

This article was downloaded by:

On: 24 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597273>

CHIRAL SEPARATION OF SEVERAL THIOBARBITURATES ON A CELLULOSE TRIS(4-METHYLBENZOATE) PHASE UNDER REVERSED-PHASE CONDITIONS

Hassan Y. Aboul-Enein^a; Martin G. Schmid^b; Claudia Tuscher^b; Gerald Gübitz^b; Maria Laffranchini^b; Jacek Bojarski^c

^a Pharmaceutical Analysis Laboratory, Biological and Medical Research Department (MBC-03), King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia ^b Institute of Pharmaceutical Chemistry, Karl-Franzens University of Graz, Graz, Austria ^c Department of Organic Chemistry, College of Medicine, Jagiellonian University, Krakow, Poland

Online publication date: 22 January 2001

To cite this Article Aboul-Enein, Hassan Y. , Schmid, Martin G. , Tuscher, Claudia , Gübitz, Gerald , Laffranchini, Maria and Bojarski, Jacek(2001) 'CHIRAL SEPARATION OF SEVERAL THIOBARBITURATES ON A CELLULOSE TRIS(4-METHYLBENZOATE) PHASE UNDER REVERSED-PHASE CONDITIONS', *Journal of Liquid Chromatography & Related Technologies*, 24: 1, 69 – 77

To link to this Article: DOI: 10.1081/JLC-100000327

URL: <http://dx.doi.org/10.1081/JLC-100000327>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

CHIRAL SEPARATION OF SEVERAL THIOBARBITURATES ON A CELLULOSE TRIS(4-METHYLBENZOATE) PHASE UNDER REVERSED-PHASE CONDITIONS

Hassan Y. Aboul-Enein,^{1,*} Martin G. Schmid,² Claudia
Tuscher,² Gerald Gübitz,² Maria Laffranchini,²
and Jacek Bojarski³

¹Pharmaceutical Analysis Laboratory, Biological and
Medical Research Department (MBC-03), King Faisal
Specialist Hospital and Research Centre,
Riyadh 11211, Saudi Arabia

²Institute of Pharmaceutical Chemistry, Karl-Franzens
University of Graz, A-8010 Graz, Austria

³Department of Organic Chemistry, College of Medicine,
Jagiellonian University, 30-688 Krakow, Poland

ABSTRACT

The chiral separation of several thiobarbiturates on a tris(4-methylbenzoate) phase (Chiralcel OJ-R, 15 × 0.46 cm and Chiralcel OJ-R 15 × 0.20 cm) under reversed-phase conditions is described. The separation properties of a normal sized and a newly developed narrow-bore column were compared. The influence of buffer and organic modifiers on enantioselectivity was investigated. The three mobile phases used were water/acetonitrile (70:30, v/v), water/acetonitrile/methanol (50:25:25, v/v), and water/acetonitrile/

*Corresponding author.

methanol (55:15:30, v/v). Of seven thiobarbiturates investigated, five have been resolved. The values of α (1.11–1.85) and R_s (0.71–5.75) have been reported.

INTRODUCTION

Thiobarbiturates are the drugs commonly used as sedative, hypnotic, and anticonvulsant agents (1). There are differences in the pharmacological activities of the thiobarbiturates enantiomers (1). The quantitative differences in the activities of the enantiomers of thiopental (2,3) and thiohexital have been reported (4). The chirality of these compounds is based on the presence of an asymmetric C-atom at position 5 of the pyrimidine ring or/and in the side chain situated at C-5 (Fig. 1).

Various chiral stationary phases, i.e., cyclodextrins, proteins, and polysaccharides, have been used for the enantiomeric resolution of barbiturates (1). However, the cellulose-based phases were found to exhibit good enantioselectivity for barbiturates. The racemic barbiturates were resolved on microcrystalline cellulose triacetate (5–9) and on cellulose tris(4-benzoate) (Chiracel OJ) (10,11). It was also shown that enantiomeric resolution of barbiturates is possible under reversed-phase conditions using a Chiracel OJ-R column (12,13).

