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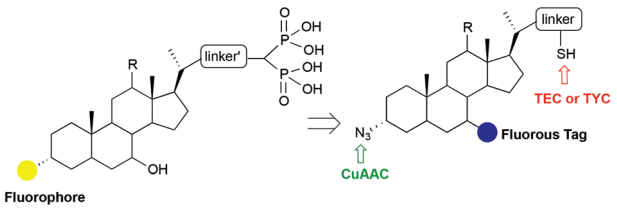
1

Fluorous-tag assisted synthesis of bile acid–bisphosphonate conjugates *via* orthogonal click reactions: an access to potential anti-resorption bone drugs

Chiara Massarenti, Olga Bortolini, Giancarlo Fantin, Dario Cristofaro, Daniele Ragno, Daniela Perrone,* Elena Marchesi, Gianluca Toniolo and Alessandro Massi*



Click reactions and fluorous separations allow for the generation of a small collection of bile acid–bisphosphonate conjugates.

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Organic & Biomolecular Chemistry

PAPER

Fluorous-tag assisted synthesis of bile acid–bisphosphonate conjugates *via* orthogonal click reactions: an access to potential anti-resorption bone drugs†

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The synthesis of a small collection of novel bile acid–bisphosphonate (BA–BP) conjugates as potential drug candidates is reported. The disclosed methodology relied on the installation of azide and thiol functionalities at the head and tail positions, respectively, of the BA scaffold and its subsequent decoration by orthogonal click reactions (copper-catalyzed azide–alkyne cycloaddition, thiol–ene or thiol–yne coupling) to introduce BP units and a fluorophore. Because of the troublesome isolation of the target conjugates by standard procedures, the methodology culminated with the functionalization of the BA scaffold with a light fluorous tag to rapidly and efficiently purify intermediates and final products by fluorous solid-phase extraction.

Introduction

Geminal bisphosphonates (BPs) are stable analogues of pyrophosphate and represent an important class of bioactive compounds, which are currently employed for the treatment and prevention of several bone disorders such as bone metastasis, myeloma, rheumatoid arthritis, osteoporosis, and Paget's disease.[1] BPs mainly act by decreasing osteoclast activity and inducing osteoclast apoptosis.[2] Structure–activity relationship (SAR) studies highlighted that bioactivity of BPs is strictly dependent on the nature of substituents installed at the geminal position of the bisphosphonic moiety, as confirmed by the evolution of this class of drugs across three generations of active molecules.[3] Despite their successful use, bioavailability remains a critical feature of BPs since these highly hydrophilic derivatives are poorly absorbed from the gastrointestinal tract after oral administration.[4] A number of delivery systems have been investigated to overcome bioavailability limitations of BPs and improve patient compliance including liposome encapsulation, use of nanoparticles and co-administration with adsorption enhancing agents such as surfactants, salicylates, and bile acids (BAs).[5] Indeed, the unique features of facial amphiphilic BAs have been successfully exploited by Park and co-workers for improving the intestinal permeability of ibandronate and zoledronic acid through the generation of molecular complexes with lysine-linked deoxycholic acid.[6] Our group also contributed to this area of research reporting on the synthesis and biological evaluation of the chenodeoxycholic-derived bisphosphonate **1** (Fig. 1).[7] This BA–BP conju-

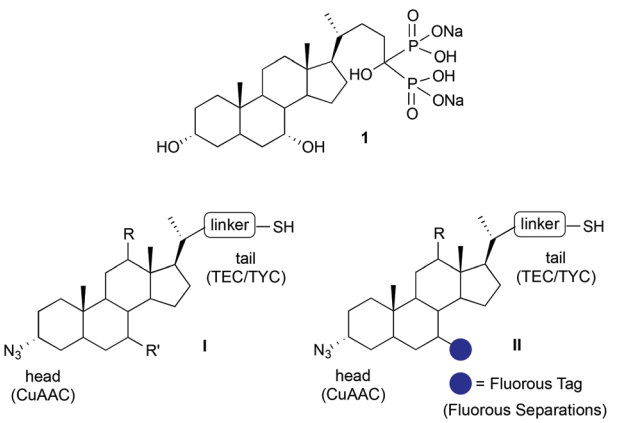

Fig. 1 Biologically active chenodeoxycholic-derived bisphosphonate **1** (ref. 7) and the multi-functional bile acid scaffolds **I** and **II** designed for this study.

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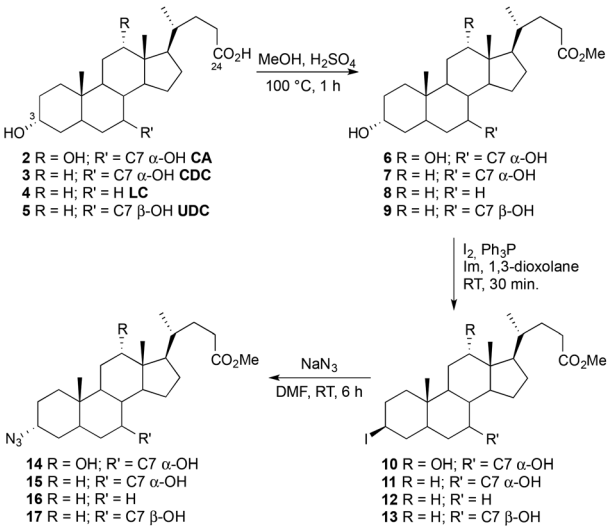
gate was prepared by a stepwise synthesis strategy and exhibited high affinity toward hydroxyapatite. Significantly, together with the lower cytotoxicity compared to neridronate in L929 murine fibroblast culture cells, the conjugate **1** displayed a higher activity in inhibition of osteoclastogenesis.[7] These promising results encouraged us to set-up a modular and general synthetic strategy to rapidly explore the chemical space around the BA scaffold through the efficient generation of a small collection of BA–BP conjugates eventually functionalized with additional molecular portions such as fluorophores. Therefore, herein we report on the design and synthesis of the multi-functional BA scaffold **I** suitably equipped with azide and thiol groups at the 'head' and 'tail' positions of the BA unit, respectively, for further elaborations *via* orthogonal click reactions (Fig. 1). The copper-catalyzed azide–alkyne cycloaddition (CuAAC),[8] thiol–ene coupling (TEC),[9] and the thiol–yne coupling (TYC)[9c,10] were selected for the scope. On the other hand, the high potential and versatility of the above click reactions performed sequentially or simultaneously have been amply documented in materials science and bioconjugation studies.[11] Although not initially planned, the post-reaction phase of BA–BP conjugates synthesis was addressed in this study by taking advantage of the incorporation of a light fluorous tag onto the BA moiety (scaffold **II**) to facilitate the purification of click intermediates and the target conjugates. In fact, tagged substrates can be efficiently separated from nonfluorous-tagged side-products by fluorous solid-phase extraction (F-SPE).[12] Noteworthy, while fluorous technology has been widely utilized for the synthesis of biomolecules such as peptides and carbohydrates,[13] its application to the elaboration of BA and BP derivatives is unprecedented to the best of our knowledge.

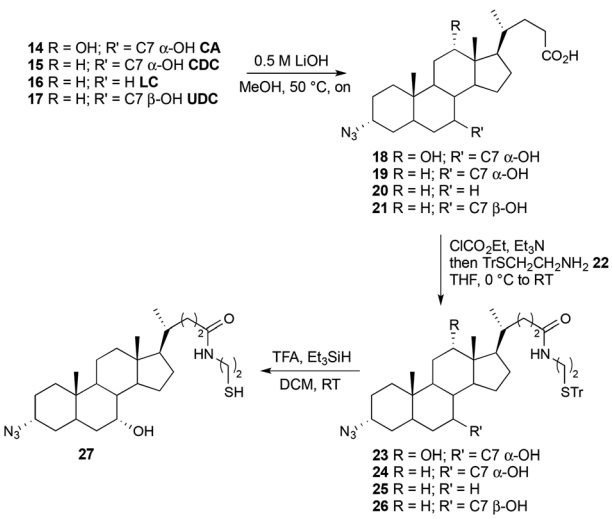
Results and discussion

The synthesis of the orthogonally functionalized BA scaffolds of type **I** commenced with the selection of four bile acids with different hydrophobic nature, namely the two human primary BAs cholic (**C**) and chenodeoxycholic (**CDC**) acids **2–3**, the secondary human BA lithocholic (**LC**) acid **4**, and the ursodeoxycholic (**UDC**) acid **5** (Scheme 1). Introduction at the head position (C3) of the 3α-azide functionality was accomplished through a double nucleophilic displacement with retention of configuration following our previously disclosed procedure with some improvements.[14] The synthetic sequence is detailed in the Experimental section for the hitherto unreported *lithocholic* series. Accordingly, the BAs **2–5** were initially converted into the corresponding methyl esters **6–9** by the classical Fisher esterification method, then the 3β-iodides **10–13** were obtained by treatment with I_2 and Ph_3P in the presence of imidazole and 1,3-dioxolane. The second nucleophilic substitution was finally performed with sodium azide in DMF at room temperature to give the 3α-azide derivatives **14–17** in satisfactory overall yields (44–63%, Scheme 1).



Scheme 1 Synthesis of the head-functionalized BA derivatives **14–17**.

The synthesis of the di-functional BA scaffolds of type **I** was completed with the introduction at the tail position (C24) of a trityl-protected thiol group through a cysteamine linker (Scheme 2). Hence, the methyl esters **14–17** were hydrolyzed with 0.5 M LiOH to the corresponding free acids **18–21** (quantitative yields), which in turn were converted into the amides **23–26** by coupling with *S*-trityl cysteamine **22** through the mixed anhydride method in the presence of ethyl chloroformate (45–83%). The derivatives **23–26** constituted a set of BA scaffolds suitably functionalized for subsequent CuAAC and TEC/TYC orthogonal click reactions upon removal of the *S*-trityl protecting group. This step was planned to be performed just before the TEC/TYC to avoid undesired sulphur oxidation side-reactions.

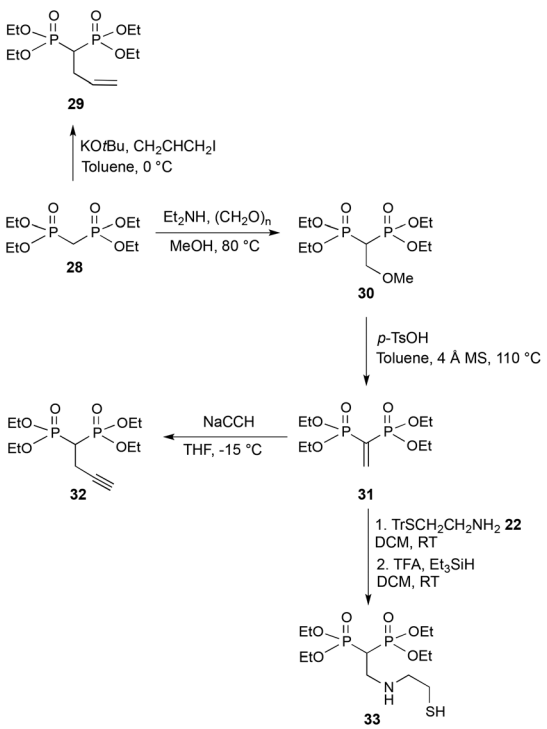


Scheme 2 Synthesis of the *S*-protected BA derivatives **23–26** and of the orthogonally functionalized chenodeoxycholic scaffold **27**.

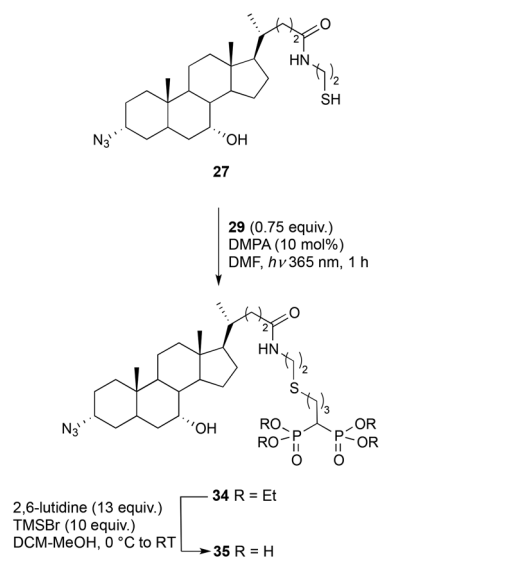
The feasibility of *S*-trityl deprotection was successfully tested using the chenodeoxycholic acid compound **24**, which was selected as the model substrate for this study in virtue of the biological activity displayed by the previously synthesized chenodeoxycholic-derived bisphosphonate **1**.[7] Therefore, treatment of protected **24** with trifluoroacetic acid/triethylsilane in dichloromethane (room temperature, 15 min) afforded the target thiol derivative **27** in 93% isolated yield (Scheme 2).

