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Design and optimization of aspartate *N*-acetyltransferase inhibitors for the potential treatment of Canavan disease

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ABSTRACT

Canavan disease is a fatal neurological disorder caused by defects in the metabolism of *N*-acetyl-L-aspartate (NAA). Recent work has shown that the devastating symptoms of this disorder are correlated with the elevated levels of NAA observed in these patients, caused as a consequence of the inability of mutated forms of aspartoacylase to adequately catalyze its breakdown. The membrane-associated enzyme responsible for the synthesis of NAA, aspartate *N*-acetyltransferase (ANAT), has recently been purified and examined (Wang et al., *Prot Expr Purif*. 2016;119:11). With the availability, for the first time, of a stable and soluble form of ANAT we can now report the identification of initial inhibitors against this biosynthetic enzyme, obtained from the screening of several focused compound libraries. Two core structures of these moderate binding compounds have subsequently been optimized, with the most potent inhibitors in these series possessing sub-micromolar inhibition constants (K_i values) against ANAT. Slowing the production of NAA *via* the inhibition of ANAT will lower the elevated levels of this metabolite and can potentially serve as a treatment option to moderate the symptoms of Canavan disease.

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1. Introduction

Defects in the metabolism of *N*-acetyl-L-aspartate (NAA) (Fig. 1) have been shown to be the underlying cause of a fatal neurological disorder called Canavan disease (CD).[1,2] Multiple mutations in the *aspA* gene that codes for aspartoacylase (www.hgmd.org), the enzyme responsible for the deacetylation of NAA in the brain, lead to mutant enzyme forms[3] with inadequate catalytic potential to produce sufficient levels of acetate or to lower the accumulation of NAA that is the diagnostic hallmark of CD.[4]

For many years the prevailing opinion among researchers in this field had linked this acetate deficiency to the brain developmental disorders that are observed in CD patients.[5,6] Structural and mechanistic characterization of aspartoacylase[7] and several clinical mutants of this enzyme[3] have led to the exploration of enzyme replacement as a possible treatment therapy for Canavan disease.[8] However, more recent work has shown that a knockout of the *Nat8l* gene that codes for aspartate *N*-acetyltransferase (ANAT), the enzyme which catalyzes NAA synthesis, leads to a reversal of the CNS demyelination in an animal model of CD.[9] On the basis of these results an approach in which selective ANAT inhibitors are designed and synthesized can be used to adjust the elevated brain NAA levels back into the physiological range. Such an approach has the potential to serve as a treatment for the symptoms of CD.

The connection between elevated brain NAA levels and CD has been well established.[1] In addition, recent work has identified some intriguing links between NAA levels and several different cancers. Overexpression of ANAT and the subsequent elevation of NAA has been detected in a large percentage of adenocarcinoma and squamous cell carcinoma cells isolated from patients with late-stage lung cancer.[10] Elevated NAA and high expression of ANAT was also identified in high-grade ovarian cancer tissue samples,[11] and has been correlated with worse overall patient survival in this and in several other forms of cancers.

Identifying and developing effective enzyme inhibitors requires the availability of a selective enzyme activity assay and the isolation and purification of the target enzyme. A fixed-time ANAT

Abbreviations: ANAT, aspartate *N*-acetyltransferase; CD, Canavan disease; DCM, dichloromethane; DTNB, dithionitrobenzoate; EDCI, 1-ethyl-3-(3-(dimethylamino) propyl)carbodiimide; HEPES, 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid; IPTG, isopropyl-β-thiogalactoside; LB, Luria broth; LE, ligand efficiency; MBP, maltose binding protein; NAA, *N*-acetyl-L-aspartate; NBS, *N*-bromosuccinimide; TLC, thin layer chromatography; THF, tetrahydrofuran.

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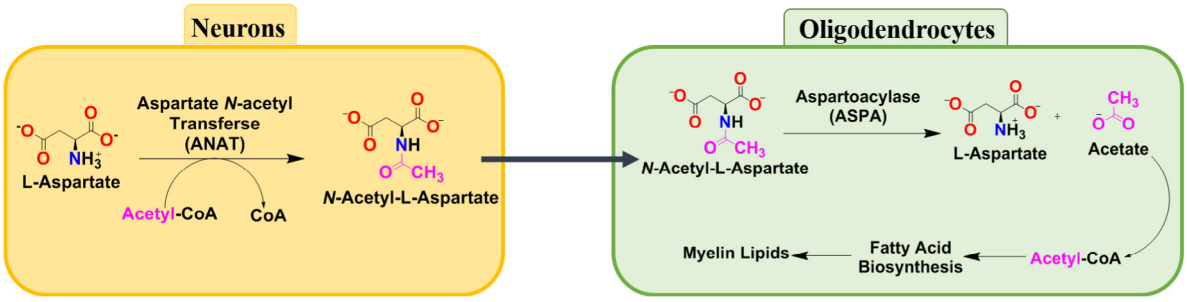


Fig. 1. Metabolism of *N*-acetyl-L-aspartate (NAA) in the brain. NAA is produced from L-aspartate and acetyl-coenzyme A catalyzed by aspartate *N*-acetyltransferase (ANAT). After transport from neurons to oligodendrocytes NAA is hydrolyzed to release acetate catalyzed by aspartoacylase (ASPA), with acetate serving as the building block for the synthesis of the fatty acid component of the myelin sheath.

assay that relies on the detection of radiolabeled NAA had previously been developed,[12] but the membrane-associated nature of ANAT had made enzyme purification a significant challenge.[13,14] Several acyl-coenzyme A derivatives had been found to inhibit the activity of ANAT in crude brain extracts,[15] but without access to purified enzyme there has been no progress to identify selective and potent ANAT inhibitors. We recently developed the application of fusion enzyme constructs to successfully solubilize and purify ANAT as a maltose binding protein (MBP) fusion,[16] and have used an optimized, continuous thiol detection assay[17] to kinetically characterize this newly produced enzyme form. We now report the results from a focused library screening effort to identify initial ANAT inhibitors, followed by the systematic optimization of several hits identified from these screens to yield selective, sub-micromolar inhibitors of this key regulator of brain NAA metabolism.

2. Results

2.1. Initial library screening

ANAT, the membrane-associated enzyme which catalyzes the synthesis of NAA, has been found to be highly selective for L-aspartate as the acetyl group acceptor. The introduction of a methyl group at carbon-3 or the incorporation of a second amino group are each somewhat tolerated, but the extension of the dioic acid structure by one carbon (glutamate *vs.* aspartate) causes a drop in catalytic efficiency to less than 1% that of the physiological substrate.[16] While the prevention of catalytic turnover is the goal of inhibitor design, this inhibition must also possess improved affinity that will enable an inhibitor to successfully compete with the substrate for active site binding.

Our search for an initial set of inhibitors of ANAT started with the screening of several focused compound libraries. The initial libraries were composed of amino acids, amino acid derivatives, various metabolites and metabolite analogs. Examination of a library of 256 compounds in these structural groups yielded a total of 17 weak inhibitors that showed at least 50% inhibition of ANAT when examined at a concentration of 2 mM (Table 1). The most potent initial inhibitor identified from these libraries, an *N*-chloroacetyl derivative of the substrate, has a K_i value of 200 μM. Next, a library of constrained amino acid analogs was examined, producing an additional set of five weak ANAT inhibitors. However, one of these compounds, the *N*-carbobenzyloxy derivative of L-aspartate, gave an unexpectedly potent K_i value of 17 μM (Table 1) and provided an excellent starting point for further inhibitor optimization.

2.2. Production of dioic acid derivatives (the D series)

Based on these initial screening results a core dioic acid structure that produced the best ANAT inhibitor was then systematically modified to determine the geometric properties that would lead to optimal inhibitor binding. For this process of optimization a 2-amino dioic acid was utilized as the parent structure, with the chain length starting from that of the substrate, L-aspartic acid (n = 1), increasing to L-glutamic acid (n = 2) and finally to 2-aminoadipic acid (n = 3) (Scheme 1).

The length of the *N*-acylated side chain (indicated in red) attached to these dioic acid core structures was also varied from m = 0–3, as was the nature of the linker between the amino group and the acyl side chain (Table 2). Each of these compounds was synthesized as described in the Section 5, and were then kinetically evaluated as potential inhibitors of ANAT.

The inhibition constants (K_i) determined from these studies identified a systematic variation in binding affinity that is associated with changes in the values of **n** and **m** (Table 2). The kinetic evaluation of this series of *N*-acyl dioic acid compounds against ANAT suggests that a value for **n** of 1–2 (aspartate or glutamate) and a value of **m** = 2, with either an acyl or an ester linkage, provides the optimal inhibition among this series of compounds. A shorter dioic acid, aminomalonate, containing the reasonably potent phenylpropanoyl side chain was also examined, but was found to be a much less effective inhibitor than the longer dioic acids (Table 2).

2.3. Optimization of dioic acid inhibitor design

Now that the geometric constraints of the ANAT inhibitor binding site has been systematically explored by varying the length of the dioic acid structure and the length of the *N*-linked side chain (Scheme 1), next the nature of the *N*-linkage, the requirement for an aryl *vs.* alkyl side chain, and the effect of substituents on the aryl side chain were explored.

2.3.1. Evaluation of the importance of the amide linkage in the optimized structure

To determine if the amide linkage plays an important role in the binding of these dioic acid inhibitors a series of structurally related *N*-aryl derivatives of DL-aspartate were prepared and tested as potential inhibitors of ANAT. While each of the *N*-aryl derivatives were found to be inhibitors of ANAT (Table S1), these compounds have only moderate affinities, even when adjusted for the correct L-isomer concentration, and the best inhibitors of this series (compounds **D19** and **D20**) were found to be significantly weaker inhibitors than the corresponding *N*-acyl and *N*-ester linked compounds (compounds **D4** and **D5**) (Table 2).

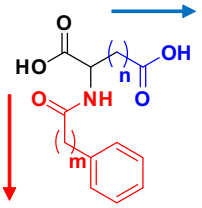
2.3.2. Evaluation of the importance of an aromatic ring in the side chain

To determine if the presence of an aryl side chain plays a significant role in the binding affinity of these dioic acid inhibitors

Table 1
Summary of compound library screening and hit optimization against ANAT.

Library	Number of compounds	Weak inhibitors[a]	Strong inhibitors[b]	Most potent (K_i value)
Amino acids	96	3	0	*N*-Chloroacetyl-L-aspartic acid (K_i = 200 μM)
Metabolites	96	13	0	2-Bromofumarate (K_i = 367 μM)
Amino acids II	64	1	0	*N*-Alanyl-L-aspartic acid (K_i = 1.6 mM)
Constrained analogs	77	5	1	*N*-Carbobenzyloxy-L-aspartic acid (K_i = 17 μM)
Screened Compounds (hit rate)	333	22 (6.6%)	1 (0.3%)	
Dioic acids (D series)	41		4	*N*-Carbobenzyloxy-L-glutamic acid (K_i = 12 μM)
Phthalates (P series)	60		11	4-Aminomethyl(*N*-carboethyl,*N*-4-carboxy-2,6-dichlorobenzyl) phthalate (K_i = 0.6 μM)
Total number	434		16	

[a] At least 50% inhibition when tested at 2 mM concentration.
[b] Compounds with K_i values less than 100 μM.



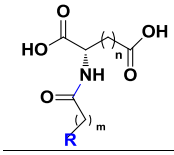
Scheme 1. Optimization of the dioic acid core structure.

a series of *N*-alkyl derivatives of L-aspartate, L-glutamate, and L-aminoadipate were prepared and tested. Several of these *N*-alkyl compounds did not show any inhibition against ANAT when tested at 2 mM, while some were found to be only weak inhibitors (Table S2). However, the best inhibitors among these *N*-alkyl derivatives achieved K_i values in the several hundred micromolar range, significantly weaker than the *N*-acyl side chains on the same dioic acid structures.

2.3.3. Evaluation of the effect of aryl substitutions on inhibitor binding

Once the important of the *N*-aryl side chain had been confirmed, a series of substituents were introduced into the aromatic ring to determine if either electronic or steric effects play an important role in the binding of the aryl side chain to ANAT. The addition of hydrophobic substituents on the *N*-benzyl ring does lead to some alterations in the inhibition of ANAT relative to the parent *N*-aryl-L-aspartate structure, with the *ortho*-CF_3 substituent (**D33**) showing the best inhibition among this group of derivatives (Table S3). However, these changes are relatively small and did not identify a significant trend for these modified derivatives in their interactions with ANAT.

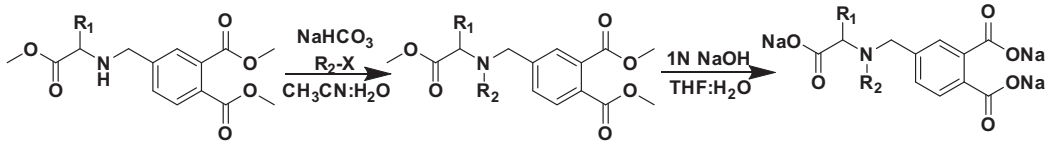
Table 2
N-Acylation derivatives of aminomalonic, aspartic, glutamic, and aminoadipic acids.



	Cpd. No.	m	Linker	R group	K_i (mM)
Aminomalonate derivative (n=0)	D1	2	Acyl	3-Phenylpropanoyl	1.4 ± 0.2
Aspartate derivatives (n = 1)	D2	0	Acyl	Benzoyl	>2
	D3	1	Acyl	2-Phenylacetyl	0.46 ± 0.08
	D4	2	Acyl	**3-Phenylpropanoyl** [a]	**0.031 ± 0.002**
	D5	2	Ester	**Benzylcarbanoyl** [a]	**0.017 ± 0.002**
	D6	3	Acyl	4-Phenylbutanoyl	0.20 ± 0.02
Glutamate derivatives (n = 2)	D7	0	Acyl	Benzoyl	0.13 ± 0.01
	D8	1	Acyl	2-Phenylacetyl	0.25 ± 0.03
	D9	2	Acyl	**3-Phenylpropanoyl** [a]	**0.038 ± 0.002**
	D10	2	Ester	**Benzylcarbanoyl** [a]	**0.012 ± 0.001**
	D11	3	Acyl	4-Phenylbutanoyl	0.61 ± 0.12
2-Aminoadipate derivatives (n = 3)	D12	0	Acyl	Benzoyl	1.60 ± 0.34
	D13	1	Acyl	2-Phenylacetyl	1.16 ± 0.24
	D14	2	Acyl	3-Phenylpropanoyl	2.90 ± 0.30
	D15	2	Ester	Benzylcarbanoyl	2.35 ± 0.29
	D16	3	Acyl	4-Phenylbutanoyl	0.74 ± 0.17

[a]Substitutions shown in red indicate the best inhibitors in this compound class.

Table 3
Synthesis and kinetic evaluation of 4-aminomethylphthalate and 4-aminoethylphthalate derivatives.



