Pergamon

Tetrahedron, Vol. 53, No. 3, pp. 1167-1176, 1997

0040-4020/97 $17.00 + 0.00

PII: S0040-4020(96)01047-2

Synthesis of N-Fmoc-α-Amino Acids Carrying the Four DNA Nucleobases in the Side Chain.

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Abstract: Four new N-Fmoc α–amino acids carrying a nucleobase in the side chain were prepared starting from L-glutamic acid. N-Boc-glutamic acid α benzyl ester underwent a radical decarboxylation in the presence of $CBrCl_3$ to give the corresponding 2-amino-4-bromobutanoic acid derivative. The four nucleobases (adenine, cytosine, thymine and guanine) were introduced "via" nucleophilic substitution of the bromide using a different synthetic protocol for each base.The Boc protection was changed to Fmoc and the benzyl ester deprotected to give the four amino acids in good yields and in a suitable form for solid phase peptide synthesis. The preparation of the insecticidal dipeptide NK 374200 is also described. Copyright © 1996 Elsevier Science Ltd

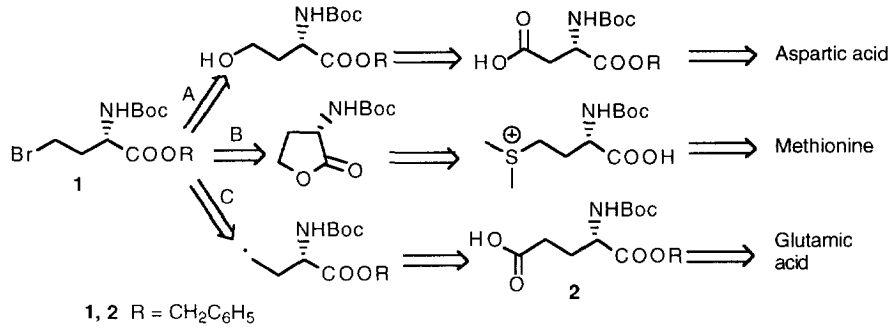
The synthesis of exotic α-amino acids is becoming more and more popular nowadays.[1] The variety of functionalities that can be introduced in the side chain of α-amino acids gives access to a large choice of possibilities to prepare products with increased lipophylicity,[2] potential metal chelation,[3] conformational constrains[4] etc. In the special case of the introduction in the amino acid side chain of groups belonging to one of the classes of biomolecules (except amino acids itself, of course), the obtained compound is called a "chimerical" amino acid.[5] Some interesting applications of amino acid chimeras carrying the structure of coenzymes in the side chain has been recently reported.[6]

We communicated our preliminary results on the preparation of α-amino acids carrying nucleobases in the side chain[7] and their use in the preparation of chiral peptidic nucleic acids.[8] Due to the continuing interest in this fascinating field of un-natural molecules constructed with pieces of natural products,[9] we report here the optimised procedure for the synthesis of N-Fmoc-α-amino acids carrying the four DNA nucleobases in the side chain.[10] These new amino acids can be utilised for the synthesis of new peptides or for the preparation of peptide libraries. [11] The amino acid carrying the adenine in the side chain has been also employed for the synthesis of the naturally occurring dipeptide NK 374200 (product **25** in scheme 7), a novel insecticidal agent isolated the culture broth of the fungus *Talaromyces* sp.[12]

We planned 2-amino 4-bromobutanoic acid (in a protected form) **1** as the key product for our synthesis. This compound can be considered an ideal intermediate for the introduction of any kind of chimerical frame in the side chain of an optically active amino acid. As this product should undergo a nucleophilic substitution (a basic environment is expected), we decided to start the synthesis with the *tert*-butoxycarbonyl protecting group (Boc) at the nitrogen, planning to replace it with the Fmoc group during a subsequent step.

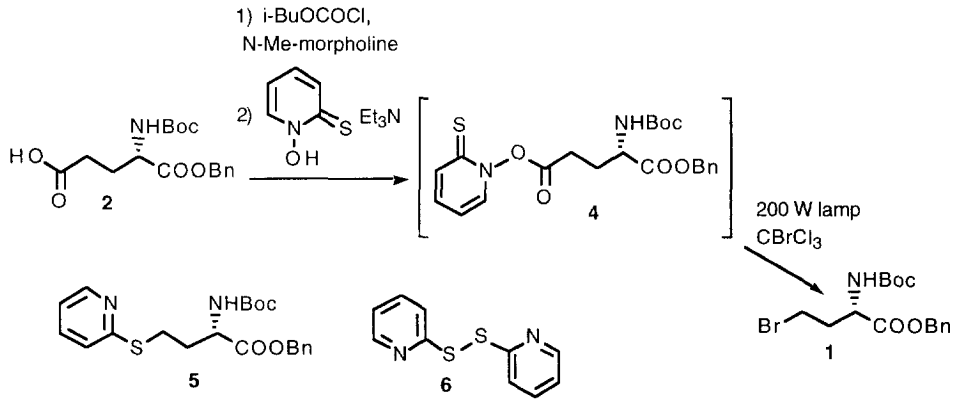
From several possible retrosynthetic analyses[13] for compound **1** (Scheme 1) we decided to follow the path C which provides the desired compound in a single step from commercially available N-Boc-glutamic acid α-benzyl ester **2**.[14]

We attempted to follow the path A or B in Scheme 1, obtaining disappointing results in the case of the transformation of protected homoserine or its lactone into the bromide.



