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PII: S0223-5234(18)30234-4

DOI: 10.1016/j.ejmech.2018.02.087

Reference: EJMECH 10264

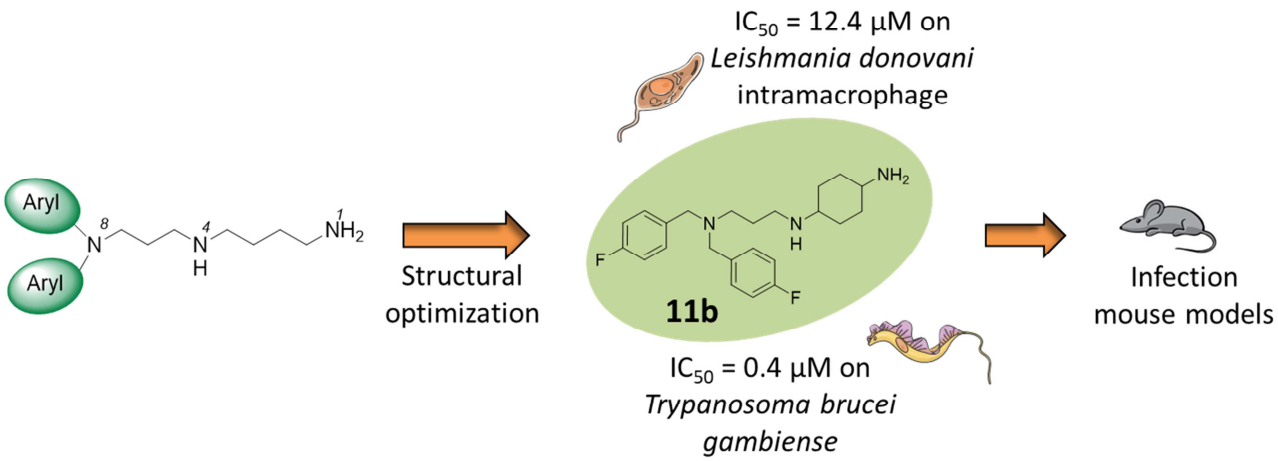
To appear in: *European Journal of Medicinal Chemistry*

Received Date: 29 November 2017

Revised Date: 27 February 2018

Accepted Date: 28 February 2018

Please cite this article as: E. Jagu, Sé. Pomel, A. Diez-Martinez, E. Rascol, Sté. Pethe, P.M. Loiseau, Raphaë. Labruère, Synthesis and antikinetoplastid evaluation of bis(benzyl)spermidine derivatives, *European Journal of Medicinal Chemistry* (2018), doi: 10.1016/j.ejmech.2018.02.087.



Synthesis and antikinetoplastid evaluation of bis(benzyl)spermidine derivatives

Elodie Jagu[a], Sébastien Pomel[b], Alba Diez-Martinez[a], Estelle Rascol[a], Stéphanie Pethe[a], Philippe M. Loiseau[b]*, Raphaël Labruère[a]*

a- Institut de Chimie Moléculaire et des Matériaux d'Orsay (ICMMO), CNRS, Univ Paris Sud, Université Paris-Saclay, 15 rue Georges Clemenceau, 91405 Orsay Cedex, France

b- Chimiothérapie antiparasitaire, UMR 8076 BioCis, CNRS, Univ Paris Sud, Université Paris-Saclay, 5 rue Jean-Baptiste Clément, 92290 Châtenay-Malabry, France

Keywords: Antikinetoplastids, *Trypanosoma brucei*, *Leishmania donovani*, Polyamines

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ABSTRACT

This study describes the synthesis and the biological evaluation of twenty-four original bis(benzyl)spermidines. Structural modifications of the polyamine scaffold were performed in order to avoid easily metabolized bonds. Some bis(benzyl)polyamine derivatives have demonstrated promising activity in vitro against *Trypanosoma brucei gambiense* and *Leishmania donovani*. From the enzymatic experiments on trypanothione reductase, we observed that this enzyme was not targeted by our compounds. In vivo evaluation on Swiss mice model infected by *T. b. gambiense* or *L. donovani* was done with the most interesting compound of the series.

INTRODUCTION

Infections by *Trypanosoma brucei gambiense*, *Trypanosoma brucei rhodesiense* and *Leishmania* sp. cause more than 100,000 deaths per year and the World Health Organization (WHO) has classified these infections as challenging neglected disease.[1] These kinetoplastids are responsible for Human African Trypanosomiasis (HAT) and leishmaniasis, respectively. Only few drugs are currently used for the treatment of these infectious diseases and the development of resistant strains to these existing drugs is quite concerning. Moreover, the drugs

administrated to patients are essentially old and present serious side effects.[2] In regard of the lack of efficacy of the current treatments, the discovery of new and safe antikinetoplastid molecules is clearly needed.[3,4] In order to limit the adverse effects, the new drugs should target specifically the parasite by preferably impairing a metabolic pathway only found in kinetoplastids.

In kinetoplastids, polyamine and trypanothione metabolisms are essential for parasite survival and infectivity.[5,6] The trypanothione is a metabolite made of two glutathione molecules linked to one spermidine, which plays a key role against oxidative stress in these protozoan parasites. The trypanothione synthetase (TryS) is involved in the trypanothione anabolism by catalyzing the coupling of glutathione and spermidine. Furthermore, trypanothione reductase (TryR) allows trypanothione to be preserved in its reduced form in the presence of NADPH. Since this compound is not biosynthesized in mammalian cells, the trypanothione pathway is a promising target for drug discovery in kinetoplastids.[7]

Numerous natural polyamine analogs or conjugates have been studied for their antikinetoplastid activity.[8] In most cases, the target was not studied nor mentioned, but, when a target was assayed, TryR was often disrupted by the polyamine derivatives. However, other target recently revealed such as methionyl-tRNA synthetase could be also impaired by the same polyamine derivatives.[9] From the literature, we noted that antikinetoplastid activity was significant when a polyamine chain was linked to a benzyl moiety.[8] Among them, the bis(benzyl)polyamines were outstanding inhibitors of TryR often associated with a good in vitro activity against both *L. donovani* and *T. cruzi*.[10] These compounds displayed a high selectivity index and were markedly effective when the two benzyl groups were attached to the same nitrogen of the polyamine. In our previous efforts regarding the development of new antikinetoplastid agents,[11,12] spermidine-aryl conjugates exhibited the most interesting antiparasitic activity, in particular, derivatives bearing aromatic substituent on the N^8-nitrogen atom.[12] With these observations, we decided to design spermidine disubstituted on the N^8-nitrogen by different aryl groups as potential antikinetoplastid compounds. Some bisbenzyl derivatives were inspired from existing literature. Indeed, thiophene,[13] benzyloxybenzyl, diphenyl[14] and naphthalene[15] have also been identified as efficient substitutents for trypanocidal activity. Therefore, we elaborated different spermidine derivatives bearing these scaffolds (Figure 1).

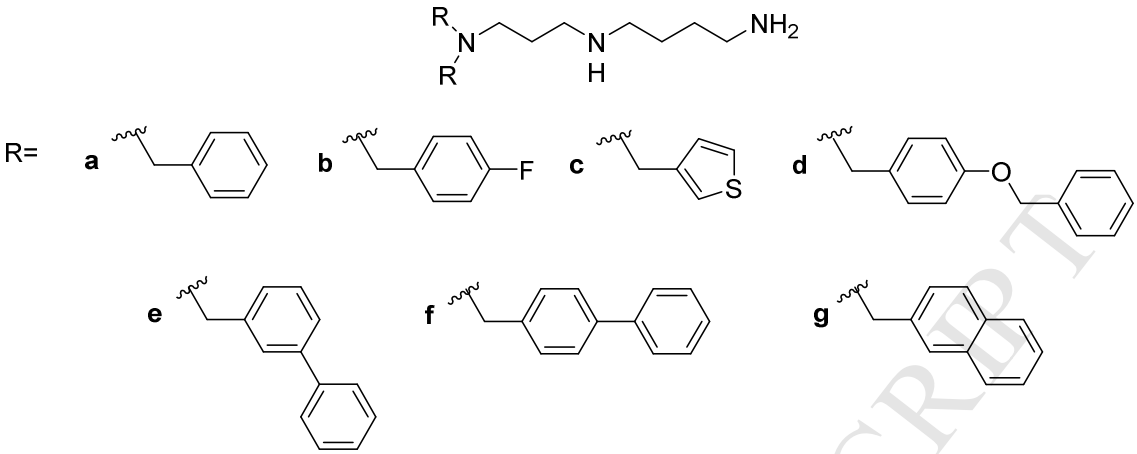


Figure 1: General structure of linear bis(benzyl)spermidines.

Additionally, the different aryl-polyamine previously tested in vivo were described as rapidly metabolized or excreted.[16,17,18,19] We hypothesized that adding structural modification to these derivatives could increase the bioavailability: (i) we introduced a fluorine atom on the para position of the phenyl ring which is well known to avoid rapid metabolization;[20] (ii) we also decided to prepare some polyamine derivatives bearing a rigidified polyamine chain since constraint structures generate better bioavailability.[21] It has been shown that polyamine derivatives bearing substituent in α-position of the amine were not hydroxylated by the spermine oxidase in mammals cells.[22,23] Consequently, we introduced a cyclohexyl ring on the polyamine skeleton (Figure 2) in order to combine rigidity of the structure and prevent fast degradation of the compound. Furthermore, we will be able to compare the linear and the cyclic polyamine structure toward the biological effect.

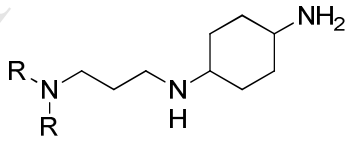


Figure 2: General structure of the rigidified spermidine derivatives.

Thus, we investigated each of the compounds for potential activity against both *T. brucei* and *L. donovani* in vitro, and the most promising compound was tested in vivo in mice models infected either by trypanosomes or *Leishmania*. Preliminary experiments in regard of the in vitro metabolism of selected compounds were carried out. We evaluated the cytotoxicity of the compounds on human macrophages in order to establish the selectivity index. We also tested all

the compounds for activity against *T. brucei* TryR since this enzyme was a probable target of this series.

RESULTS AND DISCUSSION

Synthesis. Spermidine derivatives **5a-g** were synthesized in four steps, starting from commercially available benzyl chloride or benzyl bromide **1a-g** (Figure 3). Firstly, preparation of *N*-diaryl-aminoalcohol **2a-g** was carried out using 3-aminopropanol **2** and benzyl halides **1a-g** in presence of potassium carbonate in different solvent according to the benzyl halides **1a-g** solubility (acetone, DCM or DMF, see experimental section). The hydroxyl group of **2a-g** was mesylated to provide activated compounds **3a-g**. The diaminobutane monoprotected with a Boc group **4** was prepared according to protocol described by Roblot et al.[24] Nucleophilic replacement of mesylate with the primary amine of **4** in the presence of Hünig's base provided **4a-g** in 12-41% yield on two steps. Disubstitution was occasionnally observed during this reaction and afforded byproducts **6a** and **6b**. These compounds were isolated and included in the study for biological evaluation. The Boc protecting group was then removed by treatment with 4M HCl in dioxane. Thus, the final compounds **5a-g, 7a and 7b** were obtained as hydrochloride salts in quantitative yield.

