



## Design, synthesis and in vitro cytotoxicity of novel dinuclear platinum(II) complexes

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### ABSTRACT

Five dinuclear platinum(II) complexes with a novel chiral ligand, 2-(((1*R*,2*R*)-2-aminocyclohexylamino)methyl)phenol (HL), were designed, prepared and spectrally characterized. In vitro cytotoxicity of all the resulting platinum(II) compounds was evaluated against human HEPG-2, A549 and HCT-116 cell lines, respectively. Results indicated that all compounds showed positive biological activity. Particularly, compound **D4** has lower IC<sub>50</sub> values than carboplatin toward HEPG-2 and A549, while compound **D5** shows better activity than carboplatin against A549.

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Cisplatin is one of the most successful and frequently used drugs in the cancer chemotherapy at present, especially for testicular cancer, for which the overall cure rate exceeds 90%, and is nearly 100% for early-stage disease.<sup>1–3</sup> However, the clinical application of cisplatin is greatly limited by its side effects including nephrotoxicity and neurotoxicity,<sup>4,5</sup> narrow range of activity, intrinsic and/or acquired resistance, and low aqueous solubility.<sup>6–8</sup> So far, much effort has been devoted to designing cisplatin analogues with reduced toxicity, improved clinical efficacy and broader anticancer spectrum, which has resulted in successful developments of several new anticancer platinum drugs. Among these drugs, carboplatin and oxaliplatin are their representatives.

Breaking through the set of classical structure–activity relationships (SAR) summarized by Cleare and Hoeschele,<sup>9</sup> more recently there have been efforts focused on the design of non-classical platinum complexes, such as orally active platinum(IV) complexes,<sup>10–12</sup> sterically hindered platinum(II) complexes,<sup>13–16</sup> trans-platinum complexes,<sup>17–19</sup> multinuclear platinum(II) complexes<sup>20–23</sup> and sulfur containing platinum(II) complexes,<sup>24–26</sup> etc.

Among those non-classical anticancer platinum multinuclear platinum complexes have attracted much attention. For instance, BBR3464, shown in Figure 1, is a trinuclear platinum complex owning 1,6-diaminohexyl chains as bridges to connect metal ions.<sup>27,28</sup> Much recently, Zhang et al. have reported a number of dinuclear platinum(II) complexes with iodide anions as the bridges, in which some compounds exhibited good antitumor activity.<sup>29</sup> The corresponding studies indicate that multinuclear platinum complexes,

as compared with conventional mononuclear platinum complexes, could not only deliver more platinum containing drugs to the tumor, but also form mutable-binding with tumor cell DNA and increase the activity to block DNA replication.<sup>30–32</sup>

In this letter, we report a set of dinuclear platinum(II) complexes with a new tridentate chiral ligand, 2-(((1*R*,2*R*)-2-aminocyclohexylamino)methyl)phenols (HL), which is derived from 1*R*,2*R*-diaminocyclohexane by several-step synthetic processes. With L<sup>−</sup> as a carrier ligand, and organic dicarboxylates or sulfate as leaving groups, five novel dinuclear platinum(II) complexes have been designed and synthesized, whose structures are illustrated in Figure 2.

For the synthesis of the ligand (HL), it is difficult to directly get the monosubstituted derivative due to the equivalent reactivity of the two amino groups in DACH. Thus, mono-Boc protecting DACH (**1**) was used as the starting material which was recently reported.<sup>33</sup> Detailed processes are as followed. Reaction of **1** with salicylaldehyde offered a mono-Schiff base which was then reduced by NaBH<sub>4</sub> to give intermediate **2**, then **2** was treated with HCl/EtOAc to remove the Boc group, leading to **3** of HL hydrochloride, which was finally neutralized by aqueous Na<sub>2</sub>CO<sub>3</sub> solution to give free HL (Scheme 1).