Scheme 1

On the other hand, the photochemical (radical) decarboxylation of the mercaptopyridine oxide ester **4** in the presence of bromotrichloromethane[15] worked very well, provided that the temperature of the mixture was maintained below -10°C during the reaction. Ester **4** was obtained by reaction of the acid **2** and mercaptopyridine N-oxide in the presence of isobutyl chloroformate and 4-methyl-morpholine. From the resulting crude bright yellow-green[16] THF solution, **4** was isolated by evaporation of the solvent under vacuum (in the dark). A solution of **4** in bromotrichloromethane was irradiated using a domestic 200 Watt lamp, taking care to refrigerate the solution using an internal "cold finger" containing a liquid cooled to 0°C and maintaining the irradiation until evolution of CO_2 stopped. The main by-products in this reaction were the thiopyridine derivative **5** and the disulphide **6.**

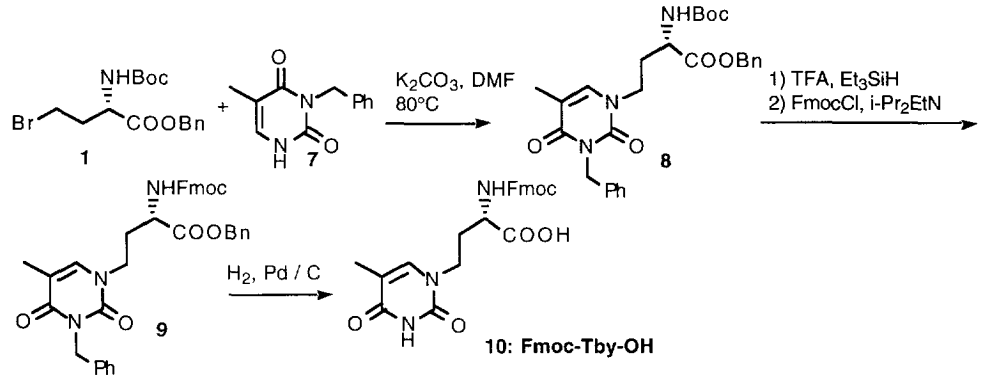


Scheme 2

Although **1** can react directly with any of the four nucleobases to give the substitution compound,[7] we needed to execute a proper protocol for each base in order to obtain better yields.

Reaction of **1** with thymine was first performed in DMF at 70°C for 6 h in the presence of potassium carbonate and tetrabutylammonium iodide (TBAI). Unfortunately a mixture of substitution products at the position 1 and 3 of the thymine ring was obtained in a 7 : 3 ratio. The desired regioisomer could be separated by column chromatography on alumina. To overcome this tedious separation we reacted compound **1** with 3-benzylthymine[17] **7** obtaining exclusively the alkylation product at N-1(**8**). Product **8** was deprotected at the

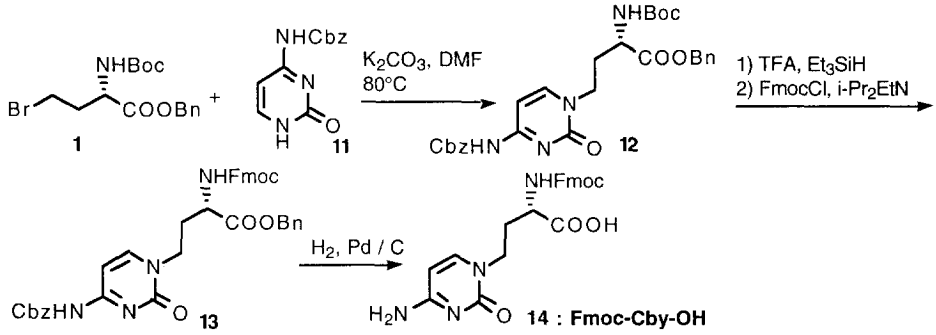
nitrogen using trifluoroacetic acid and triethylsilane (as *tert*-butyl cation scavenger) in dichloromethane and, immediately after, reprotected with fluorenylmethyl chlorocarbonate and diisopropylethylamine to give product **9** in high yield.



Scheme 3

Finally, the benzyl ester was cleaved by hydrogenation at atmospheric pressure (balloon) in the presence of Pd on charcoal, taking care to extract the obtained acid **10** from the residual catalyst with hot methanol (42% overall yield).

For the synthesis of the cytosine derivative, better results were obtained using N-4-Cbz-cytosine **11** prepared according with a reported method.[18] The presence of the protecting group at the 4-NH_2 allowed a better solubility of the reaction products **12** and **13** in organic solvents and prevented the formation of the N-3 substitution product, as previously observed in the case of thymine.



