Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters 14 (2004) 2927–2930
Bioorganic & Medicinal Chemistry Letters

Synthesis and DNA binding properties of dioxime–peptide nucleic acids ☆

Andriy Mokhir,[a,*] Roland Krämer,[a] Yan Z. Voloshin[b] and Oleg A. Varzatskii[b]

[a]*Anorganisch-Chemisches Institut, Ruprecht-Karls-Universität Heidelberg, Im Neuenheimer Feld 270, 69120 Heidelberg, Germany*
[b]*Karpov Institut of Physical Chemistry, 10 Vorontsovo Pole, 105064 Moscow, Russia*

Received 8 January 2004; revised 9 March 2004; accepted 12 March 2004

Abstract—Peptide nucleic acids (PNAs) C- or N-modified with dioxime ligands were prepared by solid-phase synthesis using iron(II)-clathrochelates as protected dioxime building blocks. These PNA bind complementary DNA sequence specifically, though with much reduced affinity in comparison with nonmodified PNA. The dioxime–PNA conjugates bind Cu^{2+} and Ni^{2+} at μM concentration.

Peptide nucleic acids (PNAs) are DNA analogues, in which the sugar–phosphate backbone has been substituted by N-(2-aminoethyl)-glycine units. PNAs advantages over DNAs include their higher chemical stability and affinity and specificity in binding of oligonucleotides. Therefore, PNA finds applications in analysis of nucleic acids and in control of gene expression in vitro[1] and in some cases in vivo.[2] Conjugation of metal complexes to termini of PNA has been used to alter optical and electronic properties of PNA,[3] for PNA attachment to surfaces[4] and more recently for tuning binding properties of PNA to DNA[5] and in DNA-templated metal catalysed reactions.[6]

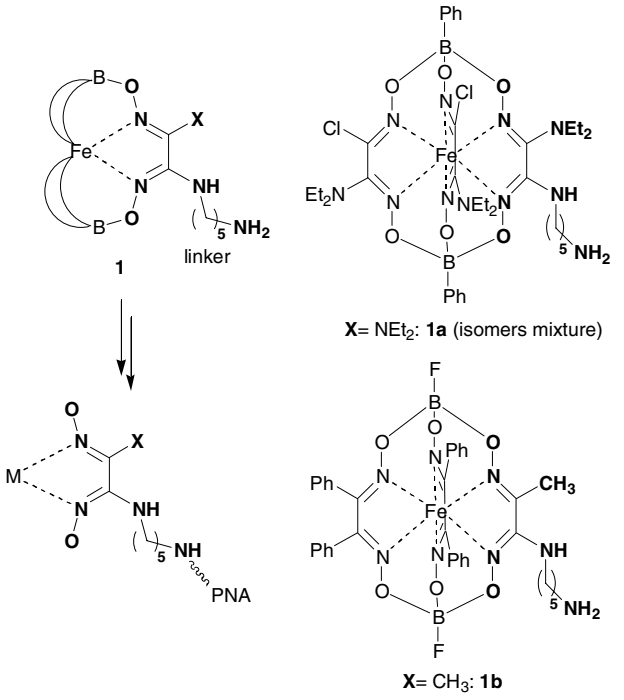
The aim of this study was to develop synthesis of new C- or N-terminally modified PNA–dioxime conjugates and their metal complexes. Conjugates of dioximes and their metal complexes with neither oligonucleotides nor their analogues were prepared up to date. Since dioximes form stable ML_2 and ML_3 complexes (with e.g., $M = Cu^{2+}$, Fe^{2+}),[7] the dioxime–PNA conjugates can potentially be organised in solution by metal ions into multi-stranded structures. Analogous compounds were prepared for ligand–DNA conjugates.[8] Such multi-stranded PNAs can be used as building blocks in preparation of nano-structures via their self-assembly in the presence of complementary oligonucleotides.

