Synthesis of Urethane Derivatives of Mono- and Diacylglycerols for Use as HPLC Standards in the Enantiomeric Separation

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Abstract This paper presents a convenient method for the preparation of referential standards for high-performance liquid chromatography (HPLC) used in stereospecific analysis of triacyl-*sn*-glycerols *via* monoacylglycerol or diacylglycerol intermediates. In the analysis, these partial acylglycerols are separated into their respective positional and enantiomeric isomer classes by chiral HPLC as their 3,5-dinitrophenylurethane derivatives or by silicic acid HPLC as their (*S*)- or (*R*)-1-(1-naphthyl)ethyl urethane derivatives. In this study, these urethane derivative standards were synthesized by the following novel procedure: first, partial urethane derivatives of glycerol were prepared by carbamoylation of glycerol with isocyanates; secondly, the products were separated into positional isomer classes by silicic acid HPLC, and; finally, a fatty acid was added to the partial urethanes using *N*,*N*'-dicyclohexylcarbodiimide. The identities of the resulting urethane derivatives of glycerol were verified by mass spectrometry and HPLC. This new procedure is advantageous in that standard urethane derivatives of partial acylglycerols can be synthesized from no more than 50 μg of fatty acids. This benefit is especially important in the case of rare and expensive fatty acids, such as very long chain polyunsaturated fatty acids, tetracosahexaenoic acid, and hexacosaheptaenoic acid, found in marine lipids.

Keywords 3,5-Dintrophenylurethane, 1-(1-naphthyl)ethyl urethane, triacylglycerol, diacylglycerol, monoacylglycerol, enantiomer, diastereomer, HPLC, stereospecific analysis

Introduction

One of the most fundamental lipid analytical techniques is fatty-acid analysis of the *sn*-1, *sn*-2, and *sn*-3 positions of triacyl-*sn*-glycerols (TAG)—so called stereospecific analysis of TAG [1]. In this method, which employs high-performance liquid chromatography (HPLC), TAG are partially degraded to monoacylglycerols (MAG) or diacylglycerols (DAG) by the addition of a Grignard reagent, derivatized to 3,5-dinitrophenylurethane (3,5-DNPU), and then resolved by chiral HPLC into their respective *sn*-1-, *sn*-2-, and *sn*-3-MAG [2,3] or *sn*-1,2- and *sn*-2,3-DAG derivatives [4,5]. Fatty acids of each isomer class are analyzed by gas chromatography. In another method, which employs inexpensive silicic acid HPLC, MAG or DAG are transformed into diastereomeric (*S*)- or (*R*)-1-(1-naphthyl)ethyl urethane derivatives, which are then resolved into the corresponding *sn*-1-, *sn*-2-, and *sn*-3-MAG [6] or *sn*-1,2- and *sn*-2,3-DAG derivatives [7,8]. However, in both HPLC methods, it is essential to precisely separate each stereoisomer of MAG and DAG. Urethane derivatives of MAG and DAG standards containing various kinds of fatty acids are useful for determining optimal resolution conditions for separating the isomer classes of MAG and DAG.

The most straightforward procedure for synthesis of MAG and DAG urethane standards involves the carbamoylation of the parent MAG or DAG with 3,5-dinitrophenyl isocyanate or 1-(1-naphthyl)ethyl isocyanate in a manner similar to those carried out in the stereospecific analysis [2-8]. Standard *sn*-1(3)-MAG and *sn*-1,2(2,3)-DAG can be synthesized by starting with 1,2(2,3)-*O*-isopropylidene-*sn*-glycerol and free fatty acid [9,10], and *sn*-1,3-DAG by starting with 1,3-dihydroxyacetone [11]. MAG and DAG standards can be also formed from the parent TAG. However, these procedures are time-consuming as they involve multiple reaction and purification steps. Another disadvantage of these procedures is that most need at least milligram quantities of fatty acids, especially in the case of synthesis from TAG. Because of rapid acyl migration [12], *sn*-2-MAG derivatives have to be prepared from TAG by Grignard degradation

and immediately converted to the urethane derivative [3]. However, yields of *sn*-2-MAG formed by reaction of TAG with ethylmagnesium bromide are only about 2–10 wt% of the TAG starting material [13,14].

In this report, a novel procedure for the synthesis of referential standard MAG and DAG urethane derivatives is presented. This procedure, shown in Scheme 1, includes the partial carbamoylation of glycerol with isocyanates, isolation of mono- and di-urethanes of glycerol by HPLC, and acylation of their hydroxyl groups with fatty acids. This paper describes the procedure, reaction conditions, HPLC separation, and application to synthesis of standards containing rare fatty acids.

