Bifunctional Bisamphiphilic Transmembrane Building Blocks for Artificial Signal Transduction

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Received 24 May 2008

Dedicated to Prof. R. W. Hoffmann on the occasion of his 75th birthday

Abstract: A convergent modular total synthesis is described which affords bisamphiphilic bifunctional transmembrane building blocks useful for artificial signal transduction across membranes. The concept relies on direct Glaser–Hay coupling of lithocholic or cholenic acid propargyl esters, carrying recognition or signaling units on their opposite hydroxy functionalities. For catechol recognition, a 2-(aminomethyl)phenylboronic acid was introduced, while the ammonium alcohol was targeted by a bisphosphonate dianion. Signaling units comprised an N-protected cysteine (thiol nucleophile) and an N-protected cysteine/pyridine disulfide, respectively (disulfide substrate). All these polar headgroups were connected to the hydroxy of the lipid core by ester formation.

Key words: signal transduction, phosphonate, Glaser–Hay coupling, disulfide coupling

All living organisms rely on the fundamental process of signal transduction across their cell membranes.[1] Although ~60% of all manufactured drugs target one of the large family of G-protein-coupled receptors (GPCRs), a functional synthetic biomimetic version has proven elusive to date. Building on the creative approach delineated by the Hunter group, the authors of this manuscript have designed unsymmetrical bisamphiphilic transmembrane building blocks that can be embedded in lipid bilayers.[2] Two recognition head groups for catechol and ammonium alcohol recognition are tailored for ditopic adrenaline binding bringing both transmembrane units into close proximity in the fluidic membrane. At the opposite end, a thiol nucleophile is attached to one unit, which is designed to nucleophilically displace thiopyridine from an adjacent disulfide substrate at the other unit. Adrenaline injection into a suspension of unilamellar liposomes with embedded transmembrane building blocks, should exclusively result in thiopyridine displacement from the 'intracellular' disulfides, detectable by a specific absorption intensity rise at 432 nm.[3] This overall strategy requires the total synthesis of large bisamphiphilic building blocks, which exactly span a lipid bilayer (typically DMPC or DPPC). These must carry on one end a recognition head group and on the other end a reactive group for the S_N2 signaling reaction.

The synthetic concept should involve a modular approach, allowing exchange of recognition and signaling tips at will and at the same time be convergent, i.e., minimize the number of consecutive steps and avoid excessive protecting group manipulations.

Lithocholic acid has already been successfully used for extracellular membrane signaling, but introduces a kink and this brings potential destabilization just outside the phosphatidylcholine headgroups. Replacement of this commercially available steroid by a cholenic acid template requires three additional steps but, nevertheless, was realized, because of its perfectly flat overall topology with an equatorial placement of its secondary hydroxy group (Figure 1).[4]

The overall strategy is as follows: each steroid is first elongated at its carboxy terminus (C1) to a propargyl ester or less reactive amide derivative, then adorned at its O-terminus (C3′) with an appropriate functional head group, either for primary messenger recognition, or for second messenger generation. In the key step, two of these lipids are connected by alkyne coupling, before in the final steps, all protecting groups are cleaved off.

The propargyl ester or amide formation from both steroids proceeds smoothly with *N*,*N*′-dicyclohexylcarbodiimide/4-(dimethylamino)pyridine and affords spacer elements **2a**–**c** in high yields (70–81%). In order to avoid intermo-

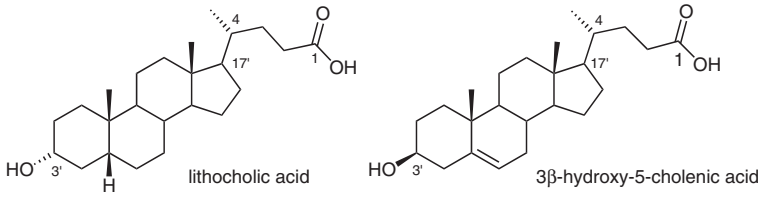
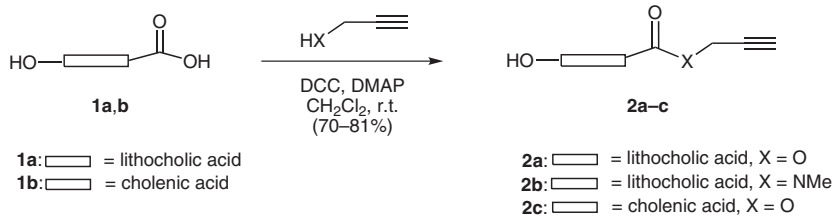
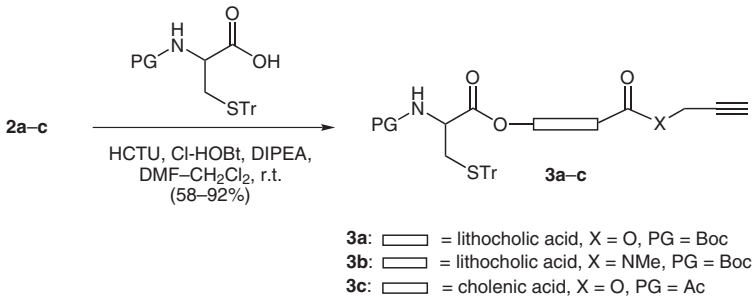


Figure 1 Rigid lipid templates for all transmembrane building blocks with two orthogonal attachment points

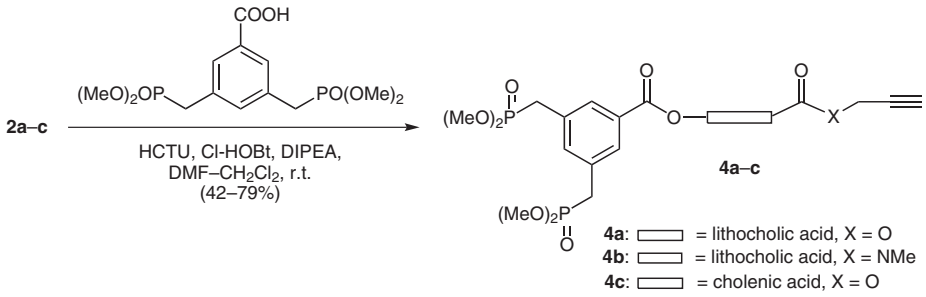
SYNTHESIS 2008, No. 14, pp 2247–2256
Advanced online publication: 02.07.2008
DOI: 10.1055/s-2008-1067155; Art ID: C03208SS



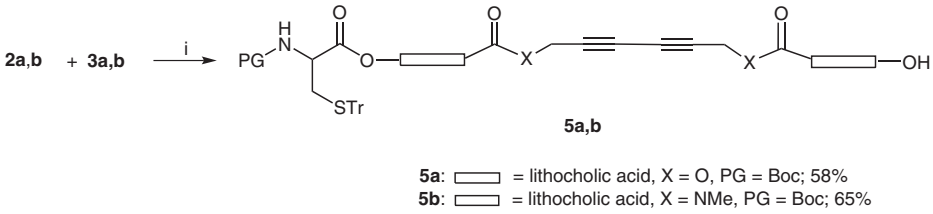
Scheme 1 Steroid elongation to propargyl esters or *N*-methylamides



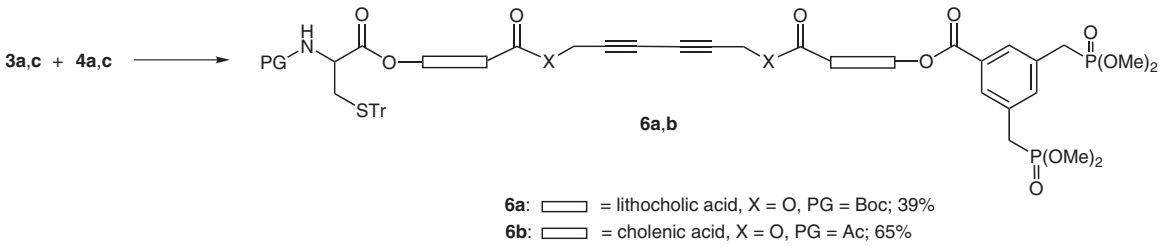
Scheme 2 Cysteine attachment by esterification



Scheme 3 Bisphosphonate attachment by esterification



Scheme 4 Directed Glaser–Hay coupling towards true membrane-spanning building blocks with boronic acid head groups. *Reagents and conditions*: (i) **2a**,**b** (2 equiv), **3a**,**b** (1 equiv), CuCl (5 equiv), TMEDA (5 equiv), O_2, CH_2Cl_2, r.t.



Scheme 5 Directed Glaser–Hay coupling towards true membrane-spanning building blocks with bisphosphonate head groups. *Reagents and conditions*: **3a**,**c** (2 equiv), **4a**,**c** (1 equiv), CuCl (5 equiv), TMEDA (5 equiv), O_2, CH_2Cl_2, r.t.

lecular hydrogen bonding between adjacent lipid moieties, *N*-methylpropargylamine was employed in this reaction, so that the resulting amides retain their mobility within the fluidic lipid phase, a prerequisite for effective signaling (Scheme 1).

