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# SYNTHESIS AND BIOLOGICAL EVALUATION OF BOTH ENANTIOMERS OF L-761,000 AS INHIBITORS OF CYCLOOXYGENASE 1 AND 2

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**Abstract:** Both enantiomers of L-761,000 were prepared and evaluated for their cyclooxygenase activities.

In the preceding paper[1] we have demonstrated that it is possible to develop a selective cyclooxygenase-2 (COX-2) inhibitor from a non-selective class of cyclooxygenase inhibitors. The structural modifications of indomethacin have led to the discovery of L-761,000, a potent and selective COX-2 inhibitor.

It is well established that enantiomers can possess different intrinsic activities against enzymes and receptors.[2] In addition, the pharmacokinetics of enantiomers might differ considerably.[3] For these reasons, the synthesis of both antipodes of L-761,000 was undertaken in order to compare their in vitro and in vivo activities.

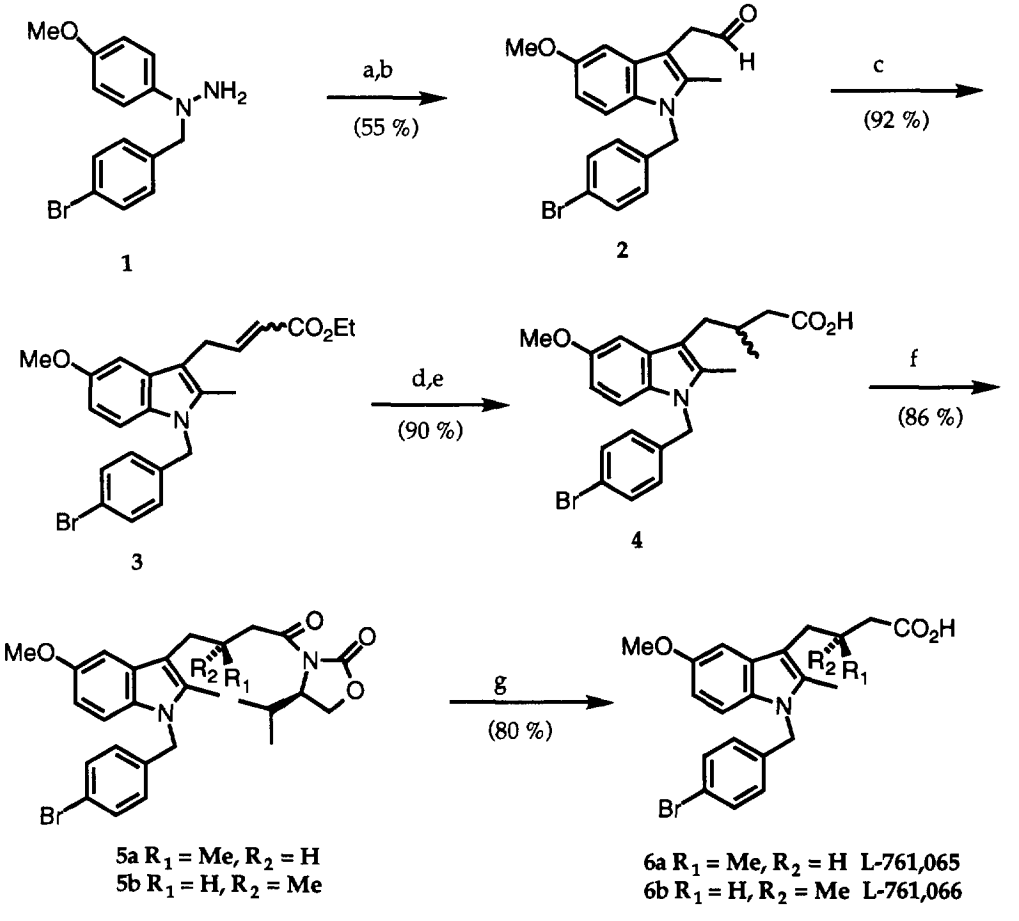
## Chemistry

### Resolution

The indole nucleus was produced in large quantities from the Fischer indole synthesis using the hydrazine **1** and ethyl levulinate (Scheme 1). The resulting ester was then reduced with DIBALH (-100 °C) to afford the aldehyde **2** in 55% yield. Condensation of (carbethoxymethylene) triphenylphosphorane with the aldehyde **2** afforded the ester **3** as a mixture of *cis* and *trans* isomers. The desired