LETTERS

Total synthesis of a chlorosulpholipid cytotoxin associated with seafood poisoning

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Each year, there are many cases of seafood poisoning in humans worldwide[1]. Among the various toxins isolated that contribute to these poisonings[2,3], the chlorosulpholipids are particularly intriguing because of their structural and stereochemical complexity[4–12]. The mechanism of biological activity remains unknown and, although chlorosulpholipids are associated with membranes in the organisms from which they are isolated, little is understood about their role within biological membranes. The lack of availability of the natural products has impaired more in-depth biochemical studies. So far, none of the chlorosulpholipids have been obtained from total synthesis, and efficient routes to their synthesis would be desirable for the preparation of material for pharmacological characterization and proper evaluation of the risk to human health. Despite the notable advances in the science of organic synthesis, reliable methods for stereoselective construction of polychlorinated acyclic substrates are lacking, although some preliminary investigations have appeared[13–15]. Here we report the synthesis of a chlorosulpholipid cytotoxin, leading to confirmation of the proposed structure and the discovery of unanticipated reactivity of polychlorinated hydrocarbons. The concise synthetic approach should enable the preparation of material in sufficient quantities to facilitate biological studies.

Marine toxins have attracted the interest of investigators as a consequence of the associated health risk to human populations and the accompanying socioeconomic impact[3]. Seafood poisoning is partly associated with the consumption of shellfish contaminated with viral and bacterial microorganisms. Additionally, a significant percentage of cases is caused by toxins produced by microalgae, especially dinoflagellates, and transferred to humans by marine bivalve molluscs[1,2]. The ensuing food poisoning can be severe and even life threatening[1]. Marine toxins such as the tetrodotoxins, saxitoxins, ciguatera toxins and brevetoxins[2,3] have been classified according to their chemical structures, and have been subjects of enormous interest to the chemical, biological and medical communities (see refs 16 and 17 and references within). A class of marine toxins that has received less attention is the chlorosulpholipids, which bear stereochemically complex, polychlorinated acyclic carbon chains and one or two *O*-sulphate esters. Chlorosulpholipids were first isolated in the late 1960s from the phytoflagellate *Ochromonas danica*[4]; the most abundant of these was later identified as compound **1** (Fig. 1)[5].

Although a large number of algae have been examined[7,8], only a few chlorosulpholipids have been structurally characterized, such as malhamensilipin A (**2**) (Fig. 1)[9]. This compound is responsible for the major antimicrobial activity in the extract of the chrysophyte *Poteriochromonas malhamensis* and displays activity in kinase assays[9]. The first chlorosulpholipids (**3**–**5**) whose relative and absolute configuration have been assigned using *J*-based configuration analysis (see ref. 18 and references within) were isolated only a few years ago from organisms in the Adriatic Sea and probably originate from harmful microalgae accumulated in mussels (Fig. 1)[10–12]. They have also been screened for their antiproliferative activity and shown to inhibit cell growth[10–12].

Chlorosulpholipid **5** drew our attention as a target for synthetic studies because of its stereochemical complexity and, more significantly, the embedded issues of reactivity that these structures present. The result of initial synthetic efforts involving model systems led us to conclude that construction of such systems would have to take into account the unique behaviour and properties of a polychlorinated backbone with electron-withdrawing groups. As a relevant benchmark, sucralose, the key ingredient of Splenda, incorporates two 1° and one 2° chlorides on a disaccharide core and is sufficiently stable and safe to be widely used as an artificial sweetener. In a similar fashion, in preliminary investigations we observed that displacement reactions of activated alcohol derivatives to furnish the corresponding alkyl chlorides proved unworkable when the carbinol bears two methine substituents with electron-withdrawing groups, such as chlorides. Additionally, we noted that α- and β-chlorinated alde-