The clickable BP reagents **29**, **31–32** utilized in the present investigation were prepared as reported in the literature with some modifications (Scheme 3). Accordingly, the tetraethyl but-3-ene-1,1-diyl-bisphosphonate **29**[15] was synthesized by treatment of the commercially available methylene bisphosphonate **28** with KO*t*Bu and allyl iodide in dry toluene at 0 °C; although the formation of the double alkylation product could not be avoided by this strategy, the reduction of reaction time and temperature allowed to recover the target mono-allyl derivative **29** in acceptable 40% yield after column chromatography. The tetraethyl ethene-1,1-diyl-bisphosphonate **31**[16] was obtained in 80% yield by a two-step sequence involving the initial formation of the BP intermediate **30** (paraformaldehyde, Et_2NH, MeOH, 80 °C) followed by elimination under acidic conditions in the presence of molecular sieves (*p*-TsOH, toluene, 4 Å MS, 110 °C). The alkyne-functionalized bisphosphonate **32** was synthesized as described by selective addition of sodium acetylide to **31** (THF, −15 °C) but recovered in lower yield (40%) compared to that reported in the literature.[16e,17] The ethylidene bisphosphonate **31** was a suitable precursor for the synthesis of the thiol-functionalized BP **33** as well. Indeed, this clickable reagent was easily prepared by Michael addition of *S*-trityl cysteamine **22** to **31** (DCM, room temperature) followed by standard trityl group deprotection (95% overall yield, Scheme 3).

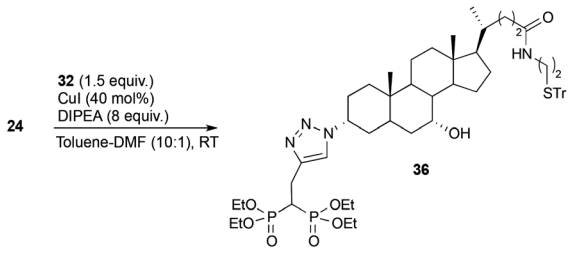
Next, having in hands the orthogonally functionalized BA derivatives **24**, **27** and the set of clickable reagents **29**, **31–33**, we investigated the efficiency of the planned click chemistry approach towards the generation of a small collection of BA–BP conjugates. The tail functionalization of the BA scaffold was initially considered by coupling **27** with the allyl-substituted BP **29** under standard photoinduced TEC conditions (Scheme 4). Thus, an equimolar mixture of **27** and **29** in DMF was irradiated with a UV-lamp (λ_{max} 365 nm) using 2,2-dimethoxy-2-phenylacetophenone (DMPA, 10 mol%) as a radical initiator. Since ^1H and ^{13}P NMR analysis showed only a partial conversion of the substrates, the TEC was next attempted using a slight excess (1.5 equiv.) of the alkene **29**. While these conditions guaranteed the full consumption of thiol **27**, the chromatographic separation of the target conjugate **34** (>95% NMR yield) from the allyl BP **29** was troublesome and impracticable; in fact, the lengthy elution from the column even with high polar mobile phases (this is typical of BP-containing products) resulted in the collection of **34** contaminated by **29**. Isolation of pure **34** was made possible using an excess (1.5 equiv.) of thiol **27** in the TEC since the disulphide by-product (MS-analysis) could be easily separated in virtue of its lower polarity. Nevertheless, the isolated yield of **34** (>95% NMR yield) could not exceed the value of 64% likely because of a partial irreversible adsorption of this polar compound on silica gel. A similar result was detected using alumina as stationary phase. Subsequently, the conversion of the tetraethyl bisphosphonate **34** into the corresponding bisphosphonic acid **35** was investigated using the McKenna conditions.[18] This deprotection reaction was optimized in



Scheme 3 Synthesis of clickable reagents **29**, **31–33**.



Scheme 4 Tail functionalization of the BA scaffold **27** by TEC and tetraethyl BP **34** deprotection.



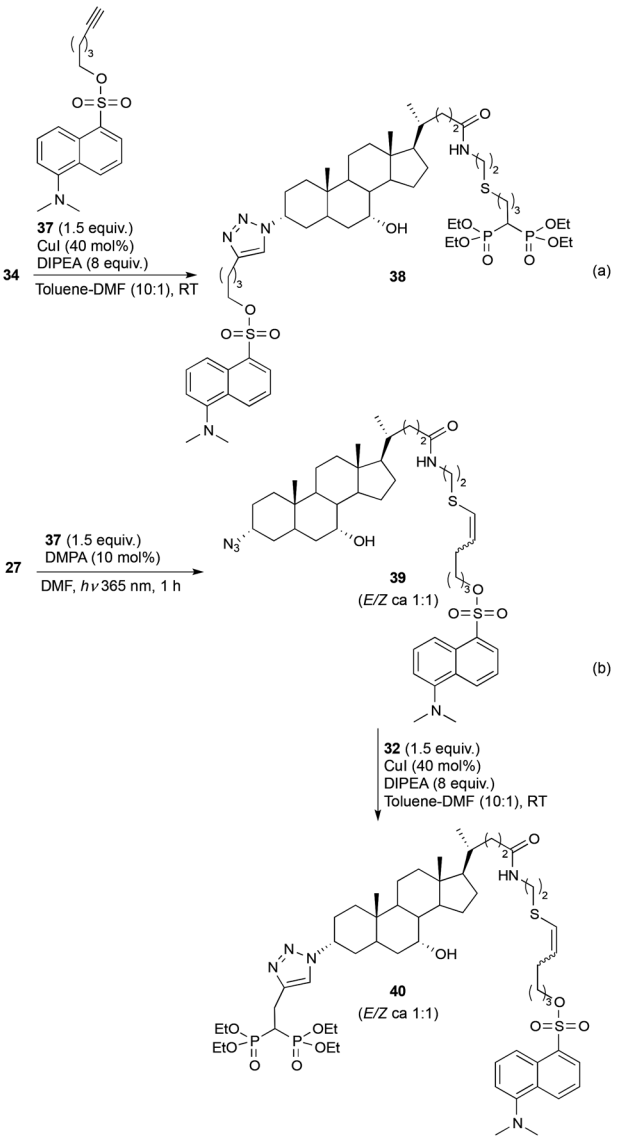
Scheme 5 Head functionalization of the BA scaffold **24** by CuAAC.

DCM-MeOH using an excess of trimethylsilyl bromide (10 equiv.) in the presence of 2,6-lutidine (13 equiv.). It is important to stress that the absence of the base resulted in the formation of a complex reaction mixture, which contained by-products arising from the partial hydrolysis of the amide bond of the cysteine linker, as confirmed by MS analysis. Isolation of the bisphosphonic acid **35** (53% yield) was finally accomplished by size exclusion chromatography employing Sephadex LH-20 as stationary phase.

The head functionalization of the *S*-protected BA scaffold **24** was performed by adopting the standard procedure for the regioselective CuAAC using the alkyne-functionalized BP **32** (Scheme 5). After some experimentation seeking efficiency and purification simplicity, optimized reaction conditions consisted in dissolving a slight excess of **32** (1.5 equiv.) and **24** in a mixture of toluene/DMF (10 : 1) and then adding at room temperature *N*,*N*-diisopropylethylamine (DIPEA, 8.0 equiv.) and copper(I)-iodide (40 mol%) in two portions over a period of 24 h. The above protocol furnished the target BA–BP conjugate **36** in quantitative NMR yield and 65% isolated yield after column chromatography.

Functionalization of the BA scaffold **27** by the designed orthogonal click reactions was applied to the synthesis of fluorescent analogues of BA–BP conjugates **34** and **36**. Indeed, fluorescently-labelled bisphosphonates are useful probes to inspect skeletal and cellular distribution as well as to investigate the BP localization in both hard and soft tissues for a better understanding of their major side effects.[19] Therefore, the tail-functionalized BA **34** was subjected to CuAAC with the dansyl alkyne fluorophore **37**[20] under the previously optimized conditions (Scheme 6, eqn (a)). Again, the target conjugate **38** was produced in almost quantitative yield (NMR analysis) but recovered in much lower yield (55%) after chromatography. As expected, the increasing complexity on the BA unit further complicated the purification phase of this class of compounds.

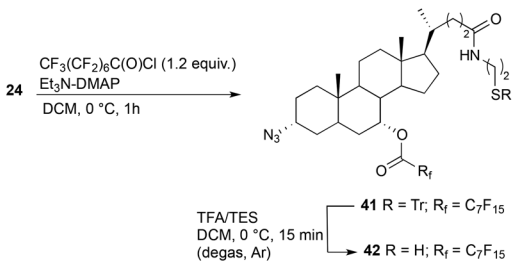
Optimal results in the synthesis of the conjugate **40** were achieved when the photoinduced radical coupling was performed before the CuAAC (eqn (b)). Actually, the effectiveness of this reaction sequence was further confirmed in subsequent coupling experiments (*vide infra*, Table 2). Hence, the photoinduced TYC between **27** and **37** was carried out under the conditions disclosed for the TEC affording the vinyl sulfide adduct **39** (~1 : 1 mixture of *E*/*Z* isomers), which in turn was



Scheme 6 Synthesis of fluorescently-labelled BA–BP conjugates **38**, **40**.

engaged in the CuAAC with the alkyne **32** to give the fluorescently-labelled BA–BP conjugate **40** in 23% overall yield.

It clearly appears from the above results that the disclosed synthetic methodology is hampered by difficulties associated with the purification of the BA–BP conjugates. Therefore, we envisaged the opportunity to introduce a perfluoroalkyl group at the C7 position of the BA scaffold **24** and thus exploit the advantages of fluorous separations. With the fluorous tag in place, fluorous solid-phase extraction (F-SPE)[12] may be employed to isolate intermediates without recourse to conventional chromatography. F-SPE involves filtration of the reaction mixture through a pad of fluorous silica, eluting first with a fluorophobic solvent to remove untagged impurities and then with a fluorophilic solvent to recover the tagged product. Accordingly, the synthesis of the tagged BA derivative **41** was initially attempted with **24** and heptadecafluorononanoic acid



Scheme 7 Synthesis of the fluorous tagged BA scaffolds **41**, **42**.

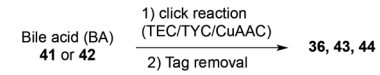
using the Yamaguchi esterification method[21] but without success.

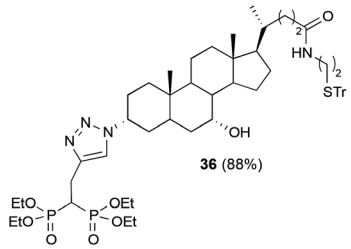
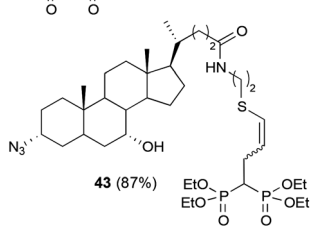
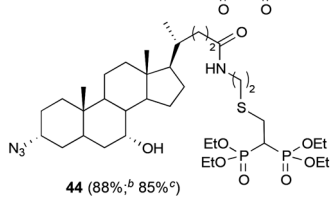
Fortunately, the reaction of **24** with a slight excess of commercially available pentadecafluorooctanoyl chloride (1.2 equiv.) in the presence of Et_3N and catalytic 4-dimethylaminopyridine (DMAP; DCM, 0 °C) afforded the tri-functional BA scaffold **41** in 71% yield (Scheme 7). Crucial in this transformation were the reaction temperature and the amount of the acylating agent, which were suitably controlled to avoid the formation of complex reaction mixtures; under the optimized conditions, instead, the esterification proceeded smoothly in one hour as confirmed by the shift of the 7β-H broad singlet from 3.85 ppm to 5.19 ppm (NMR analysis). Afterward, detritylation of **41** was run as described before for **24**. Contrary to what it is typically observed in fluorous tag-assisted syntheses where the presence of the fluorous tag does not alter the reactivity of the substrate molecule, we found that the C7 fluorous portion on the BA derivative **42** deeply affected the stability of the free thiol group towards sulphur oxidation side-reactions. Thus, the detritylation step was optimized under strictly degassed conditions (Argon) furnishing the tagged thiol compound **42** (90% yield), which was used in its crude form (contamination by triphenylmethane) to avoid undesired disulphide formation during purification (Scheme 7).

The synthesis of the head-functionalized BA–BP conjugated **36** was reproduced to test the effectiveness of the envisaged fluorous-tag assisted strategy (Table 1, entry 1). Hence, the tagged scaffold **41** was coupled with the alkyne BP **32** to give the corresponding CuAAC-adduct (not shown; see the Experimental section), which was purified by F-SPE. As far as the purification of tagged derivatives is concerned, in this study a typical F-SPE involved the loading of the crude reaction mixture with a minimum amount of organic solvent (DCM) on the cartridge packed with FluoroFlash™ silica gel, followed by elution with 80 : 20 MeOH–H_2O (fluorophobic solvent) and then with pure MeOH (fluorophilic solvent).

The subsequent detagging step was optimized using 0.5 M NaOH in a mixture of EtOH–H_2O (room temperature, 1 h). The selective removal of the fluorous tag was verified by the shift back of the 7β-H NMR signal and ESI-MS analysis. The final F-SPE furnished the target conjugate **36** in 88% overall yield and 95% purity as judged by ^{31}P NMR analysis. Therefore, it appeared that the two extra steps required by the tag-assisted strategy were well compensated by the increase of yield (24%) and the straightforward isolation of **36**.

