Caffeoyl and cinnamoyl clusters with anti-inflammatory and anti-cancer effects. Synthesis and structure–activity relationship

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The syntheses of twelve caffeoyl/cinnamoyl clusters and their anti-inflammatory and anti-cancer effects are described. Synthesis of the title compounds involved a multiple copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition. Azide or alkyne functionalized cinnamoyl or caffeoyl moieties are attached to the selected core molecules to allow variation of the introduced cinnamoyl or caffeoyl moieties in order to compare their effects on 5-lipoxygenase (5-LO) inhibition and on cell proliferation in cancerous (MCF7) and non cancerous (MCF10A) human mammary epithelial cell lines. Caffeoyl dimer **13**, trimer **17**, and tetramer **19**, inhibited 5-LO product synthesis in a cell-free assay with IC_{50} values ranging from 0.66 to 0.79 μM. These compounds surpassed the inhibitory activity of caffeic acid by more than 10-fold. Monomer **11** caused almost 95% inhibition of 5-LO and surpassed the known 5-LO inhibitor zileuton in a cell-based assay. Trimer compounds **15**, **17** and tetramer **19** decreased proliferation rates of MCF-7 cells by 36, 23 and 47%, respectively, but had no effect on MCF10A proliferation.

Introduction

Strong efforts have been made within the past years to develop potent pharmacological agents that interfere with leukotriene (LT) biosynthesis or action.[1] To achieve reduction of LT formation, reasonable targets include phospholipase A_2 (PLA_2) enzymes, 5-lipoxygenase (5-LO), membrane-bound 5-LO activating protein (FLAP), LTA_4 hydrolase and LTC_4 synthase, with 5-LO being the preferred target.[2] 5-LO is the key enzyme in the conversion of arachidonic acid (AA) to the bioactive LTs.[3] LTs are potent vasoconstrictors, but also function as pro-inflammatory mediators by acting as chemotactic and chemokinetic agents toward granulocytes.[4]

In addition to its pro-inflammatory effects, accumulating evidence suggests a role for the 5-LO pathway in tumor cell proliferation and survival, implying that cancer may become a novel indication for anti-LTs therapy.[5] 5-LO is overexpressed not only in cancer cell lines but also in tissue samples of patients suffering from prostate adenocarcinoma,[6] or esophageal,[7] breast[8] and pancreatic cancer.[9] The underlying molecular mechanism of how 5-LO expression is upregulated in transformed cells is yet unknown; however, the 5-LO product 5(S)-HETE enhanced the growth of human pancreatic cancer cells,[10] breast cancer cells,[11] lung cancer cell lines,[12] and protects human prostate cancer cells from apoptosis.[13]

Similarly, numerous studies have shown that the chemo-preventive effect of the anti-inflammatory Non-Steroidal Anti-inflammatory drugs (NSAIDs) on several cancers is mediated through inhibition of cell growth and induction of apoptosis.[14] Understanding the link between carcinogenesis, inflammation and mediators of inflammation is of considerable interest in order to develop prevention or treatment strategies.

LT biosynthesis inhibitors are grouped into direct 5-LO inhibitors classified as redox-active compounds, iron-ligand inhibitors, and non-redox-type inhibitors, and into inhibitors of FLAP.[15] Several modified caffeic acid derivatives were recently demonstrated for anti-lipoxidation and exhibited more stable characteristics.[16] Furthermore, some caffeic acid amide analogues, such as *N*-caffeoyl-β-phenethylamine were reported to have inhibitory effects on prostaglandin H synthase and have potential for the inhibition of 5-LO.[17] Finally, natural phenolic acids including caffeic acid have also been shown to possess anti-tumor and anti-proliferative properties.[18]

Multivalent interactions have several advantages over monomeric ones and are often used by nature to control a wide variety of cellular processes.[19] Several attempts to quantify the effects of multivalent presentation have been reported,[19] but the detailed mechanism at the molecular level still remains unclear. Dam and Brewer recently described the potential source of the increased binding using entropic arguments and the "bind and slide" model.[20] Thus, we anticipated that clusters bearing caffeic acid moieties will lead to compounds of biomedical importance.

Here we present the design and synthesis of novel caffeoyl and cinnamoyl clusters that were recently shown to be potent inhibitors of human 5-LO.[21] The compounds were evaluated and compared for the inhibition of 5-LO activity in both a cell-free assay utilizing crude recombinant human 5-LO expressed in HEK293 cells *and* a cell-based test system using stimulated intact HEK293 cells. Additionally, the effects of the compounds on cell proliferation of cancerous (MCF7) and non-cancerous (MCF10A) breast epithelial cell lines were measured.

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Results and discussion

Strategy

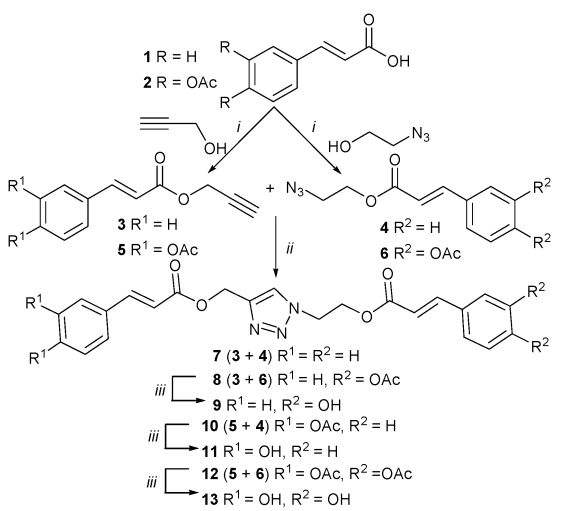
A fundamental approach to synthetic, potent, and multivalent clusters bearing ligands is the attachment of biologically active moieties to structurally simple hyper-branched molecules. Several studies have described examples of cluster and dendrimer syntheses using multivalent scaffolds.[22] Our synthesis strategy was based on the preparation of triazole-bearing clusters *via* the copper(I)-catalyzed modern version of the classical Huisgen 1,3-dipolar cycloaddition.[23] Since the hydroxyl groups within the caffeic acid catechol moiety may also play an important role in the inhibitory activity, we also examined the effect of the presence of these groups by the systematic synthesis of the cinnamoyl cluster analogs.

Synthesis

As the design of smaller molecules with high inhibitory properties would be of value, first we report herein the synthesis of two divalent clusters bearing one or two cinnamic or caffeic moieties for comparison purposes. All divalent targets were designed to share common structural similarities by using a triazole core which was generated after copper(I)-catalyzed Huisgen 1,3-dipolar cycloaddition by the propargyl **3**, **5** and azido **4**, **6** esters (Scheme 1).

As shown in Scheme 1, the novel **3**, **4**, **5**, and **6** esters were synthesized from propargyl alcohol or azidoethanol[24] with cinnamic or acetylated caffeic acid, in presence of $SOCl_2$ and pyridine, in good yields. The corresponding triazole linked derivatives **7**, **8**, **10**, and **12** were then obtained after copper-catalyzed cycloaddition. De-*O*-acetylation in **8**, **10**, **12** to afford **9**,**11**, **13** was accomplished under catalytic transesterification conditions (Scheme 1).

The azide functionalised pentaerythritol core **14**[25] provided, as expected, a trimeric cluster **15** or **16** according to Scheme 2.



Scheme 1 *Reagents and conditions*: (i) $SOCl_2$, reflux, 4 h, pyridine, benzene, rt, 12 h, **3** (83%), **4** (78%), **5** (81%), **6** (76%); (ii) $CuSO_4$, ascorbic acid, THF–H_2O, rt, 12 h, **7** (69%), **8** (80%), **10** (76%), **12** (78%); (iii) K_2CO_3 in CH_2Cl_2–MeOH (1 : 1, v/v), rt, 4 h, **9** (74%), **11** (78%), **13** (82%).

Thus, copper(I)-catalyzed 1,3-dipolar cycloaddition with triazide **14** and alkyne **3** or **5** provided trimeric clusters **15** or **16** after circular silica gel chromatography. De-*O*-acetylation of the ester protecting groups in **16** under catalytic transesterification conditions gave fully deprotected cluster **17**.

