incubation, the fraction with $t_R = 10.0$ min yielded, after lyophilization, **17a,b** · HCOOH (unlabeled, 36.5 mg, 0.089 mmol, 4.5 %), LC–ESI-MS (gradient: % MeOH (t [min]) 5(0)-95(30–35)-5(40–45), cone voltage 40 V). **17a**: $t_R = 8.3$ min; m/z (%): 401 (3) $[M\mathrm{K}]^+$, 385 (4) $[M\mathrm{Na}]^+$, 363 (100) $[M\mathrm{H}]^+$, 219 (8), 187 (17); **17b**: $t_R = 8.5$ min; m/z (%): 401 (4) $[M\mathrm{K}]^+$, 385 (6) $[M\mathrm{Na}]^+$, 363 (100) $[M\mathrm{H}]^+$, 219 (12), 187 (14); accurate mass (mean of 11 measurements ± standard deviation): **17a,b**: calculated for $C_{18}H_{27}N_4O_4$: 363.2032, found: m/z 363.2034 ± 0.0007 $[M\mathrm{H}]^+$. Preparation (scaled down to one seventh) was repeated with [1-^{13}C]D-glucose, and the products were isolated analogously. For the D-arabinose incubation, fractions with $t_R = 10.5$ and 11.3 min yielded, after lyophilization, **11** · HCOOH (12.3 mg, 0.033 mmol, 1.6 %) and **12** · HCOOH (4.8 mg, 0.013 mmol, 0.6 %), respectively, LC–ESI-MS (for gradient and cone voltage, see above). **11**: $t_R = 7.5$ min; m/z (%): 371 (1) $[M\mathrm{K}]^+$, 355 (2) $[M\mathrm{Na}]^+$, 333 (45) $[M\mathrm{H}]^+$, 175 (100), 159 (30); **12**: $t_R = 8.3$ min; m/z (%): 371 (1) $[M\mathrm{K}]^+$, 355 (1) $[M\mathrm{Na}]^+$, 333 (100) $[M\mathrm{H}]^+$, 315 (5), 189 (5), 187 (4); accurate mass (mean of 10 measurements ± standard deviation): **11**:, calculated for $C_{17}H_{25}N_4O_3$: 333.1927, found: m/z 333.1931 ± 0.0007 $[M\mathrm{H}]^+$; **12**: calculated for $C_{17}H_{25}N_4O_3$: 333.1927, found: m/z 333.1935 ± 0.0010 $[M\mathrm{H}]^+$.

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[1] F. Ledl, E. Schleicher, *Angew. Chem.* **1990**, *102*, 597–626; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 565–594.

[2] K. M. Biemel, O. Reihl, J. Conrad, M. O. Lederer, *J. Biol. Chem.* **2001**, *276*, 23405–23412.

[3] a) D. B. Shin, F. Hayase, H. Kato, *Agric. Biol. Chem.* **1988**, *52*, 1451–1458; b) M. A. Glomb, C. Pfahler, *Carbohydr. Res.* **2000**, *329*, 515–523; c) S. Vasan, X. Zhang, A. Kapurniotu, J. Bernhagen, S. Teichberg, J. Basgen, D. Wagle, D. Shih, I. Terlecky, R. Bucala, A. Cerami, J. Egan, P. Ulrich, *Nature* **1996**, *382*, 275–278; d) R. H. Nagaraj, I. N. Shipanova, F. M. Faust, *J. Biol. Chem.* **1996**, *271*, 19338–19345; e) M. O. Lederer, R. G. Klaiber, *Bioorg. Med. Chem.* **1999**, *7*, 2499–2507; f) H. Odani, T. Shinzato, J. Usami, Y. Matsumoto, E. Brinkmann-Frye, J. W. Baynes, K. Maeda, *FEBS Lett.* **1998**, *427*, 381–385.

[4] V. M. Monnier, R. R. Kohn, A. Cerami, *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 583–587.

[5] a) A. M. Schmidt, O. Hori, J. Brett, S. D. Yan, J. L. Wautier, D. Stern, *Arterioscler. Thromb.* **1994**, *14*, 1521–1528; b) T. Kislinger, C. F. Fu, B. Huber, W. Qu, A. Taguchi, S. D. Yan, M. Hofmann, S. F. Yan, M. Pischetsrieder, D. Stern, A. M. Schmidt, *J. Biol. Chem.* **1999**, *274*, 31740–31749; c) A. M. Schmidt, S. D. Yan, S. F. Yan, D. M. Stern, *Biochim. Biophys. Acta* **2000**, *1498*, 99–111.