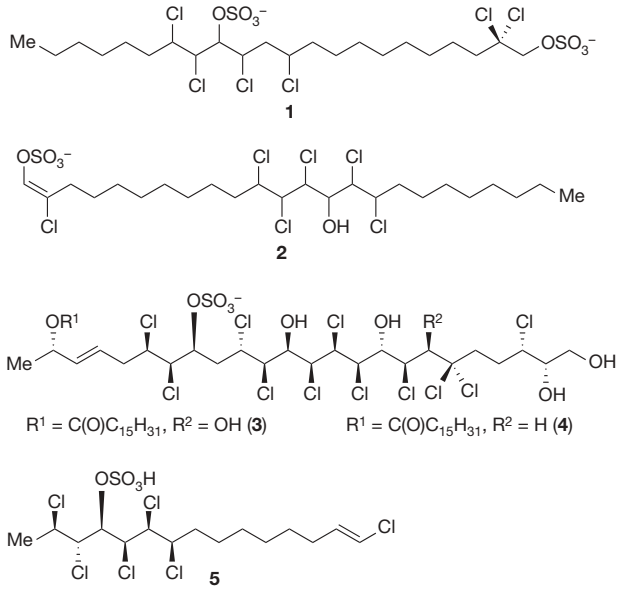

Figure 1 | Structurally characterized docosane, tetracosane and pentadecane chlorosulpholipids that originate from microalgae. Disulphate **1** and vinyl sulphate **2** have been isolated from *Ochromonas danica* and *Poteriochromonas malhamensis*, respectively, and their configurations have not been assigned. Lipids **3**–**5** have been extracted from digestive glands of contaminated shellfish *Mytilus galloprovincialis* along the Adriatic coast of Italy and are associated with seafood poisoning. They have been configurationally assigned by NMR spectroscopy using *J*-based configuration analysis. Me, H_3C.

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hydes are fleeting intermediates, which undergo enolization, hydration or elimination too rapidly. Thus, we sought to implement strategic approaches that would circumvent these limitations in crafting a synthesis route to chlorosulpholipid **5**.

The synthesis began with stereospecific dichlorination of ethyl sorbate (**6**) using $(H_5C_2)_4NCl_3$[19]. This step was followed by reduction of the ester in **7** (diisobutylaluminium hydride (DIBAL), 72% yield) and protection of the hydroxyl function in the ensuing alcohol **8** to furnish **9**. Dihydroxylation of **9** with *N*-methylmorpholine-*N*-oxide (NMO) and 5 mol% OsO_4 afforded a mixture of diastereomers in a selectivity of 5.6:1, with a 68% yield of the major diastereomer. At this point in the synthesis, we assumed that the configuration of the major diol corresponded to that expected from Kishi's empirical rule, which has proven to be highly reliable in predicting the relative configuration for the dihydroxylation reaction of allylic alcohols[20]. Treatment of the major diol diastereomer with triflic anhydride and 1,4-diazabicyclo[2.2.2]octane (DABCO) and subsequent desilylation led to *cis*-epoxide **10** (74% yield over two steps). The structure of the epoxide was determined by careful analysis of the ^{1}H NMR spectrum of **10** and later by *J*-based configuration analysis of **15** and **16** (see Supplementary Information), which indicated an *anti*-relationship between C3 and C4.

Epoxide **10** was converted into **12** as a mixture of alkenes (*Z*/*E* isomeric ratio, 4.2:1) through the sequence of reactions including oxidation and Wittig olefination with phosphonium bromide **11**. We were then able to examine a key transformation involving regioselective opening of the epoxide with chloride to furnish the targeted chlorohydrin. As discussed below, this had unexpected results.

