A Catalytic Antibody against a Tocopherol Cyclase Inhibitor

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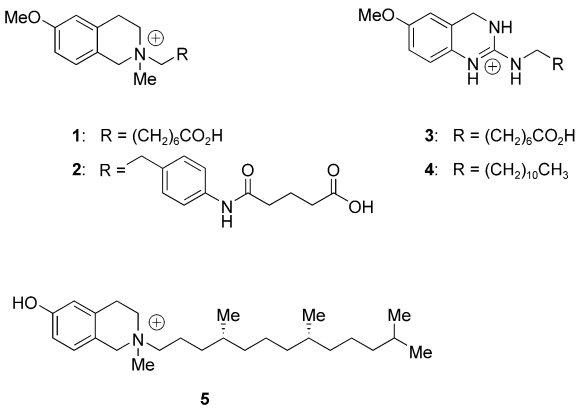
Abstract: The cyclic ammonium cation **5** and its guanidinium analogue **4** are inhibitors of tocopherol cyclase. Monoclonal antibodies were raised against protein conjugates of the haptens **1**–**3** and screened for catalytic reactions with alkene **8**, a short chain analogue of the natural substrate phytyl-hydroquinone **6**, and its enol ether analogues **10a**,**b**. Antibody 16E7 raised against hapten **3** was found to catalyze the hydrolysis of *Z* enol ether **10a** to form hemiacetal **12** with an apparent rate acceleration of $k_{cat}/k_{uncat} = 1400$. Antibody 16E7 also catalyzed the elimination of Kemp's benzisoxazole **59**. The absence of cyclization in the reaction of enol ether **10a** was attributed to the competition of water molecules for the oxocarbonium cation intermediate within the antibody binding pocket. Hapten and reaction design features contributing to this outcome are discussed. Antibody 16E7 provides the first example of a carboxyl group acting both as an acid in an intrinsically acid-catalyzed process and as a base in an intrinsically base-catalyzed process, as expected from first principles. In contrast to the many examples of general-acid-catalyzed processes known to be catalyzed by catalytic antibodies, the specific-acid-catalyzed cyclization of phytyl-hydroquinone **6** or its analogue **8** still eludes antibody catalysis.

Keywords: acid catalysis · base catalysis · catalytic antibodies · cyclization · transition-state theory

Introduction

The concept of transition-state analogy has provided an incredibly productive guiding principle for making enzyme inhibitors[1] and has also led to the discovery of catalytic antibodies as tailor-made catalysts.[2] The majority of catalytic antibodies reported to date accelerate either ester/amide-bond hydrolysis or pericyclic reactions.[3] By contrast, only a very few examples of catalytic antibodies for acid-catalyzed processes have been reported. Herein we report our investigation towards a tocopherol cyclase catalytic antibody. We describe the preparation of catalytic antibody 16E7, obtained from immunizations against transition-state analogues **1**–**3** of the tocopherol cyclase reaction, and its catalytic reactivity (Scheme 1).



Scheme 1. Transition–state analogues **1**–**3** of the tocopherol cyclase reaction and inhibitors **4** and **5** of the enzyme tocopherol cyclase.

Results

The acid-promoted cyclization of phytyl-hydroquinone **6** to γ-tocopherol (**7**) is the key step in the biosynthesis of the

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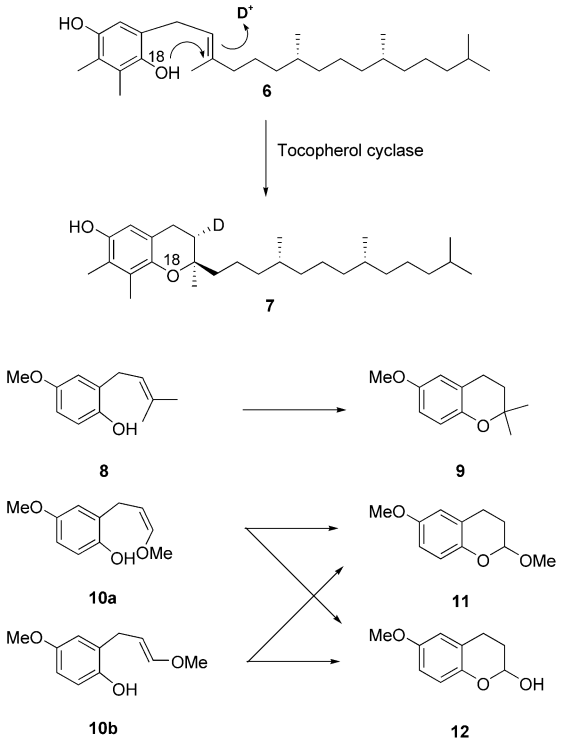
chromanol substructure of the vitamin E family (Scheme 2).[4] This reaction is of potential industrial interest for the preparation of this important food additive. This enzyme-catalyzed ring closure proceeds by *Si* protonation of the double bond of **6** and concomittant *Re* attack of the phenolic oxygen atom.[5] Although the exact nature of the catalytic machinery of this enzyme is not known, the reaction mechanism clearly requires an acidic residue for catalysis.



Scheme 2. Cyclization of phytyl-hydroquinone **6** to γ-tocopherol (**7**) catalyzed by the enzyme tocopherol cyclase and corresponding model reactions with olefin **8**, enol ether **10a** and enol ether **10b** that were used for the selection of catalytic antibodies.

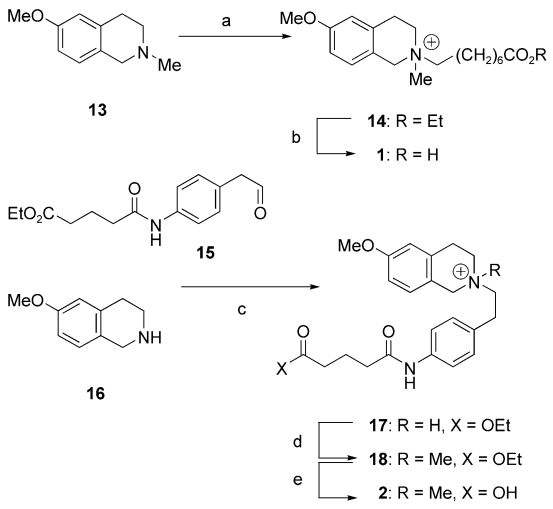
Reaction and hapten design: Due to its long isoprene side chain, phytyl-hydroquinone **6** is strongly hydrophobic and only poorly soluble in water. We initially decided to delete this sesquiterpene side chain, which is only important for recognition by the enzyme,[6] in order to enhance water solubility and therefore enable a reaction setup in the homogeneous phase, where most catalytic antibodies have been described. The second problematic element in the natural substrate **6** is the dimethylhydroquinone nucleus, which is both synthetically demanding and highly sensitive to air oxidation. Substitution by a simple *ortho*-substituted *p*-methoxyphenol would allow a shorter synthesis and would essentially suppress oxidation sensitivity, while preserving the chemical reactivity of the system towards cyclization. Modification of the γ-tocopherol precursor **6** along these lines led to the allylic *p*-methoxyphenol **8** as a model substrate (Scheme 2).

The cyclization of phytyl-hydroquinone **6** is specific-acid catalyzed, which means that in solution only strong acids can promote the reaction.[7] Preliminary investigations showed that this was also the case for the cyclization of substrate **8** to form product **9**. By contrast, antbodies have been almost exclusively described for general-acid-catalyzed processes, which are promoted by weak acids, usually a carboxylate side chain within the antibody binding pocket.[8] We therefore also prepared *Z* and *E* enol ethers **10a** and **10b**. These substrates undergo a cyclization similar to that of substrate **8** to form acetal **11** or hemiacetal **12**, with the advantage that the reaction can be promoted by weak acids such as acetic acid.[9] Additional test substrates were designed to back up the search for catalysis. These included methylene derivative **35**, enol ethers **44a**,**b** and **45a**,**b**, vinyl ether **50**, and unsaturated esters **55** and **56**, all of which might potentially undergo an intramolecular cyclization reaction with the phenolic hydroxy group.

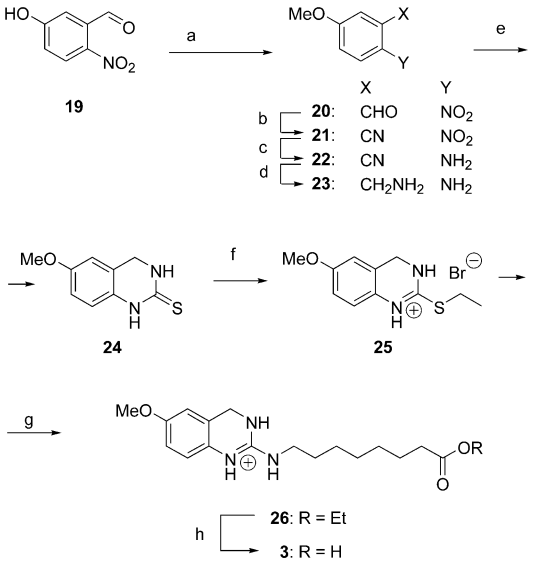
The design of haptens possibly leading to antibodies promoting specific-acid catalysis of the tocopherol cyclase reaction followed the same principles as those used in previous systems for acid catalysis. The design should emphasize electrostatic complementarity to the transition state, that is, positively charged functional groups in the hapten, since specific-acid catalysis should be triggered not by the presence of a carboxyl group but by a local electrostatic effect stabilizing positively charged intermediates more strongly than the solvent water.[10] The quaternary tetrahydroquinolinium cations **1** and **2** and the guanidinium analogue **3** were selected as transition-state analogues for the reaction. The guanidinium cation **4** and the ammonium cation **5** are nanomolar inhibitors of the enzyme tocopherol cyclase[11] and related ammonium cations have been used frequently to induce catalytic antibodies for related acid-catalyzed processes.[8a,c,12] The guanidinium cation **3** was designed along the lines of related compounds used previously as haptens to raise catalytic antibodies.[13] A carboxylate was expected on the antibody as a charge-neutralizing group against the guanidinium group.

Synthesis: Tetrahydroquinolinium hapten **1** was obtained by quaternization of 2-methyl-6-methoxytetrahydroquinoline (**13**) with ethyl 8-bromo-octanoate[14] (Scheme 3). It was conjugated to carrier proteins KLH (keyhole limpet hemocyanin) and BSA (bovine serum albumin) through its *N*-hydroxysuccinimide ester. Hapten **2** was synthesized by reductive amination of 6-methoxytetrahydroquinoline (**16**) with aldehyde **15**. Alkylation of the resulting amine **17** with dimethylsulfate gave ammonium compound **18**. Acidic hydrolysis of the ester gave hapten **2**. Conjugation to carrier proteins was carried out as for hapten **1**. Guanidinium hapten **3** was prepared from 5-hydroxy-2-nitrobenzaldehyde (**19**), which was first protected as the corresponding methyl ether **20** and then transformed into the benzonitrile **21**[15] (see Scheme 4). Reduction of the nitro group with stannous chloride gave **22**, and reduction of the cyano group with $LiAlH_4$/$AlCl_3$ yielded *o*-aminobenzylamine **23** in moderate yields. The thione **24** was prepared by condensation between benzylamine **23** and thiophosgene and was alkylated to give **25**. Substitution with ethyl 8-amino-octanoate[16] gave **26**, which was hydrolyzed under acidic conditions to give hapten **3**. Conjugation to carrier proteins was carried out as above.

Substrate **8** was prepared according to a reported method[17] and its cyclization product **9** was obtained by

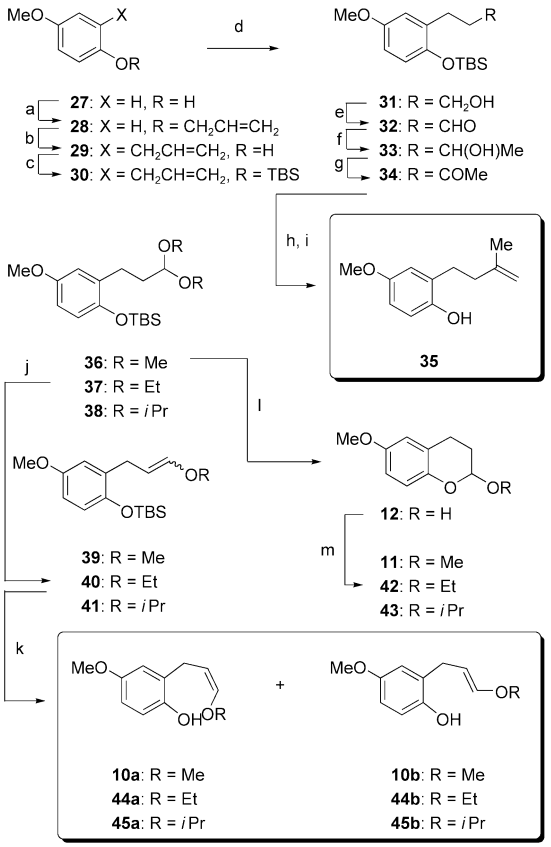


Scheme 3. Synthesis of haptens **1** and **2**. a) $Br(CH_2)_7CO_2Et$, DMF, 60 °C, 3 h, 84 %; b) 1 N HCl/DMF 1:1, RT, 24 h, RP18 HPLC, 71 %; c) $NaBH(OAc)_3$, DCE, RT, 2 h, 74 %; d) dimethylsulfate, CH_3CN, RT, 16 h, RP18 HPLC, 55 %; e) 4 M HCl in dioxane, H_2O, RT, 6 h, RP18 HPLC 71 %. DCE = 1,2-dichloroethane.



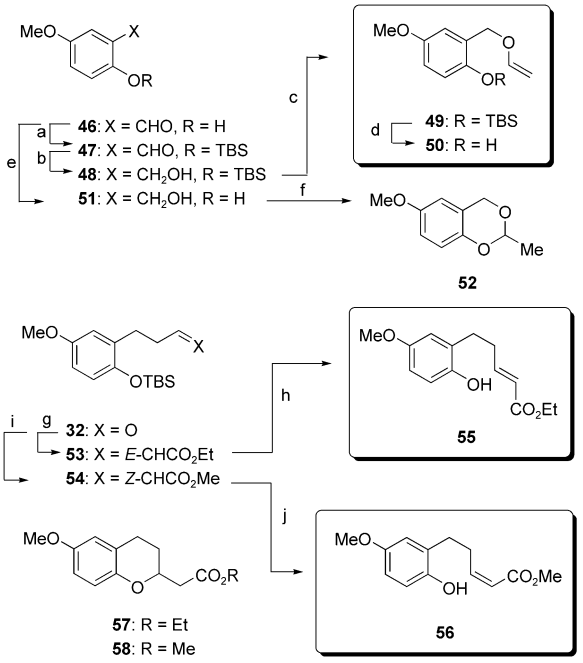
Scheme 4. Synthesis of hapten **3**. a) Cs_2CO_3, DMF, 90 min; MeI, RT, 15 h, 96 %; b) $H_2NOH{\cdot}HCl$, $MgSO_4$, *p*-TSA, toluene, Δ, 15 h, 88 %; c) $SnCl_2{\cdot}2H_2O$, MeOH, Δ, 3 h, 56 %; d) $LiAlH_4$, $AlCl_3$, Et_2O, RT, 5 h, 78 %; e) thiophosgene, Et_3N, THF, −78 °C→RT, 8 h, 84 %; f) EtBr, EtOH, Δ, 14 h, 98 %; g) $H_2N(CH_2)_7CO_2Et$, EtOH, Δ, 24 h, 65 %; h) 1 N HCl/THF, RT, 24 h, RP18 HPLC, 87 %. *p*-TSA = *para*-toluenesulfonic acid.

treatment of **8** with boron trifluoride etherate. Its methylene analogue **35**, poised for *exo-trig* cyclization, was prepared from anisole **27** (Scheme 5). O-allylation, Claisen rearrangement, and silylation gave ether **30**, which was transformed in four steps into methyl ketone **34**. Wittig reaction with methyltriphenylphosphonium bromide and deprotection gave substrate **35**. Enol ethers **10a**,**b**, **44a**,**b**, and **45a**,**b** were obtained from the corresponding acetals **36–38** by reaction with trimethylsilyl triflate[18] or trimethylsilyl iodide[19] followed by desilylation. Classical desilylation procedures with fluoride reagents failed in this case due to the reactivity of the enol ether function. Nevertheless, desilylation proceeded smoothly by treatment with ethanolamine, to give the desired hydroxy enol ethers. Purification of the isomeric mixtures **10a**,**b** and **44a**,**b** by preparative RP-HPLC provided the isomerically pure enol ethers. In the case of the enol ether **45a**,**b**, the separation of the *E*,*Z* isomers was performed easily by simple column chromatography. Treatment of acetal **36** with aqueous hydrochloric acid afforded the hydrolysis product **12**, which in turn was transformed into the corresponding cyclization acetals **11**, **42**, and **43** according to a known procedure.[20]



Scheme 5. Synthesis of substrates **35**, **10**, **44**, and **45** as well as the corresponding reference compounds **12**, **11**, **42**, and **43**. a) NaH, THF, allyl bromide, Δ, 4 h, 95 %; b) neat, 240 °C, 45 min, 89 %; c) TBSCl, imidazole, DMF, RT, 1 h, 96 %; d) $BH_3{\cdot}THF$, THF, RT, 2 h; NaOH, H_2O_2, 0 °C→RT, 89 %; e) DMSO, Et_3N, CH_2Cl_2, oxalyl chloride, −78 °C→RT, 90 min, 94 %; f) CH_3MgCl, THF, RT, 1 h, 89 %; g) DMSO, Et_3N, CH_2Cl_2, oxalyl chloride, −78 °C→RT, 1 h, 95 %; h) methyltriphenylphosphonium bromide, *n*BuLi, THF, 0 °C, 60 min, 75 %; i) TBAF, THF, 20 min, 93 %; j) for **39**: $(iPr)_2NEt$, TMSTf, CH_2Cl_2 −78→−20 °C for 4 h, 59 %; for **40**: $(iPr)_2NEt$, TMSTf, CH_2Cl_2 −78→−20 °C for 4 h, 63 %; for **41**: HMDS, TMSI, CCl_4, −15 °C for 30 min, 75 °C for 5 h, 29 %; k) for **10**: ethanolamine, 90 °C, 4 h, prep. RP18 HPLC, 46 % **10b** and 44 % **10a**; for **44**: ethanolamine, 90 °C, 3 h, prep. RP18 HPLC, 46 % **44b** and 37 % **44a**; for **45**: ethanolamine, 90 °C, 3 h, 47 % **45b** and 45 % **45a**; l) dioxane/18 % HCl 25:1, 50 °C, 18 h, 94 %; m) for **11**: thionyl chloride, CH_2Cl_2, 0 °C, 15 min; MeOH, 0 °C, 15 min, 72 %; for **42**: thionyl chloride, CH_2Cl_2, 0 °C, 15 min; EtOH, 0 °C, 15 min, 86 %; for **43**: thionyl chloride, CH_2Cl_2, 0 °C, 15 min; iPrOH, 0 °C, 15 min, 76 %. TBS = *tert*-butyldimethylsilyl, TBAF = tetrabutylammonium fluoride, Tf = triflate = trifluoromethanesulfonyl, HMDS = 1,1,1,3,3,3-hexamethyldisilazane.

Vinyl ether **50** was synthesized from 2-hydroxy-4-methoxybenzaldehyde **46** by silylation to **47**, reduction with sodium borohydride to **48**, transetherification catalyzed by $Hg(OAc)_2$[21] to form **49**, and mild desilylation with ethanolamine (Scheme 6). Reduction of **46** with lithium aluminium hydride gave benzylalcohol **51**, which was transformed into acetal **52** by acidic treatment with acetaldehyde.[22] Wittig–Horner olefination of aldehyde **32** gave the *E*-configured α,β-unsaturated ester **53**, which was desilylated with TBAF to yield **55** together with its cyclization product **57** in 56 and 27% yield, respectively. Application of the Still–Gennari variant of the Wittig–Horner olefination to aldehyde **32** gave the *Z*-configured α,β-unsaturated ester **54**. Deprotection with TBAF provide the pure *Z* isomer **56** together with its cyclization product **58** in 38 and 39% yield, respectively.



Scheme 6. Synthesis of substrates **50**, **55**, and **56** as well as the corresponding reference compounds **51**, **52**, **57**, and **58**. a) $(iPr)_2NEt$, DMF, TBSCl, 45 min, RT, 97%; b) $NaBH_4$, EtOH, RT, 45 min, 91%; c) ethyl vinyl ether, $Hg(OAc)_2$ (cat), Δ, 72%; d) ethanolamine, RT, 16 h, 94%; e) $LiAlH_4$, THF, RT, 15 min, 84%; f) acetaldehyde, H_2SO_4, 0°C, 4 h, 91%; g) phosphonoacetic acid triethyl ester, NaH, THF, 0°C→RT, 14 h, 77%; h) TBAF, THF, RT, 5 min, 56% **55** and 27% **57**; i) phosphonoacetic acid *P,P*-bis(2,2,2-trifluoroethyl)methyl ester, [18]crown-6, KHDMS, THF, −78°C, 90 min, 96%; j) TBAF, THF, RT, 5 min, 38% **56** and 39% **58**. KHDMS = potassium hexamethyldisilazanide.

Immunizations and hybridoma selection: The KLH conjugates of the haptens were used for immunization following standard procedures by using either single-hapten or mixed immunizations (Table 1). Hybridoma generation and initial screening by ELISA against hapten–BSA conjugates gave a total of 131 positives. Further cell culture up to a volume of 5 mL gave 27 hybridoma cell lines, which were assayed for catalysis of the cyclization of olefin **8** and enol ethers **10a** and **10b** by HPLC. Hybridoma 16E7 (anti-**3**) and 16G7 (anti-**2**) catalyzed the hydrolysis of enol ether **10a** and their activity was quantitatively inhibited by addition of the respective haptens. Both hybridomas were subcloned twice to give stable and homogeneous cell lines. The catalytic activity and the hapten binding activities were preserved during the cloning process. Antibodies were produced from the cell lines by in vitro cell culture up to a volume of 1 L and were purified by ammonium sulfate precipitation followed by protein G affinity-column purification.

Table 1. Data for immunization with haptens **1**–**3** and screening for catalysis with substrates **8**, **10a**, and **10b**.

Hapten[a]	Number of mice[b]	Serum titers (conjugate)[c]	Number of binders[d]	Number of screened cell lines[e]	Number of catalytic hybridoma[f]	Clone name and subtype
1/1/1	2	1 mouse 6400 and 1 mouse 3200 (**1**–BSA)	22	4	0	–
2/2/2	4	3 mice 25600 and 1 mouse 12800 (**2**–BSA)	5	1	1	16G7 κγ1
3/3/3	2	2 mice 12800 (**3**–BSA)	40	9	1	16E7 κγ2a
1/3/3	2	1 mouse 12800–25600 and 1 mouse 6400 (**1**–BSA)	34	5	0	–
3/1/1	2	1 mouse 12800 and 1 mouse 0 (**3**–BSA)	30	8	1	–

[a] First, second, and final boost of the corresponding hapten conjugates used for the immunization. [b] In total, twelve 129GIX+ mice were immunized according to standard procedures. [c] Dilution factor of blood serum (after immunization with the hapten–KLH conjugates) for 50% reduction of ELISA signal against the hapten–BSA conjugates. A high number indicates a strong immune response. [d] Number of cell lines that test positive for binding by ELISA against the hapten–BSA conjugate after fusion. [e] Number of cell lines that were grown up to a volume of 5 mL for catalysis testing. [f] Number of fully subcloned and stabilized hybridoma producing antibodies with catalytic properties.

Kinetic characterization: Antibody 16E7 was studied in detail since assays with purified antibodies showed it to be the more active protein. The antibody catalyzed the hydrolysis of the *Z* enol ether **10a** to form hemiacetal **12** with multiple turnover. The reaction was quantitatively inhibited by hapten **3**, with the indication of two active sites per antibody molecule. Isomeric *E* enol ether **10b** was not accepted as a substrate. The antibody showed a pronounced hysteresis effect, in that it required up to eight hours to reach its full catalytic activity (Figure 1). Similar kinetic retardation effects have been described in other catalytic antibodies.[23] The kinetics of antibody 16E7 followed Michaelis–Menten kinetics in both the initial phase and the steady-state phase of conversion, with a result indicating a rate acceleration in the range of $k_{cat}/k_{uncat} = 1400$ (Table 2), which is a typical value for antibody catalysis.

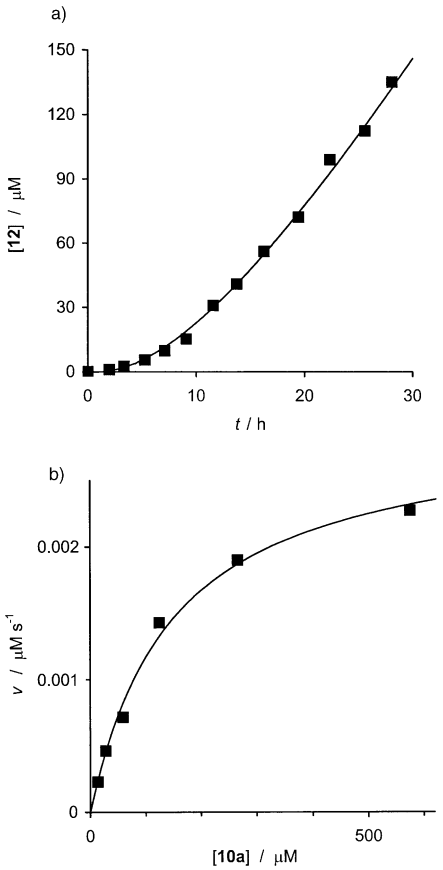


Figure 1. Kinetic measurements of the hydrolysis of enol ether **10a** by antibody 16E7. All data shown have already been corrected for the uncatalyzed reaction. All assays were performed at an antibody concentration of 0.2 mg mL^{-1} (3.33 μM active-site concentration) in 104 mM NaCl and 29 mM BisTris (bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane) in water with 10% acetonitrile at 37°C and pH 6.24. a) The time-course plot observed for hydrolysis of enol ether **10a** (300 μM) catalyzed by antibody 16E7. In general, three parameters can be graphically estimated from a time-course plot.[42] The initial velocity v_i and the steady-state velocity v_{ss} can each be obtained by simple linear regression and the apparent rate constant τ^{-1} can be calculated from the intercept of the v_{ss} slope on the time axis (see Equation (1) in the Experimental Section). b) Michaelis–Menten plot for the hydrolysis of the enol ether **10a** in the advanced stages of the catalysis, with constant v_{ss} velocities.

