Pergamon

Tetrahedron Letters 43 (2002) 6665–6667

TETRAHEDRON LETTERS

Solid-phase synthesis of a folate conjugate of a DNA binding polyamide

Sanjay K. Sharma and J. William Lown*

Department of Chemistry, University of Alberta, Edmonton, AB, Canada T6G 2G2

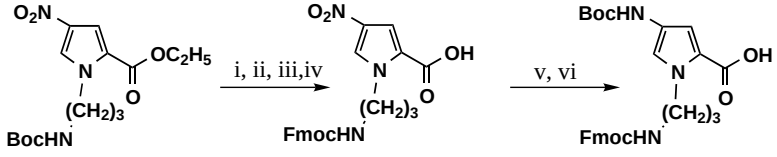
Received 22 May 2002; accepted 3 July 2002

Abstract—Solid-phase synthesis of a folate tripyrrolocarboxamide conjugate **8** of a DNA binding polyamide is described. The synthesis of a new building block monomer Boc-Py-[$(CH_2)_3$-NHFmoc] acid **3** is also reported.

Cell permeable small molecules that target predetermined DNA sequences offer a potential approach for the regulation of gene expression.[1] Although oligonucleotides and their analogues have been shown to interfere with gene expression,[2] the triple helix approach is limited to recognition of purines and suffers from poor cellular uptake. The subsequent development of pairing rules for minor groove binding polyamides containing pyrrole (Py) and imidazole (Im) moieties offers a second code to control sequence specificity.[3] A considerable amount of synthetic and biological work has been carried out on polyamides related to distamycin and netropsin in our group and in Dervan's group, which have shown promising and encouraging results on various biological targets.[4]

An ongoing research programme in our group is aimed at the nondestructive delivery of these exogenous molecules through the plasma membrane into living cells. Recently we have tagged these polyamides with a fluorescent label and studied the subcellular distribution within cells using confocal laser scanning microscopy.[5] Receptor-mediated uptake of folic acid could also be exploited to facilitate entry of attached macromolecules into both cultured cells and living cells.[6] Antitumor agents are likely to be the most suitable for conjugating to folate, since the folate receptor is known to be overexpressed on epithelial malignancies such as ovarian, colorectal and breast cancer, whereas in normal tissue it is expressed at very low levels.[7] Attaching a folate moiety to the polyamides could allow the entry of such conjugates into the cells. Therefore in order to facilitate cellular uptake of these polyamides, we herein describe the synthesis of a folic acid conjugate to tripyrrolocarboxamide polyamide **8** by a solid phase method.

The synthesis of *N*-Boc-propyl-nitropyrrole-ethyl ester **1** (Scheme 1) was carried out as described in our previous paper.[5] One of the monomers required for the solid-phase synthesis **3** was synthesized using **1**. The Boc protection on the amino chain in **1** was favored over Fmoc protection initially because in the subsequent step alkaline hydrolysis of the ester group would deblock the Fmoc group.



1 **2** **3**

Scheme 1. (i) EtOH, 0.1N NaOH, 0.1N HCl; (ii) 80% TFA, CH_2Cl_2, 0°C to rt, 2 h; (iii) Fmoc succinimide, 1:1 acetone/aqueous Na_2CO_3, 12 h, 60%; (iv) H_2/Pd–C, MeOH, 2 h; (vi) Boc anhydride, MeOH, rt, 2 h, 80%.

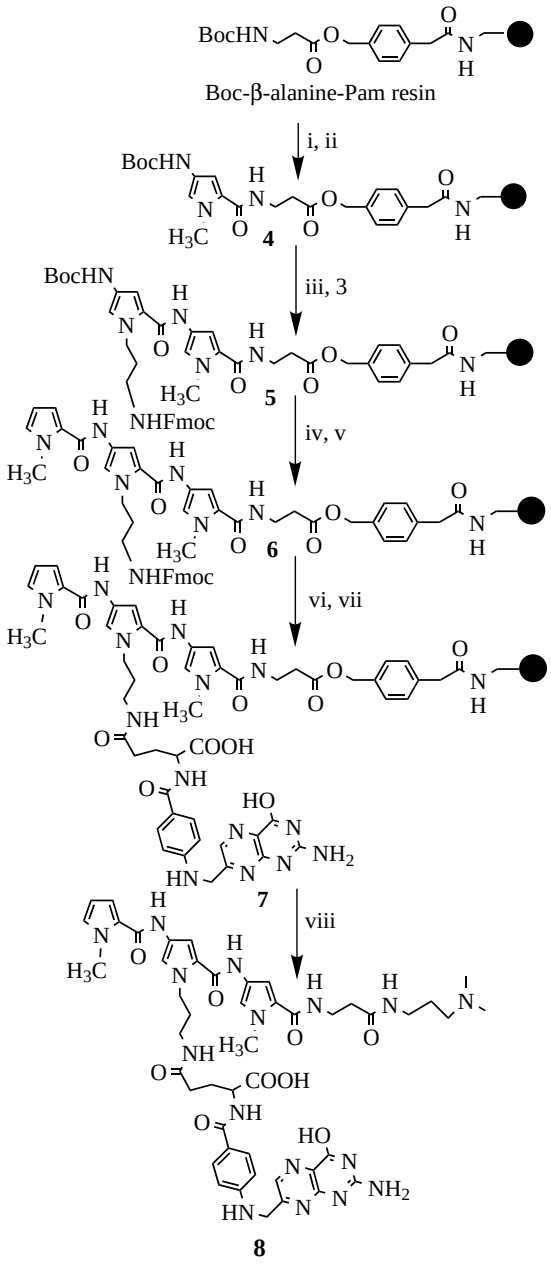
Keywords: DNA; polyamides; folic acid; solid-phase; conjugate.

