Note

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Solid supports for the synthesis of 3'-aminooxy deoxy- or ribo-oligonucleotides and their 3'-conjugation by oxime ligation

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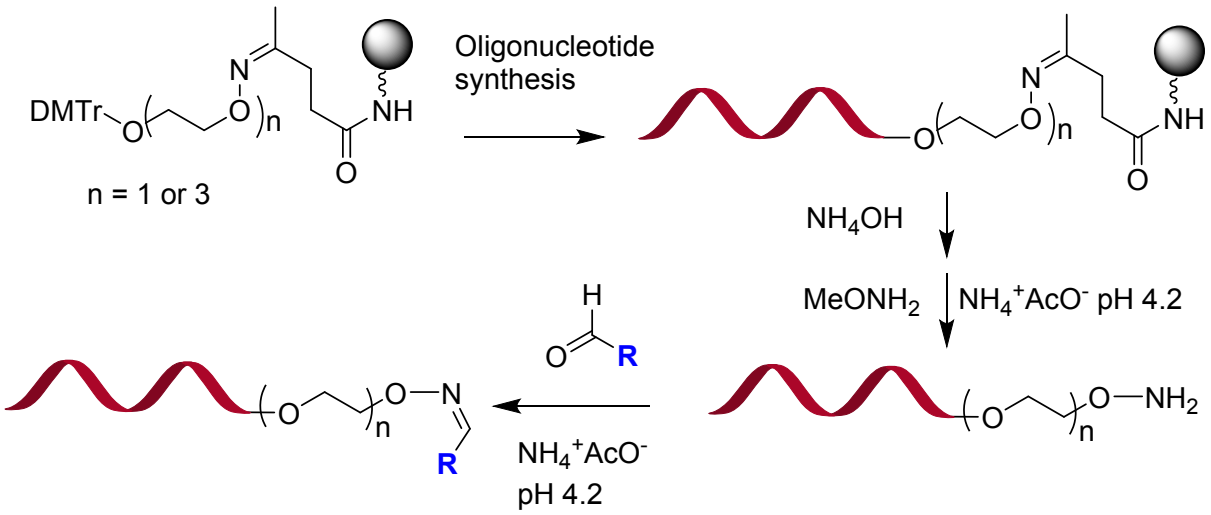
Graphical Abstract



ABSTRACT

Mono- and tri-ethyleneglycol aminooxy derivatives were reacted with levulinic acid, protected with dimethoxytrityl and immobilized on solid support. The resulting solid supports were used for elongation of oligonucleotides. Then, a mild ammonia treatment was applied to remove the oligonucleotide protecting groups, followed by a treatment with 50 mM methoxyamine at pH 4.2, releasing the 3'-aminooxy oligonucleotides by an oxime exchange reaction. The resulting

3'-aminooxy deoxy- or ribo-oligonucleotides were conjugated to various ketones and aldehydes with high efficiency by oxime ligation.

Keywords: Oligonucleotides, Oxime, solid support, click, conjugates

Conjugation of oligonucleotides to diverse molecules has attracted substantial research interest as it offers an easy way to modulate their biological properties. Most of the conjugations are made either at the 5' or the 3' extremity of the oligonucleotides. The 3'-modification has a great interest as it also increases the nuclease resistance.[1]

Different chemistries have been applied to conjugate oligonucleotides with different biomolecules or dyes including Diels-Alder ligation,[2,3] Staudinger ligation[4] copper catalyzed alkyne–azide cycloaddition,[5-9] copper free alkyne–azide cycloaddition, [10-13] thiol Michael addition,[14-18] thioether ligation,[19] inverse-electron-demand Diels–Alder reaction[20,21] or Oxime ligation.[22-30]

With regards to oxime ligation, several phosphoramidite derivatives have been reported for the introduction of the aminooxy function at the 5'-end of oligonucleotides for their subsequent conjugation or cyclization.[23-25,31-35] In contrast, the 3'-conjugation of oligonucleotides by oxime ligation has been poorly explored. The usual method reported in the literature to do so is to introduce a glycerol group at the 3'-end which is further oxidize to an aldehyde to eventually reacts with a aminooxy derivative. Thus, the modulation of the spacer between the oligonucleotide and the modification is not possible. Only one recent publication, from our group, reported the synthesis of a solid support allowing for the introduction of the aminooxy function at the 3'-end of oligonucleotides (Figure 1).[35] The resulting 3'-modified oligonucleotides were then conjugated with different aldehyde derivatives or circularized by means of bis-oxime ligation after the introduction of another aminooxy function at the 5'-end and reaction with a dialdehyde.[35]

In order to introduce some versatility by easily modulating the length of the spacer between an oligonucleotide and its 3'-aminooxy function for subsequent oxime conjugation, we designed a new type of solid supports built from ethylene glycol derivatives (Figure 1). Herein, we report the synthesis of two solid supports allowing the 3'-conjugation of deoxy- and ribo-

oligonucleotides with different ketones and aldehydes. This novel approach allows for enhanced versatility in generating a variety of tailored oligonucleotide constructs, where the different solid supports can be utilized to efficiently modulate the target designs of 3'-oligonucleotide conjugates.

