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Synthesis of Aminophosphonate Haptens for an Aminoacylation Reaction Between Methyl Glucoside and a β-alanyl Ester

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Abstract—Two 2-aminophosphonate haptens derived from methyl α-D-glucopyranoside were synthesized to mimic the transition-state of a transesterification reaction between methyl α-D-glucopyranoside and 4-nitrophenylester of *tert*-BOC-β-alanine. Two sets of monoclonal antibodies were generated against these haptens.

Only a few reports of catalytic monoclonal antibodies with aminoacylase properties have hitherto been published. In one example, an antibody raised against a charged benzylphosphonate monoester catalysed the transesterification reaction between vinyl ester and various alcohols.[1] Antibodies generated against a neutral thymidylphosphonate diester hapten catalysed the aminoacylation, i.e., the transesterification reaction of the 3′-hydroxyl group of thymidine with activated phenyl esters of phenylethyl carbamate-protected D- and L-alanine.[2] We have been interested in modifying carbohydrates with amino acids by the transesterification reaction of mono-, di- and oligosaccharides with activated *N*-protected amino acid esters. We chose the transesterification reaction between methyl α-D-glucopyranoside **1** and 4-nitrophenyl ester of *tert*-BOC-β-alanine **2** as a model system for the antibody catalysis study (Scheme 1). The aminophosphonate haptens **5** and **6** were designed and synthesized to elicit the monoclonal antibodies. The tribenzoyl hapten **5** incorporates the charged phosphonate monoester moiety as a transition-state analogue and three benzoyl groups for additional immunogenicity. The trihydroxy hapten **6** contains not only the phosphonate transition-state element but also the unprotected 2-, 3- and 4- hydroxyl groups providing structural similarity with the substrate **1**.

Preparation of the tribenzoyl hapten **5** was rather straightforward (Scheme 2).[3] The starting material methyl 2,3,4-tri-*O*-benzoyl-α-D-glucopyranoside **7** was synthesized from **1** in three steps (DMAP-catalysed tritylation in DMF, benzoylation in pyridine and detritylation with HBF_4 in acetonitrile) in 54% total yield.[4] The tribenzoyl glucopyranoside **7** was allowed to react with *N*-benzyloxycarbonyl (CBZ) and *O*-benzyl-protected (2-aminoethyl)-phosphonochloridate **8** in the presence of Hünig's base in CH_2Cl_2 to produce the neutral phosphonate diester **9**. The CBZ as well as the benzyl protecting groups were removed by catalytic hydrogenation in 1,4-dioxane, using palladium on activated carbon as a catalyst, to give the (2-aminoethyl)phosphonic acid **10** as the key intermediate in 81% yield. The bifunctional *N*-hydroxysuccinimide glutaryl chloride linker **11** was subsequently attached to the monophosphonic acid **10** in the presence of Hünig's base in CH_2Cl_2 to give the tribenzoyl hapten **5** in 64% yield.

The intermediate phosphonochloridate **8** was conveniently prepared in five steps from ethanolamine **12**, starting with the protecting of its amino portion with a benzyloxycarbonyl group (Scheme 3). The CBZ-protected ethanolamine **13** was converted to tosylate **14**, which was subsequently allowed to react with sodium dibenzyl phosphinate in THF at room temperature to provide the CBZ-protected *O,O*-dibenzyl (2-aminoethyl)phosphonate **15** in 78% yield.[5] Next, dibenzyl phosphonate **15** was monodeprotected with quinuclidine in refluxing toluene. The resulting quinuclidinium salt **16** was chlorinated with oxalyl chloride using a catalytic amount of DMF to yield the CBZ-protected (2-aminoethyl)phosphonochloridate **8** in 56% yield (five steps).[6,7]

Finally, the trihydroxy hapten **6** was synthesized by coupling phosphonochloridate **8** and methyl 2,3,4-*O*-tris (phenylmethyl)-α-D-glucopyranoside[8] **17** in the presence of Hünig's base in CH_2Cl_2 (Scheme 2). The CBZ- and benzyl-protected phosphonate **18** were subjected to catalytic

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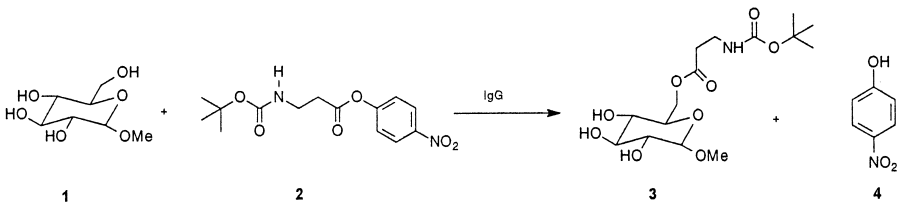
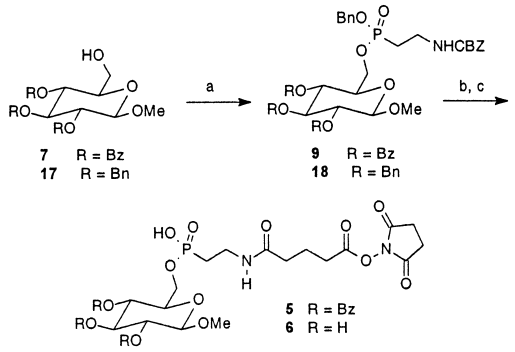
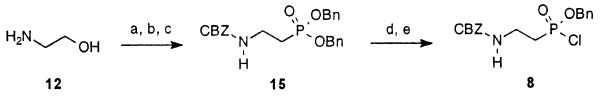


Scheme 1.