For the signaling units the steroid's O-terminus was subsequently esterified with N/S-diprotected cysteine (Scheme 2), assisted by the powerful combination of *O*-(6-chlorobenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate/6-chloro-1-hydroxybenzotriazole (HCTU) with 6-chloro-1-hydroxybenzotriazole (Cl-HOBt), furnishing **3a–c** in high yields (58–92%).

Ditopic adrenaline recognition requires the attachment of an amino alcohol and a catechol host unit to **2a–c**. To this end, the steroid's secondary hydroxy was esterified with a 3,5-bisphosphonate-functionalized benzoic acid (Scheme 3), following almost the same HCTU/Cl-HOBt protocol, described above, to give **4a–c** (42–79%).

Alternatively, a phenylboronic acid was intended to be attached to the elongated steroid by way of a compact glycine spacer. However, although this by itself proved feasible, the subsequent key alkyne coupling could not be effected in the presence of the free boronic acid and the order of coupling steps had to be inverted, placing the introduction of the sensitive boronic acid at the end of the total synthesis.

Thus, the next step for all target molecules is the alkyne coupling, which transforms the monosteroidal lipids into disteroidal, true transmembrane units. Since they are all unsymmetric, a directed coupling procedure would be the most elegant solution. A prominent example in the literature is the palladium-catalyzed cross-coupling of alkynes with 1-iodoalkynes.[5] However, all attempts to perform the coupling step under Cadiot–Chodciewiczk conditions produced complex mixtures, which contained the desired unsymmetrical dialkynes in unacceptable low amounts (<30%). In sharp contrast, symmetrical Glaser–Hay couplings proceeded smoothly with several terminal alkynes **3** and **4**.[6] It was therefore attempted to shift the 1:2 ratio between hetero- and homodimers by employing a twofold excess of the least valuable species, whose excess could be recovered during the chromatographic purification step. This procedure was, indeed, successful and furnished the desired heterocoupled dialkynes in up to 65% isolated yields.

Scheme 4 shows the preparation of dialkynes **5a**,**b** with only one protected cysteine headgroup, which served for the consecutive construction of the boronic acid recognition unit from lithocholic acid's secondary free hydroxy group (58–65%).

Scheme 5 similarly depicts the heterodimer formation between cysteine- and bisphosphonate-carrying steroid propargyl esters **6a**,**b** (39–65%).

From **5**, the synthetic route towards the boronic acid target **10** runs quite straightforward: *N*-ethyl-*N*′-[3-(dimethylamino)propyl]carbodiimide (EDCI) assisted coupling of Fmoc-protected glycine is followed by Fmoc removal with piperidine and reductive amination with 2-formylphenylboronic acid in the presence of molecular sieves.[7] Finally, trifluoroacetic acid/triisopropylsilane completely deprotects the cysteine and releases the catechol-selective functional transmembrane unit **10b** (10% from **5b**, Scheme 6).

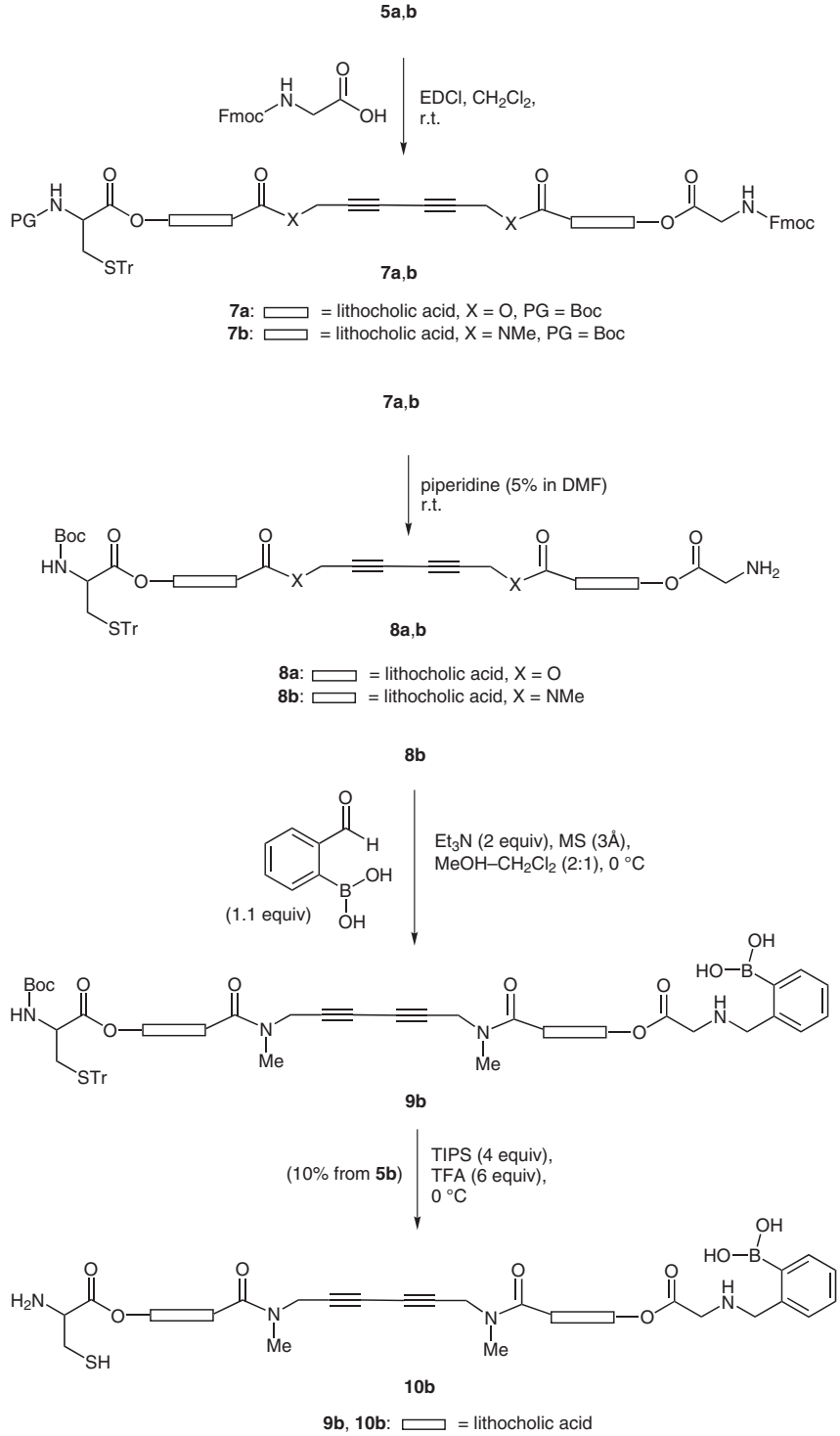
The bisphosphonate targets **12** and **13** emerge from heterodimers **6** in short sequences: it is critical to begin with phosphonate dealkylation (Scheme 7), most conveniently with S_N2 attack by lithium bromide in dipolar aprotic acetonitrile to give **11a**,**b** (99%).[8]

Subsequent acid cleavage of all cysteine protecting groups leads to bisphosphonate target **12a**,**b** with a nucleophilic thiol on one end (99%). From **11** the corresponding disulfide target **13a**,**b** can also be synthesized by an elegant short procedure (Scheme 8). Although it requires deprotection of the thiol and subsequent acid-catalyzed displacement of thiopyridine from the dimeric aldrithiol, both steps can be carried out in one pot, starting with detritylation by trifluoroacetic acid/triisopropylsilane, followed by addition of the disulfide reagent after two hours at 0 °C (99% over both steps).[9]

For all important combinations, the synthetic concept, outlined in the beginning, could be realized: Starting from well-accessible steroidal carboxylic acids, propargyl ester or *N*-methylamide formation and subsequent esterification at the opposite hydroxy end generates lipids with attached recognition and signaling moieties in protected form. The key process is a directed Glaser–Hay coupling to furnish the desired heterodimeric products in good yields. Further elaboration to the dianionic bisphosphonate[10] or free 2-(aminomethyl)phenylboronic acid head groups proceeds in a straightforward manner. A final cysteine deprotection and/or disulfide coupling generates the reactive nucleophile and its substrate in excellent yields.

Preliminary experiments with extruded unilamellar DPPC liposomes of ~200 nm diameter, carrying these transmembrane units, indeed show a reproducible increase in UV/vis absorption at 342 nm, followed by a second thiopyridine displacement after injection of a water-soluble reducing agent (sulfonated phosphine) for extracellular disulfides.

