A versatile method for the preparation of conjugates of peptides with DNA/PNA/analog by employing chemo-selective click reaction in water

Khirud Gogoi, Meenakshi V. Mane, Sunita S. Kunte and Vaijayanti A. Kumar*

Division of Organic Chemistry, National Chemical Laboratory, Pune, India, 411008

Received August 14, 2007; Revised September 21, 2007; Accepted October 11, 2007

ABSTRACT

The specific 1,3 dipolar Hu¨isgen cycloaddition reaction known as ‘click-reaction’ between azide and alkyne groups is employed for the synthesis of peptide–oligonucleotide conjugates. The peptide nucleic acids (PNA)/DNA and peptides may be appended either by azide or alkyne groups. The cycloaddition reaction between the azide and alkyne appended substrates allows the synthesis of the desired conjugates in high purity and yields irrespective of the sequence and functional groups on either of the two substrates. The versatile approach could also be employed to generate the conjugates of peptides with thioacetamido nucleic acid (TANA) analog. The click reaction is catalyzed by Cu (I) in either water or in organic medium. In water, ~3-fold excess of the peptide-alkyne/azide drives the reaction to completion in 2 h with no side products.

INTRODUCTION

Uncharged, achiral peptide nucleic acids (PNAs) are DNA mimics that show unprecedented affinity towards complementary RNA and DNA sequences(1,2). PNA and other modified oligonucleotides (ONs) (3–7) are currently being developed as DNA mimics to target disease-causing mRNA (8,9), using the principle of antisense action. This is gaining further importance because of corrective antisense therapies that do not require activation of RNase H enzyme or cleavage of the target mRNA (10). The success or failure of any such candidate in antisense therapeutics depends on a number of factors such as sequence-specific recognition of target mRNA, intracellular stability, water solubility (3) and cell penetration (11). Application of PNA and analogous uncharged DNA mimics is stymied by the fact that PNAs show very low cell penetration for any observable antisense effect (12). Several strategies are being developed for the delivery of modified ONs into cells (13,14). For uncharged ON mimics such as PNAs, the best option seems to be the covalent conjugation of PNA oligomers with cell-penetrating peptides (CPP) (15–17). Conjugation of peptides to DNA and DNA analogs with sugar-phosphate backbone ONs is also gaining importance for their biological applications (13,14). The CPPs are mostly positively charged peptides containing lysine (18) or arginine (19,20) or other peptides having specific cell receptors (21). Several other uses of peptide conjugation with ONs are known in the literature (13). The conjugation is achieved by tedious continuous solid-phase synthesis of PNA and peptide (22–25). The other methods for conjugation could be post-synthetic via disulfide bridge (26,27) or more stable thioether linkages (21) at either C- or N- terminus. A recent review (13) not only summarizes the present methods of synthesizing ON–peptide conjugates that are common to ONs and their analogs but also points out the need to develop straightforward methods to synthesize such conjugates. The highly functionalized nature of these biomolecules and their mimics such as PNA, render them susceptible for side reactions during conjugation and yield and purity of structurally defined conjugated biomolecules is often low. The (4+2) Diels-Alder cycloaddition approach was employed recently for the conjugation of DNA and CPP (28). This involved the reaction between diene and dienophile present on the respective biomolecules to get the conjugates. The maleimide dienophile used in this reaction is susceptible for Michael addition reactions with other nucleophilic centers on peptides or ONs and may give rise to side reactions. The current literature clearly indicates the need for a simple and straightforward strategy for generating highly pure ON/PNA–peptide conjugates in high yield (13).

Meanwhile, we find that the applications of highly selective orthogonal Cu (I) catalyzed Hu¨isgen 1, 3 dipolar cycloaddition reaction, recognized as ‘click chemistry’ (29–32), are expanding the scope of synthesis of variety of other bioconjugates such as DNA-glycoconjugates (33), peptide–protein conjugates (34), carbohydrate–vaccine conjugates (35) or protein modification and protein...
Aha, 6-aminohexanoic acid (or 6-trimethylbenzenesulfonyl; Boc, tert-PNA backbone) and DNA/RNA.

