Appendix A

Progress Towards Incorporation of Furan Rings into Pyrrole-Imidazole Polyamides

The work outlined in this chapter was performed in collaboration with Daniel A. Gubler (California Institute of Technology).

A.1 Introduction

Lipophilicity plays a role in the pharmacokinetic (PK) properties of lead structures for animal studies, including absorption, distribution, clearance, and metabolism. Recent *in vitro* ADMET analysis of a hairpin Py/Im polyamide revealed high levels of plasma protein binding characteristic of a highly lipophilic compound.[1] As our research program using Py-Im polyamides moves from cell culture into small animal models, new monomers with comparable DNA-binding affinities to *N*-methylpyrrole (Py) and *N*-methylimidazole (Im) and decreased lipophilicity/increased water solubility may be desirable. The development and incorporation of such monomers into Py-Im polyamides would allow the ability to fine-tune the desired lipophilicity of a given polyamide by selectively mixing and matching rings.

In this regard, the recently discovered proximicin A-C natural products have drawn our attention due to their unique 4-aminofuran-2-carboxlic acid ring system and overall similarity to DNA minor groove-binding natural products netropsin (**1**) and distamycin (**2**) (**Figure A.1**). Proximicins A-C (**3**-**5**) possess dual amide-linked furan amino acids and an *N*-terminal methyl carbamate functionality. *C*-terminal functionalities consisting of an amide (proximicin A, **3**), tyramine (proximicin B, **4**), or tryptamine (proximicin C, **5**) characterize each member of the proximicin family. Evaluations of **3**-**5** against multiple cancer cell lines revealed promising antitumor activities, with GI_{50} values in the low micromolar range against gastric adenocarcinoma (AGS), hepatocellular carcinoma (HepG2), and breast adenocarcinoma (MCF7) cell lines. These activities were significantly better than netropsin, distamycin, and analogous compounds to **3**-**5** that possess Py monomers in place of both furans.[2, 3]

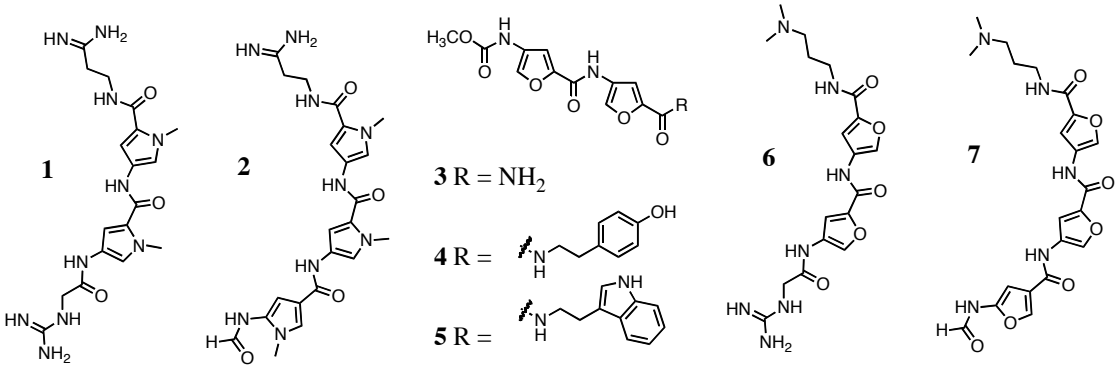


Figure A.1 DNA-binding molecules with furan heterocycle constituents

*Structures of netropsin (***1***), distamycin (***2***), proximicin A-C (***3-5***), and furan analogues of netropsin (6) and distamycin (7)*

The furan amino acid is identical in shape to Py with the exception of the methylated ring nitrogen, which is replaced by oxygen, and this replacement is predicted to increase the water solubility of polyamide oligomers. Incorporation of the furan (Fn) monomer into polyamides designed to inhibit gene regulation *in vivo* may provide a polyamide that demonstrates a better set of physical properties with regard to efficacy in small animal models. Since the *N*-methyl nitrogen of Py units within a polyamide are positioned away from the H-bonding edges of the aromatic amino acid ring pairs, we expect that oxygen at this position will be tolerated in terms of minor-groove binding as well as sequence recognition.

It is speculated the electronics and overall polarity of the furan amino acid in comparison to Py contributes to their enhanced antitumor activities, thus there is even stronger reasoning to evaluate this heterocycle as a potential Py replacement in hairpin polyamides. Recent efforts by Süssmuth to incorporate the furan heterocycle into netropsin and distamycin analogues produced compounds whose binding affinity for DNA has been considerably compromised, as assessed by thermal DNA denaturation stabilization experiments.[3] Süssmuth suggests that the lower stabilizing effect might be due to altered

stacking interactions resulting from the different electronic structures of the two heterocyclic cores. An additional suggestion for this result is that the N-methyl pyrrole of netropsin or distamycin is bulky and nonpolar within the proximity of the anionic backbone of the DNA whereas the furan ring's free electron pair in this same position may lead to a repulsive interaction with the DNA backbone. We anticipate that these unfavorable interactions of the furan ring will be mitigated by incorporation within the context of a Py-Im polyamide, allowing the furan rings to increase polyamide solubility while the Im and Py rings maintain high DNA-binding character.