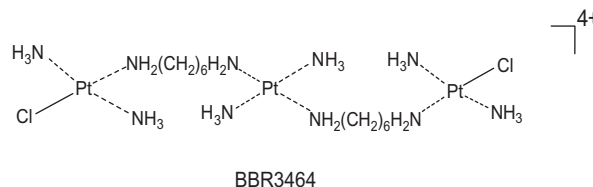
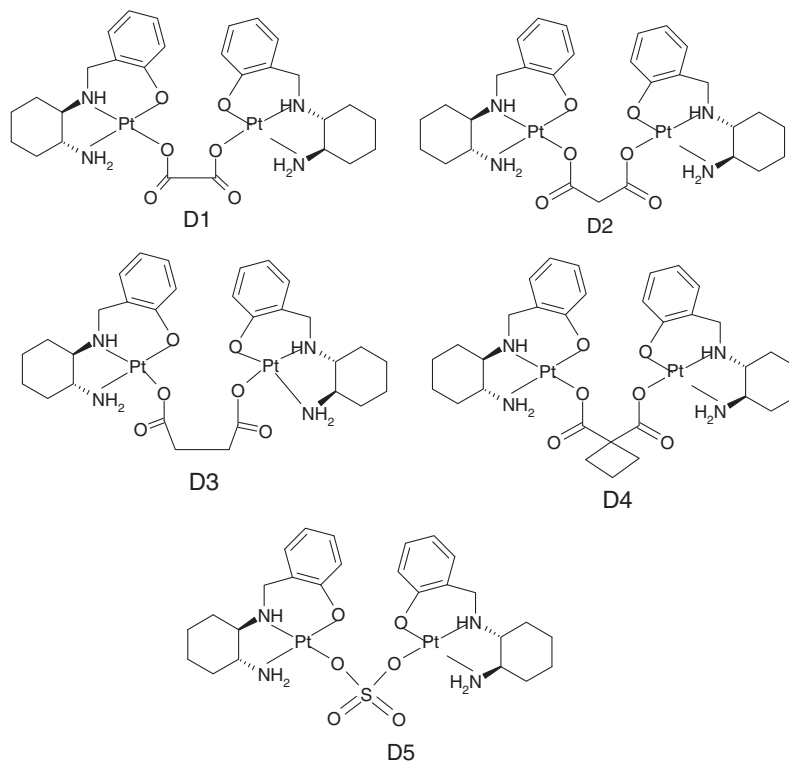


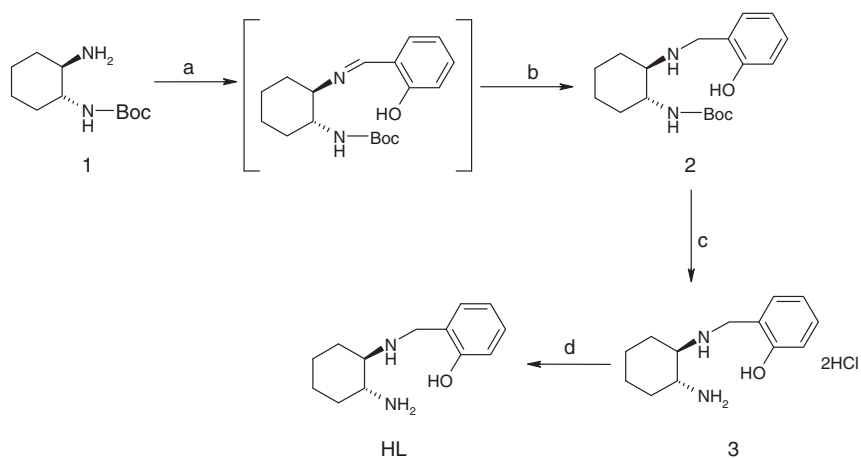
Figure 1. Structure of BBR3464.

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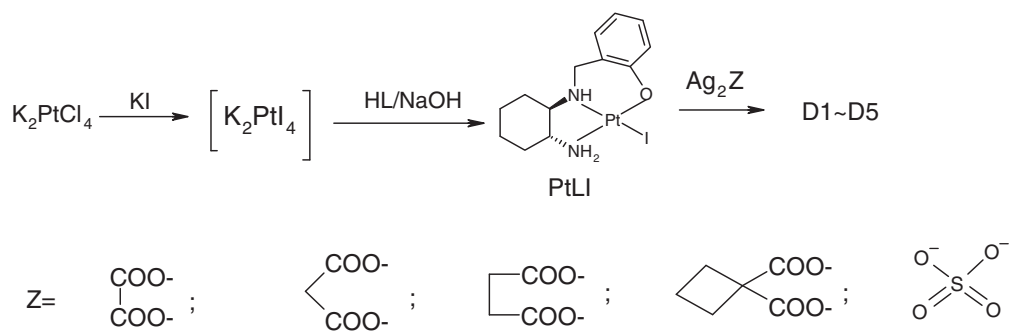
E-mail address: [sgou@seu.edu.cn](mailto:sgou@seu.edu.cn) (S. Gou).



