Bioorganic & Medicinal Chemistry Letters 19 (2009) 3811–3815

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and evaluation of a netropsin–proximicin-hybrid library for DNA binding and cytotoxicity

Falko E. Wolter [a], Lise Molinari [a], Elke R. Socher [b], Kathrin Schneider [a], Graeme Nicholson [c], Winfried Beil [d], Oliver Seitz [b], Roderich D. Süssmuth [a,*]

[a] FG Organische Chemie, Institut für Chemie, Technische Universität Berlin, Straße des 17. Juni 124, 10623 Berlin, Germany
[b] Institut für Chemie, Humboldt Universität Berlin, Brook-Taylor-Str. 2, 12489 Berlin, Germany
[c] Institut für Chemie, Eberhard Karls Universität Tübingen, Auf der Morgenstelle 18, 72076 Tübingen, Germany
[d] Institut für Pharmakologie, Medizinische Hochschule Hannover, Carl-Neuberg-Str. 1, 30625 Hannover, Germany

ARTICLE INFO

Article history:
Received 15 February 2009
Revised 7 April 2009
Accepted 8 April 2009
Available online 18 April 2009

Keywords:
Netropsin–proximicin-hybrid
DNA binding
Cytotoxicity

ABSTRACT

The proximicins A–C (**1**–**3**) are novel naturally occurring γ-peptides with a hitherto unknown 2,4-disubstituted furan amino acid as a core structure. They show a moderate cytotoxic activity and induce upregulation of cell cycle regulating proteins (p53 and p21) and lead to cell cycle arrest in G0/G1-phase. Hybrid molecules combining structural motifs of the proximicins and of netropsin (**4**), a structurally related natural product, seem to have similar effects. Herein we describe the synthesis of a netropsin–proximicin-hybrid library and its evaluation regarding cytotoxicity and minor groove binding activity.

Proximicin A, B, and C (**1**–**3**) are natural occurring γ-peptides which were isolated from a marine Actinomycete strain of the genus *Verrucosispora* (Fig. 1).[1,2] As a main structural feature these compounds bear a hitherto unknown 2,4-disubstituted furan amino acid as a dipeptide core. This heterocyclic core is structurally closely related to the prominent natural products netropsin (**4**) and distamycin (**5**) (Fig. 1).[3–5] These were arguably the first compounds for which AT-selective DNA minor groove binding was demonstrated.[6] Furthermore, these compounds served as scaffolds for a large variety of synthetic minor groove binding polyamides capable to specifically recognize DNA sequences.[6–14] In contrast, no DNA binding activity could be shown for the proximicins.[2] Furthermore, cell cycle analysis and analysis of the level of cell cycle regulating proteins (p53, p21, and cyclinE) in comparison with distamycin revealed that the proximicins might have a different cellular target than netropsin and distamycin despite their close structural relationship.[2] Similar results were obtained with a first set of synthetic netropsin–proximicin-hybrids (**6–8**) which combine structural features of both natural products (Fig. 1).[2] This led to the assumption that the difference in the biological activity between the proximicins (**1–3**) and netropsin (**4**) is caused by the different N- and C-terminal residues attached to the central dipeptide.

Toward this end, we synthesized a 19-membered library of netropsin–proximicin hybrid molecules incorporating various N- and C-terminal modifications attached to the dipeptide core of netropsin and examined the cytotoxicity as well as potential minor groove binding of these derivatives. We reported previously, that netropsin–proximicin-hybrid C (**8**) is the most active hybrid.[2] As a consequence, its N-terminal methyl carbamate and its C-terminal tryptamine served as template for the synthesis of a second generation of hybrids. The herein presented hybrid molecules can be divided into two subsets: One group bears the methyl carbamate as a constant element but differs in C-terminal modifications (**13a–i**). Several biogenic amines, amino acids, and an ester function were introduced at this position (Table 1). The other subset of hybrids consists of members possessing the tryptamine moiety at the C-terminus but different N-terminal residues (**16a–j**), like alkyl or aryl carbamates, urea derivatives, and acylations (Table 2).

With these modifications at the N- and C-termini of the *N*-methylpyrrol dipeptide the influence of different electronic and steric effects on the biological activity should be determined.

The synthesis of all netropsin–proximicin-hybrids (**13**, **16**) starts off with commercially available compound **9** (Schemes 1 and 2). The various modifications in the respective subset of the hybrid molecules were introduced in the final reaction step in order to simplify the overall synthesis. The amide bonds of all hybrids were mainly synthesized by peptide couplings

* Corresponding author.
E-mail address: suessmuth@chem.tu-berlin.de (R.D. Süssmuth).

