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Synthesis of oligonucleotides carrying amino lipid groups at the 3ø-end for RNA interference studies

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ABSTRACT: Novel lipid derivatives carrying amino and triazolyl groups were efficiently synthesized and covalently anchored at the 3ø-termini of oligonucleotides. The desired amino lipid-conjugates were fully characterized by reverse phase HPLC and MALDI-TOF mass spectrometry. The methodology was applied to the synthesis of lipid-siRNA designed to inhibit tumor necrosis factor (TNF-α) in order to obtain siRNAs with anti-inflammatory properties. The siRNA duplex carrying amino-lipids at the 3ø-end of the passenger strand has similar inhibitory properties to unmodified RNA duplexes in HeLa cells indicating that the new lipid derivatives are compatible with the RNA interference machinery.

KEYWORDS: RNA interference, oligonucleotides, chemically modified siRNA, lipids, click chemistry, guanidinium, post-synthetic approach, covalent strategy, TNF-

INTRODUCTION

RNA interference (RNAi) plays an important role in host defense and regulation of gene expression. Since RNAi was discovered a decade ago by Fire et al.,[1] RNA research has seen intense growth. During RNA interference, double-stranded RNAs are processed into small fragments of approximately 21 nucleotides by the enzyme Dicer[2] that are incorporated into the RNA-induced silencing complex (RISC) directing the degradation of specific complementary mRNA sequences.[3] The introduction of short interfering RNAs (siRNA) in mammalian cells[3] results in a selective silencing of the protein encoded by the specific mRNA targeted by siRNA. Since then, researchers have been actively looking for different approaches to enhance this powerful tool in order to be used in therapeutics.[4] However, successful therapeutic applications have been delayed due to delivery problems since native siRNAs do not freely diffuse across the cell membrane due to their relatively large molecular weight and their polyanionic

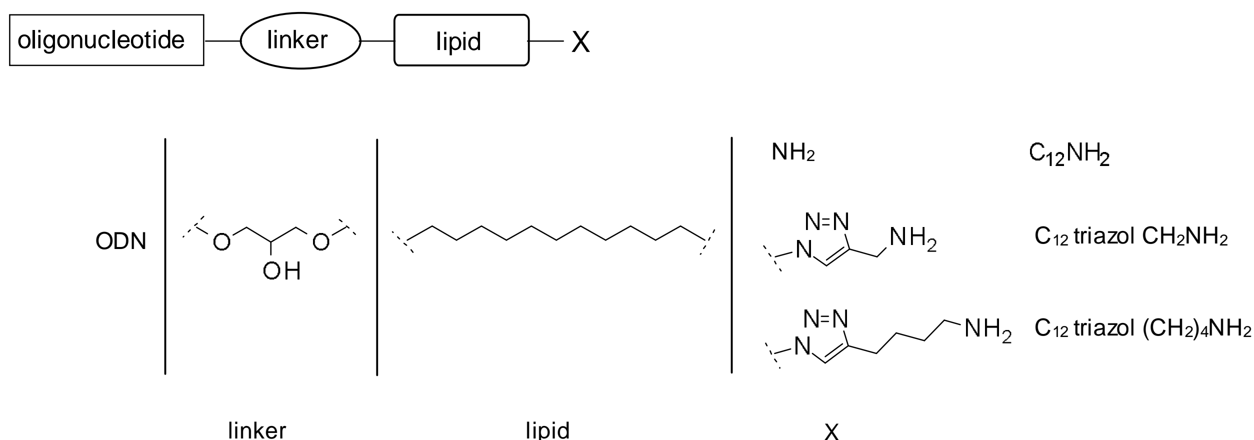
nature. To overcome these limitations, different strategies have been employed and non-viral vectors have emerged as a promising alternative to gene delivery. [5]

The most used carriers for DNA and RNA oligonucleotides are cationic lipids and liposomes which promote cellular uptake of antisense and RNAi therapies[6] although their mechanism is still unclear and controversial. In general, the use of targeted lipoplexes as systems to mediate siRNA delivery has become the most reported method.[7,8] These units, which maintain an electrostatic association, are often unstable[9] and should be prepared before use, so other alternative and robust linkage methods between siRNAs and non-viral vectors may be developed and validated. Recently it has been demonstrated that nanoparticles can be used for the efficient delivery of siRNA.[10]

Lipid-oligonucleotide conjugates are an interesting alternative. Some years ago, lipid-oligonucleotide conjugates were found to improve biological activity of triplex-forming and antisense oliognucleotides.[11] Recently, a few examples on the use of siRNA covalently conjugated to lipids have been described in the literature. Cholesterol,[12] fatty acids derivatives[12] and -tocopherol[13] have been efficiently linked to the 5ø-ends (phosphoramidite chemistry) and 3ø-ends (CPG supports) of the siRNA guide and passenger strands. Further biological evaluations of these conjugates showed that the introduction of these groups did not affect the RNAi machinery obtaining high levels of inhibition in the gene expression.

As a part of our ongoing interest in the development of chemically modified DNA and siRNAs to inhibit gene expression,[14] we have been looking into the possibility of synthesizing siRNA conjugates by linking lipid molecules with different polar and / or protonatable groups (such as amines and triazoles) at the 3ø-termini of the siRNA duplex (Scheme 1). We aimed to mimic the effect produced by lipoplex complexes. As far as we know, such complex lipids have never been introduced into siRNA. Finally, the effects of such modifications on the RNAi machinery were measured through the evaluation of these chemically modified lipid-siRNA conjugates in the regulation of the TNF- gene expression.

SCHEME 1. Structure of the lipid oligonucleotide conjugates described in this study.