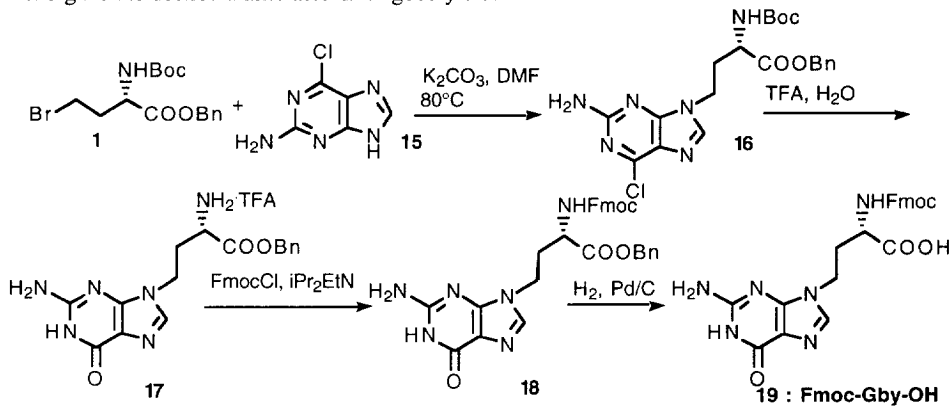
Scheme 4

In accordance with Scheme 4, product **12** was obtained in high yields and further deprotected, protected again with Fmoc-Cl, and finally the benzylic and Cbz protecting groups were removed using H_2 and Pd/C to give product **14** in 62% yield (27% yield starting from **1**).

In order to obtain the guanine derivative, we needed to start from the relatively expensive 2-amino-6-chloropurine **15** (Scheme 5). Any attempt to use guanine itself gave unsatisfactory reults in terms of yields and purity of the reaction products.

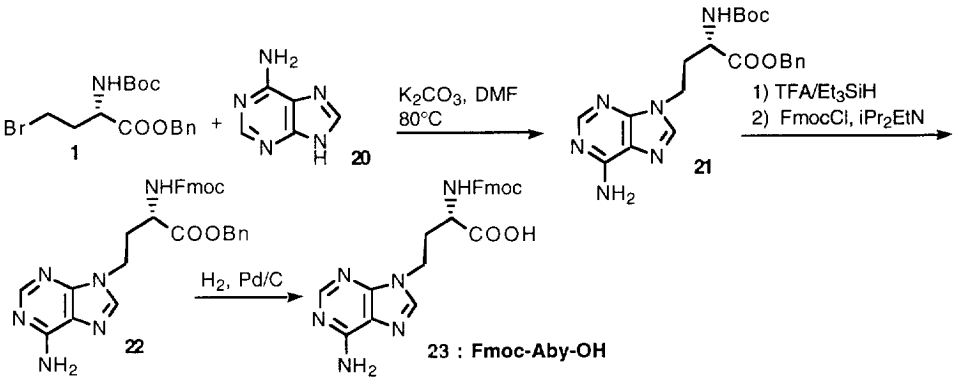
Deprotection of **16** was carried out with an excess of trifluoro acetic acid in aqueous medium to perform the contemporary hydrolysis at position 6 of the purine ring. Fmoc protection was carried out directly on the

crude reaction mixture to give product **18** in good yields. Finally debenzylation of the carboxylic group with H_2 and Pd/C gave the desired amino acid **19** in good yield.



Scheme 5

The introduction of adenine followed the original general procedure[7] giving no problems until the deprotection of the benzyl group, when only 10-30% weight of the product was recovered after filtration of the Pd/C (Scheme 6). Several extractions of the residue with hot methanol were needed to isolate product **23** in 65% yield.

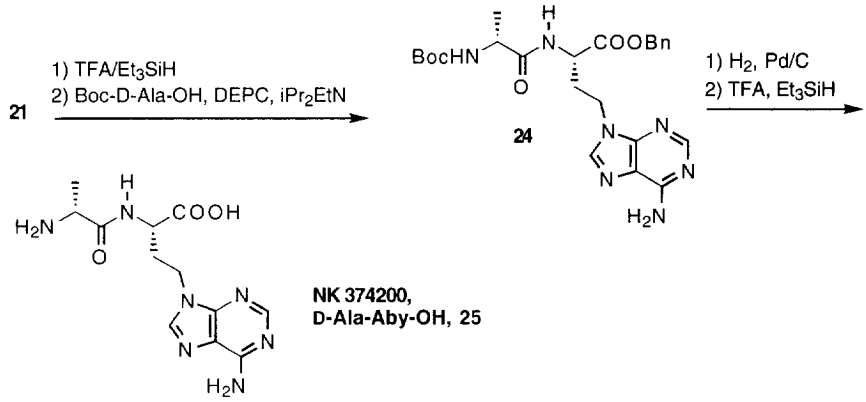


Scheme 6

As described in the above paragraphs, the benzyl deprotection in the presence of Fmoc resulted as the low yielding step. Although a possible cleavage of the Fmoc under these conditions was expected, we had no evidence of it (we cannot exclude that some zwitterions remain on the catalyst after methanol extraction). It is noteworthy to see that deprotection carried out at pH 3 resulted in lower yields of products (Fmoc is supposed to be more stable under those conditions) and that the same results occurred using a "real catalytic" amount of Pd/C (almost one equivalent on gram basis was normally required). In all four cases, reaction conditions leading to **8**, **12**, **16**, **21** were fairly alkaline. The optical purity of the products after this step was controlled transforming the amines, after Boc deprotection, into the corresponding MTPA amides and recording the ^1H and ^{19}F NMR spectra which showed a level of racemisation under 2-3%.

