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# New Insights into Poly(lactic-*co*-glycolic acid) Microstructure: Using Repeating Sequence Copolymers To Decipher Complex NMR and Thermal Behavior

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***Abstract:*** Sequence, which Nature uses to spectacular advantage, has not been fully exploited in synthetic copolymers. To investigate the effect of sequence and stereosequence on the physical properties of copolymers, a family of complex isotactic, syndiotactic, and atactic repeating sequence poly(lactic-*co*-glycolic acid) copolymers (RSC PLGAs) were prepared and their NMR and thermal behavior was studied. The unique suitability of polymers prepared from the bioassimilable lactic and glycolic acid monomers for biomedical applications makes them ideal candidates for this type of sequence engineering. Polymers with repeating units of LG, GLG and LLG (L = lactic, G = glycolic) with controlled and varied tacticities were synthesized by assembly of sequence-specific, stereopure dimeric, trimeric, and hexameric segmer units. Specifically labeled deuterated lactic and glycolic acid segmers were likewise prepared and polymerized. Molecular weights for the copolymers were in the range $M_n = 12-40$ kDa by size exclusion chromatography in THF. Although the effects of sequence-influenced solution conformation were visible in all resonances of the $^1$H and $^{13}$C NMR spectra, the diastereotopic methylene resonances in the $^1$H NMR ($CDCl_3$) for the glycolic units of the copolymers proved most sensitive. An octad level of resolution, which corresponds to an astounding 31-atom distance between the most separated stereocenters, was observed in some mixed sequence polymers. Importantly, the level of sensitivity of a particular NMR resonance to small differences in sequence was found to depend on the sequence itself. Thermal properties were also correlated with sequence.

## Introduction

The creation of polymers with a high degree of sequence and/or stereocontrol represents an exciting frontier in materials science as a result of both the paucity of examples of polymers with high values in these two categories and the potential benefits to be realized from the creation of new polymers from readily available and well-understood monomers.[1−11] A valuable lesson on the key role of sequence/stereochemical control can be learned from Nature, which exploits stereospecific sequences of amino acid monomers to form proteins with a myriad of properties and functions. This lesson is further echoed in the recent reports describing the unique behavior of sequenced foldamers[12] and in the benefits already derived from the relatively modest degrees of sequence control present in conventional polymer architectures, e.g., block copolymers, and stereocontrol, e.g., isotactic polypropylenes.[13−16] Finally, there are clear advantages to be realized from the rearrangement of "old" monomers to create new polymers: *economic*, in that the infrastructure that already exists for the large-scale synthesis of these old monomers translates into low cost and high availability, and *pragmatic*, in that the knowledge base that already exists regarding the suitability of these monomers for particular applications translates into an efficient path to application.

The extensive investigation of poly(lactic-*co*-glycolic acid)s (PLGAs) for *in vivo* applications requiring slow polymer degradation renders them ideal candidates for the investigation of the role of sequence in polymer properties. The popularity of these polymers for applications such as stem cell scaffolding and drug delivery vehicles stems from the fact that bulk structures made from PLGAs hydrolyze at a moderate rate in

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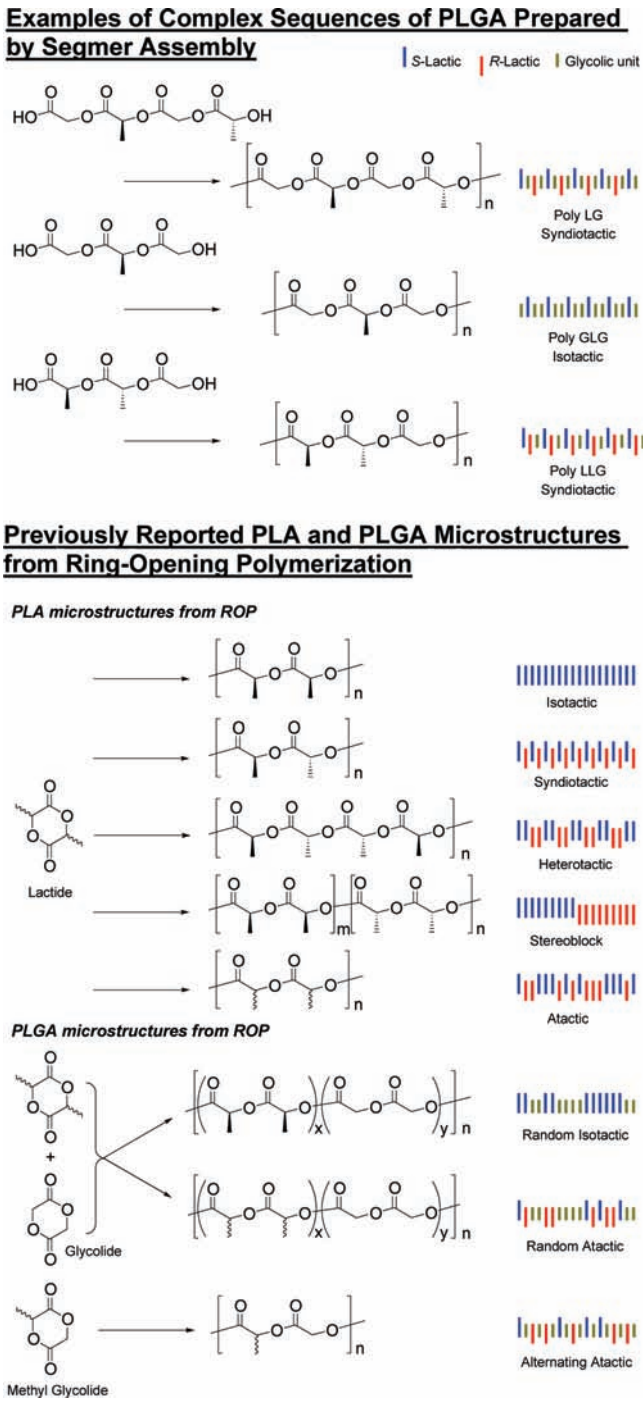


***Figure 1.*** Comparison of complex sequences prepared by segmer assembly with simpler PLA and PLGA microstructures prepared by ring-opening polymerization.[26−31,40]

the body and the products, lactic and glycolic acid, are bioassimilable.[17−25] Although other polymers are employed for these applications, studies on PLGAs and poly(lactic acid)s (PLAs) represent a significant proportion of all work in the area. The ubiquity of the materials and the special suitability of the monomers to bioengineering applications make the idea of creating repeating sequence copolymers (RSCs) from these monomers particularly attractive.

Our approach to the investigation of the role of sequence on the properties of PLGAs is to create and then analyze a family of RSCs. Our methodology, which involves the condensation of preformed segmers, allows for the synthesis of polymers with

***Table 1.*** Naming Conventions for Segmers and Polymers

| Symbol | Definition |
|---|---|
| **L** | L-Lactic unit (*S* configuration) |
| $\mathbf{L}_R$ | D-Lactic unit (*R* configuration) |
| $\mathbf{L}_{rac}$ | *rac*-Lactic unit |
| $\mathbf{L}_{d,rac}$ | α-*d*-*rac*-Lactic unit |
| **G** | Glycolic unit |
| $\mathbf{G}_{d2}$ | α-*d*,*d*-Glycolic unit |
| **Bn** | Benzyl protecting group |
| $\mathbf{SiR}_3$ | Silyl protecting group |

structural and stereosequences more complex than those previously prepared. Selected examples are presented in Figure 1. Previous efforts to prepare sequenced PLAs and PLGAs have relied on the ring-opening polymerization (ROP) of the cyclic lactide, glycolide, and methyl glycolide dimers.[26−40] Despite the laudable success achieved though the sophisticated design of selective catalysts and the exploitation of chain-end control, the number and types of sequences that can be prepared is limited by the dimeric form of the ROP monomer and by challenges inherent in programming catalysts to deliver a pattern more complex than alternation. While the strategy we employ in this paper of preassembling a sequenced segmer by a condensation mechanism is arguably less efficient than ROP, it is convergent and molecular weights >20 kDa are routinely achievable. Most importantly this approach is general: *any RSC PLGA envisioned can be prepared.*

The first benefit realized from our creation of a family of PLGA RSCs and the primary focus of this paper is the creation of a partial "Rosetta Stone" for the interpretation of NMR data of polymers with complex stereochemical patterns in general

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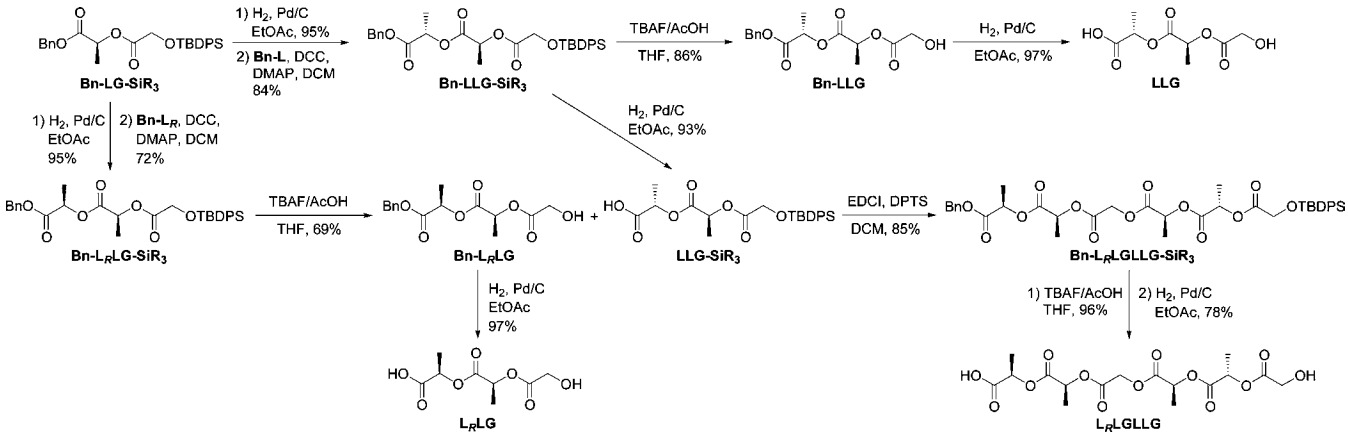
*Scheme 1*




and for PLGAs in particular. The most relevant precedents for this work are the extensive investigations of the NMR for PLA of varying tacticities.[29−31,33,41−53] It is worth noting that in the PLA system the $^{13}C$ NMR spectroscopic shifts have been found to be most sensitive to the relationships between distant stereocenters; differences in relative stereochemistries up to 5−6 units away from the nucleus under observation can be detected.[41,43,44,47,49,51,54] Although the NMR data for PLGAs has likewise been studied, the added complexity introduced by having two variables, sequence and stereochemistry, as well as the fairly modest control of these variables achievable using the common ROP synthetic approach, has inhibited progress. These studies have primarily been limited to the partial assignment of local sequence within otherwise random copolymers.[34,36,39,40,45,55−58] Our approach to preparing PLGAs, which allows for nearly perfect sequence and stereocontrol, greatly expands the database and offers thereby a significant advance in the understanding of PLGA NMR data, as well as some interesting new conclusions of a more general nature pertaining to sequenced copolymers.

