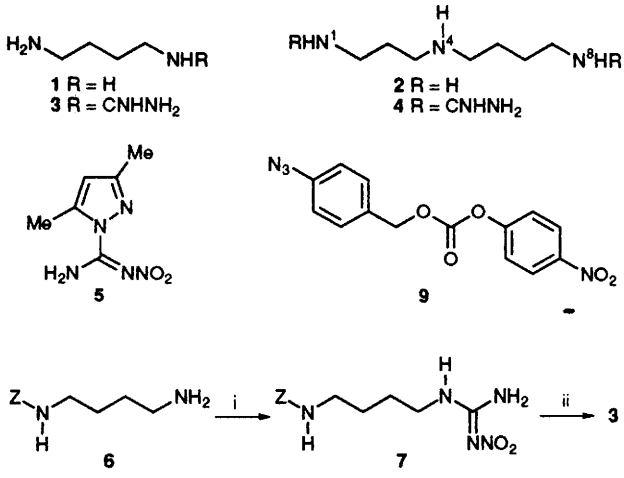
Discrimination of Spermidine Amino Functions by a New Protecting Group Strategy; Application to the Synthesis of Guanidinylated Polyamines, Including Hirudonine

Andrew Mitchinson, Bernard T. Golding,* Roger J. Griffin and Mary C. O'Sullivan

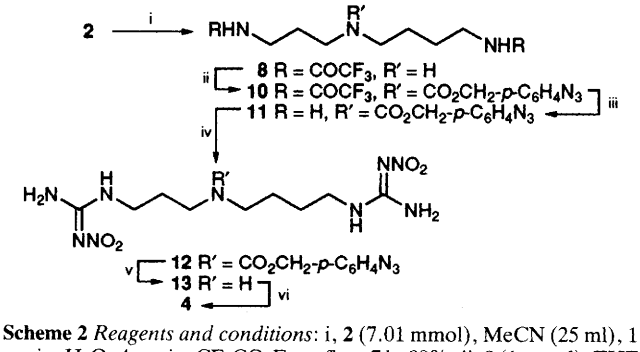
Department of Chemistry, Bedson Building, University of Newcastle upon Tyne, Newcastle upon Tyne, UK NE1 7RU

Agmatine and hirudonine, guanidine derivatives of putrescine and spermidine, respectively, are synthesised by the application of a new protecting group strategy for polyamines, which uses *N*-nitroguanidinyl as a precursor of guanidine functions and selectively blocks spermidine at *N*-1 and *N*-8 with trifluoroacetyl and at *N*-4 by 4-azidobenzyloxycarbonyl.

The participation of polyamines (*e.g.* putrescine, **1** and spermidine, **2**) in cellular growth and proliferation[1] has created much interest in the development of chemotherapeutic agents to modulate these functions.[2] Most mammalian cells possess a polyamine biosynthetic pathway, but many also exhibit a specific polyamine uptake system.[3,4] Such systems are potential vehicles for delivering cytotoxic agents into cells, and attempts have already been made to exploit this in cancer chemotherapy.[5–7] The substrate-binding sites of polyamine transporters are thought to contain carboxylate groups, which interact with protonated polyamines.[8] Strong guanidinium–carboxylate interactions have been observed in enzyme–substrate complexes,[9] suggesting that guanidinylated polyamine derivatives will be readily accepted by polyamine uptake mechanisms. Indeed, the anti-tumour agent methylglyoxal-bis(guanylhydrazone) (MGBG) and analogues are known to be taken up by polyamine transporters, and inhibit polyamine biosynthesis.[10]



Scheme 1 *Reagents and conditions*: i, **6** (2.69 mmol), MeOH (10 ml), DMNPC (1 equiv.), room temp., 3 days, 85%; ii, 7 (0.81 mmol), 5% w/v HCO_2H–MeOH; 20 ml, 10% Pd/C (1 mass equiv.), room temp., 2 h, 52% **3** (Z = $PhCH_2OCO$)