Compared to normal-phase OJ columns, handling of the Chiracel OJ-R columns is more convenient with regard to the choice of a variety of mobile phases. This study deals with the enantiomeric separation of thiobarbiturates under reversed-phase conditions, comparing a 15×0.46 cm Chiracel OJ-R column with a newly developed 15×0.2 cm narrow-bore column of the same type.

EXPERIMENTAL

Chemicals and Materials

All reagents were of analytical grade. Methanol and acetonitrile (gradient grade) were obtained from Merck (Darmstadt, Germany). Compounds 1–3 were from Abbott (North Chicago, IL, USA). Compound 4 was obtained by treatment of diethyl ethylmalonate with *N*-ethylthiourea, and compounds 5–7 were obtained by methylation of 2-thiophenobarbital (4).

High Performance Liquid Chromatography (HPLC) Conditions

HPLC was performed using a HP 1090 system (Hewlett-Packard, Palo Alto, CA, USA) equipped with a diode array ultraviolet detector. The samples were



CHIRAL SEPARATION OF THIOBARBITURATES

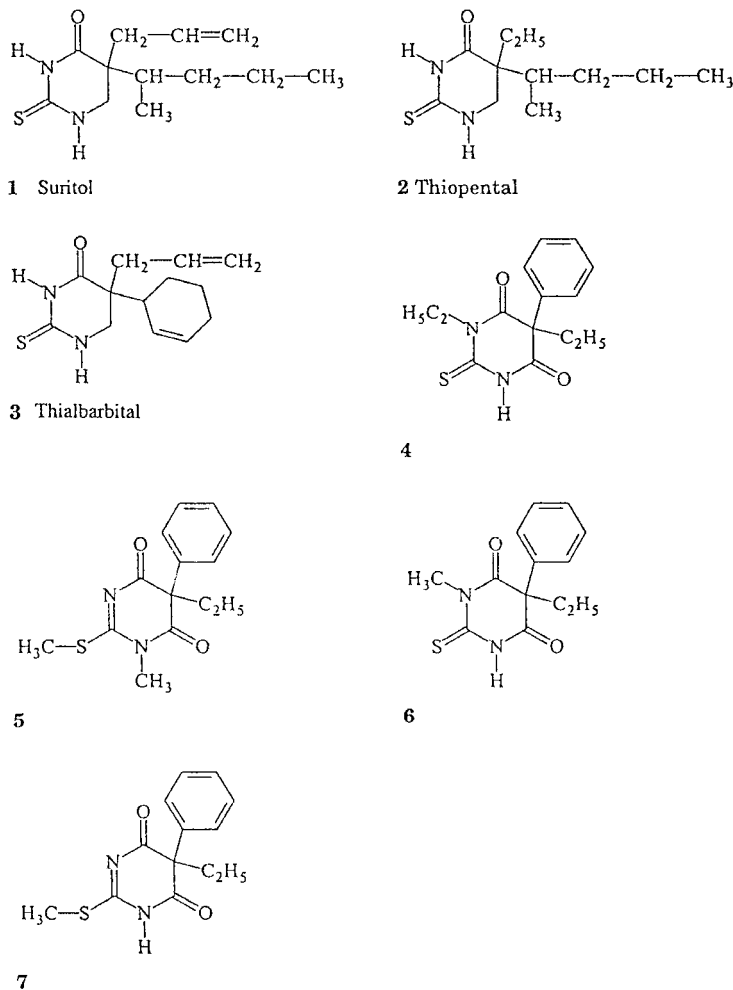


Figure 1. Structures of the compounds investigated.

injected by an autosampler, and the amount injected was 8 μ L. The detection was performed at 235 nm. The OJ-R 15 \times 0.2 cm and OJ-R 15 \times 0.46 cm chiral stationary-phase columns were obtained from Daicel Co. (Tokyo, Japan).

The mobile phases used were different mixtures of methanol, acetonitrile, and water. The flow rate of the mobile phases were 0.2 and 0.5 mL/min for the OJ-R 15 \times 0.2 cm and OJ-R 15 \times 0.46 cm columns, respectively. All separations were carried out at room temperature.