## Results

**Naming Conventions.** Segmers are named by listing the monomers in sequence order from the C-side to the O-side using the abbreviations in Table 1. **Bn**$\mathbf{LL}_{rac}\mathbf{G}$ is, therefore, a trimer of stereopure L-lactic acid, *rac*-lactic acid, and glycolic acid that bears a benzyl protecting group on the carboxylic acid (C-side) terminus. Additionally, the deuterium-labeled lactic and glycolic acids are labeled $\mathbf{L}_{\mathbf{d},rac}$ and $\mathbf{G}_{\mathbf{d2}}$, respectively. Polymers are named from the exact segmer used. Thus, the polymer prepared from **LLG** is named **poly LLG** rather than **poly GLL** despite the homology of the two sequences after polymerization, i.e., ... LLGLLGLLGLLGLLGLLG....

**Synthesis.** A series of PLGA RSCs based on dimeric, trimeric, tetrameric, and hexameric repeating units have been prepared by condensation of the preformed segmers. Segmers were assembled in a convergent fashion by reacting partly protected subunits to form completely protected products, which after subsequent deprotection of both termini yielded the desired segmers as α-hydroxy carboxylic acids. The synthesis of the **LLG** and $\mathbf{L}_R\mathbf{LG}$ segmers are presented as representative examples.

Starting from the previously reported dimer, **Bn-LG-SiR$_3$**,[59] catalytic hydrogenation was used to remove the benzyl group and generate the partially deprotected **LG-SiR$_3$** unit in a 95% yield (Scheme 1). Benzyl (*S*)-lactate (**Bn-L**) or benzyl (*R*)-lactate (**Bn-L$_R$**) were coupled to **LG-SiR$_3$** to generate diprotected trimers **Bn-LLG-SiR$_3$** and **Bn-L$_R$LG-SiR$_3$** in 84% and 72% yields, respectively. Removal of both protecting groups gave segmers **LLG** and $\mathbf{L}_R\mathbf{LG}$ in 83% and 67% yields. Using the same approach, the segmers **LG**, $\mathbf{L}_{rac}\mathbf{G}$, **GLG**, $\mathbf{GL}_{rac}\mathbf{G}$, $\mathbf{L}_{rac}\mathbf{L}_{rac}\mathbf{G}$, $\mathbf{L}_{rac}\mathbf{LG}$, $\mathbf{LL}_{rac}\mathbf{G}$, and $\mathbf{LL}_R\mathbf{G}$ were prepared. Hexameric units such as $\mathbf{L}_R\mathbf{LGLLG}$ were synthesized by coupling partially deprotected trimeric segmers **Bn-L$_R$LG** and **LLG-SiR$_3$** followed by subsequent deprotections in 75% yield overall. $\mathbf{LLGLL}_R\mathbf{G}$ and $\mathbf{GLGL}_R$ were prepared similarly from the appropriate trimeric and dimeric precursors. It should be noted that in the preparation of $\mathbf{L}_{rac}\mathbf{L}_{rac}\mathbf{G}$ from the racemic precursors, there is a slight preference, 60:40, for the formation of LLG units with different stereocenters, i.e., $\mathbf{LL}_R\mathbf{G}$ and $\mathbf{L}_R\mathbf{LG}$ are

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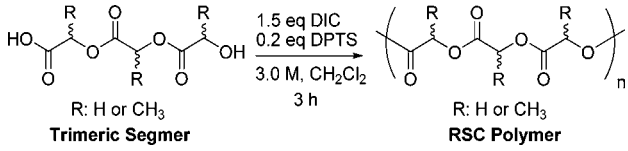
***Table 2.*** PLGA Repeating Sequence Copolymer Characterization Data

| Polymer | Repeating Pattern[a] | Yield (%)[b] | THF $M_n$ (kDa)[c] | THF PDI[c] | $CHCl_3$ $M_n$ (kDa)[c] | $CHCl_3$ PDI[c] | $CHCl_3$ Absolute $M_n$[d] | $CHCl_3$ DP[e,f] |
|---|---|---|---|---|---|---|---|---|
| **LG** | | 63 | 27.4 | 1.3 | 33.3 | 1.3 | 13.4 | 512 (206) |
| **L$_{rac}$G** | | 52 | 28.8 | 1.3 | 34.3 | 1.4 | --- | 527 |
| **GLG** | | 78 | 26.2 | 1.2 | 36.2 | 1.4 | 19.4 | 577 (309) |
| **GL$_{rac}$G** | | 60 | 21.4 | 1.3 | 27.5 | 1.4 | --- | 439 |
| **LLG** | | 70 | 41.2 | 1.2 | 41.8 | 1.3 | 23.1 | 620 (343) |
| **LL$_R$G** | | 71 | 29.0 | 1.4 | 42.3 | 1.3 | --- | 628 |
| **L$_R$LG** | | 59 | 30.6 | 1.4 | 39.8 | 1.4 | --- | 591 |
| **L$_{rac}$L$_{rac}$G** | | 65 | 30.5 | 1.4 | 35.2 | 1.3 | --- | 522 |
| **LL$_{rac}$G** | | 50 | 17.8 | 1.4 | 19.3 | 1.6 | --- | 286 |
| **L$_{rac}$LG** | | 83 | 27.4 | 1.4 | 40.5 | 1.4 | 25.9 | 601 (384) |
| **L$_{d,rac}$LG** | | 99 | 32.8 | 1.3 | 31.7 | 1.5 | --- | 468 |
| **LL$_{d,rac}$G** | | 62 | 29.6 | 1.4 | 33.7 | 1.4 | --- | 498 |
| **GLG$_{d2}$** | | 52 | 15.2 | 1.4 | 25.3 | 1.5 | --- | 400 |
| **GLGL$_R$** | | 65 | 12.3 | 1.5 | 21.1 | 1.4 | --- | 324 |
| **LLGLL$_R$G** | | 70 | 30.0 | 1.4 | 32.0 | 1.5 | --- | 475 |
| **L$_R$LGLLG** | | 63 | 30.1 | 1.4 | 39.8 | 1.3 | --- | 591 |

[a] (*S*)-lactic unit, (*R*)-lactic unit, *rac*-lactic unit, glycolic unit deuterated unit; [b]Isolated after 2x precipitation in MeOH; [c]Determined by SEC relative to PS standards; [d]Determined by SEC-MALLS; [e]DP from SEC data based on number of lactic and glycolic monomers; [f](DP) from SEC-MALLS data based on number of lactic and glycolic monomers.

***Scheme 2***




favored over **LLG** and **L$_R$L$_R$G**. This preference can be clearly seen in the copolymer spectrum as well. The synthesis of the oligomer **L$_{rac}$LG** also results in a slight bias toward **L$_R$LG** over **LLG**, but it is difficult to detect by NMR once polymerized. Complete synthetic schemes for all segmers and deuterium-labeled compounds can be found in the Supporting Information.

Polymerization conditions, utilizing mild esterification reagents 1,3 diispropylcarbodiimide (DIC) and 4-(dimethylamino)pyridinium *p*-toluenesulfonate (DPTS), were adapted from Stupp and Akutsu (Scheme 2).[60,61] In this way, for example, **poly LLG** was prepared by the DIC/DPTS-mediated condensation of the fully deprotected **LLG** segmer. All polymers were isolated as colorless solids by precipitation into methanol and purified by reprecipitation from methylene chloride into methanol to give yields ranging from 50% to 99% (Table 2). It should be noted that we did not observe significant sequence preferences when assembling segmers with racemic units into polymers. We have found that the inherent but slight preferences for alternation of stereochemical centers that were observed in selected segmer preparations can be minimized in the polymer preparation by coupling segmers bearing L groups on one terminus and G groups on the other.

Molecular weights for the polymers were determined by both size exclusion chromatography (SEC) and multiangle laser light scattering (MALLS). Relative molecular weights were obtained by SEC in both THF and $CHCl_3$ versus polystyrene standards with the average molecular weights ($M_n$) ranging from 12.0 to 41.2 kDa in THF and 19.3 to 42.3 kDa in $CHCl_3$. Absolute

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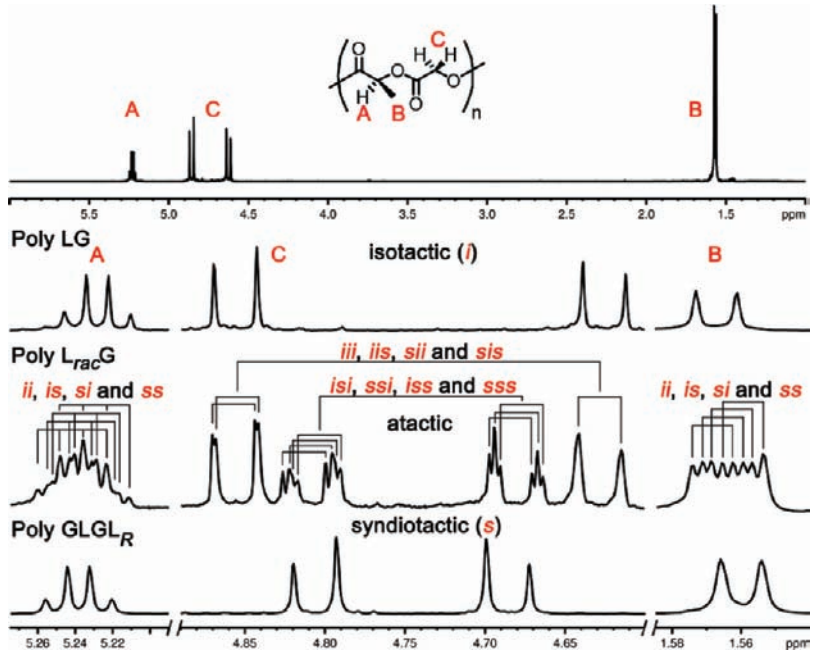


***Figure 2.*** (Top) Full spectrum of **poly LG**. (Bottom) Expansions of selected regions for **poly LG**, **L$_{rac}$G**, and **GLGL$_R$**. $^1$H NMR spectra at 600 MHz in $CDCl_3$.

molecular weights were determined for **poly LG**, **GLG**, **LLG**, and **L$_{rac}$LG** using SEC-MALLS. The dramatic lack of correlation between the SEC molecular weights in the two solvents and the differences between these molecular weights and the absolute molecular weights determined by MALLS makes it clear that the $R_g$ of the polymers is extremely sequence- and solvent-dependent. The SEC molecular weights must therefore be regarded with special care. By interpolation of the SEC and SEC-MALLS data obtained, however, we can say with a high degree of confidence that the majority of the polymers prepared have an absolute molecular weight >15 kDa, which corresponds to a DP > 200 (based on the count of glycolic and lactic units). This conclusion is further substantiated by the lack of end groups observed in the NMRs of these polymers. Previous studies by others on related polymers have shown microstructure-dependent SEC behavior.[62,63] A more detailed study of $R_g$ versus sequence is underway but is not the focus of this paper.