**Figure 2.** Molecular structures of the resulting dinuclear platinum(II) complexes.



**Scheme 1.** Preparation of HL. Reagents and conditions: (a) salicylaldehyde, toluene, refluxing and water separating for 2 h; (b) NaBH<sub>4</sub>; (c) HCl/EtOAc, Et<sub>2</sub>O; (d) Na<sub>2</sub>CO<sub>3</sub>.



**Scheme 2.** Synthetic scheme for dinuclear platinum(II) complexes **D1–D5**.

When preparing the targeted platinum complexes, we first prepared the important intermediate [PtLI], which was then used to react with related silver dicarboxylates and silver sulfate, respectively, to afford compounds **D1–D5** (Scheme 2).

All compounds were spectrally characterized by IR,  $^1\text{H}$  NMR and ESI-MS spectra together with microanalyses.<sup>34,35</sup> Elemental analysis for each compound was in good agreement with the empirical formula proposed. In the IR spectra, the amino group participation in binding with Pt(II) was confirmed by the examination of  $\nu\text{NH}_2/\nu\text{NH}$  and  $\delta\text{NH}_2/\delta\text{NH}$  frequencies, which were shifted to lower frequencies comparing with the free amino group, due to Pt(II)- $\text{NH}_2$ /Pt(II)- $\text{NH}$  coordinations. The shifts of the C=O absorption from free carboxylic acids near  $1700\text{ cm}^{-1}$  to a band near  $1640\text{--}1593\text{ cm}^{-1}$  proved that the carboxylate anion was coordinated to  $\text{Pt}^{2+}$  in each case.<sup>36</sup> All dinuclear compounds showed peaks of  $[\text{M}-\text{Z}^{2-}+\text{CH}_3\text{O}^-]^+$  in their positive ESI mass spectra, several compounds gave  $[\text{M}+\text{Na}]^+$  or  $[\text{M}+\text{K}]^+$  peaks, which are in agreement with their molecular formula weights. For three isotopes of Pt element:  $^{194}\text{Pt}$ (33%),  $^{195}\text{Pt}$ (34%),  $^{196}\text{Pt}$ (25%), all the mass spectra of platinum compounds were found with three isotopic peaks. The molecular structures of all compounds showed in Figure 1 were also proved by their related  $^1\text{H}$  NMR spectral data.

To explore the stability of our compounds, compound **D4** in  $\text{D}_2\text{O}$  at room temperature was studied by  $^1\text{H}$  NMR.  $^1\text{H}$  NMR spectra were subsequently recorded at 0, 3, 12 and 24 h. All resulting spectra were compared each other and found that there were not any obvious differences, indicating that compound **D4** kept stable in water at room temperature.

Some platinum anticancer drugs, such as cisplatin, are limited due to their poor aqueous solubility. Thus, the aqueous solubility of all compounds was measured at  $25^\circ\text{C}$ . Compared with cisplatin whose aqueous solubility is only  $1\text{ mg/mL}$ , our platinum complexes have been improved, with aqueous solubility ranging from  $4.1$  to  $7.5\text{ mg/mL}$ . Due to the introduction of a chiral ligand, specific rotations of these dinuclear platinum complexes were tested by an automatic digital polarimeter. All compounds turned out to be optical with values of specific rotations from  $+32.0^\circ$  to  $+74.2^\circ$ , similar to oxaliplatin( $+76.1^\circ$ ). The corresponding values of aqueous solubility and specific rotations for the complexes are showed in Table 1.

In vitro cytotoxicity of the dinuclear platinum complexes was tested by MTT assay<sup>37,38</sup> using A549, HEPG-2 and HCT-116 cell lines. The  $\text{IC}_{50}$  values of these compounds as well as positive controls, cisplatin, carboplatin and oxaliplatin, are given in Table 2.

