New Method of Synthesis and Biological Evaluation of Some Combretastatin A-4 Analogues

Yulia B. Malysheva,[a] Sebastien Combes,[b] Alexey Yu. Fedorov,[a] Paul Knochel,[c] Andrei E. Gavryushin*[c]

[a] Department of Organic Chemistry, Lobachevsky State University of Nizhni Novgorod, 23 Gagarin avenue, 603950 Nizhni Novgorod, Russian Federation
Fax +7(831)4658592; E-mail: afnn@rambler.ru

[b] UMR-CNRS 6264, Université d'Aix-Marseille, Faculté des Sciences Saint-Jérôme, Case 521, 13397 Marseille Cedex 20, France
Fax +33(4)9128829; E-mail: sebastien.combes@univ-provence.fr

[c] Department Chemie, Ludwig Maximilians Universität München, Butenandtstr. 5-13, Haus F, 81377 München, Germany
Fax +49(89)218077680; E-mail: knoch@cup.uni-muenchen.de; E-mail: gavryushin@nanoscape.de

Received: 02.12.2011; Accepted after revision: 13.01.2012

Abstract: A series of novel combretastatin A-4 analogues was synthesized in 36–64% yields by Negishi cross-coupling reaction under mild conditions. The prepared compounds exhibit good cytotoxicity against HBL100 epithelial cell lines (IC_{50} = 0.022–10.31 μM).

Key words: antitumor agents, cross-coupling, organometallic reagents, magnesium–zinc exchange, cytotoxicity

Combretastatin A-4 (CA-4, Figure 1), isolated from the South African tree *Combretum caffrum*, is one of the most potent antivascular and antimitotic agents acting at the colchicine binding site of tubulin, that has shown excellent activity against multidrug-resistant cancer cells.[1] The water-soluble combretastatine derivatives – disodium salt CA4P and ombrabulin derivative AVE-8062 (Figure 1) are currently in clinical trials as antitumor agents in USA and Europe.[2] Due to their unique anticancer properties and simple structures, these (*Z*)-stilbenes have drawn significant attention from medicinal chemists as lead structures for the design of novel antitumor agents.[3]

It has been demonstrated that (*Z*)-combretastatins manifest much higher biological activity than the corresponding *E*-isomers.[4] However, CA-4 and its analogues are prone to thermal *Z*/*E* isomerisation, even during the course of synthesis. This indicates a strong need for the development of convenient, mild, and stereoselective methods for the preparation of such electron-rich (*Z*)-stilbenes.

Reported synthetic routes[5] to (*Z*)-stilbenes are based on Horner–Wittig reaction,[6] alkyne hydroboration,[7] selective reduction of alkynes on the Lindlar catalyst,[8] by hydrosilylation–protodesilylation process[9] or via titanium(II)–alkyne complexes,[10] the Barbier reaction,[11] the Perkin condensation,[12] Suzuki cross-coupling,[12a,13] the Kumada–Corriu cross-coupling[14] or Ramberg–Bäcklund reaction.[15]

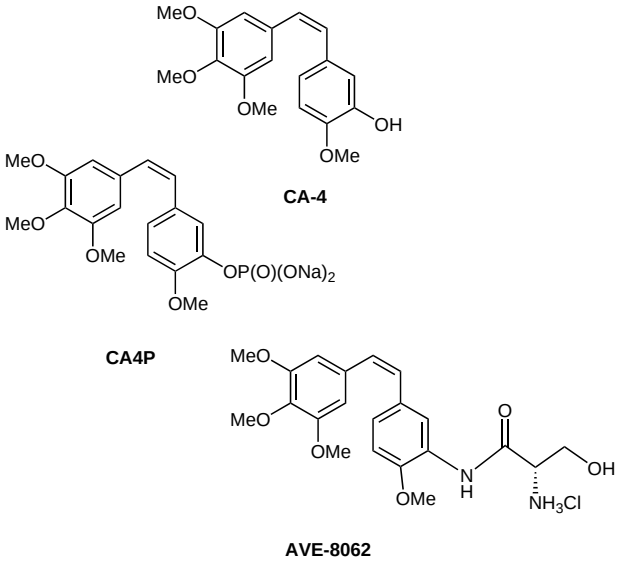
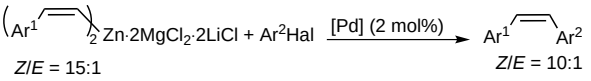