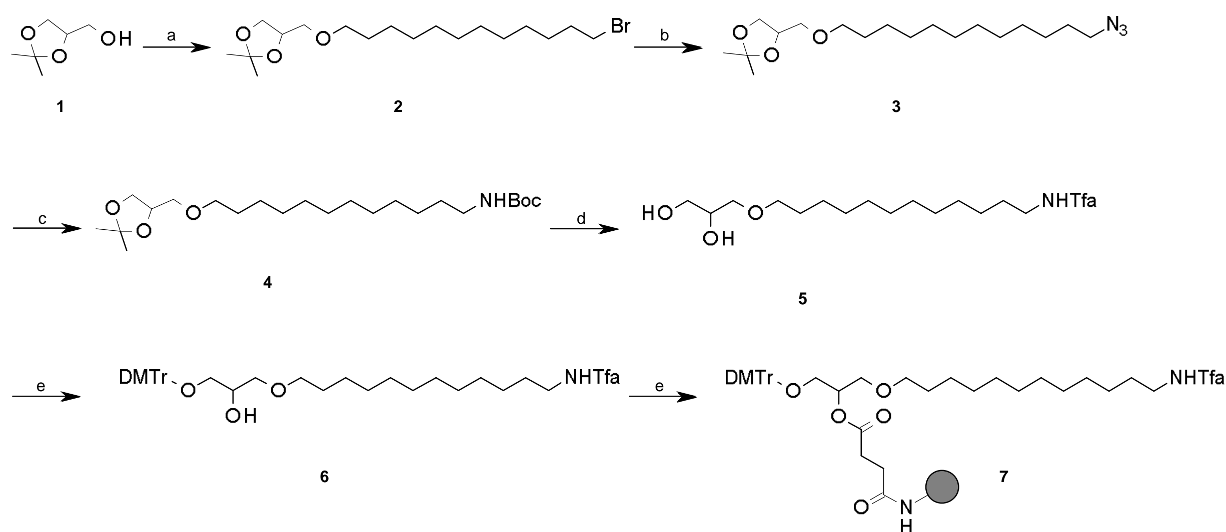


RESULTS AND DISCUSSION

Synthesis of the lipid derivatives. The introduction of lipids to the 3øend of oligonucleotides needs the use of a linker that connects the lipid to the growing DNA chain. Aminodiols such 4-aminoprolinol[12a, 12d] and trihydroxy compounds such as glycerol[12c] have been used for this purpose. Aminodiols are used to incorporate fatty acids by formation of an amide bond that is stable to oligonucleotide synthesis conditions[12d]. Moreover, aminodiols such as 4-aminoprolinol[12d] have the advantage of well defined chirality. But the use of this type of linkers needs the use of fatty acids functionalized at the other end of the hydrocarbon chain for the preparation of amino-lipids. These compounds are not easily available. In contrast, trihydroxy compounds can be used if ether or urethane bonds are used to connect the lipid to the triols. In these cases the resulting lipid derivatives usually generate diastereoisomeric mixtures, but the presence of isomers are not considered a serious problem for biological activity of the resulting siRNA. Moreover disubstituted alkanes needed for the preparation of the lipid derivatives are available. For these reasons we considered the use of a glycerol backbone (Scheme 1) as suitable linker for introducing our chemical modifications at the 3ø-end of the siRNA duplexes. This linker when reacted with phosphoramidites will produce a phosphate bond that will be stable to ammonia deprotection

conditions used in oligonucleotide synthesis. Also, it allows the incorporation of the lipid derivative to a solid support. The synthesis of lipidic solid-supports **7**, **15** and **16** is outlined in Schemes 2 and 3. All compounds were obtained from the same intermediate azide **3** which was synthesized from commercially available (*rac*)-(2,2-dimethyl-1,3-dioxolan-4-yl)methanol as starting material. The synthesis started with the alkylation reaction between solketal **1** and commercially available 1,12-dibromododecane in DMF in the presence of sodium hydride (60%) which primarily yielded the alkylated compound **2.** However, the presence of a side compound was also observed. This side compound may arise from the E2 elimination of HBr (dehydrohalogenation). To avoid this, we started an alkylation reaction of **1** via di-*n*-butylstannylene in the presence of 2.0 equiv of cesium fluoride[15] in the presence of DMF, providing **2** in 47% yield after purification by flash chromatography and without detecting any trace of that impurity.

SCHEME 2. Synthesis of solid-support **7** functionalized with amino C_{12}



Reagents and Conditions: a. i. di-n-butyltin oxide, MeOH, reflux; ii. CsF, 1,12-Dibromododecane, 60 ºC; b. NaN_3, DMF, 70 ºC; c. Boc_2O, TEA, DCM, r.t.; d. i. DCM/TFA 10%, r.t.; ii. ETFA, TEA, DCM, 0ºC; e. DMTr, DMAP, Py, 40 ºC; e. CPG derivatization

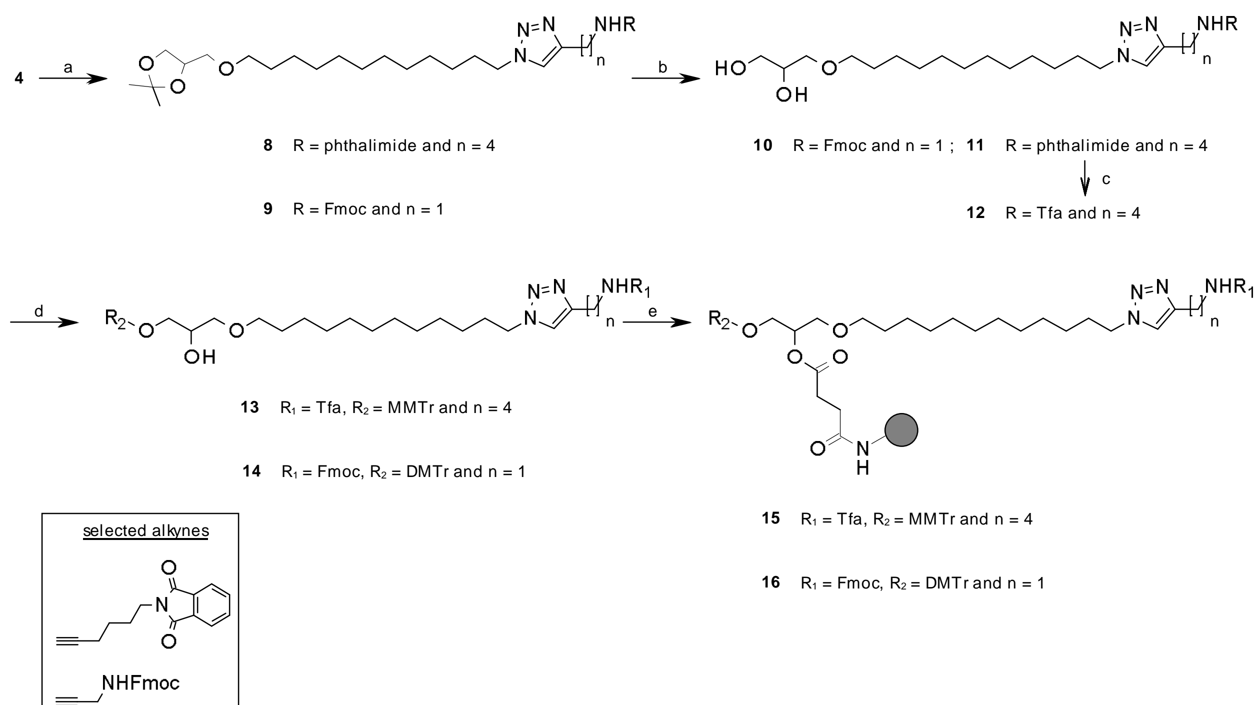
The nucleophilic displacement of the bromide group with sodium azide in DMF yielded the desired azide **3** in 93% yield. Having on hand azide **3**, subsequent reduction under Staudinger conditions gave

the expected amine which was conveniently protected with Boc_2O in standard conditions achieving the *N*-Boc-protected **4** in 65% yield. Finally, the introduction of the 4,4ø-dimethoxytrityl (DMTr) protecting group was easily obtained by removing acetonide and *N*-Boc moieties simultaneously under acidic conditions (DCM:TFA 3%) followed by *N*-protection with ethyl trifluoroacetate thereby yielding the *N*-protected compound **5** in 87% yield. DMTr protection with DMAP in the presence of pyridine afforded the desired trityl derivative **6** in 58% yield after purification by flash chromatography.

Click chemistry, on the other hand, is considered to be a modular approach that is increasingly found in all aspects of drug discovery, combinatorial chemistry and recently, in nucleic acid chemistry[16], easily obtaining triazolyl rings.[17] Taking this into account, we considered exploring the use of the 1,3-dipolar cycloadditions between the previously synthesized azide **3** and some commercially available alkynes[18] in order to study the subsequent effect that these kind of rings could exercise on the RNAi machinery. Then, the click reactions were carried out under standard conditions[19] to give the desired triazoles compounds **8** and **9** as only regioisomers in 89% and 59% yield respectively after purification by flash chromatography (Scheme 3).

