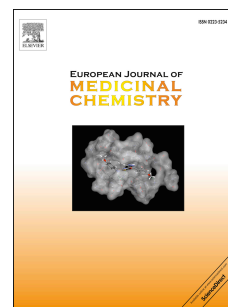


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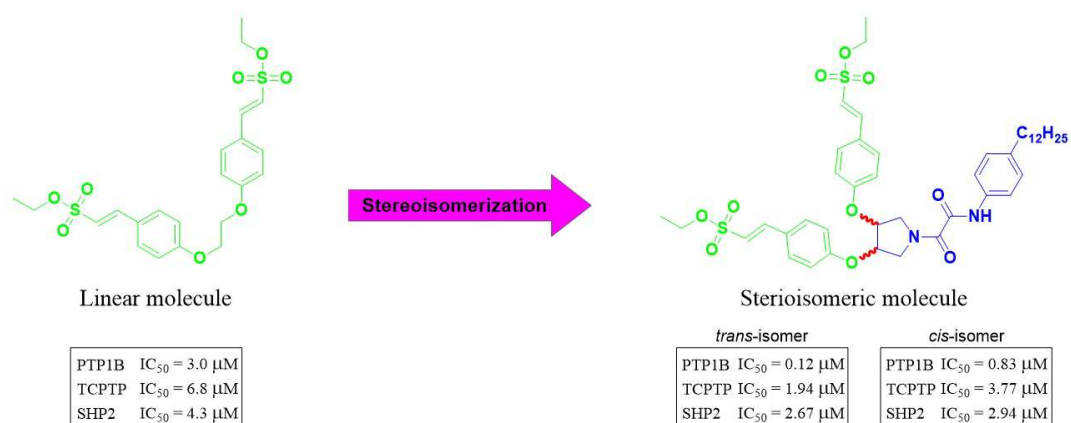
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Graphical Abstract



Investigation of Stereoisomeric Bisarylethenesulfonic Acid Esters for Discovering Potent and Selective PTP1B Inhibitors

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Protein tyrosine phosphatase 1B (PTP1B) has been considered as a promising therapeutic target for type 2 diabetes mellitus (T2DM) and obesity due to its key regulating effects in insulin signaling and leptin receptor pathways. In this work, a series of *cis*- and *trans*-pyrrolidine bisarylethenesulfonic acid esters were prepared and their PTP1B inhibitory potency, selectivity and membrane permeability were evaluated. These novel stereoisomeric molecules especially *trans*-isomers exhibited remarkable inhibitory activity, significant selectivity as well as good membrane permeability (e.g. compound **28a**, IC₅₀ = 120, 1940 and 2670 nM against PTP1B, TCPTP and SHP2 respectively, and P_{app} = 1.74 × 10⁻⁶ cm/s). Molecular simulations

indicated that *trans*-pyrrolidine bisarylethanesulfonic acid esters yielded the stronger binding affinity than their *cis*-isomers by constructing more interactions with non-catalytic sites of PTP1B. Further biological activity studies revealed that compound **28a** could enhance insulin-stimulated glucose uptake and insulin-mediated insulin receptor β (IR β) phosphorylation with no significant cytotoxicity.

Key words: PTP1B Inhibitors, Selectivity, Pyrrolidine bisarylethanesulfonic acid esters, Type 2 diabetes

1. Introduction

Protein tyrosine kinases (PTKs) and protein tyrosine phosphatases (PTPs), which together maintain proper levels of tyrosine phosphorylation, are crucial for cellular processes including growth, differentiation, metabolism, migration, and survival [1]. PTKs and PTPs provide potential molecular targets for the therapeutic interventions of associated diseases such as autoimmune disorders, diabetes, obesity and cancers [2-5]. So far, more than dozens of PTKs inhibitors have been already approved for clinical use, but only a few of PTPs inhibitors went through deep investigations and came into clinical trials. There is a huge space for PTPs to develop novel chemotherapeutic agents for the associated diseases [6,7].

Protein tyrosine phosphatase 1B (PTP1B), the first isolated enzyme of PTPs family, is a key negative regulator of insulin signaling and leptin receptor pathways [8-10]. PTP1B deficient rodents were viable and healthy, and displayed improved insulin sensitivity and resistance to diet-induced obesity [11], which had provided a strong evidence of PTP1B to be a promising drug target for the treatment of type 2 diabetes and obesity [12].

Various PTP1B inhibitors have been developed in the past decades for further pharmacological research [13,14]. However, the most typically potent PTP1B inhibitors with anionic pharmacophores [15-17] suffered a drawback of poor membrane permeability. High ionization of these inhibitors in physiological situation prevent them from penetrating cellular membranes, although the anionic

pharmacophores could bind specifically to the catalytic site of PTP1B and offer potent inhibitory activity *in vitro*. Another knotty problem is the poor selectivity of existing inhibitors for PTP1B over other members of PTPs family, particularly T-cell PTP (TCPTP) which is vital for rodent survival [18,19].

In view of these, some of recent researches on PTP1B inhibitors are focusing on the improvement of molecular selectivity and membrane permeability [20-22]. We previously reported a number of linear arylenesulfonic acid ester compounds (**Fig.1**, compounds **I** and **II**) as nonionic PTP1B inhibitors, revealing that sulfonic acid ester was an effective bioisostere of phosphoric acid and carboxylic acid moiety in inhibition of PTP1B [23,24]. Some of Y-shaped bisarylenesulfonic acid esters (e.g. compound **III**), which probably interact with multiple secondary binding sites (B, C, D or E site) in addition to the catalytic site (A site) of PTP1B [25], showed high potency, good membrane permeability, and PTP1B selectivity in some degree. Hence, compared with linear structures, more complex stereo-chemical structure resulting from extra branching could contribute to increasing binding affinity and improving bisarylenesulfonic acid esters' selectivity in inhibition of PTP1B.

Fig. 1

To further explore the SARs of bisarylenesulfonic acid esters and search for potent, selective and membrane permeable PTP1B inhibitors, we designed, synthesized and biologically evaluated a series of stereoisomeric pyrrolidine bisarylenesulfonic acid esters (*cis*- and *trans*-isomers here). With amyloxalyl as the preferred linker, some of derivatives, particularly the *trans*-isomers, exhibited potent PTP1B inhibitory activity and significant selectivity. Furthermore, the differences in PTP1B activity and selectivity between the two kinds of stereoisomers were analyzed and explained by docking simulations. As the most potent and selective PTP1B inhibitor in this presentation, compound **28a** could enhance insulin-stimulated glucose uptake and insulin-mediated insulin receptor β (IR β) phosphorylation without significant cytotoxicity. This research gives us valuable insights on discovering novel PTP1B inhibitors with higher potency and better selectivity.

2. Chemistry

The key intermediates (**7a** and **7b**) of stereoisomeric pyrrolidine bisarylethenesulfonic acid ester derivatives were synthesized according to the method outlined in **Schemes 1** and **2**, respectively. Compound **7a** was prepared from L(+)-tartaric acid (**1a**) via 7 steps (**Scheme 1**). Firstly, a ring-closing reaction was conducted by heating **1a** with benzylamine, and then the cyclization product (**2a**) was reduced to the diol (**3a**) by LiAlH₄. Intermediate **3a** reacted with methanesulfonic anhydride to afford the dimethanesulfonate (**4a**) [26], which underwent a nucleophilic substitution to give the dialdehyde (**5a**). In the presence of NaH, **5a** was converted to **6a** through Wittig-Horner reaction by treatment with ethyl (diethoxyphosphoryl) methanesulfonate [24]. Finally, **6a** was debenzylated via two steps to afford **7a** as the key intermediate of *trans*-derivatives [27].

Scheme 1

Compound **7b**, the common intermediate of *cis*-derivatives, was prepared from *cis*-butenedioic anhydride (**1b**) via 9 steps (**Scheme 2**). Compound **1b** was treated with benzylamine and then experienced a *cis*-dihydroxylation by hydrogen peroxide to give **2b** [28]. Intermediate **2b** underwent the same procedure to intermediate **2a** in **Scheme 1** to provide **3b** and **4b**. Via two separated steps of nucleophilic substitution at different temperatures, **4b** was converted to **5b**. Also following the same procedure in **Scheme 1**, **5b** was converted to **6b** and finally **7b**.

Scheme 2

Strategies for synthesis of stereoisomeric pyrrolidine bisarylethenesulfonic acid ester derivatives (**8a-28a** and **16b-28b**) from intermediate **7a** or **7b** are depicted in **Schemes 3**. Compound **8a** and **9a** were synthesized from **7a** by reductive amination with corresponding aldehydes. Intermediate **7a** condensed with various arylformic acids using HBTU to yield compounds **10a-12a**. Intermediate **7a** also reacted with triphosgene and various arylamines to give **13a-15a** [29]. Compounds **16a-28a** and

16b-28b were prepared from intermediate **7a** or **7b** by acylation with ethyl oxalyl monochloride, hydrolysis with aqueous LiOH [30,31] and condensation with arylamines in sequence.

Scheme 3

3. Result and discussion

3.1. PTP1B enzyme inhibitory activities and structure activity relationships

We have already realized that branching especially with big and long chains could change the stereochemistry environment of linear bisarylethenesulfonic acid esters, and construct richer and differentiated interactions with PTP1B, endowing these molecules with potent and to some extent selective PTP1B inhibition. In fact, a more effective strategy to change stereochemistry environment of molecules is to design stereoisomers. For the convenience of chemical synthesis, we took pyrrolidine-3,4-bisarylethenesulfonic acid esters as the core scaffold for discovering potent and selective PTP1B inhibitors. Pyrrolidine-3,4-bisarylethenesulfonic acid esters including *cis*- and *trans*-stereoisomers are easily branched by alkylation and acylation at the 1-amino. Four kinds of linkers, which are methylene, carbonyl, amylacetyl, and amyloxalyl, were investigated in this presentation. As shown in the **Table 1**, all pyrrolidine bisarylethenesulfonic acid esters, whichever the linker is, were more potent than their parent molecule (i.e. compound **7a**, $IC_{50} = 16.8 \mu M$), indicating that branched chains of bisarylethenesulfonic acid esters can provide additional interactions with PTP1B. Consistent with our previous research, bigger or longer hydrophobic chains contributed to more significant inhibitory activity (e.g. compound group **6a/8a/9a**, **13a/14a/15a**, and **16a/17a/18a**). In addition, compound **6a**, **10a**, **13a**, and **16a** showed gradual increasing PTP1B inhibitory activity ($IC_{50} = 9.34$, 7.81, 3.51 and 2.52 μM respectively), and similar phenomena were also observed in compound group **8a/11a/14a/17a** and **9a/12a/15a/18a**, suggesting that amyloxalyl is an active linker for high PTP1B inhibitory activity. Therefore, both linker's structure and hydrophobic surface area of branched chains influenced the PTP1B inhibitory activity of bisarylethenesulfonic acid esters.

Table 1

With amyloxalyl as the optimized linker, more pyrrolidine bisarylethenesulfonic acid esters were subsequently designed, and their *cis*- and *trans*-isomers were investigated by pairwise comparison. As shown in **Table 2**, both for *trans*- and *cis*-derivatives, those with bigger or longer hydrophobic chains exhibited more potent PTP1B inhibitory activity, e.g., the IC₅₀s of compound pair **16a/16b**, **17a/17b** and **18a/18b** followed the sequences of **16a** > **17a** > **18a** and **16b** > **17b** > **18b**. Also, the activity of compound pair **26a/26b**, **27a/27b** and **28a/28b** increased in sequence with the increase of hydrophobic groups. With the biggest hydrophobic chains in this research, biphenyloxyphenyl derivatives (**24a/24b**) and dodecylphenyl derivatives (**28a/28b**) provided the most potent PTP1B inhibition. Nevertheless, more importantly, all of the *trans*- derivatives showed folds higher PTP1B inhibitory activity than their *cis*-isomers without any exception. In the cases of compound pair **26a/26b**, **27a/27b** and **28a/28b**, their differences in activity between two stereoisomers became more and more significant with the increase of hydrophobic groups (the ratio of IC₅₀s are 2.5, 4.3 and 6.9, respectively). These clearly indicated that *trans*-pyrrolidine bisarylethenesulfonic acid esters have more potent binding affinity with PTP1B than the *cis*-isomers, and branching plus stereoisomerisation of bisarylethenesulfonic acid esters is an effective way to discover potent PTP1B inhibitors.

Table 2

3.2. Selectivity over TCPTP, SHP2 and membrane permeability

Another question we were eager to know was that if stereoisomeric pyrrolidine bisarylethenesulfonic acid esters particularly those *trans*-isomers could interact differentially with PTPs, and show PTP1B selectivity. For those compound pairs in which *trans*-isomers' IC₅₀s were below 300 nM against PTP1B (i.e. **19a/19b**, **24a/24b**, **27a/27b** & **28a/28b**) and some individual compound pairs (**7a/7b** & **16a/16b**), we further evaluated their inhibitory activity against TCPTP and SHP2. As shown in **Table 3**, these stereoisomeric molecules showed different inhibitory activity against

the three PTPs, but more significant selectivity for PTP1B over TCPTP and SHP2 was observed in the *trans*-derivatives.

Similar to the compound **II** (Fig. 1), nearly no PTP1B selectivity was observed in compound pair **7a/7b**. Compound **16a**, in comparison to its *cis*-derivative (**16b**), just demonstrated very weak PTP1B selectivity. Nevertheless, the situation changed quickly with the increase of hydrophobic groups' size. Compound **19a**, **24a** and **27a** showed 8.5-12.7 folds selectivity for PTP1B over TCPTP and SHP2, and their *cis*-isomers (**19b**, **24b** & **27b**) showed 2.8-6.5 folds PTP1B selectivity. Compound **28a** showed 16.2 and 22.2 folds selectivity for PTP1B over TCPTP and SHP2 respectively, while its *cis*-derivative (**28b**) showed 3.5-5.5 folds PTP1B selectivity over other 2 PTPs. Compared with the precursors without stereoisomerism (compound **I** & **II**, Fig. 1), *trans*-pyrrolidine bisarylethenesulfonic acid esters could provide PTP1B selectivity more effectively. Therefore, branching plus stereoisomerisation of bisarylethenesulfonic acid esters is a more effective strategy to discover selective PTP1B inhibitors as well as potent ones.

In addition, these compounds were evaluated in a parallel artificial membrane permeability assay (PAMPA) which could predict the membrane permeability of small molecules [32,33]. Atenolol and propranolol, representing medium and high membrane permeability respectively, were used as validity controls to monitor the consistency of the assay. The permeability rates (P_{app}) of these tested compounds were listed in Table 3. All tested compounds showed sufficient membrane permeability ($P_{app} = 0.87$ to 2.26×10^{-6} cm/s, superior to atenolol), indicating that these stereoisomeric pyrrolidine bisarylethenesulfonic acid esters have great potential in penetrating cellular membrane.

Table 3

3.3. Molecular docking

To understand the binding mode of stereoisomeric pyrrolidine bisarylethenesulfonic acid esters and explain the difference of PTP1B activity between *cis*- and *trans*-stereoisomers, we performed the molecular docking for compound **28a** and **28b**.

As illustrated in **Fig. 2**, one arylenesulfonic acid ester chain of both stereoisomers stretches deep into A site (the catalytic binding site of PTP1B), constructing multiple interactions including hydrogen bonds and π - π stacking reactions (with Y47). Specifically, eight hydrogen bonds with S217, G219, I220, G221 and R222 are formed by **28a**, while five hydrogen bonds with S217, G221 and R222 are formed in the case of **28b**. The second sulfonic acid ester chain of **28a** stretches into B site and forms two hydrogen bonds with R25. By contrast, the second sulfonic acid ester chain of **28b** cannot stretch into B site due to the configuration limitation. Instead, it expands to the E site without definite interactions. In addition, both in **28a** and **28b**, the hydrophobic chain introduced by means of a linker (amyloxalyl) stretches into C site, forming multiple hydrophobic interactions (with R48 and K42 for **28a**, and with K37 and L38 for **28b** respectively). The linker in the case of **28b** also forms hydrogen bonds with R48.

