Pergamon

Tetrahedron Letters, Vol. 38, No. 18, pp. 3195-3198, 1997

0040-4039/97 $17.00 + 0.00

PII: S0040-4039(97)00610-2

Synthesis of Thymine, Cytosine, Adenine, and Guanine Containing N-Fmoc Protected Amino Acids: Building Blocks for Construction of Novel Oligonucleotide Backbone Analogs

Robert A. Goodnow, Jr.*, Anne-Roberte Richou, Steve Tam

Roche Research Center, Hoffmann-La Roche Inc., Nutley, New Jersey 07110

Abstract: A convenient synthesis is described for thymine, cytosine, adenine, and guanine containing, glucosamine-based N-Fmoc protected amino acids. These molecules are building blocks for the construction of novel oligonucleotide analogs via N-Fmoc type peptide chemistry. © 1997 Elsevier Science Ltd.

In recent years much attention has been focused on the discovery and development of modified oligonucleotide backbone structures in hopes of improving upon the properties of the presently most useful class of antisense agents, the phosphorothioates.[1] The research in this field has been driven by the desire to provide agents which are more stable, less toxic and more selective binders of ribo-oligonucleotide targets than phosphorothioates. It has been our strategy to develop a new class of antisense agents according to a common set of design principles; these new agents should be uncharged, amide linked, optically pure, cyclic and provide somewhat rigid presentations of natural nucleobases for binding to the targeted RNA strand. The announcement that PNA is an able and sequence selective binder of DNA and RNA strands demonstrates the extent to which the natural oligonucleotide structure may be modified and still retain or improve upon the binding characteristics of natural oligonucleotide systems.[2] It was along these lines that the glucosamine-based oligonucleotide analog **1** (GNA) was conceived in our laboratories. The glucosamine 6-membered ring appeared to fulfill the requirements of an optically pure and somewhat constrained conformational scaffolding for the attachment of nucleobases. Further, hoping to improve some of the physico-chemical properties of PNA, it was thought that the two hydroxyl groups per nucleobase residue would increase the aqueous solubility of the oligomer and serve as potential pro-drug functionalization sites on the oligomer.

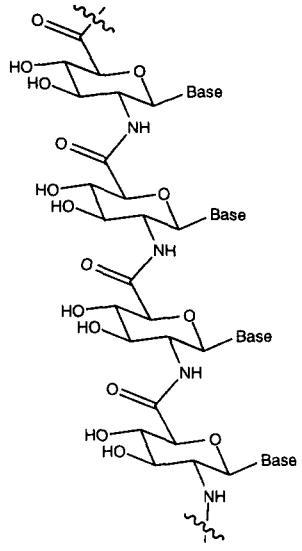


Figure 1 GNA **1**

Here we report the method by which the protected amino acid building blocks for thymine **2a**, cytosine **2b**, adenine **2c**, and guanine **2d** have been synthesized (Figure 2). We have determined a preference for Fmoc-type peptide chemistry for assembly of these oligomers on solid phase resins. This type of chemistry is advantageous for several reasons, particularly the mild and compatible nature of the conditions needed for removal of the Fmoc group. The use of *tert*-butyldimethylsilyl ether (TBDMS) as the 3' and 4' hydroxy protecting group was found to be optimal for reasons of convenience in the synthesis of the building blocks (*vide infra*), for its stability during oligomerization, and for its ease of removal after oligomerization (standard RNA synthesis protocols).

