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# Novel Hybrids of Optically Active Ring-Opened 3-*n*-Butylphthalide Derivative and Isosorbide as Potential Anti-Ischemic Stroke Agents

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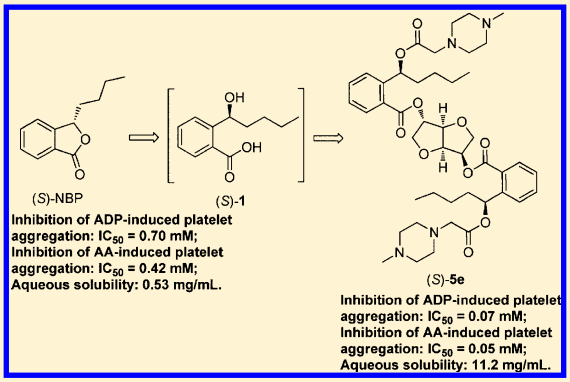
Supporting Information

**ABSTRACT:** In search of novel anti-ischemic stroke agents with higher potency than a known drug 3-*n*-butylphthalide (NBP), a series of hybrids ((*S*)- and (*R*)-**5a**–**f**) from optically active ring-opened NBP derivative and isosorbide were synthesized for evaluating their anti-ischemic stroke activity. Compound (*S*)-**5e** displayed the strongest activity in inhibiting the adenosine diphosphate (ADP) and arachidonic acid (AA)-induced platelet aggregation in vitro, with 10.0- and 8.4-fold more effectiveness than (*S*)-NBP, respectively. Furthermore, (*S*)-**5e** was stable in artificial gastrointestinal fluids and could penetrate the blood–brain barrier (BBB) with an appreciate lipid/water partition coefficient relative to (*S*)-NBP. More importantly, oral treatment with (*S*)-**5e** protected from acute thrombosis and inhibited the ischemia/reperfusion-related brain injury in animals. Our findings suggest that (*S*)-**5e** may be promising for further evaluation for the intervention of ischemic stroke.




## INTRODUCTION

Ischemic stroke is a leading cause of human morbidity and mortality worldwide.[1] Currently, stroke has approximately caused the loss of 5 million people each year, and the mortality rate of stroke is increasing.[2] Previous studies have suggested that the pathogenesis of ischemic stroke is attributed to the interaction of multiple factors, including genetic high risk, thrombosis, and chronic inflammatory diseases such as hypertension, diabetes, and others.[3−5] During the process of ischemic stroke, the platelet aggregation-related thrombosis limits sufficient blood flow in the special region of the brain and leads to ischemic inflammation and brain damage.[6,7] Although many drugs are available for the intervention of ischemic stroke, the efficacy of these drugs is not satisfactory. Hence, the development of new drugs for the effective and safe treatment of ischemic stroke will be of great significance.

The 3-*n*-butylphthalide (NBP) is an anti-ischemic stroke drug that was approved by the State Food and Drug Administration (SFDA) of China in 2002. NBP can inhibit platelet aggregation and thrombosis-associated oxidative stress, improve microcirculation, and mitigate ischemic injury in the brain.[8−10] NBP is a racemic molecule containing a chiral center. A pair of enantiomers, (*S*)- and (*R*)-NBP, have been synthesized. Pharmacologically, these two enantiomers have similar potency to NBP, but (*S*)-NBP is slightly better in a few cases.[11−15] However, the efficacy of NBP or (*S*)-NBP is moderate and is often administered together with other antiplatelet drug(s).[16] A previous study suggests that the low efficacy of NBP is attributed to its poor aqueous solubility.[17] However, the efficacy of 2-(1-hydroxypentyl)-benzoate (HPBA), a ring-opened derivative of NBP with an improved aqueous solubility, and other HPBA derivatives is still not satisfactory.[18,19] Therefore, new strategies are needed for the development of drugs for ischemic stroke.

Isosorbide (IS) is a diuretic drug and has been used for the treatment of hydrocephalus and glaucoma. Meanwhile IS is also an excellent scaffold and has been used for generating many of its derivatives with potent vasodilation and anti-inflammatory activity.[20−22] Accordingly, we hypothesize that new hybrids of optically active ring-opened NBP derivative and IS may have potent activity against ischemic stroke.

In this study, a series of novel hybrids of optically active ring-opened NBP derivative and IS were synthesized by inducing various substituted amines to improve the aqueous solubility, e.g., diethylamine, *N*-methyl piperazine, and morpholine to the side chain of HPBA via a substituted acetate linkage, respectively. We found that the promising compound, (*S*)-**5e**, displayed potent antiplatelet aggregation, antithrombotic activity, and anti-ischemic stroke activity in vitro and in vivo.

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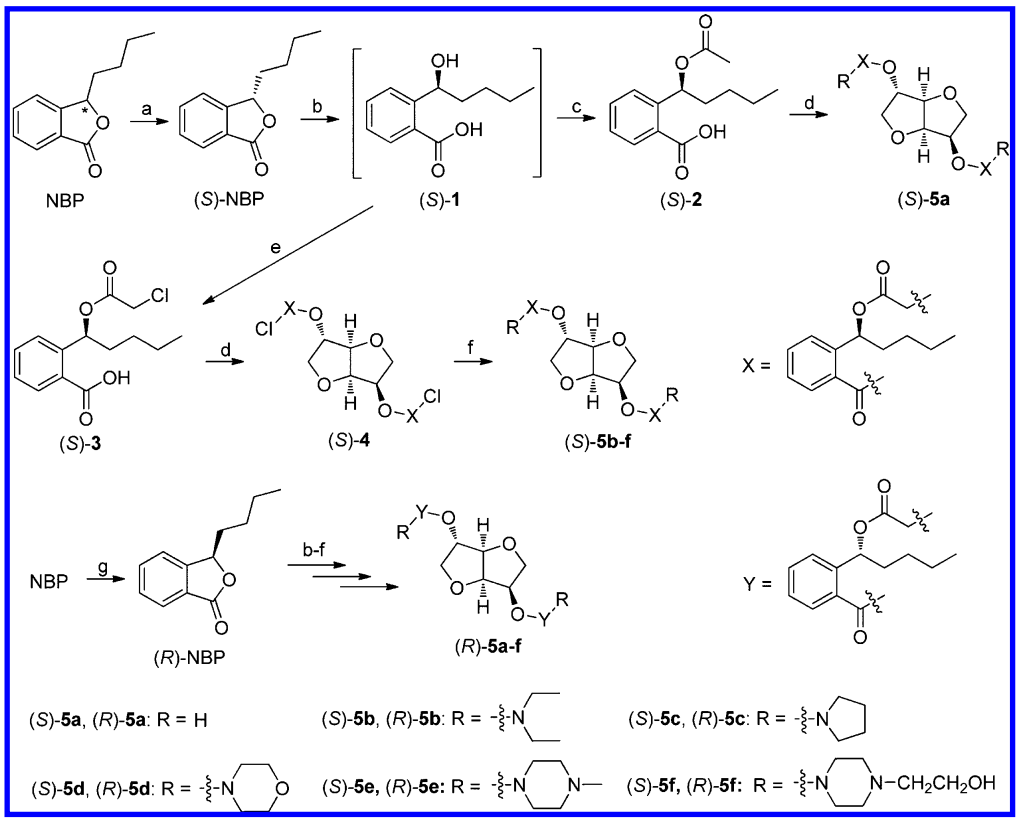
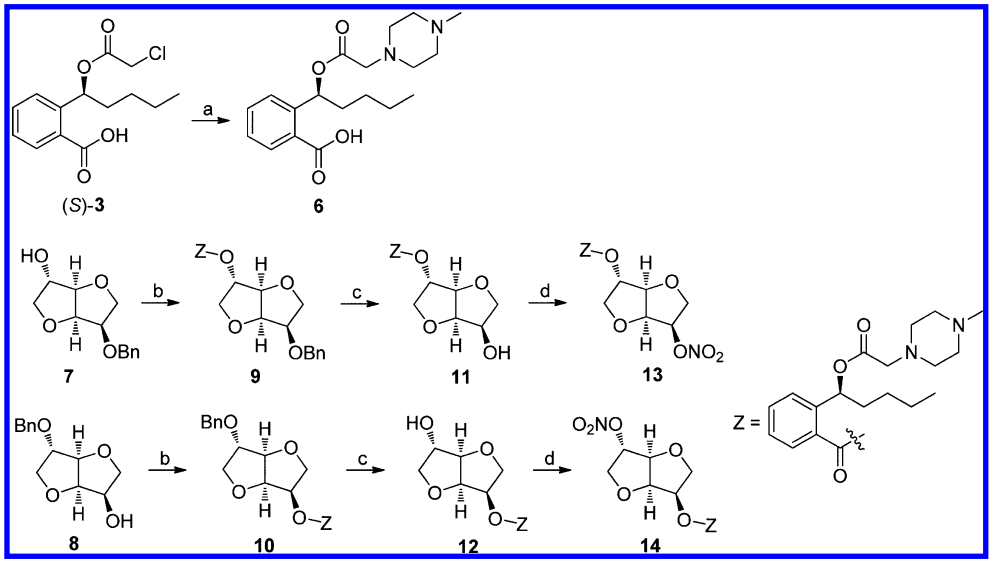
**Scheme 1. Synthesis of Compounds (*S*)- and (*R*)-5a–f[a]**




[a]Reagents and conditions: (a) (i) 2 M NaOH, $CH_3OH$–$H_2O$, 50 °C, 0.5 h; 5% HCl, −10–0 °C; (ii) (*S*)-α-methylbenzylamine, $Et_2O$, −20 to −10 °C; acetone, recrystallization repeated 2 times; (b) (i) NaOH, $CH_3OH$–$H_2O$, 50 °C, 0.5 h; (ii) 5% HCl, −10–0 °C; (c) $CH_3COCl$, $Et_3N$, DMAP, $CH_2Cl_2$, −10 °C, 5 h; (d) IS, DCC, DMAP, $CH_2Cl_2$, room temp, 8–12 h; (e) $ClCH_2COCl$, $Et_3N$, DMAP, $CH_2Cl_2$, −10 °C, 5 h; (f) corresponding amines, $K_2CO_3$, acetone, room temp, 6–10 h. (g) (i) 2 M NaOH, $CH_3OH$–$H_2O$, 50 °C, 0.5 h; 5% HCl, −10–0 °C; (ii) (*R*)-α-methylbenzylamine, $Et_2O$, −20 to −10 °C; acetone, recrystallization repeated 2 times.

**Scheme 2. Synthesis of Compounds 6–14[a]**




[a]Reagents and conditions: (a) *N*-methyl piperazine, $K_2CO_3$, acetone, reflux, 8 h; (b) **6**, DCC, DMAP, $CH_2Cl_2$, room temp, 10 h; (c) $H_2$, Pd/C, EtOH, room temp, 4 h; (d) Faming $HNO_3$, AcOH, $Ac_2O$, 0 °C to room temp, 2.5 h.