Substrate variation studies: Investigations with the pure antibody 16E7 showed that the tocopherol cyclase like cyclization of the olefinic phenol **8** to form **9** was not catalyzed by the antibody, even under very low pH conditions and in concentrated antibody solution. Even the noncatalyzed reaction could not be detected under these conditions. The formation of hemiacetal **12** instead of acetal **11** in the reaction of enol ether **10a** might be caused by a competing water molecule in the active site. Therefore, the ethyl enol ether **44a**,**b** and its isopropyl analogue **45a**,**b** were also tested with 16E7, since these more hydrophobic substrates might reduce the water content of the active site and suppress the water reaction, thereby leading to hydrolysis. Although both **44a** and **45a** were accepted as substrates, their reaction led exclusively to the formation of hemiacetal **12**. Acetal **11** was not hydrolyzed by the antibody and also did not show any competitive inhibition of the enol ether hydrolysis, a result implying that it was very poorly recognized by the antibody.

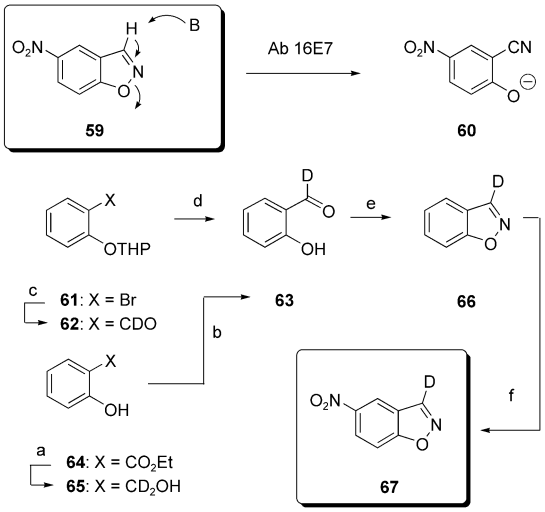
A series of alternative substrates that would potentially lead to cyclized products under acid–base catalysis was tested for catalysis by antibody 16E7. The methylene analogue **35** might be protonated to form the same carbocationic intermediate as that expected with the original substrate **8**, yet starting with an energetically higher point (a disubstituted versus a trisubstituted olefin) and following a more favorable overall *exo-trig* cyclization pathway to the desired cyclized product as opposed to a relatively disfavored *endo-trig* process for the tocopherol cyclase like cyclization of **8**. Similarly, vinyl ether **50** might be as reactive as enol ether **10a** and also lead to a cyclized product following a favored overall *exo-trig* process. Finally, the unsaturated hydroxy esters **55** and **56** were expected to undergo a facile acid–base-catalyzed 1,4-addition of the hydroxy group to form **57** and **58**, respectively. While all of these substrates indeed underwent the expected processes in organic solvent upon treatment with strong acid, only vinyl ether **50** was accepted as a substrate by antibody 16E7 to give alcohol **51** without cyclization.

Table 2. Kinetic parameters for reactions catalyzed by antibody 16E7.[a]

Substrates	Enol ethers[b]				Benzisoxazoles[c]	
	10a	**44a**	**45a**	**50**	**59**	**67**
v_i [μM s^{-1}][d]	3.22×10^{-4}[e]	3.19×10^{-4}[e]	1.56×10^{-4}[e]	n.d.	1.23×10^{-2}[f]	3.08×10^{-3}[f]
v_{ss} [μM s^{-1}][g]	1.75×10^{-3}[e]	1.68×10^{-3}[e]	1.45×10^{-3}[e]	n.d.	2.34×10^{-2}[f]	1.23×10^{-2}[f]
τ [h][h]	7.9[e]	8.1[e]	8.2[e]	n.d.	1.2[f]	9.1[f]
k_{cat} [s^{-1}]	1.11×10^{-3}	6.93×10^{-4}	5.56×10^{-4}	7.42×10^{-4}	n.d.	n.d.
k_{uncat} [s^{-1}]	7.99×10^{-7}	1.66×10^{-6}	4.78×10^{-6}	8.02×10^{-6}	3.62×10^{-6}	5.96×10^{-7}
k_{cat}/k_{uncat}	1392	417	116	92	n.d.	n.d.
K_M [μM]	171	40	107	132	n.d.	n.d.
k_{cat}/K_M [μM^{-1} s^{-1}]	6.47×10^{-6}	1.73×10^{-5}	5.20×10^{-6}	5.64×10^{-6}	1.30×10^{-4}[i]	7.19×10^{-5}[i]
K_{TS} [μM][j]	0.124	0.096	0.919	1.43	2.79×10^{-2}	8.29×10^{-3}

[a] All assays were measured in 104 mM NaCl and 29 mM BisTris in water with 10% acetonitrile at 37°C and pH 6.24. [b] All assays were performed at an active-site concentration of 3.33 μM. [c] All assays were performed at an active-site concentration of 0.32 μM. [d] v_i is the initial velocity. [e] For the determination of the values v_i, v_{ss}, and τ an initial substrate concentration of 300 μM was used. [f] For the determination of the values v_i, v_{ss}, and τ an initial substrate concentration of 671 μM was used. [g] v_{ss} is the steady-state velocity. [h] τ^{-1} is the observed rate of the transition between the initial state and the steady state. [i] The low solubility of **59** and **67** prevented measurements at substrate concentrations above 2500 μM and hence only exact values of k_{cat}/K_M could be obtained. [j] K_{TS} is defined as the dissociation constant of the complex between the catalyst and the transition state of the reaction and can be calculated by following equation: $K_{TS}=k_{uncat}/(k_{cat}/K_M)$.

Ring-opening reactions: Since our antibody 16E7 did not induce any acid-catalyzed cyclization, we set out to test its ability to promote a reverse ring-opening base-catalyzed process. The deprotonation of Kemp's benzisoxazole **59**, which undergoes precisely such a base-catalyzed ring-opening process,[24] was therefore investigated (Scheme 7). Two previous accounts of catalysis of this reaction by antibodies raised against guanidinium and amidinium haptens similar to guanidinium **3** were taken into account.[13]



Scheme 7. Deprotonation of Kemp's benzisoxazole **59** by antibody 16E7 and synthesis of deuterated benzisoxazole **67**. a) $LiAlD_4$, THF, 12 h, Δ, 86%; b) different oxidation methods, low yields; c) *n*BuLi, Et_2O, −10°C, 2 h; [D_7]DMF, RT, 8 h, 97%; d) aqueous 1 M HCl/THF 1:1; e) hydroxylamine-*O*-sulfonic acid, Na_2SO_4, H_2O, CH_2Cl_2; $NaHCO_3$, RT, 1 h, 82%; f) H_2NO_3/H_2SO_4, 0°C, 30 min, 53%. Ab = antibody, THP = tetrahydropyran.

Antibody 16E7 efficiently catalyzed the ring-opening reaction of benzisoxazole **59** to form the yellow cyanonitrophenolate **60**. As for the hydrolysis of enol ether **10a**, the reaction was quantitatively inhibited by hapten **3**, a result demonstrating that both reactions were taking place within the antibody binding pocket. A retardation effect similar to that detected with enol ether **10a** was observed (Figure 2). While there was no detectable substrate binding under the accessible substrate concentration range (Michaelis–Menten constant of $K_M > 2.5$ mM), the catalysis was efficient with a transition-state dissociation constant of $K_{TS} = 27.9$ nM, which corresponds to a rate enhancement of 8×10^6 over the corresponding acetate-catalyzed reaction, if a carboxylate catalytic group is assumed. Rate-limiting proton transfer was confirmed by comparing the kinetics of benzisoxazole **59** with its deuterated analogue **67**.

Initially, compound **67** was prepared by a reported reaction sequence,[25] which caused unexpected difficulties. Despite repeated attempts, the oxidation of the deuterated benzylalcohol **65** provided the corresponding benzaldehyde **63** only in very poor yields, presumably by a primary isotope effect. We therefore developed an alternative synthesis. Lithiation of the THP-protected bromophenol **61**[26] followed by treatment with [D_7]-*N*,*N*-dimethylformamide to form **62** and THP deprotection afforded the desired benzaldehyde **63** in good yields. Aldehyde **63** was then transformed by the known procedures[27] to the nitrobenzisoxazole **67**.

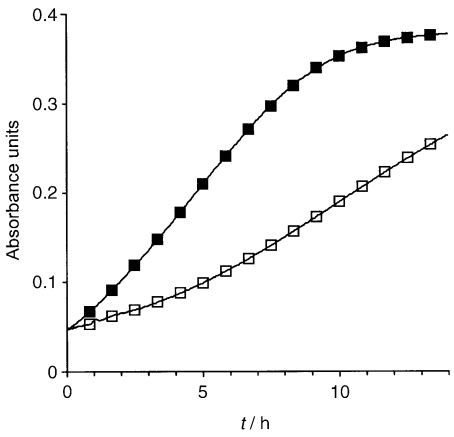


Figure 2. The time-course for the decomposition of benzisoxazole **59** (■) and its deuterated analogue **67** (□). All assays were performed at an antibody active-site concentration of 0.32 μM and at an initial substrate concentration of 671 μM in 104 mM NaCl and 29 mM BisTris in water with 10% acetonitrile at 37°C and pH 6.24.

The comparison of reaction rates between the benzisoxazole **59** and its deuterated analogue **67** was complicated by the hysteresis effect. Indeed, **59** was converted by more than 30% before the antibody had reached full activity, so the reaction rate was not approaching its true maximum at any point during the reaction. With the slower-reacting deuterated substrate **67**, the fully active state of the antibody was reached at a lower conversion, thereby allowing the rate to approach closer to a true maximum. Considering these limitations, we estimated that the kinetic isotope effect on the deprotonation reaction is approximately 2–4, which is consistent with rate-limiting proton transfer and the critical role of acid–base catalysis in the antibody-catalyzed process. Similar isotope effects have been observed with other catalytic antibodies for the same reaction.[13]

Discussion

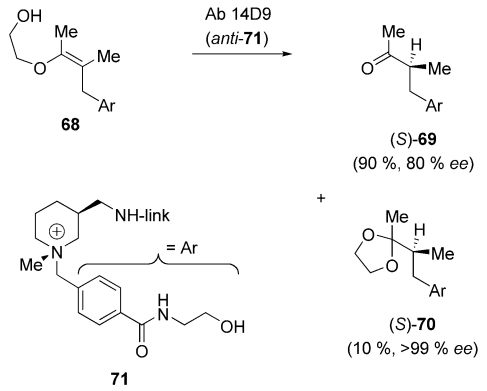
The cyclization of phytyl-hydroquinone **6** to γ-tocopherol (**7**) is, in principle, a two-step process requiring protonation of the double bond followed by trapping of the intermediate carbocation by the hydroxy group. It is believed that preionization of the phenol to a phenolate occurs in the enzyme binding pocket to favor double-bond protonation, thereby resulting de facto in a single-step process without an actual carbocation intermediate. In any case, the enantioselectivity of the enzyme-catalyzed cyclization implies that the enzyme induces and immobilizes a single enantiomeric conformation of the substrate during the reaction.

Tetrahydroquinolinium haptens **1** and **2** provide almost perfect transition-state analogues: the molecules perfectly mimic the product-like geometry of a late transition state while displaying the positive charge of the carbocationic intermediate. The cyclic nature of these transition-state ana-

logues should ensure that the complementary binding pockets of the induced antibodies are able to preorganize the acyclic precursors for cyclization. The same reasoning applies, although less perfectly, to the corresponding guanidinium hapten **3**. Within the framework of an immunization experiment, the positive charge in the haptens **1**–**3** naturally induces a charge-neutralizing carboxylate, thereby providing the necessary catalytic machinery for the reaction. The full positive charge available in the haptens at neutral pH values also ensures electrostatic complementarity in the binding pocket.

As far as can be judged from binding studies by ELISA, the isolated catalytic antibody 16E7 displays the expected binding properties, since it binds with both its original hapten **3** and the quaternary ammonium cations **1** and **2**. This suggests that the antibody binding pocket is indeed complementary to the geometry of the cyclic product. The efficient ring-opening reaction with benzisoxazole **59** probably takes place in the corresponding arrangement, as for the closely related antibody 34E4 raised against a very similar hapten.[12a] The question then arises as to why ring closure with participation of the hydroxy group is never observed with any of the cyclization precursors tested, despite of the ability of the antibody binding pocket to react with the cyclic benzisoxazole **59** in a ring-opening process.

In the case of enol ether **10a**, which was used for screening and could be considered as the "natural" substrate of antibody 16E7, ring closure would result from intramolecular trapping of the oxocarbonium cation intermediate, which occurs in competition with intermolecular trapping by a water molecule from the solvent. We have described a similar process in catalytic antibody 14D9,[8,28] in which protonation of hydroxyethyl enol ether **68** led to the formation of the optically pure cyclic acetal **70** in a 1:10 ratio to ketone **69**, which would result from the water reaction (Scheme 8).[29] In the absence of any directing effect for cyclization in the hapten design, the acetal versus ketone ratio was interpreted in terms of a low (approximately 3%) water content of the antibody binding site. Considering the known versatility of antibody 14D9, we tested whether this antibody also catalyzed reactions with enol ethers **10a** and **10b**, olefin **8**, and benzisoxazole **59**, but without success. Strikingly, antibody 14D9 was obtained from an immunization against the hydrophobic quaternary ammonium hapten **71**, which is very similar to our tetrahydroquinolinium hapten **1**. In the present series of experiments the fact that hapten **1** did not induce any catalytic antibody could be due to pure chance.[30] The guanidinium hapten **3** that induced antibody 16E7 probably induces a more hydrophilic binding site.



Scheme 8. 14D9-catalyzed hydrolysis and ring closure of hydroxyethyl enol ether **68**.

The present series of experiments to prepare antibody 16E7 was designed in close analogy to our own experience with the series leading to the hydrolytic antibody 14D9, which could be interpreted as a bias towards hydrolytic processes. Janda and co-workers have reported several catalytic antibodies towards hydroxy epoxide and olefinic tosylate cyclizations.[31] The functional groups and overall structure of their haptens is very similar to haptens **1**–**3** used here and they incorporate quaternary ammonium and guanidinium functional groups next to an immunogenic aromatic group. However, in contrast to our design, Janda and co-workers systematically placed the aromatic group of their haptens between the cationic groups and the linker to the carrier proteins, which would tend to induce binding pockets that would become readily sealed off from the solvent upon substrate binding. Yet another factor affecting the hydrolytic tendency in the system of Janda and co-workers lies in the experimental conditions used. Most of their systems, particularly those leading to olefin tosylate cyclizations, operated in a biphasic hexane/water system,[32] whereby the substrate is dissolved in the organic phase and comes into contact with a concentrated buffered antibody solution upon shaking. These reaction conditions may seem artificial but they may indeed be quite similar to the membrane-bound conditions under which many of the natural olefin–cyclization enzymes operate, including various terpene cyclases and tocopherol cyclase itself.[4–6,31f] Unfortunately, we did not observe any conversion of enol ether substrates **10a**,**b** and olefin **8** under these biphasic conditions. This lack of reactivity under the biphasic conditions might be related to the particular retardation effect in the onset of activity of 16E7 in aqueous buffer. Indeed modeling investigations suggest that the kinetic retardation effect is induced by a large conformational movement of the CDR3 loop; such a movement could well be inhibited under phase-transfer conditions.[33]

One of the striking aspects of the reactivity of antibody 16E7 is its dual reactivity consisting in general-acid catalysis of enol ether hydrolysis on one side and general-base catalysis of the benzisoxazole deprotonation on the other side. Inhibition of both reactions by hapten **3** implies that they take place within the same binding pocket. Mutagenesis of active-site residues on the recombinant chimeric Fab-16E7, expressed in *Escherichia coli*, shows that catalysis can be traced back to glutamate Glu^{L39} in the Fv region of the light protein chain. (Mutation of Glu^{L39} to either alanine or glutamine completely abolished activity and Glu^{L39} is in direct contact with the hapten as shown by molecular modeling.)[33] Antibody 16E7 therefore provides a clear experimental example that a general acid–base carboxyl group can indeed function both as an acid in an intrinsically acid-catalyzed process (enol ether hydrolysis) and as a base in an intrinsi-

cally base-catalyzed process (benzisoxazole deprotonation). Both reactions involve rate-limiting proton transfer between the substrate and the carboxyl group, as proved directly by the isotope-effect studies discussed above.

While this dual reactivity can be interpreted as a trivial manifestation of microscopic reversibility in catalysis, the particular conditions of each of the two reactions still deserves comment. Indeed both reactions show opposite behaviors towards solvent effects. Enol ether hydrolysis is strongly accelerated in polar protic solvents and the reaction essentially stops as soon as the water content of the medium decreases, as was studied in detail for the case of hydroxy enol ether **68** discussed above.[29] In contrast, the deprotonation of benzisoxazole **59** has been extensively discussed in the literature due to its unusual inverse solvent dependence, by which the reaction is strongly accelerated in aprotic media.[27b,34] The effect has been invoked as a key triggering factor in the benzisoxazole deprotonation by antibody 34E4 of Hilvert and co-workers, as well as in the related decarboxylation reaction of antibody 21D8[35] raised against a sulfonate hapten. The solvent effect on the benzisoxazole decomposition by carboxylates can be interpreted in a desolvation of the carboxylate base in aprotic media, which enhances its basicity.[36]

In contrast, enhancement of basicity of the catalytic glutamate Glu^{L49} side chain within the binding pocket of antibody 16E7 leading to benzisoxazole deprotonation catalysis cannot be interpreted in terms of medium effects. Indeed, any decrease in polarity or proticity would be fatal to the rate of enol ether hydrolysis. This favors an interpretation in terms of specific interactions within the antibody binding pocket, such as electrostatic stabilization of the carboxylate or a particular arrangement preventing the formation of strong hydrogen-bonding interactions except with the hapten. Such an arrangement should indeed be selected during the immune response selecting for the strongest and most selective hapten binding interactions.

Any effect resulting in an enhanced basicity of the carboxylate in antibody 16E7 should indeed enhance its reactivity for both the acid- and the base-catalyzed process. While it is clear that a higher pK_a value of the carboxylic acid increases the reactivity of the conjugate carboxylate as a base, it must be stressed that the effect also improves the acid-catalyzed process at pH values above the pK_a value of the acid, under which the present experiments were done. The rate enhancement of acid catalysis occurs because a higher pK_a value of the acid increases the effective concentration of the catalytically active carboxyl group at pH values higher than the pK_a. More precisely, this effect is more pronounced than the corresponding decrease in the reactivity of the acid for protonation.[14] The ratio of pK_a variation to the effect on catalysis is the value α, which can be interpreted as the extent of proton transfer occurring at the transition state. The α value is generally around 0.7 for enol ether protonation by carboxylic acids.[37]

Conclusion

Cationic tight-binding inhibitors of tocopherol cyclase were used as transition-state analogues to search for catalytic antibodies promoting the cyclization of the *o*-allylphenol substrate **8** and its more reactive enol ether analogues **10a**,**b** as short-chain models for the natural substrate phytyl-hydroquinone **6**. Antibody 16E7 was isolated against hapten **3** and catalyzed the reaction of enol ether **10a** to deliver the hydrolysis product hemiacetal **12**, without participation of the phenolic function. The antibody also catalyzed the ring-opening reaction of benzisoxazole **59**, thereby demonstrating its ability to handle cyclic substrates. The more difficult tocopherol cyclase like cyclization of substrate **8** remained out of reach. This might be attributed in part to the low reactivity of the system, as seen by the fact that there is no observable spontaneous reaction in aqueous buffered systems down to pH 2.0. Molecular modeling and mutational investigations with antibody 16E7 are in progress to explain the reaction mechanism and the unusual hysteresis effect in more detail.

Experimental Section

General: Reactions requiring anhydrous conditions were run under argon in vacuum- and flame-dried glassware. Diethyl ether and tetrahydrofuran were distilled from sodium/benzophenone prior to use. Dichloromethane was distilled from calcium hydride. Reagents were purchased from Fluka or Aldrich and were used as received. Reaction progress was monitored by TLC on Merck silica gel 60 F-254 with detection by UV light or by immersion in an acidic staining solution (ceric ammonium molybdate or phosphomolybdic acid). Merck silica gel 60 (40–63 μm) was used for column chromatography and flash chromatography. The following compounds were prepared as previously described: 6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline,[38] ethyl 8-bromo-octanoate,[14] 6-methoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride,[38] ethyl 8-amino-octanoate,[16] 4-methoxy-2-(3-methyl-2-butenyl)phenol,[17] 5-nitro-benzisoxazole,[27b] 2-hydroxy-5-nitrobenzonitrile,[39] 2-(2-bromo-phenoxy)tetrahydropyran.[26]

Instrumentation: Melting points were determined on a Kofler apparatus or with a Büchi 510 apparatus and are uncorrected. ^{1}H NMR and ^{13}C NMR spectra were recorded with Bruker DRX-600, Bruker DRX-500, Varian VXR-400, or Varian Gemini 300 spectrometers. Proton magnetic resonance (^{1}H NMR) spectra were recorded at 600, 500, 400, or 300 MHz. Chemical shifts are expressed in parts per million (δ scale) downfield from tetramethylsilane and are referenced to the residual protium signals in the solvent ($CDCl_3$, $\delta = 7.26$ ppm; $[D_4]$MeOH, $\delta = 3.31$ ppm; $[D_6]$DMSO, $\delta = 2.49$ ppm). Data are presented as follows: chemical shift, multiplicity (s = singlet, d = dublet, t = triplet, q = quartet, quin = quintet, m = multiplet), integration, and coupling constant (in Hz). Carbon magnetic resonance (^{13}C NMR) spectra were recorded at 150, 125, 100, or 75 MHz. Chemical shifts are referenced to the carbon signal for the solvent ($CDCl_3$, $\delta = 77.00$ ppm; $[D_4]$MeOH, $\delta = 49.00$ ppm; $[D_6]$DMSO, $\delta = 39.5$ ppm). IR spectroscopy was performed on a Perkin–Elmer 1600 FTIR apparatus. Data are presented as follows: frequency of transmission (cm^{-1}), intensity (s = strong, m = medium, w = weak). The mass spectra were determined on Varian VG-70–250 (EI), Varian MAT-312 (EI), or Finnigan Mat LCQ (ESI) spectrometers. HPLC was done on a Waters 600 controller with a Waters 996 photodiode array detector and using three different solvents: A (0.1% trifluoroacetic acid (TFA) in H_2O), B (H_2O/CH_3CN 1:1), and C (H_2O). HPLC conditions for selected compounds are given in Table 3. Preparative HPLC was performed on a Waters Prep LC and Delta Prep 4000 apparatus with a Waters 486 tunable absorbance detector.

Table 3. Analytical reversed-phase HPLC conditions for selected compounds.[a]

Compound	% A	% B	% C	t_R [min]
1[b]	50	50	0	4.3
2[b]	50	50	0	7.6
3[b]	50	50	0	6.5
18[b]	50	50	0	13.1
8[c]	0	100	0	7.3
9[c]	0	100	0	13.2
10a[c]	0	64	36	4.7
10b[c]	0	64	36	4.2
11[c]	0	64	36	7.1
12[c]	0	64	36	2.9
35[c]	0	100	0	7.1
44a[c]	0	64	36	6.9
44b[c]	0	64	36	6.5
45a[c]	0	72	28	7.7
45a[c]	0	72	28	7.0
42[c]	0	64	36	14.0
43[c]	0	72	28	15.3
50[b]	0	43	57	18.2
51[b]	0	43	57	3.1
52[b]	0	43	57	27.4
55[c]	0	30	70	5.5
56[c]	0	30	70	6.1
57[c]	0	30	70	10.6
58[c]	0	30	70	10.3

[a] Isocratic elution at 1.0 mL min^{-1}. A = 0.1 % TFA in H_2O, B = CH_3CN/H_2O (50:50), C = H_2O. [b] Analytical column: Vydac 218TP-54 (C18, pore size 300 Å), 0.45 × 22 cm, at 1.5 mL min^{-1}. [c] Analytical column: Bischoff LiChrospher 100 (C18, pore size 300 Å), 0.46 × 12.5 cm, at 1.0 mL min^{-1}.

Synthesis of the haptens

2-(7-Ethoxycarbonylheptyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium bromide (14): 6-Methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline (**13**; 530 mg, 2.99 mmol, 1.0 equiv) and ethyl 8-bromo-octanoate (750 mg, 3.00 mmol, 1.0 equiv) were dissolved in DMF (1 mL) and the resulting solution was heated for 3 h at 60 °C. After evaporation to dryness, the crude solid was purified by flash chromatography (CH_2Cl_2/CH_3OH 85:15) to give **14** (1.08 g, 2.51 mmol, 84 %) as an oil. R_f = 0.35 (CH_2Cl_2/CH_3OH 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.05 (d, ^3J(8,7) = 8.5 Hz, 1H; H-C(8)), 6.79 (dd, ^4J(7,5) = 2.5 Hz, 1H; H-C(7)), 6.72 (d, 1H; H-C(5)), 4.80 (d, ^2J(1,1) = 15.1 Hz, 1H; H-C(1)), 4.68 (d, 1H; H-C(1)), 4.22–4.11 (m, 1H; H-C(3)), 4.09 (q, ^3J($CO_2CH_2CH_3$,$CO_2CH_2CH_3$) = 7.1 Hz, 2H; $CO_2CH_2CH_3$), 3.88–3.83 (m, 1H; H-C(3)), 3.79 (s, 3H; OCH_3), 3.71–3.60 (m, 2H; H-C(1′)), 3.42 (s, 3H; NCH_3), 3.31–3.05 (m, 2H; H-C(4)), 2.26 (t, ^3J(7′,6′) = 7.4 Hz, 2H; H-C(7′)), 1.86–1.70 (m, 2H; H-C(2′)), 1.56 (quin, ^3J(6′,7′) = ^{3}J(6′,5′) = 7.4 Hz, 2H, H-C(6′)), 1.41–1.25 (m, 6H; H-C(5′), H-C(4′), H-C(3′)), 1.22 (t, 3H; $CO_2CH_2CH_3$) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 174.34 (C(8′)), 160.60 (C(6)), 130.89 (C(4a)), 129.35 (C(8)), 118.59 (C(8a)), 114.91 (C(7)), 114.08 (C(5)), 62.91 (C(1′)), 62.68 (C(1)), 60.87 ($CO_2CH_2CH_3$), 58.42 (C(3)), 56.09 (OCH_3), 48.90 (NCH_3), 34.76 (C(7′)), 29.39, 29.32, 26.71 (C(5′), C(4′), C(3′)), 25.26 (C(6′)), 24.88 (C(4)), 23.04 (C(2′)), 14.89 ($CO_2CH_2CH_3$) ppm; IR (NaCl): $\tilde{\nu}$ = 3180 (m), 2950 (s), 2860 (w), 1730 (s), 1610 (m), 1510 (s), 1460 (m), 1440 (m), 1380 (w), 1320 (m), 1250 (m), 1180 (m), 1160 (m), 1100 (s), 1030 (s), 910 (w), 860 (w) cm^{-1}; UV/Vis ($CHCl_3$): λ = 280 nm; MS (FAB): *m*/*z* (%): 348 [M^+−Br] (100), 214 (12), 176 (19), 147 (5), 58 (11); HRMS (ESI): *m*/*z*: calcd for $C_{21}H_{34}NO_3$: 348.2538; found: 348.2554 [M^+−Br].

