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FULL PAPER

Absolute configuration determination of (*S*)-*N*-(1-aryl-allyl)-3,5-dinitrobenzamides and elution order on brush-type chiral stationary phases

Anamarija Knežević,*[a] Jurica Novak,[b] Gennaro Pescitelli,[c] and Vladimir Vinković[a]

Abstract: A series of ten enantiomerically pure (*S*)-*N*-(1-aryl-allyl)-3,5-dinitrobenzamides (*S*-DNBs) was prepared using enzymatic resolution and chiral chromatography. Enzymatic resolution of corresponding 1-aryl-allylamines using *Candida antarctica* lipase B (CaLB) was efficient for amines with no steric hindrance near the stereogenic center and *S*-DNB amides were prepared by acylation of the obtained *S*-amine. When steric effects interrupted enzymatic resolution, racemic DNB amides were resolved using a brush-type chiral column (**CSP-A**) developed in our laboratory. Previously reported behavior of CaLB in kinetic resolution of amines was considered a starting point for the determination of absolute configuration (AC). The AC of prepared *S*-DNB amides was anticipated using the elution order of prepared DNB amides on **CSP-A** and commercial Whelk-O1 columns and comparison with DNB amides obtained after acylation of (*S*)-amines. The comparison between experimental electronic circular dichroism (ECD) spectra with those obtained by conformational analysis and ECD calculations of representative compounds allowed us to verify the AC of prepared DNB amides.

Introduction

Compounds bearing 3,5-dinitrobenzoyl (DNB) together with a second aryl (Ar) moiety are very intriguing since they posses both a π-acceptor acid (DNB) and π-donor base (Ar) group. In the 1960s and 1970s the rapid expansion of ^{1}HNMR chiral solvating agents (CSA) for the determination of optical purity[1,2] led to the development of a great number of brush-type chiral stationary phases (CSP) for enantioselective chromatography in Pirkle's laboratories.[3,4] There are several examples of 3,5-DNB derivatives of amines, amino alcohols and amino acids which are used as CSPs (**I**, **II**, **III**) or CSAs (**IV**)[5] for the separation of enantiomers (Figure 1). Particularly, CSPs with 3,5-DNB derivatives of amino acids were the first commercially available HPLC chiral stationary phases in the 1980s. Also, most commonly brush-type CSP used nowadays, such as Whelk-O1, **I** or ULMO, **II**, possess 3,5-DNB moiety together with another aromatic group.

In the past few years, compounds bearing 3,5-DNB moiety have also been investigated as potential antituberculosis (anti-TB) agents. *N*-substituted 3,5-dinitrobenzamide derivatives, such as **V** and **VI** (Figure 1), showed high activity against *Mycobacterium tuberculosis*.[6-12] These compounds share a common mechanism of action with a very potent anti-TB candidate **BZT043** (**VII**) and inhibit the enzyme decaprenylphosphoryl-*β*-D-ribose 2′-epimerase (DprE1).[7-10] Due to their potential toxicity, nitroaromatic compounds are frequently avoided in medicinal chemistry. However, it was demonstrated that some of these compounds have an acceptable safety index, in vivo stability and bio-availability.[11,12] Recently, several *N*-aryl 3,5-dinitrobenzamides, such as **VIII,** showed inhibition activity toward human 5-lipoxygenase (5-LOX), a proven target for anti-inflammatory therapy.[13]

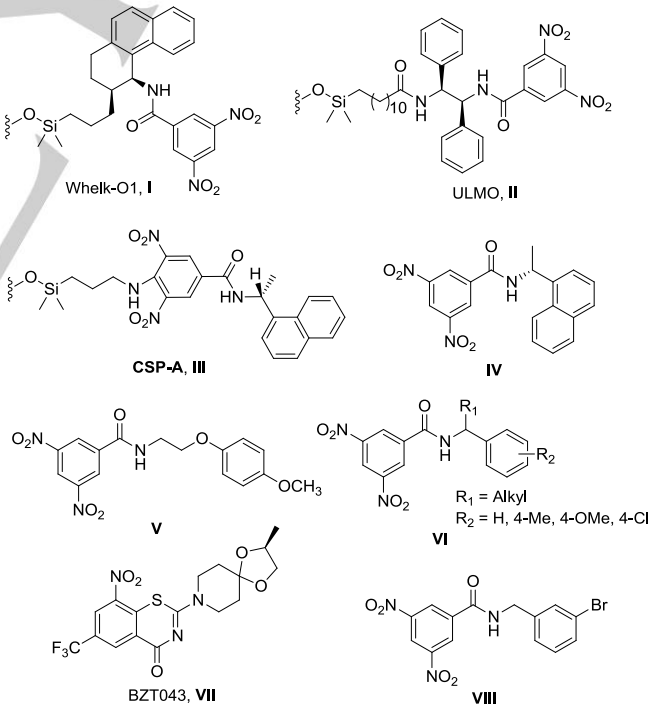

Figure 1. Structures of commercially available CSPs (**I**, **II**), CSP previously prepared in our laboratory (**III**), CSA for NMR analysis of protected amines (**IV**), known DNB based anti-TB agents (**V**-**VII**), and 5-LOX inhibitor (**VIII**).

When the allyl group is introduced into a molecule already bearing DNB and aromatic groups, it widens its synthetic potential. The terminal double bond enables the binding to a silica surface

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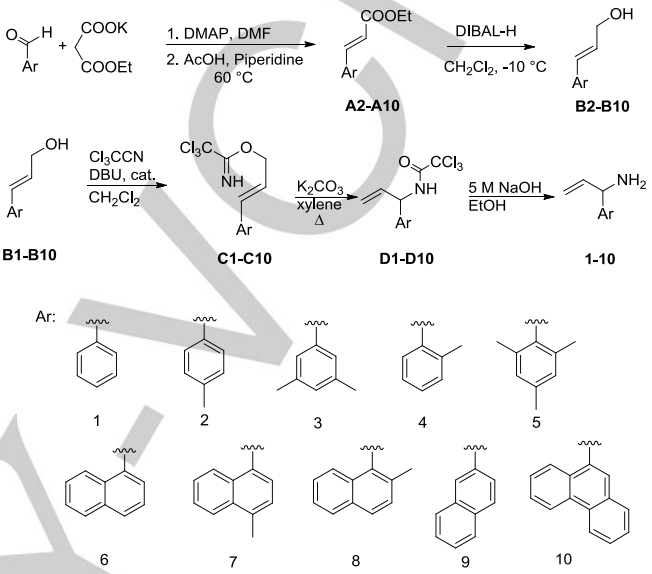
and preparation of brush-type CSP. Also, it allows the introduction of various functional groups using simple methodologies. The common feature necessary for all the above mentioned implementations of 3,5-dinitrobenzoyl-aryl-allylamines is the demand for enantiomerically pure compounds.

Previously, we reported the preparation of a series of (*S*)-1-aryl-allylamines.[14] The kinetic resolution of racemic amines using *Candida antarctica* lipase B (CaLB) in organic solvent provided amines with excellent enantiomeric excess for amines with no steric hindrance near the stereogenic center. Herein, we broaden this methodology to 1-aryl-allylamines bearing 9-phenanthryl, 2-methylnaphthalen-1-yl and naphthalen-2-yl aromatic groups and describe the preparation of enantiomerically pure *S*-3,5-DNB amides from these (*S*)-1-aryl-allylamines. Also, we demonstrate the preparation of enantiomerically pure 3,5-DNB amides from racemic amides using chiral chromatography on a brush-type CSP. The absolute configuration of prepared *S*-DNB amides was anticipated using the elution order of prepared DNB amides on brush-type chiral columns and comparison with DNB amides obtained after acylation of (*S*)-amines. The configuration was then confirmed by means of electronic circular dichroism (ECD) spectroscopy and quantum chemistry calculations.

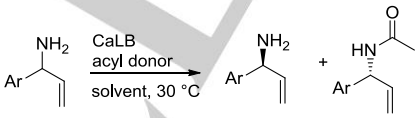
Results and Discussion

Racemic 1-aryl-allylamines were prepared from the corresponding aromatic aldehydes in five steps according to the procedure described previously (Scheme 1).[14] Acrylic esters **A2**–**A10** with (*E*)-configuration of double bond were prepared from aromatic aldehydes and reduced to allyl alcohols **B2**–**B10**. The Overman rearrangement of allyl trichloroacetimidates **C1**–**C10**, obtained from allyl alcohols, afforded 2,2,2-trichloro-*N*-(1-arylallyl)acetamides **D1**–**D10** in high yield. Racemic amines **1**–**10** were obtained after amide hydrolysis.