Scheme 2 *Reagents and conditions*: i, **2** (7.01 mmol), MeCN (25 ml), 1 equiv. H_2O, 4 equiv. CF_3CO_2Et, reflux, 7 h, 89%; ii, **8** (1 mmol), THF (9 ml), ABNPC (1.1 equiv.), dark, room temp., 24 h, 92%; iii, **10** (0.22 mmol), MeOH (8 ml), conc. NH_3 (2 ml), dark, room temp., 6 days, 82%; iv, **11** (1.7 mmol), MeOH (10 ml), DMNPC (2 equiv.), dark, room temp., 3 days, 81%; v, **12** (0.64 mmol), MeOH–H_2O (9 : 1 v/v; 5 ml), DTT (4 equiv.), Et_3N (4 equiv.), room temp., 4 h, 50%; vi, **13** (0.31 mmol), HCO_2H–H_2O (5% w/v; 7.5 ml), 10% Pd/C (0.5 mass equiv.), room temp., 3 h, 89%

We report the synthesis of polyamine derivatives, where one or more of the primary amino groups of **1** and **2** have been replaced by a guanidine function.[11] These derivatives are agmatine **3**, an arginine metabolite, and hirudonine **4**, which is located in the central nervous system of the leech *Hirudo medicinalis* L.[12] We illustrate new protecting group strategies that will be of general use for the synthesis of polyamines selectively modified at particular amino functions.

The presence of a guanidine group in a synthetic intermediate can be problematic because the basicity of guanidines often makes further synthetic transformations difficult. Primary amines can be converted into *N*-nitroguanidines by reaction with 3,5-dimethyl-*N*-nitro-1*H*-pyrazole-1-carboximidamide (DMNPC, **5**).[13] We have found that this reaction proceeds faster and more cleanly in methanol, than reported in dioxan.[14] Nitroguanidines exhibit low basicity, and so are easily carried through several stages of a synthesis before their final reduction to guanidines.[14] The use of the nitroguanidine group as a masked guanidine was initially applied to the synthesis of **3** from **1** (see Scheme 1). The monoprotected putrescine **6**[15] was converted into **7** by treatment with DMNPC in methanol. In preference to electrochemical reduction[14] we found catalytic transfer hydrogenation enabled simultaneous deprotection of the primary amine and reduction of the nitroguanidine to yield **3**, isolated as its sulfate salt.

Treatment of **2** with CF_3CO_2Et in MeCN containing traces of water gave 8 in 89% yield (Scheme 2). Compound **8** is isolated directly as its trifluoroacetate salt, with the trifluoroacetic acid produced by partial hydrolysis of CF_3CO_2Et. Similar selectivity has been reported for the ruthenium-catalysed condensation of nitriles with **2**, yielding 1,8-*N*-bis-substituted diamides,[16] and for acylations of spermidine using 3-acylthiazolidine-2-thiones[17] or acyl cyanides.[18] The free *N*-4-position of **8** was protected with 4-azidobenzyloxycarbonyl (ACBZ) using 4-azidobenzyl-4-nitrophenyl carbonate (ABNPC, **9**)[19] in THF to yield the fully protected spermidine derivative **10**. Deprotection of the terminal amino groups was achieved by treatment of **10** with conc. NH_3 in MeOH to give **11**. This was treated with DMNPC (2 equiv.) in MeOH to afford **12**. Removal of the *N*-4-protecting group was effected by treating **12** with excess of dithiothreitol (DTT) and Et_3N.[20] DTT reduces the azido group to an amine, triggering a fragmentation of the carbamate, which leads to liberation of **13**. This method of deprotection is chemoselective, and suggests great potential for the 4-azidobenzyloxycarbonyl group in polyamine syntheses, where 'orthogonal' protecting groups are often required. Finally, reduction of the nitroguanidines in **13** by catalytic transfer hydrogenation as before generated **4**, isolated as its crystalline sulfate salt. This compound has previously been obtained by direct reaction of spermidine with *S*-methylisothiourea, but no yield was given.[21]

We thank Dr L. L. Smith (MRC Toxicology Unit,

Leicester) and Lilly Pharmaceuticals for their financial support of this work.

