Easy Access to Water-Soluble Fullerene Derivatives via 1,3-Dipolar Cycloadditions of Azomethine Ylides to C_{60}

Tatiana Da Ros and Maurizio Prato*

Dipartimento di Scienze Farmaceutiche, Università di Trieste, Piazzale Europa 1, 34127 Trieste, Italy

Fabiola Novello and Michele Maggini

Centro Meccanismi di Reazioni Organiche del CNR, Dipartimento di Chimica Organica, Università di Padova, Via Marzolo 1, 35131 Padova, Italy

Elena Banfi

Dipartimento di Scienze Biomediche, Via A. Fleming 22, 34127 Trieste, Italy

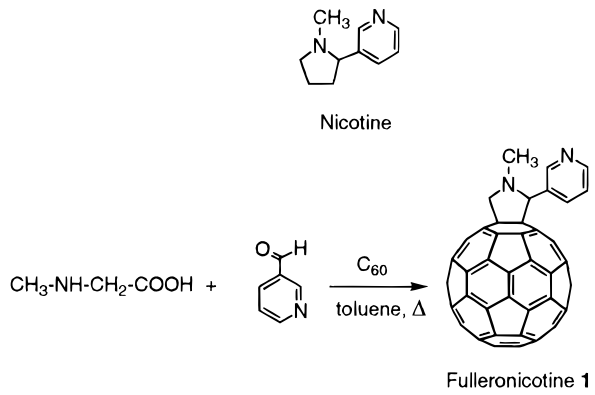
Received August 6, 1996

One of the most promising fields of applications of the fullerenes is based on their biological properties. Very exciting findings, pioneered by Wudl,[1] Nakamura,[2] and co-workers, have stimulated much interest and generated further successful investigations.[3−7] From a practical point of view, a fundamental issue that must be addressed when dealing with fullerenes is the solubilization of the candidate molecule or ion in the polar media necessary for biological tests.[7] Fullerenes, in fact, are not soluble in water or in polar organic solvents. They must be chemically modified, and a solubilizing appendage must be covalently attached.[8,9] In this paper we report a general method for the solubilization of fullerenes and a preliminary evaluation of their biological properties against some microorganisms.

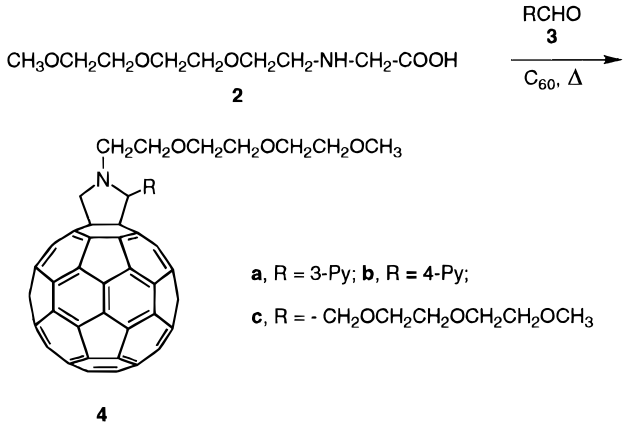
With a suitable functionalization method in hand,[10] we were attracted by the idea that a very interesting molecule, which we may call fulleronicotine for its resemblance to natural nicotine, could be obtained in a single step from commercially available reactants (Scheme 1).

Fulleronicotine (**1**) is a fullerene homologue of natural nicotine, the well-known alkaloid abundantly found in tobacco leaves. In fulleronicotine, the 3,4 bond of the pyrrolidine ring is fused to a 6,6 ring junction of C_{60}. As nicotine has a range of biological activities and is used as a pharmacological tool in a number of applications,[11] it seemed interesting to prepare a fullerene-substituted nicotine in order to check the influence of the new form of carbon on the toxicity of nicotine.

Scheme 1



Scheme 2



However, when we turned to the biological evaluation of **1**, we had to cope with its very low solubility in water-miscible solvents. Attempts to solubilize **1** in water by known methodologies[12] failed to give reproducible results.

To obtain a soluble form of fulleronicotine, it was necessary to start with an N-functionalized glycine. A triethylene glycol chain was chosen as the solubilizing appendage owing to its ready availability and high solubilizing power.