Scheme 1. Preparation of 1-aryl-allylamines.

Table 1. Enzymatic resolution of 1-aryl-allylamines using CaLB



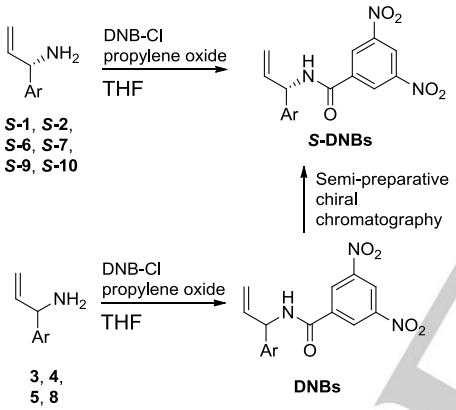
Entry	Amine	Ar	Solvent	Acyl donor[b]	Time [d]	Amine, *ee* [%]	Yield[c] [%]
1	**1**	phenyl[a]	hexane	*iso*-propyl acetate	2	> 99	45
2	**2**	4-methylphenyl[a]	hexane	ethyl methoxyacetate	3	99	39
3	**3**	3,5-dimethylphenyl[a]	hexane	*iso*-propyl acetate	7	81	43
4	**4**	2-methylphenyl[a]	MTBE	ethyl methoxyacetate	8	63	n.i.
5	**5**	2,4,6-trimethylphenyl[a]	hexane	*iso*-propyl acetate	4	-	0[d]
6	**5**	2,4,6-trimethylphenyl[a]	MTBE	ethyl methoxyacetate	4	9	n.i.
7	**6**	1-napthalenyl[a]	MTBE	ethyl methoxyacetate	5	> 99	45
8	**7**	4-methylnaphthalen-1-yl[a]	MTBE	ethyl methoxyacetate	2	> 99	48
9	**8**	2-methylnaphthalen-1-yl	MTBE	ethyl methoxyacetate	6	14	n.i.
10	**8**	2-methylnaphthalen-1-yl	hexane	*iso*-propyl acetate	3	-	0[d]
11	**9**	2-napthalenyl	hexane	*iso*-propyl acetate	11	98	49
12	**10**	9-phenanthryl	MTBE	ethyl methoxyacetate	1	99	42

[a] Described in Reference [14]. [b] Reactions in EtOAc proceed with both enantiomers of amine acylated to amides. [c] Maximum yield is 50 %. [d] Neither of the enantiomers react. n.i. = not isolated.

The enzyme-catalyzed resolutions of racemic amines were performed using CaLB immobilized on a polymer carrier (Novozym 435). Consistent with previous research, in the case of 1-aryl-allylamines with no significant steric hindrance, excellent enantiomeric excesses (*ee*'s) were obtained (Table 1). When a methyl group is attached at position 2 of the aromatic ring Ar, however, it is near the stereogenic center and this disturbs the enantiorecognition process inside the enzyme. Therefore, modest or no enantioselectivity was gained for amines **4**, **5** and **8**.

The absolute configuration of isolated amines was presumed to be (*S*), according to previously reported results in which CaLB always preferentially transformed the (*R*)-enantiomer of the amine.[15,16] This was confirmed by comparing the measured specific rotation of (*S*)-phenylallylamine **1** with literature data,[17] which we discussed in a previous paper.[14] It is reasonable to assume that structurally similar 1-aryl-allylamines behave in the same way when the reaction is catalyzed by CaLB.

Table 2. Preparation of S-DNB amides and enantioseparation using analytical **CSP-A** column.



	η [%]	Mobile phase	t_{R1} [min]	t_{R2} [min]	AC[a]	*ee*[a] [%]
***S*-DNB-1**	81	Ψ(hexane, EtOH) = 6 : 4	4.7	5.6	*S*	> 99
***S*-DNB-2**	92	Ψ(hexane, 2-PrOH) = 1 : 1	6.7	8.7	*S*	> 99
***S*-DNB-6**	90	MeOH	3.7	4.5	*S*	> 99
***S*-DNB-7**	78	MeOH	4.2	5.5	*S*	> 99
***S*-DNB-9**	73	Ψ(hexane, EtOH) = 6 : 4	7.9	9.3	*S*	99
***S*-DNB-10**	79	MeOH	5.3	9.2	*S*	> 99
DNB-3	86	Ψ(hexane, DCM, MeOH) = 50 : 100 : 1	8.6	11.3	*S*	> 99
DNB-4	97	Ψ(hexane, DCM, MeOH) = 40 : 60 : 1	6.3	7.8	*S*	> 99
DNB-5	91	Ψ(hexane, DCM, MeOH) = 65 : 35 : 1.5	7.4	8.3	*S*	> 99
DNB-8	88	Ψ(hexane, THF, DCM) = 3 : 1 : 1	5.6	7.3	*S*	99

[a] For the first eluting enantiomer after chiral chromatography.

When 1-aryl-allylamines were obtained with excellent *ee*'s using CaLB, the corresponding 3,5-DNB derivatives were easily prepared using 3,5-dinitrobenzoyl chloride. Racemic DNB amides were also prepared in order to determine the conditions for enantioseparation on a brush-type chiral column and the elution order of prepared *S*-DNB amides. Recrystallization of the prepared DNB amides increased in some cases their optical purity. Higher *ee*'s were obtained for ***S*-DNB-2**, ***S*-DNB-9** and ***S*-DNB-10** (Table 2). The preparation of brush-type chiral column (**CSP-A**) used for the analysis of DNB amides was previously described in Ranogajec *et al.* (**CSP 7**).[18] This column (Figure 1, **III**) proved suitable for the enantioseparation of compounds bearing a 3,5-dinitrobenzoyl moiety.

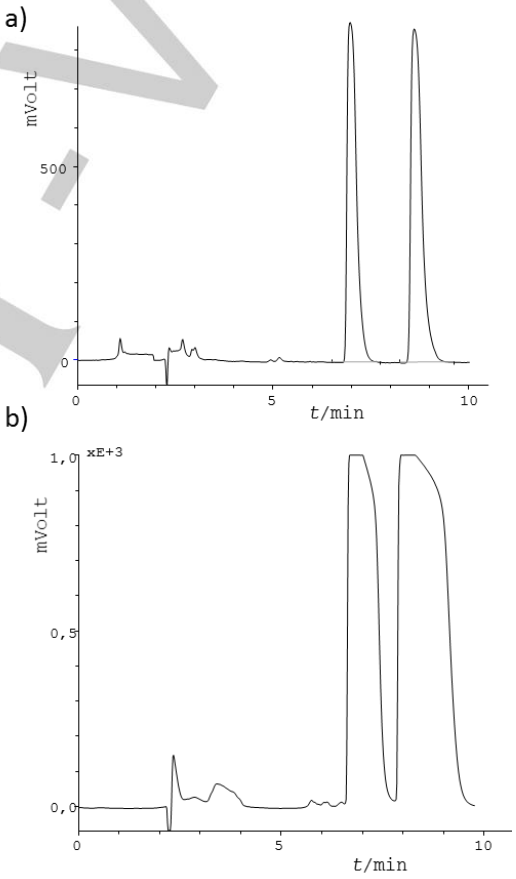


Figure 2. Enantioseparation of **DNB-4** using **CSP-A**, Ψ(hexane, DCM, MeOH) = 40 : 60 : 1; 254 nm; a) semi-preparative column (300 mm x 8 mm), 5 mL/min, injection volume 20 µL; c) semi-preparative column, 5 mL/min, injection volume 700 µL.

When enzymatic resolution of 1-aryl-allylamines did not proceed with high *ee*'s, racemic DNB amides were prepared. It was demonstrated that **CSP-A** could be used for the separation of enantiomers on a semi-preparative scale (Figure 2). Although **CSP-A** was available only in the semi-preparative form, its great

advantage is a wide range of solvents suitable for mobile phase since it is a brush-type column. Prepared DNB amides have poor solubility in nonpolar organic solvents and are soluble only in solvent mixtures with high content of DCM or THF. Eventually, all the remaining DNB amides were successfully resolved on **CSP-A,** and excellent *ee*'s were obtained for the first eluting enantiomers (Table 2).