As we can see, most dinuclear platinum complexes showed positive cytotoxicity against selected cell lines. Compared with carboplatin, the target compounds showed potent antitumor activity against HEPG-2 cell line with  $\text{IC}_{50}$  values varying from  $15.1$  to  $48.5\text{ }\mu\text{M}$ , particularly, compound **D4** somehow showed a better activity than carboplatin. Compounds **D1** and **D5** also showed comparable activity to carboplatin, the order of cytotoxicity is cisplatin > oxaliplatin > **D4** > carboplatin > **D5** > **D1** > **D2** > **D3**. As for A549 cell line, compounds **D4** and **D5** gave lower  $\text{IC}_{50}$  values than carboplatin, the order of cytotoxicity is cisplatin > oxaliplatin > **D4**

**Table 2**

In vitro cytotoxicity against selected human tumor cell lines of complexes **D1–D5**

Complex	$\text{IC}_{50}^a$ ( $\mu\text{M}$ )		
	HEPG-2 <sup>b</sup>	A549 <sup>c</sup>	HCT-116 <sup>d</sup>
<b>D1</b>	26.3	18.6	48.2
<b>D2</b>	37.2	29.2	36.6
<b>D3</b>	48.5	>50	28.2
<b>D4</b>	15.1	4.2	9.6
<b>D5</b>	24.2	5.1	7.8
Carboplatin	22.2	9.9	Not tested
Cisplatin	0.318	1.1	Not tested
Oxaliplatin	5.3	2.3	4.3

<sup>a</sup> All  $\text{IC}_{50}$  values (drug concentration giving 50% survival) calculated based on the Pt content are means  $\pm$  SD (SD <12% of the mean value) from at least three separated experiments.

<sup>b</sup> Human hepatocellular carcinoma cell.

<sup>c</sup> Human lung cancer cell.

<sup>d</sup> Human colorectal cancer cell.

> **D5** > carboplatin > **D1** > **D2** > **D3**. When the cell line is HCT-116, **D4** and **D5** also showed comparable activities to oxaliplatin.

Based on the above results, it can be found that the selection of different bridges has a clear impact on the antitumor activities. When HEPG-2 and A549 cell lines were employed, the longer the dicarboxylate bridge is the lower the antitumor activity is. Interestingly, when a cyclobutyl moiety was introduced to the dicarboxylate bridge, the antitumor activity significantly increased, indicating the cyclobutyl fragment had a positive impact on the activity. Employing inorganic sulfate as a bridge instead of the organic dicarboxylate chain also achieved a good result, as the cytotoxicity of **D5** was obviously higher than that of **D1**, **D2** and **D3**. In particular, when HCT-116 cell line was tested, sulfate-bridged dinuclear complex showed better activity than all organic dicarboxylates. Notably, the length of the bridge showed a different influence on the cytotoxicity against HCT-116 cell line: **D3** had a longer bridge chain, but its cytotoxicity was higher than that of **D1** which contained a short bridge chain, suggesting antitumor activities may increase when carbon chains are prolonged against this cell line. One of important reasons that our prepared dinuclear complexes showed positive cytotoxicity against selected cell lines may be attributed to their stereo-structures similar to oxaliplatin, because the ligand, HL, was synthetically derived from 1*R*,2*R*-diaminocyclohexane without changing its absolute configuration. Apart from the above, steric effect in the compound could also play a significant role. For example, complex **D4** with 1,1-cyclobutyl dicarboxylate as the bridge, had the minimum specific rotation of  $+32.0^\circ$  but showed the relatively good cytotoxicity. We speculated steric effect may produce between the cyclobutyl moiety and the aromatic species. When entering the tumor cell, the steric effect could make complex **D4** more easier than other dinuclear complexes lose the bridge and form positive ions to bind with DNA.

In conclusion, with a monosubstituted chiral DACH derivative (HL) as a carrier group, we designed and synthesized five novel dinuclear platinum complexes which have some organic dicarboxylates/sulfate as bridges. All of the target compounds showed better aqueous solubility than cisplatin. In vitro cytotoxicity tests showed that most prepared dinuclear complexes gave positive antitumor activity against selected cell lines. Compound **D4** which takes 1,1-cyclobutyl dicarboxylate as a bridge not only showed better antitumor activity against HEPG-2 and A549 than carboplatin, but also showed a comparable activity against HCT-116 to oxaliplatin. Besides, compound **D5** which takes sulfate as a bridge also exhibited better activity than carboplatin against A549. Consequently, the obtained dinuclear platinum complexes, especially compound **D4**, may be deserved for further investigation.

