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Novel nucleotide–calixarene conjugates via phosphoester linkage

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Dedicated to Professor M. Piattelli on the occasion of his 80th birthday

Abstract—Calix[4]arenes bearing thymine, adenine, cytosine, guanine 2′-deoxynucleotide residues have been synthesized following the phosphoramidite chemistry. Hybrid compounds **2a–d** and **3a–d** represent the first example of nucleotides linked to the calixarene lower rim by a phosphoester bond. Preliminary studies about their assembling in apolar solvent and host properties toward biologically interesting guests are also reported.

Nucleotides play an important role in regulating a variety of information-transfer functions in natural systems, therefore, there is a great interest in designing of artificial nucleotide-binding[1] and nucleotide-based receptors.[2] Due to their ability to establish a variety of interactions, complementary base pairing, multi-site hydrogen bonding, specific stacking, and generalized electrostatic interactions, nucleotides can be exploited as valid recognition motifs in the development of synthetic supramolecular systems.

Calixarenes[3] represent promising scaffolds to impose structural preorganization to nucleotide units for which nucleotide–calixarene conjugation could be a valid strategy to develop novel amphiphilic receptors and multicavity supramolecular architectures. Recently, calixarenes bearing at their upper rim nucleobase, nucleoside, and oligonucleotide units linked via amide-bond have been described.[4]

Here, we wish to report the first example of calix[4]arene derivatives in which one or two 3′-phosphorylated nucleoside moieties are anchored to the calixarene lower rim via phosphoester linkage. A preliminary study about their assembling in apolar solvent and host properties of monosubstituted derivatives is also undertaken.

The synthesis[5] of nucleotide–calixarene hybrids was accomplished by coupling of calix[4]arene **1**,[6] exposing two CH_2OH terminal groups, with protected 2′-deoxynucleoside phosphoramidite **a–d**.[7] Oxidation in the presence of I_2 and subsequent deprotection reactions (Scheme 1) afforded mono- (**2a–d**) and di-substituted (**3a–d**) compounds in 25–32% and 10–60% yield, respectively. The obtained conjugates were all characterized by NMR and ESI-MS spectra.[8]

In CD_3OD, compounds **2a–d** and **3a–d** showed well-resolved ^1H NMR spectra, displaying the characteristic signals of the nucleotide moieties in addition to those of the calixarene scaffold in *cone* conformation. According to their different symmetry, monosubstituted **2a–d** and disubstituted **3a–d** displayed two and only one AX systems for the $ArCH_2Ar$ protons, respectively.

In analogy with similar systems bearing nucleobase or nucleoside residues,[4a] these compounds are stabilized as monomeric species in hydrogen-bonding accepting solvent, whereas they tend to undergo intermolecular self-association in apolar solvents. ^1H NMR chemical shift of nucleobase resonances provided sensitive probe for chemical environment. For example, the change of solvent from $CDCl_3$ to a polar solvent able to establish solute–solvent interactions (DMSO-d_6) resulted in the shift of the imido-H peak of **2a** from 8.05 to 11.31 ppm.

In $CDCl_3$, the concentration dependence of nucleobase protonic resonances evidenced the existence of