Using brush-type column **CSP-A** for the separation of enantiomers, also enables the prediction of absolute configuration (AC) of enantiomers. The assumption is that CaLB preferentially acylates the (*R*)-amine of the prepared 1-aryl-allylamines according to Kazlauskas rule.[15] The progress of these kinetic resolutions was monitored using Chiralcel OD-H. Polysaccharide CSPs have a very complicated enantioseparation mechanism and the elution order of enantiomers is hard to predict.[19] Contrary, the mechanism of brush-type CSP is often plain and more studied.[20] For the set of compounds differing only in the methyl substituents on the aromatic ring, the elution order of enantiomers at room temperature should remain unchanged.[21-23] Indeed, the *S*-DNB amides prepared by acylation of *S*-amines obtained after enzymatic resolution, were always the first eluting enantiomers on **CSP-A** column. Also, prepared DNBs were analyzed on a commercial brush-type column Whelk-O1 (Table 3). It is evident that the resolution of prepared DNBs on Whelk-O1 is excellent. More importantly, the elution order remains the same with the first eluting enantiomer being the one obtained after acylation of *S*-amine prepared by enzyme resolution. Regarding the previous analysis, it is reasonable to conclude that with brush-type **CSP-A** and Whelk-O1 for the set of prepared DNB amides, the first eluting enantiomer is always the (*S*)-enantiomer.

Table 3. Analysis of DNB amides on Whelk-O1.

	Mobile phase	t_{R1} [min]	t_{R2} [min]	t_{R*}[a] [min]	AC[b]
DNB-1	Ψ(hexane, EtOH) = 6 : 4	14.1	19.3	13.9	*S*
DNB-2	Ψ(hexane, EtOH) = 6 : 4	14.6	22.1	14.2	*S*
DNB-3	Ψ(hexane, EtOH) = 6 : 4	15.0	28.0	14.9	*S*
DNB-4	Ψ(hexane, EtOH) = 6 : 4	11.1	18.4	11.2	*S*
DNB-5	Ψ(hexane, EtOH) = 6 : 4	10.5	27.8	10.3	*S*
DNB-6	Ψ(hexane, EtOH) = 2 : 8	11.3	34.7	11.2	*S*
DNB-7	Ψ(hexane, EtOH) = 2 : 8	13.2	53.2	13.0	*S*
DNB-8	Ψ(hexane, EtOH) = 2 : 8	9.8	26.2	10.0	*S*
DNB-9	Ψ(hexane, EtOH) = 2 : 8	18.0	66.1	17.4	*S*
DNB-10	Ψ(hexane, EtOH) = 2 : 8	18.8	59.2	18.8	*S*

[a] retention time of compounds for which ECD spectra are presented in Figure 3 – DNB amides prepared by acylation of amines obtained after enzymatic resolution or first eluting enantiomer in chiral chromatography on **CSP-A**. [b] AC of the first eluting enantiomer after chiral chromatography.

In order to substantiate this finding, electronic circular dichroism (ECD) spectra of obtained DNB amides were recorded in methanol at room temperature (Figure 3). It is clear from the recorded spectra that all DNBs bearing a substituted phenyl ring (**DNB-1** to **DNB-5**) follow the same pattern. The *S*-enantiomer is characterized by a positive signal near 260 nm and a negative signal below 240 nm. The spectra of DNBs bearing substituted 1-naphthalenyl ring are much more complicated and diverse. When the methyl group is at position 4, the spectrum is analogous to the spectrum of ***S*-DNB-6**, a species with no substituents on the ring. On the other hand, the spectrum of ***S*-DNB-8** (with methyl attached at position 2) demonstrates a completely different shape of the curve. The ECD of ***S*-DNB-9**, featuring a 2-naphthalenyl substituent, behaves analogous to ECDs of phenyl series of DNB amides, while ECD of ***S*-DNB-10** has again an entirely different ECD curve. Since these species possess unique chromophores in our set of compounds, it is understandable that their ECD spectra are not comparable to other prepared DNBs. However, these compounds were obtained after enzymatic resolution of 1-aryl-allylamines. Supported by the fact that the enantiomers whose spectra are shown in Figure 3 were also the first eluting enantiomers on the **CSP-A** and Whelk-O1 columns, we can confidently assume that these enantiomers are *S*-DNBs. This assumption was definitely proved by ECD calculations.

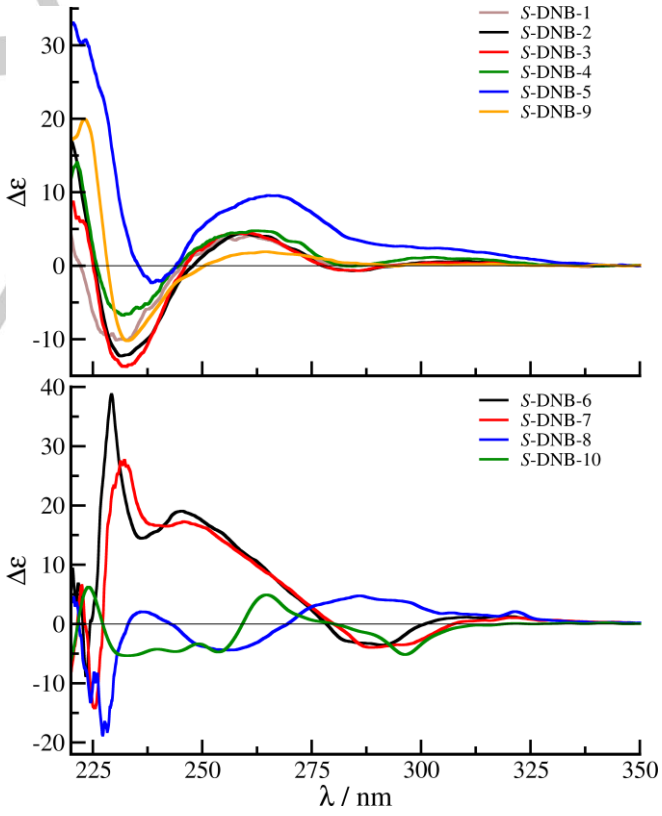


Figure 3. Experimental ECD spectra of synthesized compounds recorded in methanol, at room temperature.

As demonstrated in Figure 3, the ECD spectra of our DNBs are mainly dictated by the specific aromatic chromophore attached at C-3. In fact, ECD is a consequence of the chromophore(s) present in the chiral molecule, and it is strongly dependent on both the absolute configuration and the conformation assumed by the molecule in the solution.[24] Therefore, in order to obtain a calculated ECD spectrum to compare with the experimental one, first it is necessary to investigate the molecular conformational space, followed by the calculation of ECD of relevant conformers. The aim of the ECD study was not only to confirm the absolute configuration of DNBs, but also to rationalize better the impact of steric factors on the ECD spectra, with special attention to the presence of bulky groups near the stereogenic center. Especially intriguing is the fact that for a given chromophore, a different substitution pattern leads to very different ECD spectra. Our set of compounds includes two families with phenyl and 1-naphthyl substituent represented by 5 and 3 members, respectively. Therefore, we focused on ***S*-DNB-2** and ***S*-DNB-5** as representative of the phenyl-substituted DNB family, exemplifying respectively the compound with the smaller and larger steric hindrance of the series. For the same reason, ***S*-DNB-6** and ***S*-DNB-8** were chosen as representative of the 1-naphthyl-substituted series.

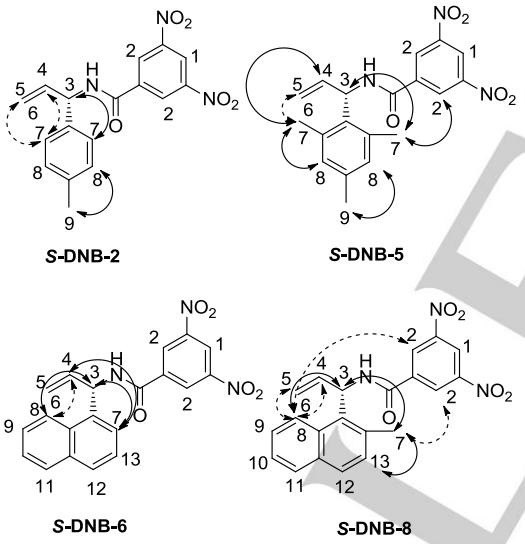


Figure 4. NOESY correlations for **DNB-2**, **DNB-5**, **DNB-6** and **DNB-8**. Dashed lines represent the correlations of lower magnitude in NOESY spectrum.

