Journal of Labelled Compounds and Radiopharmaceuticals
J. Labelled Cpd. Radiopharm. **XLI**, 935-939 (1998)

Preparation of [^{14}C]G2.5 and [^{14}C]G5.5 Starburst® PAMAM Dendrimers: The First Example of Dendrimer Radiosynthesis

Brad D. Maxwell*, Hideji Fujiwara, Sohrab Habibi-Goudarzi,
Justin P. Ortiz, and Sherry J. Logusch*

Monsanto Life Sciences Company
700 Chesterfield Parkway North
St. Louis, Missouri USA 63198

SUMMARY

A procedure for preparing ^{14}C-labelled half-generation Starburst® PAMAM dendrimers is described. Controlled incorporation of radioisotope was achieved by Michael additions of amines with ^{14}C-labelled methyl acrylate, resulting in dendrimers with specific activities between 30 and 45 mCi/mmol in very high radiochemical purity.

Key words: ^{14}C-labelled half-generation Starburst® PAMAM dendrimers, synthesis

INTRODUCTION

Dendrimers are highly branched macromolecules that are constructed from small polyfunctional cores via generational branching reactions (1,2). The physical properties of dendrimers can be varied by altering the surface morphology during synthesis (3,4). Such dendrimers have a range of reported applications, including their uses as molecular micelles (5), "nano-reactor" catalysis agents (6), and antiviral agents (7). In addition, there is a growing interest in dendrimers as delivery systems for small molecules (8), radiation contrast agents (9), and DNA in gene transfection (10,11,12). With the growing interest in dendrimers as delivery vehicles, techniques for studying these polymers in biological systems are needed. Labeling molecules of interest with radioisotopes coupled with radiochemical detection is a very effective method for studying the uptake and fate of compounds in vivo. For this purpose, a procedure for incorporating radioisotopes into dendrimer structures is described.

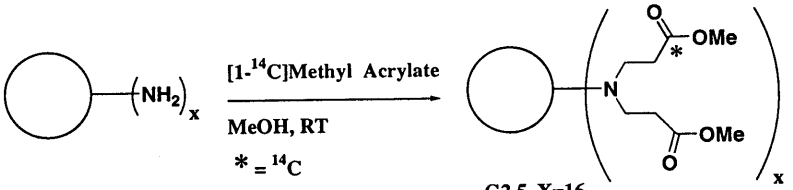
RESULTS AND DISCUSSION

Our research interests include the evaluation of half-generation (G) dendrimers in biological systems. We therefore synthesized [^{14}C]G2.5 and [^{14}C]G5.5 dendrimers by reacting the

CCC 0362–4803/98/100935–05$17.50

Received 8 April 1998
Revised 16 June 1998
Accepted 16 June 1998

corresponding whole generation Starburst® PAMAM (PolyAMidoAMine) polymers with [1-^{14}C] methyl acrylate (**Scheme 1**). Because the number of reactive amines varies with dendrimer size, the concentration of [1-^{14}C]methyl acrylate was adjusted in each synthesis using unlabelled methyl acrylate. The prepared ^{14}C-labelled dendrimers had specific activities between 30 and 45 mCi/mmol. This corresponds to one enriched carbon for every two dendrimer molecules for [^{14}C]G2.5 (34 mCi/mmol) and two enriched carbons for every three dendrimer molecules for [^{14}C]G5.5 (41 mCi/mmol). The reactions were monitored via reverse-phase HPLC using paired-ion chromatographic conditions, and product molecular weights were determined using mass spectrometric analysis.