N-Substituted glycine **2** was obtained by condensation of glycine benzyl ester with aldehyde **3c** under reductive conditions ($NaBH_3CN$), followed by hydrogenolysis of the benzyl ester. Glycine **2**, a very useful reactant for preparing water-soluble derivatives, readily condensed with a number of aldehydes and C_{60} to give the novel C_{60}-fused functionalized pyrrolidines **4a**−**c** (Scheme 2, yields 34−39%, see Experimental Section).

All compounds **4a**−**c** exhibit moderate solubility in a 9:1 ratio of water−DMSO. It was found that the best procedure for preparing the aqueous solutions was to dissolve the compounds in DMSO and to dilute 1:9 with pure water. In this binary solvent an upper limit concentration was obtained for **4c** (ca. 3×10^{-5} M), whereas the solubility for **4a**,**b** was slightly lower (ca. 1.5×10^{-5} M). A UV−vis absorption spectrum of **4a** in H_2O−DMSO (9:1, 1×10^{-5} M) is reported in Figure 1. It shows that the main absorptions of the fullerene core are still evident, although, in analogy to other fullerene

(1) Sijbesma, R.; Srdanov, G.; Wudl, F.; Castoro, J. A.; Wilkins, C.; Friedman, S. H.; DeCamp, D. L.; Kenyon, G. L. *J. Am. Chem. Soc.* **1993**, *115*, 6510.
(2) Tokuyama, H.; Yamago, S.; Nakamura, E.; Shiraki, T.; Sugiura, Y. *J. Am. Chem. Soc.* **1993**, *115*, 7918.
(3) Boutorine, A.; Tokuyama, H.; Takasugi, M.; Isobe, H.; Nakamura, E.; Hélène, C. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 2462.
(4) Toniolo, C.; Bianco, A.; Maggini, M.; Scorrano, G.; Prato, M.; Marastoni, M.; Tomatis, R.; Spisani, S.; Palù, G.; Blair, E. D. *J. Med. Chem.* **1994**, *37*, 4558.
(5) An, Y.-Z.; Chen, C.-H. B.; Anderson, J. L.; Sigman, D. S.; Foote, C. S.; Rubin, Y. *Tetrahedron* **1996**, *52*, 5179.
(6) Nakamura, E.; Tokuyama, H.; Yamago, S.; Shiraki, T.; Sugiura, Y. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 2143.
(7) For a recent review, see: Jensen, A. W.; Wilson, S. R.; Schuster, D. I. *Bioorg. Med. Chem.* **1996**, *4*, 767.
(8) Hirsch, A. *The Chemistry of the Fullerenes*; Thieme: Stuttgart, Germany, 1994.
(9) Diederich, F.; Thilgen, C. *Science* **1996**, *271*, 317.
(10) Maggini, M.; Scorrano, G.; Prato, M. *J. Am. Chem. Soc.* **1993**, *115*, 9798.
(11) *The Pharmacological Basis of Therapeutics*; Hardman, J. G., Goodman Gilman, A., Limbird, L. E., Eds.; McGraw-Hill: New York, 1996; 9th ed., p 192.
(12) Yamakoshi, Y. N.; Yagami, T.; Fukuhara, K.; Sueyoshi, S.; Miyata, N. *J. Chem. Soc., Chem. Commun.* **1994**, 517.

S0022-3263(96)01522-8 CCC: $12.00

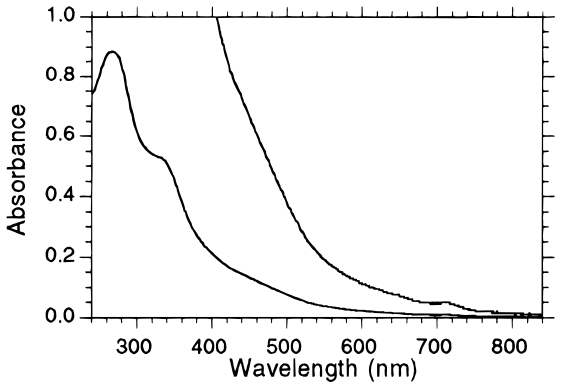


Figure 1. UV−vis absorption spectrum for a solution of **4a** in H_2O−DMSO (9:1, 1×10^{-5} M).

derivatives dissolved in polar solvents, bands are broader and less structured, especially in the low-energy region.[13]

In preliminary biological tests, **4c** was found to be active against a variety of microorganisms. Different species of bacteria and different fungal strains were killed in a slightly modified agar diffusion test: two strains, clinical isolates CA1 and Z11, of *Candida albicans*, a fastidious pathogenic eukariote; strain ATCC 6633 of *Bacillus subtilis*, a spore-forming, Gram-positive bacterium; strain AB1153 of *Escherichia coli*, a Gram-negative enteric bacterium; a clinical isolate, strain 261/6 of *Mycobacterium avium*, an acid fast, emerging pathogen resistant to most antimicrobial drugs. In the latter case, 70% inhibition was observed with a concentration of 26 μg/mL, whereas complete inhibition was achieved with concentrations 10 times higher.

