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Short communication

Preparation of novel fluoroalkyl end-capped oligomeric nanoparticles-encapsulated hibitane

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Abstract

Fluoroalkyl end-capped *N*-(1,1-dimethyl-3-oxobutyl)acrylamide oligomer [R_F–(DOBAA)$_n$–R_F] reacted with hibitane in methanol at 90 °C to afford R_F–(DOBAA)$_n$–R_F oligomeric nanoparticles-encapsulated hibitane in good isolated yields. These fluorinated oligomeric particles-encapsulated hibitane were nanometer size-controlled very fine particles, and were found to exhibit a good dispersibility and stability in a wide variety of traditional organic solvents including fluorinated aliphatic solvents. Each dispersed solution with fluorinated nanoparticles afforded transparent colorless solution. These fluorinated nanoparticles were also found to exhibit a good antibacterial activity, and were applied to the surface modification of traditional organic polymers such as poly(methyl methacrylate).

Keywords: Fluorinated oligomer; Oligomeric nanoparticle; Hibitane; Anti-bacterial activity; DLS; SEM; Surface modification

1. Introduction

In recent years, polymeric nanoparticles have great interest for their potential applications in a wide variety of fields such as medicine and nanotechnological devices [1]. For example, colloidal stable polymeric nanoparticles have been prepared by cross-linking of polymeric assemblies constructed from amphiphilic block copolymers [2]. Hitherto, it has been demonstrated that ABA triblock-type fluoroalkyl end-capped oligomers are attractive fluorinated polysoaps due to exhibit a variety of unique properties such as surface active properties, good solubilities, and nanometer size-controlled self-assembled molecular aggregates that set them apart from the corresponding non-fluorinated and randomly fluoroalkylated ones [3]. Therefore, it is in particular interest to prepare new fluorinated polymeric nanoparticls possessing a biological activity by the use of fluoroalkyl end-capped oligomers, from the applicable viewpoints of fluorinated polymeric materials into biomedical fields. Usually, it is well-known that hibitane [chlorhexidine dihydrochloride {1,1′-hexamethylenebis[(5-(4-chlorophenyl)biguanide) dihydrochloride]} is a derivative of guanidine and this compound is one of the potent low-molecular weight biocides [4]. It is expected that fluoroalkyl end-capped oligomers containing carbonyl groups such as fluoroalkyl end-capped *N*-(1,1-dimethyl-3-oxobutyl)acrylamide oligomers [R_F–($CH_2CHC(=O)NHCMe_2CH_2C(=O)$-O)Me)$_n$–$R_F$:$R_F$–(DOBAA)$_n$–$R_F$; R_F = fluoroalkyl groups] should interact with some guanidine units in hibitane to afford new fluorinated oligomeric nanoparticles-encapsulated hibitane. In fact, new fluoroalky end-capped oligomeric nanoparticles-encapsulated hibitane possessing a good antibacterial activity have been prepared by reaction of the corresponding fluorinated oligomer with hibitane. In this communication, we would like to demonstrate on the preparation and antibacterial activity of new fluorinated oligomeric nanoparticles-encapsulated hibitane. We believe that this finding is a first report on the preparation of fluorinated polymeric nanoparticles by the use of hibitane.

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2. Results and discussion

A methanol solution of R_F–(DOBAA)$_n$–R_F oligomer containing hibitane was stirred well at room temperature for 1 h, and then placed in a water bath at 90 °C for 1 h to evaporate the solvent under reduced pressure. A well-dispersed colloidal solution was obtained after the addition of a 1,2-dichloroethane solution to the obtained crude products. The expected R_F–(DOBAA)$_n$–R_F oligomeric nanoparticles-encapsulated hibitane can be easily isolated by the simple filtration of this colloidal solutions to remove the unreacted hibitane, because the parent hibitane has no solubility in 1,2-dichoroethane. These results are shown in Scheme 1.

As shown in Scheme 1, R_F–(DOBAA)$_n$–R_F oligomeric nanoparticles-encapsulated hibitane were obtained from 46 to 92% isolated yields. The yields of the nanoparticles were found to increase with decreasing the feed ratios of hibitane/R_F–(DOBAA)$_n$–R_F. The highest yield (92%) of the nanoparticles was obtained in the ratio of 1.3/4.0 (μmol/μmol), indicating that the moderate amounts of guanidine units in hibitane should interact with carbonyl groups in oligomers to afford the expected fluorinated oligomeric nanoparticles-encapsulated hibitane.

