Recognition of β-Ketoalcohol-derived Haptens by Tailor-made Antibodies

Alain Krief,*[a] Jean-Jacques Letesson,[b] Dominique Swinnen, [a,c,d] Denis Billen[a,c]

[a]Laboratoire de Chimie Organique de Synthèse, Département de Chimie, Facultés Universitaires Notre-Dame de la Paix, 61 rue de Bruxelles, B-5000 Namur, Belgium

[b]Unité de Recherche en Biologie Moléculaire, Facultés Universitaires Notre-Dame de la Paix, 61 rue de Bruxelles, B-5000 Namur, Belgium

[c]Fonds pour la Formation à la Recherche dans l'Industrie et dans l'Agriculture, 5 Rue d'Egmont, B-1050 Bruxelles, Belgium

[d]Present Address : Serono Pharmaceutical Research Institute, 14 chemin des Aulx, Plan-Les-Ouates, CH-1228 Geneva, Switzerland

Fax +32 81 724536; E-mail: alain.krief@fundp.ac.be

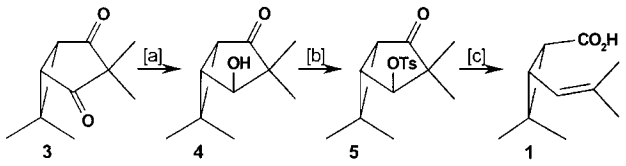
Received 25 December 2000

With great appreciation to Professor Ryoji Nogori's work

Abstract: Antibodies steamed from mice immunized towards three different conjugates do not recognize the β-ketoalcohol for which they have been elaborated. They however recognize the haptens, which bear the side chain linking them to the carrier proteins. We have synthesized and tested for recognition a series of compounds related to those haptens, especially stereoisomers and compounds bearing part of their structures. Results and hypotheses are presented below.

Key words: antibodies, β-ketoalcohols, lipases, recognition, sulfur ylides

Few years ago we showed that the bicyclic dione **3** was a key intermediate in the stereoselective synthesis of racemic (Scheme 1)[1] and of optically pure[2] chrysanthemic acids **1**.



[a] 1 mol. eq. $NaBH_4$, 1.1 eq. $CeCl_3$, MeOH, 4 h, -78°C, 88% [b] 1.2 eq. TsCl, 2.5 eq. Pyr., 0.2 eq. DMAP, CH_2Cl_2, 16 h, 20°C, 95% [c] (i) 6 eq. KOH, DMSO, 4 h, 70 °C; (ii) H_3O^+, 69%.

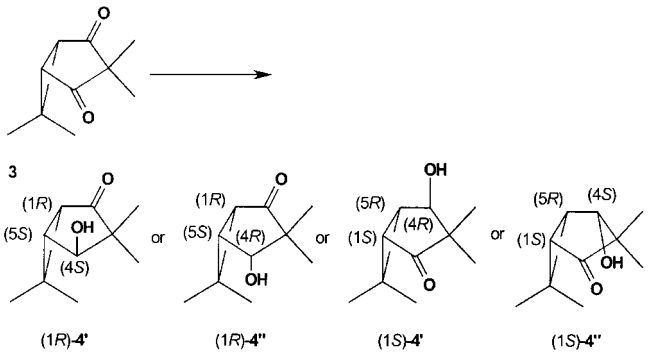
Scheme 1 Synthesis of rac-*cis*-chrysanthemic Acid

It is readily available from dimethyl dimedone **2**[1] and possesses all the features required : (i) it has a cyclopropane ring bearing two substituents that are *cis*-one to each other allowing the stereoselective synthesis of the *cis*-chrysanthemic acid or its *trans*-stereoisomer by performing a further isomerization reaction (ii) it is prochiral and allows the synthesis of the (1*R*)- as well as the (1*S*)-series of chrysanthemic acid from which commercially valuable (1*R*)-*cis*- or (1*R*)-*trans*-chrysanthemic acids can be produced respectively.[3]

The stereoselective reduction of **3** to the β-hydroxy ketone (1*R*)-**4',** which can either occur on (i) the *exo*- or *endo*-face of the concave bicyclic diketone **3** but also on (ii) either of the two carbonyl groups, is the key step of the whole process.[1,2]

Although each couple of *endo*/*exo*-stereoisomers is formally able to generate one enantiomer of chrysanthemic acid, only those bearing an *exo*-hydroxyl group lead, in practice, to chrysanthemic acid after hydroxyl group activation (as a sulfonate) and further Grob fragmentation (Scheme 1).[1,2]

