Synthetic Communications

ISSN: 0039-7911 (Print) 1532-2432 (Online) Journal homepage: http://www.tandfonline.com/loi/lsyc20

Synthesis of 4-Benzidinyl Butyric Acid: A Key Intermediate for Antibodies Production Against Benzidin

Nathalie Fischer-Durand , Samy Ben Rejeb & François Le Goffic

To cite this article: Nathalie Fischer-Durand , Samy Ben Rejeb & François Le Goffic (1998) Synthesis of 4-Benzidinyl Butyric Acid: A Key Intermediate for Antibodies Production Against Benzidin, Synthetic Communications, 28:6, 963-970, DOI: 10.1080/00397919808003065

To link to this article: http://dx.doi.org/10.1080/00397919808003065

Published online: 20 Aug 2006.

SYNTHETIC COMMUNICATIONS, 28(6), 963-970 (1998)

SYNTHESIS OF 4-BENZIDINYL BUTYRIC ACID: A KEY INTERMEDIATE FOR ANTIBODIES PRODUCTION AGAINST BENZIDIN

Nathalie Fischer-Durand*, Samy Ben Rejeb and François Le Goffic

Laboratoire de Bioorganique et Biotechnologies associé au CNRS
E.N.S.C.P. 11, rue P. et M. Curie, 75231 Paris cedex 05, France

Abstract: a four-step synthesis of monoalkylated benzidin is reported for immunogen preparation against benzidin dye. Use of 10% H_2SO_4/ dioxan for N-Boc cleavage avoids the cyclisation of 4-benzidinyl butyric acid into the corresponding lactam observed with the classical TFA/ CH_2Cl_2 system.

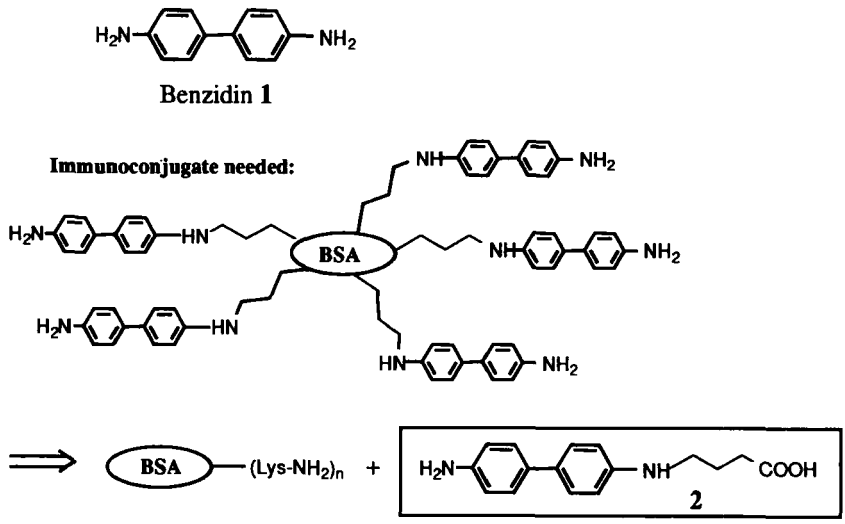
In the course of our studies devoted to the development of immunotitration techniques in view of the monitoring of traces of pollutants in the environment at low level, we were interested in the detection of benzidin **1** in waste waters and soil suspected of contamination near industrial areas. Industrial uses of azo-dyes, especially those derived from benzidin, are harshly controled due to their potent human carcinogenicity[1,2].

We have previously described an immunopreconcentration procedure based on antigen-antibody recognition for the selective solid-phase extraction clean-up step

* To whom correspondence should be addressed

before HPLC analysis [3,4]. This technique allowed the detection of triazines and phenylureas pesticides in water at the 0.1 $\mu g.L^{-1}$ level required by the European guideline[5] .The preparation of such immunosorbents needs the production of polyclonal antibodies against the target compound to be analysed.

