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Plant Stress Efficacy

Insights into the in Vitro and in Vivo SAR of Abscisic Acid – Exploring Unprecedented Variations of the Side Chain via Cross-Coupling-Mediated Syntheses

Jens Frackenpohl,*[a] Erwin Grill,*[e] Guido Bojack,[a] Rachel Baltz,[c] Marco Busch,[a] Jan Dittgen,[a] Jana Franke,[a] Jörg Freigang,[b] Susana Gonzalez,[a] Ines Heinemann,[a] Hendrik Helmke,[a] Martin Hills,[a] Sabine Hohmann,[a] Pascal von Koskull-Döring,[a] Jochen Kleemann,[a] Gudrun Lange,[a] Stefan Lehr,[a] Thomas Müller,[a] Elisabeth Peschel,[a] Fabien Poree,[d] Dirk Schmutzler,[a] Arno Schulz,[a] and Lothar Willms,[a]Christian Wunschel

Abstract: Novel analogues of the plant hormone abscisic acid (ABA) were designed and prepared to explore the impact that modifications of its terpenoid side chain have on receptor affinity and in vivo efficacy against drought stress in selected crops. Their efficient and versatile synthesis proceeded via Stille or Sonogashira couplings, shortening the synthetic route significantly. In line with molecular modelling and X-ray crystallography studies novel ABA-derivatives with small alkyl, cycloalkyl or haloalkyl substituents showed strong effects in vitro and in vivo against drought stress in crops, particularly canola and wheat.

Introduction

Abiotic stress adversely affects crop production in various parts of the world, decreasing average yields for most of the crops significantly.[1] Among various abiotic stresses affecting agricultural production, drought stress is considered to be the main source of crop losses around the globe. There are various strategies for reducing the impact that drought has on crop yield. These include exploiting beneficial effects of crop protection agents,[2] developing drought tolerant crops through transgenic approaches or breeding,[3] but also exploring novel chemical active ingredients inspired by naturally occurring plant hormones. Abscisic acid [**1**, (*S*)-ABA], a chiral sesquiterpenoid first discovered in the 1960s,[4] is an important plant hormone that regulates developmental signals such as seed maturation or dormancy and mediates the adaptation of plants to environmental stress such as drought or salinity stress. Studies on abscisic acid (ABA) mediated signaling have progressed rapidly since the discovery of RCAR/(PYR/PYL) receptor proteins as soluble ABA-receptors.[5] These crucial findings have granted new insights into the structure activity relationship of ABA. The catabolism of ABA has been well explored prior to identifying the RCAR/(PYR/PYL) receptor proteins. Its principal pathway of oxidation is through monooxygenase-mediated hydroxylation, i.e. by the CYP707A-family, of the 8′-methyl group affording 8′-hydroxy-abscisic acid which is in equilibrium with the cyclized form phaseic acid.[6] Thus, various chemical modifications of the cyclohexenone headgroup of ABA have been investigated within recent years. It has been shown that metabolism-resistant analogues altered at position C8′ were more stable towards oxidation than ABA itself with 8′-ethynyl-ABA **2a**, 8′-vinyl-ABA **2b** and 8′-trifluoromethyl-ABA **2c** exhibiting promising activity in vitro (Figure 1).[7]

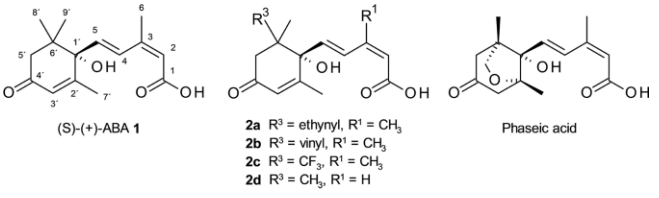


Figure 1. The structure of (*S*)-abscisic acid (**1**) with formal atom numbering and selected closely related analogues with modified headgroup.

Furthermore, tetralone analogues of ABA have been prepared,[8] as well as 3′-sulfanyl-ABA analogues,[9] ABA-analogues with modified 7′-methyl group[10] or 3′-methyl- and 3′-haloalkyl-ABA.[11] Although varying the cyclohexenone moiety has attracted significant synthetic interest, surprisingly few syn-

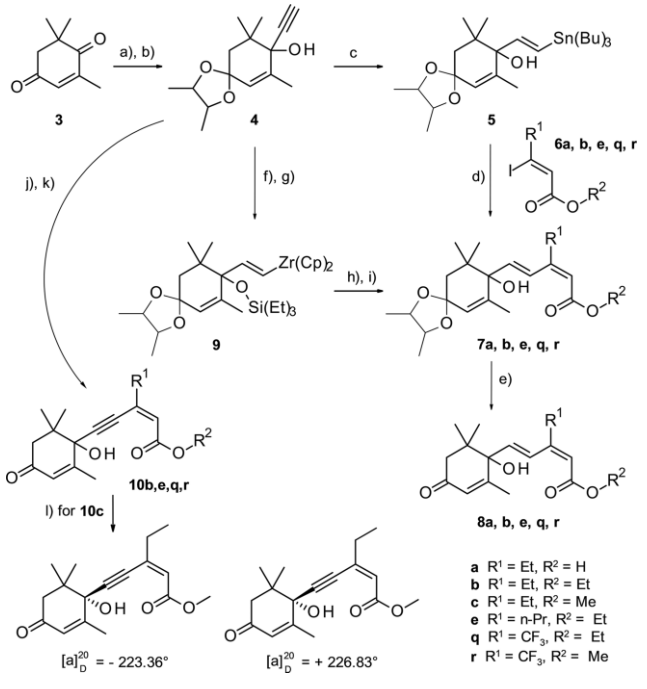
[a] *Research & Development, Weed Control, Bayer AG, CropScience Division, Industriepark Höchst*
Geb. G836, 65926 Frankfurt am Main, Germany
E-mail: jens.frackenpohl@bayer.com
www.bayer.com
[b] *Research & Development, Research Technology, Bayer AG, CropScience Division, Gebäude 6240, Alfred-Nobel-Straße 50, 40789 Monheim, Germany*
[c] *Bayer S.A.S. Centre de Recherche de La Dargoire 14 Impasse Pierre Baizet, 69263 Cedex 09, Lyon France*
[d] *Bayer SAS, Toxicology, Toxicology Research, 355, rue Dostoievski, CS 90153 Valbonne, 06906 Sophia-Antipolis Cedex, France*
[e] *Lehrstuhl für Botanik, Wissenschaftszentrum Weihenstephan, Technische Universität München*
Emil-Ramann-Straße 4, 85354 Freising
E-mail: grill@wzw.tum.de
Supporting information and ORCID(s) from the author(s) for this article are available on the WWW under https://doi.org/10.1002/ejoc.201701687.

thetic changes have been made regarding the (*E*,*Z*)-diene side chain of ABA. 6-nor-ABA **2d**, which lacks ABA's C6 methyl group, has shown significantly reduced activity,[12] indicating the potential impact of side chain modifications on receptor binding and in vivo efficacy. Herein, we outline results of our in-depth evaluation of ABA derivatives with modified diene side chains, and we discuss the structural basis of the effects of ABA derivatives on drought stress observed within our in vivo-studies.

Results and Discussion

Chemistry

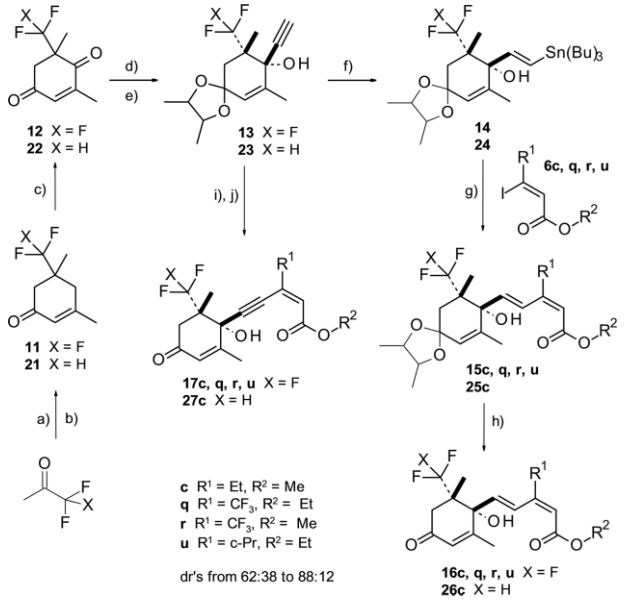
Several syntheses of ABA have been reported,[13] however, not all of them match the criteria crucial for efficiently preparing a broad range of analogues for in vivo structure-activity relationship (SAR) studies in greenhouse or field trials. Namely, low number of overall steps, versatility, satisfying yield and selective formation of the (*E*,*Z*)-diene side chain. Likewise, most syntheses starting from commercially available 4-oxoisophorone **3** proceeded via hydride-mediated reduction of an intermediate enyne system featuring i) additional steps due to re-oxidation of the terminal alcohol moiety formed or incorporated, as well as ii) insufficient (*E*)-selectivity as major drawbacks.[14] We thus decided to explore a novel, more flexible approach using Stille coupling as the key step. Protecting the less hindered carbonyl group of 4-oxoisophorone **3** with 2,3-butanediol,[15] followed by direct alkynylation using ethylenediamine lithium acetylide complex afforded crystalline alkynol **4** in good yield (Scheme 1).



Scheme 1. Cross coupling mediated syntheses of ABA-analogues. a) 2,3-butanediol, $(H_3CO)_3CH$, TsOH, dioxane; b) Li-acetylide ethylene diamine complex, THF, room temp.; c) Bu_3SnH, $Pd(PPh_3)_2Cl_2$, THF, room temp.; d) **6a**, **b**, **e**, **q**, **r**, $Pd(MeCN)_2Cl_2$, CuI, DMF, 50 °C; e) acetone, aq. HCl, room temp.; f) Et_3SiOTf, 2,6-lutidine, CH_2Cl_2, 0 °C to room temp.; g) Cp_2ZrHCl, CH_2Cl_2, room temp.; h) 1. $Pd(PPh_3)_2Cl_2$, DIBAL-H, $ZnCl_2$, THF; i) TBAF, CH_2Cl_2; j) 1. $Pd(PPh_3)_2Cl_2$, CuI, diisopropylamine, toluene, room temp.; k) acetone, aq. HCl, room temp.; l) chiral prep. HPLC.

4 could then be transformed selectively into (*E*)-configured key intermediate **5** via $Pd(PPh_3)_2Cl_2$-mediated hydrostannylation.

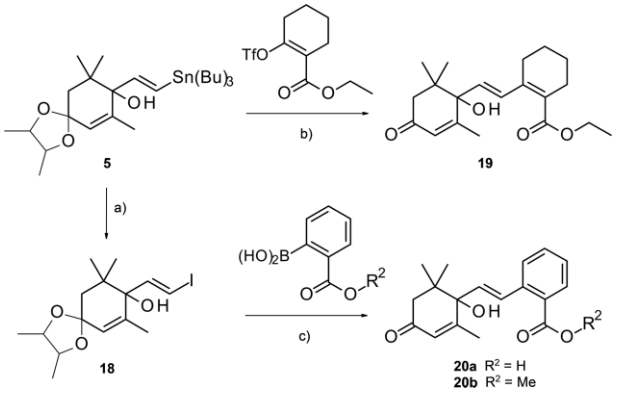
Coupling of (*E*)-stannane **5** with a suitable freshly prepared vinyl iodide **6a**,**b**,**e**,**q**,**r** via an optimized Stille-coupling protocol using $Pd(MeCN)_2Cl_2$ and CuI in *N*,*N*-DMF at slightly elevated temperatures (40–50 °C) afforded protected ABA-analogues **7a**,**b**,**e**,**q**,**r**.[16] Subsequent HCl-mediated deprotection yielded ABA-desired analogues **8a**,**b**,**e**,**q**,**r**. (*Z*)-Vinyl iodides **6a**,**b**,**e**,**q**,**r** were prepared starting from substituted alkynes within two steps via a carboxylation and subsequent hydroiodination.[17] In our hands, this methodology proved to be clearly superior towards related approaches using hydrozirconation[18] and a Negishi-type cross-coupling of **9** to establish the (*E*,*Z*)-diene side chain (e.g. **7q**, yield < 10 %). Using (*E*)-stannane **5** as the key intermediate thus allowed us to proceed without protecting the tertiary alcohol group, while this proved to be mandatory for preparing **7q** via Zr-intermediate **9**. As a result, ABA-analogues **8a**,**b**,**e**,**q**,**r** could be prepared in 5 linear steps with overall yields in the range of 30–40 %. Furthermore, upscaling of the Stille coupling to a 30 mmol scale was feasible, allowing us to prepare **8b**,**e**,**q**,**r** in multigram quantities. Alkynol **4** also served as key intermediate to prepare ABA-related enynes **10b**,**c**,**e**,**q**,**r** via Sonogashira coupling and subsequent acetal deprotection. Both enantiomers of enyne **10c** could be obtained via prep. chiral HPLC, whereas the corresponding free enyne carboxylic acid **10a** was obtained via cleavage of the parent ester to avoid formation of a butenolide.