Melting points were determined on a Reichert Kofler Thermoplan melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Bruker Avance ARX-200, AMX-300, DRX-400, and DRX-500; the residual hydrogen signal of the deuterated solvent was used as reference. ^{13}C NMR spectra were obtained on the same spectrometers, ^{31}P NMR spectra on a Bruker Advance ARX-200 and ^{11}B NMR spectra on a Bruker Avance DRX-400 and DRX-500. MS (including HRMS) were obtained on a Finnegan MAT 711 (EI) and Finnegan MAT95 S (ESI). Reaction progress was monitored by analytical TLC on precoated aluminum plates (silica gel 60 F_{254}, Merck) and products were visualized by CAM reagent [$Ce(SO_4)_2$ (2 g), $(NH_4)_2MoO_4$ (50 g), concd H_2SO_4 (50 mL), H_2O (400 mL)]. The solvent systems are stated for each procedure. Column chromatography was carried out on silica gel 60 (Merck). All compounds



Scheme 6 Elaboration of the core transmembrane unit **5a**,**b** with free steroidal hydroxy group into the boronic acid building block **10b** by glycine coupling, deprotection, and reductive amination

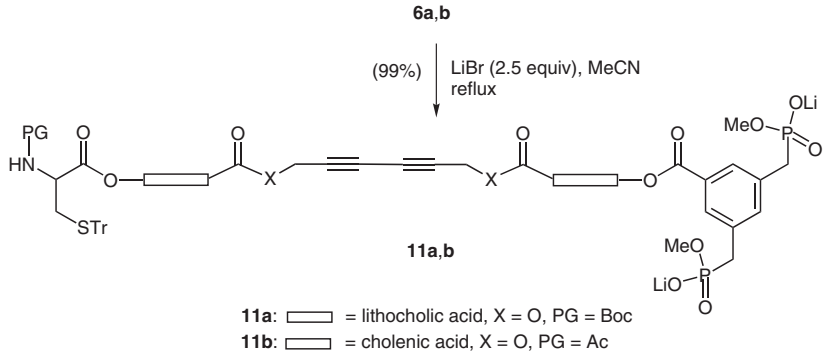
were obtained as colorless crystals with the exception of a few lightly yellow crystals.

Esterification/Amidation with Propargylic Alcohol/*N*-Methylpropargylamine; General Procedure 1

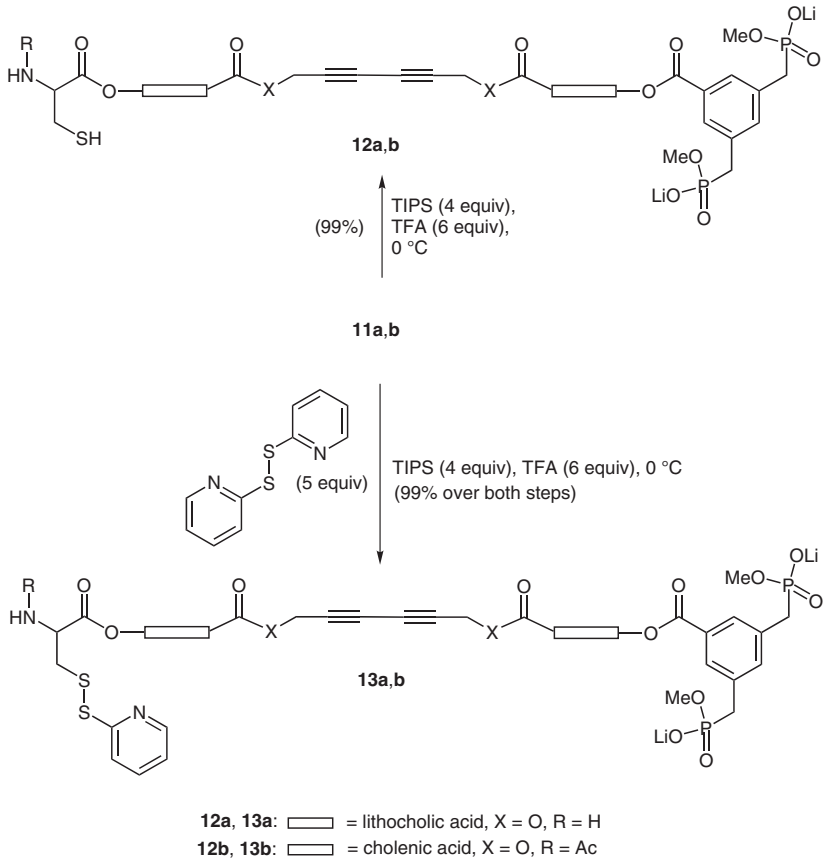
To a stirred mixture of carboxylic acid (13.28 mmol), propargylic alcohol/*N*-methylpropargylic amine (19.92 mmol), and DMAP (2.66 mmol) in CH_2Cl_2 (30 mL), was added dropwise a soln of DCC (14.61 mmol) in CH_2Cl_2 (15 mL) over 2 h and the mixture was stirred at r.t. for 16 h. The precipitate was collected by filtration and washed thoroughly with CH_2Cl_2. The solvent was evaporated and the resulting highly viscous oil was dried in vacuo. The crude product was then purified by column chromatography.

Prop-2-ynyl 4-(3-Hydroxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)pentanoate (2a)

Yield: 3.84 g (70%); mp 93 °C; R_f = 0.40 (hexane–EtOAc, 1:1).



Scheme 7 Phosphonate methyl ester cleavage in bisphosphonates **6**



Scheme 8 Final acidic trityl cleavage towards the free thiol nucleophiles **12**, optionally followed by disulfide formation to yield the S_N2 substrates **13**

^{1}H NMR ($CDCl_3$): δ = 0.64 (s, 3 H), 0.88–2.42 (m, 35 H), 2.46 (t, $^4J_{H,H}$ = 2.3 Hz, 1 H), 3.57–3.67 (m, 1 H), 4.67 (d, $^4J_{H,H}$ = 2.3 Hz, 2 H).

^{13}C NMR ($CDCl_3$): δ = 12.2, 18.4, 21.0, 23.5, 24.4, 26.6, 27.3, 28.3, 29.8, 30.7, 31.0, 31.1, 34.7, 35.5, 35.5, 36.0, 36.6, 40.3, 40.6, 42.3, 42.9, 51.9, 53.6, 56.1, 56.6, 72.0, 74.8, 78.0, 173.6.

MS (EI): *m*/*z* = 396 [$M^+ - H_2O$].

4-(3-Hydroxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)-*N*-methyl-*N*-(prop-2-ynyl)pentanamide (2b)

Yield: 4.60 g (81%); R_f = 0.17 (hexane–EtOAc, 2:1).

^{1}H NMR ($CDCl_3$): δ = 0.58 (s, 3 H), 0.86 (m, 6 H), 0.87–2.37 (m, 29 H), 2.13 (t, ^4J = 2.5 Hz, 1 H), 2.92 + 3.00 (s, 3 H), 3.50 (m, 1 H), 3.98 + 4.16 (s, 2 H).

^{13}C NMR ($CDCl_3$): δ = 12.1, 18.6, 20.9, 23.5, 24.3, 25.1, 25.7, 26.5, 27.3, 28.3, 30.2, 30.4, 30.6, 31.0, 31.3, 33.4, 34.0, 34.5, 34.6, 35.5, 35.6, 35.9, 36.2, 36.5, 39.6, 40.3, 40.5, 42.2, 42.8, 49.0, 56.1, 56.6, 71.8, 72.8, 78.3, 79.1, 173.4.

HRMS (ESI+, MeOH): *m*/*z* [M + Na]$^+$ calcd for $C_{28}H_{45}NNaO_2$: 450.3343; found: 450.3351.

Prop-2-ynyl 4-(3-Hydroxy-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)pentanoate (2c)

Yield: 4.44 g (82%); mp 81 °C; R_f = 0.21 (hexane–EtOAc, 2:1).

^{1}H NMR ($CDCl_3$): δ = 0.66 (s, 3 H), 0.91 (d, $^3J_{H,H}$ = 6.2 Hz, 3 H), 0.99 (s, 3 H), 1.00–2.43 (m, 26 H), 2.46 (t, $^4J_{H,H}$ = 2.5 Hz, 1 H), 3.42–3.58 (m, 1 H), 4.64 (d, $^4J_{H,H}$ = 2.5 Hz, 2 H), 5.32 (d, $^3J_{H,H}$ = 5.3 Hz, 1 H).

^{13}C NMR ($CDCl_3$): δ = 11.9, 18.3, 19.4, 21.1, 24.2, 28.1, 30.8, 31.0, 31.6, 31.9, 31.9, 35.3, 36.5, 37.3, 39.8, 42.3, 42.4, 50.1, 51.7, 55.8, 56.7, 71.7, 74.7, 77.8, 121.6, 140.8, 173.4.

Esterification with Acyl-Cys(Trt)-OH/5-Carboxy-*m*-xylylenebisphosphonic Acid Tetramethyl Ester; General Procedure 2

Ac-Cys(Trt)-OH, Boc-Cys(Trt)-OH, or 5-carboxy-*m*-xylylenebisphosphonic acid tetramethyl ester (1.67 mmol) was dissolved in CH_2Cl_2–DMF (2:1, 25 mL) and DIPEA (4.16 mmol) was added. The soln was cooled to 0 °C in ice water. HCTU (1.67 mmol) and Cl-HOBt (3.47 mmol) were added. The mixture was stirred at 0 °C for 10 min. The activated carboxylic acid was treated with **2a–c** (1.39 mmol). Stirring was continued for 24 h and the mixture was allowed to warm up to r.t. It was diluted with CH_2Cl_2 (10 mL) and washed with sat. aq $NaHCO_3$, 1 M AcOH, and brine. The separated organic extract was dried ($MgSO_4$). The filtrate was evaporated to dryness and the resulting crude product was purified by column chromatography.

