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Intramolecular 1,3-dipolar cycloaddition of unsaturated nitrones derived from methyl α-D-glucopyranoside

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Abstract—The intramolecular 1,3-dipolar cycloaddition of unsaturated nitrones derived from methyl α-D-glucopyranoside with 2-furaldehyde has been studied. This cycloaddition was found to afford three 9-oxa-1-azabicyclo[4.2.1]nonane diastereomers in a 3:1:1 ratio [with the principal isomer possessing a (3*S*,4*R*,5*S*,6*S*,8*S*) configuration, determined by NMR spectroscopy]. The effects of different Lewis acid catalysts ($MgCl_2$, $ZnCl_2$ and $BF_3 \cdot OEt_2$) on yields and diastereomeric ratios have been examined in detail. The best result (90% yield) was achieved when $MgCl_2$ was present (in toluene, 120 °C bath temperature, 12 h). The stereoselectivity of the 1,3-dipolar cycloaddition was not significantly altered under the conditions investigated.

1. Introduction

Peptide nucleic acids (PNAs) are nucleic acid mimics bearing a pseudopeptide backbone (Fig. 1).[1,2] They possess very favourable hybridisation properties with nucleic acid targets, display high chemical and biological stabilities and have the potential to be used as both antisense and antigene therapeutic agents. Unfortunately, PNA has low lipid penetration and, consequently, poor cellular uptake. In an attempt to overcome these undesirable attributes, we have designed a conformationally restricted oligonucleotide analogue whose backbone should be positively charged under physiological conditions. These new chiral nucleoside analogues are termed azetidine nucleic acids (ANAs, Fig. 1). The pivotal step in the synthesis of the ANA monomers, needed for construction of the oligomers, is a diastereoselective intramolecular 1,3-dipolar cycloaddition involving unsaturated nitrones derived from carbohydrate precursors. Subsequent transformations on the corresponding isoxazolidines obtained should then afford the desired azetidine derivatives (Fig. 2).

We envisage that it will be possible to control the stereochemical outcome of the intramolecular 1,3-dipolar cycloaddition by virtue of steric constraint so that the actual number of isoxazolidine isomers produced would be reduced compared to the theoretical. The use of different Lewis acid catalysts is anticipated to improve both the stereoselectivity and reactivity of the nitrone. The mechanism of such 1,3-dipolar cycloadditions has been extensively studied by several authors.[3] Nitrones are nucleophiles which

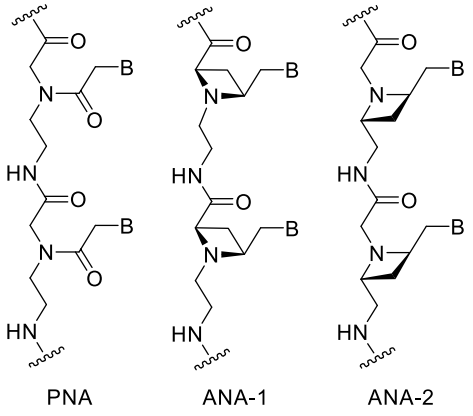

Figure 1. The structure of PNA and ANA oligomers (B, nucleobase). Only one diastereoisomer is shown for each ANA structure.

Keywords: 1,3-Dipolar cycloaddition; Isoxazolidines; Bicyclic 1,2-oxazepanes; 9-Oxa-1-azabicyclo[4.2.1]nonanes; Asymmetric synthesis.
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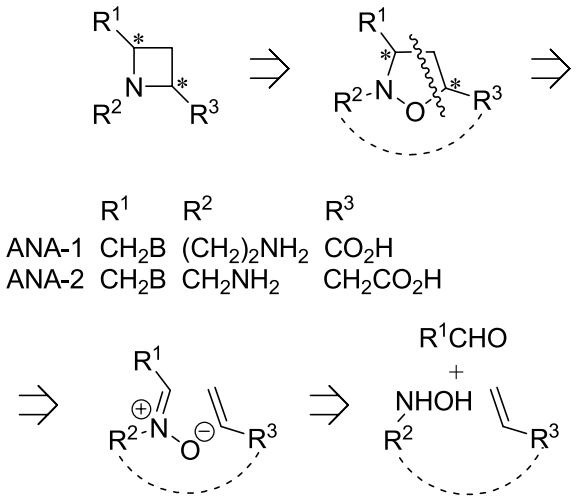

Figure 2. Retrosynthetic analysis of ANA monomers.

co-ordinate strongly to Lewis acids to form nitrone/Lewis acid complexes. These complexes are generated easily and they serve to assist in 1,3-dipolar cycloadditions through stabilization of the corresponding transition state and decreasing the energy gap between the LUMO and HOMO of one of the substrates.[4,5] Thus, a series of catalysts (metals and their complexes) has been developed for use in either normal or inverse electron demand 1,3-dipolar cycloadditions, for example, Mg^{2+}, Ti^{2+}, Zn^{2+}, Ni^{2+}, Pd^{2+}, Yb^{3+}, B(III), Al^{3+}, Cu^{2+}.[3] The 1,3-dipolar cycloaddition of nitrones bearing heteroaryl rings with electron-deficient alkenes has been investigated in detail by Merino et al.[6–8]

Our interest in the development of an efficient route for the synthesis of chiral isoxazolidines and, ultimately, azetidines led us to therefore consider using a similar strategy, utilizing unsaturated nitrones derived from carbohydrates, for their preparation. The starting material employed for the work reported herein was methyl α-D-glucopyranoside (**1**) (Fig. 3). In this case, the 1,3-dipolar cycloaddition investigated was a model reaction in order that suitable reaction procedures for performing such cycloadditions could be identified. It is envisaged that future employment of appropriate carbohydrate derivatives from the D-*manno* and D-*galacto* series in place of **1** will permit formation of isoxazolidines in which the hydroxyl groups in the carbohydrate portion are differentiated. The preparation of such compounds is an integral part of our synthetic approach to the desired ANA monomers.

