Langmuir
Article
pubs.acs.org/Langmuir

Triacetone Triperoxide (TATP): Hapten Design and Development of Antibodies

Maria Astrid Walter,[†] Dietmar Pfeifer,[†] Werner Kraus,[†] Franziska Emmerling,[†] Rudolf J. Schneider,[†] Ulrich Panne,[†,‡] and Michael G. Weller*,[†]

[†]*BAM Federal Institute for Materials Research and Testing, Richard-Willstätter-Strasse 11, D-12489 Berlin, Germany, and* [‡]*Humboldt-Universität zu Berlin, Chemistry Department, Brook-Taylor-Strasse 2, D-12489 Berlin, Germany*

Received May 7, 2010. Revised Manuscript Received June 30, 2010

Triacetone triperoxide (TATP), an improvised explosive, is a potential security threat because of its cost-efficient synthesis and the difficulty in detecting it. A highly selective antibody could provide the necessary specificity to the detection process. To obtain antibodies, a hapten made from acetone, hydrogen peroxide, and 7-oxooctanoic acid has been designed, synthesized, and confirmed by NMR that displays the utmost similarity to the analyte. The single-crystal X-ray structures of the solvated species TATP·methanol (1:1) and the TATP derivate were determined. In both compounds, the molecules exhibit D_3 symmetry and adopt a twisted boat−chair conformation. The hapten was coupled to bovine serum albumin, and mice were immunized. An immune response against TATP was elicited, and selective antibodies were detected in the mouse serum, which should be very useful for the development of a TATP biosensor system. An ELISA with a limit of detection for TATP of 65 μg L^{-1} is shown.

Introduction

Triacetone triperoxide (TATP) is an improvised explosive that, because of its properties, is a potential security threat. The simple, inexpensive synthesis and the lack of direct detection methods make TATP the foremost amateur explosive. Because of its atypical structure (i.e., the absence of any nitro groups and its low density), the identification within a short time frame has been hitherto troublesome using conventional methods. Several systems based on luminescence, Raman spectroscopy, mass spectrometry, electrochemical detection and other methods have been described.[1,2] An antibody-based sensing system could be a reliable and advantageous method given that the antibody will specifically distinguish the structure of TATP from that of innocuous substances.

The selectivity of antibodies is difficult to reach within groups of structurally closely related compounds, which is the case with parent compound/metabolite systems, such as in nitroaromatic compounds,[3] chlorophenols,[4] compound families (e.g., the triazine herbicides,[5,6] acetanilide herbicides,[7] phenylurea herbicides[8]), and lead compound variations with drugs (e.g., with sulfonamide antimicrobials[9−11]). Following the general rule of not modifying important/discriminating parts of the target molecule during the attachment of a spacer,[12] selective antibodies have been obtained,[13,14] sometimes with the help of molecular modeling[9,11] or using theoretical models.[4]

TATP can be easily synthesized from acetone and hydrogen peroxide.[15] The product does not contain nitrogen, but it forms a nonagonal ring without distinctive side chains and/or electron-rich substituents, which are usually required for excellent immunological recognition. As an additional complication, different conformations have to be considered. There are two stable (and separable) TATP conformers[16,17] at room temperature. One forms the regular and more stable twisted boat−chair (D_3) conformation, and the other forms the twisted chair−chair conformation (C_2) (ΔE = 1.85 kcal mol^{-1} = 7.75 kJ mol^{-1}).

The objective of this work was to synthesize a conjugate, which should be almost identical to the 3D structure of TATP, in order to obtain highly selective and high-affinity monoclonal or polyclonal anti-TATP antibodies. The structure should be confirmed through NMR spectroscopy and X-ray diffraction analysis.

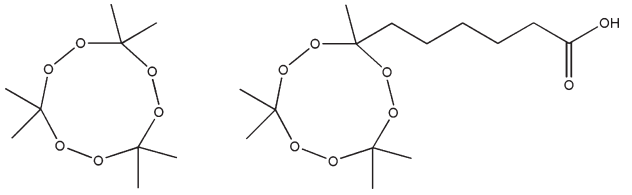
Experimental Section

Synthesis of TATP. TATP ($C_9H_{12}O_6$; CAS no. 17088-37-8; 3,3,6,6,9,9-hexamethyl-1,2,4,5,7,8-hexaoxacyclononane) was synthesized following the procedure discovered by Wolffenstein[15] in 1895 by taking into account the improvements of Milas and Golubovic,[18]

*Corresponding author. E-mail: michael.weller@bam.de.