Scheme 2. Synthesis of ABA-analogues with R^3 = haloalkyl. a) Ph_3P=$CHC(O)CH_3$, Et_2O, room temp., 20 h; b) $CH_3C(O)CH_2CO_2Et$, *t*BuOK, Et_2O, 0 °C to room temp.; c) $H_3Mo_{12}O_{48}P$, MoO_3, $CuSO_4$, H_2O, air, toluene, reflux, 4 d; d) 2,3-butanediol, $(H_3CO)_3CH$, TsOH, dioxane; e) Li-acetylide ethylene diamine complex, THF, room temp.; f) Bu_3SnH, $Pd(PPh_3)_2Cl_2$, THF, room temp.; g) **6c**,**q**,**r**,**u**, $Pd(MeCN)_2Cl_2$, CuI, DMF, 50 °C; h) acetone, aq. HCl, room temp.; i) $Pd(PPh_3)_2Cl_2$, CuI, **6c**,**q**,**r**,**u**, diisopropylamine, toluene, room temp.; j) acetone, aq. HCl, room temp.

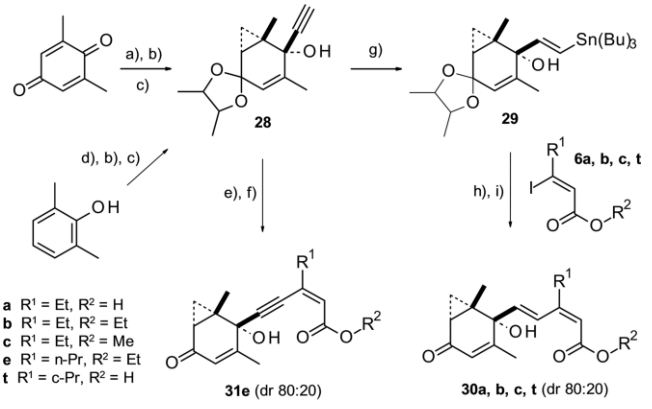
With this robust synthetic route in hand we could prepare a broad range of ABA-analogues with modified side chain (entries 3–33, Table 1). To further broaden our SAR-study we aimed to

introduce these specific novel side chain variations into metabolically stable ABA-analogues **2a**–**c**. Having chosen C8′-CF_3-ABA **2c** as a representative core structure, we decided to re-investigate and optimize the initial synthetic approaches towards **2c** to provide a scalable synthesis (Scheme 2).[7e] With particular emphasis on reducing the number of steps, we chose a direct allylic oxidation of **11** and its difluoromethyl analogue **21** to prepare haloalkyl-oxoisophorones **12** and **22**.[19] Starting from a suitable fluorinated acetone cyclohexenones **11**, **21** were prepared in two steps. MoO_3/P-molybdate-mediated aerobic oxidation of **11**, **21** using $CuSO_4$ as an additive proved to be the best method in our hands affording **12**, and **22** respectively, in satisfying yields (65–75 %). In line with the transformations of 4-oxoisophorone **3** outlined in Scheme 1, alkynols **13** and **23**, as well as stannanes **14** and **24** were then prepared as intermediates. $Pd(MeCN)_2Cl_2$-mediated Stille coupling with subsequent acidic acetal cleavage afforded desired target compounds **16c**,**q**,**r**,**u** and **26c**, again in good yield. C8′-haloalkyl ABA-derivatives **16c** and **26c** could thus be prepared in 8 steps from triflouro or difluoro acetone in overall yields from 4–10 % as mixtures of diastereomers. Typically, diastereomeric ratios (*dr*) obtained were in the range of 88:12 (**16c**) to 62:38 (**26c**). Likewise, haloalkyl-ABA related enynes **17c**,**q**,**r**,**u** and **27c** were obtained via Sonogashira coupling and subsequent acetal deprotection.



Scheme 3. Synthesis of ABA-analogues with cycloalkenyl and aryl moieties stabilizing the terpenoid side chain. a) NIS, CH_2Cl_2; b) 1. $Pd(MeCN)_2Cl_2$, CuI, DMF, 50 °C; 2. aq. HCl, acetone; c) 1. $Pd(PPh_3)_4$, $NaHCO_3$, dioxane, H_2O, reflux; 2. acetone, aq. HCl, room temp.

The utility of stannane **5** as a versatile key intermediate could be demonstrated further by preparing cyclized ABA-analogues **19** and **20a**–**b** via Stille and Suzuki couplings. By converting **5** into (*E*)-vinyl iodide **18** a $Pd(PPh_3)_4$-mediated Suzuki coupling could be used to prepare **20a** without need for further protecting groups (Scheme 3). The free carboxylic acid moiety was tolerated, and ABA-analogue **20a** could be obtained in 58 % yield (3 steps from **5**) enabling us to evaluate the impact of a cyclohexenyl (**19**) or phenyl group (**20a**–**b**) as rigid replacements for the (*Z*)-configured double bond (Scheme 3). Additionally, we applied our cross-coupling approach to a further ABA analogue bearing a stabilized headgroup that exhibited confirmed in vitro activity, namely C5′-C8′-cyclopropyl ABA.[20] Following the reaction sequences outlined in Scheme 1–Scheme 2 we prepared C5′-C8′-cyclopropyl ABA analogues **30a**–**c**,**t** starting from dimethyl-benzoquinone or 2,6-dimethylphenol (Scheme 4).[21] Key intermediate **28** was formed in three steps via acetal formation, cyclopropanation and subsequent lithium acetylide-mediated alkynylation in good yield, albeit as a mixture of diastereomers. **28** then served as starting point for Sonogashira and Stille coupling reactions affording desired target compounds **31e** and **30a**–**c**,**t**. (Scheme 4). In accordance with results obtained for ABA-analogues **8a**–**r** $Pd(MeCN)_2Cl_2$-mediated Stille coupling proved again to be superior over hydride-based approaches via reduction of related alkynes **31** and over other (*E*)-selective cross coupling reactions. As shown for fluorinated ABA-derivatives **16c**,**q**,**r**,**u** and **26c** desired dienes **30a**–**c**,**t**. and enyne **31e** were obtained as mixtures of diastereomers (80:20 ratio). With this additional set of double-modified ABA-analogues in hand, we had broadened the basis of our SAR-study further to assess the impact of side chain modifications at position C6 (R^1).



Scheme 4. Synthesis of C5′-C8′-cyclopropyl ABA-analogues. a) 2,3-butanediol, $(H_3CO)_3CH$, *p*TsOH, dioxane; b) NaH, $Me_3SO^+I^-$, DMF, room temp.; c) Li-acetylide ethylene diamine complex, THF, room temp.; d) 2,3-butanediol, $PhI(OAc)_2$; e) $Pd(PPh_3)_2Cl_2$, CuI, diisopropylamine, toluene, room temp.; f) acetone, aq. HCl, room temp.; g) Bu_3SnH, $Pd(PPh_3)_2Cl_2$, THF, room temp.; h) **6a**–**c**,**t**, $Pd(MeCN)_2Cl_2$, CuI, DMF, 50 °C; i) acetone, aq. HCl, room temp.

In Vitro and in Vivo Structure–Activity Relationship Study

A detailed analysis of the published structure of the ABA receptor isoform RCAR12 and ABI1 in complex with ABA (3kdj), and the respective structure of RCAR11 in complex with ABA (3kdi)[22a] indicated that there might be additional space in the binding pocket into which the C6 methyl group of ABA is directed (R^1). Hence, we decided to explore this pocket by varying the size and the shape of the R^1-substituents. Mainly hydrophobic substituents were chosen since the protein pocket is lined mostly with hydrophobic side chains suggesting that small hydrophobic moieties such as ethyl or cyclopropyl should fit into the binding pocket whilst maintaining or even increasing the affinity of the compounds to the target receptor. Likewise, rigidified ABA-analogues **19** and **20a**–**b** were prepared to explore the limits of this particular binding pocket. All ABA-analogues prepared were tested for target affinity, as well as for beneficial effects in vivo under drought stress conditions upon foliar application on plants. Wheat and canola were chosen as

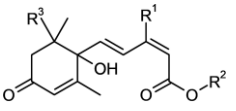
model plants for monocotyledonous and dicotyledonous species, whereas in vitro tests were carried out at the ABA-receptor system RCAR11 in *Arabidopsis thaliana*. All in vivo tests outlined herein were carried out with several replicates using dose rates based on our long-term experience in crop protection research. The very high sequence identity between ABA receptors from *A. thaliana* and canola was expected to lead to a good correlation between in vitro binding at RCAR11 in *A. thaliana* and in vivo effects measured in canola. However, subtle differences in ABA binding sites between RCAR11 from *A. thaliana* and corresponding ABA receptor isoforms in wheat may lead to different in vivo efficacy in wheat. Based on our experiences regarding herbicidal antidotes being used as Safeners[23] we also laid emphasis on exploring the impact of esters on in vivo efficacy. Corresponding in vitro activities of esters were measured in the presence of pig liver esterase to ensure consistent assessment of structural changes at R^1 and R^3.

In accordance with our initial expectations, in vitro and in vivo efficacy correlated nicely, particularly in the case of in vitro activity of *A. thaliana* and the in vivo efficacy in canola. In addition, there is an excellent agreement between in vitro activity and the in vivo efficacies of acids and their respective esters demonstrating the robustness of the test systems. Interestingly, the stereoconfiguration at carbon C1′ did not have a significant impact on in vivo efficacy as both enantiomers of ABA (Entries 1 and 2; Table 1) showed comparable effects. These findings prompted us to use compounds **8**, **16** and **26** as racemates or diastereomeric mixtures in our SAR study. However, *trans*-configured ABA (***E,E***)-**1** (entry 34, Table 1) did not show beneficial effects in canola and wheat emphasizing the importance of an in-depth SAR of ABA-side chain variations.

Firstly, strong effects against drought stress were observed for compounds with C6-ethyl moiety, i.e. **8a**–**d** (entries 3–6, Table 1), in line with good binding affinity to RCAR11. Interestingly, esters and parent acid showed consistent effects in canola and wheat. Derivatives with longer unbranched alkyl side chains, such as *n*-propyl (**8e–f**, entries 7 and 8), *n*-butyl (**8g–h**, entries 9, 10) and *n*-hexyl (**8i**, entry 11) showed a moderate decline in in vitro activity due to increasing chain length, whereas in vivo efficacy dropped significantly. Electron-withdrawing groups at C6 were also well tolerated since ABA-analogues with C6-CF_3-group, i.e. **8q**–**s** (entries 10–21, Table 1) also showed strong effects in vivo with **8q** exhibiting better efficacy, both in wheat and canola, than ABA **1** (entry 1, Table 1). Whilst small branched alkyl substituents (e.g. isopropyl **8j** and *sec*-butyl **8k**–**l**) were still active in vitro and in vivo, substituents with a quaternary carbon at C6 (e.g. *tert*-butyl **8n** and isopentyl **8o**,**p**) led to a loss in activity suggesting a clash within the narrow pocket. Likewise, similar effects could be observed upon introducing a phenyl or furyl group (**8y, z**, entries 27 and 28, Table 1). Hence, we introduced cycloalkyl substituents ranging from small cyclopropyl groups in **8t**, **8u**, **16u** (entries 23, 24 and 32, Table 1) to a rather spacious cyclohexyl moiety in **8x** (entry 16). C6-cyclopropyl ABA **8t** showed the highest target affinity observed among all analogues prepared within our SAR study in line with very strong effects against drought stress in vivo which were superior to ABA **1**. C6-cyclobutyl ABA **8v** also showed promising results, whereas related cyclopentyl and cyclohexyl analogues only gave low activity, both in vitro and in vivo. In contrast to conclusions from earlier studies,[24] we observed good receptor affinity as well as promising efficacy against drought stress in vivo for ABA-derivatives with modified side chain substituents R^1 at position C6.[25]

To further investigate the influence of substituents at position C6 on receptor binding, we determined the crystal structure of **8t** in complex with RCAR11 and the phosphatase hab1.

