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Synthesis of novel deuterium labelled derivatives of *N*-alkylated polyamines

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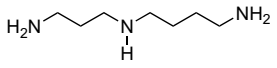
ABSTRACT

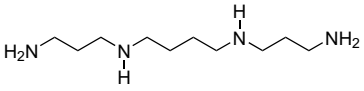
Novel di-, tetra- and octadeuterated derivatives of mono-*N*-alkylated diaminopropanes, spermidines, spermines, symmetrically bis-*N*-alkylated spermines and unsymmetrically bis-*N*-alkylated spermines were synthesized. Deuterium labels were introduced into the $RHNCH_2CH_2CN$ intermediate either by exchanging the protons next to the nitrile group under basic conditions with D_2O–EtOD mixture or/and by reducing the nitrile group to a CD_2–NH_2 fragment with $LiAlD_4$.

1. Introduction

Biogenic polyamines (PAs), spermidine (SPD), spermine (SPM) and their diamine precursor putrescine (butane-1,4-diamine) are small organic bases, which are widely distributed in nearly every prokaryotic and eukaryotic cell type, and are essential for cell growth, division and differentiation. In mammalian cells, the PAs are present at micro- to millimolar concentrations, and their intracellular levels are known to be strictly regulated by several enzymes and the cell membrane transport system.[1–3] Natural PAs and their analogues have been investigated as chemopreventative and antiparasitic agents, NMDA receptor modulators, polyamine-based venoms, metal chelators and as potential carriers for drug delivery.[4–6] It is known that relatively modest structural modifications in the PA backbone or incorporation of terminal *N*-alkyl substituent(s) may evoke significant differences in their chemical and therapeutic behaviour.[5–10]



SPD



SPM

Metabolic studies play an important role in drug discovery, development, testing and approval,[11] and represent the basic knowledge needed prior to clinical drug use by providing information on the drug safety and efficacy. This may prevent unnecessary expense in drug development as unsuitable projects can be interrupted as quickly as possible. In addition, metabolic studies may also identify novel possible targets for therapeutic intervention and new avenues for research.[5,6]

Despite intensive investigations into the biochemistry and molecular biology of PAs, there is relatively sparse data describing details of the interaction between PAs with macromolecules and receptors at the molecular level. In other words, the actual intracellular roles of PAs and the specificity of their interactions with receptor molecules are still largely unknown.[1,3,6,9] One reason for this problem is that it is biochemically challenging due to the fact that different PAs compete for the same binding site with varying degrees of specificity for their many predicted target molecules,

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and there can also be competition with divalent metal ions or other polyamine mimetics in the study systems. Moreover, the cellular distribution and compartmentalization of polyamines is not understood in detail. Furthermore, in vivo studies are hampered due to the metabolism of polyamines and their structural analogues. Thus, novel tools are required for in depth studies to identify key cellular targets as well as understanding details of the substrate properties of polyamine(analogue) metabolizing enzymes.

Today, liquid chromatography (LC) coupled to tandem mass spectrometry (MS/MS) represents a powerful tool for metabolite profiling studies since it can perform metabolite identification, as well as structural characterization and quantitation of the metabolites.[12] Recently, we have developed a novel method in which PAs, and also their biologically active derivatives and metabolites, are separated and quantitatively analyzed by LC–MS/MS using deuterium labelled compounds as internal standards.[13,14]

To achieve acceptable reproducibility and reliability, internal standards are used to correct for the variations in sample preparation and to compensate for the variability in MS detection. Stable isotopically labelled analogues are the most appropriate internal standards in quantitative LC–MS/MS assays, since virtually the only distinguishing feature from the analyte is the difference in the *m*/*z* ratio of the ions being studied. However, their use has been hampered due to the time-consuming and expensive syntheses involved in their production.[12,15] Deuterium is the most widely accessible and the least expensive stable isotope for use in the preparation of labelled internal standards. It is also often synthetically straightforward to incorporate more than one deuterium atom into the molecule, which may be necessary to sufficiently distinguish the labelled internal standards from the isotope peaks of the unlabelled analytes. In addition, deuterium labelled compounds are invaluable tools in studying the mechanisms of enzymes and the site of biotransformation, establishing the mechanism of organic reactions, and they are also useful in structural determinations, for example, in MS and NMR analyses.[16,17]

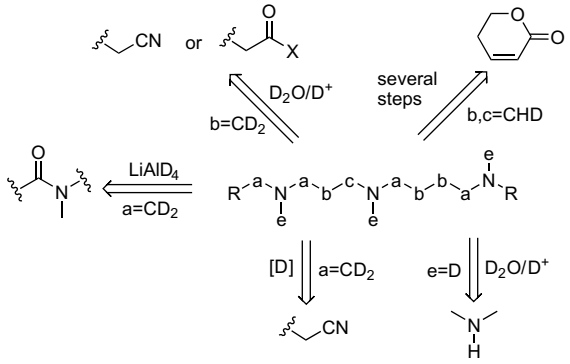
Various protocols have been developed for the synthesis of PA analogues and conjugates,[4,6,7,18] but the synthetic strategies to obtain deuterium labelled polyamines have been much more limited, and there is no general method to prepare *N*-alkylated PA derivatives labelled with deuterium.[19–22] Previously, only putrescine-d_4 (1,1,4,4-$^2H_4$-butane-1,4-diamine),[21] putrescine-d_8,[20,23] cadaverine-d_4 (1,1,5,5-$^2H_4$-pentane-1,4-diamine),[21] 1,1,3,3-$^2H_4$-propane-1,3-diamine[22] and 1,1,2,3-$^2H_4$-propane-1,3-diamine[20] have been used to prepare deuterated spermine, spermidine, N^1-acetylspermine and N^1-acetylspermidine.[19–21,24] However, the selective derivatization of diamines is not straightforward, even in the simplest cases for the synthesis of *N*-monosubstituted alkanediamines,[22] and it is especially complicated with non-symmetrical diamines.

The aim of this study was to develop a simple and straightforward method to synthesize *N*-alkylated polyamines **1**–**4**, in which R=H, Et, ^{i}Pr, Bn or *c*-Hex, and X, Y, X_1 and Y_1 are deuterium labelling positions, in order to use these compounds as standards in quantitative analysis of polyamines by LC–MS/MS,[13,14] as well as biochemical probes to study the metabolism of PAs.

2. Results and discussion

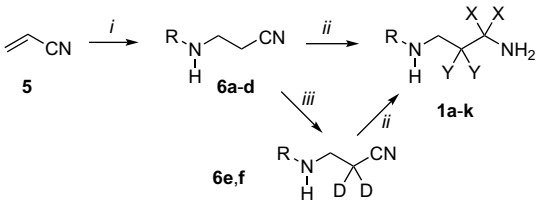
2.1. Chemistry

The most straightforward method to synthesize deuterium labelled molecules is to use commercially available labelled building blocks, but this approach is often limited due to the low availability and also the high cost of these compounds. It is possible to attempt exchange reactions adjacent to an activation group accelerated by various catalytic methods, and by using deuterated solvents as the labelling source.[22,25] However, the most common and a selective deuteration method is reduction with deuterium gas or deuterated solvents with the appropriate catalyst, or with metal deuterides.[20,21,26,27] Some possible approaches, which could be used in the synthesis of deuterium labelled polyamines are summarized in Scheme 1.



Scheme 1. Possible retrosynthetic strategies to prepare deuterated polyamine derivatives.

Our synthetic strategies to deuterated PA analogues **1**–**4** are based on the methods described above. As shown in Scheme 2, the starting compound was acrylonitrile (**5**), since it allows not only the introduction of the desired *N*-alkyl group to the double bond, but also the deuterium labelling of either X or Y positions, or both. The key intermediate **1**, after sufficient protection, can undergo chain elongation to yield the corresponding deuterium labelled SPD and SPM derivatives. The structures of prepared target compounds **1**–**4**, their overall yields and the isotopic purities of the deuterated compounds are summarized in Tables 1–3.



Scheme 2. (i) R–NH_2 (R=Et, ^{i}Pr, *c*-Hex, Bn), MeOH, reflux; (ii) Method A: $LiAlH_4$ or $LiAlD_4$, THF, reflux, Method B: H_2/PtO_2, EtOH, HCl; (iii) 0.1 M NaOD, D_2O, EtOD; X and Y=H or D.

Preparation of PA **1**–**4** was started from acrylonitrile (**5**) and the appropriate *N*-alkylamine using the Michael addition to obtain the desired *N*-alkylamino-propionitriles **6a**–**d** with 79–91% yields.[28] In

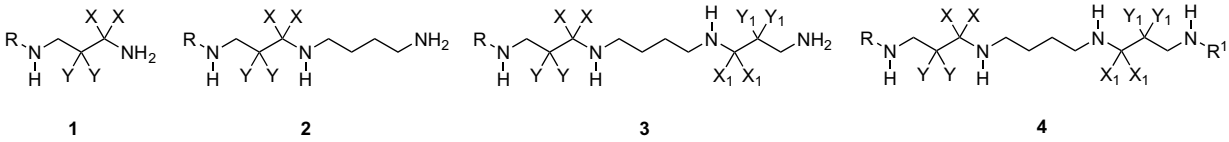


Table 1
Synthesized DAP derivatives, their overall yields and the isotopic purities of the deuterium labelled compounds

Compound					Overall yield %[a]			Isotopic purity %
DAP derivatives	R	X	Y	Intermediate compound	Method			
					A	B	C	
1a	Et	H	H	**6a**	39	73		—
1b	Et	D	H	**6a**	43			99
1c	Et	H	D	**6e**	18			94
1d	Et	D	D	**6e**	20			92
1e	Bn	H	H	**6d**	62		56	—
1f	Bn	D	H	**6d**	70			98
1g	Bn	D	D	**6f**	49			98
1h	^{i}Pr	H	H	**6b**	46	66		—
1i	^{i}Pr	D	H	**6b**	47			99
1j	*c*-Hex	H	H	**6c**	56	72		—
1k	*c*-Hex	D	H	**6c**	60			98
1l	H	D	H	**1f**	54			98

[a] Method A and method B, starting from alkylamine and acrylonitrile. Method A with $LiAlH_4$ or $LiAlD_4$ and Method B with H_2/PtO_2. Method C, starting from aldehyde. Yields are for recrystallized compounds and are not optimized.

Table 2
Synthesized SPD derivatives, their overall yields and the isotopic purities of the deuterium labelled compounds

Compound					Overall yield %[a]			Isotopic purity %
SPD derivatives	R	X	Y	Intermediate compound	Method			
					A	B	C	
2a	Et	H	H	**12a**	25	46		—
2b	Et	D	H	**12b**	21			99
2c	Et	D	D	**12c**	15			93
2d	Bn	H	H	**12d**	31		28	—
2e	Bn	D	H	**12e**	39			97
2f	^{i}Pr	H	H	**12f**	14	20		—
2g	*c*-Hex	H	H	**12g**	19	25		—
2h	H	D	H	**2e**	21			99

[a] Method A and method B, starting from alkylamine and acrylonitrile. Method C, starting from aldehyde. Yields are for recrystallized compounds and are not optimized.

the next step, it was possible to exchange $-CH_2CN$ protons with deuterium or to reduce the nitrile group to the CD_2-NH_2 or CH_2-NH_2 unit. The base catalyzed hydrogen exchange reactions of the simple cyanoalkanes are much faster than the corresponding hydrolysis to carboxylate ions,[29] which enables labelling at the

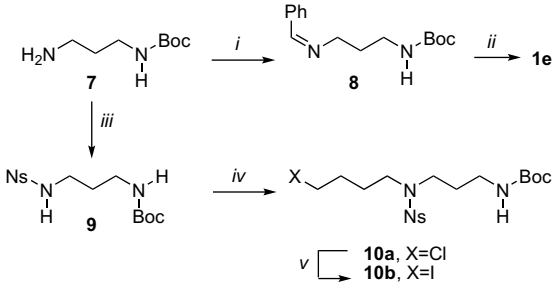
Table 3
Synthesized SPM derivatives, their overall yields and the isotopic purities of the deuterium labelled compounds

Compound								Overall yield %[a]			Isotopic purity %
SPM derivatives	R	R_1	X	Y	X_1	Y_1	Intermediate compound	Method			
								A	B	C	
3a	Et	H	H	H	H	H	**13a**	13	25		—
3b	Et	H	D	H	H	H	**13b**	15			97
3g	Et	H	H	H	D	D	**4f**	16			93
3c	Bn	H	H	H	H	H	**13c**	28		26	—
3d	Bn	H	D	D	H	H	**13d**	27			92
3e	^{i}Pr	H	H	H	H	H	**13e**	11	15		—
3f	*c*-Hex	H	H	H	H	H	**13f**	12	16		—
4a	Et	Et	H	H	H	H	**14a**	16	30		—
4b	Et	Et	D	H	D	H	**14b**	14			97
4c	^{i}Pr	^{i}Pr	H	H	H	H	**14c**	10	15		—
4d	*c*-Hex	*c*-Hex	H	H	H	H	**14d**	18	24		—
4e	Et	Bn	H	H	H	H	**14e**	21	40		—
4f	Et	Bn	H	H	D	D	**14f**	20			92
4g	Et	Bn	D	D	D	D	**14g**	10			87
4h	H	H	D	D	H	H	**3d**	22			92

[a] Method A and method B, starting from alkylamine and acrylonitrile. Method C, starting from aldehyde. Yields are for recrystallized compounds and are not optimized.

α-position. The deuteration stage achieved >97% for **6e**,**f** after the exchange reaction was repeated twice with a mixture of 0.1 M NaOD in D_2O and EtOD by using dry diethylether as the extraction solvent. Ethanol is of crucial importance for this reaction, since in its absence after the first cycle, only about 82–84% hydrogen was exchanged, whereas with ethanol, the deuterium stages were 92–95%. Moreover, chloroform was a poor extraction solvent, since both nitrogen deuteriums and α-deuteriums to nitrile were converted back to hydrogens to some extent, even when the traces of water and ethanol (as a stabilizer in chloroform) were removed with P_2O_5.[30]

The obtained nitriles **6a**–**f** were reduced to the corresponding amines **1a**–**k** either with $LiAlD_4$ (method A, 43–88% yields) or with PtO_2 catalysed hydrogenation (method B, 80–84% yields). However, the latter method is not suitable for benzyl derivatives **1e**–**g**, and as an example, compound **1e** was prepared from the commercially available Boc-protected amine **7** and benzaldehyde via imine **8** using reductive amination (method C) as shown in Scheme 3. Also the chain elongation unit **10**, which was used as a building block to the SPM derivatives **3a**–**g**, was prepared from **7** via protected **9**. Previously, **10** (X=Br) was prepared from **9** and 1,4-dibromobutane but we used 1-bromo-4-chlorobutane as an alkylating agent to avoid dimerization.[31]

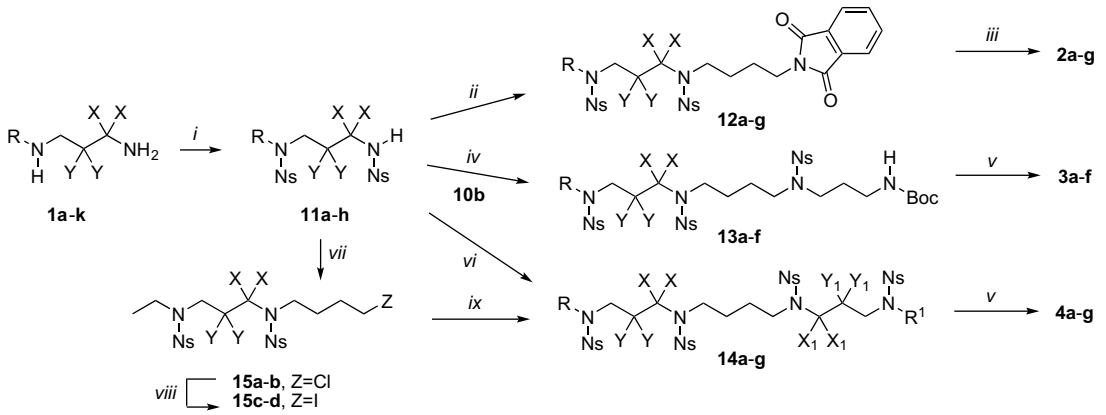


Scheme 3. (i) PhCHO, 3 Å molecular sieves, Et_2O; (ii) (1) $NaBH_4$, EtOH; (2) HCl, EtOH; (iii) NsCl, Et_3N, CH_2Cl_2; (iv) $Br(CH_2)_4Cl$, K_2CO_3, DMF; (v) NaI, acetone, reflux.

Mono-*N*-alkylated SPD analogues were selectively prepared from propanediamines **1a**–**k** after protection as the *o*-nitrophenylsulfonyl (Ns) derivatives **11a**–**k** (intermediate structures in Tables 7–10), since the only remaining NH group was readily alkylated with *N*-(4-iodobutyl)phthalimide[32] to **12a**–**k** under basic conditions with 81–95% yields as shown in Scheme 4. It was notable that the protection of the sterically hindered *N*-isopropyl and *N*-cyclohexyl derivatives to nosylated **11g**–**h** required more robust reaction conditions, chromatographic purification and yields (40–48%) were only half of that of those obtained with the ethyl and benzyl derivatives. The target SPD hydrochlorides **2a**–**g** were obtained from **12a**–**k** after deprotection and recrystallization from ethanol–water–EtOAc with 51–80% yields.

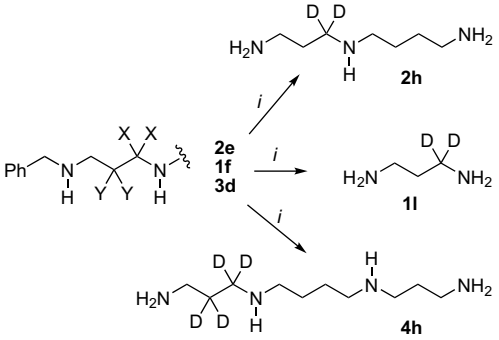
Mono-*N*-alkylated SPM analogues **3a**–**f** were also prepared from nosylated **11** via **13a**–**f** using **10b** (see Scheme 3) as an alkylating agent with 35–60% yields following the strategy having been earlier developed for spermidines.[32,33] Using the same approach and with 1,4-diiodobutane as the alkylating agent, the corresponding symmetric di-*N*,*N*-alkyl SPMs **4a**–**d** were obtained via **14a**–**d** with 32–69% yields. Surprisingly, the purification of **14a**–**c** was straightforward, since after addition of EtOAc and water to the reaction mixtures, the products crystallized out. Thus, chromatographic purification was needed only for the compound **14d**.

Using this strategy it is also possible to prepare non-symmetric *N*,*N*′-dialkyl SPMs. In this case, as shown in Scheme 4, we used **11a** (R=Et, X=Y=H) or **11c** (R=Et, X=Y=D) as starting materials to prepare alkylating agents **15c** and **15d**, respectively. The compounds **11d** (R=Bn, X=Y=H) or **11f** (R=Bn, X=Y=D) were selected



Scheme 4. (i) NsCl, Et_3N, CH_2Cl_2; (ii) $PhtN(CH_2)_4I$,[32] K_2CO_3, DMF; (iii) (1) PhSH, K_2CO_3, DMF; (2) $N_2H_4{\cdot}H_2O$, EtOH, reflux; (3) HCl, 1,4-dioxane; (iv) **10b**, K_2CO_3, DMF; (v) (1) PhSH, K_2CO_3, DMF; (2) HCl, 1,4-dioxane; (vi) $I(CH_2)_4I$, K_2CO_3, DMF; (vii) $Br(CH_2)_4Cl$, K_2CO_3, DMF; (viii) NaI, acetone, reflux; (ix) **11**, K_2CO_3, DMF.

