Pergamon

Tetrahedron Letters 43 (2002) 171–174

TETRAHEDRON LETTERS

Synthesis of an aminoalcohol hapten for the generation of catalytic antibodies

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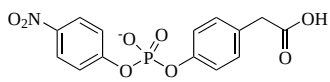
Received 27 July 2001; revised 22 October 2001; accepted 5 November 2001

Abstract—The conditions under which the reduction of α-hydroxy azides by triphenylphosphine to aziridines is diverted to produce the corresponding aminoalcohol have been established.

The production and study of enzyme-like catalysts promise advances in understanding molecular recognition and the nature of transition states. Such advances are anticipated to aid the design of tailor-made catalysts with valuable potential applications in biotechnology and medicine. Following the first demonstration of antibody-mediated catalysis in 1986,[1] a principal objective of the early work was the investigation of the wide range of chemical reactions for which antibody catalysts could be produced. This range included difficult and unfavourable chemical reactions including some for which enzymes do not exist in nature.[2] Although in most cases rate accelerations were smaller than those typical of enzyme catalysis, rate accelerations comparable to those of the analogous enzyme have been achieved by 'reactive immunisation'.[3] This involves design of the hapten such that a chemical reaction occurs in the binding pocket of the antibody during its induction. This approach is one method of attempting to recruit specific catalytic groups in the antibody. The hapten whose synthesis is described in the present paper was designed to achieve an analogous objective.

Although most investigations have been concerned with monoclonal catalytic antibodies, work published from our laboratories during the 1990s[4] demonstrated that substantial catalytic activities can be generated reproducibly in polyclonal preparations. The particular value of such preparations includes: (i) the relative simplicity of producing polyclonal as against monoclonal antibodies, (ii) their potential value as catalysts for technological applications, (iii) the rapid and cost-effective applications in evaluating a range of haptens for catalyst production and (iv) their potential value in the development of novel therapies when produced by active immunisation. Because polyclonal IgG necessarily represents the entirety of the immune response, the relative immunogenic capabilities of a series of haptens may be assessed more effectively than by studies on a small selection of isolated monoclonal antibodies.



hapten 1

Our previous work[4] used anionic phosphate haptens such as **1** to produce sheep polyclonal catalytic antibody preparations with hydrolytic activity towards carbonate, ester and amide substrates.

These preparations effect the catalysis of the reactions of these substrates with hydroxide ion assisted by hydrogen bond donation at the reaction centre by high pK_a sidechains (tyrosine and arginine).[4] In an attempt to produce antibodies with an active centre carboxy group to supply acid/base or nucleophilic catalysis in acidic media, we are investigating the use of cationic haptens containing an α-aminoalcohol motif. The protonated amino group might recruit a negatively charged sidechain in the antibody. The hydroxy group, which mimics part of the postulated tetrahedral transition state, might recruit a hydrogen bonding donor/acceptor system. One example of the type of catalytic system envisaged is the carboxylate/carboxylic acid couple of the aspartic proteinases.[5]

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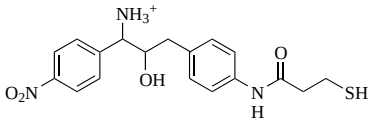
0040-4039/02/$ - see front matter
PII: S0040-4039(01)02101-3

The choice of **2** as our initial aminoalcohol was prompted by the work of Suga et al.[6] who reported what they considered to be its synthesis and use as a hapten for the generation of monoclonal catalytic antibodies.



hapten 2 (*anti*)

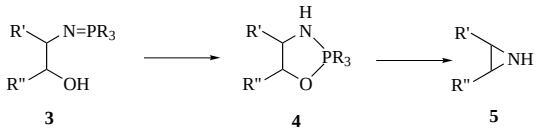
As in the case of our anionic haptens such as **1**, the presence of the 4-nitrophenyl group provides for the use of a chromophoric leaving group in the corresponding ester and anilide substrates. This group and the other aromatic ring endow the hapten with good immunogenic characteristics.

