Pergamon

Tetrahedron Letters 39 (1998) 1279–1282

TETRAHEDRON
LETTERS

Synthesis of a Novel Pyrene-Containing Nucleoside and Its Incorporation into Oligonucleotides

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Received 28 October 1997; revised 2 December 1997; accepted 3 December 1997

Abstract: The synthesis of the aryl nucleoside analogue β-2'-deoxy-D-ribofuranosyl-1'-*N*-pyrenylcarboxamide (**4**) is described. This compound can be converted into a phosphoramidite reagent and used in solid phase oligonucleotide synthesis. © 1998 Elsevier Science Ltd. All rights reserved.

Recently, there has been much interest in the synthesis of nucleoside derivatives bearing aromatic moieties at the anomeric position. For example, aryl C-nucleosides have been prepared as potential non-hydrogen bonding "universal" bases[1] and have recently been examined by Kool and coworkers[2,3] as isosteres of natural nucleosides. A number of fluorescent chromophores have also been incorporated at or near the glycosidic position of deoxyribose. Placement of these aromatic groups at the site normally occupied by the purine and pyrimidine bases allows for their direct contact with the stacked pi system in helical nucleic acid structures. The synthesis of aryl C-nucleosides bearing carbocyclic chromophores such as pyrene and phenanthrene has been reported.[3] Other efforts have included the synthesis of a pyrene-bearing glycerol derivative that serves as an acyclic mimic of a ribose C-nucleoside[4] and the synthesis of a hydroxyprolinol nucleoside mimic with a pyrenyl unit attached to a position analogous to O-4 of 3-deoxyribofuranose.[5] While these approaches have all been used to successfully incorporate aromatic nucleoside derivatives into DNA, they are limited. Specifically, the aryl C-nucleoside approach requires the use of organometallic reagents that restrict the number of functional groups that can be tolerated,[3] while the other approaches introduce perturbations from the natural nucleoside structure that may result in unforeseen changes in the structures of the oligonucleotides and their complexes.

We have recently undertaken the development of a general and efficient method for the incorporation of aromatic groups into the anomeric position of deoxyribose nucleosides. This approach is designed to tolerate many functional groups while retaining the cyclic structure, point of attachment, and glycosidic configuration of natural nucleosides. Herein is described the synthesis of the first example of a novel class of aryl nucleosides: the β-2'-deoxy-D-ribofuranosyl-1'-*N*-arylcarboxamides (aryl carboxamides). These derivatives of allonic acid maintain the furanose structure found in deoxynucleosides while possessing a carboxyl group at the anomeric position which provides a site for the attachment of a variety of amines.[6]

The synthesis of the aryl carboxamides begins with the 3,5-di-*O*-toluoyl-β-1-cyano-2-deoxy-D-ribofuranose (**1**).[7] This material is available in four steps from 2-deoxy-D-ribose; the key step involves conversion of 3,5-di-*O*-toluoyl-α-1-chloro-2-deoxy-D-ribofuranose[8] into **1** using diethylaluminum cyanide as described by Iyer and coworkers.[7] These workers reported a 2:1 ratio of β- to α-anomers;[7] in our hands, this ratio can be as high as 5:1, albeit in a lower chemical yield. The anomers can be separated using silica gel chromatography. With **1** in hand, its conversion into aryl nucleoside derivatives was examined (Scheme 1).

