Quantitative ^{1}H NMR: Development and Potential of an Analytical Method – an Update

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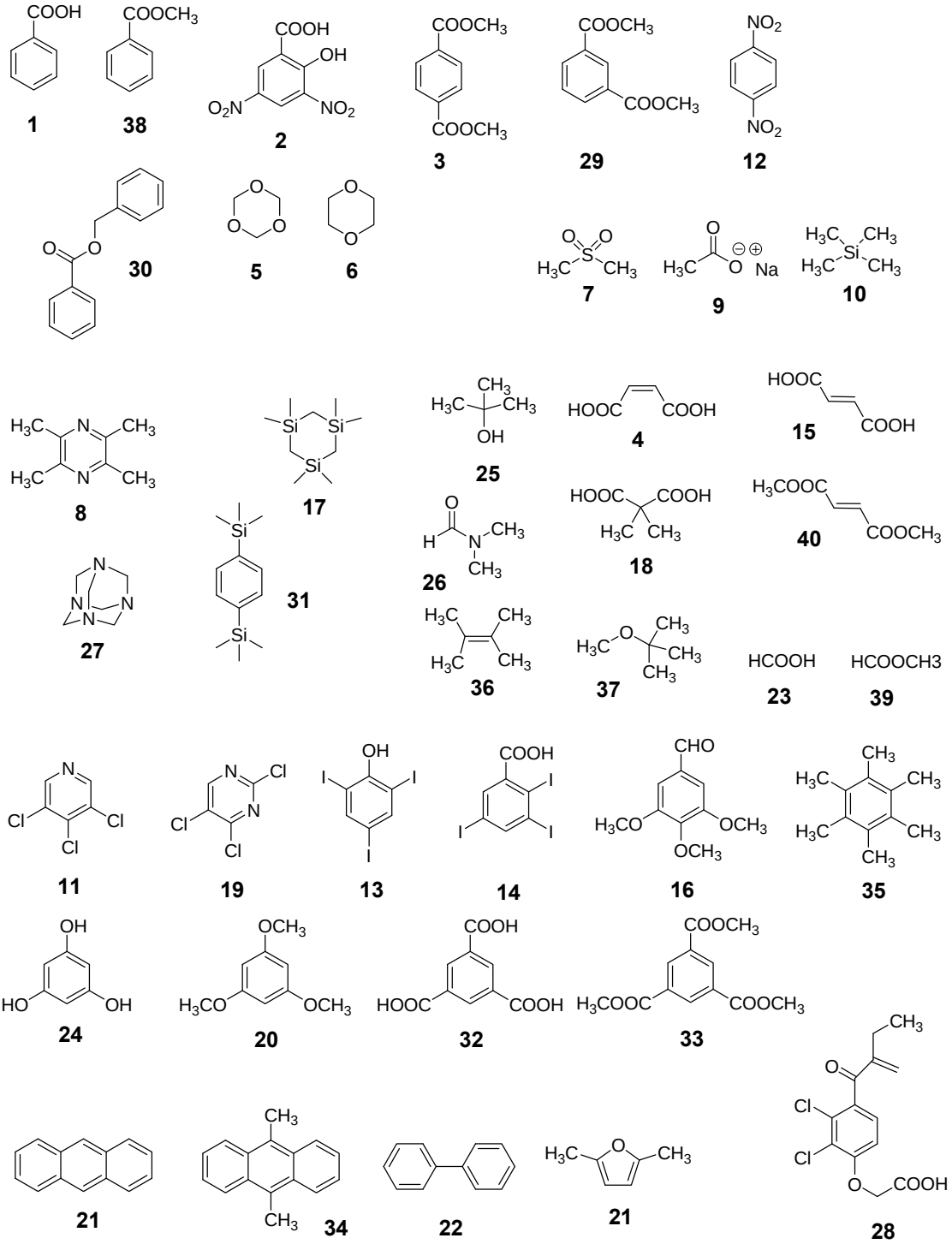
Supporting Information

S1. *Chemical Abstracts* (SciFinder©) section titles chosen to represent the NPs (NPs) literature. An index term search revealed a total of 10,936 terms, which indicates that each NPs-related qNMR publication contains ~4-5 index terms. An analysis of these terms revealed that only 2% were related to protein work, which confirmed the proper choice of the selected section titles, i.e., they were appropriate to define the small molecule portion of the NPs literature while leaving out the majority of the literature on protein and peptide work. The table shows the selected terms and their counts within the 2,400 references on qNMR of NPs.