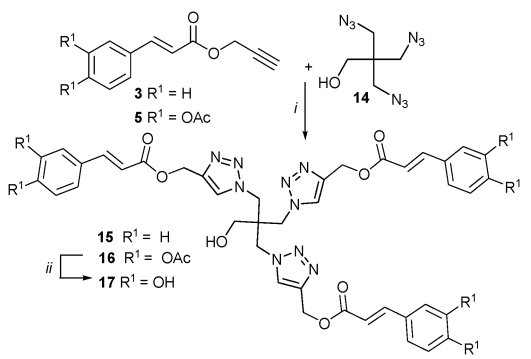
It is worth noting that considering the multifunctional and commercial attributes of the pentaerythrityl starting point, it is understandable that it has been employed in a variety of roles related to dendritic materials. These include its use as a core component for chiral[26] and nonchiral[27] constructs as well as its use for both a core and/or branch junctures.[28] Thus, copper(I)-catalyzed cycloaddition between alkyne **3** or **5** and known tetraazide **18**[25] under standard conditions (Scheme 3) provided fully protected tetramer clusters **19** and **20** in good yields.

As observed in Scheme 3, it was remarkable that the efficiency of the copper(I)-catalyzed modern version of the classical Huisgen 1,3-dipolar cycloaddition could provide multiple alkyne attachments in a single step reaction. Finally, complete deprotection of the acetyl ester protecting group in **20** to afford **21** was accomplished under catalytic transesterification conditions using K_2CO_3.

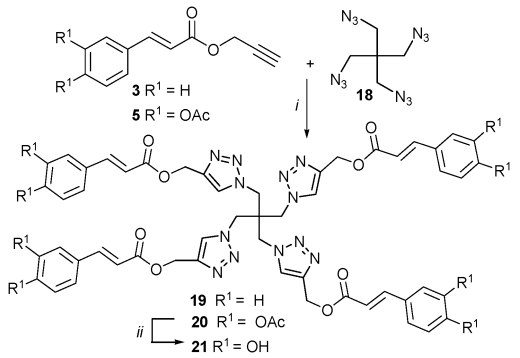
A retrosynthetic analysis reveals two possibilities for the synthesis of our clusters bearing 1,2,3-triazole linkages: the azide or the alkyne functions can be located on either the pentaerythritol core or on the caffeic or cinnamic moiety. Thus, treatment of known tetrakis(2-propynyloxymethyl)-methane **22**[29] with azides **4** or **6** using the same Cu(I) catalysed cycloadditions described above, provided tetramers **23** and **24** in good yields. As shown in Scheme 4, complete deprotection of protecting group in **24** to afford **25** was accomplished with standard catalytic transesterification conditions (Scheme 4).

The syntheses of new kinds of clusters that will open the route to novel libraries exhibiting varied active molecules should offer new opportunities for a better understanding of multivalent processes. In line with this idea, we report herein the synthesis of hexameric clusters **27** and **29** assembled using hexaazide **26**[30] as central core (Scheme 5).

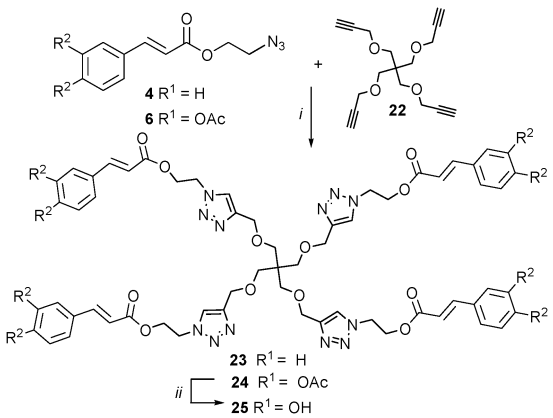
Introduction of the six cinnamoyl or caffeoyl groups was achieved by a multiple copper(I)-catalyzed 1,3-dipolar cycloaddition, as described above, with azide **26** and alkyne **3** or **5** to provide the hexameric clusters **27** and **28** in good



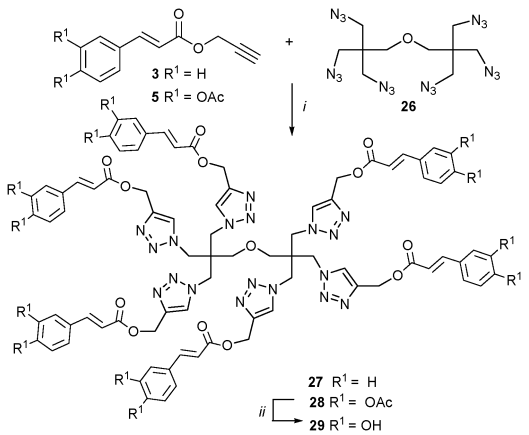
Scheme 2 *Reagents and conditions*: (i) $CuSO_4$, ascorbic acid, THF–H_2O, rt, 12 h, **15** (80%), **16** (78%); (ii) K_2CO_3 in CH_2Cl_2–MeOH (1 : 1, v/v), rt, 4 h, **17** (69%).



Scheme 3 *Reagents and conditions*: (i) $CuSO_4$, ascorbic acid, THF–H_2O, rt, 12 h, **19** (82%), **20** (79%); (ii) K_2CO_3 in CH_2Cl_2–MeOH (1 : 1, v/v), rt, 4 h, **21** (72%).



Scheme 4 *Reagents and conditions*: (i) $CuSO_4$, ascorbic acid, THF–H_2O, rt, 12 h, **23** (82%), **24** (75%); (ii) K_2CO_3 in CH_2Cl_2–MeOH (1 : 1, v/v), rt, 4 h, **25** (69%).



Scheme 5 *Reagents and conditions*: (i) $CuSO_4$, ascorbic acid, THF–H_2O, rt, 12 h, **27** (82%), **28** (78%); (ii) K_2CO_3 in CH_2Cl_2–MeOH (1 : 1, v/v), rt, 4 h, **29** (69%).

yield. The subsequent deacetylation conditions in cluster **28** gave the hexameric cluster **29** (Scheme 5).

The chemical structures of all intermediates and clusters were confirmed with NMR (^1H, ^{13}C) and HR-MS. As expected, integration of the triazole proton (7–8 ppm) was always equal to the integral of the α-carbonyl vinylic proton (5.5–6 ppm) in our clusters which confirms the multiple alkyne or azide attachments.

Inhibition of 5-LO

The clusters prepared in this study displayed some structural differences that are governed by the pentaerythritol and bis-pentaerythritol scaffolds as well as by the number of cinnamoyl/caffeoyl units and their relative orientation. These disparities offer a unique opportunity to estimate and compare their 5-LO inhibitory activities in both cell-free and cell-based assays. As previously described, measurement of 5-LO activity in cell lysates showed that clusters bearing the cinnamic acid moiety **7**, **15**, **19**, **23** and **27**, showed less inhibitory activity than the corresponding clusters bearing the caffeic acid moiety **9**, **11**, **13**, **17**, **21**, **25** and **29** (Fig. 1).[21] This reinforces the pharmacophoric contribution of the catechol entity to the mechanism of action against 5-LO activity.

In concentration–response studies, four selected compounds showed concentration-dependent inhibition of 5-LO product synthesis with IC_{50} values below 1 μM (**9**: 0.68 μM, **13**: 0.74 μM, **17**: 0.79 μM, **21**: 0.66 μM),[21] that were comparable to the inhibitory activity of zileuton (Fig. 2, IC_{50} = 0.5–1 μM[31]).

On the basis of corrected values on a per caffeoyl residue, dimer **13**, trimer **17**, and tetramer **21**, readily surpassed the activity of caffeic acid (IC_{50} = 25 μM) by more than 10-fold.

