Strong Binding of Alkylguanidinium Ions by Molecular Tweezers: An Artificial Selective Arginine Receptor Molecule with a Biomimetic Recognition Pattern

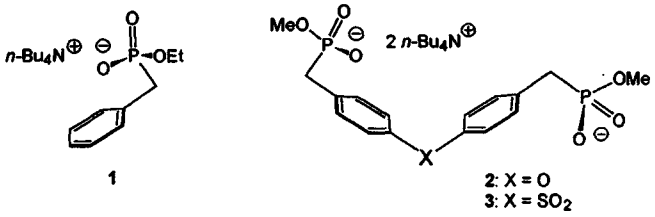
Thomas Schrader*

Abstract: Bisphosphonates **2** and **3** represent the first artificial receptor molecules for alkylguanidinium ions. They bind to the guanidinium moiety by forming a 1:1 chelate complex, stabilized by a planar network of electrostatic interactions and hydrogen bonds. This hydrogen bonding configuration is identical to the "arginine fork" postulated by Frankel as a key element in RNA–protein recognition of the AIDS virus. Our guanidinium–bisphosphonate complexes thus constitute the first synthetic model for this important biological interaction and demonstrate that the high binding energy can be a driving force for a conformational change in the receptor (induced fit, e.g., in the RNA). Although binding of monosubstituted alkylguanidines is generally strong ($K_a \approx 10000$ in DMSO), molecular tweezer **3** recognizes *N*- and *C*-amide-protected arginine derivatives especially well ($K_a \approx 300000$ in DMSO), because an additional hydrogen bond is formed between the amide and the phosphonate. Since **3** does not bind amines effectively, it is highly selective for arginine, even in the presence of lysine or other amino acids. For di-, tri-, and tetrasubstituted guanidines the association constant remains low ($K_a \leq 1000$ in DMSO) reflecting the increase in the steric bulk of the guest.

Keywords
arginine · guanidines · molecular recognition · receptors · supramolecular chemistry

Introduction

Alkylguanidinium ions play a dominant role in biological systems, especially in form of the amino acid arginine, because they act as binding sites for anionic biomolecules in enzymes and antibodies.[1] Arginine residues are also involved in the interactions of the nucleic acid phosphodiesters with the highly charged protamines and histones.[2] Many antiviral natural compounds presently being tested against HIV infection are guanidines.[3] In recent years several artificial receptors based on bisguanidines have been developed for phosphodiester binding.[4] However, to the best of our knowledge no synthetic receptor is known that binds to alkylguanidines themselves. All systems that have been developed for guanidine recognition are macrocycles. For steric reasons these can only bind the unsubstituted guanidinium cation well. Hence, with only a single exception,[5a] investigations have been confined to simple guanidinium complexes.[5] Lehn demonstrated convincingly that even the exchange of guanidine for methylguanidine causes a twentyfold drop in the association constant for his "tartrato crown".[5a] To solve this problem we have developed molecular tweezers **2** and **3** (Scheme 1), which strongly and selectively bind to monoalkylguanidines. The binding scheme may be called biomimetic inasmuch as it relies mainly on electrostatic interactions and hydrogen bonds.[1, 2]



Scheme 1. Synthetic phosphonate receptor molecules.

Results and Discussion

According to force-field calculations for the synthetic receptors **2** and **3** the bisphosphonates can be arranged to form an almost completely planar network of hydrogen bonds with guanidinium cations (Scheme 2, left).[6] This array shows a striking similarity to a key structural element in RNA–protein recognition of the AIDS virus. Its replication depends critically on complex formation between the TAR (= trans-acting responsive element) region of the mRNA and a regulatory protein called Tat (= transcriptional activator).[7] Frankel and coworkers have presented ample evidence for a simple sequence-selective binding mode.[8] They show that the protein recognizes

[*] Dr. T. Schrader
Institut für Organische Chemie und Makromolekulare Chemie
Universitätsstr. 1, 40225 Düsseldorf (Germany)
Fax: Int. code +(211)811-4788
e-mail: schrat@iris-oc2.oc2.uni-duesseldorf.de