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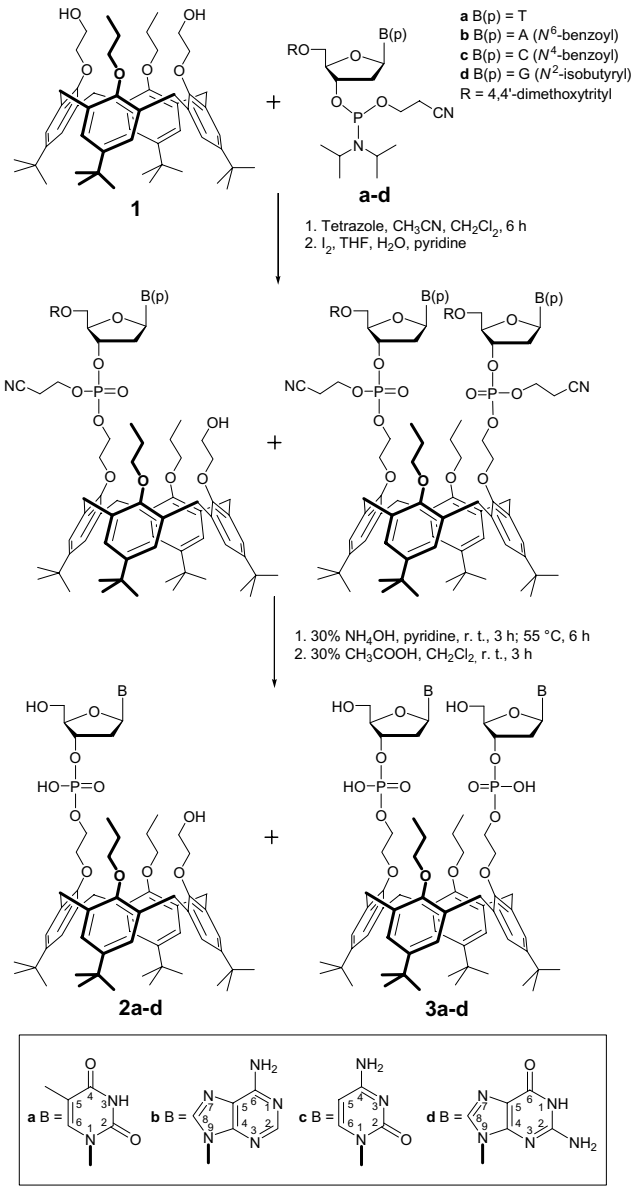

Scheme 1. Synthesis of compounds **2a**–**d** and **3a**–**d**.

intermolecular self-association for mononucleotide calixarenes **2a**–**c**. In particular, downfield shift of the thymine imido-H ($\Delta\delta = 0.37$ ppm) and adenine-NH_2 ($\Delta\delta = 0.21$ ppm) protons was observed increasing the concentration of **2a** and **2b** from 5 to 22 mM, whereas downfield shift of the cytosine C6-H ($\Delta\delta = 0.42$ ppm) and C5-H ($\Delta\delta = 0.44$ ppm) protons was recorded diluting **2c** from 22 to 4.4 mM.

Since aromatic protons undergo an upfield shift in interacting nucleobases,[9] the observed downfield shift during dilution of **2c** could be attributed to self-aggregation disruption. In contrast with derivatives **2a**–**c**, nucleotide–calixarene **2d** showed ill-resolved NMR spectra in $CDCl_3$, at both high and low concentrations. This is in agreement with a different aggregation of **2d**, probably due to multiple and more efficient H-bonding involving the guanine group.

As expected, when we mixed a solution of **2a** with equimolar solution of **2b** (10 mM each, in $CDCl_3$) downfield shifts of both thymine imido-H ($\Delta\delta = 0.39$ ppm) of **2a**, and adenine C2-H ($\Delta\delta = 0.28$ ppm) and C8-H ($\Delta\delta = 0.03$ ppm) of **2b** were observed. Significant shift of the adenine C2-H resonance in comparison to the weak C8-H one, indicated that **2a** and **2b** interact mainly by Watson–Crick type hydrogen bonding[10] that is known to be predominant in systems presenting the constrains of a sugar phosphate group. In this connection, the ability of compound **2b** to bind AZT (3′-azido-3′-deoxythymidine),[11] important antiviral drug, was considered. A-T base pairing was indicated by the downfield shift ($\Delta\delta = 0.49$ ppm) of the imido-H resonance of AZT in an equimolar host–guest mixture in $CDCl_3$.