Cpd. No.	R_1	R_2	K_i (μM)
P3	H	H	n.i.[b]
P4[a]	H	H	1710 ± 333
P5	(*S*)-CH_3	H	n.i.[b]
P6	(*S*)-CH_2OH	H	n.i.[b]
P10	H	CH_3	n.i.[b]
P11	H	Propionitrile	n.i.[b]
P12	H	Acetaldehyde	1290 ± 240
P13	H	Allyl	719 ± 143
P14	H	Benzyl	420 ± 86
P30	H	4-Biphenyl	953 ± 173
P31	H	*N*-Ethylmorpholino	1080 ± 380
P32	H	2-Naphthyl	538 ± 75
P33	H	1-Naphthyl	115 ± 12
P48[a]	H	4-Biphenyl	550 ± 103
P49[a]	H	1-Naphthyl	34 ± 5

[a] *N*-Carboxymethyl group replaced with *N*-carboxyethyl.
[b] No inhibition observed when examined at concentrations up to 2 mM.

2.4. Production of phthalate derivatives (the P series)

A related series of dioic acid structures, built on a conformationally-constrained phthalic acid core, had previously been produced and optimized as selective inhibitors of a Gram-positive form of aspartate β-semialdehyde dehydrogenase obtained from *Streptococcus pneumonia*.[18] Given the similarity between the structures of the amino acid substrate of this enzyme to that of ANAT, several members of this class of compounds were examined as potential inhibitors of this enzyme. Compounds **P3**, with an *N*-carboxymethyl side chain, as well as compounds **P5** and **P6**, with substitutions in the carboxymethyl branch, did not show any measureable inhibition of ANAT. Compound **P4** with a longer *N*-carboxyethyl side chain does inhibit ANAT, but with a very weak K_i of 1.7 mM (Table 3). To improve the affinity of the parent compound **P3** as a potential inhibitor of ANAT several additional derivatives were synthesized through coupling with various halides at the secondary amine position. The introduction of simple alkyl or nitrile groups (**P10** & **P11**) did not confer any inhibitory properties to the parent compound, while the introduction of either aldehyde or allyl groups (**P12** & **P13**) produced only weak inhibitors of ANAT with K_i values of 1.2 and 0.71 mM, respectively (Table 3).

2.4.1. Introduction of aromatic functional groups

Based on the importance of *N*-aryl groups in conferring improved binding affinity among the dioic acid inhibitors (the D series), several *N*-aryl substituents were incorporated into the

Table 4
Synthesis and kinetic evaluation of 4-(*N*-substituted)benzylaminoalkylphthalates.

R group	Aminomethylphthalates (n = 1)		Aminoethylphthalates (n = 2)	
	Cpd. No.	K_i (μM)	Cpd. No.	K_i (μM)
2-Methyl	P15	1400 ± 260	P43	724 ± 91
3-Methyl	P16	1700 ± 220	P44	563 ± 89
4-Methyl	P17	4230 ± 770	P45	2750 ± 450
2-Bromo	P18	114 ± 15	P37	173 ± 21
3-Bromo	P19	269 ± 31	P38	101 ± 4
4-Bromo	P20	1200 ± 50	P39	370 ± 60
2-Trifluroromethyl	P21	390 ± 50	P40	88 ± 6
3-Trifluoromethyl	P22	104 ± 9	P41	37 ± 1
4-Trifluoromethyl	P23	791 ± 51	P42	186 ± 16
4-*tert*-Butyl	P28	687 ± 66	P46	1660 ± 330
4-(2-Perfluoropropyl)	P29	426 ± 68	P47	587 ± 72
4-Carboxy	P34	104 ± 9	P50	29 ± 2

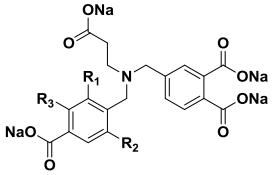
4-aminomethylphthalate core structure. Each of these aryl derivatives were observed to be moderate inhibitors of ANAT, with the benzyl (**P14**) and naphthyl (**P32** & **P33**) derivatives leading to the greatest improvement in affinity (Table 3). Introducing aryl substituents into the homologous 4-aminoethylphthalate core structure led to a further increase in binding affinity, with the 1-naphthyl substituent (**P49**) showing a 50-fold greater inhibition (K_i = 34 μM) when compared to the parent compound (**P4**) (Table 3).

2.4.2. Incorporation of benzyl substituents

To further explore the binding affinity of the *N*-aryl derivatives a series of substituted benzyl groups were introduced at this amine position in both the 4-aminomethylphthalate and the 4-aminoethylphthalate core structures. Coupling with benzyl halides substituted with methyl groups at either the *ortho*-, *meta*- or *para*-positions (**P15**–**P17**) actually led to weaker binding affinity of the 4-aminoalkylphthalates (Table 4) relative to the unmodified benzyl derivative. The effect of changes in the nature of the methyl substituent was also examined. However, replacement of the methyl group with either $–OCF_3$ or $–OCHF_2$ groups (**P24**–**P27**) did not substantially increase the affinity of these derivatives regardless of their position on the benzyl ring (Table S4). Substitutions with either a bromo or a trifluoromethyl group at the *para*-position of the benzyl ring (**P20** and **P23**) also resulted in a weaker inhibitor. Changing the *para*-group to a bulkier substituent, such as *p*-*tert*-butyl (**P28**) or *p*-perfluoropropyl (**P29**), also lead to only weak inhibitors with no enhancement in their K_i values (Table 4). However, substitution with either bromo or trifluoromethyl groups at the *ortho*- or *meta*-positions (**P18**, **P19**, **P21** and **P22**) lead to inhibitors with some improvement in their K_i values against ANAT (Table 4) relative to the unsubstituted benzyl derivative.

While the incorporation of hydrophobic substituents in the *ortho*- and *meta*-positions of the *N*-benzyl derivative resulted in up to a 4-fold improvement in binding affinity, the presence of hydrophobic substituents in the *para*-position proved to have a deleterious effect on inhibitor binding. To examine the effect of a hydrophilic substituent at this position, the *p*-carboxybenzyl derivative (**P34**) was synthesized and tested against ANAT. Unlike any of the other *para*-substituted derivatives this compound shows enhanced inhibition, with a K_i value of 104 μM (Table 4). Because this compound is the only improved inhibitor in the *para*-substituted series, it seems reasonable to suggest that this improvement is likely due to an electrostatic interaction with a positively-charged amino acid side chain within the binding pocket for this aromatic side chain. To test this hypothesis the negatively-charged *p*-carboxyl group was replaced with two different polar but uncharged substituents, a carboxamide group (**P35**) and also a ketone (**P36**). As predicted, these derivatives were found to be much less effective inhibitors (Table S4), with K_i values around 2 mM that are significantly worse than even the unsubstituted benzyl derivative.

Table 5
Optimization of benzylaminoethylphthalates.



Cpd. No.	R_1	R_2	R_3	K_i (μM)
P52	-H	-H	$-CF_3$	930 ± 151
P53	$-CF_3$	-H	-H	17.0 ± 0.4
P54	-Phenyl	-H	-H	47.4 ± 1.4
P51	-Naphthyl	-H	-H	187 ± 22
P57	-F	-H	-H	4.06 ± 0.08
P56	-Cl	-H	-H	1.70 ± 0.04
P55	-Br	-H	-H	2.01 ± 0.07
P59	**-Cl**	**-Cl**	**-H**	**0.61 ± 0.01**
P58	**-Br**	**-Br**	**-H**	**0.77 ± 0.01**

2.5. Development of the longer aminoethylphthalate structure

The parent aminomethylphthalate compound itself (**P3**) had no inhibitory properties against ANAT until the introduction of an *N*-aryl side chain. Now that the *N*-benzyl derivatives with either bromo or trifluoromethyl group at the *ortho*- and *meta*-position or with a *p*-carboxyl group, have been found to be good inhibitors, several homologs of these derivatives were synthesized starting from the parent aminoethylphthalate compound **P4** which had already been shown to be a moderate ANAT inhibitor. The *o*- and *m*-methyl substituted derivatives (**P43** and **P44**) of this series also showed improved inhibition relative to their shorter homologs (**P15** and **P16**), however these derivatives were still weaker inhibitors than the unsubstituted benzyl compound (**P14**).

2.6. Substituent effects on aminoethylphthalate binding

Both the nature and the position of different benzyl substituents were then varied in an attempt to improve the affinity of 4-(*N*-benzyl)aminoethylphthalates as ANAT inhibitors. The *o*- and *m*-derivatives of either bromo (**P37** and **P38**) or trifluoromethyl (**P40** and **P41**) not only lead to better inhibitors than the parent compound (Table 4), but these derivatives also have better K_i values when compared to the same derivatives of the shorter chain parent compound. For substituents at the *para*-position, more hydrophobic alkyl groups such as *t*-butyl (**P46**) or perfluoropropyl (**P47**) were substantially weaker inhibitors than the parent compound (Table 4). Further increases in the bulk of this benzyl side chain led to mixed results. Replacing benzyl with biphenyl (**P48**) had no significant effect on binding affinity, but replacement with a 1-naphthyl ring (**P49**) resulted in a 10-fold improvement in binding (Table 3). The *p*-bromobenzyl derivative (**P39**) gave comparable inhibition as the parent compound, while the *p*-trifluoromethyl substituent (**P42**) lead to a slight improvement in affinity (Table 4). Based on the improvement observed with a *p*-carboxyl group for the aminomethylphthalates, a similar derivative was synthesized for aminoethylphthalate. This compound (**P50**) gave the lowest inhibition constant that had been observed at this stage among the phthalate derivatives (Table 4).

2.7. Optimization of phthalic acid inhibitor design

After completion of the initial derivatization studies the two best inhibitors obtained from the phthalate series of compounds were the 1-napthyl derivative (**P49**) and the 4-carboxybenzyl derivative (**P50**) with K_i values of 34 and 29 μM, respectively. This observation led to the idea of combining the features of both derivatives into a single molecule, by retaining the charged *p*-carboxyl group and then introducing hydrophobic groups at either the *ortho*- or *meta*-positions. Unfortunately, the incorporation of a CF_3 group at the *meta*-position (**P52**) resulted in a significant loss of potency. However, placing this same group in the *ortho*-position (**P53**) lead to a 2-fold improvement in binding relative to the starting *p*-carboxyl derivative (Table 5). Expanding the size of the *ortho*-

hydrophobic group (**P51** and **P54**) caused a decrease in affinity, but replacement with an *ortho*-bromo substituent (**P55**) led to an additional 7-fold enhancement in affinity, to a K_i value of 2.4 μM (Table 5). Decreasing the size of the *ortho*-halo substituent from bromo to chloro (**P56**) resulted in a further improvement in binding, while the further decrease to fluoro (**P57**) was less effective. The dihalogenated derivatives showed the same trend, with the dichloro substituent (**P59**) giving a slight improvement over the dibromo derivative (**P58**). Each of these compounds now represent the first sub-micromolar inhibitors of ANAT that have been produced (Table 5).

3. Discussion

3.1. Importance of ANAT as a drug development target

The overproduction of NAA has been shown to not only be a consequence of the disruption of its subsequent metabolism, but the underlying basis for the symptoms of Canavan disease.[9,19] Because even the most impaired clinical mutants of aspartoacylase have been demonstrated to still possess some residual catalytic activity,[4] slowing the production of NAA through the design of selective ANAT inhibitors can potentially restore the balance in NAA metabolism in the brain of CD patients (Fig. 1). With this goal as a proposed therapeutic approach, we have screened some focused compound libraries to identify inhibitors of the NAA biosynthetic enzyme (ANAT), and have then proceeded to optimize the structures of these initial inhibitors.

The amino acid binding site of ANAT has evolved to be specific for L-aspartate as the acceptor of an acetyl group from acetyl-CoA, and this site is highly selective for the binding of dicarboxylic acids. Screening of an extensive group of amino acids and derivatives found that only structures with an additional side chain carboxyl group (or carboxylate mimic) showed any reasonable affinity for binding to ANAT. The additional screening of a metabolite library found that a wide variety of dicarboxylate structures were capable of binding to the enzyme with moderate affinities, despite variations in the length of the core structure or the presence of additional functional groups. While the requirement of unmodified carboxylate-type groups appears to be essential for binding to ANAT, the amino group of aspartic acid is the acceptor for the acetyl group in this reaction and must therefore remain accessible to serve as the nucleophile for this group transfer reaction. We have taken advantage of the required accessibility of this functional group by designing and then elaborating a series of *N*-derivatized dicarboxylic acids as more potent ANAT inhibitors.

3.2. Synthesis and optimization of dioic acid inhibitors of ANAT

Geometric optimization of a 2-amino dioic acid core structure has led to either L-aspartate or L-glutamate derivatives as the best dioic acid inhibitors, with either an acyl or an ester linkage to an aromatic side chain. The most potent inhibitors in this series have K_i values in the low micromolar range (Table 2), and have ligand efficiency (LE) values between 0.3 and 0.4 kcal/mol/heavy atom. When compared to typical drugs these are still relatively small molecules. Further structural elaborations through the incorporation of new functional groups would make these inhibitors more drug-like, and would likely lead to additional enhancements in binding affinity.

3.3. Synthesis and optimization of phthalic acid inhibitors of ANAT

A series of conformationally-constrained dioic acids built on a phthalic acid core structure were also examined as possible ANAT inhibitors. Unlike the acyclic dioic acids, many of which were already weak inhibitors even without extensive derivatization (Table 1), the parent *N*-carboxyalkylphthalates were either non-inhibitors or extremely weak inhibitors of ANAT (Table 3). However, the introduction of an *N*-aryl side chain into these core structures conferred increasing affinity for our target enzyme. The synthesis of a series of substituted benzyl derivatives clearly defined the *N*-carboxyethyl side chain as the optimal length to best position this acyl group into a binding pocket on ANAT, with most derivatives showing a 2- to 4-fold enhancement in binding when attached to the aminoethylphthalate (n = 2) structure compared to the same derivatives of the aminomethylphthalates (n = 1) (Table 4). This series of compounds also allowed the systematic exploration of the role of the aryl side chain in binding. The introduction of *p*-hydrophobic substituents on the benzyl ring were detrimental to binding, but *o*- or *m*-hydrophobic substituents led to as much as a 10-fold improvement in binding affinity (Table 4). In contrast, the presence of a charged *p*-carboxyl group caused a 14-fold increased affinity relative to the parent *N*-benzyl derivative. The additional combination of *o*-dichloro groups with the *p*-carboxyl substituent resulted in a nearly 700-fold improvement in affinity to a K_i value of 0.6 μM (Table 5). This best ANAT inhibitor (**P59**) also has a very good ligand efficiency (LE) value of 0.27 kcal/mol/heavy atom,[20] allowing the possibility of some additional optimization that can lead to further enhancements in affinity, as well as structural modifications to improve the bioavailability of this compound series.

4. Conclusions

A knock-out of the *Nat8l* gene completely blocked the synthesis of NAA, and resulted in substantial recovery from the brain developmental defects that are the hallmark of CD.[9] However, this approach does come with some enhanced risks. The survival times of the mice with this gene knock-out are significantly reduced, even when the *aspA* gene that codes for aspartoacylase is not eliminated. When only one copy of the *Nat8l* gene is removed then the survival times improve significantly.[19] These results suggest that extremely potent inhibitors of ANAT may not be desirable or even advantageous as a drug. Slowing the production of NAA through the interactions of moderately potent inhibitors with ANAT may be sufficient to lower the abnormally high levels of this metabolite and restore the balance to this pathway. Through systematic elaborations two different core structures have been converted into reasonably potent inhibitors of the human ANAT enzyme that is responsible for the overproduction of *N*-acetylaspartate in the brain. With the overexpression of ANAT and elevated NAA levels recently identified as correlating with poor patient survival in both advanced stage lung cancer[10] and in ovarian cancer,[11] the effectiveness of our ANAT inhibitors can also be tested in models of these forms of cancer. The next step will be to convert these potent enzyme inhibitors into more drug-like molecules, either by replacement of the carboxylate functional groups with uncharged isosteres or through the modification of these carboxylates with hydrolysable derivatives.

