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Tetrahedron: *Asymmetry* 16 (2005) 1403–1408

Biocatalytic asymmetric reduction of 3-acetylisoxazoles

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Received 18 January 2005; accepted 21 February 2005

Abstract—The reduction of the carbonyl group in 3-acetylisoxazole derivatives by algae (Cyanobacterium: *Synechococcus elongatus* PCC 7942 and *Synechosystis* sp. PCC 6803) and plant cells (*Caragana chamlagu*) gave the corresponding (*S*)-alcohols with high enantioselectivities.

1. Introduction

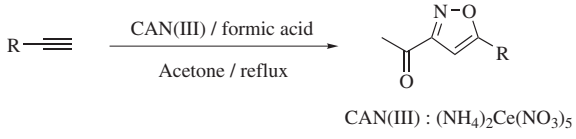
Isoxazole heterocycles have been employed in a wide variety of starting materials in organic chemistry. Isoxazoles can be converted into synthetically useful functional building blocks such as α,β-unsaturated oximes,[1] β-hydroxy ketones,[2] and γ-amino alcohols.[3] Additionally, isoxazole derivatives,[4,5] in particular, those with hydroxyl functionalities,[6] express a wide range of interesting pharmacological properties. Recently, we have reported a novel one-pot synthesis of 3-acetylisoxazole derivatives using ammonium cerium nitrate (CAN).[7] The reaction of alkenes and alkynes with CAN in acetone under reflux gave the corresponding 3-acetylisoxazole derivatives in good yields.

From the viewpoint of 'green chemistry', biocatalysts have been used for the transformation of natural or artificial substrates into useful compounds.[8] Among them, we have so far developed the use of plant cultured cells for the biotransformation of natural products such as thujopsene[9] and ionone[10] and unnatural substrates such as 1,2-diketone.[11] Among photosynthetic organisms such as microalgae and higher plants, other than conventional yeasts and fungi, are especially very useful for biocatalytic reduction, a method for preparing optically active compounds. Acetophenone derivatives,[12] camphorquinones,[13] and 1,3-diketones[14] could be reduced with high enantioselectivities by applying a cyanobacterium and the plant-cultured cells of *Caragana chamlagu*. Herein, we report an efficient asymmetric reduction of the heterocyclic carbonyl compounds, 3-acetylisoxazoles with these biocatalysts.

2. Results and discussion

2.1. Reduction of 3-acetylisoxazole derivatives by cyanobacterium

The substrates for biotransformation were prepared from the corresponding alkynes according to Scheme 1.[7]



Scheme 1. One-pot synthesis of 3-acetylisoxazoles.

First, the reaction of 3-acetyl-5-propylisoxazole **1a** with *S. elongatus* PCC 7942 was investigated. These results are summarized in Table 1 and Scheme 2. When 3-acetyl-5-propylisoxazole **1a** was added to the suspension of *S. elongatus* PCC 7942 and shaken under dark conditions, no reduction occurred (Table 1, run 12). However, the reduction of **1a** proceeded when the reaction was done under illumination with fluorescence light (2000 lux). After 96 h, the corresponding (*S*)-alcohol **1b** was obtained in 67% yield (92% ee) (Table 1, run 8).

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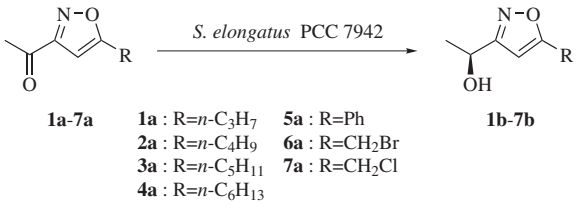
doi:10.1016/j.tetasy.2005.02.024

Table 1. Reduction of 3-acetyl-5-propylisoxazole **1a** by *Synechococcus elongatus* PCC 7942

