Natural Products

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Determination of the Absolute Configuration of the Pseudo-Symmetric Natural Product Elatenyne by the Crystalline Sponge Method

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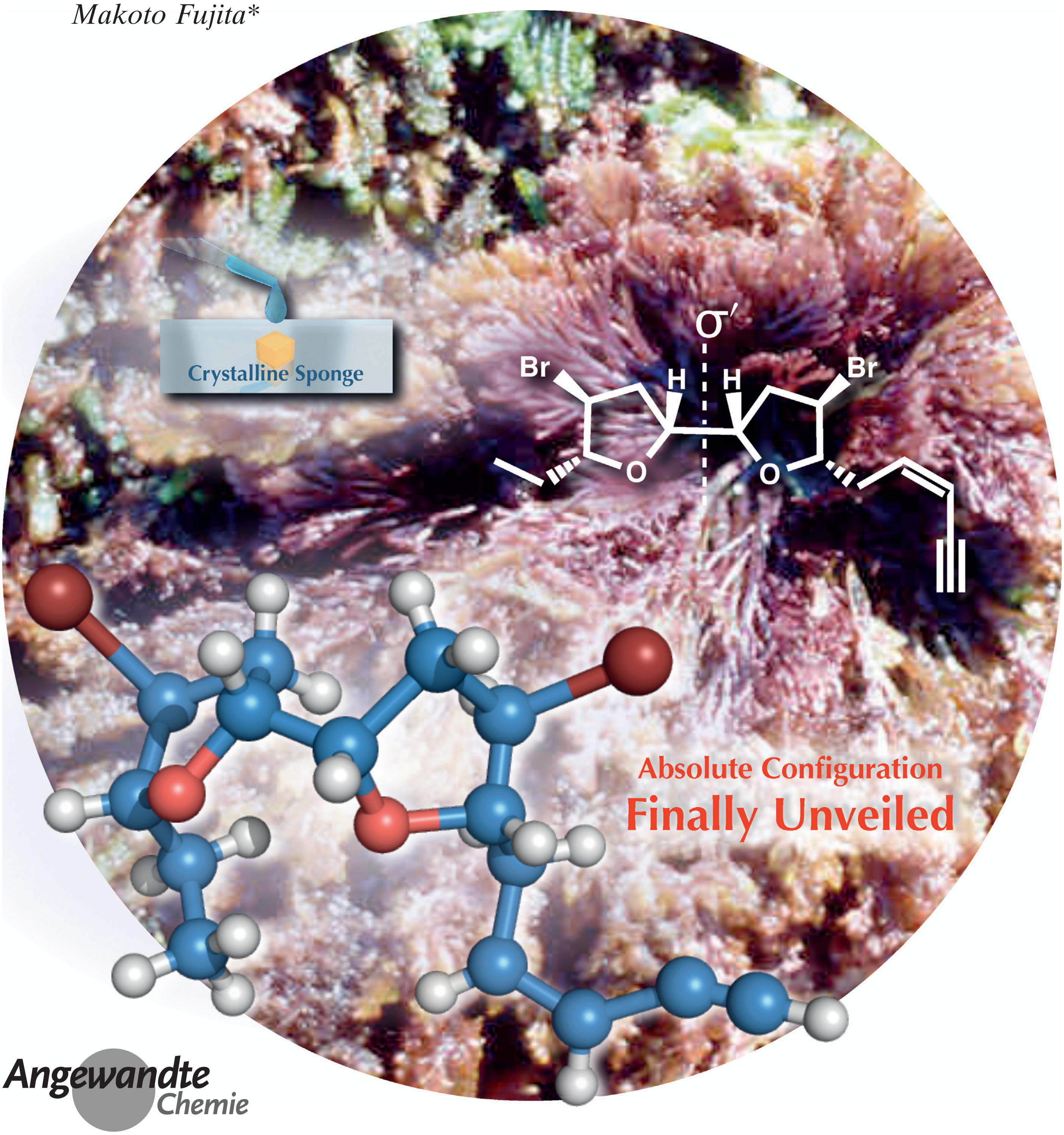
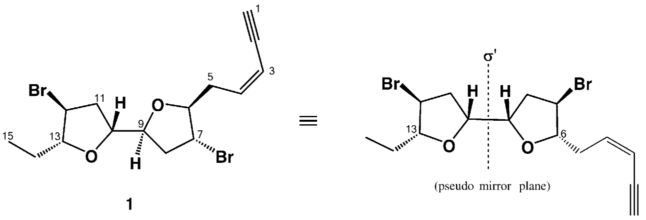