In order to obtain the corresponding trityl derivatives, compounds **8** and **9** were subjected to acetonide hydrolysis in different acid conditions (DCM:TFA 3% for acetonide **8**; *p*TsOH in the presence of methanol for acetonide **9**, respectively) yielding the requisite diols **10** and **11** in 72% and 99% yield respectively. Trityl derivative **13** was directly obtained from protecting alcohol **10** with DMTr under the same conditions previously mentioned in 39% yield. Finally, the synthesis of last trityl derivative **14** was carried out as follows: the phthalimide **11** was removed under basic hydrolysis to give the expected amine which was then subsequently protected with ethyl trifluoroacetate yielding the *N*-protected amine **12**. Finally, the selective protection of primary alcohol **12** with 4-monomethoxytrityl (MMTr) afforded the corresponding protected trityl alcohol **14** in 45% yield.

SCHEME 3. Click chemistry and synthesis of the solid-supports **15** and **16**



Reagents and Conditions: a. selected alkynes, 10% sodium ascorbate, $CuSO_4 \cdot 5H_2O$, ${}^tBuOH:H_2O$ (1:1), r.t., overnight; b. i. (for **8**): DCM:TFA 3%, r.t.; (for **9**): *p*TsOH, MeOH, r.t.; c. i. $MeNH_2$, EtOH, 40 ºC, overnight; ii. ETFA, TEA, DCM, 0 ºC; d. (for **13**): DMTr, DMAP, pyridine, 40 ºC, overnight; (for **14**): MMTr, DMAP, pyridine, 40 ºC, overnight.; e. CPG derivatization

The three trityl compounds **6**, **13** and **14** were coupled with CPG supports using the succinyl linker as described.[20] For this purpose, the DMTr and MMTr-derivatives described above reacted with succinic anhydride followed by coupling the resulting hemisuccinates with amino-functionalized CPG which yielded glass beads containing lipids **7** (21 μmol/g), **15** (25 μmol/g) and **16** (23 μmol/g), respectively. CPG functionalization was determined by the measure of the absorbance of the DMTr / MMTr cations released from the support upon acid treatment.

Oligodeoxyribonucleotide synthesis. First, we synthesized a short oligodeoxynucleotide sequence to demonstrate the stability of the lipid derivative to phosphoramidite synthesis conditions. The self-complementary *Dickerson-Drew* dodecamer sequence (5ø-CGCGAATTCGCG-3ø) was used as a model sequence. The sequence was assembled on CPG solid supports **7**, **15** and **16** using standard protocols in

order to generate the corresponding lipid-oligonucleotide conjugates by using *DMT off*- based protocols. After cleavage with ammonia solution (32%) followed by HPLC purification the corresponding modified aminolipid-oligonucleotide conjugates (**17-19**) were obtained and confirmed by MALDI-TOF mass spectrometry (MS) (Table 1).

TABLE 1. Mass spectrometry and melting temperatutes (T_m) of lipid-modified oligonucleotides prepared in this study.

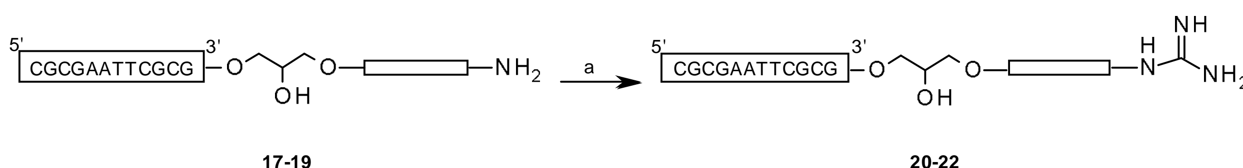
Oligonucleotide	Sequence 5ø→ 3ø	MW calc	MW found	T_m (ºC)*
17	CGCGAATTCGCG-$C_{12}NH_2$	3983	3982.5	55
18	CGCGAATTCGCG-C_{12}-triazol-CH_2NH_2	4066	4065	58
19	CGCGAATTCGCG-C_{12}-triazol-$(CH_2)_4NH_2$	4106	4105	57
20	CGCGAATTCGCG-C_{12}NHC(NH)-NH_2	4027	4029	61
21	CGCGAATTCGCG-C_{12}-triazol-CH_2NHC(NH)-NH_2	4108	4109	56
22	CGCGAATTCGCG-C_{12}-triazol-$(CH_2)_4$NHC(NH)-NH_2	4150	4151	55
23	GUGCCUAUGUCUCAGCCUCTT-$C_{12}NH_2$	6896	6899	n.d.
24	GUGCCUAUGUCUCAGCCUCTT- C_{12}-triazol-CH_2NH_2	6977	6991 [$M+Na^+$]	n.d.
25	GUGCCUAUGUCUCAGCCUCTT- C_{12}-triazol-$(CH_2)_4NH_2$	7019	7054 [$M+2Na^+$]	n.d.

*conditions: sodium phosphate 10 mM, NaCl NaCl, pH 7.0, in these conditions unmodified *Dickerson-Drew* dodecamer melted at 49 ºC; n.d. not determined.

In addition, we studied the conversion of the amino group to the guanidinium group in order to extend the possibility of cationic charge enrichment in our synthesized aminolipid-oligonucleotide conjugates **17**-**19**. The synthesis of certain guanidinium derivatives of nucleic acids has been described in the literature[21] and some of them have been synthesized through post-synthetic modification.[14a,21e] The guanidinium-modified ONs synthesized during this study are summarized on Table 1. In all cases, selective and quantitative guanidinylation were observed following a classical post-synthetic

approach[21e] on oligonucleotides **17**-**19** which were reacted with *O*-methylisourea for 16 hr at 55ºC. After desalting, the guanidinylated compounds **20**-**22** (Scheme 4) were analyzed by analytical HPLC and characterized by MALDI experiments. Melting temperatures of the synthesized lipid-modified dodecamers were higher than unmodified dodecamer (see Table 1). A similar effect has been also observed recently with the dodecamer modified with arginine and lysine residues as well as lipids.[14b].

SCHEME 4. Synthesis of the amine and guanidinium oligonucleotides



Reagents and Conditions. a. i. *O*-Methylisourea chloride, NH_3 (32%), 55 ºC, overnight

Oligoribonucleotide synthesis. All oligoribonucleotides were synthesized using solid supports **7**, **15** and **16** (1.0 µmol each) using a DNA/RNA synthesizer (sequences of guide and passenger strands are shown in Tables 1 and 2). Modified RNA oligonucleotides linked to the aforementioned solid supports were released according to *DMT on-* based protocols and then crude modified oligoribonucleotides were first purified by HPLC followed by DMTr deprotection with AcOH 80%. Finally, amino lipid-RNA conjugates (**23**-**25**) were again analyzed by reversed phase HPLC and characterized by MALDI-TOF mass spectrometry (Table 1). siRNA duplexes (**siRNA2**, **siRNA3** and **siRNA4**, respectively) were obtained by annealing of equal molar amounts of passenger (sense) and guide (antisense) strands, which were purified by ethanol precipitation protocol (see Experimental Part).