Attachment of dioximes to PNA requires protection of oxime groups, since they can be acylated during PNA synthesis in, for example, monomer coupling or final acylation with acetic anhydride (capping).[9] Since dioximes are strong ligands for 3d-metal ions,[7] they can be protected via metal complex formation. Metal ion coordination has been used before in protection of amino groups in, for example, α-amino acids or bleomycin,[12] but not in protection of oximes. Using a recently reported method, Fe^{2+} complexes (clathrochelates[10]) with a variety of unsymmetrically substituted dioximes having different '**X**' and 'linker' substituents (Scheme 1) may be obtained.[11] In these compounds the nitrogen atom of the oxime group is protected via coordination with Fe^{2+}, and the oxygen atom via co-valent interaction with the boron atom. The 'linker' component can serve for attachment of the complexes to PNA (Scheme 2). For these preliminary studies we have selected two examples of such protected dioximes: clathrochelates **1a** and **1b** with $\mathbf{X} = CH_3$, NEt_2 (Scheme 1). These compounds are fully cleaved by TFA/*m*-cresol (4/1) within ~4 min giving unprotected dioximes.

Cleavage of **1b** is shown in Figure 1. Importantly, after 90 min of the cleavage no products of degradation of the formed dioximes could be detected. Therefore, the dioxime fragment in clathrochelate–PNA conjugates can be fully and cleanly deprotected during standard PNA cleavage/deprotection step.[9] Moreover, clathrochelates

Keywords: Peptide nucleic acids; Oxime; Conjugate.
☆ Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2004.03.028
* Corresponding author. Tel.: +49-6221-54-8458; fax: +49-6221-54-8439; e-mail: andriy.mokhir@urz.uni-heidelberg.de

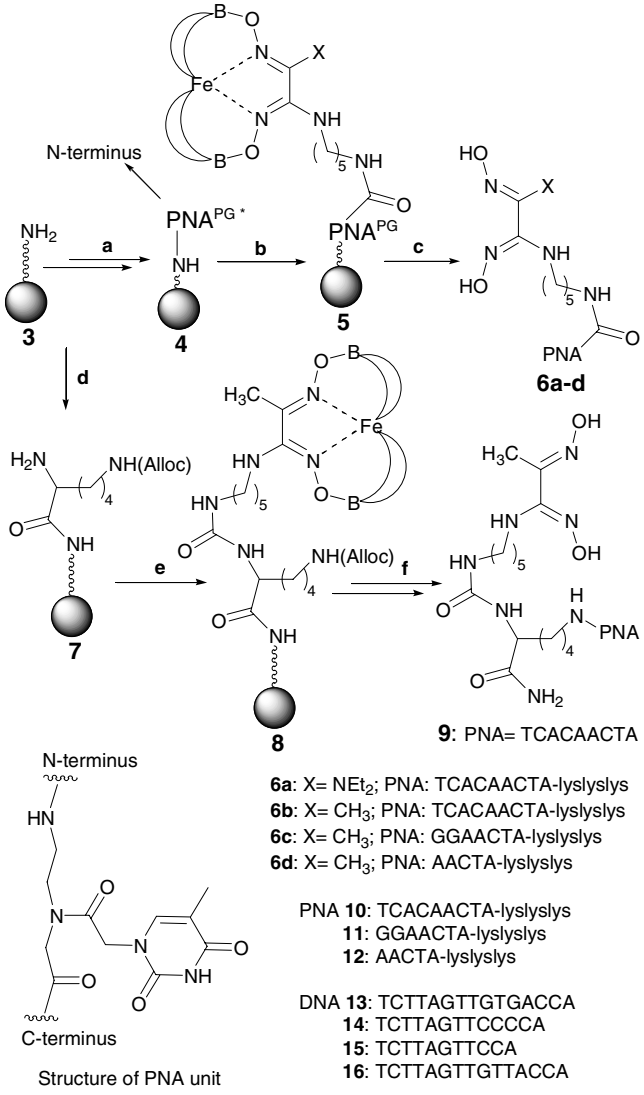
0960-894X/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2004.03.028



Scheme 1. Structures of clathrochelates, which were used for synthesis of dioxime–PNA conjugates; M: metal ion—Cu^{2+}, Ni^{2+}, Zn^{2+}. Complexes **1a** and **1b** are shown schematically in the reaction scheme.

