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PHENYLPHOSPHONATE MONOESTER ANALOGS OF COCAINE. POTENTIAL HAPTENS FOR THE GENERATION OF CATALYTIC ANTIBODIES

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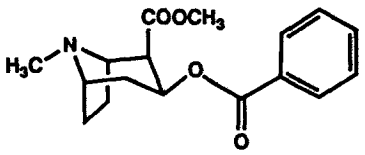
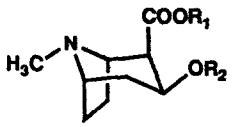
Abstract: A simple method for the stereo-controlled preparation of phenylphosphonate monoesters which are transition state mimics for the hydrolysis of the benzoic acid ester moiety of cocaine is described. Some preliminary results on the use of these mimics for the development of antibodies are also presented.

Use of cocaine (**1**), has increased dramatically in recent years and is of concern for social and health reasons.[1] Current approaches to the treatment of cocaine/crack addictions are inadequate and a change is necessary from the traditional subjective approach to a more organized and objective treatment.[2] Cocaine (**1**) contains two ester functionalities which are the primary sites of metabolism in man and other species.[3] The major metabolite, benzoylecgonine (**2a**) results from the hydrolysis of the carbomethoxy group of cocaine by serum and liver esterases. Hydrolysis of the benzoate ester linkage to the relatively non-toxic ecgonine (**2b**) and benzoic acid occurs at a much slower rate.[3] A potential method for detoxification of cocaine overdose would be administration of an antibody that could catalyze the rapid degradation of cocaine (**1**) to (**2b**) or its methyl ester (**3**). A recent report[2] on the development of antibodies for cocaine by immunization with cocaine conjugated with keyhole limpet hemocyanin prompts the disclosure of our work in this area.