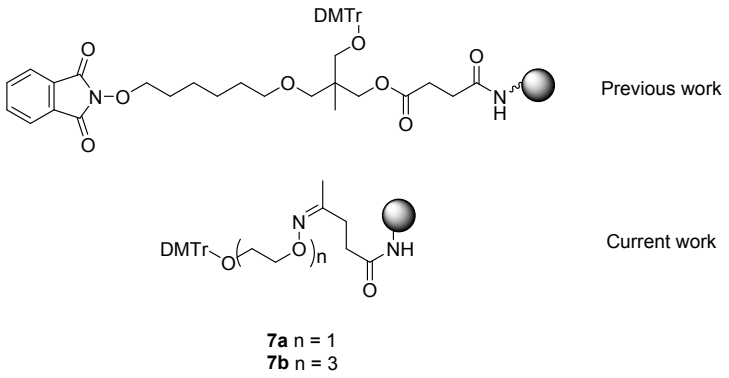
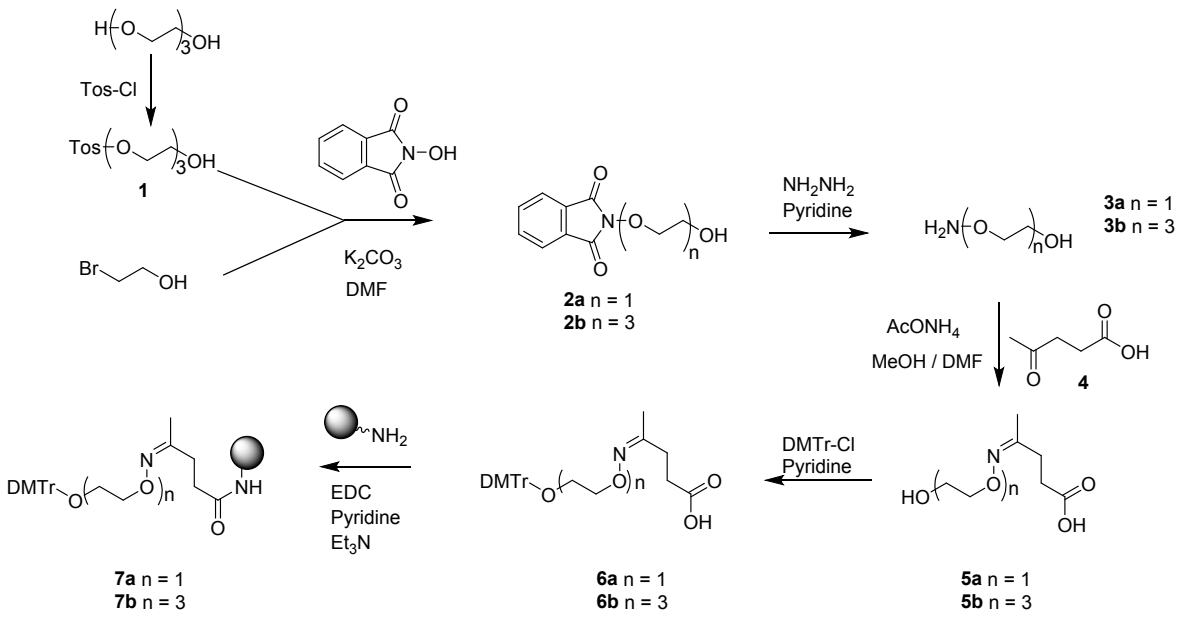


Figure 1. Structure of previous phthalimide solid support[35] and new solid supports **7a-b**.

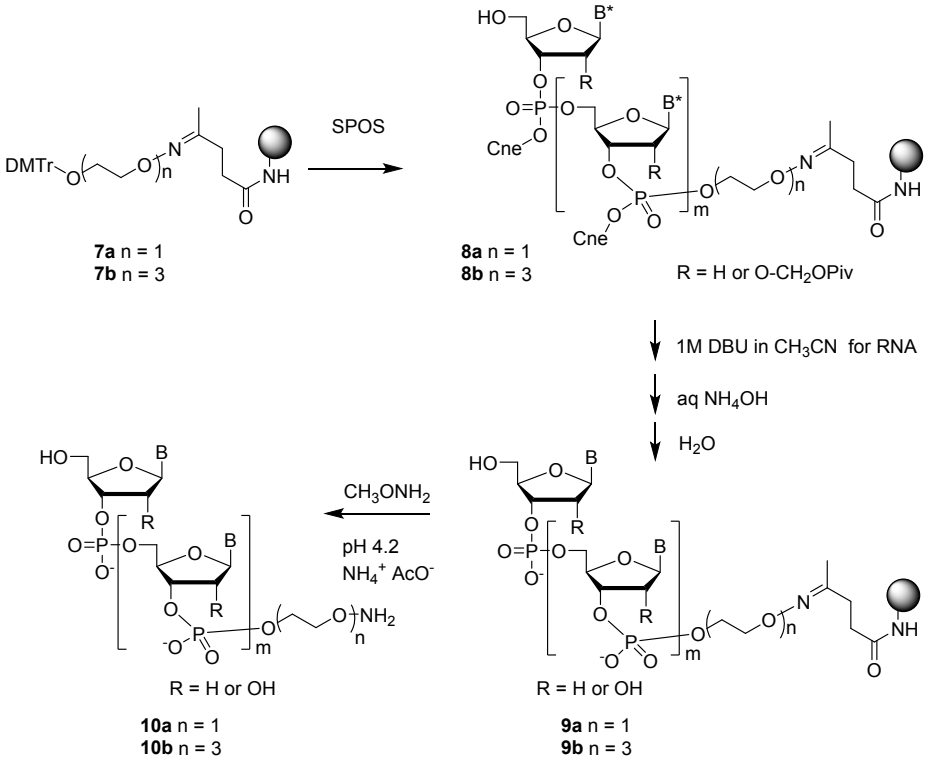
To demonstrate the usefulness of this approach, we selected mono- and tri-ethyleneglycol as the starting compounds. However, it is worth noting that this synthetic strategy can be readily applied to ethyleneglycols of any length or to any diols. The synthesis of the solid supports **7a-b** leading to 3'-aminooxy oligonucleotides with a mono- or a tri-ethylene glycol spacer proceeded in few steps (Scheme 1). Firstly, 2-bromo-ethanol, or a previously synthesized monotosyl-triethyleneglycol (**1**)[36] were substituted with hydroxyl-phthalimide in the presence of K_2CO_3 affording the phthalimide ethyleneglycol derivatives **2a-b**.[23,35] Secondly, the aminooxy function was deprotected with hydrazine, and after evaporation, levulinic acid was added to form the oxime derivatives **5a-b**. After work-up, the crude was directly treated with dimethoxytrityl chloride to protect the hydroxyl function providing derivatives **6a-b** after chromatography with an overall yield of 50% for the last three steps. Finally, **6a-b** were immobilized by amide formation on long-chain alkylamino controlled-pore glass (LCAA-CPG 1000 Å), using EDC as the coupling reagent, affording the two solid supports **7a-b**. The loading was measured by trityl assay (~40 μmol/g).