Compounds **10**, **14**, **19**, and **23** underwent complete spectroscopical and elemental analyses, which were correct in relation to the proposed structures. Nevertheless, during the first attempts to use these products in a

solid phase peptide synthesis we discovered that the so obtained material resulted positive to the ninhydrin test.[19] This problem was overcome by washing the solid products with boiling water, filtering and drying again. This procedure did not affect the melting points, nor the elemental analyses. Actually the ninhydrin test resulted negative and the products could be effectively employed in synthesis of polypeptides as confirmed by the simple preparation of FK 374200, a novel metabolite from *Talaromyces* sp. that showed low toxicity and a promising activity as an isecticidal agent.[12]



Scheme 7

Deprotection of **21** with TFA and Et_3SiH gave the amine which reacted with Boc-D-Ala using diethyl-cyano phosphonate (DEPC) as the coupling agent. (79% yield). The dipeptide **24** was easily deprotected using standard techniques and the final product obtained in 65% overall yield.

Experimental Section.

(*2S*)-4-Bromo-2-(*tert*-butoxycarbonylamino)-butanoic acid benzyl ester 1. To a solution of **2** (5 g, 14.8 mmol) in dry THF (40 mL) cooled to -15°C, under nitrogen and mechanical stirring, 4-methyl-morpholine (1.65 mL, 14.8 mmol) was slowly added followed by isobutyl chloroformate (1.93 mL, 14.8 mmol). The mixture was stirred for 30 min at -15°C and a solution of mercaptopyridine N-oxide (2.26 g, 17.8 mmol) and Et3N (2.5 mL, 17.8 mmol), previously cooled to -15°C, was added. The reaction flask was covered with aluminium foil to prevent exposure to light and the reaction mixture was stirred at -15°C for 1 h. The solution was filtered under nitrogen and the THF was evaporated (still covering the flask with aluminium foil). The residue was dissolved in $CBrCl_3$ (145 mL, 1480 mmol) and the bright yellow solution poured into a Schlenk tube where a cold element was inserted in which circulated a liquid cooled to 0°C. The tube was placed in front of a 200W lamp (no more than 10 cm far away) to promote the decarboxylation. After 3-4 h of stirring the evolution of CO_2 stopped, the $CBrCl_3$ was evaporated and the residue purified by flash chromatography (eluent hexane : EtAc 3 : 1) to give product **1** (5.01 g, 91% yield) as an oil which solidified after long standing in a refrigerator.$[\alpha]_D^{25}$ = -40.5 (c = 1 in MeOH) lit.[20] $[\alpha]_D^{25}$ =-34.0° (c=1.0 in MeOH). ^1H NMR (300 MHz, $CDCl_3$) δ 1.43 (s, 9H,), 2.22 (m, 1H,), 2.41 (m, 1H,), 3.40 (t-like, 2H,), 4.40 (m collapsing to a dd after 24 h in D_2O, 1H,), 4.91 (bs, 1H, NH), 5.16 and 5.19 (AB system, 2H,), 7.35 (5H, m,). Anal. Calcd for $C_{16}H_{22}NO_4Br$ (372.26): C, 51.62; H, 5.96; N, 3.76; O, 17.19; Br, 21.46. Found: C, 51.87; H, 5.99; N, 3.80.

(*3'S*)-3-Benzyl-1-[3'-(*tert*-butoxycarbonylamino)-3'-(benzyloxycarbonyl)-propyl]-5-methyl-2,4-pyrimidinedione, 8. Bromide **1** (5.16 g, 13.8 mmol) was mixed with 3-benzylthymine **7** (6.6 g, 30.5 mmol), potassium carbonate (4.2 g, 30.5 mmol) and TBAI (0.5 g) in dry DMF (150 mL), the mixture heated to 70°C and stirred for 1 h at this temperature. Water was added (100 mL) and the solution extracted several times with ethyl acetate (portions of 50 mL). The organic layer was separated, dried over anhydrous Na_2SO_4 and the solvent evaporated. Product **8** was isolated after flash chromatography on silica gel (eluent Hexane : EtAc 2 : 1). Obtained 4.3 g (65% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.43 (s, 9H), 1.87 (s, 3H), 1.97-2.13 (m, 1H), 2.10-2.26 (m, 1H,), 3.6-3.74 (m, 1H), 3.76-3.95 (m, 1H), 4.25-4.45 (m, 1H), 5.09 (s-like, 4H), 5.40 (bd, 1H), 6.93 (s, 1H), 7.16-7.51 (m, 10H). ^{13}C NMR (75MHz, $CDCl_3$) δ 20.3, 28.2 (3C), 31.7, 44.8, 46.6, 51.3, 67.2, 80.9, 109.1, 127.2 (2C), 128.1 (4C), 128.3 (2C), 128.4, 128.7, 134.5, 136.4, 138.6, 151.7, 155.9, 163.0, 171.6. Anal. Calcd for $C_{28}H_{33}N_3O_6$ (507.59) C, 66.26; H, 6.55; N, 8.28; O, 18.91. Found C, 66.38; H, 6.59, N, 8.20.