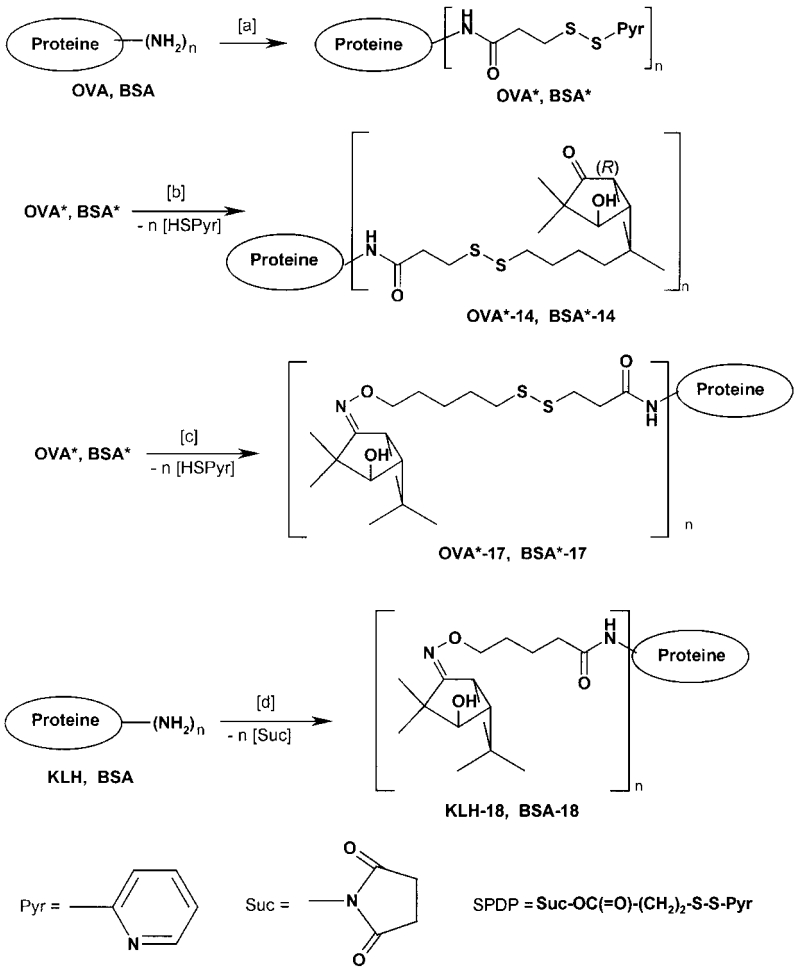
We have been able to generate[2] the required optically pure (1*R*)-**4'** *exo*-stereoisomer (Scheme 2) and therefore the corresponding optically pure (1*R*)-chrysanthemic acids but the process involves few more steps. As an alternative, we thought of using tailor-made antibodies that catalyze the chemo- and enantioselective reduction of **3** to (1*R*)-**4'** by its most hindered face.



Scheme 2 Stereoselective Synthesis of the Ketoalcohols **4**

A successful strategy requires generation of antibodies that would discriminate the four transition states leading to each of the stereoisomers.[4,5] In order to get acquainted with this approach, we decided to generate antibodies able to recognize the (1*R*)-**4'** stereoisomer, precursor of (1*R*)-*cis* chrysanthemic acid, among a mixture of the four possible stereoisomers **4** (Scheme 2).

Antigens were designed in which the hapten, bearing the β-ketoalcohol possessing the *exo*-hydroxyl group and the bicyclic structure, is linked to a carrier protein[5,6] by a saturated hydrocarbon linker (C-3 to C-8) attached: at the bicyclic structure side (i) directly to the *endo*-cyclopropyl methyl group or (ii) via a (O)-alkyloxime derived from the carbonyl group and at the protein side via (i) a disulfide linkage or (ii) a stronger amido group.

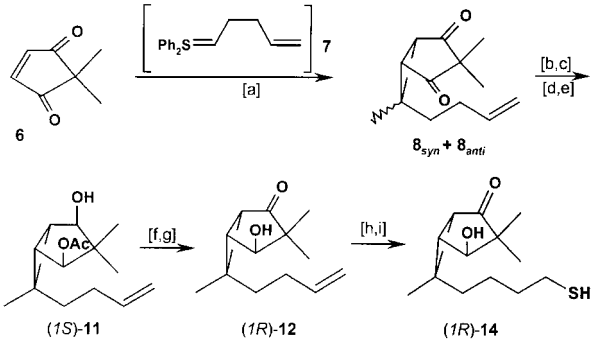


[a] Protein = **OVA**, buffer pH 7.5, 10 eq. SPDP, EtOH, 20°C, 0.5 h, (n=~5); Protein = **BSA**, buffer pH 7.5, 20 eq. SPDP, EtOH, 20°C, 0.5 h, (n=~10) [b] production of **OVA*-14** (n=~4) : **OVA*** and 100 eq. **14**, distilled water, 20°C, 15 h; production of **BSA*-14** (n=~4) : **BSA*** and 100 eq. **14**, distilled water, 20°C, 15 h [c] production of **OVA*-17** (n =~4) : **OVA*** and 100 eq. **17**, distilled water, 20°C, 15 h ; production of **BSA*-17** (n=~5) : **BSA*** and 100 eq. **17**, distilled water, 20°C, 15 h [d] production of **KLH-18** (n =~150) : **KLH**, distilled water and 500 eq. **18**, DMSO, 20°C, 4 h; production of **BSA-18** (n =~10) **BSA**, distilled water and 100 eq. **18**, DMSO, 20°C, 4 h.

