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Herbicides

Inhibitors of the Herbicidal Target IspD: Allosteric Site Binding**

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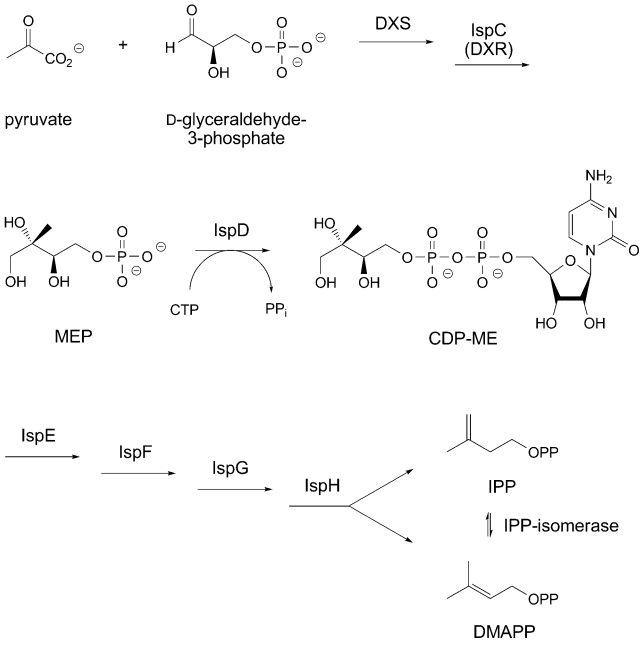
In memoriam Viktoriya Illarionova

After the identification of the non-mevalonate pathway for isoprenoid biosynthesis (Scheme 1) in the mid 1990s,[1] major efforts in academia and industry were started to use this essential pathway for the identification of new antibacterials, antimalarials, and also herbicides.[2] The emerging resistance problems of many weeds and pathogens reflect the urgent need for new actives with novel modes of action (MOA). The enzymes of the non-mevalonate pathway are especially interesting targets for anti-parasitic drugs or agrochemicals, as there exist no homologs in mammals and therefore no target related toxicological side-effects of inhibitors of this pathway are expected.

For commercial herbicides, the last novel MOA was introduced in 1980 with *p*-hydroxyphenyl pyruvate dioxygenase (HPPD).[3] Due to increasing resistance of weeds to almost all available herbicides, especially also to the commercially most important herbicide glyphosate,[4] new MOAs in the herbicide market are of high importance for the future world food production. Therefore, a program has been initiated to identify inhibitors of the non-mevalonate pathway in plants.

Besides antisense-based validation of the herbicidal potential of several of the enzymes in the pathway,[5] two herbicidal inhibitors were already known: Ketoclomazone for the enzyme DXS (1-deoxy-D-xylulose 5-phosphate synthase, EC 2.2.1.7)[6] and Fosmidomycin for the enzyme DXR (1-deoxy-D-xylulose 5-phosphate reductoisomerase, also called IspC, EC 1.1.1.267).[7] For the subsequent enzymes of the pathway, IspD-IspH, inhibitors for IspE[8] and IspF[9] have been reported recently.

As IspD has been validated by antisense experiments to be essential in plants, inhibitors of this enzyme were anticipated to be potentially herbicidal.[10] Therefore, at BASF a proprietary library of about 100000 diversity selected compounds was tested for the inhibition of the enzyme IspD of the plant *Arabidopsis thaliana* in a high throughput



Scheme 1. The non-mevalonate pathway for the biosynthesis of isoprenoids.[1] DXS: 1-deoxy-D-xylulose-5-phosphate synthase (EC 2.2.1.7), IspC/DXR: 1-deoxy-D-xylulose-5-phosphate reductoisomerase (EC 1.1.1.267), MEP: 2*C*-methyl-D-erythritol 4-phosphate, IspD: 4-diphosphocytidyl-2*C*-methyl-D-erythritol synthase (EC 2.7.7.60), CTP: cytidine triphosphate, CDP-ME: 4-diphosphocytidyl-2*C*-methyl-D-erythritol, IspE: 4-diphosphocytidyl-2*C*-methyl-D-erythritol kinase (EC 2.7.1.148), IspF: 2*C*-methyl-D-erythritol-2,4-cyclodiphosphate synthase (EC 4.6.1.12), IspG: 2*C*-methyl-D-erythritol-2,4-cyclodiphosphate reductase (EC 1.17.4.3), IspH: 1-hydroxy-2(*E*)-methylbutenyl-4-diphosphate reductase (EC 5.3.3.2), IPP: isopentenyl diphosphate, DMAPP: dimethylallyl diphosphate.