(*3'S*)-4-(Benzyloxycarbonylamino)-1-[3'-(*tert*-butoxycarbonylamino)-3'-(benzyloxycarbonyl)-propyl]-2-pyrimidinone, 12. Bromide **1** (5.18 g, 13.8 mmol), Cbz-Cytosine (7.7 g, 31.4 mmol), potassium carbonate (7.7 g, 31.4 mmol) and TBAI (0.5 g) were stirred in dry DMF (140 mL) for 48 h at room temperature. The solid was filtered off and DMF evaporated under high vacuum. From the residue, product **12** was separated by flash chromatography (4.5 g, 61% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.41 (s, 9H), 2.14 (m, 1H), 2.30 (m, 1H), 3.83 (m, 1H), 3.98 (m, 1H), 4.31 (m, 1H), 5.13 (s-like, 2H), 5.20 (s-like, 2H), 5.44 (bs, 1H), 7.14 (broad d, 1H, J = 5 Hz), 7.28-7.40 (large m, 10H), 7.59 (broad d, 1H, J = 5 Hz), 7.80 (bs, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 28.2 (3C), 31.2, 47.6, 51.2, 67.3, 67.4, 80.1, 95.2, 128.1 (2C), 128.3 (2C), 128.4 (2C), 128.6 (2C), 129.1, 135.0 (2C), 148.7 ,149.3, 152.5, 155.7 (2C), 162.3, 171.8. Anal. Calcd for $C_{28}H_{32}N_4O_7$, (536.59): C, 62.68; H, 6.01; N, 10.44; O, 20.87. Found: C, 62.70; H, 5.97; N, 10.50.

(*3'S*)-9-[3'-(*tert*-Butoxycarbonylamino)-3'-(benzyloxycarbonyl)propyl]-2-amino-6-chloropurine, 16. Bromide **1** (4.6 g, 12.4 mmol), 2-amino-6-chloropurine (5.68 g, 33.5 mmol), potassium carbonate (4.6 g, 33.5 mmol) and TBAI (0.5 g) were stirred in dry DMF (120mL) for 24 h at room temperature. After the usual work-up product **16** was isolated by flash chromatography (eluent EtAc : hexane 3 : 1). Obtained 4.13 g, 73% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.36 (s, 9H), 2.07 (m, 1H), 2.29 (m, 1H), 3.97 (m, 1H), 4.11 (m, 2H), 5.05 (m, 2H), 5.1 (bs, 1H), 6.91 (s, 2H), 7.33 (m, 5H, Ar), 8.00 (s, 1H).^{13}C NMR (75 MHz, d_6-DMSO) δ 27.9 (3C), 29.8, 47.6, 51.3, 66.0, 78.8, 123.3, 127.8, 128.1 (2C), 128,5 (2C), 135.8, 142.4, 149.6, 154.3, 155.2, 159.8, 171.7. Anal. Calcd. for $C_{21}H_{25}N_6O_4Cl$ (460.92): C, 54.72; H, 5.47; N, 18.23; O, 13.88; Cl, 7.69. Found: C, 57.65; H, 5.63; N, 15.29.

(*3'S*)-9-[3'-(*tert*-Butoxycarbonylamino)-3'-(benzyloxycarbonyl)propyl]-adenine, 21. Bromide **1** (5.2 g, 13.9 mmol), adenine (5.07 g, 37.53 mmol), potassium carbonate (5.18 g, 37.5 mmol) and TBAI (0.5 g) were stirred in dry DMF (150 mL) for 24 h at room temperature. After the usual work-up, product **22** was isolated by flash chromatography on silica gel (eluent EtAc : EtOH 10 : 1). Obtained 3.84 gr, 65% yield. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.45 (s, 9H), 2.13-2.30 (m, 1H), 2.32-2.58 (m, 1H), 4.2-4.5 (m, 3H), 5.05 (s-like, 2H), 5.50 (bd, 1H), 5.60 (s, 2H), 7.18-7.36 (m, 5H, Ar), 7.82 (s, 1H), 8.35, (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 27.9 (3C), 31.0, 40.0, 51.2, 66.9, 79.8, 119.3, 127.4 (2C), 127.9 (2C) , 128.6, 135.4, 150.5, 153.8, 155.0, 156.6, 162.7, 172.9. Anal. Calcd for $C_{21}H_{26}N_6O_4$ (426.48): C, 59.14; H, 6.15; N, 19.71; O, 15.01. Found C, 59.24; H, 6.10; N, 19.91.

(*3'S*)-3-Benzyl-1-[3'-(benzyloxycarbonyl)-3'-(9-fluorenylmethoxy-carbonylamino)-propyl]-5-methyl-2,4-pyrimidinedione, 9. Compound **8** (4 g, 7.9 mmol) was dissolved in dry

CH_2Cl_2 (16 mL, 253 mmol) containing triethylsilane (3.1 mL, 19.75 mmol). To this solution TFA (7.9 mL, 102.7 mmol) was added and the mixture stirred at room temperature for about 4 h. The solvent was evaporated under vacuum and ether (10 mL) was added and subsequently evaporated three times until the residue become a white solid. The solid was dissolved in dry THF, diisopropylethylamine (5.5 mL, 31.6 mmol) was added followed by 9-fluorenylmethyl chloroformate (2.05 g, 7.9 mmol) in dry THF (20 ml). After stirring at room temperature for 12 h, NH_4Cl (saturated solution) was added, followed by ethyl acetate (100 mL). The organic layer was separated, washed two times with brine, dried over anhydrous Na_2SO_4 and the solvent evaporated. Flash chromatography (eluent hexane:EtAc (2:1)) gave product **9** (4.3 g, 86% yield). ^1H NMR (300 MHz, $CDCl_3$) δ 1.90 (s, 3H), 2.05-2.27 (serie of m, 2H), 3.47-3.73 (m, 1H), 3.75-3.90 (m, 1H), 4.22 (t-like, 1H), 5.03-5.22 (m, 3 H), 5.10 (s-like, 4H), 5.69 (bs, 1H), 6.90 (s, 1H), 7.26-7.79 (serie of m, 18 H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.5, 31.8, 44.8, 46.3, 47.3, 51.6, 67.1, 67.7, 110.5, 120.8 (2C), 125.3 (2C), 127.1 (2C), 127.6 (2C), 127.8 (2C), 128.4 (2C), 128.7 (2C), 129.1 (3C), 134.8, 136.9, 138.5, 141 (2C), 143.6 (2C), 143.8, 151, 156, 163.6, 171. Anal. Calcd for $C_{38}H_{35}N_3O_6$ (629.72): C, 72.48; H, 5.60; N, 6.67; O, 15.24. Found: C, 72.55; H, 5.50; N, 6.61.