Scheme 3 Synthesis of Activated **OVA*,** Activated **BSA*** and of the Conjugates **OVA*-14**, **BSA*-14**, **OVA*-17**, **BSA*-17**, **KLH-18**, **BSA-18**

The (1*R*)β-ketoalcohol **14** was first selected as the hapten because it possesses the bicyclic structure bearing the same functional groups and the same stereochemistry as the product (1*R*)-**4'** to be recognized. The presence of a thiol at the terminus of the side chain should allow to link it to the activated carrier protein **OVA*** produced from **OVA** by the SPDP method (Scheme 3).[7]

The synthesis of **14** involves (Scheme 4) the cyclopropanation of 5,5-dimethyl-cyclopent-2-en-1,3-dione **6**[8] with the alkenylidenediphenylsulfurane **7**[9] (DME, -78 °C, 1 h then 20 °C, 2 h, Scheme 4, step a). This reaction leads to a (80/20) stereoisomeric mixture of $\mathbf{8}_{syn}$ and $\mathbf{8}_{anti}$ in which the stereoisomer $\mathbf{8}_{syn}$, bearing the alkyl chain in *endo*-position, prevails. Isolation of the major compound, whose stereochemistry was confirmed at a later stage by Xray,[10] was readily achieved by preparative chromatography on SiO_2.[11]



[a] 1.3 eq. $Ph_2S{=}CMe(CH_2)_2CH{=}CH_2$ $LiBF_4$, DME, 1 h, -78°C then 1 h, 20°C, 50% ($\mathbf{8}_{syn}$ /$\mathbf{8}_{anti}$ 80/20 [b] separation of $\mathbf{8}_{syn}$ from $\mathbf{8}_{anti}$ [c] 2.2 mol. eq. $NaBH_4$, 2.2 mol. eq. $CeCl_3$, MeOH, 4 h, -78°C, 76% [d] 2.2 eq. Ac_2O, 2.2 eq. Pyr., 0.2 eq. DMAP, 96% [e] PLE, DMSO-phosphate buffer pH 7.0, 96 h, 20°C, 80% [f] 1.2 eq. PDC, M.S. 4A, CH_2Cl_2, 3 h, 20°C, 98% [g] 1.2 eq. K_2CO_3, MeOH, 3 h, 20°C, 98% [h] 2,5 eq. AcSH, 0.2 eq. AIBN, CCl_4, reflux 4 h, 60% [i] PLAP, phosphate buffer pH 7.0, 20°C, 14 h, 62 %.

Scheme 4 Synthesis of the hapten **14**

The synthesis of optically active (1*R*)-ketoalcohol **12** (Scheme 4, steps c-g) has been performed according to the procedure already disclosed for the synthesis of (1*R*)-**4'** from **3**.[2] It was achieved by (i) di-reduction of $\mathbf{8}_{syn}$ from its more hindered *endo*-face using sodium borohydride in the presence of cerium trichloride (Scheme 4, step c), (ii) diacetylation of the resulting diol **9** and (iii) enzymatic desymetrization of the resulting diacetate **10** to **11** using Pig Liver Esterase (PLE, Scheme 4, step e). The transformation of **12** to **14** was achieved in two steps which involve (i) the addition of acetylthiol via a radical process to terminal C,C double bond of **12** (Scheme 4, step h)[12] and (ii) removal of the acetyl group from the resulting thiolacetate **13** (Scheme 4, step i). The last step was the most difficult of the whole process due to the sensitivity of the aldol moiety to bases and the great aptitude of the resulting thiol to be oxidized to the corresponding disulfide. Removal of the acetyl group leading to **14** has been nevertheless very cleanly achieved with PLAP (Scheme 4, step i).[14] The hapten (1*R*)-**14** was finally linked (3 equiv /mol protein)[7] to the activated **OVA***[15] to produce the conjuguate **OVA*-14** (Scheme 3).

Four Balb/c mice were immunized with **OVA*-14** (i) once with 100 μg in the presence of complete Freund adjuvant then (ii) three times consecutively with 50 μg of the same **OVA*-14** in the presence of incomplete Freund adjuvant. Two week after the last immunization, their sera have been collected and tested by enzyme linked immunosorbent assay (ELISA),[16a] by antibody binding to solid-phase bound **BSA*-14** antigen. **BSA*-14** was in turn produced by coupling of (1*R*)-**14** to activated bovine serum albumine (**BSA***), instead of **OVA*** (Scheme 3).

