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Absolute Configuration Assignment from Optical Rotation Data by Means of Biphenyl Chiroptical Probes

Stefania Vergura[a], Patrizia Scafato[a], Sandra Belviso[a], and Stefano Superchi*[a]

In memory of Carlo Rosini, who pioneered the use of biphenyl chiroptical probes for configurational assignments.

Abstract: A novel non-empirical approach for the assignment of absolute configuration of chiral 2-alkyl substituted carboxylic acids and primary amines by $[\alpha]_D$ measurements has been developed. The method requires the conversion of the chiral acids or amines into the corresponding 4,4'-disubstituted biphenylamides or biphenylazepines, respectively. In these derivatives a central-to-axial chirality transfer induces a preferred torsion in the biphenyl moiety revealed by the sign of the biphenyl A band in the ECD spectrum. By 4,4'-substitution on the biphenyl moiety a redshift of the A band is obtained, leading to an increase of its relative contribution to optical rotation. This allows to reliably establish a direct correlation between the $[\alpha]_D$ sign, the biphenyl twist and, then, the substrate absolute configuration. This approach thus constitutes a really practical and reliable method to assign the absolute configuration of chiral carboxylic acids and primary amines by simple and straightforward $[\alpha]_D$ measurement, readily obtainable by a routine instrumentation like the polarimeter.

Introduction

Optical rotation (OR) has been the first physical property to be linked to the molecular absolute configuration[1] and since XIX century it has been the commonest parameter employed to characterize (and distinguish) a couple of enantiomers. As a consequence, since the beginning, many efforts have been devoted to employ this chiroptical property to assign the absolute configuration of chiral molecules. Its direct use for assignment of absolute configuration dates back to the seminal studies of Kuhn in 1938,[2] but the most important achievements were reached with the pioneering work of Djerassi who developed the octant rule,[3] i.e. the first general, albeit empirical, approach to assign by OR measurements the absolute configuration of a whole class of chiral molecules, like the ketones. Despite these relevant historical achievements, since the sixties of XX century OR and its measurement at different wavelengths, *i.e.* Optical Rotatory Dispersion (ORD), have been largely replaced in configurational assignments by Electronic Circular Dichroism (ECD), which permits easier Cotton effects detection and then more reliable spectra interpretation. This prompted the development of several empirical and nonempirical (e.g. the exciton chirality method)[4] ECD approaches for the absolute configuration assignment, while the use of OR for this purpose was substantially abandoned. Only the advent of computational methods for OR/ORD calculation[5] allowed to overcome the problems allied to the ORD spectra interpretation, thus opening new perspectives to this technique for absolute configuration assignment.[6] However, despite the many successful applications reported in the literature,[7] this approach still remains difficult to apply for highly flexible molecules, displaying many similarly populated conformers and/or giving rise to low chiroptical response. In these cases long and time-consuming conformational analyses are required and often the use of only OR or even ORD data is not sufficient to provide reliable results.[8] These drawbacks of the computational approaches can be overcome by resorting to the so-called "chiroptical probes", i.e. achiral chromophoric moieties which, when linked to a chiral non racemic substrate, give rise to diagnostic chiroptical signal(s) from the sign of which the absolute configuration of the substrate can be determined. This approach, unlike the computational methods, does not require a precise spectral prediction and often the sign of a single chiroptical data (e.g. the sign of a diagnostic ECD Cotton effect) is sufficient for the absolute configuration assignment.[9] The chiroptical probes described so far are all chromophoric systems and the chiral induction is commonly revealed by the ECD spectra,[10] while no examples are reported till now in which OR/ORD data are the output of the chiroptical probe used for the configurational assignment. On the contrary, it will be really valuable to obtain a chiroptical probe system in which the absolute configuration detection could be achieved by very readily available OR measurements. In fact, such measurements require the use of a cheap and very common instrument, i.e. the polarimeter, and are routinely carried out in any chemical laboratory dealing with chiral non-racemic molecules. Moreover, the use of the now commercially available HPLC OR detectors could allow, at the same time, to separate two enantiomers on a chiral stationary phase and to determine their absolute configuration online by OR measurement, allowing a great saving of time with respect to the traditional approaches.

The 2,2'-bridged biphenyl chiroptical probes, that we introduced some years ago for the absolute configuration assignment to chiral diols,[11] carboxylic acids,[12] and, more recently, to primary amines,[13] appear a quite suitable system to reach the goal of OR detection of absolute configuration. In these biphenyl probes, thanks to the low aryl-aryl rotational barrier displayed,[14] a central-to-axial chirality transfer occurs between the chiral substrate and the biphenyl moiety which, in turns, assumes a preferred *M* or *P* twist depending on the substrate absolute configuration. Moreover, the sense of the biphenyl twist is easily revealed by the sign of the ECD Cotton effect at around 250 nm, in correspondence to the biphenyl A band absorption.[15] A positive sign of such Cotton effect corresponds to *aR*

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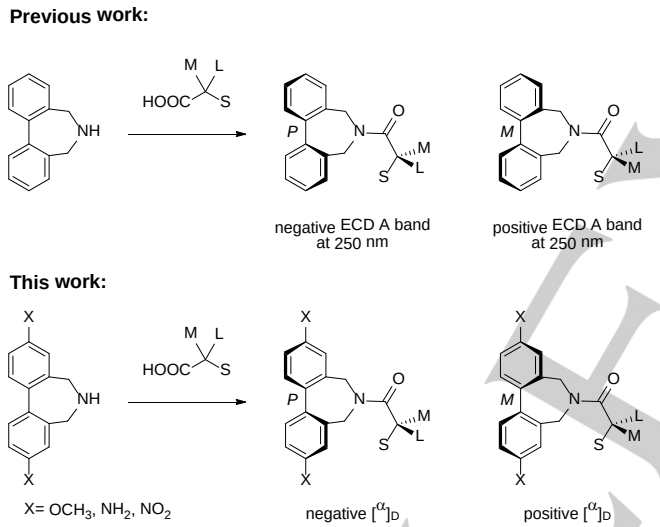
configuration (*M* torsion), reversely, a *aS* configuration (*P* torsion) is allied to a negative A band in the ECD spectrum.[16] Therefore, once determined the mechanism of twist induction in the biphenyl moiety, the absolute configuration of the chiral substrate can be assigned simply looking at the ECD spectrum of the biphenyl derivative.