UV–vis spectra of R_F–(DOBAA)$_n$–R_F oligomeric nanoparticles-encapsulated hibitane in methanol showed an absorption band around 257 nm, although original R_F–(DOBAA)$_n$–R_F oligomer in methanol shows an absorption band around 282 nm related to the carbonyl groups in oligomer. The blue-shift of UV–vis spectra from 282 to 257 nm levels suggests that R_F–(DOBAA)$_n$–R_F oligomer should form the nanometer size-controlled oligomeric aggregates with the aggregation of terminal fluoroalkyl segments in 1,2-dichloroethane to interact with hibitane as guest molecules. Thus, the carbonyl groups in oligomeric aggregates could interact with some guanidine units

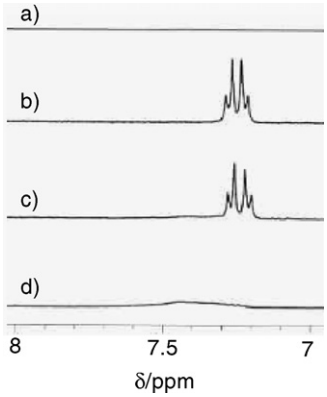
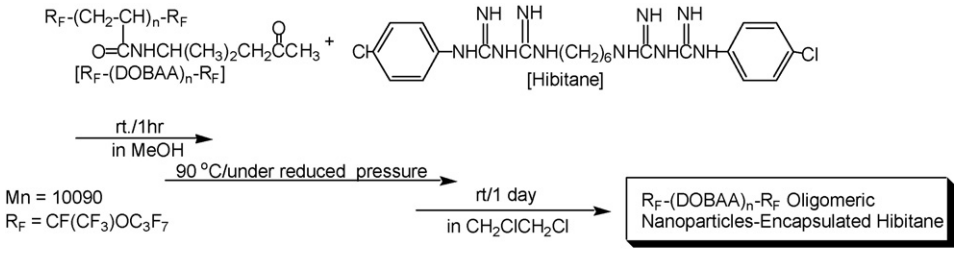


Fig. 1. ^{1}H NMR spectra of CD_3OD solutions of (a) R_F–(DOBAA)$_n$–R_F oligomer; (b) hibitane; (c) CD_3OD solution containing hibitane (1.9 μg/ml) and R_F–(DOBAA)$_n$–R_F (9.4 mg/ml) at room temperature; (d) R_F–(DOBAA)$_n$–R_F oligomeric nanoparticles-encapsulated hibitane.

in hibitane at 90 °C under reduced pressure (dehydration conditions) to afford the colloidal stable fluorinated oligomeric nanoparticles-encapsulated hibitane as a crosslinker as shown in Scheme 1.

^{1}H NMR spectra show that the parent hibitane exhibits the aromatic proton signals (AB quartet) at 7.15–7.30 ppm (see Fig. 1b). CD_3OD solution containing hibitane and R_F–(DOBAA)$_n$–R_F oligomer, of which solution was stirred well at room temperature for 1 h, afforded the same aromatic proton signals as that of original hibitane [see Fig. 1c). R_F–(DOBAA)$_n$–R_F oligomeric nanoparticles-encapsulated hibitane could not afford such aromatic proton signals, indicating that hibitane should be incorporated into the pendant moieties in oligomers. As shown in Fig. 1d, broad aromatic proton signals were newly observed from 7.20 to 7.60 ppm in fluorinated oligomeric nanoparticles-encapsulated hibitane, although R_F–(DOBAA)$_n$–R_F oligomer has no such proton signals around



Run	R_F-(DOBAA)$_n$-R_F mg (μmol)	Hibitane (μmol)	Isolate yield[a)] (%)
1	40 (4.0)	0.9	87
2		1.3	92
3		1.7	85
4		5.2	81
5		6.9	84
6		10.4	68
7		15.6	64
8		20.8	64
9		30.8	59
10		42.0	46

a) Yields were based on R_F-(DOBAA)$_n$-R_F and used Hibitane

Scheme 1.