In vacuo conformational analysis followed by DFT geometry optimizations indicates the presence of several conformers with sizable Boltzmann population at 298 K (see Table S1 in Supporting Information), ranging from two to six conformers respectively for ***S*-DNB-6** and ***S*-DNB-8**. When solvent effects are included in the DFT optimizations, only slight changes (below 5%) in conformer populations are observed. The only exception is **DNB-2**, where MIN-B becomes the lowest energy structure, being 0.28 kcal mol^{-1} below MIN-A structure, and conformer MIN-D of **DNB-8**, which doubled its population. In the most cases the main geometrical difference between close-lying structures is a slightly different relative orientation of two chromophores inside molecule (Figure S2). NOESY spectra were recorded in d4-methanol to further support calculated conformers (Figure 4). Interaction of H3 with H7 and absence of interaction between H3 and H2 in **DNB-2** and **DNB-5** is an indicator of the position of both aryl moieties in relation to H3 and is consistent with the calculated structures. Also, NOESY correlations for **DNB-8** between H3 and both H7 and H8 clearly demonstrate the presence of two types of rotamers, differing in the rotation around C*-C^{aryl} bond (Figure S3). These interactions are not possible simultaneously in a single conformer. According to DFT calculations, the most stable conformer of **DNB-8** is a structure with π stacking interactions ('sandwich' conformation, Figure S4). However, there are no correlations in the NOESY spectrum proving interactions between H2 and H3 hydrogens (only 1.88 Å apart in the DFT minimum) and H2 and H8 (3.22 Å). It must be stressed that, in the presence of multiple conformers, NOE measurements tend to underestimate the effective distance because of the $\langle r^{-6} \rangle$ dependence. This means that the importance of conformers with the shorter contacts is amplified at the expense of those with longer contacts. Therefore, in the absence of NOE correlations, at least the former ones may be safely excluded. It appears that DFT calculations with both B3LYP and M06-2X functionals overestimate stability of this conformer. Therefore, this geometry was excluded from subsequent computations, otherwise it would definitely worsen the agreement between experimental and calculated spectra (see Figure S5). This finding emphasizes the necessity of checking the reliability of calculated structures with all available experimental means.[25,26]

ECD calculations may be run with different computational methods.[27] The time-dependent DFT method (TD-DFT) has intrinsic problems with unphysically low-lying charge transfer states expected in multi-chromophore systems like the present ones.[28] This is especially true for hybrid functionals like the popular B3LYP because of their incorrect asymptotic behavior.[29] The problem may be relieved by using range-separated functionals like CAM-B3LYP[30] and ωB97XD.[31] A more computationally costly but more accurate approach requires the use of correlated wavefunction-based second-order approximate coupled cluster singles and doubles method (CC2) for calculation of ECD spectra.[29,32,33] Calculation details are reported in the Experimental Section. The level of agreement between calculated and experimental ECD spectra of compounds ***S*-DNB-2**, ***S*-DNB-5**, ***S*-DNB-6** and ***S*-DNB-8**, supported with previous experimental evidences, permits us to determine the AC (Figure 5). This is especially true by looking at CC2 calculation results and, at a lesser extent, to TDDFT results with CAM-B3LYP and ωB97XD functionals. For **DNB-5** both CAM-B3LYP and ωB97XD outperform the computationally demanding RI-CC2 method, but for all other compounds RI-CC2 is the method of choice for the ECD spectra simulation. Minor differences between RI-CC2 calculated and experimental spectra concern ***S*-DNB-5** (missing shoulder). The overall satisfactory agreement of spectral line shapes and relative intensities of the ECD spectra predicted with RI-CC2, backed with experimental evidences, support our AC determination for the series of substituted DNBs.

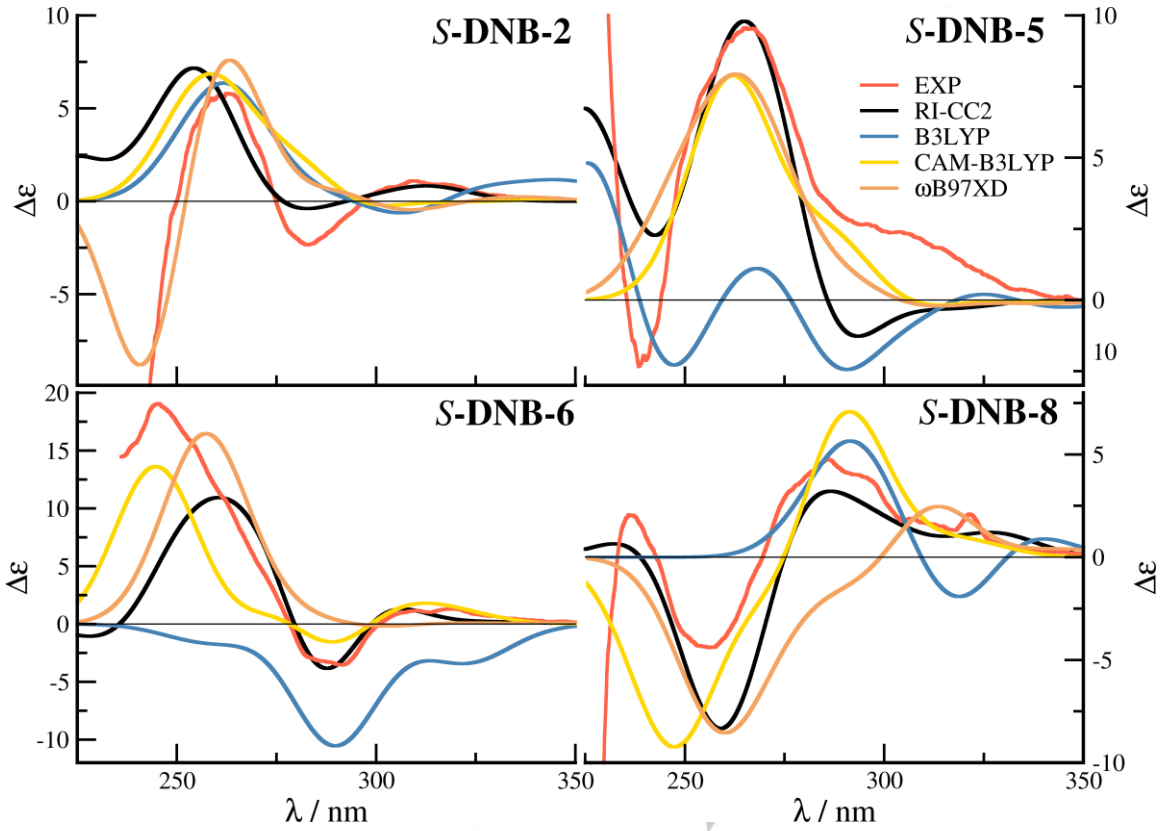


Figure 5. Experimental (red) and calculated ECD spectra of ***S*-DNB-2** (upper panel, left), ***S*-DNB-5** (upper panel, right), ***S*-DNB-6** (lower panel, left) and ***S*-DNB-8** (lower panel, right). See Computational Methods for scaling factors of theoretical spectra.

On the contrary, the use of B3LYP was largely unsatisfactory for all compounds, as it cannot reproduce neither the shape nor the relative intensities of recorded spectra. The analysis of the character of transitions confirms the well documented enormous underestimation of the charge transfer excitations by B3LYP.[29]

All ***S*-DNBs** have weak positive ECD signal above 300 nm, independent of the aryl substituent on the stereogenic carbon atom. According to our calculations and orbital analysis of the most stable conformer, these signals originate from the excitation from a nonbonding orbitals of nitro groups to the π* orbital of the DNB moiety and are common for all DNBs studied. Systems with substituted phenyl or naphthyl moieties on asymmetric carbon atom have strong positive (***S*-DNB-2**, ***S*-DNB-5**, ***S*-DNB-6**) or negative (***S*-DNB-8**) ECD signals with maxima in the range from 254 nm to 260 nm. These excitations are of ππ* type, with dominant contributions from π orbital of phenyl (naphthyl) and DNB moiety to the π* orbital of DNB (Figures S6-S13).