A.2 Experimental Design

I. Design and partial synthesis of a Py-Im-Fn polyamide library to bind 5'-WGGWCW-3'

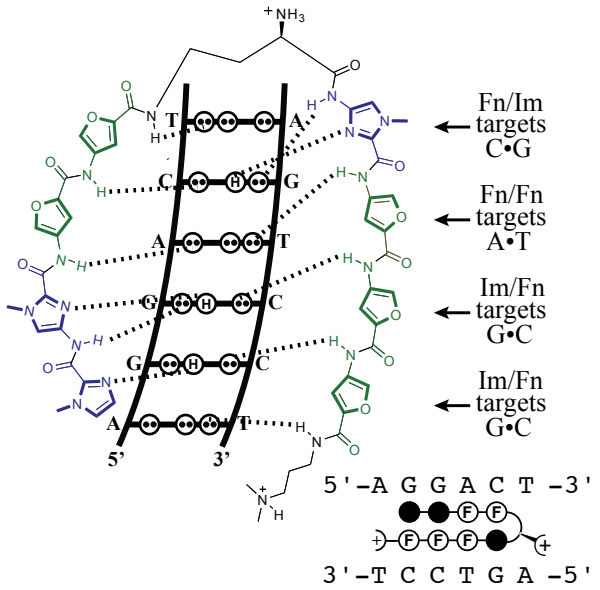


Figure A.2 Furan monomers incorporated into 5'-WGGWCW-3'targeted polyamide scaffold

We designed a library of polyamides targeting the sequence 5'-WGGWCW-3' (where W = A or T) (Figure). This scaffold was chosen because it allows for the assessment of several characteristics of the furan amino acid within a series of analogs (**Figure A.2**). As shown in **Figure A.3**, by altering which position of the parent Py-Im polyamide is altered to a furan we can assess the unit's specificity in all combinations of ring pairs: Im/Fn (**2**), Py/Fn (**3**), Fn/Im (**4**), Fn/Py (**5**) and Fn/Fn (**6**). Also, by incrementally increasing the number of furan rings incorporated into the polyamide (**7-9**), we can assess the combinatorial effect of multiple rings on lipophilicity.

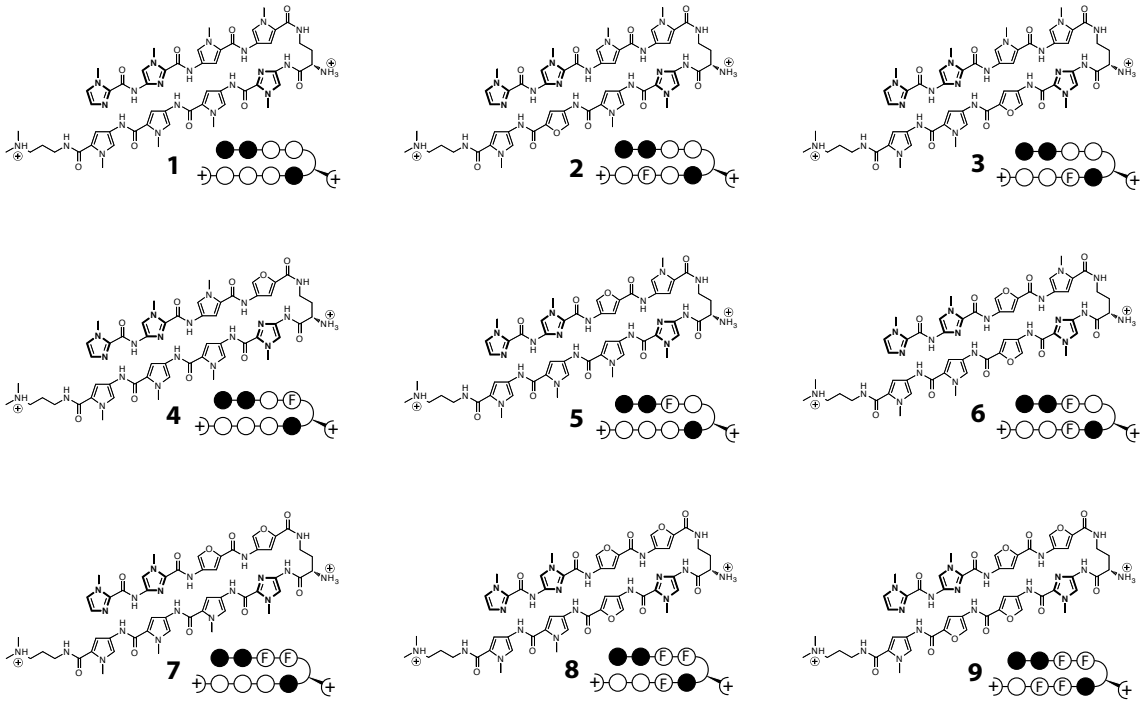


Figure A.3 Py-Im-Fn polyamide library designed to test specificity and lipophilicity

*Compounds **2-9** are based on the Im-Py scaffold 1. **2-6** allow for the measurement of specificity of Fn rings in the context of polyamide pairing rules. **7-9** allow for the measurement of lipophilicity in the context of increasing furan monomer content.*

The synthesis of the Boc-protected furan monomer unit reported by Süssmuth[3] is robust and we were able to report similar yields and purity in the synthesis of this monomer unit. Unfortunately, incorporation of the Fn monomer unit into Py-Im polyamides by solid-phase synthesis on oxime resin was not trivial, as the furan monomer was sensitive to the acidic conditions used for Boc removal. Excessive decomposition of the growing polymer chain was encountered following each trifluoroacetic acid deprotection. Attempts to use Lewis acid deprotection strategies (boron trifluoride diethyl etherate, trimethylsilyl chloride) were more successful, however, successive exposure of the oxime resin to the Lewis acid conditions appeared to destroy the oxime resin itself and resulted in decomposition or loss of the polyamide from the resin. Critically, polyamides containing Fn rings, once synthesized, appear to be as stable to light and heat as typical Py-Im polyamides

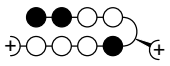
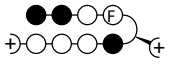
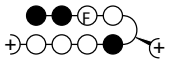
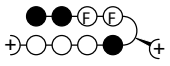
and remain pure by HPLC analysis even after being in solution at room temperature for several days. Because compounds **4, 5,** and **7** require the least cycles of exposure of the furan monomer to acidic conditions, small amounts of these compounds were successfully obtained following repeated preparatory HPLC purification of the crude product cleaved from resin. These compounds along with the parent compound **1** were analyzed for binding affinity and lipophilicity.

