Bifunctional Bisamphiphilic Transmembrane Building Blocks for Artificial Signal Transduction

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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
Scheme 1  Steroid elongation to propargyl esters or N-methylamides

\[
\begin{align*}
1a, b & \quad = \text{lithocholic acid} \\
1b & \quad = \text{cholenic acid} \\
\text{HO} & \quad = \text{lithocholic acid} \\
\text{O} & \quad = \text{cholenic acid} \\
\text{HX} & \quad = \text{DCC, DMAP} \\
\text{CH}_2\text{Cl}_2, \text{ r.t.} & \quad = (70\text{-}81\%) \\
2a, c & \quad = \text{lithocholic acid, } X = \text{O} \\
2b & \quad = \text{lithocholic acid, } X = \text{NMe} \\
2c & \quad = \text{cholenic acid, } X = \text{O} \\
\end{align*}
\]

Scheme 2  Cysteine attachment by esterification

\[
\begin{align*}
2a, c & \quad = \text{lithocholic acid, } X = \text{O} \\
2b & \quad = \text{lithocholic acid, } X = \text{NMe} \\
3a, c & \quad = \text{cholenic acid, } X = \text{O} \\
\text{PG} & \quad = \text{Boc} \\
\text{STr} & \quad = \text{HCTU, Cl-HOBt, DIPEA, DMF–CH}_2\text{Cl}_2, \text{ r.t.} \\
(58\text{-}92\%) & \quad = (58\text{-}92\%) \\
3a & \quad = \text{lithocholic acid, } X = \text{O, PG = Boc} \\
3b & \quad = \text{lithocholic acid, } X = \text{NMe, PG = Boc} \\
3c & \quad = \text{cholenic acid, } X = \text{O, PG = Ac} \\
\end{align*}
\]

Scheme 3  Bisphosphonate attachment by esterification

\[
\begin{align*}
4a, c & \quad = \text{lithocholic acid, } X = \text{O} \\
4b & \quad = \text{lithocholic acid, } X = \text{NMe} \\
4c & \quad = \text{cholenic acid, } X = \text{O} \\
2a, c & \quad = \text{lithocholic acid, } X = \text{O} \\
3a & \quad = \text{lithocholic acid, } X = \text{NMe} \\
3b & \quad = \text{cholenic acid, } X = \text{O} \\
\text{PG} & \quad = \text{Boc} \\
\text{STr} & \quad = \text{HCTU, Cl-HOBt, DIPEA, DMF–CH}_2\text{Cl}_2, \text{ r.t.} \\
(42\text{-}79\%) & \quad = (42\text{-}79\%) \\
5a, b & \quad = \text{lithocholic acid, } X = \text{O, PG = Boc; 58\%} \\
5b & \quad = \text{lithocholic acid, } X = \text{NMe, PG = Boc; 65\%} \\
\end{align*}
\]

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), O₂, CH₂Cl₂, r.t.

\[
\begin{align*}
6a & \quad = \text{lithocholic acid, } X = \text{O, PG = Boc; 39\%} \\
6b & \quad = \text{cholenic acid, } X = \text{O, PG = Ac; 65\%} \\
3a, c & \quad = \text{lithocholic acid, } X = \text{O} \\
4a, c & \quad = \text{lithocholic acid, } X = \text{O} \\
\text{PG} & \quad = \text{Boc} \\
\text{STr} & \quad = \text{HCTU, Cl-HOBt, DIPEA, DMF–CH}_2\text{Cl}_2, \text{ r.t.} \\
(39\%) & \quad = (39\%) \\
\end{align*}
\]

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), O₂, CH₂Cl₂, 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-HO\(Bt\)), 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-HO\(Bt\) 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–Chodciewicz 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\(^\prime\)-[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/trisopropylsilane 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 \(S\)/\(2\) 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 aldriothiol, both steps can be carried out in one pot, starting with detritylation by trifluoroacetic acid/trisopropylsilane, 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 heterodimensional 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 Koffer Thermoplan melting point apparatus and are uncorrected. \(^1H\) 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. \(^13C\) NMR spectra were obtained on the same spectrometers. \(^31P\) NMR spectra on a Bruker Advance ARX-200 and \(^19B\) NMR spectra on a Bruker Avance DRX-400 and DRX-500; the residual hydrogen signal of the deuterated solvent was used as reference.
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-methylpropargylamine and DMAP (2.66 mmol) in CH$_2$Cl$_2$ (30 mL), was added dropwise a soln of DCC (14.61 mmol) in CH$_2$Cl$_2$ (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$_2$Cl$_2$. The solvent was evaporated and the resulting highly viscous oil was dried in vacuo. The crude product was then purified by column chromatography.

