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A Versatile and Convenient Synthesis of ^{34}S-Labeled Phosphorothioate Oligonucleotides

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A synthetic protocol for ^{34}S-labeled phosphorothioate oligonucleotides (PS ONs) was developed to facilitate MS-based assay analysis. This was enabled by a highly efficient, two-step, one-pot synthesis of ^{34}S-labeled phenylacetyl disulfide (^{34}S-PADS), starting from ^{34}S-enriched elemental sulfur ($^{34}S_8$). ^{34}S-PADS was subsequently used for stable isotope labeling (SIL) of oligonucleotides containing a phosphorothioate backbone. The ^{34}S-SIL PS ONs are shown to retain the same melting temperature, antisense activity, and secondary structure as those of the corresponding unlabeled ^{32}S PS ONs.

Introduction

Oligonucleotide (ON) therapeutics show great promise in their ability to modulate gene expression through numerous mechanisms.[1] Antisense oligonucleotides (ASOs) and short interfering RNA (siRNA) specifically inhibit gene expression by Watson–Crick base pairing to the complementary mRNA, and hence, prevent expression of the encoded protein. In contrast, ONs such as anti-microRNAs (anti-miRs) inhibit microRNAs and allow protein expression to be restored; hence modulating gene pathways rather than a single target. ONs are also applied for splicing correction to prevent incorrect splicing or redirect splicing, rather than cleaving the target mRNA, leading to expression of an alternative protein isoform.[2] Several ON-based drugs have been successfully developed and launched, including pegaptanib (Macugen, 2004), mipomersen (Kynamro, 2013), nusinersen (Spinraza, 2016), and eteplirsen (Exondys51, 2016). Natural ONs contain a phosphodiester (PO) backbone and are highly charged, water-soluble, polyanionic macromolecules with poor cell penetration.[3] Unmodified ONs are cleared from the blood in minutes due to nuclease cleavage and glomerular filtration.[4] Consequently, a wide variety of chemical approaches, with the aim of improving the properties of ONs, have been developed, focusing on modifying the nucleotide backbone, the ribose moiety, or the nucleobase. Early work by Eckstein gave rise to phosphorothioate (PS) ONs,[5] wherein one of the nonbridging oxygen atoms in the PO linkages is replaced by sulfur.[6] This leads to increased stability and plasma protein binding, and consequently, improved tissue uptake and half-life.[7] Recently, methods for the synthesis of stereochemically pure 2′-*O*-methoxyethyl (2′MOE) ONs became available, which indicated a next generation of therapeutic ONs.[8] To support increasing efforts in drug discovery, the development of new analytical techniques is highly important.[9] In small-molecule drug discovery, it is conventional practice to use stable isotope labeled (SIL) compounds for multiple purposes, specifically in MS techniques.[10] Exhibiting a different molecular weight, while maintaining identical chemical, biological, and physical properties, allows for precise quantification and imaging in various assays.[10a] SIL ONs are envisioned to be useful in a broad range of applications, for example, as internal standards for quantification in biological matrices, for exploration in biotransformation and biodistribution studies, MS imaging, and quantitative concentration determination in tissues and cells. A few labeled ON building blocks, containing ^{2}H, ^{15}N, ^{13}C, or ^{17}O, are commercially available. However, none of the much used 2′MOE or locked nucleic acid (LNA) amidites, for gapmer synthesis, are available with SIL labeling. De novo synthesis of, for example, SIL 2′MOE phosphoramidites would necessitate extensive synthetic efforts. In addition, commercially available SIL DNA building blocks are very expensive and would not enable a general and flexible synthetic procedure useful for general stable isotope labeling of any desired sequence. Consequently, the PS backbone became a logical target for SIL in ONs. There are no other stable phosphorus isotopes than ^{31}P; therefore, phosphorous labeling falls outside the scope of our work. Labeling of the thiophosphate with ^{18}O would require, for example, ^{18}O-labeled 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (CEP chloride), which unfortu-

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nately is not commercially available. Only a few examples of backbone isotope labeling in PS ONs are known. Previous studies employ the H-phosphonate method for ON synthesis to incorporate ^{35}S.[11] However, H-phosphonate-based ON syntheses are currently less established than the industry standard that use the more efficient phosphoramidite method;[12] additionally, suitable building blocks (other than for DNA) are not commercially available. Finally, modern automatic ON synthesizers are usually optimized for the phosphoramidite method, and therefore, also the preferred choice for the synthesis of SIL ONs.

