THE USE OF PHOSPHONITRILIC DICHLORIDE CYCLIC TRIMER IN OLIGOPEPTIDE SYNTHESIS.[1] SYNTHESIS OF ISOXAZOLYL-PRODRUGS OF NETROPSIN AND DISTAMYCIN

Erik J. Verner, Bradford J. Oliver, Ludwig Schlicksupp, and Nicholas R. Natale*

Department of Chemistry, University of Idaho

Moscow, Idaho 83843, U.S.A.

Abstract -- Phosphonitrilic dichloride cyclic trimer has been found to be an effective activating agent for the synthesis of oligopeptides containing N-terminal isoxazole-4-carboxamide groups.

The family of antitumor, antibiotic, antiviral 4-aminopyrrole-2-carboxylate peptides,[2] including netropsin and distamycin-A, has recently attracted considerable attention due to their ability to bind A-T rich regions of B-DNA.[3] Unfortunately, these natural products are too toxic for agricultural or clinical application; therefore, we have initiated a program for site-specific drug delivery via the use of a promoiety.[4] As our initial promoiety we have selected the isoxazole-4-carboxamides, familiar to medicinal chemists in the oxacillin class of antibiotics.[5] A promoiety could be especially useful if it could mask a key functional group, and thus improve drug delivery. However, it is also known that replacement of a pyrrole ring with imidazole or pyridine alters the A-T sequence binding specifity of netropsin and distamycin derivatives. Therefore, we were intrigued by the possibility of using the isoxazole as a synthetic hybrid intercalator-groove binder in an overall strategy of coupling G,C words and A,T words into sentences for recognition of sequences of B-DNA. We present herein the synthesis of isoxazole prodrugs of netropsin and distamycin as well as structural evidence, based on a simple model system, that the isoxazole moiety holds significant promise in both respects. We had previously reported that the Schotten-Baumann reaction for the synthesis of isoxazole-4-carboxamides was more efficient than the use of dicyclohexylcarbodiimide (DCC).[6] Limitations of the Schotten-Baumann arise due to the severe reaction conditions required. For sterically hindered 3,5-disubstituted isoxazoles the DCC coupling appears to stop at the acyl imine. Thus we desired an activating agent for direct coupling of aminopyrrole-2-carboxylates with isoxazoles which is efficient, yet sufficiently mild to allow for the toleration of a wide variety of functional groups. We now report that

Figure 1.

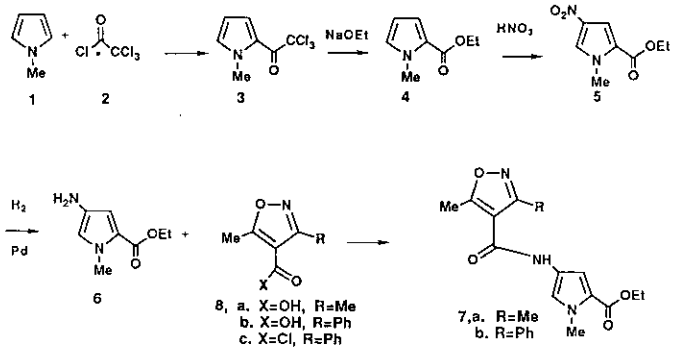


Figure 2, Iterative approach to isoxazolylaminopyrrole carboxylate peptides.

