Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1313–1316

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

DESIGN AND SYNTHESIS OF ISOXAZOLE AND ISOXAZOLINE LINKAGE FOR REPLACEMENT OF NUCLEOTIDE PHOSPHODIESTER

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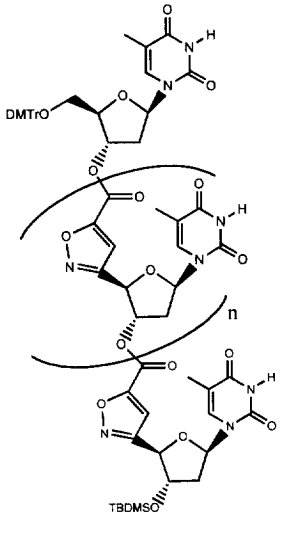
Received 20 March 1998; accepted 16 April 1998

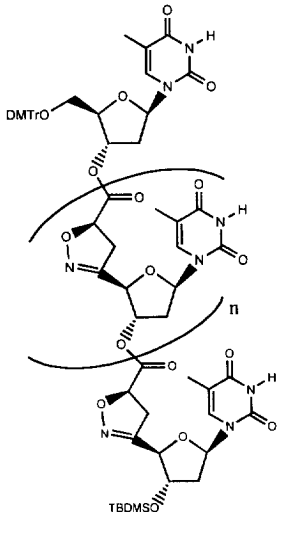
Abstract : Synthetic routes for oligonucleotides which have isoxazoline or isoxazole linkage have been developed, and di- and tri-nucleotides with thymine bases have been prepared.

Modified oligonucleotides at the phosphodiester linkage are important class of compounds especially in the field of antisense and antigene oligonucleotides.[1] We are interested in the replacement of the phosphodiester linkage of the natural oligonucleotide with novel, neutral, and heterocyclic linkages based on their stability and regidity and report here an efficient synthesis of the monomeric units, and facile chain-extension steps to prepare di- and tri-nucleotides **1-4**.