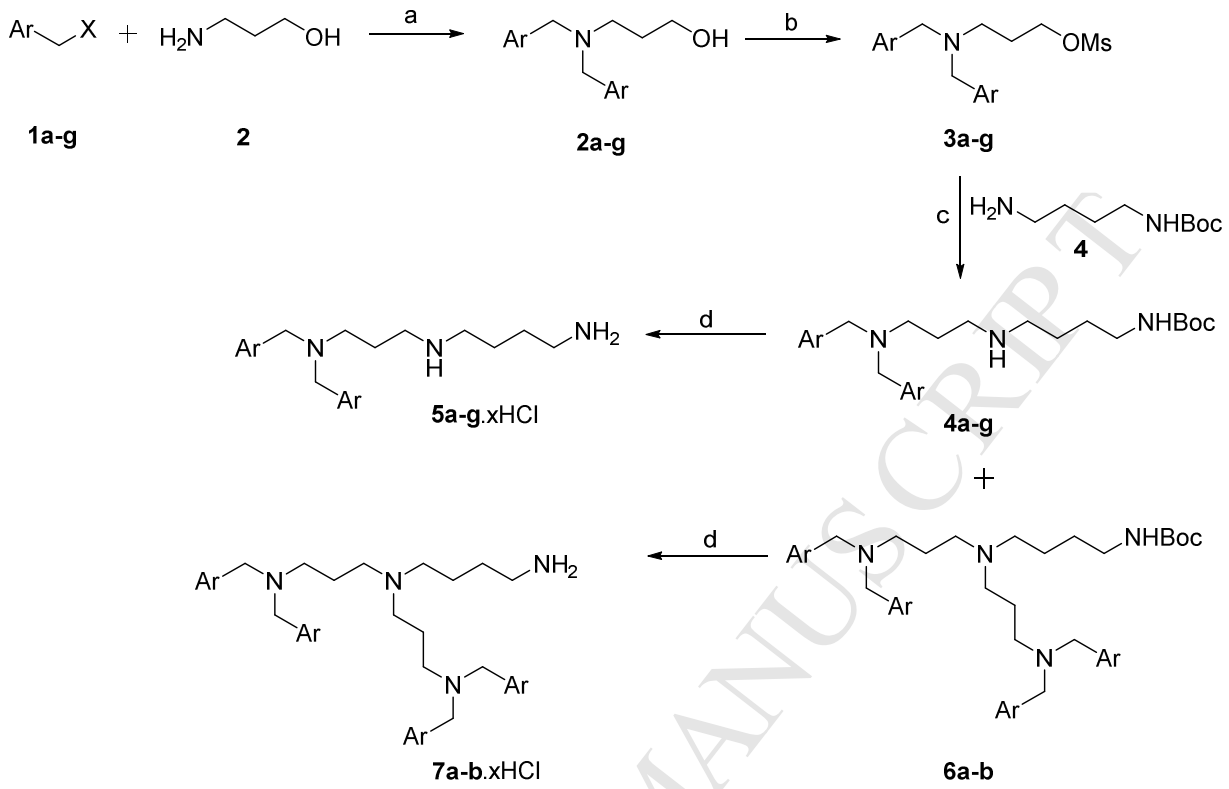


Figure 3: Synthesis of diaryl derivatives. *Reagents and conditions*: (a) K_2CO_3, acetone or DCM or DMF, rt, 12-16 h; (b) CH_3SO_2Cl, DIEA, DMAP, DCM, 0 ℃ to rt, 16h; (c) DIEA, ACN , reflux, 16h; (d) 4M HCl/Dioxane, rt, 16 h.

In an attempt to confer a degree of structural rigidity, analogs of **5a** and **5b** featuring restricted rotation in the central polyamine chain were designed. This was accomplished by incorporating a trans-cyclohexane ring into the polyamine skeleton (Figure 4). Thus, trans-1,4-diaminocyclohexane **8** was monosubstituted by a Boc protecting group following a slightly modified method previously described.[25] Boc-diaminohexane **9** was then coupled to two different mesylated intermediates **3a** and **3b** to give diarylated cyclohexane-1,4-diamine **10a** and **10b**. During this substitution reaction with the fluorinated derivative **3b**, a second product was obtained. This latter compound is derived from disubstitution of the mesylate **3b** conducting to the tertiary amine **12b**. This byproduct was isolated, then deprotected in acidic condition as mentioned above to afford compound **13b** which was included in the biological assay. Likewise, **10a** and **10b** were also deprotected to give the hydrochloride salts **11a** and **11b**.

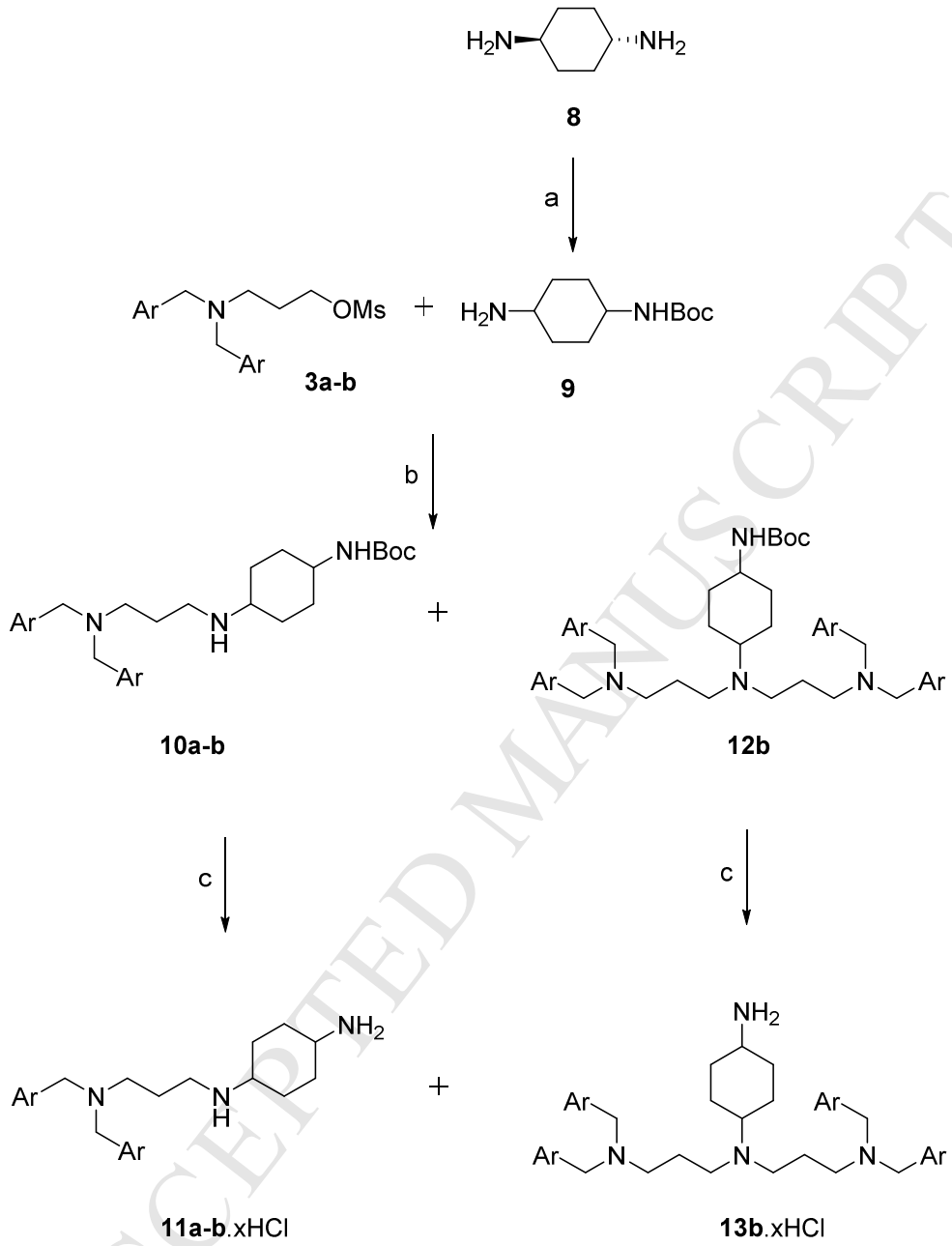


Figure 4: Synthesis of ((diaryl)aminopropyl)cyclohexanediamine. *Reagents and conditions*: (a) Boc_2O, DCM, 0 ℃ to rt, 16 h; (b) DIEA, ACN, reflux, 16 h; (c) 4M HCl/Dioxane, rt, 16 h.

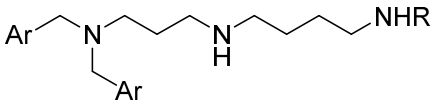
In vitro antikinetoplastid evaluation. Boc-protected and -deprotected molecules have been evaluated in vitro against both *T. b. gambiense* trypomastigotes and *Leishmania donovani* axenic and intramacrophage amastigotes. The results of the in vitro screening are shown in Tables 1, 2 and 3.

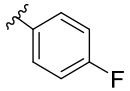
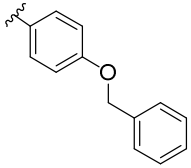
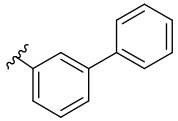
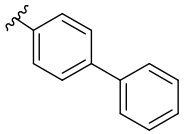
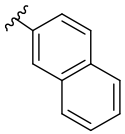
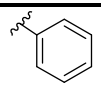
Regarding the difference of activity between the Boc protected and the free amine compounds, it is difficult to infer a general trend that either increase or not the activity. In most cases, the protecting group did not influence the biological effect, except for the couples Boc-protected polyamine/free polyamine**: 4a**/**5a**, **6b**/**7b** and **4f**/**5f** on *T. b. gambiense* (Tables 1 and 2). Against the same parasite, ten compounds of this series have displayed an IC_{50} below 1 µM and five below 0.5 µM (**5f, 5g, 6a, 7b, 11b**). Furthermore, these latter molecules were assayed on non-infected macrophages and all were moderately cytotoxic with only **7b** displaying an IC_{50} in the low micromolar range (Table 2). Hence, all these derivatives had a selectivity index (SI) over 10. Substituents on the aromatic ring were never detrimental on the trypanocidal activity except with the *meta*-phenyl group (IC_{50} (**4e**/**5e**) > 8 µM) (Table 1). We further noted that attaching a second aryl nucleus (naphthalene **5g** and para-biphenyl **5f**) was rather enhancing the trypanocidal activity providing that this additional ring is attached linearly in a rigid configuration to the first one. Indeed, **5g** (naphtyl substituent) was 20 times more active than the corresponding phenyl derivatives **5a** and, *para*-biphenyl **5f** was at least 20 times more trypanocidal than both *meta*-biphenyl **5e** (non-linear/rigid) and *para*-benzyloxyphenyl **5d** (linear/non-rigid). Interestingly, O'Sullivan et al. reported a positional isomer of N^1,N^1-bis(2-naphtylmethyl)-spermidine **5g** where each naphtyl group were borne by one terminal nitrogen of a spermidine molecule (i.e. N^1,N^8-bis(2-naphtylmethyl) isomer) that was shown as active as **5g** against four strains of *T. brucei* (IC_{50} = 0.2-0.6 µM).[17] When compound **5a** is compared with the rigidified cyclohexane-containing analog **11a**, an improvement of the antikinetoplastid activity could be noted as well as an enhancement of the cytotoxic effect. However, the corresponding fluorobenzyl derivative **11b** was 5 times more active against *T. b. gambiense* than **11a** with an IC_{50} of 0.4 µM and a selectivity index of 12 (Table 3).

