Covalent cholesterol conjugates have been prepared by iso- 
xazole-generating [3+2] nitrile oxide alkyne cycloaddition 
(NOAC) chemistry. Steroidal building blocks functionalised 
with either an alkyne or a nitrile oxide precursor were evalu-
ated. The reaction has been demonstrated for the tethering 
to cholesterol of groups capable of bioreporting, and for the 
formation of cholesterol–biomolecule conjugates.

Introduction

Cholesterol is one of the most common natural lipids, 
and due to its bioavailability and structural rigidity, func-
tionalised cholesterol building blocks have attracted interest 
in areas ranging from materials to medicinal chemistry. 
There have been a number of reports on triazole-mediated 
conjugation by copper(I)-promoted dipolar cycloaddition 
using both azido lipids and cholesterol–alkynes. This 
powerful click chemistry approach[1] has opened access to 
new cholesterol pharmacophores,[2a,2b] and has allowed the 
creation of specialist materials, including cholesterol-incor-
porating organogels,[2c,2d] nanoparticles,[2e] liquid crys-
tals,[2f] and orthopaedic medical devices.[2g] It has also facil-
itated access to cholesterol–dye conjugates for biomem-
brane[2h] or metabolic studies,[2i] to steroidal glycosides,[2j] 
and to lipid–oligonucleotide conjugates for drug delivery 
applications.[2k–2m] In addition, catalyst-free routes to tri-
azole-linked cholesterol–oligonucleotide conjugates have 
been reported.[3] However, aside from exploitation of the 
masked functionality of the isoxazole nucleus to access 
polyhydroxylated steroids, cholesterol conjugates with this 
heterocyclic linker are relatively unknown. Thermally pro-
moted cycloadditions to C-17-anchored steroidal alkynes 
have yielded important reaction intermediates.[4] In recent 
years, the regioselectivity of such cycloadditions has been 
enhanced through the use of copper[5a–5c] or ruthenium ca-
talyis.[5d] Transient nitrile oxide dipoles have been gener-
ated by the action of PhNCO on steroidal nitroalkane pre-
cursors,[6] or from C-22 aldoximes by the NCS/NEt3 proto-
col.[7] The work described in this paper demonstrates the 
versatility of nitrile oxide alkyne cycloaddition (NOAC) 
chemistry[8] for the formation of cholesterol conjugates an-
chored by way of a polar, aromatic, metabolically stable 
isoazole nucleus.

Results and Discussion

To maximize structural diversity, and to avoid potential 
stereochemical complications, we required the alkyne or 
nitrile oxide precursors to be anchored to the C-3β oxygen 
atom rather than directly to the tetracyclic core.[6b] The 
Montmorillonite K-10 (MK-10)[9] promoted route to steroi-
dal ethers 1–3 is shown in Scheme 1. The reported pro-
cedure for the formation of propargyl ether 1, which re-
quires a solution of cholesterol and the corresponding 
alcohol in CHCl3 to be stirred for 7 d at 55 °C,[10] was 
modified so that the product could be obtained with mini-
mal compromise in yield after just 17 h at 90 °C in a micro-
wave (MW) reactor. Following the modified procedure, ho-

mologous alkynyl ethers 2 and 3 were obtained in 62 and 
70% yields, respectively.

Model NOAC reactions were explored between 1–3 and 
phenyl nitrile oxide, which was generated in situ from benz-
aldehye oxime on exposure to an ethanolic solution of 
chloramine-T (Ch-T). While there was no evidence for a 
compromise in regiochemical integrity, the yields of 3,5-di-
substituted isoxazoles 4a–6a were greatly influenced by the 
spacing between the bulky lipid and the reacting alkyne. 
Thus, compound 6a, with a 4-carbon linker, was obtained 
in 90% yield after 1 h at room temperature. Compound 5a, 
with a shorter ethylene spacer, was obtained in only 44% 
after the same reaction time. Significantly, compound 4a, 
with a methylene spacer, was formed in a mere 19% yield, 
even after 17 h. The same reactivity trend was seen with the 
putative dipoles generated from 1-naphthyl and 1-pyrenyl 
oximes. Gratifyingly, we found that the sluggish reactivity of 
propargylated steroid 1a could be circumvented by con-
ducting the reaction in a microwave reactor. In this way,
the product yields for 4a–4c increased to 78, 96, and 71%, respectively.

The potential of the NOAC reaction to access biologically relevant cholesterol probes is illustrated by the preparation of steroid–coumarin 7 and steroid–azobenzene 8 conjugates. Cholesterol is implicated in a vast array of biological processes, and fluorescent derivatives are useful tools that can be used for imaging in cells and tissues,[11] azo-derivatised molecules are attractive for the construction of thermal and photoresponsive materials,[12a,12b] including phototriggered drug-delivery vehicles.[12c] Fluorescent coumarin conjugate 7 was formed in 75% yield by the cycloaddition reaction between 3 and the nitrile oxide generated from coumarin 6-aldoxime.[13a] Photoresponsive azo-conjugated steroid 8 was obtained by a parallel reaction sequence involving 4-(phenylazo)benzaldehyde oxime[13b] as the dipole precursor (Figure 1). Initially obtained almost exclusively as the trans-isomer, 8 was transformed into an 84:16 mixture of trans and cis isomers after exposure to sunlight for 2 h. When the sample was returned to darkness, the original exclusively trans geometry was restored.

