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# Synthesis of Oligonucleotides Carrying Amino Lipid Groups at the 3′-End for RNA Interference Studies

Santiago Grijalvo,[†] Sandra M. Ocampo,[†,‡] José C. Perales,[‡] and Ramon Eritja*[,†]

[†]*Institute for Research in Biomedicine* (*IRB Barcelona*), *Networking Center on Bioengineering, Biomaterials and Biomedicine* (*CIBER-BBN*) *and Institute for Advanced Chemistry of Catalonia* (*IQAC*), *Spanish Research Council* (*CSIC*), *Cluster Building, Baldiri Reixac 10, E-08028 Barcelona, Spain, and* [‡]*Biophysics Unit, Department of Physiological Sciences II, Universitat de Barcelona, Feixa Llarga s/n, E-08907 L'Hospitalet del Llobregat, Barcelona, Spain*

*recgma@cid.csic.es*

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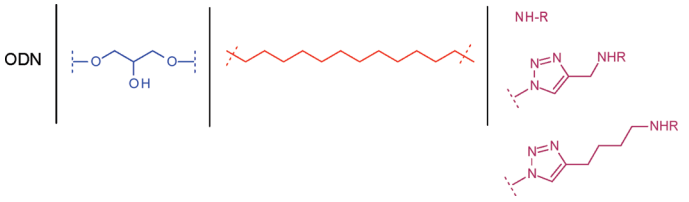


Novel lipid derivatives carrying amino and triazolyl groups were efficiently synthesized and covalently anchored at the 3′-termini of oligonucleotides. The desired amino-lipid conjugates were fully characterized by reversed-phase HPLC and MALDI-TOF mass spectrometry. The methodology was applied to the 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 aminolipids at the 3′-end of the passenger strand has inhibitory properties similar to those of unmodified RNA duplexes in HeLa cells, indicating that the new lipid derivatives are compatible with the RNA interference machinery.

## 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 nonviral 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,

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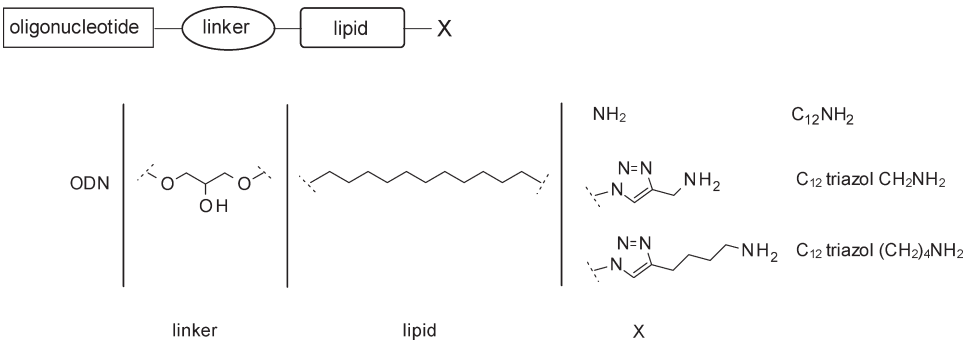
**SCHEME 1. Structure of the Lipid Oligonucleotide Conjugates Described in This Study**




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 nonviral 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 oligonucleotides.[11] Recently, a few examples on the use of siRNA covalently conjugated to lipids have been described in the literature. Cholesterol,[12] fatty acid derivatives,[12] and α-tocopherol[13] have been efficiently linked to the 5′-ends (phosphoramidite chemistry) and 3′-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 3′-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 tumor necrosis factor-α (TNF-α) gene expression.

## Results and Discussion

**Synthesis of the Lipid Derivatives.** The introduction of lipids to the 3′ end of oligonucleotides requires the use of a linker that connects the lipid to the growing DNA chain. Aminodiols such 4-aminoprolinol[12a,d] 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 is not considered a serious problem for biological activity of the resulting siRNA.[12c] 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 3′-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