Concerning the activity against *Leishmania donovani*, we observed that the vast majority of the compounds displayed similar or better activity on intramacrophage than on axenic amastigotes. Among the synthesized compounds, the tetra-aryl derivatives were the most potent, **6a** was indeed remarkable since it displayed IC_{50} value of 2.5 µM against *L. donovani* axenic amastigotes and, interestingly, an IC_{50} of 1.5 µM against the intramagrophage ones (Table 2). **7b**, a Boc-deprotected and fluorinated analog of **6a**, was equally active against axenic amastigotes and slightly less active on intramacrophage amastigotes, but this compound had a strong cytotoxicity on macrophages (IC_{50} = 1.5 µM). The thiophenes **4c**, **5c** and the benzyloxybenzyls **4d**, **5d** did not show any activity against *L. donovani*. Remarkably, we observed that the *meta*-biphenyl derivative **5e** was not leishmanicidal whereas the *para*-biphenyl **5f** had a moderate activity against both axenic and intramacrophage amastigotes (IC_{50} of 3.9

and 7.1 µM respectively) (Table 1). In regards of reported compounds displaying a structure close to our derivatives, a series of N^1,N^1-bisaryl-diamines were described by Labadie et al.[14] In the latter study, two bisbenzyl-diamines (2- or 3-carbon chain) were as leishmanicidal as the N^1,N^1-bisbenzyl-spermidine **5a** (IC_{50} ~ 25 µM). However, N^1,N^1-bisbenzyloxybenzyl-diamines (2- or 3-carbon chain) were active in the low micromolar range[14] whereas N^1,N^1-bisbenzyloxybenzyl-spermidine **5d** was inactive (IC_{50} > 3.9 µM).

Table 1: Antikinetoplastid activity and cytotoxicity of the linear bis(benzyl)spermidines.
[a] SI: Selectivity Index expressed as the ratio CC_{50}/IC_{50} ; SI_{Tb}: relative to IC_{50} on *T. b. gambiense*; SI_{aa}: relative to IC_{50} on *L. donovani* axenic amastigotes; SI_{ia}: relative to *L. donovani* intramacrophage amastigotes
[b] 50% growth inhibitory concentration



Compounds	Ar	*T. b. g.* IC_{50} (µM) ± SD	SI_{Tb} [a]	*L. d.* LV9 axenic amastigotes IC_{50} (µM) ± SD	SI_{aa} [a]	*L. d.* LV9 intramacrophage amastigotes IC_{50} ±SD (µM) [b]	SI_{ia} [a]	Cytotoxicity on macrophages CC_{50} ± SD (µM) [b]
4 R = Boc	**a** 	0.6 ± 0.1	20.8	14.6 ± 3.6	-	3.4 ± 1.2	3.7	12.5
	b 	2.0 ± 0.2	4.3	14.7 ± 0.5	-	14.5 ± 0.9	-	8.7 ± 0.2
	c 	1.5 ± 0.1	10.9	25.3 ± 0.5	-	21.1 ± 1.0	-	16.4 ± 0.2
	d 	4.7 ± 0.2	7.1	26.4 ± 1.6	1.3	42.0 ± 6.2	-	33.3 ± 2. 8
	e 	33.9 ± 0.6	> 2.9	79.5 ± 5.5	> 1.2	> 100	-	> 100
	f 	14.4 ± 2.2	> 6.9	38.5 ± 4.6	> 2.6	41.7 ± 1.	> 2.4	> 100
	g 	0.8 ± 0.0	5.4	3.8 ± 0.1	1.1	6.1 ± 2.9	-	4.3 ± 0.1
5 R = H	**a** 	8.8 ± 2.2	2.0	> 100	-	27.4 ± 2.7	-	17.7 ± 0.6

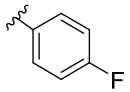
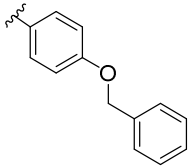
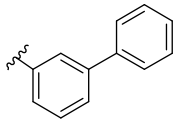
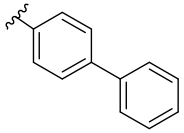
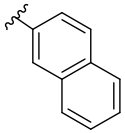
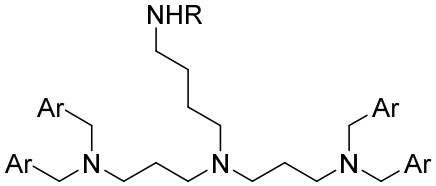
b		3.5 ± 0.5	9.4	70.3 ± 2.3	-	35.8 ± 4.2	-	33.1 ± 0.2
c		6.1 ± 0.9	8.6	>100	-	59.1 ± 3.3	-	52.8 ± 1.1
d		35.5 ± 0.5	> 2.8	72.2 ± 8.5	> 1.4	> 100	-	> 100
e		8.7 ± 0.6	3.5	35.7 ± 1.3	-	27.8 ± 5.4	1.1	30.3 ± 3. 0
f		0.2 ± 0.0	21.0	3.9 ± 0.1	1.1	7.1 ± 1.4	-	4.2 ± 0.0
g		0.4 ± 0.0	12.2	5.0 ± 0.4	-	8.7 ± 0.3	-	4.9 ± 1.1

Table 2: Antikinetoplastid activity and cytotoxicity of the linear disubstituted bis(benzyl)spermidines.
[a] SI: Selectivity Index expressed as the ratio CC_{50}/IC_{50} ; SI_{Tb}: relative to IC_{50} on *T. b. gambiense*; SI_{aa}: relative to IC_{50} on *L. donovani* axenic amastigotes; SI_{ia}: relative to *L. donovani* intramacrophage amastigotes
[b] 50% growth inhibitory concentration



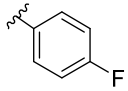
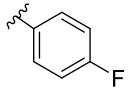
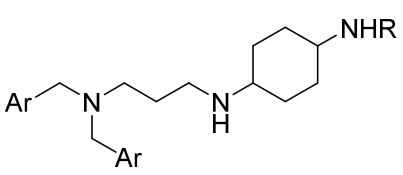
Compounds	Ar	*T. b. g.* IC_{50} (µM) ± SD	SI_{Tb} [a]	*L. d.* LV9 axenic amastigotes IC_{50} (µM) ± SD	SI_{aa} [a]	*L. d.* LV9 intramacrophage amastigotes IC_{50} ±SD (µM) [b]	SI_{ia} [a]	Cytotoxicity on macrophages CC_{50} ± SD (µM) [b]
6 R = Boc	**a** 	0.3 ± 0.1	41.7	2.5 ± 0.7	5.0	1.5 ± 0.2	8.3	12.5
	b 	1.9 ± 0.2	6.3	6.6 ± 0.1	1.8	16.6 ± 4.2	-	12.0 ± 3.2
7 R = H	**a** 	0.9 ± 0.2	1.3	57.3 ± 4.1	-	22.4 ± 5.8	-	1.2 ± 0.2
	b 	0.1 ± 0.0	15.0	2.0 ± 0.8	-	6.5 ± 1.6	-	1.5 ± 0.3

Table 3: Antikinetoplastid activity and cytotoxicity of the rigidified bis(benzyl)spermidines.
[a] SI: Selectivity Index expressed as the ratio CC_{50}/IC_{50} ; SI_{Tb}: relative to IC_{50} on *T. b. gambiense*; SI_{aa}: relative to IC_{50} on *L. donovani* axenic amastigotes; SI_{ia}: relative to *L. donovani* intramacrophage amastigotes
[b] 50% growth inhibitory concentration



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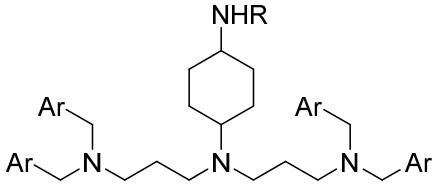
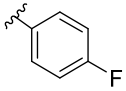
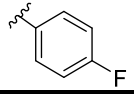
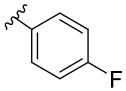
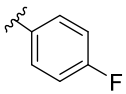


12, 13

Compounds	Ar	*T. b. g.* IC_{50} (µM) ± SD	SI_{Tb}[a]	*L. d.* LV9 axenic amastigotes IC_{50} (µM) ± SD	SI_{aa}[a]	*L. d.* LV9 intramacrophage amastigotes IC_{50} ±SD (µM)[b]	SI_{ia}[a]	Cytotoxicity on macrophages CC_{50} ± SD (µM)[b]
10 R = Boc	**a** 	0.7 ± 0.1	7.3	14.2 ± 2.1	-	5.4 ± 1.8	-	5.1 ± 1.4
	b 	0.5 ± 0.0	8.4	6.8 ± 0.2	-	6.8 ± 0.4	-	4.2 ± 0.0
11 R = H	**a** 	2.1 ± 0.0	3.6	>100	-	6.6 ± 1.2	1.1	7.6 ± 2.2
	b 	0.4 ± 0.0	11	44.4 ± 2.0	-	12.4 ± 3.5	-	4.4 ± 0.0
12 R = Boc	**b** 	4.2 ± 0.2	> 23.8	10.2 ± 0.3	> 9.8	17.2 ± 6.6	> 5.8	> 100
13 R = H	**b** 	10.4 ± 1.2	-	15.7 ± 1.3	-	4.2 ± 1.6	1.1	4.5 ± 1.0

Enzymatic assay. As mentioned earlier, TryR is a potential drug target for polyamine derivatives since large number of different TryR inhibitors reported so far displayed such scaffold attached to a hydrophobic aryl nucleus. All the compounds have been evaluated on TryR from *T. brucei*. A first screening at a fixed concentration of inhibitor at 40 µM in assay buffer containing 5% DMSO has been carried out. The activity was measured in the presence of 40 and 100 µM TS_2, respectively, and the percentage of inhibition was calculated. The results of assay using 40 µM TS_2 were depicted in the Figure 5.