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screening (HTS, see the Supporting Information for the enzyme assay).[11]

Only few hits in the sub-micromolar and in the micromolar range could be identified, with the 7-hydroxy-[1,2,4]triazolo[1,5-*a*]pyrimidine **1** being the most active inhibitor with an IC_{50} value of (140 ± 10) nM. Based on the published crystal structure of the *A. thaliana* IspD enzyme (protein data bank (PDB) code: 1W77),[12] no reasonable docking mode of hit **1** in the active site could be proposed. Thus, it was decided to attempt a co-crystal structure of **1** with the enzyme, and a structure with 1.4 Å resolution (PDB code: 2YC3) could be obtained (Figure 1, and Supporting Information, Figures 1SI and 2SI).

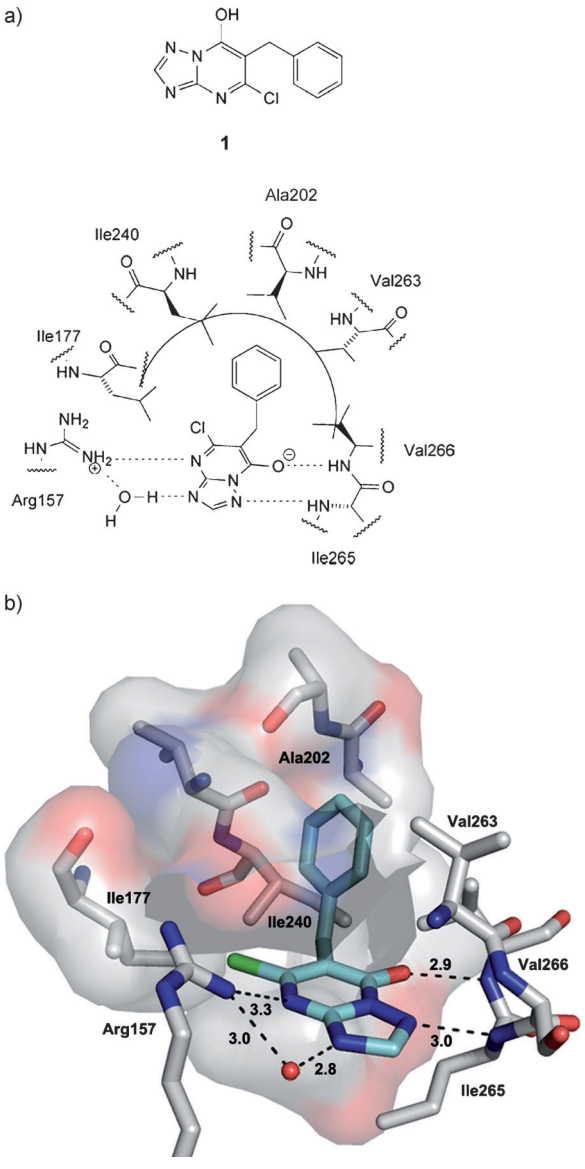


Figure 1. X-ray crystal structure of *A. thaliana* IspD co-crystallized with **1** at 1.4 Å resolution (PDB code: 2YC3). a) Chemical formula of **1** and schematic overview of its binding mode. b) Surface of the allosteric pocket. Distances are given in Å. C gray (enzyme) and cyan (inhibitor), N blue, O red, Cl green.

Surprisingly, the inhibitor does not bind to the presumed active site, but rather in a newly formed allosteric pocket in close proximity, resulting in a reduction in size of the active site.