2. Results and discussion

The starting material, methyl α-D-glucopyranoside **1**, was successfully converted into the benzoylated (**2**) and benzylated (**9**) 6-deoxy-6-iodo derivatives according to the methods described by Garegg[9] and Vasella,[10] respectively (Fig. 3). The subsequent Boord reaction on the halo derivatives [**2**→**3**, **9**→**10** (Fig. 3)] was accomplished upon treatment with zinc followed by sonication.[11] However, it was discovered that acceptable yields of the products **3** and **10** were only obtained for small scale

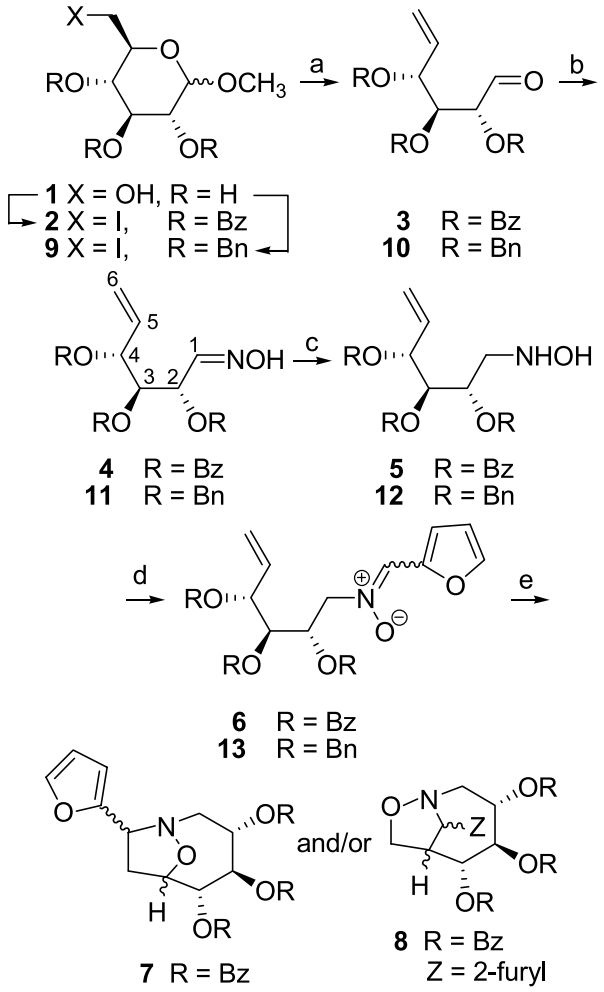

Figure 3. a: (**2**→**3**, **9**→**10**): Zn, sonication (1.6 g scale: 90%, 5 g scale: 30%) or Zn and Co(II)phthalocyanine (5 g scale: 70%), b: $NH_2OH{\cdot}HCl$, $NaHCO_3$ (70%), c: $NaBH_3CN$, HCl/dioxane, d: 2-furaldehyde, toluene (c + d: 30–40%), 4 Å MS, 50 °C, 18 h, e: toluene, 120 °C, 4 Å MS, Lewis acid catalyst, **7**: 9-oxa-1-azabicyclo-[4.2.1]nonane, **8**: 8-oxa-1-azabicyclo-[4.2.1]nonane skeletons.

reactions (up to 1.6 g of **2** or **9** afforded **3** or **10** in ca. 90% yields). Thus, several attempts were made to increase the scale of this reaction by employing activated zinc instead. This was prepared according to established methods,[13] for example, zinc–copper alloy[14] or the reduction of anhydrous zinc chloride with various alkali metals in the presence of naphthalene.[15,16] Heating solutions of **2** or **9** in ethanol at reflux in the presence of activated zinc produced by either method, afforded the same result, with respect to scale and yield (1 g scale ca. 70%, 5 g scale ca. 30% yield). When more than 5 g of the starting 6-deoxy-6-iodo derivative (**2** or **9**, respectively) was used and the activated zinc was prepared in situ from zinc chloride and lithium, the reaction also failed to go to completion. In this case, though, the remaining lithium in the reaction mixture caused decomposition of the unsaturated aldehyde and, also, prevented addition of water to the reaction mixture, which is necessary to dissolve zinc salts from the surface of zinc. Thus, all these procedures gave optimum product yields up to a maximum 1 g scale. Upon conducting further investigations into this Boord reaction, we found that, for large scale reactions, reasonable yields of the products **3** and **10** could be obtained

when zinc and cobalt(II) phthalocyanine was utilised (Kleban et al.[12] employed zinc and vitamin B_{12} for the same purpose) rather than zinc and sonication (5 g scale, ca. 70% yield).

Having prepared unsaturated aldehydes **3** and **10**, the next step in our synthetic pathway involved treatment with hydroxylamine at room temperature[17] to give oximes **4** and **11**, respectively (*E*/*Z* isomers in 1:1 ratio) (Fig. 3). Subsequently, **4** or **11**[18] were reduced with sodium cyanoborohydride and HCl/1,4-dioxane at the appropriate pH, depending on the protecting groups present, to afford **5** or **12**, respectively (Fig. 3). These hydroxylamines were used in the next step without further purification in order to avoid their decomposition. The mixture of HCl/1,4-dioxane had to be added slowly due to the acid sensitive nature of the benzoyl protecting groups and because further reduction of the hydroxylamine could easily occur at low pH which would result in formation of the amine instead. It was envisaged that this amine by-product would hinder the subsequent condensation step as it could react with 2-furaldehyde to give a Schiff's base, drastically reducing the yield of the cycloaddition reaction. Therefore, in an attempt to overcome this limitation, we have investigated performing the reduction of **4** and **11** in phosphate buffer solutions at various pHs, ranging from 4 to 8. Unfortunately, to date, all attempts have proved unsuccessful and so our original approach for preparing hydroxylamines **5** and **12** has been retained for the present work. Finally, crude hydroxylamines **5** and **12** were condensed with 2-furaldehyde to furnish the desired nitrones, **6** and **13**, required for investigation of the 1,3-dipolar intramolecular cycloaddition reaction (Fig. 3). These were afforded in overall yields of 30–40% for the two steps, after purification.

