Tetrahedron Vol. 48, No. 42, pp. 9277-9282, 1992
Printed in Great Britain

0040-4020/92 $5.00+.00

SYNTHESIS OF A SPERMIDINE-DERIVED CONJUGATING AGENT.

Christophe Morin*, Michel Vidal.

LEDSS, Chimie Recherches, Université de Grenoble,
38402 St Martin d'Hères (France).

(*Received in Belgium* 20 *July* 1992)

Abstract : a short synthesis of a spermidine conjugating agent is presented.

Spermidine, 1, is implicated in diverse physiological processes that share as a common thread a close relationship to cell proliferation and growth; in particular, it associates with polyanions such as DNA and RNAs . Spermidine derivatives are usually accessible by way of multi-step elongation of lower homologues [1,2] . The recent announcement [3] of the selective protection of spermidine primary amino groups, resulting in the preparation of a spermidine conjugate, prompted us to disclose herein our own results in this area.

Various procedures for selective reactions of terminal nitrogen atoms of spermidine have been disclosed [4-9] , but none of them is directly applicable to transient protection ; however, a direct N^1,N^8 acetylation process, based on a ruthenium-catalysed chemoselective amidation [10], has been achieved ; to obtain this diacetamide for the present work, the recently described [11] (and experimentally more convenient) selective acetylation by means of N-methoxydiacetamide has been applied to 1. The efficiency of this procedure is shown by the fact that a single product, 2, is formed, which could be isolated in 91 % yield .

Although 2 could be further reacted at its unprotected secondary amino group to introduce a pendant side chain, subsequent removal of the N-acetyl groups could not be achieved in a satisfactory manner [12]. Therefore another selective protection / deprotection sequence had to be used.

Triphenylmethyl (trityl) protecting groups [13] have been extensively used for the protection of alcohols , in particular during oligonucleotides syntheses, but, to the best of our knowledge, they have received limited attention for the selective protection of amino groups [14]. In this work, the p-methoxy derivative [15-16] has been used, due to its more facile cleavage when deprotection is needed [17]. Thus, upon reaction with 2.5 equivalents of p-methoxy trityl chloride

in the presence of 4-dimethylaminopyridine[18], 1 gave the bis(tritylated) derivative 3 in 50 - 65 % isolated yield. Alkylation of the remaining central secondary amino group (trityl groups did indeed shield their neighbouring amino groups from alkylation) could then be achieved from 3, in this case with bromoester 4.

$$R\text{-}NH\text{-}(CH_2)_3\text{-}N(R')\text{-}(CH_2)_4\text{-}NH\text{-}R$$

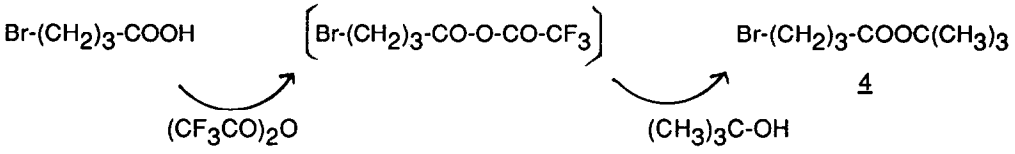
1 : R = R' = H

2 : R = CH_3CO, R' = H

3 : R = p-CH_3O-C_6H_4-$(C_6H_5)_2C$-, R' = H

5 : R = p-CH_3O-C_6H_4-$(C_6H_5)_2C$-, R' = -$(CH_2)_3$-$COOC(CH_3)_3$

6 : R = H, R' = -$(CH_2)_3$-COOH



Preparation of the latter deserves comment as introduction of the tert-butyl group from commercially available 4-bromobutyric acid was carried out by way of the tert-butanol cleavage of an *in situ* -formed mixed anhydride [19] . This procedure avoids the classical use of isobutene and competes favorably with literature methods as 4 [20-22] could be obtained in over 80 % yield. Alkylation of 3 to give 5 was then performed under conventional conditions. That the central secondary amino group of 3 had indeed been alkylated was revealed by the presence in the carbon-13 spectrum of three resonances between 52 and 54 ppm, two of them being shifted downfield when compared with the spectrum of 3. Final, simultaneous deprotection of both amine and carboxylic acid protecting groups under acidic conditions afforded 6, the desired spermidine conjugating [23] agent in good yield .

The whole scheme is straightforward and it is hoped that the two particular procedures presented here (selective protection of amino groups and "mixed anhydride" introduction of a t-butyl ester) will find other useful synthetic applications.