It is interesting to examine how is the addition of methyl groups on the aromatic ring reflected on the conformational equilibrium and subsequently on ECD spectra. Substituting two hydrogen atoms of phenyl group of ***S*-DNB-2** at *ortho* position results in ***S*-DNB-5**. MIN-A conformer of ***S*-DNB-2** is dominant conformer (Boltzmann factor N = 0.367 at 298 K), with N-C*-C^{1}-C^{2} dihedral angle -93°, and N-C*-C$^{\alpha}$-C$^{\beta}$ of only -7° (Figure S14). ***S*-DNB-5** has two close lying conformers, MIN-A (N = 0.335) and MIN-B (N = 0.304). N-C*-C^{1}-C^{2} dihedral angle of MIN-A conformer is -55°, while the ethenyl group is oriented towards the phenyl moiety (N-C*-C$^{\alpha}$-C$^{\beta}$ dihedral angle is 138°, Figure S14). All those geometrical differences are mirrored in ECD spectra. In the spectrum of ***S*-DNB-5**, a negative signal centered at 280 nm is missing, replaced by a broad positive shoulder. The same effect is even more pronounced for compounds with naphthyl substituents (Figure 6). Comparing the minimum energy structures of ***S*-DNB-6** and ***S*-DNB-8** one can see that a single methyl moiety on position 2 causes reorientation of naphth-1-yl ring. Dihedral angle N-C*-C^{1}-C^{2} is -100° in ***S*-DNB-6** and 122° in ***S*-DNB-8**, with the ethenyl group on asymmetric carbon atom adjusted to minimize steric repulsions. 1-Naphthyl rings easily adjust the rotamerism around sp^{2}–sp^{3} junctions between the limiting *syn*- and *anti*-periplanar conformations.[34] ECD spectra of these two compounds are almost completely inverted. In fact, the strong positive signal around 260 nm of ***S*-DNB-6** turns to a weak negative signal of ***S*-DNB-8**; the weak negative signal centered at 280 nm for ***S*-DNB-6** becomes a broad, structured, strong and positive signal in ***S*-DNB-8**.

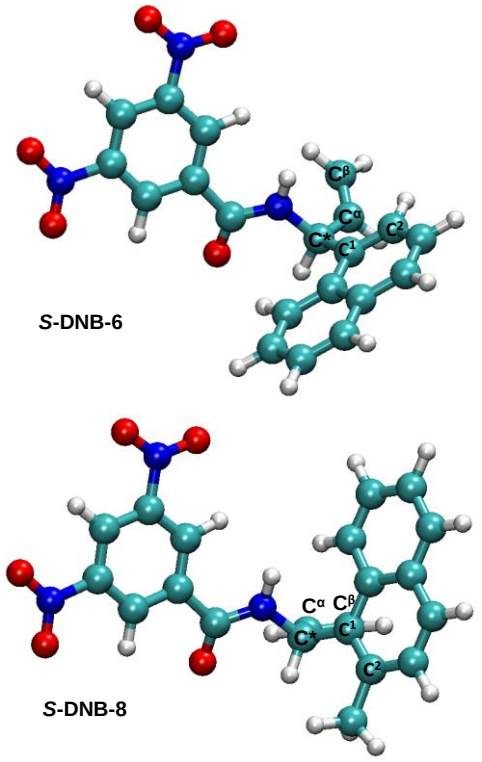


Figure 6. Structures of the lowest energy conformer (MIN-A) of **S-DNB-6** and **S-DNB-8** calculated with DFT, showing the different reciprocal orientation of the DNB and naphthalene chromophores.

The influence of solvent on ECD spectra was estimated by the change of relative populations of conformers which is to be reflected in the total spectrum (see Table S1). But, as can be seen from Figure S15, this approach demonstrates that in our case for the determination of the AC we can neglect the solvent effects.

This analysis demonstrates the success of a multidisciplinary approach combining experimental techniques with state-of-the-art methods of computational chemistry to determine the absolute configuration of a series of molecules with potential anti-TB activity, and to explain how a small structural change like methylation can induce a large conformational rearrangement and cause major differences in the overall ECD spectra.

Conclusions

In conclusion, we described the preparation of ten enantiomerically pure (*S*)-*N*-(1-aryl-allyl)-3,5-dinitrobenzamides using enzymatic resolution of corresponding 1-aryl-allylamines or resolution of racemic DNB amides on chiral stationary phases. The set of prepared DNBs include an aryl moiety with substituted phenyl and naphthalen-1-yl rings, naphthalen-2-yl and phenanthryl groups. Enzymatic resolution was achieved with *Candida antarctica* lipase B (CaLB) in organic solvent and was suitable for compounds with no substituents or methyl substituent at position 4 on the aromatic ring. CaLB preferentially acylates the *R*-amine according to Kazlauskas rule, leaving the *S*-amine in the solution. We demonstrated that racemic 3,5-DNB amides can be resolved using brush-type **CSP-A**, previously created in our laboratory, as well as commercial Whelk-O1 column. The absolute configuration of prepared *S*-DNB amides was presumed using the elution order of prepared DNB amides on **CSP-A** and Whelk-O1 columns and comparison with retention times of DNB amides obtained after acylation of (*S*)-amines. In order to confirm the proposed absolute configuration, ECD spectra of all prepared compounds were recorded and simulated by CC2 calculations. The agreement of experimental ECD spectra and calculated ECD of representative compounds allowed us to verify the absolute configuration of prepared DNB amides. The evaluation of CSPs obtained after attachment of DNB amides onto silica is in progress. Also, prepared compounds are being tested for anti-TB activity at the time.

Experimental Section

General Methods: ^{1}H and ^{13}C NMR were recorded on a Bruker AV 300 spectrometer. Chemical shifts (δH and δC) are quoted in parts per million (ppm), referenced to TMS. Melting points were determined on Electrothermal 9100 apparatus in open capillaries and are not corrected. IR spectra were recorded on a Bruker ABB Bowen instrument. Elemental analyses were done on Perkin-Elmer 2400 CHNS Elemental Analyzer. Optical rotations were measured using Optical Activity AA-10 automatic polarimeter. Chiral HPLC analysis were performed using a Shimadzu Prominence System (Pump LC-20AT, DGU-20A5 Degasser, UV detector SPD-20A) or Knauer system (Pump Knauer 64, 4-Port Knauer Degasser, UV detector Knauer Variable Wavelength Monitor, Interface Knauer, and CD detector Jasco CD-2095). For chiral HPLC analysis of prepared 3,5-dinitrobenzamides the brush-type **CSP-A** (Figure 1) and Whelk-O1 columns were used. For *ee* determination the analytical **CSP-A** column (250 mm x 4.6 mm) was used and for preparative HPLC the dimensions of semi-preparative column used were 300 mm x 8 mm. ECD spectra were recorded on Jasco J-815 Circular Dichroism (CD) Spectropolarimeter at room temperature. The samples (1 mg) were dissolved in MeOH (1 mL) and 60 µL of the solution was dilluted with MeOH to the volume of 2 mL.

General procedure for the synthesis of 3,5-dinitrobenzamides: To a solution of corresponding amine (1 equiv) in dry THF (7 mL per 1 mmol of amine) under argon 3,5-dinitrobenzoyl chloride (1.1 equiv) was added. The resulting solution was cooled to 0 °C and propylene oxide (1 mL per 1 mmol of amine) was added dropwise. The reaction mixture was stirred for 1 h at 0 °C, then for 2 h at room temperature and concentrated under reduced pressure. The crude product was purified by recrystallization or column chromatography.