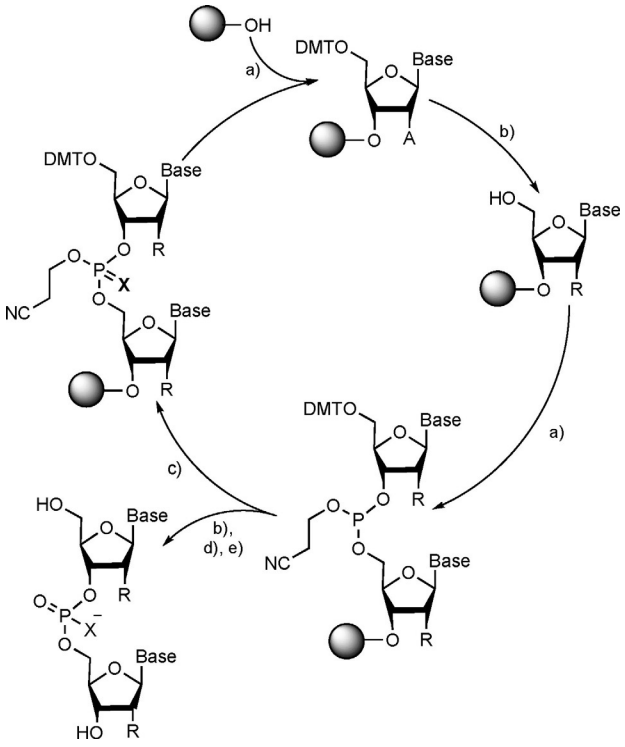
Herein, we present an optimized two-step, one-pot synthesis of ^{34}S-phenylacetyl disulfide (^{34}S-PADS) that was successfully used for general, convenient, high-yielding, and cost-effective automatic ON syntheses of ^{34}S-labeled gap- and mixmers. The ^{34}S-SIL ONs were also shown to retain the melting temperatures, secondary structure, internalization, and knockdown efficiencies of oligos targeting metastasis associated lung adenocarcinoma transcript 1 (MALAT1), compared with the corresponding unlabeled ^{32}S analogues.

Results and Discussion

In solid-phase ON syntheses, the growing ON chain is bound to an insoluble support, typically controlled pore glass or polystyrene. The chain is elongated from 3′ towards 5′ by incorporating new nucleotides as phosphoramidates. Before proceeding with the next coupling step, the reactive P^{III} center is oxidized to a P^{V} center. This can be achieved by using iodine in water to give PO linkages. This reaction was used by Lelyveld et al. to introduce backbone ^{18}O labeling into PO ONs.[13] Alternatively, a sulfur transfer reagent is employed, resulting in PS linkages. This step (step c, Scheme 1) was considered optimal to incorporate stable isotope labels because it makes the synthesis highly modular and no SIL nucleotide building blocks need to be synthesized.

During the preparation of this manuscript, it came to our attention that Mergelsberg et al. developed a method of using elemental $^{34}S_8$ to incorporate stable isotope labeling into the backbone of a PS trinucleotide.[14] However, the slow reaction speed, low overall yield, and known solubility issues of S_8 limit this approach to very few SIL incorporations and is not a suitable approach for automated ON synthesis with labeling of the entire backbone. To enable the automatic synthesis of ^{34}S-SIL PS ONs, the synthesis of a labeled sulfur transfer reagent is necessary. In a study by Stein et al., isotope-labeled 3*H*-benzo-1,2-dithiol-3-one 1,1-dioxide (Beaucage's reagent) was used to introduce a ^{35}S label into a PS dinucleotide.[15] This synthesis was not considered to be practical for our purposes due to low yields and the requirement for a large excess of sulfur during the preparation of the reagent. PADS is a sulfur transfer reagent that is routinely used in ON chemistry. Due to its high sulfur transfer efficiency and uncomplicated synthesis, it was chosen as the reagent of choice to synthesize ^{34}S SIL ONs.