substituted acid side chain was easily obtained in 90% yield from the conjugate addition of the Gilman reagent to the ester **3**, in the presence of TMSCl,[4] followed by hydrolysis. The resolution was achieved by the incorporation of the Evans chiral auxiliary into the acid **4** to produce **5a** and **5b** in 86% combined yield. Using *(4R)-(+)*-isopropyl-2-oxazolidone as the auxiliary, the more polar diastereoisomer **5a** and the less polar one **5b** were separated by chromatography using 15% EtOAc in hexane. The two diastereomers were then converted separately to **6a**

(L-761,065) and **6b** (L-761,066) respectively in 80% yield. The absolute stereochemistry of the two enantiomers was established in correlation with the chiral synthesis described in the next section.

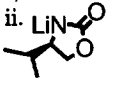
**SCHEME 1**




**1** **2** **3** **4**

**5a** $R_1$ = Me, $R_2$ = H
**5b** $R_1$ = H, $R_2$ = Me

**6a** $R_1$ = Me, $R_2$ = H **L-761,065**
**6b** $R_1$ = H, $R_2$ = Me **L-761,066**

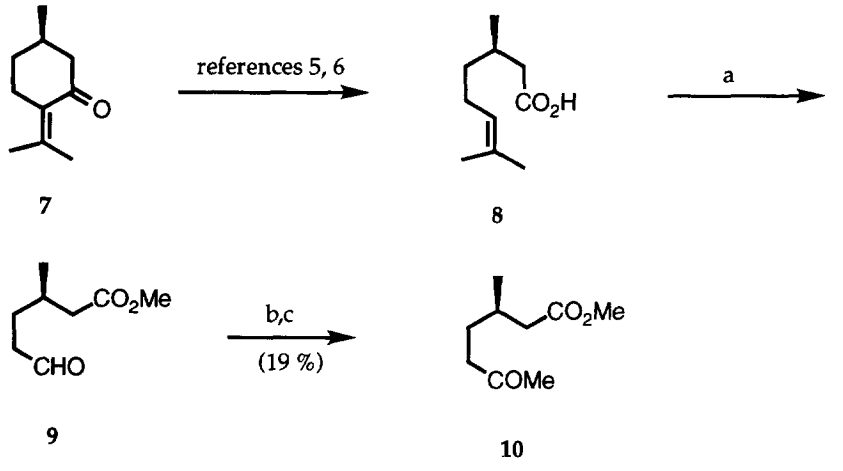
Reagents: (a) i. Ethyl levulinate, toluene, HOAc, rt; ii. EtOH,HCl,reflux; (b)i.DIBALH, $CH_2Cl_2$, toluene, -100 °C; ii. MeOH, tartaric acid; (c) $Ph_3PCHCO_2Et$, toluene, 80 °C; (d) i. MeLi, CuI, 0 °C; ii. TMSCl, -78 °C; iii. **3**, $Et_2O$, THF, -78 °C to RT; (e) NaOH, MeOH, THF, reflux, 2 h; (f) i. KHMDS, pivaloyl chloride, 0 °C; ii.  , THF, -78 °C to 0°C. (g) LiOH, $H_2O_2$, THF -10 to 0 °C.