To reach the same result by measuring OR or, more practically, specific optical rotation $[\alpha]_\lambda$, that is its value normalized for sample concentration (in g/100mL) and cell pathlength (in dm), this chiroptical property has to be unequivocally related to the sign of the ECD biphenyl A band, then to biphenyl torsion and, ultimately, to the absolute configuration of the investigated chiral substrate. In this work we demonstrate the feasibility of this approach taking as an example the absolute configuration assignment to chiral carboxylic acids, for which the use of the biphenyl probes through the transformation in the corresponding biphenylamides, proved to be particularly straightforward and reliable (Scheme 1).[12] The application to the assignment of absolute configuration of α-substituted chiral primary amines is also provided.



Scheme 1. Biphenyl probes for the absolute configuration determination to chiral acids by either ECD or OR chiroptical properties.

In the biphenylamides derived from aliphatic α-chiral carboxylic acids the two diastereomeric conformations depicted in Figure 1, having opposite biphenyl twist, are in a thermodynamic equilibrium and the major one is then the most stable one. For the absolute configuration depicted in Figure 1 it can be clearly seen that in the diastereomer having *M* torsion the largest group R_L is located in a more sterically crowded area, owing to the presence of one of the biphenyl rings, while the medium-size group R_M is in a less hindered region. The opposite holds for the *P* diastereomer, where the R_M group is closer to the biphenyl aromatic ring and the R_L group lies in a less crowded area. Therefore, the *P* diastereomer, giving rise to less steric interactions, is more stable than the *M* one.[12]

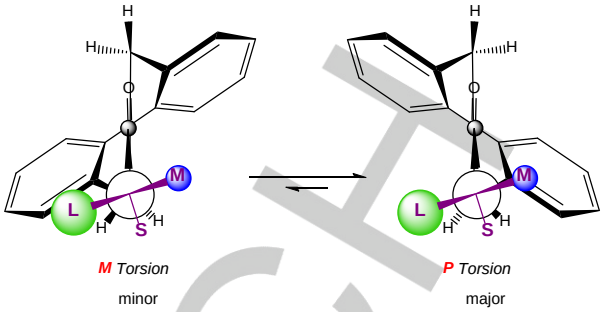


Figure 1. Schematic representation of the conformational equilibrium in biphenyl amides. L = largest group, M = medium size group, S = smallest group. (See ref.12 for details)

For α-chiral carboxylic acids a non-empirical rule was then established allowing to determine the absolute configuration by inspecting the sign of the biphenyl A band at ~250 nm in the ECD spectrum of their biphenylamides. According to such rule, a *P* biphenyl twist is preferred for 2-substituted aliphatic chiral carboxylic acids having absolute configuration such that a clockwise rotation leads from the largest to the smallest substituent on the acid moiety, and thus a negative A band is expected at around 250 nm in the ECD spectrum of the corresponding biphenyl derivatives. Vice versa, an *M* twist is preferred for a counterclockwise disposition of the substituents, and a positive A band arises in the ECD spectrum (Figure 2). An opposite rule was demonstrated to hold for aryl substituted α-chiral carboxylic acids.[12] To apply the present model, the group size priority is inferred from commonly employed steric parameters reported in the literature, such as the cyclohexane's A values,[17] or the so-called B values derived from free energies of the aryl–aryl bond rotations.[18] Obviously, the model does not take into account the possible effect on the conformational equilibria of non-covalent bonds (H-bond, π-π stacking).

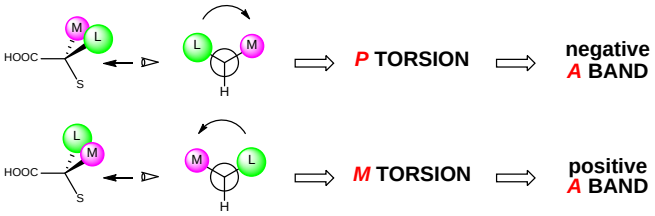


Figure 2. Mnemonic scheme relating the absolute configuration of alkyl-substituted α-chiral carboxylic acids and the sign of the A band in the ECD spectrum of their biphenylamides. (See ref.12 for details)

As reported above, to employ $[\alpha]_\lambda$ for the absolute configuration assignment we have to link its sign to the ECD A band sign and then to the biphenyl twist. $[\alpha]_\lambda$ and ECD are two physical properties linked by the Kramers-Kronig (KK) transform,[19] so that, if one of these two properties is known as a function of wavelength, the second one can be in principle obtained via KK transform. The KK transform can be in turn approximated by a sum-over-states expression[20] which at 589 nm (sodium D line

wavelength) assumes the simplified form in Equation 1, where M is the molecular weight and *Ri* and λ_i are the rotatory strength and wavelength of the *i-th* electronic transition, respectively. From this mathematical relationship it results that each ECD transition (band) gives a contribution $[\alpha]_D$ to the value of $[\alpha]_\lambda$ at the sodium D line ($[\alpha]_D$) directly proportional to its rotatory strength R_i and inversely proportional to its distance (in wavelength) from 589 nm. In other words, the more intense a band or the closer its wavelength to the sodium D line, the higher its contribution to $[\alpha]_D$.

$$[\alpha]_D = \left(\frac{9.14\times10^{43}}{M}\right)\sum_i R_i \frac{\lambda_i^2}{589^2-\lambda_i^2} = \sum_i [\alpha_i]_D$$

Equation 1. Simplified sum-over-states expression at 589 nm (see text).