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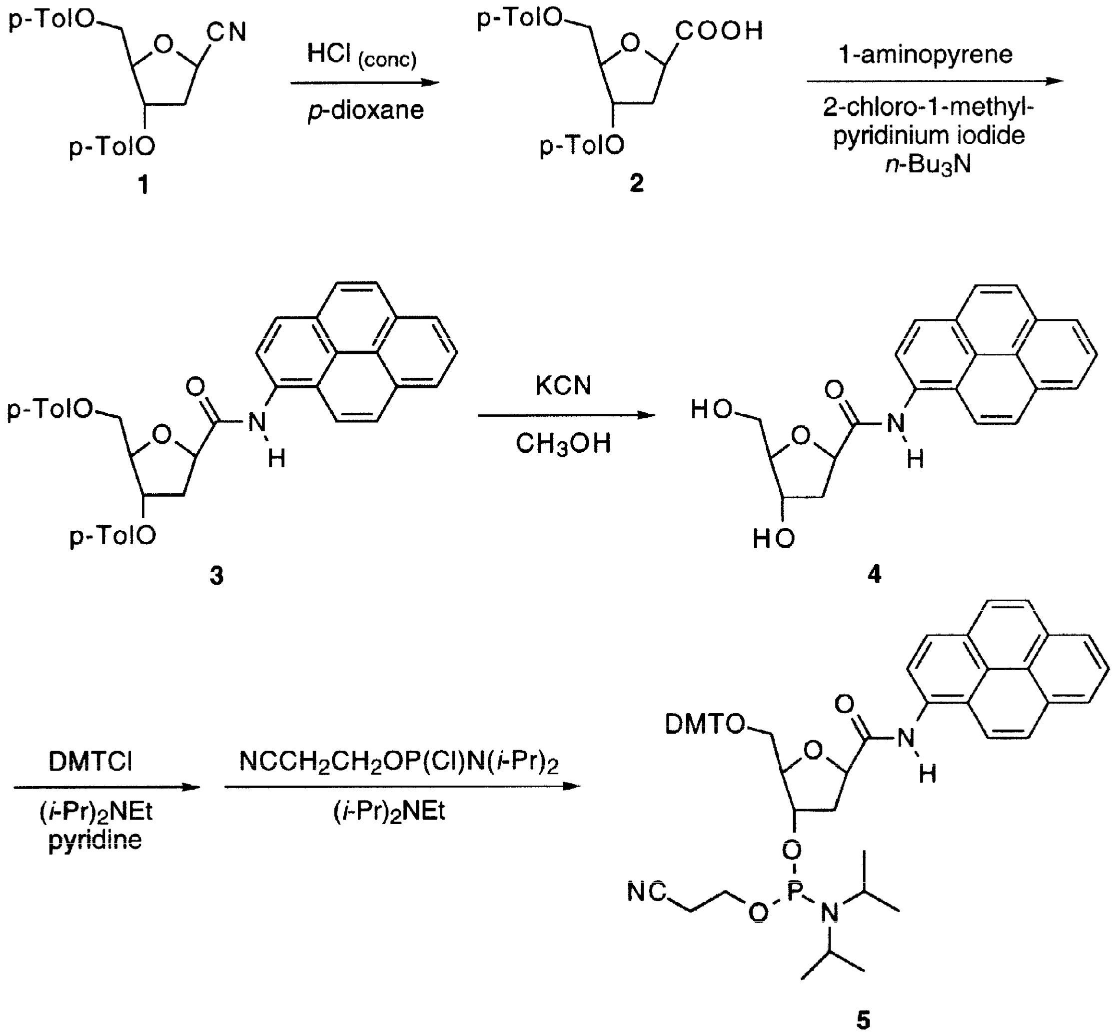
PII: S0040-4039(97)10853-X

Scheme 1



Thus, the nitrile group of **1** was hydrolyzed using concentrated HCl in *p*-dioxane (50 °C, 16 h), producing the carboxylic acid **2**[9] in quantitative yield. The proton NMR spectrum showed the presence of a single anomer which has been assigned as the β-configuration by comparison to a similar compound described previously.[10]

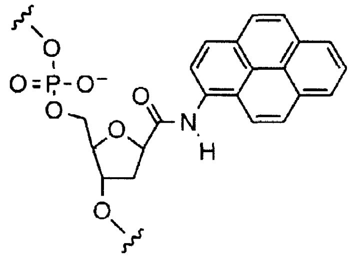
The carboxylic acid group provides a handle for the attachment of a wide variety of aromatic moieties through amide linkages. This was demonstrated by the synthesis of the pyrene carboxamide derivative **4**. The amide coupling was accomplished by condensation of **2** with commercially available 1-aminopyrene using the method of Mukaiyama (2-chloro-1-methylpyridinium iodide, tri-*n*-butylamine, CH_2Cl_2, reflux, 1 h, 53% yield after chromatography).[11,12] Selective hydrolysis of the toluoyl esters of **3** in the presence of the aryl amide was accomplished using potassium cyanide in methanol,[13] producing **4** in 73% yield.[14,15] Protection of the 5'-hydroxyl group as its dimethoxytrityl (DMT) ether and phosphitylation of the 3'-hydroxyl group produced a monomer (**5**) for oligonucleotide synthesis.