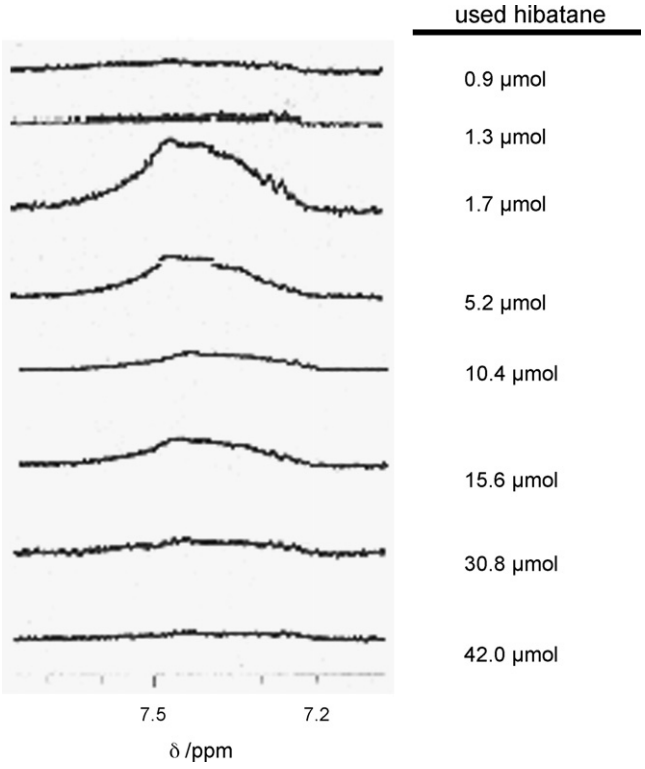


Fig. 2. Relationship between the aromatic proton signals in R_F–$(DOBAA)_n$–R_F oligomeric nanoparticles-encapsulated hibitane in CD_3OD and the used hibitane (μmol) in the nanoparticles.

these areas (see Fig. 1a). We could observe the higher broad aromatic proton signals in the nanoparticles, which were prepared under the feed ratio [hibitane/R_F–$(DOBAA)_n$–R_F: 1.7 μm/4.0 μm] condition, indicating that the carbonyl groups in R_F–$(DOBAA)_n$–R_F oligomers should interact with the guanidine units in hibitane under such conditions to afford the fluorinated oligomeric nanoparticles-encapsulated hibitane as a crosslinker (see Fig. 2). This finding corresponds well to the results for the absorbance of these fluorinate nanoparticles, and a higher absorbance (0.39) was obtained in the case of the feed ratio: hibitane/R_F–$(DOBAA)_n$–R_F: 1.7 μm/4.0 μm (see run 3 in Table 1).

On the other hand, fluoroalkyl end-capped *N*,*N*-dimethylacrylamide oligomers [R_F–$(CH_2CHC(=O)NMe_2)_n$–R_F:R_F–$(DMAA)_n$–R_F; $R_F = CF(CF_3)OC_3F_7$; Mn = 1690]/hibitane composites (Fig. 3d), which were prepared under the similar conditions to that of Scheme 1, exhibited the aromatic protons as well as the parent hibitane (Fig. 3b) and the mixture (Fig. 3c). This finding suggests that since R_F–$(DMAA)_n$–R_F oligomer has less reactive amide carbonyl groups as compared with much more reactive ketone carbonyl groups in R_F–$(DOBAA)_n$–R_F oligomer, R_F–$(DMAA)_n$–R_F oligomers could not interact tightly with hibitane to afford the corresponding fluorinated oligomeric nanoparticles.

R_F–$(DOBAA)_n$–R_F oligomeric nanoparticles-encapsulated hibitane were found to exhibit a good dispersibility and stability in a variety of organic media such as MeOH, THF, AcOEt, acetone, chloroform, 1,2-dichloroethane, and fluorinated aliphatic solvents (AK-225: 1:1 mixed solvents of 1,1-dichloro-2,2,3,3,3-pentafluoropropane and 1,3-dichloro-1,2,2,3,3-pentafluoropropane) to afford transparent colorless solutions. Thus, the size of these nanoparticles in Scheme 1 was measured by the use of dynamic light scattering (DLS) measurements at 20 °C in methanol. The size of R_F–$(DOBAA)_n$–R_F oligomeric aggregates was also measured under similar conditions, for comparison, these results are shown in Table 1.