II. Assessment of relative binding affinities and specificities of partial library

Polyamides **1, 4, 5,** and **7** were screened for favorable binding affinities and specificities by melting temperature analysis of duplex DNA[4] containing the sequence 5'-TGGXCA -3' where X was cycled through the four base pair possibilities. On each of the four

Table A.1 Assessment of binding affinities and specificities of compounds **1, 4, 5,** and **7**

5'– C G A T **G G X C** A A G C –3'
3'– G C T A **C C X G** T T C G –5'

		A–T	**T–A**	**G–C**	**C–G**
DNA only		58.0 ± 0.2	58.2 ± 0.5	59.6 ± 0.1	59.8 ± 0.3
	**1**	76.2 ± 1.9 [18.2]	76.2 ± 2.3 [17.9]	71.2 ± 0.2 [11.7]	72.3 ± 0.3 [12.4]
	**4**	73.8 ± 0.3 [15.8]	75.2 ± 1.5 [17.0]	69.9 ± 0.1 [10.3]	70.0± 0.4 [10.1]
	**5**	76.6 ± 1.1 [18.6]	77.1 ± 0.8 [18.9]	70.8 ± 0.6 [11.9]	72.9 ± 0.3 [13.1]
	**7**	73.2 ± 1.3 [15.2]	74.6 ± 1.0 [16.3]	68.5 ± 0.5 [8.6]	69.1 ± 0.3 [9.3]

Melting temperature studies of duplex DNA (2 nmoles/oligo) treated with polyamides (2.0-2.4 nmoles). Averages and S.D. were calculated from at least three analyses. Values in brackets represent ΔTm versus untreated DNA duplex.

duplexes tested, the furan-containing polyamides display similar duplex stabilization to the parent compound, indicating that the binding affinity and specificity of the polyamide is likely not adversely affected by the incorporation of furan rings. This is particularly exciting considering the severe loss of DNA stabilization demonstrated by furan analogs of netropsin.[3]

III. Assessment of relative lipophilicity of partial library as analyzed by $LogD_{octanol/water}$ at pH 7.4.

Small molecule lipophilicity is most commonly described by its octanol/water partition coefficient ($Log^{oct/water}$). The partition coefficient is a ratio of concentrations of un-ionized compound between the two solutions. To measure the partition coefficient of ionizable solutes, the pH of the aqueous phase is adjusted such that the predominant form of the compound is un-ionized. The logarithm of the ratio of the concentrations of the un-ionized solute in the solvents is the LogP (Eq 1).

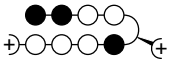
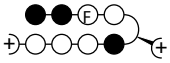
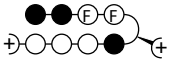
$$\log P_{oct/wat} = \log \left(\frac{[solute]^{un\text{-}ionized}_{octanol}}{[solute]^{un\text{-}ionized}_{water}} \right) \quad (1)$$

The distribution coefficient is the ratio of the sum of the concentrations of all forms of the compound (ionized plus un-ionized) in each of the two phases.. The logarithm of the ratio of the sum of concentrations of the solute's various forms in one solvent, to the sum of the concentrations of its forms in the other solvent is called LogD (Eq 2). In addition, LogD is pH dependent, hence one must specify the pH at which the LogD was measured.

$$\log D_{oct/wat} = \log \left(\frac{[solute]^{un\text{-}ionized}_{octanol} + [solute]^{ionized}_{octanol}}{[solute]^{un\text{-}ionized}_{water} + [solute]^{ionized}_{water}} \right) \quad (2)$$

Lipophilicity, as predicted by LogP and LogD, is an important predictor of oral bioavailability for test compounds. Of particular interest in the context of lead structures for animal studies is the LogD at pH = 7.4 (the physiological pH of blood serum). A variety of protocols have been reported for measuring LogP or LogD both experimentally and computationally. (**5-7**) The LogD and pH 7.4 of compounds **1, 5,** and **7** was determined via a miniaturized octanol/water shake-flask assay through the commercial services of Analiza, Inc.

Table A.2 LogD at pH 7.4 of compounds **1, 4, 5,** and **7** at 0.1 mM concentration

		LogD	LogD*
	**1**	-0.95	-0.75
	**2**	n.d.	n.d.
	**3**	-0.97	-0.77
	**4**	-1.01	-0.80

LogD values have been corrected for DMSO background*

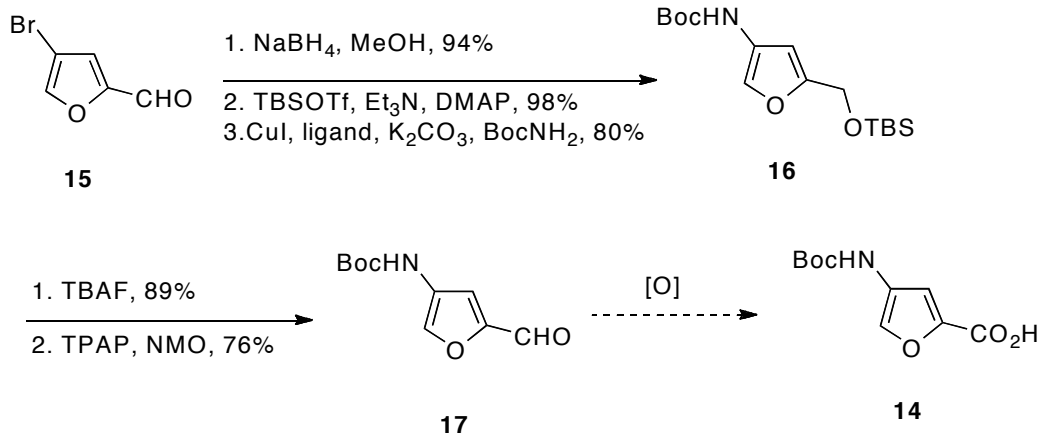
For octanol/buffer partitioning, Analiza's standard two phase system plates were used. Octanol in equilibrium with universal buffer adjusted to pH 7.4 were used to prepare partitioning plates for the assay. The initial assay was designed to measure the LogD of the compounds starting with a 10 mM stock solution, with a final working concentration of 1 mM. At this concentration, the compounds displayed significant aggregation in the octanol layer, a behavior often noted in compounds with higher molecular weights. To compensate, the compounds were diluted to 1 mM stock solution, providing a final working concentration of 0.1 mM. At this concentration, the compounds were able to be measured with the exception of compound **4**, which still displayed aggregation. Unexpectedly, the decrease in LogD over this series of increasing furan content is quite modest.