(1) Schulte-Ladbeck, R.; Vogel, M.; Karst, U. *Anal. Bioanal. Chem.* **2006**, *386*, 559.
(2) Burks, R. M.; Hage, D. S. *Anal. Bioanal. Chem.* **2009**, *395*, 301.
(3) Zeck, A.; Weller, M. G.; Niessner, R. *Fresenius J. Anal. Chem.* **1999**, *364*, 113.
(4) Galvé, R.; Camps, F.; Sanchez-Baeza, F.; Marco, M.-P. *Anal. Chem.* **2000**, *72*, 2246.
(5) Goodrow, M. H.; Harrison, R. O.; Hammock, B. D. *J. Agric. Food Chem.* **1990**, *38*, 990.
(6) Wortberg, M.; Goodrow, M. H.; Gee, S. J.; Hammock, B. D. *J. Agric. Food Chem.* **1996**, *44*, 2210.
(7) Schlaeppi, J.-M.; Moser, H.; Ramsteiner, K. *J. Agric. Food Chem.* **1991**, *39*, 1533.
(8) Karu, A. E.; Goodrow, M. H.; Schmidt, D. J.; Hammock, B. D.; Bigelow, M. W. *J. Agric. Food Chem.* **1994**, *42*, 301.
(9) Muldoon, M. T.; Font, I. A.; Beier, R. C.; Holtzapple, C. K.; Young, C. R.; Stanker, L. H. *Food Agric. Immunol.* **1999**, *11*, 117.
(10) Haasnoot, W.; Cazemier, G.; Du Pré, J.; Kemmers-Voncken, A.; Bienenmann-Ploum, M.; Verheijen, R. *Food Agric. Immunol.* **2000**, *12*, 15.
(11) Fránek, M.; Diblikova, I.; Cernoch, I.; Vass, M.; Hruska, K. *Anal. Chem.* **2006**, *78*, 1559.
(12) Szurdoki, F.; Bekheit, H. K. M.; Marco, M.-P.; Goodrow, M. H.; Hammock, B. D. In *New Frontiers in Agrochemical Immunoassays*; Kurtz, D. A., Skerritt, J. H., Stanker, L., Eds.; AOAC International: Arlington, VA, 1995; pp 39−63.
(13) Winklmair, M.; Weller, M. G.; Mangler, J.; Schlosshauer, B.; Niessner, R. *Fresenius J. Anal. Chem.* **1997**, *358*, 614.
(14) Schneider, C.; Schöler, H. F.; Schneider, R. J. *Steroids* **2004**, *69*, 245.
(15) Wolffenstein, R. *Ber. Dtsch. Chem. Ges.* **1895**, *28*, 2265.
(16) Denekamp, C.; Gottlieb, L.; Tamiri, T.; Tsoglin, A.; Shilav, R.; Kapon, M. *Org. Lett.* **2005**, *7*, 2461.
(17) Widmer, L.; Watson, S.; Schlatter, K.; Crowson, A. *Analyst* **2002**, *127*, 1627.
(18) Milas, N. A.; Golubovic, A. *J. Am. Chem. Soc.* **1959**, *81*, 6461.

Chart 1. Chemical Structures of TATP and TATP Hapten



Matyas and Pachman,[19] and Dubnikova et al.[20] Acetone (3 mmol) and hydrogen peroxide (1.5 mmol, 30%) were mixed and cooled to 0 °C, and then sulfuric acid (0.015 mmol, 2 M) was added as a catalyst. After 24−48 h of incubation at room temperature, the formed white TATP precipitate was vacuum filtered and recrystallized three times from hot methanol. For NMR experiments and the preparation of standard solutions for ELISA, pure TATP was dried carefully under reduced pressure overnight. X-ray crystallographic studies were performed with TATP crystals that were kept in mother liquor (methanol) to stay clear−transparent until the measurement. Crystals of different purities (directly after synthesis and after each recrystallization step) were examined. For the kinetic studies via HPLC, a saturated solution in methanol was used.

