Myosin Inhibition

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# Total Synthesis of Pentabromo- and Pentachloropseudilin, and Synthetic Analogues—Allosteric Inhibitors of Myosin ATPase**

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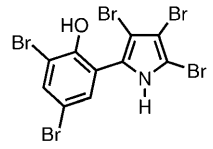
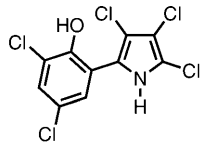
The pyrrole ring is a pivotal structural unit of many pharmacologically active natural products, for example, the lamellarins[1] and prodigiosins.[2] Recently, we have developed a novel silver(I)-promoted oxidative cyclization of homopropargylamines to generate pyrroles,[3] which has been applied to the synthesis of the anti-leishmania active (±)-harmicine and the antitumor active (±)-crispine A.[4] Homopropargylamines are most conveniently prepared by the addition of trimethylsilylpropargylmagnesium bromide to Schiff bases and thus, the silver(I)-promoted cyclization provides a short access to 2-arylpyrroles.

Over the last decades, a broad range of bioactive halogenated natural products has been isolated from diverse natural sources.[5] Pentabromo- (**1**) and pentachloropseudilin (**2**) are highly halogenated natural products containing the 2-arylpyrrole moiety. Pentabromopseudilin (**1**) was isolated in



Pentabromopseudilin (**1**)



Pentachloropseudilin (**2**)

1966 from *Pseudomonas bromoutilis*[6] and several years later from *Chromobacteria*.[7] Pentabromopseudilin (**1**) exhibits a range of useful pharmacological activities (antibiotic, antitumor, phytotoxic) and has been subject of detailed biosynthetic studies.[7–9] As a result of the strong interest in **1**, several total syntheses of **1** have been described.[8,10] The weaker antibiotic, pentachloropseudilin (**2**), was obtained by synthesis in 1978,[11] and in the same year it was isolated by Cavalleri et al. from *Actinoplanes* ATCC 33002.[12]

To achieve a silver(I)-catalyzed cyclization of the homopropargylamines, we introduced a protecting group at the nitrogen center. The N-protected homopropargylamines have been cyclized previously to give the corresponding pyrroles by treatment with iodine or various transition metals.[13] Moreover, Reissig and others have shown that buta-2,3-dienyl-*N*-tosylamines react with catalytic amounts of silver(I) nitrate to give 2,5-dihydropyrroles, which can then be converted into pyrroles.[14]

Starting from commercially available 2-methoxybenzaldehyde (**3a**), 3,5-dichloro-2-methoxybenzaldehyde (**3b**), and 3,5-difluoro-2-methoxybenzaldehyde (**3c**) the corresponding *N*-tosylaldimines **4a**–**4c** were prepared by adaptation of a literature procedure (Scheme 1a).[15] Addition of trimethylsilylpropargylmagnesium bromide to the *N*-tosylaldimines **4a**–**4c** provided the *N*-tosylhomopropargylamines **5a**–**5c**.[16] Desilylation of **5a**–**5c** using TBAF afforded, almost quantitatively, the terminal acetylenes **6a**–**6c**. Treatment of the terminal acetylenes **6a**–**6c** with catalytic amounts (10–15 mol%) of silver(I) acetate in acetone or dichloromethane under reflux temperatures provided the 2-aryl-2,3-dihydropyrroles **7a**–**7c** in excellent yields. The structure of the 2-aryl-2,3-dihydropyrrole framework has been unambiguously confirmed by an X-ray crystal structure analysis of the parent compound **7a** (Figure 1).[17]

In an investigation of the silver(I)-catalyzed cyclization reaction of **6a** giving the 2,3-dihydropyrrole **7a**, we varied the amount of catalyst and the metal salt. Lower amounts of silver(I) acetate still gave high product yields by using extended reaction times (5 mol% of AgOAc, 4 days, 86% yield of **7a**). Among the different transition-metal salts tested, only cuprous acetate led to the 2,3-dihydro-1*H*-pyrrole **7a**, albeit in low yield. Treatment of **6a** with palladium(II) acetate, gold(I) chloride, and gold(III) chloride resulted in decomposition or re-isolation of starting material.