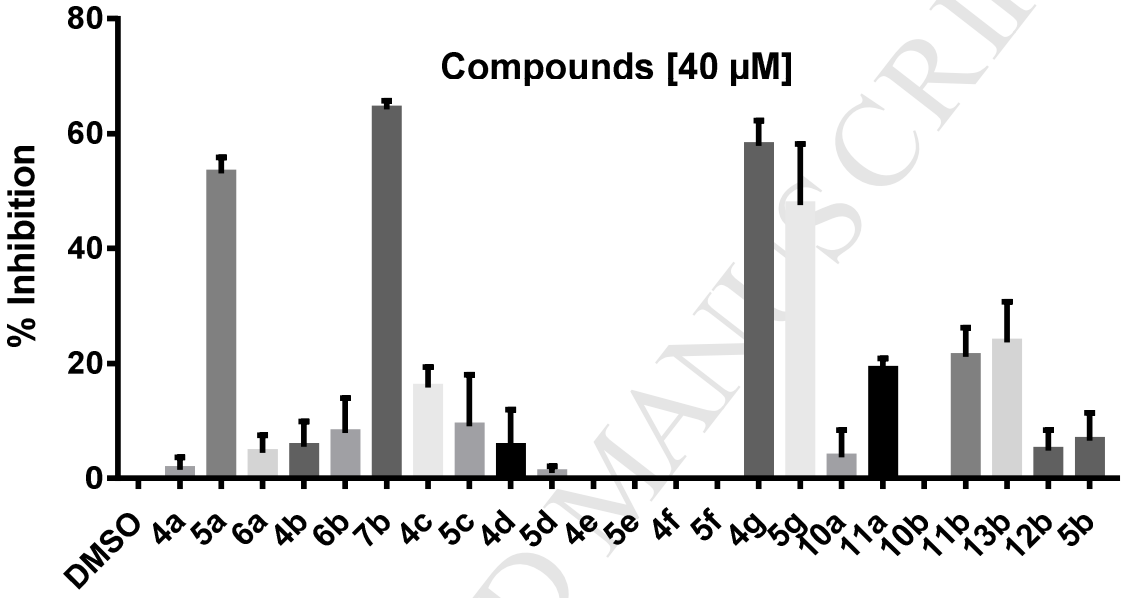


Figure 5: Inhibition of *T. b.* TryR. The activity was measured following the NADPH consumption at 340 nm as described in the Experimental Section. The assays contained a fixed concentration of 100 or 40 µM of TS_2 and 40 µM of inhibitor. Figure shows the average percentage inhibition at the two different substrate concentrations. The controls contained the same amount of DMSO used to dissolve the compounds.

Three compounds displayed an activity higher than 50% inhibition at 40 µM (**5a**, **7b and 4g**) and compound **5g** has shown 47% inhibition at the same concentration. However, compound **7b** seems to present substrate dependent inhibition. Indeed, **7b** showed more than 60% of inhibition at a TS_2 concentration of 40 µM, and around 30% of inhibition when substrate concentration is reaching 100 µM. Compound **4g** seems to have the same behavior when concentration substrate increases (60% inhibition at 40 µM against 45% at 100 µM). In contrast, percentage of inhibition for compound **5a** was independent of the substrate concentration which means that this derivative acts as pure competitive ligand.

In view of this preliminary screening, we determined the inhibitory constant and the type of inhibition of compound **5a**. We observed that **5a** behaves as mixed-type inhibitors (data not

shown), with calculated Ki- and Ki'-value of 7.5 µM and 20 µM, respectively. This activity on *T. b.* TryR is relatively low and could explain the poor IC_{50} of 8.8 µM against *T. b. gambiense* in vitro. In light of the weak inhibition obtained on *T. b.* TryR with this series of compounds, their trypanocidal effect is therefore due to their action on a different target.

In vivo evaluation. Among the most active compounds, three deprotected polyamines **5a**, **5f** and **11b** were assayed for their in vitro metabolic stability both in plasma (hydrolytic enzymes) and liver S9 fraction (phase I and II drug metabolizing enzymes). It was observed that the three molecules were stable over 12 h in human plasma. Compound **11b** which has a fluorine atom on the benzyl groups and for which the polyamine chain has been rigidified was among the most trypanocidal derivative in vitro, bearing rather hydrophobic moieties and arduously breakable bond by classical metabolic transformations. The ability of this compound to remain stable in the S9 fraction was therefore evaluated and no metabolic transformations were noted in presence or absence of NADPH. The microsome-predicted hepatic extraction ratios obtained based on the relative rates of test compound degradation was relatively high (= 0.9). In view of these results, we decided to evaluate **11b** in vivo on the *Trypanosoma brucei*/Swiss mice model. Compound **11b** was administered by intraperitoneal injection at doses of 100 mg/kg and 10 mg/kg. The results are gathered in Table 4.

Table 4 : In vivo evaluation of compound 11b at the single dose of 10 mg/kg administered by intraperitoneal route on mice infected with *Trypanosoma brucei*. (+) 1-50 parasites / µL blood; (++) 50-500 parasites / µL blood; (+++) 500-5000 parasites / µL blood; (++++) 5000-50000 parasites / µL blood. Mean survival time of excipient treated mice = 3.5 ± 0.5 days.

Compound	Dose		Parasitemia at			Mean survival time minus mean control survival time (Days ± SD)	Toxicity: number of dead mice [day of death post-treatment]
	µmol/kg	mg/kg	Day 2	Day 3	Day 4		
11b	217	100	/	/	/	-2.3 ± 0.5	2[Day 1]; 4[Day 2]
	21.7	10	+	++	++++	0.0 ± 1.1	1[Day 2]; 3[Day 4]; 2[Day 5]
Pentamidine di-isethionate	100	60	-	-	-	> 30	No toxicity
Melarsamine dihydrochloride	100	50	-	-	-	> 30	No toxicity

Excipient	/	/	+++	++++	8 mice dead	0	No toxicity

Although no prolongation of survival has been observed, the polyamine derivative **11b** induces a delay in the development of parasitemia rate, proving a trypanocidal activity at 10 mg/kg even though parasites are still present at this dose. However, this compound was toxic at 100 mg/kg. Afterwards, **11b** has been also tested in vivo on Balb/c mice model infected with *Leishmania donovani*. Considering that no toxicity was observed on the *T. b. brucei* mice model at 10 mg/kg in a single dose, we used a dose at 2 mg/kg/day by intraperitoneal route on five consecutive days, corresponding to the classical protocol for the in vivo leishmaniasis model (protocol and data not shown). Therefore, the total dose evaluated for the in vivo model of *Leishmania donovani* was 10 mg/kg and no specific action on parasites was observed at this dose.

CONCLUSION

Twenty-four new polyamines derivatives have been synthesized and evaluated in vitro against *T. b. gambiense* and *L. donovani*. Many of them displayed a good trypanocidal activity on *T. b. gambiense*. In spite of a high level of toxicity against non-infected macrophages, some derivatives were promising for the development of new antikinetoplastid compounds, in particular, compounds **6a** and **11b** which displayed an IC_{50} against *T. b. gambiense* below 0.5 µM and significant selectivity indices. Some compounds showed an antikinetoplastid activity without inhibitory effect on TryR. Therefore, further studies are required to determine the mechanism of action, and the target(s) of these compounds. Regarding the activity against *L. donovani*, we highlighted new structures which were efficient against the intramacrophage form of the parasite. The major disadvantage mentioned in the literature for the polyamine derivatives is the weak in vivo activity. It was suggested that lack of trypanocidal activity on infected animal is probably due to either rapid excretion of polyamine derivatives or fast metabolism in mammalian host. In our study, we tried to optimize the polyamine structure but unfortunately compound **11b** was not sufficiently effective in vivo on infected mice although it causes a slowdown in *T. brucei* development. The next challenge will be now the possibility to develop new antikinetoplastid polyamines displaying no toxicity toward the host.

EXPERIMENTAL SECTION

Chemistry. All chemical reagents were of analytical grade, obtained from Acros, Alfa Aesar, or Aldrich, and used without further purification. Solvents were obtained from SDS or VWR-Prolabo. Dichloromethane was dried on molecular sieves and used immediately. Chromatography was performed using silica gel (35-70 µm, Merck). Concentration of solutions was performed under reduced pressure at temperature below 40°C using rotary evaporator. Analytical TLC was performed using Silica Gel 60 F_{254} pre-coated aluminum plates (Merck). Spots were visualized by treatment with ninhydrine revelator followed by heating and/or by absorbance of UV light at 254 nm. NMR spectra were collected on Bruker DRX 250 (^{1}H at 250 MHz and ^{13}C at 62.5 MHz), DRX 300 (^{1}H at 300 MHz and ^{13}C at 75 MHz) or DRX 360 (^{1}H at 300 MHz and ^{13}C at 90 MHz) spectrometers using MestReNova software. Chemical shifts are reported in ppm (δ) and coupling constants in Hz (J). ^{1}H NMR spectra were performed in $CDCl_3$, MeOD or D_2O. High-resolution mass spectrometry (HRMS) analyses were performed by electrospray with positive (ESI^+). The purity of all compounds used for biological activity test was checked by reverse phase analytical HPLC and confirmed to be ≥ 95%.

General procedure for the synthesis of the aminoalcohols 2a-g:

A mixture of the corresponding benzyl chloride/bromide (2.1 eq.), 3-aminopropanol (1.0 eq.) and K_2CO_3 (5.0 eq.) in acetone, dichloromethane or dimethylformamide was stirred at room temperature. After completion of the reaction (12-16 h) monitored by TLC, the reaction mixture was filtered and the filtrate was concentrated under reduced pressure. The residue was purified by silica gel chromatography to afford the corresponding aminoalcohol.

2a: 3-(dibenzylamino)propan-1-ol

Conditions: Benzylbromide in acetone. Yellow oil, (70%). R_f (Cyclohexane/EtOAc 8:2) = 0.26. ^{1}H NMR (300 MHz, MeOD): δ = 7.35-7.21 (m, 10H), 3.56-3.53 (m, 6H), 2.55 (t, J = 6.5 Hz, 2H), 1.76 (m, 2H) ppm. ^{13}C NMR (75 MHz, D_2O): δ = 138.1, 128.9, 128.2, 63.4, 58.3, 52.7, 27.9 ppm. HRMS-ESI(+): calcd for $C_{17}H_{21}NO$: 255.1623, found: 256.1629 $[M+H]^+$.