(*3'S*)-4-(Benzyloxycarbonylamino)-1-[3'-(benzyloxycarbonyl)-3'-(9-fluorenylmethyl-carbonylamino)-propyl]-2-pyrimidinone 13. Compound **12** (4.35 g, 8.1 mmol) was dissolved in dry CH_2Cl_2 (16.7 mL, 260 mmol) containing triethylsilane (3.2 mL, 20.25 mmol). To this solution TFA (8.1 mL, 105 mmol) was added and the mixture stirred at room temperature for 4 h. The solvent was evaporated under vacuum and ether (10 mL) was added and subsequently evaporated three times until the residue became a white solid. The solid was dissolved in dry THF, diisopropylethylamine (3.9 mL, 22.6 mmol) was added followed by 9-fluorenylmethyl chloroformate (2.1 g, 8.2 mmol) in dry THF (10 mL). After stirring at room temperature for 12 h, NH_4Cl (saturated solution) was added followed by ethyl acetate (100 mL). The organic layer was separated, washed two times with brine, dried over anhydrous Na_2SO_4 and the solvent evaporated. Flash chromatography (eluent Et Ac : hexane 3 : 1) gave product **13** (3.8 g, 72% yield). ^1H NMR (300 MHz, $CDCl_3$) δ 2.17 (m, 1H), 2.31 (m, 1H), 3.77 (m, 1H), 3.96 (m, 1H) 4.19 (m, 1H), 4.39, (m, 1H), 4.44 (d-like, 2H), 5.13 (s-like, 2H), 5.19 (s-like, 2H), 5.76 (bd, 1H), 7.22-7.80 (series of m, 18H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 40.0, 56.0, 56.3, 60.1, 60.6 (2C), 85.1, 85.7, 86.0, 128.5 (2C), 128.6 (2C), 133.6 (2C), 133.7 (2C), 135.6 (2C), 135.7 (4C), 136.3 (2C), 136.9 (2C), 137.0 (2C), 137.2 (2C), 137.3 (2C), 150.8, 155.8, 157.6, 161.2, 175.3. Anal Calcd. for $C_{38}H_{34}N_3O_8$ (658.71): C, 69.29; H, 5.20; N, 8.51; O, 17.00. Found C,, 69.10; H, 5.09; N, 8.40.

(*3'S*)-9-[3'-(Benzyloxycarbonyl)-3'-(9-fluorenylmethoxycarbonylamino)-propyl]-guanine, 18. Compound **16** (2.6 g, 5.65 mmol) was mixed with a solution of TFA (19 mL) in water (6 mL) and stirred at room temperature for 72 h. The solvent was evaporated and diethyl ether added to the residue to give a white solid that was dissolved in dry THF (50 mL). Diisopropylethylamine (3.9 mL, 22.6 mmol) was added followed by Fmoc-Cl (1.41 g, 5.65 mmol) in dry THF (10 mL). After 24 h of stirring at room temperature, the usual work-up gave a crude from which product **18** was isolated by flash chromatography with EtAc: MeOH 10 : 1. Obtained 2.3 g, 72% yield. ^1H NMR (300 MHz, $CDCl_3$) δ 2.09 (m, 1H), 2.29 (m, 1H), 4.02 (m, 3H), 4.23 (m, 1H), 4.34 (m, 2H), 5.09 (s-like, 2H), 6.30-6.45 (bs, 3H), 7.25-7.93 (series of m, 11H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 30.5, 46.6, 51.5, 59.5, 65.5, 66.0, 109.2, 119.7, 119.9, 121.0, 124.8, 126.7, 127.0, 127.3, 127.6, 128.1 (2C), 128.3 (2C), 128.6, 136.5, 137.1, 140.5, (2C), 143.5 (2C), 150.9, 153.5 (2C), 156.6, 171.1. Anal. Calcd for $C_{31}H_{28}N_6O_5$ (564.61): C, 65.95; H, 5.00; N, 14.88; O, 14.17. Found C, 66.01, H, 5.07; N, 14.72.

