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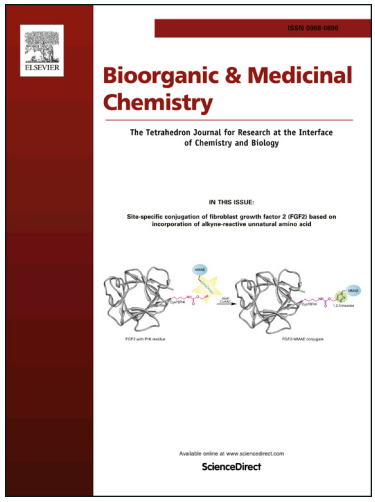

Mapping the Substrate Scope of Monoamine Oxidase (MAO-N) as a Synthetic Tool for the Enantioselective Synthesis of Chiral Amines

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Mapping the Substrate Scope of Monoamine Oxidase (MAO-N) as a Synthetic Tool for the Enantioselective Synthesis of Chiral Amines.

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KEYWORDS: Monoamine oxidase (MAO-N), enantiomerically pure chiral amines, borane, deracemisation, enzyme catalysis, chirality.

Abstract

A library of 132 racemic chiral amines (-substituted methylbenzylamines, benzhydrylamines, 1,2,3,4-tetrahydronaphthylamines (THNs), indanylamines, allylic and homoallylic amines, propargyl amines) was screened against the most versatile monoamine oxidase (MAO-N) variants D5, D9 and D11. MAO-N D9 exhibited the highest activity for most substrates and was applied to the deracemisation of a comprehensive set of selected primary amines. In all cases, excellent enantioselectivity was achieved (*e.e.* > 99%) with moderate to good yields (55-80%). Conditions for the deracemisation of primary amines using a MAO-N/borane system were further optimised using THN as a template addressing substrate load, nature of the enzyme preparation, buffer systems, borane sources, and organic co-solvents.

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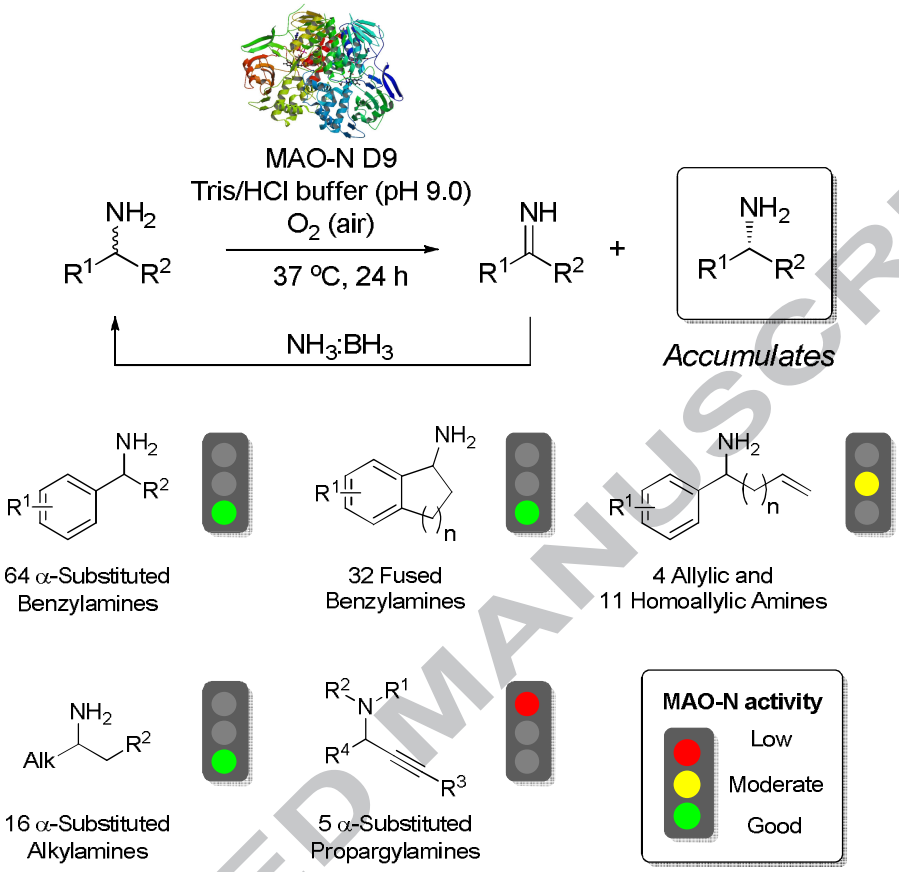


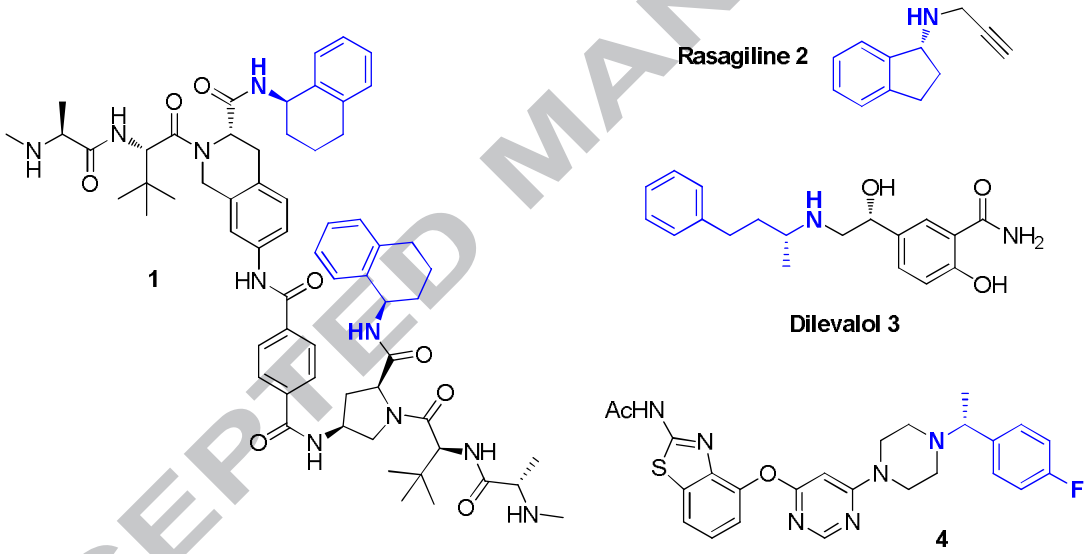
Activity screening against 3 MAO-N variants
Deracemisation of 22 selected amines (*e.e.* > 99%, 55-80% yield)

Introduction

The synthesis of enantiomerically pure compounds employing biocatalysts in place of conventional chemocatalysts has recently gained momentum.[1] The use of biocatalysis offers a sustainable alternative technology for the preparation of a broad range of highly functionalised intermediates and building blocks. In this context, the development of efficient and low waste biocatalytic methodologies is of high interest in both academia and industry.[2] Enantiomerically pure amines constitute an important class of chiral building blocks with 40% of Active Pharmaceutical Ingredients (APIs) containing a chiral amine moiety (**Figure**

1).[1] For instance, **1** was found to be a potent anti-cancer drug inhibiting apoptosis proteins[3] and rasagiline **2** has been commercialised for the treatment of Parkinson's disease.[4] Furthermore, dilevalol **3** is administered against hypertension[5] and compound **4** has found application in pain treatments.[6] Hence, synthetic methods for controlling stereochemistry in organic synthesis are of great importance in the development of new drugs. In this context, chirality is an essential dimension in pharmacology thus guiding industries to focus on the synthesis of single enantiomeric drug entities.