From above analysis, newly introduced hydrophobic branches did bring additional interactions with PTP1B compared with the non-substituted molecules (**7a** & **7b**). That is why they can greatly enhance the PTP1B activity of pyrrolidine bisarylenesulfonic acid esters. On the other hand, the configurations of *cis*-pyrrolidine bisarylenesulfonic acid esters limit their interactions with the target protein, particularly the second sulfonic acid ester chain. As the result, *cis*-isomers of pyrrolidine bisarylenesulfonic acid esters are less potent than their *trans*-isomers. As a class of potential ABC type PTP1B inhibitors, *trans*-pyrrolidine bisarylenesulfonic acid ester derivatives may interact with multiple secondary binding sites in addition to the catalytic binding site of PTP1B, which can also explain why they have better PTP1B selectivity than their *cis*-isomers and precursors without stereoisomerism.

Fig. 2

3.4. Effects of selected PTP1B inhibitors on cell viability

To determine the non-toxic concentrations of stereoisomeric bisarylenesulfonic acid esters, we selected four compounds (**24a**, **24b**, **28a** and **28b**) and evaluated their

effects on HepG2 and A549 cell viability by means of CCK8 assay. As shown in **Fig. 3**, all of the tested compounds showed no inhibitory effect on HepG2 and A549 cells at 1.56 μ M. From 6.25 μ M to 100 μ M, inhibitory effects on HepG2 were observed in a dose dependent manner, but the HepG2 cell viability was still over 75% even at 100 μ M of tested drugs. Three of the four tested compounds (**24a**, **24b** & **28b**) can hardly inhibit the viability of A549 cell at 100 μ M, and only less than 10% cell viability was inhibited by compound **28a** at 100 μ M which is over 800 folds of the IC_{50} value. These results indicated that the newly found PTP1B inhibitors have no significant cytotoxicity.

Fig. 3

3.5. Effects of selected PTP1B inhibitors on insulin-stimulated glucose uptake

It has been proved that PTP1B inhibition results in an improvement in insulin sensitivity and glucose metabolism by inhibiting insulin signaling pathways [34]. To test this effect with newly obtained PTP1B inhibitors, we evaluated compounds **24a** and **28a** on 2-NBDG uptake in HepG2 cell. As shown in **Fig. 4**, insulin-stimulated glucose uptake in HepG2 cells was increased by Pioglitazone which was used as the positive control, and the increased percentages were 51.1%, 70.2%, and 78.9% at the concentrations of 5, 10 and 20 μ M respectively. The glucose uptake in HepG2 cells was also significantly increased by treatment with tested compounds (**24a** and **28a**). The increased percentages were 49.1%, 60.5% and 65.1% for **24a**, and 59.1%, 83.4% and 81.1% for **28a** at 5, 10 and 20 μ M respectively. The latter was better than the former, and even slightly better than the positive control. The better effect of compound **28a** on 2-NBDG uptake was possibly related to its stronger PTP1B inhibitory activity (two folds potent than compound **24a**).

Fig. 4

3.6. Effect of compound 28a on phosphorylation of insulin receptor β (IR β)

Previous researches have suggested a critical role of the insulin receptor β (IR β) in insulin-mediated signaling pathways. PTP1B directly inactivates IR β by

dephosphorylating tyrosine residues, and attenuate insulin signaling. Inhibition of PTP1B by inhibitors can improve the phosphorylation level of IR β [35]. Thus we further evaluated the effect of compound **28a** on the phosphorylation level of IR β in HepG2 cell. As shown in **Fig. 5**, compound **28a** dose-dependently increased the expression of phosphorylated IR β (p-IR β) of HepG2 cells at concentrations of 1 μ M, 2 μ M and 5 μ M, and the relative density of p-IR β to β -actin which was used as internal control was increased by 15%, 73% and 308% respectively. These results demonstrated that **28a**, as a new PTP1B inhibitor, could induce insulin signaling on the cellular level.

Fig. 5

4. Conclusions

In this study, a series of novel stereoisomeric pyrrolidine bisarylethenesulfonic acid ester derivatives were systematically investigated as potential highly potent and selective PTP1B inhibitors. Some of *trans*-pyrrolidine bisarylethenesulfonic acid esters demonstrated potent and selective PTP1B inhibitory activity over TCPTP and SHP2 as well as good membrane permeability. *Trans*-derivative **28a** with the most potent PTP1B activity ($IC_{50} = 120$ nM) provided significantly improved PTP1B selectivity (16 and 22 folds over TCPTP and SHP2 respectively) compared with the precursors without stereoisomerism. Molecular docking calculated the binding mode and explained the reasons for better inhibitory activity and selectivity of *trans*-pyrrolidine bis-arylethenesulfonic acid esters. We also tested and verified the effects of compound **28a** on insulin-stimulated glucose uptake and insulin-mediated insulin receptor β (IR β) phosphorylation. This study indicated that branching and stereoisomerisation of bisarylethenesulfonic acid esters is a more effective strategy to discover potent, selective and membrane permeable PTP1B inhibitors.

5. Experimental section

5.1. Chemistry

5.1.1 General synthetic methods

All chemicals and solvents were obtained from commercial sources and purified using standard methods according to the need. And the solvents used were redistilled and dried by standard procedures whenever required.

Melting points were recorded on RY-1Gmeltingpoint apparatus and are uncorrected. All reactions were monitored by thin-layer chromatography (TLC) on silica gel plates. Column chromatography was performed on silica gel 200-300 mesh. ^1H NMR (400 MHz) and ^{13}C NMR (100 MHz) was recorded on a Bruker 400 MHz. The spin multiplicities are indicated by the symbols, s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and brs (broad singlet). The chemical shifts were given in δ (ppm) refer to the signal of CDCl_3 (δ 7.26, ^1H NMR and δ 77.00, ^{13}C NMR) and the signal of $(\text{CD}_3)_2\text{SO}$ (δ 2.54, ^1H NMR and δ 39.52, ^{13}C NMR). Chemical shift values are given in parts per million and coupling constants (J) in Hertz. High resolution mass spectroscopy was conducted using Agilent 6230 LC-MS. Optical rotations were measured with a SPSI SGW-1 polarimeter.

5.1.2 (3R,4R)-1-benzyl-3,4-dihydropyrrolidine-2,5-dione (**2a**)

To a suspension of L(+)-tartaric acid (**1a**) (20 g, 134 mmol) in 200 mL oxylene was added benzylamine (14.4 g, 134 mmol). The reaction was then heated to reflux for 5 h. The reaction mixture was cooled to room temperature, evaporated in vacuum and then washed with DCM to afford **2a** as light yellow solid (24.2 g, 85%). M.p.: 197-200 °C. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 4.43 (d, J = 5.2 Hz, 2H), 4.60 (dd, J = 8.0 Hz, 14.4 Hz, 2H), 6.35 (d, J = 5.2 Hz, 2H), 7.28-7.37 (m, 5H); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 41.2, 74.5, 127.5, 127.5, 128.6, 136.0, 174.6; HRMS (ESI) m/z calcd for $\text{C}_{11}\text{H}_{11}\text{NO}_4$ $[\text{M}+\text{Na}]^+$ 244.0580, found 244.0582. $[\alpha]_{23}^{\text{D}}$ = +144.2 (c = 0.86, MeOH).

5.1.3 (3S,4S)-1-benzylpyrrolidine-3,4-diol (**3a**)

To a suspension of LiAlH_4 (5.1 g, 135 mmol) in 200 mL dry THF was added dropwise a solution of (3R,4R)-1-benzyl-3,4-dihydropyrrolidine-2,5-dione (**2a**) (10 g, 45 mmol) in 300 mL dry THF at 0 °C. The reaction was then heated to reflux for another 24 h. The reaction mixture was cooled to 0 °C and quenched with H_2O (5.1

mL) / 15% NaOH (5.1 mL) / H₂O (15.3 mL) in turn, followed by the addition of 300 mL EtOAc. After stirring at R.T. for 15 min, the obtained suspension was filtered on a pad of celite (washing with EtOAc) and the filtrate was evaporated in vacuum, purified by flash chromatography (DCM:MeOH = 30:1) to afford **3a** as light brown solid (6.2 g, 73%). M.p.: 94-96 °C. ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.34 (dd, *J* = 4.4 Hz, 9.6 Hz, 2H), 2.77-2.81 (m, 2H), 3.49-3.63 (m, 2H), 3.89 (t, *J* = 4.0 Hz, 2H), 4.89 (brs, 2H), 7.25-7.36 (m, 5H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 60.0, 60.8, 77.6, 126.8, 128.1, 128.6, 139.0; HRMS (ESI) *m/z* calcd for C₁₁H₁₅NO₂ [M+H]⁺ 194.1176, found 194.1178. [α]₂₀^D = +7.2 (*c* = 0.56, CHCl₃).

5.1.4 (3*S*,4*S*)-1-benzylpyrrolidine-3,4-diyl dimethanesulfonate (**4a**)

To a solution of (3*S*,4*S*)-1-benzylpyrrolidine-3,4-diol (**3a**) (6 g, 31 mmol) and Et₃N (12.8 mL, 93 mmol) in 200 mL dry DCM was added dropwise a solution of methanesulfonic anhydride (12.9 g, 74 mmol) in 100 mL dry DCM at 0 °C. The reaction was allowed to warm at R.T. for 6 h. The reaction mixture was washed with saturated aqueous NaHCO₃, 5% HCl and brine in sequence, dried over anhydrous Na₂SO₄, evaporated in vacuum, and purified by flash chromatography (petroleum ether:EtOAc = 3:1) to afford **4a** as light yellow oil (8.5 g, 78%). ¹H NMR (400 MHz, CDCl₃) δ 2.75 (dd, *J* = 4.0 Hz, 11.2 Hz, 2H), 3.05 (s, 6H), 3.07-3.11 (m, 2H), 3.60-3.67 (m, 2H), 5.12 (t, *J* = 4.0 Hz, 2H), 7.26-7.34 (m, 5H); ¹³C NMR (100 MHz, CDCl₃) δ 38.3, 57.8, 59.1, 82.4, 127.5, 128.5, 128.7, 136.7; HRMS (ESI) *m/z* calcd for C₁₃H₁₉NO₆S₂ [M+H]⁺ 350.0727, found 350.0732. [α]₂₀^D = +24.6 (*c* = 0.56, CHCl₃).

5.1.5 4,4'-(((3*R*,4*R*)-1-benzylpyrrolidine-3,4-diyl)bis(oxy))dibenzaldehyde (**5a**)

A mixture of (3*S*,4*S*)-1-benzylpyrrolidine-3,4-diyl dimethanesulfonate (**4a**) (7 g, 20 mmol), *p*-hydroxybenzaldehyde (5.4 g, 44 mmol), KI (0.3 g, 2 mmol) and K₂CO₃ (8.6 g, 60 mmol) in 20 mL DMF was heated to 100 °C for 8 h. The reaction mixture was cooled to room temperature, diluted with H₂O and then extracted with EtOAc. The combined organic layers were washed with brine and dry over anhydrous Na₂SO₄. The solvent was removed to afford the crude product. It was purified by flash

chromatography (petroleum ether:EtOAc = 6:1) to afford **5a** as light yellow oil (4.0 g, 44%). ¹H NMR (400 MHz, CDCl₃) δ 2.81 (dd, *J* = 4.0 Hz, 10.4 Hz, 2H), 3.24 (dd, *J* = 6.0 Hz, 10.4 Hz, 2H), 3.67-3.75 (m, 2H), 4.97 (t, *J* = 4.0 Hz, 2H), 7.01 (d, *J* = 8.4 Hz, 4H), 7.26-7.33 (m, 5H), 7.81 (d, *J* = 8.4 Hz, 4H), 9.87 (s, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 58.1, 59.7, 81.9, 115.5, 127.4, 128.4, 128.8, 130.4, 132.1, 137.2, 162.1, 190.6; HRMS (ESI) *m/z* calcd for C₂₅H₂₃NO₄ [M+H]⁺ 402.1700, found 402.1710. [α]_D²¹ = -45.1 (c = 0.11, CHCl₃).

5.1.6 diethyl 2,2'-((((3R,4R)-1-benzylpyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1E,1'E)-bis(ethene-1-sulfonate) (**6a**)

To a solution of 4,4'-((((3R,4R)-1-benzylpyrrolidine-3,4-diyl)bis(oxy))dibenzaldehyde (**5a**) (3.6 g, 8.2 mmol) and ethyl (diethoxyphosphoryl) methanesulfonate (4.5 g, 19.3 mmol) in 100 mL dry THF, NaH (60% in mineral oil) (1.3 g, 33 mmol) was added in small portions at 0 °C. Then the reaction was stirred at room temperature for 6 h. TLC showed the reaction was completed. The reaction mixture was quenched with saturated aqueous NH₄Cl, and extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, concentrated under vacuum, and purified by flash chromatography (petroleum ether:EtOAc = 4:1) to afford **6a** as white solid (4.5 g, 92%). M.p.: 108-110 °C. ¹H NMR (400 MHz, CDCl₃) δ 1.35 (t, *J* = 7.2 Hz, 6H), 2.76 (dd, *J* = 2.8 Hz, 10.4 Hz, 2H), 3.18 (dd, *J* = 5.6 Hz, 10.4 Hz, 2H), 3.63-3.72 (m, 2H), 4.17 (q, *J* = 7.2 Hz, 4H), 4.87-4.89 (m, 2H), 6.56 (d, *J* = 15.6 Hz, 2H), 6.91 (d, *J* = 8.4 Hz, 4H), 7.23-7.30 (m, 5H), 7.40 (d, *J* = 8.4 Hz, 4H), 7.48 (d, *J* = 15.6 Hz, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 14.8, 58.1, 59.6, 66.6, 81.7, 115.8, 118.7, 125.1, 127.3, 128.4, 128.8, 130.4, 137.2, 144.0, 159.7; HRMS (ESI) *m/z* calcd for C₃₁H₃₅NO₈S₂ [M+H]⁺ 614.1877, found 614.1888. [α]_D²¹ = -19.3 (c = 0.25, CHCl₃).

5.1.7 diethyl

2,2'-((((3R,4R)-pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1E,1'E)-bis(ethane-1-sulfonate) (**7a**)

To a solution of diethyl 2,2'-((((3R,4R)-1-benzylpyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1E,1'E)-bis(ethene-1-sulfonate) (**6a**) (4.0 g, 6.5 mmol) in 100 mL DCM was added 1-chloroethyl chloroformate (1.4 g, 9.75 mmol). The mixture was heated to reflux for 4 h before concentration to move the solvent under vacuum. The residue was dissolved in 100 mL MeOH, and allowed to heat to reflux for 1 h. The reaction mixture was cooled to room temperature, and concentrated to afford the crude product **7a** as brown semi-solid (3.3 g, 95%) which was used for next step without further purification. ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.33 (t, *J* = 7.2 Hz, 6H), 3.55-3.58 (m, 2H), 3.77-3.79 (m, 2H), 4.21 (q, *J* = 7.2 Hz, 4H), 5.36 (d, *J* = 3.2 Hz, 2H), 7.15 (d, *J* = 8.4 Hz, 4H), 7.37 (d, *J* = 15.6 Hz, 2H), 7.61 (d, *J* = 15.6 Hz, 2H), 7.83 (d, *J* = 8.4 Hz, 4H), 9.72 (s, 2H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 14.7, 48.7, 66.9, 78.3, 116.2, 120.3, 126.2, 131.0, 143.4, 158.1; HRMS (ESI) *m/z* calcd for C₂₄H₂₉NO₈S₂ [M+H]⁺ 524.1407, found 524.1404.