Figure 1. DNA/RNA and PNA and TANA.

microarray fabrication (36). This reaction between high-energy organic azides and terminal alkynes can give rise to unlimited array of inert triazole containing architectures (Scheme 1). The reaction is highly predictable, fast and resistant to side reactions. Addition of Cu(I) accelerates the reaction (31,32). Some of these reactions could be carried out in aqueous medium and can be employed post-synthetically on purified units decorated with a variety of functional groups, without additional functional group protecting strategies. Recently, click reaction has also found applications for the synthesis of circular DNA (37,38), and DNA-template-directed ON strand ligation (38). With this background we envisaged a very simple possibility of synthesizing peptide–DNA/PNA conjugates using click chemistry i.e. a specific cycloaddition reaction between terminal azide and alkynyl functionalities on PNA, DNA or peptide as per the synthesis design. These two functional groups are absent in the biomolecules of current interest and no predictable side reaction can be envisaged. This approach has not been previously applied to the synthesis of such conjugates and has potential to lead to a variety of CPP–DNA/PNA conjugates from various combinations of azide and alkynyl derivatized peptides-ON substrates in aqueous solution without going into the rigors of continuous synthesis. In this paper, we report successful application of this proposal to generate the CPP conjugates with DNA/PNA ONs and thioacetamido nucleic acid (TANA, Figure 1) (39,40).

MATERIALS AND METHODS

N₂,Fmoc l-aminocaproic acid and resins for peptide and PNA synthesis were obtained from Novabiochem(Fmoc, 9-fluorenylethoxycarbonyl). The Fmoc amino acid used were Fmoc-Lys(Boc)-OH, Fmoc-Arg(Mtr)-OH and N₂,Fmoc-Aha-OH,(Mtr, 4-methoxy-2,3,6-trimethylbenzenesulfonyl; Boc, tert-butoxycarbonyl; Aha, 6-aminoheptanoic acid (or ε-aminoacapric acid). 6-aminoheptanoic acid was obtained from Aldrich chemicals and N-Fmoc protection was done using standard procedure. The concentrations of DNA/PNAs and their conjugates were determined spectrophotometrically and the concentration of the peptides was assumed approximately. Propynoic acid was procured from Trade (TCI) Mark, Tokyo Kasei. N-Boc-(2S, 4S)-4-azidopropine was synthesized according to the reported procedure (41). The Boc and Fmoc protected PNA monomers were obtained from Applied Biosystems, USA. TANA monomers were synthesized in the laboratory following the reported procedures (39). Propyn substituted 19-mer DNA sequence was synthesized using the phosphoramidite approach and an Applied Biosystems 3900 DNA synthesizer.

Reverse phase high-performance liquid chromatography analyses were carried out on VARIAN Analytical Semi-prep HPLC system consisting of Varian Pro-star 210 Binary solvent delivery system. Linear gradients of A: 0.1% TFA in water and B: 55/45: Acetonitrile/Water, 0.1% TFA (Linear gradient from A to B in 30 min Flow-1.5 ml/min.). Rainin Dynamax UV D-II Absorbance Detector Star Ver.5 at detection wavelength 254 nm or 220 nm was employed during the experimentation. Chromatography Workstation Rheodyne 7725I with manual injector and Lichrocart Lichrispher 100RP-18 250 x 4 mm id. Particle size-5 μm column were used.

Mass spectral analysis was performed on a Voyager-De-STR (Applied Biosystems) MALDI-TOF. A nitrogen laser (337 nm) was used for desorption. The matrixes used for analysis were CHCA (2-Cyano-4-hydroxycinnamic Acid), THAP (2’, 4’,and 6’-trihydroxyacetophenone) and HPA (3-hydroxypicolinic acid). Di ammonium citrate was used as additive when THAP and HPA were used as matrix.

Solid-phase synthesis of azide functionalized PNA oligomers 2, 3 and 4

The azide functionalized PNA oligomers 2 and 3 were synthesized on Rink-amide resin (100 mg, loading 0.3 mmol/g) following the standard procedures of solid-phase peptide synthesis (42) (20 min treatment with 20% piperidine in N,N-dimethyl formamide and reaction with 3 equivalents of Fmoc-PNA monomer, HBTU, HOBt and DIPEA for 6 h, were used for the deprotection and coupling steps, respectively). The last coupling reaction was done with N-Boc-(2S,4S)-4-azidopropine. The progress of the coupling reaction was tested by Kaiser test at each step. Cleavage and deprotection were effected by reaction with TFA/DCM/TIS (10:85:5) for 30 min. The resulting oligomer was precipitated by addition of cold ether and purification was done by gel filtration followed by RP-HPLC and characterized by MALDI-TOF mass spectrometry (Table 1, entry 3, 6).