2-(7-Carboxyheptyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinolinium trifluoroacetate (1): A solution of compound **14** (45.5 mg, 107 mmol) in DMF (10 mL) was treated with 1 N aqueous HCl (10 mL) and the reaction mixture was stirred at 25 °C for 24 h. The solution was neutralized by adding solid KOH and the resulting mixture was lyophilized. Purification by preparative HPLC gave product **1** (32.9 mg, 76.0 mmol, 71 %). R_f = 0.24 (CH_2Cl_2/CH_3OH/CH_3COOH 8:2:0.1); ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 11.92 (s, 1H; COOH), 7.10 (d, ^3J(8,7) = 8.1 Hz, 1H; H-C(8)), 6.88 (dd, ^4J(7,5) = 2.2 Hz, 1H; H-C(7)), 6.85 (d, 1H; H-C(5)); 4.50 (d, ^2J(1,1) = 15.2 Hz, 1H; H-C(1)), 4.44 (d, 1H; H-C(1)), 3.75 (s, 3H; OCH_3), 3.68–3.58 (m, 2H; H-C(3)), 3.31 (m, 2H; H-C(1′)), 3.11 (m, 2H; H-C(4)), 3.02 (s, 3H; NCH_3), 2.19 (t, ^3J(7′,6′) = 7.4 Hz, 2H; H-C(7′)), 1.80–1.70 (m, 2H; H-C(2′)), 1.52–1.46 (m, 2H; H-C(6′)), 1.37–1.21 (m, 6H; H-C(5′), H-C(4′), H-C(3′)) ppm; ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 174.92 (C(8′)), 159.40 (C(6)), 131.71 (C(4a)), 128.73 (C(8)), 119.36 (C(8a)), 114.08 (C(7)), 113.64 (C(5)), 62.77 (C(1′)), 61.14 (C(1)), 57.14 (C(3)), 55.66 (OCH_3), 47.13 (NCH_3), 34.01 (C(7′)), 28.66, 28.62 (C(5′), C(3′)), 26.07 (C(4′)), 24.75 (C(6′)), 23.88 (C(4)), 21.67 (C(2′)) ppm; IR (NaCl, CH_3Cl): $\tilde{\nu}$ = 3180 (m), 2950 (s), 2860 (w), 1730 (s), 1610 (m), 1500 (m), 1460 (m), 1440 (m), 1370 (w), 1100 (s), 1010 (s), 910 (w), 860 (w) cm^{-1}; UV/Vis (EtOH): λ = 282 nm; MS (FAB): *m*/*z* (%): 320 [M^+−CF_3COO] (100), 200 (19), 162 (26), 58 (19); HRMS (ESI): *m*/*z*: calcd for $C_{19}H_{30}NO_3$: 320.2226; found: 320.2224 [M^+−CF_3COO].

Carrier protein conjugates with hapten 1: The KLH and BSA conjugates of hapten **1** were prepared according to the previously described method.[40]

5-{[4-(Ethyl-2-one)phenyl]amino}-5-oxo-pentanoic acid ethyl ester (15): A solution of Dess–Martin periodinane (961 mg, 2.26 mmol, 2 equiv) in CH_2Cl_2 (30 mL) was added to a stirred suspension of 5-{[4-(ethyl-2-ol)-phenyl]amino}-5-oxo-pentanoic acid ethyl ester (300 mg, 1.13 mmol, 1 equiv) in CH_2Cl_2 (10 mL) at −78 °C and the resulting suspension was allowed to warm to room temperature over a period of 1 h with vigorous stirring. After 16 h, additional Dess–Martin periodinane (471.2 mg, 1.1 equiv) was added and the reaction mixture was stirred for an additional 24 h at room temperature. The mixture was then poured into a vigorously stirred mixture of a saturated solution of $NaHCO_3$ (100 mL) and a saturated solution of $Na_2S_2O_3$ (100 mL). The organic layer was separated and washed with brine (100 mL), dried (Na_2SO_4), and concentrated. The residue was purified by chromatography (EtOAc/hexane 3:1) to give pure product **15** (248.2 mg, 0.94 mmol, 83 %). R_f = 0.40 (EtOAc/hexane 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 9.71 (t, ^3J(2″,1″) = 2.4 Hz, 1H; H-C(6)), 7.52 (d, ^3J(2′,3′) = 8.0 Hz, 2H; H-C(2′)), 7.17 (d, 2H; H-C(3′)), 4.14 (q, ^3J(CH_2CH_3,CH_2CH_3) = 7.2 Hz, 2H; (OCH_2CH_3)), 3.65 (d, 2H; H-C(1″)), 2.42 (m, 4H; H-C(4),H-C(2)), 2.04 (m, 2H; H-C(3)), 1.26 (t, 3H; (OCH_2CH_3)) ppm; ^{13}C NMR (300 MHz, $CDCl_3$): δ = 199.66 (C(2“)), 173.73 (C(1)), 170.92 (C(2′)), 137.59 (C(1′)), 130.49 (C(4′)), 127.77 (C(3′)), 120.60 (C(2′)), 60.88 (OCH_2CH_3), 50.26 (C(1”)), 36.73 (C(4)), 33.50 (C(2)), 21.12 (C(3)), 14.53 (OCH_2CH_3) ppm; IR (KBr): $\tilde{\nu}$ = 3290 (s), 3130 (w), 3060 (w), 2940 (w), 2820 (w), 1740 (s), 1670 (s), 1600 (m), 1540 (s), 1410 (m), 1320 (m), 1260 (w), 1180 (m), 1170 (m), 1120 (m), 1070 (m), 1000 (s), 820 (m) cm^{-1}; UV/Vis (MeOH): λ (%) = 208 (72), 248 (100) nm; MS (EI): *m*/*z* (%): 278 (5), 277 [M^+] (26), 248 (14), 232 (32), 231 (7), 202 (12), 143 (44), 135 (31), 115 (25), 106 (100), 87 (22), 55 (16); HRMS (EI): *m*/*z*: calcd for $C_{15}H_{19}NO_4$: 277.1314; found: 277.1319 [M^+].

2-{5-[(4-Ethyl)phenylamino]-5-oxo-pentanoic acid ethyl ester}-6-methoxy-1,2,3,4-tetrahydroisoquinolium acetate (17): 6-Methoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (**16**; (803 mg, 3.17 mmol, 4 equiv) was dissolved in a 50 % aqueous K_2CO_3 solution (35 mL) and extracted with *tert*-butyl methyl ether (MTBE) (3 × 50 mL). The combined organic layers were dried (Na_2SO_4), and evaporated under reduced pressure. The residue was mixed with aldehyde **15** (220 mg, 0.793 mmol, 1 equiv), dissolved in 1,2-dichloroethane (20 mL) and then treated with sodium triacetoxyborohydride (252 mg, 1.18 mmol, 1.5 equiv). The mixture was stirred at room temperature for 30 min, then the reaction was quenched by adding aqueous saturated $NaHCO_3$ (100 mL) and the product was extracted with EtOAc (4 × 100 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by chromatography (MeOH/CH_2Cl_2 1:9) to give pure product **17** (285.0 mg, 0.588 mmol, 74 %). R_f = 0.50 (MeOH/CH_2Cl_2 1:9); m.p. 88–90 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.48 (s, 1H; NH), 7.45 (d, J(2″,3″) = 8.5 Hz, 2H; H-C(2″)), 7.18 (d, 2H; H-C(3″)), 6.96 (d, ^3J(8,7) = 8.7 Hz, 1H; H-C(8)), 6.74 (dd, ^4J(7,5) = 2.5 Hz, 1H; H-C(7)), 6.66 (d, 1H; H-C(5)), 4.15 (q, ^3J(OCH_2CH_3,OCH_2CH_3) = 7.6 Hz, 2H; (OCH_2CH_3)), 3.78 (s, 3H; (OCH_3)), 3.65 (s, 2H; H-C(1)), 3.05 (m, 4H; H-C(3), H-C(4)), 2.64 (m, 4H; (NCH_2CH_2-C(4″)), 2.42 (m, 4H; H-C(4′), H-C(2′)), 2.09 (s, 3H; CH_3COO), 2.05 (m, 2H, H-C(3′)), 1.24 (t, 3H; (OCH_2CH_3)) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 175.96 (CH_3COO), 173.79 (C(1′)), 170.92 (C(5′)), 158.72 (C(6)), 136.72 (C(1″)), 135.59 (C(3″)), 134.89 (C(4a)), 129.59 (C(2″)), 128.10 (C(8)), 125.21 (C(2″)), 120.45 (C(8a)), 113.61 (C(7)), 112.96 (C(5)), 60.91 (NCH_2CH_2-C(4″)), 59.11 (OCH_2CH_3),

55.65 (C(1)), 54.64 (CH_3O-C(6)), 50.38 (C(3)), 36.77 (C(4′)), 33.64 (NCH_2CH_2-C(4″)), 28.25 (C(4)), 22.21 (C(3′)), 21.25 (CH_3COO), 14.62 (OCH_2CH_3) ppm; IR (KBr): $\tilde{\nu}$ = 3450 (m), 3310 (m), 3020 (w), 2940 (m), 1730 (s), 1690 (s), 1660 (s), 1610 (s), 1540 (m), 1510 (s), 1410 (m), 1380 (w), 1310 (m), 1240 (s), 1160 (w), 1030 (m), 830 (w) cm^{-1}; UV/Vis (MeOH): λ (%) = 208 (100), 246 (86) nm; MS (FAB): *m/z* (%): 426 (20), 425 [M^+] (72), 424 (8), 423 (19), 192 (10), 177 (12), 176 (100), 175 (6), 162 (14), 160 (5), 146 (5), 143 (11), 134 (4), 120 (17), 115 (8), 106 (5), 55 (10); elemental analysis calcd (%) for $C_{27}H_{36}N_2O_6$: C 66.92, H 7.49, N 5.78, O 19.81; found: C 66.68, H 7.68.

2-Methyl-2-{5-[(4-ethyl)phenylamino]-5-oxo-pentanoic acid ethyl ester}-6-methoxy-1,2,3,4-tetrahydroisoquinolinium trifluoroacetate (18): Compound **17** (50 mg, 0.117 mmol) was dissolved in a 50% aqueous K_2CO_3 solution (35 mL) and extracted with TBME (3 × 50 mL). The combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure. The residue was redissolved in CH_3CN (5 mL) and then dimethylsulfate (14 μL, 0.106 mmol, 1.1 equiv) was added to this solution. The reaction mixture was stirred for 15 h at room temperature and then the solvent was evaporated under reduced pressure. The residue was purified by preparative HPLC to yield **18** (29.1 mg, 0.053 mmol, 55%). R_f = 0.35 CH_2Cl_2/CH_3OH/CH_3COOH 8:2:0.1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.45 (d, ^3J(2″,3″) = 8.6 Hz, 2H; H-C(2″)), 7.01 (d, 2H; H-C(3″)); 6.98 (d, ^3J(8,7) = 7.8 Hz, 1H; H-C(8)), 6.81 (dd, ^4J(7,5) = 2.4 Hz, 1H; H-C(7)), 6.71 (d, 1H; H-C(5)), 4.56 (d, ^2J(1,1) = 15.2 Hz, 1H; H-C(1)), 4.39 (d, 1H; H-C(1)), 4.08 (q, $^3J(OCH_2CH_3,OCH_2CH_3)$ = 7.4 Hz, 2H; (OCH_2CH_3)), 3.78 (s, 3H; (CH_3O)), 3.72 (m, 2H; H-C(3)), 3.60 (s, 3H; (NCH_3)), 3.50 (m, 2H; (NCH_2CH_2-C(4″))), 3.13 (m, 2H; H-C(4)), 2.96 (m, 2H; (NCH_2CH_2-C(4″))), 2.39 (t, ^3J(2′,3′) = 7.5 Hz, 2H; H-C(2′)), 2.34 (t, ^3J(4′,3′) = 7.4 Hz, 2H; H-C(4′)), 1.92 (m, 2H; H-C(3′)), 1.21 (t, 3H; (OCH_2CH_3)) ppm; ^{13}C NMR (400 MHz, $CDCl_3$): δ = 173.74 (C(1′)), 172.06 (C(5′)), 160.45 (C(6)), 138.35 (C(1″)), 130.34 (C(4″)), 129.60 (C(4a)), 129.05 (C(2″)), 121.20 (C(8)), 117.95 (C(8a)), 114.82 (C(7)), 113.78 (C(5)), 64.91 (NCH_2CH_2-C(4″)), 62.55 (C(1)), 60.78 (C(3)), 58.45 (OCH_2CH_3), 55.78 (CH_3O)), 47.26 (NCH_3)), 36.32 (C(4′)), 33.84 (C(2′)), 28.41 (NCH_2CH_2-C(4″)), 24.41 (C(4)), 1.19 (C(3′)), 14.56 (OCH_2CH_3)) ppm; IR ($CHCl_3$, NaCl): $\tilde{\nu}$ = 3570 (w), 3160 (w), 2990 (w), 2960 (w), 2840 (w), 2250 (s), 1790 (m), 1730 (m), 1690 (s), 1610 (m), 1560 (w), 1510 (w), 1460 (s), 1380 (s), 1320 (w), 1280 (w), 1100 (s), 1010 (m), 910 (s), 820 (w) cm^{-1}; UV/Vis (MeOH): λ (%) = 210 (82), 246 (100) nm; MS (ESI): *m/z* (%): 440 (28), 439 [M^+] (100), 305 (29); HRMS (EI): *m/z*: calcd for $C_{26}H_{35}N_2O_4$: 279.1471; found: 279.1470 [$M^+ - CF_3COO$].

2-Methyl-2-{5-[(4-ethyl)phenylamino]-5-oxo-pentanoic acid}-6-methoxy-1,2,3,4-tetrahydroisoquinolinium trifluoroacetate (2): Compound **18** was dissolved in a mixture of 4 M HCl in dioxane (2 mL) and water (250 μL) and the resulting reaction mixture was stirred for 6 h. With aqueous K_2CO_3 solution the pH value was adjusted to 7. The mixture was then purified by preparative HPLC to yield **2** (19.6 mg, 0.037 mmol, 71%). R_f = 0.22 (CH_2Cl_2/CH_3OH/CH_3COOH 8:2:0.1); ^{1}H NMR (500 MHz, [D$_4$]MeOH): δ = 7.54 (d, ^3J(2″,3″) = 8.5 Hz, 2H; H-C(2″)), 7.25 (d, 2H; H-C(3″)), 7.11 (d, ^3J(8,7) = 7.7 Hz, 1H; H-C(8)), 6.91 (m, 1H; H-C(7)), 6.89 (m, 1H; H-C(5)), 4.62 (d, ^2J(1,1) = 15.2 Hz, 1H; H-C(1)), 4.54 (d, 1H; H-C(1)), 3.81 (s, 3H; (CH_3O)), 3.78 (m, 2H; H-C(3)), 3.60 (m, 2H; NCH_2CH_2-C(4″)), 3.26 (m, 2H; H-C(4)), 3.22 (s, 3H; (NCH_3)), 3.19 (m, 2H; NCH_2CH_2-C(4″)), 2.43 (t, ^3J(2′,3′) = 7.5 Hz, 2H; H-C(2′)), 2.38 (t, ^3J(4′,3′) = 7.4 Hz, 2H; H-C(4′)), 1.96 (m, 2H; H-C(3′)) ppm; ^{13}C NMR (500 MHz, [D$_4$]MeOH): δ = 176.79 (C(1′)), 173.79 (C(5′)), 161.55 (C(6)), 139.21 (C(1″), 132.45 (C(4″)), 132.06 (C(4a)), 130.38 (C(3″)), 129.57 (C(8)), 121.74 (C(2″)), 119.42 (C(8a)), 115.28 (C(7)), 114.37 (C(5)), 65.52 (NCH_2CH_2-C(4″)), 63.04 (C(1)), 59.26 (C(3)), 55.85 (CH_3O), 47.80 (NCH_3)), 36.84 (C(4′)), 34.06 (C(2′)), 28.96 (NCH_2CH_2-C(4″)), 24.99 (C(4)), 2.05 (C(3′)) ppm; UV/Vis (MeOH): λ (%) = 208 (100), 248 (87) nm; IR ($CHCl_3$, NaCl): $\tilde{\nu}$ = 3570 (w), 3160 (w), 2990 (w), 2960 (w), 2840 (w), 2250 (s), 1790 (m), 1730 (m), 1680 (s), 1610 (m), 1560 (w), 1510 (w), 1470 (s), 1380 (s), 1320 (w), 1280 (w), 1100 (s), 1010 (m), 910 (s), 820 (w) cm^{-1}; MS (ESI): *m/z* (%): 412 (25), 411 [$M^+ - CF_3COO$] (100), 277 (21); HRMS (ESI): *m/z*: calcd for $C_{24}H_{31}N_2O_4$: 411.2284; found: 411.2286 [$M^+ - CF_3COO$].

Carrier protein conjugates of hapten 2: The KLH and BSA conjugates of hapten **2** were prepared according to the previously described method.[40]

5-Methoxy-2-nitrobenzaldehyde (20): Methyl iodide (80.0 mL, 182.4 g, 1.285 mol, 21 equiv) was added to a suspension of 5-hydroxy-2-nitrobenzaldehyde (**19**; 10.0 g, 59.8 mmol, 1.0 equiv) and Cs_2CO_3 (27.3 g, 83.7 mmol, 1.4 equiv) in DMF (60 mL). After stirring for 15 h at room temperature, the reaction was quenched by adding H_2O (300 mL) and the aqueous solution was extracted with EtOAc (7 × 60 mL). The organic layers were dried (Na_2SO_4) and evaporated, then the crude residue was purified by column chromatography (hexane/MTBE 1:1) to give **20** (10.3 g, 56.8 mmol, 96%) as a yellow solid. R_f = 0.34 (hexane/MTBE 1:1); m.p. 85 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ = 10.50 (s, 1H; CHO), 8.17 (d, ^3J(3,4) = 9.1 Hz, 1H; H-C(3)), 7.34 (d, ^4J(6,4) = 2.8 Hz, 1H; H-C(6)), 7.16 (dd, 1H; H-C(4)), 3.97 (s, 3H; OCH_3) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 188.53 (CHO), 164.00 (C(5)), 142.31 (C(2)), 134.34 (C(1)), 127.26 (C(3)), 118.68 (C(4)), 113.19 (C(6)), 56.35 (OCH_3) ppm; IR (KBr): $\tilde{\nu}$ = 3100 (w), 3030 (m), 2980 (m), 2940 (w), 1740 (s), 1580 (s), 1520 (s), 1500 (m), 1480 (m), 1440 (m), 1420(m), 1250 (m), 1160 (m), 1080 (m), 1030 (m), 930 (s), 900 (m) cm^{-1}; UV/Vis ($CHCl_3$): λ (%) = 248 (100), 322 (74) nm; MS (EI): *m/z* (%): 181 [M^+] (56), 151 (60), 123 (39), 108 (82), 106 (58), 95 (54), 63 (100); HRMS (EI): *m/z*: calcd for $C_8H_7NO_4$: 181.0375; found: 181.0377; elemental analysis calcd (%) for $C_8H_7NO_4$: C 53.04, H 3.90, N 7.73, O 35.33; found: C 52.86, H 4.05, N 7.61, O 35.59.

5-Methoxy-2-nitrobenzonitrile (21): A suspension of compound **20** (9.50 g, 52.4 mmol, 1.0 equiv), H_2NOH·HCl (4.00 g, 57.7 mmol, 1.1 equiv), $MgSO_4$ (25.2 g, 210 mmol, 4.0 equiv), and *p*-toluenesulfonic acid monohydrate (2.00 g, 10.5 mmol, 0.2 equiv) in toluene (200 mL) was stirred under reflux conditions. After stirring for 15 h, $CHCl_3$ (100 mL) was added to the warm suspension. The warm suspension was filtered and the filtrate was washed with H_2O (3 × 100 mL), dried (Na_2SO_4), and evaporated. The crude residue was purified by flash chromatography (hexane/EtOAc 1:1) to give **21** (8.21 g, 46.1 mmol, 88%) as a yellow solid. R_f = 0.34 (hexane/EtOAc 1:1); m.p. 96 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.32 (d, ^3J(3,4) = 9.2 Hz, 1H; H-C(3)), 7.33 (d, ^4J(6,4) = 2.8 Hz, 1H; H-C(6)), 7.22 (dd, 1H; H-C(4)), 3.98 (s, 3H; OCH_3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 163.67 (C(5)), 141.39 (C(2)), 127.84 (C(3)), 120.60 (C(4)), 118.14 (C(6)), 114.98 (CN), 109.87 (C(1)), 56.62 (OCH_3) ppm; IR (NaCl, $CHCl_3$): $\tilde{\nu}$ = 3120 (w), 3030 (w), 2950 (w), 2850 (w), 2210 (m), 1580 (s), 1520 (s), 1490 (m), 1460 (m), 1440 (w), 1340 (s), 1280 (s), 1130 (m), 1100 (m), 1090 (s), 1080 (m), 1030 (m), 890 (w) cm^{-1}; UV/Vis (CH_2Cl_2): λ = 314 nm; MS (EI): *m/z* (%): 178 [M^+] (100), 148 (68), 117 (65), 89 (31); elemental analysis calcd (%) for $C_8H_6N_2O_3$: C 53.94, H 3.39, N 15.72, O 26.94; found C 53.78, H 3.53, N 15.53, O 26.95.

2-Amino-5-methoxybenzonitrile (22): A mixture of compound **21** (5.73 g, 32.2 mmol, 1.0 equiv) and $SnCl_2$·H_2O (32.0 g, 141.9 mmol, 4.4 equiv) in MeOH (30 mL) was heated at 70 °C under argon. After 3 h the solution was allowed to cool and then poured into ice (400 g). The pH value was made slightly basic (pH 8) by addition of saturated aqueous $NaHCO_3$ before being extracted with ethyl acetate (3 × 60 mL). The organic phase was thoroughly washed with brine, dried (Na_2SO_4), and evaporated. The crude residue was purified by column chromatography (hexane/MTBE 1:1) to give **22** (2.67 g, 18 mmol, 56%) as a white solid. R_f = 0.30 (hexane/MTBE 1:1); m.p. 46 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.95 (dd, ^3J(4,3) = 8.9, ^4J(4,6) = 2.9 Hz, 1H; H-C(4)), 6.86 (d, 1H; H-C(6), 6.66 (d, 1H; H-C(3)), 4.15 (s, 2H; NH_2), 3.72 (s, 3H; OCH_3) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 151.69 (C(5)), 144.32 (C(2)), 122.77 (C(4)), 117.67 (CN), 117.20 (C(6)), 114.73 (C(3)), 96.20 (C(1)), 55.94 (OCH_3) ppm; IR (KBr): $\tilde{\nu}$ = 3460 (s), 3440 (s), 3370 (s), 3240 (m), 3060 (w), 3010 (w), 2920 (w), 2840 (w), 2220 (s), 1640 (m), 1620 (w), 1510 (s), 1470 (m), 1430 (m), 1310 (m), 1280 (s), 1250 (m), 1180 (m), 1160 (m), 1130 (m), 1040 (s), 930 (w), 890 (m), 810 (m) cm^{-1}; UV/Vis (CH_2Cl_2): λ (%) = 244 (100), 308 (40) nm; MS (EI): *m/z* (%): 148 [M^+] (78), 134 (15), 133 (100), 105 (32); elemental analysis calcd (%) for $C_8H_8N_2O$: C 64.85, H 5.44, N 18.91, O 10.80; found: C 64.64, H 5.44, N 18.71, O 10.91.

2-Amino-5-methoxybenzylamine (23): $LiAlH_4$ (133 mg, 3.50 mmol, 12.0 equiv) and $AlCl_3$ (467 mg, 3.50 mmol, 3.0 equiv) were suspended in dry Et_2O (15 mL) at 0 °C. After 15 min, a solution of compound **22** (173 mg, 1.17 mmol, 1.0 equiv) in Et_2O (15 mL) was added dropwise. After 5 h at room temperature, the reaction was quenched with water (60 mL) until no further evolution of hydrogen was observed. 6 N aqueous H_2SO_4 (120 mL) was then added and the mixture was stirred for a further 30 min. The grey suspension was then washed with Et_2O (3 × 50 mL). The grey aqueous layer was basicified by addition of solid KOH, which resulted in the formation of a white suspension. This suspension

was filtered over celite and the filtrate was extracted with Et_2O (4× 50 mL). The combined organic layers were dried (Na_2SO_4) and filtered, then the solvent was removed by evaporation to yield product **23** (138 mg, 0.91 mmol, 78%). R_f = 0.51 (EtOH/16% aq NH_3 17:3); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.68–6.59 (m, 3H; H-C(6), H-C(4), H-C(3)), 3.89 (s, 2H; CH_2NH_2), 3.84 (s, 3H; OCH_3), 1.80 (s, 4H; NH_2, CH_2NH_2) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 152.36 (C(5)), 139.57 (C(2)), 127.82 (C(1)), 116.81 (C(3)), 115.12 (C(6)), 113.16 (C(4)), 55.75 (OCH_3), 44.86 (CH_2NH_2) ppm; IR (NaCl, $CHCl_3$): $\tilde{\nu}$ = 3320 (w), 3000 (m), 2960 (s), 2860 (m), 2830 (m), 1600 (s), 1500 (s), 1470 (m), 1440 (m), 1380 (w), 1160 (w), 1100 (s), 1010 (m), 860 (w) cm^{-1}; UV/Vis (MeOH): λ (%) = 246 (100), 308 (41) nm; MS (EI): *m*/*z* (%): 152 [M^+] (55), 136 (75), 135 (100), 134 (41), 120 (52), 93 (18); HRMS (EI): *m*/*z*: calcd for $C_8H_{12}N_2O$: 152.0950; found: 152.0948 [M^+].

