# Preparation of Enantiopure Ketones and Alcohols Containing a Quaternary Stereocenter through Parallel Kinetic Resolution of β-Keto Nitriles

Juan R. Dehli and Vicente Gotor*

*Departamento de Química Orgánica e Inorgánica, Universidad de Oviedo, c/ Julián Clavería, 8. 33006 Oviedo, Spain*

*vgs@sauron.quimica.uniovi.es*

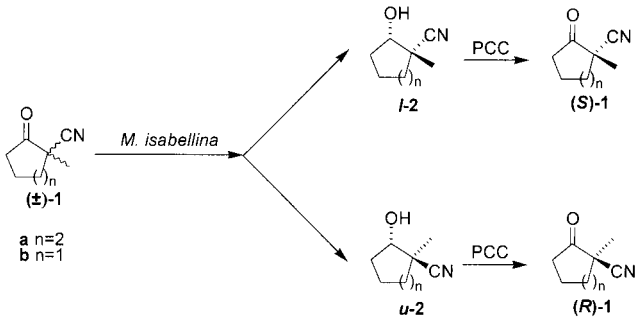
*Received November 20, 2001*

**Abstract:** Racemic 1-methyl-2-oxocycloalkanecarbonitriles have been subjected to bioreduction by the fungus *Mortierella isabellina* NRRL 1757 through a parallel kinetic-resolution process. The *u* and *l* alcohols thus obtained (up to >99% ee) were easily separated and oxidized to the *R* and *S* ketones, respectively. The process can be then repeated so that both enantiomers of the ketone and two epimers of the alcohol can be obtained in their enantiopure forms.

The asymmetric construction of molecules with quaternary carbon stereocenters has represented a very challenging and dynamic area in organic synthesis over the past decade.[1] Despite the numerous reports on bioreduction of ketones, mainly by baker's yeast,[2] reported in the literature, very few examples have made use of bioreduction as a means to obtain optically active compounds with fully substituted carbons, and in most cases with only moderate success.[3]

On the other hand, the importance of optically active β-hydroxy nitriles as suitable synthons for the preparation of γ-amino alcohols (like the antidepressant fluoxetine)[4] is steadily growing. Thus, recently, methodologies have been developed to prepare these alcohols via classical kinetic resolution,[5] dynamic kinetic resolution,[6] reduction,[7] alkylation−reduction,[8] and addition[9] processes.

**Scheme 1**




We decided then to merge both targets, and we chose racemic 1-methyl-2-oxocycloalkanecarbonitriles **1** as substrates for the bioreduction process. According to Prelog's rule,[10] the formal introduction of the hydride would take place from the *re* face of the carbonyl group, yielding the corresponding alcohol of 2*S* configuration, which would represent a parallel kinetic resolution (PKR): (*R*)-**1** would give *u*-**2** and (*S*)-**1**, *l*-**2** (see Scheme 1). Although this kind of resolution is not completely new,[11] the scarce biocatalytic examples described thus far do not allow for the recuperation of the optically active substrate.[12] In our case, mild oxidation of the alcohol would let us recover the enantioenriched ketone, which is what gives our process additional value because compounds such as **1** are interesting building blocks for the preparation of natural products[13] and conformationally restricted amino acids.[14]

Among the microorganisms tested at analytical scale[15] for the bioreduction of (±)-**1a**, the fungus *Mortierella isabellina* NRRL 1757 gave the most satisfactory results. Thus, after 2 h, a mixture of *u*-**2a** (>99% ee) and *l*-**2a** (73% ee) was obtained in a 42:58 diastereomeric ratio.

To achieve efficient preparative-scale experiments at gram scale, the substrate concentration was increased to 10 g/L (75 mM) without any change as far as stereochemistry was concerned. In a typical example, 1 g of (±)-**1a** was subjected to bioreduction using resting cells grown in 100 mL of culture for 48 h[16] and resuspended in 100 mL of distilled water.[17] After 7 h at 28 °C and 200

* Corresponding author.

(1) (a) Fuji, K. *Chem. Rev.* **1993**, *93*, 2037. (b) Corey, E. J.; Guzman-Perez, A. *Angew. Chem., Int. Ed.* **1998**, *37*, 388.

