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Total Synthesis of Proximicin A−C and Synthesis of New Furan-Based DNA Binding Agents

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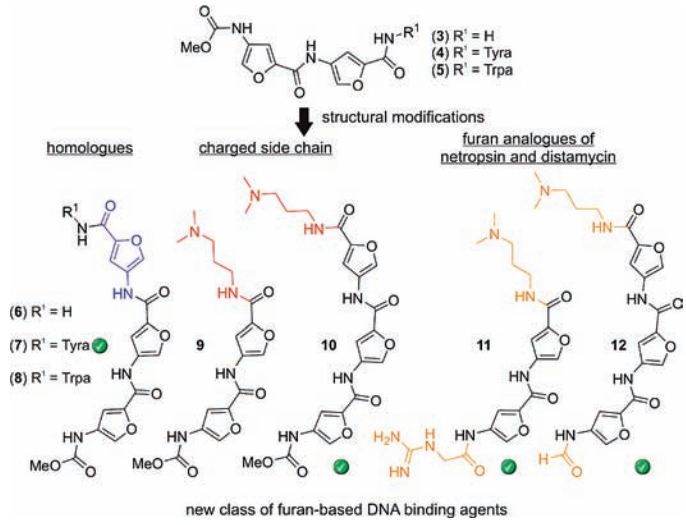
ABSTRACT



The total synthesis of the natural occurring polyamides proximicin A−C (3−5) has been accomplished. A short and efficient synthesis of a thus far unknown 4-amino-2-furan carboxylic acid was developed. Furthermore, this unique heterocyclic γ-amino-acid was used for the synthesis of a new class of AT-selective DNA-binding agents derived from the natural products combining structural features of the proximicins with those from the known DNA-binding natural products netropsin (1) and distamycin (2).

The polyamide antibiotics netropsin (**1**) and distamycin (**2**) synthesized by *Streptomyces* were arguably the first natural products for which AT-selective DNA binding was demonstrated (Figure 1).[1,2] Characteristic features of peptides **1** and **2** are the *N*-methyl pyrrol core amino acid and the modification with charged amidino or guanidino functions. Using these structures as a molecular scaffold, a considerable number of synthetic DNA ligands has been derived from these natural products.[2] A lot of effort has been made incorporating various other heterocyclic amino acids into the peptide backbone.[2b,f]

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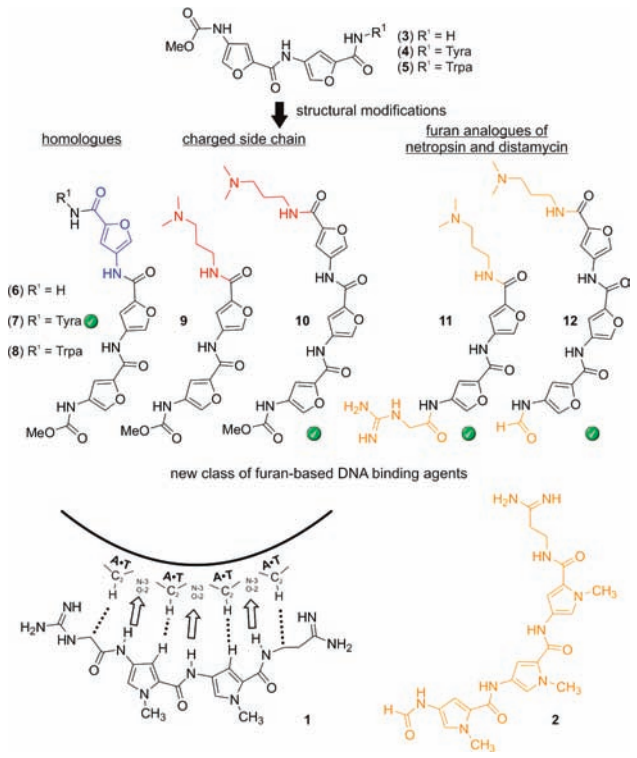
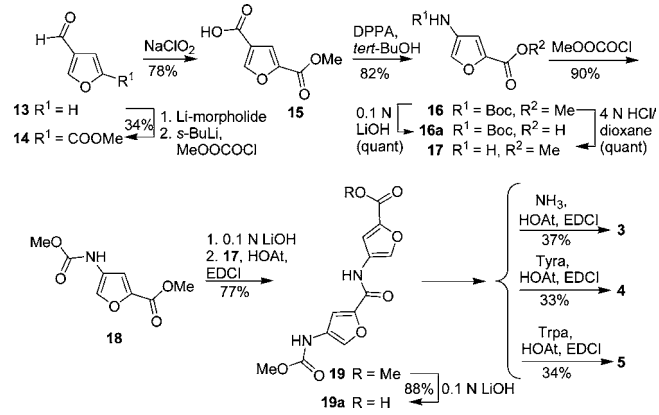


Figure 1. Structures of netropsin (**1**), distamycin (**2**), proximicin A−C (**3**−**5**), and of newly designed potential minor groove binders (**6**−**12**). Structures of compounds capable to act as DNA-binding agents are marked with a green check.