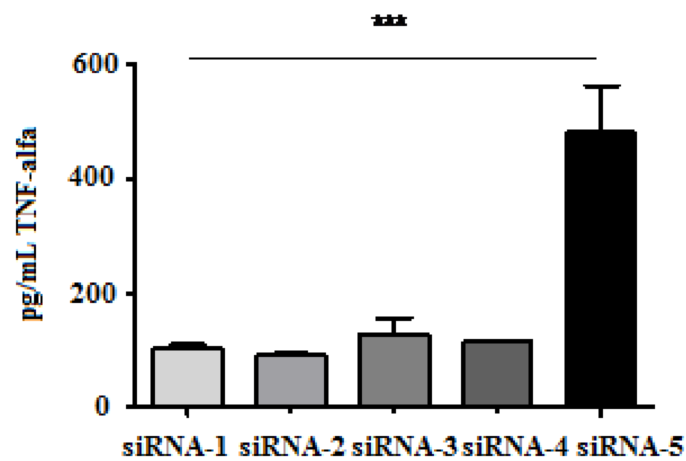
TABLE 2. Sequences of unmodified, lipid-modified and scrambled siRNA sequences used in this study

siRNA	Sequences*
Unmodified (siRNA-1)	GUGCCUAUGUCUCAGCCUCTT TTCACGGAUACAGAGUCGGAG
siRNA-2	GUGCCUAUGUCUCAGCCUCTT-$C_{12}NH_2$ TTCACGGAUACAGAGUCGGAG
siRNA-3	GUGCCUAUGUCUCAGCCUCTT-C_{12}-triazol-CH_2NH_2 TTCACGGAUACAGAGUCGGAG
siRNA-4	GUGCCUAUGUCUCAGCCUCTT-C_{12}-triazol-$(CH_2)_4NH_2$ TTCACGGAUACAGAGUCGGAG
Scrambled (siRNA-5)	CAGUCGCGUUUGCGACUGGTT TTGUCAGCGCAAACGCUGACC

*siRNAs are shown with the passenger strand above (5ø ó 3ø) and the guide strand below (3ø ó 5ø). T stands for thymidine.

Gene silencing by modified siRNAs. The gene silencing effect on the TNF- messenger RNA of several siRNAs: one native **siRNA1**, three chemically modified siRNAs (**siRNA2**, **siRNA3** and **siRNA4**) and one scrambled **siRNA5** were studied. HeLa cells were first co-transfected with plasmid pCAm TNF- by using commercial cationic liposomes (Lipofectamine-2000). We then carried out the transfection of the aforementioned siRNA derivatives with oligofectamine. Forty-eight hours after transfection, the amount of TNF- produced by the cells was analyzed by enzyme-linked immunosorbent assay (ELISA). The silencing activities are depicted on Figure 1. In general, all chemically modified siRNAs previously obtained maintained their abilities to down-regulate TNF- protein expression levels to around 65% at 50 nM concentration compared with the scrambled control **siRNA5** duplex. These results indicate that the introduction of all the proposed modifications at the 3ø-termini of the passenger strand of an RNA duplex is compatible with the RNAi machinery in HeLa cells.

FIGURE 1. Efficiency of silencing of chemically modified siRNAs with lipids in the passenger strand



Plot of gene-specific silencing activities for native (siRNA-**1**), chemically modified conjugates (siRNA-**2**, siRNA-**3** and siRNA-**4**) and scrambled sequence (siRNA-**5**) (50 nM per well). Transfection of siRNA conjugates was carried out by using Lipofectamine 2000 and Oligofectamine (see above). Values are represented the average ±ES, n = 3 and are compared to a scrambled sequence. ***p<0.001, ANOVA Test, Bonferrini post-test.

CONCLUSIONS

In general, the most common method of administration of siRNAs in cell culture is based on the use of non-viral vectors such as cationic lipids. The interaction between siRNA and cationic lipids is due to the existence of electrostatic interactions and the formation of lipoplexes. Notwithstanding, the use of a covalent strategy between the aforementioned siRNA and cationic lipids is also possible, though in most of the cases only neutral lipids have been linked to siRNA. Following this approach, we have reported a convenient synthesis by which glycerolipid based structures with different polar groups have been efficiently synthesized and introduced into the 3ø-termini of the siRNA sense strand being the first time that oligonucleotides carrying cationic lipids are reported. The amino group of the lipid can be used for the generation of guanidinium groups and also for further functionalization to proteins, liposomes, nanoparticles and so on. Finally, we were able to confirm all these proposed modifications did not affect

the RNAi machinery through silencing TNF- gene expression.

EXPERIMENTAL SECTION

Abbreviations: ACN, acetonitrile; AcOEt, ethyl acetate; Boc, *t*-butoxycarbonyl; Boc_2O, di-*t*-butyl dicarbonate; tBuOH, *t*-butanol; Ch, cyclohexane; CPG, controlled pore glass; DCM, dichloromethane; DMF, *N*,*N*-dimethylformamide, DMAP: *N*,*N*-dimethylaminopyridine; DMTr: 4,4ø-dimethoxytrityl; EtOH, ethanol; ETFA, ethyl trifluoroacetate; Fmoc, 9-fluorenylmethoxycarbonyl; MeOH, methanol; MMTr, 4-monomethoxytrityl; Py: pyridine; TEA, triethylamine; TNF-α, tumor necrosis factor; TFA, trifluoroacetic acid; Tfa: trifluoroacetyl; TsOH: *p*-toluenesulfonic acid.

Materials and Methods. All reactions were carried out under argon positive pressure in anhydrous solvents. Commercially available reagents and anhydrous solvents were used without further purification. Solvents were distilled prior to use and dried using standard methods. Analytical samples were homogeneous as confirmed by TLC and yielded spectroscopic results were consistent with the assigned structures. Chemical shifts are reported in parts per million (ppm) relative to the singlet at = 7.24 ppm of $CHCl_3$ for ^1H NMR and to the centre line of the triplet at = 77.0 ppm of $CDCl_3$ for ^{13}C NMR. IR spectra were measured in film. Thin-layer chromatography (TLC) was performed on silica gel (Alugram Sil G/UV).

HPLC conditions. Conditions for semipreparative HPLC: HPLC solutions were solvent A: 5% acetonitrile (ACN) in 100 mM triethylammonium acetate (TEAA), pH 6.5 and solvent B: 70 % ACN in 100 mM triethylammonium acetate (pH 6.5). Column: PRP-1 (Hamilton) 250x 10 mm. Flow rate 3 ml/min linear gradient from 15 to 100% in B (DMTon) and 0 to 50% in B (DMToff) was used with UV detection at 260 nm. Conditions for analytical HPLC: HPLC solutions were solvent A: 5% acetonitrile

(ACN) in 100 mM triethylammonium acetate (TEAA), pH 6.5 and solvent B: 70 % ACN in 100 mM triethylammonium acetate (pH 6.5). Column: XBridge OST C18 2.5 m. Flow rate 1ml/min linear gradient from 0 to 50% in B (DMToff) were used with UV detection at 260 nm.