Table 1 shows that the number-average diameter of R_F–$(DOBAA)_n$–R_F oligomeric aggregates is 112 nm, and the size (number-average diameter) of R_F–$(DOBAA)_n$–R_F oligomeric nanoparticles-encapsulated hibitane is from 165 to 293 nm. The size of the fluorinated nanoparticles were found to increase compared to that of the parent oligomeric aggregates, indicating that R_F–$(DOBAA)_n$–R_F oligomeric aggregates should interact with hibitane to afford fluorinated oligomeric nanoparticles-encapsulated hibitane. Scanning electron microscopy (SEM) image of freshly prepared sample by the use of fluorinated DOBAA oligomeric nanoparticles-encapsulated

Table 1
Size, absorbance (λ_{max} in methanol), thermal stability (T_{dec}) of R_F–$(DOBAA)_n$–R_F oligomeric nanoparticles-encapsulated hibitane [$R_F = CF(CF_3)OC_3F_7$], and the content of hibitane in these nanoparticles

Run[a]	Size of the particles (nm)[b]	Absorbance (λ_{max}, nm)	T_{dec} (°C) of nanoparticles[c]	Content of hibitane in particles (%)
1	292	0.16 (257)	244	12
2	293	0.17 (257)	237	10
3	243	0.39 (257)	234	10
4	230	0.27 (257)	242	11
5	165	0.33 (257)	237	10
6	237	0.24 (257)	250	14
7	165	0.26 (257)	236	10
8	249	0.26 (257)	254	15
9	187	0.24 (257)	243	11
10	228	0.22 (257)	243	11
R_F–$(DOBAA)_n$–R_F oligomer	112		159	0

[a] Each different from those of Scheme 1.
[b] Determined by DLS in methanol at 20 °C.
[c] Defined by a 10% mass loss 10 °C/min heating rate.

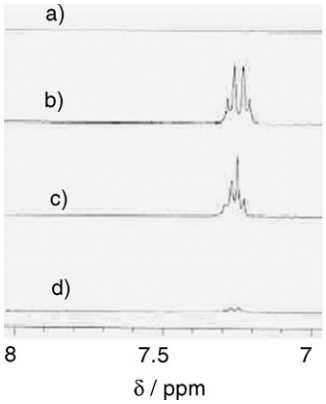


Fig. 3. ^{1}H NMR spectra of (a) CD_3OD solutions of R_F–$(DMAA)_n$–R_F oligomer; (b) hibitane; (c) CD_3OD solution containing hibitane (1.9 μg/ml) and R_F–$(DOBAA)_n$–R_F (9.4 mg/ml) at room temperature; (d) R_F–$(DMAA)_n$–R_F oligomer/hibitane composites.

hibitane (run 3 in Table 1) shows that the fluorinated nanoparticles are well dispersed and possess a similar average diameter of 380 nm as that of DLS measurement (see Fig. 4).

The contents of hibitane in the fluorinated nanoparticles were estimated by the use of TGA (thermogravimetric analyses), in which the weight loss of these nanocomposites was measured by raising the temperature around to 800 °C, and the results are shown in Table 1.

Thermal stability: T_{dec} (defined by a 10% mass loss at a 10 °C/min heating rate) of fluorinated nanoparticles was found to increase effectively, compared to that of the corresponding parent R_F–$(DOBAA)_n$–R_F oligomer. The contents of hibitane in the nanocomposites were estimated to be 10–15% by the use of the TGA data for the thermal stabilities of the parent R_F–$(DOBAA)_n$–R_F oligomer.