Materials and Methods

Materials

Glycerol obtained from Wako Pure Chemical (Osaka, Japan) was stored over 4Å molecular sieves (beads, 8–14 mesh) for dryness. 3,5-Dinitrophenyl isocyanate was obtained from Sumika Chemical Analysis Service (Osaka, Japan), and (*S*)-(+)-1-(1-naphthyl)ethyl isocyanate (99%; optical purity, 96%) and (*R*)-(−)-1-(1-naphthyl)ethyl isocyanate (98%; optical purity, 95%) from Aldrich (Sigma-Aldrich Japan, Tokyo, Japan). Dry toluene, dry pyridine, and *N*,*N*′-dicyclohexylcarbodiimide (DCC) were obtained from Kanto Chemical (Tokyo, Japan). 4-Dimethylaminopyridine (4-DMAP) was purchased from Merck (Darmstadt, Germany). Palmitic acid (16:0; free form), eicosapentaenoic acid (20:5; ethyl ester), and docosahexaenoic acid (22:6; ethyl ester) were obtained with purities higher than 99%. Tetracosahexaenoic acid (24:6) and hexacosaheptaenoic acid (26:7) were concentrated from total fatty acids of roughscale sole in the form of methyl esters [15]. The ethyl and methyl esters were used after saponification.

Enantiomeric 1- and 3-palmitoyl-*sn*-glycerols (*sn*-1- and *sn*-3-16:0-MAG) were synthesized by addition of 16:0 to (*R*)-(−)-2,3- and (*S*)-(+)-1,2-*O*-isopropylidene-*sn*-glycerols

(optical purity, > 98%; Tokyo Chemical Industry, Tokyo, Japan), respectively, followed by acidification using trifluoroacetic acid [9,10]. 1,2-Dipalmitoyl-*sn*-glycerol (*sn*-1,2-16:0-DAG) and 1,2(2,3)-dipalmitoyl-*rac*-glycerol (*rac*-1,2(2,3)-16:0-DAG) were obtained from Sigma (Sigma-Aldrich Japan). Their positional isomers, *sn*-2-16:0-MAG and *sn*-1,3-16:0-DAG, were isolated using boric acid TLC from the products of partial degradation of tripalmitoylglycerol by reaction with ethylmagnesium bromide [2].

Partial Carbamoylation of Glycerol with Isocyanates

The glassware used in this step were preliminarily dried over a flame and cooled in a desiccator to room temperature.

Glycerol mono- and di-3,5-DNPU derivatives (**3a–8a**). To a 10 mL screw-capped test tube were added dry glycerol (**1**; 0.12 mmol = 11.0 mg), dry toluene/dry pyridine (10:3, v/v; 1 mL), and 3,5-dinitrophenyl isocyanate (**2a**; 0.06 mmol = 12.5 mg). The solid block of isocyanate was crushed after its addition to the reaction mixture to prevent it from being exposed to the atmospheric moisture. The test tube was allowed to stand at room temperature for 1 h in the dark. After 1-propanol (10 μL) was added to stop the reaction (with standing for 10 min), the solvents were removed in a stream of nitrogen. The products were dissolved in 1 mL of ethanol.

Glycerol mono- and di-(S)- or (R)-1-(1-naphthyl)ethyl urethane derivatives (**3b,c–8b,c**). To a 10 mL screw-capped test tube were added dry glycerol (**1**; 0.06 mmol = 5.5 mg), dry toluene/dry pyridine (10:3, v/v; 2 mL), 4-DMAP (4 mg), and (*S*)- or (*R*)-1-(1-naphthyl)ethyl isocyanate (**2b,c**; 0.12 mmol = 23.7 mg), and held at 50 °C overnight in the dark. After 1-propanol (10 μL) was added to stop the reaction (with standing over 10 min), solvents were removed under a stream of nitrogen. The products were dissolved in 1 mL of chloroform.

Preparation of Fatty Acid Adduct of Glycerol Urethane Derivatives

MAG and DAG urethane derivatives (**10–15**). To a 1 mL reaction vial containing isomers of glycerol mono-urethane derivatives (**3–5**; 0.015 μmol = 4.3–4.5 μg) or di-urethane derivatives (**6–8**; 0.03 μmol = 14.6–15.3 μg) were added fatty acid (**9**; 0.033 μmol = 10 μg for di-urethanes and 0.165 μmol = 50 μg for mono-urethanes), a solution of 4-DMAP (0.03 μmol = 3.7 μg) in carbon tetrachloride (0.2 mL), and a solution of DCC (1.1 molar equivalent of fatty acids) in carbon tetrachloride (10 μL). After the mixture was stirred at room temperature for 2 h, 1-propanol (10 μL) was added to stop the reaction. The solution was filtered through a small cotton-wool plug. The solvents were removed in a stream of nitrogen. The products were dissolved in 0.1 mL of chloroform.

HPLC

Silicic acid HPLC separation of the glycerol urethane derivatives (**3–8**) was carried out with a Hitachi L-6200 pump (Hitachi, Tokyo, Japan), a Shimadzu CTO-10ASvp column oven (Shimadzu, Kyoto, Japan), a Jasco 875-UV/VIS detector (Jasco, Tokyo, Japan), and a Shimadzu C-R6A integrator. A LiChrospher Si 60 column (25 cm × 4.6 mm id, 5 μm particles; Merck) was used at 25 °C eluting with HPLC-grade hexane (A) and hexane/dichloromethane/ethanol (40:12:3, v/v/v) (B) as the mobile phase at a flow rate of 1 mL/min. Elution starting with A/B (75:25) (0 min) was gradually changed to A/B (0:100) over 30 min and then held at B for 20 min. UV detection was at 254 and 280 nm for the 3,5-DNPU and 1-(1-naphthyl)ethyl urethane derivatives, respectively.

