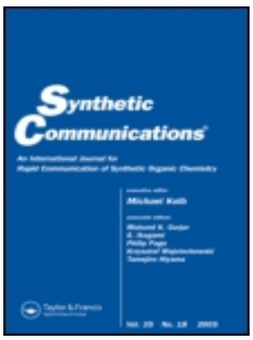

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Synthesis and Resolution of Dinucleotide(TpAZT) Phosphoramidates

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ABSTRACT

Dinucleotide (TpAZT) phosphoramidates were synthesized through Atherton-Todd reaction of dinucleoside H-phosphonates and amino acid methyl esters, and their diastereomers (R_p and S_p) were separated by crystallization. It was showed that the cheap methyl esters of natural alanine and phenylalanine could act as new chiral auxiliaries for large-scale synthesis of dinucleotide analogs.

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Key Words: Dinucleotide; Phosphoramidate; anti-HIV; Chiral separation.

INTRODUCTION

Despite the recent introduction of HIV protease and integratase inhibitors, reverse transcriptase represents an attractive target for the chemotherapy of human immunodeficiency virus (HIV).[1–3] Nucleoside analogues are widely used as antiviral agents in the treatments of AIDS and the AIDS related complex. 3′-Azido-5′-deoxythymidine (AZT), initially tested as anticancer agent, is an inhibitor of HIV-1 reverse transcriptase(RT) and the first drug clinically used for the treatment of AIDS.[4,5]

However, it has been proved that AZT must be phosphorylated intracellularly to their active triphosphate form before acting as competitive inhibitor or alternate substrate (chain terminators) of HIV RT.[6] Because the cellular kinases involved in activiting the nucleoside prodrugs are usually specific,[7] it is thought the replacement of ddNs with natural nucleoside, such as thymidine, could improve the rate of phosphorylation and inhibit the HIV-RT. Dinucleoside phosphate derivatives have attracted great attentions as anti-HIV drugs. Various homo- and heterdinucleoside, such as AZT-P-AZT, AZT-P-ddI, AZT-P-ddA, have been synthesized and tested for HIV-infected MT-2 cells.[8] It was found that dinucleside analogues showed enhanced anti-HIV potency relative to monomers. In addition, AZT-P-ddI was 10 times less toxic than AZT to human granulocytemacrophage progenitor cells.[9]

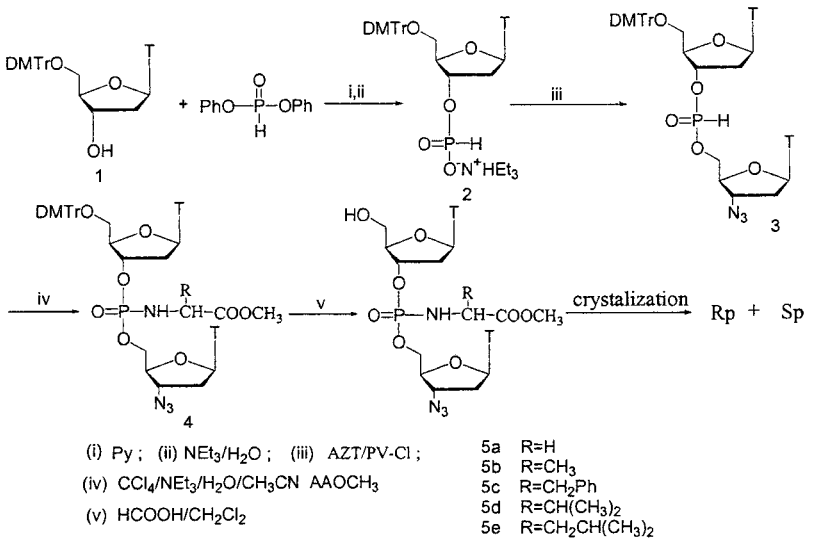
By far, most of the dinucleoside prodrugs were constructed by two unnatural nucleosides, i.e., 2′,3′-dideoxynucleside (ddNs). Hakimelahi et al. also found that dinucleotide phosphoramidates conjugated with methyl ester of alanine were completely resistant to snake venom and spleen enzyme, and these phosphoramidates showed superior bioavailability and profound antiviral activity.[10] On the other hand, a pair of diastereomers are usually formed for the synthesis of dinucleotide analogs due to phosphorus chiral center, however, these diastereomers show great difference in biological activity and cellular toxity for specificity of enzymes. For example, Meier et al. observed the difference of anitiviral activity between two diastereomers of cyclosal pronuclesides is 3–80 folds.[11] Dinucleotide phosphonamidates bearing with a D-amino acid ester moiety exhibited lower activity and less cellular toxity than the corresponding phosphoramidates bearing with a L-amino acid ester moiety.[10]