To increase the flexibility of the NOAC approach to cholesterol conjugation, steroidal building blocks bearing nitrile oxide precursors were pursued. The reported successes of cholesterol chloroformate as a handle for the introduction of alkyne[14a] or azido[14b] functionalities suggested that it would be an appropriate building block for oxime introduction by way of an amidocarbamate linker. However, after sequential acetal deprotection and oxime formation, as outlined in Scheme 2, the target oxime (i.e., 12) was obtained in just 2% overall yield, primarily as a result of a low-yielding deprotection step. Fortunately, we discovered that aldehyde regeneration and oximation could be achieved in a one-pot procedure. Thus, direct exposure of acetal 10 to NH₂OH·HCl in ethanolic pyridine in a microwave reactor (125 °C, 1 h, P_max 300 W) yielded steroidal oxime 12 in 91% yield. Putative steroidal nitrile oxide 13, derived from 12 upon treatment with chloramine-T, was trapped with both propargyl alcohol and propargyl phenyl ether, however, the yields of the cholesterol conjugates (i.e., 14a and 14b) were modest (21 and 37%). The low yields of the cycloadducts may be a consequence of solubility issues arising from a suboptimal balance of hydrophilic and hydrophobic character of the microenvironments of the lipid–amidocarbamate–isoxazole conjugate. Stable long-chain hydrophilic linkers are attractive for bioconjugation, and for this reason, amidocarbamate substrates were abandoned in favour of ether-linked analogues 16 (Scheme 3).

Figure 1. Structures of fluorescent and photoreponsive cholesterol conjugates 7 and 8 (n = 4).
Scheme 2. Amidocarbamate steroidal oximes and conjugates formed by NOAC chemistry. DCC = N,N'-dicyclohexylcarbodiimide; DMAP = 4-(dimethylamino)pyridine.

Scheme 3. Steroidal oximes and conjugates formed by NOAC chemistry.
anolic solution of oxime 16 and chloramine-T in the presence of phenyl propargyl ether, 18a–18c were isolated in 29–58 % yield. It is likely that the variation in yield of the α-, m-, and p-substituted isomeric cycloadducts reflects the effect of the bulky steroidal core on the transition state leading to isoxazole formation. Thus, the steric demand is greatest for the alignment leading to α-18, and the yield of the cycloadduct is lowest (29%); the bulk of the steroidal core has a minimal impact on the transition state leading to p-18, which results in a significantly improved yield of the cycloadduct (58%). A further improvement in the yield of p-18 to an attractive 80% was achieved by adding the steroidal oxime and chloramine-T portionwise to a solution of the dipolarophile in ethanol.

Aryl ethers of natural alcohols are inherently interesting both as end products and as intermediates en route to functional materials. Their synthesis is non-trivial and has attracted significant interest.[15] To validate NOAC chemistry as a vehicle for the assembly of cholesterol conjugates like 21, aldehyde-functionalised aryl ether 19 was required. It is known that MK-10-promoted aryl etherification of cholesterol in CHCl₃ at 50–70 °C is suited to electron-rich, but not to electron-deficient phenols.[9] However, we are pleased to report that the use of microwave irradiation in the reaction between cholesterol and p-hydroxybenzaldehyde (90 °C, 8 h, Pmax 300 W) resulted in the formation of 19 (62%). With the key intermediate to hand, subsequent oximation and cycloaddition resulted in the formation of isoxazole-linked aryl cholesterol ether 21 (Scheme 4).

Covalently functionalised cholesterol bioconjugates including sugar–cholesterol,[16a] amphiphilic DNA–cholesterol,[16b] and nucleoside–cholesterol[16c] conjugates have valuable roles as components of model biological membranes,[16d] and as specialist materials for drug-delivery applications. Thus, the potential of NOAC chemistry to deliver cholesterol bioconjugates is an effective marker of its utility. Steroidal glycoconjugate formation is demonstrated by the regioselective formation of 23 (85%) by the reaction of putative nitrile oxide p-17 with protected β-galactose 22 as a model sugar dipolarophile.[17] The selective tethering of one or two cholesterol units to a thymidine skeleton was demonstrated by trapping of the same dipole by 5′-protected mono- or bis-propargylated thymidines 24 or 26[18] to give conjugates 25 and 27 in 81 and 68% yields, respectively (Scheme 5). The mono- and bis-steroidal conjugates are potentially useful building blocks for the formation of synthetic oligonucleotides with tuneable amphiphilic properties.

Conclusions

In conclusion, NOAC chemistry has been verified as a straightforward and convenient method for the formation of diverse cholesterol conjugates including those of poten-
tial value as biochemical probes. The NOAC tethering method avoids catalysts and optimisation of numerous reagents as well as the potentially problematic task of separating such reagents from the crude reaction products. The reaction is flexible in that either of the two functionalities needed for the [3+2] cycloaddition can be introduced at the 3β position of the steroidal core. This facilitates the introduction of the conjugating partner as either the dipole or the dipolarophile, without incurring any stereochemical penalties. The hydrophobic/hydrophilic balance of the steroidal reactants and products is important for the success of the reaction, as is the length of the linker that separates the rigid cholesterol core from the cycloaddition partner. In a future communication, we will describe the use of NOAC on the solid phase for the synthesis of oligonucleotide–cholesterol conjugates.

