Inorg. Chem. **2005**, *44*, 5370–5378

Synthesis, Characterization, Biocide and Toxicological Activities of Di-*n*-butyl- and Diphenyl-tin^{IV}-Salicyliden-β-Amino Alcohol Derivatives

Luis S. Zamudio-Rivera,*,† Rocio George-Tellez,† Gerson López-Mendoza,† Adela Morales-Pacheco,† Eugenio Flores,† Herbert Höpfl,‡ Victor Barba,‡ Francisco J. Fernández,§ Nathalie Cabirol,|| and Hiram I. Beltrán*,†

Programa de Ingeniería Molecular, Instituto Mexicano del Petróleo, Eje Central Lázaro Cárdenas No.152, Apartado Postal 14-805, 07730 México, D.F, Centro de Investigaciones Químicas, Universidad Autónoma del Estado de Morelos, Av. Universidad 1001, C. P. 62210 Cuernavaca, México, Departamento de Biotecnología. Universidad Autónoma Metropolitana-Iztapalapa. Apdo. Postal 55-535, 09340 México, D.F, and Instituto de Ingeniería. Universidad Nacional Autónoma de México, Circuito Escolar S/N, Ciudad Universitaria, 04510 Coyoacán, México, D.F.

Received September 30, 2004

The one pot reaction of salicylaldehyde **1**, β-amino alcohols **2a**–**2c**, and di-*n*-butyltin^{IV} oxide **3a** or diphenyltin^{IV} oxide **3b** produced five diorganotin^{IV} compounds, **4a**–**4c, 5a**, and **5c**, in good yields. All compounds were characterized by IR, ^{1}H, ^{13}C, and ^{119}Sn NMR spectroscopy, and elemental analysis; furthermore, compounds **4b**, **4c**, **5a**, and **5c** were characterized by X-ray diffraction analysis. After the structural characterization, all of the compounds were tested *in vitro* against *Bacillus subtilis* (Gram-positive, strain ATCC 6633), *Escherichia coli* (Gram-negative, strain DH5α), *Pseudomonas aeruginosa* (Gram-negative, strain BH3), *Desulfovibrio longus* (strain DSM 6739), and *Desulfomicrobium aspheronum* (strain DSM 5918) to assess their antimicrobial activity. Compounds **4** and **5** demonstrated a wide range of bactericidal activities against the tested aerobic (one Gram-positive and two Gram-negative subtypes) and anaerobic bacteria (two sulfate-reducing bacteria, SRB). Compound **5** had better bactericidal performances than compound **4**. For all of the compounds, the acute toxicity was measured using luminescent bacteria toxicity (LBT-Microtox) tests to track their further environmental impact. According to these results and in order to fulfill environmental regulations, the toxicity of the compounds studied herein can be modulated through the proper selection of the disubstituted tin^{IV} moiety.

Introduction

The problems of bio-corrosion and fouling in the oil industry are commonly controlled through the use of biocides and dispersants.[1−3] The main groups of microorganisms associated with the bio-corrosion and fouling problems are Gram-positive, Gram-negative, and sulfate-reducing bacteria (SRB).[2] Different molecular prototypes containing tin atoms in their structure have shown good activity against these bacterial groups.[4−8] According to Lascourrèges *et al.*, the mono- and diorganotin^{IV} derivatives are more active than the corresponding triorganotin^{IV} derivatives against the SRB.[4] Moreover, the use of some triorganotin^{IV} molecules as biocides has been banned recently because of their high toxicity toward marine organisms.[9] Hence, the future tendencies

* To whom correspondence should be addressed. E-mail: lzamudio@imp.mx (L.S.Z.-R.); hbeltran@imp.mx. (H.I.B.).
† Programa de Ingeniería Molecular, Instituto Mexicano del Petróleo.
‡ Centro de Investigaciones Químicas, Universidad Autónoma del Estado de Morelos.
§ Departamento de Biotecnología, Universidad Autónoma Metropolitana-Iztapalapa.
|| Instituto de Ingeniería, Universidad Nacional Autónoma de México.

(1) O'Reilly, K. T.; Moir, M. E.; O'Rear, D. J. *US Pat.* **2003**, 2003/0162845 A1.
(2) Chan, K. Y.; Xu, L. C.; Fang, H. P. F. *Environ. Sci. Technol.* **2002**, *36*, 1720.
(3) Whale, G. F.; Whitham, T. S. In *SPE Health*, Proceedings of the Safety and Environment in Oil and Gas Exploration and Production Conference, the Hague, the Netherlands, November 11−14 1991; Society of Petroleum Engineers, Inc.: Richardson, TX, 1991; 23357, p 355.
(4) Lascourrèges, J. F.; Caumette, P.; Donard, O. X. F. *Appl. Organomet. Chem.* **2000**, *14*, 98.
(5) Gielen, M. *Appl. Organomet. Chem.* **2002**, *16*, 481.
(6) Nath, M.; Yadav, R.; Gielen, M.; Dalil, H.; de Vos, D.; Eng, G. *Appl. Organomet. Chem.* **1997**, *11*, 727.
(7) Blunden, S. J.; Cusack, P. A.; Hill, R. *The Industrial Uses of Tin Chemicals*; Royal Society of Chemistry: London, 1985.
(8) Goh, N. N.; Chu, C. K.; Khoo, L. E.; Whalen, D.; Eng, G.; Smith, F. E.; Hynes, R. C. *Appl. Organomet. Chem.* **1998**, *12*, 457.

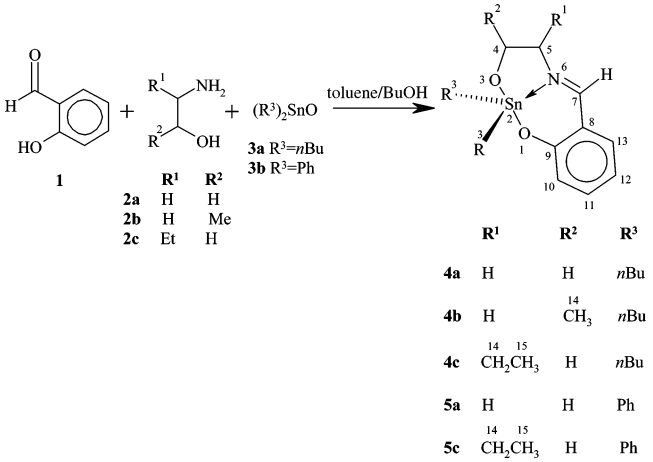
10.1021/ic048628o CCC: $30.25
Published on Web 06/21/2005

direct the research lines to new organotin^{IV} compounds for which molecular design should take care of the balance between toxicity and specific biocide activity.[1,9]

A search of the CCDB[10] revealed only 33 X-ray structures of diorganotin^{IV} compounds with methyl, vinyl, *n*-butyl, and phenyl substituents attached to the tin atoms that have been constructed from rigid O, N, O-tridentate ligands with five- or six-membered fused rings. From these X-ray data, there are 24 amino acid-containing structures and only 9 aminophenol derivatives. These compounds are mainly monomeric, even in solution, and in the solid state.[11−14] The Sn−O···Sn−O or solvent···Sn coordination modes are mostly preferred in the di-*n*-butyltin^{IV} derivatives.[11−16] In one of the most recent studies, intermolecular Sn···O interactions were shown to result in dimeric aggregates when the tin moiety is nBu$_2$Sn^{IV};[15,16] in contrast, compounds with Ph$_2$Sn^{IV} functionality barely show such interactions.[15,16] These differences would be important for the balance of the biocide activity and the toxicity in new tin prototypes. Moreover, the strength of such Sn···O interactions was shown to be modulated through chemical substitution with electro-withdrawing or electro-releasing groups, which when used in such a way are chemical balancers of a specific activity.

Herein, we describe the structural and spectroscopic characterization of a series of nBu$_2$Sn^{IV} and Ph$_2$Sn^{IV} salicylidene β-amino alcoholates whose activities have been measured against aerobic bacteria (*Bacillus subtilis*, Gram-positive, strain ATCC 6633; *Escherichia coli*, Gram-negative, strain DH5α; and *Pseudomonas aeruginosa*, Gram-negative, strain BH3) and against anaerobic bacteria (*Desulfovibrio longus*, strain DSM 6739 and *Desulfomicrobium aspheronum*, strain DSM 5918). The acute toxicological activity of each compound was measured using the Microtox luminescent bacteria toxicity (LBT-Microtox) test; the sensitive *Photobacterium phosphoreum* (*Vibrio fischering*) was used as the standard,[17a] instead of other mammalian cell lines,[17b] according to the simplicity of the organisms. The comparative study allowed us to identify a relationship between activity and structure.