RESULTS AND DISCUSSION

The chromatographic parameters, capacity factor (k'), separation factor (α), and resolution factor (R_s) for the resolved enantiomers of thiobarbiturates under the reversed-phases mode are presented in Table 1. The typical chromatograms of some of the resolved enantiomers are given in Figures 2a–2e. The comparative studies on the chiral separation of thiobarbiturates were carried out using 15×0.46 cm and a 15×0.2 cm narrow-bore Chiracel OJ-R columns. Both columns exhibited comparable enantioselectivity; however, the narrow-bore column showed several advantages, as the separations were faster with significantly sharper peak shapes and with less solvent consumption.

While the normal-sized column was operated at 0.5 mL/min, a flow rate of 0.2 mL/min was chosen for the narrow-bore column to reducing backpressure and increase the lifetime of the column. However, since the pressure limit recommended by the supplier was not reached at this flow rate, a slight increase in flow rate would be still possible.

Table 1. Separation Data for Thiobarbiturates on a OJ-R 15×0.2 cm Column (Flow = 0.2 mL/min) and a OJ-R 15×0.46 cm Column (Flow = 0.5 mL/min)

Compounds	OJ-R (15×0.2 cm)				OJ-R (15×0.46 cm)			
	k'_1	k'_2	α	R_s	k'_1	k'_2	α	R_s
Mobile phase: water/acetonitrile (70:30, v/v)								
1	8.27	9.40	1.14	0.96	9.76	10.97	1.21	0.99
2	7.55	8.48	1.12	0.77	8.51	9.48	1.11	0.97
3	8.93	11.04	1.24	1.41	10.04	12.37	1.23	1.95
4	n.e. ^a				n.e.			
5	7.84	13.12	1.67	4.06	9.05	14.44	1.60	4.76
6	n.e.				n.e.			
7	6.55	—	1.00	0.00	14.56	—	1.00	0.00
Mobile phase: water/acetonitrile/methanol (50:25:25, v/v)								
1	3.63	4.13	1.14	0.71	3.72	4.22	1.13	0.90
2	3.68	4.24	1.15	0.74	3.93	4.51	1.15	0.90
3	4.39	5.68	1.29	1.22	4.38	5.65	1.29	1.67
4	23.08	42.21	1.83	5.75	n.e.			
5	4.43	8.18	1.85	3.10	4.70	8.68	1.85	4.01
6	44.33	—	1.00	0.00	n.e.			
7	4.78	—	1.00	0.00	10.11	—	1.00	0.00
Mobile phase: water/acetonitrile/methanol (55:15:30, v/v)								
2	1.46	2.00	1.37	0.98				

^an.e., not eluted within 120 min.



CHIRAL SEPARATION OF THIOBARBITURATES

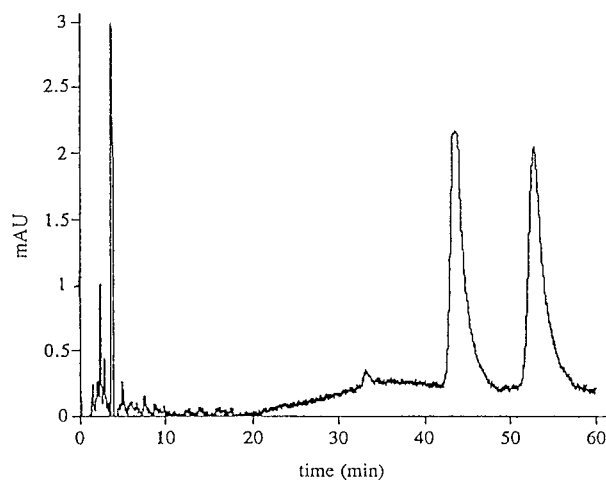


Figure 2a. Separation of compound 3 on the 15 × 0.46 cm Chiracel OJ-R column. Mobile phase: water/acetonitrile (70:30, v/v) with flow rate 0.5 mL/min.