Run	Substrate (**1a**/mg)	Time (h)	Yield (%)[a,b]	Ee (%) (Config.)
1	10	144	62	91 (*S*)
2	25	144	66	92 (*S*)
3	50	144	44	92 (*S*)
4	75	144	12	89 (*S*)
5	25	24	11	86 (*S*)
6	25	48	24	89 (*S*)
7	25	72	49	89 (*S*)
8	25	96	67	92 (*S*)
9	25	120	67	92 (*S*)
10[c]	25	120	No reaction	—
11[d]	25	120	No reaction	—
12[e]	25	120	No reaction	—

[a] Compound **1b** was obtained.
[b] Determined by GC analysis using *n*-dodecane as the internal hydrocarbon standard.
[c] The reaction without the microbe.
[d] The dried cells of *S. elongatus* PCC 7942 were used.
[e] The reaction proceeded in the dark.



Scheme 2.

Upon increasing the amount of **1a**, the chemical yield decreased according to the amount of **1a**. The time course of the reaction is shown in Figure 1. The reaction required 96 h to obtain a high yield. Since the amount of microbe used in the reaction was 20–30 mg (dry weight), the catalyst/substrate ratio of the reaction was 1:2. This ratio was superior to that in the conventional use of Baker's yeast.[15]

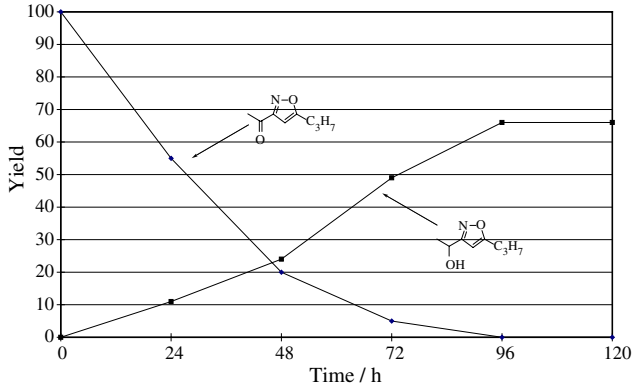


Figure 1. Time course for reduction of 3-acetyl-5-propylisoxazole by *Synechococcus elongatus* PCC 7942.

On the basis of these reaction conditions, reductions using other 3-acetylisoxazoles **2a**–**7a** by *S. elongatus* PCC 7942 were carried out (Scheme 2, Table 2). It is noteworthy that all substrates were reduced to the corresponding (*S*)-alcohol in moderate chemical yields with 91–96% ee. The substrate specificities of the cyanobacterium are wide. Thus, propyl, hexyl, phenyl, and chloromethyl derivatives were reduced in high yields, with 92–96% ee, however, the yields of 5-bromomethyl derivatives **6b** were low (21%). Ketones **6a** seemed to be poor substrates, as no significant degradation of either **6a** or the product **6b** was observed under incubation conditions. Another cyanobacterium, *Synechocystis* sp. PCC 6803 could be used[13] with the same isomers obtained in high ee (>91%) (Table 3).

Table 2. Reduction of 3-acetylisoxazoles (**2a**–**7a**) with *Synechococcus elongatus* PCC 7942

Run	Substrate	Yield (%)[a]	Ee (%) (Config.)
1	**2a**	**2b** (55)	92 (*S*)
2	**3a**	**3b** (54)	95 (*S*)
3	**4a**	**4b** (47)	96 (*S*)
4	**5a**	**5b** (57)	93 (*S*)
5	**6a**	**6b** (21)	91 (*S*)
6	**7a**	**7b** (53)	92 (*S*)

[a] Determined by GC analysis using *n*-dodecane as the internal hydrocarbon standard.