Therefore, the contribution to $[\alpha]_D$ of the ECD A band can be increased either shifting it at longer wavelengths or increasing its intensity. The former result can be reached more easily because it can be obtained modifying the biphenyl probes with the introduction of appropriate substituents. In fact, several studies have shown that the introduction in 4,4' positions of substituents extending the biphenyl conjugation leads to a bathochromic shift of the A band transition of the biphenyl chromophore.[21,22]

Results and Discussion

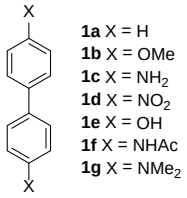
Spectral analysis of 4,4'-disubstituted biphenyl chromophores.

First, to sort out the most suitable substituents for the biphenyl substitution, we carried out a detailed analysis on the absorption spectra of 4,4'-disubstituted biphenyls. Therefore, the UV spectra of biphenyls **1a-g** (Chart 1) were measured in the 200-400 nm range (Table 1 and Figure S1 in Supporting Information, SI). As inferred from Table 1, methoxy and hydroxy substituents bathochromically shift the A band absorption of ~22÷24 nm (entries 2 and 3), amino and acetamido of ~48÷50 nm (entries 4 and 5), while the highest shift of ~65÷67 nm is provided by dimethylamino and nitro substitution (entries 6 and 7).

Table 1. Experimental A band wavelength and intensity in 4,4'-disubstituted biphenyls.[a]

Entry	Biphenyl	X	λ_{max} (nm)[b]	$\Delta\lambda$[c]	$\varepsilon\cdot10^{-3}$
1	**1a**	H	244.0[d]	/	
2	**1e**	OH	268.0	24.0	19.6
3	**1b**	OCH_3	266.4	22.4	22.5
4	**1f**	NH(Ac)	294.0	50.0	27.8
5	**1c**	NH_2	292.0	48.0	25.8
6	**1g**	$N(Me)_2$	309.3	65.3	28.5
7	**1d**	NO_2	310.9	66.9	66.9

[a]Spectra recorded in THF (c ~ 10^{-3} *M*). [b]Wavelength of A band maximum. [c]Wavelength difference in respect to **1a**. [d]Data taken from ref. [19b].



1a X = H
1b X = OMe
1c X = NH_2
1d X = NO_2
1e X = OH
1f X = NHAc
1g X = NMe_2

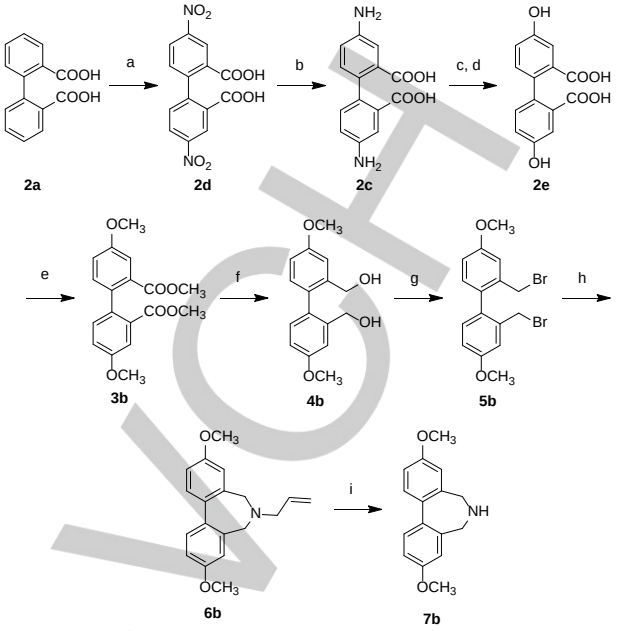
Chart 1.

These results experimentally confirm that 4,4'-substitution with groups extending the biphenyl conjugation redshift the A band wavelength. To apply the same substitutions to enhance the contribution of A band to $[\alpha]_D$ in the biphenyl probes we shall first to verify whether or not such substitution changes the nature of the A band, i.e. the type of the electron transitions underneath. This investigation was carried out by a DFT computational analysis of the biphenyls electronic transitions in **1a-g**. Accordingly, for each compound a conformational analysis was performed first by Molecular Mechanics (MM)[23] and then at DFT/B3LYP/TZVP level.[24] For each conformer the UV spectrum was calculated by Time Dependent DFT (TDDFT), B3LYP functional, and TZVP basis set considering the first 30 states. Theoretical spectra were obtained as averages over the conformers Boltzmann populations at room temperature. The obtained spectra were compared to the experimental ones (Figures S2÷S8 in SI) and for each absorption band molecular orbitals involved in the corresponding electronic transitions were analyzed (Figures S24÷S30 in SI). Notably, TDDFT computations very well reproduce the experimental absorption spectra in the 200-400 nm range, thus providing a good proof of computation reliability and robustness. The molecular orbitals analysis shows that in all the biphenyls the absorption of the A band is always due to the HOMO-LUMO electronic transition. In all the cases HOMO electronic distribution is very similar to the one of the unsubstituted biphenyl, while LUMO shape is slightly

affected by the substituents. Therefore, we can conclude that 4,4'-substitution redshifts the A band wavelength, not affecting its nature, and then presumably not affecting its direct relationship with the biphenyl twist. Moreover, inspection of theoretical spectra shows that the presence of these 4,4'-substituents on the biphenyl system affects essentially the A band energy, leaving almost unchanged the wavelength position of the other bands (Figure S2 in SI). Thus, 4,4'-substitution essentially increases only the relative contribution of the A band to $[\alpha]_D$. Dynamic chiral gas chromatography studies have also shown that the presence of substituents in 4,4'-positions even influences the rotational barrier of the biphenyl aryl-aryl bond.[25] Therefore we computationally studied this effect carrying out a relaxed potential energy surface (PES) scansion on 4,4'-disubstituted 2,2'-dimethyl-1,1'-biphenyls, taken as model compounds (see Figure S34 and Table S4 in SI), at DFT/B3LYP/TZVP level of theory, rotating the dihedral angle between the two aryl moieties of each biphenyl.[26] Computations confirm that the rotational barrier energy indeed depends on the electronic nature of the substituents, following the order NH_2>OCH_3>H>NO_2. However, in general, the rotational barrier found is not much different from that of the unsubstituted derivative, showing that the 4,4'-disubstitution does not limit the free rotation of the aryl-aryl bond and then interconversion of the two atropisomeric torsions.

