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Purines Nucleosides as Cholinesterase inhibitors

F. Marcelo,[a,b] A. P. Rauter,[a] Y. Blériot,[b] P. Sinaÿ,[b] F. V. M. Silva,[a,c] M. Goulart,[a,c] J. Justino[c]

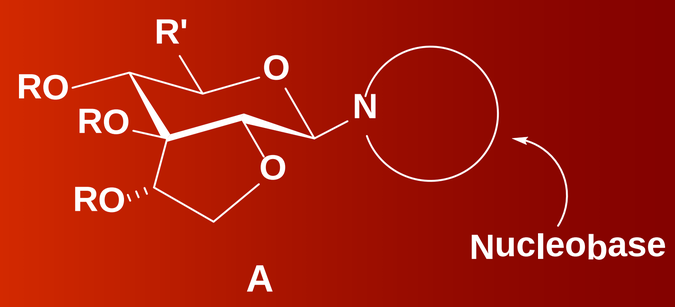
[a]Grupo de Química dos Glúcidos do centro de Química e Bioquímica / Departamento de Química e Bioquímica da Faculdade de Ciências da Universidade de Lisboa, Edifício C8, 5º Piso, 1749-016, Lisboa, Portugal
[b]Équipe de Glycochimie Organique, Biologique et Supramoléculaire ,UMR 7611, Université Pierre et Marie Curie, 4 Place de Jussieu, Paris, F-75005, France
[c]Escola Superior Agrária de Santarém, Quinta do Galinheiro, Apartado 310, 2001-904, Santarém, Portugal
jorge.justino@ipsantarem.pt

INTRODUCTION

In Alzheimer's disease (AD), there is a severe loss of cholinergic cells in the brain that leads to diminished levels of the neurotransmitter acetylcholine.[1,2] Acetylcholinesterase (AChE, EC 3.1.1.7) and butyrylcholinesterase (BuChE, EC 3.1.1.8) are serine hydrolase enzymes that effect the breakdown of acetylcholine.[3] Therefore, inhibition of these enzymes has proven to be a successful approach to treat some of the symptoms of AD.[4] However, in AD, AChE levels in the brain are already decreased, while BuChE activity is elevated suggesting that acetylcholine hydrolysis in AD may occur to a greater extent via BuChE catalysis.[5] In this regard, it has been reported that the specific inhibition of BuChE is important in raising acetylcholine levels and improving cognition. Cholinesterase inhibitors currently in use to treat AD inhibit both AChE and BuChE,[6] and with these drugs it is difficult to determine whether the positive effects observed are a result of inhibition of AChE, BuChE or both enzymes. For this reason, it is important to test selective, potent and well-tolerated inhibitors of each individual cholinesterase in order to determine which enzyme needs to be the focus for maximum effect in treating AD.[7]

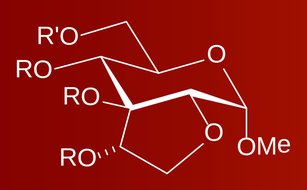
ABSTRACT

In this communication we report the synthesis of new protected purine nucleosides in which an unusual bicyclic sugar moiety is present (compounds type **A**). These nucleosides, as well as their precursors, were evaluated as AChE and BuChE inhibitors. A strong and selective inhibition for BuChE was observed for some of the compounds tested when compared to the control (rivastigmine). N^7 nucleosides were the most promising compounds and for that purpose, stereo- and regioselective coupling conditions were devised in order to obtain only the N^7 regioisomers.



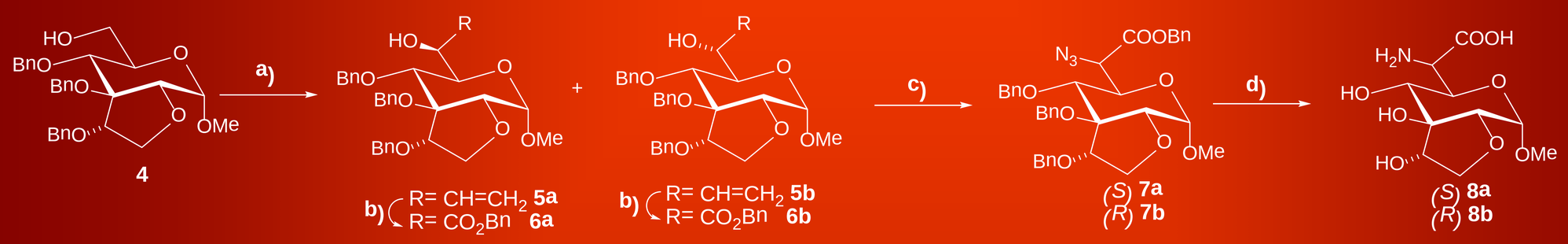
A

SYNTHESIS AND BIOLOGICAL EVALUATION



R = R' = H **1**
R = R' = Ac **2**
R = R' = Bz **3**
R = Bn R' = H **4**

Each synthetic compound was evaluated for its ability to inhibit BChE human serum and AChE bovine erythrocytes using Ellman's spectrophotometric method.[8] None of the compounds tested showed a significant inhibition of AChE. However, inhibition of BChE was observed and for some compounds achieved the nanomolar concentration range. Compound **19** showed a IC_{50} of same order of magnitude as that of the control. Observing the table we can conclude that the inclusion of benzyl groups and purine nucleobase strongly influence this activity. Toxicity studies were also performed and the low values for cytotoxicity and genotoxicity encourage the investigation of these compounds for the control of AD.