[6] J. W. Baynes, S. R. Thorpe, *Diabetes* **1999**, *48*, 1–9.

[7] C. A. L. S. Colaco, *The Glycation Hypothesis of Atherosclerosis*, Springer, Heidelberg, **1997**.

[8] a) S. D. Yan, X. Chen, J. Fu, M. Chen, H. Zhu, A. Roher, T. Slattery, L. Zhao, M. Nagashima, J. Morser, A. Migheli, P. Nawroth, D. Stern, A. M. Schmidt, *Nature* **1996**, *382*, 685–691; b) A. Takeda, T. Yasuda, T. Miyata, Y. Goto, M. Wakai, M. Watanabe, Y. Yasuda, K. Horie, T. Inagaki, M. Doyu, K. Maeda, G. Sobue, *Acta Neuropathol.* **1998**, *95*, 555–558.

[9] See Supporting Information for: a) ^{1}H and ^{13}C NMR data for **7a–d**, (Table 1); b) reasoning for the number of observable diastereoisomers for **7** and assignment of their relative configuration (Tables 1 and 2 and Figure 1); c) ^{1}H and ^{13}C NMR data for **17a,b** (Table 3); d) LC chromatograms (Figure 2); e) ^{1}H and ^{13}C NMR data for **11** and **12** (Table 3).

[10] M. O. Lederer, H. P. Bühler, *Bioorg. Med. Chem.* **1999**, *7*, 1081–1088.

[11] B. Huber, F. Ledl, *Carbohydr. Res.* **1990**, *204*, 215–220.

[12] M. S. Feather, T. G. Flynn, K. A. Munro, T. J. Kubiseski, D. J. Walton, *Biochim. Biophys. Acta* **1995**, *1244*, 10–16.

A Method for Thermal Generation of Aryloxyl Radicals at Ambient Temperatures: Application to Low-Density Lipoprotein (LDL) Oxidation**

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Antioxidant phenols (ArOH) react with peroxyl radicals (ROO•) and form relatively unreactive aryloxyl radicals (ArO•) [Eq. (1)] which, in homogeneous solutions, then trap a second peroxyl [Eq. (2)].[1, 2] α-Tocopherol (TocH, vitamin E) is the most active lipid-soluble antioxidant in

$$\mathrm{ArOH} + \mathrm{ROO}^{\bullet} \longrightarrow \mathrm{ArO}^{\bullet} + \mathrm{ROOH} \quad (1)$$

$$\mathrm{ArO}^{\bullet} + \mathrm{ROO}^{\bullet} \longrightarrow \text{nonradical products} \quad (2)$$

mammals[2] but, surprisingly, it acts as a prooxidant in human low-density lipoproteins (LDL).[3] Oxidatively modified LDL may initiate atherosclerosis.[4] Various agents (e.g., enzymes, transition metals) have been suggested to be responsible for this modification of LDL in vivo.[5] The free radical initiated oxidation of LDL in which TocH transfers radical character from water-soluble peroxyls into the LDL has been studied extensively. The resulting tocopheroxyl radical (Toc•) then carries a lipid peroxidation chain within the LDL in a process christened tocopherol-mediated peroxidation (TMP).[6] The aryloxyl radical, tyrosyl, which is formed (in 25 % yield) by reaction of myeloperoxidase with tyrosine during the immune response, can also initiate LDL peroxidation.[7] These two examples of aryloxyl radical-induced biological damage highlight the need for quantitative, in vitro studies of their reactions using thermolabile compounds which would provide "clean" and well-defined ArO• fluxes. To design an aryloxyl radical thermal source (ARTS) which would generate *any* ArO• and *only* that ArO• radical is therefore a worthwhile and exciting challenge.[8]

Hyponitrites, which are not subject to metal ion- or radical-induced decomposition,[9, 10] decompose at ambient temperatures to give N_2 and alkoxyl radicals. It appeared probable that aryloxyalkoxyl radicals would undergo very fast β-scission[11] to yield aryloxyl radicals [Eq. (3)]. A synthetic route to aryloxyalkyl hyponitrites suitable for many different

$$[\mathrm{ArOCR_2ON{=}}]_2 \xrightarrow[-N_2]{\Delta} \mathrm{ArOCR_2O}^{\bullet} \xrightarrow{\beta\text{-scission}} \mathrm{ArO}^{\bullet} + \mathrm{R_2C{=}O} \quad (3)$$