Table 3. Reduction of **2a**–**7a** with *Synechocystis* sp. PCC 6803

Run	Substrate	Yield (%)[a]	Ee (%) (Config.)
1	**1a**	**1b** (53)	91 (*S*)
2	**2a**	**2b** (50)	91 (*S*)
3	**3a**	**3b** (49)	95 (*S*)
4	**4a**	**4b** (48)	95 (*S*)
5	**5a**	**5b** (52)	91 (*S*)
6	**6a**	**6b** (20)	91 (*S*)
7	**7a**	**7b** (50)	92 (*S*)

[a] Determined by GC analysis using *n*-dodecane as the internal hydrocarbon standard.

The reduction of the carbonyl groups in the methyl heterocyclic ketones such as acetylpyridine [85% ee (*S*)][16] and 2-isoxazoline [98% ee (*S*)][17] with Baker's yeast was reported. The acetylpyridine derivatives [97% ee (*R*)][18] and acetylthiophenes [>99% ee (*S*)][19] were reduced with *Candida maris* and *Geotrichum candidum*, respectively. With the exception for *Candida maris*, the other microbes afforded (*S*)-alcohols upon reduction. Although the enantioselectivities of the product alcohols in the present reduction are slightly low compared to the reduction of 2-isoxazoline with baker's yeast, the biocatalyst/substrate ratio is superior when a cyanobacterium was used as the biocatalyst (cyanobacterium = 1 and Baker's yeast = 20).

2.2. Reduction of 3-acetylisoxazole derivatives by *C. chamlagu*

In contrast to cyanobacterium, the reduction of ketone **1a** and other substrates (Table 4) by applying the plant-cultured cells of *C. chamlagu* proceeded more easily than those by cyanobacterium. For example, the alcohol (*S*)-**1b** was obtained in 72% yield and 94% ee

Table 4. Reduction of **1a** with *Caragana chamlagu*

Run	Substrate (**1a**/mg)	Time (h)	Yield (%)[a,b]	Ee (%) (Config.)
1	10	24	70	94 (*S*)
2	20	24	72	94 (*S*)
3	40	48	59	94 (*S*)
4	60	48	46	94 (*S*)
5	80	48	16	91 (*S*)
6	20	1	18	92 (*S*)
7	20	3	48	93 (*S*)
8	20	6	66	94 (*S*)
9	20	9	72	94 (*S*)
10	20	12	72	94 (*S*)
11	20	48	71	94 (*S*)

[a] Compound **1b** was obtained.
[b] Determined by GC analysis using *n*-dodecane as the internal hydrocarbon standard.

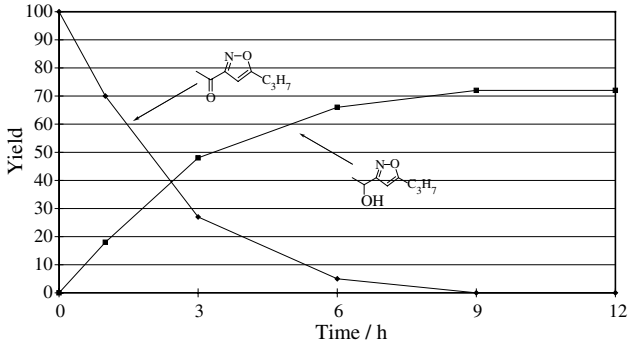


Figure 2. Time course for reduction of 3-acetyl-5-propylisoxazole by *Caragana chamlagu*.

(Table 4, run 9). The time course of the progress of this reaction is shown in Figure 2.

Next we attempted the elaboration of the incubation conditions (Table 5). The reduction by *C. chamlagu* was independent of either light or initial pH conditions. Various 3-acetylisoxazoles **2a**–**7a** were incubated (Table 6), and the corresponding (*S*)-alcohols obtained; for example, (*S*)-**5b** in 88% yield and 99% ee (Table 6, run 4). The reduction of sterically hindered ketone **6a** proceeded more efficiently (**6b**: 41%) than those by cyanobacterium as described above (Table 6, run 5).