5.1.8 (3R,4S)-1-benzyl-3,4-dihydroxypyrrolidine-2,5-dione (**2b**)

To a suspension of *cis*-butenedioic anhydride (**1b**) (49 g, 0.5 mol) in 600 mL AcOH was added benzylamine (54 g, 0.5 mmol). The reaction was then heated to reflux for 5 h. The reaction mixture was concentrated and the residue was dissolved in 300 mL EtOAc. The EtOAc layer was washed with saturated aqueous NaHCO₃ and brine. The organic layer was dried over anhydrous Na₂SO₄, concentrated in vacuum, purified by flash chromatography (petroleum ether:EtOAc = 8:1) to afford 1-benzyl-1H-pyrrole-2,5-dione as a white solid (26.9g, 29%). M.p.: 58-60 °C. ¹H NMR (400 MHz, CDCl₃) δ 4.65 (s, 2H), 6.68 (s, 2H), 7.24-7.30 (m, 5H); ¹³C NMR (100 MHz, CDCl₃) δ 41.4, 127.8, 128.3, 128.7, 134.2, 136.1, 170.4; HRMS (ESI) *m/z* calcd for C₁₁H₉NO₂ [M+H]⁺ 188.0706, found 188.0709.

A stock solution containing Mn(ClO₄)₂•6H₂O (220 mg, 0.6 mmol) and pyridine-2-carboxylic acid (450 mg, 3.6 mmol) in 200 mL acetone was prepared. 120

mL this stock solution (360 μmol $\text{Mn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$, 0.3 mol%, and 2.2 mmol pyridine-2-carboxylic acid, 1.8 mol%) was added to the solution of 1-benzyl-pyrrole-2,5-dione (22.6 g, 120 mmol) in 240 mL acetone while stirring the mixture at room temperature. After addition of 6 mL of a 0.6 M aqueous NaOAc (3.6 mmol, 3.0 mol%), the mixture was cooled to 0 °C, and to it H_2O_2 (30 wt% in water, 22.7 mL, 240 mmol, 2.0equiv.) was added dropwise. After stirring for 16 h at 0 °C, the mixture was allowed to warm to room temperature, and excess solid NaHSO_3 was added to remove residual peroxides (verified by peroxide test strips). The mixture was filtered, and the filtrate was concentrated to give **2b** as a white solid (26.5 g, 99%). M.p.: 122-124 °C. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 4.48 (s, 2H), 4.60 (s, 2H), 6.10 (brs, 2H), 7.28-7.36 (m, 5H); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 41.0, 68.1, 127.5, 127.5, 128.5, 136.0, 176.4; HRMS (ESI) m/z calcd for $\text{C}_{11}\text{H}_{11}\text{NO}_4$ $[\text{M}+\text{Na}]^+$ 244.0580, found 244.0583.

5.1.9 (3S,4R)-1-benzylpyrrolidine-3,4-diol (**3b**)

According to the procedure described for **3a**, **2b** (25 g, 113 mmol) was treated with LiAlH_4 (12.5 g, 330 mmol) to afford **3b** (7.2 g, 33%) as brown oil. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) δ 3.07 (d, $J = 8.4$ Hz, 2H), 3.39 (d, $J = 8.4$ Hz, 2H), 4.19 (s, 2H), 4.38 (s, 2H), 5.56 (s, 2H), 7.45(s, 3H), 7.65-7.66 (m, 2H); ^{13}C NMR (100 MHz, $\text{DMSO}-d_6$) δ 56.2, 59.2, 69.4, 128.8, 129.3, 130.7, 131.3; HRMS (ESI) m/z calcd for $\text{C}_{11}\text{H}_{15}\text{NO}_2$ $[\text{M}+\text{H}]^+$ 194.1176, found 194.1178.

5.1.10 (3S,4R)-1-benzylpyrrolidine-3,4-diyl dimethanesulfonate (**4b**)

According to the procedure described for **4a**, **3b** (7 g, 36 mmol) was treated with Et_3N (14.9 mL, 108 mmol) and methanesulfonic anhydride (15 g, 86 mmol) to afford **4b** (9.2 g, 72%) as brown oil. ^1H NMR (400 MHz, CDCl_3) δ 2.78-2.81 (m, 2H), 3.06 (s, 6H), 3.16-3.20 (m, 2H), 3.68 (s, 2H), 5.07 (s, 2H), 7.24-7.31 (m, 5H); ^{13}C NMR (100 MHz, CDCl_3) δ 38.4, 56.6, 59.6, 75.7, 127.5, 128.5, 128.6, 137.3; HRMS (ESI) m/z calcd for $\text{C}_{13}\text{H}_{19}\text{NO}_6\text{S}_2$ $[\text{M}+\text{H}]^+$ 350.0727, found 350.0729.

5.1.11 4,4'-(((3*S*,4*R*)-1-benzylpyrrolidine-3,4-diyl)bis(oxy))dibenzaldehyde (5b)

A mixture of (3*S*,4*R*)-1-benzylpyrrolidine-3,4-diyl dimethanesulfonate (**4b**) (8 g, 23 mmol), *p*-hydroxybenzaldehyde (5.6 g, 46 mmol), KI (0.3 g, 2 mmol) and K₂CO₃ (5.7 g, 40 mmol) in 25 mL DMF was heated to 100 °C for 4 h. The reaction mixture was cooled to room temperature, diluted with H₂O and then extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated to afford the crude product which was purified by flash chromatography (petroleum ether:EtOAc = 5:1) to afford (3*S*,4*S*)-1-benzyl-4-(4-formylphenoxy)pyrrolidin-3-yl methanesulfonate as light yellow oil (5.3 g, 62%). ¹H NMR (400 MHz, CDCl₃) δ 2.66-2.69 (m, 1H), 2.83-2.86 (m, 1H), 2.97 (s, 3H), 3.06-3.19 (m, 2H), 3.51-3.73 (m, 2H), 4.93 (s, 1H), 5.11 (s, 1H), 6.95 (d, *J* = 8.4 Hz, 2H), 7.24-7.27 (m, 5H), 7.77 (d, *J* = 8.4 Hz, 2H), 9.81 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 38.1, 57.2, 57.5, 59.2, 81.0, 83.0, 115.3, 127.2, 128.2, 128.5, 130.3, 131.8, 137.0, 161.6, 190.4; HRMS (ESI) *m/z* calcd for C₁₉H₂₁NO₅S [M+H]⁺ 376.1213, found 376.1220.

A mixture of (3*S*,4*S*)-1-benzyl-4-(4-formylphenoxy)pyrrolidin-3-yl methanesulfonate (5 g, 13 mmol), *p*-hydroxybenzaldehyde (3.2 g, 26 mmol), KI (0.45 g, 3 mmol) and Cs₂CO₃ (6.5 g, 20 mmol) in 20 mL DMF was heated to 120 °C for 24 h. The reaction mixture was cooled to room temperature, diluted with H₂O and then extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄ and concentrated to afford the crude product. It was purified by flash chromatography (petroleum ether:EtOAc = 3:1) to afford **5b** as light yellow oil (1.6 g, 30%). ¹H NMR (400 MHz, CDCl₃) δ 2.82-2.84 (m, 2H), 3.36-3.38 (m, 2H), 3.75 (s, 2H), 5.02 (s, 2H), 6.88 (d, *J* = 8.4 Hz, 4H), 7.26-7.33 (m, 5H), 7.74 (d, *J* = 8.4 Hz, 4H), 9.84 (s, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 57.7, 59.9, 75.1, 115.4, 127.4, 128.4, 128.8, 130.2, 131.8, 137.5, 162.8, 190.6; HRMS (ESI) *m/z* calcd for C₂₅H₂₃NO₄ [M+H]⁺ 402.1700, found 402.1707.

5.1.12*diethyl*

2,2'-((((3*S*,4*R*)-1-benzylpyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(

ethene-1-sulfonate) (6b)

According to the procedure described for **6a**, **6b** (2.1 g, 93%) was prepared from **5b** (1.5 g, 3.8 mmol) as brown semi-solid. The crude product was used for the next step without further purification.

5.1.13*diethyl*

2,2'-((((3*R*,4*R*)-pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethane-1-sulfonate) (**7b**)

According to the procedure described for **7a**, **7b** (1.3 g, 61%) was prepared from **6b** (2.0 g, 3.5 mmol) as brown semi-solid via 2 steps. ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.32 (t, *J* = 7.2 Hz, 6H), 3.51-3.53 (m, 2H), 3.74-3.76 (m, 2H), 4.19 (q, *J* = 7.2 Hz, 4H), 5.46 (s, 2H), 7.08 (d, *J* = 8.4 Hz, 4H), 7.35 (d, *J* = 15.6 Hz, 2H), 7.56 (d, *J* = 15.6 Hz, 2H), 7.78 (d, *J* = 8.4 Hz, 4H), 9.46 (s, 1H), 9.71 (s, 1H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 14.7, 66.9, 96.6, 109.6, 115.9, 120.0, 125.8, 128.3, 130.9, 143.5; HRMS (ESI) *m/z* calcd for C₂₄H₂₉NO₈S₂ [M+H]⁺ 524.1407, found 524.1403.

5.1.14 General procedure for 8a and 9a:

To a stirred solution of diethyl 2,2'-((((3*R*,4*R*)-pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethane-1-sulfonate) (**7a**) (1.0 equiv.), substituted phenylaldehyde (1.2 equiv.) and NaBH(OAc)₃ (3 equiv.) in DCM under argon at room temperature was added catalytic amount of acetic acid. The reaction mixture was stirred at room temperature for 12 h before concentration. The residue was dissolved in EtOAc and washed with saturated aqueous NaHCO₃ and brine, dried over anhydrous Na₂SO₄, concentrated, and purified by flash chromatography (petroleum ether: EtOAc = 3:1) to afford the target product.

5.1.14.1*diethyl*

2,2'-((((3*R*,4*R*)-1-(4-phenoxybenzyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**8a**)

White semi-solid (74%). ¹H NMR (400 MHz, CDCl₃) δ 1.37 (t, *J* = 7.2 Hz, 6H),

2.76 (dd, $J = 2.8$ Hz, 10.4 Hz, 2H), 3.18-3.22 (m, 2H), 3.62-3.70 (m, 2H), 4.18 (q, $J = 7.2$ Hz, 4H), 4.90 (s, 2H), 6.59 (d, $J = 15.6$ Hz, 2H), 6.93-6.99 (m, 8H), 7.08-7.10 (m, 1H), 7.25-7.34 (m, 4H), 7.41-7.52 (m, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.8, 58.0, 59.0, 66.6, 81.7, 115.8, 118.6, 118.8, 118.9, 123.3, 125.2, 129.7, 130.1, 130.4, 132.1, 143.9, 156.5, 157.0, 159.8; HRMS (ESI) m/z calcd for $\text{C}_{37}\text{H}_{39}\text{NO}_9\text{S}_2$ $[\text{M}+\text{H}]^+$ 706.2139, found 706.2146. $[\alpha]_{21}^{\text{D}} = -18.1$ ($c = 0.36$, CHCl_3).

5.1.14.2

diethyl

2,2'-((((3R,4R)-1-(4-(4-isopropylphenoxy)benzyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1E,1'E)-bis(ethene-1-sulfonate) (**9a**)

White semi-solid (78%). ^1H NMR (400 MHz, CDCl_3) δ 1.24 (d, $J = 6.4$ Hz, 6H), 1.37 (t, $J = 7.2$ Hz, 6H), 2.76 (dd, $J = 2.8$ Hz, 10.4 Hz, 2H), 2.86-2.91 (m, 1H), 3.18-3.22 (m, 2H), 3.62-3.70 (m, 2H), 4.18 (q, $J = 7.2$ Hz, 4H), 4.87-4.89 (m, 2H), 6.59 (d, $J = 15.6$ Hz, 2H), 6.93-6.95 (m, 8H), 7.18 (d, $J = 8.0$ Hz, 2H), 7.25 (d, $J = 8.0$ Hz, 2H), 7.43 (d, $J = 8.4$ Hz, 4H), 7.51 (d, $J = 15.6$ Hz, 2H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.8, 24.0, 33.4, 58.0, 59.0, 66.5, 81.8, 115.8, 118.2, 118.8, 118.9, 125.2, 127.5, 130.0, 130.3, 131.7, 143.9, 144.0, 154.7, 156.9, 159.7; HRMS (ESI) m/z calcd for $\text{C}_{40}\text{H}_{45}\text{NO}_9\text{S}_2$ $[\text{M}+\text{H}]^+$ 748.2608, found 748.2621. $[\alpha]_{21}^{\text{D}} = -17.9$ ($c = 0.53$, CHCl_3).

5.1.15 General procedure for 10a-12a:

To a stirred solution of diethyl 2,2'-((((3R,4R)-pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1E,1'E)-bis(ethane-1-sulfonate) (**7a**) (1.0 equiv.) and HBTU (1.2 equiv.) in DCM under argon at room temperature were added substituted benzoic acid (1.2 equiv.) and DMAP (1.5 equiv.) sequentially. The reaction mixture was stirred at room temperature for 24 h before concentration. The residue was dissolved in EtOAc, washed with 5% HCl, saturated aqueous NaHCO_3 and brine, dried over anhydrous Na_2SO_4 , concentrated, and purified by flash chromatography (petroleum ether:EtOAc:DCM = 4:1:1) to afford the target product.

5.1.15.1

diethyl

2,2'-((((3*R*,4*R*)-1-benzoylpyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**10a**)

White solid (45%). M.p.: 79-81 °C. ¹H NMR (400 MHz, CDCl₃) δ 1.37 (t, *J* = 7.2 Hz, 6H), 3.75 (d, *J* = 10.8 Hz, 1H), 3.97-4.10 (m, 2H), 4.16-4.24 (m, 5H), 4.97-5.03 (m, 2H), 6.58-6.65 (m, 2H), 6.92 (dd, *J* = 8.4 Hz, 28.0 Hz, 4H), 7.38-7.56 (m, 11H); ¹³C NMR (100 MHz, CDCl₃) δ 14.9, 49.8, 52.5, 66.7, 78.8, 115.9(2), 119.5(2), 126.0, 127.2, 128.4, 130.4, 130.5, 135.7, 143.6(2), 158.5(2), 170.3; HRMS (ESI) *m/z* calcd for C₃₁H₃₃NO₉S₂ [M+H]⁺ 628.1669, found 628.1689. [α]₂₂^D = -8.2 (*c* = 0.18, CHCl₃).

5.1.15.2

diethyl

2,2'-((((3*R*,4*R*)-1-(4-phenoxybenzoyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**11a**)

White solid (42%). M.p.: 84-86 °C. ¹H NMR (400 MHz, CDCl₃) δ 1.39 (t, *J* = 7.2 Hz, 6H), 3.80 (d, *J* = 10.8 Hz, 1H), 3.98 (d, *J* = 10.8 Hz, 1H), 3.98-4.12 (m, 2H), 4.20-4.22 (m, 4H), 4.99 (d, *J* = 12.0 Hz, 2H), 6.60-6.64 (m, 2H), 6.88-7.04 (m, 8H), 7.17 (t, *J* = 7.2 Hz, 1H), 7.37 (t, *J* = 8.4 Hz, 2H), 7.45-7.55 (m, 8H); ¹³C NMR (100 MHz, CDCl₃) δ 14.9, 49.9, 52.6, 66.7, 78.8, 115.9, 117.7, 119.6, 119.7, 124.3, 126.0, 129.4, 129.9, 130.0, 130.5, 143.6, 155.8, 158.6(2), 159.6, 169.7; HRMS (ESI) *m/z* calcd for C₃₇H₃₇NO₁₀S₂ [M+H]⁺ 720.1932, found 720.1943. [α]₂₁^D = -7.7 (*c* = 0.77, CHCl₃).