In this article, a series of dinucleotide phosphoramidates were synthesized by Atherton-Todd reaction, the single diastereomers containing alanine and phenylalanine methyl esters were separated by crystallization.

RESULTS AND DISCUSSION

Dinucleotide phosphoramidates were prepared as shown in Sch. 1. Reaction of 5′-DMTr-thymidine and diphenyl phosphite in dry pyridine at room temperature under nitrogen atmosphere and following hydrolysis in triethylamine and water led to **2**, dinucleoside H-phosphate **3** was obtained after coupling of **2** and AZT by pivaloyl chloride. Atherton-Todd reaction of **3** and amino acid methyl ester in $CCl_4/NEt_3/H_2O/CH_3CN$ solution at room temperature gave product **4**. Product **5** was obtained as white foam after 5′-deprotection of **4** in formic acid and purification on silica gel column chromatography.

Compounds **5** were obtained as mixtures of diastereoisomers as it is difficult to isolate and their structures were confirmed by ^{31}P, ^{1}H, and ^{13}C NMR. When these compounds containing methyl ester of alanine or phenylalanine were dissolved in methanol and kept in refrigerator (−5°C)



Scheme 1. Synthetic route of dinucleotide phosphoramidates.

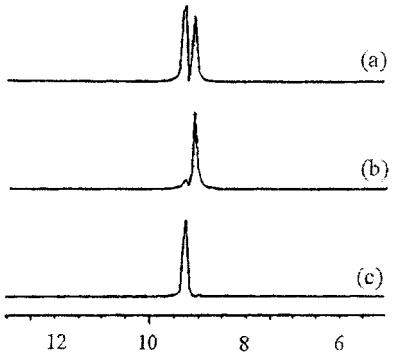


Figure 1. ^{31}P NMR spectra of **5b** (a), the mother solution (b), and the precipitate redissolved in methanol (c).

for about one week, these diastereoisomers can be completely separated by crystallization. For example, Fig. 1 shows ^{31}P NMR spectra of diastereoisomers **5b**, the mother solution and the precipitate redissolved in methanol. **5b** in methanol shows two peaks at ^{31}P NMR 9.23 and 9.04 ppm, the precipitated white solid was filtered and redissolved in methanol, its ^{31}P NMR showed a single peak at 9.23 ppm, the remained mother solution exhibited another single peak at 9.04 ppm. ^{1}H and ^{13}C NMR spectroscopies also confirmed each structures of separated diastereomers. In addition dinucleotide phosphoramidates containing phenylalanine methyl ester showed the same results.

It is worthwhile noting that the above results only occurred for dinucleotide phosphoramidate containing methyl esters of alanine and phenylalanine. For dinucleotide phosphoramidates containing glycine methyl ester, structural difference between R_p and S_p diastereomers is minor because of absence of chiral carbon in glycine, it is difficult to isolate diastereomers by crystallization. For dinucleotide phosphoramidates containing valine, leucine and iso-leucine methyl esters, they could not be precipitated from the solution because of highly hydrophobic side chains of these amino acids. So methyl esters of alanine and phenylalanine can be better chiral auxiliaries for separation of the diastereomeric dinucleotide phosphoramidates (R_P and S_P).