6-Methoxy-3,4-dihydro-(1*H*)-quinazoline-2-thione (24): A solution of benzylamine **23** (117 mg, 0.77 mmol, 1.0 equiv) and Et_3N (250 µL, 181 mg, 1.79 mmol, 2.3 equiv) in dry Et_2O (3.5 mL) was cooled to −78 °C. A solution of thiophosgene (70 µL, 103 mg, 0.93 mmol, 1.2 equiv) dissolved in dry Et_2O (1.2 mL) was added to this mixture. After 30 min, the heterogeneous mixture was allowed to warm to 25 °C and stirred at this temperature for an additional 9 h. The mixture was concentrated to half the original volume and the remaining solution was purified by column chromatography (CH_2Cl_2/MeOH 37:3) to give **24** (126 mg, 0.65 mmol, 84%) as a brown solid. R_f = 0.54 (CH_2Cl_2/MeOH (37:3)); m.p. 178 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 10.24 (s, 1H; H-N(1)), 8.40 (s, 1H; H-N(3)), 6.85 (d, ^3J(8,7) = 8.6 Hz, 1H; H-C(8)), 6.73 (dd, ^4J(7,5) = 2.9 Hz, 1H; H-C(7)), 6.70 (dd, 1H; H-C(5)), 4.31 (s, 2H; H-C(4)), 3.67 (s, 3H; OCH_3) ppm; ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 175.00 (C(2)), 155.16 (C(6)), 128.86 (C(4a)), 118.63 (C(8a)), 114.98 (C(8)), 113.54 (C(5)), 111.12 (C(7)), 55.28 (OCH_3), 42.94 (C(4)) ppm; IR (KBr): $\tilde{\nu}$ = 3600 (m), 3240 (s), 3020 (s), 2830 (m), 1570 (s), 1540 (s), 1380 (m), 1270 (s), 1240 (m), 1200 (s), 960 (m), 900 (m), 880 (w), 840 (w) cm^{-1}; UV/Vis (MeOH): λ (%) = 218 (49), 288 (100) nm; MS (EI): *m*/*z* (%): 194 [M^+] (100), 193 (31), 136 (59), 120 (24); HRMS (EI): *m*/*z*: calcd for $C_9H_{10}N_2OS$: 194.0514; found: 194.0515 [M^+].

2-(Ethylsulfanyl)-6-methoxy-3,4-dihydroquinazolinium bromide (25): Ethylbromide (7.0 mL) was added to a solution of compound **24** (120 mg, 0.62 mmol) in dry EtOH (5.0 mL). The mixture was stirred under reflux conditions for 14 h. The resulting solution was concentrated on the rotary evaporator, whereupon product **25** crystallized (185 mg, 0.64 mmol, 98%). R_f = 0.27 (CH_2Cl_2/MeOH 19:1); m.p. 194 °C; ^{1}H NMR (500 MHz, [D$_6$]DMSO): δ = 12.08 (s, 1H; H-N(1)), 10.24 (s, 1H; H-N(3)), 7.03 (d, ^3J(8,7) = 8.5 Hz, 1H; H-C(8)), 6.90 (dd, ^4J(7,5) = 2.8 Hz, 1H; H-C(7)), 6.85 (dd, 1H; H-C(5)), 4.72 (s, 2H; H-C(4)), 3.67 (3× s, 3H; OCH_3), 3.29 (q, ^3J(SCH_2CH_3,SCH_2CH_3) = 7.3 Hz, 2H; SCH_2CH_3), 1.31 (t, 3H; SCH_2CH_3) ppm; ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 160.40 (C(2)), 157.87 (C(6)), 125.44 (C(8a)), 119.78 (C(4a)), 117.53 (C(8)), 114.19 (C(7)), 111.69 (C(5)), 55.51 (OCH_3), 43.12 (C(4)), 25.92 (SCH_2CH_3), 14.29 (SCH_2CH_3) ppm; IR (NaCl, $CHCl_3$): $\tilde{\nu}$ = 3410 (s), 2960 (s), 2840 (w), 1600 (s), 1500 (m), 1460 (w), 1400 (w), 1260 (s), 1100 (s), 1010 (s), 865 (m), 640 (w) cm^{-1}; UV/Vis (EtOH): λ = 296 nm; MS (FAB): *m*/*z* (%): 223 [M^+] (100), 102 (51), 57 (30), 55 (29); HRMS (ESI): *m*/*z*: calcd for $C_{11}H_{15}N_2OS$: 223.0910; found: 223.0917 [M^+−Br].

2-(*N*-Aminocapryl acid ethyl ester)-6-methoxy-3,4-dihydroquinazolinium acetate (26): A solution of compound **25** (130 mg, 0.43 mmol, 1.0 equiv) and ethyl 8-amino-octanoate (160 mg, 0.86 mmol, 2.0 equiv) in dry EtOH (10.0 mL) was heated under reflux conditions for 24 h. After evaporation of the solvent, the residue was purified by flash chromatography (CH_2Cl_2/MeOH/CH_3COOH 19:1:0.2) to give **26** (115 mg, 0.28 mmol, 65%) as an oil. R_f = 0.34 (CH_2Cl_2/MeOH/CH_3COOH 19:1:0.2); ^{1}H NMR (600 MHz, $CDCl_3$): δ = 7.02 (d, ^3J(8,7) = 8.6 Hz, 1H; H-C(8)), 6.75 (dd, ^4J(7,5) = 2.7 Hz, 1H; H-C(7)), 6.52 (d, 1H; H-C(5)), 4.52 (s, 2H; H-C(4)), 4.13 (q, ^3J($CO_2CH_2CH_3$,$CO_2CH_2CH_3$) = 7.2 Hz, 2H; $CO_2CH_2CH_3$), 3.76 (s, 3H; OCH_3), 3.17 (m, 2H; H-C(2′)), 2.29 (t, ^3J(8′,7′) = 7.4 Hz, 2H; H-C(8′)), 2.07 (s, 3H; CH_3COO), 1.73–1.64 (m, 4H; H-C(7′), H-C(3′)), 1.62–1.58 (m, 2H; H-C(4′)), 1.38–1.24 (m, 4H; H-C(6′), H-C(5′)), 1.22 (t, 3H; $CO_2CH_2CH_3$) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 181.53 (CH_3COO), 173.88 (C(9′)), 156.66 (C(6)), 151.78 (C(2)), 126.58 (C(8a)), 118.60 (C(4a)), 116.94 (C(8)), 114.02 (C(7)), 111.22 (C(5)), 60.23 ($CO_2CH_2CH_3$), 55.58 (OCH_3), 42.42 (C(4)), 41.89 (C(2′)), 34.21 (C(8′)), 28.85, 28.79, 28.68 (C(6′), C(5′), C(4′)), 26.60 (CH_3COO), 26.48 (C(3′)), 24.79 (C(7′)), 14.23 ($CO_2CH_2CH_3$) ppm; IR (NaCl, $CHCl_3$): $\tilde{\nu}$ = 3190 (m), 2960 (s), 2940 (s), 2860 (m), 1720 (s), 1670 (s), 1630 (s), 1600 (w), 1510 (s), 1460 (m), 1380 (w), 1350 (w), 1170 (w), 1100 (m), 1040 (m), 870 (w) cm^{-1}; UV/Vis ($CHCl_3$): λ = 262 nm; MS (FAB): *m*/*z* (%): 348 [M^+ −CH_3COO] (100), 347 (14); HRMS (ESI): *m*/*z*: calcd for $C_{19}H_{30}N_3O_3$: 348.2287; found: 348.2302 [M^+−CH_3COO].

2-(*N*-Aminocapryl acid)-6-methoxy-3,4-dihydroquinazolinium trifluoroacetate (3): A solution of compound **26** (45.5 mg, 111 µmol) in THF (5 mL) was treated with 1 N aqueous HCl (15 mL) and the reaction mixture was stirred at 25 °C for 24 h. The solution was neutralized by adding solid KOH and the resulting mixture was lyophilized. Purification by preparative HPLC gave **3** (41.8 mg, 96.6 µmol, 87%). R_f = 0.72 (CH_2Cl_2/MeOH/CH_3COOH 24:16:0.5); ^{1}H NMR (600 MHz, [D$_4$]MeOH): δ = 7.1 (d, ^3J(8,7) = 8.6 Hz, 1H; H-C(8)), 6.85 (dd, ^4J(7,5) = 2.7 Hz, 1H; H-C(7)), 6.77 (d, 1H; H-C(5)), 4.52 (s, 2H; H-C(4)), 3.81 (s, 3H; OCH_3), 3.28 (t, ^3J(2′,3′) = 7.2 Hz, 2H; H-C(2′)), 2.29 (t, ^3J(8′,7′) = 7.4 Hz, 2H; H-C(8′)), 1.66–1.60 (m, 4H; H-C(7′), H-C(3′)), 1.42–1.28 (m, 6H; H-C(6′), H-C(5′), H-C(4′)) ppm; ^{13}C NMR (125 MHz, [D$_4$]MeOH): δ = 177.84 (C(9′)), 158.50 (C(6)), 154.87 (C(2)), 127.69 (C(8a)), 121.02 (C(4a)), 117.71 (C(8)), 115.03 (C(7)), 112.31 (C(5)), 56.06 (OCH_3), 42.77 (C4), 42.59 (C(2′)), 35.21 (C(8′)), 30.98, 30.68, 30.56 (C(6′), C(5′), C(4′)), 27.48 (C(3′)), 25.79 (C(7′)) ppm; IR (KBr): $\tilde{\nu}$ = 3440 (s), 3080 (m), 2940 (s), 2860 (m), 2800 (m), 1600 (s), 1510 (w), 1470 (m), 1380 (m), 1350 (m), 1280 (w), 1250 (w), 1120 (m), 1020 (m) cm^{-1}; UV/Vis (MeOH): λ = 258 nm; MS (FAB): *m*/*z* (%): 320 [M^+−CF_3COO] (100); HRMS (ESI): *m*/*z*: calcd for $C_{17}H_{26}N_3O_3$: 320.1974; found: 320.1961 [M^+−CF_3COO].

Carrier protein conjugates of hapten 3: The KLH and BSA conjugates of hapten **3** were prepared according to the previously described method.[40]

Synthesis of the substrates and reference compounds

4-Methoxy-2-(3-methyl-2-butenyl)phenol (8): Olefin **8** was prepared according to a known procedure.[17]

2,2-Dimethyl-6-methoxychroman (9): Olefin **8** (1.39 g, 7.23 mmol, 1.0 equiv) and BF_3·Et_2O (7.23 mmol, 1.0 equiv) in CH_2Cl_2 (5 mL) were stirred for 24 h at RT. The reaction mixture was partitioned between water (50 mL) and MTBE (50 mL). The aqueous layer was separated and extracted further with MTBE (2×50 mL). The combined organic layers were washed with 2 M aqueous HCl, water, saturated aqueous Na_2CO_3 solution, and finally water. The organic layers were dried over Na_2SO_4 and the solvent was removed by evaporation. The residue was purified by flash chromatography (hexane/EtOAc 9:1) to give **9** (1.19 g, 6.22 mmol, 86%) as a colorless oil. R_f = 0.32 (hexane/EtOAc 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.80–6.56 (m, 3H; H-C(8), H-C(7), H-C(5)), 3.75 (s, 3H; OCH_3), 2.78 (t, ^3J(3,4) = 7.2 Hz, 2H; H-C(3)), 1.77 (t, 2H; H-C(4)), 1.25 (s, 6H; $(CH_3)_2$-C(2)) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ = 152.88 (C(6)), 147.95 (C(8a), 121.45 (C(4a)), 117.73 (C(8)), 113.96 (C(5)), 113.39 (C(7)), 73.78 (C(2)), 55.72 (OCH_3), 32.83 (C(3)), 26.76 (($CH_3)_2$-C(2)), 22.83 (C(4)) ppm; IR (NaCl, $CHCl_3$): $\tilde{\nu}$ = 2980 (m), 2940 (m), 2900 (m), 2880 (m), 1500 (s), 1450 (m), 1440 (w), 1410 (w), 1310 (w), 1300 (m), 1280 (s), 1240 (m), 1200 (s), 1150 (m), 1100 (s), 1040 (s), 910 (m), 890 (s) cm^{-1}; UV/Vis ($CHCl_3$): λ = 292 nm; MS (EI): *m*/*z* (%): 193 (16), 192 [M^+] (100), 177 (20), 163 (6), 138 (11), 137 (98), 136 (74), 108 (21), 79 (6), 78 (9), 77 (11), 65 (11), 55 (5); HRMS (EI): *m*/*z*: calcd for $C_{12}H_{16}O_2$: 192.1150, found: 192.1150 [M^+]; elemental analysis calcd (%) for $C_{12}H_{16}O_2$: C 74.97, H 8.39, O 16.64; found: C 74.99, H 8.41, O 16.54.

1-Allyloxy-4-methoxybenzene (28): A solution of hydroquinone monomethyl ether (**27**; 87.0 g, 701 mmol, 1 equiv) in THF (200 mL) was added to a suspension of sodium hydride (25.2 g, 1.05 mol, 1.5 equiv) in THF (1.5 L) at 0 °C. The mixture was stirred for 30 min prior to the addition of allyl bromide (90 mL, 128.7 g, 1.06 mol, 1.5 equiv). The mixture was heated under reflux conditions for 4 h and then the reaction was quenched by addition of saturated aqueous NH_4Cl solution. The volume of the reaction was reduced to 400 mL and the remaining mixture was extracted with MTBE (5×80 mL). The organic phases were combined, washed with brine, dried (Na_2SO_4), and evaporated. The crude residue was purified by distillation at reduced pressure (114 °C/10 mbar) to afford pure **28** (109 g, 664 mmol, 95%) as a colorless oil. R_f = 0.46 (hexane/MTBE 9:1); b.p. 114 °C/10 mbar; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.88–6.81 (m, 4H; H-C(3), H-C(2)), 6.05 (ddt, ^3J(2′,3′a) = 17.3, ^3J(2′,3′b) = 10.6, ^3J(2′,1′) = 5.1 Hz, 1H; H-C(2′)), 5.40 (dq, ^2J(3′,3′) and

$^4J(3',1')=1.5$ Hz, 1H; H-C(3′)), 5.27 (dq, 1H; H-C(3′)), 4.49 (dt, 2H; H-C(1′)), 3.93 (s, 3H; OCH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=154.34$, 153.18 (C(4), C(1)), 134.04 (C(2′)), 117.86 (C(3′)), 116.16, 115.03 (C(3), C(2)), 69.95 (C(1′)), 56.12 (OCH_3) ppm; IR (NaCl, $CHCl_3$): $\tilde{\nu}=3010$ (m), 2960 (m), 2940 (m), 2910 (m), 2870 (w), 2840 (m), 1650 (w), 1590 (w), 1510 (s), 1470 (m), 1440 (m), 1430 (w), 1410 (w), 1360 (w), 1290 (m), 1110 (m), 1040 (s), 1000 (m), 930 (m), 830 (s) cm^{-1}; UV/Vis (EtOH): λ (%) = 228 (100), 290 (37) nm; MS (EI): *m/z* (%): 164 [M^+] (29), 124 (11), 123 (100), 95 (23), 41 (17), 39 (10); HRMS (EI): *m/z*: calcd for $C_{10}H_{12}O_2$: 164.0837; found: 164.0836 [M^+].

2-Allyl-4-methoxyphenol (29): Allyl phenyl ether **28** was heated at 240 °C until the starting material had been consumed. The crude residue was purified by distillation at reduced pressure (102 °C/10^{-1} mbar) to yield **29** (85.9 g, 523 mmol, 89 %) as an oil. $R_f=0.27$ (hexane/EtOAc 9:1); b.p 102 °C/10^{-1} mbar; ^1H NMR (400 MHz, $CDCl_3$): $\delta=6.78$–6.66 (m, 3H; H-C(6), H-C(5), H-C(3)), 6.01 (ddt, $^3J(2',3'a)=17.6$, $^3J(2',3'b)=9.8$, $^3J(2',1')=6.3$ Hz, 1H; H-C(2′)), 5.19–5.12 (m, 2H; H-C(3′)), 4.56 (s, 1H; OH), 3.76 (s, 3H; OCH_3), 3.38 (d, 2H; H-1′) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=154.24$ (C(4)), 148.38 (C(1)), 136.55 (C(2′)), 126.81 (C(2)), 116.97 (C(6)), 116.92 (C(3)), 116.36 (C(3′)), 113.06 (C(5)), 56.12 (OCH_3), 35.75 (C(1′)) ppm; IR (NaCl, $CHCl_3$): $\tilde{\nu}=3530$ (m), 3360 (s), 3080 (m), 3000 (s), 2980 (s), 2940 (s), 2910 (m), 2840 (s), 1850 (w), 1640 (m), 1610 (m), 1500 (s), 1470 (s), 1450 (s), 1430 (s), 1330 (s), 1280 (s), 1110 (m), 1100 (m), 1040 (s), 1000 (s), 920 (s), 870 (m), 850 (m) cm^{-1}; UV/Vis (EtOH): λ (%) = 228 (100), 294 (87) nm; MS (EI): *m/z* (%): 164 [M^+] (100), 149 (66), 91 (20), 77 (27); HRMS (EI): *m/z*: calcd for $C_{10}H_{12}O_2$: 164.0837; found: 164.0834 [M^+].

(2-Allyl-4-methoxyphenoxy)-*tert*-butyldimethylsilane (30): Imidazole (68.9 g, 1.01 mol, 2.0 equiv) and *tert*-butyldimethylsilyl chloride (100 g, 658 mmol, 1.3 equiv) were added sequentially to a solution of compound **29** (83.1 g, 506 mmol, 1.0 equiv) in DMF (350 mL). After being stirred for 1 h at room temperature, the reaction mixture was partitioned between water (600 mL) and MTBE (200 mL). The aqueous layer was separated and extracted further with MTBE (3 × 200 mL). The combined organic layers were dried (Na_2SO_4) and concentrated. The residue was purified by distillation at reduced pressure (117 °C/10^{-1} mbar) to yield **30** (135.4 g, 486 mmol, 96 %) as a colorless oil. $R_f=0.64$ (hexane/EtOAc 5:1); b.p. 117 °C/10^{-1} mbar; ^1H NMR (400 MHz, $CDCl_3$): $\delta=6.78$–6.69 (m, 2H; H-C(6), H-C(3)), 6.64 (dd, $^3J(5,6)=8.6$, $^4J(5,3)=3.3$ Hz, 1H; H-C(5)), 5.97 (ddt, $^3J(2',3'a)=17.7$, $^3J(2',3'b)=9.6$, $^3J(2',1')=6.6$ Hz, 1H; H-C(2′)), 5.11–5.04 (m, 2H; H-C(3′)), 3.90 (s, 3H; OCH_3), 3.35 (dt, $^4J(1',3')=1.5$ Hz, 2H; H-C(1′)), 1.03 (s, 9H; $SiC(CH_3)_3$), 0.21 (s, 6H; $Si(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$); $\delta=154.24$ (C(4)), 147.58 (C(1)), 137.21 (C(2′)), 131.94 (C(2)), 119.33 (C(6)), 116.12 (C(3′)), 116.07 (C(3)), 112.16 (C(5)), 55.99 (OCH_3), 35.01 (C(1′)), 26.25 ($SiC(CH_3)_3$), 18.64 ($SiC(CH_3)_3$), −3.78 ($Si(CH_3)_2$) ppm; IR (NaCl, $CHCl_3$): $\tilde{\nu}=3080$ (w), 3010 (s), 2960 (s), 2930 (s), 2900 (m), 2860 (s), 2840 (w), 1640 (m), 1610 (w), 1590 (w), 1500 (s), 1470 (s), 1430 (m), 1390 (w), 1360 (w), 1150 (m), 1100 (w), 1040 (s), 1000 (m), 950 (m), 920 (s), 880 (s), 840 (s) cm^{-1}; UV/Vis (MTBE): $\lambda=290$ nm; MS (EI): *m/z* (%): 278 [M^+] (48), 222, (26), 221 (100), 193 (31), 181 (42); HRMS (EI): *m/z*: calcd for $C_{16}H_{26}O_2Si$: 278.1702; found: 278.1703 [M^+]; elemental analysis calcd (%) for $C_{16}H_{26}O_2Si$: C 69.01, H 9.41, O 11.49, Si 10.09; found: C 68.87, H 9.63.

3-[2-(*tert*-Butyldimethylsilanyloxy)-5-methoxyphenyl]propan-1-ol (31): BH_3·THF (56.4 mL of a 1 M solution in THF, 56.4 mmol, 1.1 equiv) was added dropwise to a stirred solution of alkene **30** (14.5 g, 52.1 mmol, 1.0 equiv) in THF (200 mL) at 0 °C over a period of 15 min. After stirring for 10 min, the ice bath was removed and stirring was continued for an additional 2 h at room temperature. The reaction mixture was cooled to 0 °C and then aqueous NaOH (20 mL of a 3 M solution) and H_2O_2 (20 mL of a 30 % solution) were slowly added. After stirring for 15 min, the cooling bath was removed and stirring was continued for additional 1 h. Then the aqueous solution was extracted with MTBE (3 × 200 mL). The organic layers were dried and evaporated, then the crude residue was purified by column chromatography (hexane/EtOAc 3:1) to give **31** (13.8 g, 46.4 mmol, 89 %) as a colorless oil. $R_f=0.16$ (hexane/EtOAc 4:1); ^1H NMR (400 MHz, $CDCl_3$): $\delta=6.72$ (d, $^3J(3,4)=8.7$ Hz, 1H; H-C(3)), 6.70 (d, $^3J(6,4)=3.3$ Hz, 1H; H-C(6)), 6.61 (dd, 1H; H-C(4)), 3.92 (s, 3H; OCH_3), 3.60 (t, $^3J(3',2')=6.3$ Hz, 2H; H-C(3′)), 2.66 (t, $^3J(1',2')=7.4$ Hz, 2H; H-C(1′)), 1.90–1.85 (m, 2H; H-C(2′)), 1.71 (s, 1H; OH), 1.02 (s, 9H; $SiC(CH_3)_3$), 0.21 (s, 6H; $Si(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=154.33$ (C(5)), 147.74 (C(2)), 133.49 (C(1)), 119.51 (C(3)), 116.20 (C(6)), 111.94 (C(4)), 62.51 (C(1′)), 55.97 (OCH_3), 33.38 (C(2′)), 27.07 (C(3′)), 26.24 ($SiC(CH_3)_3$), 18.63 ($SiC(CH_3)_3$), −3.79 ($Si(CH_3)_2$) ppm; IR (NaCl): $\tilde{\nu}=3360$ (s), 2990 (s), 2900 (m), 2860 (s), 1490 (s), 1470 (s), 1420 (m), 1390 (m), 1360 (m), 1250 (s), 1220 (s), 1150 (m), 1120 (w), 1060 (s), 1030 (s), 950 (s), 900 (s), 840 (s), 800 (m) cm^{-1}; UV/Vis (MTBE): $\lambda=289$ nm; MS (EI): *m/z* (%): 296 [M^+] (15), 223 (6), 222 (22), 221 (100), 211 (13); HRMS (EI): *m/z*: calcd for $C_{16}H_{28}O_3Si$: 296.1808; found: 296.1808 [M^+]; elemental analysis calcd (%) for $C_{16}H_{28}O_3Si$: C 64.82, H 9.52, O 16.19, Si 9.47; found: C 64.60, H 9.75.

3-[2-(*tert*-Butyldimethylsilanyloxy)-5-methoxyphenyl]-propionaldehyde (32): Oxalyl chloride (1.2 mL, 1.78 g, 13.6 mmol, 1.3 equiv) was added dropwise to a solution of DMSO (2.0 mL, 2.18 g, 27.3 mmol, 2.6 equiv) in CH_2Cl_2 (100 mL) at −78 °C. After 10 min, a solution of alcohol **31** (3.09 g, 10.5 mmol, 1.0 equiv) in CH_2Cl_2 (5 mL) was added dropwise. Triethylamine (8.6 mL, 6.28 g, 62 mmol, 5.0 equiv) was added after stirring at −78 °C for 15 min and the reaction mixture was allowed to warm to room temperature. The reaction mixture was partitioned between water (300 mL) and MTBE (300 mL). The aqueous layer was separated and extracted further with MTBE (2 × 150 mL). The combined organic layers were washed with 2 M aqueous HCl, water, saturated aqueous Na_2CO_3 solution, and finally water. The organic layers were dried over Na_2SO_4 and the solvent was removed by evaporation to give **32** (2.90 g, 9.85 mmol, 94 %) as a colorless oil. An analytical sample was purified by column chromatography over basic aluminium oxide (hexane/EtOAc 12:1). $R_f=0.22$ (hexane/MTBE 9:1); ^1H NMR (400 MHz, $CDCl_3$): $\delta=9.81$ (t, $^3J(1',2')=1.5$ Hz, 1H; H-C(1′)), 6.71 (d, $^3J(3,4)=8.8$ Hz, 1H; H-C(3)), 6.69 (d, $^4J(6,4)=2.8$ Hz, 1H; H-C(6), 6.62 (dd, 1H; H-C(4)), 3.88 (s, 3H; OCH_3), 2.88 (t, $^3J(3',2')=7.9$ Hz, 2H; H-3′), 2.72 (td, 2H; H-C(2′)), 1.03 (s, 9H; $SiC(CH_3)_3$), 0.21 (s, 6H; $Si(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=202.35$ (C(1′)), 154.21 (C(5)), 147.78 (C(2)), 132.07 (C(1)), 119.35 (C(3)), 116.20 (C(6)), 112.38 (C(4)), 56.01 (OCH_3), 44.36 (C(2′)), 26.20 ($SiC(CH_3)_3$), 24.23 (C(3′)), 18.59 ($SiC(CH_3)_3$), −3.78 ($Si(CH_3)_2$) ppm; IR (NaCl): $\tilde{\nu}=2960$ (s), 2930 (s), 2860 (s), 1730 (s), 1610 (w), 1580 (w), 1500 (s), 1470 (m), 1430 (m), 1390 (w), 1360 (w), 1260 (m), 1230 (s), 1160 (m), 1120 (w), 1040 (m), 940 (m), 900 (s), 840 (m), 780 (m) cm^{-1}; UV/Vis (MTBE): $\lambda=290$ nm; MS (EI): *m/z* (%): 294 [M^+] (12), 238 (20), 237 (89), 209 (23), 208 (20), 207 (100), 181 (31); HRMS (EI): *m/z*: calcd for $C_{16}H_{26}O_3Si$: 294.1651; found: 294.1655 [M^+].