With nitrones **6** and **13** to hand, it was now possible to investigate the intramolecular 1,3-dipolar cycloaddition. This was simply accomplished by heating a solution of the appropriate nitrone in toluene at reflux in the presence of 4 Å molecular sieves (Fig. 3). Unfortunately, for the benzyl protected nitrone **13**, this reaction proved to be sluggish (toluene, reflux, 1 week) and very low yielding (<10%); therefore it was abandoned. For the benzoyl nitrone **6**, this reaction was found to be more successful and gave isoxazolidine **7** (Fig. 3) as a mixture of diastereoisomers in 17–90% yield as expected. These isomers were subsequently separated by column chromatography and characterised by NMR spectroscopy as the 9-oxa-1-azabicyclo[4.2.1]nonane diastereoisomers **7b–7d** (Fig. 4).

We were unable to isolate the fourth diastereisomer from the 9-oxa series (**7a**) (Fig. 4) as its yield was negligible. We assume that the other alternative product from this cycloaddition, 8-oxa-1-azabicyclo[4.2.1]nonane **8** (Fig. 3), did not form because of steric hindrance between the furyl side chain and the oxazepane ring.

Tables 1 and 2 show selected proton and carbon chemical shifts recorded in the ^{1}H and ^{13}C NMR spectra of compounds **7b–7d**. Upon assignment of the individual resonances by means of ^{1}H, ^{13}C, HSQC and HMBC NMR measurements, it was shown that protons H-6 and H-8 adopt a relative *trans* arrangement in the principal isomer **7b**

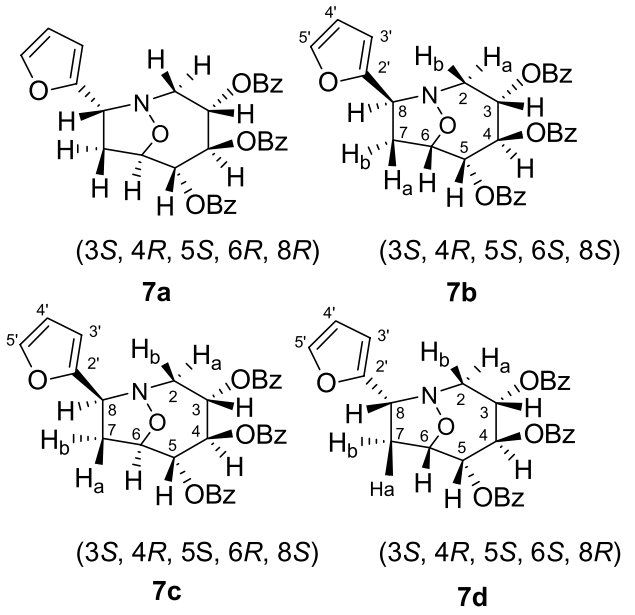

Figure 4. The structures of 9-oxa-1-azabicyclo[4.2.1]nonanes **7a–7d**.

whereas the equivalent protons in isomers **7c** and **7d** assume a *cis* arrangement. The NOESY spectrum of **7b** (Fig. 5) indicates the spatial proximity of protons H-2a, H-4, and H-8 and that of H-7b, H-4, H-8. Protons H-7b and H-2a reside on the bottom face of the structure relative to the oxazepane ring. In addition, it appears that proton H-7b is located far from its neighbours, H-2a, H-4 and H-8, as no coupling between H-7b and H-2a was detected (Table 4).

Table 1. Selected ^{1}H NMR chemical shifts of compounds **7b–7d**

Compound/ atom	7b	7c	7d
H-2a (dd)[a]	3.22	3.60	3.02
H-2b (dd)	4.22	2.89	3.53
H-3	5.85 (m)	5.96 (ddd)	5.89 (ddd)
H-4 (dd)	6.00	6.21	5.98
H-5	5.68 (dd)	5.51 (d)	5.69 (dd)
H-6	5.02 (m)	4.82 (dd)	4.96 (ddd)
H-7a (ddd)	2.75	2.83	2.67
H-7b (ddd)	3.18	3.14	2.86
H-8 (dd)	4.63	4.74	4.71
H-3′ (d)	6.28	6.60	6.51
H-4′(dd)	6.32	6.44	6.42

[a] Multiplicities in parenthesis.

Table 2. Selected ^{13}C NMR chemical shifts of compounds **7b–7d**

Compound/ atom	7b	7c	7d
C-2	58.7	54.7	52.2
C-3	68.8	68.1	66.8
C-4	73.1	74.7	74.6
C-5	73.1	79.2	73.2
C-6	77.7	81.8	77.6
C-7	34.2	38.1	31.2
C-8	65.4	60.9	64.1
C-2′	154.3	147.1	148.1
C-3′	106.2	111.2	111.1
C-4′	110.3	110.8	110.7
C-5′	142.3	143.6	143.4

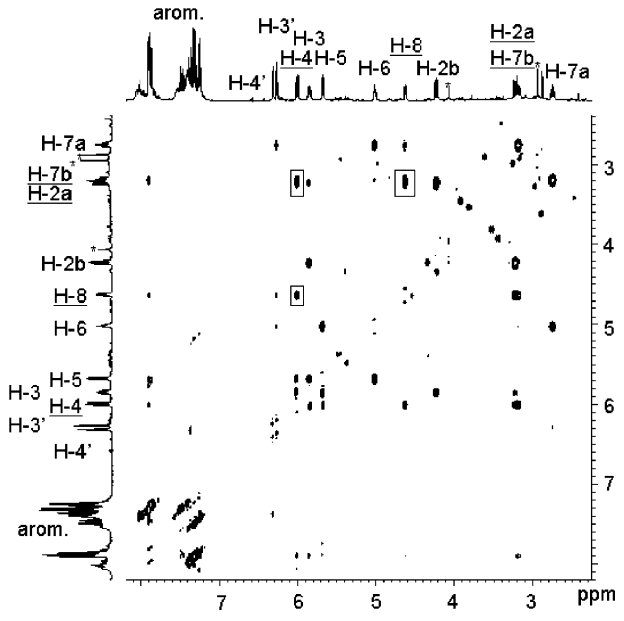

Figure 5. NOESY spectrum of compound **7b**. Crucial correlations between underlined protons are shown in boxes.