5.1.15.3

diethyl

2,2'-((((3*R*,4*R*)-1-(4-(4-isopropylphenoxy)benzoyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**12a**)

White solid (55%). M.p.: 81-83 °C. ¹H NMR (400 MHz, CDCl₃) δ 1.23 (d, *J* = 6.8 Hz, 6H), 1.35 (t, *J* = 7.2 Hz, 6H), 2.83-2.91 (m, 1H), 3.79 (d, *J* = 10.8 Hz, 1H), 3.96 (d, *J* = 10.8 Hz, 1H), 3.98-4.12 (m, 2H), 4.17-4.19 (m, 4H), 5.00 (d, *J* = 12.0 Hz, 2H), 6.60-6.64 (m, 2H), 6.91-6.95 (m, 8H), 7.18-7.20 (m, 2H), 7.47-7.52 (m, 8H); ¹³C NMR (100 MHz, CDCl₃) δ 14.7, 23.9, 33.3, 49.8, 52.4, 66.6, 78.7, 115.8, 117.2, 119.4,

119.5, 125.8, 127.6, 129.2, 129.5, 130.4, 143.5, 144.8, 153.4, 158.5, 159.8, 169.6; HRMS (ESI) m/z calcd for $C_{40}H_{43}NO_9S_2 [M+H]^+$ 762.2401, found 762.2423. $[\alpha]_{22}^D = -6.1$ ($c = 0.63$, $CHCl_3$).

5.1.16 General procedure for 13a-15a:

To a stirred solution of diethyl 2,2'-((((3R,4R)-pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1E,1'E)-bis(ethane-1-sulfonate) (**7a**) (1.0 equiv.) and Et_3N (2.2 equiv.) in DCM under argon at $0^\circ C$ was added triphosgene (0.35 equiv.). The reaction mixture was stirred for 30 min at room temperature before cooled to $0^\circ C$ again, and then substituted arylamine (1.0 equiv.) was added. The mixture was allowed to warm to room temperature and stirred for another 1 h. Then the mixture was washed with 5% HCl, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4 , concentrated, and purified by flash chromatography (petroleum ether: EtOAc:DCM = 4:1:1) to afford the target product.

5.1.16.1

diethyl

2,2'-((((3R,4R)-1-(phenylcarbamoyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1E,1'E)-bis(ethene-1-sulfonate) (**13a**)

White solid (35%). M.p.: $88-90^\circ C$. 1H NMR (400 MHz, $CDCl_3$) δ 1.37 (t, $J = 7.2$ Hz, 6H), 3.82 (d, $J = 12.0$ Hz, 2H), 3.96-4.00 (m, 2H), 4.19 (q, $J = 7.2$ Hz, 4H), 5.01 (d, $J = 4.0$ Hz, 2H), 6.29 (s, 1H), 6.59 (d, $J = 15.0$ Hz, 2H), 6.91 (d, $J = 8.4$ Hz, 4H), 7.00 (t, $J = 7.2$ Hz, 1H), 7.22-7.26 (m, 2H), 7.37 (d, $J = 8.4$ Hz, 2H), 7.43-7.51 (m, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.9, 49.5, 66.7, 78.3, 115.9, 119.4, 119.7, 123.3, 125.9, 128.9, 130.5, 138.5, 143.7, 153.8, 158.6; HRMS(ESI) m/z calcd for $C_{31}H_{34}N_2O_9S_2 [M+H]^+$ 643.1778, found 643.1790. $[\alpha]_{22}^D = -13.4$ ($c = 0.16$, $CHCl_3$).

5.1.16.2

diethyl

2,2'-((((3R,4R)-1-((4-phenoxyphenyl)carbamoyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1E,1'E)-bis(ethene-1-sulfonate) (**14a**)

White solid (37%). M.p.: $91-93^\circ C$. 1H NMR (400 MHz, $CDCl_3$) δ 1.37 (t, $J = 7.2$

Hz, 6H), 3.82 (d, $J = 12.0$ Hz, 2H), 3.96-4.00 (m, 2H), 4.19 (q, $J = 7.2$ Hz, 4H), 5.01 (d, $J = 3.6$ Hz, 2H), 6.31 (s, 1H), 6.59 (d, $J = 15.2$ Hz, 2H), 6.91 (d, $J = 8.4$ Hz, 8H), 7.04 (t, $J = 7.2$ Hz, 1H), 7.24-7.33 (m, 4H), 7.43-7.51 (m, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.9, 49.5, 66.7, 78.4, 115.9, 118.2, 119.6, 119.7, 121.7, 122.9, 126.0, 129.7, 130.5, 134.0, 143.6, 152.8, 154.0, 157.6, 158.6; HRMS (ESI) m/z calcd for $\text{C}_{37}\text{H}_{38}\text{N}_2\text{O}_{10}\text{S}_2$ $[\text{M}+\text{H}]^+$ 735.2041, found 735.2052. $[\alpha]_{22}^{\text{D}} = -12.1$ ($c = 0.14$, CHCl_3).

5.1.16.3

diethyl

2,2'-((((3R,4R)-1-((4-(4-isopropylphenoxy)phenyl)carbamoyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1E,1'E)-bis(ethene-1-sulfonate) (**15a**)

White solid (30%). M.p.: 90-91 °C. ^1H NMR (400 MHz, CDCl_3) δ 1.26-1.41 (m, 12H), 2.84-2.92 (m, 1H), 3.84 (d, $J = 12.0$ Hz, 2H), 3.99-4.03 (m, 2H), 4.22 (q, $J = 7.2$ Hz, 4H), 5.05 (d, $J = 3.6$ Hz, 2H), 6.20 (s, 1H), 6.62 (d, $J = 15.2$ Hz, 2H), 6.88-6.96 (m, 8H), 7.15 (d, $J = 8.0$ Hz, 2H), 7.33 (d, $J = 8.0$ Hz, 2H), 7.47-7.56 (m, 6H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.9, 24.1, 29.7, 49.5, 66.7, 78.4, 115.9, 118.2, 119.4, 119.6, 121.7, 126.0, 127.5, 130.5, 133.7, 143.6, 143.6, 153.3, 154.0, 155.4, 158.6; HRMS (ESI) m/z calcd for $\text{C}_{40}\text{H}_{44}\text{N}_2\text{O}_{10}\text{S}_2$ $[\text{M}+\text{H}]^+$ 777.2510, found 777.2516. $[\alpha]_{22}^{\text{D}} = -12.3$ ($c = 0.26$, CHCl_3).

5.1.17 General procedure for 16a-28a:

To a stirred solution of diethyl 2,2'-((((3R,4R)-pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1E,1'E)-bis(ethane-1-sulfonate) (**7a**) (1.5 g, 2.68 mmol) and Et_3N (0.41 g, 4.02 mmol) in 50 mL DCM under argon at 0 °C was added ethyl oxalyl monochloride (0.40 g, 2.91 mmol). The reaction mixture was stirred for 8 h at room temperature, then washed with 5% HCl, saturated aqueous NaHCO_3 and brine, dried over anhydrous Na_2SO_4 , and concentrated. The residue was redissolved in 10 mL THF and to it was added 2M aqueous of LiOH (2.7 mL, 5.40 mmol). The mixture was stirred for 30 min at room temperature, and then evaporated to move most of THF. The resulted precipitate was filtered and purified by flash chromatography (DCM:MeOH = 30:1) to afford

2-((3R,4R)-3,4-bis(4-((E)-2-(ethoxysulfonyl)vinyl)phenoxy)pyrrolidin-1-yl)-2-oxoacetic acid as yellow solid (1.26 g, 72%).

To a stirred solution of 2-((3R,4R)-3,4-bis(4-((E)-2-(ethoxysulfonyl)vinyl)phenoxy)pyrrolidin-1-yl)-2-oxoacetic acid (1.0 equiv.) and HBTU (1.2 equiv.) in DMF under argon at room temperature was added substituted arylamine (1.2 equiv.) and DMAP (1.5 equiv.) sequentially. The reaction mixture was stirred at room temperature for 24 h, then washed with 5% HCl and brine, dried over anhydrous Na₂SO₄, concentrated, and purified by flash chromatography (petroleum ether:EtOAc:DCM = 4:1:1) to afford the target product.

5.1.17.1

diethyl

2,2'-((((3R,4R)-1-(2-oxo-2-(phenylamino)acetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1E,1'E)-bis(ethene-1-sulfonate) (**16a**)

White solid (39%). M.p.: 92-94 °C. ¹H NMR (400 MHz, CDCl₃) δ 1.36-1.40 (m, 6H), 4.07 (s, 2H), 4.20-4.25 (m, 4H), 4.47 (dd, *J* = 3.6 Hz, 14.0 Hz, 1H), 4.65 (d, *J* = 14.0 Hz, 1H), 5.00 (s, 1H), 5.10 (d, *J* = 3.6 Hz, 1H), 6.63 (dd, *J* = 4.4 Hz, 15.6 Hz, 2H), 6.92-6.96 (m, 4H), 7.15 (t, *J* = 7.6 Hz, 1H), 7.34 (t, *J* = 7.6 Hz, 2H), 7.46-7.52 (m, 5H), 7.55 (d, *J* = 4.0 Hz, 1H), 7.58 (d, *J* = 7.6 Hz, 2H), 9.40 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 14.8, 51.6, 52.2, 66.7, 75.9, 79.2, 115.9(2), 119.6(2), 119.7, 125.2, 126.0(2), 129.1, 130.5, 136.4, 143.6, 157.3, 158.4, 159.4; HRMS (ESI) *m/z* calcd for C₃₂H₃₄N₂O₁₀S₂ [M+H]⁺ 671.1728, found 671.1730. [α]_D²⁵ = -23.3 (c = 0.66, CHCl₃).

5.1.17.2

diethyl

2,2'-((((3R,4R)-1-(2-oxo-2-((4-phenoxyphenyl)amino)acetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1E,1'E)-bis(ethene-1-sulfonate) (**17a**)

White solid (24%). M.p.: 93-95 °C. ¹H NMR (400 MHz, CDCl₃) δ 1.39 (t, *J* = 7.2 Hz, 6H), 4.07 (s, 2H), 4.20-4.25 (m, 4H), 4.48 (dd, *J* = 3.6 Hz, 14.0 Hz, 1H), 4.65 (d, *J* = 14.0 Hz, 1H), 5.00 (s, 1H), 5.10 (s, 1H), 6.63 (dd, *J* = 4.4 Hz, 15.6 Hz, 2H), 6.92-7.00 (m, 8H), 7.09 (t, *J* = 7.6 Hz, 1H), 7.32 (t, *J* = 7.6 Hz, 2H), 7.47-7.57 (m,

8H), 9.40 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.9, 51.6, 52.2, 66.7, 75.9, 79.2, 115.9(2), 118.6, 119.4, 119.7(2), 121.4, 123.3, 126.1(2), 129.7, 130.5, 131.9, 143.5, 154.3, 157.1, 157.2, 158.4, 159.4; HRMS (ESI) m/z calcd for $\text{C}_{38}\text{H}_{38}\text{N}_2\text{O}_{11}\text{S}_2$ $[\text{M}+\text{Na}]^+$ 785.1809, found 785.1814. $[\alpha]_{23}^{\text{D}} = -22.7$ ($c = 0.53$, CHCl_3).

5.1.17.3

diethyl

2,2'-((((3*R*,4*R*)-1-(2-((4-(4-isopropylphenoxy)phenyl)amino)-2-oxoacetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**18a**)

Light yellow solid (27%). M.p.: 92-94 °C. ^1H NMR (400 MHz, CDCl_3) δ 1.24 (d, $J = 7.2$ Hz, 6H), 1.37-1.41 (m, 6H), 2.87-2.91 (m, 1H), 4.06 (s, 2H), 4.20-4.25 (m, 4H), 4.47 (dd, $J = 3.6$ Hz, 14.0 Hz, 1H), 4.65 (d, $J = 14.0$ Hz, 1H), 5.00 (s, 1H), 5.10 (d, $J = 3.6$ Hz, 1H), 6.63 (dd, $J = 4.8$ Hz, 15.6 Hz, 2H), 6.90-6.99 (m, 8H), 7.17 (d, $J = 8.4$ Hz, 2H), 7.47-7.55 (m, 8H), 9.38 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.9, 24.1, 33.4, 51.6, 52.2, 66.7, 75.9, 79.2, 115.9(2), 118.6, 119.1, 119.7(2), 121.3, 126.1(2), 127.6, 130.5, 131.5, 143.5, 144.0, 154.8, 154.8, 157.1, 158.4, 159.5; HRMS (ESI) m/z calcd for $\text{C}_{41}\text{H}_{44}\text{N}_2\text{O}_{11}\text{S}_2$ $[\text{M}+\text{Na}]^+$ 827.2279, found 827.2277. $[\alpha]_{23}^{\text{D}} = -21.4$ ($c = 0.55$, CHCl_3).

5.1.17.4

diethyl

2,2'-((((3*R*,4*R*)-1-(2-((4-(4-ethylphenoxy)phenyl)amino)-2-oxoacetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**19a**)

Brown solid (22%). M.p.: 91-93 °C. ^1H NMR (400 MHz, CDCl_3) δ 1.23 (t, $J = 7.2$ Hz, 3H), 1.37-1.41 (m, 6H), 2.62 (q, $J = 7.2$ Hz, 2H), 4.07 (s, 2H), 4.20-4.25 (m, 4H), 4.47 (dd, $J = 3.6$ Hz, 14.0 Hz, 1H), 4.65 (d, $J = 14.0$ Hz, 1H), 5.00 (s, 1H), 5.10 (d, $J = 3.6$ Hz, 1H), 6.63 (dd, $J = 4.8$ Hz, 15.6 Hz, 2H), 6.89-6.98 (m, 8H), 7.14 (d, $J = 8.0$ Hz, 2H), 7.47-7.55 (m, 8H), 9.38 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.9, 15.6, 28.1, 51.6, 52.2, 66.7, 75.9, 79.2, 115.9(2), 118.8, 119.0, 119.7(2), 121.3, 126.1(2), 129.0, 130.5, 131.5, 139.4, 143.5, 154.8, 154.8, 157.1, 158.4, 159.4; HRMS (ESI) m/z calcd for $\text{C}_{40}\text{H}_{42}\text{N}_2\text{O}_{11}\text{S}_2$ $[\text{M}+\text{Na}]^+$ 813.2122, found 813.2130. $[\alpha]_{23}^{\text{D}} = -21.5$ ($c = 0.40$, CHCl_3).