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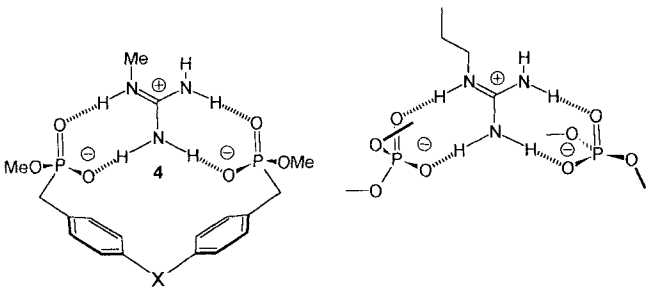

Scheme 2. Left: Complexes of the molecular tweezers **2** or **3** (X = O or SO_2) with methylguanidine (**4**) according to force-field calculations [6]. Right: the "arginine fork" [8].

an RNA conformation that allows a single arginine residue to bind simultaneously to two phosphates. Thus a structure is formed that they call the "arginine fork" (Scheme 2, right). Its hydrogen-bonding configuration is identical to that of our molecular model. Binding studies with receptor molecules **2** and **3** should therefore provide valuable information about the stability of such complexes. The hinge groups O and SO_2 provide a wide and a narrow tweezer opening according to their different bond angles. In this way we hoped to create selective hosts that do not bind cations similar to guanidinium, such as alkylammonium ions.

Addition of one equivalent of phosphonates **2** or **3** to methylguanidinium chloride (**4**) in [D_6]DMSO resulted in large shifts of host and guest signals in both the ^1H and the ^{31}P NMR spectra. We performed NMR titrations with receptors **1**–**3** (**1** serves as a reference) of various guanidines (**4**–**6**) in [D_6]DMSO and calculated the association constants from the binding curves (e.g., Figure 1) by nonlinear regression methods.[9] The results are summarized in Table 1.

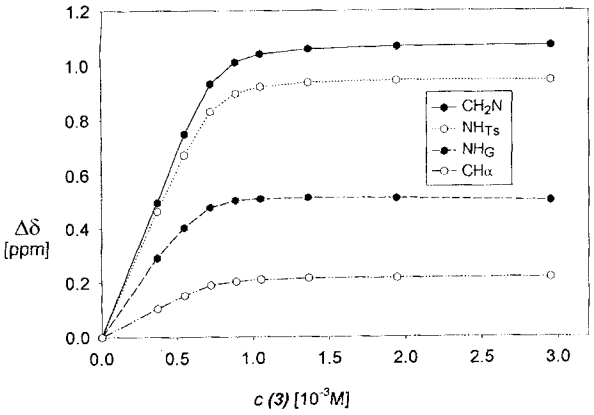

Figure 1. Dependence of the change in chemical shift $\Delta\delta$ of characteristic NMR signals of α-*N*-tosylarginine methyl ester **5** (c = 0.7 mM) on the concentration of bisphosphonate **3** in [D_6]DMSO at 20 °C.

Direct comparison of mono- and bisphosphonate (**1** vs. **2** and **3**) proves that both phosphonate anions in **2** and **3** are indeed involved in a chelate-type interaction with the guanidinium cation, because the binding constants for methylguanidine increase by a factor of 56 and 93, respectively, with respect to that for **1** (Table 1). Job's method of continuous variations[10] confirms the postulated 1:1 stoichiometry of all complexes. These results constitute strong experimental support for the high thermodynamic stability of the arginine fork postulated by Frankel for RNA–protein recognition. In the unbound phosphonates six single bonds can rotate freely, so that their conformation will be completely different from that of the complex, in order to avoid electrostatic repulsion between the phosphonate groups. This is also the case in the unbound RNA molecule; only the approach of a specific arginine moiety in the Tat protein induces a distinct conformational change that brings the phosphates close to each other (induced fit). Our model confirms that the double electrostatic attraction in the arginine fork can more than compensate for the destabilization energy necessary for reorientation of the phosphates.

