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Highly Potent Inhibitors of TNF-α Production. Part II: Metabolic Stabilization of a Newly Found Chemical Lead and Conformational Analysis of an Active Diastereoisomer

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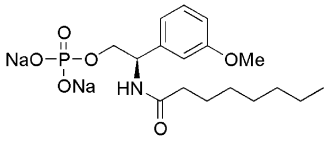
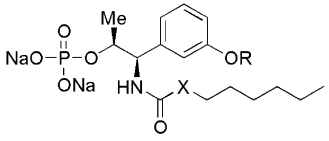
Abstract—Design and synthesis of metabolically stabilized inhibitors of TNF-α production, which could be new drug candidates, are reported. Conformational analysis of an active diastereoisomer was performed based on biological evaluations of the conformationally fixed indane derivatives **17** and **18**. Structure–activity relationships (SARs) based on biological evaluations of the optically active derivatives are also discussed. Full details including chemistry are reported.

Introduction

TNF-α plays a central role in the initiation and maintenance of many inflammatory and autoimmune diseases such as rheumatoid arthritis (RA), multiple sclerosis, sepsis, ulcerative colitis, congestive heart failure, inflammatory bowel disease and Crohn's disease.[1a,b] In the preceding paper,[2a–c] we reported on the discovery of a new chemical lead followed by its optimization. Since the final goal of our project is to identify and develop an inhibitor of TNF-α production which is a clinically useful drug candidate, we next focused our attention on the metabolic stabilization of the newly discovered chemical leads without loss of their highly potent activity because one of the crucial drawbacks of them as a drug candidate was found to be the rapid metabolic hydrolysis of their phosphate moiety as found in our in vitro study (Fig. 1 and Scheme 1). Reported in this publication are the full details of the discovery



1 : ID_{50} 0.26 mg/kg, iv in rats



2 : R = Me, X = CH_2, (ID_{50} 0.03 mg/kg, iv in rats)

3 : R = ^{i}Pr, X = CH_2, (ID_{50} 0.05 mg/kg, iv in rats)

4: R = Me, X = O, (ID_{50} 0.02 mg/kg, iv in rats)

Figure 1. Design of the metabolically stabilized molecules.

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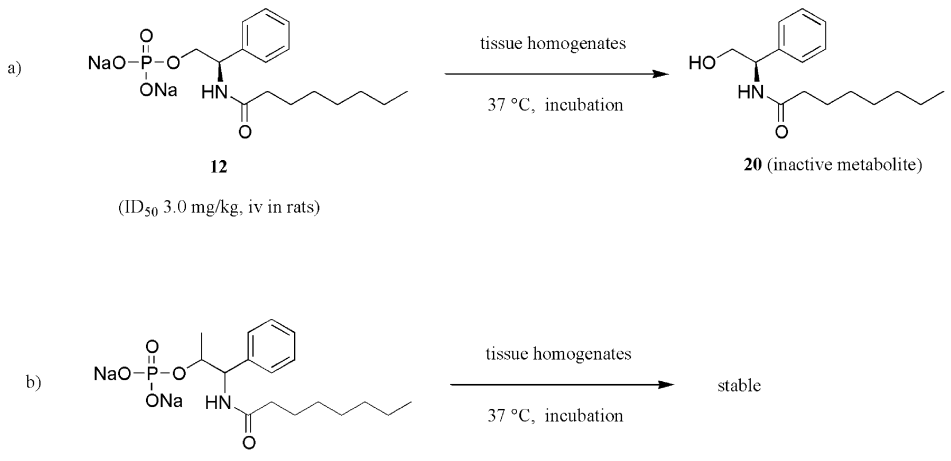
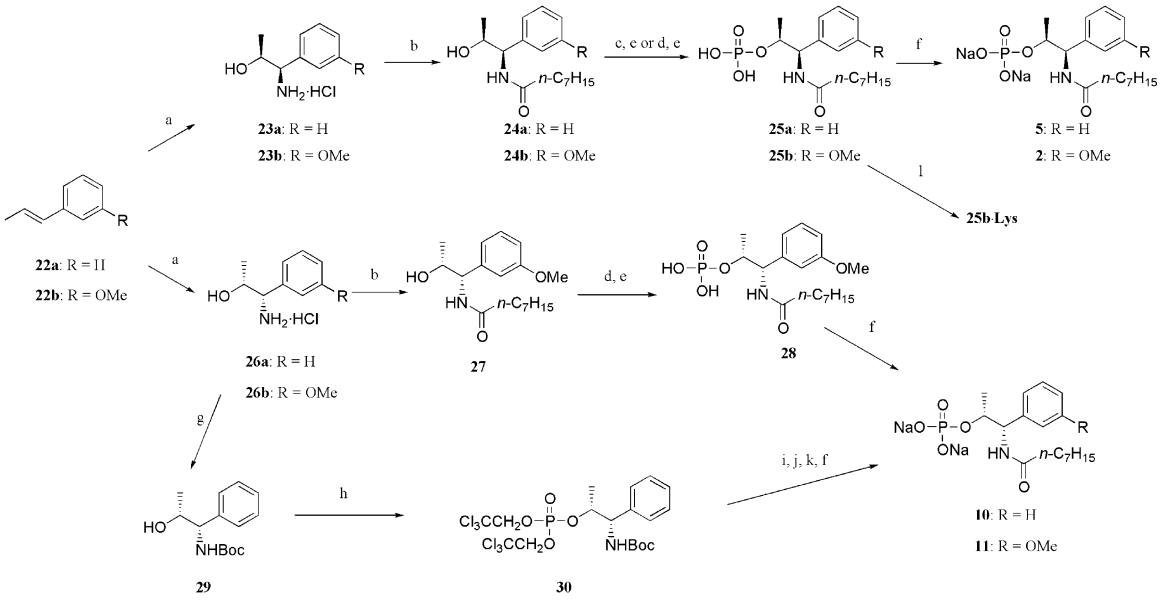
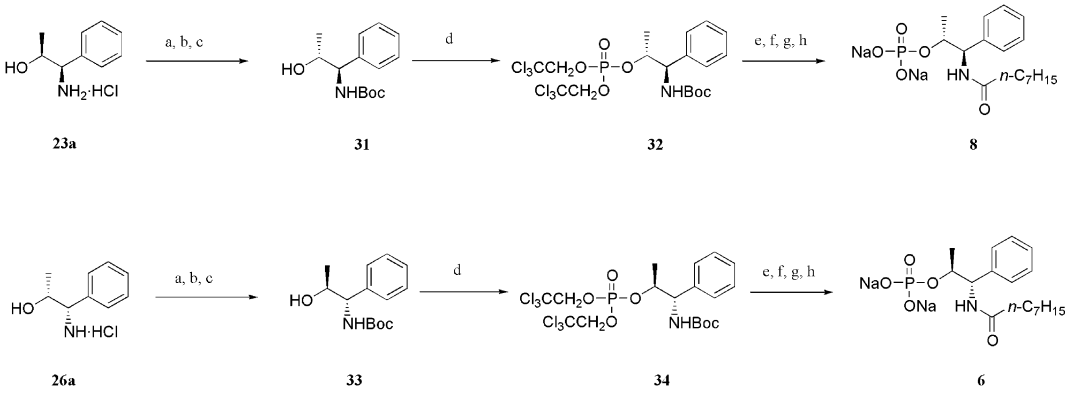


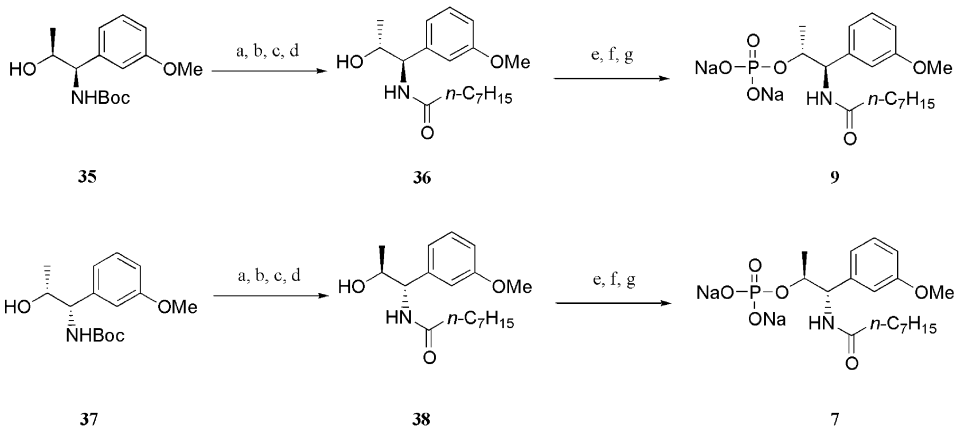
Scheme 1. Metabolic hydrolysis of the phosphates **12** and **21**.



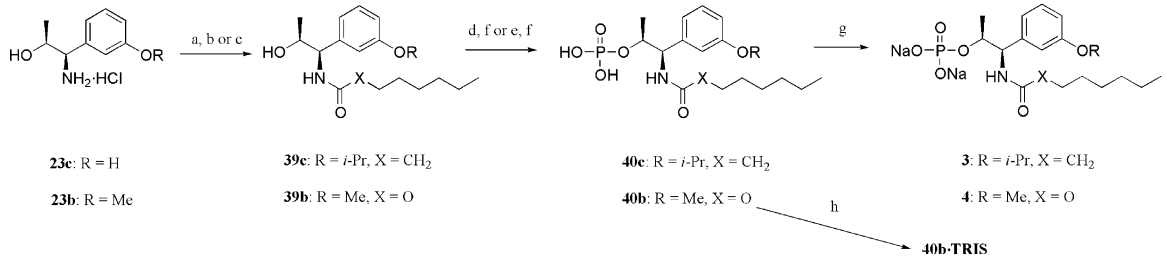
Scheme 2. Synthesis of compounds **2**, **5**, **10** and **11.** Reagents and conditions; (a) see ref 3a,b (b) n-$C_7H_{15}COCl$, $NaHCO_3$aq, dioxane; Method A (c, e, f): (c) (i) n-BuLi, THF; (ii) $(BnO)_2P(O)Cl$; Method B (d–f): (d) (i) $^iPr_2NP(OBn)_2$, tetrazole, CH_3CN; (ii) m-CPBA, CH_2Cl_2; (e) H_2, Pd–C, MeOH; (f) NaOHaq, EtOH; (g) $(Boc)_2O$, 1 N-NaOH, dioxane; Method C (h–k, f): (h) $(Cl_3CCH_2O)_2P(O)Cl$, DMAP, Py; (i) 4 N-HCl, dioxane; (j) n-$C_7H_{15}COCl$, Py, CH_2Cl_2; (k) Zn, Py, AcOH; (l) L-lysine, EtOH, H_2O.



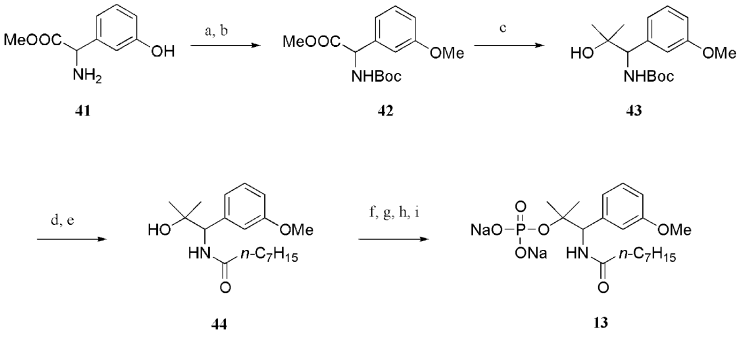
Scheme 3. Synthesis of compounds **8** and **6**. Reagents and conditions: (a) $(Boc)_2O$, 1 N-NaOH, dioxane; (b) PhCOOH, DEAD, Ph_3P, THF; (c) NaOMe, MeOH; Method C (d–h): (d) $(Cl_3CCH_2O)_2P(O)Cl$, DMAP, Py; (e) 4 N-HCl, dioxane; (f) n-$C_7H_{15}COCl$, Py, CH_2Cl_2; (g) Zn, Py, AcOH; (h) NaOHaq, EtOH.



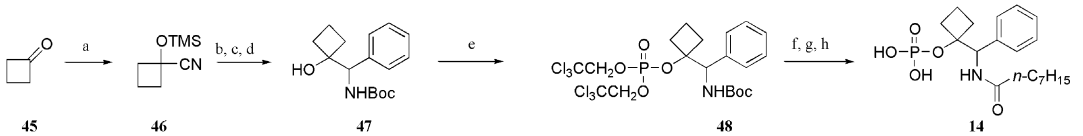
Scheme 4. Synthesis of compounds **9** and **7**. Reagents and conditions: (a) PhCOOH, DEAD, Ph_3P, THF; (b) NaOHaq, THF, MeOH; (c) 4 N-HCl, dioxane; (d) n-$C_7H_{15}COCl$, $NaHCO_3$aq, THF; Method B (e–g): (e) (i) i-$Pr_2NP(OBn)_2$, tetrazole, CH_3CN; (ii) m-CPBA, CH_2Cl_2; (f) H_2, Pd–C, MeOH; (g) NaOHaq, EtOH.



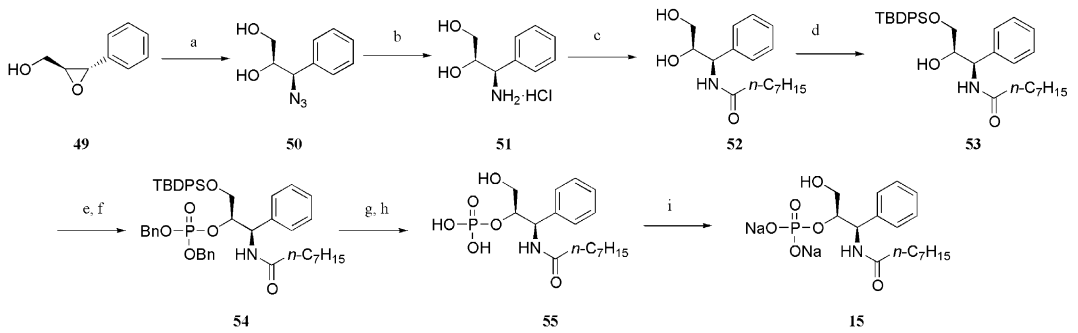
Scheme 5. Synthesis of compounds **3** and **4.** Reagents and conditions; (a) n-$C_7H_{15}COCl$, $NaHCO_3$aq, THF; (b) i-PrI, K_2CO_3, acetone; (c) n-$C_6H_{13}OCOCl$, $NaHCO_3$aq, THF; Method A (d, f, g): (d) (i) n-BuLi, THF; (ii) $(BnO)_2P(O)Cl$; Method B (e–g): (e) (i) i-$Pr_2NP(OBn)_2$, tetrazole, CH_3CN; (ii) m-CPBA, CH_2Cl_2; (f) H_2, Pd-C, MeOH; (g) NaOHaq, EtOH; (h) tris(hydroxymethyl)aminomethane, EtOH, H_2O.



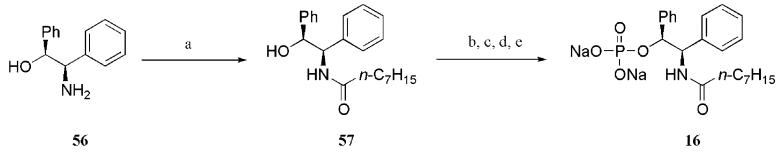
Scheme 6. Synthesis of compound **13**. Reagents and conditions: (a) $(Boc)_2O$, $CHCl_3$; (b) MeI, K_2CO_3, DMF; (c) MeMgBr, THF; (d) 4 N-HCl/dioxane; (e) n-$C_7H_{15}COCl$, $NaHCO_3$aq, dioxane; Method B (f–i): (f) i-$Pr_2NP(OBn)_2$, tetrazole, CH_3CN; (g) m-CPBA, CH_2Cl_2; (h) H_2, Pd–C, MeOH; (i) NaOHaq, EtOH.