Similarly to monosubstituted calixarene **2a**, disubstituted calixarene **3a** showed a resolved ^{1}H NMR spectrum in $CDCl_3$. Upfield shift of the thymine imido-H ($\Delta\delta = 0.46$ ppm) following dilution from 10 to 2 mM was indicative of hydrogen-bonding interaction disruption. The exclusive shift of the NH-imido signal with respect to the other protons of **3a** that remained almost constant, and the relative simplicity and sharpness of this signal, suggested that compound **3a** at 10 mM concentration in $CDCl_3$ forms a discrete self-assembly rather than a polymer.[12] This was corroborated by VT-NMR experiments. No splitting of the protonic signals when cooling **3a** up to 230 K indicated the presence of only one aggregated species in solution. When temperature was decreased from 300 to 230 K, downfield shift of the NH-imido resonance ($\Delta\delta = 0.75$ ppm) was also observed, further confirming the self-association of **3a** by intermolecular hydrogen bond.

In contrast with **3a**, nucleotide–calixarenes **3b**–**d** showed poor solubility and ill-resolved spectra in $CDCl_3$, it could be connectable to stronger and non-discrete intermolecular aggregation.

More deeper studies are in course to characterize self-assembled structures of the here reported nucleotide–calixarenes.

Considering that vital events are based on nucleic acid–protein association involving the recognition between nucleotides and basic and acid amino acids present on the protein surface, we decided to explore the affinity of nucleotide–calixarene **2a** and **2d** versus N_α-tosyl-L-arginine methylester hydrochloride and Na butyrate, respectively.

Exploiting guanidinium-phosphate association,[13] compound **2a** (in saline form) formed a 1:1 complex with arginine. By nonlinear least-squares fit of the NMR titration[15] curve following the shift of the arginine guanidinium-NH resonance as a function of equivalents of **2a**, a binding constant of $492 \pm 73\ M^{-1}$ in DMSO-d_6 was calculated (Fig. 1).

Since guanine has two hydrogen bond donor groups in the correct position to form a pair of hydrogen bonds with an ionized carboxylic group,[14] derivative **2d** formed

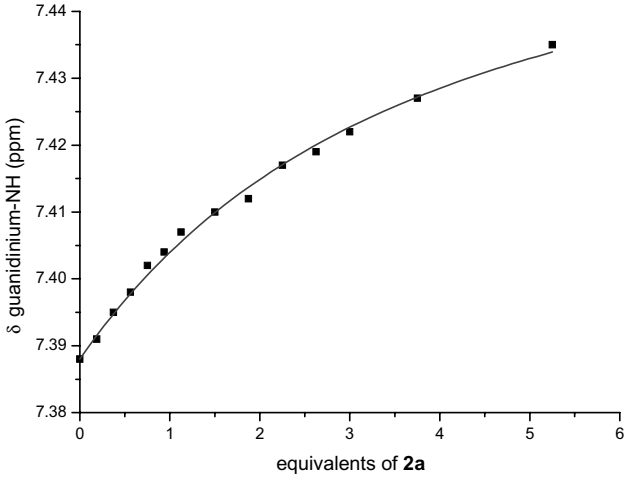

Figure 1. Titration curve of N_α-tosyl-L-arginine methylester hydrochloride in the presence of **2a**.

a 1:1 complex with Na butyrate, used as a model for the side chain of glutamate or aspartate. By ^{1}H NMR titration,[15] a binding constant of $99 \pm 11\ M^{-1}$ in DMSO-d_6 was calculated following the downfield shift of the guanine-NH_2 resonance of **2d** (Fig. 2).

In conclusion, novel nucleotide–calix[4]arene hybrids in which one or two nucleotide moieties are connected to the calixarene lower rim by phosphoester linkage have been synthesized. These compounds differ from analogous nucleoside–calixarene hybrids in containing an additional hydrophilic phosphodiester functionality capable of further non-covalent interactions. As predictable, the presence of nucleotide recognition motifs leads the assembly of calixarenes in high order structures and confers them novel host properties. Deeper studies have been started to better understand the features of the assembled structures and to verify prospective cooperation of the nucleotide moieties in recognition phenomena.