Figure 1 Combretastatin A-4 analogues

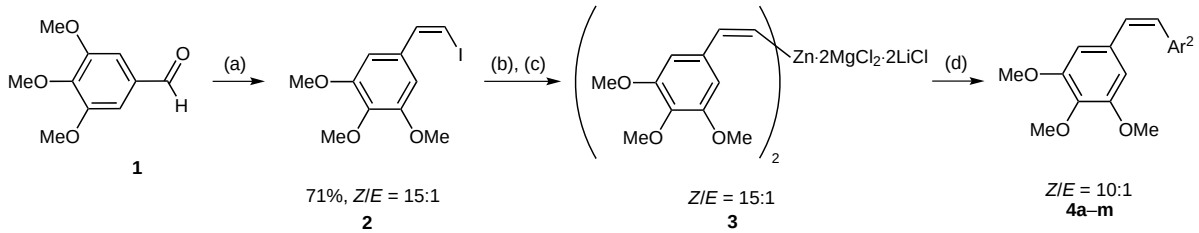
We report herein a new direct, stereoselective, and mild approach to (*Z*)-stilbenes using the palladium-catalyzed Negishi cross-coupling[16] reaction as a key step and newly developed complex organozinc reagents (Scheme 1).



Scheme 1 New route to (*Z*)-stilbenes

Since the substituted 3,4,5-trimethoxyaryl fragment can be considered as an important pharmacophore moiety in colchicine site-binding antimitotic ligands (such as derivatives of combretastatins, colchicines,[17] and 4-arylcoumarins[18]), 3,4,5-trimethoxy-β-iodostyrene (**2**) was chosen as a key intermediate for the synthesis of the target (*Z*)-stilbenes. Compound **2** was readily prepared from commercially available 3,4,5-trimethoxybenzaldehyde (**1**) and Zhao reagent in the presence of NaHMDS[19]

SYNLETT 2012, 23, 1205–1208
Advanced online publication: 26.04.2012
DOI: 10.1055/s-0031-1290899; Art ID: ST-2011-B0742-L



Scheme 2 Synthesis of combretastatin A-4 analogues. *Reagents and conditions*: (a) $^{-}IPh_3P^{+}CH_2I$, NaHMDS (1.9 M in THF), THF, –20 °C, 45 min, –78 °C, 3 h, 71% (*Z*/*E* = 15:1); (b) *i*-PrMgCl·LiCl (1.18 M in THF), –40 °C, 15 min, (*Z*/*E* = 15:1); (c) $ZnCl_2$ (1 M in THF), NMP, –40 °C, 1 min, (*Z*/*E* = 15:1); (d) Ar^2X, (A-^{ta}Phos)$_2PdCl_2$ (2 mol%), r.t., 0.5–17 h, (*Z*/*E* = 10:1); (e) The conversion was determined by GC and GC–MS analysis of reaction aliquots; (f) at the stage of the cross-coupling reaction phenolic groups were protected as TBS ethers, free amino groups as Boc amides.

(Scheme 2) in 71% yield with excellent stereoselectivity (*Z*/*E* = 15:1). When (*Z*)-iodostyrene **2** was treated with a Zn/LiCl/TMSCl system, the desired organozinc reagent was observed in low yield accompanied with the significant loss of stereoselectivity (*Z*/*E* = 1:1.4). It is known that the direct magnesium insertion to alkenyl iodides normally provides *Z*/*E* mixtures of corresponding alkenylmagnesium compounds.[20] On the other hand, acyclic alkenyl iodides could be converted to the corresponding alkenylmagnesium derivatives by the reaction with *i*-PrMgCl·LiCl under mild conditions (≤ –20 °C) with high stereoselectivity.[21] Indeed, styrene **2**[22] smoothly undergoes I–Mg exchange with *i*-PrMgCl·LiCl in THF at –40 °C over 15 minutes (Scheme 2). This reaction proceeded with excellent level of stereocontrol (*Z*/*E* = 15:1). The needed Mg–Zn transmetallation was accomplished by the addition of 1 M $ZnCl_2$ solution in a THF–NMP mixture at –40 °C over one minute and took place (as shown by iodolysis of an aliquot) with complete retention of the double bond geometry. Organozinc reagent **3** was subjected to *in situ* Negishi cross-coupling with the corresponding aryl iodides or bromides in the presence of palladium catalyst. A number of palladium complexes were tested during the optimization of this cross-coupling reaction, including DPEphosPd(OAc)$_2$, S-PhosPd(dba)$_2$, (A-^{ca}Phos)$_2PdCl_2$, and (A-^{ta}Phos)$_2PdCl_2$. The highest yield of the desired product was achieved using 2 mol% of (A-^{ta}Phos)$_2PdCl_2$ complex (Scheme 2). This reaction occurs at room temperature within 0.5–17 hours, affording the desired (*Z*)-stilbenes **4a**–**m** in 36–64% yields and very good stereoselectivity (*Z*/*E* > 10:1 for all cases, Table 1), regardless on the substitution pattern of the aryl halide reagent.

