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Discovery of Novel Phosphonic Acid Derivatives as New Chemical Leads for Inhibitors of TNF-α Production

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Abstract—2-(Acylamino)benzylphosphonic acid **6** derived from an artificial substrate of sphingomyelinase was found to show inhibitory activity of TNF-α production. Structural optimization was started with the chemical modification of **6**. The discovery of another chemical leads **7**, **8**, **10** and **16** for the development of structurally new inhibitors of TNF-α production is reported.

Introduction

The inhibition of TNF-α production has emerged as a target in the treatment of a multitude of disorders including rheumatoid arthritis (RA), multiple sclerosis, cachexia, sepsis, ulcerative colitis, congestive heart failure and Crohn's disease because of its therapeutic potential.[1] Particular attention has been paid to small molecules possessing activity to inhibit the production of TNF-α.[2] As shown in Figure 1, we reported the discovery of a new class of compound, 2-(acylamino)-2-phenylethyl disodium phosphate,[3,4] as a chemical lead. Structural optimization was started with the chemical modification of the chemical lead **1** which inhibits LPS-induced TNF-α expression in the liver and spleen of mice.[5] However, this series of compounds, 2-(acylamino)-2-phenylethyl disodium phosphates, does not show any inhibitory activity in vitro according to our biological evaluations although they exhibited very potent inhibitory activity in vivo. For example, they did not exhibit the inhibitory activity in LPS-induced TNF-α production in vitro: (i) in whole blood from rat, mouse or human; (ii) in peripheral blood mononuclear cells (PBMC) from rat, mouse or human; and (iii) in spleen cells from rat or mouse. Metabolic stabilization of the phosphate moiety of **1**[6,7] followed by conformational analysis led to the discovery of the highly potent inhibitors **2** and **3**, which exhibited efficacy in animal models of disease (in LPS-induced shock model of mice or in D-(+)-galactosamine/LPS-induced hepatitis model of rats).[7] These compounds are expected to be clinically useful although no specialized uses are yet intended. Because of their two asymmetric centers, they are predicted to be problematic from the point of view of synthetic cost. Screening was further continued to identify more cost-effective molecules as drug candidates. We performed rapid screening at the dose of 10 mg/kg, iv, in mice because we could not detect any inhibitory activity in vitro assay systems in phosphonic acid series as well as 2-(acylamino)-2-phenylethyl disodium phosphates as described above. In the process, compound **6**, derived from an artificial substrate[8] of sphingomyelinase[9] (Fig. 2), was found to show moderate activity to inhibit the production of TNF-α. This information prompted us to carry out the molecular design of new chemical leads as shown in Figure 3. We here report the discovery of 2-(acylamino)benzylphosphonic acids **7**, **8**, **10** and **16**, as more cost-effective chemical lead.

Chemistry

The synthesis of all the biologically evaluated compounds **7**–**19** described in Tables 1 and 2 is outlined in Scheme 1. Appropriately substituted 2-nitrobenzyl

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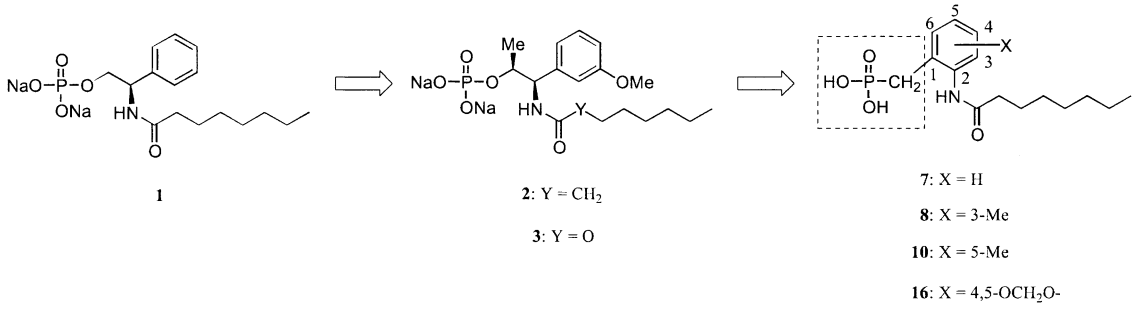


Figure 1. Discovery of new inhibitors **7**, **8**, **10** and **16**.

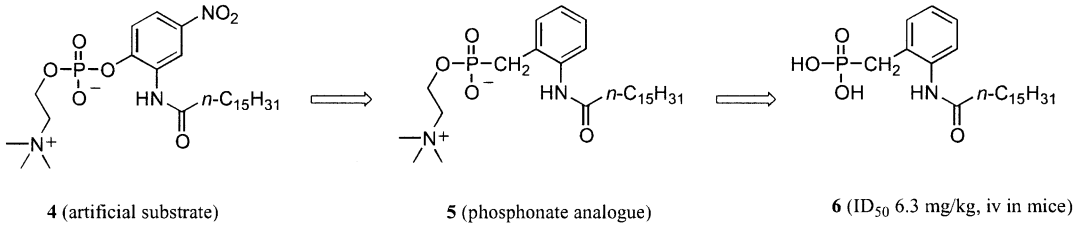


Figure 2. Discovery of a new chemical lead **6**.

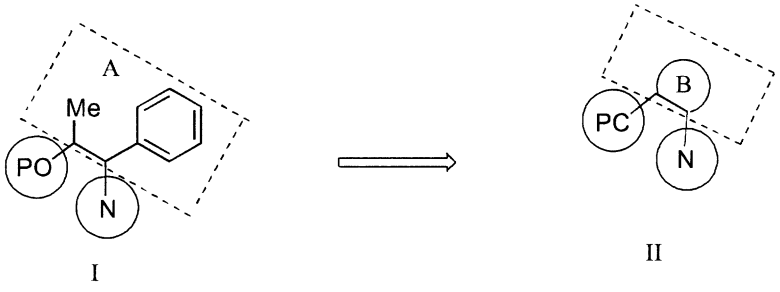
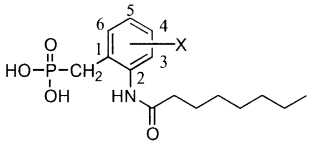


Figure 3. Molecular design of cost-effective molecules.

Table 1. Biological evaluation of 2-(octanoylamino)benzylphosphonic acids **7**–**16**



Compd	X	Inhibition of TNF-α production[a] ID_{50} (mg/kg, iv) in mice
7	H	2.8
8	3-Me	1.5
9	4-Me	(59)[b]
10	5-Me	0.9
11	6-Me	(50)[b]
12	3-OMe	(36)[b]
13	4-OMe	(39)[b]
14	5-OMe	6.8
15	6-OMe	(−21)[b]
16	4,5-OCH_2O–	1.6

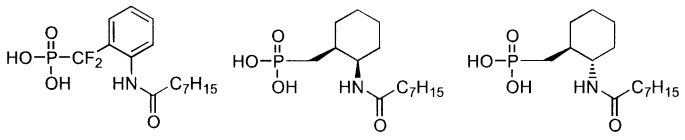
[a]See Experimental.
[b]Inhibition (%) at 10mg/kg, iv.

halides **20a**–**j**[13–16] were subjected to Arbuzov reaction to afford the corresponding diethyl phosphonates **21a**–**j**, reduction of the nitro group of which provided **22a**–**j**. Acylation of the anilines **22a**–**j** with octanoyl chloride followed by dealkylation of the diethyl phosphonates of **23a**–**j** provided the phosphonic acids **7**–**16**. Difluoromethylation was successfully carried out by a known procedure[10] to afford **24**. Catalytic hydrogenation of **24** followed by the acylation of **25** provided **26**. Dealkylation of the phosphonate **26** afforded **17**. Saturated derivatives **18** and **19** were prepared by the catalytic reduction of **23a** as described in Scheme 1. Chemical yields of all the reactions described above are given in Table 3.