Scheme 1. Numbering and Preparation of Compounds **4** and **5**



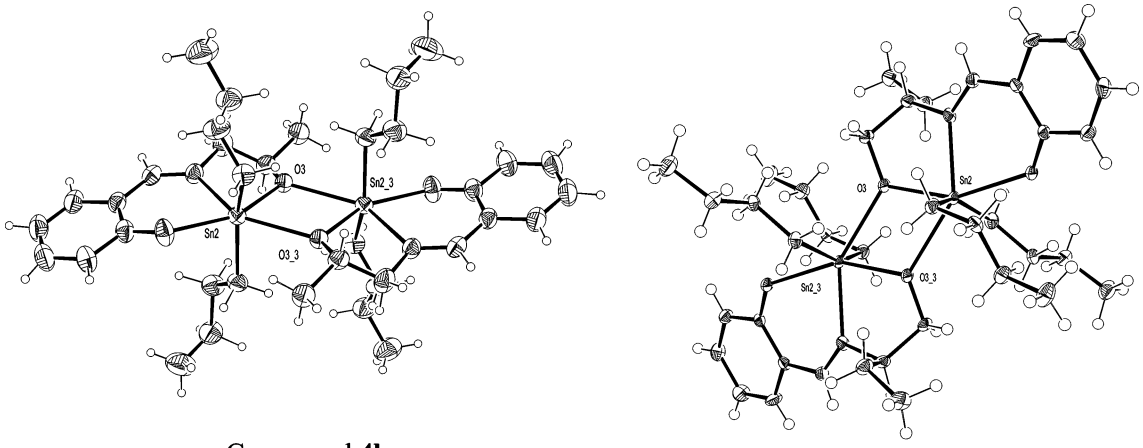
Results and Discussion

Diorganotin^{IV} derivatives of β-amino alcohols of the type proposed herein were not found in the literature; therefore, their preparation, full spectroscopic characterization, X-ray structures (four derivatives), biocide activity, and acute toxicity tests are condensed within the present contribution.

Synthetic Procedure. The method involves 1:1:1 stoichiometric addition of salicylaldehyde **1**, β-amino alcohols **2a**−**2c**, and di-*n*-butyltin^{IV} oxide **3a** or diphenyltin^{IV} oxide **3b** to a 4:1 solvent mixture of toluene and butanol, which was refluxed for 8 h to produce **4a**−**4c**, **5a**, and **5c** in yields of 70 to 92% (Scheme 1). The structural elucidation of the resulting tin compounds was accomplished by ^{1}H, ^{13}C, and ^{119}Sn NMR, IR, mass spectrometry, elemental analysis, and single-crystal X-ray crystallographic studies for **4b**, **4c**, **5a**, and **5c**. In the case of **5b** (R^1 = H, R^2 = CH_3, R^3 = Ph), the ^{1}H, ^{13}C, and ^{119}Sn NMR spectra provided evidence of oligomeric structures both in coordinating and noncoordinating deuterated solvents. Attempts to obtain monomeric **5b** were unsuccessful, and further chromatographic purification led to the hydrolysis reaction of the tin^{IV} derivative.[7] As a consequence, the structure of **5b** could not be established with certainty, therefore banning further systematic studies.

Solution NMR of 4a, 4b, 4c, 5a, and 5c. The coupling types, such as one bond, and the long range heteronuclear coupling constant for the NMR spectra were systematically determined for all of the compounds reported herein, in analogy to previous studies already published for diorganotin derivatives.[5−9,11−16] The ^{1}H NMR spectra shows single signals for H7 that appears between 8.38 and 8.25 ppm, as well as the corresponding signals for the iminic salicylidene moiety.[15,16] The ^{1}H signals for the β-imino alcohol moiety appear shielded and coupled depending on the substitution. The ^{13}C NMR data show that signals for C7 appear between 171.5 and 170.6 ppm, indicating a deshielding caused by the polarization of the C═N bond.[15,16] For compounds **4b**, **4c**, and **5c**, the di-*n*-butyl and di-phenyl, ^{1}H and ^{13}C signals are anisochronous because of the presence of stereogenic centers.[15,16] Meanwhile, for compounds **4a** and **5a**, the respective signals are isochronous.[15,16]

(9) Dubey, S. K.; Roy, U. *Appl. Organomet. Chem.* **2003**, *17*, 17.
(10) (a) http://www.ccdc.cam.ac.uk. (b) Allen, F. M. *Acta Crystallogr.* **2002**, *B58*, 380. (c) Bruno, I. J.; Cole, J. C.; Edington, P. R.; Kessler, M.; Macrae, C. F.; McCabe, P.; Pearson, J.; Taylor, R. *Acta Crystallogr.* **2002**, *B58*, 389. (d) Bruno, I. J.; Cole, J. C.; Lommerse, J. P. M.; Rowland, R. S.; Taylor, R.; Verdonk, M. *J. Comput.-Aided Mol. Des.* **1997**, *11*, 525.
(11) Narula, S. P.; Bharadwaj, S. K.; Sharda, Y.; Day, R. O.; Howe, L.; Holmes, R. R. *Organometallics* **1992**, *11*, 2206.
(12) Dakternieks, D.; Jurkschat, K.; van Dreumel, S.; Tiekink, E. R. T. *Inorg. Chem.* **1997**, *36*, 2023.
(13) Dakternieks, D.; Baul, T. S. B.; Dutta, S.; Tiekink, E. R. T. *Organometallics* **1998**, *17*, 3058.
(14) Narula, S. P.; Kaur, S.; Shankar, R.; Verma, S.; Venugopalan, P.; Sharma, S. K.; Chadha, R. K. *Inorg. Chem.* **1999**, *38*, 4777.
(15) (a) Reyes, H.; García, C.; Farfán, N.; Santillan, R.; Lacroix, P. G.; Lepetit, C.; Nakatani, K. *J. Organomet. Chem.* **2004**, *689*, 2303. (b) Farfan, N.; Mancilla, T.; Santillan, R.; Gutierrez, A.; Zamudio-Rivera, L. S.; Beltrán, H. I. *J. Organomet. Chem.* **2004**, *689*, 3481.
(16) Beltrán, H. I.; Zamudio-Rivera, L. S.; Mancilla, T.; Santillan, R.; Farfán, N. *Chem.−Eur. J.* **2003**, *9*, 2291.
(17) (a) Bulich, A. A.; Tung, K. K.; Scheibner, G. *J. Biolumin. Chemilumin.* **1990**, *5*, 71. (b) Kahru, A.; Tomson, K.; Pall, T.; Külm, I. *Water Sci. Technol.* **1996**, *33*, 147.



Compound **4b** Compound **4c**

Figure 1. X-ray structures of **4b** and **4c**. The numbering is the same as that in Figure 2.

The ^{119}Sn NMR measurements were obtained in noncoordinating ($CDCl_3$) and coordinating (DMSO-d_6) solvents for the **4** series. The chemical shifts range from −194.6 to −189.5 ppm, in $CDCl_3$, indicative of pentacoordinated tin atoms.[15,16] For experiments in DMSO-d_6, the observed shifts range from −206.1 to −201.7 ppm and since the solvent molecules coordinate to tin, shifting the signal to the region corresponding to hexacoordinated species.[15,16] The **5** series show the ^{119}Sn NMR signals between −337.3 and −334.6 ppm in $CDCl_3$;[15−17] meanwhile in DMSO-d_6, the signals remain almost unmoved, indicating the same coordination number, even in coordinating solvents.