Although the presence of immunoglobulins specific to the hapten **14** or part of it, in the sera of immunized mice was confirmed by indirect ELISA (Figure 1, ♦),[17b] we were somewhat rather surprised, using an inhibition ELISA,[16b] to find that the related β-ketoalcohol (1*R*)-**4'** did not inhibits the fixation of **AB-OVA*-14** antibodies to the **BSA*-14** antigen coated on the plate ($IC_{50:}$ > 500 μM).[18]

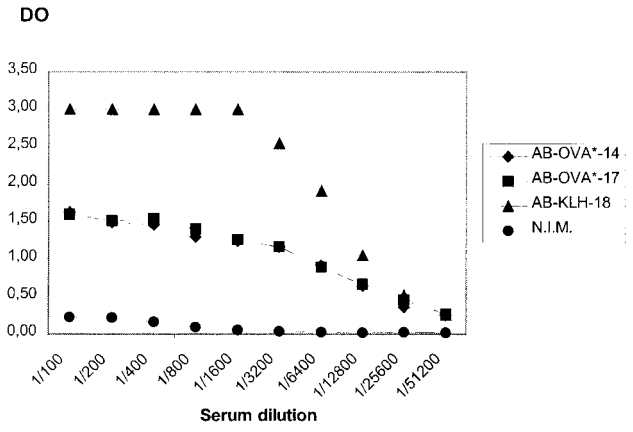


Figure 1 Indirect ELISA: Titration Curves for Various Antibodies

The same antibodies binds more efficiently the β-ketoalcohol (1*R*)-**12,** which possesses part of the linker joining (1*R*)-**4'** to **OVA** ($IC_{50:}$ 250 μM).

We have then repeated the above mentioned series of experiments using instead for immunization the **OVA*-17** antigen derived from the hapten **17** which possesses the same linker as the one present in (1*R*)-**14** but attached at another site on the bicyclic structure (Scheme 3 and 5) and found similar results.[17c] The β-ketoalcohol (1*R*)-**4'** is unable to inhibit the fixation of antibodies **AB-OVA*-17** on **BSA*-17** ($IC_{50:}$ > 500 μM) whereas the alkoximinoalcohol (1*R*)-**19c** (R = Pent, Scheme 5), which possesses the bicyclic structure as well as a side chain of critical length, is able to inhibit **AB-OVA*-17** (IC_{50}: 50 μM).

The conjuguates **OVA*-17** and **BSA*-17**, needed for that work, have been in turn prepared from activated **OVA** (**OVA***) or **BSA** (**BSA***) (see above), and the β-alkoximinoalcohol (1*R*)-**17** prepared stepwise from (1*R*)-**4'** and hydroxylamine, 1,5-dibromopentane, potassium thiolacetate and PLAP (Scheme 5, steps a-e).[19]

We have also carried out related experiments involving antigens **KLH-18** possessing a more stable linker than the disulfide group, used for **OVA*-14** and **OVA*-17**, and using a more immunogenic carrier protein : the Keyhole Limplet Hemocyanin (**KLH**).[21]

The conjuguate **KLH-18** was designed and prepared (Scheme 3) from **KLH** and the β-alkoximinoalcohol (1*R*)-**18** produced stepwise from (1*R*)-**4'**, hydroxylamine, 5-bromovaleric acid and *N*-hydroxysuccinimide (Scheme 5, steps b,f,g). **BSA-18** which was also required for ELISA was produced accordingly.

Antibodies **AB-KLH-18** raised against **KLH-18** and assayed on the conjugate **BSA-18**[17d] possess a very poor affinity toward the β-ketoalcohol (1*R*)-**4'** either (IC_{50} > 500 μM, Figure 2, •).[18] However, they were highly specific to the alkoximinoalcohol (1*R*)-**19b** (R = *n*-Bu, Scheme 5) which possesses the bicyclic structure as well as a side chain of critical length related to the linker (IC_{50}: 8 μM, Figure 2, ■).[18]