The molecular weights of the RSCs, although lower than those routinely achieved by ring opening of lactides and glycolides, are respectable for a condensation polymerization on the 1−2 g scale that we are currently employing. Moreover, it worth noting again that *polymers with the sequence complexity of those reported herein cannot be produced by any known ROP catalytic system.*

**Microstructural Analysis of RSCs Prepared from Dimeric Segmers.** Although this article focuses on the polymers with trimer-based sequences, **poly LLG** and **poly GLG**, we will begin with a brief discussion of the simpler, dimeric RSCs based on **poly LG**. Although a portion of this work was reported in a previous article,[59] we have since prepared a key member of the series, **poly GLGL$_R$**, that was not available at the time of that publication.

Our major discovery in the study of the alternating **poly L$_x$G** series polymers for **poly LG**, **L$_{rac}$G** and **GLGL$_R$** was the surprisingly high sensitivity of the glycolic methylene protons for the relative stereochemistry of the neighboring lactic units (Figure 2). The difference in the methylene signals of isotactic **poly LG** ($\delta$ 4.86 and 4.63, $\Delta$ = 0.23 ppm) and syndiotactic **poly GLGL$_R$** ($\delta$ 4.81 and 4.69, $\Delta$ = 0.12 ppm) are particularly illustrative. Although each spectrum contains a pair of doublets, as would be predicted for the clearly diastereotopic methylene protons, the chemical shifts are dramatically different.

Each of these alternating copolymers possesses a unique stereosequence that can be encoded using the widely accepted convention of assigning relative stereochemistries in polymers as either *i* for neighboring units with the same absolute stereochemistry or *s* for the opposite. Using this coding system and focusing on a *tetrad* level of resolution, the stereochemistry

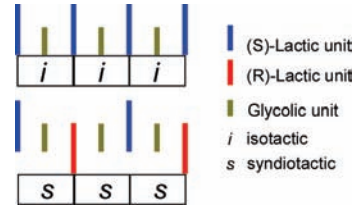


***Figure 3.*** Example of tetrad stereosequence encoding for **poly LG** (all isotactic) and **GLGL$_R$** (all syndiotactic).

***Table 3.*** Tetrad Assignments for Stereosequences of Polymers from the **poly LG** Series

| | LG | L$_{rac}$G | GLGL$_R$ | 60% LG + 40% L$_{rac}$G | |
|---|---|---|---|---|---|
| **Tetrads** | *iii* | *iii* | *sss* | *iii* | Major |
| | | *iis* | | *iis* | Minor |
| | | *sii* | | *sii* | Minor |
| | | *sis* | | *ssi* | Minor |
| | | *isi* | | *iss* | Minor |
| | | *ssi* | | | |
| | | *iss* | | | |
| | | *sss* | | | |

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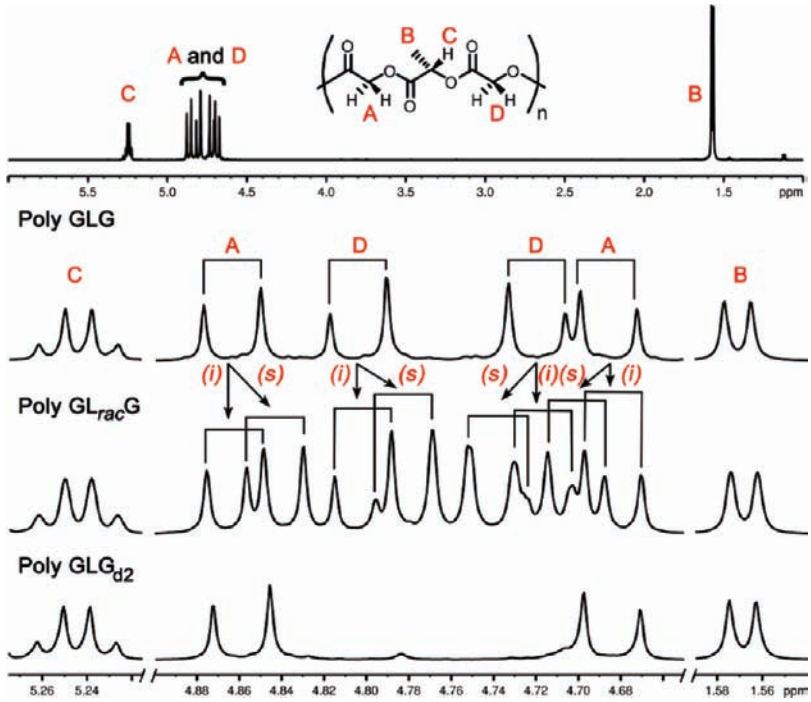


***Figure 4.*** (Top) Full spectrum of **poly GLG**. (Bottom) Expansions of selected regions for **poly GLG**, **GL$_{rac}$G**, and **GLG$_{d2}$**. $^{1}$H NMR spectra at 600 MHz in $CDCl_3$.

of **poly LG** can, for example, be expressed as *iii* and that of **poly GLGL$_R$** as *sss* (Figure 3 and Table 3).

We focus on the tetrad level because we can clearly see this level of resolution for some sequences present in the more complex spectrum of atactic **poly L$_{rac}$G** (Figure 2). Using the data from the stereopure polymers and from a sequence-weighted copolymer prepared by mixing **LG** and **L$_{rac}$G** in a 60:40 ratio (see ref 59 for details on this copolymer; see Table 3 for the expected/observed tetrads), we were able to make a nearly complete assignment of the signals. We see that the methylene resonances divide into two regions based on the relative stereochemistry of the closest neighboring lactic units: *i*-centered (outer signals) and *s*-centered (inner signals). The resonances for each of the *s*-centered tetrads, *sss*, *ssi*, *iss*, and *isi*, can be clearly differentiated, while those for the *i*-centered tetrads, *iii*, *iis*, *sii*, and *sis*, overlap such that the effective resolution is expressed at a lesser diad or triad level.

The other resonances in the spectra of the dimeric polymers also show stereosequence-dependent chemical shifts, though none exhibit as high a level of sensitivity/interpretability as the methylene protons. A clear chemical shift difference was noted, for example, between the methine and methyl groups from syndiotactic **poly GLGL$_R$** and isotactic **poly LG**. The smaller chemical shift range, however, limits the resolution of the stereosequences present in **poly L$_{rac}$G** to the triad level. In the $^{13}$C NMR spectra (see Supporting Information) the L-carbonyl resonances were clearly resolved to the triad level while the G-carbonyl, L-methyl, and L-methine resonances were nearly resolved triads. The *s*-carbonyl resonances for both L and G appeared upfield relative to the *i*-stereoisomer. In contrast, the *s*-methine and *s*-methyl resonances were downfield of the *i*-versions. Interestingly, the methylene resonance, which was so sensitive in the $^{1}$H NMR data, was only a broad singlet in the $^{13}$C NMR spectrum.

**Microstructural Analysis of RSCs Prepared from Trimeric GL$_x$G Segmers.** In moving from a dimer-based **LG** copolymer to a trimer-based copolymer, additional complexity was introduced both in the architecture and in the resulting NMR spectra. The third monomer presents the possibility of two different structural sequences, **GLG** or **LLG**, and multiplies the number of possible stereoisomers. It should be noted again that the choice to refer to a particular sequence as **GLG** rather than **GGL** was based on the unit actually used in the synthesis. Once polymerized, of course, such differences are irrelevant since **poly GLG** would necessarily be the same in all respects as **poly GGL** except in the identity of the end groups; both would have a repeating sequence of -GGLGGLGGLGGLGGLGGL- in the backbone.

Analysis of the NMR spectra of **poly GLG** in both the stereopure and racemic forms required that we first assign the resonances for the two chemically distinct G monomers. The G units are inequivalent due to the intrinsic lack of symmetry of the polyester chain, which has a distinct C- and O-terminus for each polymer and for each sequence within the polymer. We can differentially label the Gs then as either being connected to the C-terminus of the lactic residue (G$^{C}$) or the O-terminus (G$^{O}$). This inequivalence was expressed both in the $^{1}$H NMR spectrum (two pairs of doublets, Figure 4) and in the $^{13}$C NMR spectrum (C═O, $\delta$ 166.5 and 166.4; $\delta$ 60.9 and 60.7, see Supporting Information for $^{13}$C NMR spectra). The assignment of the resonances of **poly GLG** was facilitated by comparison with the spectrum of the polymer that was selectively deuterated at G$^{O}$, **poly GLG$_{d2}$**. The inner pair of doublets were absent from the $^{1}$H NMR spectrum, and the downfield methylene resonance was not present in the $^{13}$C NMR spectrum. Further confirmation of the assignment came from the 2D heteronuclear multiple bond coherence (HMBC) NMR spectrum of **poly GLG** in which a 3-bond correlation of the L-carbonyl with the outer pair of doublets from the methylene protons of

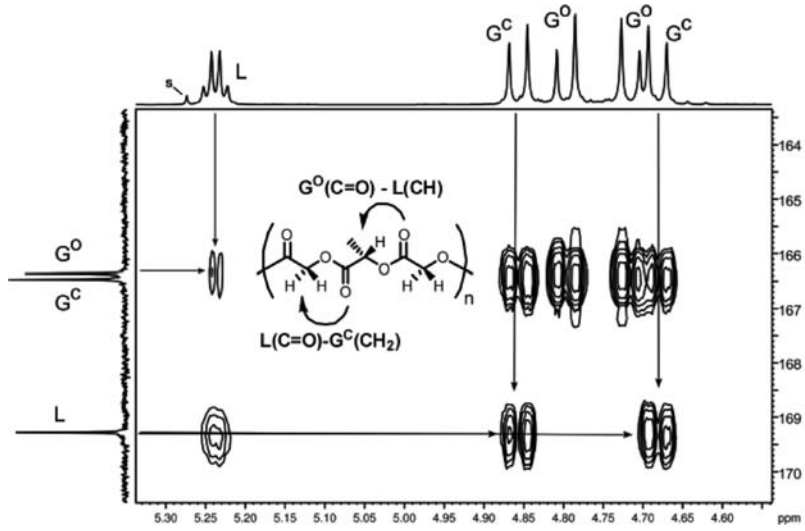


***Figure 5.*** 2D HMBC $^{1}H-^{13}C$ correlation NMR spectrum for **poly GLG** in $CDCl_3$ (700 MHz, $^{1}H$; 175 MHz $^{13}C$). The detailed cross peaks correspond to 3-bond correlations between the L-carbonyl with the $G^C$ methylenes and the $G^O$ carbonyl with the L-methine.