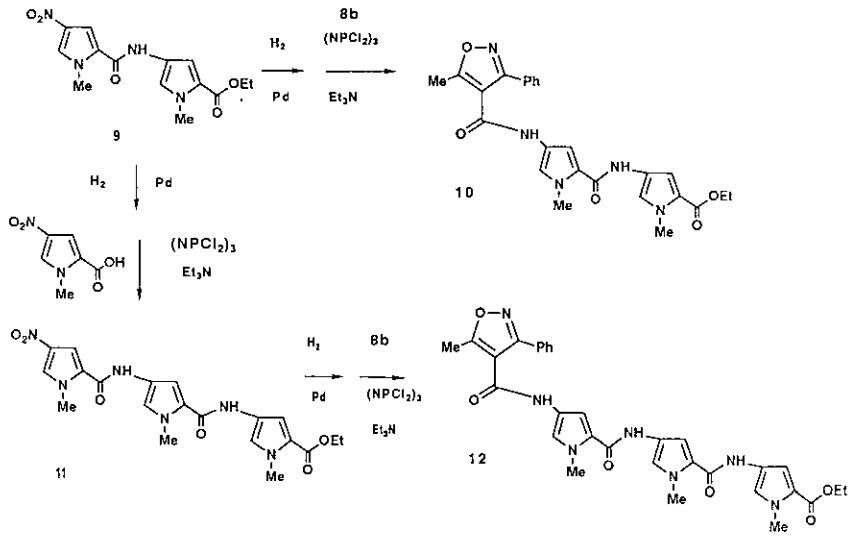
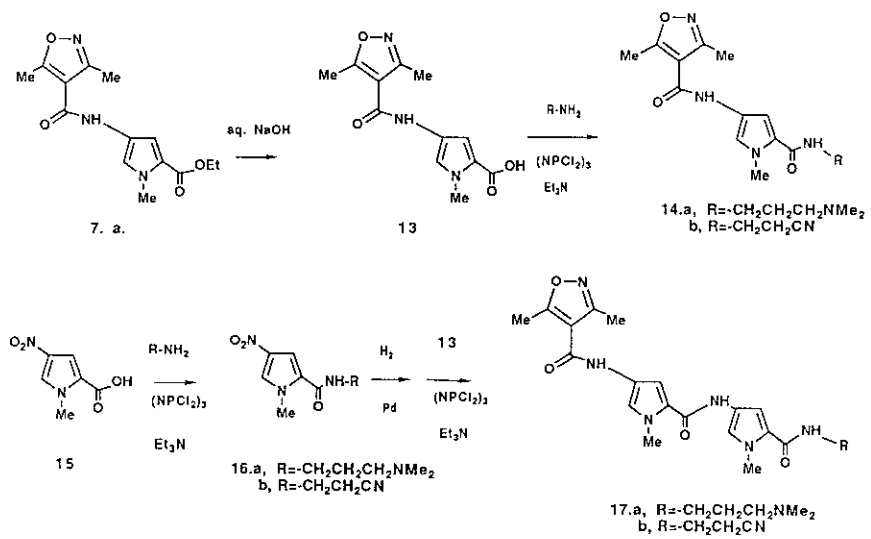


Figure 3, Convergent approach to isoxazolylaminopyrrole carboxylate peptides.



we have examined the use of phosphonitrilic dichloride cyclic trimer $(NPCl_2)_3$,[7] and found that it meets the prescribed criteria for use in oligopeptide synthesis.

The repeating unit used for this synthetic route, 4-amino-2-carboxy-1-methylpyrrole (**6**), is available in multigram quantities by literature techniques, as shown in Figure 1. 1-Methylpyrrole (**1**) undergoes Friedel-Crafts acylation with trichloroacetyl chloride (**2**) to give the trichloroketone (**3**) in over 70 percent yield in one mole batches.[14] Sodium alkoxide treatment provides the ester (**4**) in over 80 percent yield after distillation. Nitration, however, gives only a 40 percent yield of (**5**) after the necessary recrystallizations. Even given this modest yield, 50 grams of nitro ester (**5**) are readily attainable in a single run. Hydrogenations are routinely performed in 10 gram batches. We have found that monitoring the reaction by tlc gives optimum results and the amine (**6**) can be used immediately for coupling via the phosphonitrilic dichloride route. In this manner multigram quantities of the isoxazole-monopyrrole (**7**) can be obtained.

We then critically compared the synthesis of isoxazole-monopyrroles (**7**) by Shotten-Baumann and $(NPCl_2)_3$ methodology. Although the yields are comparable and synthetically useful for both processes (ca. 80% after isolation and purification), the latter procedure is more convenient. Our experience with the formation of acid chlorides (**8c**) from 3,5-disubstituted isoxazole 4-carboxylic acids (**8b**) is that the reaction is relatively slow, requires a large excess of thionyl chloride to proceed at a reasonable rate, and the product requires distillation for optimum results.