Prop-2-ynyl 4-{3-[2-(*tert*-Butoxycarbonylamino)-3-(tritylthio)propanoyloxy]-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl}pentanoate (3a)

Yield: 1.32 g (92%); mp 75 °C; R_f = 0.51 (hexane–EtOAc, 3:1).

^{1}H NMR ($CDCl_3$): δ = 0.65 (s, 3 H), 0.92–2.43 (m, 43 H), 2.45 (t, $^4J_{H,H}$ = 2.4 Hz, 1 H), 2.50 (dd, $^2J_{H,H}$ = 11.7 Hz, $^3J_{H,H}$ = 3.9 Hz, 1 H), 2.58 (dd, $^2J_{H,H}$ = 11.8 Hz, $^3J_{H,H}$ = 5.5 Hz, 1 H), 4.24 (br s, 1 H), 4.66 (d, $^4J_{H,H}$ = 2.2 Hz, 2 H), 4.66–4.75 (m, 1 H), 5.09 (d, $^3J_{H,H}$ = 7.9 Hz, 1 H), 7.20 (t, $^3J_{H,H}$ = 7.3 Hz, 3 H), 7.27 (t, $^3J_{H,H}$ = 7.5 Hz, 6 H), 7.39 (d, $^3J_{H,H}$ = 7.7 Hz, 6 H).

^{13}C NMR ($CDCl_3$): δ = 12.2, 18.4, 21.0, 23.4, 24.3, 26.4, 26.5, 27.1, 28.3, 28.4, 31.0, 31.1, 32.2, 34.5, 34.7, 35.0, 35.4, 35.9, 40.3, 40.6, 42.0, 42.9, 51.9, 52.8, 56.2, 56.7, 66.7, 74.8, 76.0, 77.9, 79.9, 126.9, 128.1, 129.6, 144.5, 155.1, 170.3, 173.4.

HRMS (ESI+, MeOH): *m*/*z* [M + Na]$^+$ calcd for $C_{54}H_{69}NNaO_6S$: 882.4738; found: 882.4730.

4-{3-[2-(*tert*-Butoxycarbonylamino)-3-(tritylthio)propanoyloxy]-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl}-*N*-methyl-*N*-(prop-2-ynyl)pentanamide (3b)

Yield: 0.88 g (60%); R_f = 0.1 (hexane–EtOAc, 3:1).

^{1}H NMR ($CDCl_3$): δ = 0.59 (s, 3 H), 0.85 (m, 6 H), 0.84–2.55 (m, 27 H), 1.37 (s, 12 H), 2.13 (t, ^4J = 2.5 Hz, 1 H), 2.92 + 3.00 (s, 3 H), 3.97 + 4.15 (s, 2 H), 4.21 (m, 1 H), 4.67 (m, 1 H), 5.03 (d, ^3J = 6.3 Hz, 1 H), 7.08–7.36 (m, 15 H).

^{13}C NMR ($CDCl_3$): δ = 12.2, 14.3, 18.6, 21.0, 23.4, 24.3, 26.4, 26.5, 27.1, 28.4, 30.3, 30.5, 31.1, 31.4, 32.2, 33.4, 34.5, 34.7, 35.0, 35.7, 35.9, 36.3, 39.6, 40.3, 40.6, 42.0, 42.9, 52.7, 56.3, 56.7, 71.7, 72.8, 76.0, 79.1, 79.9, 126.9, 128.1, 129.6, 144.5, 155.1, 170.3, 173.4.

HRMS (ESI+, MeOH): *m*/*z* [M + Na]$^+$ calcd for $C_{55}H_{72}N_2NaO_5S$: 895.5054; found: 895.5039.

Prop-2-ynyl 4-{3-[2-Acetamido-3-(tritylthio)propanoyloxy]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetrahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl}pentanoate (3c)

Following general procedure 2 using Ac-Cys(Trt)-OH (1.22 mmol) and the respective relative amounts of DIPEA (4.58 mmol), HCTU (1.68 mmol) and Cl-HOBt (3.36 mmol) as well as **2c** (1.53 mmol); yield: 0.56 g (58%); mp 74 °C; R_f = 0.58 (hexane–EtOAc, 3:1).

^{1}H NMR ($CDCl_3$): δ = 0.71 (s, 3 H), 0.80–2.47 (m, 35 H), 2.49 (t, $^4J_{H,H}$ = 2.5 Hz, 1 H), 2.55 (dd, $^2J_{H,H}$ = 12.3 Hz, $^3J_{H,H}$ = 4.5 Hz, 1 H), 2.70 (dd, $^2J_{H,H}$ = 12.3 Hz, $^3J_{H,H}$ = 2.1 Hz, 1 H), 4.55–4.67 (m, 1 H), 4.69 (d, ^4J = 2.5 Hz, 2 H), 5.40 (t, $^3J_{H,H}$ = 6.8 Hz, 1 H), 6.01 (d, $^3J_{H,H}$ = 6.6 Hz, 1 H), 7.20–7.35 (m, 9 H), 7.42 (d, $^3J_{H,H}$ = 8.1 Hz, 6 H).

^{13}C NMR ($CDCl_3$): δ = 12.1, 18.5, 19.5, 21.2, 23.3, 24.4, 27.8, 27.8, 28.3, 31.1, 31.2, 32.1, 34.3, 35.5, 36.8, 37.1, 38.0, 38.1, 39.9, 42.6, 50.2, 51.4, 51.9, 56.0, 56.9, 67.0, 74.9, 75.9, 78.1, 123.1, 127.1, 128.2, 129.7, 139.5, 144.6, 169.7, 170.1, 173.5.

HRMS (ESI+, MeOH): *m*/*z* [M + Na]$^+$ calcd for $C_{51}H_{61}NNaO_5S$: 822.4163; found: 822.4180.

10,13-Dimethyl-17-[1-methyl-4-oxo-4-(prop-2-ynyloxy)butyl]-hexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl 3,5-Bis[(dimethoxyphosphoryl)methyl]benzoate (4a)

Yield: 0.72 g (68%); mp 87 °C; R_f = 0.24 (CH_2Cl_2–MeOH, 19:1).

^{1}H NMR ($CDCl_3$): δ = 0.64 (s, 3 H), 0.90 (d, $^3J_{H,H}$ = 6.4 Hz, 3 H), 0.93 (s, 3 H), 1.00–2.41 (m, 28 H), 2.45 (t, $^4J_{H,H}$ = 2.1 Hz, 1 H), 3.18 (d, $^2J_{H,P}$ = 21.8 Hz, 4 H), 3.67 (d, $^3J_{H,P}$ = 10.8 Hz, 12 H), 4.64 (s, 2 H), 4.88–4.97 (m, 1 H), 7.43 (s, 1 H), 7.82 (s, 2 H).

^{13}C NMR ($CDCl_3$): δ = 12.2, 18.4, 21.0, 23.4, 24.3, 26.4, 26.8, 27.2, 28.3, 31.0, 31.1, 32.7 (d, $^1J_{C,P}$ = 138.4 Hz), 32.4, 34.7, 35.2, 35.4, 35.9, 40.2, 40.6, 42.1, 42.9, 51.8, 53.1 (d, $^2J_{C,P}$ = 7.1 Hz), 56.1, 56.5, 74.8, 75.4, 78.0, 129.6–129.7 (m, 1 C), 131.9, 132.2–132.3 (m, 1 C), 135.2–135.3 (m, 1 C), 165.6, 173.4.

^{31}P NMR ($CDCl_3$): δ = 28.6.

HRMS (ESI+, MeOH): *m*/*z* [M + Na]$^+$ calcd for $C_{40}H_{60}NaO_{10}P_2$: 785.3554; found: 785.3583.

10,13-Dimethyl-17-{1-methyl-4-[methy](prop-2-ynyl)amino]-4-oxobutyl}-hexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl 3,5-Bis[(dimethoxyphosphoryl)methyl]benzoate (4b)

Yield: 0.85 g (79%); mp 92 °C; R_f = 0.16 (CH_2Cl_2–MeOH, 19:1).

^{1}H NMR ($CDCl_3$): δ = 0.57 (s, 3 H,), 0.84 (m, 6 H), 0.90–0.98 (m, 1 H), 1.00–1.21 (m, 8 H), 1.23–1.42 (m, 9 H), 1.44–1.47 (m, 1 H), 1.60–1.63 (m, 1 H), 1.66–1.88 (m, 5 H), 1.90–1.93 (m, 1 H), 2.14–2.21 (m, 1 H), 2.14 (t, $^4J_{HH}$ = 2.47 Hz, 1 H), 2.23–2.39 (m, 1 H), 2.89 (s, 1 H), 2.98 (s, 2 H), 3.12 (d, $^2J_{PH}$ = 21.8 Hz, 4 H), 3.59 (d, $^2J_{PH}$ = 10.8 Hz, 12 H), 3.96 (m, 2 H,), 4.80–4.89 (m, 1 H), 7.41 (s, 1 H), 7.73 (s, 2 H).