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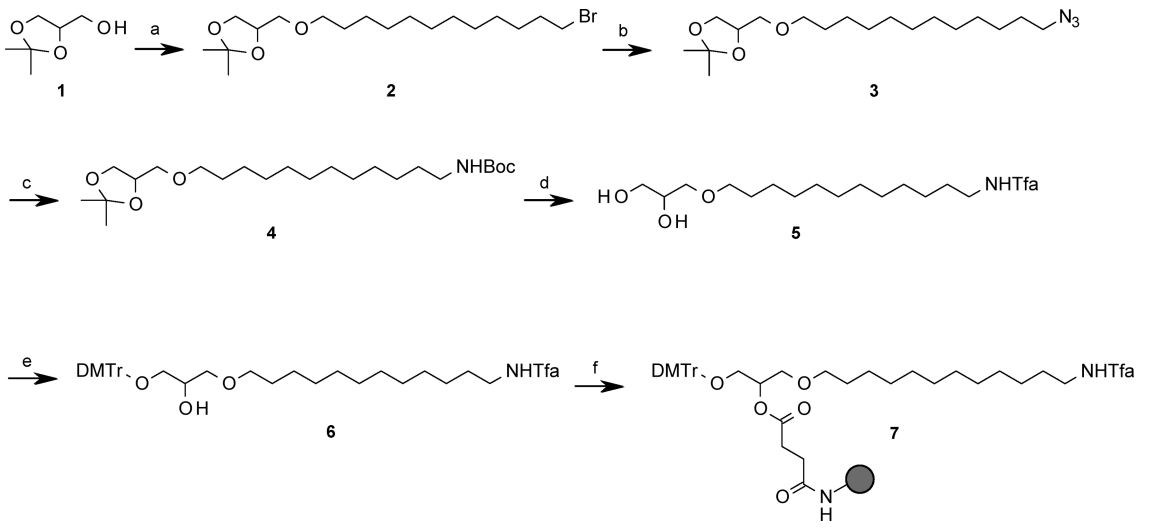
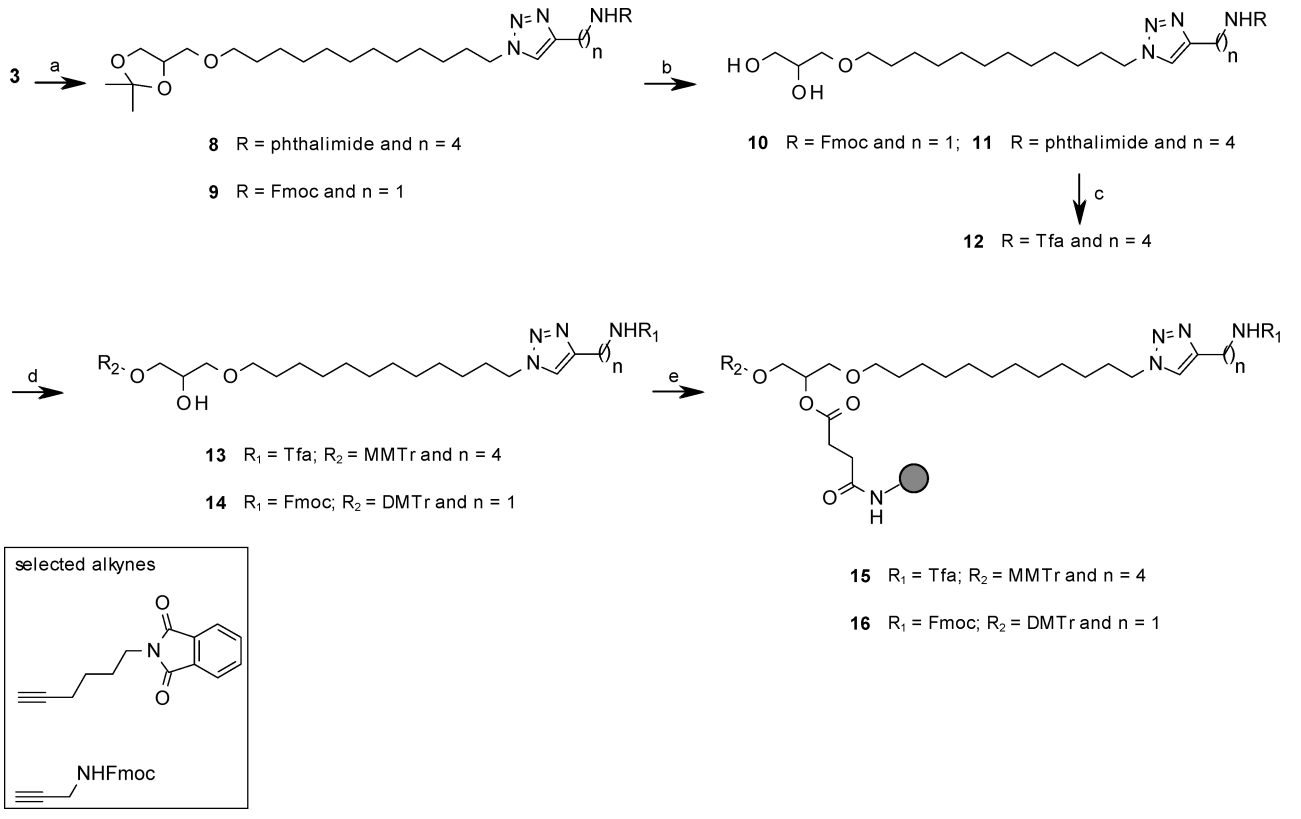
**SCHEME 2. Synthesis of Solid Support 7 Functionalized with Amino $C_{12}$**[a]