Acknowledgments : Cis Biointernational is thanked for financial support.

Experimental

Except for tetrahydrofuran which was distilled on sodium, other solvents were dried with molecular sieves. The spectra were recorded on Bruker instrument at the field and in the solvent indicated for each compound. Standard abbreviations were used for nmr description. Microanalyses were performed by the Service Central d'Analyses du CNRS, Vernaison (France).

N^1,N^8 -bis (p-methoxytrityl) spermidine 3.
Under argon, spermidine (145 mg - 1 mmol) was dissolved in a mixture of dry dichloromethane (2 mL) and dry pyridine (1 mL) at 4° C. After addition of a crystal of 4-dimethylaminopyridine, 4-methoxytrityl chloride (772 mg - 2.5 mmol) was added and stirring of the deep red-orange solution was continued at room temperature for 2 days.The mixture was quenched with ice and extraction was performed with dichloromethane. The organic layer was washed with water, and dried (sodium sulfate) and the volatiles were removed under reduced pressure without heating. Column chromatography of the crude residue was performed on silica gel 60 starting with dichloromethane with increasing amounts of methanol; elution with dichloromethane-methanol 90 - 10 afforded pure 3 as a white foam (480 mg - 67 %).

Alternatively, for a shorter reaction time, this reaction was conducted as follows : under argon, spermidine (1.45 g - 10 mmol) was dissolved in a mixture of dichloromethane (15 mL) and pyridine (10 mL) and this solution was stirred while 4-methoxytrityl chloride (7.72 g - 25 mmol) was added in 3 portions followed by triethylamine (3.5 mL - 25 mmol). After 4 hours stirring, the reaction mixture was processed as above. MPLC [24] purification (on silica gel 60 H, same eluant) which is more convenient on this scale, afforded 3 (3.68 g - 50 %).
Anal. for 3 - 1.5 H_2O. [25] Calc. C : 78.74, H : 7.59; N : 5.86.
Found. C : 78.73, H : 7.55; N : 5.88.

^{1}H (300 MHz, $CDCl_3$) : 7.1 - 7.5 (several m , 24 H : o, m, p and o'); 6.8 (d, J=8.4, 4H : m'); 3.74 and 3.73 (s, 3H each : OCH_3); 2.80, 2.65, 2.25 and 2.15 (t, J=7.0, 7.0, 6.3 and 6.7 respectively, 2H each : 1,3, 5 and 8); 1.8, 1.65 and 1.5 (m, 2H each : 2, 6 and 7).
^{13}C (75 MHz, $CDCl_3$) : 157.9 : p'; 146.4 : i; 138.4 : i'; 129.7 : o'; 128.5 : o; 127.7 : m; 126.1 : p; 113.0 : m'; 70.3 : C(Ar)$_3$; 55.1 : OCH_3; 49.4 and 48.4 : 3 and 5; 43.4 and 42.1 : 1 and 8; 30.6, 28.6 and 22.7 : 2, 6 and 7.

Tert-butyl 4-bromobutyrate 4.

To a stirred solution of 4-bromobutyric acid (3.34 g - 20 mmol) in dry tetrahydrofuran (30 mL) at -40° C under argon was added dropwise trifluoroacetic anhydride (6 mL - 42.5 mmol) ; the temperature was maintained at -40 °C for 30 minutes; an excess of tert-butyl alcohol (25 mL) in dry tetrahydrofuran (5 mL) was then slowly added. The cooling bath was then removed and the reaction mixture was stirred overnight. It was then slowly poured into cold aqueous sodium bicarbonate (foaming) and then extracted with diethyl ether. After having been washed with water, the organic layer was dried (sodium sulfate) and volatiles were removed under reduced pressure. The residue was submitted to bulb to bulb distillation (Eb. $_{0.3}$ = ca. 50 °C (oven temperature) ; litt. : Eb. $_{11}$ = 65-67 °C [22]) to afford pure 4 (3.30 g - 81 %).

As its ^{13}C nmr cannot be found in the literature [20-22], these data are now given .
^{13}C (50 MHz, $CDCl_3$) : 171.5 : 1 ; 80.5 : C(CH_3)$_3$; 33.5 and 32.6 : 2 and 4;27.9:C(CH_3)$_3$; 27.8 : 3 .

N^1,N^8-bis(p-methoxytrityl) N^4 -(t.-butyloxycarbonylpropyl) spermidine 5.