A.3 Future Directions

The binding measurements of the compounds synthesized show promise, however the modest decrease in lipophilicity observed is dissapointing. It may be that compound containing a large percentage of furans will show more promise, thus the remaining compounds of the library should be synthesized. Considering the instability of the furan monomer unit to the acid conditions required by Boc peptide chemistry, a promising route is to instead utilize Fmoc-based peptide chemistry. This alternate protecting group methodology has been successfully utilized in synthesizing Py-Im polyamides and we suspect will be much milder on the furan monomer during synthesis.[8] Once the entire library has been synthesized, lipophilicity measurements will be performed and, following this, the compounds will be tested in a protein-binding assay to determine if this alteration has the desired effect of decreasing the protein binding characteristic of Py-Im polyamides.

A.4 Synthetic Efforts

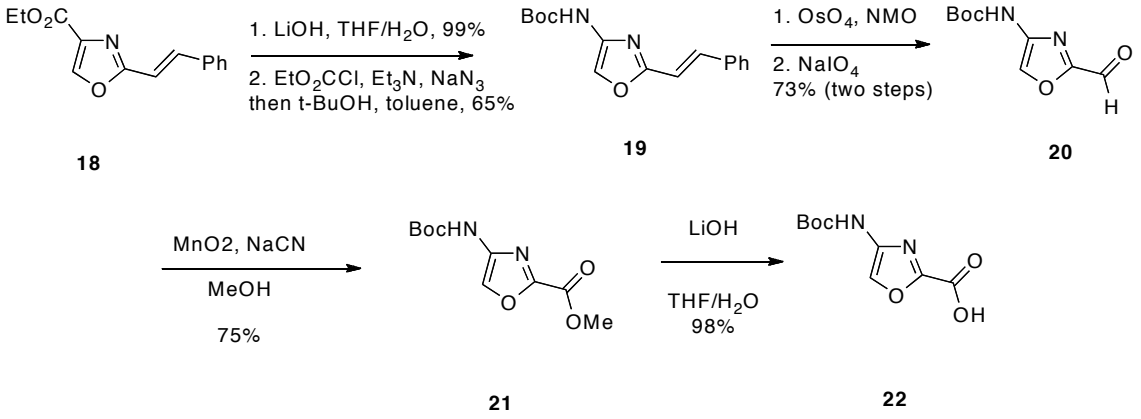
At the beginning of this project, the synthesis of furan amino acid **F** had not been reported. Accordingly, we set out to synthesize this monomer; a summary of our progress is shown in Scheme 2. The synthesis, developed by D. Gubler, began from commercially available 4-bromo-2-furaldehyde **16** and converted to carbamate **17** in three steps. The key step of this sequence, and of the entire synthesis, was a copper-catalyzed coupling of the 4-bromofuran intermediate with Boc carbamate. Unfortunately, the copper-catalyzed coupling reaction did not work on starting material **16** or its carboxylic acid derivative. Consequently, the coupling reaction was performed at the alcohol oxidation state, which, unfortunately, required two steps for reduction and protection of the alcohol followed by two steps for oxidation to give aldehyde **18**.



Scheme A.1 Attempted synthesis by D. Gubler of furan monomer Fn.

About the same time we obtained aldehyde **18**, the synthesis of 4-aminofuran-2-carboxylic acid **F** was reported by Süssmuth along with the total synthesis of the proximicins.[3] This synthesis of Fn proved to be more amendable for rapid, gram scale synthesis than our route.

In addition to the furan monomer analog of Py, 4-amino-1,3-oxazole-2-carboxylic acid (Ox) has been proposed as a more hydrophilic analogue of Im. D. Gubler developed a synthetic route to the oxazole amino acid monomer which is very robust and can be done on multi-gram scale. The synthesis of oxazole monomer **22** starts from known oxazole **18**.[9] Saponification of oxazole **18** followed by Curtius rearrangement gave Boc carbamate **19** in 65% yield. Dihydroxylation of Boc carbamate **19** using osmium tetroxide followed by cleavage of the resultant diol with sodium periodate furnished aldehyde **20** in 73% yield for the two steps. Oxidation of aldehyde **20** with manganese dioxide and sodium cyanide provided methyl ester **21**. Saponification of ester **21** smoothly furnished oxazole monomer **22** in good yield.



Scheme A.2 Synthesis of oxazole monomer Ox by D. Gubler

Unfortunately, initial attempts to couple oxazole monomer **22** into Py/Im polyamides has not been successful to date. Decomposition is seen in the coupling step under the standard coupling conditions of either PyBop/DIEA or EDCI/DMAP. Although initial forays into this area have been discouraging, more effort is needed for an accurate assessment of the feasibility of working with this monomer.