**Chiral Synthesis**

In order to establish the absolute stereochemistry of the acids **6a** (L-761,065) and **6b** (L-761,066), a chiral synthesis of the keto ester **10** was undertaken. The relatively inexpensive (*R*)-(+)-pulegone **7** was converted to (*R*)-(+)-citronellic acid **8** using the conditions described by Zwanenburg (Scheme 2).[5] The aldehyde **9** was obtained from the acid **8** using the method of Overberger.[6] Addition of methylmagnesium bromide to aldehyde **9** followed by PCC oxidation yielded the desired keto ester **10**. Alternatively, the keto ester **10** could be prepared in large quantities from (*R*)-(+)-citronellic acid, using a method described by Kulkarni for citronellol (Scheme 3).[7]

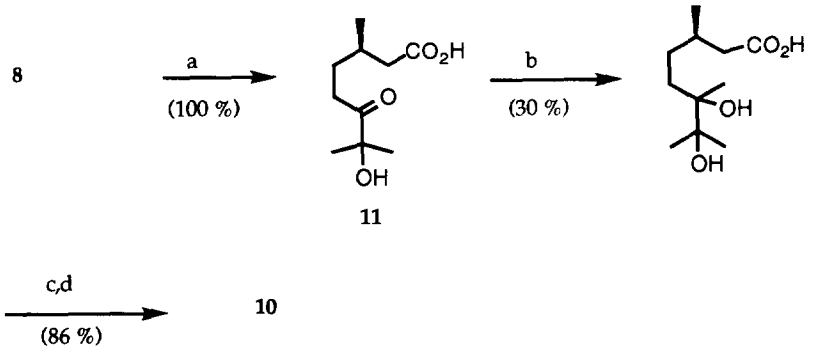
The hydrazine **1** was then condensed with the keto ester **10** to give, after hydrolysis, the acid **6b** (Scheme 4). The value and the sign of the optical rotation[8] of the acid thus obtained corresponded to the acid obtained from the less polar amide **5b**. This proves that the chiral center of L-761,066 has the (*R*) configuration.

**SCHEME 2**



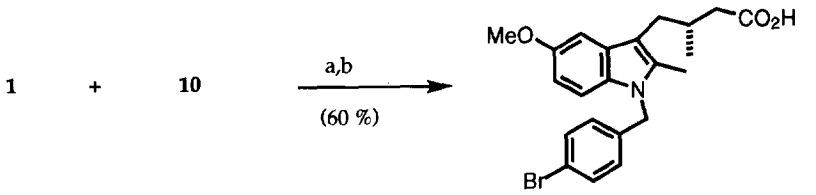

Reagents: (a)i. $O_3$, MeOH; ii. $PPh_3P$; (b) MeMgBr, THF, -78 °C; (c) PCC, $CH_2Cl_2$

SCHEME 3




Reagents: (a) $KMnO_4$, acetone, $H_2O$, HOAc;( b) MeMgI, $Et_2O$; (c) $NaIO_4$, acetone, $H_2O$ (c) $CH_2N_2$, $Et_2O$, $CH_2Cl_2$.

SCHEME 4




**6b L-761,066**

Reagent: (a) EtOH, HCl; (b) NaOH, MeOH, THF.

## Discussion

The racemate and the corresponding enantiomers were tested in parallel in the COX-1 and COX-2 whole cell assay.[9,10] As summarized in Table 1, within the experimental errors, the in vitro activities of the (R)-enantiomer (L-761,066) and the (S)-enantiomer (L-761,065) are comparable to the racemate L-761,000. However, L-761,066 was found to be more potent in vivo. This compound is active in the rat paw assay with an $ED_{30}$ of 0.4 mg/kg (Scheme 5) and in the rat pyresis assay with an $ED_{50}$ of 1.9 mg/kg while the enantiomer L-761,065 has poor activity in the rat paw edema assay ($ED_{30}$ > 3.0 mg/kg). In the rat, L-761,066 has superior

pharmacokinetics than L-761,065 with a $C_{max}$ of 24.3 μM (14.4 μM for L-761,065) and a clearance of 2.8 mL/kg/min (21mL/kg/min for L-761,065) as determined by analysis of drug levels in plasma samples.[11] The lower plasma levels of L-761,065 compared to L-761,066 may be partially responsible for the relatively lower activity of the former in the in vivo model. The observed selectivity for L-761,066 is reflected in the rat $^{51}Cr$ excretion assay[9] where no chromium leakage is observed after 5 days of treatment at 100 mg/kg BID. This represents a degree of gastrointestinal tolerance that is not available with non-selective NSAIDs such as indomethacin where this compound traced its origin.