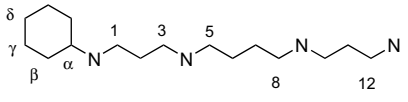
as the precursors of another three-carbon fragment of the SPM molecule. The use of these benzyl derivatives not only allowed the preparation of mixed *N*-ethyl-*N*-benzyl SPMs **4e**–**g**, but also after the removal of benzyl group by catalytic hydrogenation, it proved possible to obtain SPM derivatives labelled at the non-alkylated terminus (see compound **3g** in Table 3). The same strategy was used to prepare deuterated propanediamine-d_2 **1l**, spermidine-d_2 **2h** and spermine-d_4 **4h** starting from **1f**, **2e** and **3d**, respectively, as depicted in Scheme 5.



Scheme 5. (i) H_2/10% Pd–C, EtOH–H_2O.

2.2. NMR, MS and IR studies

All of the compounds were identified by ^1H and ^{13}C NMR spectroscopy. The complicated ^{13}C NMR spectra were assigned based on 2D techniques and via the information obtained from deuterated analogues. All the backbone carbons are quoted in Tables 4–6 (target compounds **1**–**4**) and in Tables 7–10 (intermediates). In the experimental part and in Tables 4–10, the backbone carbons are assigned with a number and *N*-alkyl substituents by using Greek letters as shown below.



During the syntheses, the overall deuterium contents were estimated from the ^1H NMR spectra and localizations were verified from the ^{13}C NMR spectra. The presence of deuterium atoms in the target compounds were identified without doubt, since in the ^1H NMR spectra the exchanged proton signals disappeared, remaining coupling patterns were simplified and in the ^{13}C NMR spectra, the low intensity CD_2 quintets closely upfield to the corresponding CH_2 signals were observed as shown in Figure 1. When the protons are replaced by deuterium the relaxation time (T_1) for the deuterated carbon may be more than an order of magnitude greater than that of the comparable protonated carbon. This may mean that with normal recycle delay for carbon measurements (D1=3 s) deuterated carbons may either not show up or appear with much reduced intensity. This was the reason to collect ^{13}C NMR data over a long period of time with a longer recycle delay (D1=10 s). In addition, the intensity of the carbon signal will be split into a multiplet due to ^{13}C–^2H *J* coupling depending on how many deuterons are attached to the carbon (1:1:1 triplet for CD and 1:2:3:2:1 quintet for CD_2). Measured ^{13}C–^2H *J* couplings ($^1J_{CD} \approx 19$–22 Hz) for quintets are

Table 4
^{13}C NMR chemical shifts for DAP derivatives **1a**–**l**[a,b,c]

Compd	R	C-1	C-2	$^1J_{CD}$	C-3	$^1J_{CD}$
1a	Et	46.8	26.6		39.5	
1b	Et	46.8	26.4		38.9*	22 Hz
1c	Et	46.7	26.0*	20 Hz	39.3	
1d	Et	46.7	25.8*	20 Hz	38.8*	22 Hz
1e	Bn	46.8	26.6		39.4	
1f	Bn	46.8	26.5		39.0*	22 Hz
1g	Bn	46.6	25.7*	20 Hz	38.8*	22 Hz
1h	^{i}Pr	44.5	26.9		39.5	
1i	^{i}Pr	44.5	26.6		39.0*	22 Hz
1j	*c*-Hex	44.2	26.8		39.5	
1k	*c*-Hex	44.1	26.6		39.0*	22 Hz
1l	H	39.4	27.5		38.9*	22 Hz

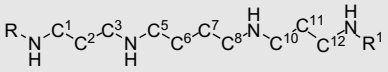
[a] NMR solvent was D_2O with TSP and products were measured as their HCl salts.
[b] ^{13}C NMR chemical shifts for R are presented in Section 4.
[c] Shifts marked with * are for CD_2 carbons, which were observed as 1:2:3:2:1 quintets.

Table 5
^{13}C NMR chemical shifts for SPD derivatives **2a**–**h**[a,b,c]

Compd	R	C-1	C-2	$^1J_{CD}$	C-3	$^1J_{CD}$	C-5	C-6	C-7	C-8
2a	Et	46.8	25.7		47.4		50.0	25.7	26.9	41.7
2b	Et	46.7	25.4		46.8*	22 Hz	49.9	25.6	26.8	41.7
2c	Et	46.6	24.7*	20 Hz	46.6*	22 Hz	49.9	25.6	26.7	41.7
2d	Bn	46.8	25.6		47.4		50.0	25.7	26.8	41.7
2e	Bn	46.7	25.3		46.8*	22 Hz	49.8	25.5	26.7	41.7
2f	^{i}Pr	44.5	25.8		47.4		50.0	25.6	26.8	41.7
2g	*c*-Hex	44.2	25.8		47.5		50.0	25.7	26.8	41.7
2h	H	39.4	26.4		46.8*	22 Hz	49.9	25.6	26.7	41.7

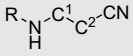
[a] NMR solvent was D_2O with TSP and products were measured as their HCl salts.
[b] ^{13}C NMR chemical shifts for R are presented in Section 4.
[c] Shifts marked with * are for CD_2 carbons, which were observed as quintets.

Table 6
^{13}C NMR chemical shifts for SPM derivatives **3a–g** and **4a–h**[a,b,c]

Compd															
	R	R^1	C-1	C-2	$^1J_{CD}$	C-3	$^1J_{CD}$	C-5	C-6	C-7	C-8	C-10	$^1J_{CD}$	C-11	$^1J_{CD}$	C-12
3a	Et	H	46.7	25.5		47.4		49.9	25.6	25.6	49.9	47.4		26.6		39.5
3b	Et	H	46.7	25.4		46.8*	22 Hz	49.8	25.6	25.6	49.9	47.4		26.6		39.4
3g	Et	H	46.7	25.5		47.3		49.8	25.6	25.6	49.8	46.7*	22 Hz	25.8*	20 Hz	39.2
3c	Bn	H	46.7	25.5		47.4		49.9	25.6	25.6	49.9	47.4		26.6		39.4
3d	Bn	H	46.6	24.7*	19 Hz	46.6*	22 Hz	49.8	25.6	25.6	49.9	47.4		26.6		39.4
3e	^{i}Pr	H	44.5	25.8		47.4		49.9	25.6	25.6	49.9	47.4		26.6		39.4
3f	*c*-Hex	H	44.1	25.8		47.4		49.9	25.6	25.6	49.9	47.4		26.6		39.4
4a	Et	Et	46.7	25.6		47.4		49.9	25.6	25.6	49.9	47.4		25.6		46.7
4b	Et	Et	46.7	25.4		46.8*	22 Hz	49.8	25.6	25.6	49.8	46.8*	22 Hz	25.4		46.7
4c	^{i}Pr	^{i}Pr	44.5	25.8		47.4		49.9	25.6	25.6	49.9	47.4		25.8		44.5
4d	*c*-Hex	*c*-Hex	44.1	25.8		47.4		49.9	25.6	25.6	49.9	47.4		25.8		44.1
4e	Bn	Et	46.7	25.5		47.4		49.9	25.6	25.6	49.9	47.4		25.6		46.7
4f	Bn	Et	46.6	24.7*	19 Hz	46.6*	(m)	49.8	25.6	25.6	49.9	47.4		25.5		46.7
4g	Bn	Et	46.6	24.7*	(m)	46.6*	(m)	49.8	25.6	25.6	49.8	46.6*	(m)	24.7*	(m)	46.5
4h	H	H	39.2	25.8*	20 Hz	46.7*	22 Hz	49.8	25.6	25.6	49.8	47.4		26.6		39.4

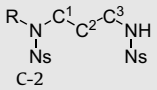
[a] NMR solvent was D_2O with TSP and products were measured as their HCl salts.
[b] ^{13}C NMR chemical shifts for R, R^1 are presented in Section 4.
[c] Shifts marked with * are for CD_2 carbons, which were observed as quintets except **4f**, C-3 which was under the other shifts, and overlaid **4g** shifts. See also Figure 2.

Table 7
^{13}C NMR chemical shifts for intermediates **6a–f**[a,b,c]

Compd					
	R	C-1	C-2	$^1J_{CD}$	CN
6a	Et	44.9	18.7		118.8
6e	Et	44.7	18.3*	21 Hz	118.8
6d	Bn	44.3	18.8		118.7
6f	Bn	44.2	18.4*	21 Hz	118.7
6b	^{i}Pr	42.6	19.1		118.8
6c	*c*-Hex	42.2	19.2		118.9

[a] NMR solvent was $CDCl_3$ with TMS.
[b] ^{13}C NMR chemical shifts for R are presented in Section 4.
[c] Shifts marked with * are for CD_2 carbons, which were observed as quintets.

Table 8
^{13}C NMR chemical shifts for intermediate DAP derivatives **11a–h**[a,b,c]

Compd						
	R	C-1	C-2		C-3	
11a	Et	44.2	28.9		40.6	
11b	Et	44.1	28.7		40.1*	(m)
11c	Et	44.0	28.0*	(m)	40.0*	(m)
11d	Bn	45.1	28.5		40.5	
11e	Bn	45.1	28.4		40.0*	(m)
11f	Bn	45.0	27.6*	(m)	39.8*	(m)
11g	^{i}Pr	41.1	32.0		40.4	
11h	*c*-Hex	41.2	32.1		41.1	

[a] NMR solvent was $CDCl_3$ with TMS.
[b] ^{13}C NMR chemical shifts for R and for protecting groups (Ns) are presented in Section 4.
[c] Shifts marked with * are for CD_2 carbons, which were observed as multiplets due to dynamic effects at room temperature.

shown in Tables 4–7. However, ^{13}C NMR spectra of the protected intermediates were more complex due to dynamic effects caused by the neighbouring protecting groups at room temperature and only multiplets were observed for the CD_2 groups (Tables 8–10). A typical example of the isotope effects generated by deuterium atoms is shown in Figure 2. Negative (upfield to the corresponding CH_2) deuterium induced isotope shifts were observed over one, two and three bonds, as can be estimated also from Tables 4–10. The isotopic shifts are additive, since deuterated C-3 resonance is shifted more upfield when also C-2 is deuterated (see Tables 4,5,8,9). In some cases, ^{1}H and ^{13}C NMR chemical shifts for the known compounds were re-evaluated, since the ^{13}C NMR chemical shifts differences in the literature compared to our shifts were 1–3 ppm (literature shifts measured in D_2O without internal standard) or in some cases chemical shifts were missing.

The isotopic distribution was estimated for all the deuterated target compounds. The isotopic purity was 87% for **4g** containing eight deuterium atoms, 91–97% for compounds with four deuterium atoms and 94–99% for compounds with two deuterium atoms, as shown in Tables 1–3. In addition, according to the ^{1}H NMR and ESI mass spectra, the overall deuterium stage of all the labelled compounds (detected deuterium amount in the molecule versus expected) was over 97%.

IR was used to study the C–D stretches in deuterated compounds. According to literature the range for C–D stretches is 2200–2080 cm^{-1}, which is beyond 'normal' IR vibrations.[34] However, only compound **11** clearly showed bands around this region (~2230 cm^{-1}). In the other spectra, there were no such clear vibrations in this region, or the bands are so weak or they may remain under the highly structured ammonium (NH_3^+, NH_2^+) vibrations (3000–2000 cm^{-1}).

3. Conclusions

A general method was developed to synthesize several deuterium labelled *N*-alkylated diaminopropanes containing two or four deuteriums in the non-exchangeable positions. These key intermediates were used to prepare mono-, di- and unsubstituted, sterically hindered, as well as symmetrically and non-symmetrically substituted polyamine derivatives, and also their symmetrically or non-symmetrically deuterium labelled analogues, having two to eight deuterium atoms in the PA backbone. According to the ESI-MS and ^{1}H NMR data, the isotopic purities were high (87–99%) for all the labelled compounds. Benzyl alkylated derivatives were found to be convenient precursors in the synthesis of a variety of labelled, non-*N*-alkylated diaminopropanes, spermidines and spermines, and they served also as an alternative route to monosubstituted polyamines.

4. Experimental

4.1. Reagents

Dimethylformamide (DMF) was distilled and stored over 4 Å molecular sieves, triethylamine (TEA) was dried over KOH and

Table 9
^{13}C NMR chemical shifts for intermediate SPD derivatives **12a–g** and alkylating agents **15a–d** and **10a–b**[a,b,c]

Compd	R	X	Y	C-1	C-2		C-3		C-5	C-6	C-7	C-8
12a	Et	Ns	NPht	44.3	27.5		45.2		47.3	25.4	25.7	37.1
12b	Et	Ns	NPht	44.3	27.3		44.6*	(m)	47.2	25.4	25.7	37.1
12c	Et	Ns	NPht	44.1	26.6*	(m)	44.5*	(m)	47.2	25.4	25.7	37.1
12d	Bn	Ns	NPht	45.1	26.9		44.9		46.8	25.2	25.6	37.1
12e	Bn	Ns	NPht	45.1	26.7		44.3*	(m)	46.7	25.2	25.6	37.1
12f	^{i}Pr	Ns	NPht	40.5	30.3		45.1		46.8	25.2	25.7	37.2
12g	*c*-Hex	Ns	NPht	41.4	30.4		45.1		46.8	25.2	25.7	37.2
15a	Et	Ns	Cl	44.4	27.6		45.1		47.2	25.4	29.3	44.4
15b	Et	Ns	Cl	44.3	26.7*	(m)	44.4*	(m)	47.1	25.4	29.3	44.4
15c	Et	Ns	I	44.4	27.6		45.1		46.7	29.0	30.1	5.9
15d	Et	Ns	I	44.3	26.7*	(m)	44.3*	(m)	46.7	28.9	30.1	6.0
10a	H	Boc	Cl	37.4	28.6		45.0		46.8	25.4	29.3	44.3
10b	H	Boc	I	37.4	28.6		45.0		46.4	28.9	30.1	5.8

[a] NMR solvent was $CDCl_3$ with TMS.
[b] ^{13}C NMR chemical shifts for R and for protecting groups (Ns, Pht and Boc) are presented in Section 4.
[c] Shifts marked with * are for CD_2 carbons, which were observed as multiplets due to dynamic effects at room temperature.

Table 10
^{13}C NMR chemical shifts for intermediate SPM derivatives **13a–f** and **14a–g**[a,b,c]

Compd	R	R^1	X	C-1	C-2		C-3		C-5	C-6	C-7	C-8	C-10		C-11		C-12
13a	Et	H	Boc	44.4	27.5		44.9		47.1	25.0	25.1	47.0	45.2		28.6		37.4
13b	Et	H	Boc	44.4	27.3		44.4*	(m)	47.0	25.0	25.1	47.0	45.2		28.6		37.4
13c	Bn	H	Boc	45.2	26.9		44.7		46.7	24.8	25.0	47.0	45.2		28.6		37.4
13d	Bn	H	Boc	45.1	26.0*	(m)	44.0*	(m)	46.6	24.8	25.0	47.0	45.2		28.6		37.4
13e	^{i}Pr	H	Boc	40.5	30.2		44.9		46.6	24.8	25.1	46.9	45.2		28.6		37.4
13f	*c*-Hex	H	Boc	41.4	30.4		44.8		46.6	24.7	25.1	46.9	45.1		28.6		37.4
14a**	Et	Et	Ns	44.4	27.5		45.0		47.2	25.0	25.0	47.2	45.0		27.5		44.4
14b*	Et	Et	Ns	44.4	27.2		44.5*	(m)	47.0	24.9	24.9	47.0	44.5*	(m)	27.2		44.4
14c**	^{i}Pr	^{i}Pr	Ns	40.2	29.8		44.4		46.5	24.5	24.5	46.5	44.4		29.8		40.2
14d**	*c*-Hex	*c*-Hex	Ns	41.4	30.3		44.8		46.6	24.7	24.7	46.6	44.8		30.3		41.4
14e	Bn	Et	Ns	45.2	26.9		44.8		46.8	24.8	25.0	47.1	45.0		27.5		44.4
14f	Bn	Et	Ns	45.1	26.0*	(m)	44.1*	(m)	46.7	24.8	25.0	47.1	45.0		27.5		44.4
14g	Bn	Et	Ns	45.1	26.0*	(m)	44.3*	(m)	46.7	24.8	24.9	47.1	44.3*	(m)	26.6*	(m)	44.3

[a] NMR solvent was $CDCl_3$ with TMS, except for **, which were measured in $CDCl_3$+DMSO-d_6 with TMS.
[b] ^{13}C NMR chemical shifts for R, R^1 and for protecting groups (Ns and Boc) are presented in Section 4.
[c] Shifts marked with * are for CD_2 carbons, which were observed as multiplets due to dynamic effects at room temperature.

distilled, diethylether was dried over sodium-benzophenone ketyl and distilled, dichloromethane (DCM) and chloroform were dried over P_2O_5 and distilled. All other reagents were used without further purification. Reactions were followed with TLC using analytical pre-coated silica gel 60 F_{254} plates, while silica gel (63–200 μm, Merck) was used for column chromatographic separations.

^1H and ^{13}C NMR spectra were measured on a Bruker Avance (Bruker, Rheinstetter, Germany) 500 DRX spectrometer operating at 500.13 and 125.76 MHz, respectively. TMS was used as an internal standard in organic solvents, and sodium 3-(trimethylsilyl)-1-propionic acid (TSP) in D_2O. Relaxation delay (D1) was set to 60 s for ^1H measurements and to 10 s for carbon experiments. ^1H and ^{13}C NMR chemical shifts were assigned based on standard 2D techniques (^1H–^1H COSY, ^1H–^{13}C HSQC and HMBC).

Isotope distribution measurements and high-resolution mass spectra were conducted on a QSTAR XL hybrid quadrupole TOF instrument (Applied Biosystems, Foster City, CA) using the positive electrospray ionization mode. Isopropylamine, amino acids and peptides were used as the internal standards in HRMS measurements.

IR spectra as KBr pelleting were recorded on Nexus 470 FT-IR. Melting points were measured in open capillary tubes and are uncorrected.

4.2. Syntheses of polyamines

4.2.1. 3-Ethylamino-propionitrile **6a**[28,35]

Prepared by the known method[28] from 70% ethylamine (20.0 g, 311 mmol) and acrylonitrile (10.6 g, 200 mmol) to give **6a** (17.93 g, 91%) as a colourless liquid, bp 75 °C/10 mbar. IR (neat): 3313 (br), 2969–2834, 2247, 1455, 1375, 1130 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.94 (2H, t, J=6.6 Hz, H-1), 2.69 (2H, q, J=7.1 Hz, H-α), 2.53 (2H, t, J=6.6 Hz, H-2), 1.68 (1H, br, NH), 1.13 (3H, t, J=7.1 Hz, H-β); ^{13}C NMR ($CDCl_3$): δ 43.5 (C-α), 15.2 (C-β), rest in Table 7; HRMS (ESI-MS): calcd for (M+H) $C_5H_{11}N_2$ 99.0922, found 99.0922.