For some of the compounds, the measurement of $|^nJ(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})|$, where $n = 1$, 2, and 3, was feasible through aromatic and aliphatic systems. These measurements are a method for determining important structural details among different derivatives and should be taken into account for the present study. The coupling constants for the aromatic salicylidene system behave in a manner very similar to that found for the analogous amino acid derivatives.[16] The coupling constants for the β-imino alcohol moiety, including the C4 and C5 positions, are not directly comparable to those observed for the α-iminoacid fragment where the values of $|^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C}\text{-})|$ for an sp^2 carbon atom and a bridging sp^3 oxygen atom are between 21.6 and 14.6 Hz.[16] The values of $|^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C5})|$ for an sp^3 carbon atom and a bridging sp^2 nitrogen atom are between 16.9 and 12.8 Hz.[16] For the compounds from this study, the magnitude of $|^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C4})|$ for an sp^3 carbon atom and a bridging sp^3 oxygen atom are between 22.5 and 18.4 Hz. They are very similar to the values found for the α-iminoacid derivatives. The magnitudes for $|^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C5})|$ for an sp^3 carbon atom and a bridging sp^2 nitrogen atom are between 44.4 and 33.1 Hz. These values are somewhat divergent from those found for the α-iminoacid derivatives. Keep in mind that at this position there is more chemical similarity between the fragments. The $|^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C}\beta)|$ values were measured (taken as reference for a free aliphatic system and therefore without cyclic restraints) to assess more evidence within these observations; their range was found to be between 34.6 and 30.5 Hz. The $|^3J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C}\gamma)|$ values were also measured to ascertain such a comparison, and their range is between 96.9 and 86.2 Hz. Therefore, a $^2J-^3J$ mixed coupling system for the C4 and C5 positions in compounds **4a**, **4b**, **4c**, **5a**, and **5c** is discarded. From the analysis of the latter statements, two main contributions have been found: the former is highly dependent on the length of the coupling ($n = 2$ and 3) and the latter is mainly dependent on the chemical system through which the coupled atoms are being connected.

X-ray Structures. Compounds **4b**, **4c**, **5a**, and **5c** were successfully crystallized, and their structures were determined by single-crystal X-ray diffraction analysis. Figures 1 and 2 show the examples for the penta- and hexacoordinated tin-containing structures. The collection data details are condensed in Table 1, and selected geometrical parameters are shown in Table 2.

Compounds **4b** and **4c** crystallized with distorted octahedral (DOC) geometry around the tin atom.[11−13] The O, N, O-tridentate ligand is in a *mer* orientation to the tin octahedron. The two *n*-butyl substituents are *trans* to each other, allowing the intramolecular coordination of the O3 with tin to build a dimeric species. The resulting Sn2···O3 bond has a magnitude of 2.500(2) Å for **4b** and 2.457(3) Å for **4c**. These values are smaller than the sum of the van der Waals radii (2.17 Å for tin and 1.52 Å for oxygen).[16] Additionally, the Sn−O···Sn−O torsion angles show evidence of coplanarity because there is a 1° deviation of the two different dimeric molecules.[15,16] The dimeric assembly occurs via the formation of a Sn_2O_2 four-membered ring.[15,16] The O···Sn bonding occurs with the oxygen atom at the five-membered ring.

Compounds **5a** and **5c** crystallized with trigonal bipyramidal (TBP) geometry surrounding the tin atom.[15,16] The two oxygen-donating atoms of the O, N, O-tridentate ligand are in axial positions, and the nitrogen atom occupies one equatorial position. The two phenyl groups attached to tin occupy the other two equatorial positions. Compound **5c** cocrystallized with 0.5 equiv of a water molecule per asymmetric unit, forming a hydrogen bond between the O3 and the hydrogen atom of the water with a bond length of 2.017(3) Å.

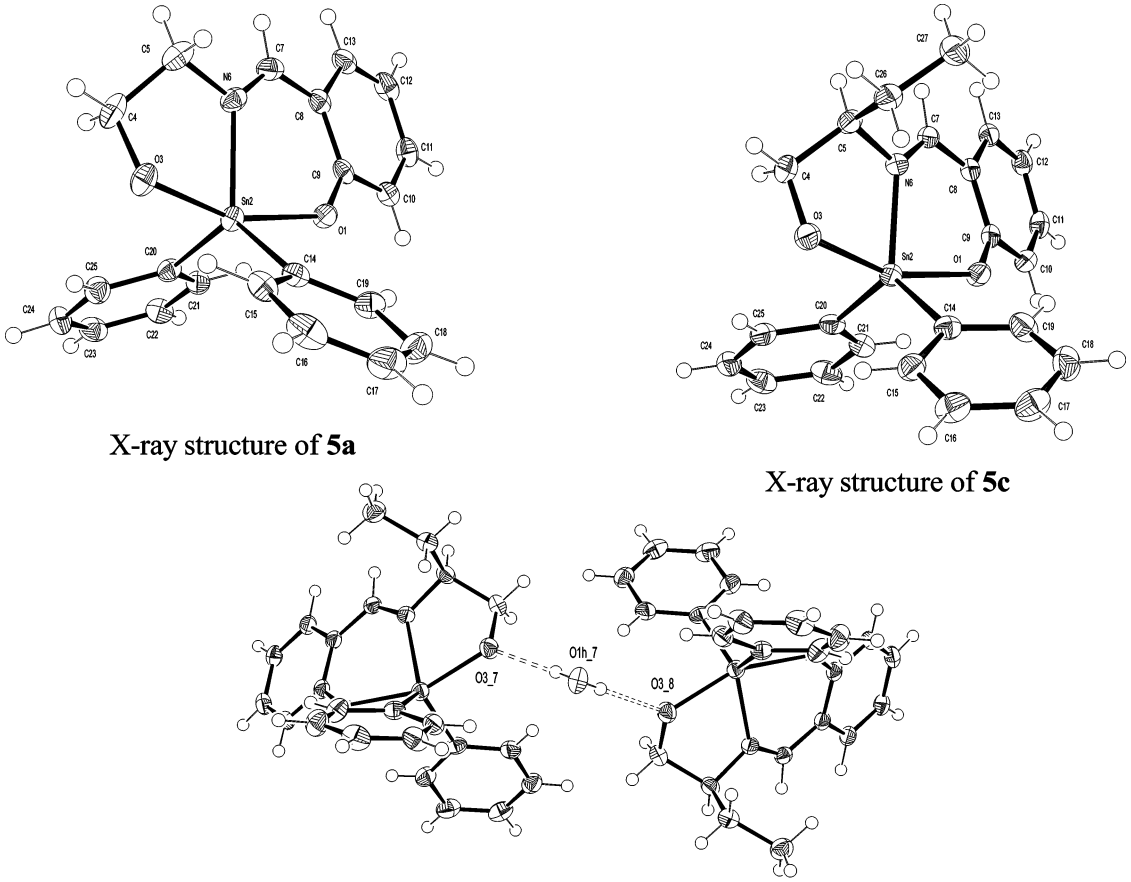


Figure 2. X-ray structures of **5a** and **5c**.

Table 1. Collection Data and Refinement Parameters for Compounds **4b**, **4c**, **5a**, and **5c**

	4b	**4c**	**5a**	**5c**
molecular formula	$C_{18}H_{29}NO_2Sn$	$C_{19}H_{31}NO_2Sn$	$C_{21}H_{19}NO_2Sn$	$C_{23}H_{23}NO_2Sn\cdot{}^1/_2H_2O$
mol wt	410.11	424.14	436.06	473.12
cryst syst	monoclinic	monoclinic	triclinic	monoclinic
space group	$P2_1/c$	$P2_1/c$	$P\bar{1}$	$C2/c$
a (Å)	12.1690(12)	9.4206(12)	9.0118(11)	16.3162(14)
b (Å)	17.9743(17)	17.690(2)	9.4311(11)	10.1829(9)
c (Å)	8.7980(9)	11.6191(15)	11.2241(13)	24.627(2)
α (deg)	90	90	86.643(2)	90
β (deg)	99.699(2)	100.862(2)	78.387(2)	93.682(2)
γ (deg)	90	90	70.979(2)	90
vol (Å^3)	1896.6(3)	2083.9(3)	883.38(18)	4083.3(6)
Z	4	4	2	8
ρ (mg/m^3)	1.436	1.481	1.639	1.539
μ (mm^{-1})	1.354	1.353	1.460	1.272
θ range (deg)	1.70 to 25.00	2.12 to 25.00	1.85 to 24.99	1.66 to 25.00
collected reflns	18142	18214	6992	9955
individual reflns [R(int)]	3343 [0.028]	3342 [0.059]	3104 [0.029]	3603 [0.025]
θ completeness [%]	25.00° [100]	25.00° [100]	24.99° [99.5]	25.00° [100]
data/restrictions/params	3343/38/279	3342/0/332	3104/0/226	3603/1/253
GOF on F^2	1.060	1.083	1.042	1.098
final indices [$I > \sigma(I)$] R1	0.024	0.035	0.022	0.026
final indices (all data) wR2	0.061	0.074	0.052	0.059
$\Delta\rho_{min}$(e·Å^3)	−0.25	−0.79	−0.37	−0.27
$\Delta\rho_{max}$(e·Å^3)	0.34	1.00	0.60	0.55