Table 1 Synthesis of the BA–BP conjugates **36**, **43**, **44** by the fluorous-tag assisted strategy



Entry	BA and clickable reagent	Product[a]
1	**41**, **32**	 **36** (88%)
2	**42**, **32**	 **43** (87%)
3	**42**, **31**	 **44** (88%;[b] 85%[c])

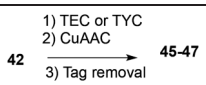
[a] Isolated yield. [b] Prepared by thiol-Michael reaction. [c] Prepared by photoinduced TEC.

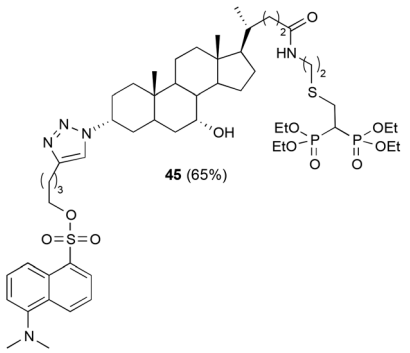
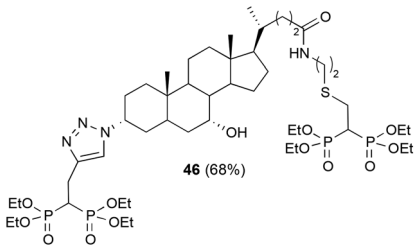
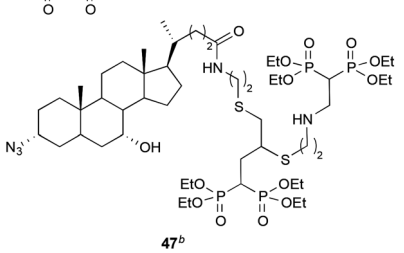
The same coupling/purification/detagging sequence was executed for the synthesis of the new tail-functionalized conjugates **43** and **44**. The former was obtained in 87% yield by TYC of the thiol **42** and the alkyne **32** (entry 2). The latter was synthesized by coupling **42** with the ethylidene bisphosphonate **31** by either radical (photoinduced) or ionic mechanisms with comparable level of efficiency (entry 3). In particular, the thiol-Michael addition[22] of **42** to the acceptor **31** was optimized in DCM (room temperature, 16 h) using Et_3N (1.5 equiv.) as the base.

Orthogonal click reactions were finally performed with the tagged BA scaffold **42** (Table 2). The synthetic strategy entailed F-SPE after each coupling step, monitoring of conversion by MS analysis, fluorous tag removal, and final F-SPE to recover the target conjugate. As anticipated, optimal results were obtained by first performing the radical couplings (TEC or TYC) rather than CuAAC. Accordingly, the synthesis of the fluorescently-labelled BA–BP conjugate **45** started with the photoinduced TEC of **42** with **31** and ended-up with the CuAAC in the presence of the dansyl alkyne fluorophore **37** (entry 1, 65% yield).

The synthesis of 'bis armed' BA derivatives functionalized with two BP moieties was also explored in this study. Hence, the conjugate **46** was rapidly prepared through the TEC/CuAAC

Table 2 Synthesis of the BA–BP conjugates **45–47** by the fluorous-tag assisted strategy



Entry	Clickable reagents	Product[a]
1	**31**, **37**	 **45** (65%)
2	**31**, **32**	 **46** (68%)
3	**32**, **33**	 **47**[b]

[a] Isolated yield. [b] Detected by ESI-MS analysis.

sequence with the alkene **31** and the alkyne **32** (entry 2, 68% yield). The synthesis of the 'bis armed' conjugate **47** was finally attempted from the BA **42** by sequential TYC/TEC (entry 3). Thus, the vinyl thioether intermediate (**43F** not shown; see the Experimental section) was first prepared by TYC of the alkyne **32** with thiol **42** under optimized conditions, and then subjected to photoinduced TEC with the thiol-functionalized BP **33** (2 equiv.) in diluted water/toluene (10 : 1 v/v) (0.01 M) according to a known procedure.[23] Unfortunately, the target adduct **47** could only be detected by MS analysis because of failure of the challenging second TEC step very likely determined by the steric hindrance of the vinyl thioether intermediate.

Conclusions

In summary, we have developed an efficient general approach for the rapid synthesis of a small collection of novel bile acid–bisphosphonate conjugates as potential drug candidates based on the execution of orthogonal click reactions (TEC/TYC and CuAAC) and fluorous separations (F-SPE). The method allowed for the straightforward incorporation onto the BA scaffold of two molecular portions, that in this study were the bisphosphonate unit and a fluorophore. Nevertheless, we believe that the disclosed click strategy might be employed as an efficient tool to explore diversity around the BA scaffold by considering functionalization with different bioactive moieties such as amino acids and carbohydrates. This part of research together with the biological evaluation of the synthesized BA–BP conjugates is currently underway in our laboratories.

Experimental section

General information

Reactions were monitored by TLC on silica gel 60 F_{254} with detection by charring with phosphomolybdic acid. Flash column chromatography was performed on silica gel 60 (230–400 mesh). ^{1}H (300 MHz), ^{13}C (75 MHz), ^{31}P (121 MHz), and ^{19}F (282 MHz) NMR spectra were recorded in $CDCl_3$ solutions at room temperature unless otherwise stated. Peaks assignments were aided by ^{1}H–^{1}H COSY and gradient-HMQC experiments. Photoinduced reactions were carried out in a glass vial (diameter, 1 cm; wall thickness, 0.65 mm), sealed with a natural rubber septum, located 2.5 cm away from the UVA lamp (irradiation on sample: 365 nm, 1.04 W m^{-2}).

ESI-MS routine analyses were performed in positive ion mode with samples dissolved in 10 mM solution of ammonium formate in 1 : 1 MeCN/H_2O. For accurate mass measurements, the compounds were detected in positive ion mode by HPLC-Chip Q/TOF-MS (nanospray) analysis using a quadrupole, a hexapole, and a time-of-flight unit to produce spectra. FluoroFlash® silica gel (40 μm, particle size ~40 μm) was purchased from Sigma-Aldrich. Bile acids **2–5**, *S*-trityl cysteamine **22**, and bisphosphonate **28** are commercially available compounds. Bile acid derivatives **6**,[14] **7**,[14] **9**,[14] **10**,[14] **11**,[14] **13**,[14] **14**,[14] **15**,[14] **17**[14] and bisphosphonates **29**,[15] **30**,[16] **31**,[16] **32**[17] are known compounds.

General procedure for the synthesis of 3β-iodides 10–13

To a stirred solution of bile acid ester **6–9** (12.30 mmol) in 1,3-dioxolane (100 mL), PPh_3 (4.84 g, 18.45 mmol) and imidazole (2.51 g, 36.90 mmol) were added. After 5 min, I_2 (4.68 g, 18.45 mmol) was added portion-wise. The resulting solution was stirred at room temperature for 30 min. The reaction mixture was poured into water (30 mL) containing a few drops of 30% H_2O_2 and extracted with EtOAc (3 × 75 mL). The combined organic layers were washed with saturated aqueous $Na_2S_2O_5$ (30 mL), dried (Na_2SO_4), filtered, and concentrated. The crude 3β-iodides **10–13** were dissolved in EtOAc (with a few drops of MeOH) and allowed to stand overnight. The crystallized product was recovered as a white amorphous solid.

Methyl 3β-iodo-5β-cholan-24-oate (12). Crystallization from EtOAc (with a few drops of MeOH) afforded **12** (5.66 g, 92%) as a white amorphous solid. ^{1}H NMR: δ = 4.99 (bs, 1 H, H-3α), 3.65 (s, 3 H, OCH_3), 2.38–2.30 (m, 1 H, H-23a), 2.24–2.17 (m,

1 H, H-23b), 2.01–1.69 (m, 8 H), 1.67–1.02 (m, 18 H), 1.01 (s, 3 H, H-19), 0.90 (d, 3 H, J = 6.4 Hz, 3H-21), 0.64 (s, 3 H, 3H-18); $^{13}C\{^1H\}$: δ = 174.8, 56.6, 55.9, 51.5, 42.7, 41.7, 40.1, 39.5, 39.0, 36.9, 35.7, 35.7, 35.4, 32.70, 31.2, 31.0, 30.9, 28.6, 27.0, 26.4, 24.1, 23.7, 20.8, 18.7, 12.0. HRMS (ESI) m/z calcd for $C_{25}H_{41}IO_2$ $[M]^+$ 500.2151, found 500.2201.

General procedure for the synthesis of 3α-azides 14–17

To a stirred solution of the 3β-iodide **10–13** (1.94 mmol) in DMF (10 mL) NaN_3 (378 mg, 5.82 mmol) was added in one portion. The reaction mixture was stirred at room temperature for 6 h and then poured into water (8 mL) and extracted twice with a mixture of Et_2O (12 mL) and EtOAc (3 mL). The combined organic layers were dried (Na_2SO_4), filtered and concentrated to give the azide **14–17**, which was used without further purifications.

Methyl 3α-azido-7α,12α-dihydroxy-5β-cholan-24-oate (14). White solid, (730, 80%); mp 108–110 °C; IR: ν (cm^{-1}) 3520–3320 (O–H), 2950–2868 (C–H), 2090 (N_3), 1722 (C═O); ^{1}H-NMR: δ = 4.00–3.97 (m, 1 H, H-12β), 3.87–3.83 (m, 1 H, H-7β), 3.66 (s, 3 H, OCH_3), 3.20–3.10 (m, 1 H, H-3α), 2.42–1.11 (m, 24 H), 0.97 (d, 3 H, J = 6.44 Hz, 3H-21), 0.90 (s, 3 H, 3H-19), 0.68 (s, 3 H, 3H-18); $^{13}C\{^1H\}$: δ = 174.8, 72.9, 68.2, 61.3, 51.6, 47.2, 46.5, 41.9, 41.8, 39.5, 35.4, 35.3, 35.2, 34.7, 43.4, 31.0, 30.8, 28.3, 26.9, 26.8, 26.6, 23.2, 22.6, 17.31, 12.5. HRMS (ESI) m/z calcd for $C_{25}H_{41}N_3O_4Na$ $[M + Na]^+$ 470.2995, found 470.2997.

Methyl 3α-azido-7α-hydroxy-5β-cholan-24-oate (15). Oil which solidified on standing, (760 mg, 86%); mp 87–89 °C; IR: ν (cm^{-1}) 3518 (O–H), 2947–2868 (C–H), 2089 (N_3), 1720 (C═O); ^{1}H-NMR: δ = 3.85–3.80 (m, 1 H, H-3β), 3.64 (s, 3 H, OCH_3), 3.17–3.09 (m, 1 H, H-7β), 2.39–2.15 (m, 2 H, 2H-23), 1.99–0.93 (m, 24 H), 0.91–0.88 (m, 6 H, 3H-19, 3H-21), 0.64 (s, 3 H, 3H-18); $^{13}C\{^1H\}$: δ = 174.7, 68.2, 61.3, 55.7, 51.5, 50.3, 42.6, 41.8, 39.6, 39.5, 39.3, 35.5, 35.4, 35.3, 35.1, 34.4, 32.7, 30.9, 28.1, 26.8, 23.7, 22.8, 20.5, 18.2, 11.7; HRMS (ESI) m/z calcd for $C_{25}H_{41}N_3O_3Na$ $[M + Na]^+$ 454.3046, found 454.3051.

Methyl 3α-azido-5β-cholan-24-oate (16). Crystallization from MeOH/EtOAc afforded **16** as a white amorphous solid (700 mg, 87%). IR: ν (cm^{-1}) 2934–2863 (C–H), 2091 (N_3), 1736 (C═O); ^{1}H NMR: δ = 3.66 (s, 3 H, OCH_3), 3.36–3.25 (m, 1 H, H-3β), 2.40–2.29 (m, 1 H, H-23a), 2.26–2.16 (m, 1 H, H-23b), 2.02–0.94 (m, 26 H), 0.93 (s, 3 H, 3H-19), 0.91 (d, 3 H, J = 6.4 Hz, 3H-21), 0.64 (s, 3 H, 3H-18); $^{13}C\{^1H\}$: δ = 174.8, 61.6, 56.4, 55.9, 51.5, 42.7, 42.3, 40.4, 40.0, 35.8, 35.5, 35.4, 34.6, 32.4, 31.0, 30.9, 28.2, 27.1, 26.7, 26.3, 24.2, 23.4, 20.8, 18.3, 12.0. HRMS (ESI) m/z calcd for $C_{25}H_{42}N_3O_2$ $[M + H]^+$ 416.3277, found 416.3205.