Caution! *TATP is an extremely dangerous material, especially when dried crystals are present, which can detonate unpredictably, particularly under impact or friction. The synthesis and handling of TATP should be carried out by highly qualified staff with proper safety precautions and small amounts of less than 100 mg of product.*

Synthesis of TATP Hapten. Analogous to the production of TATP, a carboxylated derivative of TATP (TATP hapten, $C_{14}H_{26}O_8$) was synthesized from acetone, hydrogen peroxide, and 7-oxooctanoic acid (CAS no. 14112-98-2, Aldrich) (Chart 1). The reactants were used in equimolar ratios of 0.5 mmol each, with a catalytic amount of sulfuric acid (0.005 mmol, 2 M). The mixture was kept at room temperature for at least 24 h up to several days before the desired product was separated from educts and byproducts such as TATP using an HPLC system with diode array detection. For this purpose, a binary gradient consisting of water (containing 0.1% trifluoroacetic acid) (A) and acetonitrile/methanol (9:1) (B) was run on a C18 column (250 mm × 3 mm, 5 μm, Phen, UltraSep ES, SepServ, Berlin, Germany) under the following conditions: 40% B, isocratic for 3 min, increasing to 95% B within 17 min, and keeping this ratio for 10 min. The initial conditions (40% B) were reached within 1 min and held for 8 min. The flow rate was kept constant at 0.45 mL min^{-1}, and the oven temperature was 30 °C. The elution peak containing the TATP hapten was collected, and the solvent was evaporated. The resulting crystals were used for NMR and X-ray crystallography as well as for immunogen and tracer synthesis.

NMR Studies. NMR spectra have been recorded under standard conditions on a Bruker Avance 600 NMR spectrometer operating at 600.2 MHz for ^{1}H. The substances have been dissolved in methanol-D_4 (99.8% D, Merck) and were referenced to the solvent shifts (Table 1).

X-ray Diffraction. Single-crystal X-ray data collection was carried out on a Bruker AXS SMART diffractometer at room temperature using Mo K_α radiation (λ = 0.71073 Å), which was monochromatized by a graphite crystal. Owing to the high vapor pressure of TATP, the measurements were carried out at −80 °C. TATP hapten measurements were made at room temperature. Data reduction was performed by using Bruker AXS SAINT and SADABS packages. The structure was solved by direct methods and refined by a full-matrix least-squares calculation using SHELX.[21] Anisotropic thermal parameters were employed for non-hydrogen atoms. The hydrogen atoms were treated isotropically with U_{iso} = 1.2 times the U_{eq} value of the parent atom. In the case of methylene groups, U_{iso} = 1.5 times the U_{eq} value was chosen. Crystal data and refinement details are summarized in Table 2. CCDC-769685 and CCDC-769686 contain the supplementary crystallographic data for TATP and TATP hapten, respectively, as described in this article. The data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Kinetic Studies of the Interconversion between D_3-TATP and C_2-TATP Conformers. As shown before by Widmer et al.,[17] HPLC is an appropriate method for separating the two TATP conformers. Hence, the kinetic experiments were carried out using an HPLC system and a monolithic reversed-phase column (50 mm × 2.0 mm, 2 μm, Onyx C18, Phenomenex, Aschaffenburg, Germany). A binary gradient consisting of water (A) and acetonitrile/methanol (9:1) (B) was used under the following conditions: 40% B, isocratic for 0.5 min, increasing to 95% B within 8.5 min, and keeping this ratio for 3 min. The initial conditions (40% B) were reached within 0.5 min and held for 3.5 min. The flow rate was kept constant at 0.45 mL min^{-1}, and the oven temperature was 30 °C. Seven microliters of a highly concentrated TATP solution (methanolic mother liquor, ∼40 g/L) was injected, and the C_2-TATP conformer was collected in a cooled vial. C_2-TATP had a retention time of 2.96 min, whereas D_3-TATP appeared earlier at 2.49 min. The C_2-TATP fraction of 150 μL was diluted with 350 μL water and brought to 23 °C, and 50 μL of this solution was reinjected every 16 min. The same procedure was performed at 37 °C; here 90 μL was reinjected. The resulting decrease in the C_2-TATP signal peak area was observed until equilibrium was reached.

