Bioconjugation

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Double-Clicking Peptides onto Phosphorothioate Oligonucleotides: Combining Two Proapoptotic Agents in One Molecule**

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Abstract: *Described here is a method for the conjugation of phosphorothioate oligonucleotides (PSOs) with peptides. PSOs are key to antisense technology. Peptide–PSO conjugates may improve target specificity, tissue distribution, and cellular uptake of PSOs. However, the highly nucleophilic phosphorothioate structure poses a challenge to conjugation chemistry. Herein, we introduce a new method which involves a sequence of oxime ligation and strain-promoted [2+3] cycloaddition. The usefulness of the method was demonstrated in the synthesis of peptide–PSO conjugates that targeted two suppressors of both the intrinsic and the extrinsic pathway of apoptosis. It is shown that the activity of a PSO sequence targeted against mRNA from c-Flip can be enhanced by conjugation with a peptide mimetic designed to inhibit the X-linked inhibitor of apoptosis protein (XIAP).*

Synthetic oligonucleotides enable therapeutic intervention by using, amongst others, RNA interference[1] and antisense methods.[2] Recently, the first systemically active antisense oligonucleotide was admitted to the market.[3] This compound contains several phosphorothioate linkages, which confer the required stability against degradation by nucleases.[4] Despite the recent reports of success, there are still concerns about target specificity, tissue distribution, and cellular uptake of the phosphorothioate antisense oligonucleotides (PSAOs).[5] These problems prompted the exploration of peptide–PSAO conjugates.[6] However, the nucleophilicity of phosphorothioates poses a challenge. The reported conjugation methods have mostly been restricted to disulfide exchange, peptide coupling reactions, and Diels–Alder reactions.[6] Low conjugation yields and the need for laborious multistep procedures are recurring problems which probably explain the scarcity of reports describing the conjugation of peptides with PSAOs. Herein, we introduce a new method involving a combination of oxime ligation and strain-promoted click reactions which allows rapid and efficient conjugation reactions between unprotected phosphorothioate oligonucleotides (PSOs) and peptides after solid-phase synthesis.

Usually, the peptide sequences in peptide–PSAO conjugates are selected with the aim of improving the cellular uptake, guiding intracellular localization, or adjusting ADME properties.[7] Given the recent achievements in the development of peptide therapeutics,[8] we envisioned that the peptide part could play a more active role. We explored peptide–PSAO conjugates in which the peptide and the PSAO sequences target two different regulators of critical importance for a particular biological process.

The cellular flice inhibitory protein (c-Flip) is a master anti-apoptotic regulator of the extrinsic pathway of apoptosis (see Figure S1 in the Supporting Information), which supresses, amongst other actions, the apoptosis of cancer cells induced by chemotherapy and cytokines.[9] Oligonucleotides directed against c-Flip enable reactivation of the apoptotic machinery blocked in tumor cells.[10] We wondered whether the potency of an anti-c-Flip PSO could be enhanced through conjugation with a peptide acting on the intrinsic pathway of apoptosis. The X-linked inhibitor of apoptosis protein (XIAP)[11] prevents the activation of the intrinsic apoptosis pathway, and upregulation of XIAP is a mechanism used by cancer cells to evade apoptosis.[12] Most high-affinity XIAP inhibitors mimic the N-terminal part of Smac/DIABLO, the natural opponent of XIAP, and other inhibitor of apoptosis proteins (cIAP-1/2).[13]

Before we embarked on the synthesis of peptide–PSO conjugates capable of targeting XIAP and c-Flip, we considered the multivalency of XIAP. Bivalent IAP inhibitors show high inhibitory activity, presumably through simultaneous interactions with two of the three BIR domains of XIAP.[13a,14] In pursuit of a peptide–oligonucleotide conjugate (**Pep-ON**) that has high affinity for XIAP, we characterized the multivalency of XIAP by means of DNA-programmed spatial screening. This technique involves the use of monovalent peptide–oligonucleotide conjugates which anneal to varied DNA templates and form defined bivalent peptide displays.[15] We combined copper-promoted and strain-promoted [2+3] click reactions for the synthesis of the peptide–oligonucleotide conjugates.[16] A dibenzocyclooctyne (Dibo)[17] unit was tethered to the peptide-based XIAP inhibitor AEG 40730 (Scheme 1; **Pep1**, **Pep2**).[18] It is difficult to access the required reaction partners, the azido-modified oligonucleotides, directly by solid-phase oligonucleotide synthesis because of the incompatibility of the chemistry of azide and phosphoramidite groups.[19] Instead, we used the supported alkynyl-oligonucleotide **ON-CPG** to install the azido group through Cu-catalyzed 1,3 cycloaddition with di(4-azidophenyl)-ether.[20] After cleavage with ammonia and purification by HPLC, the azide-containing oligonucleotides **N_3-ON** were applied in strain-promoted click reactions with the Dibo-peptide conjugates (**Pep1a**, **Pep1b**, **Pep2a**). The solution-phase coupling provided facile access to the peptide–oligo-