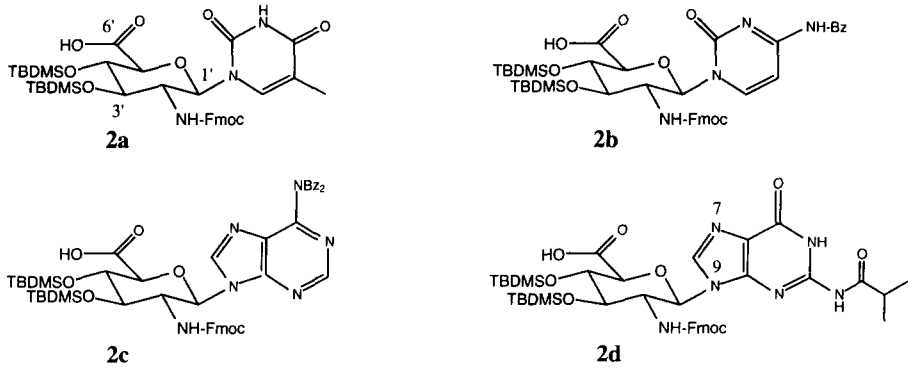
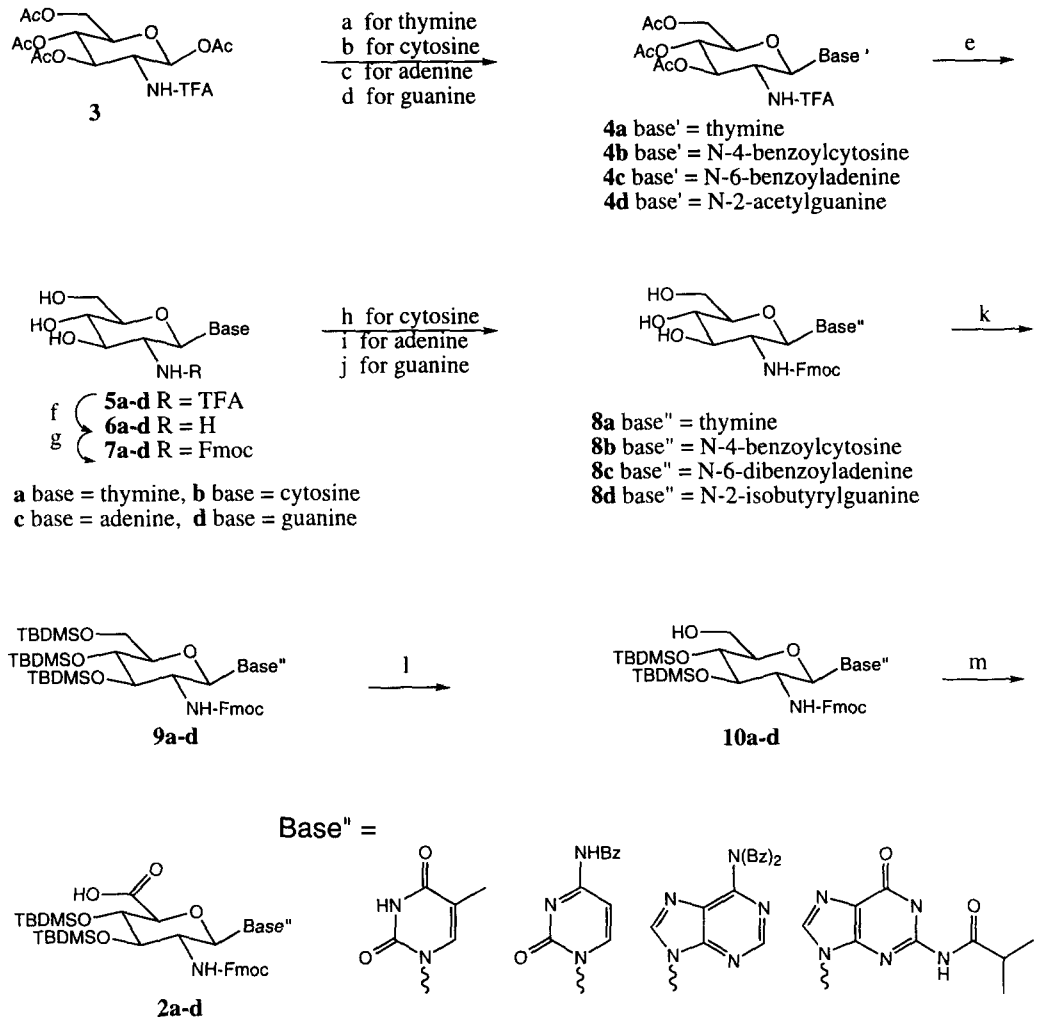