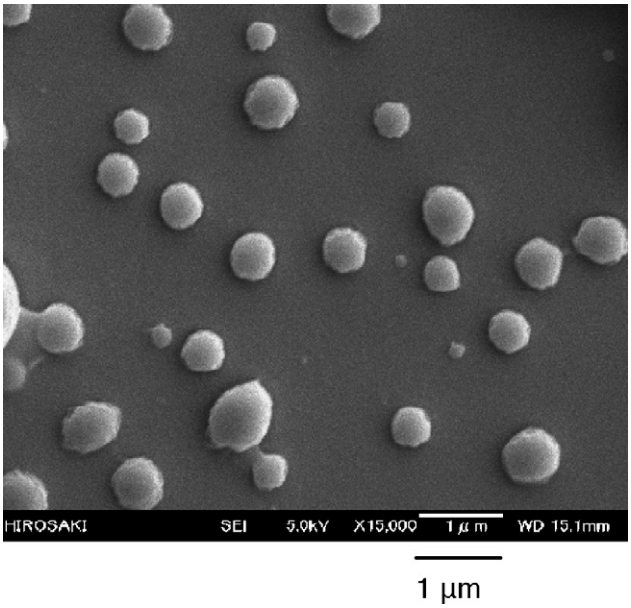


Fig. 4. SEM images of methanol solution of R_F–$(DOBAA)_n$–R_F oligomeric nanoparticles-encapsulated hibitane (sample: run 3 in Table 1).

Table 2
Antibacterial activity of R_F–$(DOBAA)_n$–R_F oligomeric nanoparticles-encapsulated hibitane against *Staphylococcus aureus*

Run[a]	*Staphylococcus aureus* (cfu)[b]
Control	1.6×10^7
6	>10
8	>10
9	>10
10	20

[a] Each different from those of Scheme 1.
[b] cfu indicates colony-forming unit.

In this way, it was verified that the present fluorinated oligomeric nanoparticles could possess hibitane. Therefore, these new fluorinated oligomeric nanoparticles are expected to exhibit an antibacterial activity since hibitane is a popular low-molecular weight biocide. We investigated the antibacterial activity of these fluorinated composites against *Staphylococcus aureus* by the vial cell counting method. About 10^7 cells/ml of *S. aureus* were exposed to 1.42 mg/ml of the nanoparticles in saline, and Table 2 shows the colony-forming units (cfu) versus exposure of these nanoparticles against *S. aureus*.

As shown in Table 2, each fluorinated nanoparticle was found to exhibit high antibacterial activity against *S. aureus*, and these nanoparticles are capable of killing the bacterial cells from 10^7 to below 10 cfu at 1.42 mg/ml of nanoparticles. This interesting result suggests that the bacteria are unable to colonize the fluorinated nanoparticle surface due to the strong interaction of bacteria with hibitane in the nanoparticle. The compounds, which are capable of killing bacterial cells from 10^7 to 10^4 cfu, are in general considered to possess antibacterial activity. Therefore, the present fluorinated nanoparticles are an attractive functional material possessing a potent antibacterial activity.

Previously, we reported that fluoroalkyl end-capped oligomers could be arranged regularly above the PMMA [poly(methyl methacrylate)] surface to exhibit a strong oleophobicity imparted by fluorine [5]. This suggests that these fluorinated nanoparticles should be also dispersed above the PMMA surface. From the developmental viewpoint of fluorinated nanoparticles into the material sciences, it is of considerable interest to apply these fluorinated nanoparticles to the surface modification of traditional polymeric materials such as PMMA. The PMMA film (film thickness: 174 μm) was prepared by casting the homogeneous 1,2-dichloroethane PMMA (0.99 g) solutions (32 ml) containing R_F–$(DOBAA)_n$–R_F oligomeric nanoparticles-encapsulated hibitane (10 mg: run 6 in Table 2) on a glass plate. We have measured the contact angle values for dodecane on the surface and reverse sides of this film at room temperature. An extremely higher value for the contact angle of dodecane (24°) was observed on the surface side of this cast film, compared to that of the reverse side (0°). Contact angle measurements show that fluorinated nanoparticles-encapsulated hibitane could exhibit a markedly strong oleophobicity imparted by fluoroalkyl segments above the surface.

3. Conclusion

We have succeeded in preparing R_F–(DOBAA)$_n$–R_F oligomeric nanoparticles-encapsulated hibitane by reaction of the corresponding oligomer with hibitane in methanol at 90 °C. These fluorinated nanoparticles thus obtained were demonstrated to be very fine particles by DLS measurements and SEM. These fluorinated nanoparticles have a good dispersibility and stability in a wide variety of organic media including fluorinated aliphatic solvents to afford transparent colorless solutions. These fluorinated nanoparticles were also found to exhibit a good antibacterial activity, and were applied to the surface modification of traditional organic polymers. Further studies are actively in progress.

4. Experimental

NMR spectra and Fourier-transform infrared (FTIR) spectra were measured using JEOL JNM-400 (400 MHz) FT NMR SYSTEM (Tokyo, Japan).