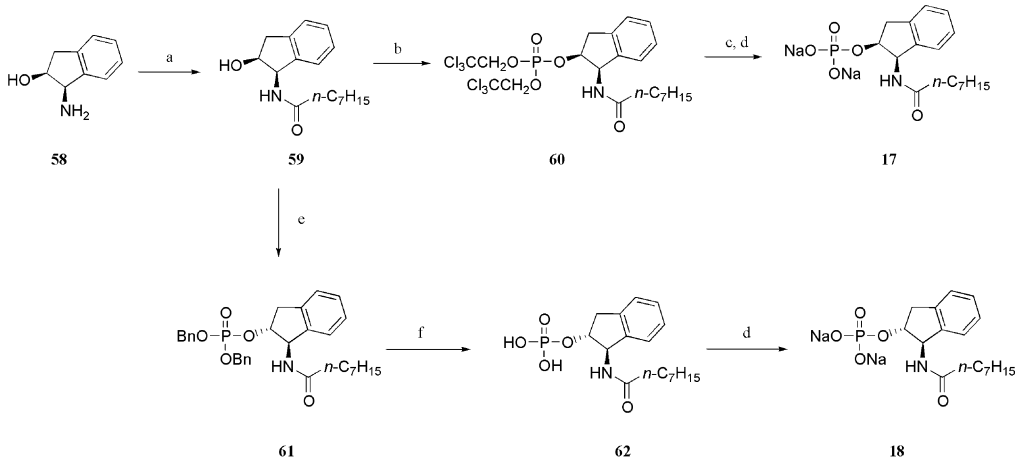
Scheme 7. Synthesis of compound **14**. Reagents and conditions: (a) TMSCN, TMSOTf, CH_2Cl_2; (b) PhMgI, Et_2O; (c) $NaBH_4$, MeOH; (d) $(Boc)_2O$; Method C (e–h): (e) $(Cl_3CCH_2O)_2P(O)Cl$, DMAP, LiI, Py; (f) 4 N-HCl/dioxane; (g) n-$C_7H_{15}COCl$, Py, CH_2Cl_2; (h) Zn, Py, AcOH.



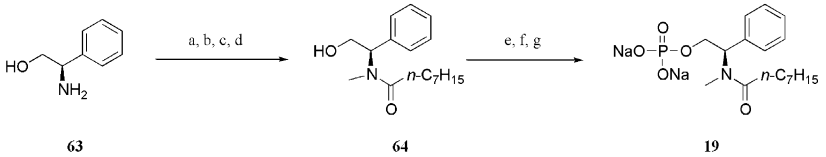
Scheme 8. Synthesis of compound **15**. Reagents and conditions: (a) NaN_3, NH_4Cl, dioxane, H_2O; (b) H_2, Pd–C, 6 N-HCl, EtOH; (c) n-$C_7H_{15}COCl$, $NaHCO_3$aq, THF; (d) TBDPSCl, imidazole, DMF; Method B (e–i): (e) i-$Pr_2NP(OBn)_2$, tetrazole, CH_3CN; (f) m-CBPA, CH_2Cl_2; (g) H_2, Pd–C, MeOH; (h) TBAF, THF; (i) NaOHaq, EtOH.



Scheme 9. Synthesis of compound **16**. Reagents and conditions: (a) n-$C_7H_{15}COCl$, $NaHCO_3$aq, dioxane; Method D (b–e): (b) i-$Pr_2NP(OCH_2CH_2CN)_2$, tetrazole, CH_3CN, CH_2Cl_2; (c) m-CPBA, CH_2Cl_2; (d) 50%Me_2NHaq, EtOH; (e) NaOHaq, EtOH.



Scheme 10. Synthesis of compounds **17** and **18**. Reagents and conditions: (a) n-$C_7H_{15}COCl$, $NaHCO_3$aq, dioxane; (b) $(Cl_3CCH_2O)_2P(O)Cl$, DMAP, Py; (c) Zn, AcOH, Py; (d) NaOHaq, EtOH; Method E (e–d): (e) $(BnO)_2P(O)OH$, DEAD, Ph_3P, THF; (f) H_2, Pd–C, MeOH.



Scheme 11. Synthesis of compound **19.** Reagents and conditions: (a) EtOCOCl, $NaHCO_3$aq, dioxane; (b) $LiALH_4$, THF; (c) 4 N-HCl, dioxane; (d) n-$C_7H_{15}COCl$, $NaHCO_3$aq, dioxane; Method A (e–g): (e) (i) n-BuLi, THF; (ii) $(BnO)_2P(O)Cl$; (f) H_2, Pd–C, MeOH; (g) NaOHaq, EtOH.

process for the metabolically stabilized derivatives as well as conformational analysis of an active diastereoisomer which was based on the SARs from the conformationally fixed indane derivatives that possessed spatially fixed functional groups.

Chemistry

Synthesis of all the compounds listed in the Tables 2–4 are described in Schemes 2–11. As outlined in Scheme 2, compounds **5** and **10** were prepared from **22a**, while

2 and **11** were prepared from **22b**. Styrene derivatives **22a**, **b** were converted to **23a**, **b** according to the reported procedure.[3a,b] *N*-Acylation of **23a**, **b** afforded **24a**, **b**, which were converted to **25a**, **b**, respectively according to the following successive procedures: (1) phosphorylation;[4a–c] and (2) deprotection by hydrogenolysis. Compounds **25a**, **b** were converted to their corresponding disodium salts **5** and **2**, respectively according to the usual procedure. Compounds **26a**, **b** were also prepared from **22a**, **b** according to the reported procedure.[3a,b] Compound **26a** could be converted to **10** via **29** and **30** according to the following successive procedures: (1) *N*-*tert*-butoxycarbonylation; (2) phosphorylation;[5,6] (3) acidic deprotection; (4) *N*-acylation; (5) reductive deprotection;[5,6] and 6) treatment with NaOH aq. Compound **26b** was transformed to **11** via **27** and **28** according to the same procedures as described above. The L-lysine salt of **25b** was produced as the crystalline compound **25b·Lys**. As described in Scheme 3, compound **31** and **33** were prepared from **23a** and **26a**, respectively according to the following successive reactions: (1) *N*-*tert*-butoxycarbonylation; (2) Mitsunobu reaction;[7] and (3) alkaline hydrolysis. Compounds **8** and **6** were prepared from **31** and **33**, respectively according to the same procedures as described for the preparation of **10** from **29** via **30**. As described in Scheme 4, compounds **9** and **7** were prepared from **36** and **38** respectively according to the same procedures as described for the preparation of **2** from **24b**. Compounds **36** and **38** were prepared from **35** and **37**, respectively according to the following successive reactions: (1) Mitsunobu reaction;[7] (2) alkaline hydrolysis; (3) acidic deprotection; and (4) *N*-acylation. As described in Scheme 5, compounds **3** and **4** were also prepared from **23c** and **23b**, respectively according to essentially the same procedures as described for the preparation of **5** and **2** from **23a** and **23b**, respectively. The diammonium salt of **40b** was produced as the crystalline bis[tris(hydroxymethyl)aminomethane] salt (**40b·TRIS**). Synthesis of compound **13** is described in Scheme 6. Protection of the amino group of **41**[8] followed by *O*-alkylation provided **42**, dimethylation of which with methyl magnesium bromide afforded **43**. Deprotection followed by *N*-acylation provided **44**, which was transformed to **13** by the above-described method. Synthesis of **14** is described in Scheme 7. Cyclobutanone **45** was converted to **46** by the conventional method.[9] Compound **46** was converted to **47** by the following successive reactions: (1) treatment with phenyl magnesium iodide followed by sodium borohydride reduction;[10] and (2) protection of the amino group with *tert*-butoxycarbonyl group. Compound **47** was converted to **14** via **48** according to the usual procedures.[5,6] Synthesis of the hydroxymethyl derivative **15** is outlined in Scheme 8. A ring opening reaction of the epoxide **49** with sodium azide afforded **50**, catalytic hydrogenation of which gave the aminoalcohol **51**. *N*-Acylation of **51** followed by the selective monosilylation of the diol **52** provided **53**, which was converted to **54** by the usual procedure. Catalytic hydrogenation of **54** followed by the treatment with TBAF provided **55**, which was converted to **15** by the usual method. As described in Scheme 9, compound **16** was prepared from the aminoalcohol **56** via **57**. *N*-Acylation of **56** gave **57**, which was converted to the disodium salt **16** according to the usual procedure. Synthesis of conformationally fixed isomers **17** and **18** is outlined in Scheme 10. *N*-Acylation of the aminoalcohol **58** with octanoyl chloride afforded **59**, bis(2-trichloroethoxy)phosphorylation[5,6] of which provided **60**. Reductive deprotection[5,6] of **60** followed by the treatment with sodium hydroxide afforded **17**. Dibenzyl phosphorylation of the hydroxy group of **59** with a S_N2 inversion gave **61**,[11] deprotection of which afforded **62**. The free acid form **62** was converted to **18** by the usual method. The *N*-methyl derivative **19** was prepared as described in Scheme 11. Compound **64** was prepared from (2*R*)-2-amino-2-phenylethanol **63** by the following successive reactions: (1) *N*-ethoxycarbonylation; (2) reduction with $LiAlH_4$; (3) treatment with acid; and (4) *N*-acylation with octanoyl chloride. Compound **19** was prepared from **64** according to the usual procedure.

Results and Discussion

To identify an inhibitor of TNF-α production which could be a clinically useful drug candidate, the design and synthesis of metabolically stable derivatives was carried out and their ability to inhibit TNF-α production was evaluated biologically. Plasma TNF-α production and the potency of the test compounds were evaluated according to the same procedures as described in the preceding papers.[2a–c] A pharmacological evaluation of the optimized derivatives **2**–**4** in the animal disease models was also carried out.

Conformational analysis of the optically active diastereoisomers **2** and **9** was performed based on the information obtained from the SARs of the conformationally fixed indane derivatives **17** and **18**.

According to our in vitro experimental data regarding the metabolism of the test compounds, the newly discovered inhibitor **12** was very sensitive to metabolic hydrolysis with tissue homogenates as described in Scheme 1 and Table 1. The formed metabolite **20** did not show any inhibitory activity as shown in Table 4. The metabolic inactivation of **12** with tissue homogenates from the small intestine and liver was very fast as described in Table 1 while with the plasma it was relatively slow. As such, it was thought that the metabolic stabilization of the chemical lead **1** could be accomplished by blocking the rapid hydrolysis of the phosphate moiety in **1**. In fact, 1-methyl-2-octanoylamino-2-phenylethyl disodium phosphate **21** consisting of four isomers **5**, **6**, **8** and **10** was found to be stable after its treatment with tissue homogenates as described in Scheme 1 and Table 1.

Based on the above-described results, the design and synthesis of the metabolically stabilized inhibitors was started with the introduction of a methyl group into the optically active backbone. As shown in Table 2, two classes of four isomers **5**, **6**, **8** and **10** as well as **2**, **7**, **9** and **11** were synthesized and biologically evaluated individually. According to our intact data, the meta-

Table 1. Results of the in vitro metabolism of **12** and **21**[d]

	Remaining% after incubation (30 min)[a]	
Tissue homogenates	**12**	**21**
Mouse liver	N.D.[b]	93
Mouse small intestine	N.D. [b]	97
Mouse plasma	89	95
Rat liver	N.D.[b]	95
Rat small intestine	N.T.[c]	97
Rat plasma	74	91

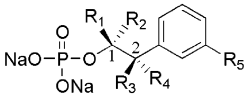
[a]In vitro stability of compounds **12** and **21** was analyzed by HPLC after treatment with tissue homogenates. To a solution of the test compound in PBS (−) (15 μg/100 μL) 1% of tissue homogenate was added. The mixture was incubated at 37 °C for 30 min, and the reaction quenched with MeOH (2 mL). [*iso*-Butyl paraben (10 μg/mL) was used as an internal standard for **12**. *iso*-Amyl paraben (10 μg/mL) was used as an internal standard for **21**]. After centrifugation at 3000 rpm for 10 min, the organic layer was evaporated. A solution of the residue in CH_3CN/H_2O (1/1, 200 μL) was analyzed by HPLC [column: Inertsil ODS-3, 4.6×150 mm (GL Science Inc.); detection: UV at 210 nm; Eluent: A = 20 mM KH_2PO_4 (pH2) / B = CH_3CN, (gradient)[d]; Flow rate: 1.0 mL/min; 25 °C; Sample: 50 μL was injected].
[b]ND: Not Detected.
[c]NT : Not Tested.
[d]Gradient for analyzing **12**.

time (min)	A/B
0	60/40
20.0	60/40
20.5	20/80
35.5	20/80
36.0	60/40
46.0	60/40

gradiant for analyzing **21**.

Time (min)	A/B
0	60/40
10	60/40
10.1	50/50
25.0	50/50
25.1	60/40
35.0	60/40

Table 2. Biological evaluation of the metabolically stabilized derivatives



Compd	R_1	R_2	R_3	R_4	R_5	Inhibition of TNF-α production[a] ID_{50} (mg/kg, iv) rats
5	Me	H	–NHCO–*n*-C_7H_{15}	H	H	4.5
2	Me	H	–NHCO-*n*-C_7H_{15}	H	OMe	0.03
6	Me	H	H	–NHCO–*n*-C_7H_{15}	H	(17)[c]
7	Me	H	H	–NHCO–*n*-C_7H_{15}	OMe	3.7
8	H	Me	–NHCO–*n*-C_7H_{15}	H	H	(23)[c]
9	H	Me	–NHCO–n-C_7H_{15}	H	OMe	(−13)[b]
10	H	Me	H	–NHCO–*n*-C_7H_{15}	H	(5)[c]
11	H	Me	H	–NHCO–*n*-C_7H_{15}	OMe	(38)[b]
3	Me	H	–NHCO–*n*-C_7H_{15}	H	O^{i}Pr	0.05
4	Me	H	–NHCOO–*n*-C_6H_{13}	H	OMe	0.02

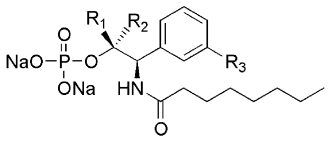
[a]See Experimental.
[b]Inhibition (%) at 10 mg/kg, iv.
[c]Inhibition (%) at 30 mg/kg, iv.

bolically stabilized compound **5** exhibited more potent activity than **12** although their ID_{50} values in rats appeared to be close to each other (**5**: 4.5 mg/kg, iv; **12**: 3.0 mg/kg, iv). For example, intravenous administration of **5** (3 mg/kg) demonstrated a more than 50% inhibition of TNF-α production (58%) while that of **12** (3 mg/kg) demonstrated less than 50% inhibition (43%). Also in their evaluation in mice, **5** exhibited a more potent ID_{50} value (0.4 mg/kg, iv) than **12** (0.8 mg/kg, iv). Thus, metabolic stabilization of **5** was found to be effective at increasing its activity.

This successful results of the metabolic stabilization was thought to be beneficial for the discovery of the highly potent inhibitors **2**–**4** described in Table 5.