The azide functionalized mixed PNA oligomer 4 was synthesized using side chain N-Cbz protected Boc-Lysine functionalized MBHA resin (100 mg, loading 0.2 mmol/g) following the standard procedures of solid-phase peptide synthesis (30 min treatment with 50% TFA in DCM and reaction with three equivalents of Boc-PNA by guest on 29 July 2018

Downloaded from https://academic.oup.com/nar/article-abstract/35/21/w139/2376604
monomer, HBTU, HOBt and DIPEA for 6 h, were used for the deprotection and coupling steps, respectively). The last coupling reaction was done with N-Boc-(2S,4S)-4-azidoproline. Cleavage and deprotection were carried out by the reaction with TFA-TFMSA, thioanisole, ethanedithiol for 2 h. The resulting oligomer was precipitated by addition of cold ether and purification was done by gel filtration followed by RP–HPLC and characterized by MALDI-TOF mass spectrometry (Table 1, entry 9).

Solid-phase synthesis of alkyne functionalized lysine peptide HO-(Lys)_6-alkyne 1

The alkyne functionalized lysine peptide was synthesized on Rink-amide resin (100 mg, loading 0.3 mmol/g) following the standard procedures of solid-phase peptide synthesis (42) (20 min treatment with 20% piperidine in N,N-dimethyl formamide and the reaction with 3 equivalent of Fmoc-Lys(Boc)-OH, HBTU, HOBt and DIPEA for 4 h, were used for the deprotection and coupling steps, respectively). The last coupling was done with propynoic acid using HBTU, HOBt and DIPEA as the coupling agent. The peptide was cleaved from the resin by treatment with TFA/DCM/TIS (10:85:5) for 30 min and the deprotection of side chain was effected by 50% TFA-DCM (TIS used as scavenger) for 1 h. The resulting peptide was precipitated by addition of cold ether.

Click reaction on solid phase: synthesis of HO-(Lys)_6-triazole-proline 5

Twenty milligrams of resin bound to protected (Lys)_6-alkyne 1, N-Boc-(2S,4S)-4-azidoproline (5 mg) (37), CuI(1 mg), DIPEA(5 µl) and DMF(0.3 ml) were reacted together in a reaction vessel for 8 h (Scheme 2). The excess

![Scheme 2. Peptide-alkyne conjugation with 4-azidoproline using click chemistry on solid support.](https://academic.oup.com/nar/article-abstract/35/21/e139/2376604)
reagents were washed out with DMF followed by DCM. Five milligrams of resin was cleaved by using the standard conditions and the product formed was purified by HPLC and characterized by MALDI-TOF mass spectrometry (Table 1, entry 2).

**Solution phase reaction between PNA-proline azides 2, 3 and 4 and HO-(Lys)₆-alkyne 1: synthesis of conjugates 6, 7 and 8**

**General procedure.** HO-β-ala-TTTTTTT-Pro-N₃ 2 (or HO-Lys-TCACTAGATG-Pro-N₃ 4 or HO-β-ala-t-t-t-t-t-Pro-N₃ 3) (1 μmol) and HO-(Lys)₆-alkyne 1 (~3 μmol) were dissolved in 50 μl water:tert. butanol(1:1). CuSO₄.5H₂O (1.0 equivalent, 1 μmol, 10 μl of a 100 mM solution in water) was then added. The mixture was stirred in a spinix vortex at room temperature (Scheme 3). The reaction mixture was analyzed by HPLC after 2 h. HPLC showed complete consumption of the starting material in the case of either 2 or 4 and reaction with 3 equivalent of Fmoc-Arg(Mtr)-OH (or Arg-Aha-OH), HBTU, HOBt and DIPEA for 4 h, were used for the deprotection and coupling steps, respectively. The final coupling was done with propynoic acid using HBTU, HOBt and DIPEA as the coupling agent. Cleavage of the peptide from resin was effected by reaction with TFA/DCM/TIS (10:85:5) for 30 min. The deprotection of side-chain protecting group (Mtr) was effected by 100% TFA and thioanisole as the scavenger for 5 h. The resulting peptide was precipitated by addition of cold ether and oligomer was purified by RP–HPLC. The product was further characterized by MALDI-TOF mass spectrometry (Table 1, entry 12).