1, n = 0

2, n = 1



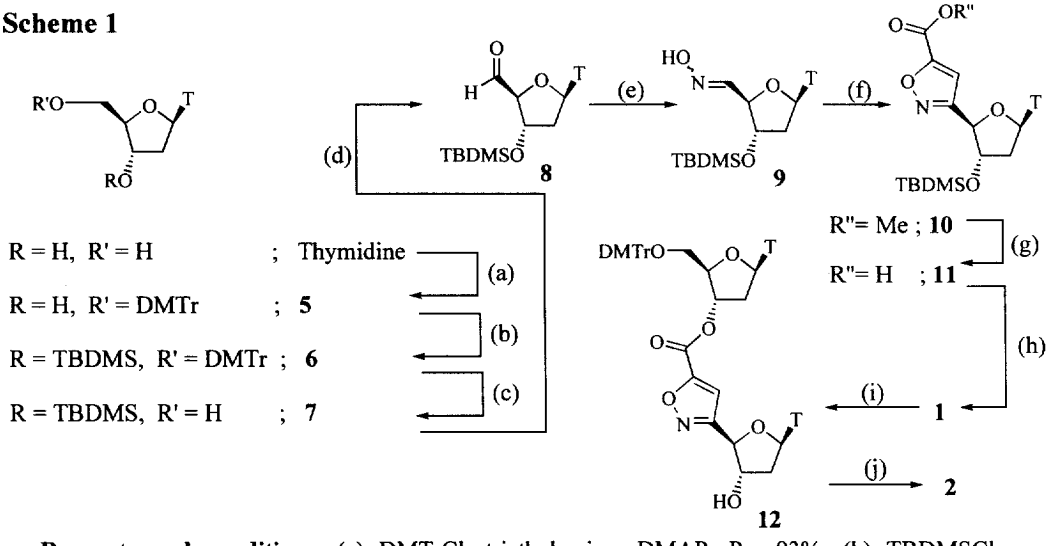
3, n = 0

4, n = 1

PII: S0960-894X(98)00210-8

The synthesis of the dimer **1** and trimer **2** is depicted in Scheme 1. Selective protection of 5′-hydroxyl group of thymidine with 4,4′-dimethoxytrityl (DMTr) group followed by silylation of 3′-hydroxyl group with *t*-butyl dimethyl silyl chloride (TBDMSCl) provided the fully protected compound **6**. Selective deprotection of 5′-position with $ZnBr_2$, and the modified Moffatt oxidation with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) gave the key intermediate **8**.[2] The aldehyde **8** was further reacted with hydroxylamine hydrochloride to give the mixture of *syn* and *anti* oxime **9**. Nitrile oxide was *in situ* generated from the crude mixture of oxime **9** using 4% aqueous NaOCl solution and cycloadded with methyl propiolate to afford the cycloadduct **10** and its *N*-chlorinated compound. This *N*-chlorinated compound was easily converted to the desired product **10** by the treatment with methyl sulfide. The monomeric building block **11** was prepared by hydrolysis of the ester **10** using $LiOH{\cdot}H_2O$. Coupling between the monomer **11** and 5′-DMTr thymidine using 2,4,6-trichlorobenzoyl chloride (TBC)[3] provided the dinucleotide analog **1**[4] in 8 steps (overall yield=31%) from thymidine. Deprotection of 3′-TBDMS group with tetrabutyl ammonium fluoride (TBAF) followed by repetitive coupling with the monomeric unit **11** provided trinucleotide analog **2**[4] in 10 steps (overall yield=15%) from thymidine. There is every reason to believe that further elaboration of the oligonucleotides beyond the trimer stage will be possible through the deprotection/coupling sequences in repetitive fashion.

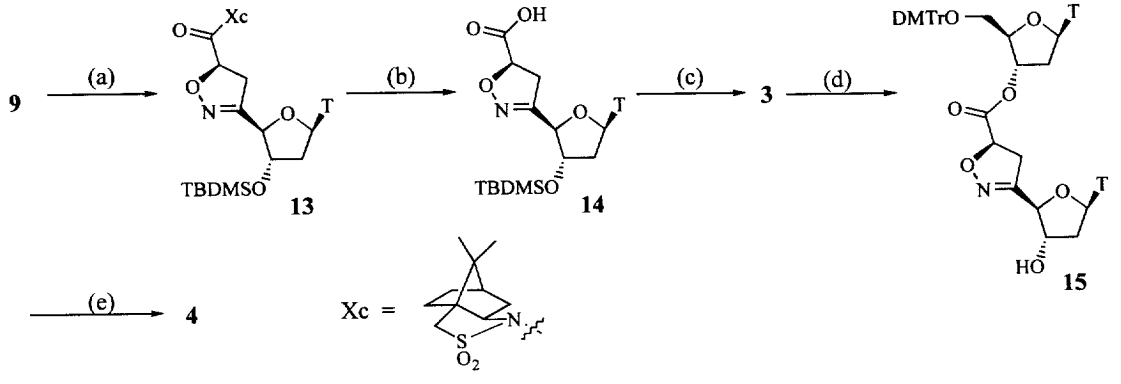
Scheme 1



Reagents and conditions: (a) DMTrCl, triethylamine, DMAP, Py, 93%; (b) TBDMSCl, diisopropylethylamine, DMF, 97%; (c) $ZnBr_2$, $CHCl_3$/MeOH (9:1), 89%; (d) EDC, DMSO, Py, Trifluoroacetic acid; (e) $NH_2OH{\cdot}HCl$, Na_2CO_3, MeOH/H_2O (1:1), 70% [(d) and (e), overall]; (f) 1. NaOCl, Methyl propiolate, CH_2Cl_2, (0°C), 2. CH_3SCH_3, 70% (g) $LiOH{\cdot}H_2O$, THF/H_2O (5:1), 89%; (h) **5**, TBC, DMAP, CH_2Cl_2, (0°C), 88%; (i) TBAF, THF, (0°C), 60%; (j) **11**, TBC, DMAP, DMSO, CH_2Cl_2, (0°C), 82%.

Synthesis of oligonucleotides **3** and **4** which possess a novel, neutral and chiral internucleoside linkage is outlined in Scheme 2. Starting from the oxime **9** prepared through the previous synthetic route in Scheme 1, diastereoselective cycloaddition of *in situ* generated nitrile oxide to *N*-acryloyl (2*R*)-bornane-10,2-sultam was carried out.[5] The major cycloadduct **13** with *R* configuration in the 5 position of isoxazoline ring was purified by recrystallization of the 90:10 diastereomeric mixture from dichloromethane-diethyl ether-hexane (1:1:1) solution. Removal of the chiral auxiliary from compound **13** afforded the enantiomerically pure monomeric unit **14** in good yield. As previously described, coupling between the monomer **14** and 5′-DMTr thymidine **5** provided the dinucleotide analog **3**[4] in 3 steps (overall yield=53%) from the oxime **9**. Further deprotection and repetitive coupling afforded the trinucleotide analog **4**[4] in 5 steps (overall yield=38%) from the oxime **9**.