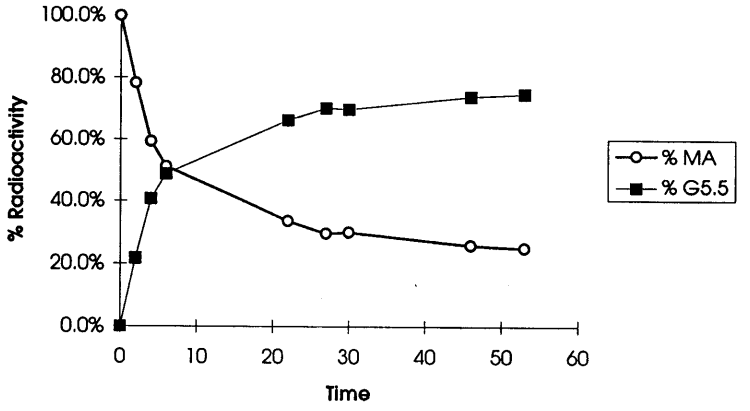


Scheme 1

EXPERIMENTAL

In general, a 5.0 mL solution of [1-^{14}C]methyl acrylate in methanol (5 mCi; 6.8 mCi/mmol), supplied by Amersham Life Science Inc, is mixed thoroughly with unlabelled methyl acrylate to adjust the initial specific activity. Solutions of G2.0 (MW 3,256) or G5.0 (MW 28,826) Starburst® PAMAM dendrimers in methanol (Dendritech, Inc., Midland, MI) are added to solutions containing 20% excess [1-^{14}C]methyl acrylate over a two hour period at room temperature. The reactions are monitored via reverse-phase HPLC using paired-ion chromatographic conditions (13). Using these conditions, methyl acrylate has a retention time of 13.8 min, G2.5 dendrimer has a retention time of 38.6 min, and G5.5 dendrimer has a retention time of 40.5 min. Disappearance of [1-^{14}C]methyl acrylate and concurrent formation of ^{14}C-labelled dendrimer are followed via radiochemical detection. The data for the synthesis of [^{14}C]G5.5 is representative for the general method (**Scheme 2**). The reactions are stopped by removing excess [^{14}C]methyl acrylate and methanol under full vacuum, resulting in a viscous product. Specific activities were determined by dissolving known

weights of each of the dendrimers in known volumes of methanol. Aliquots of these solutions were counted using LSC to determine the amount of radioactivity per unit volume. The specific activities were calculated from the measured concentrations of radioactivity (dpm / μl) and the molecular weight of each dendrimer.



Formation of [^{14}C]G5.5 Starburst® PAMAM Dendrimer Over Time
(MA = [1-^{14}C]methyl acrylate)

Scheme 2

[^{14}C]G2.5 dendrimer was prepared by mixing G2.0 dendrimer (0.314 g; 0.0963 mmol; 16 reactive amines) in methanol (26.13 wt %) with [1-^{14}C]methyl acrylate (3.67 mmol; 1.36 mCi/mmol) in 5 mL methanol. The reaction was stirred for 72 hours at room temperature. The product was characterized using positive ion electrospray mass spectrometry. Detection of a molecular ion peak at m/z 6,013 confirmed the product as [^{14}C]G2.5 dendrimer. Two additional peaks at m/z 5,927 and m/z 5,841 were also observed in the mass spectrum, corresponding to dendrimers missing one and two methyl acrylate units, respectively. The yield for the reaction was 95%, and the specific activity of [^{14}C]G2.5 dendrimer was 41 mCi/mmol.

The procedure for synthesizing [^{14}C]G5.5 dendrimer was similar to that used to prepare [^{14}C]G2.5 dendrimer. A solution of G5.0 dendrimer (3.01 g; 0.1043 mmol; 128 reactive amines) in methanol (25.05 wt %) was mixed with [1-^{14}C] methyl acrylate (32.1 mmol; 0.156 mCi/mmol) in 5

mL methanol. The reaction was stirred for 53 hours at room temperature. The product was characterized using positive ion MALDI-TOF (Matrix-Assisted Laser Desorption/Ionization - Time Of Flight) mass spectrometry. A molecular ion peak was detected at m/z 51,117, matching that of a G5.5 synthetic standard (Dendritech, Inc., Midland, MI). The yield for the reaction was 98%, and the specific activity of the [^{14}C]G5.5 dendrimer was 34 mCi/mmol.

CONCLUSION

Synthesis of ^{14}C-labelled half-generation dendrimers was achieved in excellent yields via Michael addition of amine-surfaced dendrimers to [1-^{14}C]methyl acrylate. The structures of the desired products were confirmed using HPLC chromatography and mass spectrometry. The synthetic procedure allows for controlled incorporation of radioisotope into dendrimer molecules, thus minimizing the amount of costly ^{14}C-labelled methyl acrylate needed to generate ^{14}C-labelled polymers.

ACKNOWLEDGEMENTS

The authors wish to thank Mr. Mark Kaiser of Dendritech, Inc. and Drs. Ralph Spindler, David Hedstrand, June Klimash, and Donald Tomalia of the Michigan Molecular Institute for helpful discussions.

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13. A Beckman Ultrasphere C_{18} reverse-phase column (4.6 x 250 mm) was used. The aqueous phase was 5 mM hexanesulfonic acid /5 mM triethylamine in 1% acetic acid, and the organic phase was 5 mM hexanesulfonic acid /1 mM triethylamine in methanol. The column was eluted with 15% methanol for the first five minutes followed by a ten minute linear gradient to 60% methanol. The gradient was held for 10 minutes followed by a second linear gradient to 100% methanol. After a five minute hold, the column was equilibrated back to the initial conditions.