Experimental Section

General Remarks: Analytical TLC was carried out on precoated (250 mm) silica gel 60 F-254 plates from Merck. Plates were visualized by UV irradiation, and/or staining with H2SO4 (5% in ethanol) followed by heating. Flash-chromatography-grade silica gel 60 (230–400 mesh) was obtained from Merck. High-resolution mass spectra were recorded with a Bruker BioApex 70 eV spectrometer. Reactions using microwave irradiation were carried out with a Discover SP (CEM, Matthews North Carolina, USA) apparatus, with an Explorer, comp. for 1H and 13C NMR: δ = 7.1 Hz, 2 H, OCH2), 3.19–3.05 (m, 1 H, OCH), 2.45 (td, J = 7.1, 2.6 Hz, 2 H, OCH2CH2), 1.88–0.80 (m, 40 H), 0.68 (s, 3 H, Me) ppm.

(3β)-3-(5-Hexyn-1-xyloxy)cholesterol-5-ene (3): Compound 3 (423 mg, 70%) was isolated as a white solid. Rf = 0.80 (hexane/EtOAc, 9:1), m.p. 112–114 °C. 1H NMR: δ = 5.34 (br. d, J = 5.2 Hz, 1 H, CH=H), 3.47 (t, J = 7.1 Hz, 2 H, OCH2), 3.19–3.05 (m, 1 H, OCH), 2.45 (td, J = 7.1, 2.6 Hz, 2 H, OCH2CH2), 1.97 (t, J = 2.6 Hz, 1 H, CHCCH3), 1.88–0.80 (m, 40 H), 0.68 (s, 3 H, Me) ppm.

(3β)-3-(2-Propynyloxy)cholest-5-ene (1):calendar compound

Chemical reagents were purchased from Aldrich Chemical Company unless otherwise noted, and were used without further purification.

General Etherification Procedure: A mixture of cholesterol (500 mg, 1.29 mmol), alkynyl alcohol (6.47 mmol, 5 equiv.), and MK-10 was added. The mixture was stirred for 1 h, then the solvent was removed. The residue was purified by flash column chromatography (hexane/EtOAc 0–5%).

Method 1: The oxime (2 equiv.), Ch-T (2.5 equiv.), and EtOH (1 mL per 0.1 mmol oxime) were put into a round-bottomed flask, and alkylne 1, 2, or 3 (1 equiv.) was added. The mixture was stirred at room temp. To overcome solubility issues with pyrene oxime, the mixture was sonicated for 10 min before the reaction. The mixture was stirred for 1 h, then the solvent was removed. The residue was dissolved in chloroform, and this solution was washed with H2O. The organic layer was dried with anhydrous Na2SO4, and the solvent was removed. The crude reaction products were purified by flash column chromatography (hexane/EtOAc 0–5%).

Method 2 (MW Activation): The oxime (2 equiv.), Ch-T (2.5 equiv.), and EtOH (3 mL per 0.5 mmol oxime) were put into a 10 mL microwave vessel, and cholesteryl propargyl ether 1 (1 equiv.) was added. The mixture was heated (100 °C for 1 h with benzaldehyde oxime; 60 °C for 30 min for 1-naphthaldehyde or 1-pyrene oxime) in a microwave reactor (Pmax = 300 W). The solvent was removed, and the residue was dissolved in chloroform and washed with H2O. The organic layer was dried with anhydrous Na2SO4, and the solvent was removed under reduced pressure. The crude reaction products were purified by flash column chromatography (petroleum ether/EtOAc 0–10%).

Method 3: The oxime (2 equiv.) was put into a round-bottomed flask, and Ch-T (2.5 equiv.) in EtOH (25%/aq.; 1 mL per 0.1 mmol oxime) was added. The resulting solution was allowed to stir for 10 min at room temp, after which the alkylene (1 equiv.) was added. The mixture was stirred for 1 h, then the solvent was removed. The residue was dissolved in chloroform and washed with H2O. The organic layer was dried with anhydrous Na2SO4, and the solvent was removed under reduced pressure. The crude reaction products were purified by flash column chromatography (petroleum ether/EtOAc 0–5%).
solid. \( R_2 = 0.60 \) (hexane/\( \text{EtOAc} \), 9:1), m.p. 112–114 °C. \( ^1\text{H\ NMR:} \delta = 7.83–7.74 \) (2 H, Ar-H), 7.49–7.42 (m, 3 H, Ar-H), 6.39 (s, 1 H, isoxazole-H), 5.35 (br. s, 1 H, CH=C), 3.86 (t, \( J = 6.6 \) Hz, 2 H, OCH\(_2\)), 3.18–3.08 (m, 1 H, OCH), 3.11 (t, \( J = 6.6 \) Hz, 2 H, isoxazole-CH\(_2\)), 2.45–2.80 (m, 40 H), 0.67 (s, 3 H, Me) ppm. \( ^13\text{C\ NMR:} \delta = 170.6 \) (C=\( \text{N} \)), 162.2 (isoxazole-CO), 140.1 (C=\( \text{CH} \)), 130.0, 129.2, 128.8, 126.9 (Ar-C and 5 Ar-CH), 122.0 (C=\( \text{CH} \)), 103.4 (isoxazole-CH), 79.6 (OCH), 65.0 (OCH\(_2\)), 56.8, 56.2, 50.2, 42.4, 39.8, 39.5, 39.2, 37.2, 36.9, 36.2, 35.8, 32.1, 31.9, 28.4, 28.2, 24.0, 23.8, 22.8, 22.6, 21.1, 19.4, 18.8, 11.9 ppm. HRMS (ESI): calcd. for \( \text{C}_{40}\text{H}_{56}\text{NO}_2 \left[ \text{M} + \text{H}\right] \) 558.4306; found 558.4306 (0 ppm).