Enzyme-Linked Immunosorbent Assay (ELISA). TATP hapten was coupled directly to bovine serum albumin (BSA, Serva) and horseradish peroxidase (HRP, Roche) via *N*-hydroxysuccinimide chemistry with carbodiimide to produce haptenated proteins according to Tatake et al.[22] Coupling ratios for both conjugates were determined via MALDI-TOF-MS[23] to be almost 14 hapten molecules per BSA and below 1 hapten molecule per HRP, respectively. The TATP-BSA conjugate was employed to immunize BALB/c mice at Charles River Laboratories, Kisslegg, Germany. Mice were immunized nine times with 10−50 μg of immunogen every 4 or 6 weeks. The immunization progress was monitored by testing the 1:10 000 diluted mouse sera using ELISA carried out under analogous conditions to those described before.[23] For this purpose, the 1:10 000 diluted HRP conjugate was needed as an enzyme tracer to catalyze the oxidation of the substrate, 3,3′,5,5′-tetramethylbenzidine (TMB), to a colored product. A four-parameter logistic function was utilized to interpolate the relation between the absorbance and the analyte concentration in this competitive assay.[24]

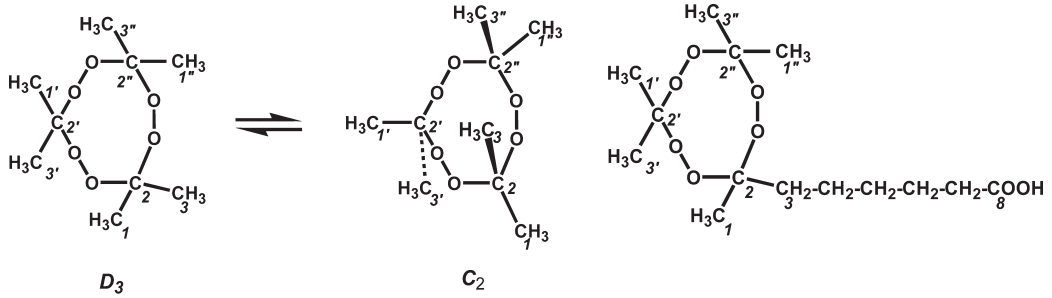
Results and Discussion

The molecular structure of TATP has been proven by assigning the signals of its ^{1}H NMR spectrum. The ^{1}H NMR spectrum of TATP hapten was found to be overlapped strongly by signals of the educt, whose molar ratio with respect to TATP hapten could be estimated to be 0.25:1. Because of the mutual overlap of adjacent signals in the ^{1}H NMR spectrum of the hapten, its structural assignment (Table 1) was drawn by means of cross-peak information from H,C-HSQC, H,C-HMBC, and H,H-COSY correlation spectra.

The X-ray structure of the cyclic TATP molecule has been described previously.[20,25,26] In the crystal structure, the nonagonal ring

(19) Matyas, R.; Pachman, J. *Sci. Technol. Energ. Mater.* **2007**, *68*, 111.
(20) Dubnikova, F.; Kosloff, R.; Almog, J.; Zeiri, Y.; Boese, R.; Itzhaky, H.; Alt, A.; Keinan, E. *J. Am. Chem. Soc.* **2005**, *127*, 1146.
(21) Sheldrick, G. M. *Acta Cryst.* **2008**, *A64*, 112.
(22) Tatake, J. G.; Knapp, M. M.; Ressler, C. *Bioconjugate Chem.* **1991**, *2*, 124.
(23) Bahlmann, A.; Weller, M. G.; Panne, U.; Schneider, R. J. *Anal. Bioanal. Chem.* **2009**, *395*, 1809.
(24) Carvalho, J. J.; Weller, M. G.; Panne, U.; Schneider, R. J. *Anal. Bioanal. Chem.* **2010**, *396*, 2617.
(25) Groth, P. *Acta Chem. Scand.* **1969**, *23*, 1311.
(26) Jensen, L.; Mortensen, P. M.; Trane, R.; Harris, P.; Berg, R. W. *Appl. Spectrosc.* **2009**, *63*, 92.

Table 1. Structural Assignment of the ^{1}H Resonances and 2D Cross Peaks for the Recorded NMR Spectra of TATP and TATP Hapten