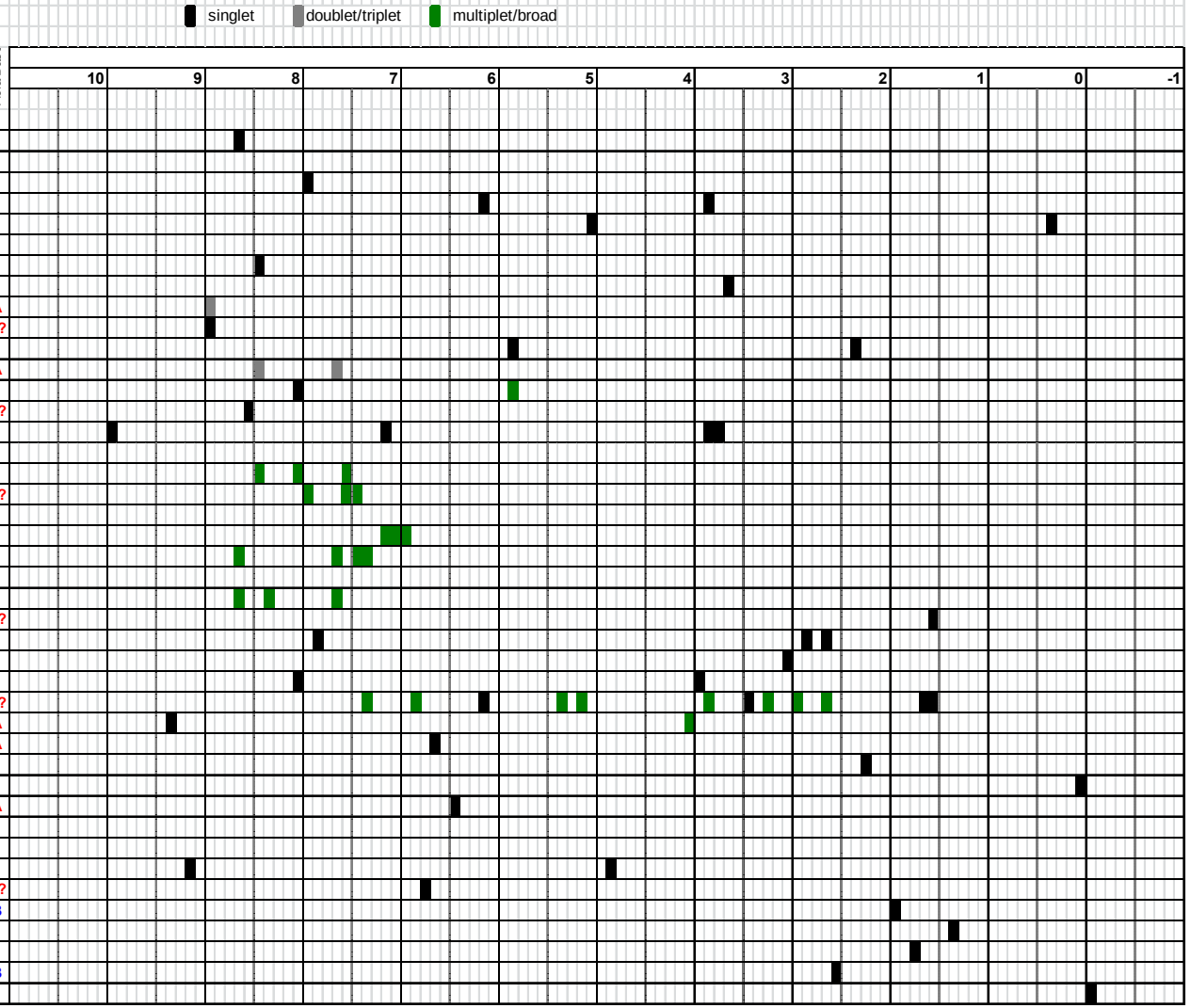
Section Title	Counts
Biochemical Methods	1301
Food and Feed Chemistry	343
General Biochemistry	342
Pharmaceutical Analysis	290
Essential Oils and Cosmetics	28
Microbial Biochemistry	24
Terpenes and Terpenoids	18
Alkaloids	17
Steroids	15
Analytical Chemistry	13
Terpenes	5
Petroleum and Petroleum Derivatives	3
Petroleum, Petroleum Derivatives, and Related Products	3
Fats and Waxes	1
Terpenoids	1

S2. Potential qHNMR Reference Materials for Use as Calibrants. The table lists the compound number and names, their molecular weight, selected higher order spin systems that represent caveats for quantitation, and acid/based properties that predictably lead to analyte interference. The right portion of the table represents a chemical shift map, mapping the resonances in 0.1 ppm bins on the 10 to -1 ppm scale. The multiplicity of the signals is coded as black (singlets), gray (doublets or triplets) and multiplets/broad signals (green).