In conclusion, we synthesized some dinucleotide (TpAZT) phosphoramidates. The introduction of alanine or phenylalanine methyl etser improved structural difference of diastereomers (R_p and S_p) that made a single diastereomer firstly precipitate from solution, and another one remained in mother solution. The attractive and cheap method could be used for chiral separation of large-scale PS-dinucleotides. Activity for

separated diastereomers (R_p and S_p) of dinucleotide phosphoramidates containing methyl esters of alanine and phenylalanine is being tested, chiral separation of PS-dinucleotide phosphoramidates is in progress.

EXPERIMENTAL

General Procedure

All reactions were monitored by TLC on silica gel GF_{254}, Column chromatography was performed using silica gel 230–400 mesh. Pyridine were dried over CaH_2 by reflux 4∼5 h. ^{1}H NMR and ^{13}C NMR spectra were recorded (internal standard tetramethylsilane) on Bruker AM 500 type spectrometer using CD_3 OD as the solvent. ^{31}P NMR spectra were taken on Bruker AC 200 spectrometer at 81 MHz under ^{1}H decoupled conditions. ^{31}P NMR chemical shift are reported in ppm downfield (+) or upfield (−) from external 85% H_3PO_4 as reference. Mass spectra were obtained using a Bruker Esquire ion-trap mass spectrometer in positive ion mode.

Synthesis of 5′-dimethoxytritylthymidine 1. The starting material DMTrT was prepared according to the published procedure.[15]

Synthesis of 5′-dimethoxytritylthymidine-3′-H-phosphonate 2. According to the Lit.[16] 5′-Dimethoxytritylthymidine-3′-H-phosphonate was synthesized by adding 5′-dimethoxytritylthymidine to the solution of diphenyl phosphite in dry pyridine at room temperature, and reacted for 20 min. Ten milliliters of triethylamine and water (1:1) was added to the reaction solution, the reaction lasted another 20 min. After evaporation of the solvent, **2** was obtained as brittle yellow foam after column chromatography. Yield: 84%.

Synthesis of *O*-(5′-dimethoxytrityl-2′-deoxythmidin-3′-yl)-*O*′-(3′-azido-2′-deoxythymidin-5′-yl)H-phosphonate 3. Zero point eight gram (1.1 mmol) 5′-*O*-dimethoxytritylthymidine-3′-phosphonate **2** and 0.267 g (1 mmol) AZT were dissolved in anhydrous pyridine and coevaporated twice. The residue was then dissolved in 10 mL anhydrous pyridine, 406 μL PivCl (3.06 mmol) was added dropwise, the reaction mixture was stired at room temperature for 10 min; after addition of a few drops of water, the solvent was removed under reduced pressure. The residue was dissolved in 25 mL EtOAc and washed with saturated NaCl solution; the organic phase was separated and dried with anhydrous Na_2SO_4. The crude product was purified with column chromatography ($CHCl_3$/MeOH 40:1). After concentration, the product was obtained as

white foam. Yield: 72.7%. ^{31}P NMR (methanol): δ 8.88, 8.04 ppm, J_{P-H} 721 Hz (mixture of diastereoisomers).

Synthesis of *O*-(2′-deoxythmidin-3′-yl) -*O*′-(3′-azido-2′- deoxythymidin-5′-yl)N-amino acid methyl ester phosphoramidate 5. Two millimolars compound **3** in 2 mL CH_3CN was added dropwise to 0.22 mmol L-amino acid methyl ester hydrochloride in solution (100 μL H_2O, 70 μL NEt_3, 100 μL CCl_4, and 5 mL CH_3CN) and stirred at room temperature until **3** disappeared (^{31}P NMR determination). The crude product **4** was obtained after the solvents were removed by rotary evaporation, and then treated with HCOOH and CH_2Cl_2 until the deprotection wad completed (TLC dertermination). The solution was neutralized with a few drops of saturated $NaHCO_3$, and then concentrated under reduced pressure. The product **5** was purified by column chromatography ($CHCl_3$:MeOH from 40:1 to 20:1).