2b: 3-(bis(4-fluorobenzyl)amino)propan-1-ol

Conditions: 4-Fluorobenzyl chloride in acetone. Yellow oil, (32%). R_f (Cyclohexane/EtOAc 8:2) = 0.32. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.28 (m, 4H), 7.04 (t, J = 8.7 Hz, 4H), 4.46 (brs, 1H), 3.65 (t, J = 6.5 Hz, 2H), 3.54 (s, 4H), 2.64 (t, J = 6.5 Hz, 2H), 1.81-1.74 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 162.1 (d, J = 244 Hz), 133.8, 130.7 (d, J = 10 Hz), 115.3 (d, J =

21 Hz), 63.8 57.7, 52.9, 29.7. HRMS-ESI(+): calcd for $C_{17}H_{19}F_2NO$: 291.1435, found: 292.1516 $[M+H]^+$.

2c: 3-(bis(thiophen-3-ylmethyl)amino)propan-1-ol

Conditions: 3-(bromomethyl)thiophene in DCM. White solid, (74%). R_f (Cyclohexane/EtOAc 1:1) = 0.43. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.29 (dd, *J* = 4.9, 3.0 Hz, 2H), 7.14 (dd, *J* = 3.0, 0.8 Hz, 2H), 7.06 (dd, *J* = 4.9, 0.8 Hz, 2H), 5.06 (br s, 1H), 3.70 (t, *J* = 5.2 Hz, 2H), 3.61 (s, 4H), 2.65 (t, *J* = 5.8 Hz, 2H), 1.76 (qt, *J* = 5.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.9, 128.3, 126.0, 123.2, 64.2, 53.5, 53.0, 28.0 ppm. HRMS-ESI(+): calcd for $C_{13}H_{17}NOS_2$: 268.0752, found: 268.0815 $[M+H]^+$.

2d: 3-(bis(4-(benzyloxy)benzyl)amino)propan-1-ol

Conditions: 4-benzyloxy(benzyl) chloride in DMF. White solid, (47%). R_f (Cyclohexane/EtOAc 1:1) = 0.51. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.46-7.38 (m, 10H), 7.27 (d, *J* = 8.6 Hz, 4H), 6.97 (d, *J* = 8.6 Hz, 4H), 5.08 (s, 4H), 3.67 (m, 2H), 3.54 (s, 4H), 2.67 (m, 2H), 1.81 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 158.1, 137.0, 130.4, 128.6, 127.9, 127.5, 114.8, 70.0, 64.1, 57.7, 53.0, 27.8 ppm. HRMS-ESI(+): calcd for $C_{31}H_{33}NO_3$: 467.2460, found: 468.2513 $[M+H]^+$.

2e: 3-(bis([1,1'-biphenyl]-3-ylmethyl)amino)propan-1-ol

Conditions: 3-phenylbenzyl bromide in DMF. White solid, (78%). R_f (Cyclohexane/EtOAc 6:4) = 0.54. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.64-7.60 (m, 8H), 7.46-7.38 (m, 10H), 4.70 (brs, 1H), 3.72 (s, 4H), 2.76 (m, 2H), 1.85 (m, 2H), 1.61 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 144.7, 140.6, 138.9, 130.1, 128.8, 127.9, 128.1, 128.0, 127.5, 125.3, 60.2, 59.4, 53.8, 28.8 ppm. HRMS-ESI(+): calcd for $C_{29}H_{29}NO$: 407.2249, found: 408.2313 $[M+H]^+$.

2f: 3-(bis([1,1'-biphenyl]-4-ylmethyl)amino)propan-1-ol

Conditions: 4-phenylbenzyl bromide in DMF. White solid, (58%). R_f (Cyclohexane/EtOAc 65:35) = 0.55. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.63 - 7.54 (m, 8H), 7.50 - 7.29 (m, 10H), 4.70 (brs, 1H), 3.73 (t, *J* = 5.3 Hz, 2H), 3.67 (s, 4H), 2.72 (t, *J* = 5.7 Hz, 2H), 1.83 (qt, *J* = 5.7 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 141.0, 140.3, 137.49, 129.7, 128.9, 127.3, 127.2, 64.1, 58.5, 53.5, 28.2. HRMS-ESI(+): calcd for $C_{29}H_{29}NO$: 407.2249, found: 408.2309 $[M+H]^+$.

2g: 3-(bis(naphthalen-2-ylmethyl)amino)propan-1-ol

Conditions: 2-(bromomethyl)naphthalene in DCM. White solid, (86%). R_f (Cyclohexane/EtOAc 65:35) = 0.46. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.91 – 7.75 (m, 8H), 7.56 – 7.46 (m, 6H), 4.68 (br s, 1H), 3.79 (s, 4H), 3.67 (t, *J* = 5.3 Hz, 2H), 2.74 (t, *J* = 5.8 Hz, 2H), 1.83 (qt, *J* = 5.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 136.0, 133.4, 132.9, 128.4, 128.2, 127.8, 127.2, 126.2, 125.9, 63.9, 59.0, 53.3, 28.3. HRMS-ESI(+): calcd for $C_{25}H_{25}NO$: 355.1936, found: 356.2000 $[M+H]^+$.

General procedure for the synthesis of the methanosulfonates 3a-g:

Methanesulfonyl chloride (2.0 eq.) in DCM (0.22 M) was cooled at 0 ℃ using an ice bath. A mixture of the corresponding aminoalcohol (1.0 eq.), di-isopropylethylamine (5.0 eq.) and DMAP (10 mol%) in DCM (0.44 M) was added dropwise under stirring. The reaction mixture was stirred at low temperature for 5 min and then, at room temperature for 16 h. Then, the dark red-brown reaction mixture was poured into a mixture of ice-water and DCM and the organic layer was separated. The aqueous phase was extracted with DCM three times. The combined organic layers were washed with water and brine, dried with $MgSO_4$ and evaporated to give the mesylated intermediate as pale yellow gummy solid. The product was used without further purification.

3a: 3-(dibenzylamino)propyl methanesulfonate

R_f (Cyclohexane/EtOAc 8:2) = 0.28. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.35-7.32 (m, 10H), 4.24 (t, 2H, *J* = 6.5 Hz), 3.55 (s, 4H), 2.82 (s, 3H), 2.55 (t, 2H, *J* = 6.5 Hz), 1.92 (qt, 2H, *J* = 6.5 Hz). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.6, 128.8, 127.4, 126.7, 67.6, 61.0, 52.8, 37.6, 26.7.

3b: 3-(bis(4-fluorobenzyl)amino)propyl methanesulfonate

R_f (Cyclohexane/EtOAc 8:2) = 0.33. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.28 (m, 4H), 7.01 (t, J = 8.7 Hz, 4H), 4.23 (t, *J* = 6.5 Hz, 2H), 3.51 (s, 4H), 2.88 (s, 3H), 2.53 (t, *J* = 6.5 Hz, 2H), 1.90 (qt, *J* = 6.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.9 (d, *J* = 244 Hz), 130.3, 115.2 (d, *J* = 21 Hz), 67.8, 57.6, 49.2, 37.3, 26,7. HRMS-ESI(+): calcd for $C_{18}H_{21}F_2NO_3S$: 369.1210, found: 370.1312 $[M+H]^+$.

3c: 3-(bis(thiophen-3-ylmethyl)amino)propyl methanesulfonate

R_f (Cyclohexane/EtOAc 1:1) = 0.62. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.35 - 7.25 (m, 2H), 7.17 - 7.11 (m, 2H), 7.09 - 7.04 (m, 2H), 4.27 (t, *J* = 6.4 Hz, 2H), 3.60 (s, 4H), 2.92 (s, 3H), 2.56 (t, *J* = 6.6 Hz, 2H), 1.91 (qt, *J* = 6.6Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 140.2, 128.4, 125.8, 122.6, 68.2, 53.3, 49.4, 37.4, 27.3. HRMS-ESI(+): calcd for $C_{14}H_{19}NO_3S_3$: 345.0527, found: 346.0582 $[M+H]^+$.

3d: 3-(bis(4-(benzyloxy)benzyl)amino)propyl methanesulfonate

R_f (Cyclohexane/EtOAc 6:4) = 0.73. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.47-7.38 (m, 10H), 7.36-7.23 (m, 4H), 6.96-6.93 (m, 4H), 5.07 (s, 4H), 4.24 (t, *J* = 6.4 Hz, 2H), 3.49 (s, 4H), 2.82 (s, 3H), 2.53-2.48 (m, 2H), 1.91-1.83 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 158.3, 137.4, 131.2, 129.6, 125.7, 122.6, 69.9, 61.5, 54.0, 49.0, 38.4, 26.0. HRMS-ESI(+): calcd for $C_{32}H_{35}NO_5S$: 545.2236, found: 546.2208 $[M+H]^+$.

3e: 3-(bis([1,1'-biphenyl]-3-ylmethyl)amino)propyl methanesulfonate

R_f (Cyclohexane/EtOAc 6:4) = 0.77. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.78-7.44 (m, 18H), 4.30-4.24 (m, 2H), 3.69 (s, 4H), 2.76 (s, 3H), 2.67-2.61 (m, 2H), 2.02-1.93 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 154.8, 141.4, 137.1, 132.3, 129.8, 126.7, 125.0, 124.8, 124.5, 123.9, 64.2, 62.1, 52.0, 37.6, 27.9. HRMS-ESI(+): calcd for $C_{30}H_{31}NO_3S$: 485.2025, found: 486.2065 $[M+H]^+$.

3f: 3-(bis([1,1'-biphenyl]-4-ylmethyl)amino)propyl methanesulfonate

R_f (Cyclohexane/EtOAc 65:35) = 0.42. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.65 - 7.54 (m, 8H), 7.51-7.41 (m, 8H), 7.39-7.30 (m, 2H), 4.30 (t, *J* = 6.4 Hz), 3.65 (s, 4H), 2.82 (s, 3H), 2.63 (t, *J* = 6.5 Hz, 2H), 1.97 (qt, *J* = 6.5 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 141.0, 140.1, 138.5, 129.4, 128.9, 127.3, 127.2, 127.1, 68.2, 58.4, 49.5, 37.3, 27.2. HRMS-ESI(+): calcd for $C_{30}H_{31}NO_3S$: 485.2025, found: 486.2077 $[M+H]^+$.

3g: 3-(bis(naphthalen-2-ylmethyl)amino)propyl methanesulfonate

R_f (Cyclohexane/EtOAc 65:35) = 0.50. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.89 - 7.72 (m, 8H), 7.54 (m, 2H), 7.50 - 7.44 (m, 4H), 4.24 (t, *J* = 6.4 Hz, 2H), 3.76 (s, 4H), 2.65 (t, *J* = 6.6 Hz, 2H), 2.61 (s, 3H), 1.95 (qt, *J* = 6.6 Hz, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 137.0, 133.5, 132.9, 128.2, 127.8, 127.7, 127.3, 126.2, 125.8, 68.1, 59.1, 49.5, 37.1, 27.3. HRMS-ESI(+): calcd for $C_{26}H_{27}NO_3S$: 433.1712, found: 434.1776 $[M+H]^+$.