$G^C$ was observed along with the correlation between the $G^O$ carbonyl and the L-methine (Figure 5).[41,46,64,65]

Spectra of **poly GL$_{rac}$G** established that the chemical shifts of the G resonances were only modestly sensitive to the tacticity of the polymer. More sensitive than the methyl and methine resonances, which exhibited only a slight broadening, the methylene region exhibited 8 pairs of doublets, which given the inequivalence of the two G units is consistent with a dyad level of resolution. In other words the chemical shifts of the G resonances depend only upon the relative stereochemistries of the two closest L units. The peaks labeled as *i*-centered resonances corresponded well with the isotactic **poly GLG** standard. The slightly shifted pattern of two pairs of doublets was assigned to the *s*-dyad. Partially resolved dyads were likewise visible in the $^{13}C$ NMR spectrum where the lactic carbonyl appeared to broaden while 3 resonances, two at ca. $\delta$ 166.5 and a broadened resonance about $\delta$ 166.4, were observed for the glycolic carbonyls.

**Microstructural Analysis of RSCs Prepared from Trimeric L$_x$L$_x$G Segmers.** In moving from the **poly GLG** series to the other trimeric series, **poly LLG**, we must again address the nomenclature and consider the stereochemical implications of the second L unit in the series. The two L units are necessarily inequivalent and are designated as $L^O$ or $L^C$ in relation to the G unit. $L^O$ is connected to the G through the O-terminus, and the $L^C$ is connected through the C-terminus. Since the monomer used to prepare these polymers is **LLG** rather than **LGL**, however, the $L^O$ unit will be listed first in the series, i.e., $L^OL^CG$. With these designations in mind, the stereochemical variants of **poly L$_x$L$_x$G** can be considered. In Figure 6, our approach to assigning tacticity patterns is illustrated. In the case of **poly L$_R$LGLLG**, for example, the sequence yields two types of G-centered octads, one of which has an *issiiss* pattern. A subset tetrad pattern of *sii* is recognized from the larger pattern if the relationships from only the nearest four neighbor Ls are considered. Interestingly, the same pattern can be reasonably used as a reference for either $L^O$ or $L^C$ despite the shift in the "center" of the sequence. The lack of symmetry in the number

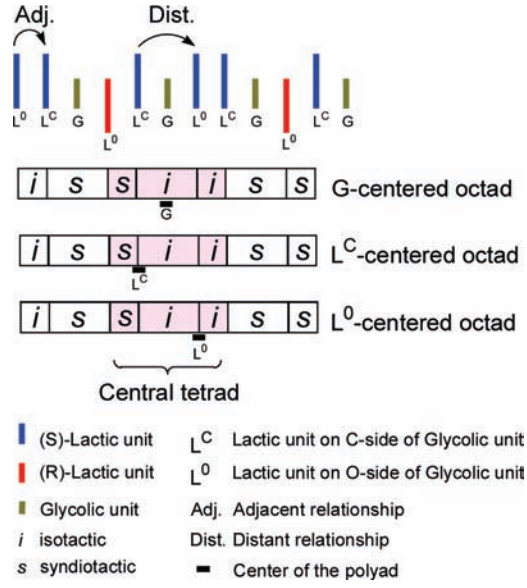


***Figure 6.*** Example of octad-level stereosequence encoding for **poly L$_R$L$_S$GL$_S$L$_S$G**. The central tetrads for each type of unit are defined as including one distant and two adjacent relationships.

of relationships considered relevant on each side of the L center is countered by the fact that the relationships chosen for the polyad maximize the number of adjacent versus distant interactions, on the assumption (substantiated by the data) that the adjacent relationships will be more influential than the distant ones.

In considering the stereochemical possibilities for the **poly L$_x$L$_x$G** series we have found it useful to divide the polymers into three families: $L^O$-variable (**poly L$_x$LG**); $L^C$-variable (**poly LL$_x$G**); and ($L^O$ + $L^C$)-variable (**poly L$_x$L$_x$G**). Table 4 shows the stereochemical possibilities for the first two families at the tetrad, hexad, and octad levels. It is important to note that **poly LLG, L$_R$LG**, and **L$_R$LGLLG** exhibit all of the stereochemical variations possible in **poly L$_{rac}$LG** at the tetrad level. Likewise **poly LLG**, **LL$_R$G**, and **LLGLL$_R$G** exhibit the possible tetrad level variations in **poly LL$_{rac}$G**. Stereosequence-specific shading in Table 4 illustrates the hierarchical relationship of the hexads and octads to their parent tetrads.

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***Table 4.*** Listing of Select Polyads for Specific **poly $L_xL_xG$** Repeating Sequence Copolymers[a]

| | Poly LLG | Poly $L_RLG$ ($LL_RG$) | Poly $L_RLGLLG$ | Poly $L_{rac}LG$ | Poly $LLGLL_RG$ | Poly $LL_{rac}G$ |
|---|---|---|---|---|---|---|
| **Tetrads** | *iii* | *sss* | *sii*<br>*iss* | *iii*<br>*sii*<br>*iss*<br>*sss* | *iis*<br>*ssi* | *iii*<br>*iis*<br>*ssi*<br>*sss* |
| **Hexads** | *i iii i* | *s sss s* | *i sii s*<br>*s iss i* | *i iii i*<br>*i iii s*<br>*s sii i*<br>*s sii s*<br>*i iss i*<br>*i iss s*<br>*s sss i*<br>*s sss s* | *s iis s*<br>*i ssi i* | *i iii i*<br>*s iii i*<br>*i iis s*<br>*s iis s*<br>*i ssi i*<br>*s ssi i*<br>*i sss s*<br>*s sss s* |
| **Octads** | *ii iii ii* | *ss sss ss* | *is sii ss*<br>*si iss i i* | *si iii ii*<br>*si iii ss*<br>*ii iii ss*<br>*ii iii ii*<br>*ss sii ii*<br>*is sii ii*<br>*ss sii ss*<br>*is sii ss*<br>*si iss ii*<br>*ii iss ii*<br>*ii iss ss*<br>*si iss ss*<br>*ss sss ss*<br>*is sss ss*<br>*is sss ii*<br>*ss sss ii* | *ss iis si*<br>*ii ssi is* | *ii iii ii*<br>*ss iii ii*<br>*ss iii is*<br>*ii iii is*<br>*ii iis si*<br>*ss iis si*<br>*ss iis ss*<br>*ii iis ss*<br>*ii ssi ii*<br>*ss ssi ii*<br>*ss ssi is*<br>*ii ssi is*<br>*ii sss si*<br>*ii sss ss*<br>*ss sss si*<br>*ss sss ss* |

[a] Tetrads subsets are identified by color within the hexad and octad tacticity patterns.

The $^1$H NMR spectra of the glycolic methylene regions of these trimeric series are spectacularly informative: *octad-level resolution of individual stereosequences is observed in some cases*. By comparison with the spectra of **poly LLG**, **$L_R$LG**, **$L_R$LGLLG**, and **$L_{rac}$LG** for the $L^O$-variable family (Figure 7) and **poly LLG**, **$LL_R$G**, **$LLGLL_R$G**, and **$LL_{rac}$G** for the $L^C$-variable family (Figure 8), it was determined that the chemical shifts of these resonances were inherently hierarchical and facile to interpret.

Beginning by focusing on the $L^O$-variable family (Figure 7), we can assign the resonances from the stereopure standards. The spectrum of the simplest sequence, isotactic **poly LLG**, exhibits a pair of well-separated doublets for the diastereotopic G methylene protons. **Poly $L_R$LG**, the fully syndiotactic sequence, also exhibits a single pair of doublets, and **poly $L_R$LGLLG** exhibits two pairs of doublets, which is consistent with expectations for this more complex sequence (see Table 4). Although these are stereopure standards with an "infinite" pattern, the resonances are labeled in the figures at the tetrad level for reasons that become clear in the analysis of the racemic variants (*vide infra*).

Analysis of the spectrum of **poly $L_{rac}$LG** reveals the useful hierarchical nature of the shift pattern in the methylene region: the gross shifts are determined by the central tetrad relationships, while the fine shifts are determined by more distant ones. The methylene region of this polymer manifests as well-separated sets of pairs of doublets, one pair for each of the resolved stereosequences. By comparison of the stereosequence standards and the spectrum of **poly $L_{rac}$LG**, it is clear that the resonances are grouped by the central tetrad relationships, *iii*, *sss*, *iss*, and *sii*, into four well-separated regions. Within these tetrad-controlled shift regions, the "fine" chemical shifts are then determined by the relative stereochemistries of L units beyond the central four.

The degree of resolution of longer range relationships in the methylene region is clearly sequence-dependent. By focusing on the upfield proton of the G methylene of **poly $L_{rac}$LG**, several levels of resolution can be identified (Figure 9). In the case of the *iii* set, for example, there are four nearly resolved doublets, the number that would be expected if there was a doublet present for each of the four possible octad level sequences. Although we do not have standards with sufficient complexity to label the individual sequences, the extremely high level of resolution is striking. The other three sets of doublets in the same region of this spectrum exhibit varying sensitivities ranging from the tetrad to hexad to octad level.

A similar hierarchical pattern is observed in the methylene region of **poly $LL_{rac}$G** (Figure 8). Sets of pairs of doublets corresponding to the expected tetrads, *iii*, *sss*, *ssi*, and *iis*, can be easily assigned. Within these sets, further resolution of certain sequences is observed. It should be noted that the spectra of **poly $L_R$LG** and **$LL_R$G** (Figures 7 and 8) are identical, as would be predicted from their enantiomeric relationship.

Changing the NMR solvent from $CDCl_3$ to $d_6$-DMSO dramatically decreases the resolution of the spectra (see Supporting Information for figure depicting the difference). The chemical shift range of the methylene (and other regions) is decreased, and sequence resolution is reduced.