Table 1. SAR-results of selected diene-analogues of ABA.



		Substituents[a]				In vitro activity ABI1-(AtRCAR11)[b]		In vivo efficacy against drought stress
Entry	No.	R^1	R^2	R^3	Activity [%] 5 µM	pI_{50}	Wheat[d]	[250 g/ha][c] Canola[d]
1	(*S*)-**1**	Me	H	Me	100	7.1	+++	+++
2	(*R*)-**1**	Me	H	Me	97	6.8	+++	+++
3	**8a**	Et	H	Me	99	5.8	+++	+++
4	**8b**	Et	Et	Me	99[b]	5.9[b]	++++	+++
5	**8c**	Et	Me	Me	100[b]	6.1[b]	+++	++++
6	**8d**	Et	*n*Pr	Me	95	5.7	++	+++
7	**8e**	*n*Pr	Et	Me	95[b]	5.8[b]	+++	+++
8	**8f**	*n*Pr	H	Me	97	5.9	+++	++
9	**8g**	*n*Bu	H	Me	96	5.6	+	++
10	**8h**	*n*Bu	Et	Me	97[b]	5.6[b]	+	++
11	**8i**	*n*Hex	H	Me	83	5.2	+	0
12	**8j**	*i*Pr	Et	Me	96[b]	5.4[b]	+++	++
13	**8k**	*s*Bu	H	Me	83	4.8	++	++
14	**8l**	*s*Bu	Me	Me	81[b]	4.7[b]	+++	+
15	**8m**	*s*Pent	Et	Me	80[b]	4.7[b]	++	++
16	**8n**	*t*Bu	Et	Me	42[b]	n.d.[b]	+++	0
17	**8o**	*i*Pent	H	Me	10	n.d.	+	0
18	**8p**	*i*Pent	Et	Me	12[b]	n.d.[b]	0	0
19	**8q**	CF_3	Et	Me	96[b]	5.9[b]	++++	+++
20	**8r**	CF_3	Me	Me	96[b]	5.9[b]	++++	++++
21	**8s**	CF_3	H	Me	98[b]	5.7[b]	+++	++++
22	**8t**	*c*Pr	H	Me	100	6.6	++++	++++
23	**8u**	*c*Pr	Et	Me	100[b]	6.2[b]	++++	++++
24	**8v**	*c*Bu	H	Me	100	5.9	+++	++++
25	**8w**	*c*Pent	Et	Me	41[b]	n.d.[b]	++	0
26	**8x**	*c*Hex	Et	Me	13[b]	n.d.[b]	+	++
27	**8y**	Ph	Et	Me	34[b]	n.d.[b]	+	0
28	**8z**	Furyl	H	Me	45	4.8	0	0
29	**16c**	Et	Me	CF_3	88[b]	5.3[b]	+++	++
30	**16d**	Et	*n*Pr	CF_3	82[b]	5.2[b]	+++	++
31	**16r**	CF_3	Me	CF_3	86[b]	5.4[b]	+++	++
32	**16u**	*c*Pr	Et	CF_3	95[b]	5.5[b]	+++	++
33	**26c**	Et	Me	CHF_2	80[b]	4.9[b]	++	++
34	(*E,E*)-**1**	Me	H	Me	80	6.1	0	0

[a] *c*Pr = cyclopropyl, *s*Bu = but-2-yl, *c*Pent = cyclopentyl, *s*Pent = pent-2-yl, *i*Pent = 2-methylbut-2-yl, *c*Hex = cyclohexyl. [b] Target affinity of esters was measured after addition of pig liver esterase. [c] Standard application rate for crop protection greenhouse trials. [d] A final expert assessment of efficacies was made according to the following classification: i.e. "0" = no effect, "+" = slight beneficial effect, "++" = significant beneficial effect, "+++" = strong beneficial effect against drought stress, "++++" = very strong effect superior to internal standard ABA (comparative visual assessment of greenmass); please note: compounds **16** and **26** have been used as mixtures of diastereomers (e.g. **16c** with *dr* of 88:12 and **26c** with *dr* of 62:38).

The superposition with published ABA complex structure (3QN1)[22b] is shown in Figure 2 and demonstrates that introducing a cyclopropyl group does not impact the principal binding mode significantly.

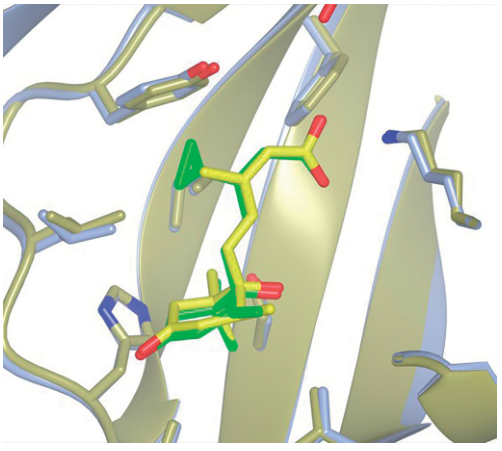


Figure 2. Crystal structure of **8t** (green) in complex with RCAR11-hab1 and superposition with ABA **1** (yellow) in complex structure (3QN1).

Furthermore, metabolically more stable derivatives **16c**, **16d**, **16r**, **16u**, **26c** (entries 29–33) carrying a CF_3- or CHF_2-group at C8′ showed comparable results, although on a slightly lower level. Likewise, C5′-C8′-cyclopropyl analogues afforded moderate target affinities in line with good effects in greenhouse experiments when bearing small alkyl and cycloalkyl substituents as in compounds **30a**–**c**,**t**. (cf. Scheme 4). However, these results were on a lower level than for parent compounds **8** (e.g. **30a**: pI_{50} 5.1, wheat: +++, canola: ++; **30t**: pI_{50} 5.0, wheat: ++, canola: +++; **31e**: pI_{50} 4.9, wheat: ++, canola: ++). Introducing additional substituents in the cyclohexenone moiety thus did not improve in vivo efficacy. Interestingly, more rigid ABA-analogues **19** and **20a**,**b** showed only weak target affinity correlating with minor effects against drought stress in our in vivo tests. These results emphasize our observation that small substituents are tolerated at C6 suggesting a cavity in the ABA-receptor of limited size. To broaden our SAR study further, as well as to evaluate the impact of the (*E*)-C4-C5-alkene linker, we have prepared a set of corresponding enynes bearing modified substituents R^1 at C6 (entries 1–22, Table 2).

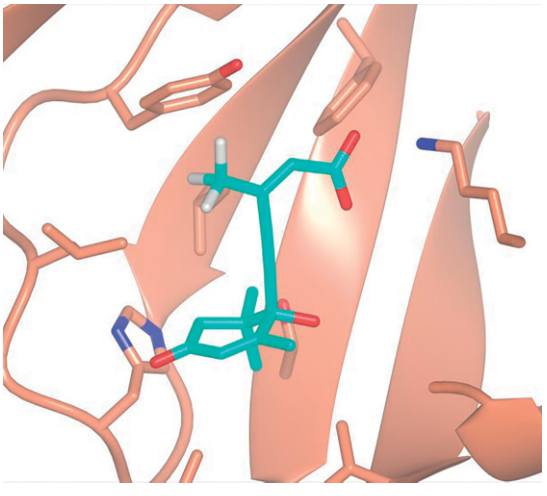
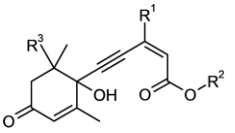


Figure 3. Crystal structure of **10s** in complex with RCAR11-hab1.

We also determined a crystal structure with this class of ligands, namely with enyne **10s**, in a complex with RCAR11 and the phosphatase hab1. Accordingly, the crystal structure of **10s** (Figure 3) shows that the enyne binds in a very similar binding mode than ABA. With the protein crystal structures of **8t** and **10s** in hand, further modelling studies were initiated to evaluate the impact of stereocentres within the cyclohexenyl headgroup. In line with results observed within our in vivo screening campaign it was shown that structural variations (i) of the diene side chain or (ii) of key elements responsible for receptor binding (e.g. the cyclohexanone carbonyl group) had a stronger impact on receptor binding and efficacy than changes in the absolute configuration of stereocentres in the cyclohexanone moiety (cf. also Table 1, entries 1, 2, 34).

Table 2. SAR-results of selected enyne-analogues of abscisic acid.



		Substituents[a]			In vitro activity ABI1-(AtRCAR11)[b]		In vivo efficacy against drought stress	
Entry	No.	R^1	R^2	R^3	Activity [%] 5 µM	pI_{50}	250 g/ha Wheat[c]	Canola[c]
1	**10b**	Et	Et	Me	100[b]	5.9[b]	+++	+++
2	**10c**	Et	Me	Me	100[b]	6.1[b]	+++	+++
3	(*S*)-**10c**	Et	Me	Me	100[b]	6.1[b]	+++	+++
4	(*R*)-**10c**	Et	Me	Me	98[b]	5.9[b]	+++	+++
5	**10e**	*n*Pr	Et	Me	92[b]	5.4[b]	+++	+++
6	**10h**	*n*Bu	Et	Me	88[b]	5.3[b]	+++	+++
7	**10j**	*i*Pr	Et	Me	97[b]	5.8[b]	++	++
8	**10n**	*t*Bu	Et	Me	15[b]	n.d.[b]	0	0
9	**10q**	CF_3	Et	Me	100[b]	6.2[b]	+++	++
10	**10r**	CF_3	Me	Me	97[b]	5.7[b]	++	++
11	**10s**	CF_3	H	Me	100[b]	6.3[b]	+++	++
12	**10u**	*c*Pr	Et	Me	100[b]	6.2[b]	++++	+++
13	**10w**	*c*Pent	Et	Me	42[b]	n.d.[b]	++	0
14	**17b**	Et	Et	CF_3	93[b]	5.2[b]	+	++
15	**17c**	Et	Me	CF_3	86[b]	5.4[b]	+++	++
16	**17e**	*n*Pr	Et	CF_3	75[b]	n.d.[b]	+	+
17	**17j**	*i*Pr	Et	CF_3	93[b]	5.3[b]	+++	+
18	**17q**	CF_3	Et	CF_3	88[b]	5.4[b]	++	+
19	**17u**	*c*Pr	Et	CF_3	85[b]	5.2[b]	++	+
20	**27b**	Et	Et	CHF_2	78[b]	4.9[b]	+	+
21	**27c**	Et	Me	CHF_2	80[b]	4.9[b]	+	++
22	**27r**	CF_3	Me	CHF_2	97[b]	5.7[b]	++	++

[a] *c*Pr = cyclopropyl, *c*Pent = cyclopentyl. [b] Target affinity of esters was measured after addition of pig liver esterase. [c] A final assessment of the respective efficacy was made according to the following classification: i.e. "0" = no effect, "+" = slight beneficial effect, "++" = significant beneficial effect, "+++" = strong beneficial effect against drought stress, "++++" = very strong effect superior to internal standard ABA; please note: compounds **17** and **27** have been used as mixtures of diastereomers (*dr* 80:20).

A similar pattern of in vitro and in vivo activity could be observed for compounds **10a**–**w**, albeit on a slightly lower level. In good accordance with results obtained for diene-analogues with ethyl and cyclopropyl groups at C6 (Table 1) enynes **10b**,

10c and **10u** (entries 1, 2, 10, Table 2) showed the best activities, both in vitro and in vivo. Furthermore, unbranched alkyl groups and the trifluoromethyl group were well tolerated exhibiting good target affinity, as well as good effects in vivo against drought stress. Derivatives with additional modifications at carbon C8′ gave weaker results, correlating with what we had observed for related dienes with modified C8′-groups. In line with results obtained for both enantiomers of ABA (entries 1,2; Table 1) also both separated enantiomers of **10c** showed comparable effects in vivo against drought stress in wheat and canola (entries 3, 4, Table 2). To validate our in vivo screening results further, we have chosen selected ABA-analogues for advanced green house trials (i.e. more replicates, additional crops such as corn and barley, different corn varieties and moderate stress levels). Accordingly, significant beneficial effects against drought stress could also be observed in corn, barley and wheat compared to untreated controls upon treatment with **8b** and **10b** as representative examples (Figure 4).