Table 5. Reaction conditions for the reduction of **1a** with *Caragana chamlagu*

Run	Condition	pH in culture medium	Yield (%)[a,b]	Ee (%) (Config.)
1[c]	Dark	5.8	No reaction	—
2[d]	Dark	5.8	No reaction	—
3	Dark	4.0	70	94 (*S*)
4	Dark	7.0	68	94 (*S*)
5	Dark	8.5	69	94 (*S*)
6	Light	4.0	69	94 (*S*)
7	Light	5.8	70	94 (*S*)
8	Light	7.0	70	94 (*S*)
9	Light	8.5	67	94 (*S*)

[a] Compound **1b** was obtained.
[b] Determined by GC analysis using *n*-dodecane as the internal hydrocarbon standard.
[c] The reaction without the plant-cultured cells.
[d] The cells of *C. chamlagu* were dried.

Table 6. Reduction of **2a**–**7a** with *Caragana chamlagu*

Run	Substrate	Time (h)	Yield (%)[a]	Ee (%) (Config.)
1	**2a**	12	**2b** (72)	95 (*S*)
2	**3a**	12	**3b** (76)	95 (*S*)
3	**4a**	12	**4b** (75)	97 (*S*)
4	**5a**	24	**5b** (88)	99 (*S*)
5	**6a**	15	**6b** (41)	98 (*S*)
6	**7a**	15	**7b** (82)	97 (*S*)

[a] Determined by GC analysis using *n*-dodecane as the internal hydrocarbon standard.

There is an advantage in the use of cultured plant cells. Although the efficiency (biocatalyst/substrate ratio) in the reduction with the cyanobacterium was better than that with the plant cells, the increase of cyanobacterial cell concentration is very difficult because of necessity of light for the progress of the reduction. The problem caused by the low activity of the plant cells can be overcome by increasing the concentration.

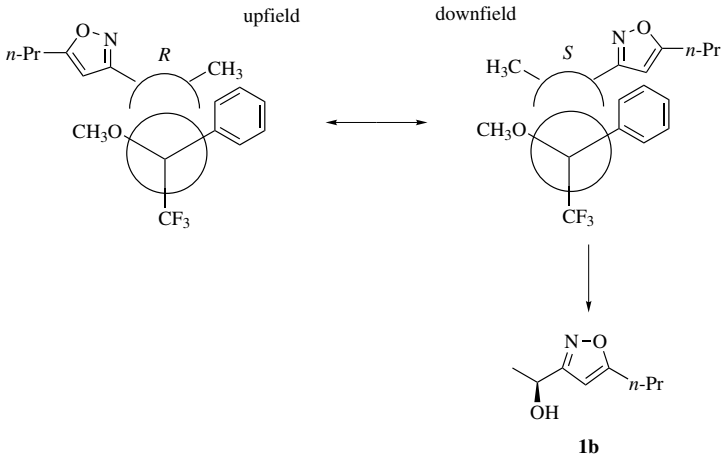
2.3. Absolute configuration of 3-(1-hydroxyethyl)-5-alkyllisoxazole 1b–7b

The absolute configuration of product alcohols **1b**–**7b** from the reduction of **1a**–**7a** with biocatalysts was determined as follows. The Mosher esters[20] of **1b**–**7b** (racemic and biocatalytic product) with (*S*)-(+)-MTPA-Cl were prepared and their ^{1}H NMR spectra measured with the results listed in Table 7. The Mosher esters derived from (*S*)-(+)-MTPA-Cl with the (*R*)- or (*S*)-isomer

Table 7. ^{1}H NMR chemical shift of Mosher esters

Mosher esters[a]	–CH_3 (methyl)	–CH_2– (methylene)
(±)-**1b**[b]	1.63	2.71
	1.71	2.66
1b	1.71	2.66
(±)-**2b**[b]	1.63	2.73
	1.7	2.69
2b	1.7	2.69
(±)-**3b**[b]	1.63	2.72
	1.7	2.68
3b	1.7	2.68
(±)-**4b**[b]	1.63	2.72
	1.7	2.68
4b	1.7	2.68
(±)-**5b**[b]	1.7	—
	1.77	—
5b	1.77	—
(±)-**6b**[b]	1.65	4.59
	1.72	4.55
6b	1.72	4.55
(±)-**7b**[b]	1.65	4.59
	1.72	4.55
7b	1.72	4.55