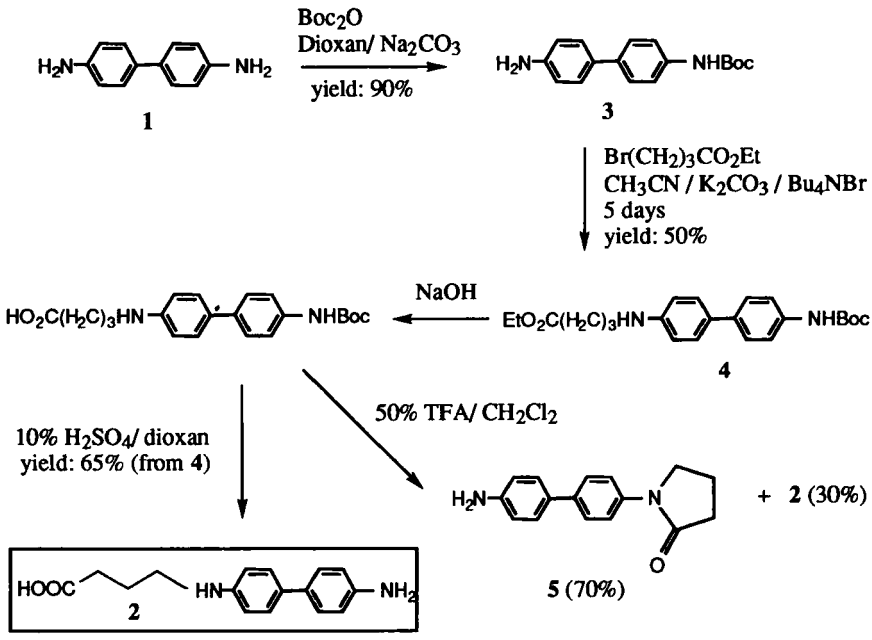
Benzidin **1** is a too small molecule to be immunogenic (184 mol.wt). Therefore it must be attached to a carrier protein, typically Bovine Serum Albumine (BSA, 66000 mol. wt), before injection to rabbits for polyclonal antibodies production[6]. Usually, coupling reaction takes place between the NH_2 terminal function of lysine residues in BSA (35 accessible lysines) and a carboxylic function introduced into the target compound through a spacer arm (scheme 1):



Scheme 1

The purpose of this paper is to report the synthesis of benzidin hapten **2**, as we were faced to a lack of litterature references devoted to mono N-alkylation of this molecule.

Direct mono N-alkylation of benzidin using large excess of the diamine in dilute medium, with slow addition of the alkylating agent ($Br(CH_2)_3CO_2Et$) lead to only traces of monoalkylated material. So we turned to a different strategy based on protection of one amino group before alkylation of the unprotected function (scheme 2):



Scheme 2

N-Boc protection was readily achieved in 90% yield after chromatography using conventionnal method[7]. The carbamate functionality deactivated the second NH_2 group, allowing only monoprotection. Initial alkylation was carried out in refluxing anhydrous CH_3CN with K_2CO_3 and 18-C-6. Starting material disappearance was monitored by TLC and needed 8 days. Only 21% of the expected compound **4** was

obtained after chromatography, as well as numerous by-products. Changing from crown ether to phase transfert catalyst (10% mol Bu_4NBr) raised dramatically the yield to 50%, and shortened the reaction time to 5 days. This reaction was not more optimized due to the high toxicity of benzidin derivatives. After saponification of the ester , N-Boc deprotection was undertaken with 50% TFA/ CH_2Cl_2. Surprisingly lactam **5** was the major product [8], and various attempts to hydrolyse it in acidic or basic medium were unsuccessful. Fortunately, changing from TFA/ CH_2Cl_2 to 10% H_2SO_4/ dioxan[9] lead to the desired compound instantaneously, in 65% yield from **4**.

This particular reactivity of benzidinyl butyric acid towards one of the most classical N-Boc cleavage conditions (50% TFA/ CH_2Cl_2), gave us the opportunity to show that 10% H_2SO_4/ dioxan is a good alternative for this deprotection when handling such derivatives.

Experimental

NMR spectra were recorded on a Bruker AM 200 (200MHz) and on a Bruker AM 250 (250 MHz) spectrometers. Chemical shifts are reported in ppm, relative to Me_4Si. Mass spectra were recorded on a NERMAG R10-10C mass analyser. Melting point were determined on a Mettler FP 62 apparatus. Silica gel 60 (230-400 Mesh) from Merck was used for flash chromatography. Analytical TLC were performed on sheets coated with silica gel containing a luminescer (254 nm) (Riedel-de Haën), or on sheets coated with RP-18 (Merck). Benzidin was purchased from Fluka.

