

## Lysinyl macrocyclic hexaoxazoles: Synthesis and selective G-quadruplex stabilizing properties

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Received 21 November 2007; revised 17 December 2007; accepted 19 December 2007

Available online 14 January 2008

**Abstract**—Macrocyclic hexaoxazoles having one or two lysinyl side chains in which the terminal nitrogen is either a primary amine, *N,N*-dimethylamine, or an acetamide have been synthesized. Sodium ion has been found to be beneficial to the macrocyclization step by acting as a template around which the linear polyoxazole can organize. Each of the targeted compounds selectively stabilizes G-quadruplex versus duplex DNA. Compounds with one valine and one lysine residue display the best combination of G-quadruplex stabilizing ability with no detectable stabilization of duplex DNA.

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The stabilization of G-quadruplex DNA represents a new strategy for the treatment of cancer.<sup>1</sup> G-quadruplex structures have been identified in the G-rich tails of telomeres as well as in the promoter regions of certain oncogenes, including *c-myc* and *KRAS*.<sup>2</sup> Telomerase cannot access the telomere while it is in the G-quadruplex conformation. Compounds that stabilize telomeric G-quadruplexes therefore indirectly inhibit telomerase and allow the telomere to become truncated until a critical length is reached, at which point DNA damage responses are initiated, including cell cycle arrest, senescence, and apoptosis.<sup>3</sup> Stabilization of G-quadruplex conformations formed within the promoter region of oncogenes is associated with the decreased expression of such oncogenes.<sup>2e,f,4</sup>

A large number of compounds are reported to stabilize G-quadruplex DNA although most lack selectivity over duplex and triplex DNA. Telomestatin, a 24-membered macrocycle comprised of seven oxazoles and one thiazoline, is a natural product isolated from *Streptomyces anulatus* 3533-SV4.<sup>5</sup> Until recently, telomestatin was

the most potent and selective G-quadruplex stabilizer identified. Computational studies suggest that the exceptional activity of telomestatin may be associated with  $\pi$ -stacking interactions and hydrogen bonding with the G-quadruplex, with which it is comparable in size.<sup>6</sup> Recently a 24-membered macrocycle containing six oxazole moieties and two valine residues (HXDV) was synthesized and found to stabilize G-quadruplex DNA with no detectable levels of duplex or triplex DNA stabilization.<sup>7</sup> It has been determined that HXDV binds with a stoichiometry of 2:1 to G-quadruplex DNA by capping at both ends as opposed to intercalation between G-tetrads.<sup>8</sup> HXDV is cytotoxic toward human lymphoblastoma RPMI 8402 cells with an  $IC_{50}$  value of 0.4  $\mu$ M.<sup>7</sup>

One disadvantage of HXDV is its limited solubility in aqueous solution. It was reasoned that the replacement of one or both isopropyl groups of the macrocycle with basic side chains would allow for the preparation of more water-soluble derivatives. These basic side chains might also exhibit increased G-quadruplex stabilizing activity by providing an additional point(s) of interaction with the quadruplex, namely the formation of salt bridges with the phosphate backbone. In this report the synthesis of macrocyclic hexaoxazoles having one or two 4-aminobutyl (lysyl) side chains is described. For the monolysinyl compound **1a** the *N,N*-dimethylamino and acetamide derivatives were also prepared

**Keywords:** Macrocyclic hexaoxazoles; G-quadruplex stabilizers; Synthesis; Lysine; Selective.

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(Fig. 1). The effect of these structural modifications on selectivity for G-quadruplex DNA and on the degree of stabilization of the quadruplex has been determined.