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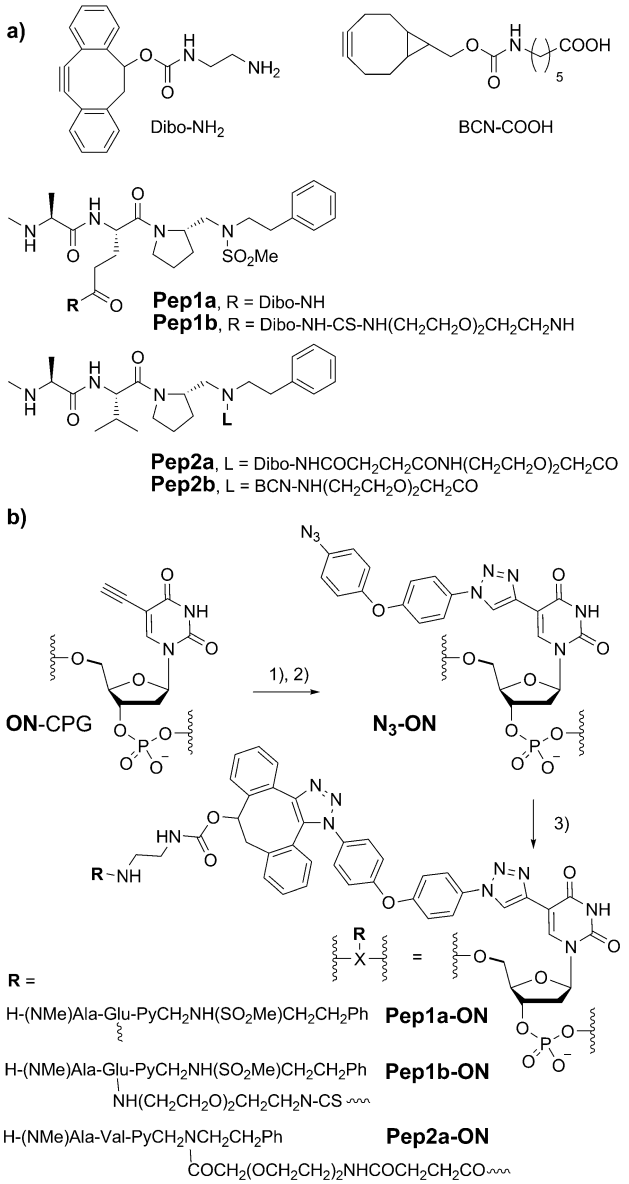


Scheme 1. a) Structures of the cyclooctyne-modified Smac-mimetic peptide conjugates (**Pep**) used in this study. b) Synthesis of peptide–oligonucleotide conjugates: 1) 20 equiv $(N_3C_6H_4)_2O$, 0.2 equiv CuBr, 0.4 equiv 3-[tris(3-hydroxypropyltriazolylmethyl)amine), THF/DMF (1:1), 20 °C, 18 h; 2) ammonia cleavage; 3) 5–10 equiv **Pep1a**, **Pep1b**, or **Pep2a**, pH 7.5 triethylammonium acetate buffer: MeCN (10:1), 20 °C, 18 h. For the conditions, see the Supporting Information.

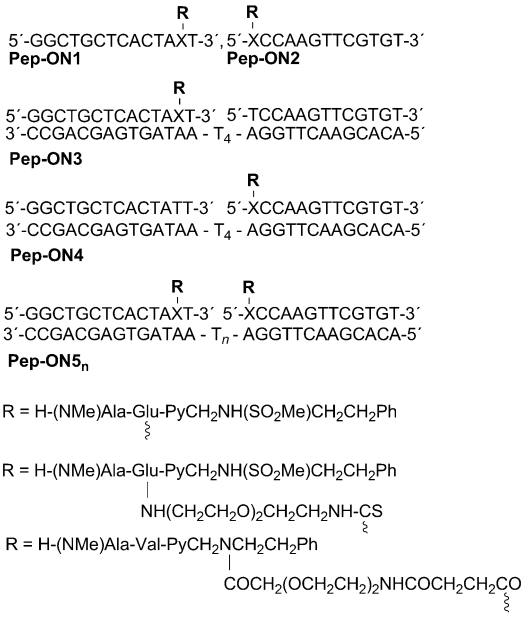


Figure 1. Complexes used in the DNA-based spatial screening.

