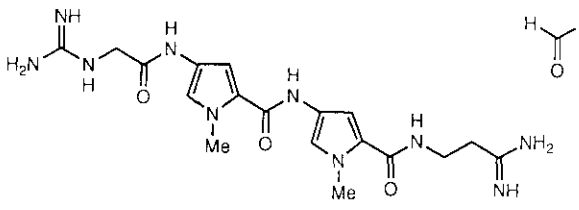
SYNTHESIS OF NOVEL 1,2,4-TRIAZOLE-CONTAINING OLIGOPEPTIDES

Toyomi Matsumoto, Kouhei Toyooka, Eiji Nishiwaki, and Masayuki Shibuya*
Faculty of Pharmaceutical Sciences, University of Tokushima,
Sho-machi 1, Tokushima 770, Japan

Abstract–Novel 1,2,4-triazole-containing oligopeptides (**12** ~ **17**) were synthesized from 1-methyl-3-nitro-1,2,4-triazole-5-carboxylic acid (**10**).

Low molecular weight substances which possess nonintercalative binding ability to double-stranded B-DNA are of particular interest. To date, oligo-N-methylpyrrolecarboxamides such as netropsin (**1**) and distamycin (**2**) and their derivatives have attracted attention because not only of their noteworthy biological activities but also of their strong minor groove nonintercalative binding ability to double-stranded B-DNA at specific AT rich region.[1] This has led to the development of synthetic analogues of natural oligopeptides for a search of the substances valuable as tools in molecular biology and as potential therapeutic agents.[2] Several oligo-N-methylpyrrole-carboxamide analogues possessing various kinds of heterocyclic moieties capable of specific DNA recognition by hydrogen bond formation have been synthesized. These include imidazole,[3] thiazole,[4] and isoxazole derivatives.[5] Here we report the synthesis of novel 1,2,4-triazole-containing oligopeptides (**12** ~ **17**).



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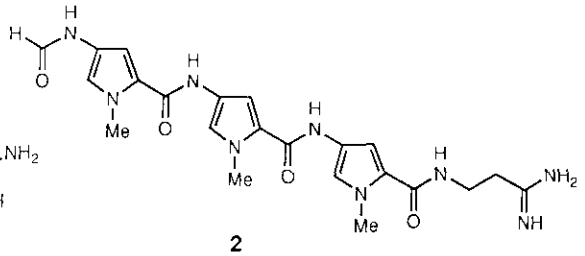

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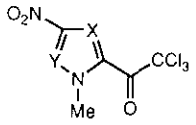
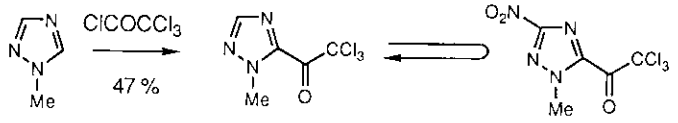
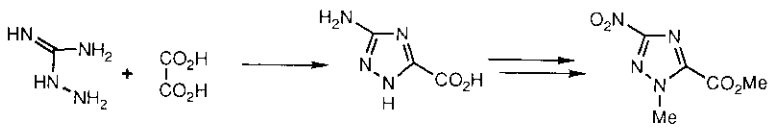