The synthetic plan for these target molecules requires the coupling of two teroxazoles followed by macrocyclization. In each instance a teroxazole having a lysinyl moiety at the 2-position is required. It was reasoned that protection of the C6-amino group of lysine as a *tert*-butyl carbamate (Boc) would ensure that amidation reactions occur selectively at the C2-amino. *N*<sup>6</sup>-Boc-*N*<sup>2</sup>-Cbz-L-lysine was prepared from *N*-Cbz lysine using Boc<sub>2</sub>O (Scheme 1). This was coupled with serine methyl ester hydrochloride using BOP to give amide **3** in high yield. Treatment with DAST afforded the oxazoline which was aromatized by treatment with BrCCl<sub>3</sub> and DBU.<sup>9,10</sup> Hydrolysis of the ester with LiOH afforded acid **4** in 73% overall yield (five steps) from *N*<sup>2</sup>-Cbz-L-lysine.

Acid **4** was coupled with O-TIPS serine-derived amino oxazole **5**<sup>7</sup> using EDC and HOBT to afford a bis(oxazolyl)amide. This was subjected to desilylation using HF/pyridine and then cyclization/aromatization using the DAST/BrCCl<sub>3</sub> protocol to give teroxazole **6** in 45% yield for the four steps (Scheme 2).

Ester **6** was hydrolyzed with LiOH to afford acid **7** in quantitative yield and this was then coupled with valine-derived amino teroxazole **8**<sup>7</sup> to yield a linear hexaoxazole in 85% yield (Scheme 3). The Cbz group was removed by transfer hydrogenolysis and the remaining ester was then hydrolyzed with LiOH to give an amino acid. When macrocyclization of this hexaoxazole was affected using HATU with *N*-methylmorpholine in DMF at high dilution, cyclic hexaoxazole **9** was formed in 54% yield, accompanied by a small amount of a by-product tentatively identified as imide **10**. The imide was likely formed by deprotonation of the amide and intramolecular attack on the *tert*-butyl carbamate moiety of the side chain, with displacement of *tert*-butanol. Attempts to hydrolyze the imide under acidic or basic conditions led to decomposition. Treatment of **9** with TFA removed the Boc group to afford amine **1a** (HXLV) in quantitative yield. A portion of **1a** was treated with Ac<sub>2</sub>O in pyridine to give HXLV-AC, **1b** in 72% yield. Treatment of another portion of **1a** with aqueous formaldehyde and sodium borohydride gave HXLV-DM, **1c**, in 67% yield.<sup>11</sup>

The synthesis of dilysinyl analog **2** is outlined in Scheme 4. A portion of teroxazole **6** was subjected to hydrogenolysis and the resulting amine was coupled with acid **7** to

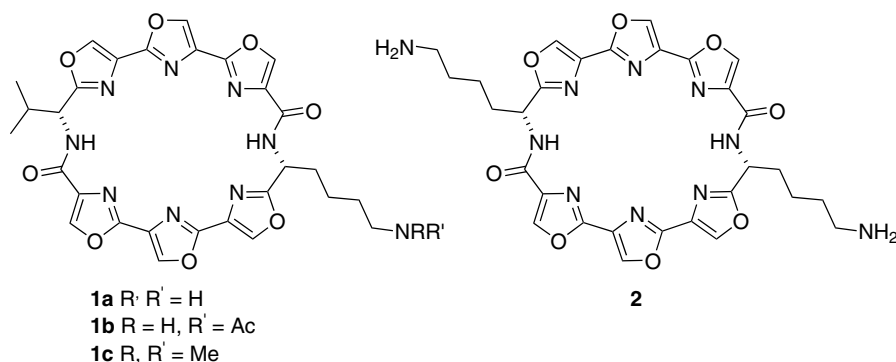
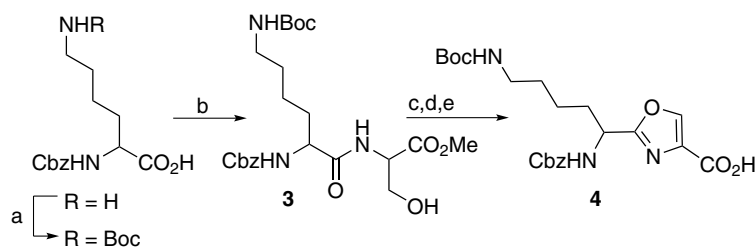
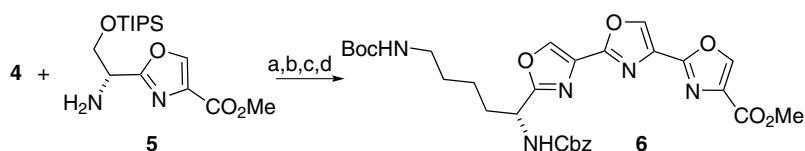