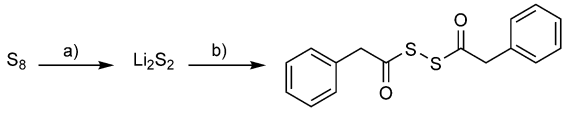
The only source of ^{34}S that is commercially available in reasonable quantities is $^{34}S_8$, which rules out the most common methods of disulfide formation for this synthetic challenge.



Scheme 1. Synthetic cycle of chemical ON synthesis R = H: DNA nucleotide. R = O-methoxyethyl: 2′-MOE nucleotide. X = O: PO bond, X = S: PS bond. a) Phosphoramidite, 5-benzylthio 1-*H* tetrazole, MeCN; b) dichloroacetic acid, toluene; c) for X = O: I_2, pyridine, H_2O; for X = S: phenylacetyl disulfide (PADS), 2-picoline, MeCN; d) Et_2NH, MeCN; e) NH_4OH (26%), 55 °C.

Various methods for the synthesis of disulfides starting from elemental sulfur are available.[16] Generally, X_2S_2 species are generated in situ followed by treatment with an alkyl halide or acyl halide to yield the desired disulfide. Reduction of elemental sulfur with sodium in liquid ammonia, to afford sodium sulfide, is a widely employed reaction in inorganic chemistry.[17] Treatment of the sodium sulfide with elemental sulfur yields sodium disulfide, which readily undergoes addition to acyl chlorides under phase-transfer conditions.[18] However, the use of large volumes of liquid ammonia was reasoned to be impractical for scaling up. In a similar manner, the naphthalene-catalyzed reduction of S_8 with sodium, reported by Takata et al. in 2003,[16b] and other approaches, including the reaction of sulfur with sodium hydroxide.[16c,d] None of these methods resulted in sufficient yields and purity of ^{34}S-PADS from a cost of goods perspective. Hexamethylphosphoramide (HMPA)-mediated reduction of elemental sulfur to Sm_2S_2 by SmI_2 was reported by Jia et al. in acceptable yields,[19] but the carcinogenic properties of HMPA prevented us from utilizing this procedure. Unfortunately, all attempts with alternative additives, such as tris(pyrrolidinephosphine) oxide,[20] were unsuccessful. After numerous attempts to identify an efficient route to ^{34}S-PADS in high yield and purity based on published procedures, we finally decided to redesign a method described by Gladysz et al. in 1979.[21] This method described the reduction of elemental sulfur by Et_3BHLi (Super-Hydride) to lithium disulfide, followed by reaction with alkyl halides, to give alkyl disulfides. Although

no reaction with acyl chlorides was reported, we found that in situ prepared lithium disulfide efficiently underwent a reaction with phenylacetyl chloride to yield PADS. To our delight, ^{34}S-PADS **1** could now be synthesized in 85% crude yield from $^{34}S_8$ by reduction with Super-Hydride and subsequent in situ treatment of the resulting Li_2S_2 species with phenylacetyl chloride (Scheme 2).



Scheme 2. Synthesis of ^{34}S-PADS **1**: a) $LiHEt_3B$ (1 equiv), 0 °C, 30 min, THF; b) phenylacetyl chloride (2 equiv), 0 °C, 4 h, THF.

PADS synthesized by this method contained varying amounts of dibenzoyl sulfide, which was easily detected by NMR spectroscopy.[22] It was established that strict control of the stoichiometry was necessary to avoid the formation of this byproduct. Excess of Super-Hydride led to an increased formation of dibenzoyl sulfide. Nonetheless, this impurity was later shown not to affect the sulfurizing efficiency or impurity profile of the produced ONs and it could, optionally, be removed by recrystallization.

