## Supporting Information

# Langkolide, a 32-membered Macrolactone Antibiotic produced by *Streptomyces* sp. Acta 3062

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**Table S1. Growth and Cultural Characteristics of Strain Acta 3062 on Selected Agar Media**

| Medium | Growth | Spore chain morphology | Spore surface ornamentation | Color of spore mass | Pigmentation of substrate | Diffusible pigment |
|---|---|---|---|---|---|---|
| Yeast extract-malt extract agar | +++ | Spiral (3-5 tight spirals)* | Sparse spines | White (1A1) | Golden wheat (4B4) | None |
| Oatmeal agar | +++ | nd | nd | White (1A1) | Colorless | None |
| Inorganic salts-starch agar | +++ | nd | nd | White (1A1) | Colorless | None |
| Glycerol-asparagine agar | +++ | nd | nd | White (1A1) | Colorless | None |
| Peptone-yeast extract agar | ++ | nd | nd | None | Wax yellow (3B5) | None |
| Tyrosine agar | ++ | nd | nd | Grey (4B1) | Colorless | Black |

* 14 day old culture ; nd: not determined ; ++: moderate growth, +++: good growth; color coding done according to ref. 28; *S. longwoodensis*: open spirales and smooth spores;[5] *S. galbus*: spirals and smooth spores;[5] *S. bungoensis* - loose spirals and spiny spores.[6]

**Table S2. Biochemical Characteristics of Strain Acta 3062, *S. galbus*, *S. longwoodensis* and *S. bungeonsis***

| | Acta 3062 | *S. galbus* | *S. longwoodensis* | *S. bungeonsis* |
|---|---|---|---|---|
| NaCl tolerance (%) | 0-4 | | | |
| Growth temperature ($^{o}$C) | 17-27 | 10-45 | 10-50 | na |
| pH range | 4.5-10.5 | | | |
| Decomposition of gelatin | - | - | - | + |
| Production of hydrogen sulphide and melanin | - | + | + | ± |
| Coagulation of skim milk | - | na | na | - |
| Decomposition of starch | + | + | + | - |
| Reduction of nitrate | - | - | - | na |
| Carbon utilisation | | | | |
| Basal medium | - | na | na | na |
| Arabinose | + | + | + | + |
| Xylose | + | + | + | + |
| Fructose | + | + | + | + |
| Glucose | + | + | + | + |
| Mannose | + | na | na | na |
| Rhamnose | + | - | _ | - |
| Inositol | + | + | + | - |
| Sucrose | + | - | _ | ± |
| Cellobiose | + | na | na | na |
| Raffinose | + | - | ± | - |

+: good growth; ±: poor growth; -: no growth; na: not available

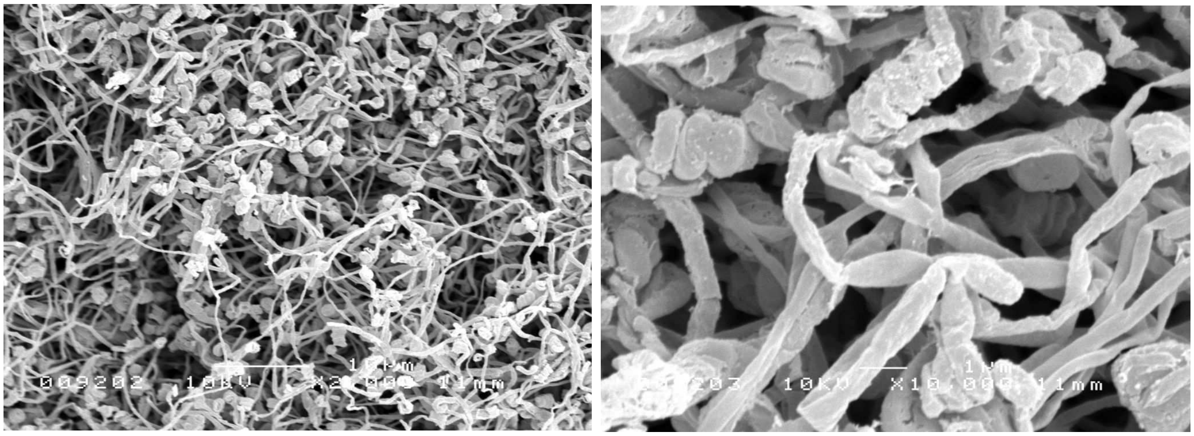

**Figure S1.** Scanning electron micrographs (2,000× and 10,000× magnification) of a 14 d *Streptomyces* sp. Acta 3062 cultured on yeast extract-malt extract agar at 27 °C.

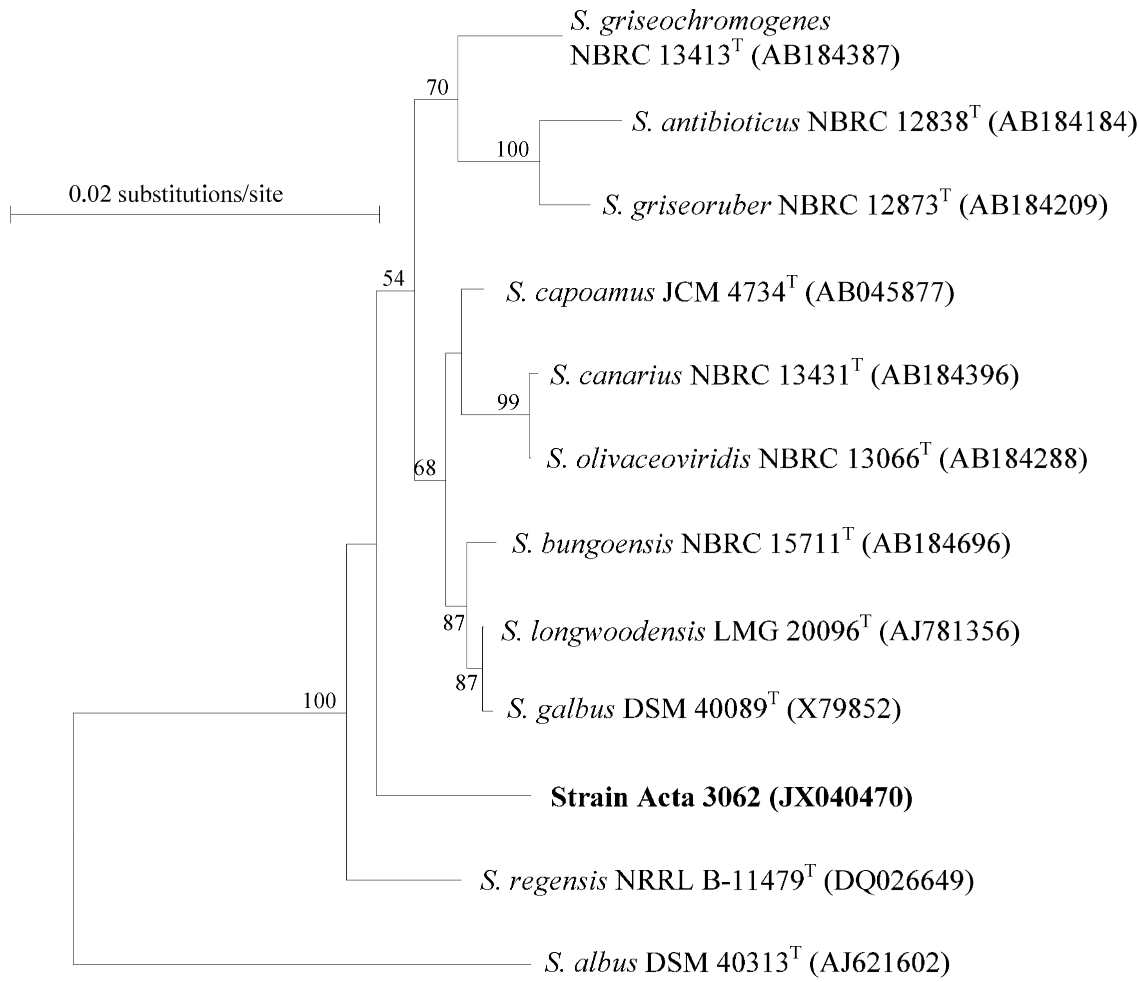


**Figure S2.** Neighbour-joining tree based on nearly complete 16S rRNA gene sequences showing relationships between strain Acta 3062 and representatives of closely related *Streptomyces* species. The numbers at the nodes indicate the level of bootstrap support (%) based on the analysis of 1,000 resampled datasets; the bar represents substitutions per nucleotide position.