As shown in Table 2, the (*S*)-configuration of the newly introduced methyl group and (*R*)-configuration of the *N*-acyl moiety were needed for potent inhibitory activity as illustrated in compounds **5**, **2** and **7**. Introduction of a *meta*-methoxy group into the phenyl moiety of **5** afforded **2** with a marked increase in activity. The same tendency was observed upon the chemical modification of **6**

Table 3. Attempted study to search for a substitute for the (1*S*)-methyl group



Compd	R_1	R_2	R_3	Inhibition of TNF-α production[a] ID_{50} (mg/kg, iv) rats
12	H	H	H	3.0
5	Me	H	H	4.5
13 (*dl*)	Me	Me	OMe	(12)[b]
14 (*dl*)	$-CH_2-CH_2-CH_2-$		H	9.5[c]
15	CH_2OH	H	H	(42)[b]
16		H	H	(3)[b]

[a]See Experimental.
[b]Inhibition (%) at 10 mg/kg, iv.
[c]Tested in mice.

to **7** while **6** exhibited relatively weak inhibitory activity. A marked reduction of inhibitory activity was observed in all the (*R*)-methyl derivatives **8**–**11**. Therefore, the configuration of the methyl group was thought to have a dominant effect on the ability to inhibit TNF-α production. The configuration of the *N*-acyl moiety also plays an important role, although it dose not seem to be as critical as that of the methyl group (R_1), as illustrated in **5** and **7**. Introduction of a *meta*-methoxy group also increased the inhibitory activity as observed upon the chemical modification of **5** to **2** and **6** to **7**. Replacement of the *meta*-methoxy group of **2** with a *meta*-isopropyloxy group provided **3** which retained the highly potent activity. Replacement of the *N*-octanoyl moiety of **2** with a *N*-hexyloxycarbonyl moiety afforded **4** also with strong activity to inhibit the TNF-α production. Thus, the (1*S*,2*R*)-configuration was found to be a structural requirement for the potent inhibitory activity in this series of compounds.

As shown in Table 3, further chemical modification was continued to identify another substituent which was acceptable instead of the (1*S*)-methyl group. The synthesized compounds **13**–**16** were evaluated biologically and their potentials compared with the chemical leads **12** and **5**. Introduction of another methyl group at the geminal position of **2** afforded **13** with a marked reduction in activity presumably because of the bulkiness of the newly constructed 2,2-dimethyl moiety, while the less hindered 2,2-trimethylene derivative **14**, which was biologically evaluated as a *dl*-mixture, had an ID_{50} value of 9.5 mg/kg, iv in mice. Replacement of the methyl group of **12** with a hydroxymethyl group provided **15** with a decrease in activity. Introduction of a phenyl moiety to **5** instead of a methyl group afforded **16** with a marked reduction in activity. Thus, it was concluded that (1*S*)-methyl is the optimized partial structure.

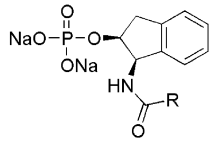
To elucidate the three dimensional active conformation, compounds **17**–**19**, in which free rotations are restricted and/or blocked, were synthesized as the optically active forms and evaluated biologically as described in Table 4. Interestingly, the *trans*-isomer **18** was much more potent than the *cis*-isomer **17**. The *trans*-isomer **18** was estimated to occupy a three dimensional structure close to the real active conformer. This SAR strongly suggested an active conformation of the optimized compounds **2**–**4**.

As illustrated in Fig. 2, the more active compound **2** is able to occupy the favored conformation more easily than the less active compound **9** because of a less hindered intramolecular repulsion between the methyl group and the *ortho*-hydrogen of the phenyl moiety. The *N*-methyl derivative **19** retained quite a good activity as shown in Table 4. The *N*-methyl group did not appear to prevent **19** from occupying the favored conformation.

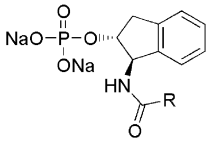
As shown in Fig. 3, increased production of the plasma TNF-α after the intravenous administration of LPS (30 μg/kg) was significantly suppressed by administration of compounds **2**–**4** in a dose-dependent manner. The ID_{50} values of **2**–**4** are 0.03, 0.05 and 0.02, respectively.

The efficacy in disease models[12a–e] and safety of **2**–**4** were evaluated.

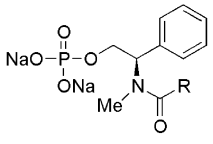
Table 4. Analysis of the structural requirements for the inhibitory activity



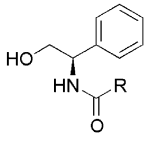
17



18



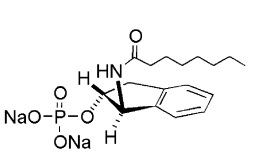
19



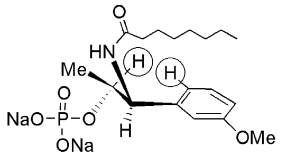
20

Compd	R	Inhibition of TNF-α production[a] ID_{50} (mg/kg, iv) rats
17	C_7H_{15}	(34)[b]
18	C_7H_{15}	2.4
19	C_7H_{15}	9.8
20	C_7H_{15}	(14)[c]

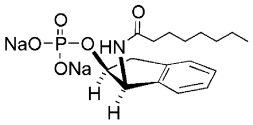
[a]See Experimental.
[b]Inhibition (%) at 10 mg/kg, iv.
[c]Inhibition (%) at 10 mg/kg, iv in mice.



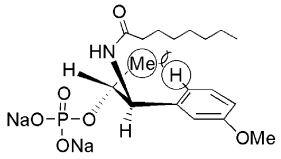
18 (*trans*-isomer: more active than *cis*-isomer **17**)



2 (1*S*, 2*R*)



17 (*cis*-isomer: less active than *trans*-isomer **18**)



9 (1*R*, 2*R*)

Figure 2. Proposed active conformation based on the more active *trans*-isomer **18**.

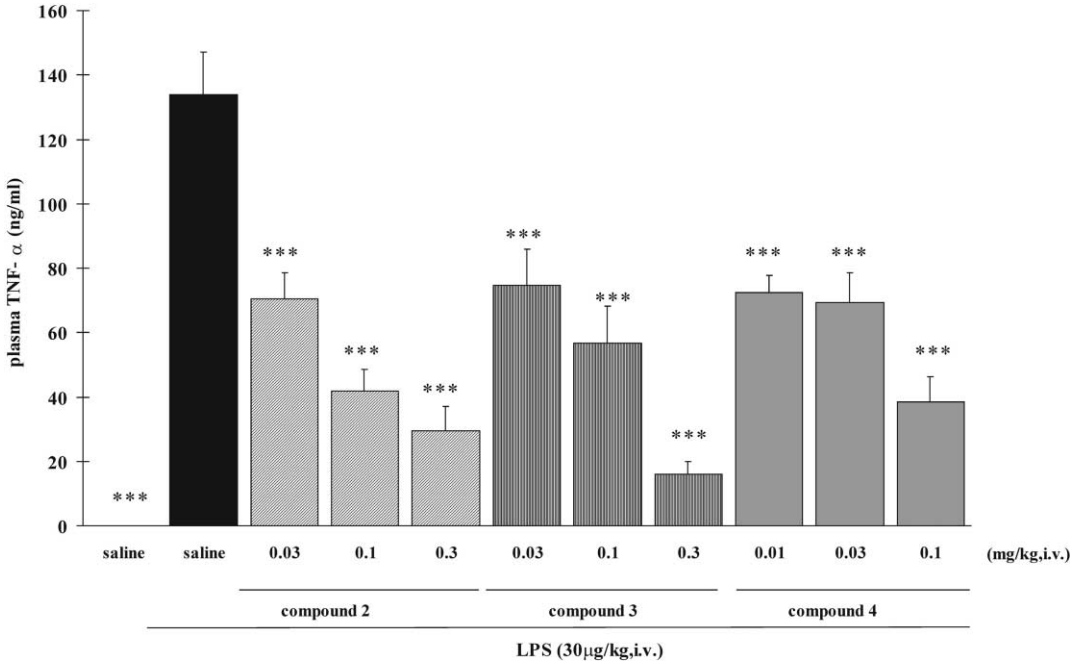


Figure 3. Effect of compound **2**, **3**, and **4** on LPS-induced TNF-α production in rats. Test compound was administered intravenously just before an intravenous injection of LPS. After 90 min of LPS injection, heparinized blood was obtained. Plasma TNF-α concentration was determined by ELISA. The data were expressed as the mean ± SEM ($n = 5$; ***, significantly different from LPS control, $P < 0.001$, ANOVA-Dunnett's *t*-test).

Table 5. Biological evaluation of **2**–**4**

Compd	LPS-induced shock model in mice ($n = 18$ or 20)[a] MED[c] mg/kg, iv (survival rate)	D-(+)-Galactosamine/LPS-induced hepatitis model in rats ($n = 18$)[b] MED[c] mg/kg, iv (survival rate)	Safety in rats ($n = 3$) MLD[d] mg/kg, iv (mortality rate)
2	0.1 (10/18)	0.3 (7/18)	> 100 (0/3)
3	0.3 (10/18)	0.3 (8/18)	100 (1/3)
4	0.1 (14/20)	0.1 (6/18)	> 100 (0/3)

[a]See Experimental.
[b]See Experimental.
[c]MED (minimum effective dose): survival rates are described in parentheses.
[d]MLD (minimum lethal dose): at least one of the tested animals died at this dose. Mortality rates are described in parentheses.

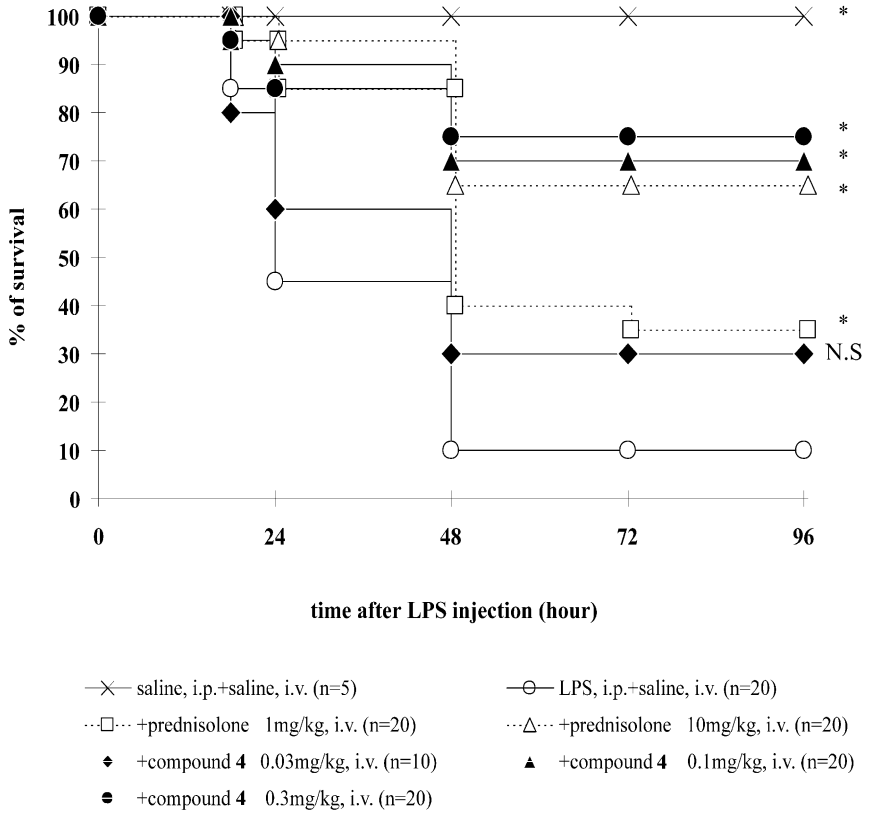


Figure 4. Effect of compound **4** on LPS-induced shock model in mice. BALB/c mice were injected intravenously with compound **4** and then immediately given an intraperitoneal injection of LPS (20 mg/kg). The survival rate of the mice was evaluated after 96 h ($n = 5$–20; *, significantly different from LPS control, $P < 0.01$; N.S., not significant, Mantel–Cox test).

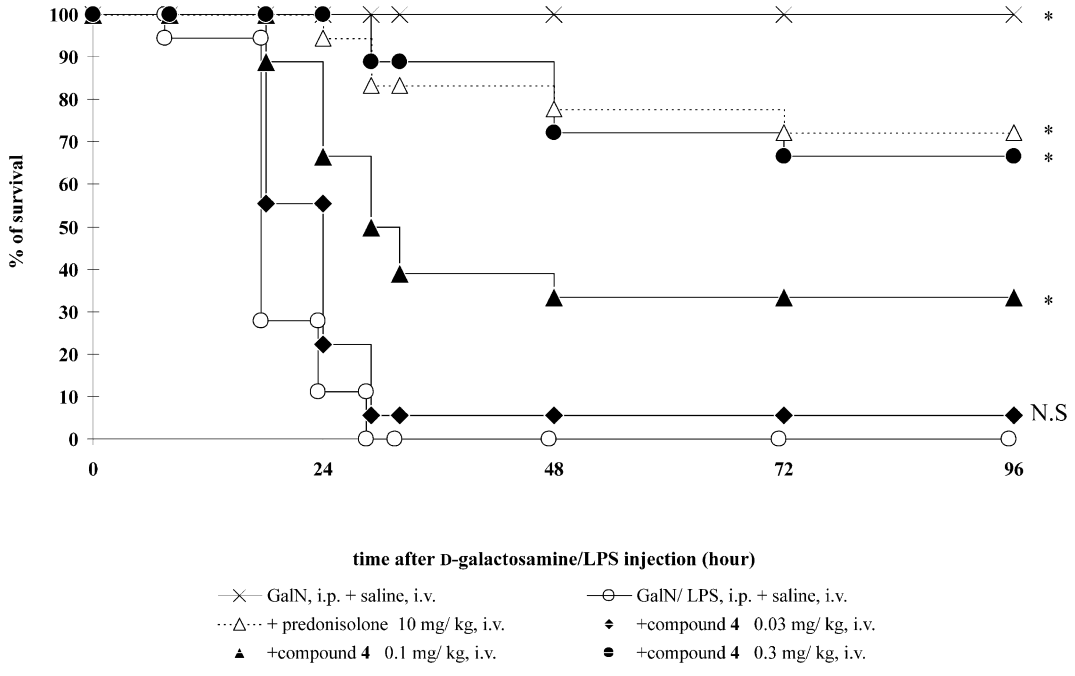


Figure 5. Effect of compound **4** on D-(+)-galactosamine/LPS-induced hepatitis model in rats. SD rats were injected intravenously with compound **4** and then immediately given an intraperitoneal injection of D-(+)-galactosamine/LPS (1 g/7.5 mg/kg). The survival rate was evaluated after 96 h ($n = 18$/group; *, significantly different from D-(+)-galactosamine/LPS control, $P < 0.01$; N.S., not significant, Mantel–Cox test).

Table 6. Oral activity of the representative compounds

Compd	Inhibition of TNF-α production ID_{50} (mg/kg, rats, $n=5$)		
	iv	po	
		Fed	Fasted
12	3.0	>300	104
2	0.03	53	16
3	0.05	63	9
4	0.02	20	4

As described in Table 5, the minimum effective doses (MEDs) of compounds **2**–**4** in the LPS-induced shock model of mice were 0.1, 0.3 and 0.1 mg/kg, iv, respectively. The minimum effective doses (MEDs) of compounds **2**–**4** in the D-(+)-galactosamine/LPS-induced hepatitis model of rats were 0.3, 0.3 and 0.1 mg/kg, iv, respectively. Among the tested, compound **4** was the most hopeful one. As shown in Figs 4 and 5, compound **4** demonstrated an efficacy equivalent to predonisolone (10 mg/kg, iv), which was used as the positive control, at the dose of 0.3 mg/kg, iv, significantly[13] in both of the above described disease models.

With respect to the safety concern, the minimum lethal dose (MLD) of **3** in rats was 100 mg/kg, iv, while the MLDs of **2** and **4** were more than 100 mg/kg, iv, respectively. All three compounds, **2**–**4**, demonstrated a sufficient margin of safety for pharmacological evaluation.

The representative compounds **12** and **2**–**4** were also evaluated for their oral activity. As shown in Table 6, much higher dosages than expected were needed to reproduce the same extent of ID_{50} values as those obtained after their intravenous administration. These results were ascribed to their presumed poor oral absorption. Three to seven-fold ID_{50} values were obtained in fed rats compared to fasted rats. Their site of action was also estimated to be in the liver and spleen according to the experimental results reported in the preceding paper.[2c]

Conclusion

In summary, through the design and synthesis of metabolically stabilized inhibitors of TNF-α production, we have discovered the drug candidates **2**–**4** for use in the treatment of diseases caused by the overexpression of TNF-α. These three compounds demonstrated efficacy in animal models of disease and are expected to be clinically useful although no specialized uses are yet intended.

