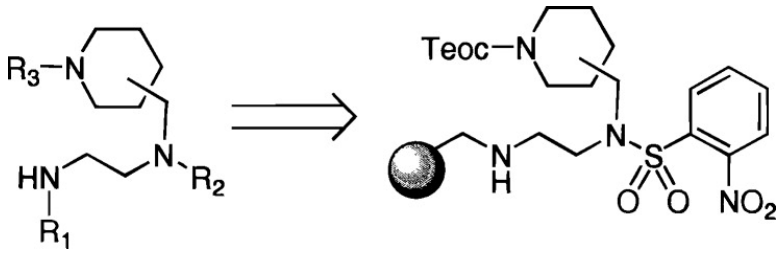
Report

Solid-Phase Synthesis of a Heterocyclic Ethylenediamine-Derivatized Library

Elizabeth A. Jefferson, Kelly G. Sprankle, and Eric E. Swayze

J. Comb. Chem., **2000**, 2 (5), 441-444• DOI: 10.1021/cc000027e • Publication Date (Web): 14 July 2000



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

Solid-Phase Synthesis of a Heterocyclic Ethylenediamine-Derivatized Library

Elizabeth A. Jefferson,* Kelly G. Sprankle, and Eric E. Swayze

Ibis Therapeutics, A Division of Isis Pharmaceuticals, Inc., 2292 Faraday Avenue, Carlsbad, California 92008

Received April 14, 2000

RNA molecules are known to play key roles in vital biological processes, and as a result, they are emerging as attractive drug targets.[1] Since therapeutic targeting of RNA with small molecules is not as well-developed as with proteins, the discovery of new RNA small molecule binders is a challenging task. Combinatorial chemistry[2] may serve as a useful tool to uncover new lead structures with high affinity and selectivity for RNA.

A combinatorial library based on piperidine- and pyrrolidine-substituted[3] ethylenediamine scaffolds were prepared (**1a**−**c** and **5**, Scheme 1).[4] These templates are attractive as they can be easily assembled using readily accessible building blocks (N-protected ethylenediamine and heterocyclic amino alcohols) and display a diverse array of functionality at three sites. In addition, the flexible nature of the scaffolds allows the diversity elements to occupy a variety of spatial orientations.

Since the Fukuyama−Mitsunobu reaction[5] is known to proceed in excellent yield on solid support, it was chosen as the key scaffold assembly reaction. The 2-nitrobenzenesulfonyl group of the ethylenediamine unit serves as both an activating group for the Fukuyama−Mitsunobu reaction as well as a protecting group for a latent diversity site. Building block 2-nitrobenzenesulfonamide **3** (HCl salt) was prepared according to literature procedures,[6] and the amino alcohol components (**4a**−**c** and **7**) were prepared by trimethylsilylethoxycarbonyl(Teoc)-protecting commercially available amino alcohols.[7] The amino terminus of **3** was immobilized on an aldehyde-bearing resin via a reductive amination procedure.[8] The resin attachment procedure is attractive as the secondary amine generated in this step can serve as a combinatorial diversity site through N-functionalization. The orthogonal protecting groups on the scaffolds enable the combinatorial synthesis to take place by the sequential deprotection of amino groups.