Spectral Data for Compounds 5a–e

T-*p*-AZT-GlyOCH$_3$ 5a. Yield: 53.4%. ^{31}P NMR (CD_3OD, 81 MHz): δ 10.55□10.35. ^{1}H NMR (CD_3OD, 500 MHz): δ 7.82, 7.80 (d, 1H, H-6), 7.55–7.54 (d, 1H, H-6), 6.31–6.28 (m, 1H, H-1′), 6.17–6.14 (m, 1H, H-1′), 5.14–5.11 (m, 1H, H-3′), 4.46–4.45 (m, 1H, H-3′), 4.33–4.18 (m, 4H, 2 × H-5′), 3.81, 3.79 (d, 2H, CH_2), 3.77, 3.74 (d, 2H, 2 × H-4′), 3.71 (d, 3H, OCH_3), 2.53–2.32 (m, 4H, 2 × H-2′), 1.89–1.87 (dd, 6H, 2 × C5-CH_3). ^{13}C NMR (CD_3OD, 125 MHz): δ 173.28 (COOMe), 166.30 (2 × C-4), 152.35, 150.18 (2 × C-2), 137.88 (2 × C-6), 11.97□111.85, 111.81 (2 × C-5), 87.39, 87.36, 87.22, 87.17, 86.58, 86.50, 83.74, 83.67 (2 × C-4′, 2 × C-1′), 79.24–78.98 (q, C-3′), 67.15, 66.79 (C-3′), 62.67–61.60 (q, 2 × C-5′), 52.68 (CH_2), 51.35 (OMe), 39.78, 37.65, 39.62 (2 × C-2′), 12.55, 12.46 (2 × C5-CH_3). ESI-MS $[M+H]^+$ m/z: 643, $[M+Na]^+$ m/z: 665.

T-*p*-AZT-AlaOCH$_3$ 5b. Yield: 55.8%. ^{31}P NMR (CD_3OD, 81 MHz): δ 9.03, 9.24. ^{1}H NMR (CD_3OD, 500 MHz): δ 7.83, 7.80 (d, 1H, H-6), 7.55, 7.52 (d, 1H, H-6), 6.31–6.29 (m, 1H, H-1′), 6.16–6.13 (m, 1H, H-1′), 5.16–5.02 (m, 1H, H-3′), 4.50–4.41 (m, 1H, H-3′), 4.32–4.04 (m, 4H, 2 × H-5′), 3.95–3.88 (m, 1H, C*H*), 3.80–3.78 (2s, 2H, 2 × H-4′), 3.72, 3.71 (d, 3H, OCH_3), 2.56–2.30 (m, 4H, 2 × H-2′), 1.90–1.87 (m, 6H, 2 × C5-CH_3), 1.41, 1.39, 1.37 (t, 3H, CH_3). ^{13}C NMR (CD_3OD, 125 MHz): δ 175.73 (COOMe), 166.29 (2 × C-4), 152.36, 150.17 (2 × C-2), 137.99, 137.92, 137.89 (137.82) (2 × C-6), 112.04, 111.90, 111.80 (2 × C-5), 87.36, 87.25, 87.20, 86.62, 86.07, 83.61 (2 × C-4′, 2 × C-1′), 79.46, 79.36, 79.02 (C-3′), 67.04, 66.64, 66.60 (C-3′), 62.71, 62.57, 61.75, 61.48

(2 × C-5′), 52.85 (CH), 51.35 (OMe), 39.74, 37.61 (2 × C-2′), 20.42, 20.36 (CH_3), 12.56, 12.47 (2 × C5-CH_3). ESI-MS $[M+H]^+$ m/z: 657, $[M+Na]^+$ m/z: 679.