The inhibitory capacities of these compounds were then evaluated in intact HEK293 cells. HEK293 cells that were stably transfected with 5-LO and FLAP were utilized. These cells have the cellular machinery required for leukotriene biosynthesis. Although human neutrophils have been used by several other groups investigating 5-LO inhibition, the cells used in the present study represent a highly reproducible model of 5-LO product biosynthesis devoid of variations encountered when using leukocyte preparations from different donors. Cells were pre-incubated with the prepared compounds, were then stimulated and the resulting biosynthesis of 5-LO products was measured as described in the Experimental section. As shown in Fig. 3, compound **9** caused no inhibition of 5-LO activity whereas the corresponding isomer **11** caused almost complete 5-LO inhibition at 10 μM, and was more effective than the known 5-LO inhibitor zileuton.[31] The main difference between the two compounds resides in the presence of a single methylene between the caffeoyl moiety and the

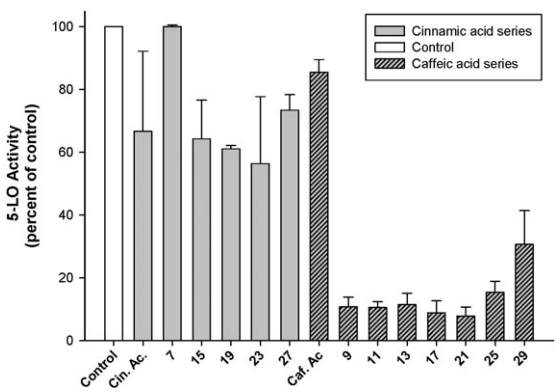


Fig. 1 5-LO activity in cell lysates preincubated with the different test compounds (1 μM).

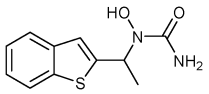


Fig. 2 Zileuton structure.

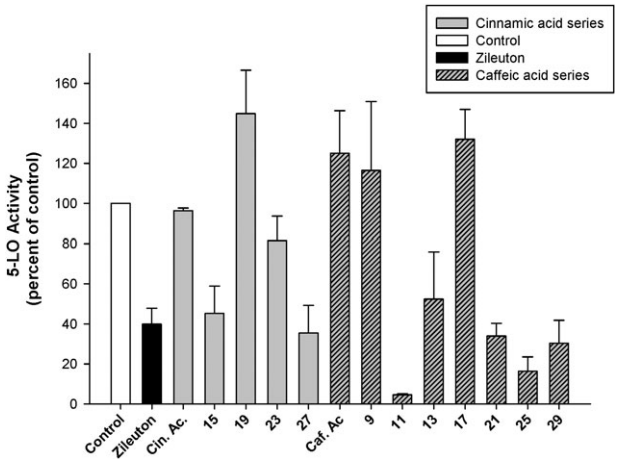


Fig. 3 5-LO activity in intact cells preincubated with the different test compounds (10 μM).

triazole core in **11** and the two methylene bridge in **9**. Possibly, the more flexibility in **9** causes a loss in the ability to suppress cellular 5-LO product synthesis, which does not apply to 5-LO inhibition in cell-free assays. In this assay system zileuton gave a little more than 60% inhibition. This is slightly less than the approximately 80% inhibition initially reported with 10 μM zileuton in rat leukocytes,[31] but is within the expected inhibition at this concentration.

The dimer **13** and trimer **15** were approximately equivalent to zileuton and were more effective inhibitors of 5-LO activity compared to caffeic acid itself. However, there were some marked differences between the two assay systems used to evaluate 5-LO inhibition. As shown in Fig. 3, compound **17** was not effective in intact HEK293 cells but significantly suppressed 5-LO activity in cell-free assays (Fig. 1). Such differences in the potencies of 5-LO inhibitors depending on the assay conditions and/or experimental settings are frequently observed.[32] The measurement of 5-LO metabolites using compound **7** in the whole cell assay was not possible because of the presence of contaminating peaks on HPLC chromatograms. Attempts to resolve 5-LO products from these peaks were not successful, but the result suggests that compound **7** was metabolised differently in intact cells compared to cell lysates. Inhibition tests with diacetylated precursors of molecules **9** and **11** (**8** and **10**) resulted in the complete inhibition of 5-LO metabolites in intact cells. These results could suggest that molecules **8** and **10** infiltrate into intact cells more efficiently than **9** and **11**, resulting in a more efficient inhibition of 5-LO following esterase action, or a possible interference with 5-LO associated proteins (*e.g.* coactosin-like protein or FLAP). As a fact of pro-drug principle, further tests, such as bioavailability of parent drugs and sustained release characteristics are also needed. These parameters will be investigated in future experiments.

Cell proliferation assay

Commonly used mammary epithelial cell models were utilized to investigate anti-proliferative properties of the prepared compounds as well as their potential selectivity for cancerous cells compared to immortalized non-cancerous cells (Fig. 4 and Fig. 5). Cell proliferation of a breast cancer (MCF7) and a non-cancerous cell line (MCF10A) were measured after incubation for 4 days in the presence or absence of the prepared compounds. All compounds were without effect on the growth profiles of the MCF10A non-cancerous cell line in relation to the untreated control. In contrast, the MCF7 breast cancer cell line demonstrated more sensitivity to the various treatments.

Growth induction MCF7 was measured in response to caffeic acid and compounds **11** and **13**. However, treatments with compounds **15**, **17** and **19** decreased proliferation rates by 36, 31 and 47%, respectively, in comparison to untreated MCF7 cells. All other compounds tested did not have any significant effect on the viability of either MCF10A or MCF-7 cells. This result is highly encouraging since selective toxicity or growth inhibition toward cancerous compared to non-cancerous cells is a crucial characteristic of compounds in the development of anti-cancer drugs.

Conclusions

In conclusion, we have synthesized and tested a series of new compounds for the inhibition of 5-LO and for anti-proliferative activity. We showed that clusters with one and four caffeic acid moieties act as novel and potent inhibitors of 5-LO product synthesis in intact stimulated HEK293 cells. Although the biochemical mechanisms by which these compounds suppress cellular 5-LO product biosynthesis are not entirely clear. Previous studies have suggested that phenolic compounds possessing the most potency in inhibiting 5-LO are those with catechol functions.[33] The mechanisms by which these compounds act have not been elucidated but could include interception of the arachidonoyl peroxy radical, the binding of iron ions and/or their reduction to the catalytically inactive ferrous form. Nevertheless, the effectiveness of the present compounds in intact cells is promising and encourages further

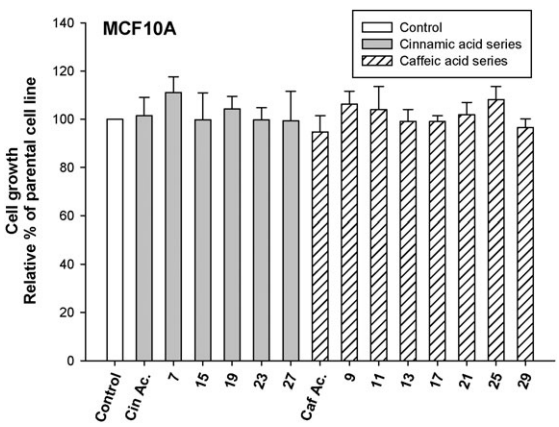


Fig. 4 Relative growth rates of the non-cancerous breast epithelial cell line MCF10A incubated with different test compounds (0.1 μM).

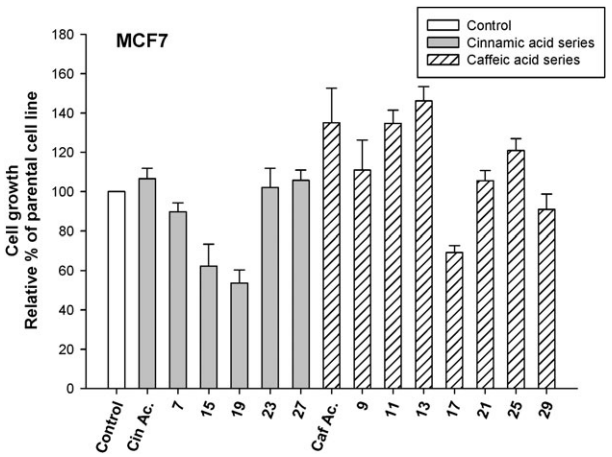


Fig. 5 Relative growth rates of the cancerous breast epithelial cell line MCF7 incubated with different test compounds (0.1 μM).

investigations of their anti-inflammatory effects. In addition, trimer and tetramer compounds were identified that present a significant effect on the viability of the cancerous epithelial breast cell line MCF7, but which were without effect on the viability of the non-cancerous breast cell line MCF10A. Although MCF-7 cells have been shown produce leukotrienes,[34] it would be premature to suggest that the inhibitory effects of these compounds on 5-LO are related to their anti-proliferative properties.