0960-894X/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2009.04.042

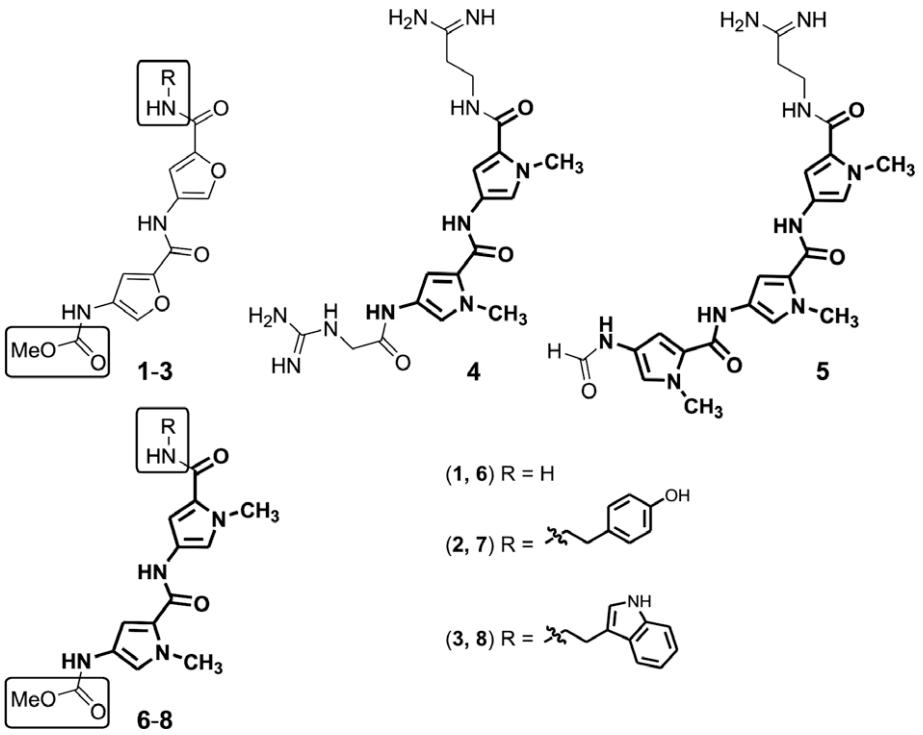


Figure 1. Structures of proximicin A–C (**1–3**), netropsin (**4**), distamycin (**5**), and of the three netropsin–proximicin-hybrids A–C (**6–8**).

applying an optimized protocol using EDCI and DMAP as activating reagents.[15] This allows an easy purification by simple acid/base liquid–liquid extracting procedures in order to avoid column chromatography. Thus obtained products were pure enough for the subsequent reaction steps.[16] Only after the last reaction step all compounds were purified by RP-HPLC for biological evaluation.[17]

The antitumor activity of **13a–i** and **16a–j** was evaluated against three carcinoma cell lines up to a maximum concentration of 20 μg/mL.[18] The growth inhibition (GI_{50}) values of the compounds which were cytotoxic below this concentration are shown (Table 3). The corresponding data of the proximicins (**1–3**) and of netropsin–proximicin-hybrid C (**8**) are also given in comparison.[2] Whereas the antitumor activity of the hybrids (**13i**, **16c–f**, **h**, **j**) listed in Table 3 is higher or at least of the same order as hybrid C (**8**), all other new synthesized derivatives (**13a–h**, 16a+b, 16g, 16i) did not show a noteworthy antitumor activity.

Remarkably, ester **13i** is the only active compound from the subset of C-terminally modified hybrids. Its cytoxicity is significantly higher than that of hybrid C (**8**) and comparable to that of the proximicins (**1–3**). The subset of N-terminal modifications consists beside the derivative with the free amino group (**16a**) out of carbamates (**16b–f**), ureas (**16i+j**), and acylated derivatives (**16g+h**). Active derivatives can be found in all three chemical classes, which are **16c–f**, **16h**, and **16j** showing a moderate cytotoxicity (Table 3).