Table 1: Binding of peptide–oligonucleotide complexes to XIAP (BIR_2-BIR_3).

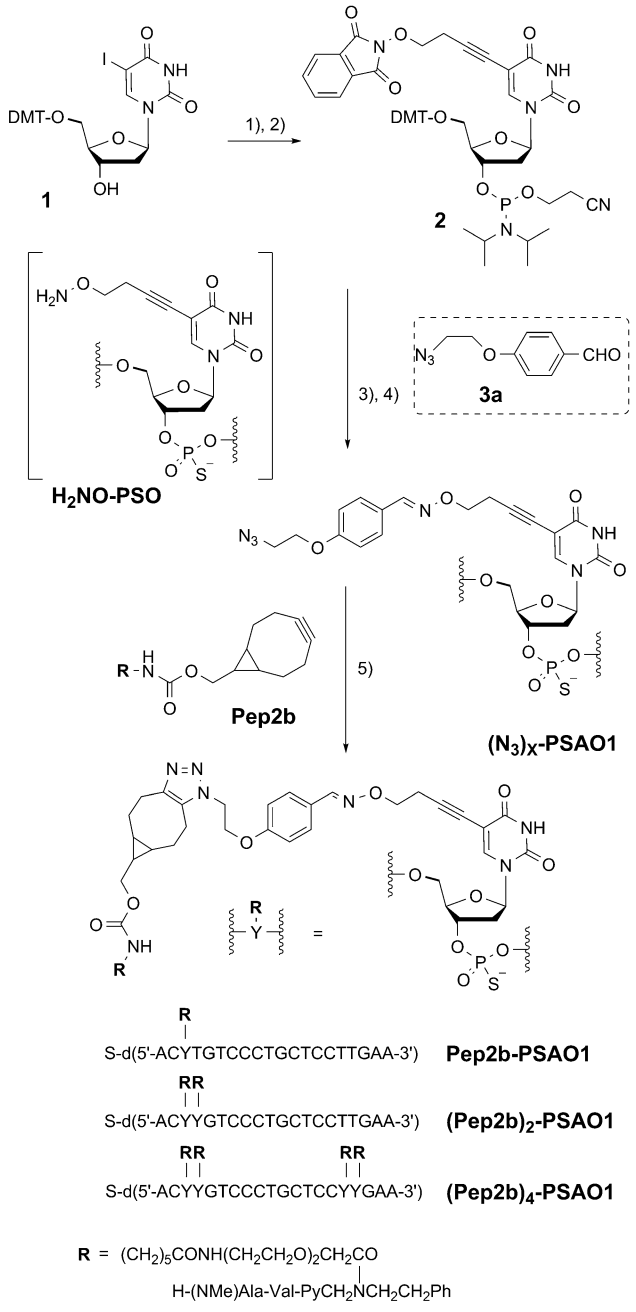
Compound	**Pep1a**[a]	**Pep1b**[a]	**Pep2a**[a]
Monovalent			
Pep-ON1	1000	1300	700
Pep-ON2	500	490	500
Pep-ON3	>2000	2000	1100
Pep-ON4	>2000	2000	1600
bivalent			
Pep-ON5$_0$	260	150	100
Pep-ON5$_1$	310	170	130
Pep-ON5$_3$	290	150	170
Pep-ON5$_5$	260	180	160
Pep-ON5$_7$	290	240	260
Pep-ON5$_{10}$	520	130	300
Pep-ON5$_{16}$	550	300	490

[a] Binding affinity characterized by IC_{50} values [nM] represented as the mean of triplicate measurements. For standard deviations, see Table S1 in the Supporting Information.

nucleotide conjugates **Pep-ON** in 68–84% yield after purification (Scheme 1b).

A competition assay was used to assess the affinity of the peptide–oligonucleotide complexes for the BIR_2-BIR_3 domain of XIAP (Figure 1, Table 1).[21] The bivalent complexes (**Pep2a-ON5$_n$**) showed higher binding affinities than the monovalent conjugates (IC_{50} = 100–500 nM versus 500–2200 nM). The comparison of three different peptide conjugates exposed a trend: The highest affinity was observed for bivalent complexes with the smallest distance between the two peptide appendages (e.g. **Pep2a-ON5$_0$**, IC_{50} = 100 nM).