4.2.2. 3-Isopropylamino-propionitrile **6b**[36,37]

Isopropylamine (5.00 g, 85 mmol) was added dropwise with stirring to a solution of acrylonitrile (6.73 g, 127 mmol) in methanol (10 mL) within 20 min. The stirring was continued for 30 min at room temperature and for 1 h under reflux. Solvents were evaporated in vacuo and the residue was distilled (bp 100 °C/35 mbar) to give **6b** (7.48 g, 79%) as a colourless liquid. IR (neat): 3313 (br), 2966–2870, 2247, 1472, 1381, 1175 cm^{-1}; ^1H NMR ($CDCl_3$): δ 2.92 (2H, t, J=6.7 Hz, H-1), 2.85 (1H, sep., J=6.2 Hz, H-α), 2.51 (2H, t, H-2), 1.09 (1H, br, NH), 1.07 (6H, d, J=6.2 Hz, H-β); ^{13}C NMR ($CDCl_3$):

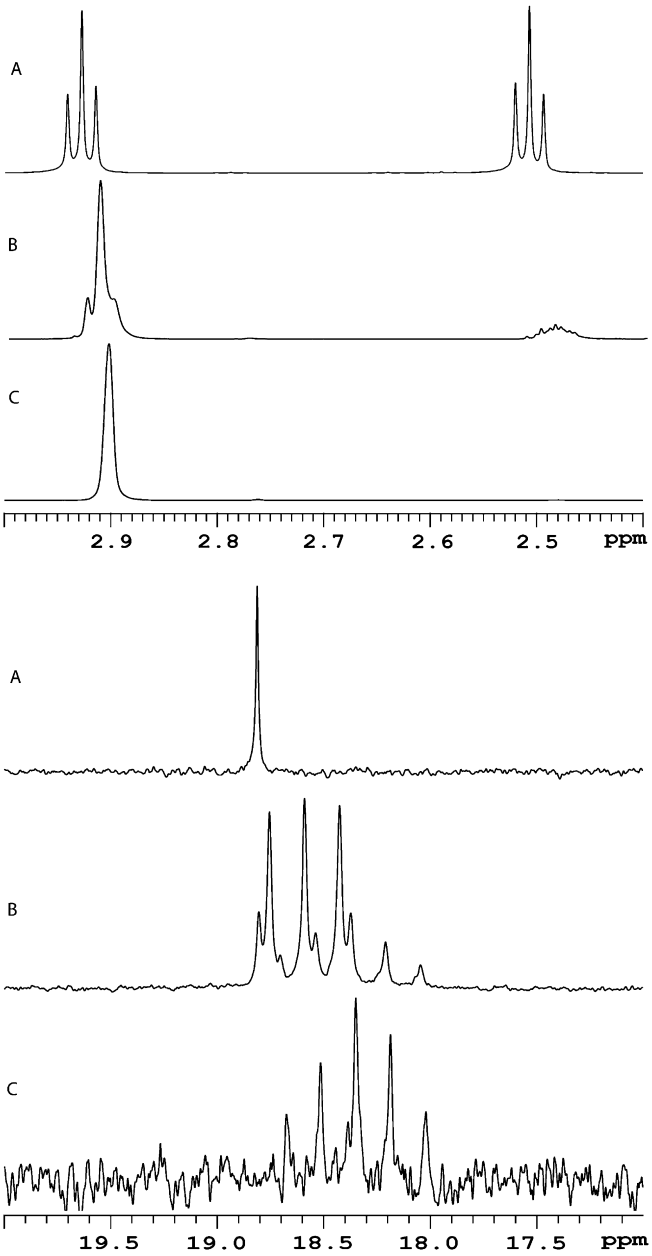


Figure 1. Deuterium induced changes in ^{1}H and ^{13}C NMR chemical shifts during the deuteration of **6d** to **6f**. Top: ^{1}H NMR; the exchanged proton signals disappear and the remaining coupling pattern is simplifying. Bottom: ^{13}C NMR; the carbon signal is splitting into a multiplet due to $^{13}C–^{2}H$ *J* coupling ($^{1}J_{CD} \approx 21$ Hz) depending on how many deuteriums are attached to the carbon. Singlet for CH_2, 1:1:1 triplet for CHD and 1:2:3:2:1 quintet for CD_2. A: unlabelled (**6d**), B: labelling in progress, C: labelled (**6f**).

δ 48.1 (C-α), 22.9 (2C-β), rest in Table 7; HRMS (ESI-MS): calcd for (M+H) $C_6H_{13}N_2$ 113.1079, found 113.1074.

4.2.3. 3-Cyclohexylamino-propionitrile **6c**[35,38,39]

Prepared as **6b** from cyclohexylamine (7.40 g, 75 mmol) and acrylonitrile (5.94 g, 112 mmol) to give **6c** (9.76 g, 86%) as a colourless liquid, bp 140 °C/12 mbar. IR (neat): 3422 (br), 2927, 2853, 2247, 1450, 1130 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.95 (2H, t, *J*=6.7 Hz, H-1), 2.53–2.43 (3H, m, H-2,α), 1.90–1.83 (2H, m, H-β_{eq}), 1.77–1.70 (2H, m, H-γ_{eq}), 1.65–1.58 (1H, m, H-δ_{eq}), 1.31–1.01 (6H, m, H-γ_{ax}, δ_{ax}, β_{ax}, NH); ^{13}C NMR ($CDCl_3$): δ 56.0 (C-α), 33.6 (2C-β), 26.0 (C-δ), 24.9 (2C-γ), rest in Table 7; HRMS (ESI-MS): calcd for (M+H) $C_9H_{17}N_2$ 153.1392, found 153.1391.

4.2.4. 3-Benzylamino-propionitrile **6d**[36,40]

Prepared as **6b** from benzylamine (8.57 g, 80 mmol) and acrylonitrile (6.37 g, 120 mmol) to give **6d** (10.75 g, 84%) as a colourless liquid, bp 125 °C/2 mbar. IR (neat): cm^{-1} as reported earlier;[36] ^{1}H NMR ($CDCl_3$): δ 7.36–7.22 (5H, m, Ph), 3.81 (2H, s, H-α), 2.90 (2H, t, *J*=6.6 Hz, H-1), 2.48 (2H, t, *J*=6.6 Hz, H-2), 1.54 (1H, br, NH); ^{13}C NMR ($CDCl_3$): δ 139.5 (Ph), 128.6 (2C, Ph), 128.1 (2C, Ph), 127.3 (Ph), 53.2 (C-α), rest in Table 7; HRMS (ESI-MS): calcd for (M+H) $C_{10}H_{13}N_2$ 161.1079, found 161.1082.

4.2.5. 3-Ethylamino-2,2-$^{2}H_2$-propionitrile **6e**

Nitrile **6a** (4.0 g, 40.75 mmol) in the mixture of 0.1 M NaOD in D_2O (26 mL) and EtOD (26 mL) was stirred at room temperature for 24 h. The reaction mixture was saturated with NaCl and extracted with dry Et_2O. Combined organic extracts were dried over K_2CO_3, solvents were removed by distillation, after the product was distilled under reduced pressure (2.65 g, 65%, 92% d, bp 72 °C/10 mbar). The reaction cycle was repeated once to give **6e** (1.91 g, 47%, 98% d) as a colourless liquid. IR (neat): 3312 (br), 2969–2340, 2248, 1453, 1384, 1126 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 2.92 (2H, s, H-1),

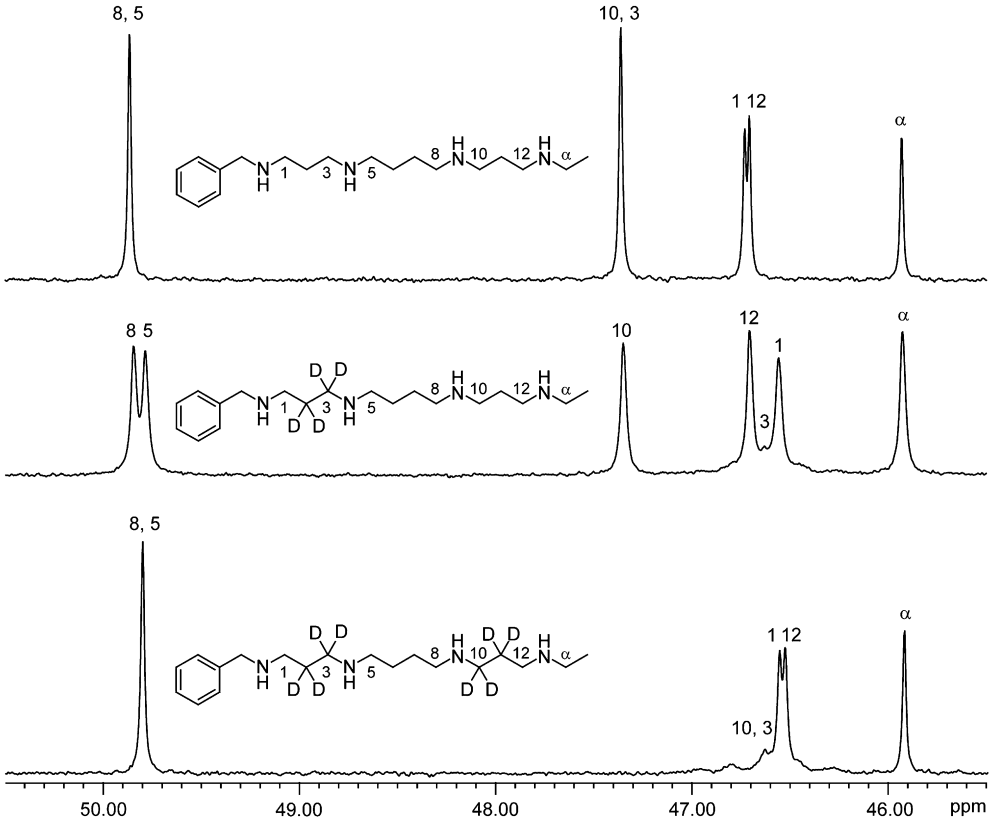


Figure 2. Negative deuterium induced isotope shifts, $\Delta\delta$, in ^{13}C NMR spectra were observed over one (0.7 ppm), two (0.17 ppm) and three bonds (0.06 ppm), as demonstrated for compounds **4e**, **4f** and **4g**.

2.69 (2H, q, J=7.1 Hz, H-α), 1.13 (3H, t, J=7.1 Hz, H-β); ^{13}C NMR ($CDCl_3$): δ 43.4 (C-α), 15.2 (C-β), rest in Table 7; HRMS (ESI-MS): calcd for (M+H) $C_5H_9D_2N_2$ 101.1048, found 101.1044.

4.2.6. 3-Benzylamino-2,2-$^2H_2$-propionitrile ***6f***

Prepared as **6e** from **6d** (3.2 g, 20 mmol) to give **6f** (2.62 g, 81%, 95% d, bp 120 °C/0.9 mbar) after the first cycle. The reaction cycle was repeated once to give **6f** (2.16 g, 66%, 99% d) as a colourless liquid. IR (neat): 3336 (br), 3085–2843, 2248, 1454, 743, 700 cm^{-1}; ^1H NMR ($CDCl_3$): δ 7.36–7.22 (5H, m, Ph), 3.82 (2H, s, H-α), 2.91 (2H, s, H-1); ^{13}C NMR ($CDCl_3$): δ 139.5 (Ph), 128.6 (2C, Ph), 128.1 (2C, Ph), 127.3 (Ph), 53.2 (C-α), rest in Table 7; HRMS (ESI-MS): calcd for (M+H) $C_{10}H_{11}D_2N_2$ 163.1204, found 163.1209.

4.3. General protocols to prepare 1a–k

Method A. To a cooled (0 °C) suspension of $LiAlH_4$ or $LiAlD_4$ (20.4 mmol) in dry Et_2O (40 mL), a solution of nitrile **6** (500 mg, 10 mmol) in dry Et_2O (10 mL) was added dropwise with stirring over a period of 45 min. The reaction mixture was allowed to warm to room temperature, heated at reflux for 3 h and further stirred at room temperature for 20 h. The reaction was quenched at 0–4 °C by dropwise addition of water (1.2 mL) and NaOH (5 M, 7.1 mL) with stirring. The organic phase was separated and the residue was extracted with hot chloroform (4×20 mL). The combined organic extracts were dried over $MgSO_4$ and the crude product was precipitated by treating with dry HCl. The subsequent recrystallization from ethanol–EtOAc yielded **1**.

Method B. To a solution of **6** (10.2 mmol) in ethanol (30 mL) was added concd HCl (2 mL) and the mixture was deoxygenated with argon for 10 min. Adam's catalyst (PtO_2, 0.44 mmol) was added and the mixture was hydrogenated at 3.5 bar pressure for 20 h. The catalyst was filtered off through Celite, washed with hot ethanol and water, and the combined filtrates were evaporated to dryness in vacuo. The residue was recrystallized from ethanol–EtOAc to give **1**.

Method C. Following the published procedure for N^1-benzylbutane-1,4-diamine,[41] compound **1** was prepared from the corresponding aldehyde (20.5 mmol) and (3-amino-propyl)carbamic acid *tert*-butyl ester (**7**, 22.6 mmol) after reduction and amino group deprotection.

4.3.1. N^1-Ethylpropane-1,3-diamine dihydrochloride ***1a***[39]

Prepared from **6a** to give **1a** (method A: 43%, method B: 80%) as colourless solid, mp 224–225 °C. IR (KBr): 3000–2387, 1606, 1459, 1379, 804 cm^{-1}; ^1H NMR (D_2O): δ 3.17–3.10 (6H, m, H-α,1,3), 2.12–2.06 (2H, m, H-2), 1.30 (3H, t, J=7.3 Hz, H-β); ^{13}C NMR (D_2O): δ 45.9 (C-α), 13.3 (C-β), rest in Table 4; HRMS (ESI-MS): calcd for (M+H) $C_5H_{15}N_2$ 103.1235, found 103.1229.

4.3.2. N^1-Ethyl-3,3-$^2H_2$-propane-1,3-diamine dihydrochloride ***1b***

Prepared from **6a** to give **1b** (method A, 47%, 99% d) as a colourless solid, mp 219–221 °C. IR (KBr): 3000–2386, 1604, 1460, 1382, 1144, 855, 804 cm^{-1}; ^1H NMR (D_2O): δ 3.17–3.10 (4H, m, H-α,1), 2.11–2.05 (2H, m, H-2), 1.30 (3H, t, J=7.3 Hz, H-β); ^{13}C NMR (D_2O): δ 45.9 (C-α), 13.4 (C-β), rest in Table 4; HRMS (ESI-MS): calcd for (M+H) $C_5H_{13}N_2D_2$ 105.1361, found 105.1354. Isotope distribution: 0% d_0, 1% d_1, 98% d_2.

4.3.3. N^1-Ethyl-2,2-$^2H_2$-propane-1,3-diamine dihydrochloride ***1c***

Prepared from **6e** to give **1c** (method A, 43%, 97% d) as a colourless solid, mp 222–224 °C. IR (KBr): 3000–2369, 1594, 1462, 1374, 1185, 802 cm^{-1}; ^1H NMR (D_2O): δ 3.17–3.08 (6H, m, H-α,1,3), 1.30 (3H, t, J=7.3 Hz, H-β); ^{13}C NMR (D_2O): δ 45.9 (C-α), 13.4 (C-β),

rest in Table 4; HRMS (ESI-MS): calcd for (M+H) $C_5H_{13}N_2D_2$ 105.1361, found 105.1356. Isotope distribution: 0% d_0, 6% d_1, 94% d_2.

4.3.4. N^1-Ethyl-2,2,3,3-$^2H_4$-propane-1,3-diamine dihydrochloride **1d**

Prepared from **6e** to give **1d** (method A, 47%, 98% *d*) as a colourless solid, mp 218–220 °C. IR (KBr): 3000–2388, 1603, 1463, 1382, 1171, 837, 806 cm^{-1}; ^1H NMR (D_2O): δ 3.17–3.10 (4H, m, H-α,1), 1.30 (3H, t, *J*=7.3 Hz, H-β); ^{13}C NMR (D_2O): δ 46.0 (C-α), 13.4 (C-β), rest in Table 4; HRMS (ESI-MS): calcd for (M+H) $C_5H_{11}N_2D_4$ 107.1486, found 107.1480. Isotope distribution: 0% d_0–d_2, 8% d_3, 92% d_4.

4.3.5. N^1-Benzyl-propane-1,3-diamine dihydrochloride **1e**[42,43]

Prepared from **6d** to give **1e** (method A, 74%) as a colourless solid, mp 271–272 °C.

Prepared from benzaldehyde (2.2 g, 20.5 mmol) and (3-aminopropyl)carbamic acid *tert*-butyl ester (3.9 g, 22.6 mmol) following method C, which after recrystallization from ethanol–EtOAc gave **1e** in the overall (3 steps) with a 56% yield. IR (KBr): 3050–2410, 1599, 1484, 1174, 744, 697 cm^{-1}; ^1H NMR (D_2O): δ 7.57–7.48 (5H, m, Ph), 4.29 (2H, s, H-α), 3.24–3.17 (2H, m, H-1), 3.14–3.08 (2H, m, H-3), 2.17–2.07 (2H, m, H-2); ^{13}C NMR (D_2O): δ 133.3 (Ph), 132.7 (2C, Ph), 132.6 (Ph), 132.2 (2C, Ph), 54.1 (C-α), rest in Table 4; HRMS (ESI-MS): calcd for (M+H) $C_{10}H_{17}N_2$ 165.1392, found 165.1389.

4.3.6. N^1-Benzyl-3,3-$^2H_2$-propane-1,3-diamine dihydrochloride **1f**

Prepared from **6d** to give **1f** (method A, 83%, 99% *d*) as a colourless solid, mp 275 °C. IR (KBr): 3050–2387, 1601, 1478, 854, 744, 697 cm^{-1}; ^1H NMR (D_2O): δ 7.57–7.48 (5H, m, Ph), 4.29 (2H, s, H-α), 3.24–3.17 (2H, m, H-1), 2.16–2.07 (2H, m, H-2); ^{13}C NMR (D_2O): δ 133.5 (Ph), 132.7 (2C, Ph), 132.6 (Ph), 132.1 (2C, Ph), 54.1 (C-α), rest in Table 4; HRMS (ESI-MS): calcd for (M+H) $C_{10}H_{15}N_2D_2$ 167.1517, found 167.1513. Isotope distribution: 0% d_0, 2% d_1, 98% d_2.

4.3.7. N^1-Benzyl-2,2,3,3-$^2H_4$-propane-1,3-diamine dihydrochloride **1g**

Prepared from **6f** to give **1g** (method A, 88%, 98% *d*) as a colourless solid, mp 274–275 °C. IR (KBr): 3050–2429, 1600, 1468, 836, 744, 697 cm^{-1}; ^1H NMR (D_2O): δ 7.57–7.46 (5H, m, Ph), 4.29 (2H, s, H-α), 3.19 (2H, s, H-1); ^{13}C NMR (D_2O): δ 133.3 (Ph), 132.7 (2C, Ph), 132.6 (Ph), 132.2 (2C, Ph), 54.1 (C-α), rest in Table 4; HRMS (ESI-MS): calcd for (M+H) $C_{10}H_{13}N_2D_4$ 169.1643, found 169.1641. Isotope distribution: 0% d_0–d_2, 7% d_3, 92% d_4.

4.3.8. N^1-Isopropylpropane-1,3-diamine dihydrochloride **1h**[39,44]

Prepared from **6b** to give **1h** (method A: 58%, method B: 83%) as a colourless solid, mp 184–186 °C. IR (KBr): 3000–2385, 1597, 1473, 1379, 1136, 766 cm^{-1}; ^1H NMR (D_2O): δ 3.45 (1H, sep., *J*=6.6 Hz, H-α), 3.19–3.09 (4H, m, H-1,3), 2.13–2.03 (2H, m, H-2), 1.33 (6H, d, *J*=6.6 Hz, H-β); ^{13}C NMR (D_2O): δ 53.9 (C-α), 21.0 (2C-β), rest in Table 4; HRMS (ESI-MS): calcd for (M+H) $C_6H_{17}N_2$ 117.1392, found 117.1386.