Experimental

General

Flash chromatography was performed using Merck silica gel 60 (0.04020.063 mm, 2302400 mesh). TLC was performed on Kieselgel 60 F254 plates from Merck. Detection was carried out under UV light or by spraying with 20% ethanolic sulfuric acid or molybdate solution followed by heating. Infrared spectra were recorded on a Perkin-Elmer 1600 FTIR instrument. NMR spectra were measured with a Varian 300 MHz and Bruker Ac200 MHz spectrometers. Accurate mass measurements were performed on a LC-MSD-TOF instrument in positive electrospray. Either protonated ions $(M + nH)^{n+}$ or sodium adducts $(M + Na)^{n+}$ were used for empirical formula confirmation.

5-LO inhibition

Cell-free assay. Following lysis of HEK293 cells, 5 mM of $CaCl_2$ was added to cell lysates which were then preincubated with each of the test compounds at the indicated concentration for 5 min at 37 °C. The 5-LO reaction was initiated with the addition of 1 mM ATP and 40 μM arachidonic acid followed by incubation at 37 °C for 20 min.[35] Reactions were stopped with addition of 0.5 volumes of cold methanol–acetonitrile (1 : 1) and 50 ng of PGB_2 as internal standard. Samples were stored at −20 °C for a minimum of 3 h. After centrifugation to remove cell debris, samples were then added to a preconditioned octadecyl (C18) column, were washed with 3 ml acidified water (0.1% acetic acid) and 5-LO products were eluted with methanol. After evaporation of solvents with nitrogen, products were resuspended in 20% methanol and quantification of 5-LO products (5-HETE, LTB_4 and its trans isomers) by RP-HPLC with UV detection was performed as previously described.[32]

Cell-based assay

HEK293 cells were co-transfected with a pcDNA3.1 vector expressing 5-LO and a pBUDCE4.1 vector expressing 5-lipoxygenase activating protein (FLAP) using Polyfect reagent (QIAGEN, Mississauga, ON, Canada) according to the manufacturer's protocol. Stable transfections of HEK293 cells were obtained following cell culture in the presence of Geneticin and Zeocin (Invitrogen, Burlington, ON, Canada). For cell stimulation of 5-LO products, transfected HEK293 cells were collected following trypsinization, washed and the cell pellet was resuspended in HBSS containing 1.6 mM $CaCl_2$ at a concentration of 5×10^5 cells mL^{-1}. Cells were pre-incubated with each compound at the indicated concentration for 5 min at 37 °C. Cells were then stimulated for 15 minutes at 37 °C with the addition of 10 μM calcium ionophore A23187 (Sigma–Aldrich, Oakville, ON, Canada) and 10 μM arachidonic acid (Cayman Chemical, Ann Arbor, MI). Stimulations were stopped and processed on RP-HPLC as described in the cell-free assay.

Cell proliferation assays

Non-cancerous (MCF10A) and cancerous (MCF7) human mammary epithelial cell lines were obtained from the American Type Culture Collection (Rockville, MD, USA). Cell lines were maintained in DMEM media supplemented with 10% fetal bovine serum (FBS; Hyclone Laboratories, Logan, UT, USA), L-glutamine (2 mM), penicillin G (100 U mL^{-1}), and streptomycin (100 μg mL^{-1}) (Invitrogen). Cells were either left untreated or were incubated with 0.1 μM of synthesized compounds for 4 days prior to analysis of cell proliferation.

Cell proliferation assays were performed on seeded cells (4 × 104 cells/well) in 96-well plates and analyzed for cellular viability using a multiplexed assay of CellTiter Blue (Promega, Madison, WI) kits according to the manufacturer's instructions. Briefly, 20 μL of the CellTiter Blue substrate was added to 100 μL of cell suspension and incubated for 1 hour at room temperature on a plate shaker. Thereafter, the plate was subjected to a colorimetric analysis and read on a fluorescence microplate reader (λ_{exc} = 560 nm/λ_{em} = 590 nm).

Syntheses

Procedure 1: ester derivatives sythesis. A mixture of cinnamic or diacetylcaffeic acid,[36] 5 mL of thionyl chloride, and two drops of DMF was heated at reflux for 4 h. The excess thionyl chloride was removed on a Rotovap, and the residue was dissolved in 4 mL of dry benzene. To this solution was slowly added 1 mL of pyridine and the appropriate alcohol derivative (1.2 eq.). The resulting mixture was stirred overnight at room temperature. After removal of solvents, the residue was dissolved in dichloromethane (25 mL), the organic extract was washed with water (2 × 20 mL), brine (2 × 20 mL), and then dried over $MgSO_4$. The residue was then purified by silica

gel circular chromatography (chromatotron®, model 7924, Harrison Research) to afford the required ester derivative.

Procedure 2: click reaction catalyzed by $CuSO_4$. To a magnetically stirred solution of azide (1 eq.) and propargyl derivative (1.2 eq.), dissolved in a 1 : 1 mixture of water and THF (3 mL), were sequentially added $CuSO_4$ (5% per azide), and ascorbic acid (5% per azide). The mixture was stirred for about 12 h until disappearance of the starting material (TLC, 5% MeOH–CH_2Cl_2). After addition of water (15 mL), the crude reaction was repeatedly extracted with ethyl acetate (4 × 10 mL). The combined organic extracts were washed with saturated NH_4Cl (2 × 20 mL), brine (2 × 20 mL), and then dried over $MgSO_4$. The residue was then purified by silica gel circular chromatography (chromatotron®, model 7924, Harrison Research) to afford the required triazole derivative.

Procedure 3: de-*O*-acetylation. To a solution of acetylated triazole derivative in a 1 : 1 mixture of MeOH and CH_2Cl_2 (5 mL) was added K_2CO_3 (1.5 eq. per OAc). The reaction mixture was stirred for 5 h. After removal of solvents, the residue was dissolved in EtOAc (25 mL), washed with brine, dried over $MgSO_4$, concentrated, and purified by silica gel circular chromatography (chromatotron®, model 7924, Harrison Research) to afford the required free hydroxyl derivative.

Compound 3: procedure 1. Cinnamic acid (2.17 g, 0.014 mol, 1 eq.), thionyl chloride (5 mL), pyridine (1 mL), propargyl alcohol (0.98 g, 0.017 mol), and benzene (10 mL) were used. Ester **3** (2.26 g, 0.012 mol, 83%) was obtained after silica gel circular chromatography (2% EtOAc–hexane) as a colorless oil; ^{1}H NMR ($CDCl_3$): δ 7.75 (d, 1H, J = 18.6 Hz, =CHC_{ar}), 7.60–7.51 (m, 2H, H_{ar}), 7.48–7.35 (m, 3H, H_{ar}), 6.45 (d, 1H, J = 18.6 Hz, =CHCO), 4.82 (d, 2H, J = 2.3 Hz, CH_2CCH), 2.53 (t, 1H, J = 2.3 Hz, CH_2CCH); ^{13}C NMR ($CDCl_3$): δ 166.0, 145.4, 134.2, 130.6, 128.9, 128.2, 117.0, 77.8, 74.9, 52.0; HRMS m/z calc. for $C_{12}H_{10}O_2$ + (H^+): 187.0759; found: 187.0754.