[a] Alcohol (0.1 mmol), (*S*)-(+)-MTPA-Cl (0.4 mmol), pyridine (0.1 mL) and tetrachloride (1.0 mL) were employed.
[b] Alcohol (0.1 mmol), $NaBH_4$ (0.3 mmol) and Et_2O (10.0 mL) were employed.



Scheme 3. Absolute configuration.

would give configurations as shown in Scheme 3. In this configuration, the methyl group (CH–CH_3) in the (*R*)-isomer faces the phenyl ring in the (*S*)-MTPA and should exhibit an upfield chemical shift relative to the (*S*)-isomer in the NMR spectrum.[21]

In the present experiment, all of the (*S*)-(+)-MTPA esters of the alcohols from the biocatalytic reduction of the 3-acetylisoxazoles afforded downfield methyl signals than those of the isomers in the ^{1}H NMR spectra. The configurations of the products were then determined to be (*S*). Also, the NMR spectrum in the methylene group of the side chain shows a characteristic upfield chemical shift.

3. Conclusion

In conclusion, the present study revealed some characteristic feature and potency of cyanobacterium and cultured cell, in the biocatalytic reduction of various heterocyclic, isoxazole ketones to provide the optically active forms of the corresponding alcohols.

4. Experimental

4.1. General

The IR spectra were recorded using a Jasco FT-IR 230 spectrometer. The ^{1}H and ^{13}C NMR spectra were measured using a JEOL GSX 400 Model spectrometer in deuteriochloroform solutions with tetramethylsilane as the internal standard. The gas chromatographic analyses were performed using a chiral GC-column (CP-cyclodextrin-B-2,3,6-M-19 [CPCD]; 25 m) attached to a Shimazu GC-17A and a GC-column (DB-1, 25 m) attached to a Shimazu GC-14A. *S. elongatus* PCC 7942 and *Synechosystis* sp. PCC 6803 were obtained from the Institu Pasteur.

4.2. Cultivation

S. elongatus PCC 7942 and *Synechosystis* sp. PCC 6803 were grown in a BG-11 medium (pH 8.0) under continuous illumination provided by fluorescent lamps (2000 lux) with air-bubbling at 25 °C. The callus tissues used in this experiment were derived from the leaves of *C. chamlagu* and maintained in our laboratory for eight years on agar plate containing an MS medium (pH 5.8) plus 3% sucrose and 2,4-dichlorophonoxacetic acid at 25 °C in the dark.[9a]

4.3. Typical procedures: reduction of 3-acetylisoxazole derivatives by cyanobacterium (*S. elongatus* PCC 7942 and *Synechosystis* sp. PCC 6803)

3-Acetylisoxazole derivatives **1a**–**7a** (25 mg) were added to the suspended culture of *S. elongatus* PCC 7942 or *Synechosystis* sp. PCC 6803 (1 g/L as dry weight) in a BG-11 medium (50 mL). The mixture was shaken at 120 rpm and 25 °C in the fluorescence light illumination (2000 lux). The resulting mixture was extracted with EtOAc. The chemical and enantiomeric purities were determined by GC analyses. For the enantiomeric purities, the GC conditions and retention times of the products are as follows: t_R/min; **1b**: (120 °C) 5.3 (*R*), 5.4 (*S*); **2b**: (120 °C) 6.7 (*R*), 6.8 (*S*); **3b**: (120 °C) 8.9 (*R*), 9.0 (*S*); **4b**: (120 °C) 13.0 (*R*), 13.2 (*S*); **5b**: (160 °C) 5.8 (*R*), 6.5 (*S*); **6b**: (120 °C) 5.8 (*R*), 6.5 (*S*); **7b**: (120 °C) 11.7 (*R*), 12.2 (*S*).