^{13}C NMR ($CDCl_3$): δ = 11.9, 18.4, 20.8, 23.2, 24.1, 26.2, 26.9, 28.1, 29.9, 30.1, 30.8, 31.3, 32.1, 33.2 (d, $^1J_{PC}$ = 115.3 Hz), 33.3, 34.3, 34.5, 35.0, 35.3, 35.7, 36.0, 38.5, 39.3, 39.9, 40.3, 41.8, 42.6, 52.9 (d, $^2J_{PC}$ = 6.1 Hz), 55.9, 56.3, 71.6, 72.7, 75.1, 78.1, 78.8, 129.4, 131.6, 131.9, 135.1, 165.6, 173.2.

^{31}P NMR ($CDCl_3$): δ = 28.6.

HRMS (ESI+, MeOH): *m*/*z* [M + Na]$^+$ calcd for $C_{41}H_{63}NNaO_9P_2$: 798.3876; found: 798.3881.

10,13-Dimethyl-17-[1-methyl-4-oxo-4-(prop-2-ynyloxy)butyl]-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl 3,5-Bis[(dimethoxyphosphoryl)-methyl]benzoate (4c)

Yield: 0.45 g (42%); mp 136 °C; R_f = 0.24 (CH_2Cl_2–MeOH, 19:1).

^{1}H NMR ($CDCl_3$): δ = 0.66 (s, 3 H), 0.91 (d, $^3J_{H,H}$ = 6.6 Hz, 3 H), 0.93–2.47 (m, 29 H), 3.16 (d, $^2J_{H,P}$ = 21.8 Hz, 4 H), 3.66 (d, $^3J_{H,P}$ = 10.8 Hz, 12 H), 4.64 (d, $^4J_{H,H}$ = 2.2 Hz, 2 H), 4.76–4.86 (m, 1 H), 5.38 (d, $^3J_{H,H}$ = 4.4 Hz, 1 H), 7.43 (s, 1 H), 7.81 (s, 2 H).

^{13}C NMR ($CDCl_3$): δ = 11.9, 18.4, 19.4, 21.1, 24.3, 27.9, 28.2, 30.9, 31.0, 31.9, 32.0, 32.7 (d, $^1J_{C,P}$ = 138.3 Hz), 35.4, 36.7, 37.1, 38.3, 39.8, 42.5, 50.1, 51.8, 53.0 (d, $^2J_{C,P}$ = 7.4 Hz), 55.8, 56.7, 74.8, 74.9, 77.9, 122.8, 129.6–129.6 (m, 1 C), 131.7–131.8 (m, 1 C), 132.2–132.3 (m, 1 C), 135.3, 139.7, 165.4, 173.4.

^{31}P NMR ($CDCl_3$): δ = 28.6.

HRMS (ESI+, MeOH): *m/z* [M + Na]$^+$ calcd for $C_{40}H_{58}NaO_{10}P_2$: 783.33974; found: 783.33953.

Glaser–Hay Coupling; General Procedure 3

The acetylene compounds and CuCl (exact amounts see below) were dissolved/suspended in CH_2Cl_2 (25 mL). TMEDA was added to the stirred mixture, resulting in slow dissolution of all CuCl. Upon completion, the atmosphere above the green soln was saturated with O_2 (balloon). Stirring was continued at r.t. for 6 h, until a deep blue color was reached. The reaction was quenched with sat. aq NH_4Cl. The organic soln was subsequently washed with sat. aq NH_4Cl (3 ×), the separated organic extract was dried ($MgSO_4$), and the solvent was evaporated in vacuo. The slightly green crude product was purified by column chromatography.

6-[4-(3-Hydroxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)pentanoyloxy]hexa-2,4-diynyl 4-{3-[2-(*tert*-Butoxycarbonylamino)-3-(tritylthio)propanoyloxy]-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl}pentanoate (5a)

Following general procedure 3 using **2a** (0.21 mmol), **3a** (0.11 mmol), CuCl (0.80 mmol), and TMEDA (0.8 mmol); yield: 0.09 g (65%); R_f = 0.14 (hexane–EtOAc, 3:1).

^{1}H NMR ($CDCl_3$): δ = 0.63 (s, 3 H), 0.65 (s, 3 H), 0.91 (m, 12 H), 0.84–2.00 (m, 53 H), 1.43 (d, ^3J = 8.02 Hz, 9 H), 2.25–2.41 (m, 4 H), 2.51–2.56 (m, 2 H), 3.62 (m, 1 H), 4.25 (m, 1 H), 4.73 (m, 1 H), 4.74 (s, 4 H), 5.09 (d, ^3J = 6.87 Hz, 1 H), 7.21 (t, ^3J = 7.22 Hz, 3 H), 7.28 (t, ^3J = 8.36 Hz, 6 H), 7.39 (d, ^3J = 7.79 Hz, 6 H).

17-{4-[(6-{[4-(3-Hydroxy-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)pentanoyl](methyl)amino}hexa-2,4-diynyl)(methyl)amino]-1-methyl-4-oxobutyl}-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl 2-(*tert*-Butoxycarbonylamino)-3-(tritylthio)propanoate (5b)

Following general procedure 3 using **2b** (0.39 mmol), **3b** (0.39 mmol), CuCl (1.94 mmol), and TMEDA (1.94 mmol); yield: 0.29 g (58%); R_f = 0.12 (hexane–EtOAc, 1:1).

^{1}H NMR ($CDCl_3$): δ = 0.57 (s, 6 H), 0.84 (m, 12 H), 0.83–2.55 (m, 68 H), 2.89 + 2.98 (s, 6 H), 3.53 (m, 1 H), 4.04 + 4.21 (s, 4 H), 4.17 (m, 1 H), 4.66 (m, 1 H), 5.08 (d, ^3J = 8.3 Hz, 1 H), 7.08–7.36 (d, ^3J = 6.8 Hz, 15 H).

^{13}C NMR ($CDCl_3$): δ = 12.1, 14.3, 18.6, 19.8, 20.9, 21.0, 21.1, 21.4, 23.4, 23.5, 24.3, 26.4, 26.5, 27.1, 27.3, 28.3, 28.4 30.2, 30.4, 30.6, 31.0, 31.3, 32.2, 33.6, 34.4, 34.7, 35.1, 35.0, 35.5, 35.9, 36.5,36.9, 40.3, 40.5, 41.9, 42.1, 42.8, 52.7, 56.1, 56.3, 56.4, 56.6, 60.5, 66.7, 67.4, 67.9, 69.0, 71.8, 72.5, 73.7, 74.8, 76.0, 77.5, 78.0, 125.6, 125.8, 126.9, 127.9, 128.2, 128.4, 129.0, 129.6, 130.0, 137.8, 144.5, 155.1, 170.3, 173.4.

HRMS (ESI+, MeOH): *m/z* [M + Na]$^+$ calcd for $C_{83}H_{115}N_3NaO_7S$: 1320.8348; found: 1320.8342.

17-{4-[6-(4-{3-[2-(*tert*-Butoxycarbonylamino)-3-(tritylthio)propanoyloxy]-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl}pentanoyloxy)hexa-2,4-diynyloxy]-1-methyl-4-oxobutyl}-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl 3,5-Bis[(dimethoxyphosphoryl)methyl]benzoate (6a)

Following general procedure 3 using **3a** (0.05 mmol), **4a** (0.05 mmol), CuCl (0.25 mmol), and TMEDA (0.25 mmol); yield: 0.05 g (65%); R_f = 0.05 (CH_2Cl_2–MeOH, 19:1).

^{1}H NMR ($CDCl_3$): δ = 0.67 (s, 3 H), 0.67 (s, 3 H), 0.94–2.45 (m, 76 H), 2.53 (dd, $^2J_{H,H}$ = 21.8 Hz, $^3J_{H,H}$ = 4.1 Hz, 1 H), 2.60 (dd, $^2J_{H,H}$ = 21.9 Hz, $^3J_{H,H}$ = 5.3 Hz, 1 H), 3.22 (d, $^2J_{H,P}$ = 21.9 Hz, 4 H), 3.71 (d, $^3J_{H,P}$ = 10.8 Hz, 12 H), 4.26 (br s, 1 H), 4.71–4.78 (m, 5 H), 4.97 (m, 1 H), 5.11 (d, $^3J_{H,H}$ = 7.6 Hz, 1 H), 7.22 (t, $^3J_{H,H}$ = 7.1 Hz, 3 H), 7.29 (t, $^3J_{H,H}$ = 7.2 Hz, 6 H), 7.41 (d, $^3J_{H,H}$ = 7.6 Hz, 6 H), 7.47 (s, 1 H), 7.85 (s, 2 H).