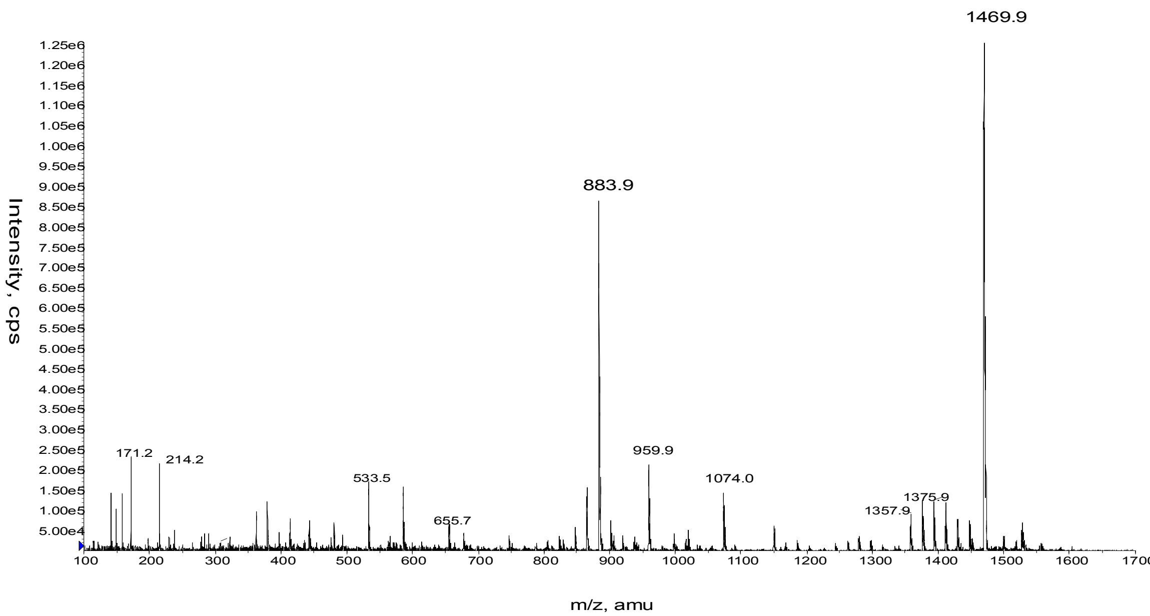


**Figure S3.** ESI-MS spectrum of lankolide in positive ionization mode.

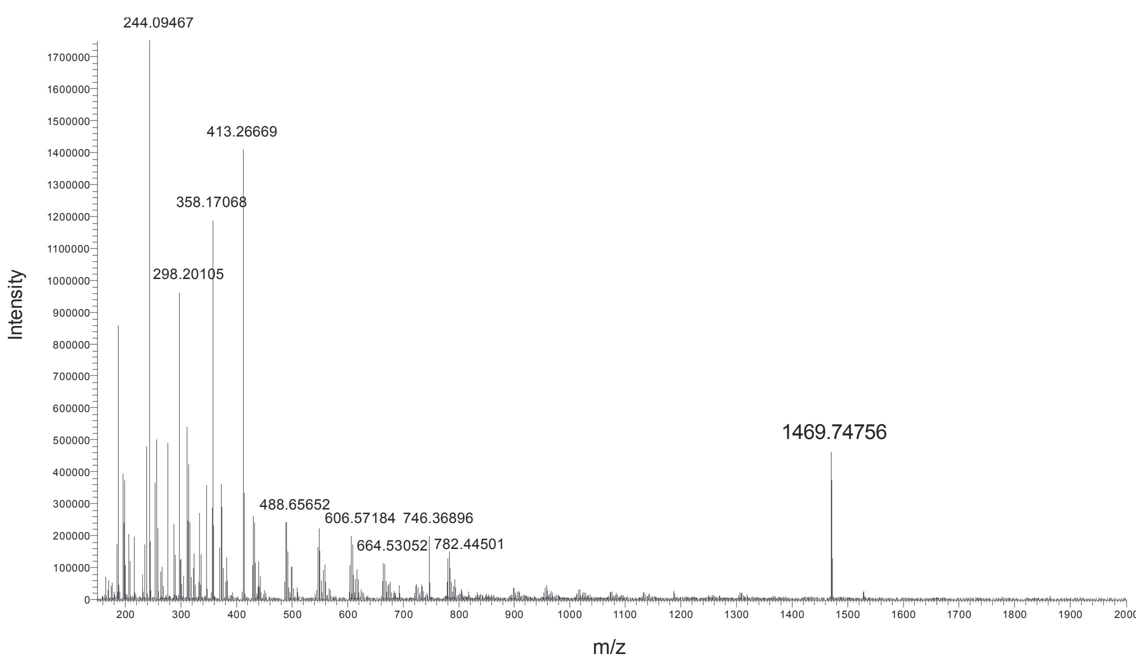


**Figure S4.** High-resolution Orbitrap-ESI-MS spectrum of lankolide.

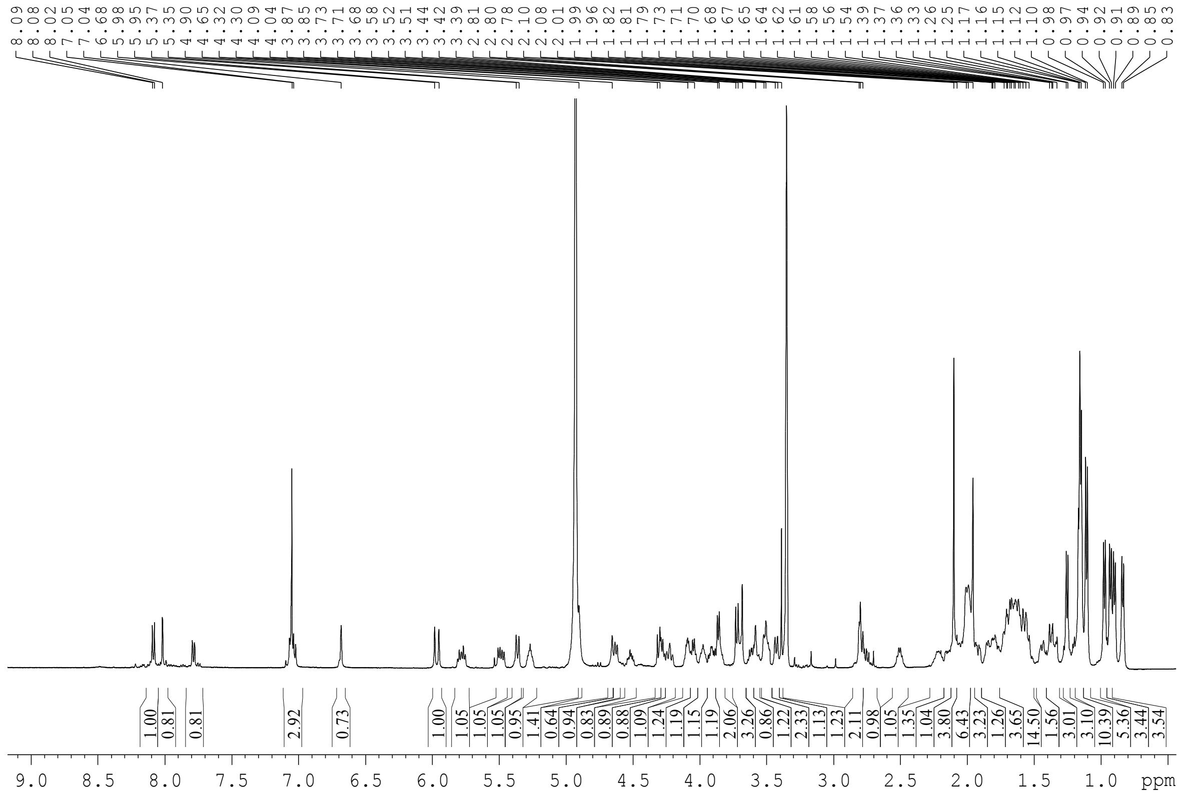


**Figure S5.** [1]H-NMR spectrum of langkolide in methanol-$d_4$ (600 MHz).

a)