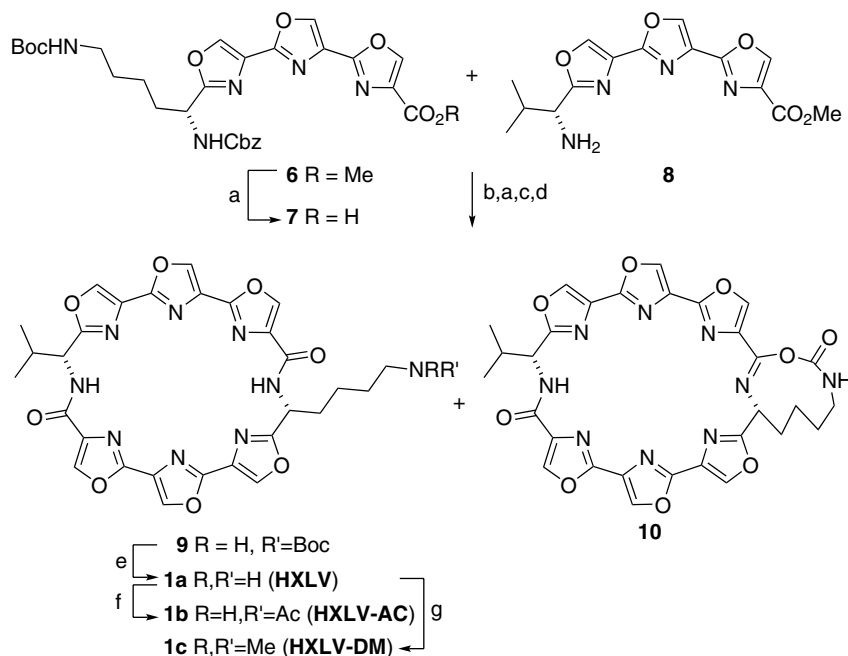
Figure 1. Structures of target molecules.



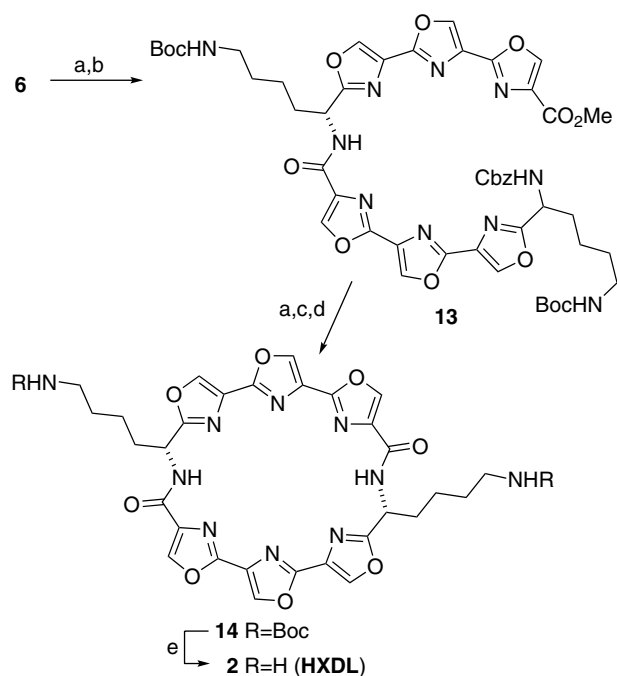
Scheme 1. Reagents: (a) Boc<sub>2</sub>O, NaHCO<sub>3</sub>; (b) serine Me ester HCl, BOP, Et<sub>3</sub>N, CH<sub>3</sub>CN; (c) DAST, K<sub>2</sub>CO<sub>3</sub>; (d) BrCCl<sub>3</sub>, DBU; (e) LiOH, THF/H<sub>2</sub>O.