[a]Reagents and Conditions: (a) (i) di-*n*-butyltin oxide, MeOH, reflux, (ii) CsF, 1,12-dibromododecane, 60 °C; (b) $NaN_3$, DMF, 70 °C; (c) $Boc_2O$, TEA, DCM, rt; (d) (i) DCM/TFA 10%, rt, (ii) ethyl trifluoroacetate (ETFA), triethylamine (TEA), DCM, 0 °C; (e) DMTr, DMAP, Py, 40 °C; (f) (i) DMAP, succinic anhydride, DCM, rt, overnight, (ii) CPG derivatization.

**SCHEME 3. Click Chemistry and Synthesis of the Solid Supports 15 and 16**[a]




[a]Reagents and conditions: (a) selected alkynes, 10% sodium ascorbate, $CuSO_4 \cdot 5H_2O$, tBuOH/$H_2O$ (1:1), rt, overnight; (b) (i) (for **8**) DCM/TFA 3%, rt, (ii) (for **9**): *p*TsOH, MeOH, rt; (c) (i) $MeNH_2$, EtOH, 40 °C, overnight, (ii) ETFA, TEA, DCM, 0 °C; (d) (for **13**): DMTr, DMAP, pyridine, 40 °C, overnight; (for **14**): MMTr, DMAP, pyridine, 40 °C, overnight; (e) (i) DMAP, succinic anhydride, DCM, rt, overnight, (ii) CPG derivatization.

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.

The nucleophilic displacement of the bromide group with sodium azide in DMF yielded the desired azide **3** in 93%

**TABLE 1. Mass Spectrometry and Melting Temperatutes ($T_m$) of Lipid-Modified Oligonucleotides Prepared in This Study**

| oligonucleotide | sequence 5′ → 3′ | MW calc | MW found | $T_m$[a] (°C) |
|---|---|---|---|---|
| **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-$CH_2$NHC(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. |

[a]Conditions: sodium phosphate 10 mM, NaCl NaCl, pH 7.0, in these conditions unmodified Dickerson−Drew dodecamer melted at 49 °C; n.d., not determined.

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 4,4′-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, 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 expected diols **10** and **11** in 72% and 99% yield, respectively. DMTr derivative **14** was directly obtained from protected alcohol **10** in 45% yield under the same conditions used for the synthesis of compound **6**. Finally, the synthesis of last trityl derivative **13** 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 **13** in 39% overall yield (three steps).

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 $\mu$mol/g), **15** (25 $\mu$mol/g), and **16** (23 $\mu$mol/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 (5′-CGCGAATTCGCG-3′) 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).

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 postsynthetic modification.[14a,21e] The guanidinium-modified ONs synthesized during this study are summarized in Table 1. In all cases, selective and

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(18) A 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 under neutral conditions by reaction with TMSOTf in the presence of 2,6-lutidine. For experimental details, see the Supporting Information.

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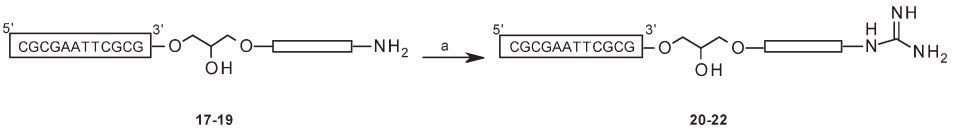
**SCHEME 4. Synthesis of the Amine and Guanidinium Oligonucleotides**




[a]Reagents and conditions: (a) (i) *O*-methylisourea chloride, $NH_3$ (32%), 55 °C, overnight.