The hydroxytriazolopyrimidine has a measured pK_a value of 3.9, and hence binds in the deprotonated form, bridging between the side chain of Arg157 and the backbone NH of Val266. Moreover, one nitrogen atom of the triazole substructure interacts with the backbone NH of Ile265. The second nitrogen is bound to Arg157 by a highly localized water molecule, so that the inhibitor is fixed by a network of four H-bonds. The heterobicycle is sandwiched between a H-bonding array involving the side chain NH of Gln238 and the backbone C=O of Asp262 on top, and an array of lipophilic side chains of Leu245, Ile265, and Val259 on the bottom (Supporting Information, Figure 2SI).

The benzyl moiety of **1** fits tightly into the lipophilic rear part of the pocket, composed of Val266, Val263, Ala202, and Ile240. This benzyl pocket provides almost no space for further derivatization. The *ortho* positions of the aromatic core point towards the lipophilic side chains of Ile240 and Val263, with C···C distances of 3.8 Å and 3.9 Å, respectively. Potential substituents in *meta* position would run into repulsion with the backbone carbonyl group of Val239 (distance C_{Bn}···O: 3.5 Å) and the side chain of Val263 (distance C_{Bn}···C: 3.7 Å). The *para* position is blocked by the side chain of Val204 with a C_{Bn}···C distance of 3.9 Å. This explicitly apolar environment is completed by the side chain of Ile177, interacting closely with the chlorine substituent of the ligand (distance C···Cl: 2.9 Å, see Supporting Information, Figure 2SI).

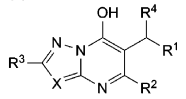
We became intrigued by the possibility to further enhance binding affinity through proper substitution of the crystallographically defined water molecule at the entrance channel to the binding pocket, which forms H-bonds to N(3) of **1** (for atom labeling, see Scheme 2) and the side chain of Arg157 (Figure 1). To explore this opportunity and to test the hypotheses derived from the examination of the X-ray co-crystal structure of **1**, a set of 17 derivatives (**2**–**18**, Table 1) was synthesized.

The synthesis of all 7-hydroxy[1,2,4]triazolo[1,5-*a*]pyrimidines and 7-hydroxypyrazolo[1,5-*a*]pyrimidines follows the same route illustrated in Scheme 2 for ligand **1** (experimental details are shown in the Supporting Information). Benzyl malonate **19** was condensed with aminotriazole **20**, resulting in the dihydroxytriazolopyrimidine **5**. Subsequent chlorination with $POCl_3$ gave the dichloropyrimidine **21**, which was regioselectively hydrolyzed in the 7-position with NaOH to yield the targeted 5-chloro-7-hydroxypyrimidine **1**.

The benzyl pocket is really tight. Introduction of an additional methyl group in the benzylic position ((±)-**14**) reduces the activity by a factor greater than 100 compared to **2**. Similarly, substituents on the aromatic ring of the benzyl moiety lead to a decrease in activity (comparison of **2** with **15**–**17**).

All changes on the heterobicyclic core besides the 3-position resulted in significant reduction of target activity (see **4**–**7**, **13**). Replacing the N(3) of **1** by C-Me (**8**) resulted in the expected drastic reduction in activity due to disruption of the

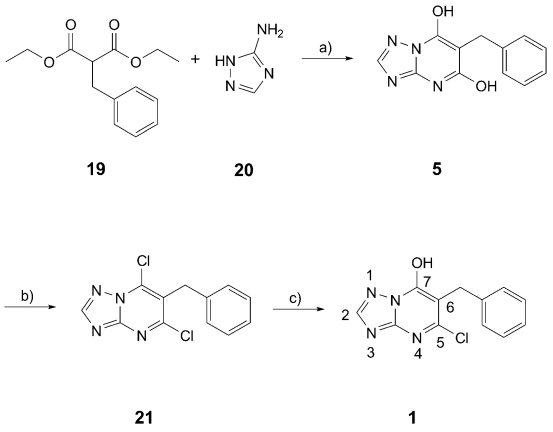
Table 1: Inhibition data of *A. thaliana* IspD and herbicidal activity on *Abutilon theophrasti* and *Setaria faberi* at 3 kg/ha by various azolopyrimidines.