The nature of the activity of the fullerene derivatives **4a**−**c** is yet to be determined. However, the rather wide range of biological activity exhibited makes these new water-soluble fullerene derivatives interesting candidates for further investigations. Work along these lines is underway.

Experimental Section

Materials and Methods. Details regarding instrumentation used in this paper have been described elsewhere.[14] All other reagents were used as purchased from Fluka and Aldrich. All solvents were distilled prior to use.

Synthesis of 3,6,9-Trioxadecanaldehyde (3c). To a solution of oxalyl chloride (3 mL) in dichloromethane (75 mL) under nitrogen and cooled in a dry ice−acetone bath were carefully added 5 mL of dimethyl sulfoxide in 15 mL of CH_2Cl_2. The solution was stirred for 10 min, and then a solution of 5 mL (29.7 mmol) of triethylene glycol monomethyl ether in 30 mL of CH_2Cl_2 was added dropwise. The mixture was stirred for 15 min, and then triethylamine (20 mL) was added dropwise over a period of 20 min. The reaction mixture was left for 30 min at −78 °C and then allowed to reach rt. After a standard workup with a saturated aqueous NaCl solution, the crude was purified by flash chromatography (eluant: ethyl acetate−methanol (9:1), $R_f = 0.54$) affording 2.7 g (56%) of pure aldehyde **3c**. Bulb-to-bulb distillation at 90 °C (0.01 Torr) gave a clear homogeneous oil, which gave a single peak in GC−MS analysis. ^{1}H-NMR (250 MHz, C_6D_6): δ (ppm) 9.74 (t, $J = 0.7$ Hz, 1H), 4.16 (d, $J = 0.7$ Hz, 2H), 3.72 (m, 2H), 3.64 (m, 2H), 3.56 (m, 2H), 3.38 (s, 3H). ^{13}C-NMR (62.5 MHz, C_6D_6): δ (ppm) 200.1, 76.8, 72.2, 71.3, 71.0, 70.8, 58.6. EI-HRMS: *m*/*z* 162 (M^+, <0.3%, exact mass not measurable), 133.0899 ([M − CHO]$^+$, 3%; $C_6H_{13}O_3$ requires 133.0861).

Synthesis of *N*-(3,6,9-Trioxadecyl)glycine (2). To a solution of 265 mg (1.63 mmol) of aldehyde **3c** and 552 mg (1.63 mmol) of glycine benzyl ester 4-toluenesulfonate in 12 mL of methanol−acetic acid (99:1) was added 127 mg (2.02 mmol) of sodium cyanoborohydride in 30 min. The solution was stirred at rt for 2.5 h and then was poured into 30 mL of saturated $NaHCO_3$ solution. The product was extracted with ethyl acetate, the organic phase was washed with brine and dried with anhydrous $NaSO_4$, and the solvent was removed in vacuo. Since the reaction mixture consisted of an unseparable mixture of glycine benzyl ester, the desired *N*-(3,6,9-trioxadecyl)glycine (**2**) (ethyl acetate−methanol (9:1), $R_f = 0.40$), and the dialkylated *N*,*N*-bis(3,6,9-trioxadecyl)glycine, the residue was dissolved in dioxane and treated with 342 mg (2.00 mmol) of benzyl chloroformate. The *N*-Cbz-*N*-(3,6,9-trioxadecyl)glycine benzyl ester was then purified by column chromatography (ethyl acetate−petroleum ether (1:1), $R_f = 0.36$). Yield: 355 mg (49% from aldehyde **3c**). ^{1}H-NMR (200 MHz): δ 7.35 (s, 5H), 7.29 (m, 5H), 5.25 (s, 2H), 5.18 (m, 2H), 4.21 and 4.16 (two s, 2H), 3.55 (m, 12 H), 3.35 (s, 3H). ^{13}C-NMR (50 MHz): δ 169.7, 156.2, 155.8, 136.3, 136.4, 128.5, 128.4, 128.3, 128.3, 128.1, 127.9, 127.8, 127.7, 71.8, 70.4, 70.3, 67.5, 67.3, 66.7, 66.6, 58.9, 50.2, 48.5, 47.9. EI-MS: *m*/*z* 311 (M − $COOCH_2Ph)^+$, 267, 149, 91, 68 (100). IR (film, cm^{-1}): 2975, 2940, 1750, 1715, 1700. *N*-Cbz-*N*-(3,6,9-trioxadecyl)glycine (750 mg) was dissolved in methanol (40 mL), and 50 mg of Pd/C was added. The suspension was stirred at rt under H_2 atmosphere for 18 h and then was filtered on Celite, and the solvent evaporated. Amino acid **2** was obtained in quantitative yield and recrystallized from methanol−ethyl ether. Mp: >250 °C. ^{1}H-NMR (200 MHz, $CDCl_3$): δ 3.78 (broad t, 2H), 3.51 (m, 12H), 3.30 (s, 3H), 3.18 (broad t, 2H). ^{13}C-NMR (50 MHz, $CDCl_3$): δ 170.5, 71.8, 70.4, 70.4, 70.3, 66.5, 58.9, 49.9, 46.7. IR (cm^{-1}): 3350, 1650, 1111.