4-((12-bromododecyloxy)methyl)-2,2-dimethyl-1,3-dioxolane, 2. Alcohol **1** (200 mg; 1.51 mmol) was reacted with dibutyltin oxide (751 mg, 3.02 mmol; 2.0 eq) in dry methanol (10 mL) at reflux for 3h, followed by removing the methanol and any traces of water using azeotropic distillation with toluene. The reaction mixture was concentrated and then, 1,12-dibromododecane (991 mg, 3.02 mmol; 2.0 eq), cesium fluoride (458 mg, 3.02 mmol; 2.0 eq) and dry DMF (10 mL) were added. The reaction mixture was kept at room temperature overnight. The solvent was evaporated and the resulting residue was purified by flash chromatography (Ch:AcOEt 1% to 4%): Yield: 47%; IR (film) 2989, 2931, 2858, 1455, 1367, 1255, 1212, 1115, 1054, 845 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) 4.24 (q, *J* = 6.0 Hz, 1H, C$\underline{H}$-O), 4.03 (dd, *J* = 14.6 Hz, 8.2 Hz; 1H, O-C$\underline{H}$-CH), 3.71 (dd, *J* = 8.2 Hz, 14.6 Hz; 1H, O-C$\underline{H}$-CH), 3.49 (m, 2H, C$\underline{H}_2$-O), 3.40 (m, 2H, C$\underline{H}_2$-O), 3.38 (t, *J* = 6.8 Hz, 2H, C$\underline{H}_2$-Br), 1.83 (q, *J* = 7.1 Hz, 2H, C$\underline{H}_2$-CH_2), 1.55 (m, 2H), 1.40 (s, 3H, C$\underline{H}_3$-C), 1.34 (s, 3H, C$\underline{H}_3$-C), 1.25 (m, 16H); ^{13}C-NMR (125 MHz, $CDCl_3$) 109.9 (O-$\underline{C}$-CH_3), 75.4 ($\underline{C}$H-O), 72.5 (O-$\underline{C}H_2$), 72.4 ($\underline{C}H_2$-O), 67.5 ($\underline{C}$H2-O), 34.6 ($\underline{C}H_2$-Br), 33.4, 30.2, 30.1, 30.1, 30.0, 30.0, 29.4, 28.8, 27.4, 26.6, 26.0; HR ESI MS: *m/z* calcd for $C_{18}H_{35}BrO_3$ Na (M + Na), 401.1661; found, ; *m/z* 401.1662; calcd for $C_{36}H_{70}O_6NaBr_2$ Na (2M + Na), 779.3427; found, 779.3431.

4-((12-azidododecyloxy)methyl)-2,2-dimethyl-1,3-dioxolane, 3. Compound **2** (100 mg, 0.264 mmol) was dissolved in 5 mL of anhydrous DMF. NaN_3 (103 mg, 1.58 mmol; 6.0 eq) was then added and the mixture was stirred and heated to 70 ºC for 48 h. The reaction was cooled at 0ºC and water was added carefully. The solvent was evaporated and the resulting oil was then purified by flash chromatography (Ch:AcOEt 1% to 3%): Yield: 93%; IR (film) 2935, 2858, 2363, 2344, 2093, 1251, 1077 cm^{-1}; ^{1}H-NMR

(400 MHz, $CDCl_3$)   4.26 (q, *J* = 6.1 Hz, 1H, CH-O), 4.06 (dd, *J* = 14.6 Hz, 8.23 Hz; 1H, O-CH-CH), 3.72 (dd, *J* = 8.22 Hz, 14.6 Hz; 1H, O-CH-CH), 3.51 (m, 2H, CH_2-O), 3.43 (m, 2H, CH_2-O), 3.25 (t, *J* = 6.97 Hz, 2H, CH_2-N_3), 1.59 (m, 6H), 1.42 (s, 3H, CH_3-C), 1.36 (s, 3H, CH_3-C), 1.27 (m, 14H); ^{13}C-NMR (125 MHz, $CDCl_3$)   109.3 (O-C-CH_3), 74.7 (CH-O), 71.8 (O-CH_2), 71.7 (O-CH_2), 66.9 (O-CH_2), 51.4 (CH_2-N_3), 29.5, 29.4, 29.4, 29.4, 29.4, 29.1, 28.7, 26.7, 26.6, 26.0, 25.3; HR ESI MS: *m/z* calcd for $C_{18}H_{36}N_3O_3$ (M^+), 342.2755; found, *m/z* 342.2751; calcd for $C_{18}H_{35}N_3O_3K$ (M + K), 380.2312; found, 380.2310.

***tert*-butyl 12-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)dodecylcarbamate, 4.** Azide **3** (85 mg, 0.249 mmol) along with PPh_3 (131 mg, 0.498 mmol; 2.0 eq) were dissolved in 3 mL of anhydrous THF. The reaction was stirred for 2 hours at room temperature. Subsequently, water (500 uL) was added dropwise. The mixture was stirred overnight at room temperature. The solvent was evaporated and the crude was dried by pressure, yielding the anticipated amine. This amine was dissolved in 2.0 mL of anhydrous DCM; TEA (69   L, 0.498 mmol; 2.0 eq) was added dropwise along with Boc_2O (82 mg, 0.373 mmol; 1.5 eq). The reaction was stirred for 5 hours at room temperature. The organic layer was extracted with more DCM (5 mL) and rinsed with water (3 x 10 mL). The organic layer was dried on anhydrous Na_2SO_4. The solvent evaporated and the resulting residue was purified by flash chromatography (Ch:AcOEt 4% to 9 %): Yield: 65%; IR (film) 3020, 2935, 2850, 1699, 1506, 1371, 1216, 1166, 1046 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$)   4.49 (broad s, 1H), 4.26 (m, 1H, CH-O), 4.04 (dd, *J* = 14.6 Hz, 8.2 Hz; 1H, O-CH-CH), 3.72 (dd, *J* = 14.6 Hz, 8.2 Hz; 1H, O-CH-CH), 3.50 (m, 2H, CH_2-O), 3.42 (m, 2H, CH_2-O), 3.08 (m, 2H, CH_2-N), 1.56 (m, 2H), 1.43 (s, 9H, 3 CH_3-C), 1.41 (s, 3H, CH_3-C), 1.35 (s, 3H, CH_3-C), 1.24 (m, 18H); ^{13}C-NMR (125 MHz, $CDCl_3$)   158.8 (CO), 110.0 (OCCH3), 75.4 (CH-O), 72.5 (O-CH_2), 72.4 (O-CH_2), 67.6 (O-CH_2), 30.7 (N-CH_2), 30.2, 30.1, 29.9, 29.1, 27.4, 27.4, 26.7, 26.1; HR ESI MS: *m/z* calcd for $C_{23}H_{45}NO_5$ (M + H^+), 416.3370; found, *m/z* 416.3370.

***N*-(12-(2,3-dihydroxypropoxy)dodecyl)-2,2,2-trifluoroacetamide, 5.** Compound **4** (60.0 mg, 0.161

mmol) was dissolved in a mixture of DCM/TFA 10% (5 mL) at room temperature for 20 minutes. The solvent evaporated yielding the corresponding trifluoroacetate in its salt form. The resulting oil was then dissolved in anhydrous DCM (5.0 mL) and TEA was added dropwise (45 L, 0.322 mmol). The reaction was cooled at 0°C and ethyl trifluoroacetate (22.0 L, 0.177 mmol) was added. The reaction was stirred for 30 minutes, extracting the organic layer with more DCM (3 x 10 mL) and rinsed with water (3 x 10 mL). The organic layer was dried with anhydrous Na_2SO_4. The solvent evaporated and the resulting oil was purified by flash chromatography (DCM:MeOH 2%): Yield: 87 %; IR (film) 3333, 2924, 2854, 1694, 1552, 1471, 1185 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) 6.42 (broad s, 1H), 3.05 (m, 1H, CH-O), 3.72 (dd, *J* = 11.4 Hz, 3.8 Hz; 1H, O-CH-CH), 3.65 (dd, *J* = 11.4 Hz, 5.2 Hz; 1H, O-CH-CH), 3.51 (m, 2H, CH_2-O), 3.49 (m, 2H, CH_2-O), 3.35 (m, 2H, CH_2-NHCO), 1.57 (m, 2H), 1.26 (m, 20H); ^{13}C-NMR (125 MHz, $CDCl_3$) 157.8 (q, *J* = 36.9 Hz, $COCF_3$), 117.9 (q, *J* = 287.6 Hz, CF_3), 73.2 (CH-O), 72.5 (O-CH_2), 71.0 (O-CH_2), 64.9 (O-CH_2), 40.6 (NHCO-CH_2), 30.2, 30.1, 30.1, 30.1, 30.0, 29.7, 29.6, 27.3, 26.7; ^{19}F-NMR ($CDCl_3$) -76.3 (reference $CFCl_3$); HR ESI MS: *m/z* calcd for $C_{17}H_{32}F_3NO_4Na$ (M + Na^+), 394.2175; found, *m/z* 394.2176.