Results and Discussion

In the course of further screening for inhibitors of TNF-α production, 2-(hexadecanoylamino) benzylphosphonic acid **6**, which was derived from an artificial substrate of

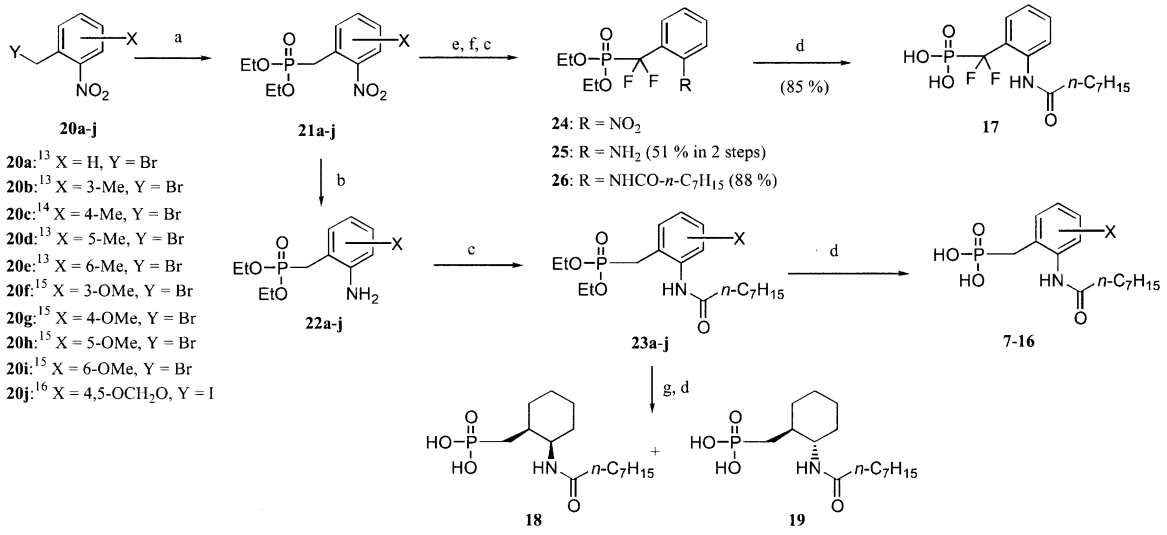
Table 2. Biological evaluation of the miscellaneous compounds **17**–**19**



17 **18** **19**

Compd	Inhibition of TNF-α production in mice[a] (% inhibition at 10 mg/kg, iv)
17	47
18 (racemic)	25
19 (tacemic)	16

[a]See Experimental.



Scheme 1. Synthesis of **7**–**19**. Reagents: (a) $P(OEt)_3$, toluene, reflux; (b) H_2, Pd-C, EtOH or $SnCl_2{\cdot}2H_2O$, EtOH; (c) n-$C_7H_{15}COCl$, Et_3N, CH_2Cl_2; (d) TMSBr, $CHCl_3$ or TMSI, $CHCl_3$; (e) NaHMDS, $(PhSO_2)_2NF$, THF; (f) H_2, Pd-C, MeOH; (g) H_2, PtO_2, AcOH, separation, *cis* (42%), *trans* (15%); Chemical yields of the above-described reactions (unless noted in parentheses) are listed in Table 3.

sphingomyelinase as shown in Figure 2, was found to exhibit moderate inhibitory activity (ID_{50} = 6.3 mg/kg, iv in mice). Based on this information, the molecular design of a cost-effective inhibitor of TNF-α production was started. As described in one of our full papers,[7] oral dosing of phosphate analogues exhibited much less potency relative to their iv dosing. Based on their structural similarity, phosphonic acid analogues were also considered to show much more activity by their iv dosing rather than by their oral dosing. Rapid screening was performed in duplicate or more at a dose of 10 mg/kg, iv, only (n = 5). The values of ID_{50} determination were then carried out for the compounds which showed more than 50% inhibition at the dose of 10 mg/kg, iv. When all experiments for the values of ID_{50} determination of compounds were performed, chemical lead **1** was used as a positive control (ID_{50} = 0.8 mg/kg, iv, in mice).

Table 3. Chemical yields of the reactions described in Scheme 1

Compd	Yield (%)			
	20a–j→21a–j	**21a–j→22a–j**	**22a–j→23a–j**	**23a–j→7–16**
7	70	Quantity	97	70
8	Quantity	Quantity	60	94
9	82	97	Quantity	59
10	30	Quantity	85	96
11	Quantity	99	89	59
12	83	99	45	53
13	69	88	85	76
14	65	94	88	61
15	98	99	92	93
16	94	99	Quantity	67

According to the process described in Figure 3, compounds **7**–**19**, in which the metabolically labile phosphate moiety is replaced with a presumed metabolically stable phosphonate moiety[11] and the two asymmetric centers are removed, were synthesized and evaluated as to their ability to inhibit TNF-α production in mice (Tables 1 and 2).

The outcome of the biological evaluation of compounds **7**–**16** is described in Table 1. Based on the information described in the preceding papers,[3,4] *N*-octanoyl was favored in the chemical modification of the phenyl moiety. The compound **7** was more than twice as active as the chemical lead **6**. Introduction of a 3-methyl group into the phenyl moiety of **7** afforded **8** with enhanced activity. 4-Methyl derivative **9** also retained the activity. 5-Methyl derivative **10** was the most potent inhibitor among this class of compounds **7**–**16** while 6-methyl

derivative **11** was the least active of all the methyl-substituted derivatives **8**–**11**. Introduction of a methoxy group into the phenyl moiety of **7** was predicted to optimize the inhibitory activity because of the excellent potential shown by **1**–**3**. Consequently, compounds **12**–**15** were synthesized and evaluated biologically. Unexpectedly, **12**–**15** were less potent than the corresponding methyl derivatives **8**–**11**, respectively. In both cases, the 5-substituted derivatives **10** and **14** were the most active compounds in each class. 4,5-Methylenedioxy derivative **16** demonstrated more potent activity than either of the corresponding monosubstituted compounds **13** and **14**. The formation of a five-membered ring fused to position-4 and-5 of the phenyl moiety of **7** was effective in increasing the inhibitory activity. As a result, introduction of a substituent into position-6, which is the *ortho*-position of the methylphosphonic acid moiety, markedly reduced the activity whichever substituent, lipophilic or hydrophilic, was introduced as illustrated in **11** and **15**. The introduction of a relatively hydrophilic substituent such as a methoxy group into position-6 was particularly deleterious, more so than that of a lipophilic substituent such as a 6-methyl group.

Miscellaneous modifications of **7** produced **17**–**19**, the biological evaluations of which are described in Table 2. Replacement of the methylene moiety attached directly to the phosphonic acid group of **7** with a difluoromethyl moiety afforded **17** with a significant reduction in inhibitory activity. The increased acidity of **17** caused by the conversion of the methylene moiety to a more electron-attracting difluoromethylene moiety was estimated to be one of the factors responsible for the reduced activity because of the greater chance of nonspecific interactions with proteins other than the target. As described in one of the preceding papers,[4] the three dimensional arrangement of the acidic phosphate moiety and *N*-acyl moiety was considered to be critical to the activity. Based on this information, two geometrical isomers of 2-(acylamino)cyclohexylmethylphosphonic acid, **18** and **19**, were synthesized as racemates and evaluated biologically. Both compounds showed less than 50% inhibition of TNF-α production at 10 mg/kg, iv in mice. Thus, the aromatic moiety was thought to be indispensable to the biological activity of this class of derivatives.