For the conjugation of peptides with phosphorothioate oligonucleotides, we took recourse to [2+3] cycloaddition click reactions. However, phosphorothioate linkages inhibit the "copper-click" reaction used to install the azide group in the alkyne-oligonucleotides **ON**-CPG. We developed a new method which enables the introduction of the azide during the treatment of solid-supported phosphorothioate oligonucleotides with ammonia. A Sonogashira reaction was used to attach a phthaloyl-protected aminooxyalkine to uridine **1** (Scheme 2). The resulting building block **2** was incorporated during the automated solid-phase assembly of phosphorothiate oligonucleotides. The supported oligonucleotide was treated with triethylamine in acetonitrile to remove the cyanoethyl esters prior to global deprotection with ammonia. This avoided the exposure of the alkoxyamino group (**H_2NO-PSO**) liberated during ammonia cleavage to acrylonitrile



Scheme 2. 1) $PhthNOCH_2CH_2CCH$, $[PdP(Ph_3)]_4$, CuI, NEt_3, DMF; 2) $(iPr_2N)_2POC_2H_4CN$, benzylmercaptotetrazole, $CHCl_3$, 63%; 3) phosphorothioate DNA synthesis; 4) NEt_3/MeCN (1:1), 3 h, then wash; then NH_3/EtOH (9:1), 20–100 equiv **3a**, aniline; 5) **Pep2b** (5 equiv per modification) in buffer (10 mM Na_2HPO_4, 140 mM NaCl in H_2O/MeCN (9:1), pH 7.5), 20°C, 18 h.

generated during elimination of the cyanoethyl esters. The global deprotection with ammonia was carried out in the presence of a large amount of the azido-containing benzaldehyde **3a** and aniline. The intermediate (**H_2NO-PSO**) was immediately trapped by the azido-containing benzaldehyde **3a**. This led to the formation of the oxime (**$(N_3)_x$-PSO**. A large excess of the inexpensive benzaldehyde **3a** (20–100 equiv) enabled the quantitative introduction of the azido group

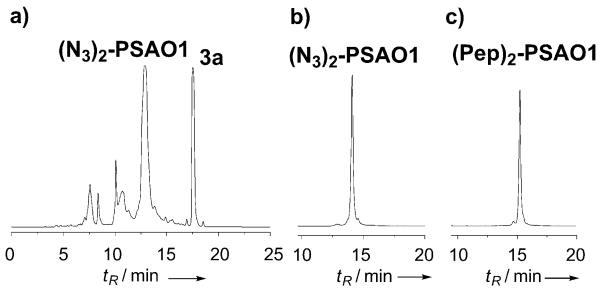


Figure 2. a) Preparative HPLC purification of crude azido-modified DMT-on PSO with UV detection at 260 nm ((**$(N_3)_2$-PSAO1**, signals at < 13 min arise from truncations and small molecule debris from global deprotection) and HPLC analysis of b) purified (**$(N_3)_2$-PSAO1**, and c) the ligation reaction between **Pep2b** (10 equiv) and (**$(N_3)_2$-PSAO1** after 18 h at 20°C. For the conditions, see the Supporting Information.

(Figure 2a). After cleavage of the dimethoxytrityl group (DMT) the azido-modified phosphorothiate oligonucleotides were subjected to the strain-promoted click reactions with the [6.1.0]bicyclononine (BCN)[22] armed peptide **Pep1b**. Formally, the conjugation proceeds through a sequence of oxime[23] and click ligations, yet practically the method involves only a single reaction step with a purified conjugate.

The method was used for the modification of a known c-Flip antisense oligonucleotide (ISIS 23296) optimized for downregulation of c-Flip in cancer cells.[24] Thymidine residues flanking a central 11 mer segment required to recruit RNase-H were replaced by phthalimidooxide-uridine **2**. The resulting conjugates carried a single or two adjacent peptide chains in **Pep2b-PSAO1** and (**Pep2b**)$_2$**-PSAO1**, respectively. In addition, four peptide chains were appended in a winglike design ((**Pep2b**)$_4$**-PSAO1**). Notably, the yields obtained in the synthesis of the azido-modified phosphorothiate oligonucleotides **N_3-PSAO1** and (**N_3**)$_2$**-PSAO1** were similar to the yields of unmodified phosphorothiate oligonucleotides (34–43%, DMT-on). A slightly reduced yield (29%) was observed for tetravalent (**N_3**)$_4$**-PSAO1**. Click ligation was quantitative (Figure 2c).