5. Experimental

5.1. General chemistry

Solvents and reagents were used as purchased without further purification. The fragment compound libraries were assembled from commercially available compounds and prepared as 200 mM stock solutions with the pH adjusted as necessary using either HCl or NaOH. For the newly synthesized enzyme inhibitors

the reaction time courses were monitored by TLC using an ethyl acetate/hexane solvent system on Silica gel HLF plates (Analtech, Inc., Newark, DE). The final products were purified by silica gel (230–400 mesh) flash column chromatography. ^{1}H and ^{13}C NMR spectra were recorded on either a Varian VXRS 400 MHz or a Varian INOVA 600 MHz spectrometer. Mass spectra were measured on a Bruker Esquire Liquid Chromatograph – Ion Trap Mass Spectrometer by using electrospray ionization and detection in positive ion mode. Compound purity was assessed by HPLC, using an Alltech alltima HP C18 amide 5μ 150 × 4.6 mm column; with a linear gradient from 90% water:10% methanol to 5% water:95% methanol in 20 min at a flow rate of 1.0 mL/min. For the compounds found to be <95% pure, in most cases the primary impurity was the non-hydrolyzed ester precursor. The esters of this series of compounds were found not to be inhibitors of ANAT.

5.2. Enzyme production and activity assay

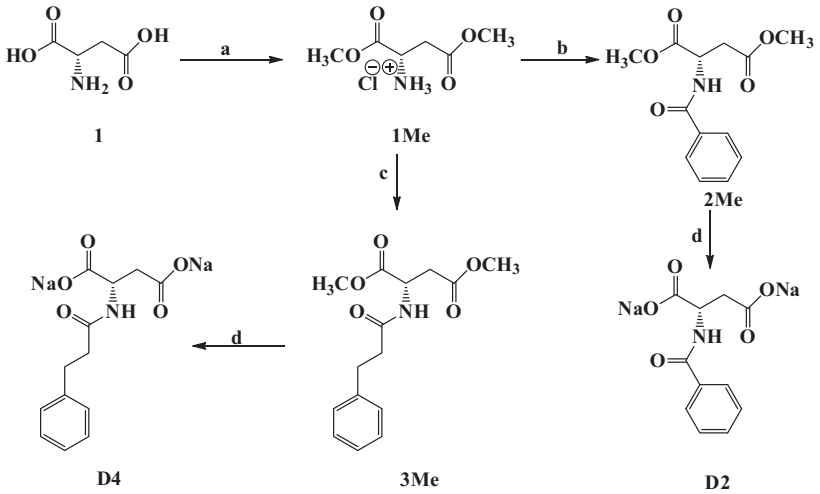
The recombinant maltose-binding protein-human aspartate *N*-acetyltransferase (MBP-ANAT) fusion enzyme was expressed and purified as reported previously.[16] Briefly, NiCo21 (DE3) competent *E. coli* cells (New England Biolabs, Ipswich, MA) were transformed with an MBP-ANAT-his construct and selected on LB plates with 30 μg/ml kanamycin. Colonies from these plates were used to inoculate starter cultures in LB media. After diluting each starter culture by 100-fold in 1 L of LB media, cell growth was continued for about 2 h at 37 °C until A_{600} reached 0.6. IPTG was then added to a final concentration of 0.5 mM and protein expression was induced at 16 °C for 20 h. To purify MBP-ANAT, a Ni Sepharose 6 Fast Flow column (GE Healthcare, Pittsburgh, PA) was equilibrated with Buffer A (20 mM potassium phosphate, pH 7.4, 300 mM sodium chloride, 10% glycerol and 20 mM imidazole). Cell lysate was loaded on to the column and partially purified ANAT was then eluted with a linear gradient of Buffer B (buffer A containing 400 mM imidazole). The active fractions were pooled and loaded onto an amylose column and highly purified ANAT fusion was then obtained by elution with a 0–10 mM linear maltose gradient. ANAT activity was measured by an established DTNB-based assay[17] using a SpectraMax 190 spectrophotometer plate reader (Molecular Devices, CA). A typical activity assay mixture contains 20 mM HEPES, pH 7.4, 150 mM NaCl, 5% glycerol, 40 μM DTNB, 40 μM acetyl-CoA, 2 mM L-aspartate, 25 μg of enzyme and various amount of inhibitors in a total volume of 200 μl. Each compound was prepared as 200 mM stock in water and compounds from in-house library was kept at 4 °C. Acetyl-CoA was prepared as 10 mM stock in water and stored at −20 °C to minimize degradation. Before each set of assays, acetyl-CoA was diluted into a 2 mM working solution using HEPES buffer. 2 mM DTNB was freshly prepared and kept on ice during the kinetic assay. For compound library screening, 2 μl of 200 mM L-aspartate, 2 μl of inhibitor, and 116 μl of buffer were added into each reaction well. 80 μl of freshly prepared pre-mix, which includes 4 μl of acetyl-CoA, 4 μl of DTNB, 5 μl of enzyme and 67 μl of buffer, was added to start the reaction. The reaction time course was monitored at 412 nm ($\varepsilon = 14.15\ mM^{-1}\ cm^{-1}$) for at least 15 min. For K_i determinations a twofold serial dilution of each inhibitor was performed starting from either a 2 mM, 0.2 mM, or 0.02 mM stock solution, with the starting concentration determined according to the potency of the inhibitors. A negative control, in which no inhibitor was added, and a background signal control, in which neither L-aspartate nor inhibitor were added, were included in each set of assays. The pH of the reaction wells with highest concentration of inhibitor was tested to rule out any possible false positive due to pH variations.

5.3. Compound library screening

Four different compound libraries were used for the initial screening of ANAT inhibitors. These fragment compound libraries were assembled in-house: an amino acids library contained 96 compounds; a metabolite library contained 96 compounds and a second amino acids library contained 64 compounds. An additional constrained analog library contained 77 compounds that were also purchased from commercial sources. Once the dioic acid core structure was identified as a viable inhibitor of ANAT a library of dioic acid compounds was synthesized for testing and optimization.

5.4. General procedure for the preparation of N-acylated amino acids

The general synthetic scheme for the preparation of *N*-acylated derivatives of aminomalonate (n = 0), aspartate (n = 1), glutamate (n = 2) and 2-aminoadipate (n = 3) involves esterification to protect the carboxylic acid groups, followed by coupling to different acyl halides and then hydrolysis to yield the final products. The production of specific dioic acid derivatives is illustrated for the synthesis of two different *N*-acyl derivatives of L-aspartic acid (Scheme 2).



Scheme 2. Synthetic scheme for representative *N*-acyl-L-aspartate derivatives. Reagents and conditions: (a) $SOCl_2$ in MeOH at 0 °C to RT 14 h; (b) benzoyl chloride, $NaHCO_3$ in DCM and H_2O, RT, 8 h; (c) 3-phenylpropanoic acid, EDCl, Et_3N in DCM, 0 °C to RT, 3 h; (d) NaOH in THF and H_2O, RT, 5 h.

*5.4.1. Synthesis of dimethyl L-aspartate hydrochloride (**1Me**)*

To an oven-dried round bottom flask, L-aspartic acid (**1**; 1 eq) was added and placed under a dry nitrogen gas atmosphere. The material is then dissolved in anhydrous methanol and stirred at 0 °C for a few minutes until the desired temperature is attained. Next, thionyl chloride (3 eq) was added dropwise until the reaction mixture became homogeneous, then the reaction mixture was warmed slowly to room temperature and left stirring for 14 h. The reaction mixture was then concentrated under reduced pressure to obtain compound **2** in the form of a pure white solid with no addition purification required

*5.4.2. Synthesis of dimethyl benzoyl-L-aspartate (**2Me**)*

Dimethyl L-aspartate hydrochloride (**1Me**; 1 eq) was added to a round bottom flask, to which 5 ml of dichloromethane (DCM) and 2 ml of water was added. Sodium bicarbonate (1.5 eq) was added to the resulting biphasic solution and the reaction mixture was stirred for 2 min. Benzoyl chloride (1.4 eq) was added dropwise to this solution and the reaction mixture was stirred at room temperature overnight. After confirming completion of the reaction by TLC, the organic layer was separated and the aqueous layer was extracted three times with 50 ml of DCM. The organic extracts were combined, washed with 75 ml of brine solution (aq), dried over solid sodium sulfate, and then concentrated under reduced pressure to obtain the crude product. Silica gel column chromatography, with elution using 20% ethyl acetate in hexanes, afforded pure compound **2Me** as a white solid.

*5.4.3. Synthesis of dimethyl (3-phenyl)propanoyl)-L-aspartate (**3Me**)*

To a mixture of dimethyl L-aspartate hydrochloride (**1Me**; 1 eq), EDCl (1.5 eq) and trimethylamine (1.4 eq), DCM was added and the solution stirred for 5 min. 3-phenylpropanoic acid (1 eq) was then added to this solution with stirring for 3 h. After quenching the reaction with saturated ammonium chloride, the reaction mixture was extracted thrice with ethyl acetate. The organic extracts were combined, washed with brine solution (aq), dried over solid sodium sulfate, and then concentrated under reduced pressure to obtain the crude product in the form of reddish oil. Purification was performed by silica gel column chromatography with elution using 50% ethyl acetate in hexanes to afford pure amide **3Me** as a yellow viscous liquid.

*5.4.4. Hydrolysis of dimethyl-2-amino dioates (**D2** or **D4**)*

To the mixture of dimethyl-2-amino dioate (**2Me** or **3Me**) (1 eq) in THF and water (9:1 ratio), sodium hydroxide (2 eq) was added and stirred for 5–8 h. After confirming the completion of reaction by TLC, the reaction solution was concentrated for several hours under reduced pressure to obtain the corresponding *N*-derivatized sodium dioates (**D2** or **D4**) as white/light yellow solids.

*5.5. General protocol for the synthesis of N-acyl dioic acid derivatives (**D1–D16**)*

A similar protocol to that described in Scheme 2 was used for the synthesis of a series of *N*-acyl dioic acid derivatives, starting with aspartic, glutamic or 2-aminoadipic acid and coupling with different phenyl halides.

*5.5.1. Diethyl 2-(3-phenylpropanamido)malonate (**D1**)*

Diethylester: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.32 (t, J = 7.16 Hz, 6H), 2.60 (t, J = 7.64, 2H), 3.02 (m, 2H), 4.06 (d, J = 4.90 Hz, 1H) 4.24 (q, J = 7.13, 14.27 Hz, 4H), 6.06 (s, 1H), 7.24–7.35 (m, 5H).

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.45 (dt, J = 2.41, 7.93, 1H), 2.59 (dt, J = 2.41, 7.93 Hz, 1H), 2.90 (m, 2H), 3.72 (m, 1H), 7.23–7.28 (m, 3H), 7.32 (m, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 24.97, 31.14, 31.90, 37.35, 39.19, 43.15, 67.79, 126.04, 126.37, 128.26, 140.71, 142.00, 168.27, 175.55, 176.67, 182.61.

Purity: HPLC – (>91%).

*5.5.2. Benzoyl aspartic acid (**D2**)*

Dimethylester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.97–3.01 (dd, J = 4.77, 17.66 Hz, 1H), 3.14–3.17 (dd, J = 4.77, 17.66 Hz, 1H), δ = 3.71 (s, 1H), 3.80 (s, 1H), 5.07 (m, 1H), 7.22 (d, 1H), 7.46 (t, 1H), 7.53 (t, 2H), 7.81 (d, 2H).

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.63 (m, 1H), 2.78 (m, 1H), 4.59 (q, J = 9.9 Hz, 1H), 7.51 (t, 1H), 7.60 (t, 2H), 7.78 (d, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 39.55, 53.70, 127.17, 128.65, 132.0, 133.59, 170.27, 178.63, 179.01.

Purity: HPLC – (>99%).

*5.5.3. (2-Phenylacetyl) aspartate (**D3**)*

Dimethylester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.82 (dd, J = 4.65, 16.96 Hz, 1H), 2.98 (dd, J = 4.65, 16.96 Hz, 1H), 3.60 (s, 2H), 3.61 (s, 3H), 3.72 (s, 3H), 4.83 (p, 1H), 6.45 (d, J = 7.57 Hz, 1H), 7.26–7.31 (m, 3H), 7.36 (t, J = 7.08 Hz, 2H).

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.48 (dd, J = 9.54, 15.41 Hz, 1H), 2.66 (dd, J = 9.54, 15.41 Hz, 1H), 3.64 (q, 2H), 4.39 (q, J = 3.67, 9.54 Hz, 1H), 7.31–7.334 (m, 3H), 7.39 (t, J = 7.7 Hz, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 39.58, 42.13, 53.28, 127.11, 128.81, 129.25, 135.00, 171.01, 173.87, 178.55, 178.74.

Purity: HPLC – (>99%).

*5.5.4. Dimethyl (3-phenylpropanoyl)aspartate (**D4**)*

Dimethylester: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.55 (m, 2H), 2.75 (dd, J = 4.60 Hz, 17.65 Hz, 1H) 2.98 (t, J = 7.81 Hz, 2H), 3.01 (dd, J = 4.15, 17.58 Hz, 1H), 3.67 (s, 3H), 3.75 (s, 1H), 4.86 (m, 1H), 6.40 (d, J = 8.06 Hz, 1H), 7.20 (m, 3H), 7.29 (t, J = 7.57 Hz, 2H).

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.38–2.43 (m, 2H), 2.49–2.52 (m, 2H), 2.85 (dt, J = 2.93, 7.69 Hz, 2H), 4.25 (q, J = 4.03 Hz, 1H), 7.16–7.21 (m, 3H), 7.27 (t, J = 7.69 Hz, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 25.29, 31.21, 37.44, 39.52, 52.75, 58.29, 126.34, 128.38, 128.67, 140.76, 175.00, 178.58, 178.64

Purity: HPLC – (98.5%).

*5.5.5. (4-Phenylbutanoyl)-L-aspartic acid (**D6**)*

Dimethylester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.95 (m, 2H), 2.21 (t, 2H), 2.43 (t, 2H), 2.81 (m, 2H), 3.03 (m, 2H), 3.65 (s, 3H), 3.74 (s, 3H), 4.85 (t, 1H), 6.43 (d, 1H), 7.15–7.18 (m, J = 7.2 Hz, 3H), 7.23–7.27 (m, J = 7.2 Hz, 2H). MS (ESI) m/z: (obs) $[M+Na]^+$ 330.1, (calc) 330.1.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 1.85 (m, 3H), 2.22 (t, 2H), 2.43 (m, 1H), 2.60 (m, 3H), 4.34 (t, 1H), 7.21 (t, J = 7.2 Hz, 2H), 7.25 (m, J = 7.2 Hz, 2H), 7.31 (m, J = 7.8 Hz, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 26.94, 34.19, 35.03, 39.67, 53.03, 126.02, 128.54, 128.57, 128.65, 142.12, 175.92, 178.72, 178.77.

Purity: HPLC – (>99%).