As shown in Figure 4a and b, increased production of the plasma TNF-α after the intraperitoneal (ip) administration of LPS (5 mg/kg) was significantly suppressed by administration of compounds **1** (positive control), **8**, **16** and **10** in a dose-dependent manner. The ID_{50} values of **8**, **16** and **10** are 1.5, 1.6 and 0.9, mg/kg, iv, respectively. These data showed the possibility of the newly discovered phosphonic acid derivatives as a more cost-effective chemical lead for the inhibitors of TNF-α production. Regarding the toxicity or side-effects of these compounds, hypotension activity, which was observed in some of the phosphate analogues,[12] was not observed after iv administration of **16** up to 10 mg/kg in rats. All the test compounds exhibited no in vitro TNF-α inhibition in the known assay systems. Mechanism of their action remains to be clarified. According to our experimental data, compound **1** was found to inhibit LPS-induced increase of TNF-α mRNA expression in mouse liver and spleen.[5] As a result, these phosphonate analogues was speculated to show TNF-α inhibition by the same mechanism of action as that of **1** because of their structural similarity.

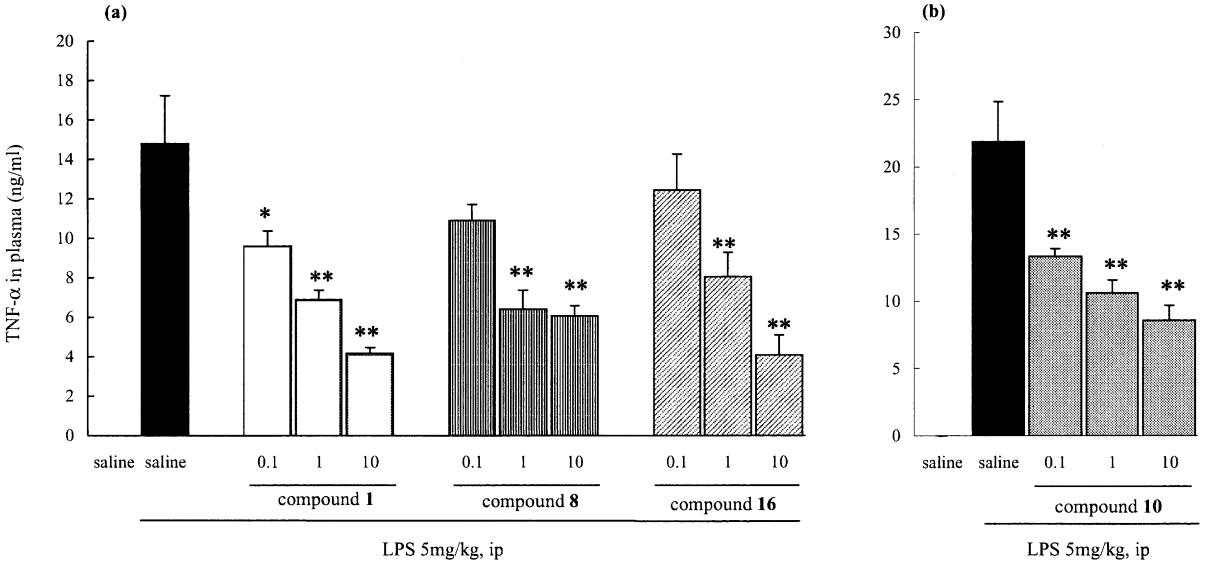


Figure 4. Effect of compound **1**, **8**, **16** and **10** on LPS-induced TNF-α production in mice. Compound was administrated intraperitonealy just before an intraperitoneal injection of LPS. After 90 minuets of LPS injection, heparinized blood was obtained. Plasma TNF-α concentration was determined by ELISA. The data were expressed as the mean±SEM ($n=5$/group; **, significantly different from LPS control, $P<0.01$, *, $P<0.05$, ANOVA-dunnett's *t*-test).

Conclusion

In summary, further screenings of a chemical lead followed by optimization were attempted in an effort to obtain more cost-effective inhibitors of TNF-α production and identify drug candidate. Based on the molecular design described in Figure 3, we have produced several chemical leads, most notably **7**, **8**, **10** and **16**, with the potential for further optimization. An increase in the acidity of the phosphonic acid moiety and the saturation of the aromatic moiety to the cyclohexyl moiety reduced the inhibitory activity as illustrated in **17**, **18** and **19**. Mechanism of their action remains to be clarified. Further optimization of these chemical leads is in progress in our laboratory.

Experimental

General directions

Analytical samples were homogeneous as confirmed by TLC, and afforded spectroscopic results consistent with the assigned structures. All ^{1}H NMR spectra were taken on a Varian Gemini-200, VXR-200s, Unity-INOVA 500 or Mercury 300 spectrometer. MS spectra were obtained on a Hitachi M1200H, JMS-DX303HF or PerSeptive Voyager Elite spectrometer. 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. 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 F254). The following abbreviations for solvents and reagents are used: THF, tetrahydrofuran; EtOAc, ethyl acetate; MeOH, methanol; EtOH, ethanol; CH_2Cl_2, dichloromethane; $CHCl_3$, chloroform.

General procedure A. Diethyl 2-nitrobenzylphosphonate (21a). To a stirred solution of 1-(bromomethyl)-2-nitrobenzene **20a** (6.48 g, 30 mmol) in toluene (60 mL) was added triethyl phosphite (5.14 mL, 30 mmol) and stirring was continued under reflux for 5 h. Removal of the solvent by evaporation gave a residue, which was purified by column chromatography on silica gel (Merck 7734, *n*-hexane/EtOAc, 1/1–1/4) to afford **21a** as a pale yellow oil (5.76 g, 70% yield): TLC R_f = 0.26 (*n*-hexane/EtOAc, 1/4); MS (APCI, Pos.) *m*/*z* 274 $(M+H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.96 (d, *J* = 7.8 Hz, 1H), 7.50–7.36 (m, 3H), 4.10–3.96 (m, 4H), 3.71 (d, *J* = 23.0 Hz, 2H), 1.24 (t, *J* = 7.2 Hz, 6H).

Diethyl 2-aminobenzylphosphonate (22a). A mixture of **21a** (5.75 g, 21 mmol) in EtOH (30 mL) and 5% Pd-C (500 mg) was stirred at room temperature under an atmospheric pressure of hydrogen for 6 h. Removal of the catalyst by filtration through a pad of Celite followed by evaporation afforded **22a** quantitatively as a pale yellow oil (5.12 g): TLC R_f = 0.48 ($CHCl_3$/MeOH, 1/4); MS (APCI, Pos.) *m*/*z* 244 $(M+H)^+$; ^{1}H NMR (200 MHz, $CDCI_3$) δ 7.16–7.00 (m, 2H), 6.81–6.74 (m, 2H), 4.30 (brs, 2H), 4.18–3.92 (m, 411), 3.16 (d, *J* = 23.0 Hz, 2H), 1.25 (t, *J* = 7.2 Hz, 611).