Silicic acid HPLC analysis of MAG and DAG urethane derivatives (**10–15**) was carried out with a Shimadzu LC-6A pump, a Hitachi L-4200 UV/VIS detector, and a Shimadzu C-R3A integrator. A column of Inertsil SIL-100A (25 cm × 4.0 mm id, 5 μm particles; GL Sciences, Tokyo, Japan) was used at 30 °C with hexane/2-propanol in 97:3 (v/v) and 99:1 (v/v)

compositions as the mobile phase for MAG and DAG 3,5-DNPU derivatives, respectively. For the analyses of MAG and DAG 1-(1-naphthyl)ethyl urethanes, hexane/2-propanol in 97:3 (v/v) and 99.2:0.8 (v/v) compositions were used as the mobile phase, respectively. The flow rate of the mobile phases was at 1 mL/min.

Chiral HPLC analysis of MAG and DAG 3,5-DNPU derivatives (**10a–15a**) was carried out with the same system as described above. Analyses were performed with a Sumichiral OA-4100 column (25 cm × 4.6 mm id, 5 μm particles; Sumika Chemical Analysis Service) at 30°C with a hexane/dichloromethane/ethanol mobile phase of 40:12:3 (v/v/v) and 40:10:1 (v/v/v) compositions for MAG and DAG urethane derivatives, respectively, at a flow rate of 1.0 mL/min.

Mass Spectrometry (MS)

Mass spectra of the isomers of glycerol urethane derivatives were obtained with a Thermo Scientific Exactive mass spectrometer (Thermo Fisher Scientific, Yokohama, Japan) equipped with an atmospheric pressure chemical ionization (APCI) source, operating in the positive and negative ion modes. The MS data were acquired in the full-scan mode in the mass-to-charge ratio (*m*/*z*) range of 150–2000. The conditions for APCI spectra were as follows: sheath gas flow rate, 20 units; auxiliary gas flow rate, 0 units; vaporizer temperature, 250 °C; discharge current, 5.0 μA; and skimmer voltage, 30 V.

Results and Discussion

HPLC Separation of Isomers of Glycerol Mono- and Di-urethane Derivatives

Figure 1 shows the chromatograms of glycerol urethane derivatives with 3,5-dinitrophenyl isocyanate and (*S*)- and (*R*)-1-(1-naphthyl)ethyl isocyanates. The urethane peaks were identified by APCI-MS after collection as described below. In the silicic acid HPLC, the isomers eluted in

the order of glycerol *sn*-1,3-di-urethane, *sn*-1,2(2,3)-di-urethanes, *sn*-2-mono-urethane, and finally *sn*-1(3)-mono-urethanes. In the HPLC of 3,5-DNPU derivatives (Fig. 1a), enantiomeric *sn*-1,2- and 2,3-di-urethanes, and *sn*-1- and 3-mono-urethanes co-eluted, with a total of four peaks corresponding to the partial glycerol urethanes appearing in the chromatogram. In the HPLC of 1-(1-naphthyl)ethyl urethanes, diastereomeric *sn*-1,2- and 2,3-di-urethanes resolved into two peaks in this order for the (*S*)-isomer (Fig. 1b) and in the reversed order for the (*R*)-isomer (Fig. 1c). The diastereomers of *sn*-1- and 3-monourethanes could not be separated.

APCI-MS operating in negative-ion mode gave $[M-H]^-$ ions at *m*/*z* 509 and 300, corresponding to di- and mono-3,5-dinitrophenyl urethanes, respectively, and that in positive-ion mode gave $[M+H]^+$ ions at *m*/*z* 487 and 290 corresponding to di- and mono-1-(1-naphthyl)ethyl urethanes, respectively. Fragment ions diagnostic of the urethanes also appeared in the spectra. These results show that the components indicated in Fig. 1 are glycerol urethanes, and confirm the successful partial carbamoylation of glycerol with the isocyanates.

Conditions for Partial Carbamoylation of Glycerol

Partial carbamoylation was conducted with various glycerol/isocyanate ratios. A portion of the products was analyzed by silicic acid HPLC. Figure 2 shows the absolute yield (mg) of each isomer, which was determined using the calibration plots of peak areas *vs*. known amounts of mono- and di-urethanes. The yields of glycerol mono-urethanes were higher at a higher glycerol/isocyanate ratio.

The yields of di-urethanes were highest at a glycerol/isocyanate ratio of 2 in the reaction with 3,5-dinitrophenyl isocyanate (Fig. 2a), and at 0.33–0.55 in those with 1-(1-naphthyl)ethyl isocyanates (Figs. 2c and 2c). Compared with 3,5-dinitrophenyl isocyanate, 1-(1-naphthyl)ethyl isocyanates needed a lower proportion of glycerol to the reagents. Reactivities of 1-(1-naphthyl)ethyl isocyanates were found to be lower than that of 3,5-dinitrophenyl isocyanate.