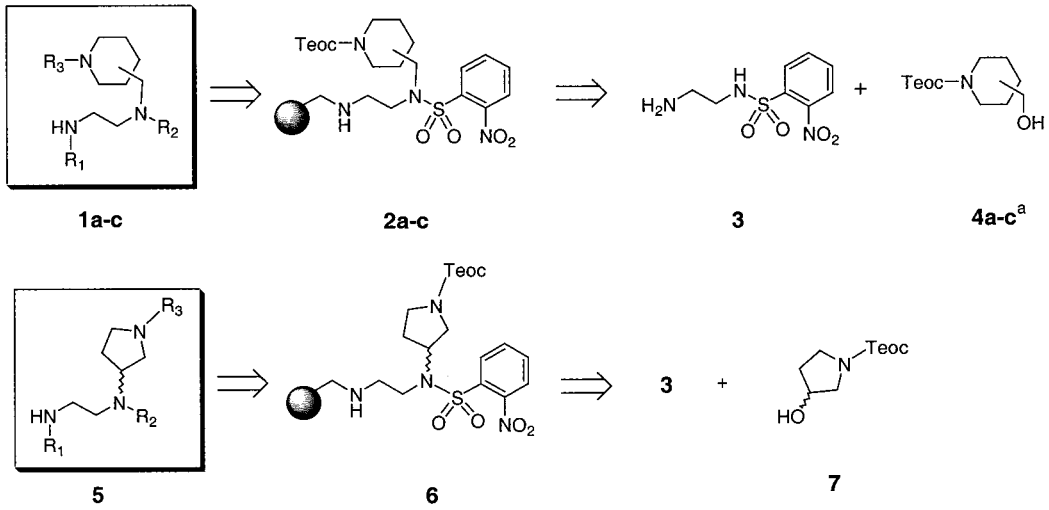
The general synthetic plan is shown in Scheme 2. To explore synthesis feasibility, model library member **14a** was prepared using *p*-toluenesulfonyl chloride, *m*-toluic acid, and phenylacetic acid as diversity reagents. ArgoGel-MB-CHO resin[9] was reacted with neutralized 2-nitrobenzenesulfonyl-protected ethylenediamine (**8**) via a borane-pyridine mediated reductive amination procedure[8] to afford sulfonamide **9**. According to gel-phase ^{13}C NMR spectroscopy, the yield of resin loading is >95%, based upon the initial resin substitution. The secondary amine of resin **9** was reacted with *p*-toluenesulfonyl chloride/diisopropylethylamine (DIEA) to afford resin **10**. A Mitsunobu reaction employing Fukuyama's protocol[5] with heterocyclic amino alcohol **4a** provided scaffold **11** in essentially quantitative yield, according to HPLC and mass spectrometric analysis of the residue liberated from solid support with trifluoroacetic acid (TFA). Orthogonally protected scaffold **11** was then consecutively functionalized at two nitrogen atoms following selective unmasking. First, the 2-nitrobenzenesulfonyl group was removed with mercaptoacetic acid/1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), and the resultant amine acylated with *m*-toluic acid using *o*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorphosphate (HATU)[10] activation. The Teoc-protecting group of resin **12** was then removed with tetrabutylammonium fluoride (TBAF) and the piperidine nitrogen acylated with phenylacetic acid to provide resin **13**. Ethylenediamine-derivatized library member **14a** was liberated from resin with TFA, in 94% HPLC purity and 81% crude mass recovery yield.

A combinatorial library of 180 members ($5 \times 4 \times 3 \times 3$ array) was prepared using the IRORI radio frequency-encoded split-mix synthesis technology[11] in order to examine the scope and generality of the synthetic scheme. The secondary amine site of resin-bound diamine **9** was reacted with a variety of reagents (*p*-toluenesulfonyl chloride, dansyl chloride, 4-methoxybenzene isocyanate, Boc-3-aminobenzoic acid, and Boc-isonipecotic acid). Other heterocyclic amino alcohol building blocks (**4b**, **4c,** and **7**) were then incorporated into the Fukuyama−Mitsunobu reaction. It was found that the Mitsunobu reaction tolerated a variety of functionality at the substituted amine site in structure **10** according to the high purity of final library members. There was interest in expanding the library to incorporate 3-hydroxy- and 4-hydroxypiperidines; however, the yields of Mitsunobu products were too low in these experiments to give sufficiently pure final library members. Presumably, steric constraints in the Fukuyama−Mitsunobu reaction accounts for the low reactivity of these heterocyclic secondary alcohols. The N-protected diveristy sites of scaffolds **11** were selectively deprotected and functionalized. The 2-nitrobenzenesulfonyl group was removed with mercaptoacetic acid/DBU, and the secondary amine was reacted with either 2-pyrazinecarboxylic acid or isopropylisocyanate. Alternatively, isobutyraldehyde was reacted at this amine site via a reductive amination protocol. After the Teoc protection of resin **12** was removed with TBAF, the secondary amine was acylated with either thymine-1-acetic acid or cyclopropanecarboxylic acid. Alternatively, the secondary amine was reacted with 3-hydroxybenzaldehyde via a reductive amination procedure. Library members **14** were cleaved from solid support in excellent purity, sufficient for direct biological screening.