A key step in the synthetic route to **2** (*anti*) is the formation of the amino group of the required α-aminoalcohol motif by reduction of an azide precursor. Unexpectedly, consideration of the ^{1}H NMR data of Suga et al.[6] together with literature ^{1}H NMR data for aminoalcohols[7] suggested that the hapten used by Suga et al.[6] might not have been the aminoalcohol. The present paper reports (a) evidence that this hapten was in fact an aziridine and (b) the modifications to the published procedure necessary to produce the required aminoalcohol.

The reduction of azides to amines has been extensively investigated and several reducing agents are able to effect this reaction in simple compounds.[8] Some of these methods, such as the use of H_2/Pd or $LiAlH_4$ cannot be used in the synthesis of **2**, however, because of the presence of the nitro group. Suga et al.[6] used triphenylphosphine (Staudinger reduction)[9] in THF at room temperature. In simple compounds this reaction proceeds via the iminophosphorane compound from which the amine is produced by hydrolysis.

$$R_3P{=}N{-}R' \rightarrow R_3P{=}O{+}H_2N{-}R'$$

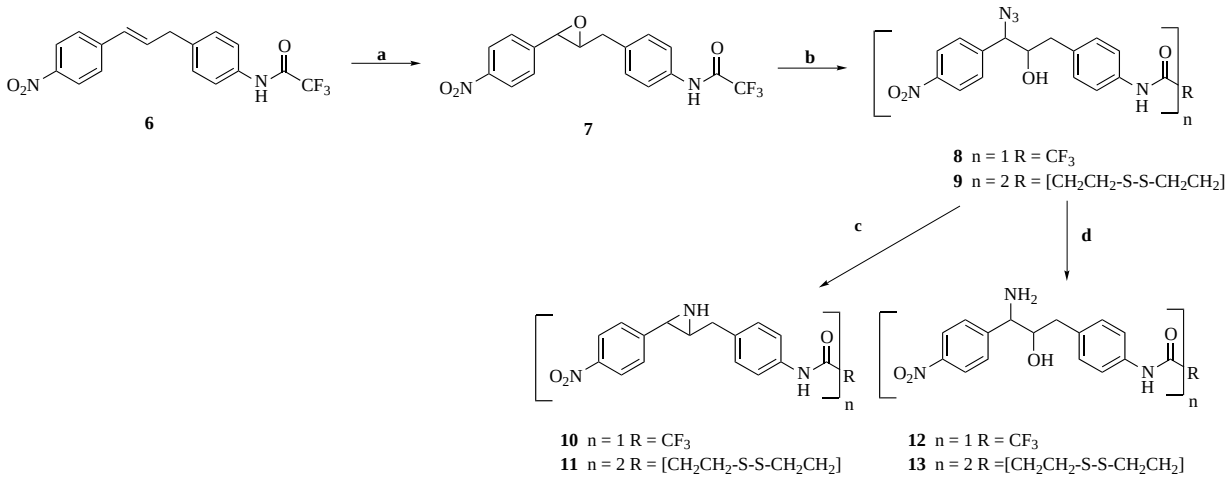
The existence of a hydroxy group on a carbon atom adjacent to that bearing the azide substituent, however, can result in intramolecular cyclisation of the hydroxyiminophosphorane **3** to form the oxazaphospholidine **4**, which decomposes on heating to give the aziridine **5**.[7,10] The relative stereochemistry of **4** and **5** depends on the stereochemistry of the starting material **3**. It was considered that this type of cyclisation might have occurred in the reaction of the azide used by Suga et al.[6]



3 **4** **5**

This possibility was investigated initially using **8** (*anti*), one of the intermediates of the synthetic route to **2**. Azide **8** (*anti*) is readily obtained in high yield from the *trans* alkene **6** via the epoxide **7** (*anti*) as shown in Fig. 1.[11] Table 1 contains ^{1}H and ^{13}C NMR, elemental analysis, and mass spectral data for **8** and the two derived products, aziridine **10** and aminoalcohol **12**.