Figure 1. Representative examples of biologically active compounds featuring enantiomerically pure amine scaffolds.

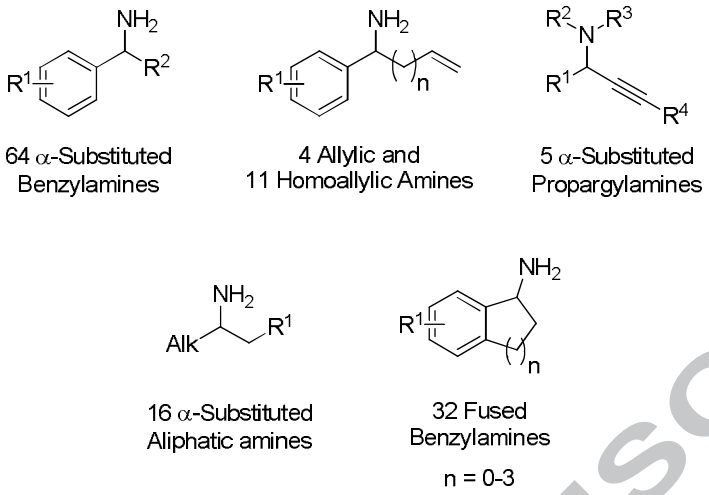


A variety of strategies such as diastereomeric resolution, homogeneous catalysis or biocatalytic approaches have been reported for the preparation of chiral amines.[7] The use of biocatalysis has grown remarkably in the past decades as exemplified by the use of -transaminases,[8] phenylalanine ammonia lyases,[9] amine dehydrogenases[10] and imine reductases.[11] An attractive alternative to the kinetic resolution of amines involves the use of monoamine oxidase, an enzyme class which only requires aerial oxygen with no need for additional or sacrificial co-factors.[12] We have previously developed variants of the

Aspergillus niger monoamine oxidase N (MAO-N) such as D5 which was, unlike the wild-type enzyme, the first MAO-N generated in our laboratories to accept cyclic secondary and tertiary amines.[13] For example, (*R*)-1-phenylethylamine, (*R*)-coniine and (*R*)-1-methyl-2-phenylpyrrolidine were obtained in an enantioselective fashion with MAO-N D5 demonstrating a high (*S*)-enantiopreference. In further rounds of rational protein design targeting the generation of MAO-N mutants with increased volume of the active-site pocket, variant D9 was generated and found to display significantly enhanced activity towards more bulky amines.[14] Several natural products such as crispine A, leptaflorin and eleagnine were synthesised with MAO-N D9 in an enantiomerically pure fashion while retaining the parental enantioselectivity of MAO-N D5. Furthermore, MAO-N D11 has been established as the 'high-end' biocatalyst in the MAO-N family tree with the largest active-site and entrance channel. This variant was successfully applied to the deracemisation of primary, secondary and tertiary hindered amines such as substituted benzhydrylamines, 1-phenyl-tetrahydroisoquinoline and tetrahydrocarbolines.[14,15]

With this versatile set of MAO-N biocatalysts in hand, we set out to investigate the applicability of variants D5, D9 and D11 in the enantioselective synthesis of amines frequently found in APIs (**Figure 1**). To this end, we firstly turned our attention towards the screening of 5 amine libraries comprising (i) -substituted benzylamines, (ii) -substituted allyl-, homoallyl- and propargylamines, (iii) -substituted aliphatic amines and (iv) 1,2,3,4-tetrahydronaphthylamine (THN) and aminoindane derivatives *via* a previously established high-throughput assay[16] (**Figure 2**).

Figure 2: General structure of the different amine libraries screened in this study.



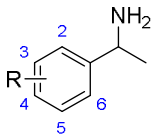
Results and Discussion

In order to gain deeper insight into a broader variety of previously untested amines that could be accessed by MAO-N-catalysed oxidations, we firstly conducted a structure-activity relationship study starting from simple mono- and disubstituted -substituted benzylamines (1-phenylethan-1-amine related compounds, MBAs) (**Tables 1 and 2**).

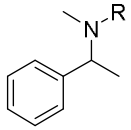
The prototype substrate MBA **5** was oxidised with high activity by MAO-N D9 and D11 which showed a 3- to 5-fold increased specific activity when compared to variant D5 (**Table 1, entry 1**). The specific activity displayed by MAO-N variant D9 towards MBA **5** was set to 100% (relative activity) and henceforth used as the benchmark for both the activities shown by MAO-N D5 and D11 and all subsequent substrates tested. Fluorine substitution at C-2 gave the highest activities for the three enzymes but D9 was the only variant to produce good activity with the three fluorinated substrates **6-8** (**Table 1, entry 2**). The *ortho*-chlorinated and brominated compounds **9** and **12** were only moderately oxidised by MAO-N D9 and low to moderate activity was displayed in all cases with the *meta*-substituted compounds **10** and **13**. In contrast, both MAO-N D9 and D11 displayed excellent activities towards the *para*-

chloro and *para*-bromo substituted amines **11** and **14**, respectively (**Table 1, entries 3** and **4**). Iodine substitution was only accepted in the *para*-position with a good activity obtained with both MAO-N D9 and D11 confirming the higher tolerance of those enzymes for bulky substrates (**Table 1, entry 5**). In general, methyl substituents were not tolerated at all by MAO-N D5 but high activities were obtained for MAO-N D9 and D11 with the *para*-methyl-substituted compound **19**. As previously described for the chloro- and bromo-substituted compounds, *ortho*- and *meta*-methylated substrates (**17** and **18**) produced moderate to low activities with both MAO-N D9 and D11 (**Table 1, entry 6**). Similarly, replacing the methyl by a trifluoromethyl group at the 4-position gave excellent activity whereas a CF_3 substituent at C-2 yielded no activity (**Table 1, entry 7**). MBAs bearing a methoxy group at the 2- or the 4-position were efficiently oxidised by MAO-N D9 while no activity was displayed towards 3-methoxy MBA with any of the variants (**Table 1, entry 8**). Furthermore, hydroxy groups on the aromatic ring did not lead to any oxidation of the substrates by the three variants (**Table 1, entry 9**). Regarding the influence of a substituent on the nitrogen atom, *N*-methyl substituted MBAs were included into the screening with compound **27** revealing lower activities than model substrate **5** with the three variants and no activity in case of the tertiary amine **28** (**Table 1, entry 10**), stressing the importance of the nitrogen atom for an efficient interaction with the flavin co-factor.[17]

Table 1. Influence of substituents on the aromatic ring of α-methylbenzylamines MBAs 5-28 on the relative activities of MAO-N D5, D9 and D11.