	TATP conformers			TATP hapten		
		H, C in the molecular segment		δ ^{1}H [ppm]	δ ^{13}C [ppm]	conformer ratio
TATP	D_3	H-1,1′,1″,3,3′,3″	CH_3	1.394		97.8%
	C_2	H-1,1′,1″	CH_3(eq)	1.711		1.1%
		H-3,3′,3″	CH_3(ax)	1.228		1.1%
TATP hapten	D_3	H-1′,1″,3′,3″	CH_3	1.394	21.74	∼100%
		C-2′,2″	C		108.5	
	C_2	H-1′,1$''^{aa}$	C-1′,1$''^{aa}$	CH_3(ax)	23.5	trace
		H-3′,3$''^{aa}$	C-3′,3$''^{aa}$	CH_3(eq)	1.23	20.9	trace
		H-1	C-2′,2$''^{aa}$	C	1.71	110.14	trace
			C-1	CH_3(ax)	1.346	19.1	
			C-2	C		110.43	
		H-1	C-1	CH_3(eq)	1.389	21.74	
			C-2	C		108.5	
		H-3[a]	C-3	CH_2	1.81		
		H-3[b]	C-3	CH_2	1.57	34.7	
		H-4[a]	C-4	CH_2	1.484		
		H-4[b]	C-4	CH_2	1.391	24.76	
		H-5	C-5	CH_2	1.371	30.41	
		H-6	C-6	CH_2	1.624	26.05	
		H-7	C-7	CH_2	2.294	35.04	
			C-8	COOH		177.75	
CD_3OD		CHD_2OD			3.31	49.15	

[aa] H:TATP hapten−triperoxide fraction in C_2 symmetry.

Table 2. Crystal Data and Structure Refinement of TATP and TATP Hapten

	TATP	TATP hapten
empirical formula	$C_9H_{18}O_6 \cdot CH_3OH$	$C_{14}H_{26}O_8$
formula weight	250.24	322.35
temperature (K)	216(2)	296(2)
wavelength (Å)	0.71073	0.71073
crystal system, space group	hexagonal, $R3\bar{c}$ (no. 167)	triclinic, $P\bar{1}$ (no. 2)
unit cell dimensions (Å, deg)	a = 11.8111(7)	a = 5.951(5), α = 97.56(3)
	c = 16.8071(13)	b = 11.882(7), β = 93.92(2)
		c = 12.470(9), γ = 98.934(16)
volume (Å^3)	2030.5(2)	860.0(11)
Z/calculated density (g cm^{-3})	6/1.228	2/1.245
absorption coefficient (mm^{-1})	0.105	0.102
F(000)	802.4	348
crystal size (mm)	0.34 × 0.30 × 0.18	0.04 × 0.025 × 0.003
θ range for data collection (deg)	3.14 to 27.49	1.65 to 25.35
limiting indices	$-15 \le h \le 14$,	$-7 \le h \le 6$,
	$-13 \le k \le 15$,	$-14 \le k \le 12$,
	$-21 \le l \le 21$	$-14 \le l \le 14$
reflections collected/unique	5087/522	6436/3137
R(int)	0.1043	0.0833
absorption correction	multiscan	empirical
refinement method	full-matrix least-squares on F^2	full-matrix least-squares on F^2
data/restraints/parameters	522/2/35	31137/0/199
goodness-of-fit on F^2	1.113	0.838
final R indices ($I > 2\sigma(I)$)	R1 = 0.0518, wR2 = 0.1528	R1 = 0.0390, wR2 = 0.0529
R indices (all data)	R1 = 0.0549, wR2 = 0.1564	R1 = 0.1516, wR2 = 0.0653
largest diff. peak and hole (e/Å^3)	0.38 and −0.17	0.14 and −0.12

Table 3. Selected Bond Lengths of TATP and TATP Hapten

distance (Å) CCDC−	TATP 241 973	TATP 769 685	TATP hapten 769 686
C−O	1.4159	1.4209(14)	1.412(3)
	1.4174		1.422(3)
	1.4180		1.423(3)
	1.4190		1.425(3)
	1.4207		1.432(3)
	1.4226		1.433(3)
O−O	1.4660	1.4677(19)	1.462(2)
	1.4697		1.466(2)
	1.4733		1.468(2)
C−C	1.5047	1.516(2)	1.508(3)
	1.5107		1.514(4)
	1.5114		1.516(4)
	1.5123		1.518(3)
	1.5141		1.526(3)
	1.5159		1.522(3)