Treatment of **7a**–**7c** with potassium *tert*-butoxide in DMSO led, via elimination of *p*-toluenesulfinic acid, to the pyrroles **8a**–**8c**.[14] Cleavage of the methyl ether **8a** with sodium sulfide to pseudilin (**9**) and subsequent electrophilic bromination using pyridinium tribromide[18] afforded pentabromopseudilin (**1**; Scheme 1b).[19] Electrophilic chlorination of **8b** with NCS to afford *O*-methylpentachloropseudilin (**10**) and subsequent treatment with boron tribromide provided pentachloropseudilin (**2**) in high yield.[19] The spectroscopic data of our products[19] were in good agreement with those reported for **1**[8,10] and **2** in the literature.[11] Electrophilic bromination of the pyrrole **8b** and then ether cleavage of **11**

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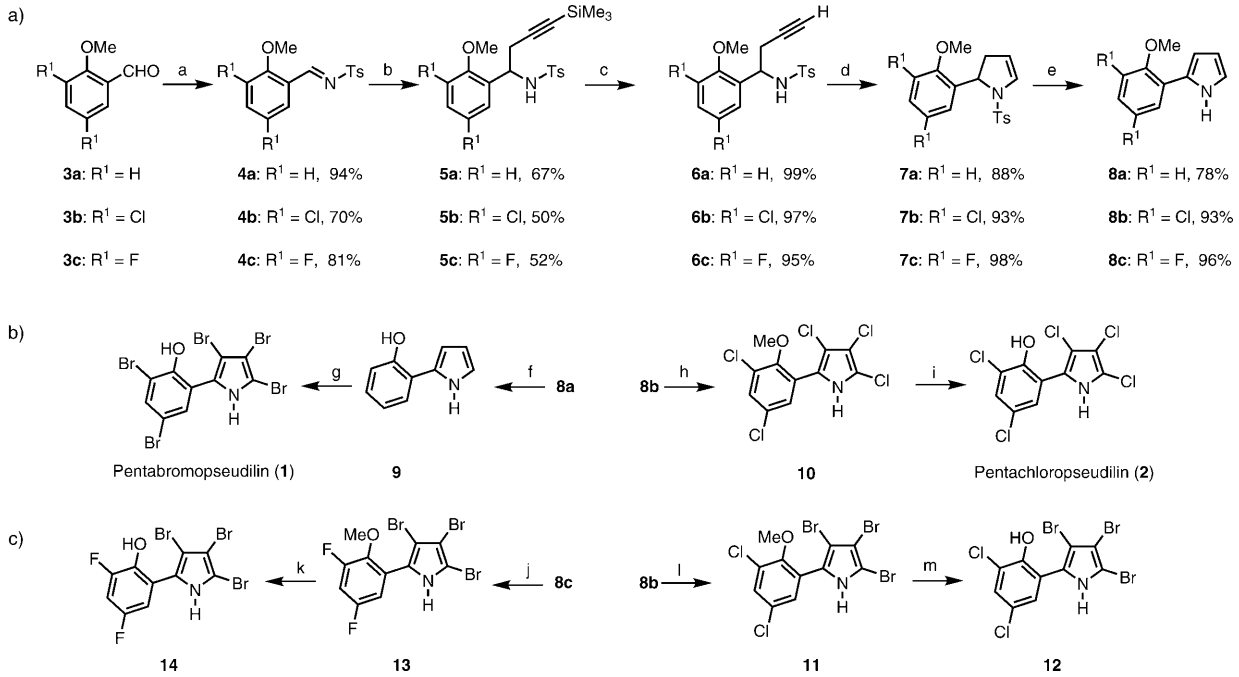



***Scheme 1.*** Total synthesis of pentabromopseudilin (**1**), pentachloropseudilin (**2**) and synthetic derivatives **12** and **14**. Reagents and conditions: a) *p*-$TsNH_2$, $Si(OEt)_4$, 160 °C; b) $BrMgCH_2C{\equiv}CSiMe_3$, $CH_2Cl_2$, RT; c) $Bu_4NF$, THF, RT; d) 10–15 mol% AgOAc, acetone, 56 °C or $CH_2Cl_2$, 40 °C; e) KO*t*Bu, DMSO, RT or 50 °C; f) $Na_2S$, NMP, 160 °C, 2.5 h (93%); g) Py·HBr·$Br_2$, EtOH, RT, 3 d (59%); h) NCS, MeCN, −40 °C to RT (81%); i) $BBr_3$, $CH_2Cl_2$, −78 to 0 °C, 2 h (79%); j) Py·HBr·$Br_2$, EtOH, RT, 30 min (91%); k) $BBr_3$, $CH_2Cl_2$, −78 to 0 °C, 1.5 h (78%); l) Py·HBr·$Br_2$, EtOH, RT, 60 min (87%); m) $BBr_3$, $CH_2Cl_2$, −78 to 0 °C, 2.5 h (76%). NMP = 1-methyl-2-pyrrolidinone, py = pyridine, NCS = *N*-chlorosuccinimide.