Scheme 1. Synthesis of solid supports for the preparation of 3'-aminooxy oligonucleotides.

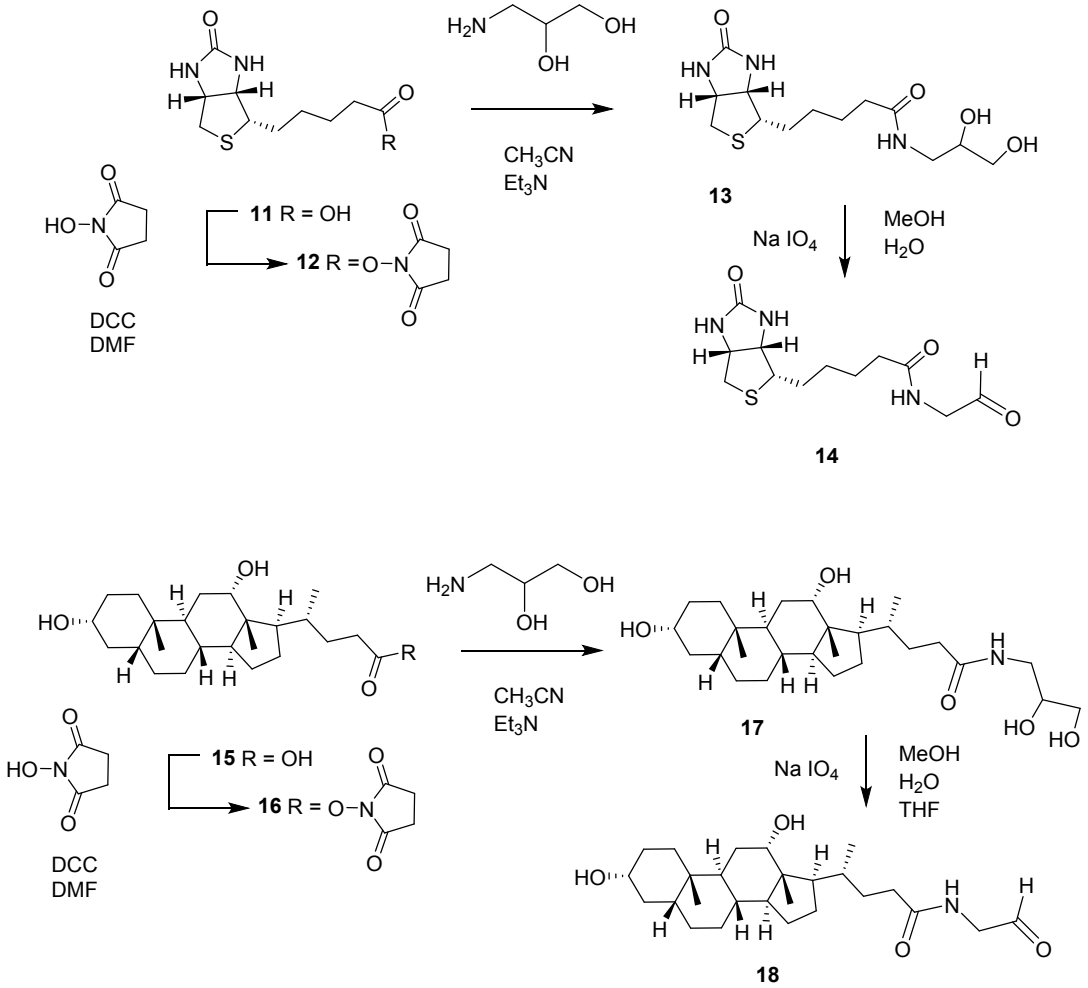
The elongation to full-length oligonucleotides (DNA or RNA) was performed on a DNA/RNA synthesizer, at the 1 μmol scale, by means of solid phase oligonucleotide synthesis (SPOS) using standard 3'-phosphoramidite chemistry with fast-labile nucleobase protecting groups and 2'-*O*-pivaloyloxymethyl (PivOM) groups for the 2'-OH protection of the ribonucleotides,[37] (Scheme 2). After elongation, the oligodeoxynucleotides **8a-b** were first deprotected with concentrated aqueous ammonia for 1 h at room temperature to remove the cyanoethyl groups on the phosphates and the acyl groups on the nucleobases. For oligoribonucleotides a first-step treatment with 1 M DBU was applied to remove the cyanoethyl groups and then the second-step ammonia treatment for 3 h at 37 °C was performed to allow removal of the PivOM groups on the 2' position of ribose.[37] The ammonia solution was collected, and after evaporation, the residue was analyzed confirming no undesired cleavage of the oligonucleotides from the solid supports. Finally, the 3'-aminooxy-oligonucleotides **10a-b** were released from the solid supports by treatment with a 0.4 M ammonium acetate buffer solution of 50 mM methoxyamine (pH 4.2) for 3 h at rt. The 3'-aminooxy-oligonucleotides were purified by size exclusion chromatography on G25-sephadex (Nap-10) to remove the methoxyamine and the salts. The 3'-aminooxy-oligonucleotides were obtained in the range of 300 to 500 nmoles that is a similar amount in comparison with oligonucleotides synthesized on a standard succinyl solid support. HPLC and MALDI-TOF MS analyses showed the efficiency of the syntheses and confirmed the nature of the 3'-modified oligonucleotides (See SI). It has been reported that methoxyamine