1a and **1b** are stable in the presence of piperidine (conditions of Fmoc-group cleavage) and Ac_2O/2,6-lutidine (capping step in PNA synthesis, data not shown).[9] This favourably distinguishes our protection scheme from the one based on oxime acylation. In particular, acylated oximes are only partially deprotected in TFA and, moreover, they are very sensitive to nucleophiles.[13] Compounds **1a** and **1b** were attached to the N-terminus of PNA via urea group using CDI as a coupling reagent (Scheme 2). After final PNA cleavage and deprotection the desired dioxime–PNAs (**6a**–**d**) were obtained in high yields as it was shown by analysis of the reaction mixtures by HPLC and MALDI-TOF mass spectrometry. To show generality of this reaction it was reproduced for three different PNAs using **1b** clathrochelate (**6b**–**d**). C-(dioxime)–PNA could be synthesised by a modified method. In particular, orthogonally protected lysine was coupled to Rink-resin using HBTU activator, then, after deprotection of α-amino-group, **1b** was attached using CDI. Finally, ε-amino group of the lysine was deprotected in the presence of $Pd(PPh_3)_4$, PNA synthesised off this amino group and the resulting conjugate cleaved from the resin and deprotected under standard conditions[9] (**9**, Scheme 2).

The dioxime–PNA conjugates (**6a**–**c**) bind their complementary DNA significantly weaker in comparison with unmodified DNA. This is reflected in strongly decreased T_m's of the corresponding duplexes ($\Delta T_m = -21.5\,^{\circ}C$, Table 1). One can speculate that the possible reason of this effect is stabilisation of a single-stranded form of the PNAs by the dioxime group. Binding of the dioxime–PNAs to DNA is sequence



Scheme 2. Synthesis of dioxime–PNA conjugates: (a) PNA synthesis; (b) (1) CDI, (2) **1a** or **1b**; (c) TFA, *m*-cresol; (d) Fmoc-Lys(ε-Alloc)-OH, HBTU, HOBT, DIEA; (e) (1) CDI, (2) **1b**; (f) (1) PNA synthesis, (2) TFA, *m*-cresol. (*) PG = protecting group, Bhoc for PNA bases: A, C and G and Boc for ε-NH_2 of terminal lysin residues.

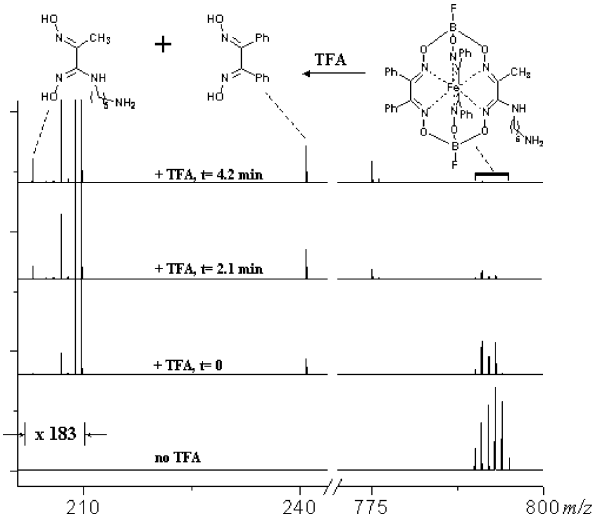


Figure 1. Monitoring of cleavage of **1b** in TFA by ESI-MS.