Molecular weights were measured using a Shodex DS-4 (pomp) and Shodex RI-71 (detector) gel permeation chromatography (GPC) calibrated with polystyrene standard using tetrahydrofuran (THF) as the eluent. Thermal analyses were recorded on Bruker axs TG-DTA2000SA differential thermobalance (Kanagawa, Japan). Dynamic light-scattering (DLS) measurements were measured using Otsuka Electronics DLS-7000 HL (Tokyo, Japan). Ultraviolet–visible (UV–vis) spectra were measured using Shimadzu UV-1600 UV–vis spectrophotometer (Kyoto, Japan). Scanning electron microscopy (SEM) images were obtained using a JEOL JSM-5300-electron microscopy (Tokyo, Japan). The contact angles were measured by the use of Kyowa Interface Science Drop Master 300 (Saitama, Japan).

Following is a typical experimental procedure for the preparation of fluorinated oligomeric nanoparticles-encapsulated hibitane. To a methanol solution (10 ml) of fluoroalkyl end-capped *N*-(1,1-dimethyl-3-oxobutyl)acrylamide oligomer [R_F–($CH_2CHC(=O)NHCMe_2CH_2COMe)_n$–$R_F$ [R_F–(DOBAA)$_n$–R_F]; $R_F = CF(CF_3)OC_3F_7$; Mn = 10,090 (40 mg)], which was prepared by the reaction of fluoroalkanoyl peroxide with the corresponding monomer according to our previously reported method, [6] were added hibitane (1.7 μmol). The mixture was stirred well with a magnetic stirring bar at room temperature for 1 h, and then placed in a water bath at ∼90 °C for 1 h. After the solvent was evaporated off at 90 °C under reduced pressure, the obtained crude product was stirred with magnetic stirring bar at room temperature in 1,2-dichloroethane (DE: 10 ml) for 1 day, and then the solution was filtered through a 0.45 μm PTFE membrane to obtain a transparent colorless solution. The expected fluorinated nanoparticles were easily isolated after the evaporation of solvents, and fluorinated nanoparticle white colored powders thus obtained were dried in vacuo at 50 °C for 2 day to afford purified particle powders (35 mg; isolated yield: 85%). The yield is based on the used R_F–(DOBAA)$_n$–R_F oligomer and hibitane. The size of the obtained composites was measured by dynamic light scattering measurements in methanol at 20 °C.

The modified PMMA film was prepared by casting the 1,2-dichloroethane solution (10 ml) of PMMA (0.99 g) and the 1,2-dichlroethane solution (10 ml) containing R_F–(DOBAA)$_n$–R_F oligomeric nanoparticles-encapsulated hibitane(10 mg: average particle size: 237 nm) on glass plates. The solvent was evaporated at room temperature, and the film formed peeled off and dried at 50 °C for 24 h under vacuum to afford the modified PMMA film (film thickness: 174 μm).

The contact angle values for dodecane were measured with a Drop Master 300 (Kyowa Interface Science Co.) by depositing a drop of dodecane (2 μl) on the modified PMMA film (10 mm × 10 mm; film thickness: 203 μm) at room temperature.

The bacterial activity of the nanoparticles was evaluated against *S. aureus* by viable cell counting method as described previously [7].

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[6] A typical experiment for the synthesis of R_F–(DOBAA)$_n$–R_F oligomers is as follows: perfluoro-2-methyl-3-oxahexanoyl peroxide (5 mmol) in 1: 1 mixed solvents (AK-225) of 1,1-dichloro-2,2,3,3,3-pentafluoropropane – 1,3-dichloro-1,2,2,3,3-pentafluoropropane (30 g) was added to a mixture of *N*-(1,1-dimethyl-3-oxobutyl)acrylamide (15 mmol) and AK-225 (100 g). The solution was stirred at 45 °C for 5 h under nitrogen. After evaporating the solvent, the crude products were reprecipitated from AK-225 – hexane to give bis(perfluoro-1methyl-3-oxapentyl)-*N*-(1,1-dimethyl-3-oxobutyl)acrylamide oligomer (3.01 g): see H. Sawada, Y. Yoshino, Y. Ikematsu, T. Kawae, Eur. Polym. J. 36 (2000) 231–240;
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