Diethyl 2-(octanoylamino)benzylphosphonate (23a). To a stirred solution of **22a** (1.53 g, 4.94 mmol) and Et_3N (1.76 mL, 12.6 mmol) in CH_2C1_2 (20 mL) was added octanoyl chloride (1.29 mL, 8.56 mmol) at 0 °C. Stirring was continued at that temperature for 30 min and at room temperature for 2 h. The reaction mixture was poured into ice-cold 1 M HCl and extracted with EtOAc. The organic layer was successively washed with H_2O saturated $NaHCO_3$ aq, brine and 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, 3/1) to afford **23a** as a colorless oil (2.26 g, 97% yield): TLC R_f = 0.41 (*n*-hexane/EtOAc, 1/1); MS (APCI, Pos.) *m*/*z* 370 $(M+H)^+$; ^{1}H NMR (200 MHz, $CDC1_3$) δ 9.44 (brs, IH), 7.78 (d, *J* = 7.8 Hz, 1H), 7.29 (m, 1H), 7.18–7.02 (m, 2H), 4.18–3.84 (m, 4H), 3.12 (d, *J* = 23.0 Hz, 2H), 1.42–1.20 (m, 14H), 0.88 (t, *J* = 6.2 Hz, 3H).

2-(Octanoylamino)benzylphosphonic acid (7). To a stirred solution of **23a** (2.25 g, 6.1 mmol) in $CHCl_3$ (10 mL) was added bromotrimethylsilane (2.42 mL. 18.3 ml) at room temperature and stirring was continued for 20 h at that temperature. Removal of the solvent by evaporation gave a residue, which was purified by column chromatography on silica gel (Merck 7734, $CHCl_3$/MeOH, 20/1) to afford an oily residue, which was solidified by Et_2O to obtain **7** as white powder: 70% yield; TLC R_f = 0.31 ($CHCl_3$/MeOH/AcOH, 8/1/1); IR (KBr) 3266, 2926, 2855, 1658, 1589, 1534, 1452, 1418, 1294, 1269, 1195, 1087 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.2 (s, 1H), 7.63 (d, *J* = 7.5 Hz, 1H), 7.21–6.98 (m, 3H), 2.96 (d, *J* = 21.0 Hz, 2H), 2.27 (t, *J* = 7.5 Hz, 2H), 1.61 (m, 2H), 1.40–1.18 (m, 8H), 0.87 (t, *J* = 6.5 Hz, 3H); MS (FAB, Pos) *m*/*z* 352 $(M+K)^+$, 314 $(M+H)^+$, 296; HRMS (MALDI-TOF, Pos.) calcd for $C_{15}H_{24}NO_4P$ + Na: 366.1341; found: 366.1354.

Preparation of **9**–**11** and **13**–**16**. These compounds were prepared according to the essentially same procedures as described for the preparation of **7** from **20a**.

4-Methyl-2-(octanoylamino)benzylphosphonic acid (9). The title compound **9** was prepared from 1-(bromomethyl)-4-methyl-2-nitrobenzene **20c** according to the same procedure as described for the preparation of **7** from **20a**. **21c**: 82% yield; TLC R_f = 0.30 (*n*-hexane/EtOAc, 1/3); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.77 (s, 1H), 7.35 (s, 2H), 4.12–3.96 (m, 4H), 3.65 (d, *J* = 23.0 Hz, 2H), 2.41 (d, *J* = 2.0 Hz, 3H), 1.24 (t, *J* = 7.0 Hz, 6H). **22c**: 97% yield; TLC R_f = 0.24 ($CHCl_3$/MeOH, 50/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.92 (dd, *J* = 8.0. 3.0 Hz, 1H), 6.63–6.55 (m, 2H), 4.43–4.25 (m, 2H), 4.10–3.89 (m, 4H), 3.11 (d, *J* = 21.0 Hz, 2H), 2.25 (d, *J* = 3.0 Hz, 3H), 1.25 (t, *J* = 7.0 Hz, 6H). **23c**: quant.; TLC R_f = 0.43 (*n*-hexane/EtOAc, 1/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.39 (s, 1H), 7.61 (s, 1H), 7.02 (dd, *J* = 8.0, 3.0 Hz, 1H), 6.90 (d, *J* = 8.0 Hz, 1H), 4.16–3.86 (m, 4H), 3.08 (d, *J* = 21.0 Hz, 2H), 2.41 (t, *J* = 7.0 Hz, 2H), 2.33

(d, $J=3.0$ Hz, 3H), 1.82–1.65 (m, 2H), 1.50–1.20 (m, 8H), 1.25 (t, $J=8.0$ Hz, 6H), 0.88 (t, $J=7.0$ Hz, 3H). 9: 59% yield; white powder; TLC $R_f=0.80$ ($CHCl_3$/MeOH/AcOH, 5/1/1); IR (KBr) 3265, 2927, 1654, 1538 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 7.33 (s, 1H), 7.18 (dd, $J=3,8$ Hz, 1H), 6.98 (d, $J=8$ Hz, 1H), 3.08 (d, $J=21.0$ Hz, 2H), 2.40 (t, $J=8.0$ Hz, 2H), 2.30 (d, $J=2.0$ Hz, 3H), 1.81–1.62 (m, 2H), 1.59–1.16 (m, 8H), 0.91 (t, $J=7.0$ Hz, 3H); MS (MALDI-TOF, Pos.) m/z 366$(M+K)^+$, 350 $(M+Na)^+$, 328 $(M+H)^+$; HRMS (MALDI-TOF, Pos.) calcd for HRMS (MALDI-TOF, Pos.) calcd for $C_{16}H_{26}NO_4P+Na$: 350.1497; found: 350.1493.

5-Methyl-2-(octanoylamino)benzylphosphonic acid (10). The title compound **10** was prepared from 1-(bromomethyl)-5-methyl-2-nitrobenzene **20d** according to the same procedure as described for the preparation of **7** from **20a**. **21d**: 30% yield; TLC $R_f=0.20$ (*n*-hexane/EtOAc, 1/3); ^1H NMR (200 MHz, $CDCl_3$) δ 7.89 (d, $J=8.0$ Hz, 1H), 7.27–7.23 (m, 2H), 4.03 (dq, $J=8.0$. 7.0 Hz, 4H), 3.69 (d, $J=23.0$ Hz, 2H), 2.41 (s, 3H), 1.23 (t, $J=7.0$ Hz, 6H). **22d**: quant.; TLC $R_f=0.62$ ($CHCl_3$/MeOH, 9/1); ^1H NMR (200 MHz, $CDCl_3$) δ 6.87–6.83 (m, 2H), 6.63 (d, $J=8.0$ Hz, 1H), 4.26–3.88 (m, 4H), 3.10 (d, $J=21.0$ Hz, 2H), 2.22 (s, 3H), 1.25 (t, $J=7.0$ Hz, 6H). **23d**: 85% yield; TLC $R_f=0.73$ (EtOAc); ^1H NMR (200 MHz, $CDCl_3$) δ 9. 27 (s, 1H), 7.63 (d, $J=8.0$ Hz, 1H), 7.13–7.07 (m, 1H), 6.95 (s, 1H), 4.12–3.90 (m, 4H), 3.08 (d, $J=21.0$ Hz, 2H), 2.40 (t, $J=7.0$ Hz, 2H), 2.29 (s, 3H), 1.83–1.64 (m, 2H), 1.47–1.20 (m, 8H), 1.25 (t, $J=8.0$ Hz, 6H), 0.88 (t, $J=6.0$ Hz, 3H). **10**: 96% yield; white powder; TLC $R_f=0.31$ ($CHCl_3$/MeOH/AcOH, 8/1/1); IR (KBr) 3263, 2927, 2856, 1652, 1532 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 7.36 (d, $J=8.0$ Hz, 1H), 7.16–7.03 (m, 2H), 3.08 (d, $J=21.0$ Hz, 2H), 2.39 (t, $J=8.0$ Hz, 2H), 2.30 (s, 3H), 1.80–1.62 (m, 2H), 1.44–1.18 (m, 8H), 0.91 (t, $J=7.0$ Hz, 3H); MS (MALDI-TOF, Pos.) m/z 350 $(M+Na)^+$; HRMS (MALDI-TOF, Pos.) calcd for $C_{16}H_{26}NO_4P+Na$: 350.1497; found: 350.1481.