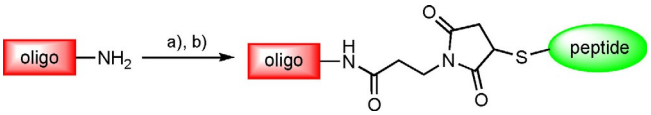
Next, three ^{34}S-SIL ONs were synthesized by using ^{34}S-PADS as a sulfur transfer reagent (Table 1). ON **1** binds complementary to MALAT1; a noncoding RNA present in the vast majority of mammalian cells. Our tailored design of this sequence builds on a sequence previously described by Hung et al.[23] ON **2** has the same binding region as that of **1**, but also contains a hexylamine linker at the 5′-end by using a PO-bridged T-C-A trinucleotide. This T-C-A linkage is rapidly cleaved in the cytosol by nucleases to release the active 20mer.[24] ON **3** was synthesized by using a 1:1 mixture of commercially available ^{32}S-PADS and our ^{34}S-PADS. The commercial product showed very similar sulfurization efficiency to that of the isotope-labeled material, giving an ON labeled with the expected 50% ^{34}S PSs in the backbone (Section S1-3 in the Supporting Information). ONs **4**, **5**, and **6** are analogues of **1**, **2**, and **3**, but with natural ^{32}S labeling, and hence, used as controls. The yields and purity of the crude materials were not significantly different upon employing ^{34}S-labeled PADS or commercially available ^{32}S reagent.

Table 1. Sequences of synthesized ONs.[a]

ON	Sequence	Yield [mg] ([%])
1[b]	^{34}S-**ATAGT**ACTATAGCAT**CTGTG**	84 (26)
2[b]	^{34}S-Hex*T*C*A***ATAGT**ACTATAGCAT**CTGTG**	145 (39)
3[c]	$^{32/34}$S-GGAACCTT	67 (75)
4	^{32}S-**ATAGT**ACTATAGCAT**CTGTG**	81 (25)
5	^{32}S-Hex*T*C*A***ATAGT**ACTATAGCAT**CTGTG**	150 (39)
6	^{32}S-GGAACCTT	63 (76)
7[b]	^{34}S-MALAT1-GLP1bp conjugate	3.0 (10)
8	^{32}S-MALAT1-GLP1bp conjugate	1.4 (14)

[a] Bold letters represent 2′-MOE nucleotides, standard letters are DNA. *: PO linkages. Hex: a hexylamine linker. All oligos contain Et_3N counterions, and yields were calculated based on resin substitution. [b] Fully ^{34}S labeled. [c] 50% ^{34}S-labeled.

ONs **2** and **5** were conjugated to a cysteine-modified GLP1 binding (GLP1bp) peptide, which was reported to enhance uptake of ONs in cells expressing the GLP1 receptor.[24] The hexylamine modifier on the 5′-end of **2** was treated with 3-(maleimido)propionic acid *N*-hydroxysuccinimide ester. The resulting maleimide underwent Michael addition with a C-terminal cysteine residue on the GLP1-binding peptide, which yielded **7**. Similar conditions, starting from **5**, yielded the corresponding ^{32}S-labeled ON-GLP1bp conjugate **8** (Scheme 3).



Scheme 3. Synthesis of ON peptide conjugates. The oligo is a hexylamine-modified oligo and peptide represents GLP1bp with a C-terminal cysteine. a) Maleimidopropionic acid *N*-hydroxylsuccinimide (5 equiv), $NaHPO_3/Na_2PO_3$ buffer, 0.1 M, pH 7.1, 4 h; b) cysteine-containing peptide (1.5 equiv), $NaHPO_3/Na_2PO_3$ buffer, 0.1 M, pH 7.1, 6 h.

To confirm that labeling of the thiophosphate backbone did not affect the secondary structure of the ON, NMR spectra of ONs **1** and **3**, and their respective unlabeled derivatives **4** and **6**, were recorded. No significant difference could be observed between the labeled and unlabeled ONs (Section S3-1).

Melting temperatures of ^{34}S-labeled ONs bound to the commercially available complementary RNA sequence were also measured. Incorporation of ^{34}S-thiophosphates did not change the melting points of **1** and **4** bound to a commercially available RNA decamer complementary to the DNA gap region (Section S3-2). The secondary structures of **3** and **6** were compared by ^{1}H NMR spectroscopy, whereas **1** and **4**, as duplexes with the same RNA as that used in the T_m studies, were compared by circular dichroism (CD). No significant differences in secondary structure were observed (Sections S3-1 and S3-3).