Scheme 2. Reagents: (a) EDC, HOBT, 2,6-lutidine, CH<sub>2</sub>Cl<sub>2</sub>; (b) HF/pyridine, THF; (c) DAST, K<sub>2</sub>CO<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>; (d) BrCCl<sub>3</sub>, DBU, CH<sub>2</sub>Cl<sub>2</sub>.



**Scheme 3.** Reagents and conditions: (a) LiOH, THF/H<sub>2</sub>O; (b) EDC, HOBT, 2,6-lutidine, DMF; (c) 1,4-cyclohexadiene, 20% Pd(OH)<sub>2</sub>/C, THF/EtOH, Δ, 2d; (d) HATU, NMM, DMF; (e) TFA, CH<sub>2</sub>Cl<sub>2</sub>; (f) Ac<sub>2</sub>O, pyridine; (g) MeOH, HCl, HCHO/H<sub>2</sub>O, NaBH<sub>4</sub>.



**Scheme 4.** Reagents and conditions: (a) 20% Pd(OH)<sub>2</sub>/C, cyclohexadiene, Δ, 3d; (b) 7, EDC, HOBT, 2,6-lutidine, DMF; (c) LiOH, THF/H<sub>2</sub>O; (d) HATU, NMM, DMF/CH<sub>2</sub>Cl<sub>2</sub>; (e) TFA, CH<sub>2</sub>Cl<sub>2</sub>.

give amide **13**. After sequential deprotection of the ester and Cbz groups, macrocyclization was achieved using HATU to give the desired product **14** in low yield (7%). A significant amount of a by-product, similar to cyclic imide **10**, was also obtained from this reaction. This by-product could not be converted into **14**. Changing the reagent from HATU to BOP led to an improvement in the yield of **14** without forming the by-product.

A chance observation in which a linear hexaoxazole was found by high resolution mass spectrometry to be tightly bound to a sodium ion suggested that organization of the cyclization precursor around a metal cation template might enhance the rate and yield of the macrocyclization process. The template effect of sodium and potassium ions on the formation of **14** was evaluated. These results are summarized in Table 1. The addition of 5 equiv of NaI resulted in a 60% increase in the yield of **14**. Additional NaI did not significantly improve the yield. Potassium ions actually had a deleterious effect on the macrocyclization. The Boc groups were removed from **14** in quantitative yield upon treatment with TFA in CH<sub>2</sub>Cl<sub>2</sub> to give **2** (HXDL).<sup>11</sup>

HXDV, HXLV (**1a**), HXLV-AC (**1b**), HXLV-DM (**1c**), and HXDL (**2**) were evaluated for their abilities to bind and thermally stabilize G-quadruplex and duplex DNA in the presence of K<sup>+</sup> ions. We used salmon testes DNA (ST-DNA) and d(T<sub>2</sub>AG<sub>3</sub>)<sub>4</sub> as representative models for duplex and G-quadruplex DNA, respectively. Patel and coworkers have shown that the structure adopted by d(T<sub>2</sub>AG<sub>3</sub>)<sub>4</sub> in K<sup>+</sup> solution is an intramolecular (3 + 1)

**Table 1.** Effect of salts on the formation of **14**<sup>a</sup>

Additive	Equivalents	Yield of <b>14</b> (%)
None	—	30
NaI	5	48
	10	50
KI	5	20
	10	27

<sup>a</sup> Reactions were performed by slow addition (syringe pump, 1 h) of a solution of deprotected **13** (27 μmol) in DMF (10 mL) to a solution of BOP (1.5 equiv), HOBT (1.5 equiv), the salt, and *i*-Pr<sub>2</sub>NEt (3 equiv) in 20 mL of DMF at 0 °C.