reacts with certain cytosines of tRNA. [38] Such degradation occurred after a 20 h treatment at 37 °C with a 3 M methoxyamine concentration and 10 mM $MgCl_2$ pH 5.5. In our case, no similar side reaction was observed, this could be explained by the short treatment (3 h) at room temperature with a low concentration of methoxyamine (50 mM) applied for the release of the aminooxy-oligonucleotides from the solid support.



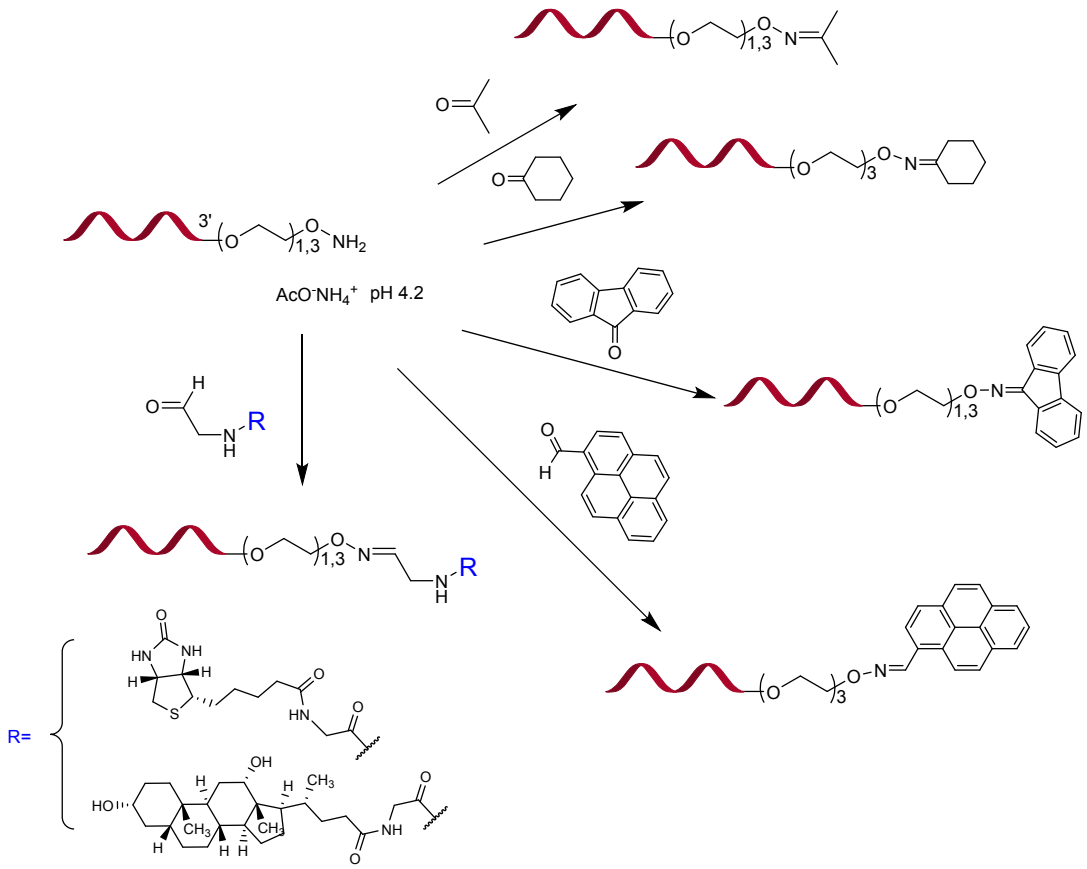
Scheme 2: Synthesis of 3'-aminooxy-oligonucleotides. B* represents the protected nucleobase A^{Pac}, $G^{-tbu-Pac}$, C^{-Ac}. Cne: cyanoethyl, Piv: pivaloyl. SPOS: Solid phase oligonucleotide synthesis.

To confirm the reactivity of the 3'-aminooxy-oligonucleotides, they were then engaged in derivatization reactions using different ketones and aldehydes, like acetone, cyclohexanone, fluorenone, pyrene carboxaldehyde, biotin or deoxycholic acetamide aldehydes (Scheme 4 and table 1). The aldehyde derivatives of biotin and deoxycholic acid **14** and **18** were synthesized in three steps, as follows: first, activation of the carboxylic function of biotin and deoxycholic acid as a *N*-hydroxysuccinimide derivative; second, conjugation with 3-amino-2-hydroxy-propan-1-ol; and, third, oxidation of the diol by treatment with sodium periodate to generate the aldehyde function (Scheme 3).[39]



Scheme 3: Synthesis of aldehyde derivatives of biotin **14** and deoxycholic acid **18**.