General Procedure for the Synthesis of Fullerene Derivatives 1 and 4a−c. To a solution of 100 mg (0.14 mmol) of C_{60} in 50 mL of toluene were added 0.70 mmol of aldehyde and 0.14 mmol of N-substituted glycine. The mixture was heated to reflux for 2 h, brought to rt, poured on top of a silica gel column, eluted with toluene, and then eluted with toluene−acetate (9:1). The compounds were dissolved in dichloromethane and precipitated by addition of methanol.

Derivative 1: 39% (toluene−ethyl acetate (8:2), $R_f = 0.24$). ^{1}H-NMR (250 MHz): δ 9.00 (m, 1H), 8.60 (m, 1H), 8.16 (m, 1H), 7.38 (dd, $J = 6.0$ Hz, $J = 7.5$ Hz, 1H), 5.02 (d, $J = 9.5$ Hz, 1H), 4.99 (s, 1H), 4.32 (d, $J = 9.4$ Hz, 1H), 2.83 (s, 3H). ^{13}C NMR (62.5 MHz): δ 155.5, 153.2, 152.2, 151.8, 150.3, 149.6, 147.0, 146.0, 145.9, 145.8, 145.4, 145.2, 145.1, 145.0, 144.9, 144.3, 142.9, 142.8, 142.4, 142.3, 142.0, 141.7, 141.6, 140.0, 139.9, 137.0, 136.2, 136.1, 135.8, 123.3, 80.8, 69.8, 68.6, 39.7. UV−vis (cyclohexane): λ_{max} nm 430, 317, 256, 212. MALDI *m*/*z*: 855 $(M + H)^+$, 720 $(C_{60})^+$. Anal. Calcd for $C_{68}H_{10}N_2$: C, 95.54; H, 1.18; N, 3.28. Found: C, 93.71; H, 0.97; N, 3.19.

Derivative 4a: 37% (toluene−ethyl acetate (1:1), $R_f = 0.12$). ^{1}H-NMR (200 MHz): δ 9.01 (m, 1H), 8.58 (dd, $J = 4.8$ Hz, $J = 1.6$ Hz, 1H), 8.19 (broad d, $J = 8.1$ Hz, 1H), 7.37 (dd, $J = 8.1$ Hz, $J = 4.8$ Hz, 1H), 5.21 (d, $J = 9.8$ Hz, 1H), 5.21 (s, 1H) 4.33 (d, $J = 9.8$ Hz, 1H), 3.90−4.15 (m, 2H), 3.75 (m, 6H), 3.45 (m, 6H), 2.92 (m, 1H). ^{13}C-NMR (50 MHz, CS_2−$CDCl_3$): δ 156.2, 153.8, 152.7, 152.3, 150.7, 150.0, 147.3, 146.3, 146.3, 16.2, 146.2, 146.2, 146.1, 146.1, 145.9, 145.7, 145.7, 145.6, 145.5, 145.5, 145.4, 145.4, 145.3, 145.3, 145.2, 144.7, 144.5, 144.4, 144.3, 143.1, 143.0, 142.7, 142.6, 142.5, 142.2, 142.1, 142.1, 142.0, 141.9, 141.8, 141.7, 141.6, 140.2, 140.1, 139.5, 137.3, 137.0, 136.3, 136.0, 135.6, 133.1, 123.6, 79.7, 76.0, 72.0, 70.8, 70.7, 70.1, 69.2, 67.6, 59.1, 52.1. UV−vis (cyclohexane): λ_{max} nm 701, 430, 323, 309, 255, 212. MALDI *m*/*z*: 987 $(M + H^+)$. Anal. Calcd for $C_{74}H_{22}N_2O_3$: C, 90.05; H, 2.25; N, 2.84. Found: C, 88.85; H, 2.06; N, 2.76.