In vitro cytotoxicity of the synthesized combretastatin A-4 (**4a**)[24] and its analogues **4b**–**m** was investigated toward HBL100 human mammary cell line. A tetrazolium-based assay was used for the determination of the drug concentration required to inhibit cell growth by 50% after the incubation in the culture medium for 72 hours. The obtained values are summarized in Table 1. Several new (*Z*)-stilbenes **4c**–**m** exhibited promising antiproliferative activity (IC_{50} = 0.022–10.3 μM).

In conclusion, we have developed a mild and stereoselective approach to (*Z*)-stilbenes, using palladium-mediated Negishi cross-coupling reaction of (*Z*)-alkenylzinc reagents with different arylhalogenides. The proposed method permits the synthesis of CA-4 analogues bearing different substitution patterns in good yield and high stereoselectivity via a sequence of three-step one-pot reactions. Newly prepared compounds manifested promising cytotoxic properties (IC_{50} = 0.022–10.3 μM).

Acknowledgment

This work was supported by German Academic Research Service (A/08/81119) and the Council on Grants at the President of the Russian Federation (MD-5606.2010.3), Federal Targeted Programme 'Scientific and Scientific-Pedagogical Personnel of the Innovative Russia in 2009–2013' (16.740.11.0476).

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synlett.

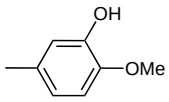
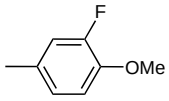
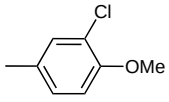
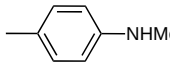
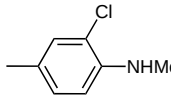
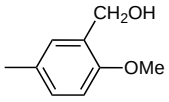
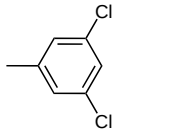
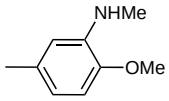
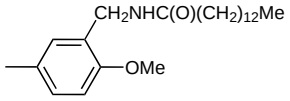
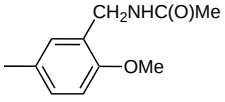
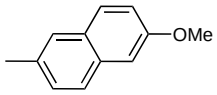
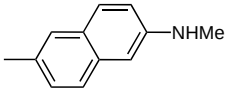
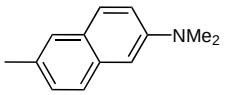
References and Notes

(1) (a) Pettit, G. R.; Cragg, G. M.; Herald, D. L.; Schmidt, J. M.; Lobavanijaya, P. *Can. J. Chem.* **1982**, *60*, 1347. (b) Hamel, E.; Lin, C. M. *Biochem. Pharmacol.* **1983**, *32*, 3864. (c) Lin, C. M.; Singh, S. B.; Chu, P. S.; Dempcy, R. O.; Schmidt, J. M.; Pettit, G. R.; Hamel, E. *Mol. Pharmacol.* **1988**, *34*, 200. (d) Lin, C. M.; Ho, H. H.; Pettit, G. R. *Biochemistry* **1989**, *28*, 6984. (e) Pettit, G. R.; Singh, S. B.; Niven, M. L.; Hamel, E.; Schmidt, J. M. *J. Nat. Prod.* **1987**, *50*, 119. (f) Pettit, G. R.; Singh, S. B.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Schmidt, J. M.; Hogan, F. *J. Med. Chem.* **1995**, *38*, 1666. (g) Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendall, D. *Experientia* **1989**, *45*, 209.