G-quadruplex in which three strands are oriented in one direction and the fourth strand is oriented in the opposite direction.<sup>12</sup> Figure 2 shows the UV melting profiles (depicted in their first-derivative forms) of d(T<sub>2</sub>AG<sub>3</sub>)<sub>4</sub> and ST-DNA in the absence and presence of HXDV, HXLV, HXLV-AC, and HXDL. The transition temperatures ( $T_{\text{tran}}$ ) corresponding to the maxima or minima of these first-derivative melting profiles are listed in Table 2, as are the corresponding  $T_{\text{tran}}$  values derived from the melting profile (not shown) conducted in the presence of HXDV, HXLV-DM. The presence of HXDV, HXLV, HXLV-DM, or HXLV-AC does not alter the thermal stability of ST-DNA, with any observed changes in  $T_{\text{tran}}$  being within the experimental uncertainty. This observation is consistent with little or no duplex DNA binding on the part of these four compounds. With regard to HXDL, the observed behavior confirms

that which we have previously reported.<sup>7,8</sup> The presence of HXDL increases the  $T_{\text{tran}}$  value of ST-DNA by 2.5 °C. Thus, unlike the monolysiny compounds, the dilysiny compound appears to exhibit some degree of duplex DNA binding under the solution conditions employed.

In marked contrast to their negligible impacts on ST-DNA thermal stability, HXDV, HXLV, HXLV-DM, and HXLV-AC increase the  $T_{\text{tran}}$  value of d(T<sub>2</sub>AG<sub>3</sub>)<sub>4</sub> by 24.5, 37.5, 28.5, and 26.5 °C, respectively. Although HXDL does increase the thermal stability of ST-DNA ( $\Delta T_{\text{tran}} = 2.5$  °C), the extent to which it increases the thermal stability of d(T<sub>2</sub>AG<sub>3</sub>)<sub>4</sub> is significantly greater ( $\Delta T_{\text{tran}} = 49.0$  °C). Taken together, our UV melting data indicate that the divaliny hexaoxazole HXDV, the monolysiny and dilysiny hexaoxazoles bind to G-quadruplex DNA with a high degree of specificity.

In summary, macrocyclic hexaoxazoles having one or two lysiny side chains in which the terminal nitrogen is present as either a primary amine, *N,N*-dimethylamine, or an acetamide have been synthesized. Sodium ion has been found to be beneficial to the macrocyclization step by acting as a template around which the linear polyoxazole can organize. All four targeted compounds selectively stabilize G-quadruplex DNA. HXLV provides the greatest degree of G-quadruplex stabilization with no detectable stabilizing effect on duplex DNA.