Table 3. Coupling constants for compounds **7b–7d** (Hz)

Compd/coupling constant	**7b**	**7c**	**7d**
$J_{2a,2b}$	13.8	15.0	12.5
$J_{2a,3}$	7.9	10.9	10.3
$J_{2b,3}$	4.9	4.0	4.0
$J_{3,4}$	9.2	10.9	10.1
$J_{4,5}$	7.9	7.6	8.7
$J_{5,6}$	6.0	—	5.8
$J_{6,7a}$	8.8	6.1	8.7
$J_{6,7b}$	3.8	10.0	6.2
$J_{7a,7b}$	13.1	13.5	13.2
$J_{7a,8}$	3.6	11.0	7.2
$J_{7b,8}$	8.5	9.5	11.2
$J_{3',4'}$	3.2	3.2	3.1
$J_{4',5'}$	1.9	2.0	1.9

Table 4 reveals that compounds **7b** and **7d** adopt similar structures. Naturally, though, the position of protons H-8 and H-3′ in the furyl ring are reversed for isomer **7d** compared to isomer **7b** [**7b**: (8*S*), **7d**: (8*R*)]. This was confirmed by the coupling constants measured between protons H-6, H-7 and H-8 (Table 3). The only real difference between their structures is that proton H-7b is located closer to protons H-2a and H-4 in isomer **7d** (evaluated from the dihedral angles). Thus, for isomer **7d**, a cross-correlation peak was visible in the NOESY spectrum (H-7b/H-2a/H-4). In the case of isomer **7c**, protons H-3′/H-3/H-7a and H-5/H-3/H-7a on the top face of the oxazepane ring are found to be in close proximity to each other, according to the NOESY spectrum recorded. By taking into account the coupling constants for all the isoxazolidine and 1,2-oxazepane ring protons in all three isomers, we have been able to determine the configuration of each of the newly formed chiral centres [**7b**: (6*S*,8*S*); **7c**: (6*R*,8*S*); and **7d**: (6*S*,8*R*) (Fig. 4)]. The stereochemistry of the remaining chiral centres have been deduced from D-glucose and the conformation of the 1,2-oxazepane ring.

The effects of different Lewis acid catalysts ($BF_3 \cdot OEt_2$, $ZnCl_2$, $MgCl_2$), solvents, absence or presence of 4 Å molecular sieves and reflux time on yields and diastereomeric ratios for this intramolecular 1,3-dipolar cycloaddition with nitrone **6** (Fig. 3) have been examined in detail (Table 5). The diastereomeric ratios of **7b–7d** obtained from each reaction were initially determined by the combined use of TLC and RP-HPLC. However, as this method proved cumbersome and inaccurate, alternatives were sought. The fortunate finding that, in the ^1H NMR spectra, the peaks assigned to protons H-3′ and H-4′ of the furyl ring were located in unique positions for each of the three isomers, that is, **7b–7d**, led us to investigate using ^1H NMR spectroscopy instead for these measurements. This afforded ratios which were in good agreement with those obtained previously from RP-HPLC experiments and so, due to its convenience and accuracy, it became the method of choice.

From Table 5, it can be seen that when the intramolecular 1,3-dipolar cycloaddtion was performed in toluene, the reflux time (24 or 48 h) had no effect on yield or diastereomeric ratio. However, since nitrone **6** decomposed quickly at temperatures above 100 °C, even under an argon atmosphere, it was not advantageous to heat the reaction in toluene at reflux for more than 24 h. 1,4-Dioxane was found to be an unsuitable solvent for this reaction; the mixture of isomers **7b–7d** was afforded in only 17% yield.

We have established that the main diastereoisomer obtained from these cycloaddition reactions (except when 1,4-dioxane and $ZnCl_2$ was used) was **7b**, bearing the (6*S*,8*S*) configuration at the newly formed chiral centres (Table 5). The maximum yield for this intramolecular 1,3-dipolar cycloaddition was achieved when $MgCl_2$ was added [90%, toluene, 120 °C (bath temperature), 12 h, Table 5]. Unexpectedly, in the presence of the harder Lewis acid catalyst, $BF_3 \cdot OEt_2$, most of the starting nitrone **6** decomposed after only a few hours to give an undesired product which contained one less benzoyl group (as determined from MS data). As a result of this finding, we propose that this also attributed to the reduced yield observed for the reaction performed in the presence of $ZnCl_2$, although here decomposition of the nitrone was slower.

In conclusion, we have ascertained that the optimum conditions for performing this 1,3-dipolar cycloaddition

Table 4. NOESY data of compounds **7b–7d**

Compd	Connected protons (upside positions)[a]	Connected protons (downside positions)[a]	Connected protons (peripheral positions)[a]
7b	H-3···H-2b; H-5···H-6	H-7b···H-8···H-4; H-8···H-2a···H-4	H-6···H-7a; H-7a···H-3′
7c	H-3′···H-3···H-7a H-3···H-5···H-7a H-3···H-2b	H-2a···H-4	H-7b···H-8; H-7b···H-6
7d	H-3···H-2b; H-5···H-6	H-7b···H-2a···H-4···H-3′	H-7a···H-6; H-7a···H-8

[a] Relative to the 9-oxa-1-azabicyclo[4.2.1]nonane skeleton as shown in Figure 6.

Table 5. The effect of Lewis acids and solvents on yields and diastereomeric ratios

Solvent, mol sieves	Catalyst	Time (h)	Temperature (°C)[a]	Yield (%)	Diastereomeric ratios (HPLC)[b]	Diastereomeric ratios (NMR)[b,c]
Toluene, 4 Å	—	24	120	70	2.8:1:1	3:1:1
Toluene	—	48	120	75	2.8:1:1.2	3:1:1
Benzene, 4 Å	—	24	80	32	2.1:1:1	2:1:1
1,4-Dioxane, 4 Å	—	24	100	17	1:1.1:1.2	—[d]
Toluene, 4 Å	$ZnCl_2$	24	120	50	4:1:1[e]	0.5:1:1
Toluene, 4 Å	$MgCl_2$	12	120	90	2:1:1	2:1:1
Toluene, 4 Å	$BF_3 \cdot OEt_2$	24	120	—[f]	—	—

[a] Bath temperature.
[b] Ratio of isomers **7b**:**7c**:**7d** isolated from the reaction mixture.
[c] Determined from the integral of protons H-3′ and H-4′.
[d] Not determined due to the presence of impurities.
[e] Diastereomer **7b** could not be separated from an impurity.
[f] The starting material decomposed and a by-product was formed (see text).

reaction with nitrone **6** (Fig. 3) involve using toluene as the solvent and $MgCl_2$ as the Lewis acid catalyst. It appears that if the Lewis acid catalyst added is hard, a side reaction involving elimination of a benzoyl protecting group from the starting nitrone becomes significant and this may be accompanied by decomposition and conversion of the furyl group, too. The stereoselectivity of the cycloaddition was found not to alter much under the conditions investigated here.