After separation of *Z*-**12** from the obtained *Z*/*E* mixture, we treated it with $(H_3C)_3SiCl$, which led to a mixture of diastereomeric allylic chlorides *Z*-**15** in 39% yield (56% based on recovered starting material), minor amounts of *Z*-**16** (4%) and a 10% yield of S_N2' products. The major isolated chlorohydrin diastereomer (*Z*-**15**) was initially assumed to have the 4,5-*syn* relative configuration found in the natural product. Given the proximity to the end of the synthesis, we were confident that comparison to the natural product would prove decisive. Thus, allylic chloride **15** was transformed to chlorosulpholipid **17** in a few steps involving desilylation, oxidation, Takai–Utimoto olefination[21] and a final *O*-sulphation (Fig. 2). Surprisingly, the spectral data for **17** did not match that reported for the natural product **5** in the characteristic region of 4–5 p.p.m. of the ^{1}H NMR spectrum.

This unexpected result compelled us to re-examine and re-evaluate the various steps in the sequence described above. Studies conducted in parallel involving the isolation and characterization of various diastereomers of crystalline polychlorinated diols with subsequent *J*-based configuration analysis (see ref. 18 and references within) enabled the spectroscopic determination of the relative configuration of polychlorinated structure **15**. The key data were extracted using HETLOC (analysis of heteronuclear long-range couplings), HECADE (heternuclear couplings from ASSCI-domain experiments with E.COSY-type cross peaks)[22], refocused PS-HMBC (phase-sensitive heteronuclear multiple-bond correlation), as well as NOESY (nuclear Overhauser enhancement spectroscopy). The small ^3J(H,H) coupling constant of 2.3 Hz between H4 and H5, in combination with the corresponding pairwise large and small ^2J(H,C) and ^3J(H,C) coupling constants in allylic chloride *Z*-**15**, indicated that the stereogenic centres C4 and C5 possessed *anti*-configuration and not the anticipated *syn*-arrangement. Inevitably, this meant that opening of allylic epoxide *Z*-**12** (**12** → **15**) had preferentially occurred with retention of configuration rather than the predicted inversion. Additional data (see Supplementary Information) allowed us to identify the minor diastereomeric product *Z*-**16** as possessing the desired C4-C5