Fig. 2 indicates the position of the significant protons H_A, H_B and H_C, and carbon atoms C_A, C_B and C_C in structures **8**–**13**. Reaction of **8** with triphenylphosphine in THF with stirring at 69°C for 24 h, followed by addition of water, produced aziridine **10** in >60% yield after purification. This molecule is characterised by having chemical shifts for protons H_A, H_B and H_C+H_D below δ 3.0. When the same reaction was carried out at room temperature, a mixture of different products including the aziridine was observed. Inhibition of the formation of aziridine **10** and promotion of the formation of aminoalcohol **12** was achieved by carrying out the reaction of azide **8** with Ph_3P in the presence of acetic acid. This was predicted to suppress intramolecular nucleophilic attack by the ring N atom in the oxazaphospholidine **4**.



6 **7**

8 n = 1 R = CF_3
9 n = 2 R = [CH_2CH_2-S-S-CH_2CH_2]

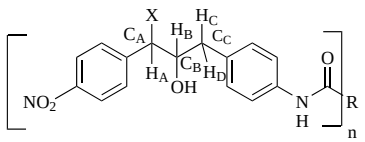
10 n = 1 R = CF_3
11 n = 2 R = [CH_2CH_2-S-S-CH_2CH_2]

12 n = 1 R = CF_3
13 n = 2 R =[CH_2CH_2-S-S-CH_2CH_2]

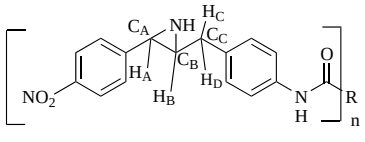
Figure 1. (a) *m*-CPBA, CH_2Cl_2/KH_2PO_4 buffer (pH 8.0), (80%); (b) For **8**: (i) NaN_3, NH_4Cl, acetone/H_2O, 75°C, (82%); For **9**: (i) (85%) followed by (ii) K_2CO_3, MeOH/H_2O, (79%); (iii) 3,3′-dithiodipropionic acid, bis(succinimido) ester, CH_2Cl_2, (81%); (c) PPh_3, THF, 69°C, (62%) for **10**, (36%) for **11**; (d) PPh_3, AcOH, THF, (53%) for **12**, (33%) for **13**. For all compounds **8**–**13** the relative configuration is *anti*.

Table 1. Spectroscopical data for compounds **8**, **10** and **12**

Compound	^{1}H NMR chemical shift			^{13}C NMR chemical shift			Mass	Elemental analysis
	H_A	H_B	H_C+H_D	C_A	C_B	C_C		
8 ($CDCl_3$)	4.65	4.07	2.85, 2.67	68.9	75.0	38.6	410 (M+1) (13.51%), 367 (12.16%), 307 (13.17%), 289 (13.51%), 232 (31.75%), 202 (100%)	Calcd: C, 50.0; N, 17.1; H, 3.4. Found: C, 50.3; N, 16.9; H, 3.5
10 ($CDCl_3$)	2.87	2.32	2.99	43.3	39.2	38.1	366 (M+1) (26.35%), 279 (13.51%), 231 (10.13%), 215 (10.13%), 202 (100%)	Calcd: C, 55.9; N, 11.5; H, 3.8. Found: C, 53.5; N, 10.9; H, 3.6
12 (CD_3OD)	3.98	4.02	2.76, 2.44	61.0	76.8	40.6	384 (M+1) (50.34%), 279 (17.57%), 242 (14.53%), 232 (11.48%), 202 (100%)	Calcd: C, 53.2; N, 10.9; H, 4.2. Found: C, 52.9; N, 10.8; H, 4.2



8 : X = N_3 n = 1 R = CF_3
9 : X = N_3 n = 2 R = $CH_2CH_2SSCH_2CH_2$
12 : X = NH_2 n = 1 R = CF_3
13 : X = NH_2 n = 2 R = $CH_2CH_2SSCH_2CH_2$



10 : n = 1 R = CF_3
11 : n = 2 R = $CH_2CH_2SSCH_2CH_2$

Figure 2.