Experimental

General directions

Analytical samples were homogeneous as confirmed by TLC, and the spectroscopic results obtained were consistent with the assigned structures. All ^{1}H NMR spectra were taken on a Varian Gemini-200, VXR-200s or Mercury 300 spectrometer. MS spectra were obtained on a Hitachi M1200H, JMS-DX303HF or PerSeptive Voyager Elite spectrometer. The matrix assisted laser desorption ionization-time of flight high-resolution mass spectra (MALDI-TOF, HRMS) were obtained on a PerSeptive Voyager Elite spectrometer. IR spectra were measured on a Perkin–Elmer FT-IR 1760X or Jasco FT/IR-430 spectrometer. Elemental analyses for carbon, hydrogen and nitrogen were carried out by the Analytical Section of ONO Pharmaceutical Co., Ltd. on a Perkin–Elmer PE2400 SeriesII CHNS/O analyzer. Melting points were uncorrected. Optical rotations were measured on a Jasco DIP-1000 polarimeter. Column chromatography was carried out on silica gel (Merck silica gel 60 (0.063–0.200 mm) or Fuji Silysia FL60D). Thin layer chromatography was performed on silica gel (Merck TLC plate, silica gel 60 F_{254}). The following abbreviations for solvents and reagents are used: THF, tetrahydrofuran; EtOAc, ethyl acetate; MeOH, methanol; EtOH, ethanol; DMF, dimethylformamide; CH_2Cl_2, dichloromethane; $CHCl_3$, chloroform; EDC·HCl, 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride; HOBt·H_2O, *N*-hydroxybenzotriazole hydrate; *m*-CPBA, *meta*-chloroperbenzoic acid; DEAD, diethyl azodicarboxylate; TBDPSCl, *tert*-butylchlorodiphenylsilane; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; TBAF, tetrabutylammonium fluoride; DMAP, *N*,*N*-dimethylpyridin-4-amine

***N*-[(1*R*,2*S*)-2-Hydroxy-1-(3-methoxyphenyl)propyl]octanamide (24b).** (1*R*,2*S*)-1-Amino-1-(3-methoxyphenyl) propan-2-ol hydrochloride **23b** was prepared from 1-methoxy-3-[(1*E*)-prop-1-enyl]benzene **22b** by following the known procedure:[3a,b] **23b**: white solid; TLC $R_f=0.47$ ($CHCl_3$/MeOH/H_2O, 10/5/1); MS (MALDI-TOF, Pos.) *m*/*z* 182 $(M+H)^+$; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.53 (brs, 3H), 7.23 (t, $J=8.1$ Hz, 1H), 7.06 (s, 1H), 6.97 (d, $J=7.8$ Hz, 1H), 6.83 (dd, $J=8.4$, 2.4 Hz, 1H), 4.85–4.60 (br, 1H), 4.46–4.32 (m, 2H), 3.70 (s, 3H), 0.99 (d, $J=6.0$ Hz, 3H). To a stirred mixture of **23b** (20.0 g, 92.0 mmol) and $NaHCO_3$ (23.2 g, 275.9 mmol) in THF (92 mL)–H_2O (92 mL) was added dropwise octanoyl chloride (15.9 mL, 92.9 mmol) at 0 °C and stirring was continued at that temperature for 7 h. The reaction mixture was poured into H_2O and extracted with EtOAc. The organic layer was successively washed with H_2O and brine before being dried over $MgSO_4$. Removal of the solvent by evaporation afforded a white solid, which was washed with *n*-hexane and dried under reduced pressure to obtain **24b** as a white powder (24.7 g, 87%): TLC $R_f=0.32$ (EtOAc/toluene, 2/1); MS (APCI, Pos. 20eV) *m*/*z* 308 $(M+H)^+$, 290; IR (KBr) 3308, 2927, 1645, 1604, 1543, 1261 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.27 (t, $J=7.8$ Hz, 1H), 6.89–6.82 (m, 3H), 6.33 (brd, $J=7.8$ Hz, 1H), 4.90 (dd, $J=7.8$, 3.9 Hz, 1H), 4.11–4.03 (m, 1H), 3.80 (s, 3H), 2.24–2.19 (m, 2H), 1.90 (brs, 1H), 1.68–1.57 (m, 2H), 1.31–1.24 (m, 8H), 1.09 (d, $J=6.3$ Hz, 3H), 0.89–0.85 (m, 3H).

***N*-[(1*R*,2*S*)-2-Hydroxy-1-phenylpropyl]octanamide (24a).** (1*R*,2*S*)-1-Amino-1-phenylpropan-2-ol hydrochloride **23a** was prepared from (1*E*)-prop-1-enylbenzene **22a**

according to the known procedure.[3a,b] The title compound **24a** was prepared from **23a** according to essentially the same procedure as described for the preparation of **24b** from **23b**: white powder; TLC R_f = 0.40 (*n*-hexane/EtOAc, 1/2); MS (MALDI, Pos.) *m*/*z* 300 $(M+Na)^+$, 278 $(M+H)^+$;IR (KBr) 3305, 1644, 1549, 1454, 1123 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 7.40–7.23 (m, 5H), 6.28 (d, *J* = 8.0 Hz, 1H), 4.95 (dd, *J* = 8.0, 3.6 Hz, 1H), 4.09 (m, 1H), 2.23 (t, *J* = 6.6 Hz, 2H), 1.95 (d, *J* = 7.5 Hz, 1H), 1.63 (m, 2H), 1.27 (m, 8H), 1.09 (d, *J* = 6.3 Hz, 3H), 0.87 (t, *J* = 6.6 Hz, 3H).

Hexyl (1*R*,2*S*)-2-hydroxy-1-(3-methoxyphenyl)propylcarbamate (39b). To a stirred mixture of **23b** (14.5 g, 66.7 mmol) and $NaHCO_3$ (16.8 g, 200 mmol) in THF/H_2O (70 mL/70 mL) was added hexyl chloridocarbonate (11.0 mL, 67.3 mmol) at 0 °C. Stirring was continued at that temperature for 30 min and at room temperature for 30 min. The reaction mixture was poured into ice-water and extracted with EtOAc. After the organic layer was successively washed with H_2O and brine, it was dried over $MgSO_4$. Removal of the solvent by evaporation gave a solid which was washed with *n*-hexane to afford **39b** as a white solid (19.7 g, 96% yield): TLC R_f = 0.28 (*n*-hexane/EtOAc, 1/1); MS (MALDI-TOF, Pos.) *m*/*z* 310 (M + H) +; ^1H NMR (300 MHz, $CDCl_3$) δ 7.32–7.22 (m, 1H), 6.92–6.81 (m, 3H), 5.50 (brd, *J* = 6.9 Hz, 1H), 4.67–4.56 (br, 1H), 4.13–3.95 (m, 3H), 3.81 (s, 3H), 1.80–1.47 (m, 2H), 1.42–1.20 (m, 6H), 1.11 (d, *J* = 6.3 Hz, 3H), 0.88 (t, *J* = 6.9 Hz, 3H).

***N*-[(1*R*,2*S*)-2-Hydroxy-1-(3-isopropoxyphenyl)propyl]octanamide (39c).** 3-[(1*R*,2*S*)-1-Amino-2-hydroxypropyl]phenol hydrochloride **23c** was prepared from 1-(benzyloxy)-3-[(1*E*)-prop-1-enyl]benzene by following the known procedure.[3a,b] **23c**: TLC R_f = 0.45 ($CHCl_3$/MeOH/H_2O, 65/35/8); ^1H NMR (300 MHz, $CDCl_3$) δ 7.23 (t, *J* = 8.4 Hz, 1H), 6.92–6.88 (m, 2H), 6.84–6.81 (m, 1H), 4.20–4.12 (m, 2H), 1.04 (d, *J* = 6.0 Hz, 3H). *N*-[(1*R*,2*S*)-2-hydroxy-1-(3-hydroxyphenyl)propyl]octanamide was prepared from **23c** according to essentially the same procedure as described for the preparation of **24b** from **23b**. Purification was performed by column chromatography on silica gel (Merck 7734, *n*-hexane/EtOAc, 1/1) to give *N*-[(1*R*,2*S*)-2-Hydroxy-1-(3-hydroxyphenyl)propyl]octanamide as a colorless viscous oil (80% yield): TLC R_f = 0.43 (EtOAc/toluene, 2/1); ^1H NMR (300 MHz, $CDCl_3$) δ 8.05 (brs, 1H), 7.14 (t, *J* = 7.5 Hz, 1H), 6.78–6.70 (m, 3H), 6.65 (d, *J* = 8.1 Hz, 1H), 4.81 (dd, *J* = 8.1, 4.2 Hz, 1H), 4.08–3.98 (m, 1H), 2.85 (brs, 1H), 2.20–2.15 (m, 2H), 1.65–1.52 (m, 2H), 1.30–1.20 (m, 8H), 1.06 (d, *J* = 6.6 Hz, 3H), 0.90–0.80 (m, 3H). To a stirred mixture of *N*-[(1*R*,2*S*)-2-hydroxy-1-(3-hydroxyphenyl)propyl]octanamide (5.28 g, 18.0 mmol) and K_2CO_3 (6.22 g, 45.0 mmol) in acetone (40 mL) was added 2-iodopropane (2.70 mL, 27.0 mmol). Stirring was continued under reflux for 12 h. The reaction mixture was poured into ice–water and extracted with Et_2O. The organic layer was successively washed with H_2O and brine before being dried over Na_2SO_4. Removal of the solvent by evaporation gave a residue which was purified by column chromatography on silica gel (Merck 7734, *n*-hexane/EtOAc, 1/2) to give **39c** as a colorless oil (4.82 g, 80% yield): TLC R_f = 0.56 (EtOAc/toluene, 2/1); MS (APCI, Pos. 20eV) *m*/*z* 336 $(M+H)^+$; IR (KBr) 3307, 2928, 1641, 1544, 1258, 1118 cm^{-1}; ^1H NMR; (300 MHz, $CDCl_3$) δ 7.24 (dd, *J* = 8.7, 7.8 Hz, 1H), 6.85–6.80 (m, 3H), 6.29 (d, *J* = 7.8 Hz, 1H), 4.90 (dd, *J* = 7.8, 3.9 Hz, 1H), 4.58–4.50 (m, 1H), 4.12–4.01 (m, 1H), 2.24–2.19 (m, 2H), 2.13 (brd, *J* = 6.6 Hz, 1H), 1.68–1.58 (m, 2H), 1.33 (d, *J* = 6.0 Hz, 3H), 1.33 (d, *J* = 6.0 Hz, 3H), 1.32–1.23 (m, 8H), 1.10 (d, *J* = 6.3 Hz, 3H), 0.89–0.85 (m, 3H).

General method A

(1*S*,2*R*)-2-(3-Isopropyloxyphenyl)-1-methyl-2-(octanoylamino)ethyl disodium phosphate (3). To a stirred solution of **39c** (4.0 g, 11.9 mmol) in THF (100 mL) was added dropwise *n*-butyllithium in *n*-hexane (1.53 M, 21.0 mL, 32.1 mmol) at −78 °C under an argon atmosphere. Dibenzylphosphorochloridate in THF[4a] (1 M, 35.7 mL, 35.7 mmol) was added to the resulting mixture at that temperature and stirring was continued for 10 min at −20 °C. The reaction was quenched with 1M NaOH and the resulting mixture was warmed to room temperature with stirring. After the reaction mixture was diluted with EtOAc, the organic layer was successively washed with saturated $NaHCO_3$ aq and brine, it was dried over $MgSO_4$. Removal of the solvent by evaporation gave a residue which was purified by column chromatography on silica gel (Merck 7734, *n*-hexane/EtOAc, 4/1) to afford dibenzyl (1*S*,2*R*)-2-(3-isopropyloxyphenyl)-1-methyl-2-(octanoylamino)ethyl phosphate as a colorless oil (4.54 g, 64% yield): TLC R_f = 0.58 (EtOAc/toluene, 1/1). A mixture of dibenzyl (1*S*,2*R*)-2-(3-isopropyloxyphenyl) - 1 - methyl - 2 - (octanoylamino)ethyl phosphate (4.54 g) and 10% Pd-C (400 mg) in MeOH was stirred at room temperature under an atmospheric pressure of hydrogen for 1 h. Removal of the catalyst by filtration through a pad of Celite followed by evaporation gave an oily residue which was dissolved in saturated $NaHCO_3$ aq and extracted with EtOAc. The aqueous layer was acidified by 1 M HCl and then extracted with EtOAc. The organic layer was successively washed with H_2O and brine before being dried over $MgSO_4$. Removal of the solvent by evaporation gave **40c** as a white amorphous powder (2.56 g, 81%). To a stirred solution of **40c** (2.24 g, 5.4 mmol) in EtOH (50 mL) was added 1 M NaOH (10.8 mL, 10.8 mmol) at room temperature. Removal of the solvent by evaporation followed by the dissolution of the residue in EtOH was repeated several times to remove the H_2O azeotropically. The addition of Et_2O followed by evaporation afforded **3** as a white amorphous powder: TLC R_f = 0.33 ($CHCl_3$/MeOH/H_2O, 65/35/8); IR (KBr) 3423, 1637, 1089, 984 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.12 (t, *J* = 7.8 Hz, 1H), 6.99 (brd, *J* = 7.8 Hz, 1H), 6.96 (brs, 1H), 6.73–6.69 (m, 1H), 4.64–4.54 (m, 3H), 2.35–2.19 (m, 2H), 1.61–1.49 (m, 2H), 1.29 (d, *J* = 6.0 Hz, 3H), 1.26 (d, *J* = 6.0 Hz, 3H), 1.30–1.20 (m, 8H), 1.06 (d, *J* = 6.3 Hz, 3H), 0.89–0.84 (m, 3H); optical rotation $[\alpha]_D^{24}$ −53.3 (*c* 1.05, MeOH); MS (FAB, Pos.) *m*/*z* 460 $(M+H)^+$, 438, 416; HRMS (MALDI-TOF, Pos.) calcd for $C_{20}H_{32}NO_6P{\cdot}2Na+H^+$: 460.1841; found: 460.1805.

(1*S*,2*R*)-1-Methyl-2-octanoylamino-2-phenylethyl disodium phosphate (5). The title compound **5** was prepared from **24a** according to the general method A: TLC R_f = 0.24 ($CHCl_3$/MeOH/H_2O, 65/25/4); IR (KBr) 3336, 1637, 1535, 1454, 1385, 1089 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.42 (brd, J = 6.6 Hz, 2H), 7.25–7.14 (m, 3H), 4.62–4.57 (m, 2H), 2.33–2.18 (m, 2H), 1.60–1.50 (m, 2H), 1.35–1.15 (m, 8H), 1.03 (d, J = 6.6 Hz, 3H), 0.86 (brt, J = 6.6 Hz, 3H); optical rotation $[\alpha]_D^{25}$ −64.5 (*c* 0.96, MeOH); MS (FAB, Pos.) *m*/*z* 424 $(M+Na)^+$, 402 $(M+H)^+$; HRMS (MALDI-TOF, Pos.) calcd for the free acid form $C_{17}H_{28}NO_5P+Na^+$: 380.1603; found: 380.1599.