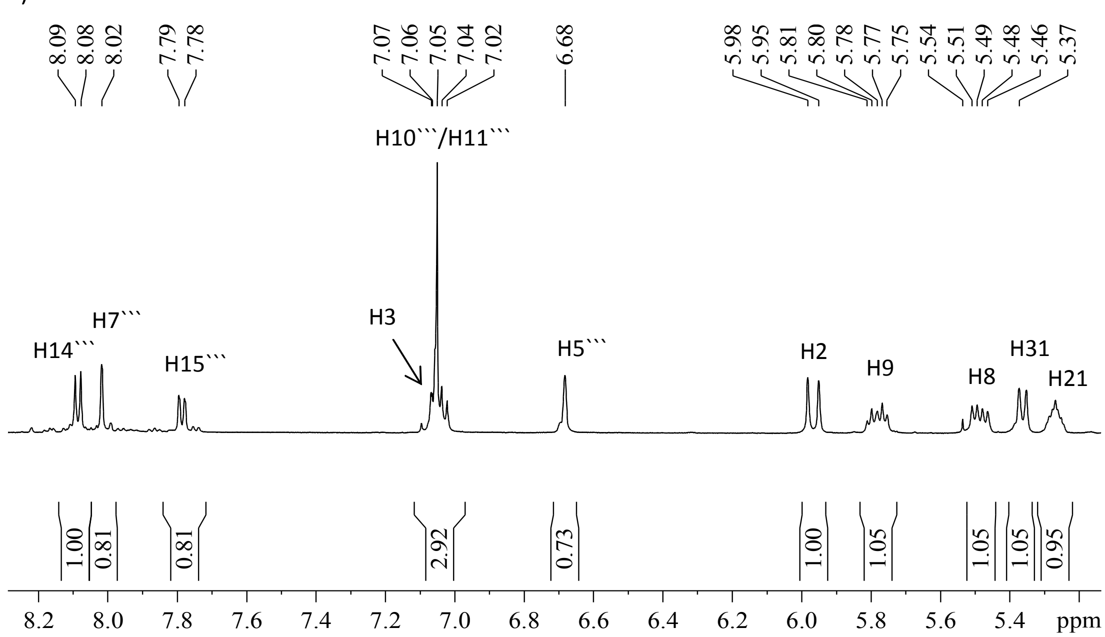


**Figure S5a.** Expansions of the [1]H-NMR spectrum of langkolide in methanol-$d_4$ (600 MHz): a) expansion between 5 and 8.5 ppm.

b)

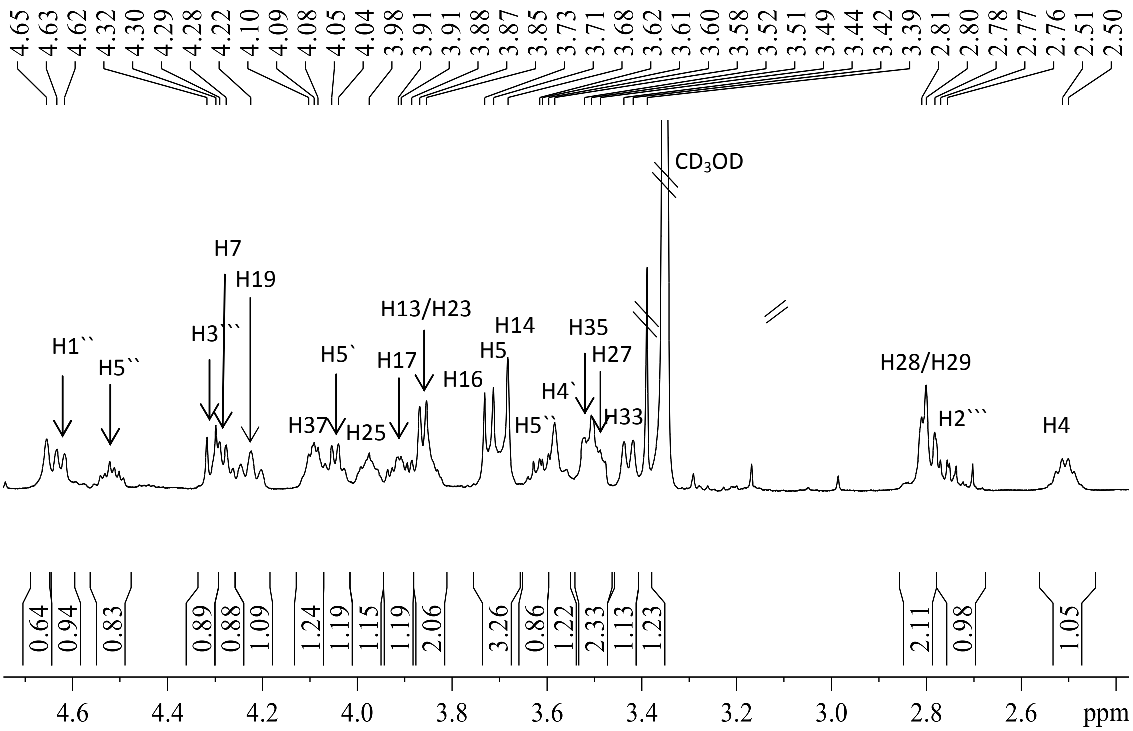


**Figure S5b.** Expansions of the [1]H-NMR spectrum of langkolide in methanol-$d_4$ (600 MHz): b) expansion between 2.5 and 4.8 ppm.

c)

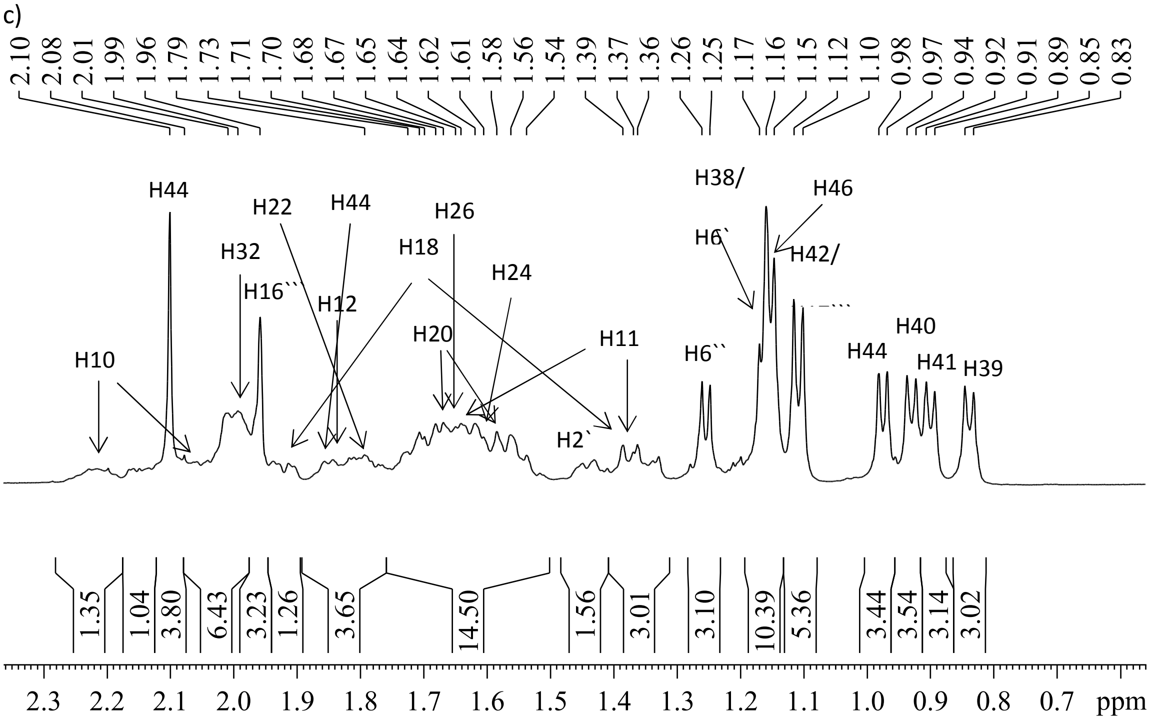


**Figure S5c.** Expansions of the [1]H-NMR spectrum of langkolide in methanol-$d_4$ (600 MHz): c) expansion between 0.8 and 2.4 ppm.