**Table 1**  
Aqueous solubility and specific rotations of complexes **D1–D5**

Complex	Aqueous solubility (mg/mL, $25^\circ\text{C}$ )	$[\alpha]_D^{15}$ (c 1, MeOH)
<b>D1</b>	5.6	+74.2
<b>D2</b>	6.2	+46.2
<b>D3</b>	5.0	+85.4
<b>D4</b>	4.1	+32.0
<b>D5</b>	7.5	+59.5

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- Synthesis of complex [PtLI]*: To a stirring aqueous solution (40 ml) of KI (80 mmol), K<sub>2</sub>PtCl<sub>4</sub> (12 mmol) in water (40 ml) was added. The blending solution was stirred at 25 °C for 30 min under a nitrogen atmosphere to get a black solution of K<sub>2</sub>PtI<sub>4</sub>. Then an aqueous solution (40 ml) of HL (12 mmol) and NaOH (12 mmol) was added dropwise under stirring in the dark at 25 °C. After 6 h, the dark yellow precipitate was filtered, washed sequentially with water, ethanol and ether, then dried in vacuum.  
*Data for [PtLI]*: yield: 95%, dark yellow solid. IR( $\nu$ , cm<sup>-1</sup>): 3418m(br), 3219m, 3180m, 2933m, 2859m, 1594m, 1562m, 1480s, 1448s, 1289m, 1266m, 1038w, 876w, 748m, 591w, 457m. <sup>1</sup>H NMR (DMSO/TMS):  $\delta$  6.72–7.09 (m, 4H, 4H of C<sub>6</sub>H<sub>4</sub>), 5.83–6.54 (m, 3H, 3H of NH<sub>2</sub> and NH), 3.88–4.09 (m, 2H of CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 1.06–2.74 (m, 10H of DACH). ESI-MS:  $m/z$  [M+H]<sup>+</sup> = 541 (60%), [M+Na]<sup>+</sup> = 564 (100%). Anal. (C<sub>13</sub>H<sub>19</sub>N<sub>2</sub>O<sub>6</sub>PT) C, H, N.
- Synthesis of complexes: D1, D2, D3, D4 and D5.*  
A suspension of the corresponding silver dicarboxylate or Ag<sub>2</sub>SO<sub>4</sub> (1 mmol) and [PtLI] (2 mmol) in 100 ml water was stirred at 60 °C under a nitrogen atmosphere in the dark for 24 h, the resulting AgI deposit was filtered off and washed with water for two times. The filtrate was evaporated to nearly dryness and some white solids precipitated, which were washed with water and ethanol for several times, and dried in vacuum.  
*Data for D1*: Yield: 32%, white solid. IR( $\nu$ , cm<sup>-1</sup>): 3403s(br), 3185s, 2935s, 2860s, 1640vs, 1594vs, 1480s, 1448s, 1385m, 1292vs, 1148m, 1115m, 1038m, 877m, 763s, 516m, 464m. <sup>1</sup>H NMR (DMSO/TMS):  $\delta$  6.51–6.90 (m, 8H of 2C<sub>6</sub>H<sub>4</sub>), 5.10–6.36 (m, 6H of 2NH<sub>2</sub> and 2NH), 3.73–4.18 (m, 4H of 2CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 0.85–2.73 (m, 20H, 20H of 2DACH). ESI-MS:  $m/z$  [M–C<sub>2</sub>O<sub>4</sub><sup>2-</sup>+CH<sub>3</sub>O<sup>-</sup>]<sup>+</sup> = 859(60%), [M+K]<sup>+</sup> = 955(40%). Anal. (C<sub>28</sub>H<sub>38</sub>N<sub>4</sub>O<sub>6</sub>PT<sub>2</sub>) C, H, N.  