4-[2-(*tert*-Butyldimethylsilanyloxy)-5-methoxyphenyl]-butan-2-ol (33): Methyl magnesium chloride (15 mL of a 3 M solution in THF, 45 mmol, 4.5 equiv) was added dropwise to a solution of aldehyde **32** (2.91 g, 9.87 mmol, 1.0 equiv) in anhydrous THF (50 mL). After stirring for 1 h at room temperature, saturated aqueous NH_4Cl solution (200 mL) was added. The aqueous mixture was extracted with MTBE (3 × 150 mL) and the combined organic layers were dried over Na_2SO_4. The solvent was removed by evaporation and the residue was purified by flash chromatography (hexane/EtOAc 4:1) to give **33** (2.73 g, 8.81 mmol, 89 %) as a colorless oil. $R_f=0.19$ (hexane/EtOAc 4:1); ^1H NMR (400 MHz, $CDCl_3$): $\delta=6.72$ (d, $^3J(3,4)=8.8$ Hz, 1H; H-C(3)), 6.70 (d, $^3J(6,4)=3.1$ Hz, 1H; H-C(6)), 6.61 (dd, 1H; H-C(4)), 3.76 (s, 3H; OCH_3), 3.72 (tq, $^3J(2',3')$ and $^3J(2',1')=6.3$ Hz, 1H; H-C(2′)), 2.76–2.59 (m, 2H; H-C(4′)), 2.66 (t, $^3J(1',2')=7.4$ Hz, 2H; H-C(1′)), 1.94 (s, 1H; HO-C(2′)), 1.75–1.68 (m, 2H; H-C(3′)), 1.19 (d, 3H; H-C(1′)), 1.02 (s, 9H; $SiC(CH_3)_3$), 0.21 (s, 6H; $Si(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta=154.38$ (C(5)), 147.65 (C(2)), 133.69 (C(1)), 119.58 (C(3)), 116.10 (C(6)), 111.90 (C(5)), 67.61 (C(2′)), 55.98 (OCH_3), 40.05 (C(3′)), 27.13 (C(4′)), 26.26 ($SiC(CH_3)_3$), 23.71 (C(1′)), 18.66 ($SiC(CH_3)_3$), −3.79 ($Si(CH_3)_2$) ppm; IR (NaCl, $CHCl_3$): $\tilde{\nu}=3600$ (s), 3490 (s), 3010 (s), 2960 (s), 2940 (s), 2860 (s), 2840 (s), 1600 (m), 1580 (m), 1490 (s), 1460 (s), 1420 (m), 1390 (w), 1370 (w), 1150 (m), 1120 (w), 1060 (s), 1040 (s), 940 (s), 900 (s), 860 (s), 840 (m) cm^{-1}; UV/Vis (MTBE): $\lambda=290$ nm; MS (EI): *m/z* (%): 310 [M^+] (21), 235 (15), 211 (100), 193 (33); HRMS (EI): *m/z*: calcd for $C_{17}H_{30}O_3Si$: 310.1964; found: 310.1968 [M^+].

4-[2-(*tert*-Butyldimethylsilanyloxy)-5-methoxyphenyl]-butan-2-one (34): Oxalyl chloride (570 μL, 864 mg, 6.8 mmol, 1.4 equiv) was added dropwise to a solution of DMSO (1.0 mL, 1.09 g, 13.6 mmol, 2.8 equiv) in CH_2Cl_2 (50 mL) at −78 °C. After 10 min, a solution of alcohol **33** (1.51 g, 4.86 mmol, 1.0 equiv) in CH_2Cl_2 (5 mL) was added dropwise. After stirring at −78 °C for 15 min, triethylamine (4.0 mL, 2.95 g, 29.1 mmol,

6.0 equiv) was added and the reaction mixture was allowed to warm to room temperature. The reaction mixture was partitioned between water (300 mL) and MTBE (300 mL). The aqueous layer was separated and extracted further with MTBE (2×150 mL). The combined organic layers were washed with 2 M aqueous HCl, water, saturated aqueous Na_2CO_3 solution, and finally water. The organic layers were dried over Na_2SO_4, and the solvent was removed by evaporation. The residue was purified by flash chromatography (hexane/MTBE 4:1) to give **34** (1.42 g, 4.59 mmol, 95%) as a colorless oil. R_f=0.22 (hexane/MTBE 4:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.70 (d, ^3J(3,4)=8.8 Hz, 1H; H-C(3)), 6.69 (d, ^4J(6,4)=3.0 Hz, 1H; H-C(6)), 6.61 (dd, 1H; H-C(4)), 3.86 (s, 3H; OCH_3), 2.81 (m, 2H; H-C(4′)), 2.72 (m, 2H; H-C(3′)), 2.02 (s, 3H; H-C(1′)), 1.04 (s, 9H; $SiC(CH_3)_3$), 0.21 (s, 6H; $Si(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ=208.59 (C(2′)), 154.19 (C(5)), 147.78 (C(2)), 132.74 (C(1)), 119.31 (C(3)), 116.18 (C(6)), 112.23 (C(4)), 56.00 (OCH_3), 44.08 (C(3′)), 30.33 (C(1′)), 26.18 ($SiC(CH_3)_3$), 25.84 (C(4′)), 18.56 ($SiC(CH_3)_3$), −3.79 ($Si(CH_3)_2$) ppm; IR (NaCl, $CHCl_3$): $\tilde{\nu}$=3010 (m), 2960 (s), 2930 (s), 2860 (s), 2840 (w), 1710 (s), 1610 (m), 1580 (m), 1490 (s), 1470 (m), 1440 (w), 1430 (w), 1360 (m), 1160 (m), 1120 (w), 1060 (m), 1030 (s), 940 (s), 890 (s), 840 (m) cm^{-1}; UV/Vis ($CHCl_3$): λ= 288 nm; MS (EI): *m*/*z* (%): 308 [M^+] (8), 251 (100), 235 (15), 207 (36), 181 (28); HRMS (EI): *m*/*z*: calcd for $C_{17}H_{28}O_3Si$: 308.1808; found: 308.1812 [M^+]; elemental analysis calcd (%) for $C_{17}H_{28}O_3Si$: C 66.19, H 9.15, O 15.56, Si 9.10; found: C 66.14, H 9.36.

4-Methoxy-2-(3-methylbut-3-enyl)phenol (35): *n*BuLi (1.7 mL of a 1.6 M *n*BuLi solution in hexane, 2.75 mmol, 2.2 equiv) was added to a suspension of methyltriphenylphosphonium bromide (893 mg, 2.5 mmol, 2 equiv) in THF (50 mL) at 0°C. The mixture was stirred for 15 min at room temperature and then ketone **34** (385 mg, 1.25 mmol, 1.0 equiv) dissolved in THF (5 mL) was added dropwise. After stirring for 60 min, the reaction was quenched with water (100 mL) and extracted with MTBE (3×50 mL). The combined organic layers were dried over Na_2SO_4 and and the solvent was removed by evaporation. The residue was purified by flash chromatography (hexane/MTBE 9:1) to yield 4-[2-(*tert*-butyldimethylsilanyloxy)-5-methoxyphenyl]-2-methyl-but-1-enyl (287 mg, 937 μmol, 75%) as an oil. R_f=0.45 (hexane/MTBE 4:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.73 (d, ^4J(6,4)=3.0 Hz, 1H; H-C(6)), 6.70 (d, ^3J(3,4)=8.8 Hz, 1H; H-C(3)), 6.61 (dd, 1H; H-C(4)), 4.71 (s, 2H; H-C(1′)), 3.86 (s, 3H; OCH_3), 2.71 (t, ^3J(4′,3′)=8.3 Hz, 2H; H-(4′)), 2.72 (t, 2H; H-C(3′)), 1.83 (s, 3H; $CH_3C(2')$), 1.02 (s, 9H; $SiC(CH_3)_3$), 0.23 (s, 6H; $Si(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ=154.12 (C(5)), 147.74 (C(2)), 146.15 (C(2′)), 132.07 (C(1)), 119.20 (C(3)), 116.06 (C(6)), 111.67 (C(4)), 110.25 (C(1′)), 55.99 (OCH_3), 38.34 (C(3′)), 29.64 (C(4′)), 26.22 ($SiC(CH_3)_3$), 23.09 ($CH_3C(2')$), 18.61 ($SiC(CH_3)_3$), −3.79 ($Si(CH_3)_2$) ppm; IR (NaCl, $CHCl_3$): $\tilde{\nu}$=3090 (w), 3000 (w), 2940 (m), 2830 (m), 1660 (m), 1610 (w), 1500 (s), 1460 (m), 1440 (m), 1430 (m), 1380 (w), 1340 (w), 1260 (m), 1150 (m), 1100 (w), 1040 (s), 890 (s) cm^{-1}; UV/Vis (MeOH): λ=288 nm; MS (EI): *m*/*z* (%): 306 [M^+] (100), 251 (33), 249 (31), 207 (67), 193 (83); HRMS (EI): *m*/*z*: calcd for $C_{18}H_{30}O_2Si$: 306.2015; found: 306.2012 [M^+]. A solution of tetrabutylammonium fluoride (250 μL of a 1 M TBAF in THF, 250 μmol, 1.3 equiv) was added to a solution of 4-[2-(*tert*-butyldimethylsilanyloxy)-5-methoxyphenyl]-2-methyl-but-1-enyl (59.1 mg, 192 μmol, 1 equiv) in THF (2 mL) at 0°C. After being stirred at 0°C for 20 min, the reaction mixture was partitioned between saturated aqueous $NaHCO_3$ solution (20 mL) and MTBE (20 mL). The aqueous layer was separated and extracted further with MTBE (2×20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated. The oily residue was purified by flash chromatography (hexane/MTBE 2:1) to give product **35** (34.4 mg, 179 μmol, 93%) as a colorless oil. R_f=0.33 (hexane/MTBE 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.75 (d, ^4J(3,5)=3.0 Hz, 1H; H-C(3)), 6.71 (d, ^3J(6,5)=8.8 Hz, 1H; H-C(6)), 6.59 (dd, 1H; H-C(5)), 4.81 (s, 2H; H-C(4′)), 4.40 (s, 1H; OH), 3.77 (s, 3H; OCH_3), 2.73 (t, ^3J(1′,2′)=8.2 Hz, 2H; H-(1′)), 2.42 (t, 2H; H-C(2′)), 1.83 (s, 3H; $CH_3C(3')$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ= 154.13 (C(4)), 147.83 (C(1)), 146.10 (C(3′)), 129.84 (C(2)), 116.34 (C(6)), 116.22 (C(3)), 112.21 (C(5)), 110.69 (C(4′)), 56.14 (OCH_3), 38.14 (C(2′)), 29.24 (C(1′)), 23.06 ($CH_3C(3')$) ppm; IR (NaCl, $CHCl_3$): $\tilde{\nu}$=3600 (m), 3350 (m), 3080 (w), 3010 (w), 2940 (m), 2840 (m), 1650 (m), 1610 (w), 1510 (s), 1470 (m), 1450 (m), 1430 (m), 1380 (w), 1330 (w), 1260 (m), 1150 (m), 1100 (w), 1040 (s), 890 (s) cm^{-1}; UV/Vis ($CHCl_3$): λ=292 nm; MS (EI): *m*/*z* (%): 192 [M^+] (60), 137 (100), 109 (10); HRMS (EI): *m*/*z*: calcd for $C_{12}H_{16}O_2$: 192.1150; found: 192.1146 [M^+].

3-[2-(*tert*-Butyldimethylsilanyloxy)-5-methoxyphenyl]-1,1-dimethoxypropane (36): Aldehyde **32** (2.78 g, 9.49 mmol, 1.0 equiv) was treated with methanol (30 mL), trimethyl orthoformate (15 mL), and (±)-camphorsulfonic acid (175 mg, 0.76 mmol, 8 mol%) at 45°C for 30 min. The mixture was evaporated to dryness and the crude solid was purified by flash chromatography (hexane/MTBE 85:15) to yield **36** (2.75 g, 8.09 mmol, 85%) as an oil. R_f=0.26 (hexane/MTBE 9:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ= 6.71 (d, ^4J(6,4)=3.2 Hz, 1H; H-C(6)), 6.69 (d, ^3J(3,4)=8.8 Hz, 1H; H-C(3)), 6.61 (dd, 1H; H-C(4)), 4.37 (t, ^3J(1′,2′)=5.8 Hz, 1H; H-(C1′)), 3.88 (s, 3H; OCH_3), 3.33 (s, 6H; $(CH_3O)_2C(1')$), 2.65–2.55 (m, 2H; H-C(3′)), 1.88 (td, ^3J(2′,3′)=5.8 Hz, 2H; H-C(2′)), 1.01 (s, 9H; $SiC(CH_3)_3$), 0.20 (s, 6H; $Si(CH_3)_2$) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ=153.71 (C(5)), 147.41 (C(2)), 133.04 (C(1)), 118.93 (C(3)), 115.67 (C(6)), 111.53 (C(4)), 104.22 (C(1′)), 55.60 (OCH_3), 52.79 ($CH_3O)_2C(1')$), 32.55 (C(2′)), 26.05 (C(3′)), 25.84 ($SiC(CH_3)_3$), 18.24 ($SiC(CH_3)_3$), −4.10 ($Si(CH_3)_2$) ppm; IR (NaCl, $CHCl_3$): $\tilde{\nu}$=3010 (s), 2960 (s), 2930 (s), 2900 (s), 2860 (s), 2830 (m), 1610 (w), 1580 (w), 1500 (s), 1470 (s), 1450 (m), 1430 (w), 1390 (w), 1360 (w), 1160 (m), 1130 (s), 1080 (s), 1050 (s), 940 (m), 900 (s), 840 (s) cm^{-1}; UV/Vis (CH_3OH): λ (%)=222 (100), 288 (68) nm; MS (EI): *m*/*z* (%): 340 [M^+] (39), 251 (28), 225 (69), 196 (22), 195 (100), 89 (24), 75 (20); elemental analysis calcd (%) for $C_{18}H_{32}O_4Si$: C 63.49, H 9.47, O 18.79, Si 8.25; found: C 63.53, H 9.41.

3-[2-(*tert*-Butyldimethylsilanyloxy)-5-methoxyphenyl]-1,1-diethoxypropane (37): Aldehyde **32** (2.49 g, 8.46 mmol, 1.0 equiv) was treated with ethanol (30 mL), triethyl orthoformate (15 mL), and (±)-camphorsulfonic acid (181 mg, 0.78 mmol, 9 mol%) at 45°C for 30 min. The mixture was evaporated to dryness and the crude solid was purified by flash chromatography (hexane/MTBE 9:1) to yield **37** (2.34 g, 6.36 mmol, 76%) as an oil. R_f=0.21 (hexane/MTBE 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ= 6.73 (d, ^4J(6,4)=3.1 Hz, 1H; H-C(6)), 6.70 (d, ^3J(3,4)=8.5 Hz, 1H; H-C(3)), 6.63 (dd, 1H; H-C(4)), 4.73 (t, ^3J(1′,2′)=5.7 Hz, 1H; H-C(1′)), 3.78 (s, 3H; OCH_3), 3.74–3.46 (m, 4H; $C(OCH_2CH_3)_2$), 2.70–2.60 (m, 2H; H-C(3′)), 1.98–1.86 (m, 2H; H-C(2′)), 1.24 (t, ^3J($C(OCH_2CH_3)_2$,-$C(OCH_2CH_3)_2$)=5.7 Hz, 6H; $C(OCH_2CH_3)_2$), 1.04 (s, 9H; $SiC(CH_3)_3$), 0.24 (s, 6H; $Si(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ=154.10 (C(5)), 147.79 (C(2)), 133.59 (C(1)), 119.29 (C(3)), 115.93 (C(6)), 111.83 (C(4)), 103.08 (C(1′)), 61.46 ($C(OCH_2CH_3)_2$), 55.98 (OCH_3), 33.93 (C(2′)), 26.54 (C(3′)), 26.29 ($SiC(CH_3)_3$), 18.62 ($SiC(CH_3)_3$), 15.79 ($C(OCH_2CH_3)_2$), −4.16 ($Si(CH_3)_2$) ppm; IR (NaCl, $CHCl_3$): $\tilde{\nu}$=3010 (s), 2960 (s), 2930 (s), 2900 (s), 2880 (m), 1610 (m), 1580 (m), 1490 (s), 1470 (s), 1440 (m), 1420 (w), 1390 (w), 1370 (m), 1340 (w), 1130 (s), 1060 (s), 1040 (s), 1000 (m), 940 (m), 900 (s), 840 (s) cm^{-1}; UV/Vis (MeOH): λ (%)=222 (100), 288 (62) nm; MS (EI): *m*/*z* (%): 368 [M^+] (36), 252 (17), 251 (26), 239 (21), 195 (100), 73 (29); elemental analysis calcd (%) for $C_{20}H_{36}O_4Si$: C 65.17, H 9.84, O 17.36, Si 7.62; found: C 64.91, H 9.61.

3-[2-(*tert*-Butyldimethylsilanyloxy)-5-methoxyphenyl]-1,1-diisopropoxypropane (38): Aldehyde **32** (895 mg, 3.03 mmol, 1.0 equiv) was treated with isopropanol (10 mL), triisopropyl orthoformate (5 mL), and (±)-camphorsulfonic acid (60.0 mg, 0.26 mmol, 10 mol%) at 45°C for 50 min. The mixture was evaporated to dryness and the crude solid was purified by flash chromatography (hexane/MTBE 19:1) to yield **38** (985 mg, 2.49 mmol, 83%) as an oil. R_f=0.52 (hexane/MTBE 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.71 (d, ^4J(6,4)=3.1 Hz, 1H; H-C(6)), 6.68 (d, ^3J(3,4)=8.6 Hz, 1H; H-C(3)), 6.60 (dd, 1H; H-C(4)), 4.58 (t, ^3J(1′,2′)= 5.6 Hz, 1H; H-C(1′)), 3.87 (septet, ^3J($OCH(CH_3)_2$,$OCH(CH_3)_2$)=6.2 Hz, 2H; $OCH(CH_3)_2$), 3.78 (s, 3H; OCH_3), 2.64–2.58 (m, 2H; H-C(3′)), 1.90–1.83 (m, 2H; H-C(2′)), 1.19 (d, 3H; $OCH(CH_3)_2$), 1.13 (d, 3H; $OCH(CH_3)_2$), 1.02 (s, 9H; $SiC(CH_3)_3$), 0.20 (s, 6H; $Si(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ=154.10 (C(5)), 147.81 (C(2)), 133.83 (C(1)), 119.23 (C(3)), 115.94 (C(6)), 111.72 (C(4)), 100.51 (C(1′)), 67.99 ($OCH(CH_3)_2$), 55.98 (OCH_3), 35.65 (C(2′)), 26.74 (C(3′)), 26.27 ($SiC(CH_3)_3$), 23.85, 23.03 ($OCH(CH_3)_2$), 18.62 ($SiC(CH_3)_3$), −3.78 ($Si(CH_3)_2$) ppm; IR (NaCl): $\tilde{\nu}$=2970 (s), 2930 (s), 2900 (m), 2860 (m), 2840 (w), 1610 (w), 1580 (w), 1490 (s), 1460 (m), 1380 (m), 1370 (m), 1330 (w), 1250 (m), 1220 (s), 1180 (w), 1160 (m), 1130 (m), 1030 (s), 990 (w), 950 (m), 890 (m), 840 (s), 800 (m) cm^{-1}; UV/Vis (MeOH): λ (%)= 225 (100), 286 (68) nm; MS (EI): *m*/*z* (%): 396 [M^+] (58), 336 (25), 293 (63), 251 (44), 237 (100), 211 (34), 193 (20), 75 (38); elemental analysis

calcd (%) for $C_{22}H_{40}O_4Si$: C 66.62, H 10.16, O 16.14, Si 7.08; found: C 66.41, H 10.09.

(*E*,*Z*)-[2-(3-Methoxy-2-buten-1-yl)-4-methoxyphenoxy]-*tert*-butyldimethylsilane (39): Compound **36** (2.58 g, 7.59 mmol, 1 equiv) and *N*-ethyldiisopropylamine (1.8 mL, 1.38 g, 10.63 mmol, 1.4 equiv) were dissolved in CH_2Cl_2 (50 mL). After the solution was cooled to −78 °C, TMSOTf (1.6 mL, 2.03 g, 9.11 mmol, 1.2 equiv) was added dropwise through a syringe with stirring. The pale yellow mixture was stirred at −20 °C for 4 h. The reaction was quenched by the addition of a saturated aqueous $NaHCO_3$ solution (200 mL) and the aqueous layer was separated and extracted further with MTBE (2×50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated. The crude solid was purified by flash chromatography (hexane/MTBE 47:3) to yield **39** (1.37 g, 4.48 mmol, 59%) as a yellow oil. R_f=0.30 (hexane/MTBE 47:3); ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.77 (d, ^4J(*Z*-3,*Z*-5)=3.3 Hz, 1H; H-C(*Z*-3)), 6.73 (d, ^4J(*E*-3,*E*-5)=3.1 Hz, 1H; H-C(*E*-3)), 6.69 (d, ^3J(*E*-6,*E*-5)=8.6 Hz, 1H; H-C(*E*-6)), 6.68 (d, ^3J(*Z*-6,*Z*-5)=8.6 Hz, 1H; H-C(*Z*-6)), 6.62–6.56 (m, 2H; H-C(*E*-5), H-C(*Z*-5)), 6.37 (d, ^3J(*E*-3′,*E*-2′)=12.6 Hz, 1H; H-C(*E*-3′)), 6.02 (d, ^3J(*Z*-3′,*Z*-2′)=6.3 Hz, 1H; H-C(*Z*-3′)), 4.88 (td, ^3J(*E*-2′,*E*-1′)=7.3 Hz, 1H; H-C(*E*-2′)), 4.52 (td, ^3J(*Z*-2′,*Z*-1′)=7.3 Hz, 1H; H-C(*Z*-2′)), 3.75 (s, 3H; *E*-(C(4)-CH_3)), 3.74 (s, 3H; *Z*-(C(4)-OCH_3)), 3.62 (s, 3H; *E*-(C(3′)-OCH_3)), 3.52 (s, 3H; *Z*-(C(3′)-OCH_3)), 3.38 (d, 2H; H-C(*Z*-1′)), 3.22 (d, 2H; H-C(*E*-1′)), 1.01 (s; 9H; *E*-($SiC(CH_3)_3$), *Z*-($SiC(CH_3)_3$)), 0.19 (s, 9H, *E*-($SiC(CH_3)_3$), *Z*-($SiC(CH_3)_3$)) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ=154.303 (C(*E*-4), C(*Z*-4)), 147.52 (C(*E*-1), C(*Z*-1)), 147.07 (C(*E*-3′), C(*Z*-3′)), 133.52 (C(*E*-2), C(*Z*-2)), 119.25 (C(*Z*-6)), 119.24 (C(*E*-6)), 115.86 (C(*E*-3), C(*Z*-3)), 111.77 (C(*E*-5)), 111.66 (C(*Z*-5)), 105.24 (C(*Z*-2′)), 102.10 (C(*E*-2′)), 59.9 (*Z*-(C(3′)-OCH_3)), 56.28 (*E*-(C(3′)-OCH_3)), 55.98 (*E*-(C(4)-OCH_3)), 55.96 (*Z*-(C(4)-OCH_3)), 28.81 (C(*E*-1′)), 26.26 (*E*-($SiC(CH_3)_3$), *Z*-($SiC(CH_3)_3$)), 25.15 (C(*Z*-1′)), 18.99 (*E*-$SiC(CH_3)_3$), *Z*-$SiC(CH_3)_3$)), −3.80 (*E*-($Si(CH_3)_2$), *Z*-($Si(CH_3)_2$)) ppm; IR (NaCl): $\tilde{\nu}$=2960 (s), 2930 (s), 2910 (w), 2860 (m), 2830 (w), 1650 (w), 1600 (w), 1560 (w), 1500 (s), 1470 (m), 1460 (m), 1430 (w), 1390 (w), 1370 (w), 1150 (m), 1110 (s), 1040 (w), 1010 (w), 940 (m), 900 (m), 840 (s) cm^{-1}; UV/Vis ($CHCl_3$): λ= 290 nm; MS (EI): m/z (%): 308 [M^+] (63), 251 (51), 236 (16), 219 (45), 195 (22), 145 (25), 89 (100), 59 (22); HRMS (EI): *m*/*z*: calcd for $C_{17}H_{28}O_3Si$: 308.1808; found: 308.1811 [M^+]; elemental analysis calcd (%) for $C_{17}H_{28}O_3Si$: C 66.19, H 9.15, O 15.56, Si 9.10; found: C 66.34, H 9.25.