3: X = Y = CH
4: X = N, Y = CH
5: X = Y = N



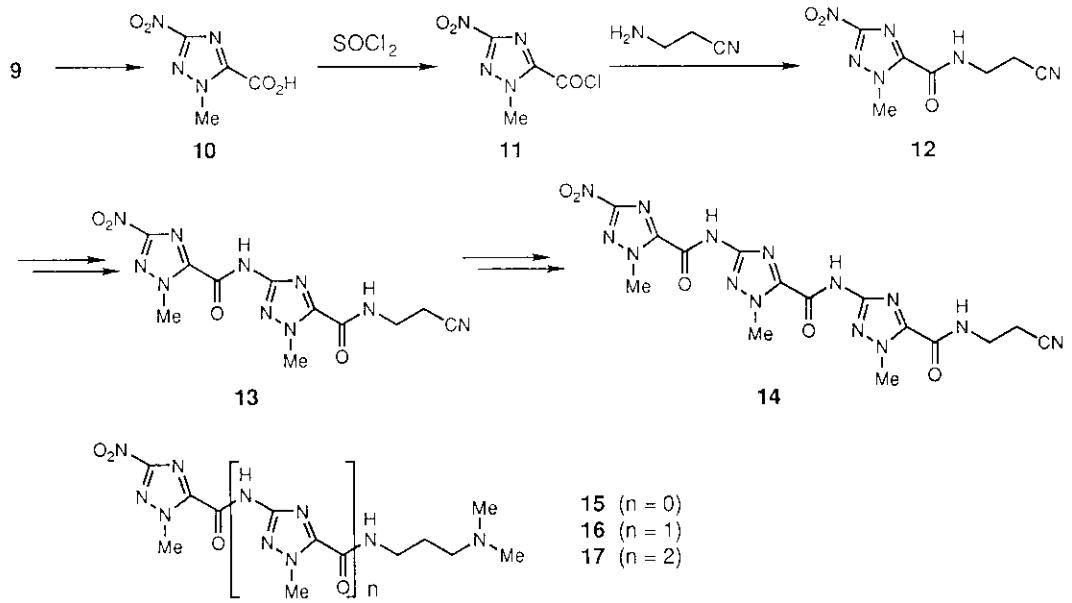
6 **7** **5**



8 **9**

In the synthesis of the oligopeptides (**12** ~ **17**), the key problem is the preparation of a triazole synthon that contains elements for the oligomer linking amide moiety. In a previous work, we have demonstrated the development of 1-methyl-4-nitro-2-trichloroacetylpyrrole (**3**) and 1-methyl-4-nitro-2-trichloroacetylimidazole (**4**) and their versatility for the syntheses of oligopeptide antibiotics and their analogues.[6] Although the trichloroacetylation process of the previous synthesis was applicable to 1-methyl-1,2,4-triazole (**6**)[7] affording the trichloroacetyltriazole (**7**),[8] the following nitration failed in various reaction conditions to recover the starting trichloroacetyltriazole. We decided therefore to utilize the nitro ester (**9**) which could be synthesized from aminoguanidine via the amino acid (**8**) by the method known in the literature.[9]

Hydrolysis of the ester (**9**) with sodium hydroxide afforded the carboxylic acid (**10**), which was then treated with thionyl chloride to give the acid chloride (**11**) in 80% overall yield from **9**. Condensation of the acid chloride (**11**) with 3-aminopropiononitrile in dichloromethane afforded the amide (**12**) in 87 % yield. The nitro group of the amide (**12**) was reduced to the amine which was condensed with **11** to give the dipeptide (**13**). The reduction-condensation process was then repeated, leading to the tripeptide (**14**). Similarly, the peptides (**15** ~ **17**) were also synthesized by employing 3-dimethylaminopropylamine in place of 3-aminopropiononitrile. Investigations on DNA cleaving activities of the oligopeptides (**12** ~ **17**) under near uv (UV-A) light[10] and their affinity efficiencies on DNA are now in progress.



EXPERIMENTAL

Melting points were determined by the capillary method and were uncorrected. Infrared (Ir) spectra were determined with a Hitachi 215 spectrophotometer and ^{1}H-nmr spectra were measured with a JEOL JNM FX-200 spectrometer using tetramethylsilane as an internal reference. Mass spectra were recorded on a JEOL JMS-D 300 mass spectrometer generally at 20 ev using a direct insertion probe.