(*3'S*)-9-[3'-(Benzyloxycarbonyl))-3'-(9-fluorenylmethoxycarbonylamino)-propyl]-adenine, 22. Compound **21** (2.6 g, 6.1 mmol) was dissolved in dry CH_2Cl_2 (13 mL, 195 mmol) containing triethylsilane (2.4 mL, 15.3 mmol). To this solution TFA (6.1 mL, 79.3 mmol) was added and the mixture stirred at room temperature for 2 h. The solvent was evaporated under vacuum and ether (10 mL) was added and subsequently evaporated for three times until the residue became a white solid. The solid was dissolved in dry THF, diisopropyl ethylamine (4.3 mL, 24.4 mmol) was added, followed by 9-fluorenylmethyl chloroformate (1.58 g, 6.1 mmol) in dry THF (10 mL). After stirring at room temperature for 12 h, NH_4Cl (saturated solution) was added followed by ethyl acetate (100 mL). The organic layer was separated, washed two times with brine, dried over anhydrous Na_2SO_4 and the solvent evaporated. Flash chromatography (eluent Et Ac : MeOH 10 : 1) gave product **22** (2.0 g, 68% yield). ^1H NMR (300 MHz, $CDCl_3$) δ 2.20-2.50 (two large m, 2H), 4.10-4.28 (m, 3H), 4.35-4.61 (large m, 3H), 5.08 (s-like, 2H), 6.30 (bs, 2H), 6.49 (bs, 1H), 7.21-7.55 (series of m, 14 H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 32.2, 40.2, 47.0, 51.5, 66.8, 67.4, 119.4, 119.8 (2C), 124.9 (3C), 126.9 (2C), 127.6 (2C), 128.2 (2C), 128.5 (2C), 134.7, 140.7, 141.2 (2C), 143.5 (2C), 149.7, 152.8, 155.6, 156.2, 171.2. Anal. Calcd. for $C_{31}H_{28}N_6O_4$ (548.61): C, 67.87; H, 5.14; N, 15.32; O, 11.67. Found: C, 67.90; H, 5.17; N, 15.27.

Fmoc-Tby-OH, 10. General procedure for the removal of the benzyl ester. Pd /C 10% (about 1-2 g of catalyst for 2-5 g of product) was dissolved in methanol (40 mL) and activated with three cycles of vacuum / H_2. The flask was connected with a hydrogenation line (for reaction performed on smaller scale a balloon filled of H_2 was used) and the benzyl ester dissolved in methanol (10 mL) was added. The mixture was stirred until the proper amount of H_2 was adsorbed, (30-60 min for compounds **9**, **13** and **18,** 2 h for compound **22**). The catalyst was filtered off on Celite and the residue extracted several times with hot methanol (Warning, the solid has to be filtered when the solvent is still hot). The MeOH solutions were collected and evaporated to give the desired product as a solid.(75% yield) The solid was treated with boiling water, filtered and driied to give an analytical sample that gave a negative ninhydrin test. M.p. = 194-196°C (dec.). $[\alpha]_D^{25}$ = -6.4 (c = 1 in DMF). ^1H NMR (300 MHz, DMSO-d_6, 45°C) δ 1.86 (s, 3H, CH_3), 1.95 (m, 1H, CH_2), 2.08 (m, 1H), 3.61-3.81 (m, 1H), 3.84 (m, 1H), 4.01 (m, 1H CH), 4.25 (m, 1H), 4.43 (d-like, 2H), 5.76 (bd, 1H), 6.93 (s, 1H), 7.24, 7.81 (series of m, 8 H), 8.71 (bs, 1H), 9.7 (bs, 1H).^{13}C NMR (75 MHz, DMSO-d_6, 45°C) δ 11.5, 29.8, 44.4, 46.6, 65.6, 108.3, 119.7 (2C), 123.7, 124.9, 126.9 (2C), 127.5 (2C), 140.5, 140.9 (2C), 143.5 (2C), 150.5, 155.11, 163.9, 172.6..Anal. Calcd. for $C_{24}H_{23}N_3O_6$ (449.47): C, 64.14; H, 5.16; N, 9.35; O, 21.36. Found: C, 64.21; H, 5.19; N, 9.30.

Fmoc-Cby-OH, 14. 62% yield. M.p. = 214-218°C (dec.). $[\alpha]_D^{25}$ = -16.4 (c = 1 in DMF). ^1H NMR (300 MHz, DMSO-d_6, 45°C) δ 1.76-1.98 (m, 1H), 2.01-2.22 (m, 1H), 3.62-3.79 (m, 2H), 3.82-4.1 (m, 1H), 4.15-4.34 (m, 3H), 5.65 (bd, 1H), 7.21-7.98 (serie of m, 10H). ^{13}C NMR (75 MHz, DMSO-d_6, 45°C) δ 30.6, 46.2, 46.7, 51.5, 65.7, 92.9, 120.2 (2C), 125.3 (2C), 127.2 (2C), 127.7 (2C), 140.8 (2C), 142.1,143.8 (2C), 155.7, 156.2, 165.8, 173.7. Anal. calcd. for $C_{23}H_{22}N_4O_5$ (434.45): C, 63.59; H, 5.1; N, 12.9; O, 18.41. Found: C, 63.82; H, 4.96, N, 12.76.

Fmoc-Gby-OH, 19. 85% yield. M.p. = 220-224°C (dec.). $[\alpha]_D^{25}$ = -19.0 (c = 1 in DMF). ^1H NMR (300 MHz, DMSO-d_6, 45°C) δ 2.02 (m, 1H), 2.26 (m, 1H), 3.85-4.28 (series of m, 6H), 6.44 (bs, 1H), 7.20-7.94 (series of m, 11H), 10.6 (bm, 2H). ^{13}C NMR (75 MHz, DMSO-d_6, 45°C) δ 30.7, 45.6, 51.2, 65.6, 66.0, 116.6, 120.2 (2C), 125.0 (2C), 127.0 (2C), 127.7 (2C), 136.7, 140.5 (2C), 143.5 (2C), 150.7, 153.5, 156.5, 156.9, 172.5. Anal. calcd. for $C_{24}H_{22}N_6O_5$ (474.48): C, 60.75; H, 4.67; N, 17.71; O, 16.86. Found: C, 60.59, H, 4.57; N, 17.81.