5-{(Cholest-5-en-3-\( \text{yloxy})\)-1-butyl}-3-(1-pyrenyl)isoxazole (6a): Following Method 1, compound 6a (115 mg, 90%) was isolated as a white solid. \( R_2 = 0.60 \) (hexane/\( \text{EtOAc} \), 9:1), m.p. 128–130 °C. \( ^1\text{H\ NMR:} \delta = 7.83–7.74 \) (m, 2 H, Ar-H), 7.49–7.42 (m, 3 H, Ar-H), 6.32 (s, 1 H, isoxazole-H), 5.35 (br. s, 1 H, CH=C), 3.83 (t, \( J = 6.3 \) Hz, 2 H, OCH\(_2\)), 3.27–3.08 (m, 1 H, OCH), 2.90 (t, \( J = 6.3 \) Hz, 2 H, isoxazole-CH\(_2\)), 2.44–2.80 (m, 40 H), 0.67 (s, 3 H, Me) ppm. \( ^13\text{C\ NMR:} \delta = 170.6 \) (C=\( \text{N} \)), 162.5 (isoxazole-CO), 140.7 (C=\( \text{CH} \)), 132.5, 131.3, 130.6, 128.7, 128.4, 126.2, 125.9, 125.6, 125.1, 125.0, 124.9, 122.7 (7 Ar-C and 9 Ar-CH), 121.8 (C=\( \text{CH} \)), 104.3 (isoxazole-CH), 82.0 (OCH), 62.0 (OCH), 56.8, 56.2, 50.9, 50.2, 42.3, 42.3, 39.8, 39.5, 37.2, 36.5, 36.2, 35.8, 31.9, 31.6, 28.2, 28.0, 24.2, 23.8, 22.8, 22.6, 21.1, 19.4, 18.8, 11.9 ppm. HRMS (ESI): calcd. for \( \text{C}_{43}\text{H}_{58}\text{NO}_2 \left[ \text{M} + \text{H}\right] \) 566.4642; found 566.4637 (3.7 ppm).

5-{(Cholest-5-en-3-\( \text{yloxy})\)-1-butyl}-3-(1-pyrenyl)isoxazole (5c): Following Method 1, compound 5c (57 mg, 35%) was isolated as a white solid. \( R_2 = 0.70 \) (hexane/\( \text{EtOAc} \), 9:1), m.p. 160–162 °C. \( ^1\text{H\ NMR:} \delta = 8.57 \) (d, \( J = 7.6 \) Hz, 1 H, Ar-H), 8.32 (d, \( J = 8.1 \) Hz, 1 H, Ar-H), 8.20–7.93 (m, 7 H, Ar-H), 6.49 (s, 1 H, isoxazole-H), 5.37 (br. s, 1 H, CH=C), 3.79 (t, \( J = 6.7 \) Hz, 2 H, OCH\(_2\)), 3.27–3.09 (m, 3 H, OCH, isoxazole-CH\(_2\)), 2.38–2.24 (m, 2 H), 2.10–1.74 (m, 6 H), 1.66–0.80 (m, 32 H), 0.68 (s, 3 H, Me) ppm. \( ^13\text{C\ NMR:} \delta = 162.5 \) (isoxazole-CO), 140.4 (C=\( \text{CH} \)), 133.9, 131.0, 130.2, 128.5, 127.8, 126.9, 126.3, 125.7 (3 Ar-C and 7 Ar-CH), 122.2 (C=\( \text{CH} \)), 104.1 (isoxazole-CH), 79.9 (OCH), 65.2 (OCH\(_2\)), 56.8, 56.2, 50.2, 42.3, 39.8, 39.5, 38.2, 37.3, 36.9, 36.2, 31.9, 31.0, 30.3, 29.6, 28.9, 28.5, 26.7, 24.3, 23.8, 22.8, 22.6, 21.1, 18.8, 11.9 ppm. HRMS (ESI): calcd. for \( \text{C}_{43}\text{H}_{58}\text{NO}_2 \left[ \text{M} + \text{H}\right] \) 668.4651; found 668.4651 (5.5 ppm).
(isoxazole-CO), 160.2 (coumarin-CO), 159.2 (coumarin-COOC=), 143.9 (coumarin-COC=), 140.8 (C=CH), 133.5, 132.5, 130.1, 120.1, 119.0 (2 Ar-C and 4 Ar-CH), 121.7 (C=CH), 101.6 (isoxazole-CH), 79.3 (OCH), 69.6 (OCH2), 56.8, 56.2, 50.1, 42.3, 42.3, 39.8, 39.5, 37.3, 36.5, 36.2, 35.8, 31.9, 31.6, 28.2, 28.0, 24.8, 23.8, 22.8, 21.5, 20.8, 19.4, 18.7, 11.8 ppm. HRMS (ESI): calcld. for C46H64N3O2 [M + H]+ 690.4993; found 690.5018 (3.6 ppm).