general ligand structure

Ligand	R^1	R^2	R^3	R^4	X	IC_{50} [μM]	Herbicidal activity[a]
1	Ph	Cl	H	H	N	0.14 ± 0.01	+++
4	Ph	Me	H	H	N	20 ± 3	+++
5	Ph	OH	H	H	N	> 500	n.t.
6	Ph	Me	CO_2H	H	N	> 500	+
7	Ph	Me	CH_2OH	H	N	> 500	0
8	Ph	Cl	H	H	C-Me	43 ± 2	n.t.
3	Ph	Cl	H	H	C-CO_2H	274 ± 15	0
9	Ph	Cl	H	H	C-CO_2tBu	38 ± 3	0
10	Ph	Cl	H	H	C-CO_2Et	9.5 ± 0.6	0
11	Ph	Cl	H	H	C-CONH (*n*-pentyl)	18 ± 1	n.t.
12	Ph	Cl	H	H	C-$CONH_2$	6 ± 0.3	n.t.
2	Ph	Cl	H	H	C-CN	0.035 ± 0.007	+
13	Ph	OH	H	H	C-CN	> 500	0
(±)-**14**	Ph	Cl	H	Me	C-CN	3.1 ± 0.3	0
15	2-F-Ph	Cl	H	H	C-CN	0.25 ± 0.01	0
16	3-F-Ph	Cl	H	H	C-CN	0.27 ± 0.02	0
17	3-Cl-Ph	Cl	H	H	C-CN	0.45 ± 0.05	0
18	*i*Pr	Cl	H	H	N	91 ± 12	n.t.

[a] n.t. = not tested.



Scheme 2. Synthesis of triazolopyrimidine **1**. a) NBu_3, 150 °C, 8 h, 25%; b) $POCl_3$, $HNMe_3Cl$, 150 °C, 15 h, 48%; c) NaOH, THF, 25 °C, 14 h, 57%.

hydrogen-bonding network. However, introducing a H-bond acceptor that can interact with the positively charged Arg157 side chain and thus replace the water molecule, should be favorable.[13] Figure 2 shows the structures of two such derivates, nitrile **2** and carboxylic acid **3**, as well as the respective co-crystal structures with the *A. thaliana* IspD enzyme (**2**: 1.6 Å resolution, PDB code: 2YC5; **3**: 1.8 Å resolution, PDB code: 2YMC). Both ligands adopt a very similar binding geometry in the allosteric site to the one seen for **1**.

The nitrile and the carboxylate participate in the H-bonding network and thus replace the water molecule that was present in the co-crystal structure with **1**. As a result, nitrile **2** exhibits an IC_{50} value of (35 ± 7) nM, a fourfold increase in activity compared to **1**, which can be explained by the energetically favorable water replacement.[13] The activity of carboxylate **3** (IC_{50} value: (274 ± 15) μM), however, is lowered by a factor of almost 2000. This result is rather surprising, considering that the pK_a values of the carboxylic acid and the aromatic OH group were determined to be 2.32, and 5.22, respectively, and that **3** would therefore potentially bind as a di-anion. We explain the low activity of **3** with the high energetic costs to be paid for the desolvation of the carboxylate moiety upon binding. Carboxylic esters and amides (**9**–**12**) are more potent than the acid, but much less than the lead ligands **1** and **2**.

It is noteworthy to discuss that in the crystal structure of *A. thaliana* IspD with cytidine monophosphate (CMP) (PDB code: 1W77), the allosteric pocket filled by ligands, such as **1**–**3**, is not occupied and actually not fully developed. This is a result of the geometry and flexibility of the loop between Glu255 and Tyr268 with partly unresolved side chains. The loop fully opens the pocket and adopts an ordered conformation only in the presence of a suitable ligand, such as **2**. Figure 3 shows an overlay of the two co-crystal structures of *A. thaliana* IspD with CMP and **2**, respectively.