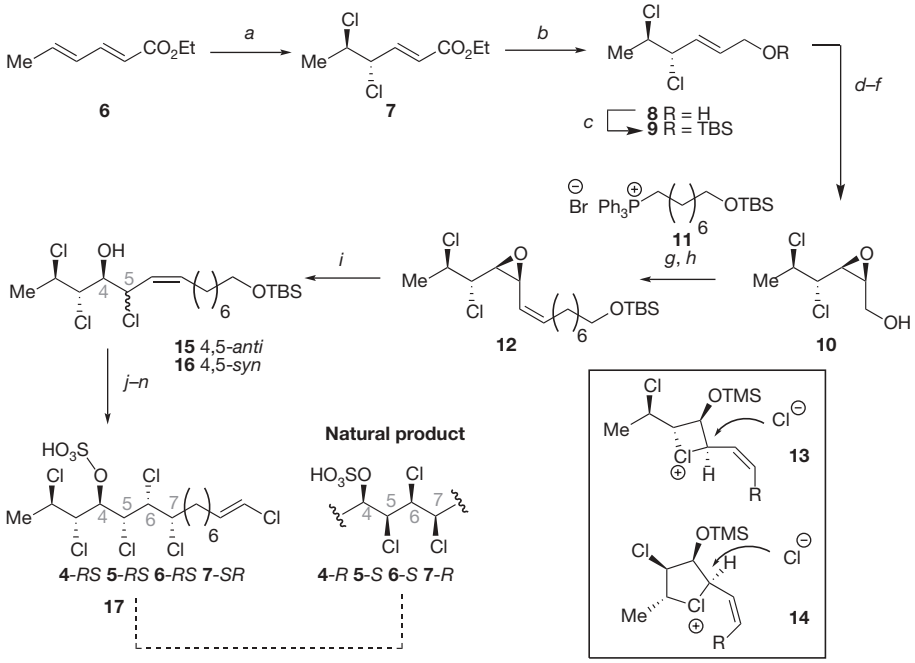

Figure 2 | Initial synthetic studies of chlorosulpholipid 5. Reagents and conditions: (*a*) $(H_5C_2)_4NCl_3$, CH_2Cl_2, 0 °C, 45 min, 68%; (*b*) DIBAL (2.3 equiv.), $H_5C_2H_5C_6$, 0 °C, 10 min, 72%; (*c*) imidazole (1.5 equiv.), *t*-Bu$(H_3C)_2SiCl$ (1.2 equiv.), CH_2Cl_2, 0 °C to room temperature (RT, 20 °C), 30 min, 87%; (*d*) OsO_4 (5 mol%), NMO (1.1 equiv.), acetone/H_2O, RT, 19 h, 68%; (*e*) DABCO (3.0 equiv.), $(F_3CSO_2)_2O$ (1.0 equiv.), −78 °C, 10 min, then diol, −78 °C to RT, 15 h, 75% (96% based on recovered starting material); (*f*) (+)-CSA (0.1 equiv.), $(H_3C)OH$, RT, 3 h, 98%; (*g*) $(COCl)_2$ (1.3 equiv.), $(H_3C)_2SO$ (2.5 equiv.), CH_2Cl_2, −78 °C, 10 min, then **10** (1.0 equiv.), −78 °C, 30 min, then $(H_5C_2)_3N$ (5.4 equiv.), −78 °C to RT, 1 h; (*h*) **11** (1.05 equiv.), *n*-BuLi (1.05 equiv.), THF, −78 °C, then RT, 10 min, followed by aldehyde (1.0 equiv.) at −78 °C, 5 min, then RT, 30 min, 62% over two steps; (*i*) $(H_3C)_3SiCl$ (2.0 equiv.), CH_2Cl_2, $H_3CCO_2C_2H_5$, 11.5 h, 39% **15**, 4% **16**, 10% mixture of S_N2' products (31% starting material recovered); (*j*) $(H_5C_2)_4NCl_3$ (3.0 equiv.), CH_2Cl_2, 0 °C, 10 min, 51%; (*k*) (+)-CSA (10 mol%), H_3COH, 12 h, 80%; (*l*) DAIB (1.1 equiv.), TEMPO (0.1 equiv.), CH_2Cl_2, RT, 16.5 h; (*m*) $CrCl_2$ (6.9 equiv.), $CHCl_3$ (2.6 equiv.), THF, 65 °C, 49% over two steps; (*n*) SO_3-pyridine (6.0 equiv.), THF, 30 min, 27% (66% starting material recovered). Bu, H_9C_4; CSA, 10-camphorsulphonic acid; THF, tetrahydrofuran; DAIB, (diacetoxyiodo)benzene; TEMPO, 2,2,6,6-tetramethylpiperidine-1-oxyl; TBS, *t*-$H_9C_4(H_3C)_2Si$; TMS, $(H_3C)_3Si$.