Methyl 3α-azido-7β-hydroxy-5β-cholan-24-oate (17). Amorphous white solid, (828 mg, 94%). IR: ν (cm^{-1}) 3526–3315 (O–H), 2947–2867 (C–H), 2092 (N_3), 1736 (C═O); ^{1}H-NMR: δ = 3.64 (s, 3 H, OCH_3), 3.58–3.52 (m, 1 H, H-3β), 3.30–3.21 (m, 1 H, H-7α), 2.38–2.28 (m, 1 H, H-23a), 2.25–2.15 (m, 1 H, H-23b), 2.02–0.94 (m, 24 H), 0.94 (s, 3 H, 3H-19), 0.90 (d, 3 H, J = 6.44 Hz, 3H-21), 0.65 (s, 3 H, 3H-18); $^{13}C\{^1H\}$: δ = 174.7, 71.1, 60.8, 55.6, 54.8, 51.5, 43.7, 43.6, 42.7, 40.0, 39.1, 36.6, 35.2, 35.1, 34.1, 33.4, 31.0, 31.0, 28.6, 26.8, 26.6, 23.4, 21.1, 18.4, 12.1; HRMS (ESI) m/z calcd for $C_{25}H_{41}N_3O_3Na$ $[M + Na]^+$ 454.3046, found 454.3001.

General procedure for the synthesis of acids 18–21

To a stirred solution of ester **14–17** (1.74 mmol) in MeOH (10 mL), 1.5 M LiOH (19 mL) was added in one portion. The reaction mixture was heated at 50 °C for 24 h, cooled to room temperature, diluted with 1 N HCl until pH = 4, and then extracted with EtOAc (2 × 20 mL). The combined organic layers were washed with water (2 × 15 mL), dried (Na_2SO_4), filtered and concentrated to give the acid **18–21**, which was used without further purifications.

3α-Azido-7α,12α-dihydroxy-5β-cholan-24-oic acid (18). Crystallization from EtOAc (with a few drops of MeOH) afforded **18** as a white amorphous solid (580 mg, 77%). ^{1}H NMR: δ = 4.00 (bs, 1 H, H-12β), 3.87–3.86 (m, 1 H, H-7β), 3.18–3.11 (m, 1 H, H-3β), 2.49–1.01 (m, 23 H), 0.98 (d, 3 H, J = 6.0 Hz, 3H-21), 0.90 (s, 3 H, 3H-19), 0.69 (s, 3 H, 3H-18); $^{13}C\{^1H\}$: δ = 179.3, 73.1, 68.4, 61.3, 47.1, 46.5, 41.9, 41.8, 39.3, 35.4, 34.8, 34.5, 31.0, 30.7, 28.1, 27.6, 26.8, 26.5, 23.2, 22.5, 17.3, 12.5. HRMS (ESI) m/z calcd for $C_{24}H_{40}N_3O_4$ $[M + H]^+$ 434.3019, found 434.3108.

3α-Azido-7α-hydroxy-5β-cholan-24-oic acid (19). Crystallization from EtOAc (with a few drops of MeOH) afforded **19** as a white amorphous solid (689 mg, 95%). ^{1}H NMR: δ = 3.87–3.85 (m, 1 H, H-7β), 3.19–3.11 (m, 1 H, H-3β), 2.45–0.94 (26 H), 0.93 (d, 3 H, J = 6.4 Hz, 3H-21), 0.91 (s, 3 H, 3H-19), 0.66 (s, 3 H, 3H-18); $^{13}C\{^1H\}$: δ = 179.8, 68.4, 61.23, 55.72 50.3, 42.7, 41.8, 39.5, 39.4, 35.5, 35.4, 35.3, 35.1, 34.4, 32.7, 30.9, 30.7, 28.1, 26.8, 23.7, 22.8, 20.6, 18.2, 11.8. HRMS (ESI) m/z calcd for $C_{24}H_{40}N_3O_3$ $[M + H]^+$ 418.3070, found 418.3112.

3α-Azido-5β-cholan-24-oic acid (20). Crystallization from EtOAc (with a few drops of MeOH) afforded **20** as a white amorphous solid (551 mg, 79%). ^{1}H NMR: δ = 3.33–3.26 (m, 1 H, H-3α), 2.45–2.34 (m, 1 H, H-23a), 2.31–2.19 (m, 1 H, H-23b), 1.99–0.93 (m, 26 H), 0.92–0.90 (m, 6 H, 3H-21, 3H-19), 0.64 (s, 3 H, 3H-18); $^{13}C\{^1H\}$: δ = 180.2, 61.2, 56.3, 55.9, 42.7, 42.3, 40.4, 40.0, 35.8, 35.5, 35.3, 34.6, 32.4, 30.9, 30.7, 28.1, 27.1, 26.7, 26.3, 24.2, 23.4, 20.8, 18.2, 12.0. HRMS (ESI) m/z calcd for $C_{24}H_{40}N_3O_2$ $[M + H]^+$ 402.3121, found 402.3179.

3α-Azido-7β-hydroxy-5β-cholan-24-oic acid (21). Crystallization from EtOAc (with a few drops of MeOH) afforded **21** as a white amorphous solid (570 mg, 80%) slightly contaminated by an uncharacterized alkene byproduct. ^{1}H NMR: δ = 3.61–3.55 (m, 1 H, H-7α), 3.31–3.23 (m, 1 H, H-3β), 2.44–2.34 (m, 1 H, H-23a), 2.30–2.20 (m, 1 H, H-23b), 2.10–0.97 (m, 24 H), 0.96 (s, 3 H, 3H-19), 0.93 (d, 3 H, J = 6.4 Hz, 3H-21), 0.67 (s, 3 H, 3H-18); $^{13}C\{^1H\}$: δ = 179.6, 71.2, 60.8, 55.6, 54.8, 43.7, 43.6, 42.7, 39.9, 39.1, 36.6, 35.2, 35.1, 34.1, 33.4, 30.9, 30.8, 28.6, 26.8, 23.4, 22.3, 21.2, 18.3, 12.1. ESI-MS (417.2): 418.3 $[M + H]^+$.

General procedure for the synthesis of *S*-trityl protected bile acid derivatives 23–26

To a cooled (0 °C), stirred solution of acid **18–21** (0.96 mmol) in THF (12 mL) triethylamine (201 μL, 1.44 mmol) and ethyl chloroformate (138 μL, 1.44 mmol) were added dropwise. The

resulting mixture was stirred at 0 °C for 45 min, then 2-(tritylsulfanyl)ethanamine (442 mg, 1.44 mmol) was added and the stirring continued for 20 h at room temperature. The mixture was concentrated and the resulting crude material was dissolved in CH_2Cl_2 (30 mL) and washed with water (2 × 15 mL), 0.1 N HCl solution (2 × 15 mL), water (2 × 15 mL), and brine (2 × 15 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated, and eluted from a column of silica gel with the suitable elution system to give the *S*-trityl protected bile acid derivative **23–26**.

***N*-(2-Tritylsulfanylethyl)-3α-azido-7α,12α-dihydroxy-5β-cholan-24-amide (23).** Column chromatography with 2 : 1 (1% MeOH) CH_2Cl_2–AcOEt afforded **23** (536 mg, 76%) as a white amorphous solid. ^{1}H NMR: δ = 7.43–7.38 (m, 6 H, Ar), 7.32–7.19 (m, 9 H, Ar), 5.71 (bs, 1 H, NH), 3.98–3.96 (bs, 1 H, H-12β), 3.84–3.82 (m, 1 H, H-7β), 3.20–3.03 (m, 3 H, H-3β, 2H-25), 2.41 (t, 2 H, J = 6.3 Hz, 2H-26), 2.38–0.97 (m, 24 H), 0.95 (d, 3 H, J = 6.4 Hz, 3H-21), 0.90 (s, 3 H, 3H-19), 0.67 (s, 3 H, 3H-18); ^{13}C{^{1}H}: δ = 173.6, 144.6, 132.5, 129.5, 127.8, 126.6, 72.9, 68.1, 61.3, 46.8, 46.5, 41.9, 41.8, 39.4, 38.1, 35.5, 35.3, 34.7, 34.5, 32.9, 31.9, 31.4, 28.2, 27.5, 26.8, 26.6, 23.2, 22.6, 17.4, 12.5. HRMS (ESI) m/z calcd for $C_{45}H_{59}N_4O_3S$ $[M + H]^+$ 735.4308, found 735.4381.

***N*-(2-Tritylsulfanylethyl)-3α-azido-7α-hydroxy-5β-cholan-24-amide (24).** Column chromatography with 3 : 1 (5% CH_2Cl_2) cyclohexane–AcOEt afforded **24** (573 mg, 83%) as a white amorphous solid. ^{1}H NMR: δ = 7.43–7.39 (m, 6 H, Ar), 7.31–7.26 (m, 6 H, Ar), 7.24–7.19 (m, 3 H, Ar), 5.47–5.45 (m, 1 H, NH), 3.85 (bs, 1 H, H-7β), 3.20–3.02 (m, 3 H, H-3β, 2H-25), 2.41 (t, 2 H, J = 6.3 Hz, 2H-26), 2.37–0.94 (m, 26 H), 0.92 (d, 3 H, J = 2.6 Hz, 2H-21), 0.91 (s, 3 H, 3H-19), 0.64 (s, 3 H, 3H-18); ^{13}C{^{1}H}: δ = 173.2, 144.6, 129.5, 127.9, 126.8, 68.3, 61.4, 55.8, 50.3, 42.7, 41.8, 39.5, 39.7, 38.0, 35.6, 35.4, 35.1, 34.4, 33.5, 32.8, 32.1, 31.6, 28.2, 26.8, 23.7, 22.9, 20.6, 18.4, 11.8. HRMS (ESI) m/z calcd for $C_{45}H_{59}N_4O_2S$ $[M + H]^+$ 719.4359, found 719.4412.

***N*-(2-Tritylsulfanylethyl)-3α-azido-5β-cholan-24-amide (25).** Column chromatography with 5 : 1 (5% CH_2Cl_2) cyclohexane–AcOEt afforded **25** (291 mg, 43%) as a white amorphous solid. ^{1}H NMR: δ = 7.43–7.39 (m, 6 H, Ar), 7.31–7.26 (m, 6 H, Ar), 7.24–7.19 (m, 3 H, Ar), 5.46 (bs, 1 H, NH), 3.35–3.37 (m, 1 H, H-3β), 3.10–3.06 (m, 2 H, 2H-25), 2.41 (t, 2 H, J = 6.3 Hz, 2H-26), 2.20–0.94 (m, 28 H), 0.93 (s, 3 H, 3H-19), 0.90 (d, J = 6.5 Hz, 3 H, 3H-21), 0.63 (s, 3H, 3H-18); ^{13}C{^{1}H}: δ = 173.3, 144.6, 129.5, 127.9, 126.8, 61.3, 56.4, 55.9, 42.7, 42.4, 40.4, 40.1, 38.0, 35.8, 35.5, 35.4, 34.6, 33.5, 32.4, 32.1, 31.6, 28.2, 27.1, 26.7, 26.3, 24.2, 23.4, 20.8, 18.4, 12.1. HRMS (ESI) m/z calcd for $C_{45}H_{59}N_4OS$ $[M + H]^+$ 703.4410, found 703.4485.

***N*-(2-Tritylsulfanylethyl)-3α-azido-7β-hydroxy-5β-cholan-24-amide (26).** Column chromatography with 1.5 : 1 (5% CH_2Cl_2) cyclohexane–AcOEt afforded **26** (414 mg, 60%) as a white amorphous solid. ^{1}H NMR: δ = 7.43–7.39 (m, 6 H, Ar), 7.31–7.26 (m, 6 H, Ar), 7.22–7.20 (m, 3 H, Ar), 5.51 (bs, 1 H, NH), 3.60–3.54 (m, 1 H, H-7α), 3.31–3.23 (m, 1 H, H-3β), 3.07–3.00 (m, 2 H, 2H-25), 2.41 (t, 2 H, J = 6.3 Hz, 2H-26), 2.20–0.98 (m, 26 H), 0.96 (s, 3 H, 3H-19), 0.91 (d, 3 H, J = 6.5 Hz, 3H-21), 0.66 (s, 3 H, 3H-18); ^{13}C{^{1}H}: δ = 173.3, 144.6, 129.5, 127.9, 126.8, 71.1, 60.9, 55.6, 54.9, 43.7, 43.7, 42.7, 40.0, 39.1, 38.1, 36.6, 35.3, 35.1, 34.1, 33.5, 33.4, 32.1, 31.7, 28.7, 26.8, 26.6, 23.5, 21.2, 18.5, 12.2. HRMS (ESI) m/z calcd for $C_{45}H_{59}N_4O_2S$ $[M + H]^+$ 719.4359, found 719.4495.