(2) (a) Servi, S. *Synthesis* **1990**, 1. (b) Csuk, R.; Glänzer, B. I. In *Stereoselective Biocatalysis*; Patel, R. N., Ed.; Marcel Dekker, Inc.: New York, **2000**; Chapter 19, p 527.

(3) (a) Brooks, D. W. *J. Org. Chem.* **1982**, *47*, 2820. (b) Brooks, D. W.; Woods, K. W. *J. Org. Chem.* **1987**, *52*, 2036. (c) Brooks, D. W.; Mazdiyasni, H.; Grothaus, P. G. *J. Org. Chem.* **1987**, *52*, 3223. (d) Fuhshuku, K.; Funa, N.; Akeboshi, T.; Ohta, H.; Hosomi, H.; Ohba, S.; Sugai, T. *J. Org. Chem.* **2000**, *65*, 129.

(4) Koenig, T. M.; Mitchell, D. *Tetrahedron Lett.* **1994**, *35*, 1339.

(5) (a) Itoh, T.; Takagi, Y.; Nishiyama, S. *J. Org. Chem.* **1991**, *56*, 1521. (b) Itoh, T.; Takagi, Y.; Murakami, Y. *J. Org. Chem.* **1996**, *61*, 2158.

(6) Pàmies, O.; Bäckvall, J.-E. *Adv. Synth. Catal.* **2001**, *343*, 726.

(7) (a) Itoh, T.; Fukuda, T.; Fujisawa, T. *Bull. Chem. Soc. Jpn.* **1989**, *62*, 3851. (b) Mehmandoust, M.; Buisson, D.; Azerad, R. *Tetrahedron Lett.* **1995**, *36*, 6461. (c) Florey, P.; Smallridge, A. J.; Ten, A.; Trewhella, M. A. *Org. Lett.* **1999**, *1*, 1879. (d) Dehli, J. R.; Gotor, V. *Tetrahedron: Asymmetry* **2000**, *11*, 3693.

(8) (a) Itoh, T.; Takagi, Y.; Fujisawa, T. *Tetrahedron Lett.* **1989**, *30*, 3811. (b) Gotor, V.; Dehli, J. R.; Rebolledo, F. *J. Chem. Soc., Perkin Trans. 1* **2000**, 307. (c) Dehli, J. R., Gotor, V. *Tetrahedron: Asymmetry* **2001**, *12*, 1485.

(9) Soai, K.; Hirose, Y.; Sakata, S. *Tetrahedron: Asymmetry* **1992**, *3*, 677.

(10) Prelog, V. *Pure Appl. Chem.* **1964**, *9*, 119.

(11) (a) Vedejs, E.; Chen, X. *J. Am. Chem. Soc.* **1997**, *119*, 2584. (b) Eames, J. *Angew. Chem., Int. Ed.* **2000**, *39*, 885. (c) Bertozzi, F.; Crotti, P.; Macchia, F.; Pineschi, M.; Feringa, B. L. *Angew. Chem., Int. Ed.* **2001**, *40*, 930.

(12) See, for example: (a) Konigsberg, K.; Alphand, V.; Furstoss, R.; Griengl, H. *Tetrahedron Lett.* **1991**, *32*, 499. (b) Petit, F.; Furstoss, R. *Tetrahedron: Asymmetry* **1993**, *4*, 1341. (c) Mischitz, M.; Faber, K. *Tetrahedron Lett.* **1994**, *35*, 81.

(13) Enders, D.; Zamponi, A.; Raabe, G.; Runsink, J. *Synthesis* **1993**, 725 and references therein.

(14) Westermann, B.; Walter, A.; Diedrichs, N. *Angew. Chem., Int. Ed.* **1999**, *38*, 3384.

(15) Typically, the substrate (1 g/L), solvated in ethanol (1% v/v), was added to a suspension of resting cells in 35 mL of distilled water. This mixture was then shaken (200 rpm) at 28 °C, and the reaction was monitored by chiral GC.

(16) (a) Quirós, M.; Rebolledo, F.; Liz, R.; Gotor, V. *Tetrahedron: Asymmetry* **1997**, *8*, 3035. (b) Quirós, M.; Rebolledo, F.; Gotor, V. *Tetrahedron: Asymmetry* **1999**, *10*, 473.

(17) Recently, special emphasis has been placed on the substitution of plain water for buffers, which simplifies the downstream processing of a hypothetical industrial application. See, for example: Genzel, Y.; Archelas, A.; Broxterman, Q. B.; Schulze, B.; Furstoss, R. *J. Org. Chem.* **2001**, *66*, 538.