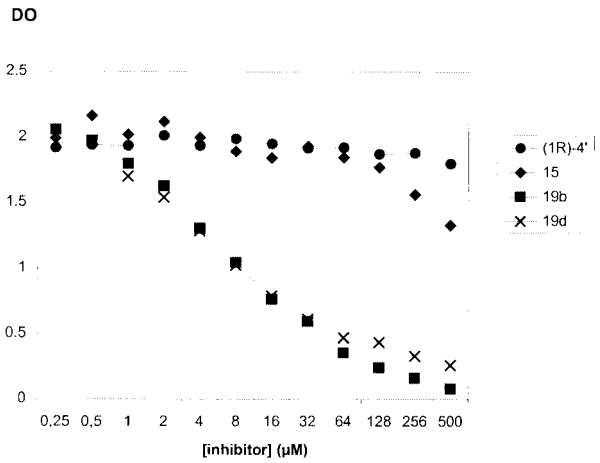
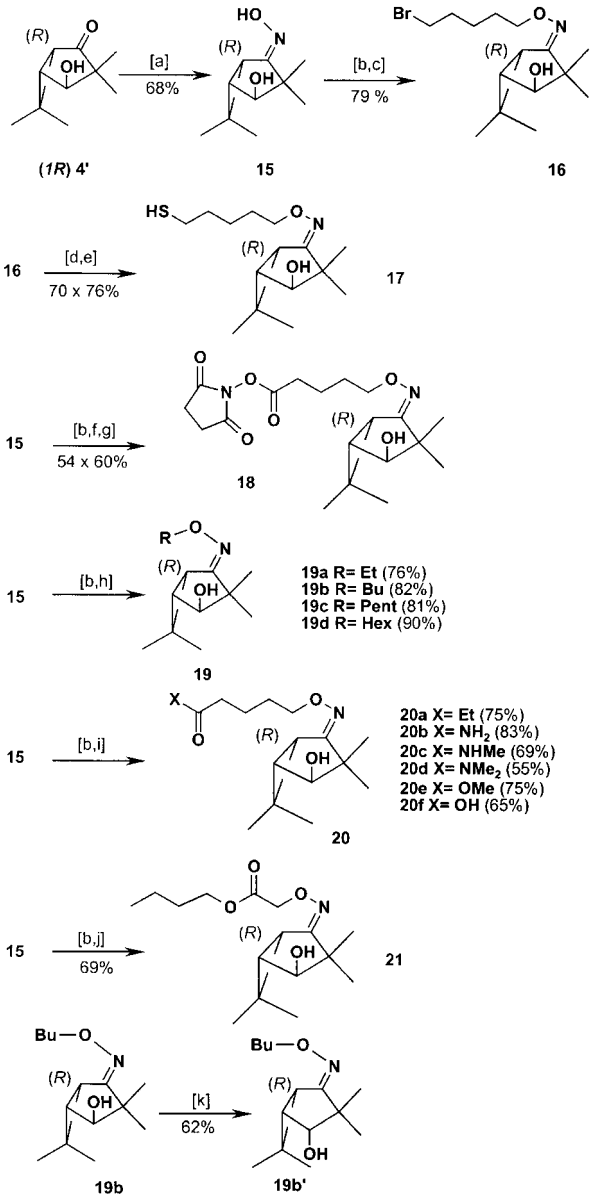


Figure 2 Inhibitive ELISA: Inhibition Curves for Various Inhibitors Implying AB-KLH-18

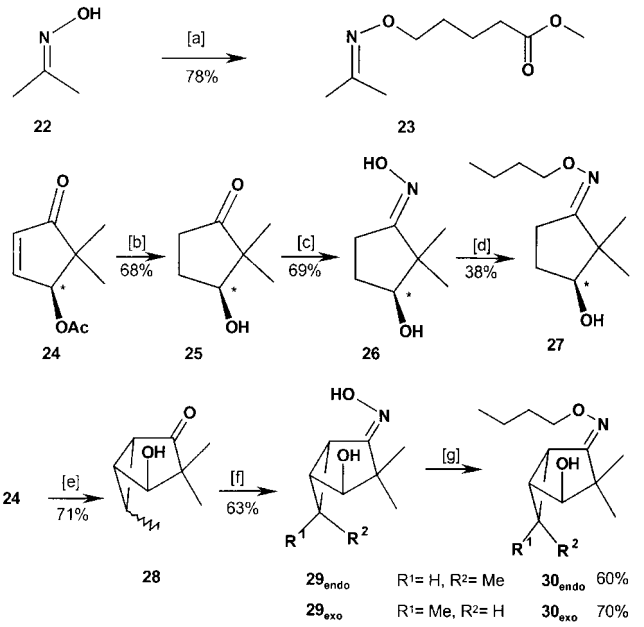


[a] 5 eq. $H_2NOH.HCl$, 5 eq. AcONa, MeOH, reflux, 60 h [b] 1.1 eq. *t*-BuOK, THF, 0°C [c] 1.5 eq. 1,5-dibromopentane, THF, 20°C, 3 h [d] AcSK, slow addition 20°C, 1 h, then 20°C, 12 h [e] PIAP (8 g/mol), phosphate buffer pH 7, MeOH, 20°C, 48 h [f] $Br(CH_2)_4C(=O)ONa$, THF, 0°C, 12 h [g] $_4$ eq. Suc-OH, 1 eq. DCC , cat DMAP, 0°C, 12 h [h] 2 eq. EtBr,*n*-BuBr, *n*-PentBr or *n*-hexylBr, THF-DMF, 20°C, 20 h [i] 1.2 eq. $Br(CH_2)_3C(=O)X$: X= NH_2, NHMe, NMe_2, OMe, OH, Et, THF, 20°C, 12 h [j] 1.2 eq. $ICH_2C(=O)O(CH_2)_3Me$, THF, 12 h [k] (i) 1.05 eq. pyridinium dichromate (PDC), CH_2Cl_2, 0°C, 0.5 h (ii) 1 eq. $LiBHEt_3$, THF, -78°C, 1 h.