General method B

(1*S*,2*R*)-1-Methyl-2-(3-methoxyphenyl)-2-(octanoylamino)ethyl disodium phosphate (2). To a stirred mixture of **24b** (25.5 g, 83 mmol) and tetrazole (11.64 g, 166 mmol) in CH_3CN (330 mL) was added dibenzyl diisopropylphosphoramidite (33.5 mL, 99.7 mmol) at room temperature and stirring was continued at that temperature for 3 h. The reaction mixture was poured into saturated $NaHCO_3$ aq and the resultant precipitates were removed by filtration. The filtrate was then diluted with EtOAc and washed with brine before being dried over Na_2SO_4. Removal of the solvent by evaporation gave dibenzyl (1*S*,2*R*)-1-methyl-2-(3-methoxyphenyl)-2-(octanoylamino)ethyl phosphite which was used for the next reaction without further purification (52.0 g, >100%, pale yellow oil): TLC R_f = 0.60 (*n*-hexane/EtOAc, 1/2). To a stirred solution of dibenzyl (1*S*,2*R*)-1-methyl-2-(3-methoxyphenyl)-2-(octanoylamino)ethyl phosphite (52.0 g) in CH_2Cl_2 (415 mL) was added *m*-CPBA (77%, 20.5 g, 91.4 mmol) at 0 °C and stirring was continued at that temperature for 1 h. The reaction mixture was treated with saturated $Na_2S_2O_3$ aq and extracted with CH_2Cl_2. The organic layer was successively washed with H_2O, saturated $NaHCO_3$ aq and brine before being dried over Na_2SO_4. The *m*-chlorobenzoic acid was removed by passing the mixture through a pad of Al_2O_3 (100 g). Removal of the solvent by evaporation gave dibenzyl (1*S*,2*R*)-1-methyl-2-(3-methoxyphenyl)-2-(octanoylamino)ethyl phosphate which was used for the next reaction without further purification (50.6 g, >100%, colorless oil): TLC R_f = 0.35 (*n*-hexane/EtOAc, 1/2). A mixture of dibenzyl (1*S*,2*R*)-1-methyl-2-(3-methoxyphenyl)-2-(octanoylamino) ethyl phosphate (50.6 g) and 10% Pd-C (50% wet, 10 g) in MeOH (850 mL) was vigorously stirred at room temperature under an atmospheric pressure of hydrogen for 5.5 h. Removal of the catalyst by filtration through a pad of Celite followed by evaporation afforded an oily residue which was dissolved in saturated $NaHCO_3$ aq and extracted with EtOAc. The aqueous layer was acidified by 1 M HCl and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Removal of the solvent by evaporation gave **25b** (23.5 g, 73% in 3 steps, beige amorphous powder): TLC R_f = 0.28 ($CHCl_3$/MeOH/H_2O, 65/35/8); ^1H NMR (300 MHz, CD_3OD) δ 7.22 (t, J = 8.1 Hz, 1H), 6.96–6.90 (m, 2H), 6.84–6.81 (m, 1H), 5.01 (d, J = 4.2 Hz, 1H), 4.71–4.60 (m, 1H), 3.78 (s, 3H), 2.26 (t, J = 7.2 Hz, 2H), 1.65–1.55 (m, 2H), 1.30–1.25 (m, 8H), 1.21 (d, J = 6.3 Hz, 3H), 0.90–0.85 (m, 3H). To a stirred solution of **25b** (2.13 g, 5.5 mmol) in EtOH (50 mL) was added 1 M NaOH (11 mL, 11 mmol) at 0 °C. Removal of the solvent by evaporation followed by the dissolution of the residue in EtOH was repeated several times to remove the H_2O azeotropically. Then addition of Et_2O followed by evaporation afforded **2** as a white amorphous powder (5.18 g, 98%): TLC R_f = 0.28 ($CHCl_3$/MeOH/H_2O, 65/35/8); IR (KBr) 3389, 1638, 1534, 1087, 984 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.14 (t, J = 8.4 Hz, 1H), 7.01–6.99 (m, 2H), 6.74 (ddd, J = 8.4, 2.7, 1.2 Hz, 1H), 4.65–4.53 (m, 2H), 3.78 (s, 3H), 2.35–2.19 (m, 2H), 1.61–.49 (m, 2H), 1.30–1.20 (m, 8H), 1.05 (d, J = 6.3 Hz, 3H), 0.88–0.84 (m, 3H); optical rotation $[\alpha]_D^{24}$ −63.3 (*c* 1.02, MeOH); MS (FAB, Pos.) *m*/*z* 432 $(M+H)^+$, 454, 410; HRMS (MALDI-TOF, Pos.) calcd for $C_{18}H_{28}NO_6P{\cdot}2Na+H^+$: 432.1528; found: 432.1524.

L-Lysine salt of compound 2 (25·Lys). Compound **2** was one of the best inhibitors for TNF-α production, so an alternative salt was prepared. To a stirred solution of **25b** (15.6 g, 40.2 mml) in EtOH/H_2O (20/1, 130 mL) was added L-lysine (5.94 g, 40.6 mmol). Stirring was continued under reflux until the reaction mixture changed from a suspension to a clear solution. After cooling to room temperature, the resultant precipitates were collected by filtration and dried under reduced pressure to give **25·Lys** as a white powder (17.7 g, 82%): TLC R_f = 0.22 ($CHCl_3$/MeOH/H_2O, 10/5/1); MS (FAB, Pos.) *m*/*z* 534 $(M+H)^+$, 426, 410, 388, 290, 147; IR (KBr) 3296, 2927, 2856, 1643, 1602, 1545, 1467, 1416, 1379, 1346, 1258, 1165, 1124, 1049 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.18 (t, J = 8.1 Hz, 1H), 6.99–6.92 (m, 2H), 6.82–6.76 (m, 1H), 4.84 (d, J = 3.3 Hz, 1H), 4.64–4.51 (m, 1H), 3.78 (s, 3H), 3.56 (t, J = 6.0 Hz, 1H), 2.92 (t, J = 7.5 Hz, 2H), 2.34–2.17 (m, 2H), 1.95–1.75 (m, 2H), 1.75–1.41 (m, 6H), 1.38–1.17 (m, 8H), 1.12 (d, J = 6.6 Hz, 3H), 0.87 (t, J = 6.9 Hz, 3H); mp 194.5–196. 5 °C; optical rotation $[\alpha]_D^{26}$ −45.99 (*c* 1.0, MeOH); Anal calcd for $C_{18}H_{30}NO_6P{\cdot}C_6H_{14}N_2$: C, 54.02; H, 8.31; N, 7.87; Found: C, 54.04; H, 8.37; N, 7.79.

(1*S*,2*R*)-1-Methyl-2-[(hexyloxycarbonyl)amino]-2-(3-methoxyphenyl) ethyl disodium phosphate (4). The title compound **4** was prepared from **39b** according to the general method B: TLC R_f = 0.30 ($CHCl_3$/MeOH/H_2O, 65/35/4); IR (KBr) 3239, 2932, 1699, 1602, 1491, 1456, 1436, 1343, 1255, 1105 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.12 (*t*, J = 8 Hz, 1H), 7.04–6.94 (m, 2H), 6.78–6.72 (m, 1H), 4.66–4.52 (m, 1H), 4.49 (m) and 4.38 (brs) total 1H, 3.93 (t, J = 6.6 Hz, 2H), 3.78 (s, 3H), 1.64–1.48 (m, 2H), 1.40–1.20 (m, 6H), 1.05 (d, J = 6.6 Hz, 3H), 0.88 (m, 3H); optical rotation $[\alpha]_D^{26}$ −48.83 (*c* 1.2, MeOH); mp >300 °C (decomposed); MS (FAB, Pos.) *m*/*z* 434 $(M+H)^+$, 412, 390; HRMS (MALDI-TOF, Pos.) calcd for $C_{17}H_{26}NO_7P{\cdot}2Na+H^+$: 434.1321; found: 434.1363.

Bis[tris(hydroxymethyl)aminomethane] salt of compound 40b (40b·TRIS). Compound **4** was one of the best inhibitors for TNF-α production, so an alternative salt was prepared. A mixture of **40b** (21.8 g, 56.2 mmol) and

Tris(hydroxymethyl)aminomethane (13.6 g, 112 mmol) in EtOH/H_2O (20/1, 200 mL) was heated under reflux until the reaction mixture changed from a suspension to a clear solution. After cooling to room temperature, the resultant precipitates were collected by filtration and dried under reduced pressure to give **40b·TRIS** as a white powder (24.7 g, 70% yield): TLC $R_f = 0.20$ ($CHCl_3$/MeOH/H_2O, 65/25/4); MS (FAB, Pos.) m/z 511, 412, 390, 122; IR (KBr) 2931, 1691, 1611, 1541, 1491, 1466, 1077 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.18 (t, $J = 8.0$ Hz, 1H), 6.69 (s) and 6.95 (d, $J = 8.0$ Hz) total 2H, 6.80–6.75 (m, 1H), 4.66–4.52 (m, 2H), 3.96 (t, $J = 6.6$ Hz, 2H), 3.78 (s, 3H), 3.58 (s, 12H), 1.64–1.50 (m, 2H), 1.42–1.24 (m, 6H), 1.08 (d, $J = 6.3$ Hz, 3H), 0.94–0.84 (m, 3H); optical rotation $[\alpha]_D^{26}$ −32.16 (c 1.0, MeOH); mp 130–133 °C; Anal calcd for $C_{17}H_{28}NO_7P{\cdot}2C_4H_{11}NO_3{\cdot}H_2O$: C, 46.22; H, 8.07; N, 6.47; Found: C, 46.41; H, 8.19; N, 6.49.

***tert*-Butyl (1*S*,2*S*)-2-hydroxy-1-phenylpropylcarbamate (33).** (1*S*,2*R*)-1-Amino-1-phenylpropan-2-ol hydrochloride **26a** was prepared from (1*E*)-prop-1-enylbenzene **22a** according to the known procedure:[3a,b] **26a**: white powder; TLC $R_f = 0.50$ (EtOAc/AcOH/H_2O 3/1/1); MS (APCI, Pos, 20eV) m/z 152, 135, 106; ^1H NMR (200 MHz, $CDCl_3 + CD_3OD$) δ 7.50–7.30 (m, 5H), 4.43–4.28 (m, 1H), 4.21 (d, $J = 3.6$ Hz, 1H), 1.04 (d, $J = 6.6$ Hz, 3H). To a stirred solution of **26a** (1.26 g, 6.72 mmol) in dioxane (35 mL) were added 2 M NaOH (6.8 mL, 13.4 mmol) and di-*tert*-butyl dicarbonate (1.76 g, 8.06 mmol) at 0 °C and stirring was continued at that temperature for 3 h. The reaction mixture was diluted with EtOAc. After the organic layer was successively washed with H_2O and brine, it was dried over Na_2SO_4. Removal of the solvent by evaporation gave a residue which was solidified with *n*-hexane to afford *tert*-butyl (1*S*,2*R*)-2-hydroxy-1-phenylpropylcarbamate **29** as a white powder (92% yield): TLC $R_f = 0.53$ (*n*-hexane/EtOAc, 1/1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.40–7.25 (m, 5H), 5.45–5.30 (br, 1H), 4.70–4.52 (br, 1H), 4.15–4.00 (m, 1H), 1.90–1.70 (br, 1H), 1.41 (s, 9H), 1.09 (d, $J = 6.3$ Hz, 3H). To a stirred mixture of **29** (1.54 g, 6.12 mmol), PPh_3 (1.60 g, 6.12 mmol) and benzoic acid (747 mg, 6.12 mmol) in THF (60 mL) was added DEAD (2.3 M in toluene, 2.7 mL, 6.12 mmol) at 0 °C and stirring was continued at that temperature for 2 h. Removal of the solvent by evaporation gave a residue which was purified by column chromatography on silica gel (Merck 7734, *n*-hexane/EtOAc, 3/1) to afford an oil, which was solidified by *n*-hexane to obtain (1*S*, 2*S*)-2-[(*tert*-butyloxycarbonyl)amino]-1-methyl-2-phenylethyl benzoate as a white powder (83% yield): TLC $R_f = 0.58$ (*n*-hexane/EtOAc, 3/1); ^1H NMR (200 MHz, $CDCl_3$) δ 8.08–7.98 (m, 2H), 7.62–7.20 (m, 8H), 5.50–5.35 (m, 1H), 5.20 (d, $J = 9.2$ Hz, 1H), 4.98–4.80 (m, 1H), 1.40–1.20 (m, 12H). To a stirred solution of (1*S*,2*S*)-2-[(tert-butyloxycarbonyl)amino]-1-methyl-2-phenylethyl benzoate (1.74 g, 4.9 mmol) in MeOH (50 mL) was added NaOMe (53 mg, 0.98 mmol) at room temperature and stirring was continued at that temperature for 20 h. Removal of the solvent by evaporation gave a residue which was purified by column chromatography on silica gel (Merck 7734, *n*-hexane/EtOAc, 3/1–1/1) and recrystallization from EtOAc/*n*-hexane to afford **33** as a white crystal (1.09 g, 88% yield): TLC $R_f = 0.33$ (*n*-hexane/EtOAc, 2/1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.40–7.25 (m, 5H), 5.32 (d, $J = 8.1$ Hz, 1H), 4.62–4.50 (m, 1H), 4.10–3.95 (m, 1H), 2.10–1.95 (br, 1H), 1.43 (s, 9H), 1.23 (d, $J = 6.3$ Hz, 3H).

General method C

(1*S*,2*S*)-1-Methyl-2-octanoylamino-2-phenylethyl disodium phosphate (6). To a stirred mixture of **33** (873 mg, 3.48 mmol) and DMAP (636 mg, 5.22 mmol) in pyridine (10 mL) was added bis(2,2,2-trichloroethyl) chloridophosphate (1.98 g, 5.22 mmol) at 0 °C. Stirring was continued at room temperature for 18 h. The reaction mixture was diluted with EtOAc. The organic layer was successively washed with 1 M HCl, H_2O and brine before being dried over Na_2SO_4. Removal of the solvent by evaporation gave **34** as a colorless oil (3.15 g, quant) which was used for the next reaction without further purification: TLC $R_f = 0.50$ (*n*-hexane/EtOAc, 2/1). A solution of **34** (3.15 g, 3.48 mmol) in 4M HCl/dioxane (20 mL) was stirred at room temperature for 2 h. Removal of the solvent by evaporation gave a residue which was solidified by Et_2O to afford (1*S*,2*S*)-2-amino-1-methyl-2-phenylethyl bis(2,2,2-trichloroethyl) phosphate hydrochloride as a white powder (1.73 g, 94% yield): TLC $R_f = 0.50$ ($CHCl_3$/MeOH); ^1H NMR (200 MHz, $CDCl_3 + CD_3OD$) δ 7.62–7.50 (m, 2H), 7.50–7.38 (m, 3H), 5.25–5.05 (m, 1H), 4.82–4.55 (m, 4H), 4.40 (d, $J = 8.8$ Hz, 1H), 1.40 (d, $J = 6.2$ Hz, 3H). To a stirred suspension of (1*S*,2*S*)-2-amino-1-methyl-2-phenylethyl bis(2,2,2-trichloroethyl) phosphate hydrochloride (1.72 g, 3.24 mmol) in CH_2Cl_2 (16 mL) were added octanoyl chloride (631 mg, 3.89 mmol) and pyridine (1.31 mL, 16.2 mmol) at 0 °C. Stirring was continued at room temperature for 45 min. Removal of the solvent by evaporation gave (1*S*,2*S*)-1-methyl-2-phenyl-2-(octanoylamino)ethyl bis(2,2,2-trichloroethyl) phosphate which was used for the next reaction without further purification. To a stirred solution of (1*S*,2*S*)-1-methyl-2-phenyl-2-(octanoylamino)ethyl bis(2,2,2-trichloroethyl) phosphate (1.96 g, 3.24 mmol) in pyridine (15 mL)/AcOH (3 mL) was added Zn powder (1.90 g, 29.2 mmol) at 0 °C. Stirring was continued at room temperature for 2 h. Removal of the Zn powder by filtration through a pad of Celite followed by evaporation afforded an oily residue which was dissolved in 5M NaOH and extracted with EtOAc. The aqueous layer was acidified by 2M HCl and extracted with EtOAc. The organic layer was successively washed with H_2O and brine before being dried over Na_2SO_4. Removal of the solvent by evaporation gave a residue, which was purified by column chromatography on silica gel (Merck 7734, $CHCl_3$/MeOH/NH_4OH, 65/25/2-$CHCl_3$/MeOH/H_2O, 65/25/4) to afford a solid. The solid was dissolved in 2M HCl/EtOAc. After the organic layer was successively washed with H_2O and brine, it was dried over Na_2SO_4. Removal of the solvent by evaporation gave a residue, which was solidified by *n*-hexane to obtain (1*S*,2*S*)-1-methyl-2-octanoylamino-2-phenylethyl dihydrogen phosphate as a white powder (697 mg, 60% yield from **34**). The title compound **6** was prepared from

(1*S*,2*S*)-1-methyl-2-octanoylamino-2-phenylethyl dihydrogen phosphate according to the same procedure as described for the preparation of **2** from **25b**: white powder; TLC R_f = 0.43 ($CHCl_3$/MeOH/H_2O, 65/35/8); IR (KBr) 3399, 2928, 2857, 2359, 1637, 1549, 1496, 1455, 1378, 1301, 1204, 1090 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.40–7.30 (m, 2H), 7.30–7.15 (m, 3H), 4.41–4.25 (m, 2H), 2.38–2.15 (m, 2H), 1.63–1.45 (m, 2H), 1.38–1.12 (m, 8H), 1.06 (d, J = 6.0 Hz, 3H), 0.87 (t, J = 6.8 Hz, 3H); optical rotation $[\alpha]_D^{25}$ +17.3 (*c* 1.0, MeOH); MS (FAB, Pos.) *m*/*z* 424 $(M+Na)^+$, 402 $(M+H)^+$, 380; HRMS (MALDI-TOF, Pos.) calcd for $C_{17}H_{26}NO_5P{\cdot}2Na+H^+$: 402.1422; found: 402.1437.

