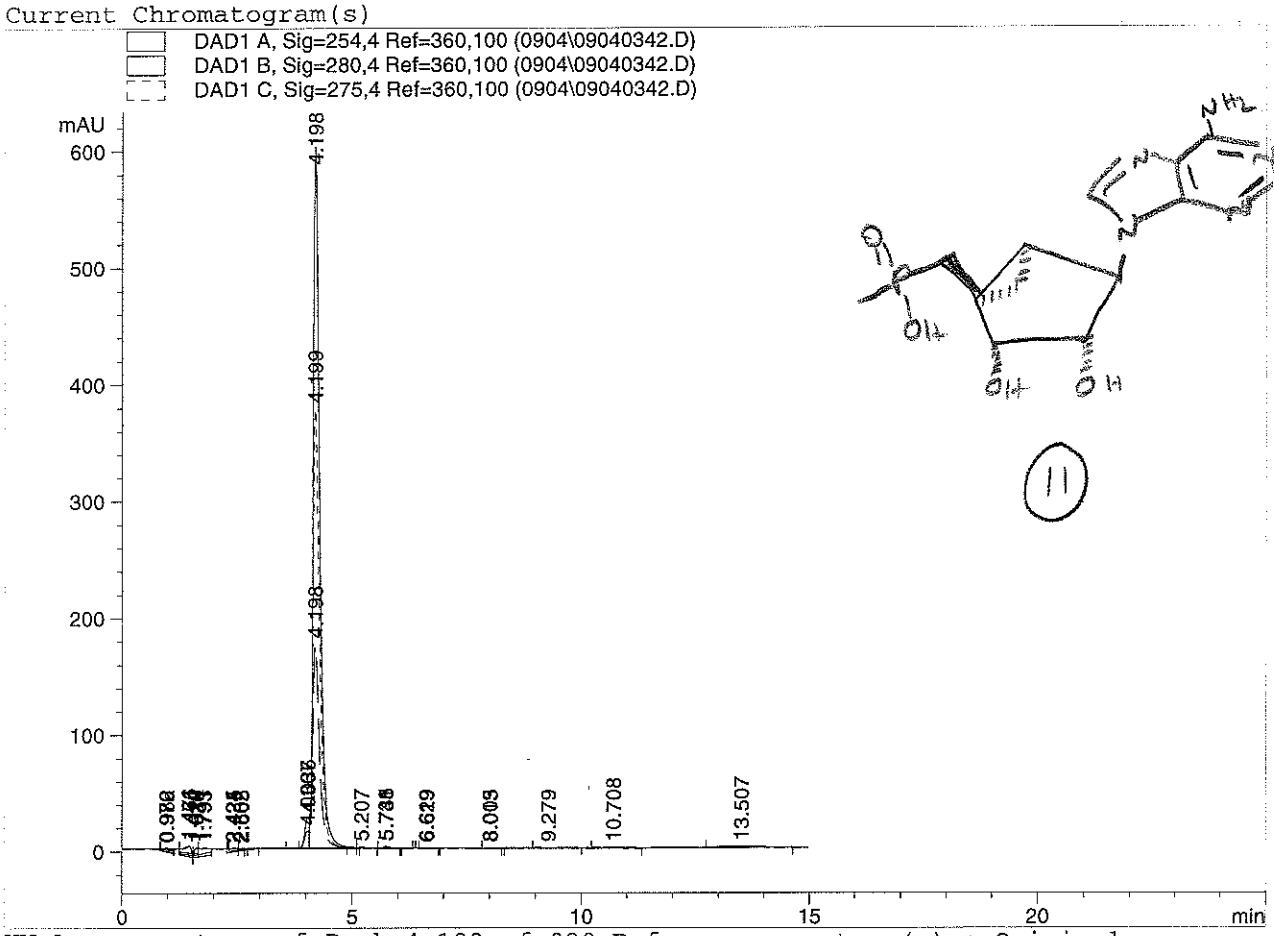
UV Apex spectrum of Peak 8.224 of SIG Reference spectrum(a) + Original