Recently, proximicin A, B, and C (**3**-**5**) composed of a dipeptide of a 2,4-disubstituted furan amino acid were isolated from a marine actinomycete of the genus *Verrucosispora* (Figure 1).[3] Besides the apparent structural similarities of the proximicin core to netropsin (**1**) and distamycin (**2**) the central dipeptide of 4-amino-furan-2-carboxylic acids has neither been reported for natural products nor from synthetic studies. In proximicins the charged residues of (**1**) and (**2**) are formally replaced by an *N*-terminal methyl carbamate and *C*-terminal amides, respectively. These structural differences of proximicins apparently lead to a change of the molecular target.[3a] Remarkably, it was found through cell cycle analysis and the expression level of cell cycle regulating proteins (p53, p21, and cyclinE) that, in contrast to distamycin (**2**), the proximicins address the G0/G1 phase of the cell cycle.[3a] Previous experimental work has reported on the synthesis, cytotoxicity, and DNA binding of netropsin-proximicin-hybrids combining the *N*-methyl pyrrol amino acid of netropsin with *N*- and *C*-terminal residues derived from the proximicins.[4] In this contribution, we present the first total synthesis of the proximicins (**3**−**5**) and examine the influence of structural modifications of analogues (**6**−**12**) with a furan amino acid core on DNA-binding.

A considerable number of syntheses toward variously substituted furans have been reported to date, assembled either by condensation and metal-catalyzed cyclization of linear precursors or by directly performing substitutions at the furan ring. However, methods for preparation of 2,4-disubstituted furans are rare.[5] Our attempts were directed at a three step synthesis of the central core amino acid of the proximicins beginning with commercially available 3-furaldehyde (**13**), which was selectively lithiated at the C-5 position (Scheme 1).[6] Subsequent trapping of the lithiated

Scheme 1. Total Synthesis of Proximicin A (**3**), B (**4**), and C (**5**)



species with methyl chloroformate led to derivative **14**. The aldehyde **14** was oxidized under mild conditions to the corresponding carboxylic acid (**15**) which was further converted by a Curtius rearrangement to the central 2,4-disubstituted furan amino acid in a suitably protected form (**16**) in good yields. Removal of the Boc-group and introduction of the methyl carbamate functionality led to **18**. The methyl ester was easily saponified and the resulting free acid was coupled without further purification to previously synthesized H-Fu-OMe•HCl (**17**) with HOAt/EDCI to yield Meoc-Fu-Fu-OMe (**19**) After saponification of dipeptide **19**

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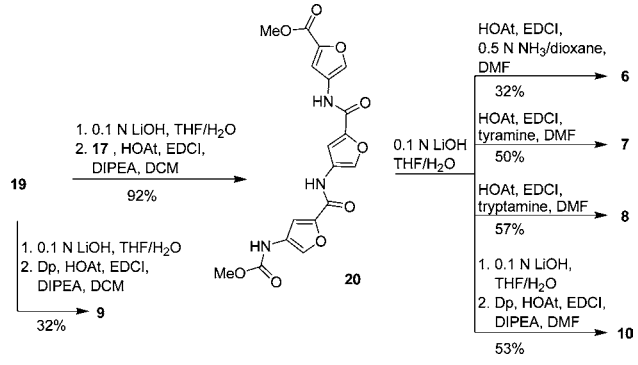
the free acid **19a** was used for the syntheses of the three proximicins A-C. Ammonolysis of an OBt-ester led to proximicin A (**3**), whereas HOAt/EDCI-mediated coupling with the biogenic amines tyramine and tryptamine furnished proximicin B (**4**) and C (**5**), respectively. The synthetic proximicins were identical in all aspects to natural samples.