(1*R*,2*R*)-1-Methyl-2-octanoylamino-2-phenylethyl disodium phosphate (8). The title compound **8** was prepared from **23a** according to the general method C; TLC R_f = 0.24 ($CHCl_3$/MeOH/H_2O, 65/25/4); IR (KBr) 3408, 1636, 1455, 1091, 985 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.35 (brd, J = 7.2 Hz, 2H), 7.26 (brt, J = 7.2 Hz, 2H), 7.18 (m, 1H), 4.40–4.24 (m, 2H), 2.38–2.10 (m, 2H), 1.55 (m, 2H), 1.35–1.10 (m, 8H), 1.05 (d, J = 5.7 Hz, 3H), 0.87 (t, J = 6.6 Hz, 3H); optical rotation $[\alpha]_D^{24}$ −14.9 (*c* 1.0, MeOH); MS (FAB, Pos.) *m*/*z* 424 $(M+Na)^+$, 402 $(M+H)^+$, 380; HRMS (MALDI-TOF, Pos.) calcd for $C_{17}H_{26}NO_5P{\cdot}2Na+H^+$: 402.1422; found: 402.1428.

(1*R*,2*S*)-1-Methyl-2-octanoylamino-2-phenylethyl disodium phosphate (10). The title compound **10** was prepared from **29** according to the general method C; off-white powder; TLC R_f = 0.23 ($CHCl_3$/MeOH/H_2O, 65/25/4); IR (KBr) 3346, 1639, 1538, 1454, 1093 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.44 (d, J = 6.9 Hz, 2H), 7.28–7.12 (m, 3H), 4.61 (m, 2H), 2.35–2.18 (m, 2H), 1.55 (m, 2H), 1.25 (m, 8H), 1.04 (d, J = 6.3 Hz, 3H), 0.87 (t, J = 6.3 Hz, 3H); optical rotation $[\alpha]_D^{25}$ +60.9 (*c* 1.25, MeOH); MS (FAB, Pos.) *m*/*z* 402 $(M+H)^+$, 380, 358; HRMS (MALDI-TOF, Pos.) calcd for $C_{17}H_{26}NO_5P{\cdot}2Na+H^+$: 402.1422; found: 402.1416.

***N*-[(1*S*,2*S*)-2-Hydroxy-1-(3-methoxyphenyl)propyl]octanamide (38).** *tert*-Butyl (1*S*,2*R*)-2-hydroxy-1-(3-methoxyphenyl)propylcarbamate **37** was prepared from **26b** according to the same procedure as described for the preparation of *tert*-butyl (1*S*,2*R*)-2-hydroxy-1-phenylpropylcarbamate from **26a**. (1*S*,2*R*)-2-[(*tert*-Butyloxycarbonyl)amino]-1-methyl-2-(3-methoxyphenyl)ethyl benzoate was prepared from **37** according to the same procedure as described for the preparation of (1*S*,2*S*)-2-[(*tert*-butyloxycarbonyl)amino]-1-methyl-2-phenylethyl benzoate from *tert*-butyl (1*S*,2*R*)-2-hydroxy-1-phenylpropylcarbamate (96% yield). To a stirred solution of (1*S*,2*R*)-2-[(*tert*-butyloxycarbonyl)amino]-1-methyl-2-(3-methoxyphenyl)ethyl benzoate (3.48 g, 9.5 mmol) in THF (50 mL)/MeOH (20 mL) was added 1 M NaOH (20 mL, 20 mmol) at room temperature and stirring was continued at that temperature for 20 h. Removal of the solvent by evaporation gave a reside, which was extracted with EtOAc. The organic layer was successively washed with 1 M NaOH and brine before being dried over $MgSO_4$. Removal of the solvent by evaporation afforded a residue which was purified by column chromatography on silica gel (Merck 7734, *n*-hexane/EtOAc, 2/1) to obtain *tert*-butyl (1*S*,2*S*)-2-hydroxy-1-(3-methoxyphenyl)propylcarbamate (1.36 g, 51% yield): TLC R_f = 0.52 (*n*-hexane/EtOAc, 1/1); ^1H NMR (300 MHz, CD_3OD) δ 7.28 (dd, J = 9.0, 7.2 Hz, 1H), 6.87–6.79 (m, 3H), 5.30 (brs, 1H), 4.53 (brs, 1H), 4.03 (brs, 1H), 3.80 (s, 3H), 1.43 (brs, 9H), 1.22 (d, J = 6.3 Hz, 3H). A solution of *tert*-butyl (1*S*,2*S*)-2-hydroxy-1-(3-methoxyphenyl)propylcarbamate (1.34 g, 6.72 mmol) in 4 M HCl/dioxane (20 mL) was stirred at 0 °C for 2 h. Removal of the solvent by evaporation followed by the dissolution of the residue in toluene was repeated several times to give (1*S*,2*S*)-1-amino-1-(3-methoxyphenyl)propan-2-ol hydrochloride as an oil. To a stirred mixture of (1*S*,2*S*)-1-amino-1-(3-methoxyphenyl)propan-2-ol hydrochloride in THF (50 mL) and 0.5M $NaHCO_3$ (50 mL) was added octanoyl chloride (1.1 g, 6.79 mmol) in dioxane (5 mL) at 0 °C and stirring was continued at that temperature for 4 h. The reaction mixture was diluted with EtOAc. The organic layer was successively washed with 1M HCl and brine before being dried over $MgSO_4$. Removal of the solvent by evaporation afforded a residue, which was purified by column chromatography on silica gel (Merck 7734, *n*-hexane/EtOAc, 2/1–1/1) to afford **38** (1.53 g, 100% yield): TLC R_f = 0.22 (*n*-hexane/EtOAc, 1/1); ^1H NMR (200 MHz, CD_3OD) δ 7.26 (dd, J = 8.4, 8.4 Hz, 1H), 6.87–6.78 (m, 3H), 6.29 (brd, J = 8.4 Hz, 1H), 4.87 (dd, J = 8.4, 4.0 Hz, 1H), 4.09 (m, 1H), 3.80 (s, 3H), 2.26 (brt, J = 8.2 Hz, 2H), 1.70–1.60 (m, 2H), 1.40–1.25 (m, 8H), 1.20 (d, J = 6.6 Hz, 3H), 0.87 (brt, J = 6.6 Hz, 3H).

(1*S*,2*S*)-1-Methyl-2-(3-methoxyphenyl)-2-(octanoylamino)ethyl disodium phosphate (7). The title compound **7** was prepared from compound **38** according to the General Method B: 94% yield; TLC R_f = 0.22 ($CHCl_3$/MeOH/H_2O, 65/35/4); IR (KBr) 3412, 2929, 1638, 1457, 1266, 1092, 984, 801 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 7.16 (dd, J = 8.0, 8.0 Hz, 1H), 6.92 (m, 2H), 6.74 (ddd, J = 8.0, 2.4, 1.2 Hz, 1H), 4.35–4.29 (m, 2H), 3.76 (s, 3H), 2.38–2.12 (m, 2H), 1.53 (m, 2H), 1.30–1.20 (m, 8H), 1.05 (d, J = 6.0 Hz, 3H), 0.86 (brt, J = 6.6 Hz, 3H); optical rotation $[\alpha]_D^{25}$ +17.0 (*c* 1.0, MeOH); MS (FAB, Pos.) *m*/*z* 454 $(M+Na)^+$, 432 $(M+H)^+$; HRMS (MALDI-TOF, Pos.) calcd for $C_{18}H_{28}NO_6P{\cdot}2Na+H^+$: 432.1528; found: 432.1524.

(1*R*,2*R*)-1-Methyl-2-(3-methoxyphenyl)-2-(octanoylamino)ethyl disodium phosphate (9). The title compound **9** was prepared from **36** according to the general method B: white powder; TLC R_f = 0.28 ($CHCl_3$/MeOH/H_2O, 65/25/4); IR (KBr) 3328, 2929, 1638, 1457, 1265, 1091, 801 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 7.16 (dd, J = 8.0, 8.0 Hz, 1H), 6.92 (m, 2H), 6.74 (ddd, J = 8.0, 2.4, 1.2 Hz, 1H), 4.35–4.29 (m, 2H), 3.76 (s, 3H), 2.38–2.12 (m, 2H), 1.53 (m, 2H), 1.30–1.20 (m, 8H), 1.05 (d, J = 6.0 Hz, 3H), 0.86 (brt, J = 6.6 Hz, 3H); optical rotation $[\alpha]_D^{25}$ −16.6 (*c* 1.0, MeOH); MS (FAB, Pos.) *m*/*z* 454 $(M+Na)^+$, 432 $(M+H)^+$; HRMS (MALDI-TOF, Pos.) calcd for $C_{18}H_{28}NO_6P{\cdot}2Na+H^+$: 432.1528; found: 432.1568.

(1*R*,2*S*)-1-Methyl-2-(3-methoxyphenyl)-2-(octanoylamino)ethyl disodium phosphate (11). The title compound **11** was prepared from **27** according to the General Method

B: white powder; TLC $R_f = 0.20$ ($CHCl_3$/MeOH/H_2O, 65/25/4); IR (KBr) 3333, 2928, 1638, 1535, 1256, 1088, 984 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.13 (dd, $J = 8.1$, 8.1 Hz, 1H), 7.01–6.99 (m, 2H), 6.73 (ddd, $J = 8.1$, 2.4, 1.2 Hz, 1H), 4.63–4.57 (m, 2H), 3.77 (s, 3H), 2.36–2.18 (m, 2H), 1.55 (m, 2H), 1.35–1.20 (m, 8H), 1.05 (d, $J = 6.3$ Hz, 3H), 0.86 (brt, $J = 6.6$ Hz, 3H); optical rotation $[\alpha]_D^{25}$ +65.4 (*c* 1.0, MeOH); MS (FAB, Pos.) *m*/*z* 432 $(M+H)^+$; HRMS (MALDI-TOF, Pos.) calcd for $C_{18}H_{28}NO_6P{\cdot}2Na + H^+$: 432.1528; found: 432.1505.

Methyl [(*tert*-butoxycarbonyl)amino](3-methoxyphenyl)acetate (42). Methyl amino(3-hydroxyphenyl)acetate **41** was prepared from 3-(benzyloxy)benzaldehyde according to the same procedure as described in the preceding paper.[2c,8] To a stirred solution of **41** (5.0 g, 27.6 mmol) in $CHCl_3$ (150 mL) was added dropwise a solution of di-*tert*-butyl dicarbonate (5.96 g, 27.3 mmol) in $CHCl_3$ (20 mL) at room temperature and stirring was continued at that temperature for 5 h. The reaction was quenched with NH_4OH and extracted with $CHCl_3$. The organic layer was successively washed with saturated $NaHCO_3$, H_2O and brine before being dried over Na_2SO_4. Removal of the solvent by evaporation gave methyl [(*tert*-butoxycarbonyl)amino](3-hydroxyphenyl)-acetate which was used for the next reaction without further purification: TLC $R_f = 0.39$ (*n*-hexane/EtOAc, 1/1). To a stirred mixture of methyl [(*tert*-butoxycarbonyl)amino](3-hydroxyphenyl)acetate (27.6 mmol) and K_2CO_3 (11.5 g, 82.9 mmol) in DMF (54 mL) was added iodomethane (5.16 mL, 82.9 mol) at room temperature and stirring was continued at that temperature for 2 h. The reaction mixture was treated with H_2O and then extracted with EtOAc. After the organic layer was successively washed with 1 M HCl, H_2O and brine, it was dried over Na_2SO_4. Removal of the solvent by evaporation gave **42** (7.91 g, 97% yield in 2 steps): TLC $R_f = 0.63$ (*n*-hexane/EtOAc, 1/1); ^1H NMR (200 MHz, $CDCl_3$) δ 7.31–7.22 (m, 1H), 6.95–6.82 (m, 3H), 5.52–5.48 (m, 1H), 5.28 (d, $J = 7.0$ Hz, 1H), 3.79 (s, 3H), 3.71 (s, 3H), 1.42 (s, 9H).

***tert*-Butyl 2-hydroxy-1-(3-methoxyphenyl)-2-methylpropylcarbamate (43).** To a stirred solution of **42** (4.07 g, 13.8 mmol) in THF (64 mL) was added dropwise methylmagnesium bromide (0.84 M in Et_2O 63.9 mL, 53.4 mmol) at 0 °C. Stirring was continued at room temperature for 2 h. The reaction was quenched with 1 M HCl and extracted with EtOAc, and the organic layer was washed with brine before being dried over Na_2SO_4. Removal of the solvent by evaporation gave **43**: TLC $R_f = 0.50$ (*n*-hexane/EtOAc, 1/1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.27–7.22 (m, 1H), 7.11–6.79 (m, 3H), 5.50 (d, $J = 6.9$ Hz, 1H), 4.48 (d, $J = 6.9$ Hz, 1H), 3.83 (s, 3H), 1.40 (s, 9H), 1.32 (s, 3H), 1.06 (s, 3H).

***N*-[2-hydroxy-1-(3-methoxyphenyl)-2-methylpropyl]octanamide (44).** The title compound **44** was prepared from **43** according to essentially the same procedures as described for the preparation of **38** from *tert*-butyl (1*S*,2*S*)-2-hydroxy-1-(3-methoxyphenyl)propylcarbamate: TLC $R_f = 0.48$ (*n*-hexane/EtOAc, 1/2); ^1H NMR (300 MHz, $CDCl_3$) δ 7.24 (t, $J = 7.2$ Hz, 1H), 6.88–6.79 (m, 3H), 6.43 (d, $J = 8.7$ Hz, 1H), 3.79 (s, 3H), 2.24–2.19 (m, 2H), 1.68–1.58 (m, 2H), 1.30–1.07 (m, 8H), 0.86 (t, $J = 6.6$ Hz, 3H).

1,1-Dimethyl-2-(3-methoxyphenyl)-2-(octanoylamino)ethyl disodium phosphate (13). The title compound **13** was prepared from **44** according to the general method B: TLC $R_f = 0.31$ ($CHCl_3$/MeOH/H_2O, 65/25/4); IR (KBr) 3321, 2928, 1621, 1538, 1490, 1455, 1388, 1368, 1243, 1088 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.13 (t, $J = 7.8$ Hz, 1H), 7.09–7.05 (m, 2H), 6.73–6.69 (m, 1H), 4.49 (d, $J = 1.5$ Hz, 1H), 3.78 (s, 3H), 2.27 (t, $J = 7.2$ Hz, 2H), 1.66 (s, 3H), 1.62–1.53 (m, 2H), 1.32–1.23 (m, 8H), 1.16 (s, 3H), 0.86 (t, $J = 6.3$ Hz, 3H); MS (FAB, Pos.) *m*/*z* 446 $(M+H)^+$; HRMS (MALDI-TOF, Pos.) calcd for $C_{19}H_{30}NO_6P{\cdot}2Na + H^+$: 446.1684; found: 446.1651.