Scheme 5 Synthesis of hapten **17** and **18** and oximes **19**, **20**, **21** derived from the ketoalcohol **4** and of the *endo* diastereoisomeric oxime **19'**

This behavior is similar to those reported above from **AB-OVA*-14** and **AB-OVA*-17** instead. The presence on the substrates of the carbon framework of the linker was essential, in all the three cases reported (**AB-OVA*-14, AB-OVA*-17** and **AB-KLH-18)** for their recognition.

We therefore decided to test the affinity of the antibodies raised against **KLH-18**, to complex a series of substrates such as (1*S*)-**4'**, (1*R*)-**4"**, **15**, **19**, **19b'**, **20**, **21**, **23, 27** and **30** (Schemes 5[22,23] and 6[9,24]), whose structures are somewhat related to that of the ketoalcohol (1*R*)-**4'**. Some of their syntheses are gathered in Schemes 5[22,23] and 6.[24]



[a] (i) 1,2 eq. *t*-BuOK, DMF, 0°C, 0.5 h (ii) 1.2 eq. $Br(CH_2)_4CO_2Me$, DMF, 0-20 °C, 15 h [b] (i) H_2, Pd/C, EtOH, 48 h (ii) 1,2 eq. K_2CO_3 , MeOH, 1 h [c] 5 eq. $NH_2OH.HCl$, 5 eq. AcONa, MeOH, 65°C, 72 h [d] (i) 1,2 eq. *t*-BuOK, THF, 0°C, 0.5 h (ii) 2 eq. *n*-Bu-I, THF, 0-20 °C, 24 h [e] (i) 1,2 eq. $MeCHLi\text{-}SPh_2$ BF_4, DME, 1 h, -78 °C, intractable mixture of stereoisomers (ii) 1,2 eq. K_2CO_3, MeOH, -20 °C, 1 h [f] 5 eq. $NH_2OH.HCl$, 5 eq. AcONa, MeOH, 65°C, 72 h (ii) separation of stereoisomers [g] (i) 1,2 eq. *t*-BuOK, THF, 0°C, 0.5 h (ii) 2 eq. *n*-Bu-I, THF, 0-20 °C, 24 h.

Scheme 6 Synthesis of the Oxime **23** Derived from Acetone, the Monocyclic Oxime **27** and of the Bicyclic Oximes **30**

We first found that antibodies **AB-KLH-18** neither recognize the regioisomeric *endo*-ketoalcohol (1*R*)-**4"** nor the enantiomeric *exo*-ketoalcohol (1*S*)-**4'** (Scheme 2, IC_{50} > 500 μM).

We also found that these antibodies did not bind efficiently the O-unsubstituted oxime **15**, which possesses all the functional groups present on the hapten (IC_{50} > 500 μM, Figure 2, ♦), but bind effectively its O-ethyl-substituted homologue **19a** (IC_{50}: 32 μM). Increasing the length of the side chain to O-Bu as in **19b** (IC_{50}: 8 μM, Figure 2, ■) or O-Hex as in **19d** (IC_{50}: 8 μM, Figure 2, **x**) leads to an increasing inhibition. This clearly confirms, as we described above, that the linker plays an important role in the recognition process.

We have also observed that the presence of a carbonyl group on the side chain of sufficient length (as in **20a**), has a little effect on the inhibition and this is also the case for compounds **20b-d,f** bearing a carboxy group at the terminus of the side chain (IC_{50}: 32 μM for X = $C(=O)NH_2$, $C(=O)NMe_2$ and C(=O)OH; 16 μM for X = C(=O)NHMe and C(=O)OMe).

Interestingly **20e**, bearing the ester group at the terminus of the side chain is a better ligand (IC_{50}: 16 μM) than **21** (IC_{50}: 128 μM), in which the same functional group is internal.

As expected from the previous results, neither **19b'**, the diastereoisomer of **19b,** bearing an hydroxy group in *endo*-position**,** nor **19b"** the enantiomer of **19b** are recognized by the antibodies (IC_{50} > 500 μM).

We have finally observed that the latter were highly specific to the whole structure present on the hapten **KLH-18** since they did not recognize **23** missing the bicyclic structure present on **4**, nor **27** missing the cyclopropane ring. Even more surprising, they were unable to bind $\mathbf{30}_{endo}$ and $\mathbf{30}_{exo}$ on which a single *exo*- or *endo*-methyl group is missing (IC_{50} > 500 μM).