The effect of modification of the ON part of a MALAT1-GLP1bp conjugate on its capability to activate and induce endocytosis of the GLP1 receptor was measured by using an internalization assay. As expected, receptor internalization was not significantly affected by exchanging natural sulfur isotopes to ^{34}S-SIL analogous with essentially identical potency and efficacy for conjugates with ^{32}S- and ^{34}S-labeled ONs (Figure 1, Section S4-1, and Table S1).

As a reference, the known peptide agonist exenatide (Byetta, used in the treatment of T2D) was included, as was unconjugated MALAT1 ASO. The ASO conjugates displayed similar potency to that of exenatide, but with a reduced efficacy. Importantly, this was of almost identical magnitude to that of conjugates with ^{32}S- and ^{34}S-labeled ONs. Unconjugated MALAT1 ASO did not by itself promote receptor internalization.

To further prove that the properties for ^{32}S and ^{34}S ONs remained similar, the corresponding RNA knockdown of these ASOs were compared (Figure 2). ONs **7** and **8** were tested in a

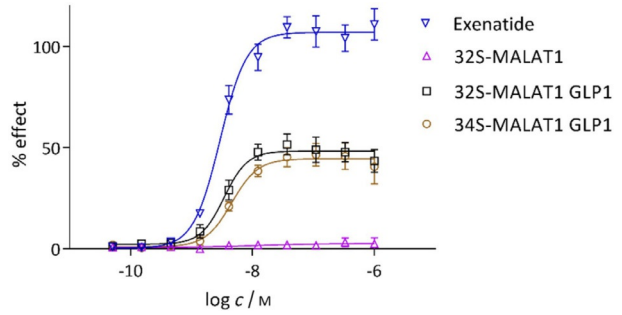


Figure 1. Concentration response curves for ligand-induced GLP1 receptor internalization. Data presented as the mean ± standard error of the mean (SEM) from three independent experiments with exenatide as a reference for 100% effect.

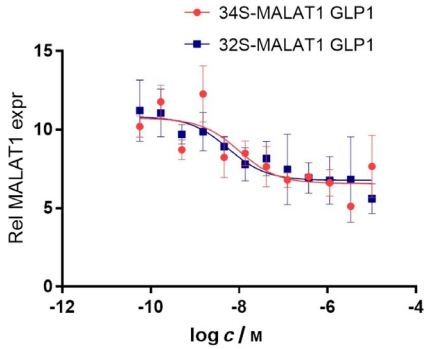


Figure 2. Concentration response curves for knockdown of ASO target gene MALAT1. Data are representative of three independent experiments, and values are expressed as the mean ± SEM.

knockdown assay by employing HEK cells with overexpressed GLP1 receptor. Degradation of MALAT1 RNA was measured by qPCR (Section S5-1). The labeled ONs displayed biological activity equal to that of their unlabeled counterparts (both at $pIC_{50} = -7.5$). The ^{34}S labeling was well tolerated and modified ONs showed the same reduction in RNA levels as that of their respective parent ^{32}S ONs.

Conclusion

We reported on a novel, efficient, general, and versatile method for stable isotope labeling of oligonucleotides with the dominant phosphorothioate modification by introducing ^{34}S labels. This was enabled by a new synthesis of the sulfurizing agent ^{34}S-PADS from elemental sulfur, $^{34}S_8$. This reagent was used to synthesize stable isotope-labeled antisense ONs, that is, ^{34}S-SIL ASOs, which could be utilized independently of the building blocks used if a PS backbone was present. It was also shown that the T_m, uptake, and knockdown potency and efficacy of SIL ONs was on a par with those of the corresponding ^{32}S versions. The data suggests that the chemical and biological properties of ONs are equivalent upon substituting ^{32}S for ^{34}S in the PS backbone; therefore, these compounds are excellent candidates for SIL internal standards in, for example, LC–MS-based assays. Stable isotope labeling is also a promising way to label ASOs for detection in, for example, NanoSIMS imaging, which would allow tracking of intracellular trafficking of ONs and conjugates with excellent resolution. We are currently looking into this technology and the results will be published in due course.