**TABLE 2. Sequences of Unmodified, Lipid-Modified and Scrambled siRNA Sequences Used in This Study**

| siRNA | sequences[a] |
|---|---|
| unmodified (siRNA-1) | GUGCCUAUGUCUCAGCCUCTT<br>TTCACGGAUACAGAGUCGGAG |
| siRNA-2 | GUGCCUAUGUCUCAGCCUCTT-$C_{12}NH_2$<br>TTCACGGAUACAGAGUCGGAG |
| siRNA-3 | GUGCCUAUGUCUCAGCCUCTT-$C_{12}$-triazole-$CH_2NH_2$<br>TTCACGGAUACAGAGUCGGAG |
| siRNA-4 | GUGCCUAUGUCUCAGCCUCTT-$C_{12}$-triazole-$(CH_2)_4NH_2$<br>TTCACGGAUACAGAGUCGGAG |
| scrambled (siRNA-5) | CAGUCGCGUUUGCGACUGGTT<br>TTGUCAGCGCAAACGCUGACC |

[a]siRNAs are shown with the passenger strand above (5′ − 3′) and the guide strand below (3′ − 5′). T stands for thymidine.

quantitative guanidinylation were observed following a classical postsynthetic approach[21e] on oligonucleotides **17**−**19**, which were reacted with *O*-methylisourea for 16 h at 55 °C. 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 for the 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] Preliminary experiments to insert guanidinium functions at the end of RNA oligomers using the conditions described above yielded RNA degradation. Further attempts to extend such modifications in RNA oligomers are in progress.

**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 the Experimental Section).

**Gene Silencing by Modified siRNAs.** The gene silencing effect on the TNF-α mRNA of several siRNAs: one native **siRNA1**, three chemically modified siRNAs (**siRNA2**, **siRNA3**, and **siRNA4**), and one scrambled **siRNA5** were studied. HeLa cells were first cotransfected with plasmid pCAm TNF-α by using commercial cationic liposomes (lipofectin). We then carried out the transfection of the aforementioned siRNA derivatives with oligofectamine. Forty-eight hours after transfection, the amount of TNF-α produced by the cells was analyzed by enzyme-linked immunosorbent assay (ELISA). The silencing activities are depicted in Figure 1. In general, all chemically modified siRNAs previously obtained maintained their abilities to down-regulate TNF-α 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 3′-termini of the passenger strand of an RNA duplex is compatible with the RNAi machinery in HeLa cells.

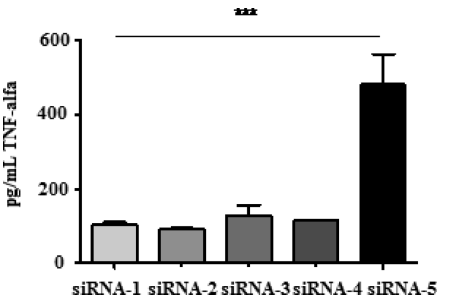



**FIGURE 1.** Efficiency of silencing of chemically modified siRNAs with lipids in the passenger strand. Plot of gene-specific silencing activities for native (siRNA-**1**), chemically modified conjugates (siRNA-**2**, siRNA-**3** and siRNA-**4**), and scrambled sequence (siRNA-**5**) (50 nM per well). Transfection of siRNA conjugates was carried out by using 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 nonviral 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 3′-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 containing amine tails did not affect the RNAi machinery through silencing TNF-α gene expression.