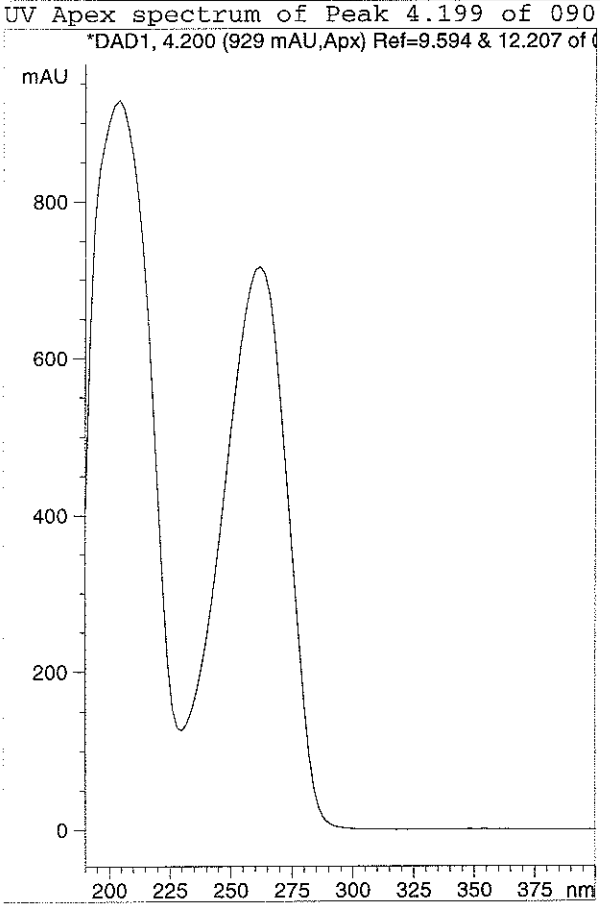
558

Current Chromatogram(s)

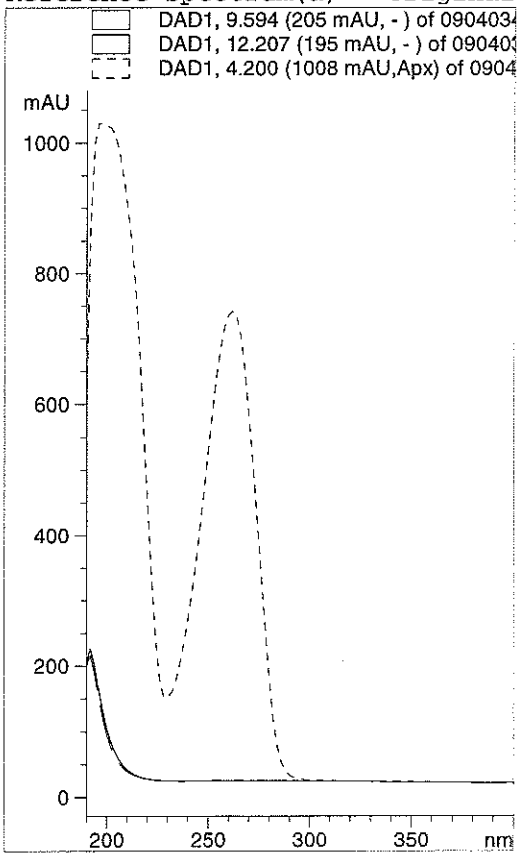


(11)