195 The best yield for the partial carbamoylation of glycerol was dependent on the number of
196 urethanes and the structure of urethane residues.

197 The yields of *sn*-1(3)-mono-urethanes were much higher than those of *sn*-2-
198 monourethanes. The primary 1,3-positions of glycerol appeared to react with isocyanates faster
199 than the secondary 2-position, as primary alcohols are generally lower in pK_a than secondary
200 alcohols. The lower yields of *sn*-1,2(2,3)-di-urethanes than those of *sn*-1,3-di-urethanes
201 probably resulted from steric hindrance. It is also possible that the urethanes need an adjacent
202 secondary hydroxyl group to form a stable transition form or intermediate that promote
203 formation of the final product. In the present study, it was impossible to boost the yields of
204 1,2(2,3)-di-urethanes to levels as found for the 1,3-isomers.

205

206 HPLC of MAG and DAG Urethanes

207 Each mono- and di-urethane glycerol derivative collected from silicic acid HPLC (Fig. 1) was
208 acylated with palmitic acid in the presence of DCC. The products were subjected to chiral and
209 silicic acid HPLC without purification. Figure 3 shows chromatograms of the MAG and DAG
210 urethane products. Simple peaks were observed for the isomers in the HPLC analysis. Retention
211 times of the peaks were close to those of MAG and DAG urethanes that were prepared by
212 carbamoylation of 16:0-MAG and 16:0-DAG with 3,5-dinitrophenyl and 1-(1-naphthyl)ethyl
213 isocyanates in the typical procedures [2-8]. Co-injection with the MAG and DAG urethanes
214 gave a single peak for each isomer. These results show that the MAG and DAG urethane
215 standards can be synthesized by the present new procedure. Additionally, the peaks of glycerol
216 urethanes in silicic acid HPLC are identified, as indicated in Fig. 1.

217 The new procedure was applied to the preparation of MAG and DAG urethanes
218 containing highly polyunsaturated fatty acids. Acylation of glycerol urethanes was possible even
219 with bulky fatty acids such as 20:5 and 22:6. Table 1 summarizes relative retention times of the

MAG and DAG urethanes observed in the chiral and silicic acid HPLC analysis. As found in the previous study [16,17], 3,5-DNPU derivatives of 20:5- and 22:6-MAG were almost co-eluted at a later retention time than 16:0-MAG derivatives on the Sumichiral OA-4100 column. Isomeric *sn*-1- and *sn*-2-MAG were inseparable on this column [2,3,17]. In contrast, these isomers could be separated on the Inertsil SIL100A silica column. Similar results were observed for the 3,5-DNPU derivatives of DAG.

Diastereomeric 1-(1-naphthyl)ethyl urethane derivatives originating from enantiomeric *sn*-1(3)-MAG and *sn*-1,2(2,3)-DAG can be resolved by achiral silicic acid HPLC [6-8]. The present study confirmed the previous findings. Under the HPLC conditions used in this study, 20:5- and 22:6-MAG and DAG eluted ahead of 16:0-MAG and DAG, respectively.

This is the first report providing retention data on rare 24:6 and 26:7-containing MAG and DAG urethanes. In our previous study [18], stereospecific analyses of 24:6-containing triacylglycerols were carried out with the assumption that the 3,5-DNPU derivative of 24:6-MAG behaves chromatographically in a manner similar to that of 22:6-MAG on the Sumichiral OA-4100 chiral column. In TAG obtained from flathead flounder, 24:6 was preferentially located in the *sn*-2 position, followed by *sn*-3 and *sn*-1 positions [18]. The present results shown in Table 1 indicate that the assumption is likely true. All retention data of 24:6-MAG and DAG on OA-4100 were observed to lie between those of 16:0 and 22:6. In addition, the retention times of 26:7-MAG and DAG urethanes were somewhat lower than those of 24:6-MAG and DAG. Commonly found 20:5 and 22:6-containing MAG and DAG derivatives are found to be usable as markers for separation of rare very-long-chain polyunsaturated fatty acid–containing MAG and DAG compounds.

Conclusion

In conclusion, MAG and DAG urethane standards can be prepared by way of mono- and di-

urethanes of glycerol. Once the intermediates are synthesized and correctly fractionated by silicic acid HPLC, they are easily converted to MAG and DAG urethanes containing desirable fatty acid substituents. The intermediates can also be stored for a long period without the worry of isomerization, in this way differing from the free forms of MAG and DAG. This new procedure is convenient and can be applicable to the preparation of rare fatty acid–containing urethanes.