Scheme 2



Reagents and conditions: (a) 1. NaOCl, *N*-acryloyl (2*R*)-bornane-10,2-sultam, CH_2Cl_2, (0°C), 2. CH_3SCH_3, 72%; (b) $LiOH{\bullet}H_2O$, THF/H_2O (5:1), 100%; (c) **5**, TBC, DMAP, CH_2Cl_2, (0°C), 74%; (d) TBAF, THF, (0°C), 74%; (e) **14**, TBC, DMAP, DMSO, CH_2Cl_2, (0°C), 96%.

In summary, we have developed efficient synthetic routes for oligonucleotides with isoxazole/isoxazoline heterocyclic linkage. These synthetic pathways which consist of high yielding monomer formation and chain extension provide a unique oligonucleotide system with heterocyclic linkage for the first time. The resulting oligonucleotides should be useful as bioactive compounds and their biological activities will be reported in due course.

Acknowledgments:

We are grateful to the Ministry of Education (BSRI-97-3437) for financial support. We thank Mr. Ki Hwan Ko for the exploratory experiments.

References and Notes:

1 (a) Uhlmann, E.; Peyman, A. *Chem. Rev*, **1990**, *90*, 543. (b) De Mesmaeker, A.; Häner, R.; Martin, P.; Moser, H. E. *Acc. Chem. Res.*, **1995**, *28*, 366. (c) Egli, M. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1894.

2 (a) Wang, G.; Middleton, P. J. *Tetrahedron Lett.* **1996**, *37*, 2739. (b) O-Yang, C.; Wu, H. Y.; Fraser-Smith, E. B.; Walker, K. A. M. *Tetrahedron Lett.* **1992**, *33*, 37. (c) Bousquié, I.; Madiot, V.; Florent, J.-C.; Monneret, C. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1815. (d) Lebreton, J.; De Mesmaeker, A.; Waldner, A.; Fritsch, V.; Wolf, R. M.; Freier, S. M. *Tetrahedron Lett.* **1993**, *34*, 6383.

3 (a) Inanaga, J.; Hirata, K.; Saeki, H.; Katsuki, T.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1979**, *52*, 1989. (b) Hikota, M.; Tone, H.; Horita, K.; Yonemitsu, O. *Tetrahedron* **1990**, *46*, 4613. (c) Martin, S. F.; Yamashita, M. *J. Am. Chem. Soc.* **1991**, *113*, 5478.