The N6−Sn2 bond distances are 2.244(2), 2.237(3), 2.186(2), and 2.175(2) Å, the Sn2−O1 bond distances are 2.234(2), 2.268(2), 2.107(2), and 2.100(2) Å, and the Sn2−O3 bond distances are 2.087(2), 2.100(2), 2.049(2), and 2.074(2) Å for **4b**, **4c**, **5a**, and **5c**, respectively. By analyzing the three sets of distances, one can see that the first two values are longer because they are related to hexacoordinated tin atoms, and the latter values are shorter because of the bonding to pentacoordinated tin atoms. The bond angles between both **α** carbons and the tin atoms (C−Sn−C) also depend on the coordination number of tin. They are 145.89(13)° for **4b**, 151.90(16)° for **4c**, 126.14(8)° for **5a**, and

Table 2. Selected Geometrical Parameters for Compounds **4b**, **4c**, **5a**, and **5c**

	4b	4c	5a	5c
		bond lengths (Å)		
N(6)−Sn(2)	2.244(2)	2.237(3)	2.186(2)	2.175(2)
C−Sn(2)	2.116(3)	2.138(4)	2.119(2)	2.120(3)
C−Sn(2)	2.122(3)	2.120(4)	2.130(2)	2.125(3)
O(1)−Sn(2)	2.234(2)	2.268(2)	2.107(2)	2.100(2)
O(3)−Sn(2)	2.087(2)	2.100(2)	2.049(2)	2.074(2)
O(3)···Sn(2)	2.500(2)	2.457(3)	−	−
N(6)−C(7)	1.286(3)	1.285(5)	1.291(3)	1.290(3)
C(9)−O(1)	1.292(3)	1.298(4)	1.318(3)	1.315(3)
C(4)−O(3)	1.397(4), 1.432(11)[a]	1.414(4)	1.402(3)	1.410(3)
C(4)−C(5)	1.447(5), 1.381(12)[a]	1.527(5)	1.527(4)	1.523(4)
N(6)−C(5)	1.467(4)	1.475(5)	1.470(3)	1.477(3)
C(7)−C(8)	1.424(8)	1.443(5)	1.433(3)	1.436(3)
C(8)−C(9)	1.411(4)	1.417(5)	1.421(3)	1.424(3)
C(7)−H(7)	0.918(3)	0.916(39)	0.893(29)	0.938(26)
		bond angles (deg)		
C−Sn(2)−C	145.89(13)	151.90(16)	126.14(8)	124.73(9)
O(1)−Sn(2)−O(3)	153.51(7)	152.94(9)	159.26(7)	159.21(7)
N(6)−Sn(2)−O(1)	78.07(8)	77.59(10)	82.20(7)	81.75(7)
N(6)−Sn(2)−O(3)	75.44(7)	75.54(10)	78.04(7)	78.21(7)
N(6)−Sn(2)−C	105.28(11)	103.63(14)	124.71(8)	123.10(9)
N(6)−Sn(2)−C	105.45(10)	100.65(14)	109.07(8)	112.08(8)
O(1)−Sn(2)−C	85.81(11)	83.26(13)	93.67(8)	90.36(9)
O(1)−Sn(2)−C	86.35(11)	88.36(13)	91.14(8)	94.55(9)
O(3)−Sn(2)−C	100.87(11)	100.03(13)	94.91(8)	98.04(9)
O(3)−Sn(2)−C	100.85(11)	99.55(13)	98.66(8)	95.91(9)
Sn(2)−O(1)−C(9)	132.68(18)	127.3(2)	131.97(14)	128.73(15)
Sn(2)−N(6)−C(5)	110.95(16)	112.9(2)	111.65(16)	111.86(15)
Sn(2)−N(6)−C(7)	130.13(19)	128.0(3)	128.20(16)	127.60(17)
Sn(2)−O(3)−C(4)	116.67(18), 115.6(5)[a]	115.9(2)	112.73(15)	111.99(15)
O(1)−C(9)−C(8)	123.2(3)	122.7(3)	123.0(2)	122.9(2)
C(4)−C(5)−N(6)	110.1(3), 115.6(5)[a]	105.2(3)	107.0(2)	105.4(2)
O(3)−C(4)−C(5)	111.2(3), 113.1(8)[a]	110.1(3)	109.5(2)	110.2(2)
C(7)−C(8)−C(9)	123.0(2)	123.2(3)	123.3(2)	122.4(2)
N(6)−C(7)−C(8)	128.0(3)	127.0(4)	127.2(2)	126.8(2)
Sn(2)−O(3)···Sn(2)	110.17(7)	111.70(10)	−	−
O(3)−Sn(2)···O(3)	69.83(7)	68.30(10)	−	−
		dihedral angles [deg]		
Sn(2)−O(1)-C(9)−C(10)	156.2(3)	145.2(3)	−159.19(16)	−147.57(19)
N(6)−C(7)-C(8)−C(13)	−173.3(3)	−170.8(4)	174.4(2)	170.8(3)
Sn(2)−O(3)-C(4)−C(5)	38.9(5), −35.7(12)[a]	41.7(4)	−48.0(2)	−46.4(3)
C(7)−N(6)-C(5)−C(4)	177.0(8), −145.0(4)[a]	−132.3(4)	148.9(2)	140.8(2)
		deviation from mean C(7)−C(8)−C(9)−C(10)-C(11)−C(12)-C(13)−O(1) plane (Å)		
Sn(2)	0.580(2)	−0.989(4)	−0.505(2)	−0.786(2)
N(6)	0.185(1)	−0.199(5)	−0.145(3)	−0.261(3)

[a] There are two values reported because disorder occurred for this fragment.

124.73(9)° for **5c** (the average value for TBP compounds is 126.99°, denying molecules which contain abnormal packing effects that significantly modify the geometry of tin).[15,16] The O1−Sn2−O3 bond angle values are 153.51(7)° for **4b**, 152.94(9)° for **4c**, 159.26(7)° for **5a**, and 159.21(7)° for **5c**. The first two values are smaller because of the steric and electronic demands of the sixth ligand.

As a consequence of ring fusion, the O3−Sn2−N6 bond angles in the five-membered rings have values of 75.44(7)° for **4b**, 75.54(10)° for **4c**, 78.04(7)° for **5a**, and 78.21(7)° for **5c**, while the angles of the O1−Sn2−N6 bonds are 78.07(8)° for **4b**, 77.59(10)° for **4c**, 82.20(7)° for **5a**, and 81.75(7)° for **5c**. The first two values for each angle are smaller than the following two values. This trend is also caused by the increment in coordination number and the proximity of a second molecule.

Biocide Activity. The bactericidal efficiencies of compounds **4** and **5** have been determined to examine their applicability as biocides. They have shown good activity. The compounds exhibited varying degrees of inhibitory effects on the growth of *B. subtilis*, *E. coli*, *P. aeruginosa*, *D, longus*, and *D. aspheronum* strains (Table 3).

From the data compiled in Table 4, it is evident that compound **5** exhibited better antibacterial activity against aerobic bacteria at concentrations ranging from 1×10^{-2} to 25 ppm than that exhibited by compound **4**, the concentrations of which range from 1×10^{-2} to 50 ppm. The data show that there is an increased activity against *P. aeruginosa*, whereby three different compounds result in the inhibition of the growth of the microorganism at concentrations as low as 1×10^{-2} ppm. In the case of the *B. subtilis* strain, the effect is moderate with only two compounds having a minimum inhibitory concentration (MIC, defined in the Experimental Section) of around 1 ppm. Finally, the tests with *E. coli* are indicative that all of the compounds have higher inhibitive doses with MICs near 25 ppm.