## RESULTS AND DISCUSSION

**Chemistry.** The target compounds (*S*)- and (*R*)-**5a–f** were synthesized from chemical resolution of racemic NBP (Scheme 1).[23] NBP was subjected to saponification and sequential acidification to form the ring-opened acid (HPBA), which was reacted with (*S*)-α-methyl-benzylamine to yield the diastereoisomeric salt, followed by alkali treatment and lactonization under acid conditions to give (*S*)-NBP (ee >99%). Similarly,


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Table 1. The $IC_{50}$ Values of (*S*)- and (*R*)-5a–f against Platelet Aggregation in Vitro[a]

| compd | $IC_{50}$ (mM) ADP (10 μM) | $IC_{50}$ (mM) AA (1 mM) | compd | $IC_{50}$ (mM) ADP (10 μM) | $IC_{50}$ (mM) AA (1 mM) |
|---|---|---|---|---|---|
| control | | | (*R*)-**5b** | 0.68 ± 0.11 | 0.56 ± 0.07 |
| IS | 1.35 ± 0.12 | 1.68 ± 0.31 | (*S*)-**5c** | 0.80 ± 0.13 | 0.64 ± 0.05 |
| (*S*)-NBP | 0.70 ± 0.10 | 0.42 ± 0.05 | (*R*)-**5c** | 1.00 ± 0.16 | 0.91 ± 0.12 |
| (*R*)-NBP | 1.15 ± 0.22 | 0.68 ± 0.08 | (*S*)-**5d** | 0.45 ± 0.05 | 0.32 ± 0.06 |
| Ticlid[b] | 0.36 ± 0.04 | | (*R*)-**5d** | 0.94 ± 0.17 | 0.80 ± 0.09 |
| ASP[c] | | 0.14 ± 0.01 | (*S*)-**5e** | 0.07 ± 0.01 | 0.05 ± 0.01 |
| (*S*)-**5a** | 0.33 ± 0.05 | 0.21 ± 0.03 | (*R*)-**5e** | 0.36 ± 0.07 | 0.27 ± 0.04 |
| (*R*)-**5a** | 0.71 ± 0.06 | 0.57 ± 0.09 | (*S*)-**5f** | 0.49 ± 0.09 | 0.36 ± 0.06 |
| (*S*)-**5b** | 0.31 ± 0.04 | 0.20 ± 0.01 | (*R*)-**5f** | 0.75 ± 0.12 | 0.63 ± 0.10 |

[a]$IC_{50}$ values (a dose achieved 50% inhibition of platelet aggregation) are expressed as mean ± SD. [b]Ticlid is an inhibitor of ADP-induced platelet aggregation. [c]ASP is an inhibitor of AA-induced platelet aggregation.

(*R*)-NBP (ee >99%) was obtained with (*R*)-α-methyl-benzylamine. Subsequently, (*S*)-NBP underwent alkali hydrolysis and acidification to form acid (*S*)-**1** that was treated with acetyl chloride or chloroacetyl chloride to give the acylated compounds (*S*)-**2** or (*S*)-**3**, which was esterified with IS in the presence of 1,3-dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) to offer ester (*S*)-**5a** or (*S*)-**4**. Compound (*S*)-**4** was condensated with corresponding amines to generate the target compounds (*S*)-**5b–f**. Similarly, (*R*)-**5a–f** were synthesized from (*R*)-NBP. The synthesis of **6–10** was illustrated in Scheme 2. Compound **6** was obtained by condensation of (*S*)-**2** with *N*-methyl piperazine. Treatment of **6** with **7** or **8** led to ester **9** or **10**. Debenzylation of **9** or **10** with Pd/C in hydrogen atmosphere gave hydroxy compound **11** or **12**, which was then treated with fuming $HNO_3$, AcOH, and $Ac_2O$ to provide mononitrate **13** or **14**. Compounds **7** and **8** were prepared by benzylation of IS, as described previously.[24] All of the new compounds were purified by column chromatography and characterized by IR, ESI-MS, $^1H$ NMR, $^{13}C$ NMR, and HRMS. The individual compounds with a chemical purity of >95% and optical purity (ee) of >99% (determined by chiral HPLC analysis) were used for subsequent experiments.

**Pharmacology: Antiplatelet Aggregation Effect In Vitro.** The inhibitory effects of target compounds on platelet aggregation in vitro were determined using Born's turbidimetric method.[25] Ticlopidine hydrochloride (Ticlid) and aspirin (ASP) are inhibitors of adenosine diphosphate (ADP)- and arachidonic acid (AA)-induced platelet aggregation, respectively[26,27] and were used as positive controls. The IS, (*S*)-, and (*R*)-NBP were used as control compounds. At least five different concentrations of individual compounds were tested, respectively, and data were calculated and expressed as the $IC_{50}$ values.

As shown in Table 1, (*S*)-**5a–f** displayed near 2-fold higher inhibitory activity than that of (*R*)-**5a–f** in ADP- and AA-induced platelet aggregation, indicating (*S*)-isomers were more potent than (*R*)-isomers in inhibition of both ADP- and AA-induced platelet aggregation, which was consistent with (*S*)- and (*R*)-NBP. In addition, the (*S*)-isomers, except for (*S*)-**5c**, were more potent than (*S*)-NBP. Notably, (*S*)-**5e** was the most potent, significantly superior to IS, (*S*)-NBP, Ticlid, and ASP, and its $IC_{50}$ value (0.07 mM) on ADP-induced platelet aggregation was 19.3-, 10.0-, and 5.1-fold less than that of IS (1.35 mM), (*S*)-NBP (0.70 mM), and Ticlid (0.36 mM), respectively, and the $IC_{50}$ value (0.05 mM) on AA-induced platelet aggregation was 33.6-, 8.4-, and 2.8-fold less than that of IS (1.68 mM), (*S*)-NBP (0.42 mM), and ASP (0.14 mM), respectively.

Analysis of individual moieties (IS, **6**, **11**, and **12**) of (*S*)-**5e** as well as mononitrate **13** and **14** revealed that they at 1 mM had similar levels of inhibitory activity (6.94–24.10%), which were significantly lower than that of (*S*)-**5e** (50.10%) in inhibiting the ADP-induced platelet aggregation in vitro ($P < 0.001$, Figure 1A). Interestingly, compounds **11–14** had comparable levels of inhibitory activity, suggesting that the nitrate moiety may not be necessary and can be replaced by HPBA.

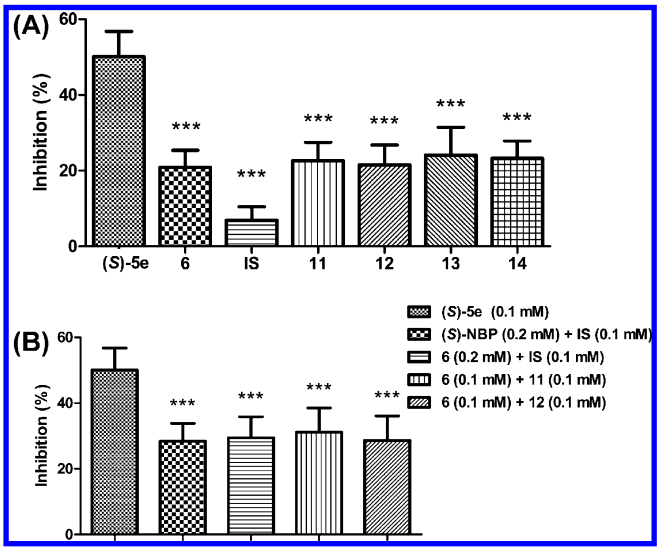


**Figure 1.** (A) Inhibitory effects of (*S*)-**5e**, IS, **6**, and **11–14** (0.1 mM each) on ADP-induced platelet aggregation in vitro. Data are expressed as mean ± SD of each group ($n = 6$) and analyzed by one-way analysis of variance (ANOVA) followed by post hoc Tukey test. ***$P < 0.001$ vs the (*S*)-**5e** group. (A) Inhibitory effects of (*S*)-**5e** and combinations of its moieties on ADP-induced platelet aggregation in vitro. Data are expressed as mean ± SD of each group ($n = 6$) and analyzed by ANOVA followed by post hoc Tukey test. ***$P < 0.001$ vs the (*S*)-**5e** group.

Next, we investigated whether administration of (*S*)-**5e** alone is better than combination administration of its corresponding moieties in inhibition of ADP-induced platelet aggregation in vitro. The results indicated that the inhibitory activity of (*S*)-**5e** (0.1 mM) was significantly more potent than that of (*S*)-NBP (0.2 mM) together with IS (0.1 mM), **6** (0.2 mM) with IS (0.1


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Table 2. The Concentrations of (*S*)-5e in the Artificial Gastric and Intestinal Juices[a]

| | concentrations ($\mu$M) | | | | |
|---|---|---|---|---|---|
| gastrointestinal fluid | 0 h | 1 h | 2 h | 4 h | 8 h |
| artificial gastric juice | 4.92 ± 0.21 | 4.89 ± 0.29 | 5.00 ± 0.11 | 4.79 ± 0.20 | 4.96 ± 0.28 |
| artificial intestinal juice | 4.97 ± 0.14 | 4.86 ± 0.11 | 5.04 ± 0.19 | 4.89 ± 0.10 | 4.92 ± 0.17 |

[a]Data are expressed as mean ± SD of the concentrations of (*S*)-**5e** at each time point of each group ($n$ = 6) and analyzed by ANOVA and post hoc Tukey test.

mM), **6** (0.1 mM) with **11** (0.1 mM), or **6** (0.1 mM) with **12** (0.1 mM) in inhibiting the ADP-induced platelet aggregation in vitro ($P$ < 0.001, Figure 1B).

**Stability of (*S*)-5e in Artificial Gastrointestinal Fluids.** Theoretically, an ideal anti-ischemic stroke drug should be rapidly absorbed into blood circulation and enter the target tissues following oral administration. Alternatively, the drug has to be tolerant to gastrointestinal fluids for later absorption. Thus, we tested the stability of (*S*)-**5e** in artificial gastric juice (pH 1.5) and artificial intestinal juice (pH 6.8) by high-performance liquid chromatography (HPLC).[28] We found that incubation of (*S*)-**5e** in these types of fluids at 37 °C for 8 h did not significantly alter the contents of (*S*)-**5e** (Table 2), suggesting that (*S*)-**5e** was stable in these extreme conditions.

**Antithrombotic Activities of (*S*)-5e In Vivo.** We next evaluated the antithrombotic effects of (*S*)-**5e** in vivo using two models of thrombosis in which platelet activation is critical factor.

The first one was acute vascular occlusion initiated by intravascular platelet induced aggregation through infusion of a mixture of collagen and adrenaline into the jugular vein.[29] Male ICR mice were randomized and treated orally with vehicle, ASP, (*S*)-NBP, or (*S*)-**5e** for seven days. Two hours after the last administration, individual mice were injected intravenously with a mixture of collagen (0.21 mg/kg) and adrenaline (44.5 $\mu$g/kg) to induce acute systemic vascular thromboembolism. We observed that treatment with (*S*)-**5e** at equal mass (160 mg/kg) or molar (680 mg/kg) to (*S*)-NBP had similar protection to that of (*S*)-NBP and ASP (Figure 2). The protective effect of (*S*)-**5e** at 680 mg/kg was slightly higher than that of others and significantly greater than that of vehicle alone ($P$ < 0.05, Log-rank test). Hence, treatment with (*S*)-**5e** efficiently inhibited acute thrombosis in mice.

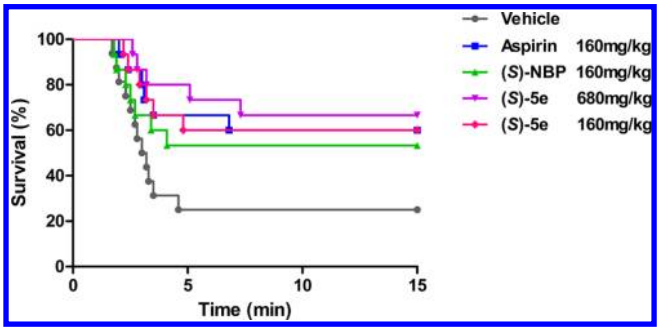


**Figure 2.** The survival of mice. Male ICR mice were treated with the indicated doses of each drug or vehicle for seven days. Two hours after the last treatment, individual mice were injected intravenously with a mixture of collagen and adrenaline for inducing acute thrombosis. The survival of individual mice was monitored up to 15 min post injection. Data are expressed as mean % of the surviving mice in individual groups ($n$ = 15). *$P$ < 0.05, **$P$ < 0.01 vs the vehicle group.