Compound 4: procedure 1. Cinnamic acid (500 mg, 3.37 mmol, 1 eq.), thionyl chloride (5 mL), pyridine (1 mL), 2-azidoethanol (353 mg, 4.05 mol), and benzene (10 mL) were used. Ester **4** (570 mg, 2.63 mmol, 78%) was obtained after silica gel circular chromatography (2% EtOAc–hexane) as a yellow oil; IR (cm^{-1}): 2110.9 (N_3); ^{1}H NMR ($CDCl_3$): δ 7.53 (d, 1H, J = 19.1 Hz, =CHC_{ar}), 7.45 (m, 5H, H_{ar}), 6.45 (d, 1H, J = 19.1 Hz, =CHCO), 4.38 (t, 2H, J = 4.7 Hz, CH_2OCO), 3.53 (t, J = 4.7 Hz, 2H, CH_2N_3); ^{13}C NMR ($CDCl_3$), δ 166.5, 145.8, 134.2, 130.5, 128.9, 128.2, 117.2, 63.1, 49.9; HRMS m/z calc. for $C_{11}H_{11}O_2N_3$ + (H^+): 218.0929; found: 218.0922.

Compound 5: procedure 1. Diacetylcaffeic acid (1 g, 3.78 mmol, 1 eq.), thionyl chloride (5 mL), pyridine (1 mL), propargyl alcohol (254.6 mg, 4.54 mmol), and benzene (10 mL) were used. Ester **5** (923 mg, 3.05 mmol, 81%) was obtained after silica gel circular chromatography (10% EtOAc–hexane) as a colorless oil ^{1}H NMR ($CDCl_3$): δ 7.68 (d, 1H, J = 18.7 Hz, =CHC_{ar}), 7.44–7.31 (m, 2H, H_{ar}), 7.23 (d, 1H, J = 6.1 Hz, H_{ar}), 6.43 (d, 1H, J = 18.7 Hz, =CHCO), 4.78 (d, 2H, J = 2.3 Hz, CH_2CCH), 2.51 (t, 1H, J = 2.3 Hz, CH_2CCH), 2.33 (s, 6H, 2 × OAc); ^{13}C NMR ($CDCl_3$): δ 168.0, 167.9, 143.9, 142.5, 132.9, 126.5, 123.9, 122.8, 118.2, 86.4, 77.7, 52.1, 20.6; HRMS m/z calc. for $C_{16}H_{14}O_6$ + (H^+): 303.0868; found: 303.0863.

Compound 6: procedure 1. Diacetylcaffeic acid (2 g, 7.57 mmol, 1 eq.), thionyl chloride (5 mL), pyridine (1 mL), 2-azidoethanol (792 mg, 9.08 mmol), and benzene (10 mL) were used. Ester **6** (1.91 g, 5.73 mmol, 76%) was obtained after silica gel circular chromatography (10% EtOAc–hexane) as a white solid; mp 71–73 °C; ^{1}H NMR ($CDCl_3$): δ 7.7 (d, 1H, J = 19.1 Hz, =CHC_{ar}), 7.52–7.38 (m, 2H, H_{ar}), 7.24 (d, 1H, J = 8.6 Hz, H_{ar}), 6.53 (d, , 1H, J = 19.1 Hz, =CHCO), 4.42 (t, 2H, J = 4.77 Hz, CH_2OCO), 3.56 (t, 2H, J = 4.77 Hz, CH_2N_3), 2.34 (s, 6H, 2 × OAc); ^{13}C NMR ($CDCl_3$), δ 168.0, 167.9, 166.1, 143.8, 143.7, 142.4, 133.0, 126.5, 123.9, 122.8, 118.4, 63.2, 49.8, 20.6; HRMS m/z calc. for $C_{15}H_{15}O_6N_3$ + (H^+): 334.1039; found: 334.1033.

Compound 7: procedure 2. Alkyne **3** (228 mg, 1.22 mmol, 1.2 eq.), azide **4** (202 mg, 0.93 mmol, 1 eq.), $CuSO_4$ (11.6 mg, 0.05 mmol), ascorbic acid (8.2 mg, 0.05 mmol), THF (1.5 ml), and H_2O (1.5 ml) were used. Triazole derivative **7** (260 mg, 0.65 mmol, 69%) was obtained after silica gel circular chromatography (2% MeOH–CH_2Cl_2) as a white solid; mp 104–105 °C; ^{1}H NMR ($CDCl_3$): δ 7.77 (s, 1H, =CHN), 7.66 (d, 2H, J = 19.1 Hz, =CHC_{ar}), 7.60–7.25 (m, 10H, H_{ar}), 6.45 (d, 2H, J = 19.1 Hz, =CHCO), 5.44 (s, 2H, CH_2OCO), 4.72–4.70 (m, 2H, NCH_2CH_2O), 4.62–4.66 (m, 2H, NCH_2CH_2O); ^{13}C NMR ($CDCl_3$): δ 166.7, 166.2, 146.1, 145.5, 143.2, 134.2, 133.9, 130.6, 130.4, 128.9, 128.8, 128.2, 128.1, 124.6, 117.4, 116.7, 62.4, 57.6, 49.2; HRMS m/z calc. for $C_{23}H_{21}O_6N_3$ + (H^+): 404.1610; found: 404.1603.

Compound 8: procedure 2. Alkyne **3** (225 mg, 1.2 mmol, 1.2 eq.), azide **6** (336 mg, 1 mmol, 1 eq.), $CuSO_4$ (12.5 mg, 0.05 mmol), ascorbic acid (8.8 mg, 0.05 mmol), THF (1.5 ml), and H_2O (1.5 ml) were used. Triazole derivative **8** (416 mg, 0.8 mmol, 80%) was obtained after silica gel circular chromatography (5% MeOH–CH_2Cl_2) as a white solid; mp 124–126 °C; ^{1}H NMR ($CDCl_3$): δ 7.81 (s, 1H, =CHN), 7.73 (d, 1H, J = 19.1 Hz, =CHC_{ar}), 7.64 (d, 1H, J = 19.1 Hz, =CHC_{ar}), 7.50–7.32 (m, 7H, H_{ar}), 7.21 (d, 1H, J = 9.1 Hz, H_{ar}), 6.44 (d, 1H, J = 19.1 Hz, =CHCO), 6.36 (d, 1H, J = 19.1 Hz, =CHCO), 5.48 (s, 2H, CH_2OCO), 4.80–4.70 (m, 2H, NCH_2CH_2O), 5.20–5.10 (m, 2H, NCH_2CH_2O), 2.30 (s, 6H, 2 × OAc); ^{13}C NMR ($CDCl_3$): δ 167.8, 166.7, 165.8, 145.6, 144.2, 143.8, 143.273, 142.5, 134.2, 132.8, 130.4, 128.1, 126.6, 124.6, 124.0, 122.9, 117.9, 117.4, 62.5, 57.6, 49.2, 20.6; HRMS m/z calc. for $C_{27}H_{25}O_8N_3$ + (H^+): 520.1719; found: 520.1712.

Compound 9: procedure 3. Triazole **8** (200 mg, 0.38 mmol), K_2CO_3 (160 mg, 1.15 mmol), MeOH (2.5 mL), and CH_2Cl_2 (2.5 mL) were used. The free hydroxyl derivative **9** (124 mg, 0.28 mmol, 74%) was obtained after silica gel circular chromatography (10% MeOH–CH_2Cl_2) as a white solid; mp 144–146 °C; ^{1}H NMR (DMSO-d_6): δ 8.28 (s, 1H, =CHN), 6.68 (d, 1H, J = 19.1 Hz, =CHC_{ar}), 7.55 (d, 1H, J = 19.1 Hz, =CHC_{ar}), 7.52–7.28 (m, 5H, H_{ar}), 7.21 (br s, 1H, H_{ar}), 7.08 (d, 1H, J = 8.7 Hz, H_{ar}), 6.86 (d, 1H, J = 8.7 Hz, H_{ar}), 6.51

(d, 1H, J = 19.1 Hz, =CHCO), 6.28 (d, 1H, J = 19.1 Hz, =CHCO), 5.29 (s, 2H, CH_2OCO), 4.80 (t, 2H, J = 4.7 Hz, NCH_2CH_2O), 4.60 (t, 2H, J = 4.7 Hz, NCH_2CH_2O); ^{13}C NMR (DMSO-d_6): δ 166.9, 166.8, 148.9, 146.5, 146.3, 145.8, 143.5, 135.2, 131.2, 129.7, 129.0, 127.4, 125.8, 122.7, 118.5, 116.3, 115.3, 114.8, 63.1, 61.4, 58.2, 49.8; HRMS m/z calc. for $C_{23}H_{21}O_6N_3$ + (H^+): 436.1508; found: 436.1502.