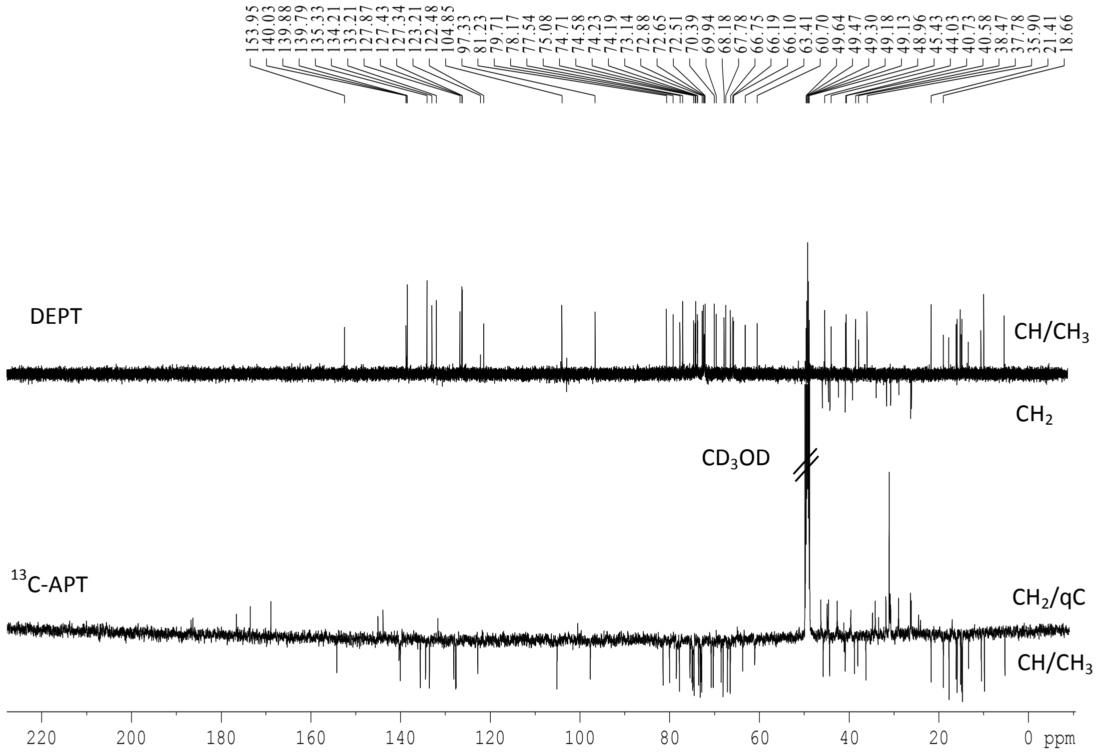


**Figure S6.** $^{13}$C-NMR and DEPT spectra of langkolide in methanol-$d_4$ (500 MHz).

a)

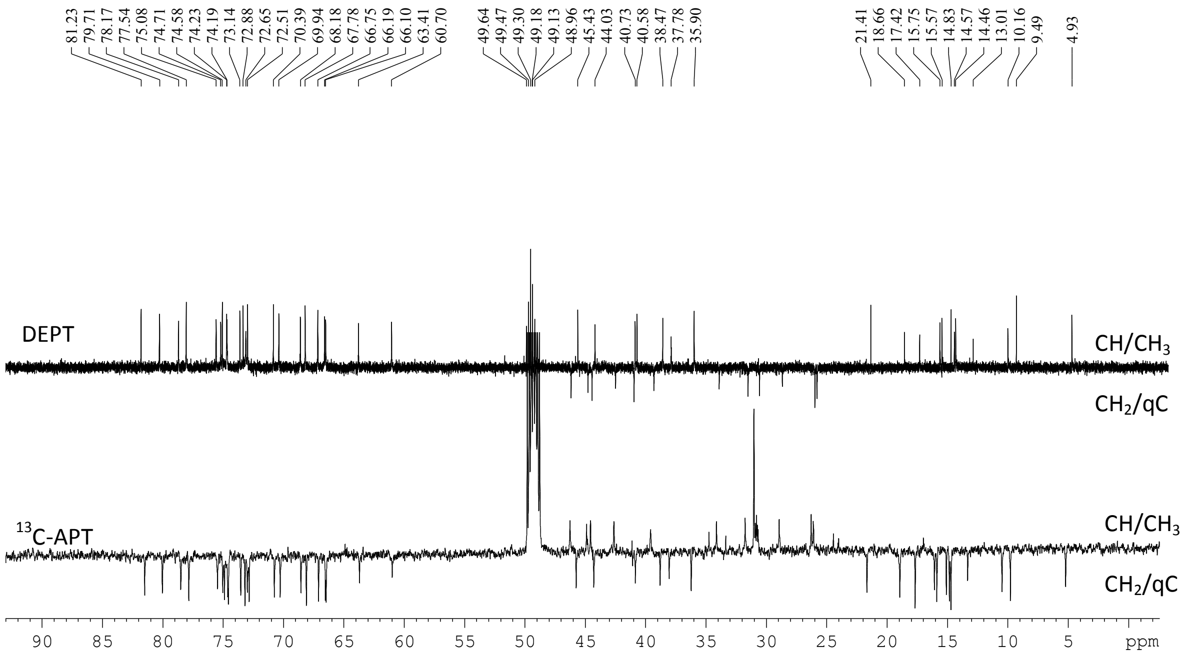


**Figure S6a.** Comparative expansions of $^{13}$C-NMR and DEPT spectra of langkolide in methanol-$d_4$ (500 MHz): a) expansion between 0 and 95 ppm.

b)

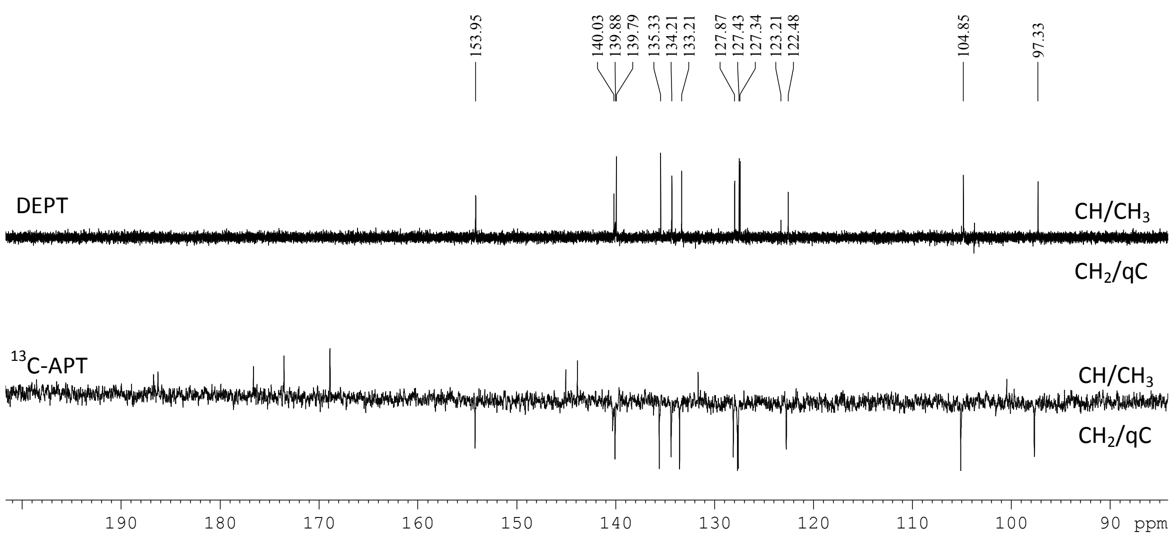


**Figure S6b.** Comparative expansions of $^{13}$C-NMR and DEPT spectra of langkolide in methanol-$d_4$ (500 MHz): b) expansion between 90 and 200 ppm.