(*E*,*Z*)-[2-(3-Ethoxy-2-buten-1-yl)-4-methoxyphenoxy]-*tert*-butyldimethylsilane (40): Compound **37** (2.10 g, 5.70 mmol, 1 equiv) and *N*-ethyldiisopropylamine (1.4 mL, 1.03 g, 7.98 mmol, 1.4 equiv) were disolved in CH_2Cl_2 (50 mL). After the solution was cooled to −78 °C, TMSOTf (1.2 mL, 1.52 g, 6.84 mmol, 1.2 equiv) was added dropwise through a syringe with stirring. The pale yellow solution was stirred at −20 °C for 4 h. The reaction was quenched by the addition of a saturated aqueous $NaHCO_3$ solution (200 mL) and the aqueous layer was separated and extracted further with MTBE (2×50 mL). The combined organic layers were dried over Na_2SO_4 and concentrated. The crude solid was purified by flash chromatography (hexane/MTBE 19:1) to yield **40** (1.158 g, 3.59 mmol, 63%) as a yellow oil. R_f=0.40 (hexane/MTBE 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.79 (d, ^4J(*Z*-3,*Z*-5)=3.0 Hz, 1H; H-C(*Z*-3)), 6.74 (d, ^4J(*E*-3,*E*-5)=3.0 Hz, 1H; H-C(*E*-3)), 6.69 (d, ^3J(*E*-6,*E*-5)=8.6 Hz, 1H; H-C(*E*-6)), 6.68 (d, ^3J(*Z*-6,*Z*-5)=8.6 Hz, 1H; H-C(*Z*-6)), 6.62–6.56 (m, 2H; H-C(*E*-5), H-C(*Z*-5)), 6.30 (d, ^3J(*E*-3′,*E*-2′)=12.7 Hz, 1H; H-C(*E*-3′)), 6.05 (d, ^3J(*Z*-3′,*Z*-2′)=6.1 Hz, 1H; H-C(*Z*-3′)), 4.92 (td, ^3J(*E*-2′,*E*-1′)=7.3 Hz, 1H; H-C(*E*-2′)), 4.53 (td, ^3J(*Z*-2′,*Z*-1′)=7.3 Hz, 1H; H-C(*Z*-2′)), 3.81 (q, ^3J(OCH_2CH_3,OCH_2CH_3)=7.1 Hz, 2H; *Z*-(OCH_2CH_3)), 3.75 (s, 3H; *E*-(OCH_3)), 3.74 (s, 3H; *Z*-(OCH_3)), 3.72 (q, ^3J(OCH_2CH_3,OCH_2CH_3)=7.1 Hz, 2H; *E*-(OCH_2CH_3)), 3.38 (d, 2H; H-C(*Z*-1′)), 3.19 (d, 2H; H-C(*E*-1′)), 1.27 (t, 6H; *E*-(OCH_2CH_3), *Z*-(OCH_2CH_3)), 1.01 (s, 6H; *E*-($SiC(CH_3)_3$), *Z*-($SiC(CH_3)_3$)), 0.19 (s, 12H; *E*-($SiC(CH_3)_3$), *Z*-($SiC(CH_3)_3$)) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ=154.25 (C(*E*-4), C(*Z*-4)), 147.59 (C(*E*-1), C(*Z*-1)), 145.77 (C(*E*-3′), C(*Z*-3′)), 133.58 (C(*E*-2), C(*Z*-2)), 119.25 (C(*Z*-6)), 119.21 (C(*E*-6)), 115.79 (C(*E*-3), C(*Z*-3)), 111.76 (C(*E*-5)), 111.62 (C(*Z*-5)), 105.22 (C(*Z*-2′)), 102.41 (C(*E*-2′)), 67.98 (*Z*-(OCH_2CH_3)), 65.00 (*E*-(OCH_2CH_3)), 56.02 (*E*-(OCH_3)), 55.97 (*Z*-(OCH_3)), 28.84 (C(*E*-1′)), 26.25 (*E*-($SiC(CH_3)_3$), *Z*-($SiC(CH_3)_3$)), 25.17 (C(*Z*-1′)), 18.64 (*E*-$SiC(CH_3)_3$), *Z*-$SiC(CH_3)_3$)), 15.71 (*Z*-(OCH_2CH_3)), 15.17 (*E*-(OCH_2CH_3)), −3.80 (*E*-($Si(CH_3)_2$), *Z*-($Si(CH_3)_2$)) ppm; IR (NaCl): $\tilde{\nu}$=3030 (w), 2960 (s), 2930 (s), 2900 (w), 2860 (m), 2830 (w), 1660 (m), 1610 (w), 1590 (w), 1500 (s), 1470 (m), 1440 (m), 1390 (w), 1370 (w), 1360 (w), 1250 (s), 1220 (s), 1150 (m), 1110 (s), 1040 (m), 950 (m), 900 (s), 840 (s), 800 (m), 780 (s) cm^{-1}; UV/Vis (MeOH): λ=288 nm; MS (EI): *m*/*z* (%): 322 [M^+] (73), 237 (66), 236 (41), 209 (72), 207 (46), 181 (100), 161 (19); HRMS (EI): *m*/*z*: calcd for $C_{18}H_{30}O_3Si$: 322.1964, found: 322.1959 [M^+]; elemental analysis calcd (%) for $C_{18}H_{30}O_3Si$: C 67.03, H 9.38, O 14.88, Si 8.71; found: C 67.29, H 9.52.

(*E*,*Z*)-[2-(3-Propoxy-2-buten-1-yl)-4-methoxyphenoxy]-*tert*-butyldimethylsilane (41): Compound **38** (165 mg, 416 μmol, 1 equiv) was dissolved in CCl_4 (5 mL) and treated at −10 °C with anhydrous hexamethyldisilazane (140 μL, 86.7 mg, 666 μmol, 1.6 equiv) and iodotrimethylsilane (74 μL, 108 mg, 540 μmol, 1.3 equiv). After stirring for 1 h at −10 °C, the mixture was heated at 75 °C for an additional 15 h. The reaction was quenched by the addition of a saturated aqueous $NaHCO_3$ solution (15 mL) and the aqueous layer was separated and extracted further with CH_2Cl_2 (2×20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated. The crude solid was purified by flash chromatography (hexane/MTBE 48:2) to yield **41** (40.5 mg, 120 μmol, 29%) as a yellow oil. R_f=0.25 and 0.22 (hexane/MTBE 96:4) for *Z*-**41** and *E*-**41**, respectively; ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.80–6.56 (m, 6H; H-C(*E*-6), H-C(*Z*-6), H-C(*E*-5), H-C(*Z*-5), H-C(*E*-3), H-C(*Z*-3)), 6.15 (d, ^3J(*E*-3′,*E*-2′)=12.4 Hz, 1H; H-C(*E*-3′)), 6.09 (d, ^3J(*Z*-3′,*Z*-2′)=6.1 Hz, 1H; H-C(*Z*-3′)), 5.00 (dt, ^3J(*E*-2′,*E*-1′)=7.3 Hz, 1H; H-C(*E*-2′)), 4.54 (td, ^3J(*Z*-2′,*Z*-1′)=7.3 Hz, 1H; H-C(*Z*-2′)), 3.97 (septet, ^3J($OCH(CH_3)_2$,$OCH(CH_3)_2$)= 6.2 Hz, 1H; *Z*-($OCH(CH_3)_2$)), 3.92 (septet, ^3J($OCH(CH_3)_2$,$OCH(CH_3)_2$)=6.2 Hz, 1H; *E*-($OCH(CH_3)_2$)), 3.74 (s, 3H; *E*-(OCH_3)), 3.73 (s, 3H; *Z*-(OCH_3)), 3.37 (d, 2H; H-C(*Z*-1′)), 3.19 (d, 2H; H-C(*E*-1′)), 1.24 (d, 6H; *Z*-($OCH(CH_3)_2$)), 1.22 (d, 6H; *E*-($OCH(CH_3)_2$)), 1.01 (s, 9H; *Z*-($SiC(CH_3)_3$)), 0.99 (s, 9H; *E*-($SiC(CH_3)_3$)), 0.19 (s, 12H; *E*-($SiC(CH_3)_3$), *Z*-($SiC(CH_3)_3$)) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ=154.22 (C(*E*-4), C(*Z*-4)), 147.55 (C(*E*-1′), C(*Z*-1′)), 146.42 (C(*E*-3′)), 144.72 (C(*Z*-3′)), 133.75 (C(*E*-2)), 133.57 (C(*Z*-2)), 119.27 (C(*Z*-6)), 119.20 (C(*E*-6)), 115.73 (C(*Z*-3)), 115.67 (C(*E*-3)), 111.76 (C(*E*-5)), 111.60 (C(*Z*-5)), 105.31 (C(*Z*-2′)), 104.18 (C(*E*-2′)), 74.25 (*Z*-($OCH(CH_3)_2$)), 72.76 (*E*-($OCH(CH_3)_2$)), 56.00 (*E*-(OCH_3)), 55.94 (*Z*-(OCH_3)), 28.73 (C(*E*-1′)), 26.27 (*Z*-($SiC(CH_3)_3$)), 26.25 (*E*-($SiC(CH_3)_3$)), 25.17 (C(*Z*-1′)), 22.88 (*Z*-($OCH(CH_3)_2$)), 22.55 (*E*-($OCH(CH_3)_2$)), 18.66 (*E*-$SiC(CH_3)_3$), *Z*-$SiC(CH_3)_3$)), −3.77 (*E*-($Si(CH_3)_2$)), −3.80 (*Z*-($Si(CH_3)_2$)) ppm; IR (NaCl): $\tilde{\nu}$=3040 (w), 2960 (s), 2930 (s), 2910 (w), 2900 (w), 2860 (m), 2830 (w), 1660 (m), 1600 (w), 1500 (s), 1470 (m), 1460 (m), 1430 (m), 1370 (w), 1340 (m), 1250 (s), 1220 (s), 1150 (m), 1120 (m), 1100 (m), 1050 (s), 950 (m), 900 (s), 840 (s), 800 (m) cm^{-1}; UV/Vis (MeOH): λ=288 nm; MS (EI): *m*/*z* (%): 336 [M^+] (39), 237 (53), 181 (100); HRMS (EI): *m*/*z*: calcd for $C_{19}H_{32}O_3Si$: 336.2121; found: 336.2125 [M^+].

(*E* and *Z*)-2-(3-Methoxy-2-buten-1-yl)-4-methoxyphenol (10a and 10b): Enol ether **39** (34.6 mg, 112 μmol) was dissolved in ethanolamine (4 mL) and heated at 90 °C for 3 h. Evaporation of the solvent at reduced pressure (39 °C/4×10^{-1} mbar) and column chromatography (hexane/MTBE/NEt_3 16:4:0.2) of the crude residue gave a mixture of the two isomers. Purification of the isomeric mixture by preparative RP18 HPLC provided isomerically pure *E* enol ether **10b** (10.2 mg, 51.5 μmol; 46%) and *Z* enol ether **10a** (9.5 mg, 49.2 μmol, 44%). Analytical data for (*E*)-2-(3-methoxy-2-buten-1-yl)-4-methoxyphenol (**10b**). R_f=0.14 (hexane/MTBE 8:2); ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.84 (d, ^3J(6,5)=8.4 Hz, 1H; H-C(6)), 6.69 (d, ^4J(3,5)=3.0 Hz, 1H; H-C(3)), 6.56 (dd, 1H; H-(5)), 6.41 (d, ^3J(3′,2′)=12.8 Hz, 1H; H-C(3′)), 4.79 (dt, ^3J(2′,1′)=7.2 Hz, 1H; H-C(2′)), 4.36 (s, 1H; OH), 3.78 (s, 3H; C(4)-OCH_3), 3.58 (s, 3H; C(3′)-OCH_3), 3.26 (d, 2H; H-C(1′)) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ= 154.10 (C(4)), 149.12 (C(1)), 148.88 (C(3′)), 128.45 (C(2)), 116.72 (C(6)), 116.06 (C(3)), 112.70 (C(5)), 100.74 (C(2′)), 56.44 (C(3′)-OCH_3), 56.13 (C(4)-OCH_3), 29.54 (C(1′)) ppm; IR (NaCl): $\tilde{\nu}$=3410 (s), 3060 (w), 3040 (w), 3000 (w), 2940 (m), 2920 (m), 2840 (m), 1660 (s), 1620 (w), 1510 (s), 1470 (m), 1440 (m), 1350 (w), 1270 (m), 1210 (s), 1150 (m), 1110 (m), 1040 (m), 940 (m), 870 (w), 810 (m), 790 (w), 720 (m) cm^{-1}; UV/Vis (EtOH): λ (%)=224 (100), 292 (73) nm; MS (EI): *m*/*z* (%): 195 (11), 194 [M^+] (84), 163 (5), 162 (13), 161 (35), 147 (9), 137 (13), 136 (100), 135 (5), 108 (33), 107 (4), 91 (10), 79 (7), 78 (10), 77 (9), 65 (11), 63 (3), 55

(5), 51 (6); HRMS (EI): *m/z*: calcd for $C_{11}H_{14}O_3$: 194.0943; found: 194.0947 [M^+].

(*Z*)-2-(3-Methoxy-2-buten-1-yl)-4-methoxyphenol (10a): $R_f = 0.17$ (hexane/MTBE 8:2); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.82$ (d, $^3J(6,5) = 8.7$ Hz, 1H; H-C(6)), 6.62 (dd, $^4J(5,3) = 3.2$ Hz, 1H; H-C(5)), 6.64 (d, 1H; H-C(3)), 6.31 (s, 1H; OH), 6.19 (d, $^3J(3',2') = 6.2$ Hz, 1H; H-C(3')), 4.46 (td, $^3J(2',1') = 8.6$ Hz, 1H; H-C(2')), 3.78 (s, 3H; C(4)-OCH_3), 3.52 (s, 3H; C(3')-OCH_3), 3.30 (d, 2H; H-C(1')) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 153.66$ (C(4)), 148.89 (C(1)), 146.45 (C(3')), 127.55 (C(2)), 117.08 (C(6)), 116.01 (C(3)), 113.12 (C(5)), 105.52 (C(2')), 60.31 (C(3')-OCH_3), 56.43 (C(4)-OCH_3), 26.29 (C(1')) ppm; IR (NaCl): $\tilde{\nu} = 3420$ (s), 3060 (w), 3040 (w), 3010 (m), 2950 (m), 2930 (m), 2830 (m), 1660 (s), 1620 (w), 1520 (s), 1480 (m), 1440 (m), 1370 (w), 1260 (m), 1210 (s), 1150 (m), 1110 (m), 1040 (m), 940 (m), 870 (w), 840 (m), 810 (m), 770 (m), 720 (m) cm^{-1}; UV/Vis (EtOH): λ (%) = 224 (100), 292 (43) nm; MS (EI): *m/z* (%): 195 (12), 194 [M^+] (87), 163 (5), 162 (12), 161 (35), 147 (9), 137 (11), 136 (100), 119 (6), 108 (33), 91 (10), 79 (9), 78 (11), 77 (16), 65 (18), 55 (7), 45 (12), 41 (8), 39 (10); HRMS (EI): *m/z*: calcd for $C_{11}H_{14}O_3$: 194.0943; found: 194.0948 [M^+].

(*E* and *Z*)-2-(3-Ethoxy-2-buten-1-yl)-4-methoxyphenol (44a and 44b): Enol ether **40** (19.4 mg, 60.1 μmol) was dissolved in ethanolamine (4 mL) and heated at 90 °C for 3 h. Evaporation of the solvent at reduced pressure (39 °C/4×10^{-1} mbar) and column chromatography (hexane/MTBE/NEt_3 15:5:0.2) of the crude residue gave a mixture of the two isomers. Purification of the isomeric mixture by preparative RP18 HPLC provided isomerically pure *E* enol ether **44b** (5.8 mg, 27.8 μmol, 46%) and *Z* enol ether **44a** (4.6 mg, 22.1 μmol, 37%). **44b**: $R_f = 0.18$ (hexane/MTBE 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.73$ (d, $^3J(6,5) = 8.6$ Hz, 1H; H-C(6)), 6.70 (d, $^4J(3,5) = 2.9$ Hz, 1H; H-C(3)), 6.66 (dd, 1H; H-(5)), 6.41 (d, $^3J(3',2') = 12.6$ Hz, 1H; H-C(3')), 4.93 (dt, $^3J(2',1') = 7.1$ Hz, 1H; H-C(2')), 4.81 (s, 1H; OH), 3.75 (s, 3H; OCH_3), 3.73 (q, ^3J(OCH_2-CH_3,OCH_2CH_3) = 6.9 Hz, 2H; OCH_2CH_3), 3.26 (d, 2H; H-C(1')), 1.27 (t, 1H; OCH_2CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 154.10$ (C(4)), 148.52 (C(1)), 148.37 (C(3')), 128.24 (C(2)), 116.76 (C(6)), 116.00 (C(3)), 112.74 (C(5)), 101.50 (C(2')), 65.24 (OCH_2CH$_3$), 56.13 (OCH_3), 27.37 (C(1')), 15.12 (OCH_2CH_3) ppm; IR (NaCl): $\tilde{\nu} = 3420$ (s), 3060 (w), 3030 (w), 2980 (s), 2930 (s), 2900 (w), 2830 (m), 1650 (m), 1610 (w), 1500 (s), 1460 (m), 1450 (m), 1430 (m), 1390 (w), 1350 (w), 1260 (m), 1200 (s), 1150 (s), 1110 (m), 1040 (s), 930 (m), 870 (m), 850 (s), 800 (m), 720 (m) cm^{-1}; UV/Vis (EtOH): λ (%) = 224 (100), 292 (73) nm; MS (EI): *m/z* (%): 208 [M^+] (82), 161 (31), 137 (26), 136 (100), 108 (29); HRMS (EI): *m/z*: calcd for $C_{12}H_{16}O_3$: 208.1099; found: 208.1098 [M^+].

(*Z*)-2-(3-Ethoxy-2-buten-1-yl)-4-methoxyphenol (44a): $R_f = 0.23$ (hexane/MTBE 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.80$ (d, $^3J(6,5) = 8.8$ Hz, 1H; H-C(6)), 6.67 (dd, $^4J(5,3) = 3.0$ Hz, 1H; H-C(5)), 6.64 (d, 1H; H-C(3)), 6.30 (s, 1H; (OH)), 6.06 (d, $^3J(3',2') = 6.1$ Hz, 1H; H-C(3')), 4.66 (td, $^3J(2',1') = 8.6$ Hz, 1H; H-C(2')), 3.93 (q, ^3J(OCH_2CH$_3$,OCH_2CH_3) = 7.1 Hz, 2H; OCH_2CH$_3$), 3.75 (s, 3H; OCH_3), 3.30 (d, 2H; H-C(1')), 1.35 (t, 1H; OCH_2CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 153.59$ (C(4)), 149.10 (C(1)), 144.90 (C(3')), 127.57 (C(2)), 117.10 (C(6)), 115.97 (C(3)), 113.09 (C(5)), 105.48 (C(2')), 68.74 (OCH_2CH$_3$), 56.13 (OCH_3), 27.38 (C(1')), 15.46 (OCH_2CH_3) ppm; IR (NaCl): $\tilde{\nu} = 3400$ (s), 3040 (w), 2980 (s), 2930 (s), 2900 (w), 2840 (m), 1670 (w), 1650 (m), 1620 (w), 1610 (w), 1510 (s), 1470 (m), 1430 (m), 1390 (w), 1350 (w), 1260 (s), 1200 (s), 1150 (s), 1110 (m), 1040 (s), 930 (m), 870 (m), 850 (s), 800 (m), 720 (m) cm^{-1}; UV/Vis (EtOH): λ (%) = 224 (100), 292 (43) nm; MS (EI): *m/z* (%): 208 [M^+] (87), 161 (30), 137 (23), 136 (100); HRMS (EI): *m/z*: calcd for $C_{12}H_{16}O_3$: 208.1099; found: 208.1098 [M^+].

(*E* and *Z*)-2-(3-Isopropoxy-2-buten-1-yl)-4-methoxyphenol (45a and 45b): Enol ether **41** (17.4 mg, 51.7 μmol) was dissolved in ethanolamine (3 mL) and heated at 90 °C for 4 h. Evaporation of the solvent at reduced pressure (39 °C/4×10^{-1} mbar) and column chromatography (hexane/MTBE/NEt_3 15:5:0.2) of the crude residue gave isomerically pure *E* enol ether **45b** (5.4 mg, 24.3 μmol, 47%) and *Z* enol ether **45a** (5.2 mg, 23.4 μmol, 45%). **45b**: $R_f = 0.27$ (hexane/MTBE 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.74$ (d, $^3J(6,5) = 8.6$ Hz, 1H; H-C(6)), 6.69 (d, $^4J(3,5) = 2.8$ Hz, 1H; H-C(3)), 6.66 (dd, 1H; H-C(5)), 6.26 (d, $^3J(3',2') = 12.4$ Hz, 1H; H-C(3')), 5.02 (dt, $^3J(2',1') = 7.1$ Hz, 1H; H-C(2')), 4.83 (s, 1H; OH), 3.98 (septet, ^3J(OCH(CH_3)$_2$,OCH(CH_3)$_2$) = 6.2 Hz, 1H; OCH(CH_3)$_2$), 3.75 (s, 3H; OCH_3), 3.25 (d, 2H; H-C(1')), 1.22 (d, 6H; OCH(CH_3)$_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 154.10$ (C(4)), 148.58 (C(1)), 147.27 (C(3')), 128.09 (C(2)), 116.77 (C(6)), 115.98 (C(3)), 112.79 (C(5)), 103.23 (C(2')), 73.17 (OCH(CH_3)$_2$), 56.13 (OCH_3), 29.83 (C(1')), 22.51 (OCH(CH_3)$_2$) ppm; IR (NaCl): $\tilde{\nu} = 3410$ (s), 3080 (w), 2970 (s), 2920 (s), 2850 (m), 2830 (m), 1670 (s), 1650 (s), 1500 (s), 1460 (m), 1430 (m), 1390 (w), 1370 (m), 1340 (m), 1260 (m), 1200 (s), 1160 (s), 1120 (s), 1040 (s), 930 (m) cm^{-1}; UV/Vis (MeOH): $\lambda = 292$ nm; MS (EI): *m/z* (%): 222 [M^+] (49), 180 (100), 137 (61), 136 (96); HRMS (EI): *m/z*: calcd for $C_{13}H_{18}O_3$: 222.1256; found: 222.1258 [M^+].

(*Z*)-2-(3-Isopropoxy-2-buten-1-yl)-4-methoxyphenol (45a): $R_f = 0.39$ (hexane/MTBE 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 6.80$ (d, $^3J(6,5) = 8.6$ Hz, 1H; H-C(6)), 6.67 (dd, $^4J(5,3) = 3.0$ Hz, 1H; H-C(5)), 6.64 (d, 1H; H-C(3)), 6.40 (s, 1H; OH), 6.09 (d, $^3J(3',2') = 6.1$ Hz, 1H; H-C(3')), 4.66 (td, $^3J(2',1') = 8.6$ Hz, 1H; H-C(2')), 4.07 (septet, ^3J(OCH-CH_3)$_2$,OCH(CH_3)$_2$) = 6.2 Hz, 1H; C(OCH(CH$_3$)$_2$)), 3.75 (s, 3H; OCH_3), 3.27 (d, 2H; H-C(1')), 1.33 (d, 6H; C(OCH(CH_3)$_2$)) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 153.55$ (C(4)), 149.15 (C(1)), 143.61 (C(3')), 127.68 (C(2)), 117.13 (C(6)), 115.98 (C(3)), 113.08 (C(5)), 105.55 (C(2')), 75.51 (OCH(CH$_3$)$_2$), 56.14 (OCH_3), 26.71 (C(1')), 22.61 (OCH(CH_3)$_2$) ppm; IR (NaCl): $\tilde{\nu} = 3380$ (s), 3080 (w), 2970 (s), 2930 (s), 2870 (w), 2840 (w), 1660 (s), 1640 (w), 1610 (w), 1500 (s), 1470 (m), 1450 (m), 1430 (m), 1380 (m), 1370 (m), 1340 (w), 1230 (s), 1200 (m), 1180 (m), 1150 (s), 1100 (s), 1050 (s), 910 (w) cm^{-1}; UV/Vis (MeOH): $\lambda = 292$ nm; MS (EI): *m/z* (%): 222 [M^+] (55), 180 (100), 161 (28), 136 (89); HRMS (EI): *m/z*: calcd for $C_{13}H_{18}O_3$: 222.1256; found: 222.1259 [M^+].

6-Methoxychroman-2-ol (12): An 18% aqueous HCl solution (1.5 mL) was added to a solution of compound **36** (321 mg, 943 μmol) in dioxane (7 mL). The resulting mixture was heated to 55 °C for 10 h. The mixture was diluted with water (300 mL) and the aqueous layer was extracted with MTBE (3 × 75 mL). The organic layers were combined, dried over Na_2SO_4, and evaporated to dryness. The crude residue was purified by flash chromatography (hexane/MTBE 3:1) to yield product **12** (159 mg, 886 μmol, 94%) as an oil. $R_f = 0.17$ (hexane/MTBE 3:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 6.75$ (d, $^3J(8,7) = 8.8$ Hz, 1H; H-C(8)), 6.69 (dd, $^4J(7,5) = 3.1$ Hz, 1H; H-C(7)), 6.62 (d, 1H; H-C(5)), 5.64–5.58 (m, 1H; H-C(2)), 3.71 (s, 3H; OCH_3), 3.05–2.95 (m, 1H; H-C(3)), 2.91 (s, 1H; OH), 2.78 (dt, $^2J(3,3) = 16.4$, $^3J(3,4) = 5.3$ Hz, 1H; H-C(3)), 2.11–1.90 (m, 2H; H-C(4)) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 154.12$ (C(6)), 146.30 (C(8a)), 122.97 (C(4a)), 117.89 (C(8)), 114.27 (C(5)), 113.88 (C(7)), 92.38 (C(2)), 56.13 (OCH_3), 27.40 (C(3)), 20.97 (C(4)) ppm; IR (NaCl): $\tilde{\nu} = 3420$ (s), 2940 (m), 2900 (m), 2830 (m), 1720 (w), 1610 (m), 1590 (w), 1500 (s), 1470 (m), 1430 (m), 1350 (m), 1320 (m), 1300 (m), 1270 (s), 1180 (m), 1150 (m), 1100 (m), 1060 (s), 1000 (m), 950 (s), 910 (m), 870 (m) cm^{-1}; UV/Vis (EtOH): λ (%) = 227 (100), 292 (54) nm; MS (EI): *m/z* (%): 180 [M^+] (100), 161 (20), 136 (59), 108 (32), 77 (20); HRMS (EI): *m/z*: calcd for $C_{10}H_{12}O_3$: 180.0786; found: 180.0787 [M^+].