*5.5.6. (2-Phenylacetyl)-L-glutamic acid (**D8**)*

Dimethylester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.89 (m, 1H), 2.13 (m, 1H), 2.22–2.33 (m, 2H), 3.57 (s, 2H), 3.61 (s, 3H), 3.68 (s, 3H), 4.58 (m, 1H), 6.09 (d, 1H), 7.23–7.28 (m, J = 7.8 Hz, 3H), 7.32–7.35 (m, J = 7.8 Hz, 2H). MS (ESI) m/z: (obs) $[M+Na]^+$ 316.1, (calc) 316.0.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 1.85 (m, 2H), 1.99 (d, 1H), 2.13 (m, 2H), 3.61 (m, 1H), 4.34 (d, 1H), 7.28 (m, 3H), 7.34 (m, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 28.43, 34.01, 42.19, 55.30, 127.16, 128.84, 129.12, 129.14, 135.06, 135.09, 174.10, 178.76, 182.13.

Purity: HPLC – (>99%).

*5.5.7. (3-Phenylpropanoyl)-L-glutamic acid (**D9**)*

Dimethylester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.90 (m, 1H), 2.13 (m, 3H), 2.47–2.53 (m, 2H), 2.93 (m, 2H), 3.64 (s, 3H), 3.71 (s, 3H), 4.58 (m, 1H), 6.06 (d, 1H), 7.18 (d, *J* = 7.8 Hz, 3H), 7.23–7.27 (m, *J* = 7.8 Hz, 2H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 330.1, (calc) 330.1.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 1.52–1.69 (m, 4H), 2.42 (t, 2H), 2.71–2.20 (m, 2H), 3.84 (m, 1H), 4.34 (t, 1H), 7.06–7.09 (m, *J* = 7.2 Hz, 3H), 7.17 (m, *J* = 7.2 Hz, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 28.42, 31.28, 33.77, 37.40, 54.86, 126.49, 128.42, 128.68, 140.36, 175.07, 178.73, 182.11.

Purity: HPLC – (98.0%).

*5.5.8. (4-Phenylbutanoyl)-L-glutamic acid (**D11**)*

Dimethylester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.94 (m, 3H), 2.18 (m, 3H), 2.33–2.41 (m, 2H), 2.62 (t, 2H), 3.63 (s, 3H), 3.72 (s, 3H), 4.60 (m, 1H), 6.06 (d, 1H), 7.16 (d, *J* = 8.4 Hz, 3H), 7.23–7.27 (m, *J* = 7.8 Hz, 2H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 344.1, (calc) 344.1.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 1.79 (m, 1H), 1.86 (m, 2H), 1.95 (m, 1H), 2.14 (t, 2H), 2.24 (t, 2H), 2.59 (t, 2H), 4.34 (m, 1H), 7.20–7.24 (m, *J* = 7.8 Hz, 3H), 7.30 (t, *J* = 7.2 Hz, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 26.87, 28.44, 34.07, 0.4.32, 35.06, 55.12, 126.06, 128.57, 128.61, 142.01, 176.11, 178.89, 182.15.

Purity: HPLC – (>99%).

*5.5.9. 2-Benzamidohexanedioic acid (**D12**)*

Dimethylester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.38–1.82 (m, 3H), 1.98 (m, 1H), 2.36 (m, 2H), 3.64 (s, 3H), 3.77 (s, 3H), 4.81 (m, 1H), 6.81 (d, 1H), 7.41–7.51 (m, *J* = 8.4 Hz, 5H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 316.1, (calc) 316.1.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 1.62–1.84 (m, 4H), 2.17 (m, 2H), 4.34 (m, 1H), 7.47 (m, 2H), 7.55 (m, 1H), 7.74 (m, *J* = 6.6 Hz, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 22.67, 31.40, 37.14, 55.91, 127.16, 128.63, 131.99, 133.63, 170.61, 179.28, 183.20.

Purity: HPLC – (>99%).

*5.5.10. 2-(2-Phenylacetamido)hexanedioic acid (**D13**)*

Dimethylester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.50–1.61 (m, 3H), 1.84 (m, 1H), 2.26 (m, 2H), 3.58 (s, 2H), 3.62 (s, 3H), 3.68 (s, 3H), 4.56 (m, 1H), 6.81 (d, 1H), 7.26 (m, *J* = 6.6 Hz, 3H), 7.34 (m, *J* = 7.8 Hz, 2H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 330.1, (calc) 330.1.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 1.48–1.70 (m, 4H), 2.09 (s, 2H), 3.55 (m, 2H), 4.05 (m, 1H), 7.27 (m, 3H), 7.33 (m, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 22.43, 31.35, 37.07, 42.22, 44.42, 55.31, 127.18, 128.86, 129.07, 129.08, 135.09, 174.05, 179.18, 183.10.

Purity: HPLC – (99.4%).

*5.5.11. 2-(3-Phenylpropanamido)hexanedioic acid (**D14**)*

Dimethylester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.48–1.66 (m, 4H), 1.78–1.84 (m, 1H), 2.28 (dt, *J* = 2.69, 7.32 Hz, 2H), 2.49–2.59 (m, 2H), 2.96–2.99 (m, 2H), 3.67 (s, 3H), 3.73 (s, 3H), 4.58–4.61 (m, 1H), 5.96 (d, *J* = 7.57 Hz, 1H), 7.18–7.22 (m, 3H), 7.28 (t, *J* = 7.32 Hz, 2H).

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 1.01 (m, 2H), 1.23–1.40 (m, 2H), 1.81 (dt, *J* = 1.69, 5.81HZ, 2H), 2.40 (m, 2H), 2.72 (m, 2H), 3.77 (q, *J* = 4.17, 8.67 Hz, 1H), 7.03 (m, 3H), 7.12 (t, *J* = 7.48 Hz, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 22.08, 31.31, 31.35, 37.06, 37.36, 54.95, 126.49, 128.40, 128.65, 140.36, 171.01, 175.08, 179.23, 182.95.

Purity: HPLC – (98.5%).

*5.5.12. 2-(4-Phenylbutanamido)hexanedioic acid (**D16**)*

Dimethylester: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.66–1.82 (m, 2H), 1.92–2.09 (m, 4H), 2.32 (t, *J* = 7.46 Hz, 2H), 2.40–2.47 (m, 2H), 2.74 (q, *J* = 7.34, 2H), 3.73 (s, 3H), 3.82 (s, 3H), 4.67–4.72 (m, 1H), 6.09 (d, *J* = 6.83 Hz, 1H), 7.25 (m, 3H), 7.36 (m, 2H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 358.4, (calc) 358.4.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 1.5–1.75 (m, 4H), 1.89 (p, *J* = 7.48, 15.04 Hz, 2H), 2.11–2.19 (m, 2H), 2.27 (t, *J* = 7.46 Hz, 2H), 2.62 (t, *J* = 7.67 Hz, 2H), 4.04 (q, *J* = 4.54, 8.80 Hz, 2H), 7.23 (t, *J* = 7.31 Hz, 1H), 7.26 (d, *J* = 7.17 Hz, 2H), 7.33 (t, *J* = 7.74 Hz, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 22.54, 26.90, 31.40, 34.29, 35.01, 37.13, 39.93, 55.15, 126.05, 128.62, 142.02, 171.01, 176.10, 179.31, 183.12.

Purity: HPLC – (94.4%).

5.6. General phthalate derivative procedures

Based on the observation of the dioic acid series and their potency to bind with the ANAT, phthalate analogs were introduced into the inhibitor design. This core structure allowed additional structural elaborations and added complexity to the inhibitor structure. To produce the parent compounds we followed our previously published protocol,[18] for the production of the amine-containing 4-(carboxymethylaminomethyl) and 4-(2-carboxyethylaminomethyl)phthalate analogs **P3** and **P4**, respectively, and also for the amine-containing alanine methyl ester or serine methyl ester analogs **P5** and **P6**, respectively (Scheme 3).

A similar protocol was used to produce compound **P19** which is analogous to compound **P3** except for moving the position of the side chain to carbon-3. Unfortunately, these 3-aminoalkylphthalate derivatives rapidly cyclized through attack by the adjacent carboxyl group. Protecting the amino group by *N*-methylation did prevent cyclization, but this parent compound was not an effective ANAT inhibitor. The affinities of the parent 4-aminoalkylphthalate compounds were examined against ANAT and then improved through the production of an extensive series of derivatives at the introduced secondary amine.

*5.6.1. 4-Methylphthalate dimethyl ester (**4Me**)*

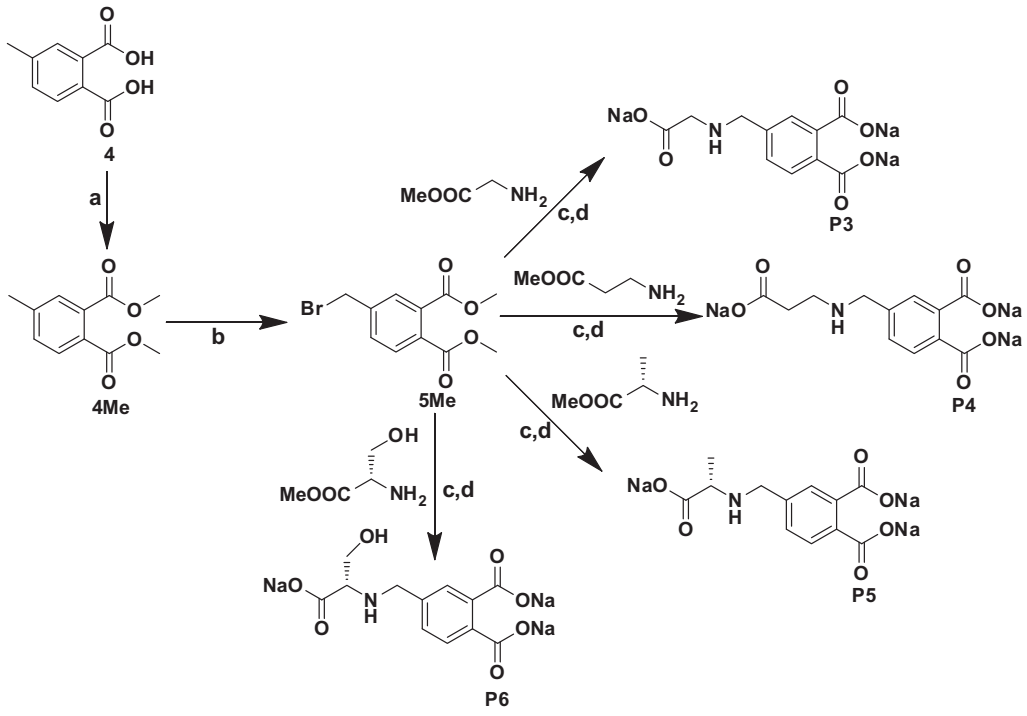
To a solution of compound **4**, 4-methyl-1,2-dicarboxylic acid (5 g, 27.7 mmol) in 12 mL of methanol, 5 mL of sulfuric acid was added at r. t., the reaction mixture was allowed to stir in a microwave reactor at 80 °C for 8 h. Saturated $NaHCO_3$ solution was added to the reaction mixture until slightly basic, with the product precipitating as an oily liquid. The product was extracted with several portions of dichloromethane (DCM), was dried over anhydrous sodium sulfate and concentrated in vacuum. The compound was purified by column chromatography using 0–10% ethyl acetate: hexanes to yield ester **4Me**, yield: 90%. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.39 (s, 3H), 3.87 (s, 3H), 3.88 (s, 3H), 7.30 (d, *J* = 7.8 Hz, 1H), 7.45 (s, 1H), 7.65 (d, *J* = 7.8 Hz, 1H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 231.1, (calc) 231.2.

*5.6.2. 4-Bromomethylphthalate dimethyl ester (**5Me**)*

To a solution of compound **4Me** (5 g, 24.8 mmol) in 25 mL dichloromethane was added NBS (4.9 g, 27.3 mmol). The reaction mixture was stirred in the presence of light for 3 h, concentrated under vacuum and purified by flash column chromatography using 0–20% ethyl acetate: hexanes to obtain compound **5Me**, yield: 78%. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.89 (s, 6H), 4.46 (s, 2H), 7.53 (d, *J* = 6.6 Hz, 1H), 7.68–7.71 (m, 2H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ (isotopic) 309.3, 311.1 (calc) (isotopic) 309.0, 311.0.

*5.6.3. N-Carboxymethyl-3,4-dicarboxybenzylamine (**P3**)*

To a solution of compound **5Me** (3.1 g, 10.7 mmol) in DMF was added $NaHCO_3$ (4.6 g, 54 mmol) and glycine methyl ester.HCl (6.8 g, 54 mmol). The reaction mixture was stirred at r. t. for 8 h. DMF was air evaporated and the reaction mixture was purified by flash column chromatography using 10–50% ethyl acetate: hexanes to obtain compound **P3Me**, yield: 64% ^{1}H NMR (600 MHz,



Scheme 3. Synthetic route to 4-aminomethylphthalate derivatives. Reagents and conditions: (a) H_2SO_4/MeOH, microwave, 80 °C, 8 h; (b) NBS/DCM, light, 3 h; (c) $NaHCO_3$/DMF, r.t., 8 h; (d) 1 N NaOH, r.t., 3 h.

$CDCl_3$): δ = 3.38 (s, 2H), 3.70 (s, 3H) 3.84 (s, 2H), 3.86–3.87 (s, 6H), 7.48 (d, J = 7.8 Hz, 1H), 7.64 (s, 1H), 7.68 (d, J = 7.8 Hz, 2H). MS (ESI) m/z: (obs) $[M+Na]^+$ 318.4, (calc) 318.3. This ester was hydrolyzed by adding H_2O (0.1 mL) to a solution of compound **P3Me** (0.04 g, 0.14 mmol) in THF (0.4 mL). Then 1 N NaOH (0.4 mL, 0.42 mmol) was added to the reaction mixture and stirred at r. t. for 3 h. The reaction mixture was concentrated and dried for several hours under high vacuum to yield pure compound **P3**, yield: 100% ^{1}H NMR (400 MHz, D_2O): δ = 3.12 (s, 2H), 3.66 (s, 2H), 7.48 (d, J = 7.8 Hz, 1H), 7.27–7.32 (m, 2H), 7.40 (s, 1H). ^{13}C NMR (600 MHz, D_2O): δ = 51.44, 51.62, 126.91, 127.54, 128.46, 136.26, 138.23, 139.58, 177.20, 177.58, 179.46.

Purity: HPLC – (89.9%).