It is remarkable that the bacteria of the genus *Pseudomonas* are a group of microorganisms that show very high metabolic versatility, and therefore a large number of biocides feed

Table 3. Antibacterial Activity of Compounds **4** and **5** Varying the Concentration[a]

	concentration (ppm)									
compound	0.01	0.1	1	2.5	5	10	25	50	75	100
					B. subtilis					
4a	(- - -)	(- - -)	(- - -)	(- - -)	16.6	17.9	20.4	21.4	21.9	22.4
4b	(- - -)	(- - -)	(- - -)	14.9	16.8	18.1	20.0	21.4	22.03	22.4
4c	(- - -)	(- - -)	(- - -)	13.3	14.8	16.0	18.1	20.4	21.3	23.3
5a	(- - -)	(- - -)	13.4	14.1	19.5	19.4	22.1	22.9	22.6	22.8
5d	(- - -)	(---)	(- - -)	13.0	16.7	18.4	20.0	20.1	22.2	22.9
ligand	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)
					E. coli					
4a	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	10.45	11.5	12.3	13.03
4b	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	11.3	12.3	13.6	14.6
4c	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	11.2	12.5	13.6
5a	(- - -)	(- - -)	(- - -)	(- - -)	13.1	13.6	(- - -)	18.1	17.9	20.7
5d	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	10.6	10.6	11.3	11.4
ligand	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)
poly-**1**[b]								25.2		
					P. aeruginosa					
4a	(- - -)	(- - -)	(- - -)	11.0	11.7	12.9	12.9	14.3	17.0	20.4
4b	(- - -)	(- - -)	(- - -)	14.2	15.1	16.0	16.8	18.8	21.9	26.2
4c	10.5	11.2	10.8	12.3	13.0	14.8	14.8	15.0	23.7	24.3
5a	10.1	10.8	11.5	11.8	13.9	15.9	21.9	24.8	26.3	27.0
5d	11.2	11.9	11.2	12.2	13.4	13.3	17.8	22.6	24.7	26.0
ligand	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)	(- - -)
poly-**1**[b]								20.0		
					D. longus					
4b	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(−)	(−)
4c	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(−)	(−)
5a	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(−)	(−)
					D. aspheronum					
4b	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(−)	(−)
4c	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(−)	(−)
5a	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(−)	(−)

[a] **(**- - -) denotes no inhibition zone formation. For *B. subtilis*, *E. coli*, and *P. aeruginosa*, the diameter of the inhibition zone (mm) was measured after 16 h of incubation at 30 °C. For *D. longus* and *D. aspheronum*, the presence (+) and absence (−) of growth was measured. Measurements taken from Sun et al. (ref 21a). [b] Reported organotin compounds (ref 21b) have shown inhibition zones between 18 and 8 mm at 500 and 1000 ppm concentrations and therefore they are more active than those of the Streptomycin reference (ref 21b and 21c)

Table 4. Agar MICs of the Antimicrobial Agents Following Incubation at 30 °C for a Period of 16 h[a]

	average (mode) MIC (ppm)		
compound	*B. subtilis*	*E. coli*	*P. aeruginosa*
4a	5	25	2.5
4b	2.5	25	2.5
4c	2.5	50	**1** × 10^{-2}
5a	1	5	**1** × 10^{-2}
5c	1	25	**1** × 10^{-2}
ligand	−	−	−

these bacteria.[19] Compounds **4** and **5** are specifically inhibitive against its growth.

To clarify that the inhibitory efficiency is a result of the R_2Sn^{IV} (R = *n*Bu and Ph) moiety presence, we tested the ligand corresponding to **4a** and **5a**, and the results show no inhibitory effect for all of the bacteria used (as measured by MIC determination).

By taking the latter results as definite criteria, we performed the further anaerobic bacteria (SRB) tests only for **4b**, **4c**, and **5a**, which show the same efficacy at 75 ppm.

The variation in the effectiveness of the biocide compounds against different microorganisms depends on, among other things, the permeability across the bacterial cell wall.[20] A number of biocides have limited effects against specific Gram-positive or -negative subsets of microorganisms. *P. aeruginosa* and *E. coli*, both Gram-negative bacteria, showed very different susceptibility to the biocides described in this work. On the other hand, there is not a great difference between the data obtained from *P. aeruginosa* (Gram-negative) and *B. subtilis* (Gram-positive). Thus, the differences in composition and ultrastructure (at the cell wall of the bacteria) are not the key to explain the different activities of the biocides against the different microorganisms. A mechanism of action, based on the chemical structure of the compounds, involves the formation of X−Sn bonds (X = $N_{\text{puric and pyrimidinic}}$, $O_{\text{carboxylate}}$) at active biological centers resulting in an interference with the normal cell processes.[7−9]

Acute Toxicity Assays. The luminescent bacteria toxicity (LBT-Microtox) tests were performed for all diorganotin compounds. It is worth mentioning that the LBT-Microtox assay was first used for lixiviates and residual water solutions for industrial field applications.[17] In the remnant references, acute toxicity tests performed in several mammalian cell lines were often trackers of such activities.[22] Nevertheless, the latter reviews stated that within this branded Microtox, plenty of other new protocols were developed to test different types

(18) Bondi, A. *J. Phys. Chem.* **1964**, *68*, 441.
(19) Spiers, A. J.; Buckling, A.; Rainey, P. B. *Microbiology* **2000**, *146*, 2345.
(20) Saxena, C.; Singh, R. V. *Phosphorus, Sulfur Silicon Relat. Elem.* **1994**, *97*, 17.

Table 5. Acute Toxicity of Compounds **4** and **5** in *P. phosphoreum*[a]

compound	EC_{50} (5 min)	toxicity rating	EC_{50} (15 min)	toxicity rating
4a	2.39	moderately toxic	2.06	moderately toxic
4b	80.42	slightly toxic	67.66	slightly toxic
4c	23.70	slightly toxic	72.41	slightly toxic
5a	4.50	moderately toxic	−	−
5c	0.43	highly toxic	−	−
ligand	617.43	nontoxic	318.66	nontoxic
fatty diamine[b,c]	−	−	1.1	moderately toxic
chlorinated aromatic[b,c]	−	−	0.52	highly toxic

[a] (−) indicates no measurement recorded because of the observed toxicity. [b] See ref 3 for further details. [c] Commercial product cited for comparison.

of toxicity for pure chemical compounds.[23] This alternative protocol was performed also to reduce or refine some of the mammalian cell procedures with high proficiency in the obtained results, and therefore, it has became one of the streamlined tests for this means.[17,24]

Herein, the LBT-Microtox tests (lately called Microtox) for specific acute toxicity measurements were carried out to select the most environmentally benign compound as a biocide prototype. The 5 and 15 min EC_{50} values for **4a**, **4b**, **4c**, **5a**, and **5c** obtained in a commercial LBT-Microtox assay are listed in Table 5. The values for the 5 min Microtox EC_{50} for compounds **4** and **5** ranged from 0.426 mg/L (**5d**) to 80.42 mg/L (**4b**). The less toxic compounds were the **4** series, specifically **4b** and **4c** which display only slightly toxic values at both 5 and 15 min. On the other hand, the ligand exhibited practically nontoxic behavior (617.43 mg/L). The toxicity levels were maintained, even through 30 min kinetic tests.

Qualitative Structure Activity Relationships. In solution, the coordination number of the tin^{IV} atom, in compound **4**, can be incremented from five to at least six with the addition of coordinating solvents such as DMSO-d_6. Meanwhile, with noncoordinating solvents, such as $CDCl_3$, the coordination number is maintained. This statement gives further insight into the understanding of the lower toxicity and lower biocide activity of the **4** series compared to that of the **5** series. The series **4** compounds are dimeric in the solid state through Sn_2O_2 four-member ring formation, which is not observed for series **5** compounds. These molecular modes can be a first principle model of intermolecular interaction and, in a superlative mode, analogous to the supramolecular recognition of microorganisms, often called molecular-macromolecular docking.