The XIAP binding assay showed, again, the increase in the inhibitory activity of bivalent as opposed to monovalent presentation (Figure 3a). The XIAP affinity of dual peptide modified (**Pep2b**)$_2$**-PSAO1** was comparable to the best peptide–oligonucleotide complexes identified in the DNA-programed spatial screen. Further increases of multivalency in the tetravalent conjugate (**Pep2b**)$_4$**-PSAO1** had little effect on the XIAP affinity. Next we assessed the ability of peptide–PSAOs to induce apoptosis in the human lung adenocarcinoma cell line A549, which is resistant against apoptosis induced by TRAIL (tumor necrosis factor alpha related inducing ligand).[9] The compounds were transfected at 500 nM with lipofectamine LTX. The viability of the cell line after incubation for 72 h was moderately affected by treatment with the antisense compound **N_3-PSAO1** (77% viability) which lacked the peptide (Figure 3b). By contrast, only 20% of the cells survived after the treatment with the monovalent peptide–PSO conjugate **Pep2b-PSAO1**, which suggests a significant contribution of the peptide part. The

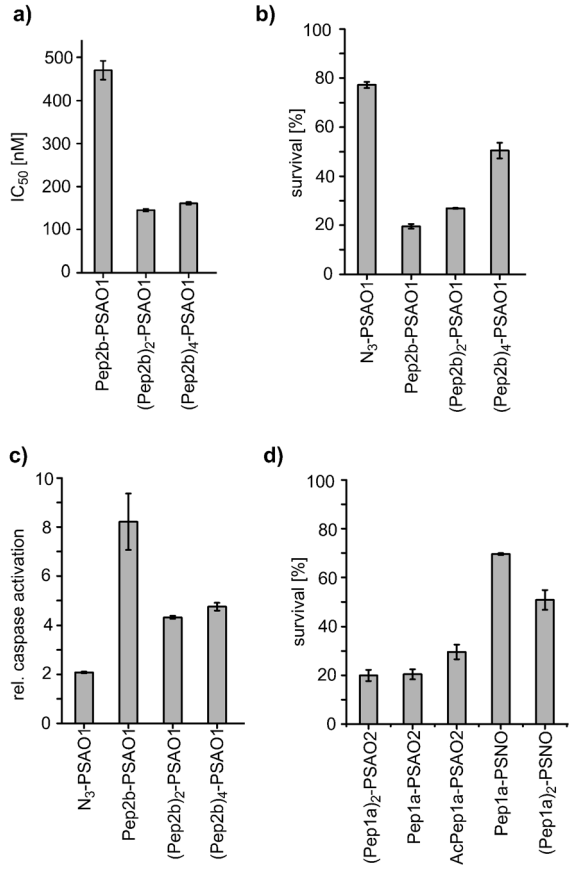


Figure 3. a) In vitro binding affinity (IC_{50}) of peptide–PSAO conjugates for XIAP (Bir_2-Bir_3). b) Viability of A549 cells after treatment with 500 nM peptide—PSAO for 72 h. c) Increase of caspase-3,7 activity relative to untreated cells upon after treatment with 300 nM compound for 72 h. d) Viability of A549 cells after treatment for 72 h with 300 nM of the PSO conjugates terminally modified with peptide (antisense, PSAO, or nonsence sequence, PSNO).

bivalent and tetravalent conjugates (**Pep2b**)$_2$**-PSAO1** and (**Pep2b**)$_4$**-PSAO1** had lower inhibitory activity than the monovalent conjugate, which stands in contrast to the results of the XIAP in vitro binding assay.

A caspase assay suggested that cell death was induced by apoptosis (Figure 3c). Treatment with the monovalent conjugate **Pep2b-PSAO1** resulted in the highest (8-fold) increase in caspase activity. The activation of caspase 3 and 7 was less efficient with the bi- and tetravalent conjugates and even lower with the nonpeptide control **N_3-PSAO1**. Apparently, the dual pathway directed peptide–PSO conjugates were better inductors of apoptosis than the **N_3-PSAO1** alone. To better understand the discrepancy between the XIAP in vitro binding assay and the cellular viability and caspase assays, we determined the efficiency of downregulation of the c-Flip mRNA targeted by the **PSO** sequence (see Figure S4 in the Supporting Information). The RT-PCR assay suggested that the peptide appendages impaired the ability to decrease the expression levels of c-Flip mRNA. We therefore moved the peptide ligation site to the 5′-terminal end. A phthaloyl-protected alkoxyamino terminal modifier (M in Figure S2 in the Supporting Information) was introduced at the 5′-terminus. The global deprotection and in situ ligation with monovalent azido-aldehyde **3a** or the bivalent derivative **3b** (see Figure S3 in the Supporting Information) and subsequent strain-promoted click reactions with peptide **Pep1a** yielded the 5′-modified peptide–PSO conjugates (Figure 4). Conjugates containing the apoptotically inactive, N-acetylated peptide **AcPep1a** were synthesized to assess the specificity of the effects exerted by the peptide appendage.