Different mobile phase compositions, such as water or buffer/acetonitrile mixtures, and ternary mixtures consisting of water or buffer, acetonitrile, and methanol were investigated. Extremely high retention times were observed with mobile phases containing only 20% acetonitrile. As is typical for reversed phases,

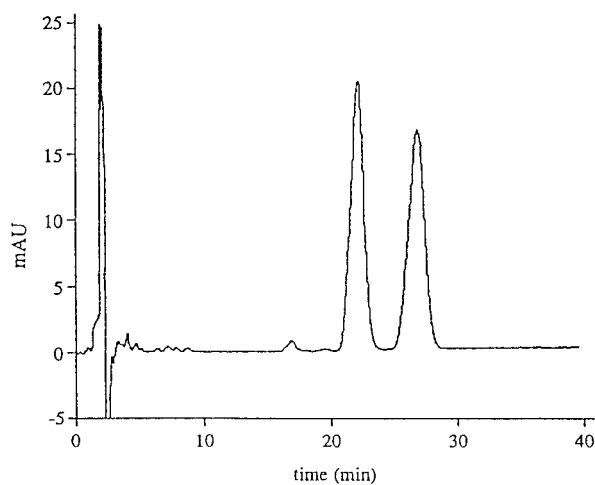


Figure 2b. Separation of compound 3 on the 15 × 0.20 cm Chiracel OJ-R column. Mobile phase: water/acetonitrile (70:30, v/v) with flow rate 0.2 mL/min.



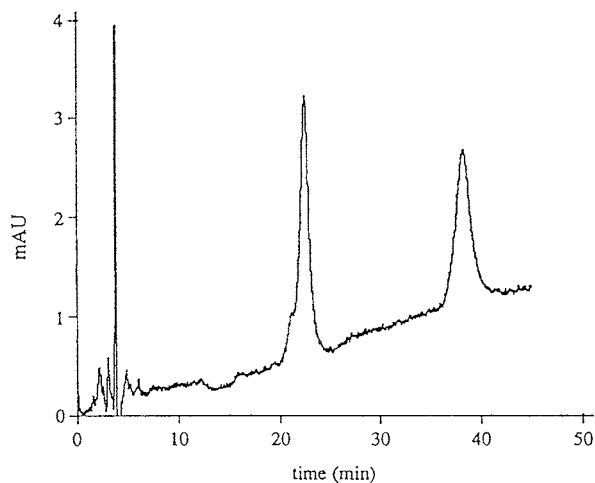


Figure 2c. Separation of compound 5 on the 15×0.46 cm Chiracel OJ-R column. Mobile phase: water/acetonitrile/methanol (50:25:25, v/v) with flow rate 0.50 mL/min.

a decrease in retention time was observed with increasing acetonitrile content. Therefore, the acetonitrile content was systematically varied. Figure 3 shows the influence of acetonitrile percentage on resolution of thiobarbiturates. It is clear from Figure 3 that any slight increase in the acetonitrile content resulted in the decrease

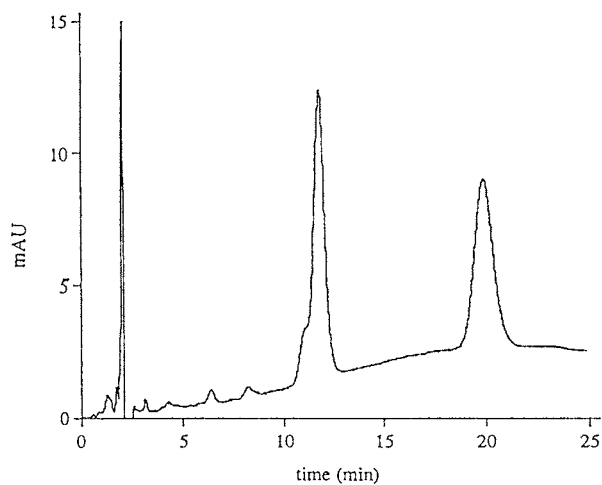


Figure 2d. Separation of compound 5 on the 15×0.20 cm Chiracel OJ-R column. Mobile phase: water/acetonitrile/methanol (50:25:25, v/v) with flow rate 0.20 mL/min.