(*S*)-*N*-(1-Phenylallyl)-3,5-dinitrobenzamide (*S*-DNB-1): White solid (0.20 g, 81 %) starting from (*S*)-1-phenyl-prop-2-en-1-amine (0.10 g). Purified by recrystallization from methanol. ν_{max}/cm^{-1} 3300, 1637, 1539, 1342, 1078, 731, 702; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.15 (1H, t, *J* = 2.0 Hz, DNB*Ar*), 8.95 (2H, d, *J* = 2.0 Hz, DNB*Ar*), 7.42–7.29 (5H, m, *Ar*), 6.72, (1H, br s, N*H*), 6.14 (1H, ddd, *J* = 5.5, 10.4, 17.1 Hz, C*H*=CH_2), 5.84 (1H, m, C*H*NH), 5.38 (1H, d, *J* = 10.4 Hz, CH_2=CH), 5.32 (1H, d, *J* = 17.1 Hz, CH_2=CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.98, 148.81, 139.46, 137.80, 136.26, 129.22, 128.49, 127.50, 127.36, 121.36, 117.42, 56.67; $C_{16}H_{13}N_3O_5$ requires: C, 58.72; H, 4.00; N, 12.84, found: C, 58.54; H, 4.12;

N, 12.65; *ee* > 99 %, **CSP-A** column, hexane-EtOH, 6 : 4, 1 mL/min, 254 nm, rt, $t_{R(S)}$ = 4.7 min., $t_{R(R)}$ = 5.6 min, $[\alpha]_D^{25}$ – 21.2 (*c* 1.0, THF).

(*S*)-*N*-(1-(4-methylphenyl)allyl)-3,5-dinitrobenzamide (*S*-DNB-2): White solid (1.19, 92%) starting from (*S*)-1-(4-methylphenyl)-prop-2-en-1-amine (0.56 g). Purified by recrystallization from methanol. mp 191.9–192.8 °C; ν_{max}/cm^{-1} 3317, 1639, 1543, 1342, 1077, 921, 730, 722; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.14 (1H, t, *J* = 2.0 Hz, DNB*Ar*), 8.97 (2H, d, *J* = 2.0 Hz, DNB*Ar*), 7.25 (2H, d, *J* = 8.1 Hz, *Ar*), 7.17 (2H, d, *J* = 8.1 Hz, *Ar*), 6.95 (1H, br s, N*H*), 6.14 (1H, ddd, *J* = 17.1, 10.4, 5.5 Hz, C*H*=CH_2), 5.79 (1H, m, C*H*NH), 5.37 (1H, d, *J* = 10.4 Hz, CH_2=CH), 5.31 (1H, d, *J* = 17.1 Hz, CH_2=CH), 2.34 (3H, s, ArCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.76, 148.66, 138.21, 137.75, 136.36, 136.27, 129.74, 127.30, 127.20, 121.17, 116.95, 56.29, 21.11; $C_{17}H_{15}N_3O_5$ requires: C, 59.82; H, 4.43; N, 12.31, found: C, 59.44; H, 4.49; N, 12.28; *ee* > 99 %, **CSP-A** column, hexane-2-propanol, 1:1, 1 mL/min, 254 nm, rt, $t_{R(S)}$ = 6.7 min, $t_{R(R)}$ = 8.7 min, $[\alpha]_D^{25}$ -24,2 (*c* 1.0, DCM).

(*S*)-*N*-(1-(3,5-dimethylphenyl)allyl)-3,5-dinitrobenzamide (*S*-DNB-3): White solid (0.20, 86%) starting from 1-(3,5-dimethylphenyl)-prop-2-en-1-amine (0.10 g). Purified by recrystallization from methanol. mp 202.7–204.2 °C; ν_{max}/cm^{-1} 3313, 1649, 1543, 1345, 1080, 917, 849, 729, 698; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.16 (1H, t, *J* = 2.1 Hz, DNB*Ar*), 8.95 (2H, d, *J* = 2.1 Hz, DNB*Ar*), 6.98 (3H, s, *Ar*), 6.56 (1H, br s, N*H*), 6.12 (1H, ddd, *J* = 17.0, 10.3, 5.5 Hz, C*H*=CH_2), 5.77 (1H, m, C*H*NH), 5.36 (1H, d, *J* = 10.3 Hz, CH_2=CH), 5.31 (1H, d, *J* = 17.0 Hz, CH_2=CH), 2.33 (6H, s, ArCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.68, 148.70, 139.27, 138.82, 137.82, 136.36, 130.02, 127.17, 125.15, 121.13, 116.70, 56.52, 21.30; $C_{18}H_{17}N_3O_5$ requires: C, 60.84; H, 4.82; N, 11.83, found: C, 61.14; H, 5.08; N, 11.53 (*S*)-*N*-(1-(3,5-dimethylphenyl)allyl)-3,5-dinitrobenzamide was prepared using semi-preparative chiral HPLC chromatography, **CSP-A** column, hexane-DCM-MeOH, 50 : 100 : 1, 5 mL/min, 254 nm, rt. Racemic amide (100 mg) was dissolved in DCM-hexane, 4 : 1 (20 mL) and 0.8 – 0.9 mL was injected. Approximately 23 runs were performed to resolve 100 mg of amide. mp 175.0–175.6 °C; *ee* > 99 %, **CSP-A** column, hexane-DCM-methanol, 50:100:1, 1 mL/min, 254 nm, rt, $t_{R(S)}$ = 8.6 min, $t_{R(R)}$ = 11.3 min, $[\alpha]_D^{25}$ -33.7 (*c* 0.9, DCM).

(*S*)-*N*-(1-(2-methylphenyl)allyl)-3,5-dinitrobenzamide (*S*-DNB-4): White solid (0.70 g, 97 %) starting from 1-(2-methylphenyl)-prop-2-en-1-amine (0.31 g). Purified by column chromatography in DCM-MeOH, 100 : 1 (R_f = 0.63). mp 160.2–161.5 °C; ν_{max}/cm^{-1} 3289, 1641, 1540, 1344, 1078, 920, 731, 720; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.15 (1H, t, *J* = 2.0 Hz, DNB*Ar*), 8.95 (2H, d, *J* = 2.0 Hz, DNB*Ar*), 7.33–7.28 (1H, m, *Ar*), 7.25–7.20 (3H, m, *Ar*), 6.68 (1H, br s, N*H*), 6.14 (1H, ddd, *J* = 16.9, 10.2, 4.9 Hz, C*H*=CH_2), 6.03 (1H, m, C*H*NH), 5.37 (1H, d, *J* = 10.2 Hz, CH_2=CH), 5.25 (1H, d, *J* = 16.9 Hz, CH_2=CH), 2.41 (3H, s, ArCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.71, 148.68, 137.55, 137.27, 136.53, 136.02, 131.08, 128.31, 127.17, 126.65, 126.53, 121.20, 116.74, 53.09, 19.23; $C_{17}H_{15}N_3O_5$ requires: C, 59.82; H, 4.43; N, 12.31, found: C, 59.63; H, 4.29; N, 11.99 (*S*)-*N*-(1-(2-methylphenyl)allyl)-3,5-dinitrobenzamide was prepared using semi-preparative chiral HPLC chromatography, **CSP-A** column, hexane-DCM-MeOH, 40 : 60 : 1, 5 mL/min, 254 nm, rt. Racemic amide (100 mg) was dissolved in DCM-hexane, 4 : 1 (15 mL) and 0.7 mL was injected. Approximately 21 runs were performed to resolve 100 mg of amide. mp 202.3–203.2 °C; *ee* > 99 %, **CSP-A** column, hexane-DCM-methanol, 40:60:1, 1 mL/min, 254 nm, rt, $t_{R(S)}$ = 6.3 min, $t_{R(R)}$ = 7.8 min, $[\alpha]_D^{25}$ -23.5 (*c* 1.0, DCM)