As already reported,[25] aminooxy function reacts easily with aldehydes and ketones, since such species could be in the air or glassware (e.g. acetone) or in some solvents (e.g. acetaldehyde). Hence, it is strongly recommended to avoid a HPLC purification of the crude aminooxy-oligonucleotides. It is safer to proceed rapidly to the oxime ligation between the crude 3'-aminooxy-oligonucleotides and the desired aldehydes or ketones to introduce. After ligation, the conjugates can be easily purified by HPLC. The different 3'-aminooxy-deoxy- and ribo-oligonucleotides were dissolved in 0.4 M ammonium acetate buffer (pH 4.2) and ketones or aldehydes were added at rt. After 1 to 4 h of contact time, the conjugates were obtained and characterized by HPLC and MALDI-TOF MS (See SI). The HPLC profiles showed total conversion of the starting material and the formation of the conjugate as a single peak, except for the pyrene conjugate, where the two *E* and *Z* isomers were visualized.



Scheme 4: Conjugation of 3'-aminooxy-(deoxy and ribo)-oligonucleotides with ketone or aldehyde derivatives.

Table 1: 3-aminooxy-deoxy or ribo-oligonucleotides and their conjugates.

Sequences	MALDI- TOF	
	calcd	found
Deoxy-ribonucleotides		
T_6-EG_3-ONH_2	1989.36	1989.79
T_6-EG_3-ON=CMe_2	2029.43	2029.57
T_6-EG_3-ON=CH-pyrene	2201.61	2201.73
T_6-EG_3-ON=$C(CH_2)_5$	2069.49	2069.51
T_{12}-EG_3-ONH_2	3814.54	3414.60
T_{12}-EG_3-ON=CMe_2	3854.60	3854.84
T_{12}-EG_3-ON=$C(CH_2)_5$	3894.67	3894.65
T_{12}-EG_3-ON=CH-pyrene	4026.72	4026.13
d(TTG TTC GTT GAC CTC CAC T) EG-ONH_2	5858.80	5858.29

d(TTG TTC GTT GAC CTC CAC T) EG-ON=CMe_2	5898.87	5899.06
d(TTG TTC GTT GAC CTC CAC T) EG-ON=CH-fluorene	6021.00	6019.21
d(TTG TTC GTT GAC CTC CAC T) EG-ON=CH-dchol	6274.42	6274.21
d(TTG TTC GTT GAC CTC CAC T) EG-ON=CH-biot	6126.16	6126.36
d(TTG TTC GTT GAC CTC CAC T) EG_3-ONH_2	5946.91	5947.18
d(TTG TTC GTT GAC CTC CAC T) EG_3-ON=CMe_2	5986.98	5985.32
d(TTG TTC GTT GAC CTC CAC T) EG_3-ON=CH-fluorene	6109.10	6108.99
d(TTG TTC GTT GAC CTC CAC T) EG_3-ON=CH-dchol	6362.53	6361.46
d(TTG TTC GTT GAC CTC CAC T) EG_3-ON=CH-biot	6214.26	6214.29
Ribo-oligonucleotides		
r(GUA CCA UGA ACG AU) EG_3-ONH_2	4679.91	4680.68
r(GUA CCA UGA ACG AU) EG_3-ON=CMe_2	4719.88	4719.08
r(GUA CCA UGA ACG AU) EG_3-ON=CH-pyrene	4892.17	4891.40
r(GUA CCA UGA ACG AU) EG_3 ON=CH-dchol	5095.53	5094.60
r(GUA CCA UGA ACG AU) EG_3 ON=CH-biot	4947.26	4946.71

CONCLUSION

Solid supports allowing the preparation of oligonucleotides with a 3'-aminooxy function were rapidly and easily synthesized in few steps from bromoethanol or triethyleglycol. According to this strategy, it is possible to modulate the length of the spacer between the oligonucleotide and the aminooxy function since any diol derivatives and ethyleneglycols of any length can be chosen as a spacer. After elongation by standard phosphoramidite chemistry, a two-step protocol was applied to first deprotect the oligonucleotides and second to release the 3'-aminooxy oligonucleotides from the solid support. The corresponding 3'-modified deoxy- and ribo-oligonucleotides were conjugated to various ketones and aldehydes with high efficiency by oxime ligation.

This solid supports could be used in combination with other building blocks like special phosphoramidites to introduce another orthogonal modification at the 5'-end of oligonucleotides allowing their bis-conjugation. Along this lane, we previously demonstrated that combination of oxime ligation and CuAAC conjugation are orthogonal.[28] The data reported in this manuscript increases the toolbox to synthesize oligonucleotide conjugates.

EXPERIMENTAL SECTION

General Methods: All commercial chemicals were reagent grade and were used without further purification. The DNA synthesis reagents and phosphoramidites were commercially available from Link Technologies or ChemGenes. Flash column chromatography was performed on silica gel 60 (40–63 μm). ^{1}H NMR (400 or 500 MHz) and ^{13}C NMR spectra (100 or 126 MHz) were recorded in $CDCl_3$ or DMSO-d6 at room temperature. Compounds **1**,[36] **2a**[33] and **2b**[35] were prepared according to literature protocols.

General protocol of deprotection of aminooxy ethyleneglycol derivatives. To a solution of phthalimide derivative **2a-b** (3.4 mmol) in 12 mL of absolute ethanol, hydrazine hydrate (900 μL, 18 mmol) was added. A white precipitate appeared after 5 min and the reaction was stirred for 3 h. The precipitate was removed by filtration and the solution was evaporated followed by 3 coevaporations with ethanol affording **3a-b** that were used without purification.