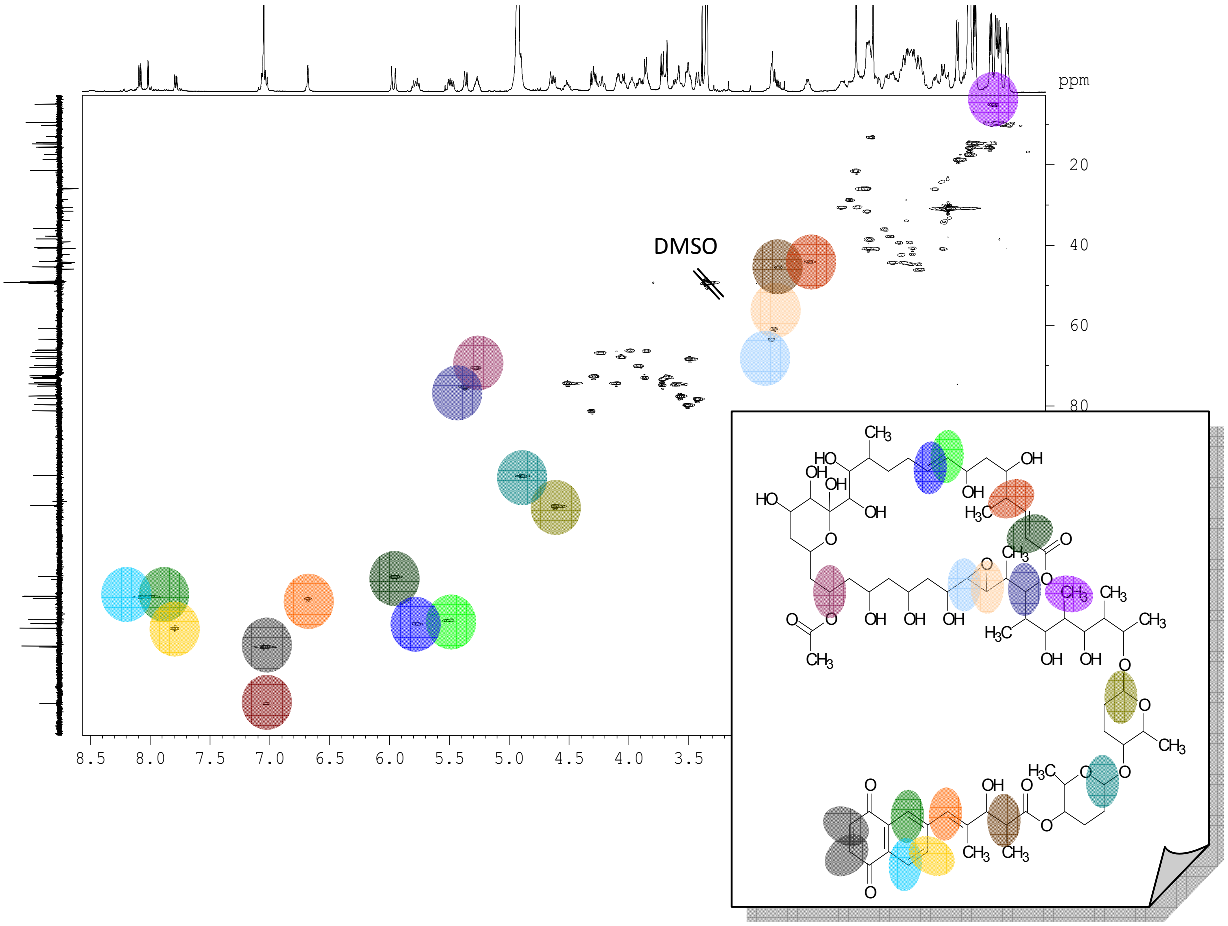


**Figure S7.** $^{1}$H-$^{13}$H-HSQC-NMR spectrum of langkolide (methanol-$d_4$, 500 MHz). Selected correlations are highlighted by colors circles in the spectrum as well as in the structure.

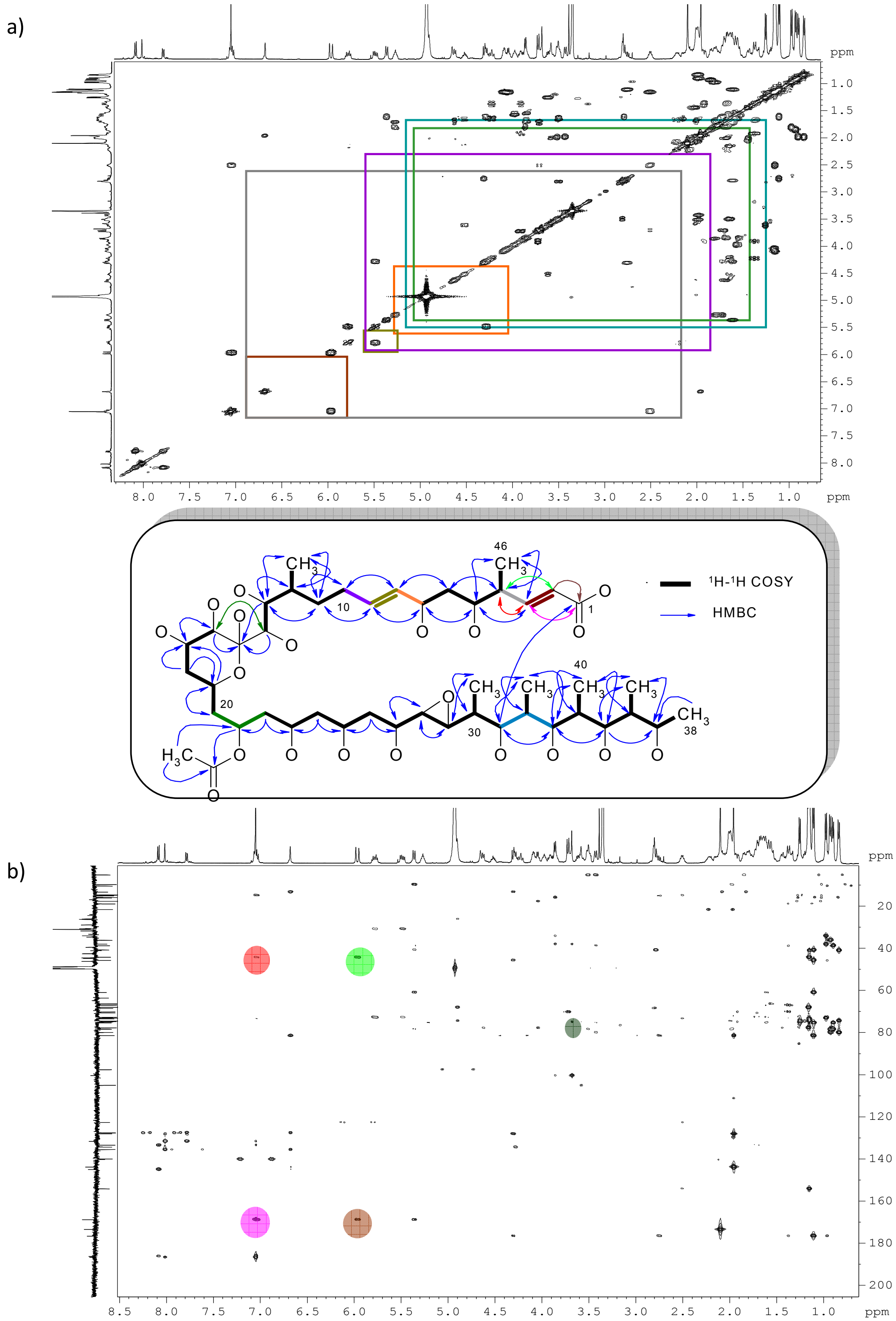


**Figure S8.** a) $^1$H-$^1$H-COSY-NMR spectrum of langkolide, b) $^1$H-$^{13}$C-HMBC-NMR spectrum of langkolide. Selected correlations are highlighted by colors in both spectra as well as in the structural moiety C1-C46 of langkolide.