The results give an estimate of the influence of N- and C-terminal modifications on the cytotoxicity of the netropsin–proximicin-hybrids. Compound **13i**, the only active hybrid out of the subset of C-terminal modifications, reveals that an ester function at the C-terminus raises the activity in comparison to an amide function. This structural modification might have two distinctive influences. One possibility is a shift from a hydrogen bond donor (NH-function of an amide) to a hydrogen bond acceptor (O-atom of an ester). Alternatively, the ester moiety lowers the overall polarity of the molecule and might simplify penetration of the cell membrane.

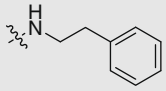
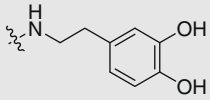
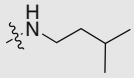
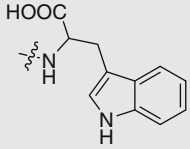
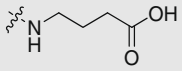
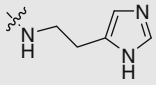
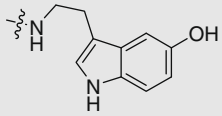
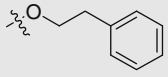
With regard to antitumor activity a great variety of N-terminal modifications is tolerated. The comparison of active with inactive members of this subset reveals subtle differences in the effects caused by the different residues. Among the carbamates it seems that a methylene group at the α-position next to the oxygen is mandatory for biological activity. This is confirmed by the inactive *tert*-butyl carbamate **16b**. But steric effects in the carbamate part appear negligible with regard to the bulky residues of **16d–f**.

Whereas **16e**, a benzyl carbamate, shows a growth inhibiting activity of tumor cells the corresponding urea derivative **16i** is inactive. Again, this corresponds to a shift from a hydrogen bond acceptor (O-atom, **16e**) to a hydrogen bond donor (NH, **16i**). Interestingly derivative **16j**, which lacks a methylene group compared with derivative **16i**, displays a moderate cytotoxicity. In case of an acetylation of the N-terminus (**16g**) no antitumor activity was observed and only a moderate cytotoxicity for the corresponding pivaloyl derivative (**16h**). These data support the view that an overall non-polar structure of the molecule appears to be beneficial for cytotoxicity.

The stem compounds **13a** and **16a** reveal that hybrids with a polar free N-terminus or C-terminus lack cytotoxic activity. Neither the derivative with free amino group (**16a**) nor with the carboxylic acid (**13a**) displays cytotoxic effects. Other polar functional groups like phenols (**13c**, **h**) or carboxylic acids (**13e**, **f**) in the C-terminal residues seem to prevent an activity, too.

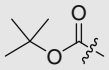
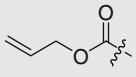
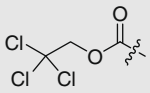
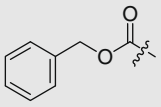
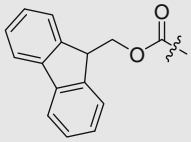
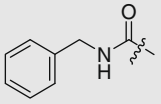
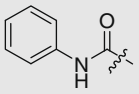
Based on the data obtained from cytotoxicity measurements, we were interested in investigating the potential for minor groove binding activity of our synthetic derivatives. The library

Table 1
Structures, reaction conditions and yields of the C-terminally modified library members (Scheme 1)

Compd	Structure, R=[a]	Reagents, conditions	Yield[b]
13a	OH	H_2/Pd	—
13b		Phenylethylamine[c]	8 mg, 12%
13c		Dopamine[c]	5 mg, 8%
13d		Isoamylamine[c]	18 mg, 29%
13e		H-L-Trp-OMe·HCl[d]	4 mg. 5%
13f		H-GABA-OMe·HCl[d]	6 mg, 10%
13g		Histamine[e]	6 mg, 10%
13h		Serotonine[c]	24 mg, 32%
13i		Phenylethanol[c]	17 mg, 26%

[a] Referring to compound **13** in Scheme 1.
[b] After purification by preparative RP-HPLC; conditions (cf. Scheme 1).
[c] EDCI/DMAP, DMF, 16 h, rt.
[d] (1) EDCI/DMAP, DMF, 16 h, rt; (2) 0.1 N LiOH, rt.
[e] PyBOP, DIPEA, DCM/DMF, 0 °C→rt.