***N*-(2-Mercaptoethyl)-3α-azido-7α-hydroxy-5β-cholan-24-amide (27)**

To a stirred solution of the trityl derivative **24** (100 mg, 0.14 mmol) in CH_2Cl_2 (2 mL), triethylsilane (67 μL, 0.42 mmol) and trifluoroacetic acid (200 μL, 2.61 mmol) were added portion-wise. The mixture was stirred at room temperature for 15 min, and then concentrated. The resulting residue was triturated three times with toluene (3 × 2 mL) and after each step it was evaporated to dryness. The crude thiol was eluted from a short plug of silica gel with CH_2Cl_2 and then with 95 : 5 CH_2Cl_2–MeOH to give **27** (62 mg, 93%) as a white amorphous solid. ^{1}H NMR: δ = 5.85 (bs, 1 H, NH), 3.85 (bs, 1 H, H-7β), 3.46–3.39 (m, 2 H, 2H-25), 3.19–3.10 (m, 1 H, H-3β), 2.71–2.64 (m, 2 H, 2H-26), 2.38–0.94 (m, 9 H), 0.93 (d, 3 H, J = 6.5 Hz, 3H-21), 0.90 (s, 3 H, 3H-19), 0.66 (s, 3 H, 3H-18); ^{13}C{^{1}H}: δ = 173.7, 68.3, 61.3, 55.8, 50.3, 42.7, 42.3, 41.8, 39.5, 39.4, 35.5, 35.4, 35.1, 34.4, 33.5, 32.8, 31.7, 28.2, 26.8, 24.7, 23.7, 22.8, 20.6, 18.4, 11.8. HRMS (ESI) m/z calcd for $C_{12}H_{26}NaO_6P_2$ $[M + Na]^+$ 351.1102, found 351.1178.

Tetraethyl but-3-ene-1,1-diylbis(phosphonate) (29)

A solution of methylene bisphosphonate **28** (0.62 mL, 3.47 mmol) in dry toluene (7 mL) was slowly added under N_2 to a cooled (0 °C), stirred suspension of potassium *tert*-butoxide (428 mg, 3.82 mmol) in toluene (7 mL). After stirring for 1 h, allyl iodide (0.32 mL, 3.47 mmol) was added to the reaction mixture and stirring was continued at 0 °C for an additional 3 h. The reaction mixture was then quenched with pH 7 phosphate buffer (30 mL) and extracted with AcOEt (45 mL). The organic layer was then washed with brine (10 mL), dried (Na_2SO_4), filtered, concentrated, and eluted from a column of silica gel with cyclohexane–acetone (from 2 : 1 to 1 : 2) to give compound **29**[15] as a colorless oil (455 mg, 40%). ^{1}H NMR: δ = 6.02–5.90 (m, 1 H, HC═CH_2), 5.16–5.01 (m, 2 H, HC═CH_2), 4.17 (q, 8 H, J = 6.8 Hz, OCH_2CH$_3$), 2.75–2.60 (m, 2 H, CH_2), 2.38 (tt, 1 H, J = 6.2, 23.9 Hz, CH), 1.30 (t, 12 H, J = 6.8 Hz, OCH$_2$CH_3); ^{13}C{^{1}H}: δ = 135.9 (t, J_{CP} = 7.1 Hz), 116.6, 62.5 (dd, J_{CP} = 6.9, 9.7, Hz), 37.1 (t, J = 133.3 Hz), 29.8, 16.4, ^{31}P NMR: δ = 23.1. HRMS (ESI) m/z calcd for $C_{26}H_{45}N_4O_2S$ $[M + Na]^+$ 477.3263, found 477.3301.

Tetraethyl ethene-1,1-diylbis(phosphonate) (31)

To a stirred solution of paraformaldehyde (521 mg, 17.35 mmol) and diethylamine (0.36 mL, 3.47 mmol) in methanol (6 mL) tetraethyl methylene bisphosphonate **28** (0.86 mL, 3.47 mmol) was added in one portion. The mixture was heated to 80 °C and refluxed for two hours. The clear solution was then stirred overnight at 65 °C. The solvent was removed under reduced pressure, toluene (10 mL) was added, and removed again under reduced pressure in order to co-

evaporate residual paraformaldehyde and methanol. This dissolution and evaporation process was repeated twice. The resulting residue was eluted from a column of silica gel with 95 : 5 CH_2Cl_2–MeOH to give **30**[16] as a clear viscous oil (1.08 g, 97%). ^{1}H NMR: δ = 4.03 (m, 8 H, OCH_2CH$_3$), 3.74 (dd, 2 H, J = 5.5, J = 16.2 Hz, CH_2), 3.22 (s, 3 H, OCH_3), 2.54 (tt, 1 H, J = 5.5, 23.8 Hz, CH), 1.19 (t, J = 7.1 Hz, 12 H, OCH_2CH_3).

To a stirred mixture of **30** (505 mg, 1.52 mmol), 4 Å molecular sieves (100 mg) in toluene (10 mL), *p*-toluenesulfonic acid (8.94 mg, 0.05 mmol) was added in one portion. The mixture was refluxed overnight at 110 °C, then cooled to room temperature, filtered over a pad of Celite, and concentrated. The residue was dissolved in CH_2Cl_2 (5 mL) and extracted with H_2O (5 mL). The organic layer was dried (Na_2SO_4), filtered, and concentrated to afford **31**[16] (366.8 mg, 80%) as colorless oil and at least 95% pure as judged by ^{1}H NMR analysis. ^{1}H NMR: δ = 6.99 (2d, 2 H, J = 4.0 Hz, J = 71.6 Hz, CH_2), 4.22–4.06 (m, 8 H, OCH_2CH$_3$), 1.35 (t, 12 H, J = 7.1 Hz, OCH_2CH_3); ^{13}C{^{1}H}: δ = 149.3, 131.9 (t, J_{CP} = 166.7 Hz) 62.6, 16.3; ^{31}P NMR δ = 13.1.

Tetraethyl but-3-yne-1,1-diylbis(phosphonate) (32)

To a cooled (−15 °C), stirred solution of **31** (347 mg, 1.16 mmol) in THF (3.5 mL) sodium acetylide (18% in xylene, 0.45 mL, 1.51 mmol) was added dropwise over a period of one hour. The mixture was stirred at room temperature for 18 h, quenched with pH 7 phosphate buffer (15 mL), and then the volatiles were evaporated. The resulting residue was diluted with EtOAc (15 mL) and extracted with H_2O (10 mL). The aqueous phase was extracted again with EtOAc (15 mL). The combined organic layers were dried (Na_2SO_4), filtered, concentrated, and eluted from a column of silica gel with 4.5 : 4.5 : 0.5 CH_2Cl_2–EtOAc–MeOH to give **32**[17] (151 mg, 40%) as a pale yellow oil. ^{1}H NMR: δ = 4.22–4.18 (m, 8 H, OCH_2CH$_3$), 2.83 (ddd, J = 2.7, 6.1, 16.3, 1 H, CH_2–C≡C–H), 2.80 (ddd, J = 2.7, 6.1, 16.3, 1 H, CH_2≡C–H), 2.54 (tt, J = 6.2, 23.4, Hz, 1 H, CHP), 2.05 (t, 1 H, J = 2.7 Hz, C≡C–H), 1.33 (t, J = 7.1 Hz, 12 H, OCH_2CH_3); ^{13}C{^{1}H}: δ = 81.2 (t, J_{CP} = 11.1 Hz), 62.9, 62.8 (d, J_{CP} = 7.0 Hz), 36.5 (t, J_{CP} = 134.3 Hz), 16.3 (d, J_{CP} = 5.1 Hz), 15.5 (t, J_{CP} = 4.9 Hz); ^{31}P NMR: δ = 21.4.

Tetraethyl (2-((2-mercaptoethyl)amino)ethane-1,1-diyl)bis(phosphonate) (33)

To a stirred solution of **31** (175 mg, 0.58 mmol) in anhydrous CH_2Cl_2 (0.7 mL) amine **22** (186 mg, 0.58 mmol) was added under an Argon atmosphere. The mixture was stirred at room temperature for 2 h and then concentrated to give the crude tetraethyl (2-((2-(tritylthio)ethyl)amino)ethane-1,1-diyl) bis(phosphonate) intermediate (358 mg, >95%), at least 95% pure as judged by ^{1}H NMR analysis. ^{1}H NMR: δ = 7.44–7.35 (m, 6 H, Ar), 7.31–7.22 (m, 6 H, Ar), 7.22–7.14 (m, 3 H, Ar), 4.20–4.09 (m, 8 H, OCH_2CH$_3$), 3.01 (td, 2 H, J = 5.8, 16.6, Hz, HC–CH_2–N), 2.56 (tt, 1 H, J = 5.8, 23.5 Hz, CH), 2.48 (t, 2 H, J = 6.7 Hz, N–CH_2CH$_2$), 2.33 (t, 2 H, J = 6.8 Hz, CH_2–S), 1.37–1.21 (m, 12 H, OCH_2CH_3). ^{13}C{^{1}H}: δ = 129.7, 127.9, 126.7, 66.6, 62.9, 62.8, 62.7, 62.6, 47.8, 45.5, 37.7 (t, J_{CP} = 132.4 Hz), 32.2, 16.50, 16.5. ^{31}P NMR δ = 22.5. ESI-MS (619.7): 620.9 $[M + H]^+$.

To a stirred solution of the above *S*-trityl derivative (68 mg, 0.11 mmol) in CH_2Cl_2 (2 mL), triethylsilane (54 μL, 0.34 mmol) and trifluoroacetic acid (100 μL, 1.31 mmol) were added portion-wise. The mixture was stirred at room temperature for 60 min, and then concentrated. The resulting residue was triturated three times with toluene (3 × 2 mL) and after each step it was evaporated to dryness. The crude thiol was eluted from a short plug of silica gel with 95 : 5 CH_2Cl_2–MeOH to give **33** in the form of trifluoroacetic salt (50 mg, 93%) as a with solid and slightly contaminated by uncharacterized byproduct. ^{1}H NMR: δ = 10.21 (bs, 2 H, NH_2), 4.30–4.14 (m, 8 H, OCH_2CH$_3$), 3.60–3.44 (m, 2 H, HC–CH_2–N), 3.35–3.15 (m, 3 H, CH, N–CH_2CH$_2$), 2.95–2.80 (m, 2 H, CH_2–S), 1.76 (bs, 1 H, SH), 1.34 (t, 12 H, J = 7.1 Hz, OCH_2CH_3). ^{13}C{^{1}H}: δ = 163.7–156.7 (m), 131.2–111.2 (m), 64.8, 50.8, 44.1, 33.5 (t, J_{CP} = 133.8 Hz), 20.9, 16.2. ^{31}P NMR: δ = 18.8. ESI-MS (377.1): 378.2 $[M + H]^+$.

Tail-functionalized BA–BP conjugate 34

To a stirred solution of **27** (67 mg, 0.14 mmol) in DMF (0.7 mL), bisphosphonate **29** (34 mg, 0.09 mmol) and 2,2-dimethoxy-2-phenyl-acetophenone (3.6 mg, 0.014 mmol) were added. The resulting mixture was irradiated at room temperature for 1 h under vigorous magnetic stirring, then concentrated and eluted from a column of silica gel with 1 : 2 CH_2Cl_2–acetone to give the conjugate **34** as a light yellow oil (46 mg, yield 64%). ^{1}H NMR: δ = 6.30 (bs, 1 H, NH), 4.24–4.12 (m, 8 H, OCH_2CH$_3$), 3.85 (bs, 1 H, H-7β), 3.45–3.40 (m, 2 H, 2H-25), 3.18–3.10 (m, 1 H, H-3β), 2.70–2.65 (m, 2 H, 2H-26), 2.57–2.50 (m, 2 H, 2H-27), 2.38–0.96 (m, 40 H), 0.92 (d, J = 6.4 Hz, 3 H, 3H-21), 0.91 (s, 3 H, 3H-19), 0.88 (t, 3 H), 0.66 (s, 3 H, 3H-18); ^{13}C{^{1}H}: δ = 174.3, 68.5, 62.9, 62.8, 62.6 (dd, J_{CP} = 20.8, 5.4 Hz), 61.6, 56.0, 50.6, 42.9, 42.0, 39.8, 39.6, 38.6, 38.5 (t, J_{CP} = 132.7 Hz), 36.6, 35.7, 35.3, 34.6, 33.4, 32.9, 32.2, 31.9, 31.6, 29.9, 28.4, 27.0, 24.8, 23.9, 23.1, 20.8, 18.6, 16.6, 14.3, 12.0; ^{31}P NMR: δ = 23.5. HRMS (ESI) m/z calcd for $C_{38}H_{71}N_4O_8P_2S$ $[M + H]^+$ 805.4468, found 805.4501.