Table 1. UV melting points of PNA–DNA duplexes[a]

No.	PNA–DNA	T_m (°C)				Ref.
		No metal	Zn^{2+}	Ni^{2+}	Cu^{2+}	
1	**6a**:**13**	40.5 ± 1.3	39.5 ± 1.4	39.6 ± 0.9	40.2 ± 1.1	[b]
2	**6b**:**13**	39.9 ± 1.2	39.7 ± 0.9	40.0 ± 0.7	39.2 ± 0.7	[b]
3	**6b**:**16**	33.5 ± 1.5	34.0 ± 1.2	33.0 ± 2.1	32.9 ± 1.9	[b]
4	**6c**:**14**	35.9 ± 2.0	34.2 + 0.8	34.1 ± 1.0	35.0 ± 1.1	[b]
5	**6d**:**15**	<15	<15	<15	<15	[b]
6	**9**:**13**	[c]	[c]	[c]	[c]	[b]
7	**10**:**13**	61.4 ± 0.8	60.3 + 1.2	61.5 ± 0.7	59.6 ± 0.7	5
8	**10**:**16**	52.9 ± 2.3	—	—	—	14
9	**11**:**14**	55.9 ± 0.7	—	—	—	14
10	**12**:**15**	<15	—	—	—	14

[a] Average of at least four melting points ± SD at 2 μM (**6a**–**d**, **10**–**16**) strand concentration, MOPS pH 7 10 mM, NaCl 50 mM, $[M^{2+}] = 2$ μM.
[b] This work.
[c] No transition.

specific, as it is evident from 6.4 °C decrease of the T_m for the duplex of PNA **6b** with DNA **16** having a single mismatch base in its interior (Table 1, entry 3). Notwithstanding destabilising effect of the dioxime group, the conjugates with longer PNA strands (PNA **6b**,**c**) are fully bound to their complementary DNAs at room temperature (Table 1, entries 2 and 4). Surprisingly, affinity of the PNA conjugates towards the complementary DNAs is not affected by the nature of substituent **X** (Scheme 2) at the C-atom of the dioxime group (Table 1, entries 1 and 2). In the presence of equivalent amounts of divalent metal ions (Zn^{2+}, Ni^{2+}, Cu^{2+}), T_m's of the duplexes are not changed as well. This may indicate that either a metal complex of the dioxime does not interact with the DNA in the PNA–DNA duplex or the metal ions do not bind the ligand at our experimental conditions. The latter possibility could be excluded at least for Ni^{2+} and Cu^{2+}, since in MALDI-TOF mass spectra of mixtures containing equimolar amounts (μM) of the corresponding metal ions and PNA **6b**, peaks of M^{2+}–PNA complexes were observed. The complexes of Zn^{2+} are sensitive to acidic conditions of probe preparation for MALDI-TOF mass spectrometry, therefore, they could not be detected. In MALDI-TOF mass spectra of analogous mixtures containing nonmodified PNA **10** in place of PNA **6b** no peaks corresponding to M^{2+}–PNA are seen, which indicates that the dioxime group rather than PNA donor atoms binds metal ions.

Studies of formation of ML_2 and ML_3 complexes of the PNA–dioxime conjugates with 3d-metal ions will be reported separately.

In summary, we have developed an efficient and versatile method for conjugation of dioxime ligands to both termini of PNA. The dioxime–PNA conjugates bind their DNA targets sequence specifically, though with much reduced affinity in comparison with nonmodified PNA.

Notes: HPLC purification of PNA: HPLC was on EC 250 × 4.6 mm Nucleosil 300-5 C4 column at 45 °C. Gradients of 0.1% trifluoroacetic acid (TFA) in CH_3CN (solvent B) in 0.1% TFA in water (solvent A) were used: 5 min 0% B, in 23 min to 20% B, in 7 min to 95% B, 10 min at 95% B. Characterisation data for **6a**: Yield 7.3%; MALDI-TOF MS for $C_{123}H_{175}N_{62}O_{31}$ $[M+H]^+$: calcd 3016.4, found 3015.0; **6b**: yield 9.4%; MALDI-TOF MS for $C_{126}H_{182}N_{63}O_{31}$ $[M+H]^+$: calcd 3073.5, found 3074.7; **6c**: yield 7.7%; MALDI-TOF MS for $C_{103}H_{148}N_{55}O_{25}$ $[M+H]^+$: calcd 2555.2, found 2556.1; **6d**: yield 4.9%; MALDI-TOF MS for $C_{81}H_{122}N_{41}O_{19}$ $[M+H]^+$: calcd 1973.0, found 1974.7; **9**: yield 2.1%; MALDI-TOF MS for $C_{111}H_{151}N_{58}O_{29}$ $[M+H]^+$: calcd 2760.2, found 2759.7; probe preparation for MALDI-TOF MS: a mixture of analyte (1 μL) and matrix (sinnapinic acid, 6 mg/mL in 0.1% trifluoroacetic acid in $H_2O/CH_3CN/CH_3OH$ 1/1/1, 2 μL) was prepared on the stainless steel plate and allowed to dry at 0.01 mbar. UV-melting points: heating and cooling rates −0.5 °C/min, pre-equilibration: 5 min at 90 °C, in 70 min from 90 to 20 °C.