In contrast, the use of $(NPCl_2)_3$ eliminates the extra step required in acid chloride formation, gives rapid reaction (usually less than half an hour), and requires only stoichiometric amounts of the activator. We then examined the usual iterative procedure for the synthesis of netropsin (**10**) and distamycin (**12**) congeners shown in Figure 2. This sequence of reactions demonstrates that the reaction conditions tolerate nitro groups and peptide linkages. Figure 3 illustrates the incorporation of end-groups capable of hydrogen bonding (the a series), and which also may serve as useful functional handles for formation of amidines (the b series). This can be readily accomplished both by iterative and convergent pathways as shown. All new products were characterized by CI- or FAB-ms and/or combustion analysis after purification to homogeneity by preparative tlc. We feel that this method represents a definite improvement over the previously reported methods, especially for hindered systems (e.g., the 3-phenyl-5-alkylisoxazole-4-carboxylic acid), is convenient and relatively inexpensive (Shinnisso Kako currently offers the $(NPCl_2)_3$ at $70 per kilo), and can be scaled

Table 1. Characterization data for Isoxazolyl-peptides.

Structure	R	mp	^{1}H nmr	ir	ms	Anal. Found (calcd) C	H	N	Formula
7a	Me	120-121	8.3 (br s , 1H); 7.25 (d, J=2Hz, 1H); 6.7 (d, J=2Hz, 1H); 4.2 (q, J=7Hz, 2H); 3.8 (s, 3H); 2.6 (s, 3H); 2.4 (s, 3H); 1.3 (t, J=7Hz, 3H).	3396.6, 3182.5, 2983.9, 1712.8, 1643.3, 1423.5, 1253.7, 1095.6, 783.1.	291 (100); 246 (26.4); 168 (27.5); 124 (81.6); 82 (42.3).	57.60 (57.72)	5.88 (5.88)	14.32 (14.42)	$C_{14}H_{17}N_3O_4$
7b	Ph	173-174	7.55 (s, 5H); 7.3 (d, J=2Hz, 1H); 6.35 (d, J=2H, 1H); 4.25 (q, J=7Hz, 2H); 3.85 (s, 3H); 2.7 (s, 3H); 1.3 (t, J=7Hz, 3H).	3269.3, 3117.0, 2980.0, 1708.9, 1672.3, 1585.5, 1421.5, 1265.3, 1199.7, 1087.8, 758.0.	353 (100); 308 (13.2); 186 (12.8); 168 (19.4); 144 (72.6); 77 (11.4).	64.37 (64.58)	5.3 (5.42)	11.8 (11.89)	$C_{19}H_{19}N_3O_4$
10	Ph	164-166	7.5 (s, 5H); 7.3 (d, J=2Hz, 1H); 7.2 (br s, 1H); 6.95 (d, J=2Hz, 1H); 6.7 (d, J=2Hz, 1H); 6.3 (d, J=2Hz, 1H); 4.25 (q, J=7Hz, 2H); 3.8 (s, 3H); 2.7 (s, 3H); 1.3 (t, J=7Hz, 3H).	3242.3, 3176.8, 2978.1, 1705.1, 1440.8, 1253.7, 1109.1, 783.1.	475 (100); 308 (86.4); 1683.9, 1651.1, 186 (5.3); 144 (22.6).	63.12 (63.15)	5.12 (5.29)	14.61 (14.73)	$C_{25}H_{25}N_5O_5$
12	Ph	b	7.75 (br s, 1H); 7.45 (s, 5H); 7.3 (d, J=2Hz, 1H); 7.0 (d, J=2Hz, 1H); 6.8 (d, J=2Hz, 1H); 6.7 (d, J=2Hz, 1H); 6.5 (d, J=2Hz, 1H); 6.3 (d, J=2Hz, 1H); 4.2 (q, J=6Hz, 2H); 3.75 (s, 9H); 2.6 (s, 3H); 1.3 (s, 3H).	3292.5, 3130.5, 2981.9, 1703.1, 1666.5, 1651.1, 1583.6, 1550.8, 1469.8, 1442.8, 1408.0, 1253.7, 1111.0, 779.2.	FAB - 598 (7.6,m+1); 552 (0.2); 430 (30); 308 (29.6); 186 (17.8); 144 (76.3).	60.4 (60.47)	5.00 (5.4)	15.45 (15.92)	$C_{31}H_{31}N_7O_6 \cdot H_2O$

a) CI, unless otherwise noted b) TLC -- R_f 0.18 (SiO_2, EtOAc)

up to multigram quantities. Thus, one of the critical bond forming reactions for further work in the development of isoxazolyl-prodrugs has been demonstrated by what we feel is an improved method.