6-Methyl-2-(octanoylamino)benzylphosphonic acid (11). The title compound **11** was prepared from 1-(bromomethyl)-6-methyl-2-nitrobenzene **20e** according to the same procedure as described for the preparation of **7** from **20a**. **21e**: quant.; TLC $R_f=0.24$ (*n*-hexane/EtOAc, 1/3); ^1H NMR (200 MHz, $CDCl_3$) δ 7.71(d, $J=8.0$ Hz, 1H), 7.44 (d, $J=8.0$ Hz, 1H), 7.28 (dt, $J=8.0$, 2.0 Hz, 1H), 4.01 (dq, $J=8.0$, 7.0 Hz, 4H), 3.77 (d, $J=23.0$ Hz, 2H), 2.53 (d, $J=2.0$ Hz, 3H), 1.24 (t, $J=7.0$ Hz, 6H). **22e**: 99% yield; TLC $R_f=0.69$ ($CHCl_3$/MeOH, 9/1); ^1H NMR (200 MHz, $CDCl_3$) δ 6.96 (dt, $J=8.0$, 3.0 Hz, 1H), 6.69–6.56 (m, 2H), 4.10–3.91 (m, 4H), 3.19 (d, $J=21.0$ Hz, 2H), 2.31 (d, $J=2.0$ Hz, 3H), 1.25 (t, $J=7.0$ Hz, 6H). **23e**: 89% yield; TLC $R_f=0.80$ (EtOAc); ^1H NMR (200 MHz, $CDCl_3$) δ 9.39 (s, 1H), 7.57 (d, $J=8.0$ Hz, 1H), 7.18 (d, $J=2.8$ Hz, 1H), 7.00 (d, $J=8.0$ Hz, 1H), 4.16–3.72 (m, 4H), 3.17 (d, $J=21.0$ Hz, 2H), 2.42 (t, $J=8.0$ Hz, 2H), 2.34 (d, $J=1.0$ Hz, 3H), 1.84–1.18 (m, 10H), 1.25 (t, $J=7.0$ Hz, 6H), 0.88 (t, $J=7.0$ Hz, 3H). **11**: 59% yield; white powder; TLC $R_f=0.47$ ($CHCl_3$/MeOH/AcOH, 8/1/1); IR (KBr) 3269, 2856, 1651, 1536 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 7.36 (d, $J=7.0$ Hz, 1H), 7.22–7.03 (m, 2H), 3.17 (d, $J=22.0$ Hz, 2H), 2.41 (t, $J=8.0$ Hz, 2H), 2.40 (s, 3H), 1.82–1.64 (m, 2H), 1.50–1.18 (m, 8H), 0.91 (t, $J=7.0$ Hz, 3H); MS (MALDI-TOF, Pos.) m/z 350 $(M+Na)+$; HRMS (MALDI-TOF, Pos.) calcd for $C_{16}H_{26}NO_4P+Na$: 350.1497; found: 350.1470.

4-Methoxy-2-(octanoylamino)benzylphosphonic acid (13). The title compound **13** was prepared from 1-(bromomethyl)-4-methoxy-2-nitrobenzene **20g** according to the same procedure as described for the preparation of **7** from **20a**. **21g**: 69% yield; TLC $R_f=0.44$ (EtOAc); ^1H NMR (200 MHz, $CDCl_3$) δ 7.48 (d, $J=3.0$ Hz, 1H), 7.37 (dd, $J=9.0$, 3.0 Hz, 1H), 7.10 (ddd, $J=9.0$, 3.0, 1.0 Hz, 1H), 4.03 (dq, $J=8.0$, 7.0 Hz, 4H), 3.86 (s, 3H), 3.62 (d, $J=22.0$ Hz, 2H), 1.24 (t, $J=7.0$ Hz, 6H). **22g**: 88% yield; TLC $R_f=0.28$ (EtOAc); MS (MALDI-TOF, Pos.) m/z 296 $(M+Na)^+$, 274 $(M+H)^+$; ^1H NMR (200 MHz, $CDCl_3$) δ 6.93 (dd, $J=8.0$, 3.0 Hz, 1H), 6.36–6.25 (m, 2H), 4.44–4.18 (m, 2H), 4.14–3.91 (m, 4H), 3.75 (s, 3H), 3.05 (d, $J=20.0$ Hz, 2H), 1.25 (t, $J=7.0$ Hz, 6H). **23g**: 85% yield; TLC $R_f=0.75$ (EtOAc); ^1H NMR (200 MHz, $CDCl_3$) δ 9.51 (s, 1H), 7.02 (dd, $J=8.0$, 2.0 Hz, 1H), 6.66 (dd, $J=8.0$, 3.0 Hz, 1H), 4.17–3.89 (m, 4H), 3.80 (s, 3H), 3.06 (d, $J=21.0$ Hz, 2H), 2.42 (t, $J=8.0$ Hz, 2H), 1.83–1.06 (m, 10H), 1.25 (t, $J=7.0$ Hz, 6H), 0.88 (t, $J=7.0$ Hz, 3H). **13**: 76% yield; white powder; TLC $R_f=0.18$ ($CHCl_3$/MeOH/AcOH, 8/1/1); IR (KBr) 3257, 2931, 1651, 1537, 1293, 1057 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 7.21–7.13 (m, 2H), 6.74 (dd, $J=3.0$, 8.0 Hz, 1H), 3.76 (3H,S), 3.05 (d, $J=21.0$ Hz, 2H), 2.40 (t, $J=8.0$ Hz, 2H), 1.80–1.62 (m, 2H), 1.48–1.20 (m, 8H), 0.90 (t, $J=7.0$ Hz, 3H); MS (APCI, Neg.) m/z 342 $(M-H)^-$; HRMS (MALDI-TOF, Pos.) calcd for $C_{16}H_{26}NO_5P+Na$: 366.1446; found: 366.1399.