5.1.17.5

diethyl

2,2'-((((3*R*,4*R*)-1-(2-((4-(4-(*tert*-butyl)phenoxy)phenyl)amino)-2-oxoacetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**20a**)

Brown solid (18%). M.p.: 95-97 °C. ¹H NMR (400 MHz, CDCl₃) δ 1.31 (s, 9H), 1.37-1.41 (m, 6H), 4.06 (s, 2H), 4.20-4.25 (m, 4H), 4.47 (dd, *J* = 3.6 Hz, 14.0 Hz, 1H), 4.65 (d, *J* = 14.0 Hz, 1H), 5.00 (s, 1H), 5.10 (d, *J* = 3.6 Hz, 1H), 6.63 (dd, *J* = 4.8 Hz, 15.5 Hz, 2H), 6.91-7.00 (m, 8H), 7.33 (d, *J* = 8.4 Hz, 2H), 7.47-7.55 (m, 8H), 9.39 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 14.9, 31.4, 34.3, 51.6, 52.2, 66.7, 75.9, 79.2, 115.9(2), 118.2, 119.2, 119.7(2), 121.3, 126.1(2), 126.5, 130.5, 131.6, 143.5, 146.3, 154.6, 154.7, 157.1, 158.4, 159.5; HRMS (ESI) *m/z* calcd for C₄₂H₄₆N₂O₁₁S₂ [M+Na]⁺ 841.2435, found 841.2430. [α]₂₃^D = -21.1 (*c* = 0.39, CHCl₃).

5.1.17.6

diethyl

2,2'-((((3*R*,4*R*)-1-(2-((4-(4-fluorophenoxy)phenyl)amino)-2-oxoacetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**21a**)

White solid (28%). M.p.: 96-97 °C. ¹H NMR (400 MHz, CDCl₃) δ 1.37-1.40 (m, 6H), 4.07 (s, 2H), 4.18-4.24 (m, 4H), 4.47 (dd, *J* = 3.6 Hz, 14.0 Hz, 1H), 4.65 (d, *J* = 14.0 Hz, 1H), 5.00 (s, 1H), 5.10 (d, *J* = 3.6 Hz, 1H), 6.63 (dd, *J* = 4.8 Hz, 15.5 Hz, 2H), 6.92-7.01 (m, 10H), 7.47-7.56 (m, 8H), 9.39 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 14.9, 51.6, 52.2, 66.7, 75.9, 79.2, 115.9(2), 116.2 and 116.4, 118.8, 119.7(2), 120.2(2), 121.4, 126.1(2), 130.5, 131.8, 143.5, 152.8(2), 154.7, 157.2, 157.6 and 160.0, 158.4, 159.4; HRMS (ESI) *m/z* calcd for C₃₈H₃₇FN₂O₁₁S₂ [M+Na]⁺ 803.1715, found 803.1706. [α]₂₃^D = -20.2 (*c* = 0.64, CHCl₃).

5.1.17.7

diethyl

2,2'-((((3*R*,4*R*)-1-(2-((4-(4-chlorophenoxy)phenyl)amino)-2-oxoacetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**22a**)

White solid (34%). M.p.: 92-94 °C. ¹H NMR (400 MHz, CDCl₃) δ 1.37-1.41 (m, 6H), 4.06 (s, 2H), 4.20-4.25 (m, 4H), 4.47 (dd, *J* = 3.6 Hz, 14.0 Hz, 1H), 4.65 (d, *J* =

14.0 Hz, 1H), 5.00 (s, 1H), 5.10 (d, $J = 3.6$ Hz, 1H), 6.63 (dd, $J = 4.8$ Hz, 15.6 Hz, 2H), 6.89-7.00 (m, 8H), 7.07-7.32 (m, 2H), 7.48-7.58 (m, 8H), 9.40-9.41 (m, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.9, 51.6, 52.2, 66.7, 75.9, 79.2, 115.9(2), 118.6, 119.5(2), 119.7, 119.8, 121.4(2), 123.3, 126.1, 128.3, 129.7(2), 130.5, 131.9(2), 143.5, 153.9, 154.3 and 155.9, 157.1(2), 158.4, 159.4(2); HRMS (ESI) m/z calcd for $\text{C}_{38}\text{H}_{37}\text{ClN}_2\text{O}_{11}\text{S}_2$ $[\text{M}+\text{Na}]^+$ 819.1420, found 819.1428. $[\alpha]_{23}^{\text{D}} = -20.1$ ($c = 0.74$, CHCl_3).

5.1.17.8

diethyl

2,2'-((((3*R*,4*R*)-1-(2-((4-(4-bromophenoxy)phenyl)amino)-2-oxoacetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene)))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**23a**)

Yellow solid (38%). M.p.: 94-95 °C. ^1H NMR (400 MHz, CDCl_3) δ 1.37-1.41 (m, 6H), 4.08(s, 2H), 4.19-4.24 (m, 4H), 4.48 (dd, $J = 3.6$ Hz, 14.0 Hz, 1H), 4.65 (d, $J = 14.0$ Hz, 1H), 5.00 (s, 1H), 5.10 (d, $J = 3.6$ Hz, 1H), 6.63 (dd, $J = 4.8$ Hz, 15.6 Hz, 2H), 6.84-7.00 (m, 8H), 7.40-7.58 (m, 10H), 9.40 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.9, 51.6, 52.2, 66.7, 75.9, 79.2, 115.7, 115.9(2), 119.6, 119.7(2), 120.2, 121.4, 126.1(2), 130.5, 132.3, 132.7, 143.5, 153.7, 156.4, 157.2, 158.4, 159.4; HRMS (ESI) m/z calcd for $\text{C}_{38}\text{H}_{37}\text{BrN}_2\text{O}_{11}\text{S}_2$ $[\text{M}+\text{Na}]^+$ 863.0914, found 863.0923. $[\alpha]_{23}^{\text{D}} = -19.7$ ($c = 0.52$, CHCl_3).

5.1.17.9

diethyl

2,2'-((((3*R*,4*R*)-1-(2-((4-([1,1'-biphenyl]-4-yloxy)phenyl)amino)-2-oxoacetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene)))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**24a**)

White solid (34%). M.p.: 109-111 °C. ^1H NMR (400 MHz, CDCl_3) δ 1.37-1.41 (m, 6H), 4.09 (s, 2H), 4.19-4.25 (m, 4H), 4.48 (dd, $J = 3.6$ Hz, 14.0 Hz, 1H), 4.67 (d, $J = 14.0$ Hz, 1H), 5.01 (s, 1H), 5.10 (d, $J = 3.6$ Hz, 1H), 6.63 (dd, $J = 4.8$ Hz, 15.5 Hz, 2H), 6.93-7.06 (m, 8H), 7.30-7.60 (m, 15H), 9.41 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.9, 51.6, 52.2, 66.7, 75.9, 79.2, 115.9(2), 118.8, 119.6, 119.7(2), 121.4, 126.1(2), 126.8, 127.0, 128.4, 128.7, 130.5, 132.0, 136.4, 140.4, 143.6, 154.2, 156.7, 157.2, 158.4, 159.4; HRMS (ESI) m/z calcd for $\text{C}_{44}\text{H}_{42}\text{N}_2\text{O}_{11}\text{S}_2$ $[\text{M}+\text{Na}]^+$ 861.2122, found 861.2118. $[\alpha]_{23}^{\text{D}} = -21.6$ ($c = 0.37$, CHCl_3).

5.1.17.10*diethyl*

2,2'-((((3*R*,4*R*)-1-(2-((4-(naphthalen-2-yloxy)phenyl)amino)-2-oxoacetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**25a**)

White solid (31%). M.p.: 111-113 °C. ¹H NMR (400 MHz, CDCl₃) δ 1.37 (t, *J* = 7.2 Hz, 6H), 4.06 (s, 2H), 4.20 (q, *J* = 7.2 Hz, 4H), 4.46 (dd, *J* = 14.4 Hz, 4.0 Hz, 1H), 4.64 (d, *J* = 14.4 Hz, 1H), 4.99 (s, 1H), 5.08 (d, *J* = 4.0 Hz, 1H), 6.61 (dd, *J* = 15.6 Hz, 2.4 Hz, 2H), 6.88-6.97 (m, 4H), 7.00-7.08 (m, 2H), 7.18-7.28 (m, 2H), 7.35-7.61 (m, 10H), 7.66 (d, *J* = 8.0 Hz, 1H), 7.80 (dd, *J* = 8.4 Hz, 4.4 Hz, 2H), 9.43 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 14.9, 51.6, 52.2, 66.7, 75.8, 79.2, 113.8, 115.8, 119.6, 119.7, 121.4, 124.7, 126.0, 126.5, 127.0, 127.7, 129.9, 130.1, 130.5, 132.1, 134.2, 143.6, 154.1, 155.0, 157.2, 158.4, 159.4; HRMS (ESI) *m/z* calcd for C₄₂H₄₀N₂O₁₁S₂ [M+Na]⁺ 835.1966, found 835.1958. [α]₂₃^D = -23.9 (*c* = 0.36, CHCl₃).

5.1.17.11*diethyl*

2,2'-((((3*R*,4*R*)-1-(2-((4-hexylphenyl)amino)-2-oxoacetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**26a**)

White solid (34%). M.p.: 84-85 °C. ¹H NMR (400 MHz, CDCl₃) δ 0.86 (m, 3H), 1.27-1.29 (m, 6H), 1.40 (td, *J* = 7.2 Hz, 2.4 Hz, 6H), 1.53-1.59 (m, 2H), 2.56 (t, *J* = 8.4 Hz, 2H), 4.07 (s, 2H), 4.22 (qd, *J* = 7.2 Hz, 2.4 Hz, 4H), 4.47 (dd, *J* = 14.0 Hz, 3.6 Hz, 1H), 4.65 (d, *J* = 14.0 Hz, 1H), 5.01 (s, 1H), 5.10 (d, *J* = 3.6 Hz, 1H), 6.63 (dd, *J* = 4.4 Hz, 15.6 Hz, 2H), 6.93 (t, *J* = 8.4 Hz, 4H), 7.14 (d, *J* = 8.4 Hz, 2H), 7.47-7.56 (m, 8H), 9.35 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 14.0, 14.9, 22.5, 28.8, 31.4, 31.6, 35.3, 51.5, 52.2, 66.7, 75.8, 79.1, 115.8, 119.5(2), 119.6, 125.9(2), 129.0, 130.5, 134.0, 140.1, 143.6, 157.1, 158.4(2), 159.5; HRMS (ESI) *m/z* calcd for C₃₈H₄₆N₂O₁₀S₂ [M+Na]⁺ 777.2486, found 777.2474. [α]₂₃^D = -22.6 (*c* = 0.68, CHCl₃).

5.1.17.12*diethyl*

2,2'-((((3*R*,4*R*)-1-(2-((4-decylphenyl)amino)-2-oxoacetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**27a**)

White solid (36%). M.p.: 76-78 °C. ^1H NMR (400 MHz, CDCl_3) δ 0.87 (t, $J = 7.2$ Hz, 3H), 1.21-1.28 (m, 14H), 1.39 (td, $J = 7.2$ Hz, 2.4 Hz, 6H), 1.54-1.58 (m, 2H), 2.56 (t, $J = 8.4$ Hz, 2H), 4.07 (s, 2H), 4.22 (qd, $J = 7.2$ Hz, 2.4 Hz, 4H), 4.47 (dd, $J = 14.0$ Hz, 3.6 Hz, 1H), 4.65 (d, $J = 14.0$ Hz, 1H), 5.00 (s, 1H), 5.10 (d, $J = 3.6$ Hz, 1H), 6.63 (dd, $J = 4.4$ Hz, 15.6 Hz, 2H), 6.94 (t, $J = 8.4$ Hz, 4H), 7.15 (d, $J = 8.0$ Hz, 2H), 7.47-7.56 (m, 8H), 9.34 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.1, 14.9, 22.6, 29.2, 29.3, 29.4, 29.5, 29.6, 31.4, 31.8, 35.4, 51.6, 52.2, 66.7, 75.8, 79.1, 115.8, 119.5(2), 119.6, 126.0(2), 129.0, 130.5, 134.0, 140.1, 143.6, 157.1, 158.4(2), 159.5; HRMS (ESI) m/z calcd for $\text{C}_{42}\text{H}_{54}\text{N}_2\text{O}_{10}\text{S}_2$ $[\text{M}+\text{Na}]^+$ 833.3112, found 833.3090. $[\alpha]_{23}^{\text{D}} = -21.8$ ($c = 0.50$, CHCl_3).

5.1.17.13

diethyl

2,2'-((((3*R*,4*R*)-1-(2-((4-dodecylphenyl)amino)-2-oxoacetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**28a**)

White solid (33%). M.p.: 74-76 °C. ^1H NMR (400 MHz, CDCl_3) δ 0.87 (t, $J = 7.2$ Hz, 3H), 1.24-1.29 (m, 18H), 1.40 (td, $J = 7.2$ Hz, 2.4 Hz, 6H), 1.55-1.60 (m, 2H), 2.57 (t, $J = 8.4$ Hz, 2H), 4.08 (s, 2H), 4.22 (qd, $J = 7.2$ Hz, 2.4 Hz, 4H), 4.47 (dd, $J = 14.0$ Hz, 3.6 Hz, 1H), 4.66 (d, $J = 14.0$ Hz, 1H), 5.01 (s, 1H), 5.10 (d, $J = 3.6$ Hz, 1H), 6.63 (dd, $J = 4.4$ Hz, 15.6 Hz, 2H), 6.94 (t, $J = 8.4$ Hz, 4H), 7.15 (d, $J = 8.0$ Hz, 2H), 7.47-7.56 (m, 8H), 9.33 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.1, 14.9, 22.7, 29.2, 29.3, 29.5, 29.5, 29.6, 29.6, 29.6, 31.4, 31.9, 35.4, 51.6, 52.2, 66.7, 75.8, 79.1, 115.9(2), 119.5(2), 119.7, 126.0(2), 129.0, 130.5, 134.0, 140.2, 143.6, 157.1, 158.4(2), 159.5; HRMS (ESI) m/z calcd for $\text{C}_{44}\text{H}_{58}\text{N}_2\text{O}_{10}\text{S}_2$ $[\text{M}+\text{Na}]^+$ 861.3425, found 861.3392. $[\alpha]_{23}^{\text{D}} = -21.5$ ($c = 0.87$, CHCl_3).

5.1.18 General procedure for **16b-28b**:

Compounds **16b-28b** were synthesized according to the above method for the synthesis of **16a-28a**.

5.1.18.1

diethyl

2,2'-((((3*S*,4*R*)-1-(2-oxo-2-(phenylamino)acetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**16b**)

White solid (46%). M.p.: 86-88 °C. ¹H NMR (400 MHz, CDCl₃) δ 1.40 (t, *J* = 7.2 Hz, 6H), 3.97-4.02 (m, 1H), 4.10-4.13 (m, 1H), 4.21-4.23 (m, 4H), 4.40-4.46 (m, 1H), 4.69-4.72 (m, 1H), 5.11 (d, *J* = 3.6 Hz, 2H), 6.58-6.63 (m, 2H), 6.95-6.97 (m, 4H), 7.16-7.19 (m, 1H), 7.35-7.61 (m, 10H), 9.36 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 14.9, 50.1, 51.2, 66.7, 73.4, 75.9, 116.1, 119.3(2), 119.7, 125.3, 125.8, 129.2, 130.3, 136.4, 143.8, 157.1, 159.3, 159.6(2); HRMS (ESI) *m/z* calcd for C₃₂H₃₄N₂O₁₀S₂ [M+Na]⁺ 693.1547, found 693.1534.

5.1.18.2

diethyl

2,2'-((((3*S*,4*R*)-1-(2-oxo-2-((4-phenoxyphenyl)amino)acetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**17b**)

Light brown solid (54%). M.p.: 94-96 °C. ¹H NMR (400 MHz, CDCl₃) δ 1.39 (t, *J* = 7.2 Hz, 6H), 3.97-4.00 (m, 1H), 4.10-4.14 (m, 1H), 4.20-4.22 (m, 4H), 4.41-4.45 (m, 1H), 4.68-4.71 (m, 1H), 5.11 (s, 2H), 6.60 (d, *J* = 14.8 Hz, 2H), 6.93-7.01 (m, 8H), 7.10 (t, *J* = 8.0 Hz, 1H), 7.31-7.57 (m, 10H), 9.36 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 14.9, 50.1, 51.2, 66.7, 73.4, 75.9, 116.2, 118.6, 119.3, 119.5, 121.3, 123.4, 125.8, 129.8, 130.3, 131.8, 143.8, 154.3, 157.0, 157.1, 159.3, 159.6(2); HRMS (ESI) *m/z* calcd for C₃₈H₃₈N₂O₁₁S₂ [M+Na]⁺ 785.1809, found 785.1800.