## Experimental Section

**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 $\delta$ = 7.24 ppm of $CHCl_3$ for $^1$H NMR and to the center line of the triplet at $\delta$ = 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 $\mu$m. Flow rate 1 mL/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 equiv) in dry methanol (10 mL) at reflux for 3 h, 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 equiv), cesium fluoride (458 mg, 3.02 mmol; 2.0 equiv), 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, $CDCl_3$) $\delta$ 4.24 (q, $J$ = 6.0 Hz, 1H, C*H*-O), 4.03 (dd, $J$ = 14.6, 8.2 Hz; 1H, O-C*H*-CH), 3.71 (dd, $J$ = 8.2, 14.6 Hz; 1H, O-C*H*-CH), 3.49 (m, 2H, C$H_2$-O), 3.40 (m, 2H, C$H_2$-O), 3.38 (t, $J$ = 6.8 Hz, 2H, C$H_2$-Br), 1.83 (q, $J$ = 7.1 Hz, 2H, C$H_2$-CH$_2$), 1.55 (m, 2H), 1.40 (s, 3H, C$H_3$-C), 1.34 (s, 3H, C$H_3$-C), 1.25 (m, 16H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 109.9 (O-*C*-CH$_3$), 75.4 (*C*H-O), 72.5 (O-*C*H$_2$), 72.4 (*C*H$_2$-O), 67.5 (*C*H$_2$-O), 34.6 (*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 (2 M + 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 equiv) was then added, and the mixture was stirred and heated to 70 °C for 48 h. The reaction was cooled at 0 °C, 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, $CDCl_3$) $\delta$ 4.26 (q, $J$ = 6.1 Hz, 1H, C*H*-O), 4.06 (dd, $J$ = 14.6, 8.23 Hz; 1H, O-C*H*-CH), 3.72 (dd, $J$ = 8.22, 14.6 Hz; 1H, O-C*H*-CH), 3.51 (m, 2H, C$H_2$-O), 3.43 (m, 2H, C$H_2$-O), 3.25 (t, $J$ = 6.97 Hz, 2H, C$H_2$-N$_3$), 1.59 (m, 6H), 1.42 (s, 3H, C$H_3$-C), 1.36 (s, 3H, C$H_3$-C), 1.27 (m, 14H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 109.3 (O-*C*-CH$_3$), 74.7 (*C*H-O), 71.8 (O-*C*H$_2$), 71.7 (O-*C*H$_2$), 66.9 (O-*C*H$_2$), 51.4 (*C*H$_2$-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 equiv) were dissolved in 3 mL of anhydrous THF. The reaction was stirred for 2 h 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 dichloromethane (DCM); TEA (69 $\mu$L, 0.498 mmol; 2.0 equiv) was added dropwise along with $Boc_2O$ (82 mg, 0.373 mmol; 1.5 equiv). The reaction was stirred for 5 h at room temperature. The organic layer was extracted with more DCM (5 mL) and rinsed with water (3 × 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, $CDCl_3$) $\delta$ 4.49 (broad s, 1H), 4.26 (m, 1H, C*H*-O), 4.04 (dd, $J$ = 14.6, 8.2 Hz; 1H, O−C*H*-CH), 3.72 (dd, $J$ = 14.6, 8.2 Hz; 1H, O-C*H*-CH), 3.50 (m, 2H, C$H_2$-O), 3.42 (m, 2H, C$H_2$-O), 3.08 (m, 2H, C$H_2$-N), 1.56 (m, 2H), 1.43 (s, 9H, 3C$H_3$-C), 1.41 (s, 3H, C$H_3$-C), 1.35 (s, 3H, C$H_3$-C), 1.24 (m, 18H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 158.8 (CO), 110.0 (O*C*CH$_3$), 75.4 (*C*H-O), 72.5 (O-*C*H$_2$), 72.4 (O-*C*H$_2$), 67.6 (O-*C*H$_2$), 30.7 (N-*C*H$_2$), 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 min. The solvent was 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 $\mu$L, 0.322 mmol). The reaction was cooled at 0 °C, and ethyl trifluoroacetate (22.0 $\mu$L, 0.177 mmol) was added. The reaction was stirred for 30 min, extracting the organic layer with more DCM (3 × 10 mL), and rinsed with water (3 × 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, $CDCl_3$) $\delta$ 6.42 (broad s, 1H), 3.05 (m, 1H, C*H*-O), 3.72 (dd, $J$ = 11.4, 3.8 Hz; 1H, O-C*H*-CH), 3.65 (dd, $J$ = 11.4, 5.2 Hz; 1H, O-C*H*-CH), 3.51 (m, 2H, C$H_2$-O), 3.49 (m, 2H, C$H_2$-O), 3.35 (m, 2H, C$H_2$-NHCO), 1.57 (m, 2H), 1.26 (m, 20H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 157.8 (q, $J$ = 36.9 Hz, COCF$_3$), 117.9 (q, $J$ = 287.6 Hz, CF$_3$), 73.2 (*C*H-O), 72.5 (O-*C*H$_2$), 71.0 (O-*C*H$_2$), 64.9 (O-*C*H$_2$), 40.6 (NHCO-*C*H$_2$), 30.2, 30.1, 30.1, 30.1, 30.0, 29.7, 29.6, 27.3, 26.7; $^{19}$F NMR ($CDCl_3$) $\delta$ −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 4,4′-dimethoxytrityl chloride (DMTrCl) (48 mg, 0.141 mmol; 1.2 equiv) and DMAP (8 mg, 0.07 mmol; 0.5 equiv) 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, $CDCl_3$) $\delta$ 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, C*H*-O), 3.76 (s, 6H, 2 C$H_3$), 3.52 (dd, $J$ = 9.7 Hz, 4.2 Hz; 1H, CH-C$H_2$-O), 3.43 (m, 3H, CH-C$H_2$-O), 3.35 (m, 2H, C$H_2$-O), 3.17 (m, 2H, C$H_2$-NH), 2.41 (broad d, 1H), 1.85 (m, 2H), 1.57 (m, 9H), 1.25 (m, 9H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 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 $\delta$ −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 equiv) and selected alkynes (1.0 equiv) were suspended in a 1:1 mixture of water and *tert*-butyl alcohol (1.0 mL). Sodium ascorbate (10%) and copper(II) sulfate 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 (3 × 5 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, $CDCl_3$) $\delta$ 7.81 (m, 2H, phenyl), 7.69 (m, 2H, phenyl), 7.27 (s, 1H, C*H*═C), 4.27 (t, $J$ = 7.24 Hz; 2H, C*H*2-O), 4.22 (m, 1H, C*H*-O), 4.04 (dd, $J$ = 14.6 Hz, 8.2 Hz; 1H, CH-C$H_2$-O), 3.69 (m, 3H), 3.49 (m, 2H), 3.41 (m, 2H), 2.74 (t, $J$ = 7.0 Hz, 2H, C$H_2$-N), 1.85 (m, 2H), 1.72 (m, 4H), 1.54 (m, 2H), 1.40 (s, 3H, C$H_3$-C), 1.34 (s, 3H, C$H_3$-C), 1.26 (m, 16H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 168.6 (CO), 134.1 (phenyl), 132.3 (phenyl), 123.3 (phenyl), 121.0 (*C*═N), 120.6 (*C*H-N) 109.5 (O-*C*-$CH_3$), 74.9 (*C*H-O), 72.1 (CH-C$H_2$-O), 72.0 (CH-C$H_2$-O), 67.1 (CH-C$H_2$-O), 50.4 (*C*$H_2$-C═), 37.8 (*C*$H_2$-NCO), 30.5 (*C*$H_2$-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.