Compound 10: procedure 2. Alkyne **5** (125 mg, 0.41 mmol, 1.2 eq.), azide **4** (75 mg, 0.34 mmol, 1 eq.), $CuSO_4$ (5 mg, 0.02 mmol), ascorbic acid (4 mg, 0.02 mmol), THF (1.5 ml), and H_2O (1.5 ml) were used. Triazole derivative **10** (136 mg, 0.26 mmol, 76%) was obtained after silica gel circular chromatography (5% MeOH–CH_2Cl_2) as a white solid; mp 125–126 °C; ^{1}H NMR ($CDCl_3$): δ 7.82 (s, 1H, =CHN), 7.68 (d, 1H, J = 19.1 Hz, 1H, =CHC_{ar}), 7.53 (d, 1H, J = 19.1 Hz, 1H, =CHC_{ar}), 7.51–7.29 (m, 8H, H_{ar}), 6.47 (d, J = 19.1 Hz, 1H, HC=CH_{ar}), 6.33 (d, J = 19.1 Hz, 1H, =CHCO), 5.37 (s, 2H, CH_2OCO), 4.75–4.66 (m 2H, NCH_2CH_2O), 4.58–4.49 (m 2H, NCH_2CH_2O), 2.31 (s, 6H, 2 × OAc); ^{13}C NMR ($CDCl_3$): δ 168.0, 167.9, 166.3, 166.2, 146.1, 143.6, 143.5, 142.1, 133.4, 132.9, 130.9, 128.7, 128.9, 126.2, 124.3, 123.7, 122.9, 118.8, 116.6, 85.7, 62.4, 57.7, 49.2, 20.6, 20.5; HRMS m/z calc. for $C_{27}H_{25}O_8N_3$ + (H^+): 520.1503; found: 520.1502.

Compound 11: procedure 3. Triazole **10** (102 mg, 0.19 mmol), K_2CO_3 (81 mg, 0.6 mmol), MeOH (2.5 mL), and CH_2Cl_2 (2.5 mL) were used. The free hydroxyl derivative **11** (66.5 mg, 0.15 mmol, 78%) was obtained after silica gel circular chromatography (10% MeOH–CH_2Cl_2) as a colorless oil; ^{1}H NMR (DMSO-d_6): δ 8.19 (s, 1H, NCH=C), 7.68 (d, 1H, J = 18.2 Hz, =CHC_{ar}), 7.64 (d, 1H, J = 18.1 Hz, =CHC_{ar}), 7.60–7.38 (m, 5H, H_{ar}), 7.22–7.18 (m, 1H, H_{ar}), 7.01 (d, 1H, J = 9.2 Hz, H_{ar}), 6.84 (d, 1H, J = 9.2 Hz, H_{ar}), 6.55 (d, J = 19.1 Hz, 1H, =CHCO), 6.29 (d, J = 19.1 Hz, 1H, =CHCO), 5.29 (s, 2H, CH_2OCO), 4.86 (t, J = 4.8 Hz, 1H, NCH_2CH_2O), 4.63 (t, J = 4.8 Hz, 1H, NCH_2CH_2O); ^{13}C NMR (DMSO-d_6): δ 167.2, 166.6, 148.9, 146.3,146.1, 143.8, 135.2, 131.3, 129.8, 129.1, 127.5, 125.7, 122.6, 118.3, 116.3, 115.2, 115.0, 63.3, 58.0, 49.8; HRMS m/z calc. for $C_{23}H_{21}O_6N_3$ + (H^+): 436.1508; found: 436.1500.

Compound 12: procedure 2. Alkyne **5** (138 mg, 0.45 mmol, 1.2 eq.), azide **6** (127 mg, 0.38 mmol, 1 eq.), $CuSO_4$ (12.5 mg, 0.05 mmol), ascorbic acid (8.8 mg, 0.05 mmol), THF (1.5 ml), and H_2O (1.5 ml) were used. Triazole derivative **12** (189 mg, 0.29 mmol, 78%) was obtained after silica gel circular chromatography (10% MeOH–CH_2Cl_2) as a white solid; mp 152–154 °C; ^{1}H NMR ($CDCl_3$): δ 7.81 (s, 1H, =CHN), 7.65 (d, 2H, J = 19.1 Hz, =CHC_{ar}), 7.46–7.28 (m, 4H, H_{ar}), 7.25–7.12 (m, 2H, H_{ar}), 6.39 (d, 2H, J = 19.1 Hz, =CHCO), 5.38 (s, 2H, CH_2OCO), 4.72–4.70 (m, 2H, NCH_2CH_2O), 4.60–4.64 (m, 2H, NCH_2CH_2O), 2.30 (s, 12H, 4 × OAc); ^{13}C NMR ($CDCl_3$), δ 168.0, 167.9, 166.3, 165.8, 144.1, 143.8, 143.6, 143.5, 143.0, 142.5, 142.4, 132.9, 132.7, 126.5, 126.4, 124.7, 124.0, 123.9, 122.9, 122.8, 118.6, 117.9, 62.5, 57.7, 49.2, 20.6, 20.5; HRMS m/z calc. for $C_{31}H_{29}O_{12}N_3$ + (H^+): 636.1824; found: 636.1818.

Compound 13: procedure 3. Triazole **12** (185 mg, 0.29 mmol), K_2CO_3 (240 mg, 1.74 mmol), MeOH (2.5 mL), and CH_2Cl_2 (2.5 mL) were used. The free hydroxyl derivative **13** (110 mg, 0.23 mmol, 82%) was obtained after silica gel circular chromatography (16% MeOH–CH_2Cl_2) as a white solid; mp 102–105 °C; ^{1}H NMR (DMSO-d_6): δ 8.07 (s, 1H, =CHN), 7.56 (d, 2H, J = 19.1 Hz, =CHC_{ar}), 7.25 (d, 2H, J = 3.4 Hz, H_{ar}), 6.93 (td, 2H, J = 8.7, 3.4 Hz, H_{ar}), 6.83 (d, 2H, J = 8.7 Hz, H_{ar}), 6.30 (d, 2H, J = 19.1 Hz, =CHCO), 5.28 (s, 2H, CH_2OCO), 4.79 (t, 2H, J = 4.7 Hz, NCH_2CH_2O), 4.55 (t, 2H, J = 4.7 Hz, NCH_2CH_2O); ^{13}C NMR (DMSO-d_6): 167.2, 166.9, 148.9, 148.8, 146.5, 146.2, 143.7, 127.4, 125.7, 122.7, 122.6, 116.3, 115.3, 115.2, 115.1, 114.8, 63.1, 57.9, 49.8; HRMS m/z calc. for $C_{23}H_{21}O_8N_3$+(H^+): 468.1406; found: 468.1401.

Compound 15: procedure 2. Alkyne **3** (222 mg, 1.2 mmol, 1.2 eq.), triazide **14** (70 mg, 0.33 mmol, 1 eq.), $CuSO_4$ (12.5 mg, 0.05 mmol), ascorbic acid (8.8 mg, 0.05 mmol), THF (1.5 ml), and H_2O (1.5 ml) were used. Triazole derivative **15** (204 mg, 0.26 mmol, 80%) was obtained after silica gel circular chromatography (1.5% MeOH–CH_2Cl_2) as a white solid; mp 90–92 °C; ^{1}H NMR ($CDCl_3$): δ 8.28 (s, 3H, =CHN), 7.64 (d, 3H, J = 18.6 Hz, =CHC_{ar}), 7.52–7.46 (m, 6H, H_{ar}), 7.38–7.29 (m, 9H, H_{ar}), 6.43 (d, 3H, J = 18.6 Hz, =CHCO), 5.36 (s, 6H, CH_2OCO), 4.38 (s, 6H, NCH_2), 3.22-3.11 (m, 2H, CH_2OH); ^{13}C NMR ($CDCl_3$): δ 166.5, 145.7, 143.0, 134.1. 130.5, 128.9, 128.2, 127.1, 117.3, 59.8, 57.4, 48.8, 47.12; HRMS m/z calc. for $C_{41}H_{39}O_7N_9$ + (H^+): 770.3045; found: 770.3034.