General procedure for the synthesis of the *N*HBoc amines 4a-g, 6a, 6b:

To a solution of the corresponding methanesulfonate (**3a-g**) (1.0 eq.) and di-isopropylethylamine (2.0 eq.) in ACN (0.2 M) at room temperature, the diaminobutane **4** (1.5 eq.) was added dissolved in the minimum amount of ACN. The reaction mixture was stirred at 60 ℃ overnight. After evaporation of the solvent at reduced pressure, the residue was

dissolved in water and DCM and the organic layer was separated. The aqueous phase was extracted with DCM three times. The combined organic layers were washed with water and brine, dried with $MgSO_4$ and evaporated. The crude product was purified by silica gel chromatography to afford the corresponding Boc-protected compounds. For compounds **4a** and **4b** the first fraction of purification was isolated, concentrated and characterized to afford compounds **6a** and **6b**.

4a: *tert*-butyl (4-((3-(dibenzylamino)propyl)amino)butyl)carbamate

Yellow oil, (37% in two steps). R_f (EtOAc/MeOH 9:1) = 0.60. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.34-7.24 (m, 10H), 5.18 (s, 1H), 3.54 (s, 4H), 3.09 (s, 2H), 2.63 (t, 2H, *J* = 6.5 Hz), 2.49 - 2.44 (m, 4H), 1.78 - 7.72 (m, 2H), 1-57 - 1.53 (m, 4H), 1.45 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 164.2, 138.2, 128.9, 128.2, 126.9, 76.9, 58.5, 51.4, 49.1, 48.0, 36.2, 28.5, 27.4, 27.1, 25.5. HRMS-ESI(+) calcd for $C_{26}H_{39}NO_2$: 425.3042, found: 426.3126 $[M+H]^+$.

6a: *tert*-butyl (4-(bis(3-(dibenzylamino)propyl)amino)butyl)carbamate

Yellow oil, (16% in two steps). R_f (EtOAc/MeOH 9:1) = 0.68. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.43 - 7.27 (m, 20H), 4.98 (s, 1H), 3.60 (s, 8H), 3.11-3.09 (m, 2H), 2.49-2.37 (m, 10H), 1.70-1.64 (m, 4H), 1.51 (s, 9H), 1.42-1.34 (m, 2H). HRMS-ESI(+): calcd for $C_{43}H_{58}N_4O_2$: 662.4560, found: 663.4655 $[M+H]^+$.

4b: *tert*-butyl (4-((3-(bis(4-fluorobenzyl)amino)propyl)amino)butyl)carbamate

Yellow oil, (12% in two steps). R_f (EtOAc/MeOH 9:1) = 0.80. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.26 (dd, *J* = 8.2, 5.8 Hz, 4H), 7.00 (t, *J* = 8.6 Hz, 4H), 4.98 (s, 1H), 3.48 (s, 4H), 3.10 (d, *J* = 4.8 Hz, 2H), 2.79 - 2.59 (m, 4H), 2.46 (t, *J* = 6.3 Hz, 2H), 1.88 (t, *J* = 6.7 Hz, 2H), 1.63 (d, *J* = 7.0 Hz, 2H), 1.52 (d, *J* = 7.0 Hz, 2H), 1.43 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 162.1 (d, *J* = 244 Hz), 155.8, 135.3, 130.9 (d, *J* = 6 Hz), 115.2 (d, *J* = 17 Hz), 78.4, 59.0, 50.7, 49.8, 47.9, 32.5, 28.7, 28.3, 27.2, 24.9. HRMS-ESI(+) calcd for $C_{26}H_{38}F_2N_3O_2$: 461.2854, found: 462.2926 $[M+H]^+$.

6b: *tert*-butyl (4-(bis(3-(bis(4-fluorobenzyl)amino)propyl)amino)butyl)carbamate

Yellow oil, (13% in two steps). R_f (Cyclohexane/EtOAc 5:5) = 0.55. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.28 (dd, *J* = 8.4, 5.7 Hz, 8H), 6.99 (t, *J* = 8.7 Hz, 8H), 4.85 (brs, 1H), 3.48 (s, 8H), 3.08 (m, 2H), 2.35 (m, 10H), 1.57 (m, 4H), 1.45 (s, 9H), 1.37 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 161.9 (d, *J* = 204 Hz), 155.8, 135.3, 130.1 (d, *J* = 6 Hz), 115.0 (d, *J* = 17

Hz*)*, 79.5, 57.5, 53.7, 52.0, 51.7, 40.2, 28.4, 28.1, 24.60, 24.40. HRMS-ESI(+) calcd for $C_{43}H_{54}F_4N_4O_2$: 734.4183, found: 735.4354 $[M+H]^+$.

4c: *tert*-butyl (4-((3-(bis(thiophen-3-ylmethyl)amino)propyl)amino)butyl)carbamate

Yellow oil, (29% in two steps). R_f (EtOAc/MeOH 9:1) = 0.20. ^{1}H NMR (360 MHz, $CDCl_3$): δ = 7.28 (dd, *J* = 4.3, 3.0 Hz, 2H), 7.11 (dd, *J* = 2.8, 0.8 Hz, 2H), 7.05 (dd, *J* = 4.5, 1.0 Hz, 2H), 4.98 (brs, 1H), 3.57 (s, 4H), 3.20 - 3.03 (m, 2H), 2.61 (t, *J* = 6.8 Hz, 2H), 2.54 (t, *J* = 6.7 Hz, 2H), 2.46 (t, *J* = 6.7 Hz, 2H), 1.70 (qt, *J* = 6.6 Hz, 2H), 1.53-1.42 (m, 13H). ^{13}C NMR (90 MHz, $CDCl_3$): δ = 156.2, 140.6, 128.4, 125.5, 122.3, 79.7, 53.1, 51.4, 49.7, 48.2, 40.6, 28.6, 28.1, 27.7, 27.2. HRMS-ESI(+) calcd for $C_{22}H_{35}N_3O_2S_2$: 438.2171, found: 438.2242 $[M+H]^+$.

4d: *tert*-butyl (4-((3-(bis(4-(benzyloxy)benzyl)amino)propyl)amino)butyl)carbamate

Yellow oil, (14% in two steps). R_f (EtOAc/MeOH 9:1) = 0.13. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.48 - 7.32 (m, 10H), 7.26 (d, *J* = 8.5 Hz, 4H), 6.92 (d, *J* = 8.6 Hz, 4H), 5.04 (s, 4H), 3.48 (s, 4H), 3.07 (s, 2H), 2.40-2.36 (m, 6H), 1.66-1.60 (m, 4H), 1.44 (s, 9H), 1.28-1.24 (m, 2H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 157.7, 156.0, 137.2, 132.1, 129.9, 128.5, 127.9, 127.5, 114.5, 70.0, 57.5, 53.2, 52.8, 50.1, 37.3, 28.4, 26.5, 25.2 ppm. HRMS-ESI(+): calcd for $C_{40}H_{51}N_3O_4$: 637.3880, found: 638.3921 $[M+H]^+$.

4e: *tert*-butyl(4-((3-(bis([1,1'-biphenyl]-3-ylmethyl)amino)propyl)amino)butyl)carbamate

Yellow oil, (40% in two steps). R_f (EtOAc/MeOH 8:2) = 0.24. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.69-7.33 (m, 18H), 4.75 (s, 1H), 3.63 (s, 4H),3.14-3.12 (m, 2H), 2.67-2.64 (m, 2 H), 2.55-2.42 (m, 4H), 1.76-1.70 (m, 4H), 1.37-1.23 (m, 2H), 151-1.47 (m, 2H), 1.44 (s, 9H). ^{13}C NMR (75 MHz, $CDCl_3$): δ = 155.8, 141.2, 141.0, 140.4, 128.7, 128.6, 127.7, 127.5, 127.2, 127.1, 125.7, 58.4, 52.1, 51.8, 47.2, 28.4, 26.8, 25.0 ppm. HRMS-ESI(+): calcd for $C_{38}H_{47}N_3O_2$: 577.3668, found: 578.3731 $[M+H]^+$.

4f: *tert*-butyl (4-((3-(bis([1,1'-biphenyl]-4-ylmethyl)amino)propyl)amino)butyl)carbamate

Yellow oil, (34% in two steps). R_f (EtOAc/MeOH 9:1) = 0.23. ^{1}H NMR (300 MHz, MeOD-d_4): δ = 7.66 - 7.53 (m, 8H), 7.48 - 7.36 (m, 8H), 7.35 - 7.26 (m, 2H), 3.58 (s, 4H), 3.04-2.88 (m, 2H), 2.60-2.39 (m, 6H), 1.78 - 1.66 (m, 2H), 1.50 - 1.32 (m, 13H) ppm. ^{13}C NMR (75 MHz, MeOD-d_4): δ = 160.7, 142.2, 141.3, 140.0, 130.7, 129.9, 128.3, 127.9, 59.2, 52.5, 50.3, 48.8, 41.1, 28.8, 27.8, 27.3 ppm. HRMS-ESI(+): calcd for $C_{38}H_{47}N_3O_2$: 577.3668, found: 578.3733 $[M+H]^+$.

4g: *tert*-butyl (4-((3-(bis(naphthalen-2-ylmethyl)amino)propyl)amino)butyl)carbamate

Yellow oil, (25% in two steps). R_f (EtOAc/MeOH 9:1) = 0.18. ^{1}H NMR (360 MHz, $CDCl_3$): δ = 7.86 - 7.74 (m, 8H), 7.58 - 7.51 (m, 2H), 7.51 - 7.40 (m, 4H), 4.84 (brs, 1H), 3.73 (s, 4H), 3.07 - 2.95 (m, 2H), 2.63 - 2.50 (m, 4H), 2.43 (t, *J* = 6.9 Hz, 2H), 1.75 (qt, *J* = 6.5 Hz, 2H), 1.44 (s, 9H), 1.40 - 1.28 (m, 4H). ^{13}C NMR (90 MHz, $CDCl_3$): δ = 156.1, 137.5, 133.5, 132.9, 128.0, 127.8, 127.6, 127.4, 126.1, 125.7, 58.9, 51.4, 49.6, 48.0, 40.6, 28.6, 27.9, 27.5, 27.1. HRMS-ESI(+): calcd for $C_{34}H_{43}N_3O_2$: 525.3355, found: 526.3422 $[M+H]^+$.

10a: *tert*-butyl (4-((3-(dibenzylamino)propyl)amino)cyclohexyl)carbamate

Yellowish solid, (41%). R_f (EtOAc/MeOH 9:1) = 0.19. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.46 - 7.16 (m, 10H), 4.35 (m, 1H), 3.52 (s, 4H), 3.39 (m, 1H), 2.59 (t, *J* = 6.8 Hz, 2H), 2.44 (t, *J* = 6.6 Hz, 2H), 2.28 (brs, 1H), 1.99-1.95 (m, 2H), 1.85-1.81 (m, 2H), 1.71-1.65 (m, 2H), 1.44 (s, 9H),1.21-0.98 (m, 2H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 155.1, 139.7, 128.8, 128.2, 126.8, 59.6, 58.4, 55.6, 51.3, 45.0, 32.2, 31.9, 28.4, 27.1. HRMS-ESI(+) calcd for $C_{28}H_{41}N_3O_2$: 451.3199, found: 452.3297 $[M+H]^+$.