***Abstract:** Elatenyne is a marine natural product that was isolated in 1986. Despite its simple 2,2′-bifuranyl backbone, its relative structure was only recently determined. The absolute configuration of elatenyne has still not been unequivocally confirmed because of its pseudo-meso core structure, which results in a specific rotation, $[\alpha]_D$, of almost zero. In this work, the structure of natural elatenyne was determined by the crystalline sponge method and the use of a porous coordination network (a crystalline sponge) capable of absorbing organic guests; in the sponge, the absorbed guests are ordered and crystallographically observable. The crystalline sponge could differentiate between the two very similar alkyl side chains, and the absolute structure of elatenyne was thus reliably determined. The total amount required for the experiments was only approximately 100 μg, and the majority (95 μg) could be recovered after the experiments.*

The synthesis of natural products represents a major challenge, but offers a reliable way to confirm and often determine the complete structure of natural products.[1] Comparing the spectroscopic data and specific rotation values of synthetic and isolated compounds can enable the determination of the structures of natural products, including their absolute configuration. However, this is not the case for the marine natural product elatenyne (**1**).[2] In 2012, Burton, Kim, and co-workers determined the relative structure of **1** by accomplishing the total synthesis of the most probable diastereoisomer of **1**, as elucidated by a computation-assisted ^{13}C NMR study.[3–5] The spectroscopic data of synthetic **1** were identical to those of natural **1**, which had been isolated from the marine red alga, *Laurencia elata*, by the Urban group.[6,7] However, synthetic **1** showed an $[\alpha]_D$ specific rotation of almost zero,[5] which is inconsistent with the curious observation that positive and negative specific rotations have been reported for the first and second isolated sample of **1**, respectively ($[\alpha]_D = +19$ and -10, recorded in 1986 and 2013, respectively).[2,7] Whereas an $[\alpha]_{365}$ value was recorded for synthetic **1** (-17.4),[5] whose sign is consistent with that of the second isolated sample of **1** (-20.1),[7] the two are within experimental error because, given the almost zero $[\alpha]_D$ specific rotation of synthetic **1**, the observed $[\alpha]_D$ value for the second sample of **1** is due to chiral impurities; this means that the specific rotation becomes easily deviated either in the positive or negative direction, making the observed $[\alpha]_{365}$ value invalid. Furthermore, the structure was still questioned by the elucidation of another possible diastereomer by a further NMR study.[7] The inconsistent specific rotations and the structural ambiguity have urged researchers to undertake further structure analysis; however, the structure cannot be concluded by comparison with synthetic compounds.[7] The absolute configuration of **1** can only be unequivocally determined by direct analysis of isolated natural **1**. Given that **1** is an oily compound, this can presumably only be reliably achieved by the crystalline sponge method, which enables the crystallographic analysis of non-crystalline compounds on the microgram scale.[8–13]



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The small specific rotation of **1** is largely due to its pseudo-mirror-symmetric structure with two bromine and two alkyl substituents on the *meso* 2,2′-bifuranyl skeleton. As a result, all six chiral centers on the skeleton are defined only by the two different alkyl chains at the C6 and C13 positions. The crystalline sponge employed here is based on $[(ZnI_2)_3(tpt)_2\cdot(cyclohexane)_x]$ (**2**, tpt = 1,3,5-tris(4-pyridyl)triazine).[14]