CRYSTAL STRUCTURE OF (7b)

Figure 4 shows the thermal ellipsoids of the (7b). The crystallographic data is summarized in Table 2. As in other 3-phenylisoxazoles, the benzene ring is tilted vs. the isoxazole by 31.2°. Both rings are planar with C(18) (-0.0094Å, benzene) and N(1) (-0.0056Å, isoxazole) showing the highest deviation from planarity. The conformation of the amide with respect to the isoxazole ring is such that the carbonyl oxygen is syn with respect to the C-5 methyl group, C(19), (C(3)-C(2)-C(4)-O(2) dihedral angle, -53.7°) but anti with respect to the 3-phenyl substituent and almost synperiplanar with respect to the pyrrole ring (-5.0°). On the other hand, the amide nitrogen, N(2), is anti with respect to the carbon-carbon double bond of the isoxazole C(3)-C(2)-C(4)-N(2) dihedral angle, (128.7°) but syn relative to the carbon-carbon single bond (-53.5°).

Both ester and amide show a high degree of delocalization, as evidenced by the C-O and C-N bond lengths. The C(4)-N(2) bond length is 1.34Å as compared to 1.32Å for a typical double bond and 1.41Å for a C-N single bond. The C(10)-O(4) ether bond length of the ester is 1.33Å as compared to 1.19Å for a double bond and 1.48Å for a carbon-oxygen single bond.

The carbonyl oxygen of the amide is in close enough proximity to one of the hydrogens of the isoxazole methyl group to allow hydrogen bonding (O(2) to C(19), 2.86Å).

The ethyl group of the ester is disordered. Since the disorder only affected the ethyl group, it can be stated with confidence that the carbonyl group is virtually antiperiplanar with the pyrrole ring (O(3)-C(10)-C(8)-C(7) dihedral angle 176.1°) but completely synperiplanar with respect to the N-methyl group, C(9). Lists of atomic coordinates and isotropic thermal parameters, bond lengths, bond angles, anisotropic thermal parameters, and H-atom coordinates are given in Tables 2-7, respectively.

It has been found that substitution for pyrrole by other heterocycles (i.e., imidazole,[2c] pyridine[3b]) changes the sequence specificity of the lexitropsin from A-T to G-C if the pyrrole C-3 hydrogen is replaced by a group bearing a lone pair. This had been earlier predicted by Dickerson based on isohelical analysis and the crystal structure of a netropsin-oligonucleotide complex.[3d] Rapoport had observed that methyl substituents at the pyrrole C-3 reduced biological activity, presumably by interrupting the hydrogen bonding required for

Table 2. Crystallographic Data for (7b)

Formula	$C_{19}H_{19}N_3O_4$
Molecular Weight	353.38
Diffractometer	Nicolet R3/m
Crystal Class	monoclinic
a,	23.104 (14)
b,	7.144 (1)
c,	25.299 (8)
β, deg.	119.45 (3)
V, A^3	3636.25
Z	8
D, g cm^{-1}	1.29
Space Group	C 2/c
μ, cm^{-1}	0.86
F(000)	1488
T, °K	298
2 Θ range, deg.	3-40
no. of measured reflections	1260
no. of unique reflections with $F > 3\sigma(F)$	1225
Largest peaks on final Fourier map, e-/A^3	+0.35, -0.27
R=	0.068
Rw=	0.068
Δ/σ	0.041

Figure IV. Thermal ellipsoids for (7b)