**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.

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

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$_3$); $\delta$ = 0.64 (s, 3 H), 0.88–2.42 (m, 35 H), 2.46 (t, $^3J_{HH} = 2.3$ Hz, 1 H), 3.57–3.67 (m, 1 H), 4.67 (d, $^3J_{HH} = 2.3$ Hz, 2 H).

$^{13}$C NMR (CDCl$_3$); $\delta$ = 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, 40.3, 40.6, 42.3, 42.9, 51.9, 53.1, 56.1, 56.6, 72.0, 74.8, 78.0, 173.6.

MS (EI); m/z = 396 [M$^+$ – H$_2$O].

4-(3-Hydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[α]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$_3$); $\delta$ = 0.58 (s, 3 H), 0.86 (m, 6 H), 0.87–2.37 (m, 29 H), 2.13 (t, $^3J = 2.5$ 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$_3$); $\delta$ = 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$^+$] calcld for C$_{29}$H$_{46}$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-1H-cyclopenta[α]phenanthren-17-yl)pentanoate (2c)
Yield: 4.44 g (82%); mp 81 °C; $R_f$ = 0.21 (hexane–EtOAc, 2:1).
Yield: 0.88 g (60%); mp 75 °C; Rf = 0.51 (hexane–EtOAc, 3:1).

1H NMR (CDCl3): δ = 0.66 (s, 3 H), 0.91 (d, JHH = 6.2 Hz, 3 H), 0.99 (s, 3 H), 1.00–2.45 (m, 26 H), 2.46 (t, JHH = 2.5 Hz, 1 H), 3.42–3.58 (m, 1 H), 4.64 (d, JHH = 2.5 Hz, 2 H), 5.32 (d, JHH = 5.3 Hz, 1 H).

13C NMR (CDCl3): δ = 11.9, 18.3, 19.4, 21.1, 24.2, 28.1, 30.8, 31.0, 31.6, 31.9, 31.9, 35.5, 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-xylenebisphosphonic Acid Tetramethyl Ester; General Procedure 2: Ac-Cys(Trt)-OH, Boc-Cys(Trt)-OH, or 5-carboxy-m-xylenebisphosphonic acid tetramethyl ester (1.67 mmol) was dissolved in CHCl3–DMF (2:1, 25 mL) and DIPEA (4.16 mmol) was added. The solution 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 CH2Cl2 (10 mL) and washed with sat. aq NaHCO3, 1 M AcOH, and brine. The separated organic extract was dried (MgSO4). The filtrate was evaporated to dryness and the resulting crude product was purified by column chromatography.

Prop-2-ynyl-4-{3-[2-(tert-Butylocarbamino)-3-(tritylthio)propenylxoy]-10,13-dimethylhexadecahydro-III-cyclopenta[l]phenanthren-17-yl}-penta[2]phenanthrene (3a) Yield: 1.32 g (92%); mp 75 °C; Rf = 0.51 (hexane–EtOAc, 3:1).

1H NMR (CDCl3): δ = 0.65 (s, 3 H), 0.92–2.43 (m, 43 H), 2.45 (t, JHH = 2.4 Hz, 1 H), 2.50 (dd, JHH = 11.7 Hz, JHP = 3.9 Hz, 1 H), 2.58 (dd, JHH = 11.8 Hz, JHP = 5.5 Hz, 1 H), 4.24 (br s, 1 H), 4.66 (d, JHH = 2.2 Hz, 2 H), 4.66–4.75 (m, 1 H), 5.09 (d, JHH = 7.9 Hz, 1 H), 7.20 (t, JHH = 7.3 Hz, 3 H), 7.27 (t, JHH = 7.5 Hz, 6 H), 7.39 (d, JHH = 7.7 Hz, 6 H).