Deprotected B–BP conjugate 35

To a cooled (0 °C), stirred solution of **34** (54 mg, 0.07 mmol) in CH_2Cl_2 (0.5 mL) 2,6-lutidine (106 μL, 0.91 mmol) and trimethylsilyl bromide (92 μL, 0.70 mmol,) were added dropwise. The reaction mixture was stirred at room temperature for 24 h. After cooling at 0 °C, MeOH (1 mL) was added and the resulting mixture was allowed to reach room temperature. The solution was then concentrated and the resulting residue was dissolved in MeOH (1 mL) and subsequently concentrated. This dissolution and evaporation process was repeated twice. The resulting residue was eluted from a column of Sephadex LH-20 with MeOH to give **35** as a white amorphous solid (26 mg, 53%). ^{1}H NMR (CD_3OD): δ = 3.80 (bs, 1 H, H-7β), 3.37–3.31 (m, 2 H, H-25), 3.22–3.11 (m, 1 H, H-3β), 2.67–2.60 (m, 2 H, 2H-26), 2.60–2.54 (m, 2 H, 2H-27), 2.43–1.11 (m, 31 H), 0.97 (d, 3 H, J = 6.5 Hz, 3H-21), 0.94 (s, 3 H, 3H-19), 0.69 (s, 3 H, 3H-18); ^{13}C{^{1}H}(CD_3OD): δ = 176.8, 68.9, 62.7, 61.5, 57.3, 51.4, 43.6, 43.3, 40.9, 40.7, 40.1 (t, J_{CP} = 132.2 Hz), 36.8, 36.6, 36.6,

36.2, 35.6, 34.1, 34.0, 33.3, 32.3, 31.9, 30.4, 29.2, 27.9, 25.7, 23.3, 21.7, 20.8, 18.9, 12.2; ^{31}P NMR (CD_3OD): δ = 21.6. HRMS (ESI) *m/z* calcd for $C_{30}H_{54}N_4O_8P_2S$ $[M]^+$ 692.3138, found 692.3195.

Head-functionalized BA–BP conjugate 36

Method A (Scheme 5). To a stirred mixture of **24** (40 mg, 0.05 mmol), bisphosphonate **32** (27 mg, 0.08 mmol), toluene (0.5 mL), and DMF (0.05 mL), DIPEA (35 μL, 0.20 mmol) and CuI (2 mg, 0.01 mmol) were sequentially added. The resulting mixture was stirred in the dark for 18 h, then fresh portions of DIPEA (35 μL, 0.20 mmol) and CuI (2 mg, 0.01 mmol) were added and the mixture stirred in the dark for an additional 18 h. Then, the mixture was concentrated and eluted from a column of silica gel with 9 : 1 CH_2Cl_2–MeOH to give **36** (37 mg, 65%) as a white amorphous solid. ^{1}H NMR: δ = 7.53 (s, 1 H, H-triazole), 7.44–7.38 (m, 6 H, Ar), 7.33–7.27 (m, 6 H, Ar), 7.25–7.18 (m, 3 H, Ar), 5.47 (bs, 1 H, NH), 4.33 (bs, 1 H, H-3β), 4.23–4.03 (m, 8 H, OC*H*$_2$CH$_3$), 3.86 (bs, 1 H, H-7β), 3.32 (td, 2 H, *J* = 6.3, 16.1 Hz, 2H-27), 3.13–2.81 (m, 3 H, 2H-25, H-28), 2.41 (t, 2 H, *J* = 6.2 Hz, 2H-26), 2.29–1.03 (m, 40 H), 0.98 (s, 3 H, 3H-19), 0.93 (d, 3 H, *J* = 6.4 Hz, 3H-21), 0.66 (s, 3 H, H-18); ^{13}C{^{1}H}: δ = 173.2, 146.8, 144.6, 129.5, 127.9, 127.9, 127.2, 126.8, 120.7, 68.2, 66.8, 63.1, 62.8 (dd, J_{CP} = 35.0, 6.4 Hz), 61.5, 55.9, 55.5, 50.3, 42.7, 42.1, 39.4, 38.1, 37.8, 36.9, 36.6 (t, J_{CP} = 130.2 Hz), 35.7, 35.4, 35.2, 35.1, 34.2, 33.5, 33.1, 32.8, 32.1, 31.6, 28.2, 28.1, 23.7, 22.8, 21.9, 20.6, 18.4, 16.3, 11.8; ^{31}P NMR: δ = 22.2. HRMS (ESI) *m/z* calcd for $C_{57}H_{83}N_4O_8P_2S$ $[M + H]^+$ 1045.5407, found 1045.5486.

Method B (Table 1). To a stirred mixture of the fluorous tagged BA scaffold **41** (56 mg, 0.05 mmol), bisphosphonate **32** (27 mg, 0.08 mmol), toluene (0.5 mL), and DMF (0.05 mL), DIPEA (35 μL, 0.20 mmol) and CuI (2 mg, 0.01 mmol) were sequentially added. The resulting mixture was stirred in the dark for 18 h, then fresh portions of DIPEA (35 μL, 0.20 mmol) and CuI (2 mg, 0.01 mmol) were added and the mixture stirred in the dark for an additional 18 h, and then concentrated. The residue was taken up in CH_2Cl_2 (0.2 mL) and applied to a small column containing FluoroFlash® silica gel (4 g), which was pre-eluted with 8 : 2 MeOH–H_2O. The column was eluted with 8 : 2 MeOH–H_2O until all the non fluorous by-products were removed. Subsequently, the column was eluted with pure MeOH to obtain the fluorous tagged derivative **36F** as a white amorphous solid (68 mg, yield 95%). ^{1}H NMR: δ = 7.49 (s, 1 H, H-triazole), 7.44–7.37 (m, 6 H, Ar), 7.31–7.26 (m, 6 H, Ar), 7.23–7.19 (m, 3 H, Ar), 5.44 (bs, 1 H, NH), 5.20 (bs, 1 H, H-7β), 4.48–4.32 (m, 1 H, H-3β), 4.21–4.00 (m, 8 H, OC*H*$_2$CH$_3$), 3.37–3.23 (m, 2 H, 2H-27), 3.11–2.94 (m, 3 H, 2H-25, H-28), 2.41 (t, 2 H, *J* = 6.3 Hz, 2H-26), 2.37–1.05 (m, 40 H), 1.03 (s, 3 H, 3H-19), 0.91 (d, *J* = 6.5 Hz, 3 H, 3H-21), 0.65 (s, 3 H, 3H-18); ^{13}C{^{1}H}: δ = 173.0, 157.5 (t, *J* = 23.6 Hz), 144.6, 129.5, 127.9, 126.8, 119.9, 62.8, 62.5, 60.8, 55.9, 49.7, 42.7, 41.4, 39.1, 38.1, 38.0, 36.6 (t, J_{CP} = 130.2 Hz), 35.8, 35.6, 35.4, 34.9, 34.0, 33.7, 32.0, 31.4, 31.3, 29.7, 28.4, 27.8, 23.4, 22.7, 21.9, 20.5, 18.3, 16.2, 11.7; ^{31}P NMR: δ = 22.3; ^{19}F NMR: δ = −80.6 (s, 3 F, CF_3), −118.13 (s, 2 F, CF_2), −121.39 (s, 2 F, CF_2), −121.94 (s, 2 F, CF_2), −122.11 to −122.37 (m, 2 F, CF_2), −122.67 (s, 2 F, CF_2), −126.01 (s, 2 F, CF_2). ESI MS (1441.3): 1464.9 $[M + Na]^+$.

To a stirred mixture of **36F** (68 mg, 0.05 mmol), EtOH (0.8 mL), H_2O (0.1 mL), 0.5 M NaOH (0.2 mL, 0.10 mmol) was added in one portion. The resulting mixture was stirred at room temperature for 1 hour and then concentrated under reduced pressure. The residue was taken up in CH_2Cl_2 (0.2 mL) and applied to a small column containing FluoroFlash® silica gel (4 g), which was pre-eluted with 8 : 2 MeOH–H_2O. The column was eluted with 8 : 2 MeOH–H_2O to collect **36** (45 mg, 92%) at least 95% pure as judged by ^{31}P NMR analysis. Subsequently, the column was eluted with pure MeOH to obtain the fluorous tagged by-product.

Fluorescently-labelled BA–BP conjugate 38

To a stirred mixture of **34** (40 mg, 0.05 mmol), dansyl alkyne **37** (25 mg, 0.08 mmol), toluene (0.5 mL), and DMF (0.05 mL), DIPEA (35 μL, 0.20 mmol) and CuI (2 mg, 0.01 mmol) were sequentially added. The resulting mixture was stirred in the dark for 18 h, then fresh portions of DIPEA (35 μL, 0.20 mmol) and CuI (2 mg, 0.01 mmol) were added and the mixture stirred in the dark for an additional 18 h. Then, the mixture was concentrated and eluted from a column of silica gel with 9 : 1 CH_2Cl_2–MeOH to give **38** (31 mg, 55%) as a yellow oil. ^{1}H NMR: δ = 8.60–8.58 (m, 1 H, Ar), 8.28–8.21 (m, 2 H, Ar), 7.62–7.49 (m, 2 H, Ar), 7.27 (s, 1 H, H-triazole), 7.27–7.18 (m, 1 H, Ar), 6.16–6.10 (m, 1 H, NH), 4.36–4.26 (m, 1 H, H-3β), 4.23–4.10 (m, 8 H, OC*H*$_2$CH$_3$), 4.00–3.97 (m, 2 H, CH_2O), 3.87 (bs, 1 H, H-7β), 3.44–3.39 (m, 2 H, 2H-25), 2.88 (s, 6 H, NCH_3), 2.70–2.65 (m, 2 H, 2H-26), 2.57–2.50 (m, 2 H, 2H-27), 2.39–1.03 (m, 49 H), 0.98 (s, 3 H, 3H-19), 0.93 (d, 3 H, *J* = 6.4 Hz, 3H-21), 0.66 (s, 3 H, 3H-18); ^{13}C{^{1}H}: δ = 173.8, 146.9, 131.7, 130.7, 128.9, 123.3, 119.6, 118.5, 115.8, 70.8, 68.5, 62.81 (dd, J_{CP} = 14.9, 6.5 Hz), 61.1, 56.1, 50.6, 45.7, 42.9, 42.4, 39.7, 39.6, 38.6 (t, J_{CP} = 131.4 Hz), 38.4, 37.2, 36.6, 36.1, 35.7, 35.5, 34.8, 34.6, 33.7, 33.1, 32.2, 31.9, 31.5, 28.9, 28.5, 25.4, 25.1, 24.8, 23.9, 23.1, 22.8, 20.9, 18.6, 16.7, 16.6, 12.0; ^{31}P NMR: δ = 23.5. HRMS (ESI) *m/z* calcd for $C_{56}H_{91}N_5NaO_{11}P_2S_2$ $[M + Na]^+$ 1158.5529, found 1158.5502.

Fluorescently-labelled BA 39

To a stirred solution of **27** (67 mg, 0.14 mmol) in DMF (0.7 mL), dansyl alkyne **37** (70 mg, 0.21 mmol) and 2,2-dimethoxy-2-phenyl-acetophenone (3.6 mg, 0.014 mmol) were added. The resulting mixture was irradiated at room temperature for 1 h under vigorous magnetic stirring, then concentrated and eluted from a column of silica gel with 100 : 0 to 98 : 2 CH_2Cl_2–MeOH to give **39** (49 mg, 43%) as a yellow oil and as a ~1 : 1 mixture of *E*/*Z* isomers. ^{1}H NMR: δ = 8.60 (d, 1 H, *J* = 8.6 Hz, Ar), 8.30–8.22 (m, 2 H, Ar), 7.63–7.51 (m, 2 H, Ar), 7.20 (d, 1 H, *J* = 7.5 Hz, Ar), 5.93–5.73 (m, 2 H, H-27, NH), 5.61–5.36 (m, 1 H, H-28), 4.04–3.95 (m, 2 H, CH_2O), 3.84 (bs, 1 H, H-7β), 3.46–3.37 (m, 2 H, 2H-25), 3.20–3.08 (m, 1 H, H-3β), 2.89 (s, 6 H, NCH_3), 2.80–2.62 (m, 2 H, 2H-26), 2.40–0.93 (m, 33 H), 0.91 (d, 3 H, *J* = 3.1 Hz, 3H-21), 0.90 (s, 3 H, 3H-19), 0.63 (s, 3 H, 3H-18); ^{13}C{^{1}H}: δ = 173.6, 131.4, 131.3, 130.4, 129.9,

129.5, 128.6, 124.9, 123.1, 122.3, 119.4, 115.5, 70.6, 68.3, 61.4, 55.7, 50.3, 45.4, 42.3, 42.2, 41.8, 39.5, 39.4, 39.3, 38.4, 35.5, 35.4, 35.1, 34.4, 33.7, 33.5, 33.4, 32.8, 32.2, 31.6, 28.2, 26.8, 24.9, 24.6, 23.7, 22.8, 20.6, 18.4, 11.8. HRMS (ESI) *m*/*z* calcd for $C_{44}H_{66}N_5O_5S_2$ $[M + H]^+$ 808.4505, found 808.4573.