10b: *tert*-butyl (4-((3-(bis(4-fluorobenzyl)amino)propyl)amino)cyclohexyl)carbamate

Yellow oil. (29%) R_f (EtOAc/MeOH 8:2) = 0.09. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.11 - 7.08 (m, 4H), 6.87 - 6.81 (m, 4H), 4.21 - 4.20 (m, 1H), 3.31 (s, 2H), 3.25 (s, 1H), 2.28 (t, *J* = 6.9 Hz, 2H), 2.28 (t, *J* = 6.2 Hz, 2H), 1.74 - 1.62 (m, 6H), 1.28 (s, 9H), 1.14 - 1.10 (m, 4H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 161.9 (d, *J* = 244 Hz*)*, 155.1, 134.9, 130.3 (d, *J* = 7 Hz*)*, 115.1 (d, *J* = 20 Hz*)*, 79.3, 57.5, 56.1, 51.1, 49.0, 44.6, 31.7, 30.4, 28.4, 25.9 HRMS-ESI(+): calcd for $C_{28}H_{39}F_2N_3O_2$: 487.3010, found: 488.3080 $[M+H]^+$.

12b: *tert*-butyl (4-(bis(3-(bis(4-fluorobenzyl)amino)propyl)amino)cyclohexyl)carbamate

Yellow oil, (25% in two steps). R_f (Cyclohexane/EtOAc 5:5) = 0.22. ^{1}H NMR (250 MHz, $CDCl_3$): δ = 7.29 (m, 8H), 7.00 (m, 8H), 4.38 (m, 1H), 3.48 (s, 8H), 3.33 (m, 1H), 2.37 (m, 6H), 2.01 (m, 4H), 1.67 (m, 6H), 1.47 (s, 9H), 1.28 (m, 4H). ^{13}C NMR (62.5 MHz, $CDCl_3$): δ = 161.9 (d, *J* = 244 Hz*)*, 155.2, 135.3, 130.1 (d, *J* = 7 Hz*)*, 115.0 (d, *J* = 21 Hz*)*, 79.4, 57.6, 51.6, 48.7, 42.3, 32.9, 31.5, 28.4, 26.5. HRMS-ESI(+): calcd for $C_{45}H_{56}F_4N_4O_2$: 760.4339, found: 761.4440 $[M+H]^+$.

General procedure for synthesis of compounds 5a-g, 7a-b, 11a-b and 13b:

Boc-protected polyamines were deprotected using an excess of 4M HCl in dioxane. The mixture was stirred overnight at room temperature and the solvent was removed under reduced pressure to afford the final product as pure hydrochloride salt.

5a: N^1-(3-(dibenzylamino)propyl)butane-1,4-diamine

Yellowish solid, (quantitative yield). ^{1}H NMR (300 MHz, MeOD): δ = 7.67 - 7.58 (m, 10H), 3.55 (s, 4H), 2.80 - 2.76 (m, 6H), 1.76 - 1.59 (m, 6H), 1.42 - 1.37 (m, 2H) ppm. ^{13}C NMR (75 MHz, MeOD): δ = 139.2, 128.7, 128.5, 126.9, 59.1, 52.4, 49.7, 48.4, 40.8, 31.14, 27.3, 27.0 ppm. HRMS-ESI(+): calcd for $C_{21}H_{31}N_3$: 325.2518, found: 326.2611 $[M+H]^+$.

7a: N^1,N^1-bis(3-(dibenzylamino)propyl)butane-1,4-diamine

Yellowish solid, (quantitative yield). ^{1}H NMR (300 MHz, MeOD): δ = 7.76 - 7.71 (m, 20H), 3.59 (s, 8H), 2.91-2.88 (m, 8H), 1.65 - 1.52 (m, 10H), 1.36 - 1.29 (m, 2H) ppm. ^{13}C NMR (75 MHz, MeOD): δ = 140.2, 138.2, 129.0, 126.3, 60.5, 59.5, 58.5, 55.3, 53.09, 52.9, 40.2, 31.3, 25.0, 24.2 ppm. HRMS-ESI(+): calcd for $C_{38}H_{50}N_4$: 562.4035, found: 563.4121 $[M+H]^+$.

5b: N^1-(3-(bis(4-fluorobenzyl)amino)propyl)butane-1,4-diamine

Yellowish solid, (quantitative yield). ^{1}H NMR (300 MHz, MeOD): δ = 7.70 - 7.66 (m, 4H), 7.24 (t, J = 8.4 Hz, 4H), 4.46 (s, 4H), 3.36-3.30 (m, 2H), 3.07 - 3.01 (m, 6H), 2.35 - 2.33 (m, 2H), 1.83 - 1.80 (m, 4H) ppm. ^{13}C NMR (75 MHz, MeOD): δ = 163.6 (d, J = 244 Hz), 133.6, 125.2, 115.9 (d, J = 21 Hz), 56.2, 49.3, 44.5, 38.6, 24.1, 22.8, 20.6 ppm. HRMS-ESI(+): calcd for $C_{21}H_{29}F_2N_3$: 361.2330, found: 362.2391 $[M+H]^+$.

7b: N^1,N^1-bis(3-(bis(4-fluorobenzyl)amino)propyl)butane-1,4-diamine

Yellowish solid, (quantitative yield). ^{1}H NMR (300 MHz, MeOD): δ = 7.50 - 7.47 (m, 8H), 7.26 - 7.23 (m, 8H), 4.43 (s, 8H), 3.19 - 3.09 (m, 12H), 2.18 - 2.13 (m, 4H), 1.75 - 1.70 (m, 4H). ^{13}C NMR (75 MHz, MeOD): δ = 164.0 (d, J = 246 Hz), 133.8, 125.3, 116.9 (d, J = 21 Hz), 57.5, 52.9, 50.2, 49.6, 39.2, 24.3, 21.0, 19.4. HRMS-ESI(+): calcd for $C_{38}H_{46}F_4N_4$: 634.3659, found: 635.3742 $[M+H]^+$.

5c: N^1-(3-(bis(thiophen-3-ylmethyl)amino)propyl)butane-1,4-diamine

Yellowish solid, (quantitative yield). ^{1}H NMR (360 MHz, MeOD): δ = 7.87 (d, J = 1.9 Hz, 2H), 7.61 (dd, , J = 4.9, 2.8 Hz, 2H), 7.35 (d, J = 4.9 Hz, 2H), 4.45 (s, 4H), 3.24 - 3.13 (m, 2H), 3.13 - 2.93 (m, 6H), 2.39 - 2.24 (m, 2H), 1.91 - 1.71 (m, 4H). ^{13}C NMR (90 MHz, MeOD): δ = 130.8, 130.5, 130.2, 128.9, 52.4, 50.3, 48.2, 45.8, 40.0, 25.5, 24.2, 22.0. HRMS-ESI(+): calcd for $C_{17}H_{27}N_3S_2$: 337.1646; found: 338.1713 $[M+H]^+$.

5d: N^1-(3-(bis(4-(benzyloxy)benzyl)amino)propyl)butane-1,4-diamine

Yellowish solid, (quantitative yield). ^{1}H NMR (300 MHz, MeOD): δ = 7.45 - 7.33 (m, 14H), 7.11 - 7.08 (m, 4H), 5.14 (s, 4H), 4.35 (s, 4H), 3.04 - 2.72 (m, 6H), 2.41 - 2.38 (m, 4H), 1.83 - 1.78 (m, 4H). ^{13}C NMR (75 MHz, MeOD): δ = 162.3, 139.5, 133.7, 130.7, 129.7, 125.7, 117.8, 117.6, 72.2, 59.3, 57.0, 48.6, 44.9, 29.9, 28.4, 24.3. HRMS-ESI(+): calcd for $C_{35}H_{43}N_3O_2$: 537.3355, found: 538.3417 $[M+H]^+$.

5e: *N*^{1}-(3-(bis([1,1'-biphenyl]-3-ylmethyl)amino)propyl)butane-1,4-diamine

Yellowish solid, (quantitative yield). ^{1}H NMR (300 MHz, MeOD): δ = 7.68 - 7.34 (m, 18H), 4.66-4.55 (m, 4H), 3.36 - 3.32 (m, 4H), 2.98 - 2.87 (m, 2H), 2.56 - 2.53 (m, 4H), 1.86 - 1.74 (m, 4H). ^{13}C NMR (75 MHz, MeOD): δ = 142.1, 139.7, 129.8, 129.5, 128.6, 128.2, 127.5, 126.8, 57.5, 52.7, 50.6, 49.6, 38.6, 24.1, 20.7, 19.0. HRMS-ESI(+): calcd for $C_{33}H_{39}N_3$: 477.3144, found: 478.3179 $[M+H]^+$.

5f: *N*^{1}-(3-(bis([1,1'-biphenyl]-4-ylmethyl)amino)propyl)butane-1,4-diamine

Yellowish solid, (quantitative yield). ^{1}H NMR (360 MHz, MeOD): δ = 7.81-7.51 (m, 12H), 7.48-7.23 (m, 6H), 4.48 (s, 4H), 3.29-3.21 (m, 2H), 3.13-2.87 (m, 6H), 2.56-2.30 (m, 2H), 1.96-1.65 (m, 4H). ^{13}C NMR (90 MHz, MeOD): δ = 144.0, 140.1, 133.1, 130.0, 129.3, 129.0, 128.8, 128.0, 58.2, 50.8, 48.3, 45.9, 40.0, 25.5, 24.2, 22.0. HRMS-ESI(+): calcd for $C_{33}H_{39}N_3$: 477.3144, found: 478.93 $[M+H]^+$.

5g: *N*^{1}-(3-(bis(naphthalen-2-ylmethyl)amino)propyl)butane-1,4-diamine

Yellowish solid, (quantitative yield). ^{1}H NMR (300 MHz, MeOD): δ = 8.15 - 8.09 (m, 2H), 8.01 - 7.85 (m, 6H), 7.68 (d, *J* = 7.8 Hz, 2H), 7.61 - 7.50 (m, 4H), 4.66 (s, 4H), 3.43 - 3.21 (m, 2H), 3.16 - 2.90 (m, 6H), 2.51 - 2.33 (m, 2H), 1.94 - 1.66 (m, 4H). ^{13}C NMR (75 MHz, MeOD): δ = 135.2, 134.6, 132.8, 130.4, 129.4, 128.8, 128.6, 128.0, 127.8, 58.9, 51.1, 48.3, 45.9, 40.0, 25.5, 24.2, 22.1. HRMS-ESI(+): calcd for $C_{29}H_{35}N_3$: 425.2831, found: 426.2904 $[M+H]^+$.