A.5 Materials and Methods

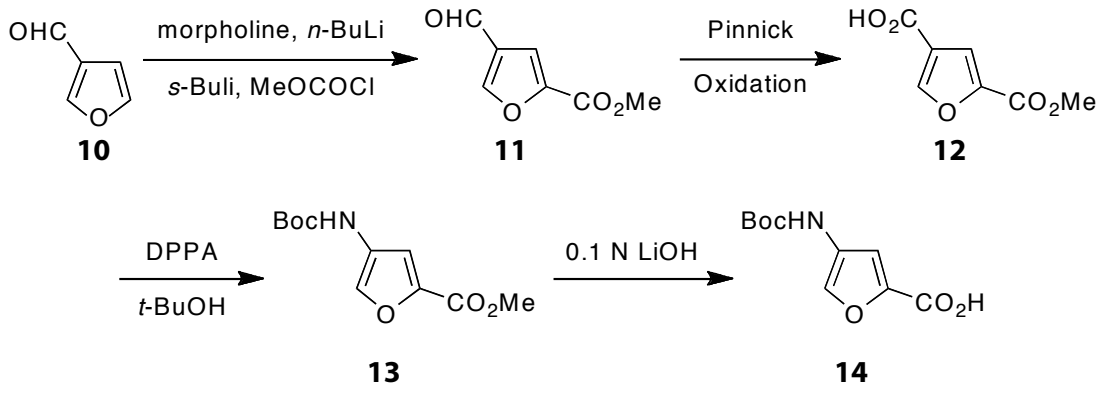
Materials

Unless otherwise stated, DNA oligonucleotides were ordered HPLC-purified from Integrated DNA Technologies. Unless otherwise stated, reagents were purchased from Sigma-Aldrich. All solvents were purchased from Aldrich or EMD Biosciences. Commercially obtained reagents were used as received. Reaction temperatures were controlled by an IKAmag temperature modulator. Thin-layer chromatography (TLC) was performed using E. Merck silica gel 60 F254 precoated plates (0.25 mm) and visualized by UV fluorescence quenching. ICN Silica gel (particle size 0.032-0.063 mm) was used for flash column chromatography.

Methods

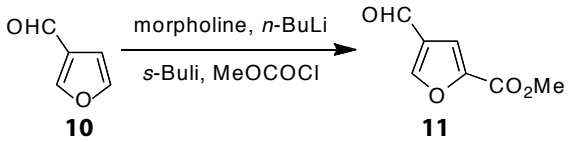
UV spectra were recorded using an Agilent 8453 UV-Vis spectrophotometer. Polyamide concentrations were measured as a solution in water at λ=310 nm using an estimated extinction coefficient of ε=69,500 M-1 cm-1 for 8-ring polyamides.[39] LASER desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) was performed using an Applied Biosystems Voyager DE Pro spectrometer. Analytical and preparative high-pressure liquid chromatography (HPLC) were performed with a Beckman Gold system equipped with a diode array (analytical) or single-wavelength (preparative) detector. ^{1}H NMR spectra were recorded on a Varian Mercury 300 (at 300 MHz) or a Varian Inova 500 (at 500 MHz) and are reported relative to solvent peak. Data for ^{1}H NMR spectra are reported as follows: chemical shift (ppm) (multiplicity, integration, coupling constant (Hz)). ^{13}C NMR spectra were recorded on a Varian Inova 500 (at 125 MHz) and are reported relative to solvent peak. Data for ^{13}C NMR spectra are reported in terms of chemical shift. High-resolution mass spectra were obtained from the California Institute of Technology Mass Spectral Facility.

i. Furan monomer synthesis



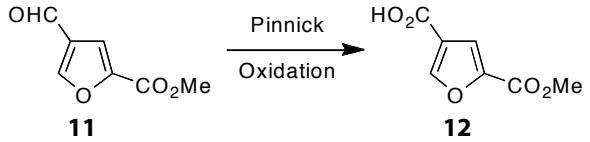
Scheme A.3 Synthesis of Boc-protected Fn monomer as reported by Süssmuth.

Synthesis of furan monomer was performed exactly as described by the literature procedure[3]:

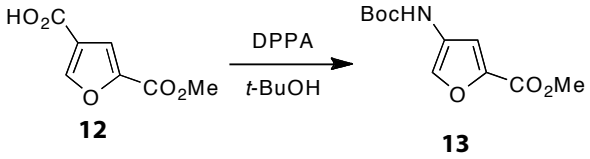


10 **11**

4-Formyl-furan-2-carboxylic acid methyl ester (**11**). Morpholine (952 mg, 10.93 mmol, 1.05 eq) was dissolved in THF (75 mL) and cooled to -78 °C under argon. To this solution n-BuLi (6.830 mL, 1.6 M in hexane, 1.05 eq) was added dropwise and the resulting slightly yellow solution was stirred for further 20 min and then 3-furaldehyde (**10**, 1000 mg, 10.41 mmol, 1.0 eq) dissolved in THF (2 mL) was added dropwise. After another 20 min s-BuLi (8.406 mL, 1.3 M in cyclohexane, 1.05 eq) was added dropwise. The resulting suspension was stirred at -78 C for 2.5 h. Methyl chloroformate (1082 mg, 11.45 mmol, 1.1 eq) was dissolved in THF (2 mL) and added dropwise and the reaction mixture was stirred for 45 min at this temperature an additional 40 min at room temperature. The solution was poured into ice cold 10 % HCl and the layers were separated and the aqueous phase was extracted with Et_2O (4 x 50 mL). The combined organic phases were dried over $MgSO_4$, filtered, and concentrated under reduced pressure to give the crude product, which was purified by column chromatography (silica gel, hexanes/EtOAc 7/1) to yield 460 mg (30%) of the desired compound. ^{1}H NMR δH (300 MHz; $CDCl_3$) 3.93 (s, 3H), 7.49 (d, 1H, J=0.6), 8.17 (d, 1H, J=0.8), 9.96 (s, 1H).

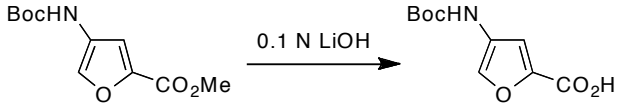


Furan-2,4-dicarboxylic acid 2-methyl ester (**12**). Compound **11** (860 mg, 5.58 mmol, 1.0 eq) was dissolved in t-BuOH (138 mL) and 2-methyl-2-butene (34 mL). 80 % $NaClO_2$ (5.93 g, 52.45 mmol, 9.4 eq) and $NaH_2PO_4 \bullet 2H_2O$ (6.094g, 39.06 mmol, 7.0 eq) were dissolved in H_2O (54 ml) and slowly added to a stirring solution of the aldehyde. The reaction was stirred for 50 min at room temperature. Then 2-methyl-2-butene and t-BuOH were removed under reduced pressure and the residue was diluted with water and extracted with hexane (3 x 50 mL). The aqueous phase was acidified to pH 2 with 10 % HCl and extracted with EtOAc (3 x 50 mL). The organic phases were combined and dried over Na_2SO_4, filtered and concentrated under reduced pressure. This slightly yellow solid was washed with $CHCl_3$, yielding the title compound as a colorless solid (710 mg, 75 %). ^{1}H NMR δH (300 MHz; DMSO-d6) 3.82 (s, 1H), 7.40 (d, 2H, J=), 8.54 (d, 2H, J=0.83), 13.10 (brs, 1H).