1-Methyl-5-trichloroacetyl-1,2,4-triazole (7). A solution of 1-methyl-1,2,4-triazole (**6**)[7] (1.000 g, 12.05 mmol) in CH_2Cl_2 (5 ml) was added dropwise to a solution of trichloroacetyl chloride (1.35 ml, 12.05 mmol) in CH_2Cl_2 (15 ml) at room temperature, and the mixture was stirred overnight. The resulting suspension was cooled to 0°C, and a solution of Et_3N (1.68 ml, 12.05 mmol) in CH_2Cl_2 (2 ml) was added in one portion and then the mixture was stirred at the same temperature for 2 h. Upon evaporation of the solvent, the residue was chromatographed on silica gel column (10:1 $CHCl_3$/acetone) to give the trichloroacetate (**7**, 1.300 g, 47 %) as a colorless solid; mp 110-111°C. Ir (KBr): 1720 cm^{-1}; ^1H nmr (200 MHz, $CDCl_3$):δ 4.30 (3H, s) and 8.10 (1H, s) ppm. Anal. Calcd for $C_5H_4N_3OCl_3 \cdot H_2O$: C,24.37; H,2.45; N,17.05. Found: C,24.31; H,2.39; N,17.13. The trichloroacetate (**7**) was further identified as methyl 1-methyl-1,2,4-triazole-5-carboxylate. To a stirred solution of **7** (200 mg, 0.875 mmol) in MeOH (2 ml), a catalytic amount of NaOMe was added at 0°C. The reaction mixture was warmed up to room temperature, stirred for 10 min, and then concentrated *in vacuo*. The residue was purified with column chromatography on silica gel (10:1 $CHCl_3$/acetone) to give the ester (121 mg, 98 %); mp 74-75°C (lit.[8] mp 74-75°C). Spectroscopic data of this compound were identical with the reported values of methyl 1-methyl-1,2,4-triazole-5-carboxylate.[8]

1-Methyl-3-nitro-1,2,4-triazole-5-carboxylic acid (10). Aqueous solution of NaOH (10 %, 18.1 ml) was added to a stirred suspension of the ester (**9**, 7.0 g, 37.6 mmol) in H_2O (35 ml) at room temperature, and the stirring was continued for 30 min. The reaction mixture was acidified by adding 6N HCl at 0 °C and the precipitated solid was collected and washed with water. The crude solid was recrystallized from MeOH to give the acid (**10**, 5.2 g, 80 %) as colorless plates; mp 109-111°C. Ir (KBr): 1710, 1560, and 1320 cm^{-1}; ^1H nmr (200 MHz, DMSO-d_6):δ 4.23 (3H, s) and 7.45 (1H, br, CO_2H) ppm. Anal. Calcd for $C_4H_4N_4O_4$: C,27.92; H,2.34; N,32.55. Found: C,28.17; H,2.21; N,32.80.

1-Methyl-3-nitro-1,2,4-triazole-5-carbonyl chloride (11). A mixture of the acid (**10**, 3.44 g, 20 mmol), thionyl chloride (17.2 ml, 236 mmol), and a catalytic amount of DMF was refluxed for 30 min and then evaporated to give a colorless solid. The crude acid chloride without further purification was used for the next step.

1-Methyl-3-nitro-1,2,4-triazole-5-carboxamidopropiononitrile (12). A solution of 3-aminopropiononitrile (1.44 g, 20 mmol) and N,N-diisopropylethylamine (3.8 ml, 22 mmol) in CH_2Cl_2 (8 ml) was added dropwise to a stirred solution of the acid chloride (**11**, 3.81 g, 20 mmol) in CH_2Cl_2 (20 ml) at -20°C. The mixture was warmed up to room temperature and the stirring was continued for 30 min. The solvent was removed *in vacuo*, and then water was added to the residue. The resultant crystalline solid was collected and washed with water to afford the amide (**12**, 3.88 g, 87 %). Recrystallization of **12** from AcOEt gave an analytical sample; mp 174-176°C. Ir (KBr): 2240, 1682, 1560, 1540, and 1320 cm^{-1}; ^1H nmr (200 MHz, DMSO-d_6):δ 2.81 (2H, t, J=6.0), 3.52 (2H, q, J=6.0), 4.26 (3H, s), and 9.52 (1H, t, J=5.0, NH) ppm. Anal. Calcd for $C_7H_8N_6O_3$: C,37.50; H,3.60; N,37.49. Found: C,37.74; H,3.54; N,37.69.