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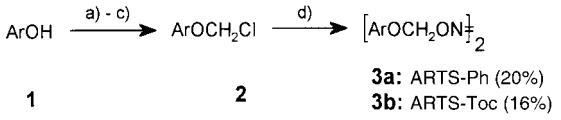
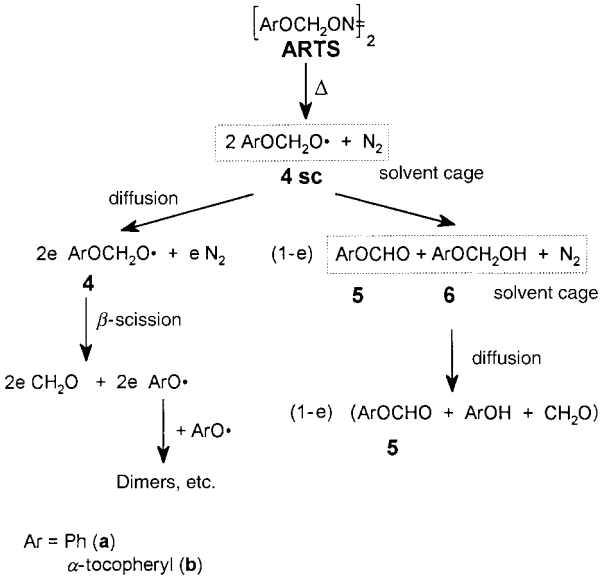
phenols was designed (Scheme 1)[9, 12] and used to prepare **3a** (Ar = Ph; **ARTS-Ph**) as a potential phenoxyl radical source and **3b** (Ar = tocopheryl; **ARTS-Toc**) as a potential Toc• source.[13] Decomposition rates of **3a** and **3b** were measured by ^{1}H NMR spectroscopy and found to be almost identical,



Scheme 1. Synthesis of **ARTS**s. a) NaH in DMF, 0 °C, 30 min, $ClCH_2SCH_3$, 20 °C, 6 h; b) *m*-$ClC_6H_4CO_3H$ in CH_2Cl_2, 0 °C, 10 min; c) CH_3COCl in CH_2Cl_2, 0 → 20 °C, 3 h; d) $Ag_2N_2O_2$, 0 → 20 °C, 1–5 h. For steps a–c see ref. [12] and for step d see ref. [9]. Physical data for new compounds are given in the Supporting Information.

for example, at 37 °C: 9.1×10^{-4} s^{-1} for **ARTS-Ph** (in CD_3CN) and 10×10^{-4} s^{-1} for **ARTS-Toc** (in $CDCl_3$). **ARTS-Ph** decomposed at the same rate in $CDCl_3$ and CD_3CN:D_2O (1:1) as in dry CD_3CN, that is, its decomposition is solvent independent. The Arrhenius parameters for **ARTS-Ph** decomposition (based on measurements at three temperatures between 23 and 37 °C) were: $E_A = 106$ kJ mol^{-1} and $\log(A/s^{-1}) = 14.8$.

The expected decomposition pathways for an **ARTS** are outlined in Scheme 2. The initial geminate pair of alkoxyls, **4sc**, will partition between diffusion from the solvent cage (efficiency *e*) and an in-cage disproportionation to **5** and **6**, with the latter probably decomposing to ArOH and formaldehyde. Under most circumstances the vast majority of free alkoxyls, **4**, would be expected to undergo β-scission to yield the desired aryloxyls, ArO•, with very little H-atom abstraction to form **6**. Consistent with this scheme, thermolysis of **3a** gave PhOH, **5a**, formaldehyde, biphenols, phenoxyphenols, and polyphenols and thermolysis of **3b** gave TocH, **5b**, formaldehyde, tocopheryl quinone, and various tocopherol dimers.[14] These products are consistent with the formation of



Scheme 2. Expected thermal decomposition pathways of **ARTS**.