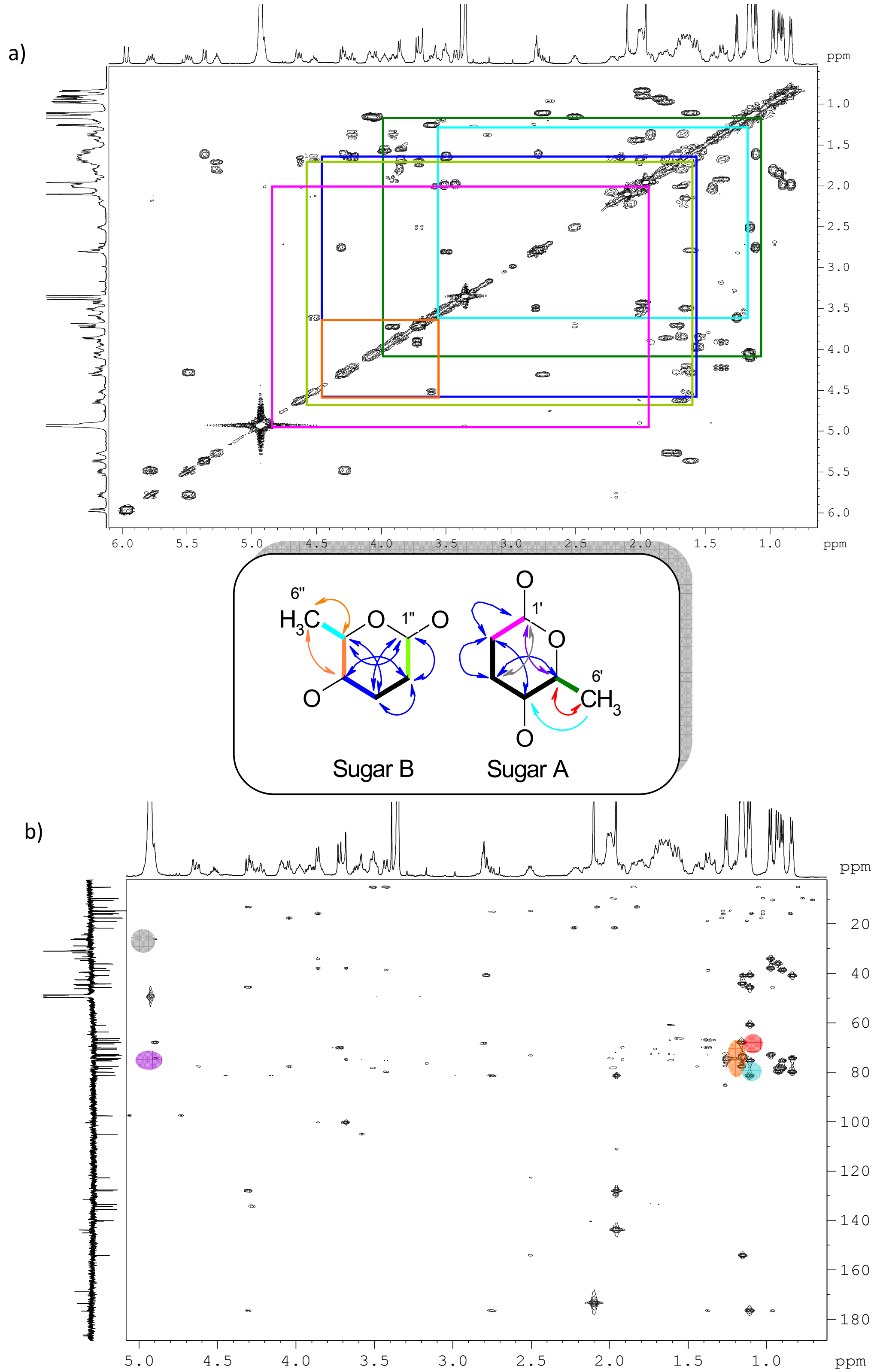


**Figure S9.** a) $^{1}$H-$^{1}$H-COSY-NMR spectrum of langkolide, b) $^{1}$H-$^{13}$C-HMBC-NMR spectrum of langkolide. Selected correlations are highlighted by colors in both spectra as well as in the structural moieties C1´-C6´ and C1´´-C6´´ of langkolide.

a)

b)

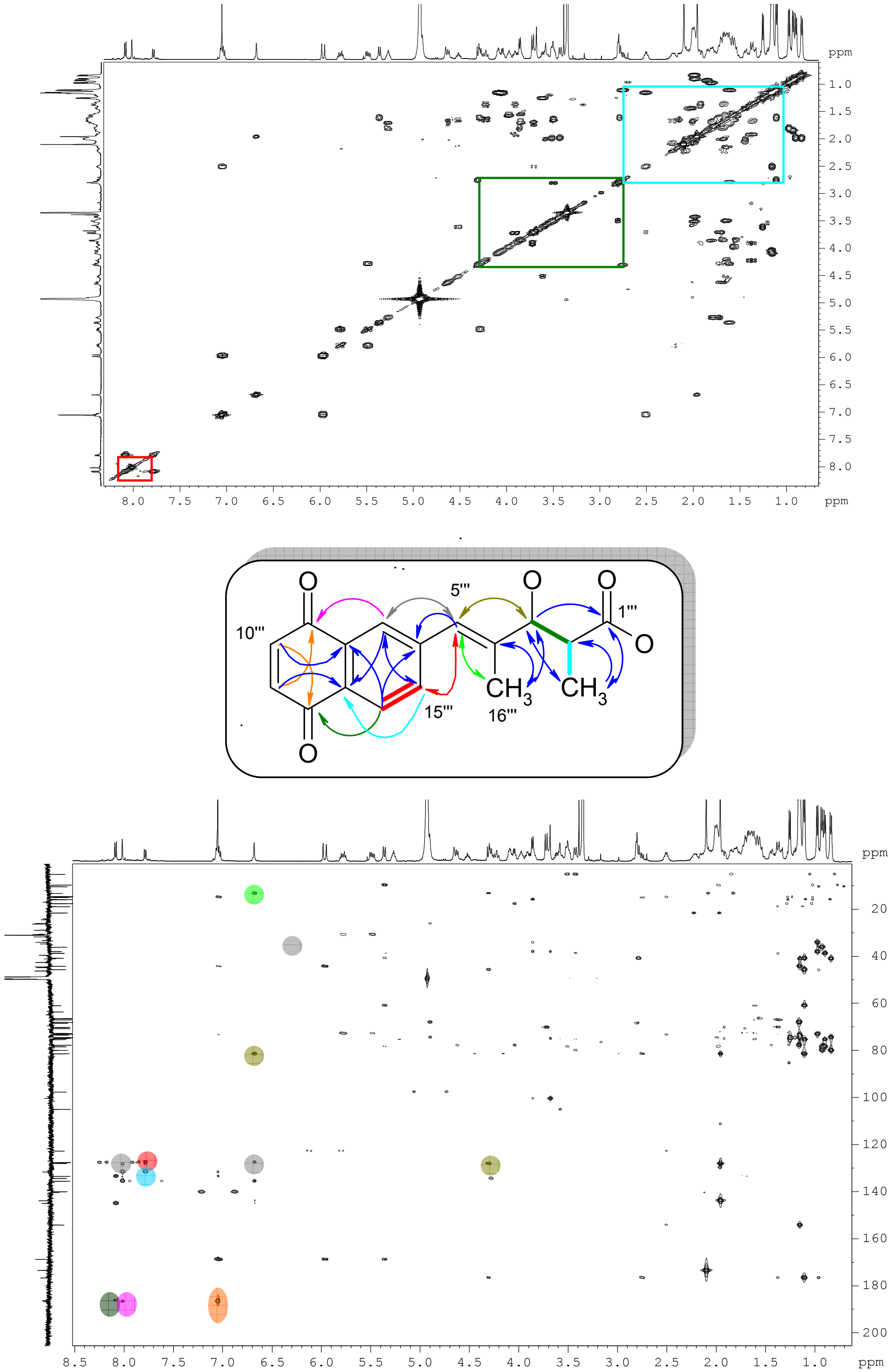


**Figure S10.** a) $^1$H-$^1$H-COSY-NMR spectrum of langkolide, b) $^1$H-$^{13}$C-HMBC-NMR spectrum of langkolide. Selected correlations are highlighted by colors in both spectra as well as in the structural moiety C1´´´-C17´´´ of langkolide.