5.1.18.3

diethyl

2,2'-((((3*S*,4*R*)-1-(2-((4-(4-isopropylphenoxy)phenyl)amino)-2-oxoacetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**18b**)

Yellow solid (39%). M.p.: 97-99 °C. ¹H NMR (400 MHz, CDCl₃) δ 1.24 (d, *J* = 7.2 Hz, 6H), 1.39 (t, *J* = 7.2 Hz, 6H), 2.87-2.91 (m, 1H), 3.97-4.00 (m, 1H), 4.09-4.14 (m, 1H), 4.18-4.23 (m, 4H), 4.41-4.45 (m, 1H), 4.67-4.72 (m, 1H), 5.11 (d, *J* = 3.6 Hz, 2H), 6.58-6.62 (m, 2H), 6.91-7.19 (m, 10H), 7.43-7.56 (m, 8H), 9.34 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 14.9, 24.1, 33.4, 50.1, 51.2, 66.7, 73.4, 75.9, 116.1, 118.7, 119.1, 119.3, 121.3, 125.8, 127.6, 130.3, 131.5, 143.7, 144.0, 154.7, 154.8, 157.0,

159.4, 159.6(2); HRMS (ESI) m/z calcd for $C_{41}H_{44}N_2O_{11}S_2 [M+Na]^+$ 827.2279, found 827.2285.

5.1.18.4

diethyl

2,2'-((((3*S*,4*R*)-1-(2-((4-(4-ethylphenoxy)phenyl)amino)-2-oxoacetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**19b**)

White solid (44%). M.p.: 93-95 °C. 1H NMR (400 MHz, $CDCl_3$) δ 1.23 (t, J = 7.2 Hz, 3H), 1.39 (t, J = 7.2 Hz, 6H), 2.62 (q, J = 7.2 Hz, 2H), 3.97-4.00 (m, 1H), 4.11-4.14 (m, 1H), 4.18-4.24 (m, 4H), 4.41-4.45 (m, 1H), 4.67-4.72 (m, 1H), 5.11 (d, J = 3.6 Hz, 2H), 6.58-6.62 (m, 2H), 6.90-7.24 (m, 10H), 7.42-7.55 (m, 8H), 9.34 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.9, 15.7, 28.1, 50.1, 51.2, 66.7, 73.4, 75.9, 116.2, 118.8, 119.0, 119.3, 121.3, 125.8, 129.0, 130.3, 131.5, 139.5, 143.8, 154.8, 154.9, 157.0, 159.3, 159.6(2); HRMS (ESI) m/z calcd for $C_{40}H_{42}N_2O_{11}S_2 [M+Na]^+$ 813.2122, found 813.2108.

5.1.18.5

diethyl

2,2'-((((3*S*,4*R*)-1-(2-((4-(4-(*tert*-butyl)phenoxy)phenyl)amino)-2-oxoacetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**20b**)

Brown solid (42%). M.p.: 96-98 °C. 1H NMR (400 MHz, $CDCl_3$) δ 1.31 (s, 9H), 1.39 (t, J = 7.2 Hz, 6H), 3.97-4.00 (m, 1H), 4.10-4.14 (m, 1H), 4.18-4.24 (m, 4H), 4.41-4.45 (m, 1H), 4.68-4.71 (m, 1H), 5.11 (d, J = 3.6 Hz, 2H), 6.60 (d, J = 14.8 Hz, 2H), 6.91-7.01 (m, 8H), 7.33 (d, J = 8.4 Hz, 2H), 7.43-7.56 (m, 8H), 9.35 (s, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 14.9, 31.4, 34.3, 50.1, 51.2, 66.7, 73.4, 75.9, 116.2, 118.2, 119.2, 119.3, 121.3, 125.8, 126.6, 130.3, 131.5, 143.8, 146.3, 154.6, 154.7, 157.0, 159.3, 159.6(2); HRMS (ESI) m/z calcd for $C_{42}H_{46}N_2O_{11}S_2 [M+Na]^+$ 841.2435, found 841.2442.

5.1.18.6

diethyl

2,2'-((((3*S*,4*R*)-1-(2-((4-(4-fluorophenoxy)phenyl)amino)-2-oxoacetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**21b**)

White solid (47%). M.p.: 102-103 °C. ^1H NMR (400 MHz, CDCl_3) δ 1.39 (t, J = 7.2 Hz, 6H), 3.97-4.00 (m, 1H), 4.19-4.13 (m, 1H), 4.18-4.24 (m, 4H), 4.42-4.45 (m, 1H), 4.68-4.71 (m, 1H), 5.11 (d, J = 3.6 Hz, 2H), 6.60 (d, J = 14.8 Hz, 2H), 6.95-7.04 (m, 10H), 7.43-7.57 (m, 8H), 9.35 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.9, 50.1, 51.2, 66.7, 73.4, 75.9, 116.2, 116.2, 116.5, 118.9, 119.4, 120.3, 120.4, 121.4, 125.9, 130.3, 131.7, 143.7, 154.8, 157.0, 159.3, 159.6(2); HRMS (ESI) m/z calcd for $\text{C}_{38}\text{H}_{37}\text{FN}_2\text{O}_{11}\text{S}_2$ $[\text{M}+\text{Na}]^+$ 803.1715, found 803.1718.

5.1.18.7

diethyl

2,2'-((((3*S*,4*R*)-1-(2-((4-(4-chlorophenoxy)phenyl)amino)-2-oxoacetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**22b**)

White solid (34%). M.p.: 89-91 °C. ^1H NMR (400 MHz, CDCl_3) δ 1.39 (t, J = 7.2 Hz, 6H), 3.97-4.00 (m, 1H), 4.10-4.14 (m, 1H), 4.18-4.24 (m, 4H), 4.41-4.45 (m, 1H), 4.68-4.71 (m, 1H), 5.10 (m, 2H), 6.60 (d, J = 14.8 Hz, 2H), 6.91-7.00 (m, 8H), 7.10-7.35 (m, 2H), 7.43-7.59 (m, 8H), 9.35-9.36 (m, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.9, 50.1(2), 51.2, 66.7, 73.4, 75.9, 116.2, 118.6, 119.4(3), 119.8, 121.3(2), 123.4, 125.9, 129.7(2), 130.3, 143.7, 153.9, 154.3, 155.8, 157.0(3), 159.3(2), 159.6(2); HRMS (ESI) m/z calcd for $\text{C}_{38}\text{H}_{37}\text{ClN}_2\text{O}_{11}\text{S}_2$ $[\text{M}+\text{Na}]^+$ 819.1420, found 819.1406.

5.1.18.8

diethyl

2,2'-((((3*S*,4*R*)-1-(2-((4-(4-bromophenoxy)phenyl)amino)-2-oxoacetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**23b**)

White solid (49%). M.p.: 85-88 °C. ^1H NMR (400 MHz, CDCl_3) δ 1.39 (t, J = 7.2 Hz, 6H), 3.98-4.01 (m, 1H), 4.10-4.13 (m, 1H), 4.18-4.24 (m, 4H), 4.41-4.45 (m, 1H), 4.67-4.72 (m, 1H), 5.11 (d, J = 3.6 Hz, 2H), 6.60 (d, J = 14.8 Hz, 2H), 6.85-7.01 (m, 8H), 7.43-7.59 (m, 10H), 9.37 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.9, 50.1, 51.2, 66.7, 73.4, 75.9, 115.8, 116.2, 119.4, 119.7, 120.2, 121.4, 125.9, 130.3, 132.2, 132.7, 143.7, 153.8, 156.4, 157.0, 159.3, 159.6(2); HRMS (ESI) m/z calcd for $\text{C}_{38}\text{H}_{37}\text{BrN}_2\text{O}_{11}\text{S}_2$ $[\text{M}+\text{Na}]^+$ 863.0914, found 863.0919.

5.1.18.9

diethyl

2,2'-((((3*S*,4*R*)-1-(2-((4-([1,1'-biphenyl]-4-yloxy)phenyl)amino)-2-oxoacetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**24b**)

White solid (42%). M.p.: 104-106 °C. ¹H NMR (400 MHz, CDCl₃) δ 1.39 (t, *J* = 7.2 Hz, 6H), 3.97-4.01 (m, 1H), 4.11-4.14 (m, 1H), 4.18-4.23 (m, 4H), 4.42-4.45 (m, 1H), 4.68-4.72 (m, 1H), 5.11 (d, *J* = 3.6 Hz, 2H), 6.60 (d, *J* = 14.8 Hz, 2H), 6.95 (t, *J* = 7.6 Hz, 4H), 7.05 (d, *J* = 7.2 Hz, 4H), 7.33-7.61 (m, 15H), 9.40 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 14.9, 50.1, 51.2, 66.7, 73.4, 75.9, 116.2, 118.8, 119.3, 119.6, 121.4, 125.8, 126.8, 127.1, 128.4, 128.8, 130.3, 132.0, 136.4, 140.3, 143.8, 154.2, 156.7, 157.0, 159.3, 159.6(2); HRMS (ESI) *m/z* calcd for C₄₄H₄₂N₂O₁₁S₂ [M+Na]⁺ 861.2122, found 861.2110.

5.1.18.10

diethyl

2,2'-((((3*S*,4*R*)-1-(2-((4-(naphthalen-2-yloxy)phenyl)amino)-2-oxoacetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**25b**)

White solid (40%). M.p.: 109-111 °C. ¹H NMR (400 MHz, CDCl₃) δ 1.36 (t, *J* = 7.2 Hz, 6H), 3.95-3.98 (m, 1H), 4.08-4.12 (m, 1H), 4.16-4.22 (m, 4H), 4.39-4.43 (m, 1H), 4.65-4.70 (m, 1H), 5.09 (d, *J* = 3.6 Hz, 2H), 6.58 (d, *J* = 14.8 Hz, 2H), 6.92 (t, *J* = 7.6 Hz, 4H), 7.05 (d, *J* = 8.8 Hz, 2H), 7.20-7.82 (m, 15H), 9.38 (s, 1H); ¹³C NMR (100 MHz, CDCl₃) δ 14.9, 50.1, 51.2, 66.7, 73.4, 75.9, 113.8, 116.1(2), 119.3, 119.7, 119.8, 121.4, 124.8, 125.8, 126.6, 127.0, 127.7, 130.0, 130.1, 130.3(2), 132.0, 134.2, 143.8, 154.2, 155.0, 157.0, 159.3, 159.6(2); HRMS (ESI) *m/z* calcd for C₄₂H₄₀N₂O₁₁S₂ [M+Na]⁺ 835.1966, found 835.1958.

5.1.18.11

diethyl

2,2'-((((3*S*,4*R*)-1-(2-((4-hexylphenyl)amino)-2-oxoacetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))(1*E*,1'*E*)-bis(ethene-1-sulfonate) (**26b**)

Light yellow solid (58%). M.p.: 87-88 °C. ¹H NMR (400 MHz, CDCl₃) δ 0.87 (m, 3H), 1.24-1.28 (m, 6H), 1.38 (t, *J* = 7.2 Hz, 6H), 1.53-1.59 (m, 2H), 2.56 (t, *J* = 8.4 Hz, 2H), 3.97-4.00 (m, 1H), 4.10-4.14 (m, 1H), 4.18-4.24 (m, 4H), 4.41-4.44 (m, 1H),

4.67-4.71 (m, 1H), 5.11 (d, $J = 3.6$ Hz, 2H), 6.60 (d, $J = 14.8$ Hz, 2H), 6.94 (t, $J = 7.6$ Hz, 4H), 7.15 (d, $J = 7.2$ Hz, 2H), 7.41-7.52 (m, 8H), 9.33 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.0, 14.8, 22.5, 28.8, 31.4, 31.6, 35.4, 50.1, 51.1, 66.7, 73.4, 75.9, 116.1, 119.2(2), 119.7, 125.7, 129.0, 130.3, 134.0, 140.2, 143.8, 157.0, 159.4, 159.6(2); HRMS (ESI) m/z calcd for $\text{C}_{38}\text{H}_{46}\text{N}_2\text{O}_{10}\text{S}_2$ $[\text{M}+\text{Na}]^+$ 777.2486, found 777.2478.

5.1.18.12

diethyl

2,2'-((((3*S*,4*R*)-1-(2-((4-decylphenyl)amino)-2-oxoacetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))((1*E*,1'*E*)-bis(ethene-1-sulfonate) (**27b**)

Brown solid (52%). M.p.: 79-80 °C. ^1H NMR (400 MHz, CDCl_3) δ 0.87 (t, $J = 7.2$ Hz, 3H), 1.24-1.28 (m, 14H), 1.38 (t, $J = 7.2$ Hz, 6H), 1.55-1.59 (m, 2H), 2.57 (t, $J = 8.4$ Hz, 2H), 3.97-4.00 (m, 1H), 4.10-4.14 (m, 1H), 4.18-4.24 (m, 4H), 4.41-4.44 (m, 1H), 4.67-4.72 (m, 1H), 5.11 (d, $J = 3.6$ Hz, 2H), 6.60 (d, $J = 14.8$ Hz, 2H), 6.94 (t, $J = 7.6$ Hz, 4H), 7.15 (d, $J = 7.2$ Hz, 2H), 7.41-7.53 (m, 8H), 9.32 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.1, 14.9, 22.6, 29.2, 29.3, 29.5, 29.6, 29.6, 31.4, 31.8, 35.4, 50.1, 51.2, 66.7, 73.4, 75.9, 116.2, 119.2, 119.7, 125.7, 129.0, 130.3, 134.0, 140.2, 143.8, 157.0, 159.4, 159.6(2); HRMS (ESI) m/z calcd for $\text{C}_{42}\text{H}_{54}\text{N}_2\text{O}_{10}\text{S}_2$ $[\text{M}+\text{Na}]^+$ 833.3112, found 833.3091.

5.1.18.13

diethyl

2,2'-((((3*S*,4*R*)-1-(2-((4-dodecylphenyl)amino)-2-oxoacetyl)pyrrolidine-3,4-diyl)bis(oxy))bis(4,1-phenylene))((1*E*,1'*E*)-bis(ethene-1-sulfonate) (**28b**)

White solid (57%). M.p.: 76-78 °C. ^1H NMR (400 MHz, CDCl_3) δ 0.87 (t, $J = 7.2$ Hz, 3H), 1.24-1.28 (m, 18H), 1.39 (t, $J = 7.2$ Hz, 6H), 1.55-1.59 (m, 2H), 2.57 (t, $J = 8.4$ Hz, 2H), 3.97-4.00 (m, 1H), 4.10-4.14 (m, 1H), 4.18-4.23 (m, 4H), 4.41-4.45 (m, 1H), 4.68-4.72 (m, 1H), 5.11 (d, $J = 3.6$ Hz, 2H), 6.60 (d, $J = 14.8$ Hz, 2H), 6.95 (t, $J = 7.6$ Hz, 4H), 7.15 (d, $J = 7.2$ Hz, 2H), 7.42-7.53 (m, 8H), 9.31 (s, 1H); ^{13}C NMR (100 MHz, CDCl_3) δ 14.1, 14.9, 22.7, 29.2, 29.3, 29.5, 29.5, 29.6, 29.6, 29.6, 31.4, 31.9, 35.4, 50.1, 51.2, 66.7, 73.4, 75.9, 116.2, 119.3(2), 119.7, 125.8, 129.0, 130.3,

134.0, 140.2, 143.8, 157.0, 159.4, 159.6(2); HRMS (ESI) m/z calcd for $C_{44}H_{58}N_2O_{10}S_2 [M+Na]^+$ 861.3425, found 861.3447.