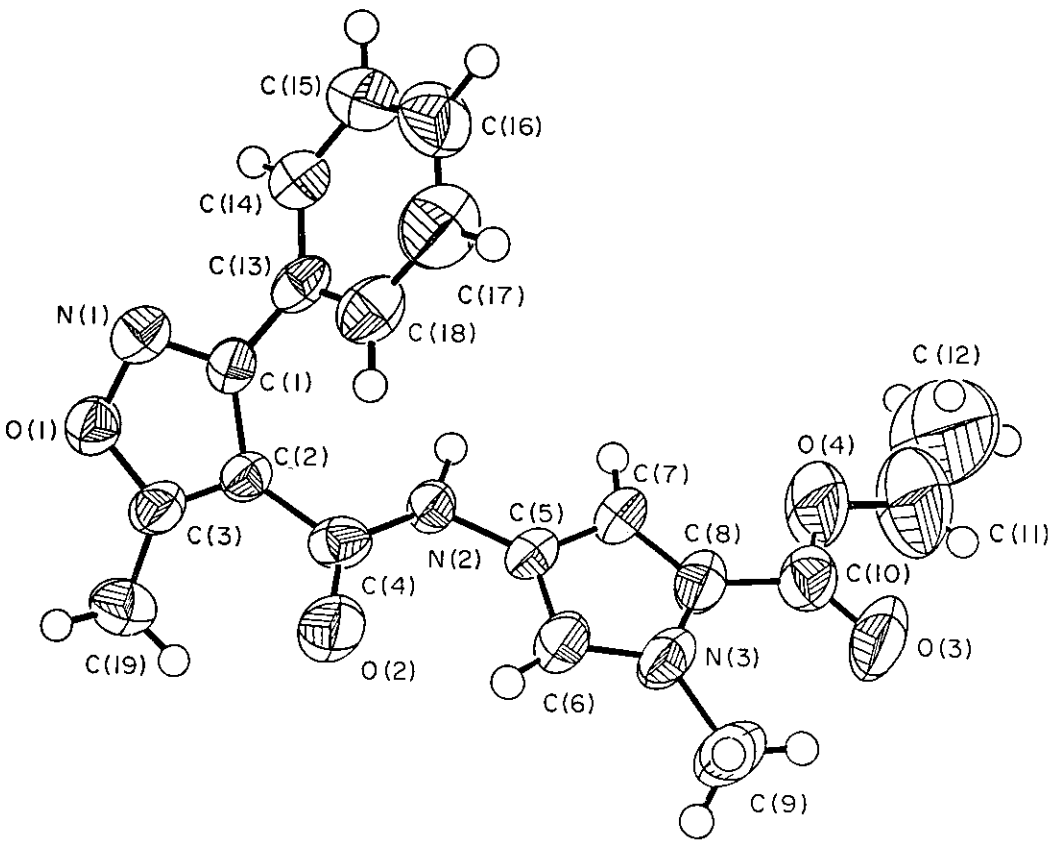


Table 3. Atomic coordinates and isotropic thermal parameters

	x	y	z	U
O(1)	1865(2)	5201(5)	2947(2)	63(2)*
N(1)	2444(2)	5067(6)	2896(2)	59(2)*
C(1)	2259(3)	5455(7)	2326(2)	46(3)*
C(2)	1567(3)	5816(7)	1991(2)	46(3)*
C(4)	1152(3)	6354(8)	1337(2)	54(3)*
N(2)	1380(2)	7845(6)	1176(2)	48(2)*
C(5)	1086(3)	8614(7)	585(2)	50(3)*
C(6)	552(3)	7947(8)	56(2)	59(3)*
C(7)	1323(3)	10216(8)	432(2)	58(3)*
C(3)	1346(3)	5641(7)	2396(2)	56(3)*
C(19)	686(3)	5796(9)	2355(3)	77(4)*
C(13)	2762(3)	5403(7)	2123(2)	52(3)*
C(15)	3892(3)	5748(9)	2342(3)	81(4)*
O(4)	1410(3)	13274(8)	-228(2)	128(4)*
C(9)	-77(3)	8842(9)	-1026(2)	85(3)*
N(3)	456(2)	9118(7)	-393(2)	61(2)*
C(8)	917(3)	10517(8)	-167(2)	62(3)*
C(14)	3420(3)	5834(8)	2523(3)	63(3)*
C(16)	3722(3)	5196(9)	1765(3)	92(4)*
C(17)	3072(4)	4759(10)	1361(3)	91(4)*
C(18)	2588(3)	4827(8)	1538(3)	67(3)*
C(10)	943(4)	12027(11)	-552(3)	80(4)*
C(11)	1426(7)	14917(19)	-570(6)	191(12)*
C(12)	1906(7)	15529(25)	-443(8)	302(16)*
O(3)	580(2)	12160(7)	-1095(2)	105(3)*
O(2)	646(2)	5504(5)	1000(2)	87(2)*

Equivalent isotropic U defined as one third of the trace of the orthogonalized tensor