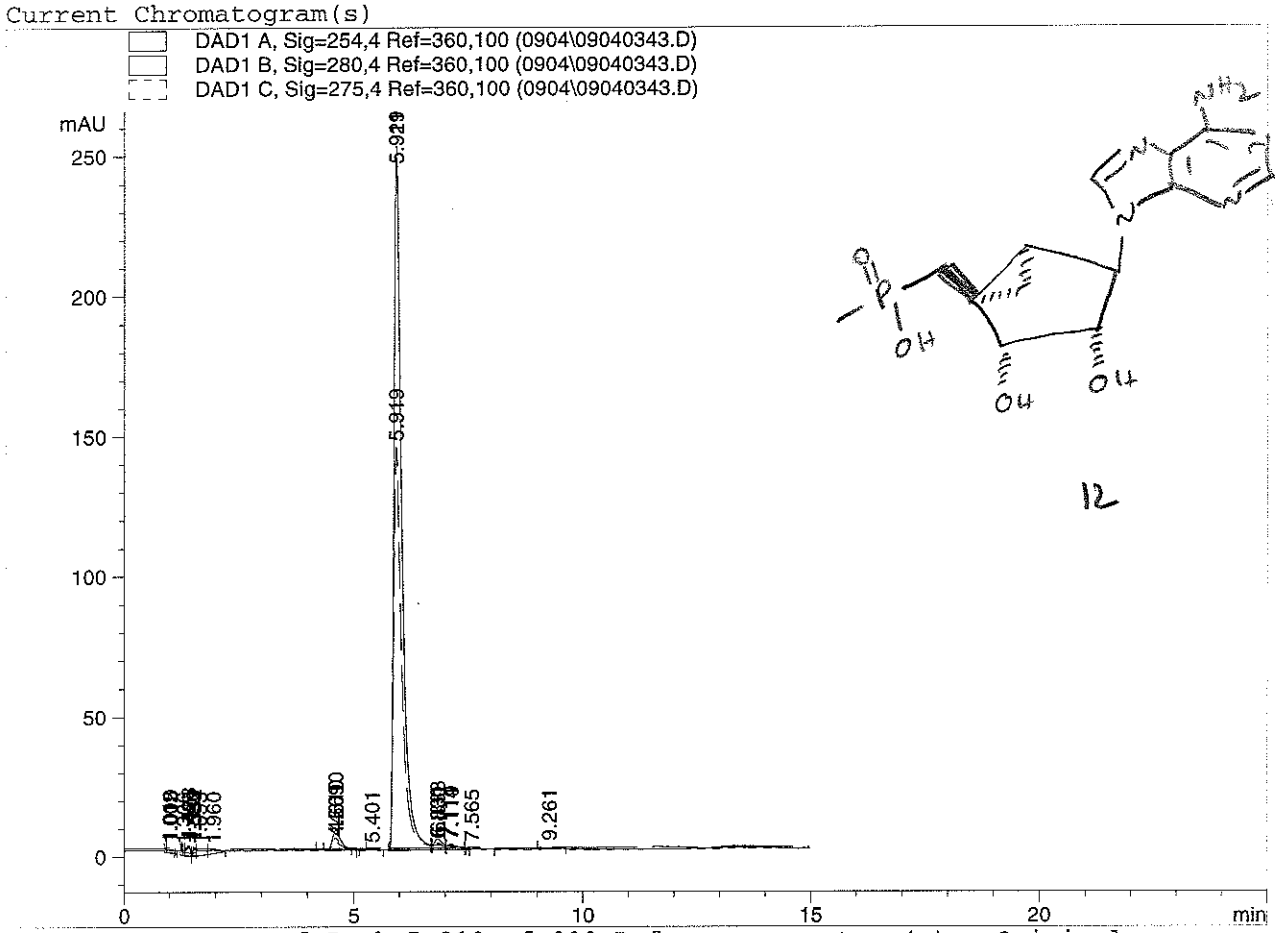
UV Apex spectrum of Peak 4.199 of 090



Reference spectrum(a) + Original

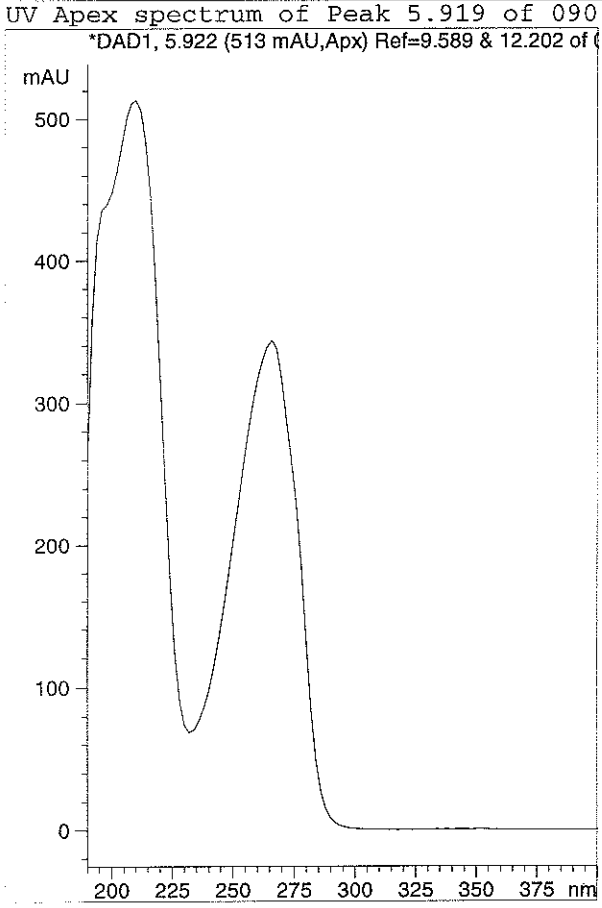


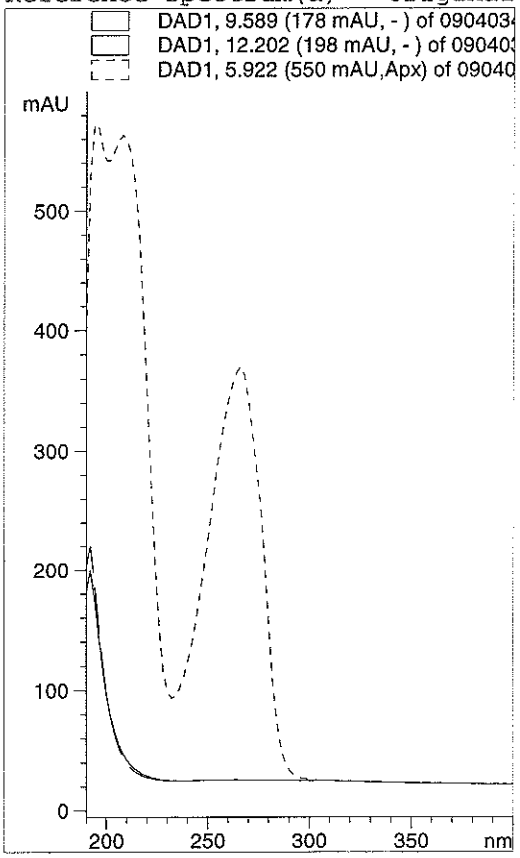
Current Chromatogram(s)