**Solution phase reaction between PNA-proline azides 3, 4 and HO-(Arg-Aha-Arg)₄-alkyne 9: synthesis of conjugates 10 and 11**

**General procedure.** HO-Lys-TCACTAGATG-Pro-N₃ 4 or HO-β-ala-t-t-t-t-t-t-Pro-N₃ 3 (1 μmol) and (Arg-Aha-Arg)₄-alkyne 9 (~3 μmol) were dissolved in 50 μl water:tert. Butanol (1:1). CuSO₄.5H₂O (1.0 equivalent, 1 μmol, 8 μl of 500 mM solution in water) and freshly prepared solution of sodium ascorbate (4 equivalents, 4 μmol, 8 μl of 500 mM solution in water) was then added. The mixture was stirred in a spinix vortex at room temperature. The reaction mixture was analyzed by RP–HPLC after 2 h. HPLC showed complete conversion of starting materials to the products. The products (10 and 11) formed were purified by RP–HPLC and characterized by MALDI-TOF mass spectrometry (Table 1, entry 13 and 14).

**Synthesis of alkyne substituted DNA oligomer 12**

The DNA sequence 12 was synthesized using commercially available monomeric units using phosphoramidite chemistry on automated DNA synthesizer. The last coupling was done using 5′-O-(propynyl-N,N-benzoyl-thymidin)-3′-O-(N,N-diisopropylamino-O-cyanoethyl-phosphoramidite) (Figure 2). (Supplementary Data). The ON 12 was deprotected and cleaved from the solid support. It was further purified by RP–HPLC and characterized by MALDI-TOF mass spectrometry (Table 1, entry 15).

**Solid-phase synthesis of the peptide**

HO-(Arg-Aha-Arg)₄-alkyne 9

The arginine peptide 9 was synthesized on Rink-amide resin (200 mg, loading 0.3 mmol/g) following the standard procedures of solid-phase peptide synthesis: 20 min treatment with 20% piperidine in N,N-dimethyl formamide and reaction with 3 equivalent of Fmoc-Arg(Mtr)-OH (or N⁵-Fmoc-Aha-OH), HBTU, HOBt and DIPEA for 4 h, were used for the deprotection and coupling steps, respectively. The final coupling was done with propynoic acid using HBTU, HOBt and DIPEA as the coupling agent. Cleavage of the peptide from resin was effected by reaction with TFA/DCM/TIS (10:85:5) for 30 min. The deprotection of side-chain protecting group (Mtr) was effected by 100% TFA and thioanisole as the scavenger for 5 h. The resulting peptide was precipitated by addition of cold ether and oligomer was purified by RP–HPLC. The product was further characterized by MALDI-TOF mass spectrometry (Table 1, entry 12).

**Figure 2.** Alkyne and azide carrier units.
for 4 h, were used for the deprotection and coupling steps, respectively. The final coupling was done with N-Boc-4-azido-proline using HBTU, HOBI and DIPEA as the coupling agent. Cleavage of the peptide from resin was effected by reaction with TFA/DCM/TIS (10:85:5) for 30 min. The deprotection of side chain protecting group (Mtr) was effected by 100% TFA and thioisoureas as the scavenger for 5 h. The resulting peptide was precipitated by addition of cold ether and was purified by RP–HPLC.

The product was further characterized by MALDI-TOF mass spectrometry (Table 1, entry 16).

**Solution phase reaction between DNA-alkyne 12 and HO-(Arg-Aha-Arg)₄-azide 13: synthesis of conjugate 14**

Alkyne functionalized ON 12 (1 µmol) and azide functionalized peptide 13 (~3 µmol) were dissolved in 50 µl water:tert. Butanol (1:1). CuSO₄·5H₂O (0.6 equivalent, 0.6 µmol, 6 µl of a 100 mM solution in water) and freshly prepared solution of sodium ascorbate (2.4 equivalents, 4 µmol, 6 µl of 500 mM solution in water) was then added (Scheme 4). The mixture was stirred in a spin vortex at room temperature. After 1 h, a small portion of the reaction mixture was analyzed by RP–HPLC. HPLC showed complete conversion of starting materials to product. The product 14 formed was purified by RP–HPLC and characterized by MALDI-TOF mass spectrometry (Table 1, entry 17).