The lower part of the Figure shows an enlargement of the loop in both structures. Whereas Glu255 of the 1W77 structure (green) and Glu255* of the structure co-crystallized with **2** (gray) still show a comparable alignment, it can be seen that the loop adopts a different conformation starting with the following amino acid residues. Especially the side chain of Glu258*, pointing outside of the protein in the complex of **2**, points inside in the 1W77 structure, into the space of the allosteric binding pocket. Moreover, the side chain of Asp262* turns from the outside of the protein (in 1W77) into the direction of the MEP-binding site (in 2YC5) and thus contributes to the allosteric inhibition effect of **2** by a reduction in size of the active site on the one hand, presumably by electrostatic repulsion with the anionic substrate on the other.

The original lead **1** showed very good herbicidal activity (+++) in the greenhouse at a dose of 3 kg/ha on two key weeds in corn, *Abutilon theophrasti* and *Setaria faberi* (Table 1). The analogues with lower target activity showed weaker or no observable herbicidal activity. The highly target-active nitrile derivative **2** displayed in the same assay also some activity in the greenhouse (+), but on a lower level than lead structure **1**. Changing the adjuvant to the more acidic

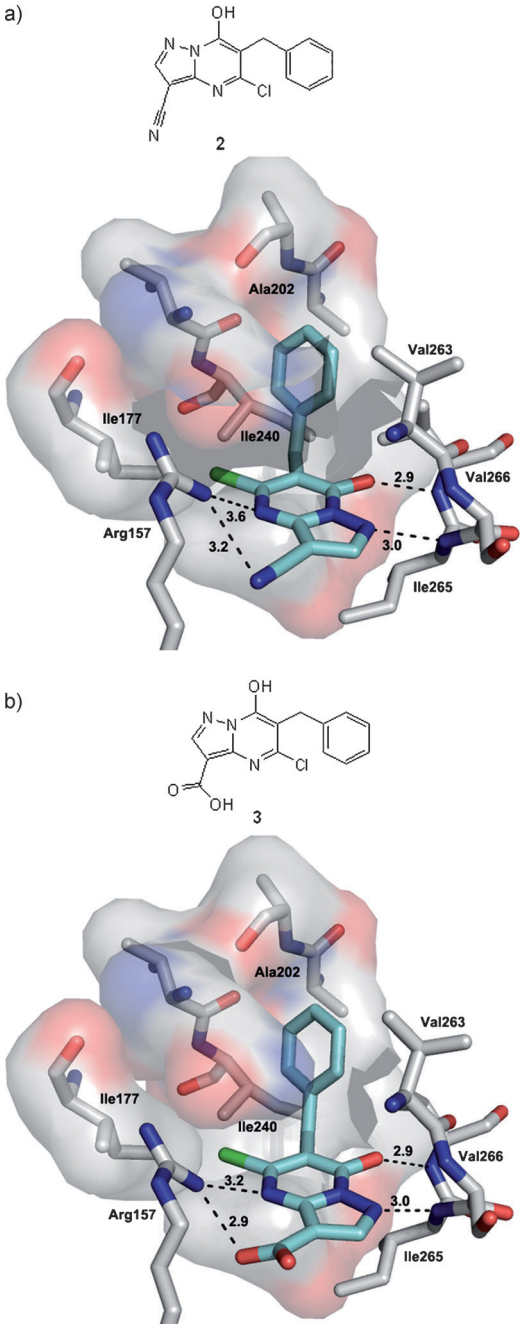


Figure 2. X-ray crystal structures of a) **2** (1.6 Å resolution, PDB code: 2YC5) and b) **3** (1.8 Å resolution, PDB code: 2YMC) co-crystallized with *A. thaliana* IspD. Distances are given in Å. C gray (enzyme) and cyan (inhibitor), N blue, O red, Cl green.