5-Methoxy-2-(octanoylamino)benzylphosphonic acid (14). The title compound **14** was prepared from 1-(bromomethyl)-5-methoxy-2-nitrobenzene **20 h** according to the same procedure as described for the preparation of **7** from **20a**. **21h**; 65% yield; TLC $R_f=0.29$ (*n*-hexane/EtOAc, 1/4); ^1H NMR (200 MHz, $CDCl_3$) δ 8.06 (d, $J=9.0$ Hz, 1H), 6.96–6.82 (m, 2H), 4.07 (dq, $J=8.0$, 7.0 Hz, 4H), 3.89 (s, 3H), 3.76 (d, $J=23.0$ Hz, 2H), 1.25 (t, $J=7.0$ Hz, 6H). **23h**: 88% yield; TLC $R_f=0.61$ (*n*-hexane/EtOAc, 1/3); ^1H NMR (200 MHz, $CDCl_3$) δ 9.17 (s, 1H), 7.62 (d, $J=9.0$ Hz, 1H), 6.84 (dt, $J=9.0$, 3.0 Hz, 1H), 6.70 (t, $J=3.0$ Hz, 1H), 4.16–3.88 (m, 4H), 3.78 (s, 3H), 3.09 (d, $J=21.0$ Hz, 2H), 2.40 (t, $J=8.0$ Hz, 2H), 1.83–1.66 (m, 2H), 1.51–1.20 (m, 8H), 1.26 (t, $J=7.0$ Hz, 6H), 0.88 (t, $J=6.0$ Hz, 3H). **14**: 61% yield; white powder; TLC $R_f=0.48$ ($CHCl_3$/MeOH/AcOH, 8/1/1); IR (KBr) 3270, 2927, 2854, 1651, 1537, 1239, 1057 cm^{-1}; ^1H NMR (200 MHz, CD_3OD) δ 7.32 (d, $J=9.0$ Hz, 1H), 6.89 (t, $J=3.0$ Hz, 1H), 6.82 (dt, $J=9.0$, 3.0 Hz, 1H), 3.77 (s, 3H), 3.08 (d, $J=22.0$ Hz, 2H), 2.38 (t, $J=8.0$ Hz, 2H), 1.82–1.63 (m, 2H), 1.49–1.20 (m, 8H), 0.90 (t, $J=7.0$ Hz, 3H); MS (APCI, Neg.) m/z 342 $(M-H)^-$; HRMS (MALDI-TOF, Pos.) calcd for $C_{16}H_{26}NO_5P+Na$: 366.1446; found: 366.1414.

6-Methoxy-2-(octanoylamino)benzylphosphonic acid (15). The title compound **15** was prepared from 1-(bromo-

methyl)-6-methoxy-2-nitrobenzene **20i** according to the same procedure as described for the preparation of **7** from **20a**. **21i**: 98% yield; TLC R_f = 0.23 (EtOAc); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.50 (d, J = 8.0 Hz, 1H), 7.35 (dt, J = 8.0, 2.2 Hz, 1H), 7.12 (d, J = 8.0 Hz, 1H), 4.15–3.90 (m, 7H), 3.84 (d, J = 23.2 Hz, 2H), 1.27–1.18 (m, 6H). **22i**: The product was used for the next reaction without further purification; 99% yield; TLC R_f = 0.57 ($CHCl_3$/MeOH, 10/1). **23i**: 92% yield; TLC R_f = 0.33 (*n*-hexane/EtOAc, 1/1); ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.51 (s, 1H), 7.43 (d, J = 8.0 Hz, 1H), 7.24 (dt, J = 8.0, 2.6 Hz, 1H), 6.70 (d, J = 8.0 Hz, 1H), 4.14–3.93 (m, 4H), 3.83 (s, 3H), 3.26 (d, J = 21.4 Hz, 2H), 2.41 (t, J = 7.2 Hz, 2H), 1.85–1.60 (m, 2H), 1.50–1.21 (m, 14H), 0.91–0.85 (m, 3H). **15**: 93% yield; white powder; TLC R_f = 0.31 ($CHCl_3$/MeOH/AcOH, 8/1/1); IR (KBr) 3263, 2929, 2857, 1656, 1594, 1537, 1477, 1441, 1414, 1314, 1265, 1197, 1148, 1077, 1012 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 10.3 (s, 1H), 7.31 (d, J = 8.0 Hz, 1H), 7.15 (dt, J = 8.0, 2.2 Hz, 1H), 6.77 (d, J = 8.0 Hz, 1H), 3.77 (s, 3H), 3.04 (d, J = 21.2 Hz, 2H), 2.26 (t, J = 7.4 Hz, 2H), 1.65–1.59 (m, 2H), 1.28 (brs, 8H), 0.90–0.83 (m, 3H); MS (MADLI-TOF, Pos.) m/z 382 $(M+K)^+$, 366 $(M+Na)^+$, 344 $(M+H)^+$; HRMS (MALDI-TOF, Pos.) calcd for $C_{16}H_{26}NO_5P$ + Na: 366.1446; found: 366.1440.

[6-(Octanoylamino)-1,3-benzodioxol-5-yl]methylphosphonic acid (16). The title compound **16** was prepared from 5-(iodomethyl)-6-nitro-1,3-benzodioxole **20j** according to the same procedure as described for the preparation of **7** from **20a**. **21j**: 94% yield; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.51 (s, 1H), 6.86 (d, J = 2.4 Hz, 1H), 6.11 (s, 2H), 4.10–4.00 (m, 4H), 3.67 (d, J = 22.5 Hz, 2H), 1.26 (t, J = 6.6 Hz, 6H). **23j**: quant.; TLC R_f = 0.55 (*n*-hexane/EtOAc, 1/3); ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.26 (s, 1H), 7.23 (s, 1H), 6.75 (d, J = 2.0 Hz, 1H), 5.95 (s, 2H), 4.20–3.91 (m, 4H), 3.01 (d, J = 21.0 Hz, 2H), 2.39 (t, J = 8.0 Hz, 2H), 1.81–1.52 (m, 2H), 1.44–1.18 (m, 8H), 1.27 (t, J = 7.0 Hz, 6H), 0.88 (t, J = 7.0 Hz, 3H). **16**: 67% yield; white powder; TLC R_f = 0.35 ($CHCl_3$/MeOH/H_2O, 65/25/4); IR (KBr) 3253, 2927, 1650, 1536, 1506, 1488, 1038 cm^{-1}; ^{1}H NMR (300 MHz, CD_3OD) δ 6.95 (s, 1H), 6.78 (d, J = 2.1 Hz, 1H), 5.94 (s, 2H), 3.02 (d, J = 21.3 Hz, 2H), 2.38 (t, J = 7.0 Hz, 2H), 1.72 (q, J = 7.0 Hz, 2H), 1.45–1.20 (m, 8H), 0.91 (t, J = 6.9 Hz, 3H); MS (MALDI-TOF, Pos) m/z 380 $(M+Na)^+$, 358 $(M+H)^+$; HRMS (MALDI-TOF, Pos.) calcd for $C_{16}H_{26}NO_6P$ + Na: 380.1239; found: 380.1256.