1-Methyl-3-(1-methyl-3-nitro-1,2,4-triazole-5-carboxamido)-1,2,4-triazole-5-carboxamidopropiononitrile (13). A suspension of 10% Pd-C (600 mg) in a solution of **12** (2.24 g, 10 mmol) in MeOH (100 ml) was stirred for 2 h under a current of H_2 at room temperature, and then filtered. The residual catalyst was washed thoroughly with MeOH and the combined filtrate and washings were concentrated *in vacuo* to give the crude amino compound. To a stirred solution of this amine and N,N-diisopropylethylamine (1.8 ml, 10 mmol) in CH_2Cl_2 (20 ml), a solution of the acid chloride (**11**, 1.9 g, 10 mmol) in CH_2Cl_2 (10 ml) was added dropwise at -20°C. The mixture was warmed up to room temperature, and the stirring was continued for 2 h. The solvent was removed *in vacuo,* and then water was added to the residue. The resultant crystalline solid was collected and washed with water to afford the amide (**13**, 1.60 g, 46 %). Recrystallization of **13** from MeOH gave an analytical sample; mp 85-115°C. Ir (KBr): 2240, 1710, 1685, 1560, and 1320 cm^{-1}; ^1H nmr (200 MHz, $CDCl_3$): δ 2.75 (2H, t, J=6.5), 3.73 (2H, q, J=6.5), 4.28, 4.47(2X3H, s), 7.71 (1H, t, J=6.4, NH) and 9.70 (1H, br, NH) ppm. Anal. Calcd for $C_{11}H_{12}N_{10}O_4 \cdot 1/2H_2O$: C,36.98; H,3.67; N,39.20. Found: C,36.75; H,3.48; N,38.84.

1-Methyl-3-[1-methyl-3-(1-methyl-3-nitro-1,2,4-triazole-5-carboxamido)-1,2,4-triazole-5-carboxamido]-1,2,4-triazole-5-carboxamidopropiononitrile (14). A suspension of PtO_2 (10 mg) in a solution of **13** (1.5 g, 4.3 mmol) in DMF (20 ml) and MeOH (20 ml) was stirred for 3 h under a current of H_2 at room temperature. The catalyst was removed by filtration, then the solvent was removed *in vacuo.* The residual crude amino compound was acylated with **11** prepared from **10** (741 mg, 4.3 mmol), by the same procedure as that described for **13**. Recrystallization of the product from DMF gave **14** (540 mg, 27 %) as a 1:1 DMF adduct; mp 285-286°C. Ir (KBr): 2240, 1710, 1685, 1650, and 1565 cm^{-1}; ^1H nmr (200 MHz, $CDCl_3$): δ 2.80 (2H, br), 3.70 (2H, br), 4.13, (3H, s), 4.51 (6H, s), 6.95 (1H, br, NH), 8.78 (1H, br, NH), and 11.24 (1H, br, NH) ppm. Anal. Calcd for $C_{15}H_{16}N_{14}O_5 \cdot C_3H_7NO$(DMF): C,39.63; H,4.25; N,38.52. Found: C,39.95; H,4.48; N,38.80.