N- tertbutoxycarbonyl benzidin, 3

0.5g (2.71 mmol) of benzidin were dissolved in dioxan (30 ml), and Na_2CO_3

(0.28g, 1 eq) was added. 0.59g (2.71 mmol) of Boc_2O dissolved in dioxan (30 ml) were added slowly with stirring using a dropping funnel, and the reaction mixture was allowed to stir at room temperature overnight [TLC monitoring AcOEt: $CHCl_3$ / 50:50; R_f benzidine= 0.4 (blue-green after I_2 exposure; R_f N-Boc benzidine= 0.6 (brown)]. Dioxan was then partially evaporated, and ethyl acetate was added. The organic layer was washed 2 times with a saturated aqueous NaCl solution, dried over Na_2SO_4, and evaporated to dryness. The crude was purified by flash chromatography with AcOEt: $CHCl_3$ / 50:50 as eluent to give an orange powder (0.69g, 90%). m.p.= 145°C. ^1H NMR (DMSO) δ: 1.48 (s, 9H, *t*Bu), 5.16 (2 H, NH_2), 6.61 (d, J= 8.5 Hz, 2 H_{ar}), 7.3 (d, J= 8.5 Hz, 2 H_{ar}), 7.43 (m, 4 H_{ar}), 9.33 (1 H, N*H*tBu). ^{13}C NMR (DMSO) δ: 28.4 (*t*Bu), 79.2 (C_q *t*Bu), 114.5, 118.7, 125.7, 126.9, 127.5, 134.8, 137.7, 148.1, 153 (C_{ar}).

MS (IC+NH_3) m/z: 302 [M-NH_4^+], 285 [MH^+].

***N-tertbutoxycarbonyl-N'(4-ethyl butyrate) benzidin,* 4**

0.35g (1.23 mmol) of **3** were dissolved in dry CH_3CN (25 ml) under Argon. Dry K_2CO_3 (0.34g, 2eq) and Bu_4NBr (40 mg, 10% mol) were added. The reaction vessel was protected from light and heated under reflux, then ethyl 4-bromobutyrate (0.21 ml, 1.2eq) were added. The mixture was kept with stirring under reflux and argon atmosphere 5 days (TLC monitoring AcOEt: $CHCl_3$ / 5: 95). After cooling, ethyl acetate was added and the organic layer was washed successively with a dilute aqueous solution of 1N $KHSO_4$, water, and saturated aqueous NaCl solution. The organic solution was dried (Na_2SO_4), evaporated to dryness and purified by flash chromatography using AcOEt: $CHCl_3$ / 5: 95 as eluent to give compound **4** as orange powder (0.24g, 50%). R_f= 0.5 (green spot then purple after I_2 exposure). m.p.= 139°C. ^1H NMR ($CDCl_3$) δ: 1.28 (t, 3 H,

CH_3 ester), 1.55 (s, 9 H, *t*Bu), 1.99 (quint., J= 6.95 Hz, 2 H, $CH_2CH_2CH_2$), 2.46 (t, J= 7.26 Hz, 2 H, CH_2CO), 3.24 (t, J= 6.85 Hz, 2 H, CH_2NH), 4.17 (q, 2 H, CH_2 ester), 6.51 (br s, NH), 6.68 (d, J= 8.7 Hz, 2 H, H_{ar}), 7.4 (m, 6 H, H_{ar}). ^{13}C NMR ($CDCl_3$) δ: 14 (CH_3 ester), 24.1 ($CH_2CH_2CH_2$), 28 (*t*Bu), 31.65 (CH_2CO), 43.8 (CH_2N), 60.35 (CH_2O ester), 80.25 (C_q *t*Bu), 113.6, 118.75, 126.5, 127.4, 130.5, 135.7, 136.5, 145.9, 152.6 (C_{ar}), 173.2 (CO ester).

***4-benzidinyl-butyric acid,* 2**

0.19 g (0.447 mmol) of the diprotected compound **4** were dissolved in dioxan (7 ml), and a solution of 0.25 N NaOH in water (3 ml) was added. The reaction mixture was allowed to stir overnight at room temperature. The solution was then diluted in ethyl acetate and water was added. The aqueous layer was acidified with a diluted 1N $KHSO_4$ solution, and extracted 3 times with ethyl acetate. The organic layer was dried over Na_2SO_4, and evaporated to dryness to give an orange oil (0.16 g , 93%).