**RESULTS AND DISCUSSION**

The aim of this study was to develop a simple and chemoselective method for the conjugation of peptides and ON mimics such as PNA and TANA. The azide and acetylene carrier units were chosen so that these could be installed online during the solid supported peptide or PNA/TANA synthesis at the N/C-terminus through an amide linkage that is stable towards conditions employed for the deprotection and cleavage of the peptide/ON from the solid support. The candidates chosen for this purpose were propynoic acid and (2S,4S)-4-azidoproline (Figure 2) (41). DNA ON was functionalized at 5’-end using 5’-O-propynyl-thymidine-phosphoramidite (Figure 2), (Supplementary Data). The 4-azidoproline was chosen in this strategy, as it could also open up the possibility to introduce the azide group at either C/N-terminus or in the center of the PNA or peptide sequences as desired. This could enable the conjugation of peptide at either C- or N-terminus of PNA or peptide. To assess the methodology and its application potential, we chose three PNA sequences, a homopyrimidine PNA-T8, a mixed purine-pyrimidine 10-mer PNA sequence and a recently developed homothyminyl-oligomer with uncharged thioacetamido nucleic acid (TANA) backbone (39). The reaction medium was tert.-butanol:water that solubilizes all the reactants and is also capable of scavenging hydroxy radicals (44). The peptide sequence chosen was the lysine (18) or the arginine rich peptide (19) that were known to act as vehicle for PNAs to penetrate through the cell membrane.

The azide functionalized PNA oligomers 2 and 3 were synthesized on Rink-amide resin following the standard procedures (42) of Fmoc based solid-phase peptide synthesis. The last coupling reaction was done using N-Boc-(2S,4S)-4-azidoproline monomer unit. The azide functionalized mixed purine-pyrimidine PNA oligomer 4 was synthesized following the standard procedures of Boc-based solid-phase peptide synthesis (42). The final coupling reaction was done with N-Boc-(2S,4S)-4-azidoproline. The alkyne functionalized lysine peptide 1 and arginine-rich peptide 9, were synthesized on Rinkamide resin following the standard procedures of solid-phase peptide synthesis. The final coupling was done with propynoic acid. To install the azide functional group on DNA oligomer, we attempted to synthesize 5’deoxy-5’-azido-thymidinyl-3’-O-phosphoramidate, but this was found to be quite unstable. We then synthesized 5’-O-propynyl-N3-benzoylthymidinyl-3’-O-phosphoramidate (Supplementary Data). The 5’-end alkyn substituted DNA sequence 12 was synthesized using automated DNA synthesizer when the last coupling was with 5’-O-propynyl-N3-benzoylthymidinyl-3’-O-phosphoramidate. N-terminal azide containing arginine-rich peptide 13 was synthesized as 9, and N-Boc-(2S,4S)-4-azidoproline was used for the last coupling reaction. The installation of reactive alkyne and azide functionalities on either the peptide or ON, was followed by the experiments to study the feasibility of the click cyclodaddition reaction. The peptide 1, attached to the solid support with all the side chain lysine amino- and terminal carboxy- groups protected, was treated with 3 equivalents of N-Boc(2S,4S)-4-azidoproline in DMF in the presence of CuI. The product 5 was isolated after cleavage from the support. There was negligible difference in RP–HPLC tR but mass showed conjugation of proline unit through the triazole linkage (Scheme 2, Table 1, entry 5).
After the formation of the desired product was established, the solution phase reactions of PNA s with N-terminal azidoprol ine (2 and 4) or TANA (3) and N-terminal-alkyne substituted lysine peptide 1 and arginine-rich peptide 9, were carried out in tert. butanol: water medium in presence of CuSO 4 and sodium ascorbate as a catalyst (Scheme 3). The CuI catalyst was found to be less efficient as compared to CuSO 4-sodium ascorbate under these conditions. The reactions were followed by HPLC. The reaction took longer time for PNA, TANA substrates (16–32 h) to completion with- approximately one equivalent of each substrate. With approximately three equivalents excess of 1 or 9 complete conversion to the conjugated products (6–8 and 10, 11) was observed within 2 h. The DNA substrate 12 was converted to the peptide–DNA conjugate 14 within 1 h with 3 equivalents of the peptide (Scheme 4). Cu(1) catalysis is known to degrade DNA due to the presence of hydroxy radicals and addition of Cu(I) stabilizing ligands such as polytriazoles is often recommended to circumvent the DNA degradation (38). Arginine side chain imine nitrogen is also known to efficiently stabilize Cu(I) (43). It is therefore possible that the arginine peptide used in this reaction could have contributed to the stabilization of Cu(I) similar to the Cu(I)-binding ligands as small percentage of DNA degradation was observed. Additionally, presence of tert.-butanol as solvent in this reaction would circumvent DNA degradation as tert.-butanol is known scavenger of hydroxy radicals (44). The yields of peptide–DNA conjugates may be further improved by the use of Cu(I)-stabilizing ligands such as polytriazoles (38) along with tert.-butanol as hydroxy radical scavenger (44). The results are tabulated in Table 1 (entry 4, 7, 10, 13 and 14). The peptide–ON conjugates with either the DNA (14), aegPNA backbone (6, 8 and 11) or TANA backbone (7, 10) were obtained in high purity using this simple cycloaddition reaction. The reaction seems to be very specific without interfering with other functional groups present, either on peptide or DNA/ PNA/TANA segments of the conjugates.