The mass spectrometric (electrospray ionization) and analytical reversed-phase HPLC data (light-scattering detection) for a representative set of library members is summarized in Table 1. Automated high-performance liquid chromatography and mass spectrometry (LC/MS)[12] featuring atmospheric chemical ionization (APCI) was recently inte-

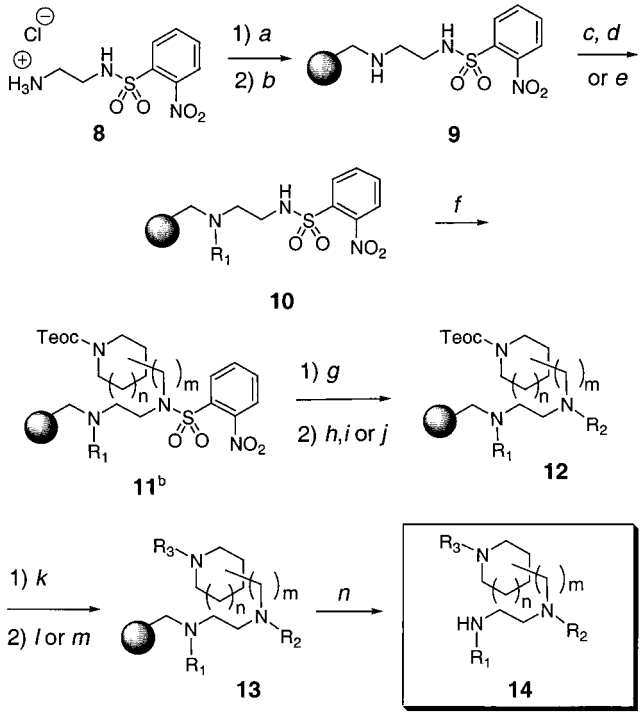
10.1021/cc000027e CCC: $19.00
Published on Web 07/14/2000

Scheme 1



[a] **4a** = 4-hydroxymethylpiperidine, **4b** = 3-hydroxymethylpiperidine, **4c** = 2-hydroxymethylpiperidine.

Scheme 2[a]



[a] Reagents and conditions for library synthesis: (a) 3.0 equiv **8**, 3.1 equiv DIEA, 1.0 equiv ArgoGel-MB-CHO, 4:1 MeOH/$CH(OMe)_3$, rt, 24 h; (b) 2.0 equiv BH_3-pyridine, 2.0 equiv AcOH, rt, 24 h; (c) 0.20 M *p*-toluenesulfonyl chloride or dansyl chloride, 0.20 M DIEA, DCM, rt, 24 h; (d) 0.11 M Boc-isonipecotic acid or Boc-3-aminobenzoic acid, 0.11 M HATU, 0.22 M collidine, DMF, rt, 24 h; (e) 0.20 M 4-methoxybenzyl isocyanate, DMF, rt, 24 h; (f) 0.33 M **4a**−**c** or **7**, 0.33 M triphenylphospine, 0.33 M diisopropylazodicarboxylate, DCM, 0 °C to room temperature, 24 h; (g) 0.50 M $HSCH_2CO_2H$, 1.0 M DBU, DMF, rt, 2 h; (h) 0.11 M *m*-toluic acid or 2-pyrazine carboxylic acid/0.11 M HATU, 0.22 M collidine, DMF, rt, 24 h; (i) 0.20 M isopropylisocyanate, DMF, rt, 24 h; (j) 0.50 M isobutyraldehyde, 0.50 M BH_3-pyridine, MeOH/trimethylorthoformate (3/1, v/v) + 5% AcOH, rt, 24 h; (k) 0.2 M TBAF, NMP, rt, 2 h; (l) 0.11 M phenyl acetic acid, thymine-1-acetic acid or cyclopropane carboxylic acid, 0.11 M HATU, 0.22 M collidine, DMF; (m) 0.5 M 3-hydroxybenzaldehyde, 0.5 M BH_3-pyridine, MeOH/trimethylorthoformate (3/1, v/v) + 5% AcOH, rt, 24 h; (n) TFA/triisopropylsilane (95/5, v/v), rt, 5 h. [b]When $n = 0$, $m = 0$ (3-substituted) and when $n = 1$, $m = 1$ (2-, 3-, and 4-substituted).

grated into our drug discovery program for both structure characterization and purity assessment of library members. Characterization of the heterocyclic ethylenediamine library by LC/MS showed formation of the expected products in comparable amounts (see Supporting Information).