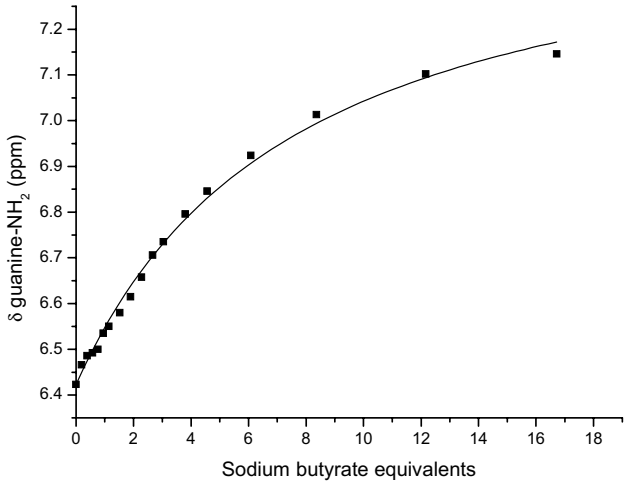

Figure 2. Titration curve of compound **2d** in the presence of sodium butyrate.

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5. *Procedure for the preparation of compounds* **2a**–**d** and **3a**–**d**: A solution of protected 2′-deoxynucleoside phosphoramidite **a**, **b**, **c**, or **d** (0.6 mmol) in anhydrous CH_3CN (4 mL) and tetrazole 0.45 M in the same solvent (2 mL) was added dropwise to a stirring solution of compound **1** (100 mg, 0.12 mmol) dissolved in a mixture of anhydrous CH_2Cl_2/CH_3CN 3:1 (8 mL). The mixture was stirred at room temperature for 6 h and then treated with an excess of 0.1 M iodine solution in THF/H_2O/pyridine (9:1:0.1). After evaporation under vacuum, the residue was dissolved in CH_2Cl_2 (20 mL) and the solution was washed by freshly prepared 5% aqueous sodium metabisulfite and water. The organic layer was dried in vacuo, then the residue was dissolved in pyridine (2 mL) and concd ammonia (40 mL) was added. The suspension was stirred at room temperature for 3 h and then (except for compound **2a** and **3a**) at 55 °C for 6 h. After removal of the solvent under vacuum, the residue was dissolved in CH_2Cl_2 (2 mL), 30% aqueous HOAc (25 mL) was added and the mixture was stirred at room temperature for 3 h. Remotion of the solvent under vacuum left a residue for compounds **2a**–**b** and **3a**–**b** that was dissolved in EtOAc/MeOH (95:5), washed with water, and dried in vacuo; whereas for compounds **2c**–**d** and **3c**–**d** the residue was suspended in water, collected by filtration, and dried. Pure compounds **2a**–**d** and **3a**–**d** were obtained by silica gel preparative TLC (*i*PrOH/concd ammonia 95:5 or *i*PrOH/H_2O/concd ammonia 85:15:5).
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8. ^{1}H NMR (CD_3OD) and ESI-MS data for compounds **2a**–**d** and **3a**–**d**. Compound **2a** (25% yield): δ 0.94 (t, 6H, $J = 7.4$ Hz, $2\times OCH_2CH_2CH_3$), 1.06 (s, 18H, $2\times C(CH_3)_3$), 1.23 (s, 9H, $C(CH_3)_3$), 1.24 (s, 9H, $C(CH_3)_3$), 1.88 (s, 3H, thymine-CH_3), 1.92 (q, 4H, $J = 7.5$ Hz, $2\times OCH_2CH_2CH_3$), 2.30 (ddd br, 1H, $J_{2',2''} = -13.5$ Hz, $J_{2',1'} = 7.1$ Hz, H-2′), 2.49 (br dd, 1H, $J_{2'',3'} = 3.4$ Hz, H-2″), 3.35 and 4.41 (AX system, 4H, $J = 12.3$ Hz, $2\times ArCH_2Ar$), 3.38 and 4.46 (AX system, 4H, $J = 12.3$ Hz, $2\times ArCH_2Ar$), 3.84 (br s, 2H, H-5′), 3.97–4.10 (m br overlapped signals, 6H, $2\times OCH_2CH_2CH_3$+OCH_2CH_2OH), 4.19 (m br overlapped signals, 3H, H-4′+OCH_2CH_2OH), 4.28 (br m, 2H, OCH_2CH_2OP), 4.41 (overlapped signal, 2H, OCH_2CH_2OP), the H-3′ resonance is obscured by the residual HOD signal, 6.37 (dd, 1H, $J_{1',2''} = 6.4$ Hz, $J_{1',2'} = 7.1$ Hz, H-1′), 7.01 (br s, 4H, $4\times ArH$), 7.25 (s, 4H, $4\times ArH$), 7.89 (s, 1H, H-6); ESI-MS (m/z): calcd for $C_{64}H_{88}N_2O_{13}P$ $[M-H]^-$ 1123.60, found 1123.6. Compound **3a** (60% yield): δ 0.99 (t, 6H, $J = 7.4$ Hz, $2\times OCH_2CH_2CH_3$), 1.08 (s, 18H, $2\times C(CH_3)_3$), 1.13 (s, 18H, $2\times C(CH_3)_3$), 1.88 (s, 6H, $2\times$ thymine-CH_3), 1.99 (q, 4H, $J = 7.5$ Hz, $2\times OCH_2CH_2CH_3$), 2.26 (ddd, 2H, $J_{2',2''} = -13.5$ Hz, $J_{2',1'} = 7.1$ Hz, $2\times$ H-2′), 2.37 (br dd, 2H, $J_{2'',2'} = -13.5$ Hz, $J_{2'',1'} = 6.5$ Hz, $2\times$ H-2″), 3.13 and 4.48 (AX system, 4H, $J = 12.3$ Hz, $2\times ArCH_2Ar$), 3.15 and 4.50 (AX system, 4H, $J = 12.3$ Hz, $2\times ArCH_2Ar$), 3.80 (br s, 4H, $2\times$ H-5′), 3.99 (br m, 4H, $2\times OCH_2CH_2CH_3$), 4.17 (br s, 2H, $2\times$ H-4′), 4.31 (br m, 4H, $2\times OCH_2CH_2OP$), 4.37 (br m, 4H, $2\times OCH_2OP$), the H-3′ resonance is obscured by the residual HOD signal, 6.31 (dd, 2H, $J_{1',2''} = 6.4$ Hz, $J_{1',2'} = 7.1$ Hz, $2\times$ H-1′), 6.92 (br s, 4H, $4\times ArH$), 7.00 (s, 4H, $4\times ArH$), 7.87 (br s, 2H, $2\times$ H-6); ESI-MS (m/z): calcd for $C_{74}H_{101}N_4O_{20}P_2$ $[M-H]^-$ 1427.65, found 1428.0. Compound **2b** (30% yield): δ 0.92 (t, 6H, $J = 7.4$ Hz, $2\times OCH_2CH_2CH_3$), 1.05 (s, 18H, $2\times C(CH_3)_3$), 1.20 (s, 9H, $C(CH_3)_3$), 1.24 (s, 9H, $C(CH_3)_3$), 1.92 (q, 4H, $J = 7.6$ Hz, $2\times