Conclusions

An efficient one-step procedure was established to prepare a series of 2,2-di-*n*-butyl- and 2,2-diphenyl-6-aza-1,3-dioxa-2-stanna-benzocyclononenes (**4a**−**d**, **5a**, and **5d**) which consists of the in situ formation of a tridentate Schiff base ligand followed by the addition of di-*n*-butyl- or diphenyltin^{IV} oxide.

The ^1H, ^{13}C, and ^{119}Sn NMR data allowed us to establish the formation of the products that supported the N−Sn bond formation and specify the coordination number of the tin atom as five when $CDCl_3$ was employed as solvent. Meanwhile, when DMSO-d_6 was used as the solvent, the coordination number of the tin atom increases to six.

The solid-state structures of compounds **5a** and **5d** have a TBP tin atom, while compounds **4b** and **4c** crystallized within dimeric arrangements via the intramolecular self-assembly of tin atoms with donor oxygen atoms to build a Sn_2O_2 four-membered ring moiety.

The title compounds were tested in vitro against *B. subtilis* (strain ATCC 6633), *E. coli* (strain DH5α), *P. aeruginosa* (strain BH3), *D. longus* (strain DSM 6739), and *D. asperonum* (strain DSM 5918) demonstrating a wide range of bactericidal activity.

Moreover, compound **5** displayed an enhanced bactericidal activity in comparison to that of compound **4** indicating that the $Sn^{IV}Ph_2$ moiety is responsible for this increase.

Acute toxicity tests were also performed to evaluate the ecological impact of compounds **4** and **5**; the results show moderate toxicity in all cases.

A brief discussion and qualitative connection between the solution and solid-state coordination modes of diorganotin molecules with the biocide activities and the acute toxicity tests is also presented, showing a modulated biologic effect depending on chemical structure.

Experimental Section

All reagents and solvents were purchased as reagent grade quality and were used as received. Salicylaldehyde (**1**), β-amino alcohols (**2a**−**d**), di-*n*-butyltin^{IV} oxide (**3a**), and diphenyltin^{IV} oxide (**3b**) were purchased from Aldrich, and toluene and butanol were purchased from Fermont.

The solution NMR experiments were performed on Varian Mercury 200-4 nucleus and 200-BB spectrometers at room temperature; chemical shifts (ppm) are relative to TMS and $SnMe_4$, and the coupling constants are quoted in Hz. Infrared spectra were recorded as KBr pellets on a Bruker Tensor 27 FT-IR spectrophotometer. Melting points were measured in open capillary tubes on a Gallenkamp MFB 595 apparatus and have not been corrected. Elemental analysis determinations were obtained on a Perkin Elmer CHNO/S apparatus.

The X-ray single-crystal structure determinations for **4b**, **4c**, **5a**, and **5c** were performed on a BRUKER-AXS APEX diffractometer with a CCD area detector (λ(Mo Kα) = 0.71073 Å, graphite monochromator). Frames were collected at $T = 173$ or 298 K via ω and φ rotation at 10 s per frame (SMART).[25] The measured intensities were reduced to F^2 and corrected for absorption with SADABS (SAINT-NT).[26] Structure solution, refinement, and data output were carried out with the SHELXTL-NT[27] program package.

(21) (a) Sun, G.; Wheatley, W. B.; Worley S. D. *Ind. Eng. Chem. Res.* **1994**, *33*, 168. (b) Jain, M.; Gaur, S.; Singh, V. P.; Singh, R. V. *App. Organomet. Chem.* **2004**, *18*, 73. (c) Nagpal, P.; Singh, R. V. *Appl. Organomet. Chem.* **2004**, *18*, 221.
(22) US Congress, Office of Technology Assessment. *Alternatives to Animal Use in Research, Testing and Education*; OTA-BA-273; US Government Printing Office: Washington, DC, 1986.
(23) *Microtox Tabulations of Pure Chemical Compounds*; Strategic Diagnostics Inc.: Newark, DE, 2003.
(24) (a) Ren, S. J.; Frymier, P. D. *Chemosphere* **2005**, *58*, 543. (b) Steinberg, S. M.; Poziomek, E. J.; Engelmann, W. H.; Rogers, K. R. *Chemosphere* **1995**, *30*, 2155.
(25) *SMART*, version 5.618; Bruker AXS: Madison, WI, 2000.
(26) *SAINT + NT*, version 6.04; Bruker AXS: Madison, WI, 2001.
(27) *SHELXTL-NT*, version 6.10; Bruker AXS: Madison, WI, 2000.

All software manipulations were done in the WIN-GX[28] environment. Molecular perspectives were drawn using the ORTEP 3[29] drawing application. All of the heavier atoms were found using Fourier difference maps and refined with an anisotropic scheme. Some hydrogen atoms were also found using Fourier difference maps and were refined with an isotropic scheme; the remaining hydrogen atoms were geometrically modeled and calculated for the refinement.

General Synthetic Procedure. Preparation of 2,2-di-*n*-butyl-6-aza-1,3-dioxa-2-stanna-benzocyclononene (4a). Salicylaldehyde (**1**, 0.5 g, 4.09 mmol), ethanolamine (**2a**, 0.447 g, 4.09 mmol) and di-*n*-butyltin^{IV} oxide (**3**, 1.02 g, 4.09 mmol) were added to 100 mL of a 4:1 mixture of toluene and butanol in a previously dried flask; the resulting saturated solution was refluxed for 8 h. Compound **3** was not soluble under the reaction conditions, but after 2 or 3 h, it condenses with the in situ formed tridentate ligand. After the reaction was completed, the crude product was evaporated under reduced pressure, and the solid was dissolved in 10 mL of dichloromethane and precipitated by solvent polarity change with hexane or petroleum ether to yield compound **4a** as a yellow powder.

All of the compounds were prepared using the same molar ratios described for **4a**.

2,2-Di-*n*-butyl-6-aza-1,3-dioxa-2-stanna-benzocyclononene (4a). Yellow powder. Yield: 84%. mp: 72−74 °C. ^{1}H NMR: δ 8.35 (sb, 1H, $^3J(^{119/117}\mathrm{Sn}-^1\mathrm{H})$ = 39.8, H7), 7.27 (ddd, J_o = 8.4, 7.0, J_m = 1.4, 1H, H11), 7.04 (dd, J_o = 7.6, J_m = 1.4, 1H, H13), 6.69 (d, J_o = 8.4, 1H, H10), 6.58 (dd, J_o = 7.6, 7.0, 1H, H12), 4.05 (t, J = 5.4, $^3J(^{119/117}\mathrm{Sn}-^1\mathrm{H})$ = 26.8, 2H, H4), 3.62 (t, J = 5.4, $^3J(^{119/117}\mathrm{Sn}-^1\mathrm{H})$ = 20.4, 2H, H5), 1.34 (t, J = 7.4, 4H, α-CH_2−), 1.62 (sq, J = 7.4, 4H, β-CH_2−), 1.30 (sx, J = 7.4, 4H, γ-CH_2−), 0.85 (t, J = 7.4, 4H, δ-CH_3). ^{13}C NMR: δ 171.4 ($^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$ = 8.4, C7), 170.0 (C9), 136.3 (C11), 134.7 (C13), 122.7 (C10), 117.3 (C8), 115.8 (C12), 63.4 ($^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$=22.1, C4), 60.7 ($^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$ = 37.0, C5), 27.9 ($^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$ = 30.5, β-CH_2−), 27.3 ($^3J(^{119}\mathrm{Sn}-^{13}\mathrm{C})$ = 90.0, $^3J(^{117}\mathrm{Sn}-^{13}\mathrm{C})$ = 86.2, γ-CH_2−), 21.5 ($J(^{119}\mathrm{Sn}-^{13}\mathrm{C})$ = 628.3, $J(^{117}\mathrm{Sn}-^{13}\mathrm{C})$ = 599.8, α-CH_2−), 14.2 (δ-CH_3). ^{119}Sn NMR: δ −194.6 ($CDCl_3$), −202.0 (DMSO-d_6). IR (KBr): ν 2935, 2921, 2850, 2860, 1626 (C═N), 1527, 1450, 1466, 1146, 1060, 869, 759, 600 cm^{-1}. Calcd for $C_{17}H_{27}NO_2Sn$: C, 51.55; H, 6.87; N, 3.54. Found: C, 51.74; H, 7.01; N, 3.38.