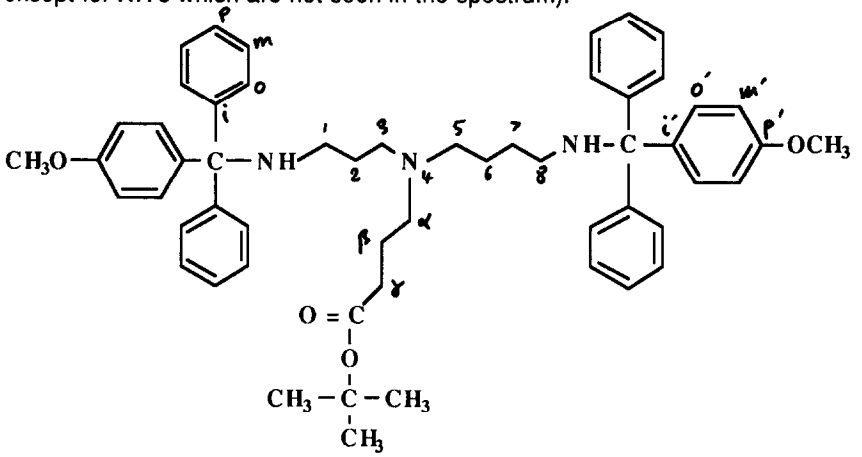
To a solution of 3 (3.0 g - 2.39 mmol) in dry acetonitrile (20 mL) at 4°C under argon, were added successively with stirring, potassium carbonate (875 mg 6.34 mmol, 2.65 eq.), sodium iodide (955 mg, 6.36 mmol , 2.66 eq.), acetone (250 µL) and freshly distilled 4 (1.42 g, 6.36 mmol , 2.66 eq.). The cooling bath was removed and the mixture was stirred overnight at 70°C. The cooled mixture was filtered and the residue was abundantly washed with acetonitrile. The volatiles were removed under reduced pressure without heating and the residue was chromatographed by MPLC on silica gel 60 H [24] to afford, using dichloromethane - methanol (100 : 0 to 98 :2) as the eluant, pure 5 as an hygroscopic oil (1.98 g - 55 %).

Anal. for 5 - 1.5 H_2O. [25] Calc. C : 76.89, H : 7.63; N : 4.89.

Found. C : 76.96, H : 8.05; N : 4.89.

IR (film)1720cm^{-1} : C=O.

^{1} H (200 MHz, $CDCl_3$) : 7.1-7.5 (M , 24H : o, m, p and o'); 6.8 (two d , J = 8.9, 4H : m'); 3.75 and 3.74 (s, 3H each : OCH_3), 1.3-2.5 (M with s at 1.45 ($C(CH_3)_3$), 20 H: all other protons except for NH's which are not seen in the spectrum).



^{13} C (50 MHz, $CDCl_3$) : 173.0 : C=O; 157.7 : p'; 146.5 : i; 138.5 : i'; 129.7 : o'; 128.5 : o; 127.7 : m; 126.0 : p; 113.0 : m'; 80.0 : $\underline{C}(CH_3)_3$; 70.3 : $\underline{C}(Ar)_3$; 55.1 : OCH_3 ; 53.9, 52.9 and 52.5 : 3, 5 and α; 44.0 and 42.3 : 1 and 8; 33.3, 29.7, 28.8, 24.9 and 22;3 : 2, 6, 7, β and γ; 28.1 : $C(\underline{C}H_3)_3$.

N^4 (-carboxypropyl) spermidine 6.

To a solution of 5 (1.98 g - 2.3 mmol) in tetrahydrofuran (40 mL) was added hydrochloric acid (200 mL of an 0.5 M aqueous solution). The mixture was stirred at 70 °C for 2 hours and cooled to room temperature. The pH was then adjusted to 7.0 with diluted aqueous sodium hydroxide and the mixture concentrated to half volume. It was then extracted twice with diethyl ether and the aqueous layer was evaporated under reduced pressure to dryness. The product was taken up in methanol, the salts filtered and washed with methanol; concentration of the methanolic solution gave pure 6 (0.47 g - 88 %) as a very hygroscopic solid [26] .

^{1} H (300 MHz, CD_3OD) : 2.5-2.9 (M, 10H : 1,3,5,8 and α); 1.9-2.1 (m, 2H,) γ; 1.1-1.8 (M, 8H : 2,6,7 and β).

^{13} C (75 MHz, CD_3OD) : 180.9 : CO; 55.8, 53.6 and 51.3 : 3, 5 and α; 40.1, 38.3 and 36.5 : 1, 8 and γ; 25.7, 23.6, 22.5 and 21.8 : 2,4, 6 and β.