OCH_2CH_2CH_3$), 2.65 (dd, 1H, $J_{2'',2'} = -13.6$ Hz, $J_{2'',1'} = 5.9$ Hz, H-2″), 2.93 (ddd, 1H, $J_{2',2''} = -13.6$ Hz, $J_{2',1'} = 7.1$ Hz, $J_{2',3'} = 6.0$ Hz, H-2′), 3.32 and 4.41 (AX system, 4H, $J = 12.6$ Hz, $2\times ArCH_2Ar$), 3.35 and 4.46 (AX system, 4H, $J = 12.6$ Hz, $2\times ArCH_2Ar$), 3.85 (dd, H, $J_{5'',5'} = -12.4$ Hz, $J_{5'',4'} = 2.9$ Hz, H-5″), 3.89 (dd, H, $J_{5',5''} = -12.4$ Hz, $J_{5',4'} = 2.3$ Hz, H-5′), 4.04 (m overlapped signals, 6H, $2\times OCH_2CH_2CH_3$+OCH_2CH_2OH), 4.19 (br s, 2H, OCH_2CH_2OH), 4.31 (m, 2H, OCH_2CH_2OP), 4.36 (d, 1H, $J_{4',5'} = 2.3$ Hz, H-4′), 4.47 (m overlapped, 2H, OCH_2CH_2OP), 5.10 (t, 1H, $J_{2',3'} = 6.0$ Hz, H-3′), 6.48 (dd, 1H, $J_{1',2'} = 7.1$ Hz, $J_{1',2''} = 5.9$ Hz, H-1′), 7.00 (br s, 4H, $4\times ArH$), 7.23 (s, 2H, $2\times ArH$), 7.25 (s, 2H, $2\times ArH$), 8.18 (s, 1H, H-2), 8.36 (s, 1H, H-8); ESI-MS (m/z): calcd for $C_{64}H_{87}N_5O_{11}P$ $[M-H]^-$ 1132.61, found 1132.7. Compound **3b** (27% yield): δ 0.96 (t, 6H, $J = 7.4$ Hz, $2\times OCH_2CH_2CH_3$), 1.03 (s, 18H, $2\times C(CH_3)_3$), 1.10 (s, 18H, $2\times C(CH_3)_3$), 1.99 (q, 4H, $J = 7.6$ Hz, $2\times OCH_2CH_2CH_3$), 2.56 (dd, 2H, $J_{2'',2'} = -13.7$ Hz, $J_{2'',1'} = 5.2$ Hz, $2\times$ H-2″), 2.80 (br ddd, 2H, $J_{2',2''} = -13.7$ Hz, $J_{2',1'} = 7.1$ Hz, $2\times$ H-2′), 3.27-3.30 (signals partially obscured by methanol, 4H, $2\times ArCH_2Ar$), 3.78 (dd, 2H, $J_{5'',5'} = -12.2$ Hz, $J_{5'',4'} = 2.7$ Hz, $2\times$ H-5″), 3.85 (dd, 2H, $J_{5',5''} = -12.2$ Hz, $J_{5',4'} = 2.1$ Hz, $2\times$ H-5′), 4.07 (br m, 4H, $2\times OCH_2CH_2CH_3$), 4.32 (br m, 6H, $2\times OCH_2CH_2OP$+$2\times$ H-4′), 4.41 (br m, 4H, $2\times OCH_2CH_2OP$), 4.50 (d, 2H, $J = 12.1$ Hz, $ArCH_2Ar$), 4.51 (d, 2H, $J = 12.1$ Hz, $ArCH_2Ar$), 5.02 (br t, 2H, $2\times$ H-3′), 6.43 (dd, 2H, $J_{1',2'} = 7.1$ Hz, $J_{1',2''} = 5.2$ Hz, $2\times$ H-1′), 6.99 (br s, 4H, $4\times ArH$), 7.00 (br s, 4H, $4\times ArH$), 8.18 (s, 2H, $2\times$ H-2), 8.34 (s, 2H, $2\times$ H-8); ESI-MS (m/z): calcd for $C_{74}H_{99}N_{10}O_{16}P_2$ $[M-H]^-$ 