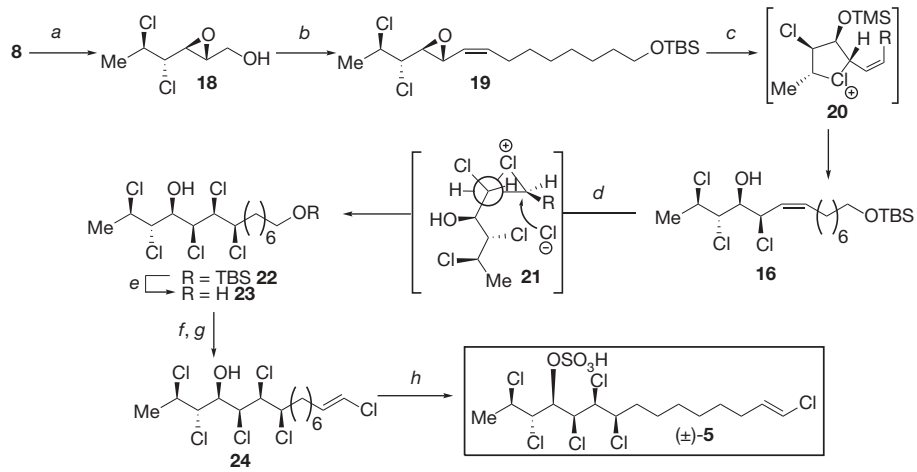

Figure 3 | Total synthesis of chlorosulpholipid cytotoxin 5. Reagents and conditions: (*a*) *m*-CPBA, CH_2Cl_2, 0 °C to RT, d.r. = 1:1, 95% overall; (*b*) 4 Å molecular sieves, NMO (1.1 equiv.), TPAP (5 mol%), CH_2Cl_2, 6 h; **11** (1.6 equiv.), *n*-BuLi (1.6 equiv.), THF, −78 °C, RT, 10 min; then addition of the aldehyde solution to the phosphonium ylide at −78 °C, 1 h, then RT, 1.5 h, 34% (56% based on recovered starting material); (*c*) $(H_3C)_3SiCl$ (2.0 equiv.), CH_2Cl_2, $H_3CCO_2C_2H_5$, 9 h, 43% (73% based on recovered starting material); (*d*) $(H_5C_2)_4NCl_3$ (3.0 equiv.), CH_2Cl_2, −78 °C, 2 h, d.r. = 10:1, 93% overall; (*e*) (+)-CSA (10 mol%), H_3COH, 12 h, 98%; (*f*) DAIB (1.3 equiv.), TEMPO (0.2 equiv.), CH_2Cl_2, RT, 16.5 h; (*g*) $CrCl_2$ (6.8 equiv.), $CHCl_3$ (2.5 equiv.), THF, 65 °C, 47% over two steps; (*h*) SO_3-pyridine (3.0 equiv.), THF, 20 min, 99%. TPAP, tetra-*n*-propylammonium perruthenate(VII).

syn-configuration. We hypothesize that anchimeric participation of one of the chlorides at C2 and C3 over the course of the ring-opening reaction is key to understanding the unexpected stereochemical outcome.

A small number of mechanistic studies from the 1960s and 1970s suggest the intervention of four- and five-membered ring chloronium ions as intermediates in solvolytic displacement reactions in F_3CCO_2H, H_3CCO_2H or HCO_2H (ref. 23; for anchimeric participation of chloride in epoxide opening processes, see ref. 24). It was shown that solvolysis of *erythro* and *threo* 5-chloro-2-hexyl tosylates occurs with up to 92% retention, depending on the solvent[23]. This suggested anchimeric participation through formation of a chloronium intermediate in these simple systems and was supported by kinetic studies[23]. On the basis of this data, it may seem that the five-membered chloronium ion **14** should be favoured over the four-membered chloronium ion **13**. However, in the absence of additional data for the more complicated systems discussed here, we are not able to distinguish unambiguously between the two alternatives (Fig. 2).

The finding that ring opening of the allylic epoxide can suffer interference from neighbouring chlorides necessitated alteration of the strategy. Because the problem is associated with one of the epoxide termini, the solution required investigating the ring-opening reaction of the corresponding *trans*-epoxide. However, it is important to note that for this solution to be workable, it is a necessary requirement that opening of the *trans*-epoxide diastereomer proceeds with retention of configuration, following the mechanistic pathway observed for the *cis*-stereoisomer.