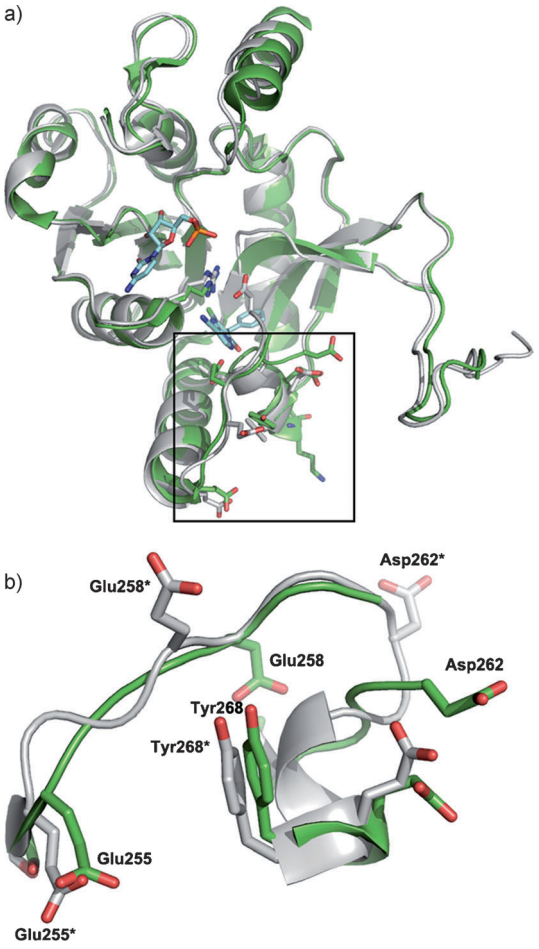


Figure 3. Schematic overlay representation of the two co-crystal structures of *A. thaliana* IspD. Green: with CMP (PDB code: 1W77), gray: with **2** (PDB code: 2YC5). a) Overview of the complete protein. b) Flexible loop between Glu255 and Tyr268 (residues marked with an asterix belong to the co-crystal structure with **2**). Color code for the protein side chains and ligands: C gray or green (enzyme) and cyan (CMP and inhibitor **2**), N blue, O red, Cl green, P orange.

DASH,[14] the one-week rating could be improved, for example, for **2** and **15** to good activity (++), indicating limitations in the uptake of these compounds. The activity of these compounds is at an interesting level for a lead structure. An improvement in target- and greenhouse activity by a factor of at least ten will still be required to approach commercially acceptable level.

To our knowledge, the azolopyrimidines are the first described inhibitors of the enzyme IspD. Analysis of the binding mode in the newly formed allosteric pocket and subsequent structure optimization has resulted in an enhanced activity down to IC_{50} values of (35 ± 7) nM. For several target active analogues, we could also show herbicidal activity. A potential feedback regulatory function of the allosteric pocket by binding of later products of the non-mevalonate-pathway is currently under investigation.

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[1] a) M. Rohmer, M. Knani, P. Simoni, B. Sutter, H. Sahm, *Biochem. J.* **1993**, *295*, 517–524; b) M. K. Schwarz, Dissertation No. 10951, ETH Zürich, **1994**; c) S. T. J. Broers, Dissertation No. 10978, ETH Zürich, **1994**; d) W. Eisenreich, B. Menhard, P. J. Hylands, M. H. Zenk, A. Bacher, *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 6431–6436; e) W. Eisenreich, M. Schwarz, A. Cartayrade, D. Arigoni, M. H. Zenk, A. Bacher, *Chem. Biol.* **1998**, *5*,

R221 – R233; f) W. Eisenreich, F. Rohdich, A. Bacher, *Trends Plant Sci.* **2001**, *6*, 78 – 84; g) W. Eisenreich, A. Bacher, D. Arigoni, F. Rohdich, *Cell. Mol. Life Sci.* **2004**, *61*, 1401 – 1426.

[2] H. Jomaa, J. Wiesner, S. Sanderbrand, B. Altincicek, C. Weidemeyer, M. Hintz, I. Turbachova, M. Eberl, J. Zeidler, H. K. Lichtenthaler, D. Soldati, E. Beck, *Science* **1999**, *285*, 1573 – 1576.

[3] H. Matsumoto, M. Mitzutani, T. Yamaguchi, J. Kadotani, *Weed Biol. Manage.* **2002**, *2*, 39 – 45.

[4] S. C. Weller, M. D. K. Owen, W. G. Johnson in *Glyphosate Resistance in Crops and Weeds* (Eds.: V. K. Nandula), Wiley, Hoboken, **2010**, pp. 213 – 232.