4-tert-Butoxycarbonylamino-furan-2-carboxylic acid methyl ester (**13**). To a solution of the acid **12** (1.3 g, 7.68 mmol, 1.0 eq) in CH_3CN (1 mL/mmol) were added sequentially triethylamine (1.069 mL, 7.68 mmol, 1.0 eq) and DPPA (1.657 mL, 7.68 mmol, 1.0 eq). This solution was stirred at RT for 3.5 h. Then t-BuOH (0.95 mL/mmol) was added in one portion and the resulting mixture was stirred under reflux for 24 h. The mixture was cooled to room temperature, poured into saturated $NaHCO_3$ (50 mL), and extracted with Et_2O (3 x 50 mL). The combined organic phases were dried over Na_2SO_4, filtered, and concentrated

under reduced pressure. The crude product was purified by flash chromatography (silica gel, 7/1, hexane/EtOAc), yielding the title compound as a slightly yellow solid (1.4 g, 78 %).[1]H NMR δH (300 MHz; $CDCl_3$) 1.49 (s, 9H), 3.87 (s, 3H), 6.36 (brs, 1H), 7.00 (s, 1H), 7.88 (brs, 1H).



13 **14**

4-tert-Butoxycarbonylamino-furan-2-carboxylic acid (**14**). To a solution of ester **13** (374 mg, 1.55 mmol, 1.0 eq) in THF (78 mL), LiOH (186 mg, 7.75, 5.0 eq) dissolved in H_2O (78 mL) was added dropwise. After stirring for 1.5 h at room temperature the reaction mixture was concentrated under reduced pressure and diluted with water. The solution was acidified to pH 3 by adding 10% HCl and extracted with EtOAc (3 x 40 mL). The combined organic phases were dried over Na_2SO_4, filtered, and the solvent was removed under reduced pressure, yielding the title compound as a yellow solid (345 mg, quant. yield). [1]H NMR δH (500 MHz; DMSO-d6) 1.44 (s, 9H), 6.97 (s, 1H), 7.85 (s, 1H), 9.43 (s, 1H), 13.08 (brs, 1H). [13]C NMR δC (125 MHz; DMSO-d6) 28.46, 79.92, 111.47, 127.71, 133.60, 143.32, 153.00, 159.58. EI-HRMS m/z 227.0791 [M+•, $C_{10}H_{13}O_5N_1$ requires 241.0794].