We have shown that the sera steamed from mice immunized towards three different conjugates: **OVA*-14**, **OVA*-17** and **KLH-18**, do not recognize (1*R*)-**4'** for which they have been elaborated. We do not understand the real reasons of such observation. Haptens **14**, **17** and **18** present on the conjugates **OVA*-14**, **OVA*-17** and **KLH-18** are all small molecules. Although some, even closely related conjugates, have been successfully used to produce antibodies which recognize small molecules without apparent trouble related to the linkers,[25] it remains that most of the published examples involve not only substrates but also haptens bearing aromatic rings and polar groups.[4,5a,26] This is not the case of our conjugates. We are pursuing our study towards these goals.

References and Notes

(1) (a) Krief, A.; Surleraux, D.; Frauenrath, H. *Tetrahedron Lett.* **1988**,*29*, 6157. (b) Krief, A.; Surleraux, D.; Robson, M. J. *Synlett* **1991**, 276.

(2) Krief, A.; Surleraux, D.; Ropson, N. *Tetrahedron: Asymmetry* **1993**, *4*, 289.

(3) Krief, A. in "Stereocontrolled Organic Synthesis. A 'Chemistry for the 21st Century' Monograph", International Union of Pure and Applied Chemistry, Trost, B.M., Ed; Blackwell Scientific Publications **1994**, 337.

(4) (a) Lerner, R. A.; Benkovic, S. J.; Schultz, P. G. *Science* **1991**, *252*, 659. (b) Schultz, P. G.; Lerner,R. A. *Acc. Chem. Res.* **1993**, *26*, 391 (c) Raymond, J. L. *Topics in current Chemistry* **1999**, 200, 59.

(5) For an example of stereoselective, antibody-catalyzed, reduction of a ketone carbonyl group[5a] (a) Hsien, L. C.; Yonkovich, S.; Kochersperger, L.; Schultz, P. G. *Science* **1993**, *260*, 337.

(6) (a) Fujii, I.; Tanaka, F.; Miyashita, H.; Tanimura, R.; Kinoshita, K. *J. Am. Chem. Soc.* **1995**, *117*, 6199. (b) Tramontano, A.; Schoeder, D. *Methods in Enzymology* **1989**, *178*, 531 (c) The degree of conjugation to the carrier protein was determined using the trinitrobenzene assay of HABEEB.[6d] The number of hapten per protein was estimated to be approximately 150 (d) Habeeb, A. F. S. A. *Analytical Biochemistry* **1966**, *14*, 328.

(7) This method offers the advantage to measure, by fluorimetry, the number of hapten attached to the protein.[7a] (a) Carlsson, J.; Drevin, H.; Axen, R. *Biochem. J.* **1978**, *173*, 723.

(8) (a) Krief, A.; Swinnen, D. *Tetrahedron Lett.* **1996**, *37*, 7123 (b) Kreiser, W.; Wiggermann, A.; Krief, A.; Swinnen, D. *Tetrahedron Lett.* **1996**, *37*, 7119.

(9) The diphenyl sulfurane **7** was prepared by metallation of the corresponding sulfonium tetrafluoroborate with LDA (1equiv, CH_2Cl_2, DME, -78 °C, 0.5 h) The latter salt was prepared by methylation of 1-(4-pentenylidene)diphenyl sulfurane and was directly used in the next step.

(10) X-ray has been performed on the camphanoate of **11** (Scheme 4, Mp 117 °C).

(11) The reaction of the same ylide **7** on 6,6-dimethyl-(*R*)4-acetoxy-cyclopent-2-en-1-one is more convergent and provides directly after deacetylation to optically active **12** in high yield but as an intractable mixture of *syn*- and *anti*-stereoisomers.

(12) (a) Borwell, F.G.; Hewett, W. A. *J. Org. Chem.* **1957**, 22, 980 (b) Goering, H.L.; Releyea, D.I.; Larsen, D.W. *J. Am. Chem. Soc.* **1956**, *78*, 348.

(13) Deprotection of the thiolacetate moiety cannot be properly achieved in basic media because bases also react on the aldol functional group present on the bicycle. Furthermore the thiolate produced, in such medium, is very air sensitive. It is therefore very difficult, since the reaction is carried out on small quantities, to avoid the concomitant formation of the corresponding disulfide.

(14) For previous enzymatic hydrolysis of thiolacetates:[13] (a) Bianchi, D.; Cesti, P. *J. Org. Chem.* **1990**, *55*, 5657 (b) Kiefer, M.; Vogel, R.; Helmchen, G. *Tetrahedron* **1994**, *50*, 7109 (c) Hof, R. P.; Kellog, R. M. *J. Chem. Soc. Perkin Trans. 1* **1995**, 1247.