T-*p*-AZT-PheOCH$_3$ 5c. Yield: 57.2%. ^{31}P NMR (CD_3OD, 81 MHz): δ 9.12, 8.65. ^{1}H NMR (CD_3OD, 500 MHz): δ 7.79, 7.77 (d, 1H, H-6), 7.47, 7.42 (d, 1H, H-6), 7.30–7.19 (m, 5H, Ph), 6.23–6.21 (m, 1H, H-1′), 6.13–6.07 (m, 1H, H-1′), 4.96–4.93 (m, 1H, H-3′), 4.33–4.22 (m, 1H, H-3′), 4.11–3.93 (m, 4H, 2 × H-5′), 3.85–3.84 (m, 1H, C*H*), 3.74, 3.73 (2s, 2H, 2 × H-4′), 3.72, 3.71 (d, 3H, OCH_3), 3.19–2.81 (m, 2H, CH_2Ph), 2.40–2.18 (m, 4H, 2 × H-2′), 1.88–1.86 (m, 6H, 2 × C5-CH_3). ^{13}C NMR (CD_3OD, 125 MHz): δ 174.89, 174.80 (COOMe), 166.29 (2 × C-4), 152.30, 150.13 (2 × C-2), 138.64, 138.48, 137.87, 137.78 (2 × C-6), 130.60–127.97 (Ph), 111.99, 111.94, 111.82, 111.77 (2 × C-5), 87.28, 87.25, 87.15, 86.55, 86.46, 85.97, 83.51 (2 × C-4′, 2 × C-1′), 79.61, 79.57, 78.84 (C-3′), 66.80, 66.75, 66.47 (C-3′), 62.70, 62.47, 61.83, 61.50 (2 × C-5′), 57.87, 57.65 (CH), 52.86, 52.83 (OMe), 40.76, 39.67 (2 × C-2′), 37.74, 37.64 (CH_2Ph), 12.62, 12.47 (2 × C5-CH_3). ESI-MS $[M+H]^+$ m/z: 733, $[M+Na]^+$ m/z: 755.

T-*p*-AZT-ValOCH$_3$ 5d. Yield: 51.9%. ^{31}P NMR (CD_3OD, 81 MHz): δ 9.93. ^{1}H NMR (CD_3OD, 500 MHz): δ 7.83, 7.82 (d, 1H, H-6), 7.55, 7.52 (d, 1H, H-6), 6.32–6.29 (m, 1H, H-1′), 6.17–6.14 (m, 1H, H-1′), 5.11–5.05 (m, 1H, H-3′), 4.50–4.41 (m, 1H, H-3′), 4.32–4.06 (m, 4H, 2H-5′), 3.80–3.78 (2s, 2H, 2 × H-4′), 3.74, 3.73 (d, 3H, OCH_3), 3.65–3.59 (m, 1H, C*H*), 2.58–2.33 (m, 4H, 2 × H-2′), 2.10–2.05 (CH(CH_3)$_2$) 1.91–1.88 (m, 6H, 2 × C5-CH_3), 0.98–0.94 (m, 6H, 2 × CH_3). ^{13}C NMR (CD_3OD, 125 MHz): δ 174.98 (COOMe), 166.27 (2 × C-4), 152.36, 152.15 (2 × C-2), 138.04, 137.98, 137.89, 137.83 (2 × C-6), 112.06, 112.03, 111.90, 111.81 (2 × C-5), 87.35, 87.25, 87.20, 86.72, 86.09, 83.64, 83.53 (2 × C-4′, 2 × C-1′), 79.57, 79.53, 79.06 (C-3′), 67.33, 67.29, 66.75, 66.71 (C-3′), 62.73, 62.60, 61.80, 61.56 (2 × C-5′), 53.03 (CH), 52.67 (OMe), 39.76, 37.58 (2 × C-2′), 32.02, 32.96 (CH(CH_3)$_2$), 19.71, 18.60, 18.30, 18.23 (2 × CH_3), 12.63, 12.54 (2 × C5-CH_3). ESI-MS $[M+H]^+$ m/z: 685, $[M+Na]^+$ m/z: 707.

T-*p*-AZT-LeuOCH$_3$ 5e. Yield: 54.3%. ^{31}P NMR (CD_3OD, 81 MHz): δ 9.43, 9.24. ^{1}H NMR (CD_3OD, 500 MHz): δ 7.83, 7.81 (d, 1H, H-6), 7.55, 7.51 (d, 1H, H-6), 6.31–6.27 (m, 1H, H-1′), 6.17–6.12 (m, 1H, H-1′), 5.12–5.02 (m, 1H, H-3′), 4.41–4.40 (m, 1H, H-3′), 4.30–4.04 (m, 4H, 2 × H-5′), 3.89–3.84 (m, 1H, CHCOO), 3.79–3.78 (2s, 2H, 2 × H-4′), 3.72, 3.71 (d, 3H, OCH_3), 2.55–2.33 (m, 4H, 2 × H-2′), 1.90–1.86 (m, 6H, 2 × C5-CH_3), 1.79–1.76 (m, 1H, CH(CH_3)$_2$), 1.58–1.54 (m, 2H, CH_2), 0.93–0.92 (m, 6H, 2 × CH_3). ^{13}C NMR (CD_3OD, 125 MHz): δ 175.92 (COOMe), 166.28 (2 × C-4), 152.33, 152.15 (2 × C-2), 137.93, 137.98,