NMR data of the crude acid: ^1H NMR (DMSO) δ: 1.48 (s, 9 H, *t*Bu), 1.79 (quint., J= 7 Hz, 2 H, $CH_2CH_2CH_2$), 2.33 (t, J= 7.4 Hz, 2 H, CH_2CO), 3.05 (t, J= 6.9 Hz, 2 H, CH_2N), 6.61 (d, J= 8.6 Hz, 2 H, H_{ar}), 7.33 (d, J= 8.6 Hz, 2 H, H_{ar}), 7.4 (m, 4 H_{ar}), 9.26 (br s, NH). ^{13}C NMR (DMSO) δ: 24.34 ($CH_2CH_2CH_2$), 28.43 (*t*Bu), 31.5 (CH_2CO), 42.4 (CH_2N), 79.2 (C_q, *t*Bu), 112.6, 118.7, 125.66, 126.9, 134.7, 137.8, 148, 153 (C_{ar}), 174.6 (CO_2H).

The crude acid was then dissolved in 2.5 ml dioxan, and cooled in a cold water bath, while 0.25 ml of concentrated H_2SO_4 (> 95%) was added dropwise. The solution turned red instantaneously. After 10 min (TLC monitoring: CH_3CN: H_2O/ AcOH / 5.5: 4.5: 0.1 on RP-18 sheet), water was added, and the solution was

treated with NaOH 30% until a solid separated (between pH 3 and 6). The compound was collected, washed with water, and dried to afford 78 mg (65%) of **2** as a beige powder. m.p.> 250°C (decomp.). ^{1}H NMR (DMSO) δ: 1.77 (quint., J= 6.9 Hz, 2 H, $CH_2CH_2CH_2$), 2.33 (t, J= 7.1 Hz, CH_2CO), 3.03 (t, J= 7 Hz, CH_2N), 6.58 (d, J=7.9 Hz, 4 H_{ar}), 7.23 (m, 4 H_{ar}). ^{13}C NMR (DMSO) δ: 24.3 ($CH_2CH_2CH_2$), 31.45 (CH_2CO), 42.6 (CH_2N), 112.5, 114.7, 126.2, (C_{ar}), 174.6 (CO_2H).

MS (IC+NH_3) m/z: 271 [MH^+].

Acknowledgement

This work was supported by the Environment R& D Program 1996-1999 on the analysis of different groups of organic pollutants in industrial effluents and waste waters from the commission of the European Communities (contract N° ENV4-CT95-0016).

References and notes

1. Jones, T. C. "Preliminary risk assessment phase I on benzidin, its congeners and their derivative dyes and pigments", Washington, EPA, **1980**.

2. Haley, T. J. *clinical toxicology,* **1975**, *8*, 13.

3. Pichon V., Chen L., Hennion M-C., Daniel R., Martel A., Le Goffic F., Abian J., Barcelo D. *Anal. Chem.* (**1995**), *67*, 2451.

4. Pichon V., Chen L., Durand N., Le Goffic F., Hennion M-C. *J. Chromatogr., A* (**1996**), *725*, 107.

5. EC Directive relating quality of water intended for human consumption (80/778/EEC), *Off. J. Eur. Commun.*, L229/11.

6. Kerr M. A., Thorpe R. editors "Immunochemistry labFax", Bios Scientific Publishers Limited, Oxford, **1994**, pp. 63-81.

7. Greene T. W., Wuts P. G. M. "Protective groups in organic synthesis" 2nd edition, Wiley-interscience, N.Y., **1991**.

8. Data of lactam **5**:

^{1}H NMR (DMSO) δ: 2.08 (quint., J= 7.5 Hz, 2 H, $CH_2CH_2CH_2$), 2.5 (CH_2CO, with DMSO), 3.86 (t, J= 6.8 Hz, 2H, CH_2N), 7 (d, J= 8.6 Hz, 2 H_{ar}), 7.6 (m, 6 H_{ar}).

MS (IC+NH_3) m/z: 253 [MH^+].

9. Houghten R. A., Beckman A., Ostresh J. M. *Int. J. Pept. Protein Res.* (**1986**), *27*, 653.

(Received in the UK 15th July, 1997)