Receptor Specificity

Selected ABA analogues were analyzed for their receptor specificity, i.e. **8t**, **8v** and **30a**. *Arabidopsis thaliana* has a total of 14 ABA receptor isoforms (RCAR 1–14) and 9 isoforms of the interacting phosphatase (e.g. HAB1 and ABI1) and is the plant species in which the ABA receptor is extensively characterized.[26]

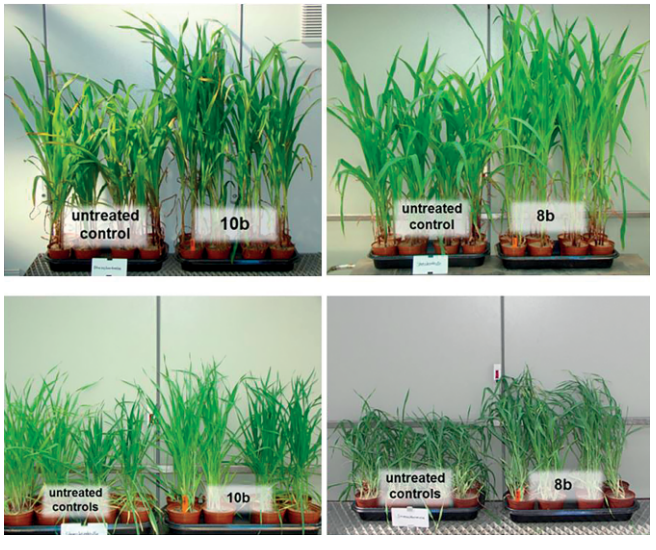


Figure 4. Advanced drought stress trials with **10b** and **8b** in corn, barley and wheat under moderate stress conditions, i.e. 40 % damage for corn, 48 % damage for barley and 40 % for wheat, respectively.

Ectopic expression of RCARs in cell wall-free leaf cells, so-called protoplasts, of *A. thaliana* stimulates ABA-responsive reporter expression in an ABA-dependent manner.[5a] All ABA receptors were transiently expressed in protoplasts either in the presence or absence of ABA and respective novel analogues **8t** or **30a**. Subsequently, the ABA response was recorded (Figure 5).

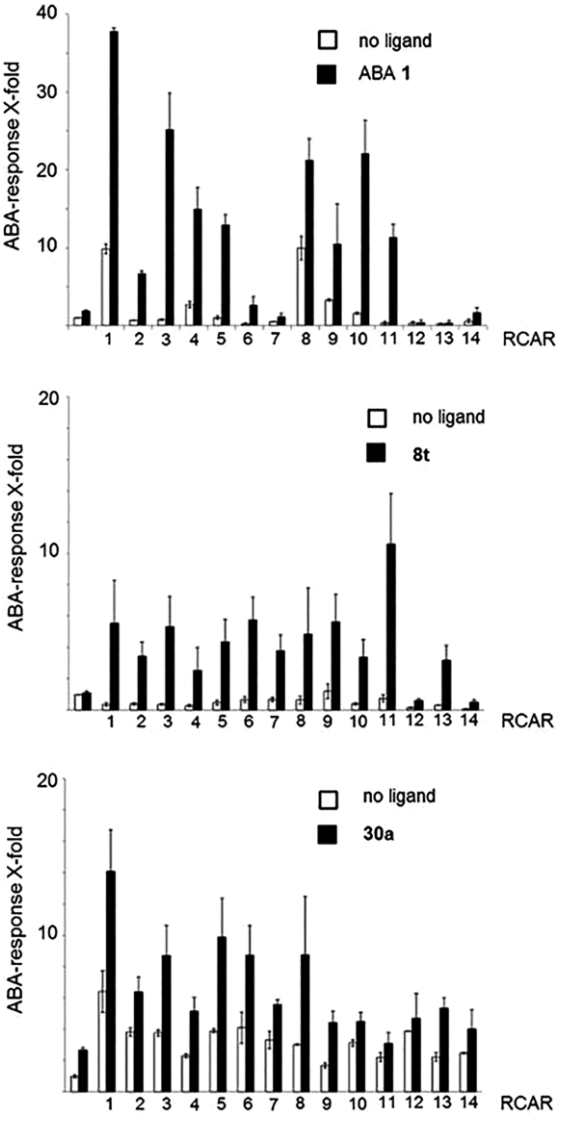


Figure 5. Ligand- and ABA receptor-specific stimulation of ABA signalling in Arabidopsis cells. The ABA receptors RCAR1 to RCAR14 were ectopically expressed in protoplasts and the activation of the ABA-responsive luciferase reporter expressed as fold induction.

Interestingly, ABA analogues **8t** and **30a** revealed varying ABA receptor specificities, since **8t** preferentially stimulated the ABA response via RCAR11, whereas **30a** also stimulated the ABA response via RCAR1, RCAR5, and RCAR10. To further corroborate the ABA receptor specificity, we selected potent ABA analogues with free acid moieties **8v** and **30a**, as well as *n*Pr ester **8d** for in vitro analysis of ABA co-receptor regulation.[5a] Inhibition of the protein phosphatase activity of ABA co-receptors is strictly dependent on the presence of RCAR proteins and ABA or ABA agonists.

All receptors were separately purified and analyzed for regulation of PP2CA (ABI1) in the presence of 100 μM ABA analogues and ABA (Figure 6). Free acids **8v** and **30a** emerged as potent ABA agonists in these in vitro tests, whereas ester **8d** was less active in line with our expectations as ester derivatives had shown their full potential mainly in vivo.

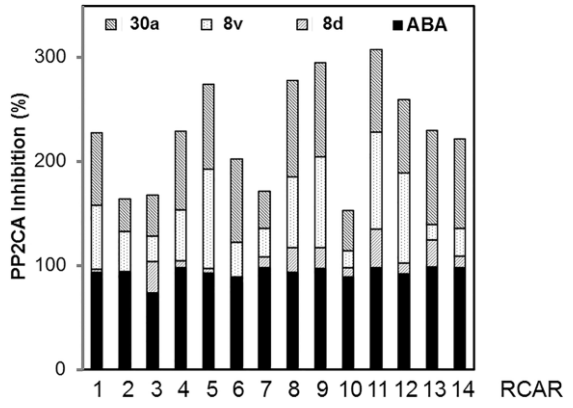


Figure 6. Ligand- and ABA receptor-specific inhibition of the protein phosphatase activity of PP2CA (ABI1). For the in vitro ABA receptor assay, 50 nM PP2CA and 100 nM RCAR (RCAR1 to RCAR14) were incubated in the presence of 100 µM ligand. The analyses were performed in triplicates.

Determination of IC_{50} values for all *A. thaliana* RCARs further emphasized differences in selectivity between **8v** and **30a** at low µM concentrations (Table 3). **8v** sensitively regulated the RCAR5-, RCAR9-, and RCAR11-PP2CA holo-receptor complex (IC_{50} values between 0.5–1.3 µM), **30a** regulated these protein complexes approximately 4–20 times less efficiently. However, **30a** was more effective in regulating RCAR4, RCAR6, RCAR8, RCAR13, and RCAR14 respectively, by a factor of up to 15 compared to **8v** emphasizing its broad activity observed in protoplast tests (Figure 5).

Table 3. Receptor specificity of ABA agonists. The IC_{50}-values of PP2CA inhibition are given in micromolar ligand concentration for distinct ABA receptors RCAR1 to RCAR14 (+/– SE < 5 %).

RCAR	**8v** IC_{50} [µM]	**30a** IC_{50} [µM]	RCAR	**8v** IC_{50} [µM]	**30a** IC_{50} [µM]
1	45	25	8	50	3.8
2	> 100	> 100	9	0.9	4.0
3	> 100	> 100	10	> 100	> 100
4	> 100	10	11	1.3	20
5	0.5	10	12	4.7	11
6	> 100	19	13	> 100	6.5
7	> 100	> 100	14	> 100	6.1

Conclusions

We have developed a highly versatile and robust synthetic route towards derivatives of abscisic acid, giving rise to a broad variety of closely related analogues with particular focus on structural changes at positions C6 and C8′. This synthetic approach enabled us to investigate in vivo activities of a large set of ABA-analogues against drought stress. In summary, we have identified six highly potent analogues of abscisic acid with improved in vivo efficacy against drought stress in canola and wheat. Results from in vitro measurements and crystal structure analyses have confirmed a cavity in the ABA-receptor of limited size tolerating small substituents. The best substituents at C6 were small lipophilic substituents like CF_3, ethyl and cyclopropyl, while larger moieties appeared to be too large for the binding pocket.

Experimental Section

In Vivo biology: Seeds of monocotyledonous and dicotyledonous crop plants were laid out in sandy loam in wood-fiber pots, covered with soil and cultivated in a greenhouse under good growth conditions. The test plants were treated at the early leaf stage (BBCH10 – BBCH13). To ensure uniform water supply before commencement of stress, the potted plants were supplied with the maximum amount of water immediately beforehand by dam irrigation and, after application, transferred into plastic inserts in order to prevent subsequent, excessively rapid drying. The respective compounds, formulated in the form of wettable powders (WP), wettable granules (WG), suspension concentrates (SC) or emulsion concentrates (EC), were sprayed onto the green parts of the plants as an aqueous suspension at an equivalent water application rate of 600 L/ha with addition of 0.2 % wetting agent (agrotin). Substance application is followed immediately by drought stress treatment of the plants. Drought stress was induced by gradual drying out under the following conditions: "day" = 14 hours with illumination at 26 °C; "night" = 10 hours without illumination at 18 °C. The duration of the respective stress phases was guided mainly by the state of the untreated (treated with blank formulation but without test compound), stressed control plants and thus varied from crop to crop. It was ended (by re-irrigating or transfer to a greenhouse with good growth conditions) as soon as irreversible damage was observed on the untreated, stressed control plants. In the case of dicotyledonous crops, for example oilseed rape, the duration of the drought stress phase varied between 3 and 5 days; in the case of monocotyledonous crops, for example wheat, it varied between 6 and 10 days. The end of the stress phase was followed by an approx. 5–7-day recovery phase, during which the plants were once again kept under good growth conditions in a greenhouse. In order to rule out any influence of the effects observed by any fungicidal action of the test compounds, it was additionally ensured that the tests proceeded without fungal infection and without infection pressure. After the recovery phase had ended, the intensities of damage were rated visually in comparison to untreated, unstressed controls of the same age (in the case of drought stress) or the same growth stage (in the case of cold stress). The intensity of damage was first recorded as a percentage (100 % = plants have died, 0 % = like control plants). These values were then used to calculate the efficacy of the test compounds (= percentage reduction in the intensity of damage as a result of substance application), and a final assessment of the respective efficacy was made, i.e. "0" = no effect, "+" = slight beneficial effect, "++" = significant beneficial effect, "+++" = strong beneficial effect against drought stress, "++++" = very strong effect superior to internal standard ABA.

In Vitro Biology: *ABA signalling*: Preparation and analysis of Arabidopsis protoplasts was performed as described in earlier studies.[27] Briefly, protoplasts (105) were transfected with 5 µg DNA of reporter construct (pRD29B::LUC), 3 µg of p35S::GUS plasmid as a control for internal normalization of expression, and 3 µg of RCAR effector expression cassettes. The effector cassettes drive expression of RCAR coding sequences under the control of the 35S promoter.[27] All constructs used were verified by DNA sequence analysis. Total amount of transfected DNA per assay remained constant by supplementing with DNA of the empty effector cassette. The protoplast suspensions were incubated in the presence or absence of ABA and ABA analogues immediate after transfection for 16–18 hours. Assays were done in three replicates per data point. In vitro *activity ABI1-(AtRCAR11)* – The assay described hereinafter utilizes the inhibition of the phosphatase ABI1 via the co-regulator RCAR11/PYR1 from *Arabidopsis thaliana*. Expression and purification of RCARs and

PP2Cs was performed as described.[5a] For the determination of activity, the dephosphorylation of 4-methylumbelliferyl phosphate (MUP) was measured at 460 nm. The in vitro assay was conducted in Greiner 384-well PS microplates F-well, using two controls: a) dimethyl sulfoxide (DMSO) 0.5 % (f.c.) and b) 5 μM (f.c.) abscisic acid (ABA). The assay described here was generally conducted with substance concentrations of the appropriate chemical test substances in a concentration range of 0.1 μM to 100 μM in a solution of DMSO and water. The substance solution thus obtained, if necessary, was stirred with esterase from porcine liver (EC 3.1.1.1) at room temperature for 3 h and centrifuged at 4000 rpm for 30 min. A total volume of 45 μL was introduced into each cavity of the microplate, featuring the following composition:
(1) 5 μL of substance solution, i.e. a) DMSO 5 % or b) abscisic acid solution or c) the corresponding example compound of the general formula (I) dissolved in 5 % DMSO.
(2) 20 μL of enzyme buffer mix, composed of a) 40 % by vol. of enzyme buffer [10 mL contain equal proportions by volume of 500 mM Tris-HCl pH8, 500 mM NaCl, 3.33 mM $MnCl_2$, 40 mM dithiothreitol (DTT)], b) 4 % by vol. of ABI1 dilution (protein stock solution was diluted so as to give, after addition, a final concentration in the assay of 0.15 μg ABI1/well), c) 4 % by vol. of RCAR11 dilution (enzyme stock was diluted so as to give, on addition of the dilution to the enzyme buffer mix, a final concentration in the assay of 0.30 μg enzyme/well), d) 5 % by vol. of Tween20 (1 %), e) 47 % by vol. H_2O bi-dist.
(3) 20 μL of substrate mix, composed of a) 10 % by vol. of 500 mM Tris-HCl pH8, b) 10 % by vol. of 500 mM NaCl, c) 10 % by vol. of 3.33 mM $MnCl_2$, d) 5 % by vol. of 25 mM MUP, 5 % by vol. of Tween20 (1 %), 60 % by vol. of H_2O bi-dist.