4.4. Typical procedures: reduction of 3-acetylisoxazole derivatives by *C. chamlagu*

The callus tissues (5 g) of *C. chamlagu* were transferred to an MS medium (50 mL) containing 2 ppm of 2,4-D and 3% sucrose. Then the 3-acetylisoxazole derivatives **1a**–**7a** (20 mg) were added to this suspension. The mixture was shaken at 120 rpm at 25 °C in the dark. The resulting mixture was filtered to separate the callus tissues and extracted with EtOAc. The chemical and enantiomeric purities were determined by GC analyses.

4.4.1. 3-(1-Hydroxyethyl)-5-propylisoxazole 1b. Pale-yellow oil; IR (NaCl) 3387 and 1601 cm^{-1}; $[\alpha]_D = -17.4$ (*c* 0.38, $CHCl_3$); ^{1}H NMR ($CDCl_3$) $\delta = 6.00$ (s, 1H), 4.99–5.00 (m, 1H), 2.71 (t, $J = 7.56$, 2H), 2.34 (br s, 1H), 1.68–1.77 (m, 2H), 1.54 (d,

$J = 6.56$, 3H) and 0.99 (t, $J = 7.56$, 3H); ^{13}C NMR ($CDCl_3$) $\delta = 174.0$, 167.4, 98.2, 63.5, 28.7, 22.8, 20.9 and 13.7; HRMS found: *m/z* 155.0947 $[M]^+$. Calcd for $C_8H_{13}NO_2$: 155.0946.

4.4.2. 3-(1-Hydroxyethyl)-5-butylisoxazole 2b. Pale-yellow oil; IR (NaCl) 3395 and 1601 cm^{-1}; $[\alpha]_D = -23.6$ (*c* 0.28, $CHCl_3$); ^1H NMR ($CDCl_3$) $\delta = 6.01$ (s, 1H), 4.95–5.00 (m, 1H), 2.72 (t, $J = 7.32$, 2H), 2.17 (br s, 1H), 1.64–1.71 (m, 2H), 1.53 (d, $J = 6.59$, 3H), 1.32–1.44 (m, 2H) and 0.94 (t, $J = 7.32$, 3H); ^{13}C NMR ($CDCl_3$) $\delta = 174.1$, 167.6, 98.2, 63.3, 29.6, 26.5, 22.8, 22.2 and 13.7; HRMS found: *m/z* 169.1102 $[M]^+$. Calcd for $C_9H_{15}NO_2$: 169.1102.

4.4.3. 3-(1-Hydroxyethyl)-5-pentylisoxazole 3b. Pale-yellow oil; IR (NaCl) 3387 and 1601 cm^{-1}; $[\alpha]_D = -17.6$ (*c* 0.41, $CHCl_3$); ^1H NMR ($CDCl_3$) $\delta = 6.01$ (s, 1H), 4.95–5.00 (m, 1H), 2.72 (t, $J = 7.56$, 2H), 2.17 (br s, 1H), 1.65–1.73 (m, 2H), 1.53 (d, $J = 6.59$, 3H), 1.31–1.39 (m, 4H) and 0.91 (t, $J = 7.56$, 3H); ^{13}C NMR ($CDCl_3$) $\delta = 174.1$, 167.6, 98.2, 63.2, 31.3, 27.2, 26.7, 22.8, 22.3, and 13.9; HRMS found: *m/z* 183.1242 $[M]^+$. Calcd for $C_{10}H_{17}NO_2$: 183.1249.