1445.67, found 1445.6. Compound **2c** (32% yield): δ 0.90 (t, 6H, $J = 7.3$ Hz, $2\times OCH_2CH_2CH_3$), 1.02 (s, 18H, $2\times C(CH_3)_3$), 1.10 (s, 9H, $C(CH_3)_3$), 1.12 (s, 9H, $C(CH_3)_3$), 1.86 (m, 4H, $2\times OCH_2CH_2CH_3$), 2.21 (br ddd, 1H, $J_{2',2''} = -13.2$ Hz, $J_{2',1'} = 7.2$ Hz, H-2′), 2.56 (br dd, 1H, $J_{2',2''} = -13.2$ Hz, H-2″), 3.36 and 4.42 (AX system, 4H, $J = 12.7$ Hz, $2\times ArCH_2Ar$), 3.40 and 4.45 (AX system, 4H, $J = 12.7$ Hz, $2\times ArCH_2Ar$), 3.80 (br s, 2H, H-5′), 4.01 (s br overlapped signals, 6H, $2\times OCH_2CH_2CH_3$+OCH_2CH_2OH), 4.14 (br s, 2H, OCH_2CH_2OH), 4.20 (br s, 1H, H-4′), 4.26 (br m, 2H, OCH_2CH_2OP), 4.43 (m overlapped, 2H, OCH_2CH_2OP), 4.87 (br s, 1H, H-3′), 5.87 (d, 1H, $J = 7.4$ Hz, H-5), 6.30 (t, 1H, $J_{1',2''} = 6.5$ Hz, H-1′), 6.97 (br s, 4H, $4\times ArH$), 7.19 (s, 4H, $4\times ArH$), 7.98 (d, 1H, $J = 7.4$ Hz, H-6); ESI-MS (m/z): calcd for $C_{63}H_{87}N_3O_{12}P$ $[M-H]^-$ 1108.60, found 1108.5. Compound **3c** (22% yield): δ 0.95 (t, 6H, $J = 7.4$ Hz, $2\times OCH_2CH_2CH_3$), 1.11 (s, 18H, $2\times C(CH_3)_3$), 1.14 (s, 18H, $2\times C(CH_3)_3$), 1.99 (q, 4H, $J = 7.6$ Hz, $2\times OCH_2CH_2CH_3$), 2.14 (br ddd, 2H, $J_{2',2''} = -13.2$ Hz, $J_{2',1'} = 6.8$ Hz, $2\times$ H-2′), 3.53 (dd, 2H, $J_{2'',2'} = -13.2$ Hz, $J_{2'',3'} = 3.2$ Hz, $2\times$ H-2″), 3.36 and 4.49 (AX system, 4H, $J = 12.5$ Hz, $2\times ArCH_2Ar$), 3.39 and 4.51 (AX system, 4H, $J = 12.5$ Hz, $2\times ArCH_2Ar$), 3.79 (d overlapped signals, 4H, $2\times$ H-5′), 4.06 (br s, 4H, $2\times OCH_2CH_2CH_3$), 4.15 (br d, 2H, $2\times$ H-4′), 4.35 (m overlapped signals, 8H, $2\times OCH_2CH_2OP$+$2\times OCH_2CH_2OP$), the H-3′ resonance is obscured by the residual HOD signal, 5.90 (d, 2H, $J = 7.5$ Hz, $2\times$ H-5), 6.34 (t, 2H, $J_{1',2'} = 6.8$ Hz, $2\times$ H-1′), 7.09 (br s, 8H, $8\times ArH$), 8.02 (d, 2H, $J = 7.5$ Hz, $2\times$ H-6); ESI-MS (m/z): calcd for $C_{72}H_{99}N_6O_{18}P_2$ $[M-H]^-$ 1397.65, found 1397.9. Yield 15%. Compound **2d** (25% yield): δ 0.94 (t, 6H, $J = 7.4$ Hz, $2\times OCH_2CH_2CH_3$), 