*Data for D2* yield: 32%, white solid. IR( $\nu$ , cm<sup>-1</sup>): 3184s(br), 2934s, 2859s, 1593vs, 1480s, 1448s, 1357s, 1296s, 1267s, 1114m, 1039m, 963m, 877w, 752m, 622w, 466m. <sup>1</sup>H NMR (DMSO/TMS):  $\delta$  6.50–6.96 (m, 8H of 2C<sub>6</sub>H<sub>4</sub>), 5.02–6.31 (m, 6H of 2NH<sub>2</sub> and 2NH), 3.87–4.08 (m, 4H of 2CH<sub>2</sub>C<sub>6</sub>H<sub>4</sub>), 2.68–2.70 (m, 2H of COOCH<sub>2</sub>COO), 1.08–2.63 (m, 20H of 2DACH). ESI-MS:  $m/z$  [M–C<sub>3</sub>O<sub>4</sub>H<sub>2</sub><sup>2-</sup>+CH<sub>3</sub>O<sup>-</sup>]<sup>+</sup> = 859 (100%). Anal. (C<sub>29</sub>H<sub>40</sub>N<sub>4</sub>O<sub>6</sub>PT<sub>2</sub>) C, H, N.  
*Data for D3* yield: 21%, white solid. IR( $\nu$ , cm<sup>-1</sup>): 3391s, 3184s(br), 2936s, 2860s, 1594vs, 1560s, 1480s, 1449s, 1385s, 1294s, 1268s, 1151m, 1039m, 877m, 753m, 570m, 467m. <sup>1</sup>H NMR (DMSO/TMS):  $\delta$  6.32–7.12 (m, 8H of 2C<sub>6</sub>H<sub>4</sub>), 4.98–6.31 (m, 6H of 2NH<sub>2</sub> and 2NH), 3.70–4.12 (m, 4H of 2CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 0.90–2.45 (m, 24H, 20H of 2DACH and 4H of –C<sub>4</sub>O<sub>4</sub>H<sub>4</sub><sup>2-</sup>). ESI-MS:  $m/z$  [M–C<sub>4</sub>O<sub>4</sub>H<sub>4</sub><sup>2-</sup>+CH<sub>3</sub>O<sup>-</sup>]<sup>+</sup> = 859 (100%), [M+H]<sup>+</sup> = 945(45%), [M+Na]<sup>+</sup> = 967(13%). Anal. (C<sub>30</sub>H<sub>42</sub>N<sub>4</sub>O<sub>6</sub>PT<sub>2</sub>) C, H, N.  
*Data for D4* yield: 45%, white solid. IR( $\nu$ , cm<sup>-1</sup>): 3434s, 3185s(br), 2938s, 2861s, 1596vs, 1565s, 1481s, 1449s, 1361s, 1294s, 1268s, 1115m, 1039m, 877m, 754s, 568m, 449m. <sup>1</sup>H NMR (DMSO/TMS):  $\delta$  6.39–7.05 (m, 8H of 2C<sub>6</sub>H<sub>4</sub>), 4.86–6.26 (m, 6H of 2NH<sub>2</sub> and 2NH), 3.74–4.18 (m, 4H of 2CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 0.98–2.81 (m, 26H, 20H of 2DACH and 6H of C<sub>6</sub>O<sub>4</sub>H<sub>6</sub><sup>2-</sup>). ESI-MS:  $m/z$  [M–C<sub>4</sub>O<sub>4</sub>H<sub>6</sub><sup>2-</sup>+CH<sub>3</sub>O<sup>-</sup>]<sup>+</sup> = 859 (100%), [M+Na]<sup>+</sup> = 993(40%). Anal. (C<sub>32</sub>H<sub>44</sub>N<sub>4</sub>O<sub>6</sub>PT<sub>2</sub>) C, H, N.  
*Data for D5* yield: 36%, white solid. IR( $\nu$ , cm<sup>-1</sup>): 3432s(br), 3185s, 2936s, 2861s, 1596s, 1565s, 1481s, 1449s, 1294s, 1268s, 1118vs, 1115m, 1038s, 950m, 877m, 753s, 618m, 465m. <sup>1</sup>H NMR (DMSO/TMS):  $\delta$  6.52–7.02 (m, 8H of 2C<sub>6</sub>H<sub>4</sub>), 4.93–6.47 (m, 6H of 2NH<sub>2</sub> and 2NH), 3.75–4.20 (m, 4H of 2CH<sub>2</sub>C<sub>6</sub>H<sub>5</sub>), 1.05–3.01 (m, 20H of 2DACH). ESI-MS:  $m/z$  [M–SO<sub>4</sub><sup>2-</sup>+CH<sub>3</sub>O<sup>-</sup>]<sup>+</sup> = 859 (100%). Anal. (C<sub>26</sub>H<sub>38</sub>N<sub>4</sub>SO<sub>6</sub>PT<sub>2</sub>) C, H, N.
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