2,6-Dimethoxychroman (11): A solution of compound **12** (36.63 mg, 203 μmol, 1 equiv) in CH_2Cl_2 (1.5 mL) was stirred with ice-bath cooling while a solution of thionyl chloride (1.3 mL of a 267 mM thionyl chloride solution in CH_2Cl_2, 374 μmol, 1.9 equiv) was added dropwise. After being stirred in the cold for 30 min, the green solution was treated with dry methanol (3 mL). The resulting bluish solution was stirred in the cold for an additional 90 min and then poured into saturated aqueous $NaHCO_3$ (75 mL). The organic layer was separated and the aqueous layer was extracted with MTBE (3 × 30 mL). The combined organic extracts were dried over Na_2SO_4 and evaporated under reduced pressure. The crude residue was purified by flash chromatography (hexane/MTBE 7:1) to yield product **11** (28.11 mg, 145 μmol, 72%) as an oil. $R_f = 0.5$ (hexane/MTBE 6:1); ^{1}H NMR (500 MHz, $CDCl_3$): $\delta = 6.77$ (d, $^3J(8,7) = 8.8$ Hz, 1H; H-C(8)), 6.68 (dd, $^4J(7,5) = 3.0$ Hz, 1H; H-C(7)), 6.60 (d, 1H; H-C(5)), 5.14–5.11 (m, 1H; H-C(2)), 3.71 (s, 3H; (CH_3O)-C(6)), 3.47 (s, 3H; (CH_3O)-C(2)), 2.97–2.90 (m, 1H; H-C(3)), 2.61 (ddd, $^2J(3,3) = 16.2$, $^3J(3,4) = 5.9$, $^3J(3,4) = 3.1$ Hz, 1H; H-C(3)), 2.06–1.90 (m, 2H; H-C(4)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): $\delta = 153.60$ (C(6)), 145.75 (C(8a)), 123.14 (C(4a)), 117.53 (C(8)), 113.87 (C(5)), 113.29 (C(7)), 97.94 (C(2)), 55.68 (C(6)-OCH_3), 55.51 (C(2)-OCH_3), 26.23 (C(3)), 20.59 (C(4)) ppm; IR (NaCl): $\tilde{\nu} = 2990$ (m), 2940 (m), 2830 (m), 1610 (w), 1500 (s), 1470 (m), 1450 (m), 1430 (m), 1370 (m), 1320 (w), 1270 (m), 1250 (m), 1200 (s), 1150 (m), 1110 (m), 1060 (s), 1050 (s), 1000 (s), 910 (s), 870 (m), 850 (w), 810 (m) cm^{-1}; UV/Vis (EtOH): λ (%) = 228 (100), 290 (54) nm; MS (EI): *m/z* (%): 194 [M^+] (96), 163 (64), 161 (70), 136 (100),

108 (72), 91 (30), 65 (38); HRMS (EI): *m/z*: calcd for $C_{11}H_{14}O_3$: 194.0943; found: 194.0944 [M^+].

2-Ethoxy-6-methoxychroman (42): Following the procedure for compound **11** with the use of ethanol instead of methanol afforded product **42** (23.2 mg, 111 μmol, 86%). R_f=0.42 (hexane/MTBE 4:6); ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.75 (d, ^3J(8,7)=8.8 Hz, 1H; H-C(8)), 6.68 (dd, ^4J(7,5)=3.0 Hz, 1H; H-C(7)), 6.60 (d, 1H; H-C(5)), 5.23–5.19 (m, 1H; H-C(2)), 3.87 (q, $^3J(OCH_2CH_3,OCH_2CH_3)$=7.08 Hz, 2H; (CH_3CH_2O)-C(2)), 3.74 (s, 3H; (CH_3O)-C(6)), 2.97–2.90 (m, 1H; H-C(3)), 2.61 (ddd, ^2J(3,3)=16.2, ^3J(3,4)=5.8, ^3J(3,4)=3.2 Hz, 1H; H-C(3)), 2.06–1.88 (m, 2H; H-C(4)), 1.18 (t, 3H; (CH_3CH_2O)-C(2)) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ=153.92 (C(6)), 146.50 (C(8a)), 123.61 (C(4a)), 117.84 (C(8)), 114.29 (C(5)), 113.61 (C(7)), 97.16 (C(2)), 63.93 (C(2)-OCH_2CH_3), 56.08 (C(6)-OCH_3), 26.95 (C(3)), 21.31 (C(4)), 15.55 (C(2)-OCH_2CH_3) ppm; IR (NaCl): $\tilde{\nu}$=2970 (m), 2940 (m), 2910 (m), 2830 (m), 1730 (w), 1610 (m), 1590 (w), 1500 (s), 1470 (m), 1450 (m), 1430 (m), 1370 (m), 1350 (m), 1320 (m), 1300 (m), 1270 (m), 1200 (s), 1150 (m), 1120 (s), 1110 (s), 1060 (s), 1000 (s), 970 (s), 930 (m), 910 (m), 880 (m), 840 (m), 810 (m) cm^{-1}; UV/Vis (EtOH): λ (%)=228 (100), 290 (54) nm; MS (EI): *m/z* (%): 208 [M^+] (72), 194 (23), 162 (41), 136 (100), 108 (37); HRMS (EI): *m/z*: calcd for $C_{12}H_{16}O_3$: 208.1100; found: 208.1103 [M^+].

2-Isopropoxy-6-methoxychroman (43): Following the procedure for compound **11** with the use of isopropanol instead of methanol afforded product **43** (26.84 mg, 121 μmol, 76%). R_f=0.58 (hexane/MTBE 6:1); ^{1}H NMR (300 MHz, $CDCl_3$): δ=6.73 (d, ^3J(8,7)=8.8 Hz, 1H; H-C(8)), 6.67 (dd, ^4J(7,5)=3.0 Hz, 1H; H-C(7)), 6.60 (d, 1H; H-C(5)), 5.28–5.21 (m, 1H; H-C(2)), 4.43 (septet, $^3J(OCH(CH_3)_2,OCH(CH_3)_2)$=6.2 Hz, 2H; C(2)-($OCH(CH_3)_2$)), 3.74 (s, 3H; ($CH_3O$)-C(6)), 3.02–2.84 (m, 1H; H-C(3)), 2.61 (m, 1H; H-C(3)), 2.04–1.76 (m, 2H; H-C(4)), 1.18 (d, 3H; C(2)-($OCH(CH_3)_2$)), 1.13 (d, 3H; C(2)-($OCH(CH_3)_2$)) ppm; ^{13}C NMR (75 MHz, $CDCl_3$): δ=153.51 (C(6)), 146.38 (C(8a)), 123.35 (C(4a)), 117.53 (C(8)), 113.96 (C(5)), 113.22 (C(7)), 95.15 (C(2)), 69.52 (C(2)-$OCH(CH_3)_2$), 55.78 (C(6)-OCH_3), 27.04 (C(3)), 23.55 (C(4)), 21.95, 21.12 (C(2)-$OCH(CH_3)_2$) ppm; IR (NaCl): $\tilde{\nu}$=2970 (m), 2940 (m), 2830 (w), 1610 (m), 1500 (s), 1470 (m), 1430 (m), 1380 (m), 1340 (m), 1320 (m), 1270 (m), 1250 (m), 1200 (s), 1150 (m), 1120 (m), 1100 (m), 1060 (s), 1040 (s), 1000 (s), 910 (m), 870 (m), 810 (m) cm^{-1}; UV/Vis (EtOH): λ (%)=230 (100), 290 (53) nm; MS (EI): *m/z* (%): 222 [M^+] (57), 180 (100), 161 (32), 136 (67); HRMS (EI): *m/z*: calcd for $C_{13}H_{18}O_3$: 222.1256; found: 222.1256 [M^+].

2-(*tert*-Butyldimethylsilanyloxy)-5-methoxybenzaldehyde (47): A stirred solution of 2-hydroxy-5-methoxybenzaldehyde (**46**; 2.0 mL, 2.44 g, 16.0 mmol, 1.0 equiv) and *tert*-butyldimethylsilyl chloride (3.80 g, 25.6 mmol, 1.6 equiv) in DMF (40 mL) was treated with *N*,*N*-diisopropylethylamine (6.0 mL, 4.45 g, 29.0 mmol, 1.8 equiv). After 30 min at room temperature, the reaction solution was poured into saturated aqueous $NaHCO_3$ (500 mL) and this mixture was extracted with MTBE (4× 150 mL). The organic layers were combined, dried over Na_2SO_4, and concentrated at reduced pressure. The oily residue was purified by column chromatography (hexane/MTBE 9:1) yielding silyl ether **47** (4.96 g, 15.6 mmol, 97%) as a yellowish oil. R_f=0.39 (hexane/MTBE 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=10.40 (s, 1H; CHO), 7.27 (d, ^4J(6,4)=3.3 Hz, 1H; H-C(6)), 7.04 (dd, ^3J(4,3)=9.1 Hz, 1H; H-C(4)), 6.82 (d, 1H; H-C(3)), 3.78 (s, 3H; OCH_3), 1.01 (s, 9H; $SiC(CH_3)_3$), 0.23 (s, 6H; $Si(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ=190.28 (CHO), 154.46 (C(5)), 153.72 (C(2)), 127.53 (C(1)), 124.29 (C(3)), 122.00 (C(4)), 109.90 (C(6)), 56.11 (OCH_3), 26.08 ($SiC(CH_3)_3$), 18.71 ($SiC(CH_3)_3$), −3.97 ($Si(CH_3)_2$) ppm; IR (NaCl): $\tilde{\nu}$=3070 (w), 3000 (w), 2960 (s), 2930 (s), 2890 (m), 2860 (s), 1680 (m), 1610 (w), 1580 (w), 1490 (s), 1470 (s), 1420 (s), 1390 (s), 1360 (w), 1280 (s), 1220 (m), 1190 (w), 1150 (s), 1040 (s), 1010 (w), 960 (w), 940 (m), 900 (s), 840 (s), 820 (m), 800 (m), 780 (m), 750 (m), 690 (m) cm^{-1}; UV/Vis (MeOH): λ (%)=224 (100), 256 (50), 346 (25) nm; MS (FAB): *m/z* (%): 267 [M^++H] (49), 209 (100), 73 (46); elemental analysis calcd (%) for $C_{14}H_{22}O_3Si$: C 63.12, H 8.32, Si 10.54, O 18.02; found: C 63.15, H 8.22.

2-(*tert*-Butyldimethylsilyanyloxy)-5-methoxybenzylalcohol (48): Sodium borohydride (195 mg, 5.15 mmol, 1.3 equiv) was added to an ice-cold solution of aldehyde **47** (1.05 g, 3.94 mmol, 1.0 equiv) in absolute ethanol (40 mL). The reaction mixture was stirred at 0°C for 45 min and then was carefully partitioned between saturated aqueous NH_4Cl solution (350 mL) and Et_2O (200 mL). The aqueous layer was separated and extracted further with Et_2O (2×200 mL). The combined organic layers were dried over Na_2SO_4 and then concentrated at reduced pressure. The residue was purified by column chromatography (hexane/MTBE 2:1) to provide **48** (961 mg, 3.58 mmol, 91%) as a colorless oil. R_f=0.26 (hexane/MTBE 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.89 (d, ^4J(6,4)=2.8 Hz, 1H; H-C(6), 6.73 (d, ^3J(3,4)=8.6 Hz, 1H; H-C(3)), 6.70 (dd, 1H; H-C(4)), 4.65 (d, $^3J(CH_2,OH)$=8.6 Hz, 2H; CH_2OH), 3.78 (s, 3H; CH_3O), 2.10 (t, 1H; $HOCH_2$), 1.01 (s, 9H; $SiC(CH_3)_2$), 0.23 (s, 6H; $Si(CH_3)$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ=154.43 (C(5)), 147.44 (C(2)), 132.62 (C(1)), 119.47 (C(3)), 114.37 (C(4)), 113.87 (C(6)), 62.32 (CH_2OH), 56.07 (CH_3O), 26.16 ($SiC(CH_3)_3$), 18.55 ($SiC(CH_3)_3$), −3.82 ($Si(CH_3)_2$) ppm; IR (NaCl): $\tilde{\nu}$=3420 (m), 3000 (w), 2960 (s), 2930 (s), 2890 (w), 2860 (m), 1500 (s), 1460 (m), 1430 (m), 1390 (w), 1360 (w), 1270 (s), 1220 (s), 1150 (m), 1110 (w), 1040 (m), 1010 (w), 940 (m), 900 (s), 840 (s), 820 (w), 800 (w), 780 (m), 690 (w) cm^{-1}; UV/Vis (MeOH): λ (%): 228 (100), 288 (60) nm; MS (EI): *m/z* (%): 268 [M^+] (7), 212 (22), 211 (100), 193 (42), 75 (85); HRMS (EI): *m/z*: calcd for $C_{14}H_{24}O_3Si$: 268.1495; found: 268.1494 [M^+].

2-(*tert*-Butyldimethylsilanyloxy)-5-methoxybenzyl vinyl ether (49): A stirred solution of benzyl alcohol **48** (441 mg, 1.65 mmol), ethyl vinyl ether (50 mL), and $Hg(OAc)_2$ (60.0 mg, 188 μmol, 11 mol%) was heated at 90°C for 16 h. $Hg(OAc)_2$ (52.0 mg, 163 μmol, 10 mol%) was added and the mixture was heated to 90°C for an additional 16 h. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (hexane/MTBE 9:1) to yield **49** (348 mg, 1.18 mmol, 72%) as a colorless oil. R_f=0.51 (hexane/MTBE 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.92 (m, 1H; H-C(3)), 6.72 (m, 2H; H-C(6), H-C(4)), 6.56 (dd, 1H; ^3J(3′,4′a)=14.2, ^3J(3′,4′b)=6.8 Hz; H-C(3′)), 4.76 (s, 2H; H-C(1′)), 4.28 (dd, 1H; ^2J(4′a,4′b)=2.2 Hz; H-C(4′a)), 4.07 (dd, 1H; H-C(4′b)), 3.77 (s, 3H; OCH_3), 1.03 (s, 9H; $SiC(CH_3)_3$), 0.18 (s, 6H; $Si(CH_3)_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ=154.39 (C(5)), 152.11 (C(3′)), 147.24 (C(2)), 128.68 (C(1)), 119.62 (C(3)), 114.48 (C(4)), 114.47 (C(6)), 87.47 (C(4′)), 65.89 (C(1′)), 56.07 (OCH_3), 26.17 ($SiC(CH_3)_3$), 18.60 ($SiC(CH_3)_3$), −3.89 ($Si(CH_3)_2$) ppm; IR (NaCl, $CHCl_3$): $\tilde{\nu}$=3000 (w), 2960 (m), 2930 (m), 2900 (w), 2860 (m), 1640 (w), 1610 (m), 1500 (s), 1460 (m), 1430 (w), 1320 (m), 1150 (m), 1050 (m), 1010 (w), 940 (m), 900 (s), 840 (s) cm^{-1}; UV/Vis (MeOH): λ (%)=228 (100), 290 (50) nm; MS (EI): *m/z* (%): 294 [M^+] (11), 251 (60), 237 (29), 207 (40), 195 (100), 181 (15), 75 (21); HRMS (EI): *m/z*: calcd for $C_{16}H_{26}O_3Si$: 294.1651; found: 294.1650 [M^+].

2-Hydroxy-5-methoxybenzyl vinyl ether (50): Compound **49** (25.1 mg, 85.2 μmol) was dissolved in ethanolamine (10 mL) and stirred at room temperature for 16 h. The reaction mixture was diluted with saturated aqueous NH_4Cl solution and the resulting mixture was extracted with MTBE (4×80 mL). The combined organic layers were dried over Na_2SO_4 and then concentrated at reduced pressure. The residue was purified by column chromatography (hexane/MTBE 3:1) to provide product **50** (14.4 mg, 80.0 μmol, 94%) as a colorless oil. R_f=0.22 (hexane/MTBE 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ=6.82 (d, ^3J(3,4)=8.6 Hz, 1H; H-C(3)), 6.78 (dd, ^4J(4,6)=2.8 Hz, 1H; H-C(4)), 6.72 (d, 1H; H-C(6)), 6.53 (dd, ^3J(3′,4′a)=14.5, ^3J(3′,4′b)=6.8 Hz, 1H; H-C(3′)), 5.83 (s, 1H; HO-C(2)), 4.86 (s, 2H; H-C(1′)), 4.42 (dd, ^2J(4′a,4′b)=2.5 Hz, 1H; H-C(4′a)), 4.18 (dd, 1H; H-C(4′b)), 3.76 (s, 3H; OCH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ=153.80 (C(5)), 150.95 (C(3′)), 149.27 (C(2)), 123.12 (C(1)), 117.61 (C(3)), 115.23 (C(4)), 114.60 (C(6)), 89.54 (C(4′)), 68.89 (C(1′)), 56.20 (OCH_3) ppm; IR (NaCl, $CHCl_3$): $\tilde{\nu}$=3420 (s), 3000 (w), 2920 (s), 2900 (w), 2850 (m), 1620 (s), 1500 (s), 1460 (s), 1450 (s), 1430 (s), 1370 (m), 1320 (s), 1110 (w), 1040 (m), 1010 (w), 990 (w), 960 (w), 900 (w) cm^{-1}; UV/Vis (EtOH): λ (%)=220 (100), 294 (40) nm; MS (EI): *m/z* (%): 180 [M^+] (26), 138 (14), 137 (100), 108 (17); HRMS (EI): *m/z*: calcd for $C_{10}H_{12}O_3$: 180.0786; found: 180.0786 [M^+].

2-Hydroxy-5-methoxybenzylalcohol (51): A solution of 2-hydroxy-5-methoxybenzaldehyde (**46**; 1.0 mL, 1.22 g, 8.02 mmol, 1.0 equiv) in THF (5 mL) was added to a stirred ice-cold suspension of $LiAlH_4$ (114 mg, 3.00 mmol, 1.5 equiv) in THF (35 mL). This mixture was stirred for 15 min and the reaction was quenched by addition of H_2O (50 mL). The suspension was slowly poured into ice-cold aqueous 1 M HCl solution (150 mL) to dissolve the precipitated aluminium hydroxide. The resulting solution was extracted with MTBE (3×150 mL). The organic layers were combined, dried over Na_2SO_4, and filtered through a celite pad. The removed solids were washed thoroughly with Et_2O and the filtrate was con-

centrated. The residue was purified by flash chromatography (hexane/MTBE 4:6) to yield **51** (1.03 g, 6.65 mmol, 84%) as a colorless oil. R_f = 0.22 (hexane/MTBE 4:6); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.95 (s, 1H; HO-(C(2)); 6.78 (d, $^3J(3,4)$ = 8.7 Hz, 1H; H-C(3)); 6.73 (dd, $^4J(4,6)$ = 2.5 Hz, 1H; H-C(4)); 6.56 (d, 1H; H-C(6)); 4.82 (s, 2H; C*H*$_2$OH); 3.78 (s, 3H; OCH_3); 2.68 (s, 1H; CH_2O*H*) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 153.50 (C(5)), 150.06 (C(2)), 126.05 (C(1)), 117.46 (C(3)), 114.74 (C(4)), 113.97 (C(6)), 64.74 (CH_2OH), 56.25 (OCH_3) ppm; IR (KBr): $\tilde{\nu}$ = 3440 (s), 3180 (s), 3010 (s), 2980 (m), 2900 (m), 2840 (m), 1620 (w), 1520 (s), 1480 (s), 1460 (m), 1440 (s), 1400 (w), 1380 (w), 1300 (m), 1280 (m), 1260 (m), 1230 (m), 1200 (s), 1160 (s), 1110 (w), 1040 (s), 1010 (s), 980 (s), 930 (m), 860 (s), 820 (s), 780 (m), 760(m), 700 (m) cm^{-1}; UV/Vis (MeOH): λ (%) = 230 (100), 294 (86) nm; MS (EI): *m*/*z* (%): 154 [*M*$^+$] (22), 136 (100), 108 (47), 78 (36); HRMS (EI): *m*/*z*: calcd for $C_8H_{10}O_3$: 154.0630; found: 154.0632 [*M*$^+$]; elemental analysis calcd (%) for $C_8H_{10}O_3$: C 62.33, H 6.54, O 31.13; found: 62.31, H 6.54, O 30.92.

6-Methoxy-2-methyl-4*H*-benzo[1,3]dioxane (52): Two drops of concentrated H_2SO_4 were added to a solution of compound **51** (110 mg, 710 μmol) in acetaldehyde (10 mL). The reaction mixture was stirred at 4°C for 3 h and then diluted with H_2O (200 mL). After extraction with MTBE (3×150 mL), the combined organic layers were washed sequentially with a 3M aqueous NaOH solution (100 mL) and H_2O (100 mL) and dried over Na_2SO_4, then the solvent was removed. The residue was purified by flash chromatography (hexane/MTBE 9:1) to yield **52** (116 mg, 644 μmol, 91%) as a colorless solid. R_f = 0.24 (hexane/MTBE 9:1); m.p. 98°C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.79 (d, $^3J(8,7)$ = 9.1 Hz, 1H; H-C(8)), 6.72 (dd, $^4J(7,5)$ = 3.0 Hz, 1H; H-C(7)), 6.49 (d, 1H; H-C(5)), 5.12 (q, $^3J(2,CH_3)$ = 5.1 Hz, 3H; H-C(2)), 4.98 (d, $^2J(4,4)$ = 14.7 Hz, 1H; H-C(4)), 4.80 (d, 1H; H-C(4)), 3.80 (s, 3H; CH_3O), 1.52 (d, 3H; H_3C-C(2)) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 154.33 (C(6)), 147.34 (C(8a)), 121.67 (C(4a)), 117.74 (C(8)), 114.37 (C(7)), 109.91 (C(5)), 97.38 (C(2), 66.78 (C(4), 56.12 (CH_3O), 21.04 (H_3C-C(2)) ppm; IR (KBr): $\tilde{\nu}$ = 2990 (m), 2940 (m), 2910 (m), 2890 (m), 1500 (s), 1460 (m), 1450 (w), 1400 (w), 1320 (w), 1300 (m), 1270 (s), 1250 (m), 1220 (s), 1150 (m), 1100 (s), 1030 (s), 930 (m), 900 (s), 860 (m), 820 (m), 790 (m) cm^{-1}; UV/Vis (MeOH): λ (%) = 223 (100), 292 (23) nm; MS (EI): *m*/*z* (%): 180 [*M*$^+$] (20), 136 (100), 108 (40); HRMS (EI): *m*/*z*: calcd for $C_{10}H_{12}O_3$: 180.0786; found: 180.0786 [*M*$^+$].

(*E*)-5-[2-(*tert*-Butyldimetylsilanyloxy)-5-methoxyphenyl]-pent-2-enoic acid ethyl ester (53): A suspension of triethyl phosphonoacetate (0.73 mL, 3.65 mmol, 2 equiv) and NaH (79 mg, 3.29 mmol, 1.8 equiv) in anhydrous THF (15 mL) was cooled to 0°C. Aldehyde **32** (537 mg, 1.83 mmol, 1 equiv) dissolved in THF (5 mL) was added dropwise to the cold suspension. The mixture was then stirred for 12 h at room temperature. Saturated aqueous NH_4Cl solution (150 mL) was added and the product was extracted into MTBE (3×75 mL). The organic extracts were dried over Na_2SO_4 and evaporated. The crude product was purified by flash chromatography (hexane/EtOAc 9:1) to give pure *E* isomer **53** (534 mg, 1.46 mmol, 77%) as a colorless oil. R_f = 0.37 (hexane/MTBE 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.00 (dt, $^3J(3,2)$ = 16.2, $^3J(3,4)$ = 7.2 Hz, 1H; H-C(3)), 6.71–6.65 (m, 2H; H-C(3′), H-C(4′)), 6.64–6.60 (m, 1H; H-C(6′)), 5.83 (d, 1H; H-C(2)), 4.18 (q, ^3J(OC*H*$_2$CH$_3$,OCH$_2$C*H*$_3$) = 7.1 Hz, 2H; OC*H*$_2$CH$_3$), 3.74 (s, 3H; OCH_3), 2.70 (t, $^3J(5,4)$ = 7.3 Hz, 2H; H-C(5)), 2.47 (dt, 2H; H-C(4)), 1.28 (t, 3H; OCH$_2$C*H*$_3$), 0.99 (s, 9H; SiC(CH$_3$)$_3$), 0.20 (s, 6H; Si(CH$_3$)$_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.05 (C(1)), 154.15 (C(5′)), 148,85 (C(3)), 147.77 (C(2′)), 132.65 (C(1′)), 121.98 (C(2)), 119.28 (C(3′)), 116.13 (C(6′)), 112.19 (C(4′)), 60.53 (O*C*H$_2$CH$_3$), 56.01 (OCH_3), 32.99 (C(4)), 29.89 (C(5)), 26.21 (SiC(*C*H$_3$)$_3$), 18.59 (Si*C*(CH$_3$)$_3$), 14.66 (OCH$_2$*C*H$_3$), −3.78 (Si(CH$_3$)$_2$) ppm; IR (NaCl, $CHCl_3$): $\tilde{\nu}$ = 2955 (s), 2950 (s), 2857 (m), 1721 (s), 1653 (w), 1499 (s), 1471 (m), 1426 (w), 1390 (w), 1367 (w), 1317 (w), 1268 (s), 1225 (s), 1193 (m), 1151 (m), 1044 (s), 895 (m), 839 (m), 779 (m) cm^{-1}; UV/Vis (MeOH): λ = 289 nm; MS (EI): *m*/*z* (%): 364 [*M*$^+$] (12), 307 (85), 251 (16), 211 (59), 195 (100), 193 (40), 75 (54); HRMS (EI): *m*/*z*: calcd for $C_{20}H_{32}O_4Si$: 364.2070; found: 364.2072 [*M*$^+$]; elemental analysis calcd (%) for $C_{20}H_{32}O_4Si$: C 65.89, H 8.85, O 17.56, Si 7.70; found: C 65.72, H 8.82.