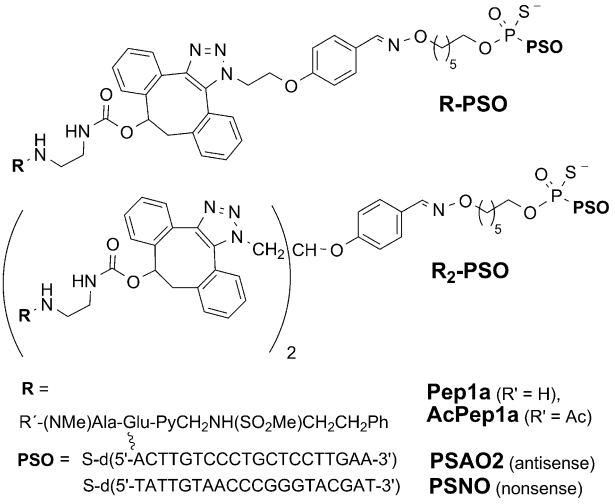


Figure 4. Terminal peptide click reaction on phosphorothiate oligonucleotides.

The peptide–PSO conjugates (**Pep1a**)$_2$**-PSAO2** and **Pep1a-PSAO2** decreased the viability of the A549 cells to 20% (Figure 3d). In comparison, 30% of the cells remained viable after treatment with **AcPep1a-PSAO2** which contained the inactive peptide conjugated to an antisense sequence. In **Pep1a-PSNO**, the active peptide was conjugated with a nonsense sequence. This compound showed modest apoptotic activity. Notably, an increase in the peptide load in the bivalent conjugate (**Pep1a**)$_2$**-PSNO** led to more efficient apoptosis. This suggests that the peptide contributed to the apoptotic activity. We concluded that, in internally labeled peptide conjugates, there is a trade-off between the gain of apoptotic activity obtained upon attachment of the Smac-mimetic peptides to PSAO and a loss of the RNase H activation. The cellular assays suggested that in monovalent peptide–PSO conjugate **Pep2b-PSAO1** the peptide-mediated inhibition of XIAP overcompensated the impairment of RNase H induction. From this and the gain in activity of terminally modified peptide–PSO conjugates (Figure 3d), we inferred that a PSO-linked peptide is able to reach its intracellular target protein.

In conclusion, we have developed a new conjugation method that allows the convergent coupling of unprotected peptides with unprotected antisense phosphorothioate oligonucleotides. The method relies on a sequence of oxime and strain-promoted click ligation reactions and can be applied to both internal and terminal positions. Click reactions based on [2+3] cycloaddition or inverse electron demand Diels–Alder reactions have been used to modify oligonucleotides and

in vitro transcribed RNA,[16b,20a,25] but, to the best of our knowledge, reactions with phosphorothioate oligonucleotides have not been possible so far. The method proposed by us provides high conjugation yields and requires no additional HPLC purification. This facilitates the synthesis of peptide-enhanced antisense molecules. We demonstrated the usefulness of the method in the synthesis of conjugates targeted against two different pathways of apoptosis. The combination of a nucleic acid sequence designed to downregulate mRNA from c-Flip (a repressor of the extrinsic apoptosis pathway) and a peptide mimetic for the inhibition of XIAP (a repressor of the intrinsic apoptosis pathway) led to enhanced apoptotic activity. The design extends beyond the previous applications of oligonucleotide conjugates which focused on the use of cell-penetrating peptides. The presented data shows that the peptide can play a more active role by targeting an intracellular protein that is involved in the biological process to be perturbed by the antisense oligonucleotide. At this stage, it is unclear how the conjugate reaches the two different targets. However, it should be considered that the peptide–PSAO conjugate would be able to access the target protein in both the RNA-bound form as well as in the free form (after RNase H induced mRNA degradation). Regardless of the mechanism involved, given the availability of powerful antisense molecules and powerful peptide-based inhibitors, it seems worthy to explore other combinations.

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[1] a) G. J. Hannon, *Nature* **2002**, *418*, 244 – 251; b) A. Fire, S. Xu, M. K. Montgomery, S. A. Kostas, S. E. Driver, C. C. Mello, *Nature* **1998**, *391*, 806 – 811; c) C. V. Pecot, G. A. Calin, R. L. Coleman, G. Lopez-Berestein, A. K. Sood, *Nat. Rev. Cancer* **2011**, *11*, 59 – 67.

[2] a) C. F. Bennett, E. E. Swayze, *Annu. Rev. Pharmacol. Toxicol.* **2010**, *50*, 259 – 293; b) R. Kole, A. R. Krainer, S. Altman, *Nat. Rev. Drug Discovery* **2012**, *11*, 125 – 140; c) N. Dias, C. A. Stein, *Mol. Cancer Ther.* **2002**, *1*, 347 – 355.

[3] R. G. Lee, J. Crosby, B. F. Baker, M. J. Graham, R. M. Crooke, *J. Cardiovasc. Transl. Res.* **2013**, *6*, 969 – 980.