A single crystal of **2** (typically ca. 300 × 150 × 100 μm^3) was placed in a vial and immersed in a small amount of cyclohexane (50 μL). Ten vials with the same content were prepared, and a 1,2-dichloroethane solution (10 μL) of **1** (1 $\mu g\,\mu L^{-1}$, 10 μg) was added to each of them. The vials were equipped with syringe needles for slow solvent evaporation (Figure 1 A, a). Guest soaking was carried out at room temperature for the first five vials and at 50 °C for the remaining five vials. After two days, all guest-absorbed crystals were transferred to a crystallization plate and subjected to sample screening with an X-ray scanner that can rapidly provide preliminary X-ray diffraction images for the crystals (Figures 1 A, b and c). Cracked or weakly diffracting crystals were eliminated, and the crystal that gave the best diffraction pattern with sharp and non-split spots and the highest resolution was selected. One of the crystals (400 × 160 × 120 μm^3) soaked at 50 °C gave the best diffraction pattern, and after cutting into a suitable size (200 × 160 × 120 μm^3), the crystal was subjected to X-ray crystallographic data collection (Figure 1 A, d). The single-crystal X-ray diffraction experiment was performed on an in-house X-ray diffractometer with Cu K_α radiation. Strong absorption effects that are due to the Cu K_α radiation were properly corrected with the numerical absorption correction method.

Owing to multiple weak interactions with the chiral guest, the net host–guest structure is chiral, and the inherent centrosymmetric *C*2/*c* space group of host **2** is turned into a non-centrosymmetric *C*2 space group,[12] which was confirmed based on the extinction rule: The appearance of *h0l* (*l* = odd number) reflections after guest inclusion clearly indicated the space-group conversion. The crystal structure of the guest-absorbed complex was solved by dual-space iter-

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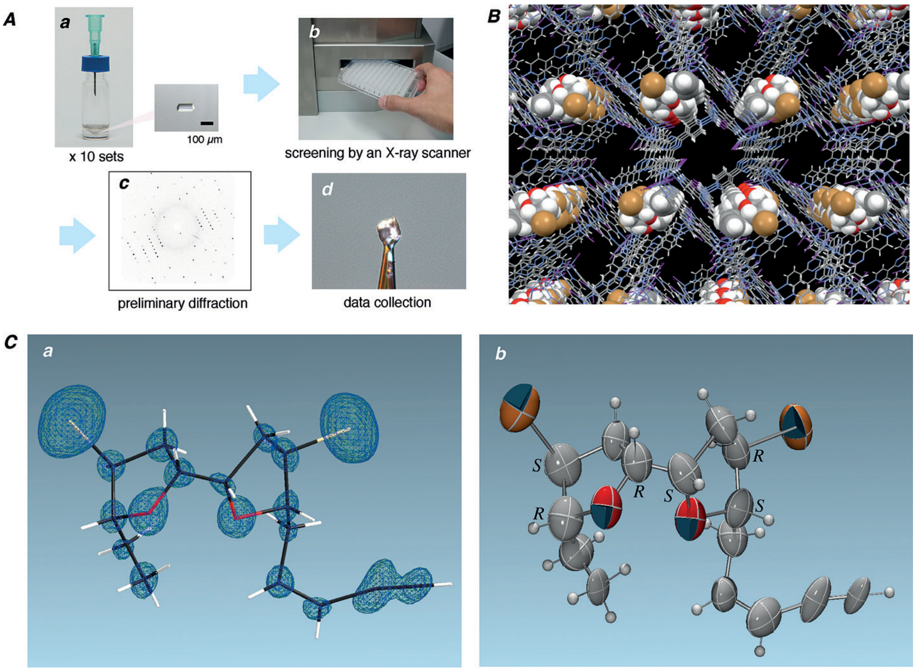

Figure 1. X-ray structure analysis of elatenyne. A) Sample preparation: a) Guest soaking in a vial. The crystalline sponge crystal (inset) was treated with elatenyne (10 µg) in a vial equipped with a needle for slow solvent evaporation. b–d) All crystals were analyzed with an X-ray scanner, and the crystal with the best diffraction pattern was subjected to data collection. B) Crystal structure of the guest-absorbed crystalline sponge, $[(ZnI_2)_3(tpt)_2{\cdot}\mathbf{1}]$ (**3**). Solvents in the pores omitted for clarity. C) Molecular structure of elatenyne found in **3**, superimposed with a) the Fourier electron density map (σ level = 1.0), and b) the ORTEP drawing with 30% probability. Somewhat large thermal ellipsoids, though quite common for host–guest complexes, may be attributed to guest occupancies slightly lower than 100%.