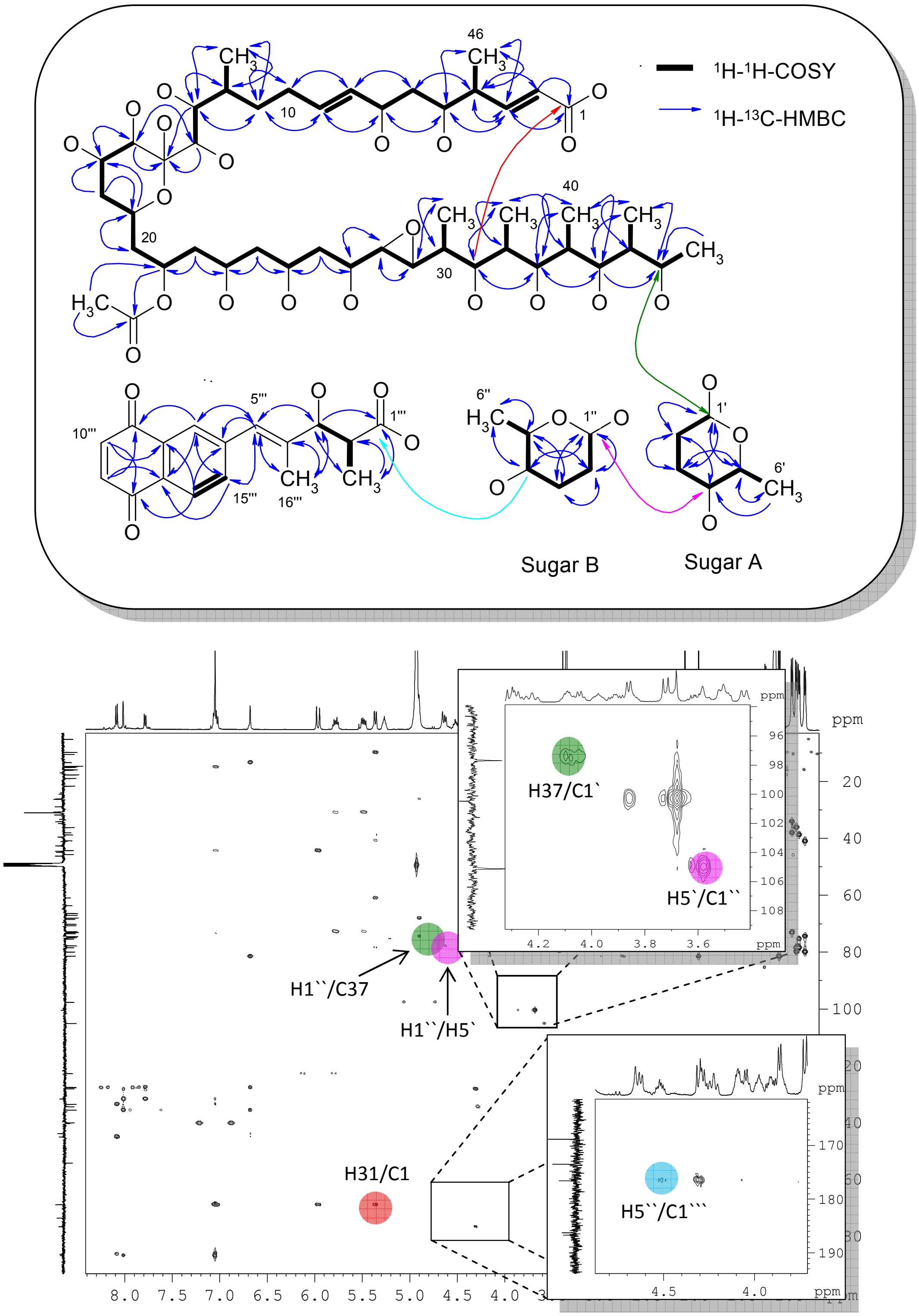


**Figure S11.** $^1$H-$^{13}$C-HMBC-NMR spectrum of langkolide and the full 2D NMR correlations of langkolide. The three glycosidic linkages in langkolide are highlighted in colors in the spectrum as well as in the structure.

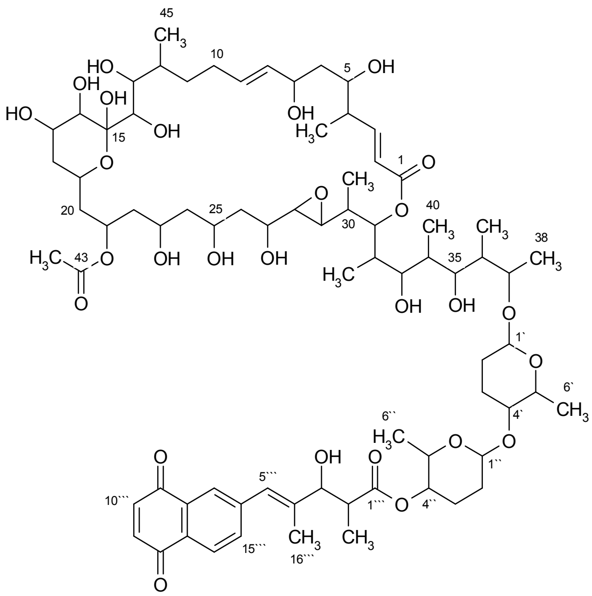

**Figure S12.** Constitutional formula of langkolide according to the data obtained from 1D- ($^{1}$H-NMR, $^{13}$C-NMR and DEPT) and 2D- ($^{1}$H-$^{1}$H-COSY, $^{1}$H-$^{13}$C-HSQC and $^{1}$H-$^{13}$C-MHBC) NMR experiments.

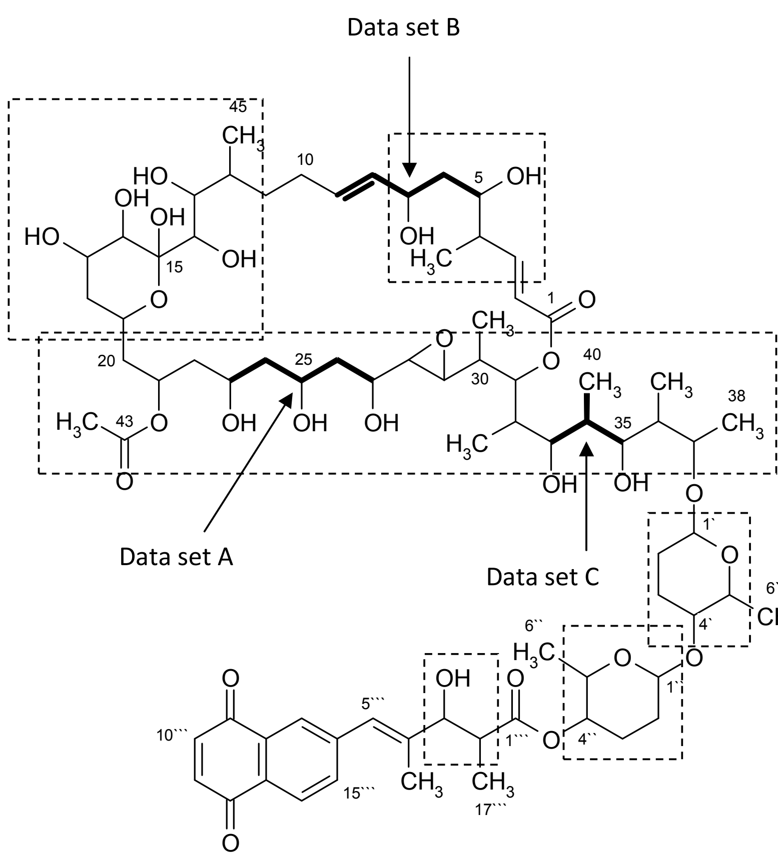


**Figure S13.** Stereoclusters of langkolide including 32 chiral centers.

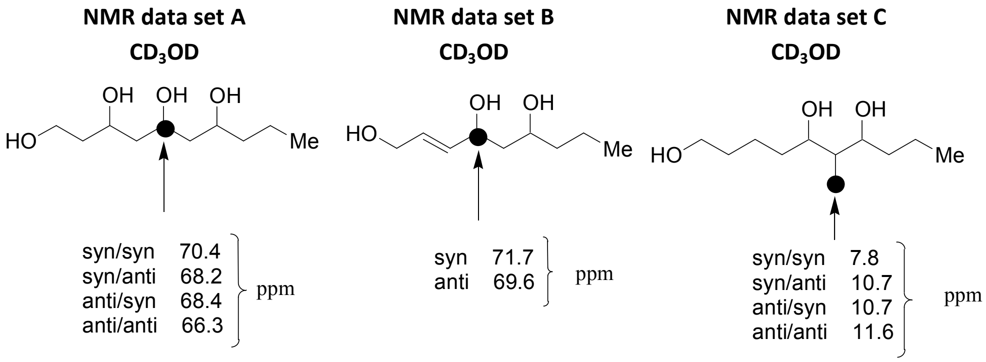


**Figure S14.** Kishi NMR data sets for elucidation of the relative configuration of 1,3,5-triols, 1,3-diols and 2-methy-1,3-diols (for comparison $^{13}$C chemical shifts of data set C in $CDCl_3$ *syn*/*syn* (4.40 ppm), *syn*/*anti* (11.7 ppm), *anti*/*syn* (11.6 ppm) and *anti*/*anti* (12.8 ppm)).[1,2]

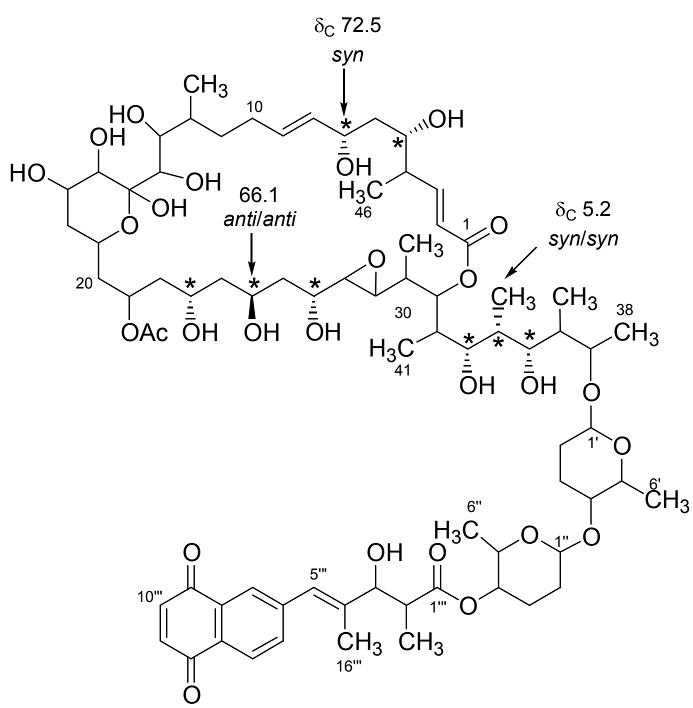


**Figure S15.** The relative stereochemistry of eight chiral centers assigned using Kishi NMR data sets (C5-C7, C23-C27, C33-C35).