2,2-Di-*n*-butyl-6-aza-1,3-dioxa-4-methyl-2-stanna-benzocyclononene (4b). Yellow powder. Yield: 72%. mp: 119−121 °C.^{1}H NMR: δ 8.34 (sb, 1H, $^3J(^{119/117}\mathrm{Sn}-^1\mathrm{H})$ = 39.8, H7), 7.28 (ddd, J_o = 8.6, 6.8, J_m = 1.5, 1H, H11), 7.04 (dd, J_o = 7.8, J_m = 1.5, 1H, H13), 6.71 (d, J_o = 8.6, 1H, H10), 6.59 (dd, J_o = 7.8, 6.8, 1H, H12), 4.01 (m, 1H, H5), 3.62 (t, J = 5.4, $^3J(^{119/117}\mathrm{Sn}-^1\mathrm{H})$ = 20.4, 2H, H5), 1.44−1.29 (m, 4H, α-CH_2−), 1.73−1.48 (m, 4H, β-CH_2−), 1.42−1.15 (m, 4H, γ-CH_2−), 1.23 (d, J = 6, 3H, H14), 0.87 (t, J = 7.4, 2H, δ-CH_3), 0.84 (t, J = 7.4, 2H, δ-CH_3). ^{13}C NMR: δ 170.9 ($^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$ = 8.0, C7), 170.2 (C9), 136.2 (C11), 134.7 (C13), 122.8 (C10), 117.3 (C8), 115.7 (C12), 68.15 ($^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$ = 22.5, C4), 66.5 ($^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$ = 36.2, C5), 27.9 ($^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$ = 30.5, β-CH_2−), 27.3 ($^3J(^{119}\mathrm{Sn}-^{13}\mathrm{C})$ = 90.0, $^3J(^{117}\mathrm{Sn}-^{13}\mathrm{C})$ = 86.2, γ-CH_2−), 21.5 ($J(^{119}\mathrm{Sn}-^{13}\mathrm{C})$ = 628.3, $J(^{117}\mathrm{Sn}-^{13}\mathrm{C})$ = 599.8, α-CH_2−), 14.2 (δ-CH_3). ^{119}Sn NMR: δ −189.5 ($CDCl_3$), −206.1 (DMSO-d_6). IR (KBr): ν 2955, 2923, 2852, 1624 (C═N), 1527 (C═N), 1464, 1451, 1145, 861, 596 cm^{-1}.Calcd for $C_{18}H_{29}NO_2Sn$: C, 52.72; H, 7.13; N, 3.42. Found: C, 52.87; H, 7.22, N, 3.46. Crystals suitable for X-ray diffraction analysis were obtained from a 6:1 solvent mixture of hexane and dichloromethane.

2,2-Di-*n*-butyl-6-aza-1,3-dioxa-5-ethyl-2-stanna-benzocyclononene (4c). Yellow powder. Yield: 78%. mp: 56−58 °C.^{1}H NMR: δ 8.25 (sb, 1H, $^3J(^{119/117}\mathrm{Sn}-^1\mathrm{H})$ = 40.8, H7), 7.24 (ddd, J_o = 8.4, 7.0, J_m = 1.4, 1H, H11), 7.03 (dd, J_o = 7.0, J_m = 8.4, 1H, H13), 6.66 (d, J_o = 8.4, 1H, H10), 6.55 (dd, J_o = 8.4, 7.0, 1H, H12), 4.04 (d, J = 9.9, $^3J(^{119/117}\mathrm{Sn}-^1\mathrm{H})$ = 48.8, 1H, H4a), 3.90 (dd, J = 9.9, 3.6, 1H, H4b), 3.17 (dt, J = 7.1, 3.6, $^3J(^{119/117}\mathrm{Sn}-^1\mathrm{H})$ = 35.8, 1H, H5), 1.88 (sx, J = 7.1, 1H, H14a), 1.73−1.17 (m, 13H, α-CH_2−, β-CH_2−, γ-CH_2−), 0.93 (t, J = 7.1, 3H, H15), 0.86 (t, J = 7.4, 2H, δ-CH_3), 0.78 (t, J = 7.4, 2H, δ-CH_3). ^{13}C NMR: δ 170.6 ($^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$ = 8.0, C7), 170.0 ($^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$ = 29.5, C9), 136.2 (C11), 134.8 (C13), 122.7 (C10), 117.4 ($^3J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$ = 23.1, C8), 115.8 (C12), 70.6 ($^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$ = 33.1, C5), 66.5 ($^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$ = 21.9, C4), 27.7 (β-CH_2−), 27.6 (β-CH_2−), 27.4 (γ-CH_2−), 27.3 (γ-CH_2−), 27.2 (C14), 21.9 ($J(^{119}\mathrm{Sn}-^{13}\mathrm{C})$ = 648.6, $J(^{117}\mathrm{Sn}-^{13}\mathrm{C})$ = 614.5, α-CH_2−), 20.7 ($J(^{119}\mathrm{Sn}-^{13}\mathrm{C})$ = 609.6, $J(^{117}\mathrm{Sn}-^{13}\mathrm{C})$ = 582.2, α-CH_2−), 14.2 (δ-CH_3), 11.2 (C15). ^{119}Sn NMR: δ −192.8 ($CDCl_3$), −201.7 (DMSO-d_6). IR (KBr): ν 2958, 2918, 2852, 1606 (C═N), 1584 (C═N), 1532, 1476, 1464, 1438, 1386, 1288, 1276, 1210, 1148, 836, 526 cm^{-1}. Calcd for $C_{19}H_{31}NO_2Sn$: C, 53.80; H, 7.37; N, 3.30. Found: C, 53.62; H, 7.20, N, 3.07. Crystals suitable for X-ray diffraction analysis were obtained from an 8:1 solvent mixture of hexane and dichloromethane.

2,2-Di-phenyl-6-aza-1,3-dioxa-2-stanna-benzocyclononene (5a). Yellow powder. Yield: 92%. mp: 203 °C (dec). ^{1}H NMR: δ 8.38 (sb, 1H, $^3J(^{119/117}\mathrm{Sn}-^1\mathrm{H})$ = 50.4, H7), 7.93 (dd, J_o = 7.8, J_m = 4.4, $^3J(^{119/117}\mathrm{Sn}-^1\mathrm{H})$ = 75.6, 4H, H-*ortho*-Sn−Ph), 7.44 (ddd, J_o = 7.8, 7.0, J_m = 1.8, 1H, H11), 7.41−7.36 (m, 6H, H-*meta*-, H-*para*-Sn−Ph), 7.09 (dd, J_o = 7.8, J_m = 1.8, 1H, H13), 7.08 (d, J_o = 7.8, 1H, H10), 6.58 (dd, J_o = 7.8, 7.0 1H, H12), 4.26 (t, J = 5.3, $^3J(^{119/117}\mathrm{Sn}-^1\mathrm{H})$ = 35.8, 2H, H4), 3.74 (t, J = 5.3, $^3J(^{119/117}\mathrm{Sn}-^1\mathrm{H})$ = 22.0, 2H, H5). ^{13}C NMR: δ 171.5 ($^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$ = 9.6, C7), 169.6 ($^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$ = 32.1, C9), 140.4 ($J(^{119}\mathrm{Sn}-^{13}\mathrm{C})$ = 982.3, $J(^{117}\mathrm{Sn}-^{13}\mathrm{C})$ = 939.4, C-*ipso*-SnPh), 136.6 (C11), 136.6 ($^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$ = 50.5, C-*ortho*-SnPh), 134.8 (C13), 129.9 ($^4J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$ = 16.8, C-*para*-SnPh), 128.5 ($^3J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$ = 81.1, C-*meta*-SnPh), 122.8 (C10), 117.3 ($^3J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$ = 26.4, C8), 116.5 (C12), 63.0 ($^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$ = 18.4, C4), 60.3 ($^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$ = 44.4, C5). ^{119}Sn NMR: δ −337.3 ($CDCl_3$), −339.7 (DMSO-d_6). IR (KBr): ν 2909, 2865, 2811, 1628 (C═N), 1541 (C═N), 1467, 1448, 1318, 1099, 1055, 793, 756, 739, 699, 662, 580, 522 cm^{-1}. Calcd for $C_{21}H_{19}NO_2Sn$: C, 57.84; H, 4.39; N, 3.21. Found: C, 57.69; H, 4.28, N, 3.10. Suitable crystals for the X-ray diffraction analysis were obtained from the mother liquors after one week of evaporation.