(2) (a) Mooney, C. J.; Nagaiah, G.; Fu, P.; Wasman, J. K.; Cooney, M. M.; Savvides, P. S.; Bokar, J. A.; Dowlati, A.; Wang, D.; Agarwala, S. S.; Flick, S. M.; Hartman, P. H.; Ortiz, J. D.; Lavertu, P. N.; Remick, S. C. *Thyroid* **2009**, *19*, 233. (b) Kingston, D. G. I. *J. Nat. Prod.* **2009**, *72*, 507. (c) Lippert, J. W. *Bioorg. Med. Chem.* **2007**, *15*, 605. (d) Dumontet, C.; Jordan, M. A. *Nat. Rev.* **2010**, *9*, 790.

(3) (a) Pettit, G. R.; Toki, B.; Herald, D. L.; Verdier-Pinard, P.; Boyd, M. R.; Hamel, E.; Pettit, R. K. *J. Med. Chem.* **1998**, *41*, 1688. (b) Getahun, Z.; Jurd, L.; Chu, P. S.; Lin, C. M.; Hamel, E. *J. Med. Chem.* **1992**, *35*, 1058. (c) Siles, R.; Ackley, J. F.; Hadimani, M. B.; Hall, J. J.; Mugabe, B. E.; Guddneppanavar, R.; Monk, K. A.; Chapuis, J.-C.; Pettit, G. R.; Chaplin, D. J.; Edvardsen, K.; Trawick, M. L.; Garner, C. M.; Pinney, K. G. *J. Nat. Prod.* **2008**, *71*, 313. (d) Simoni, D.; Romagnoli, R.; Baruchello, R.; Rondanin, R.; Rizzi, M.;

Table 1 Yields and Cytotoxicity of Combretastatin A-4 Analogues

Ar^2	Product	Yield (%)	IC_{50} (μM)
	**4a** (CA-4)	55[a]	0.002
	**4b**	51	0.007
	**4c**	55	0.022
	**4d**	42[b]	0.040
	**4e**	39[b]	0.047
	**4f**	37[a]	0.398
	**4g**	36	5.51
	**4h**	38[b]	10.3
	**4i**	42	0.462
	**4g**	64	0.489
	**4k**	56	0.085
	**4l**	60[b]	0.359
	**4m**	56	0.862

[a] Overall yield for (*Z*)-stilbene preparation including the stage of TBS-group cleavage

[b] Overall yield for (*Z*)-stilbene preparation including the stage of Boc-group cleavage

Pavani, M. G.; Alloatti, D.; Giannini, G.; Marcellini, M.; Riccioni, T.; Castorina, M.; Guglielmi, M. B.; Bucci, F.; Carminati, P.; Pisano, C. *J. Med. Chem.* **2006**, *49*, 3143.

(4) (a) Nam, N. H. *Curr. Med. Chem.* **2003**, *10*, 1697. (b) Tron, G. C.; Pirali, T.; Sorba, G.; Pagliai, F.; Busacca, S.; Genazzani, A. A. *J. Med. Chem.* **2006**, *49*, 3033.

(5) Singh, R.; Kaur, H. *Synthesis* **2009**, 2471.

(6) (a) Pettit, G. R.; Singh, S. B.; Boyd, M. R.; Hamel, E.; Pettit, R. K.; Schmidt, J. M.; Hogan, F. *J. Med. Chem.* **1995**, *38*, 1666. (b) Roberti, M.; Pizzirani, D.; Simoni, D.; Rondanin, R.; Baruchello, R.; Bonora, C.; Buscemi, F.; Grimaudo, S.; Tolomeo, M. *J. Med. Chem.* **2003**, *46*, 3546. (c) Pettit, G. R.; Singh, S. B. *J. Org. Chem.* **1989**, *54*, 4105. (d) Harrowven, D. C.; Guy, I. L.; Howell, M.; Packham, G. *Synlett* **2006**, 2977.