qHNMR Reference Standards

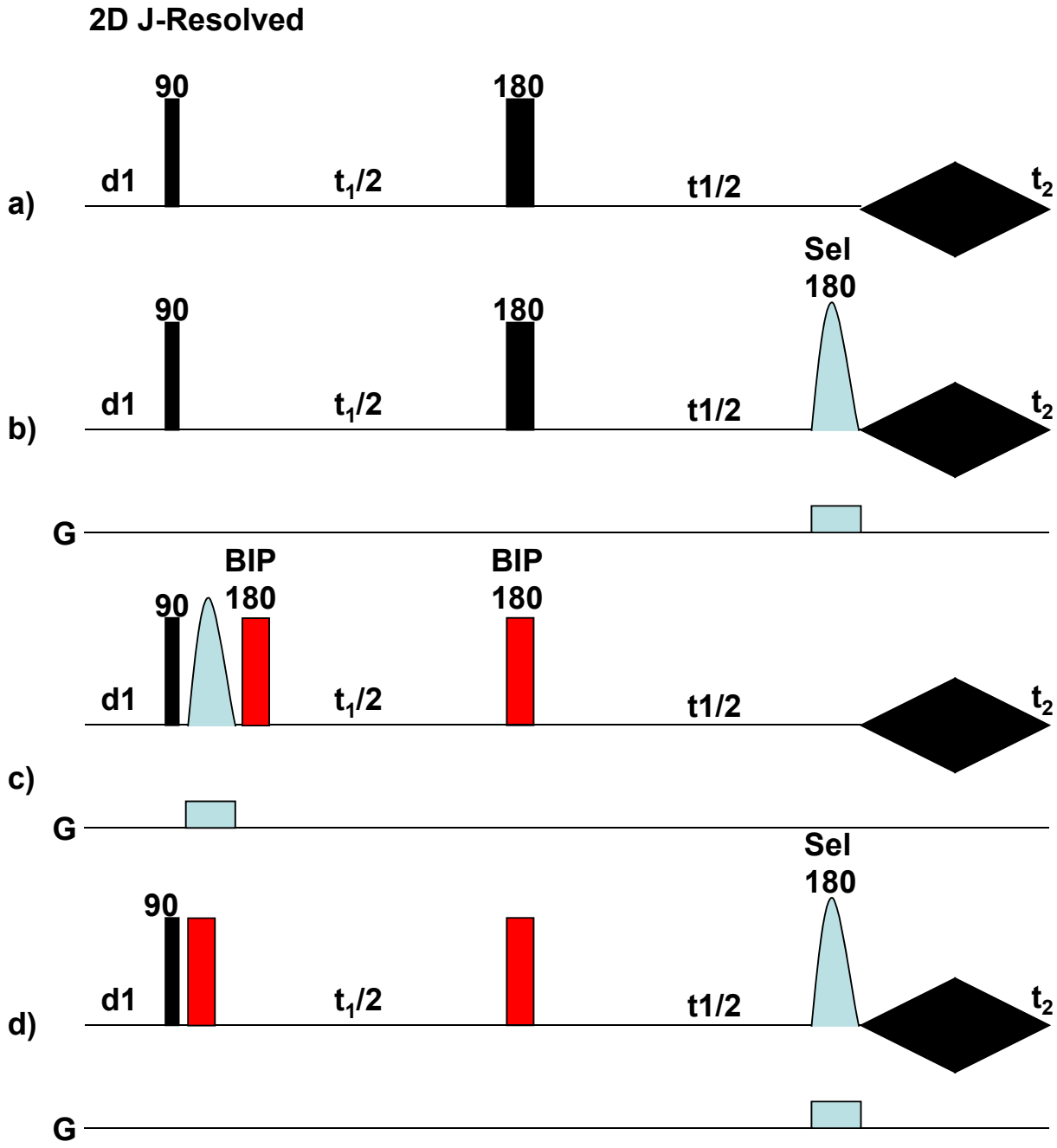
Pauli/Gödecke/Jaki/Lankin JNatProd (2012)