General protocol for oxime formation. To a solution of aminooxy ethyleneglycol derivatives **3a-b** (3.4 mmol) in 10 mL of methanol and 5 mL of DMF, 1 mL of 0.1M ammonium acetate aqueous buffer, 2 drops of acetic acid and levulinic acid (306 μL, 3.4 mmol) were added. The solution was stirred at rt for 5 h and the reaction was monitored by RP18 TLC. The solvents were evaporated. The residue was coevapored 3 times with dry pyridine and compounds **5a-b** were used without purification.

General protocol for dimethoxytritylation. To a solution of compounds **5a-b** (3.4 mmol) in 20 mL of dry pyridine, dimethoxytrityl chloride (1.4g, 4.1 mmol) was added. After 2 h under magnetic stirring, 1 mL of methanol was added. After 5 min, saturated $NaHCO_3$ aqueous solution (50 mL) was added and the product was extracted with CH_2Cl_2 (3 × 50 mL). The organic layers were dried over Na_2SO_4 and evaporated under vacuum. The residue was purified by silica gel chromatography from CH_2Cl_2:Et_3N, 98:2, (v/v) to CH_2Cl_2:Et_3N:MeOH 83:2:15, (v/v/v), to afford compounds **6a-b**.

4-((2-dimethoxytrityl-oxyethoxy)imino)-pentanoic acid 6a: Starting from 823 mg (4.0 mmol) of **2a** gave **6a** 1.15 g 50% (3 steps). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.22 (t, J = 7.3 Hz, 6H, CH_3 of Et_3N), 1.88 and 1.93 (2s, 3H, CH_3), 2.46-2.49 and 2.69-2.73 (2m, 4H, $OCNCH_2CH_2CO_2$), 2.99 (q, J = 7.3 Hz, 4H, CH_2 of Et_3N), 3.24-3.26 (m, 2H, CH_2ODMTr), 3.77 (s, 6H, OCH_3), 4.17-4.19 (m, 2H, CH_2ON=), 6.79-6.82 (m, 4H, Ar), 7.16-7.48 (m, 9H, Ar). ^{13}C{^{1}H}-NMR ($CDCl_3$, 100 MHz): δ 8.5, 14.4, 19.9, 32.0, 32.6, 44.8, 55.2, 62.7, 72.9,

85.7, 113.3, 126.9, 128.0, 128.6, 130.3, 136.8, 145.4, 157.1, 158.6, 177.9. HRMS (ESI/Q-TOF): $[M+Na]^+$ calcd. for $C_{28}H_{31}NO_6Na$ 500.2049, found 500. 2053.

4-((8-dimethoxytrityl-oxy-3,6-dioxaoctanoxy)imino)-pentanoic acid 6b: Starting from 1.0 g (3.4 mmol) of **2b** gave **6b** 1.10 g 49% (3 steps). ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 1.23 (t, J = 7.3 Hz, 6H, CH_3 of Et_3N), 1.83 and 1.87 (2s, 3H, CH_3), 2.40-2.63 (m, 4H, CCH_2-CH_2C), 2.97 (q, J = 7.3 Hz, 4H, CH_2 of Et_3N), 3.21-3.24 (m, 2H, CH_2ODMTr), 3.66-3.74 (m, 8H, CH_2OCH_2), 3.78 (s, 6H, OCH3), 4.14-4.17 (t, J = 5.0 Hz, 2H, CH_2ON=), 6.80-6.82 (m, 4H, Ar), 7.17-7.47 (m, 9H, Ar). $^{13}C\{^1H\}$-NMR ($CDCl_3$, 100 MHz): δ 8.7, 14.5, 20.0, 31.9, 32.3, 44.9, 55.2, 63.2, 69.8, 70.6, 70.7, 70.8, 72.5, 85.9, 113.0, 126.6, 127.7, 128.2, 130.1, 136.2, 145.1, 157.2, 158.6. HRMS (ESI/Q-TOF): $[M + H]^+$ calcd. for $C_{38}H_{55}N_2O_8$ 667.3958, found 667.3958.

General protocol for immobilization on solid support. Compound **6a** or **6b** (0.46 mmol), LCAA-CPG (500 mg, 1000 Å), DMAP (56 mg, 0.46 mmol) were dried by coevaporation with dry pyridine (three times). Dry pyridine (5 mL) was added with EDC (397 mg, 2.1 mmol) and Et_3N (190 μL, 1.4 mmol). The mixture was shaken for 24 h at room temperature. The solid support was filtered off, washed with MeOH and CH_2Cl_2, and dried. A capping step with standard Cap A and Cap B solutions was applied for 2 h, and the solid support was filtered off, washed with MeOH and CH_2Cl_2, and dried. The trityl assay indicated a loading of 40 μmol/g for **7a** and 41 μmol/g for **7b**.

Synthesis of oligonucleotides 8a-b. The oligonucleotides were elongated from solid support **7a-b** (1 μmol scale) on an ABI 394 DNA synthesizer according to standard phosphoramidite chemistry protocols. Detritylation was performed for 65 s using 3 % trichloroacetic acid (TCA) in CH_2Cl_2. For the coupling step: benzylmercaptotetrazole (0.3 M in anhydrous CH_3CN) was used as the activator along with commercially available 2′-deoxyribonucleoside-3′-*O*-2-cyanoethyl, *N*,*N*-diisopropylphosphoramidites phenoxyacetyl or tert-butylphenoxyacetyl (0.1 M in CH_3CN, 30 s coupling time) or 2′-*O*-pivaloyloximethyl-yribonucleoside-3′-*O*-2-cyanoethyl, *N*,*N*-diisopropylphosphoramidites (0.1 M in CH_3CN, 180 s coupling time). The capping step was performed using commercially available solutions (Cap A: phenoxyacetic anhydride:pyridine:THF 10:10:80 v/v/v, and Cap B: 10 % *N*-methylimidazole in THF) for 60 s. The oxidation step was performed with a standard, diluted iodine solution (0.1 M I_2, THF:pyridine:water 90:5:5, v/v/v) for 15 s.