5.2. Biological assays

5.2.1 PTP1B, SHP2 and TCPTP inhibition assays

The inhibitory activities of substituted bisarylethanesulfonic acid ester derivatives against PTP1B, TCPTP and SHP2 were determined with our published procedure [23]. Briefly, *p*-nitrophenylphosphonic acid (pNPP) was hydrolyzed by PTP1B, TCPTP or SHP2 to pNP that can be detected at 405 nm. Tested compounds were predisposed in 96-well micro plates as 1.0 μ L aliquots per well in DMSO. The protein enzymatic assay was carried out in a total volume of 100 μ L per well in assay plates with 75 nM recombinant protein (PTP1B, TCPTP or SHP2), 2.5 mM pNPP, 10 mM Tris, 25 mM NaCl and 1 mM EDTA (pH = 7.1). After being incubated at room temperature for 30 min, the reaction was terminated by addition of 50 μ L 2.0 M NaOH. Then the amount of hydrolysis product (pNP) was monitored by detection of the absorbance at 405 nm. The IC_{50} values of the compounds were shown in the **Table 1**, **Tabel 2** and **Tabel 3**.

5.2.2 Parallel artificial membrane permeability assay (PAMPA)

Parallel artificial membrane permeability assay was tested by our previous reported method with PAMPA Lipid Blend I [24]. Briefly, 5 μ L PAMPA Lipid Blend I (Avanti 888787) was added to the filter in the donor (upper) compartment and an artificial membrane will be formed. 200 μ L physiological saline (pH = 7.4) was added to the acceptor (lower) compartment in triplicates, and the donor compartment was placed on the acceptor compartment. Then 200 μ L (10 μ M final concentration, diluted with physiological saline) of tested compound was added to the donor compartment. Cover the lid and incubate the plate at room temperature for 12 h. The permeation of compound across an artificial membrane was quantified by LC-MS.

The apparent permeability for each compound (P_{app}) was calculated from the following equation:

$$P_{app} = \left\{ -\ln \left(1 - \frac{[drug]_{acceptor}}{[drug]_{equilibrium}} \right) \right\} * \frac{V_D * V_A}{(V_D + V_A) * area * time}$$

Where

$[drug]_{acceptor}$ - the concentration of test compound in acceptor compartments,

$[drug]_{equilibrium}$ - the concentration of test compound in the total volume of the donor and acceptor compartments,

V_D - the volumes of the donor compartments,

V_A - the volumes of the acceptor compartments,

Area - surface area of the membrane multiplied by the porosity (0.31 cm²),

Time – time of the assay,

P_{app} was expressed in 10⁻⁶ cm/s

5.2.3 Cell cytotoxicity assay

HepG2 and A549 cell line were cultured in DMEM supplemented with 10% fetal bovine serum for 24 h with 5% CO₂ at 37 °C. For cell cytotoxicity assay, 1×10⁴ cells were seeded in each well of 96-well plates and treated with varying concentrations of compounds for 72 h before addition of 10 µL CCK-8 solution (Cell Counting Kit-8, Beyotime) to each well. Cells were incubated for another 1-2 h in the incubator at 37 °C in dark place. Wells containing only media were used for background correction. The optical density was measured spectrophotometrically at 490 nm by using a micro plate reader.

5.2.4 Effect of compound on 2-NBDG uptake by HepG2 cell line

Glucose uptake into HepG2 cells was measured using 2-NBDG as previously described with minor revision [36]. Cells were cultured in DMEM supplemented with 10% fetal bovine serum for 24 h with 5% CO₂ at 37 °C. For 2-NBDG uptake assay, 1×10⁵ cells were seeded in each well of 24-well plates. After treating cells with compounds for 24 h, the medium was removed, and the cells were washed twice with PBS buffer. HepG2 cultures were treated with insulin (100 nM, final concentration)

for 10 min, followed by the addition of the fluorescent glucose analog 2-NBDG (50 μ M, final concentration) for 60 min. Uptake of 2-NBDG was measured by a FACSCalibur (BD) set at an excitation wave length of 485 nm and an emission wave length of 535 nm.

5.2.5 Effect of PTP1B inhibitors on the phosphorylation level of HepG2 cells

HepG2 cells were cultured in DMEM supplemented with 10% fetal bovine serum for 24 h with 5% CO₂ at 37 °C. Cells were serum free starved for 4 h and incubated with or without compounds for 1 h, then insulin (100 nM, final concentration) was added for stimulation for 15 min before harvested. For total protein extraction, HepG2 cells were washed with ice-cold PBS and lysed with RIPA lysis buffer (Beyotime) on ice for 30 min. Cell debris was removed by centrifugation at 14000 rpm for 5 min at 4 °C. The protein concentrations were determined by the BSA assay (BSA Kit, Beyotime) and stored at -80 °C until the Western blotting analyses. Total proteins (30 μ g) were electrophoresed on SDS polyacrylamide gels, and then transferred to PVDF membranes. Membranes were blocked with 1.5% BSA and then incubated with appropriated primary antibodies (p-IR β and β -actin, Santa Cruz) overnight at 4 °C, followed by incubation with appropriate secondary antibodies for 1 h at room temperature. Membranes were washed three times with TBST for 5 min each time. Finally, the chemiluminescence method was employed to detect the signals using ECL Western Blotting Substrate (Beyotime), and protein bands were detected by autoradiography, and the intensities were analyzed by Image J.

5.3. Molecular docking

The docking simulations were performed on GOLD 5.1. The default parameters were used except for otherwise stated. 5 different structures of PTP1B (1AAX, 1G1H, 1LQF, 1Q6T and 2CNE) were loaded into GOLD, followed by superimposition to protein structure 1AAX. Hydrogen was added, and water molecules and ligands were deleted from the protein structure. The binding site was determined using point (45.484, 14.573 and 5.329) and a radius of 22.5 Å. All possible ligand flexibility

options were turned on, and early termination was turned off. Every possible form of the inhibitor molecule was docked into the protein. Generated poses were checked manually. Finally, the acceptable pose with high score and reasonable binding mode was selected and presented with Pymol 1.7.

Conflicts of interest

The authors state no conflict of interest.

Acknowledgments

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Table/Figure/Scheme captions:

Table 1. PTP1B inhibitory activities of pyrrolidine bis-arylethenesulfonic acid esters.

Table 2. PTP1B inhibitory activities of stereoisomeric pyrrolidine bis-arylethenesulfonic acid ester derivatives.

Table 3. The membrane permeability and inhibitory activities of partial stereoisomeric bis-arylethenesulfonic acid esters against PTP1B, TCPTP and SHP2.

Fig. 1. Representative arylethenesulfonic acid ester PTP1B inhibitors.

Fig. 2. Docking simulation of compound **28a** and **28b** with PTP1B (PDB code 2CNE).

Fig. 3. Inhibitory effects of compound **24a**, **24b**, **28a** and **28b** on cell viability.

Fig. 4. Effects of compound **24a** and **28a** on insulin-stimulated glucose uptake.

Fig. 5. Effect of compound **28a** on tyrosine phosphorylation of insulin receptor β (IR β).

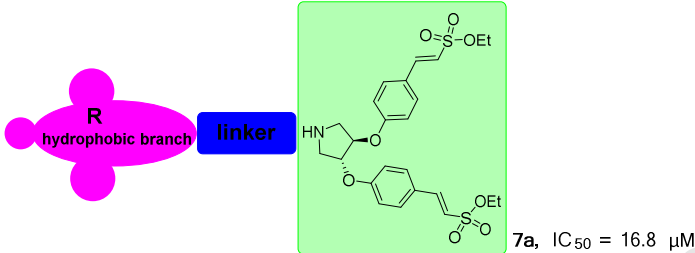
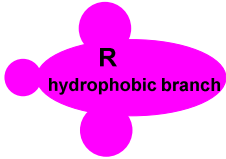
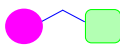
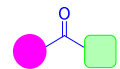
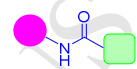
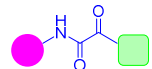

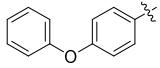
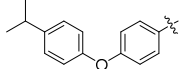
Scheme 1. Synthesis of intermediate **7a**.

Scheme 2. Synthesis of intermediate **7b**.

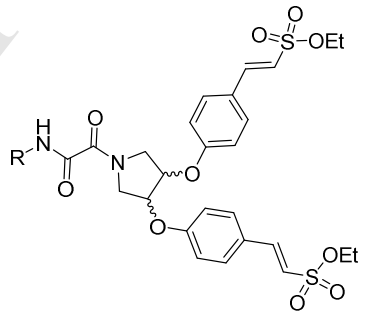

Scheme 3. Synthesis of stereoisomeric pyrrolidine bisarylethenesulfonic acid esters with different linkers.

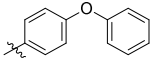
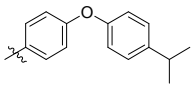
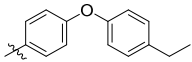
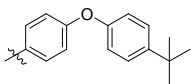
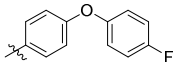
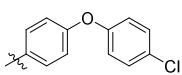
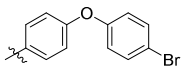
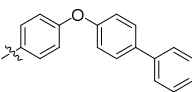
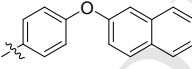
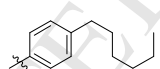
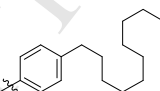
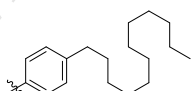
Table(s)

Table 1. PTP1B inhibitory activities of pyrrolidine bis-arylethenesulfonic acid esters^a.

				
7a, IC ₅₀ = 16.8 μM				
	Compound, IC ₅₀ (μM)			
	Methylene 	Carbonyl 	Amylacyl 	Amyloxalyl 
	6a , 9.34	10a , 7.81	13a , 3.51	16a , 2.52
	8a , 7.42	11a , 2.95	14a , 1.93	17a , 1.07
	9a , 6.87	12a , 3.36	15a , 1.37	18a , 0.42

^a Values represent triplicate determinations.**Table 2.** PTP1B inhibitory activities of stereoisomeric pyrrolidine bis-arylethenesulfonic acid ester derivatives^a.

				
<i>trans</i> -isomers		<i>cis</i> -isomers		
16a-28a		16b-28b		
Cmpd	R	IC ₅₀ (μM)		
		<i>trans</i>	<i>cis</i>	<i>cis/trans</i>
16a/16b		2.52	6.84	2.7

17a/17b		1.07	2.79	2.6
18a/18b		0.42	1.39	3.3
19a/19b		0.30	1.18	3.9
20a/20b		0.43	1.17	2.7
21a/21b		0.46	1.22	2.6
22a/22b		0.38	1.45	3.8
23a/23b		0.44	1.51	3.4
24a/24b		0.23	0.77	3.3
25a/25b		0.36	1.26	3.5
26a/26b		0.61	1.54	2.5
27a/27b		0.25	1.08	4.3
28a/28b		0.12	0.83	6.9

^a Values are means of triplicates, repeated two to three times.

Table 3. The membrane permeability and inhibitory activities of partial stereoisomeric bis-arylethanesulfonic acid esters against PTP1B, TCPTP and SHP2.

Cmpd	PTP1B (μM) ^a	TCPTP (μM) ^a	SHP2 (μM) ^a	P_{app} (10^{-6} cm/s) ^b
Na_3VO_4	0.062	0.018	0.081	ND

7a/7b	16.8/27.4	29.6/47.8	23.5/55.2	0.93/0.87
16a/16b	2.52/6.84	7.84/11.3	5.65/7.83	1.27/1.09
19a/19b	0.30/1.18	3.81/5.62	3.28/3.10	1.62/1.77
24a/24b	0.23/0.77	2.63/5.04	1.95/2.18	1.68/1.48
27a/27b	0.25/1.08	2.16/3.44	2.28/3.29	1.93/1.82
28a/28b	0.12/0.83	1.94/3.77	2.67/2.94	1.74/2.26
Atenolol ^c	-	-	-	0.84
Propranolol ^c	-	-	-	8.71

^a Values are means of triplicates, repeated two to three times.

^b Values of parallel artificial membrane permeation assay (PAMPA)

^c Controls in PAMPA. Atenolol represents medium membrane permeability, and propranolol represents high membrane permeability.

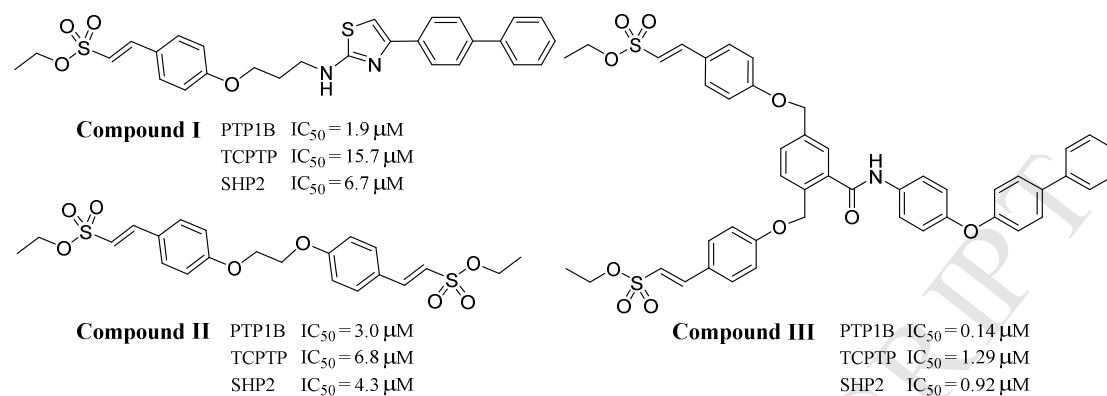
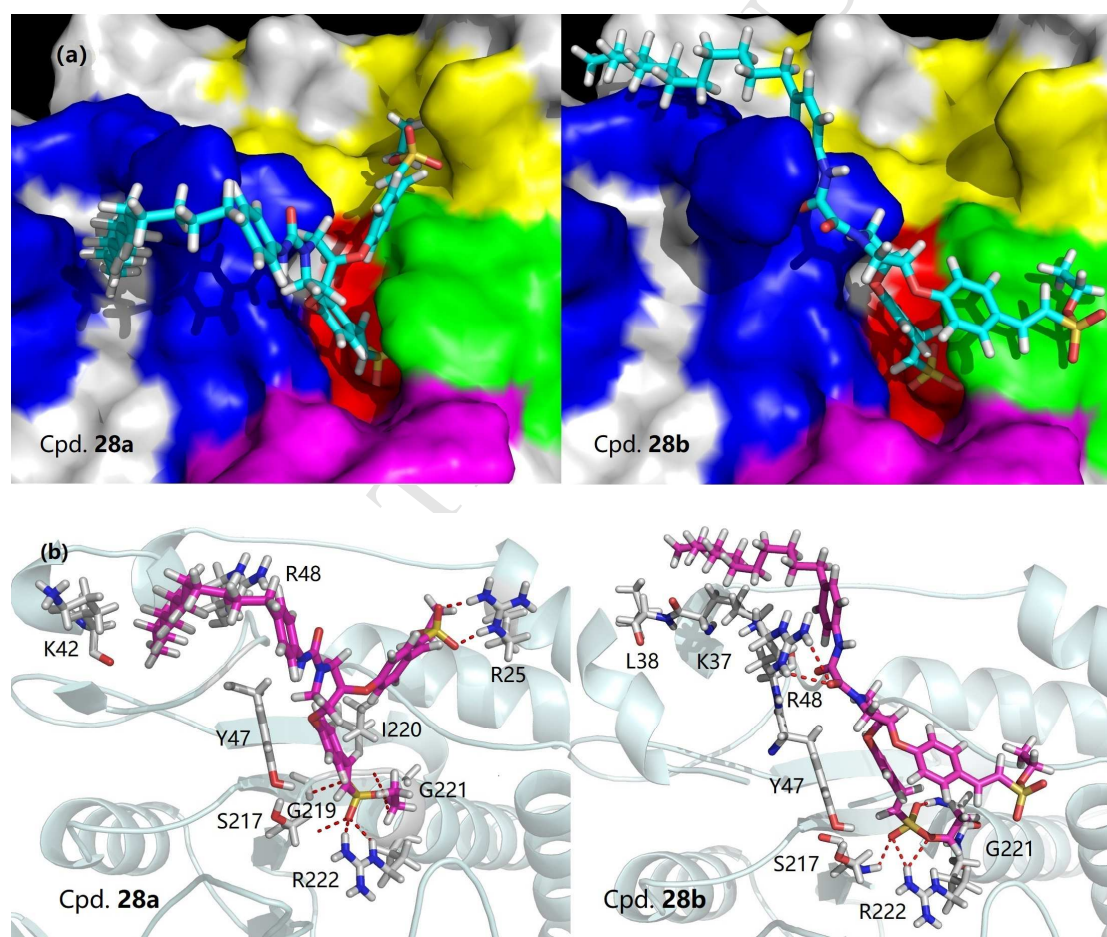
Fig (s)**Fig. 1.** Representative arylethanesulfonic acid ester PTP1B inhibitors.