(7) (a) Lawrence, N. J.; Ghani, F. A.; Hepworth, L. A.; Hadfield, J. A.; McGown, A. T.; Pritchard, R. G. *Synthesis* **1999**, 1656. (b) Brown, H. C.; Zweifel, G. *J. Am. Chem. Soc.* **1961**, *83*, 3834.

(8) Fürstner, A.; Nikolakis, K. *Liebigs Ann.* **1996**, 2107.

(9) (a) Giraud, A.; Provot, O.; Hamze, A.; Brion, J.-D.; Alami, M. *Tetrahedron Lett.* **2008**, *49*, 1107. (b) Hamze, A.; Provot, O.; Brion, J.-D.; Alami, M. *Synthesis* **2007**, 2025.

(10) Lara-Ochoa, F.; Espinosa-Pérez, G. *Tetrahedron Lett.* **2007**, *48*, 7007.

(11) Pettit, G. R.; Singh, S. B.; Cragg, G. M. *J. Org. Chem.* **1985**, *50*, 3404.

(12) (a) Gaukroger, K.; Hadfield, J. A.; Hepworth, L. A.; Lawrence, N. J.; McGown, A. T. *J. Org. Chem.* **2001**, *66*, 8135. (b) Zou, Y.; Xiao, C.-F.; Zhong, R.-Q.; Wei, W.; Huang, W.-M.; He, S.-J. *J. Chem. Res.* **2008**, 354.

(13) Bazin, M.-A.; Jouanne, M.; El-Kashef, H.; Rault, S. *Synlett* **2009**, 2789.

(14) Camacho-Davila, A. A. *Synth. Commun.* **2008**, *38*, 3823.

(15) (a) Robinson, J. E.; Taylor, R. J. K. *Chem. Commun.* **2007**, 1617. (b) Chan, T.-L.; Fong, S.; Li, Y.; Man, T.-O.; Poon, C.-D. *J. Chem. Soc., Chem. Commun.* **1994**, 1771. (c) Yang, G.; Franck, R. W.; Byun, H.-S.; Bittman, R.; Samadder, P.; Arthur, G. *Org. Lett.* **1999**, *1*, 2149. (d) Meyers, C. Y.; Malte, A. M.; Matthews, W. S. *J. Am. Chem. Soc.* **1969**, *91*, 7510.

(16) (a) Negishi, E. *Acc. Chem. Res.* **1982**, *15*, 340. (b) Klement, I.; Rottlander, M.; Tucker, C. E.; Majid, T. N.; Knochel, P.; Venegas, P.; Cahiez, G. *Tetrahedron* **1996**, *52*, 7201.

(17) (a) Cragg, G. M.; Newman, D. J. *J. Nat. Prod.* **2004**, *67*, 232. (b) Nicolaou, K. C.; Dai, W.-M.; Guy, R. K. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 15. (c) Abal, M.; Andreu, J. M.; Barasoain, I. *Curr. Cancer Drug Targets* **2003**, *3*, 193.

(18) (a) Bailly, C.; Bal, C.; Barbier, P.; Combes, S.; Finet, J.-P.; Hildebrand, M.-P.; Peyrot, V.; Wattez, N. *J. Med. Chem.* **2003**, *46*, 5437. (b) Rappl, C.; Barbier, P.; Bourgarel-Rey, V.; Gregoire, C.; Gilli, R.; Carre, M.; Combes, S.; Finet, J.-P.; Peyrot, V. *Biochemistry* **2006**, *45*, 9210. (c) Ganina, O. G.; Daras, E.; Bourgarel-Rey, V.; Peyrot, V.; Andresyuk, A. N.; Finet, J.-P.; Fedorov, A. Y.; Beletskaya, I. P.; Combes, S. *Bioorg. Med. Chem.* **2008**, *16*, 8806.

(19) Cheung, L. L. W.; Yudin, A. K. *Org. Lett.* **2009**, *11*, 1281.

(20) Knochel, P.; Normant, J. F. *Tetrahedron Lett.* **1986**, *27*, 4431.

(21) Ren, H.; Krasovskiy, A.; Knochel, P. *Org. Lett.* **2004**, *6*, 4215.