In parallel, we investigated the antithrombotic activity of (*S*)-**5e** in a rat arterio-venous (A-V) shunt model.[30] Male Sprague–Dawley (SD) rats were randomized and treated with 80 or 340 mg/kg (which was equal mass or equal molar to that of (*S*)-NBP) of (*S*)-**5e**, 80 mg/kg of (*S*)-NBP, or vehicle by gavage daily for seven days. Two hours after the last treatment, all animals were subjected to the arteriovenous shunt using a 14 cm polyethylene catheter containing a 6 cm silk thread for 15 min. We found that the wet and dry thrombus weights in the (*S*)-**5e**-treated rats, similar to other treatment groups of rats, were significantly less than that in the vehicle controls ($P$ < 0.001, Figure 3). Interestingly, the wet thrombus weights in the (*S*)-**5e**-treated (340 mg/kg) rats were significantly less than that in the (*S*)-NBP-treated rats ($P$ < 0.01), indicating that (*S*)-**5e** had stronger antithrombotic activity in this model. These two independent lines of evidence demonstrate that (*S*)-**5e** has potent antithrombotic activity in vivo.

**Lipid/Water Partition Coefficient and Aqueous Solubility of (*S*)-5e.** The BBB protects the brain from other toxicants and infections, and it is well-known that whether a drug could pass the BBB depends on an appropriate lipid/water partition coefficient. We calculated the log $D$ values of (*S*)-**5e** and (*S*)-NBP using ACD/Laboratories Professional Software[31] and tested their aqueous solubility, as described previously.[32] Interestingly, the log $D$ value of (*S*)-**5e** (log $D_{3.6}$ 1.87) was less than that of (*S*)-NBP (log $D_{7.0}$ 3.19), whereas the aqueous solubility of (*S*)-**5e** (11.2 mg/mL) was greater than that of (*S*)-NBP (0.53 mg/mL), indicating that (*S*)-**5e** had an appropriate lipid/water partition coefficient[33] and a better aqueous solubility relative to (*S*)-NBP.

**The BBB Penetration of (*S*)-5e.** To test the BBB penetration of (*S*)-**5e**, we determined the concentration of (*S*)-**5e** in mouse brain and plasma at different time points (0.5, 1.0, 2.0, 4.0, and 8.0 h) following oral administration (680 mg/kg) and calculated the ratios of the concentrations of (*S*)-**5e** in the brain to plasma ($C_{brain}/C_{plasma}$).[34] As shown in Table 3 and Figure 4A, the concentrations of (*S*)-**5e** in the brain, similar to that in plasma, rapidly increased and gradually declined. The mean ratios of the concentrations of (*S*)-**5e** in the brain to plasma gradually decreased (Figure 4B). These results suggest that (*S*)-**5e** may be rapidly absorbed and penetrate to the BBB in mice.

**Treatment with (*S*)-5e Inhibits Ischemia/Reperfusion (I/R)-Related Brain Injury in Rats.** The occlusion of middle cerebral artery (MCAO) is the most common reason for inducing I/R-related brain injury in the clinic. To gain insight into the therapeutic potential of (*S*)-**5e**, we examined the impact of treatment with (*S*)-**5e** on I/R-related brain injury in a rat I/R model. Male SD rats were randomly treated orally with 0.5% sodium carboxyl methyl cellulose (CMC-Na) (sham group and vehicle group), (*S*)-NBP (80 mg/kg), and (*S*)-**5e** (80 mg/kg and 340 mg/kg, which were equal mass and equal molar to (*S*)-NBP, respectively) for seven days. Two hours after the last treatment, the rats were subjected to MCAO for


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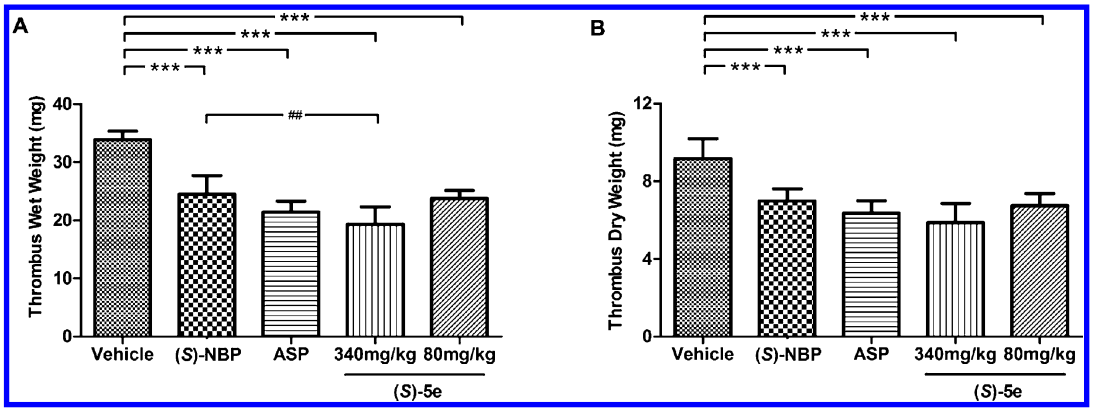


**Figure 3.** Effects of (*S*)-**5e**, (*S*)-NBP, and ASP on thrombus wet weights (A) and dry weights (B) in rats. SD rats were treated with the indicated doses of each drug by gavage daily for seven days. Two hours after the last treatment, the rats were subjected to the A-V shunt using a silk thread for 15 min. The wet and dry weights were measured. Data are expressed as mean ± SD of each group ($n$ = 12) and analyzed by ANOVA and post hoc Tukey test. ***$P$ < 0.001 vs the sham group, $^{\#\#}P$ < 0.01 vs the (*S*)-NBP group.

**Table 3. The Concentrations of (*S*)-5e in the Brain and Plasma**[a]

| | concentrations (ng/mL) | | | | |
|---|---|---|---|---|---|
| distribution | 0.5 h | 1.0 h | 2.0 h | 4.0 h | 8.0 h |
| brain | 408.3 ± 32.7 | 260.2 ± 31.7 | 173.7 ± 26.5 | 54.3 ± 14.4 | 16.7 ± 8.54 |
| plasma | 320.2 ± 63.5 | 241.0 ± 63.5 | 186.2 ± 25.8 | 126.5 ± 28.9 | 69.7 ± 14.1 |

[a]Data are expressed as mean ± SD of the concentrations of (*S*)-**5e** in the brain and plasma at each time point after a single oral dose of 680 mg/kg in mice ($n$ = 6).

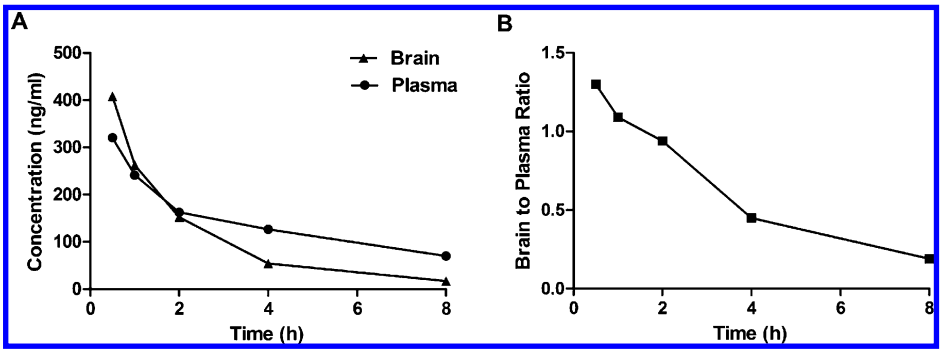


**Figure 4.** (A) Concentrations of (*S*)-**5e** in brain and plasma after a single oral dose of 680 mg/kg in mice. (B) The mean brain-to-plasma ratio of (*S*)-**5e** after a single oral dose of 680 mg/kg in mice. Data are presented as mean ± SD ($n$ = 6).

two hours, followed by reperfusion. The neurological function of individual rats was evaluated using Longa's method[35] 24 h after reperfusion (Figure 5). As expected, there was no obvious neuronal abnormality detected in the sham-operated group while the neurological deficit scores in the vehicle-treated I/R rats significantly increased ($P$ < 0.001). In contrast, the neurological deficit scores in the (*S*)-**5e**-treated I/R rats, but not in the (*S*)-NBP-treated group, were significantly reduced, as compared with the vehicle-treated controls ($P$ < 0.01).

Similarly, analysis of the infarct size by the 2,3,5-triphenyltetrazolium chloride (TTC) assay indicated that in comparison with that in the vehicle-treated controls, the infarct sizes in the brains of the (*S*)-NBP or (*S*)-**5e**-treated I/R rats were significantly reduced, particularly in those with higher dose of (*S*)-**5e** ($P$ < 0.001) (Figure 6A). In addition, treatment with (*S*)-**5e** (80 and 340 mg/kg) and (*S*)-NBP (80 mg/kg) significantly reduced the water contents in rat brain (Figure 6B).

Histologically, to examine the protective effects of (*S*)-**5e** against cortical neuronal damage, experiments on hematoxylin and eosin (H&E) staining were performed. While necrotic neurons, neuronal perikarya shrinkage, perivascular inflamma-

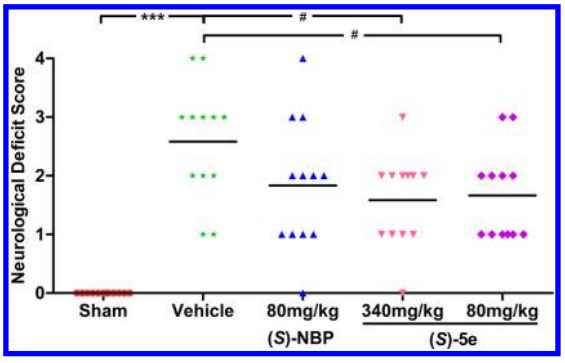


**Figure 5.** Effects of (*S*)-**5e** and (*S*)-NBP on neurological deficit score in rats subjected to I/R ($n$ = 12). Data are assessed 24 h after reperfusion and analyzed by Kruskal–Wallis test, followed by the Mann–Whitney $U$ test. ***$P$ < 0.001 vs the sham group, $^{\#}P$ < 0.05 vs the vehicle group.