Table 2
Structures, reaction conditions and yields of the N-terminally modified library members (Scheme 2)

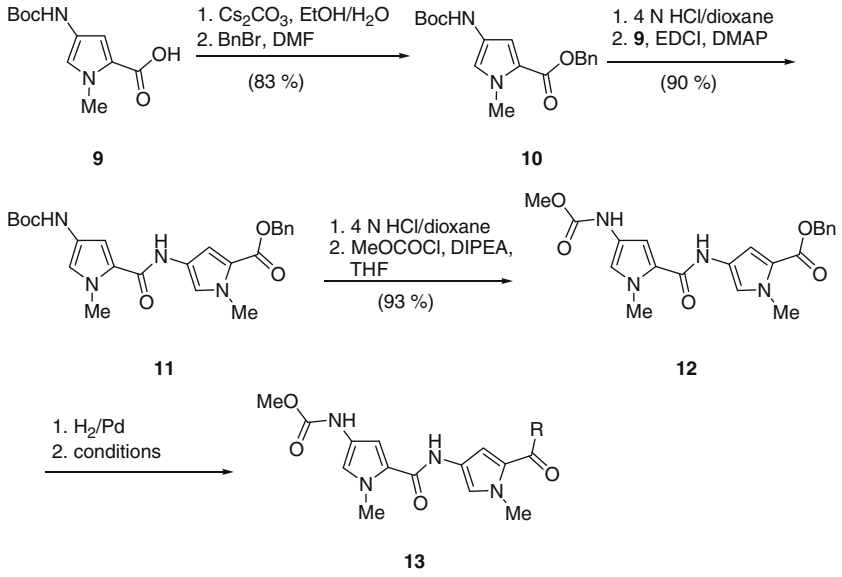
Compd	Structure, R=[a]	Reagents, conditions	Yield[b]
16a	H	—	—
16b		Boc_2O[c]	7 mg, 12%
16c		Alloc-Cl[d]	13 mg, 23%
16d		Troc-Cl[d]	8 mg, 13%
16e		Cbz-Cl[d]	8 mg, 13%
16f		Fmoc-OSu[d]	9 mg, 13%
16g		AcCl[d]	11 mg, 22%
16h		Piv-Cl[d]	6 mg, 12%
16i		BIC[d]	9 mg, 15%
16j		PIC[d]	6 mg, 10%

[a] Referring to compound **16** in Scheme 2.
[b] After purification by preparative RP-HPLC; conditions (cf. Scheme 2).
[c] 1.3 equiv reagent, $NaHCO_3$, dioxane/H_2O, rt.
[d] 1.0 equiv reagent, TEA, THF, rt.

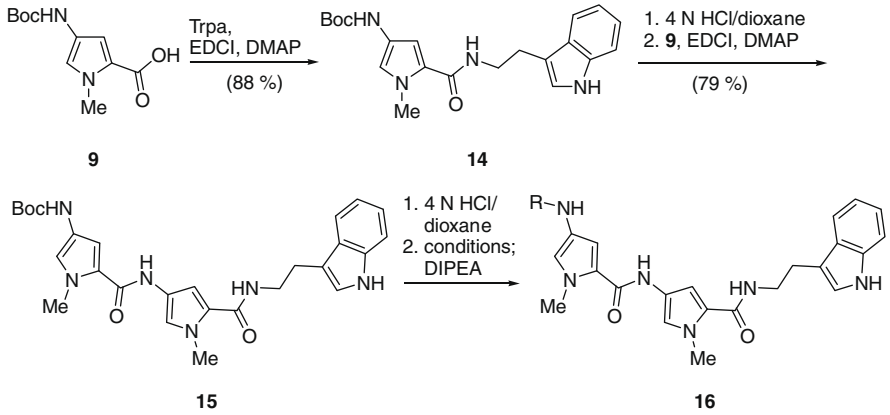
members of both subsets were incubated in various concentrations with an AT-rich (cg*caaatttg*cg) and with a GC-rich (cat*ggcc*atg) oligonucleotide (Table 4, only compounds are shown which display DNA interactions).[19] Within our test panel we found that not all compounds displaying cytotoxicity also showed DNA binding capabilities. Incubation with **13i** and **16j** result in a shift of T_m by 25 °C and 6 °C, which is selective for AT-rich DNA. In contrast **16d** and **e** lead to a stabilizing effect of both AT-rich and GC-rich DNA sequences. Their stabilizing effect is even stronger for GC-rich DNA sequence represented by a shift in T_m of 21 °C (**16d**) and 23 °C (**16e**), respectively. It seems that both compounds unspecifically bind into the minor groove of DNA.

In summary, we presented the synthesis and biological evaluation of new netropsin–proximicin-hybrids (Fig. 2). Compared to the first generation of hybrids[2] the antitumor activity of some new hybrids could be increased to the same order of magnitude of proximicin A, B, and C. In addition we were able to generate a set of new minor groove binders based on the core structure of netropsin. These DNA binding agents partially show selectivity for an AT-rich oligonucleotide and lead to a very strong stabilization of the double helix as determined by DNA melting point analyses.