With α-*N*-tosylarginine methyl ester (**5**) instead of methylguanidine a significantly higher binding constant is produced (Table 1). Force-field calculations for α-*N*-tosyl- and for α-*N*-acylarginine esters demonstrate that after guanidine "docking" the amino acid side chain can be reoriented so that the amide function is brought close to the phosphonate (Scheme 3).[6] The large downfield shift of the amide protons of **5** (Figure 1) indicates formation of an additional intermolecular hydrogen bond to the phosphonate. The binding constant with **3** reaches 62800 M^{-1} as a result of this cooperative effect. Titrations with **3** in pure methanol produce an association constant K_a of 490 M^{-1} for **4** and 570 M^{-1} for **5**. These values for alkylguanidines exceed those for all neutral crown ethers by at least one order of magnitude ($K_{a(max)}$ = 68 for the unsubstituted guanidinium cation).[11] Finally, the narrow diphenyl sulfone **3** binds to all monoalkylguanidines more strongly than the wider diphenyl ether **2** (Table 1). In contrast, benzylamine and 1,1-dimethylguanidine (**6**) are bound much more weakly. Encouraged by these results we examined **3** closer for its potential to act as a guanidine or even arginine selector.

How much better does **3** recognize arginine derivatives over other amino acids? In Table 2a the binding constants for α-*N*-acyl-protected alanine, lysine, and arginine esters, which were produced under identical conditions, are compared: alanine is not complexed at all; lysine is bound 14 times more weakly than arginine derivative **7**. The conformational lock of the arginine molecule due to the additional amide binding site is so strong that on complexation of the *N*-benzoyl derivative **7** the methylene protons of the highly symmetrical artificial receptor become diastereotopic (Scheme 3).[12]

Table 1. Association constants ($K_{1:1}$) from NMR titrations in DMSO at 20 °C [a].

Guest	$K_{1:1}$(**1**) $\times 10^{-3}$ [M^{-1}]	ΔG^{293}(**1**) [kcal mol^{-1}]	$K_{1:1}$(**2**) $\times 10^{-3}$ [M^{-1}]	ΔG^{293}(**2**) [kcal mol^{-1}]	$K_{1:1}$(**3**) $\times 10^{-3}$ [M^{-1}]	ΔG^{293}(**3**) [kcal mol^{-1}]
methylguanidine (**4**)	0.1	2.7	5.6	5.0	9.3	5.3
α-*N*-tosylarginine methyl ester (**5**)	–	–	21.8	5.8	62.8	6.5
benzylamine	0.2	3.1	3.7	4.8	1.9	4.4
1,1-dimethylguanidine (**6**)			6.6	5.1	2.9	4.7

[a] Because of the strongly hygroscopic character of both titration partners the [D_6]DMSO solution contained about 0.1% of water. Errors in K_a are estimated at ±6–40%.

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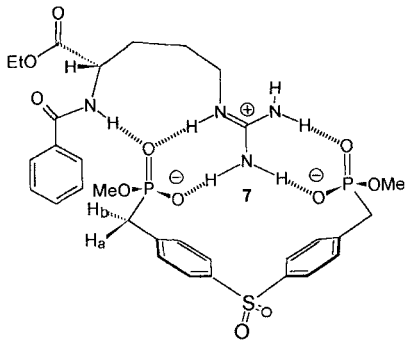

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Scheme 3. Conformational lock of α-*N*-benzoylarginine ethyl ester (**7**) in its complex with **3** according to force-field calculations [6].

In order to simulate the electronic environment in a peptide we examined the *N*- and *C*-amide-protected arginine derivatives **8** and **9** (Scheme 4a). The additional hydrogen bond switches completely to the carboxamide side of the arginine[13] and the association constant increases markedly again to a value around 300000 M^{-1}. Table 2a documents the sensitivity with which **3** recognizes the different side chains of even similar proteinogenic amino acids. Especially in an amidic environment, as in a protein, the bisphosphonate is highly selective for arginine.

To further characterize the recognition profile of **3** we investigated its affinity towards several amines and guanidines with a different degree of substitution (Table 2b,c; Scheme 4b,c). Generally, amines are complexed weakly (Table 2b); even the amino alcohol **10** (a model compound for adrenaline) gives only a moderate association constant of 4000 M^{-1}. This is in sharp contrast to the effective binding by a *m*-xylylene bisphosphonate (K_a = 55000 M^{-1}), which we introduced recently as a highly efficient synthetic receptor molecule for amino alcohols.[14]

Table 2. Association constants ($K_{1:1}$) from NMR titrations with **3** in DMSO at 20 °C. For formulas see Scheme 4 [a].

a) Amino acids.