Enzyme buffer mix and substrate mix were made up 5 minutes prior to the addition and warmed to a temperature of 35 °C. On completion of pipetting of all the solutions and on completion of mixing, the plate was incubated at 35 °C for 20 min. Finally, a relative fluorescence measurement was made at 35 °C with a BMG Labtech "POLARstar Optima" microplate reader using a 340/10 nm excitation filter and a 460 nm emission filter.

Chemistry:

General: All reagent-grade solvents and chemicals were purchased from standard commercial suppliers and used without further purification. All non-aqueous reactions were carried out under anhydrous conditions using dry solvents. Reactions were monitored by LC-MS or TLC carried out on 0.25 mm silica gel plates (60F-254). TLC plates were visualized using UV light. Flash chromatography was carried out using Biotage Isolera One systems with pre-packed column cartridges (Biotage KP-Sil [40+M] or KP-Sil [25+M]), with typical gradients starting from an ethyl acetate/heptane ratio of 20:80, to a final ratio of 80:20. The ^{1}H NMR, ^{13}C NMR and ^{19}F NMR spectroscopy data reported for the chemical examples described below (400 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR and 375 MH z for ^{19}F NMR, solvent: $CDCl_3$, CD_3OD or $[D_6]$DMSO, internal standard: tetramethylsilane δ = 0.00 ppm), were obtained on a Bruker instrument, and the signals listed have the meanings given below: br = broad; s = singlet, d = doublet, t = triplet, dd = doublet of doublets, ddd = doublet of a doublet of doublets, m = multiplet, q = quartet, quint = quintet, sext = sextet, sept = septet, dq = doublet of quartets, dt = doublet of triplets. The abbreviations used for chemical groups are defined as follows: Me = CH_3, Et = CH_2CH_3, *t*Hex = $C(CH_3)_2CH(CH_3)_2$, *t*Bu = $C(CH_3)_3$, *n*Bu = unbranched butyl, *n*Pr = unbranched propyl, *c*Hex = cyclohexyl. In the case of diastereomeric mixtures, either the significant signals for each of the diastereomers or the characteristic signal of the main diastereomer are reported. Further experimental details have been described in the Supporting Information, as well as in references.[21,25]

8-Ethynyl-2,3,7,9,9-pentamethyl-1,4-dioxaspiro[4.5]dec-6-en-8-ol (4): In a round-bottom flask under argon, 2,2,6-trimethyl-1,4-cyclohexanedione (15.40 g, 101.19 mmol) was dissolved in 2,3-butanediol (90 mL), and abs. toluene (90 mL), and trimethyl orthoformate (33.21 mL, 303.56 mmol) and *p*-toluenesulfonic acid (1.22 g, 7.08 mmol) were added. The resulting reaction mixture was stirred at 50 °C for 7 h. After cooling to room temperature, water and toluene were added and the aqueous phase was extracted repeatedly with toluene. The combined organic phases were dried with magnesium sulfate, filtered and concentrated under reduced pressure. By column chromatography purification of the resulting crude product (ethyl acetate/heptane gradient), 2,3,7,9,9-pentamethyl-1,4-dioxaspiro[4.5]dec-6-en-8-one (20.01 g, 88 % of theoretical) was obtained. In a round-bottom flask under argon, 2,3,7,9,9-pentamethyl-1,4-dioxaspiro[4.5]dec-6-en-8-one (10.00 g, 44.58 mmol) was then dissolved in tetrahydrofuran (50 mL) and added dropwise to a solution of a lithium acetylide/ethylenediamine complex (6.28 g, 57.96 mmol, 85 % pure) in tetrahydrofuran (70 mL). On completion of addition, the reaction mixture was stirred at room temperature for 4 h, then water was added and the mixture was concentrated under reduced pressure. The remaining residue was admixed with water and dichloromethane, and the aqueous phase was extracted repeatedly with dichloromethane. The combined organic phases were dried with magnesium sulfate, filtered and concentrated under reduced pressure. By column chromatography purification of the crude product obtained (ethyl acetate/heptane gradient), 8-ethynyl-2,3,7,9,9-pentamethyl-1,4-dioxaspiro[4.5]dec-6-en-8-ol (10.02 g, 85 % of theoretical) was isolated as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5 ppm.41/5.39 (s, 1 H), 4.24–4.20/3.62–3.54 (m, 2 H), 2.49 (s, 1 H), 2.08/2.01 (d, 1 H), 1.92/1.90 (s, 3 H), 1.88/1.81 (d, 1 H), 1.26–1.21 (m, 3 H), 1.18–1.06 (m, 9 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 139.9/139.1; 125.5/125.3; 103.8/103.6; 84.4; 78.07; 77.90; 74.5/74.3; 73.9/73.7; 46.1/45.6; 39.1/38.9; 25.4; 22.5/21.9; 18.8/18.4; 17.0/16.8; 15.6/15.4. LC-MS (ret.-time, min, [M^+/log p]) 1.47 [250.16/3.03]; 1.49 [250.16/3.10]; 1.51 [250.16/3.17]. HRMS-ESI: calcd. for $C_{15}H_{21}O_2^+$ [M + H]$^+$ – H_2O 233.1542, found 233.1543.

2,3,7,9,9-Pentamethyl-8-[(*E*)-2-(tributylstannyl)vinyl]-1,4-dioxaspiro [4.5]dec-6-en-8-ol (5): Under argon, tetrakis(triphenylphosphine) palladium(0) (231 mg, 0.20 mmol) was initially charged in a round-bottom flask that had been dried by heating, and abs. tetrahydrofuran (25 mL) and 8-ethynyl-2,3,7,9,9-pentamethyl-1,4-dioxaspiro[4.5]dec-6-en-8-ol **4** (1.0 g, 3.99 mmol) were added. Stirring at room temperature for 5 min was followed by the addition of tributyltin hydride (1.29 mL, 4.79 mmol). The resulting reaction mixture was stirred at room temperature for 1 h and then water was added. The aqueous phase was repeatedly extracted thoroughly with dichloromethane, and the combined organic phases were then dried with magnesium sulfate, filtered and concentrated under reduced pressure. By final column chromatography purification of the resulting crude product (ethyl acetate/heptane gradient), it was possible to obtain 2,3,7,9,9-pentamethyl-8-[(*E*)-2-(tributylstannyl)vinyl]-1,4-dioxaspiro[4.5]dec-6-en-8-ol **5** (1.50 g, 66 % of theoretical) in the form of a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.13 (d, 1 H), 5.93 (d, 1 H), 5.42 (s, 1 H), 4.22/3.63 (m, 2 H), 1.61 (s, 3 H), 1.59 (d, 1 H), 1.52 (d, 1 H), 1.49 (m, 6 H), 1.32–1.24 (m, 12 H), 1.09 (s, 3 H), 0.89 (m, 18 H). HRMS-ESI: calcd. for $C_{27}H_{50}O_3Sn^+$ [M + H]$^+$ 543.2782, found 543.2771.

Ethyl (2*Z*)-3-[(*E*)-2-(8-hydroxy-2,3,7,9,9-pentamethyl-1,4-dioxaspiro [4.5]dec-6-en-8-yl)vinyl]hex-2-enoate (7e): Under

argon, 2,3,7,9,9-pentamethyl-8-[(*E*)-2-(tributylstannyl)vinyl]-1,4-dioxaspiro[4.5] dec-6-en-8-ol **5** (300 mg, 0.55 mmol) and ethyl (2*Z*)-3-iodohex-2-enoate **6c** (156 mg, 0.55 mmol) in a round-bottom flask that had been dried by heating were dissolved in *N*,*N*-dimethylformamide (4 mL), dichlorobis(acetonitrile)palladium(II) (7 mg, 0.03 mmol) was added and the mixture was stirred at room temperature for 3 h. After the addition of potassium fluoride solution, the reaction mixture was stirred further at room temperature overnight, and water was added. The aqueous phase was repeatedly extracted thoroughly with CH_2Cl_2. The combined organic phases were then dried with magnesium sulfate, filtered and concentrated under reduced pressure. Purification of the resulting crude product by column chromatography (ethyl acetate/heptane gradient) gave ethyl (2*Z*)-3-[(*E*)-2-(8-hydroxy-2,3,7,9,9-pentamethyl-1,4-dioxaspiro[4.5]-dec-6-en-8-yl)vinyl]hex-2-enoate **7e** (140 mg, 41 % of theoretical) in the form of a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.68 (d, 1 H), 6.09 (d, 1 H), 5.97 (s, 1 H), 5.48 (s, 1 H), 4.19 (m, 3 H), 3.61 (m, 1 H), 2.29 (m, 1 H), 2.22 (m, 2 H), 2.02 (m, 1 H), 1.92 (m, 3 H), 1.67 (m, 2 H), 1.62 (m, 1 H), 1.28 (m, 6 H), 1.20–1.08 (m, 9 H), 0.91 (t, 3 H) ppm. HRMS-ESI: calcd. for $C_{23}H_{36}O_5^+$ [M]$^+$ 392.2562, found 392.2556.

Ethyl (2*E*,4*E*)-5-(8-Hydroxy-2,3,7,9,9-pentamethyl-1,4-dioxaspiro[4.5]dec-6-en-8-yl)-3-(trifluoromethyl)penta-2,4-dienoate (7q): Under argon, 2,3,7,9,9-pentamethyl-8-[(*E*)-2-(tributylstannyl)vinyl]-1,4-dioxa-spiro[4.5] dec-6-en-8-ol 5 (300 mg, 0.55 mmol) and ethyl (2*Z*)-4,4,4-trifluoro-3-iodobut-2-enoate **6q** (163 mg, 0.55 mmol) in a round-bottom flask that had been dried by heating were dissolved in *N*,*N*-dimethylformamide (4 mL), dichlorobis(acetonitrile)palladium(II) (7 mg, 0.03 mmol) was added and the mixture was stirred at room temperature for 3 h. After the addition of potassium fluoride solution, the reaction mixture was stirred further at r.t. overnight. The aqueous phase was then repeatedly extracted thoroughly with diethyl ether, and the combined organic phases were then dried with $MgSO_4$, filtered and concentrated under reduced pressure. By final column chromatography purification of the resulting crude product (ethyl acetate/heptane gradient), ethyl (2*E*,4*E*)-5-(8-hydroxy-2,3,7,9,9-pentamethyl-1,4-dioxaspiro[4.5]dec-6-en-8-yl)-3-(trifluoromethyl)penta-2,4-dienoate **7q** (150 mg, 61 % of theoretical) was obtained in the form of a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.47 (d, 1 H), 6.29 (d, 1 H), 6.25 (s, 1 H), 5.48 (s, 1 H), 4.26 (q, 2 H), 3.68 (m, 1 H), 3.59 (m, 1 H), 1.93 (d, 1 H), 1.83 (br. m, 1 H, OH), 1.77 (d, 1 H), 1.69 (s, 3 H), 1.32 (t, 3 H), 1.25 (m, 3 H), 1.18 (m, 3 H), 1.10 (s, 3 H), 0.91 (s, 3 H) ppm. HRMS-ESI: calcd. for $C_{21}H_{29}O_5F_3^+$ [M]$^+$ 418.1971, found 418.1980.