5-26



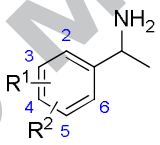
27 (R = H)
28 (R = Me)

Entry	Amine	R	Relative activity [%] D5	D9	D11
1	**5**	H	20	100	65
2	**6**	2-F	48	175	131
	7	3-F	13	30	31
	8	4-F	3	123	6
3	**9**	2-Cl	8	19	4
	10	3-Cl	2	2	1
	11	4-Cl	1	246	145
4	**12**	2-Br	1	3	1
	13	3-Br	0	1	0
	14	4-Br	37	147	99
5	**15**	2-I	6	3	0
	16	4-I	0	39	60
6	**17**	2-CH_3	1	23	14
	18	3-CH_3	0	3	5
	19	4-CH_3	0	211	149
7	**20**	2-CF_3	0	1	0
	21	4-CF_3	6	125	80
8	**22**	2-OCH_3	13	59	29
	23	3-OCH_3	0	0	0
	24	4-OCH_3	0	111	58
9	**25**	3-OH	0	0	0
	26	4-OH	0	3	2
10	**27**	H	3	14	0
	28	CH_3	0	0	0

Enzyme activities were measured as described in the Supporting Information in a total volume of 200 µL and with 0.025 mg/mL of enzyme. The specific activity determined for MAO-N D9 towards MBA 5 (0.91 U mg^{-1}) was set to 100 % relative activity and used as benchmark for MAO-N D5 and D11 and further compounds tested. Relative activity scale: •1 = low activity; 2-10 = moderate; 11-100 = good; >100 = excellent.

In general, di-halogenated substrates produced better activity in the case of fluorine compared to chlorine with MAO-N D9 revealing the highest activities in most cases (**Table 2, entry 1**). Given the high activity of MAO-N D9 towards fluorinated substrates, we wondered if the presence of an additional fluorine substituent on the aromatic ring would increase the activity of the enzymes for a given MBA (**Table 2, entries 2-5**). With MAO-N D9, a fluorine atom in the *para*-position led in most cases to higher activities. To our delight, a 5- to 9-fold increase in activity was observed for substrates **34** and **35** bearing halogens at C-2 (**Table 2, entries 2** and **3**). Fluorinated substrates **36** and **37** bearing methyl substituents respectively gave a 20% decrease and a 25-fold increase in activity with MAO-N D9 (**Table 2, entries 4** and **5**).

Table 2. Influence of di-substitution of the aromatic ring of α-methylbenzylamines on the relative activities of MAO-N D5, D9 and D11.



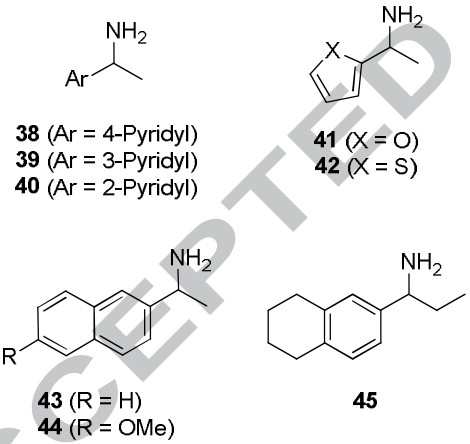
29-39

Entry	Amine	R^1	R^2	Relative activity [%] D5	D9	D11
1	**6**	2-F	H	48	175	131
	29	2-F	4-F	10	148	25
	30	3-F	5-F	4	8	8
	31	2-F	6-F	18	48	1
	32	2-Cl	4-Cl	0	1	7
	33	2-Cl	5-Cl	0	2	0
2	**9**	2-Cl	H	8	19	4
	34		4-F	5	96	10
3	**12**	2-Br	H	1	3	1
	35		4-F	0	28	0
4	**17**	2-CH_3	H	1	23	14
	36		4-F	8	19	0
5	**18**	3-CH_3	H	0	3	5
	37		4-F	0	74	2

Enzyme activities were measured as described in the Supporting Information in a total volume of 200 µL and with 0.025 mg/mL of enzyme. The specific activity determined for MAO-N D9 towards MBA **5** (0.91 U mg^{-1}) was set to 100 % relative activity and used as benchmark for MAO-N D5 and D11 and further compounds tested. Relative activity scale: •1 = low activity; 2-10 = moderate; 11-100 = good; >100 = excellent.

We next turned our attention towards heteroaromatic rings and naphthyl derivatives and found that MAO-N D5 and D9 were able to oxidise the 4-pyridyl substrate **38** as well as the furan **41** and the thiophene **42** to a moderate extent (**Table 3, entries 1** and **2**). Methoxy-substituted 2-naphthyl and 1,2,3,4-tetrahydro-2-naphthyl substrates **44** and **45** gave moderate to good activities with MAO-N D9 and D11 but were unreactive with the D5 variant (**Table 3, entry 3**).

Table 3. Influence of the nature of substituents on the aromatic ring of α-methylbenzylamines on the relative activities of MAO-N D5, D9 and D11.



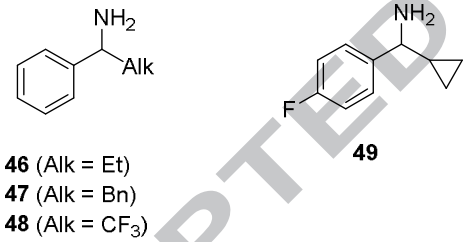
Entry	Amine	Relative activity [%] D5	D9	D11
1	**38**	12	11	11
	39	0	0	0
	40	1	2	0
2	**41**	9	9	3
	42	14	10	0
3	**43**	0	0	0
	44	0	4	3
	45	0	18	9

Enzyme activities were measured as described in the Supporting Information in a total volume of 200 µL and with 0.025 mg/mL of enzyme.. The specific activity determined for MAO-N D9 towards MBA **5** (0.91 U mg^{-1}) was set to 100 % relative activity and used as benchmark for MAO-N D5 and D11 and further compounds tested. Relative activity scale: •1 = low activity; 2-10 = moderate; 11-100 = good; >100 = excellent.