^{13}C NMR ($CDCl_3$): δ = 12.2, 18.5, 21.1, 21.1, 23.5, 23.5, 24.4, 26.5, 26.6, 26.9, 27.2, 27.2, 28.4, 28.5, 31.1, 31.1, 31.1, 32.8 (d, $^1J_{C,P}$ = 138.4 Hz), 32.5, 34.6, 34.8, 34.8, 35.1, 35.3, 35.5, 36.0, 36.0, 40.3, 40.4, 40.7, 42.1, 42.2, 43.0, 52.2, 52.9, 53.1 (d, $^2J_{C,P}$ = 6.1 Hz), 56.2, 56.3, 56.6, 56.7, 66.8, 70.4, 70.4, 73.9, 74.0, 75.5, 76.1, 80.0, 127.0, 128.1, 129.7, 132.0, 132.3–132.4 (m, 1C), 135.3–135.4 (m, 1C), 144.6, 155.2, 165.7, 170.4, 173.3, 173.3.

^{31}P NMR ($CDCl_3$): δ = 28.6.

HRMS (ESI+, MeOH): *m/z* [M + H]$^+$ calcd for $C_{94}H_{128}NO_{16}P_2S$:1621.84571; found: 1621.85069.

17-{4-[6-(4-{3-[2-Acetamido-3-(tritylthio)propanoyloxy]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl}pentanoyloxy)hexa-2,4-diynyloxy]-1-methyl-4-oxobutyl}-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl 3,5-Bis[(dimethoxyphosphoryl)methyl]benzoate (6b)

Following general procedure 3 using **3c** (0.08 mmol), **4c** (0.08 mmol), CuCl (0.61 mmol), and TMEDA (0.61 mmol); yield: 0.05 g (39%); R_f = 0.08 (CH_2Cl_2–MeOH, 19:1).

^{1}H NMR ($CDCl_3$): δ = 0.70 (s, 6 H), 0.71 (s, 6 H), 0.88–2.51 (m, 59 H), 2.55 (dd, $^2J_{H,H}$ = 12.3 Hz, $^3J_{H,H}$ = 4.9 Hz, 1 H), 2.60 (ddd, $^2J_{H,H}$ = 12.3 Hz, $^3J_{H,H}$ = 5.6 Hz, $^3J_{H,H}$ = 2.5 Hz, 1 H), 3.22 (d, $^2J_{H,P}$ = 21.8 Hz, 4 H), 3.71 (d, $^3J_{H,P}$ = 10.9 Hz, 12 H), 4.55–4.69 (m, 2 H), 4.74 (s, 4 H), 4.80–4.92 (m, 1 H), 5.36–5.46 (m, 2 H), 6.06 (d, $^3J_{H,H}$ = 7.7 Hz, 1 H), 7.23 (t, $^3J_{H,H}$ = 7.4 Hz, 3 H), 7.29 (t, $^3J_{H,H}$ = 7.4 Hz, 6 H), 7.40 (d, $^3J_{H,H}$ = 8.4 Hz, 6 H), 7.47 (s, 1 H), 7.86 (s, 2 H).

^{13}C NMR ($CDCl_3$): δ = 11.9, 18.4, 19.4, 21.1, 24.3, 27.9, 28.2, 30.9, 31.0, 31.9, 32.0, 32.7 (d, $^1J_{C,P}$ = 138.3 Hz), 35.4, 36.7, 37.1, 38.3, 39.8, 42.5, 50.1, 51.8, 53.0 (d, $^2J_{C,P}$ = 7.4 Hz), 55.8, 56.7, 74.8, 74.9, 77.9, 122.8, 129.6–129.6 (m, 1 C), 131.7–131.8 (m, 1 C), 132.2–132.3 (m, 1 C), 135.3, 139.7, 165.4, 173.4.

^{31}P NMR ($CDCl_3$): δ = 28.9.

HRMS (ESI+, MeOH): *m/z* [M + Na]$^+$ calcd for $C_{91}H_{117}NO_{15}P_2SNa$: 1580.7511; found: 1580.7467.

Esterification with Fmoc-Gly-OH; General Procedure 4

Fmoc-Gly-OH (0.15 mmol) and the hydroxy compounds (0.1 mmol) were dissolved in CH_2Cl_2 (25 mL). A soln of EDCl (0.125 mmol) in CH_2Cl_2 (15 mL) was added dropwise over a period of 6 h. The mixture was stirred at r.t. for 16 h. The clear soln was then washed H_2O (2 ×) and the aqueous phase was extracted with CH_2Cl_2. The combined organic extracts were dried ($MgSO_4$) and the solvent evaporated in vacuo. The crude product was purified by column chromatography.

6-(4-{3-[2-(*tert*-Butoxycarbonylamino)-3-(tritylthio)propanoyloxy]-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl}pentanoyloxy)hexa-2,4-diynyl 4-(3-{2-[(9*H*-Fluoren-9-ylmethoxy)carbonylamino]acetoxy}-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)pentanoate (7a)

Yield: 0.09 g (55%); R_f = 0.1 (hexane–EtOAc, 3:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.63 + 0.66 (s, 6 H), 0.92 (m, 12 H), 0.93–2.58 (m, 68 H), 3.96 (d, ^3J = 5.7 Hz, 2 H), 4.25 (t, ^3J = 7.1 Hz, 1 H), 4.38 (d, ^3J = 7.6 Hz, 2 H), 4.74 (s, 4 H) 4.82 (m, 2 H), 5.10 (d, ^3J = 7.9 Hz, 1 H), 5.33 (t, ^3J = 5.4 Hz, 1 H), 7.27–7.76 (m, ^3J = 7.6 Hz, 23 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.3, 18.5, 21.1, 21.1, 23.5, 24.4, 26.5, 26.6, 26.7, 27.2, 28.6, 29.9, 31.1, 31.2, 32.4, 34.6, 34.8, 35.2, 35.5, 36.0, 36.1, 40.3, 40.4, 40.7, 42.2, 43.0, 43.3, 47.2, 52.2, 52.9, 56.2, 56.3, 56.6, 56.8, 66.9, 67.5, 70.5, 74.0, 76.2, 77.5, 120.3, 125.4, 127.0, 127.3, 128.0, 128.2, 129.8, 169.7, 170.4, 173.4.

17-(4-[(6-{[4-(3-{2-[(9*H*-Fluoren-9-ylmethoxy)carbonylamino]acetoxy}-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)pentanoyl](methyl)amino}hexa-2,4-diynyl)(methyl)amino]-1-methyl-4-oxobutyl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl 2-(*tert*-Butoxycarbonylamino)-3-(tritylthio)propanoate (7b)

Yield: 0.13 g (85%); R_f = 0.3 (hexane–EtOAc, 1:1).

^{1}H NMR ($CDCl_3$): δ = 0.56 (s, 6 H), 0.83–2.55 (m, 82 H, steroid), 2.89 + 2.97 (s, 6 H), 3.30–4.00 (m, 4 H), 4.04 + 4.22 (s, 4 H), 4.30 (m, 2 H), 4.71 (m, 2 H), 5.06 (d, ^3J = 8.3 Hz, 1 H), 5.39 (t, ^3J = 5.0 Hz, 1 H), 7.08–7.72 (m, 19 H).

^{13}C NMR ($CDCl_3$): δ = 12.1, 14.3, 18.6, 20.9, 21.1, 23.4, 24.2, 26.3, 26.6, 27.0, 28.4, 30.4, 31.0, 32.2, 34.4, 34.6, 34.7, 34.9, 35.6, 35.8, 36.9, 40.2, 40.4, 41.9, 42.7, 43.1, 47.2, 52.7, 56.1, 56.3, 56.6, 60.4, 66.6, 67.2, 67.9, 73.7, 75.9, 77.2, 79.9, 120.1, 125.2, 126.8, 127.1, 127.8, 128.1, 129.5, 141.3, 143.9, 144.4, 155.0, 156.4, 169.5, 170.2, 171.2, 173.3.

HRMS (ESI+, MeOH): *m*/*z* [M + Na]$^+$ calcd for $C_{100}H_{128}N_4NaO_{10}S$:1600.9277; found: 1600.9275.

Fmoc Deprotection; General Procedure 5

The Fmoc-protected amine (0.09 mmol) was dissolved in DMF (5 mL). Piperidine (0.2 mL) was added at r.t. Stirring was continued for 20 min. The mixture was diluted with the same amount of CH_2Cl_2 and piperidine was extracted with 0.001 M aq HCl. The organic phase was dried ($MgSO_4$), the solvent was evaporated in vacuo and the crude product was purified by column chromatography.

6-(4-{3-[2-(*tert*-Butoxycarbonylamino)-3-(tritylthio)propanoyloxy]-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl}pentanoyloxy)hexa-2,4-diynyl 4-[3-(2-Aminoacetoxy)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl]pentanoate (8a)

Yield: 0.11 g (92%); R_f = 0.1 (hexane–EtOAc, 1:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.62 (s, 6 H), 0.90 (m, 12 H), 0.93–2.56 (m, 57 H), 1.42 (s, 12 H), 3.37 (s, 2 H), 4.24 (m, 1 H), 4.71 (s, 4 H), 4.72 (m, 2 H), 5.12 (d, ^3J = 8.0 Hz, 1 H), 7.10–7.38 (m, ^3J = 7.0 Hz, 15 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 12.1, 18.4, 21.0, 23.4, 24.3, 26.4, 27.1, 28.3, 28.4, 31.0, 32.2, 32.4, 34.5, 34.7, 35.1, 35.4, 35.9, 40.3, 40.6, 42.0, 42.9, 52.1, 52.7, 56.1, 56.2, 56.7, 58.3, 66.7, 70.4, 73.9, 75.3, 76.0, 77.4, 80.0, 126.9, 128.1, 129.6, 144.5, 155.1, 170.3, 173.3.