**(9*H*-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, $CDCl_3$) $\delta$ 7.74 (d, $J$ = 7.5 Hz; 2H, phenyl), 7.57 (d, $J$ = 7.5 Hz; 2H, phenyl), 7.47 (s, 1H, C*H*═C), 7.38 (t, $J$ = 7.3 Hz; 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$H_2$-OCO), 4.40 (d, $J$ = 7.0 Hz; 2H, CH-C$H_2$-O), 4.31 (t, $J$ = 7.2 Hz; 1H, O-C*H*-C), 4.25 (t, $J$ = 6.0 Hz; 2H, C$H_2$-O), 4.22 (m, 2H), 4.05 (dd, $J$ = 14.6 Hz, 8.2 Hz; 1H, C*H*-O-C), 3.72 (dd, $J$ = 14.6 Hz, 8.2 Hz; 1H, C*H*-O-C), 3.50 (m, 2H), 3.42 (m, 2H), 1.87 (m, 2H), 1.55 (m, 2H), 1.41 (s, 3H, C$H_3$-C), 1.35 (s, 3H, C$H_3$-C), 1.27 (m, 16H); $^{13}$C NMR (125 MHz, $CDCl_3$) $\delta$ 156.3 (CO), 143.8 (phenyl), 141.2 (phenyl), 127.6 (phenyl), 127.0 (*C*H═C), 125.0 (*C*═C), 119.9 (phenyl), 109.3 (O-*C*-O), 74.7 (*C*H-O), 71.9 (CH-C$H_2$-O), 71.8 (CH-C$H_2$-O), 66.9 (C$H_2$-O), 50.3 (O-*C*$H_2$-C), 47.2 (C-*C*$H_2$-NCO), 36.5 (C-*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 min. 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, $CDCl_3$) $\delta$ 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, $CDCl_3$) $\delta$ 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 equiv) along with *p*-TsOH (5 mg, 0.026 mmol; 0.5 equiv) were dissolved in methanol (1.0 mL). The reaction was stirred at room temperature for 5 h. 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, $CDCl_3$) $\delta$ 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, $CDCl_3$) $\delta$ 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 $\mu$L of $MeNH_2$ was added dropwise. The mixture was stirred overnight at 40 °C. The solvent was reduced to dryness, and the resulting product was dissolved again in anhydrous DCM (1.5 mL). TEA (38 $\mu$L) was added dropwise at room temperature. The reaction was cooled at 0 °C, and EFTA (8.0 uL) was added dropwise. The reaction was stirred for 30 min at 0 °C. The organic layer was washed with water and brine (3 × 10 mL), 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 equiv) and DMAP (4.0 mg, 0.03 mmol; 0.5 equiv) were dissolved in 1.0 mL of pyridine. The reaction was heated at 40 °C 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, $CDCl_3$) $\delta$ 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, $CDCl_3$) $\delta$ 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 $\delta$ −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.