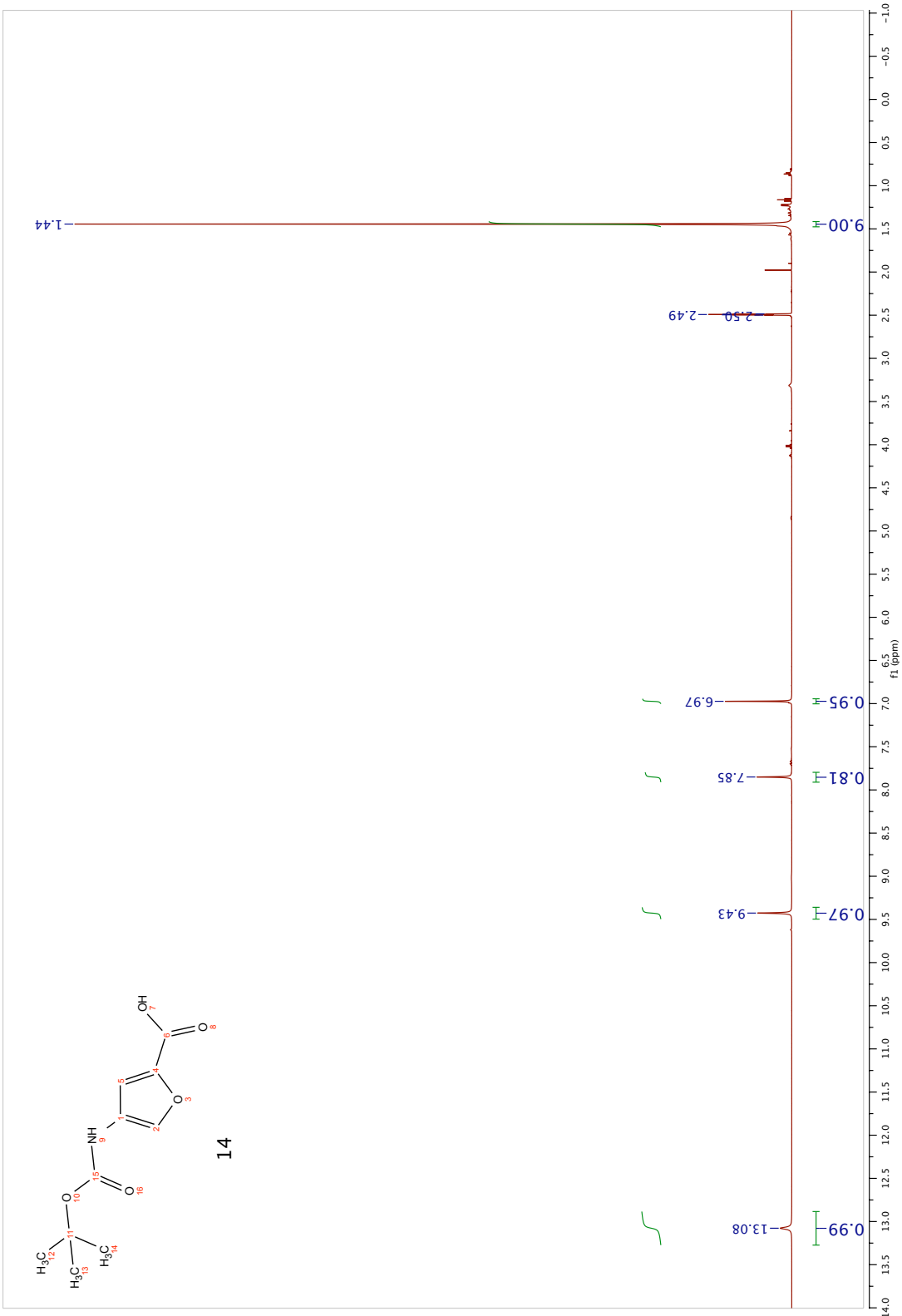


Figure A.4 ^{1}H NMR (500 MHz, DMSO) of compound **14**

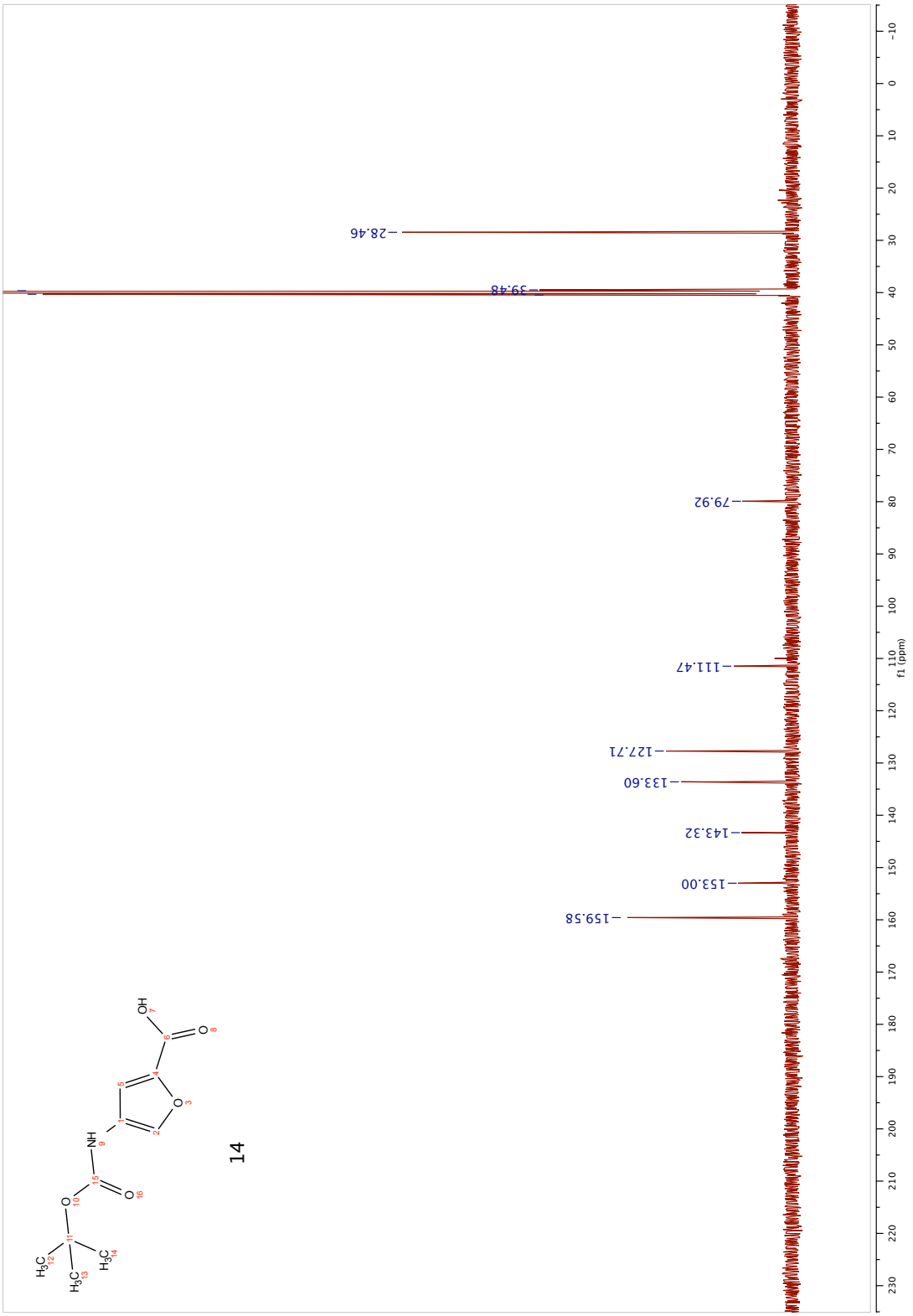


Figure A.5 ^{13}C NMR (500 MHz, DMSO) of compound **14**

ii. Polyamide synthesis

Pyrole and imidazole monomer units [Boc-Py-OBt (1,2,3-Benzotriazol-1-yl 4-[(tert-Butoxycarbonyl)amino]-1-methylpyrrole-2-carboxylate) and Boc-Im-OH (4-[(tert-Butoxycarbonyl)amino]-1-methylimidazole-2-carboxylic acid) respectively] are synthesized according to established protocols[10] and maintained as general group stock. Fmoc-D(Dab)-Boc-OH turn monomer unit was purchased from Peptides International.

Polyamides **1, 4, 5,** and **7** were synthesized by solid-phase methods on Kaiser oxime resin (Nova Biochem, Darmstadt, Germany) following established protocols,[11] with the exception of the Boc deprotection step. In a typical synthesis, 4 molar equivalents (relative to resin loading for the lot of oxime resin used) of monomer unit were activated with 4 equivalents of PyBOP (NovaBiochem) and 4 equivalents of diisopropyletheylamine (DIEA) in a solution of dimethylformamide (DMF) (solution was approximately 0.3-0.5 M in monomer unit). In a glass peptide synthesis vessel, the resin was shaken with this solution for 2-4 hours at room temperature until the reaction was complete, as assessed by analytical HPLC of a cleaved sample of resin. Resin was deprotected by shaking in a solution of 5 equivalents boron trifluoride diethyl etheraate (BF_3•Et_2O) in methylene chloride for 5 minutes at room temperature. After completion of synthesis the polyamide was cleaved by incubation in neat 3-dimethylamino-1-propylamine (0.3 mL) at 37 °C for 16 h. Products were purified by preparatory reverse-phase HPLC on a Beckman Gold system using either a Waters Delta-Pak 25°—100 mm, 15 μm 300 Å C18 PrepPak Cartridge reverse-phase column or a Varian Dynamax 21.4°—250 mm Microsorb 8 μm 300 Å C8 reverse-phase column in 0.1% (w/v) TFA with acetonitrile as the eluent. The appropriate fractions were lyophilized after characterization by analytical HPLC, UV-visible spectroscopy, and MALDI-TOF mass spectrometry.