* Corresponding author. Tel.: (780) 492-3646; fax: (780) 492-8231; e-mail: annabelle.wiseman@ualberta.ca

0040-4039/02/$ - see front matter
PII: S0040-4039(02)01448-X

Alkaline hydrolysis of **1** followed by Boc deprotection (80% TFA/CH_2Cl_2) afforded the corresponding amino acid, which was then successfully reprotected with Fmoc-succinimide in acetone–aqueous Na_2CO_3 to give **2**[†] in 60% overall yield.[8] The nitro group on **2** was reduced by hydrogenation with 10% Pd/C to give unstable amine, which was immediately protected with Boc-anhydride to give **3**[‡] in 80% yield. The monomer building block **3** has two amino protecting group and a free acid. The free acid group and the NHBoc group were used for the chain elongation of the basic unit of the polyamide with carboxamide bonds, while the NHFmoc group was used in the final step for the coupling of folic acid.

The solid-phase synthesis of **8** is depicted in Scheme 2. The resin used for the solid-phase synthesis, Boc-β-alanine-Pam-resin **4**[9] (230 mg, 0.046 mmol) was packed into a 20 mL filtration column fitted with a filter at one end and stopper at the other end. Dry DMF (2 mL) was added to the resin and the mixture was shaken for 5 min and then the solvent was drained, this procedure was repeated twice. Then the resin was washed with dry CH_2Cl_2 (2×5 mL). The resin was dried by vacuum in the column and after drying it was treated with 2 ml of 80% TFA/CH_2Cl_2, 1 mL of 0.5 M PhSH and shaken for 30 min. The resin was washed thoroughly with DMF (3×5 mL) and then with CH_2Cl_2 (5×5 mL) and dried. The Boc-Py-Obt activated ester of the pyrrole moiety (53 mg, 0.15 mmol)[9] was then transferred to the column containing the free NH_2 group on the resin with DMF (1 mL) and 0.1 mL of diisopropylethylamine. The mixture was shaken for 90 min. A small portion of the resin was taken for analysis (deprotection from the resin and then analyzing it with mass spectroscopy). The resin **5** was treated with 80% TFA, washing and drying as for the first cycle and then the activated ester of **3** prepared in a separate vial (75 mg of **3** (0.15 mmol), HOBt (20 mg, 0.15 mmol), DCC (30.9 mg, 0.15 mmol) stirring at rt for 30 min), was transferred to the deprotected resin (free amino group of) **5** with DMF (1 mL) and 0.1 mL DIEA. The mixture was shaken for 90 min. A small portion of the resin was taken for analysis. The resin **6** was washed with CH_2Cl_2 and DMF and dried. The resin **6** was treated with 80% TFA (2 mL), washed and dried as in the earlier two cycles and then activated ester of pyrrole-2-carboxylic acid[9] was added to the free amino group of **6** on the resin with DMF (1 mL) and 0.1 mL of DIEA. The mixture was shaken for 90 min. A small portion of the resin was taken for analysis. The tripyrrole unit **7** on the resin was washed with CH_2Cl_2 and DMF and dried, followed by removal of the Fmoc group with 20% piperidine/NMP, washing with CH_2Cl_2 and DMF and then the addition of the activated ester of folic acid [folic acid (71 mg, 0.15 mmol), HOBt (33



Scheme 2. (i) 80% TFA, CH_2Cl_2, 0.4 M PhSH; (ii) Boc-Py-OBt, DMF, DIEA, 90 min; (iii) 80% TFA, CH_2Cl_2, 0.4 M PhSH; (iv) Boc-Py-[$(CH_2)_3$-Fmoc)]-acid **3**, HOBt, DCC, DMF, DIEA, 90 min; (v) 80% TFA, CH_2Cl_2, 0.4 M PhSH; (vi) Py-OBt, DMF, DIEA, 90 min; (vii) 20% piperidine, NMP, folic acid, DCC, HOBt, DIEA, 5 h; (viii) (*N*,*N*′-dimethylamino)propylamine, 55°C, 5 h.