4.4.4. 3-(1-Hydroxyethyl)-5-hexylisoxazole 4b. Pale-yellow oil; IR (NaCl) 3387 and 1601 cm^{-1}; $[\alpha]_D = -22.0$ (*c* 0.30, $CHCl_3$); ^1H NMR ($CDCl_3$) $\delta = 6.00$ (s, 1H), 4.95–5.00 (m, 1H), 2.72 (t, $J = 7.56$, 2H), 2.17 (br s, 1H), 1.65–1.72 (m, 2H), 1.53 (d, $J = 6.59$, 3H), 1.29–1.39 (m, 6H) and 0.89 (t, $J = 7.56$, 3H); ^{13}C NMR ($CDCl_3$) $\delta = 174.1$, 167.6, 98.2, 63.2, 31.4, 28.8, 27.5, 26.8, 22.8, 22.5 and 14.0; HRMS found: *m/z* 197.1406 $[M]^+$. Calcd for $C_{11}H_{19}NO_2$: 197.1406.

4.4.5. 3-(1-Hydroxyethyl)-5-phenylisoxazole 5b. Pale-yellow oil; IR (NaCl) 3387 and 1601 cm^{-1}; $[\alpha]_D = -24.0$ (*c* 0.25, $CHCl_3$); ^1H NMR ($CDCl_3$) $\delta = 7.79$–7.88 (m, 2H), 7.43–7.53 (m, 3H), 6.78 (s, 1H), 4.94–4.99 (m, 1H), 2.15 (br s, 1H) and 1.55 (d, $J = 6.59$, 3H); ^{13}C NMR ($CDCl_3$) $\delta = 171.2$, 170.2, 131.3, 130.1, 128.8, 126.8, 98.5, 63.7 and 23.1; HRMS found: *m/z* 189.0791 $[M]^+$. Calcd for $C_{11}H_{11}NO_2$: 189.0790.

4.4.6. 3-(1-Hydroxyethyl)-5-bromomethylisoxazole 6b. Pale-yellow oil; IR (NaCl) 3380 and 1605 cm^{-1}; $[\alpha]_D = -12.0$ (*c* 0.35, $CHCl_3$); ^1H NMR ($CDCl_3$) $\delta = 6.49$ (s, 1H), 4.86–4.91 (m, 1H), 4.61 (s, 2H), 2.15 (br s, 1H) and 1.48 (d, $J = 6.59$, 3H); ^{13}C NMR ($CDCl_3$) $\delta = 169.9$, 169.5, 102.6, 63.6, 23.0 and 19.3; HRMS found: *m/z* 204.9741 $[M]^+$. Calcd for $C_6H_8NO_2Br$: 204.9738.

4.4.7. 3-(1-Hydroxyethyl)-5-chloromethylisoxazole 7b. Pale-yellow oil; IR (NaCl) 3374 and 1607 cm^{-1}; $[\alpha]_D = -15.8$ (*c* 0.39, $CHCl_3$); ^1H NMR ($CDCl_3$) $\delta = 6.49$ (s, 1H), 4.87–4.92 (m, 1H), 4.74 (s, 2H), 2.15 (br s, 1H) and 1.48 (d, $J = 6.59$, 3H); ^{13}C NMR ($CDCl_3$) $\delta = 169.8$, 169.4, 102.7, 63.6, 35.0 and 23.0; HRMS found: *m/z* 161.0245 $[M]^+$. Calcd for $C_6H_8NO_2Cl$: 161.0244.

4.5. Preparation of Mosher ester (α-methoxy-α-trifluoromethylphenylacetyl derivatives) [22]

(+)-α-Methoxy-α-trifluoromethylphenylacetyl chloride [(*S*)-(+)-MTPA-Cl, 100 mg, 0.40 mmol] was added to a mixture of alcohol (0.1 mmol) and pyridine (0.1 mL) in carbon tetrachloride (1.0 mL) at 0–5 °C. The resulting mixture was stirred at room temperature for 2 h and then extracted with diethyl ether (30 mL).

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