We were also able to make significant progress in assigning the methyl and methine regions of the trimeric series of polymers. These regions are inherently more difficult to interpret because of the presence of the two inequivalent lactic units,

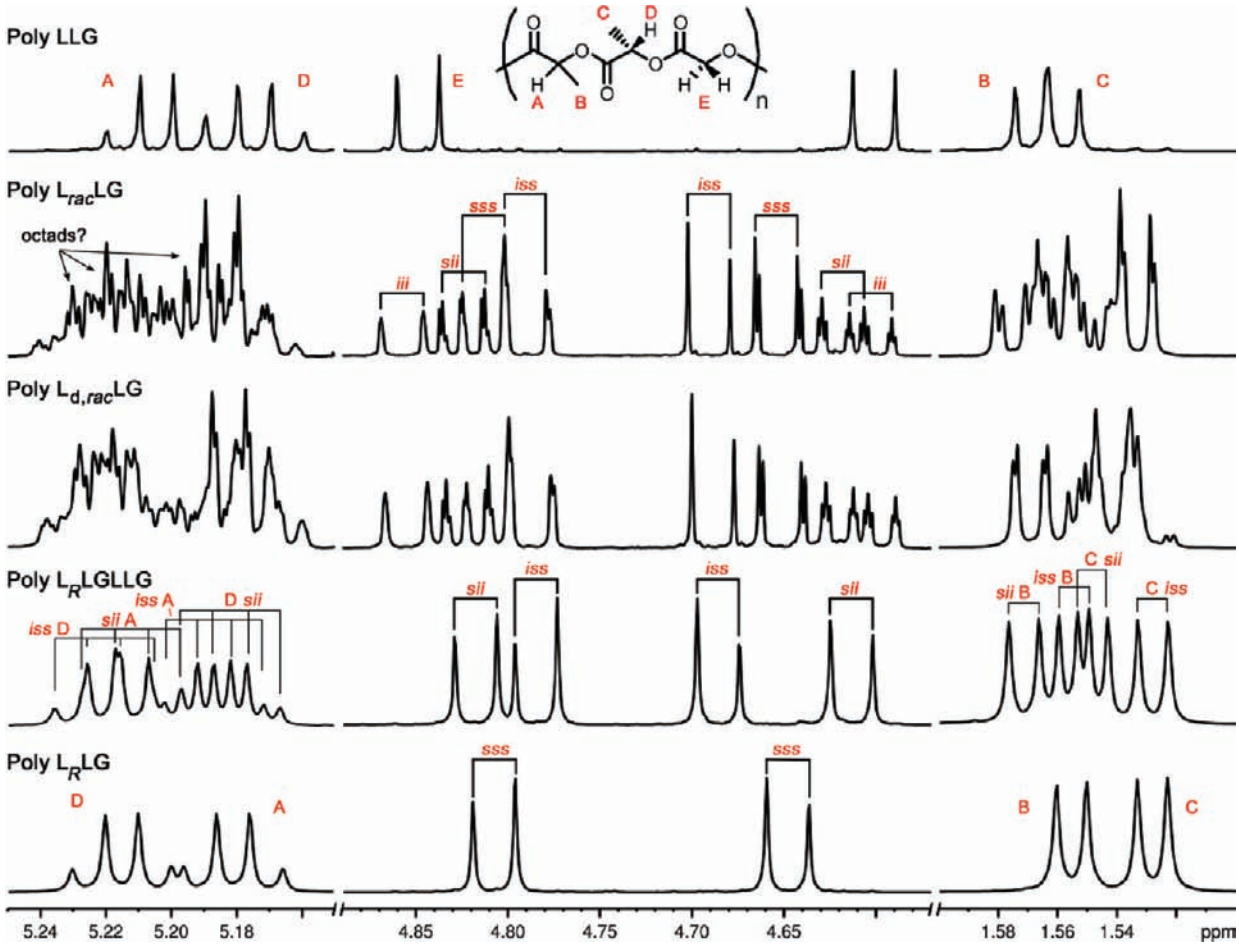


***Figure 7.*** $^1$H NMR spectra of $L^O$-variable LLG polymers at 700 MHz in $CDCl_3$. Comparisons of the expansions of selected regions for **poly LLG**, **L$_{rac}$LG**, **L$_{d,rac}$LG**, **L$_R$LGLLG**, and **L$_R$LG**.

the relatively small chemical shift range, and the lack of an hierarchical shift pattern. Primary differentiation of $L^O$ versus $L^C$ in the fully sequenced polymers **poly LLG**, **L$_R$LG**, and **L$_R$LGLLG** was accomplished using a combination of 2D NMR techniques. Methine resonances were assigned by 2D HMBC NMR spectra where a correlation between the G carbonyl and the $L^C$ methine was observed. Frustrating facile assignment, the relative chemical shifts of the $L^O$ and $L^C$ methines were not consistent throughout the stereochemical series. As Figure 10 illustrates, in isotactic **poly LLG** the $L^C$ resonance is upfield of the $L^O$ resonance, but the order is reversed in syndiotactic **poly L$_R$LG**. In polymers that contain mixed stereochemical relationships, **poly L$_R$LGLLG**, and multiple mixed relationships, **poly L$_{rac}$LG** and **L$_{rac}$L$_{rac}$G**, the 2D correlations showed that the chemical shifts of the $L^O$ and $L^C$ methines were extremely sequence-dependent and likely to invert chemical shift order from sequence to sequence. By coupling the assignments of the methine region with 2D COSY NMR spectroscopy, however, the methyl region could be assigned. Unlike the methine resonances, the $L^O$ methyl resonances were always downfield of the corresponding $L^C$ resonances.

Although complete interpretation of the methine and methyl regions for **poly L$_{rac}$LG** and **LL$_{rac}$G** could not be accomplished, it was clear that the sequence resolution of both regions was greater than a tetrad level. It should be noted that neither homonuclear decoupling nor 2D NMR was of much use in interpreting these spectra further. The chemical shift envelope for each type of signal was large enough to render the complete homonuclear decoupling of the regions impractical, while the small chemical shift range and sequence-dependent shift inversions (*vide supra*) hindered the interpretation of 2D spectra. The relatively modest simplification of the spectra for the selectively deuterated polymers **poly L$_{d,rac}$LG**, and **LL$_{d,rac}$G**, however, establishes the sensitivity of these signals for the stereochemical relationships of lactic units beyond the tetrad level in the chain. Moreover, by simple counting of the resonances and comparison of the spectra with the stereosequence standards, it is clear that some sequences are actually resolved to a hexad or even octad level.

Chemical shifts in the $^{13}$C NMR spectra of **poly L$_{rac}$LG** and **LL$_{rac}$G** showed primarily a tetrad level of resolution. Partial assignment was accomplished by comparison with the stereopure and deuterated RSCs (Figures 11 and 12) and by 2D HMBC NMR as described earlier. Analysis of the stereopure RSCs established that the $L^O$ carbonyl resonance was generally downfield of the $L^C$ resonance and the L carbonyl resonances were more sensitive to stereochemistry than the $^{13}$C NMR resonances for other groups. The presence of >8 lactic carbonyl signals in the spectra of both **poly L$_{rac}$LG** and **LL$_{rac}$G** was consistent with a higher than tetrad (mixed hexad/octad) level of resolution, although it was not possible to make individual assignments due to overlap. It is also of note that, in both the L- and G-carbonyl regions, the *i*-centered resonances were downfield of the *s*-centered resonances. Methine $L^O$ and $L^C$ resonances were assigned by comparison to the deuterium-labeled polymers, as the deuterium-substituted carbons exhibited

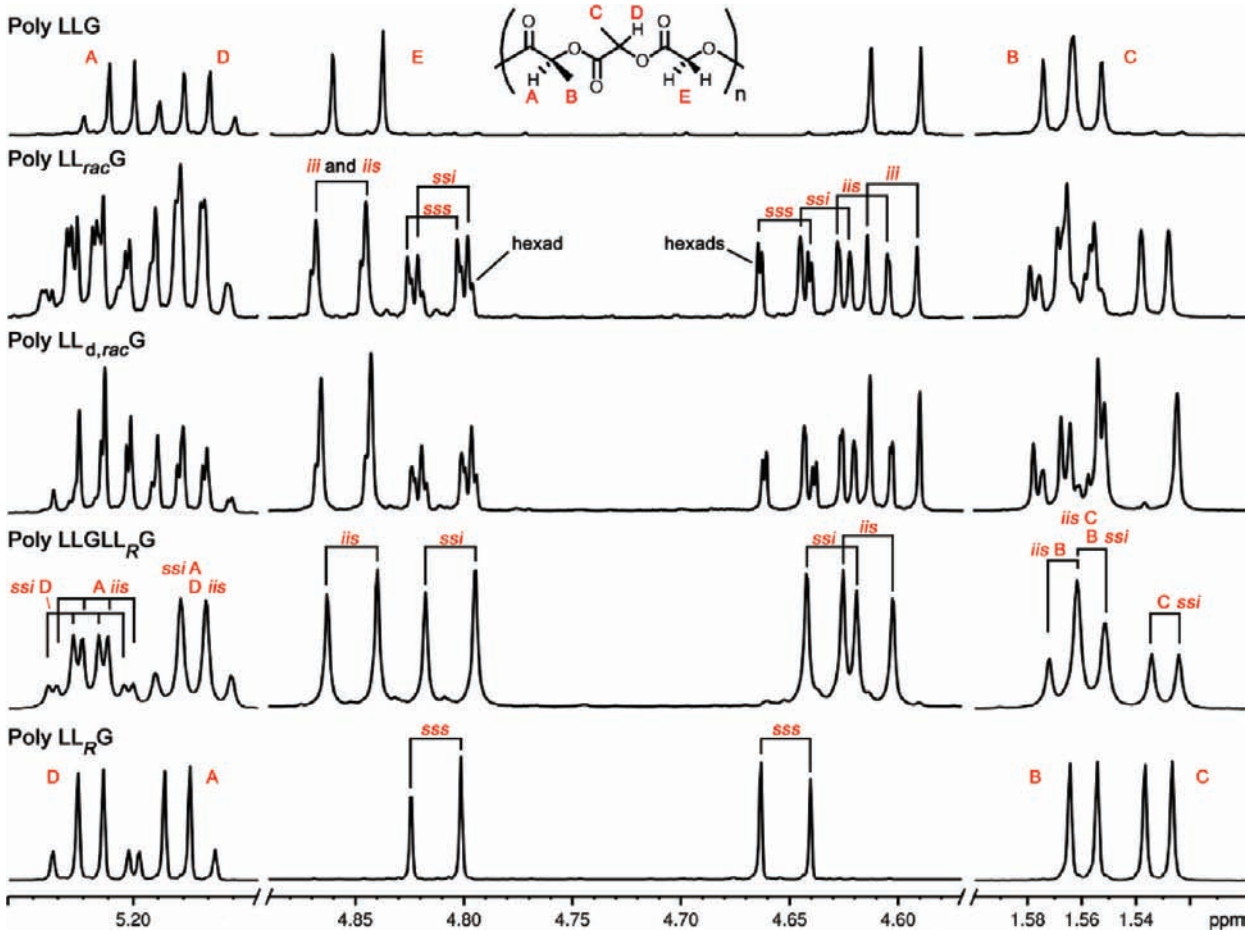


***Figure 8.*** $^1$H NMR spectra of L$^C$-variable LLG polymers at 700 MHz in $CDCl_3$. Comparisons of the expansions of selected regions for **poly LLG**, **LL$_{rac}$G**, **LL$_{d,rac}$G**, **LLGLL$_R$G**, and **LL$_R$G**.