Derivative 4b: 38% (toluene−ethyl acetate (1:1), $R_f = 0.08$). ^{1}H-NMR (250 MHz): δ 8.68 (m, 2H), 7.81 (m, 2H), 5.23 (d, $J = 7.8$ Hz, 1H), 5.21 (s, 1H), 4.35 (d, $J = 7.8$ Hz, 1H), 4.01 (2m, 2H), 3.78 (m, 6H), 3.57 (m, 2H), 3.38 (m, 4H), 2.99−2.90 (m, 1H). ^{13}C-NMR (62.5 MHz): δ 155.9, 153.7, 152.4, 152.0, 149.5, 147.4, 147.3, 147.3, 146.3, 146.3, 146.2, 146.2, 146.2, 146.1, 146.1, 145.9, 145.9, 145.6, 145.6, 145.5, 145.4, 145.4, 145.3, 145.3, 145.3, 145.2, 145.2, 144.7, 144.5, 144.4, 144.3, 143.1, 143.0, 142.7, 142.6, 142.5, 142.2, 142.1, 142.1, 142.0, 141.9, 141.8, 141.7, 141.6, 141.5, 140.2, 139.9, 139.4, 137.1, 136.3, 136.1, 135.5, 124.5, 80.9, 75.5, 71.9, 70.7, 70.6, 70.0, 69.3, 67.4, 59.0, 52.1. UV−vis (cyclohexane):

(13) Diederich, F.; Jonas, U.; Gramlich, V.; Herrmann, A.; Ringsdorf, H.; Thilgen, C. *Helv. Chim. Acta* **1993**, *76*, 2445.

(14) Bianco, A.; Maggini, M.; Scorrano, G.; Toniolo, C.; Marconi, G.; Villani, C.; Prato, M. *J. Am. Chem. Soc.* **1996**, *118*, 4072.

λ_{max} nm 700, 430, 320, 309, 255, 212. MALDI *m/z*: 987 ($M + H^+$). Anal. Calcd for $C_{74}H_{22}N_2O_3$: C, 90.05; H, 2.25; N, 2.84. Found: C, 89.68; H, 1.99; N, 2.80.

Derivative 4c: 34% (toluene−methanol (9:1), $R_f = 0.22$). ^{1}H-NMR (200 MHz): δ 4.97 (d, $J = 9.8$ Hz, 1H), 4.64 (m, 1H), 4.38 (s, 1H), 4.36 (m, 1H), 4.26 (d, $J = 9.8$ Hz, 1H), 4.04 (t, $J = 5.5$ Hz, 2H), 3.66 (m, 18H), 3.36 (s, 3H), 3.35 (s, 3H). ^{13}C-NMR (50 MHz) δ 156.1, 154.8, 154.2, 152.6, 147.2, 147.2, 147.1, 146.6, 146.3, 146.2, 146.2, 146.1, 146.0, 145.9, 145.8, 145.7, 145.6, 145.5, 145.4, 145.3, 145.2, 144.7, 145.3, 144.6, 144.4, 143.2, 143.0, 142.7, 142.6, 142.2, 142.1, 142.1, 142.0, 141.7, 141.6, 140.2, 140.1, 139.6, 139.3, 137.3, 136.4, 135.9, 135.5, 74.8, 73.5, 72.2, 72.0, 71.9, 70.9, 70.8, 70.6, 70.6, 70.3, 70.1, 67.7, 59.1, 52.3. UV−vis (cyclohexane): λ_{max} nm 702, 430, 321, 306, 255, 213. MALDI *m/z*: 1041 (M^+). Anal. Calcd for $C_{75}H_{31}NO_6$: C, 86.44; H, 3.00; N, 1.34. Found: C, 85.73; H, 2.70; N, 1.30.