Methyl-(2*E*,4*E*)-5-(8-hydroxy-2,3,7,9,9-pentamethyl-1,4-dioxaspiro [4.5]dec-6-en-8-yl)-3-(trifluoromethyl)penta-2,4-dienoate (7r): Under argon, 2,3,7,9,9-pentamethyl-8-[(*E*)-2-(tributylstannyl)vinyl]-1,4-dioxa-spiro[4.5] dec-6-en-8-ol 5 (15.0 g, 27.71 mmol) and methyl (2*Z*)-4,4,4-trifluoro-3-iodobut-2-enoate **6r** (7.76 g, 27.71 mmol) in a round-bottom flask that had been dried by heating were dissolved in *N*,*N*-dimethylformamide (100 mL), dichlorobis(acetonitrile)palladium(II) (0.22 g, 0.83 mmol) and copper(I) iodide (4.22 g, 22.17 mmol) were added, and the mixture was stirred at 50 °C for 5 h. After cooling to room temperature and subsequent addition of potassium fluoride solution, the reaction mixture was stirred further at room temperature overnight. The aqueous phase was then repeatedly extracted thoroughly with ethyl acetate, and the combined organic phases were then dried with magnesium sulfate, filtered and concentrated under reduced pressure. By final column chromatography purification of the resulting crude product (ethyl acetate/heptane gradient), methyl (2*E*,4*E*)-5-(8-hydroxy-2,3,7,9,9-pentamethyl-1,4-dioxaspiro[4.5]dec-6-en-8-yl)-3-(trifluoromethyl)penta-2,4-dienoate **7r** (6.72 g, 60 % of theoretical) was obtained in the form of a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.47 (d, 1 H), 6.32 (d, 1 H), 6.28/6.26 (s, 1 H), 5.59/5.39 (s, 1 H), 4.28–4.22/3.68–3.55 (m, 2 H), 3.79 (s, 3 H), 2.02 (d, 1 H), 1.83 (d, 1 H), 1.69/1.66 (s, 3 H), 1.65/1.60 (br. m, 1 H, OH), 1.25 (m, 3 H), 1.18 (m, 3 H), 1.10 (s, 3 H), 0.91 (s, 3 H).. LC-MS (ret.-time, min, [M$^+$/log p]) diastereomer 1: 1.85 [404.18/4.16]; diastereomer 2: 1.87 [404.18/4.25]. HRMS-ESI: calcd. for $C_{20}H_{27}O_5F_3^+$ [M + H]$^+$ 405.1814, found 405.1819.

Ethyl (2*Z*)-3-[(*E*)-2-(1-Hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)vinyl]hex-2-enoate (8e): Ethyl (2*Z*)-3-[(*E*)-2-(8-hydroxy-2,3,7,9,9-pentamethyl-1,4-dioxaspiro[4.5]-dec-6-en-8-yl)vinyl]hex-2-enoate **7e** (180 mg, 0.46 mmol) was dissolved in acetone (5 mL) under argon in a round-bottom flask, and 3 drops of conc. hydrochloric acid were added. The resulting reaction solution was stirred at room temperature for 30 min, and water was then added. After removing acetone under reduced pressure, the aqueous phase was extracted repeatedly with dichloromethane. The combined organic phases were dried with magnesium sulfate, filtered and concentrated under reduced pressure. By final column chromatography purification of the resulting crude product (ethyl acetate/heptane gradient), ethyl (2*Z*)-3-[(*E*)-2-(1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)vinyl]hex-2-enoate **8e** (114 mg, 74 % of theoretical) was obtained in the form of a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.77 (d, 1 H), 6.15 (d, 1 H), 6.05 (s, 1 H), 5.93 (s, 1 H), 4.19 (q, 2 H), 2.96 (br. s, 1 H, OH), 2.47 (d, 1 H), 2.31 (d, 1 H), 2.24 (t, 2 H), 1.92 (s, 3 H), 1.58 (m, 2 H), 1.27 (t, 3 H), 1.24 (s, 3 H), 1.12 (s, 3 H), 0.92 (t, 3 H) ppm. LC-MS (retention time, min [M$^+$/log p]) 1.53 [320.20/3.20]. HRMS-ESI: calcd. for $C_{19}H_{28}O_4^+$ [M + H]$^+$ – H_2O 321.1990, found 321.1997.

Ethyl (2*E*,4*E*)-5-(1-Hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-(trifluoromethyl)penta-2,4-dienoate (8q): Ethyl (2*E*,4*E*)-5-(8-hydroxy-2,3,7,9,9-pentamethyl-1,4-dioxaspiro[4.5]dec-6-en-8-yl)-3-(trifluoromethyl)penta-2,4-dienoate **7q** (150 mg, 0.36 mmol) was dissolved in acetone (5 mL) under argon in a round-bottom flask, and 10 % hydrochloric acid was added. The resulting reaction solution was stirred at room temperature for 40 min, and water was then added. After removing acetone under reduced pressure, the aqueous phase was extracted repeatedly with dichloromethane. The combined organic phases were dried with magnesium sulfate, filtered and concentrated under reduced pressure. By column chromatography purification of the resulting crude product (ethyl acetate/heptane gradient), ethyl (2*E*,4*E*)-5-(1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-(trifluoromethyl)-penta-2,4-dienoate **8d** (80 mg, 82 % of theoretical) was obtained in the form of a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.55 (d, 1 H), 6.37 (d, 1 H), 6.33 (s, 1 H), 5.97 (s, 1 H), 4.25 (q, 2 H), 2.47 (d, 1 H), 2.34 (d, 1 H), 1.92 (s, 3 H), 1.90 (br. s, 1 H, OH), 1.33 (t, 3 H), 1.11 (s, 3 H), 1.02 (s, 3 H) ppm. LC-MS (ret. time, min, [M$^+$/log p]) 1.46 [346.14/3.00]. HRMS-ESI: calcd. for $C_{17}H_{19}O_3F_3^+$ [M + H]$^+$ – H_2O 329.1834, found 329.1830.

Methyl (2*E*,4*E*)-5-(1-Hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-(trifluoromethyl)penta-2,4-dienoate (8r): Methyl (2*E*,4*E*)-5-(8-hydroxy-2,3,7,9,9-pentamethyl-1,4-dioxaspiro[4.5]dec-6-en-8-yl)-3-(trifluoromethyl)penta-2,4-dienoate **7r** (6.84 g, 16.91 mmol) was dissolved in acetone (15 mL) under argon in a round-bottom flask, and 10 % hydrochloric acid was added (5 mL). The resulting reaction solution was stirred at room temperature for 40 min, and water was then added. After removing acetone under reduced pressure, the aqueous phase was extracted repeatedly with dichloromethane. The combined organic phases were dried with magnesium sulfate, filtered and concentrated under reduced pressure. By column chromatography purification of the resulting crude product (ethyl acetate/heptane gradient), methyl (2*E*,4*E*)-5-(1-

hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-(trifluoromethyl)-penta-2,4-dienoate **8r** (4.72 g, 84 % of theoretical) was obtained in the form of a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.56 (d, 1 H), 6.39 (d, 1 H), 6.33 (s, 1 H), 5.97 (s, 1 H), 3.80 (s, 3 H), 2.47 (d, 1 H), 2.35 (d, 1 H), 1.92 (s, 3 H), 1.88 (br. s, 1 H, OH), 1.11 (s, 3 H), 1.02 (s, 3 H) ppm. LC-MS (ret. time, min, [M$^+$/log p]) 1.47 [332.12/2.67]. HRMS-ESI: calcd. for $C_{16}H_{19}O_4F_3^+$ [M + H]$^+$ – H_2O 332.1244, found 332.1235.

Ethyl (2*E*)-5-(1-Hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-(trifluoromethyl)pent-2-en-4-ynoate (10q): Copper(I) iodide (46 mg, 0.24 mmol) and bis(triphenylphosphine)palladium(II)-chloride (126 mg, 0.18 mmol) were initially charged under argon in a round-bottom flask which had been dried by heating, and toluene (9 mL) and ethyl (2*Z*)-4,4,4-trifluoro-3-iodobut-2-enoate **6q** (388 mg, 1.32 mmol) were added. Stirring at room temperature for 10 min was followed by the dropwise addition of a solution of 8-ethynyl-2,3,7,9,9-pentamethyl-1,4-dioxaspiro[4.5]dec-6-en-8-ol **4** (300 mg, 1.19 mmol) in toluene (3 mL) and of diisopropylamine (0.34 mL, 2.39 mmol). The resulting reaction mixture was stirred at room temperature for 3 h and then water was added. The aqueous phase was extracted repeatedly with dichloromethane. The combined organic phases were dried with magnesium sulfate, filtered and concentrated under reduced pressure. By final column chromatography purification of the crude product obtained (using an ethyl acetate/heptane gradient), ethyl (2*E*)-5-(8-hydroxy-2,3,7,9,9-pentamethyl-1,4-dioxaspiro[4.5]dec-6-en-8-yl)-3-(tri-fluoromethyl)pent-2-en-4-ynoate (300 mg, 57 % of theoretical) was isolated in the form of a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.61 (s, 1 H), 5.56 (s, 1 H), 4.22 (m, 3 H), 3.58 (m, 1 H), 2.21 (br. s, 1 H, OH), 1.99 (m, 1 H), 1.92 (m, 4 H), 1.31 (t, 3 H), 1.22–1.13 (m, 12 H) ppm. Ethyl (2*E*)-5-(8-hydroxy-2,3,7,9,9-pentamethyl-1,4-dioxaspiro[4.5]dec-6-en-8-yl)-3-(tri-fluoromethyl)pent-2-en-4-ynoate (200 mg, 0.48 mmol) was dissolved in acetone (5 mL) under argon in a round-bottom flask, and 10 % hydrochloric acid was added (3 mL). The resulting reaction solution was stirred at room temperature for 45 min, and water was then added. After removing acetone under reduced pressure, the aqueous phase was extracted repeatedly with dichloromethane. The combined organic phases were dried with magnesium sulfate, filtered and concentrated under reduced pressure. By column chromatography purification of the resulting crude product (ethyl acetate/heptane gradient), ethyl (2*E*)-5-(1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-(trifluoromethyl)pent-2-en-4-ynoate **10q** (130 mg, 79 % of theoretical) was obtained in the form of a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 6.68 (s, 1 H), 5.90 (s, 1 H), 4.26 (q, 2 H), 2.87 (s, 1 H, OH), 2.58 (d, 1 H), 2.45 (d, 1 H), 2.16 (s, 3 H), 1.33 (t, 3 H), 1.25 (s, 3 H), 1.15 (s, 3 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 197.8; 162.8; 129.9; 126.9; 125.1; 121.6; 119.8; 102.4; 78.2; 75.2; 61.7; 24.9; 19.6; 14.1 ppm. LC-MS (ret. time, min, [M$^+$/log p]) 1.46 [344.12/3.00]. HRMS-ESI: calcd. for $C_{17}H_{20}F_3O_4^+$ [M + H]$^+$ 345.1314, found 345.1302.