Table 4. Bond Lengths

O(1)-N(1)	1.409(7)
O(1)-C(3)	1.354(5)
N(1)-C(1)	1.317(6)
C(1)-C(2)	1.417(7)
C(1)-C(13)	1.483(10)
C(2)-C(4)	1.498(6)
C(2)-C(3)	1.356(10)
C(4)-N(2)	1.336(8)
C(4)-O(2)	1.218(6)
N(2)-C(5)	1.414(6)
C(5)-C(6)	1.385(6)
C(5)-C(7)	1.402(8)
C(6)-N(3)	1.338(7)
C(7)-C(8)	1.353(6)
C(3)-C(19)	1.482(10)
C(13)-C(14)	1.386(7)
C(13)-C(18)	1.392(8)
C(15)-C(14)	1.376(12)
C(15)-C(16)	1.370(10)
O(4)-C(10)	1.326(9)
O(4)-C(11)	1.469(17)
C(9)-N(3)	1.477(6)
N(3)-C(8)	1.364(8)
C(8)-C(10)	1.474(10)
C(16)-C(17)	1.374(8)
C(17)-C(18)	1.395(13)
C(10)-O(3)	1.210(7)
C(11)-C(12)	1.084(23)

Table 5. Bond Angles

N(1)-O(1)-C(3)	108.8(5)
O(1)-N(1)-C(1)	105.9(4)
N(1)-C(1)-C(2)	110.9(6)
N(1)-C(1)-C(13)	119.0(4)
C(2)-C(1)-C(13)	130.1(4)
C(1)-C(2)-C(4)	128.9(6)
C(1)-C(2)-C(3)	105.3(4)
C(4)-C(2)-C(3)	125.8(5)
C(2)-C(4)-N(2)	114.0(4)
C(2)-C(4)-O(2)	121.2(6)
N(2)-C(4)-O(2)	124.8(5)
C(4)-N(2)-C(5)	125.2(4)
N(2)-C(5)-C(6)	128.6(5)
N(2)-C(5)-C(7)	124.0(4)
C(6)-C(5)-C(7)	107.3(4)
C(5)-C(6)-N(3)	107.6(5)
C(5)-C(7)-C(8)	106.8(5)
O(1)-C(3)-C(2)	109.1(5)
O(1)-C(3)-C(19)	116.7(6)
C(2)-C(3)-C(19)	134.2(4)
C(1)-C(13)-C(14)	120.5(5)
C(1)-C(13)-C(18)	120.6(5)
C(14)-C(13)-C(18)	118.8(7)
C(14)-C(15)-C(16)	120.5(6)
C(10)-O(4)-C(11)	114.9(6)
C(6)-N(3)-C(9)	122.6(5)
C(6)-N(3)-C(8)	109.5(4)
C(9)-N(3)-C(8)	127.9(5)
C(7)-C(8)-N(3)	108.7(5)
C(7)-C(8)-C(10)	128.7(6)
N(3)-C(8)-C(10)	122.5(4)
C(13)-C(14)-C(15)	120.8(6)
C(15)-C(16)-C(17)	119.7(8)
C(16)-C(17)-C(18)	120.5(7)
C(13)-C(18)-C(17)	119.6(5)
O(4)-C(10)-C(8)	111.7(5)
O(4)-C(10)-O(3)	123.1(7)
C(8)-C(10)-O(3)	125.2(6)
O(4)-C(11)-C(12)	117.8(13)