Received, 17th August 1994; Com. 4/05052E

References

1 A. E. Pegg, *Cancer Res.*, 1988, **48**, 759.
2 C. W. Porter and J. R. Sufrin, *Anticancer Res.*, 1986, **6**, 525.
3 N. Seiler and F. Dezeure, *Int. J. Biochem.*, 1990, **22**, 211; D. M. L. Morgan, *Biochem. J.*, 1992, **286**, 413.
4 M. C. O'Sullivan, B. T. Golding, L. L. Smith and I. Wyatt, *Biochem. Pharmacol.*, 1991, **41**, 1839.
5 C. W. Porter, P. F. Cavanaugh, N. Stolowich, B. Ganis, E. Kelly and R. J. Bergeron, *Cancer Res.*, 1985, **45**, 2050.
6 W. D. W. Heston, K. A. Watanabe, K. W. Pankiewicz and D. F. Covey, *Biochem. Pharmacol.*, 1987, **36**, 1849.
7 J. L. Holley, A. Mather, R. T. Wheelhouse, P. M. Cullis, J. A. Hartley, J. P. Bingham and G. M. Cohen, *Cancer Res.*, 1992, **52**, 4190; G. M. Cohen, P. M. Cullis, J. A. Hartley, A. Mather, M. C. R. Symons and R. T. Wheelhouse, *J. Chem. Soc., Chem. Commun.*, 1992, 298.
8 B. T. Golding, I. K. Nassereddin and M. C. O'Sullivan, *Biochem. Soc. Trans.*, 1990, **18**, 1087.
9 K. W. Hart, A. R. Clarke, D. B. Wigley, A. D. B. Waldman, W. N. Chia, D. A. Barstow, T. Atkinson, J. B. Jones and J. J. Holbrook, *Biochim. Biophys. Acta*, 1987, **914**, 294; D. B. Wigley, A. Lyall, K. W. Hart and J. J. Holbrook, *Biochem. Biophys. Res. Commun.*, 1987, **149**, 927.
10 U. Regenass, G. Caravatti, H. Mett, J. Stanek, P. Schneider, M. Müller, A. Matter, P. Vertino and C. W. Porter, *Cancer Res.*, 1992, **52**, 4712.
11 A brief account of these results was given at the 651st meeting of the Biochemical Society, Lancaster, UK, July 1994 (see B. T. Golding, R. J. Griffin, A. Mitchinson and M. C. O'Sullivan, *Biochem. Soc. Trans.*, 1994, **22**, 3915).
12 Y. Robin, N. van Thoai and L.-A. Pradel, *Biochim. Biophys. Acta*, 1957, **24**, 381.
13 A. F. S. A. Habeeb, *Biochim. Biophys. Acta*, 1964, **93**, 533.
14 J. Boukouvalas, B. T. Golding, R. W. McCabe and P. K. Slaich, *Angew. Chem. Suppl.*, 1983, 860.
15 W. B. Lawson, M. D. Leafer, A. Tewes and G. J. S. Rao, *Z. Physiol. Chem.*, 1968, **349**, 251.
16 S.-I. Murahashi, T. Naota and E. Saito, *J. Am. Chem. Soc.*, 1986, **108**, 7846.
17 Y. Nagao, K. Seno, T. Miyasaka and E. Fujita, *Chem. Lett.*, 1980, 159.
18 S.-I. Murahashi, T. Naota and N. Nakajima, *Chem. Lett.*, 1987, 879.
19 A. Robinson, E. L. Evers, R. J. Griffin and W. J. Irwin, *J. Pharm. Pharmacol.*, 1988, **40**, 61; R. J. Griffin, E. L. Evers, R. Davison, D. Layton and W. J. Irwin, manuscript to be submitted to *J. Chem. Soc., Perkin Trans. 1*.
20 J. V. Staros, H. Bayley, D. N. Standring and J. R. Knowles, *Biochem. Biophys. Res. Commun.*, 1978, **80**, 568.
21 Y. Robin and N. V. Thoai, *C. R. Séances Acad. Sci.*, 1961, **252**, 1224.