Experimental Section

General: All starting materials, reagents, and solvents were used as received. Unless otherwise stated, solvents and reagents were obtained from Sigma Aldrich. Phase separators were obtained from Biotage. CD spectra were measured on a Jasco J-810 spectropolarimeter. RNA of the sequence 5′-AUGCUAUAGU-3′ was obtained from Sigma–Aldrich (purified by HPLC) and used in the T_m measurements. T_m was measured at $\lambda = 260$ nm on a Cary 300 UV/Vis dual-beam spectrophotometer (Varian). ^1H and ^{13}C NMR spectra were recorded at 300 K on a Bruker 500 MHz system equipped with a CryoProbe, operating at 500 and 126 MHz, respectively. The chemical shifts were recorded in ppm relative to the solvent residual signals: $CDCl_3$.

Preparation of ^{34}S-PADS: $^{34}S_8$ (0.823 g, 14.7 mmol) was suspended in dry THF (100 mL) and cooled to 0 °C under argon. Super-Hydride (1 M in THF, 18.32 mL, 1 equiv) was added dropwise to the suspension. After stirring at RT for 1 h, phenylacetyl chloride (2.91 mL, 1.2 equiv) was added dropwise. The resulting yellow solution was stirred for another 3 h at RT. The reaction mixture was concentrated under reduced pressure, then water and dichloromethane were added, and the layers were separated. The organic layer was washed sequentially with 1 M NaOH, 1 M HCl, and water before it was finally dried by passage through a phase separator. The organic layer was once again concentrated to give a yellow oil, which was treated with cold 2-propanol to accomplish precipitation. The resulting off-white solid was filtered and washed with cold 2-propanol. The product typically contains 15–20% benzoyl sulfide, but could be effectively used in automated ON synthesis without further purification. Higher purity was obtained by recrystallization from hot cyclohexane, yielding high-purity PADS as white crystals (68%). The NMR shifts matched those of commercially available ^{32}S-PADS (S2). ^1H NMR (500 MHz, $CDCl_3$): $\delta = 7.37$–7.28 (m, 10H), 3.99 ppm (s, 4H); ^{13}C NMR (126 MHz, $CDCl_3$): $\delta = 49.3$, 128.1, 129.0, 129.9, 132.2, 191.6 ppm.

ON synthesis: ONs were synthesized on a 32 μmol scale on an ÄKTA Oligopilot 10 system by using commercially available DNA phosphoramidate buildings blocks and PS 5G UnyLinker support (GE Healthcare). 2′-MOE phosphoramidites (3 equiv used in each step) were obtained from CarboSynth. Detritylation was performed by using 3% dichloroacetic acid in toluene. 5-(Benzylthio)-1*H*-tetrazole (BTT) was used as activating agent. CE backbone deprotection was performed with diethylamine/toluene (20% *v/v*). Phosphoramidites were dissolved to a final concentration to 0.1 M in DNA-grade acetonitrile prior to use. ^{34}S- and/or ^{32}S-PADS was dissolved in 1:1 (v/v) MeCN/3-picoline (0.2 M) and aged for 24 h before use. Recirculation times for phosphoramidites were 5 min for DNA building blocks, 10 min for 2′-MOE building blocks, and 40 min for the MMT-hexylamine building block. ONs were cleaved from the solid support by treatment with aqueous ammonia (26%) at 55 °C for 15–20 h.

MMT-hexylamine ONs were deprotected directly after backbone deprotection by passing a 5% solution of dichloroacetic acid in dichloromethane through the solid support bound ON until no more yellow color was observed. Finally, the solid support was washed

with pure CH_2Cl_2 before global deprotection and cleavage from resin by treatment with aqueous ammonia (26%) at 55 °C for 15–20 h was performed.

LC–MS was performed on a Xbridge C_{18} column by using mixtures of 0.1 M triethylammonium acetate (pH 7)/acetonitrile and an appropriate gradient.

ONs were purified on an XBridge C_{18}, 5 μm 19×150 mm column by using a gradient from 5 to 22% acetonitrile in 0.1 M triethylammonium acetate (pH 7.6) in 20 min at 60 °C.