***tert*-Butyl (1-hydroxycyclobutyl)(phenyl)methylcarbamate (47).** To a stirred mixture of cyclobutanone (5.0 g, 71 mmol) and trimethylsilyl cyanide (7.03 g, 71 mmol) in CH_2Cl_2 (100 mL) was added dropwise a solution of trimethylsilyl trifluoromethanesulfonate (1.27 mL, 7.1 mmol) in CH_2Cl_2 (50 mL) at −78 °C and stirring was continued at that temperature for 2.5 h. The reaction was quenched with a few drops of pyridine. The resulting mixture was poured into saturated $NaHCO_3$ aq and extracted with CH_2Cl_2. The organic layer was successively washed with H_2O and brine before being dried over $MgSO_4$. Removal of the solvent by evaporation gave **46** as a colorless oil (7.08 g, 59% yield): ^1H NMR (300 MHz, $CDCl_3$) δ 3.09 (t, $J = 8.1$ Hz, 2H), 2.63–2.58 (m, 1H), 2.40–2.23 (m, 1H), 2.03–1.80 (m, 2H), 0.22 (s, 6H), 0.15 (s, 3H). To a stirred solution of PhMgI in Et_2O (2M, 23.1 mL, 46.1 mmol) was added dropwise a solution of 46 (7.08 g, 41.9 mmol) in Et_2O (50 mL) at 0 °C. Stirring was continued at room temperature for 2 days. To this resulting mixture a solution of $NaBH_4$ (1.71 g, 45.3 mmol) in MeOH (50 mL) was added at 0 °C and stirring was continued at room temperature for 4.5 h. Next H_2O (25 mL) and 1 M HCl (100 mL) were added to the stirred reaction mixture. Stirring was continued at room temperature for 1 h. After removal of the organic layer, the aqueous layer was adjusted to pH 9 by 1 M NaOH and extracted with $CHCl_3$. The organic layer was dried over K_2CO_3. Removal of the solvent by evaporation gave 1-[amino(phenyl)methyl]cyclobutanol which was used for the next reaction without further purification. The title compound **47** was prepared from 1-[amino(phenyl)methyl]cyclobutanol according to essentially the same procedure as described for the preparation of methyl [(*tert*-butoxycarbonyl)amino](3-hydroxyphenyl)acetate from **41**: 13% yield in 3 steps; off-white solid; TLC $R_f = 0.37$ (*n*-hexane/EtOAc, 3/1), MS (APCI, Pos. 40eV) *m*/*z* 278 $(M+H)^+$, 178, 161; ^1H NMR (300 MHz, $CDCl_3$) δ 7.40–7.20 (m, 5H), 5.51 (br, 1H), 4.76 (d, $J = 8.4$ Hz, 1H), 2.40–2.23 (m, 1H), 2.20–2.00 (m, 2H), 1.93–1.63 (m, 3H), 1.43 (s, 9H).

1-(Phenylmethyl)-1-(octanoylamino)cyclobutyl dihydrogen phosphate (14). The title compound **14** was prepared from **47** according to the general method C: TLC $R_f = 0.44$ ($CHCl_3$/MeOH/H_2O, 65/25/4); IR (KBr) 3252, 2930, 2858, 1557, 1456, 1010 cm^{-1}; ^1H NMR (300 MHz,

CD_3OD) δ 7.45–7.38 (m, 2H), 7.35–7.20 (m, 3H), 5.15 (d, $J=2.4$ Hz, 1H), 2.88 (q, $J=12$ Hz, 1H), 2.45 (q, $J=12$ Hz, 1H), 2.32–2.14 (m) and 2.27 (t, $J=6$ Hz) total 4H, 1.94–1.80 (m, 1H), 1.78–1.52 (m, 3H), 1.40–1.16 (m, 8H), 0.88 (t, $J=6.6$ Hz, 3H); MS (FAB, Pos.) m/z 406 $(M+Na)^+$, 384 $(M+H)^+$; HRMS (MALDI-TOF, Pos.) calcd for $C_{19}H_{30}NO_5P+Na^+$: 406.1759; found: 406.1731.

(2*R*,3*R*)-3-Amino-3-phenylpropane-1,2-diol hydrochloride (51). A mixture of [(2*S*,3*S*)-3-phenyloxiran-2-yl]methanol **49** (5.6 g, 37.3 mmol), NaN_3 (4.86 g, 74.6 mmol) and NH_4Cl (991 mg, 17.8 mmol) in dioxane (50 mL)/H_2O (5 mL) was heated at 80 °C for 2 days with stirring. After cooling, the reaction mixture was diluted with EtOAc. The organic layer was successively washed with H_2O and brine before being dried over Na_2SO_4. Removal of the solvent by evaporation gave a residue which was purified by column chromatography on silica gel (Merck 7734, *n*-hexane/EtOAc, 3/1–1/1) to afford **50** as a colorless oil (4.28 g, 59% yield): TLC $R_f=0.38$ (*n*-hexane/EtOAc, 1/1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.50–7.35 (m, 5H), 4.65 (d, $J=6.9$ Hz, 1H), 3.60 (m, 1H), 3.80–3.65 (m, 2H), 2.19 (br, 1H), 1.97 (br, 1H). A mixture of **50** (4.28 g, 22 mmol) and 10% Pd-C (430 mg) in EtOH (60 mL)/6 M HCl (3.7 mL) was stirred at room temperature under an atmospheric pressure of hydrogen for 14 h. Removal of the catalyst by filtration through a pad of Celite followed by evaporation afforded **51** as a white solid: ^1H NMR (300 MHz, CD_3OD) δ 7.54–7.46 (m, 2H), 7.45–7.39 (m, 3H), 4.44 (d, $J=3.9$ Hz, 1H), 4.08–4.00 (m, 1H), 3.40 (dd, $J=11.0$, 5.5 Hz, 1H), 3.28 (dd, $J=11.0$, 6.0 Hz, 1H).

***N*-[(1*R*,2*R*)-2,3-dihydroxy-1-phenylpropyl]octanamide (52).** The title compound **52** was prepared from **51** according to essentially the same procedure as described for the preparation of **24b** from **23b**: white solid; 83% yield; TLC $R_f=0.55$ (EtOAc); ^1H NMR (300 MHz, $CDCl_3$) δ 7.43–7.25 (m, 5H), 6.16 (d, $J=7.2$ Hz, 1H), 5.01 (t, $J=7.8$ Hz, 1H), 3.86 (dt, $J=7.8$, 3.0 Hz, 1H), 3.66 (dq, $J=12.6$, 3.0 Hz, 2H), 2.21 (t, $J=7.5$ Hz, 2H), 1.62 (m, 2H), 1.40–1.10 (m, 8H), 0.87 (m, 3H).

(1*R*,2*R*)-1-Hydroxymethyl-2-octanolyamino-2-phenylethyl disodium phosphate (15). The title compound **15** was prepared from **55** according to the general method B: to a stirred mixture of **52** (1.3 g, 4.3 mmol) and imidazole (589 mg, 8.7 mmol) in DMF (20 mL) was added TBDPSCl (1.4 g, 5.2 mmol) at room temperature and stirring was continued at that temperature for 18 h. The reaction mixture was diluted with Et_2O. The organic layer was successively washed with saturated $NaHCO_3$ aq, H_2O and brine before being dried over Na_2SO_4. Removal of the solvent by evaporation gave **53** quantitatively as a colorless oil (2.3 g): TLC $R_f=0.35$ (*n*-hexane/EtOAc, 2/1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.73 (m, 1H), 7.60 (m, 3H), 7.45–7.30 (m, 6H), 7.28–7.20 (m, 5H), 6.70 (d, $J=8.1$ Hz, 1H), 5.19 (dd, $J=8.1$, 4.2 Hz, 1H), 3.99 (dd, $J=9.6$, 4.2 Hz, 1H), 3.59 (dd, $J=10.8$, 4.2 Hz, 1H), 3.48 (dd, $J=10.8$, 5.7 Hz, 1H), 2.18 (t, $J=7.2$ Hz, 2H), 1.60 (m, 2H), 1.38–1.15 (m, 8H), 1.09 (s, 9H), 0.87 (t, $J=6.6$ Hz, 3H). The compound **54** was prepared from **53** according to the same procedure as described for the preparation of dibenzyl (1*S*,2*R*)-1-methyl-2-(3-methoxyphenyl)-2-(octanoylamino)ethyl phosphate from **24b**: colorless oil; 70% yield from **53**. **54**: ^1H NMR (300 MHz, $CDCl_3$) δ 7.63–7.58 (m, 5H), 7.40–7.20 (m, 20H), 5.49 (dd, $J=8.1$, 3.0 Hz, 1H), 5.20–4.85 (m, 5H), 4.65–4.55 (m, 1H), 3.65–3.0 (m, 2H), 2.20–2.10 (m, 2H), 1.65–1.50 (m, 2H), 1.38–1.20 (m, 8H), 1.07 (s, 9H), 0.85 (m, 3H). (1*R*,2*R*)-1-*tert*-Butyldiphenylsilyloxymethyl-2-octanonyamino-2-phenylethyl dihydrogen phosphate was prepared from **54** according to the same procedure as described for the preparation of **25b** from dibenzyl (1*S*,2*R*)-1-methyl-2-(3-methoxyphenyl)-2-(octanoylamino)ethyl phosphate: TLC $R_f=0.50$ ($CHCl_3$/MeOH/H_2O, 65/25/4); ^1H NMR (300 MHz, $CDCl_3$) δ 7.70–7.60 (m, 4H), 7.45–7.20 (m, 1H), 5.50 (d, $J=3.3$ Hz, 1H), 4.63–4.58 (m, 1H), 3.74 (dd, $J=10.5$, 4.2 Hz, 1H), 3.42 (dd, $J=10.5$, 7.8 Hz, 1H), 2.24 (t, $J=7.2$ Hz, 2H), 1.65–1.53 (m, 2H), 1.38–1.20 (m, 8H), 1.09 (s, 9H), 0.89 (t, $J=6.3$ Hz, 3H). To a stirred solution of (1*R*,2*R*)-1-*tert*-butyldiphenylsilyloxymethyl-2-octanolyamino-2-phenylethyl dihydrogen phosphate (1.8 g, 3 mmol) in THF (20 mL) was added TBAF (1 M in THF, 7.5 mL, 7.5 mmol) at room temperature and stirring was continued at that temperature for 15 min. The reaction was quenched with H_2O and the pH adjusted to 12 with 2 M NaOH before the organic layer was extracted with Et_2O. The aqueous layer was acidified with 2 M HCl and extracted with EtOAc. Next, the organic layer was successively washed with H_2O and brine before being dried over Na_2SO_4. Removal of the solvent by evaporation gave **55** as an amorphous powder (700 mg, 63% yield): TLC $R_f=0.23$ ($CHCl_3$/MeOH/H_2O, 65/25/4); ^1H NMR (300 MHz, $CDCl_3$) δ 7.24–7.20 (m, 5H), 5.28 (d, $J=5.0$ Hz, 1H), 4.60–4.43 (m, 1H), 3.60–3.40 (m, 2H), 2.28 (t, $J=7.5$ Hz, 2H), 1.60 (m, 2H), 1.40–1.20 (m, 8H), 0.88 (m, 3H). 15: TLC $R_f=0.23$ ($CHCl_3$/MeOH/H_2O, 65/25/4); IR (KBr) 3418, 2928, 2856, 1644, 1539, 1455, 1094 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.44 (d, $J=7.2$ Hz, 2H), 7.26–7.14 (m, 3H), 4.93 (d, $J=2.7$ Hz, 1H), 4.48 (m, 1H), 3.38–3.25 (m, 2H), 2.36–2.18 (m, 2H), 1.55 (m, 2H), 1.38–1.18 (m, 8H),0.86 (t, $J=6.3$ Hz, 3H); MS (FAB, Pos.) m/z 418 $(M+H)^+$, 396, 374; HRMS (MALDI-TOF, Pos.) calcd for $C_{17}H_{26}NO_6P{\cdot}2Na+H^+$: 418.1371; found: 418.1407.

***N*-[(1*R*,2*S*)-2-Hydroxy-1,2-dipenylethyl]octanamide (57).** The title compound **57** was prepared from (1*S*,2*R*)-2-amino-1,2-diphenylethanol **56** according to essentially the same procedure as described for the preparation of **24b** from **23b**: white powder; 84% yield; ^1H NMR (300 MHz, $CDCl_3$) δ 8.11 (d, $J=9.6$ Hz, 1H), 7.35–7.10 (m, 10H), 5.35 (d, $J=4.8$ Hz, 1H), 4.94 (dd, $J=9.4$, 7.8 Hz, 1H), 4.70 (m, 1H), 1.93 (t, $J=7.0$ Hz, 2H), 1.34–0.90 (m, 10H), 0.85 (t, $J=6.2$ Hz, 3H).

Method D

(1*S*,2*R*)-1,2-Diphenyl-2-octanoylaminoethyl disodium phosphate (16). To a stirred mixture of **57** (770 mg, 2.3 mmol) and tetrazole (318 mg, 4.5 mmol) in CH_3CN (25 mL)–CH_2Cl_2 (25 mL) was added a solution of bis(2-cyanoethyl)diisopropylamidophosphite (678 mg, 2.5

mmol) in CH_3CN (5 mL) at room temperature and stirring was continued at that temperature for 1.5 h. The reaction mixture was poured into saturated $NaHCO_3$ aq and extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. Removal of the solvent by evaporation gave bis(2-cyanoethyl) (1*S*,2*R*)-1,2-diphenyl-2-(octanoylamino)ethyl phosphite as a white powder which was used for the next reaction without further purification (1.2 g, quant): TLC R_f = 0.43 (*n*-hexane/EtOAc, 1/1). To a stirred solution of bis(2-cyanoethyl) (1*S*,2*R*)-1,2-diphenyl-2-(octanoylamino)ethyl phosphite (1.2 g) in CH_2Cl_2 (30 mL) was added *m*-CPBA (57%, 685 mg, 2.3 mmol) at 0 °C and stirring was continued at that temperature for 30 min. To the reaction mixture saturated $Na_2S_2O_3$ aq was added and extracted with CH_2Cl_2. The organic layer was successively washed with H_2O, saturated $NaHCO_3$ aq and brine before being dried over Na_2SO_4. The *m*-chlorobenzoic acid was removed by passing the solution through a pad of Al_2O_3. Removal of the solvent by evaporation gave bis(2-cyanoethyl) (1*S*,2*R*)-1,2-diphenyl-2-(octanoylamino)ethyl phosphate as a white powder which was used for the next reaction without further purification (1.0 g, 84% yield): TLC R_f = 0.20 (CH_2Cl_2/EtOAc, 1/1). To a stirred solution of bis(2-cyanoethyl) (1*S*,2*R*)-1,2-diphenyl-2-(octanoylamino)ethyl phosphate (1.0 g, 1.9 mmol) in EtOH (20 mL) was added 50% Me_2NH aq and stirring was continued under reflux for 8 h. After cooling, removal of the solvent by evaporation gave a residue, which was dissolved in 1 M NaOH and extracted with Et_2O. The aqueous layer was acidified with 1 M HCl and extracted with EtOAc. The organic layer was successively washed with H_2O and brine before being dried over Na_2SO_4. Removal of the solvent by evaporation gave (1*S*,2*R*)-1,2-diphenyl-2-(octanoylamino)ethyl dihydrogen phosphate as a white solid (641 mg, 80% yield), which was converted to the disodium salt **16** according to the same procedure as descried for the preparation of **2** from **25b**: TLC R_f = 0.41 ($CHCl_3$/MeOH/H_2O, 65/25/4); IR (KBr) 3062, 2927, 1652, 1520, 1453, 1114 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.20–7.05 (m) and 7.00 (m) total 10H, 5.68 (dd, *J* = 10.2, 2.4 Hz, 1H), 4.96 (d, *J* = 2.4 Hz, 1H), 2.40–2.30 (m, 2H), 1.65–1.55 (m, 2H), 1.40–1.20 (m, 8H), 0.88 (t, *J* = 6.3 Hz, 3H); MS (FAB, Pos.) *m*/*z* 486 $(M+Na)^+$, 464 $(M+H)^+$, 442; HRMS (MALDI-TOF, Pos.) calcd for $C_{22}H_{28}NO_5P{\cdot}2Na+H^+$: 464.1579; found: 464.1557.