ation[15] and refined by full-matrix least-squares[16] methods (Figure 1B). The structure of one independent molecule of **1** per asymmetric unit was clearly observed, and its population was estimated to be 100% according to the structure refinement. Therefore, the guest-absorbed complex can be written as $[(ZnI_2)_3(tpt)_2{\cdot}\mathbf{1}]$ (**3**). The data quality was high, and the structure of **1** was refined directly and easily from the observed electron density as revealed by the Fourier map that nicely overlapped with the refined structure of **1** (Figure 1C). No unusual bond angles were observed for the 2,2′-bifuranyl skeleton of **1**. In the crystal structure, five intermolecular interactions between **1** and the framework were observed, namely one C–H···O (3.35(2) Å) and four C–H···I (3.68(4), 3.95(5), 4.04(3), and 4.15(5) Å) interactions. The latter C–H···I interactions create chiral environments around the iodine atoms (see Figure S3 and the detailed discussion in the Supporting Information). No other intermolecular contacts within the sum of the van der Waals radii were observed.

The Flack parameter calculated by Parsons' method was 0.074(7), and to our delight, we could determine the absolute structure of **1**, which had not be solved by the specific rotation studies. The Hooft parameter of 0.083(6) was also sufficient to confirm the absolute structure. The absolute configurations of the chiral centers of **1** were thus determined to be 6*S*, 7*R*, 9*S*, 10*R*, 12*S*, and 13*R*. The relative configuration is consistent with that of synthesized **1** as reported by Burton and Kim in 2012. The crystalline sponge method concludes that the compound synthesized by Burton and Kim is indeed the correct diastereoisomer, and the nearly zero $[\alpha]_D$ specific rotation of **1** is attributed to its inherent pseudo-*meso* structure. The previously reported positive and negative specific rotation values for isolated **1** are presumably due to small amounts of impurities and unavoidable experimental errors associated with the very low concentrations used to obtain the specific rotation data.

The molecular chirality of the pseudo-mirror-symmetric skeleton of **1** was clearly recognized by the binding pockets of sponge **2**. If the chirality of **1** was not recognized and the enantiomeric binding pockets of host **2** bound **1** with similar affinity, unfavorable electron density overlap at the side chains would be anticipated. However, no significant residual electron density was observed at the extension of the terminal C14–C15 bond, indicating that the electron densities of the C2 and C5 fragments do not overlap. The success of the absolute-structure determination is attributed to the remarkably high molecular recognition of the crystalline sponge, which differentiates between the side chains of the pseudo-*meso* elatenyne core.

The total amount of **1** that was used for the soaking experiment was approximately 100 µg. As every crystal (ca. 5 µg) can absorb only approximately 0.5 µg of **1** (as estimated from the crystal density and the molecular weight of the crystal structure), a large excess of pure **1** was still in the vials and easily recovered. All of the vials were washed with small portions of fresh dichloromethane. NMR analysis (600 MHz, CryoProbe, $CDCl_3$ with 1,3,5-tribromobenzene in a sealed capillary as an internal standard) quantified the recovery of 95 µg of **1** from the vials (see the Supporting Information). Though experimentally possible, we did not attempt the further recovery of **1** from the guest-absorbed crystals because the expected amount is only approximately 5 µg, and the sample might be rather contaminated with the components of sponge **2** (leached zinc iodide and the ligand). We were thus able to carry out all experiments without any substantial loss of natural elatenyne.