at least some free ArO• from both **ARTS**, this was confirmed with **3b** by the direct detection of Toc• by ESR and UV/Vis spectroscopy (Figure 1). The maximum steady-state concentration of Toc• ($[Toc^•]_{mss}$) was solvent dependent, with 1.1 mM **3b** at 20 °C $[Toc^•]_{mss} \approx 5.5$ μM in chlorobenzene but was only

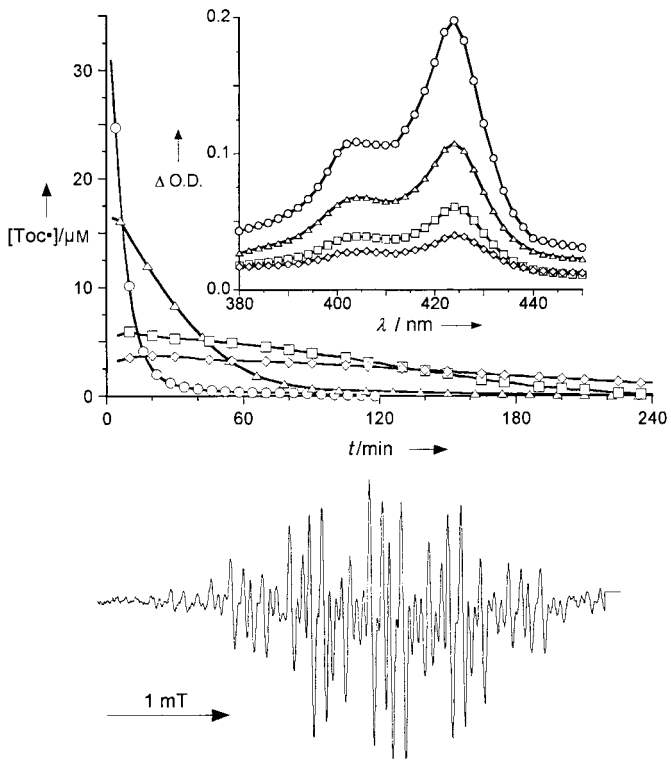


Figure 1. Thermal decomposition of **ARTS-Toc**. Top: Concentration of Toc• as determined by UV/Vis spectroscopy ($\lambda = 424$ nm)[14] during decomposition of 1 mM **ARTS-Toc** in chlorobenzene. Inset: UV/Vis spectra recorded at the maximum [Toc•]. Key: 20 °C (◇), 25 °C (□), 37 °C (△), and 50 °C (○). Bottom: ESR spectrum recorded during decomposition of **ARTS-Toc** (10 mM) at room temperature in benzene. Hyperfine splitting constants determined by simulation: $a(CH_3) = 0.644$ mT, $a(CH_3) = 0.492$ mT, $a(CH_3) = 0.104$ mT, $a(CH_2) = 0.162$ mT, $a(CH_2) = 0.016$ mT (linewidth: 0.015 mT).

half as large in cyclohexane and no Toc• could be detected in 1,4-cyclohexadiene. The rate constant for β-scission (k_β) in nonpolar solvents can now be estimated since the rate of β-scission in cyclohexane must be *roughly* equal to the rate of H-atom abstraction from the cyclohexane, $k_s \times [c\text{-}C_6H_{12}] = 1.2 \times 10^6$ M^{-1} s^{-1}[15] thus $k_s \times [c\text{-}C_6H_{12}] = 1.2 \times 10^6$ M^{-1} s^{-1}[15] $\times$ 9.3 M $= 1.1 \times 10^7$ s^{-1}. More precisely, we estimate $k_\beta \approx 1.8 \times 10^7$ s^{-1} in nonpolar solvents (see Supporting Information) and this reaction will be even faster in polar solvents.[11, 15] Indeed, $[Toc^•]_{mss}$ in cyclohexene containing 1 M methanol ($[Toc^•]_{mss} = 4$ μM) was twice as large as in neat cyclohexene. It is likely that all **4** would undergo β-scission in chlorobenzene. The minimum cage escape efficiency (*e*) in chlorobenzene at 20 °C can therefore be calculated to be 5% based on the **3b** decomposition rate constant (1.8×10^{-4} s^{-1}), $[Toc^•]_{mss}$, and the smallest reported rate constant for the Toc•/Toc• reaction ($2k = 1000$ M^{-1}s^{-1}).[14] The presence of a TocH dimer as a minor impurity causes a dramatic increase in the apparent rate constant of the Toc•/Toc• reaction[14] and since this dimer

may be formed during **3b** decomposition the true value of *e* is likely to be $>5\%$.