^{1}H NMR spectra were obtained on selected library members to provide additional structural characterization (see Supporting Information). The ^{1}H NMR spectra were found to be rather complex due to the conformational rotomers of the flexible scaffolds. For these reasons, the use of ^{1}H NMR spectroscopy as a technique to determine compound purity proves difficult for this class of compounds. The technique of APCI-LC/MS was found to be better suited as an indicator of library member purity.

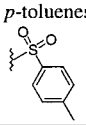
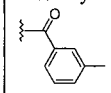
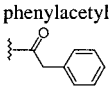
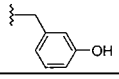
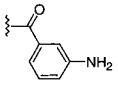
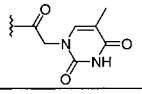
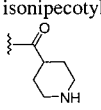
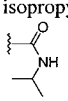
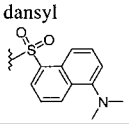
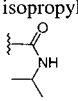
In summary, a versatile solid-phase route to a heterocyclic ethylenediamine-derivatized combinatorial library has been developed from readily accessible building blocks. Three independent sets of diversity elements can be introduced around the scaffolds. The synthesis scheme was applied to a mixture-based synthesis of >20 000 library members,[13] using an automated parallel array synthesis technology.[14] Alternatively, the synthesis of a larger combinatorial library using the IRORI directed sorting technology is feasible.

Library members were screened in various high-throughput RNA-targeted functional assays, and no high-affinity lead compounds were found. It can be suggested that the ethylenediamine-derivatized library members are too flexible to support an induced fit binding to structured RNA, which is relatively dynamic in nature. Combinatorial libraries supporting more rigid, constrained structures have recently led to compounds with more promising RNA-binding activity.[15]

Acknowledgment. This work was supported in part by grants from the National Institute of Standards and Technology Advanced Technology Program (97-01-0135) and the Defense Advanced Projects Research Agency (BAA 98-25-544).

Supporting Information Available. Experimental details for the synthesis of **14a** and the combinatorial library. Analytical reversed-phase HPLC, EI-MS, APCI-LC/MS, and ^{1}H NMR data of a representative set of library members. This material is available free of charge via the Internet at http://pubs.acs.org.