13C NMR (CDCl3): δ = 12.2, 18.4, 21.0, 23.4, 24.3, 26.4, 26.5, 27.1, 28.3, 28.4, 31.0, 32.1, 32.2, 34.5, 37.5, 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.


4-3-[2-(tert-Butylocarbamino)-3-(tritylthio)propenylxoy]-10,13-dimethylhexadecahydro-III-cyclopenta[l]phenanthren-17-yl]-N-methyl-N′-(prop-2-ynyl)pentanamide (3b) Yield: 0.88 g (66%); mp 92 °C; Rf = 0.1 (hexane–EtOAc, 3:1).

1H NMR (CDCl3): 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, JHH = 2.5 Hz, 1 H), 2.92 + 3.00 (s, 3 H), 3.97 + 4.45 (s, 4 H), 2.41 (m, 1 H), 4.67 (m, 1 H), 5.03 (d, JHH = 6.3 Hz, 1 H), 7.08–7.56 (m, 15 H).

13C NMR (CDCl3): 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, 36.9, 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, 129.6, 144.5, 155.1, 170.3, 173.4.


Prop-2-ynyl-4-{3-[2-Acetamido-3-(tritylthio)propenylxoy]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-III-cyclopenta[l]phenanthren-17-yl}pentanamide (3e) 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; Rf = 0.58 (hexane–EtOAc, 3:1).

1H NMR (CDCl3): δ = 0.71 (s, 3 H), 0.80–2.47 (m, 35 H), 2.49 (t, JHH = 2.5 Hz, 1 H), 2.55 (dd, JHH = 12.3 Hz, JHP = 4.5 Hz, 1 H), 2.70 (dd, JHH = 12.3 Hz, JHP = 2.1 Hz, 1 H), 4.55–4.67 (m, 1 H), 4.69 (d, JHH = 2.5 Hz, 2 H), 5.40 (t, JHH = 6.8 Hz, 1 H), 6.01 (d, JHH = 6.6 Hz, 1 H), 7.20–7.35 (m, 9 H), 7.42 (d, JHH = 8.1 Hz, 6 H).

13C NMR (CDCl3): 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, 60.7, 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]+ calc for C23H52N2O2S: 822.4163; found: 822.4180.
31P NMR (CDCl3): δ = 28.6.

Glaser–Hay Coupling; General Procedure 3
The acetylene compounds and CuCl (exact amounts see below) were dissolved/suspended in CH2Cl2 (25 mL). TMEDA was added to the stirred mixture, resulting in slow dissolution of all CuCl. Upon completion, the atmosphere above the green solution was saturated with O2 (balloon). Stirring was continued at r.t. for 6 h, until a deep blue color was reached. The reaction was quenched with sat. aq NHCl. The organic soln was subsequently washed with sat. aq NHCl (3 ×), the separated organic extract was dried (MgSO4), and the solvent was evaporated in vacuo. The slightly green crude product was purified by column chromatography.

6-(4-[3-Hydroxy-10,13-dimethylhexadecahydro-1H-cyclopenta[a][phenanthren-17-yl]pentanoyloxy]hexa-2,4-diylnyl)-4-[3-[2-(tert-Butyloxy carbonylamino)-3-(tritylthio)propanoate (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 (65%); Rf = 0.14 (hexane–EtOAc, 3:1).

1H NMR (CDCl3): 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, J = 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, J = 6.87 Hz, 1 H), 7.21 (t, J = 7.22 Hz, 3 H), 7.28 (t, J = 8.36 Hz, 6 H), 7.39 (d, J = 7.79 Hz, 6 H).

17-(4-[6-(4-{3-[2-(tert-Butyloxy carbonylamino)-3-(tritylthio)propano)-10,13-dimethylhexadecahydro-1H-cyclopenta[a][phenanthren-17-yl]pentanoyloxy]hexa-2,4-diylnyl)-1-methyl-4-oxobuty]pentyl)-10,13-dimethylhexadecahydro-1H-cyclopenta[a][phenanthren-3-yl]-2-(tert-Butyloxy carbonylamino)-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 (58%); Rf = 0.12 (hexane–EtOAc, 1:1).