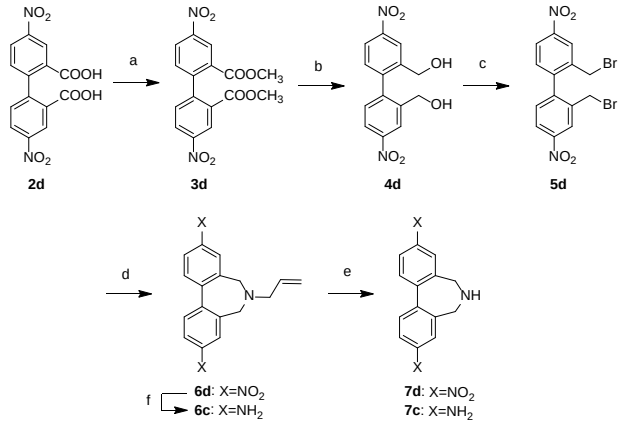
Synthesis and UV spectral analysis of 4,4'-disubstituted biphenylazepines probes.

Driven by these results we then prepared, starting from diphenic acid, the three chiroptical probes **7b-d**, one for each shift range (*vide supra*) and having OCH_3, NH_2 and NO_2 substitution, respectively. The 4,4'-dimethoxy substituted biphenyl azepine **7b** was synthesized as reported in Scheme 2. Bis-nitration of diphenic acid (**2a**) provided the 4,4'-dinitrodiphenic acid **2d** and the subsequent reduction of the nitro groups with Sn and HCl aq gave aminoacid **2c** as a precipitate after ammonia neutralization.[27] Then, **2c** was converted to the 4,4'-dihydroxydiphenic acid **2e** through diazo chemistry: compound **2c** was treated with hydrochloric acid and sodium nitrite to form the corresponding diazonium salt, which was decomposed in phosphoric acid to afford bis-phenol **2e**.[25] A simultaneous methylation of phenolic and acid moieties afforded the diester **3b**, which was then reduced to diol **4b** by $LiAlH_4$.[28] A standard bromination of **4b** with PBr_3 led to formation of the benzyl dibromide **5b**, which was cyclized to afford the seven-membered allylazepine **6b** by reaction with allylamine.[29] Finally the 4,4'-dimethoxy-substituted chiroptical probe **7b** was obtained after allyl removal from **6b** by treatment with 1,3-dimethylbarbituric acid (NMBA), in the presence of $Pd(OAc)_2$ catalyst and PPh_3.[30]



Scheme 2. (a) HNO_3, H_2SO_4, H_2O, -15°C; (b) Sn, 9M HCl aq, rt; (c) HCl aq, $NaNO_2$; (d)H_3PO_4 aq; (e) CH_3I, K_2CO_3, DMF, rt; (f) $LiAlH_4$, dry THF; (g) PBr_3, CH_3Cl; (h) allylamine, CH_3CN, 50°C; (i) NMBA, $Pd(OAc)_2$, PPh_3, CH_2Cl_2, 35°C.

The biphenyl azepines **7c** and **7d**, bearing amino and nitro substituents, respectively, were synthesized from diphenic acid as well, following the procedures reported in Scheme 3. Accordingly, the dimethyl 4,4'-dinitro-2,2'-biphenyldicarboxylate **3d** was obtained heating at reflux the 4,4'-dinitrodiphenic acid **2d** in methanol in the presence of catalytic H_2SO_4. Compound **2d** was then treated with DIBAL-H in order to reduce only the ester moieties, leaving unmodified the nitro groups, thus obtaining diol **4d**. After bromination of the hydroxy groups with PBr_3 to provide **5d**, the resulting dibromide was reacted with allylamine giving rise to the formation of the seven-membered ring in **6d**.[29] The 4,4'-dinitro-substituted probe **7d** was obtained after reaction of **6d** with NMBA, $Pd(OAc)_2$ and PPh_3 to remove the allyl moiety.[30] Compound **6d** was also reduced to the diamino compound **6c** by reaction with Sn and 9M HCl aq and the latter de-allylated to the 4,4'-diamino-substituted chiroptical probe **7c**.



Scheme 3. (a) H_2SO_4, CH_3OH, reflux; (b) DIBAL-H, CH_2Cl_2, reflux; (c) PBr_3, CH_3Cl, reflux; (d) allylamine, CH_3CN, 50°C; (e) NMBA, $Pd(OAc)_2$, PPh_3, CH_2Cl_2, 35°C; (f) Sn, 9M HCl aq, rt.

The UV spectra of **7b**, **7c** and **7d** were then measured in the 200-400 nm range (Figure 3), observing in all the three compounds approximately the same redshift of the A band in respect to the A band position in the unsubstituted biphenylazepine **7a** displayed by the parent 4,4'-disubstituted biphenyls **1b-d** (~24, ~47 and ~62 nm, respectively).