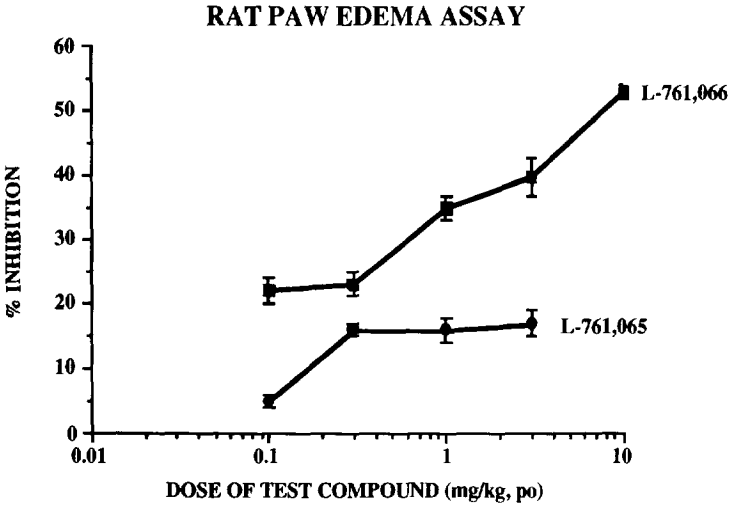
**TABLE 1**

In vitro and in vivo comparison of L-761,066, L-761,065 and the racemate L-761,000

| | COX-2 Whole Cell $IC_{50}$ (nM) | COX-1 Whole Cell $IC_{50}$ (nM) | Rat Paw Edema $ED_{30}$ (mg/kg) | Rat Pyresis $ED_{50}$ (mg/kg) |
|---|---|---|---|---|
| **6b (L-761,066)** | 60 | > 10,000 | 0.4 | 1.9 |
| **6a (L-761,065)** | 20 | > 10,000 | >3 | not tested |
| **6 (L-761,000)** | 50 | > 10,000 | 0.4 | not tested |
| **Indomethacin** | 10 | 6 | 0.4 | 1.0 |

**SCHEME 5**

Activities of L-761,065 and L-761,066 in the rat paw edema assay.



In conclusion, structural transformation of indomethacin has led to the discovery of a potent and selective COX-2 inhibitor (L-761,066) which is free of gastrointestinal side effects. This result suggests that other classes of NSAIDs could potentially be modified and designed into selective COX-2 inhibitors.

**References and Notes**

1. See preceding paper
2. (a) Lien, E. J. *J. Drug Targeting*, **1995**, *2*, 527. (b) Brossi, A. *Med. Res. Rev.*, **1994**, *14*, 665. c) Waldeck, B. *Chirality*, **1993**, *5*, 350.
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4. Corey, E. J.; Boaz, N. W. *Tetrahedron Lett.* **1985**, *26* , 6015.
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7. Randad, R. S.; Bhat, N. G.; Kulkarni, G. H. *Indian J. Chem.* **1984**, *23B*, 947.
8. The value of the optical rotation for L-761,066 is -7.1° (c = 1, acetone).
9. Chan, C.-C.; Boyce, S.; Brideau, C.; Ford-Hutchinson, A. W.; Gordon, R.; Guay, D.; Hill, R. G.; Li, C.-S.; Mancini, J.; Penneton, M.; Prasit, P.; Rasori, R.; Riendeau, D.; Roy, P.; Tagari, P.; Vickers, P.; Wong, E.; Rodger, I. W. *J. Pharmacol. Exp. Ther.* **1995**, *274*, 1531.
10. For direct comparison the racemate and both enantiomers were tested in parallel in the COX-1 and COX-2 assay. Some variations in $IC_{50}$'s values have been observed in the COX-2 induction of different cell cultures. This partially explains why the $IC_{50}$ value for the racemate (L-761,000) is different than in the preceeding paper.
11. A clearance of 5.7 mL/kg/min was observed for the racemate L-761,000.

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