ON peptide conjugation: 5′-Hexylamine-modified ON (10 mg, 0.9 μmol) was dissolved in 0.1 M potassium phosphate buffer (350 μL; pH 7.1). 3-(Maleimido)propionic acid *N*-hydroxysuccinimide (2 mg, 8 equiv) was dissolved in DMSO (300 μL) and added to the ON solution. The reaction mixture was left to stand at RT for 1 h. The oligo was precipitated from sodium acetate/ethanol (1:4; 0.3 M). The precipitate was redissolved in a minimal amount of 0.1 M potassium phosphate buffer (0.2 mL; pH 7.1) then the GLP1-cysteine peptide, dissolved in a minimal amount of DMSO (0.1 mL), was added. The reaction mixture was left to stand at RT overnight and then directly subjected to HPLC purification on an Xbridge C_{18} column by using a gradient from 5% acetonitrile in 0.1 M triethylammonium acetate (pH 7) to 80% acetonitrile in 30 min at 20 °C.

ON 1: ON **1** was obtained as a white powder after preparative HPLC (Et_3N salt, 84 mg). Yield based on initial resin substitution: 26%; MS: *m/z* calcd: 1805.818 (*z* = 4); found: 1805.50.

ON 2: ON **2** was obtained as a white powder after preparative HPLC (Et_3N salt, 145 mg). Yield based on initial resin substitution: 39%; MS: *m/z* calcd (*z* = 5): 1661.602; found: 1661.49.

ON 3: ON **3** was synthesized by using a 1:1 mixture of ^{34}S (aged overnight) and ^{32}S PADS (freshly prepared). The ON was obtained as a white powder after preparative HPLC (Et_3N salt, 67.3 mg). A mixture of isotope incorporation was observed. Most of the material contained three or four isotope labels. Yield based on initial resin substitution: 75%; MS: *m/z* calcd (*z* = 2, 3 isotopes incorporated): 1276.918; found: 1276.23.

ON 4: ON **4** was obtained as a white powder after preparative HPLC (Et_3N salt, 81 mg). Yield based on initial resin substitution: 25%; MS: *m/z* calcd (*z* = 5): 1442.815; found: 1443.2.

ON 5: ON **5** was obtained as a white powder after preparative HPLC (Et_3N salt, 150 mg). Yield based on initial resin substitution: 39%; MS: *m/z* calcd (*z* = 5): 1654.35; found: 1653.89.

ON 6: ON **6** was obtained as a white powder after preparative HPLC (Et_3N salt, 60 mg). Yield based on initial resin substitution: 56%; MS: *m/z* calcd (*z* = 2): 1275.055; found: 1273.48.

ON 7: ON **7** was obtained as a white powder after preparative HPLC (Et_3N salt). Yield of conjugation (over two steps): 1.4 mg, 10%; MS: *m/z* calcd (*z* = 7): 1803.2511; found: 1803.1257.

ON 8: ON **8** was obtained as a white powder after preparative HPLC (4.1 mg, Et_3N salt). Yield of conjugation (over two steps): 14%; MS: *m/z* calcd (*z* = 5): 1800.070; found: 1799.4768.

LC–MS spectra for ONs **1**–**8** are available in the Supporting Information.

GLP1R internalization assay: Receptor internalization was measured by using the PathHunter eXpress GLP1R Activated GPCR Internalization Assay (#93-0724E3CP0L; DiscoverX Corporation, Fremont, CA) in human glucagon-like peptide 1 receptor (GLP1R) overexpressing U2OS cells. Serial dilutions of the GLP1 peptide conjugated ASOs were incubated with cells for 3 h at 37 °C and ligand-induced GLP1 receptor internalization quantified. (Further details are given in Section S4-1.)

Knockdown assay

MALAT1 knockdown was assessed in HEK293 cells stably overexpressing human glucagon-like peptide 1 receptor (GLP1R). Serial dilutions of the GLP1-peptide-conjugated ASOs were incubated with cells for 24 h at 37 °C. Cells were then lysed, and quantitative real-time PCR analysis was performed. Data are presented as MALAT1 expression levels normalized against the reference gene HPRT1 ($2^{-\Delta Cq}$). (Further details are given in Section S5-1.)

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: antisense agents · isotopic labeling · oligonucleotides · sulfur · synthesis design

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