The required allylic *trans*-epoxide **18** was prepared as shown in Fig. 3. Allylic alcohol **8** was treated with *m*-chloroperbenzoic acid (*m*-CPBA) to afford a diastereomeric mixture of epoxy alcohols (diastereomeric ratio (d.r.) = 1:1), which could be separated by chromatography on silica gel. The two regioisomeric diols obtained by treatment of **18** with $TiCl(O\textit{i}\text{-}C_3H_7)_3$ were crystalline, and therefore allowed the epoxide configuration to be established unambiguously using X-ray structural analysis (Fig. 4). Ley oxidation of alcohol **18** and *in situ* Wittig olefination[25] gave alkene **19**. Notably, the selectivity (*Z*/*E*, 7:1) was considerably improved over that observed for **12**. Treatment of **19** with $(H_3C)_3SiCl$ gave chlorohydrin **16** in 73% yield (based on recovered starting material). The opening was demonstrated to proceed exclusively with retention of configuration (judged using ^1H NMR), in analogy to that observed above for *cis*-epoxide **12**. Dichlorination of alkene **16** with $(H_5C_2)_4NCl_3$ at −78 °C gave pentachloride **22** together with a minor diastereomer (d.r. = 10:1) in 93% yield. As investigated using *J*-based configuration analysis, the diastereoselectivity is in agreement with model **21** (ref. 26). Deprotection of the silyl ether with acid, selective oxidation of the primary alcohol with TEMPO and $H_5C_6I(O_2CCH_3)_2$ and subsequent Takai–Utimoto olefination[21] produced alcohol **24**. Treatment of **24** with py-SO_3-pyridine afforded **5** in 99% yield. The spectroscopic data for **5** are in agreement with that reported for the natural product in both *d*6-acetone as well as *d*4-methanol (see Supplementary Information)[10]. To further confirm the identity of the synthetic and natural cytotoxin **5**, we independently analysed the homonuclear (H,H) and heteronuclear (H,C) coupling constants of **5**, which led to the assignment of the same relative configuration as that originally proposed in ref. 10.

In conclusion, we have presented the diastereoselective synthesis of a member of the chlorosulpholipid cytotoxin family. Our studies led to the confirmation of the proposed relative configuration and, more broadly, contribute to a database to facilitate *J*-based configuration analysis of polychlorinated structures, which may assist in the

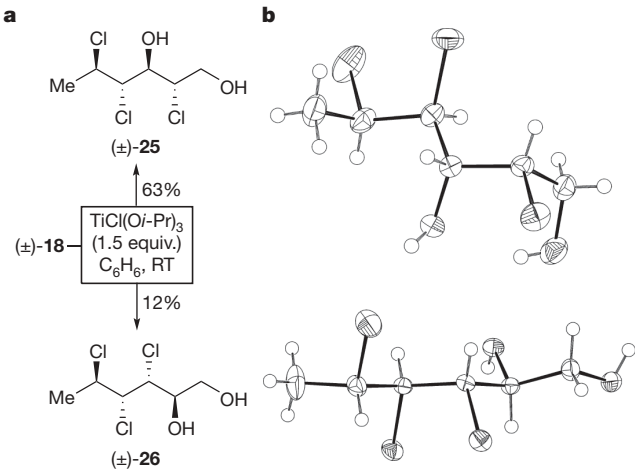

Figure 4 | Determination of the relative configuration of epoxyalcohol 18. a, Treatment of **18** with $TiCl(O\textit{i}\text{-}C_3H_7)_3$ provided a mixture of crystalline diols **25** and **26**. **b**, The X-ray structures allowed the determination of the relative configuration of diols **25** and **26**, which in turn permitted the configurational assignment of epoxyalcohol **18**.

stereochemical assignment of numerous other chlorosulpholipids such as **1** and **2**. A key observation in the synthetic studies is the retentive epoxide-opening reaction that most likely involves an intermediate cyclic chloronium ion. In this respect, the proposed anchimeric participation of chlorides in these complex systems will need to be given careful consideration in any future efforts towards synthesizing the chlorosulpholipids. Additionally, the concise synthesis can provide enough material for biological and pharmacological studies and, thus, the evaluation of the role of chlorosulpholipid cytotoxins in marine ecosystems and their effects on human health.