We then evaluated the influence of the α-substituent and tested benzylamine derivatives **46-49** (**Table 4, entry 1**). Ethyl and cyclopropyl groups, respectively, led to good and high

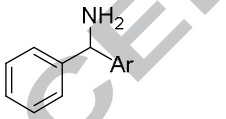
activity with MAO-N D9 but no oxidation could be observed with a benzylic moiety. Also, α-trifluoromethyl benzylamine **48** was presumably too electron-deficient to be a suitable substrate. The bulkier benzhydrylamine **50** was accepted by the three enzymes and MAO-N D11 was the only one accepting 4-substituted benzhydrylamines **51-53** (**Table 4, entry 2**).[14] However, the less hindered substrate **55** containing a furan ring was only moderately oxidised by MAO-N D9. We also tested substrates **56-64** (structures detailed in supporting information) that were synthesised in a single step *via* the Petasis multicomponent reaction (**Table 4, entry 3**).[18] Regardless of the substitution pattern, no activity could be measured in all cases, probably due to the substrates' hindered benzylic position and/or the phenolic moiety.

Table 4. Influence of the nature of the α-substituent of α-methylbenzylamines on the relative activities of MAO-N D5, D9 and D11.

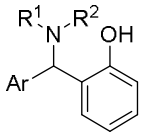


46 (Alk = Et)
47 (Alk = Bn)
48 (Alk = CF_3)

49



50 (Ar = Ph)
51 (Ar = 4-MeC_6H_4)
52 (Ar = 4-$MeOC_6H_4$)
53 (Ar = 4-FC_6H_4)
54 (Ar = 4-ClC_6H_4)
55 (Ar = furan)



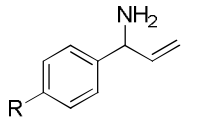
56-64

Entry	Amine	Relative activity [%] D5	D9	D11
1	**46**	0	34	0
	47/48	0	0	0
	49	0	184	42
2	**50**	11	9	10
	51	0	0	2
	52	0	0	2
	53	0	0	7
	54	0	0	0
	55	0	3	1
3	**56/57-64**	0	0	0

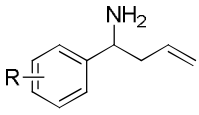
Enzyme activities were measured as described in the Supporting Information in a total volume of 200 µL and with 0.025 mg/mL of enzyme.. The specific activity determined for MAO-N D9 towards MBA **5** (0.91 mU mg^{-1}) was set to 100 % relative activity and used as benchmark for MAO-N D5 and D11 and further compounds tested. Relative activity scale: •1 = low activity; 2-10 = moderate; 11-100 = good; >100 = excellent.

To further assess the substrate scope of the MAO-N variants, we then tested amines with synthetic potential such as α-substituted allyl-[19] and homoallylamines[20] as well as propargylamines[21] (**Table 5**). 2-Phenyl allylamine **65** generally gave only moderate activities with MAO-N D5 and D9 (**Table 5, entry 1**). *para*-Substituents were not tolerated by the three variants and only a weak activity was measured for amine **68** using D5. Similarly, homoallylamine **69** gave moderate activities with the three variants and a chlorine atom on the aromatic ring was tolerated only when using MAO-N D9 and D11 (**Table 5, entries 2 and 3**).[22] Regardless of the substitution pattern, low to moderate activities could be measured for homoallylamines **73-76**. However, we were pleased to find moderate to good activities of MAO-N D9 and D11 for 4-pyridyl-, 2-naphthyl- and cinnamyl-substituted homoallylamines **77-79**, further stressing the high substrate tolerance of these variants (**Table 5, entry 5**). Propargylamines are a known class of MAO-inhibitors that, once oxidised, form a covalent bond with the flavin co-factor *via* Michael addition (**Figure 1**, Rasagiline **2**).[23] We wondered if the presence of a substituent at the - position or at the terminal carbon of the triple bond would prevent the enzyme inhibition while allowing the oxidation of the propargylic position. However, no oxidation could be detected for any of the five substrates tested (**Table 5, entry 6**) as additionally confirmed by inhibition studies (**see Supporting information for details and structures of 80-84**).

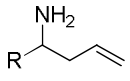
Table 5. Relative activities of MAO-N D5, D9 and D11 towards allylamine, homoallylamine and propargylamine derivatives.



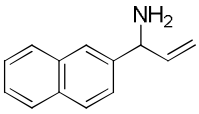
65 (R = H)
66 (R = OMe)
67 (R = Cl)



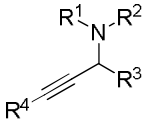
69 (R = H)
70 (R = 2-Cl)
71 (R = 3-Cl)
72 (R = 4-Cl)
73 (R = 3-Me)
74 (R = 3-NO_2)
75 (R = 4-MeO)
76 (R = 3-F, 4-Br)



77 (R = 4-pyridyl)
78 (R = 2-naphthyl)
79 (R = cinnamyl)



68



80-84

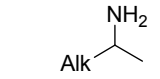
Entry	Amine	Relative activity [%] D5	D9	D11
1	**65**	3	4	0
	66/67	0	0	0
	68	2	0	0
2	**69**	2	5	2
3	**70**	0	2	1
	71	0	4	3
	72	0	20	9
4	**73**	1	9	6
	74	1	4	3
	75	0	0	0
	76	0	16	11
5	**77**	0	28	14
	78	3	22	11
	79	0	3	2
6	**80-84**	0	0	0

Enzyme activities were measured as described in the Supporting Information in a total volume of 200 µL and with 0.025 mg/mL of enzyme.. The specific activity determined for MAO-N D9 towards MBA **5** (0.91 U mg^{-1}) was set to 100 % relative activity and used as benchmark for MAO-N D5 and D11 and further compounds tested. Relative activity scale: •1 = low activity; 2-10 = moderate; 11-100 = good; >100 = excellent.

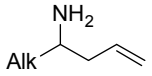
Next we investigated simple aliphatic amines to study whether the oxidation would take place in the absence of an aromatic ring (**Table 6**). Gratifyingly, MAO-N D9 gave by far the best activities for 2-amino linear alkyl chains (**85-86**) but also for bulky amines such **87**, **88** and **89**. *tert*-Butylamine **90** was only weakly oxidised by MAO-N D9 and α-trifluoromethyl ethylamine **91** was incompatible with the three variants (**Table 6, entry 1**). Homoallylamine **92** gave good activity but its analogue **93** was less reactive possibly due to the longer alkyl chain (**Table 6, entry 2**). We next tested substrates featuring an aromatic on the alkyl chain and found that the homobenzylic substrate **94** was only weakly oxidised by the three variants. A longer alkyl chain was well tolerated by MAO-N D9 but an additional fluorine substituent

at the *ortho*-position produced weaker activity (**Table 6, entry 3**). Finally, we evaluated amino alcohols derivatives **97-100** and found only weak activities in all cases (**Table 6, entry 4**).