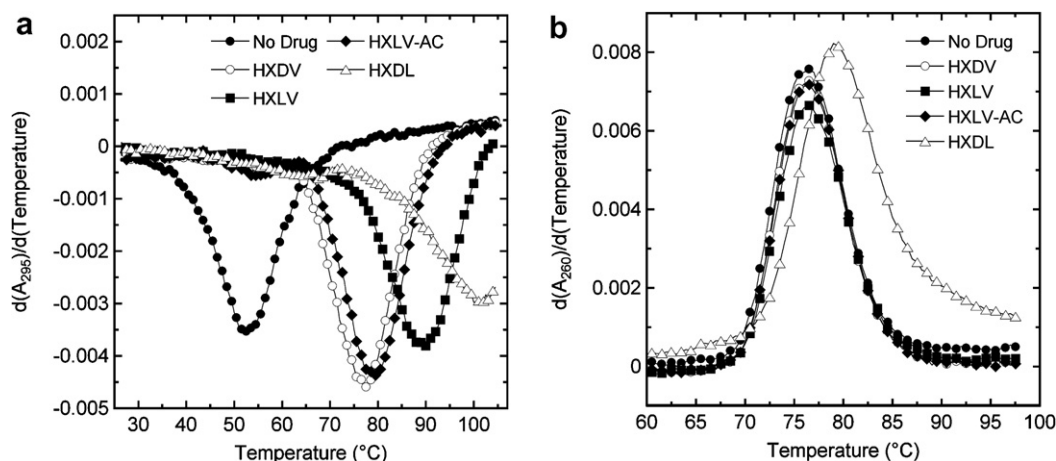
### Acknowledgments

This study was supported by NIH Grants CA098127 (E.J.L.) and CA097123 (D.S.P.). Mass spectrometry was provided by the Washington University Mass Spec-

**Table 2.** Transition temperatures for the melting of ST-DNA and d(T<sub>2</sub>AG<sub>3</sub>)<sub>4</sub> in the absence and presence of macrocyclic lysiny-containing hexaoxazoles

Sample	$T_{\text{tran}}^a$ (°C)
ST-DNA	76.5
ST-DNA + HXDV	76.5
ST-DNA + HXLV	76.5
ST-DNA + HXLV-DM	76.0
ST-DNA + HXLV-AC	76.5
ST-DNA + HXDL	79.0
d(T <sub>2</sub> AG <sub>3</sub> ) <sub>4</sub>	52.5
d(T <sub>2</sub> AG <sub>3</sub> ) <sub>4</sub> + HXDV	77.0
d(T <sub>2</sub> AG <sub>3</sub> ) <sub>4</sub> + HXLV	90.0
d(T <sub>2</sub> AG <sub>3</sub> ) <sub>4</sub> + HXLV-DM	81.0
d(T <sub>2</sub> AG <sub>3</sub> ) <sub>4</sub> + HXLV-AC	79.0
d(T <sub>2</sub> AG <sub>3</sub> ) <sub>4</sub> + HXDL	101.5

<sup>a</sup> DNA transition temperatures ( $T_{\text{tran}}$ ) reflect the maxima or minima of the first derivatives of UV melting profiles acquired as described in the legend to Figure 2. The uncertainty in the  $T_{\text{tran}}$  values is  $\pm 0.5$  °C.



**Figure 2.** First derivatives of the UV melting profiles of d(T<sub>2</sub>AG<sub>3</sub>)<sub>4</sub> (profile A) and ST-DNA (profile B) in the absence and presence of macrocyclic lysiny-containing hexaoxazoles. Profiles were acquired on an AVIV model 14NT-UV-VIS spectrophotometer using quartz cuvettes with a 1 cm pathlength. The temperature was raised in 0.5 °C increments and the samples were allowed to equilibrate for 1.5 min at each temperature setting, whereupon absorbances were recorded over a period of 5 s and averaged. When present, ST-DNA was used at a base pair concentration of 15  $\mu$ M, while d(T<sub>2</sub>AG<sub>3</sub>)<sub>4</sub> was used at a strand concentration of 4  $\mu$ M. Macrocyclic ligands were used at a concentration of 15  $\mu$ M in the ST-DNA experiments and 20  $\mu$ M in the d(T<sub>2</sub>AG<sub>3</sub>)<sub>4</sub> experiments. The solution conditions were 10 mM EPPS (pH 7.5) and sufficient KCl to bring the total K<sup>+</sup> concentration to 50 mM. In the ST-DNA experiments, the acquisition wavelength was 260 nm, while being 295 nm in the d(T<sub>2</sub>AG<sub>3</sub>)<sub>4</sub> experiments.

trometry Resource with support from the NIH National Center for Research Resources (Grant No. P41RR0954).