10.1021/jo011092t CCC: $22.00 
Published on Web 02/02/2002

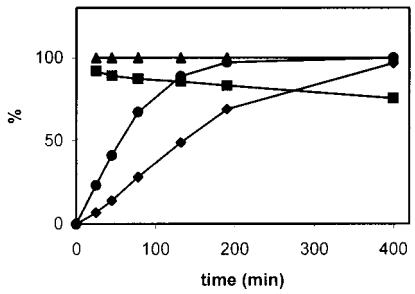
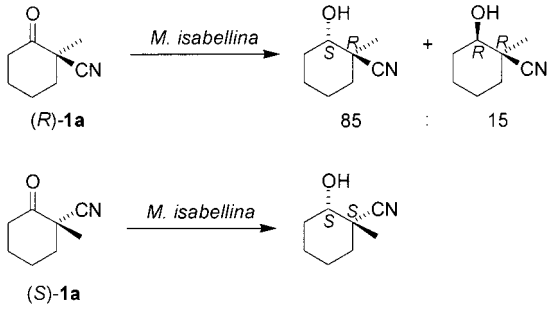



**Figure 1.** Preparative bioreduction of (±)-**1a** by the fungus *M. isabellina*: (●) conversion of (*S*)-**1a**, (♦) conversion of (*R*)-**1a**, (▲) ee of *u*-**2a**, (■) ee of *l*-**2a**.

**Scheme 2**




rpm, the reaction was complete. This reaction afforded, after flash chromatography, *u*-**2a** (38% yield, >99% ee) and *l*-**2a** (54% yield, 73% ee). As can be seen in Figure 1, the difference in the rate of reduction of both enantiomers is rather small but is no impediment to the high stereoselectivity of the process. This result confirms our initial hypothesis of a PKR because after completion the products are obtained in high ee, contrary to what happens to classical kinetic resolutions, which require conversion values close to 50%. From these products, both antipodes of ketone **1a** (*R* and *S* respectively) were obtained by mild oxidation with PCC without racemization (confirmed by GC) and in quantitative yield.

Similar results were obtained when we used (±)-**1b** as the substrate: *u*-**2b** was obtained in 34% yield and >99% ee, and *l*-**2b**, in 58% yield and 61% ee. *u*-**2b** and *l*-**2b** could also be oxidized to the corresponding enantiomers of ketone **1b**.

Although in both cases the *u* isomer was obtained in the enantiopure form, the ee of its epimer was unsatisfactory. We thought that it could then be easily increased by iterating the process, making use of the easy recovery of the enantioenriched ketone (*S*)-**1** from *l*-**2**. Thus, in a second bioreduction process, we obtained *l*-**2a** in 44% yield and 96% ee (together with a small amount of enantiopure *u*-**2a**, which increased its overall yield to 42%).

To understand this increase in the ee, we undertook an examination of the reduction of the enantiomerically pure ketones (*R*)- and (*S*)-**1a** (see Scheme 2). In the first case, the introduction of the hydride follows Prelog's rule, with moderate selectivity. Thus, the *u* alcohol was obtained as the major isomer (*u*−*l*, 85:15). In the second case, the reduction occurs with total diastereoselectivity, and the *l* diastereomer was the only product detected by GC.

By starting from (±)-**1a**, enantiopure *u*-**2a** is obtained (it consists only of the (1*R*,2*S*) enantiomer), whereas *l*-**2a** has an ee value of only 73% (it consists of both (1*S*,2*S*) from (*S*)-**1a** and (1*R*,2*R*) from (*R*)-**1a**). Therefore, starting from a ketone enriched in the *S* antipode (instead of the racemic one), the *l* alcohol reaches a higher ee value.