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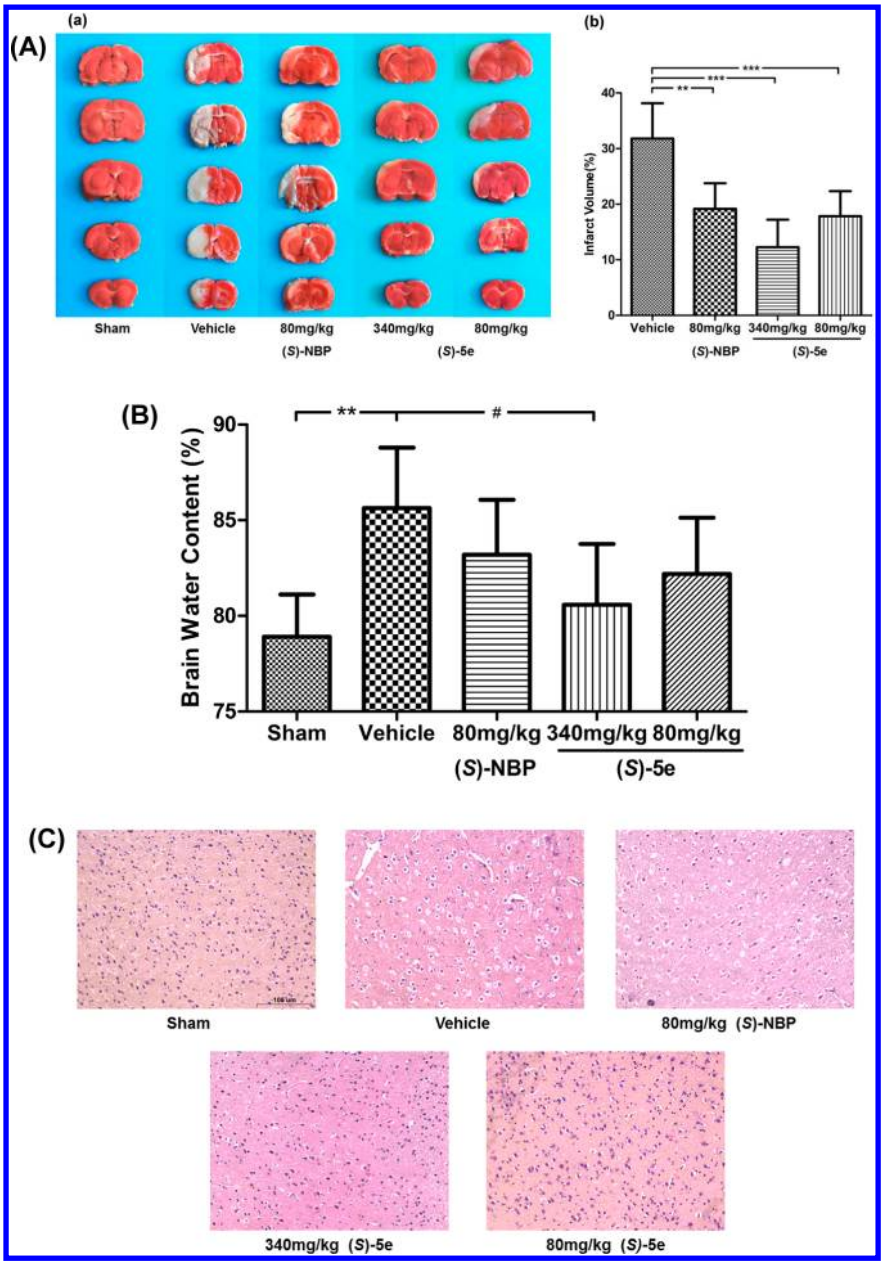


**Figure 6.** (A) Effects of (*S*)-**5e** and (*S*)-NBP on infarction after cerebral I/R in rats. (a) Infarcted brain areas were visualized using TTC staining. Representative examples from each treatment group were shown. (b) Quantitative analysis of the infracted brain regions. The ratios of infarcts area to whole brain areas were calculated. Data are expressed as mean ± SD ($n$ = 6) and analyzed by ANOVA followed by post hoc Tukey test. ***$P$ < 0.001 vs the vehicle group, **$P$ < 0.01 vs the vehicle group. (B) Effects of (*S*)-**5e** and (*S*)-NBP on brain edema after cerebral I/R in rats. Data are expressed as mean ± SD ($n$ = 6) and analyzed by ANOVA followed by post hoc Tukey test. **$P$ < 0.01 vs the sham group, $^{\#}P$ < 0.05 vs the vehicle group. (C) Protective effects of (*S*)-**5e** and (*S*)-NBP on neuronal injury in the ischemic cerebral cortex of rats ($n$ = 6). Cerebral sections were stained with hematoxylin and eosin then were examined under light microscope. Representative images from individual groups ($n$ = 6) are shown (magnification, × 200).

tory infiltration, and vacuolization were observed in the brain tissues of the vehicle-treated rats, there were a few necrotic neurons, less inflammatory infiltrates and abnormal changes in the brains of the (*S*)-**5e** or (*S*)-NBP-treated rats, particularly in those with higher dose of (*S*)-**5e**. These data indicated that treatment with (*S*)-**5e** significantly inhibited the I/R-related injury in the brains of rats.

## ■ CONCLUSIONS

Analysis of structure and antiplatelet aggregation activity relationship (SAR) revealed that the target compounds (*S*)- and (*R*)-**5a**−**f** displayed different antiplatelet aggregation activity. In general, (*S*)-isomers exhibited much stronger inhibitory effects than (*R*)-isomers in both ADP- and AA-induced platelet aggregation. Additionally, various substituted amines in the side chain of target compounds exerted variable effects. Significantly, the (*S*)-**5e** bearing an *N*-methyl piperazino moiety in side chain exhibited the strongest inhibitory activity among all compounds. Interestingly, individual moieties of (*S*)-**5e** inhibited the ADP-induced platelet aggregation but their inhibitory activity were much less than (*S*)-**5e**. Moreover, treatment with (*S*)-**5e** alone had better antiplatelet aggregation


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activity than either of the four different combinations from its corresponding moieties. These results suggest that individual moieties of (*S*)-**5e** may act synergistically.

In summary, a series of novel hybrids ((*S*)- and (*R*)-**5a−f**) of optically active ring-opened NBP derivative and IS were synthesized, and compound (*S*)-**5e** displayed the most potent activity in inhibiting the ADP- and AA-induced platelet aggregation in vitro, superior to IS, (*S*)-NBP, ASP, and Ticlid. Furthermore, (*S*)-**5e** was stable in artificial gastrointestinal fluids and rapidly absorbed and distributed into the brain. Moreover, (*S*)-**5e** exhibited higher antithrombotic activity than (*S*)-NBP and ASP in vivo. Most importantly, treatment with (*S*)-**5e** inhibited the I/R-related neurobehavioral dysfunction, the infarct brain size, the brain-water contents, and the brain damage in rats. Therefore, (*S*)-**5e** may be valuable for the intervention of ischemic stroke. We are interested in further investigation of the mechanisms underlying the action of (*S*)-**5e** in inhibiting thrombosis and ischemic stroke.

## EXPERIMENTAL SECTION

**Chemistry.** Melting points are uncorrected and were measured in open capillary tubes using a Gallenkamp melting point apparatus. $^1$H and $^{13}$C NMR spectral data were obtained from a Bruker Avance 300 MHz spectrometer at 300 K using TMS as an internal standard. MS spectra were recorded on a Mariner mass spectrometer (ESI). Analytical and preparative TLC were performed on silica gel GF/UV 254, and the chromatograms were conducted on silica gel (200−300 mesh) and visualized under UV light at 254 and 365 nm. The purities of the compounds were characterized by HPLC analysis (LC-10A HPLC system consisting of LC-10ATvp pumps and SPD-10Avp UV detector) and HRMS (Agilent technologies LC/MSD TOF). The target compounds (*S*)- and (*R*)-**5a−f** with a chemical purity of >95% and optical purity (ee) of >99% were used for subsequent experiments(see the ESI†).

All animal experiments were performed in accordance with the Animal (Scientific Procedures) Act 2009 (P. R. China), and the experimental protocols were approved by the Animal Research Protection Committee of China Pharmaceutical University, Nanjing, China.

**(*S*)-(−)-2-(1-Acetoxypentyl)benzoic Acid ((*S*)-2).** To a stirred solution of (*S*)-NBP (1.90 g, 10.00 mmol) in $CH_3OH-H_2O$ (20 mL, 1/1, v/v) was added NaOH (0.80 g, 20.00 mmol). The reaction mixture was heated at 50 °C for 0.5 h. The solvent was removed under reduced pressure and dissolved in water (20 mL), followed by acidification with 5% HCl to pH 3−4 at −10−0 °C. The mixture was extracted with cold $Et_2O$ (10 mL × 3) to get (*S*)-**1** and quickly used for the next step without any purification.

A solution of acetyl chloride (2.12 mL, 30.00 mmol) in dry $CH_2Cl_2$ (10 mL) was added dropwise to a mixture of (*S*)-**1** (2.08 g, 10.00 mmol), $Et_3N$ (4.17 mL, 30.00 mmol), and DMAP (1.00 mmol) in $CH_2Cl_2$ (150 mL) at −10 °C, and the solution was left stirring at −10 °C for 5 h. The mixture was acidified with 1 M HCl to pH 2 and stirred for 1 h at room temperature. The organic layer was washed with water, dried, filtered, and evaporated to dryness. The residue was recrystallized from *n*-hexane to obtain (*S*)-**2** as a white crystal (1.70 g, 68%, over two steps); mp 65−66 °C; $[\alpha]_D^{27}$ −39.5° (*c* 1.00 $CHCl_3$). MS (ESI): *m*/*z* 249.1 $[M - H]^-$. IR ($cm^{-1}$, KBr): $\nu_{max}$ 1412, 1691, 1734, 2958, 3450. $^1$H NMR (300 Hz, $CDCl_3$): $\delta$ 0.93 (t, 3H, $CH_3$, *J* = 8.5 Hz), 1.37−1.42 (m, 4H, 2 × $CH_2$), 1.88−1.91 (m, 2H, $CH_2$), 2.13−2.33 (m, 3H, $COCH_3$), 6.61−6.72 (m, 1H, $OC\underline{H}CH_2$), 7.37−7.40 (m, 1H, ArH), 7.56−7.62 (m, 2H, ArH), 8.05 (d, 1H, ArH, *J* = 8.1 Hz), 10.98 (brs, 1H, COOH). $^{13}$C NMR (75 Hz, $CDCl_3$): $\delta$ 172.0, 166.5, 140.8, 133.1, 130.3, 130.0, 127.1, 125.7, 74.8, 41.0, 36.3, 27.8, 22.4, 13.8.

**(*R*)-(+)-2-[1-(2-Chloroacetoxy)pentyl]benzoic Acid ((*R*)-2).** Compound (*R*)-**2** was synthesized in the same manner as compound (*S*)-**2**. Starting from compound (*R*)-**1** (2.08 g, 10.00 mmol), compound (*R*)-**2** was obtained as a white crystal (1.63 g, 65%, over two steps); mp 65−66 °C; $[\alpha]_D^{27}$ +37.8° (*c* 1.00 $CHCl_3$). IR (KBr), ESI-MS, $^1$H NMR, and $^{13}$C NMR spectral data were identical to those of (*S*)-**2**.

**(*S*)-(−)-2-[1-(2-Chloroacetoxy)pentyl]benzoic Acid ((*S*)-3).** Compound (*S*)-**3** was synthesized in the same manner as compound (*S*)-**2**. Starting from compound (*S*)-**1** (2.08 g, 10.00 mmol) and 2-chloroacetyl chloride (4.16 mL, 30.00 mmol), compound (*S*)-**3** was obtained as a white crystal (1.85 g, 65%, over two steps); mp 67−68 °C; $[\alpha]_D^{27}$ −40.8° (*c* 1.00 $CHCl_3$). MS (ESI): *m*/*z* 283 $[M - H]^-$. IR ($cm^{-1}$, KBr): $\nu_{max}$ 1412, 1691, 1734, 2958, 3450. $^1$H NMR (300 Hz, $CDCl_3$): $\delta$ 0.93 (t, 3H, $CH_3$, *J* = 4.2 Hz), 1.37−1.42 (m, 4H, 2 × $CH_2$), 1.88−1.91 (m, 2H, $CH_2$), 4.11−4.32 (m, 2H, $COCH_2Cl$), 6.71−6.78 (m, 1H, $OC\underline{H}CH_2$), 7.36−7.42 (m, 1H, ArH), 7.56−7.62 (m, 2H, ArH), 8.08 (d, 1H, ArH, *J* = 8.1 Hz), 10.89 (brs, 1H, COOH). $^{13}$C NMR (75 Hz, $CDCl_3$): $\delta$ 172.0, 166.5, 140.8, 133.1, 130.3, 130.0, 127.1, 125.7, 74.8, 41.0, 36.3, 27.8, 22.4, 13.8.