Supporting information: Description of synthesis of **1a** and **1b**.

Acknowledgements

We would like to thank Ruprecht-Karls-Universitaet Heidelberg and Ministerium für Wissenschaft, Forschung und Kunst Baden-Württemberg for financial support and Helena Wolf for help with synthesis and measurements of melting points.

References and notes

1. Nielsen, E.; Egholm, M. In *Peptide Nucleic Acids Protocols and Applications*; Nielsen, P. E., Egholm, M., Eds.; Horizon Scientific: England, 1999; pp 1–19.
2. Koppelhus, U.; Nielsen, P. E. *Adv. Drug. Deliv. Rev.* **2003**, *55*, 267–280.
3. Verheijen, J. C.; van der Marel, G. A.; van Boom, J. H.; Metzler-Nolte, N. *Bioconjug. Chem.* **2000**, *11*, 741–743; Bigey, P.; Soennichsen, S. H.; Meunier, B.; Nielsen, P. E. *Bioconjug. Chem.* **1997**, *8*, 267–270.
4. Orum, H.; Nielsen, P. E.; Joergensen, M.; Larsson, C.; Stanley, C.; Koch, T. *BioTechniques* **1995**, *19*, 472–480.
5. Mokhir, A.; Stiebing, R.; Krämer, R. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1399–1401.

6. Brunner, J.; Mokhir, A.; Krämer, R. *J. Am. Chem. Soc.* **2003**, *125*, 12410–12411.
7. Data for dimethylglyoxime from ScQuerry Vn 4.06, IUPAC stability constants database.
8. Takenaka, S.; Funatu, Y.; Kondo, H. *Chem. Lett.* **1996**, *1*, 891–892.
9. *Expedite™ 8900 PNA Chemistry User's Guide*, PerSeptive Biosystems, Inc., **1998**, Rev. 2, USA.
10. Voloshin, Y. Z.; Kostromina, N. A.; Krämer, R. *Clathrochelates: Synthesis, Structure and Properties*; Elsevier: Amsterdam, The Netherlands, 2002.
11. Voloshin, Y. Z.; Zavodnik, V. E.; Varzatskii, O. A.; Belsky, V. K.; Palchik, A. V.; Strizhakova, N. G.; Vorontsov, I. I.; Antipin, M. Y. *J. Chem. Soc., Dalton Trans*. **2002**, 1193–1202.
12. *Protecting Groups in Organic Synthesis*; Green, T. W., Wuts, P. G. M., Eds.; John Wiley and Sons: USA, 1999.
13. Oxime esters are cleaved by, for example, hydrazine: Voyer, N.; Lavoie, A.; Pinette, M.; Bernier, J. *Tetrahedron Lett.* **1994**, *35*, 355–358; and amines: Lumma, W. C.; Witherup, K. M.; Tucker, T. J.; Brady, S. F.; Sisko, J. T.; Naylor-Olsen, A. M.; Lewis, S. D.; Lucas, B. J.; Vacca, J. P. *J. Med. Chem.* **1998**, *41*, 1011–1013.
14. Mokhir, A.; Kraemer, R. *Bioconjug. Chem.* **2003**, *14*, 877–883.