1.04 (s, 18H, $2\times C(CH_3)_3$), 1.22 (s, 9H, $C(CH_3)_3$), 1.25 (s, 9H, $C(CH_3)_3$), 1.92 (m, 4H, $2\times OCH_2CH_2CH_3$), 2.57 (br dd, 1H, H-2″), 2.79 (br dd, 1H, $J_{2',2''} = -13.2$ Hz, $J_{2',3'} = 6.8$ Hz, H-2′), 3.32 and 4.44 (AX system, 4H, $J = 12.5$ Hz, $2\times ArCH_2Ar$), 3.34 and 4.46 (AX system, 4H, $J = 12.5$ Hz, $2\times ArCH_2Ar$), 3.82 (dd, 1H, $J_{5'',5'} = -12.1$ Hz, $J_{5'',4'} = 3.6$ Hz, H-5″), 3.88 (dd, 1H, $J_{5',5''} = -12.1$ Hz, $J_{5',4'} = 3.2$ Hz, H-5′), 3.91–4.12 (m overlapped, 6H, $2\times OCH_2CH_2CH_3$+OCH_2CH_2OH), 4.18 (br m, 2H, OCH_2CH_2OH), 4.25 (br d, 1H, $J_{4',3'} = 2.8$ Hz, H-4′), 4.31 (m, 2H, OCH_2CH_2OP), 4.45 (m, 2H, OCH_2CH_2OP), 5.09 (br s, 1H, H-3′), 6.29 (t, 1H, $J_{1',2''} = 6.7$ Hz, H-1′), 6.97 (br s, 4H, $4\times ArH$), 7.22 (d, 4H, $4\times ArH$), 8.04 (s, 1H, H-8); ESI-MS (m/z): calcd for $C_{64}H_{87}N_5O_{12}P$ $[M-H]^-$ 1148.61, found 1148.7. Compound **3d** (10% yield): δ 0.98 (t, 6H, $J = 7.4$ Hz, $2\times OCH_2CH_2CH_3$), 1.07 (s, 18H, $2\times C(CH_3)_3$), 1.08 (s, 18H, $2\times C(CH_3)_3$), 2.00 (q, 4H, $J = 7.6$ Hz, $2\times OCH_2CH_2CH_3$), 2.43 (ddd, 2H, $J_{2'',2'} = -13.6$ Hz, $J_{2'',1'} = 6.4$ Hz, $J_{2'',3'} = 2.7$ Hz, $2\times$ H-2″), 2.64 (ddd, 2H, $J_{2',2''} = -13.6$ Hz, $J_{2',1'} = 7.2$ Hz, $2\times$ H-2′), 3.23 and 4.48 (AX system, 4H, $J = 12.7$ Hz, $2\times ArCH_2Ar$), 3.25 and 4.49 (AX system, 4H, $J = 12.7$ Hz, $2\times ArCH_2Ar$), 3.78 (dd, 2H, $J_{5'',5'} = -12.1$ Hz, $J_{5'',4'} = 3.4$ Hz, $2\times$ H-5″), 3.85 (dd, 2H, $J_{5',5''} = -12.1$ Hz, $J_{5',4'} = 2.8$ Hz, $2\times$ H-5′), 4.19 (br m, 4H, $2\times OCH_2CH_2CH_3$), 4.23 (br d, 2H, $2\times$ H-4′), 4.31 (br m, 4H, $2\times OCH_2CH_2OP$), 4.38 (m, 4H, $2\times OCH_2CH_2OP$), 5.01 (br t, 2H, $J_{3',2'} = 6.2$ Hz, $2\times$ H-3′), 6.22 (dd, 2H, $J_{1',2'} = 7.2$ Hz, $J_{1',2''} = 6.4$ Hz, $2\times$ H-1′), 6.93 (br s, 8H, $8\times ArH$), 7.82 (s, 2H, $2\times$ H-8); ESI-MS (m/z): calcd for $C_{74}H_{99}N_{10}O_{18}P_2$ $[M-H]^-$ 1477.66, found 1477.7.
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