**(*R*)-(+)-2-[1-(2-Chloroacetoxy)pentyl]benzoic Acid ((*R*)-3).** Compound (*R*)-**3** was synthesized in the same manner as compound (*S*)-**3**. Starting from compound (*R*)-NBP (1.90 g, 10.00 mmol), compound (*R*)-**3** was obtained as a white crystal (1.79 g, 63%, over two steps); mp 67−68 °C; $[\alpha]_D^{27}$ +39.6° (*c* 1.00 $CHCl_3$). IR (KBr), ESI-MS, $^1$H NMR, and $^{13}$C NMR spectral data were identical to those of (*S*)-**3**.

**2,5-Bis{2-[(*S*)-(+)-1-(2-Chloroacetoxy)]pentyl}benzoate-1,4:3,6-dianhydro-D-glucitol ((*S*)-4).** To a stirred solution of (*S*)-**3** (1.85 g, 6.5 mmol) and isosorbide (0.48 g, 3.25 mmol) in anhydrous $CH_2Cl_2$ (60 mL) was added DMAP (0.2 mmol), and the solution was left stirring at 0 °C for 10 min. DCC (1.47 g, 7.2 mmol) was then added, and the solution was left stirring at room temperature for 6 h. The solution was then filtered, and the filtrate was washed sequentially with 1 M HCl, a saturated aqueous solution of $NaHCO_3$, water, and brine. The solution was then dried, filtered, and evaporated to dryness. The residue was purified by flash chromatography (PE/EtOAc = 5/1, v/v) to obtain the compound (*S*)-**4** as a light-yellow oil (2.03 g, 92%); $[\alpha]_D^{27}$ +48.0° (*c* 0.06 $CHCl_3$). MS (ESI): *m*/*z* 696.3 $[M + NH_4]^+$. IR ($cm^{-1}$, KBr): $\nu_{max}$ 756, 1275, 1462, 1723, 2957. $^1$H NMR (300 Hz, $CDCl_3$): $\delta$ 0.89 (t, 6H, 2 × $CH_3$, *J* = 6.6 Hz), 1.00−1.46 (m, 8H, 4 × $CH_2$), 1.70−1.89 (m, 4H, 2 × $CH_2$), 3.91−4.40 (m, 8H, 2 × $OCH_2$, 2 × $CH_2Cl$,), 4.71 (t, 1H, OCH, *J* = 4.5 Hz), 5.07 (t, 1H, OCH, *J* = 4.8 Hz), 5.33−5.50 (m, 2H, 2 × COOCH), 6.59−6.67 (m, 2H, 2 × $OC\underline{H}CH_2$), 7.26−7.94 (m, 8H, ArH). $^{13}$C NMR (75 MHz, $CDCl_3$): $\delta$ 170.34, 166.54, 162.02, 161.94, 146.04, 144.91, 134.15, 134.07, 133.97, 133.89, 131.33, 131.23, 127.85, 127.59, 126.41, 126.38, 86.13, 86.01, 81.20, 81.05, 74.85, 74.83, 72.89, 72.87, 40.97, 36.43, 36.29, 29.68, 28.09, 27.94, 22.42, 22.35, 13.94, 13.92. HRMS (ESI): *m*/*z* calcd for $C_{34}H_{40}Cl_2O_{10}$ $[M + H]^+$ 679.1999; found 679.2097.

**2,5-Bis{2-[(*R*)-(+)-1-(2-chloroacetoxy)]pentyl}benzoate-1,4:3,6-dianhydro-D-glucitol ((*R*)-4).** Compound (*R*)-**4** was synthesized in the same manner as compound (*S*)-**4**. Starting from compound (*R*)-**3** (1.79 g, 6.30 mmol), compound (*R*)-**4** was obtained as a light-yellow oil (1.92 g, 90%); $[\alpha]_D^{27}$ +23.7° (*c* 0.06 $CHCl_3$). IR (KBr), ESI-MS, $^1$H NMR, $^{13}$C NMR, and HRMS spectral data were identical to those of (*S*)-**4**.

**2,5-Bis{2-[(*S*)-(+)-2-(1-acetoxypentyl)]}benzoate-1,4:3,6-dianhydro-D-glucitol ((*S*)-5a).** To a stirred solution of (*S*)-**2** (1.70 g, 6.8 mmol) and isosorbide (0.50 g, 3.4 mmol) in anhydrous $CH_2Cl_2$ (60 mL) was added DMAP (0.3 mmol), and the solution was left stirring at 0 °C for 10 min. DCC (1.68 g, 8.2 mmol) was then added, and the solution was left stirring at room temperature for 6 h. The solution was then filtered, and the filtrate was washed sequentially with 1 M HCl, a saturated aqueous solution of $NaHCO_3$, water, and brine. The solution was then dried, filtered, and evaporated to dryness. The residue was purified by flash chromatography (PE/EtOAc = 5/1, v/v) to obtain the compound (*S*)-**5a** as a light yellow oil (1.95 g, 94%); $[\alpha]_D^{27}$ +36.7° (*c* 0.06 $CHCl_3$); ee = 99.7%. MS (ESI): *m*/*z* 628.4 $[M + NH_4]^+$. IR ($cm^{-1}$, KBr): $\nu_{max}$ 763, 1247, 1461, 1728, 2958. $^1$H NMR (300 Hz, $CDCl_3$): $\delta$ 0.89 (t, 6H, 2 × $CH_3$, *J* = 6.8 Hz), 1.10−1.55 (m, 8H, 4 × $CH_2$), 1.70−1.91 (m, 4H, 2 × $CH_2$), 2.06 (s, 6H, 2 × $CH_3$), 3.91−4.16 (m, 4H, 2 × $OCH_2$), 4.72 (t, 1H, OCH, *J* = 4.4 Hz), 5.07 (t, 1H,


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OCH, $J$ = 4.4 Hz), 5.39−5.50 (m, 2H, 2 × COOCH), 6.46−6.58 (m, 2H, 2 × OCHCH$_2$), 7.30−7.95 (m, 8H, ArH). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 170.15, 170.01, 165.39, 165.20, 142.20, 142.09, 134.15, 134.01, 132.62, 132.47, 130.50, 130.41, 127.21, 127.06, 126.39, 126.25. HRMS (ESI): $m/z$ calcd for $C_{34}H_{42}O_{10}$ [M + H]$^+$ 611.2778; found 611.2796.

**2,5-Bis{2-[(*R*)-(+)-2-(1-acetoxypentyl)]}benzoate-1,4:3,6-dianhydro-D-glucitol ((*R*)-5a).** Compound (*R*)-**5a** was synthesized in the same manner as compound (*S*)-**5a**. Starting from compound (*R*)-**2** (1.63 g, 6.52 mmol), compound (*R*)-**5a** was obtained as a light-yellow oil (1.79 g, 90%); $[\alpha]_D^{27}$ +20.0° (*c* 0.06 CHCl$_3$); ee = 99.8%. HRMS (ESI): $m/z$ calcd for $C_{34}H_{42}O_{10}$ [M + H]$^+$ 611.2778; found 611.2804. IR (KBr), ESI-MS, $^1$H NMR, and $^{13}$C NMR spectral data were identical to those of (*S*)-**5a**.

**General Method for the Preparation of Compounds (*S*)- and (*R*)-5b−f.** To a solution of (*S*)-**4** or (*R*)-**4** (0.2 mmol) and K$_2$CO$_3$ (55 mg, 0.4 mmol) in acetone (10 mL) was added the corresponding amine, and the solution was left stirring at room temperature for 6−10 h. The solution was then filtered, and the filtrate was reconstituted in EtOAc (20 mL) and solvent removed under reduced pressure. The amino derivatives were purified by flash chromatography (CH$_2$Cl$_2$/MeOH = 100/1−30/1, v/v), to give the title compounds (55−70%).

**2,5-Bis{2-[(*S*)-(+)-1-(2-(diethylamino)acetoxy)]pentyl}-benzoate-1,4:3,6-dianhydro-D-glucitol ((*S*)-5b).** The title compound was obtained as a light yellow oil, 66% yield; $[\alpha]_D^{27}$ +33.2° (*c* 0.05 CHCl$_3$); ee = 99.0%. MS (ESI): $m/z$ 753.3 [M + H]$^+$. IR (cm$^{-1}$, KBr): $\nu_{max}$ 763, 1258, 1461, 1725, 2958. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 0.89 (t, 6H, 2 × CH$_3$, $J$ = 6.3 Hz), 1.01−1.62 (m, 12H, 4 × NCH$_3$), 1.36−1.41 (m, 8H, 4 × CH$_2$), 1.81−1.88 (m, 4H, 2 × CH$_2$), 2.59−2.67 (m, 8H, 4 × NCH$_2$), 3.19−3.37 (m, 4H, 2 × NCH$_2$COO), 3.99−4.16 (m, 4H, 2 × OCH$_2$), 4.73 (t, 1H, OCH, $J$ = 4.5 Hz), 5.04 (t, 1H, OCH, $J$ = 5.0 Hz), 5.39−5.50 (m, 2H, 2 × COOCH), 6.53−6.62 (m, 2H, 2 × OCHCH$_2$), 7.27−7.95 (m, 8H, ArH). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 170.76, 169.95, 166.00, 165.81, 143.89, 143.76, 132.50, 132.38, 130.34, 130.24, 127.88, 127.76, 127.33, 127.21, 126.39, 126.25, 86.13, 81.06, 78.74, 74.60, 73.36, 72.79, 72.61, 70.68, 54.10, 47.57, 36.51, 36.46, 28.03, 27.99, 22.45, 22.42, 13.96, 13.93, 12.35. HRMS (ESI): $m/z$ calcd for $C_{42}H_{60}N_2O_{10}$ [M + H]$^+$ 753.4248; found 753.4320.

**2,5-Bis{2-[(*R*)-(+)-1-(2-(Diethylamino)acetoxy)]pentyl}-benzoate-1,4:3,6-dianhydro-D-glucitol ((*R*)-5b).** The title compound was obtained as a light-yellow oil, 60% yield; $[\alpha]_D^{27}$ +19.2° (*c* 0.05 CHCl$_3$); ee = 99.3%. HRMS (ESI): $m/z$ calcd for $C_{42}H_{60}N_2O_{10}$ [M + H]$^+$ 753.4248; found 753.4334. IR (KBr), ESI-MS, $^1$H NMR, and $^{13}$C NMR spectral data were identical to those of (*S*)-**5b**.

**2,5-Bis{2-[(*S*)-(+)-1-(2-(pyrrolidin-1-yl)acetoxy)]pentyl}-benzoate-1,4:3,6-dianhydro-D-glucitol ((*S*)-5c).** The title compound was obtained as a light yellow oil, 58% yield; $[\alpha]_D^{27}$ +26.7° (*c* 0.06 CHCl$_3$); ee = 99.9%. MS (ESI): $m/z$ 749.4 [M + H]$^+$. IR (cm$^{-1}$, KBr): $\nu_{max}$ 750, 1260, 1462, 1723, 2958. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 0.89 (t, 6H, 2 × CH$_3$, $J$ = 6.6 Hz), 1.35−1.37 (m, 8H, 4 × CH$_2$), 1.79−1.86 (m, 8H, 4 × NCH$_2$CH$_2$), 1.95−1.97 (m, 4H, 2 × CH$_2$), 2.61 (s, 8H, 4 × NCH$_2$), 3.34−3.50 (m, 4H, 2 × NCH$_2$COO), 4.11−4.32 (m, 4H, 2 × OCH$_2$), 4.72 (t, 1H, OCH, $J$ = 4.5 Hz), 5.04 (t, 1H, OCH, $J$ = 4.9 Hz), 5.35−5.50 (m, 2H, 2 × COOCH), 6.51−6.63 (m, 2H, 2 × OCHCH$_2$), 7.27−7.95 (m, 8H, ArH). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 170.12, 170.12, 165.83, 165.83, 143.77, 143.63, 132.54, 132.43, 130.34, 130.27, 127.22, 127.20, 126.39, 126.30, 126.23, 126.15, 86.13, 81.06, 76.61, 75.57, 74.61, 73.36, 72.83, 70.68, 56.80, 53.76, 36.44, 29.67, 27.98, 23.80, 22.47, 13.94. HRMS (ESI): $m/z$ calcd for $C_{42}H_{56}N_2O_{10}$ [M + H]$^+$ 749.3935; found 749.4020.