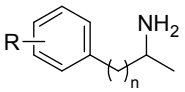
Table 6. Relative activities of MAO-N D5, D9 and D11 towards aliphatic amines.



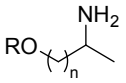
85 (Alk = Et)
86 (Alk = Pent)
87 (Alk = *i*-Bu)
88 (Alk = Cy)
89 (Alk = adamantyl)
90 (Alk = *t*-Bu)
91 (Alk = CF_3)



92 (Alk = *i*-Bu)
93 (Alk = hydrocinnamyl)



94 (n = 1, R = 4-F)
95 (n = 2, R = H)
96 (n = 2, R = 2-F)



97 (n = 1, R = H)
98 (n = 2, R = H)
99 (n = 1, R = Me)
100 (n = 1, R = Bn)

		Relative activity [%]		
Entry	Amine	D5	D9	D11
1	**85**	3	67	2
	86	3	87	2
	87	16	69	17
	88	15	202	54
	89	2	50	1
	90	0	1	0
	91	0	0	0
2	**92**	2	27	19
	93	0	3	2
3	**94**	0	0	0
	95	3	22	3
	96	0	16	1
4	**97/98**	0	1	0
	99	0	0	0
	100	0	2	0

Enzyme activities were measured as described in the Supporting Information in a total volume of 200 µL and with 0.025 mg/mL of enzyme.. The specific activity determined for MAO-N D9 towards MBA **5** (0.91 U mg^{-1}) was set to 100 % relative activity and used as benchmark for MAO-N D5 and D11 and further compounds tested. Relative activity scale: •1 = low activity; 2-10 = moderate; 11-100 = good; >100 = excellent.

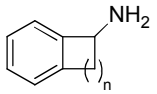
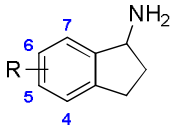
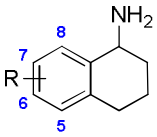
We finally investigated bicyclic compounds **101-104** bearing an amino group at the benzylic position to gain more insights on the influence of a more rigid backbone on the enzymatic activity (**Table 7**). The 4-membered ring **101** was preferentially accepted by MAO-N D5 and D9 but not at all by the D11 variant. 1-Aminoindane **102** turned out to be an excellent substrate for both MAO-N D9 and D11 together with THN **103** producing very good activity with MAO-N D9. On the contrary, only moderate activities could be detected

for bigger ring-size (**Table 7, entry 1**). In the next step, a comprehensive set of 1-aminoindanes and THNs as well as related heterocycles were screened against the three MAO-N variants (**Tables 7** and **8**).

Similar to the MBA series (cf. **Tables 1** and **2**), 1-aminoindanes harboring a fluorine substitution at C-4, C-5 and C-6 were particularly reactive with MAO-N D9 (**Table 7, entry 2**). A chlorine or a bromine atom at the 4-position gave moderate activity for MAO-N D9 whereas 6-chloro-1-aminoindane **109** turned out to be a good substrate for MAO-N D9 (**Table 7, entries 3** and **4**). A comparison between these results and the ones obtained for *meta*-chloro MBA **10** (**Table 1**) clearly showed the positive influence of a rigid backbone on the enzyme activity. A methyl substituent at the 5-position gave good activity with MAO-N D9, whereas 6-methyl-1-aminoindane was only oxidized by MAO-N D11 (**Table 7, entry 5**). The dimethoxy substituted compound **113** was only weakly oxidised by the three variants (**Table 7, entry 6**).

In the THN series, a similar trend was observed with fluorinated compounds being excellent substrates for MAO-N D9 (**Table 7, entry 7**). The presence of a methyl group at the 7-position yielded good activities but an additional methyl group at the 5-position generally suppressed the activity of the three variants (**Table 7, entry 8**). 6-methoxy- and 6-cyano-THN **118** and **121** respectively gave good and excellent activity for both MAO-N D9 and D11 (**Table 7, entries 9** and **10**). Noteworthy, the bicyclic substrates **101**-**121** turned out to be highly compatible towards MAO-N D9 or D11 with 14 substrates giving good to excellent activities with either of the two variants.

Table 7. Relative activities of MAO-N D5, D9 and D11 towards 1-aminoindane and THN derivatives.

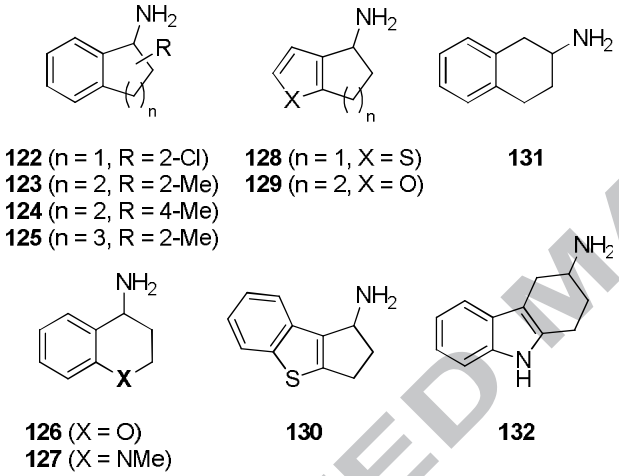
	Entry	Amine	Relative activity [%] D5	D9	D11
	1	**101** (n = 1)	20	12	0
		102 (n = 2)	5	199	165
		103 (n = 3)	1	92	10
		104 (n = 4)	0	6	4
	2	**105** (R = 4-F)	0	101	1
		106 (R = 5-F)	1	242	75
		107 (R = 6-F)	0	143	39
	3	**108** (R = 4-Cl)	0	3	0
		109 (R = 6-Cl)	0	103	13
	4	**110** (R = 4-Br)	0	4	0
	5	**111** (R = 5-Me)	23	61	20
		112 (R = 6-Me)	0	0	20
	6	**113** (R = 5,6-OMe)	1	2	1
	7	**114** (R = 6-F)	0	155	107
		115 (R = 7-F)	18	90	25
	8	**116** (R = 7-Me)	20	39	26
		117 (R = 5,7-Me)	0	0	0
	9	**118** (R = 6-OMe)	0	49	11
		119 (R = 7-OMe)	0	2	1
		120 (R = 6,7-OMe)	0	1	0
	10	**121** (R = 6-CN)	0	103	32

Enzyme activities were measured as described in the Supporting Information in a total volume of 200 µL and with 0.025 mg/mL of enzyme. The specific activity determined for MAO-N D9 towards MBA 5 (0.91 U mg^{-1}) was set to 100 % relative activity and used as benchmark for MAO-N D5 and D11 and further compounds tested. Relative activity scale: •1 = low activity; 2-10 = moderate; 11-100 = good; >100 = excellent.