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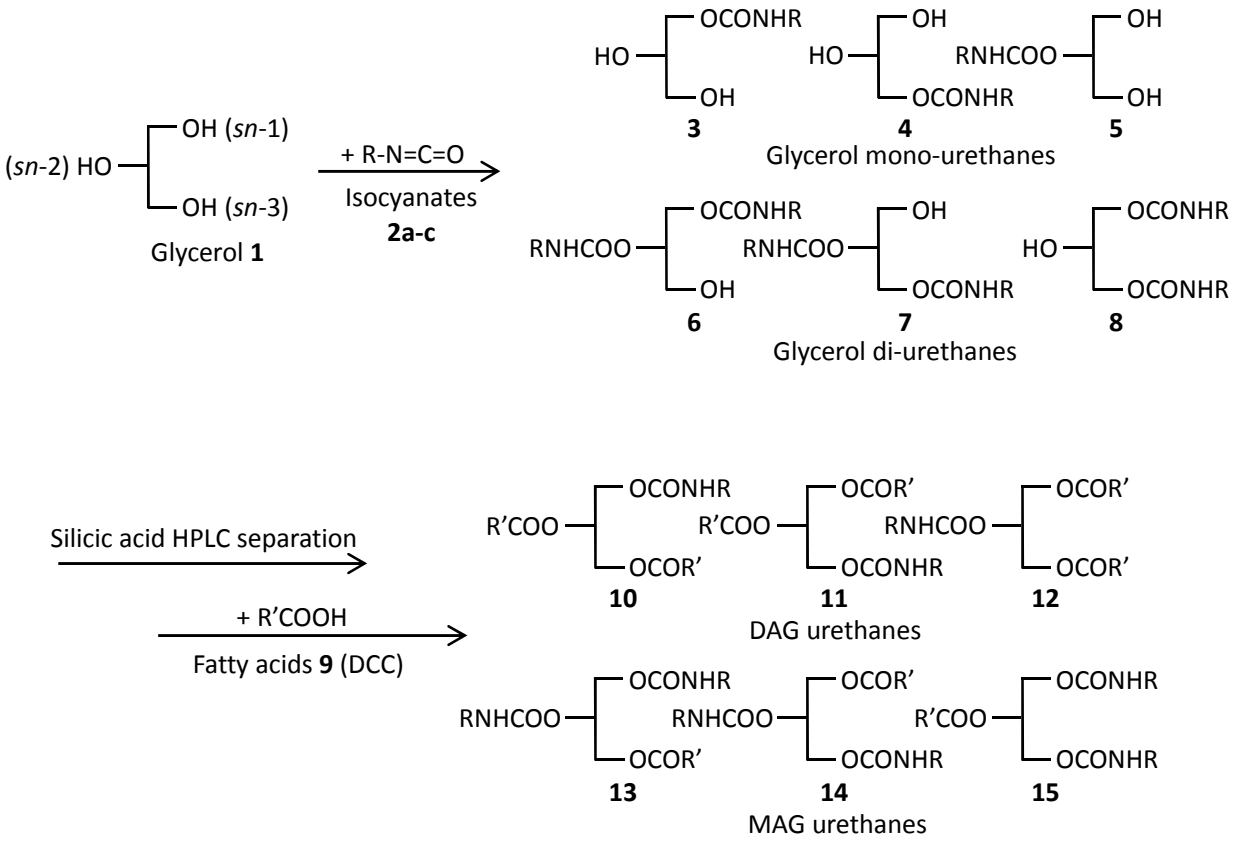
Figure captions

Fig. 1 Silicic acid HPLC of glycerol mono- and di-urethanes prepared by partial carbamoylation of glycerol with **a** 3,5-dinitrophenyl, **b** (*S*)-1-(1-naphthyl)ethyl, and **c** (*R*)-1-(1-naphthyl)ethyl isocyanates. Column, LiChrospher Si 60 (25 cm × 4.6 mm id, 5 μm particles); column temperature, 25 °C; mobile phase, hexane (A) and hexane/dichloromethane/ethanol (40:12:3, v/v/v) (B) starting with A/B (75:25) (0 min), gradually changed to A/B (0:100) over 30 min and then held at B for 20 min; flow rate, 1 mL/min; detection, **a** 254 nm and **b,c** 280 nm. The components of the non-labeled peaks were not identified in this study.

Fig. 2 Yields of glycerol mono- and di-urethanes generated by partial carbamoylation at various ratios of glycerol to **a** 3,5-dinitrophenyl, **b** (*S*)-1-(1-naphthyl)ethyl, and **c** (*R*)-1-(1-naphthyl)ethyl isocyanates.

Fig. 3 HPLC of MAG and DAG urethane derivatives prepared by acylation of glycerol mono- and di-urethanes with palmitic acid (16:0). **a** 3,5-DNPU derivatives on Sumichiral OA-4100 (25 cm × 4.6 mm id, 5 μm particles). **b** 3,5-DNPU derivatives, **c** (*S*)-1-(1-naphthyl)ethyl urethanes, and **d** (*R*)-1-(1-naphthyl)ethyl urethanes on Inertsil SIL 100A (25 cm × 4.0 mm id, 5 μm particles). Column temperature; 30 °C; mobile phase, **a** hexane/dichloromethane/ethanol (40:12:3, v/v/v) and (40:10:1, v/v/v) for MAG and DAG, respectively, **b** hexane/2-propanol (97:3, v/v) and (99:1, v/v) for MAG and DAG, respectively, **c,d** hexane/2-propanol (97:3, v/v) and (99.2:0.8, v/v) for MAG and DAG, respectively; flow rate, 1 mL/min.