The netropsin–proximicin-hybrids are accessible by a simple synthetic methodology. Further work is directed to the synthesis of other hybrids based on **13i** as a lead structure in order to exam-



Scheme 1. Synthesis of the subset with C-terminal modifications. Conditions and yields of the ultimate reaction step are shown in Table 1.



Scheme 2. Synthesis of the subset with N-terminal modifications. Conditions and yields of the ultimate reaction step are shown in Table 2.

Table 3
Antitumor activities of selected compounds against three carcinoma cell lines

Compounds	AGS[a] GI_{50}[b] (μM)	HepG2[a] GI_{50}[b] (μM)	MCF7[a] GI_{50}[b] (μM)
Proximicin A (**1**)	2.0[c]	2.8[c]	24.6[c]
Proximicin B (**2**)	3.6[c]	23.0[c]	12.1[c]
Proximicin C (**3**)	0.6[c]	1.8[c]	20.6[c]
Hybrid C (**8**)	17.7[c]	21.6[c]	24.9[c]
13i	2.8	2.0	13.0
16c	1.7	14.3	15.4
16d	10.0	4.0	10.3
16e	9.3	5.6	10.8
16f	>31.9[d]	4.6	18.8
16h	23.5	>40.9[e]	17.4
16j	16.8	14.3	19.7

Inactive derivatives (GI_{50} > 20 μg/mL) are not shown.
[a] Cell lines: AGS: gastric adenocarcinoma; HepG2: hepatocellularcarcinoma, MCF7: breast adenocarcinoma.
[b] GI_{50}: 50% growth inhibition.
[c] Data obtained from literature (Ref. 2).
[d] 47% inhibition at 20 μg/mL.
[e] 37% inhibition at 20 μg/mL.

Table 4
DNA binding of netropsin (**4**), distamycin (**5**) and of **13i** and **16d**, **e**, **j** (values given in °C)

Compounds	oligonucleotide 1 cg*caaatttg*cg			oligonucleotide 2 cat*ggccatg*		
	T_m[a] 0 μM	T_m[a] 6 μM	ΔT_m[b]	T_m[a] 0 μM	T_m[a] 6 μM	ΔT_m[b]
Netropsin (**4**)	51[c]	69[c]	18	46[c]	48[c]	~0
Distamycin (**5**)	49[c]	65[c]	16	46[c]	45[c]	~0
13i	47	71	25	47	47	0
16d	48	59	11	47	68	21
16e	48	65	17	47	70	23
16j	47	53	6	47	45	~0

Other synthetic derivatives showed no DNA binding.
[a] T_m: melting point, given T_m-value is the mean of at least three denaturation curves.
[b] Differences in T_m of pure oligonucleotide and after adding 6 μM of ligand to 2 μM oligonucleotide.
[c] Data obtained from the literature (Ref. 2).