CONCLUSIONS

The peptide–DNA/PNA/TANA conjugation chemistry presented in this paper will prove to be a unique solution for CPP conjugation to antisense/siRNA therapeutics. In this fast-developing field, application is mainly restrained by the problems faced in cellular delivery of modified ONs. The ease of conjugation and compatibility of the strategy with various substrates and functional groups will prove to be an attractive alternative to present methods for the synthesis of peptide–PNA conjugates. It is sequence-independent with respect to either peptide or PNA/DNA or other backbone modified DNA mimics (such as TANA), which are being developed for antisense applications. The reactive azide and alkyne groups can be installed on either ON or peptide and the work presented, has a potential for general application in nucleic acid–peptide conjugation. This could be a method of choice when a peptide of choice needs to be conjugated with several ON sequences or different modifications of the same sequence for studying their bioactivity. Alternatively, the chosen ON may be conjugated with several peptides to establish the biological efficacy of different peptides. Some recent work was carried out to evaluate the ON–peptide conjugates as a potential alternative to the use of transfection agents to improve the efficacy of siRNA (45). It will be very interesting to study the effects of the chemistry of this new linkage on the intracellular performance of the conjugates (9,19).

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

ACKNOWLEDGEMENTS

K.G. thanks CSIR, New Delhi and V.A.K. thanks DST, New Delhi and CEFIPRA, New Delhi for supporting this work. The initial work was presented as a poster in the Cell Penetrating Peptides meeting held at Telford, UK during 9–11 May, 2007. Funding to pay the Open Access publication charges for this article was waived by Oxford University Press.

Conflict of interest statement. None declared.

REFERENCES

1. Nielsen,P.E., Egholm,M., Berg,R.H. and Buchardt,O. (1991) Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide. Science, 254, 1497–1500.
2. Braasch,D.A. and Corey,D.R. (2002) Novel antisense and peptide nucleic acid strategies for controlling gene expression. Biochemistry, 41, 4503–4510.
3. Uhmann,E., Peymann,A., Breieohl,G. and Will,D.W. (1998) PNA: synthetic polyamide nucleic acids with unusual binding properties. Angew. Chem. Int. Ed. Engl., 37, 2796–2823.
4. Kurreck,J. (2003) Antisense technologies: improvement through novel chemical modifications. Eur. J. Biochem., 270, 1628–1644.
5. Micklefield,J. (2001) Backbone modification of nucleic acids: synthesis, structure and therapeutic applications. Curr. Med. Chem., 8, 1157–1179.
6. Kumar,V.A. (2002) Structural preorganization of peptide nucleic acids: chiral cationic analogues with five- or six-membered ring structures. Eur. J. Org. Chem., 2002–2032.
7. Kumar,V.A. and Ganesh,K.N. (2003) Conformationally constrained PNA analogs: structural evolution towards DNA/RNA binding selectivity. Acc. Chem. Res., 38, 404–412.
8. In Crooke,S.T. (ed), Antisense Research and Applications, Springer, Berlin, pp. 1–50.
9. Opalinska,J.B. and Gewirtz,A.M. (2002) Nucleic-acid therapeutics: basic principles and recent applications. Nat. Rev. Drug Discovery, 1, 503–515.
10. Dominski,Z. and Kole,R. (1993) Restoration of correct splicing in Thalassemic pre-mRNA by antisense oligonucleotides. Proc. Natl Acad. Sci. USA, 90, 8673–8677.
11. Turner,J.J., Fabani,M., Arzumanov,A.A., Ivanova,G. and Gait,M.J. (2006) Targeting the HIV-1 RNA leader sequence with synthetic oligonucleotides and si-RNA: chemistry and cell delivery. Biochim. Biophys. Acta, 1758, 290–300.
12. Manoharan,M. (2002) Oligonucleotide conjugates as potential antisense drugs with improved uptake, biodistribution, targeted delivery, and mechanism of action. Antisense Nucleic Acid Drug Dev., 12, 103–128.
13. Venkatesan,N. and Hyean Kim,B. (2006) Peptide conjugates of oligonucleotides: Synthesis and applications. Chem. Rev., 106, 3712–3761.
14. Fraley, A.W., Pons, B., Dalkara, D., Nullans, G., Behr, J.-P. and Zuber, G. (2006) Cationic oligonucleotide-peptide conjugates with aggregating properties enter efficiently into cells while maintaining hybridization properties and enzymatic recognition. J. Am. Chem. Soc., 128, 10763–10771.