***N*-(12-(3-(bis(4-methoxyphenyl)(phenyl)methoxy)-2-hydroxypropoxy)dodecyl)-2,2,2-trifluoroacetamide, 6.** Compound **5** (52 mg, 0.140 mmol) along with DMTrCl (48 mg, 0.141 mmol; 1.2 eq) and DMAP (8 mg, 0.07 mmol; 0.5 eq) were dissolved in 1.5 mL of anhydrous pyridine. The reaction was heated at 40 °C and stirred overnight. Methanol (0.5 mL) was added and the solvent evaporated. The resulting product was purified by flash chromatography (Hex/AcOEt 15%): Yield: 58%; IR (film) 3276, 3072, 2927, 2851, 1716, 1673, 1607, 1502, 1462, 1390, 1248, 1176, 1038 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) 7.67 (m, 3H, phenyl), 7.40 (m, 2H, phenyl), 7.28 (m, 3H, phenyl), 7.01 (broad s, 1H), 6.81 (d, *J* = 8.9 Hz, 4H, phenyl), 3.92 (m, 1H, CH-O), 3.76 (s, 6H, 2 CH_3), 3.52 (dd, *J* = 9.7 Hz, 4.2 Hz; 1H, CH-CH_2-O), 3.43 (m, 3H, CH-CH_2-O), 3.35 (m, 2H, CH_2-O), 3.17 (m, 2H, CH_2-NH), 2.41 (broad d, 1H), 1.85 (m, 2H), 1.57 (m, 9H), 1.25 (m, 9H); ^{13}C-NMR (125 MHz, $CDCl_3$) 162.7, 158.6, 149.9, 145.0. 136.3, 136.2, 130.2, 128.3, 127.9, 126.9, 123.9, 113.2, 86.2, 72.3, 71.8, 70.1,

64.7, 55.4, 55.3, 55.3, 50.8, 40.2, 36.6, 31.6, 29.9, 29.8, 29.7, 29.7, 29.6, 29.6, 29.6, 29.3, 29.1, 26.8, 26.2; ^{19}F-NMR -74.3 (reference $CFCl_3$); HR ESI MS: *m/z* calcd for $C_{38}H_{50}F_3NO_6Na$ (M+ Na^+)), 696.3482; found, *m/z* 696.3490.

General procedure for the click reaction (**8** and **9**). Azide **3** (1.0 eq) and selected alkynes (1.0 eq) were suspended in a 1:1 mixture of water and *tert*-butyl alcohol (1.0 mL). Sodium ascorbate (10%) and copper (II) sulphate pentahydrate (1%) were added dropwise. The heterogeneous mixture was stirred vigorously overnight. The reaction mixture was diluted with DCM and the organic layer was washed with water (3x5 mL). The organic layer was dried with anhydrous Na_2SO_4 and solvent evaporated. The resulting oil was purified by flash chromatography.

2-(4-(1-(12-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)dodecyl)-1*H*-1,2,3-triazol-4-yl)butyl)isoindoline-1,3-dione, 8. Yield: 89%; IR (film) 2919, 2854, 2363, 2342, 1710, 1053 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) 7.81 (m, 2H, phenyl), 7.69 (m, 2H, phenyl), 7.27 (s, 1H, C$\underline{H}$=C), 4.27 (t, *J* = 7.24 Hz; 2H, CH$\underline{2}$-O), 4.22 (m, 1H, C$\underline{H}$-O), 4.04 (dd, *J* = 14.6 Hz, 8.2 Hz; 1H, CH-C$\underline{H}_2$-O), 3.69 (m, 3H), 3.49 (m, 2H), 3.41 (m, 2H), 2.74 (t, *J* = 7.0 Hz, 2H, C$\underline{H}_2$-N), 1.85 (m, 2H), 1.72 (m, 4H), 1.54 (m, 2H), 1.40 (s, 3H, C$\underline{H}_3$-C), 1.34 (s, 3H, C$\underline{H}_3$-C), 1.26 (m, 16H); ^{13}C-NMR (125 MHz, $CDCl_3$) 168.6 (CO), 134.1 (phenyl), 132.3 (phenyl), 123.3 (phenyl), 121.0 ($\underline{C}$=N), 120.6 ($\underline{C}$H-N) 109.5 (O-$\underline{C}$-CH3), 74.9 ($\underline{C}$H-O), 72.1 (CH-C$\underline{H}_2$-O), 72.0 (CH-C$\underline{H}_2$-O), 67.1 (CH-C$\underline{H}_2$-O), 50.4 ($\underline{C}H_2$-C=), 37.8 ($\underline{C}H_2$-NCO), 30.5 ($\underline{C}$H2-N), 29.7, 29.7, 29.7, 29.6, 29.5, 29.2, 28.2, 26.9, 26.8, 26.7, 26.2, 25.6, 25.3; HR ESI MS: *m/z* calcd for $C_{32}H_{48}N_4O_5$ (M^++1), 569.3697; found, *m/z* 569.3697 calcd for $C_{32}H_{48}N_4O_5$ Na (M + Na^+), 591.3516; found, *m/z* 591.3516.

(9H-fluoren-9-yl)methyl-(1-(12-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)dodecyl)-1H-1,2,3-triazol-4-yl)methylcarbamate, 9. Yield: 58%; IR (film) 2930, 2858, 1721,1523, 1448, 1370, 1250, 1120, 1049, 758, 738 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) 7.74 (d, *J* = 7.5 Hz; 2H, phenyl), 7.57 (d, *J* = 7.5 Hz; 2H, phenyl), 7.47 (s, 1H, C$\underline{H}$=C), 7.38 (t, *J* = 7.3Hz; 2H, phenyl), 7.29 (t, *J* = 7.4 Hz; 2H,

phenyl), 5.48 (broad d, 1H, NH), 4.46 (d, *J* = 6.0 Hz; 2H, C$\underline{H}_2$-OCO), 4.40 (d, *J* = 7.0 Hz; 2H, CH-C$\underline{H}_2$-O), 4.31 (t, *J* = 7.2 Hz; 1H, O-C$\underline{H}$-C), 4.25 (t, *J* = 6.0 Hz; 2H, C$\underline{H}_2$-O), 4.22 (m, 2H), 4.05 (dd, *J* = 14.6 Hz, 8.2 Hz; 1H, C$\underline{H}$-O-C), 3.72 (dd, *J* = 14.6 Hz, 8.2 Hz; 1H, C$\underline{H}$-O-C), 3.50 (m, 2H), 3.42 (m, 2H), 1.87 (m, 2H), 1.55 (m, 2H), 1.41 (s, 3H, C$\underline{H}_3$-C), 1.35 (s, 3H, C$\underline{H}_3$-C), 1.27 (m, 16H); ^{13}C-NMR (125 MHz, $CDCl_3$) 156.3 (CO), 143.8 (phenyl), 141.2 (phenyl), 127.6 (phenyl), 127.0 ($\underline{C}$H=C), 125.0 ($\underline{C}$=C), 119.9 (phenyl), 109.3 (O-$\underline{C}$-O), 74.7 ($\underline{C}$H-O), 71.9 (CH-C$\underline{H}_2$-O), 71.8 (CH-C$\underline{H}_2$-O), 66.9 (C$\underline{H}_2$-O), 50.3 (O-$\underline{C}H_2$-C), 47.2 (C-$\underline{C}H_2$-NCO), 36.5 (C-$\underline{C}H_2$), 30.2, 29.5, 29.5, 29.4, 29.4, 29.3, 28.9, 26.7, 26.4, 26.0, 25.4; HR ESI MS: *m/z* calcd for $C_{36}H_{50}N_4O_5$ (M^++1), 619.3853; found, ; *m/z* 619.3858; calcd for $C_{36}H_{50}N_4O_5$ Na (M + Na^+), 641.3673; found, *m/z* 641.3675.