4 Selected spectroscopic data for **1**: ^{1}H NMR (300MHz, acetone-d_6) δ 9.36 (2H, s), 7.49-7.20 (12H, m), 6.88 (4H, d, J = 8.9 Hz), 6.34 (1H, t, J = 7.1 Hz), 6.29 (1H, t, J = 6.8 Hz), 5.64 (1H, m), 4.92 (1H, d, J = 4.1 Hz), 4.73 (1H, m), 4.26 (1H, m), 3.75 (6H, s), 3.44-3.38 (2H, m), 2.55 (2H, m), 2.45-2.30 (2H, m), 1.81 (3H, s), 1.46 (3H, s), 0.88 (9H, s), 0.05 (3H, s), 0.02 (3H, s); ^{13}C NMR (75.5MHz, acetone-d_6) δ 164.49, 163.96, 163.88, 160.81, 159.30, 156.21, 150.96, 150.93, 145.25, 137.20, 135.98, 135.82, 130.53, 128.53, 128.34, 127.39, 113.60, 111.08, 110.79, 109.14, 87.29, 86.68, 84.72, 83.68, 80.91, 77.70, 76.11, 64.19, 55.11, 39.40, 37.49, 25.63, 18.05, 12.09, 11.69, -5.15, -5.18; FAB-MS *m/z* 963.5 (M^+), calcd 963.4. Selected spectroscopic data for **2**: ^{1}H NMR (500MHz, acetone-d_6) δ 10.14 (1H, s), 10.11 (1H, s), 10.10 (1H, s), 7.66-7.24 (14H, m), 6.92 (4H, d, J = 8.8 Hz), 6.52 (1H, t, J = 7.6 Hz), 6.46 (1H, dd, J_1 = 8.6 Hz, J_2 = 5.9 Hz), 6.40 (1H, t, J = 6.9 Hz), 5.98 (1H, m), 5.82 (1H, m), 5.50 (1H, d, J = 3.1 Hz), 5.02 (1H, d, J = 3.9 Hz), 4.92 (1H, m), 4.40 (1H, d, J = 2.2 Hz), 3.79 (6H, s), 3.58 (1H, dd, J_1 = 10.3 Hz, J_2 = 3.4 Hz), 3.50 (1H, dd, J_1 = 10.5 Hz, J_2 = 3.1 Hz), 2.90 (1H, m), 2.82-2.63 (4H, m), 2.41 (1H, m), 1.83 (6H, s), 1.47 (3H, s), 0.91 (9H, s), 0.10 (3H, s), 0.07 (3H, s); ^{13}C NMR (125.8MHz, acetone-d_6) δ 164.16, 163.35, 160.63, 160.12, 158.92, 158.91, 155.68, 155.65, 144.83, 136.76, 136.33, 135.59, 135.44, 135.32, 130.11, 128.12, 127.89, 126.95, 113.18, 110.64, 110.61, 110.31, 109.03, 108.83, 86.89, 86.43, 86.31, 84.28, 83.21, 80.51, 78.40, 77.61, 77.28, 75.72, 63.77, 54.66, 38.95, 37.01, 35.35, 25.17, 17.61, 11.61, 11.58, 11.21, -5.61, -5.64; FAB-MS *m/z* 1267.9 (M^+), calcd 1268.4. Selected spectroscopic data for **3**: ^{1}H NMR (300MHz, acetone-d_6) δ 10.22 (1H, s), 10.18 (1H, s), 7.62-7.24 (11H, m), 6.91 (4H, d, J = 8.8 Hz), 6.37 (2H, m), 5.60 (1H, s), 5.20 (1H, t, J = 8.9Hz), 4.88 (1H, m), 4.63 (1H, d, J = 2.8 Hz), 4.23 (1H, s), 3.77 (6H, s), 3.52 (4H, m), 2.25-2.70 (4H, m), 1.79 (3H, s), 1.41 (3H, s), 0.89 (9H, s), 0.11 (3H, s), 0.10 (3H, s); ^{13}C NMR (75.5MHz, acetone-d_6) δ 169.98, 163.94, 163.82, 159.31, 157.56, 150.95, 150.87, 145.24, 136.87, 135.94, 135.80, 130.52, 128.52, 128.34, 127.40, 113.60, 111.05, 111.91, 87.25, 86.54, 84.64, 83.92, 82.14, 77.86, 76.94, 74.21, 64.27, 55.12, 39.44, 38.77, 37.49, 25.67, 18.06, 12.10, 11.66, -5.12; FAB-MS *m/z*: 965.5 (M^+), calcd 965.4. Selected spectroscopic data for **4**: ^{1}H NMR (300MHz, acetone-d_6) δ 9.56 (2H, s), 9.51 (1H, s), 7.58-7.34 (12H, m), 6.98 (4H, d, J = 8.9 Hz), 6.41-6.34 (2H ,m), 6.27 (1H, t, J = 6.9 Hz), 5.73 (1H, m), 5.56 (1H, s), 5.26-5.20 (2H, m), 4.92 (1H, d, J = 2.2 Hz), 4.85 (1H, m), 4.67 (1H, d, J = 3.1 Hz), 4.27 (1H, d, J = 1.9 Hz), 3.86 (6H, s), 3.57-3.40 (6H, m), 2.60-2.29 (6H, m), 1.92 (3H, s), 1.89 (3H, s), 1.51 (3H, s), 0.99 (9H, s), 0.20 (3H, s), 0.19 (3H, s); ^{13}C NMR (75.5MHz, acetone-d_6) δ 169.98, 169.87 163.99, 163.86, 159.29, 157.65, 157.03, 150.98, 150.88, 145.23, 137.09, 136.34, 135.96, 135.78, 130.53, 128.52, 128.34, 127.40, 113.60, 111.34, 111.04, 111.87, 87.25, 86.89, 86.31, 84.64, 83.85, 82.25, 79.12, 78.16, 77.73, 77.02, 76.56, 74.31, 64.25, 60.08, 55.11, 39.34, 39.03, 37.49, 35.66, 25.66, 20.38, 18.05, 14.04, 12.16, 12.12, 11.63, -5.10; FAB-MS *m/z* 1272.6 (M^+), calcd 1272.5. Compound **1-4** are all soluble in DMSO, CH_2Cl_2, and ethyl acetate.

5 (a) Chung, Y. J.; Ryu, E. J.; Keum, G.; Kim, B. H. *Bioorg. Med. Chem.* **1996**, *4*, 209. (b) Lee, J. Y.; Kim, B. H. *Tetrahedron* **1996**, *52*, 571. (c) Kim, B. H.; Curran. D. P. *Tetrahedron* **1993**, *49*, 293. (d) Kim, K. S.; Kim, B. H.; Park, W. M.; Cho, S. J.; Mhin, B. J. *J. Am. Chem. Soc.* **1993**, *115*, 7472.