3-(1-Methyl-3-nitro-1,2,4-triazole-5-carboxamido)dimethylaminopropane (15). A solution of 3-dimethylaminopropylamine (562 mg, 5.5 mmol) in CH_2Cl_2 (3 ml) was added dropwise to a stirred solution of **11**, prepared from **10** (860 mg, 5 mmol), at -40°C. The mixture was warmed up to room temperature and the stirring was continued for 1 h. Precipitated solid was collected, washed with acetone, and recrystallized from MeOH to afford **15** (1.13 g, 77 %) as a HCl salt; mp 240-241°C. Ir (KBr): 1685, 1560, and 1320 cm^{-1}; ^1H nmr (200 MHz, DMSO-d_6): δ 1.95 (2H, m), 2.72 (6H, s), 3.06 (2H, t, J=8.0), 3.34 (2H, m), 4.26 (3H, s), and 9.36 (1H, br, NH) ppm. Anal. Calcd for $C_9H_{16}N_6O_3 \cdot HCl$: C,36.93; H,5.85; N,28.71. Found: C,36.69; H,5.76; N,28.47.

3-[1-Methyl-3-(1-methyl-3-nitro-1,2,4-triazole-5-carboxamido)-1,2,4-triazole-5-carboxamido]dimethylaminopropane (16). A suspension of PtO_2 (10 mg) in a solution of **15** (420 mg, 1.43 mmol) in MeOH (15 ml) was stirred for 3 h under a current of H_2 at room temperature. The catalyst was removed by filtration, then the solvent removed *in vacuo.* To a stirred solution of this amine and N,N-diisopropylethylamine (0.268 ml, 1.54 mmol) in CH_2Cl_2 (12 ml), a solution of the acid chloride (**11**), prepared from **10** (265 mg, 1.54 mmol), in CH_2Cl_2 (12 ml) was added at 0°C. The mixture was warmed up to room temperature and then the solvent was removed *in vacuo.* The residue was taken up with acetone to give a solid. Recrystallization from EtOH afforded

the amide (**16**, 321 mg, 54 %) as a HCl salt; mp 230-232°C. Ir (KBr): 1710, 1670, 1555, and 1310 cm^{-1}; ^1H nmr (200 MHz, DMSO-d_6): δ 2.05 (2H, m), 2.90 (6H, s), 3.20, (2H, t J=8.0), 3.49 (2H, m), 4.21, 4.38 (2X3H, s), 8.99 (1H, br, NH) and 10.30 (1H, br, NH) ppm. Anal. Calcd for $C_{13}H_{20}N_{10}O_4 \cdot HCl \cdot 1/4H_2O$: C,37.06; H,5.14; N,33.24. Found: C,37.42; H,5.13; N,32.84.

3-{1-Methyl-3-[1-methyl-3-(1-methyl-3-nitro-1,2,4-triazole-5-carboxamido)-1,2,4-triazole-5-carboxamido]-1,2,4-triazole-5-carboxamido}dimethylaminopropane (17). A suspension of PtO_2 (10 mg) in a solution of **16** (208 mg, 0.5 mmol) in DMF (10 ml) and MeOH (10 ml) was stirred for 3 h under a current of H_2 at room temperature. The catalyst was removed by filtration, then the solvent was removed *in vacuo*. The residual crude amino compound was acylated with **11**, prepared from **10** (129 mg, 0.75 mmol), by the same procedure as that described for **16**. Recrystallization of the product from EtOH afforded the amide (**17**, 60 mg, 22 %) as a colorless powder; mp 173-176°C. Ir (KBr): 1710, 1675, 1560, and 1320 cm^{-1}; ^1H nmr (200 MHz, DMSO-d_6): δ 1.90 (2H, m), 2.73 (6H, s), 3.06 (2H, t, J=7.0), 3.43 (2H, m), 4.15 (6H, s), 4.27 (3H, s), 9.01 (1H, br, NH), 10.00 (1H, br, NH) and 11.05 (1H, br, NH) ppm. Ms m/z Calcd for $C_{17}H_{24}N_{14}O_5(M^+)$: 504.2051. Found: 504.2043.

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8. The structure of **7** was confirmed by leading it to known methyl 1-methyl-1,2,4-triazole-5-carboxylate.[7]
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