The phenoxyl radical could not be detected during the thermal decomposition of **3a** at 23 °C, presumably because the PhO•/PhO• reaction ($2k = 1-12\times10^{8}\,M^{-1}s^{-1}$)[16] is so much faster than the Toc•/Toc• reaction.[14] However, *e* for **3a**, could be estimated by assuming that the in-cage reaction of **4sc** would yield equal amounts of **5a** and PhOH whereas GC analysis showed an excess of PhOH and biphenols over **5a**. From the "excess" phenol, *e* was estimated to be around 20%.

The aryloxyl radical-initiated peroxidation of LDL was chosen to illustrate a biologically relevant in vitro application of **ARTS**. To a freshly prepared LDL dispersion **3a** was added and incubated at 37 °C until decomposion was virtually complete. The TocH consumption curve and the cholesteryl ester hydroperoxide (CEOOH) formation curve are characteristic of TMP in that peroxidation is faster while TocH is present than after the TocH is consumed[6] (Figure 2).

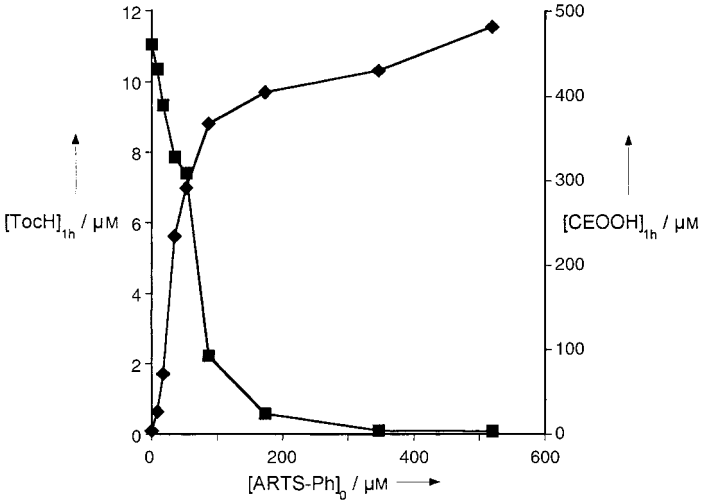


Figure 2. Concentration of cholesteryl ester hydroperoxides (CEOOH) (◆) and α-tocopherol (TocH) (■) in 1.8 μM LDL dispersed in aerated phosphate buffered saline (PBS; pH 7.4, 50 mM) after incubation for 1 h at 37 °C in the presence of the indicated initial **ARTS-Ph** concentrations. **ARTS-Ph** was added as a solution in DMSO, the amount of which did not exceed 1% of the 300 μL LDL dispersion.

Furthermore, at low initial **3a** concentrations where significant amounts of TocH remain after the 1 h incubation, the CEOOH was formed in a chain reaction, for example $d[CEOOH]/d[PhO^{\bullet}] \approx 16$ at an initial [**3a**] = 35 μM (see Supporting Information). That is, TMP is initiated in LDL by PhO• attack on TocH [Eq. (4)]. TMP explains the earlier

$$(PhO^{\bullet})_{aq} + (TocH)_{LDL} \longrightarrow (PhOH)_{aq} + (Toc^{\bullet})_{LDL} \quad (4)$$

observation that tyrosyl radicals generated by myeloperoxidase initiate LDL peroxidation in a process not inhibited by TocH.[7b]

At low **3a** concentration, approximately 0.2 molecules of TocH are consumed per PhO• generated (see Supporting Information). This value would be 0.5 if all the PhO• were destroyed by TocH and it implies that PhO•/PhO• coupling reactions are probably important under the present conditions.

Aryloxyl-radical induced oxidative stress has been largely ignored because of the lack of suitable precursors. The present synthesis of two **ARTS** overcomes this lack and provides a new tool for studying the effects of known fluxes of ArO• radicals on biologically relevant targets. Currently, we are designing a synthesis for water-soluble **ARTS**s which will enable the tyrosyl radical to be generated in a controlled manner.