1: (MALDI-TOF) $[M+H]^+$ calcd for $C_{54}H_{67}N_{22}O_9^+$ 1167.6, observed 1167.6

4: (MALDI-TOF) $[M+H]^+$ calcd for $C_{53}H_{64}N_{21}O_{10}^+$ 1154.5, observed 1154.6

5: (MALDI-TOF) $[M+H]^+$ calcd for $C_{53}H_{64}N_{21}O_{10}^+$ 1154.5, observed 1154.6

7: (MALDI-TOF) $[M+H]^+$ calcd for $C_{52}H_{61}N_{20}O_{11}^+$ 1141.5, observed 1141.7

iii. UV Absorption Spectrophotometry of of DNA thermal stabilization

Melting temperature analysis was performed on a Varian Cary 100 spectrophotometer equipped with a thermo-controlled cell holder possessing a cell path length of 1 cm. An aqueous solution of 10 mM sodium cacodylate, 10 mM KCl, 10 mM $MgCl_2$, and 5 mM $CaCl_2$ at pH 7.0 was used as analysis buffer. This buffer was degassed under vacuum and DNA duplexes and polyamides were added to a final concentration of 2 μM DNA, 3μM polyamide for each experiment. Prior to analysis, samples were heated to 90 °C and cooled to a starting temperature of 23 °C with a heating rate of 5 °C/min for each ramp. Denaturation profiles were recorded at λ = 260 nm from 23 to 90 °C with a heating rate of 0.5 °C/min. Each sample was subjected to denaturation in technical duplicate, and the reported numbers are the average of at least three experimental replicates. The reported melting temperatures were defined as the maximum of the first derivative of the denaturation profile.

iv. LogD Analysis

LogD analysis was performed by Analiza, Inc., information can be found at http://www.analiza.com. The following description regarding experimental protocol was provided by the company.

Sample Preparation:

The DMSO stock solutions were sonicated in a 40°C water bath to facilitate dissolution. Following sonication all of the samples appeared to be fully dissolved. Due to aggregation in the two phase system, the compounds were diluted to 1mM from the original 10mM DMSO stock and analyzed further. Note that for compounds **5** and **7**, some material accumulated at the interface of the octanol/water partition even at the diluted range.

Partitioning:

For octanol/buffer partitioning, Analiza's standard two phase system plates were used. Octanol in equilibrium with universal buffer (composed of 0.15 M NaCl and 0.01 M each of phosphoric, boric, and acetic acids, adjusted to pH 7.4 with NaOH) were used to prepare partitioning plates for the assay. This buffer provides uniform ionic composition across a wide pH range. DMSO stock solutions (25μL) were added to each partitioning plate to a final concentration of 10% DMSO. The plates were sealed, vortexed on our specially designed deep well plate mixer, and centrifuged to aid in phase settling. The assay was conducted on the ADW workstation using chemiluminescent nitrogen detection.

Three separate on-board performance indicating standards were assayed with the Caltech supplied compounds and all results were within the acceptable range.

Calculation of Results:

The equimolar nitrogen response of the detector is calibrated using standards which span the dynamic range of the instrument from 0.08 to 4500 μg/ml nitrogen. Both the top and bottom phases were quantitated with respect to this calibration curve and the logarithm of the ratio of the concentration in the top phase to the concentration in the bottom phase is calculated as the partition coefficient. In addition to reporting the directly observed Log D value, the observed Log D value was adjusted to a corrected Log D* based upon our previous work correlating Log D in the presence and absence of a fixed amount of DMSO in the partitioning system.

The calculated Log D and Log D* values are corrected for any background nitrogen in the DMSO. The Log D results presented assume that the samples were free of nitrogen containing impurities and are stable under the assay conditions.

A.6 References

(1) Chenoweth, D. M. ; Harki, D. A.; Phillips, J. W.; Dose, C.; Dervan, P. B. *J. Am. Chem. Soc.* **2009** *131*, 7182.

(2) Fiedler, H. -P.; Bruntner, C.; Riedlinger, J.; Bull, A. T. *et al.*, *J. Antibiot.* **2008** *61*, 158.

(3) Wolter, F. E.; Schneider, K.; Davies, B. P.; Socher, E. R. *et al.*, *Org. Lett.* **2009** *11*, 2804.

(4) Pilch, D. S.; Poklar, N.; Gelfand, C. A.; Law, S. M. *et al.*, *Proc. Natl. Acad. Sci. USA* **1996***, 93*, 8306.

(5) Lombardo, F.; Shalaeva, M. Y.; Tupper, K. A.; Gao, F.; Abraham, M. H.; *J. Med. Chem.* **2000** *43*, 2922.

(6) J. Sangster, *Octanol-water partition coefficients: fundamentals and physical chemistry* (John Wiley & Son Ltd, 1997).

(7) Smith, D. A.; Jones, B. C.; Walker, D. K.; *Med. Res. Rev.* **1996** *16*, 243.

(8) Wurtz, N. R.; Turner, J. M.; Baird, E. E.; Dervan, P. B. *Org. Let.* **2001** *3*, 1201.

(9) Celatka, C. A.; Liu, P.; Panek, J. S. *Tetrahedron Letters* **1997** *38*, 5449.

(10) Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc* **1996** *118*, 6141.

(11) Belitsky, J. M.; Nguyen, D. H.; Wurtz, N. R.; Dervan, P. B. *Bioorg. Med. Chem.* **2002** *10*, 2767.