[4] a) F. Eckstein, *Annu. Rev. Biochem.* **1985**, *54*, 367 – 402; b) S. Spitzer, F. Eckstein, *Nucleic Acids Res.* **1988**, *16*, 11691 – 11704.

[5] D. Jones, *Nat. Rev. Drug Discovery* **2011**, *10*, 401 – 402.

[6] a) S. Basu, E. Wickstrom, *Tetrahedron Lett.* **1995**, *36*, 4943 – 4946; b) M. Antopolsky, E. Azhayeva, U. Tengvall, S. Auriola, I. Jaaskelainen, S. Ronkko, P. Honkakoski, A. Urtti, H. Lonnberg, A. Azhayev, *Bioconjugate Chem.* **1999**, *10*, 598 – 606; c) A. Sánchez, E. Pedroso, A. Grandas, *Bioconjugate Chem.* **2012**, *23*, 300 – 307; d) S. M. Jirka, H. Heemskerk, C. L. Tanganyika-de Winter, D. Muilwijk, K. H. Pang, P. C. de Visser, A. Janson, T. G. Karnaoukh, R. Vermue, P. A. t Hoen, J. C. van Deutekom, B. Aguilera, A. Aartsma-Rus, *Nucleic Acid Ther.* **2014**, *24*, 25 – 36; e) I. Diala, A. Osada, S. Maruoka, T. Imanisi, S. Murao, T. Ato, H. Ohba, M. Fujii, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6576 – 6578; f) J. J. Turner, A. A. Arzumanov, M. J. Gait, *Nucleic Acids Res.* **2005**, *33*, 27 – 42.

[7] a) P. Järver, T. Coursindel, S. E. Andaloussi, C. Godfrey, M. J. Wood, M. J. Gait, *Mol. Ther. Nucleic Acids* **2012**, *1*, e27; b) R. Juliano, M. R. Alam, V. Dixit, H. Kang, *Nucleic Acids Res.* **2008**, *36*, 4158 – 4171.

[8] A. A. Kaspar, J. M. Reichert, *Drug Discovery Today* **2013**, *18*, 807 – 817.

[9] L. Zhang, B. Fang, *Cancer Gene Ther.* **2005**, *12*, 228 – 237.

[10] A. E. Logan, T. R. Wilson, C. Fenning, R. Cummins, E. Kay, P. G. Johnston, D. B. Longley, *Apoptosis* **2010**, *15*, 1435 – 1443.

[11] Q. L. Deveraux, R. Takahashi, G. S. Salvesen, J. C. Reed, *Nature* **1997**, *388*, 300 – 304.

[12] a) I. Tamm, S. M. Kornblau, H. Segall, S. Krajewski, K. Welsh, S. Kitada, D. A. Scudiero, G. Tudor, Y. H. Qui, A. Monks, M. Andreeff, J. C. Reed, *Clin. Cancer Res.* **2000**, *6*, 1796 – 1803; b) L. Yang, Z. Cao, H. Yan, W. C. Wood, *Cancer Res.* **2003**, *63*, 6815 – 6824.

[13] a) H. Sun, Z. Nikolovska-Coleska, C. Y. Yang, D. Qian, J. Lu, S. Qiu, L. Bai, Y. Peng, Q. Cai, S. Wang, *Acc. Chem. Res.* **2008**, *41*, 1264 – 1277; b) R. Mannhold, S. Fulda, E. Carosati, *Drug Discovery Today* **2010**, *15*, 210 – 219.

[14] a) J. Lu, L. Bai, H. Sun, Z. Nikolovska-Coleska, D. McEachern, S. Qiu, R. S. Miller, H. Yi, S. Shangary, Y. Sun, J. L. Meagher, J. A. Stuckey, S. Wang, *Cancer Res.* **2008**, *68*, 9384 – 9393; b) H. Sun, Z. Nikolovska-Coleska, J. Lu, J. L. Meagher, C. Y. Yang, S. Qiu, Y. Tomita, Y. Ueda, S. Jiang, K. Krajewski, P. P. Roller, J. A. Stuckey, S. Wang, *J. Am. Chem. Soc.* **2007**, *129*, 15279 – 15294; c) A. Laurent, K. E. Hewitt, S. Morris, P. Bureau, A. Boudreault, S. Jarvis, J. Jaquith, US 7547724 B2, **2009**.