*5.6.4. N-Carboxyethyl-3,4-dicarboxybenzylamine (**P4**)*

To a solution of compound **5Me** (0.52 g, 1.8 mmol) in DMF was added $NaHCO_3$ (0.8 g, 9 mmol) and β-alanine methyl ester.HCl (1.3 g, 9 mmol). The reaction mixture was stirred at r. t. for 8 h. DMF was air evaporated and the reaction mixture was purified by flash column chromatography using 10–60% ethyl acetate: hexanes to obtain compound **P4Me**, yield: 66% ^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.38 (s, 2H), 3.70 (s, 3H), 3.84 (s, 2H), 3.86 (s, 3H), 3.87 (s, 3H), 7.48 (d, J = 7.8 Hz, 1H), 7.64 (s, 1H), 7.68 (d, J = 7.8 Hz, 2H). MS (ESI) m/z: (obs) $[M+Na]^+$ 332.4, (calc) 332.3. A solution of compound **P4Me** (0.071 g, 0.229 mmol) was base-hydrolyzed as described above to yield compound **P4**, yield: 100% ^{1}H NMR (600 MHz, D_2O): δ = 2.47 (t, J = 7.2 Hz, 2H), 2.66 (t, J = 7.2 Hz, 2H), 3.59 (s, 2H), 7.18 (d, J = 7.8 Hz, 1H), 7.21 (s, 1H), 7.30 9 (d, J = 7.8 Hz, 1H). ^{13}C NMR (600 MHz, D_2O): δ = 37.03, 45.09, 51.91, 126.83, 127.51, 128.35, 136.14, 138.22, 139.79, 177.20, 177.61, 181.36.

Purity: HPLC – (>99%).

*5.6.5. N-((2-Methyl)carboxymethyl)-3,4-dicarboxybenzylamine (**P5**)*

To a solution of compound **5Me** (1.36 g, 4.7 mmol) in DMF was added $NaHCO_3$ (2.0 g, 23.7 mmol) and L-alanine methyl ester.HCl (3.3 g, 23.7 mmol). The reaction mixture was stirred at r. t. for 8 h. DMF was air evaporated and the reaction mixture was purified by flash column chromatography using 10–60% ethyl acetate: hexanes to obtain compound **P5Me**, yield: 58% ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.32 (d, J = 6.6 Hz 3H), 3.49 (m, J = 6.6 Hz 1H), 3.71 (s, 4H), 3.84 (s, 2H), 3.87–3.90 (m, 8H), 7.51 (d, J = 7.8 Hz 1H), 7.66 (s, 1H), 7.69 (d, J = 7.8 Hz, 1H). MS (ESI) m/z: (obs) $[M+Na]^+$ 332.4, (calc) 332.3. A solution of compound **P5Me** (0.071 g, 0.229 mmol) was base-hydrolyzed as described above to yield pure compound **P5**, yield: 100% ^{1}H NMR (600 MHz, D_2O): δ = 1.16 (d, J = 6.6 Hz 3H), 3.11 (m, J = 7.2 Hz 1H), 3.52 (d, J = 12.6 Hz 1H), 3.68 (d, J = 12.6 Hz 1H), 7.27 (d, J = 8.4 Hz 1H), 7.31 (s, 1H), 7.40 (d, J = 7.8 Hz, 1H). ^{13}C NMR (600 MHz, D_2O): δ = 18.27, 50.65, 58.27, 126.95, 127.54, 128.54, 138.22, 139.57, 177.22, 177.58, 183.34.

Purity: HPLC – (98.2%).

*5.6.6. N-((2-Hydroxymethyl)carboxymethyl)-3,4-dicarboxybenzylamine (**P6**)*

To a solution of compound **5Me** (1.15 g, 4.0 mmol) in DMF was added NaHCO3 (1.7 g, 20.1 mmol) and L-serine methyl ester.HCl (3.1 g, 20.1 mmol). The reaction mixture was stirred at r. t. for 8 h. DMF was air evaporated and the reaction mixture was purified by flash column chromatography using 10–60% ethyl acetate: hexanes to obtain compound **P6Me**, yield: 52% ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.43 (s, 2H), 3.39 (m, 1H), 3.64 (m, 1H), 3.74 (s, 3H), 3.80 (m, 2H), 3.81–3.89 (m, 6H), 3.95 (d, J = 14.4 Hz 1H), 7.50 (d, J = 7.8 Hz, 1H), 7.66 (s, 1H), 7.70 (d, J = 7.8 Hz, 1H). MS (ESI) m/z: (obs) $[M+Na]^+$ 348.4, (calc) 348.3. A solution of compound **P6Me** (0.071 g, 0.229 mmol) was base-hydrolyzed as described above to yield pure compound **P6**, yield: 100% ^{1}H NMR (600 MHz, D_2O): δ = 3.17 (t, J = 5.2 Hz 1H), 3.61 (d, 13.2 Hz 1H), 3.68–3.70 (m, 2H), 3.78 (d, J = 12.8 Hz 1H), 7.31 (d, J = 8 Hz, 1H), 7.35 (s, 1H), 7.42 (d, J = 7.8 Hz, 1H). ^{13}C NMR (600 MHz, D_2O): δ = 50.71, 62.96, 64.28, 126.91, 127.54, 128.54, 136.26, 138.20, 139.54, 177.21, 177.57, 179.75.

5.7. General protocol for N-derivatized dicarboxybenzylamines (P10-P59)

To a solution of compound **P3Me** or **P4Me** (1 eq.) in acetonitrile was added 1.1 eq of various alkyl halides or various benzyl halides (Tables 3–5) along with 1.1 eq of $NaHCO_3$. The reaction mixture was stirred at r. t. for 3 h. CH_3CN was evaporated in vacuum and purified by flash column chromatography on silica gel using 0–25% ethyl acetate: hexanes to obtain corresponding *N*-derivatized benzylamine esters. Ester hydrolysis was carried out by adding 0.1 mL of H_2O to a solution of *N*-derivatized benzylamine esters (1 eq) in THF (0.4 mL) with stirring. Then, 1 N NaOH (3.3 eq) was added to the reaction mixture and stirred at r. t. for 3 h. The reaction mixture was concentrated and dried for several hours under high vacuum to yield the corresponding sodium salts of the *N*-derivatized benzylamines, compounds **P10**–**P59**. The percent yield for the corresponding esters was typically about 80–90% and the hydrolysis to the corresponding acids was essentially quantitative.

*5.7.1. N-Methyl, N-carboxymethyl-3,4-dicarboxybenzylamine (**P10**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.36 9 s, 3H), 3.28 (s, 2H), 3.69 (s, 3H) 3.73 (s, 2H), 3.88 (s, 6H), 7.52 (d, *J* = 7.8 Hz, 1H), 7.66 (s, 1H), 7.68 (d, *J* = 7.8 Hz, 2H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 332.4, (calc) 332.3.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.04 (s, 3H), 2.85 (s, 2H), 3.46 (s, 2H), 7.15–7.17 (m, *J* = 7.2 Hz, 2H), 7.25 (d, *J* = 7.2 Hz, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 41.85, 58.14, 61.44, 126.86, 127.17, 130.98, 132.84, 134.45, 140.35, 175.89, 177.76, 178.80.

Purity: HPLC – (92.6%).

*5.7.2. N-Propionitrile, N-carboxymethyl-3,4-dicarboxybenzylamine (**P11**)*

Triester: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.42 (s, 2H), 3.68 (s, 2H), 3.72 (s, 3H) 3.84 (s, 2H), 3.89 (s, 6H), 7.55 (d, *J* = 7.6 Hz, 1H), 7.7 (m, *J* = 7.6 Hz, 2H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 357.4, (calc) 357.3.

Sodium salt: ^{1}H NMR (400 MHz, D_2O): δ = 2.97 (s, 2H), 2.99 (s, 2H), 3.57 (s, 2H), 7.17–7.20 (m, 2H), 7.27 (d, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 57.17, 57.63, 127.33, 128.39, 130.16, 136.55, 137.53, 137.93, 177.30, 177.61, 178.92.

Purity: HPLC – (99.2%).

*5.7.3. N-Acetaldehyde, N-carboxymethyl-3,4-dicarboxybenzylamine (**P12**)*

Triester: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.42 (s, 2H), 3.68 (s, 2H), 3.72 (s, 3H) 3.84 (s, 2H), 3.89 (s, 6H), 7.55 (d, *J* = 7.6 Hz, 1H), 7.7 (m, *J* = 7.6 Hz, 2H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 357.4, (calc) 357.3.

Sodium salt: ^{1}H NMR (400 MHz, D_2O): δ = 2.97 (s, 2H), 2.99 (s, 2H), 3.57 (s, 2H), 7.17–7.20 (m, 2H), 7.27 (d, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 51.44, 51.62, 126.92, 127.54, 128.48, 136.25, 138.23, 139.60, 171.03, 177.21, 177.59, 179.47.

Purity: HPLC – (96.8%).

*5.7.4. N-Allyl, N-carboxymethyl-3,4-dicarboxybenzylamine (**P13**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.24 (s, 2H), 3.31 (s, 2H), 3.66 (s, 3H) 3.82 (s, 2H), 3.88–3.89 (s, 6H), 5.13–5.21 (m, 2H), 5.78–5.84 (m, 1H) 7.54 (d, *J* = 7.8 Hz, 1H), 7.68 (m, *J* = 7.8 Hz, 2H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 358.4, (calc) 358.3.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.9 (s, 2H), 3.04 (s, 2H), 3.58 (s, 2H), 5.02–5.07 (m, 2H), 5.69–5.73 (m, 1H), 7.15–7.17 (m, 2H), 7.25 (d, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 55.91, 56.28, 56.69, 119.17, 127.30, 128.47, 130.10, 134.30, 136.39, 137.93, 137.98, 177.23, 177.61, 178.84.

Purity: HPLC – (>99%).

*5.7.5. N-Benzyl, N-carboxymethyl-3,4-dicarboxybenzylamine (**P14**)*

Triester: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 3.30 (s, 2H), 3.68 (s, 3H), 3.79 (s, 2H), 3.87 (s, 2H), 3.89 (s, 3H), 3.91 (s, 3H), 7.23–7.34 (m, 5H), 7.59 (d, *J* = 8 Hz, 1H), 7.72 (m, *J* = 8 Hz, 2H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 408.3, (calc) 408.4.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.88 (s, 2H), 3.62 (s, 4H), 7.20 (m, 7H), 7.29 (m, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 55.76, 56.74, 56.91, 127.35, 127.47, 128.38, 128.41, 129.97, 130.10, 136.40, 137.42, 138.05, 138.15, 177.22, 177.61, 178.79.

Purity: HPLC – (>99%).

*5.7.6. N-(2-Methylbenzyl)-N-carboxymethyl-3,4-dicarboxybenzylamine (**P15**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.31 (s, 3H), 3.24 (s, 2H), 3.65 (s, 3H), 3.80 (s, 2H), 3.87 (s, 2H), 3.88–3.89 (s, 6H), 7.13 (m, 3H), 7.28 (d, *J* = 6.6 Hz, 1H), 7.52 (d, *J* = 7.8 Hz, 1H) 7.64 (s, 1H), 7.68 (d, *J* = 7.8 Hz, 1H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 422.2, (calc) 422.4.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.13 (s, 3H), 3.04 (s, 2H), 3.73 (s, 2H), 3.82 (s, 2H), 7.17 (m, 3H), 7.31–7.33 (m, 3H), 7.41 (d, *J* = 7.8 Hz, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 18.37, 53.86, 55.85, 57.38, 125.72, 127.30, 127.43, 128.44, 130.30, 130.31, 130.51, 136.38, 136.41, 138.78, 177.31, 177.62, 179.28.

Purity: HPLC – (99.0%).

*5.7.7. N-(3-Methylbenzyl)-N-carboxymethyl-3,4-dicarboxybenzylamine (**P16**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.31 (s, 3H), 3.28 (s, 2H), 3.66 (s, 3H), 3.73 (s, 2H), 3.84 (s, 2H), 3.87–3.88 (s, 6H), 7.03 (d, *J* = 7.8 Hz, 1H), 7.12 (d, *J* = 7.8 Hz, 3H), 7.18 (m, *J* = 7.8 Hz, 1H) 7.57 (d, *J* = 7.8 Hz, 1H), 7.68 (d, *J* = 7.8 Hz, 2H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 422.2, (calc) 422.4.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.28 (s, 3H), 3.01 (s, 2H), 3.70 (s, 2H), 3.74 (s, 2H), 7.12–7.16 (m, *J* = 6 Hz, 2H), 7.16 (s, 1H), 7.24–7.26 (t, *J* = 7.2 Hz, 1H), 7.29–7.31 (m, 2H), 7.41–7.42 (d, *J* = 7.8 Hz, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 20.36, 55.86, 56.65, 56.91, 127.06, 127.33, 127.99, 128.36, 128.37, 129.93, 130.70, 136.32, 137.61, 138.04, 138.39, 138.47, 177.25, 177.65, 178.97.

Purity: HPLC – (96.6%).

*5.7.8. N-(4-Methylbenzyl)-N-carboxymethyl-3,4-dicarboxybenzylamine (**P17**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.31 (s, 3H), 3.27 (s, 2H), 3.66 (s, 3H), 3.73 (s, 2H), 3.87–3.89 (s, 6H), 7.11 (d, *J* = 7.8 Hz, 2H), 7.22 (d, *J* = 7.8 Hz, 2H), 7.58 (d, *J* = 7.8 Hz, 1H), 7.69–7.70 (m, *J* = 7.8 Hz, 2H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 422.2, (calc) 422.4.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.27 (s, 3H), 2.96 (s, 2H), 3.70 (s, 2H), 3.72 (s, 2H), 7.18–7.22 (m, *J* = 7.8 Hz, 4H), 7.28–7.30 (m, 2H), 7.40 (d, *J* = 7.8 Hz, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 20.09, 55.73, 56.54, 127.33, 128.32, 128.93, 129.93, 130.14, 134.39, 136.30, 137.54, 138.04, 138.37, 177.23, 177.64, 178.97.

Purity: HPLC – (96.9%).

*5.7.9. N-(2-Bromobenzyl)-N-carboxymethyl-3,4-dicarboxybenzylamine (**P18**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.31 (s, 2H), 3.67 (s, 3H), 3.86–3.88 (s, 6H), 3.92 (s, 4H), 7.08 (t, Hz, 1H), 7.25 (t, *J* = 7.6 Hz, 1H), 7.49 (t, *J* = 8.0 Hz, 2H), 7.54 (d, *J* = 8.0 Hz, 1H), 7.68 (d, *J* = 7.6 Hz, 2H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ (isotopic) 486.2, 488.0 (calc) (isotopic) 486.1, 488.1.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 3.07 (s, 2H), 3.89 (s, 2H), 3.90 (s, 2H), 7.13–7.17 (t, 1H), 7.31–7.36 (m, *J* = 7.2 Hz, 3H), 7.39 (d, *J* = 8 Hz, 2H), 7.56 (d, *J* = 8 Hz, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 55.47, 56.29, 57.51, 124.74, 127.28, 127.49, 128.49, 129.09, 130.06, 131.96, 132.80, 136.43, 137.30, 137.96, 138.63, 177.32, 177.60, 179.10.

Purity: HPLC – (99.3%).

*5.7.10. N-(3-Bromobenzyl)-N-carboxymethyl-3,4-dicarboxybenzylamine (**P19**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.26 (s, 2H), 3.65 (s, 3H), 3.73 (s, 2H), 3.83 (s, 2H), 3.86–3.88 (s, 6H), 7.14 (d, J = 7.8 Hz, 1H), 7.24 (d, J = 7.8 Hz, 1H), 7.33 (d, J = 7.8 Hz, 1H), 7.48 (s, 1H), 7.54 (d, J = 7.8 Hz, 1H), 7.68 (d, J = 7.8 Hz, 2H). MS (ESI) m/z: (obs) $[M+Na]^+$ (isotopic) 486.2, 488.0 (calc) (isotopic) 486.1, 488.1.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 3.01 (s, 2H), 3.70 (s, 2H), 3.74 (s, 2H), 7.22 (t, J = 7.2 Hz, 1H), 7.27–7.31 (m, 3H), 7.41 (d, J = 7.8 Hz, 1H), 7.43–7.45 (d, J = 7.8 Hz, 1H), 7.51 (d, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 56.83, 121.71, 127.36, 128.32, 128.70, 129.88, 130.07, 130.25, 132.58, 136.39, 138.04, 138.25, 140.20, 177.24, 177.60, 178.86.