	$K_{1:1} \times 10^{-3}$ [M^{-1}]	ΔG^{293} [kcal mol^{-1}]
BOC-Ala-OMe	< 0.01	< 1.3
α-Ac-Lys-OMe	4	4.8
α-Bz-Arg-OEt (**7**)	58	6.4
α-Ts-Arg-OMe (**5**)	63	6.5
8	280	7.3
9	310	7.4

b) Amines.

	$K_{1:1} \times 10^{-3}$ [M^{-1}]	ΔG^{293} [kcal mol^{-1}]
hexylamine	1	4.0
benzylamine	2	4.4
10	4	4.8

c) Guanidines.

	$K_{1:1} \times 10^{-3}$ [M^{-1}]	ΔG^{293} [kcal mol^{-1}]
methylguanidine (**4**)	9	5.3
guanethidine (**11**)	9	5.3
1,1-dimethylguanidine (**6**)	3	4.7
creatine (**12**)	0.1	2.7
creatinine (**13**)	1	4.0
14	1	4.0

[a] The [D_6]DMSO solution contained about 0.1 % of water because of the strongly hygroscopic character of both titration partners. Errors in K_a are estimated at ±3–50 %.

Among guanidinium ions the artificial receptor **3** is equally selective: while binding monoalkylguanidines (**4**, **11**) very well, its affinity towards 1,1-dialkylguanidines (**6**, **12**) already decreases markedly; the binding constant for creatine (**12**) is extremely low (K_a = 140 M^{-1}), probably because of the strong phosphonate–carboxylate repulsion between host and guest. Similarly, the tri- and tetrasubstituted guanidines **13** and **14** are only weakly bound, although creatinine (**13**) is an acylguanidine and hence much more NH-acidic than simple trialkylguanidines.

Perspectives

We intend to use the high selectivity of the artificial arginine receptor molecule **3** to design enzyme mimics that are able, like thrombin and trypsin, to cleave proteins after arginine. To this end the phosphonic acid may be linked with an appropriate nucleophile.

Scheme 4. a) Amino acids, b) amines, and c) guanidines for the NMR titrations.

Experimental procedure

General: ^{1}H NMR spectra were recorded at 20 °C on a Varian EM 390 (90 MHz) or a Varian VXR 300 spectrometer (300 MHz). ^{13}C NMR spectra were recorded on the same instruments at 75 MHz. For high binding constants NMR titrations were carried out on a Bruker 500 MHz spectrometer. Chemical shifts δ are given relative to an internal tetramethylsilane standard (TMS). ^{31}P NMR spectra were recorded on a Bruker AM 200 SY spectrometer with H_3PO_4 as external standard. $CDCl_3$ and [D_6]DMSO were purchased from Aldrich in 99.8% purity (water content of DMSO ≈ 0.03%). TLC analysis was carried out on silica gel 60 F-254 (layer thickness 0.2 mm). Preparative chromatography columns were packed with silica gel 60 (70–230 mesh) from Macherey & Nagel. All solvents were dried and freshly distilled before use.

NMR Titrations: A solution of the phosphonate (10 equiv in 0.4 mL [D_6]DMSO) was added with a 50 µL microsyringe in aliquots to a solution of the alkylguanidinium chloride (1 equiv in 0.7 mL [D_6]DMSO; 0.5–2 mM) in a sealed NMR tube. The guanidine and tetrabutylammonium phosphonate solutions contained ≈0.05–0.1% and ≈0.3–0.6% water, respectively, because of their highly hygroscopic character. Volume and concentration changes were taken into account during analysis.[7] NMR titrations on the 500 MHz spectrometer were carried out automatically by a robot. To this end nine NMR tubes were each filled with the same amount and concentration of guest solution, and increasing amounts of host solutions of uniform concentration were added successively. Errors in K_a were markedly smaller compared with the 300 MHz measurements owing to the preparation of stock solutions and their tenfold lower concentrations.