The assignment of the relative configuration of the alcohols **2** has been done on the basis of their $^{13}C$ NMR spectra, as had been previously done for similar structures.[3c] In the *l* isomers, the CN signal was substantially deshielded (with regard to that of the *u* isomers) because of a $\gamma$ steric-compression effect of the vicinal hydroxyl group.[18] As expected, the opposite effect is observed for the $CH_3$ signal (see Experimental Section). The validity of this assignment was confirmed by the transformation of *u*-**2a** into its *O*-trimethylsilyl derivative and by comparing its $^1H$- and $^{13}C$ NMR spectra with those already published.[19] The absolute configuration of (*R*)-**1a** was assigned by comparison of the sign of its optical rotation with that of the value published in the literature.[20] For the cyclopentane analogues, the following correlation was made: enantiopure *u*-**2b** was hydrolyzed to the carboxylic acid and esterified with trimethylsilyl diazomethane. Further oxidation with PCC led to the previously described $\beta$-keto ester.[21] This methodology was used to unambiguously assign the configurations of all the products reported herein.

In summary, the bioreduction of $\beta$-keto nitriles bearing a quaternary stereocenter has been studied. A parallel kinetic-resolution process takes place so that each enantiomer yields a different diastereomer. The corresponding optically active ketones have been efficiently recovered by oxidation under mild conditions. The products that were not obtained in their enantiopure forms can be subjected to reduction again so that both enantiomers of the ketone can be obtained (after a second oxidation) in very good yield and excellent ee. To the best of our knowledge, this simple and efficient method represents the first biocatalytic approach to obtaining optically active alcohols and ketones bearing quaternary centers by using a parallel kinetic resolution. Further work concerning an in-depth study of this process as well as application to other sterically hindered ketones is currently in progress in our laboratory.

## Experimental Section

**General.** Reagents were obtained from Aldrich Chemie. THF was distilled over sodium and stored under nitrogen. Precoated TLC plates of silica gel 60 F254 from Merck were used, while for column chromatography, Merck silica gel 60/230−400 mesh was used. NMR spectra were carried out in $CDCl_3$. The ee and dr values were determined by GC using a Rt-$\beta$DEXse (30 m × 0.25 mm, Restek) capillary column and nitrogen as the carrier gas (15 psia) (100 °C, 15 min; 1 °C $min^{-1}$ until 170 °C). The fungus *M. isabellina* NRRL 1757 was obtained from the NRRL culture collection and was precultured and cultured as previously described.[16]

**Syntheses of (±)-1-Methyl-2-oxocycloalkanecarbonitriles, (±)-1.** To a suspension of NaH (1.3 g, 55 mmol) in 100 mL THF was added 50 mmol of the corresponding alkanedinitrile, and the mixture refluxed until the disappearance of the

(18) Grant, D. M.; Cheney, B. V. *J. Am. Chem. Soc.* **1967**, *89*, 5315.
(19) Imi, K.; Yanagihara, N.; Utimoto, K. *J. Org. Chem.* **1987**, *52*, 1013.
(20) $[\alpha]^{20}_D$ +263.8 (*c* 2.9, benzene, >99% ee *S*), lit. $[\alpha]^{25}_D$ +232.2 (*c* 1.04, benzene, 90% ee *R*), see ref 14.
(21) $[\alpha]^{20}_D$ +11.1 (*c* 1.0, $CHCl_3$, >99% ee), lit. $[\alpha]^{25}_D$ −10.6 (*c* 1.15, $CHCl_3$, >96% ee *R*): Sato, T.; Maeno, H.; Noro, T.; Fujisawa, T. *Chem. Lett.* **1988**, 1739. It should be noted that because of the Cahn−Ingold−Prelog priority rule (*R*)-nitrile led to (*S*)-ester.

substrate (TLC monitoring, eluent hexane/AcOEt 2:1). Then, the reaction was allowed to reach room temperature, quenched with 30 mL of water, and extracted with diethyl ether. After drying and elimination of the solvent, the enamine formed was hydrolyzed at room temperature with 50 mL of 3 N $H_2SO_4$ and extracted again with diethyl ether and dried, and the solvent was removed on the rotovapor. The residual oil was solvated in a mixture of LiOH (55 mmol), water (25 mL), and methanol (25 mL). MeI (55 mmol) was added, and the solution was heated at 50 °C for 2 h. Extraction with diethyl ether, drying, and elimination of the solvent on the rotovapor led to substantially pure **1**, which was further purified by bulb-to-bulb distillation. GC analysis, $t_R$ (min): (*S*)-**1a** 15.9; (*R*)-**1a** 17.7; (*S*)-**1b** 15.5; (*R*)-**1b** 17.1.