Purity: HPLC – (98.3%).

*5.7.11. N-(4-Bromobenzyl)-N-carboxymethyl-3,4-dicarboxybenzylamine (**P20**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.25 (s, 2H), 3.65 (s, 3H), 3.71 (s, 2H), 3.82 (s, 2H), 3.87–3.89 (s, 6H), 7.20 (d, J = 7.8 Hz, 2H), 7.42 (d, J = 7.8 Hz, 2H), 7.54 (d, J = 7.8 Hz, 1H), 7.66–7.69 (m, J = 7.8 Hz, 2H). MS (ESI) m/z: (obs) $[M+Na]^+$ (isotopic) 486.2, 488.0 (calc) (isotopic) 486.1, 488.1.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.78 (s, 2H), 3.45 (s, 2H), 3.46 (s, 2H), 6.97 (d, J = 8.4 Hz, 2H), 7.05–7.07 (d, J = 7.8 Hz, 1H), 7.12 (s, 1H), 7.21–7.24 (m, J = 7.8 Hz, 3H).

^{13}C NMR (600 MHz, D_2O): δ = 55.92, 56.28, 56.77, 120.60, 127.35, 128.31, 129.90, 131.23, 131.78, 136.36, 136.77, 138.05, 138.24, 177.22, 177.61, 178.87.

Purity: HPLC – (>99%).

*5.7.12. N-(2-Trifluoromethylbenzyl)-N-carboxymethyl-3,4-dicarboxybenzylamine (**P21**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.28 (s, 2H), 3.66 (s, 3H), 3.86 (s, 2H), 3.86–3.88 (s, 6H), 3.95 (s, 2H), 7.31 (t, J = 7.8 Hz, 1H), 7.51 (t, J = 7.8 Hz, 1H), 7.57 (t, J = 7.8 Hz, 2H) 7.68 (m, J = 7.8 Hz, 2H), 7.85 (d, J = 7.8 Hz, 1H). MS (ESI) m/z: (obs) $[M+Na]^+$ 476.1, (calc) 476.4.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 3.05 (s, 2H), 3.79 (s, 2H), 3.91 (s, 2H), 7.33 (m, 2H), 7.36–7.40 (m, 2H), 7.56 (t, J = 7.8 Hz, 1H), 7.65 (d, J = 7.8 Hz, 1H), 7.78 (d, J = 7.8 Hz, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 53.23, 56.29, 57.43, 125.69, 125.73, 127.17, 127.31, 128.12, 129.67, 131.02, 132.12, 136.33, 137.61, 137.98, 138.92, 177.31, 177.61, 179.32.

Purity: HPLC – (>99%).

*5.7.13. N-(3-Trifluoromethylbenzyl)-N-carboxymethyl-3,4-dicarboxybenzylamine (**P22**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.29 (s, 2H), 3.66 (s, 3H), 3.83 (s, 2H), 3.85 (s, 2H), 3.86–3.89 (s, 6H), 7.40 (t, J = 7.8 Hz, 1H), 7.49 (d, J = 7.8 Hz, 1H), 7.53 (t, J = 7.8 Hz, 2H) 7.57 (s, J = 7.8 Hz, 1H), 7.68 (d, J = 7.8 Hz, 2H). MS (ESI) m/z: (obs) $[M+Na]^+$ 476.1, (calc) 476.4.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 3.03 (s, 2H), 3.75 (s, 2H), 3.80 (s, 2H), 7.29–7.32 (m, J = 6 Hz, 2H), 7.16 (s, 1H), 7.41 (d, J = 7.8 Hz, 1H), 7.48–7.51 (t, J = 7.8 Hz, 1H), 7.55 (d, J = 7.8 Hz, 1H), 7.59 (d, J = 7.8 Hz, 1H), 7.65 (s, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 56.09, 56.68, 56.88, 124.10, 124.13, 126.39, 126.41, 127.36, 128.33, 128.93, 129.90, 133.53, 138.05, 138.19, 138.72, 177.25, 177.59, 178.83.

Purity: HPLC – (98.6%).

*5.7.14. N-(4-Trifluoromethylbenzyl)-N-carboxymethyl-3,4-dicarboxybenzylamine (**P23**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.28 (s, 2H), 3.66 (s, 3H), 3.83 (s, 2H), 3.87 (s, 3H), 3.89 (s, 3H), 7.45 (d, J = 7.8 Hz, 2H), 7.54–7.56 (m, J = 7.8 Hz, 3H), 7.68–7.70 (m, J = 7.8 Hz, 2H). MS (ESI) m/z: (obs) $[M+Na]^+$ 476.1, (calc) 476.4.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.89 (s, 2H), 3.61 (s, 2H), 3.66 (s, 2H), 7.16 (m, J = 8.4 Hz, 2H), 7.27 (d, J = 7.2 Hz, 1H), 7.33 (d, J = 6 Hz, 2H), 7.50 (d, J = 6.6 Hz, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 56.10, 56.53, 56.89, 125.13, 125.16, 127.36, 128.29, 128.58, 128.79, 129.88, 130.18, 136.40, 138.06, 138.19, 142.09, 177.21, 177.60, 178.86.

Purity: HPLC – (85.3%).

*5.7.15. N-(4-t-Butylbenzyl)-N-carboxymethyl-3,4-dicarboxybenzylamine (**P28**)*

Triester: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.30 (s, 9H), 3.31 (s, 2H), 3.67 (s, 3H), 3.77 (s, 2H), 3.87 (s, 2H), 3.88–3.91 (s, 6H), 7.27 (d, J = 8 Hz, 2H), 7.34 (d, J = 8 Hz, 2H), 7.59 (d, J = 8 Hz, 1H), 7.61 (d, J = 7.6 Hz, 2H). MS (ESI) m/z: (obs) $[M+Na]^+$ 464.3, (calc) 464.5.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 1.26 (s, 9H), 3.01 (s, 2H), 3.73 (s, 4H), 3.66 (s, 2H), 7.16 (m, J = 8.4 Hz, 2H), 7.28–7.31 (m, 3H), 7.34 (s, 1H), 7.41–7.43 (d, J = 7.8 Hz, 1H), 7.44–7.46 (d, J = 7.8 Hz, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 30.49, 33.84, 55.79, 56.41, 56.48, 125.33, 127.35, 128.31, 129.93, 130.02, 134.57, 136.30, 138.06, 138.34, 150.92, 177.23, 177.67, 178.97.

Purity: HPLC – (85.1%).

*5.7.16. N-(4-(2-Perfluoropropyl))-N-carboxymethyl-3,4-dicarboxybenzylamine (**P29**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.30 (s, 2H), 3.66 (s, 3H), 3.84 (s, 2H), 3.87–3.88 (m, 8H), 7.47 (m, J = 7.8 Hz, 2H), 7.52 (m, J = 8.4 Hz, 2H), 7.56 (d, J = 7.8 Hz, 1H), 7.69 (m, 2H). MS (ESI) m/z: (obs) $[M+Na]^+$ 575.9, (calc) 576.4.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 3.07 (s, 2H), 3.77 (s, 2H), 3.83 (s, 2H), 7.33–7.34 (m, 1H), 7.39 (s, 1H), 7.45 (d, J = 7.8 Hz, 1H), 7.54 (d, J = 7.8 Hz, 1H), 7.68 (d, J = 7.8 Hz, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 56.13, 56.39, 56.81, 119.36, 119.52, 121.26, 121.45, 124.91, 125.05, 125.61, 125.68, 127.37, 128.27, 129.87, 130.30, 136.39, 138.08, 138.22, 141.33, 177.22, 177.64, 178.88.

Purity: HPLC – (95.4%).

*5.7.17. N-(4-Biphenyl)-N-carboxymethyl-3,4-dicarboxybenzylamine (**P30**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.33 (s, 2H), 3.68 (s, 3H), 3.83 (s, 2H), 3.88–3.91 (m, 8H), 7.31–7.33 (m, J = 7.2 Hz 1H), 7.40–7.43 (m, 4H), 7.54, (d, J = 7.2 Hz 2H), 7.58 (d, J = 7.2 Hz, 2H), 7.61 (d, J = 7.8 Hz, 1H), 7.71–7.73 (m, 2H). MS (ESI) m/z: (obs) $[M+Na]^+$ 484.2, (calc) 484.5.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 3.03 (s, 2H), 3.74 (d, J = 14.4 Hz 4H), 7.31–7.33 (m, J = 7.2 Hz 1H), 7.29 (d, 1H), 7.33–7.37, (m, J = 7.2 Hz, 2H), 7.39–7.42 (t, J = 8.4 Hz, 3H), 7.44–7.46 (t, J = 7.8 Hz, 2H), 7.60 (d, J = 7.8 Hz, 2H), 7.73 (d, J = 7.8 Hz, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 55.91, 56.58, 56.64, 126.77, 129.84, 127.36, 127.62, 128.34, 129.08, 129.93, 130.66, 136.34, 136.93, 138.07, 138.34, 139.50, 140.22, 177.23, 177.64, 178.95.

Purity: HPLC – (>99%).

*5.7.18. N-(Ethylmorpholino)-N-carboxymethyl-3,4-dicarboxybenzylamine (**P31**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.38 (s, 3H), 2.44 (t, J = 6.6 Hz, 2H), 2.77 (t, J = 6.6 Hz, 2H), 3.39 (s, 2H), 3.64 (s, 6H), 3.85–3.86 (m, 8H), 7.49 (d, J = 7.8 Hz, 2H), 7.64–7.66 (m, 3H). MS (ESI) m/z: (obs) $[M+H]^+$ 408.3, (calc) 408.4.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.40 (s, 4H), 2.46 (t, J = 8.4 Hz, 2H), 2.67 (t, J = 7.2 Hz, 2H), 3.09 (s, 2H), 3.64 (s, 4H), 3.69 (s, 2H), 7.32 (d, J = 6.6 Hz, 2H), 7.40 (d, J = 8.4 Hz, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 49.07, 25.60, 54.86, 57.41, 57.69, 65.98, 127.30, 128.29, 129.95, 136.50, 138.00, 138.21, 177.20, 177.48, 178.91.

Purity: HPLC – (90.6%).

5.7.19. N-(2-Naphthyl)-N-carboxymethyl-3,4-dicarboxybenzylamine (**P32**)

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.32 (s, 2H), 3.67 (s, 3H), 3.87 (s, 2H), 3.88–3.90 (m, 8H), 3.91 (s, 3H), 3.94 (s, 2H), 7.43–7.45 (m, 2H), 7.51 (d, J = 8.4 Hz, 2H), 7.61 (d, J = 7.2 Hz, 1H), 7.70–7.74 (m, 3H), 7.79 (d, J = 8.4 Hz, 3H). MS (ESI) m/z: (obs) $[M+Na]^+$ 458.2, (calc) 458.5.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 3.06 (s, 2H), 3.78 (s, 2H), 3.89 (s, 2H), 7.30 (d, J = 7.8 Hz, 1H), 7.34 (s, 1H), 7.41 (d, J = 7.8 Hz, 1H), 7.47–7.51 (m, 3H), 7.80 (s, 1H), 7.86–7.89 (m, J = 7.8 Hz, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 56.01, 56.73, 57.04, 126.09, 126.33, 127.36, 127.54, 127.72, 127.81, 128.18, 128.38, 128.59, 129.94, 132.34, 132.87, 135.47, 136.35, 138.07, 138.35, 177.23, 177.63, 178.99.

Purity: HPLC – (93.7%).

5.7.20. N-(1-Naphthyl)-N-carboxymethyl-3,4-dicarboxybenzylamine (**P33**)

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.28 (s, 2H), 3.67 (s, 3H), 3.87 (s, 2H), 3.89 (s, 3H), 3.91 (s, 3H), 4.26 (s, 2H), 7.38 (s, J = 7.8 Hz, 1H), 7.44–7.53 (m, 4H), 7.65 (m, J = 7.8 Hz, 2H), 7.75 (d, J = 8.4 Hz, 1H), 7.81 (d, J = 8 Hz, 1H). MS (ESI) m/z: (obs) $[M+Na]^+$ 458.2, (calc) 458.5.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 3.05 (s, 2H), 3.88 (s, 2H), 4.18 (s, 2H), 7.32 (d, 1H), 7.38 (s, 1H), 7.41–7.49 (m, 5H), 7.83 (d, J = 7.8 Hz, 1H), 7.88–7.90 (m, J = 7.8 Hz, 1H), 7.93 (d, J = 8.4 Hz, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 53.94, 56.19, 57.73, 124.36, 125.46, 125.96, 126.30, 127.35, 128.04, 128.41, 128.54, 128.73, 130.08, 133.49, 134.02, 136.50, 138.05, 138.92, 177.28, 177.54, 179.34.

Purity: HPLC – (93.3%).

5.7.21. N-(4-Carboxybenzyl)-N-carboxymethyl-3,4-dicarboxybenzylamine (**P34**)

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 3.26 (s, 2H), 3.64 (s, 3H), 3.81 (s, 2H), 3.83 (s, 2H), 3.85 (s, 3H), 3.86 (s, 3H), 3.87 (s, 3H), 7.39 (d, J = 8.4 Hz, 2H), 7.54 (d, J = 8.4 Hz, 1H), 7.66–7.68 (m, J = 8.4 Hz, 2H), 7.94 (d, J = 8.4 Hz, 2H). MS (ESI) m/z: (obs) $[M+Na]^+$ 466.2, (calc) 466.4.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.83 (s, 2H), 3.54 (s, 2H), 3.58 (s, 2H), 7.11 (d, 2H), 7.17–7.19 (m, 3H), 7.25 (d, 1H), 7.61 (d, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 55.82, 56.64, 56.78, 127.35, 128.36, 128.81, 129.74, 129.94, 135.11, 136.37, 138.04, 138.19, 140.87, 175.54, 177.23, 177.63, 178.89.

Purity: HPLC – (96.5%).

5.7.22. N-(2-Bromobenzyl)-N-carboxyethyl-3,4-dicarboxybenzylamine (**P37**)

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.49 (t, 2H), 2.80 (t, 2H), 3.58 (s, 3H), 3.63 (s, 2H), 3.65 (s, 2 H), 3.85 (s, 3H), 3.87 (s, 3H), 7.05 (t, J = 12 Hz, 1H), 7.24 (t, J = 7.2 Hz, 1H), 7.44 (m, J = 7.8 Hz, 3H), 7.61 (s, 1H), 7.65 (d, J = 7.8 Hz, 1H). MS (ESI) m/z: (obs) $[M+Na]^+$ (isotopic) 500.4, 502.4 (calc) (isotopic) 500.1, 502.1.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.50 (t, 2H), 2.81 (t, 2H), 3.69 (s, 2H), 3.71 (s, 2 H), 7.16 (t, J = 7.8 Hz, 1H), 7.33 (m, J = 8.4 Hz, 2H), 7.40 (m, J = 7.8 Hz, 3H), 7.58 (d, J = 7.8 Hz, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 33.09, 49.73, 55.88, 57.04, 124.77, 127.29, 127.48, 128.64, 129.16, 130.12, 132.06, 132.85, 137.95, 138.15, 177.27, 177.49, 181.63.