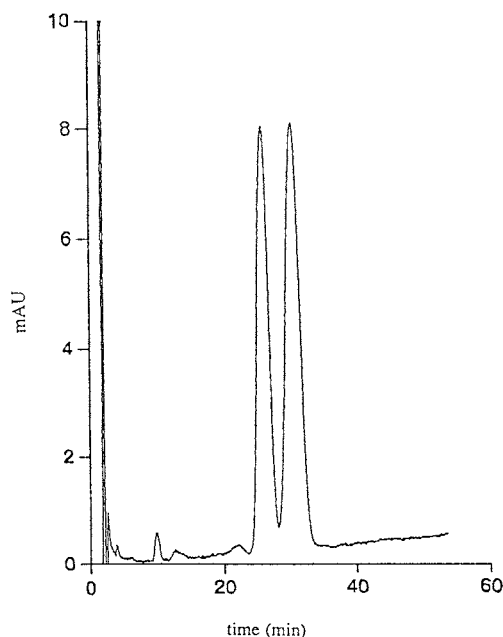


Figure 2e. Separation of compound 2 on the 15 × 0.20 cm Chiracel OJ-R column. Mobile phase: water/acetonitrile/methanol (55:15:30, v/v) with flow rate 0.20 mL/min.

of resolution factor. The substitution of water by a perchlorate buffer resulted in a slight increase in retention, coupled with more pronounced band broadening. Ishikawa and Shibata (15) reported an increase in retention and improvement of resolution of acidic compounds with increasing pH.

The influence of pH was investigated over a range of 7 to 2 using a perchlorate buffer; however, no significant change in retention and resolution was observed. This might be due to the fact that the thiobarbiturates are rather weak acids (pK_a about 8–9), except compound 5 which is neutral. Therefore, in further experiments, no buffer was used. Ternary mobile phases containing water/methanol/acetonitrile showed the best results.

Table 1 shows that compound 4 possesses an extremely high retention time. By changing to water/acetonitrile/methanol (40:40:20) as a mobile phase, baseline resolution was still obtained with a reasonable separation time. Surprisingly, compound 6, which differs from 4 only in the substituent on the nitrogen, was not resolved, despite the fact it was also strongly retained. Compound 7 was rather weakly retained and was not resolved in any of the mobile phases investigated. This may be due to the poor hydrogen bonding at the sulfur atom that is affected by the steric effect of the methyl group attached to it.



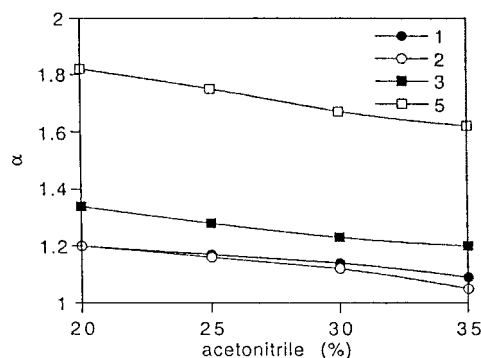


Figure 3. Influence of acetonitrile content on selectivity. Column: Chiracel OJ-R 15 \times 0.2 cm (flow = 0.2 mL/min); mobile phase: water with different amounts of acetonitrile.

The same is the case with compound 5. The baseline resolution for compound 2 can be achieved by using water/acetonitrile/methanol (55:15:30) as a mobile phase.

The chiral recognition mechanism on cellulose-based phases involves: a) the inclusion of aromatic moieties or other bulky substituents of the analytes into cavities of the chiral selectors; and b) attractive interactions such as dipole-dipole, π - π , and hydrogen bonding (15–19). In the case of thiobarbiturates, hydrogen bonding and dipole-dipole stacking between the imide group of the analytes and the ester groups of the selector might be assumed to be the main interactions responsible for chiral recognition.

Since the compounds containing an aromatic moiety, such as compound 4, showed significantly higher retention and better resolution compared to compounds with aliphatic side chains, inclusion into the cavities paired with π - π interactions between the phenyl group on C-5 and the benzoyl group of the chiral selector might be taken into account in these particular cases. The same might apply also for compound 3, which contains an alicyclic ring, except with weaker π - π interactions, as reflected in a slight decrease in resolution. These facts are in good agreement with observations made for non-sulfur-containing barbiturates (12,13).