3. Theoretical investigations

In order to analyse the geometry of all four diastereoisomers of the isozaxolidine derivative (i.e., **7a**–**7d** (Fig. 4)) produced from the intramolecular 1,3-dipolar cycloaddition with nitrone **6** (Fig. 3), we have conducted a systematic computational investigation. This involved performing molecular dynamics simulations followed by high-level ab initio calculations. For the molecular dynamics studies, the 'simulated annealing' protocol described in the SYBYL program package[19] was employed to obtain the required starting geometries for isomers **7a**–**7d** for the subsequent higher level investigations. The Merck's force field parameter set (MMFF94) was applied with its own charge distribution. The molecules were equilibrated for 2000 fs at 1200 K and then cooled to 50 K exponentially over 10,000 fs. In this way, 1000 conformations were provided for each isomer. Next a semi-empirical optimization using the PM3 method was performed and the results afforded were grouped according to their energies. Finally, ab initio calculations were conducted on a representative for each of the different energy clusters. These utilised the Hartree–Fock method with 3-21 Gaussian basis set and applied the Gaussian03 code.[20] Although this is one of the simplest methods, it was reasoned that this was sufficient for our purposes. The conformations of the oxazepane rings for isomers **7b**–**7d** derived from the computational studies were found to be in very good agreement with the structures obtained previously from NMR studies. In Figure 6, the optimized geometry of isomer **7b** is presented. The spatial proximity of protons H-7b, H-8, H-4 and H-2a on the bottom face can be clearly seen. In addition to the theoretical calculations providing information about the geometry of isomers **7a**–**7d**, we had hoped that they could also be used to predict the diastereomeric ratio afforded by the cycloaddition reaction (**7a**:**7b**:**7c**:**7d** ≈ 0:3:1:1) based on the total energy of each of the isomers.

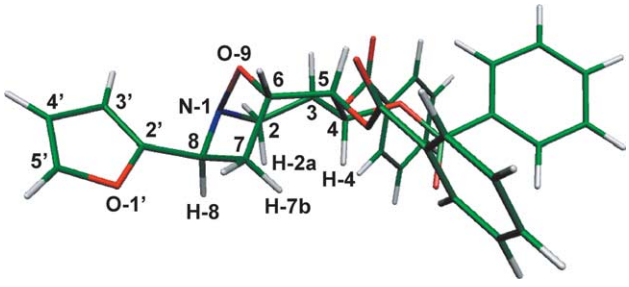

Figure 6. The lowest-energy conformation of compound **7b** calculated by HF/3-21 method. Proximal hydrogen atoms of 9-oxa-1-azabicyclo[4.2.1]-nonane skeleton, supporting the configuration and conformation of the above compound (NMR evidence), are shown with the H prefix. Carbon atoms of the above skeleton and those of the furan skeleton (primed numbers) are labelled without the C prefix for clarity.

However, it appeared that, at this level of theory, significant differences could not be observed with the total energies for diastereoisomers **7a**–**7d** being approximately the same. In Table 6, the HF/3-21G total energies of the minimized geometries for each of the different isozaxolidine isomers are presented.

Table 6. Calculated total energies of compounds **7a**–**7d**[a]

Compound	Total energy (hartree)
7a	−1870.388634
7b	−1870.389014
7c	−1870.389014
7d	−1870.389272

[a] Ab initio (HF/3-21G method).

4. Conclusion

We have successfully synthesized a variety of isoxazolidine derivatives of chiral moieties employing a Lewis acid-catalyzed 1,3-dipolar cycloaddition. In our preliminary studies, the exclusive formation of 9-oxa-1-azabicyclo-[4.2.1]nonane diastereomers **7b**–**7d** (Fig. 4) from nitrone **6** (Fig. 3) has been observed. The maximum yield for the 1,3-dipolar cycloaddition reaction was achieved with $MgCl_2$ as the Lewis acid catalyst [toluene, 120 °C (bath temperature), 12 h]. The ratio of the diastereoisomers of **7b**–**7d** was 3:1:1 and this did not alter significantly under the different

reaction conditions investigated here. The theoretical total energies of the minimized geometries for **7a–7d** obtained computationally failed to rationalise the experimentally determined diastereomeric ratios of these cycloadducts produced from the intramolecular 1,3-dipolar cycloaddition. Further studies are in progress to obtain chiral 1,2,4-substituted azetidines from the cycloadducts afforded and new cycloaddition reactions are envisaged.

5. Experimental

5.1. General procedures

The following abbreviations are employed: ACN (acetonitrile); ANA (azetidine nucleic acid(s)); anh. (anhydrous); Bn (benzyl); Bz (benzoyl); $CDCl_3$ (deuterochloroform); CH_2Cl_2 (dichloromethane); ESI (electrospray ionization); EtOAc (ethyl acetate); FAB (fast atom bombardment); HRMS (high resolution mass spectrometry); LRMS (low resolution mass spectrometry); MeOD (deuteromethanol); MeOH (methanol); PNA [peptide nucleic acid(s)]; rt (room temperature); THF (tetrahydrofuran).