Previous concepts have amply addressed the variation of heterocycles, their multiple alignment and *N*-terminal and *C*-terminal substitutions in order to evaluate preconditions for DNA-binding and to address sequence specificity.[2] We applied these concepts to the synthesis of seven compounds (**6**−**12**, Figure 1) containing the 4-amino-furan-2-carboxylic acid in order to set these proximicin analogues in context with previously reported heterocyclic polyamide antibiotics regarding DNA-binding capabilities.[2] These new furan-based derivatives can be subdivided into three groups: (a) proximicin homologues (**6**−**8**) containing a third furan amino acid (multiple alignment), (b) derivatives with basic *C*-terminal side chains (**9**−**10**), and (c) furan analogues of netropsin and distamycin (**11**−**12**). As a basic side chain in **9**−**12**, a dimethylpropylamine (Dp) residue was used instead of the amidine group found in netropsin (**1**) or distamycin (**2**). This was mainly chosen to avoid the variable yielding Pinner reaction and for the overall ease of synthesis. This substitution is admissable with regard to DNA-affinity and selectivity.[2f,7] Because of the substitution pattern of the central furan amino acid, all designed derivatives were expected to show selectivity for an AT-rich DNA sequence.[2a]

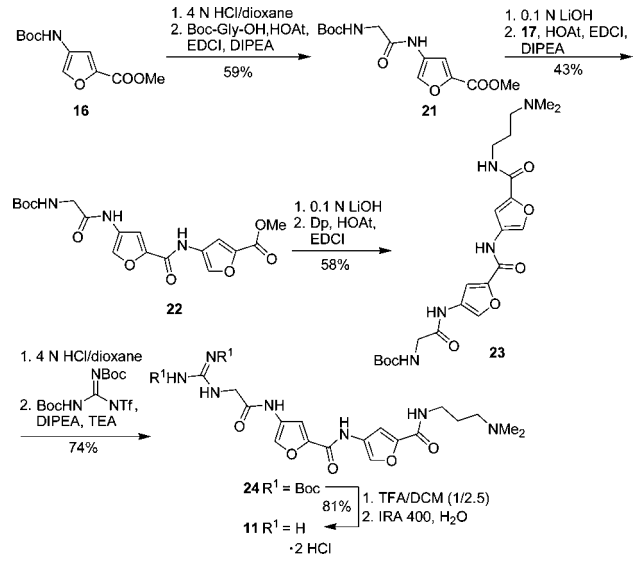
The synthesis of the polyamides **6**−**10** begins with the universal dipeptide building block **19** (Scheme 2). Saponification of the methyl ester and direct use of the free acid for introduction of either another furan amino acid or of a basic dimethylpropylamine (Dp) residue leads to **20** or to derivative **9** with a charged side chain. Upon saponification, tripeptide **20** served as the source for the synthesis of further derivatives. *C*-terminal residues characteristic of proximicin A−C were introduced, yielding the tripeptide homologues **6**−**8**. Coupling of dimethylpropylamine led to tripeptide **10**.

Scheme 2. Synthesis of Proximicin Homologues (**6**−**8**) and of Derivatives with a Charged Side Chain (**9**, **10**)



The synthesis of furan-containing netropsin analogue **11** starts with the introduction of a Boc-protected glycyl residue at the *N*-terminus of **16** yielding **21** (Scheme 3). Then **23** was assembled from **21** by a sequence of *C*-terminal deprotection and peptide coupling steps. The Boc-group of **23** was cleaved and guanidylation with Goodman's reagent yielded **24**, which was deprotected and purified by anion exchange chromatography to yield **11**.[8]

Scheme 3. Synthesis of Furan Analogue (**11**) of Netropsin



The synthesis of **12**, a furan analogue of distamycin, followed in principle the same route as described for **10**, but started with Boc-Fu-OMe (**16**) (Scheme 4). After assembly of the tripeptide composed of three furan units and a dimethylaminopropyl side chain, the Boc-group of **27** was cleaved and subsequent formylation using *N*-formyl imidazole provided **12**.

The interaction of **6**−**12** with DNA was investigated by melting analysis of a GC-rich (5′-cat*ggcc*atg-3′) and an AT-rich (5′-cgc*aaattt*gcg-3′) oligonucleotide (Table 1). In accordance with the literature, netropsin (**1**) and distamycin (**2**) had a stabilizing effect on the AT-rich oligonucleotide.[1c] The derivatives **7** and **10**−**12** also had a stabilizing effect on the melting of the DNA of the AT-rich sequence (Table 1) as observed by an increased T_M value. As expected, **6**−**12** had no effect on the GC-rich sequence (see Supporting Information).