REFERENCES

1. Bojarski, J.; Chiral Barbiturates: Synthesis, Chromatographic Resolution and Biological Activity. In *The Impact of Stereochemistry on Drug Development and Use*; Aboul-Enein, H.Y.; Wainer, I.W., Eds.; Wiley & Sons: New York, 1997; Vol. 142.



CHIRAL SEPARATION OF THIOBARBITURATES

77

2. Mark, L.C.; Brand, L.; Parel J.M.; Carroll, F.I. *Excerpta Med. Int. Cong. Ser.* **1978**, 399, 144.
3. Haley, T.J.; Gidley, J.T. *Eur. J. Pharmacol.* **1976**, 36, 211.
4. Carroll, F.I.; Phillip, A.; Naylor, D.M.; Christensen, H.D.; Goad, W.C. *J. Med. Chem.* **1981**, 24, 1241.
5. Blaschke, G. *J. Liq. Chromatogr.* **1986**, 9, 341.
6. Rizzi, A.M. *J. Chromatogr.* **1989**, 71, 478.
7. Rizzi, A.M. *J. Chromatogr.* **1989**, 87, 478.
8. Rizzi, A.M. *J. Chromatogr.* **1998**, 101, 478.
9. Rizzi, A.M. *J. Chromatogr.* **1990**, 195, 513.
10. Aboul-Enein, H.Y.; Serignese, V.; Abou-Basha, L.I.; Bojarski, J. *Die Pharm.* **1996**, 51, 159.
11. Aboul-Enein, H.Y.; Serignese, V.; Bojarski, J. *J. Liq. Chromatogr.* **1993**, 16, 2741.
12. Aboul-Enein, H.Y.; Bakar, S.A. *Enantiomer* **1996**, 1, 223.
13. van Overbeke, A.; Baeyens, W.; Oda, H.; Aboul-Enein, H.Y. *Chromatographia* **1996**, 43, 599.
14. Kubaszek, M.; Paluchowska, M.; Chmiel, E.; Bojarski, J. *Pol. J. Chem.* **1994**, 68, 117.
15. Ishikawa A.; Shibata, T. *J. Liq. Chromatogr.* **1993**, 16, 859.
16. Francotte, E.; Romain, M.; Lohmann, D.; Müller, R. *J. Chromatogr.* **1985**, 347, 25.
17. Wainer, I.W.; Alembik, M.C. *J. Chromatogr.* **1986**, 385, 358.
18. Wainer, I.W.; Stiffin, R.M.; Shibata, T. *J. Chromatogr.* **1987**, 411, 139.
19. Okamoto, Y.; Yashima, E. *Angew. Chem. Int. Ed.* **1998**, 37, 1020.

Received July 11, 2000

Author's Revisions August 3, 2000

Accepted July 30, 2000

Manuscript 5343



Request Permission or Order Reprints Instantly!

Interested in copying and sharing this article? In most cases, U.S. Copyright Law requires that you get permission from the article's rightsholder before using copyrighted content.

All information and materials found in this article, including but not limited to text, trademarks, patents, logos, graphics and images (the "Materials"), are the copyrighted works and other forms of intellectual property of Marcel Dekker, Inc., or its licensors. All rights not expressly granted are reserved.

Get permission to lawfully reproduce and distribute the Materials or order reprints quickly and painlessly. Simply click on the "Request Permission/Reprints Here" link below and follow the instructions. Visit the [U.S. Copyright Office](#) for information on Fair Use limitations of U.S. copyright law. Please refer to The Association of American Publishers' (AAP) website for guidelines on [Fair Use in the Classroom](#).

The Materials are for your personal use only and cannot be reformatted, reposted, resold or distributed by electronic means or otherwise without permission from Marcel Dekker, Inc. Marcel Dekker, Inc. grants you the limited right to display the Materials only on your personal computer or personal wireless device, and to copy and download single copies of such Materials provided that any copyright, trademark or other notice appearing on such Materials is also retained by, displayed, copied or downloaded as part of the Materials and is not removed or obscured, and provided you do not edit, modify, alter or enhance the Materials. Please refer to our [Website User Agreement](#) for more details.

[Order now!](#)

Reprints of this article can also be ordered at

<http://www.dekker.com/servlet/product/DOI/101081JLC100000327>