Methyl (2*Z*)-5-(1-Hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-(ethyl)pent-2-en-4-ynoate (10c): Copper(I) iodide (23 mg, 0.12 mmol) and bis(triphenylphosphine)palladium(II)chloride (65 mg, 0.09 mmol) were initially charged under argon in a round-bottom flask which had been dried by heating, and toluene (15 mL) and methyl (2*Z*)-3-iodopent-2-enoate **6c** (738 mg, 3.08 mmol) were added. Stirring at room temperature for 10 min was followed by the dropwise addition of a solution of 8-ethynyl-2,3,7,9,9-pentamethyl-1,4-dioxaspiro[4.5]dec-6-en-8-ol **4** (770 mg, 3.08 mmol) in toluene (5 mL) and of diisopropylamine (1.29 mL, 9.23 mmol). The resulting reaction mixture was stirred at room temperature for 3 h and then water was added. The aqueous phase was extracted repeatedly with dichloromethane. The combined organic phases were dried with magnesium sulfate, filtered and concentrated under reduced pressure. By final column chromatography purification of the crude product obtained (using an ethyl acetate/heptane gradient), methyl (2*E*)-5-(8-hydroxy-2,3,7,9,9-pentamethyl-1,4-dioxaspiro-[4.5]dec-6-en-8-yl)-3-(ethyl)pent-2-en-4-ynoate (860 mg, 78 % of theoretical) was isolated in the form of a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.99 (s, 1 H), 5.53/5.41 (s, 1 H), 4.23–4.15/3.65–3.57 (m, 2 H), 3.72 (s, 3 H), 2.55–2.45 (br. s, 1 H, OH), 2.33–2.28 (m, 2 H), 2.08–2.02 (m, 1 H), 1.95–1.87 (m, 4 H), 1.26–1.13 (m, 15 H) ppm. Methyl (2*E*)-5-(8-hydroxy-2,3,7,9,9-pentamethyl-1,4-dioxaspiro[4.5]dec-6-en-8-yl)-3-(ethyl)pent-2-en-4-ynoate (750 mg, 2.07 mmol) was dissolved in acetone (4 mL) under argon in a round-bottom flask, and 10 % hydrochloric acid (4 mL) was added. The resulting reaction solution was stirred at room temp. for 45 min, and water was then added. After removing acetone under reduced pressure, the aqueous phase was extracted repeatedly with CH_2Cl_2. The combined organic phases were dried with $MgSO_4$, filtered and concentrated under reduced pressure. By column chromatography purification of the resulting crude product (ethyl acetate/heptane gradient), methyl (2*E*)-5-(1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-(ethyl)pent-2-en-4-ynoate **10c** (470 mg, 78 % of theoretical) was obtained in the form of a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.06 (s, 1 H), 5.87 (s, 1 H), 3.72 (s, 3 H), 2.91 (br. s, 1 H, OH), 2.62 (br. d, 2 H), 2.44 (d, 1 H), 2.33 (q, 2 H), 2.16 (s, 3 H), 1.26 (s, 3 H), 1.16–1.13 (m, 6 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 198.1; 165.4; 139.9; 137.6; 126.3; 124.2; 99.5; 85.9; 74.4; 51.5; 41.9; 31.5; 25.2; 19.6; 12.6 ppm. LC-MS (ret. time, min, [M$^+$/log p]) 1.29 [290.15/2.44]. HRMS-ESI: calcd. for $C_{17}H_{22}O_4^+$ [M + H]$^+$ – H_2O 290.1524, found 290.1519.

(*R*)- and (*S*)-Methyl (2*Z*)-5-(1-Hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-(ethyl)pent-2-en-4-ynoate [(*R*)-10c], [(*S*)-10c]: Enantiomers of (2*E*)-5-(1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-(ethyl)pent-2-en-4-ynoate **10c** were separated carefully via chiral prep. HPLC to afford (*S*)-**10c**, ret. time 19.550, $[\alpha]_D^{20}$ = –223.36 and (*R*)-**10c**, ret. time 24.03, $[\alpha]_D^{20}$ = +226.83 (chiral HPLC analysis – Chiralpak IC, flow rate 0.6 mL/min, eluent *n*-heptan/isopropanol = 90:10).

(2*E*)-5-(1-Hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-(tri-fluoromethyl)pent-2-en-4-ynoic Acid (10s): In a round-bottom flask, ethyl (2*E*)-5-(1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-(trifluoromethyl)pent-2-en-4-ynoate **10q** (130 mg, 0.38 mmol) was dissolved in a mixture of water and tetrahydrofuran (1:1), and sodium hydroxide (38 mg, 0.94 mmol) was then added. The resulting reaction mixture was stirred under reflux for 2 h and, after cooling to room temperature, acidified with aqueous hydrochloric acid. The aqueous phase was repeatedly extracted thoroughly with CH_2Cl_2, and the combined organic phases were then dried with $MgSO_4$, filtered and concentrated under reduced pressure. By final column chromatography purification of the resulting crude product (ethyl acetate/heptane gradient), (2*E*)-5-(1-hydroxy-2,6,6-trimethyl-4-oxocyclohex-2-en-1-yl)-3-(trifluoromethyl)pent-2-en-4-ynoic acid **10s** (40 mg, 32 % of theoretical) was obtained in the form of a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 10.14 (br. s, 1 H, OH), 6.70 (s, 1 H), 5.92 (s, 1 H), 2.61 (d, 1 H), 2.43 (d, 1 H), 2.30 (br. s, 1 H, OH), 2.16 (s, 3 H), 1.28 (s, 3 H), 1.13 (s, 3 H) ppm. HRMS-ESI: calcd. for $C_{15}H_{15}O_4F_3^+$ [M + H]$^+$ 317.0922, found 317.0930.

8-Ethynyl-2,3,7,9-tetramethyl-9-(trifluoromethyl)-1,4-dioxaspiro[4.5]-dec-6-en-8-ol (13): Acetylmethylenetriphenylphosphorane (12.91 g, 40.57 mmol) was dissolved in a mixture of diethyl ether (30 mL) and dichloromethane (10 mL) and stirred for 5 min, then 1,1,1-trifluoroacetone (5.00 g, 44.62 mmol) was added and the

mixture was stirred at room temperature for 40 h. The precipitate formed was filtered off, the filter cake was washed with diethyl ether and the combined organic phases were concentrated cautiously under slightly reduced pressure. The crude solution of (3*Z*)-5,5,5-trifluoro-4-methylpent-3-en-2-one thus obtained was used without further purification in the next reaction step and taken up in toluene (25 mL). After the addition of ethyl acetoacetate (3.42 g, 26.29 mmol) and potassium *tert*-butoxide (0.88 g, 7.89 mmol), the resulting reaction mixture was stirred under reflux conditions for 5 h. After cooling to r.t., water was added, the mixture was stirred vigorously for 5 min and then the aqueous phase was extracted repeatedly with CH_2Cl_2. The combined organic phases were dried with $MgSO_4$, filtered and concentrated under reduced pressure. By final column chromatography purification of the resulting crude product (ethyl acetate/heptane gradient), it was possible to obtain 3,5-dimethyl-5-(trifluoromethyl)cyclohex-2-en-1-one (1.9 g, 38 % of theoretical) in the form of a colorless oil. 3,5-Dimethyl-5-(trifluoromethyl)cyclohex-2-en-1-one (1.60 g, 8.33 mmol) was then dissolved in toluene (60 mL), and molybdatophosphoric acid hydrate (30 mg, 0.02 mmol), copper(II) sulfate pentahydrate (4 mg, 0.02 mmol) and molybdenum(VI) oxide (5 mg, 0.03 mmol) were added. The resulting reaction mixture was stirred with introduction of air under reflux conditions for 4 d. After cooling to room temperature, water was added, the mixture was stirred vigorously for 5 min and then the aqueous phase was extracted repeatedly with CH_2Cl_2. The combined organic phases were dried with $MgSO_4$, filtered and concentrated under reduced pressure. By column chromatography purification of the resulting crude product (ethyl acetate/heptane gradient), it was possible to obtain 2,6-dimethyl-6-(trifluoromethyl)cyclohex-2-ene-1,4-dione **12** (300 mg, 17 % of theoretical) in the form of a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.69 (s, 1 H), 3.17 (d, 1 H), 2.80 (d, 1 H), 2.06 (s, 3 H), 1.48 (s, 3 H) ppm. 2,6-Dimethyl-6-(trifluoromethyl)cyclohex-2-ene-1,4-dione **12** (520 mg, 2.52 mmol) was dissolved in 2,3-butanediol (4 mL) under argon, and trimethyl orthoformate (0.83 mL, 7.57 mmol) and *p*-toluenesulfonic acid (30 mg, 0.18 mmol) were added. The resulting reaction mixture was stirred at 50 °C for 6 h. After cooling to room temperature, water and toluene were added and the aqueous phase was extracted repeatedly with toluene. The combined organic phases were dried with magnesium sulfate, filtered and concentrated under reduced pressure. By column chromatography purification of the resulting crude product (ethyl acetate/heptane gradient), 2,3,7,9-tetramethyl-9-(trifluoromethyl)-1,4-dioxaspiro[4.5]dec-6-en-8-one (700 mg, 98 % of theoretical) was obtained. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.70 (s, 1 H), 4.35–4.28/3.76–3.67 (m, 2 H), 2.61 (d, 1 H), 2.43 (d, 1 H), 2.16 (s, 3 H), 1.34–1.26 (m, 6 H), 1.20 (m, 3 H). In a round-bottom flask under argon, 2,3,7,9-tetramethyl-9-(trifluoromethyl)-1,4-dioxaspiro[4.5]-dec-6-en-8-one (700 mg, 2.52 mmol) was then dissolved in tetrahydrofuran (3 mL) and added dropwise to a solution of a lithium acetylide/ethylenediamine complex (376 mg, 3.27 mmol, 80 % pure) in tetrahydrofuran (5 mL). On completion of addition, the reaction mixture was stirred at r.t. for 4 h, then water was added and the mixture was concentrated under reduced pressure. The remaining residue was admixed with water and CH_2Cl_2, and the aqueous phase was extracted repeatedly with CH_2Cl_2. The combined organic phases were dried with magnesium sulfate, filtered and concentrated under reduced pressure. By column chromatography purification of the crude product obtained (ethyl acetate/heptane gradient), 8-ethynyl-2,3,7,9-tetramethyl-9-(trifluoromethyl)-1,4-dioxaspiro-[4.5]dec-6-en-8-ol **13** (550 mg, 68 % of theoretical) was isolated as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.50/5.48/5.40 (s, 1 H), 4.26–4.21/3.70–3.58 (m, 2 H), 2.63/2.60 (s, 1 H), 2.53/2.32 (m, 1 H), 2.33/2.31 (br. s, 1 H, OH), 2.02–1.96 (m, 4 H), 1.910–1.87 (m, 1 H), 1.40 (s, 3 H), 1.25 (m, 3 H), 1.17 (m, 3 H). LC-MS (ret. time, min, [M^+/log p]) 1.38 [304.13/2.90], 1.40 [304.13/3.00], 1.42 [304.13/3.07].

2,3,7,9-Tetramethyl-8-[(*E*)-2-(tributylstannyl)vinyl]-9-(trifluoromethyl)-1,4-dioxaspiro[4.5]dec-6-en-8-ol (14): Under argon, tetrakis-(triphenylphosphine)palladium(0) (19 mg, 0.02 mmol) was initially charged in a round-bottom flask that had been dried by heating, and THF (5 mL) and 8-ethynyl-2,3,7,9-tetramethyl-9-(trifluoromethyl)-1,4-di-oxaspiro[4.5]dec-6-en-8-ol **13** (100 mg, 0.33 mmol) were added. Stirring at room temperature for 5 min was followed by the addition of tributyltin hydride (0.11 mL, 0.39 mmol). The resulting reaction mixture was stirred at 55 °C for 1 h and, after cooling to r.t., water was added. The aqueous phase was repeatedly extracted thoroughly with CH_2Cl_2, and the combined organic phases were then dried with $MgSO_4$, filtered and concentrated. under reduced pressure. By final column chromatography purification of the resulting crude product (ethyl acetate/heptane gradient), it was possible to obtain 2,3,7,9-tetramethyl-8-[(*E*)-2-(tributylstannyl)-vinyl]-9-(trifluoro-methyl)-1,4-dioxaspiro[4.5]dec-6-en-8-ol **14** (160 mg, 82 % of theoretical) in the form of a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.33/6.31 (d, 1 H), 5.97/5.92 (d, 1 H), 5.51/5.42 (s, 1 H), 4.24/3.64 (m, 2 H), 2.46/2.35 (d, 1 H), 1.92 (br. s, 1 H, OH), 1.91/1.87 (d, 1 H), 1.64/1.62 (s, 3 H), 1.49 (m, 6 H), 1.32–1.26 (m, 9 H), 1.18 (m, 3 H), 0.89 (m, 18 H) ppm. HRMS-ESI: calcd. for $C_{27}H_{48}O_3F_3Sn^+$ [M + H]$^+$ 597.2504, found 597.2518.