137.83 (2 × C-6), 112.02, 111.85, 111.81 (2 × C-5), 87.40, 87.36, 87.20, 86.69, 86.61, 83.64, 83.60 (2 × C-4′, 2 × C-1′), 79.43, 79.08, 79.05 (C-3′), 66.97, 66.93 (C-3′), 62.69, 62.59, 61.82, 61.72 (2 × C-5′), 54.41, 54.30 (CHNH), 52.79 (OMe), 43.82, 43.76 ($CH(CH_3)_2$), 39.83, 37.71 (2 × C-2′), 32.02, 32.96 ($CH(CH_3)_2$), 25.73, 25.70 (CH_2), 23.28, 21.87, 21.83 (2 × CH_3), 12.63, 12.53 (2 × C5-CH_3). ESI-MS $[M+H]^+$ m/z: 699, $[M+Na]^+$ m/z: 721.

Diastereomer in mother solution of 5b. ^{31}P NMR (CD_3OD, 81 MHz): δ 9.03. ^{1}H NMR (CD_3OD, 500 MHz): δ 7.81 (s, 1H, H-6), 7.56 (s, 1H, H-6), 6.32–6.29 (m, 1H, H-1′), 6.18–6.15 (m, 1H, H-1′), 5.06–5.03 (m, 1H, H-3′), 4.51–4.48 (m, 1H, H-3′), 4.32–4.05 (m, 4H, H-5′), 3.92–3.90 (m, 1H, C*H*), 3.79, 3.78 (2s, 2H, H-4′), 3.72 (s, 3H, CO_2CH_3), 2.57–2.34 (m, 4H, H-2′), 1.89, 1.88 (2s, 6H, CH_3), 1.39, 1.38 (2s, 3H, CH_3). ^{13}C NMR (CD_3OD, 125 MHz): δ 175.73 (COOMe), 166.29 (C-2), 152.35, 150.17 (C-4), 137.89, 137.92 (C-6), 111.81, 111.80 (C-5), 87.25, 87.20 (C-4′), 86.62, 86.07 (C-1′), 83.61, 83.55 (C-4′), 79.47, 79.37 (C-3′), 66.74, 66.60 (C-3′), 62.71, 61.48 (C-5′), 52.85 (CH), 51.35 (OMe), 39.74, 37.61 (C-2′), 20.42, 20.36 (CH3), 12.56, 12.47 (CH3). ESI-MS $[M+Na]^+$ m/z: 695.

Diastereomer of precipitation of 5b. ^{31}P NMR (CD_3OD, 81 Hz): δ 9.24. ^{1}H NMR (CD_3OD, 500 Hz): δ 7.84 (s, 1H, H-6), 7.53 (s, 1H, H-6), 6.30–6.29 (m, 1H, H-1′), 6.16–6.14 (m, 1H, H-1′), 5.15–5.12 (m, 1H, H-3′), 4.44–4.41 (m, 1H, H-3′), 4.27–4.05 (m, 4H, H-5′), 3.97–3.94 (m, 1H, C*H*), 3.81, 3.80 (2s, 2H, H-4′), 3.73 (s, 3H, CO_2CH_3), 2.57–2.36 (m, 4H, H-2′), 1.91, 1.89 (2s, 6H, CH_3), 1.42, 1.40 (2s, 3H, CH_3). ^{13}C NMR (CD_3OD, 125 Hz): δ 175.75 (COOMe), 166.31 (C-2), 152.39, 152.18 (C-4), 137.95, 137.90 (C-6), 112.02, 111.82 (C-5), 87.42, 87.38 (C-4′), 86.72, 86.13 (C-1′), 83.70, 83.64 (C-4′), 79.06, 79.02 (C-3′), 67.11, 67.07 (C-3′), 62.59, 61.78 (C-5′), 52.85 (CH), 51.51 (OMe), 39.74, 37.61 (C-2′), 20.41, 20.36 (CH3), 12.53, 12.44 (CH_3). ESI-MS $[M+Na]^+$ m/z: 695.