The compatibility of the aryl carboxamide nucleosides for DNA synthesis was demonstrated by the incorporation of pyrenylphosphoramidite **5** into synthetic oligonucleotides **6-8** (Scheme 2). The average coupling yield for **5** was ≥97%.[16] The only departure from standard methods[17] was the use of commercially available nucleoside phosphoramidites bearing labile base protecting groups (*N*-acetyl for deoxycytidine, *N*-phenoxyacetyl for deoxyadenosine, and *N*-(4-isopropylphenoxy)acetyl for deoxyguanosine).[18] These reagents

Scheme 2

oligonucleotide **6** : 5'-TTT TTT TT-Pyr- TT
oligonucleotide **7** : 5'-CGT TCG -Pyr- GAC AGC T
oligonucleotide **8** : 5'-CGT TCG -Pyr-Pyr- GAC AGC T

Pyr =



permit deprotection and cleavage from the support to be accomplished in two hours at room temperature using 0.05 M K_2CO_3 in methanol.[18,19] The deprotection reaction was quenched by the addition of water, and the oligonucleotides were desalted using Sep-Pak® C18 columns and purified by denaturing polyacrylamide gel electrophoresis.

To confirm the incorporation of the pyrene unit, oligonucleotides **6-8** have been spectroscopically characterized. The UV absorbance spectra for all three oligonucleotides show peaks corresponding to the pyrenyl group. The fluorescence spectra for aqueous solutions of oligonucleotides **6-8** were also recorded. As expected, the emission spectra for all three oligonucleotides showed a group of peaks centered at approximately 400 nm that has been assigned as emission from a monomeric pyrene. Oligonucleotide **8**, which contains neighboring pyrene residues, also showed a large peak at 486 nm corresponding to the excimer emission. Interestingly, the excimer-to-monomer fluorescence intensity ratio for this single-stranded oligonucleotide was 1.86. This is considerably larger than the ratio reported for an oligonucleotide internally labeled with two pyrenes using acyclic glycerol linkers (0.72) and is comparable to the ratio observed for the same oligonucleotide when hybridized to a complementary DNA (2.56).[4] This behavior may be due to a preference for a more ordered (helical) structure by oligonucleotide **8**.

The synthesis of a pyrene-containing nucleoside derivative and its incorporation into synthetic oligonucleotides illustrates the utility of the aryl carboxamides for the introduction of aromatic groups into DNA. When one considers the availability of aryl amines, the mildness of the chemistry used, and the variety of functional groups tolerated, this approach should provide a general route for the synthesis of aryl nucleoside derivatives. Work is currently underway to examine the synthesis of other aryl nucleosides using this methodology.

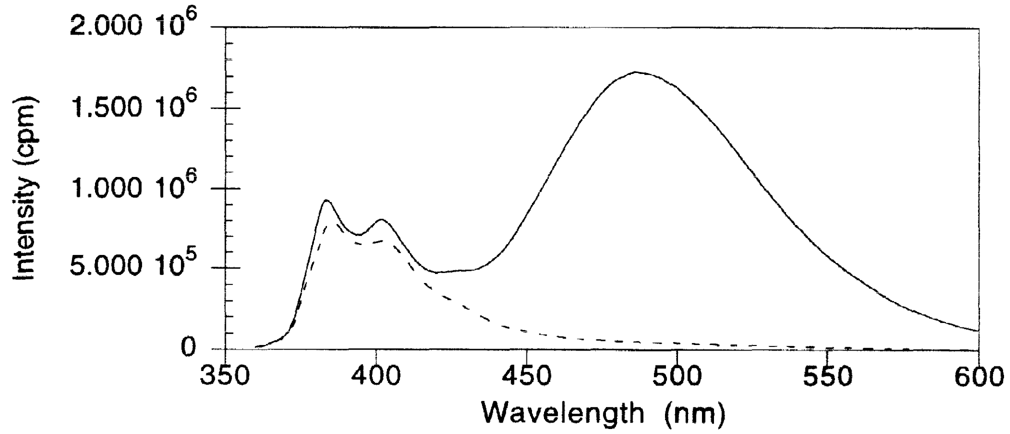


Figure 1: Fluorescence spectra for oligonucleotides **7** (-------) and **8** (———). Spectra were recorded in H_2O at room temperature with an excitation wavelength of 343 nm.