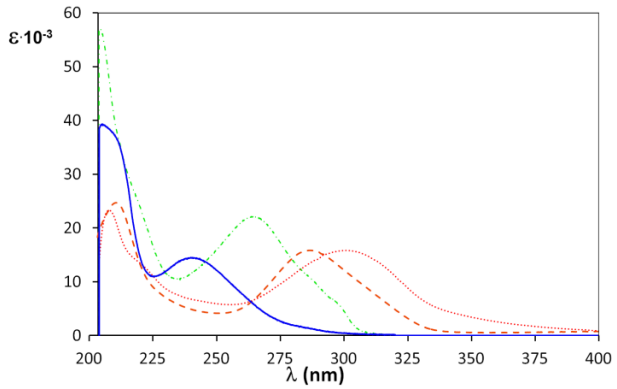
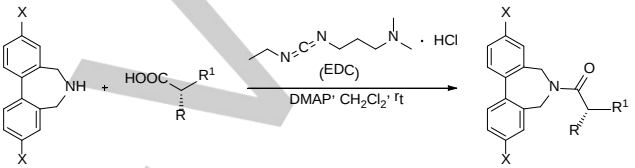


Figure 3. Experimental UV spectra (c ~10^{-3} M in THF) of **7b** (X=OMe, dashed dotted green line), **7c** (X=NH_2, dotted orange line), and **7d** (X=NO_2; red dotted line) compared to the unsubstituted azepine **7a** (X=H, solid blue line).

Again, the nature of the electronic transition(s) allied to the absorption of the A band in compounds **7b-d** was investigated by quantum mechanical calculations of their UV spectra. Considering that in the case of the biphenyls each conformer shows identical UV spectra, a single point calculation of ground state energy at DFT/B3LYP/TZVP level was carried out. The UV spectra were calculated by TDDFT/B3LYP/TZVP computations taking into account the first 30 states. The theoretical results well reproduce the experimental spectra (See Figures S9÷S13 in SI), again confirming the reliability of the calculations. As inferred from the molecular orbitals (See Figures S31÷S33 in SI), also in this case the A band nature is not affected by the 4,4'-substitution and such substitution causes a redshift of only this band, leaving almost unaffected the wavelength position of the other absorption bands (Figure S9 in SI).

Synthesis and ECD spectra of biphenyl amides from chiral carboxylic acids. We then proceeded with the synthesis of the biphenylamides of *N*-Boc-L-valine (**9a**), L-lactic acid (**9b**), and (*S*)-2-methylbutanoic acid (**9c**) following a standard procedure (Scheme 4).[31] These three acids were chosen as representative examples of amino, hydroxy, and alkyl α-substituted chiral aliphatic acids. Being all aliphatic acids they follow the same biphenyl twist induction rule reported in Figure 2 and are devoid of aryl chromophores which could give rise to spectral interferences with the biphenyl signals.



7b: X=OCH_3
7c: X=NH_2
7d: X=NO_2

9a: R=NHBoc R^1=*i*-Pr
9b: R=OH R^1=CH_3
9c: R=CH_3 R^1=CH_2CH_3

10b: X=OCH_3; R=NHBoc R^1=*i*-Pr
10c: X=NH_2; R=NHBoc R^1=*i*-Pr
10d: X=NO_2; R=NHBoc R^1=*i*-Pr
11b: X=OCH_3; R=OH R^1=CH_3
11c: X=NH_2; R=OH R^1=CH_3
11d: X=NO_2; R=OH R^1=CH_3
12b: X=OCH_3; R=CH_3 R^1=CH_2CH_3
12d: X=NO_2; R=CH_3 R^1=CH_2CH_3

Scheme 4. Synthesis of biphenyl amides from chiral aliphatic carboxylic acids.

ECD and UV spectra of the purified biphenylamides **10b-d**, **11b-d**, and **12b,d** were measured and compared with the unsubstituted derivatives.[12] As inferred from the UV and ECD spectra (Table 2, Figure 4 and Figure S15÷S16 in SI) in the 4,4'-disubstituted biphenylamides the biphenyl A band is bathochromically shifted compared to the parent unsubstituted amides.

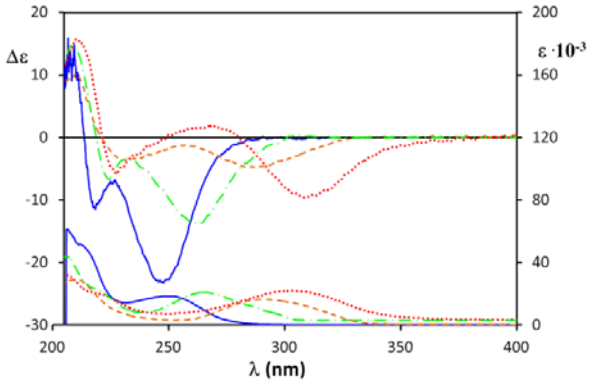


Figure 4. Experimental UV and ECD spectra (c ~10^{-3} M in THF) of **10b** (X=OMe, dashed dotted green line), **10c** (X=NH_2, dotted orange line), and **10d** (X=NO_2; dotted red line) compared to the unsubstituted azepine **10a** (X=H, solid blue line).

Table 2. ECD experimental A band wavelength and Δε of biphenylamides **10b-d**, **11b-d**, and **12b,d** compared to the corresponding unsubstituted derivatives **10a**, **11a**, and **12a**.[a]

Amide	X	λ_{max}(nm)[b][Δε][c]		
		10	**11**	**12**
a	H	247.8[-23.2][d]	246.0[-8.7][d]	246.0[-6.3][d]
b	OCH_3	263.6[-13.7]	262.8[-4.5]	261.6[-1.4]
c	NH_2	289.3[-4.7]	298.8[-1.0]	n.d.
d	NO_2	310.8[-9.3]	307.9[-4.8]	310.5[-2.2]

[a]Recorded in THF (c ~10^{-3} *M*); [b]Wavelength of A band maximum. [c]Values in L mol^{-1} cm^{-1}. [d]Data taken from ref.[12].