**Syntheses of the Diastereomeric Mixtures of (±)-2.** To a solution of 1 mmol (±)-**1** in 5 mL of ethanol was added 25 mg (0.7 mmol) of $NaBH_4$, and the mixture was shaken at room temperature for 6 h. After extraction with diethyl ether and elimination of the solvent, a pale yellowish oil was obtained (95% of theor yield) that was used without further purification as a standard for chiral GC analyses. $t_R$ (min): (1*S*,2*S*)-**2a** 34.4; (1*R*,2*R*)-**2a** 35.1; (1*R*,2*S*)-**2a** 41.6; (1*S*,2*R*)-**2a** 42.1; (1*S*,2*S*)-**2b** 28.7; (1*R*,2*R*)-**2b** 31.0; (1*R*,2*S*)-**2b** 38.2; (1*S*,2*R*)-**2b** 38.8.

**Preparative Biotransformations with *M. isabellina* NRRL 1757.** A 100 mL culture grown for 2 days at 28 °C and 200 rpm was filtered out and resuspended in the same volume of distilled water, and the substrate (1 g) and ethanol (1 mL) were added. The reaction was monitored by GC. When no more substrate remained, cells were harvested by filtration and washed with water. The combined aqueous phases were saturated with NaCl and continuously extracted with $CH_2Cl_2$ for 12 h. After drying and evaporation of the solvent, the diastereomeric mixture was efficiently separated by flash chromatography (eluent hexane/$Et_2O$ 5:1).

**(1*R*,2*S*)-2-Hydroxy-1-methylcyclohexanecarbonitrile, *u*-2a:** colorless oil; yield 42%; $[\alpha]^{20}_D$ +31.5 (*c* 2.9, $CHCl_3$, >99% ee); IR $\tilde{\nu}$: 3061 (OH) and 2234 (CN) $cm^{-1}$; $^1H$ NMR $\delta$: 1.34 (s, 3H, $CH_3$), 1.4−1.9 (m, 8H), 2.26 (bs, 1H, OH), 3.87 (m, 1H, CH); $^{13}C$ NMR $\delta$: 19.9 ($CH_3$), 20.4, 21.3, 29.6, 32.2 ($CH_2$), 38.3 (C), 71.0 (CH), 124.4 (CN); EIMS *m*/*z*: 139 ($M^+$, <1), 110 (38), 68 (100). Anal. Calcd for $C_8H_{13}NO$: C, 69.03; H, 9.41; N, 10.06. Found: C, 68.77; H, 9.68; N, 9.92.

**(1*S*,2*S*)-2-Hydroxy-1-methylcyclohexanecarbonitrile, *l*-2a:** colorless oil; yield 44%; $[\alpha]^{20}_D$ −22.4 (*c* 0.7, $CHCl_3$, 96% ee); IR $\tilde{\nu}$: 3062 (OH) and 2235 (CN) $cm^{-1}$; $^1H$ NMR $\delta$: 1.2−1.36 (m, 2H), 1.42 (s, 3H, $CH_3$), 1.5−1.7 (m, 3H), 1.75−1.87 (m, 1H), 1.9−2.02 (m, 2H), 2.44 (d, 1H, $J$ = 5.1 Hz, OH), 3.24 (m, 1H, CH); $^{13}C$ NMR $\delta$: 22.4 ($CH_2$), 23.6 ($CH_3$), 24.2, 32.3, 36.1 ($CH_2$), 42.1 (C), 75.2 (CH), 122.3 (CN); EIMS *m*/*z*: 139 ($M^+$, <1), 110 (37), 68 (100). Anal. Calcd for $C_8H_{13}NO$: C, 69.03; H, 9.41; N, 10.06. Found: C, 68.87; H, 9.62; N, 9.81.

**(1*R*,2*S*)-2-Hydroxy-1-methylcyclopentanecarbonitrile, *u*-2b:** colorless oil; yield 34%; $[\alpha]^{20}_D$ +43.8 (*c* 1.3, $CHCl_3$, >99% ee); IR $\tilde{\nu}$: 3059 (OH) and 2234 (CN) $cm^{-1}$; $^1H$ NMR $\delta$: 1.36 (s, 3H, $CH_3$), 1.6−1.85 (m, 4H), 2.0−2.25 (m, 2H), 2.57 (bs, 1H, OH), 4.25−4.35 (m, 1H, CH); $^{13}C$ NMR $\delta$: 18.0 ($CH_3$), 20.2, 32.1, 35.7 ($CH_2$), 41.9 (C), 77.8 (CH), 125.0 (CN); ESIMS *m*/*z*: 126 [$(M + H)^+$, 2], 148 [$(M + Na)^+$, 100]. Anal. Calcd for $C_7H_{11}NO$: C, 67.17; H, 8.86; N, 11.19. Found: C, 66.89; H, 8.99; N, 11.38.