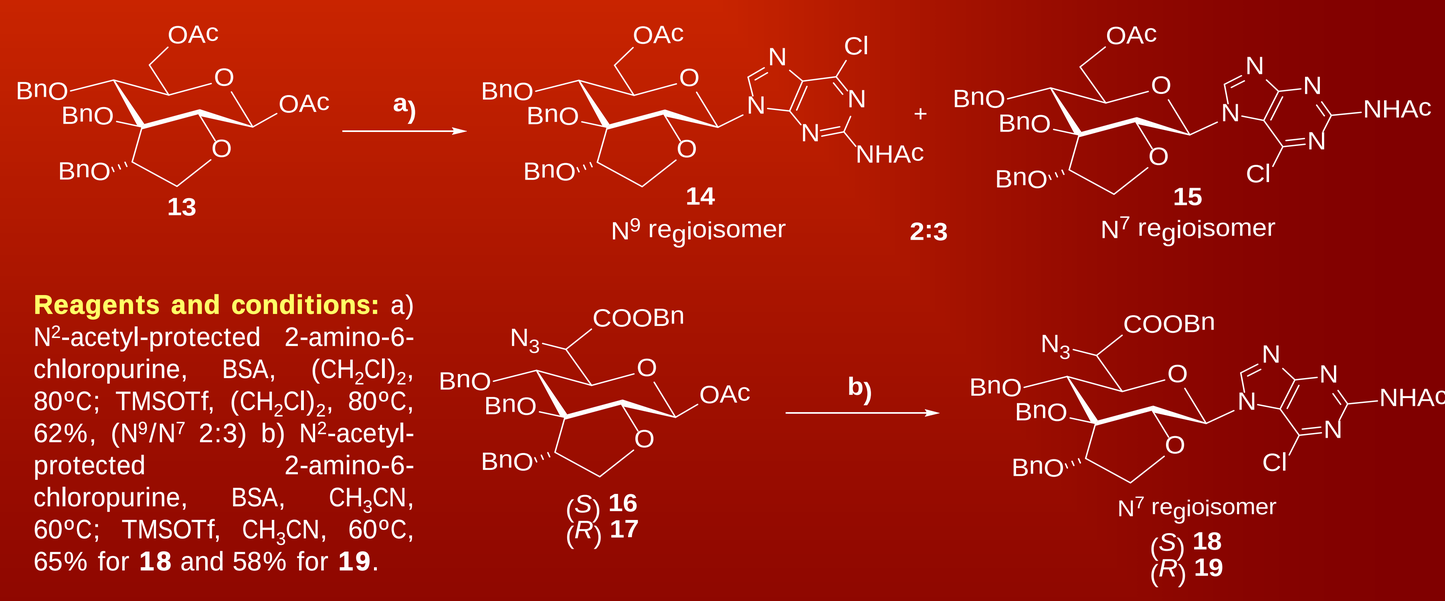


Reagents and conditions: a) $(COCl)_2$, DMSO, Et_3N, DCM, -78ºC; CH_2=CHMgBr, THF, -78ºC → r.t., 65% over 2 steps b) O_3, DMS, DCM, -78ºC; $NaClO_2$, $NaH_2PO_4.H_2O$, *t*BuOH/H_2O/2-methylbut-2-ene (2:2:1); BnBr, Bu_4NI, $KHCO_3$, DMF, 68% over 3 steps c) Tf_2O, py, DCM, -78 ºC; NaN_3, DMF, 72% for **7a** and 86% for **7b** over 2 steps d) H_2, 10% Pd/C, EtOAc/MeOH/HCl 1M, quant. For **8a** and **8b**.

Table: % Inhibition of BChE for different concentrations and IC_{50} values of test compounds and rivastigmine[b].

compound	Concentration (µg/mL)	% inhibition BChE	BChE (IC50, µM)[a]
1	100	<5	...
2	100	<5	...
3	100	7	...
4	100, 10	60***, <5	148
5a	100, 10, 1	60***, 54***, 4	63,3
5b	100, 10	73***, 23	49,8
6a	100, 10, 1	85***, 56***, 7	20,9
6b	100, 10	55***, 8	132,4
7a	100, 10, 1, 0.1	73***, 64***, 36***, 15*	7,4
7b	100, 10, 1	81***, 73***, 14**	18,5
8a	100, 10	18**, 5	...
8b	100, 10	18**, 5	...
14	100, 10	48***, 11	...
15	100, 10, 1, 0.1	84***, 85***, 70***, 20***	1,1
18	100, 10, 1	67***, 34***, 9	37,5
19	100, 10, 1, 0.1, 0.01	83***, 79***, 77***, 63***, 10	0,11
rivastigmine[b]	100, 10, 1, 0.1, 0.01	100***, 100***, 100***, 76***, ni	0,17

[a]IC_{50} is an estimate of the compound concentration which inhibits 50% of the enzyme activity. [b]Rivastigmine is a standard drug to treat AD patients and was used as positive control. *** P<0.001, **P<0.01, *P<0.05.



Reagents and conditions: a) N^2-acetyl-protected 2-amino-6-chloropurine, BSA, $(CH_2Cl)_2$, 80ºC; TMSOTf, $(CH_2Cl)_2$, 80ºC, 62%, (N^9/N^7 2:3) b) N^2-acetyl-protected 2-amino-6-chloropurine, BSA, CH_3CN, 60ºC; TMSOTf, CH_3CN, 60ºC, 65% for **18** and 58% for **19**.

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