**2,5-Bis{2-[(*R*)-(+)-1-(2-(pyrrolidin-1-yl)acetoxy)]pentyl}-benzoate-1,4:3,6-dianhydro-D-glucitol ((*R*)-5c).** The title compound was obtained as a light yellow oil, 58% yield; $[\alpha]_D^{27}$ +17.4° (*c* 0.10 CHCl$_3$); ee = 99.8%. HRMS (ESI): $m/z$ calcd for $C_{42}H_{56}N_2O_{10}$ [M + H]$^+$ 749.3935; found 749.4036. IR (KBr), ESI-MS, $^1$H NMR, and $^{13}$C NMR spectral data were identical to those of (*S*)-**5c**.

**2,5-Bis{2-[(*S*)-(+)-1-(2-morpholinoacetoxy)]pentyl}benzoate-1,4:3,6-dianhydro-D-glucitol ((*S*)-5d).** The title compound was obtained as a light-yellow oil, 67% yield; $[\alpha]_D^{27}$ +28.8° (*c* 0.05 CHCl$_3$); ee = 99.8%. MS (ESI): $m/z$ 781.1 [M + H]$^+$. IR (cm$^{-1}$, KBr): $\nu_{max}$ 763, 1258, 1462, 1723, 2956. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 0.89 (t, 6H, 2 × CH$_3$, $J$ = 6.7 Hz), 1.26−1.37 (m, 8H, 4 × CH$_2$), 1.81−1.86 (m, 4H, 2 × CH$_2$), 2.55 (t, 8H, 4 × NCH$_2$CH$_2$O, $J$ = 4.5 Hz), 3.15−3.30 (m, 4H, 2 × NCH$_2$COO), 3.71 (t, 8H, 4 × NCH$_2$CH$_2$O, $J$ = 4.4 Hz), 3.99−4.15 (m, 4H, 2 × OCH$_2$), 4.72 (t, 1H, OCH, $J$ = 4.5 Hz), 5.04 (t, 1H, OCH, $J$ = 5.0 Hz), 5.39−5.50 (m, 2H, 2 × COOCH), 6.54−6.64 (m, 2H, 2 × OCHCH$_2$), 7.29−7.95 (m, 8H, ArH). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 169.44, 169.41, 165.99, 165.79, 143.63, 143.48, 132.57, 132.47, 130.38, 130.28, 127.99, 127.88, 127.33, 127.21, 126.34, 126.19, 86.12, 81.05, 78.75, 74.63, 73.35, 73.18, 73.01, 70.67, 66.76, 59.70, 59.67, 53.26, 36.44, 36.38, 28.01, 27.96, 22.44, 22.42, 13.97, 13.94. HRMS (ESI): $m/z$ calcd for $C_{42}H_{56}N_2O_{12}$ [M + H]$^+$ 781.3833; found 781.3987.

**2,5-Bis{2-[(*R*)-(+)-1-(2-morpholinoacetoxy)]pentyl}benzoate-1,4:3,6-dianhydro-D-glucitol ((*R*)-5d).** The title compound was obtained as a light-yellow oil, 64% yield; $[\alpha]_D^{27}$ +14.6° (*c* 0.07 CHCl$_3$); ee = 99.5%. HRMS (ESI): $m/z$ calcd for $C_{42}H_{56}N_2O_{12}$ [M + H]$^+$ 781.3833; found 781.3998. IR (KBr), ESI-MS, $^1$H NMR, and $^{13}$C NMR spectral data were identical to those of (*S*)-**5d**.

**2,5-Bis{2-[(*S*)-(+)-1-(2-(4-methylpiperazin-1-yl)acetoxy)]-pentyl}benzoate-1,4:3,6-dianhydro-D-glucitol ((*S*)-5e).** The title compound was obtained as a light-yellow oil, 72% yield; $[\alpha]_D^{27}$ +20.4° (*c* 0.05 CHCl$_3$); ee = 99.4%. MS (ESI): $m/z$ 807.4 [M + H]$^+$. IR (cm$^{-1}$, KBr): $\nu_{max}$ 750, 1260, 1462, 1723, 2958. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 0.89 (t, 6H, 2 × CH$_3$, $J$ = 6.3 Hz), 1.26−1.35 (m, 8H, 4 × CH$_2$), 1.84−1.86 (m, 4H, 2 × CH$_2$), 2.53 (s, 6H, 2 × NCH$_3$), 2.79−2.82 (m, 16H, 8 × NCH$_2$), 3.19−3.37 (m, 4H, 2 × NCH$_2$COO), 3.99−4.08 (m, 4H, 2 × OCH$_2$), 4.73 (t, 1H, OCH, $J$ = 4.5 Hz), 5.05 (t, 1H, OCH, $J$ = 5.0 Hz), 5.39−5.49 (m, 2H, 2 × COOCH), 6.53−6.63 (m, 2H, 2 × OCHCH$_2$), 7.30−7.95 (m, 8H, ArH). $^{13}$C NMR (75 MHz, CDCl$_3$): $\delta$ 179.96, 169.60, 166.02, 165.91, 143.72, 143.63, 132.54, 132.43, 130.34, 130.25, 128.02, 127.90, 127.26, 127.20, 126.37, 126.23, 86.13, 81.05, 78.75, 74.61, 73.35, 73.10, 72.92, 70.68, 59.47, 54.84, 52.96, 45.94, 36.45, 28.01, 22.45, 13.93. HRMS (ESI): $m/z$ calcd for $C_{44}H_{62}N_4O_{10}$ [M + H]$^+$ 807.4466; found 807.4550.

**2,5-Bis{2-[(*R*)-(+)-1-(2-(4-methylpiperazin-1-yl)acetoxy)]-pentyl}benzoate-1,4:3,6-dianhydro-D-glucitol ((*R*)-5e).** The title compound was obtained as a light-yellow oil, 70% yield; $[\alpha]_D^{27}$ +8.6° (*c* 0.05 CHCl$_3$); ee = 99.6%. HRMS (ESI): $m/z$ calcd for $C_{44}H_{62}N_4O_{10}$ [M + H]$^+$ 807.4466; found 807.4576. IR (KBr), ESI-MS, $^1$H NMR, and $^{13}$C NMR spectral data were identical to those of (*S*)-**5e**.

**2,5-Bis{2-[(*S*)-(+)-1-(2-(4-(2-hydroxyethyl)piperazin-1-yl)-acetoxy)]pentyl}benzoae-1,4:3,6 dianhydro-D-glucitol ((*S*)-5f).** The title compound was obtained as a light yellow oil, 67% yield. $[\alpha]_D^{27}$ +12.4° (*c* 0.05 CHCl$_3$). ee = 99.5%. MS (ESI): $m/z$ 867.4 [M + H]$^+$. IR (cm$^{-1}$, KBr): $\nu_{max}$ 763, 1247, 1460, 1723, 2958. $^1$H NMR (300 MHz, CDCl$_3$): $\delta$ 0.89 (t, 6H, 2 × CH$_3$, $J$ = 6.3 Hz), 1.26−1.35 (m, 8H, 4 × CH$_2$), 1.83−1.85 (m, 4H, 2 × CH$_2$), 2.48−2.56 (m, 20H, 8 × NCH$_2$, 2 × NCH$_2$CH$_2$OH), 3.16−3.31 (m, 4H, 2 × NCH$_2$COO), 3.48−3.65 (m, 4H, 2 × NCH$_2$CH$_2$OH), 3.89−4.15 (m, 4H, 2 × OCH$_2$), 4.46−4.50 (m, 1H, OH), 4.72−4.75 (m, 1H, OH), 4.98 (t, 1H, OCH, $J$ = 4.5 Hz), 5.04 (t, 1H, OCH, $J$ = 5.0 Hz), 5.37−5.49 (m, 2H, 2 × COOCH), 6.54−6.61 (m, 2H, 2 × OCHCH$_2$), 7.30−7.95 (m, 8H, ArH). $^{13}$C NMR (75 MHz, CDCl$_3$) $\delta$ 179.96, 169.60, 166.02, 165.91, 143.72, 143.63, 132.54, 132.43, 130.34, 130.25, 128.02, 127.90, 127.26, 127.20, 126.37, 126.23, 86.13, 81.05, 78.75, 74.61, 73.35, 73.10, 72.92, 70.68, 59.55, 59.34, 54.84, 52.96, 45.94, 36.45, 28.01, 22.45, 13.93. HRMS (ESI): $m/z$ calcd for $C_{46}H_{66}N_4O_{12}$ [M + H]$^+$ 867.4677; found 867.5745.

**2,5-Bis((*R*)-(+)-1-(2-(4-(2-hydroxyethyl)piperazin-1-yl)-acetoxy)]pentyl}benzoae-1,4:3,6-dianhydro-D-glucitol ((*R*)-5f).** The title compound was obtained as a light-yellow oil, 67% yield; $[\alpha]_D^{27}$ +4.6° (*c* 0.05 CHCl$_3$); ee = 99.3%. HRMS (ESI): $m/z$ calcd for $C_{46}H_{66}N_4O_{12}$ [M + H]$^+$ 867.4677; found 867.5763. IR (KBr), ESI-MS, $^1$H NMR, and $^{13}$C NMR spectral data were identical to those of (*S*)-**5f**.

**(*S*)-2-{1-[2-(4-Methylpiperazin-1-yl)acetoxy]pentyl}benzoic Acid (6).** To a solution of compound (*S*)-**3** (0.57 g, 2.0 mmol) and K$_2$CO$_3$ (0.76 g, 4.0 mmol) in acetone (20 mL) was added *N*-methyl piperazine (0.66 mL, 6.0 mmol), and the solution was left stirring at


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room temperature for 8 h. The solvent was removed under reduced pressure and purified by flash chromatography ($CH_2Cl_2$/MeOH = 10/1, v/v) to give the **6** as a light-yellow oil (0.34 g, 48%); $[\alpha]_D^{27}$ −36.3° (*c* 1.00 $CHCl_3$). MS (ESI): *m/z* 349.2 $[M + H]^+$. IR ($cm^{-1}$, KBr): $\nu_{max}$ 1383, 1584, 1764, 2360, 2932. $^1H$ NMR (300 Hz, $CDCl_3$): $\delta$ 0.96 (t, 3H, $CH_3$, *J* = 8.2 Hz), 1.33−1.44 (m, 4H, 2 × $CH_2$), 1.86−1.90 (m, 2H, $CH_2$), 2.23−2.34 (m, 3H, $NCH_3$), 2.46 (s, 8H, 4 × $NH_2$), 3.32 (s, 2H, $NH_2COO$), 6.65−6.79 (m, 1H, OC<u>H</u>$CH_2$), 7.37−7.40 (m, 1H, ArH), 7.56−7.62 (m, 2H, ArH), 8.05 (d, 1H, ArH, *J* = 8.1 Hz), 11.02 (brs, 1H, COOH). $^{13}C$ NMR (75 Hz, $CDCl_3$): $\delta$ 172.0, 169.4, 142.6, 133.6, 129.8, 128.5, 126.7, 125.7, 73.8, 56.8, 55.2, 52.5, 43.0, 35.7, 26.8, 22.5, 14.0. HRMS (ESI): *m/z* calcd for $C_{19}H_{28}N_2O_4$ $[M + H]^+$ 349.2049; found 349.2130.