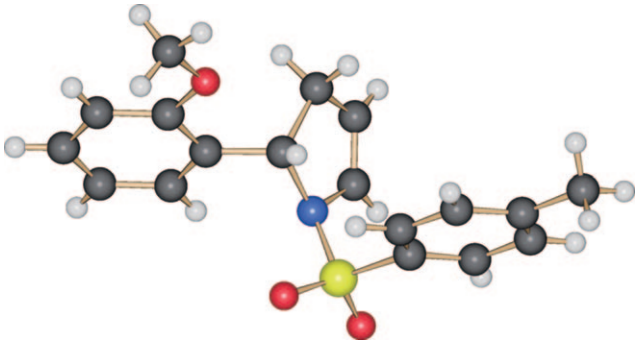
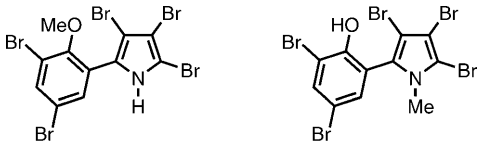


***Figure 1.*** Molecular structure of the 2,3-dihydropyrrole **7a** in the crystal.

using boron tribromide provided the tribromodichloropseudilin (**12;** Scheme 1c).[19] Electrophilic bromination of the difluoro compound **8c** and ether cleavage of **13** afforded the tribromodifluoropseudilin (**14**).[19] Compound **14** represents the first fluoro analogue of the natural antibiotics **1** and **2**. The specific effect of carbon–fluorine bonds on docking interaction with proteins have led to an increasing importance of organofluorine compounds for pharmaceuticals.[20]

For an investigation of the structure–activity relationships, pentabromopseudilin (**1**) was converted into the corresponding *O*-methyl and *N*-methyl derivatives. Both compounds would provide information as to whether hydrogen bonding of the N–H and O–H bonds of pentabromopseudilin is essential for bioactivity. *O*-Methylpentabromopseudilin (**15**) was prepared by reaction with trimethylsilyldiazomethane ($Et_2O$, RT, 15 h, 56% yield).[10d,21] *N*-Methylation was achieved by treatment of intermediate **7a** with KO*t*Bu and quenching with methyl iodide. Subsequent ether cleavage with sodium sulfide and bromination using pyridinium tribromide led to the novel *N*-methylpentabromopseudilin (**16**; 31% overall yield based on **7a**).




We achieved the total synthesis of pentabromopseudilin (**1**) and pentachloropseudilin (**2**), as well as the synthetic analogues **12** and **14–16** using a highly efficient silver(I)-catalyzed cyclization of *N*-tosylhomopropargylamines to 2,3-dihydropyrroles. The seven-step total syntheses of pentabromopseudilin (**1**; 23% overall yield) and pentachloropseudilin (**2**; 19% overall yield) are straightforward and flexible enough to provide access to a range of structural analogues.

Specific inhibitors of myosins hold great promise for the treatment of a broad range of diseases including cancer, malaria, and most obviously a range of muscle malfunctions. In a screen for motor protein inhibitors, some of the pseudilins described above proved to be very efficient inhibitors of skeletal muscle myosin-2 ATPase activity

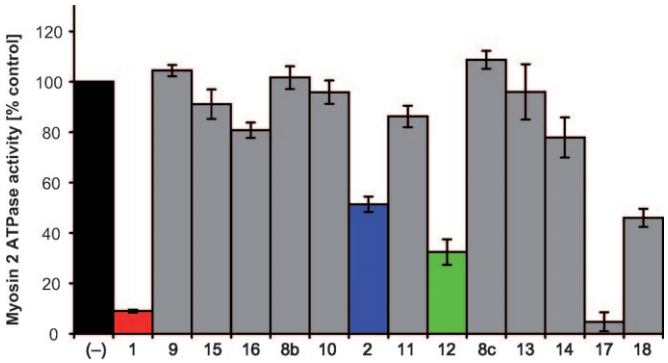
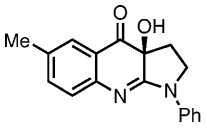
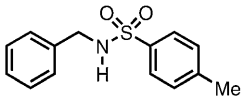