[15] a) C. Scheibe, A. Bujotzek, J. Dernedde, M. Weber, O. Seitz, *Chem. Sci.* **2011**, *2*, 770 – 775; b) H. Eberhard, F. Diezmann, O. Seitz, *Angew. Chem.* **2011**, *123*, 4232 – 4236; *Angew. Chem. Int. Ed.* **2011**, *50*, 4146 – 4150; c) F. Abendroth, A. Bujotzek, M. Shan, R. Haag, M. Weber, O. Seitz, *Angew. Chem.* **2011**, *123*, 8751 – 8755; *Angew. Chem. Int. Ed.* **2011**, *50*, 8592 – 8596; d) C. Scheibe, S. Wedepohl, S. B. Riese, J. Dernedde, O. Seitz, *ChemBioChem* **2013**, *14*, 236 – 250.

[16] a) F. Amblard, J. H. Cho, R. F. Schinazi, *Chem. Rev.* **2009**, *109*, 4207 – 4220; b) A. H. El-Sagheer, T. Brown, *Acc. Chem. Res.* **2012**, *45*, 1258 – 1267.

[17] a) X. Ning, J. Guo, M. A. Wolfert, G. J. Boons, *Angew. Chem.* **2008**, *120*, 2285 – 2287; *Angew. Chem. Int. Ed.* **2008**, *47*, 2253 – 2255; b) M. E. Jung, A. B. Mossman, M. A. Lyster, *J. Org. Chem.* **1978**, *43*, 3698 – 3701.

[18] M. J. Bertrand, S. Milutinovic, K. M. Dickson, W. C. Ho, A. Boudreault, J. Durkin, J. W. Gillard, J. B. Jaquith, S. J. Morris, P. A. Barker, *Mol. Cell* **2008**, *30*, 689 – 700.

[19] a) R. Lartia, P. Murat, P. Dumy, E. Defrancq, *Org. Lett.* **2011**, *13*, 5672 – 5675; b) C. Coppola, L. Simeone, L. De Napoli, D. Montesarchio, *Eur. J. Org. Chem.* **2011**, 1155 – 1165; c) T. Wada, A. Mochizuki, S. Higashiya, H. Tsuruoka, S. Kawahara, M. Ishikawa, M. Sekine, *Tetrahedron Lett.* **2001**, *42*, 9215 – 9219; d) A. M. Jawalekar, N. Meeuwenoord, J. G. O. Cremers, H. S. Overkleeft, G. A. van der Marel, F. Rutjes, F. L. van Delft, *J. Org. Chem.* **2008**, *73*, 287 – 290; e) G. Pourceau, A. Meyer, J. J. Vasseur, F. Morvan, *J. Org. Chem.* **2009**, *74*, 1218 – 1222.

[20] a) J. Gierlich, G. A. Burley, P. M. Gramlich, D. M. Hammond, T. Carell, *Org. Lett.* **2006**, *8*, 3639 – 3642; b) P. J. Barr, A. S. Jones, P. Serafinowski, R. T. Walker, *J. Chem. Soc. Perkin Trans. 1* **1978**, 1263 – 1267.

[21] Z. Nikolovska-Coleska, J. L. Meagher, S. Jiang, S. A. Kawamoto, W. Gao, H. Yi, D. Qin, P. P. Roller, J. A. Stuckey, S. Wang, *Anal. Biochem.* **2008**, *374*, 87 – 98.

[22] J. Dommerholt, S. Schmidt, R. Temming, L. J. Hendriks, F. P. Rutjes, J. C. van Hest, D. J. Lefeber, P. Friedl, F. L. van Delft, *Angew. Chem.* **2010**, *122*, 9612 – 9615; *Angew. Chem. Int. Ed.* **2010**, *49*, 9422 – 9425.

[23] a) D. Forget, D. Boturyn, E. Defrancq, J. Lhomme, P. Dumy, *Chem. Eur. J.* **2001**, *7*, 3976 – 3984; b) E. Trévisiol, E. Defrancq, J.

Lhomme, A. Laayoun, P. Cros, *Eur. J. Org. Chem.* **2000**, 211 – 217; c) K. Rose, *J. Am. Chem. Soc.* **1994**, *116*, 30 – 33.
[24] E. Ackermann, C. Bennett, N. Dean, W. Ricketts, A. Watt, H. Zhang, US 20040254137A1, **2004**.
[25] a) M.-L. Winz, A. Samanta, D. Benzinger, A. Jäschke, *Nucleic Acids Res.* **2012**, 10.1093/nar/gks062; b) I. S. Marks, J. S. Kang, B. T. Jones, K. J. Landmark, A. J. Cleland, T. A. Taton, *Bioconjugate Chem.* **2011**, *22*, 1259 – 1263; c) K. N. Jayaprakash, C. G. Peng, D. Butler, J. P. Varghese, M. A. Maier, K. G. Rajeev, M. Manoharan, *Org. Lett.* **2010**, *12*, 5410 – 5413.