(*Z*)-5-[2-(*tert*-Butyldimethylsilanyloxy)-5-methoxyphenyl]-pent-2-enoic acid methyl ester (54): A solution of phosphonoacetate-*P*,*P*-bis(2,2,2-trifluoroethyl)methyl ester (95 μL, 0.44 mmol, 1.3 equiv) and 18-crown-6 (448 mg, 1.69 mmol, 5 equiv) in anhydrous THF was cooled to −78°C and treated with KN(TMS)$_2$ (820 μL of a 0.5M solution in toluene, 0.41 mmol, 1.2 equiv). The aldehyde **32** (100 mg, 0.34 mmol, 1 equiv) was then added and the resulting mixture was stirred at −78°C for 90 min. Saturated aqueous NH_4Cl solution (150 mL) was added and the product was extracted into MTBE (3×75 mL). The ether extracts were dried over Na_2SO_4 and evaporated. The product was purified by flash chromatography (hexane/MTBE 9:1) to give pure *Z* isomer **54** (116 mg, 0.33 mmol, 96%) as a colorless oil. R_f = 0.35 (hexane/MTBE 9:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.73–6.68 (m, 2H; H-C(3′), H-C(4′)), 6.63–6.59 (m, 1H; H-C(6′)), 6.24 (dt, $^3J(3,2)$ = 12.2, $^3J(3,4)$ = 7.4 Hz, 1H; H-C(3)), 5.77 (dt, $^4J(2,4)$ = 2.6 Hz, 1H; H-C(2)), 3.74 (s, 3H; OCH_3), 3.69 (s, 3H; CO_2CH_3), 2.90 (td, $^3J(4,5)$ = 7.0 Hz, 2H; H-C(4)), 2.70 (t, 2H; H-C(5)), 0.99 (s, 9H; SiC(CH$_3$)$_3$), 0.20 (s, 6H; Si(CH$_3$)$_2$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.13 (C(1)), 154.13 (C(5′)), 150,12 (C(3)), 147.82 (C(2′)), 132.90 (C(1′)), 120.03 (C(2)), 119.24 (C(3′)), 116.13 (C(6′)), 112.11 (C(4′)), 56.01 (OCH_3), 51.37 (CO_2CH_3), 30.24 (C(4)), 29.51 (C(5)), 26.21 (SiC(*C*H$_3$)$_3$), 18.60 (Si*C*(CH$_3$)$_3$), −3.79 (Si(CH$_3$)$_2$) ppm; IR (NaCl, $CHCl_3$): $\tilde{\nu}$ = 2950 (s), 2857 (m), 1723 (s), 1646 (w), 1498 (s), 1471 (m), 1436 (w), 1405 (w), 1254 (w), 1224 (s), 1177 (m), 1044 (w), 897 (m), 838 (m), 779 (m) cm^{-1}; UV/Vis (MeOH): λ = 289 nm; MS (EI): *m*/*z* (%): 350 [*M*$^+$] (12), 294 (13), 293 (56), 225 (52), 196 (18), 195 (100), 89 (25), 75 (12), 73 (33), 59 (12); HRMS (EI): *m*/*z*: calcd for $C_{19}H_{30}O_4Si$: 350.1913 [*M*$^+$]; found: 350.1902; elemental analysis calcd (%) for $C_{19}H_{30}O_4Si$: C 65.10, H 8.63, O 18.26, Si 8.01; found: C 65.26, H 8.67.

(*E*)-5-[2-Hydroxy-5-methoxyphenyl]pent-2-enoic acid ethyl ester (55) and (*rac*)-(6-methoxychroman-2-yl)acetic acid ethyl ester (57): TBAF (0.55 mL of 1M solution in THF, 0.55 mmol, 1.0 equiv) was added to a stirred solution of compound **53** (202 mg, 0.55 mmol, 1.0 equiv) in THF (5 mL) and the resulting red reaction mixture was directly quenched by addition of saturated aqueous NH_4Cl solution (40 mL). The mixture was extracted with MTBE (3×25 mL), the combined organic layers were dried over Na_2SO_4, and the solvent was removed by evaporation. The crude mixture was purified by flash chromatography (hexane/EtOAc 3:1) to give the pure *E* isomer of the α,β-unsaturated ester **55** (77.4 mg, 0.31 mmol, 56%) and chroman **57** (37.3 mg, 0.15 mmol, 27%). **55**: R_f = 0.17 (hexane/MTBE 3:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.05 (dt, $^3J(3,2)$ = 16, $^3J(3,4)$ = 7.2 Hz, 1H; H-C(3)), 6.70–6.60 (m, 3H; H-C(3′), H-C(4′), H-C(6′)), 5.85 (d, 1H; H-C(2)), 4.18 (q, ^3J(OC*H*$_2$CH$_3$,OCH$_2$C*H*$_3$) = 7.1 Hz, 2H; OC*H*$_2$CH$_3$), 3.74 (s, 3H; OCH_3), 2.74 (t, $^3J(5,4)$ = 7.3 Hz, 2H; H-C(5)), 2.51 (dt, 2H; H-C(4)), 1.28 (t, 3H; OCH$_2$C*H*$_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.21 (C(1)), 154.12 (C(5′)), 148,89 (C(3)), 147.84 (C(2′)), 128.71 (C(1′)), 122.07 (C(2)), 116.30 (C(6′), C(3′)), 112.19 (C(4′)), 60.63 (O*C*H$_2$CH$_3$), 56.14 (OCH_3), 32.69 (C(4)), 29.39 (C(5)), 14.66 (OCH$_2$*C*H$_3$) ppm; IR (NaCl, $CHCl_3$): $\tilde{\nu}$ = 3400 (s), 2980 (m), 2940 (m), 2830 (w), 1690 (s), 1650 (m), 1510 (s), 1470 (w), 1450 (w), 1430 (m), 1370 (w), 1310 (m), 1270 (m), 1200 (s), 1150 (m), 1070 (w), 1040 (s), 970 (w), 800 (m), 710 (w) cm^{-1}; UV/Vis (CH_3OH): λ = 293 nm; MS (EI): *m*/*z* (%): 250 [*M*$^+$] (38), 204 (60), 176 (36), 161 (46), 137 (100); HRMS (EI): *m*/*z*: calcd for $C_{14}H_{18}O_4$: 250.1205; found: 250.1197 [*M*$^+$]; elemental analysis calcd (%) for $C_{14}H_{22}O_3Si$: C 67.18, H 7.25, O 25.57; found: C 66.98, H 7.16, O 25.58.

(*rac*)-(6-Methoxychroman-2-yl)acetic acid ethyl ester (57): R_f = 0.40 (hexane/MTBE 5:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.71 (d, $^3J(8,7)$ = 8.8 Hz, 1H; H-C(8)), 6.65 (dd, $^4J(7,5)$ = 2.9 Hz, 1H; H-C(7)), 6.58 (d, 1H; H-C(5)), 4.45–4.37 (m, 1H; H-C(2)), 4.18 (q, ^3J(OC*H*$_2$CH$_3$,OCH$_2$C*H*$_3$) = 7.2 Hz, 2H; (OC*H*$_2$CH$_3$)), 3.74 (s, 3H; (CH_3O)-C(6)), 2.92–2.83 (m, 1H; H-C(3)), 2.78 (dd, ^2J(C*H*$_2$CO$_2$Et,C*H*$_2$CO$_2$Et) = 15.4, ^3J(C*H*$_2$CO$_2$Et,2) = 6.1 Hz, 1H; (CH_2-CO_2Et)), 2.61 (ddd, $^2J(3,3)$ = 16.3, $^3J(3,4)$ = 5.9, $^3J(3,4)$ = 3.0 Hz, 1H; H-C(3)), 2.57 (dd, 1H; (CH_2-CO_2Et)), 2.10–2.02 (m, 1H; H-C(4)), 1.82–1.72 (m, 1H; H-C(4)), 1.28 (t, 3H; (OCH$_2$C*H*$_3$)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.23 (CO_2Et), 153.37 (C(6)), 148.47 (C(8a)), 122.09 (C(4a)), 117.41 (C(8)), 113.97 (C(5)), 113.32 (C(7)), 72.14 (C(2)), 60.52 (O*C*H$_2$CH$_3$), 55.71 (C(6)-O*C*H$_3$), 40.64 (*C*H$_2$CO$_2$Et), 27.46 (C(3)), 24.72 (C(4)), 14.76 (OCH$_2$*C*H$_3$) ppm; IR (NaCl, $CHCl_3$): $\tilde{\nu}$ = 2980 (m), 2950 (m), 2830 (w), 1740 (s), 1620 (m), 1500 (s), 1430 (m), 1380 (w), 1360 (m), 1320 (w), 1290 (m), 1260 (s), 1200 (m), 1150 (m), 1050 (s), 1030 (m), 1010 (m), 920 (m), 890 (w) cm^{-1}; UV/Vis (CH_3OH): λ = 293 nm; MS (EI): *m*/*z* (%): 250 [*M*$^+$] (100), 176 (16), 161 (40), 136 (41); HRMS (EI): *m*/*z*: calcd for $C_{14}H_{18}O_4$: 250.1205; found: 250.1203 [*M*$^+$].

(*Z*)-5-[2-Hydroxy-5-methoxyphenyl]pent-2-enoic acid methyl ester (56) and (*rac*)-(6-methoxychroman-2-yl)acetic acid methyl ester (58): TBAF (1.1 mL of 1 M solution in THF, 1.08 mmol, 1.1 equiv) was added to a stirred solution of compound **54** (331 mg, 0.94 mmol, 1.0 equiv) in THF (10 mL) and the resulting orange reaction mixture was directly quenched by addition of saturated aqueous NH_4Cl solution (40 mL). The mixture was extracted with MTBE (3×25 mL), the combined organic layers were dried over Na_2SO_4, and the solvent was removed by evaporation. The crude mixture was purified by flash chromatography (hexane/MTBE 2:1) to give the pure *Z* isomer of the α,β-unsaturated ester **56** (161 mg, 0.36 mmol, 38%) and chroman **58** (165 mg, 0.37 mmol, 39%). **56**: R_f = 0.18 (hexane/MTBE 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.80 (d, $^3J(3',4')$ = 7.6 Hz, 1H; H-C(3′)), 6.69–6.65 (m, 2H; H-C(4′), H-C(6′)), 6.49 (dt, $^3J(3,2)$ = 12.4, $^3J(3,4)$ = 7.6 Hz, 1H; H-C(3)), 5.86 (d, 1H; H-C(2)), 3.78 (s, 3H; OCH_3), 3.74 (s, 3H; CO_2CH_3), 2.82–2.69 (m, 4H; H-C(5), H-C(4)) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 167.70 (C(1)), 153.72 (C(5′)), 150,11 (C(3)), 148.94 (C(2′)), 127.76 (C(1′)), 120.71 (C(2)), 117.10 (C(3′)), 115.93 (C(6′)), 113.09 (C(4′)), 56.13 (OCH_3), 51.94 (CO_2CH_3), 31.04 (C(4)), 30.14 (C(5)) ppm; IR (NaCl, $CHCl_3$): $\tilde{\nu}$ = 3430 (s), 2980 (w), 2950 (m), 2830 (w), 1700 (s), 1640 (m), 1510 (s), 1440 (s), 1340 (w), 1200 (s), 1040 (m), 820 (m) cm^{-1}; UV/Vis (MeOH): λ = 293 nm; MS (EI): *m*/*z* (%): 236 [M^+] (37), 204 (49), 176 (26), 161 (29), 137 (100), 77 (11); HRMS (EI): *m*/*z*: calcd for $C_{13}H_{16}O_4$: 236.1049; found: 236.1049 [M^+].

(*rac*)-(6-Methoxychroman-2-yl)acetic acid methyl ester (58): R_f = 0.340 (hexane/MTBE 2:1); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.74 (d, $^3J(8,7)$ = 8.9 Hz, 1H; H-C(8)), 6.65 (dd, $^4J(7,5)$ = 3.0 Hz, 1H; H-C(7)), 6.58 (d, 1H; H-C(5)), 4.46–4.37 (m, 1H; H-C(2)), 3.74 (s, 3H; (CH_3O)-C(6)), 3.72 (s, 3H; CO_2CH_3), 2.92–2.67 (m, 3H; H-C(3), (CH_2-CO_2Me)), 2.62 (dd, $^2J(CH_2CO_2Me,CH_2CO_2Me)$ = 15.5, $^3J(CH_2CO_2Et,2)$ = 6.0 Hz, 1H; (CH_2-CO_2Me)), 2.10–2.02 (m, 1H; H-C(4)), 1.81–1.72 (m, 1H; H-C(4)) ppm; ^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.63 (CO_2Me), 153.38 (C(6)), 148.47 (C(8a)), 122.49 (C(4a)), 117.56 (C(8)), 113.91 (C(5)), 113.33 (C(7)), 72.09 (C(2)), 55.76 (C(6)-OCH_3), 51.79 (CO_2CH_3), 40.44 (CH_2CO_2Me), 27.21 (C(3)), 24.71 (C(4)) ppm; IR (NaCl, $CHCl_3$): $\tilde{\nu}$ = 2980 (m), 2950 (m), 2830 (w), 1740 (s), 1620 (m), 1500 (s), 1430 (m), 1380 (w), 1360 (m), 1320 (w), 1290 (m), 1260 (s), 1200 (m), 1150 (m), 1050 (s), 1030 (m), 1010 (m), 920 (m), 890 (w) cm^{-1}; UV/Vis (CH_3OH): λ = 293 nm; MS (EI): *m*/*z* (%): 236 [M^+] (100), 176 (18), 162 (42), 136 (40); HRMS (EI): *m*/*z*: calcd for $C_{13}H_{16}O_4$: 236.1049; found: 236.1051 [M^+].

2-α-Deuterio-(tetrahydropyran-2-yloxy)benzaldehyde (62): A solution of 2-(2-bromophenoxy)tetrahydropyran (**61**; 5.91 g, 22.7 mmol, 1 equiv) in Et_2O (30 mL) was cooled in an ice bath. *n*Buli (14.2 mL of a 1.6 M solution in hexane, 22.7 mmol, 1 equiv) was added dropwise. After stirring at 0 °C for 2 h, a solution of [D_7]DMF (2.0 g, 24.95 mmol, 1.1 equiv) in Et_2O (5 mL) was added dropwise and the resulting reaction mixture was stirred for 8 h at room temperature. The reaction was quenched by addition of H_2O (60 mL) and then the aqueous layer was separated and extracted further with Et_2O (3×30 mL). The combined organic layers were dried over Na_2SO_4 and concentrated. The oily residue was purified by flash chromatography (hexane/MTBE 3:1) to give benzaldehyde **62** (4.56 g, 22.0 mmol, 97%) as an oil. R_f = 0.47 (hexane/MTBE 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.83 (d, $^3J(6,5)$ = 7.8 Hz, 1H; H-C(6)), 7.48 (t, $^3J(4,5)$ and $^3J(4,3)$ = 7.3 Hz, 1H; H-C(4)), 7.22 (d, 1H; H-C(3)), 7.03 (t, 1H; H-C(5)), 5.61–5.57 (m, 1H; H-C(2′)), 3.90–3.83 (m, 1H; H-C(3′)), 3.64–3.59 (m, 1H; H-C(3′)), 2.04–1.57 (m, 6H; H-C(4′), H-C(5′), H-C(6′)) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 189.80 (t, ^2J(CDO,CDO) = 27.22 Hz; *C*DO), 159.92 (C(2)), 136.03 (C(4)), 128.40 (C(6)), 125.74 (C(1)), 121.91 (C(5)), 115.86 (C(3)), 96.98 (C(2′)), 62.49 (C(6′)), 30.49 (C(3′)), 25.41 (C(5′)), 18.89 (C(4′)) ppm; IR (NaCl): $\tilde{\nu}$ = 3060 (w), 2940 (m), 2870 (w), 2120 (m), 1670 (m), 1650 (s), 1640 (s), 1600 (m), 1580 (m), 1480 (m), 1460 (m), 1390 (w), 1350 (w), 1280 (s), 1240 (m), 1230 (m), 1200 (m), 1180 (m), 1150 (m), 1120 (m), 1070 (m), 1040 (m), 1020 (m), 960 (m), 920 (m), 870 (m), 820 (w), 760 (m), 750 (m), 720 (m), 660 (m) cm^{-1}; UV/Vis (MeOH): λ (%) = 252 (100), 320 (38) nm; MS (EI): *m*/*z* (%): 207 [M^+] (15), 123 (63), 121 (51), 85 (100), 84 (41), 67 (22); HRMS (EI): *m*/*z*: calcd for $C_{12}H_{13}DO_3$: 207.1006; found: 207.1006 [M^+].

α-Deuterio-2-hydroxybenzaldehyde (63): Benzaldehyde **62** (4.42 g, 21.3 mmol) was dissolved in THF (10 mL) and 1 N aqueous HCl solution (10 mL) was added. After stirring for 10 h at room temperature, the mixture was diluted with water (200 mL) and extracted with Et_2O (5× 50 mL). The combined organic layers were dried over Na_2SO_4 and then concentrated at reduced pressure to provide compound **63** (2.55 g, 20.7 mmol; 97%) as a colorless oil. R_f = 0.26 (hexane/MTBE 2:1); ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.56–7.48 (m, 2H; H-C(6), H-C(4)), 7.03–6.95 (m, 2H; H-C(5), H-C(3)) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 196.66 (t, ^2J(CDO,CDO) = 27.22 Hz; *C*DO), 162.10 (C(2)), 137.38 (C(4)), 134.08 (C(6)), 120.99 (C(1)), 120.23 (C(5)), 118.01 (C(3)) ppm; IR (NaCl): $\tilde{\nu}$ = 3180 (w), 3060 (w), 2940 (m), 2360 (w), 2130 (m), 2060 (w), 1650 (s), 1640 (s), 1620 (m), 1580 (m), 1490 (m), 1460 (m), 1350 (w), 1280 (s), 1250 (m), 1230 (m), 1210 (m), 1150 (m), 1110 (w), 1040 (m), 1020 (m), 870 (m), 760 (m), 750 (m), 710 (m) cm^{-1}; UV/Vis (MeOH): λ (%) = 254 (100), 325 (32) nm; MS (EI): *m*/*z* (%): 123 [M^+] (100), 121 (71), 93 (20), 76 (26); HRMS (EI): *m*/*z*: calcd for $C_7H_5DO_2$: 123.0431; found: 123.0430 [M^+].

α-Deuterio-benzo[*d*]isoxazole (66): Following the reported procedure[27a], benzaldehyde **63** (2.47 g, 20.1 mmol, 1.0 equiv) afforded benzisoxazole **66** (1.98 g, 16.5 mmol, 82%) as an oil. R_f = 0.48 (hexane/MTBE 8:1); ^{1}H NMR (100 MHz, $CDCl_3$): δ = 7.83 (d, $^3J(6,5)$ = 7.7 Hz, 1H; H-C(4)), 7.68–7.35 (m, 2H; H-C(6), H-C(5)), 7.22 (d, $^3J(7,6)$ = 7.4, 1H; H-C(7)) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 162.66 (C(7a)), 146.35 (t, ^2J(C(3),C(3)-D) = 28.71 Hz; C(3)), 130.44 (C(6)), 124.14 (C(4)), 122.39 (C(5)), 121.61 (C(3a)), 110.11 (C(7)) ppm; IR (NaCl): $\tilde{\nu}$ = 3080 (m), 2940 (m), 2870 (w), 1940 (w), 1640(s), 1620 (s), 1500 (s), 1470 (s), 1430 (s), 1340 (w), 1300 (m), 1250 (m), 1220 (s), 1200 (w), 1150 (m), 1120 (m), 1010 (m), 940 (w), 890 (m), 860 (s), 810 (s), 770 (m), 750 (s) cm^{-1}; UV/Vis (MeOH): λ (%) = 235 (100), 281 (32) nm; MS (EI): *m*/*z* (%): 120 [M^+] (89), 92 (100), 64 (51), 38 (24); HRMS (EI): *m*/*z*: calcd for $C_7H_5DO_2$: 120.0434; found: 120.0433 [M^+].

3-Deuterio-5-nitrobenzo[*d*]isoxazole (67): Following the reported procedure[27b], benzisoxazole **66** (1.64 g, 13.7 mmol) afforded product **67** (1.19 g, 7.26 mmol, 53%) as a white solid: m.p. 76 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.73 (d, $^3J(4,6)$ = 2.4 Hz, 1H; H-C(4)), 8.49 (dd, $^3J(6,7)$ = 9.1 Hz, 1H; H-C(6)), 7.76 (d, 1H; H-C(7)) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ = 164.81 (C(7a)), 147.20 (t, ^2J(C(3),C(3)-D) = 29.91 Hz; C(3)), 145.21 (C(5)), 125.99 (C(6)), 122.19 (C(3a)), 119.60 (C(4)), 110.88 (C(7)) ppm, IR (NaCl): $\tilde{\nu}$ = 3100 (m), 2920 (m), 2860 (w), 1920 (w), 1790 (w), 1620 (m), 1530 (s), 1520 (s), 1490 (s), 1450 (m), 1430 (m), 1350 (w), 1300 (m), 1260 (m), 1250 (m), 1230 (s), 1130 (m), 1080 (m), 1000 (m), 960 (w), 930 (m), 900 (s), 850 (s), 830 (s), 810 (m), 770 (m), 740 (s) cm^{-1}; UV/Vis (EtOH): λ (%) = 224 (100), 277 (32) nm; MS (EI): *m*/*z* (%): 165 [M^+] (100), 135 (30), 119 (30), 91 (61), 63 (92); HRMS (EI): *m*/*z*: calcd for $C_7H_3DN_2O_2$: 165.0285; found: 165.0285 [M^+].

Preparation of antibodies and kinetic measurements

Immunization, fusion, and cell culture: Eight mice (129GIX/boy+) were immunized against the KLH conjugates of either **1**, **3**, or a succession of **1** and **3** or **3** and **1** (two mice each) following standard procedures.[41] Four mice (129GIX/boy+) were immunized against the KLH conjugate of **2**. Each mouse received two intraperitoneal injections of hapten–KLH conjugate (100 μg in 200 μL phosphate-buffered saline (PBS; aqueous 10 mM phosphate, 160 mM NaCl, pH 7.4) emulsified with monophosphoryl-lipid A (MPL) and trehalose dicorynomycolate (TDM) adjuvants (Sigma M6536)), one injection on day 1 and one on day 14. Samples of serum were taken by means of a tail bleed on day 21 to estimate the titer. Fusion process and cell cultures were done according to the previously described procedure.[40]

Screening for catalysis: Cell-culture supernatant (5 mL) was passed onto Protein G gel (100 mg; Gammabind Plus sepharose, Pharmacia Biotech) in a cotton-plugged Pasteur pipette. The gel was washed with PBS (2× 1 mL) and 0.1 M aqueous NaCl (2×1 mL) and finally eluted with 50 mM citrate (2×150 μL, pH 2.7). BisTris solution (30 μL of a 1 M aqueous solution) was added to the eluted acidic buffer containing the antibody in order to adjust the pH value to 6.2. The antibody solution was transferred into three different vials (90 μL in each vial) and a different substrate (10 μL of a 5 mM substrate solution in CH_3CN, substrates **8**, **10a**, or **10b** were used for the screening) was added to each vial. A 50-μL aliquot of each sample was immediately transferred to another vial containing a solution of the corresponding hapten **1**–**3** (1 μL, 1 mM hapten in PBS). The capped vials were kept at 37 °C for at least 48 h. The substrate and product concentrations of each vial were determined by RP18 HPLC on a Bischoff LiChrospher 100 (4.6×125 mm) column with isocratic elution at

a rate of 1 mL min^{-1} with a premixed acetonitrile/water solution of the desired proportions. Substrate and product concentrations were determined after 24 and 48 h incubation time, respectively. The difference in apparent rate between the antibody and the inhibited antibody (with hapten) samples was used as the criterion for specific catalysis. The screening assay was performed repeatedly with each individual hybridoma at all stages of cell culture.

Kinetic measurements: A solution of antibody in BisTris buffer (pH 6.24) at 37 °C was mixed with a substrate solution (acetonitrile/buffer 1:1) to provide a final solution containing 29 mM BisTris, 104 mM NaCl, and 10 % acetonitrile. The final antibody concentration was 250 μg mL^{-1} for the hydrolysis of the enol ethers and 24 μg mL^{-1} for the Kemp elimination. Product formation was monitored by RP18 HPLC on a Bischoff LiChrospher 100 (4.6×125 mm) column with isocratic elution at rate of 1 mL min^{-1} with a premixed acetonitrile/water solution of the desired proportions. The 16E7-catalyzed elimination was monitored by measuring the absorbance increase at 380 nm with a Molecular Devices microtiter plate reader. Measurements were taken at intervals of 60, 90, or 120 s. With all substrates, the antibody-catalyzed reactions were quantitatively inhibited by the hapten.

Data treatment: For the hydrolysis of the enol ethers, the values for the initial velocity, v_i, and for the final velocity, v_{ss}, were graphically estimated from the slopes of the progress curve. From Equation (1), the value, T, of the intercept on the time axis was used to determine the apparent rate constant, τ, for the transition between the two velocities v_i and v_{ss}.[42]

$$T = \frac{(v_{ss}-v_i)}{v_{ss}}\tau \quad (1)$$

For the Kemp elimination the data were analyzed with the Kaleidagraph program (Abelbeck Software). The three parameters v_i, v_{ss}, and τ were obtained from Equation (2).[42]

$$[P] = v_{ss}t-(v_{ss}-v_i)(1-e^{-t/\tau})\tau \quad (2)$$

Michaelis–Menten kinetics for the hydrolysis of the enol ethers: The hydrolysis of the enol ethers was initiated by adding the substrate to the antibody in a capped microtiter plate. The microtiter plate was kept in a closed plastic box with a wet paper towel inside for humidity saturation at 37 °C for at least 12 h and then the substrate and product concentrations were measured by RP18 HPLC. The determination of the substrate and product concentrations was repeated four and eight hours later, respectively. During this period the catalyzed reaction is in the stable phase with constant velocity, which allows the determination of the final velocity, v_{ss}, for the different substrate concentrations used in the measurements. After correction of the rates for the uncatalyzed reaction rates in BisTris buffer, the net v_{ss} rates were obtained. These rates were used to derive the Michelis–Menten constants, K_M, and the maximum velocity, V_{max}, from the Lineweaver–Burk plot of $1/V_{max}$ versus 1/[S]. The catalytic constant, k_{cat}, was obtained by dividing V_{max} by the antibody concentration.

Michaelis–Menten kinetics for the Kemp elimination: For the Kemp elimination the obtained v_{ss} rates from Equation (2) were used to determine the value of K_M/V_{max} from the Lineweaver–Burk plot of $1/V_{max}$ versus 1/[S].

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