(9*H*-fluoren-9-yl)methyl-(1-(12-(2,3-dihydroxypropoxy)dodecyl)-1*H*-1,2,3-triazol-4-yl)methylcarbamate, 10. Compound **9** (48 mg; 0.078 mmol) was dissolved in a mixture of CH_2Cl_2 / TFA 3% (2.5 mL). The reaction was stirred at room temperature for 30 minutes. The solvent was reduced to dryness, washing three times with more CH_2Cl_2. The resulting compound was purified by flash chromatography (CH_2Cl_2 / MeOH 2%): Yield: 72%; IR (film) 3329, 2926, 2858, 1715, 1522, 1441, 1256, 1127, 1043, cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) 7.76 (d, *J* = 7.5 Hz; 2H), 7.58 (d, *J* = 7.3 Hz; 2H), 7.38 (t, *J* = 7.3 Hz; 2H), 7.28 (m, 3H), 5.83 (broad s, 1H), 5.31 (s, 2H), 4.64-4.13 (m, 5H), 3.76 (m, 4H), 3.47 (m, 4H), 1.89 (m, 2H), 1.56 (m, 2H), 1.26 (m, 16H); ^{13}C-NMR (400 MHz, $CDCl_3$) 156.4, 143.6, 141.1, 127.5, 126.8, 124.9, 119.8, 72.3, 71.6, 70.3, 70.2, 66.7, 64.0, 53.2, 50.6, 47.0, 29.9, 29.5, 29.3, 29.2, 29.2, 29.2, 29.2, 29.1, 28.7, 26.3, 25.8; HR ESI MS: *m/z* calcd for $C_{33}H_{47}N_4O_5$ (M^++1), 579.3540; found, *m/z* 579.3539 calcd for $C_{33}H_{46}N_4O_5$ Na (M + Na^+), 601.3360; found, *m/z* 601.3358.

2-(4-(1-(12-(2,3-dihydroxypropoxy)dodecyl)-1*H*-1,2,3-triazol-4-yl)butyl)isoindoline-1,3-dione, 11.

Compound **8** (30 mg, 0.052 mmol; 1.0 eq) along with *p*TsOH (5 mg, 0.026 mmol; 0.5 eq) were dissolved in methanol (1.0 mL). The reaction was stirred at room temperature for 5 hours. The methanol evaporated and the resulting oil was purified by flash chromatography (CH_2Cl_2:MeOH 2%): Yield:

99%; IR (film) 3349, 2924, 2850, 1684, 1517, 1251, 1170, 1123, 1046 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) 7.82 (m, 2H), 7.69 (m, 2H), 7.28 (s, 1H), 4.27 (t, *J* = 7.2 Hz; 2H), 3.85 (m, 1H), 3.69 (m, 3H), 3.63 (m, 1H), 3.49 (m, 2H), 3.44 (m, 2H), 2.74 (m, 2H), 2.53 (broad d, 2H), 1.85 (m, 2H), 1.72 (m, 4H), 1.55 (m, 2H), 1.23 (m, 18H); ^{13}C-NMR (400 MHz, $CDCl_3$) 168.6, 134.1, 132.3, 123.4, 72.7, 72.0, 70.6, 64.4, 50.4, 37.8, 30.5, 29.7, 29.6, 29.6, 29.6, 29.5, 29.5, 29.1, 28.2, 26.8, 26.6, 26.2, 25.3; ; HR ESI MS: *m/z* calcd for $C_{29}H_{44}N_4O_5$ (M^++1), 529.3384; found, *m/z* 529.3384; calcd for $C_{29}H_{44}N_4O_5$ Na (M + Na^+), 551.3190; found, *m/z* 551.3200.

***N*-(4-(1-(12-(3-(bis(4-methoxyphenyl)(phenyl)methoxy)-2-hydroxypropoxy)dodecyl)-1*H*-1,2,3-triazol-4-yl)butyl)-2,2,2-trifluoroacetamide, 13.** Compound **11** (30 mg, 0.060 mmol) was dissolved in 1.0 mL of ethanol. Then, 80 µL of $MeNH_2$ was added dropwise. The mixture was stirred overnight at 40 ºC. The solvent was reduced to dryness and the resulting product was dissolved again in anhydrous DCM (1.5 mL). TEA (38uL) was added dropwise at room temperature. The reaction was cooled at 0ºC and EFTA (8.0 uL) was added dropwise. The reaction was stirred for 30 minutes at 0ºC. The organic layer was washed with water and brine (3 x 10 mL) and dried with anhydrous Na_2SO_4 and evaporated. The resulting compound was used in the next step without further purification. The compound obtained, along with MMTrCl (27.8 mg, 0.09 mmol; 1.5 eq) and DMAP (4.0 mg, 0.03 mmol; 0.5 eq) were dissolved in 1.0 mL of pyridine. The reaction was heated at 40 ºC and stirred overnight. Methanol was added (1.0 mL) and the solvent evaporated. The product was then purified by flash chromatography (DCM/MeOH/TEA 98:1:1): Yield: 39%; IR (film) 3065, 2935, 2860, 1715, 1650, 1593, 1454, 1230, cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) 7.41 (m, 2H), 7.31 (m, 4H), 7.26 (m, 4H), 6.82 (d, *J* = 8.8 Hz, 4H), 4.29 (t, *J* = 7.2 Hz; 2H), 3.94 (m, 1H), 3.78 (s, 6H), 3.51 (m, 2H), 3.42 (m, 4H), 3.17 (m, 2H), 2.99 (m, 3H), 2.74 (t, *J* = 7.0 Hz, 2H), 1.87 (m, 2H), 1.75 (m, 2H), 1.67 (m, 2H), 1.53 (m, 2H), 1.28 (m, 16H); ^{13}C-NMR (400 MHz, $CDCl_3$) 158.6, 150.0, 147.4, 145.0, 136.2, 130.2, 128.3, 128.0, 126.9, 124.0, 123.8 (q, *J* = 298.1 Hz, CF_3), 120.9, 113.2, 86.2, 72.3, 71.8, 70.1, 64.6, 55.4, 50.4, 39.8, 30.5, 29.8, 29.7, 29.6, 29.5, 29.2, 28.1, 26.7, 26.4, 26.3, 24.9; ^{19}F-NMR -73.5 (reference $CFCl_3$); HR ESI MS: *m/z*

calcd for $C_{44}H_{59}F_3N_4O_6$ (M + H^+), 796.4434; found, *m/z* 796.44353 calcd for $C_{44}H_{59}F_3N_4O_6Na$ (M^++23), 819.3745; found, *m/z* 819.3742.