The reaction was carried out by dissolving azide **8** in THF containing one equivalent of acetic acid and adding triphenylphosphine. The reaction was left stirring for 48 h at room temperature followed by addition of water and stirring for a further 24 h at room temperature. After purification the aminoalcohol **12** was obtained in good yield. The ^{1}H NMR data for **12** show the signal for H_A as a doublet at δ 3.98 and H_B as a multiplet at δ 4.02. The differences in the chemical shifts for H_A and H_B of aziridine **10** and aminoalcohol **12** constitute a key observation in assigning the structures.

Table 1 shows also the chemical shifts for the three carbon atoms (C_A, C_B and C_C). Once again the differences between the aziridine and the aminoalcohol are particularly noteworthy. The C_A signal which is at 68.9 in the azide, is at 61.0 for the aminoalcohol and 43.4 for the aziridine. C_B, which is the carbon bearing the hydroxyl group, has a chemical shift of 75.0 in the azide, 76.8 in the aminoalcohol but only 39.2 in the aziridine. These data are consistent with the literature values[7] for aziridine and aminoalcohol compounds. The mass spectra and the elemental analysis data confirmed the identities of the three compounds.

Having used the model system to establish the experimental conditions for the formation of the two different products we then prepared the corresponding azide **9**, in which the trifluoroacetamido group is replaced by the disulfide spacer chain. Reactions were performed under the two sets of experimental conditions and we obtained the corresponding aziridine **11** and the aminoalcohol **13**, respectively. Both compounds were fully characterised. The ^{1}H and ^{13}C NMR data for **9**, **11** and **13** are shown in Table 2 together with the data reported by Suga et al. to be characteristic of the aminoalcohol **13**. It is evident that there is a very close resemblance between the latter and those for aziridine **11**.

It is now clear that the synthesis of aminoalcohol **13** has not been reported previously. We have now completed the synthesis of hapten **2**[12] and have used it to prepare the KLH-conjugate. This is currently being used for the first time in an immunisation programme with a view to producing catalytic antibodies.

Table 2. Spectroscopical data for compounds **9**, **11** and **13** compared with literature data

Compound	^{1}H NMR data			^{13}C NMR data		
	H_A	H_B	H_C+H_D	C_A	C_B	C_C
9 ($CDCl_3$)	4.65	4.00	2.78, 2.61	70.5	76.2	39.7
11 ($CDCl_3$)	2.75	2.22	2.88	43.3	38.9	37.4
13 (CD_3OD)	4.08	4.12	2.65, 2.52	61.0	77.1	40.6
Lit. data[6] ($CDCl_3$)	2.80	2.26	2.91		Not available	

Acknowledgements

We thank Professor K. B. Sharpless for helpful discussion on aziridine formation and the EC (ERBFMRXCT 980 193) for financial support (including a studentship for J.F.A.).

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12. ^{1}H NMR (CD_3OD, 270 MHz): δ 8.20 (d, $J=8.0$ Hz, 2H), 7.55 (d, $J=8.0$ Hz, 2H), 7.45 (d, $J=8.0$ Hz, 2H), 7.05 (d, $J=8.0$ Hz, 2H), 4.05 (m, 1H), 3.90 (d, $J=4.0$ Hz, 1H), 2.75 (t, $J=7.0$ Hz, 2H), 2.70 (dd, $J=14.0$, 4.0 Hz, 1H), 2.60 (t, $J=7.0$ Hz, 2H), 2.40 (dd, $J=14.0$, 8.0 Hz, 1H); ^{13}C (CD_3OH, 270 MHz): δ 172.2, 151.5, 148.7, 138.0, 136.4, 130.8, 130.4, 124.3, 121.5, 77.2, 61.0, 41.9, 40.6, 21.0.