Next, the influence of a substituent on the saturated alkyl chain was studied. Weak to moderate activities were measured for aminoindanes **122-124** and a moderate activity for this group of substrates was found for MAO-N D9 towards **125** (**Table 8, entry 1**). Oxygenated heterocycle **126** was oxidised by the D9 variant but low activity was found for diamine **127**. We next attempted to replace the aromatic ring with heterocycles such as thiophene **128** and

furan **129** for comparison with MBA derivatives **41** and **42** (**Table 3**, **entry 2**). To our delight, a dramatic improvement in activity was measured (up to 25-fold) with the more rigid backbones **128** and **129**. However, the oxidation of non-benzylic positions in the bulky compounds **131-132** remains a limitation of the current MAO-N variants (**Table 8, entry 4**).

Table 8. Relative activities of MAO-N D5, D9 and D11 towards 1-aminoindane and THN derivatives.



Entry	Amine	Relative activity [%] D5	D9	D11
1	**122**	0	1	1
	123	0	4	4
	124	0	5	0
	125	8	14	8
2	**126**	0	22	0
	127	1	2	0
3	**128**	1	33	1
	129	6	214	10
	130	0	12	0
4	**131**	11	0	0
	132	0	1	0

Enzyme activities were measured as described in the Supporting Information in a total volume of 200 µL and with 0.025 mg/mL of enzyme.. The specific activity determined for MAO-N D9 towards MBA **5** (0.91 U mg^{-1}) was set to 100 % relative activity and used as benchmark for MAO-N D5 and D11 and further compounds tested. Relative activity scale: •1 = low activity; 2-10 = moderate; 11-100 = good; >100 = excellent.

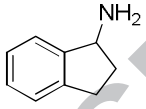
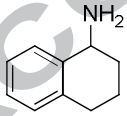
In summary, this extensive and wide-ranging investigation of the substrate scope of MAO-N has highlighted the versatility of this biocatalyst for the synthesis of enantiomerically enriched chiral amines. Although the variants D5, D9 and D11 were initially selected for activity towards simple model chiral amines, screening against a much broader range of pharmaceutically relevant amines has identified new applications beyond those originally envisaged.

Optimisation of biotransformations with 1-aminoindane and THN

Based on the results from the activity measurements, 1-aminoindanes (**102**, **105-113**) and THNs (**103**, **114-121**) were identified as new classes of substrates compatible with MAO-N catalysed oxidations. The efficient biocatalytic amination of 1-indanone and 1-tetralone still remains a challenge with transaminases[24] which makes these particular substrates of interest for enantioselective MAO-N oxidations. Accordingly, kinetic constants for 1-aminoindane **102** and THN **103** were measured with mutants D9 and D11 (**Table 9**).

Both MAO-N D9 and D11 were found to possess higher affinities (K_m) towards **103** with D9 possessing a 11-fold elevated K_m as compared to D11. As MAO-N D9 displayed the highest affinities (K_m) and catalytic efficiencies (k_{cat}/K_m) towards both amine substrates when compared to D11, we opted for this variant for subsequent experiments.

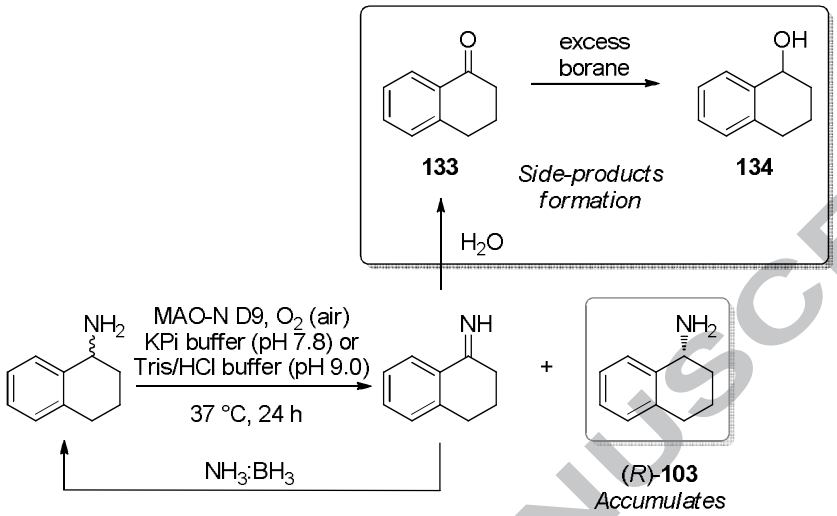
Table 9. Kinetic constants determined for MAO-N variants D9 and D11 towards 102 and 103.

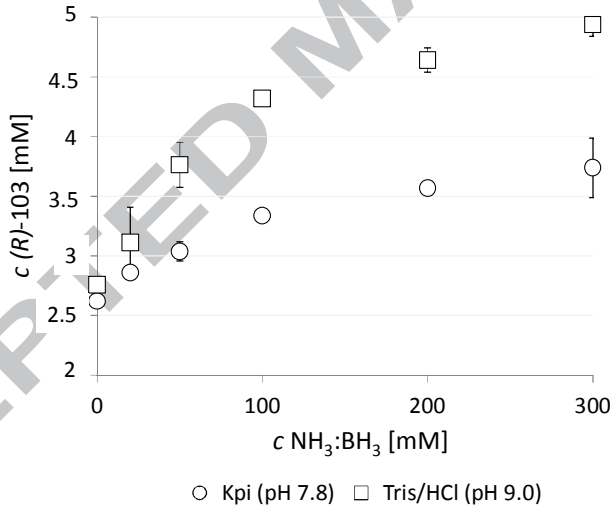
Amine	Variant	K_m [mM]	V_{max} [U mg^{-1}]	k_{cat} [s^{-1}]	k_{cat}/K_m [s^{-1}mM^{-1}]
 **102**	**D9**	1.47	3.11	2.882	1.958
	D11	7.06	1.31	1.213	0.172
 **103**	**D9**	0.51	0.53	0.491	0.963
	D11	5.85	0.369	0.342	0.058

Conditions: 4-AAP/TBHBA/horseradish peroxidase-coupled assay (200 µL) in KPi buffer (100 mM, pH 7.8) with substrates **102** and **103** dissolved in DMSO (co-solvent concentration thoroughly adjusted to 6%, v/v), **=510 nm**, 30 °C (Figure S1-S4).