(9H-fluoren-9-yl)methyl-(1-(12-(2-hydroxy-3-((4-methoxyphenyl)-diphenylmethoxy)-propoxy)-dodecyl)-1H-1,2,3-triazol-4-yl)methylcarbamate, 14. 10 (32 mg; 0.055 mmol) along with DMAP (3 mg; 0.0275 mmol; 0.5 eq) and MMTrCl (26 mg, 0.083 mmol; 1.5 eq) were dissolved in pyridine (1.5 mL). The reaction was heated at 40 ºC and stirred overnight. Methanol (1.0 mL) was added and the solvent was evaporated. The resulting compound was purified by flash chromatography (CH_2Cl_2:MeOH 1%): Yield: 45%; IR (film) 3064, 2930, 2855, 1726, 1607, 1505, 1449, 1252, 1074, 755 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) 7.69 (d, *J* = 7.5 Hz; 1H), 7.50 (d, *J* = 7.5 Hz; 1H), 7.35 (m, 4H), 7.21 (m, 4H), 7.13 (m, 1H), 6.73 (d, *J* = 8.9 Hz; 1H), 5.37 (m, 1H), 4.38 (dd, *J* = 22.9 Hz, 5.8 Hz; 4H), 4.18 (m, 4H), 3.87 (m, 1H), 3.71 (s, 3H), 3.45 (m, 2H), 3.38 (m, 2H), 3.10 (m, 2H), 2.48 (c, *J* = 7.2 Hz; 2H), 1.80 (m, 2H), 1.46 (m, 2H), 1.18 (m, 16H); ^{13}C-NMR (400 MHz, $CDCl_3$) 158.8, 156.6, 144.6, 144.0, 141.5, 135.7, 130.5, 128.7, 128.6, 128.0, 127.9, 127.2, 1271, 125.2, 122.2, 120.0, 113.3, 86.5, 72.3, 71.8, 70.0, 67.0, 64.7, 55.4, 50.6, 47.4, 46.4, 36.7, 30.4, 29.8, 29.7, 29.7, 29.6, 29.5, 29.2, 26.7, 26.7, 26.3; HR ESI MS: *m/z* calcd for $C_{53}H_{62}N_4O_6Na$ (M + Na^+), 873.4558; found, *m/z* 873.4555 calcd for $C_{106}H_{124}N_8O_{12}$ Na (2M^++23), 1723.9230; found, *m/z* 1723.9234.

Synthesis of amine oligonucleotides (17-19). Oligonucleotide synthesis was carried out in an automated DNA synthesizer on a 1.0 mol scale using CPG resins **7, 15, 16**. The synthesis followed the regular protocol for the DNA synthesizer. After a final detritylation, the oligonucleotides were removed from the universal solid support by treatment with concentrated ammonia at 55 °C for 20 h. After desalting (Sephadex G-25, NAP-10), pure aminolipid-oligonucleotide conjugates **17-19** were obtained.

Synthesis of guanidin-oligonucleotides (**20-22**). Amine oligonucleotides **20-22** (about 2.0 OD) were treated with a mixture (60 L) of a *O*-Methylisourea chloride prepared solution (50 mg, 0.40 mmol) in water (50 L) and an aqueous ammonia (30%, 60 L). The reaction mixtures were incubated overnight

at 55 ºC. The water evaporated and crude was desalted (Sephadex G-25, NAP-5), yielding the corresponding guanidine-oligonucleotides **20-22** in a pure form.

Melting Experiments. UV melting experiments were carried out on a UV spectrometer. Samples were dissolved in a medium salt buffer containing Na_2HPO_4 (5 mM), NaH_2PO4 (10 mM), NaCl (100 mM). The increase in absorbance at 260 nm as a function of time was recorded while the temperature was increased linearly from 20 to 90 °C at a rate of 0.5 °C/min by means of a Peltier temperature programmer. The melting temperature was determined by the local maximum of the first derivatives of the absorbance vs. temperature curve. All melting curves were found to be reversible. All determinations are averages of triplicates.

Oligoribonucleotide synthesis. The following RNA sequences were obtained from commercial sources (Sigma-Proligo, Dharmacon): sense or passanger scrambled 5ø-CAGUCGCGUUUGCGACUGGTT-3ø; antisense or guide scrambled 5ø-CCAGUCGCAAACGCGACUGTT-3ø; sense or passenger anti-TNF- : 5ø-GUGCCUAUGUCUCAGCCUCTT-3ø and antisense or guide anti-TNF : 5ø-GAGGCUGAGAC-AUAGGCACTT-3ø. Trityl derivatives **6**, **13** and **14** were coupled to the CPG solid supports, according to the literature.[20] Modified CPG solid supports **7**, **15** and **16** were then employed in the preparation of RNAs strands using a DNA/RNA synthesizer. Modified siRNAs sense strands were purified using DMT on- based protocols: RNAs were cleaved from the support with a mixture of concentrated ammonia and ethanol 3:1 at 55 ºC for 60 minutes. Solvent was evaporated to dryness and 1M solution of TBAF in THF was added. RNAs were incubated 24 hours at room temperature. The deprotection reaction was quenched and modified RNA conjugates were desalted (Sephadex, NAP-10) and purified by semi-preparative HPLC. Finally, DMTr groups were deprotected (AcOH 80%, 30 min), extracted them with ether and finally, chemically modified RNA conjugates **24**, **25** and **26** were desalted (Sephadex G-25, NAP-5) and characterized by MALDI-TOF mass spectrometry.

siRNA duplexes. The chemically modified sense or passanger strand and antisense or guide strand

RNAs were dissolved in a buffer 10 mM TRIS 50 mM NaCl (100µL). Different aliquots were taken. For each sample, buffer is added until having a final volume of 100 µL. Modified siRNAs (siRNA-**2**, siRNA-**3** and siRNA-**4**, respectively) were heated at 94 ºC for two minutes and they let to cool until reaching room temperature. Then, 3M NaOAc pH = 5.2 was added (10 µL) along with EtOH (96%) (275 µL). Samples were stirred, centrifuged at 4 ºC (15 minutes, 12000 rpm) and precipitated at -20 ºC. Finally, the supernatant was removed and the respective pellets were carefully dried with argon.

Cell culture and siRNA conjugates treatments. HeLa cells were cultured under standard conditions (37ºC, 5% CO_2, Dulbeccoøs Modified Eagle Medium, 10% fetal bovine serum, 2mM L-glutamine, supplemented with penicillin (100 U/ml) and streptomycin (100 mg/ml). All experiments were conducted at 40-60% confluence. HeLa cells were transfected with 250 ng of the plasmid expressing murine TNF- plasmid using lipofectin (*Invitrogen*), following the manufacturerøs instructions. One hour after transfection, m-TNF- expressing HeLa cells were transfected with 50nM of native siRNA (**23**), chemically modified siRNA-**2**, siRNA-**3**, siRNA-**4** and scrambled siRNA-**5** against TNF- , using oligofectamine (*Invitrogen*). The TNF- concentration was determined from cell culture supernatant by enzyme-linked immunoabsorbent assay kit (ELISA, *Bender MedSystems*) following the manufacturerøs instructions.

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SUPPORTING INFORMATION AVAILABLE. Complete characterization data, copies of NMR spectra for all new compounds and MALDI-TOF mass spectrometry of chemically modified siRNA conjugates. This material is available free of charge via the Internet at http://pubs.acs.org

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18. Click chemistry reaction between azide **3** and *N*-Boc-protected propargylamine was also carried out. It is worth mentioning that we made several attempts to deprotect the *N*-Boc group and the acetonide moiety simultaneously but failed, even when the hydrolysis of this group was carried out in neutral conditions by reaction with TMSOTf in the presence of 2,6-lutidine. For experimental details, see Supplementary Part.

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SYNOPSIS TOC.