Experimental Section

The Toc• EPR spectrum was recorded at room temperature under N_2 on a Varian E104 spectrometer (9.5 GHz) with microwave power = 2 mW, modulation amplitude = 0.04 mT, modulation frequence = 100 kHz, scan time = 8 min. The hyperfine splitting constants were obtained using the ESR simulation program WINSIM.[17] NMR spectroscopic data were recorded on a Bruker 400-DRX spectrometer. Freshly isolated LDL,[18] was dispersed in aerated PBS, mixed with a known amount of a **3a** stock solution in DMSO and then incubated for 1 h at 37 °C with the usual analyses for CEOOH and TocH.[19]

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[1] L. R. Mahoney, *Angew. Chem.* **1969**, *81*, 555–563; *Angew. Chem. Int. Ed. Engl.* **1969**, *8*, 547–555.
[2] G. W. Burton, K. U. Ingold, *Acc. Chem. Rev.* **1986**, *19*, 194–201.
[3] V. W. Bowry, K. U. Ingold, R. Stocker, *Biochem. J.* **1992**, *288*, 341–344.
[4] D. Steinberg, S. Parthasarathy, T. E. Carew, J. C. Khoo, J. L. Witzum, *N. Engl. J. Med.* **1989**, *87*, 915–924.
[5] J. W. Heinecke, *Curr. Opin. Lipidol.* **1997**, *8*, 268–274, and references therein.
[6] V. W. Bowry, R. Stocker, *J. Am. Chem. Soc.* **1993**, *115*, 6029–6044.
[7] a) J. W. Heinecke, *Atherosclerosis* **1998**, *141*, 1–15; b) M. I. Savenkova, D. M. Mueller, J. W. Heinecke, *J. Biol. Chem.* **1994**, *269*, 20394–20400; c) J. W. Heinecke, W. Li, H. L. Daehnke III, J. A. Goldstein, *J. Biol. Chem.* **1993**, *268*, 4069–4077.
[8] A few methods for thermal generation of ArO• are known but either they are limited to sterically hindered phenols (see for example: C. D. Cook, M. Fraser, *J. Org. Chem.* **1964**, *29*, 3716–3719) or a second, more reactive radical is formed simultaneously and stoichiometrically (see e.g. P. M. Lahti, D. A. Modarelli, F. C. Rossitto, A. L. Inceli, A. S. Ichimura, S. Ivatury, *J. Org. Chem.* **1996**, *61*, 1730–1738).
[9] C. A. Ogle, S. W. Martin, M. P. Dziobak, M. W. Urban, G. D. Mendenhall, *J. Org. Chem.* **1983**, *48*, 3728–3733.
[10] K. U. Ingold, T. Paul, M. J. Young, L. Doiron, *J. Am. Chem. Soc.* **1997**, *119*, 12364–12365; T. Paul, *Arch. Biochem. Biophys.* **2000**, *382*, 253–261.
[11] The thermochemically analogous β-scission of 2-phenylethoxyl has a rate constant in benzene of $2.3\times10^{7}\,s^{-1}$. See: G. D. Mendenhall, L. C. Stewart, J. C. Scaiano, *J. Am. Chem. Soc.* **1982**, *104*, 5109–5114.
[12] Ø. Antonsen, T. Benneche, K. Undheim, *Acta Chem. Scan. Ser. B* **1988**, *42*, 515–523.
[13] See Supporting Information for physical data of new compounds.
[14] V. W. Bowry, K. U. Ingold, *J. Org. Chem.* **1995**, *60*, 5456–5467, and references therein.
[15] D. V. Avila, C. E. Brown, K. U. Ingold, J. Lusztyk, *J. Am. Chem. Soc.* **1993**, *115*, 466–470.
[16] J. A. Howard, J. C. Scaiano in *Landolt-Börnstein*, *New Series Vol. 13d* (Ed.: H. Fischer), Springer, Berlin, **1984**, pp. 142–192.
[17] The WINSIM program was developed at the National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, NC, USA and can be downloaded at: epr.niehs.nih.gov.
[18] B. H. Chung, J. P. Segrest, M. J. Ray, J. D. Brunzell, J. E. Hokanson, R. M. Krauss, K. Baudrie, J. T. Cone, *Method Enzymol.* **1986**, *128*, 181–209.
[19] W. Sattler, D. Mohr, R. Stocker, *Method Enzymol.* **1994**, *233*, 469–489.

  1433-7851/02/4105-0806 $ 17.50+.50/0