***Figure 2.*** Assay for the inhibition of skeletal muscle myosin-2 ATPase activity. The graph shows the inhibition of the basal ATPase activity of myosin 2 relative to the control (no inhibitor added, 100% activity). (–) = control. The known inhibitors (–)-blebbistatin (**17**) and *N*-benzyl-*p*-toluenesulfonamide (= BTS) (**18**) were included as positive controls. The bars represent the mean value of three measurements. The error bars indicate the standard deviation. All substances were tested at 25 μM concentration.

(Figure 2). The efficacy of pentabromopseudilin (**1**) is in the same range as observed for (–)-blebbistatin (**17**)[22] and much superior to that of *N*-benzyl-*p*-toluenesulfonamide (BTS)




(–)-Blebbistatin (**17**)




*N*-Benzyl-*p*-toluenesulfonamide (**18**)

(**18**).[23] For comparison, both known inhibitors have been included in our assay (Figure 2). The assay results obtained with skeletal muscle myosin-2 demonstrate the effect of structural modifications at the pseudilins on the inhibitory potency of this class of compounds. We observed no inhibition with pseudilin (**9**) itself. Pentabromopseudilin (**1**) is the most potent inhibitor, followed by tribromodichloropseudilin (**12**) and then pentachloropseudilin (**2**). The residual activity of myosin-2 ATPase observed by inhibition with pentachloropseudilin (**2**) was in the same range as with BTS (**18**). Tribromodifluoropseudilin (**14**) is a weak inhibitor of myosin-2 ATPase. Methylation of pentabromopseudilin (**1**) to *O*-methylpentabromopseudilin (**15**) or to *N*-methylpentabromopseudilin (**16**) significantly reduces the inhibitory potency. Residual ATPase activities of 90% (for **15**) and 80% (for **16**) have been found. These findings indicate that the free OH and NH groups are required for hydrogen bonding to the enzyme. The *O*-methyl derivatives **10** and **11** also showed a considerably reduced inhibiting activity as compared to their non-methylated counterparts **2** and **12**.

Further investigations showed that the pseudilins inhibit the ATPase and motor activities of a range of different myosins. Individual compounds showed variations in their potency for specific myosin isoforms. In particular, the inhibitory potency of pentabromopseudilin (**1**) is 25-fold higher for vertebrate class-5 myosins than observed with skeletal muscle myosin-2 isoforms.[24] Moreover, some pseudilin derivatives appear to activate myosin function.

X-ray crystallographic analysis of the complex formed by the *Dictyostelium* myosin-2 motor domain, $Mg^{2+}$–ADP–*meta*-vanadate, and pentabromopseudilin (**1**) revealed a new allosteric binding pocket that is located 16 Å away from the nucleotide binding pocket (Figure 3).[24,25] Moreover, the

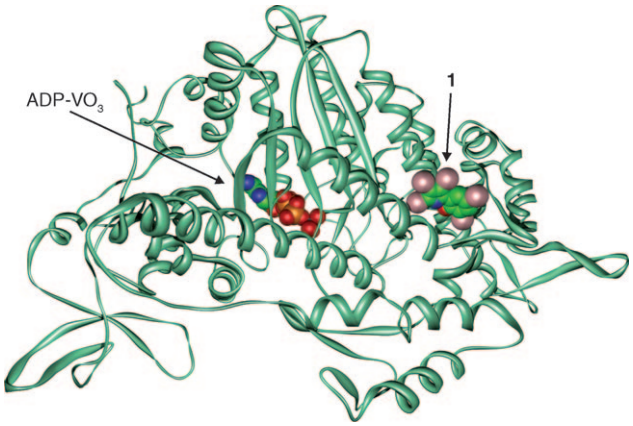



***Figure 3.*** Structure of the myosin-2 motor-domain complex with $Mg^{2+}$–ADP–*meta*-vanadate and pentabromopseudilin (**1**).

allosteric binding pocket of pentabromopseudilin (**1**) is 7.5 Å away from the allosteric binding pocket of blebbistatin (**17**) (Figure 4). The identification of a second allosteric binding pocket paves the way for structure-based design approaches leading to the development of more potent and isoform-specific myosin inhibitors.