Purity: HPLC – (95.0%).

5.7.23. N-(3-Bromobenzyl)-N-carboxyethyl-3,4-dicarboxybenzylamine (**P38**)

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.46 (t, 2H), 2.76 (t, 2H), 3.48 (s, 2H), 3.57 (s, 2H), 3.61 (s, 3H), 3.86 (s, 3H), 3.88 (s, 3H), 7.15 (m, J = 12 Hz, 2H), 7.34 (d, J = 7.8 Hz, 2H), 7.42 (s, 1H), 7.48 (d, J = 7.8 Hz, 2H), 7.61 (s, 1H), 7.66 (d, J = 7.8 Hz, 2H) MS (ESI) m/z: (obs) $[M+Na]^+$ (isotopic) 500.4, 502.4 (calc) (isotopic) 500.1, 502.1.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.43 (t, 2H), 2.73 (t, 2H), 3.54 (s, 2H), 3.58 (s, 2 H), 7.23–7.288 (m, J = 7.8 Hz, 3H), 7.32 (s, J = 8.4 Hz, 1H), 7.41–7.47 (m, J = 7.2 Hz, 3 H).

^{13}C NMR (600 MHz, D_2O): δ = 33.60, 49.65, 56.28, 56.63, 121.72, 127.40, 128.44, 128.75, 129.94, 130.11, 130.33, 132.63, 136.48, 137.97, 138.07, 139.86, 177.21, 177.52, 181.42.

Purity: HPLC – (97.3%).

5.7.24. N-(4-Bromobenzyl)-N-carboxyethyl-3,4-dicarboxybenzylamine (**P39**)

Triester: ^{1}H NMR (600 MHz, $CDCl_3$):δ = 2.46 (t, 2H), 2.74 (t, 2H), 3.47 (s, 3H), 3.56 (s, 2H), 3.59 (s, 3H), 3.86 (s, 3H), 3.88 (s, 3H), 7.13 (d, J = 7.8 Hz, 2H), 7.40 (d, J = 7.8 Hz, 2H), 7.46 (d, J = 7.8 Hz, 1H), 7.59 (s, 1H), 7.66 (d, J = 7.8 Hz, 1H). MS (ESI) m/z: (obs) $[M+Na]^+$ (isotopic) 500.1, 501.9 (calc) (isotopic) 500.1, 502.1.

Sodium salt: ^{1}H NMR (600 MHz, $CDCl_3$):δ = 2.43 (t, 2H), 2.73 (t, 2H), 3.54 (s, 2H), 3.59 (s, 2H), 7.19 (d, J = 7.8 Hz, 2H), 7.27–7.31 (m, 2H), 7.41 (d, J = 7.2 Hz, 1H), 7.48 (d, J = 7.8 Hz 2H).

^{13}C NMR (600 MHz, D_2O): δ = 33.55, 49.63, 56.00, 56.60, 120.67, 127.38, 129.92, 131.26, 131.80, 136.45, 136.53, 137.96, 138.07, 177.16, 177.51, 181.45.

Purity: HPLC – (95.8%).

5.7.25. N-(2-Trifluoromethylbenzyl)-N-carboxymethyl-3,4-dicarboxybenzylamine (**P40**)

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.48 (t, 2H), 2.77 (t, 2H), 3.58 (s, 2H), 3.59 (s, 3H), 3.71 (s, 2H), 3.85 (s, 3H), 3.85 (s, 3H), 7.30 (d, J = 7.8 Hz, 1H), 7.50 (m, J = 7.2 Hz, 2H), 7.57 (d, J = 7.8 Hz, 1H), 7.62 (s, 1H), 7.66 (d, J = 8.4 Hz, 1H), 7.77 (d, J = 7.2 Hz, 1H). MS (ESI) m/z: (obs) $[M+Na]^+$ 490.2, (calc) 490.2.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.44 (t, 2H), 2.77 (t, 2H), 3.63 (s, 2H), 3.78 (s, 2H), 7.34 (m, 2H), 7.39 (m, J = 8.4 Hz, 2H), 7.58 (t, J = 7.2 Hz, 1H), 7.66 (d, J = 7.8 Hz 1H), 7.73 (d, J = 7.8 Hz 1H).

^{13}C NMR (600 MHz, D_2O): δ = 33.90, 50.27, 53.10, 57.04, 125.74, 125.78, 127.17, 127.31, 128.11, 129.59, 130.94, 132.10, 136.36, 137.45, 137.98, 198.77, 177.27, 177.53, 181.64.

Purity: HPLC – (98.8%).

5.7.26. N-(3-Trifluoromethylbenzyl)-N-carboxymethyl-3,4-dicarboxybenzylamine (**P41**)

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.47 (t, 2H), 2.77 (t, 2H), 3.55 (s, 2H), 3.58 (s, 2H), 3.60 (s, 3H), 3.85 (s, 3H), 3.87 (s, 3H), 7.38 (t, J = 7.8 Hz, 1H), 7.45 (m, J = 7.2 Hz, 3H), 7.54 (s, 1H), 7.61 (s, 1H), 7.66 (d, J = 8.4 Hz, 1H). MS (ESI) m/z: (obs) $[M+Na]^+$ 490.2, (calc) 490.2.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.29 (t, 2H), 2.60 (t, 2H), 3.46 (s, 2H), 3.49 (s, 2H), 7.14 (d, J = 7.8 Hz, 2H), 7.17 (s, 1H), 7.27 (d, J = 7.8 Hz, 1H), 7.35 (d, J = 7.8 Hz 3H), 7.46 (d, J = 7.8 Hz 1H).

^{13}C NMR (600 MHz, D_2O): δ = 33.55, 49.72, 56.37, 56.72, 124.18, 124.20, 126.44, 126.46, 127.40, 128.46, 128.97, 129.93, 133.59, 136.51, 137.88, 138.08, 138.42, 177.19, 177.48, 181.40.

Purity: HPLC – (>99%).

*5.7.27. N-(4-Trifluoromethylbenzyl)-N-carboxymethyl-3,4-dicarboxybenzylamine (**P42**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$):δ = 2.48 (t, 2H), 2.77 (t, 2H), 3.58 (s, 2H), 3.59 (s, 2H), 3.60 (s, 3H), 3.86 (s, 3H), 3.88 (s, 3H), 7.40 (d, *J* = 7.8 Hz, 2H), 7.49 (d, *J* = 7.8 Hz, 2H), 7.52 (d, *J* = 7.8 Hz, 1H), 7.61 (s, 1H), 7.66 (d, *J* = 7.8 Hz, 1H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 490.2, (calc) 490.4.

Sodium salt: ^{1}H NMR (600 MHz, $CDCl_3$):δ = 2.44 (t, 2H), 2.75 (t, 2H), 3.61 (s, 2H), 3.64 (s, 2H), 7.33 (d, *J* = 7.8 Hz, 2H), 7.41–7.45 (m, 3H), 7.64 (d, *J* = 7.8 Hz 2H).

^{13}C NMR (600 MHz, D_2O): δ = 36.63, 49.78, 56.27, 56.71, 123.36, 125.19, 127.39, 128.39, 128.63, 128.84, 129.92, 130.23, 136.48, 137.94, 138.08, 177.16, 177.52, 181.42.

Purity: HPLC – (99.5%).

*5.7.28. N-(2-Methylbenzyl)-N-carboxyethyl-3,4-dicarboxybenzylamine (**P43**)*

Triester:^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.26 (s, 3H), 2.46 (s, 2H), 2.76 (s, 3H), 3.53 (s, 2H), 3.56 (s, 2H), 3.58 (s, 3H), 3.86 (s, 3H), 3.88 (s, 3H), 7.10 (m, *J* = 7.2 Hz, 3H), 7.23 (m, *J* = 12 Hz, 1H), 7.45 (d, *J* = 7.8 Hz, 1H) 7.56 (s, 1H), 7.64 (m, *J* = 7.8 Hz, 1H) MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 436.2, (calc) 436.2.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.09 (s, 3H), 2.47 (s, 2H), 2.78 (s, 2H), 3.56 (s, 2H), 3.63 (s, 2H), 7.16–7.19 (m, 3H), 7.16 (s, 1H), 7.30–7.33 (t, *J* = 7.8 Hz, 2H), 7.34 (s, 1H), 7.41 (d, *J* = 7.8 Hz, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 18.44, 33.19, 49.81, 53.55, 56.97, 125.71, 127.32, 127.47, 128.59, 130.08, 130.36, 130.56, 136.13, 136.49, 137.95, 137.97, 138.40, 177.26, 177.51, 181.75.

Purity: HPLC – (96.0%).

*5.7.29. N-(3-Methylbenzyl)-N-carboxyethyl-3,4-dicarboxybenzylamine (**P44**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.31 (s, 3H), 2.47 (s, 2H), 2.76 (s, 3H), 3.50 (s, 2H), 3.57 (s, 2H), 3.60 (s, 3H), 3.86 (s, 3H), 3.88 (s, 3H), 7.03 (d, *J* = 12 Hz, 1H), 7.07 (d, *J* = 7.8 Hz, 2H), 7.16 (m, *J* = 7.8 Hz, 1H) 7.49 (d, *J* = 7.8 Hz, 1H), 7.62 (d, *J* = 7.8 Hz, 1H), 7.62 (d, *J* = 8.4 Hz, 1H) MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 436.2, (calc) 436.2.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.28 (s, 3H), 2.44 (s, 2H), 2.74 (s, 2H), 3.55 (s, 2H), 3.59 (s, 2H), 7.11–7.15 (m, *J* = 7.2 Hz, 3H), 7.24–7.318 (m, *J* = 7.8 Hz, 3H), 7.42 (d, *J* = 7.8 Hz, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 20.31, 33.49, 49.59, 56.58, 56.62, 127.08, 127.35, 128.03, 128.38, 129.95, 130.73, 136.42, 137.36, 138.02, 138.06, 138.50, 177.19, 177.53, 181.53.

Purity: HPLC – (97.3%).

*5.7.30. N-(4-Methylbenzyl)-N-carboxyethyl-3,4-dicarboxybenzylamine (**P45**)*

Triester:^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.29 (s, 3H), 2.46 (s, 2H), 2.75 (s, 3H), 3.50 (s, 2H), 3.56 (s, 2H), 3.59 (s, 3H), 3.86 (s, 3H), 3.88 (s, 3H), 7.09 (t, *J* = 7.2 Hz, 2H), 7.15 (t, *J* = 7.8 Hz, 2H), 7.49 (d, *J* = 7.8 Hz, 1H) 7.60 (s, *J* = 7.8 Hz, 1H), 7.67 (d, *J* = 7.8 Hz, 1H), MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 436.2, (calc) 436.2.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.12 (s, 3H), 2.28 (s, 2H), 2.57 (s, 2H), 3.39 (s, 2H), 3.43 (s, 2H), 7.03–7.06 (m, *J* = 8.4 Hz, 4H), 7.12–7.13 (m, *J* = 8.4 Hz, 1H), 7.15 (s, 1H), 7.25 (d, *J* = 7.8 Hz, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 20.08, 33.51, 49.47, 56.30, 56.45, 127.36, 128.43, 128.96, 129.95, 130.15, 134.14, 136.39, 137.61, 138.04, 138.07, 177.18, 177.54, 181.54.

Purity: HPLC – (96.8%).

*5.7.31. N-(4-t-Butylbenzyl)-N-carboxyethyl-3,4-dicarboxybenzylamine (**P46**)*

Triester: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 1.27 (s, 9H), 2.48 (t, 2H), 2.77 (t, 2H), 3.52 (s, 2H), 3.58 (s, 2H), 3.59 (s, 3H), 3.86 (s, 3H), 3.86 (s, 3H), 7.20 (d, *J* = 8.4 Hz, 2H), 7.30 (d, *J* = 7.8 Hz, 2H), 7.50 (d, *J* = 7.8 Hz, 1H), 7.62 (s, 1H), 7.67 (d, *J* = 7.8 Hz, 1H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 478.3, (calc) 478.2.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 1.26 (s, 9H), 2.45 (t, 2H), 2.73 (t, 2H), 3.57 (s, 2H), 3.59 (s, 2H), 7.27–7.301 (m, *J* = 7.8 Hz, 3H), 7.34 (d, *J* = 7.8 Hz, 1H), 7.41–7.42 (m, *J* = 7.8 Hz, 1H), 7.44–7.46 (m, *J* = 7.8 Hz, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 30.47, 33.59, 33.83, 49.40, 56.18, 56.39, 125.36, 127.37, 128.40, 129.96, 130.02, 134.28, 136.37, 138.04, 138.08, 150.99, 177.18, 177.57, 181.52.

Purity: HPLC – (84.0%).

*5.7.32. N-(4-(2-Perfluoropropyl))-N-carboxyethyl-3,4-dicarboxybenzylamine (**P47**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.48 (t, 2H), 2.78 (t, 3H), 3.57 (s, 2H), 3.58 (s, 3H), 3.60 (s, 2H), 3.85 (s, 3H), 3.87 (s, 3H), 7.40 (d, *J* = 7.8 Hz, 2H), 7.49 (m, *J* = 8.4 Hz, 3H), 7.61 (s, 1H), 7.67 (m, *J* = 8.4 Hz, 1H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 590.2, (calc) 590.1.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.46 (t, 2H), 2.76 (t, 2H), 3.63 (s, 2H), 3.65 (s, 2H), 7.29 (d, *J* = 7.8 Hz, 1H), 7.36 (s, 1H), 7.41 (d, *J* = 7.8 Hz, 1H), 7.48 (d, *J* = 8.4 Hz, 1H), 7.66 (d, *J* = 8.4 Hz, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 33.64, 49.79, 55.98, 56.71, 124.99, 125.12, 125.64, 125.71, 127.41, 128.39, 129.89, 136.51, 137.86, 138.12, 141.01, 177.13, 177.50, 181.41.

Purity: HPLC – (95.7%).

*5.7.33. N-(4-Biphenyl)-N-carboxyethyl-3,4-dicarboxybenzylamine (**P48**)*

Triester: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.53 (s, 2H), 2.83 (s, 2H), 3.60 (s, 2H), 3.61 (s, 2H), 3.64 (s, 3H), 3.89 (s, 3H), 3.91 (s, 3H), 7.36 (m, *J* = 7.2 Hz, 3H), 7.43 (t, *J* = 7.2 Hz, 2H), 7.57, (m, *J* = 7.6 Hz 5H), 7.66 (s, 1H), 7.72 (d, *J* = 8 Hz, 1H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 498.2, (calc) 498.2.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.46 (t, 2H), 2.76 (t, 2H), 3.61 (s, 1H), 3.62 (s, 1H), 7.29 (d, *J* = 7.8 Hz, 1H), 7.34–7.39 (m, *J* = 7.8 Hz, 4H), 7.43, (d, *J* = 7.8 Hz, 1H), 7.47 (t, *J* = 7.8 Hz, 2H), 7.62 (d, *J* = 7.8 Hz, 2H), 7.66 (d, *J* = 7.2 Hz, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 33.59, 49.62, 56.30, 56.56, 126.80, 126.86, 127.39, 127.63, 128.45, 129.08, 129.96, 130.69, 136.44, 136.67, 138.01, 138.11, 136.58, 140.20, 177.18, 177.54, 181.50. Purity: HPLC – (95.2%).