Chemicals were purchased from Aldrich, Fluka, Merck or Reanal (Budapest, Hungary). 2-Furaldehyde and $BF_3 \cdot OEt_2$ were freshly distilled prior to use. Anhydrous solvents and anhydrous Lewis acid catalysts were prepared as described.[21] Organic solutions were dried using anhydrous $MgSO_4$ and evaporated in Büchi rotary evaporators. TLC: Kieselgel 60 F_{254} (Merck), solvent systems: CH_2Cl_2/MeOH, hexane/EtOAc, visualization: UV light, H_2SO_4/ethanol. Mp: Electrothermal IA 8103 apparatus. IR spectra: Bio-Rad FTS-60A (KBr pellets unless otherwise stated, ν_{max}/cm^{-1}, s, strong; m, medium; w, weak). NMR: Bruker Avance DRX 400 and 500 spectrometers (^1H: 400.13, 500.13 MHz; ^{13}C: 125.76 MHz, respectively), MeOD, $CDCl_3$ solutions, δ (ppm), J (Hz). Spectral patterns: s, singlet; d, doublet; dd, doublet of doublet; t, triplet; m, multiplet; br, broad; deut, deuterable. The superscripts *, # denote interchangeable assignments. For the 2D experiments (HSQC, HMBC, NOESY) the standard Bruker software packages (INV4GSSW, INV4GSLRNDSW) were applied. LRMS: Finnigan MAT TSQ 7000, ESI technique. HRMS: VG ZAB SEQ high resolution mass spectrometer using FAB ion source. Samples were dissolved in glycerol, the resolution of the instrument was 10,000. TLC/MS; TLC/HPLC: the analyte solution has been applied onto a 5 cm wide silica gel TLC plate as a band to obtain sufficient material. After developing in a solvent system the appropriate band was removed, the silica gel was suspended in MeOH (100 μL for MS, 1000 μL for HPLC), sonicated, centrifuged and the supernatant was used for MS analysis and HPLC (for HPLC 10 μL was injected). HPLC: SHIMADZU UV/VIS detector: SPD-10A VP, pump: LC-10AC VP, column: LiChrospher 6.1, RP select B (5 μm). Eluent system: gradient: 70–90% ACN within 25 min, flow rate: 1 mL/min.

5.1.1. (2*S*,3*S*,4*R*)-1-(Hydroxyimino)hex-5-ene-2,3,4-triyl tribenzoate (4). The unsaturated aldehyde **3**[11] (5.00 g, 10.92 mmol, 1 equiv) was dissolved in ethanol (100 mL) and distilled water (30 mL). The solution was treated with hydroxylamine hydrochloride (3.41 g, 41.19 mmol, 4.5 equiv) and sodium hydrogen carbonate (3.41 g, 49.80 mmol, 4.6 equiv). After 2 h stirring at room temperature, the ethanol was removed under reduced pressure, and the residue was dissolved in dichloromethane (100 mL). The solution was washed with water (3×50 mL), dried ($MgSO_4$) and evaporated in vacuo. Further purification was accomplished by column chromatography [10%–50% (v/v) EtOAc in hexane] to give oxime **4** as a light yellow oil (5.16 g, 70%, 1:1 mixture of *E* and *Z* isomers). R_f: 0.60; 0.53 (*E*/*Z* isomer), (1:1, hexane/EtOAc); IR (film, ν_{max}/cm^{-1}): 3426m, 3069w, 2984w, 1726s, 1601m, 1450m, 1315m, 1260s, 1246s, 1177m, 1105s, 1094s, 1069s, 1026m, 942w, 710s; δ_H (500 MHz, CH_3OD): 5.36 (m, 2H, H-6a, H-6b), 6.02 (m, 4H, H-2, H-3, H-4, H-5), 6.60 (m, 1H, H-1, *E*) 6.80 (d, 1H, $J_{1,2}$=5.4 Hz, H-1, *Z*), 7.34–7.56 (m, 9H, arom. H), 7.96–8.04 (m, 6H, arom. H), 8.63 (s, 1H, OH). δ_c (125 MHz, CH_3OD): 68.1 (C-2, *Z*), 71.8 (C-2, *E*), 73.8 (C-3, *Z*), 74.5 (C-3, *E*), 74.6 (C-4, *E*), 74.8 (C-4, *Z*), 120.4 (C-6, *E*), 121.0 (C-6, *Z*), 129.6–129.7 (6×arom. CH), 130.7 (3×arom. C_q), 130.8 (6×arom. CH), 132.7 (C-5, *Z*), 134.6 (C-5, *E*), 134.7 (3×arom. CH), 145.9 (C-1, *E*) 147.0 (C-1, *Z*), 166.6 (C=O), 166.7 (C=O), 167.1 (C=O); LRMS (ESI): *m*/*z* 474 (57%, $[M+H]^+$), 491 (100%, $[M+NH_4]^+$), 496 (75%, $[M+Na]^+$); HRMS (FAB, glycerol): Calcd for $C_{27}H_{24}NO_7$ $[M+H]^+$ *m*/*z* 474.15473, found *m*/*z* 474.15418.

5.1.2. (2*S*,3*S*,4*R*)-2,3,4-Tris(benzoyloxy)-*N*-(furan-2-ylmethylene)hex-5-en-1-amine oxide (6). To a stirred solution of oxime **4** (0.50 g, 1.06 mmol, 1 equiv) in dioxane (5 mL) was added sodium cyanoborohydride (0.22 g, 3.20 mmol, 3 equiv) in small portions, while the solution was carefully treated with HCl/dioxane (1.7 M, ~1 mL), to maintain the pH between 3 and 5. After completion of reaction (TLC), the solution was evaporated in vacuo, the residue was dissolved in EtOAc (50 mL), the organic layer was washed with aqueous sodium carbonate (40 mL), water (40 mL) and brine (40 mL), dried ($MgSO_4$) and evaporated in vacuo. The resulting hydroxylamine **5** was used without any further purification.