Compound 16: procedure 2. Alkyne **5** (516 mg, 1.7 mmol, 1.2 eq.), triazide **14** (100 mg, 0.47 mmol, 1 eq.), $CuSO_4$ (17.6 mg, 0.07 mmol), ascorbic acid (12.3 mg, 0.07 mmol), THF (1.5 ml), and H_2O (1.5 ml) were used. Triazole derivative **16** (412 mg, 0.37 mmol, 78%) was obtained after silica gel circular chromatography (5% H_2O–CH_3CN) as a white foam; ^{1}H NMR ($CDCl_3$): δ 8.32 (s, 3H, =CHN), 7.74 (d, 3H, J = 19.1 Hz, =CHC_{ar}), 7.46–7.33 (m, 6H, H_{ar}), 77.24 (d, 3H, J = 8.6 Hz, H_{ar}), 6.47 (d, 3H, J = 19.1 Hz, =CHCO), 5.42 (s, 6H, CH_2OCO), 4.37 (s, 6H, NCH_2), 3.78–3.61 (br s, 1H, OH), 3.25–3.04 (br s, 2H, CH_2OH); ^{13}C NMR ($CDCl_3$): δ 168.1, 167.9, 166.2, 143.7, 143.6, 142.9, 142.4, 133.0, 127.0, 126.5, 123.9, 122.8, 118.5, 59.8, 57.6, 48.6, 47.2, 20.6; HRMS m/z calc. for $C_{53}H_{51}O_{19}N_9$ + (H^+): 1118.3374; found: 1118.3366.

Compound 17: procedure 3. Triazole **16** (117 mg, 0.1 mmol), K_2CO_3 (130.3 mg, 0.9 mmol), MeOH (2.5 mL), and CH_2Cl_2 (2.5 mL) were used. The free hydroxyl derivative **17** (74.5 mg, 0.07 mmol, 69%) was obtained after silica gel circular chromatography (5% H_2O–CH_3CN) as a yelow solid; mp 148–150 °C; ^{1}H NMR (DMSO-d_6): 8.34 (s, 3H, =CHN), 7.72 (d, 3H, J = 19.1 Hz, =CHC_{ar}), 7.28 (br s, 3H, H_{ar}), 7.18 (d, 3H, J = 8.6 Hz, H_{ar}), 6.89 (d, 3H, J = 8.6 Hz, H_{ar}), 6.29 (d, 3H, J = 19.1 Hz, =CHCO), 5.38 (s, 6H, CH_2OCO), 4.66 (s, 6H, NCH_2), 3.34–3.29 (br s, 2H, CH_2OH); ^{13}C NMR (DMSO-d_6): δ 166.2, 145.1, 143.2, 128.1, 121.6, 118.2, 117.3, 114.9, 61.4, 60.7, 51.2, 46.8; HRMS m/z calc. for $C_{41}H_{39}O_{13}N_9$ – (H^+): 864.2595; found: 864.2487.

Compound 19: procedure 2. Alkyne **3** (265 mg, 1.4 mmol, 1.2 eq.), azide **18** (70 mg, 0.3 mmol, 1 eq.), $CuSO_4$ (15 mg, 0.06 mmol), ascorbic acid (10 mg, 0.06 mmol), THF (1.5 ml), and H_2O (1.5 ml) were used. Triazole derivative **19** (238 mg, 0.24 mmol, 82%) was obtained after silica gel circular chromatography (5% MeOH–CH_2Cl_2) as a white foam; ^{1}H NMR ($CDCl_3$): δ 8.30 (s, 4H, =CHN), 7.72 (d, 4H, J = 18.7 Hz, =CHC_{ar}), 7.54–7.44 (m, 8H, H_{ar}), 7.40–7.28 (m, 12H, H_{ar}), 6.46 (d, 3H, J = 18.7 Hz, =CHCO), 5.38 (s, 8H, $COOCH_2$), 4.41 (s, 8H, NCH_2); ^{13}C NMR ($CDCl_3$): δ 166.5, 145.8, 142.7, 134.2, 130.5, 128.9, 128.2, 127.8, 117.2, 57.4, 49.2, 46.4; HRMS m/z calc. for $C_{53}H_{48}O_8N_{12}$ + (H^+): 981.3791; found: 981.3785.

Compound 20: procedure 2. Alkyne **5** (246 mg, 0.81 mmol, 3 × 1.2 eq.), azide **18** (40 mg, 0.17 mmol, 1 eq.), $CuSO_4$ (8.5 mg, 0.034 mmol), ascorbic acid (6 mg, 0.043 mmol), THF (1.5 ml), and H_2O (1.5 ml) were used. Triazole derivative **20** (193 mg, 0.13 mmol, 79%) was obtained after silica gel circular chromatography (3% MeOH–CH_2Cl_2) as a colorless oil; ^{1}H NMR ($CDCl_3$): δ 8.30 (s, 4H, =CHN), 7.63 (d, 4H, J = 17.6 Hz, =CHC_{ar}), 7.35 (br s, 8H, H_{ar}), 7.20 (d, 4H, J = 8.2 Hz, H_{ar}), 6.41 (d, 4H, J = 17.6 Hz, =CHCO), 5.38 (s, 8H, CH_2O), 4.44 (s, 8H, CH_2N), 2.25 (s, 24H, 8 × OAc); ^{13}C NMR ($CDCl_3$): δ 168.0, 167.9, 166.1, 143.6, 142.43, 132.9, 132.4, 127.8, 126.5, 123.9, 122.8, 118.4, 86.1, 57.5, 49.2, 46.6, 20.6; HRMS m/z calc. for $C_{69}H_{64}O_{24}N_{12}$ + (H^+): 1445.4234; found: 1445.4231.

Compound 21: procedure 3. Triazole **20** (62 mg, 0.043 mmol), K_2CO_3 (36.6 mg, 0.26 mmol), MeOH (2.5 mL), and CH_2Cl_2 (2.5 mL) were used. The free hydroxyl derivative **21** (34 mg, 0.03 mmol, 72%) was obtained after silica gel circular chromatography (5% H_2O–CH_3CN) as a yellow solid; mp 168–170 °C; ^{1}H NMR (DMSO-d_6): δ 8.35 (s, 4H, =CHN), 7.60 (d, 4H, J = 18.6 Hz, =CHC_{ar}), 7.12 (br s, 8H, OH), 7.05 (m, 4H, H_{ar}), 6.85 (m, 8H, H_{ar}), 6.30 (d, 4H, J = 18.6 Hz, =CHCO), 5.30 (s, 8H, CH_2O), 4.72 (s, 8H, CH_2N); ^{13}C NMR (DMSO-d_6): δ 166.3, 145.6, 143.2, 142.9, 142.5, 129.4, 127.5, 121.3, 118.2, 117.6, 115.3, 59.3, 50.2, 44.5; HRMS calc. for $C_{53}H_{48}O_{16}N_{12}$ + (H^+): 1109.3389; found: 1109.3367.

Compound 23: procedure 2. Alkyne **22** (80.7 mg, 0.28 mmol, 1 eq.), azide **4** (292 mg, 1.34 mmol, 4 × 1.2 eq.), $CuSO_4$ (12.5 mg, 0.05 mmol), ascorbic acid (8.8 mg, 0.05 mmol), THF (1.5 ml), and H_2O (1.5 ml) were used. Triazole derivative **23** (265 mg, 0.2 mmol, 82%) was obtained after silica gel circular chromatography (5% MeOH–CH_2Cl_2) as a white solid; mp 86–88 °C; ^{1}H NMR ($CDCl_3$): δ 7.69 (s, 4H, =CHN), 7.61 (d, 4H, J = 19.1 Hz, =CHC_{ar}), 7.55–7.35 (m, 20H, H_{ar}), 6.41 (d, 4H, J = 17.6 Hz, =CHCO), 4.72–4.68 (m, 8H, CH_2OCO), 4.61–4.66 (m, 8H, NCH_2CH_2OCO), 4.52 (s, 8H, OCH_2C), 3.41 (s, 8H, CCH_2O); ^{13}C NMR ($CDCl_3$): δ 166.3, 146.0, 145.4, 134.0, 130.6, 128.9, 128.2, 123.1, 116.9, 69.05, 64.8, 62.5, 49.1, 45.2; HRMS m/z calc. for $C_{61}H_{64}O_{12}N_{12}$ + ($2H^+$): 579.2456; found: 579.2456.