General Procedure B. 3-Methyl-2-(octanoylamino)benzylphosphonic acid (8). Compound **21b** was prepared from 1-(bromomethyl)-3-methyl-2-nitrobenzene **20b** according to the essentially same procedures as described for the preparation of **21a** from **20a**. quant.; MS (MALDI-TOF, Pos.) m/z 310 $(M+Na)^+$, 288 $(M+H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.43–7.13 (m, 3H), 4.08 (dq, J = 22.0, 7.0 Hz, 4H), 3.25 (d, J = 22.0 Hz, 2H), 2.34 (s, 3H), 1.26 (t, J = 7.0 Hz, 6H). To a stirred solution of **21b** (6.2 g, 21.6 mmol) and concd HCl (18.8 mL) in EtOH (100 mL) was added $SnCl_2 \cdot H_2O$ (14.2 g, 62.9 mmol) in EtOH (20 mL) at room temperature and stirring was continued for 2 h at that temperature. The reaction mixture was neutralized with 5M NaOH and the resulting precipitates were removed by filtration. The filtrate was extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. Removal of the solvent by evaporation gave **22b**, which was used for the next reaction without further purification: TLC R_f = 0.45 ($CHCl_3$/MeOH, 20/1); MS (MALDI-TOF, Pos.) m/z 280 $(M+Na)^+$, 258 $(M+H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.01–6.88 (m, 2H), 6.65 (t, J = 7.0 Hz, 1H), 4.13–3.93 (m, 2H), 3.14 (d, J = 21.0 Hz, 2H), 2.20 (s, 3H), 1.26 (t, J = 7.0 Hz, H). Compound **23b** was prepared from **22b** according to the same procedure as described for the preparation of **23a** from **22a**. **23b**: 60% yield; white powder; TLC R_f = 0.71 (EtOAc); MS (MALDI-TOF, Pos.) m/z 422 $(M+K)^+$, 406 $(M+Na)^+$, 384 $(M+H)^+$; ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.02 (s, 1H), 7.21–7.13 (m, 1H), 7.11 (t, J = 8.0 Hz, 1H), 7.06–6.91 (m, 1H), 4.10–3.83 (m, 4H), 3.11 (d, J = 21.0 Hz, 2H), 2.43 (t, J = 8.0 Hz, 2H), 2.25 (s, 3H), 1.84–1.61 (m, 2H), 1.48–1.16 (m, 14H), 0.89 (t, J = 7.0 Hz, 3H). The title compound **8** was prepared from **23b** according to the same procedure as described for the preparation of **7** from **23a**. **8**: 94% yield; white powder; TLC R_f = 0.28 (AcOEt/AcOH/H_2O, 8/1/1); IR (KBr) 3259, 2927, 2857, 1655, 1529, 1469 cm^{-1}; ^{1}H NMR (200 MHz, CD_3OD) δ 7.23–7.11 (m, 3H), 3.08 (d, J = 22.0 Hz, 2H), 2.45 (t, J = 8.0 Hz, 2H), 2.21 (s, 3H), 1.85–1.63 (m, 2H), 1.58–1.19 (m, 8H), 0.91 (t, J = 7.0 Hz, 3H); MS (FAB, Pos.) m/z 328 $(M+H)^+$; HRMS (MALDI-TOF, Pos.) calcd for $C_{16}H_{26}NO_4P$ + Na: 350.1497; found: 350.1540.

3-Methoxy-2-(octanoylamino)benzylphosphonic acid (12). The title compound **12** was prepared from 1-(bromomethyl)-3-methoxy-2-nitrobenzene **20f** according to the same procedure as described for the preparation of **8** from **20b**. **21f**: 83% yield; TLC R_f = 0.56 (EtOAc); ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.39 (d, J = 8.0 Hz, 1H), 7.12 (ddd, J = 8.0, 2.0, 1.0 Hz, 1H), 6.96 (dd, J = 8.0, 2.0 Hz, 1H), 4.04 (dq, J = 8.0, 7.0 Hz, 4H), 3.89 (s, 3H), 3.20 (d, J = 22.0 Hz, 2H), 1.27 (t, J = 7.0 Hz, 6H). **22f**: 99% yield; TLC R_f = 0.45 (EtOAc); ^{1}H NMR (200 MHz, $CDCl_3$) δ 6.78–6.62 (m, 3H), 4.58–4.32 (m, 2H), 4.22–3.91 (m, 4H), 3.84 (s, 3H), 3.14 (d, J = 21.0 Hz, 2H), 1.25 (t, J = 7.0 Hz, 6H). **23f**: 45% yield; TLC R_f = 0.25 (*n*-hexane/EtOAc, 1/3); ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.32 (s, 1H), 7.15 (d, J = 8.0 Hz, 1H), 6.19–6.77 (m, 2H), 4.10–3.90 (m, 4H), 3.83 (s, 3H), 3.13 (d, J = 21.0 Hz, 2H), 2.42 (t, J = 8.0 Hz, 2H), 1.82–1.60 (m, 2H), 1.52–1.20 (m, 8H), 1.24 (t, J = 7.0H, 6H), 0.89 (t, J = 7.0 Hz, 3H). **12**: 53% yield; white powder; TLC R_f = 0.31 ($CHCl_3$/MeOH/AcOH, 8/1/1); IR (KBr) 3257, 2927, 2856, 1636, 1531, 267, 1006 cm^{-1}; ^{1}H NMR (200 MHz, CD_3OD) δ 7.23 (t, J = 8.0 Hz, 1H), 7.03–6.90 (m, 2H), 3.80 (s, 3H), 3.08 (d, J = 21.0 Hz, 2H), 2.42 (t, J = 8.0 Hz, 2H), 1.81–1.64 (m, 2H), 1.51–1.19 (m, 8H), 0.91 (t, J = 7.0 Hz, 3H); MS (MALDI-TOF, Pos.) m/z 382 $(M+K)^+$, 366 $(M+Na)^+$, 344 $(M+H)^+$; HRMS (MALDI-TOF, Pos.) calcd for $C_{16}H_{26}NO_5P$ + Na: 366.1446; found: 366.1472.

Difluoro[2-(octanoylamino)phenyl]methfr1phosphonic acid (17). To a stirred solution of sodium bis(-

trimethylsilyl)amide (17.6 mL, 1M in THF, 17,6 mmol) in THF (20 mL) was added dropwise a solution of **21a** (2.18 g, 7.99 mmol) in TAF (20 mL) at −78 °C and stirring was continued for l h at that temperature. To the reaction mixture was added dropwise a solution of *N*-fluorobenzenesulfonimide (6.31 g, 20 mmol) in THF (20 mL) at that temperature. Stirring was continued at that temperature for 2 h and then at −20 °C for 1 h. The reaction mixture was quenched with ice-cold 1 M HCl and extracted with EtOAc. The organic layer was successively washed with H_2O, saturated $NaHCO_3$, brine and 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, 1/2) to afford diethyl difluoro(2-nitrophenyl)methylphosphonate **24** (2.03 g). The product was used for the next reaction without further purification: TLC $R_f = 0.55$ (*n*-hexane/EtOAc, 1/4); ^{1}H NMR (200 MHz, CDC1$_3$) δ 7.90–7.50 (m, 4H), 4.40–4.20 (m, 4H), 1.40–1.30 (m, 6H). Diethyl (2-aminophenyl)-difluoromethylphosphonate **25** was prepared from **24** according to the same procedure as described for the preparation of **22a** from **21a** (51% yield from **21a**). Diethyl difluoro[2-(octanoylamino)phenyl]methylphosphonate **26** was prepared from **25** according to the same procedure as described for the preparation of **23a** from **22a**. **26**: 88% yield; TLC $R_f = 0.31$ (*n*-hexane/EtOAc, 2/1); ^{1}H NMR (200 MHz, CDCl$_3$) δ 9.36 (brs, 1H), 8.04 (d, $J = 8.5$ Hz, 1H), 7.53–7.45 (m, 2H), 7.20 (t, $J = 7.6$ Hz, 1H), 4.50–4.02 (m, 4H), 2.39 (t, $J = 7.4$ Hz, 2H), 1.74 (m, 2H), 1.48–1.20 (m, 14H), 0.88 (t, $J = 6.6$ Hz, 3H). The title compound **17** was prepared from **26** according to the essentially same procedure as described for the preparation of **7** from **23a** using iodotrimethylsilane instead of bromotrimethylsilane: 85% yield; white powder; TLC $R_f = 0.36$ (CHCl$_3$/MeOH/AcOH, 5/1/1); IR (KBr) 3301, 2929, 2858, 1646, 1590, 1452, 1296, 1140, 1030 cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6) δ 7.86 (d, $J = 8.0$ Hz, 1H), 7.40 (d, $J = 8.0$ Hz, 1H), 7.30 (t, $J = 8.0$ Hz, 1H), 7.06 (t, $J = 8.0$ Hz, 1H), 2.24 (m, 2H), 1.56 (m, 2H), 1.40–1.20 (m, 8H), 0.86 (m, 3H); MS (FAB, Neg.) *m*/*z* 348 (M–H)$^-$; HRMS (MALDI-TOF, Pos.) calcd for $C_{15}H_{22}F_2NO_4P + Na$: 372.1152; found: 372.1145.