15. Albertsbocher, K., Siwickowski, A.M., Wancewicz, E.V., Esau, C.C., Watanabe, T., Nishiura, K.C., Knitberger, G.A., Malik, L., Eldrup, A.B. et al. (2005) Structure-activity relationship study on a simple cationic peptide motif for cellular delivery of antisense peptide nucleic acid. J. Med. Chem., 48, 6741–6749.

16. Bendifallah, N., Rasmussen, F.W., Zachar, V., Ebbesen, P., Nielsen, P.E. and Koppelhus, U. (2006) Evaluation of cell-penetrating peptides (CPPs) as vehicles for intracellular delivery of antisense peptide nucleic acid (PNA). Bioconjugate Chem., 17, 750–758.

17. Maier, M.A., Esau, C.C., Siwickowski, A.M., Wancewicz, E.V., Albertsbocher, K., Kinberger, G.A., Kadaba, N.S., Watanabe, T., Manoharan, M. et al. (2006) Evaluation of basic amphipathic peptides for cellular delivery of antisense peptide nucleic acids. J. Med. Chem., 49, 2534–2542.

18. Sazanov, P., Kang, S.H., Maier, M.A., Wei, C., Dillman, J., Summerton, J., Manoharan, M. and Kole, R. (2001) Nuclear antisense effects of neutral, anionic and cationic oligonucleotide analogs. Nucleic Acids Res., 29, 3965–3974.

19. Rothbard, J.B., Kreider, E., VanDeusen, C.L., Wright, L., Wylie, B.L. and Wender, P.A. (2002) Arginine-rich, soluble translocators for drug delivery: role of backbone spacing in cellular uptake. J. Med. Chem., 45, 3612–3618.

20. Rothbard, J.B., Garlington, S., Lin, Q., Kirschberg, T., Kreider, E., McGrane, P.L., Wender, P.A. and Khavari, P.A. (2000) Conjugation of arginine oligomers to cyclosporin A facilitates topical delivery and inhibition of inflammation. Nat. Med., 6, 1253–1257.

21. Turner, J.J., Ivanova, G.D., Verbeure, B., Williams, D.A., Arzumanov, A., Abes, S., Lebleu, B. and Gait, M.J. (2005) Cell-penetrating peptide conjugates of peptide nucleic acids (PNA) as inhibitors of HIV-1 Tat-dependent trans-activation in cells. Nucleic Acids Res., 33, 6837–6849.

22. Diaz-Mochon, J.J., Bialy, L. and Bradley, M. (2004) Full orthogonality between Dde and Fmoc: the direct synthesis of PNA-peptide conjugates. Org. Lett., 6, 1127–1129.

23. Diaz-Mochon, J.J., Bialy, L., Wasten, J., Anchez-Martin, R.M. and Bradley, M. (2005) Synthesis and cellular uptake of cell delivering PNA-peptide conjugates. Chem. Commun., 3315–3318.

24. Kailhatu, K., Huffmann, K.E. and Corey, D.R. (2004) Intracellular uptake and inhibition of gene expression by PNA's and PNA-peptide conjugates. Biochemistry, 43, 14340–14347.

25. Mayfield, L.D. and Corey, D.R. (1999) Automated synthesis of peptide nucleic acid and peptide nucleic acid-PNA conjugates. Anal. Biochem., 268, 401–404.

26. Mier, W., Eritja, R., Mohammed, A., Haberkorn, U. and Eisenhut, M. (2003) Peptide-PNA conjugates: targeted transport of antisense therapeutics into tumors. Angew. Chem. Int. Ed. Engl., 42, 1968–1971.

27. Turner, J.J., Arzumanov, A.A. and Gait, M.I. (2005) Synthesis, cellular uptake and HIV-1 Tat-dependent trans-activation inhibition activity of oligonucleotide analogues disulphide-conjugated to cell-penetrating peptides. Nucleic Acids Res., 33, 27–42.

28. Marchan, V., Ortega, S., Pulido, D., Pedroso, F. and Grandas, A. (2006) Diels-Alder cycloadditions in water for the straightforward preparation of peptide oligonucleotide conjugates. Nucleic Acids Res., 34, e24.