**(9*H*-Fluoren-9-yl)methyl [1-[12-[2-Hydroxy-3-[(4-methoxyphenyl)diphenylmethoxy]propoxy]dodecyl]-1*H*-1,2,3-triazol-4-yl]methylcarbamate, 14.** Compound **10** (32 mg; 0.055 mmol) along with DMAP (3 mg; 0.0275 mmol; 0.5 equiv) and MMTrCl (26 mg, 0.083 mmol; 1.5 equiv) were dissolved in pyridine (1.5 mL). The reaction was heated at 40 °C 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, $CDCl_3$) $\delta$ 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, $CDCl_3$) $\delta$ 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 $\mu$mol scale using controlled pore glass (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 Guanidine−Oligonucleotides 20−22.** Amine oligonucleotides **20−22** (about 2.0 OD) were treated with a mixture (60 $\mu$L) of a *O*-methylisourea chloride prepared solution (50 mg, 0.40 mmol) in water (50 $\mu$L) and an aqueous ammonia (30%, 60 $\mu$L). The reaction mixtures were incubated overnight at 55 °C. The water was evaporated, and the 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), and 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 5′-CAGUCGCGUUUGCGACUGGTT-3′; antisense or guide scrambled 5′-CCAGUCGCAAACGCGACUGTT-3′; sense or passenger anti-TNF-α: 5′-GUGCCUAUGUCUCAGCCUCTT-3′ and antisense or guide anti-TNFα: 5′-GAGGCUGAGAC-AUAGGCACTT-3′. 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 °C for 60 min. Solvent was evaporated to dryness and 1 M solution of TBAF in THF was added. RNAs were incubated 24 h at room temperature. The deprotection reaction was quenched and modified RNA conjugates were desalted (Sephadex, NAP-10) and purified by semipreparative HPLC. Finally, DMTr groups were deprotected (AcOH 80%, 30 min) and extracted with ether, and finally, chemically modified RNA conjugates **24−26** 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 (100 $\mu$L). Different aliquots were taken. For each sample, buffer was added until a final volume of 100 $\mu$L was reached. Modified siRNAs (siRNA-**2**, siRNA-**3** and siRNA-**4**, respectively) were heated at 94 °C for 2 min, and they were allowed to cool until room temperature was reached. Then 3 M NaOAc pH = 5.2 was added (10 $\mu$L) along with EtOH (96%) (275 $\mu$L). Samples were stirred, centrifuged at 4 °C (15 min, 12000 rpm), and precipitated at −20 °C. Finally, the supernatant was removed, and the respective pellets were carefully dried with argon.

**Cell Culture and siRNA Conjugate Treatments.** HeLa cells were cultured under standard conditions (37 °C, 5% $CO_2$, Dulbecco's Modified Eagle Medium, 10% fetal bovine serum, 2 mM 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-α plasmid using lipofectin (Invitrogen), following the manufacturer's instructions. One hour after transfection, m-TNF-α-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-α, using oligofectamine (Invitrogen). The TNF-α concentration was determined from cell culture supernatant by enzyme-linked immunoabsorbent assay kit (ELISA, Bender MedSystems) following the manufacturer's 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