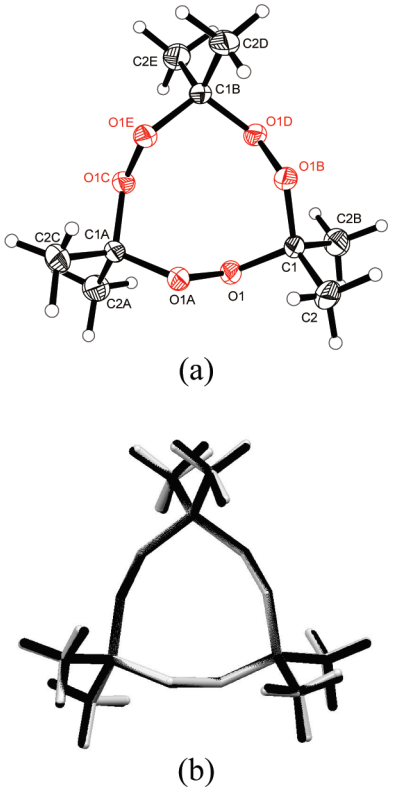


Figure 1. (a) Conformation and atomic numbering of TATP. Thermal ellipsoid plots are drawn at the 30% probability level. (b) Overlay of the nonagonal ring of TATP·methanol solvate (black) with the unsolvated TATP molecule (light gray). Only the comparisons with the left-hand conformers are shown.

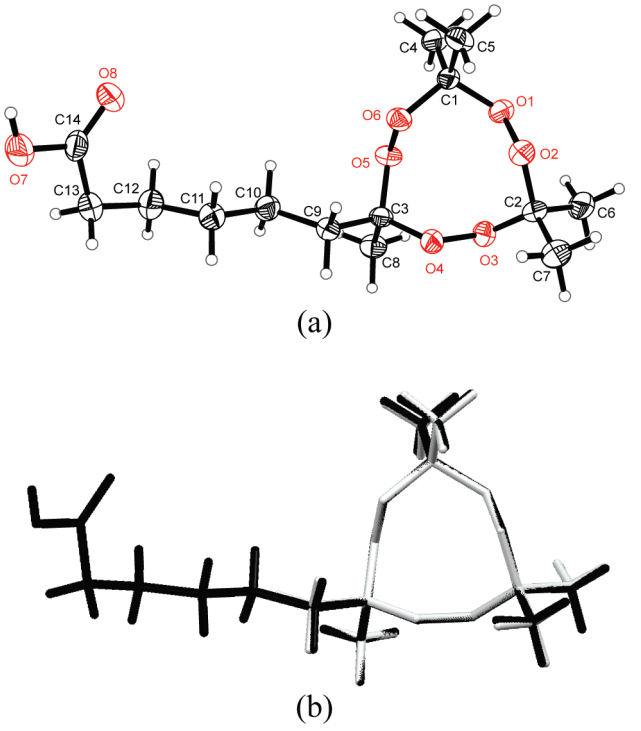


Figure 2. (a) Conformation and atomic numbering of TATP hapten. Thermal ellipsoid plots are drawn at the 30% probability level. (b) Overlay of TATP hapten (black) and the unsolvated TATP molecule (light gray). Only the comparisons with the left-hand conformers are shown.

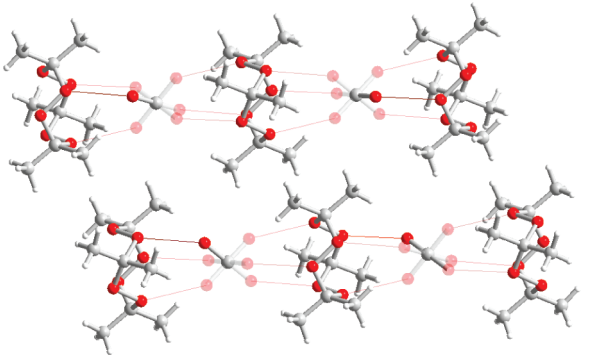


Figure 3. Arrangement of the TATP molecules in the unit cell. The methanol molecule is disordered over six positions. Gray spheres denote carbon atoms, red spheres denote oxygen atoms, and white spheres denote hydrogen atoms. Hydrogen bonds (d_{O-O} = 2.826 Å) are indicated by thin red lines.

adopts a twisted boat−chair conformation with a local symmetry close to D_3. As a result of the centrosymmetry of space group $P2_1/c$, both conformers (the right-handed (Δ) and left-handed (Λ)) are present in the crystal structure.[27]

In this work, TATP was crystallized from methanol, leading to clear block-shaped crystals of TATP that crystallize in hexagonal space group $R3\bar{c}$. TATP hapten crystallizes in triclinic space group $P1$ with one molecule in the asymmetric unit. The structure solution of TATP revealed a solvate structure consisting of TATP and methanol molecules in a molar ratio of 1:1. The TATP molecule consists of six atom positions in the asymmetric unit: one oxygen atom, two carbon atoms, and three hydrogen atoms. The molecule is completed by the symmetry operations of the trigonal space group and exhibits D_3 symmetry. The intramolecular bond lengths of the two new compounds are comparable to those in the unsolvated TATP molecule (Table 3).