Figure 2

The four building blocks were synthesized in a parallel fashion in several general steps: a) glycosylation of the nucleobase, b) interchange of protecting groups, c) selection of the primary alcohol (C-6'), d) oxidation as a final step (Scheme 1). The choice of the initial amino protecting group was a significant consideration given the requirements for *beta* stereochemistry of the nucleobase at the anomeric position. After some investigation, N-trifluoroacetamido (N-TFA) was chosen over N-phthalimido. Treatment of the common intermediate **3**[3] under Vorbrüggen conditions[4] with persilylated thymine and cytosine resulted in good coupling yields of the *beta* coupled products **4a** and **4b**. With adenine, the formation in CH_3CN of a complex with $SnCl_4$ not only solubilized the N-benzoyladenine, but also effected the desired coupling in good yield with carbohydrate **3**. When carbohydrate **3** was treated with persilylated N-acetylguanine, a 2:1 mixture of the desired N-9 to the N-7 linked nucleoside was obtained. The regioisomers were separated using silica gel flash column chromatography providing the N-9 product (**4d**) in 36-43% yield on multi-gram scale.[5] No significant quantities of the *alpha*-stereochemistry product were observed for any of the four glycosylation reactions generating **4a-d**.

Subsequent to glycosylation, the O-acetyl, N-TFA, and nucleobase protecting groups were removed under based catalyzed hydrolysis conditions to afford the amino triols **6a-d**. Shifts in UV absorption as a function of solution pH provided an opportunity to confirm the N-9 substitution pattern for the adenine and guanine containing intermediates[6]. Carbon-13 chemical shifts of the guanine and adenine moieties of **6c** and **6d** were nearly identical to those reported for similar compounds.[7,8] The greater reactivity of the 2'-deoxy-2'-amino group was advantageous for its selective reaction with N-Fmoc-O-succinimide in aqueous dioxane (**7a-d**). The protection of amino functionalities for the cytosine, adenine, and guanine heterocycles was achieved according to known procedures[9] generating triols **8b-d**. The 3', 4', and 6' hydroxyl goups of **8a-c** were protected as *tert*-butyldimethylsilyl ethers by treatment with excess *tert*-butyldimethylsilyl trifluoromethanesulfonate in presence of 2,6-lutidine. In the case of the guanine analog (**8d**) good yields of the trisilyl product (**9d**) were obtained by initial treatment with *tert*-butyldimethylsilyl chloride and imidazole in DMF, followed by work-up and subsequent treatment with 4.0 eq of *tert*-butyldimethylsilyl trifluoromethanesulfonate in presence of 8.0 eq of 2,6-lutidine in CH_2Cl_2. It was possible to crystallize **8d** from CD_3OD; an X-ray crystal structure confirmed the N-9 substitution. Tri-silyl ethers **9a-d** were treated with camphor sulfonic acid, cleanly producing the primary alcohol in each case (**10a-d**). The protected amino acid nucleobases (**2a-d**) were obtained by oxidation of the primary alcohol. Of several methods surveyed, it was best to oxidize the alcohol to the aldehyde using TEMPO (2,2,6,6-tetramethyl-1-piperidinyloxy free radical) catalyzed