4.3.9. N^1-Isopropyl-3,3-$^2H_2$-propane-1,3-diamine dihydrochloride **1i**

Prepared from **6b** to give **1i** (method A, 59%, 99% *d*) as a colourless solid, mp 182–184 °C. IR (KBr): 3000–2300, 1594, 1471, 1382, 1161, 845 cm^{-1}; ^1H NMR (D_2O): δ 3.45 (1H, sep., *J*=6.6 Hz, H-α), 3.18–3.13 (2H, m, H-1), 2.10–2.04 (2H, m, H-2), 1.33 (6H, d, *J*=6.6 Hz, H-β); ^{13}C NMR (D_2O): δ 53.9 (C-α), 21.0 (2C-β), rest in Table 4; HRMS (ESI-MS): calcd for (M+H) $C_6H_{15}N_2D_2$ 119.1517, found 119.1514. Isotope distribution: 0% d_0, 1% d_1, 99% d_2.

4.3.10. N^1-Cyclohexylpropane-1,3-diamine dihydrochloride **1j**[39,45]

Prepared from **6c** to give **1j** (method A: 65%, method B: 84%) as a colourless solid, mp 200–202 °C (lit. mp 199–202 °C).[45] IR (KBr): 3000–2368, 1606, 1464, 1164, 783 cm^{-1}; ^1H NMR (D_2O): δ 3.22–3.08 (5H, m, H-1,3,α), 2.13–2.02 (4H, m, H-2,β_{eq}), 1.90–1.79 (2H, m, H-γ_{eq}), 1.72–1.63 (1H, m, H-δ_{eq}), 1.41–1.27 (4H, m, H-β_{ax},γ_{ax}), 1.25–1.13 (1H, m, H-δ_{ax}); ^{13}C NMR (D_2O): δ 60.3 (C-α), 31.7 (2C-β), 27.3 (C-δ), 26.8 (2C-γ), rest in Table 4; HRMS (ESI-MS): calcd for (M+H) $C_9H_{21}N_2$ 157.1705, found 157.1707.

4.3.11. N^1-Cyclohexyl-3,3-$^2H_2$-propane-1,3-diamine dihydrochloride **1k**

Prepared from **6c** to give **1k** (method A, 70%, 99% *d*) as a colourless solid, mp 205–207 °C. IR (KBr): 3000–2385, 1604, 1467, 855 cm^{-1}; ^1H NMR (D_2O): δ 3.22–3.08 (3H, m, H-1,α), 2.13–2.02 (4H, m, H-2,β_{eq}), 1.90–1.79 (2H, m, H-γ_{eq}), 1.72–1.63 (1H, m, H-δ_{eq}), 1.41–1.27 (4H, m, H-β_{ax},γ_{ax}), 1.25–1.13 (1H, m, H-δ_{ax}); ^{13}C NMR (D_2O): δ 60.3 (C-α), 31.7 (2C-β), 27.3 (C-δ), 26.7 (2C-γ), rest in Table 4; HRMS (ESI-MS): calcd for (M+H) $C_9H_{19}N_2D_2$ 159.1830, found 159.1826. Isotope distribution: 0% d_0, 2% d_1, 98% d_2.

4.3.12. 1,1-$^2H_2$-Propane-1,3-diamine dihydrochloride **1l**[46]

To a mixture of **1f** (190 mg, 0.79 mmol) in ethanol (30 mL), water was added to dissolve **1f** followed with 10% Pd–C (80 mg) and the reaction mixture was hydrogenated at 3.5 bar pressure for 20 h. The catalyst was filtered off through Celite, washed with ethanol and water, and the combined filtrates were evaporated to dryness in vacuo. Recrystallization from ethanol–water–EtOAc yielded **1l** (92 mg, 78%, 99% *d*) as a colourless solid, mp 249–250 °C (lit. mp 246–250 °C).[46] IR (KBr): 3000–2300, 2227, 1610, 1463, 1167, 844 cm^{-1}; ^1H NMR (D_2O): δ 3.15–3.09 (2H, m, H-1), 2.10–2.04 (2H, m, H-2); ^{13}C NMR (D_2O): δ in Table 4; HRMS (ESI-MS): calcd for (M+H) $C_3H_9N_2D_2$ 77.1048, found 77.1051. Isotope distribution: 1% d_0, 1% d_1, 98% d_2.

4.3.13. N^1-Ethyl-bis-N^1,N^3-(2-nitrobenzenesulfonyl)propane-1,3-diamine **11a**

A suspension of **1a** (2.0 g, 11.42 mmol) and TEA (8 mL, 57 mmol) in dry DCM (100 mL) was cooled to 0 °C. NsCl (5.32 g, 23.99 mmol) in dry DCM (70 mL) was added dropwise with stirring over a period of 10 min, and stirring was continued for 1 h at 0 °C and then for 3 h at room temperature. The reaction mixture was washed with water (2×200 mL), 10% citric acid (1×200 mL), brine (1×200 mL), dried over $MgSO_4$ and evaporated to dryness in vacuo that resulted in **11a** (5.4 g, ~100%) as a pale yellow solid, mp 82–84 °C. IR (KBr): 3321, 3095–2878, 1545, 1380, 1160, 736 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.14–8.09 (1H, m, *Ph*), 8.03–7.98 (1H, m, Ph), 7.87–7.83 (1H, m, Ph), 7.78–7.67 (4H, m, Ph), 7.64–7.59 (1H, m, Ph), 5.66 (1H, t, *J*=6.2 Hz, NH), 3.42–3.31 (4H, m, H-α,1), 3.22–3.16 (2H, m, H-3), 1.89–1.81 (2H, m, H-2), 1.10 (3H, t, *J*=7.1 Hz, H-β); ^{13}C NMR ($CDCl_3$): δ 148.1, 148.0, 133.7, 133.6 (2C), 133.3, 132.9, 131.8, 130.9, 130.8, 125.5, 124.2 (totally 12C–Ph), 42.4 (C-α), 13.7 (C-β), rest in Table 8; HRMS (ESI-MS): calcd for (M+H) $C_{17}H_{21}N_4O_8S_2$ 473.0801, found 473.0793.

4.3.14. N^1-Ethyl-bis-N^1,N^3-(2-nitrobenzenesulfonyl)-3,3-$^2H_2$-propane-1,3-diamine **11b**

Prepared as **11a** from **1b** (1.20 g, 6.78 mmol) to give **11b** (3.33 g, ~100%) as a pale yellow solid, mp 79–81 °C. IR (KBr): 3323, 3095–2879, 1545, 1380, 1161, 732 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.14–8.09 (1H, m, Ph), 8.02–7.97 (1H, m, Ph), 7.87–7.84 (1H, m, Ph), 7.78–7.67 (4H, m, Ph), 7.64–7.59 (1H, m, Ph), 5.66 (1H, br, NH), 3.42–3.31 (4H, m, H-α,1), 1.86–1.81 (2H, m, H-2), 1.10 (3H, t, *J*=7.1 Hz, H-β); ^{13}C NMR ($CDCl_3$): δ 148.1, 148.0, 133.7, 133.6, 133.6, 133.3, 132.9, 131.8, 130.9, 130.8, 125.5, 124.2 (totally 12C–Ph), 42.4 (C-α), 13.7 (C-β), rest in Table 8; HRMS (ESI-MS): calcd for (M+H) $C_{17}H_{19}D_2N_4O_8S_2$ 475.0926, found 475.0925.

4.3.15. N^1-Ethyl-bis-N^1,N^3-(2-nitrobenzenesulfonyl)-2,2,3,3-$^2H_4$-propane-1,3-diamine **11c**

Prepared as **11a** from **1d** (700 mg, 3.91 mmol) to give **11c** (1.89 g, ~100%) as a pale yellow solid, mp 79–83 °C. IR (KBr): 3323,

3095–2881, 1544, 1380, 1161, 746 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.14–8.10 (1H, m, Ph), 8.04–7.99 (1H, m, Ph), 7.89–7.84 (1H, m, Ph), 7.78–7.67 (4H, m, Ph), 7.64–7.60 (1H, m, Ph), 5.63 (1H, br, NH), 3.38 (2H, s, H-1), 3.35 (2H, q, J=7.2 Hz, H-α), 1.11 (3H, t, J=7.2 Hz, H-β); ^{13}C NMR ($CDCl_3$): δ 148.1, 148.0, 133.7, 133.6, 133.6, 133.2, 132.9 131.8, 130.9, 130.8, 125.5, 124.2 (totally 12C–Ph), 42.4 (C-α), 13.7 (C-β), rest in Table 8; HRMS (ESI-MS): calcd for (M+H) $C_{17}H_{17}D_4N_4O_8S_2$ 477.1052, found 477.1046.

4.3.16. N^1-Benzyl-bis-N^1,N^3-(2-nitrobenzenesulfonyl)propane-1,3-diamine **11d**

Prepared as **11a** from **1e** (600 mg, 2.53 mmol) to give **11d** (1.25 g, 92%) as a pale yellow solid, mp 40–55 °C (amorphous solid). IR (KBr): 3342 (br), 3095–2880, 1541, 1163, 732 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.07–8.03 (1H, m, Ph), 8.01–7.98 (1H, m, Ph), 7.86–7.82 (1H, m, Ph), 7.74–7.64 (5H, m, Ph), 7.31–7.22 (5H, m, Ph), 5.39 (1H, t, J=6.3 Hz, NH), 4.47 (2H, s, H-α), 3.36–3.32 (2H, m, H-1), 3.02–2.96 (2H, m, H-3), 1.62–1.55 (2H, m, H-2); ^{13}C NMR ($CDCl_3$): δ 148.0, 148.0, 135.5, 133.8, 133.6, 133.6, 133.1, 132.8, 131.9, 131.1, 130.9, 128.9 (2C), 128.3 (2C), 128.3, 125.4, 124.3 (totally 18C–Ph), 52.3 (C-α), rest in Table 8; HRMS (ESI-MS): calcd for (M+H) $C_{22}H_{23}N_4O_8S_2$ 535.0957, found 535.0984.

4.3.17. N^1-Benzyl-bis-N^1,N^3-(2-nitrobenzenesulfonyl)-3,3-$^2H_2$-propane-1,3-diamine **11e**

Prepared as **11a** from **1f** (700 mg, 2.93 mmol) to give **11e** (1.43 g, 91%) as a pale yellow solid, mp 40–48 °C (amorphous solid). IR (KBr): 3341 (br), 3095–2880, 1541, 1163, 740, 699 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.07–8.03 (1H, m, Ph), 8.01–7.98 (1H, m, Ph), 7.86–7.82 (1H, m, Ph), 7.74–7.64 (5H, m, Ph), 7.31–7.22 (5H, m, Ph), 5.38 (1H, br, NH), 4.47 (2H, s, H-α), 3.36–3.32 (2H, m, H-1), 1.59–1.55 (2H, m, H-2); ^{13}C NMR ($CDCl_3$): δ 148.0 (2C), 135.5, 133.8, 133.7 133.6, 133.1, 132.8, 131.9, 131.1, 130.9, 128.9 (2C), 128.3 (2C), 128.2, 125.4, 124.3 (totally 18C–Ph), 52.3 (C-α), rest in Table 8; HRMS (ESI-MS): calcd for (M+H) $C_{22}H_{21}D_2N_4O_8S_2$ 537.1083, found 537.1083.

4.3.18. N^1-Benzyl-bis-N^1,N^3-(2-nitrobenzenesulfonyl)-2,2,3,3-$^2H_4$-propane-1,3-diamine **11f**

Prepared as **11a** from **1g** (2.50 g, 10.36 mmol) to give **11f** (5.16 g, 93%) as a pale yellow solid, mp 39–48 °C (amorphous solid). IR (KBr): 3340 (br), 3084–2928, 2225, 1541, 1164, 740 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.06–8.01 (1H, m, Ph), 7.99–7.95 (1H, m, Ph), 7.84–7.80 (1H, m, Ph), 7.75–7.63 (5H, m, Ph), 7.31–7.21 (5H, m, Ph), 5.41 (1H, br, NH), 4.46 (2H, s, H-α), 3.32 (2H, s, H-1); ^{13}C NMR ($CDCl_3$): δ 148.0 (2C), 135.5, 133.8, 133.7, 133.5, 133.0, 132.9, 132.0, 130.9, 130.8, 128.8 (2C), 128.3 (2C), 128.2, 125.4, 124.3 (totally 18C–Ph), 52.2 (C-α), rest in Table 8; HRMS (ESI-MS): calcd for (M+H) $C_{22}H_{19}D_4N_4O_8S_2$ 539.1208, found 539.1208.

4.3.19. N^1-Isopropyl-bis-N^1,N^3-(2-nitrobenzenesulfonyl)propane-1,3-diamine **11g**

Prepared as **11a** from **1h** (3.00 g, 15.86 mmol) but after 2 h at room temperature, the reaction mixture was heated at reflux for 20 h to give **11g** (3.08 g, 40%) as a pale yellow solid after column chromatography using EtOAc–hexane 3:2 as an eluent, mp 100–102 °C. R_f 0.52 (EtOAc–hexane 3:2); IR (KBr): 3332, 3096–2850, 1545, 1380, 1160 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.15–8.12 (1H, m, Ph), 8.05–8.01 (1H, m, Ph), 7.89–7.85 (1H, m, Ph), 7.78–7.66 (4H, m, Ph), 7.62–7.58 (1H, m, Ph), 5.61 (1H, br, NH), 4.10 (1H, sep., J=6.8 Hz, H-α), 3.36–331 (2H, m, H-1), 3.23–3.19 (2H, m, H-3), 1.94–1.87 (2H, m, H-2), 1.14 (6H, d, J=6.8 Hz, H-β); ^{13}C NMR ($CDCl_3$): δ 148.1 (2C), 133.7, 133.6, 133.6, 133.5, 132.9, 131.7, 131.0, 131.0, 125.5, 124.2 (totally 12C–Ph), 50.1 (C-α), 21.4 (2C-β), rest in Table 8; HRMS (ESI-MS): calcd for (M+H) $C_{18}H_{23}N_4O_8S_2$ 487.0957, found 487.0957.

4.3.20. N^1-Cyclohexyl-bis-N^1,N^3-(2-nitrobenzenesulfonyl)propane-1,3-diamine **11h**

Prepared as **11g** from **1j** (917 mg, 4.0 mmol) to give **11h** (1.02 g, 48%) as a pale yellow solid, mp 134–136 °C. R_f 0.50 (EtOAc–hexane 3:2); IR (KBr): 3311, 3091–2851, 1542, 1164 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.15–8.11 (1H, m, Ph), 8.04–7.99 (1H, m, Ph), 7.89–7.85 (1H, m, Ph), 7.78–7.66 (4H, m, Ph), 7.61–7.56 (1H, m, Ph), 5.62 (1H, t, J=6.3 Hz, NH), 3.70–3.62 (1H, m, H-α), 3.37–3.32 (2H, m, H-1), 3.24–3.18 (2H, m, H-3), 1.92–1.85 (2H, m, H-2), 1.78–1.72 (2H, m, H-γ_{eq}), 1.69–1.59 (3H, m, H-β_{eq},δ_{eq}), 1.40–1.25 (4H, m, H-β_{ax},γ_{ax}), 1.10–0.99 (1H, m, H-δ_{ax}); ^{13}C NMR ($CDCl_3$): δ 148.1 (2C), 133.8, 133.7, 133.6, 133.5, 132.9, 131.7, 130.9, 130.8, 125.5, 124.1 (totally 12C–Ph), 58.3 (C-α), 32.1 (2C-β), 26.0 (2C-γ), 25.2 (C-δ), rest in Table 8; HRMS (ESI-MS): calcd for (M+Na) $C_{21}H_{26}N_4O_8S_2Na$ 549.1090, found 549.1098.

4.3.21. 2-(4-{[3-(Ethyl-(2-nitrobenzenesulfonyl)amino)propyl]-(2-nitrobenzenesulfonyl)amino}-butyl)isoindole-1,3-dione **12a**

To a mixture of **11a** (1.0 g, 2.17 mmol) and K_2CO_3 (0.88 g, 6.35 mmol) in dry DMF (25 mL), *N*-(4-iodobutyl)phthalimide[32] (0.77 g, 2.33 mmol) was added with stirring and the mixture was allowed to react at room temperature for 17 h. After filtration, the solvents were evaporated in vacuo and the residue was purified on silica gel using first EtOAc–hexane 1:1 and then 5:2 as an eluent to give **12a** (1.24 g, 85%) as a colourless solid, mp 44–46 °C (amorphous solid). R_f 0.53 (EtOAc–hexane 5:2); IR (KBr): 3095, 2940 (br), 1709, 1543, 1373, 1158, 720 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.02–7.98 (2H, m, Ph), 7.86–7.81 (2H, m, Ph), 7.74–7.55 (8H, m, Ph), 3.68–3.64 (2H, m, H-8), 3.39–3.26 (8H, m, H-α,1,3,5), 1.91–1.83 (2H, m, H-2), 1.69–1.62 (2H, m, H-7) 1.60–1.52 (2H, m, H-6), 1.12 (3H, t, J=7.1 Hz, H-β); ^{13}C NMR ($CDCl_3$): δ 168.4 (2C, C=O), 148.0 (2C), 134.0 (2C), 133.5, 133.5, 133.4, 133.2, 132.1 (2C), 131.8, 131.8, 130.8 (2C), 124.2 (2C), 123.3 (2C) (totally 18C–Ph), 42.3 (C-α), 13.6 (C-β), rest in Table 9; HRMS (ESI-MS): calcd for (M+H) $C_{29}H_{32}N_5O_{10}S_2$ 674.1591, found 674.1584.

4.3.22. 2-(4-{[3-(Ethyl-(2-nitrobenzenesulfonyl)-amino)-1,1-$^2H_2$-propyl]-(2-nitrobenzenesulfonyl)-amino}butyl) isoindole-1,3-dione **12b**

Prepared as **12a** from **11b** (600 mg, 1.26 mmol) to give **12b** (803 mg, 94%) as a colourless solid, mp 38–44 °C (amorphous solid). R_f 0.53 (EtOAc–hexane 5:2); IR (KBr): 3095, 2940 (br), 1710, 1544, 1373, 1160, 721 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.02–7.97 (2H, m, Ph), 7.86–7.81 (2H, m, Ph), 7.75–7.55 (8H, m, Ph), 3.68–3.63 (2H, m, H-8), 3.39–3.27 (6H, m, H-α,1,5), 1.88–1.83 (2H, m, H-2), 1.69–1.62 (2H, m, H-7), 1.60–1.52 (2H, m, H-6), 1.11 (3H, t, J=7.1 Hz, H-β); ^{13}C NMR ($CDCl_3$): δ 168.4 (2C, C=O), 148.0 (2C), 134.0 (2C), 133.6, 133.5, 133.3, 133.1, 132.0 (2C), 131.8, 131.8, 130.7 (2C), 124.2 (2C), 123.3 (2C) (totally 18C–Ph), 42.3 (C-α), 13.6 (C-β), rest in Table 9; HRMS (ESI-MS): calcd for (M+H) $C_{29}H_{30}D_2N_5O_{10}S_2$ 676.1716, found 676.1721.