17-[4-([6-({4-[3-(2-Aminoacetoxy)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl]pentanoyl}(methyl)amino)hexa-2,4-diynyl](methyl)amino)-1-methyl-4-oxobutyl]-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl 2-(*tert*-Butoxycarbonylamino)-3-(tritylthio)propanoate (8b)

Yield: 0.12 g (98%); R_f = 0.08 (CH_2Cl_2–MeOH, 30:1).

^{1}H NMR ($CDCl_3$): δ = 0.69 (s, 6 H), 0.85–2.65 (m, 80 H), 3.02 + 3.11 (s, 6 H), 3.62 (s, 2 H), 4.22 + 4.34 (s, 4 H), 4.30 (m, 1 H), 4.67 (m, 2 H), 4.78 (m, 1 H), 5.16 (d, ^3J = 5.1 Hz, 1 H), 7.17–7.76 (m, 15 H).

^{13}C NMR ($CDCl_3$): δ = 12.2, 18.6, 21.0, 23.4, 24.4, 25.1, 26.5, 26.6, 26.8, 27.2, 28.5, 29.9, 30.5, 31.1, 32.2, 32.4, 34.1, 34.5, 34.7, 35.1, 35.2, 35.7, 35.9, 37.0, 40.3, 40.3, 40.6, 42.1, 42.9, 44.4, 52.8, 53.5, 53.5, 55.1, 56.3, 56.4, 56.6, 56.7, 66.7, 73.8, 75.2, 76.1, 126.9, 128.1, 129.7, 170.3, 173.4.

2-[(2-{17-[4-({6-[(4-{3-[(2-(*tert*-Butoxycarbonylamino)-3-(tritylthio)propanoyloxy]-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl}pentanoyl)(methyl)amino]hexa-2,4-diynyl}(methyl)amino)-1-methyl-4-oxobutyl]-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yloxy}-2-oxoethylamino)methyl]phenylboronic Acid (9b)

A mixture of the amine **8b** (0.04 mmol), 2-formylphenylboronic acid (0.04 mmol), Et_3N (0.08 mmol), and molecular sieves (3 Å) were dissolved in dry MeOH. The mixture was stirred at r.t. for 3 h, $NaB(OAc)_3H$ (0.24 mmol) was added to the soln. Stirring was continued for 1 h. Subsequently, the mixture was filtrated through Celite. The filtrate was evaporated under reduced pressure and dried in vacuo. The residue was washed several times with hot MeCN in order to remove traces of less polar byproducts; yield: 0.02 g (34%); R_f = 0.28 (CH_2Cl_2–MeOH, 9:1).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.66 (s, 6 H), 0.93 (m, 12 H), 0.80–2.59 (m, 58 H), 1.44 (s, 12 H), 2.97 + 3.09 (s, 6 H), 3.87–4.29 (m, 8 H), 4.73 + 4.83 (s, 2 H), 5.11 (d, ^3J = 8.1 Hz, 1 H), 7.10–7.79 (m, ^3J = 7.8 Hz, 15 H).

^{13}C NMR (100 MHz, $CDCl_3$): δ = 1.1, 11.1, 11.4, 12.2, 14.2, 16.2, 18.7, 21.0, 21.6, 22.6, 22.8, 23.1, 23.4, 23.5, 23.9, 24.3, 25.9, 26.4, 26.6, 26.7, 27.1, 27.3, 28.0, 28.4, 29.0, 29.5, 29.8, 30.4, 30.5, 30.7, 31.1, 31.4, 32.2, 32.6, 33.7, 34.5, 34.7, 35.1, 35.5, 35.7, 35.9, 36.6, 37.0, 38.9, 40.3, 40.5, 40.6, 42.0, 42.2, 42.9, 48.5, 52.8, 54.1, 56.2, 56.4, 56.5, 56.6, 56.7, 63.1, 66.7, 67.2, 67.5, 73.8, 76.0, 76.5, 80.0, 126.9, 128.1, 129.6, 144.5, 155.1, 170.3, 173.4.

HRMS (ESI+, MeOH–CH_2Cl_2): *m*/*z* (M^+ – H_2O) calcd for $C_{98}H_{128}N_4BO_{10}S$: 1563.9439; found: 1563.9401.

Cleavage of Phosphonic Acid Methyl Esters; General Procedure 6

The respective bisphosphonic acid tetramethyl ester (0.12 mmol) was dissolved in MeCN (25 mL) under argon. Dry LiBr (0.27 mmol) was added and the mixture was refluxed for 2–4 h. The product appeared as a colorless precipitate. The solvent was decanted carefully and the product was washed MeCN (2 ×) and Et_2O (3 ×). The colorless pure products were dried in vacuo.

17-(4-[6-(4-{3-[2-(*tert*-Butoxycarbonylamino)-3-(tritylthio)propanoyloxy]-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl}pentanoyloxy)hexa-2,4-diynyloxy]-1-methyl-4-oxobutyl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl 3,5-Bis{[hydroxy(methoxy)phosphoryl]methyl}benzoate Dilithium Salt (11a)

Yield: 0.19 g (99%); mp >300 °C.

^{1}H NMR (MeOH-d_4–$CDCl_3$): δ = 0.58 (s, 6 H), 0.71–2.58 (m, 80 H), 2.93 (d, $^2J_{H,P}$ = 20.8 Hz, 4 H), 3.45 (d, $^3J_{H,P}$ = 10.5 Hz, 6 H), 4.05–4.18 (m, 1 H), 4.50–4.65 (m, 6 H), 7.03–7.38 (m, 15 H), 7.59 (s, 3 H).

^{13}C NMR (MeOH-d_4–$CDCl_3$): δ = 11.3, 17.4, 17.5, 20.3, 22.4, 22.6, 23.6, 25.8, 26.1, 26.4, 26.5, 27.4, 27.6, 30.3, 30.4, 30.4, 31.5, 32.4 (d, $^1J_{C,P}$ = 115.3 Hz), 33.4, 34.0, 34.1, 34.3, 34.3, 34.5, 34.8, 35.4, 35.4, 39.7, 39.8, 40.0, 41.4, 41.6, 42.3, 50.8 (d, $^2J_{C,P}$ = 6.1 Hz), 51.4, 51.4, 52.7, 55.6, 55.6, 56.0, 56.2, 66.4, 69.3, 69.4, 73.2, 73.2, 74.6, 75.5, 77.3, 79.4, 126.3, 127.1, 127.4, 127.6, 129.0, 129.7, 135.4, 144.0, 155.4, 166.5, 170.2, 173.1.

^{31}P NMR (MeOH-d_4–$CDCl_3$): δ = 25.0.

HRMS (ESI–, MeOH): *m*/*z* [M]$^-$ calcd for $C_{92}H_{121}NO_{16}P_2S$: 795.3952; found: 795.3945.

17-{4-[6-(4-{3-[2-Acetamido-3-(tritylthio)propanoyloxy]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl}pentanoyloxy)hexa-2,4-diynyloxy]-1-methyl-4-oxobutyl}-10,13-dimethyl-2,3,4,7,8,9,10,11, 12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl 3,5-Bis{[hydroxy(methoxy)phosphoryl]methyl}benzoate Dilithium Salt (11b)
Yield: 0.19 g (99%); mp >300 °C.

^{1}H NMR (MeOH-d_4–$CDCl_3$): δ = 0.51 (s, 6 H), 0.62–2.54 (m, 69 H), 2.80 (d, $^2J_{H,P}$ = 20.3 Hz, 4 H), 3.36 (d, $^3J_{H,P}$ = 10.2 Hz, 6 H), 4.37 (br s, 1 H), 4.46–4.67 (m, 5 H), 5.16–5.26 (m, 2 H), 7.03–7.24 (m, 15 H), 7.41 (s, 1 H), 7.51 (s, 2 H).

^{13}C NMR (MeOH-d_4–$CDCl_3$): δ = 12.1, 15.2, 18.5, 19.6, 21.4, 22.6, 24.6, 27.9, 28.2, 28.4, 31.2, 31.3, 32.2, 34.0, 34.2 (d, $^1J_{C,P}$ = 130.2 Hz), 35.7, 35.7, 36.9, 37.0, 37.2, 37.4, 38.2, 38.5, 40.1, 40.1, 42.8, 50.4, 50.5, 51.7 (d, $^2J_{C,P}$ = 5.5 Hz), 52.2, 52.4, 56.1, 57.0, 57.1, 66.3, 67.3, 70.5, 74.1, 75.1, 76.1, 123.1, 123.3, 127.2, 128.3, 129.9, 130.6, 136.0–136.1 (m, 1C), 136.3, 139.7–139.7 (m, 1C), 140.0, 144.7, 167.2, 170.5, 171.3, 171.5, 174.0.