Fmoc-Aby-OH, 23. 65% yield. M.p. = 184-186°C (evolution of gas observed). $[\alpha]_D^{25}$ = -17.5 (c = 1 in DMF) ^{1}H NMR (300 MHz, DMSO-d_6, 45°C) δ 1.98-2.22 (m, 1H) 2.28-2.5 (m, 1H), 3.74-3.93 (m, 1H), 4.06-4.41 (m, 5H), 7.16-7.51 (series of m, 6H), 7.64-8.20 (series of m, 7H). ^{13}C NMR (75 MHz, DMSO-d_6, 45°C) δ 30.6, 40.2, 46.6, 51.2, 66.8, 118.7, 120.1 (2C), 125.3 (2C), 127.1 (2C), 127.7 (2C), 140.7 (2C), 141.2, 143.8 (2C), 149.2, 149.6, 155.9, 156.2, 173.2. Anal. calcd. for $C_{24}H_{22}N_6O_4$ (458.48): C, 62.88; H, 4.80; N, 18.34; O, 13.98. Found C, 62.62; H, 4.76; N, 18.33.

Boc-D-ALA-ABY-OBn, 24 Compound **21** (0.3 g), 0.7 mmol) was dissolved in CH_2Cl_2 (15 mL) and Et_3SiH (0.28 mL, 1.75 mmol) and TFA (0.7 mL, 9.1 mmol) subsequently added. After 2 h tlc analysis (eluent Et Ac: MeOH, 10 : 1) showed that the starting material was consumed. Diethyl ether (15 mL) was added and the volatiles removed under vacuum (rotatory film evaporator). The residue was suspended in CH_2Cl_2 (15 mL) and cooled to 0°C. iPr_2EtN (0.37 mL, 2.1 mmol) was added followed by Boc-D-Ala-OH (0.145 g, 0.77 mmol) and DEPC (0.12 g, 0.77 mmol). After 2 h of stirring at room temperature, NH_4Cl (10 mL of a saturated solution) was added followed by ethyl acetate (60 mL). After separation, the organic layer was washed with Na_2CO_3 (10 mL 10% solution), HCl (10 mL 3M solution) and brine. After drying and evaporation of the solvent, column chromatography on silica gel (eluent Et Ac followed by EtAc: MeOH 10: 1) yielded compound **24** (0.265g, 81% yield). ^{1}H NMR (300 MHz, DMSO-d_6, 45°C) δ 1.40 (d, 3H, J = 7Hz), 1.43 (s, 9H), 2.2-2.5 (m, 2H), 4.2-4.4 (m, 3H), 4.6 (m, 1H), 4.92 (s-like, 2H), 5.11 (d, J = 8 Hz, 1H), 5.89 (bs, 1H), 7.3 (m, 5H), 7.62 (d, 1H), 7.85 (s, 1H), 8.34 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6, 45°C) δ 18.1, 28.2 (3C), 32.1, 40.7, 49.2, 51.2, 67.1, 80.2, 119.3, 128.2 (3C), 129.1 (2C), 135.6, 141.1 (2C), 150.7, 152.2, 156.6, 171.2, 173.3. Anal. calcd. for $C_{27}H_{31}N_7O_5$ (533.59): C, 60.78; H, 5.86. N, 18.37; O 14.99. Found C, 61.92; H, 5.79; N, 18.43.

D-Ala-Aby-OH 25. After activation of Pd/C (100 mg) in MeOH (15 mL) as described for the preparation of compound **10**, dipeptide **24** (0.23 g, 0.4 mmol) was added and the mixture maintained under a H_2 atmosphere (balloon) for 2 h. The catalyst was filtered off on Celite and the residue extracted several times with hot methanol. The MeOH solutions were collected and evaporated to give a solid. After addition of CH_2Cl_2 (3 mL), Et_3SiH (0.09 mL, 1.0 mmol) and TFA (0.57 mL, 5.2 mmol) were subsequently added and the mixture stirred for 4 h at room temperature. The volatiles were removed under vacuum and diethyl ether added to have the formation of a solid which was dried in a dessicator in the presence of phosphorous pentoxide. ^{1}H NMR (300 MHz, DMSO-d_6, 45°C) δ 1.41 (d, 3H, J=7 Hz), 2.1-2.3 (m, 1H), 2.4-2.6 (m, 1H), 3.6-4.0 (m, 1H), 4.2 (m, 1H), 4.3 (m, 2H), 8.4 (s, 1H), 8.48 (s, 1H), 8.94 (d, J= 8 Hz, 1H), 9.22 (s, 1H). ^{13}C NMR (75 MHz, DMSO-d_6, 45°C) δ 18.5, 32.7, 40.8, 48.6, 62.5, 118.7, 141.3, 149.9, 152.8, 155.7, 172.5, 174.1. Ms, m/z (%): 308 (M^++ 1, 5), 275 (10), 130 (45), 86 (100).

Acknowledgement. *We would like to acknowledge the financial support of MURST, Rome (fondi 40%). We also acknowledge André Mann and Angèle Schoenfelder (CNRS, Strasbourg) for the technical assistance.*

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(*Received in UK* 6 *September* 1996; *revised* 11 *November* 1996; *accepted* 14 *November* 1996)