1H NMR (CDCl3): 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, J = 8.3 Hz, 1 H), 7.08–7.36 (d, J = 6.8 Hz, 15 H).

13C NMR (CDCl3): 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, 71.5, 72.2, 74.7, 76.0, 77.5, 78.0, 125.6, 125.8, 126.9, 127.9, 128.4, 128.9, 129.6, 130.0, 130.7, 134.4, 155.1, 170.3, 173.4.


Esterification with Fmoc-Gly-OH; General Procedure 4
Fmoc-Gly-OH (0.15 mmol) and the hydroxy compounds (0.1 mmol) were dissolved in CH2Cl2 (25 mL). A soln of EDCI (0.125 mmol) in CH2Cl2 (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 with H2O (2 ×) and the aq phase was extracted with CH2Cl2. The combined organic extracts were dried (MgSO4) and the solvent evaporated in vacuo. The crude product was purified by column chromatography.

6-(4-[6-(4-{3-[2-(tert-Butyloxy carbonylamino)-3-(tritylthio)propanoyloxy]hexa-2,4-diylnyl)-1-methyl-4-oxobuty]pentyl)-10,13-dimethylhexadecahydro-1H-cyclopenta[a][phenanthren-17-yl]pentanoyloxy]hexa-2,4-diylnyl)-4-[3-[2-(4H-Fluoren-9-ylmethyloxycarbonylamino)acetoxy]-10,13-dimethylhexadecahydro-1H-cyclopenta[a][phenanthren-17-yl]pentanato (7a)
Yield: 0.09 g (55%); Rf = 0.1 (hexane–EtOAc, 3:1).

1H NMR (300 MHz, CDCl3): δ = 0.63 + 0.66 (s, 6 H, 0.92) (m, 12 H), 0.93–2.58 (m, 68 H), 3.96 (d, J = 5.7 Hz, 2 H), 4.25 (t, J = 7.1 Hz, 1 H), 4.38 (d, J = 7.6 Hz, 2 H), 4.74 (s, 4 H) 4.82 (m, 2 H), 5.10 (d, J = 7.9 Hz, 1 H), 5.33 (t, J = 5.4 Hz, 1 H), 7.27–7.76 (m, 19 H), 7.17 (J = 7.6 Hz, 23 H).

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1H NMR (100 MHz, CDCl3): δ = 12.2, 18.6, 21.0, 23.4, 24.4, 25.1, 26.5, 26.6, 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, 40.6, 42.1, 42.9, 44.2, 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.

13C NMR (100 MHz, CDCl3): δ = 52.8, 53.5, 55.1, 56.3, 56.4, 56.6, 56.7, 66.7, 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.

1H NMR (300 MHz, CDCl3): 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, J = 8.3 Hz, 1 H), 5.39 (t, J = 5.0 Hz, 1 H), 7.08–7.72 (m, 19 H).

1H NMR (CDCl3): 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, 129.1, 141.3, 143.9, 144.4, 155.0, 156.4, 169.5, 170.2, 171.2, 173.3.


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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-1H-cyclopenta[a]phenanthren-17-yl]pentanoyloxy]hexa-2,4-diylnyloxy]-1-methyl-4-oxobutyl]-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl 3,5-Bis[(hydroxy(methoxy)phosphoryl)methyl]benzole Dilithium Salt (11b)

Yield: 0.19 g (99%); mp >300 °C.

1H NMR (MeOD-d$_4$-CDC$_1$); $\delta$ = 0.51 (s, 6 H), 0.62–2.54 (m, 59 H), 2.80 (d, $J_{HC} = 10.2$ 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).

13C NMR (MeOD-d$_4$-CDC$_1$); $\delta$ = 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, $J_{CP} = 130$ Hz), 35.7, 35.7, 36.9, 37.0, 37.4, 38.2, 38.5, 40.1, 40.1, 42.8, 50.4, 50.5, 51.7 (d, $J_{HP} = 5.5$ Hz), 52.2, 52.4, 56.1, 57.0, 57.1, 66.3, 67.3, 70.5, 74.1, 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.