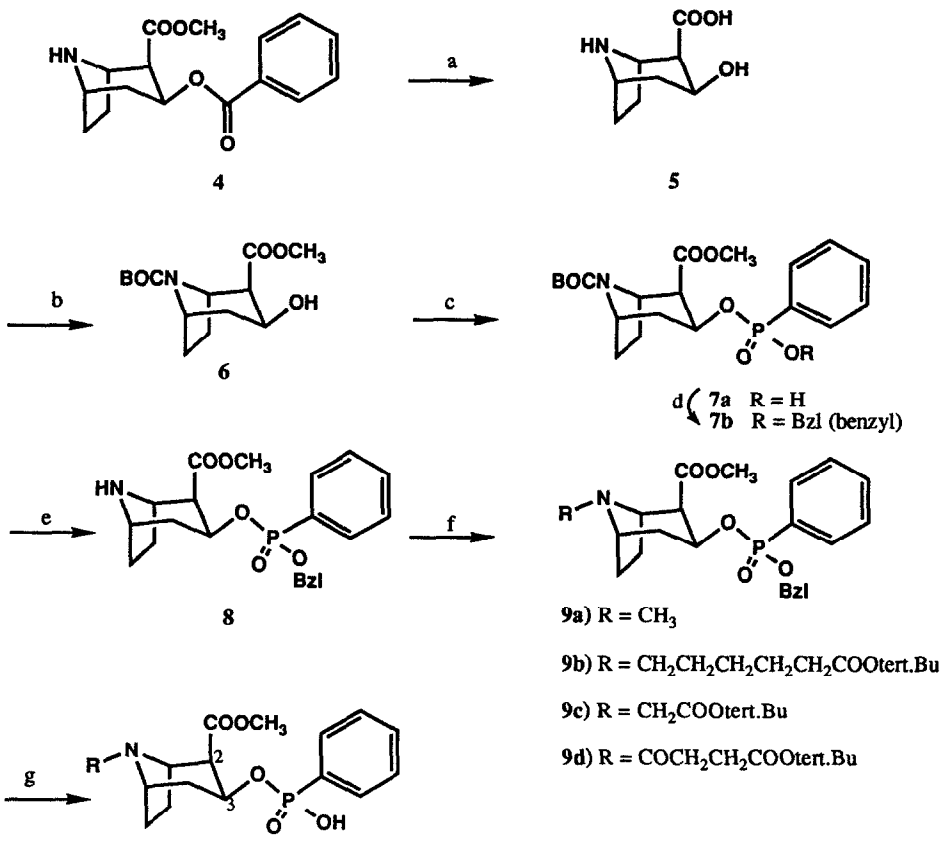
1



2a $R_1 = H$; $R_2 = COPh$
2b $R_1 = H$; $R_2 = H$
3 $R_1 = CH_3$; $R_2 = H$

The concept of transition state stabilization has been elegantly applied to the generation of antibodies with the ability to catalyze a variety of reactions.[4] Catalytic antibodies with high turnover numbers have been generated for ester hydrolysis using phosphonate monoesters as transition state analogs.[5,6] Based on these precedences, it appeared that antibodies generated against the phosphonate monoesters (**10**) could be useful for the *in vivo* hydrolysis of the benzoate ester moiety of cocaine (**1**).

Scheme - 1



a. 2N HCl (aqueous), reflux. b.(i) CH_3OH, conc. H_2SO_4; (ii) $(Boc)_2O$, Et_3N. c. (i) Phenylphosphinic acid, DCC, DMAP; (ii) $NaIO_4$. d. Benzyl bromide, K_2CO_3, DMF. e. TFA:CH_2Cl_2 (1:1), then aq. $NaHCO_3$. f. Alkyl halide, K_2CO_3, DMF (see ref.10) or $HOOCCH_2CH_2COOtBu$ (see ref.11), isobutylchloroformate,1-methylmorpholine, THF. g. (i) H_2/Pd-C, MeOH; (ii) 4N dry HCl in dioxane.

Synthetic considerations dictated that for enhanced antigenic response the necessary attachment of these haptens to a carrier macromolecule like serum albumin be accomplished by using substitutents on nitrogen of the analogs. This strategy not only simplified the chemistry but also provided two types of haptens, one with the retention of the basicity of nitrogen and another with a neutral amide nitrogen. As outlined in Scheme-1, norcocaine (**4**), prepared from commercial (Mallinckrodt) cocaine HCl using a known[7] procedure, was hydrolyzed exhaustively using dil. HCl, to give the amino acid (**5**). Acid catalysed esterification of the carboxyl group of **5** followed by protection of the nitrogen as tert.butylcarbonate gave the key intermediate alcohol (**6**).[8] The crucial phenylphosphonylation of (**6**) was achieved using a two step procedure[9] to give the phosphonic acid monoester (**7a**). Esterification of (**7a**) to the phosphonate diester (**7b**)[8] followed by treatment with acid to remove the tert.butoxycarbonyl group, gave the amine (**8**) suitable for derivatization. Alkylation of crude (**8**) with methyl iodide, tert.butyl bromoacetate or tert.butyl 5-bromo-hexanoate[10] gave compounds (**9a-9c**);[8] acylation of (**8**) with succinic acid mono tert.butyl ester[11] gave (**9d**).[10] Compound **9d** could also be prepared in three steps from (**5**) by (i) acid catalyzed esterfication, (ii) acylation with succinic acid mono tert. butyl ester[11] and (iii) phenylphosphonylation as for **6**. Debenzylation of (**9b-9d**) by hydrogenolysis followed by exposure to acid to remove the tert.butyl group provided the target haptens (**10b-10d**)[8,12] suitable for coupling to macomolecules like BSA.

Hydrogenolysis of **9a** gave **10a**.[13] The compound **10a** contains all unique elements of the predicted phosphonate monoester transition state for the hydrolysis of the benzoic acid ester moiety of cocaine. Monoclonal antibodies having affinity for **10a** can be identified by competitive inhibition binding assays.

In preliminary experiments compound **10d** was coupled via its carboxylic acid group to thyroglobulin and ovalbumin. Mice were immunized with thyroglobulin conjugate of **10d** for the production of anti-**10a** & **10d** monoclonal antibodies (mAb) using conventional techniques.[14] Nine hybridoma cell lines were generated that produced antibody that recognized the ovalbumin-conjugated **10d** (ova-**10d**). Monoclonal antibodies that recognize soluble hapten **10a** were identified in a competitive inhibition assay. Compound **10a** was tested for its ability to inhibit mAb binding to immobilized ova-**10d**; soluble **10a** inhibited the binding of seven of nine mAbs to immobilized ova-**10d**. The IC_{50} of these seven mAb ranged from 15 to 350 μM hapten **10a**. Together, these results suggest that the ova-**10d** binding mAb recognize soluble hapten **10a**, the predicted phosphonate transition state mimic. The demonstration of mAb binding to transition state mimic has been used as a method for the identification of catalytic antibodies displaying rate enhancement and specificity as well as turnover.[15] Further study of the catalytic properties of the mAbs described herein is ongoing.[16]

Acknowledgement: NSC thanks Ms. P.Finnegan (Searle, Physical Methodology) for extensive NMR investigations and interpretation of data.