Scheme 1

a) N,O-Bis(trimethylsilyl)acetamide (BSA), thymine, CH_3CN, then **3**, trimethylsilyl trifluoromethanesulfonate (TMSOTf), 80%. b) BSA, N-benzoylcytosine, $ClCH_2CH_2Cl$, then **3**, $SnCl_4$, 85%. c) N-Benzoyladenine, $SnCl_4$, CH_3CN, then **3**, 79%. d) BSA, 2-acetylamino-6-hydroxypurine, CH_3CN, then **3**, TMSOTf, 43%. e) Et_3N, H_2O CH_3OH (1:4:5 by vol), 70-100%. f) NH_4OH,70-90%. g) N-(9-Fluorenylmethoxycarbonyloxy)succinimide, $NaHCO_3$, H_2O, dioxane, 60-70%. h) Benzoic anhydride, EtOH reflux, 70%. i) Trimethylsilyl chloride TMS-Cl, pyridine, then benzoyl chloride, then aq. $NaHCO_3$, 65%. j) (TMS-Cl), pyridine, then *iso*-butyric anhydride, then aq. $NaHCO_3$, 75%. k)*tert*-Butyldimethylsilyl trifluoromethanesulfonate, 2,6-lutidine, CH_2Cl_2, 70-86%. l) Camphor sulfonic acid, CH_3OH / CH_2Cl_2 (1:1), 70-90%. m) TEMPO, KBr,$(Bu_4N)_2SO_4$, NaOCl, NaCl, $NaHCO_3$, H_2O, CH_2Cl_2; then $NaClO_2$, NaH_2PO_4, 2-methyl-2-butene, *tert*-butyl alcohol, H_2O, 50-90%.

NaClO conditions,[10] followed by treatment with $NaClO_2$. The compatibility of this mild oxidation method with both purine and pyrimidine substrates is significant.[11] The carboxylic acid products were purified in each case by silica gel column chromatography.

In conclusion, we have shown a convenient method to generate novel amino acids ready for oligomerization according to standard Fmoc based peptide chemistry. This approach should accommodate a wide variety of substituents at the anomeric position. The successful solid phase oligomerization of these building blocks and purification of the resultant oligomers is reported in the subsequent communication along with DNA and RNA binding affinity and selectivity data.

Acknowledgments: Mr. Gino Sasso is thanked for NMR spectroscopic measurements and interpretations. Dr. Louis Todaro is thanked for his efforts to solve the crystal structure of guanine intermediate **9d**.

References and Notes

1 *Carbohydrate Modification in Antisense Research* ; Sanghvi, Y. S., Cook, P. D., Eds.; ACS Symposium Series 580; American Chemical Society: Washington, DC, 1995. Milligan J. F.; Matteucci, M. D.; Martin, J. C. *J. Med. Chem.* **1993**, *36*, 1923-1937.

2 Nielsen, P. E.; Egholm, M.; Berg, R. H.; Buchardt, O. *Science* **1991**, *254*, 1497-1500.

3 Wolfrom, M. L.; Bhat, H. B. *J. Org. Chem.* **1967**, *32*, 1821-1823.

4 Vorbrüggen H.; Bennua, B. *Tetrahedron Lett.* **1978**, 1339; Vorbrüggen H.; Bennua, B.*Chem. Ber.* **1981**, *114*, 1279.