(15) Van Regenmortel, M. H. V.; Briand, J. P.; Muller, S.; Plaue, S. In *Laboratory Techniques in Biochemistry and Molecular Biology*; Burdon, R. H. and Knippenberg, P. H., Eds.; Elsevier: Amsterdam **1988**, p 95.

(16) (a) *General procedure for the indirect ELISA*[16c] : Calculated concentration of antigen[16c] was coated (24 h, 4 °C) on the wells of a polystyrene microtiter plate. After blocking of unoccupied adsorption sites on the polymer surface, mouse serum was added in various dilutions to the wells and incubated (24 h, 4 °C). After washing of the wells, enzyme-labelled antibody specific for mice antibodies was added. After further incubation (1 h, 37 °C) and washing, the corresponding chromogenic enzyme substrate was dispensed. The coloured product that indicates the amount of the first antibody bound to the solid-phase immobilized antigen was determined by simple photometrical absorption at 490 nm and 630 nm. Between all steps, excess reagent are removed by washing with a PBS-Tween 20 (0.1% solution) (b) *General procedure for the inhibition ELISA*: Calculated concentration of antigen[16c] was bound to the wells of a polystyrene microtiter plate. After blocking of unoccupied adsorption sites on the polymer surface, optimal dilution of mouse serum (determined via indirect ELISA test described above)[16a] was incubated 2 h at 37 °C with the test samples (inhibitive antigen) and was added to the wells. Next steps are the same as described above. (c) Crowter, J. R. *Methods in Molecular Biology* **1995**, p 42.

(17) Measured optical densities for : (a) Non immunized mice (NIM, Figure 1, ●) (b) **AB-OVA*-14**: Figure 1, ♦ (c) **AB-OVA*-17**: Figure 1, ■ (d) **AB-KLH-18:** Figure 1, ⇨.

(18) A single example is presented for convenience. Three others have been studied which exhibit similar patterns.

(19) Enantiomerically pure **17** has been prepared from **4** via the oxime **15**. **15** has been transformed to **16** by sequential reaction with only one equivalent of potassium *t*-butoxide and excess (1.5 equiv) of 1,5-dibromopentane.[20] **16** was then transformed to **17** on reaction with potassium thiolacetate and hydrolysis of the resulting alkylthiolacetate with PLAP as disclosed above (Scheme 4, step i). The oxime **15** has been in turn prepared on reaction of the β-ketoalcohol **4** with hydroxylamine hydrochloride and sodium acetate in methanol at reflux. Its structure has been unambiguously assessed by X-ray crystallography of its di-*N*,*O*(4-bromobenzoate) which

shows that the N-O bond lies away from the gem dimethyl group on the cyclopentane ring.
(20) Royaux, I Mémoire de Licence, *Facultés Universitaires Notre-Dame de la Paix*, Namur **1992**.
(21) Dinaut, A. N.; Chen, M. J.; Marks, A.; Batey, R. A.; Taylor, S. D. *Chem. Commun.* **2000**, 385.
(22) The key step of **18**, **19**, **20** and **21** takes advantage of the selective O-alkylation of the oxime[20] performed as described above.[19] The synthesis of the activated ester **18** has been achieved by DCC coupling of the corresponding acid with *N*-hydroxysuccinimide in the presence of catalytic amounts of DMAP.
(23) The *endo* hydroxyl derivative **19'** has been synthesized from its *exo*-stereoisomer on oxidation to a ketoxime which has been then chemo- and stereoselectively reduced from its *exo*-face by lithium triethylborohydride.[1,2]
(24) The synthesis of the cyclopentanol **27** as well as of the bicylic derivatives **30** bearing a methyl group in *endo*- or *exo*-position has been achieved from optically pure **24**[8] by catalytic hydrogenation or cyclopropanation with ethylidene diphenylsulfurane respectively. **28** is obtained as an untractable mixture (50/50) of stereoisomers. The related oximes **29** have been nevertheless easily separated and each stereoisomer has been transformed to the *O*-butyl oxime as described above.[19,20]
(25) (a) Wing, K.D.; Hammock, B. D.; Wustner, D. A. J. *Agric. Food Chem.* **1978**, *26*, 1328 (b) Wing, K.D.; Hammock, B. D. *Experientia* **1979**, *35*, 1619 (c) Hofstetter,O.; Hofstetter, H.; Schurig, V.; Wilchek, M.; Green, B. S. *J. Am. Chem. Soc.* **1998**, *120*, 3251 (d) Hofstetter, O.; Hofstetter, H.; Wilchek, M.; Schurig, V.; Green, B. S. *Chem. Commun.* **2000**, 1581.
(26) Sinha, S. C.; Keinan, E. *J. Am. Chem. Soc.* **1995**, *117*, 3653.

Article Identifier:
1437-2096,E;2001,0,SI,0931,0936,ftx,en;Y21800ST.pdf