4.3.23. 2-(4-{[3-(Ethyl-(2-nitrobenzenesulfonyl)-amino)-1,1,2,2-$^2H_4$-propyl]-(2-nitrobenzenesulfonyl)-amino}butyl)isoindole-1,3-dione **12c**

Prepared as **12a** from **11c** (715 mg, 1.50 mmol) to give **12c** (948 mg, 93%) as a colourless solid, mp 41–45 °C (amorphous solid). R_f 0.57 (EtOAc–hexane 5:2); IR (KBr): 3095, 2937 (br), 1710, 1544, 1372, 1161, 721 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.04–7.96 (2H, m, Ph), 7.86–7.81 (2H, m, Ph), 7.75–7.54 (8H, m, Ph), 3.68–3.63 (2H, m, H-8), 3.39–3.27 (6H, m, H-α,1,5), 1.69–1.61 (2H, m, H-7), 1.60–1.52 (2H, m, H-6), 1.12 (3H, t, J=7.1 Hz, H-β); ^{13}C NMR ($CDCl_3$): δ 168.4 (2C, C=O), 148.0 (2C), 134.0 (2C), 133.6, 133.5, 133.3, 133.1, 132.0 (2C), 131.8, 131.8, 130.7 (2C), 124.2 (2C), 123.3 (2C) (totally 18C–Ph), 42.3 (C-α), 13.6 (C-β), rest in Table 9; HRMS (ESI-MS): calcd for (M+H) $C_{29}H_{28}D_4N_5O_{10}S_2$ 678.1842, found 678.1848.

4.3.24. 2-(4-{[3-(Benzyl-(2-nitrobenzenesulfonyl)amino)propyl]-(2-nitrobenzenesulfonyl)amino}-butyl)isoindole-1,3-dione ***12d***

Prepared as **12a** from **11d** (1.19 g, 2.23 mmol) to give **12d** (1.32 g, 81%) as a colourless solid, mp 47–50 °C (amorphous solid). R_f 0.57 (EtOAc–hexane 5:2); IR (KBr): 3093, 2940, 1710, 1543, 1160, 721 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.01–7.98 (1H, m, Ph), 7.95–7.91 (1H, m, Ph), 7.85–7.81 (2H, m, Ph), 7.74–7.60 (7H, m, Ph), 7.56–7.52 (1H, m, Ph), 7.34–7.25 (5H, m, Ph), 4.48 (2H, s, H-α), 3.63–3.58 (2H, m, H-8), 3.23–3.18 (2H, m, H-1), 3.16–3.08 (4H, m, H-3,5), 1.68–1.61 (2H, m, H-2), 1.60–1.52 (2H, m, H-7), 1.45–1.36 (2H, m, H-6); ^{13}C NMR ($CDCl_3$): δ 168.3 (2C, C=O), 148.0, 147.9, 135.6, 134.0 (2C), 133.7, 133.5, 133.2 (2C), 132.1 (2C), 131.9, 131.7, 131.0, 130.7, 128.8 (2C), 128.4 (2C), 128.2, 124.3, 124.1, 123.3 (2C) (totally 24C–Ph), 51.9 (C-α), rest in Table 9; HRMS (ESI-MS): calcd for (M+H) $C_{34}H_{34}N_5O_{10}S_2$ 736.1747, found 736.1746.

4.3.25. 2-(4-{[3-(Benzyl-(2-nitrobenzenesulfonyl)amino)-1,1-$^2H_2$-propyl]-(2-nitrobenzenesulfonyl)-amino}butyl)isoindole-1,3-dione ***12e***

Prepared as **12a** from **11e** (1.42 g, 2.61 mmol) to give **12e** (1.83 g, 95%) as colourless solid, mp 50–52 °C (amorphous solid). R_f 0.47 (EtOAc–hexane 3:2); IR (KBr): 3093–2980, 1709, 1541, 1161, 721 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.01–7.98 (1H, m, Ph), 7.95–7.91 (1H, m, Ph), 7.85–7.81 (2H, m, Ph), 7.74–7.60 (7H, m, Ph), 7.56–7.52 (1H, m, Ph), 7.33–7.25 (5H, m, Ph), 4.48 (2H, s, H-α), 3.63–3.58 (2H, m, H-8), 3.23–3.18 (2H, m, H-1), 3.16–3.11 (2H, m, H-5), 1.66–1.61 (2H, m, H-2), 1.59–1.52 (2H, m, H-7), 1.44–1.36 (2H, m, H-6); ^{13}C NMR ($CDCl_3$): δ 168.3 (2C, C=O), 148.0, 147.9, 135.6, 134.0 (2C), 133.7, 133.5, 133.2, 133.2, 132.0 (2C), 131.9, 131.7, 131.0, 130.7, 128.8 (2C), 128.4 (2C), 128.2, 124.3, 124.1, 123.3 (2C) (totally 24C–Ph), 51.9 (C-α), rest in Table 9; HRMS (ESI-MS): calcd for (M+H) $C_{34}H_{32}D_2N_5O_{10}S_2$ 738.1873, found 738.1840.

4.3.26. 2-(4-{[3-(Isopropyl-(2-nitrobenzenesulfonyl)amino)-propyl]-(2-nitrobenzenesulfonyl)amino}-butyl)isoindole-1,3-dione ***12f***

Prepared as **12a** from **11g** (973 mg, 2.0 mmol) to give **12f** (1.28 g, 93%) as a colourless solid, mp 46–54 °C (amorphous solid). R_f 0.30 (EtOAc–hexane 2:1); IR (KBr): 3096, 2940 (br), 1710, 1543, 1373, 1159, 721 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.04–7.99 (2H, m, Ph), 7.85–7.80 (2H, m, Ph), 7.74–7.62 (6H, m, Ph), 7.60–7.54 (2H, m, Ph), 4.12 (1H, sep., J=6.7 Hz, H-α), 3.69–3.63 (2H, m, H-8), 3.39–3.32 (4H, m, H-3,5), 3.23–3.17 (2H, m, H-1), 1.96–1.88 (2H, m, H-2), 1.69–1.54 (4H, m, H-6,7), 1.12 (6H, d, J=6.7 Hz, H-β); ^{13}C NMR ($CDCl_3$): δ 168.3 (2C, C=O), 148.1, 148.0, 134.0 (2C), 133.6, 133.5, 133.4, 133.4, 132.1 (2C), 131.8, 131.7, 130.9, 130.8, 124.1, 124.0, 123.3 (2C) (totally 18C–Ph), 50.1 (C-α), 21.3 (2 C-β), rest in Table 9; HRMS (ESI-MS): calcd for (M+H) $C_{30}H_{34}N_5O_{10}S_2$ 688.1747, found 688.1751.

4.3.27. 2-(4-{[3-(Cyclohexyl-(2-nitrobenzenesulfonyl)amino)-propyl]-(2-nitrobenzenesulfonyl)amino}butyl)isoindole-1,3-dione ***12g***

Prepared as **12a** from **11h** (1.05 g, 2.0 mmol) to give **12g** (1.35 g, 93%) as a colourless solid, mp 53–63 °C (amorphous solid). R_f 0.56 (EtOAc–hexane 3:2); IR (KBr): 3095, 2935, 2858, 1711, 1544, 1157, 721 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.04–7.98 (2H, m, Ph), 7.85–7.80 (2H, m, Ph), 7.74–7.62 (6H, m, Ph), 7.59–7.54 (2H, m, Ph), 3.73–3.63 (3H, m, H-7,α), 3.39–3.31 (4H, m, H-3,5), 3.25–3.18 (2H, m, H-1), 1.95–1.86 (2H, m, H-2), 1.79–1.71 (2H, m, H-γ_{eq}), 1.69–1.54 (7H, m, H-6,7,β_{eq},δ_{eq}), 1.39–1.25 (4H, m, H-β_{ax},γ_{ax}), 1.10–0.99 (1H, m, H-δ_{ax}); ^{13}C NMR ($CDCl_3$): δ 168.3 (2C, C=O), 148.0, 148.0, 134.0 (2C), 133.9, 133.5, 133.4, 133.4, 132.1 (2C), 131.8, 131.6, 130.8, 130.7, 124.1, 124.0, 123.3 (2C) (totally 18C–Ph), 58.3 (C-α), 31.9 (2C-β), 26.0 (2C-γ), 25.2 (C-δ), rest in Table 9; HRMS (ESI-MS): calcd for (M+Na) $C_{33}H_{37}N_5O_{10}S_2Na$ 750.1880, found 750.1867.

4.3.28. N^1-(3-Ethylamino-propyl)-butane-1,4-diamine trihydrochloride ***2a***[47]

A mixture of **12a** (1.21 g, 1.79 mmol), PhSH (0.56 mL, 5.40 mmol) and K_2CO_3 (1.48 g, 10.74 mmol) in DMF (15 mL) was stirred overnight at room temperature. The reaction mixture was then evaporated to dryness in vacuo. The residue was dissolved in a mixture of DCM and 2 M KOH (2:1, 150 mL), the water layer was extracted with DCM (3×50 mL), and the combined organic extracts were dried over $MgSO_4$. Solvent was evaporated in vacuo and the residue was dissolved in ethanol (14 mL) containing $N_2H_4 \cdot H_2O$ (135 μL, 2.78 mmol). After heating for 3 h at reflux, the solvents were evaporated in vacuo, the residue was suspended in a mixture of dioxane and concd HCl (3:1, 28 mL) followed by evaporating to dryness in vacuo. After being co-evaporated once with dioxane, DCM was added to the residue and the product was filtered, washed with DCM and extracted with water. The filtrate was evaporated in vacuo, dissolved in water, the insoluble phthalyl hydrazide was filtered off and the solvent was evaporated to dryness in vacuo. The treatment with water was repeated twice. The product was washed with EtOAc, DCM and cold ethanol. After recrystallization from ethanol–water–EtOAc, **2a** (376 mg, 74%) was obtained as a colourless solid, mp >298 °C decomp. IR (KBr): 3000–2387, 1611, 1460, 1354, 1147, 805 cm^{-1}; ^1H NMR (D_2O): δ 3.18–3.08 (8H, m, H-α,1,3,5), 3.06–3.02 (2H, m, H-8), 2.15–2.06 (2H, m, H-2), 1.83–1.71 (4H, m, H-6,7), 1.29 (3H, t, J=7.3 Hz, H-β); ^{13}C NMR (D_2O): δ 46.0 (C-α), 13.4 (C-β), rest in Table 5; HRMS (ESI-MS): calcd for (M+H) $C_9H_{24}N_3$ 174.1970, found 174.1964.

4.3.29. N^1-(3-Ethylamino-1,1-$^2H_2$-propyl)-butane-1,4-diamine trihydrochloride ***2b***

Prepared as **2a** from **12b** (800 mg, 1.18 mmol) to give **2b** (172 mg, 51%, 99% d) as a colourless solid, mp >308 °C decomp. IR (KBr): 3000–2264, 1612, 1460, 1346, 1144, 844, 803 cm^{-1}; ^1H NMR (D_2O): δ 3.17–3.09 (6H, m, H-α,1,5), 3.08–3.02 (2H, m, H-8), 2.13–2.07 (2H, m, H-2), 1.84–1.72 (4H, m, H-6,7), 1.30 (3H, t, J=7.3 Hz, H-β); ^{13}C NMR (D_2O): δ 46.0 (C-α), 13.4 (C-β), rest in Table 5; HRMS (ESI-MS): calcd for (M+H) $C_9H_{22}N_3D_2$ 176.2096, found 176.2092. Isotope distribution: 0% d_0, 1% d_1, 99% d_2.

4.3.30. N^1-(3-Ethylamino-1,1,2,2-$^2H_4$-propyl)-butane-1,4-diamine trihydrochloride ***2c***

Prepared as **2a** from **12c** (927 mg, 1.37 mmol) to give **2c** (315 mg, 80%, 98% d) as a colourless solid, mp >285 °C decomp. IR (KBr): 3000–2312, 1611, 1458, 1343, 1123, 823, 806 cm^{-1}; ^1H NMR (D_2O): δ 3.18–3.09 (6H, m, H-α,1,5), 3.08–3.02 (2H, m, H-8), 1.85–1.73 (4H, m, H-6,7), 1.30 (3H, t, J=7.3 Hz, H-β); ^{13}C NMR (D_2O): δ 45.9 (C-α), 13.4 (C-β), rest in Table 5; HRMS (ESI-MS): calcd for (M+H) $C_9H_{20}N_3D_4$ 178.2221, found 178.2217. Isotope distribution: 0% d_0–d_2, 8% d_3, 92% d_4.

4.3.31. N^1-(3-Benzylamino-propyl)-butane-1,4-diamine trihydrochloride ***2d***[41]

Prepared as **2a** from **12d** (1.24 g, 1.69 mmol) to give **2d** (393 mg, 68%) as a colourless solid, mp >300 °C decomp. (lit. mp >300 °C).[41] IR (KBr): 3050–2417, 1610, 1448, 744, 698 cm^{-1}; ^1H NMR (D_2O): δ 7.54–7.47 (5H, m, Ph), 4.28 (2H, s, H-α), 3.22–3.08 (6H, m, H-1,3,5), 3.07–3.02 (2H, m, H-8), 2.17–2.07 (2H, m, H-2), 1.82–1.71 (4H, m, H-6,7); ^{13}C NMR (D_2O): δ 133.4, 132.8 (2C), 132.7, 132.3 (2C) (totally 6C–Ph), 54.2 (C-α), rest in Table 5; HRMS (ESI-MS): calcd for (M+H) $C_{14}H_{26}N_3$ 236.2127, found 236.2121.

4.3.32. N^1-(3-Benzylamino-1,1-$^2H_2$-propyl)-butane-1,4-diamine trihydrochloride ***2e***

Prepared as **2a** from **12e** (1.81 g, 2.45 mmol) to give **2e** (542 mg, 64%, 99% d) as a colourless solid, mp >300 °C decomp. IR (KBr): 3050–2380, 1611, 1447, 843, 744, 698 cm^{-1}; ^1H NMR (D_2O):

δ 7.55–7.48 (5H, m, Ph), 4.29 (2H, s, H-α), 3.22–3.17 (2H, m, H-1), 3.14–3.09 (2H, m, H-5), 3.08–3.03 (2H, m, H-8), 2.16–2.10 (2H, m, H-2), 1.84–1.72 (4H, m, H-6,7); ^{13}C NMR (D_2O): δ 133.3, 132.7 (2C), 132.6, 132.2 (2C) (totally 6C–Ph), 54.1 (C-α), rest in Table 5; HRMS (ESI-MS): calcd for (M+H) $C_{14}H_{24}N_3D_2$ 238.2252, found 238.2241. Isotope distribution: 0% d_0, 3% d_1, 97% d_2.

4.3.33. N^1-(3-Isopropylamino-propyl)-butane-1,4-diamine trihydrochloride **2f**

Prepared as **2a** from **12f** (1.26 g, 1.84 mmol), but the stirring was continued at room temperature for 70 h to give **2f** (438 mg, 80%) as a colourless solid, mp 236–239 °C. IR (KBr): 3000–2361, 1606, 1457, 1387, 1099, 964 cm^{-1}; ^{1}H NMR (D_2O): δ 3.44 (1H, sep., J=6.5 Hz, H-α), 3.22–3.02 (8H, m, H-1,3,5,8), 2.15–2.05 (2H, m, H-2), 1.84–1.71 (4H, m, H-6,7), 1.33 (6H, d, J=6.5 Hz, H-β); ^{13}C NMR (D_2O): δ 54.0 (C-α), 21.1 (2C-β), rest in Table 5; HRMS (ESI-MS): calcd for (M+H) $C_{10}H_{26}N_3$ 188.2127, found 188.2123.

4.3.34. N^1-(3-Cyclohexylamino-propyl)-butane-1,4-diamine trihydrochloride **2g**

Prepared as **2f** from **12g** (1.33 g, 1.83 mmol) to give **2g** (478 mg, 78%) as a colourless solid, mp 277–278 °C. IR (KBr): 3000–2368, 1607, 1456, 1050, 775 cm^{-1}; ^{1}H NMR (D_2O): δ 3.22–3.00 (9H, m, H-α,1,3,5,8), 2.15–2.02 (4H, m, H-2,β_{eq}), 1.89–1.71 (6H, m, H-6,7,γ_{eq}), 1.70–1.63 (1H, m, H-δ_{eq}), 1.40–1.26 (4H, m, H-β_{ax},γ_{ax}), 1.24–1.12 (1H, m, H-δ_{ax}); ^{13}C NMR (D_2O): δ 60.4 (C-α), 31.8 (2C-β), 27.4 (C-δ), 26.8 (2C-γ), rest in Table 5; HRMS (ESI-MS): calcd for (M+H) $C_{13}H_{30}N_3$ 228.2440, found 228.2434.

4.3.35. N^1-(3-Amino-1,1-$^2H_2$-propyl)butane-1,4-diamine trihydrochloride **2h**

Prepared as **1l** from **2e** (300 mg, 0.87 mmol) to give **2h** (122 mg, 55%, 99% d) as a colourless solid, mp 251–252 °C (lit. mp for unlabelled SPD: 250–254 °C).[48] IR (KBr): 3000–2370, 1596, 1451, 1171, 845 cm^{-1}; ^{1}H NMR (D_2O): δ 3.14–3.02 (6H, m, H-1,5,8), 2.11–2.05 (2H, m, H-2), 1.83–1.72 (4H, m, H-6,7); ^{13}C NMR (D_2O): δ in Table 5; HRMS (ESI-MS): calcd for (M+H) $C_7H_{18}N_3D_2$ 148.1783, found 148.1787. Isotope distribution: 0% d_0, 1% d_1, 99% d_2.

4.3.36. [3-(2-Nitro-benzenesulfonylamino)-propyl]-carbamic acid tert-butyl ester **9**

Prepared from (3-amino-propyl)-carbamic acid *tert*-butyl ester (3.5 mL, 20 mmol), TEA (3.7 mL, 26.5 mmol) and NsCl (4.7 g, 21 mmol) as described earlier for (*R*)-N^1-(*o*-nitrophenylsulfonyl)-N^3-(*tert*-butyloxycarbonyl)-1,3-diaminobutane[32,33] to give **9** (7.0 g) as yellow solid with 97% yield (lit.[49] yield 89%). ^{1}H and ^{13}C NMR chemical shift are in accordance with published data.[50]

4.3.37. 3-[(4-Chlorobutyl)-(2-nitrobenzenesulfonyl)amino]propyl}-carbamic acid tert-butyl ester **10a**

To a stirred mixture of **9** (7.2 g, 20 mmol) and K_2CO_3 (8.9 g, 64 mmol) in dry DMF (170 mL), 1-bromo-4-chlorobutane (14.9 mL, 129 mmol) was added and stirring was continued for 65 h at room temperature. The precipitates were filtered off, the filtrate was evaporated to dryness in vacuo, and the residue was suspensed in EtOAc, washed with water (350 mL), brine (350 mL) and dried over $MgSO_4$. The solvent was evaporated in vacuo to give **10a** (8.9 g, 99% yield based on **9**) as a pale yellow oil. IR (neat): 3423 (br), 3095–2850, 1701, 1365, 1163, 746 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.03–7.98 (1H, m, Ph), 7.74–7.66 (2H, m, Ph), 7.65–7.61 (1H, m, Ph), 4.78 (1H, br, NH), 3.54–3.49 (2H, m, H-8), 3.38–3.31 (4H, m, H-3,5), 3.18–3.12 (2H, m, H-1), 1.79–1.66 (6H, m, H-2,6,7), 1.44 (9H, s, Me_3C); ^{13}C NMR ($CDCl_3$): δ 156.0 (C=O), 148.1, 133.6, 133.3, 131.7, 130.7, 124.3 (totally 6C–Ph), 79.3 (O–C), 28.4 (3C–Me_3), rest in Table 9; HRMS (ESI-MS): calcd for (M+Na) $C_{18}H_{28}ClN_3O_6SNa$ 472.1285, found 472.1283.