To more extensively evaluate the DNA-binding activity of **6**−**12** binding constants were determined (Table 1) using an ethidium bromide displacement assay.[9] Binding constants

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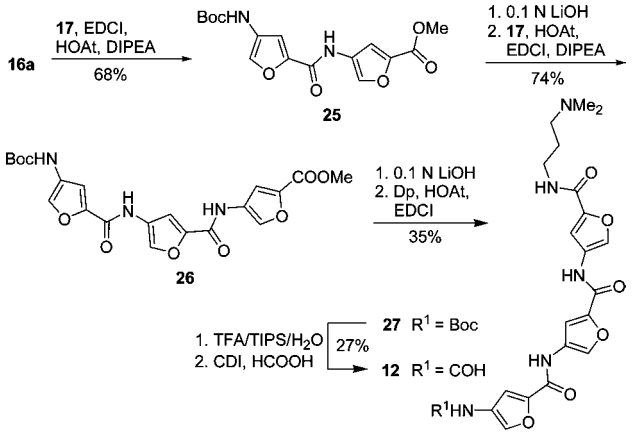
Scheme 4. Synthesis of Furan Analogue (**12**) of Distamycin



obtained for netropsin (**1**) and distamycin (**2**) were comparable to literature values.[9a] The binding constants for new derivatives **6**−**12** show a good correlation with the data found by DNA melting analysis (Table 1). A significant binding affinity was found for **10**, **11**, and **12**, which also show the highest ΔT_M values among the synthesized compounds.

From the proximicin homologues **6**−**8** only compound **7** had a DNA-binding effect with a ΔT_M of 5 °C (Table 1) indicating that *C*-terminal modifications exert a distinguishing influence. Tyramine derivative **7** showed the best interaction compared to the sterically more demanding tryptamine derivative **8**, or amide derivative **6** putatively lacking the establishment of hydrophobic interactions (Figure 1, see binding-mode of **1**).[2a,10]

The introduction of the *C*-terminal dimethylaminopropylamine leads to an increased DNA-binding activity for systems with three repetitive furan rings exemplified by **10** and **12**. Additionally, the *N*-terminal modification of **10** and **12** with a methylcarbamate or a formyl group does not significantly alter the molecule properties in terms of DNA-binding or binding constants. Moreover, the results obtained for **11** (Table 1) imply that an additional charge at the *N*-terminus can compensate for the lack of one furan with regard to DNA-binding affinity. Absence of the *N*-terminal charge, as represented by **9** results in a breakdown of DNA-binding.

Table 1. DNA Melting Analysis and Determination of Binding Constants (K_a) to an AT-Rich Oligonucleotide (cgc*aaatttg*cg) of **6**−**12** and Comparison with Netropsin (**1**) and Distamycin (**2**)

compound	ΔT_M (°C)[a]	K_a ($\times 10^6$ M^{-1})[b]
1	19	280 ± 100
2	15	190 ± 40
6	2	2.5 ± 0.8
7	5	1.5 ± 0.2
8	0	0.44 ± 0.03
9	1	0.63 ± 0.09
10	8	27 ± 10
11	9	31 ± 7
12	8	24 ± 5

[a] Difference in T_M of oligonuleotide cgc*aaatttg*cg and after adding 6 μM of compound **1**, **2**, **6**−**12** to a 2 μM solution of the oligonucleotide. [b] Nonlinear analysis of ethidium bromide displacement titration curve.

The stabilizing effect on AT-rich DNA of the furan analogues of netropsin and distamycin, **11** and **12**, are clearly lower than the effect of netropsin (**1**) and distamycin (**2**) (Table 1). The primary difference in **1** and **11** or in **2** and **12** is the furan heterocycle. The replacement of the *C*-terminal amidine by a dimethylaminopropylamine is expected to have a neglectable influence on the binding affinity.[2f] Hence, the lower stabilizing effect might be due to the different electronic structures of the two heterocyclic cores resulting in altered stacking interactions. Additionally, the *N*-methyl pyrrol of netropsin or distamycin is bulky and nonpolar and in proximity to the anionic backbone of the DNA. In contrast, the furan ring bears a free electron pair, which might lead to a repulsive interaction whith the DNA backbone.

In summary, we developed a short and efficient synthesis for a novel heterocyclic amino acid in an orthogonally protected manner, facilitating the first total synthesis of proximicin A-C. Furthermore, this amino acid was used for the first time as a building block in the synthesis of polyamides capable of interacting with AT-rich DNA. Thus, the stock of five-membered heterocyclic amino acids as scaffolds for minor groove binders was expanded and a novel element of diversity was added for generating a new class of DNA binding agents. Further work is being done using these synthetic methods to generate novel proximicin derivatives to investigate mode of action and to identify the molecular target of the proximicins.

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Supporting Information Available: Experimental procedures and characterization of all new compounds. DNA melting analysis and determination of binding constants by ethidium bromide assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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