Fluorescently-labelled BA–BP conjugate 40

To a stirred mixture of **39** (40 mg, 0.05 mmol), bisphosphonate **32** (27 mg, 0.08 mmol), toluene (0.5 mL), and DMF (0.05 mL), DIPEA (35 μL, 0.20 mmol) and CuI (2 mg, 0.01 mmol) were sequentially added. The resulting mixture was stirred in the dark for 18 h, then fresh portions of DIPEA (35 μL, 0.20 mmol) and CuI (2 mg, 0.01 mmol) were added and the mixture stirred in the dark for an additional 18 h. Then, the mixture was concentrated and eluted from a column of silica gel with 95 : 5 CH_2Cl_2–MeOH to give **40** (31 mg, 55%) as a yellow amorphous solid and as a ~1 : 1 mixture of *E*/*Z* isomers. ^{1}H NMR: δ = 8.62 (d, J = 7.9 Hz, 1 H, Ar), 8.32–8.21 (m, 2 H, Ar), 7.65–7.50 (m, 2 H, Ar), 7.25 (s, 1 H, H-triazole), 7.22 (d, 1 H, J = 6.7 Hz, Ar), 5.94–5.70 (m, 2 H, H-27, NH), 5.61–5.37 (m, 1 H, H-28), 4.39–4.26 (m, 1 H, H-3β), 4.24–4.04 (m, 8 H, OCH_2CH_3), 4.04–3.95 (m, 2 H, CH_2OS), 3.85 (bs, 1 H, H-7β), 3.49–3.23 (m, 4 H), 2.90 (s, 6 H, NCH_3), 2.81–2.70 (m, 2 H, 2H-26), 2.29–1.07 (m, 47 H), 0.97 (s, 3 H, 3H-19), 0.92 (d, 3 H, J = 6.2 Hz, 3H-21), 0.66 (s, 3 H, 3H-18); ^{13}C{^{1}H}: δ = 173.7, 144.4, 131.7, 131.3, 130.7, 130.0, 129.5, 128.6, 125.0, 123.8, 122.4, 116.1, 70.8, 68.3, 63.0, 62.6, 61.2, 55.9, 50.4, 45.8, 42.8, 42.2, 39.5, 39.4, 38.0 (t, J_{CP} = 133.4 Hz), 37.1, 35.9, 35.5, 35.3, 34.3, 33.7, 33.5, 32.9, 32.7, 32.3, 31.7, 29.8, 28.3, 28.2, 25.0, 24.6, 23.8, 23.0, 22.2, 20.7, 18.5, 16.4, 11.9; ^{31}P NMR: δ = 23.5. HRMS (ESI) *m*/*z* calcd for $C_{56}H_{91}N_5NaO_{11}P_2S_2$ $[M + Na]^+$ 1158.5529, found 1158.5502.

Fluorous tagged BA scaffold 41

To a cooled (0 °C), stirred solution of **24** (50 mg, 0.07 mmol) in CH_2Cl_2 (1 mL) pentadecafluorooctanoyl chloride (21 μL, 0.08 mmol), triethylamine (30 μL, 0.21 mmol) and 4-(dimethylamino)pyridine (0.9 mg, 0.007 mmol) were added. The resulting mixture was stirred for 1 h at 0 °C then diluted with CH_2Cl_2 (10 mL) and washed with saturated $NaHCO_3$ (2 × 5 mL), H_2O (2 × 5 mL), and brine (2 × 5 mL). The organic layer was dried (Na_2SO_4), filtered, concentrated, and eluted from a column of silica gel with 8 : 2 cyclohexane–AcOEt (acetone 2%) to give **41** (55 mg, 71%) as a white amorphous solid. ^{1}H NMR: δ = 7.43–7.38 (m, 6 H, Ar), 7.31–7.25 (m, 6 H, Ar), 7.24–7.19 (m, 3 H, Ar), 5.45 (bs, 1 H, NH), 5.19 (s, 1 H, H-7β), 3.18–3.04 (m, 3 H, H-3β, 2H-25), 2.41 (t, 2 H, J = 6.3 Hz, 2H-26), 2.20–0.98 (m, 26 H), 0.96 (s, 3 H, 3H-19), 0.91 (d, J = 6.5 Hz, 3 H, 3H-21), 0.63 (s, 3 H, 3H-18); ^{13}C{^{1}H}: δ = 173.1, 157.8 (t, J = 29.4 Hz), 144.6, 129.5, 127.9, 126.8, 77.6, 66.8, 60.5, 55.8, 49.8, 42.7, 41.0, 39.2, 38.1, 38.0, 35.4, 35.3, 34.7, 34.4, 33.8, 33.6, 32.0, 31.5, 31.4, 27.8, 26.9, 26.7, 23.4, 22.7, 20.5, 18.3, 11.7; ^{19}F NMR: δ = −80.7 (s, 3 F, CF_3), −117.8 (s, 2 F, CF_2), −118.1 (s, 2 F, CF_2), −122.0 (s, 2 F, CF_2), −122.7 (s, 2 F, CF_2), −122.9 (s, 2 F, CF_2), −126.1 (s, 2 F, CF_2). HRMS (ESI) *m*/*z* calcd for $C_{53}H_{58}F_{15}N_4O_3S$ $[M + H]^+$ 1115.3990, found 1115.3911.

Fluorous tagged BA scaffold 42

To a stirred and degassed (Ar) solution of the trityl derivative **41** (156 mg, 0.14 mmol) in CH_2Cl_2 (2 mL), triethylsilane (67 μL, 0.42 mmol) and trifluoroacetic acid (200 μL, 2.61 mmol) were added portion-wise. The mixture was degassed under vacuum and saturated with argon (by an argon-filled balloon) three times. The mixture was stirred at room temperature for 15 min, and then concentrated to give crude **42** (110 mg, 90%) as a yellow oil. ^{1}H NMR: δ = 5.85 (bs, 1 H, NH), 5.19 (bs, 1 H, H-7β), 3.44–3.40 (m, 2 H, 2H-25), 3.16–3.08 (m, 2 H, H-3β), 2.81 (t, J = 2.8 Hz, 1 H, H–S), 2.69–2.63 (m, 2 H, 2H-26), 2.30–0.97 (m, 25 H), 0.95 (s, 3 H, 3H-19), 0.91 (d, J = 6.5 Hz, 3 H, 3H-21), 0.63 (s, 3 H, 3H-18); ^{13}C{^{1}H}: δ = 173.4, 157.7 (t, J = 29.4 Hz), 157.5, 77.6, 77.2, 60.5, 55.8, 49.7, 42.7, 42.2, 40.9, 39.2, 38.4, 37.9, 37.6, 35.4, 35.2, 34.7, 34.4, 33.8, 33.6, 31.5, 31.4, 27.8, 26.8, 26.6, 24.7, 23.4, 22.8, 20.4, 18.3, 11.6; ^{19}F NMR: δ = −80.6 (s, 3 F, CF_3), −116.8 to −118.8 (m, 2 F, CF_2), −121.5 (s, 2 F, CF_2), −122.0 (s, 2 F, CF_2), −122.1 (s, 2 F, CF_2), −122.7 (s, 2 F, CF_2), −126.6 (s, 2 F, CF_2). ESI MS (895.9): 918.8 $[M + Na]^+$.

Tail-functionalized BA–BP conjugate 43

To a stirred solution of crude **42** (61 mg, 0.07 mmol) in DMF (0.5 mL), bisphosphonate **32** (34 mg, 0.11 mmol), and 2,2-dimethoxy-2-phenyl-acetophenone (1.8 mg, 0.007 mmol) were added. The resulting mixture was irradiated at room temperature for 1 h under vigorous magnetic stirring, then concentrated. The residue was taken up in CH_2Cl_2 (0.2 mL) and applied to a small column containing FluoroFlash® silica gel (4 g), which was pre-eluted with 8 : 2 MeOH–H_2O. The column was eluted with 8 : 2 MeOH–H_2O until all the non fluorous by-products were removed. Subsequently, the column was eluted with pure MeOH to obtain the fluorous tagged derivative **43F** (84 mg, 95%) as a ~1 : 1 mixture of *E*/*Z* isomers. ^{1}H NMR: δ = 6.57 and 6.36 (2 bs, 1 H, NH), 6.04–5.95 (m, 1 H, H-27), 5.84–5.75 (m, 1 H, H-28), 5.18 (bs, 1 H, H-7β), 4.25–4.11 (m, 8 H, OCH_2CH_3), 3.54–3.20 (m, 2 H, 2H-25), 3.17–3.05 (m, 1 H, H-3β), 3.02–0.97 (m, 42 H), 0.95 (s, 3 H, 3H-19), 0.92 (d, 3 H, J = 6.3 Hz, 3H-21), 0.64 (s, 3 H, 3H-18); ^{13}C{^{1}H}: δ = 173.7, 157.8, 157.5, 128.9, 127.9, 126.0, 124.4, 77.6, 77.2, 76.9, 62.7 (dd, J_{CP} = 5.7, 25.5 Hz), 62.6, 60.6, 56.1, 55.9, 49.8, 42.8, 41.0, 39.2, 38.0 (t, J_{CP} = 133.2 Hz), 35.58, 35.36, 34.82, 34.50, 33.96, 33.67, 32.12, 31.68, 31.49, 29.77, 29.42, 27.91, 26.74, 25.20, 23.55, 22.78, 20.54, 18.41, 16.49, 11.76; ^{31}P NMR: δ = 23.7; ^{19}F NMR: δ = −80.7 (s, 3 F, CF_3), −117.2 to −118.9 (m, 2 F, CF_2), −121.6 (s, 2 F, CF_2), −121.9 (s, 2 F, CF_2), −122.0 (s, 2 F, CF_2), −122.7 (s, 2 F, CF_2), −126.1 (s, 2 F, CF_2). ESI MS (1199.9): 1223.6 $[M + Na]^+$.

To a stirred mixture of **43F** (60 mg, 0.05 mmol), EtOH (0.8 mL), H_2O (0.1 mL), 0.5 M NaOH (0.2 mL, 0.10 mmol) was added in one portion. The resulting mixture was stirred at room temperature for 1 hour and then concentrated under reduced pressure. The residue was taken up in CH_2Cl_2 (0.2 mL) and applied to a small column containing FluoroFlash® silica gel (4 g), which was pre-eluted with 8 : 2

MeOH–H_2O. The column was eluted with 8 : 2 MeOH–H_2O to collect **43** (37 mg, 91%) as a yellow oil and as a ~1 : 1 mixture of *E*/*Z* isomers and at least 95% pure as judged by ^{1}H NMR analysis. Subsequently, the column was eluted with pure MeOH to obtain the fluorous tagged by-product. ^{1}H NMR: δ = 6.52 (bs, 1 H, NH), 5.96 (d, 1 H, J = 9.3 Hz, H-27), 5.80 (dd, 1 H, J = 7.0, 16.1 Hz, H-28), 4.26–4.07 (m, 8 H, OCH_2CH$_3$), 3.84 (bs, 1 H, H-7β), 3.43–3.36 (m, 2 H, 2H-25), 3.19–3.05 (m, 1 H, H-3β), 2.9–2.72 (m, 4 H, 2H-26, 2H-29), 0.92 (d, 3 H, J = 6.3 Hz, 3H-21), 0.90 (s, 3 H, 3H-19), 0.64 (s, 3 H, 3H-18); ^{13}C{^{1}H}: δ = 174.7, 129.0, 126.3, 68.4, 63.0 (dd, J_{CP} = 5.7, 25.5 Hz), 62.9, 61.6, 60.6, 56.0, 50.6, 42.9, 42.0, 39.8, 39.6, 39.5, 38.7 (t, J_{CP} = 133.2 Hz), 35.8, 35.7, 35.3, 34.6, 34.1, 33.0, 32.3, 32.0, 29.9, 29.6, 28.4, 27.0, 25.3, 23.9, 23.1, 21.3, 20.8, 18.6, 16.7, 16.6, 14.4, 12.0; ^{31}P NMR: δ = 22.7. ESI MS (803.0): 804.1 [M + H]$^+$.

Tail-functionalized BA–BP conjugate 44

Method A. To a stirred solution of crude **42** (61 mg, 0.07 mmol) in CH_2Cl_2 (2 mL), bisphosphonate **31** (32 mg, 0.11 mmol) and triethylamine (15 μL, 0.11 mmol) were added. The resulting mixture was stirred at room temperature for 18 h and then concentrated. The residue was taken up in CH_2Cl_2 (0.2 mL) and applied to a small column containing FluoroFlash® silica gel (4 g), which was pre-eluted with 8 : 2 MeOH–H_2O. The column was eluted with 8 : 2 MeOH–H_2O until all the non fluorous by-products were removed. Subsequently, the column was eluted with pure MeOH to obtain the fluorous tagged derivative **44F** (77 mg, 95%) as a yellow oil and at least 95% pure as judged by ^{1}H NMR analysis. ^{1}H NMR: δ = 6.64 (bs, 1 H, NH), 5.16 (bs, 1 H, H-7β), 4.23–4.14 (m, 8 H, OCH_2CH$_3$), 3.49–3.42 (m, 2 H, 2H-25), 3.17–2.98 (m, 3 H, H-3β, 3H-27), 2.73–2.51 (m, 3 H, 2H-26, H-28), 2.32–0.96 (m, 27 H), 0.94 (s, 3 H, 3H-19), 0.91 (d, 3 H, J = 6.4 Hz, 2H-21), 0.64 (s, 3 H, 3H-18); ^{13}C{^{1}H}: δ = 173.6, 158.1, 157.8, 157.5, 63.1, 63.0 (dd, J_{CP} = 5.7, 25.5 Hz), 62.9, 62.8, 60.6, 56.0, 49.8, 42.8, 41.0, 39.2, 38.7, 38.0 (t, J_{CP} = 133.2 Hz), 38.0, 35.6, 35.3, 34.8, 34.5, 33.9, 33.7, 32.9, 31.6, 31.5, 29.7, 27.9, 27.3, 27.2, 26.7, 23.5, 22.0, 20.5, 18.4, 16.4, 16.42, 11.7; ^{31}P NMR: δ = 21.4; ^{19}F NMR: δ = −80.6 (s, 3 F, CF_3), −117.9 to −118.9 (m, 2 F, CF_2), −121.5 (s, 2 F, CF_2), −122.0 (s, 2 F, CF_2), −122.2 (s, 2 F, CF_2), −122.7 (s, 2 F, CF_2), −126.1 (s, 2 F, CF_2). ESI MS (1172.9): 1196.2 [M + Na]$^+$.