(sn-2) HO
OH (sn-1)
OH (sn-3)
Glycerol 1
+ R-N=C=O
Isocyanates
2a-c
HO
OCONHR
OH
3
HO
OH
OCONHR
4
RNHCOO
OH
OH
5
Glycerol mono-urethanes
RNHCOO
OCONHR
OH
6
RNHCOO
OH
OCONHR
7
HO
OCONHR
OCONHR
8
Glycerol di-urethanes
Silicic acid HPLC separation
+ R'COOH
Fatty acids 9 (DCC)
R'COO
OCONHR
OCOR'
10
R'COO
OCOR'
OCONHR
11
RNHCOO
OCOR'
OCOR'
12
DAG urethanes
RNHCOO
OCONHR
OCOR'
13
RNHCOO
OCOR'
OCONHR
14
R'COO
OCONHR
OCONHR
15
MAG urethanes
R =
NO2
NO2
a
3,5-dinitrophenyl
CH3
b
(S)-1-(1-naphthyl)ethyl
CH3
c
(R)-1-(1-naphthyl)ethyl

Scheme 1

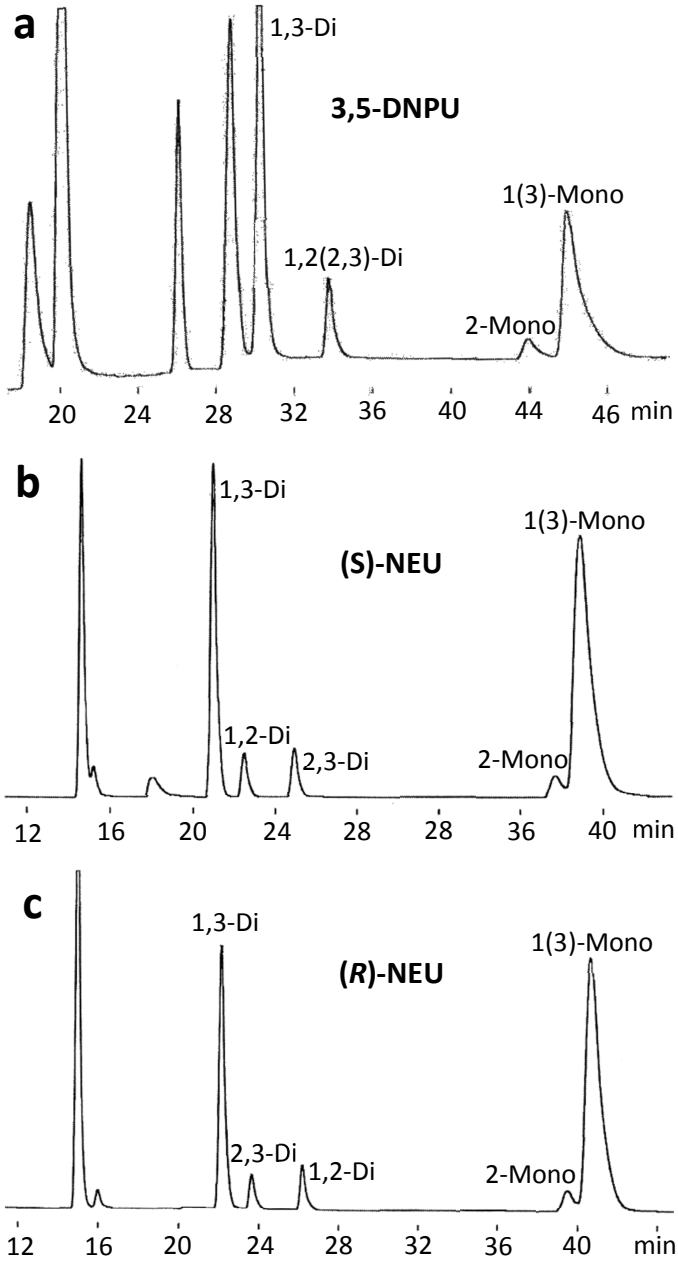

a
1,3-Di
3,5-DNPU
1(3)-Mono
1,2(2,3)-Di
2-Mono
20 24 28 32 36 40 44 46 min
b
1,3-Di
1(3)-Mono
(S)-NEU
1,2-Di
2,3-Di
2-Mono
12 16 20 24 28 28 36 40 min
c
1,3-Di
1(3)-Mono
(R)-NEU
2,3-Di
1,2-Di
2-Mono
12 16 20 24 28 32 36 40 min