***N*-[(1*R*,2*S*)-2-Hydroxy-2,3-dihydro-1*H*-inden-1-yl]octanamide (59).** The title compound **59** was prepared from (1*R*,2*S*)-1-aminoindan-2-ol **58** according to essentially the same procedure as described for the preparation of **24b** from **23b**: 86% yield; TLC R_f = 0.13 (*n*-hexane/EtOAc, 3/2); ^1H NMR (300 MHz, $CDCl_3$) δ 7.30–7.10 (m, 4H), 6.20 (brd, *J* = 8.4 Hz, 1H), 5.37 (dd, *J* = 8.4, 5.1 Hz, 1H), 4.61 (ddd, *J* = 5.1, 5.1, 2.4 Hz, 1H), 3.16 (dd, *J* = 16.5, 5.1 Hz, 1H), 2.93 (dd, *J* = 16.5, 2.4 Hz, 1H), 2.31–2.26 (m, 2H), 1.70–1.50 (m, 2H), 1.40–1.20 (m, 8H), 0.88 (brt, *J* = 6.9 Hz, 3H).

(1*R*,2*S*)-1-(Octanoylamino)-2,3-dihydro-1*H*-inden-2-yl bis(2,2,2-trichloroethyl) phosphate (60). The title compound **60** was prepared from **59** according to essentially the same procedure as described for the preparation of **34** from **33**: 50% yield; ^1H NMR (200 MHz, $CDCl_3$) δ 7.30–7.20 (m, 4H), 6.06 (brd, *J* = 9.0 Hz, 1H), 5.75–5.67 (m, 1H), 5.40–5.33 (m, 1H), 4.61 (d. *J* = 7.0 Hz, 2H), 4.59 (d, *J* = 7.0 Hz, 2H), 3.38 (dd, *J* = 17.6, 1.6 Hz, 1H), 3.24 (dd, *J* = 17.6, 4.4 Hz, 1H), 2.32 (dd, *J* = 7.8, 7.2 Hz, 2H), 1.80–1.65 (m, 2H), 1.50–1.20 (m, 8H), 0.89 (t, *J* = 6.6 Hz, 3H).

(1*R*,2*S*)-1-(Octanoylamino)-2,3-dihydro-1*H*-inden-2-yl disodium phosphate (17). The title compound **17** was prepared from **60** according to the same procedures as described for the preparation of **6** from (1*S*,2*S*)-1-methyl-2-phenyl-2-(octanoylamino)ethyl bis(2,2,2-trichloroethyl) phosphate: 60% yield; TLC R_f = 0.29 ($CHCl_3$/MeOH/H_2O, 65/25/4); IR (KBr) 3305, 1626, 1542, 1459, 1106, 986 cm^{-1}; ^1H NMR (300 MHz, CD_3OD) δ 7.25–7.05 (m, 4H), 5.27 (d, *J* = 4.8 Hz, 1H), 5.01 (m, 1H), 3.35 (m, 1H), 3.03 (dd, *J* = 16.4, 4.4 Hz, 1H), 2.50–2.25 (m, 2H), 1.70 (m, 2H), 1.50–1.20 (m, 8H), 0.90 (m, 3H).; MS (FAB, Pos.) *m*/*z* 422 $(M+Na)^+$, 400 $(M+H)^+$; HRMS (MALDI-TOF, Pos.) calcd for $C_{17}H_{24}NO_5P{\cdot}2Na+H^+$: 400.1266; found: 400.1301.

Method E

Dibenzyl (1*S*,2*R*)-1-(octanoylamino)-2,3-dihydro-1*H*-inden-2-yl phosphate (61). To a stirred mixture of **59** (1.1 g, 4.0 mmol), Ph_3P (1.3 g, 4.8 mmol) and dibenzyl hydrogen phosphate (1.3 g, 4.8 mmol) in THF (12 mL) was added DEAD (40% in toluene, 2.1 mL, 4.8 mmol) at 0 °C. Stirring was continued at room temperature for 24 h. Removal of the solvent by evaporation gave a residue which was purified by column chromatography on silica gel (FL60D, *n*-hexane/EtOAc, 5/1–4/1) twice to afford **61** (260 mg, 12%): TLC R_f = 0.55 (CH_2Cl_2/MeOH, 19/1); ^1H NMR (300 MHz, $CDCl_3$) δ 7.40–7.15 (m, 14H), 5.89 (d, *J* = 8.1 Hz, 1H), 5.53 (dd, *J* = 8.1, 7.5 Hz, 1H), 5.10–5.01 (m, 4H), 4.92 (m, 1H), 3.22 (dd, *J* = 15.6, 6.9 Hz, 1H), 3.01 (dd, *J* = 15.6, 7.8 Hz, 1H), 2.17 (dd, *J* = 8.1, 7.2 Hz, 2H), 1.80–1.55 (m, 2H), 1.40–1.20 (m, 8H), 0.87 (t, *J* = 6.9 Hz, 3H).

(1*S*,2*R*)-1-(Octanoylamino)-2,3-dihydro-1*H*-inden-2-yl dihydrogen phosphate (62). The title compound **62** was prepared from **61** according to the same procedure as described for the preparation of **25b** from dibenzyl (1*S*,2*R*)-1-methyl-2-(3-methoxyphenyl)-2-(octanoylamino)ethyl phosphate: 83% yield; ^1H NMR (200 MHz, CD_3OD) δ 7.30–7.10 (m, 4H), 5.39 (brd, *J* = 5.8 Hz, 1H), 4.85 (m, 1H), 3.40 (dd, *J* = 16.0, 7.0 Hz, 1H), 3.04 (dd, *J* = 16.0, 6.6 Hz, 1H), 2.26 (brt, *J* = 7.4 Hz, 2H), 1.70–1.55 (m, 2H), 1.45–1.20 (m, 8H), 0.90 (brt, *J* = 6.6 Hz, 3H).

(1*S*,2*R*)-1-(Octanoylamino)-2,3-dihydro-1*H*-inden-2-yl disodium phosphate (18). The title compound **18** was prepared from **62** according to the same procedure as described for the preparation of **2** from **25b**: 87% yield; TLC R_f = 0.29 ($CHCl_3$/MeOH/H_2O, 65/25/4); IR (KBr) 3430, 1631, 1543, 1461, 1096, 987 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 7.14 (m, 4H), 5.16 (d, *J* = 7.6 Hz, 1H), 4.67 (ddd, *J* = 8.0, 7.6, 7.0 Hz, 1H), 3.41 (dd, *J* = 15.8, 7.0 Hz, 1H), 2.94 (dd, *J* = 15.8, 8.0 Hz, 1H),

2.25 (m, 2H), 1.68 (m, 2H), 1.45–1.20 (m, 8H), 0.89 (m, 3H); MS (FAB, Pos.) *m/z* 422 $(M+Na)^+$, 400 $(M+H)^+$; HRMS (MALDI-TOF, Pos.) calcd for $C_{17}H_{24}NO_5P{\cdot}2Na+H^+$: 400.1266; found: 400.1275.

***N*-[(1*R*)-2-hydroxy-1-phenylethyl]-*N*-methyloctanamide (64).** Ethyl (1*R*)-2-hydroxy-1-phenylethylcarbamate was prepared from (2*R*)-2-amino-2-phenylethanol **63** according to essentially the same procedures as described for the preparation of **24b** from **23b**. To the stirred suspension of $LiAlH_4$ (760 mg, 20 mmol) in THF (50 mL) was added dropwise a solution of ethyl (1*R*)-2-hydroxy-1-phenylethylcarbamate (2.1 g, 10 mmol) in THF (10 mL) under reflux. Stirring was continued for 11 h under reflux. To this stirred reaction mixture H_2O (0.76 mL), 15% NaOH aq (0.76 mL) and H_2O (2.2 mL) were successively added at 0 °C and the vigorous stirring was continued. The precipitates were removed by filtration and the filtrate was concentrated to give a residue, which was dissolved in dioxane (5 mL). To the mixture was added 4 M HCl in dioxane (3 mL) and removal of the solvent by evaporation gave a residue, which was solidified by Et_2O/EtOH to afford (2*R*)-2-(methylamino)-2-phenylethanol (665 mg, 36% yield): TLC R_f = 0.19 (CH_2Cl_2/MeOH/AcOH, 3/1/1). The title compound **64** was prepared from (2*R*)-2-(methylamino)-2-phenylethanol according to essentially the same procedure as described for the preparation of **24b** from **23b**: 67% yield: TLC R_f = 0.26 (*n*-hexane/EtOAc, 1/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.40–7.15 (m, 5H), 5.85, 5.14 (dd, *J* = 9.4, 5.0 Hz, 1H), 4.23–4.00 (m, 2H), 2.75 (s, 3H), 2.56–2.35 (m, 3H), 1.75–1.60 (m, 2H), 1.45–1.20 (m, 8H), 0.85 (m, 3H).

(2*R*)-2-[hexanoyl(methyl)amino]-2-phenylethyl dihydrogen phosphate (19). The title compound **19** was prepared from **64** according to the general method A: 17% yield; TLC R_f = 0.23 ($CHCl_3$/MeOH/H_2O, 65/25/4); IR (KBr) 3399, 1618, 1451, 1406, 1096, 987 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD, 2 rotamers) δ 7.34–7.20 (m, 5H), 5.94 (dd, *J* = 9.0, 6.0 Hz, 0.55H), 5.32 (dd, *J* = 9.0, 5.1 Hz, 0.45H), 4.41–4.13 (m, 2H), 2.93 (s, 1.65H), 2.88 (s, 1.35H), 2.60–2.35 (m, 2H), 1.60 (m, 2H), 1.45–1.20 (m, 8H), 0.89 (m, 3H); MS (FAB, Pos.) *m/z* 402 $(M+H)^+$; HRMS (MALDI-TOF, Pos.) calcd for $C_{17}H_{26}NO_5P{\cdot}2Na+H^+$: 402.1422; found: 402.1430.

Biological assay method

Inhibition of LPS-induced plasma TNF-α production in rats (iv). LPS from the *Escherichia coli* strain 055 B5 (Difco laboratories) and the test compounds were all dissolved in saline. Male Sprague–Dawley (CD)/IGS rats (Charles River Inc., Japan) aged 6–8 weeks (*n* = 5) were injected intravenously with the test compounds (0.01–0.1 mg/ 10 mL/kg), and then immediately given an intraperitoneal injection of LPS (30 μg/kg). Plasma TNF-α production was determined by ELISA 90 min after the LPS challenge using a commercial kit (R&D Systems). ID_{50} Values were determined by log-linear regression analysis (3–4 doses per compound). ID_{50} = The dosage required to inhibit plasma TNF-α production by 50%. The data were expressed as the mean ± SEM of 5 animals per group or ID_{50} values.

% Inhibition = 100−(C−S)/(L−S)×100
C: Plasma TNF-α concentration of LPS-treated animals pretreated with a test compound.
L: Plasma TNF-α concentration of LPS-treated animals pretreated with saline.
S: Plasma TNF-α concentration of saline-treated animals also pretreated with saline.

Inhibition of LPS-induced plasma TNF- α production in rats (po). The experiments were performed using male Sprague–Dawley (CD (IGS)) rats, 6–8 weeks of age, purchased from Charles River Breeding Laboratories (Shizuoka, Japan). The animals were given access to food and water ad libitum and were maintained on 12 h light/dark cycle at 22–23 °C. All experimental procedures were conformed to the Animal Care and Use Committee protocols filed at ONO Pharmaceutical Co., Ltd. (Osaka, Japan). In experiments under fasted condition, rats were fasted for about 16 h. Test compounds and LPS from *Escherichia coli* strain 055 B5 (DIFCO LABORATORIES, Detroit, MI, USA) were dissolved in saline. Compounds were administrated orally (1–100 mg/kg) to rats 30 min prior to intravenous injection of LPS at the dose of 30 μg/kg. After 90 min of LPS injection, heparinized blood was obtained. Blood was centrifuged and plasma samples were kept frozen at −80 °C. Plasma TNF-α concentration was determined by enzyme-linked immunosorbent assay using a commercial kit (BIOSOURCE international inc., CA, USA) according to the manufacturer's instructions. The data were expressed as the mean ± SEM of 5 animals per group or ID_{50} values. ID_{50} values, which describe the effective dose with 50% inhibition of TNF-α production, were determined by log-linear regression analysis (3–4 doses per compound).

Inhibition of LPS-induced plasma TNF-α production in mice (iv). Male BALB/c mice aged 8 weeks (*n* = 5) were injected intravenously with the test compounds (0.01–0.1 mg/ 10 mL/kg), and then immediately given an intraperitoneal injection of LPS (5 mg/ 10 mL/kg). Plasma TNF-α production was determined by ELISA 90 min after the LPS challenge using a commercial kit (GENZYME). ID_{50} = The dosage required to inhibit plasma TNF-α production by 50%. The data were expressed as the mean ± SEM of 5 animals per group or ID_{50} values.

% Inhibition = 100−(C−S)/(L−S)×100
C: Plasma TNF-α concentration of LPS-treated animals pretreated with a test compound.
L: Plasma TNF-α concentration of LPS-treated animals pretreated with saline.
S: Plasma TNF-α concentration of saline-treated animals also pretreated with saline.

LPS-induced shock model in mice. LPS from *Escherichia coli* strain 055 B5 (Difco Laboratories, Detroit, MI, USA) and the test compounds were all dissolved in saline. Female BALB/c mice (Charles River Inc., Shizuoka, Japan), 7 weeks of age, were injected intravenously with the test compounds and then immediately given an

intraperitoneal injection of LPS (20 mg/kg). The survival rate of the mice was evaluated after 96 h. Predonisolone (Shionogi Pharmaceutical Co., Ltd., 10 mg/kg, iv), which was used as the positive control, demonstrated an efficacy (survival rate: 13/20) equivalent to **4** (survival rate 15/20 at 0.3 mg/kg, iv) in this model. The survival rate of the controls which were given saline was 2/20. Results are expressed as the mean ± SEM. Parameters were analyzed with ANOVA-Dunnett's *t*-test, but survival was analyzed with the Mantel-Cox test.

D-(+)-Galactosamine/LPS-induced hepatitis model in rats. D-(+)-Galactosamine (SIGMA)/LPS (Difco Laboratories, Detroit, MI, USA) and the test compounds were all dissolved in saline. Male Sprague–Dawley rats (Charles River Inc., Shizuoka, Japan), 6 weeks of age, were injected intravenously with the test compounds and then immediately given an intraperitoneal injection of D-(+)-galactosamine/LPS (1 g/ 7.5 μg/ 5 mL/kg). The survival rate of the rats was evaluated after 96 h. Predonisolone (Shionogi Pharmaceutical Co. Ltd., 10 mg/kg, iv), which was used as the positive control, demonstrated an efficacy (survival rate: 13/18) equivalent to **4** (survival rate: 12/18 at 0.3 mg/kg, iv) in this model. The survival rate of the controls which were given saline was 0/18. Results are expressed as the mean ± SEM. Parameters were analyzed with ANOVA-Dunnett's *t*-test, but survival was analyzed with the Mantel-Cox test.

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