^{31}P NMR (MeOH-d_4–$CDCl_3$): δ = 25.4.

HRMS (ESI–, MeOH): *m*/*z* [M]$^-$ calcd for $C_{89}H_{112}NO_{15}P_2S$: 1528.7222; found: 1528.7200.

Boc/Trityl Deprotection; General Procedure 7
The Boc- or trityl-protected starting material (0.1 mmol) and *i*-Pr_3SiH (0.2 mmol) were dissolved in CH_2Cl_2–TFA (1:1, 10 mL). The color of the soln immediately changed to a dark yellow. The mixture was stirred at r.t. for 10 min, at which point the yellow color had disappeared and stirring was continued for a further 4 h. The mixture was diluted with the same amount of toluene and the solvents were evaporated in vacuo; this procedure was repeated three times. Final purification was achieved by two different procedures, depending on the nature of the bisphosphonate ester groups: The dried lithium salts were extracted several times with Et_2O in order to remove Ph_3CH. After this operation the product was dried in vacuo. The neutral esters were purified by column chromatography.

1-(17-{4-[(6-{[4-(3-{2-[2-(Dihydroxyboryl)benzylamino]acetoxy}-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl)pentyl](methyl)amino}hexa-2,4-diynyl)(methyl)amino]-1-methyl-4-oxobutyl}-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yloxy)-3-mercapto-1-oxopropan-2-aminium Trifluoroacetate (10b)
Following general procedure 7 using **9b** (0.1 mmol); yield: 0.033 g (33%).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 0.64 (s, 6 H), 0.91 (m, 12 H), 0.85–2.80 (m, 58 H), 2.97 + 3.06 (s, 6 H), 3.63 (m, 1 H), 4.10–4.45 (m, 8 H), 4.85 + 4.95 (m, 2 H), 7.10–7.64 (m, 4 H).

HRMS (ESI+, MeOH–CH_2Cl_2): *m*/*z* (M^+ + Na + 2 MeOH – H_2O) calcd for $C_{70}H_{107}N_4NaBO_8S$: 1197.7800; found: 1197.7807.

1-{17-[4-(6-{4-[3-(3,5-Bis{[hydroxy(methoxy)phosphoryl]-methyl}benzoyloxy)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl]pentanoyloxy}hexa-2,4-diynyloxy)-1-methyl-4-oxobutyl]-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yloxy}-3-mercapto-1-oxopropan-2-aminium Trifluoroacetate Dilithium Salt (12a)
Following general procedure 7 using **11a** (0.1 mmol); yield: 0.13 g (99%); mp >300 °C.

^{1}H NMR (MeOH-d_4–$CDCl_3$): δ = 0.57 (s, 6 H), 0.61 (s, 6 H), 0.74–2.39 (m, 66 H), 2.74–2.84 (m, 2 H), 2.93 (d, $^2J_{H,P}$ = 20.5 Hz, 4 H), 3.55 (d, $^3J_{H,P}$ = 10.5 Hz, 6 H), 4.53–4.96 (m, 6 H), 7.54 (s, 1 H), 7.61 (s, 2 H).

^{31}P NMR (MeOH-d_4–$CDCl_3$): δ = 24.5.

HRMS (ESI–, MeOH): *m*/*z* [M]$^-$ calcd for $C_{68}H_{100}NO_{14}P_2S$: 1248.6334; found: 1248.6325.

17-[4-(6-{4-[3-(2-Acetamido-3-mercaptopropanoyloxy)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl]pentanoyloxy}hexa-2,4-diynyloxy)-1-methyl-4-oxobutyl]-10,13-dimethyl-2,3,4,7,8,9,10,11,1213,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl 3,5-Bis{[hydroxy(methoxy)phosphoryl]methyl}benzoate Dilithium Salt (12b)
Following general procedure 7 using **11b** (0.1 mmol); yield: 0.12 g (99%); mp >150 °C.

^{1}H NMR (MeOH-d_4–$CDCl_3$): δ = 0.63 (s, 6 H), 0.64 (s, 6 H), 0.85–2.45 (m, 69 H), 2.85–2.97 (m, 6 H), 3.53 (d, $^3J_{H,P}$ = 10.5 Hz, 6 H), 5.29–5.38 (m, 2 H), 7.53 (s, 1 H), 7.62 (s, 2 H).

^{31}P NMR (MeOH-d_4–$CDCl_3$): δ = 25.6.

HRMS (ESI–, MeOH): *m*/*z* [M]$^-$ calcd for $C_{70}H_{98}NO_{15}P_2S$: 1286.6127; found: 1286.6124.

Disulfide Coupling; General Procedure 8
S-Trityl cleavage was effected with TFA–TIPS in CH_2Cl_2 as described in general procedure 7. After stirring for 2 h, aldrithiole (0.1 mmol) was added to the mixture. Stirring was continued at r.t. for a further 16 h. The purification step again depends on the nature of the bisphosphonate ester group, as outlined in general procedure 7.

1-[17-(4-(6-{4-[3-(3,5-Bis{[hydroxy(methoxy)phosphoryl]methyl}benzoyloxy)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl]pentanoyloxy}hexa-2,4-diynyloxy)-1-methyl-4-oxobutyl)-10,13-dimethylhexadecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yloxy]-1-oxo-3-(pyridin-2-yldisulfanyl)propan-2-aminium Trifluoroacetate Dilithium Salt (13a)
Yield: 0.13 g (99%); mp >300 °C.

^{1}H NMR (MeOH-d_4–$CDCl_3$): δ = 0.37 (s, 6 H), 0.43 (s, 6 H), 0.62–2.17 (m, 68 H), 2.76 (d, $^2J_{H,P}$ = 20.2 Hz, 4 H), 3.33 (d, $^3J_{H,P}$ = 10.6 Hz, 6 H), 4.39–4.63 (m, 6 H), 6.96 (t, $^3J_{H,H}$ = 5.3 Hz, 1 H), 7.16 (d, $^3J_{H,H}$ = 7.6 Hz, 1 H), 7.30 (s, 1 H), 7.40 (t, $^3J_{H,H}$ = 7.6 Hz, 1 H), 7.46 (s, 2 H), 8.25 (d, $^3J_{H,H}$ = 5.3 Hz, 1 H).

^{31}P NMR (MeOH-d_4–$CDCl_3$): δ = 24.7.

HRMS (ESI–, MeOH): *m*/*z* [M]$^-$ calcd for $C_{73}H_{103}N_2O_{14}P_2S_2$: 1357.6320; found: 1357.6324.

17-{4-[6-(4-{3-[2-Acetamido-3-(pyridin-2-yldisulfanyl)propanoyloxy]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-17-yl}pentanoyloxy)hexa-2,4-diynyloxy]-1-methyl-4-oxobutyl}-10,13-dimethyl-2,3-4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1*H*-cyclopenta[*a*]phenanthren-3-yl 3,5-Bis{[hydroxy(methoxy)phosphoryl]methyl}benzoate Dilithium Salt (13b)
Yield: 0.13 g (99%); mp 163 °C.

^{1}H NMR (MeOH-d_4–$CDCl_3$): δ = 0.62 (s, 6 H), 0.64 (s, 6 H), 0.84–2.42 (m, 38 H), 2.93 (d, $^2J_{H,P}$ = 20.4 Hz, 4 H), 3.53 (d, $^3J_{H,P}$ = 10.4 Hz, 6 H), 4.53–4.80 (m, 7 H), 5.27–5.34 (m, 2 H), 7.11 (t, $^3J_{H,H}$ = 5.0 Hz, $^4J_{H,H}$ = 0.8 Hz, 1 H), 7.52–7.67 (m, 5 H), 8.42 (d, $^3J_{H,H}$ = 4.7 Hz, 1 H).

^{13}C NMR (MeOH-d_4–$CDCl_3$): δ = 11.5, 16.9, 17.9, 19.0, 19.0, 20.8, 22.1, 24.0, 26.1, 27.4, 27.6, 27.9, 30.7, 30.7, 31.6, 31.7, 32.8, 34.1, 35.1, 36.4, 36.4, 36.7, 36.8, 37.7, 37.9, 39.5, 42.2, 49.3, 49.8, 49.9, 51.2, 51.8, 54.1, 55.6, 56.5, 56.5, 69.9, 73.5, 74.6, 75.7, 77.4, 117.7, 122.5, 122.8, 128.2, 130.3, 134.8, 135.5, 139.0, 139.4, 164.0, 166.4, 169.5, 171.3, 173.5.

^{31}P NMR (MeOH-d_4–$CDCl_3$): δ = 24.6.

HRMS (ESI–, MeOH): *m/z* $[M]^-$ calcd for $C_{76}H_{102}NO_{15}P_2S_2$: 1394.6161; found: 1394.6131.

Acknowledgment

Financial support by the DFG (Deutsche Forschungsgemeinschaft) is gratefully acknowledged.

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