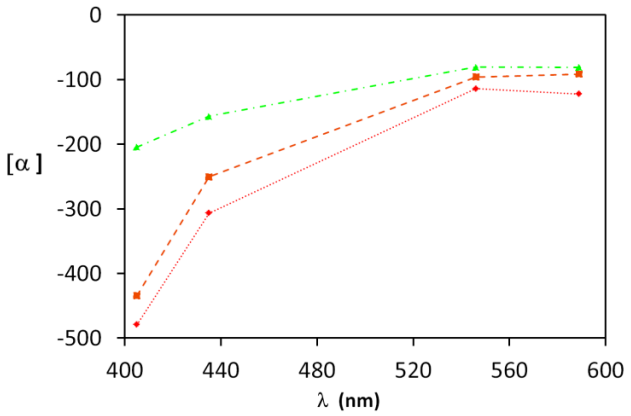


Figure 5. ORD curves of **10b** (X=OMe, dashed dotted green line), **10c** (X=NH_2, dashed orange line), and **10d** (X=NO_2, dotted red line) recorded in $CHCl_3$.

In correspondence to absorption maxima of A band, negative Cotton effects are observed in the ECD spectra, as expected from the *P* torsion predicted by the general rule in Figure 2 (Figure 4 and Figures S15÷S16 in SI).[12] The redshift of the A band was accompanied by a broadening of the same band and, although the Δε value decreased, the band area (and then the allied transition rotational strength) remained substantially unaffected, compared to the other absorption bands. The OR of **10b-d**, **11b-d** and **12b,d** was then measured at 589 nm observing a direct relationship between the sign of $[\alpha]_D$ and the sign of the Cotton effect corresponding to the A band. An increase of the absolute value of $[\alpha]_D$ was also observed with the shift of the A band at longer wavelengths (Table 3). An even stronger effect was revealed by the ORD curves (Figure 5 and Figures S17÷S19 in SI). In fact, the redshift of the A band gives rise to quite high OR values at shorter wavelengths, as inferred also from OR values at 435 nm reported Table 3. Therefore, the OR measurement at either 405 or 435 nm (readily available with polarimeters equipped with wavelength filters and tungsten or mercury lamps) makes the configurational assignment even more reliable.

Table 3. Optical rotation values of disubstituted biphenyl amides **10b-d**, **11b-d**, and **12b,d** compared to the corresponding unsubstituted derivatives **10a**, **11a**, and **12a**.

Amide	X	$[\alpha]_D^{20}$		
		10[a,b]	**11**[a,b]	**12**[a,b]
a	H	-21[c]	-40[c]	-4.0[c]
b	OCH_3	-81[-157]	-86[-165]	-9.2[-29]
c	NH_2	-91[-251]	-36[-63]	n.d.
d	NO_2	-122[-306]	-134[-347]	-11[-103]

[a]Recorded in $CHCl_3$, concentrations in Experimental Section, values in deg cm^3 g^{-1} dm^{-1}. [b]In square brackets optical rotations recorded at 435 nm. [c]Data taken from ref.[12].

Remarkably, in the case of compound **11c** (NH_2 substituted) the ECD spectrum is much less intense than in the other derivatives. The $[\alpha]_D$ value of **11c** is also smaller than in **11b** and **11d** still obtained from (*S*)-lactic acid (Table 3). The reason of this behavior was investigated by quantomechanical calculations. For each (*S*)-lactic acid amide a MM conformational analysis was performed by Montecarlo method on both *P* and *M* torsion. The minimum energy conformers found by MM were further fully optimized by DFT/B3LYP/TZVP computations and re-optimized at DFT/M062X/TZVP level, employing the M062X functional which is known to take better into account conjugation interactions between distant atoms.[32] A Boltzmann distribution of minima was calculated, and the ratio of *M* and *P* isomers was obtained (Table 4).

Table 4. Ratio of *M* and *P* isomers of biphenylamides derived from L-lactic acid **11**.

Entry	Amide	X	*P/M* twist ratio[a]
1	**11a**	H	76/24
2	**11b**	OCH_3	84/16
3	**11c**	NH_2	54/46
4	**11d**	NO_2	58/42

[a]Obtained by conformational analysis at DFT/M062X/TZVP level of theory.

It was found that in the case of compound **11c** the *P* and *M* isomers are in a 54:46 ratio, a lower value than in the other derivatives. Therefore, the absence of a strongly preferred torsion leads to a less intense ECD spectrum. In these cases, when ECD A band intensity and/or $[\alpha]_D$ absolute value are particularly low, some ambiguities in the absolute configuration assignment could arise. Measurement of $[\alpha]$ at shorter wavelengths could then help, enhancing its absolute value and then the assignment reliability.

In summary, in all the analyzed cases the presence of substituents in 4,4'-positions leads to a redshift of the UV biphenyl A band and of the corresponding Cotton effect in the ECD spectra. The latter then increases its contribution to $[\alpha]_D$ absolute value, compared to the corresponding unsubstituted biphenylamides, so that $[\alpha]_D$ sign results correlated to that of this Cotton effect. A more certain correlation of the $[\alpha]_D$ sign (whose absolute value is also increased) with that of the A band and thus with the absolute configuration then results. These results show that disubstituted biphenyl probes can be used to determine the absolute configuration of chiral carboxylic acids by simple $[\alpha]_D$ measurements.

Relative contribution to $[\alpha]_D$ of A band in 4,4'-disubstituted twisted biphenyls. The increase of the A band contribution to $[\alpha]_D$ value can be quantitatively determined by means of the sum-over-states expression (Equation 1). This equation states that $[\alpha]_D$ derives from the sum of contributions coming from all the electronic transitions and that the contribution of a single *i-th* electronic transition to $[\alpha]_D$ (i.e. $[\alpha_i]_D$) can be obtained by the corresponding wavelength (λ_i) and rotatory strength (R_i) (Equation 2). Therefore, from Equation 3 it is possible to determine the relative contribution of A band to $[\alpha]_D$ (i.e. $[\alpha_A]_D$), allowing then to estimate to what extent the A band sign determines the $[\alpha]_D$ sign.