Table 6. Anisotropic thermal parameters

O(1)	60(3)	78(3)	51(2)	13(2)	29(2)	10(2)
N(1)	59(3)	54(3)	57(3)	8(2)	23(3)	11(3)
C(1)	55(4)	41(3)	36(3)	2(2)	18(3)	-4(3)
C(2)	43(4)	50(4)	37(3)	5(3)	12(3)	-1(3)
C(4)	45(4)	47(4)	58(4)	-6(3)	17(3)	-5(3)
N(2)	48(3)	41(3)	44(2)	3(2)	14(2)	-10(2)
C(5)	45(4)	55(3)	39(3)	5(3)	14(3)	5(3)
C(6)	58(4)	68(4)	44(3)	10(3)	19(3)	7(3)
C(7)	55(4)	70(4)	41(3)	8(3)	16(3)	3(3)
C(3)	51(4)	51(4)	54(3)	11(3)	15(3)	4(3)
C(19)	62(4)	85(5)	88(4)	10(4)	40(4)	4(4)
C(13)	54(4)	40(3)	48(3)	9(3)	15(3)	12(3)
C(15)	66(5)	94(5)	86(5)	3(4)	40(4)	0(4)
O(4)	139(5)	111(4)	112(4)	47(4)	45(4)	-28(4)
C(9)	71(5)	110(5)	50(3)	4(4)	10(3)	7(4)
N(3)	60(3)	78(4)	34(2)	5(3)	13(2)	9(3)
C(8)	70(5)	65(4)	49(3)	14(3)	28(3)	10(4)
C(14)	56(4)	71(4)	57(4)	9(3)	24(3)	6(4)
C(16)	95(5)	100(5)	112(5)	20(4)	74(5)	27(4)
C(17)	109(6)	100(5)	72(4)	-3(4)	51(5)	29(5)
C(18)	75(5)	68(4)	50(4)	-5(3)	23(4)	7(4)
C(10)	75(5)	94(5)	82(5)	34(4)	46(4)	28(5)
C(11)	226(18)	161(11)	193(12)	83(9)	108(13)	-29(11)
C(12)	220(20)	267(19)	323(21)	181(15)	59(17)	-60(17)
O(3)	122(4)	125(4)	66(2)	41(3)	46(3)	39(3)
O(2)	77(3)	88(3)	60(2)	4(2)	7(2)	-37(3)

Table 7. H-Atom coordinates and isotropic thermal parameters

	x	y	z	U
H(2)	1749	8689	1374	60
H(6)	339	6823	80	67
H(7)	1705	10844	746	68
H(19A)	357	6090	1946	89
H(19B)	697	6771	2621	89
H(19C)	576	4629	2472	89
H(15)	4345	6077	2622	60
H(9A)	-55	9817	-1277	97
H(9B)	-501	8880	-1043	97
H(9C)	-20	7649	-1169	97
H(14)	3549	6197	2932	60
H(16)	4054	5116	1644	60
H(17)	2950	4403	953	60
H(18)	2137	4479	1258	60
H(11A)	1195	15900	-491	169
H(11B)	1193	14619	-995	169
H(12A)	1859	16526	-715	251
H(12B)	2162	14535	-481	77(21)
H(12C)	2128	15988	-33	251

minor groove binding.[3f] The crystal structure of the isoxazole-pyrrole is noteworthy, since in the s-cis conformation, it appears that the amide function would be shielded from the minor groove and, therefore, prevent hydrogen bonding to the A-T rich regions of double helical DNA until after metabolic release of the isoxazole. Further studies on the chemistry and biology of isoxazoles are underway.

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EXPERIMENTAL SECTION

Phosphonitrilic dichloride cyclic trimer was obtained from Shinnisso Kako Company and used as received. 3,5-Dimethylisoxazolecarboxylic acid was prepared according to McMurry and purified by sublimation.[8] 5-Methyl-3-phenylisoxazole-4-carboxylic acid was obtained from Aldrich and recrystallized from toluene before use. Ethyl N-methylpyrrole-2-carboxylate was prepared from N-methylpyrrole essentially by the method of Bailey.[9] Ethyl 4-nitropyrrole-2-carboxylate was prepared according to Grehn;[10] the corresponding acid was prepared according to Grokhovskii and co-workers.[11] Reactions were performed under inert atmosphere, the inert gas was purified by percolation through activated BASF catalyst R3-11, followed by indicator Drierite. THF was distilled immediately before use from sodium and benzophenone. Chromatography was performed on silica gel on a Harrison Research Associates Chromatotron. Chromatography solvents were distilled prior to use. Hydrogenations were performed in a Fischer-Porter aerosol dispersion tube with a pressure gauge/safety release valve assembly, according to Odle and Hegedus.[12] Proton nmr was performed either on a Jeol FX90Q at 90MHz or on an IBM AF300 at 300 MHz. 2D COSY, NOSY and CP-MAS were performed on the IBM. Mass spectra were obtained on a VG Micromass 7070 mass spectrometer. EI: electron impact; CI: chemical ionization; FAB: fast atom bombardment. Infrared spectra were obtained on a Digilab FTS-80 Fourier Transform ir, using diffuse reflectance or photoacoustic spectroscopy detection, unless otherwise indicated. Elemental analyses were performed by Desert Analytics, Tucson, AZ. Crystallography was performed at the Washington State University x-ray facility, as previously described.[13]