[†] Compound **2**. ^{1}HNMR (DMSO-d_6): δ 8.25 (s, 1H, N*H* exchanged with D_2O), 7.87 (d, 2H, Fmoc-*H*), 7.65 (d, 2H, Fmoc-*H*), 7.50 (t, 2H, Fmoc-*H*), 7.32 (t, 3H, 2 Fmoc-H and 1 Py-*H*), 7.29 (d, J=6.5 Hz, 1H, Py-*H*), 4.31 (q, J=6.9 Hz, 4H, C*H*$_2$-CH and N-C*H*$_2$-), 4.20 (t, 1H, C*H*$_2$-CH-), 2.95 (q, J=6.5 Hz, 2H, -CH$_2$-C*H*$_2$-CH$_2$-), 1.85 (q, J=6.5 Hz, 2H, -CH$_2$-CH$_2$-C*H*$_2$-Fmoc). HRMS calcd for $C_{23}H_{21}N_3O_6$ 435.143, found 458.132 (M^++Na).

[‡] Compound **3**. ^{1}HNMR (DMSO-d_6): δ 9.19 (s, 1H, N*H*Boc, exchanged with D_2O), 7.95 (s, 1H, N*H*Fmoc, exchanged with D_2O), 7.88 (d, 2H, Fmoc-*H*), 7.69 (d, 2H, Fmoc-*H*), 7.50 (t, 2H, Fmoc-*H*), 7.32 (t, 3H, 2 Fmoc-H and 1 Py-*H*), 7.12 (d, J=6.5 Hz, 1H, Py-*H*), 6.59 (d, J=6.5 Hz, 1H, Py-*H*), 4.29 (d, 2H, C*H*$_2$-CH-), 4.19 (t, 3H, CH$_2$-C*H*-, and N-C*H*$_2$), 2.91 (q, J=6.9 Hz, 2H, -CH$_2$-C*H*$_2$-CH$_2$-), 1.75 (q, J=6.9 Hz, 2H, -CH$_2$-CH$_2$-C*H*$_2$-Fmoc). HRMS calcd for $C_{28}H_{31}N_3O_6$ 505.221, found 528.211 (M^++Na).

mg, 0.25 mmol), DCC (51 mg, 0.25 mmol)] shaking the mixture for 5 h. A small portion of the resin was removed for analysis. The resin was cleaved by aminolysis with (*N*,*N*′-dimethylamino)propylamine, by heating at 55°C for 5 h. The composition of folate conjugate **8** (60% yield), precipited by adding methanol, was confirmed by HRMS of the sample [calcd for $C_{47}H_{58}N_{16}O_9$ 991.09, found 1013.09 (M^++ Na)].

In summary we have described a solid-phase synthetic approach to connect a *N*-methyloligopyrrole-polyamide to folic acid using a new monomer. The new monomer should prove flexible enough to provide a variety of derivatives with different molecules. Results from cellular uptake and subcellular distribution will be reported in due course.

Acknowledgements

We are grateful for a research grant (to J.W.L) from the Natural Sciences and Engineering Research Council of Canada.

References

1. Gottesfeld, J. M.; Neely, J.; Trauger, J. W.; Baird, E. E.; Dervan, P. B. *Nature* **1997**, *387*, 202.
2. (a) Maher, J. L.; Dervan, P. B.; Wold, B. *Biochemistry* **1992**, *31*, 70; (b) Thuong, N. T.; Helene, C. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 504.
3. (a) Wade, W. S.; Mrksich, M.; Dervan, P. B. *J. Am. Chem. Soc.* **1992**, *114*, 8783; (b) Mrksich, M.; Wade, W. S.; Dwyer, T. J.; Geierstanger, B. H.; Wemmer, D. E.; Dervan, P. B. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 7586; (c) Trauger, J. W.; Baird, E. E.; Dervan, P. B. *Nature* **1996**, *382*, 559; (d) Chan, Y. H.; Lown, J. W. *J. Am. Chem. Soc.* **1994**, *116*, 6995.
4. Sharma, S. K.; Reddy, B. S. N.; Lown, J. W. *Drugs of the Future* **2001**, *26*, 39 and references cited therein.
5. Sharma, S. K.; Morrissey, A. T.; Miller, G. G.; Gmeiner, W. H.; Lown, J. W. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 769.
6. Leamon, C. P.; Low, P. S. *J. Biol. Chem.* **1992**, *267*, 24966.
7. Konig, B.; Rodel, M. *Synth. Commun.* **1999**, *29*, 943.
8. Nomura, M.; Shuto, S.; Matsuda, A. *J. Org. Chem.* **2000**, *65*, 5016.
9. (a) Baird, E. E.; Dervan, P. B. *J. Am. Chem. Soc.* **1996**, *118*, 6141; (b) Sharma, S. K.; Tandon, M.; Lown, J. W. *J. Org. Chem.* **2001**, *66*, 1030; (c) Wurtz, N. R.; Turner, J. M.; Baird, E. E.; Dervan, P. B. *Org. Lett.* **2001**, *3*, 1201.