Deprotection affording 9a-b. For DNA: The CPG was withdrawn in a microtube and treated with aqueous conc. ammonia for 1 h at rt. The supernatant was removed and CPG was washed

with water (3 × 1 mL). For RNA: The CPG was treated with a 1 M solution of DBU in dry acetonitrile for 3 min using two syringes, and washed with dry acetonitrile (3 × 5 mL). Then the CPG was withdrawn in a microtube and treated with aqueous conc. ammonia for 3 h at 37 °C. The supernatant was removed and CPG was washed with water (3 × 1 mL).

Release from solid support affording 10a-b. The CPG with the solid-supported oligonucleotides **9a-b** were treated with a solution of 50 mM CH_3ONH_2,HCl in 0.4 M ammonium acetate pH 4.2 (2 mL) for 3 h at rt under stirring. The supernatant was applied on a Sephadex G25 column for desalting. The solution was lyophilized affording the 3'-aminooxy-(deoxy or ribo)-oligonucleotides **10a-b**. Warning: Since aminooxy is a very reactive function, **10a-b** should be used rapidly. The glassware must be washed with ethanol to avoided any trace of acetone.

Conjugation with ketones or aldehyde derivatives. Fluorenone 50 mM in MeOH, Pyrene carboxyaldehyde 50 mM in DMF, Deoxycholic amide ethanal 50 mM in MeOH, biotin amide ethanal 50 mM in MeOH. To 3'-aminooxy-EG/EG_3-deoxyoligonucleotides **10a-b** 15 nmol in 25 µL of water were added 225 µM of 0.4M $NH_4^+AcO^-$ pH 4.2 and 10 eq of ketone or aldehyde derivatives. To 3'-aminooxy-EG_3-ribooligonucleotides **10b** 5 nmol in 10 µL of water were added 20 µM of 0.4M $NH_4^+AcO^-$ pH 4.2 and 10 eq of ketone or aldehyde derivatives. After 1 h to 4 h the conjugate was purified by size exclusion chromatography on Sephadex G25. The conversion of 3'-aminooxy-oligonucleotide into its conjugate was better than 90% (See SI). HPLC purification gave between 19 to 48% of pure conjugate.

Biotin amide 1,2-propanediol 13

To a solution of biotin *N*-hydroxysuccinimide,[17] (150 mg 0.44 mmol) and Et_3N (148 µL, 1.06 mmol) in dry CH_3CN (10 mL), 3-amino-2-hydroxy-propan-1-ol (48 mg, 0.53 mmol) dissolved in 3 mL of DMF was added and stirred at rt for 2 h. After evaporation, the residue was purified by silica gel chromatography from 5% to 20% MeOH in CH_2Cl_2 affording the diol derivative **13**, 120 mg, 86%. Rf : 0.5 (MeOH/CH_2Cl_2, 2:8, v/v). ^{1}H-NMR (400 MHz, DMSO-d6): ppm 1.23-1.37 (m, 2H, (g) CH_2); 1.39-1.66 (m, 4H, (f,h) $2CH_2$); 2.09 (t, 2H, (e) CH_2, J = 7.5 Hz); 2.57 (d, 1H, (l) CH_2, J = 12.4 Hz); 2.82 (dd, 1H, (l) CH_2, J = 5.2 Hz J = 12.5 Hz); 2.93-3.29 (m, 5H, (a,c) CH_2, (i) CH); 3.43-3.49 (m, 1H, (b) CH); 4.1-4.16 (m, 1H, (k) CH); 4.27-4.33 (m, 1H, (j) CH); 4.51 (t, 1H, OH, J =5.8 Hz); 4.72 (d, 1H, OH, J=4.9 Hz); 6.35 and 6.41 (2s, 2H, (m,n) NH); 7.76 (t, 1H, (d) CONH, J = 5.7 Hz). ^{13}C{^{1}H}-NMR (100 MHz, DMSO-d6): ppm 25.2,

27.9, 28.1, 35.0, 39.4, 39.7, 41.9, 55.3, 59.1, 60.9, 63.5, 70.5, 162.6, 172.5. HRMS (ESI/Q-TOF): $[M+H]^+$ calcd. for $C_{13}H_{24}N_3O_4S$, Calcd 318.1482, found 318.1490.

Biotin amide ethanal 14

To a solution of biotin amide 1,2-propanediol (120 mg, 0.37 mmol) in CH_3OH:H_2O, 4:2, (v/v) (6 mL), sodium periodate (88 mg , 0.41 mmol) was added. After 2 h, the solution was evaporated and the residue was dissolved in methanol (10 mL). After filtration and evaporation of filtrate the crude was purified by silica chromatography using a gradient of methanol (5 to 15%) in CH_2Cl_2 affording **14**, 80 mg, 76%. Rf: 0.65 (20% MeOH/CH_2Cl_2). Mixture of ethanal (70%) and dimethylacetal (30%), ^{1}H-NMR (400 MHz, DMSO-d6): ppm 1.21-1.67 (m, 6H, (g,f,h), CH_2); 2.07 (t, 1.3H, (e) CH_2, J = 7.2 Hz); 2.17 (t, 0.7H, (e) CH_2, J = 7.2 Hz); 2.57 (d, 1H, (l) CH_2, J = 12.5 Hz); 2.82 (dd, 1H, (l) CH_2, J = 5.1 Hz J = 12.5 Hz); 3.08-3.13 (m, 1.3H, (c) CH_2 (i) CH); 3.22 (s, 1.8H, OCH_3); 3.85 (d, 0.7H, (c) CH_2, J = 5.4 Hz); 4.09-4.16 (m, 1H, (k) CH); 4.27-4.33 (m, 1H, (j) CH); 4.38-4.44 (m, 0.7H, C<u>H</u>$(OMe)_2$); 6.15, 6.18 and 6.35, 6.40 (2 x 2s, 2H, NHCONH); 7.78 and 8.24 (t and bs, 1H, CONH, J = 5.52 Hz); 9.42 (s, 0.7H, CHO). ^{13}C{^{1}H}-NMR (400 MHz, DMSO-d6): ppm 25.2, 27.9, 28.0, 34.9, 39.7, 49.1, 55.3, 59.1, 60.9, 95.7, 162.6, 172.1, 200.1. HRMS (ESI/Q-TOF): $[M+H]^+$ calcd. for $C_{12}H_{20}N_3O_3S$, 286.1220, found 286.1232.