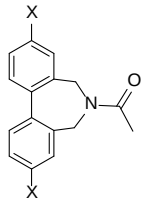
$$[\alpha_i]_D = \left(\frac{9.14\times10^{43}}{M}\right) R_i \frac{\lambda_i^2}{589^2 - \lambda_i^2}$$

Equation 2. Contribution of a *i-th* band to optical rotation.

$$weight_A\% = \frac{|[\alpha_A]_D|}{\sum_i |[\alpha_i]_D|}\times 100$$

Equation 3. Relative contribution of A band transition to optical rotation. $[\alpha_A]_D$ and $[\alpha_i]_D$ are the contribution to $[\alpha]_D$ of A band and *i*-th electronic transition, respectively.

To determine the influence of the A band bathochromic shift on $[\alpha]_D$ we then computed rotatory strengths of first 30 electronic transitions, and thus ECD spectra, of the model compounds *N*-acetyl biphenylamides **8a-d** (Chart 2), assuming a *P* twist of the biphenyl moiety. In principle, the sum-over states in Equation 1, and then summation at the denominator in Equation 3, should be carried out on infinite number of electronic transitions. This is obviously impossible and practical reasons dictate that only a finite number of electronic transitions can be used. In fact, it has been observed that the low-lying valence and Rydberg transitions usually are not of major importance for evaluation of OR in the nonresonant long wavelength region[20b] and the use of the first 25-30 transitions is generally sufficient.[20a] The computation on the first 30 excited states also allows us to take into account the contribution of transitions occurring below 200 nm, not experimentally visible because of the solvent cut-off. To make calculations general and representative of biphenyl signals allied to amides derived from any aliphatic carboxylic acid we chose these acetyl model compounds lacking of other chromophores and stereogenic centers. The geometry was optimized by a single point calculations at DFT/B3LYP/TZVP level in gas phase. The optimized structures were used for excited states calculation, performed at TDDFT/CAM-B3LYP/aug-cc-pVDZ theory level. The ECD spectra were obtained from calculated excitation energies and rotational strengths, as a sum of Gaussian functions centered at the wavelength of each transition (Figure 6).



8a: X= H
8b: X= OCH_3
8c: X= NH_2
8d: X= NO_2

Chart 2.

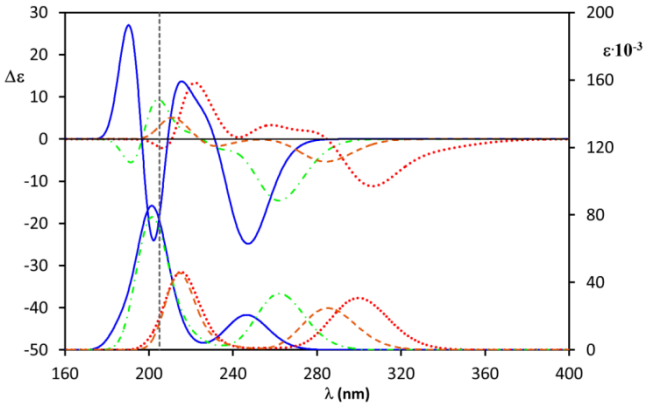


Figure 6. Theoretical UV and ECD spectra (TDDFT/CAM-B3LYP/aug-cc-pVDZ/gas phase) of **8b** (X=OMe, green dashed dotted line), **8c** (X=NH_2, orange dashed line), and **8d** (X=NO_2; red dotted line) compared to the unsubstituted biphenylamide **8a** (X=H, blue solid line). The theoretical spectra were shifted to fit the experimental maxima of **10a-d** (*vide supra*). The vertical dashed line indicates the solvent (THF) cut-off.

Calculations confirmed that also in acetylamides **8b-d** the nature of A band is unaffected by substitution. In fact, the electronic distribution of the orbitals involved in the transition is very similar to the one found before in the case of biphenyls and biphenylamines. Moreover, the theoretical ECD spectra confirms that the sign of the A band Cotton effect depends only on the biphenyl moiety torsion. Computed excitation transitions energies were shifted to fit the experimental data for A band maximum obtained for biphenylamides **10a-d**.[16] By inserting in equation 2 the rotational strengths and wavelengths computed for the first 30 electronic transitions of **8b-d**, the contribution $[\alpha_i]_D$ of any single transition is determined, included that of A band transition $[\alpha_A]_D$. From equation 3 the relative A band contribution to $[\alpha]_D$ is then obtained. As inferred from Table 5 the 4,4'-substitutions provide the desired increase of the relative A band contribution to $[\alpha]_D$. Taking also into account that many high energy ECD transitions are oppositely signed (Figure 6) and then mutually cancelling themselves, it results that the $[\alpha]_D$ sign is essentially determined by the A band sign. This confirms our assumptions and opens the way to the use of 4,4'-disubstituted biphenyl probes for the absolute configuration assignment by $[\alpha]_D$ measurement.

Table 5. Computed A band Cotton effect maximum wavelengths and relative contribution to $[\alpha]_D$ of compounds **8a-d**.

Amide	X	λ_{max} [a]	$\Delta\lambda$ [b]	A band contribution to $[\alpha]_D$ [c]
8a	H	246.0	/	15%
8b	OCH_3	261.4	15.4	30%
8c	NH_2	286.8	40.8	34%
8d	NO_2	306.0	60.0	50%

[a]Maxima were shifted to better fit the experimental ECD spectra of compounds **10a-d** (*vide supra*). [b]Wavelength difference in respect to the unsubstituted biphenyl **8a**. [c]Calculated by Equation 3.

Disubstituted biphenyl azepines as chiroptical probes for chiral primary amines. In order to extend the scope of such approach, we verified its applicability to the assignment of absolute configuration to α-substituted chiral primary amines. Also in this case a non-empirical rule was established allowing to determine the absolute configuration of the primary amines by inspecting the ECD spectrum of their corresponding biphenylazepines.[13] According to such rule an *M* biphenyl twist is preferred for α-substituted chiral primary amines having absolute configuration such that a clockwise rotation leads from the largest to the smallest substituent on the amine moiety (Figure 7), and thus a negative A band is expected at around 250 nm in the ECD spectrum of the corresponding biphenyl derivatives. Vice versa, a *P* twist is preferred for a counterclockwise disposition of the substituents, and a positive A band arises in the ECD spectrum. An opposite rule is operative in the case of aryl substituted α-chiral primary amines.[13a].