In conclusion, we developed a practical silver(I)-catalyzed synthesis of 2-arylpyrroles which has been utilized for a highly efficient route to the naturally occurring pentabromo- (**1**) and pentachloropseudilin (**2**), as well as synthetic analogues. The pentahalogenated pseudilins **1**, **2**, and **12** are members of a new class of myosin ATPase inhibitors. Further studies on the

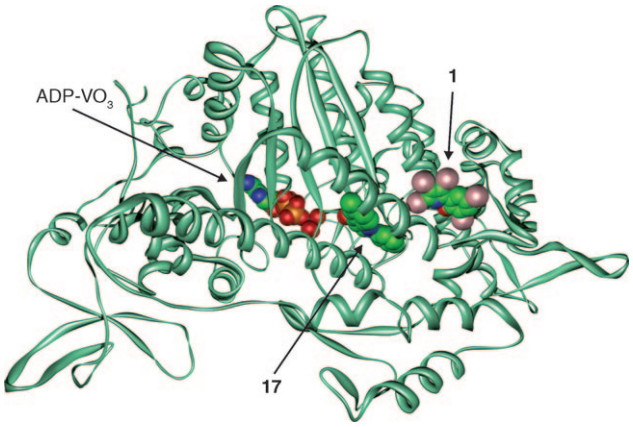



***Figure 4.*** Structure of the myosin-2 motor-domain complex with $Mg^{2+}$–ADP–*meta*-vanadate and pentabromopseudilin (**1**) in super-position with the structure of the corresponding complex with blebbistatin (**17**) [22b].

mechanism of myosin inhibition by pseudilin derivatives are currently in progress.