4.3.38. 3-[(4-Iodobutyl)-(2-nitrobenzenesulfonyl)amino]propyl}-carbamic acid tert-butyl ester **10b**

A mixture of **10a** (8.6 g, 19.1 mmol) and NaI (28.7 g, 191 mmol) in acetone (250 mL) was heated at reflux with stirring for 48 h. The precipitate was filtered off and the filtrate was evaporated to dryness in vacuo. The residue was dissolved in EtOAc, washed with water (250 mL) and brine (250 mL), and dried over $MgSO_4$. Solvent was evaporated in vacuo and the residue was purified on silica gel using EtOAc–hexane 1:1 as an eluent, to give **10b** (7.3 g, 70%) as a yellow oil. R_f 0.54 (EtOAc–hexane 1:1); IR (neat): 3422 (br), 2975, 2935, 1701, 1366, 1162, 748, 584 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.03–7.98 (1H, m, Ph), 7.74–7.67 (2H, m, Ph), 7.66–7.61 (1H, m, Ph), 4.78 (1H, br, NH), 3.40–3.29 (4H, m, H-3,5), 3.19–3.11 (4H, m, H-1,8), 1.81–1.72 (4H, m, H-2,7), 1.69–1.61 (2H, m, H-6), 1.44 (9H, s, Me_3C); ^{13}C NMR ($CDCl_3$): δ 156.0 (C=O), 148.1, 133.6, 133.3, 131.7, 130.7, 124.3 (totally 6C–Ph), 79.3 (O–C), 28.4 (3C–Me_3), rest in Table 9; HRMS (ESI-MS): calcd for (M+Na) $C_{18}H_{28}IN_3O_6SNa$ 564.0641, found 564.0633.

4.3.39. {3-[{4-[{3-[Ethyl-(2-nitrobenzenesulfonyl)-amino]-propyl}-(2-nitrobenzenesulfonyl)-amino]-butyl}-(2-nitrobenzenesulfonyl)-amino]-propyl}-carbamic acid tert-butyl ester **13a**

To a stirred mixture of **11a** (1.0 g, 2.12 mmol) and K_2CO_3 (0.88 g, 6.35 mmol) in dry DMF (21 mL) was added **10b** (1.26 g, 2.33 mmol) and the reaction mixture was stirred for 17 h at room temperature. The solids were filtered off, the filtrate was evaporated to dryness in vacuo and the residue was purified on silica gel using first EtOAc–hexane 2:1 and then 5:2 as an eluent, to give **13a** (1.3 g, 69%) as a colourless solid, mp 45–54 °C (amorphous solid). R_f 0.35 (EtOAc–hexane 2:1); IR (KBr): 3422 (br), 3096, 2940 (br), 1707, 1544, 1374, 1160, 744 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.02–7.97 (3H, m, Ph), 7.73–7.68 (6H, m, Ph), 7.64–7.59 (3H, m, Ph), 4.81 (1H, br, NH), 3.38–3.23 (12H, m, H-α,1,3,5,8,10), 3.16–3.09 (2H, m, H-12), 1.89–1.81 (2H, m, H-2), 1.76–1.69 (2H, m, H-11), 1.54–1.47 (4H, m, H-6,7), 1.43 (9H, s, Me_3C-), 1.11 (3H, t, J=7.1 Hz, H-β); ^{13}C NMR ($CDCl_3$): δ 156.1 (*C*=O), 148.0 (2C), 148.0, 133.7, 133.7, 133.6, 133.3, 133.2, 133.0, 131.9, 131.9, 131.8, 130.8, 130.7, 130.6, 124.3, 124.2, 124.2 (totally 18C–Ph), 79.3 (O–C), 42.4 (C-α), 28.4 (3C–Me_3), 13.6 (C-β), rest in Table 10; HRMS (ESI-MS): calcd for (M+Na) $C_{35}H_{47}N_7O_{14}S_3Na$ 908.2241, found 908.2234.

4.3.40. {3-[{4-[{3-[Ethyl-(2-nitrobenzenesulfonyl)-amino]-1,1-$^2H_2$-propyl}-(2-nitrobenzenesulfonyl)-amino]-butyl}-(2-nitrobenzenesulfonyl)-amino]-propyl}-carbamic acid tert-butyl ester **13b**

Prepared as **13a** from **11b** (1.0 g, 2.11 mmol) to give **13b** (1.40 g, 75%) as a colourless solid, mp 40–48 °C (amorphous solid). R_f 0.35 (EtOAc–hexane 2:1); IR (KBr): 3424 (br), 3096–2880, 1708, 1544, 1372, 1160, 748 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.02–7.96 (3H, m, Ph), 7.73–7.67 (6H, m, Ph), 7.64–7.59 (3H, m, Ph), 4.83 (1H, br, NH), 3.38–3.23 (10H, m, H-α,1,5,8,10), 3.16–3.09 (2H, m, H-12), 1.86–1.81 (2H, m, H-2), 1.75–1.68 (2H, m, H-11), 1.54–1.47 (4H, m, H-6,7), 1.43 (9H, s, Me_3C–), 1.11 (3H, t, J=7.1 Hz, H-β); ^{13}C NMR ($CDCl_3$): δ 156.1 (C=O), 148.0 (2C), 148.0, 133.7 (2C), 133.6, 133.3 133.2, 133.0, 131.9, 131.9, 131.8, 130.8, 130.7, 130.6, 124.3, 124.2, 124.2 (totally 18C–Ph), 79.2 (O–C), 42.4 (C-α), 28.4 (3C–Me_3), 13.6 (C-β), rest in Table 10; HRMS (ESI-MS): calcd for (M+Na) $C_{35}H_{45}D_2N_7O_{14}S_3Na$ 910.2366, found 910.2392.

4.3.41. {3-[{4-[{3-[Benzyl-(2-nitrobenzenesulfonyl)-amino]-propyl}-(2-nitrobenzenesulfonyl)-amino]-butyl}-(2-nitrobenzenesulfonyl)-amino]-propyl}-carbamic acid tert-butyl ester **13c**

Prepared as **13a** from **11d** (992 mg, 1.86 mmol) to give **13c** (1.42 g, 80%) as a colourless solid, mp 52–58 °C (amorphous solid). R_f 0.58 (EtOAc–hexane 3:1); IR (KBr): 3422 (br), 3096–2880, 1707, 1543, 1161, 742 cm^{-1}; ^{1}H NMR ($CDCl_3$): δ 8.00–7.90 (3H, m, Ph), 7.74–7.56 (9H, m, Ph), 7.33–7.22 (5H, m, Ph), 4.81 (1H, br, NH), 4.47 (2H, s, H-α), 3.32–3.03 (12H, m, H-1,3,5,8,10,12), 1.74–1.67 (2H, m,

H-11), 1.66–1.57 (2H, m, H-2), 1.48–1.30 (4H, m, H-6,7), 1.43 (9H, s, Me_3C–); ^{13}C NMR ($CDCl_3$): δ 156.0 (C═O), 148.0, 148.0, 147.9, 135.5, 133.7, 133.7, 133.6, 133.2, 133.1, 133.0, 131.9, 131.9 (2C), 130.9, 130.7, 130.6, 128.8 (2C), 128.4 (2C), 128.2, 124.3, 124.2, 124.2 (totally 24C–Ph), 79.3 (O–C), 52.0 (C-α), 28.4 (3C–Me_3), rest in Table 10; HRMS (ESI-MS): calcd for (M+Na) $C_{40}H_{49}N_7O_{14}S_3Na$ 970.2397, found 970.2354.

4.3.42. {3-[{4-[{3-[Benzyl-(2-nitrobenzenesulfonyl)-amino]-1,1,2,2-$^2H_4$-propyl}-(2-nitrobenzenesulfonyl)-amino]-butyl}-(2-nitrobenzenesulfonyl)-amino]-propyl}-carbamic acid tert-butyl ester ***13d***

Prepared as **13a** from **11f** (1.83 g, 3.39 mmol) to give **13d** (2.75 g, 85%) as a colourless solid, mp 44–54 °C (amorphous solid). R_f 0.58 (EtOAc–hexane 3:1); IR (KBr): 3423 (br), 3095–2880, 1707, 1544, 1162, 743 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.00–7.90 (3H, m, Ph), 7.74–7.56 (9H, m, Ph), 7.33–7.23 (5H, m, Ph), 4.82 (1H, br, NH), 4.47 (2H, s, H-α), 3.32–3.05 (10H, m, H-1,5,8,10,12), 1.74–1.66 (2H, m, H-11), 1.48–1.30 (4H, m, H-6,7) 1.43 (9H, s, Me_3C–); ^{13}C NMR ($CDCl_3$): δ 156.0 (C═O), 148.0, 148.0, 147.9, 135.5, 133.8, 133.7, 133.6, 133.2, 133.1, 133.0, 132.0, 131.9 (2C), 130.9, 130.7, 130.6, 128.8 (2C), 128.4 (2C), 128.2, 124.3, 124.2, 124.2 (totally 24C–Ph), 79.2 (O–C), 52.0 (C-α), 28.4 (3C–Me_3), rest in Table 10; HRMS (ESI-MS): calcd for (M+Na) $C_{40}H_{45}D_4N_7O_{14}S_3Na$ 974.2648, found 974.2607.

4.3.43. {3-[{4-[{3-[Isopropyl-(2-nitrobenzenesulfonyl)-amino]-propyl}-(2-nitrobenzenesulfonyl)-amino]-butyl}-(2-nitrobenzenesulfonyl)-amino]-propyl}-carbamic acid tert-butyl ester ***13e***

Prepared as **13a** from **11g** (973 mg, 2.0 mmol) to give **13e** (1.53 g, 85%) as a colourless solid, mp 50–55 °C (amorphous solid). R_f 0.44 (EtOAc–hexane 2:1); IR (KBr): 3421 (br), 3097–2880, 1707, 1544, 1161, 744 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.04–7.96 (3H, m, Ph), 7.74–7.66 (6H, m, Ph), 7.64–7.57 (3H, m, Ph), 4.84 (1H, br, NH), 4.10 (1H, sep., J=6.7 Hz, H-α), 3.35–3.26 (8H, m, H-3,5,8,10), 3.22–3.09 (4H, m, H-1,12), 1.93–1.85 (2H, m, H-2), 1.76–1.68 (2H, m, H-11), 1.55–1.46 (4H, m, H-6,7), 1.43 (9H, s, Me_3C-), 1.11 (6H, d, J=6.7 Hz, H-β); ^{13}C NMR ($CDCl_3$): δ 156.1 (*C*═O), 148.1, 148.0, 148.0, 133.7 (2C), 133.5, 133.5, 133.2, 133.2, 131.9, 131.9, 131.7, 130.8 (2C), 130.6, 124.3, 124.2, 124.1 (totally 18C–Ph), 79.2 (O–C), 50.1 (C-α), 28.4 (3C–Me_3), 21.3 (2C-β), rest in Table 10; HRMS (ESI-MS): calcd for (M+Na) $C_{36}H_{49}N_7O_{14}S_3Na$ 922.2397, found 922.2361.

4.3.44. {3-[{4-[{3-[Cyclohexyl-(2-nitrobenzenesulfonyl)-amino]-propyl}-(2-nitrobenzenesulfonyl)-amino]-butyl}-(2-nitrobenzenesulfonyl)-amino]-propyl}-carbamic acid tert-butyl ester ***13f***

Prepared as **13a** from **11h** (1.05 g, 2.0 mmol) to give **13f** (1.19 g, 63%) as a colourless solid, mp 54–63 °C (amorphous solid). R_f 0.44 (EtOAc–hexane 2:1); IR (KBr): 3442 (br), 3096, 2936, 2861, 1707, 1544, 1159, 742 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.04–7.95 (3H, m, Ph), 7.74–7.66 (6H, m, Ph), 7.64–7.56 (3H, m, Ph), 4.84 (1H, br, NH), 3.70–3.62 (1H, m, H-α), 3.37–3.25 (8H, m, H-3,5,8,10), 3.23–3.17 (2H, m, H-1), 3.16–3.09 (2H, m, H-12), 1.91–1.84 (2H, m, H-2), 1.78–1.68 (4H, m, H-11, γ_{eq}), 1.66–1.57 (3H, m, H-β_{eq},δ_{eq}), 1.54–1.44 (4H, m, H-6,7), 1.43 (9H, s, Me_3C–), 1.38–1.24 (4H, m, H-β_{ax},γ_{ax}), 1.10–0.99 (1H, m, H-δ_{ax}); ^{13}C NMR ($CDCl_3$): δ 156.0 (*C*═O), 148.1, 148.0, 148.0, 133.8, 133.7 (2C), 133.5, 133.2, 133.2, 131.9, 131.9, 131.7, 130.9, 130.6, 130.6, 124.3, 124.2, 124.0 (totally 18C–Ph), 79.2 (O–C), 58.4 (C-α), 31.9 (2C-β), 28.4 (3C–Me_3), 26.0 (2C-γ), 25.2 (C-δ), rest in Table 10; HRMS (ESI-MS): calcd for (M+Na) $C_{39}H_{53}N_7O_{14}S_3Na$ 962.2710, found 962.2683.

4.3.45. N-(3-Aminopropyl)-N′-(3-ethylamino-propyl)butane-1,4-diamine tetrahydrochloride ***3a***[51]

A mixture of **13a** (1.27 g, 1.43 mmol), PhSH (0.67 mL, 6.48 mmol) and K_2CO_3 (1.78 g, 12.9 mmol) in DMF (13 mL) was stirred for 20 h at room temperature and then evaporated to dryness in vacuo. The residue was dissolved in a mixture of DCM and 2 M KOH (4:3, 130 mL), the water layer was extracted with DCM (4×50 mL), and the combined organic extracts were dried over $MgSO_4$. The solvent was evaporated in vacuo, the residue was dissolved in 1,4-dioxane (12 mL) followed with concd HCl (3 mL) and the resulting mixture was stirred for 2 h at room temperature. The reaction mixture was evaporated to dryness in vacuo and co-evaporated once with dioxane (10 mL). DCM was added to the residue, the product was filtered and washed with DCM and cold ethanol. Recrystallization from ethanol–water–EtOAc yielded **3a** (269 mg, 50%) as a colourless solid, mp >275 °C decomp. IR (KBr): 3000–2388, 1612, 1461, 1355, 1145, 806 cm^{-1}; ^1H NMR (D_2O): δ 3.21–3.08 (14H, m, H-α,1,3,5,8,10,12), 2.16–2.06 (4H, m, H-2,11), 1.84–1.75 (4H, m, H-6,7), 1.30 (3H, t, J=7.3 Hz, H-β); ^{13}C NMR (D_2O): δ 45.9 (C-α), 13.3 (C-β), rest in Table 6; HRMS (ESI-MS): calcd for (M+H) $C_{12}H_{31}N_4$ 231.2549, found 231.2555.

4.3.46. N-(3-Aminopropyl)-N′-(3-ethylamino-1,1-$^2H_2$-propyl)-butane-1,4-diamine tetrahydrochloride ***3b***

Prepared as **3a** from **13b** (1.0 g, 1.13 mmol) to give **3b** (199 mg, 47%, 98% *d*) as a colourless solid, mp >300 °C decomp. IR (KBr): 3000–2382, 1611, 1460, 1354, 1145, 844 cm^{-1}; ^1H NMR (D_2O): δ 3.20–3.08 (12H, m, H-α,1,5,8,10,12), 2.15–2.07 (4H, m, H-2,11), 1.84–1.76 (4H, m, H-6,7), 1.30 (3H, t, J=7.3 Hz, H-β); ^{13}C NMR (D_2O): δ 46.0 (C-α), 13.4 (C-β), rest in Table 6; HRMS (ESI-MS): calcd for (M+H) $C_{12}H_{29}N_4D_2$ 233.2674, found 233.2666. Isotope distribution: 0% d_0, 3% d_1, 97% d_2.

4.3.47. N-(3-Aminopropyl)-N′-(3-Benzylamino-propyl)-butane-1,4-diamine tetrahydrochloride ***3c***[52]

Prepared as **3a** from **13c** (1.40 g, 1.48 mmol) to give **3c** (405 mg, 62%) as a colourless solid, mp >300 °C decomp. IR (KBr): 3000–2420, 1609, 1460, 874, 744, 698 cm^{-1}; ^1H NMR (D_2O): δ 7.55–7.47 (5H, m, Ph), 4.28 (2H, s, H-α), 3.25–3.05 (12H, m, H-1,3,5,8,10,12), 2.20–2.05 (4H, m, H-2,11), 1.85–1.73 (4H, m, H-6,7); ^{13}C NMR (D_2O): δ 133.3, 132.7 (2C), 132.7, 132.2 (2C) (totally 6C–Ph), 54.1 (C-α), rest in Table 6; HRMS (ESI-MS): calcd for (M+H) $C_{17}H_{33}N_4$ 293.2705, found 293.2698.

4.3.48. N-(3-Aminopropyl)-N′-(3-Benzylamino-1,1,2,2-$^2H_4$-propyl)-butane-1,4-diamine tetrahydrochloride ***3d***

Prepared as **3a** from **13d** (2.73 g, 2.86 mmol) to give **3d** (904 mg, 71%, 98% *d*) as a colourless solid, mp >300 °C decomp. IR (KBr): 3050–2309, 1610, 1450, 825, 744, 698 cm^{-1}; ^1H NMR (D_2O): δ 7.55–7.47 (5H, m, Ph), 4.28 (2H, s, H-α), 3.25–3.05 (10H, m, H-1,5,8,10,12), 2.17–2.05 (2H, m, H-11), 1.85–1.73 (4H, m, H-6,7); ^{13}C NMR (D_2O): δ 133.3, 132.7 (2C), 132.7, 132.2 (2C) (totally 6C–Ph), 54.1 (C-α), rest in Table 6; HRMS (ESI-MS): calcd for (M+H) $C_{17}H_{29}N_4D_4$ 297.2956, found 297.2947. Isotope distribution: 0% d_0–d_2, 7% d_3, 92% d_4.

4.3.49. N-(3-Aminopropyl)-N′-(3-isopropylaminopropyl)butane-1,4-diamine tetrahydrochloride ***3e***

Prepared as **3a** from **13e** (1.51 g, 1.68 mmol) to give **3e** (452 mg, 69%) as a colourless solid, mp >269 °C decomp. IR (KBr): 3000–2364, 1625, 1460, 1376, 877 cm^{-1}; ^1H NMR (D_2O): δ 3.44 (1H, sep., J=6.5 Hz, H-α), 3.22–3.00 (12H, m, H-1,3,5,8,10,12), 2.18–2.00 (4H, m, H-2,11), 1.85–1.70 (4H, m, H-6,7), 1.33 (6H, d, J=6.5 Hz, H-β); ^{13}C NMR (D_2O): δ 53.9 (C-α), 21.0 (2C-β), rest in Table 6; HRMS (ESI-MS): calcd for (M+H) $C_{13}H_{33}N_4$ 245.2705, found 245.2695.

4.3.50. N-(3-Aminopropyl)-N′-(3-cyclohexylaminopropyl)butane-1,4-diamine tetrahydrochloride ***3f***

Prepared as **3a** from **13f** (1.15 g, 1.22 mmol) to give **3f** (380 mg, 72%) as a colourless solid, mp >285 °C decomp. IR (KBr):

3000–2419, 1610, 1460, 1051, 775 cm^{-1}; ^1H NMR (D_2O): δ 3.21–3.07 (13H, m, H-α,1,3,5,8,10,12), 2.15–2.02 (6H, m, H-2,11,β_{eq}), 1.89–1.74 (6H, m, H-6,7,γ_{eq}), 1.71–1.64 (1H, m, H-δ_{eq}), 1.40–1.26 (4H, m, H-β_{ax},γ_{ax}), 1.23–1.13 (1H, m, H-δ_{ax}); ^{13}C NMR (D_2O): δ 60.4 (C-α), 31.7 (2C-β), 27.3 (C-δ), 26.7 (2C-γ), rest in Table 6; HRMS (ESI-MS): calcd for (M+H) $C_{16}H_{37}N_4$ 285.3018, found 285.3009.