5 To 7.68 g of 2-acetylamino-6-hydroxypurine suspended in 400 mL of dry acetonitrile was added 20 mL of bis-silylacetamide; the mixture was heated to reflux under inert and dry atmosphere for 30 min. At this time, to the cooled mixture was added 8.86 g of carbohydrate **3** and 7.72 mL of trimethylsilyl trifluoromethanesulfonate. The mixture was heated to reflux under inert atmosphere for 12 h. The clear solution was concentrated to 100 mL volume, before the addition of 200 mL 5% (wt/vol) $NaHCO_3$ and 400 mL of EtOAc. The resulting suspension was filtered and washed with EtOAc through celite. The aqueous layer was separated and the organic layer was extracted twice with saturated $NaHCO_3$, brine, and dried with $MgSO_4$. Upon filtration and evaporation in vacuo, the resulting residue was chromatographed on silica gel, eluting with 5:95 $CH_3OH/CHCl_3$. The N-7 substitution product eluted first (3.11 g, 27%). Selected data: ^1H NMR (CD_3OD) δ 8.37 (br, 1H, H-8), 6.13 (br, 1H, H-1'), 5.52 (t, 1H, J=9.8Hz, H-3'), 5.31 (t, 1H, J=9.8Hz, H-4'), 5.09 (br, 1H, H-2'), 4.25 (m, 2H, H-6', 6"), 4.15 (m, 1H, H-5'), 2.22 (s, 3H, $-CH_3$), 2.06 (s, 3H, $-CH_3$), 2.04 (s, 3H, $-CH_3$), 2.00 (s, 3H, $-CH_3$). ^{13}C NMR (DMSO-*d6*) δ 173.5, 170.0, 169.5, 169.3, 158.1, 156.7, 156.3 (q), 151.8, 147.6, 144.1, 115.3 (q), 110.8, 82.2 (C-1'), 73.5, 71.7, 67.8, 61.6, 52.8, 23.7, 20.5, 20.1. This was followed by the desired N-9 coupling product **4d** (4.97 g, 43%). ^1H NMR (CD_3OD) δ 8.1 (br, 1H, H-8), 5.97 (d, 1H, J=9.8Hz, C-1'), 5.54 (t, 1H, J=9.8Hz, C-3'), 5.27 (t, 1H, J=9.8Hz, C-4'), 5.01 (br, 1H, C-2'), 4.18, 4.32 (AB of ABX, 2H, J_{vic}=4.9Hz, J_{gem}=12.6 H-6', H-6"), 4.09 (ddd, 1H, J=1.9, 4.9, 9.8Hz, H-5'), 2.24 (s, 3H, CH_3), 2,06 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.00 (s, 3H, CH_3). (DMSO-*d6*) δ H-8 8.19 (br, 1H), H-1' 5.93 (s 1H); ^{13}C NMR (DMSO-*d6*) δ 174.7, 170.0, 169.5, 169.4, 156.4 (q), 154.6, 148.6, 148.1, 138.8 (C-8), 120.3, 115.3 (q), 79.7 (C-1'), 73.6, 71.3, 67.7, 61.6, 51.6, 23.8, 20.5, 20.34, 20.0.

6 UV absorption maxima observed for aqueous solutions of **6c** as a function of pH were observed: 256 nm (pH 1.5); 260 nm (pH 7); 260 nm (pH 12.5). UV absorption maxima observed for aqueous solutions of **6d** as a function of pH were observed: 256 nm (pH 1.5); 254 nm (pH 7); 260 nm (pH 12).

7 **6c**: ^{13}C NMR (DMSO-*d6*) δ 156.0 (C-6), 152.6 (C-2), 149.8 (C-4), 139.6 (C-8), 118.7 (C-5), 83.8 (C-1'), 80.0, 77.5, 69.8 (C-3', C-4', C-5'), 60.9 (C-6'), 55.4 (C-2');
6d: ^{13}C NMR (DMSO-*d6*) δ 156.8 (C-6), 153.7 (C-2), 151.5 (C-4), 135.8 (C-8), 116.5 (C-5), 83.2 (C-1'), 80.1, 77.4, 69.7 (C-3', C-4', C-5), 60.9 (C-6'), 55.3 (C-2').

8 Rosenquist, Å.; Kvarnström, I.; Svensson, S. C. T.; Classon, B.; Samuelsson. B. *J. Org. Chem.* **1994**, *59*, 1779-1782.

9 Otter, B. A.; Fox, J. J. in *Synthetic Procedures in Nucleic Acid Chemistry*, W. Werner Zorbach and R. Stuart Tipson, Eds.; Interscience Publishers: New York (1968), pp 285-287. Ti, G. S.; Gaffney, B. L.; Jones, R. A. *J. Am. Chem. Soc.* **1982**, *104*, 1316-1319.

10 Davis, N. J.; Flitsch, S. L. *Tetrahedron Lett.* **1993**, *34*, 1181-1184.

11 Singh, A. K.; Varma, R. S. *Tetrahedron Lett.* **1992**, *33*, 2307-2310. Varma, R. S.; Hogan, M. E. *Tetrahedron Lett.* **1992**, *33*, 7719-7720.

(*Received in USA* 21 *February* 1997; *revised* 14 *March* 1997; *accepted* 16 *March* 1997)