Using the MAO-N variants developed in our laboratories in presence of a non-selective chemical reductant was previously found to constitute an efficient deracemisation process for the preparation of enantiomerically pure primary, secondary and tertiary amines. In order to find improved conditions broadly applicable for MAO-N catalysed deracemisation reactions, we next revisited the influence of the reaction milieu. Primary amines are particularly challenging substrates due to their propensity for competing hydrolysis of the intermediate imine to the corresponding ketone, thereby reducing the enrichment of the desired amine enantiomer. Therefore, we considered the use of more basic buffers in place of the commonly used potassium phosphate buffer (KPi, 100 mM, pH 7.8) to produce a higher pH of the reaction milieu shifting the equilibrium to the imine side. Ammonia buffer (250 mM) was found to be not suitable in combination with MAO-N D9 as enzyme inhibition, evidenced by drastically reduced conversions of **103**, was observed (**see Supporting Information**). We next focused on the use of Tris/HCl buffer (pH 9.0) and were pleased to find a remarkable enrichment of (*R*)-**103** (**Scheme 1**). Generally, increasing ammonia borane concentrations were well tolerated by the MAO-N enzyme and gradually led to excellent and almost quantitative concentrations of the enantiomerically pure amine **103**.[25] The excess borane reduced the amount of ketone **133** to alcohol **134**, both being present in the reaction mixture at the end of the reaction.

Scheme 1. Yields of (*R*)-103 in deracemisation reactions of (±)-103 in potassium phosphate (KPi, 100 mM, pH 7.8) or Tris/HCl buffer (pH 9.0).





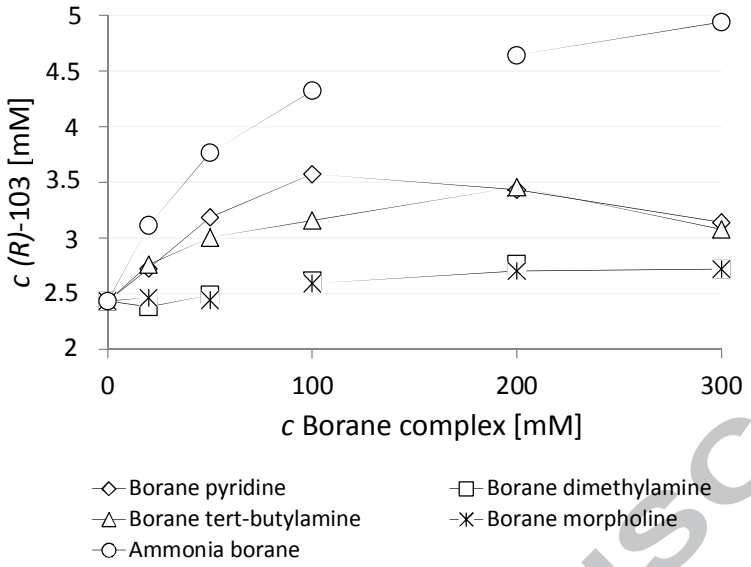
Reaction conditions: 5 mM **103** (100 mM in DMSO), 20-300 mM ammonia borane (NH_3:BH_3; 1 M stock solution made up in the respective reaction buffer), 0.5 mg/mL MAO-N D9, KPi buffer (pH 7.8) or Tris/HCl buffer (pH 9.0), 1 mL final volume, 14 mL headspace, 37 °C, 250 rpm, 24 h. Error bars correspond to three replicates. Concentration determined by HPLC.

With a view to the practicality of MAO-N catalysed deracemisation reactions for operative requirements of pharmaceutical industries, we next studied further reaction parameters

including the nature of the biocatalyst employed, the use of a bi-phasic reaction system with organic solvents, the substrate load and the use of alternative borane complexes. THN **103** was chosen as model compound based on the good kinetic constants obtained for variant D9. Whole or lyophilized cells turned out to be not applicable in this instance, delivering only moderate enrichments towards the desired enantiomer . The addition of co-solvent such as toluene, *2-methylpentane*, cyclohexane or 2-methyltetrahydrofuran to the Tris/HCl buffer in a 1:1 ratio (v/v) was attempted to prevent extensive hydrolysis of the imine intermediate in reactions with whole cells. Although the enzyme operated without any evidence of inhibition with all solvents except 2-methyltetrahydrofuran, again no clear enrichment of the desired enantiomer was achieved. Additionally, the presence of organic solvents favoured the formation of side-products. These results suggested that the imine hydrolysis is faster than its reduction by the borane complex in presence of cells. Based on this we conclude that the use of whole or lyophilised cells in deracemisation reactions of primary amines is not a feasible option although the procedure has proven successfully with the more stable imine intermediates of secondary and tertiary amines in previous studies.[14]

In the next set of experiments, we tested various borane sources at different concentrations and found that borane pyridine and borane *tert*-butylamine complexes gave levels of (*R*)-**103** up to 73% (100 mM borane pyridine) and 71% (200 mM borane *tert*-butylamine) (**Figure 2**). On the contrary, dimethylamine and morpholine borane complexes turned out to be less efficient reductants regardless of the borane concentration. Overall, ammonia borane performed best at all concentrations as compared to the other boranes tested.

Figure 2. Yields of (*R*)-103 in deracemisation reactions with different borane complexes.

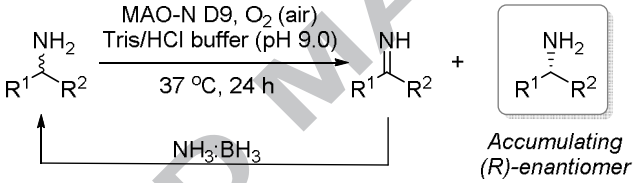


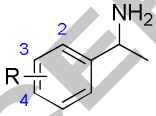
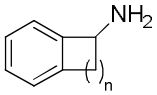
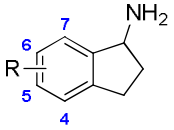
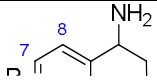
Reaction conditions: 5 mM **103** (100 mM in DMSO), 20-300 mM borane complexes (3 M stock solutions made up in Tris/HCl buffer or DMSO), 0.5 mg/mL MAO-N D9, Tris/HCl buffer (pH 9.0), 1 mL final volume, 14 mL headspace, 37 °C, 250 rpm, 24 h. Concentration determined by HPLC.