4.3.51. *N-(3-Amino-1,1,2,2-$^2H_4$-propyl)-N′-(3-aminopropyl)-butane-1,4-diamine tetrahydrochloride* ***4h***

Prepared as **1l** from **3d** (400 mg, 0.90 mmol) to give **4h** (262 mg, 82%, 98% *d*) as a colourless solid, mp >300 °C decomp. IR (KBr): 3124–2010, 1594, 1479, 1158, 812 cm^{-1}; ^1H NMR (D_2O): δ 3.22–3.08 (10H, m, H-1,5,8,10,12), 2.16–2.07 (2H, m, H-11), 1.86–1.75 (4H, m, H-6,7); ^{13}C NMR (D_2O): δ in Table 6; HRMS (ESI-MS): calcd for (M+H) $C_{10}H_{23}N_4D_4$ 207.2487, found 207.2486. Isotope distribution: 0% d_0–d_2, 8% d_3, 92% d_4.

4.3.52. *N,N′-Bis-[3-(ethyl{2-nitrobenzenesulfonyl}amino)propyl]-N,N′-bis-(2-nitrobenzenesulfonyl)butane-1,4-diamine* ***14a***

To a stirred mixture of **11a** (894 mg, 1.89 mmol) and K_2CO_3 (0.75 g, 5.4 mmol) in dry DMF (10 mL), 1,4-diiodobutane (279 g, 0.90 mmol) was added and stirring was continued for 24 h at room temperature. The solids were filtered off and the filtrate was evaporated to dryness in vacuo. The residue was treated with EtOAc (20 mL) and water (20 mL) and this resulted in precipitation of the product, which was filtered off and dried in vacuo yielding **14a** (718 mg, 80%) as a sparingly soluble colourless solid, mp 151–156 °C. IR (KBr): 3102–2850, 1542, 1376, 1161, 744 cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6): δ 8.01–7.94 (4H, m, Ph), 7.79–7.71 (8H, m, Ph), 7.70–7.64 (4H, m, Ph), 3.39–3.23 (16H, m, H-α,1,3,5,8,10,12), 1.88–1.79 (4H, m, H-2,11), 1.55–1.48 (4H, m, H-6,7), 1.11 (6H, t, *J*=7.1 Hz, H-β); ^{13}C NMR ($CDCl_3$+DMSO-d_6): δ 148.0 (2C), 148.0 (2C), 133.7 (2C), 133.6 (2C), 133.3 (2C), 132.9 (2C), 132.0 (2C), 131.9 (2C), 130.8 (2C), 130.6 (2C), 124.2 (2C), 124.2 (2C) (totally 24C–Ph), 42.4 (2C-α), 13.6 (2C-β), rest in Table 10; HRMS (ESI-MS): calcd for (M+K) $C_{38}H_{46}N_8O_{16}S_4K$ 1037.1552, found 1037.1588.

4.3.53. *N,N′-Bis-[3-(ethyl-{2-nitro-benzenesulfonyl}-amino)-1,1-$^2H_2$-propyl]-N,N′-bis-(2-nitro-benzenesulfonyl)butane-1,4-diamine* ***14b***

Prepared as **14a** from **11b** (1.50 g, 3.16 mmol) and 1,4-diiodobutane (467 mg, 1.51 mmol) to give **14b** (1.07 g, 71%) as a colourless solid, mp 155–158 °C. IR (KBr): 3102, 2941, 1541, 1374, 1160 cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6): δ 8.01–7.94 (4H, m, Ph), 7.78–7.70 (8H, m, Ph), 7.69–7.62 (4H, m, Ph), 3.39–3.23 (12H, m, H-α,1,5,8,12), 1.87–1.78 (4H, m, H-2,11), 1.56–1.46 (4H, m, H-6,7), 1.11 (6H, t, *J*=7.1 Hz, H-β); ^{13}C NMR ($CDCl_3$+DMSO-d_6): δ 147.9 (2C), 147.9 (2C), 133.8 (2C), 133.7 (2C), 133.0 (2C), 132.7 (2C), 132.0 (2C), 131.9 (2C), 130.5 (2C), 130.4 (2C), 124.2 (2C), 124.1 (2C) (totally 24C–Ph), 42.4 (2C-α), 13.6 (2C-β), rest in Table 10; HRMS (ESI-MS): calcd for (M+K) $C_{38}H_{42}D_4N_8O_{16}S_4K$ 1041.1803, found 1041.1773.

4.3.54. *N,N′-Bis-[3-(isopropyl-{2-nitrobenzenesulfonyl}amino)-propyl]-N,N′-bis-(2-nitrobenzenesulfonyl)butane-1,4-diamine* ***14c***

Prepared as **14a** from **11g** (3.08 g, 6.33 mmol) and 1,4-diiodobutane (934 mg, 3.02 mmol) to give **14c** (2.68 g, 87%) as a colourless solid, mp 202–204 °C. IR (KBr): 3090–2850, 1544, 1362, 1157 cm^{-1}; ^1H NMR ($CDCl_3$+DMSO-d_6): δ 8.03–7.91 (8H, m, Ph), 7.90–7.79 (8H, m, Ph), 3.97 (2H, sep., *J*=6.7 Hz, H-α), 3.31–3.22 (8H, m, H-3,5,8,10), 3.15–3.09 (4H, m, H-1–12), 1.79–1.71 (4H, m, H-2,11), 1.47–1.40 (4H, m, H-6,7), 1.02 (12H, d, *J*=6.7 Hz, H-β); ^{13}C NMR ($CDCl_3$+DMSO-d_6): δ 147.4 (2C), 147.4 (2C), 134.4 (4C), 132.3 (2C), 132.2 (2C), 132.0 (2C), 131.7 (2C), 129.8 (4C), 124.2 (2C), 124.1 (2C) (totally 24C–Ph), 49.6 (2C-α), 20.6 (4C-β), rest in Table 10; HRMS (ESI-MS): calcd for (M+K) $C_{40}H_{50}N_8O_{16}S_4K$ 1065.1865, found 1065.1844.

4.3.55. *N,N′-Bis-[3-(cyclohexyl-{2-nitrobenzenesulfonyl}-amino)propyl]-N,N′-bis-(2-nitrobenzenesulfonyl)butane-1,4-diamine* ***14d***

To a stirred mixture of **11h** (1.18 g, 2.24 mmol) and K_2CO_3 (0.88 g, 6.39 mmol) in dry DMF (12 mL), 1,4-diiodobutane (330 mg, 1.06 mmol) was added and stirring was continued for 24 h at room temperature. Solids were filtered off and the filtrate was evaporated to dryness in vacuo. The residue was treated with EtOAc (25 mL) and water (25 mL), the water layer was extracted with EtOAc (2×25 mL), and the combined organic phases were washed with brine (25 mL) and dried over $MgSO_4$. Solvent was evaporated in vacuo and the residue was purified on silica gel using first EtOAc–hexane 3:2 and then 3:1 as an eluent, affording **14d** (993 mg, 84% yield based on 1,4-diiodobutane) as a colourless solid, mp 62–72 °C (amorphous solid). R_f 0.31 (EtOAc–hexane 3:2); IR (KBr): 3096, 2935, 2858, 1543, 1158, 741 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.04–7.97 (4H, m, Ph), 7.74–7.66 (8H, m, Ph), 7.63–7.55 (4H, m, Ph), 3.72–3.63 (2H, m, H-α), 3.35–3.26 (8H, m, H-3,5,8,10), 3.24–3.18 (4H, m, H-1,12), 1.92–1.83 (4H, m, H-2,11), 1.78–1.71 (4H, m, H-γ_{eq}), 1.67–1.57 (6H, m, H-β_{eq},δ_{eq}), 1.52–1.45 (4H, m, H-6,7), 1.39–1.25 (8H, m, H-β_{ax},γ_{ax}), 1.09–0.98 (2H, m, H-δ_{ax}); ^{13}C NMR ($CDCl_3$): δ 148.1 (2C), 147.9 (2C), 133.8 (2C), 133.7 (2C), 133.4 (2C), 133.2 (2C), 132.0 (2C), 131.7 (2C), 130.8 (2C), 130.6 (2C), 124.2 (2C), 124.0 (2C) (totally 24C–Ph), 58.4 (2C-α), 31.9 (4C-β), 26.0 (4C-γ), 25.2 (2C-δ), rest in Table 10; HRMS (ESI-MS): calcd for (M+Na) $C_{46}H_{58}N_8O_{16}S_4Na$ 1129.2751, found 1129.2792.

4.3.56. *N,N′-Bis-(3-ethylamino-propyl)butane-1,4-diamine tetrahydrochloride* ***4a***[53]

A mixture of **14a** (700 mg, 0.70 mmol), PhSH (0.43 mL, 4.20 mmol) and K_2CO_3 (1.16 g, 8.41 mmol) in DMF (7 mL) was stirred for 20 h at room temperature and then the reaction mixture was evaporated to dryness in vacuo. The residue was dissolved in a mixture of DCM and 2 M KOH (4:3, 60 mL), the water layer was extracted with a mixture of ethanol and DCM (1:2, 4×20 mL), and the combined organic extracts were dried over $MgSO_4$. Solvents were evaporated in vacuo, the residue was dissolved in 1,4-dioxane (6 mL) followed by the addition of concd HCl (1.5 mL) and the resulting mixture was stirred for 5 min at room temperature. The reaction mixture was evaporated to dryness in vacuo and co-evaporated once with dioxane (10 mL). DCM was added to the residue and the product was filtered, washed with DCM and cold ethanol. Recrystallization from ethanol–water–EtOAc yielded **4a** (144 mg, 51%) as a colourless solid, mp >300 °C decomp. IR (KBr): 3000–2389, 1596, 1460, 1354, 1142, 806 cm^{-1}; ^1H NMR (D_2O) chemical shifts as reported earlier;[53] ^{13}C NMR (D_2O): δ 45.9 (2C-α), 13.3 (2C-β), rest in Table 6; HRMS (ESI-MS): calcd for (M+H) $C_{14}H_{35}N_4$ 259.2862, found 259.2854.

4.3.57. *N,N′-Bis-(3-ethylamino-1,1-$^2H_2$-propyl)butane-1,4-diamine tetrahydrochloride* ***4b***

Prepared as **4a** from **14b** (1.04 g, 1.04 mmol) to give **4b** (190 mg, 45%, 99% *d*) as a colourless solid, mp >300 °C decomp. IR (KBr): 3000–2265, 1595, 1461, 1348, 1145, 843, 803 cm^{-1}; ^1H NMR (D_2O): δ 3.19–3.07 (12H, m, H-α,1,5,8,12), 2.15–2.06 (4H, m, H-2,11), 1.85–1.74 (4H, m, H-6,7), 1.30 (6H, t, *J*=7.3 Hz, H-β); ^{13}C NMR (D_2O): δ 45.9 (2C-α), 13.3 (2C-β), rest in Table 6; HRMS (ESI-MS): calcd for (M+H) $C_{14}H_{31}N_4D_4$ 263.3113, found 263.3103. Isotope distribution: 0% d_0–d_2, 3% d_3, 97% d_4.

4.3.58. *N,N′-Bis-(3-isopropylaminopropyl)butane-1,4-diamine tetrahydrochloride* ***4c***[53]

Prepared as **4a** from **14c** (1.03 g, 1.0 mmol), PhSH (0.64 mL, 6.2 mmol) and K_2CO_3 (1.66 g, 12 mmol) in DMF (10 mL), but stirring was continued for 70 h to give **4c** (282 mg, 65%) as a colourless

solid, mp >281 °C decomp. IR (KBr): 3000–2360, 1636, 1478, 1386, 1150, 762 cm^{-1}; ^1H NMR (D_2O) chemical shifts as reported earlier;[53] ^{13}C NMR (D_2O): δ 53.9 (2C-α), 21.0 (4C-β), rest in Table 6; HRMS (ESI-MS): calcd for (M+H) $C_{16}H_{39}N_4$ 287.3175, found 287.3165.

4.3.59. *N,N′-Bis-(3-cyclohexylaminopropyl)butane-1,4-diamine tetrahydrochloride* ***4d***[54]

Prepared as **4c** from **14d** (968 mg, 0.87 mmol) to give **4d** (364 mg, 82%) as colourless solid, mp >300 °C decomp. IR (KBr): 3000–2420, 1596, 1456, 1052, 775 cm^{-1}; ^1H NMR (D_2O): δ 3.21–3.07 (14H, m, H-α,1,3,5,8,10,12), 2.15–2.02 (8H, m, H-2,11,β_{eq}), 1.89–1.74 (8H, m, H-6,7,γ_{eq}), 1.71–1.63 (2H, m, H-δ_{eq}), 1.40–1.26 (8H, m, H-β_{ax},γ_{ax}), 1.24–1.12 (2H, m, H-δ_{ax}); ^{13}C NMR (D_2O): δ 60.4 (2C, C-α), 31.7 (4C, C-β), 27.3 (2C, C-δ), 26.7 (4C, C-γ), rest in Table 6; HRMS (ESI-MS): calcd for (M+H) $C_{22}H_{47}N_4$ 367.3801, found 367.3785.

4.3.60. *N^1-(4-Chlorobutyl)-N^3-ethyl-N,N^3-bis-(2-nitrobenzenesulfonyl)propane-1,3-diamine* ***15a***

To a stirred mixture of **11a** (2.02 g, 4.26 mmol) and K_2CO_3 (1.76 g, 12.77 mmol) in dry DMF (34 mL) was added 1-bromo-4-chlorobutane (2.94 mL, 25.55 mmol) and stirring was continued for 65 h at room temperature. Solids were filtered off and the filtrate was evaporated to dryness in vacuo. The residue was treated with EtOAc, washed with water (35 mL), brine (35 mL) and dried over $MgSO_4$. Solvent was evaporated in vacuo affording **15a** (2.45 g, ~100%) as a yellow oil. IR (neat): 3096, 2940 (br), 1544, 1159, 745 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.04–7.98 (2H, m, Ph), 7.73–7.66 (4H, m, Ph), 7.64–7.59 (2H, m, Ph), 3.55–3.51 (2H, m, H-8), 3.41–3.27 (8H, m, H-α,1,3,5), 1.94–1.86 (2H, m, H-2), 1.80–1.66 (4H, m, H-6,7), 1.12 (3H, t, *J*=7.1 Hz, H-β); ^{13}C NMR ($CDCl_3$): δ 148.0 (2C), 133.7, 133.5, 133.3, 133.0, 131.9, 131.8, 130.9, 130.8, 124.2, 124.2 (totally 12C–Ph), 42.3 (C-α), 13.6 (C-β), rest in Table 9; HRMS (ESI-MS): calcd for (M+H) $C_{21}H_{28}ClN_4O_8S_2$ 563.1037, found 563.1038.

4.3.61. *N^1-(4-Chlorobutyl)-N^3-ethyl-1,1,2,2-$^2H_4$-N^1,N^3-bis-(2-nitrobenzenesulfonyl)propane-1,3-diamine* ***15b***

Prepared as **15a** from **11c** (953 mg, 2.0 mmol) with subsequent purification on silica gel using EtOAc–hexane 3:2 as an eluent to give **15b** (1.07 g, 95%) as a pale yellow oil. IR (neat): 3096, 2939 (br), 2226, 1544, 1161, 745 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.05–7.96 (2H, m, Ph), 7.74–7.65 (4H, m, Ph), 7.65–7.57 (2H, m, Ph), 3.55–3.51 (2H, m, H-8), 3.42–3.25 (6H, m, H-α,1,5), 1.80–1.65 (4H, m, H-6,7), 1.12 (3H, t, *J*=7.1 Hz, H-β); ^{13}C NMR ($CDCl_3$): δ 148.0 (2C), 133.7, 133.6, 133.3, 133.1, 131.9, 131.8, 130.8, 130.7, 124.2, 124.2 (totally 12C–Ph), 42.4 (C-α), 13.6 (C-β), rest in Table 9; HRMS (ESI-MS): calcd for (M+H) $C_{21}H_{24}D_4ClN_4O_8S_2$ 567.1288, found 567.1302.

4.3.62. *N^1-(4-Iodobutyl)-N^3-ethyl-N^1,N^3-bis-(2-nitrobenzenesulfonyl)-propane-1,3-diamine* ***15c***

Prepared as **10b** from **15a** (2.4 g, 4.26 mmol) to give **15c** (2.49 g, 89%) as a slightly yellowish oil. R_f 0.44 (EtOAc–hexane 3:2); IR (neat): 3096, 2939 (br), 1542, 1159, 744 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.04–7.98 (2H, m, Ph), 7.74–7.66 (4H, m, Ph), 7.65–7.58 (2H, m, Ph), 3.40–3.26 (8H, m, H-α,1,3,5), 3.17 (2H, t, *J*=6.6 Hz, H-8), 1.95–1.86 (2H, m, H-2), 1.83–1.75 (2H, m, H-7), 1.70–1.62 (2H, m, H-6), 1.12 (3H, t, *J*=7.1 Hz, H-β); ^{13}C NMR ($CDCl_3$): δ 148.0 (2C), 133.7, 133.6, 133.4, 133.0, 131.9, 131.8, 130.9, 130.8, 124.2, 124.2 (totally 12C–Ph), 42.4 (C-α), 13.6 (C-β), rest in Table 9; HRMS (ESI-MS): calcd for (M+H) $C_{21}H_{28}IN_4O_8S_2$ 655.0393, found 655.0393.

4.3.63. *N^1-(4-Iodobutyl)-N^3-ethyl-1,1,2,2-$^2H_4$-N^1,N^3-bis-(2-nitrobenzenesulfonyl)propane-1,3-diamine* ***15d***

Prepared as **10b** from **15b** (1.04 g, 1.84 mmol) to give **15d** (1.15 g, 95%) as a yellowish oil. R_f 0.44 (EtOAc–hexane 3:2); IR (neat): 3096, 2937 (br), 2224, 1541, 1772, 1160, 744 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.04–7.97 (2H, m, Ph), 7.74–7.66 (4H, m, Ph), 7.65–7.58 (2H, m, Ph), 3.40–3.26 (6H, m, H-α,1,5), 3.17 (2H, t, *J*=6.6 Hz, H-8), 1.83–1.74 (2H, m, H-7), 1.70–1.61 (2H, m, H-6), 1.12 (3H, t, *J*=7.1 Hz, H-β); ^{13}C NMR ($CDCl_3$): δ 148.0 (2C), 133.7, 133.6, 133.3, 133.0, 131.9, 131.8, 130.8, 130.7, 124.2, 124.2 (totally 12C–Ph), 42.4 (C-α), 13.6 (C-β), rest in Table 9; HRMS (ESI-MS): calcd for (M+H) $C_{21}H_{24}D_4IN_4O_8S_2$ 659.0644, found 659.0656.