Job Plots: Equimolar solutions (0.03 M) of phosphonate **1** and methylguanidinium chloride were mixed in various ratios. ^{1}H NMR spectra of the mixtures were recorded, and the change in chemical shift of host and guest signals was analyzed with Job's method modified for NMR experiments.[8]

Monoethyl Benzylphosphonate (starting material for **1**) was prepared by alkaline hydrolysis of the corresponding diester with aqueous sodium hydroxide (5 N), followed by acidification with HCl (1 N). Yield: 98%; M.p. 66 °C; ^{1}H NMR (90 MHz, [D_6]DMSO): δ = 1.25 (t, 3H, J = 7 Hz, CH_3), 3.0 (d, 2H, J = 22 Hz, CH_2P), 3.85 (dq, 2H, J = 7 Hz, CH_2O), 7.25 (m, 5ArH), 9.8 (brs, 1H, P-OH). ^{31}P NMR ([D_6]DMSO): δ = 24.74 (s). Elemental analysis: calcd. for $C_9H_{13}O_3P$: C 53.98, H 6.55, found C 53.82, H 6.55.

Tetrabutylammonium benzylphosphonic acid monoethyl ester (1): A typical pH-titration experiment was carried out with monoethyl benzylphosphonate and tetrabutylammonium hydroxide (1.50 M) in water. When the equivalence point was reached (after the addition of exactly 1 equiv of aqueous tetrabutylammonium hydroxide) the reaction mixture was evaporated to dryness and extracted with dry chloroform. After drying over magnesium sulfate and filtration the solvent was removed in vacuo and the solid was further dried over phosphorus pentoxide at 10^{-3} mbar. Yield: 95%; M.p. 48 °C; ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 0.92 (t, 12H, J = 7.3 Hz, CH_3(Bu)), 1.01 (t, 3H, J = 7.0 Hz, CH_3(Et)), 1.29 (tq, 8H, J = 7.3 Hz, $8CH_2$(Bu)), 1.55 (m, 8H, $8CH_2$(Bu)), 2.65 (d, 2H, J = 22 Hz, CH_2P), 3.20 (m, 8H, CH_2N), 3.60 (dq, 2H, J = 7.0/7.3 Hz, CH_2O), 7.03 (t, 1ArH, J = 7.4 Hz), 7.14 (t, 2ArH, J = 7.4 Hz), 7.21 (d, 2ArH, J = 7.4 Hz). ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 13.50 (s, $4CH_3$(Bu)), 16.99 (d, CH_3(Et)), 19.22 (s, $4CH_2$(Bu)), 23.14 (s, $4CH_2$(Bu)), 36.37 (d, J = 122.9 Hz, CH_2P), 57.48 (m, $4CH_2N$), 58.43 (d, J = 6.2 Hz, CH_2O), 124.20 (d, J = 2.3 Hz, CH_{arom}), 127.19 (d, J = 2.2 Hz, $2CH_{arom}$), 129.47 (d, J = 5.6 Hz, $2CH_{arom}$), 138.89 (m, $1C_{arom}$). ^{31}P NMR ([D_6]DMSO): δ = 14.89 (s). Elemental analysis: calcd. for $C_{25}H_{48}O_3P$: C 67.98, H 10.96, N 3.17, found C 67.93, H 11.19, N 3.40.

Bis(dimethyl phosphonates) (starting materials for the molecular tweezers): Di-*p*-tolyl ether and di-*p*-tolyl sulfone were converted to the corresponding dibromides with 2 molequiv of NBS and a catalytic amount of AIBN in dry tetrachloromethane by heating the reaction mixtures to 80 °C. After recrystallization the dibromides were heated for 3 h to 140 °C with 2 molequiv of trimethyl phosphite. Residual trimethyl phosphite and dimethyl methylphosphonate were evaporated in vacuo. Chromatographic purification over silica gel (ethyl acetate/methanol = 10:1) afforded analytically pure colorless oils, which crystallized during one day.

Bis[p-(dimethoxyphosphorylmethyl)phenyl] ether: Yield: 26% overall; M.p. 86–87 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.12 (d, 4H, J = 21 Hz, CH_2P), 3.63 (d, 6H, J = 10.5 Hz, CH_3O), 6.73 (d, 4ArH, J = 8 Hz), 7.18 (dd, 4ArH, J = 8/2 Hz) (AA'BB' system). ^{31}P NMR ($CDCl_3$): δ = 29.48 (s). Elemental analysis: calcd. for $C_{18}H_{24}O_7P_2$: C 52.18, H 5.84, found C 52.20, H 5.75.