Supporting Information Available: ^{1}H and/or ^{13}C NMR spectra of compounds **2**, **3c**, and **4a**−**c** (11 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO961522T

Additions and Corrections

Vol. 40, 1975

Albert Padwa,* Andrew Au, George A. Lee, and William Owens. Photochemical Ring-Opening Reactions of Substituted Chromenes and Isochromenes.

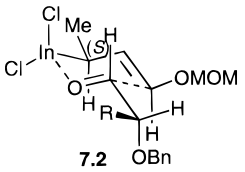
Page 1148. The NMR spectrum of *trans*-2-methoxy-3-hydroxy-2-phenylindan (**33**) should read as follows: NMR ($CDCl_3$) δ 3.10 (s, 3H), 3.90 (broad s, 2H), 4.93 (s, 1H), and 6.8−7.8 (m, 9H). The integration pattern previously reported was inadvertently switched.

JO964025L

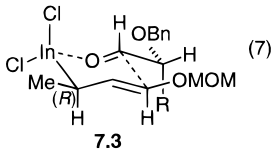
Vol. 61, 1996

James A. Marshall* and Kevin W. Hinkle. Stereoselective S_E2' Additions of Enantioenriched Allylic Tin and Indium Reagents to Protected Threose and Erythrose Aldehydes: A General Strategy for the Stereocontrolled Synthesis of Precursors to the Eight Diastereomeric Hexoses and Their Enantiomers.

Page 107. The correct structures for transition states **7.2** and **7.3** are shown below.



7.2



7.3 (7)

JO9640231

J.-M. Mattalia, M. Chanon, and C. J. M. Stirling*. A New Radical Clock for Testing the Possibility of Electron Transfer from Carbanions.

Page 1153. The kinetic relation is $k = k_H([\mathbf{5}]/[\mathbf{1}])[Bu_3SnH]_0$, where $k_H = 3.6 \times 10^6$ M^{-1} s^{-1} (80 °C).

Page 1154. **Reductions of Chlorocyclopropylmethyl Phenyl Sulfone 4 with Bu_3SnH.** The determined ratios are **5**/**1**. In refluxing cyclohexane this ratio is 92/8 when $[Bu_3SnH]_0 = 0.26$ M (5 equiv) and 0.27 M (6.6 equiv).

Chloro sulfide **3** was prepared according to W. Adam and M. Heil: Adam, W.; Heil, M. *J. Am. Chem. Soc.* **1991**, *113*, 1730. The chlorination with CCl_4 in a basic medium follows the principle given in the following: Meyers, C. Y.; Matthews, W. S.; Ho, L. L.; Kolb, V. M.; Parady, T. E. In *Catalysis in Organic Synthesis*; Smith, G. V., Ed.; Academic Press: New York, 1977.

For a precedent to this type of radical probe, see: Makosza, M.; Kwast, A. *Bull. Soc. Chim. Belg.* **1994**, *103*, 445.

JO964020O

Joseph A. Leonetti, Tim Gross, and R. Daniel Little* . Cycloaddition−Fragmentation as a Route to Bicyclic Ring Systems. Use of the Intermolecular Diyl Trapping Reaction.

Page 1787. We are pleased to acknowledge the assistance of Ms. Janette Gunther with various aspects of this research.

JO964016C

Syed A. Abbas, M. Bilayet Hossain, Dick van der Helm, Francis J. Schmitz,* Maureen Laney, Ronnel Cabuslay, and Randall C. Schatzman. Alkaloids from the Tunicate *Polycarpa aurata* from Chuuk Atoll.

Page 2709. The correct spelling is of the first author's name is Syed A. Abbas.

JO964019P

Andrew G. H. Wee* and J. Slobodian. Metal-Catalyzed Reaction of Indoline Diazoamides Possessing a C-2 CH_2X Substituent: Site-Selectivity, Diastereoselectivity, and Chemoselectivity.

Page 2899. References 15a,b cited for **12a** in the supplementary material section should read 7a,b. Reference 7a should be cited as follows: Cliff, G. R.; Jones, G.; Woollard, J. McK. *Tetrahedron Lett.* **1973**, 2401.

Under **General** in the Experimental Section, "... reference 5" should read "... reference 1".

JO9640276