Fig. 1

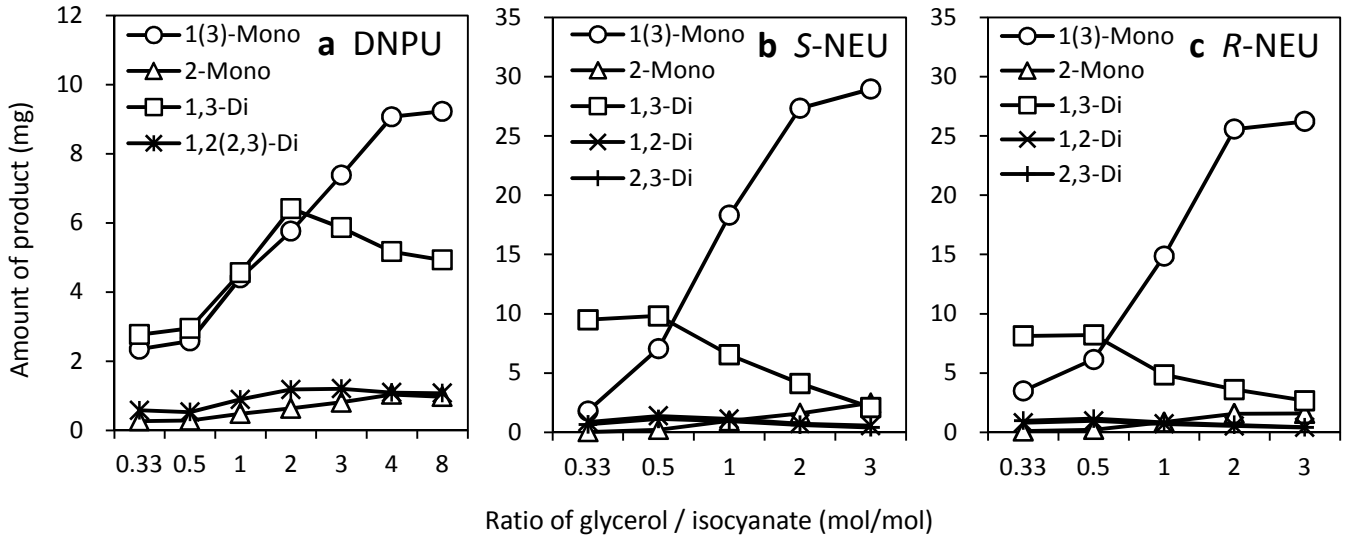

Amount of product (mg)
a DNPU
b S-NEU
c R-NEU
1(3)-Mono
2-Mono
1,3-Di
1,2(2,3)-Di
1,2-Di
2,3-Di
0.33
0.5
1
2
3
4
8
Ratio of glycerol / isocyanate (mol/mol)