11a: *N*^{1}-(3-(dibenzylamino)propyl)cyclohexane-1,4-diamine

Yellowish solid, (quantitative yield). ^{1}H NMR (300 MHz, MeOD): δ = 7.55 - 7.50 (m, 4H), 7.48 - 7.44 (m, 6H), 4.39 (s, 4H), 3.28 - 3.25 (m, 2H), 3.16 - 3.12 (m, 2H), 3.01 - 2.99 (m, 2H), 2.31 - 2.26 (m, 4H), 2.19 - 2.16 (m, 2H), 1.53 - 1.49 (m, 4H). ^{13}C NMR (75 MHz, MeOD): δ = 139.3, 129.1, 128.9, 127.9, 126.5, 59.0, 58.1, 53.3, 50.5, 49.7, 33.2, 30.6, 27.3. HRMS-ESI(+): calcd for $C_{23}H_{33}N_3$: 351.2674, found: 352.2641 $[M+H]^+$.

11b: *N*^{1}-(3-(bis(4-fluorobenzyl)amino)propyl)cyclohexane-1,4-diamine

Yellowish solid, (quantitative yield). ^{1}H NMR (300 MHz, MeOD): δ = 7.75 - 7.72 (m, 4H), 7.29 - 7.25 (m, 4H), 5.01 (s, 4H), 4.55 - 4.49 (m, 3H), 3.28 - 3.25 (m, 1H), 3.15 - 3.13 (m, 2H), 2.31 - 2.24 (m, 6H), 1.62 - 1.57 (m, 4H). ^{13}C NMR (75 MHz, MeOD): δ = 167.7 (d, *J* = 244 Hz), 137.7, 129.2, 119.9 (d, *J* = 20 Hz), 60.3, 53.3, 45.8, 32.1, 30.5, 24.7, 22.3. HRMS-ESI(+): calcd for $C_{23}H_{31}F_2N_3$: 387.2486, found: 388.2547 $[M+H]^+$.

13b: N^1,N^1-bis(3-(bis(4-fluorobenzyl)amino)propyl)cyclohexane-1,4-diamine

Yellowish solid, (quantitative yield). ^{1}H NMR (300 MHz, MeOD): δ = 7.69 (dd, *J* = 8.7, 5.2 Hz, 8H), 7.25 (t, *J* = 8.7 Hz, 8H), 4.51 (s, 1H), 4.47 (s, 8H), 3.27 (m, 6H), 2.53 (m, 4H), 2.22 (m, 4H), 1.74 (m, 4H), 1.31 (m, 2H). ^{13}C NMR (75 MHz, MeOD): δ = 167.7 (d, *J* = 244 Hz), 135.2, 130.4, 119.9 (d, *J* = 20 Hz), 58.9, 51.1, 48.3, 45.9, 40.0, 25.5, 24.2, 22.1. HRMS-ESI(+): calcd for $C_{40}H_{48}F_4N_4$: 660.3815, found: 661.3877 $[M+H]^+$.

Culture of *Leishmania donovani*. MHOM/ET/67/HU3, also called LV9, promastigote forms were grown in M-199 medium supplemented with 40 mM HEPES, 100 mM adenosine, 0.5 mg.mL^{-1} haemin, 10% heat-inactivated foetal bovine serum (FBS) and 50 mg.mL^{-1} gentamycin at 26 ℃ in a dark environment. Differentiation of promastigotes into axenic amastigotes was achieved by dilution of 1.10^6 promastigotes in 5 mL of axenic amastigote media M-199 (15 mM KCl; 8 mM glucose; 5 mM glutamine, 2.5% BBL™ trypticase™ peptone, 4 mM haemin, and 20% fetal bovine serum). The pH was adjusted to pH 5.5. Axenic amastigotes were grown at 37 ℃ under an atmosphere of 5% CO_2. All the experiments were performed with parasites in their logarithmic phase of growth.

In vitro evaluation of *Leishmania donovani* axenic amastigotes. The evaluations of activity on axenic amastigotes were adapted from the protocols previously described.[26] Two fold serial dilutions (100 to 0.02 µM) of the compounds were performed in 100 µL of complete medium (see above) in 96-well microplates. Axenic amastigotes were then added to each well at a density of 10^6/mL in a 200 µL final volume. After 72 h of incubation at 37 ℃ with 5 % CO_2, 20 µL of resazurin (450 µM) was added to each well and further incubated in the dark for 24 h at 37 ℃. In living cells, resazurin is reduced in resorufin and this conversion is monitored by measuring OD_{570nm} (resorufin) and OD_{600nm} (resazurin) (Lab systems Multiskan MS). The activity of the compounds was expressed as IC_{50} in µM (concentration of drug inhibiting 50% of the parasite growth, comparatively to the controls treated with the excipient only). Miltefosine was used as the reference drug.

In vitro evaluation on *Leishmania donovani* intramacrophage amastigotes. The evaluations of activity on intramacrophage amastigotes were adapted from the protocols

previously described.[26] RAW 264.7 macrophages (from ATCC) were plated in 96-well microplates at a density of 2.10^4 cells per well and incubated for 24 h at 37 °C with 5% CO_2. Axenic amastigotes were differenciated as described above, centrifuged at 2,000 g for 10 min, resuspended in DMEM complete medium, and added to each well to reach a 16:1 parasite to macrophage ratio. After 24 h of infection at 37 °C with 5% CO_2, extracellular parasites were removed, and DMEM complete medium (100 µL) containing two fold serial dilutions of the compounds from a maximal concentration of 100 µM was added to each well. After 48 h of treatment, the medium was removed and replaced by DirectPCR Lysis Reagent (100 µL; Euromedex) before 3 freeze-thaw cycles at room temperature, addition of 50 µg/ml proteinase K, and a final incubation at 55°C overnight to allow cell lysis. 10 µL of each cell extract was then added to 40 µL of DirectPCR Lysis reagent containing Sybr Green I (0.05 %; Invitrogen). DNA fluorescence was monitored using Mastercycler® realplex (Eppendorf). This method detects both host cell DNA and parasite DNA. Total DNA of treated infected macrophages were compared with total DNA of untreated infected macrophages. Mastercycler® realplex detects significant differences allowing a measurement of compound activities. The activity of the compounds was expressed as IC_{50}. Miltefosine was used as the reference drug.

Culture of *Trypanosoma brucei gambiense* and in vitro trypanocidal evaluation. Strain FéoITMAP/1893 was grown in a medium constituted of prepacked Iscove's modified Dulbecco's medium (Gibco, BRL) supplemented with 36 mM $NaHCO_3$, 1 mM hypoxanthine, 0.05 mM bathocuproine, 0.16 mM thymidine, 0.2 mM 2-mercaptoethanol, 1.5 mM L-cysteine, 10 % heat-inactivated foetal bovine serum, 100 IU penicillin and 100 $\mu g.mL^{-1}$ streptomycin. Two fold serial dilutions of the compounds were performed in 100 µL of the same medium in 96-well microplates. Trypanosomes were then added to each well at 4.10^4/mL in 200 µL final volume. After 72 h of incubation at 37 °C with 5% CO_2 in the dark, 20 µL of 450 µM resazurin in aqueous solution was added to each well and further incubated for 6 h at 37 °C with 5% CO_2. Cell viability was then monitored as described above. In this evaluation, the activity was expressed in IC_{50} (concentration inhibiting the parasite growth by 50%) and the reference drug used was pentamidine diisethionate.

Cytotoxicity assays. Cytotoxicity was evaluated on RAW 264.7 macrophages as previously described.[26] Briefly, cells were plated in 96-well microplates at a density of 2.10^4 cells per well. After an incubation of 24 h at 37 °C with 5% CO_2, the medium was removed in each well, and 100 µL of DMEM complete medium containing two fold serial dilutions of the compounds was added to each well. After 48 h of incubation at 37 °C with 5% CO_2, 10 µL of resazurin (450 µM) was added to each well, and further incubated in the dark for 4 h at 37 °C with 5% CO_2. Cell viability was then monitored as described above. The cytotoxicity of the

compounds was expressed as CC_{50} (Cytotoxic Concentration 50%: concentration inhibiting the macrophages growth by 50%).

Metabolic assays. Human plasma was diluted 10-fold in PBS buffer. 150 µL of 1 mM solution of compounds **5a**, **5f** and **11b** were incubated for 0, 2 and 12 h at 37 °C. After each time point, 150 µL of ice-cold acetonitrile were added. Precipitated proteins were removed by centrifugation for 20 min at 3000 rpm before analysis. The supernatants were filtered through 0.2 µm syringe filters. HPLC analyses were performed on an Agilent 1260 series using an analytical "Zorbax Eclipse Plus C18 column" (3.5 µm, 100 x 4.6 mm) maintained at 25°C.

Human pooled S9 fractions were diluted 20-fold in PBS buffer (1 mg/ml of proteins). 100 µM and 1 mM solutions of **11b** were incubated for 0 and 12 h at 37 °C for a final volume of 150 µL. Metabolization by hepatic enzymes, such as cytochromes or cytosolic enzymes was investigated in presence or absence of 1 mM NADPH.

Enzymatic assays. Recombinant *T. brucei* TryR was prepared and assayed as described previously.[27] Stock solutions of the inhibitors were prepared in DMSO. The activity was measured at 25 °C in a total volume of 1 mL of 40 mM Hepes, 1 mM EDTA, pH 7.5 (Assay Buffer) in the presence of 100 µM NADPH and 5-10 mU TryR containing 5% DMSO. The reaction was started by adding TS_2 and NADPH consumption was followed spectrophotometrically at 340 nm. To determine the percentage of inhibition, the assay contained 100 µM and 40 µM TS_2, respectively, in the absence and presence of a fixed concentration (40 µM) of inhibitor. The type of inhibition was determined by a Lineweaver-Burk plot. The experiment was performed in triplicates and the results are expressed as mean value ± standard deviation.

In vivo evaluation.

In vivo, Swiss mice were infected intraperitoneally with 10,000 trypanosomes (*T. brucei brucei* CMP strain) and then randomly allocated to groups and treated by intraperitoneal route with compound **11b** at the single dose of 100 mg/kg or 10 mg/kg. Six mice were used per batch. Control mice were treated with the reference compounds (pentamidine and melarsamine) or the excipient. The follow-up of parasitemia was monitored at days 2, 3, 4, and 30. The efficiency was evaluated with the survival time in comparison with control mice (mean survival time, 3.5 ± 0.5 days).

ACKNOWLEDGMENTS

This work was supported by Initiative d'Excellence Paris-Saclay (IdEx) and Laboratoire d'Excellence en Recherche sur le Médicament et l'Innovation Thérapeutique (LabEx LERMIT). The authors are grateful to the COST Action CM1307 for the collaboration established between Heidelberg and Paris-Sud laboratories. The authors would like to thank P. Grellier who kindly provided the strain FéoITMAP/1893 of *Trypanosoma brucei gambiense*. R. Luise Krauth-Siegel is also acknowledged for providing the *T. b.* TryR enzyme.

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A series of twenty-four aryl-polyamine cojugates were synthesized.

In vitro antiproliferative activity was determined against *T. brucei*, *L. donovani* and macrophages.

The lead compound **11b** displayed an IC_{50} around 100 nM against *T. brucei*.

11b at 10 mg/kg induced a delay in parasitic growth in vivo on mice infected by T. brucei.