(*S*)-*N*-(1-(2,4,6-trimethylphenyl)allyl)-3,5-dinitrobenzamide (*S*-DNB-5): White solid (0.66 g, 91%) starting from 1-(2,4,6-trimethylphenyl)-prop-2-en-1-amine (0.34 g). Purified by column chromatography in DCM (R_f = 0.42). mp 186.3–187.8 °C; ν_{max}/cm^{-1} 3362, 1643, 1541, 1346, 1076, 919, 731, 719; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.15 (1H, t, *J* = 1.9 Hz, DNB*Ar*), 8.92 (2H, d, *J* = 1.9 Hz, DNB*Ar*), 6.88 (2H, s, *Ar*), 6.82, (1H, br s, N*H*), 6.30–6.24 (1H, m, C*H*NH), 6.16 (1H, ddd, *J* = 17.2, 10.4, 3.8 Hz, C*H*=CH_2), 5.30 (1H, d, *J* = 10.4 Hz, CH_2=CH), 5.13 (1H, d, *J* = 17.2 Hz, CH_2=CH), 2.45 (6H, s, ArCH_3), 2.26 (3H, s, ArCH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.88, 148.70, 137.84, 137.69, 136.34, 136.31, 132.43, 130.32, 127.03, 121.09, 116.34, 52.49, 20.93, 20.76; $C_{19}H_{19}N_3O_5$ requires: C, 61.78; H, 5.18; N, 11.38, found: C, 61.39; H, 5.48; N, 10.91 (*S*)-*N*-(1-(2,4,6-trimethylphenyl)allyl)-3,5-dinitrobenzamide was prepared using semi-preparative chiral HPLC chromatography **CSP-A** column, hexane-DCM-MeOH, 65 : 35 : 1.5, 5 mL/min, 254 nm, rt. Racemic amide (100 mg) was dissolved in DCM- hexane, 2 : 3 (12 mL) and 0.3 – 0.4 mL was injected. Approximately 34 runs were performed to resolve 100 mg of amide. mp 66.2–67.5 °C; *ee* > 99 %, **CSP-A** column, hexane-DCM-MeOH, 65 : 35 : 1.5, 1 mL/min, 254 nm, rt, $t_{R(S)}$ = 7.4 min., $t_{R(R)}$ = 8.3 min, $[\alpha]_D^{25}$ -78.4 (*c* 1.2, DCM)

(*S*)-*N*-(1-Naphthalen-1-yl-allyl)-3,5-dinitrobenzamide (*S*-DNB-6): White solid (1.21 g, 90 %) starting from (*S*)-1-(1-naphthalenyl)-prop-2-en-1-amine (0.65 g). Purified by recrystallization from methanol with 1 % acetone. mp 210.1–211.7 °C; ν_{max}/cm^{-1} 3280, 1644, 1545, 1344, 1074, 922, 805, 782, 731, 717; ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.09 (1H, t, *J* = 2.2 Hz, DNB*Ar*), 8.91 (2H, d, *J* = 2.2 Hz, DNB*Ar*), 8.01 (1H, m, *Ar*), 7.88–7.83 (2H, m, *Ar*), 7.56–7.43 (4H, m, *Ar*), 6.92 (1H, br s, N*H*), 6.55 (1H, m, C*H*NH), 6.26 (1H, ddd, *J* = 17.1, 10.5, 4.6 Hz, C*H*=CH_2), 5.45 (1H, d, *J* = 10.5 Hz, CH_2=CH), 5.34 (1H, d, *J* = 17.1 Hz, CH_2=CH); ^{13}C NMR (75 MHz, $CDCl_3$) δ 161.28, 147.97, 136.68, 135.38, 134.39, 133.47, 130.51, 128.78, 128.46, 126.68, 126.61, 125.79, 124.75, 124.70, 122.48, 120.64, 116.35, 51.77; $C_{20}H_{15}N_3O_5$ requires: C, 63.66; H, 4.01; N, 11.14; found: C, 63.40; H, 3.98; N, 10.85; *ee* > 99 %, **CSP-A** column, MeOH, 1 mL/min, 254 nm, rt, $t_{R(S)}$ = 3.7 min, $t_{R(R)}$ = 4.5 min, $[\alpha]_D^{25}$ -14.3 (*c* 1.0, DCM).

(*S*)-*N*-(1-(4-methylnaphthalen-1-yl)allyl)-3,5-dinitrobenzamide (*S*-DNB-7): Light yellow solid (0.34 g, 78%) starting from (*S*)-1-(4-methylnaphthalen-1-yl)-prop-2-en-1-amine (0.22 g). Purified by recrystallization from MeOH with 1 % DCM. mp 186.0–186.7 °C; ν_{max}/cm^{-1} 3340, 1642, 1543, 1524, 1343, 921, 766, 732, 721; ^{1}H NMR (300 MHz, DMSO) δ 9.77 (1H, d, *J* = 7.9 Hz, N*H*), 9.14 (2H, d, *J* = 1.9 Hz, DNB*Ar*), 8.93 (1H, t, *J* = 1.9 Hz, DNB*Ar*), 8.17–8.13 (1H, m, *Ar*), 8.09–8.04 (1H, m, *Ar*), 7.61–7.55 (2H, m, *Ar*), 7.50 (1H, d, *J* = 7.2 Hz, *Ar*), 7.40 (1H, d, *J* = 7.2 Hz, *Ar*), 6.52 (1H, m, C*H*NH), 6.31 (1H, ddd, *J* = 17.0, 10.4, 5.4 Hz, C*H*=CH_2), 5.34 (1H, d, *J* = 10.4 Hz, CH_2=CH), 5.31 (1H, d, *J* = 17.0 Hz, CH_2=CH), 2.65 (3H, s, ArCH_3); ^{13}C NMR (75 MHz, DMSO) δ 161.34, 148.15, 137.25, 136.42, 134.33, 134.09, 132.41, 130.87, 127.79, 126.19, 125.97, 125.72, 124.86, 124.77, 123.65, 120.96, 116.24, 51.71, 19.13; $C_{21}H_{17}N_3O_5$ requires: C, 64.45; H, 4.38; N, 10.74, found: C, 63.67; H, 4.65; N, 10.54; *ee* > 99 %, **CSP-A** column, MeOH, 1 mL/min, 254 nm, rt, $t_{R(S)}$ = 4.2 min, $t_{R(R)}$ = 5.5 min, $[\alpha]_D^{25}$ -21.8 (*c* 1.2, DCM).

(*S*)-*N*-(1-(2-methylnaphthalen-1-yl)allyl)-3,5-dinitrobenzamide (*S*-DNB-8): Yellow solid (1.04 g, 88%) starting from 1-(2-methylnaphthalen-1-yl)-prop-2-en-1-amine (0.59 g). Purified by recrystallization from methanol with 5 % DCM. mp 233.4–234.2 °C; ν_{max}/cm^{-1} 3321, 1638, 1543, 1347, 1074, 819, 730, 718; ^{1}H NMR (300 MHz, DMSO) δ 9.90 (1H, d, *J* = 5.4 Hz, N*H*), 9.06 (2H, d, *J* = 2.0 Hz, DNB*Ar*), 8.91 (1H, t, *J* = 2.0 Hz, DNB*Ar*), 8.37 (1H, d, *J* = 8.4 Hz, *Ar*), 7.86 (1H, d, *J* = 7.6 Hz, *Ar*), 7.76 (1H, d, *J* = 8.4 Hz, *Ar*), 7.52–7.40 (2H, m, *Ar*), 7.36 (1H, d, *J* = 8.4 Hz, *Ar*), 6.50 (1H, ddd, *J* = 17.0, 10.4, 4.9 Hz, C*H*=CH_2), 6.41 (1H, m, C*H*NH), 5.25 (1H, d, *J* = 10.4 Hz, CH_2=CH), 5.04 (1H, d, *J* = 17.0 Hz, CH_2=CH), 2.68 (3H, s, ArCH_3); ^{13}C NMR (75 MHz, DMSO) δ 161.93, 148.04, 137.03, 136.55, 134.74, 133.09, 132.82, 130.67, 129.48, 128.65, 127.83, 127.80, 125.55, 125.11, 124.47, 120.85, 116.50, 53.07, 20.78; $C_{21}H_{17}N_3O_5$ requires: C, 64.45; H, 4.38; N, 10.74, found: C, 63.80; H, 4.49; N, 10.47 (*S*)-*N*-(1-(2-methylnaphthalen-1-yl)allyl)-3,5-dinitrobenzamide was prepared using semi-preparative chiral HPLC chromatography, **CSP-A**

column, hexane-THF-DCM, 3 : 1 : 1, 5 mL/min, 254 nm, rt. Racemic amide (100 mg) was dissolved in the mixture of THF (11 mL) and mobile phase (6 mL) and 0.6 mL was injected. Approximately 28 runs were performed to resolve 100 mg of amide. mp 191.2–192.0 °C; *ee* 99 %, **CSP-A** column, hexane-THF-DCM, 3 : 1 : 1, 1 mL/min, 254 nm, rt, $t_{R(S)}$ = 5.6 min., $t_{R(R)}$ = 7.3 min, $[\alpha]_D^{25}$ -24.6 (*c* 1.2, DCM)