METHODS SUMMARY

All reactions were carried out under argon under anhydrous conditions, unless otherwise stated. Commercially available reagents were purchased and used without further purification unless otherwise noted. Reactions were monitored by thin-layer chromatography and products identified by NMR and infrared spectroscopy as well as mass spectrometry. For experimental details and characterization of all new compounds (^{1}H NMR, ^{13}C NMR, infrared, mass spectrometry, melting points), the determination of the relative configuration of epoxyalcohol **18** including the crystallographic data of diols **25** and **26**, the data for *J*-based configuration analysis of compounds **15**, **16**, **22** and synthetic (±)-**5** and the ^{1}H NMR spectra of the key compounds **8**, **10**–**12**, **15**–**19**, **22**, **32** and synthetic (±)-**5**, see the Supplementary Information.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Information Supplementary crystallographic data for this paper has been deposited at the Cambridge Crystallographic Data Centre under deposition numbers CCDC 711103 and CCDC 711104. These data can be obtained free of charge from www.ccdc.cam.ac.uk/data_request/cif. Reprints and permissions information is available at www.nature.com/reprints. Correspondence and requests for materials should be addressed to E.M.C. (carreira@org.chem.ethz.ch).

METHODS

General methods. All reactions were carried out under an argon atmosphere in flame-dried glassware, unless stated otherwise. THF, $(H_5C_2)_2O$, H_3CCN, toluene and CH_2Cl_2 were dried by passage over activated alumina under argon atmosphere (H_2O content <30 p.p.m., Karl Fischer titration)[27]. $N(C_2H_5)_3$ was distilled under nitrogen from KOH. H_3COH was used from Fluka (*pro analysi* grade) without further purification. All other chemicals were purchased from Acros, Aldrich, Fluka, Merck or Lancaster and used as such unless noted otherwise. Reactions were monitored by thin-layer chromatography using Merck silica gel 60 F254 TLC glass plates and visualized with ceric ammonium molybdate or potassium permanganate staining solution. Chromatographic purification was performed as flash chromatography on Brunschwig silica 32-63, 60 Å, using a forced flow of eluant at 0.3 bar. Concentration under reduced pressure was performed by rotary evaporation at 40 °C at the appropriate pressure. Yields refer to chromatographically purified and spectroscopically pure compounds, unless noted otherwise.

Spectroscopic and spectrometric methods. NMR spectra were recorded on a Varian Mercury 300 spectrometer (operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C acquisitions), Bruker DRX400 and AV400 spectrometers (operating at 400 MHz for ^{1}H, 101 MHz for ^{13}C and 162 MHz for ^{31}P acquisitions) and a Bruker Avance II 600 spectrometer (operating at 600 MHz for ^{1}H and 151 MHz for ^{13}C acquisitions).

Chemical shifts δ are reported in parts per million with tetramethylsilane (0.00 p.p.m. (^{1}H NMR), 0.00 p.p.m. (^{13}C NMR)) or the solvent resonance as the internal standard (*d*1-chloroform: 7.26 (^{1}H NMR), 77.2 (^{13}C NMR); *d*4-methanol: 3.34 (^{1}H NMR), 49.0 (^{13}C NMR); *d*8-THF: 1.73, 3.58 (^{1}H NMR), 25.5, 67.7 (^{13}C NMR); *d*6-acetone: 2.05 (^{1}H NMR), 29.8 and 206.3 (^{13}C NMR)). ^{31}P NMR shifts are reported in parts per million relative to 85% H_3PO_4 in water. IR spectra were obtained on a Perkin Elmer Spectrum RX-I FT-IR as thin film or on a Perkin Elmer Spectrum One FT IR Spectrometer (ATR). Mass spectra were obtained on a Waters/Micromass AutoSpec Ultima (EI), a Waters/Micromass Q-TOF Ultima (ESI), a Varian IonSpec FT-ICR (ESI) or a Bruker maXis (ESI) spectrometer.

Melting points. Melting points were measured on a Büchi B-540 melting point apparatus using open glass capillaries and are uncorrected.

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