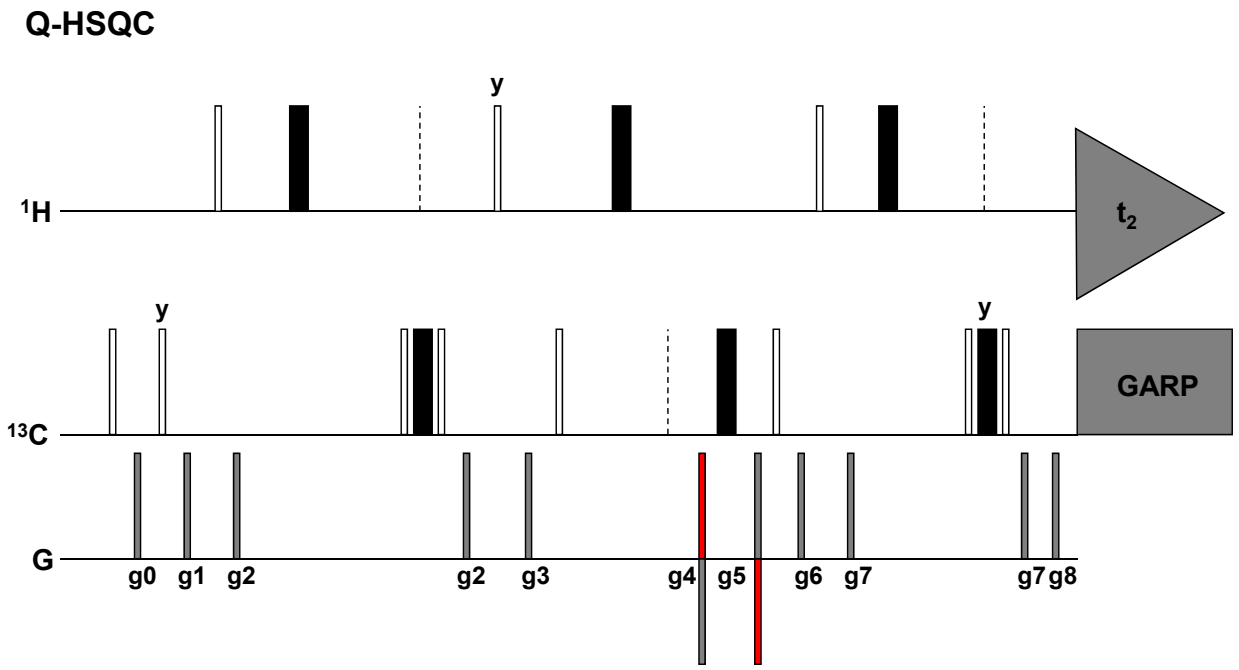
#	compound	MW	spin sys	Acid/Base
32	1,3,5-benzene tricarboxylic acid	210.14		
33	1,3,5-benzene tricarboxymethylate			
13	1,3,5-trichloro-2-nitro-benzene	226.45		
20	1,3,5-trimethoxybenzene	168.19		
5	1,3,5-trioxane	90.08		
31	1,4-bis(TMS)-benzene	222.47		
12	1,4-dinitrobenzene	168.11		
6	1,4-dioxane	88.11		
2	2-OH,3,5-dinitrobenzoic acid	228.12	AM	A
19	2,4,5-trichloropyrimidine	183.42		A?
21	2,5-dimethylfuran	96.13		
14	2,3,5-triidobenzoic acid	499.81		A
13	2,4,6-triidophenol	471.80		
11	3,4,5-trichloropyridine	182.44		A?
16	3,4,5-trimethoxybenzaldehyde	196.20		
34	9,10-dimethyl-anthracene			
21	anthracene	178.23		
1	benzoic acid	122.12	A2B2X	A?
38	methyl benzoate	136.15	A2B2X	
30	benzyl benzoate	212.24	(A2B2X)2	
22	biphenyl	154,21	A2B2X	
40	dimethyl fumarate	144.13		
29	dimethylisophthalate	194.18	AMNX	
18	dimethylmalonic acid	132.11		A?
26	dimethylformamide	73.09		
7	dimethylsulphone	94.13		
3	dimethylterephthalate	194.19		
28	ethacrynic acid	303.14		A?
23	formic acid	46.03		A
15	fumaric acid	116.07		A
35	hexamethylbenzene	162.27		
17	hexamethylcyclotrisiloxane	222.46		
4	maleic acid	116.07		A
37	methyl-t-butyl ether	88.15		
27	methenamine	140.19		
39	methyl formate	60.05		
24	phloroglucinol	126.11		A?
9	sodium acetate	82.03		B
25	tert-butanol	74.12		
36	tetramethylethylene	84.16		
8	tetramethylpyrazine	136.19		B
10	TSP/TMS			

S3. 2D qHNMR pulse sequences

The 2D *J*-resolved experiment for 2D qHNMR. The pulse sequences for the absorption mode 2D *J*-resolved experiment according to Pell and Keeler (Pell, A. J.; Keeler, J. *J. Magn. Res.* **2007**, *189*, 293-299). (a) the sequence for the standard 2D *J*-resolved experiment. (b) the sequence for the anti *J*-spectrum; by combining (a) + (b) the result is an absorption mode lineshape. (c) and (d) are used to actually record the *J* and –*J* spectra so that both spectra have the same intensity.