2,2-Di-phenyl-6-aza-1,3-dioxa-5-ethyl-2-stanna-benzocyclononene (5c). Yellow solid. Yield: 70%. mp: 117−119 °C. ^{1}H NMR: δ 8.27 (sb, 1H, $^3J(^{119/117}\mathrm{Sn}-^1\mathrm{H})$ = 51.4, H7), 8.02−7.98 (m, 2H, H-*ortho*-Sn−Ph), 7.85−7.80 (m, 2H, H-*ortho*-Sn−Ph), 7.39−7.31 (m, 7H, H-11, H-*meta*-, H-*para*-Sn−Ph), 7.08−7.02 (m, 2H, H-13, H10), 6.65 (t, J_o = 7.5, 1H, H12), 4.28 (d, J = 9.6, $^3J(^{119/117}\mathrm{Sn}-^1\mathrm{H})$ = 64.0, 1H, H4a), 4.10 (dd, J = 9.6, 3.8, 1H, H4b), 3.28 (dt, J = 7.2, 3.8, $^3J(^{119/117}\mathrm{Sn}-^1\mathrm{H})$ = 42.0, 1H, H5), 1.88 (sx, J = 7.2, 1H, H14a), 1.71 (sx, J = 7.2, 1H, H14b), 0.93 (t, J = 7.2, 3H, H15). ^{13}C NMR: δ 170.8 (C7), 169.6 ($^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$ = 30.6, C9), 140.7 ($J(^{119}\mathrm{Sn}-^{13}\mathrm{C})$ = 1005.6, $J(^{117}\mathrm{Sn}-^{13}\mathrm{C})$ = 960.5.4, C-*ipso*-SnPh), 140.2 ($J(^{119}\mathrm{Sn}-^{13}\mathrm{C})$ = 956.3, $J(^{117}\mathrm{Sn}-^{13}\mathrm{C})$ = 911.9, C-*ipso*-SnPh), 136.6 ($^2J(^{119/117}\mathrm{Sn}-^{13}\mathrm{C})$ = 50.5, C-*ortho*-

(28) Farrugia, L. *J. Appl. Crystallogr.* **1999**, *32*, 837.
(29) Farrugia, L. *J. Appl. Crystallogr.* **1997**, *30*, 565.

SnPh), 136.5 (C11), 134.9 (C13), 129.9 ($^4J(^{119/117}Sn-^{13}C) = 15.3$, C-*para*-SnPh), 128.6 ($^3J(^{119/117}Sn-^{13}C) = 78.1$, C-*meta*-SnPh), 128.5 ($^3J(^{119/117}Sn-^{13}C) = 84.2$, C-*meta*-SnPh), 122.9 (C10), 117.3 ($^3J(^{119/117}Sn-^{13}C) = 27.6$, C8), 116.6 (C12), 70.6 ($^2J(^{119/117}Sn-^{13}C) = 38.2$, C5), 66.0 ($^2J(^{119/117}Sn-^{13}C) = 18.4$, C4), 27.9 (C14), 11.7 (C15). ^{119}Sn NMR: δ −334.6 ($CDCl_3$), −337.2 (DMSO-d_6). IR (KBr): ν 3051, 2970, 2928, 2882, 2826, 1616 (C═N), 1540 (C═N), 1467, 1445, 1429, 1308, 1203, 1151, 1118, 1088, 765, 733, 699, 592 cm^{-1}. Calcd for $C_{23}H_{23}NO_2Sn$: C, 59.52; H, 4.99; N, 3.02. Found: C, 59.40; H, 4.78, N, 3.05. Suitable crystals for the X-ray diffraction analysis were obtained after the dissolution of **5c** in 20 mL of a 6:1 solvent mixture of heptanes and dichloromethane.

Microbiology. The compounds synthesized were screened in vitro for their antibacterial activity against three strains of aerobic bacteria, one Gram-positive (*B. subtilis* ATCC 6633) and two Gram-negative (*E. coli* DH5α and *P. aeruginosa* BH3) and against two anaerobic species *(D. longus*, DSM 6739, and *D. aspheronum*, DSM 5918). Antimicrobial assays were done by using the agar well diffusion method[30,31] to determine the minimum inhibitory concentrations (MICs).

Aerobic bacteria. Seeding agar was prepared in flat-bottomed 90 mm Petri dishes by cooling the TBS molten medium[32] to 40 °C and then adding the required amount of bacterial suspension (8×10^8 bacteria per mL). Four wells per dish were dug in the media with the help of a sterile metallic borer with centers of at least 24 mm. Each compound was dissolved in DMSO, at concentrations of 1×10^{-3}, 1×10^{-2}, 1.0, 2.5, 5, 10, 25, 50, 75, and 100 ppm. Fifty microliter samples of each dilution were applied by triplicate into the corresponding wells, and after that, the Petri dishes were cooled to 4 °C for 60 min to allow diffusion of the compounds through the medium. DMSO controls were also included in the assay. The plates were then incubated at a suitable growth temperature for the bacteria (30 °C ± 2 °C) for 16 h. The zone of inhibition of bacterial growth formed around each slot was accurately measured.

(30) Chohan, Z. H.; Scozzafava, A.; Supuran, C. T. *Synth. React. Inorg. Met.−Org. Chem.* **2003**, *33*, 241.
(31) Atta-ur-Rahman; Choudhary, M. I.; Thomsen, W. J. *Bioassay Techniques for Drug Development.* Harwood Academic OPA N. V.; Amsterdam, The Netherlands, 2001; p 16.
(32) Sambrook, J.; Fritsch, E. F.; Maniatis, T. *Molecular Cloning: A Laboratory Manual,* 3rd ed; Cold Spring Harbor Laboratory: New York, 2001; 3 vols.

Anaerobic bacteria. The anaerobic strains used in this work were grown in 40 mL flasks in a Postgate C modified medium as follows: The headspace, in the bottles of medium, was flushed with nitrogen, and the bottles were sealed (the bottle caps had a concentric 4.8 mm hole to allow the rubber liner to be punctured with a syringe when required). The title compounds were injected into the bottles at seven different concentrations from 1 to 75 ppm, and then the flasks were inoculated with 1 mL of stock SRBs culture. All bottles were incubated at 32 °C for 7 days. After this time, the minimum inhibitory concentration for each compound was determined by recording the presence or absence of visible growth in the bottles, compared with positive (bottles without chemicals) and negative (bottles not inoculated with culture) controls. Two parallel series with the same conditions were set up for each compound.

Acute toxicity assays. The acute toxicity of the chemicals synthesized was assessed using a Microtox analyzer. In the test, a vial of the *P. phosphoreum* (*V. fischering*) culture was reconstituted in 1 mL of solution and maintained at 3 °C in an incubator well on the analyzer. For each test, a serial dilution of the compounds was prepared in 2% brine. In addition, a series of brine solutions containing approximately 106 colony forming units of *P. phosphoreum* were prepared in glass cuvettes by pipetting 10 μL of the reconstituted bacterial suspension into 500 μL of 2% brine. As a bacterial population control, these solutions were incubated in temperature-controlled wells (6 °C) for 15 min and measured. Hence, the activity of treated compounds was recorded after 5 and 15 min of exposure. The results of Microtox test are expressed in terms of the EC_{50} value.

Acknowledgment. We thank Programa de Ingeniería Molecular-IMP for financial support from project D.00178.

Supporting Information Available: Crystallographic data in CIF format for compounds **4b**, **4c**, **5a**, and **5c**. This material is available free of charge via the Internet at http://pubs.acs.org. Full crystallographic data, CCDC numbers 269982 for **4b**, 269983 for **4c**, 269984 for **5a**, and 269985 for **5c,** is available from the Cambridge Crystallographic Data Center, CCDC, 12 Union Road, Cambridge CB21EZ, UK (Fax: +44−1223−336033. E-mail: deposit@ccdc.cam.ac.uk. Website: http://www.ccdc.cam.ac.uk).

IC048628O