13P NMR (MeOD-d$_4$-CDC$_1$); $\delta$ = 25.4.

HRMS (ESI–, MeOH); $m/z$ [M] + calcd for C$_{70}$H$_{107}$N$_4$NaBO$_8$S: 1197.7800; found: 1197.7807.

Following general procedure 7 using H$_2$N-(CH$_2$)$_2$-Trityl cleavage was effected with TFA–TIPS in CH$_2$Cl$_2$ as described in general procedure 7. After stirring for 2 h, triethylamine (0.1 mmol) was added to the mixture. Stirring was continued at rt. for a further 16 h. The purification step again depends on the nature of the bisphosphonate ester groups, as outlined in general procedure 7.

17-[4-6-(4-[3-(5-Bis[(hydroxy(methoxy)phosphoryl)methyl]benzoyloxy]-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl]pentanoyloxy]hexa-2,4-diylnyloxy]-1-methyl-4-oxo-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-3-yl]oxo-3-(pyridin-2-ylidisulfanyl)propan-2-aminopropionate Trifluoroacetate Dilithium Salt (13a)

Yield: 0.13 g (99%); mp >300 °C.

1H NMR (MeOD-d$_4$-CDC$_1$); $\delta$ = 0.37 (s, 6 H), 0.43 (s, 6 H), 0.62–2.17 (m, 68 H), 2.76 (d, $J_{HC} = 10.0$ Hz, 4 H), 3.30 (d, $J_{HP} = 10.6$ Hz, 4 H), 3.83–4.63 (m, 6 H), 6.96 (t, $J_{HP} = 5.3$ Hz, 1 H), 7.16 (d, $J_{HP} = 7.6$ Hz, 1 H), 7.30 (s, 1 H), 7.40 (t, $J_{HP} = 7.6$ Hz, 1 H), 7.46 (s, 2 H), 8.25 (d, $J_{HP} = 5.3$ Hz, 1 H).

13P NMR (MeOD-d$_4$-CDC$_1$); $\delta$ = 24.7.

HRMS (ESI–, MeOH); $m/z$ [M] + calcd for C$_{71}$H$_{103}$N$_2$O$_{14}$P$_2$S$_2$: 1357.6334; found: 1357.6325.

Disulfide Coupling: General Procedure 8

S-Trityl cleavage was effected with TFA-TIPS in CH$_2$Cl$_2$, as described in general procedure 7. After stirring for 2 h, triethylamine (0.1 mmol) was added to the mixture. Stirring was continued at rt. for a further 16 h. The purification step again depends on the nature of the bisphosphonate ester groups, as outlined in general procedure 7.

17-[4-6-(4-[3-(5-Bis[(hydroxy(methoxy)phosphoryl)methyl]benzoyloxy]-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-17-yl]pentanoyloxy]hexa-2,4-diylnyloxy]-1-methyl-4-oxo-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-3-yl]oxo-3-[pyridin-2-ylidisulfanyl]propan-2-aminopropionate Trifluoroacetate Dilithium Salt (13b)

Yield: 0.13 g (99%); mp >300 °C.

1H NMR (MeOD-d$_4$-CDC$_1$); $\delta$ = 0.62 (s, 6 H), 0.64 (s, 6 H), 0.85–2.45 (m, 68 H), 2.85–2.97 (m, 6 H), 3.53 (d, $J_{HP} = 10.5$ Hz, 6 H), 5.29–5.38 (m, 2 H), 7.53 (s, 1 H), 7.62 (s, 2 H).

13P NMR (MeOD-d$_4$-CDC$_1$); $\delta$ = 24.6.

HRMS (ESI–, MeOH); $m/z$ [M] + calcd for C$_{68}$H$_{100}$NO$_{14}$P$_2$S$_2$: 1286.6127; found: 1286.6124.
HRMS (ESI−, MeOH): $m/z$ [M]$^-$ calcld for $\text{C}_{76}\text{H}_{102}\text{NO}_{15}\text{P}_{2}\text{S}_{2}$: 1394.6161; found: 1394.6131.

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References