**2-{2-[(*S*)-(+)-1-(2-(4-Methylpiperazin-1-yl)acetoxy)]pentyl}-benzoate-5-benzyloxy-1,4:3,6-dianhydro-D-glucitol (9).** To a stirred solution of compound **7** (0.24 g, 1.00 mmol) and compound **6** (0.34 g, 0.98 mmol) in anhydrous $CH_2Cl_2$ (60 mL) was added DMAP (0.1 mmol), and the solution was left stirring at 0 °C for 10 min. DCC (0.25 g, 1.2 mmol) was then added, and the solution was left stirring at room temperature for 8 h. The solution was then filtered, and the filtrate was washed sequentially with 1 M HCl, a saturated aqueous solution of $NaHCO_3$, water, and brine. The solution was then dried, filtered, and evaporated to dryness. The residue was purified by flash chromatography (PE/EtOAc = 5/1, v/v) to obtain the compound **9** as a light-yellow oil (0.50 g, 90%); $[\alpha]_D^{27}$ +6.0° (*c* 2.00 $CHCl_3$). MS (ESI): *m/z* 567.4 $[M + H]^+$. IR ($cm^{-1}$, KBr): $\nu_{max}$ 1124, 1259, 1455, 1659, 1722, 2925, 3360. $^1H$ NMR (300 Hz, $CDCl_3$): $\delta$ 0.89 (t, 3H, $CH_3$, *J* = 7.0 Hz), 1.33−1.42 (m, 4H, 2 × $CH_2$), 1.82−1.93 (m, 2H, $CH_2$), 2.28 (s, 3H, $NCH_3$), 2.47−2.57 (m, 8H, 4 × $NCH_2$), 3.32−3.40 (m, 2H, $OCOCH_2$), 3.96−4.01 (m, 4H, 2 × $OCH_2$), 4.36 (d, 1H, COH, *J* = 4.9 Hz), 4.39 (t, 1H, OCH, *J* = 4.9 Hz), 4.59 (s, 2H, $OCH_2Ar$), 4.96 (t, 1H, OCH, *J* = 5.0 Hz), 5.36 (d, 1H, OCH, *J* = 5.7 Hz), 6.56−6.65 (m, 1H, OC<u>H</u>$CH_2$), 7.27−7.35 (m, 6H, ArH), 7.49−7.51 (m, 2H, ArH), 7.90 (d, 1H, ArH, *J* = 8.0 Hz). $^{13}C$ NMR (75 Hz, $CDCl_3$): $\delta$ 169.6, 166.0, 143.7, 137.6, 132.4, 132.0, 130.4, 130.2, 128.5, 127.9, 127.7, 127.2, 126.2, 126.1, 86.4, 83.3, 80.7, 74.6, 73.2, 73.1, 71.4, 70.3, 59.4, 54.8, 54.8, 52.9, 52.9, 46.0, 36.6, 28.1, 22.7,14.1. HRMS (ESI): *m/z* calcd for $C_{32}H_{42}N_2O_7$ $[M + H]^+$ 567.2992; found 567.2302.

**2-Benzyloxy-5-{2-[(*S*)-(+)-1-(2-(4-methylpiperazin-1-yl)-acetoxy)]pentyl}benzoate-1,4:3,6-dianhydro-D-glucitol (10).** Compound **10** was synthesized in the same manner as compound **9**. Starting from compound **8** (0.24 g, 1.00 mmol) and compound **6** (0.34 g, 0.98 mmol), compound **10** was obtained as a light-yellow oil (0.51 g, 92%). $[\alpha]_D^{27}$ +14.7° (*c* 2.00 $CHCl_3$). HRMS (ESI): *m/z* calcd for $C_{32}H_{42}N_2O_7$ $[M + H]^+$ 567.2992; found 567.2320. IR (KBr), ESI-MS, $^1H$ NMR, and $^{13}C$ NMR spectral data were identical to those of compound **9**.

**2-{2-[(*S*)-(+)-1-(2-(4-Methylpiperazin-1-yl)acetoxy)]pentyl}-benzoate-2-hydroxy-1,4:3,6-dianhydro-D-glucitol (11).** A mixture of compound **9** (0.50 g, 0.88 mmol) and 10% Pd/C in methanol (10 mL) was allowed to stir under an atmosphere of $H_2$ (45 psi) at room temperature for 4 h. The reaction mixture was filtered and evaporated to dryness. The residue was purified by flash chromatography ($CH_2Cl_2$/MeOH = 30/1, v/v) to obtain the compound **11** as a light-yellow oil (0.40 g, 95%); $[\alpha]_D^{27}$ +14.3° (*c* 2.00 $CHCl_3$). MS (ESI): *m/z* 477.3 $[M + H]^+$. IR ($cm^{-1}$, KBr): $\nu_{max}$ 170, 1260, 1456, 1721, 2955. $^1H$ NMR (300 Hz, $CDCl_3$): $\delta$ 0.89 (t, 3H, $CH_3$, *J* = 6.8 Hz), 1.34−1.45 (m, 4H, 2 × $CH_2$), 1.75−1.85 (m, 2H, $CH_2$), 2.27 (s, 3H, $NCH_3$), 2.32−2.57 (m, 8H, 4 × $NCH_2$), 3.31−3.48 (m, 2H, $OCOCH_2$), 3.72−4.03 (m, 4H, 2 × $OCH_2$), 4.05 (t, 1H, OHC<u>H</u>, *J* = 5.0 Hz), 4.43 (d, 1H, C−OH, *J* = 4.4 Hz), 4.63 (t, 1H, OCH, *J* = 4.9 Hz), 5.00 (t, 1H, OCH, *J* = 5.0 Hz), 5.38 (d, 1H, OCH, *J* = 5.2 Hz), 6.58−6.70 (m, 1H, OC<u>H</u>$CH_2$), 7.30−7.35 (m, 1H, ArH), 7.50−7.54 (m, 2H, ArH), 7.90 (d, 1H, ArH, *J* = 8.2 Hz). $^{13}C$ NMR (75 Hz, $CDCl_3$): $\delta$ 169.6, 166.1, 143.5, 132.5, 130.4, 128.0, 127.3, 126.2, 88.6, 88.2, 81.6, 80.5, 80.5, 73.4, 70.2, 59.2, 54.7, 54.7, 52.7, 52.7, 45.8, 36.4, 28.0, 22.4, 14.0. HRMS (ESI): *m/z* calcd for $C_{25}H_{36}N_2O_7$ $[M + H]^+$ 477.2523; found 477.2605.

**2-Hydroxy-5-{2-[(*S*)-(+)-1-(2-(4-methylpiperazin-1-yl)-acetoxy)]pentyl}benzoate-1,4:3,6-dianhydro-D-glucitol (12).** Compound **12** was synthesized in the same manner as compound **11**. Starting from compound **10** (0.51g, 0.90 mmol), compound **12** was obtained as a light-yellow oil (0.39 g, 91%); $[\alpha]_D^{27}$ +36.2° (*c* 2.00 $CHCl_3$). HRMS (ESI): *m/z* calcd for $C_{25}H_{36}N_2O_7$ $[M + H]^+$ 477.2523; found 477.2621. IR (KBr), ESI-MS, $^1H$ NMR, and $^{13}C$ NMR spectral data were identical to those of compound **11**.

**2-{2-[(*S*)-(+)-1-(2-(4-Methylpiperazin-1-yl)acetoxy)]pentyl}-benzoate-5-nitrooxy-1,4:3,6-dianhydro-D-glucitol (13).** A solution of compound **11** (0.40 g, 0.84 mmol) was added, dropwise, to an ice-cooled solution of fuming nitric acid (90%, 2 mL) in AcOH/$Ac_2O$ (10 mL, 1:1, v/v). The reaction was stirred in an ice-bath for 0.5 h and then at room temperature for 2 h. The reaction solution was poured to the ice water and extracted with $CH_2Cl_2$ (20 mL × 3). The combined organic phases were dried, filtered, and evaporated to dryness. The residue was purified by column chromatography ($CH_2Cl_2$/MeOH = 50/1, v/v) to obtain the compound **13** as a light-yellow oil (0.20 g, 46%); $[\alpha]_D^{27}$ +22.4° (*c* 2.00 $CHCl_3$). MS (ESI): *m/z* 522 $[M + H]^+$. IR ($cm^{-1}$, KBr): $\nu_{max}$ 1281, 1384, 1637, 1717, 2360, 2908, 3447. $^1H$ NMR (300 Hz, $CDCl_3$): $\delta$ 0.89 (t, 3H, $CH_3$, *J* = 6.3 Hz), 1.33−1.45 (m, 4H, 2 × $CH_2$), 1.70−1.85 (m, 2H, $CH_2$), 2.28−2.35 (m, 3H, $NCH_3$), 2.54−2.79 (m, 8H, 4 × $NCH_2$), 3.35−3.50 (m, 2H, $COCH_2N$), 3.72−4.03 (m, 4H, 2 × $OCH_2$), 4.05 (d, 1H, $ONO_2CH$, *J* = 5.1 Hz), 5.17 (t, 1H, OCH, *J* = 5.1 Hz), 5.40 (t, 1H, OCH, *J* = 5.0 Hz), 5.48 (d, 1H, COOCH, *J* = 5.2 Hz), 6.54−6.65 (m, 1H, OC<u>H</u>$CH_2$), 7.39−7.41 (m, 1H, ArH), 7.54−7.66 (m, 2H, ArH), 7.95 (d, 1H, ArH, *J* = 8.4 Hz). $^{13}C$ NMR (75 Hz, $CDCl_3$): $\delta$ 169.6, 166.1, 143.5, 132.5, 130.4, 128.0, 127.3, 126.2, 88.6, 88.2, 81.6, 80.5, 80.5, 73.4, 70.2, 59.2, 54.7, 54.7, 52.7, 52.7, 45.8, 36.4, 29.7, 28.0, 22.4. HRMS (ESI): *m/z* calcd for $C_{25}H_{35}N_3O_9$ $[M + H]^+$ 522.2373; found 522.2406.

**2-Nitrooxy-5-{2-[(*S*)-(+)-1-(2-(4-methylpiperazin-1-yl)-acetoxy)]pentyl}benzoate-1,4:3,6-dianhydro-D-glucitol (14).** Compound **14** was synthesized in the same manner as compound **13**. Starting from compound **12** (0.39 g, 0.82 mmol), compound **14** was obtained as a light-yellow oil (0.18 g, 43%); $[\alpha]_D^{27}$ +35.1° (*c* 2.00 $CHCl_3$). HRMS (ESI): *m/z* calcd for $C_{25}H_{35}N_3O_9$ $[M + H]^+$ 522.2373; found 522.2418. IR (KBr). ESI-MS, $^1H$ NMR, and $^{13}C$ NMR spectral data were identical to those of compound **13**.