*5.7.34. N-(1-Naphthyl)-N-carboxyethyl-3,4-dicarboxybenzylamine (**P49**)*

Triester: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.52 (s, 2H), 2.86 (s, 2H), 3.49 (s, 3H), 3.63 (s, 2H), 3.87 (s, 3H), 3.89 (s, 3H), 4.10 (s, 2H), 7.46 (m, *J* = 7.8 Hz, 5H), 7.59 (m, *J* = 7.8 Hz, 2H), 7.74 (m, *J* = 7.6 Hz, 4H), 8.14 (d, *J* = 8.8 Hz, 2H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 472.3, (calc) 472.2.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.51 (t, 2H), 2.82 (t, 2H), 3.70 (s, 2H), 3.99 (s, 2H), 7.34 (d, *J* = 6.6 Hz, 1H), 7.41–7.53 (m, 6H), 7.75 (d, *J* = 7.8 Hz, 1H), 7.84 (d, *J* = 7.2 Hz, 1H), 7.90 (d, *J* = 7.2 Hz, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 32.87, 50.03, 53.48, 57.33, 124.15, 125.42, 125.93, 126.27, 127.40, 128.09, 128.43, 128.70, 128.78, 130.17, 131.87, 133.48, 133.66, 136.61, 138.07, 138.50, 177.24, 177.47, 181.80.

Purity: HPLC – (97.3%).

*5.7.35. N-(4-Carboxybenzyl)-N-carboxymethyl-3,4-dicarboxybenzylamine (**P50**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.46 (s, 2H), 2.75 (s, 2H), 3.56–3.58 (m, 7H), 3.85–3.87 (m, 9H), 7.34 (d, *J* = 8.4 Hz, 2H), 7.48 (d, *J* = 7.8 Hz, 1H), 7.59 (s, 1H), 7.67 (d, *J* = 7.8 Hz, 1H), 7.95 (d, *J* = 8.4 Hz, 2H) MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 480.2, (calc) 480.2.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.46 (t, 2H), 2.74 (t, 2H), 3.62 (s, 2H), 3.58 (s, 2H), 7.31 (t, *J* = 7.2 Hz, 1H), 7.35–7.38 (m, *J* = 7.8 Hz, 3H), 7.43 (m, *J* = 7.8 Hz, 1H), 7.80 (m, *J* = 7.8 Hz, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 33.54, 49.56, 56.43, 56.61, 127.39, 128.46, 128.84, 129.78, 129.99, 135.17, 136.43, 137.96, 138.07, 140.63, 175.49, 177.20, 177.56, 181.49.

Purity: HPLC – (98.9%).

*5.7.36. N-(1-Naphthyl-4-carboxybenzyl)-N-carboxyethyl-3,4-dicarboxybenzylamine (**P51**)*

Triester: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.49 (s, 2H), 2.83 (s, 2H), 3.45 (s, 3H), 3.62 (s, 2H), 3.84 (s, 3H), 3.86 (s, 3H), 3.93 (s, 3H), 3.99 (s, 2H), 7.41 (d, *J* = 7.8 Hz, 2H), 7.49 (m, *J* = 7.2 Hz, 2H), 7.55 (m, *J* = 7.2 Hz, 2H), 7.61 (d, *J* = 7.8 Hz, 2H), 8.04 (d, *J* = 7.2 Hz, 1H), 8.12 (d, *J* = 8.4 Hz, 1H), 8.86 (d, *J* = 8.8 Hz, 1H) MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 530.3, (calc) 530.2.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.51 (t, 2H), 2.82 (t, 2H), 3.71 (s, 2H), 4.01 (s, 2H), 7.34–7.36 (s, *J* = 6.6 Hz, 1H), 7.44–7.47 (m, *J* = 7.2 Hz, 3H), 7.49–7.53 (m, *J* = 7.2 Hz, 3H), 7.77 (d, *J* = 8.4 Hz, 1H), 8.13 (d, *J* = 8.4 Hz, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 32.84, 49.93, 53.59, 57.31, 123.17, 124.42, 126.05, 126.21, 126.36, 127.44, 127.98, 128.74, 129.43, 130.24, 131.96, 134.47, 136.64, 137.81, 138.08, 138.45, 177.25, 177.50, 178.25, 181.80. Purity: HPLC – (94.5%).

*5.7.37. N-(2-Trifluromethyl-4-carboxybenzyl)-N-carboxyethyl-3,4-dicarboxybenzylamine (**P52**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.47 (s, 2H), 2.77 (s, 2H), 3.59 (s, 3H), 3.60 (s, 2H), 3.73 (s, 3H), 3.83 (s, 3H), 3.86–3.88 (m, 5H), 7.48 (d, *J* = 7.8 Hz, 1H), 7.60 (s, 1H), 7.64 (d, *J* = 7.8 Hz, 1H), 7.88 (d, *J* = 7.8 Hz, 1H), 8.14 (d, *J* = 8.4 Hz, 1H), 8.2 (s, 1H). MS (ESI) *m*/*z*: (obs) $[M+Na^+$ 548.2, (calc) 548.2.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.43 (t, 2H), 2.77 (t, 2H), 3.64 (s, 2H), 3.81 (s, 2H), 7.35–7.41 (d, *J* = 7.8 Hz, 3H), 7.83 (d, *J* = 7.8 Hz, 1H), 8.01 (d, *J* = 7.2 Hz, 1H), 8.11 (d, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 33.55, 49.51, 56.11, 56.61, 126.78, 127.20, 127.23, 127.42, 128.49, 129.97, 133.57, 136.52, 137.67, 137.79, 138.08, 176.72, 177.19, 177.51, 181.40.

Purity: HPLC – (98.8%).

*5.7.38. N-(3-Trifluromethyl-4-carboxybenzyl)-N-carboxyethyl-3,4-dicarboxybenzylamine (**P53**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.48 (s, 2H), 2.78 (s, 2H), 3.59–3.61 (m, 7H), 3.86–3.88 (m, 9H), 7.45 (d, *J* = 7.8 Hz, 1H), 7.50 (d, *J* = 7.8 Hz, 1H), 7.61 (s, 1H), 7.67 (m, *J* = 7.8 Hz, 2H), 7.7 (m, *J* = 7.8 Hz, 1H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 548.2, (calc) 548.2.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.43 (t, 2H), 2.77 (t, 2H), 3.64 (s, 2H), 3.81 (s, 2H), 7.35–7.41 (d, *J* = 7.8 Hz, 3H), 7.83 (d, *J* = 7.8 Hz, 1H), 8.01 (d, *J* = 7.2 Hz, 1H), 8.11 (d, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 34.05, 50.21, 53.09, 57.02, 126.29, 126.33, 127.36, 128.07, 129.55, 130.86, 132.16, 135.08, 136.35, 137.99, 138.78, 140.73, 174.03, 177.28, 177.59, 181.63.

Purity: HPLC – (98.7%).

*5.7.39. N-(2-Phenyl-4-carboxybenzyl)-N-carboxyethyl-3,4-dicarboxybenzylamine (**P54**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.29 (t, 2H), 2.61 (t, 2H), 3.45 (s, 2H), 3.54 (s, 2H), 3.58 (s, 3H), 3.86 (s, 3H), 3.87 (s, 3H), 3.88 (s, 3H), 7.22 (d, *J* = 7.8 Hz, 2H), 7.39 (m, *J* = 6.6 Hz, 4H), 7.51 (s, 1H), 7.63 (d, *J* = 7.8 Hz, 1H), 7.68 (d, *J* = 8.4 Hz, 1H), 7.86 (s, 1H), 7.98 (d, *J* = 7.8 Hz, 1H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 556.2, (calc) 556.3.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.08 (t, 2H), 2.52 (t, 2H), 3.42 (s, 2H), 3.65 (s, 2H), 7.16 (d, *J* = 8 Hz, 1H), 7.21 (s, 1H), 7.34 (m, *J* = 7.2 Hz, 3H), 7.43 (m, *J* = 7.2 Hz, 3H), 7.54 (s, *J* = 8 Hz, 1H), 7.70 (s, 1H) 7.81 (d, *J* = 8 Hz, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 32.83, 49.43, 52.71, 56.88, 127.35, 127.40, 127.75, 128.19, 128.40, 129.54, 129.59, 130.35, 130.37, 134.85, 136.32, 138.01, 138.32, 138.63, 140.58, 142.46, 175.22, 177.20, 177.47, 181.34.

Purity: HPLC – (98.9%).

*5.7.40. N-(2-Bromo-4-carboxybenzyl)-N-carboxyethyl-3,4-dicarboxybenzylamine (**P55**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.50 (t, 2H), 2.82 (t, 2H), 3.61 (s, 3H), 3.65 (s, 2H), 3.69 (s, 2H), 3.86–3.88 (m, 10*H*), 7.50 (d, *J* = 7.2 Hz, 2H), 7.56 (m, *J* = 7.2 Hz, 4H), 7.62 (s, 1H), 7.67 (d, *J* = 7.8 Hz, 1H), 7.92 (d, *J* = 8.4 Hz, 1H), 8.16 (s, 1H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ (isotopic) 558.1, 559.4 (calc) (isotopic) 558.6, 559.7.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.48 (t, 2H), 2.80 (t, 2H), 3.68 (s, 2H), 3.73 (s, 2H), 7.32 (d, *J* = 8 Hz, 1H), 7.37–7.45 (m, 3H), 7.72 (d, *J* = 8 Hz, 1H), 7.98 (d, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 33.23, 49.68, 55.77, 57.01, 124.45, 127.33, 127.64, 128.61, 130.09, 131.56, 133.15, 137.97, 138.13, 140.00, 173.87, 177.26, 177.52, 181.61.

Purity: HPLC – (99.5%).

*5.7.41. N-(2-Chloro-4-carboxybenzyl)-N-carboxyethyl-3,4-dicarboxybenzylamine (**P56**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.52 (t, 2H), 2.83 (t, 2H), 3.63 (s, 3H), 3.67 (s, 2H), 3.72 (s, 2H), 3.89 (s, 3H), 3.90–3.91 (s, 6H), 7.50 (d, *J* = 7.8 Hz, 1H), 7.57 (d, *J* = 8.4 Hz, 1H), 7.64 (s, *J* = 8.4 Hz, 1H), 7.68 (d, *J* = 7.8 Hz, 1H), 7.89 (d, *J* = 8.4 Hz, 1H), 7.99 (d, *J* = 1.2 Hz 1H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 514.3 (calc) 514.1.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.48 (t, 2H), 2.79 (t, 2H), 3.68 (s, 2H), 3.72 (s, 2H), 7.32 (d, *J* = 7.8 Hz, 1H), 7.37 (s, 1H), 7.39 (d, *J* = 7.8 Hz, 1H), 7.43 (d, *J* = 8.4 Hz, 1H), 7.67 (d, *J* = 7.8 Hz, 1H), 7.79 (s, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 33.22, 49.73, 53.28, 57.06, 127.02, 127.33, 128.59, 129.78, 130.06, 131.72, 134.25, 136.53, 136.97, 137.97, 138.05, 138.19, 174.03, 177.24, 177.52, 181.58.

Purity: HPLC – (>99%).

*5.7.42. N-(2-Fluoro-4-carboxybenzyl)-N-carboxyethyl-3,4-dicarboxybenzylamine (**P57**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.52 (t, 2H), 2.81 (t, 2H), 3.63 (s, 3H), 3.65 (s, 2H), 3.67 (s, 2H), 3.89 (s, 3H), 3.91 (s, 6H), 7.45 (t, *J* = 7.8 Hz, 1H), 7.57 (d, *J* = 7.8 Hz, 1H), 7.63–7.703 (m, 3H), 7.78 (d, *J* = 7.8 Hz, 1H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ 498.1 (calc) (isotopic) 498.1.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.45 (t, 2H), 2.74 (t, 2H), 3.64 (s, 2H), 3.65 (s, 2H), 7.29 (d, *J* = 7.8 Hz, 1H), 7.34–7.37 (m, *J* = 7.2 Hz, 2H), 7.40 (d, *J* = 7.8 Hz, 1H), 7.47 (d, *J* = 10.8 Hz, 1H), 7.57 (d, *J* = 7.8 Hz, 1H).

^{13}C NMR (600 MHz, D_2O): δ = 33.43, 49.54, 49.56, 56.93, 115.49, 115.64, 124.31, 127.05, 127.16, 128.47, 129.94, 132.19, 132.22, 136.49, 137.90, 137.96, 138.05, 160.23, 161.85, 174.13, 177.20, 177.53, 181.45.

Purity: HPLC – (97.5%).

*5.7.43. N-(2,6-Dibromo-4-carboxybenzyl)-N-carboxyethyl-3,4-dicarboxybenzylamine (**P58**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.54 (t, 2H), 2.89 (t, 2H), 3.61 (s, 3H), 3.69 (s, 2H), 3.87 (s, 2H), 3.87 (s, 3H), 3.89 (s, 3H), 3.91 (s, 3H), 3.99 (s, 2H), 7.50 (d, *J* = 7.8 Hz, 1H), 7.57 (m, 2H), 8.13 (s, 2H). MS (ESI) *m*/*z*: (obs) $[M+Na]^+$ (isotopic) 636.4, 638.0 (calc) (isotopic) 635.9, 637.9.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.55 (t, 2H), 2.77 (t, 2H), 3.72 (s, 2H), 3.96 (s, 2H), 7.32–7.39 (m, *J* = 7.2 Hz, 3H), 7.94 (s, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 32.57, 49.10, 56.32, 57.13, 126.31, 127.15, 128.79, 130.33, 132.75, 136.54, 137.79, 138.11, 138.41, 139.15, 172.17, 177.34, 177.52, 181.80.

Purity: HPLC – (>99%).

*5.7.44. N-(2,6-Dichloro-4-carboxybenzyl)-N-carboxyethyl-3,4-dicarboxybenzylamine (**P59**)*

Triester: ^{1}H NMR (600 MHz, $CDCl_3$): δ = 2.52 (t, 2H), 2.85 (t, 2H), 3.60 (s, 3H), 3.67 (s, 2H), 3.87 (s, 3H), 3.89–3.91 (m, 8H), 7.44 (d, J = 7.8 Hz, 1H), 7.58 (s, 2H), 7.61 (d, J = 7.8 Hz, 1H), 7.90 (s, 2H). MS (ESI) m/z: (obs) $[M+Na]^+$ (isotopic) 548.2, 549.9 (calc) (isotopic) 548.1, 550.0.

Sodium salt: ^{1}H NMR (600 MHz, D_2O): δ = 2.55 (t, 2H), 2.78 (t, 2H), 3.70 (s, 2H), 3.88 (s, 2H), 7.31 (d, J = 7.8 Hz, 1H), 7.37 (d, J = 8.4 Hz, 2H), 7.71 (s, 2H).

^{13}C NMR (600 MHz, D_2O): δ = 32.43, 49.52, 50.89, 57.39, 127.15, 128.59, 128.92, 130.46, 136.16, 136.44, 136.62, 137.75, 137.77, 138.09, 172.50, 177.32, 177.48, 181.73.

Purity: HPLC – (>99%).

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2016.11.060.

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