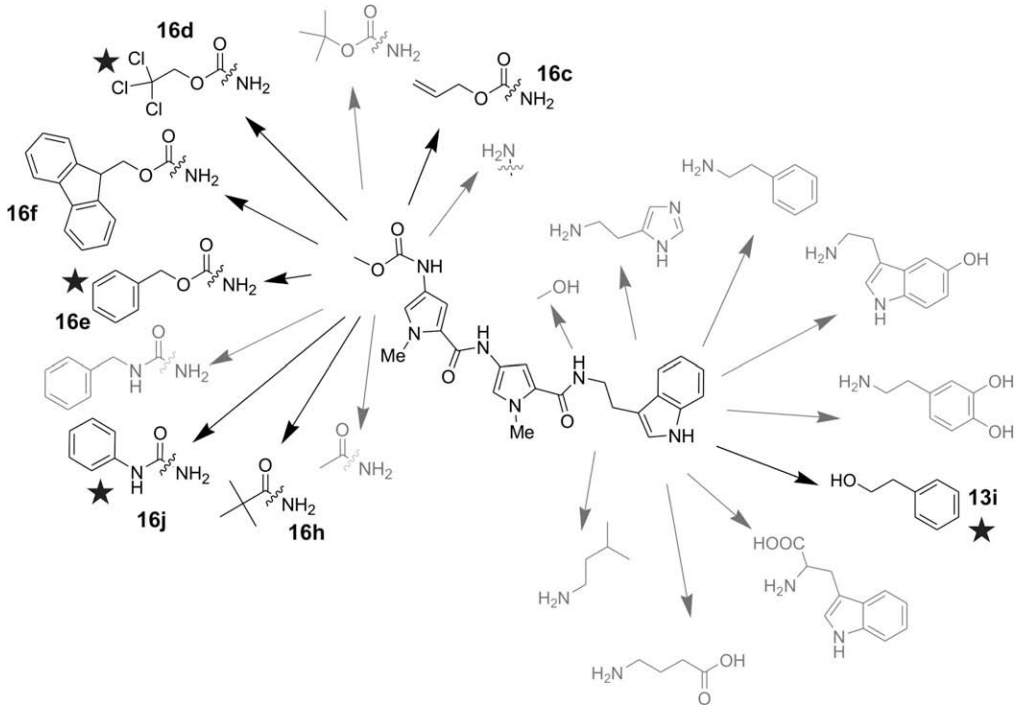


Figure 2. Overview of synthesized netropsin–proximicin-hybrids. Structures of compounds with a cytotoxicity <20 μg/mL are shown in solid black; structures of compounds with a cytotoxicity >20 μg/mL are given in light grey; structures of compounds capable to interact with double-stranded DNA are marked with an asterisk.

ine the influence of various N- and C-terminal residues on biological activity more in detail.

Acknowledgment

The work was supported by the Deutsche Forschungsgemeinschaft (DFG project SU 239-5/1) and by the Cluster of Excellence 'Unifying Concepts in Catalysis' coordinated by the Technische Universität Berlin and funded by the DFG.

References and notes

1. Fiedler, H. P.; Bruntner, C.; Riedlinger, J.; Bull, A. T.; Knutsen, G.; Goodfellow, M.; Jones, A.; Maldonado, L.; Pathom-Aree, W.; Beil, W.; Schneider, K.; Keller, S.; Süssmuth, R. D. *J. Antibiot.* **2008**, *61*, 158.
2. Schneider, K.; Keller, S.; Wolter, F. E.; Roeglin, L.; Beil, W.; Seitz, O.; Nicholson, G.; Bruntner, C.; Riedlinger, J.; Fiedler, H. P.; Süssmuth, R. D. *Angew. Chem., Int. Ed.* **2008**, *47*, 3258.
3. Finlay, A. C.; Hochstein, F. A.; Sobin, B. A.; Murphy, F. X. *J. Am. Chem. Soc.* **1951**, *73*, 341.
4. Arcamone, F.; Bizioli, F.; Canevazzi, G.; Grein, A. DE 1039198, 1957.
5. Arcamone, F.; Penco, S.; Orezzi, P.; Nicolella, V.; Pirelli, A. *Nature* **1964**, *203*, 1064.
6. Zimmer, C.; Wahnert, U. *Prog. Biophys. Mol. Biol.* **1986**, *47*, 31.
7. Wemmer, D. E. *Ann. Rev. Biophys. Biomol. Struct.* **2000**, *29*, 439.
8. Suckling, C. J. *Exp. Opin. Therap. Pat.* **2004**, *14*, 1693.
9. Pindur, U.; Jansen, M.; Lemster, T. *Curr. Med. Chem.* **2005**, *12*, 2805.
10. Bailly, C.; Chaires, J. B. *Bioconjug. Chem.* **1998**, *9*, 513.
11. Dervan, P. B. *Bioorg. Med. Chem.* **2001**, *9*, 2215.
12. Dervan, P. B.; Poulin-Kerstien, A. T.; Fechter, E. J.; Edelson, B. S. *Top. Curr. Chem.* **2005**, *253*, 1.
13. Reddy, B. S. P.; Sharma, S. K.; Lown, J. W. *Curr. Med. Chem.* **2001**, *8*, 475.
14. Reddy, B. S. P.; Sondhi, S. M.; Lown, J. W. *Pharmacol. Therap.* **1999**, *84*, 1.
15. Boger, D. L.; Fink, B. E.; Hedrick, M. P. *J. Am. Chem. Soc.* **2000**, *122*, 6382.
16. All new compounds were fully characterized by means of ^{1}H and ^{13}C NMR and HRMS.
17. Substantial mass-losses were observed after purification by RP-HPLC.
18. GI_{50}-values were determined using methods described in Ref. 2.
19. DNA melting analysis was performed using methods described in Ref. 2.