**Antiplatelet Aggregation Effect In Vitro.** Blood samples were withdrawn from the carotid artery of individual rabbits and mixed with 3.8% sodium citrate solution (9/1, v/v), followed by centrifuging at 500 rpm for 10 min at room temperature. After the collection of the resulting platelet-rich plasma (PRP), the residue was centrifuged at 3000 rpm for another 10 min at room temperature to obtain platelet-poor plasma (PPP). The PRP was adjusted with PPP in order to obtain platelet counts of 400−450 × $10^9$ Pl/L. The effects of individual compounds on the platelet aggregation were determined by Born's turbidimetric method using a four-channel aggregometer (LG-PABER-I Platelet-Aggregometer, Beijing, China) within 3 h after blood collection. Briefly, PRP (240 $\mu$L) was preincubated with vehicle DMSO, positive controls of Ticlid or ASP, or different concentrations (0.1, 0.2, 0.4, 0.8, and 1.6 mM) of individual compounds (30 $\mu$L) for 5 min at 37 °C, followed by the addition of 10 $\mu$M of adenosine 5′-diphosphate sodium salt (ADP, Sigma-Aldrich, USA) or 0.33 mM of arachidonic acid (AA, Cayman Chemical, USA), respectively, to induce the platelet aggregation. The maximum aggregation rate (MAR) was recorded within 5 min at 37 °C. The inhibition rate of the tested compounds on platelet aggregation was calculated with the following formula: Inhibition rate (%) = (100% − MAR of tested compound/MAR of vehicle). The $IC_{50}$ value of each compound was calculated accordingly.

**Stability of (*S*)-5e in the Gastrointestinal Fluids.** Artificial gastric juice (500 mL) was prepared by adding 5.0 g of pepsin to water and adjusting the pH to 1.5 with HCl. Then 500 mL of artificial intestinal juice was prepared by adding 3.4 g of potassium dihydrogen phosphate and 5.0 g of trypsin to water and then adjusting the pH to 6.8 with sodium hydroxide. (*S*)-**5e** (20 $\mu$L of 50 $\mu$M) was mixed in triplicate with 180 $\mu$L of artificial gastric juice or intestinal juice and incubated at 37 °C for 0, 1, 2, 4, and 8 h. The mixture was exposed to


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triple volumes of acetonitrile to terminate the reaction. The mixture was vortexed for 2 min and centrifuged at 12000 rpm for 5 min. The supernatant samples (20 μL each) were subjected to HPLC analysis using Shimadzu 20A series (Shimadzu, Kyoto, Japan) on Sapphire $C_{18}$ analytical column (250 mm × 4.6 mm, i.d., 5 μm, Sepax Technologies, InC., Delaware, USA). The mobile phase comprised of methanol−water (90/10, v/v) at a flow rate of 1.0 mL/min with the detection wavelength at 260 nm.

**Thromboembolism in Mice.** Male Swiss mice (30−35 g, from ICR animal colony) were purchased from B&K Universal Group, Shanghai, China. Animals were housed in specific pathogen free facility in a 12 h light/dark cycle with free access to food and water. The mice were subjected to inducing acute systemic vascular thromboembolism by infusion of a mixture of collagen and adrenaline.[29] Briefly, the mice were randomized and treated with vehicle dimethyl sulfoxide (DSMO), ASP (160 mg/kg), (*S*)-NBP (160 mg/kg), or (*S*)-**5e** (160 or 680 mg/kg) by gavage daily for seven days. Two hours after the last administration, the mice were injected intravenously with a mixture of 0.21 mg/kg of collagen and 44.5 μg/kg of adrenaline. The survival of individual mice was monitored for 15 min.

**Antithrombotic Activity Assay in Rats.** Male Sprague−Dawley (SD) rats (250−280 g) were randomized and divided into five groups ($n$ = 12 per group): (1) sham (0.5% CMC-Na), (2) vehicle (A-V + 0.5% CMC-Na), (3) A-V + (*S*)-NBP (80 mg/kg), (4) A-V + (*S*)-**5e** (340 mg/kg), (5) A-V + (*S*)-**5e** (80 mg/kg). The rats were treated with the indicated compound with the indicated dose by gavage daily for seven days. Two hours after the last treatment, the rats were anesthetized by intraperitoneally injecting with chloral hydrate (300 mg/kg) and subjected to the arteriovenous shunt procedure, as described previously with modifications.[30] The left jugular vein and the right carotid artery of individual rats were inserted with a saline-filled polyethylene catheter (14 cm in the length, containing a 6 cm silk thread (4#)) for maintaining for 15 min, allowing the formation of a thrombus along the silk thread. The shunt was subsequently removed, and the silk thread with thrombus were collected and weighed immediately. Subsequently, the silk thread was dried at room temperature for 24 h to determine the dry mass. Both the wet and dry weights of a thrombus were further subtracted by the weight of the dry 6 cm silk thread.

**Aqueous Solubility Assays.** Individual compounds at ca. 1 mg were dissolved in 10 mL of methanol and the maximum UV absorption of each compound was determined in a UV755B spectrophotometer, eventually diluting the solution (with $CH_3OH$) as necessary. A saturated solution of each compound was then prepared by stirring magnetically a small volume of normal saline in the presence of excess compound for 5 h at 25 °C. The saturated solution was filtered with a 0.45 mm filter (Millipore) and measured by UV-spectrometry at the wavelength determined. Total solubility was determined by the relationship: $C' = A'CA^{-1}$, where $C$ = the concentration of standard solution (mg/mL), $A$ = absorbance of standard solution, $A'$ = absorbance of saturated solution, and $C'$ = concentration of saturated solution (mg/mL).

**The BBB Penetration of (*S*)-5e In Vivo Assay.** The Male ICR mice (30−35 g) were treated with a single dose (680 mg/kg) of (*S*)-**5e** by gavage. The mice were sacrificed at 0.5, 1.0, 2.0, 4.0, and 8.0 h post treatment. Their blood samples were collected, and their brain tissues were dissected, washed with cold saline, and weighed. Their blood plasma samples were prepared by centrifuging and stored on ice. The individual brains were homogenized in 3 volumes of saline. Individual plasma and homogenized brain samples (50 μL each) were mixed with triple volumes of acetonitrile to precipitate proteins and centrifuged at 12000 rpm for 5 min. The supernatants (10 μL each) were used for HPLC analysis.

HPLC analysis of (*S*)-**5e** in the prepared plasma and brain lysates was performed on a LC-MS-2020 liquid chromatograph mass spectrometer (Shimadzu, Kyoto, Japan) equipped with a SIL-20A autosampler, two LC-20AD pumps, a CTO-20A column oven, and an electrospray-ionization (ESI) interface using Shimadzu LC-MS Solution (version 5.42). Separation was performed on a shim-pack VP-ODS column (5 μm, 2.1 mm × 150 mm, Shimadzu, Kyoto, Japan) coupled with a Security Guard $C_{18}$ guars column (4 mm × 3.0 mm, Phenomenex, Torrance, CA, USA) using gradient elution with water containing 0.1% formic acid as solvent A and methanol as solvent B. The gradient elution conditions were: B was kept 20% for 2 min, increased to 80% at 2.5 min and kept for 3.5 min, and returned to 20% at 6.5 min and kept for 1.5 min. The flow rate was 0.3 mL/min and the injection volume was 10 μL. The column temperature was maintained at 37 °C. The mass spectrometer equipped with an electrospray ionization source was operated with a gas ($N_2$) flow of 1.5 mL/min and detector voltage of 1.6 kV. The heat block temperature was 200 °C, and the curved desolvation line (CDL) temperature was maintained at 250 °C. LC-ESI-MS was performed in positive selected-ion monitoring mode. (*S*)-**5e** was detected at $m/z$ 807.7.

**The Cerebral Ischemia/Reperfusion (I/R) Model in Rats.** Male SD rats (280−320 g) were randomly divided into five groups ($n$ = 12 per group): (1) sham (0.5% CMC-Na), (2) vehicle (I/R + 0.5% CMC-Na), (3) I/R + (*S*)-NBP (80 mg/kg), (4) I/R + (*S*)-**5e** (340 mg/kg), (5) I/R + (*S*)-**5e** (80 mg/kg). The rats were treated with the indicated compound at the indicated dose by gavage daily for seven days. Two hours after the last treatment, the rats were anesthetized with chloral hydrate (300 mg/kg, ip) and subjected to a middle cerebral artery occlusion (MCAO), as described previously with some modification. Briefly, the right common carotid artery (CCA), internal carotid artery (ICA), and external carotid artery (ECA) were exposed. A 40 monofilament nylon suture (Beijing Sunbio Biotech, Beijing, China) was carefully inserted from the ECA stump into the ICA and was gently advanced to occlude the origin of the right middle cerebral artery (MCA) until a light resistance was felt (18−20 mm from CCA bifurcation). Two hours later, the suture was withdrawn to restore blood flow (reperfusion). The sham group of rats received a sham operation by inserting the filament into the ICA and immediately withdrawing it. The core body temperature was monitored and maintained at 37 ± 0.5 °C with a heating pad and warm light throughout surgical procedures until the rats completely recovered from anesthesia.

**Neurological Deficit Scores.** Neurological deficits of individual rats were evaluated at 24 h post reperfusion by the Longa's method in a blinded manner.[35] Tests were always performed between 10 and 11 a.m. to exclude the possible interference of circadian rhythm-related behavioral changes.

**Measurement of Infarct Size.** At 24 h post reperfusion, some rats ($n$ = 6) from each group were sacrificed and their brains were rapidly removed onto ice and coronally sectioned (2 mm). The sections were immediately incubated in 2% TTC for 30 min at 37 °C and fixed in 10% formalin overnight. The infarct areas (white) in individual sections were measured and analyzed using Image-Pro Plus (Version 6.0). The infarct size was expressed as the percentage size in the total area of whole brain.

**Measurement of Brain-Water Content.** At 24 h post reperfusion, some rats ($n$ = 6) form each group were sacrificed and their brains were rapidly removed onto ice, followed by isolating the ischemic hemispheres. The tissue samples were immediately weighed to obtain the wet weight. The brains were then dried at 100 °C for 24 h and determined for its dry weight. The percentage of brain water content was calculated as (wet weight − dry weight)/wet weight ×100%.

**Histopathological Assessment.** At 24 h post reperfusion, some rats ($n$ = 6) form each group were anesthetized with chloral hydrate and perfused transcardially with saline and 4% paraformaldehyde, followed by embedding in paraffin. The paraffin-embedded tissue sections (5 μM) were stained with hematoxylin and eosin (H&E) and examined under a light microscope.

## ■ ASSOCIATED CONTENT

### Ⓢ Supporting Information

Tables of compound purity measured by HPLC and compound optical purity measured by chiral HPLC. This material is available free of charge via the Internet at http://pubs.acs.org.

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### Notes

The authors declare no competing financial interest.

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## ABBREVIATIONS USED

ADP, adenosine diphosphate; AA, arachidonic acid; NBP, 3-*n*-butylphthalide; ASP, aspirin; BBB, blood−brain barrier; SFDA, State Food and Drug Administration; HPBA, 2-(1-hydroxypentyl) benzoic acid; IS, isosorbide; DCC, 1,3-dicyclohexylcarbodiimide; DMAP, 4-dimethylaminopyridine; ANOVA, one-way analysis of variance; A-V, arterio-venous; SD, Sprague−Dawley; I/R, ischemia/reperfusion; PRP, platelet rich plasma; PPP, platelet-poor plasma; MAR, maximum aggregation rate; SAR, structure−activity relationship; HPLC, high-performance liquid chromatography; ESI, electrospray-ionization; MTT, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide; MCAO, middle cerebral artery occlusion; CMC-Na, sodium carboxyl methyl cellulose; TTC, 2,3,5-triphenyltetrazolium chloride; H&E, hematoxylin and eosin; MDA, malondialdehyde; DSMO, dimethyl sulfoxide; CDL, curved desolvation line; CCA, common carotid artery; ICA, internal carotid artery; ECA, external carotid artery; MCA, middle cerebral artery

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