NOTES AND REFERENCES

1. Ganem, B., *Acc. Chem. Res.*, **1982**, 15, 290.
2. Bergeron, R.J. *Acc. Chem. Res.*, **1986**, 19, 105.
3. Cohen, G.M., Cullis, P.M., Hartley, J.A., Mather, A, Symons, M.C.R. and Wheelhouse, R.T., *J. Chem. Soc. , Chem. Commun.*, **1992**, 298.
4. Kunieda, T., Higuchi, T., Abe, Y. and Hirobe, M., *Tet. Letters* **1982**, 23, 1159 .
5. Husson, A., Besselièvre, R. and Husson, H.-P., *Tet. Letters*, **1983**, 24, 1031.
6. Kunieda, T., Higuchi, T., Abe, Y. and Hirobe, M., *Tetrahedron*, **1983**, 20, 3253.
7. Joshua, A.V. and Scott, J.R., *Tet. Letters*, **1984**, 25, 5725.
8. Acher, F. and Wakselman, M., *J. Org. Chem.*, **1984**, 49, 4133.
9. Samejima, K., Takeda, Y., Kawase, M., Okada, M. and Kyogoku Y., *Chem. Pharm. Bull.*,**1984**, 32 , 3428.
10. Murahashi, S.-I., Naota, T. and Saito, E., *J. Am. Chem. Soc.*, **1986**, 108, 7846.
11. Kikugawa, Y., Mitsui, K., Sakamoto, T. Kawase, M. and Tamiya, H., *Tet. Letters*, **1990**, 31, 243.
12. Note however that 2 , by way of a reductive process (see :Bergeron, R. J., Neims, A.H., McManis, J.S., Hawthorne, T.R., Vinson, J.R.T., Bortell, R. and Ingeno, M.J., *J. Med. Chem.*, **1988**, 31, 1183) could open the way to N^1,N^8 bis(ethyl)spermidine derived conjugates . For biological activity of N^1,N^8 bis(ethyl)spermidine see *inter alia* : Libby, P.R. , Bergeron, R.J., and Porter, C.W. *Biochem. Pharmacol.*, **1989**, 38, 1435.
13. Helfrich, B., Moog, L., and Junger, A., *Chem. Ber.* **1925**, 58, 82.
14. Bodansky, M., *Principles of Peptide Synthesis*, Springer Verlag, Berlin, **1984**, p. 82.
15. Smith, M., Rammler, D.H., Goldberg, I.H. and Khorana, H.G., *J. Am. Chem. Soc.*, **1962**, 84, 430.
16. Lapidot, Y., de Groot, N., Weiss, M., Peled, R. and Wolman, Y., *Biochim. Biophys. Acta*, **1967**, 138, 241.

17. Among the various possible (un)substituted trityl groups, the para methoxytrityl group is a good compromise between ease of removal and reasonable stability (chromatography on silica gel could be performed without significant deprotection).

18. Chaudhary, S.K. and Hernandez, O., *Tet. Letters*, **1979**, 95; the presence of DMAP is not necessary however; see experimental part.

19. Bonar-Law, R., Davis, A.P., and Sanders, J.K.M., *J. Chem. Soc., Perkin Trans 1*, **1990**, 2245.

20. Henry, D.E., Pentoney Jr., S., Kondrat, R.W. and Griffiths, P.R., *Chromatographia*, **1987**, 23, 547.

21. Wu, T.-C., Wehmeyer, R.M. and Rieke, R.D., *J. Org. Chem.*, **1987**, 52, 5057.

22. Inoue, K., Yatsuzuka, M., Muramatsu, T., Hirano, Y. and Inouye H., *Yakugaku Zasshi*, **1988**, 108, 951.

23. This is the purpose of the carboxylic acid pendant side chain; for use of such spermidine conjugating agents see ref.3.

24. Morin, C., *J. Chem. Educ.*, **1988**, 65, 903.

25. Retention of molecules of water, as revealed by microanalysis assays, is not surprising; it should not be forgotten indeed that these compounds can also act as "ligands", for example of tripodal type; naturally -occuring siderophores such as agrobactin or parabactin (see : Tait, G.T., *Biochem. J.*, **1975**, 146, 191 and Neilands, J.B., Ong, S.A. and Peterson T., *J. Biol. Chem.*, **1979**, 254, 1860) do bear a spermidine backbone and therefore the use of 6, or its derivatives, for metal complexation constitutes another line of application.

26. This compound is very hygroscopic and no satisfactory microanalysis could be obtained in this case; the spectroscopic data however fully support the assigned structure.