1H NMR: δ = 8.15 (s, 1 H, CHONH), 7.80 (d, J = 8.1 Hz, 2 H, Ar-H), 7.63 (d, J = 8.1 Hz, 2 H, Ar-H), 6.47 (br. s, 1 H, NH), 5.36 (br. s, 1 H, CH=CH), 4.73 (br. s, 1 H, CHONH), 4.58 (m, 1 H, OCH3), 3.56–3.44 (m, 2 H, CONHC=CH), 3.35 (br. s, 1 H, OMe), 3.33 (s, 3 H, OMe), 3.31 (s, 3 H, OMe), 3.28–3.17 (m, 2 H, CONHC=CH2), 2.41–0.96 (m, 44 H), 0.68 (s, 3 H, Me) ppm. 13C NMR: δ = 167.3 (CONH), 156.4 (CONH), 141.4 (C=CH), 139.8, 134.7 (2 Ar-C), 126.9, 126.9, 126.8 (4 Ar-CH), 122.5 (C=CH), 102.4 [CH(OMe)], 76.6 (OCH), 56.7, 56.2, 52.0, 50.0, 49.1, 42.3, 40.4, 39.8, 39.7, 39.5, 38.6, 37.0, 36.6, 36.2, 35.8, 34.0, 31.9, 28.2, 28.0, 27.7, 25.6, 25.2, 24.2, 23.8, 22.8, 22.6, 21.4, 19.4, 18.8, 11.9 ppm. HRMS (ESI): calcld. for C60H102N6O4 [M + H]+ 976.6948; found 976.6932 (6.0 ppm).

A solution of HCl (2 N; 10 mL) was added to a solution of crude protected aldehyde 10 (312 mg) in toluene (10 mL). After a few minutes in a sonication bath, the emulsion was stirred for 2 h at room temp. The reaction was carefully quenched with satd. aqueous NaHCO3 until the aqueous layer became neutral. The product was extracted with toluene. The organic phase was washed with water and dried with anhydrous Na2SO4, and the solvent was removed under reduced pressure. The residue was purified by flash column chromatography (hexane/EtOAc 30–60%) to give compound 11 (50 mg, 10% over the two steps from 9 to 11) as an off-white solid. Rf = 0.58 (hexane/EtOAc, 8:2), m.p. 152–154 °C. 1H NMR: δ = 10.08 (s, 1 H, CHO), 7.96 (s, 4 H, Ar-H), 6.70 (br. s, 1 H, CH=CH), 5.37 (br. s, 1 H, CH=CH), 4.76 (br. s, 1 H, NH), 4.56–4.42 (m, 1 H, OCH), 3.58–3.48 (m, 2 H, CONHC=CH), 3.28–3.17 (m, 2 H, OCONHC=CH), 3.06 (s, 3 H, Me) ppm. 13C NMR: δ = 191.6 (C=O), 167.8 (CONH), 157.4 (CONH), 139.8 (C=CH), 138.1, 132.5 (2 Ar-C), 130.9, 128.8, 127.7 (4 Ar-CH), 122.6 (C=CH), 76.6 (OCH), 56.7, 56.2, 50.0, 42.3, 39.8, 39.6, 39.5, 38.7, 38.6, 37.0, 36.6, 36.2, 35.8, 31.9, 30.4, 28.9, 28.2, 27.9, 24.3, 23.8, 23.0, 22.8, 22.6, 21.1, 19.3, 18.7, 11.0 ppm.

Method A – from Free Aldehyde 11: A mixture of aldehyde 11 (80 mg, 0.13 mmol), hydroxylamine hydrochloride (14 mg, 0.19 mmol, 1.5 equiv.), and pyridine (15 mg, 0.19 mmol, 1.5 equiv.) in EtOH (3 mL) was heated to 125 °C for 1 h in a microwave reactor (10 mL vessel, Pmax = 300 W). The solvent was removed under reduced pressure. The residue was dissolved in chloroform, and this solution was washed with H2O. The organic layer was dried with anhydrous Na2SO4 and the solvent was removed under reduced pressure to give oxime 12 (42 mg, 48%) as an off-white solid.