12

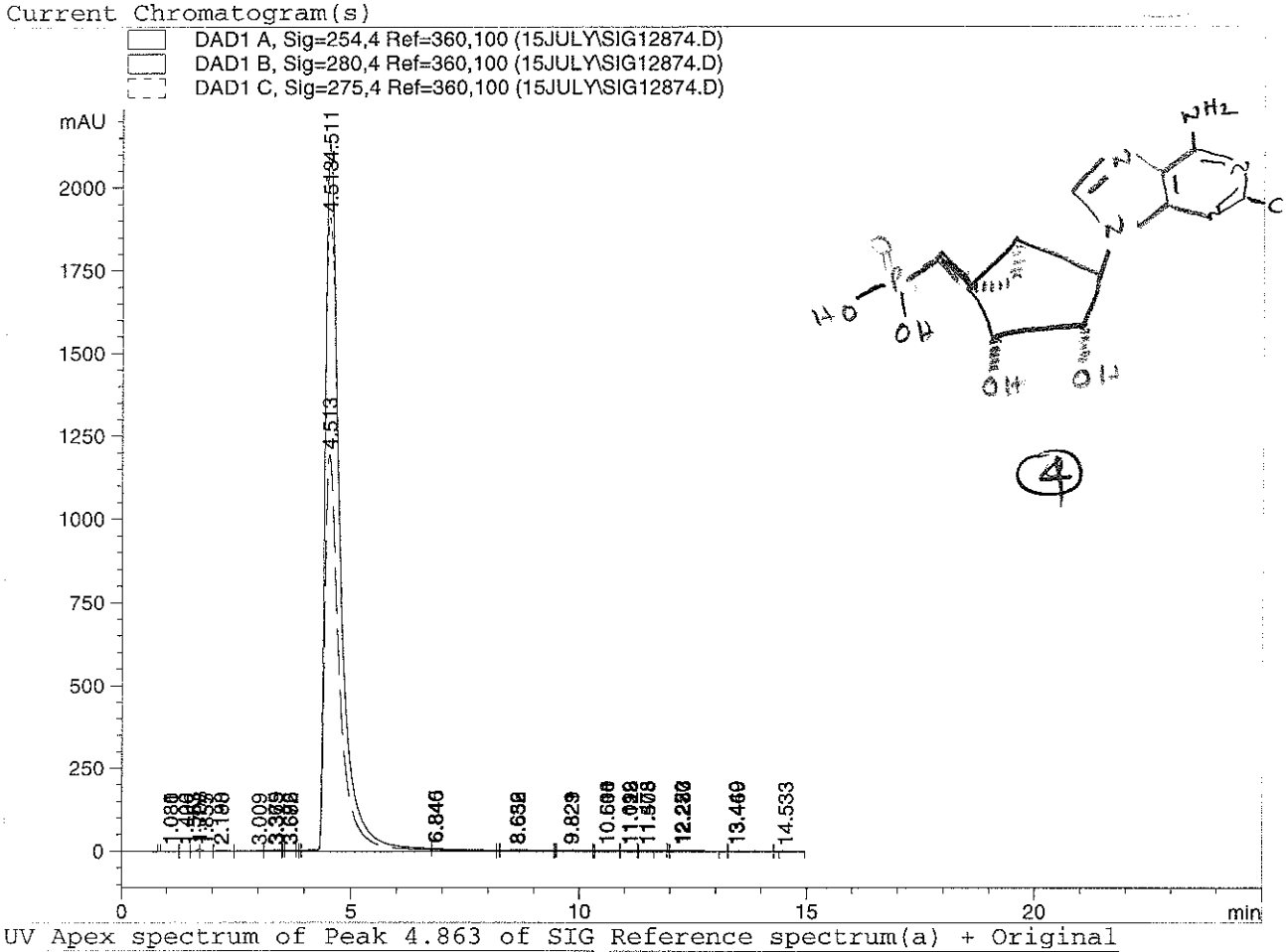
UV Apex spectrum of Peak 5.919 of 090 Reference spectrum(a) + Original





560

Current Chromatogram(s)



(4)

UV Apex spectrum of Peak 4.863 of SIG Reference spectrum(a) + Original