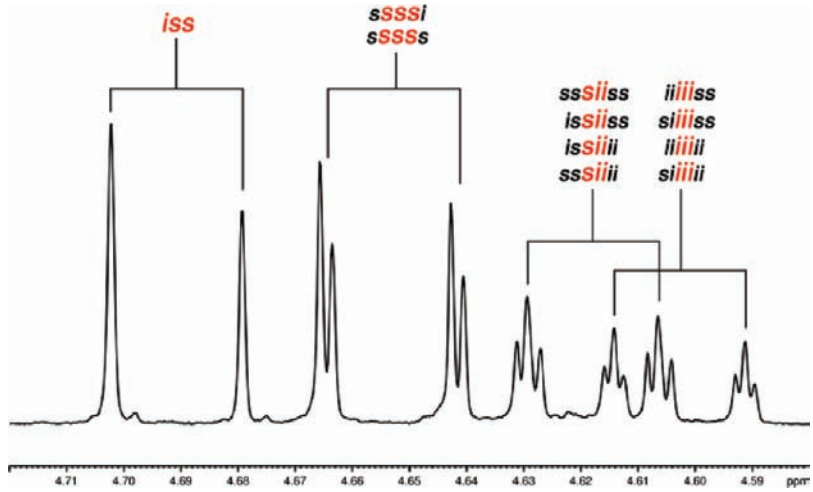


***Figure 9.*** $^1$H NMR expansion for the upfield diastereotopic proton of the glycolic methylenes of **poly L$_{rac}$LG** (full spectrum in Figure 7). The level of sensitivity for sequence ranges from tetrad (*sii, iii*) to hexad (*sss*) to octad (*iss*) depending on the core tetrad sequence.

much lower intensities due to slow relaxation. These signals clearly resolved to a slightly more than tetrad level for RSCs incorporating racemic units. The methyl region also showed a greater than tetrad level of resolution, but the small chemical shift range made individual assignments in this region impractical.

The chemical shifts for stereopure polymers are compiled in Tables S1 and S2, which are located in the Supporting Information. It should be emphasized that these are stereopure samples, and exact matches to unknowns will not be expected unless the sequences of the spectra compared are homologous at or beyond the level of resolution observed. For convenience, however, the polyads with more complex sequences have been labeled at the tetrad level.

Using these spectral tables as a guide and focusing on the methylene region, it is possible to interpret the spectra of samples that contain more than one structural sequence and of

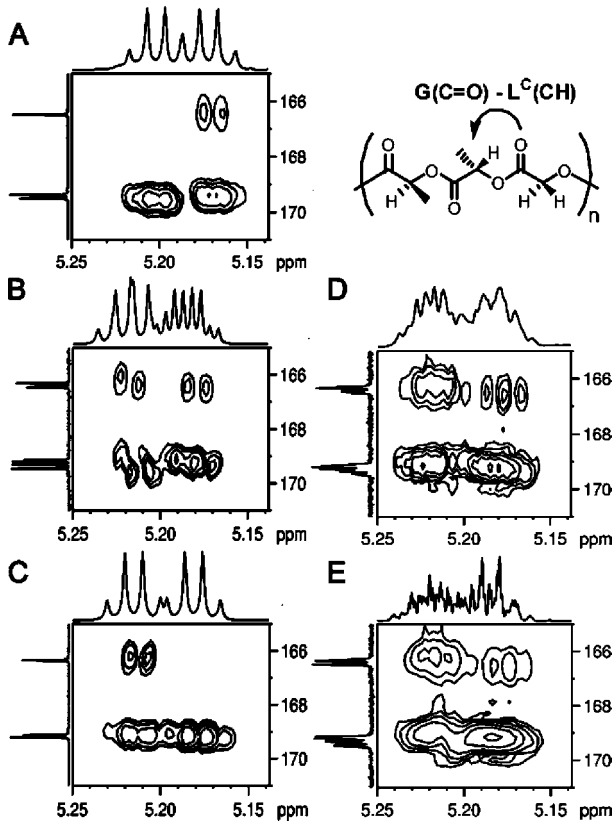


***Figure 10.*** 2D HMBC $^{1}H-^{13}C$ correlation NMR spectra at 700 MHz in $CDCl_3$ for **poly LLG** (A), $\mathbf{L}_{R}\mathbf{LGLLG}$ (B), $\mathbf{L}_{R}\mathbf{LG}$ (C), $\mathbf{L}_{rac}\mathbf{L}_{rac}\mathbf{G}$ (D), and $\mathbf{L}_{rac}\mathbf{LG}$ (E).

samples that contain multiple stereosequences. In a sample comprising a mixture of different structural sequences, in this case a mixture of **poly LG**, **GLG**, and **LLG**, resonances for each sequence can easily be distinguished (Figure 13).

A more stringent test is the assignment of the methylene region of **poly** $\mathbf{L}_{rac}\mathbf{L}_{rac}\mathbf{G}$. This spectrum is complicated because of the number of possible polyads (8 at the tetrad level) and the presence of multiple levels of resolution for each polyad. Nevertheless, it is possible to make a fairly complete assignment of the sequences present at the tetrad level (Figure 14).

**MALDI-TOF Mass Spectroscopy.** MALDI TOF analysis confirms the sequence fidelity of the PLGA RSCs. Using conditions optimized for PLGAs,[66,67] the analysis of selected copolymers was carried out. The masses of the chains present in **poly LG**, for example, differ in molecular weight by increments of exactly 58 amu, corresponding with the glycolic unit, and 72 amu, corresponding to the lactic unit (Figure 15). Moreover, chains differing by exactly one segmer weight (L + G = 130 amu) dominate, which is consistent with synthesis by assembly of segmers. Analogously, the envelope of masses present in the spectrum of **poly GLG** differ in molecular weight by a pattern of G, L, and G molecular weights with a majority of chains differing by exactly a segmer weight. Unfortunately, an analysis of the absolute molecular weights did not correspond to chemically reasonable end groups. It seems likely that there is some degradation of the samples in the experiment. The full spectra for all samples can be found in the Supporting Information.

**Thermal Properties.** Differential scanning calorimetry (DSC) was performed on polymer samples and annealed films. The glass transition temperature ($T_g$) varied slightly due to changes in the sequence composition (Table 5). When the L content increased from 50% in **poly LG** to 67% in **poly LLG** the $T_g$ remained constant around 57 °C. However, increasing the G content to 67% in **poly GLG** the $T_g$ decreased slightly to 50 °C. Within the LG and LLG series the $T_g$ was dependent on stereochemistry. Isotactic polymers possessed the highest $T_g$'s (57 °C), the atactic polymers possessed intermediate $T_g$'s (55−51 °C) and the syndiotactic polymers possessed the lowest $T_g$'s (48−50 °C). For the higher G content GLG series, changes in stereochemistry did not change $T_g$'s; **poly GLG** and $\mathbf{GL}_{rac}\mathbf{G}$ both exhibit a $T_g$ of 50 °C. Interestingly, melting transitions were only found for the syndiotactic LLG polymers, **poly** $\mathbf{L}_{R}\mathbf{LG}$ and $\mathbf{LL}_{R}\mathbf{G}$, at 154 and 158 °C, respectively.

Polymer films were prepared by drop-casting from methylene chloride into DSC pans, drying under vacuum and annealing at 85 °C for 3 h. Annealing had little effect on the transitions for **poly** $\mathbf{LL}_{R}\mathbf{G}$ and $\mathbf{L}_{R}\mathbf{LG}$, but the $T_g$'s for all of the remaining annealed samples dropped and became less sequence-sensitive. A new melting transition appeared for **poly LLG** at 114 °C, which is much lower than that observed for **poly** $\mathbf{LL}_{R}\mathbf{G}$ and $\mathbf{L}_{R}\mathbf{LG}$.

Although direct homologues of these RSCs have not been previously reported, the behavior of these sequences can be examined relative to the known PLA sequences. Coates and co-workers have reported the $T_m$ for syndiotactic PLA as 153 °C, which is close to those of **poly** $\mathbf{LL}_{R}\mathbf{G}$ and $\mathbf{L}_{R}\mathbf{LG}$. The $T_m$'s for isotactic and stereocomplexed PLA are much higher at 175 and 230 °C, respectively.[30,31]

Given the regularity of the remaining stereopure polymers, the lack of crystallization is somewhat surprising. Although annealing did promote crystallization in the case of **poly LLG**, other sequences did not exhibit melting points despite repeated efforts. We do not rule out the potential for crystallinity, however, as we suspect that we may not have discovered the proper thermal conditions to promote the longer range organization of the chains. Notably, Coates and co-workers did not observe crystal formation for the predominantly heterotactic sequenced PLA.[31]

## Discussion

We are able routinely to prepare RSC PLGAs with DPs > 200 on a per monomer basis with a greater than 95% sequence fidelity. Previously, we were successful in preparing RSC PLGAs using DCC/DMAP but the DPs were significantly lower.[59] The newly adopted DIC/DPTS method, first developed by Stupp and Moore, utilizes DPTS instead of DMAP in order to suppress chain terminating *N*-acylurea byproducts, neutralize the reaction pH, and minimize depolymerization.[60,68] Similar methods were used by Hawker and co-workers in the preparation of well-defined oligomers of lactic acid and caprolactone.[69,70] Examination of the NMR spectra establishes that under the

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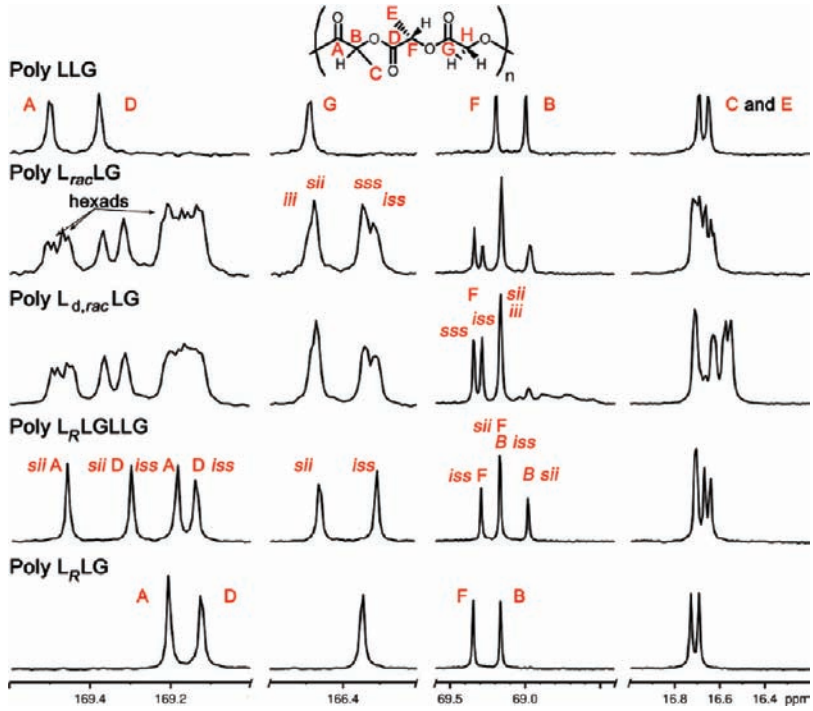


***Figure 11.*** $^{13}$C NMR spectra of L$^{O}$-variable LLG polymers at 175 MHz in $CDCl_3$. Comparisons of the expansions of selected regions for **poly LLG**, **L$_{rac}$LG**, **L$_{d,rac}$LG**, **L$_R$LGLLG**, and **L$_R$LG**.