Method B – from Protected Aldehyde 10: A mixture of 10 (80 mg, 0.12 mmol), hydroxylamine hydrochloride (52 mg, 0.71 mmol, 6 equiv.), and pyridine (28 mg, 0.35 mmol, 3 equiv.) in EtOH (3 mL) was heated to 125 °C for 1 h in a microwave reactor (10 mL vessel, Pmax = 300 W). The solvent was removed under reduced pressure. The residue was dissolved in chloroform, and this solution was washed with satd. aq. NaHCO3 and H2O. The organic layer was dried with anhydrous Na2SO4, and the solvent was removed under reduced pressure to give pure oxime 12 (75 mg, 91%) as an off-white solid. Rf = 0.60 (hexane/EtOAc, 8:2), m.p. 156–158 °C. 1H NMR: δ = 8.15 (s, 1 H, CHONH), 7.80 (d, J = 8.1 Hz, 2 H, Ar-H), 7.63 (d, J = 8.1 Hz, 2 H, Ar-H), 6.47 (br. s, 1 H, NH), 5.36 (br. s, 1 H, CH=CH), 4.73 (br. s, 1 H, NH), 4.58–4.44 (m, 1 H, OCH3), 3.56–3.45 (m, 2 H, CONHC=CH), 3.31–3.17 (m, 2 H, CONHC=CH2), 2.43–1.89 (m, 44 H), 0.68 (s, 3 H, Me) ppm. 13C NMR: δ = 166.9 (CONH), 156.5 (CONH), 149.9 (CONH) (C=CH), 135.6, 135.0 (2 Ar-C), 127.4, 127.1, 127.1 (4 Ar-CH), 122.5 (C=CH), 76.6 (OCH), 56.7, 56.2, 50.0, 42.3, 39.8, 39.5, 38.6, 37.0, 36.6, 36.2, 35.8, 31.9, 28.2, 28.2, 27.7, 26.5, 24.3, 23.8, 22.8, 22.6, 21.1, 19.3, 18.7, 11.9 ppm. IR: ν = 3280, 1765, 1620, 1563, 1498, 1461, 1405, 1382, 1211, 1041, 870, 692 cm–1. HRMS (ESI): calcld. for C40H62N4O2 [M + H]+ 648.4735; found 648.4772 (5.7 ppm).
3.19 (m, 1 H, OCH), 2.57–0.80 (m, 40 H), 0.68 (s, 3 H, Me) ppm. 13C NMR: δ = 156.5 (O3-), 129.0 (2 Ar-H), 122.5 (Ar-C), 120.7 (C=CH), 136.7, 131.1, 129.2 (2 Ar-H), 123.8 (Ar-C), 121.9, 121.7 (2 Ar-C), 107.9, 100.7 (isoxazole-CH), 79.7 (OCH), 56.1 (OCH2), 56.3, 50.4, 43.9, 39.8, 39.6, 36.8, 36.9, 35.9, 31.9, 28.2, 27.8, 26.6, 24.3, 23.4, 22.8, 21.1, 19.3, 18.7, 11.9 ppm. IR: ν = 3289, 1768, 1644, 1570, 1450, 1383, 1022, 888 cm−1. HRMS (ESI): calcd. for C48H68N2O5 [M + H]+ 778.5153; found 778.5184 (4.0 ppm).

General Procedure for the Preparation of Aldehydes 15: A mixture of cholesteryl (1.00 g, 2.95 mmol), 2-3, or 4-(2-hydroxyethyl)benzaldehyde (purchased from TCI Europe; 2.15 g, 12.93 mmol, 5 equiv), and MK-10 (activated at 120 °C overnight before use; 2.00 g) in chloroform (5 mL) was heated to 90 °C for 8 h in a microwave reactor (10 mL vessel, Pmax = 300 W). The solvent was removed under reduced pressure, and hexane (20 mL) was added.

The catalyst was removed by filtration and washed with hexane. The solvent was evaporated, and the residue was purified by flash column chromatography (hexane/EtOAc 0–10%) to give the title compounds.

2-(Cholest-5-yl)-3[3-(4-Phenoxyphenyl)isoxazol-3-yl]benzaldehyde (m-15): An off-white solid (485 mg, 35%). Rf = 0.62 (hexane/EtOAc, 8:2), m.p. 86–88 °C. 1H NMR: δ = 10.53 (s, 1 H, CHO), 7.88–7.80 (m, 1 H, Ar-H), 7.59–7.47 (m, 1 H, Ar-H), 7.11–6.95 (m, 2 H, Ar-H), 5.41–5.29 (m, 1 H, CH=C), 4.23 (t, J = 5.0 Hz, 2 H, OCH2), 3.89 (t, J = 5.0 Hz, 2 H, OCH2), 3.35–3.19 (m, 1 H, OCH2), 2.46–0.77 (m, 40 H), 0.68 (s, 3 H, Me) ppm. 13C NMR: δ = 189.9 (C=O), 161.4 (Ar-C), 160.6 (C=CH), 135.8, 128.2 (2 Ar-C), 121.5 (Ar-C), 121.9 (C=CH), 112.9 (2 Ar-C), 79.7 (OCH), 76.6, 68.5, 65.9, 56.8, 56.2, 50.2, 42.3, 39.8, 39.5, 39.1, 37.2, 36.9, 36.2, 35.8, 31.9, 28.4, 28.2, 28.0, 24.3, 23.8, 22.8, 22.6, 21.1, 19.4, 18.7, 11.9 ppm. IR: ν = 2765, 1702, 1450, 1378, 1227, 1034, 760 cm−1. HRMS (ESI): calcd. for C50H49NO4 [M + H]+ 753.3546; found 753.3541 (2.8 ppm).

4-(Cholest-5-yl)-3(3-[2-(Cholest-5-en-3-β-yloxy)ethoxy]benzaldehyde (p-15): An off-white solid (502 mg, 0.94 mmol), hydroxylamine hydrochloride (97 mg, 1.40 mmol, 1.5 equiv), and pyridine (110 mg, 1.40 mmol, 1.5 equiv.) in EtOH was heated at 125 °C for 1 h in a microwave reactor (10 mL vessel, Pmax = 300 W). The solvent was removed under reduced pressure, the residue was dissolved in chloroform, and this solution was washed with H2O. The organic layer was dried with anhydrous Na2SO4, and the solvent was removed to give the pure oximes as off-white solids in quantitative yields.