**(1*S*,2*S*)-2-Hydroxy-1-methylcyclopentanecarbonitrile, *l*-2b:** colorless oil; yield 58%; $[\alpha]^{20}_D$ +3.8 (*c* 1.0, $CHCl_3$, 61% ee); IR $\tilde{\nu}$: 3060 (OH) and 2234 (CN) $cm^{-1}$; $^1H$ NMR $\delta$: 1.35 (s, 3H, $CH_3$), 1.6−1.85 (m, 3H), 1.9−2.1 (m, 2H), 2.2−2.3 (m, 1H), 2.35 (bs, 1H, OH), 3.89 (m, 1H, CH); $^{13}C$ NMR $\delta$: 19.8 ($CH_2$), 22.0 ($CH_3$), 31.8, 35.4 ($CH_2$), 44.6 (C), 79.8 (CH), 123.0 (CN); ESIMS *m*/*z*: 126 [$(M + H)^+$, <1], 148 [$(M + Na)^+$, 100]. Anal. Calcd for $C_7H_{11}NO$: C, 67.17; H, 8.86; N, 11.19. Found: C, 66.95; H, 9.14; N, 11.12.

**Preparation of Optically Active Ketones 1.** To a solution of the corresponding optically active hydroxy nitrile **2** (2 mmol) in $CH_2Cl_2$ (5 mL), pyridinium chlorochromate (PCC) was added (3 mmol), and the mixture was shaken at room temperature for 6 h. Then, it was filtered through Florisil and eluted with $CH_2Cl_2$. This crude residue (quantitative yield) was essentially pure by TLC, GC, and NMR and could be used directly for a second bioreduction. For analytical purposes, it was purified by flash chromatography (eluent hexane/$CH_2Cl_2$ 1:1).

**1-Methyl-2-oxocyclohexanecarbonitrile, 1a:** colorless oil; yield 85% (from 1,7-heptanedinitrile) or quantitative (from **2a**); $[\alpha]^{20}_D$ +263.8 (*c* 2.9, benzene, >99% ee *R*); spectral data (IR, $^1H$- and $^{13}C$ NMR, and MS) match those already published.[13] Anal. Calcd for $C_8H_{11}NO$: C, 70.04; H, 8.08; N, 10.21. Found: C, 69.86; H, 8.16; N, 10.13.

**1-Methyl-2-oxocyclopentanecarbonitrile, 1b:** colorless oil; yield 92% (from 1,6-hexanedinitrile) or quantitative (from **2b**); $[\alpha]^{20}_D$ +28.8 (*c* 1.3, $CHCl_3$, >99% ee *R*); IR $\tilde{\nu}$: 2235 (CN) and 1756 (CO) $cm^{-1}$; $^1H$ NMR $\delta$: 1.44 (s, 3H, $CH_3$), 1.9−2.2 (m, 3H), 2.3−2.6 (m, 3H); $^{13}C$ NMR $\delta$: 19.0 ($CH_2$), 20.3 ($CH_3$), 35.9, 36.2 ($CH_2$), 43.6 (C), 119.7 (CN), 209.5 (CO); ESIMS *m*/*z*: 136 $(M + H)^+$. Anal. Calcd for $C_7H_9NO$: C, 68.27; H, 7.37; N, 11.37. Found: C, 68.01; H, 7.58; N, 11.12.

**Acknowledgment.** Financial support of this work by the Spanish Ministerio de Ciencia y Tecnología (Project BIO 98-0770) is gratefully acknowledged. J.R.D. thanks the Spanish Ministerio de Educación, Cultura y Deporte for a predoctoral scholarship.

**Supporting Information Available:** $^1H$- and $^{13}C$ NMR spectra of **1a**, **1b**, *l*-**2a**, *u*-**2a**, *l*-**2b**, *u*-**2b**. This material is available free of charge via the Internet at http://pubs.acs.org..

JO011092T