Table 1. Ethylenediamine-Derivatized Heterocyclic Library Members **14a−y**

entry	R_1	alcohol[a]	R_2	R_3	MS-EI, *m/z* (M+H)	purity (yield),[b] %
14a	*p*-toluenesulfonyl 	**4a**	*m*-toluoyl 	phenylacetyl 	549	94 (81)
14b	*p*-toluenesulfonyl	**4a**	isobutyl 	3-hydroxybenzyl 	474	84 (49)
14c	3-aminobenzoyl 	**4a**	2-pyrazinecarboxyl 	thymine-1-acetyl 	549	> 98 (71)
14d	carbamoyl 	**4a**	2-pyrazinecarboxyl	3-hydroxybenzyl	413	97 (85)
14e	isonipecotyl	**4a**	2-pyrazinecarboxyl	thymine-1-acetyl	541	97 (76)
14f	isonipecotyl 	**4b**	isopropylcarbamoyl 	cyclopropanecarboxyl 	422	> 98 (66)
14g	carbamoyl	**4b**	isobutyl	cyclopropanecarboxyl	325	> 98 (71)
14h	dansyl 	**4b**	isopropylcarbamoyl 	cyclopropanecarboxyl	544	94 (68)
14i	3-aminobenzoyl	**4b**	2-pyrazinecarboxyl	cyclopropanecarboxyl	451	>98 (89)
14j	3-aminobenzoyl	**4b**	2-pyrazinecarboxyl	thymine-1-acetyl	549	94 (95)
14k	carbamoyl	**4c**	isobutyl	cyclopropanecarboxyl	325	> 98 (43)
14l	3-aminobenzoyl	**4c**	isobutyl	cyclopropanecarboxyl	401	> 98 (60)
14m	isonipecotyl	**4c**	isopropylcarbamoyl	3-hydroxybenzyl	460	85 (83)
14n	dansyl	**4c**	2-pyrazinecarboxyl	cyclopropanecarboxyl	565	> 80 (67)
14o	dansyl	**4c**	isobutyl	cyclopropanecarboxyl	515	> 98 (35)
14p	3-aminobenzoyl	**7**	2-pyrazinecarboxyl	3-hydroxybenzyl	461	96 (91)
14q	isonipecotyl	**7**	isopropylcarbamoyl	thymine-1-acetyl	492	96 (69)
14r	dansyl	**7**	2-pyrazinecarboxyl	thymine-1-acetyl	635	96 (72)
14s	carbamoyl	**7**	2-pyrazinecarboxyl	cyclopropanecarboxyl	347	> 98 (66)
14t	carbamoyl	**7**	2-pyrazinecarboxyl	3-hydroxybenzyl	385	> 98 (95)
14u[c]	*p*-toluenesulfonyl	**7**	2-pyrazinecarboxyl	3-hydroxybenzyl	496	>95 (75)
14v[c]	*p*-toluenesulfonyl	**4a**	2-pyrazinecarboxyl	3-hydroxybenzyl	524	>95 (77)
14w[c]	*p*-toluenesulfonyl	**4b**	2-pyrazinecarboxyl	3-hydroxybenzyl	524	>95 (73)
14x[c]	*p*-toluenesulfonyl	**7**	isobutyl	3-hydroxybenzyl	446	>95 (80)
14y[c]	*p*-toluenesulfonyl	**4b**	isobutyl	3-hydroxybenzyl	474	>95 (66)

[a] **4a** = *N*-Teoc-4-hydroxymethylpiperidine, **4b** = *N*-Teoc-3-hydroxymethylpiperidine, **4c** = *N*-Teoc-2-hydroxymethylpiperidine, **7** = *N*-Teoc-3-hydroxypyrrolidine. [b] Purity determined by RP-HPLC using an evaporative light-scattering detector. Yields are derived from the average resin loading of MicroKans, using a resin-solvent slurry. Samples were dried to constant weight under high vacuum, over NaOH. [c] Synthesis of library members was carried out separately on a 100 mmol scale. In a purification step, samples were passed through short silica gel columns.

References and Notes

(1) (a) Griffey, R. H.; Hofstadler, S. A.; Sannes-Lowery, K. A.; Ecker, D. J.; Crooke, S. T. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 10129−10133. (b) Michael, K.; Tor, Y. *Chem. Eur. J.* **1998**, *4*, 2091−2098. (c) Afshar, M.; Prescott, C. D.; Varani, G. *Curr. Opin. Biotechnol*. **1999**, *10*, 59−63. (d) Wilson, W. D.; Li, K. *Curr. Med. Chem.* **2000**, *7*, 73−98.

(2) For reviews, see: (a) Lam, K. S.; Lebl, M.; Krchňák, V. *Chem. Rev.* **1997**, *97*, 411. (b) Armstrong, R. W.; Combs, A. P.; Tempest, P. A.; Brown, S. D.; Keating, T. A. *Acc. Chem. Res.* **1996**, *29*, 123. (c) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555. (d) Terrett, N. K.; Gardner, M.; Gordon, D. W.; Kobylecki, R. J.; Steele, J. *Tetrahedron* **1995**, *51*, 8135. (e) Gallop, M. A.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gordon, E. M. *J. Med. Chem.* **1994**, *37*, 1233. (f) Gordon, E. M.; Barrett, R. W.; Dower, W. J.; Fodor, S. P. A.; Gallop, M. A. *J. Med. Chem.* **1994**, *37*, 1385. (g) Desai, M. C.; Zuckermann, R. N.; Moos, W. H. *Drug Dev. Res.* **1994**, *33*, 174.