Fig.2. Docking simulation of compound **28a** and **28b** with PTP1B (PDB code 2CNE). The figures were prepared by GOLD 5.1 and PyMol. (a) Overlay of **28a** and **28b** in the PTP1B active site. The catalytic binding site (A site) was displayed in red and the secondary binding sites (B, C, D and E site) were displayed in yellow, blue, magenta

and green respectively. (b) The interactions of compound **28a** and **28b** with PTP1B.

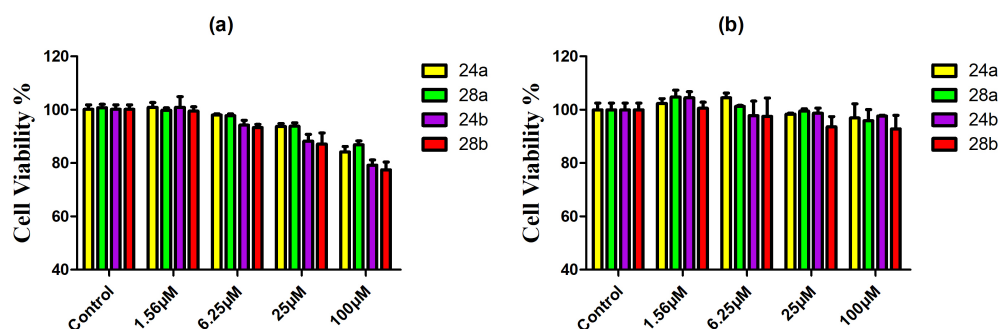


Fig.3. Inhibitory effects of compound **24a**, **24b**, **28a** and **28b** on cell viability. (a) Inhibitory effects of tested compounds on the viability of HepG2 cell. (b) Inhibitory effect of tested compounds on the viability of A549 cell. Cells were treated without or with 1.56, 6.25, 25 and 100 μ M compound **24a**, **24b**, **28a** and **28b** for 72 h. Each value was presented as mean \pm SD, n = 3.

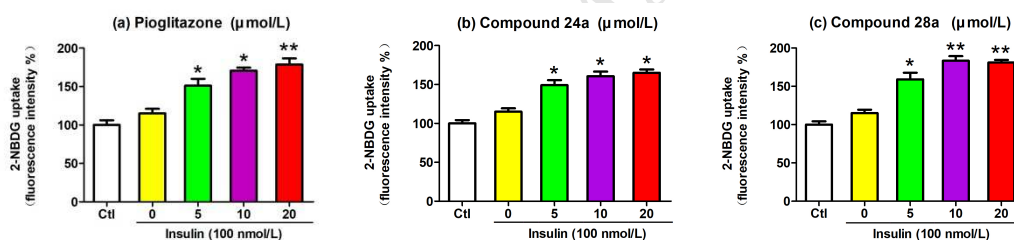


Fig. 4. Effects of compound **24a** and **28a** on insulin-stimulated glucose uptake. (a) Effects of Pioglitazone on insulin-stimulated glucose uptake. (b) Effects of compound **24a** on insulin-stimulated glucose uptake. (c) Effects of compound **28a** on insulin-stimulated glucose uptake. HepG2 cell was serum starved for 24 h and then incubated with varying concentrations of Pioglitazone, compound **24a**, and compound **28a** (0, 5, 10 and 20 μ M). After 4 h, insulin (100 nM) stimulated glucose uptake was evaluated using 2-NBDG as described in methods [36]. Each value was presented as mean \pm SD, n = 3; (*) P < 0.05 and (**) P < 0.01 vs the insulin-treated group.

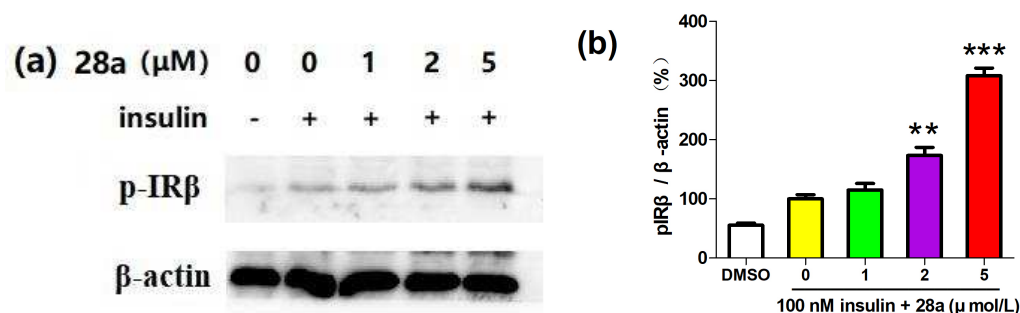
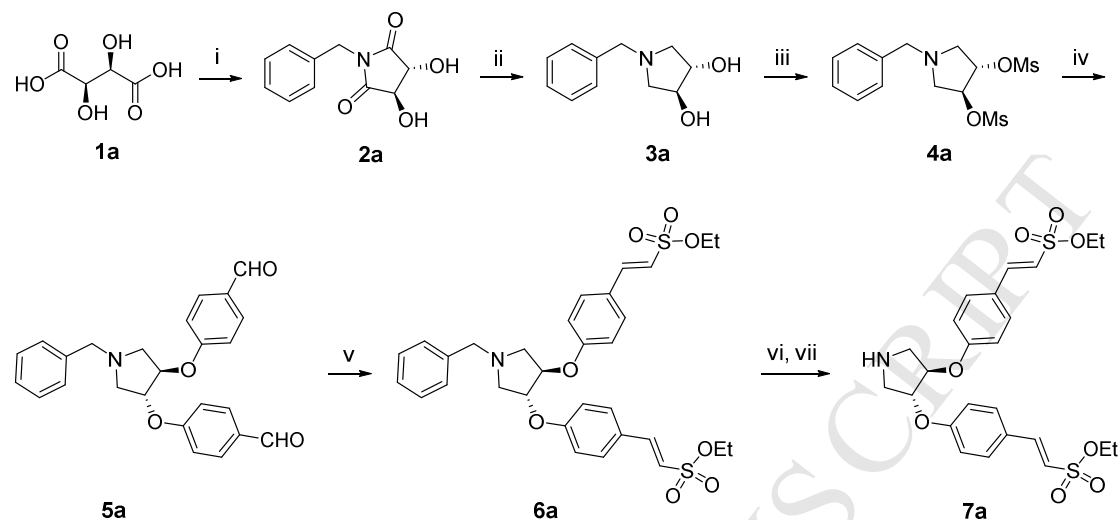
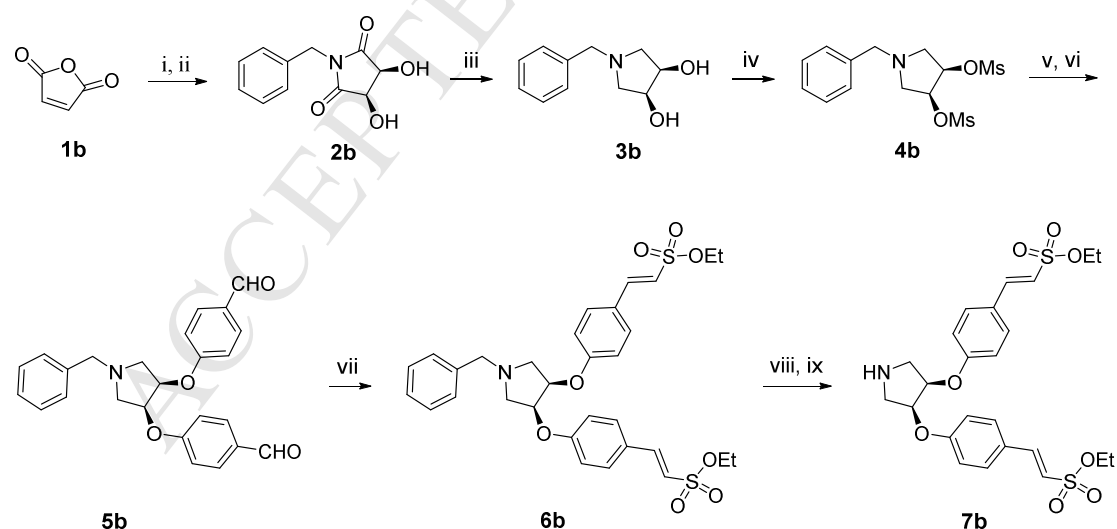


Fig. 5. Effect of compound **28a** on tyrosine phosphorylation of insulin receptor β (IR β). (a) Activation of insulin signaling during exposure of HepG2 to **28a**. (b) The relative density of p-IR β to β -actin. HepG2 cell was serum free starved for 4 h and then treated with a range of concentrations of compound **28a** (0, 1, 2 and 5 μ M) for 1 h, which was followed by a 15 min treatment with 100 nM insulin. Cell lysates were subjected to SDS-PAGE, and the resolved proteins were transferred to nitrocellulose membranes, which were incubated with anti-phospho-IR β . β -actin was used as an internal control. Each value was presented as mean \pm SD, n =3. (**) P < 0.01 vs control cells in the presence of insulin. (***) P < 0.001 vs control cells in the presence of insulin.

Scheme(s)

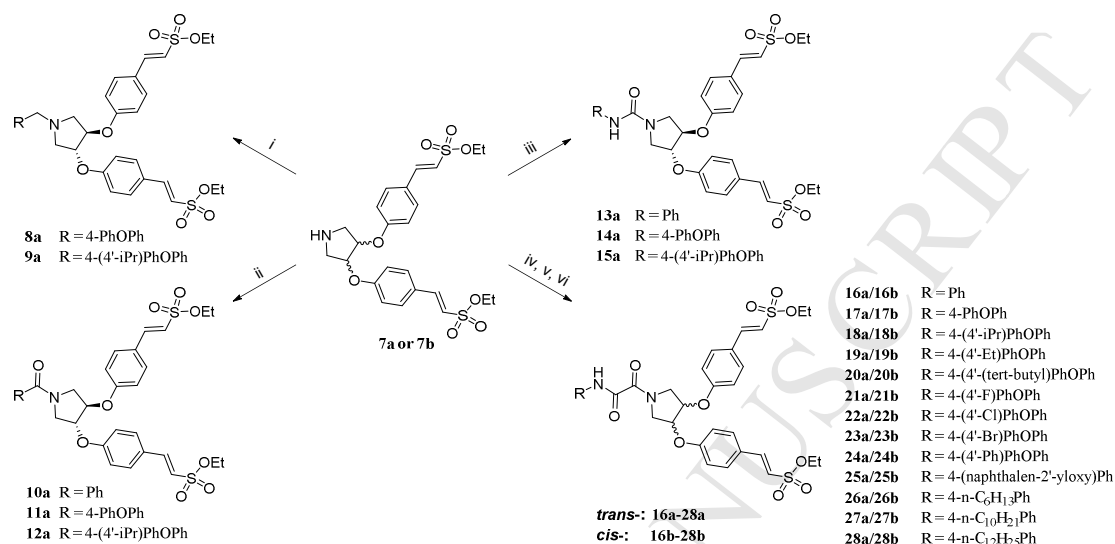


Scheme 1. Synthesis of intermediate **7a**. Reagents and conditions: (i) benzylamine, oxylene, reflux, 5 h; (ii) LiAlH_4 , THF, reflux, 24 h; (iii) $(\text{Ms})_2\text{O}$, Et_3N , DCM, rt, 6 h; (iv) *p*-hydroxybenzaldehyde, KI, K_2CO_3 , DMF, 100 °C, 8 h; (v) ethyl (diethoxyphosphoryl)methanesulfonate, NaH, THF, 0 °C-rt, 6 h; (vi) 1-chloroethyl chloroformate, 1,2-dichloroethane, reflux, 4 h; (vii) MeOH, reflux, 1 h.



Scheme 2. Synthesis of intermediate **7b**. Reagents and conditions: (i) benzylamine, AcOH, reflux, 5 h; (ii) $\text{Mn}(\text{ClO}_4)_2$, H_2O_2 , NaOAc, 2-picolinic acid, 0 °C-rt, 16 h; (iii) LiAlH_4 , THF, reflux, 24 h; (iv) $(\text{Ms})_2\text{O}$, Et_3N , DCM, rt, 6 h; (v) *p*-hydroxybenzaldehyde, KI, K_2CO_3 , DMF, 100 °C, 4 h; (vi) *p*-hydroxybenzaldehyde, KI, K_2CO_3 , DMF, 100 °C, 4 h; (vii) *p*-hydroxybenzaldehyde, KI, K_2CO_3 , DMF, 100 °C, 4 h; (viii) 1-chloroethyl chloroformate, 1,2-dichloroethane, reflux, 4 h; (ix) MeOH, reflux, 1 h.

KI, Cs₂CO₃, DMF, 120 °C, 24 h; (vii) ethyl (diethoxyphosphoryl)methanesulfonate, NaH, THF, rt, 6 h; (viii) 1-chloroethyl chloroformate, 1,2-dichloroethane, reflux, 4 h; (ix) MeOH, reflux, 1 h.



Scheme 3. Synthesis of stereoisomeric pyrrolidine bisarylethenesulfonic acid esters with different linkers. Reagents and conditions: (i) arylaldehyde, AcOH, NaBH(OAc)₃, DCM, rt, 8 h; (ii) arylformic acid, HBTU, DMAP, DCM, rt, 24 h; (iii) arylamine, triphosgene, Et₃N, DCM, rt, 1 h; (iv) ethyl oxalyl monochloride, Et₃N, DCM, rt, 8 h; (v) LiOH, THF/H₂O, rt, 0.5 h; (vi) arylamine, HBTU, DMAP, DCM, rt, 24 h.

Highlights:

1. Discovered a set of stereoisomeric PTP1B inhibitors with selectivity.
2. Explained the difference in activity/selectivity of *cis* and *trans*-isomers.
3. A preferred molecule (**28a**) demonstrated potential hypoglycemic effects.