Compound **5** was dissolved in toluene (20 mL) and treated with freshly distilled 2-furaldehyde (176 μL, 2.12 mmol) in the presence of 4 Å molecular sieves. After stirring at 50 °C for 18 h, the solution was filtered, evaporated under reduced pressure and co-evaporated with ACN (3×50 mL). The crude residue was purified by column chromatography (10–30% (v/v) EtOAc in hexane) to give nitrone isomers **6** as a pale yellow foam (0.23 g, 40%). R_f: 0.17; 0.23 (*E*/*Z* isomer, 7:3, hexane/EtOAc); IR (KBr, ν_{max}/cm^{-1}): 3429w, 3065w, 2980w, 1728s, 1612m, 1601w, 1450w, 1315m, 1261s, 1240s, 1179w, 1107s, 1096s, 1069m, 1026w, 948w, 863m, 708s; δ_H (500 MHz, $CDCl_3$): 4.31 (d, 1H, $J_{1,2}$=5.5 Hz, H-1), 5.35 (d, 1H, $J_{5,6b}$=10.1 Hz H-6b, *Z*), 5.45 (d, 1H, H-6a, $J_{5,6a}$=16.6 Hz, *E*), 5.95–6.07 (m, 4H, H-2, H-3, H-4, H-5), 6.16 (dd, 1H, $J_{3',4'}$=1.7 Hz, $J_{4',5'}$=3.4 Hz, H-4′), 6.52 (d, 1H, $J_{3',4'}$=1.7 Hz, H-3′), 7.26 (s, 1H, $^-O^+N$=CH), 7.34–7.42 (m, 9H, arom. H), 7.79 (d, 1H, $J_{4',5'}$=3.4 Hz, H-5′), 7.97–8.04 (m, 6H, arom. H). δ_C (125 MHz, $CDCl_3$): 65.3 (C-1), 69.5 (C-2), 73.0 (C-4*), 73.5 (C-3*), 112.4 (C-3′), 116.3 (C-4′), 120.7 (C-6), 127.3 (CH=N), 128.4 (3×arom. CH), 128.5 (3×arom. CH), 128.6 (arom. C_q), 129.7 (3×arom. CH), 129.9 (3×arom.

CH), 131.5 (C-5), 133.2 (arom. CH), 133.5 (2×arom. CH), 144.0 (C-5′), 146.3 (C-2′), 165.1 (C=O), 165.3 (C=O), 165.7 (C=O); LRMS (ESI): *m/z* 554 (100%, $[M+H]^+$), 576 (20%, $[M+Na]^+$); HRMS (FAB, glycerol): Calcd for $C_{32}H_{28}NO_8$ $[M+H]^+$ *m/z* 554.18094, found *m/z* 554.18324.

5.1.3. (3*S*,4*R*,5*S*,6*S*,8*S*)-8-(Furan-2-yl)-9-oxa-1-azabicyclo[4.2.1]nonane-3,4,5-triyl tribenzoate (7b); (3*S*,4*R*,5*S*,6*R*,8*S*)-8-(furan-2-yl)-9-oxa-1-azabicyclo-[4.2.1]-nonane-3,4,5-triyl tribenzoate (7c) and (3*S*,4*R*,5*S*,6*S*,8*R*)-8-(furan-2-yl)-9-oxa-1-azabicyclo[4.2.1]-nonane-3,4,5-triyl tribenzoate (7d). *General procedure*. The pure nitrone **6** (0.40 g, 0.72 mmol) was dissolved in dry toluene (20 mL) in the presence of 4 Å molecular sieves and the solution was heated at 80–100–120 °C (bath temperature) for 12–48 h under an argon atmosphere. The reaction mixture was cooled, filtered, evaporated in vacuo and co-evaporated with ACN (3×50 mL). Chromatographic purification [0–10% (v/v) EtOAc in hexane], yielded three isomers (**7b**,**7c**,**7d**) as an oil or foam, in different isomer ratios depending on the reaction conditions, in 17–90% yield (Table 6). The ratios of the three diastereisomers were determined by TLC, RP-HPLC and ^{1}H NMR (Table 6). The reactions were carried out under different conditions, for example, solvents, temperature, reflux time and with or without Lewis acid catalysts (see Table 5). The catalysts, anh. $ZnCl_2$, anh. $MgCl_2$ and freshly distilled $BF_3 \cdot OEt_2$, were used in 0.1–0.2 mol equiv with respect to nitrone. R_f: 0.32 (7:3, hexane/EtOAc as a single spot; after 5× development in an 8:2, hexane/EtOAc eluent it could be separated into three isomers).

Compound **7b**: IR (KBr, ν_{max}/cm^{-1}): 3429w, 3065w, 2961w, 1726s, 1601w, 1450w, 1315m, 1281s, 1261s, 1179w, 1107s, 1096s, 1069m, 1026w, 708s; δ_H (500 MHz, $CDCl_3$): 2.75 (ddd, 1H, $J_{7a,7b}=13.1$ Hz, $J_{6,7a}=8.8^{\#}$ Hz, $J_{7a,8}=3.6^{*}$ Hz, H-7a), 3.18 (ddd, 1H, $J_{7a,7b}=13.1$ Hz, $J_{7b,8}=8.5^{\#}$ Hz, $J_{6,7b}=3.8^{*}$ Hz, H-7b), 3.22 (dd, 1H, $J_{2a,2b}=13.8$ Hz, $J_{2a,3}=7.9$ Hz, H-2a), 4.22 (dd, 1H, $J_{2a,2b}=13.8$ Hz, $J_{2b,3}=4.9$ Hz, H-2b), 4.63 (dd, 1H, $J_{7b,8}=8.5^{\#}$ Hz, $J_{7a,8}=3.6^{*}$ Hz, H-8), 5.02 (m, 1H, H-6), 5.68 (dd, 1H, $J_{4,5}=7.9$ Hz, $J_{5,6}=6.0$ Hz, H-5), 5.85 (m, 1H, H-3), 6.00 (dd, 1H, $J_{3,4}=9.2$ Hz $J_{4,5}=7.9$ Hz, H-4), 6.28 (d, 1H, $J_{3',4'}=3.2$ Hz, H-3′), 6.32 (dd, 1H, $J_{3',4'}=3.2$ Hz, $J_{4',5'}=1.9$ Hz, H-4′), 7.24–7.50 (m, 10H, arom. H, H-5′), 7.86–8.00 (m, 6H, arom. H); δ_C (125 MHz, $CDCl_3$): 34.2 (C-7), 58.7 (C-2), 65.4 (C-8), 68.8 (C-3), 73.1 (C-4, C-5), 77.7 (C-6), 106.2 (C-3′), 110.3 (C-4′), 128.3 (3×arom. CH), 128.4 (3×arom. C), 129.6 (3×arom. C_q, 3×arom. CH), 129.7 (3×arom. CH), 133.2 (3×arom. CH), 142.3 (C-5′), 154.3 (C-2′), 165.0 (C_q), 165.1 (C_q), 165.6 (C_q); LRMS (ESI): *m/z* 554 (100%, $[M+H]^+$), 576 (50%, $[M+Na]^+$); HRMS (FAB, glycerol): Calcd for $C_{32}H_{28}NO_8$ $[M+H]^+$ *m/z* 554.18094, found *m/z* 554.18213.