Compound 24: procedure 2. Alkyne **22** (230 mg, 0.8 mmol, 1 eq.), azide **6** (1276 mg, 3.8 mmol, 4 × 1.2 eq.), $CuSO_4$ (40 mg, 0.16 mmol), ascorbic acid (28 mg, 0.16 mmol), THF (1.5 ml), and H_2O (1.5 ml) were used. Triazole derivative **24** (969 mg, 0.6 mmol, 75%) was obtained after silica gel circular chromatography (15% MeOH–CH_2Cl_2) as a white foam; ^{1}H NMR ($CDCl_3$): δ 7.71 (s, 4H, =CHN), 7.59 (d, 4H, J = 19.1 Hz, =CHC_{ar}), 7.73–7.31 (m, 8H, H_{ar}), 7.22 (d, 4H, J = 8.3 Hz, H_{ar}), 6.37 (d, 4H, J = 17.6 Hz, =CHCO), 4.69–4.52 (m, 12H, CH_2OCO, NCH_2CH_2OCO, OCH_2C), 3.72 (s, 8H, CCH_2O); ^{13}C NMR ($CDCl_3$): δ 168.1, 167.9, 165.9, 145.3, 143.9, 143.7, 142.5, 132.8, 126.6, 123.9, 123.3, 122.8, 118.1, 69.1, 64.8, 62.6, 49.0, 45.1, 20.6; HRMS m/z calc. for $C_{77}H_{80}O_{28}N_{12}$ + ($2H^+$): 811.7691; found: 811.7690.

Compound 25: procedure 3. Triazole **24** (280 mg, 0.17 mmol), K_2CO_3 (286.3 mg, 2.1 mmol), MeOH (2.5 mL), and CH_2Cl_2 (2.5 mL) were used. The free hydroxyl derivative **25** (152 mg, 0.12 mmol, 69%) was obtained after silica gel circular chromatography (8% H_2O–CH_3CN) as a yellow foam; ^{1}H NMR (DMSO-d_6): δ 8.12 (s, 4H, =CHN), 7.48 (d, 4H, J = 18.2 Hz, =CH_{ar}), 7.10 (s, 4H, H_{ar}), 7.02 (d, 4H, J = 8.3 Hz, H_{ar}), 6.83 (d, 4H, J = 8.3 Hz, H_{ar}), 6.25 (d, 4H, J = 18.2 Hz, =CHCO), 4.88–4.75 (m, 8H, CH_2OCO), 4.68–4.56 (m, 8H, NCH_2CH_2OCO), 4.47 (s, 8H, OCH_2C), 3.41 (s, 8H, CCH_2O); ^{13}C NMR (DMSO-d_6): δ 167.0, 149.1, 146.6, 146.3, 145.8, 127.3, 124.7, 122.9, 116.4, 115.2, 114.7, 69.5, 65.3, 63.1, 49.9, 46.1; HRMS m/z calc. for $C_{61}H_{64}O_{20}N_{12}$ − ($2H^+$): 642.2136; found: 642.2128.

Compound 27: procedure 2. Alkyne **3** (147.5 mg, 0.8 mmol, 6 × 1.2 eq), hexaazide **26** (47.5 mg, 0.1 mmol, 1 eq.), $CuSO_4$ (7.5 mg, 0.03 mmol), ascorbic acid (5.3 mg, 0.03 mmol), THF (1.5 ml), and H_2O (1.5 ml) were used. Triazole derivative **27** (146.5 mg, 0.09 mmol, 82%) was obtained after silica gel circular chromatography (2% MeOH–CH_2Cl_2) as a white solid; mp 104–105 °C; ^{1}H NMR ($CDCl_3$): δ 8.35 (s, 6H, =CHN), 7.73 (d, 6H, J = 18.7 Hz, =CHC_{ar}), 7.50–7.41 (m, 12H, H_{ar}), 7.39–7.28 (m, 18H, H_{ar}), 6.44 (d, 6H, J = 18.7 Hz, =CHCO), 5.36 (s, 12H, CH_2OCO), 4.38 (s, 12H, CH_2N); 3.15 (s, 4H, CH_2OC); ^{13}C NMR ($CDCl_3$): δ 166.5, 145.7, 143.1, 134.1, 130.5, 128.9, 128.1, 127.3, 117.2, 67.3, 57.5, 48.9, 46.4; HRMS m/z calc. for $C_{82}H_{76}O_{13}N_{18}$ + (H^+): 1521.5912; found: 15215.5914.

Compound 28: procedure 2. Alkyne **5** (376 mg, 1.25 mmol, 6 × 1.2 eq.), hexaazide **26** (70 mg, 0.17 mmol, 1 eq.), $CuSO_4$ (12.5 mg, 0.05 mmol), ascorbic acid (8.8 mg, 0.05 mmol), THF (1.5 ml), and H_2O (1.5 ml) were used. Triazole derivative **28** (300 mg, 0.13 mmol, 78%) was obtained after silica gel circular chromatography (5% MeOH–CH_2Cl_2) as a yellow solid; mp 110–111 °C; ^{1}H NMR ($CDCl_3$): δ 8.32 (s, 6H, =CHN), 7.61 (d, 6H, J = 19.1 Hz, 6H, =CHC_{ar}), 7.48–7.31 (m, 12H, H_{ar}), 7.21 (d, 6H, J = 8.1 Hz, H_{ar}), 6.33 (d, J (H,H) = 19.1 Hz, 6H, =CHCO), 5.38 (s, 12H, CH_2OCO), 4.46 (s, 12H, CH_2N); 3.19 (s, 4H, CH_2OC), 2.29 (s, 36H, 6 × OAc); ^{13}C NMR ($CDCl_3$): δ 168.1, 167.9, 166.1, 143.6, 142.4, 132.9, 126.5, 123.9, 122.8, 118.4, 68.3, 57.6, 49.1, 46.1, 29.7, 20.6, 20.5; HRMS m/z calc. for $C_{106}H_{100}O_{37}N_{18}$ + ($2H^+$): 1109.8336; found: 1109.8332.

Compound 29: procedure 3. Triazole **28** (151 mg, 0.06 mmol), K_2CO_3 (169.2 mg, 1.2 mmol), MeOH (2.5 mL), and CH_2Cl_2 (2.5 mL) were used. The free hydroxyl derivative **29** (80.3 mg,

0.05 mmol, 69%) was obtained after silica gel circular chromatography (8% H_2O–CH_3CN) as a yellow solid; mp 161–162 °C; ^{1}H NMR (DMSO-d_6): δ 8.40 (s, 6H, =CHN), 7.54 (d, 4H, J = 19.1 Hz, =CHC_{ar}), 7.18 (s, 6H, H_{ar}), 7.0 (d, 6H, J = 8.2 Hz, H_{ar}), 6.81 (d, 6H, J = 8.2 Hz, H_{ar}), 6.23 (d, 6H, J = 19.1 Hz, =CHCO), 5.38 (s, 12H, CH_2OCO), 4.78 (s, 12H, CH_2N), 3.36 (s, 8H, CH_2OC); ^{13}C NMR (DMSO-d_6): δ 167.3, 148.6, 146.4, 146.2, 143.6, 128.1, 127.4, 122.8, 116.3, 115.2, 114.9, 68.1, 57.9, 50.8, 46.1; HRMS m/z calc. for $C_{82}H_{76}O_{25}N_{18}$ – ($2H^+$): 855.2542; found: 855.2542.

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