Fig. 2

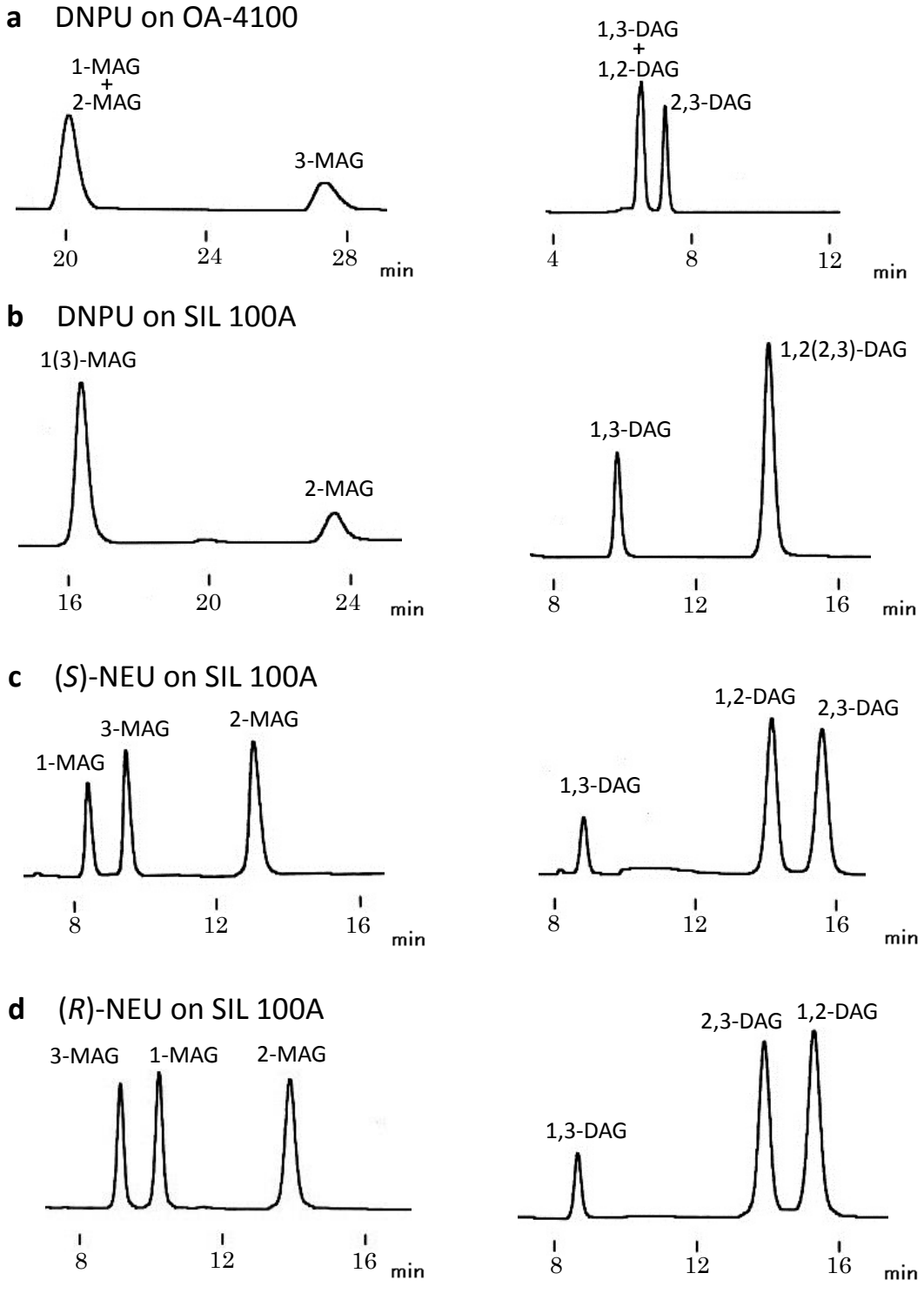

a DNPU on OA-4100
1-MAG
+
2-MAG
3-MAG
20
24
28
min
1,3-DAG
+
1,2-DAG
2,3-DAG
4
8
12
min
b DNPU on SIL 100A
1(3)-MAG
2-MAG
16
20
24
min
1,3-DAG
1,2(2,3)-DAG
8
12
16
min
c (S)-NEU on SIL 100A
1-MAG
3-MAG
2-MAG
8
12
16
min
1,3-DAG
1,2-DAG
2,3-DAG
8
12
16
min
d (R)-NEU on SIL 100A
3-MAG
1-MAG
2-MAG
8
12
16
min
1,3-DAG
2,3-DAG
1,2-DAG
8
12
16
min

Fig. 3

Table 1 Relative retention times (RRT) of MAG and DAG urethane derivatives on HPLC

Urethane on HPLC[c]	Acyl group	RRT of MAG isomer[a]			RRT of DAG isomer[b]		
		sn-1	*sn*-3	*sn*-2	*sn*-1,2	*sn*-2,3	*sn*-1,3
3,5-DNPU	16:0	1.00	1.43	1	1.00	1.23	1
on Sumichiral	20:5	1.15	1.65	1.15	1.34	1.60	1.34
OA-4100	22:6	1.14	1.66	1.14	1.34	1.57	1.34
	24:6	1.07	1.56	1.07	1.21	1.43	1.21
	26:7	1.06	1.53	1.06	1.17	1.39	1.17
3,5-DNPU	16:0	0.65	0.65	1	1.59	1.59	1
on Inertsil	20:5	0.65	0.65	1.00	1.59	1.59	0.95
SIL100A	22:6	0.66	0.66	1.00	1.50	1.50	0.96
	24:6	0.66	0.66	1.00	1.54	1.54	0.91
	26:7	0.62	0.62	0.94	1.34	1.34	0.86
(*S*)-NEU	16:0	0.52	0.63	1	1.84	2.06	1
on Inertsil	20:5	0.50	0.59	0.91	1.57	1.73	0.95
SIL100A	22:6	0.49	0.57	0.82	1.40	1.54	0.91
	24:6	0.49	0.58	0.87	1.49	1.63	0.90
	26:7	0.43	0.53	0.81	1.23	1.35	0.78
(*R*)-NEU	16:0	0.65	0.53	1	2.15	1.91	1
on Inertsil	20:5	0.61	0.53	0.92	1.77	1.60	0.94
SIL100A	22:6	0.59	0.50	0.84	1.57	1.42	0.90
	24:6	0.60	0.49	0.89	1.67	1.56	0.89
	26:7	0.57	0.43	0.83	1.38	1.25	0.76

MAG, monoacylglycerols; DAG, diacylglycerols;

DNPU, dinitrophenyl urethnaes; NEU, 1-(1-naphthyl)ethyl urethanes

[a] $RRT = (RT_{MAG} - RT_{hexane})/(RT_{sn\text{-}2\text{-}16:0\text{-}MAG} - RT_{hexane})$, where RT = retention times (min) of each MAG isomer, *sn*-2-16:0-MAG, and hexane (3.167 for OA-4100 and 2.913 for SIL100).

[b] $RRT = (RT_{DAG} - RT_{hexane})/(RT_{sn\text{-}1,3\text{-}16:0\text{-}DAG} - RT_{hexane})$, where RT = retention times (min) of each DAG isomer, *sn*-1,3-16:0-DAG, and hexane (3.167 for OA-4100 and 2.913 for SIL100).

[c] See text for the HPLC conditions.