(3) Disubstituted piperidines and pyrrolidines are frequently found as key structural elements in compounds possessing biological activity. (a) Broekkamp, C. L. E.; Leysen, D.; Peeters, B. W. M. M.; Pinder, R. M. *J. Med. Chem.* **1995**, *38*, 4615−4633. (b) Klein, S.; Molino, B. F.; Czekaf, M.; Dener, J. S.; Leadley, R. J.; Sabatino, R.; Dunwiddie, C. T.; Chu, V. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1403−1408. (c) Shilvock, J. P.; Wheatley, J. R.; Davis, B.; Nash, R. J.; Griffiths, R. C.; Jones, M. G.; Mueller, M.; Crook, S.; Watkin, D. J.; Smith, C. *Tetrahedron Lett.* **1996**, *37*, 8569−8572. (d) Byrgesen, E.; Nielson, J. *Tetrahedron Lett.* **1997**, *38*, 5697−5700. (e) Hoekstra, W. J.; Maryanoff, B. E.; Andrade-Gordon, P.; Cohen, J. H.; Costanzo, M. J.; Damiano, B. P. Haertlein, B. J.; Harris, B. D.; Kauffman, J. A.; Keane, P. M. McComsey, D. F.; Villani, F. J., Jr.; Yabut, S. C. *Bioorg. Med. Chem. Lett.* **1996**, *6*, 2371−2376.

(4) This work was presented in part at the 216th National Meeting of the American Chemical Society, Boston, August 23−27, 1998. *Abstracts of Papers*; American Chemical Society: Washington, DC, 1998; ORGN-227.

(5) (a) Fukuyama, T.; Jow, C.-K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373−6374. For examples of solid-phase Fukuyama-Mitsunobu reactions, see: (b) Swayze, E. E. *Tetrahedron Lett*. **1997**, *38*, 8643−8646. (c) Dankwardt, S. M.; Smith, D. B.; Porco, J. A.; Nguyen, C. H. *Synlett* **1997**, *7*, 854−856. (d) Yang, L.; Chiu, K. *Tetrahedron Lett*. **1997**, *38*, 7307−7310.

(6) For the preparation of 2-nitrobenzenesulfonyl-protected ethylenediamine, see: Kirsanov, A. V.; Kirsanova, N. A. *J. Gen. Chem. USSR (Engl. Transl.)* **1962**, *32*, 877−882.

(7) For the general synthesis of Teoc-protected alcohols, see: Shute, R. E.; Rich, D. H. *Synthesis* **1987**, 346−349.

(8) For reductive aminations using borane-pyridine complex, see: (a) Swayze, E. E. *Tetrahedron Lett.* **1997**, *38*, 8465−8468. (b) Kung, P.-P.; Swayze, E. E. *Tetrahedron Lett.* **1999**, *40*, 5651−5654. (c) Khan, N. M.; Arumugam, V.; Balasubramanian, S. *Tetrahedron Lett*. **1996**, *37*, 4819−4822. (d) Bomann, M. D.; Guch, I. C.; DiMare, M. *J. Org. Chem.* **1995**, *60*, 5995−5996.

(9) Argonaut Technologies, 887 Industrial Rd., Suite G, San Carlos, CA 94070.

(10) Carpino, L. A.; Elfaham, A.; Albericio, F. *Tetrahedron Lett.* **1994**, *35*, 2279.

(11) Xiao, X.-Y.; Li, R.; Zhuang, H.; Ewing, B.; Karunaratne, K.; Lillig, J.; Brown, R.; Nicolaou, K. C. *Biotechnol. Bioeng.* **2000**, *71* (1), 44−50.

(12) Lee, M. S.; Kerns, E. H. *Mass Spectrom. Rev.* **1999**, *18*, 187−279.

(13) Synthesis of a larger library was outsourced to ProtoGene Laboratories, 1454 Page Mill Rd., Palo Alto, CA 94304.

(14) Brennan, T.; Biddison, G.; Frauendorf, A.; Schwarcz, L.; Keen, B.; Ecker, D. J.; Davis, P. W.; Tinder, R.; Swayze, E. E. *Biotechnol. Bioeng*. **1998**, *61*, 33−45.

(15) Jefferson, E. A.; Swayze, E. E. *Tetrahedron Lett.* **1999**, *40*, 7757−7760.

CC000027E