References and Notes:

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3.Cocaine is metabolized by both hydrolytic and oxidative pathways. Serum pseudocholinesterase hydrolyzes the compound to give ecgonine methyl ester, a reaction that is also carried out by the liver esterases, and benzoylecgonine can be formed by chemical hydrolysis as well. Hydrolytic pathways account for 80-90% of urinary cocaine metabolites in humans. For leading references see, A.R. Jeffcoat, M. Perez-Reyes, J.M.Hill, B.M. Sadler and C.E. Cook, *Drug metabolism and disposition*, 17(2), 153-159 (**1989**).
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7. S.W. Baldwin, P.G.Jeffs and S. Natarajan, *Syn. Comm., 7(1)*, 79-84, (**1977**).
8. Compounds characterized by satisfactory spectral as well as microanalytical data; compounds **10a- 10d** were hygroscopic non-crystalline solids. (Yields: **4-->6**, 61%; **6-->7a**, 90%;**7a-->7b**, 100%;**7b-->9a**, 61%;**7b-->9b**, 71%;**7b-->9c**, 89%;**7b-->9d**, 68%;**9-->10**, 100%).
9. D.S. Karanewsky and M.C. Badia, *Tet. Letts., 27(16)*, 1751-1754, (**1986**).
10. The tert.butyl ester of 5-bromo-hexanoic acid was prepared by treatment of the acid with isobutene in CH_2Cl_2 in presence of conc. H_2SO_4 at ambient temperature. The crude product was washed with aqueous $NaHCO_3$ and used without purification since attempted vacuum distillation lead to clean decomposition leading to the acid.
11. Tert.butyl mono ester of succinic acid (b.p.130°C/2mm) was prepared by refluxing tert.butanol with succinic anhydride in the presence of 4-DMAP.
12. NMR assignments for DMSO-d_6 solutions of **10a-10d.**

Compound	H-2	H-5	H-1	H-3
10a	3.27(dd, J=7,2.5Hz)	3.83 (m)	4.16 (d, J=7 Hz)	4.86 (m)
10b	3.29 (dd, J=7.5, 1.5 Hz)	3.89 (m)	4.32 (d, J=7 Hz)	4.90 (m)
10c	3.34 (dd, J=7, 2.5 Hz)	4.00 (m)	4.22 (d, J=7.5 Hz)	4.86 (m)
10d (appears to be mixture of rotamers)	2.88 (dd, J=6, 2.5 Hz) 2.96 (dd, J=6, 2.0 Hz)	4.36 (m) 4.57 (m)	4.49 (d, J=7 Hz) 4.63-4.73 (complex)	4.63-4.73 (complex)
10e	3.09 (dd, J=7, 2.0 Hz)	3.93 (m)	4.14 (d, J=6 Hz)	4.72 (m)

13. NMR data for **10a** were also obtained in $CDCl_3/CD_3OD$, and assignments were confirmed by decoupling experiments. In DMSO-d_6, the H-1 signal appears to be a doublet (J=6 Hz). However, decoupling experiments showed that H-1 also has two small, unresolved couplings to H-2 and H-5. The stereochemistry of **10a** was assigned by comparison of the NMR data with published data for the four isomeric cocaines.[13a] ($J_{2,3}$ in $CDCl_3/CD_3OD$: Cocaine HCl, 6.8 Hz; **10a**, 6,5 Hz). [13a] F.I. Carroll, M.L.Coleman and A.H. Lewin, *J. Org. Chem., 47*, 13-20, (**1982**).
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16. Correspondence regarding the development of antibodies should be addressed to Dr. C. P. Carron, Monsanto Corporate Research, Monsanto Company, 700 Chesterfield Village Parkway, St. Louis, Mo 63198.