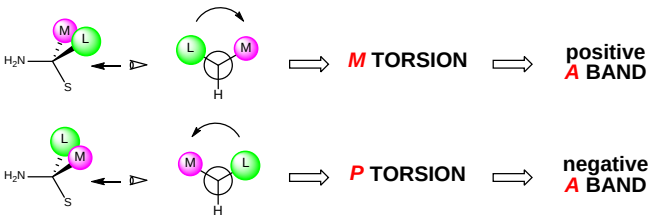
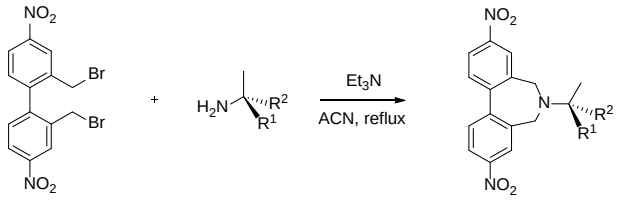


Figure 7. Mnemonic scheme relating the absolute configuration of α-alkyl-substituted primary amines and sign of the A band in the ECD spectrum of their biphenylazepines.

Therefore, the two 4,4'-dinitro substituted biphenylazepines **14d** and **15d** were prepared from amines **13a** and **13b**, respectively, following a previously described procedure (Scheme 5).[13a]



5d **13a** R_1=H R_2=*t*-Bu **13b** R_1=H R_2=Cy **14d** R_1=H R_2=*t*-Bu **15d** R_1=H R_2=Cy

Scheme 5. Synthesis of biphenyl azepine derivatives from α-chiral primary amines.

The ECD and UV spectra (See Figures S20÷S23 and Table S2 in SI) of **14d** and **15d** were recorded and the optical rotation values measured. As seen in case of the acids the A band of both the NO_2-substituted amine derivatives is bathochromically shifted (~80 nm) in respect to the corresponding unsubstituted biphenyl derivatives.[13a] Notably, no significant differences were observed in ECD spectra recorded in THF and methanol, revealing a negligible solvent effect on the conformational equilibrium (see Figures S20÷S23 in SI). Although the Δε of the A band decreases, the $[\alpha]_D$ absolute value of the disubstituted derivatives is always larger than the parent unsubstituted biphenyl probe, confirming that the redshift of A band increases its contribution to optical rotation (Table 6).

Table 6. Experimental A band wavelength and intensity in 4,4'-disubstituted biphenyls.

Entry	Derivative	X	λ_{max} (nm)	$\Delta\lambda^{[a]}$	Δε	$[\alpha]_D^{20}$[b]
1	**14a**[c]	H	247	/	-7.2	-45
2	**14d**	NO_2	327	80.0	-2.0	-54
3	**15a**[c]	H	249	/	-2.6	-27
4	**15d**	NO_2	326	77.0	-0.61	-62

[a]Wavelength difference in respect to the unsubstituted derivatives. [b]Recorded in $CHCl_3$, see concentrations in Experimental Section, values in deg cm^3 g^{-1} dm^{-1}. [c]Data taken from ref. [13a].

These results show that even the absolute configuration of chiral aliphatic primary amines can be reliably determined by looking at the sign of $[\alpha]_D$ after derivatization with a suitable 4,4'-dinitro biphenyl probe. This result then further widens the applicability of the method.

Conclusions

A novel non-empirical approach for the absolute configuration assignment of chiral 2-alkyl substituted carboxylic acids and primary amines by $[\alpha]_D$ measurements has been developed. The method requires the conversion of chiral acids or amines into the corresponding 4,4'-disubstituted biphenylamides or biphenylazepines, respectively. In these derivatives a central-to-axial chirality transfer induces a preferred axial torsion in the biphenyl moiety revealed by the sign of the biphenyl A band in the ECD spectrum. By 4,4'-substitution on the biphenyl moiety, a redshift of the A band is obtained, leading to an increase of its relative contribution to optical rotation. Therefore, the sign of $[\alpha]_D$ becomes essentially determined by the sign of the A band and a direct relationship between these two chiroptical data is thus established. This allows assignment of absolute configuration to acids and amines by to the sign of $[\alpha]_D$ of their biphenyl derivatives. The method is particularly reliable in the case of the dinitro substituted probe, in which the redshift is the largest. The stereochemical detection based on simple and straightforward optical rotation measurements has the great advantage to make it possible to assign the absolute configuration by means of a basic instrumentation like the polarimeter and through measurements routinely carried out in any chemical laboratory dealing with chiral molecules. Moreover, this approach opens the way to the simultaneous enantiomers separation and absolute configuration determination by using chiral HPLC coupled with OR detectors, thereby allowing a saving of time with respect to traditional approaches.

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Keywords: absolute configuration • optical rotation • biphenyls • circular dichroism • chiroptical probes

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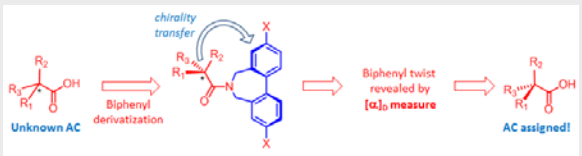

Absolute Configuration from Optical Rotation data: The use of 4,4'-disubstituted biphenyl chiroptical probes allows to determine in a non-empirical and straightforward way the absolute configuration of chiral α-substituted carboxylic acids and primary amines by simple $[\alpha]_D$ measurements. This goal is obtained by redshifting the UV and ECD A band of biphenyls by the introduction of suitable substituents at the 4,4' biphenyl position.

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Absolute Configuration Assignment from Optical Rotation Data by Means of Biphenyl Chiroptical Probes