Deoxycholamide 1,2-propanediol 17

To a solution of deoxycholic acid *N*-hydroxysuccinimide,[17] (490 mg, 1.0 mmol) and Et_3N (140 µL, 1.1 mmol) in DMF (10 mL), 3-amino-2-hydroxy-propan-1-ol (100 mg, 1.1 mmol) dissolved in 1 mL of DMF was added and stirred at 50 °C for 3 h. After evaporation the residue was purified by silica gel chromatography from 5% to 20% of MeOH in CH_2Cl_2 affording the diol derivative **17** as a clear oil, 300 mg, 64%. Rf: 0.5 (20% MeOH/CH_2Cl_2), ^{1}H-NMR (500 MHz, DMSO-d6): ppm 0.59 (s, 3H, CH_3); 0.84 (s, 3H, CH_3); 0.91 (d, 3H, CH_3, J = 6.5 Hz); 0.95-1.83 (m, 24H, CH_2, CH); 1.94-2.14 (m, 2H, C23, CH_2); 2.93-3.39 (m, 5H, propyl CH_2, CH); 3.45 (qd, 1H, C_3, CH, J = 5.4 Hz, J = 10.8 Hz); 3.78 (br, 1H, C12, CH); 4.19 (d, 1H, OH, J = 4.1 Hz); 4.46 (d, 1H, OH, J = 4.3 Hz); 4.50 (t, 1H, OH, J= 5.9 Hz); 4.71 (dd, 1H, OH, J= 1.7 and 4.8 Hz); 7.76 (br, 1H, NH). ^{13}C{^{1}H}-NMR (126 MHz, DMSO-d6): ppm 12.4, 17.0, 23.0, 23.4, 26.0, 26.9, 27.1, 28.5, 30.1, 31.6, 32.3, 32.8, 33.7, 35.0, 35.6, 36.2, 41.5, 41.9, 45.9, 46.1, 47.4, 48.5, 63.5, 69.9, 70.5, 70.9, 173.1. HRMS (ESI/Q-TOF): $[M+H]^+$ calcd. for $C_{27}H_{48}NO_5$, Calcd 466.3532, found 466.3537.

Deoxycholamide ethanal 18

To a solution of deoxycholamide 1,2-propanediol (280 mg, 0.6 mmol) in CH_3OH:H_2O:THF, 4:2:2, (v/v/v) (8 mL), sodium periodate (155 mg , 0.73 mmol) was added. After 2 h, the solution was evaporated and the residue was dissolved in methanol (10 mL). After filtration and evaporation of filtrate the crude was purified by silica chromatography using a gradient of methanol (5 to 15%) in CH_2Cl_2 affording **18** as a clear foam, 240 mg, 92%

Rf: 0.75 (20% MeOH/CH_2Cl_2), Mixture of ethanal (20%) and dimethylacetal (80%). ^{1}H-NMR (400 MHz, DMSO-d6): ppm 0.59 (s, 3H, CH_3); 0.84 (s, 3H, CH_3); 0.91 (d, 3H, CH_3, J = 6.5 Hz); 0.94-1.84 (m, 24H, CH_2, CH); 1.92-2.13 (m, 2H, C23, CH_2); 3.04 (t, 2H, CH_2, J = 5.5 Hz); 3.21 (s, 2H, OMe) 3.34-3.42 (m, 3H, C3 CH, CH_2); 3.78 (br, 1H, C12 CH); 4.16 (d, 1H, OH, J =4.1 Hz); 4.37-4.43 (m, 0.8H, C<u>H</u>$(OMe)_2$); 4.45 (d, 1H, OH, J = 4.3 Hz); 7.76 and 8.22 (2 m, 1H, NH); 9.41 (s, 0.2H, CHO). ^{13}C{^{1}H}-NMR (100 MHz, DMSO-d6): ppm 12.3, 16.9, 23.0, 23.4, 26.0, 26.9, 27.1, 28.5, 30.1, 31.6, 32.2, 32.8, 33.72, 35.0, 35.6, 36.2, 41.5, 43.9, 45.9, 46.1, 47.4, 49.1, 53.4, 54.8, 69.8, 70.9, 95.7, 172.7, 200.1. HRMS (ESI/Q-TOF): $[M+H]^+$ calcd. for $C_{26}H_{44}NO_4$, 434.3270, found 434.3279.

Associated content

* Supporting Information. NMR data of **6a-b**, **13**, **14**, **17**, **18**; HPLC and MALDI-TOF spectra of 3'-aminooxy-(deoxy or ribo)-oligonucleotides and their conjugates.

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Notes

The authors declare no competing financial interest.

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