***cis*-[2-(Octanoylamino)cyclohexyl]methylphosphonic acid (18).** A mixture of **23a** (970 mg, 2.63 mmol) in AcOH (10 mL) and $PtO_2 \cdot xH_2O$ (268 mg) was stirred at room temperature under an atmospheric pressure of hydrogen for 4 days. Removal of the catalyst by filtration through a pad of Celite and the filtrate was diluted with EtOAc. The organic layer was successively washed with H_2O, saturated $NaHCO_3$, brine and 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, 1/1–0/1) to afford *cis*-diethyl [2-(octanoylamino)cyclohexyl]methylphosphonate and *trans*-diethyl [2-(octanoylamino)cyclohexyl]methylphosphonate. Stereo chemistry of them was determined by NMR technique (NOE). *cis*-isomer: 42% yield; TLC $R_f = 0.21$ (EtOAc); ^{1}H NMR (500 MHz, CDCl$_3$) δ 6.76–6.64 (brs, 1H), 4.26–4.19 (m, 1H), 3.95–3.80 (m, 4H), 2.27–2.19 (brs, 1H), 2.15 (t, $J = 8.0$ Hz, 2H), 1.82 (ddd, $J = 21.0$, 17.0, 8.0 Hz, 1H), 1.78–1.70 (m, 2H), 1.66–1.56 (m, 2H), 1.49 (ddd, $J = 21.0$, 18.0, 7.0 Hz, 1H), 1.45–1.16 (m, 14H), 1.04 (t, $J = 8.0$ Hz, 6H), 0.85 (t, $J = 8.0$ Hz, 3H). *trans*-isomer: 15% yield; TLC $R_f = 0.39$ (EtOAc); ^{1}H NMR (500 MHz, CDCl$_3$) δ 6.23 (d, $J = 10.0$ Hz, 1H), 4.16–4.00 (m, 4H), 3.52–3.46 (rn, 1H), 2.18 (t, $J = 8.0$ Hz, 2H), 2.08–1.99 (m, 2H), 1.90 (ddd, $J = 21.0$, 16.0, 6.0 Hz, 1H), 1.78–1.60 (m, 2H), 1.51 (ddd, $J = 21.0$, 16.0, 5.0 Hz, lH), 1.39–1.18 (m, 18H), 1.17–1.06 (m, 1H), 0.85 (t, $J = 8.0$ Hz, 3H). The title compound 18 was prepared from *cis*-diethyl[2-(octanoylamino)cyclohexyl]methylphosphonate according to the same procedure as described for the preparation of **7** from **23a**: 24% yield; beige powder; TLC $R_f = 0.51$ (CHCl$_3$/MeOH/AcOH, 8/1/1); IR (KBr) 3296, 2929, 2857, 1643, 1543, 1465, 1138 cm^{-1}; ^{1}H NMR (200 MHz, CD$_3$OD) 84.13–4.02 (br, 1H), 2.24 (t, $J = 8.0$ Hz, 2H), 2.24–2.01 (m, 1H), 1.81–1.14 (m, 20H), 0.89 (t, $J = 7.0$ Hz, 3H); MS (MALDI-TOF, Pos.) *m*/*z* 364 (M + 2Na–H)$^+$, 342 (M + Na)$^+$, 320 (M + H)$^+$; HRMS (MALDI-TOF, Pos.) calcd for $C_{15}H_{30}NO_4P + Na$: 342.1810; found: 342.1795.

***trans*-12-(Octanoylamino)cyclohexyljmethylphosphonic acid (19).** The title compound **19** was prepared from trans-diethyl [2-(octanoylamino)cyclohexyl]methylphosphonate according to the same procedure as described for the preparation of **7** from **23a**: 12% yield; white amorphous powder; TLC $R_f = 0.17$ (CHCl$_3$/MeOH/AcOH, 20/1/1); IR (KBr) 3434, 2932, 2857, 1638, 1545, 1450, 1123 cm^{-1}; ^{1}H NMR (200 MHz, CD$_3$OD) 83.51–3.33 (m, 1H), 2.32–2.10 (m, 1H), 2.18 (t, $J = 7.0$ Hz, 2H), 2.06–0.97 (m, 20H), 0.89 (t, $J = 7.0$ Hz, 3H); MS (MALDI-TOF, Pos.) *m*/*z* 342 (M + Na)$^+$, 320 (M + H)$^+$; HRMS (MALDI-TOF, Pos.) calcd for $C_{15}H_{30}NO_4P + Na$: 342.1810; found: 342.1842.

Biological assay method. Inhibition of LPS-induced Plasma TNF-α production in mice. The experiments were performed using male BALB/c mice, 8 weeks of age, purchased from Charles River Breeding Laboratories (Shizuoka, Japan). 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). Test compounds and LPS from *Escherichia coli* strain 055 B5 (DIFCO LABORATORIES, Detroit, MI, USA) were dissolved in saline. Compounds were injected intravenously (0.01–0.1 mg/10 mL/kg) to mice, and then immediately given an intraperitoneal injection of LPS (5 mg/10 mL/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 (ELISA) using a commercial kit (GENZYME, 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).

$$\% \text{ Inhibition} = 100 - (C - S)/(L - S) \times 100$$

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.

References and Notes

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6. We focused our attention on the metabolic stabilization of the newly discovered chemical lead **1** without loss of its TNF-α inhibitory activity because one of the critical drawbacks of it as a drug candidate was found to be the rapid metabolic hydrolysis of its phosphate moiety as found in our in vitro study. See ref 7.
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8. 2-(*N*-Hexadecanoylamino)-4-nitorophenylphosphocholine (HNP), a water-soluble synthetic substrate for sphingomyelinase, was purchased from Sigma Chemical Co., Ltd.
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11. A series of phosphate analogues were rapidly metabolized to afford the corresponding alcohol as described in one of our previous papers.[7] Design and synthesis of phosphonic acid analogues instead of phosphate analogues were quite reasonable way of thinking in the medicinal chemistry because phosphonic acid can not be metabolically hydrolyzed although we have no experimental data of the improved metabolic stability.
12. For example, compound **1** lowered the blood pressure by 15 mmHg at the dose of 4.2 mg/kg, iv in rats.
13. Bromides **20a, b, d** and **e** were prepared from their corresponding carboxylic acids according to conventional procedures.
14. According to the conventional procedure bromide **20c** was prepared from 4-methyl-2-nitro benzoic acid which was in turn prepared from 4-chloro-3-nitro toluene: i) CuCN in DMF, ii) H_2SO_4, H_2O.
15. Bromides **20f–i** were prepared from their corresponding *ortho*-nitro toluenes by bromination with NBS and benzoyl peroxide in CCl_4.
16. Iodide **20j** was prepared by the iodination of the commercially available alcohol according to a known procedure (I_2, Ph_3P, imidazole, Et_2O–CH_3CN).