2-(Cholest-5-yl)-3(3-[2-(Cholest-5-en-3-β-yloxy)ethoxy]benzaldehyde (m-16): An off-white solid (454 mg, 0.71 mmol, 1H CHO), 7.35–7.07 (m, 4 H, Ar-H), 7.03–6.86 (m, 1 H, Ar-H), 5.40–5.25 (m, 1 H, CH=C), 4.15 (t, J = 5.1 Hz, 2 H, OCH2), 3.85 (t, J = 5.0 Hz, 2 H, OCH2), 3.35–3.20 (m, 1 H, OCH2), 2.46–0.77 (m, 40 H), 0.68 (s, 3 H, Me) ppm. 13C NMR: δ = 157.0 (Ar-C), 146.5 (C=CH), 140.8 (C=CH), 131.9, 131.1, 129.2 (2 Ar-C), 134.3 (Ar-C), 121.8 (C=CH), 112.6 (2 Ar-C), 79.8 (OCH), 76.6, 68.5, 65.3, 56.8, 56.2, 50.2, 42.3, 39.8, 39.5, 39.1, 37.2, 36.9, 36.2, 35.8, 32.9, 31.9, 28.4, 28.3, 20.0, 23.9, 22.8, 22.6, 21.1, 19.4, 18.7, 11.9 ppm. IR: ν = 2730, 1452, 1381, 1225, 1079, 765 cm−1. HRMS (ESI): calcd. for C46H67NO4 [M + H]+ 550.4255; found 550.4284 (5.4 ppm).

4-(Cholest-5-yl)-3(3-[2-(Cholest-5-en-3-β-yloxy)ethoxy]benzaldehyde (p-16): An off-white solid (516 mg, 0.94 mmol), (hexane/EtOAc, 8:2), m.p. 111–112 °C. 1H NMR: δ = 8.08 (s, 1 H, CHO), 7.35–7.07 (m, 4 H, Ar-H), 7.03–6.90 (m, 1 H, Ar-H), 5.40–5.25 (m, 1 H, CH=C), 4.13 (t, J = 5.1 Hz, 2 H, OCH2), 3.84 (t, J = 5.1 Hz, 2 H, OCH2), 3.35–3.20 (m, 1 H, OCH2), 2.48–0.77 (m, 40 H), 0.68 (s, 3 H, Me) ppm. 13C NMR: δ = 158.1 (Ar-C), 149.2 (C=CH), 138.2, 132.4, 128.7 (2 Ar-H), 124.8 (Ar-C), 120.7 (C=CH), 115.9 (2 Ar-H), 78.8 (OCH), 76.5, 66.8, 65.4, 55.8, 55.1, 49.2, 41.3, 38.8, 38.5, 38.0, 36.2, 35.9, 35.2, 34.77, 30.94, 30.9, 27.4, 27.2, 27.0, 23.3, 21.8, 21.6, 20.1, 18.4, 17.7, 17.0 ppm. IR: ν = 1773, 1500, 1408, 1384, 1227, 1052, 702 cm−1. HRMS (ESI): calcd. for C46H67NO4 [M + H]+ 550.4255; found 550.4284 (5.2 ppm).
Benzene (16 mg, 0.12 mmol) was put into a round-bottomed flask, then a solution of oxime in EtOH (1 mL per 0.1 mmol oxime) was added. The mixture was stirred for 17 h at room temp. The solvent was removed under reduced pressure, the residue was dissolved in toluene, and this solution was washed with H2O. The organic layer was dried with anhydrous Na2SO4, and the solvent was removed under reduced pressure. The crude reaction product was purified by flash column chromatography (hexane/EtOAc 0–10%) to give compound 

**General Procedure for Cycloaddition Between (Prop-2-yn-1-yloxy)-benzene and Putative Nitrile Oxides 17, derived from 16:** (Prop-2-yn-1-yloxy)benzene (16 mg, 0.12 mmol) was put into a round-bottomed flask, a solution of oxime and Ch-T was added, and then the mixture was stirred for 4 h at room temp. The crude reaction product was purified by flash column chromatography (hexane/EtOAc 0–10%) to give compound 24, 2522–2532 2530

**Method A — Following the General Procedure:** Compound p-18 was isolated in 58% yield (47 mg). The organic layer was dried with anhydrous Na2SO4, and the solvent was removed under reduced pressure. The crude reaction product was purified by flash column chromatography (hexane/EtOAc 0–10%) to give compound 24, 2522–2532 2530

**Method B — Following a Modified Procedure:** (Prop-2-yn-1-yloxy)-benzene (16 mg, 0.12 mmol) was put into a round-bottomed flask, and a solution of oxime in EtOH (1 mL per 0.1 mmol oxime) was added. The mixture was stirred for 4 h at room temp. The crude reaction product was purified by flash column chromatography (hexane/EtOAc 0–10%) to give compound 24, 2522–2532 2530

**General Procedure for the Nitrile Oxide–Alkyne Cycloaddition with Sugar and Nucleoside Substrates:** Alkylated sugar 22 (0.75 equiv.), thymidine mono-alkyne 24 (0.75 equiv.), or thymidine bis-alkyne 26 (1.5 equiv.) was put into a round-bottomed flask, and a solution of oxime p-16 and Ch-T (1.5 equiv. for 22 and 24, 2522–2532 2530

**HRMS (ESI):** calcd. for C16H20NO4 [M + H]+ 680.4673; found 680.4672 (–1.5 ppm).

**3β-[4-Formyl]phenoxystereolesteryl-5-ene (19):** Compound 19, prepared in an analogous manner to the hydroxyethoxy analogues 15, was isolated (788 mg, 62%) as an off-white solid. Rf = 0.71 (hexane/EtOAc, 8:2). 1H NMR: δ = 9.86 (s, 1 H, CHO), 7.83 (d, J = 8.7 Hz, 2 H, Ar-H), 5.49–5.37 (m, 1 H, OCH), 4.33–4.17 (m, 1 H, OCH2), 2.56–0.77 (m, 40 H), 0.69 (s, 3 H, Me) ppm. 13C NMR: δ = 189.7 (C=O), 163.1 (Ar-C), 139.8 (C=CH), 132.1, 129.6 (Ar-CH), 122.9 (Ar-C), 122.8 (C=CH), 115.6 (2 Ar-C), 77.0 (OCH), 76.6, 56.2, 56.2, 42.3, 39.8, 39.5, 38.4, 37.1, 36.8, 36.2, 35.8, 32.0, 31.9, 28.4, 28.2, 24.3, 23.8, 22.8, 22.6, 22.1, 19.4, 18.7, 11.9 ppm. HRMS (ESI): calcd. for C16H20NO4 [M + H]+ 680.4673; found 680.4672 (–1.5 ppm).