4.3.64. *N-[3-(Benzyl-{2-nitrobenzenesulfonyl}amino)-propyl]-N′-[3-(ethyl-{2-nitro-benzenesulfonyl}amino)-propyl]-N,N′-bis-{2-nitrobenzenesulfonyl}butane-1,4-diamine* ***14e***

To a stirred mixture of **11d** (535 mg, 1.0 mmol) and K_2CO_3 (414 mg, 3.0 mmol) in dry DMF (10 mL), **15c** (720 mg, 1.1 mmol) was added and stirring was continued for 24 h at room temperature. Solids were filtered off, the filtrate was evaporated to dryness in vacuo, and the precipitate was dissolved in EtOAc (20 mL) and water (20 mL). The water layer was extracted with EtOAc (20 mL) and the combined organic extracts were washed with brine (20 mL) and dried over $MgSO_4$. After evaporation of the solvent in vacuo, the residue was purified on silica gel using first EtOAc–hexane 3:2 and then 3:1 as the eluent, to give **14e** (939 mg, 88%) as a colourless solid, mp 45–56 °C (amorphous solid). R_f 0.44 (EtOAc–hexane 3:1); IR (KBr): 3096, 2940 (br), 1543, 1374, 1160, 744 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.02–7.90 (4H, m, Ph), 7.74–7.57 (12H, m, Ph), 7.33–7.23 (5H, m, Ph), 4.47 (2H, s, Ph–CH_2–), 3.35 (2H, q, *J*=7.1 Hz, H-α), 3.30–3.04 (12H, m, H-1,3,5,8,10,12), 1.88–1.80 (2H, m, H-11), 1.67–1.59 (2H, m, H-2), 1.47–1.31 (4H, m, H-6,7), 1.10 (3H, t, *J*=7.1 Hz, H-β); ^{13}C NMR ($CDCl_3$): δ 148.0, 148.0, 148.0, 147.9, 135.5, 133.7, 133.7, 133.7, 133.6, 133.3, 133.1, 133.0 (2C), 132.0, 132.0, 131.9, 131.8, 130.8, 130.8, 130.7, 130.6, 128.8 (2C), 128.4 (2C), 128.2, 124.3, 124.2 (2C), 124.2 (totally 30C–Ph), 52.0 (Ph–*C*H_2–), 42.4 (C-α), 13.6 (C-β), rest in Table 10; HRMS (ESI-MS): calcd for (M+Na) $C_{43}H_{48}N_8O_{16}S_4Na$ 1083.1969, found 1083.2011.

4.3.65. *N-[3-(Benzyl-{2-nitrobenzenesulfonyl}amino)-1,1,2,2-$^2H_4$-propyl]-N′-[3-(ethyl-{2-nitro-benzenesulfonyl}amino)propyl]-N,N′-bis-{2-nitrobenzenesulfonyl}butane-1,4-diamine* ***14f***

Prepared as **14e** from **11f** (1.62 g, 3.0 mmol) and **15c** (2.16 g, 3.3 mmol) to give **14f** (2.67 g, 83%) as a colourless solid, mp 44–56 °C (amorphous solid). R_f 0.39 (EtOAc–hexane 2:1); IR (KBr): 3096, 2938 (br), 1544, 1373, 1160, 743 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.02–7.89 (4H, m, Ph), 7.75–7.57 (12H, m, Ph), 7.33–7.23 (5H, m, Ph), 4.47 (2H, s, Ph–CH_2–), 3.35 (2H, q, *J*=7.1 Hz, H-α), 3.31–3.21 (6H, m, H-8,10,12), 3.19 (2H, s, H-1), 3.14–3.07 (2H, m, H-5), 1.88–1.79 (2H, m, H-11), 1.46–1.30 (4H, m, H-6,7), 1.10 (3H, t, *J*=7.1 Hz, H-β); ^{13}C NMR ($CDCl_3$): δ 148.0, 148.0, 148.0, 147.9, 135.5, 133.8, 133.7, 133.7, 133.6, 133.3, 133.1, 133.0, 133.0, 132.0, 132.0, 132.0, 131.9, 130.8, 130.8, 130.6, 130.6, 128.8 (2C), 128.4 (2C), 128.2, 124.3, 124.2 (2C), 124.2 (totally 30C–Ph), 52.0 (Ph–*C*H_2–), 42.4 (C-α), 13.6 (C-β), rest in Table 10; HRMS (ESI-MS): calcd for (M+K) $C_{43}H_{44}D_4N_8O_{16}S_4K$ 1103.1959, found 1103.1908.

4.3.66. *N-[3-(Benzyl-{2-nitro-benzenesulfonyl}amino)-1,1,2,2-$^2H_4$-propyl]-N′-[3-(ethyl-{2-nitro-benzenesulfonyl}amino)-1,1,2,2-$^2H_4$-propyl]-N,N′-bis-{2-nitrobenzenesulfonyl}butane-1,4-diamine* ***14g***

Prepared as **14e** from **11f** (792 mg, 1.47 mmol) and **15d** (1.07 g, 1.62 mmol) to give **14g** (1.32 g, 84%) as a colourless solid, mp 43–54 °C (amorphous solid). R_f 0.39 (EtOAc–hexane 2:1); IR (KBr): 3096, 2937 (br), 1541, 1373, 1161, 743 cm^{-1}; ^1H NMR ($CDCl_3$): δ 8.01–7.89 (4H, m, Ph), 7.74–7.56 (12H, m, Ph), 7.32–7.23 (5H, m, Ph), 4.47 (2H, s, Ph–CH_2–), 3.34 (2H, q, *J*=7.1 Hz, H-α), 3.27 (2H, s, H-12), 3.25–3.21 (2H, m, H-8), 3.19 (2H, s, H-1), 3.13–3.07

(2H, m, H-5) 1.46–1.31 (4H, m, H-6,7), 1.10 (3H, t, J=7.1 Hz, H-β); ^{13}C NMR ($CDCl_3$): δ 148.0, 148.0, 148.0, 147.9, 135.5, 133.8, 133.7, 133.7, 133.6, 133.3, 133.1, 133.0 (2C), 132.0, 132.0, 132.0, 131.9, 130.8, 130.8, 130.6, 130.6, 128.8 (2C), 128.4 (2C), 128.2, 124.3, 124.2 (2C), 124.2 (totally 30C–Ph), 52.0 (Ph–CH_2–), 42.5 (C-α), 13.6 (C-β), rest in Table 10; HRMS (ESI-MS): calcd for (M+Na) $C_{43}H_{40}D_8N_8O_{16}S_4Na$ 1091.2471, found 1091.2467.

4.3.67. N-(3-Benzylaminopropyl)-N′-(3-ethylaminopropyl)butane-1,4-diamine tetrahydrochloride ***4e***

Prepared as **4a** from **14e** (927 mg, 0.87 mmol) to give **4e** (284 mg, 70%) as a colourless solid, mp >275 °C decomp. IR (KBr): 3050–2389, 1593, 1460, 873, 743, 698 cm^{-1}; ^{1}H NMR (D_2O): δ 7.56–7.47 (5H, m, Ph), 4.28 (2H, s, PhCH_2–), 3.24–3.05 (14H, m, H-α,1,3,5,8,10,12), 2.19–2.06 (4H, m, H-2,11), 1.85–1.73 (4H, m, H-6,7), 1.30 (3H, t, J=7.3 Hz, H-β); ^{13}C NMR (D_2O): δ 133.3, 132.7 (2C), 132.7, 132.2 (2C) (totally 6C–Ph), 54.1 (Ph–CH_2–), 45.9 (C-α), 13.3 (C-β), rest in Table 6; HRMS (ESI-MS): calcd for (M+H) $C_{19}H_{37}N_4$ 321.3018, found 321.3003.

4.3.68. N-(3-Benzylamino-1,1,2,2-$^2H_4$-propyl)-N′-(3-ethylaminopropyl)butane-1,4-diamine tetrahydrochloride ***4f***

Prepared as **4a** from **14f** (2.65 g, 2.48 mmol) to give **4f** (790 mg, 68%, 98% d) as a colourless solid, mp >300 °C decomp. IR (KBr): 3050–2311, 1594, 1455, 825, 743, 698 cm^{-1}; ^{1}H NMR (D_2O): δ 7.56–7.47 (5H, m, Ph), 4.29 (2H, s, PhCH_2–), 3.24–3.06 (12H, m, H-α,1,5,8,10,12), 2.18–2.06 (2H, m, H-11), 1.86–1.75 (4H, m, H-6,7), 1.31 (3H, t. J=7.3 Hz, H-β); ^{13}C NMR (D_2O): δ 133.3, 132.7 (2C), 132.6, 132.2 (2C) (totally 6C–Ph), 54.1 (Ph–CH_2–), 45.9 (C-α), 13.3 (C-β), rest in Table 6; HRMS (ESI-MS): calcd for (M+H) $C_{19}H_{33}N_4D_4$ 325.3269, found 325.3260. Isotope distribution: 0% d_0–d_2, 8% d_3, 92% d_4.

4.3.69. N-(3-Benzylamino-1,1,2,2-$^2H_4$-propyl)-N′-(3-ethylamino-1,1,2,2-$^2H_4$-propyl)butane-1,4-diamine tetrahydrochloride ***4g***

Prepared as **4a** from **14g** (1.29 g, 1.21 mmol) to give **4g** (381 mg, 66%, 98% d) as a colourless solid, mp >300 °C decomp. IR (KBr): 3050–2312, 1593, 1452, 822, 744, 698 cm^{-1}; ^{1}H NMR (D_2O): δ 7.56–7.46 (5H, m, Ph), 4.29 (2H, s, PhCH_2–), 3.24–3.05 (10H, m, H-α,1,5,8,12), 1.85–1.74 (4H, m, H-6,7), 1.30 (3H, t, J=7.3 Hz, H-β); ^{13}C NMR (D_2O): δ 133.3 (Ph), 132.7 (2C), 132.6, 132.2 (2C) (totally 6C–Ph), 54.1 (Ph–CH_2–), 45.9 (C-α), 13.3 (C-β), rest in Table 6; HRMS (ESI-MS): calcd for (M+H) $C_{19}H_{29}N_4D_8$ 329.3520, found 329.3518. Isotope distribution: 0% d_0–d_5, 1% d_6, 12% d_7, 87% d_8.

4.3.70. N-(3-Amino-1,1,2,2-$^2H_4$-propyl)-N′-(3-ethylamino-propyl)-butane-1,4-diamine tetrahydrochloride ***3g***

Prepared as **1l** from **4f** (400 mg, 0.85 mmol) to give **3g** (259 mg, 80%, 98% d) as a colourless solid, mp >300 °C decomp. IR (KBr): 3000–2388, 1610, 1459, 1353, 1147, 823 cm^{-1}; ^{1}H NMR (D_2O): δ 3.22–3.07 (12H, m, H-α,1,3,5,8,12), 2.18–2.06 (2H, m, H-2), 1.86–1.75 (4H, m, H-6,7), 1.30 (3H, t, J=7.3 Hz, H-β); ^{13}C NMR (D_2O): δ 45.9 (C-α), 13.3 (C-β), rest in Table 6; HRMS (ESI-MS): calcd for (M+H) $C_{12}H_{27}N_4D_4$ 235.2800, found 235.2788. Isotope distribution: 0% d_0–d_2, 6% d_3, 93% d_4.

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References and notes

1. Jänne, J.; Alhonen, L.; Keinänen, T. A.; Pietilä, M.; Uimari, A.; Pirinen, E.; Hyvönen, M. T.; Järvinen, A. *J. Cell. Mol. Med.* **2005**, *9*, 865–882.
2. Wallace, H. M.; Fracer, A. V.; Hughes, A. *Biochem. J.* **2003**, *376*, 1–14.
3. Gugliucci, A. *Clin. Chim. Acta* **2004**, *344*, 23–35.
4. Karigiannis, G.; Papaioannou, D. *Eur. J. Org. Chem.* **2000**, 1841–1863.
5. Casero, R. A., Jr.; Marton, L. J. *Nat. Rev. Drug Discov.* **2007**, *6*, 373–389.
6. Casero, R. A., Jr.; Woster, P. M. *J. Med. Chem.* **2001**, *44*, 1–26.
7. Boncher, T.; Bi, X.; Varghese, S.; Casero, R. A., Jr.; Woster, P. M. *Biochem. Soc. Trans.* **2007**, *35*, 356–363.
8. Wallace, H. M.; Niiranen, K. *Amino Acids* **2007**, *33*, 261–265.
9. Seiler, N. *Pharmacol. Ther.* **2005**, *107*, 99–119.
10. Bergeron, R. J.; Wiegand, J.; Weimar, W. R.; Snyder, P. S. *Pharmacol. Res.* **1998**, *38*, 367–380.
11. Prakash, C.; Shaffer, C. L.; Nedderman, A. *Mass Spectrom. Rev.* **2007**, *26*, 340–369.
12. Kostiainen, R.; Kotiaho, T.; Kuuranne, T.; Auriola, S. *J. Mass Spectrom.* **2003**, *38*, 357–372.
13. Hakkinen, M. R.; Keinänen, T. A.; Vepsäläinen, J.; Khomutov, A. R.; Alhonen, L.; Jänne, J.; Auriola, A. *J. Pharm. Biomed. Anal.* **2007**, *45*, 625–634.
14. Hakkinen, M. R.; Keinänen, T. A.; Vepsäläinen, J.; Khomutov, A. R.; Alhonen, L.; Jänne, J.; Auriola, A. *J. Pharm. Biomed. Anal.* **2008**, *48*, 414–421.
15. Stokvis, E.; Rosing, H.; Beijnen, J. H. *Rapid Commun. Mass Spectrom.* **2005**, *19*, 401–407.
16. Cleland, W. W. *Arch. Biochem. Biophys.* **2005**, *433*, 2–12.
17. Lee, K. Y.; Na, J. E.; Lee, M. J.; Kim, J. N. *Tetrahedron Lett.* **2004**, *45*, 5977–5981.
18. Kuksa, V.; Buchan, R.; Kong Thoo Lin, P. *Synthesis* **2000**, 1189–1207.
19. Gawandi, V.; Fitzpatric, P. F. *J. Labelled Compd. Radiopharm.* **2007**, *50*, 666–670.
20. Maruyoshi, K.; Demura, T.; Sagane, T.; Matsumori, N.; Oishi, T.; Murata, M. *Tetrahedron* **2004**, *60*, 5163–5170.
21. Smith, R. G.; Daves, G. D., Jr. *Biomed. Mass Spectrom.* **1977**, *4*, 146–151.
22. Jentgens, C.; Bienz, S.; Hesse, M. *Helv. Chim. Acta* **1997**, *80*, 1133–1143.
23. Schere, G.; Limbach, H.-H. *J. Am. Chem. Soc.* **1994**, *116*, 1230–1239.
24. Van Den Berg, G. A.; Elzinga, H.; Nagel, G. T.; Kingma, A. W.; Muskiet, F. A. *J. Biochim. Biophys. Acta* **1984**, *802*, 175–187.
25. Fodor-Csorba, K.; Galli, G.; Holly, S.; Gács-Baitz, E. *Tetrahedron Lett.* **2002**, *43*, 3789–3792.
26. Moser, M.; Hudlicky, T.; Sadechi, S.; Sternin, E. *J. Labelled Compd. Radiopharm.* **2007**, *50*, 671–674.
27. Concellón, J. M.; Rodríguez-Solla, H.; Concellón, C. *Tetrahedron Lett.* **2004**, *45*, 2129–2131.
28. Whitmore, F. C.; Mosher, H. S.; Adams, R. R.; Taylor, R. B.; Chapin, E. C.; Weisel, C.; Yanko, W. *J. Am. Chem. Soc.* **1944**, *66*, 725–731.
29. Richard, J. P.; Williams, G.; Gao, J. *J. Am. Chem. Soc.* **1999**, *121*, 715–726.
30. Iwasaki, M.; Sakka, T.; Ohashi, S.; Matsushita, H. *J. Phys. Chem.* **1989**, *93*, 5139–5143.
31. Hidai, Y.; Kan, T.; Fukuyama, T. *Chem. Pharm. Bull.* **2000**, *48*, 1570–1576.
32. Järvinen, A. J.; Cerrade-Gimenez, M.; Grigorenko, N. A.; Khomutov, A. R.; Vepsäläinen, J. J.; Sinervirta, R. M.; Keinänen, T. A.; Alhonen, L. I.; Jänne, J. E. *J. Med. Chem.* **2006**, *49*, 399–406.
33. Grigorenko, N. A.; Khomutov, A. R.; Keinänen, T. A.; Järvinen, A.; Alhonen, L.; Jänne, J.; Vepsäläinen, J. *Tetrahedron* **2007**, *63*, 2257–2262.
34. Pretsch, E.; Bühlmann, P.; Affolter, C. *Structure Determination of Organic Compounds: Tables of Spectral Data* 3rd Completely revised and enlarged Engl. ed.; Springer: Berlin, 2000; p 247.
35. Rouvier, E.; Pastor, R.; Musso, J.; Cambon, A. *Org. Magn. Reson.* **1974**, *6*, 640–643.
36. Zhang, H.; Zhang, Y.; Liu, L.; Xu, H.; Wang, Y. *Synthesis* **2005**, *13*, 2129–2136.
37. Pearson, D. E.; Jones, W. H.; Cope, A. C. *J. Am. Chem. Soc.* **1946**, *68*, 1225–1229.
38. Dawber, J. G.; Massey-Shaw, J. *J. Chem. Soc., Perkin Trans. 2* **1989**, 1249–1254.
39. Compounds **1a**, **1h** and **1j** as free base: Tarbell, D. S.; Shakespeare, N.; Claus, C. J.; Bunnett, J. F. *J. Am. Chem. Soc.* **1946**, *68*, 1217–1219.
40. King, J. A.; McMillan, F. H. *J. Am. Chem. Soc.* **1946**, *68*, 1468–1470.
41. Martin, B.; Possémé, F.; Le Barbier, C.; Carreaux, F.; Carboni, B.; Seiler, N.; Moulinoux, J.-P.; Delcros, J.-G. *J. Med. Chem.* **2001**, *44*, 3653–3664.
42. Bolkenius, F. N.; Seiler, N. *Biol. Chem. Hoppe-Seyler* **1989**, *370*, 525–531.
43. Compound **1e** as free base: Surrey, A. *J. Am. Chem. Soc.* **1949**, *71*, 3354–3356.
44. Wysocka-Skrzela, B.; Cholody, W. M.; Ledochowski, A. *Pol. J. Chem.* **1981**, *55*, 2211–2214.
45. Wagner-Jauregg, T. *Helv. Chim. Acta* **1961**, *44*, 1237–1249.
46. Callery, P. S.; Subramayam, B.; Yuan, Z.-M.; Pou, S.; Geelhaar, L. A.; Reynolds, K. A. *Chem.-Biol. Interact.* **1992**, *85*, 15–26.
47. Bergeron, R. J.; Feng, Y.; Weimar, W. R.; McManis, J. S.; Dimova, H.; Porter, C.; Raisler, B.; Phanstiel, O. *J. Med. Chem.* **1997**, *40*, 1475–1494.
48. Carboni, B.; Benalil, A.; Vaultier, M. *J. Org. Chem.* **1993**, *58*, 3736–3741.
49. da Silva, E. T.; Fona, F. S.; Lima, E. L. S. *J. Braz. Chem. Soc.* **2004**, *15*, 433–436.
50. Olsen, C. A.; Jorgensen, M. R.; Witt, M.; Mellor, I. R.; Usherwood, P. N. R.; Jaroszewski, J. W.; Franzyk, H. *Eur. J. Org. Chem.* **2003**, 3288–3299.
51. Bergeron, R. J.; McManis, J. S.; Liu, C. Z.; Feng, Y.; Weimar, W. R.; Luchetta, G. R.; Wu, Q.; Ortiz-Ocasio, J.; Vinson, J. R. T.; Kramer, D.; Porter, C. *J. Med. Chem.* **1994**, *37*, 3464–3476.
52. Tomasi, S.; Le Roch, M.; Renault, J.; Corbel, J.-C.; Uriac, P.; Carboni, B.; Moncoq, D.; Martin, B.; Delcros, J.-G. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 635–640.
53. Bergeron, R. J.; Wiegand, J.; McManis, J. S.; Weimar, W. R.; Smith, R. E.; Algee, S. E.; Fannin, T. L.; Slusher, M. A.; Snyder, P. S. *J. Med. Chem.* **2001**, *44*, 232–244.
54. Weitl, F. L.; Raymond, K. N. *J. Org. Chem.* **1981**, *46*, 5234–5237.