29. Huisgen, R. (1984) In Padwa, A (ed), 1, 3-dipolar Cycloaddition Chemistry, Wiley, New York.

30. Tornae, C.W., Christensen, C. and Meldal, M. (2002) Peptidotriazoles on solid phase: [1,2,3]-triazoles by regiospecific copper(I)-catalyzed 1,3-dipolar cycloadditions of terminal alkynes to azides. J. Org. Chem., 67, 3057–3064.

31. Narayan, S., Muldoon, J., Finn, M.G., Fokin, V.V. and Kolb, H.C. and Sharpless, K.B. (2005) “On Water”: Unique reactivity of organic compounds in aqueous suspension. Angew. Chem. Int. Ed., 44, 3275–3279.

32. Kolb, H.C., Finn, M.G. and Sharpless, K.B. (2001) Click chemistry: diverse chemical function from a few good reactions. Angew. Chem. Int. Ed., 40, 2004–2021.

33. Bouillon, C., Meyer, A., Vidal, S., Jochem, A., Chevolot, Y., Cloarec, J.-P., Praly, J.-P., Vasseur, J.-J. and Morvan, F. (2006) Microwave assisted “Click” chemistry for the synthesis of multiple labeled-carbohydrate oligonucleotides on solid support. J. Org. Chem., 71, 4700–4702.

34. Meier, J.J., Mercer, A.C., Rivera, H.Jr. and Burkart, M.D. (2006) Synthesis and evaluation of bioorthogonal pantetheine analogues for in vivo protein modification. J. Am. Chem. Soc., 128, 12174–12184.

35. Wan, Q., Chen, J., Chen, G. and Danishefsky, S.J. (2006) A potentially valuable advance in the synthesis of carbohydrate-based anticancer vaccines through extended cycloaddition chemistry. J. Org. Chem., 71, 8244–8249.

36. Lin, P.-C., Ueng, S.-H., Tseng, M.-C., Ko, J.-L., Huang, K.-T., Yu, S.-C., Adak, A.K., Chen, Y.-J. and Lin, C.-C. (2006) Site-specific protein modification through Cu-catalyzed 1,2,3-triazole formation and its implementation in protein microarray fabrication. Angew. Chem. Int. Ed., 45, 4286–4290.

37. Nakane, M., Ishikawa, S. and Matsuda, A. (2005) Synthesis of cross-linked circular DNAs using Huisgen reaction. Nucleic Acids Symp. Ser., 49, 189–190.

38. Kumar, R., El-Sagheer, A., Tumpane, J., Lincoln, P., Wilmelsson, L.M. and Brown, T. (2007) Template-directed oligonucleotide strand ligation, covalent intramolecular DNA circularization and catenation using click chemistry. J. Am. Chem. Soc., 129, 6859–6864.

39. Gogoi, K., Gunjal, A. and Kumar, V.A. (2006) Sugar-thioacetic acid backbone in oligodeoxyribonucleosides for specific recognition of nucleic acids. Chem. Commun., 2373–2375.

40. Gogoi, K., Gunjal, A. and Phalune, U.D. and Kumar, V.A. (2007) Synthesis and RNA binding selectivity of oligonucleotides modified with five-atom TANA backbone structures. Org. Lett., 9, 2697–2700.

41. Gangamani, B.P., Kumar, V.A. and Ganesh, K.N. (1996) Synthesis of N4-(puinyl/pyrimidinyl acetyl)-4-aminopropionil diastereomers with potential use in PNA synthesis. Tetrahedron, 52, 15071–15030.

42. Nielsen, P.E. and Egholm, M. (1999) Peptide nucleic acids. In Nielsen, P.E. and Egholm, M. (eds), Protocols and Applications., Horizon scientific Norfolk, CT.

43. Bluhm, B. K., Shields, S. J., Bayse, C. A., Hall, M. B. and Russell, D. H. (2001) Determination of copper binding sites in peptides containing basic residues: a combined experimental and theoretical study. Int. J. Mass Spectrom., 204, 31–46.

44. Weller, R. L. and Rajski, S. R. (2005) DNA methyltransferase-mediated click chemistry. Org. Lett., 7, 2141–2144.

45. Turner, J.J., Jones, S., Fabani, M., Ivanova, G., Arzumanov, A.A. and Gait, M. (2007) RNA targeting with peptide conjugates of oligonucleotides, siRNA and PNA. Blood Cell. Mol. Dis., 38, 1–7.