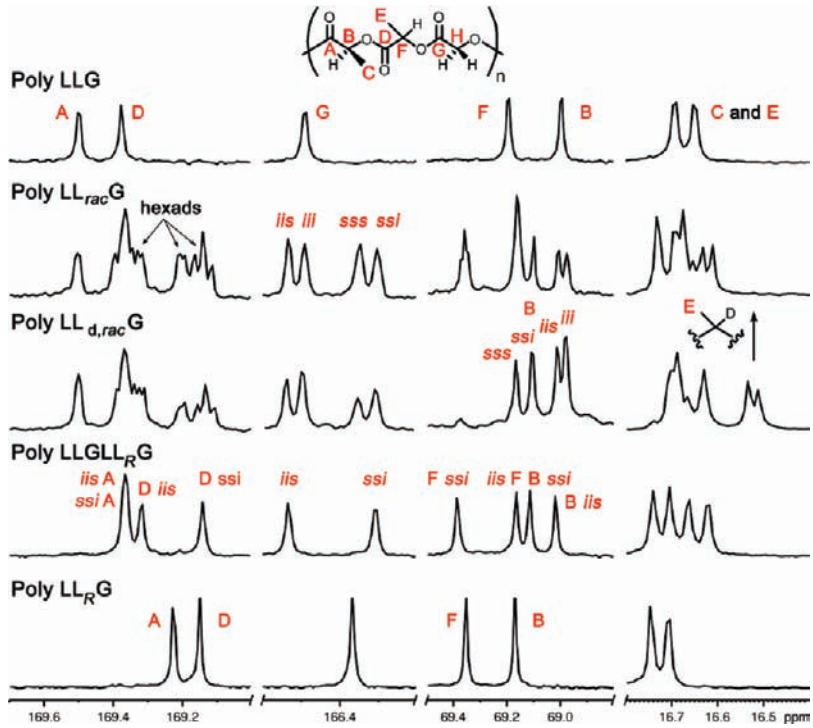


***Figure 12.*** $^{13}$C NMR spectra of L$^{C}$-variable LLG polymers at 175 MHz in $CDCl_3$. Comparisons of the expansions of selected regions for **poly LLG**, **LL$_{rac}$G**, **LL$_{d,rac}$G**, **LLGLL$_R$G**, and **LL$_R$G**.

specified reaction conditions, neither sequence scrambling nor epimerization are significant problems, although in select samples mistakes (<5%) can be observed. The spectrum of **poly LG** (Figure 2, methylene region), for example, shows contamination by syndiotactic sequences.

Sequence was found to have a dramatic effect on the thermal properties of PLGA. In random PLGAs the material properties are primarily dependent on the length of the lactic blocks that generate preferential packing domains.[39] Others have reported that as glycolic content increases, the $T_g$ drops to as low as 36 °C at a 50% glycolic unit incorporation.[39,56,71] RSC PLGAs, on the other hand, maintained $T_g$'s at or above 50 °C even when the glycolic unit content exceeded 60%. As long as the

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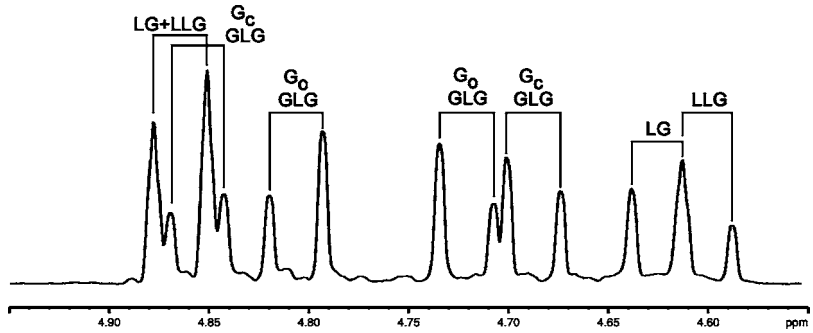


***Figure 13.*** Glycolic methylene region of a mixed $^1$H NMR spectrum for mixed sample (1:1:1) of **poly LG**, **GLG**, and **LLG** at 600 MHz in $CDCl_3$.

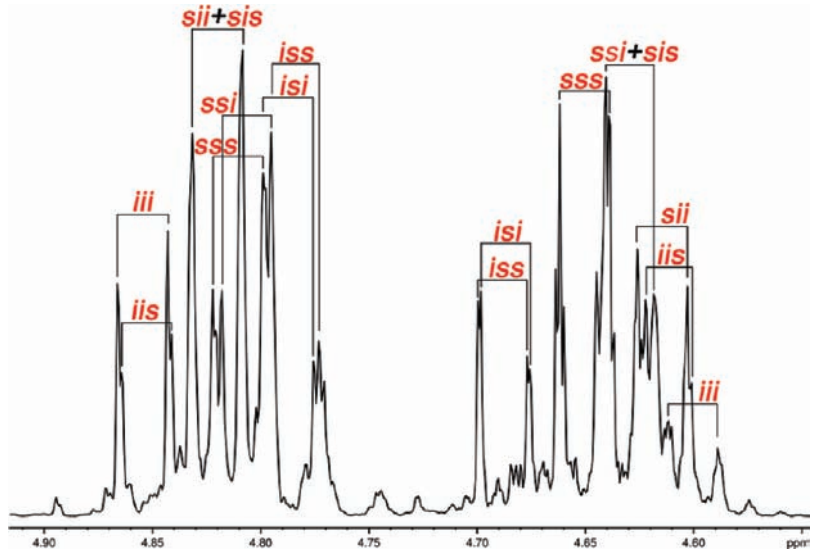


***Figure 14.*** Glycolic methylene region of **poly** $\mathbf{L}_{rac}\mathbf{L}_{rac}\mathbf{G}$ at 700 MHz in $CDCl_3$.

uniformity of the structural sequence remained, altering the stereosequence only slightly lowered the $T_g$. Most of the polymers remained amorphous even after annealing. **Poly LLG**, $\mathbf{L}_R\mathbf{LG}$, and $\mathbf{LL}_R\mathbf{G}$ were the only polymers that displayed a melting transition, and **poly LLG** only crystallized after annealing. Although sequence and stereoregularity should promote crystallinity, it is clear that it is challenging for these polymers to exhibit long-range order based on these relatively short sequences.

**Sequence and NMR.** Our synthetic approach, which allowed for the creation of an unprecedented array of polymer microstructures, facilitates the understanding of the NMR assignments for specific PLGA sequences. The polymers prepared serve as a partial Rosetta Stone for the assignment of PLGA NMR data. Prior assignments of the NMR resonances for PLGA have been based on random copolymers (and a very small set of easily prepared well-defined homo- and copolymers). While this approach has led to significant understanding for a variety of polymers, it is worth noting that controversies over assignments of stereochemistry in the closely related but obviously simpler PLA system have taken years and the input of several groups to resolve.[41,43,46,72] The fact that PLGA has the potential for both structural and stereosequence variability complicates the issue significantly. However, we have been able, using our family of RSCs, not only to make assignments but also to develop a deeper understanding of the issues involved in the interpretation of NMRs of RSCs.

Although the complexity of PLGAs makes the assignment of NMR resonances more challenging than in PLAs, our studies have also shown that the copolymer structure offers compensatory advantages that facilitate interpretation. The most important advantage offered by the copolymer is the exquisite sensitivity of the methylenic protons of the glycolic units to sequence and stereochemistry. The chemical shift range is relatively large and the shifts appear to depend on stereosequence in a hierarchical fashion: the gross chemical shift is determined by the central polyad, while the fine chemical shift depends on longer range relationships. The sensitivity of this signal to stereosequence is much greater than any other $^1$H NMR resonance or the classically more informative $^{13}$C NMR resonances. The second compensatory advantage of PLGAs over PLAs (and other well-studied vinyl polymers) is a function of the fact that the resonances for the two monomers are necessarily distributed over a larger chemical shift range than the resonances for any single monomer and the fact that the polymers are unsymmetric; each monomer and each polymer have distinct C and O termini. Using 2D NMR, these differences were exploited to allow for the definitive assignment of resonances.