**3β-[4-Hydroxyminoformyl]phenoxystereolesteryl-5-ene (20):** Compound 20, prepared in an analogous manner to the hydroxyethoxy analogues 16, was isolated (510 mg, quantitative) as an off-white solid. Rf = 0.69 (hexane/EtOAc, 8:2). 1H NMR: δ = 8.08 (s, 1 H, CH=NOH), 7.85 (br. s, 1 H, CH=NOH), 7.48 (d, J = 8.7 Hz, 2 H, Ar-H), 6.58 (d, J = 8.7 Hz, 2 H, Ar-H), 5.48–5.33 (m, 1 H, OCH), 4.25–4.06 (m, 1 H, OCH), 2.55–0.78 (m, 40 H), 0.69 (s, 3 H, Me) ppm. 13C NMR: δ = 159.3 (Ar-C), 150.0 (C=O), 140.1 (C=CH), 128.5 (2 Ar-C), 124.4 (Ar-C), 122.5 (C=CH), 115.9 (2 Ar-C), 77.0 (OCH), 76.6, 56.2, 56.2, 50.2, 42.4, 39.8, 39.5, 38.4, 37.1, 36.8, 36.2, 35.8, 32.0, 31.9, 28.4, 28.2, 24.3, 23.8, 22.8, 22.6, 22.1, 19.4, 18.7, 11.9 ppm. HRMS (ESI): calcd. for C16H20NO4 [M + H]+ 680.4673; found 680.4672 (–1.5 ppm).

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and 3.0 equiv. for 26) in EtOH (2 mL per 0.1 mmol oxime) was added. The mixture was stirred for 4 h at room temp. The same dose of oxime/Ch-T was added again, and then the mixture was stirred for a further 17 h at room temp. The solvent was removed under reduced pressure. The residue was dissolved in CH₂Cl₂, and this solution was washed with H₂O. The organic layer was dried with anhydrous sodium sulfate, and the solvent was removed under reduced pressure. The crude product was purified as described below.

3-(4-[2-(Cholest-5-en-3β,5-lyloxy)ethyl]oxyphenyl)-5-(6-O-[1,2,3,4-bis(1-methylethylidene)-α,β-dialkoxypropyl]methoxy methyl)-isoxazole (23): The crude reaction product was purified by flash column chromatography (hexane/EtOAc 10–30%) to give compound 23 (118 mg, 85% yield) as a white sticky solid. R₄ = 0.35 (hexane/EtOAc, 7:3). ¹H NMR: δ = 7.92–7.80 (m, 2 H, Ar-H), 7.06–6.99 (m, 2 H, Ar-H), 6.54 (s, 1 H, isoxazole-H), 5.54 (d, J = 5.7 Hz, 1 H, 1°,H), 5.34 (br. s, 1 H, CH=C), 5.10 (s, 2 H, OCH₃), 4.61 (d, J = 5.7 Hz, 1 H, 2°,H), 4.31–4.14 (m, 4 H, 3°,H, 4°,H, OCH₃), 4.07–3.96 (m, 3 H, 5°,H, OCH₃), 3.82–3.73 (m, 1 H, 6°,H), 3.73–3.63 (m, 1 H, 6°,H), 3.32–3.15 (m, 1 H, OCH), 2.36–0.81 (m, 52 H), 0.68 (s, 3 H, Me) ppm. ¹³C NMR: δ = 167.3 (C=N), 162.0 (C=CH), 159.8 (isoxazole-CO), 140.8 (Ar-C), 132.0 (Ar-C), 130.2 (C=CH), 121.6 (Ar-C), 114.8 (Ar-C), 109.3, 108.6 (C=CH₂), 109.9 (isoxazole-CO), 96.3 (C-1'), 79.6 (OCH), 74.6 (isoxazole-CO), 71.7, 71.2, 70.7, 70.5 (C-2', C-3', C-4', C-5'), 69.6 (OCH₃), 68.7 (OCH₃), 66.7 (C-6'), 61.0 (OCH), 58.5, 56.7, 56.1, 50.1, 42.3, 39.8, 39.5, 37.2, 36.5, 36.2, 35.8, 31.9, 31.6, 28.2, 28.0, 26.0, 24.9, 24.5, 24.3, 24.0, 23.8, 22.8, 22.6, 21.1, 19.4, 18.7, 11.9 ppm. IR: v = 1562, 1409, 1330, 1245, 1160, 1019, 832 cm⁻¹. HRMS (ESI): calcd. for C₈₀H₆₅NO₁₅ [M + H⁺] 846.5451; found 846.5558 (5.1 ppm).

Supporting Information (see footnote on the first page of this article): Copies of ¹H and ¹³C NMR spectra of new compounds.

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