Ethyl (2*Z*,4*E*)-3-Cyclopropyl-5-[8-hydroxy-2,3,7,9-tetramethyl-9-(tri-fluoromethyl)-1,4-dioxaspiro[4.5]dec-6-en-8-yl]penta-2,4-dienoate (15u): Under argon, 2,3,7,9-tetramethyl-8-[(*E*)-2-(tributyl-stannyl)vinyl]-9-(trifluoromethyl)-1,4-dioxaspiro[4.5]dec-6-en-8-ol **14** (150 mg, 0.25 mmol) and ethyl (2*Z*)-3-cyclopropyl-3-iodoacrylate 6u (67 mg, 0.25 mmol) in a round-bottom flask that had been dried by heating were dissolved in tetrahydrofuran (4 mL), dichlorobis-(acetonitrile)palladium(II) (3 mg, 0.01 mmol) was added and the mixture was stirred at room temperature for 3 h. After the addition of potassium fluoride solution, the reaction mixture was stirred further at room temperature overnight. The aqueous phase was then repeatedly extracted thoroughly with diethyl ether, and the combined organic phases were then dried with magnesium sulfate, filtered and concentrated under reduced pressure. By final column chromatography purification of the resulting crude product (ethyl acetate/heptane gradient), ethyl (2*Z*,4*E*)-3-cyclopropyl-5-[8-hydroxy-2,3,7,9-tetramethyl-9-(trifluoromethyl)-1,4-dioxaspiro[4.5]dec-6-en-8-yl]penta-2,4-dienoate 15u (40 mg, 36 % of theoretical) was obtained in the form of a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.80/7.78 (d, 1 H), 6.40/6.38 (d, 1 H), 5.62/5.60 (s, 1 H), 5.52/5.44 (s, 1 H), 4.28/3.63 (m, 2 H), 4.18 (q, 2 H), 2.52/2.41 (d, 1 H), 2.03/1.94 (d, 1 H), 2.00 (br. s, 1 H, OH), 1.72/1.69 (s, 3 H), 1.62/1.55 (m, 1 H), 1.40–1.34 (m, 3 H), 1.29–1.17 (m, 6 H), 0.92 (t, 3 H), 0.83 (m, 2 H), 0.58 (m, 2 H) ppm. HRMS-ESI: calcd. for $C_{23}H_{32}O_5F_3^+$ [M + H]$^+$ 445.2115, found 445.2109.

Ethyl (2*Z*,4*E*)-3-Cyclopropyl-5-[1-hydroxy-2,6-dimethyl-4-oxo-6-(tri-fluoromethyl)cyclohex-2-en-1-yl]penta-2,4-dienoate (16u): Ethyl (2*Z*,4*E*)-3-cyclopropyl-5-[8-hydroxy-2,3,7,9-tetramethyl-9-(trifluoromethyl)-1,4-dioxaspiro[4.5]dec-6-en-8-yl]penta-2,4-dienoate **15u** (40 mg, 0.09 mmol) was dissolved in acetone (4 mL) under argon in a round-bottom flask, and a few drops of concentrated HCl were added. The resulting reaction solution was stirred at room temperature for 4 h and then water was added. After removing acetone under reduced pressure, the aqueous phase was extracted repeatedly with CH_2Cl_2. The combined organic phases were dried with $MgSO_4$, filtered and concentrated under reduced pressure. By column chromatography purification of the resulting crude product

(ethyl acetate/heptane gradient), ethyl (2*Z*,4*E*)-3-cyclopropyl-5-[1-hydroxy-2,6-dimethyl-4-oxo-6-(trifluoro-methyl)cyclohex-2-en-1-yl]penta-2,4-dienoate **16u** (15 mg, 45 % of theoretical) was obtained in the form of a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.88 (d, 1 H), 6.37 (d, 1 H), 6.01 (s, 1 H), 5.64 (s, 1 H), 4.17 (q, 2 H), 2.92 (d, 1 H), 2.52 (d, 1 H), 2.39 (br. s, 1 H, OH), 1.98 (s, 3 H), 1.59 (m, 1 H), 1.30 (t, 3 H), 1.28 (s, 3 H), 0.88 (m, 2 H), 0.59 (m, 2 H) ppm. HRMS-ESI: calcd. for $C_{19}H_{24}O_4F_3^+$ $[M + H]^+$ 373.1547, found 373.1555.

Methyl (2*Z*,4*E*)-5-[1-Hydroxy-2,6-dimethyl-4-oxo-6-(trifluoro-methyl)cyclohex-2-en-1-yl]-3-trifluormethyl-penta-2,4-dienoate (16r): Target compound **16r** (29 mg, 98 % of theoretical in the final step) was prepared and isolated in the form of a colorless viscous oil following the synthetic procedures described for **16u**. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.63/7.52 (d, 1 H), 6.39 (s, 1 H), 6.30/6.25 (d, 1 H), 6.03 (s, 1 H), 3.81 (s, 3 H), 2.93 (d, 1 H), 2.50 (d, 1 H), 2.34 (br. s, 1 H, OH), 1.95 (s, 3 H), 1.20 (s, 3 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 193.4; 164.7; 159.9; 137.1; 127.8, 126.7, 122.1, 121.4; 121.2; 78.2; 52.4, 42.9; 18.9; 18.6; 17.9; 16.8 ppm. LC-MS (ret. time, min, [M$^+$/log p]) 1.31 [386.1/2.97], HRMS-ESI: calcd. for $C_{16}H_{17}O_4F_6^+$ $[M + H]^+$ 386.2862, found 386.2850.

Methyl (2*Z*,4*E*)-3-Ethyl-5-[1-hydroxy-2,6-dimethyl-4-oxo-6-(trifluoro-methyl)cyclohex-2-en-1-yl]penta-2,4-dienoate (16c): Target compound **16c** (147 mg, 79 % of theoretical in the final step) was prepared and isolated in the form of a colorless viscous oil following the synthetic procedures described for **16u**. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.85 (d, 1 H), 6.01 (s, 1 H), 6.00 (d, 1 H), 5.79 (s, 1 H), 3.72 (s, 3 H), 2.91 (d, 1 H), 2.50 (d, 1 H), 2.43 (br. s, 1 H, OH), 2.40–2.36 (q, 2 H), 1.96 (s, 3 H), 1.27 (s, 3 H), 1.12 (t, 3 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 193.8; 166.6; 161.4; 154.3; 132.8, 128.5, 127.3, 117.6; 78.4; 51.3, 43.2; 27.2; 21.3; 18.8; 18.1; 17.5; 13.1 ppm. HRMS-ESI: calcd. for $C_{17}H_{21}O_4F_3^+$ $[M + H]^+$ 346.3414, found 346.3422.

***n*-Propyl (2*Z*,4*E*)-3-Ethyl-5-[1-hydroxy-2,6-dimethyl-4-oxo-6-(trifluoromethyl)cyclohex-2-en-1-yl]penta-2,4-dienoate (16d):** Target compound **16d** (128 mg, 83 % of theoretical) was prepared and isolated in the form of a colorless viscous oil following the synthetic procedures described for **16u**. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.86 (d, 1 H), 6.43/6.00 (d, 1 H), 5.98 (s, 1 H), 5.80 (s, 1 H), 4.08 (q, 2 H), 2.90 (d, 1 H), 2.49 (d, 1 H), 2.41 (br. s, 1 H, OH), 2.40–2.36 (q, 2 H), 1.96 (s, 3 H), 1.71–1.65 (m, 2 H), 1.27 (s, 3 H), 1.13 (t, 3 H), 0.96 (t, 3 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 193.8; 166.3; 161.5; 153.8; 132.6, 128.7, 127.3, 118.1; 78.4; 65.8; 50.6, 43.2; 27.2; 20.0; 18.8; 18.1; 13.1; 10.5 ppm. ^{19}F NMR (376 MHz, $CDCl_3$): δ = –67.07 (s, CF_3) ppm. LC-MS (ret. time, min, [M$^+$/log p]) 1.56 [374.3/3.28], HRMS-ESI: calcd. for $C_{19}H_{26}O_4F_3^+$ $[M + H]^+$ 375.1783, found 375.1779.

Ethyl (2*E*)-5-[1-Hydroxy-2,6-dimethyl-4-oxo-6-(trifluoro-methyl)cyclo-hex-2-en-1-yl]-3-(trifluoromethyl)pent-2-en-4-ynoate (17q): Copper(I) iodide (16 mg, 0.09 mmol) and bis(triphenylphosphine)palladium(II)chloride (45 mg, 0.06 mmol) were initially charged under argon in a flame-dried round-bottom flask, and toluene (3 mL) and ethyl (2*Z*)-4,4,4-trifluoro-3-iodobut-2-enoate (126 mg, 0.43 mmol) were added. Stirring at room temperature for 10 min was followed by the dropwise addition of a solution of 8-ethynyl-2,3,7,9-tetramethyl-9-(trifluoromethyl)-1,4-dioxaspiro[4.5]dec-6-en-8-ol 13 (130 mg, 0.43 mmol) in toluene (1 mL) and of diisopropylamine (0.12 mL, 0.85 mmol). The resulting reaction mixture was stirred at room temperature for 3 h and then water was added. The aqueous phase was extracted repeatedly with CH_2Cl_2. The combined organic phases were dried with magnesium sulfate, filtered and concentrated under reduced pressure. By final column chromatography purification of the crude product obtained (using an ethyl acetate/heptane gradient), ethyl (2*E*)-5-[8-hydroxy-2,3,7,9-tetramethyl-9-(trifluoromethyl)-1,4-dioxa-spiro[4.5]dec-6-en-8-yl]-3-(trifluoromethyl)pent-2-en-4-ynoate (110 mg, 52 % of theoretical) was isolated in the form of a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.65/6.63 (s, 1 H), 5.54/5.51/5.29 (s, 1 H), 4.28/3.96 (q, 2 H), 4.27/3.60 (m, 2 H), 2.62 (br. s, 1 H, OH), 2.47/2.34 (d, 1 H), 2.01/1.99 (s, 3 H), 1.98/1.91 (d, 1 H), 1.42 (s, 3 H), 1.31 (t, 3 H), 1.28 (m, 3 H), 1.17 (m, 3 H) ppm. Ethyl (2*E*)-5-[8-hydroxy-2,3,7,9-tetramethyl-9-(trifluormethyl)-1,4-dioxaspiro[4.5]dec-6-en-8-yl]-3-(trifluoro-methyl)pent-2-en-4-ynoate (110 mg, 0.23 mmol) was dissolved in acetone (5 mL) under argon in a round-bottom flask, and 5 drops of concentrated hydrochloric acid were added. The resulting reaction solution was stirred at room temperature for 4 h and then water was added. After removing acetone under reduced pressure, the aqueous phase was extracted repeatedly with dichloromethane. The combined organic phases were dried with magnesium sulfate, filtered and concentrated under reduced pressure. By column chromatography purification of the resulting crude product (ethyl acetate/heptane gradient), ethyl (2*E*)-5-[1-hydroxy-2,6-dimethyl-4-oxo-6-(trifluoromethyl)cyclohex-2-en-1-yl]-3-(trifluoromethyl)pent-2-en-4-ynoate **17q** (70 mg, 71 % of theoretical) was isolated in the form of a colorless viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.72/6.71 (s, 1 H), 5.98/5.97 (s, 1 H), 4.28/3.93 (q, 2 H), 3.28 (br. s, 1 H, OH), 3.00 (d, 1 H), 2.66 (d, 1 H), 2.21/2.18 (s, 3 H), 1.49/1.37 (s, 3 H), 1.32/1.09 (t, 3 H) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 194.9/193.6; 166.7/166.6; 154.5; 135.0; 130.8/130.2; 127.8/126.7; 100.3/99.9; 85.6/84.3; 76.3; 74.2; 61.9/59.7; 41.4; 19.7/18.4; 14.2/14.1 ppm. ^{19}F NMR (376 MHz, $CDCl_3$): δ = –67.09/–67.47 (s, CF_3), –68.91 (s, CF_3) ppm. LC-MS (ret. time, min, [M$^+$/log p]) 1.52 [398.10/3.27], HRMS-ESI: calcd. for $C_{17}H_{15}O_3F_6^+$ $[M + H]^+ - H_2O$ 381.2245, found 381.2241.

Ethyl (2*E*)-5-[1-Hydroxy-2,6-dimethyl-4-oxo-6-(trifluoro-methyl)cyclo-hex-2-en-1-yl]-3-(ethyl)pent-2-en-4-ynoate (17b): Target compound **17b** (40 mg, 91 % of theoretical) was prepared and isolated in the form of a colorless viscous oil following the synthetic procedures described for **17q**. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 6.09/6.07 (s, 1 H), 5.94/5.92 (s, 1 H), 4.21–4.16 (q, 2 H), 3.67 (br. s, 1 H, OH), 3.02/2.97 (d, 1 H), 2.70/2.62 (d, 1 H), 2.33 (q, 2 H), 2.21/2.19 (s, 3 H), 1.50 (s, 3 H), 1.28 (t, 3 H), 1.15 (t, 3 H) ppm. ^{19}F NMR (376 MHz, $CDCl_3$): δ = –67.14/–67.42 (s, CF_3) ppm. LC-MS (ret. time, min, [M$^+$/log p]) 1.48 [358.14/3.16], HRMS-ESI: calcd. for $C_{18}H_{20}O_3F_3^+$ $[M + H]^+ - H_2O$ 340.0426, found 340.0429.

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