The most intriguing single result of the NMR studies of these RSC PLGAs, one that is applicable beyond the narrow scope of these polymers, is the unambiguous demonstration that

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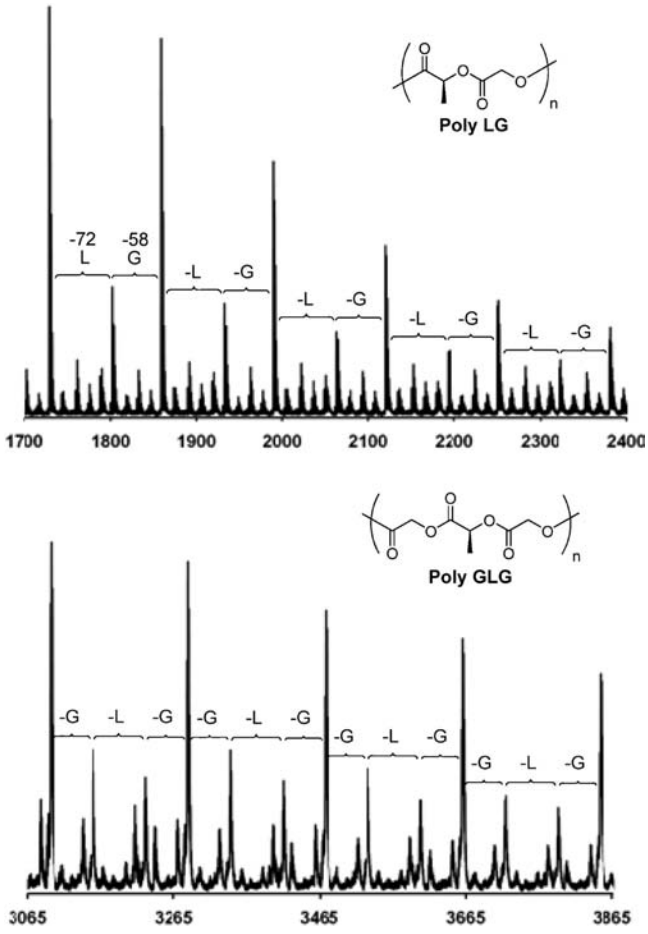


***Figure 15.*** MALDI TOF patterns for **poly LG** (top) and **poly GLG** (bottom).

***Table 5.*** Thermal Properties for RSC PLGAs

| | precipitated | | annealed[a] | |
|---|---|---|---|---|
| polymer | $T_g$ (°C) | $T_m$ (°C) | $T_g$ (°C) | $T_m$ (°C) |
| **LG** | 57[b] | ND | 49[c] | ND |
| **L$_{rac}$G** | 55[b] | ND | 48[c] | ND |
| **GLGL$_R$** | 50[b] | ND | | |
| **GLG** | 50[b] | ND | 43[c] | ND |
| **GL$_{rac}$G** | 50[b] | ND | | |
| **LLG** | 57[b] | ND | 50[c] | 114[c] |
| **L$_R$LG** | 50[b] | 154[c] | | |
| **LL$_R$G** | 48[b] | 158[c] | 48[c] | 155[c] |
| **LLGLL$_R$G** | 52[b] | ND | 48[c] | ND |
| **L$_R$LGLLG** | 52[b] | ND | 48[c] | ND |
| **L$_{rac}$L$_{rac}$G** | 51[b] | ND | 47[c] | ND |
| **L$_{rac}$LG** | 51[b] | ND | 48[c] | ND |
| **LL$_{rac}$G** | 53[b] | ND | 48[c] | ND |

[a] Polymer films were drop-cast into DSC pans, dried under vacuum, and annealed at 85 °C for 3 h. [b] Transitions were measured in the second heating cycle. [c] Transitions were measured in the first cycle.

chemical shift resolution in a polymer bearing multiple sequences *depends on the exact sequence*. Although investigators who have previously analyzed polymers with complex stereosequences have proposed shift assignments based on different levels of resolution in some cases,[42,47] the range of sequence-specific polymers available in our system gives a particularly dramatic validation of the hypothesis. The clearest illustration in our system was found in the methylene region of **poly L$_{rac}$LG**. Examining the upfield proton, four groups of doublets are visible (Figure 9). The leftmost group that corresponds to

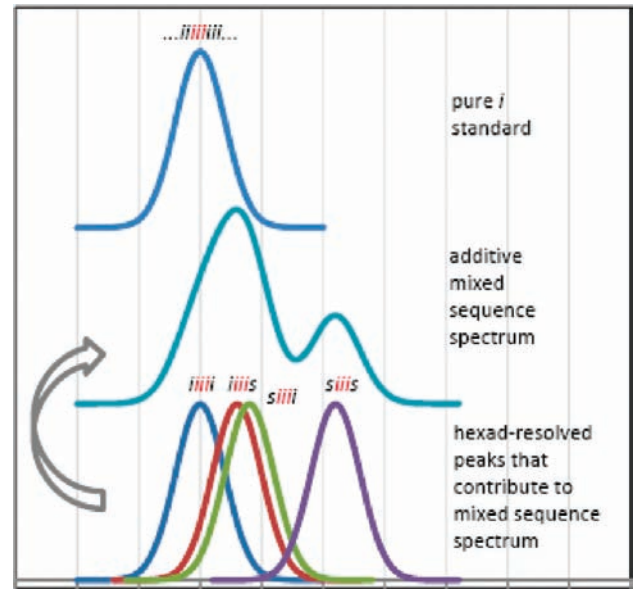


***Figure 16.*** Simulated NMR spectra highlighting the challenges inherent in comparing a standard with perfect stereosequence control to a sample with multiple resolved sequences. Although all sequences share the same "*iii*" central tetrad and exhibit similar chemical shifts, the shifts of the nearly hexad-resolved sequences create a pattern that does not show an easily interpretable correspondence with the standard.

the *iss* tetrad is a simple doublet with no fine structure; this signal is resolved only to the tetrad level. The next group is a barely resolved pair of doublets that corresponds to the gross shift of the *sss* tetrad but which is resolved to a hexad level. The final groups, which exhibit gross chemical shifts consistent with *iii* and *sii* tetrads, exhibit four nearly resolved pairs of doublets that correspond to the much higher octad level of resolution. An octad level of resolution maps to an impressive distance along the backbone of *31 atoms* between the most separated stereocenters. Such sequence-dependent resolution occurs throughout these spectra, although individual signals exhibit varying levels of sensitivity.

Another important lesson learned from the study of RSC PLGAs is that there are some inherent limitations in our ability to make accurate assignments, limitations that are general to the use of exact sequence RSCs as a key to the interpretation of the NMR spectra of samples with mixed sequence chains, no matter what polymer is involved. The first order approach to assigning spectra, one that works in some cases, is to assign the resonances for specific sequences by comparison of the chemical shifts with those of a stereopure standard. Our analysis of the extensive database of shifts for the polymers described herein, however, highlights the potential for misinterpretation in systems where the sensitivity of a resonance to stereochemistry is high and not yet understood. The difficulties are illustrated in the simulated spectrum depicted in Figure 16. If a stereosequence with only "*i*" relationships, for example, is compared to a sample that contains several stereosequences, no peak with a 1:1 shift correspondence to the standard will be found despite the fact that the sample contains a significant (25% in this case) proportion of the "*i*" sequence in the form of "*iiiii*" at a hexad level of resolution. A similar problem would occur if the mixed sample contained only *iiiis* and *siiis* sequences. Despite the fact that both share the same *iii* central tetrad, the shift of neither peak would correspond with that of the "all *i*" standard.

The lack of correlation between stereopure standards and the resonances for individual sequences can thus be attributed to a combination of two phenomena: (1) a full set of standards is not available, i.e., sequences longer than those prepared as standards can be distinguished in mixtures and (2) the ultimate sensitivity of the chemical shift for a particular sequence in a mixture, e.g., tetrad, hexad, etc., is determined by the inherent resolution of the NMR spectrum. We see the effects of both of these phenomena in our spectra in that the shifts of our standards do not exactly match those observed in our mixed sequence polymers. To achieve a 100% correlation, it would have been necessary in the case of **poly** $\mathbf{L}_{rac}\mathbf{L}_{rac}\mathbf{G}$, for example, to prepare all 32 possible octad sequences. As the synthesis of all of these sequenced polymers is impractical, it was necessary to extrapolate from the observed trends from the available standards in assigning certain sequences. The second phenomenon that results in chemical shift miscorrelation, the fact that the certain chemical shift differences are on the same order of magnitude as the peak widths, is a universally recognized spectroscopic challenge and could be overcome by a combination of expanding the database of sequences and "fitting" the mixed spectra. Although the lack of long-sequence standards prevented an analysis of this depth, these phenomena were taken into account when assignments were made.

The broad manifestation of these chemical shift correlation phenomena in our data can readily be observed in the stacked plots of $^1$H NMR spectra for the PLGA RSCs (Figures 8 and 9). The fact that peaks labeled as arising from the same tetrad do not always "line up" is due to the peak matching problems just described, not to poor chemical shift calibration. It should be noted that these issues disproportionally complicate the interpretation of the methine and methyl regions because those regions span a smaller chemical shift range and are not as hierarchical as the methylene region.

The analysis of the NMR data for these polymers has also given us some insight into the specific interpretation of the spectra of PLGAs. In particular, we note that the shift range observed for differing stereosequences within the same structural sequence overlaps exactly with the chemical shift range observed for differing structural sequences. Given the similarity of the monomers involved, it is perhaps not surprising that structural sequence does not introduce a larger perturbation. The bottom line is that, for PLGAs, stereosequence is extremely important in determining the NMR spectral pattern.

**Sequence and Conformation.** One inescapable conclusion to be drawn from the solution-phase behavior of these PLGA RSCs, both NMR and SEC, is that the conformations of these polyesters are sequence-dependent. Conformational differences must, for example, be responsible for the differences in chemical environment exhibited by the diastereotopic methylene protons imbedded in different stereosequences. Conformation is also likely to be responsible for the sequence dependence of the SEC; the $R_g$ is determined by the shape assumed by the polymer in solution.

Although the homology of the monomers in these polymers with those in amino acids, specifically alanine and glycine, renders the sequence-dependence of the conformations unsurprising, the fact that the effect is so strong, given the lack of amide-mediated hydrogen bonding, is perhaps less expected. Indeed, it is common practice to substitute the ester analogue of an amino acid into a peptide to determine the importance of a particular amino acid to the tertiary structure of a protein.[73,74]

Although we cannot yet correlate our NMR spectra with specific conformational preferences the data suggests that there is much to be learned. The fact that we observe a sequence-specific sensitivity to stereochemistry is, for example, intriguing. Such behavior could arise either because specific sequences create conformations that place the diastereotopic methylene protons in better positions to "observe" distant stereochemical relationships, or it could arise because certain sequences simply have stronger conformational preferences (or both). Also relevant to the understanding of the rules governing the preferred conformations in this system is the observation that the chemical shift difference between the two methylenic protons on a particular carbon was consistently smaller for *s*-centered polyads than *i*-centered polyads. An analogous trend has been well-documented in vinyl polymers such as polypropylene.[75,76]

## Conclusions

The solution-phase conformations for RSC PLGAs were found to be extremely sequence- and stereosequence-dependent, analogous to peptides. To access these RSC PLGAs, we have developed a methodology that allows the preparation of complex sequenced copolymers of lactic and glycolic acids from simple monoprotected building blocks. The method is general and convergent and allows for the synthesis of sequences unavailable by other methods such as ROP. Although RSC synthesis can be used to make stereopure NMR standards, the inherent resolution of specific sequences must be taken into account as the level of resolution for a particular nucleus may be sequence-dependent. Improved thermal properties were also a direct result of the uniformity of polymer sequence. We plan to extend this work by undertaking conformational modeling and bulk characterization studies.

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**Supporting Information Available:** Detailed procedures and full characterization of all synthetic intermediates and polymers are provided, along with figures for the $^{13}$C spectra of **poly GLG** and **LG** series, the $^1$H spectra for selected polymers in DMSO, and full MADLI-TOF spectra for **poly LG** and **GLG**. This material is available free of charge via the Internet at http://pubs.acs.org.

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