Diastereomer in mother solution of 5c. ^{31}P NMR (CD_3OD, 81 MHz): δ 9.12. ^{1}H NMR (CD_3OD, 500 MHz): δ 7.80 (s, 1H, H-6), 7.48 (s, 1H, H-6), 7.31–7.21 (m, 5H, Ph), 6.24–6.21 (m, 1H, H-1′), 6.14–6.10 (m, 1H, H-1′), 4.96–4.94 (m, 1H, H-3′), 4.33–4.24 (m, 1H, H-3′), 4.12–3.95 (m, 4H, 2 × H-5′), 3.85–3.84 (m, 1H, C*H*), 3.74, 3.73 (2s, 2H, 2 × H-4′), 3.71 (s, 3H, OCH_3), 3.20–2.81(m, 2H, CH_2Ph), 2.42–2.19 (m, 4H, 2 × H-2′), 1.89–1.87 (m, 6H, 2 × C5-CH_3). ^{13}C NMR (CD_3OD, 125 MHz): δ 174.89 (COOMe), 166.27 (2 × C-4), 152.30 (2 × C-2), 137.89, 137.80 (2 × C-6), 130.60–127.95 (Ph), 111.82, 111.77 (2 × C-5), 87.28, 87.21, 87.15, 86.55, 86.46, 83.53 (2 × C-4′, 2 × C-1′), 79.61, 79.57 (C-3′), 66.81, 66.75 (C-3′), 62.70, 62.48, 61.83, 61.50 (2 × C-5′), 57.87, 57.65 (CH), 52.86, 52.84 (OMe), 40.73, 39.67 (2×C-2′), 37.75, 37.67 (CH_2Ph), 12.61, 12.47 (2 × C5-CH_3). ESI-MS $[M+H]^+$ m/z: 733, $[M+Na]^+$ m/z: 755.

Diastereomer of precipitation of 5c. ^{31}P NMR (CD_3OD, 81 MHz): δ 8.65. ^{1}H NMR (CD_3OD, 500 MHz): δ 7.78 (s, 1H, H-6), 7.43 (s, 1H, H-6), 7.32–7.20 (m, 5H, Ph), 6.24–6.21 (m, 1H, H-1′), 6.14–6.11 (m, 1H, H-1′), 4.97–4.95 (m, 1H, H-3′), 4.33–4.26 (m, 1H, H-3′), 4.12–3.97 (m, 4H, 2 × H-5′), 3.86–3.84 (m, 1H, C*H*), 3.74, 3.73 (2s, 2H, 2 × H-4′), 3.72 (s, 3H, OCH_3), 3.19–2.80 (m, 2H, CH_2Ph), 2.39–2.17 (m, 4H, 2 × H-2′), 1.89–1.88 (m, 6H, 2 × C5-CH_3). ^{13}C NMR (CD_3OD, 125 MHz): δ 174.80 (COOMe), 166.24 (2 × C-4), 150.13 (2 × C-2), 138.68, 138.49 (2 × C-6), 130.62–127.97 (Ph), 111.99, 111.94 (2 × C-5), 87.29, 87.15, 86.57, 86.46, 85.93, 83.51 (2 × C-4′, 2 × C-1′), 79.58, 78.84 (C-3′), 66.77, 66.42 (C-3′), 62.74, 62.47, 61.83, 61.50 (2 × C-5′), 57.87, 57.65 (CH), 52.85, 52.82 (OMe), 40.76, 39.65 (2 × C-2′), 37.72, 37.63 (CH_2Ph), 12.62, 12.48 (2 × C5-CH_3). ESI-MS $[M+H]^+$ m/z: 733, $[M+Na]^+$ m/z: 755.

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