The 2D Q-HSQC pulse sequence for 2D qHNMR. The pulse sequence according to Heikkinen, et. al. (Heikkinen, S.; Toikka, M. M.; Karhunen, P. T.; Kilpelaeinen, I. A. *J. Amer. Chem. Soc.* **2003**, *125*, 4362-4367), for quantitative 2D-NMR which is optimized for obtaining quantitative information from the HSQC experiment by suppressing the dependence of cross peak intensity on $^{1}J_{CH}$, homonuclear coupling effects, and relaxation during the pulse sequence, and permits quantitative information to be extracted from the 2D experiment.



S4. QNMR Analysis with Nuclei Other than Protons

While ^{13}C and ^{15}N NMR are widely used in NPs analysis, the much reduced sensitivity compared to ^{1}H poses a major limitation for the implementation of qNMR protocols for these heteronuclei. The following reports are noteworthy in the context of the present focus on qHNMR, as they may inspire future "out-of-the-box" applications of qNMR. Two studies report on the ^{14}N(!) qNMR analysis of very small nitrogenous molecules: one provided evidence for the presence of nitrate in humic acids,[1] the other describes the determination of urea, nitrate and ammonium and provides a comprehensive overview of the often overlooked capabilities of ^{14}N NMR.[2] Preceding reports of ^{14}N-based quantitation deal with synthetic nitrofuroxanes[3] and nitroso-azadioxy dimerization equilibria.[4] Quantitative studies of tautomeric equilibria have also been reported with ^{15}N detection.[5]

Interesting applications of qCNMR for NPs addressed the establishment of the molecular formula of a triterpene from *Austroplenckia populnea* based on an exact carbon count by use of an inverse-gated ^{13}C NMR sequence.[6] An intriguing method for the detection of natural vs. lignin-derived vanillin also utilized a qCNMR approach which allows the precise determination of the $^{12}C/^{13}C$ ratios of the 8 carbons in the molecule at natural abundance.[7,8] The method involves curve fitting of the experimental spectra for improved quantitation. Relevant to nutritional labeling, Gao et al. demonstrated that qCNMR is a highly precise (<1% error) primary analytical method for the analysis of saturated, mono- and poly-unsaturated fats.[9] Utilizing solid-state (CPMAS) qCNMR, Wooten et al. characterized the main constituents in cured bright tobacco samples by uni- and multivariate statistical analysis.[10]

(1) Zhang, Y.-h.; Mao, X.-a. *Bopuxue Zazhi* **2000**, *17*, 449-454.
(2) Simeral, L. S. *Appl. Spectrosc.* **1997**, *51*, 1585-1587.
(3) Rakitin, O. A.; Ogurtsov, V. A.; Strelenko, Y. A.; Godovikova, T. I.; Khmel'nitskii, L. I. *Izvestiya Akademii Nauk SSSR, Seriya Khimicheskaya* **1990**, 1020-1023.
(4) Witanowski, M.; Sitkowski, J.; Biernat, S.; Kamienski, B.; Hamdi, B. T.; Webb, G. A. *Journal of Magnetic Resonance (1969-1992)* **1985**, *63*, 354-359.
(5) Schilf, W.; Stefaniak, L.; Witanowski, M.; Webb, G. A. *J. Mol. Struct.* **1986**, *140*, 311-315.
(6) Vieira Filho, S. A.; Duarte, L. P.; Silva, G. D. F.; Howarth, O. W.; Lula, I. S. *Helvetica Chimica Acta* **2003**, *86*, 3445-3449.
(7) Tenailleau, E.; Lancelin, P.; Robins, R. J.; Akoka, S. *Anal. Chem.* **2004**, *76*, 3818-3825.
(8) Tenailleau, E. J.; Lancelin, P.; Robins, R. J.; Akoka, S. *J. Agric. Food Chem.* **2004**, *52*, 7782-7787.
(9) Gao, L.; Sedman, J.; García-González, D. L.; Ehsan, S.; Sprules, T.; xvan de Voort, T. *Eur. J. Lipid Sci. Technol.* **2009**, *111*, 612-622.
(10) Wooten, J. B.; Kalengamaliro, N. E.; Axelson, D. E. *Phytochemistry* **2009**, *70*, 940-951.