The 3D structure of the TATP·methanol solvate and TATP hapten are illustrated in Figures 1a and 2a, together with the atomic labeling schemes. A comparison of the molecular conformation of the nonagonal ring of both structures reveals that the bond lengths and angles do not differ significantly and are nearly the same as in the crystal structure of the unsolvated TATP as illustrated in the structure overlay (Figures 1b and 2b).

(27) Reany, O.; Kapon, M.; Botoshansky, M.; Keinan, E. *Cryst. Growth Des.* **2009**, *9*, 3661.

The methanol molecules in TATP are disordered over six positions. From a crystallographic point of view, there are 12 positions that are symmetrically possible but only 6 positions can be realized because each of these 6 positions allows for a hydrogen bond to oxygen ring atoms. Columns of right- (Δ) and left-handed (Λ) conformers of the ring change alternately along the *c* axis (Figure 3). These columns have a hexagonal close packing arrangement. Voids within the crystal structure lead to a smaller density of 1.090 g cm^{-3}, in contrast to the value of unsolvated TATP (1.272 g cm^{-3}).

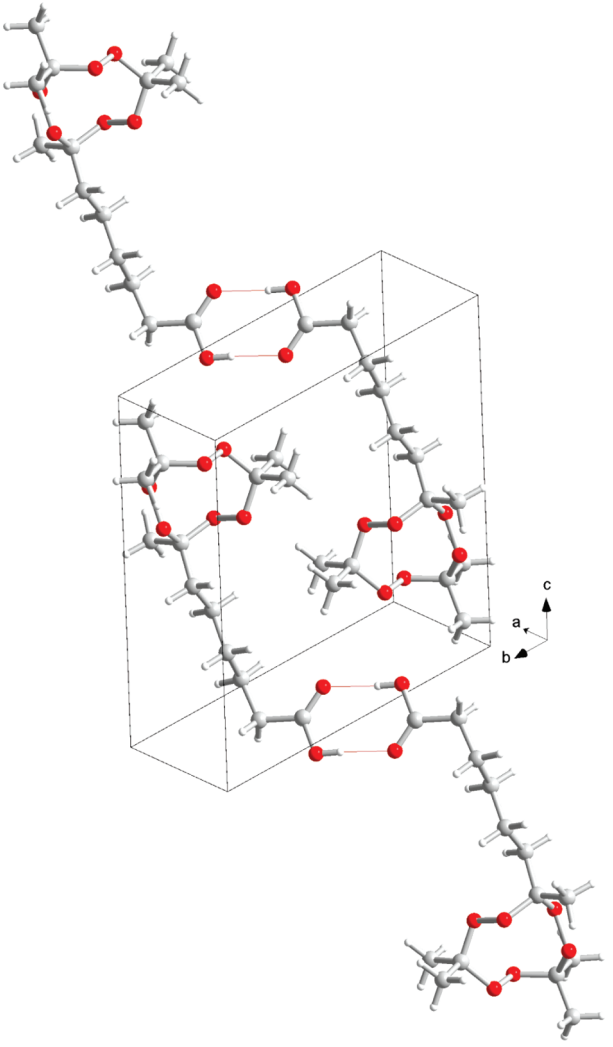


Figure 4. Arrangement of TATP hapten in the unit cell. Gray spheres denote carbon atoms, red spheres denote oxygen atoms, and white spheres denote hydrogen atoms. Hydrogen bonds (d_{O-O} = 2.708 Å) are indicated by thin red lines.

Because of the centrosymmetry ($P\bar{1}$) of TATP hapten, the unit cell contains two molecules, each of which builds a dimer structure with an adjacent molecule via hydrogen bonds (Figure 4). The density of the derivate (1.245 g cm^{-3}) is in the range of that of unsolvated TATP (1.272 g cm^{-3}).