The absolute configuration of **1** was further confirmed by the crystalline sponge analysis of click adduct **4** (Figure 2A). This adduct was previously synthesized by the Urban group as a means to obtain crystals suitable for single-crystal X-ray diffraction studies. Similar optimization experiments with **4** for the guest soaking process followed by data collection and structure refinement displayed the structure of **4** trapped in the pore of **2** (Figure 2B). The Flack (Parsons) and Hooft

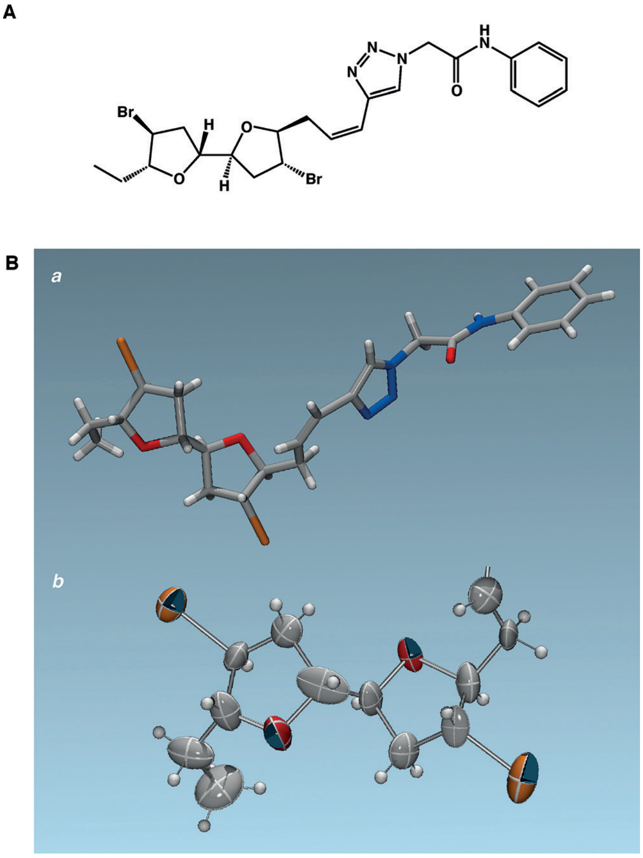

Figure 2. Crystal structure of clicked elatenyne (**4**). A) Chemical structure of **4**. B) Stick representation (a) and ORTEP drawing of the elatenyne moiety with 30% probability (b).

parameters were 0.084(5) and 0.083(5), respectively. The same absolute configuration was observed for the 2,2′-bifuranyl moiety, thereby providing independent confirmation for the absolute configuration of **1**. Around the click side chains and a part of the core, a few unnatural geometries were observed because of the merging or almost overlapping disordered structures, but these do not influence the assignment of the chemical structure of **4**.

Whereas the absolute configuration of **1** was first proposed by Burton and Kim, this was achieved with some uncertainty. Final confirmation had to be achieved by the re-isolation of pure **1** together with X-ray analysis. The ultimate determination of the absolute configuration of **1** was achieved by X-ray crystallographic analysis, which enabled the unequivocal assignment of the natural product. This analysis confirmed that Burton and Kim had indeed concluded the correct absolute configuration of **1**, but the crystalline sponge method provided the unquestionable evidence.

Furthermore, the present study represents the first real application of the crystalline sponge method to the determination of the absolute configuration of a natural product. The results open up prospects for both natural product and synthetic chemists as a new way to obtain complete crystal structures for compounds that would otherwise never be amenable to X-ray crystallographic analysis. Previously isolated natural products that possess any element of ambiguity or structurally undetermined motifs are worthy of examination by the crystalline sponge method. The typical steps and experiments employed by natural-product chemists (from the collection of bulk plants or marine invertebrates from the ocean to the extraction and purification of secondary metabolites and spectroscopic analysis) can be scaled down by one or two orders of magnitude. Furthermore, the ability to establish the absolute configuration of highly complex bioactive natural products by this method will be groundbreaking, particularly in the drug-discovery area.