Acknowledgments: J.D.F. and S.M.H. were supported by an REU grant from the NSF (CHE-9531496). We thank Prof. R. Metzger for use of his spectrofluorimeter.

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9. ^{1}H NMR of **2** (500 MHz, $CDCl_3$): 7.94-7.91 (m, 4H, toluoyl), 7.27-7.24 (m, 4H, toluoyl), 5.52 (d, J = 5.6 Hz, 1H), 4.83 (dd, J = 10.0, 6.6 Hz, 1H), 4.68 (dd, J = 12.6, 6.1 Hz, 1H), 4.60-4.53 (m, 2H), 2.66 (dd, J = 13.9, 6.4 Hz, H-2), 2.47-2.41 (m, 7H, CH_3, CH_3, H-2)
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12. ^{1}H NMR of **3** (360 MHz, $CDCl_3$): 9.32 (s, 1H, NH), 8.45 (d, J = 8.3 Hz, 1H, pyrene), 8.18 (d, J = 7.7 Hz, 1H, pyrene), 8.13 (d, J = 8.3 Hz, 1H, pyrene), 8.12 (d, J = 7.7 Hz, 1H, pyrene), 8.03-7.97 (m, 5H), 7.92 and 7.88 (ABq, J = 9.3 Hz, 2H, pyrene), 7.71 (d, J = 8.1 Hz, 2H, toluoyl), 7.30 (d, J = 8.1 Hz, 2H, toluoyl), 6.87 (d, J = 8.1 Hz, 2H, toluoyl), 5.67 (br d, J = 5.9 Hz, 1H), 5.04 (dd, J = 10.3, 6.3 Hz, 1H), 4.97-4.93 (m, 1H), 4.74-4.69 (m, 2H, H-5'), 2.76 (ddd, J = 14.0, 6.3, 1.4 Hz, 1H, H-2'), 2.60 (ddd, J = 14.0, 10.3, 5.9 Hz, 1H, H-2'), 2.45 (s, 3H, CH_3), 2.16 (s, 3H, CH_3).
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14. ^{1}H NMR of **4** (500 MHz, 1:1 $CDCl_3/CD_3OD$): 8.26 (d, J = 8.2 Hz, 1H, pyrene), 8.21-8.17 (m, 4H, pyrene), 8.12 (d, J = 9.3 Hz, 1H, pyrene), 8.05 (s, 2H, pyrene), 8.01 (t, J = 7.6 Hz, 1H, pyrene), 4.91 (apparent t, J = 7.6 Hz, 1H, H-1'), 4.49 (ddd, J = 6.5, 4.7, 3.9 Hz, 1H, H-3'), 4.12 (ddd, J = 4.1, 3.9, 3.4 Hz, 1H, H-4'), 3.95 (dd, J = 11.9, 3.4 Hz, 1H, H-5a'), 3.81 (dd, J = 11.9, 4.1 Hz, 1H, H-5b'), 2.54-2.44 (m, 2H, H-2').
15. This product was isolated as a single diastereomer which has been assigned as the β-configuration based on the "peak width rule" of Srivastava, et al. (*J. Heterocyclic Chem.* **1981**, *18*, 1659-1662). Studies to unequivocally assign the anomeric stereochemistry are currently underway.
16. Coupling yields were determined by spectrophotometric quantitation of the dimethoxytrityl cation.
17. Oligonucleotide syntheses were performed on a Perkin Elmer ABI 391 DNA synthesizer on a 0.2 μmol scale using the standard protocol supplied by the manufacturer.
18. Glen Research Corporation 1997 Catalog, p 31.
19. Nucleoside **4** was unchanged upon treatment with 0.05 M potassium carbonate in methanol for 2 h as was determined by TLC analysis.