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- The structures of all compounds were determined using  $^1\text{H}$  (200 MHz) and  $^{13}\text{C}$  NMR (50 MHz) in  $\text{CDCl}_3$  unless otherwise noted. Results are reported as ppm downfield from internal TMS: HXLV, **1a**: mp 297–300 °C (TFA salt);  $[\alpha]_{\text{D}}^{20} -20.6^\circ$  (*c* 0.18, 10% MeOH/ $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.31 (m, 8H), 5.37 (m, 2H), 2.85 (m, 2H), 2.44 (m, 1H), 1.93 (m, 2H), 1.29 (m, 2H), 0.98 (m, 8H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3/\text{CD}_3\text{OD}$ )  $\delta$  164.9, 164.0, 159.3, 155.6, 153.8, 140.4, 139.8, 139.2, 138.5, 138.3, 136.0, 135.6, 129.5, 128.2, 128.1, 63.0, 52.6, 39.3, 32.5, 32.0, 26.2, 20.1, 17.5, 17.3; HRMS (FAB) *m/z* calcd for  $\text{C}_{29}\text{H}_{27}\text{N}_9\text{O}_8\text{Li}$  (M+Li) 636.2137; found 636.2139. HXLV-AC, **1b**: mp 222–224 °C;  $[\alpha]_{\text{D}}^{20} -37.4^\circ$  (*c* 0.605, 10% MeOH/ $\text{CHCl}_3$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.60 (d, 1H, *J* = 8), 8.48 (d, 1H, *J* = 8), 8.23 (m, 6H), 5.78 (br s, 1H), 5.43 (dt, 1H, *J* = 6, 7), 5.33 (dd, 1H, *J* = 5, 8), 3.22 (m, 2H), 2.40 (m, 1H), 1.75 (m, 5H), 1.44 (m, 4H), 1.05 (d, 3H, *J* = 7), 0.99 (d, 3H, *J* = 7);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  169.3, 164.0, 163.7, 159.1, 159.0, 155.4, 155.2, 153.9, 153.8, 140.0, 139.4, 138.3, 138.1, 137.5, 136.2, 136.1, 130.24, 130.16, 128.8, 52.2, 46.8, 38.5, 33.8, 33.1, 27.8, 22.4, 21.2, 17.6, 17.3; HRMS (ESI) *m/z* calcd for  $\text{C}_{31}\text{H}_{29}\text{N}_9\text{O}_9$  (M+H) 672.2161; found 672.2199. HXLV-DM, **1c**: mp 308–310 °C (dec);  $[\alpha]_{\text{D}}^{20} +19^\circ$  (*c* 0.5, 20%  $\text{CHCl}_3/\text{MeOH}$ );  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.76 (d, 1H, *J* = 8), 8.41 (d, 1H, *J* = 8), 8.23 (m, 6H), 5.48 (dt, 1H, *J* = 6, 7), 5.34 (dd, 1H, *J* = 5, 8), 2.96 (m, 2H), 2.77 (s, 6H), 2.44 (m, 1H), 2.11 (m, 2H), 1.90 (m, 2H), 1.06 (d, 3H, *J* = 7), 1.00 (d, 3H, *J* = 7); HRMS (FAB) *m/z* calcd for  $\text{C}_{31}\text{H}_{31}\text{N}_9\text{O}_8\text{Li}$  (M+Li) 658.2368; found 658.2373. HXDL, **2**: mp 305 °C (TFA salt);  $[\alpha]_{\text{D}}^{20} +79.1^\circ$  (*c* 1.05, MeOH);  $^1\text{H}$  NMR ( $\text{CDCl}_3/\text{CD}_3\text{OD}$ )  $\delta$  8.47 (s, 4H), 8.35 (s, 2H), 5.40 (m, 2H), 3.40–2.90 (m, 4H), 2.08 (m, 4H), 1.82 (m, 4H), 1.60–1.20 (m, 6H);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  164.0, 159.9, 155.9, 154.4, 141.4, 141.3, 139.9, 135.8, 129.3, 127.8, 39.0, 37.4, 26.7, 21.2; HRMS (ESI) *m/z* calcd for  $\text{C}_{30}\text{H}_{31}\text{N}_{10}\text{O}_8$  (M+H) 659.2326; found 659.2311.
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