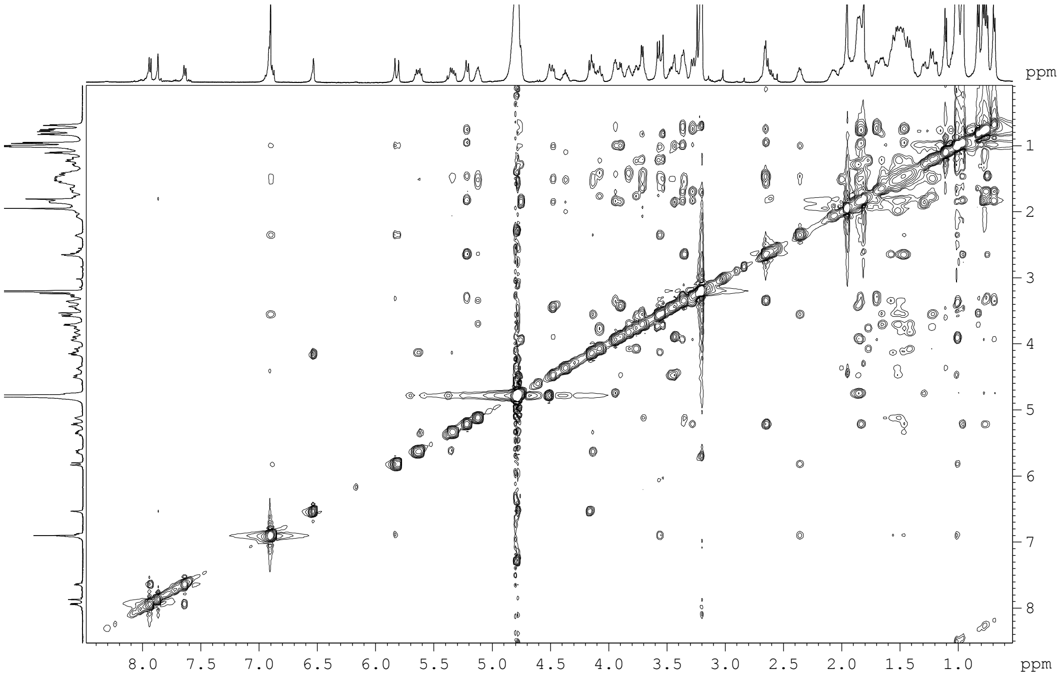


**Figure S16.** NOESY spectrum of langkolide in methanol-$d_4$ (500 MHz, relaxation time 600 ms).

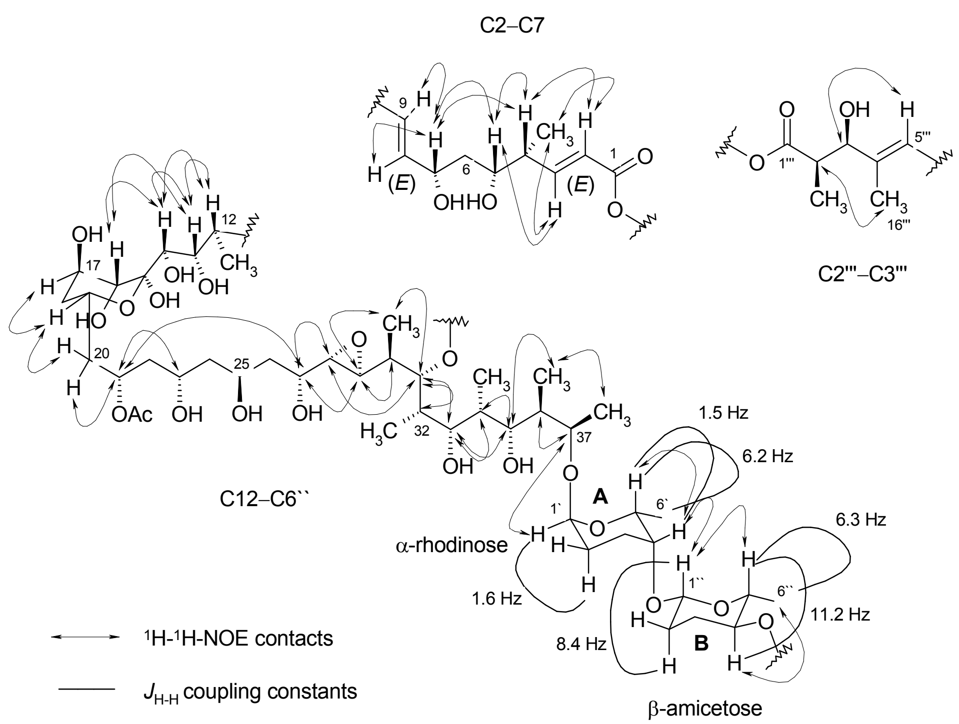


**Figure S17.** NOE correlations of the three stereoclusters of langkolide (C2-C7, C12-C6´´ and C2´´´-C3´´´) and coupling constants of the carbohydrate residues α-rhodinose and β-amicetose.

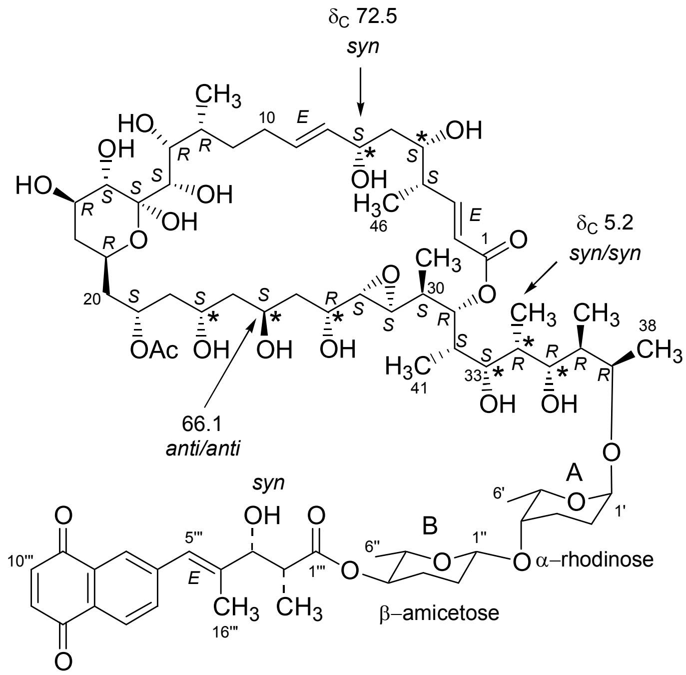


**Figure S18.** Relative stereochemistry of langkolide. Stereocenters marked with asterisk were assigned on the basis of Kishi data sets; other stereocenters were assigned on the basis of NOESY-NMR data. The proposed absolute stereochemistry has been derived from the comparative assignment with brasilinolide C[3] and the abundance of L-rhodinose and D-amicetose in natural products.

## References

1. (a) Kobayashi, Y.; Tan, C.-H.; Kishi, Y. *Angew. Chem. Int. Ed.* **2000**, *39*, 4279-4281. (b) Tan, C.-H.; Kobayashi, Y.; Kishi, Y. *Angew. Chem. Int. Ed.* **2000**, *39*, 4282-4284. (c) Kobayashi,Y.; Tan, C.-H.; Kishi, Y. *J. Am. Chem. Soc.* **2001**, *123*, 2076-2078.
2. Kobayashi, Y.; Czechtizky, W.; Kishi, Y. *Org. Lett.* **2003**, *5*, 93-96.
3. Komatsu, K.; Tasuda, M.; Tanaka, Y.; Mikami, Y.; Kobayashi. J. *J. Org. Chem.* **2004**, *69*, 1535-1541.