(*S*)-*N*-(1-Naphthalen-2-yl-allyl)-3,5-dinitrobenzamide (*S*-DNB-9): Yellow solid (0.30 g, 73 %) starting from (*S*)-1-(2-naphthalenyl)-prop-2-en-1-amine (0.20 g). Purified by recrystallization from methanol with 5 % DCM. mp 193.0–193.9 °C; ν_{max}/cm^{-1} 3298, 1636, 1541, 1346, 1077, 935, 920, 825, 731, 721; ^{1}H NMR (300 MHz, DMSO) δ 9.81 (1H, d, *J* = 8.1 Hz, N*H*), 9.15 (2H, d, *J* = 2.1 Hz, DNB*Ar*), 8.96 (1H, t, *J* = 2.1 Hz, DNB*Ar*), 7.95–7.88 (4H, m, *Ar*), 7.57 (1H, d, *J* = 8.5 Hz, *Ar*), 7.53–7.49 (2H, m, *Ar*), 6.29 (1H, ddd, *J* = 17.1, 10.3, 6.4 Hz, C*H*=CH$_2$), 5.95 (1H, m, C*H*NH), 5.34 (1H, d, *J* = 17.1 Hz, C*H*$_2$=CH), 5.32 (1H, d, *J* = 10.3 Hz, C*H*$_2$=CH); ^{13}C NMR (75 MHz, DMSO) δ 162.06, 148.64, 138.90, 137.96, 137.19, 133.28, 132.73, 128.58, 128.30, 128.21, 127.97, 126.77, 126.46, 126.09, 125.95, 121.42, 117.18, 56.59; $C_{20}H_{15}N_3O_5$ requires: C, 63.66; H, 4.01; N, 11.14; found: C, 63.35; H, 3.95; N, 10.97; *ee* 99 %, **CSP-A** column, hexane-EtOH, 6 : 4, 1 mL/min, 254 nm, rt, $t_{R(S)}$ = 7.9 min, $t_{R(R)}$ = 9.3 min, $[\alpha]_D^{25}$ -32.1 (*c* 1.1, DCM).

(S)-N-(1-Phenanthren-9-yl-allyl)-3,5-dinitrobenzamide (S-DNB-10): Yellow solid (0.19 g, 79%) starting from (*S*)-1-(phenanthren-9-yl)-prop-2-en-1-amine (0.13 g). Purified by recrystallization from MeOH. mp 252.4–253.6 °C; ν_{max}/cm^{-1} 3423, 1622, 1532, 1347, 731, 725, 717; ^{1}H NMR (300 MHz, DMSO) δ 9.82 (1H, d, *J* = 7.9 Hz, N*H*), 9.16 (2H, d, *J* = 2.1 Hz, DNB*Ar*), 8.93 (1H, t, *J* = 2.1 Hz, DNB*Ar*), 8.92–8.88 (1H, m, *Ar*), 8.83 (1H, d, *J* = 7.8 Hz, *Ar*), 8.21–8.17 (1H, m, *Ar*), 8.01 (1H, d, *J* = 7.6 Hz, *Ar*), 7.92 (1H, s, *Ar*), 7.74–7.63 (4H, m, *Ar*), 6.57 (1H, m, C*H*NH), 6.43 (1H, ddd, *J* = 16.9, 10.3, 5.3 Hz, C*H*=CH$_2$), 5.43 (1H, d, *J* = 10.3 Hz, C*H*$_2$=CH), 5.42 (1H, d, *J* = 16.9 Hz, C*H*$_2$=CH); ^{13}C NMR (75 MHz, DMSO) δ 161.49, 148.16, 136.85, 136.31, 134.35, 130.74, 130.25, 129.83, 129.66, 128.61, 127.83, 127.23, 127.18, 127.10, 126.73, 126.08, 123.89, 123.57, 122.77, 120.99, 116.62, 51.88; $C_{24}H_{17}N_3O_5$ requires: C, 67.44; H, 4.01; N, 9.83, found: C, 66.95; H, 4.27; N, 9.32; *ee* > 99 %, **CSP-A** column, MeOH, 1 mL/min, 254 nm, rt, $t_{R(S)}$ = 5.3 min, $t_{R(R)}$ = 9.2 min, $[\alpha]_D^{25}$ -172.2 (*c* 1.3, THF).

Computational Methods: Conformational analysis of ***S*-DNB-2**, ***S*-DNB-5**, ***S*-DNB-6** and ***S*-DNB-8** have been carried out using the Monte Carlo algorithm and the Merck molecular force field (MMFF)[35] with Spartan 14 package.[36] For all in vacuo MMFF minima, DFT-based optimizations exploiting B3LYP hybrid functional[37] and def-SVP basis set were performed, followed by high level optimization with Ahlrichs def2-TZVPP basis set.[38] Minnesota global hybrid functional M06-2X[39] with 54 % of the exact exchange was used to calculate relative energies of the conformers to evaluate room temperature Boltzmann populations. Only for those conformers with Boltzmann population above 1 %, 15 lowest singlet excited states and associated rotatory strengths in length gauge are calculated using three DFT functionals (B3LYP,[37] CAM-B3LYP,[30] ωB97X-D[31]) and state-of-the-art approximate coupled cluster singles and doubles model (CC2)[32] with the resolution of the identity approximation for two-electron integrals (RI)[40] and def2-TZVPP basis set. When B3LYP functional was used, 25 states were calculated. Possible change of conformer populations due to solvation was investigated by performing single point energy calculations on M06-2X/def2-TZVPP level, while the Polarizable Continuum Model (PCM)[41] was used to incorporate solvent effects. To simulate electronic circular dichroism (ECD) spectra electronic transitions are convoluted by Gaussian functions

$$\Delta\varepsilon(E) = \frac{1}{2.296\times10^{-39}} \times \frac{1}{\Delta\sqrt{\pi}} \sum_i \Delta E_i R_i e^{-\left(\frac{E-\Delta E_i}{\Delta}\right)^2}$$

where Δ*E* is transition energy, Δ is half the bandwidth at 1/e peak height expressed in energy units, and ΔE_i and R_i are the transition energy and rotatory strength for transition *i*, respectively.[42] For each conformer rotatory strengths were scaled by Boltzmann factor, and for the half the bandwidth at 1/e peak height value of 15 nm is used. The conformer with π stacking interactions ('sandwich' conformation) was excluded from subsequent computations since there are no correlations in NOESY spectrum for interactions between H2 and H3 (only 1.88 Å apart) and H2 and H8 (3.22 Å) (Figure S4). Simulated spectra were scaled and wavelength shifted to maximize overlap with experimental ECD spectra (Table 4). All geometry optimizations, B3LYP and RI-CC2 calculations were run in Turbomole 7.0,[43] with default grid size and convergence criteria. For M06-2X single point energy calculations, and CAM-B3LYP and ωB97X-D excited states calculations Gaussian 09 was used.[44]

Table 4. Scaling (y = intensity) and shifting wavelength (x) of simulated ECD spectra.

	B3LYP	CAM-B3LYP	ωB97X-D	RI-CC2
***S*-DNB-2**	x - 20 nm y*3	x + 35 nm y/3	x + 25 nm	 y*5
***S*-DNB-5**	x - 10 nm	x + 35 nm y/3	x + 30 nm y/2	x + 5 nm y*2
***S*-DNB-6**		x + 10 nm y*2	x + 35 nm y/3	x + 15 nm
***S*-DNB-8**		x + 10 nm	x + 35 nm	x + 15 nm

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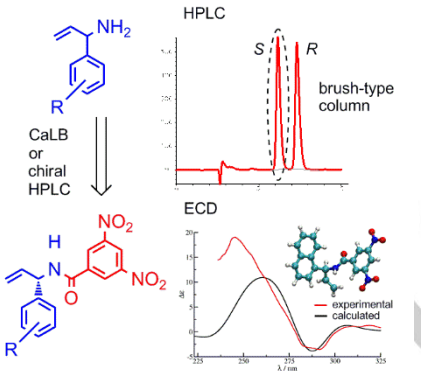
FULL PAPER

Enantiomerically pure (*S*)-*N*-(1-aryl-allyl)-3,5-dinitrobenzamides were prepared using enzymatic resolution and chiral chromatography. Absolute configuration of prepared *S*-DNB amides was anticipated using the elution order on brush-type chiral columns and verified by agreement of experimental and calculated ECD spectra.



Absolute configuration determination

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Absolute configuration determination of (*S*)-*N*-(1-aryl-allyl)-3,5-dinitrobenzamides and elution order on brush-type chiral stationary phases