To a stirred mixture of **44F** (58 mg, 0.05 mmol), EtOH (0.8 mL), H_2O (0.1 mL), 0.5 M NaOH (0.2 mL, 0.10 mmol) was added in one portion. The resulting mixture was stirred at room temperature for 1 hour and then concentrated under reduced pressure. The residue was taken up in CH_2Cl_2 (0.2 mL) and applied to a small column containing FluoroFlash® silica gel (4 g), which was pre-eluted with 8 : 2 MeOH–H_2O. The column was eluted with 8 : 2 MeOH–H_2O to collect **44** (38 mg, 92%) as a yellow oil at least 95% pure as judged by ^{1}H NMR analysis. Subsequently, the column was eluted with pure MeOH to obtain the fluorous tagged by-product. ^{1}H NMR: δ = 6.75 (bs, 1 H, NH), 4.26–4.12 (m, 8 H, OCH_2CH$_3$), 3.83 (bs, 1 H, H-7β), 3.51–3.41 (m, 2 H, 2H-25), 3.19–2.97 (m, 3 H, H-3β, 2H-27), 2.74–2.48 (m, 3 H, 2H-26, H-28), 2.44–0.94 (m, 38 H), 0.92 (d, 3 H, J = 6.4 Hz, 3H-21), 0.90 (s, 3 H, 3H-19), 0.64 (s, 3 H, 3H-18); ^{13}C{^{1}H}: δ = 173.8, 68.2, 62.9 (dd, J_{CP} = 22.1, 6.3 Hz), 61.3, 55.8, 50.3, 42.6, 41.8, 39.5, 39.4, 38.6 (t, J_{CP} = 132.5 Hz), 38.0, 35.5, 35.5, 35.4, 35.1, 34.4, 33.3, 32.8, 32.7, 31.7, 28.2, 27.2, 26.8, 23.7, 22.8, 20.6, 18.3, 16.4, 16.4, 11.8; ^{31}P NMR: δ = 21.5. ESI MS (776.9): 800.1 [M + Na]$^+$.

Method B. To a stirred solution of crude **42** (61 mg, 0.07 mmol) in DMF (0.5 mL), bisphosphonate **31** (32 mg, 0.11 mmol), and 2,2-dimethoxy-2-phenyl-acetophenone (1.8 mg, 0.007 mmol) were added. The resulting mixture was irradiated at room temperature for 1 h under vigorous magnetic stirring, then concentrated. The residue was taken up in CH_2Cl_2 (0.2 mL) and applied to a small column containing FluoroFlash® silica gel (4 g), which was pre-eluted with 8 : 2 MeOH–H_2O. The column was eluted with 8 : 2 MeOH–H_2O until all the non fluorous by-products were removed. Subsequently, the column was eluted with pure MeOH to obtain the fluorous tagged derivative **44F** (77 mg, 95%) at least 95% pure as judged by ^{1}H NMR analysis.

To a stirred mixture of **44F** (58 mg, 0.05 mmol), EtOH (0.8 mL), H_2O (0.1 mL), 0.5 M NaOH (0.2 mL, 0.10 mmol) was added in one portion. The resulting mixture was stirred at room temperature for 1 hour and then concentrated under reduced pressure. The residue was taken up in CH_2Cl_2 (0.2 mL) and applied to a small column containing FluoroFlash® silica gel (4 g), which was pre-eluted with 8 : 2 MeOH–H_2O. The column was eluted with 8 : 2 MeOH–H_2O to collect **44** (35 mg, 89%) as a yellow oil at least 95% pure as judged by ^{1}H NMR analysis. Subsequently, the column was eluted with pure MeOH to obtain the fluorous tagged by-product.

Fluorescently-labelled BA–BP conjugate 45

To a stirred mixture of **44F** (59 mg, 0.05 mmol), dansyl alkyne **37** (25 mg, 0.08 mmol), toluene (0.5 mL), and DMF (0.05 mL), DIPEA (35 μL, 0.20 mmol) and CuI (2 mg, 0.01 mmol) were sequentially added. The resulting mixture was stirred in the dark for 18 h, then fresh portions of DIPEA (35 μL, 0.20 mmol) and CuI (2 mg, 0.01 mmol) were added and the mixture stirred in the dark for an additional 18 h and finally concentrated. The residue was taken up in CH_2Cl_2 (0.2 mL) and applied to a small column containing FluoroFlash® silica gel (4 g), which was pre-eluted with 8 : 2 MeOH–H_2O. The column was eluted with 8 : 2 MeOH–H_2O until all the non fluorous by-products were removed. Subsequently, the column was eluted with pure MeOH to obtain the fluorous tagged derivative **45F** (62 mg, 83%), which was subjected to the detagging procedure. ESI MS (1504.4): 1527.8 [M + Na]$^+$.

To a stirred mixture of **45F** (75 mg, 0.05 mmol), EtOH (0.8 mL), H_2O (0.1 mL), 0.5 M NaOH (0.2 mL, 0.10 mmol) was added in one portion. The resulting mixture was stirred at room temperature for 1 hour and then concentrated under reduced pressure. The residue was taken up in CH_2Cl_2 (0.2 mL) and applied to a small column containing FluoroFlash® silica gel (4 g), which was pre-eluted with 8 : 2

MeOH–H_2O. The column was eluted with 8 : 2 MeOH–H_2O to collect **45** (49 mg, 90%) as a yellow amorphous solid and at least 90% pure as judged by ^{1}H NMR analysis. Subsequently, the column was eluted with pure MeOH to obtain the fluorous tagged by-product. ^{1}H NMR: δ = 8.75–8.65 (m, 1 H, Ar), 8.35–8.24 (m, 2 H, Ar), 7.6–7.54 (m, 2 H, Ar), 7.35–7.18 (m, 2 H, Ar, H-triazole), 6.64 (bs, 1 H, NH), 4.38–4.25 (m, 1 H, H-3β), 4.26–4.10 (m, 8 H, OCH_2CH$_3$), 4.02–3.97 (m, 2 H, SO_2OCH_2), 3.88 (bs, 1 H, H-7β), 3.51–3.42 (m, 2 H, 2H-25), 3.06 (td, 2 H, J = 6.4, 17.0 Hz, 2H-27), 2.95 (s, 6 H, NCH_3), 2.75–2.66 (m, 2 H, 2H-26), 2.62–1.02 (m, 45 H), 0.98 (s, 3 H, 3H-19), 0.92 (d, 3 H, J = 6.2 Hz, 3H-21), 0.66 (s, 3 H, 3 H-18); ^{13}C{^{1}H}: δ = 173.9, 149.4, 131.5, 130.6, 128.7, 128.4, 123.2, 118.3, 115.6, 70.7, 68.4, 68.1, 65.3, 63.0 (dd, J = 6.3, 21.2 Hz), 62.7, 61.0, 58.2, 56.0, 55.8, 50.4, 50.3, 49.0, 45.5, 42.8, 42.7, 42.2, 41.7, 39.4, 38.7 (t, J = 132.6 Hz), 38.1, 37.0, 35.6, 34.5, 34.2, 33.5, 32.9, 31.8, 30.4, 29.8, 28.3, 27.5, 27.2, 25.3, 25.0, 23.7, 23.7, 22.9, 22.6, 21.8, 20.8, 18.4, 16.5, 16.4, 11.9; ^{31}P NMR: δ = 21.5. ESI MS (1107.4): 1108.5 $[M + H]^+$.

Bis-armed BA–BP conjugate 46

To a stirred mixture of **44F** (59 mg, 0.05 mmol), alkyne bisphosphonate **32** (26 mg, 0.08 mmol), toluene (0.5 mL), and DMF (0.05 mL), DIPEA (35 μL, 0.20 mmol) and CuI (2 mg, 0.01 mmol) were sequentially added. The resulting mixture was stirred in the dark for 18 h, then fresh portions of DIPEA (35 μL, 0.20 mmol) and CuI (2 mg, 0.01 mmol) were added and the mixture stirred in the dark for an additional 18 h and finally concentrated. The residue was taken up in CH_2Cl_2 (0.2 mL) and applied to a small column containing FluoroFlash® silica gel (4 g), which was pre-eluted with 8 : 2 MeOH–H_2O. The column was eluted with 8 : 2 MeOH–H_2O until all the non fluorous by-products were removed. Subsequently, the column was eluted with pure MeOH to obtain the fluorous tagged derivative **46F** (64 mg, 85%), which was subjected to the detagging procedure. ESI MS (1499.3): 1523.0 $[M + Na]^+$.

To a stirred mixture of **46F** (75 mg, 0.05 mmol), EtOH (0.8 mL), H_2O (0.1 mL), 0.5 M NaOH (0.2 mL, 0.10 mmol) was added in one portion. The resulting mixture was stirred at room temperature for 1 hour and then concentrated under reduced pressure. The residue was taken up in CH_2Cl_2 (0.2 mL) and applied to a small column containing FluoroFlash® silica gel (4 g), which was pre-eluted with 8 : 2 MeOH–H_2O. The column was eluted with 8 : 2 MeOH–H_2O to collect **46** (50 mg, 91%) at least 90% pure as judged by ^{1}H NMR analysis. Subsequently, the column was eluted with pure MeOH to obtain the fluorous tagged by-product. ^{1}H NMR: δ = 7.63 (bs, 1 H, H-triazole), 6.51 (bs, 1 H, NH), 4.39–4.27 (m, 1 H, H-3β), 4.24–4.03 (m, 8 H, OCH_2CH$_3$), 3.85 (bs, 1 H, H-7β), 3.70–1.03 (m, 68 H), 0.98 (s, 3 H, 3H-19), 0.94 (d, 3 H, J = 6.2 Hz, 3H-21), 0.67 (s, 3 H, 3 H-18); ^{13}C{^{1}H}: δ = 174.3, 128.1, 68.2, 63.1, 62.7 (dd, J = 6.3, 21.2 Hz), 61.5, 55.7, 50.4, 42.7, 42.1, 39.5, 39.4, 38.5 (t, J = 132.6 Hz), 37.8, 37.0, 35.8, 35.5, 35.3, 34.3, 33.2, 33.0, 31.7, 29.8, 28.3, 23.7, 22.9, 20.7, 18.5, 16.4, 11.9; ^{31}P NMR: δ = 22.3. ESI MS (1103.2): 1126.8 $[M + Na]^+$.

Bis-armed BA–BP conjugate 47

To a mixture of alkene **43F** (60 mg, 0.05 mmol), thiol-functionalized bisphosphonate **33** (38 mg, 0.10 mmol), 2,2-dimethoxy-2-phenyl-acetophenone (3 mg, 0.001 mmol), and toluene (0.5 mL) was added H_2O (5 mL). The resulting dispersion was irradiated at room temperature for 2 h under magnetic stirring, and then concentrated. The residue was taken up in CH_2Cl_2 (0.2 mL) and applied to a small column containing FluoroFlash® silica gel (4 g), which was pre-eluted with 8 : 2. MeOH–H_2O. The column was eluted with 8 : 2 MeOH–H_2O until all the non fluorous by-products were removed. Subsequently, the column was eluted with pure MeOH to obtain the fluorous tagged derivative **47F** (65 mg), which was subjected to the detagging procedure. ESI MS (1576.4): 1599.9 $[M + Na]^+$.

To a stirred mixture of **47F** (65 mg), EtOH (0.8 mL), H_2O (0.1 mL), 0.5 M NaOH (0.2 mL, 0.10 mmol) was added in one portion. The resulting mixture was stirred at room temperature for 1 hour and then concentrated under reduced pressure. The residue was taken up in CH_2Cl_2 (0.2 mL) and applied to a small column containing FluoroFlash® silica gel (4 g), which was pre-eluted with 8 : 2. MeOH–H_2O. The column was eluted with 8 : 2 MeOH–H_2O to collect a crude mixture (52 mg; yellow oil), which contained **43** as the main product (^{1}H NMR analysis) together with a small amount of the target conjugate **47** as determined by MS analysis. Subsequently, the column was eluted with pure MeOH to obtain the fluorous tagged by-product.

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