Scheme 2. Reagents and conditions: (a) phosphonochloridate **8**, Hünig's base, CH_2Cl_2, 0 °C, 76% for **9**, 79% for **18**; (b) H_2, Pd-C, 1,4-dioxane, 81% for **10**, 89% for **19**; (c) 5-[(2,5-dioxo-1-pyrrolidinyl)oxy]-5-oxopentanoyl chloride **11**, Hünig's base, CH_2Cl_2, 64% for **5**, 40% for **6**. Bn = benzyl, Bz = benzoyl, CBZ = benzyloxycarbonyl.



Scheme 3. Reagents and conditions: (a) BnOCOCl, Hünig's base, CH_2Cl_2, 93%; (b) TsCl, py, 82%; (c) $(BnO)_2PNa$, THF, 78%; (d) quinuclidine, PhMe, Δ; (e), $(COCl)_2$, DMF cat., 94%.

hydrogenation to afford monophosphonic acid **19** in 89% yield. The bifunctional linker **11** was subsequently attached to **19** to give the desired trihydroxy hapten **6** in 40% yield.

The aminophosphonate haptens **5** and **6** were conjugated to keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA) for the purposes of immunisation and ELISA screening, respectively. Standard hybridoma methodology was used to elicit monoclonal antibodies of IgG class against KLH-**5** and KLH-**6**.[9] A total of 11 and 6 antibody-secreting hybridomas were isolated specifically for BSA-**5** and BSA-**6**, respectively. After rigorous purification (sodium ammonium sulphate precipitation, anion exchange chromatography with DEAE, cation exchange chromatography with mono Q and affinity chromatography with protein G), the monoclonal antibodies were investigated for their ability to enhance the rate of the transesterification reaction between methyl α-D-glucopyranoside **1** and 4-nitrophenyl ester of *tert*-BOC-β-alanine **2**.[10]

None of the antibodies elicited against KLH-**5** and KLH-**6** were found to be catalysts for the aminoacylation reaction between methyl glucoside **1** and the activated β-alanyl ester **2** in an aqueous medium. However, we are currently studying the same aminoacylation reaction in organic solvents, using the obtained KLH-**5** and KLH-**6** antibodies in reverse micelles and antibodies immobilised to the solid supports.[11–13] The results of these studies will be reported in due course.

Acknowledgements

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References and Notes

1. Wirsching, P.; Ashley, J. A.; Benkovic, S. J.; Janda, K. D.; Lerner, R. A. *Science* **1991**, *252*, 680.
2. Jacobsen, J. R.; Prudent, J. R.; Kochersperger, L.; Yonkovich, S.; Schultz, P. G. *Science* **1992**, *256*, 365.
3. All the compounds were characterised by ^1H NMR, ^{13}C NMR, FTIR and mass spectra.
4. Kováč, P.; Glaudemans, C. P. J. *J. Carbohydr. Chem.* **1988**, *7*, 317.
5. Duggan, M. E.; Karanewsky, D. S. *Tetrahedron Lett.* **1983**, *24*, 2935.
6. Saady, M.; Lebeau, L.; Mioskowski, C. *Tetrahedron Lett.* **1995**, *36*, 4785.
7. **CAUTION**. Phosphonochloridates are poisonous organophosphorus compounds.
8. Methyl 2,3,4-*O*-tris(phenylmethyl)-α-D-glucopyranoside was obtained from methyl α-D-glucopyranoside in three steps: (a) TBDPSCl, imidazole, DMF; (b) BnBr, NaH, DMF; (c) TBAF, THF; a total yield of 59%. See: Reitz, A. B.; Tuman, R. W.; Marchione, C. S.; Jordan, A. D.; Bowden, C. R.; Maryanoff, B. E. *J. Med. Chem.* **1989**, *32*, 2110.
9. Yli-Kauhaluoma, J.; Janda, K. D. *Bioorg. Med. Chem.* **1994**, *2*, 521.
10. Assay conditions: antibody 5 μM, substrates 500 μM, phosphate-buffered saline PBS (10 mM, pH 7.40, 150 mM NaCl), 5 v/v-% DMF. All reactions were carried out at 25 °C and monitored at 240 nm by HP Series 1050 HPLC equipped with an HP autosampler and an ODS Hypersil RP-18 column (100×4.6 mm, 5 μm beads) using acetonitrile/water gradient at 1.5 mL min^{-1}.
11. Durfor, C. N.; Bolin, R. J.; Sugasawara, R. J.; Massey, R. J.; Jacobs, J. W.; Schultz, P. G. *J. Am. Chem. Soc.* **1988**, *110*, 8713.
12. Janda, K. D.; Ashley, J. A.; Jones, T. M.; McLeod, D. A.; Schloeder, D. M.; Weinhouse, M. I. *J. Am. Chem. Soc.* **1990**, *112*, 8886.
13. Ashley, J. A.; Janda, K. D. *J. Org. Chem.* **1992**, *57*, 6691.