Compound **7c**: IR (KBr, ν_{max}/cm^{-1}): 3433w, 3063w, 2924w, 1726s, 1601w, 1450m, 1315m, 1281s, 1271s, 1179w, 1107s, 1069m, 1026m, 710s; δ_H (500 MHz, $CDCl_3$): 2.83 (ddd, 1H, $J_{7a,7b}=13.5$ Hz, $J_{7a,8}=11.0$ Hz, $J_{6,7a}=6.1$ Hz, H-7a), 2.89 (dd, 1H, $J_{2a,2b}=15.0$ Hz, $J_{2b,3}=4.0$ Hz, H-2b), 3.14 (ddd, 1H, $J_{7a,7b}=13.5$ Hz, $J_{6,7b}=10.0$ Hz, $J_{7b,8}=9.5$ Hz, H-7b), 3.60 (dd, 1H, $J_{2a,2b}=15.0$ Hz, $J_{2a,3}=10.9$ Hz, H-2a), 4.74 (dd, 1H, $J_{7a,8}=11.0$ Hz, $J_{7b,8}=9.5$ Hz, H-8), 4.82 (dd, 1H, $J_{6,7b}=10.0$ Hz, $J_{6,7a}=6.1$ Hz, H-6), 5.51 (d, 1H, $J_{4,5}=7.6$ Hz, H-5), 5.96 (ddd, 1H, $J_{3,4}=10.9$ Hz, $J_{2a,3}=10.9$ Hz, $J_{2b,3}=4.0$ Hz, H-3), 6.21 (dd, 1H, $J_{3,4}=10.9$ Hz, $J_{4,5}=7.6$ Hz, H-4), 6.44 (dd, 1H, $J_{3',4'}=3.2$ Hz, $J_{4',5'}=2$ Hz, H-4′), 6.60 (d, 1H, $J_{3',4'}=3.2$ Hz, H-3′), 7.19–7.54 (m, 9H, arom. H, 1H, H-5′), 7.75–8.00 (m, 6H, arom. H); δ_C (125.76 MHz, $CDCl_3$): 38.1 (C-7), 54.7 (C-2), 60.9 (C-8), 68.1 (C-3), 79.2 (C-5), 74.7 (C-4), 81.8 (C-6), 110.8 (C-4′) 111.2 (C-3′), 127.6 (3×arom. CH), 127.9 (3×arom. CH), 129.0 (3×arom. CH, 3×arom. C_q), 132.5 (3×arom. CH), 132.9 (3×arom. CH), 143.6 (C-5′), 147.1 (C-2′), 164.3 (C_q), 165.1 (C_q), 165.7 (C_q); LRMS (ESI): *m/z* 554 (100%, $[M+H]^+$), 576 (43%, $[M+Na]^+$); HRMS (FAB, glycerol): Calcd for $C_{32}H_{28}NO_8$ $[M+H]^+$ *m/z* 554.18094, found *m/z* 554.18268.

Compound **7d**: IR (KBr, ν_{max}/cm^{-1}): 3067w, 2928w, 1730s, 1601w, 1450w, 1315w, 1281s, 1260s, 1179w, 1109m, 1096m, 1070m, 1026w, 710m; δ_H (500 MHz, $CDCl_3$): 2.67 (ddd, 1H, $J_{7a,7b}=13.2$ Hz, $J_{6,7a}=8.7$ Hz, $J_{7a,8}=7.2$ Hz, H-7a), 2.86 (ddd, 1H, $J_{7a,7b}=13.2$ Hz, $J_{7b,8}=11.2$ Hz, $J_{6,7b}=6.2$ Hz, H-7b), 3.02 (dd, 1H, $J_{2a,2b}=12.5$ Hz, $J_{2a,3}=10.3$ Hz, H-2a), 3.53 (dd, 1H, $J_{2a,2b}=12.5$ Hz, $J_{2b,3}=4.0$ Hz, H-2b), 4.71 (dd, 1H, $J_{7a,8}=7.2$ Hz, $J_{7b,8}=11.2$ Hz, H-8), 4.96 (ddd, 1H, $J_{6,7a}=8.7$ Hz, $J_{6,7b}=6.2$ Hz, $J_{5,6}=5.8$ Hz, H-6), 5.89 (ddd, 1H, $J_{2a,3}=10.3$ Hz, $J_{3,4}=10.1$ Hz, $J_{2b,3}=4.0$ Hz, H-3), 5.69 (dd, 1H, $J_{4,5}=8.7$ Hz, $J_{5,6}=5.8$ Hz, H-5), 5.98 (dd, 1H, $J_{3,4}=10.1$ Hz, $J_{4,5}=8.7$ Hz, H-4), 6.42 (dd, 1H, $J_{3',4'}=3.1$ Hz, $J_{4',5'}=1.9$ Hz, H-4′), 6.51 (d, 1H, $J_{3',4'}=3.1$ Hz, H-3′), 7.20–7.25 (m, 3H, arom. H), 7.39–7.41 (m, 3H, arom. H), 7.49–7.52 (m, 4H, arom. H, H-5′), 7.78–7.82 (m, 3H, arom. H), 7.97–7.99 (m, 3H, arom. H); δ_C (125 MHz, $CDCl_3$): 31.2 (C-7), 52.2 (C-2), 64.1 (C-8), 66.8 (C-3), 73.2 (C-5), 74.6 (C-4), 77.6 (C-6), 111.1 (C-3′), 110.7 (C-4′), 128.1, 128.2 (3×arom. CH), 128.5 (3×arom. C_q,) 129.6 (3×arom. CH) 129.8 (3×arom. CH), 133.0 (3×arom. CH), 133.4 (3×arom. CH), 143.4 (C-5′), 148.1 (C-2′), 165.0 (C_q), 165.4 (C_q), 165.9 (C_q); LRMS (ESI): *m/z* 554 (100%, $[M+H]^+$), 576 (56%, $[M+Na]^+$); HRMS (FAB, glycerol): Calcd for $C_{32}H_{28}NO_8$ $[M+H]^+$ *m/z* 554.18094, found *m/z* 554.18379.

NOESY data: Table 4.

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