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[17] Crystallographic data for **7a**: $C_{18}H_{19}NO_3S$, $M = 329.40\ g\,mol^{-1}$, crystal size: $0.45 \times 0.16 \times 0.16\ mm^3$, monoclinic, space group $P2_1/c$, $a = 7.7320(10)$, $b = 20.232(2)$, $c = 12.8800(10)$ Å, $\beta = 126.540(10)°$, $V = 1618.8(3)$ Å$^3$, $Z = 4$, $\rho_{calcd} = 1.352\ g\,cm^{-3}$, $\mu = 0.214\ mm^{-1}$, $\lambda = 0.71073$ Å, $T = 198(2)$ K, $\theta_{range} = 3.28$–$30.00°$, reflections collected: 39066, independent: 4697 ($R_{int} = 0.0598$), 210 parameters. The structure was solved by direct methods and refined by full-matrix least-squares on $F^2$; final $R$ indices [$I > 2\sigma(I)$]: $R_1 = 0.0414$; $wR_2 = 0.1092$; maximal residual electron density: $0.416\ e\,Å^{-3}$. CCDC 738984 (**7a**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
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[19] Selected spectroscopic data for the pyrrole alkaloids **1**, **2**, **12**, and **14**. Pentabromopseudilin (**1**): purple solid, m. p. 152 °C (decomp.) (lit.[8] 152 °C, decomp.); $^1$H NMR (500 MHz, $CDCl_3$): $\delta = 6.03$ (s, 1H), 7.57 (d, $J = 2.3$ Hz, 1H), 8.10 (d, $J = 2.3$ Hz, 1H), 9.48 ppm (br s, 1H); $^{13}$C NMR and DEPT (125 MHz, $CDCl_3$): $\delta = 99.36$ (C), 100.97 (C), 103.85 (C), 111.90 (C), 113.21 (C), 119.29 (C), 125.05 (C), 130.86 (CH), 133.13 (CH), 147.19 (C); MS (EI, 70 eV): $m/z$ (%) = 559 (7), 557 (38), 555 (77), 553 (79), 551 (40), 549 (8) [$M^+$], 478 (17), 476 (66), 474 (100), 472 (67), 470 (17), 451 (5), 449 (21), 447 (33), 445 (23), 443 (6), 397 (22), 395 (59), 393 (57), 391 ppm (19); HRMS: $m/z$ calc. for $C_{10}H_4Br_5NO$ [$M^+$]: 548.6210, found: 548.6209. Pentachloropseudilin (**2**): grey solid, m.p. 131–132 °C (lit.[11] 126–130 °C); $^1$H NMR (500 MHz, $CDCl_3$): $\delta = 6.12$ (s, 1H), 7.28 (d, $J = 2.4$ Hz, 1H), 8.00 (d, $J = 2.4$ Hz, 1H), 9.55 ppm (br s, 1H); $^{13}$C NMR and DEPT (125 MHz, $CDCl_3$): $\delta = 110.38$ (C), 110.43 (C), 112.32 (C), 118.57 (C), 120.48 (C), 121.31 (C), 126.33 (CH), 126.36 (C), 127.06 (CH), 145.42 ppm (C); MS (EI, 70 eV): $m/z$ (%) = 337 (3), 335 (18), 333 (61) 331 (100), 329 (58) [$M^+$], 300 (8), 298 (34), 296 (77), 294 (59), 273 (6), 271 (26), 269 (59), 267 (47), 263 (6), 261 (18), 259 (18); HRMS: $m/z$ calc. for $C_{10}H_4Cl_5NO$ [$M^+$]: 328.8735, found: 328.8723. 2,3,4-Tribromo-5-(3,5-dichloro-2-hydroxyphenyl)-1*H*-pyrrole (tribromodichloropseudilin) (**12**): grey solid, m.p. 170 °C (lit.[8] 170 °C, decomp.); $^1$H NMR (500 MHz, $CDCl_3$): $\delta = 6.07$ (s, 1H), 7.31 (d, $J = 2.4$ Hz, 1H), 7.96 (d, $J = 2.4$ Hz, 1H), 9.53 ppm (br s, 1H); $^{13}$C NMR and DEPT (125 MHz, $CDCl_3$): $\delta = 99.30$ (C), 100.98 (C), 103.87 (C), 118.94 (C), 121.30 (C), 125.12 (C), 126.05 (C), 127.17 (CH), 127.58 (CH), 145.89 ppm (C); MS (EI, 70 eV): $m/z$ (%) = 471 (2), 469 (18), 467 (62), 465 (100), 463 (73), 461 (20) [$M^+$], 390 (5), 388 (32), 386 (86), 384 (93), 382 (35), 361 (14), 359 (40), 357 (45), 355 (18), 309 (8), 307 (41), 305 (84), 303 (50); HRMS: $m/z$ calc. for $C_{10}H_4Br_3Cl_2NO$ [$M^+$]: 460.7220, found: 460.7220. 2,3,4-Tribromo-5-(3,5-difluoro-2-hydroxyphenyl)-1*H*-pyrrole (tribromodifluoropseudilin)(**14**): light green solid, m.p. 132 °C (decomp.); $^1$H NMR (500 MHz, $CDCl_3$): $\delta = 5.47$ (d, $J = 4.6$ Hz, 1H), 6.85 (ddd, $J = 10.2$, 7.7, 2.7 Hz, 1H), 7.68 (ddd, $J = 10.0$, 2.7, 2.1 Hz, 1H), 9.68 ppm (br s, 1H); $^{13}$C NMR and DEPT (125 MHz, $CDCl_3$): $\delta = 99.09$ (C), 101.02 (C), 102.99 (dd, $J = 27.6$, 22.4 Hz, CH), 104.00 (C), 109.85 (dd, $J = 25.7$, 3.2 Hz, CH), 118.78 (C), 125.31 (m, C), 136.23 (d, $J = 16.6$ Hz, C), 150.70 (dd, $J = 237.6$, 13.2 Hz, C), 155.49 ppm (dd, $J = 241.3$, 12.2 Hz, C); MS (EI, 70 eV): $m/z$ (%) = 435 (29), 433 (95), 431 (98), 429 (30) [$M^+$], 354 (47), 352 (100), 350 (47), 327 (25), 325 (54), 323 (27), 273 (75), 271 (74), 192 (49); HRMS: $m/z$ calc. for $C_{10}H_4Br_3F_2NO$ [$M^+$]: 428.7811, found: 428.7793.
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[25] Coordinates for the myosin-2 motor domain–ADP–*meta*-vanadate-pentabromopseudilin complex have been deposited with accession code 2 JHR at the Protein Data Bank.

  