[5] K. Okada, H. Kawaide, T. Kuzuyama, H. Seto, I. S. Curtis, Y. Kamiya, *Planta* **2002**, *215*, 339 – 344.

[6] C. Müller, J. Schwender, J. Zeidler, H. K. Lichtenthaler, *Biochem. Soc. Trans.* **2000**, *28*, 792 – 793.

[7] a) F. Rohdich, S. Lauw, J. Kaiser, R. Feicht, P. Köhler, A. Bacher, W. Eisenreich, *FESB J.* **2006**, *273*, 4446 – 4458; b) T. Kuzuyama, T. Shimizu, S. Takahashi, H. Seto, *Tetrahedron Lett.* **1998**, *39*, 7913 – 7916; c) J. Zeidler, J. Schwender, C. Müller, J. Wiesner, C. Weidemeyer, E. Beck, H. Jomaa, H. K. Lichtenthaler, *Z. Naturforsch. C* **1998**, *53*, 980 – 986.

[8] A. K. H. Hirsch, M. S. Alphey, S. Lauw, M. Seet, L. Barandun, W. Eisenreich, F. Rohdich, W. N. Hunter, A. Bacher, F. Diederich, *Org. Biomol. Chem.* **2008**, *6*, 2719 – 2730.

[9] a) C. M. Crane, J. Kaiser, N. L. Ramsden, S. Lauw, F. Rohdich, W. Eisenreich, W. N. Hunter, A. Bacher, F. Diederich, *Angew. Chem.* **2006**, *118*, 1082 – 1087; *Angew. Chem. Int. Ed.* **2006**, *45*, 1069 – 1074; b) J. G. Geist, S. Lauw, V. Illarionova, B. Illarionov, M. Fischer, T. Gräwert, F. Rohdich, W. Eisenreich, J. Kaiser, M. Groll, C. Scheurer, S. Wittlin, J. L. Alonso-Gómez, W. B. Schweizer, A. Bacher, F. Diederich, *ChemMedChem* **2010**, *5*, 1092 – 1101.

[10] a) F. Rohdich, J. Wungsintaweekul, W. Eisenreich, G. Richter, C. A. Schuhr, S. Hecht, M. H. Zenk, A. Bacher, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 6451 – 6456; b) M. Fellermeier, M. Raschke, S. Sagner, J. Wungsintaweekul, C. A. Schuhr, S. Hecht, K. Kis, T. Radykewicz, P. Adam, F. Rohdich, W. Eisenreich, A. Bacher, D. Arigoni, M. H. Zenk, *Eur. J. Biochem.* **2001**, *268*, 6302 – 6310.

[11] a) V. Illarionova, J. Kaiser, E. Ostrozhenkova, A. Bacher, M. Fischer, W. Eisenreich, F. Rohdich, *J. Org. Chem.* **2006**, *71*, 8824 – 8834; b) A. Bacher, M. H. Zenk, W. Eisenreich, M. Fellermeier, M. Fischer, S. Hecht, S. Herz, K. Kis, H. Lüttgen, F. Rohdich, S. Sagner, C. A. Schuhr, J. Wungsintaweekul, WO 2001011055, **2001**.

[12] M. Gabrielsen, J. Kaiser, F. Rohdich, W. Eisenreich, R. Laupitz, A. Bacher, C. S. Bond, W. N. Hunter, *FESB J.* **2006**, *273*, 1065 – 1073.

[13] a) M. Ellermann, R. Jakob-Roetne, C. Lerner, E. Borroni, D. Schlatter, D. Roth, A. Ehler, M. G. Rudolph, F. Diederich, *Angew. Chem.* **2009**, *121*, 9256 – 9260; *Angew. Chem. Int. Ed.* **2009**, *48*, 9092 – 9096; b) H. Yu, S. W. Rick, *J. Am. Chem. Soc.* **2009**, *131*, 6608 – 6613.

[14] T. Whitwell, K. E. Kalmowitz, G. S. Stapleton, *J. Environ. Hortic.* **1992**, *10*, 55 – 58.