Experimental Section

Crystallographic data of **3**: $C_{122.25}H_{138.5}N_{24}O_2Zn_6Br_2I_{12}$, $M = 4050.93$, monoclinic $C2$, $a = 34.6788(6)$ Å, $b = 15.0282(2)$ Å, $c = 31.1707(9)$ Å, $\beta = 101.973(2)^\circ$, $V = 15891.5(6)$ Å^3, $Z = 4$, GoF $= 1.034$, $R_1 = 0.0673$, $wR_2 = 0.2136$. Flack parameter (Parsons' method): 0.074(7). Hooft parameter: 0.083(6).

Crystallographic data of **4**: $C_{119}H_{124}N_{28}O_3Zn_6Br_2I_{12}$, $M = 4069.29$, monoclinic $C2$, $a = 34.3028(5)$ Å, $b = 15.1781(2)$ Å, $c = 29.4820(5)$ Å, $\beta = 100.9110(10)^\circ$, $V = 15072.4(4)$ Å^3, $Z = 4$, GoF $= 1.017$, $R_1 = 0.0426$, $wR_2 = 0.1113$. Flack parameter (Parsons' method): 0.084(5). Hooft parameter: 0.083(5).

CCDC 1406701 (**3**) and 1406700 (**4**) contain the supplementary crystallographic data for this paper. These data are provided free of charge by The Cambridge Crystallographic Data Centre.

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[1] K. C. Nicolaou, S. A. Snyder, *Angew. Chem. Int. Ed.* **2005**, *44*, 1012–1044; *Angew. Chem.* **2005**, *117*, 1036–1069.
[2] J. G. Hall, J. A. Reiss, *Aust. J. Chem.* **1986**, *39*, 1401–1409.
[3] H. M. Sheldrake, C. Jamieson, J. W. Burton, *Angew. Chem. Int. Ed.* **2006**, *45*, 7199–7202; *Angew. Chem.* **2006**, *118*, 7357–7360.
[4] S. G. Smith, R. S. Paton, J. W. Burton, J. M. Goodman, *J. Org. Chem.* **2008**, *73*, 4053–4062.
[5] B. S. Dyson, J. W. Burton, T. Sohn, B. Kim, H. Bae, D. Kim, *J. Am. Chem. Soc.* **2012**, *134*, 11781–11790.
[6] D. A. Dias, S. Urban, *Photochemistry* **2011**, *72*, 2081–2089.
[7] R. Brkljača, S. Urban, *Nat. Prod. Commun.* **2013**, *8*, 729–732.
[8] Y. Inokuma, S. Yoshioka, J. Ariyoshi, T. Arai, Y. Hitora, K. Takada, S. Matsunaga, K. Rissanen, M. Fujita, *Nature* **2013**, *495*, 461–466. Corrigendum: *Nature* **2013**, *501*, 262.
[9] Y. Inokuma, S. Yoshioka, J. Ariyoshi, T. Arai, M. Fujita, *Nat. Protoc.* **2014**, *9*, 246–252.
[10] S. Yoshioka, Y. Inokuma, M. Hoshino, T. Sato, M. Fujita, *Chem. Sci.* **2015**, *6*, 3765–3768.
[11] N. Zigon, M. Hoshino, S. Yoshioka, Y. Inokuma, M. Fujita, *Angew. Chem. Int. Ed.* **2015**, *54*, 9033–9037; *Angew. Chem.* **2015**, *127*, 9161–9165.
[12] M. Hoshino, A. Khutia, H. Xing, Y. Inokuma, M. Fujita, *IUCrJ* **2016**, DOI: 10.1107/S2052252515024379.

[13] R. Boese, M. T. Kirchner, W. E. Billups, L. R. Norman, *Angew. Chem. Int. Ed.* **2003**, *42*, 1961–1963; *Angew. Chem.* **2003**, *115*, 2005–2007.
[14] K. Biradha, M. Fujita, *Angew. Chem. Int. Ed.* **2002**, *41*, 3392–3395; *Angew. Chem.* **2002**, *114*, 3542–3545.
[15] Done with SHELXT 2014/5; see: G. M. Sheldrick, *Acta Crystallogr. Sect. A* **2015**, *71*, 3–8.
[16] Done with SHELXL 2014/7; see: G. M. Sheldrick, *Acta Crystallogr. Sect. C* **2015**, *71*, 3–8.

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