The interconversion half-life of the TATP conformers was determined in 30% acetonitrile in water to be 33 ± 2.8 and 8.0 ± 0.31 min at 23 and 37 °C, respectively, using an exponential function and disregarding the back reaction. It follows that TATP has a flexible structure, especially at 37 °C. This is the natural temperature during antibody production via immunization of mammals. Because of the nonrigidity of hapten, antibodies with a lower affinity might result.

In equilibrium, a ratio of 13:1 in methanol was found for the respective D_3-TATP and C_2-TATP peak areas in the chromatograms, which was similar to the one determined before in organic solvent by Widmer et al.[17] Furthermore, the energy difference between both conformers in gas phase could be determined ($\Delta E = -RT \ln K$, $K = D_3/C_2$) to be 6.4 kJ mol^{-1}, which is comparable to the theoretical ZPE total energy calculation reported by Denekamp et al.[16]

By knowing the interconversion constant *k*, it was possible to estimate the activation energy E_A to be 77.5 kJ mol^{-1}

$$E_A = RT^2\left(\frac{d \ln k}{dT}\right) \quad (1)$$

where *R* is the gas constant and *T* is the temperature in K. Compared to the barrier for interconversion calculated by Denekamp et al.[16] (ΔE = 26.3 kcal mol^{-1} = 110 kJ mol^{-1}, gas phase), the obtained experimental value in 30% acetonitrile in water is in the same range.

Figure 5 reveals ELISA calibration curves using dilutions of sera from immunized mice. The *C* values representing the inflection points of the sigmoidal curves (which are similar to IC50 values) obtained were 2.5 and 5.6 mg L^{-1}. The limits of detection, calculated according to Bahlmann et al.,[23] were 65 and 870 μg L^{-1}, respectively.

Conclusions

The ELISA curves obtained from immunized mouse sera are evidence of the successful employment of TATP hapten to produce, to our knowledge, the first anti-TATP antibodies. However, the affinity of the antibodies from these immunizations has been

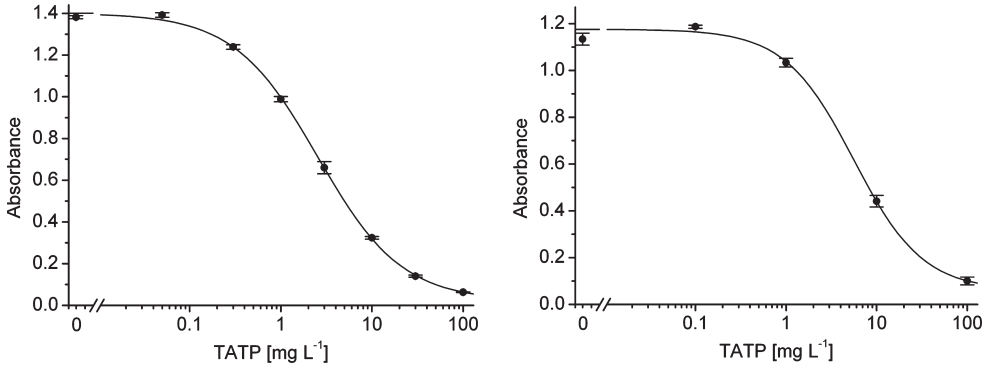


Figure 5. Calibration curves obtained using serum from two immunized mice (*C* values/inflection points are 2.5 mg L^{-1} (*n* = 4) and 5.6 mg L^{-1} (*n* = 3)). Error bars: standard deviation. Mouse sera and the enzyme tracer were both diluted 1:10 000.

rather low (5.7 μmol L^{-1}, calculated according to Zeck et al.[3]), a fact that may be owed to the flexible TATP structure. This study underlines the importance of a thorough structural characterization of both, the target compound and the hapten mimic, careful hapten design, the proper choice of a synthesis method in order to avoid structural changes, and the evaluation of target and hapten similarities and their stability under the physiological conditions of the laboratory animal to be immunized: in short, doing everything possible to achieve a successful immunization. The antibodies, especially when integrated into a suitable biosensor setup, could help to establish reliable devices with the indispensable low rate of false-positive detects. The generation of TATP antibodies is a basic achievement for the development of highly selective sensing surfaces as a basis for TATP sensors and rapid on-site tests.

Acknowledgment. M.A.W. thanks the BAM Federal Institute for Materials Research and Testing for a grant within its Ph.D. program. N. Hoffmann and S. Ramin are acknowledged for performing the HPLC studies. S. Flemig is thanked for the skillful performance of MALDI-TOF-MS measurements.