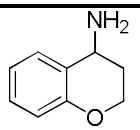
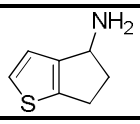
With this new protocol in hand, we tested a comprehensive set of racemic amines including MBAs, THNs, 1-aminoindanes and amino-substituted heterocycles previously identified to produce the best activities with MAO-N D9 (relative activities ranging from 17 to 417%, **Tables 1, 7 and 8**). MAO-N D9 showed excellent enantioselectivity in all oxidation and deracemisation reactions (**Table 10**, *e.e.* > 99%). The reference compound **5** was obtained in a good yield (67%) while moderate yields were achieved with MBAs bearing a fluorine substituent, regardless of its position on the aromatic ring (**Table 10, entries 1 and 2**). Several electron withdrawing and donating groups were tolerated at the 4-position, affording the corresponding enantioenriched MBAs in a 55-62% yield (**Table 10, entry 3**). As already presented in **Table 7**, MAO-N D9 exhibited only a moderate activity towards the bicyclic compound **101** containing a 4-membered ring. To our delight, an encouraging yield of 55% was reached, emphasising the broad range of applicability of this methodology (**Table 10, entry 4**). The same yield was obtained for (*R*)-1-aminoindane **102** but one of the best results

was achieved in the case of THN **103**, which could be deracemised towards the (*R*)-enantiomer in 79% yield. As observed for the MBAs, fluorinated 1-aminoindanes were efficiently deracemised by the MAO-N D9/borane system (**Table 10, entry 5**). A good enrichment was also achieved in the case of 5-methyl- and 6-chloro-1-aminoindane (**Table 10, entry 6**). 6- and 7-fluoro substituted THNs could be deracemised in an excellent 80% and 72% yield, respectively (**Table 10, entry 7**). THNs bearing a methoxy group or a cyano moiety on C-6 were also compatible with the deracemisation reaction conditions employed (**Table 10, entry 8**). Finally, heterocycles **126** and **128** could be obtained in an enantiopure fashion in up to 71% yield *via* this deracemisation approach (**Table 10, entries 9** and **10**).

Table 10. Deracemisation of selected amines with MAO-N D9/BH_3-NH_3 system.[a]



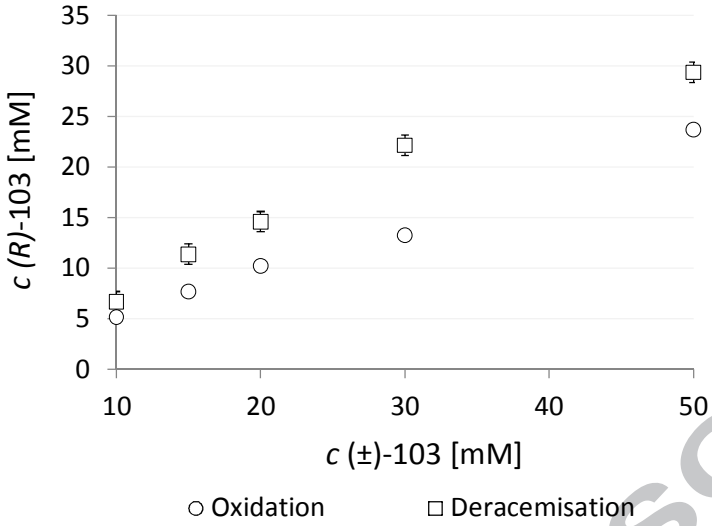
	Entry	Amine	Relative Activity [%]	Conv. [%][b]	*e.e.*[%][c]
	**1**	**5** (R = H)	100	67	> 99 *(R)*
	2	**6** (R = 2-F)	175	58	> 99
		7 (R = 3-F)	30	60	> 99
		8 (R = 4-F)	123	57	> 99
	3	**11** (R = 4-Cl)	246	62	> 99 (*R*)
		14 (R = 4-Br)	147	61	> 99
		19 (R = 4-CH_3)	211	61	> 99
		24 (R = 4-OCH_3)	111	55	> 99
	**4**	**101** (n = 1)	12	55	> 99
		102 (n = 2)	199	55	> 99 (*R*)
		103 (n = 3)	92	79	> 99 (*R*)
	**5**	**105** (R = 4-F)	101	63	> 99
		106 (R = 5-F)	242	71	> 99
		107 (R = 6-F)	143	58	> 99
	6	**109** (R = 6-Cl)	103	74	> 99
		111 (R = 5-CH_3)	61	63	> 99
	**7**	**114** (R = 6-F)	155	80	> 99
		115 (R = 7-F)	90	72	> 99

	8	**118** (R = 6-OCH_3)	49	69	> 99
		121 (R = 6-CN)	103	56	> 99
	**9**	**126**	22	71	> 99
	**10**	**128**	33	62	> 99

[a]Reaction Conditions: 5 mM amine substrate (100 mM in DMSO, 5 % v/v co-solvent), 100 mM NH_3:BH_3 (1 M in Tris/HCl buffer, pH 9.0), 0.5 mg/mL MAO-N D9, Tris/HCl buffer (pH 9.0), 1 mL final volume, 14 mL headspace, 37 °C, 250 rpm, 24 h. [b]Conversion of the respective enantiomer in deracemisation reactions. [c](*R*)-enantiomer remained (*S*-selective oxidation by MAO-N D9). Each experiment was accomplished in three replicates. Relative activity scale: •1 = low activity; 2-10 = moderate; 11-100 = good; >100 = excellent.

We finally turned our attention to higher substrate loads (10-50 mM) in order to gain an improved amine/reductant ratio and chose compound **103** as a model substrate (**Figure 3**). In all cases, MAO-D9 oxidised the (*S*)-enantiomer quantitatively (*e.e.* > 99% for the *R*-enantiomer) and the best conversion was achieved at 30 mM amine concentration with 6.6 equiv. of ammonia borane (84% conversion, 22.2 mM).

Figure 3. Yields of (*R*)-103 in oxidation and deracemisation reactions with increasing concentrations of (±)-103.



Reaction conditions: 10-50 mM **103** (1 M in DMSO), 200 mM ammonia borane (NH_3:BH_3; 1 M stock solution made up in Tris/HCl buffer), 0.5 mg/mL MAO-N D9, Tris/HCl buffer (pH 9.0), 1 mL final volume, 14 mL headspace, 37 °C, 250 rpm, 24 h. Concentration determined by HPLC.

Conclusion

In conclusion, screening extensively various types of amines with the most versatile variants of MAO-N in a high-throughput fashion using a spectrophotometric assay has allowed us to identify new classes of substrates compatible with these enzymes. The MAO-N D9 variant stands out as the most active enzyme variant and a thorough optimisation of the reaction conditions was carried out in the case of THN **103**. A selection of primary amines was then deracemised using the optimised MAO-N D9/ammonia borane system. The good results observed in the case of the moderately active amine **126** suggest that at least 56 out of the 132 amines tested are substrates and 22 of them form enantioenriched amines (Table 10) in good yield using MAO-N D9. In view of a potential application on scale, we showed that amine **103** can be obtained in an enantiomerically pure fashion using a six-fold increased substrate load and 6.6 equiv. of reductant. Research towards an application of MAO-N on large scale is currently on going in our laboratories and will be reported in due course.

ASSOCIATED CONTENT

Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

ǂThese authors contributed equally to the work.

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