Bis[p-(dimethoxyphosphorylmethyl)phenyl] sulfone: Yield: 26% overall; M.p. 88 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.21 (d, 4H, J = 22.3 Hz, CH_2P), 3.69 (d, 6H, J = 11.0 Hz, CH_3O), 7.45 (dd, 4ArH, J = 8.5/2.5 Hz), 7.89 (d, 4ArH, J = 8.5 Hz) (AA'BB' system). ^{31}P NMR ($CDCl_3$): δ = 28.29 (s). Elemental analysis: calcd. for $C_{18}H_{24}O_8P_2S$: C 46.76, H 5.23, found C 46.69, H 5.27.

Synthesis of 2 and 3: The tetramethyl phosphonates were heated at 120 °C for ca. one week with exactly 2 molequiv of aqueous tetrabutylammonium hydroxide solution (1.5 M). After evaporation to dryness and extraction with dry chloroform the solution was dried over magnesium sulfate, filtered, and again evaporated to dryness. The oily product was purified from residual solvent and traces of water by stirring at 60 °C in vacuo. After ca. one week the product crystallized slowly. Even after this drying procedure the receptor contained exactly one molecule of water per phosphonate group; obviously water is bound very tightly to this host (cf. elemental analysis).

Bis[(tetrabutylammonium)-p-(methoxyoxyphosphorylmethyl)phenyl] ether (2): Yield: 95%; M.p. 50–51 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.99 (t, 24H, J = 7.2 Hz, CH_3), 1.40 (tq, 16H, J = 7.2 Hz, $8CH_2$), 1.60 (m, 16H, $8CH_2$), 2.97 (d, 4H, J = 19.8 Hz, CH_2P), 3.26 (m, 16H, CH_2N), 3.49 (d, 6H, J = 10.1 Hz, CH_3O), 6.83 (d, 4ArH, J = 8.5 Hz), 7.33 (dd, 4ArH, J = 8.5/2.1 Hz) (AA'BB' system). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.47 (s, $8CH_3$(Bu)), 19.17 (s, $8CH_2$(Bu)), 23.08 (s, $8CH_2$(Bu)), 35.05 (d, J = 123.7 Hz, $2CH_2P$), 50.47 (m, $2CH_3O$), 57.42 (m, $8CH_2N$), 128.39 (m, $4CH_{arom}$), 134.50 (m, $2C_{arom}$). ^{31}P NMR ($CDCl_3$): δ = 19.34 (s). Elemental analysis: calcd. for $C_{48}H_{90}N_2O_7P_2 \cdot 2H_2O$: C 63.69, H 10.47, N 3.09, found C 63.53, H 10.43, N 3.08.

Bis[(tetrabutylammonium)-p-(methoxyoxyphosphorylmethyl)phenyl] sulfone (3): Yield: 95%; M.p. 67–68 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.96 (t, 24H, J = 7.1 Hz, CH_3), 1.36 (tq, 16H, J = 7.1 Hz, $8CH_2$), 1.53 (m, 16H, $8CH_2$), 3.02 (d, 4H, J = 20.1 Hz, CH_2P), 3.18 (m, 16H, CH_2N), 3.49 (d, 6H, J = 10.1 Hz, CH_3O), 7.51 (dd, 4ArH, J = 8.3/1.7 Hz), 7.73 (d, 4ArH, J = 8.3 Hz) (AA'BB' system). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 13.69 (s, $8CH_3$(Bu)), 19.72 (s, $8CH_2$(Bu)), 23.97 (s, $8CH_2$(Bu)), 36.31 (d, J = 124.0 Hz, $2CH_2P$), 51.64 (d, J = 4.8 Hz, $2CH_3O$), 58.65 (s, $8CH_2N$), 126.83 (d, J = 2.4 Hz, $4CH_{arom}$), 130.60 (d, J = 6.1 Hz, $4CH_{arom}$), 138.06 (d, J = 2.4 Hz, $2C_{arom}$ (*ipso*-C)), 145.14 (d, J = 7.3 Hz, $2C_{arom}$ (*ipso*-C)). ^{31}P NMR ($CDCl_3$): δ = 16.98 (s). Elemental analysis: calcd. for $C_{48}H_{90}N_2O_8P_2S \cdot 2H_2O$: C 60.48, H 9.94, N 2.94, found C 60.55, H 10.05, N 3.04.

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