An aerosol dispersion tube was charged with ethyl-N-methyl-4-nitropyrrole-2-carboxylate (5) (9 g, 45 mmol), 10% palladium on charcoal (3 g) and dry THF (250 ml). The vessel was evacuated and placed under 50 psi of hydrogen, after 5 h tlc indicated the absence of starting material. The slurry was filtered through Celite, and the resulting solution was placed under a nitrogen atmosphere. To the solution were added triethylamine (36 ml), 3,5-dimethyl-isoxazole-4-carboxylic acid (10 g in 20 ml of THF) and $(NPCl_2)_3$ (17.5 g in 20 ml of THF). The solution was allowed to stir for 20 h, after which time the THF was concentrated in vacuo. The residue was triturated with cold 10% aqueous hydrochloric acid (100 ml) and extracted with chloroform (5 x 100 ml). The combined organic extracts were washed with water (2 x 100 ml) and dried over anhydrous sodium sulfate. Filtration and concentration gave isoxazole-pyrrole-ester (**7a**) as a solid (10.5 g, 80%). The product after extractive isolation is usually of suitable purity for subsequent synthetic steps. An analytical sample of (**7a**) was obtained by chromatography on silica gel. Analytical data is shown in Table 1.

The Isoxazolepyrroleester (**7a**) (809.8 mg, 2.79 mmol) and 5N aqueous sodium hydroxide (1 ml) in 60% aqueous ethanol (9 ml) were warmed to 90°C for 45 mins, after which time tlc indicated absence of the ester (**7a**). The solution was cooled, the ethanol was concentrated in vacuo, and the pH was adjusted to <2 with cold 10% aqueous hydrochloric acid. The resulting white solid was filtered and dried in vacuo. The yield of isoxazole-pyrrole-acid (13) was 76% (559 mg). Tlc (SiO_2) Rf 0.212 (EtOAc) mp 202-4°C.

Nitro-pyrrole-propiononitrile (**16b**) (545 mg 2.45 mmol) was placed in an aerosol dispersion tube with 10% Palladium on Charcoal (606 mg) and dry THF (50 ml). The vessel was evacuated and placed under 15 psi of hydrogen. After 20 h tlc indicated that reaction was complete, and the slurry was filtered through Celite and placed under a nitrogen atmosphere. To the above solution was added isoxazole-pyrrole-acid (**13**) (502.7 mg, 2.139 mmol) in THF (20 ml); triethylamine (2 ml, 14 mmol) and $(NPCl_2)_3$ (855 mg, 2.5 mmol) in 20 ml of THF. The mixture was stirred for 2 h, after which time the THF was concentrated in vacuo, cold 10% aqueous hydrochloric acid was added (75 ml), and the mixture was extracted with chloroform (5 x 50 mL). The combined organic extracts were washed with water (50 ml), cold 2N aqueous sodium hydroxide (50 ml), water (50 ml) and dried over anhydrous sodium sulfate. The mixture was filtered, concentrated in vacuo and chromatographed on silica gel (ptlc, Rf 0.25 (EtOAc)) to give the isoxazole-dipyrrole (**17b**) as a transparent glass (609 mg, 57% overall from nitro-pyrrole-propiononitrile (**16**)). FAB - m/z 438 (relative intensity 2.91%, $M+1^+$); 368 (1.93), 246 (5.96), 124 (5.5), 82 (2.2). ^1H - Nmr 8.1 (brs,1H); 8.0 (br.s., 1H); 7.2 (d, 1H, J=$2H_z$);

7.15 (d, 1H, J=2Hz); 6.8 (brs, 1H); 6.7 (d, 1H, J=2Hz); 6.5 (d,1H,J=2Hz); 3.9 (s, 3H); 3.8 (s, 3H); 3.4 - 3.6 (m, 2H); 2.6 (t, J=6Hz,2H); 2.55 (s, 3H); 2.4 (s, 3H). Ir - 3300, 3100, 2940, 2240 cm^{-1} (-CN) 1630. Anal. Calcd for $C_{21}H_{23}N_7O_4$: C, 57.66; H, 5.29; N, 22.4. Found: C, 57.95;H, 5.02;N, 22.31.

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