(22) **Preparation of (*Z*)-3,4,5-Trimethoxy-β-Iodostyrene (2)**
Into a flame-dried 2 L round-bottom flask equipped with a magnetic stirrer and a septum was placed iodomethylene-triphenylphosphonium iodide (62 g, 117 mmol).[19,23] The flask was then put under vacuum for 5 min and purged with nitrogen. Dry THF (350 mL) was added, and the yellow

suspension was cooled to –20 °C. Then, NaHMDS in THF (62 mL of 1.9 M solution, 117 mmol) was added dropwise along the flask wall within 30 min. The mixture was stirred at –20 °C for 15 min, then cooled to –78 °C, and 3,4,5-trimethoxybenzaldehyde (17.6 g, 90 mmol) in THF (200 mL) was added at this temperature within 1 h with good stirring. The reaction was stirred in the cooling bath for 2 h more, and quenched while still cold with sat. aq NH_4Cl. Then Et_2O was added to the mixture, the layers were separated, and the aqueous layer was extracted with Et_2O. The combined organic layers were filtered to remove Ph_3PO, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by flash chromatography on silica gel (pentane–EtOAc, 4:1) to give **2** (20.5 g, 64 mmol, 71%, *Z*/*E* = 15:1) as a yellow oil. ^1H NMR (300 MHz, $CDCl_3$): δ = 7.20 (d, *J* = 8.6 Hz, 1 H), 6.91 (s, 2 H), 6.48 (d, *J* = 8.6 Hz, 1 H), 3.86 (s, 6 H), 3.85 (s, 3 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 152.8, 138.2, 138.1, 131.8, 105.8, 78.0, 60.8, 56.2. MS (EI): *m/z* (%) = 320 (100) $[M]^+$, 306 (5), 303 (45), 276 (5), 150 (5). HRMS (EI): *m/z* calcd for $C_{11}H_{13}IO_3$: 319.9909; found: 319.9910 $[M]^+$.

(23) Conway, J. C.; Quayle, P.; Regan, A. C.; Urch, C. J. *Tetrahedron* **2005**, *61*, 11910.

(24) **Typical Procedure for the Preparation of 4a-OTBS**
A dry nitrogen-flushed Schlenk flask, equipped with a magnetic stirrer and a septum, was charged with a solution of alkenyl iodide (320 mg, 1 mmol) in dry THF (3 mL). The solution of *i*-PrMgCl·LiCl (0.92 mL of 1.19 M in THF, 1.1 mmol) was added slowly at –40 °C, and the reaction mixture was stirred at this temperature for 15 min to complete the I–Mg exchange. A solution of $ZnCl_2$ (0.5 mL of 1 M in THF, 0.5 mmol) and NMP (0.1 mL) was added dropwise within 1 min, and the reaction was warmed to r.t. 4-Methoxy-3-(*tert*-butyldimethylsilyloxy)iodobenzene (400 mg, 1.1 mmol) and $(A\text{-}^{ta}Phos)_2PdCl_2$ (14 mg, 0.02 mmol) were added. The reaction mixture was stirred at r.t. for 30 min, poured into sat. aq NH_4Cl solution, and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated to get brown oil. It was purified by flash chromatography on silica gel (pentane–EtOAc, 5:1) to give **4a-OTBS** (267 mg, 0.62 mmol, 62%) as a yellow oil. ^1H NMR (300 MHz, $CDCl_3$): δ = 6.85 (dd, *J* = 8.3, 2.1 Hz, 1 H), 6.79 (d, *J* = 2.1 Hz, 1 H), 6.73 (d, *J* = 8.3 Hz, 1 H), 6.50 (s, 2 H), 6.47 (d, *J* = 12.1 Hz, 1 H), 6.41 (d, *J* = 12.1 Hz, 1 H), 3.83 (s, 3 H), 3.77 (s, 3 H), 3.70 (s, 6 H), 0.93 (s, 9 H), 0.06 (s, 6 H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 153.1, 150.4, 144.7, 137.2, 133.2, 130.2, 129.8, 128.9, 123.0, 121.4, 111.8, 106.0, 61., 56.0, 55.6, 25.8, 18.5, –4.7. MS (EI): *m/z* (%) = 430 (40) $[M]^+$, 373 (22), 359 (23), 358 (100), 343 (25). HRMS (EI): *m/z* calcd for $C_{24}H_{34}O_5Si$: 430.2176; found: 430.2180 $[M]^+$.

